Supporting Information

for

Trifluoromethylated 4,5-dihydro-1,2,4-triazin-6(1*H*)-ones *via* (3+3)-annulation of nitrile imines with α-amino esters

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Copies of ^{1}H and ^{13}C NMR spectra of new compounds

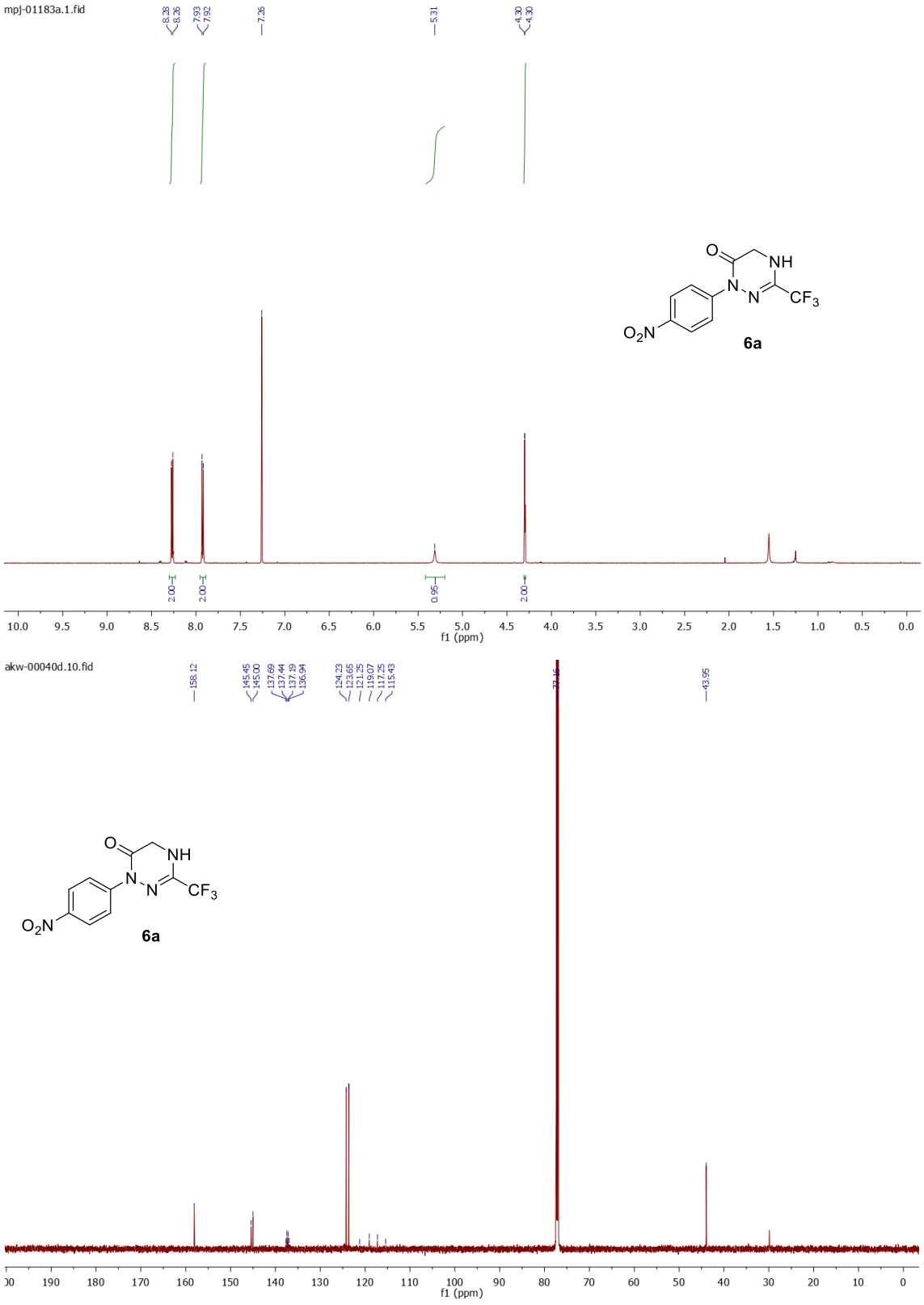


Figure S1. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **6a**.

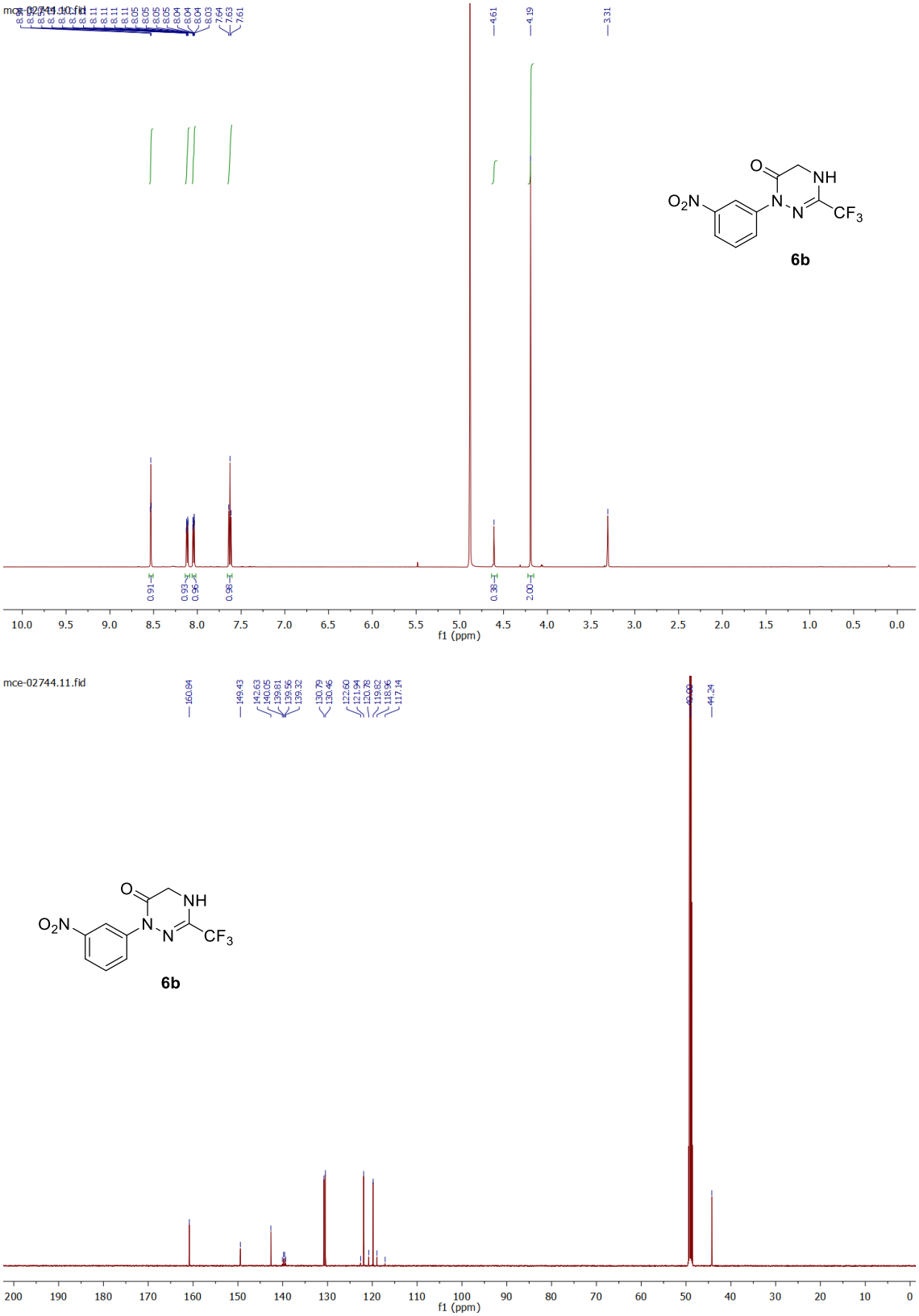


Figure S2. ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (151 MHz, CD_3OD) spectra for compound **6b**.

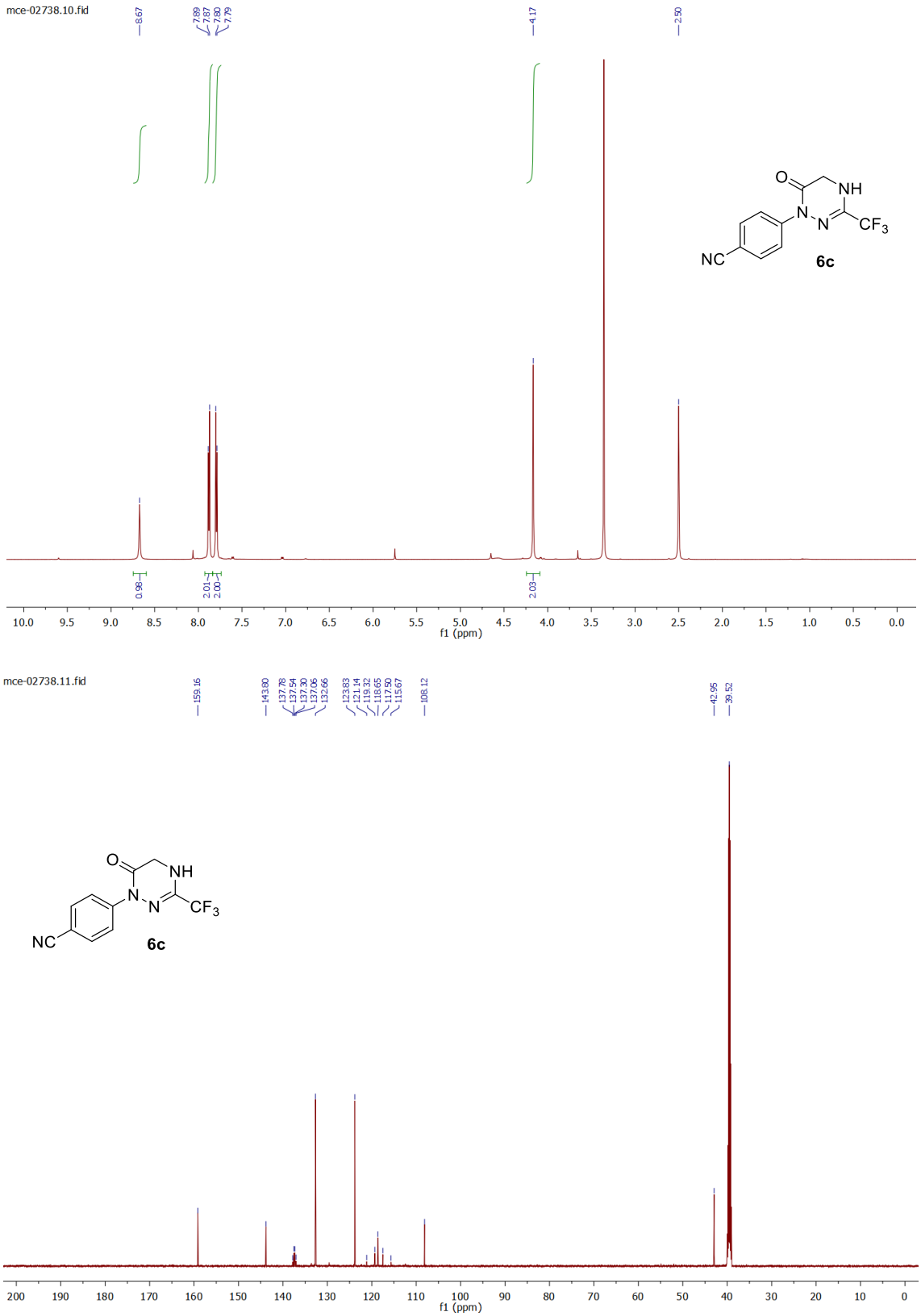


Figure S3. ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (151 MHz, DMSO-d_6) spectra for compound **6c**.

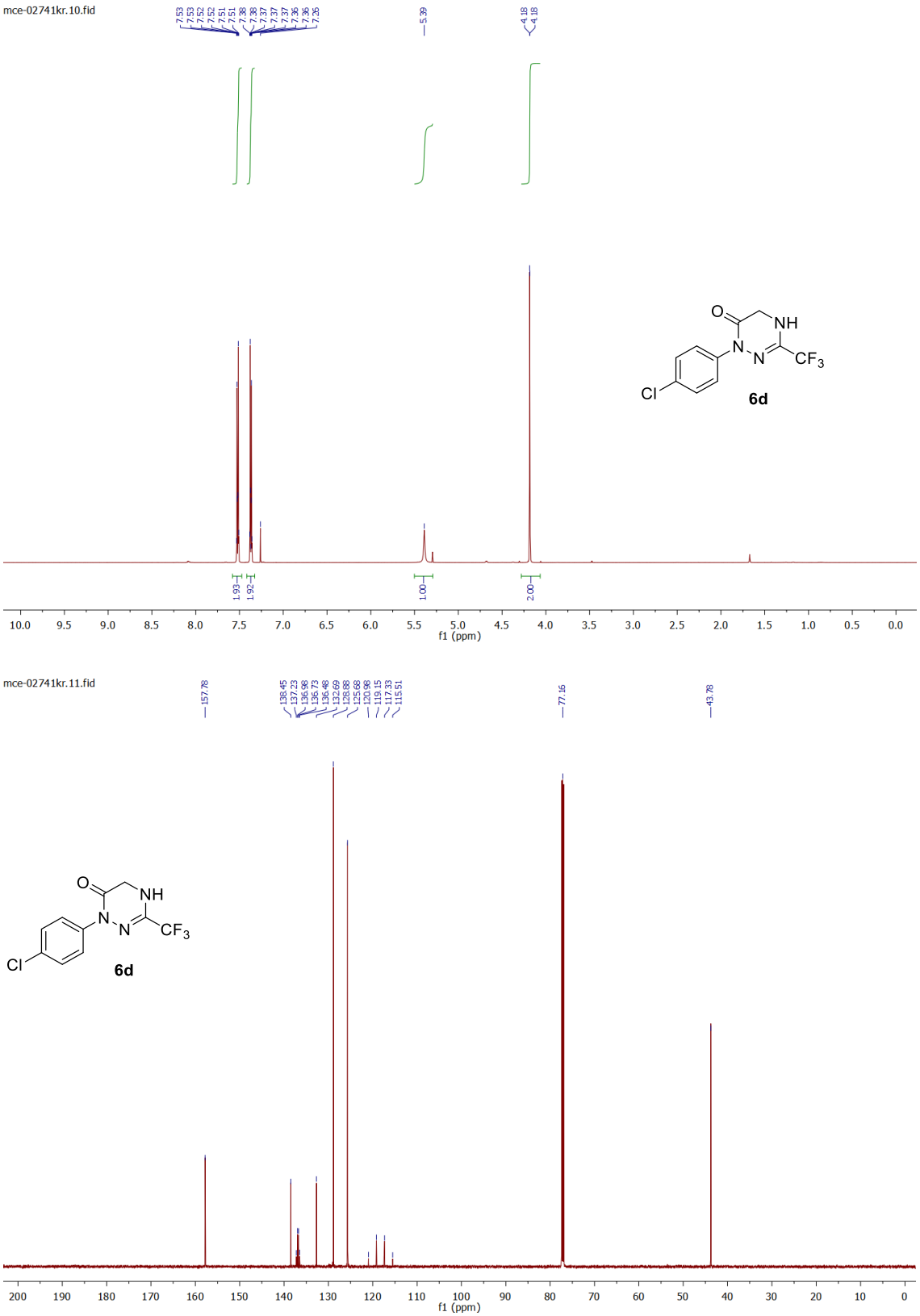


Figure S4. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **6d**.

