Phosphatase CDC25B inhibitors produced by basic alumina-supported one-pot gram-scale synthesis of fluorinated 2-alkyl- thio-4-aminoquinazolines using microwave irradiation

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Supporting Information

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Biological assays

In vitro CDC25B enzyme inhibition assay[1]

CDC25B phosphatase catalytic domain was expressed with the Glutathionine S-transferase (GST) and purified by the GSTrap affinity chromatograph. GST-CDC25B active enzyme was stored in 50 mM Tris–HCl Ph 8.0, 50 mM NaCl, 10 mM Glutathionine, 2 mM DTT and 2 mM EDTA at −80 °C. The enzyme inhibition activity of **3** was measured according to the method reported previously. Briefly, 10 μL of Cdc25B was preincubated for 20 min with the different concentration of compounds (preliminary screened compounds: 20 μg/mL concentration) or with DMSO. The reaction mixture including 5 μL of reaction buffer (100 mM Tris–HCl PH8.0, 40 mM NaCl, 1 mM DTT, 2 mM EDTA, 1% glycerol) and 10 μL of substrate assay solution (0.5 mM OMFP, 3-O-methylfluorescein phosphate) were added to initiated the reaction. Fluorescence emission from the product was measured after a 30 min incubation period at room temperature with a Spectra-MaxM5 (Excitation 485 nM/Emission 535 nM). IC_{50} concentrations were determined using Prism 4.0 (GraphPad Software Inc., San Diego, CA).

Cell cultures[2]

Human cancer cell lines HCT116, HT29, A549, HepG2 and SGC7901 were obtained from the American Type Culture Collection (Manassas, VA); HCT116 and HT29 cells were cultured in McCoy's 5A medium (Gibco), while HepG2 and SGC7901 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Hyclone, Thermo Scientific). A549 cells were grown in RPMI1640 medium (Hyclone, Thermo Scientific). All the mediums were supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cultures were maintained at 37 °C in a CO_2 incubator with a controlled humidified atmosphere composed of 95% air and 5% CO_2.

In vitro cytotoxicity assay[2]

Different human cancer cell lines were treated with compounds at various concentrations. After a 96 h incubation, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added to the wells (50 mL; 0.4 mg/mL) and incubated for another 4 h. Medium were aspirated and DMSO (150 μL) was added to each well. Absorbance was measured at 490 nm using 2030 Multi-label Reader (Perkin-Elmer Victor X5, US). Compound concentrations causing 50% growth inhibition (IC_{50}) were calculated.

Cell cycle analysis

Exponentially growing HCT116 cells were seeded (6000 cells/well) in 96-well plates. Cells were incubated overnight and treated with various concentrations of **3j**, **3n**, **3w,** and DMSO for 24 h and then harvested. After treatment, the medium was removed, and 50 μL of 4% PFA was added to each well. The cells were fixed for 10 min at RT. Then, they were washed twice with PBS, and 100 μL of 0.2% Triton X-100 was added to each well; the plates were then incubated at RT for 5 min. Next, the cells were washed twice with PBS, and 50 μL of DAPI (1 μg/mL, containing 20 μg/mL RNase A) was added to each well. The cells were stained in the dark by incubation for 20 min. After incubation, the cells were washed twice with PBS and analyzed with a GE Incell 2200 imaging system.

Western blot analysis

The protein samples were heated at 95 ℃ for 10 min, and clarified by centrifugation at 12000 rpm for 10 min at 4 ℃. Proteins were resolved on 10% SDS-PAGE gels (10 μg cell lysate per lane), transferred to nitrocellulose membranes, and blocked for 1 h at 37 ℃ in 5% dry milk in TBST (137 nM NaCl, 20 mM Tris, 0.05% Tween-20). Then, the membranes were incubated in the same buffer with primary antibodies (anti-phosphor Cdc2 (Tyr15) (#9111; Cell Signaling Technology, Inc., MA), anti-Cdc2 (POH1) mouse mAb (#9116S; Cell Signaling Technology, Inc.), and anti-β-actin (Beyotime)) overnight at 4 ℃, incubated for 1 h with horseradish peroxidase-conjugated secondary antibody, washed again with TBST, and developed using chemiluminescent substrate. The band intensity was determined using ImageJ software and a molecular imager (Amersham Imager 600, GE Healthcare).

X-ray Data[3] of 3e

Table S1. Crystal data and structure refinement for **3e.**

Empirical formula	C_{10} H_5 F_3 N_4 S
Formula weight	270.24
Temperature	293(2) K
Wavelength	0.71073 A
Crystal system, space group	Triclinic, P-1
Unit cell dimensions	a = 7.2243(10) A alpha = 110.099(2) deg.
	b = 8.0299(11) A beta = 90.348(2) deg.
	c = 9.8872(13) A gamma = 98.938(2) deg.
Volume	531.00(12) A^3
Z, Calculated density	2, 1.690 Mg/m^3
Absorption coefficient	0.333 mm^-1
F(000)	272
Crystal size	0.40 x 0.38 x 0.08 mm
Theta range for data collection	2.20 to 25.15 deg.
Limiting indices	-8<=h<=8, -9<=k<=9, -11<=l<=11
Reflections collected / unique	4234 / 1892 [R(int) = 0.0192]
Completeness to theta = 25.15	99.4 %
Max. and min. transmission	0.9739 and 0.8785
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1892 / 0 / 164
Goodness-of-fit on F^2	1.052
Final R indices [I>2sigma(I)]	R1 = 0.0355, wR2 = 0.0928
R indices (all data)	R1 = 0.0503, wR2 = 0.1094
Largest diff. peak and hole	0.170 and -0.257 e.A^-3

Table S2. Bond lengths [A] and angles [deg] for **3e**

F(1)-C(2)	1.344(2)	C(2)-C(1)-C(8)	126.31(19)
F(2)-C(4)	1.342(2)	C(6)-C(1)-C(8)	115.93(18)
F(3)-C(5)	1.342(2)	F(1)-C(2)-C(3)	117.09(19)
N(1)-C(7)	1.318(3)	F(1)-C(2)-C(1)	119.34(19)
N(1)-C(6)	1.362(3)	C(3)-C(2)-C(1)	123.57(19)
N(2)-C(8)	1.332(3)	C(2)-C(3)-C(4)	117.1(2)
N(2)-C(7)	1.355(3)	C(2)-C(3)-C(9)	120.5(2)
N(3)-C(9)	1.135(3)	C(4)-C(3)-C(9)	122.4(2)
N(4)-C(8)	1.326(3)	F(2)-C(4)-C(5)	120.44(19)
N(4)-H(4A)	0.8600	F(2)-C(4)-C(3)	117.8(2)
N(4)-H(4B)	0.8600	C(5)-C(4)-C(3)	121.78(19)
S(1)-C(7)	1.748(2)	F(3)-C(5)-C(4)	119.39(19)
S(1)-C(10)	1.797(2)	F(3)-C(5)-C(6)	119.35(19)
C(1)-C(2)	1.392(3)	C(4)-C(5)-C(6)	121.21(19)
C(1)-C(6)	1.423(3)	N(1)-C(6)-C(5)	118.39(18)
C(1)-C(8)	1.450(3)	N(1)-C(6)-C(1)	123.10(19)
C(2)-C(3)	1.381(3)	C(5)-C(6)-C(1)	118.51(19)
C(3)-C(4)	1.405(3)	N(1)-C(7)-N(2)	128.6(2)
C(3)-C(9)	1.435(3)	N(1)-C(7)-S(1)	120.88(16)
C(4)-C(5)	1.348(3)	N(2)-C(7)-S(1)	110.54(15)
C(5)-C(6)	1.413(3)	N(4)-C(8)-N(2)	116.69(19)
C(10)-H(10A)	0.9600	N(4)-C(8)-C(1)	123.54(19)
C(10)-H(10B)	0.9600	N(2)-C(8)-C(1)	119.76(18)
C(10)-H(10C)	0.9600	N(3)-C(9)-C(3)	176.9(3)
C(7)-N(1)-C(6)	114.64(18)	S(1)-C(10)-H(10A)	109.5
C(8)-N(2)-C(7)	117.97(18)	S(1)-C(10)-H(10B)	109.5
C(8)-N(4)-H(4A)	120.0	H(10A)-C(10)-H(10B)	109.5
C(8)-N(4)-H(4B)	120.0	S(1)-C(10)-H(10C)	109.5
H(4A)-N(4)-H(4B)	120.0	H(10A)-C(10)-H(10C)	109.5
C(7)-S(1)-C(10)	103.35(12)	H(10B)-C(10)-H(10C)	109.5
C(2)-C(1)-C(6)	117.76(19)		

Table S3. Torsion angles [deg] for **3e.**

C(6)-C(1)-C(2)-F(1)	179.37(19)
C(8)-C(1)-C(2)-F(1)	-0.1(3)
C(6)-C(1)-C(2)-C(3)	-0.8(3)
C(8)-C(1)-C(2)-C(3)	179.8(2)
F(1)-C(2)-C(3)-C(4)	-179.53(19)
C(1)-C(2)-C(3)-C(4)	0.6(3)
F(1)-C(2)-C(3)-C(9)	-0.5(3)
C(1)-C(2)-C(3)-C(9)	179.7(2)
C(2)-C(3)-C(4)-F(2)	178.24(18)
C(9)-C(3)-C(4)-F(2)	-0.8(3)
C(2)-C(3)-C(4)-C(5)	-1.0(3)
C(9)-C(3)-C(4)-C(5)	180.0(2)
F(2)-C(4)-C(5)-F(3)	0.0(3)
C(3)-C(4)-C(5)-F(3)	179.15(19)
F(2)-C(4)-C(5)-C(6)	-177.66(18)
C(3)-C(4)-C(5)-C(6)	1.5(3)
C(7)-N(1)-C(6)-C(5)	-178.99(19)
C(7)-N(1)-C(6)-C(1)	0.5(3)
F(3)-C(5)-C(6)-N(1)	0.3(3)
C(4)-C(5)-C(6)-N(1)	177.91(19)
F(3)-C(5)-C(6)-C(1)	-179.27(18)
C(4)-C(5)-C(6)-C(1)	-1.6(3)
C(2)-C(1)-C(6)-N(1)	-178.29(19)
C(8)-C(1)-C(6)-N(1)	1.2(3)
C(2)-C(1)-C(6)-C(5)	1.2(3)
C(8)-C(1)-C(6)-C(5)	-179.25(18)
C(6)-N(1)-C(7)-N(2)	-2.2(3)
C(6)-N(1)-C(7)-S(1)	179.51(15)
C(8)-N(2)-C(7)-N(1)	1.9(3)
C(8)-N(2)-C(7)-S(1)	-179.74(15)
C(10)-S(1)-C(7)-N(1)	5.4(2)
C(10)-S(1)-C(7)-N(2)	-173.14(17)
C(7)-N(2)-C(8)-N(4)	-178.2(2)
C(7)-N(2)-C(8)-C(1)	0.3(3)
C(2)-C(1)-C(8)-N(4)	-3.8(4)
C(6)-C(1)-C(8)-N(4)	176.8(2)
C(2)-C(1)-C(8)-N(2)	177.8(2)
C(6)-C(1)-C(8)-N(2)	-1.6(3)
C(2)-C(3)-C(9)-N(3)	-10(5)
C(4)-C(3)-C(9)-N(3)	169(5)

