Supplementary information for

Exploration of 3,4-unsubstituted coumarins as thioredoxin reductase 1 inhibitor for cancer therapy

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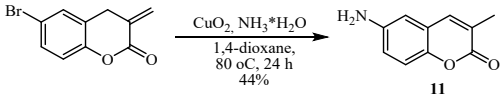
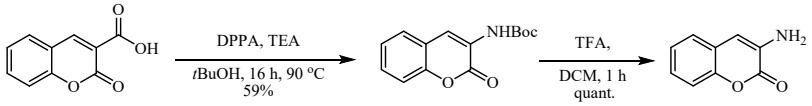
Synthesis of 6-amino-3-methyl-2H-chromen-2-one (11)



11

A 4 mL reaction vessel was charged with Cu_2O (15.6 mg, 0.10 mmol), 6-bromo-3-methylenechroman-2-one (260 mg, 1 mmol), 2 mL of 1,4-dioxane, 0.65 mL of ammonium hydroxide solution (29% NH3, 10.0 mmol) and a magnetic stir bar. The vessel was sealed with a Teflon screw cap, immersed in a preheated oil bath and the reaction mixture was stirred at 80 °C. Upon completion, the reaction mixture was cooled to rt, quenched with water, extracted with Et_2O and dried over anhydrous Na_2SO4 . The solvents were removed under vacuum and the residue was filtered through Celite pad, eluting with EtOAc. Submitted to biological assay without additional purification. Yield: 84 mg (44%), dark brown amorphous compound. **IR** spectrum ($CDCl_3$), ν, cm^{-1}: 3442, 3355 (NH_2), 3247, 1699 (CO-coumarin), 1395. **^{1}H NMR** (400 MHz, $CDCl_3$-*d*) δ 7.38 (s, 1H); 7.13 (d, *J* = 8.7 Hz, 1H); 6.80 (dd, *J* = 8.8, 2.7 Hz, 1H); 6.66 (d, *J* = 2.8 Hz, 1H); 2.19 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 162.7; 146.8; 143.0; 139.1; 126.2; 120.3; 118.5; 117.3; 111.1; 17.4. HRMS (ESI^+) m/z calculated for $C_{10}H_{10}NO_2$ $[M+H]^+$ 176.0712, found 176.0710. (obtained by modified procedure) [1]

Synthesis of 3-amino-2H-chromen-2-one (12)



Boc-12 **12**

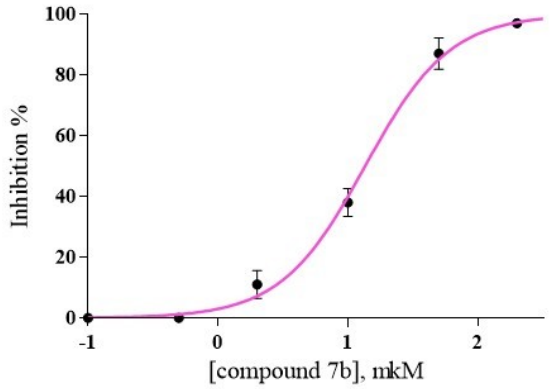
***t*Butyl (2-oxo-2H-chromen-3-yl)carbamate (Boc-12)**. Triethylanine (3.7 mmol, 1 equiv) and subsequently diphenylphosphorylazide (DPPA) (3.7 mmol, 1 equiv) were added dropwise to a refluxing solution of 2-oxo-2H-chromene-3-carboxylic acid (3.7 mmol, 0.7 g, 1 equiv) in *t*BuOH (8 mL) with stirring. The reaction mixture was refluxed for 16 h (90 °C). Upon completion, the reaction mixture was filtered and concentrated under reduced pressure, giving the oily residue, which was purified by flash chromatography, eluent EtOAc:Hexane (gradient 1:8 to 1:4) to provide a white solid. Yield: 570 mg (59%). **^{1}H NMR** (400 MHz, $CDCl_3$-*d*) δ 8.27 (s, 1H); 7.46 (dd, *J* = 7.7, 1.6 Hz, 1H); 7.45 – 7.36 (m, 2H); 7.34 – 7.23 (m, 2H, partially overlapped with the solvent signal); 1.53 (s, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 158.8; 152.6; 149.6; 129.1; 127.4; 125.2; 124.7; 120.5; 120.2; 116.4; 81.9; 28.35.

To the solution of **Boc-12** (570 mg, 2.2 mmol) in DCM (20 mL) was added TFA (2 mL). The reaction mixture was stirred at room temperature till full consumption of the starting material, (TLC control reaction, eluent EtOAc). Upon the completion, the reaction mixture was evaporated and the residue was retaken in sat. aq. NaHCO3 (10 mL) and the product was extracted with EtOAc (2*15 mL). The organic phases were combined, dried over Na2SO4, filtrated and evaporated to provide a product as white solid. Yield: 345 mg (98%), white solid (mp 135.5 °C). **IR** spectrum ($CDCl_3$), ν, cm^{-1}: 3426, 3312, 1704, 1456. **^{1}H NMR** (400 MHz, $CDCl_3$-*d*) δ 7.33 – 7.23 (m, 3H, partially overlapped with the solvent signal); 7.25 – 7.16 (m, 1H); 6.70 (s, 1H); 4.26 (brs, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 159.6; 149.2; 132.1; 126.8; 125.2; 124.8; 121.3; 116.3; 111.0.

Reaction of inhibitor 7b with methyl 3-mercaptopropanoate in physiological conditions[3]

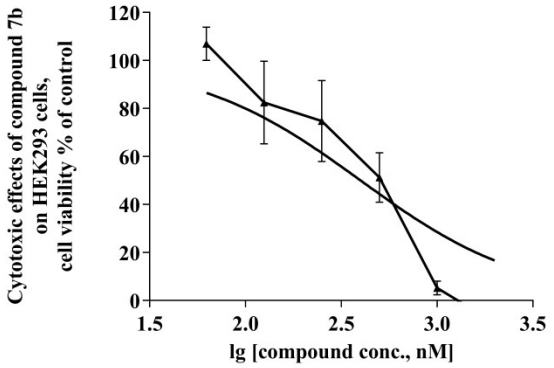
Into a 5 mL vessel, transfer 5 mL of PBS solution (pH 7.4) and a 0.05 mL of 3.0×10^{-4} mol/L of **7b** stock solution in DMF. Then appropriate volume of methyl 3-mercaptopropanoate 0.05mL of 0.6×10^{-4} mol/L in DMF was added by pipette. The resulting solution was stirred thoroughly at rt. The aliquots were taken in 30 min, 60 min and 90 min to proceed in GC-MS. (Gas chromatographic (GC) analysis was performed on Agilent Technologies gas chromatographer with triple-axis detector, heating range 40 – 280 ^{o}C, column 30 m x 0.25 mm, 0.25 μm, 7 inch cage).

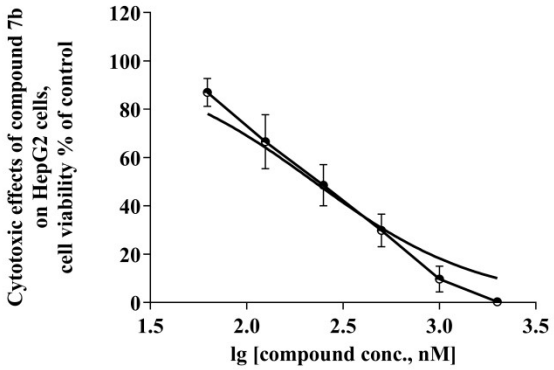
Dose- response curve for compound 7b, TrxR1 IC_{50}

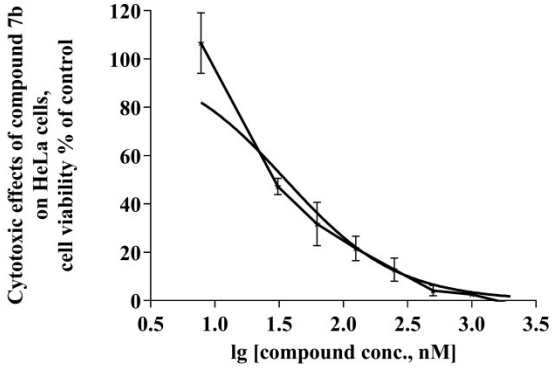


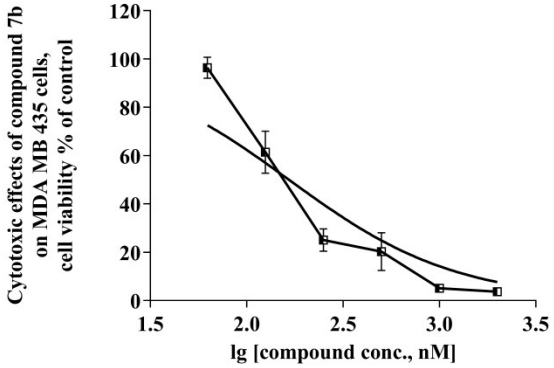
IC_{50}=13.56 μM, R squared=0.9828

Dose- response curve for compound 7b on different cell lines (representative examples)

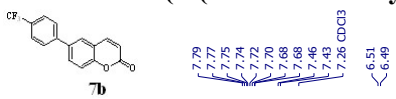


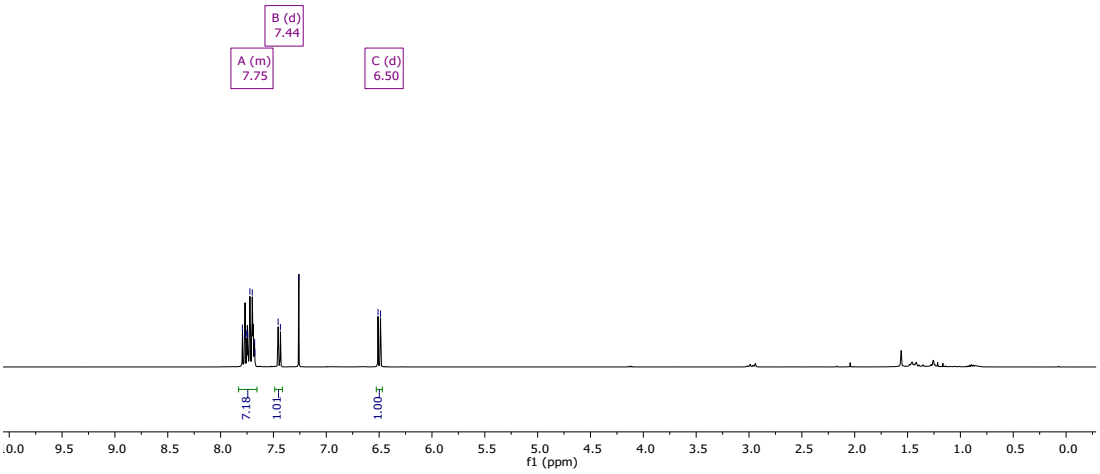


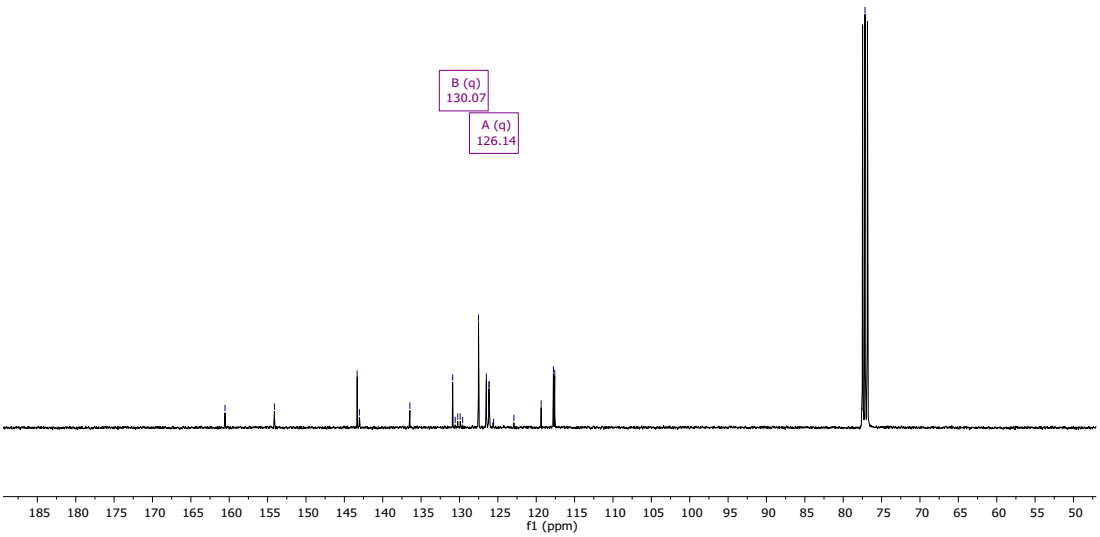


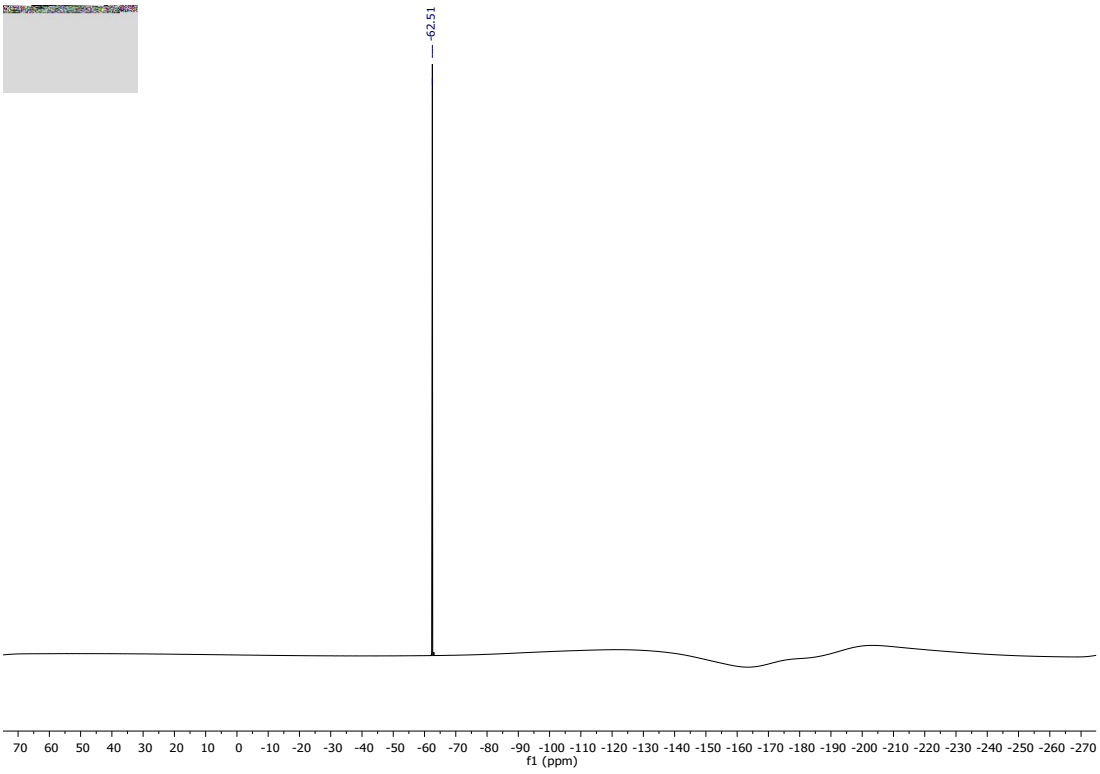


6-(4-(Trifluoromethyl)phenyl)-2H-chromen-2-one (7b) ^{1}H, ^{13}C, ^{18}F, IR, HRMS





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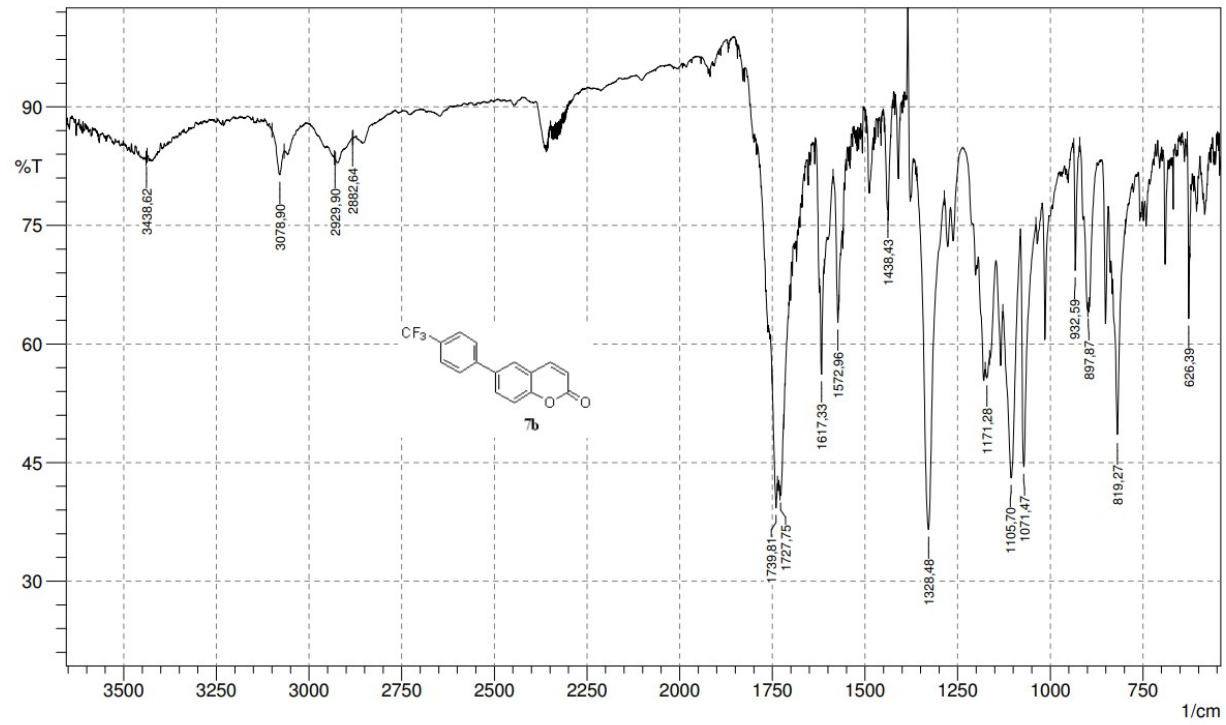
f1 (ppm)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CF3
O
O
7b
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1/cm

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5

Monoisotopic Mass, Even Electron Ions
291 formula(e) evaluated with 2 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-15 O: 0-15 F: 3-3

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
291.0640	100.00	291.0638	0.2	0.7	3.5	519.3	8.386	0.02	C H6 N12 O3 F3
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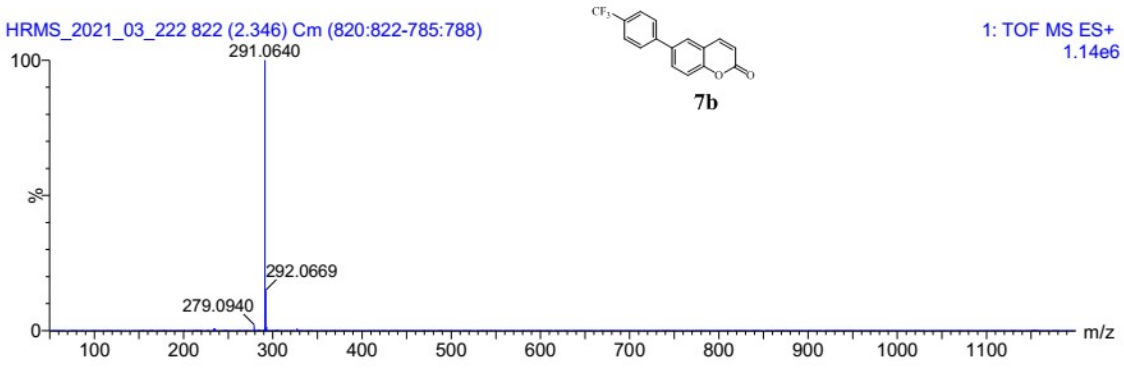
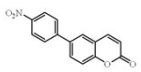


7b

6-(4-Nitrophenyl)-2H-chromen-2-one (8b) ^{1}H, ^{13}C, IR, HRMS



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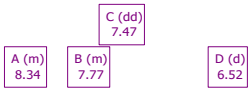
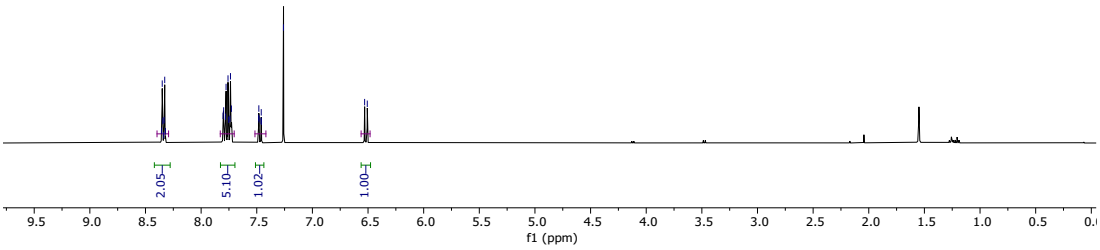
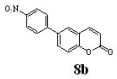
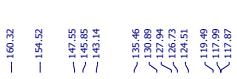
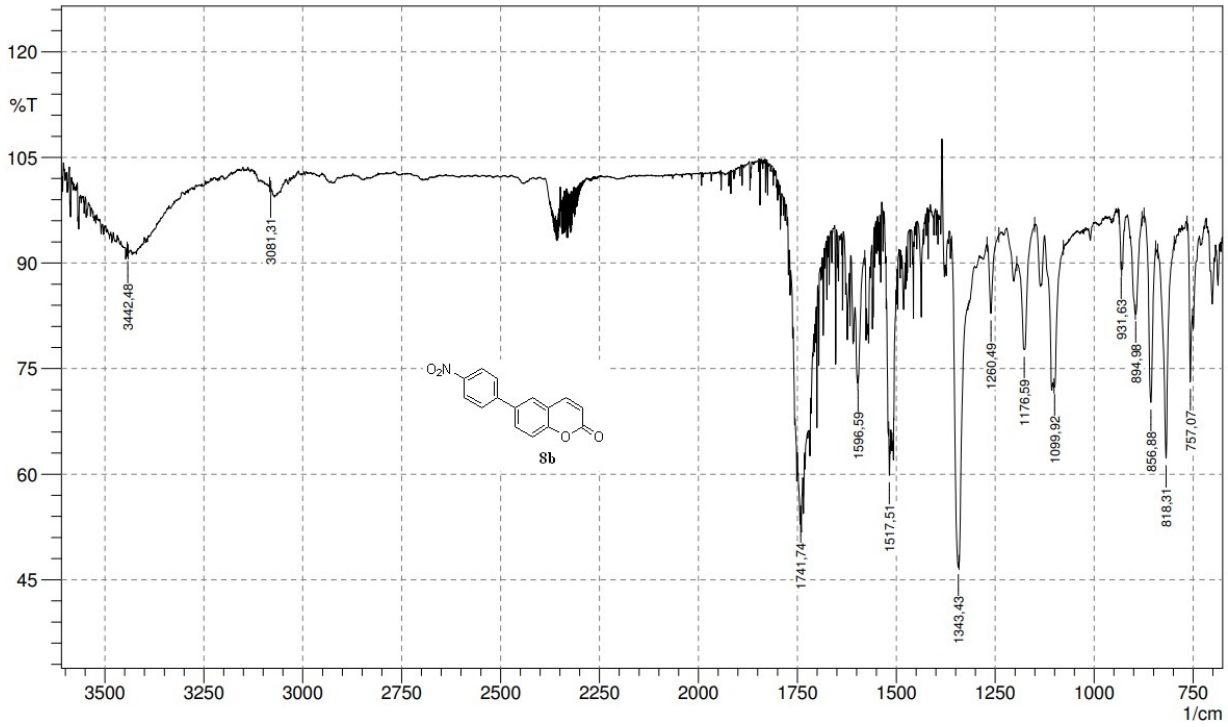