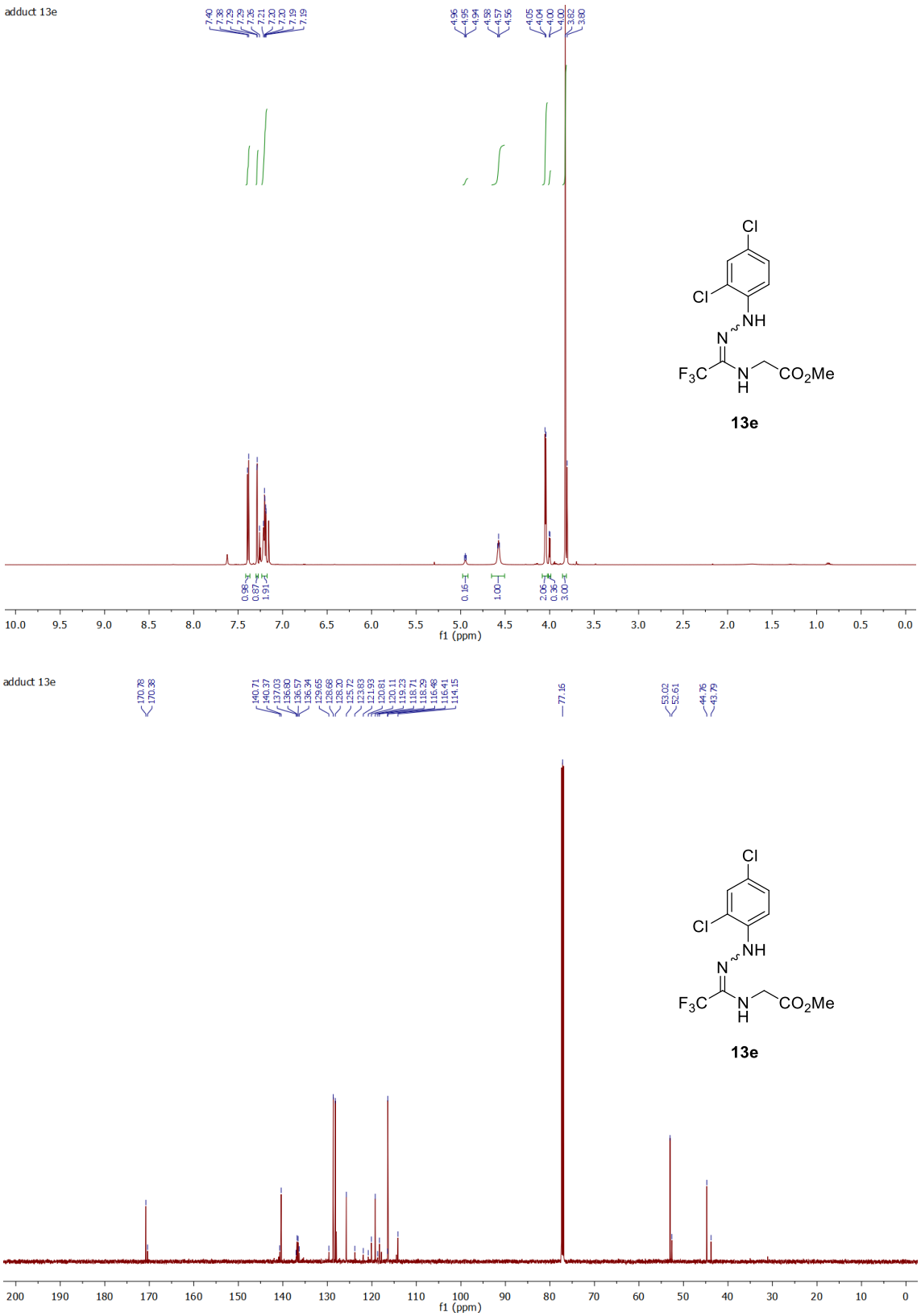


Figure S5. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **13e**.

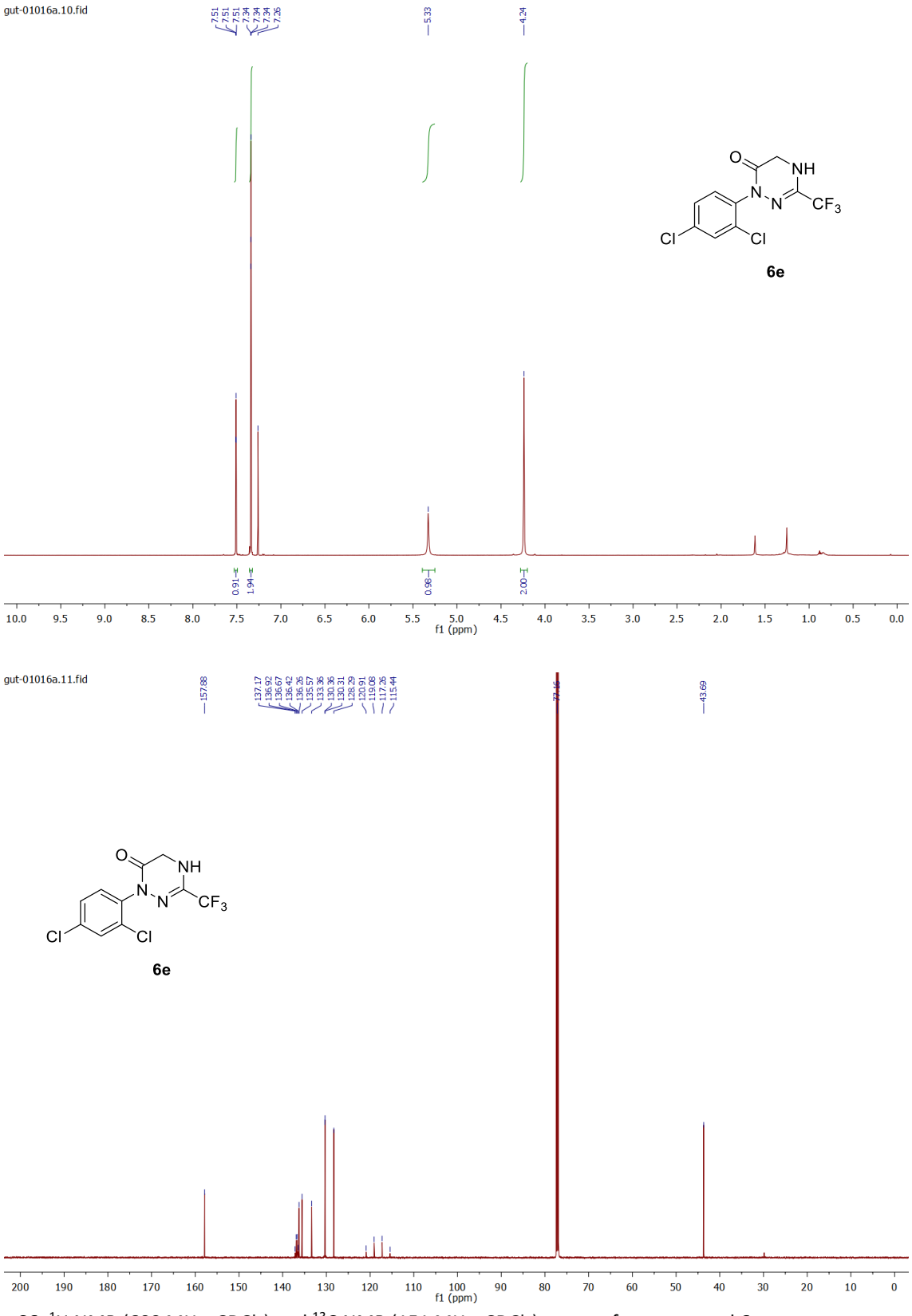


Figure S6. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **6e**.

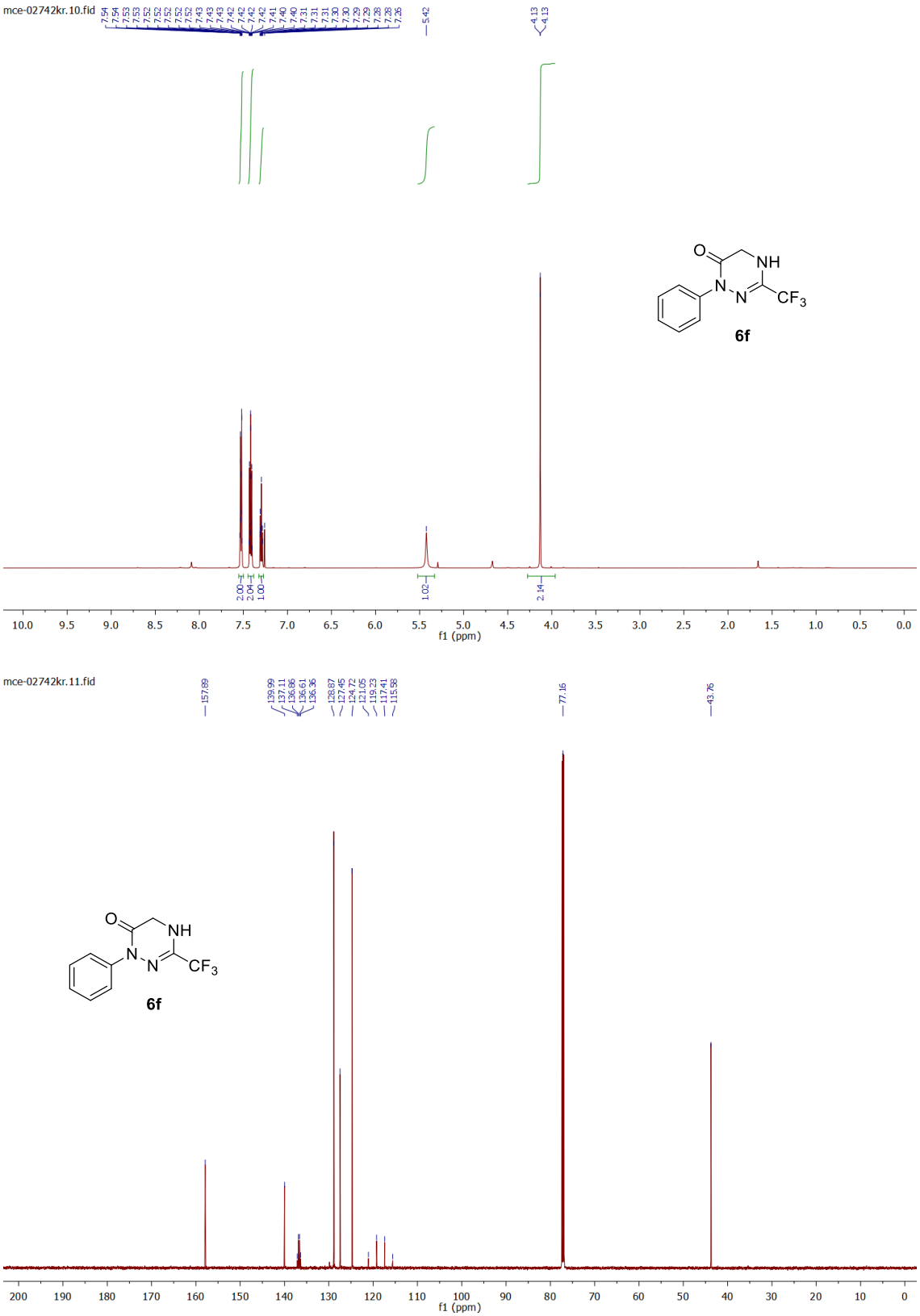


Figure S7. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **6f**.

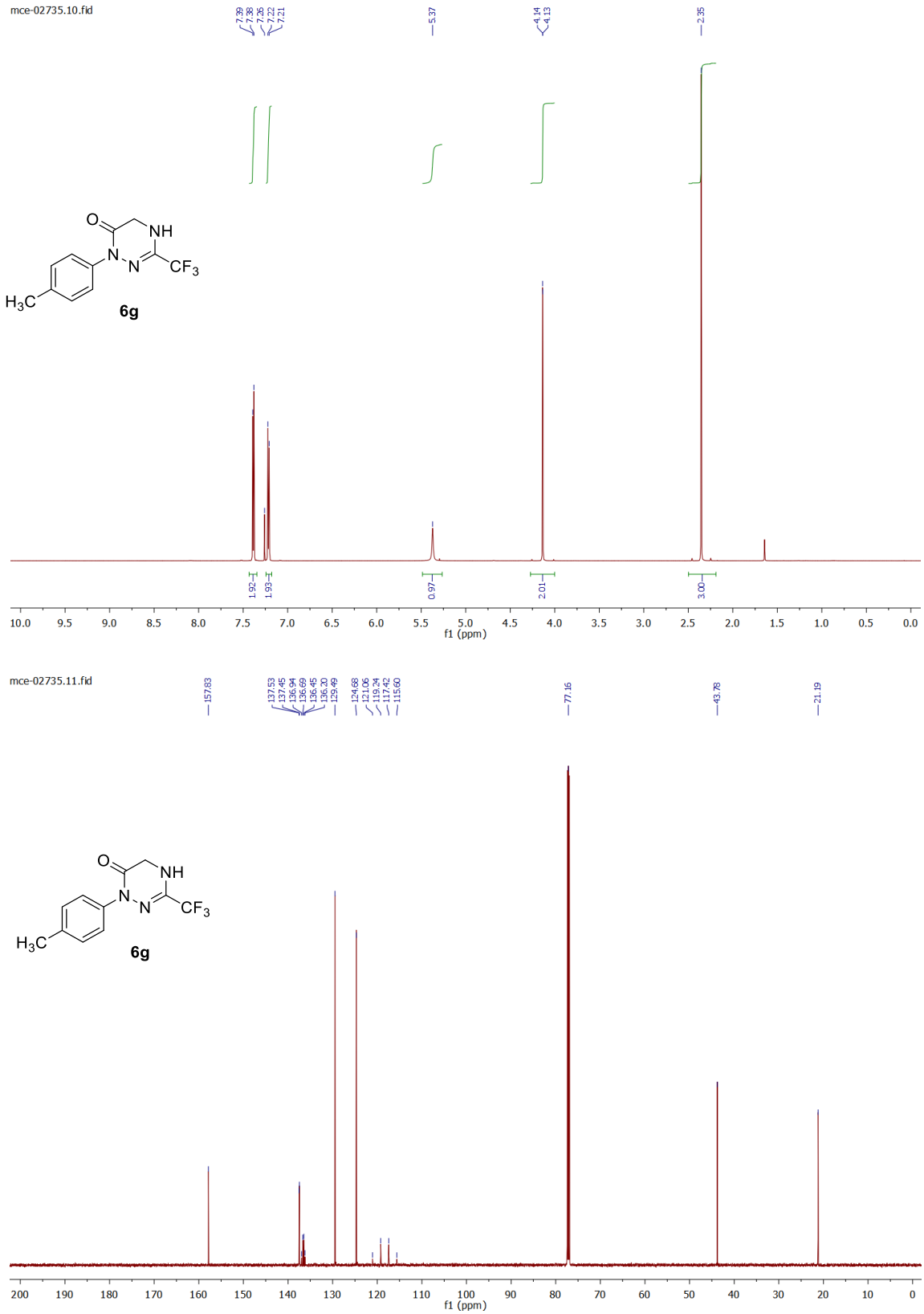


Figure S8. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **6g**.

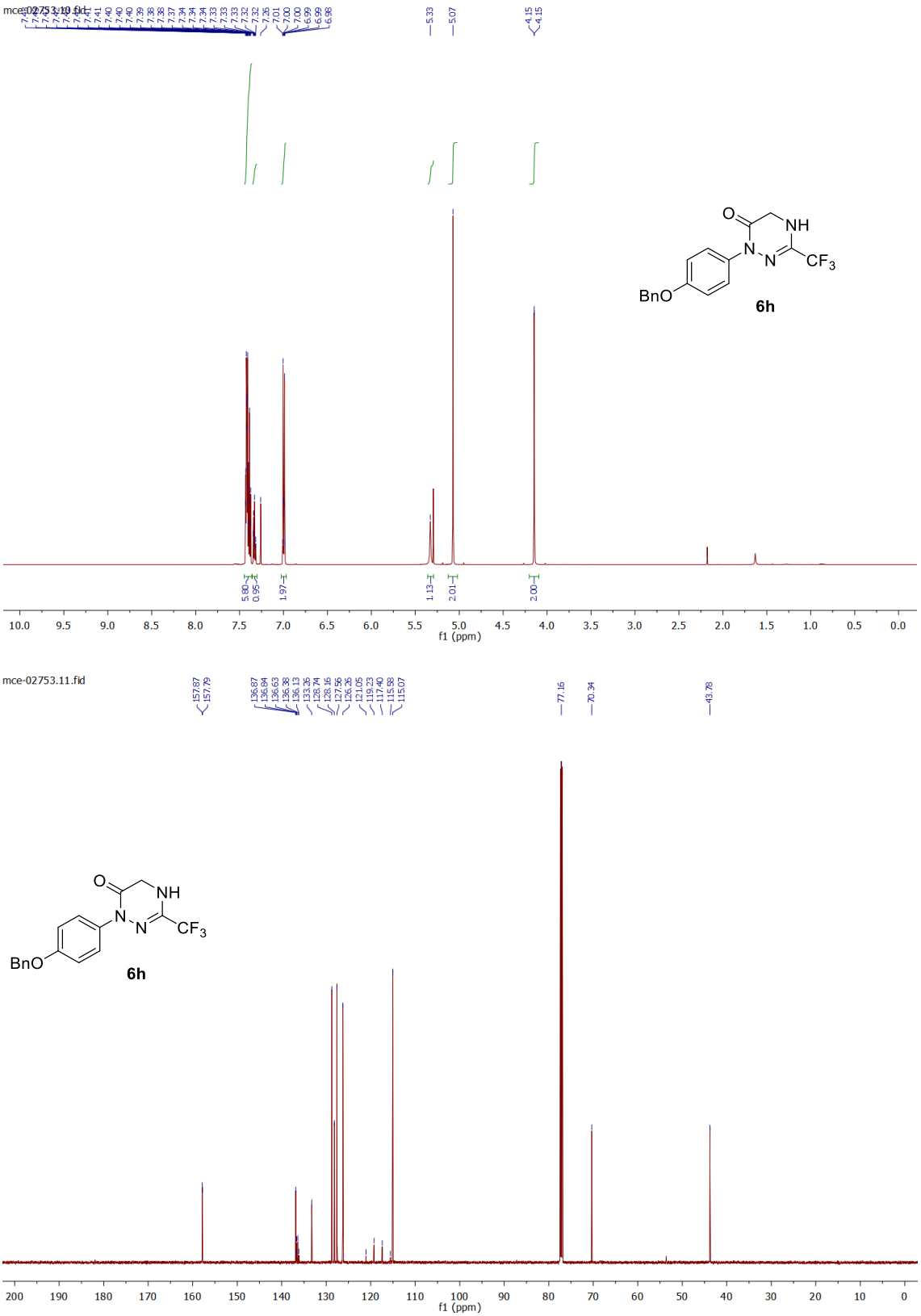


Figure S9. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **6h**.