^{1}H NMR and ^{13}C NMR spectra for compound 3

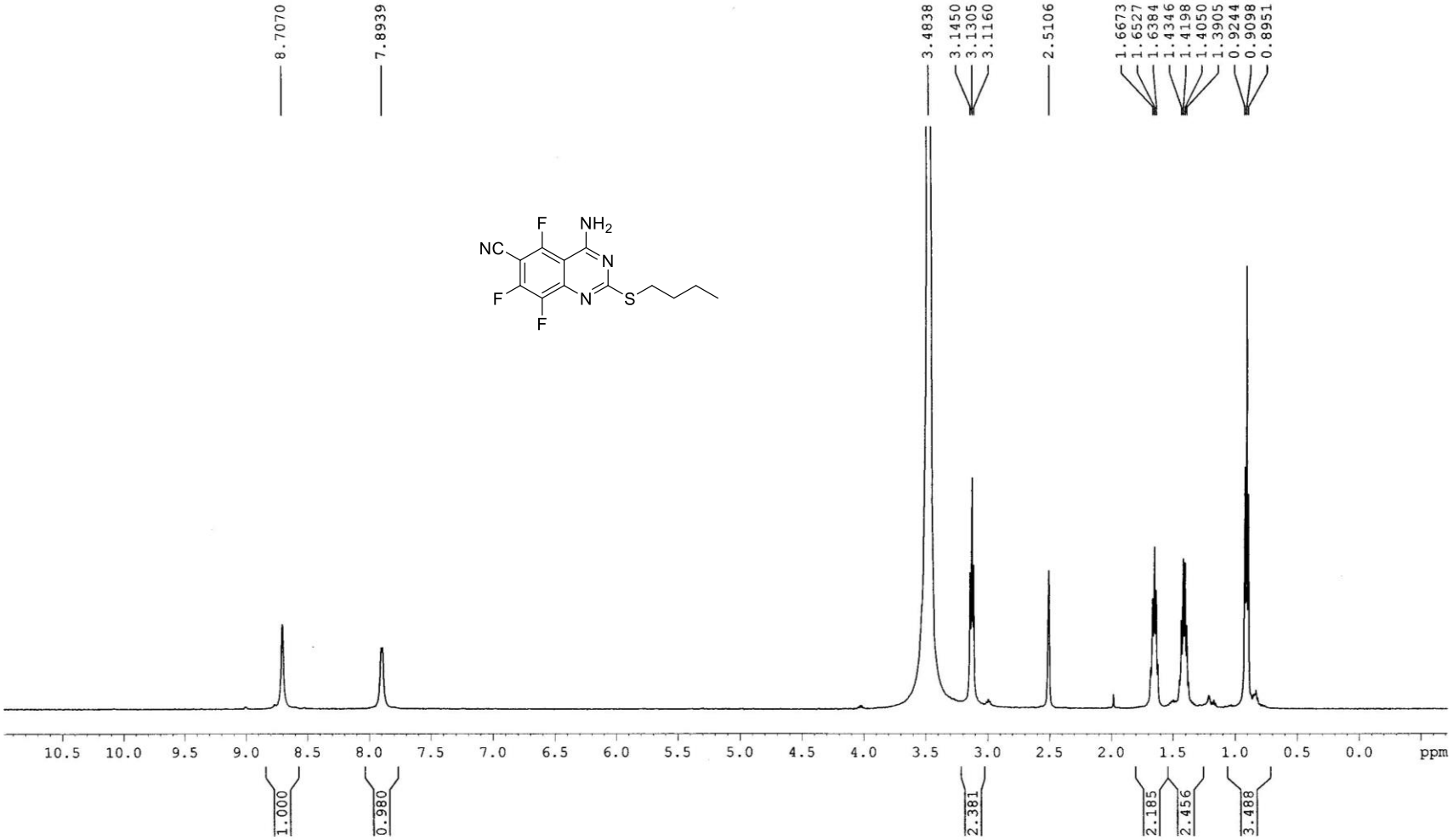

Figure S1. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3a**

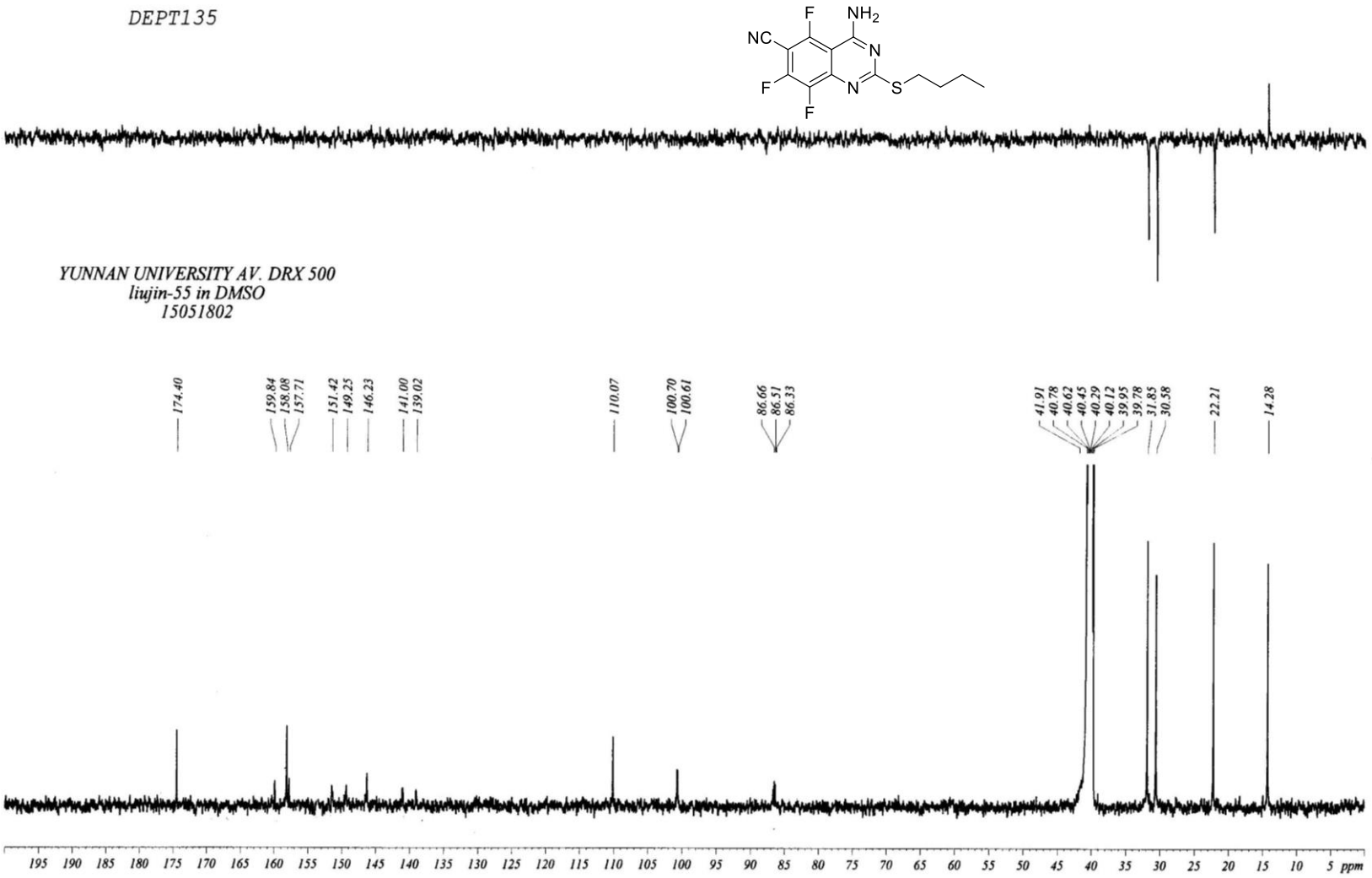

Figure S2. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3a**

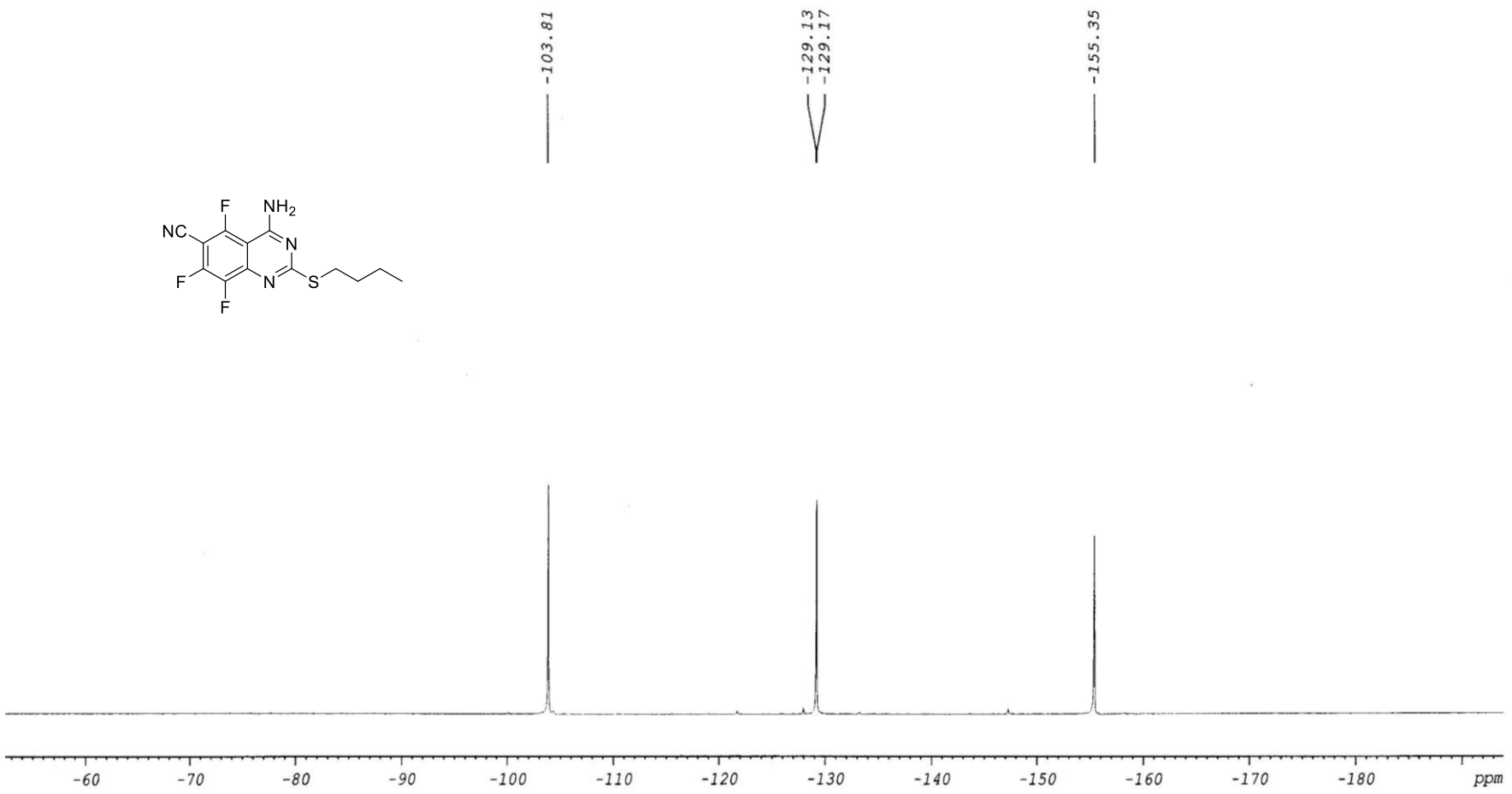

Figure S3. ^{19}F NMR (470 MHz, DMSO-d_6) spectra of compound **3a**

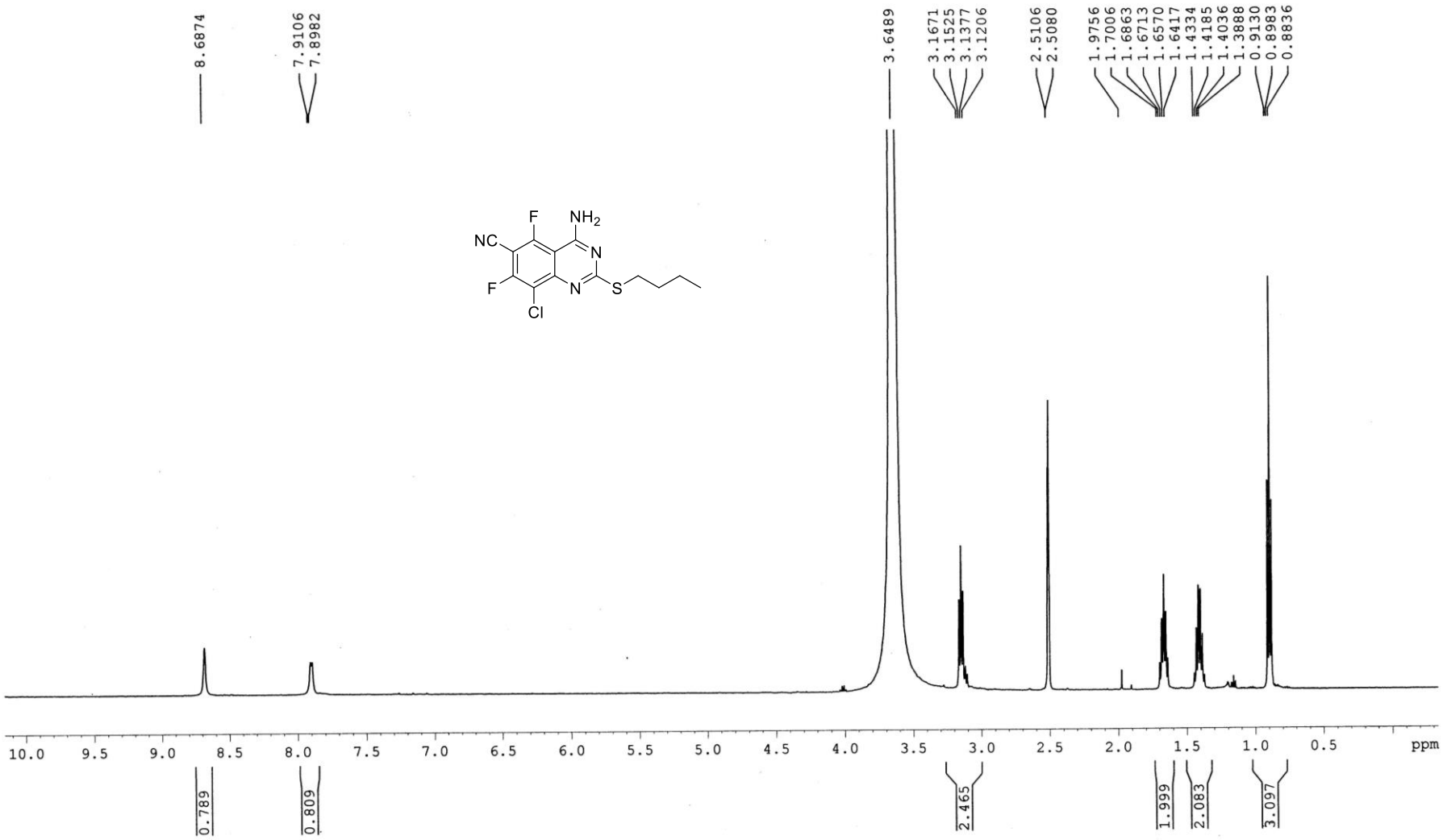

Figure S4. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3b**

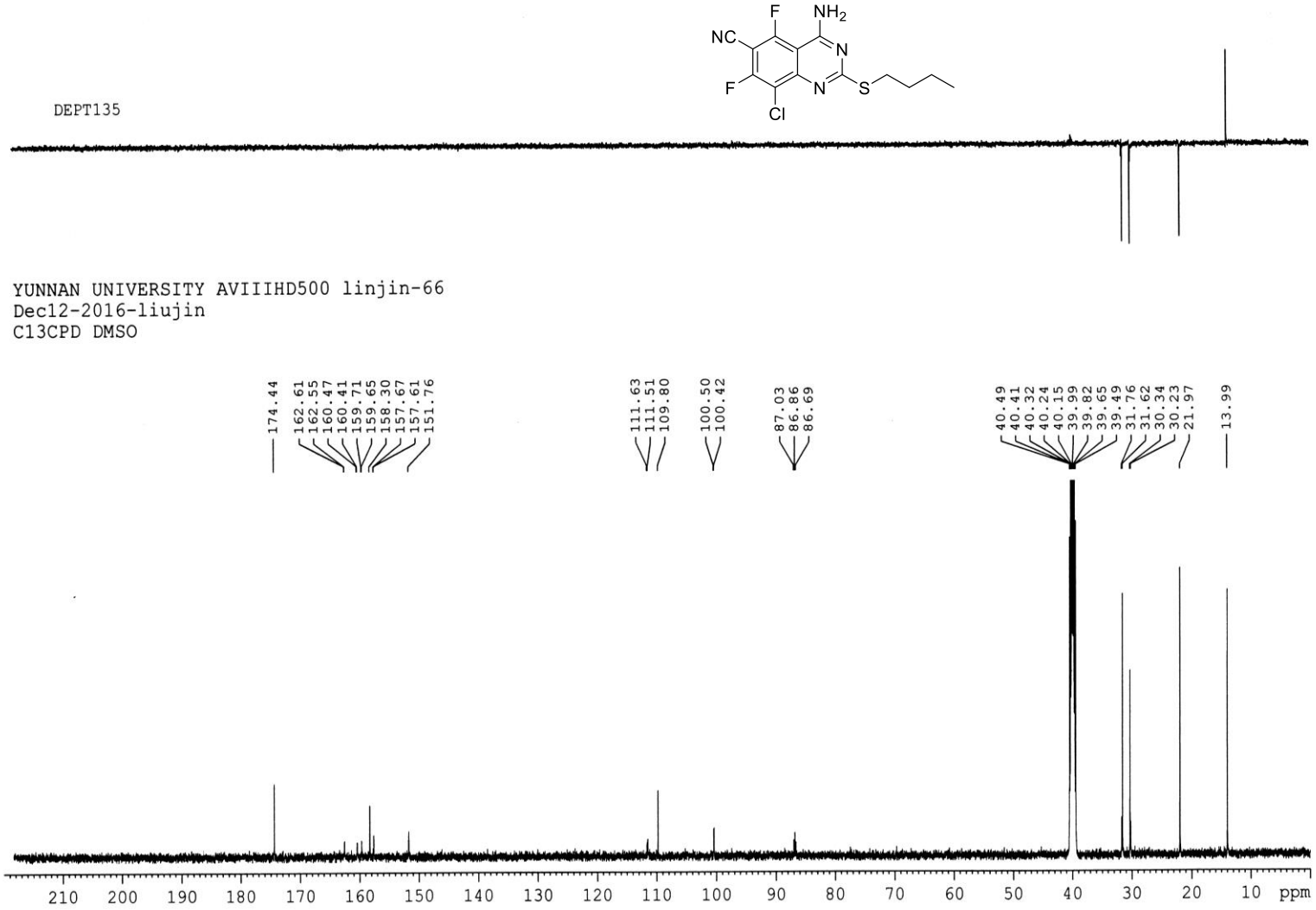

Figure S5. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3b**

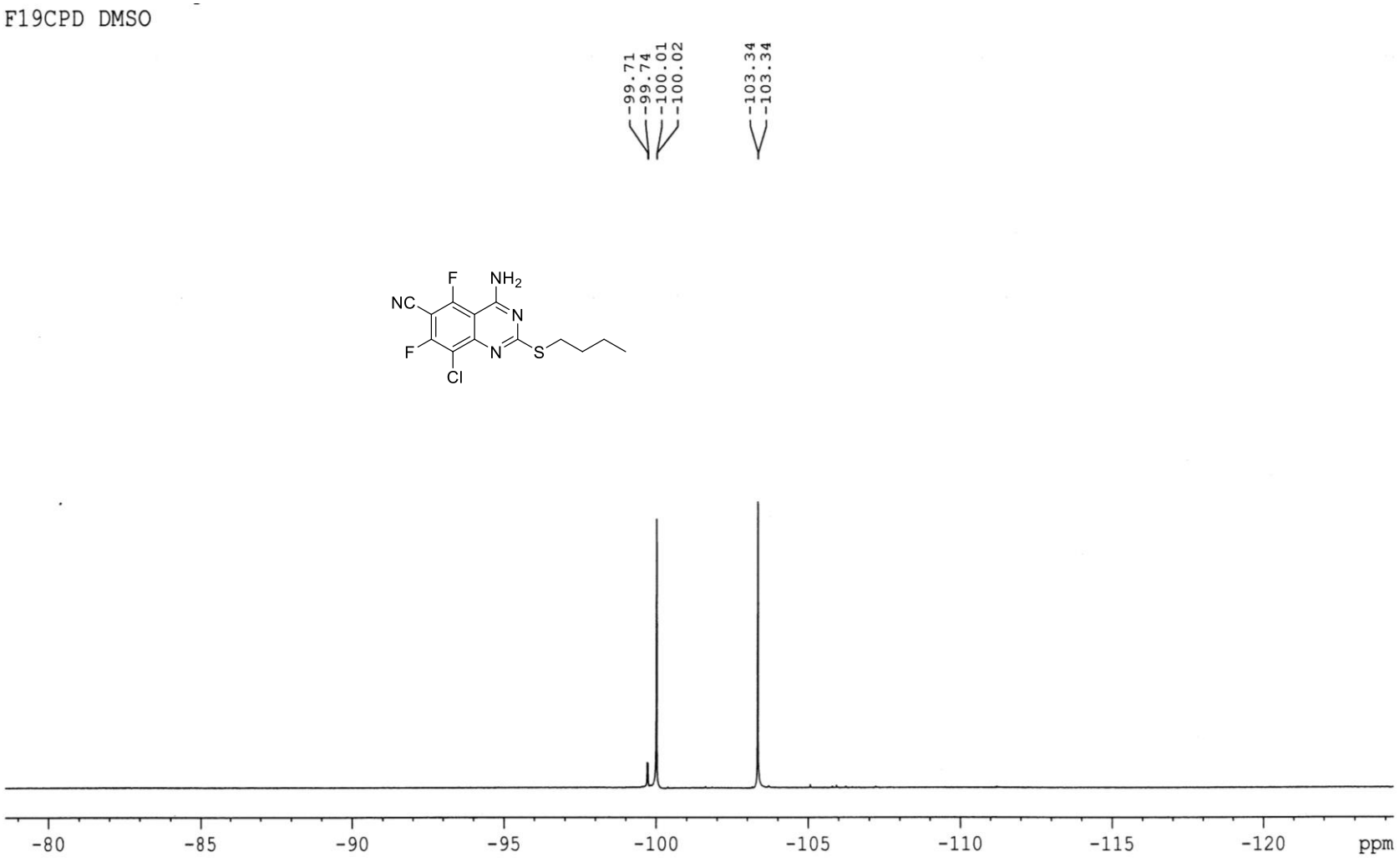

Figure S6. ^{19}F NMR (470 MHz, DMSO-d_6) spectra of compound **3b**

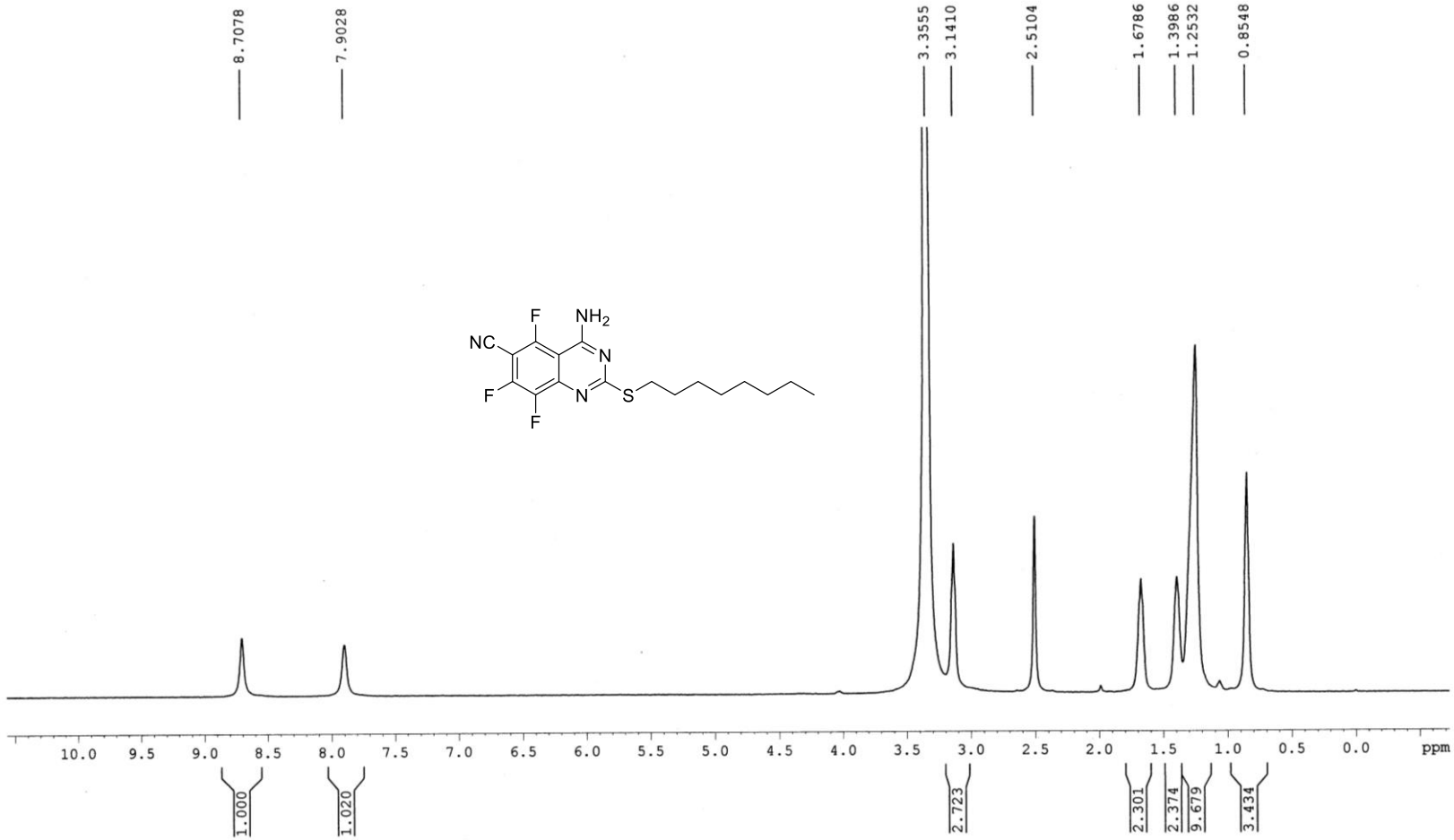

Figure S7. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3c**

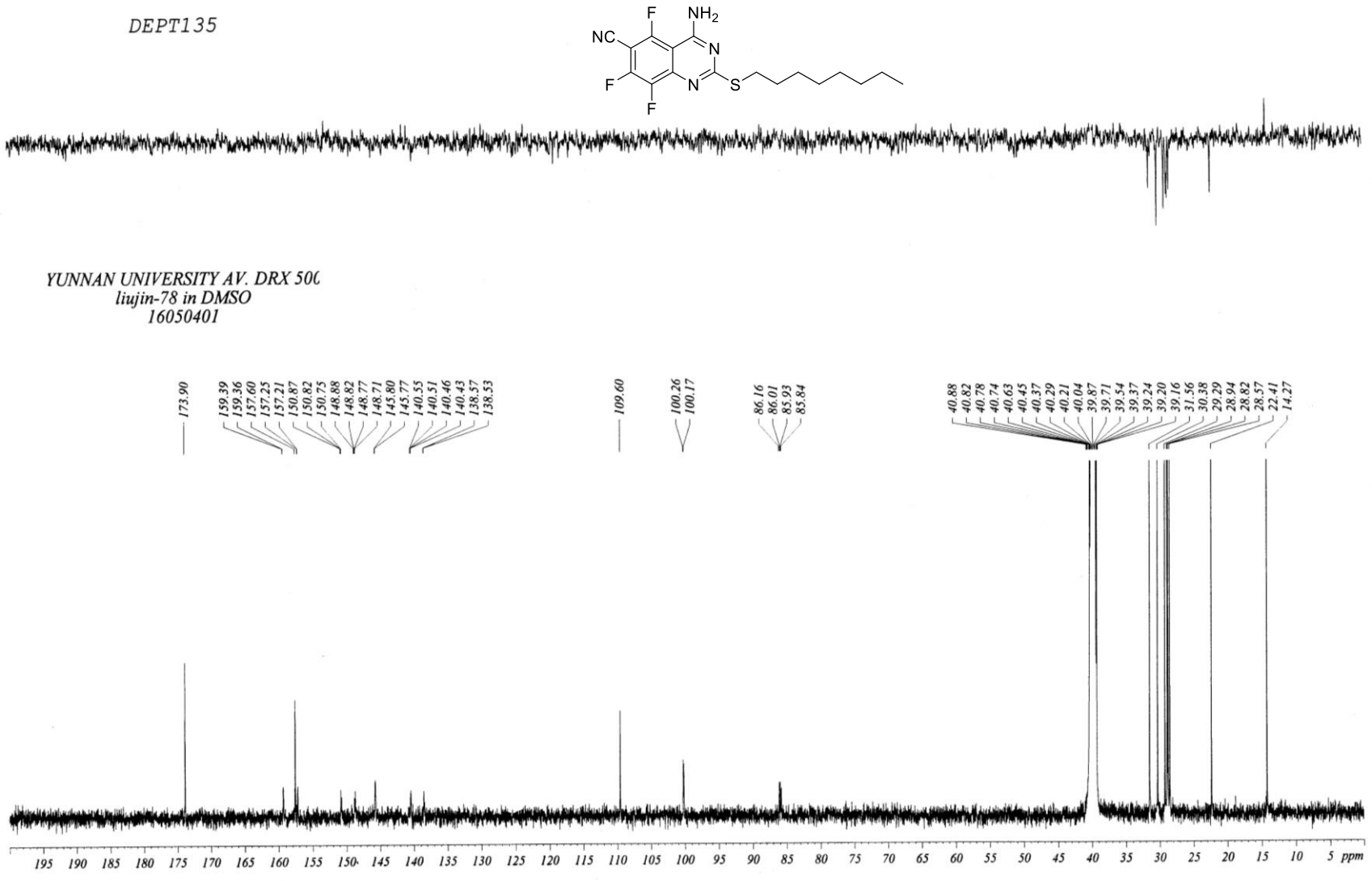