8b



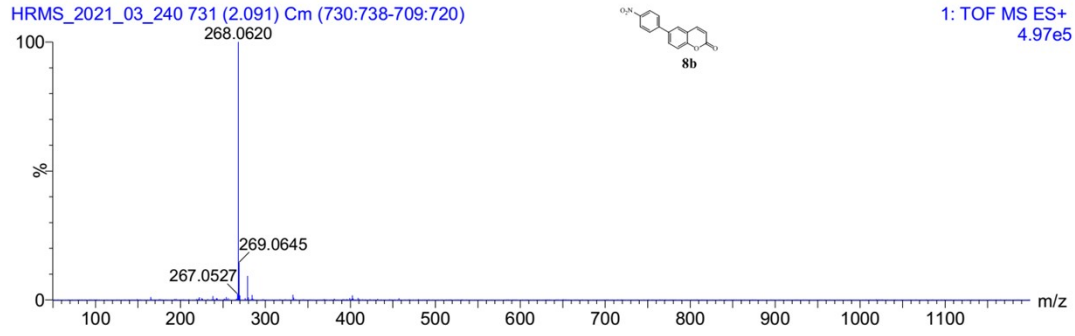


Elemental Composition Report:

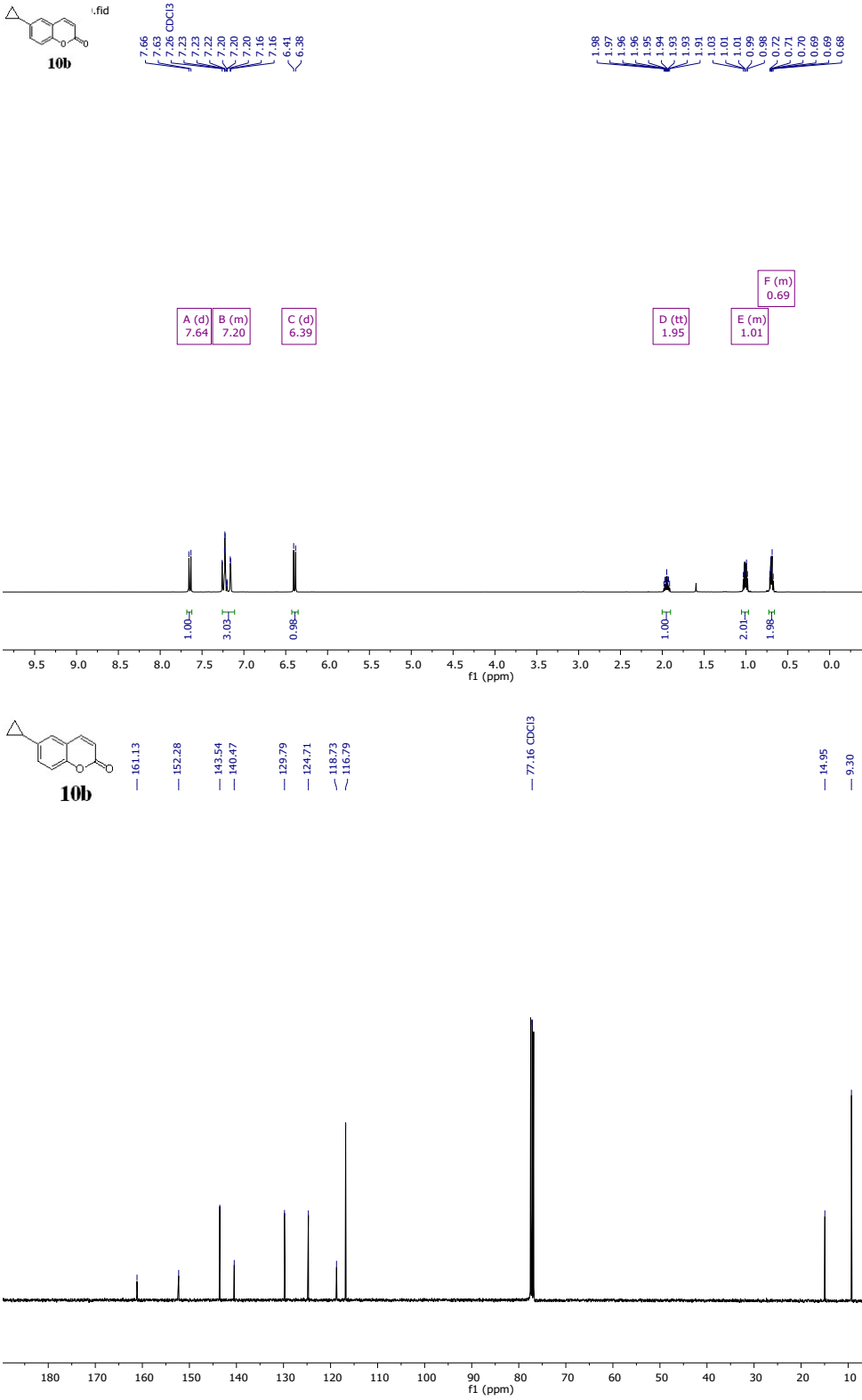
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5

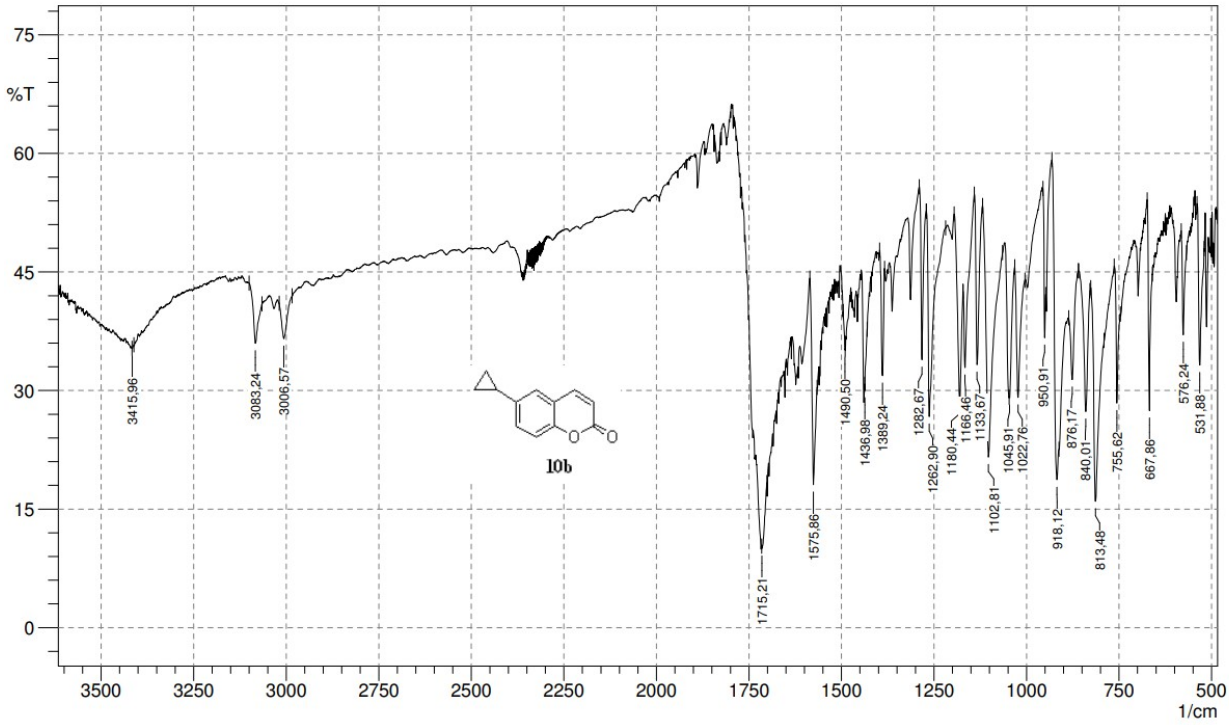
Monoisotopic Mass, Even Electron Ions
430 formula(e) evaluated with 4 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-15 O: 0-15

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
268.0620	100.00	268.0623	-0.3	-1.1	16.5	367.7	0.374	68.79	C16 H6 N5
		268.0615	0.5	1.9	4.5	372.8	5.486	0.41	H6 N13 O5
		268.0628	-0.8	-3.0	-1.5	372.3	5.020	0.66	C3 H14 N3 O11
		268.0610	1.0	3.7	11.5	368.5	1.199	30.14	C15 H10 N O4



6-Cyclopropyl-2H-chromen-2-one (10b) ^{1}H, ^{13}C, IR, HRMS





Elemental Composition Report:

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Number of isotope peaks used for i-FIT = 5

Monoisotopic Mass, Even Electron Ions
192 formula(e) evaluated with 1 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-15 O: 0-15

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
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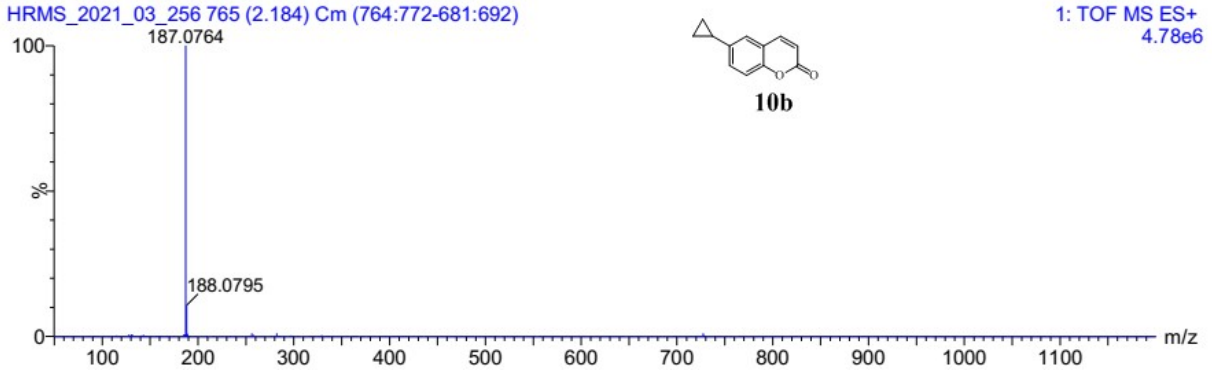
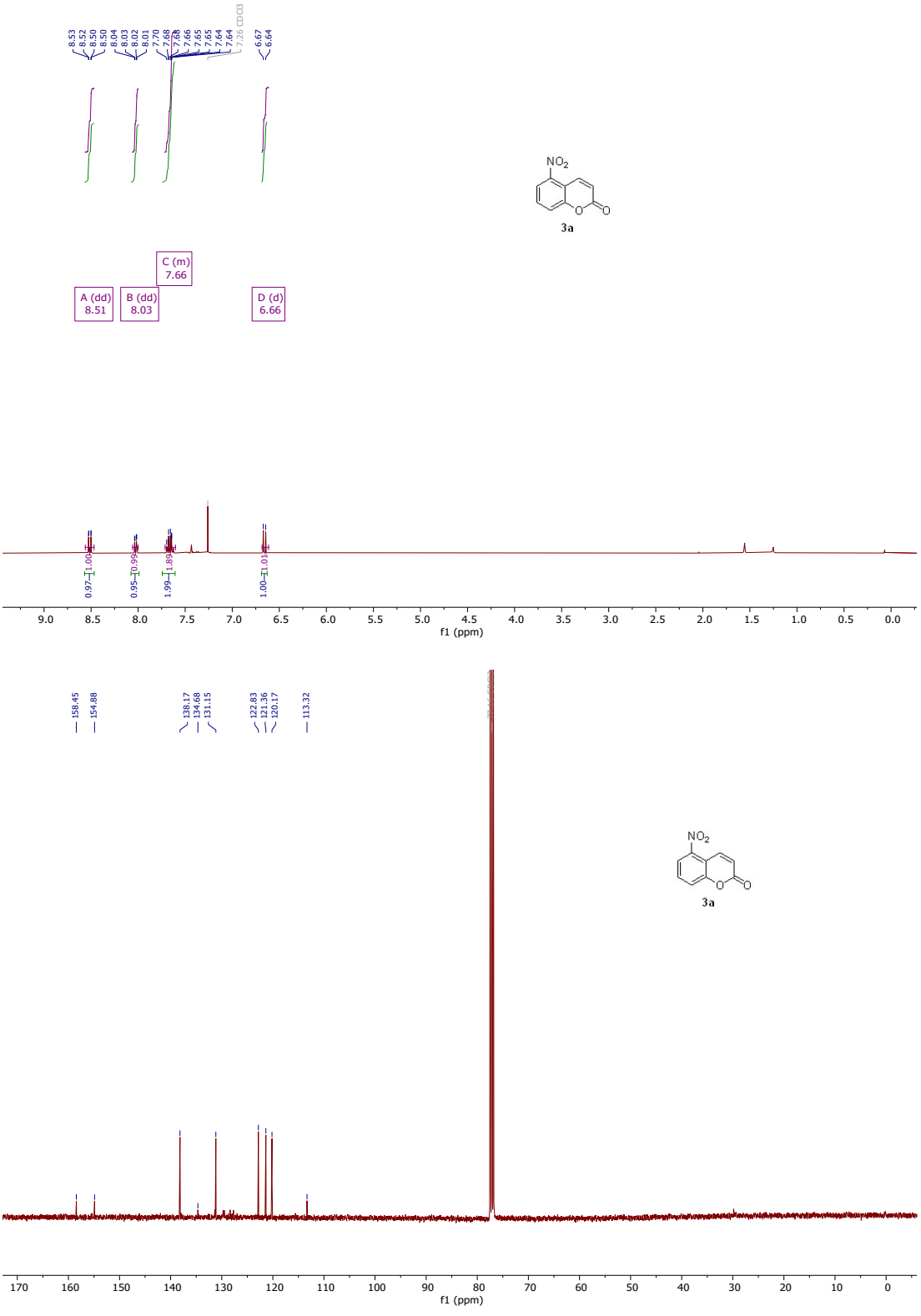
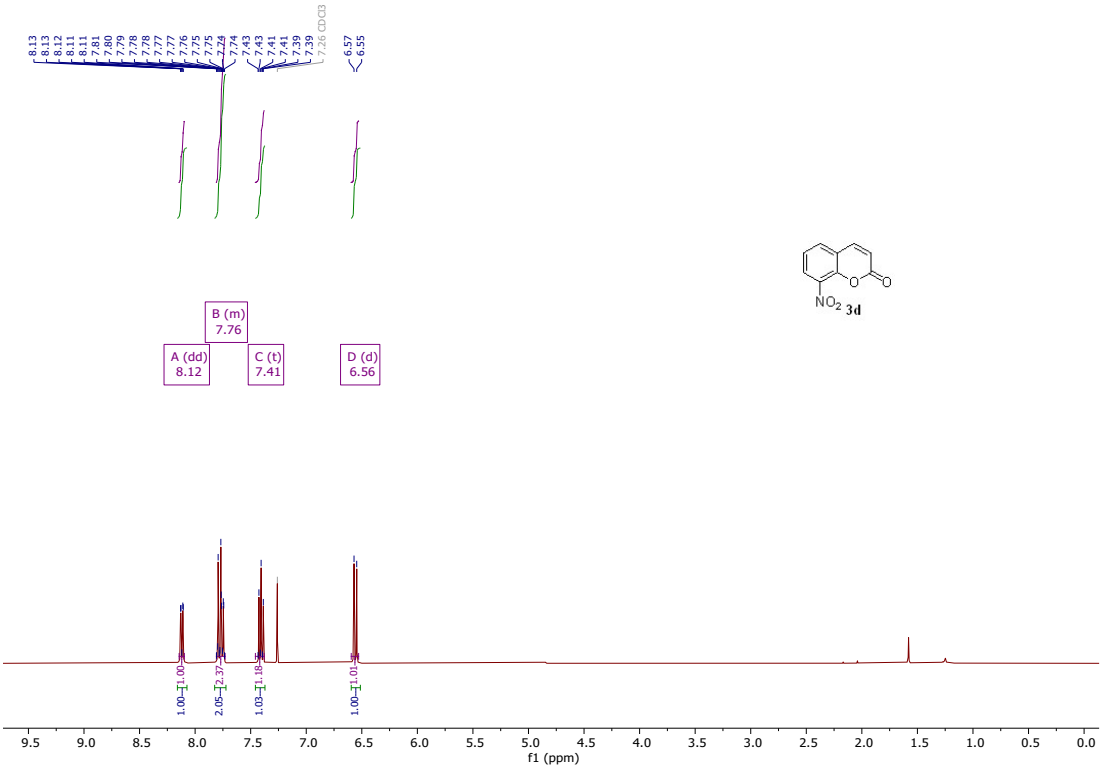
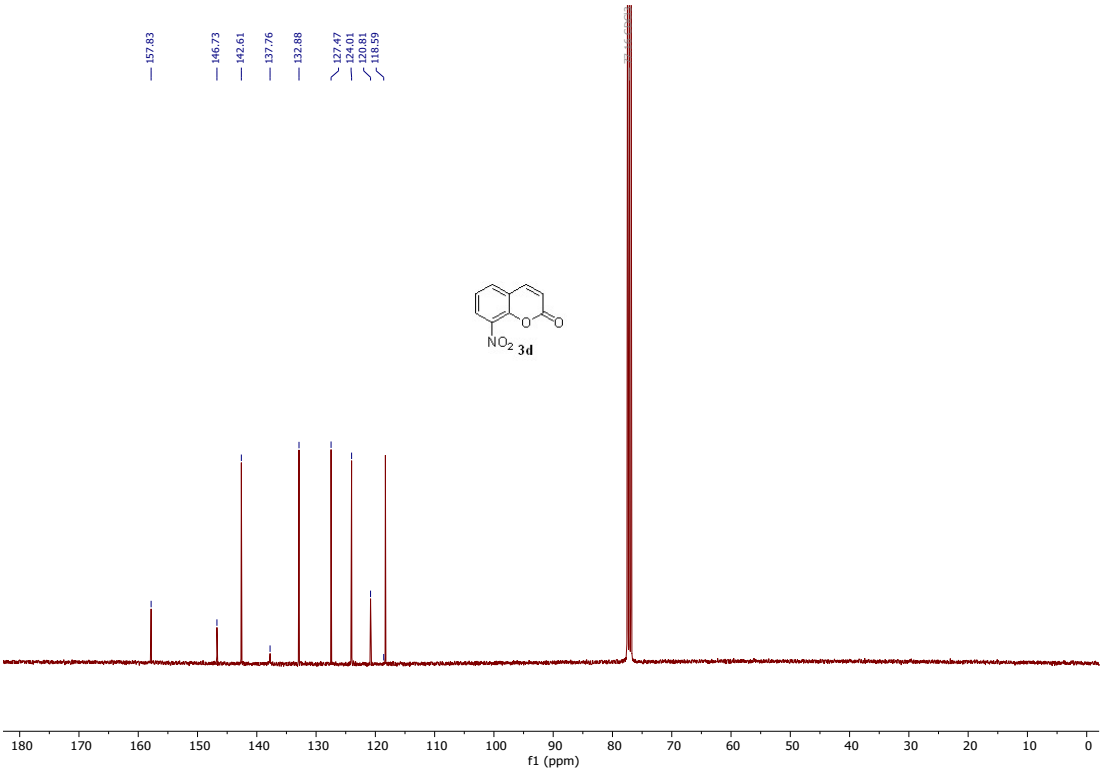