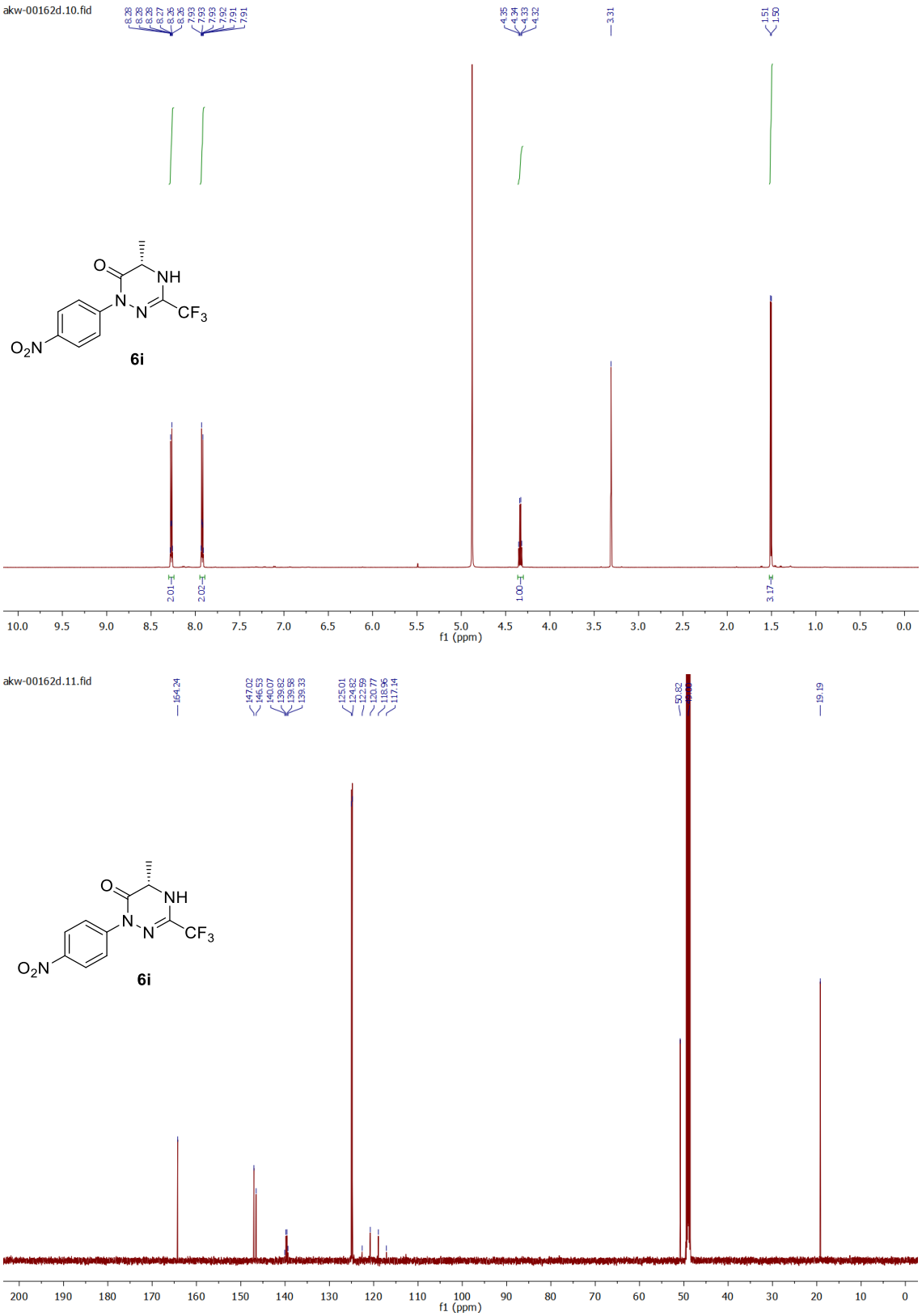


Figure S10. ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (151 MHz, CD_3OD) spectra for compound **6i**.

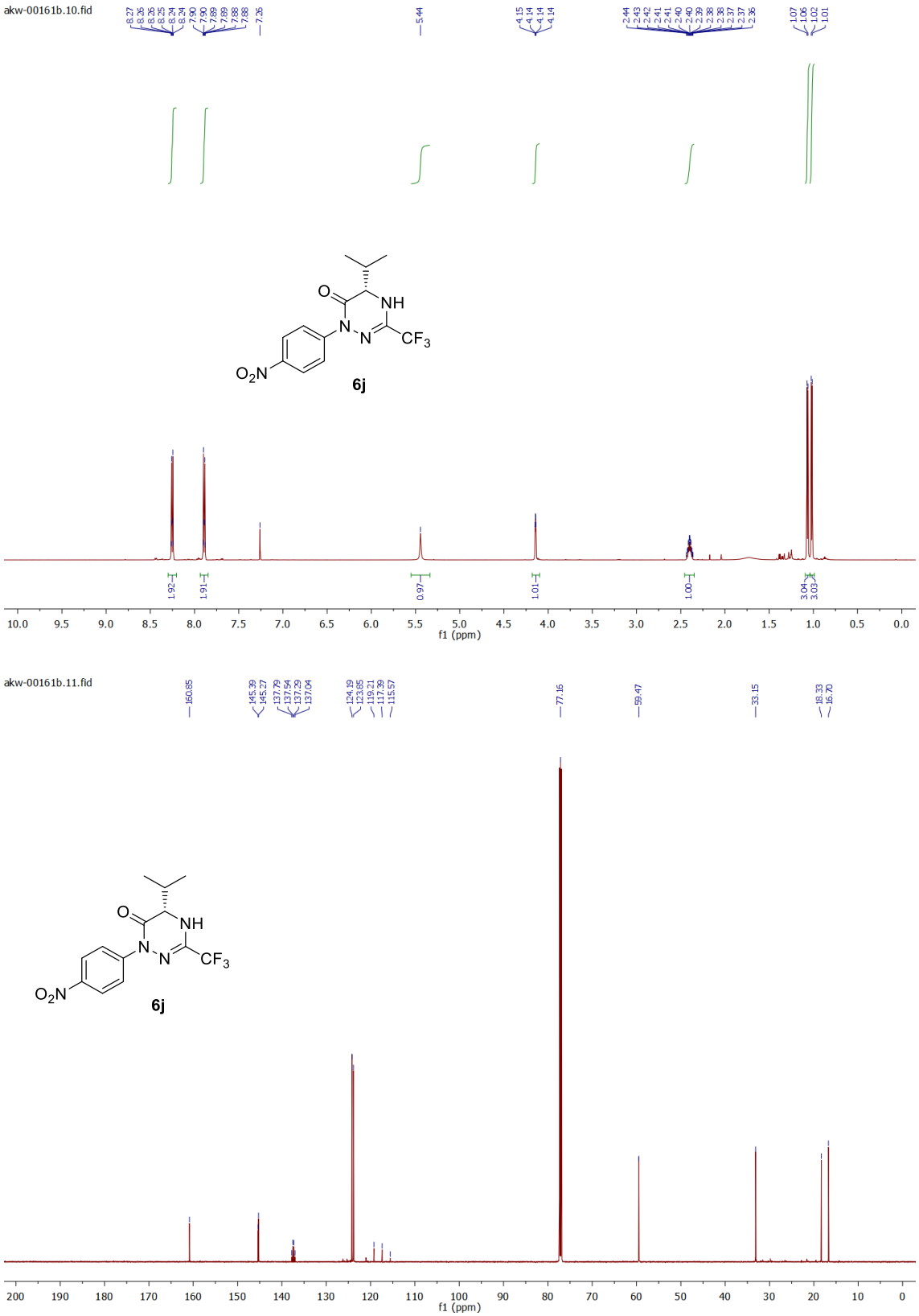


Figure S11. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **6j**.

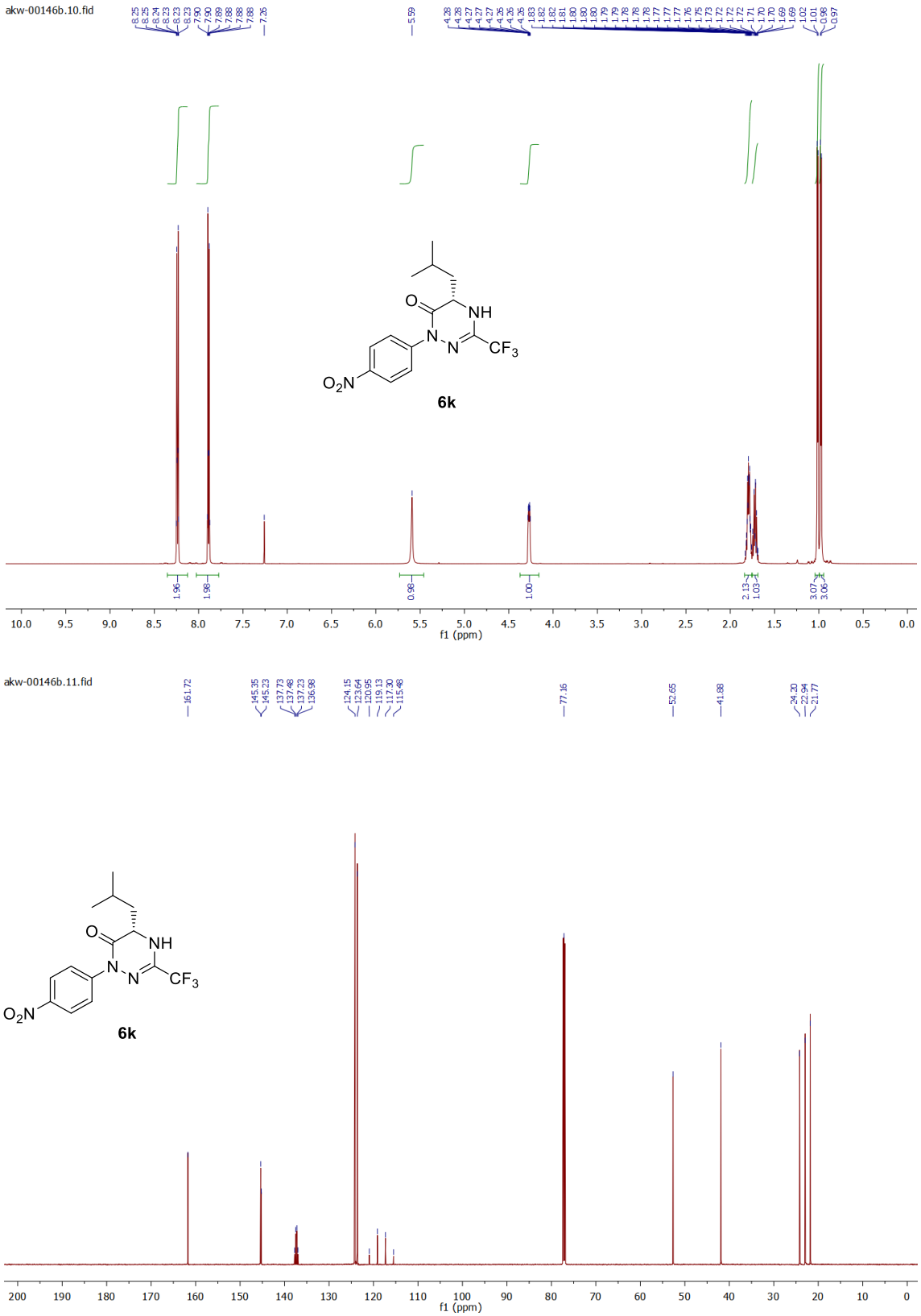


Figure S12. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **6k**.

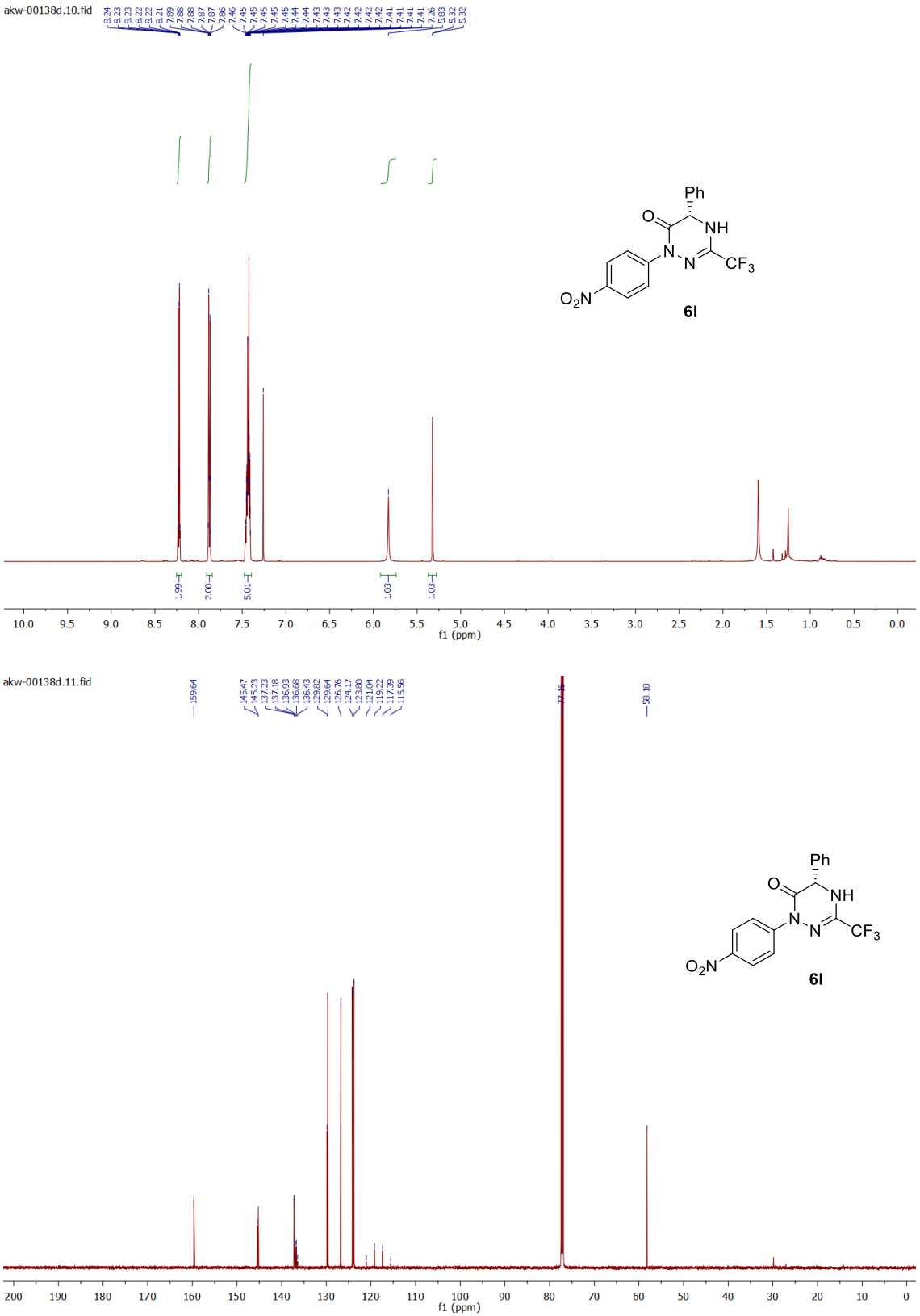


Figure S13. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **6l**.

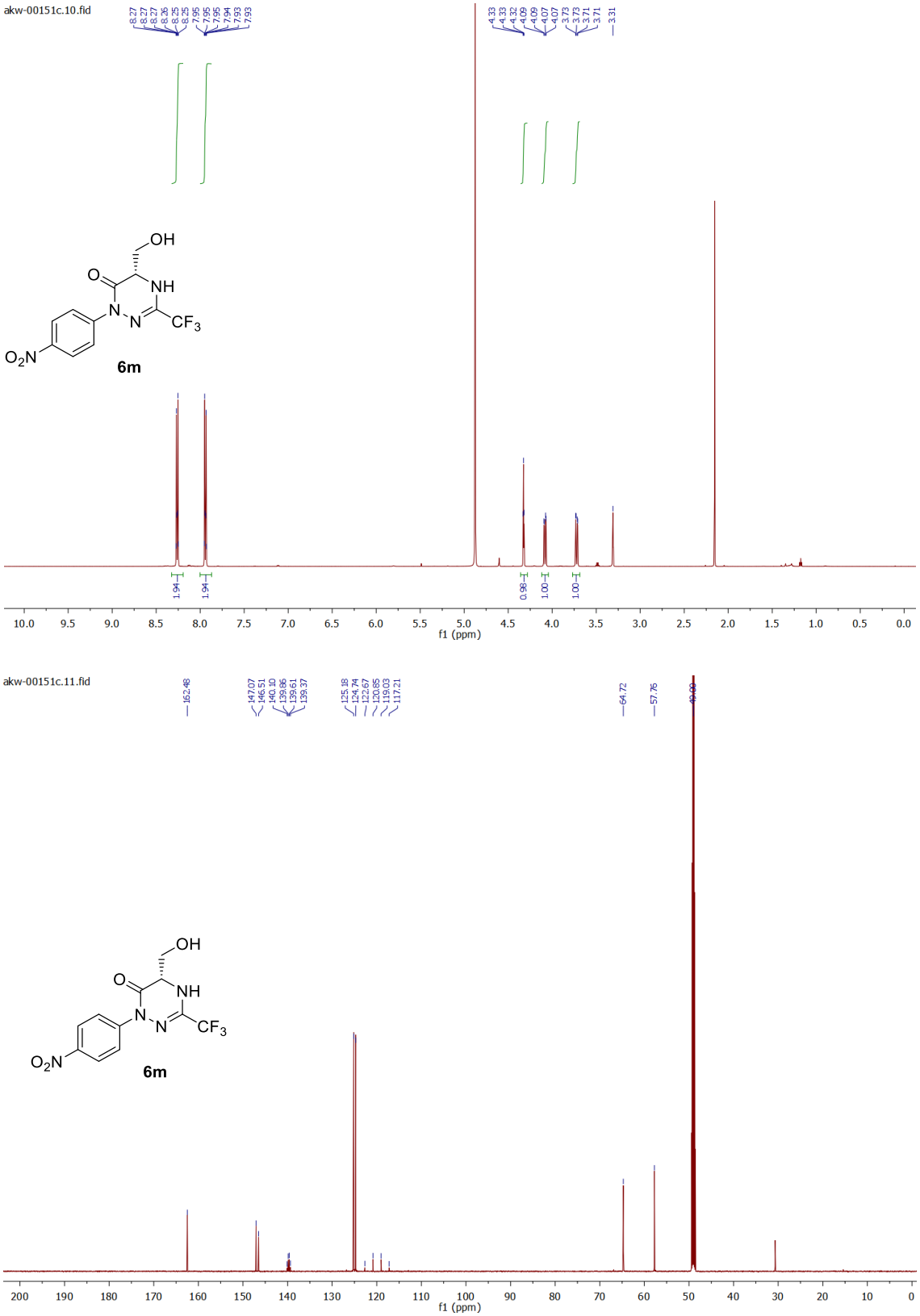


Figure S14. ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (151 MHz, CD_3OD) spectra for compound **6m**.