Figure S8. ^{13}C NMR (150 MHz, DMSO-d_6) spectra of compound **3c**

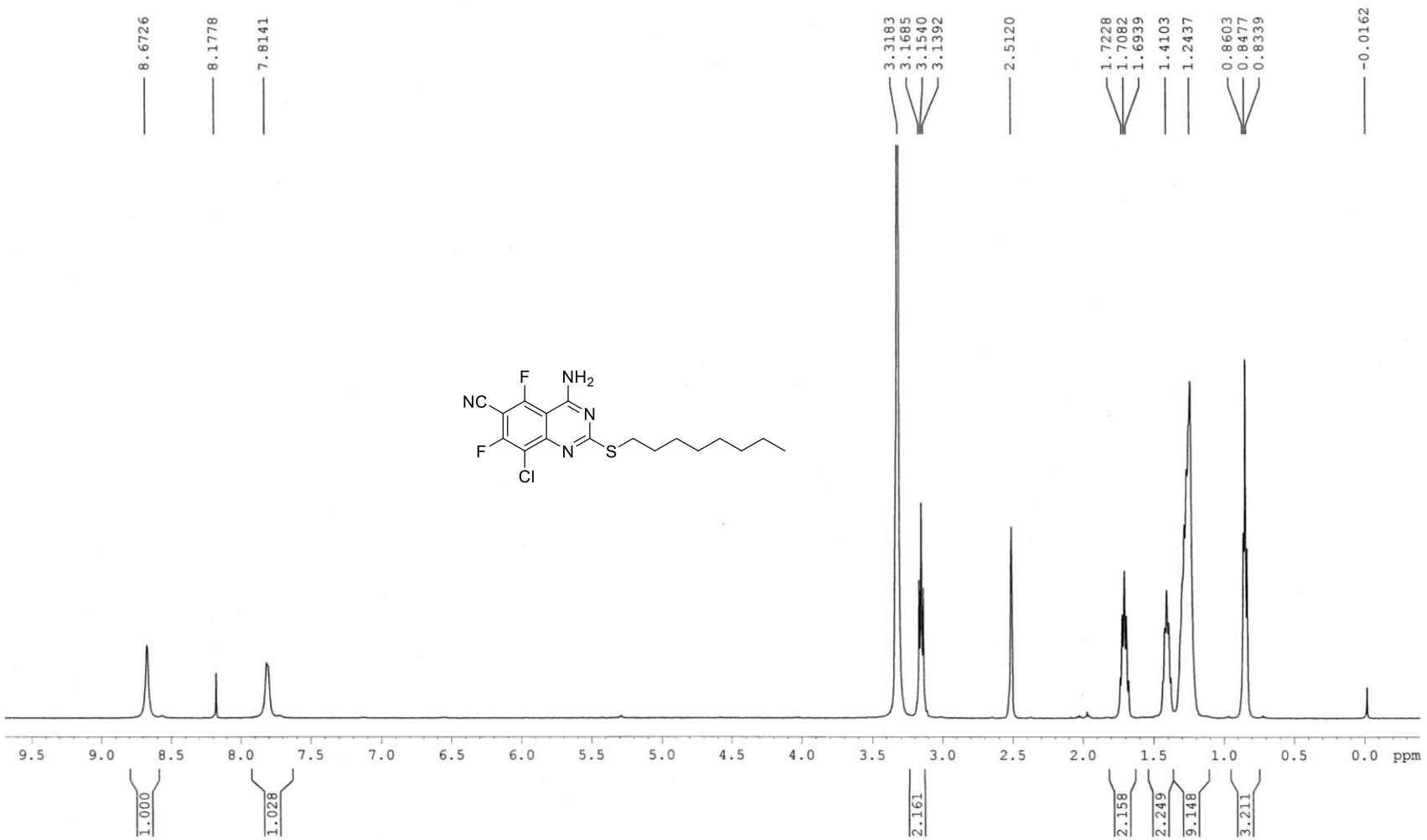

Figure S9. ^{1}H NMR (500 MHz, $CDCl_3$+DMSO-d_6) spectra of compound **3d**

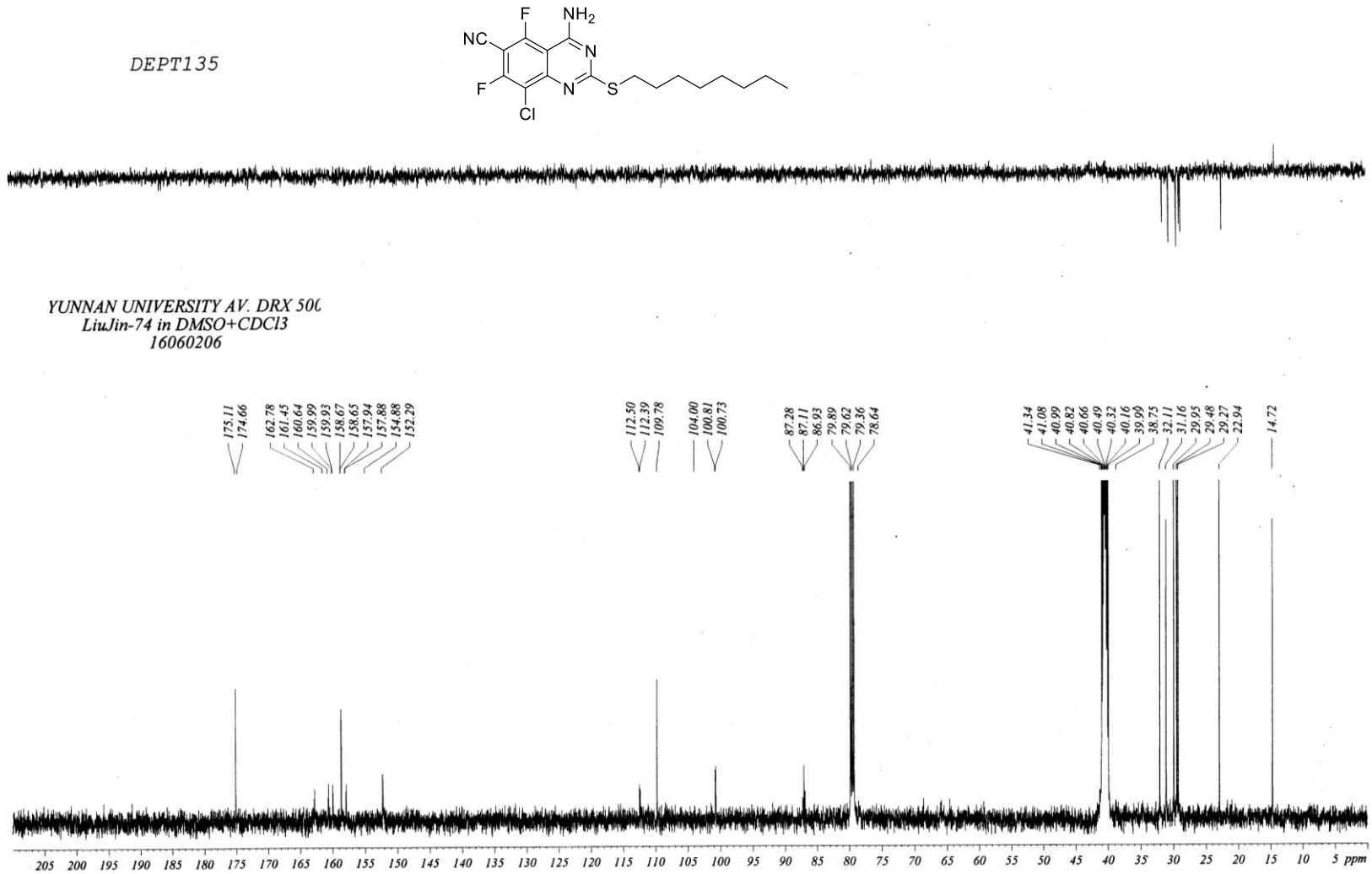

Figure S10. ^{13}C NMR (125 MHz, $CDCl_3$+DMSO-d_6) spectra of compound **3d**

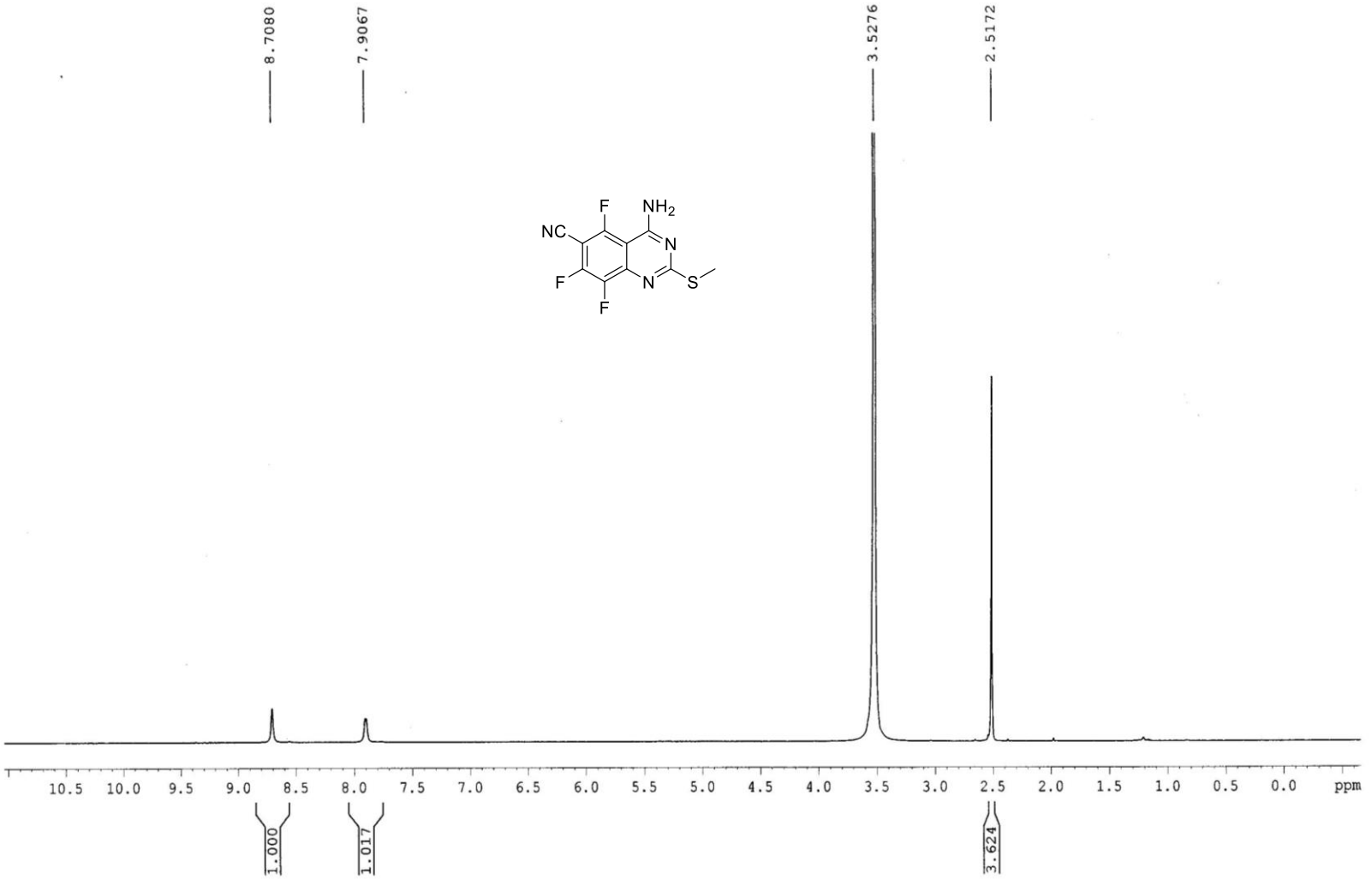

Figure S11. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3e**

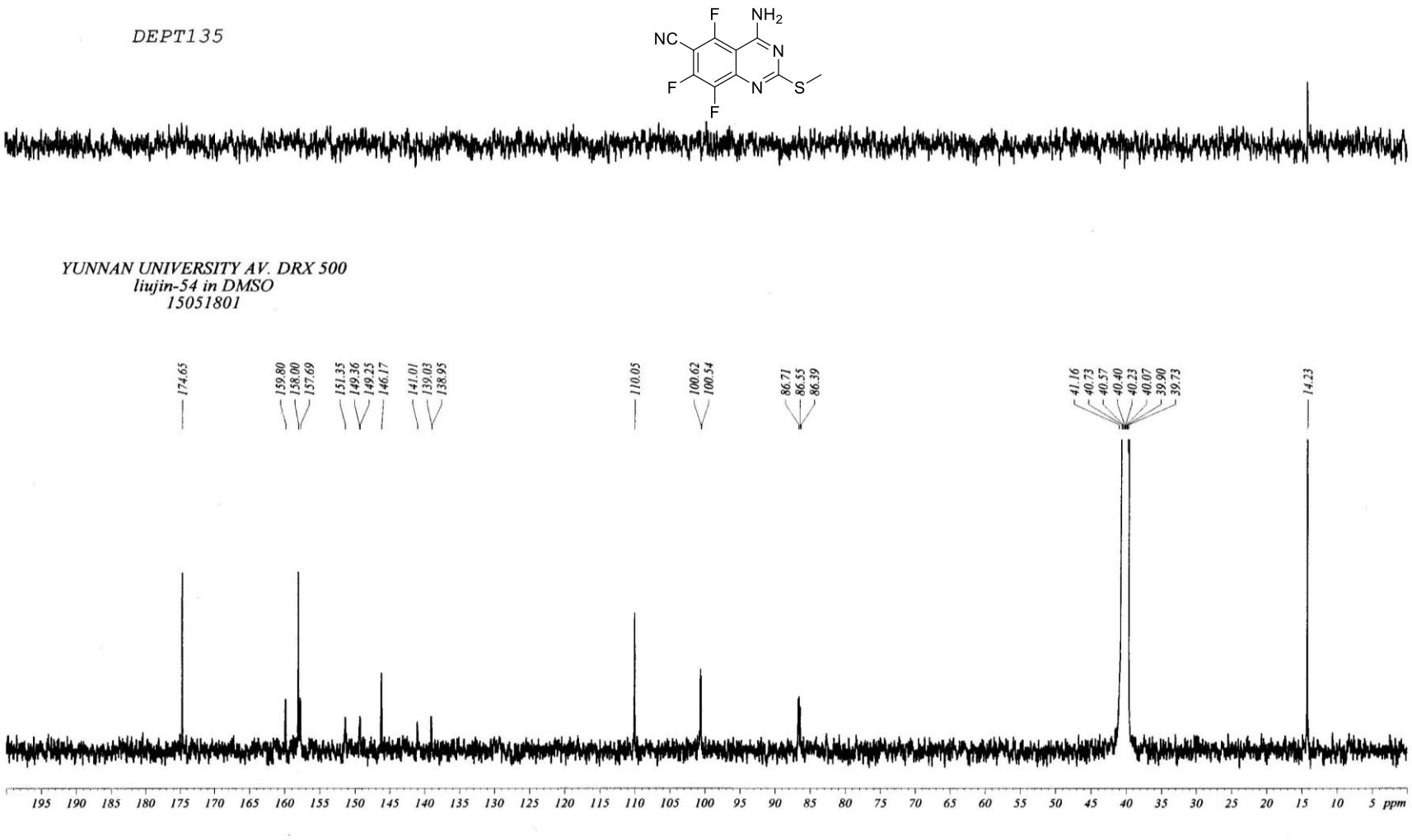

Figure S12. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3e**

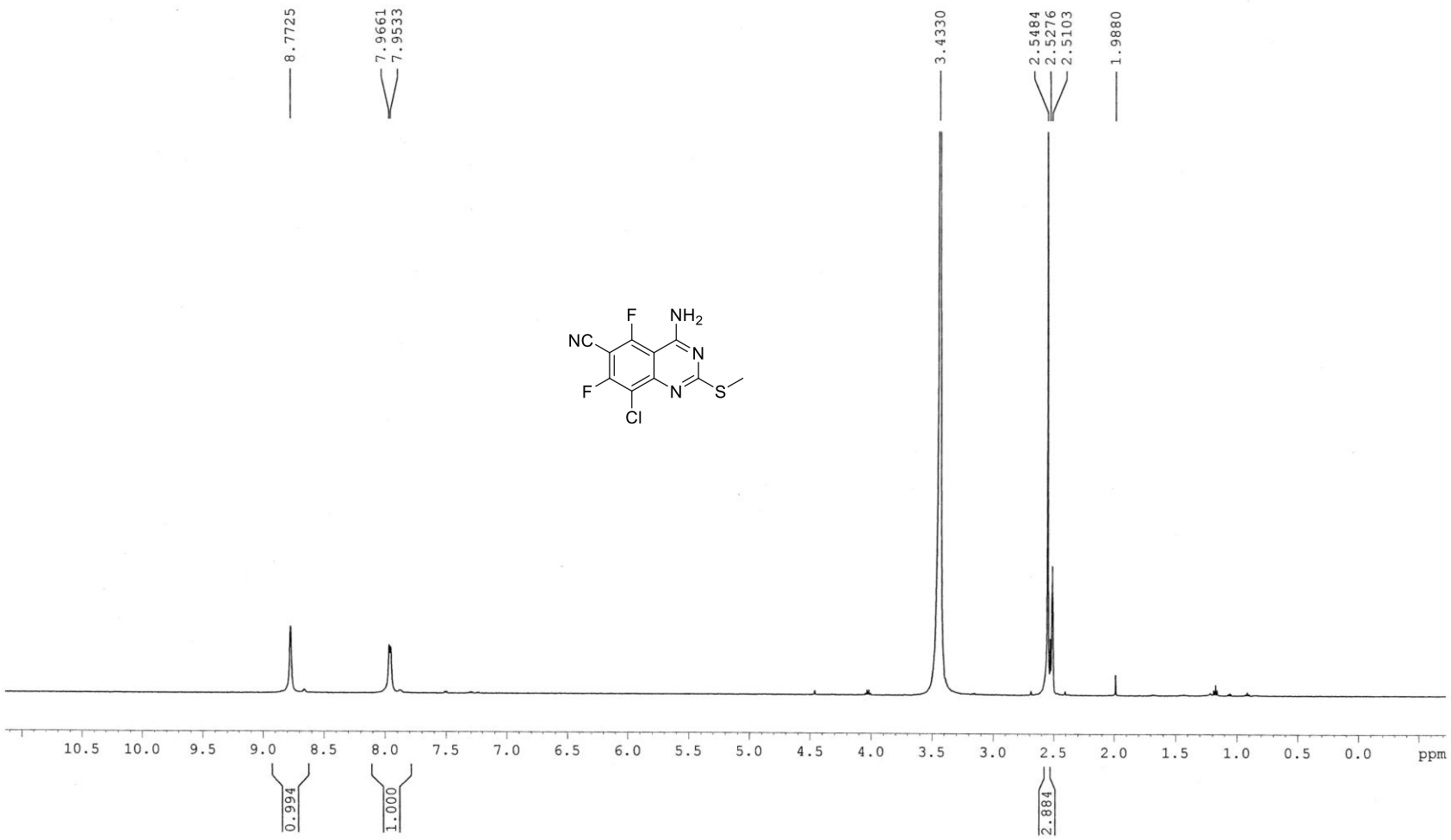

Figure S13. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3f**

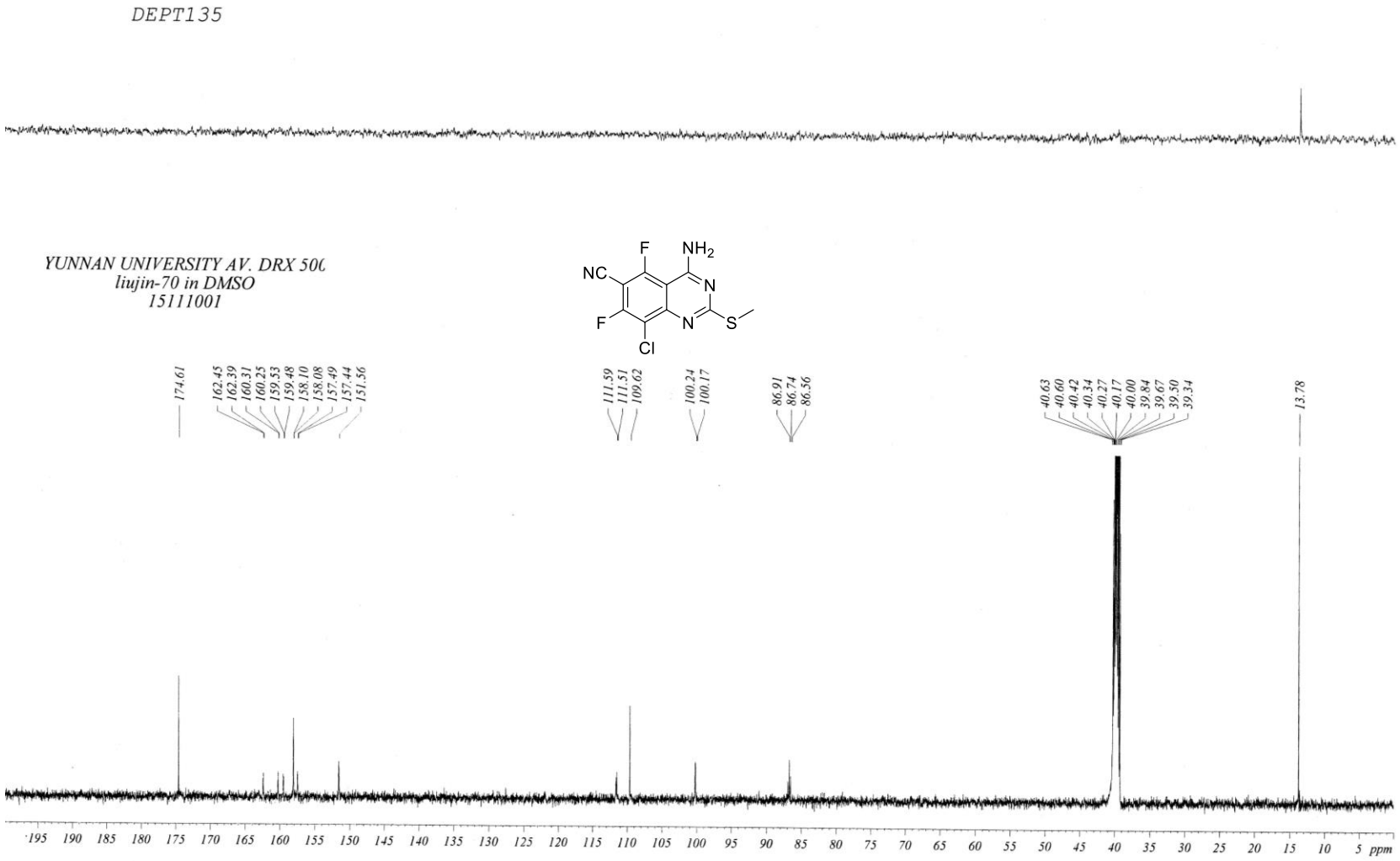

Figure S14. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3f**

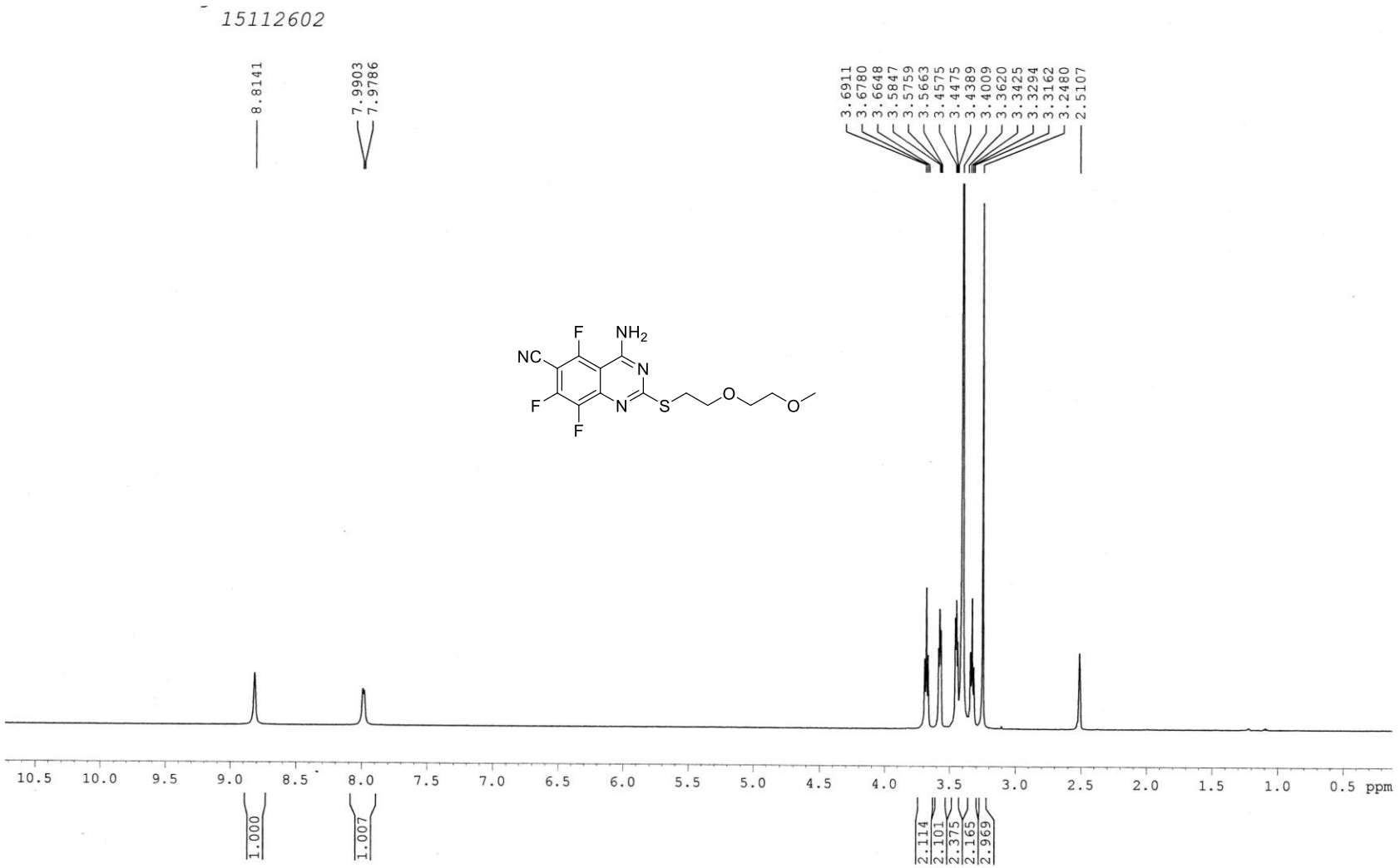

Figure S15. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3g**

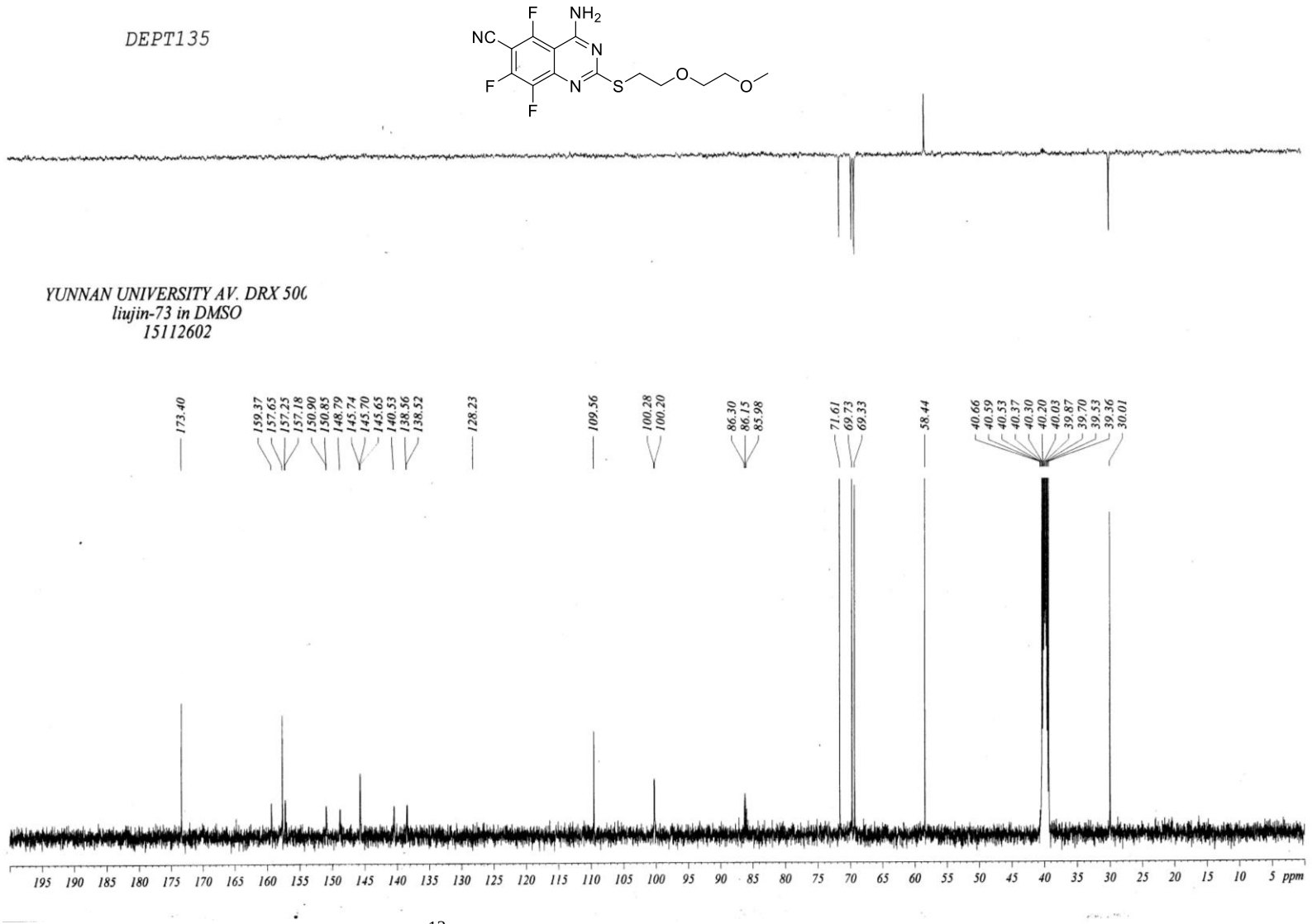