5-Nitro-2H-chromen-2-one (3a) ^{1}H and ^{13}C

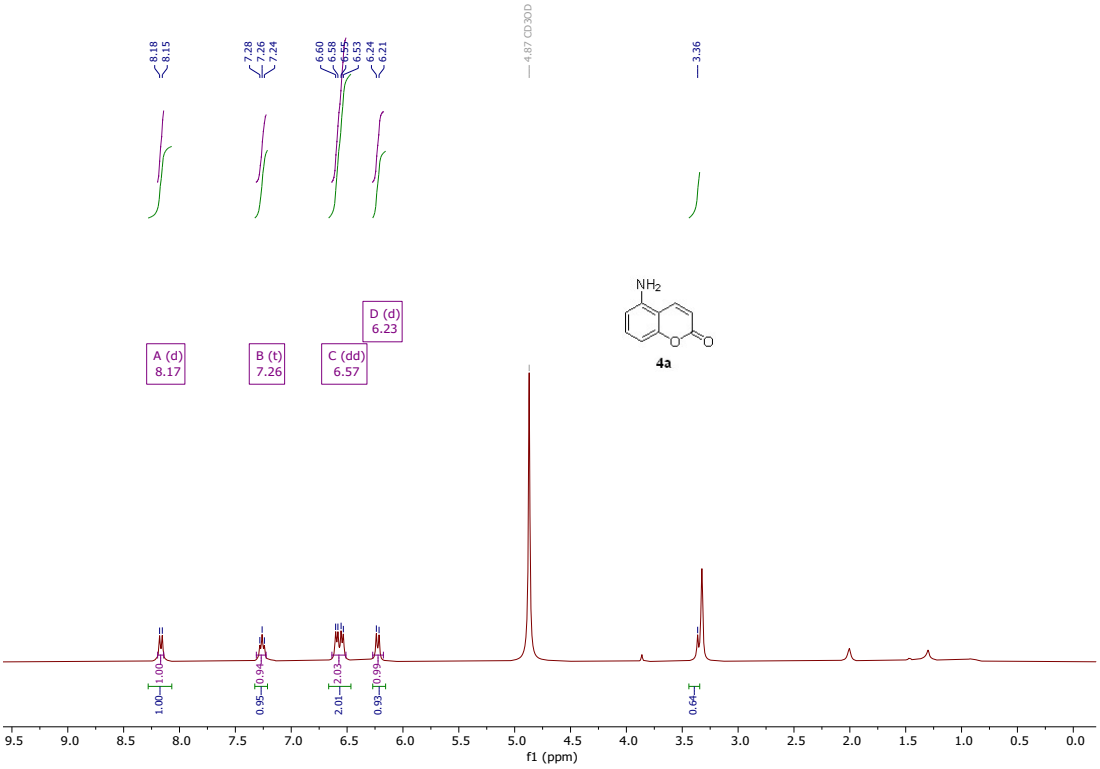


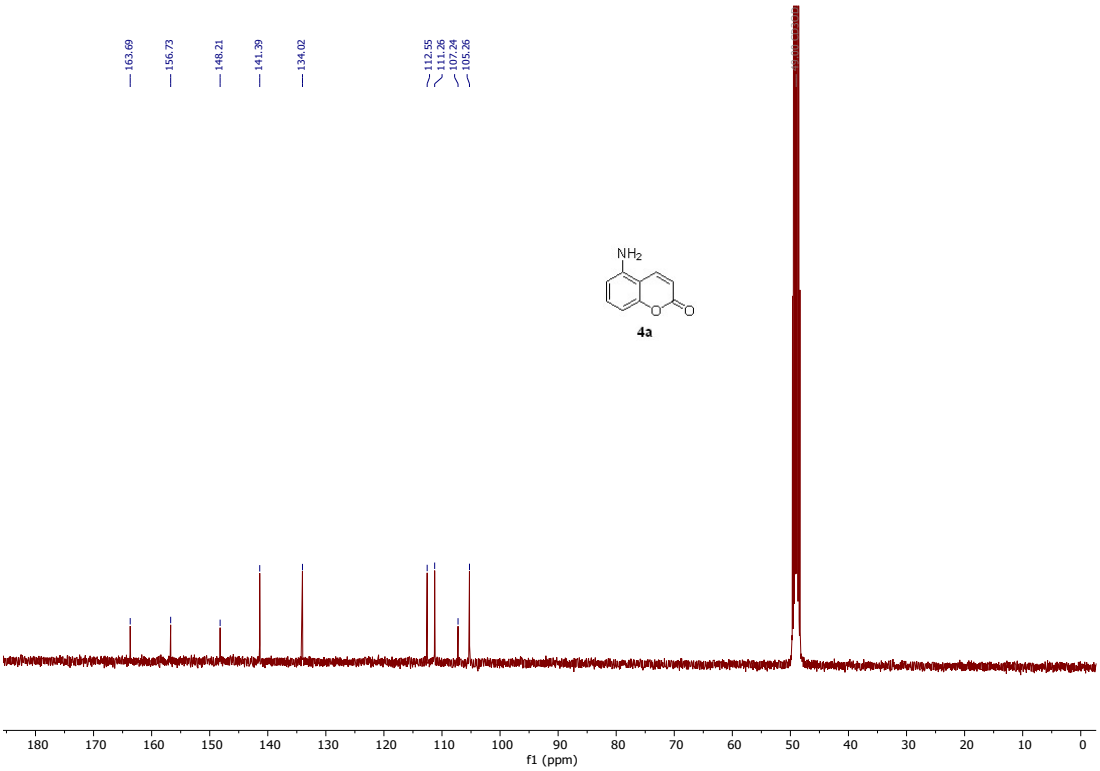
8-Nitro-2H-chromen-2-one (3d) ^{1}H and ^{13}C



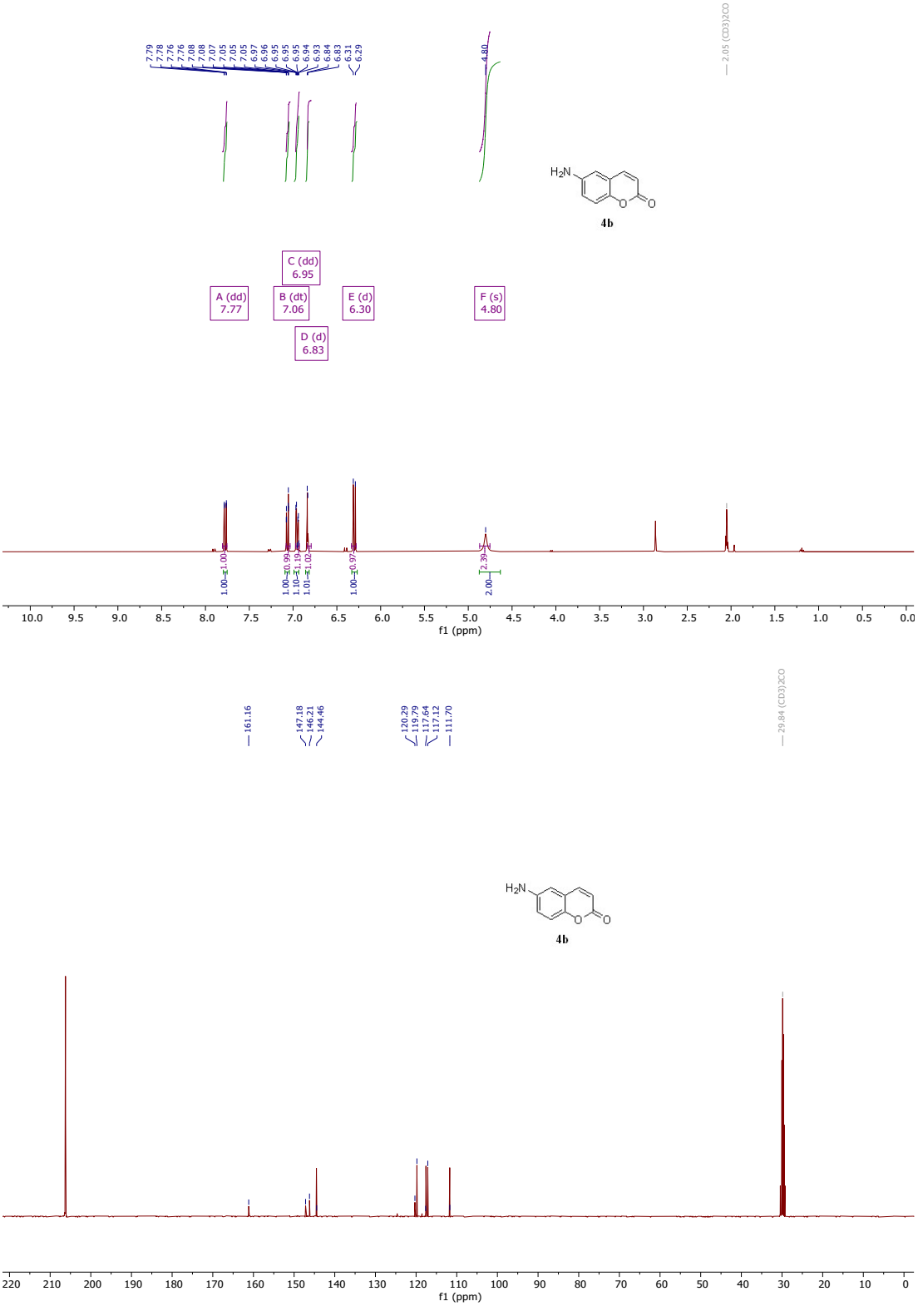


5-Amino-2H-chromen-2-one (4a) ^{1}H and ^{13}C





6-Amino-2H-chromen-2-one (4b) ^{1}H and ^{13}C



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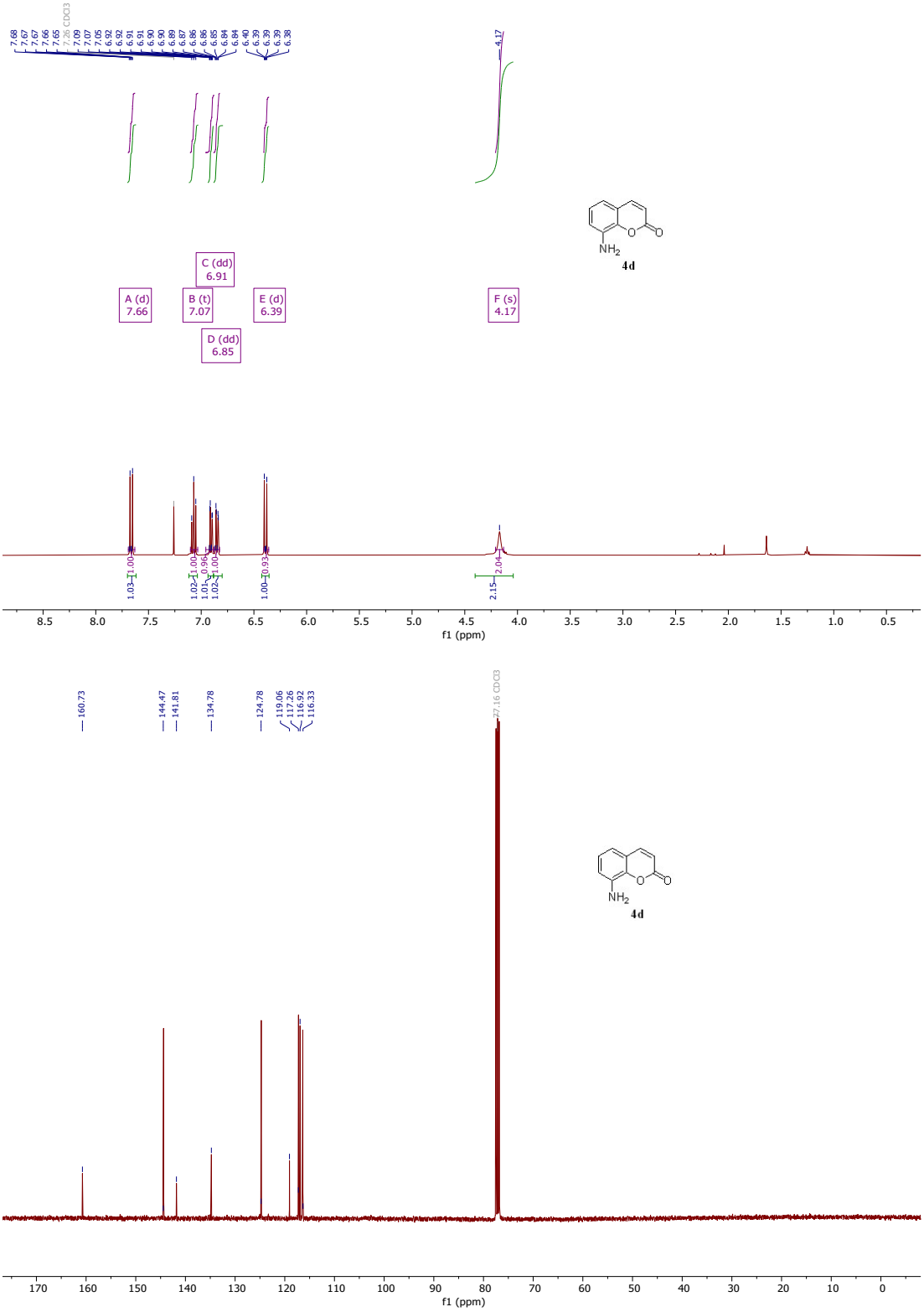

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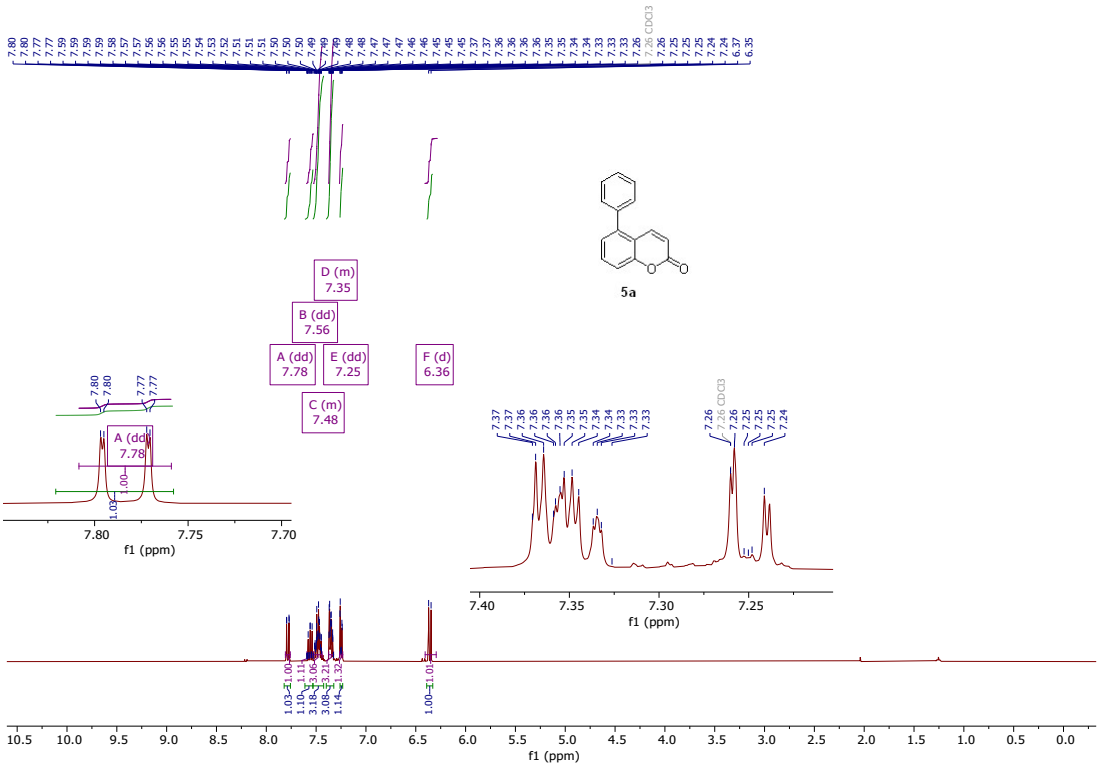
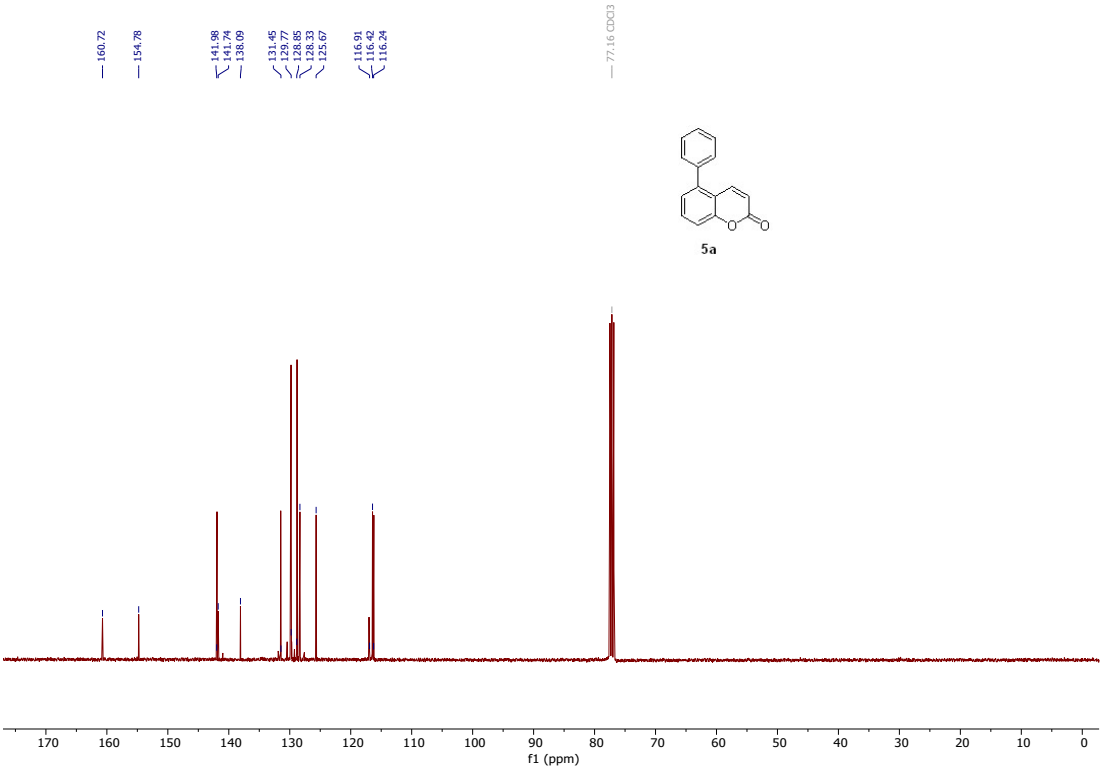
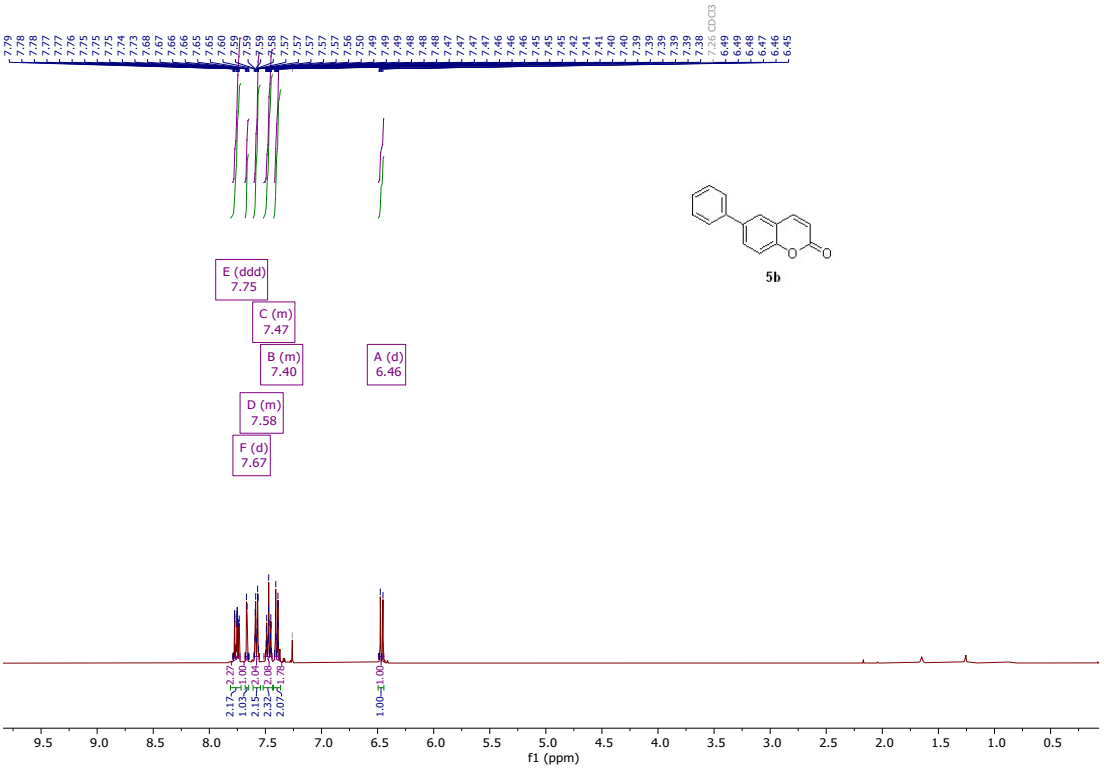
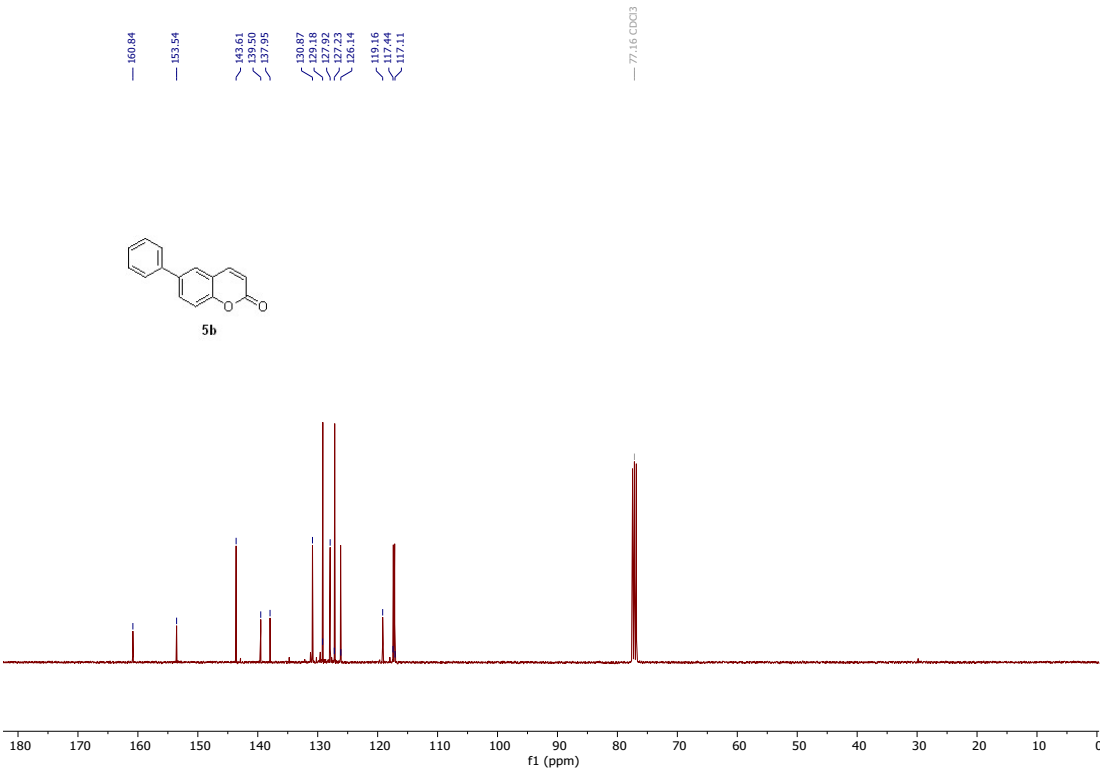