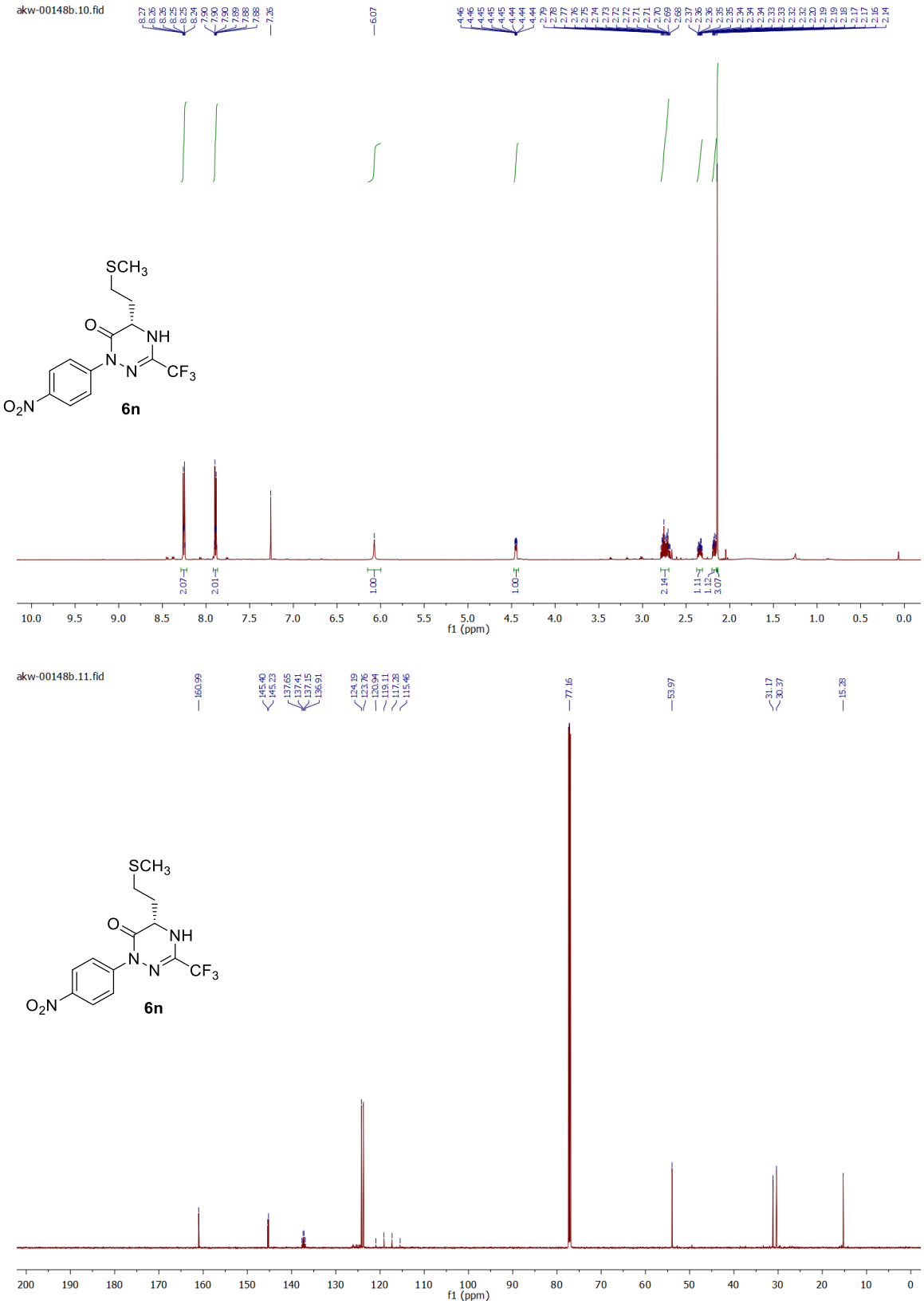


Figure S15. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **6n**.

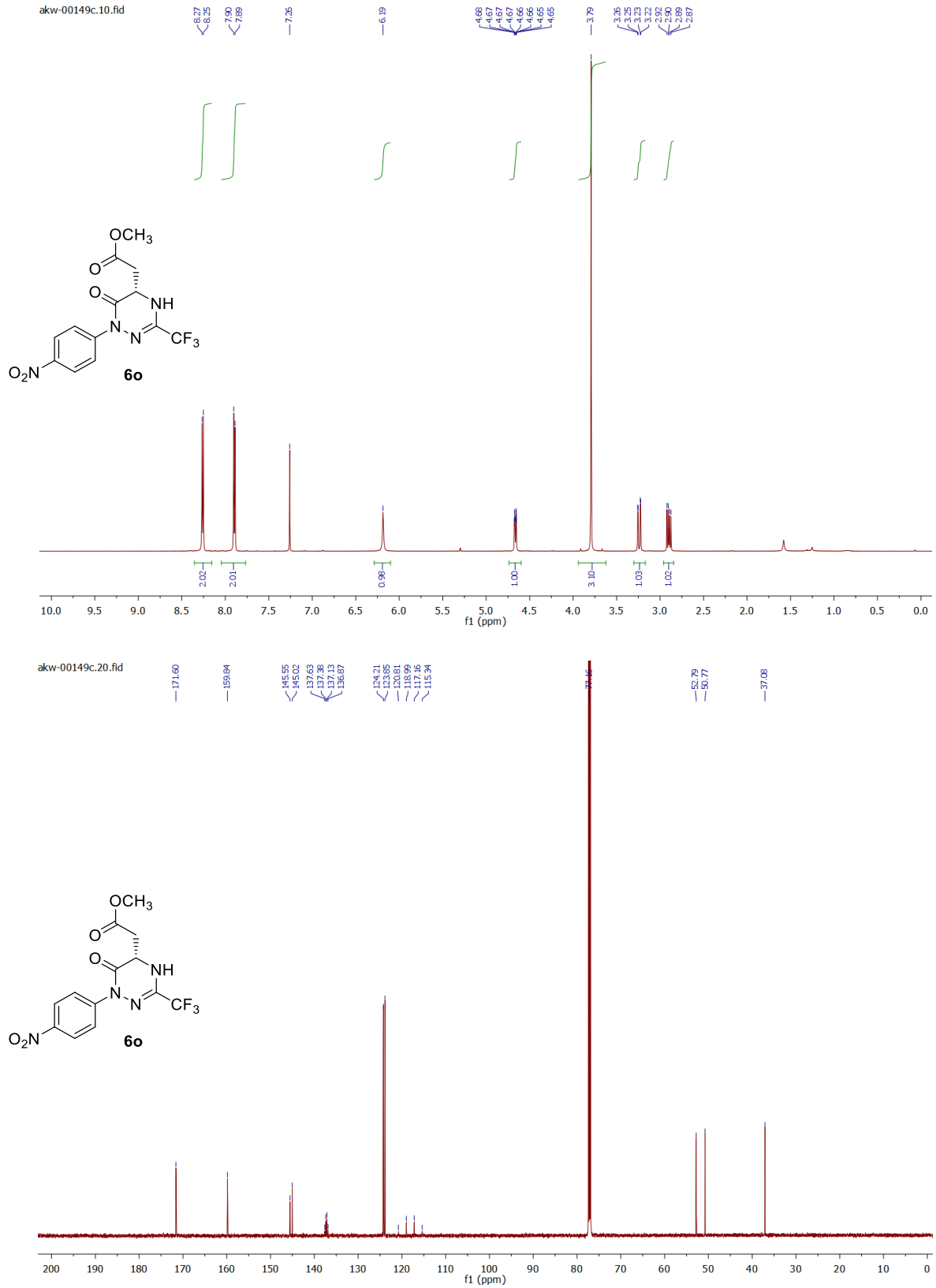


Figure S16. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **6o**.

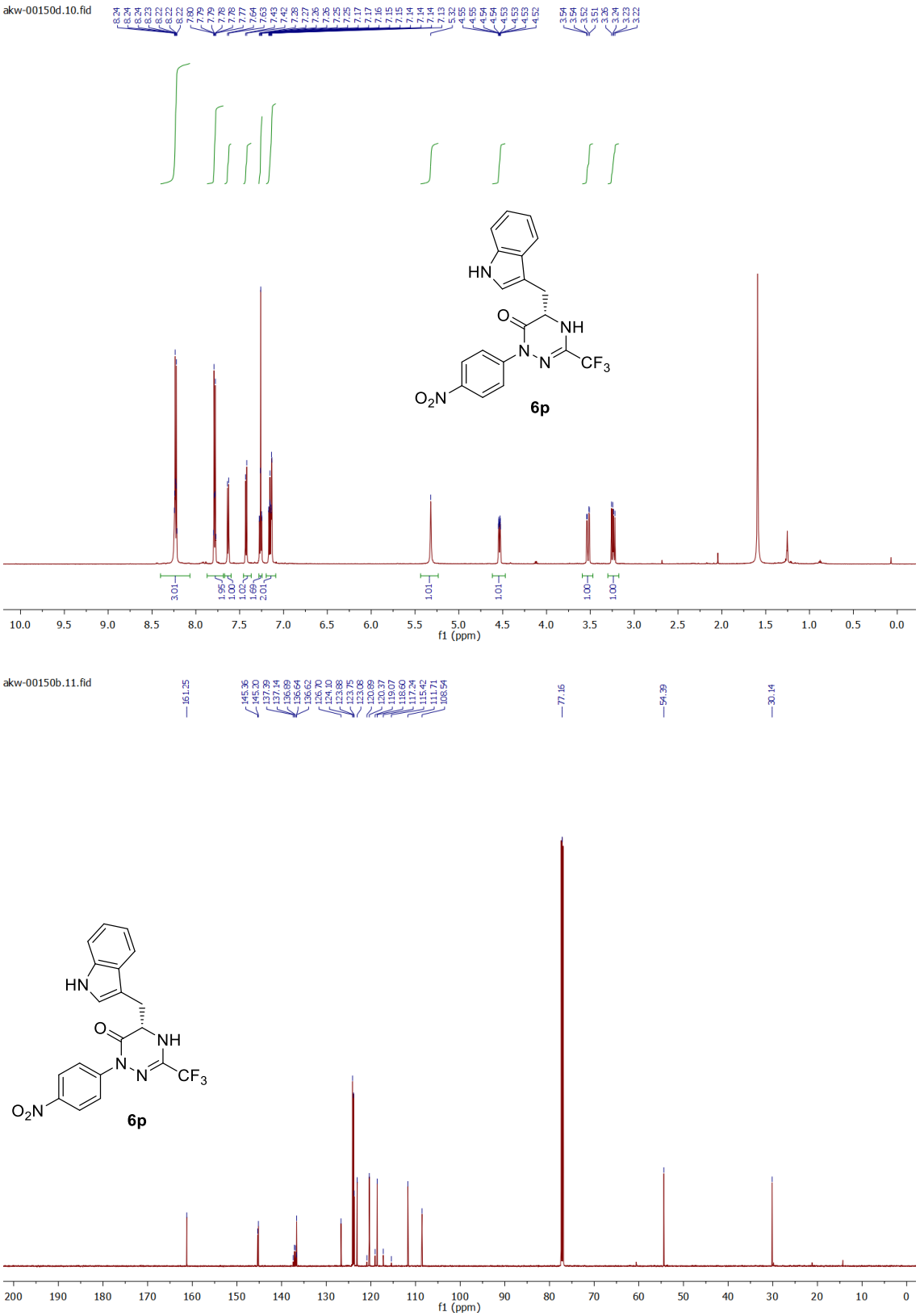


Figure S17. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **6p**.

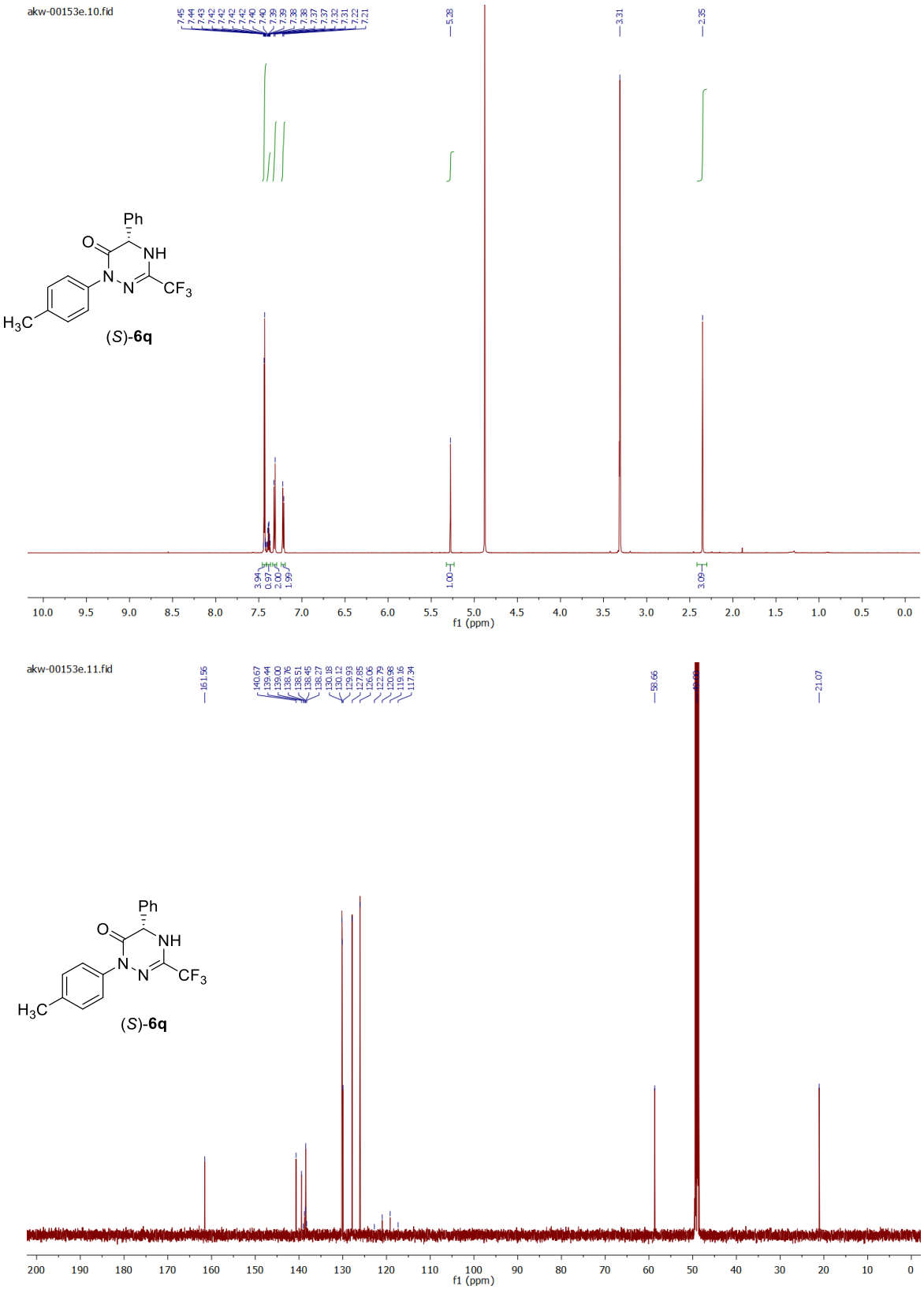


Figure S18. ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (151 MHz, CD_3OD) spectra for compound (*S*)-**6q**.