Figure S16. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3g**

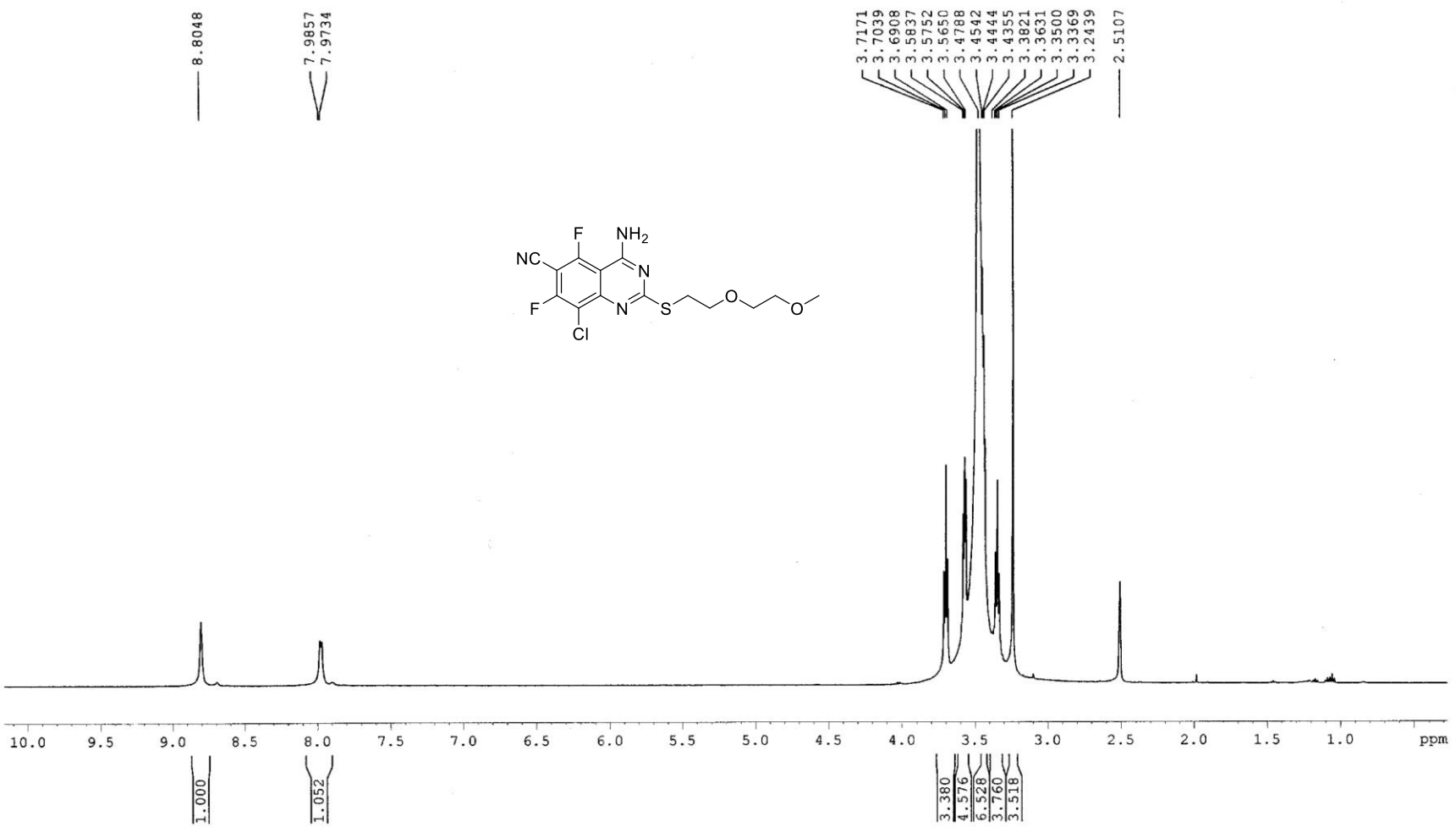

Figure S17. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3h**

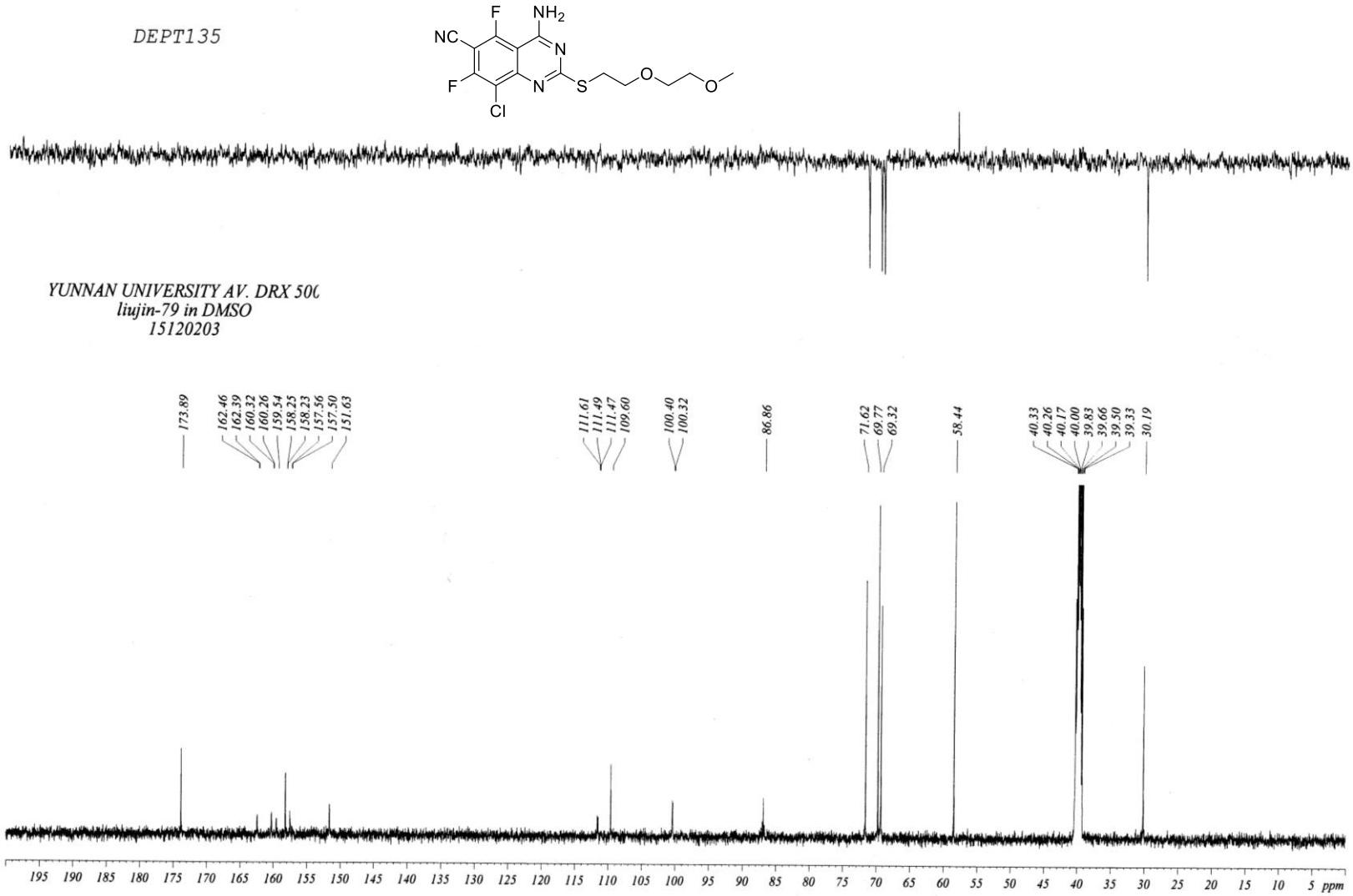

Figure S18. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3h**

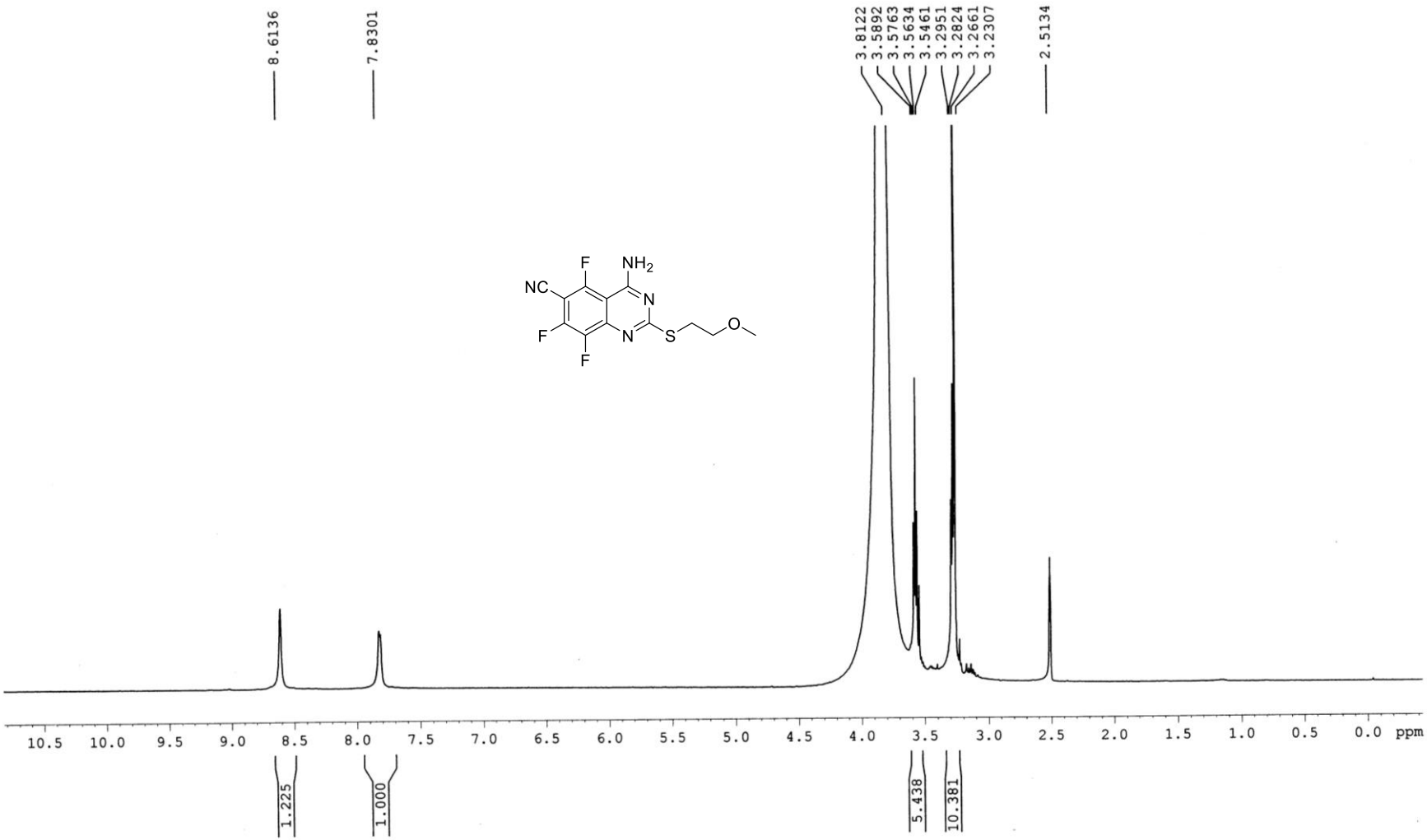

Figure S19. [1]H NMR (500 MHz, DMSO-d_6) spectra of compound **3i**

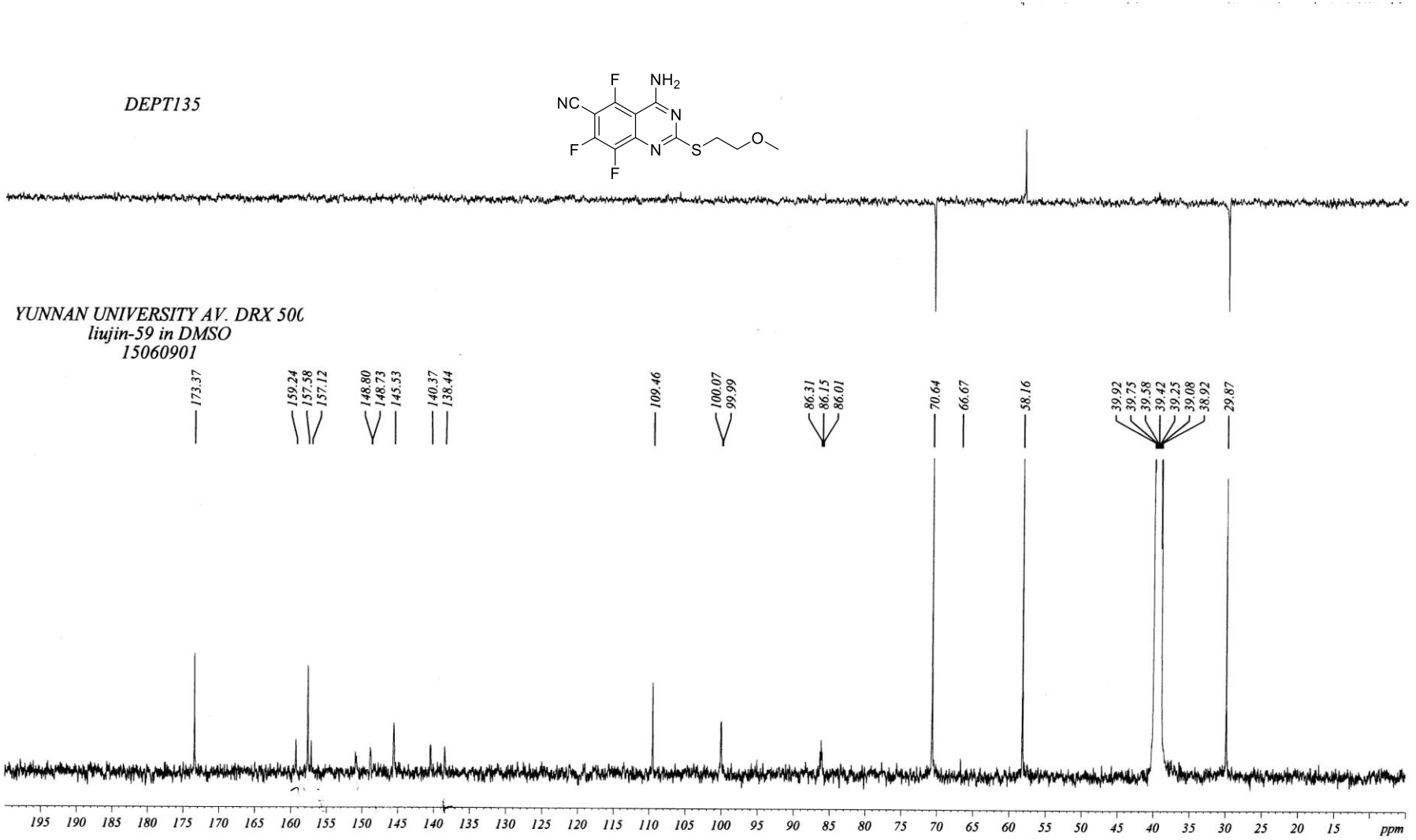

Figure S20. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3i**

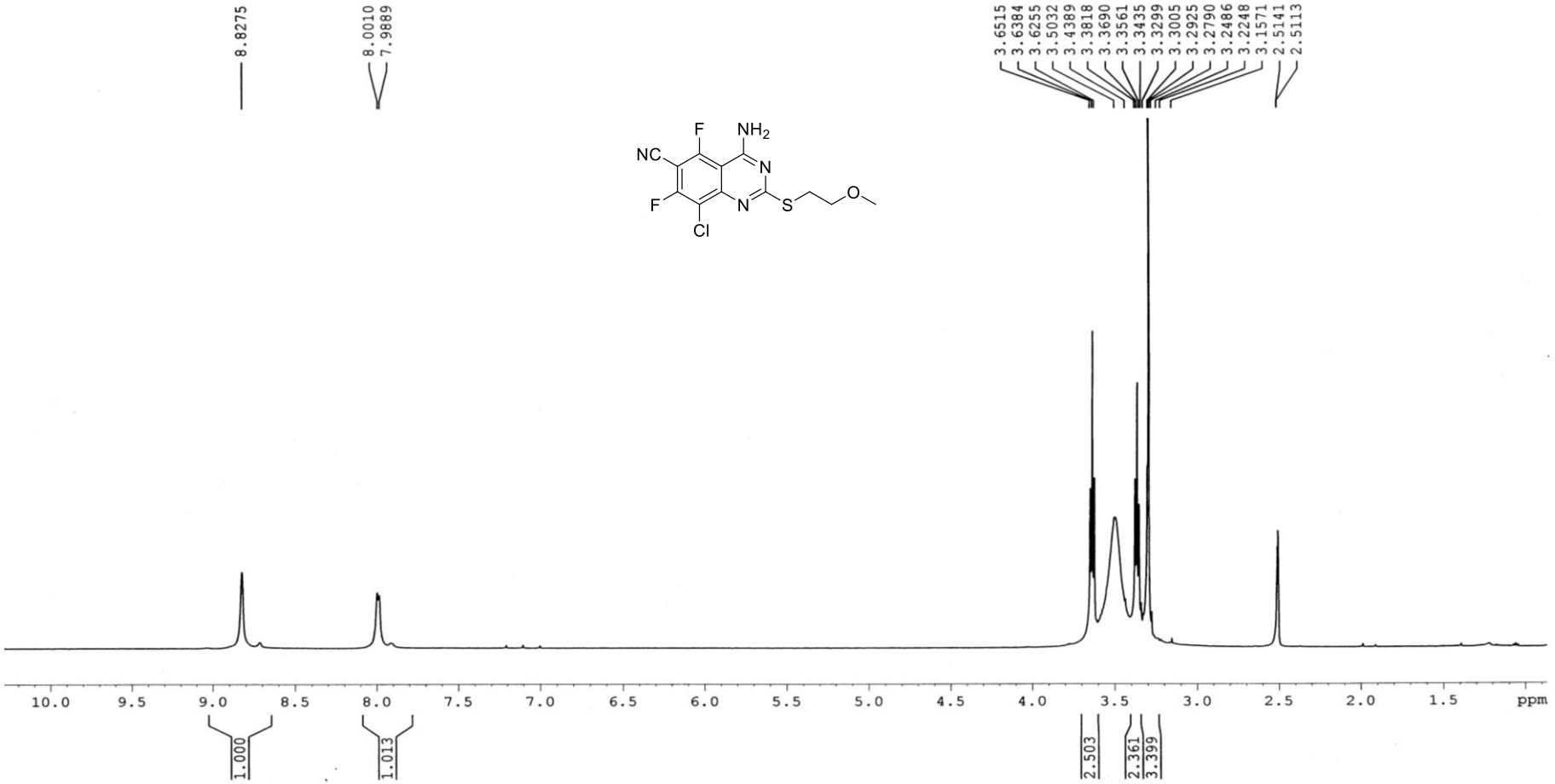

Figure S21. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3j**

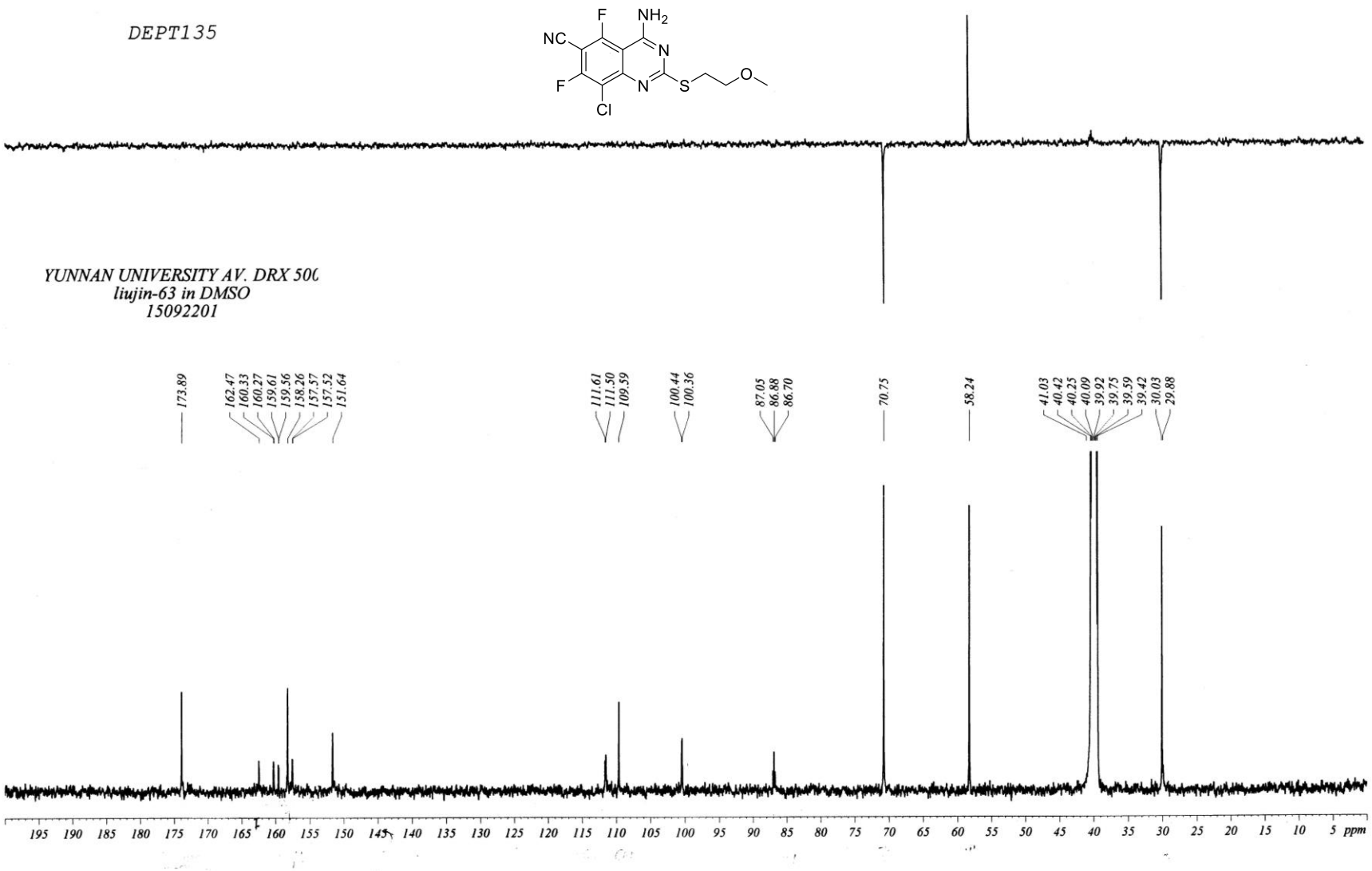

Figure S22. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3j**

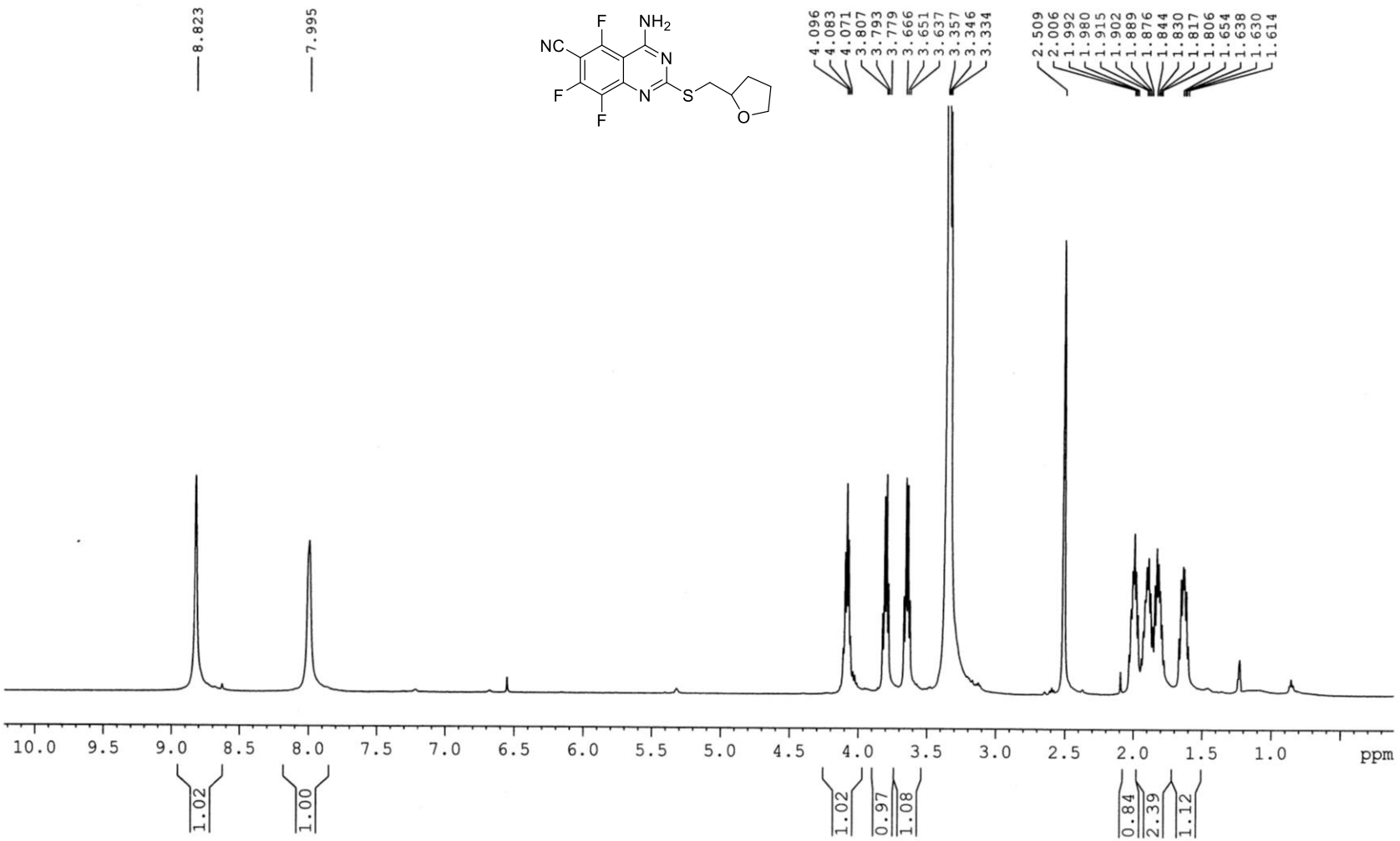

Figure S23. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3k**