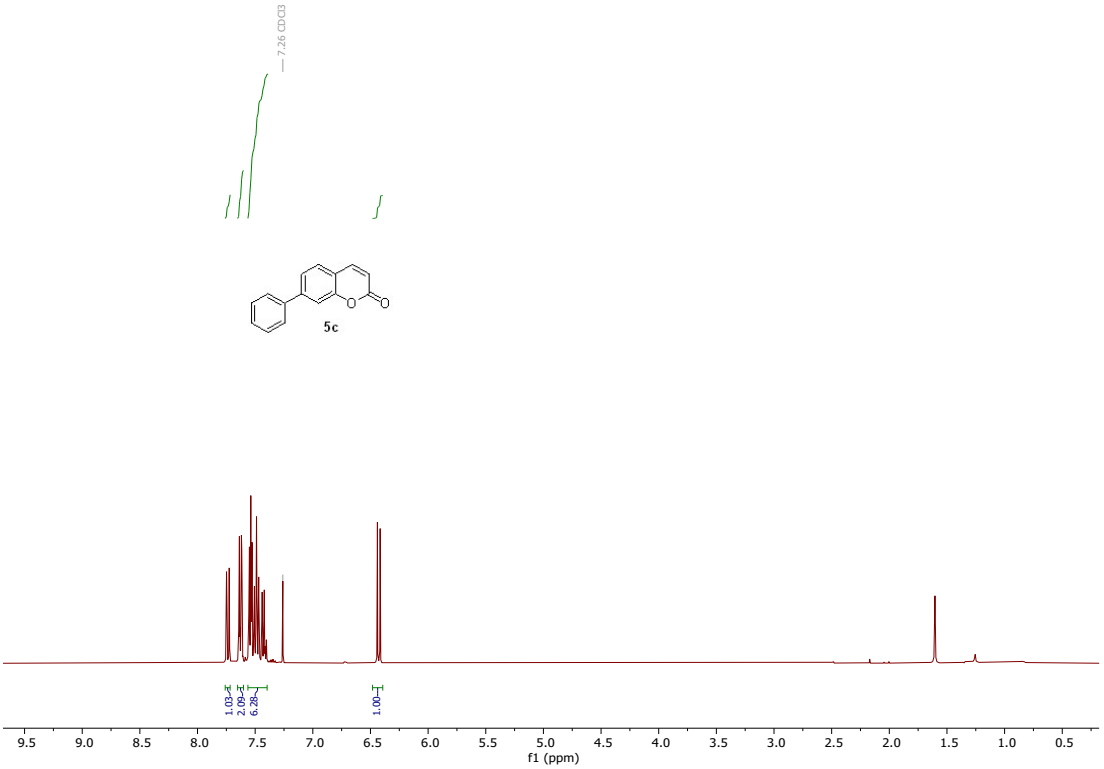


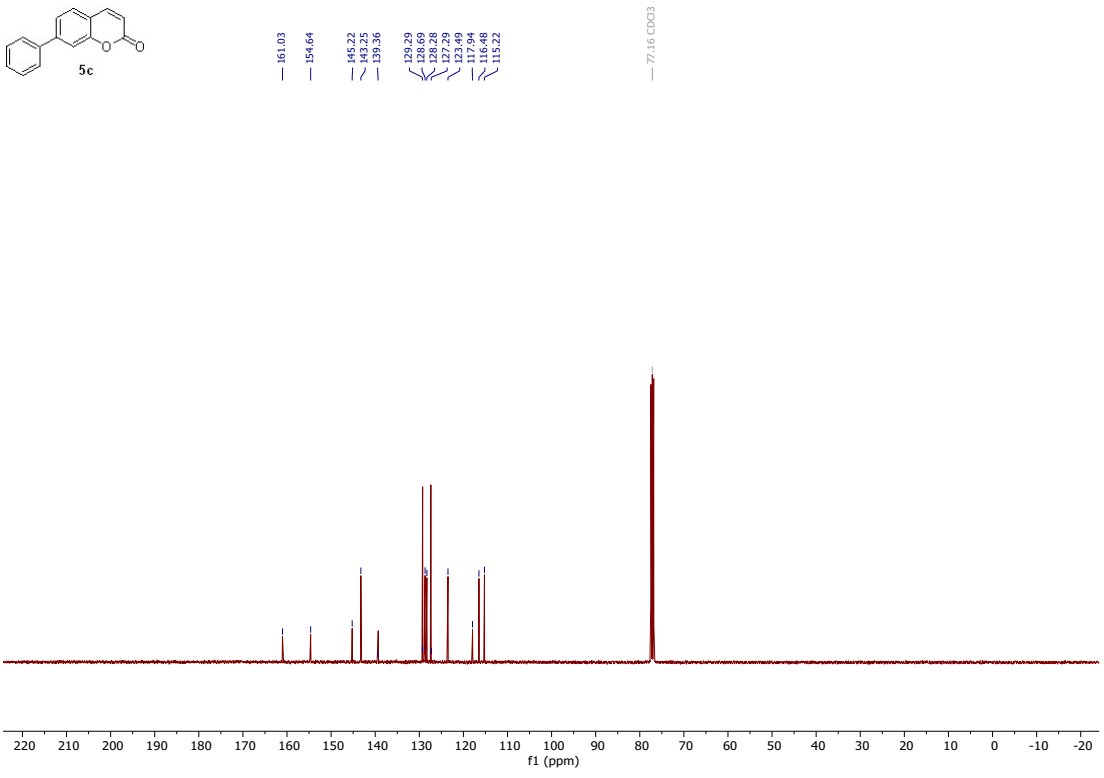
6-Phenyl-2H-chromen-2-one (5b) ^{1}H and ^{13}C



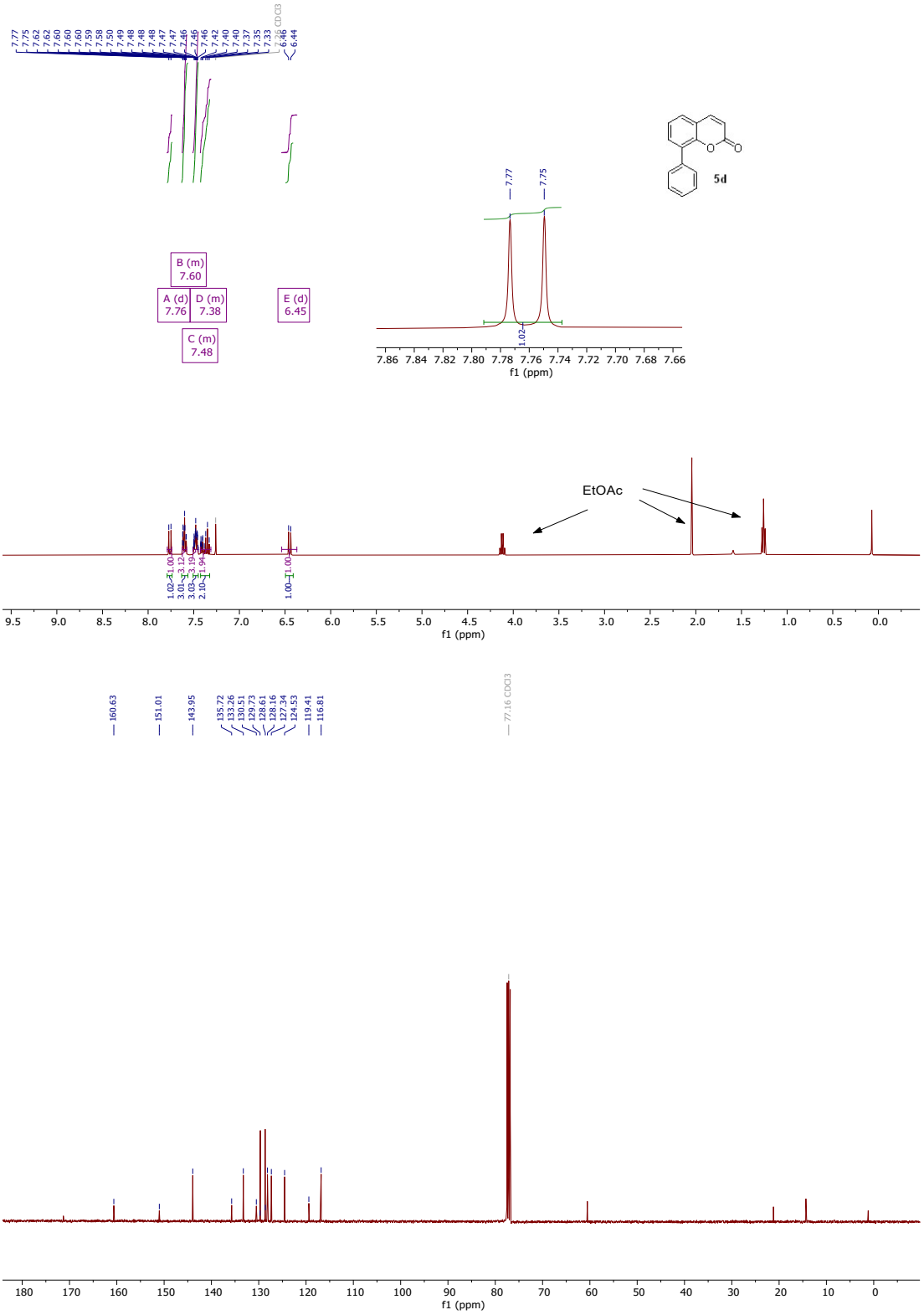


7-Phenyl-2H-chromen-2-one (5c) ^{1}H and ^{13}C





8-Phenyl-2H-chromen-2-one (5d) ^{1}H and ^{13}C



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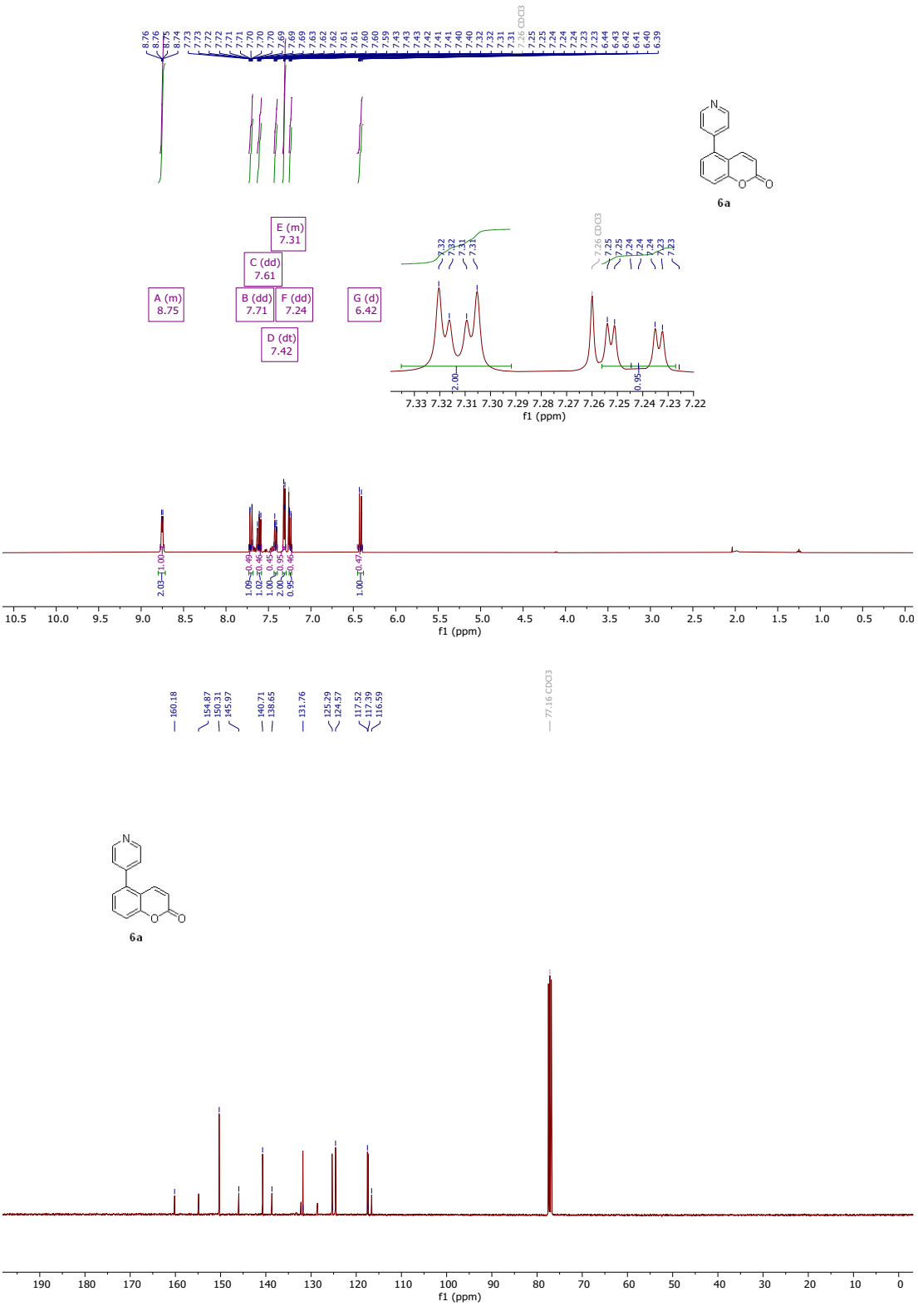
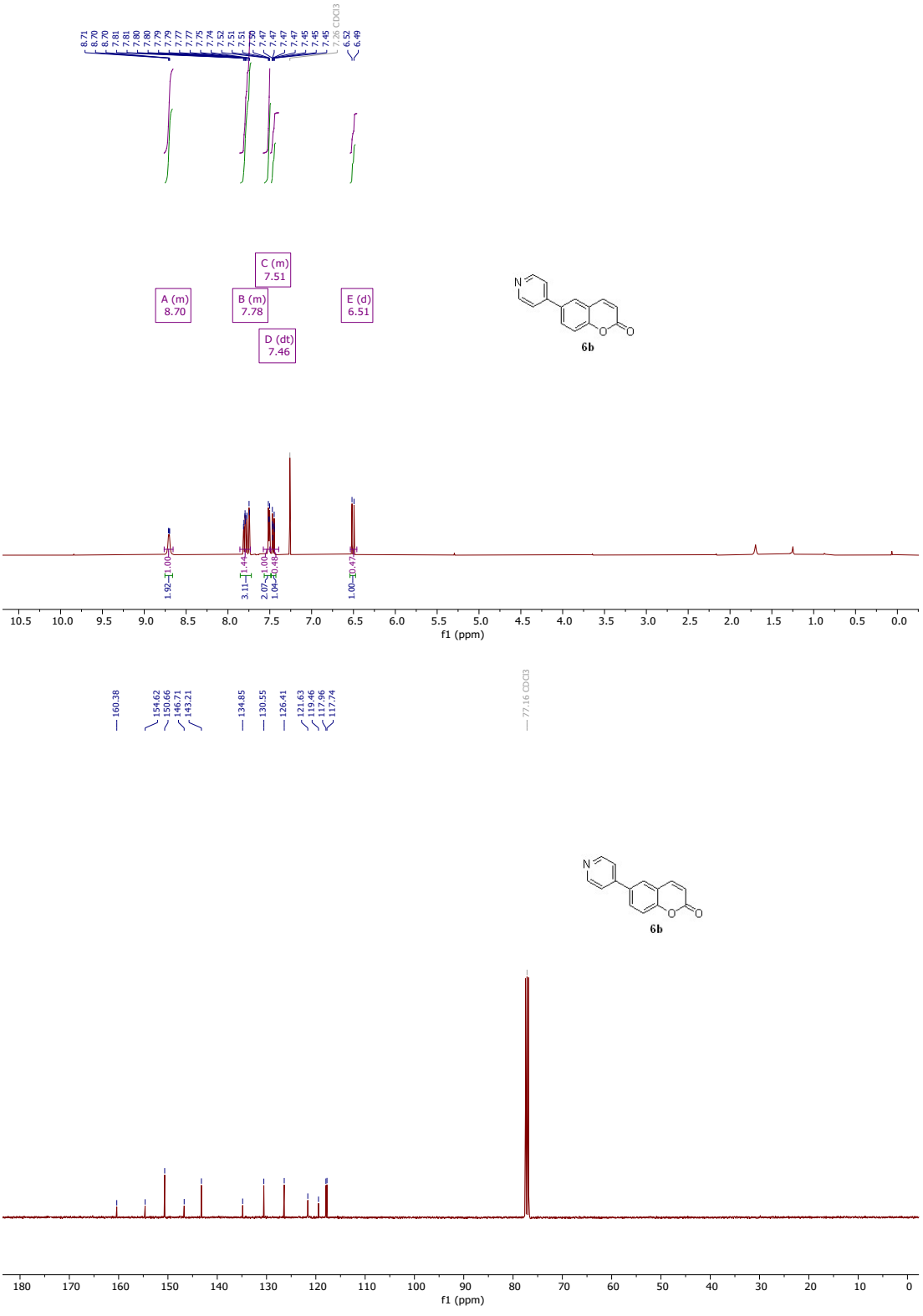
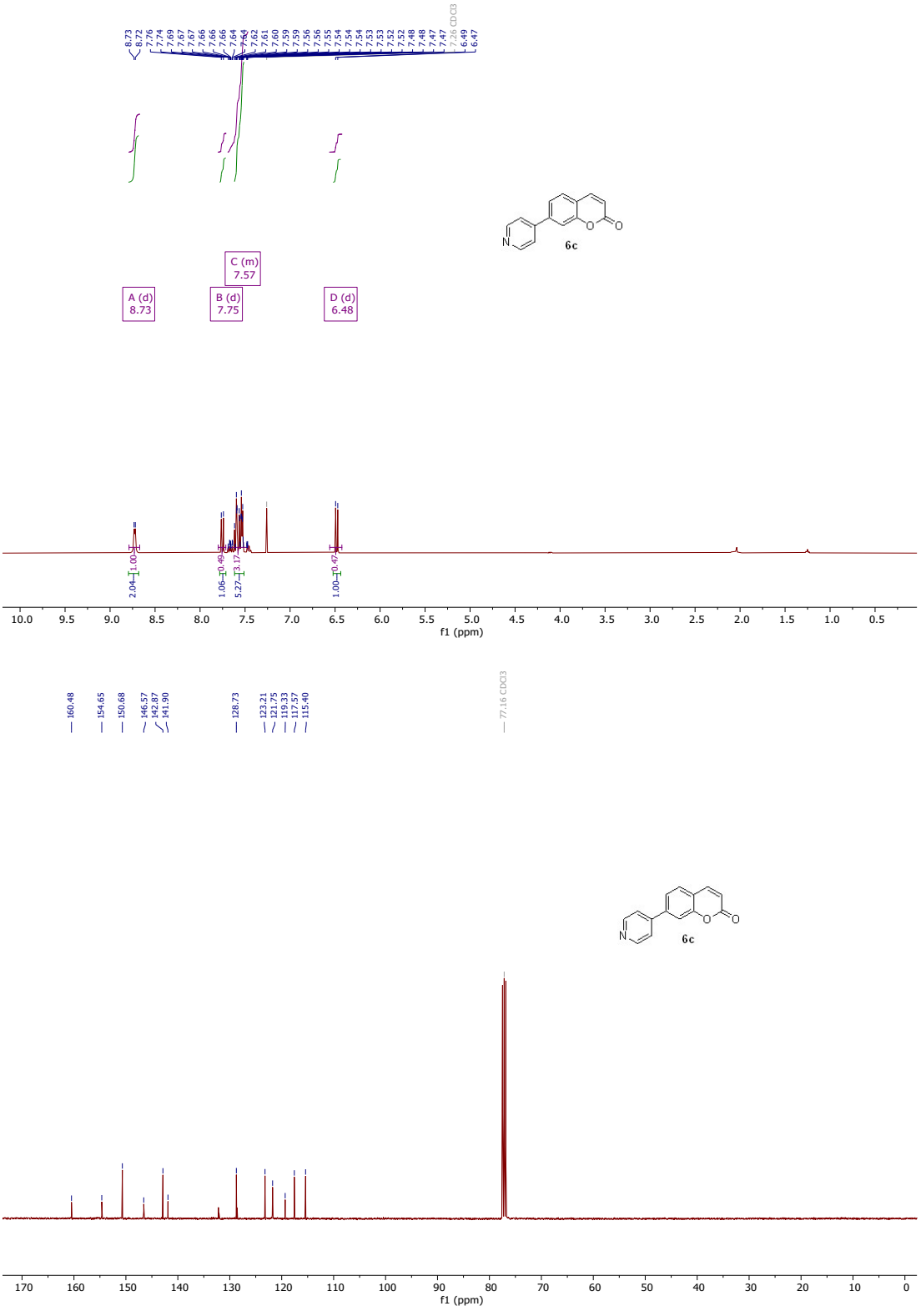


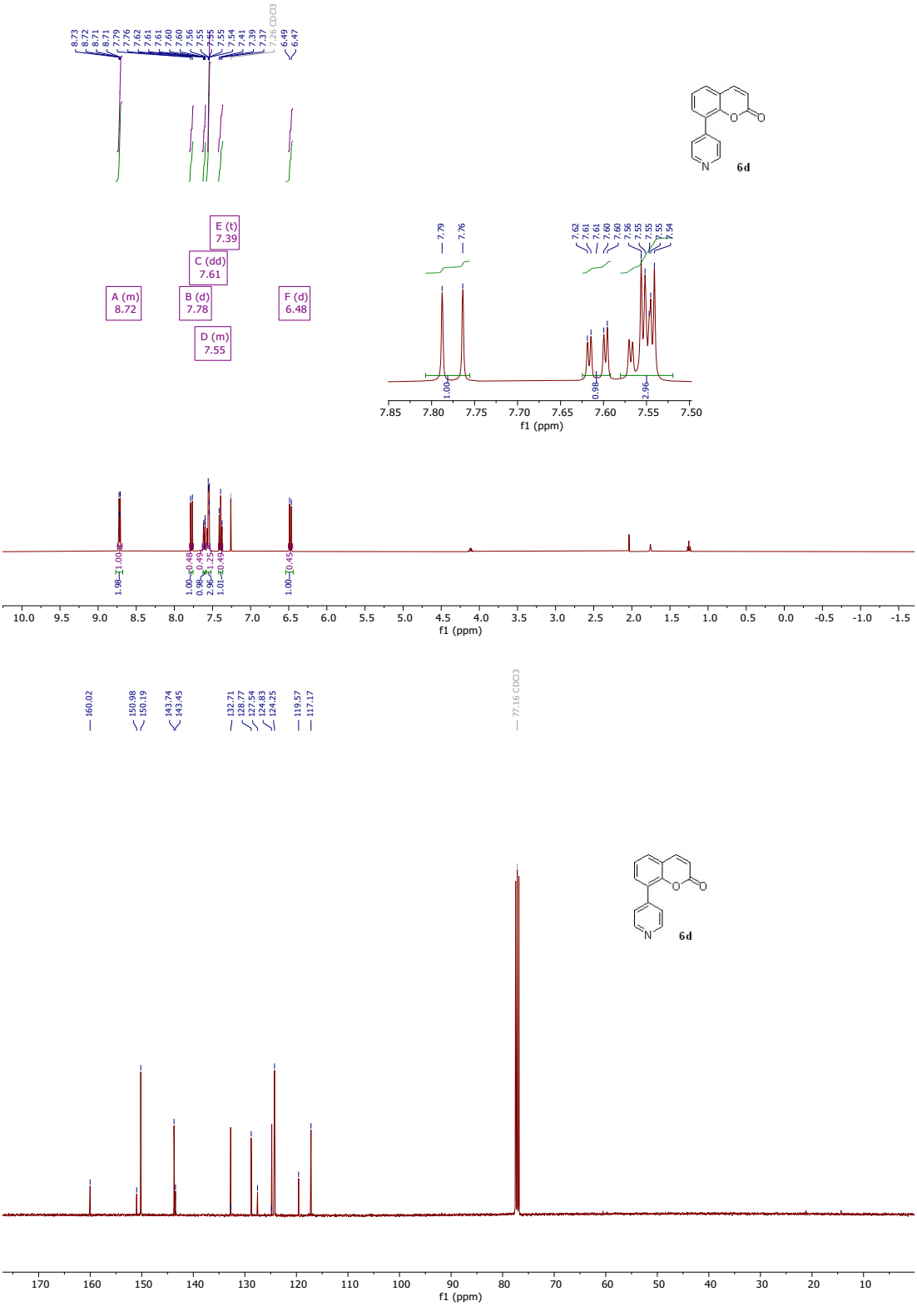
6-(Pyridin-4-yl)-2H-chromen-2-one (6b) ^{1}H and ^{13}C