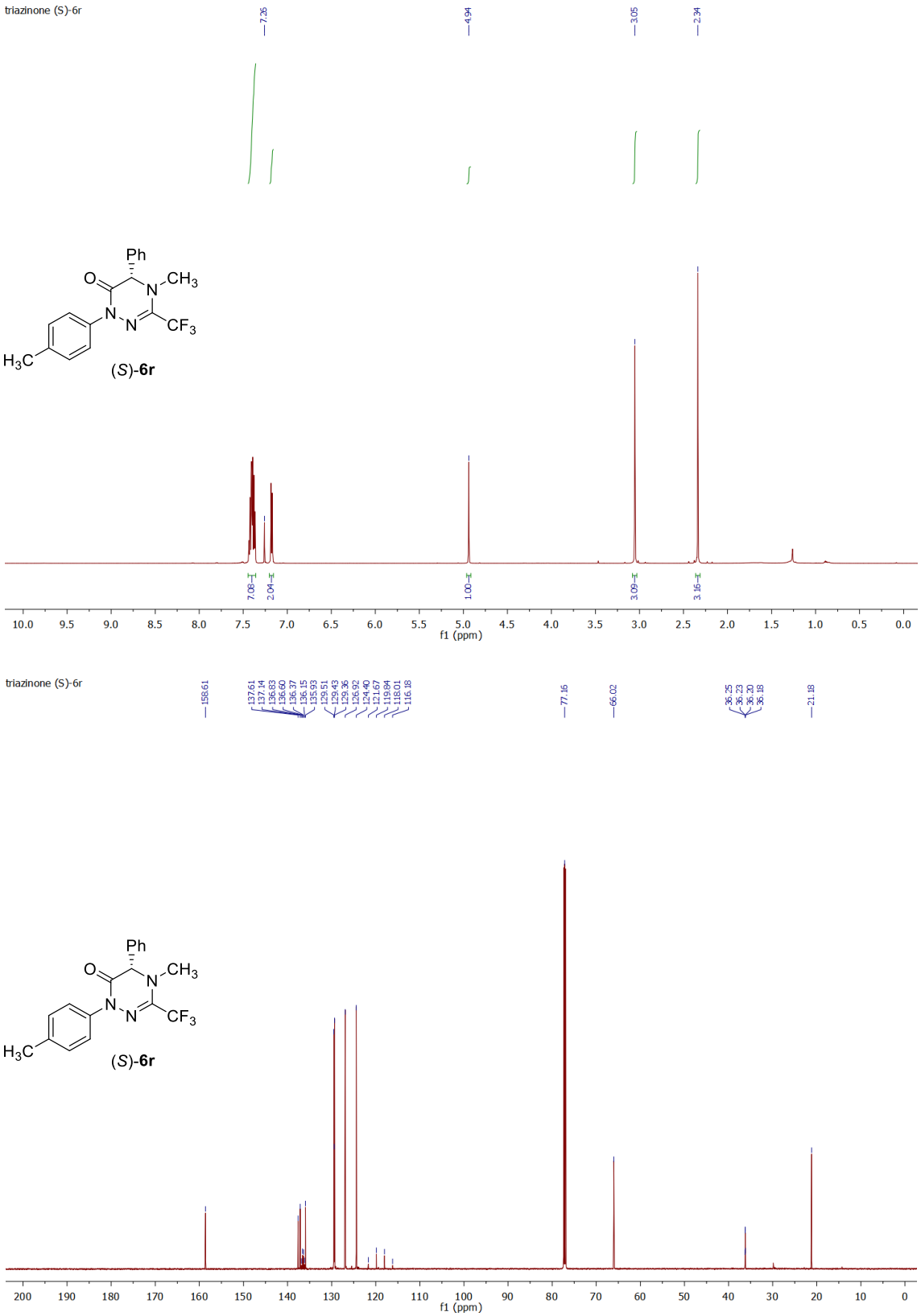


Figure S19. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound (*S*)-**6r**.

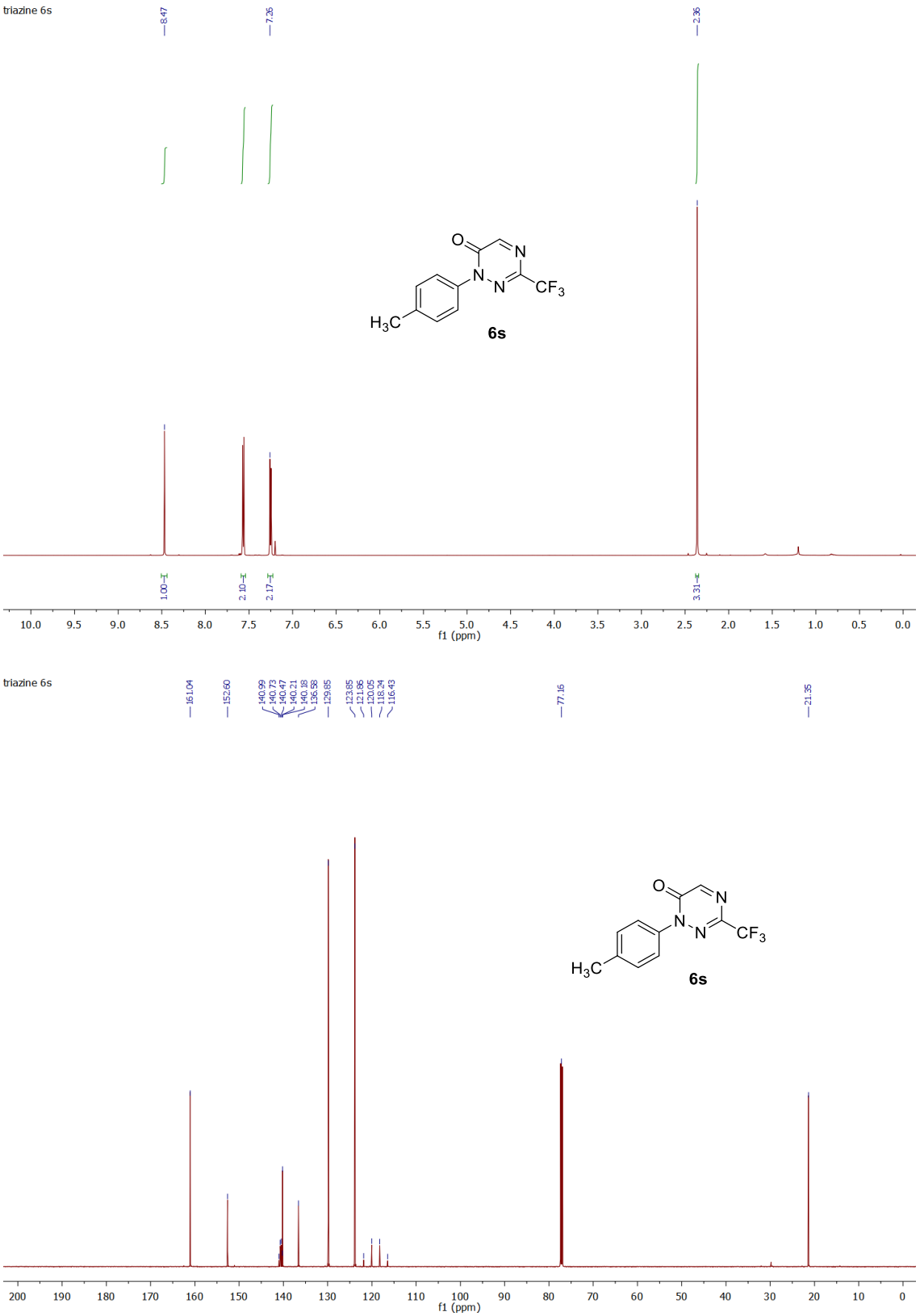


Figure S20. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **6s**.

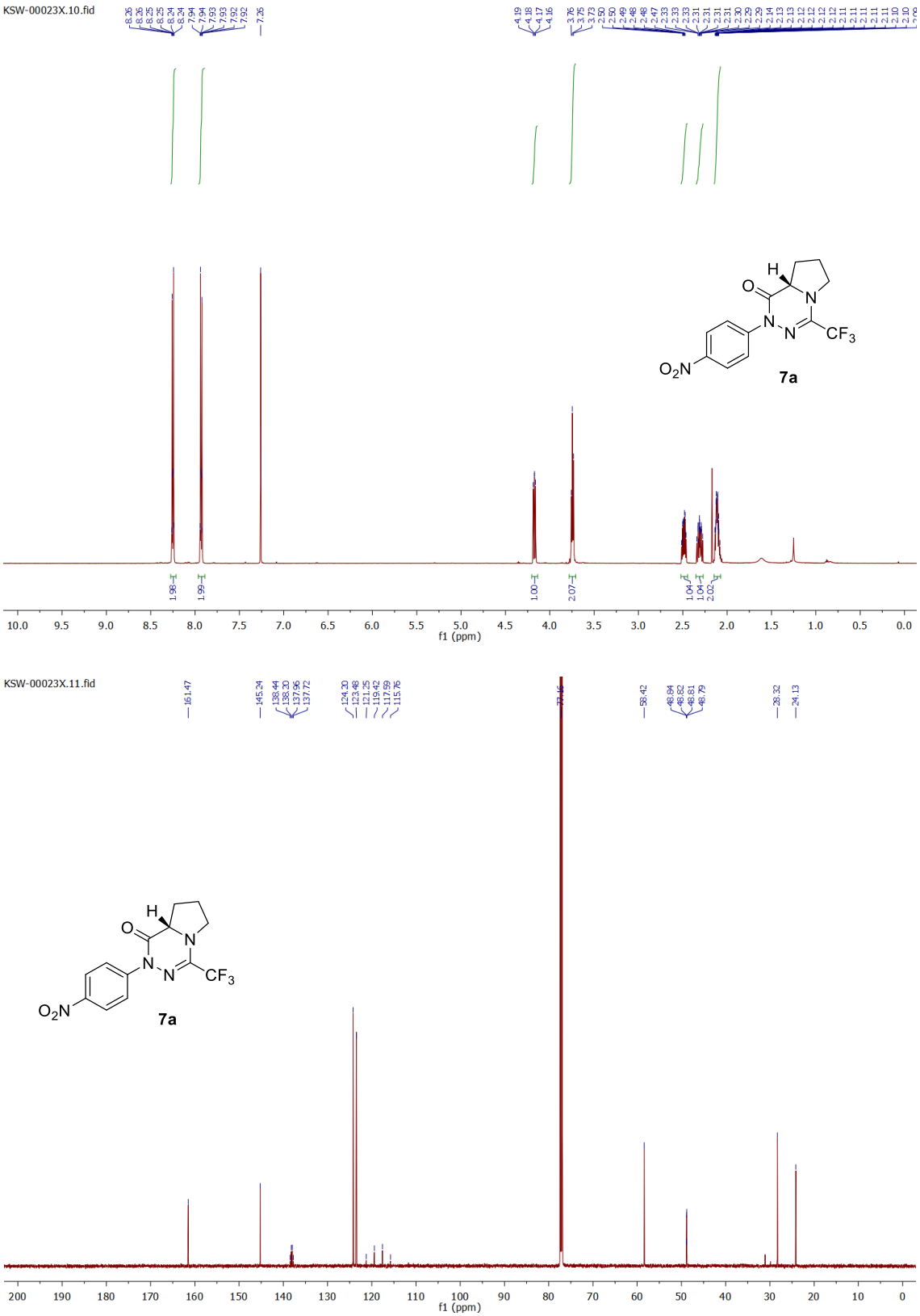


Figure S21. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **7a**.

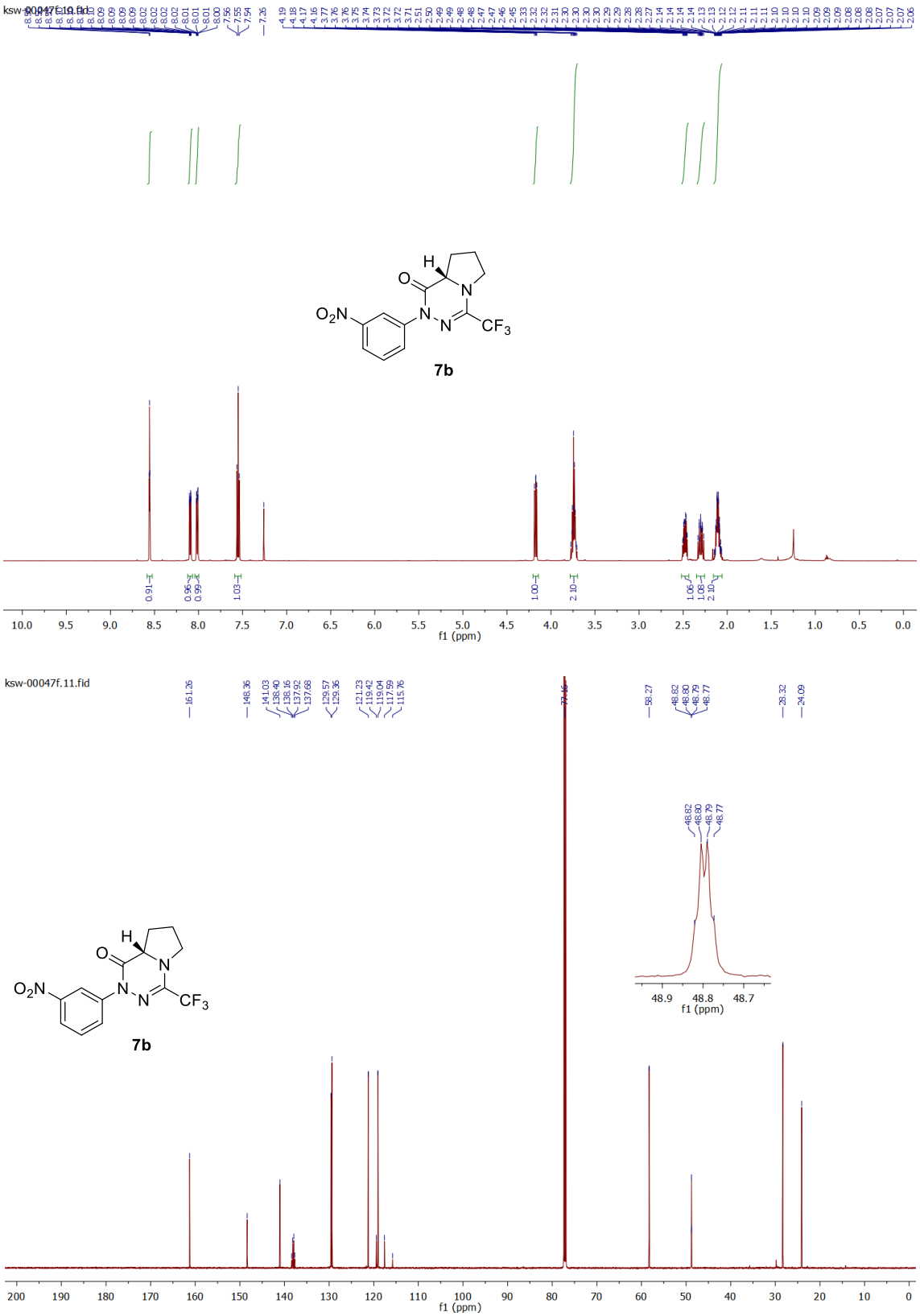


Figure S22. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **7b**.

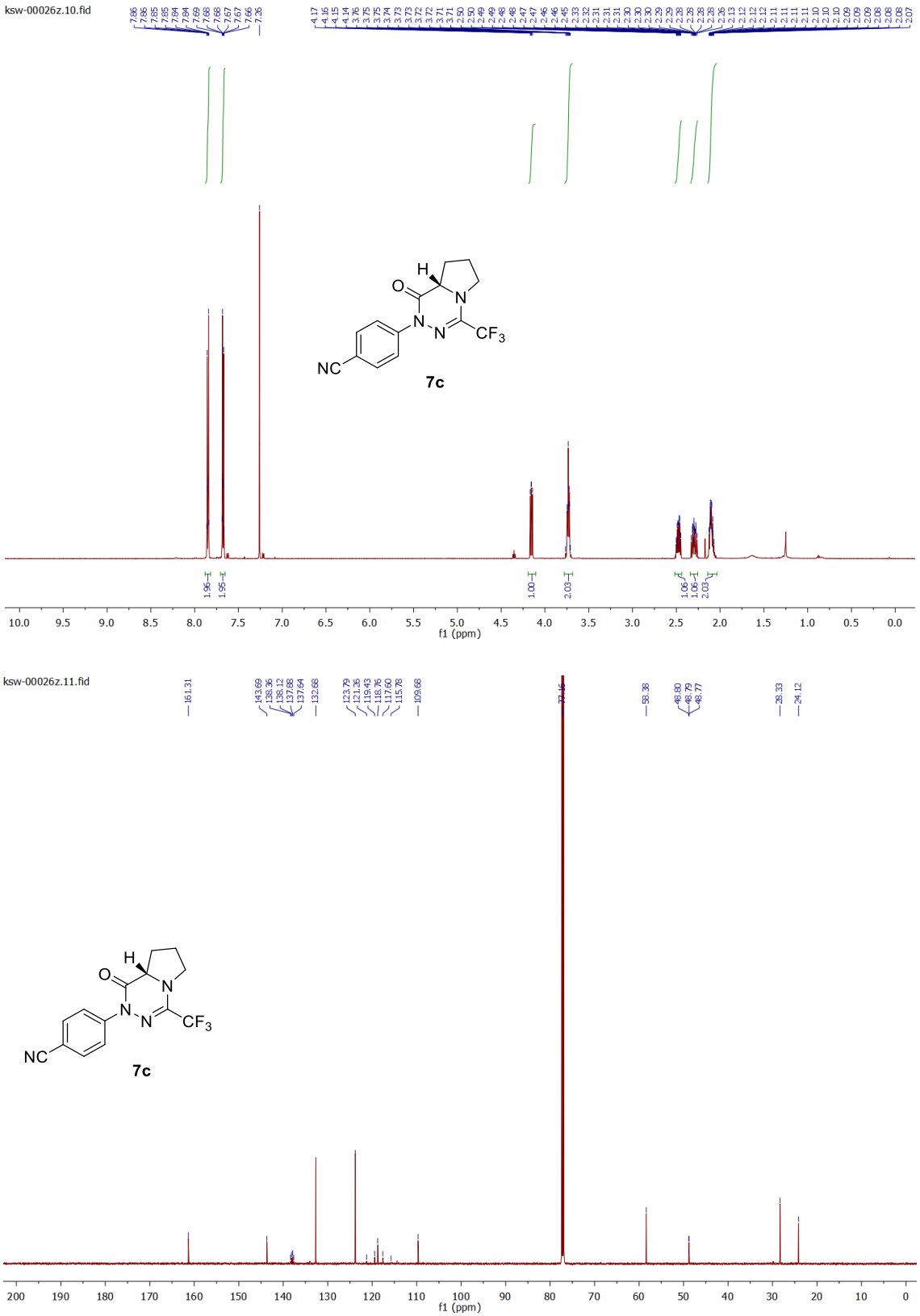


Figure S23. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **7c**.