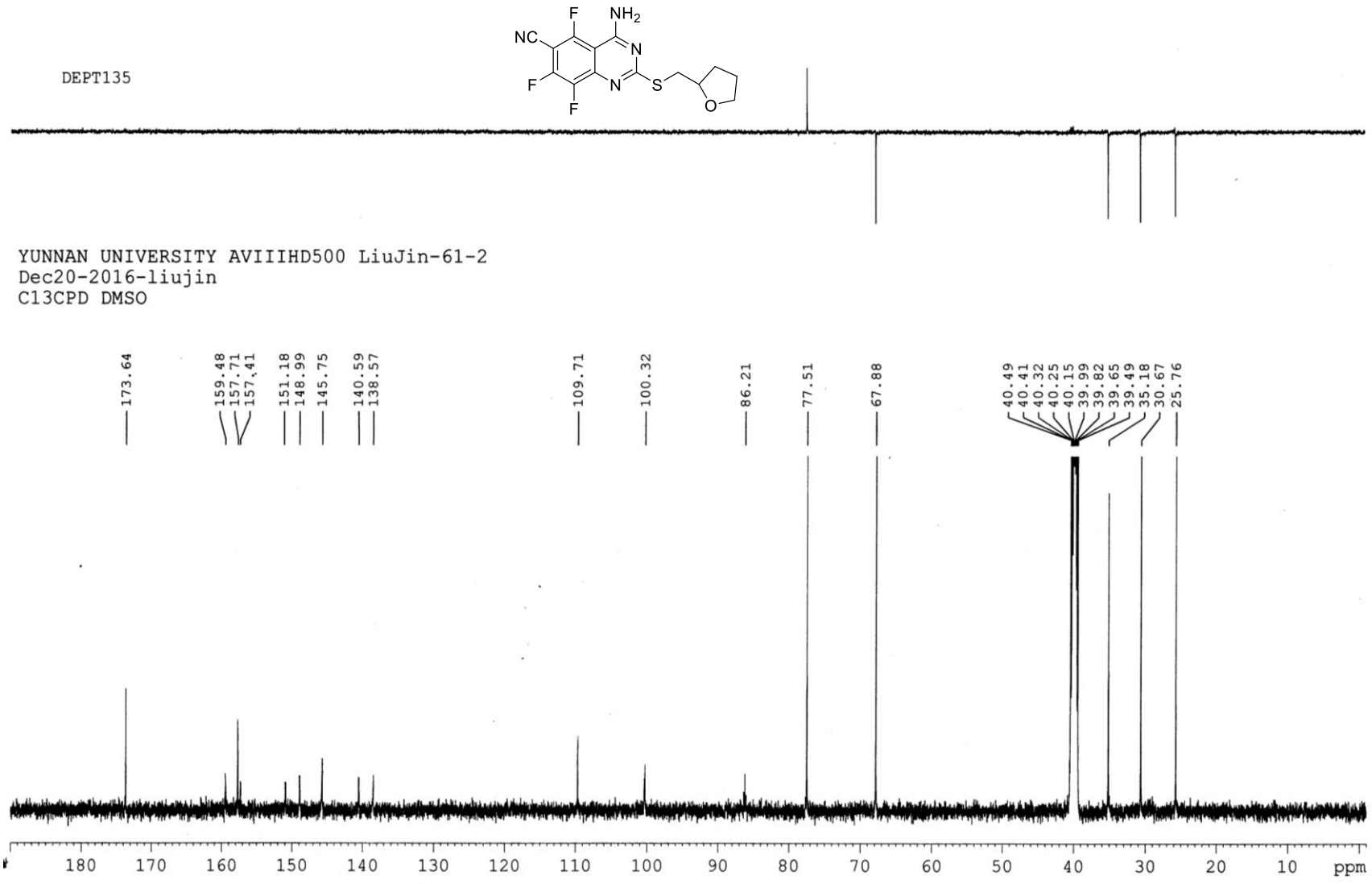

Figure S24. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3k**

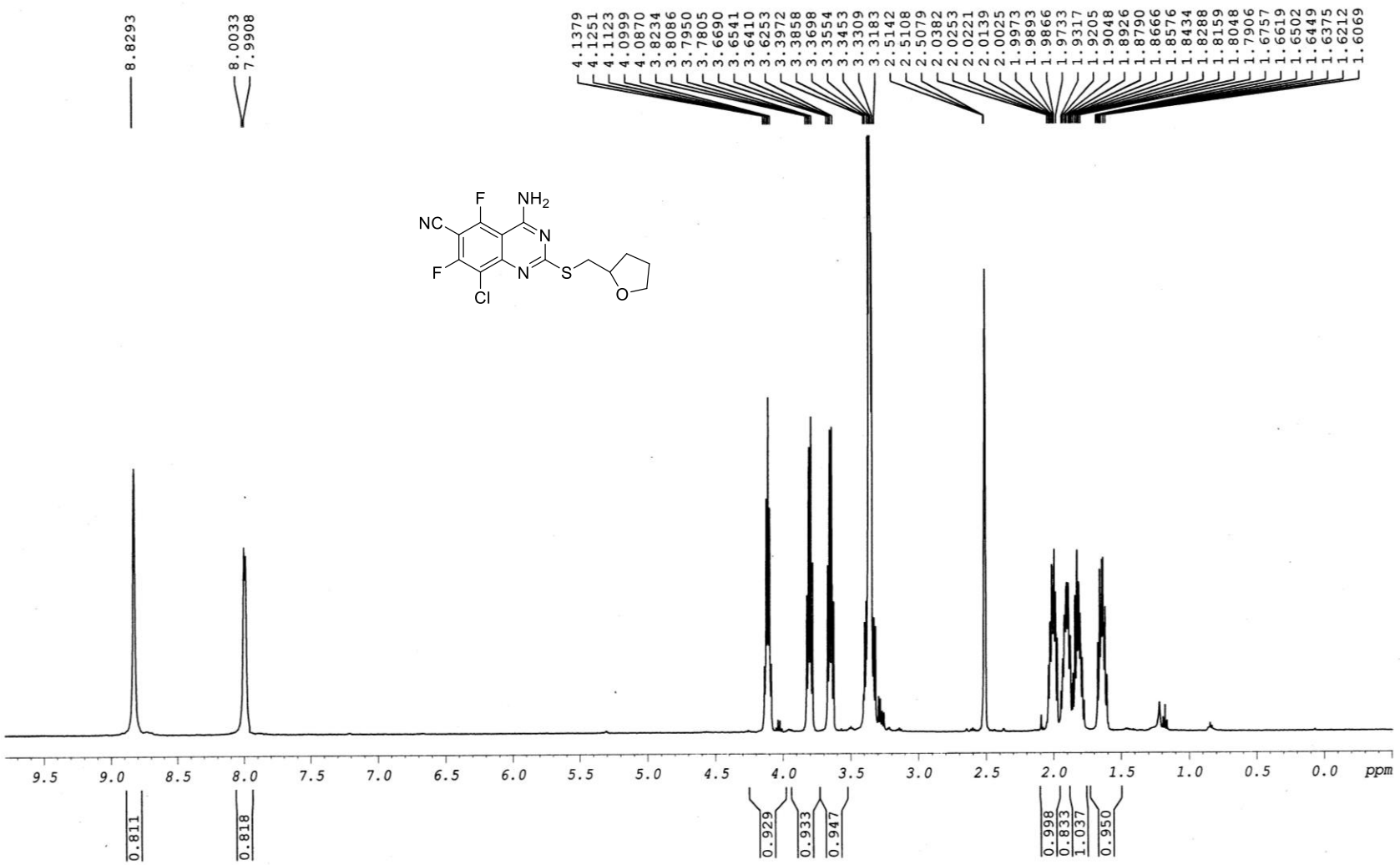

Figure S25. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3l**

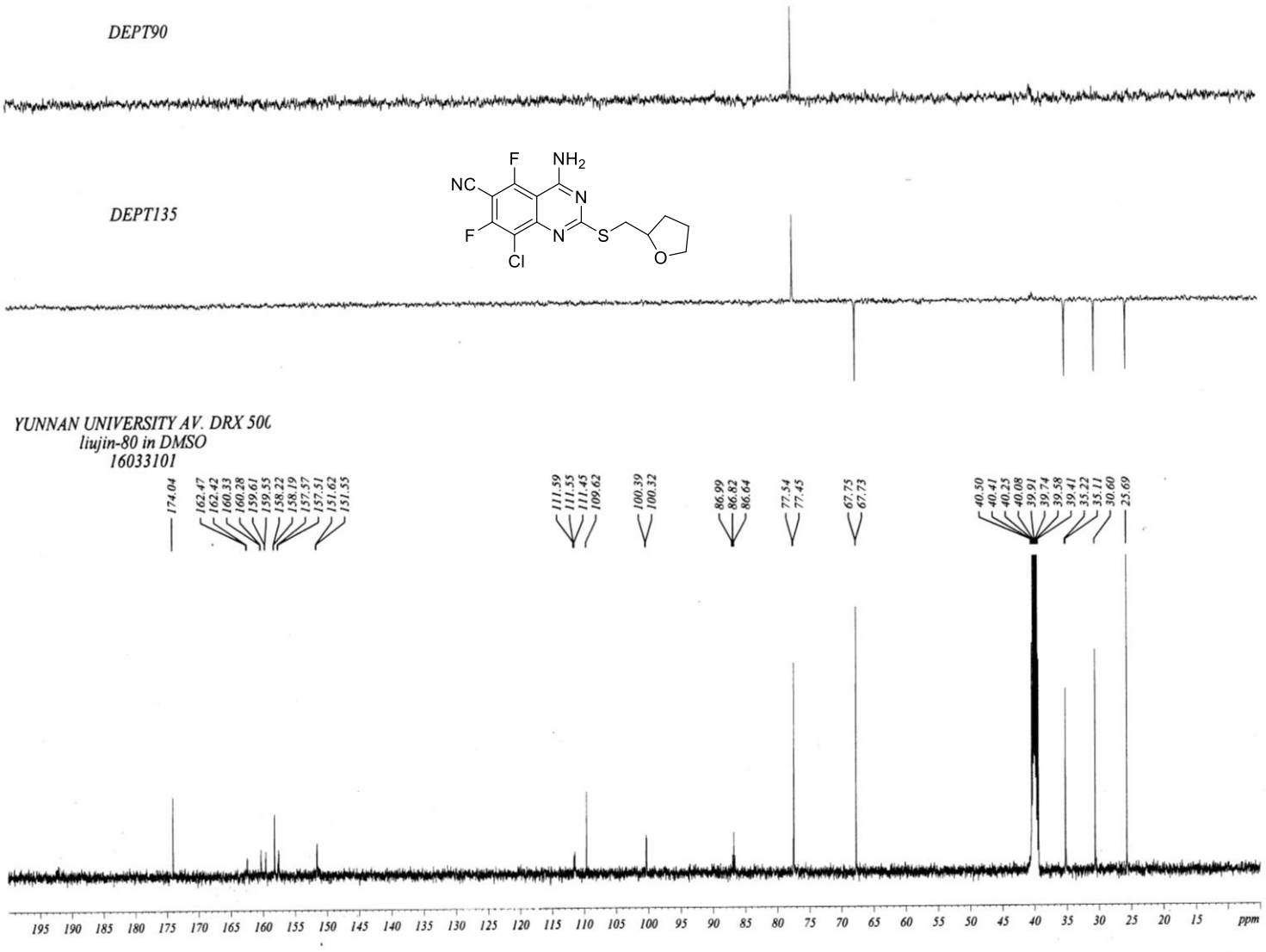

Figure S26. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3l**

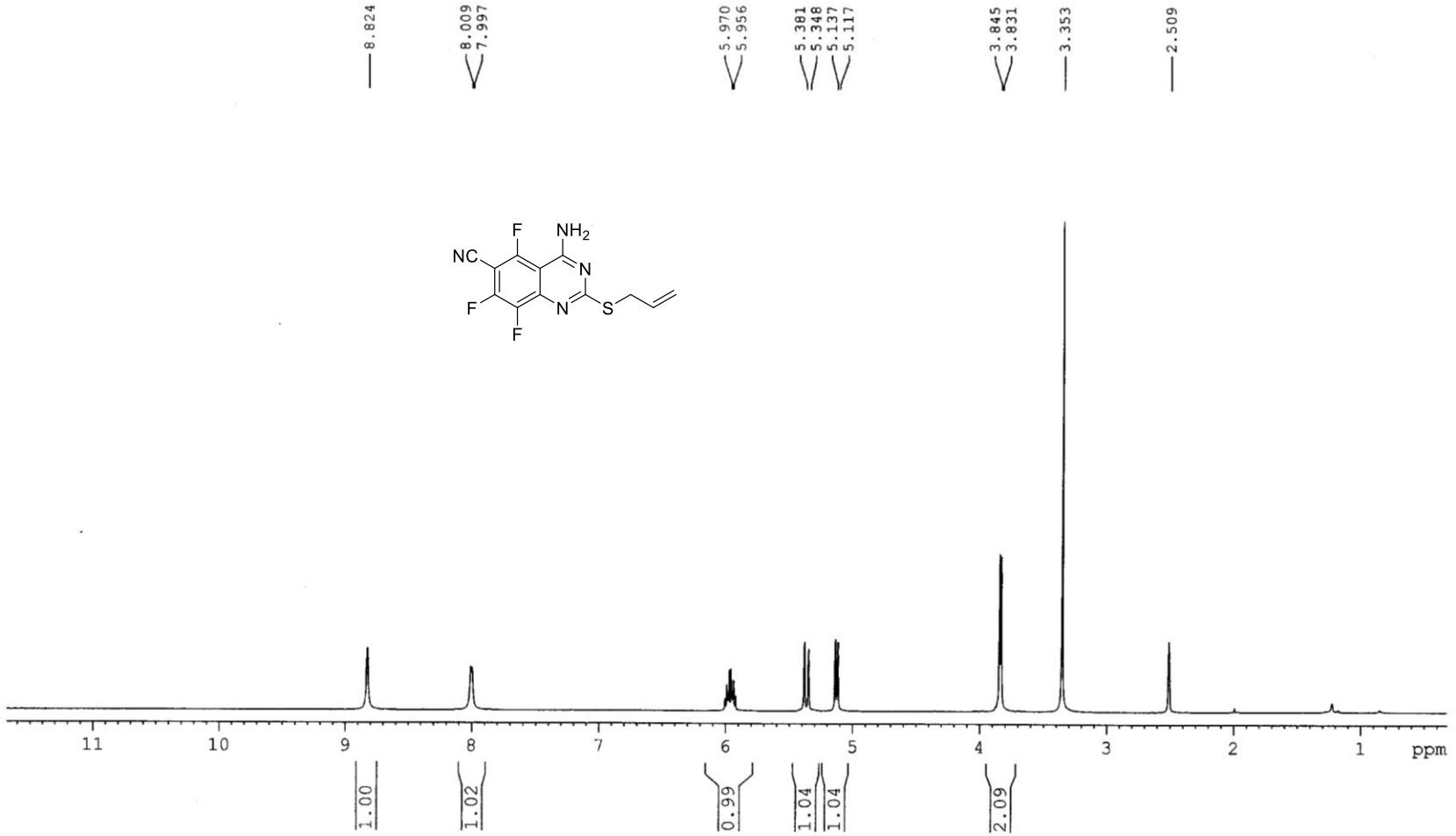

Figure S27. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3m**

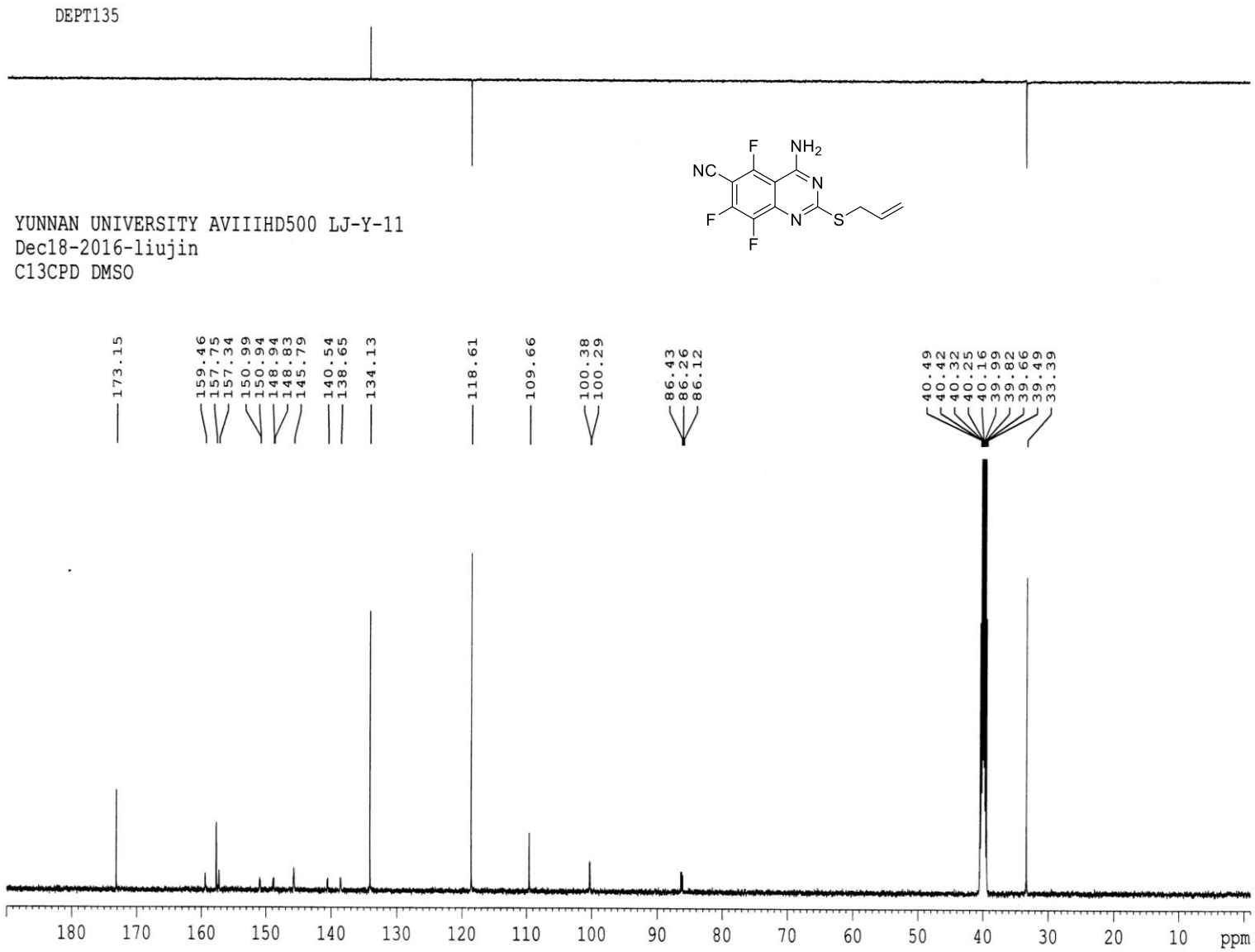

Figure S28. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3m**

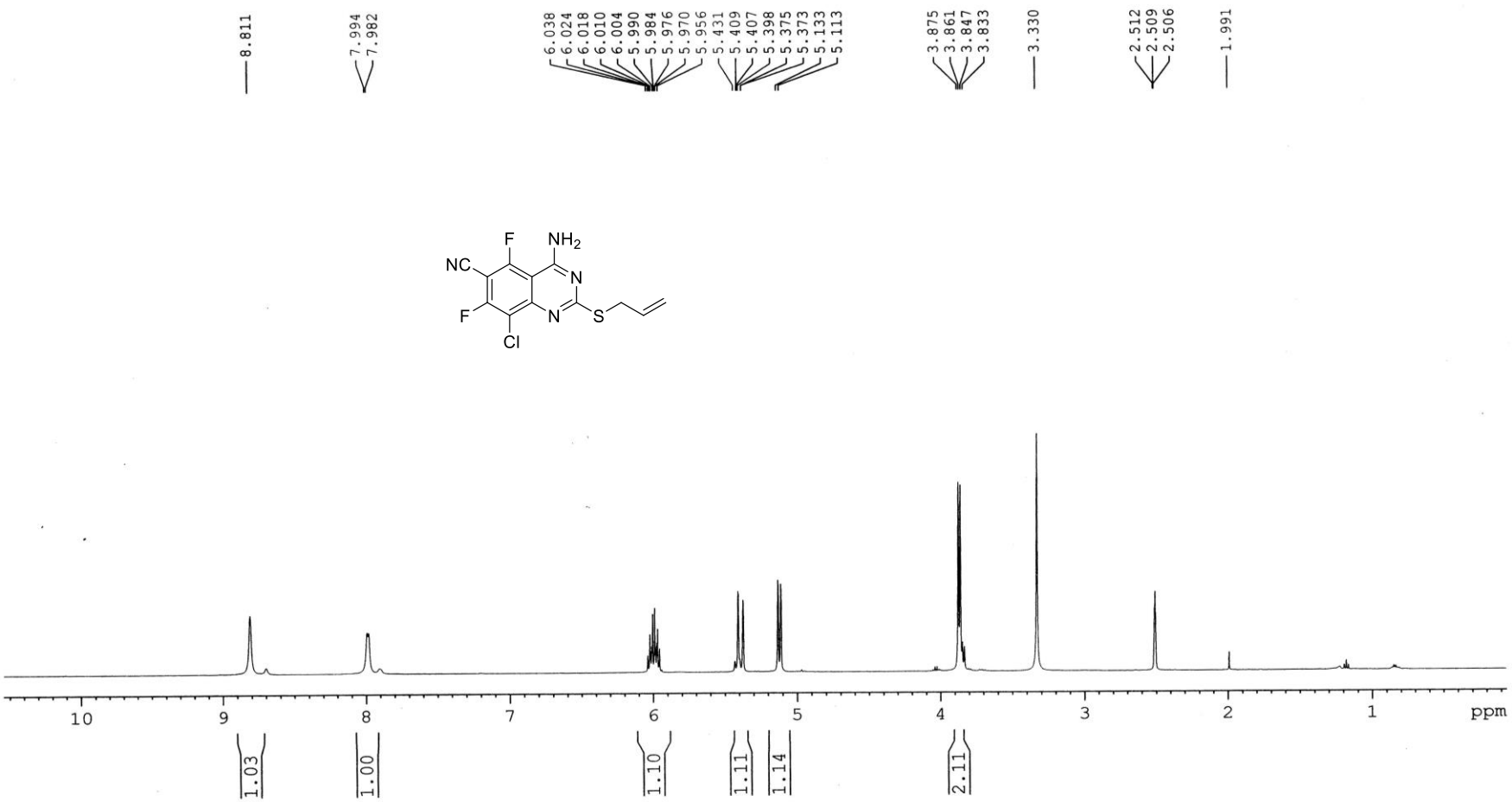

Figure S29. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3n**

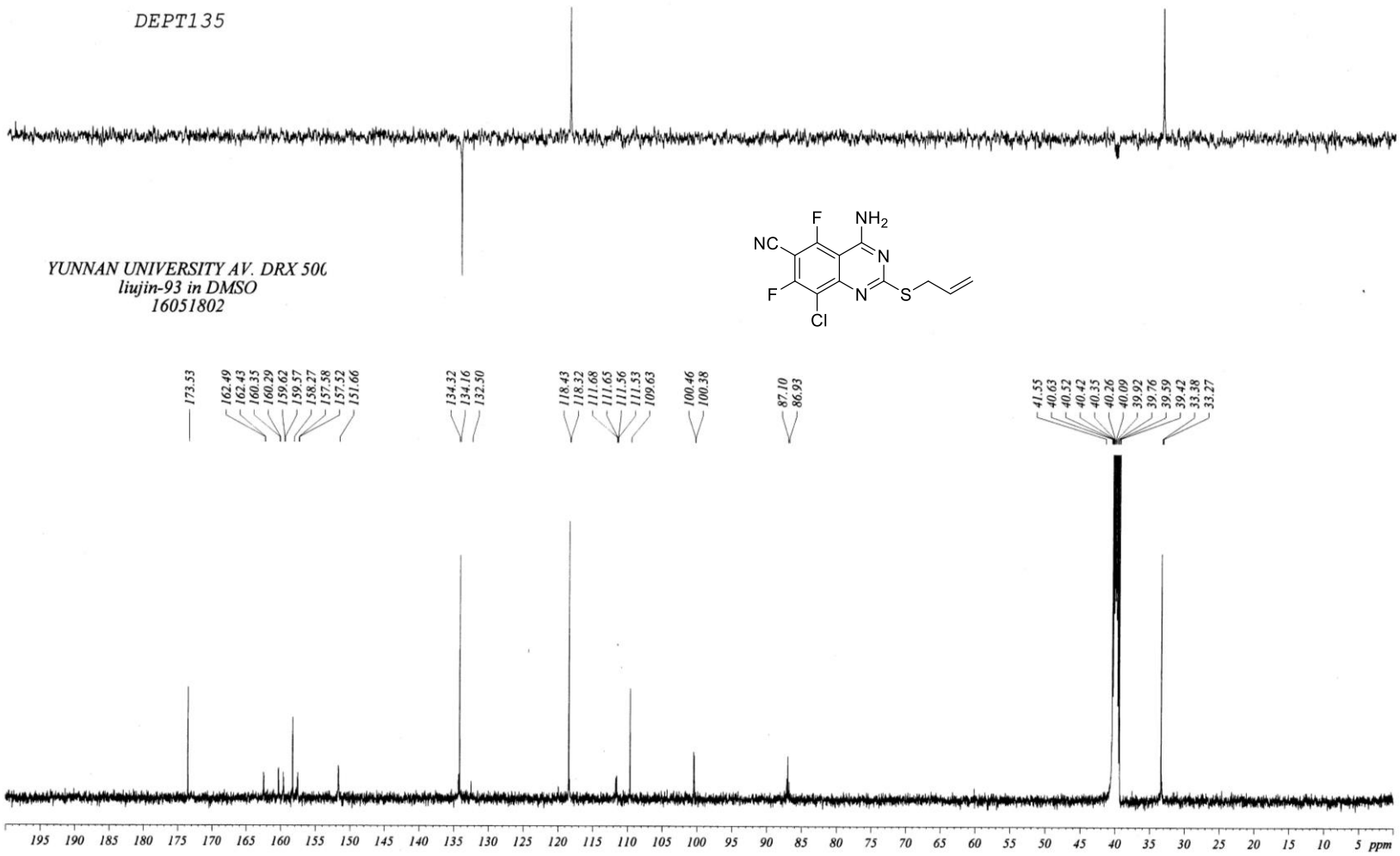

Figure S30. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3n**

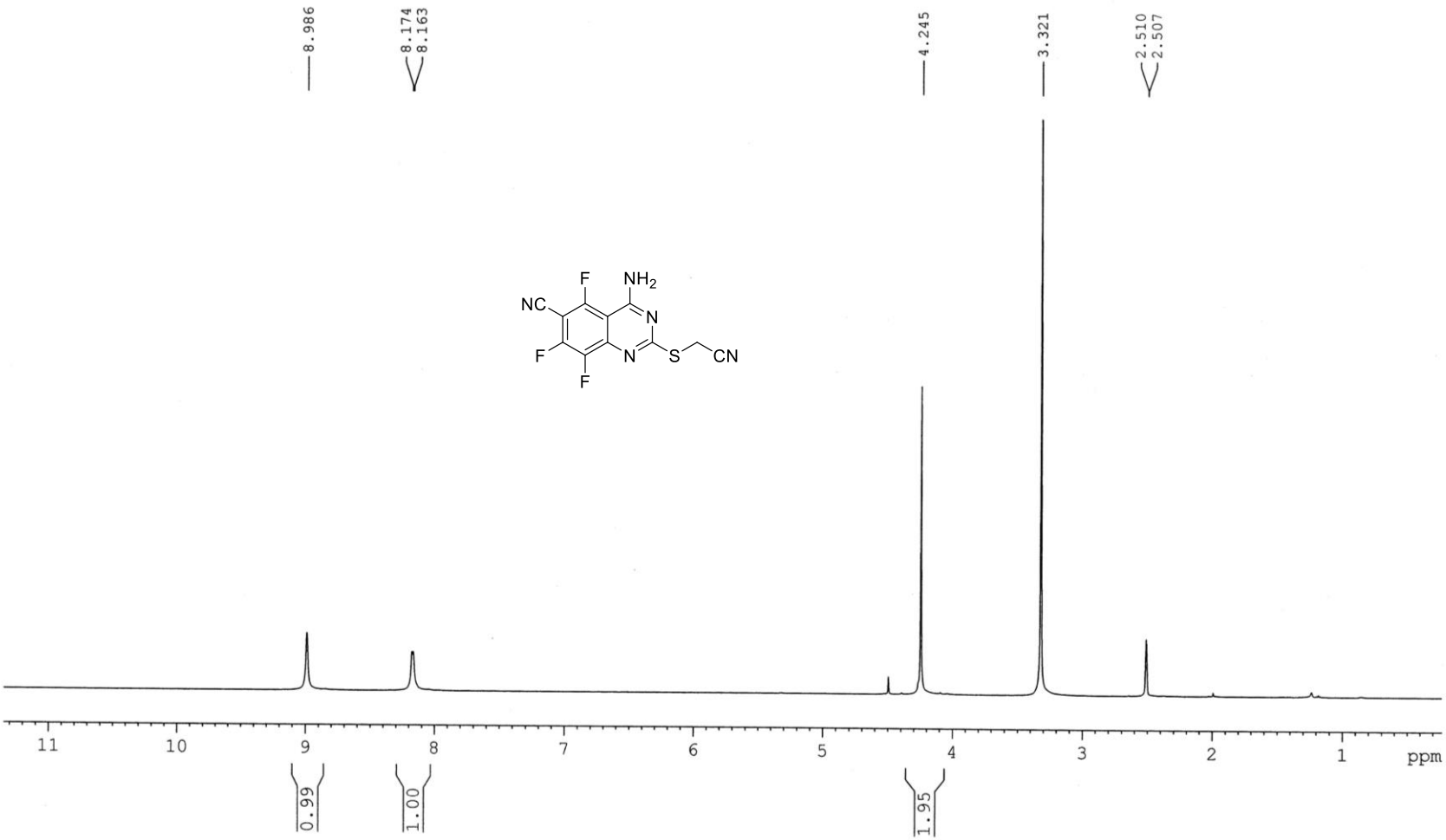

Figure S31. ^{1}H NMR (600 MHz, DMSO-d_6) spectra of compound **3o**