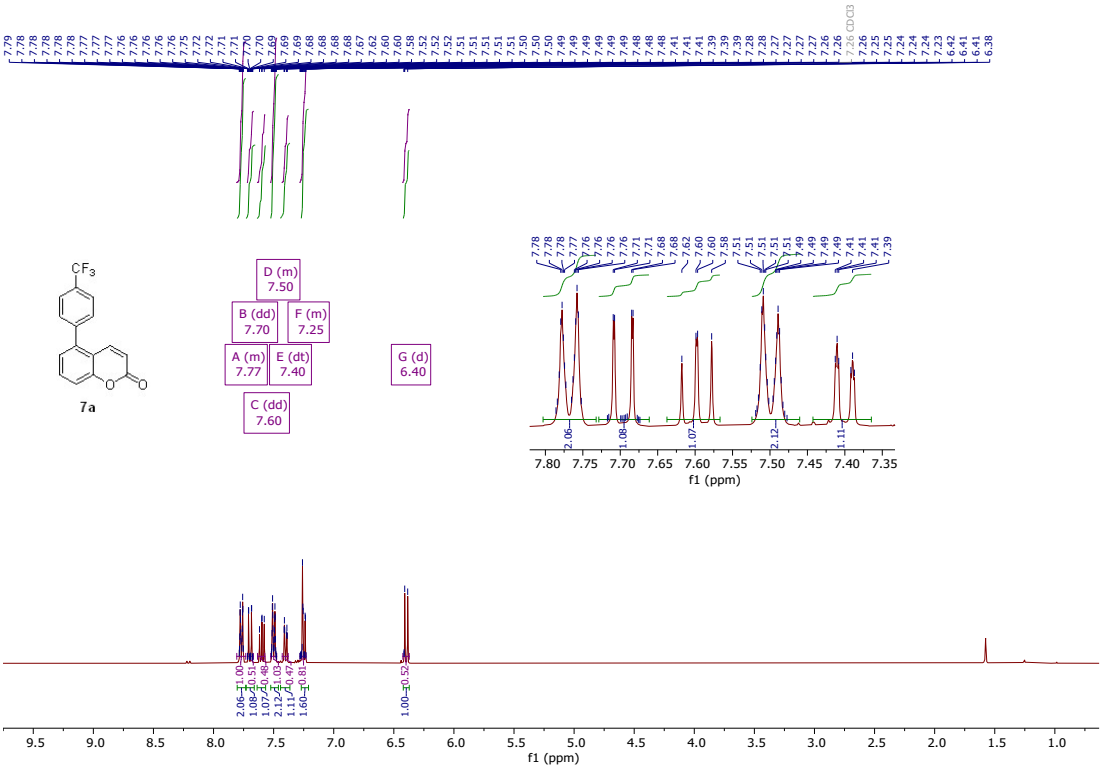
7-(Pyridin-4-yl)-2H-chromen-2-one (6c) ^{1}H and ^{13}C

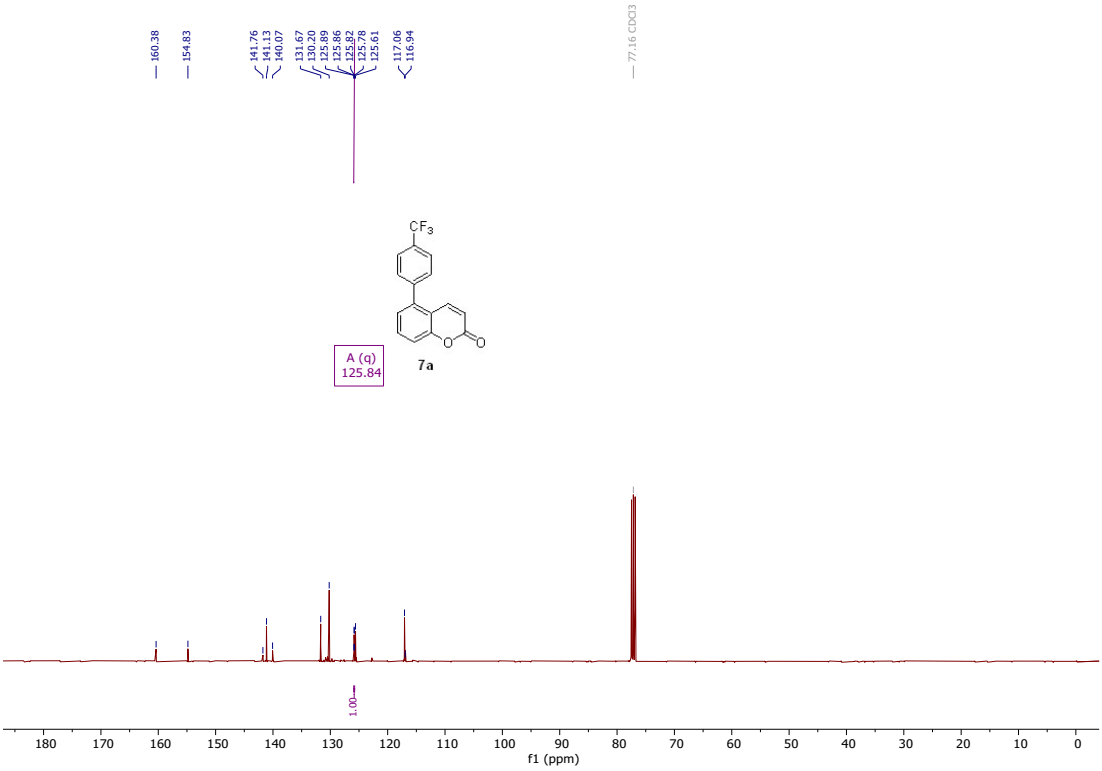


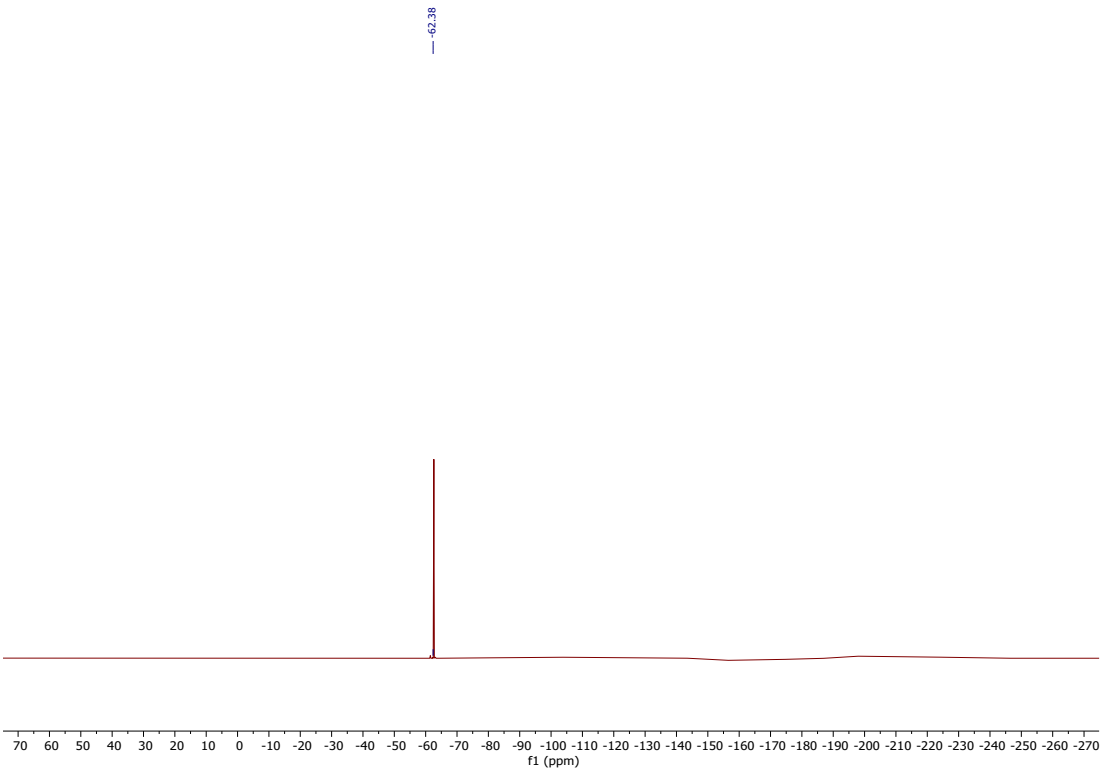
8-(Pyridin-4-yl)-2H-chromen-2-one (6d) ^{1}H and ^{13}C



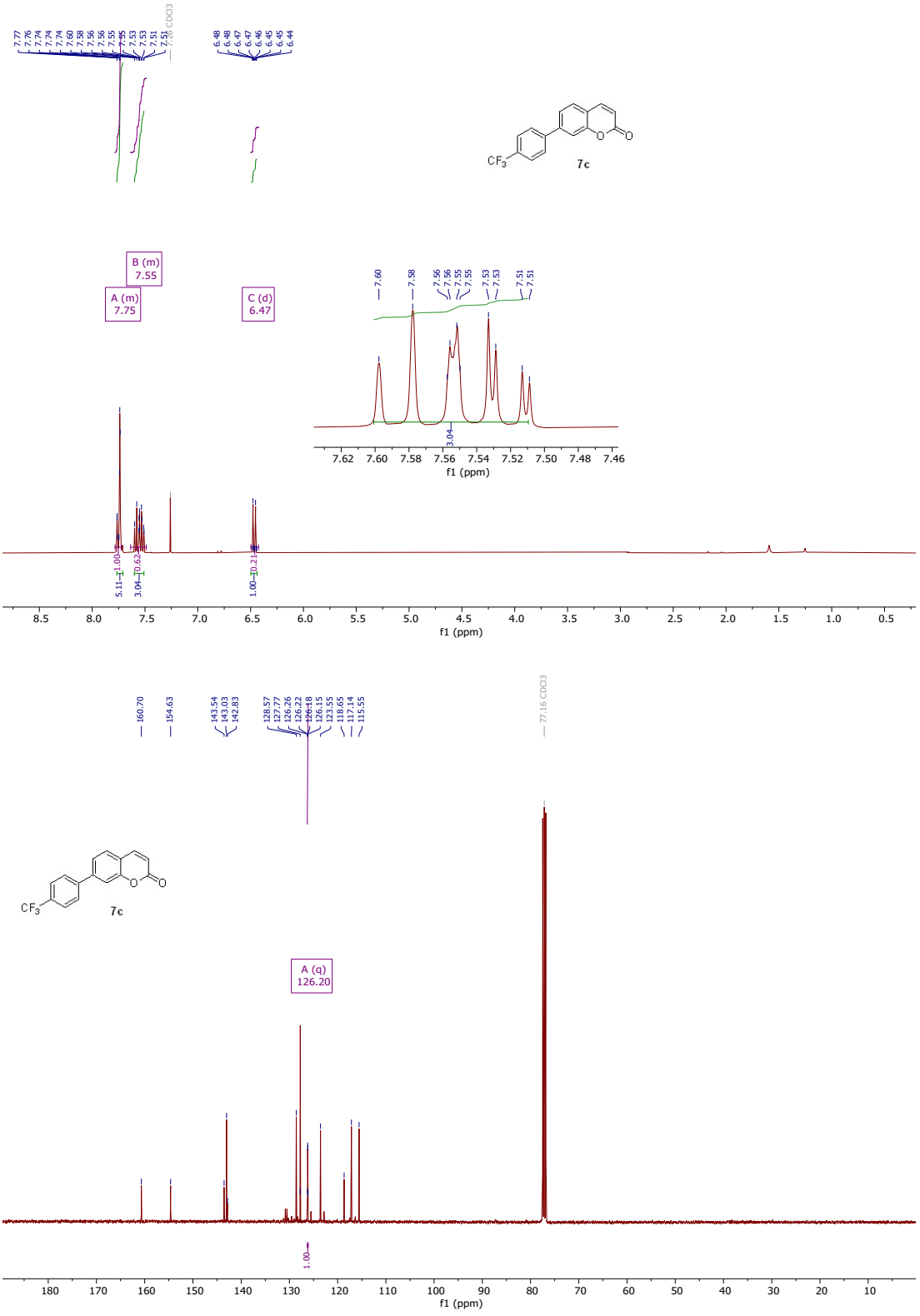
5-(4-(Trifluoromethyl)phenyl)-2H-chromen-2-one (7a) ^{1}H, ^{13}C and ^{18}F

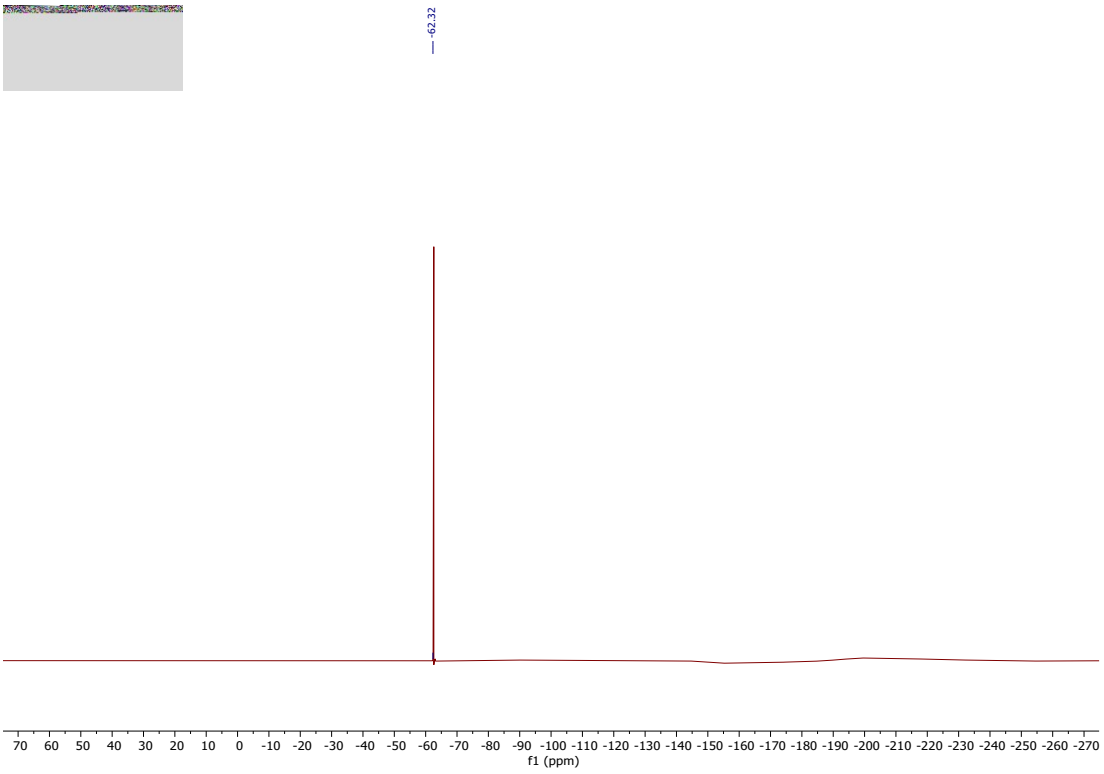




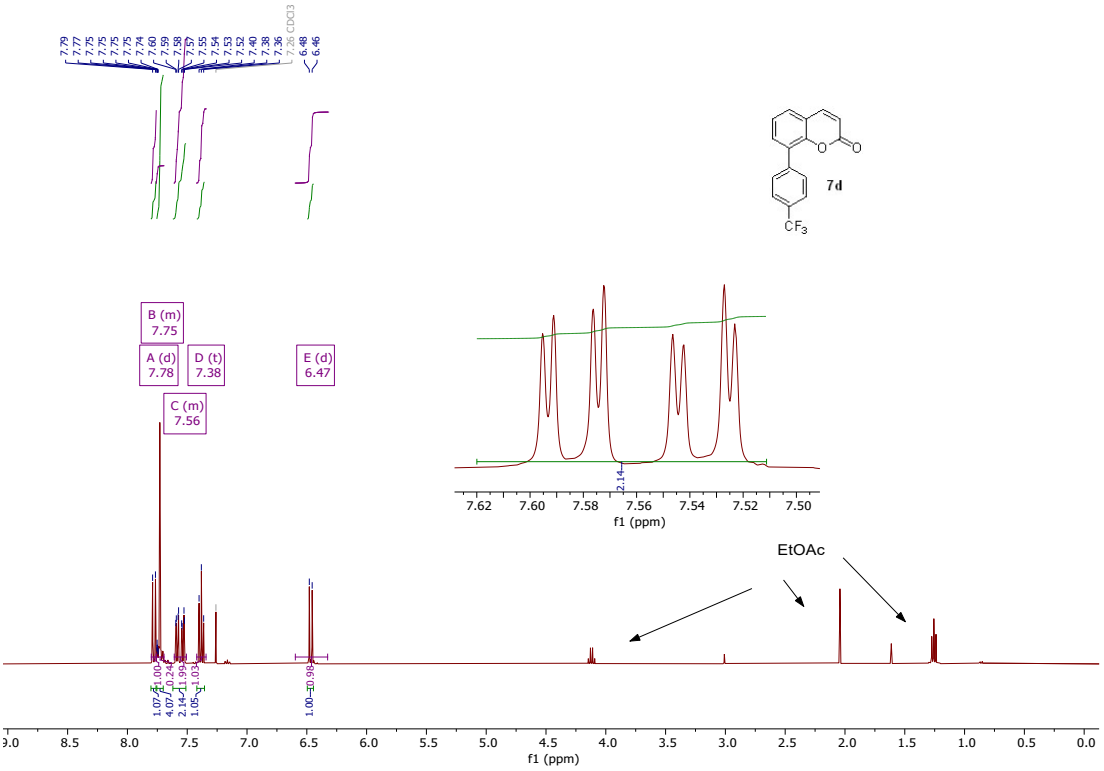

-62.38
70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220 -230 -240 -250 -260 -270
f1 (ppm)

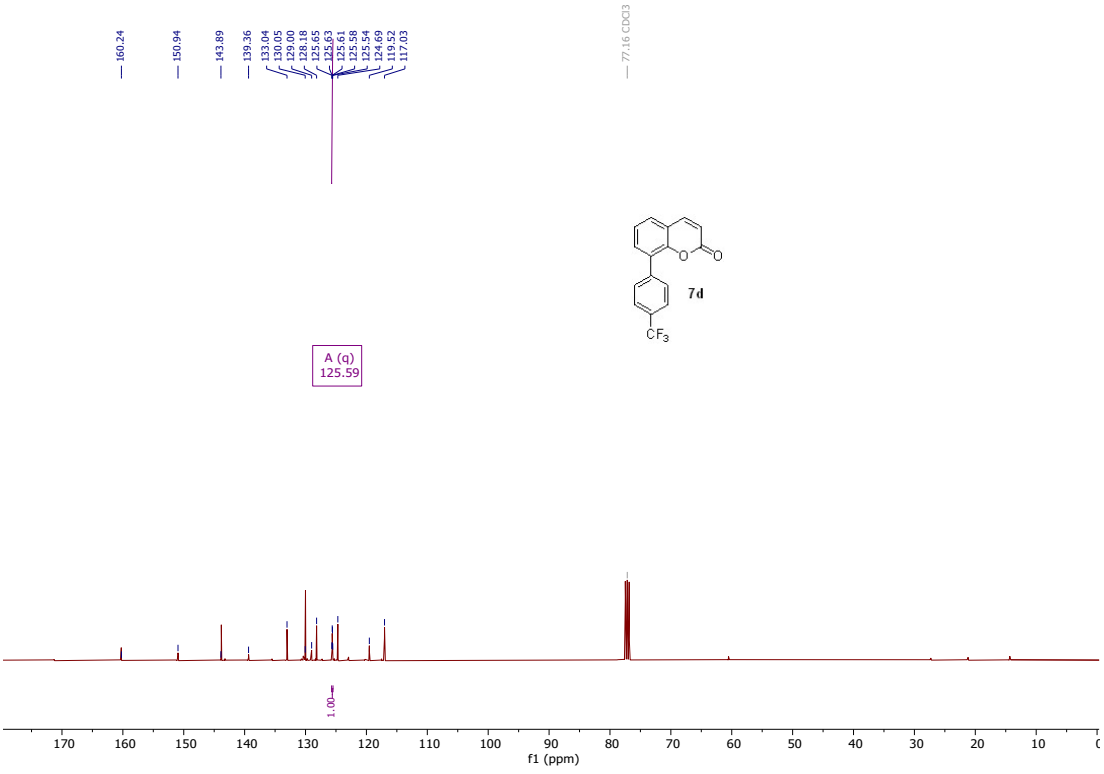
7-(4-(Trifluoromethyl)phenyl)-2H-chromen-2-one (7c) ^{1}H, ^{13}C and ^{18}F