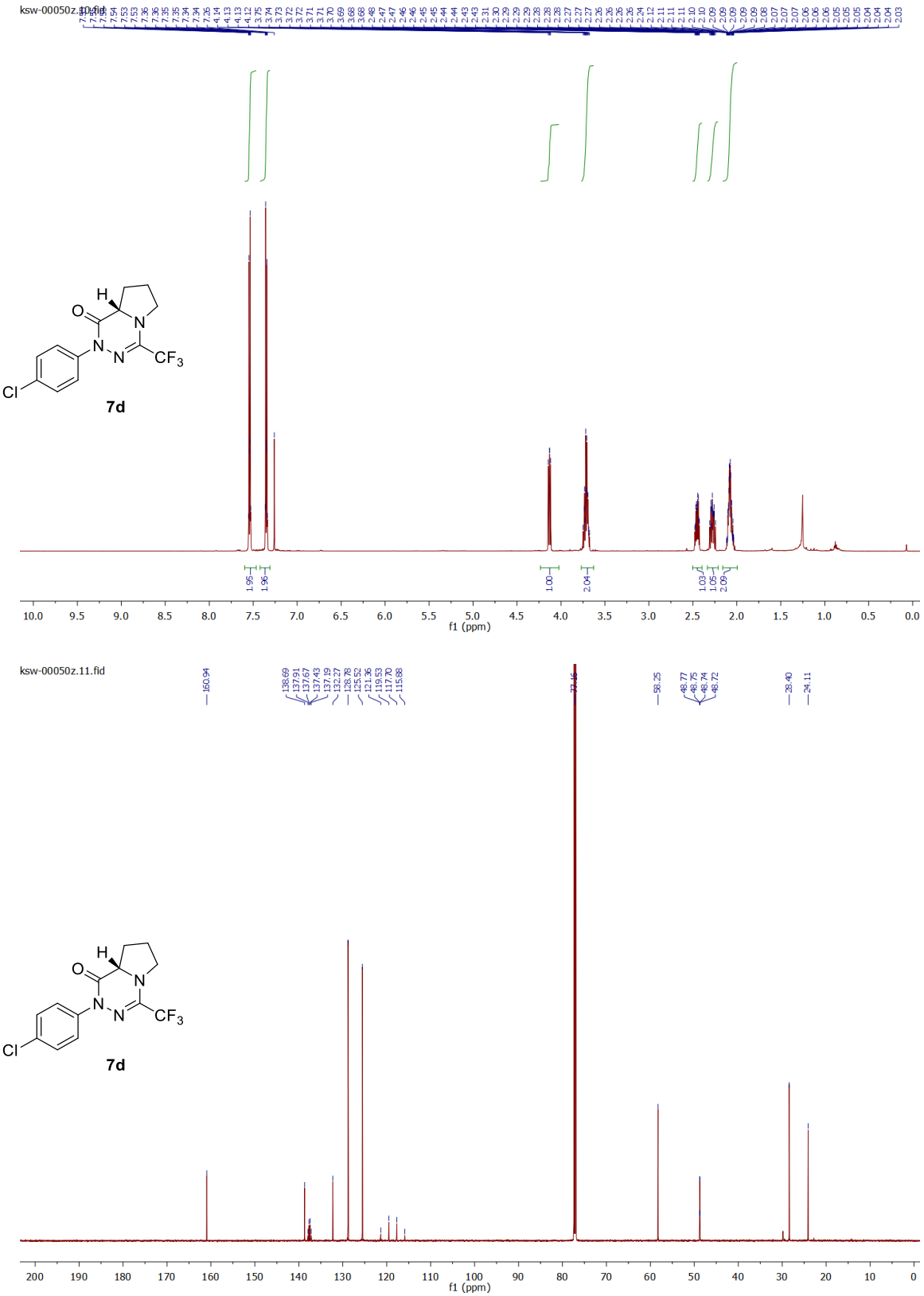


Figure S24. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **7d**.

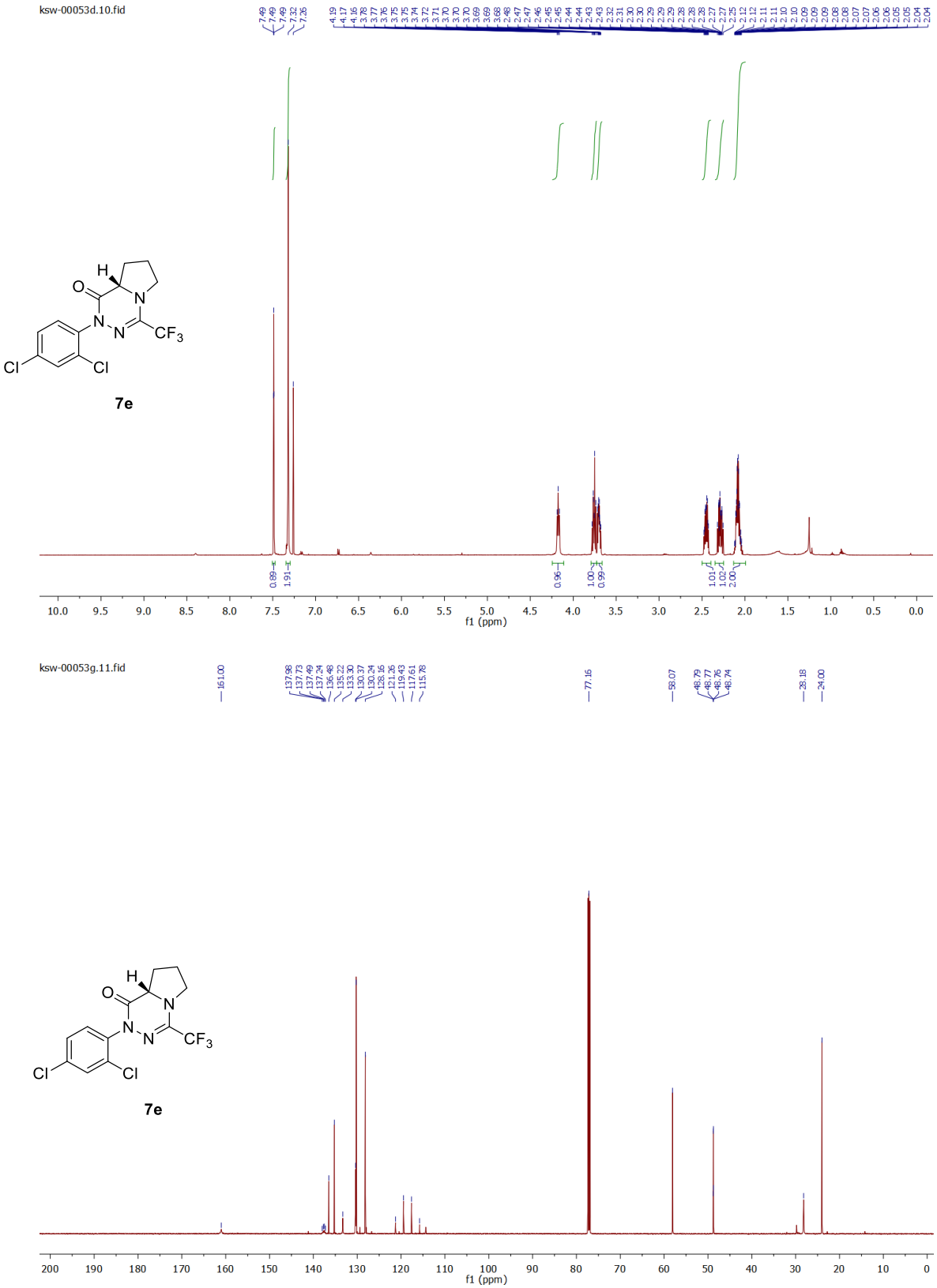


Figure S25. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **7e**.

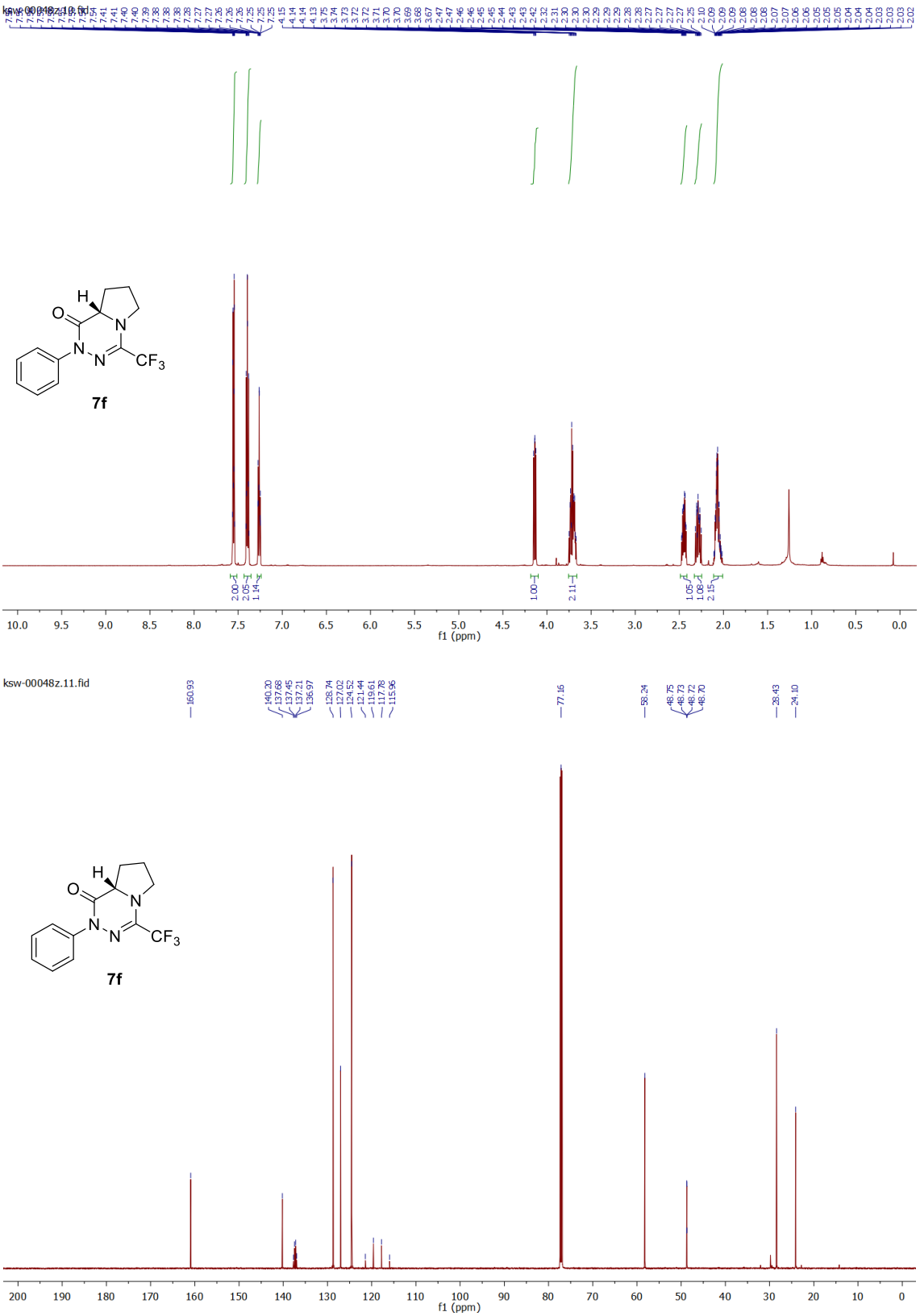


Figure S26. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **7f**.

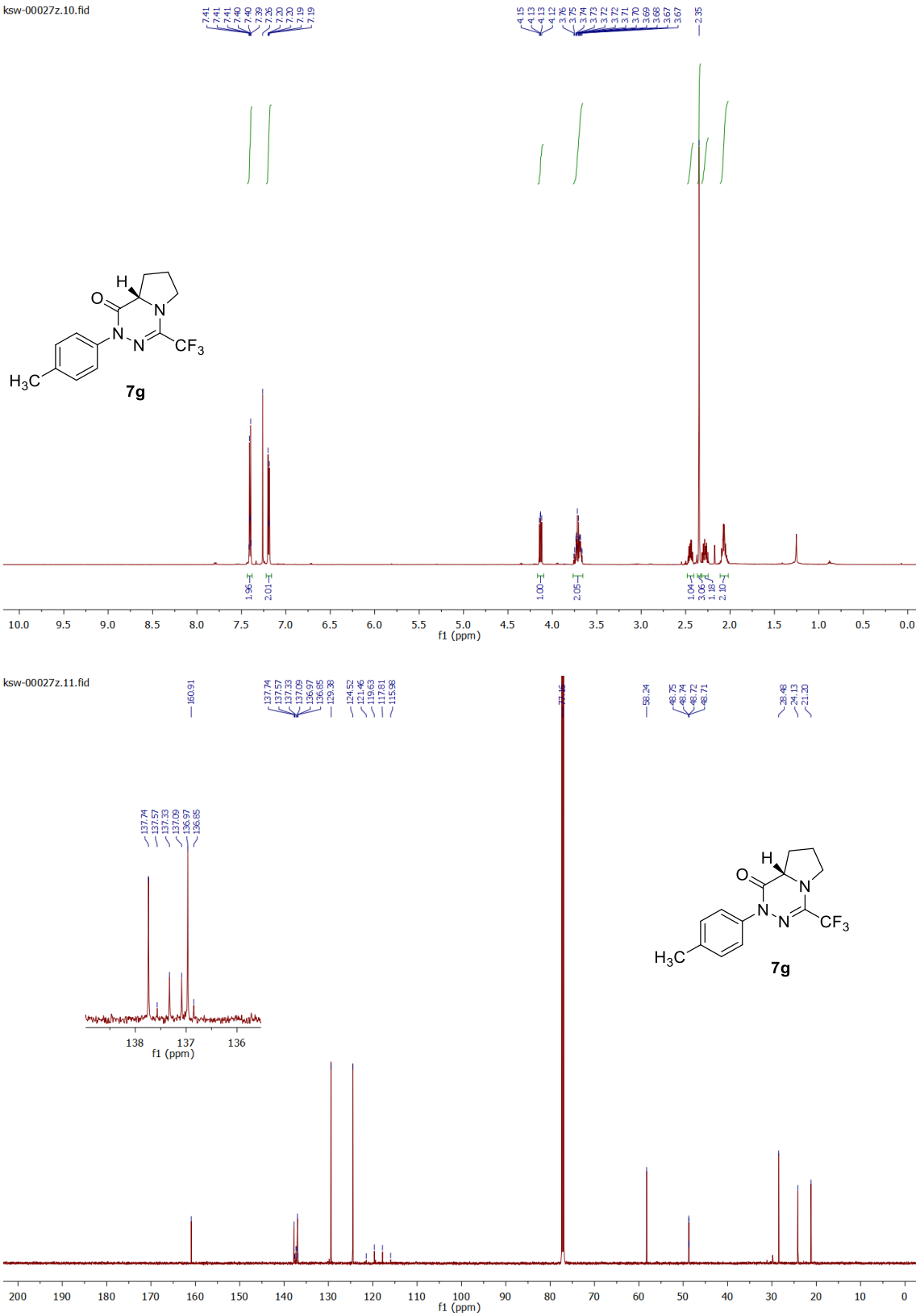


Figure S27. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **7g**.