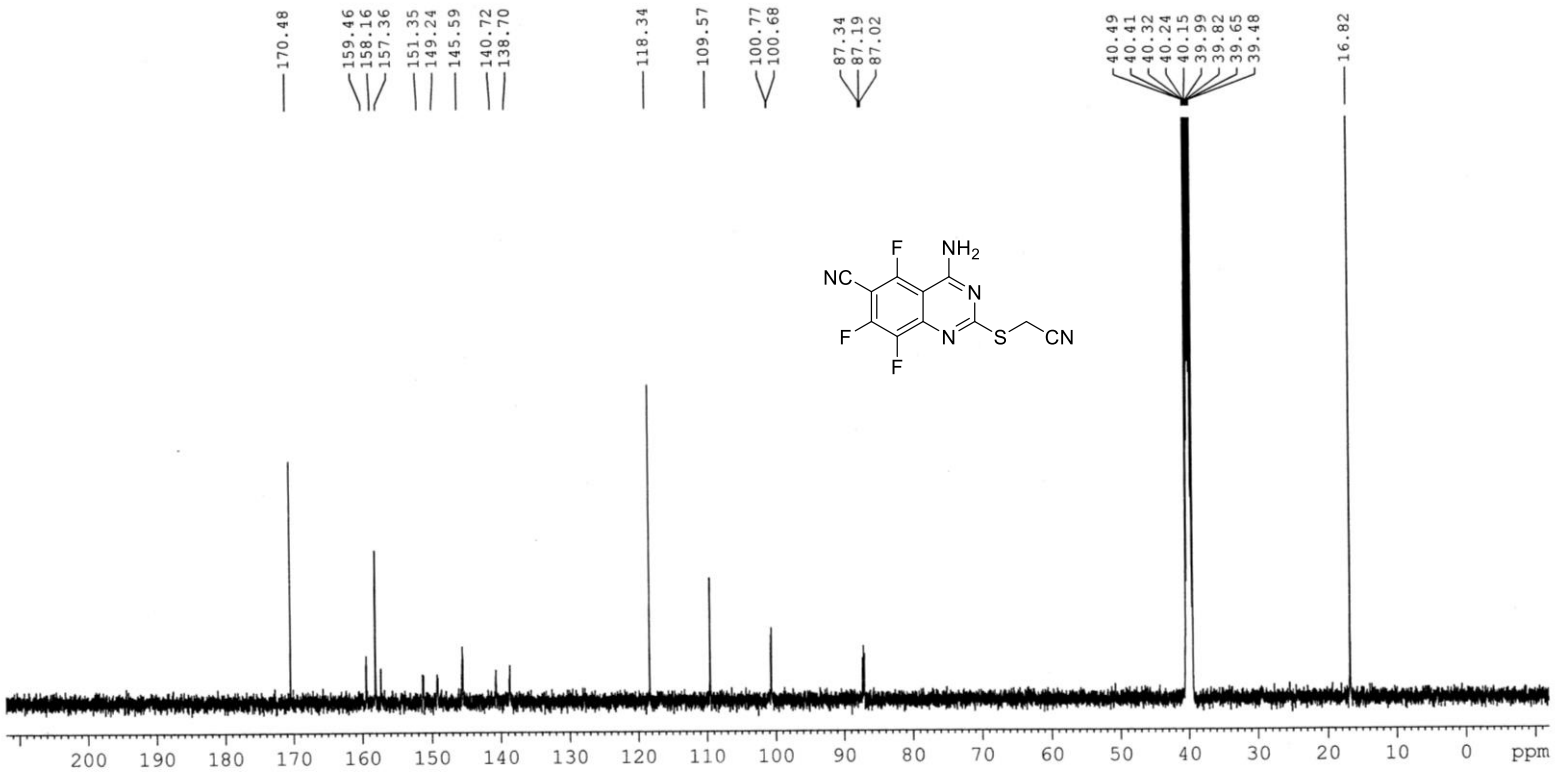

Figure S32. ^{13}C NMR (150 MHz, DMSO-d_6) spectra of compound **3o**

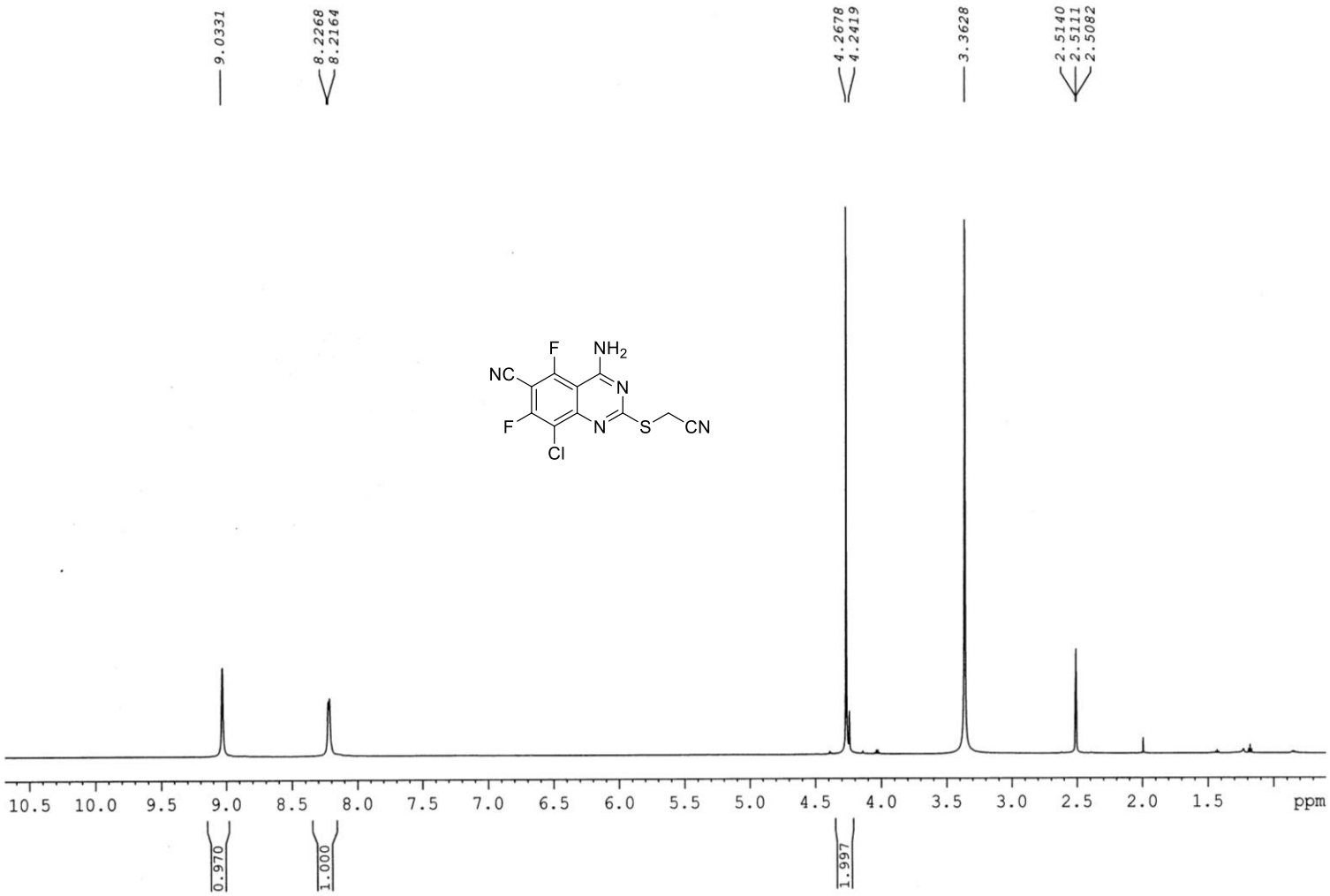

Figure S33. ^{1}H NMR (600 MHz, DMSO-d_6) spectra of compound **3p**

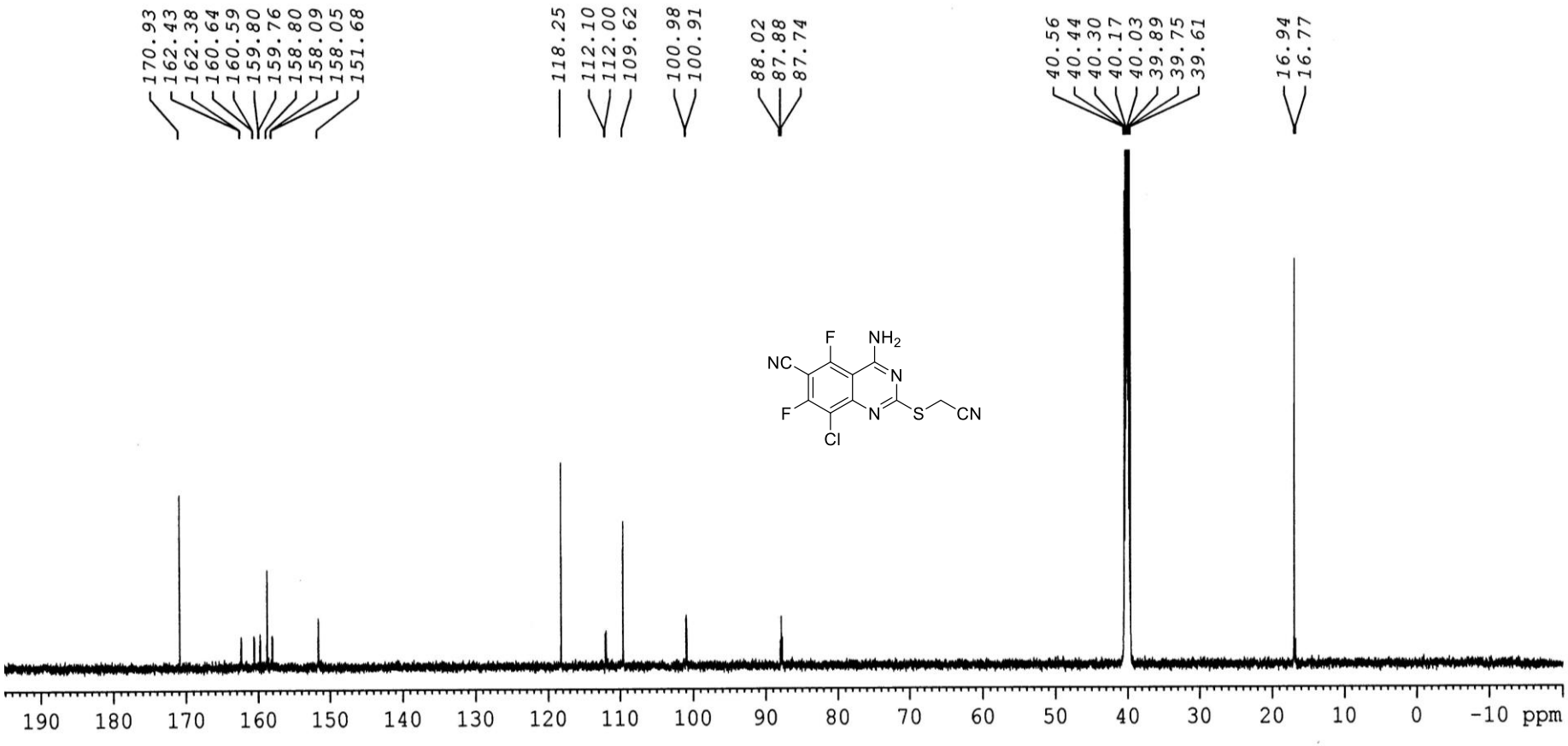

Figure S34. ^{13}C NMR (150 MHz, DMSO-d_6) spectra of compound **3p**

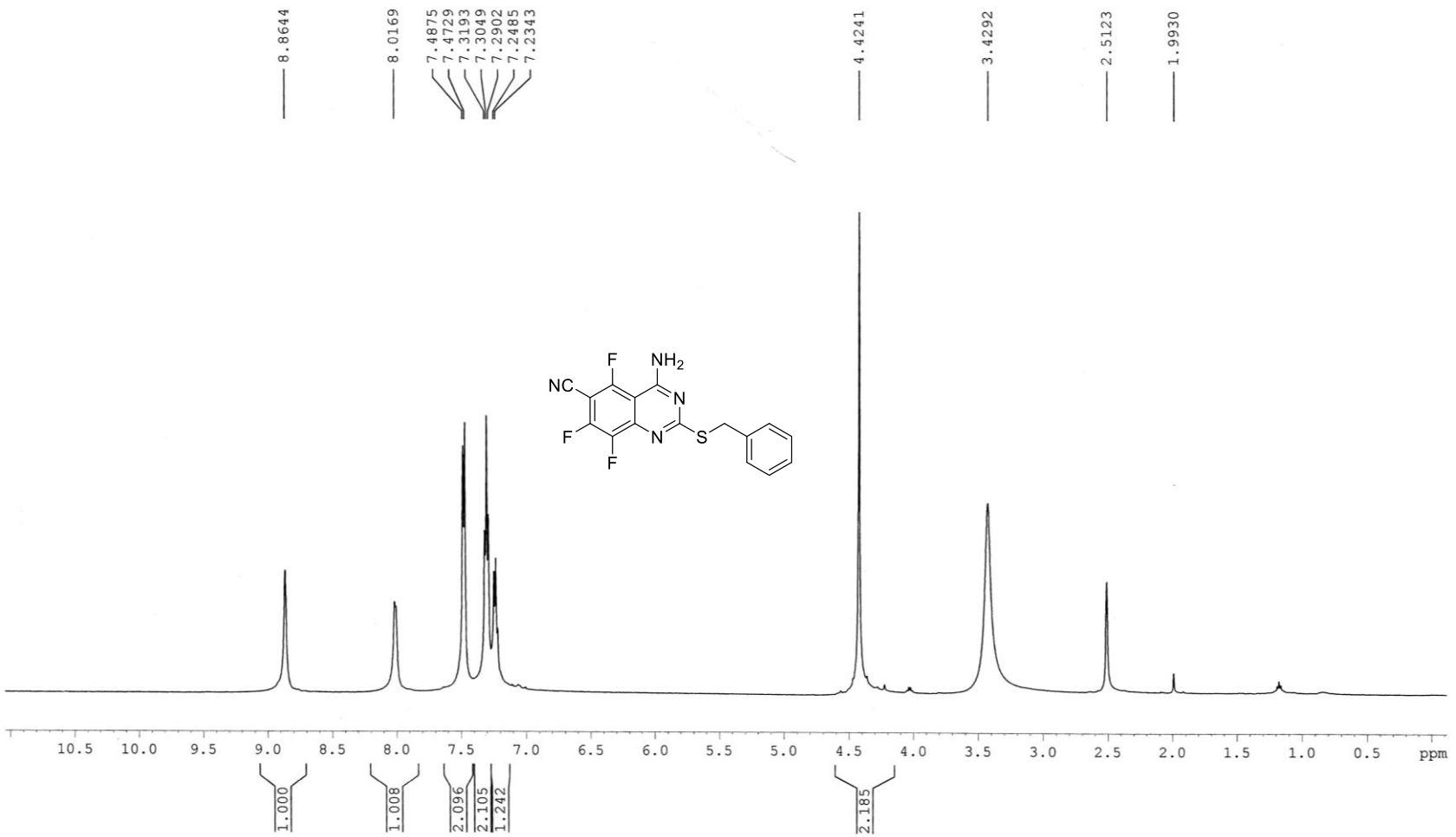

Figure S35. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3q**

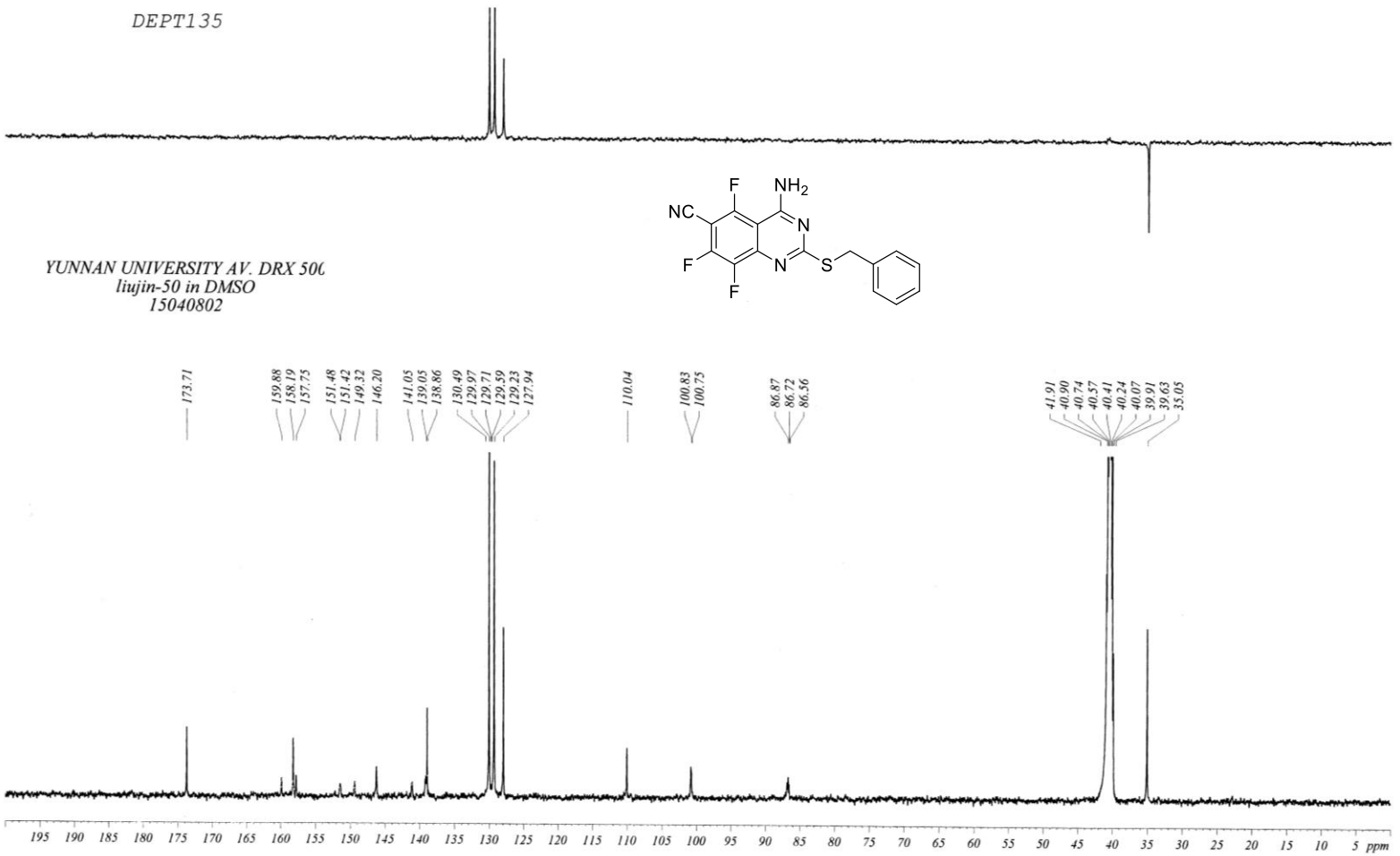

Figure S36. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3q**

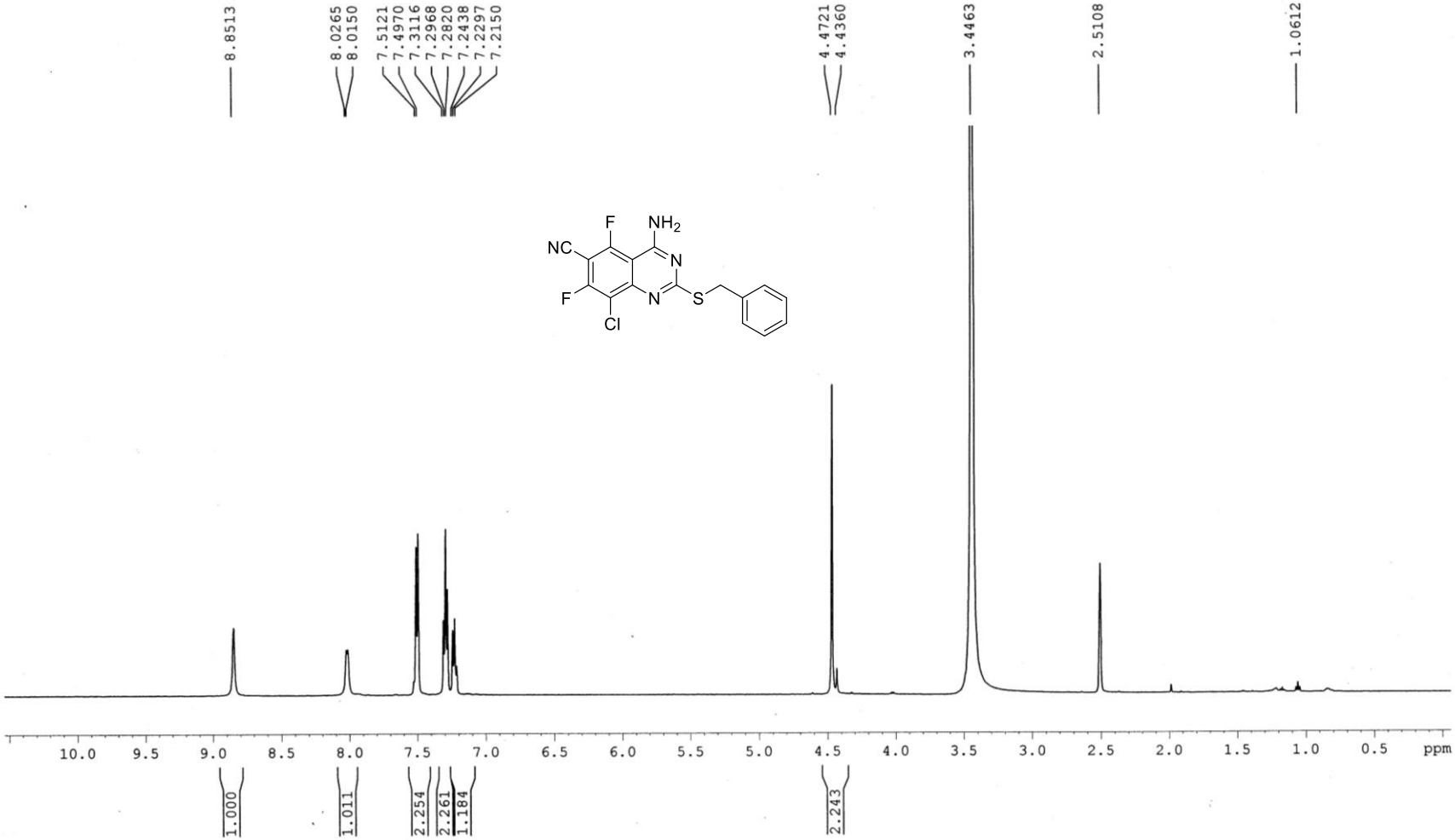

Figure S37. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3r**

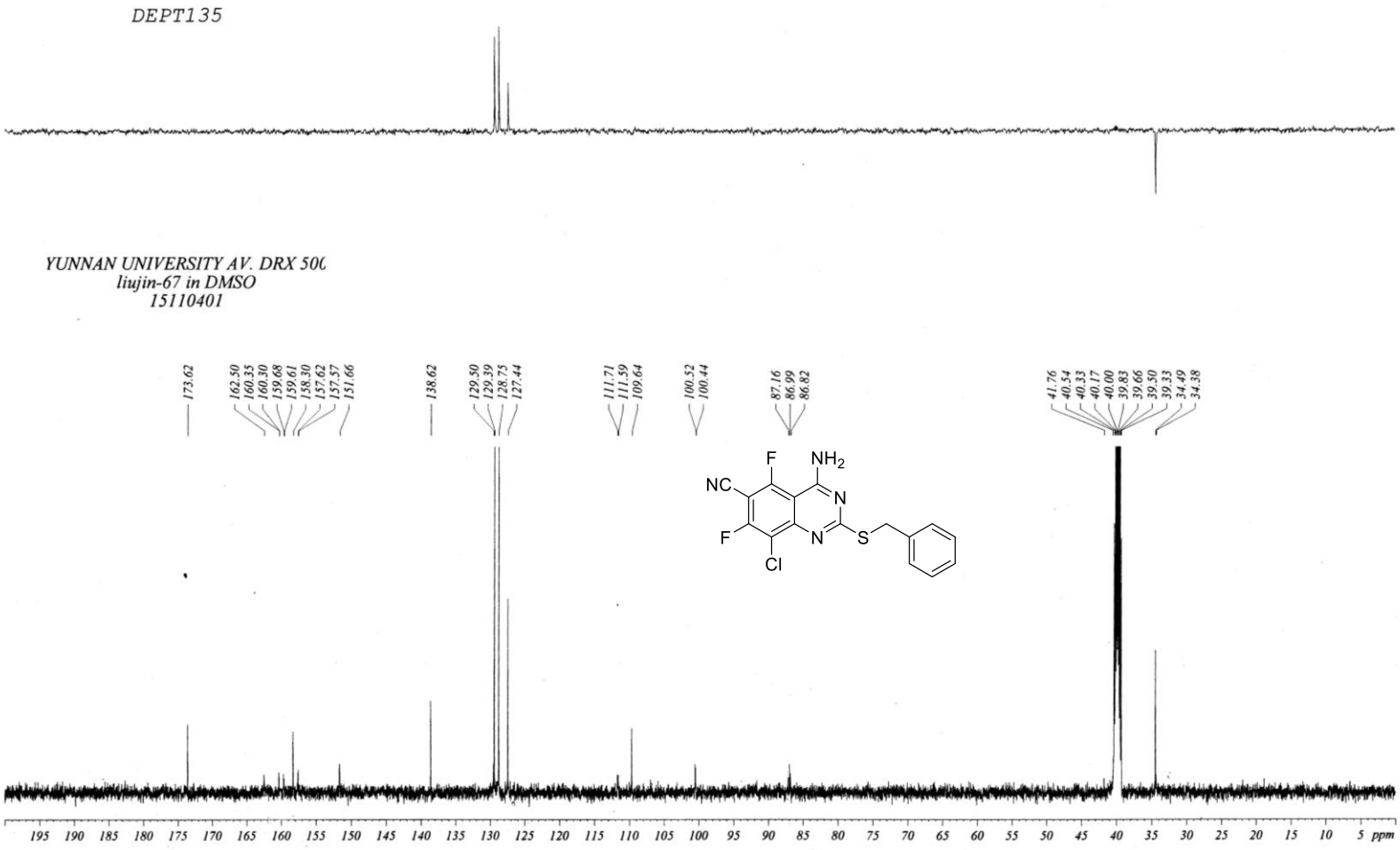

Figure S38. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3r**

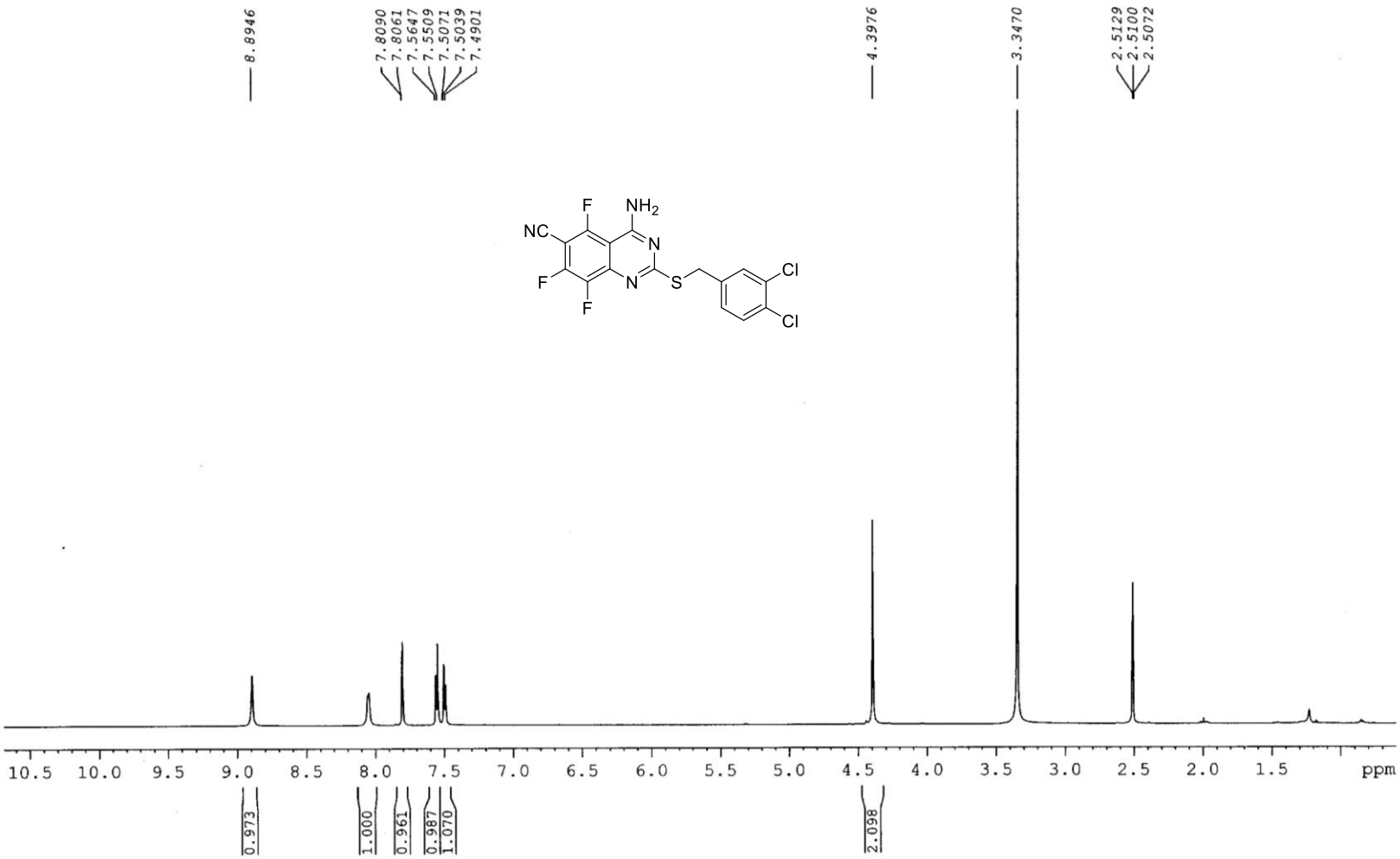

Figure S39. ^{1}H NMR (600 MHz, DMSO-d_6) spectra of compound **3s**