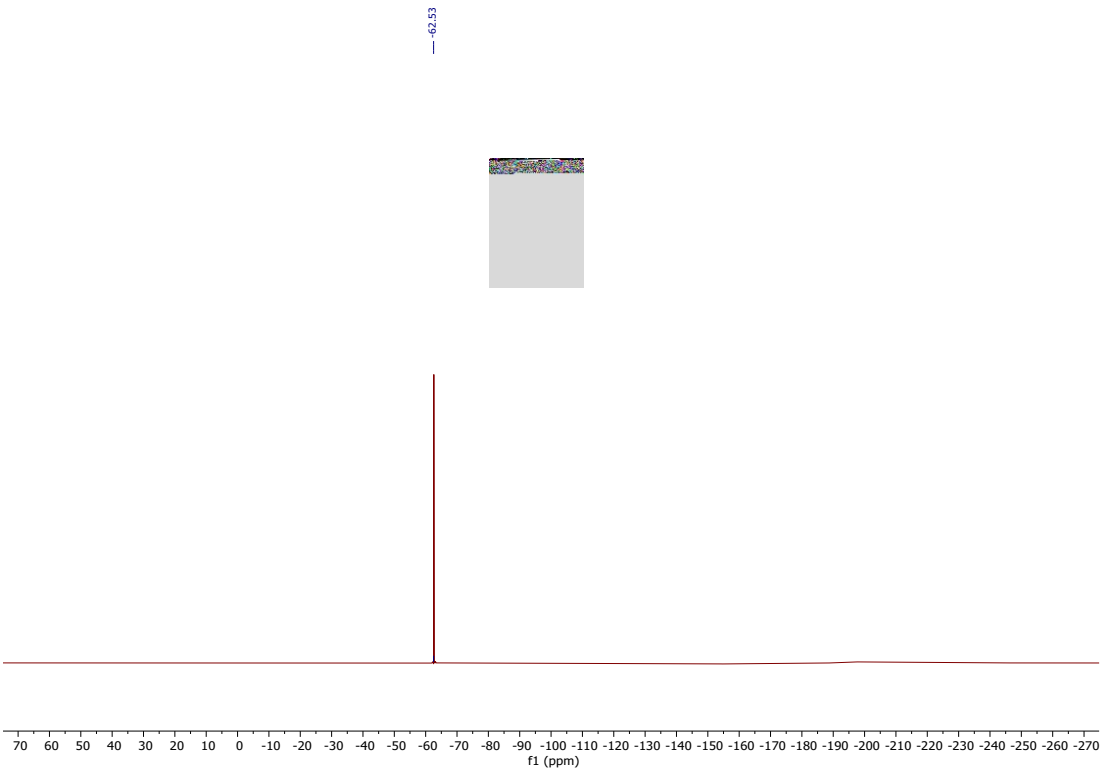



62.32
70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220 -230 -240 -250 -260 -270
f1 (ppm)

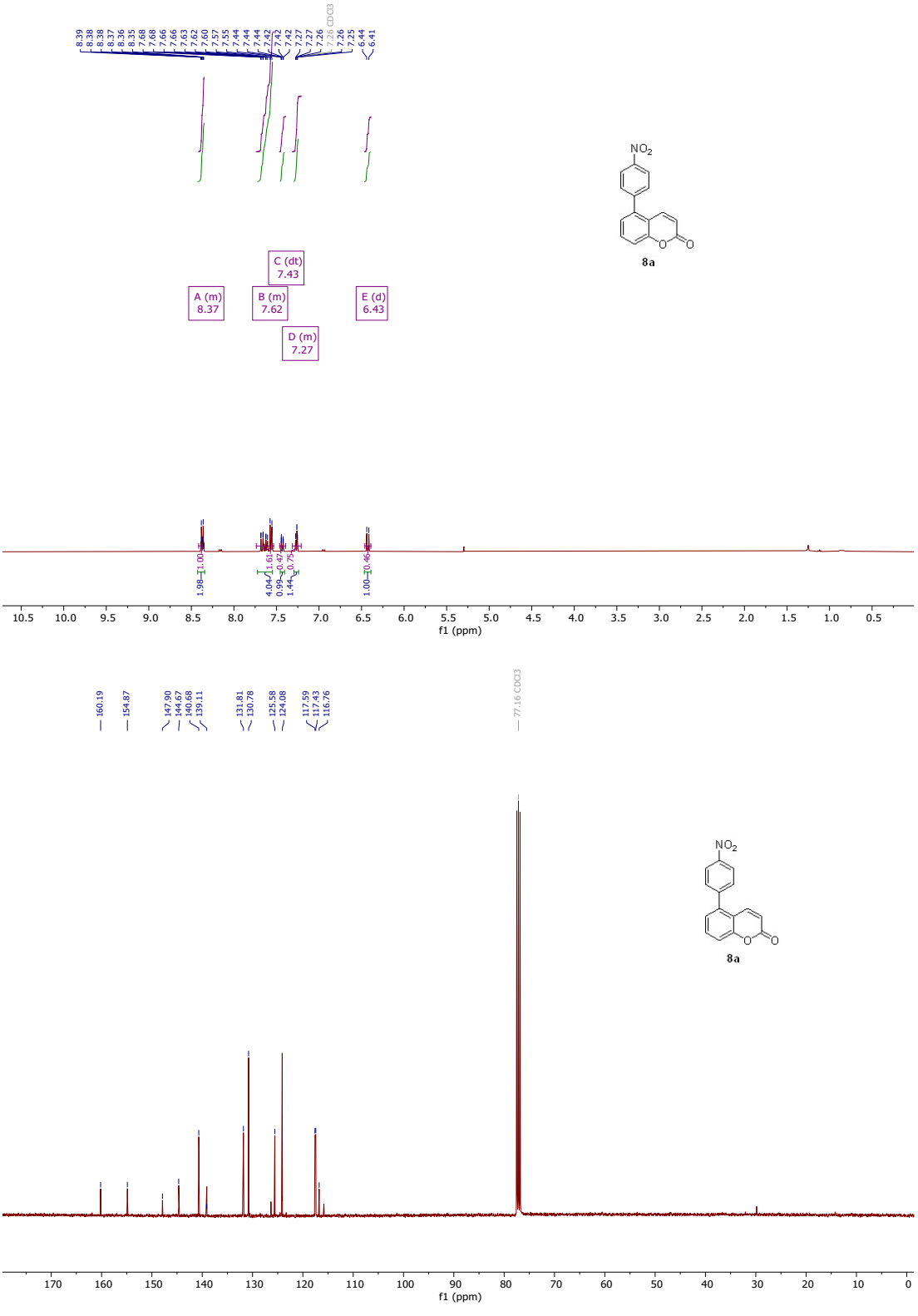
8-(4-(Trifluoromethyl)phenyl)-2H-chromen-2-one (7d) ^{1}H, ^{13}C and ^{18}F



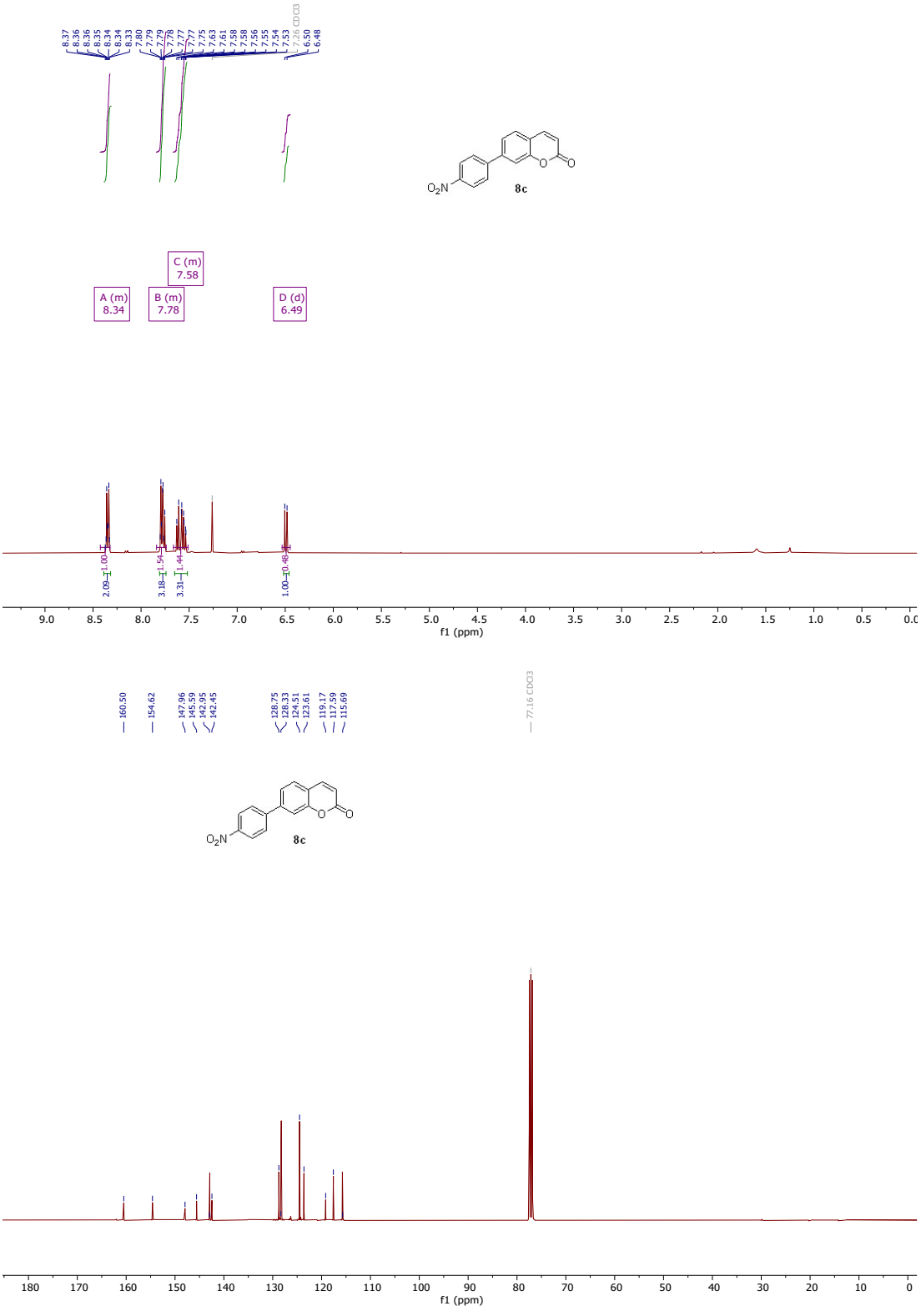



-62.53
70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220 -230 -240 -250 -260 -270
f1 (ppm)

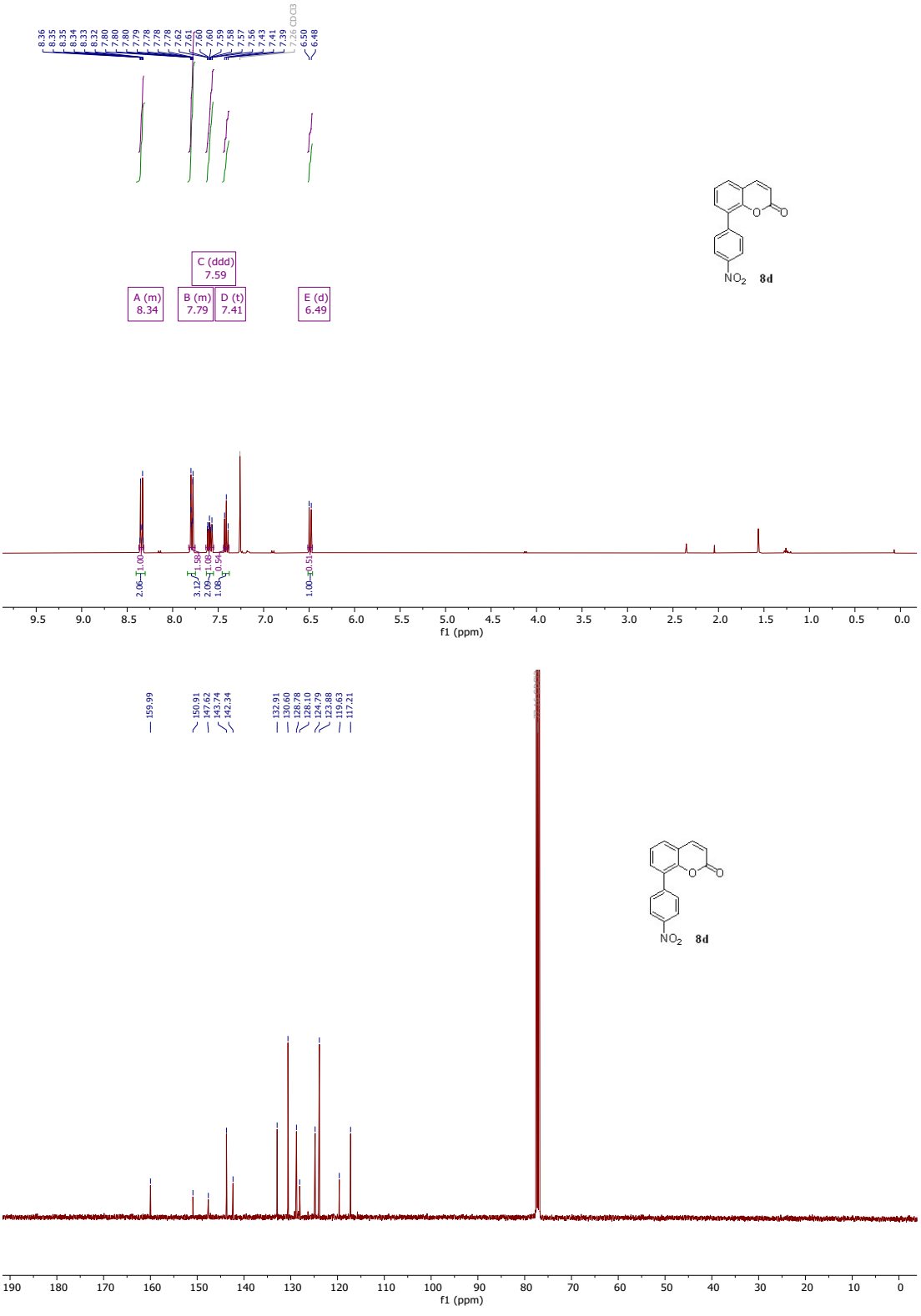
5-(4-Nitrophenyl)-2H-chromen-2-one (8a) ^{1}H and ^{13}C



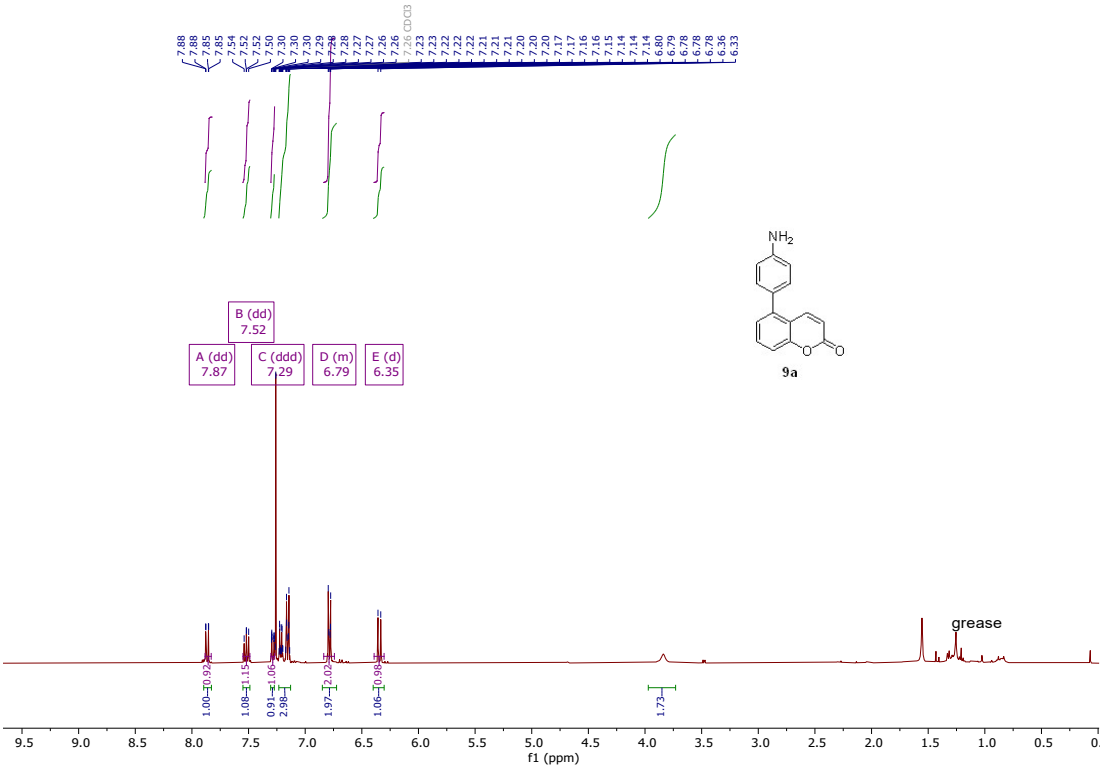
7-(4-Nitrophenyl)-2H-chromen-2-one (8c) ^{1}H and ^{13}C