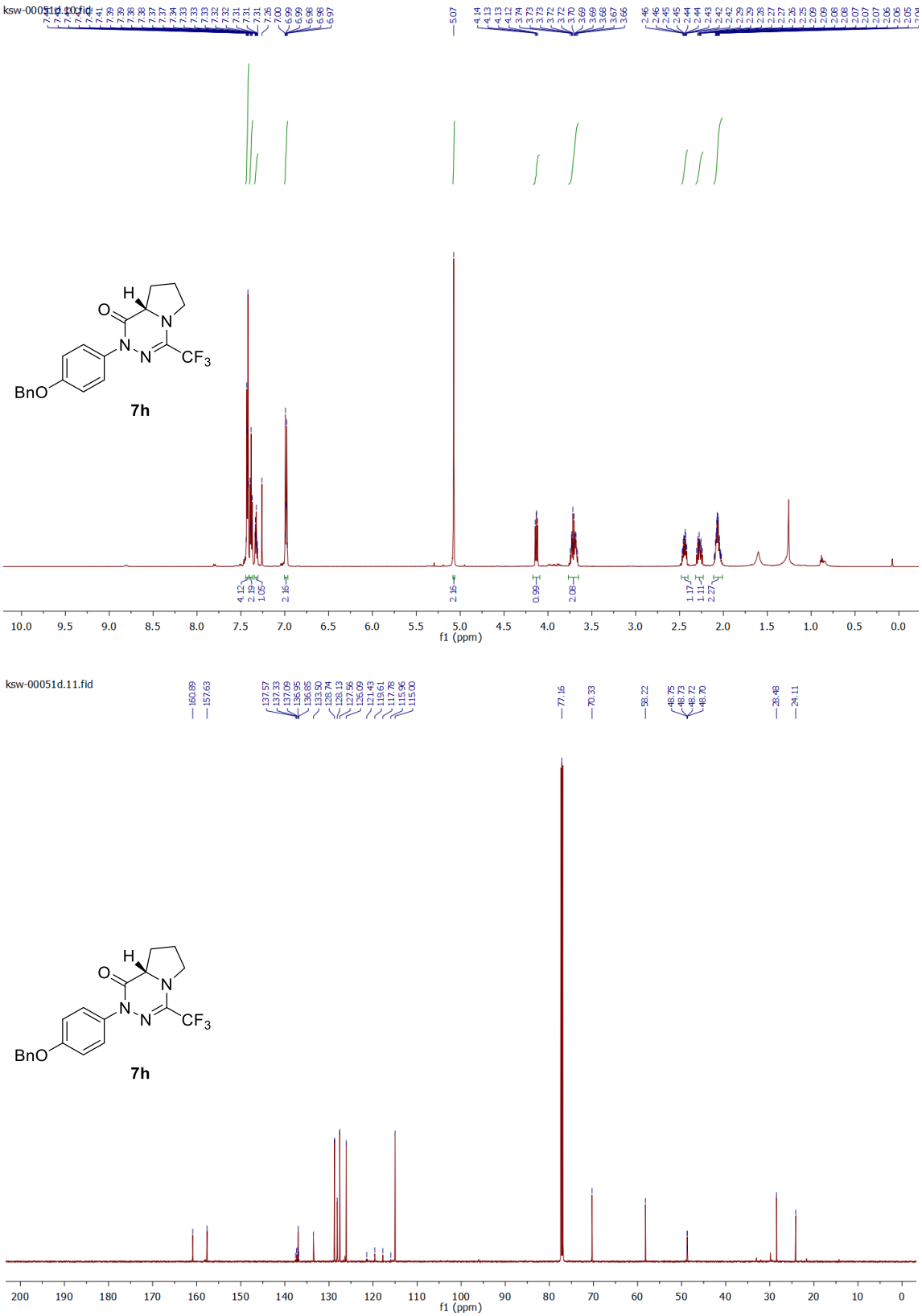


Figure S28. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **7h**.

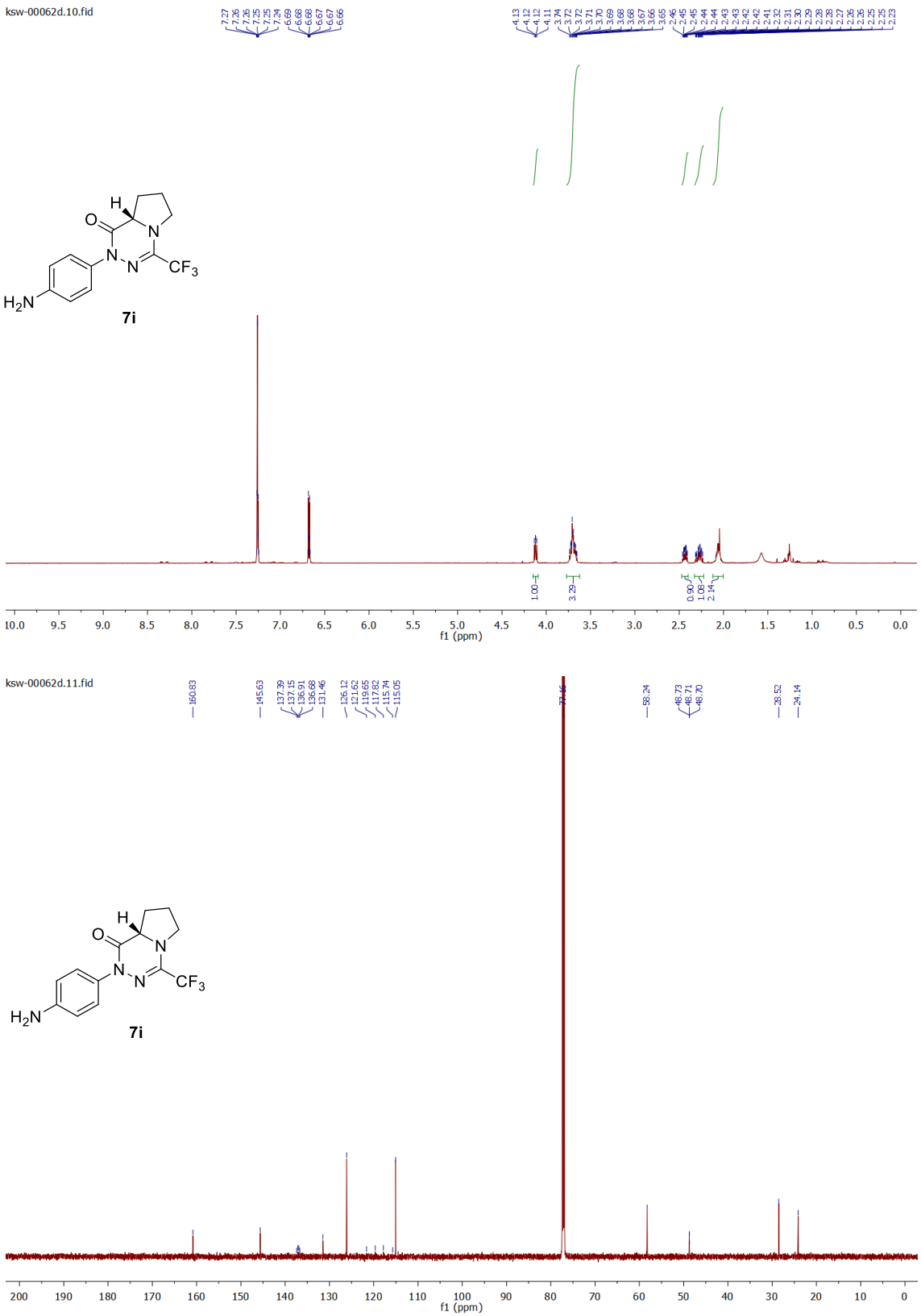


Figure S29. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **7i**.

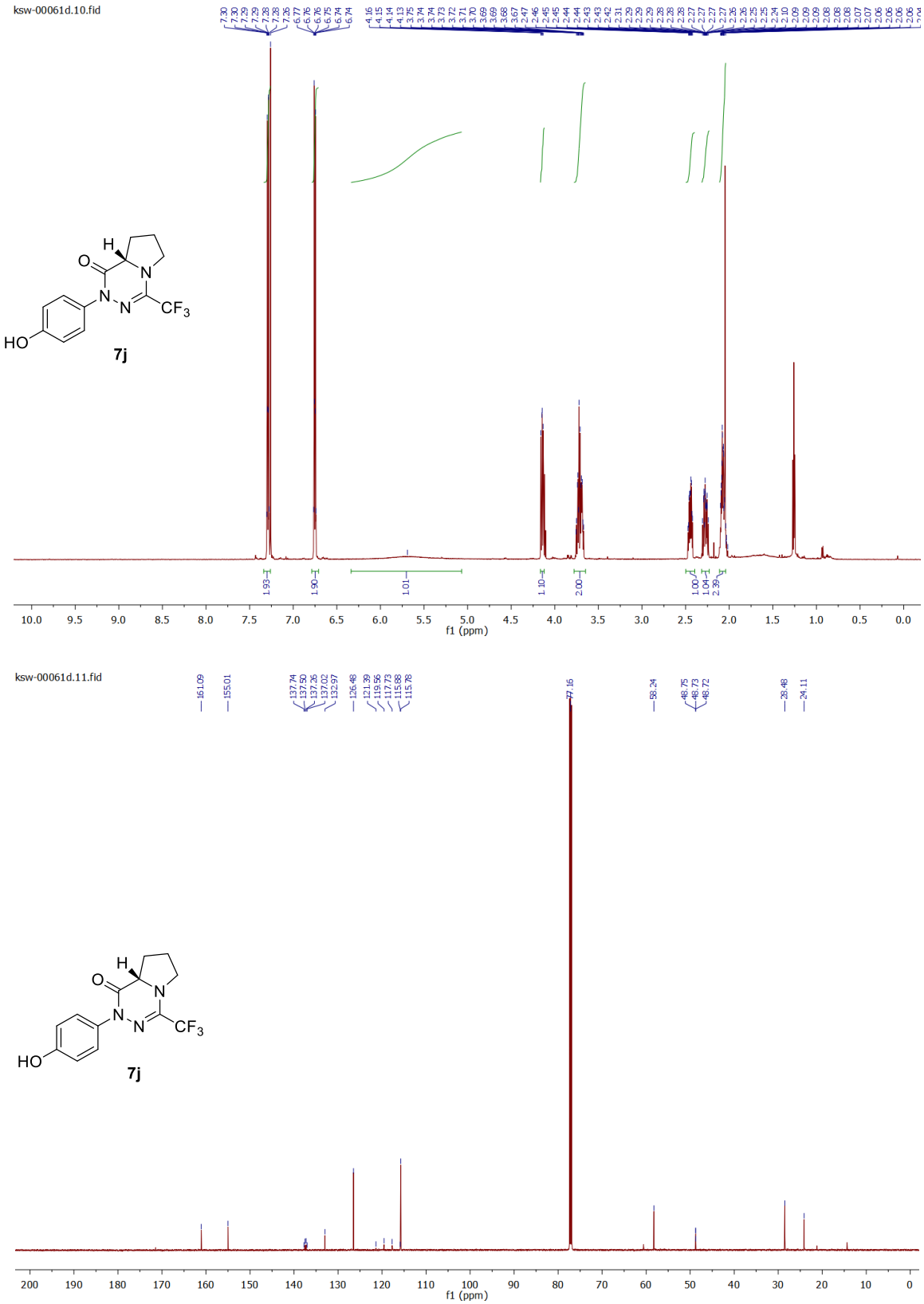


Figure S30. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **7j**.

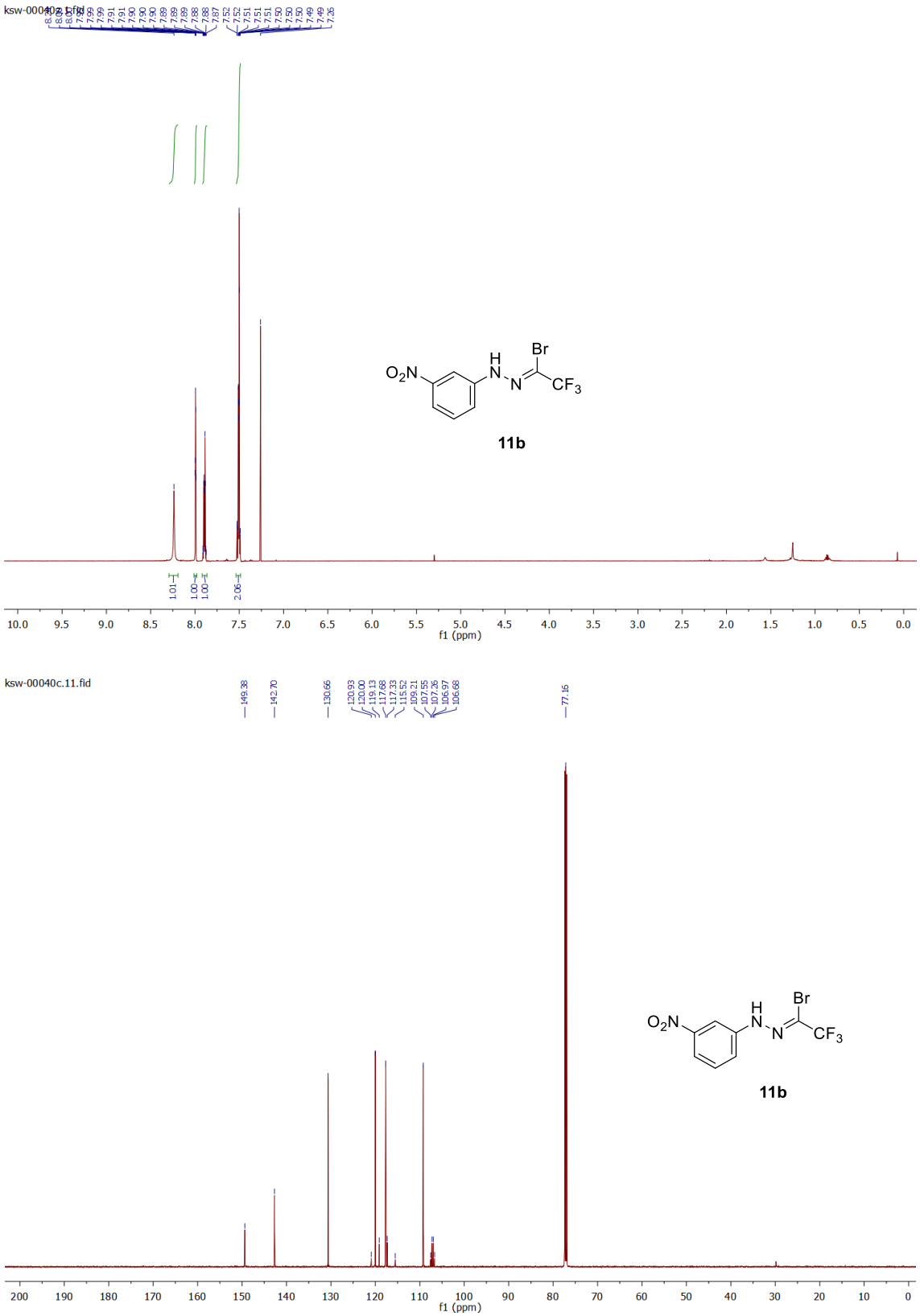


Figure S31. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **11b**.

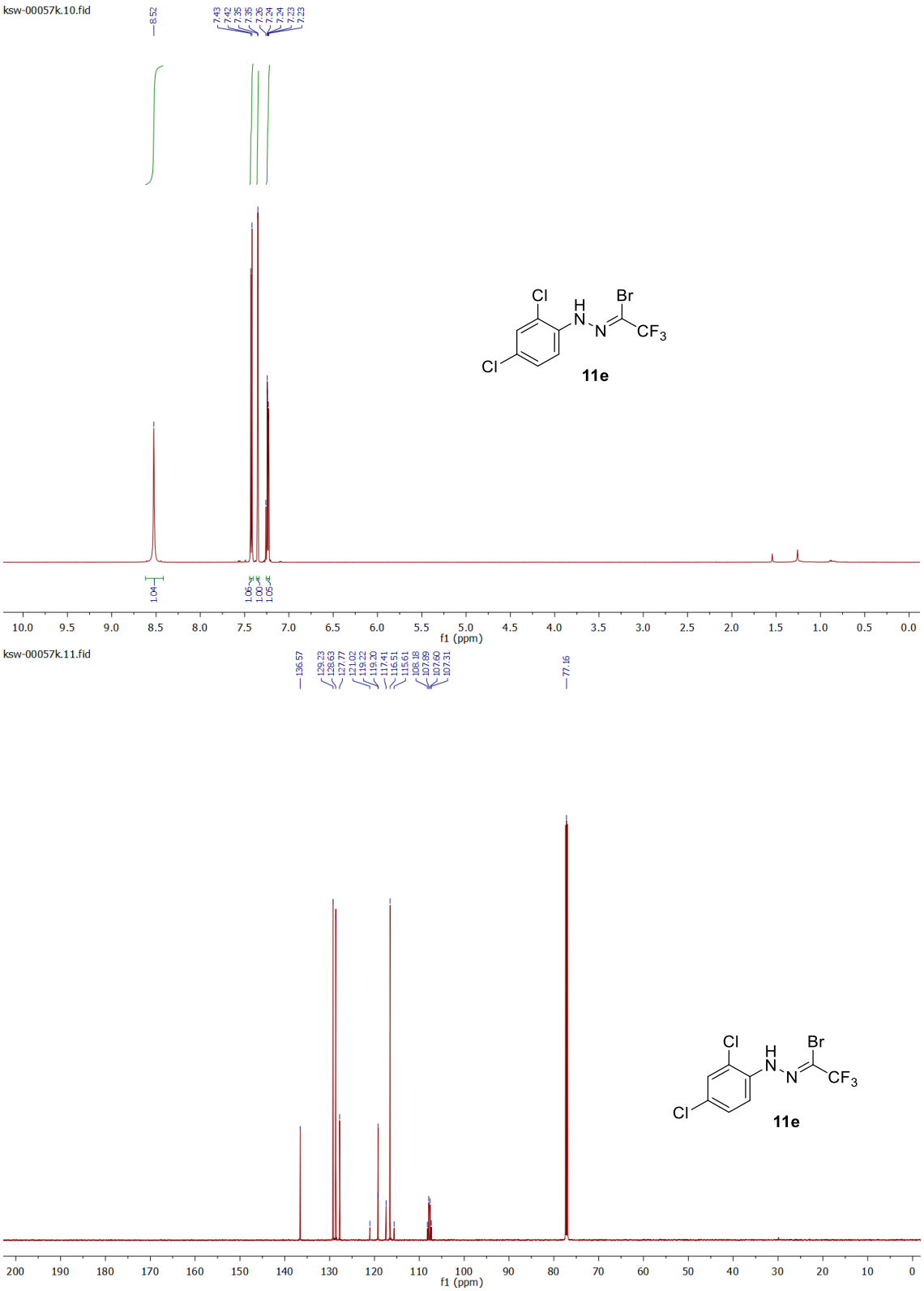


Figure S32. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **11e**.