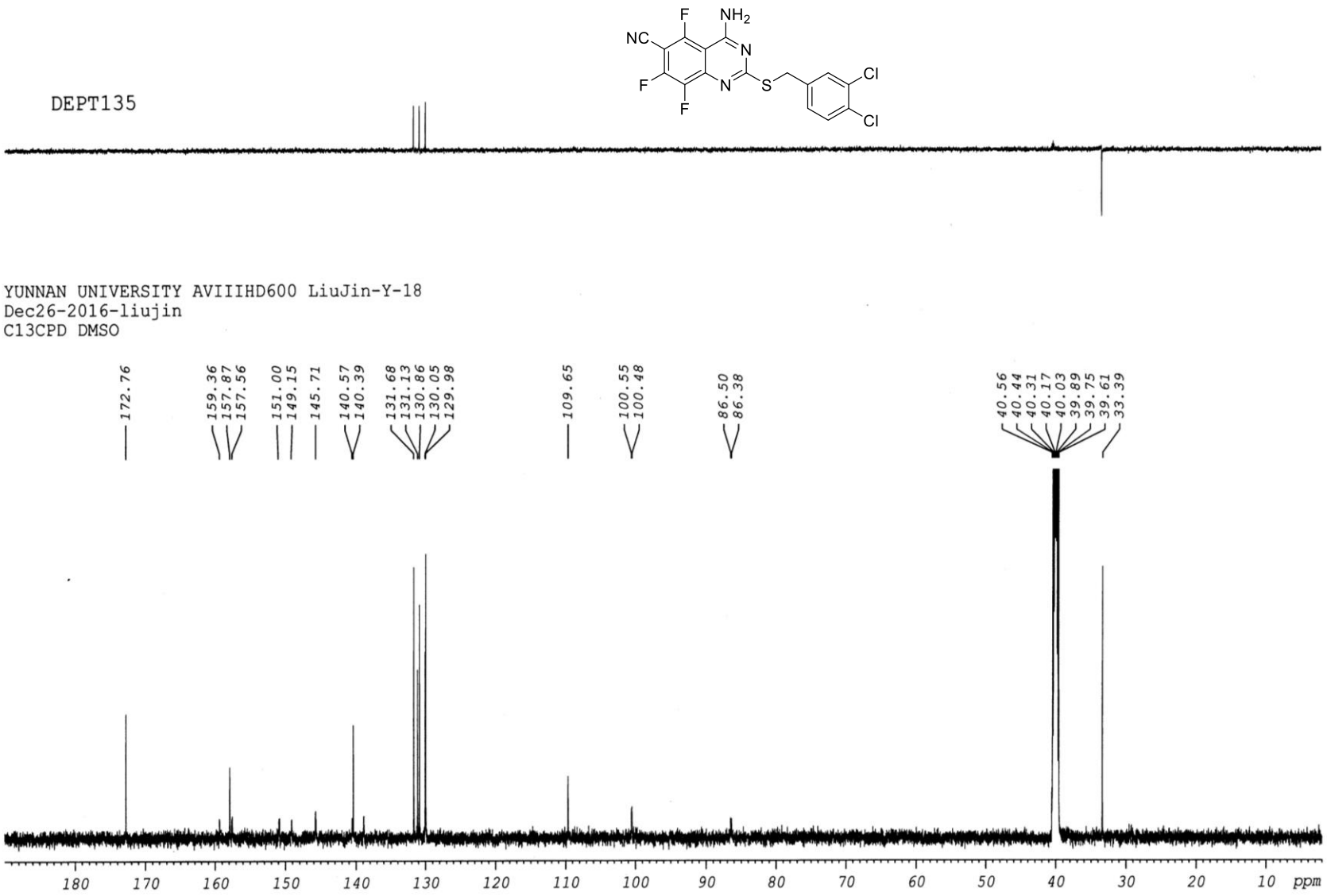

Figure S40. ^{13}C NMR (150 MHz, DMSO-d_6) spectra of compound **3s**

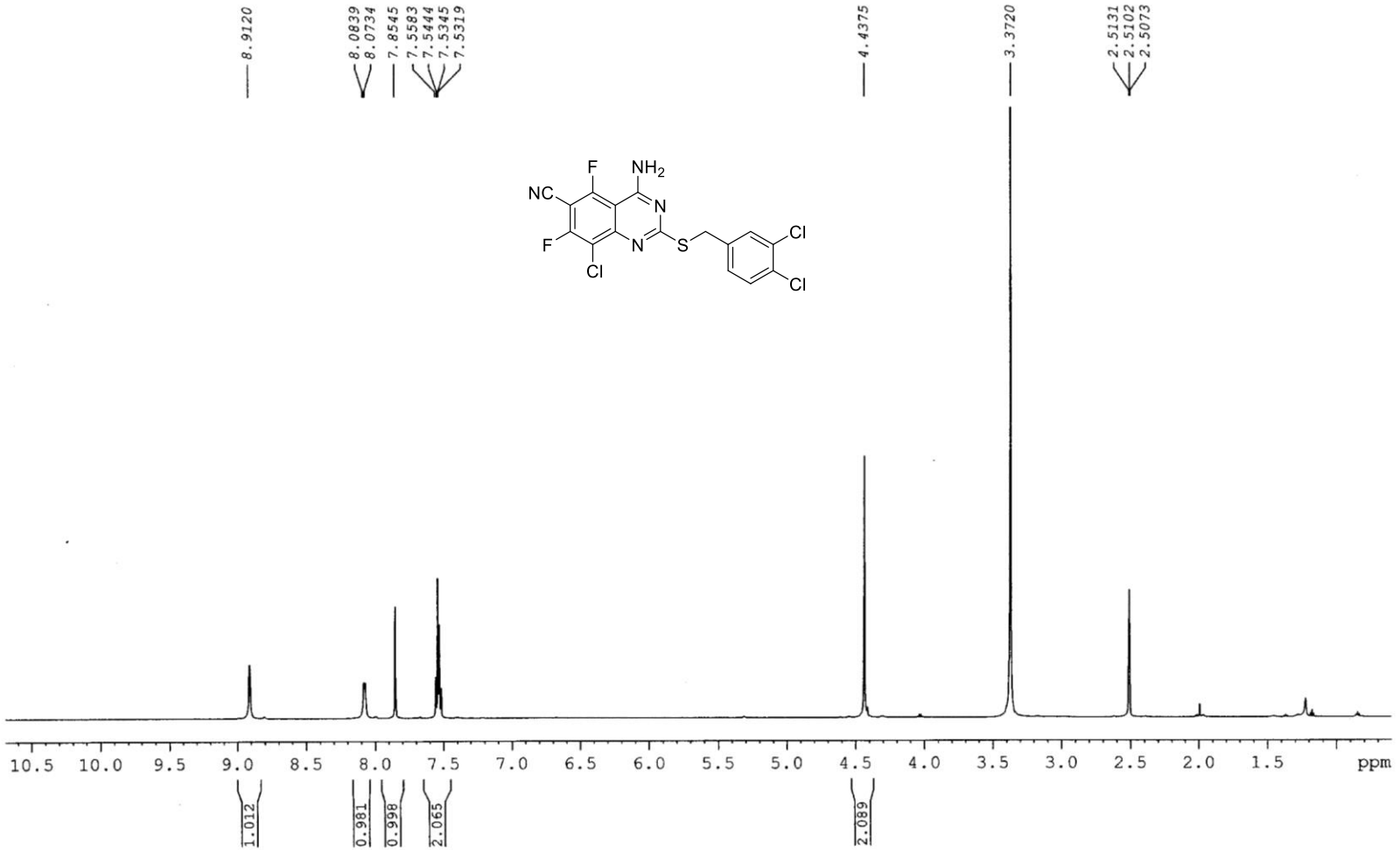

Figure S41. ^{1}H NMR (600 MHz, DMSO-d_6) spectra of compound **3t**

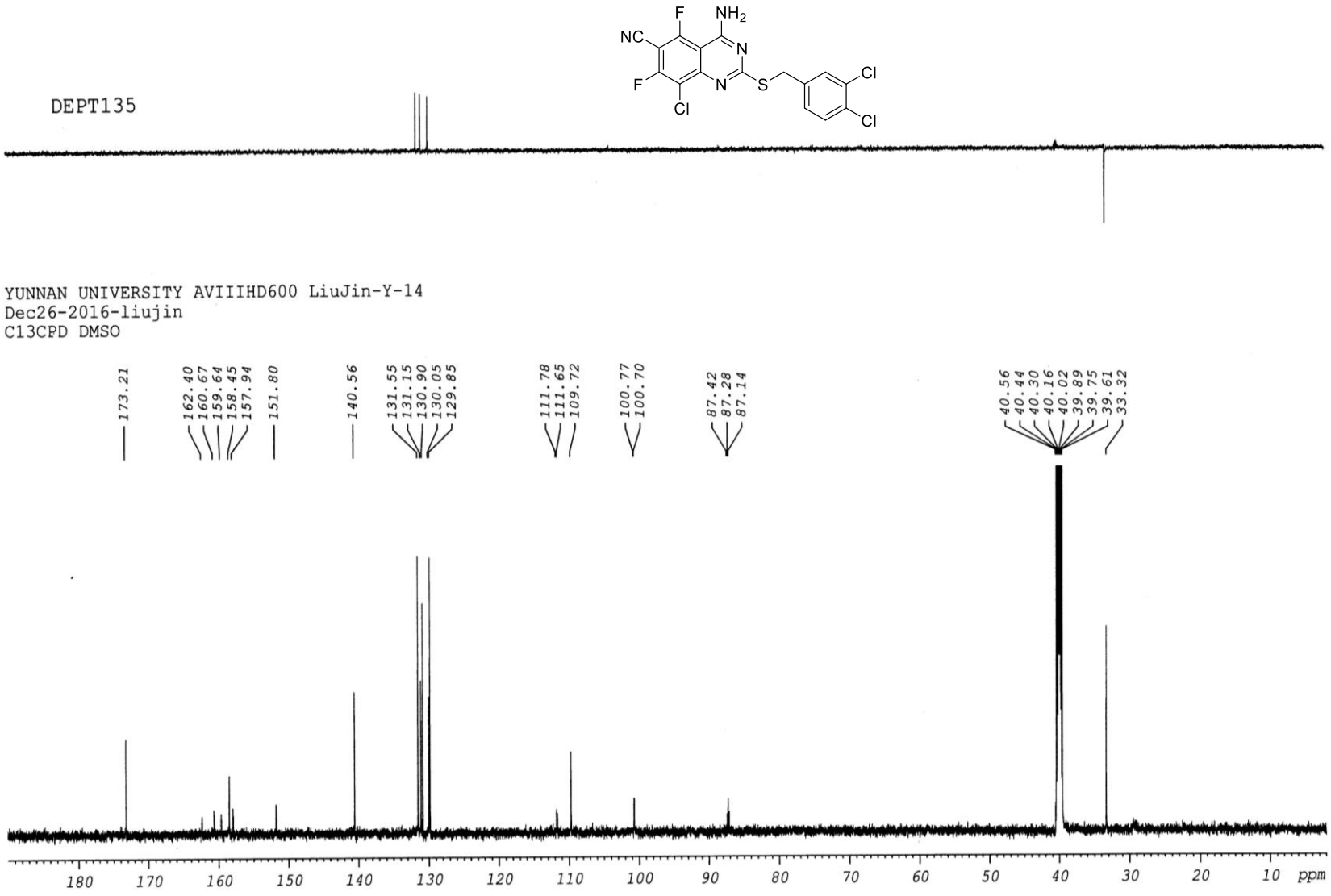

Figure **S42**. ^{13}C NMR (150 MHz, DMSO-d_6) spectra of compound **3t**

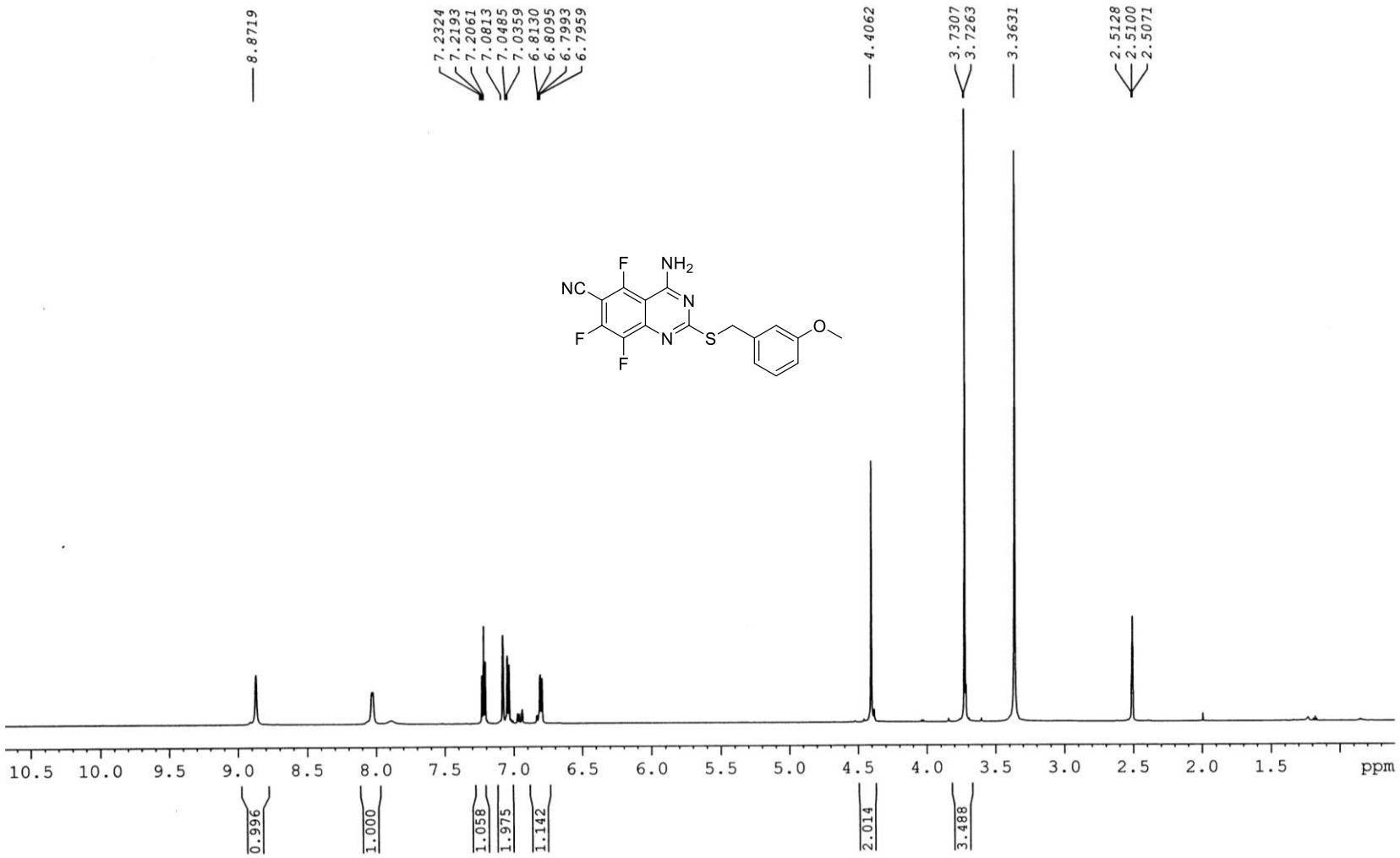

Figure S43. ^{1}H NMR (600 MHz, DMSO-d_6) spectra of compound **3u**

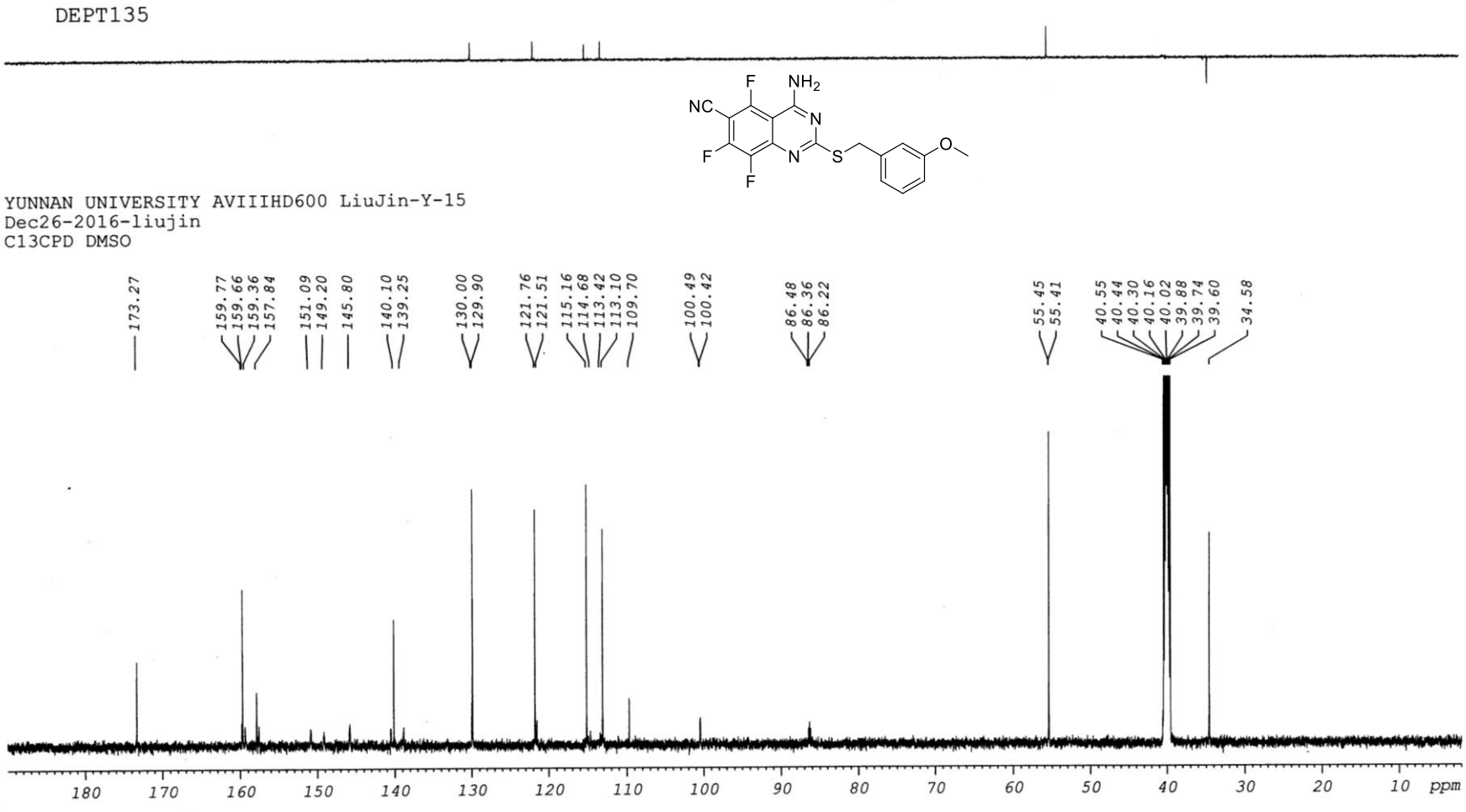

Figure S44. ^{13}C NMR (150 MHz, DMSO-d_6) spectra of compound **3u**

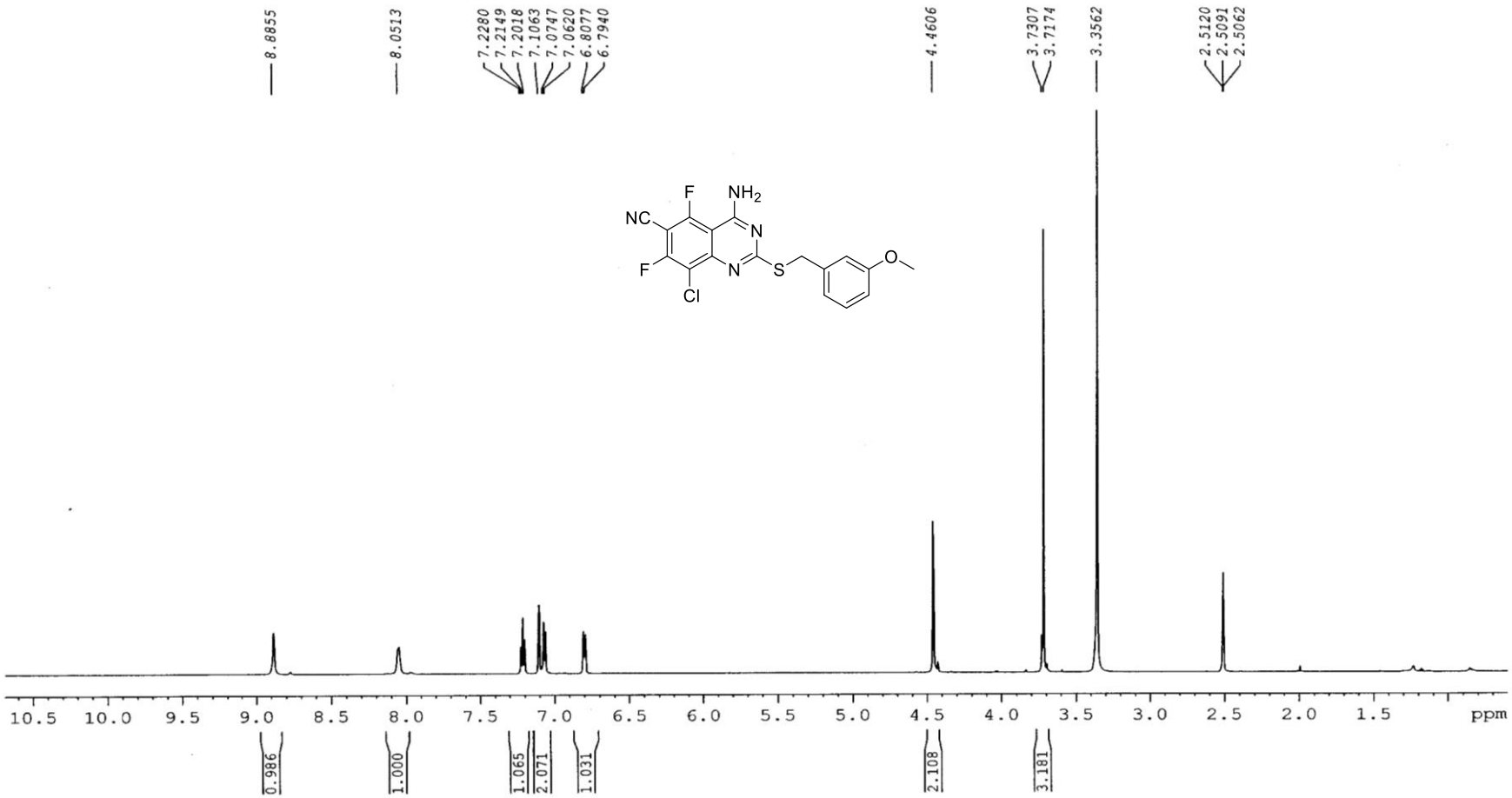

Figure S45. ^{1}H NMR (600 MHz, DMSO-d_6) spectra of compound **3v**

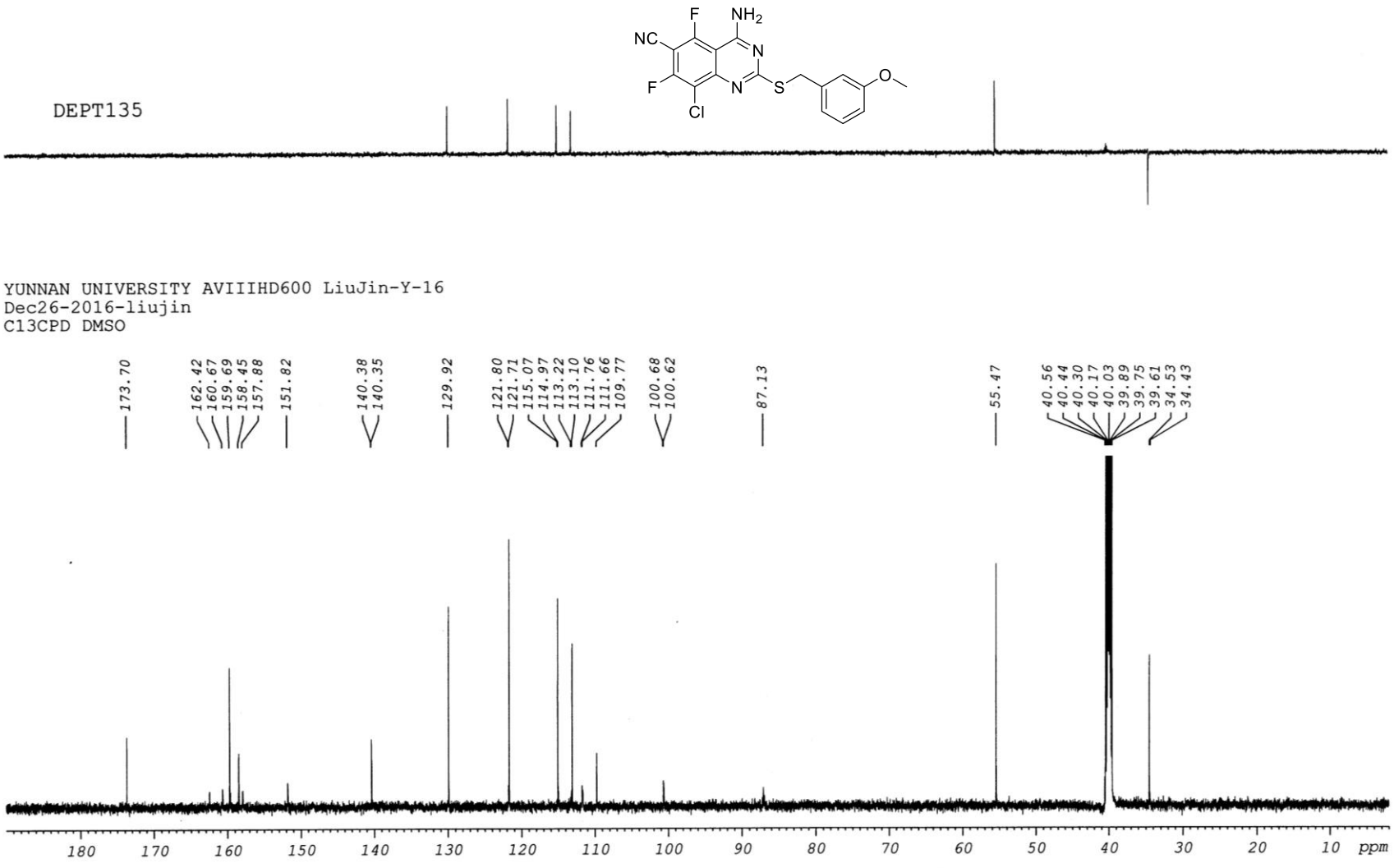

Figure S46. ^{13}C NMR (150 MHz, DMSO-d_6) spectra of compound **3v**

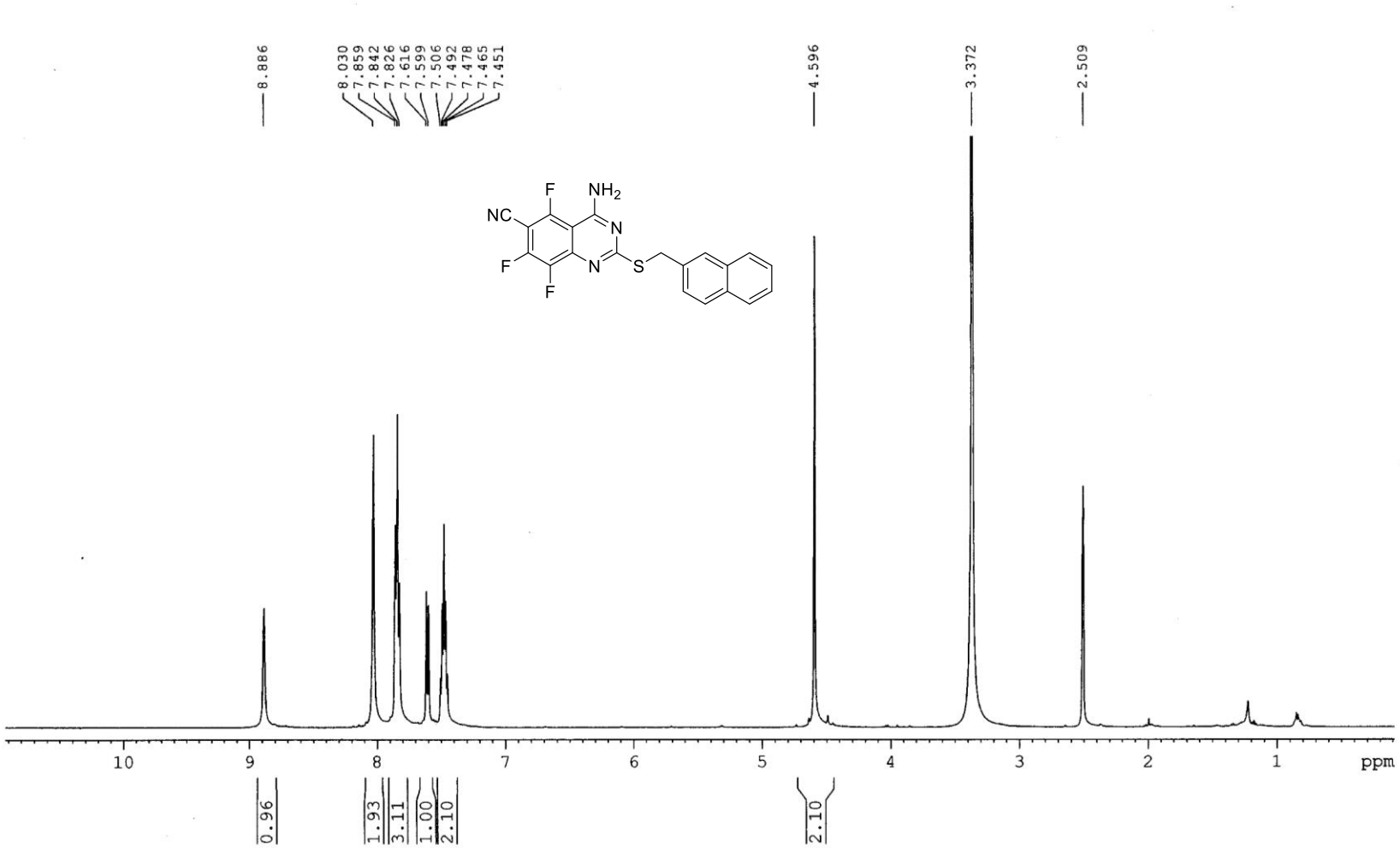

Figure S47. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3w**