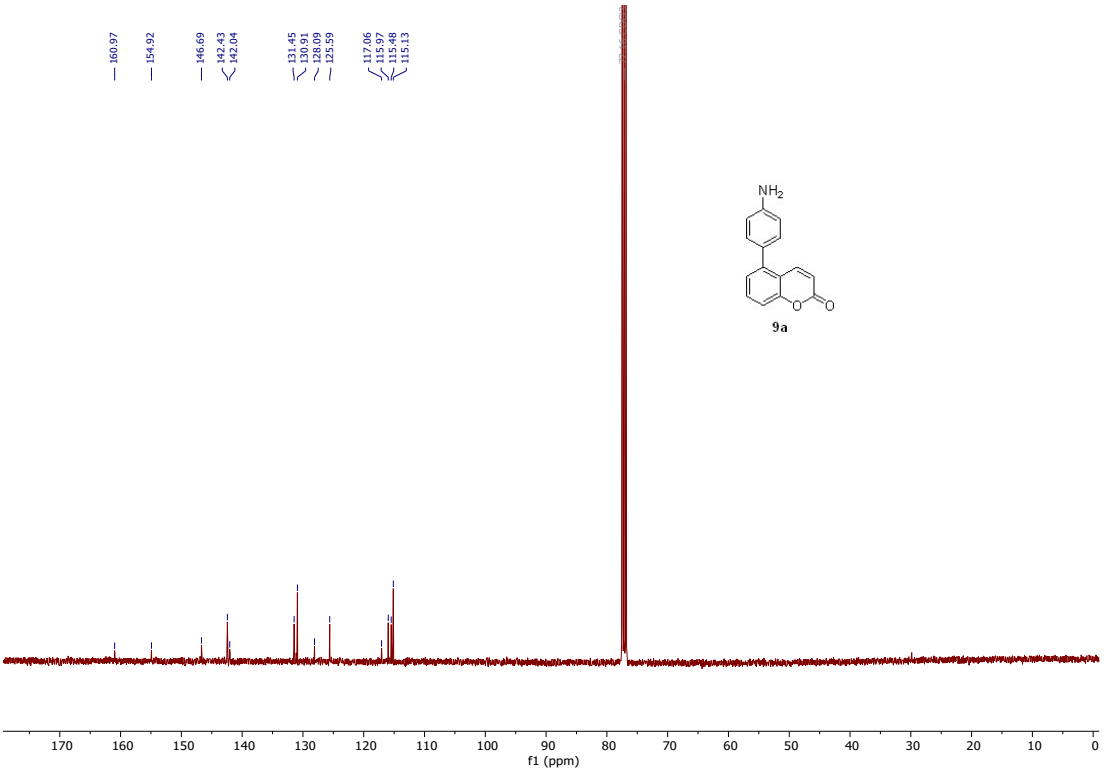


8-(4-Nitrophenyl)-2H-chromen-2-one (8d) ^{1}H and ^{13}C

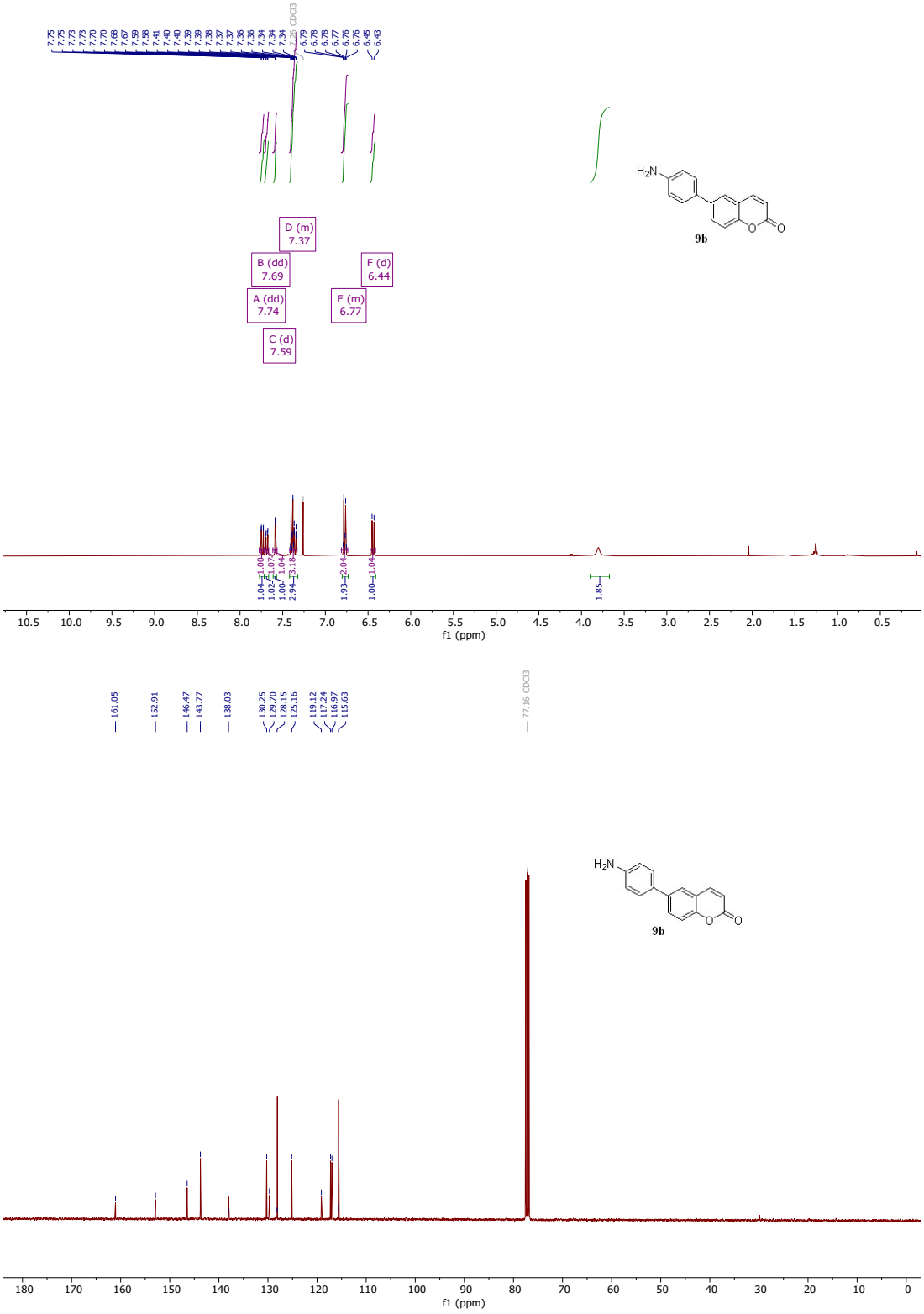


5-(4-Aminophenyl)-2H-chromen-2-one (9a) ^{1}H and ^{13}C

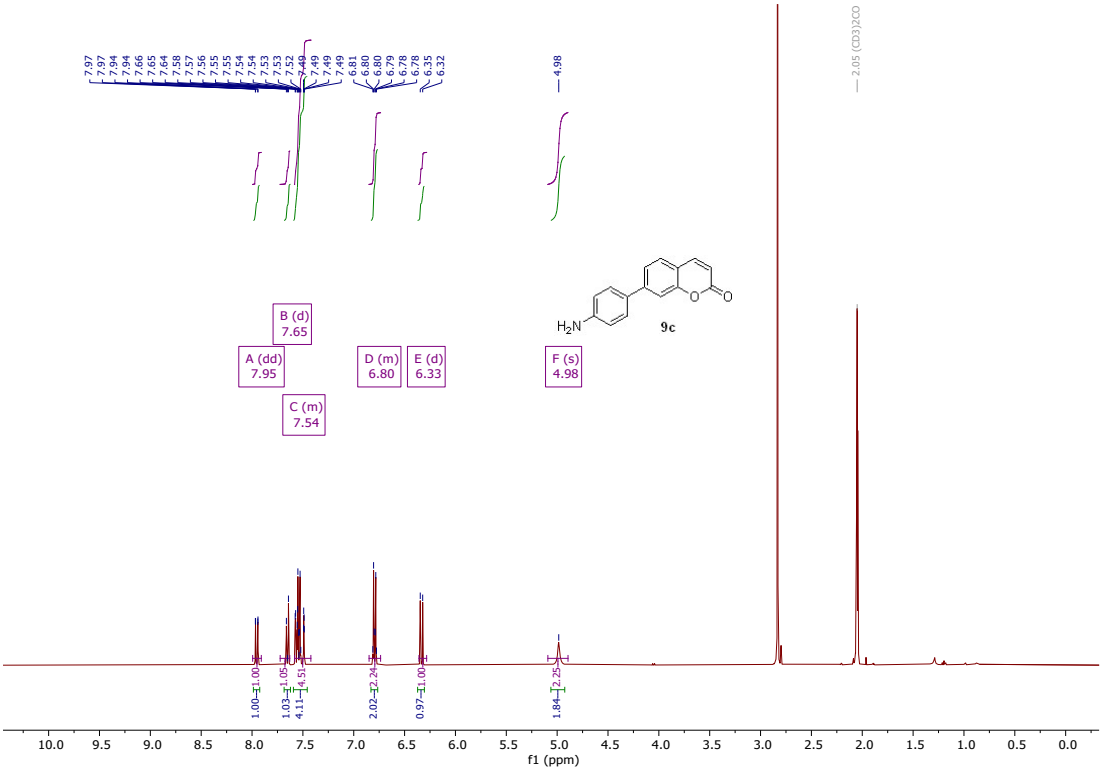


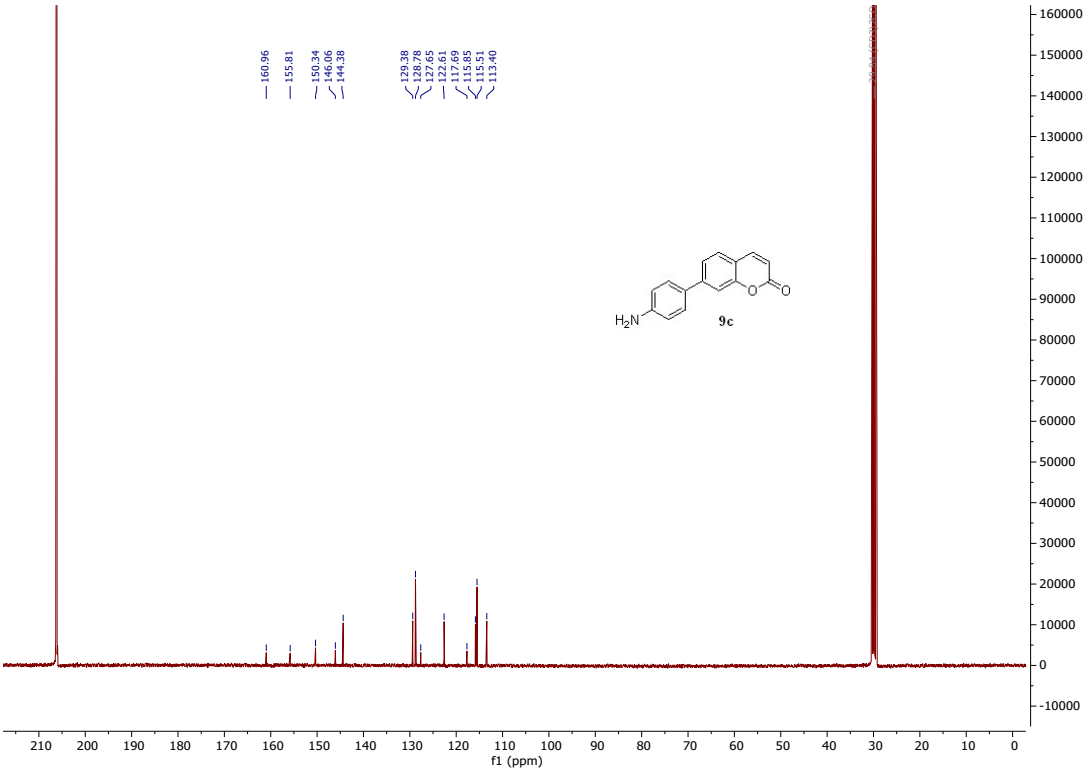


6-(4-Aminophenyl)-2H-chromen-2-one (9b) ^{1}H and ^{13}C

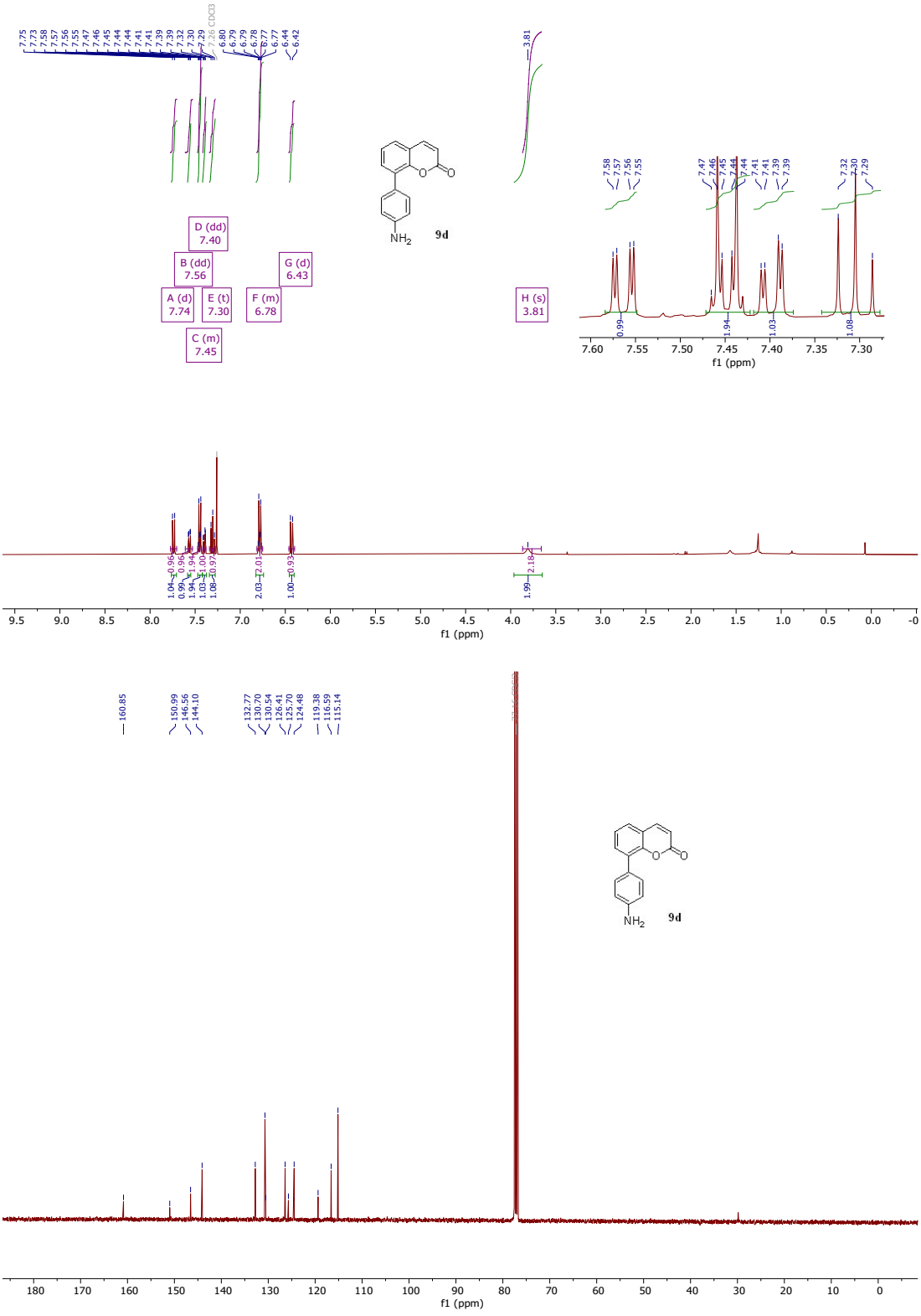


7-(4-Aminophenyl)-2H-chromen-2-one (9c) ^{1}H and ^{13}C

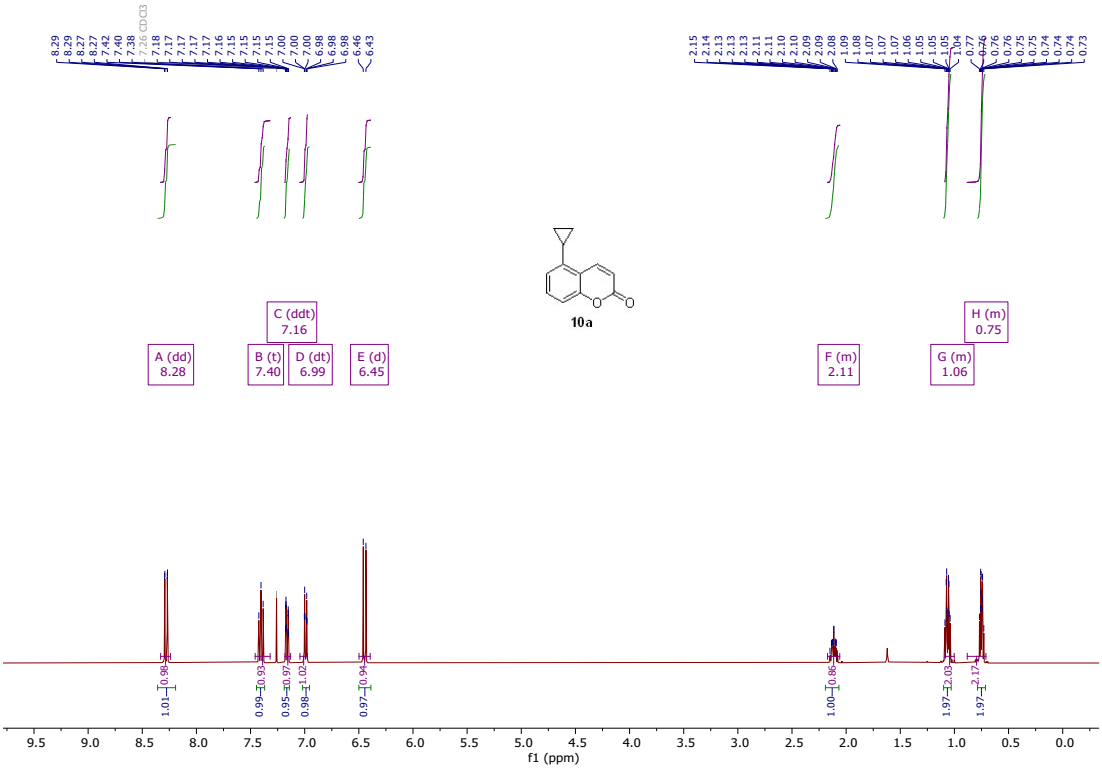


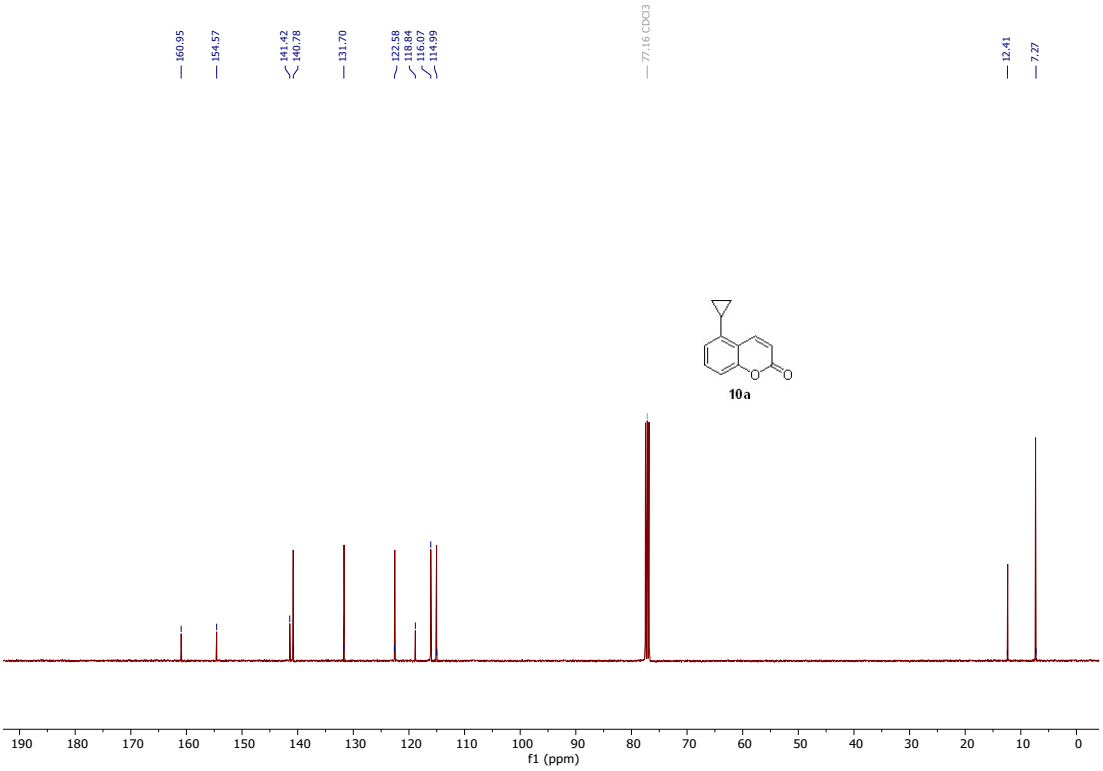


8-(4-Aminophenyl)-2H-chromen-2-one (9d) ^{1}H and ^{13}C

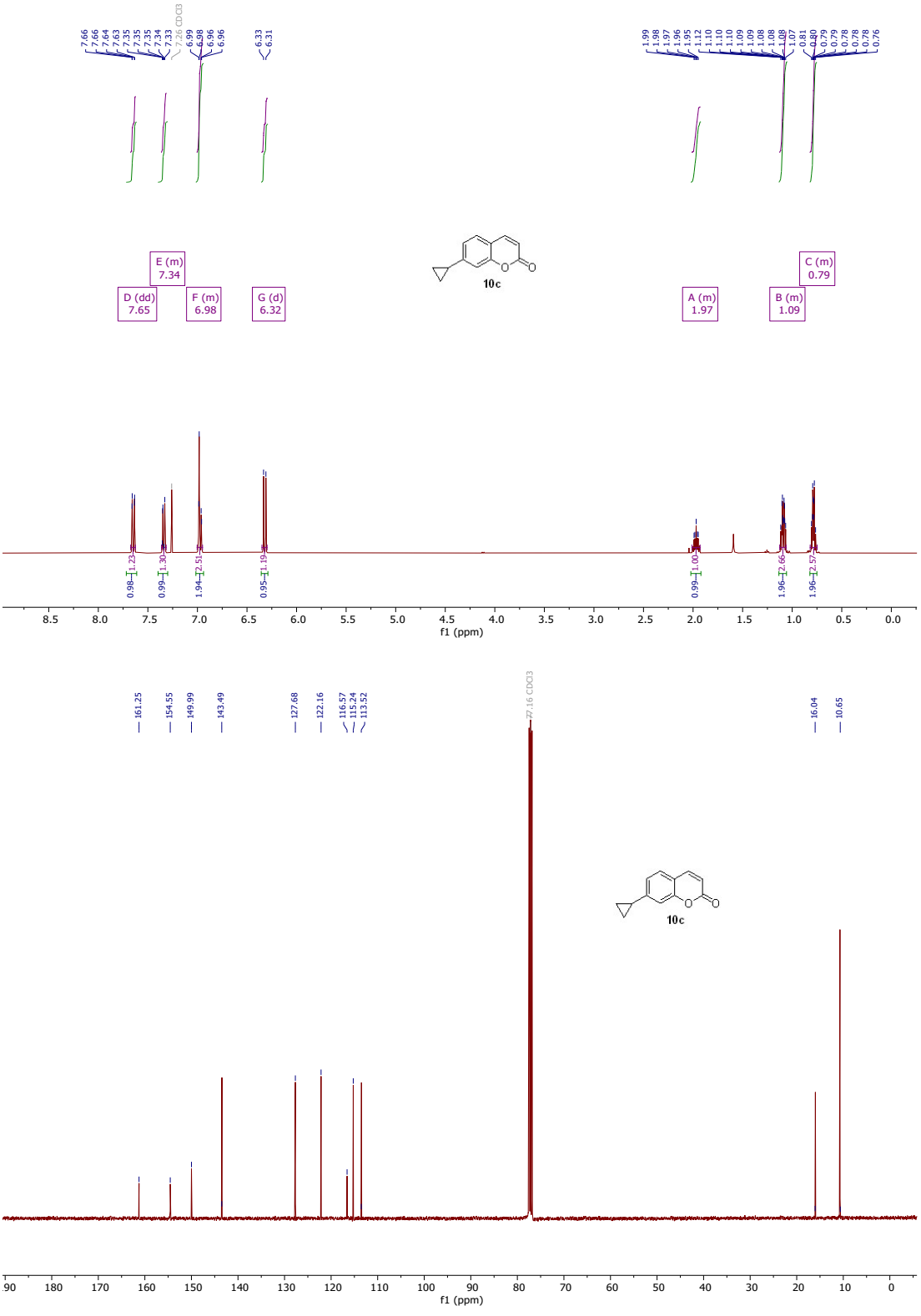


5-Cyclopropyl-2H-chromen-2-one (10a) ^{1}H and ^{13}C

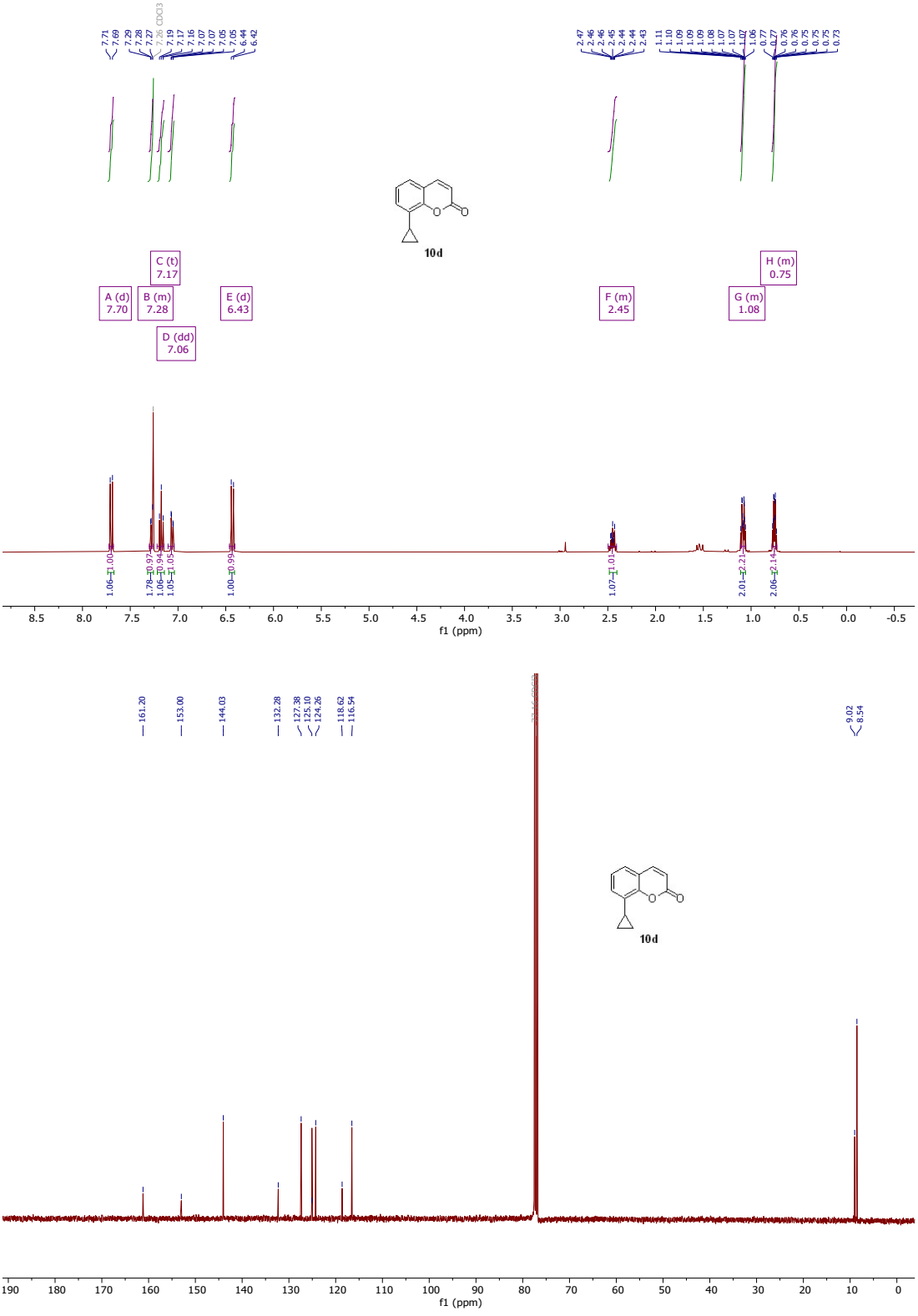




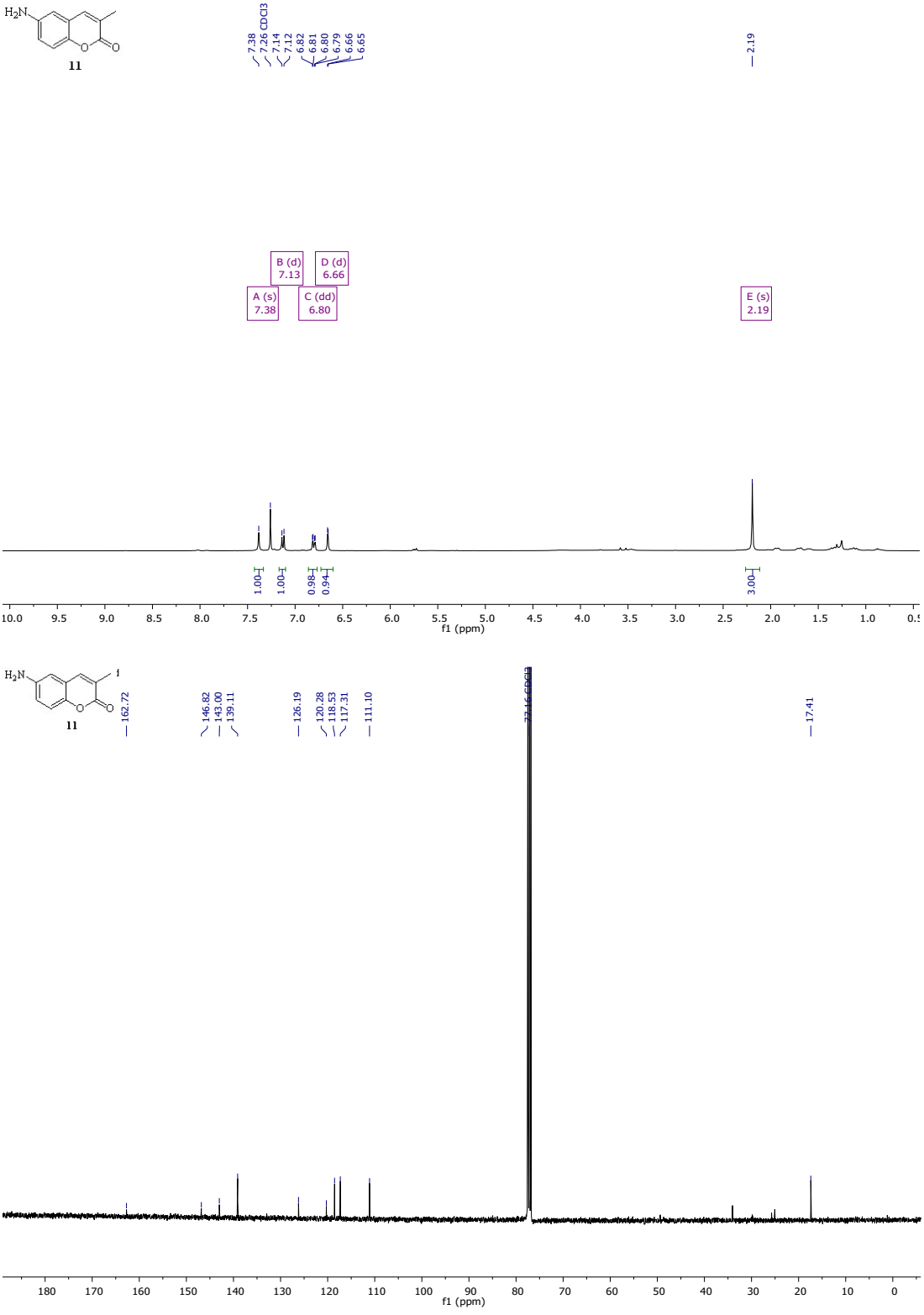
7-Cyclopropyl-2H-chromen-2-one (10c) ^{1}H and ^{13}C

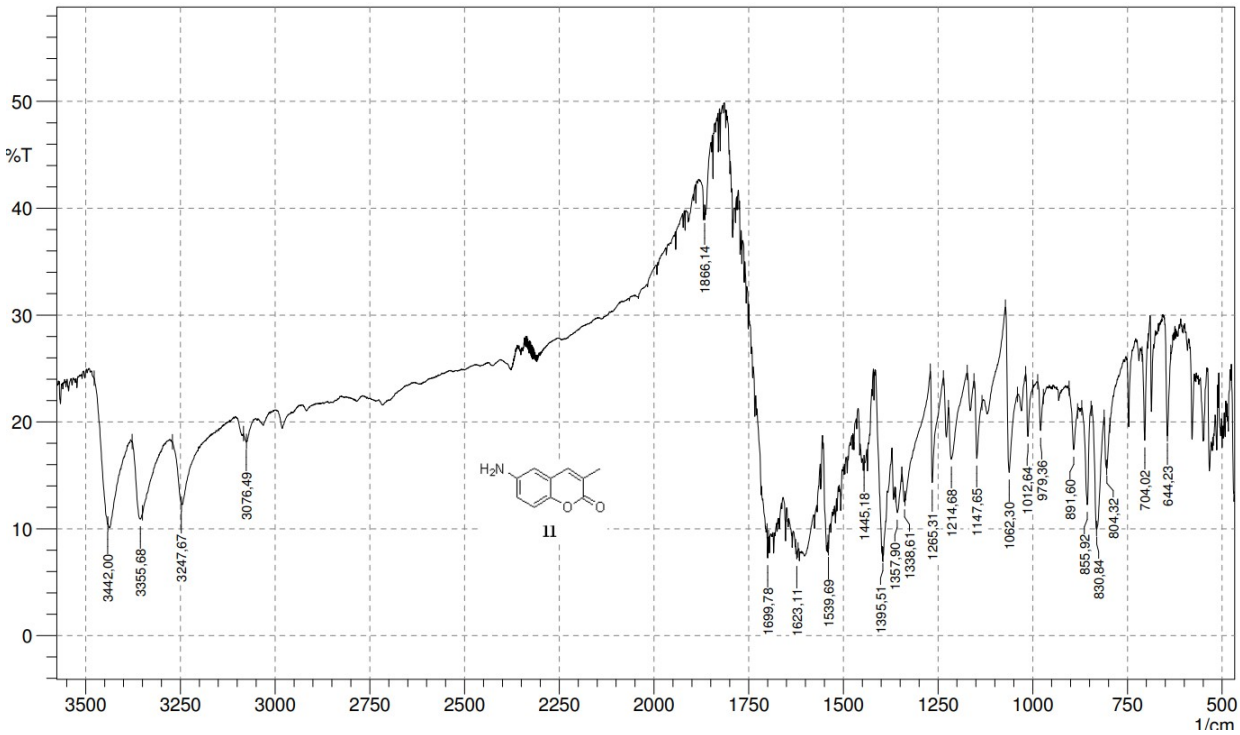


8-Cyclopropyl-2H-chromen-2-one (10d) ^{1}H and ^{13}C



6-Amino-3-methyl-2H-chromen-2-one (11) ^{1}H, ^{13}C, IR, HRMS

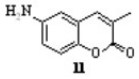




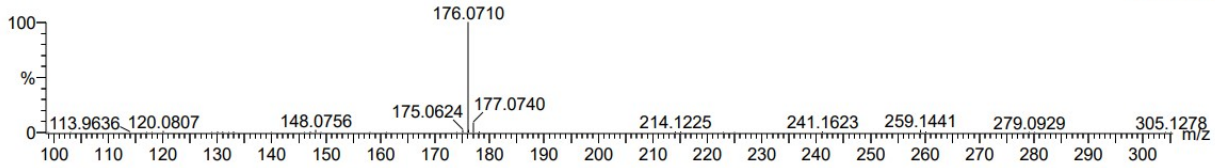
Monoisotopic Mass, Even Electron Ions
105 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-70 H: 1-150 N: 0-8 O: 0-6

1473 Nikitjuka

HRMS_2023_03_189 486 (1.398) Cm (486:506-385:405)

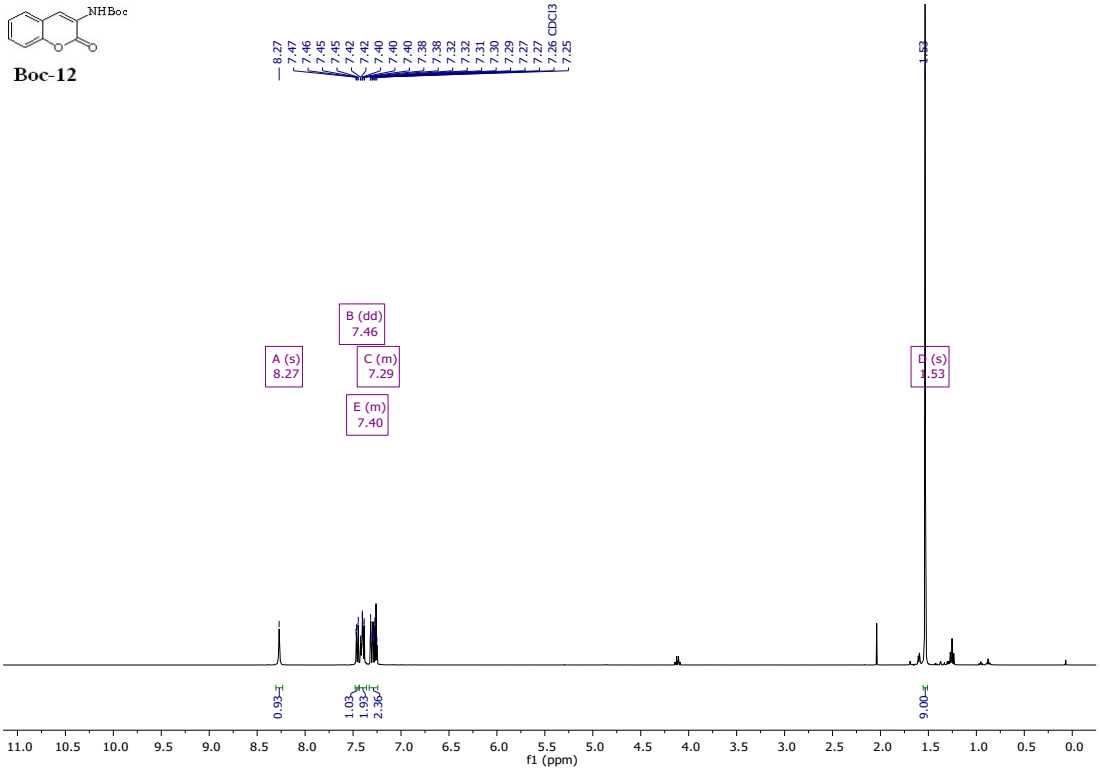


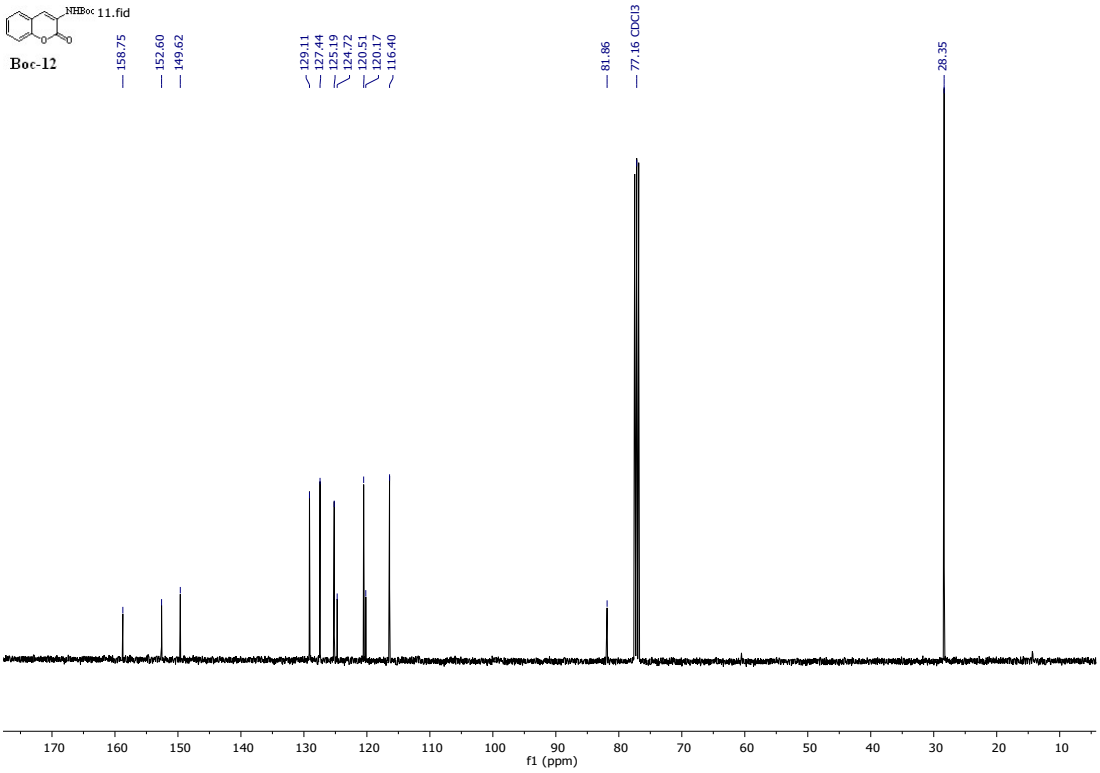
OSI/FOKL-MS
Synapt G2-Si
1: TOF MS ES+
2.67e+007



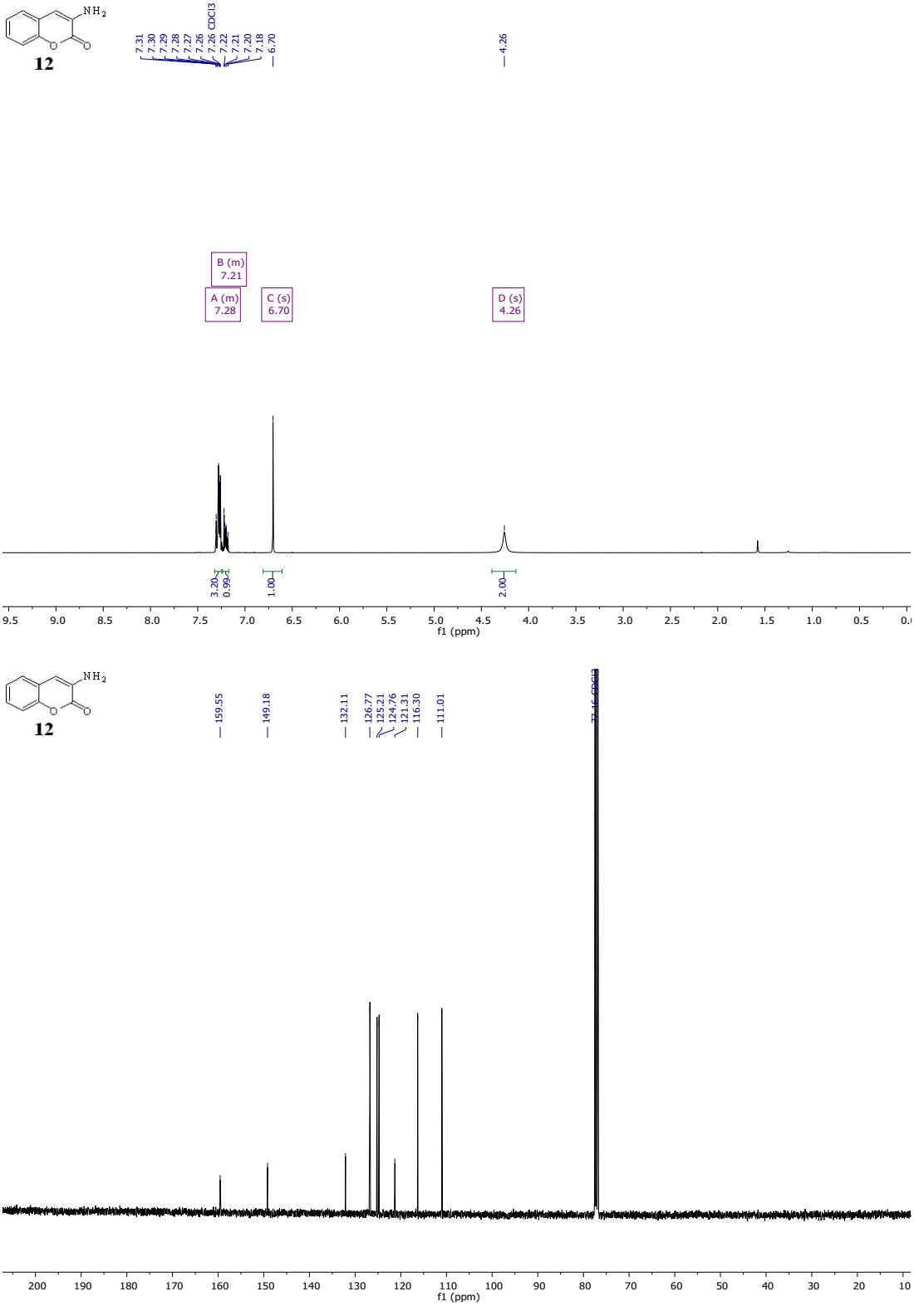
Minimum:				-0.5				