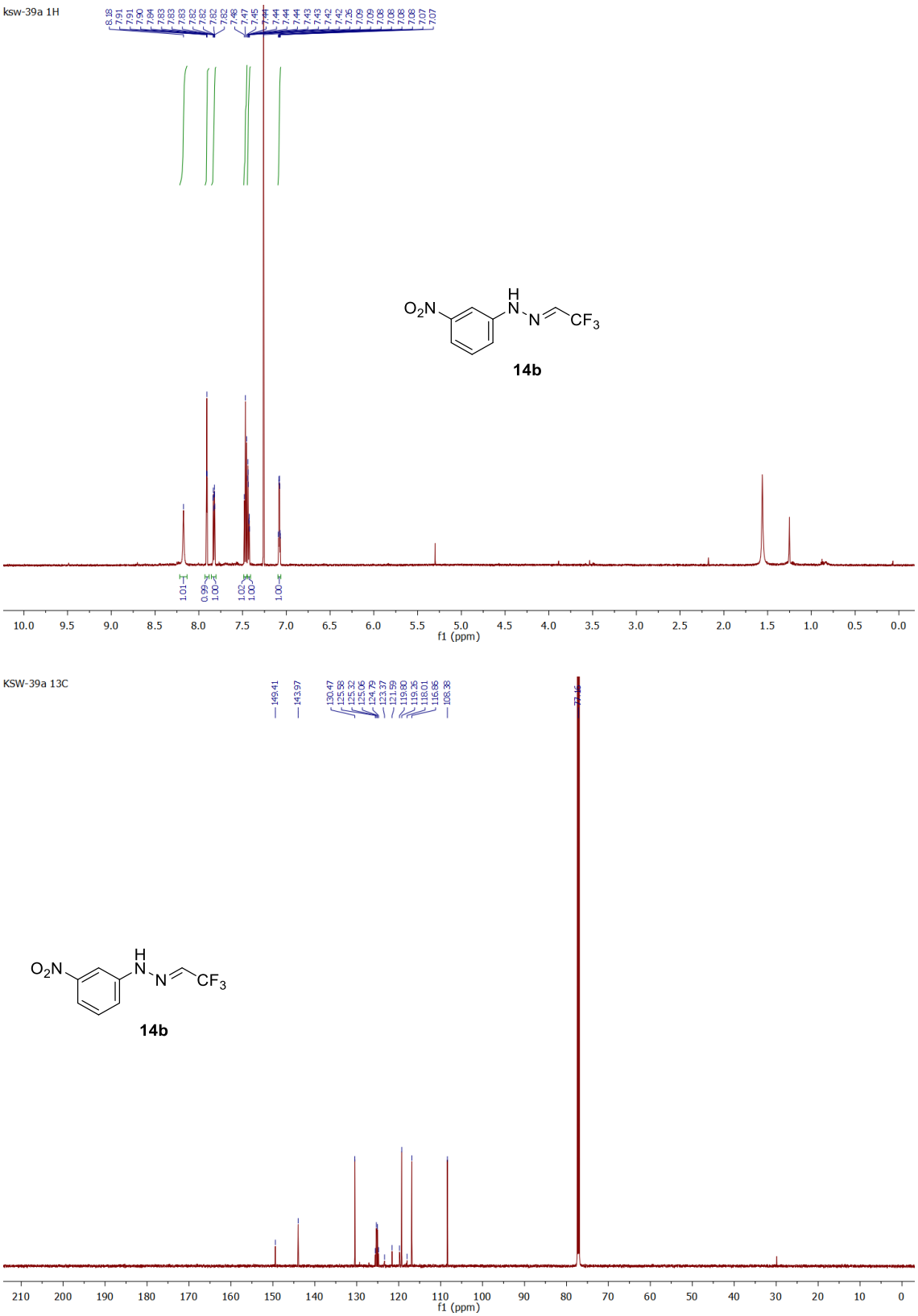


Figure S33. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **14b**.

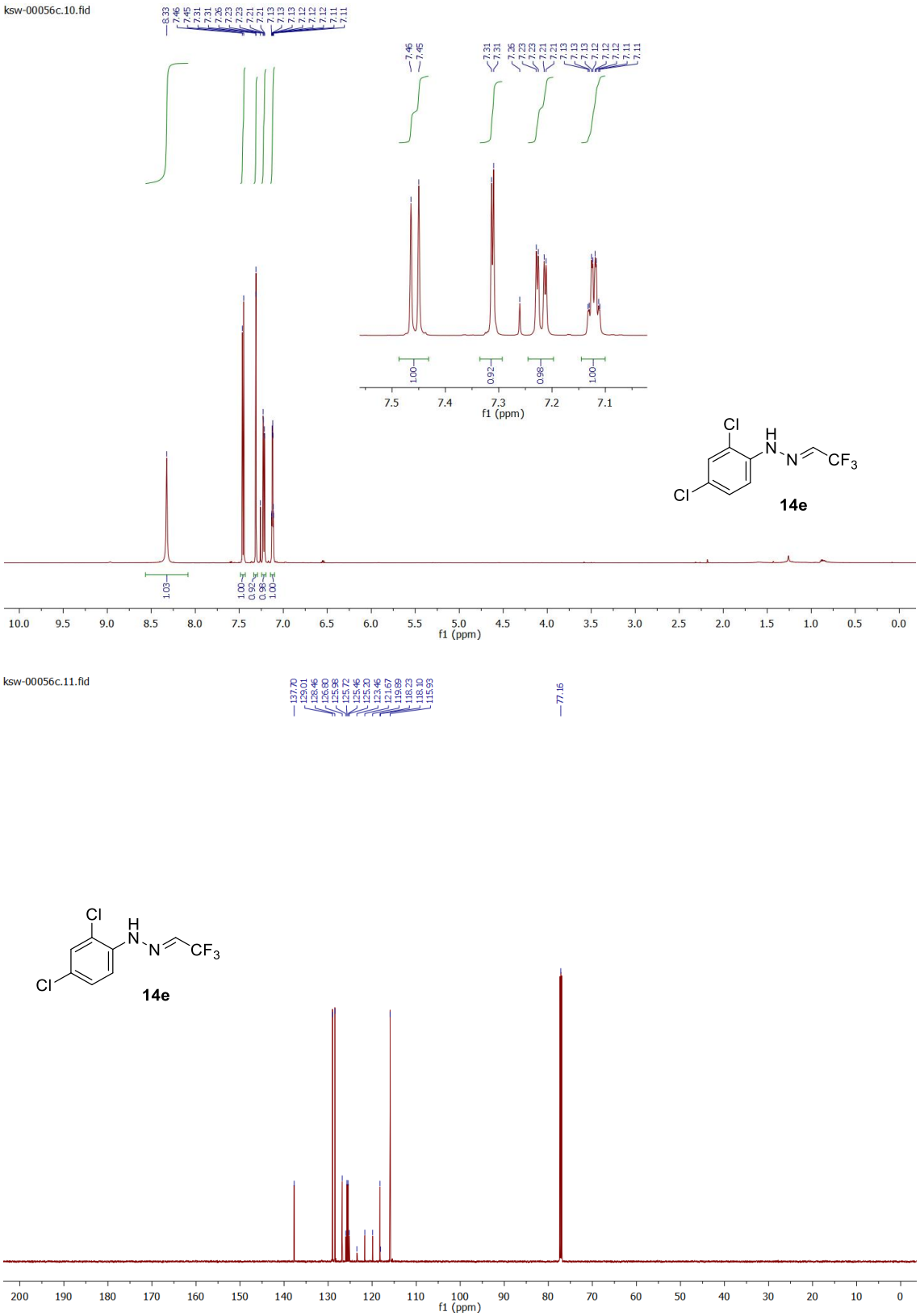
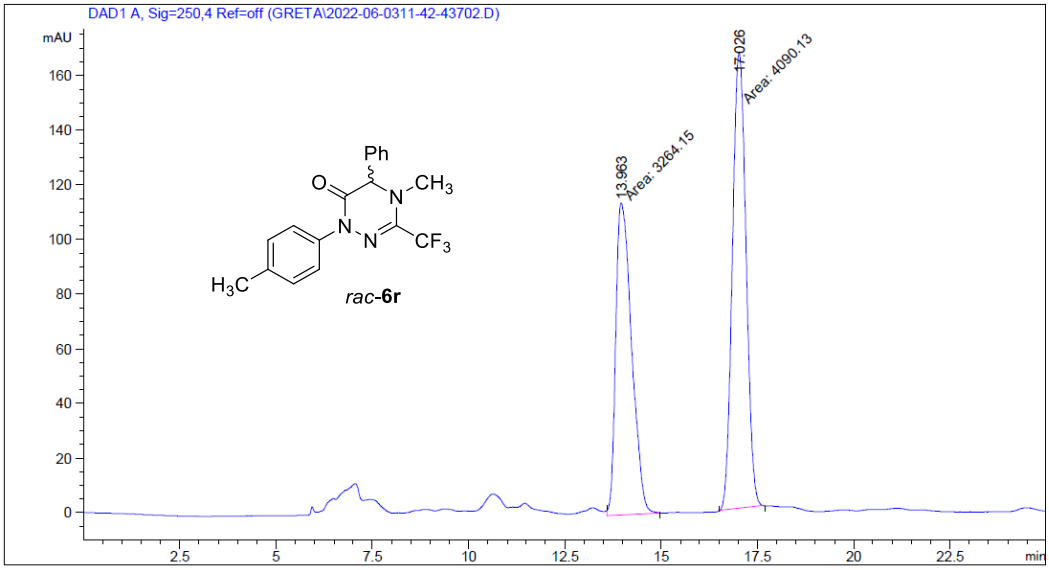
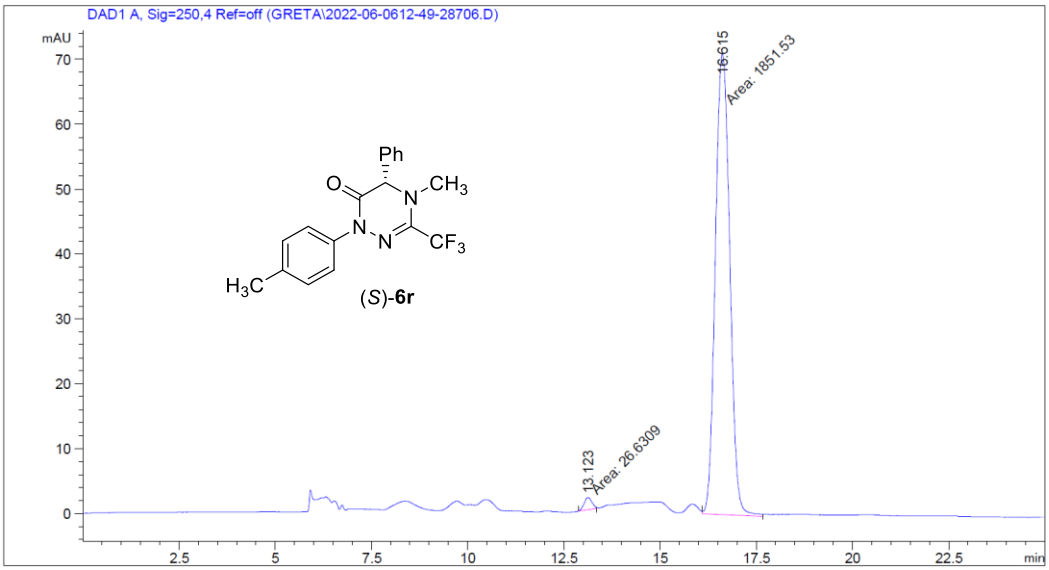


Figure S34. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) spectra for compound **14e**.

HPLC analyses

HPLC chromatograms of racemic and *S*-configured 4-methyl-5-phenyl-1-(4-tolyl)-3-trifluoromethyl-4,5-dihydro-1*H*-[1,2,4]triazin-6-one (**6r**): CHIRALPAK® AD-H column {amylose *tris*(3,5-dimethylphenyl-carbamate) coated on 5 µm silica-gel}; hexane:^{i}PrOH = 90:10, flow = 0.5 mL/min, 253 nm.



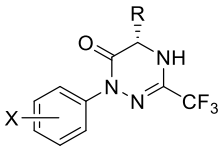


Cytotoxicity tests

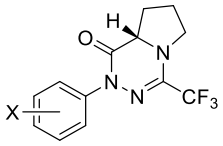
Cell culture and treatment: The promyelocytic leukemia (HL-60) and breast cancer adenocarcinoma (MCF-7) cell lines were purchased from the European Collection of Cell Cultures (ECACC). Leukemia cells were cultured in RPMI 1640 plus GlutaMax I medium (Gibco/Life Technologies, Carlsbad, CA, USA). MCF-7 cells were maintained in Minimum Essential Medium Eagle (Sigma Aldrich, St. Louis, MO, USA) supplemented with 2 mM glutamine and Men Non-essential amino acid solution (Sigma Aldrich, St. Louis, MO, USA). Both media were supplemented with 10% heat-inactivated fetal bovine serum (Biological Industries, Beit-Haemek, Israel) and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin) (Sigma-Aldrich, St. Louis, MO, USA). Cells were maintained at 37°C in 5% CO_2 atmosphere and grown until 80% confluent. The tested compounds were dissolved in DMSO and further diluted with culture medium. The final concentration of DMSO in cell cultures was less than 0.1% v/v.

In vitro cytotoxicity assay: The MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay was performed according to the known procedure [1]. Cells were seeded into 24-well plates at a density of 8×10^4/mL and left to grow for 24 h. After being cultured for 48 h with various concentrations of the tested compounds, cells were incubated with MTT solution (100 µL, 5 mg/mL in phosphate buffered saline) for 2 h. Then, the plates were centrifuged and the supernatant was discarded. DMSO (1 mL) was added to each well to dissolve the blue formazan product, whose absorbance was measured at 560 nm using FlexStation 3 Multi-Mode Microplate Reader (Molecular Devices, LLC, CA, USA). The untreated cells were used as control. The data were expressed as mean ± SEM of three independent experiments.

Table S1. *In vitro* cytotoxic activity of 1,2,4-triazinones **6** and **7** on selected cancer cell lines.



6a–6q



7a–7h

	X	R	IC_{50} [µM] [1]	
			HL-60	MCF-7
6a	4-NO_2	H	>100	>100
6b	3-NO_2	H	>100	>100
6c	4-CN	H	>100	>100
6d	4-Cl	H	>100	>100
6e	2,4-Cl_2	H	>100	>100
6f	H	H	>100	>100
6g	4-CH_3	H	>100	>100
6h	4-BnO	H	>100	>100
6i	4-NO_2	CH_3	>100	>100
6j	4-NO_2	$CH(CH_3)_2$	93.04±4.52	>100
6k	4-NO_2	$CH_2CH(CH_3)_2$	58.10±3.78	97.50±3.54

6l	4-NO_2	Ph	75.94±5.50	>100
6m	4-NO_2	CH_2CH_2OH	23.04±1.85	>100
6n	4-NO_2	$CH_2CH_2SCH_3$	>100	>100
6o	4-NO_2	CH_2COOCH_3	>100	>100
6p	4-NO_2	1*H*-indol-3-yl	86.33±2.16	99.25±2.05
6q	4-CH_3	Ph	>100	>100
7a	4-NO_2	–	>100	>100
7b	3-NO_2	–	90.58±4.32	>100
7c	4-CN	–	45.44±3.67	>100
7d	4-Cl	–	>100	>100
7e	2,4-Cl_2	–	>100	>100
7f	H	–	>100	>100
7g	4-CH_3	–	>100	>100
7h	4-BnO	–	56.23±1.23	>100

[1] Compound concentration required to inhibit metabolic activity by 50%. Values are expressed as mean ± SEM from concentration-response curves of at least three experiments using a nonlinear estimation (quasi-Newton algorithm) method.

References

[1] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63. [doi: 10.1016/0022-1759(83)90303-4]