Maximum:		5.0	5.0	200.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
176.0710	176.0712	-0.2	-1.1	6.5	135.7	n/a	n/a	C10 H10 N O2

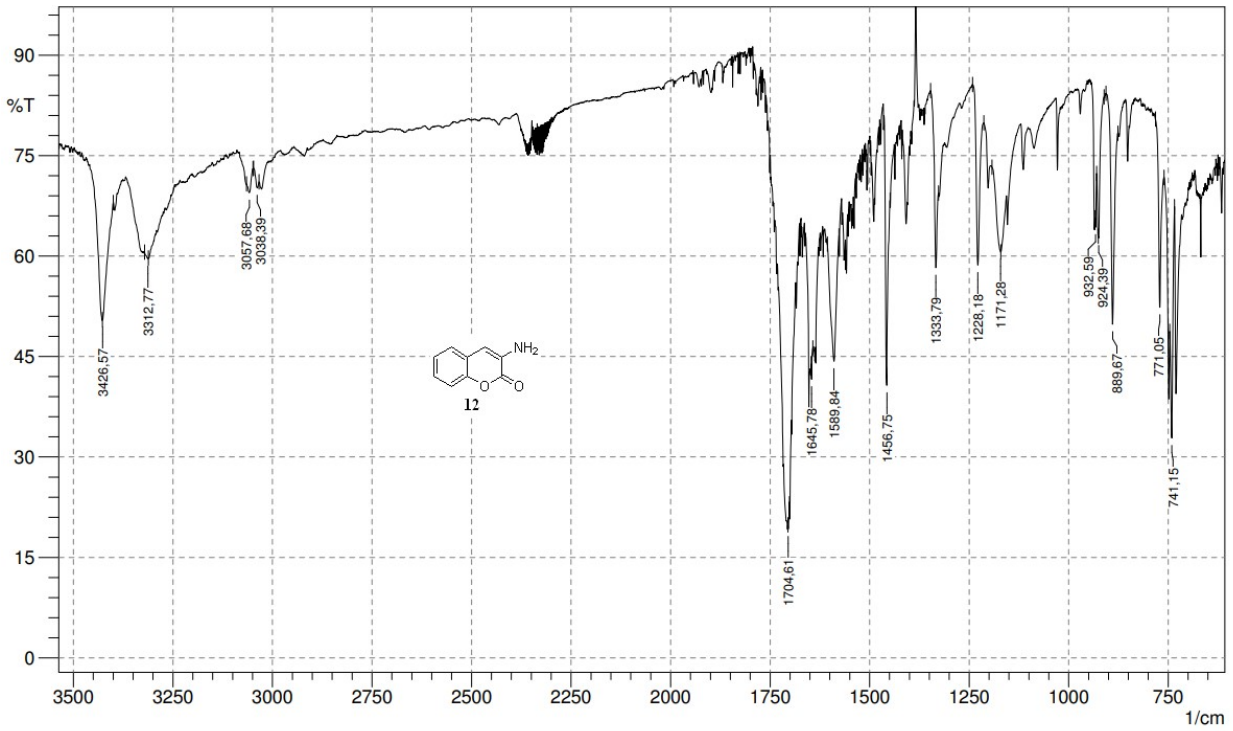
tButyl (2-oxo-2H-chromen-3-yl)carbamate (Boc-12) ^{1}H and ^{13}C





3-amino-2H-chromen-2-one (12) ^{1}H, ^{13}C, IR, HRMS

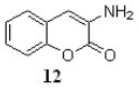




Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -0.5, max = 200.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5

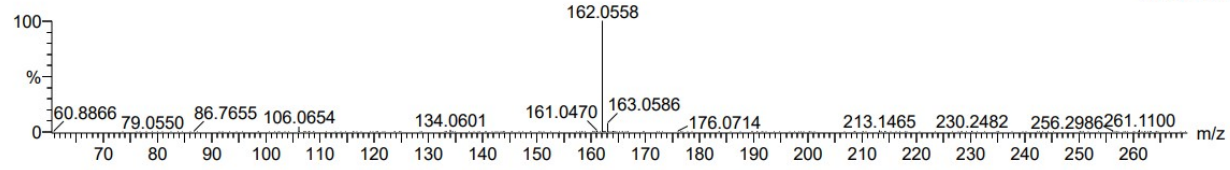
Monoisotopic Mass, Even Electron Ions
84 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-55 H: 1-150 N: 0-8 O: 0-5

1



OSI/FOKL-MS
Synapt G2-Si
1: TOF MS ES+
2.77e+006

HRMS_2023_04_417 634 (1.814) Cm (633:639-534:562)



Minimum:				-0.5				
Maximum:		5.0	5.0	200.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
162.0558	162.0555	0.3	1.9	6.5	274.3	n/a	n/a	C9 H8 N O2

References

1. Xu, H.; Wolf, C. Efficient copper-catalyzed coupling of aryl chlorides, bromides and iodides with aqueous ammonia. ***Chem. Commun.***, 2009, 3035-3037.
2. Donald, J. R.; Edwards, M. G.; Taylor, R. J. K. Tandem Oxime Formation—Epoxide Ring Opening Sequences for the Preparation of Oxazines Related to the Trichodermamides. *Tetrahedron Lett.* **2007**, *48* (30), 5201–5204.
3. Zuo, Q.-P.; Li, B.; Pei, Q.; Li, Z.; Liu, S.-K. A highly selective fluorescent probe for detection of biological samples thiol and its application in living cells. *J. Fluoresc.* **2010***, 20,* 1307-1313.