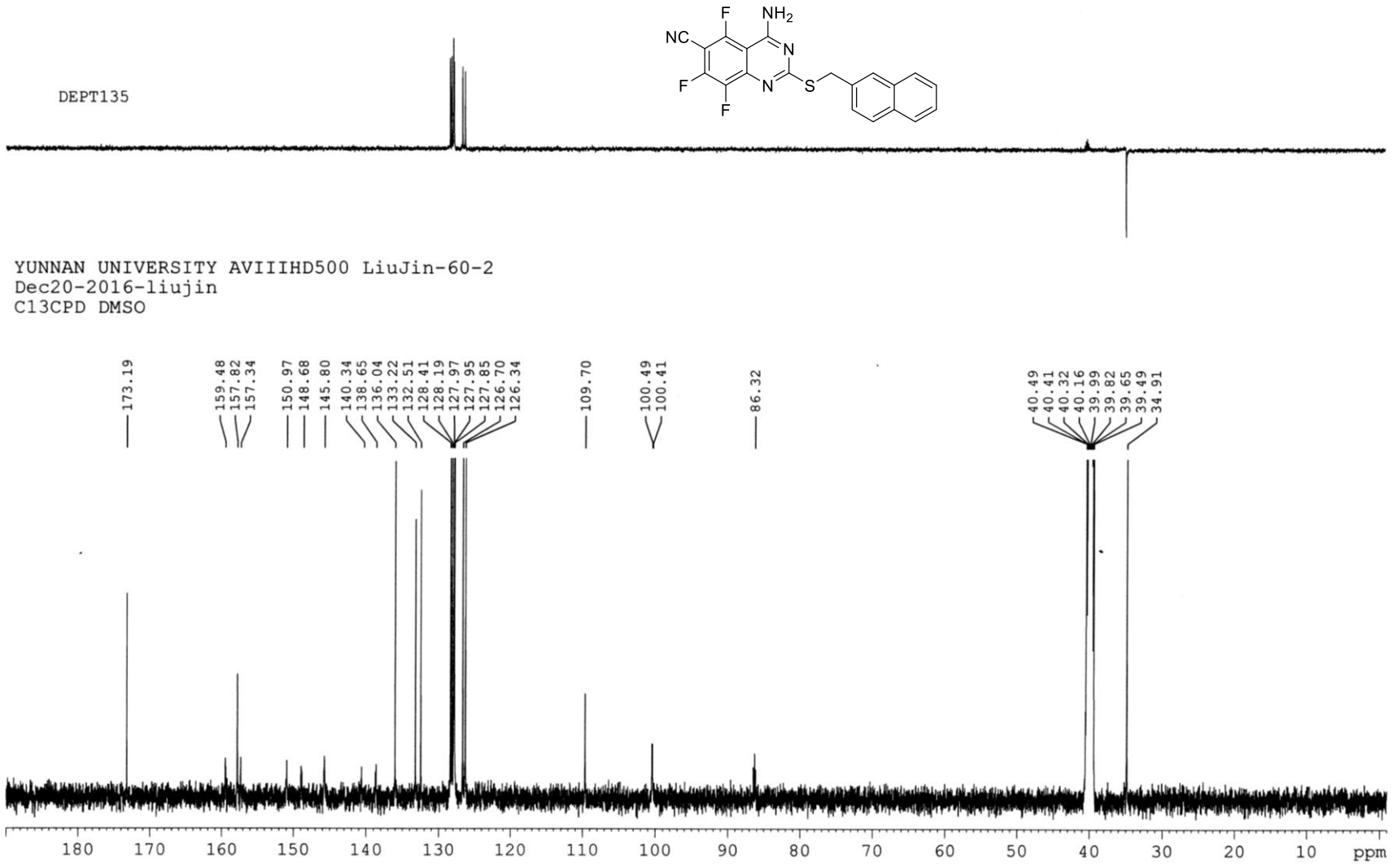

Figure S48. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3w**

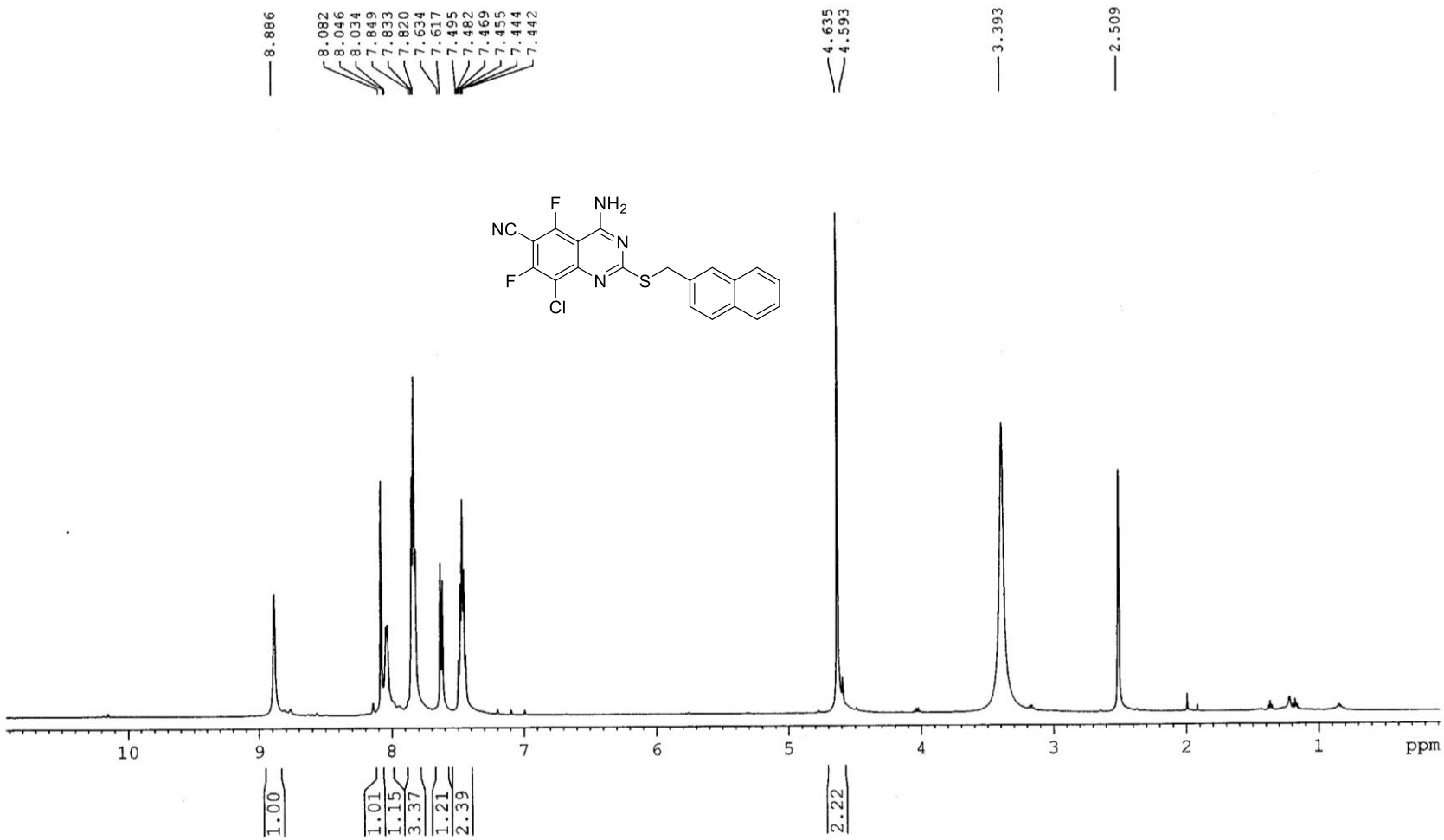

Figure S49. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3x**

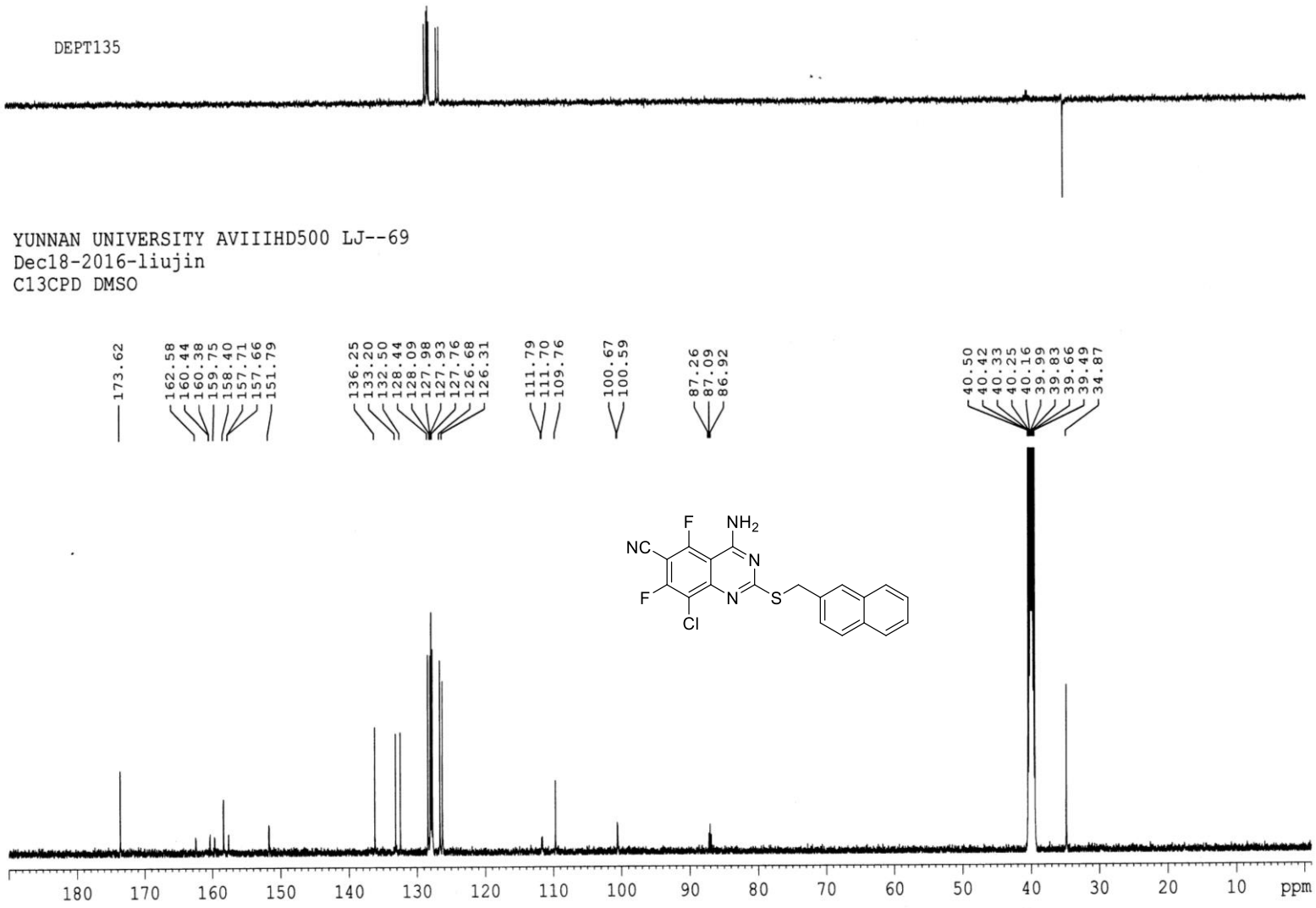

Figure S50. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3x**

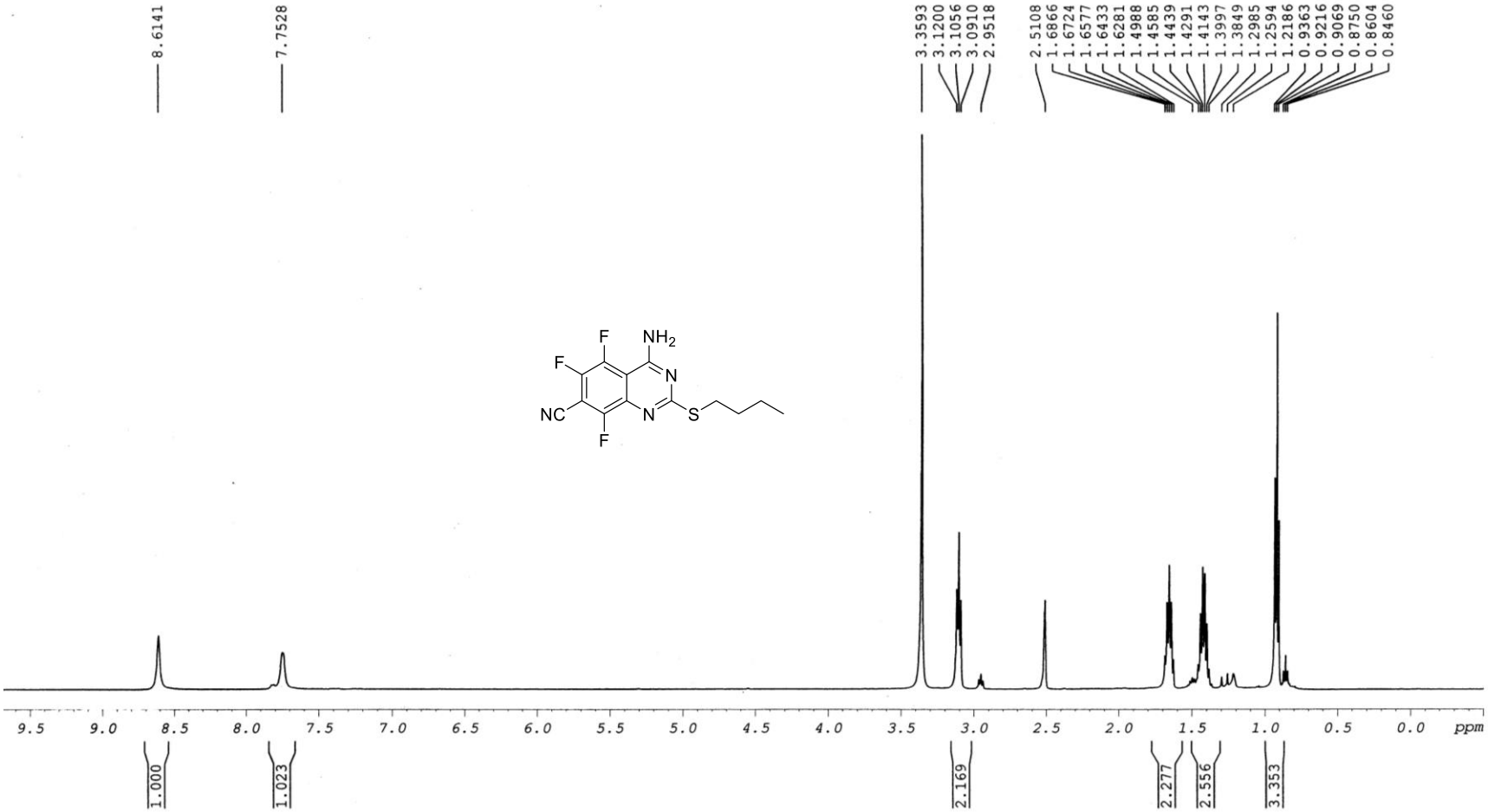

Figure S51. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3y**

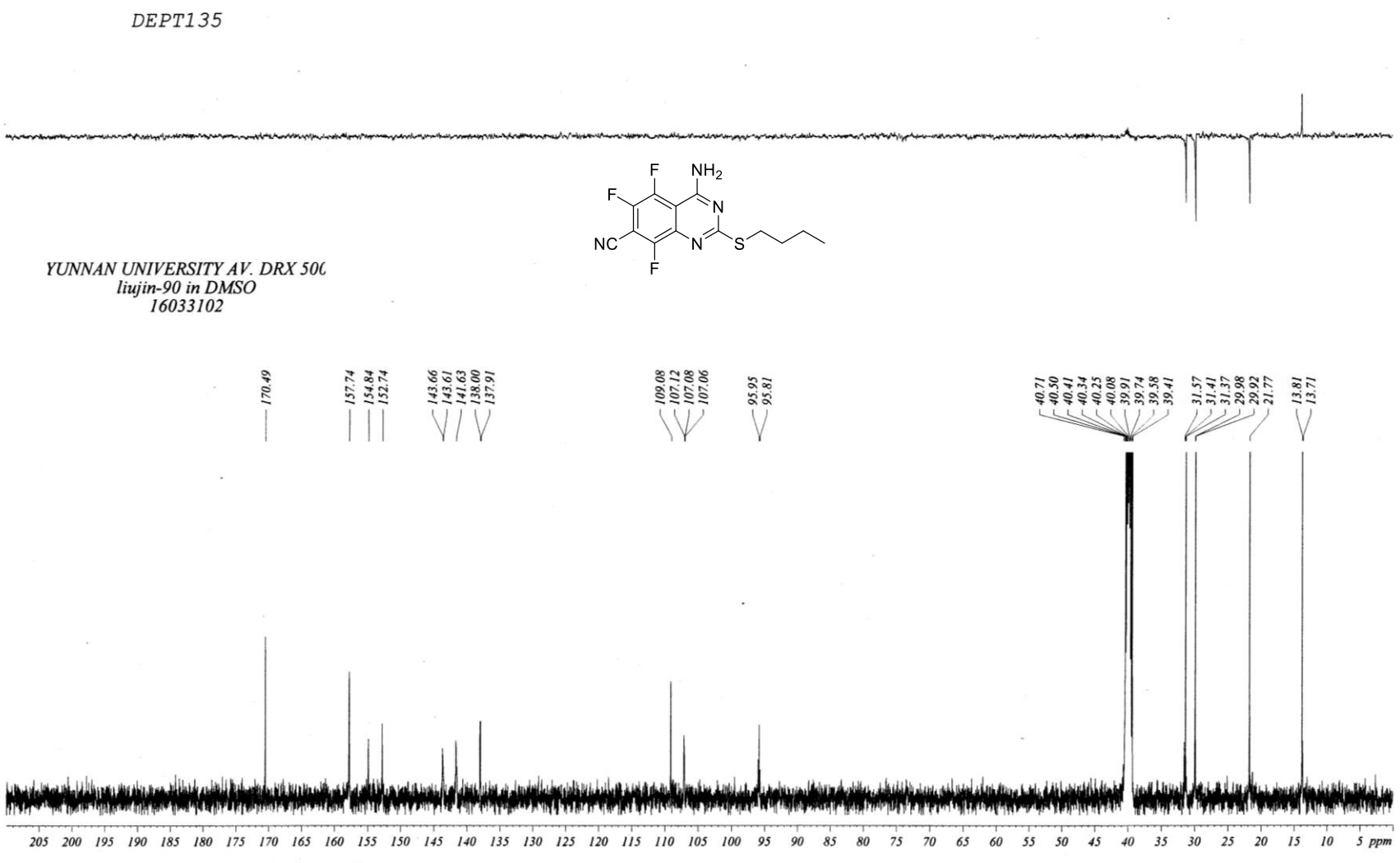

Figure S52. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3y**

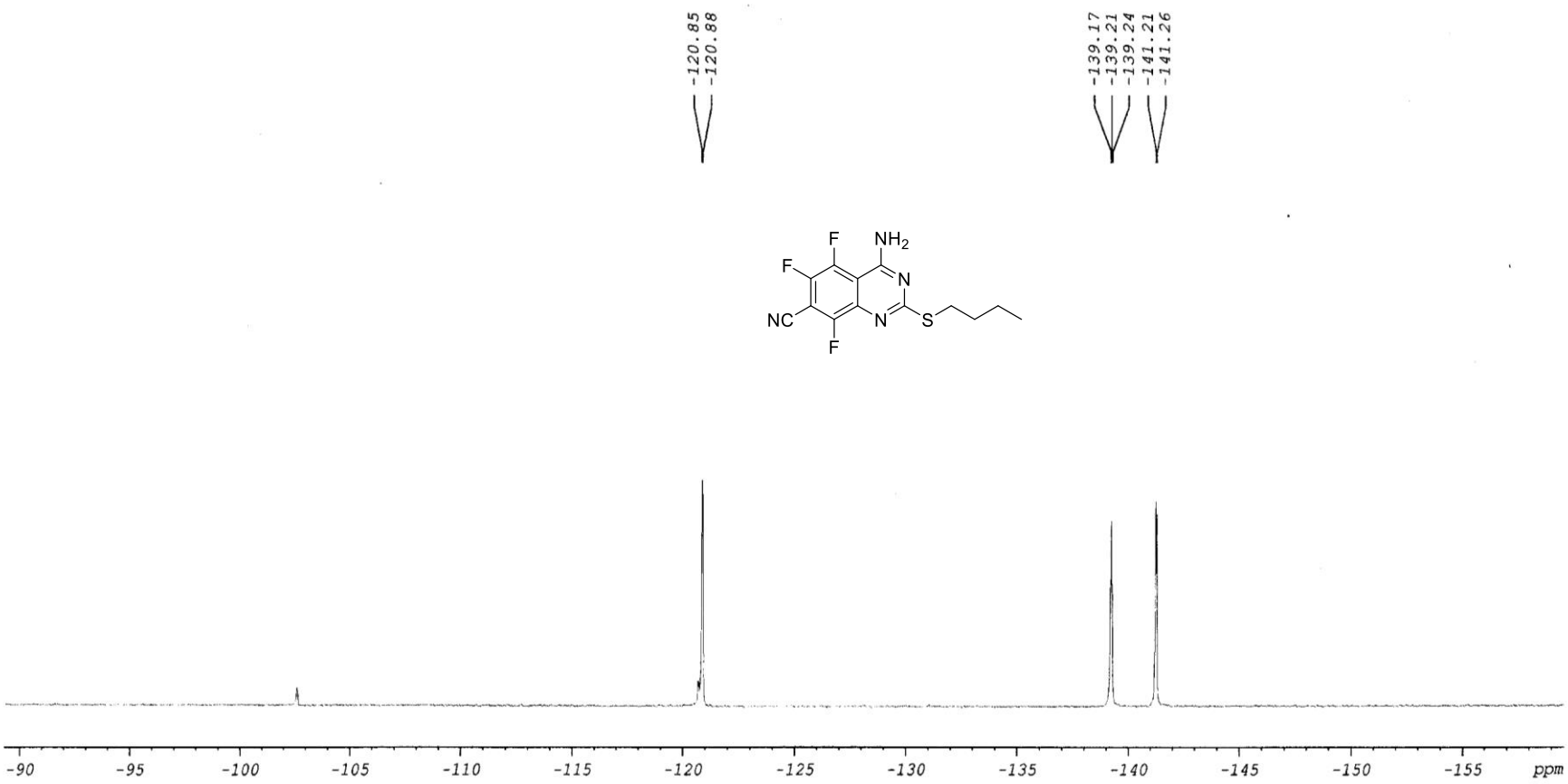

Figure S53. 19F NMR (470 MHz, DMSO-d_6) spectra of compound **3y**

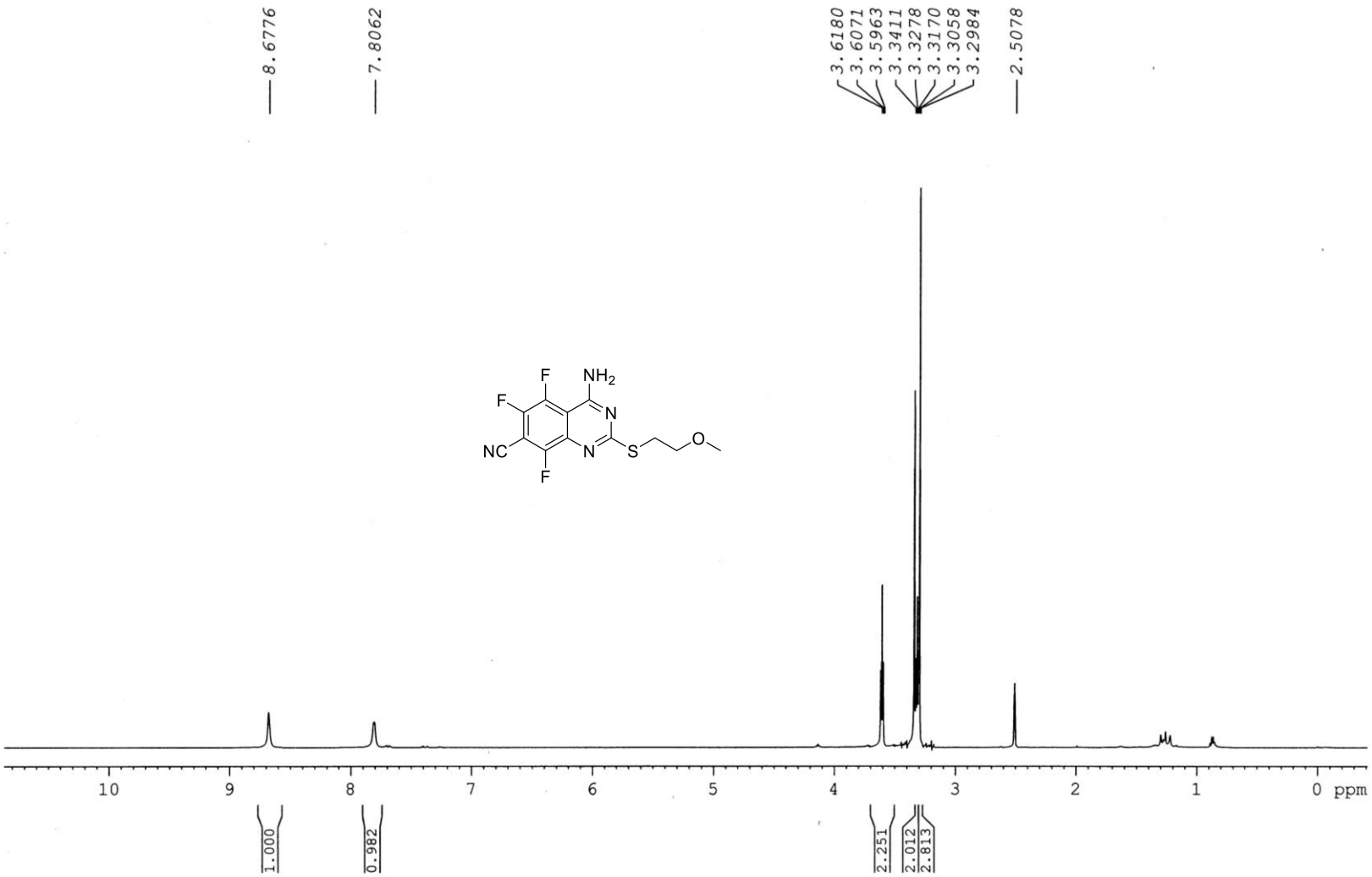

Figure S54. ^{1}H NMR (600 MHz, DMSO-d_6) spectra of compound **3z**

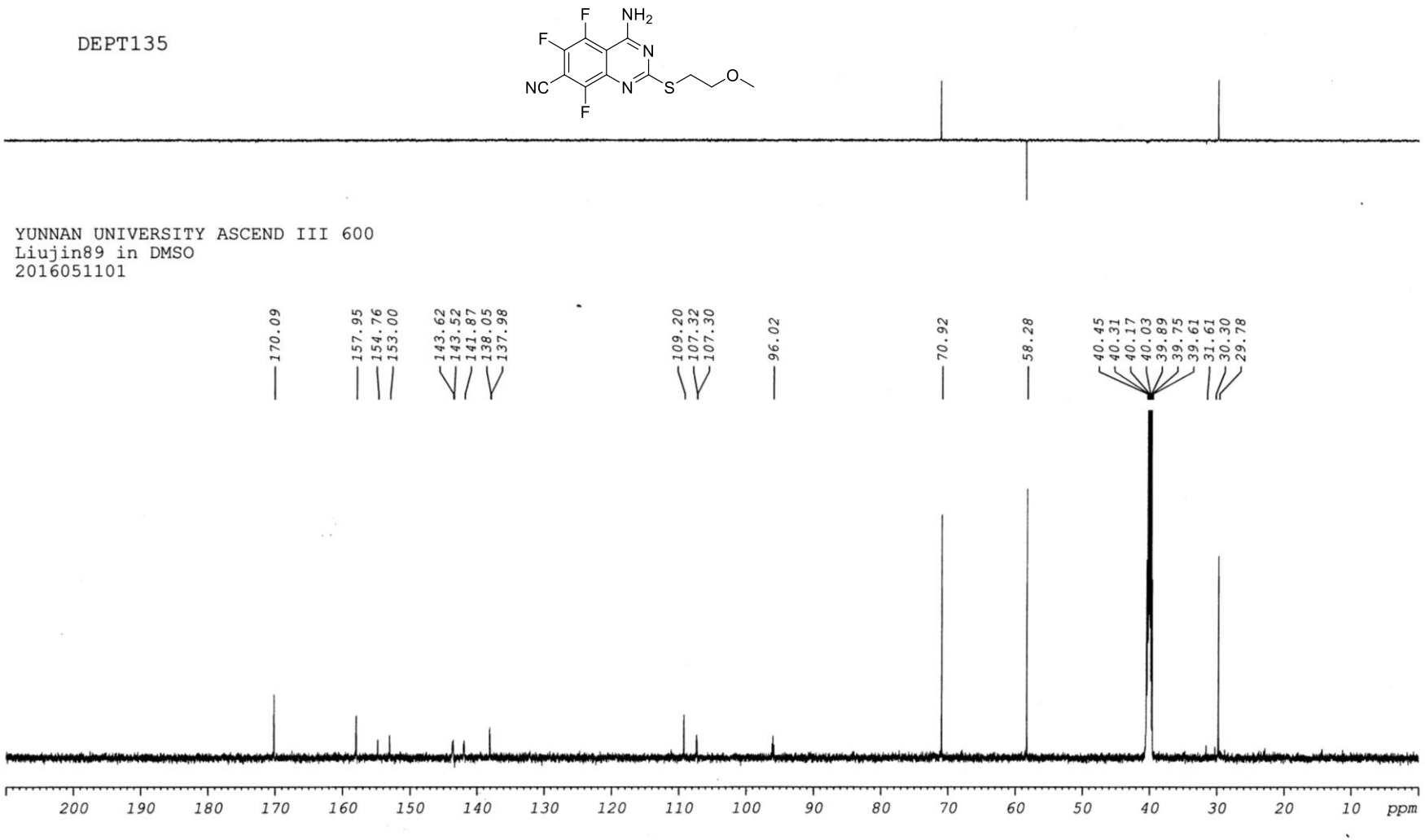

Figure S55. ^{13}C NMR (150 MHz, DMSO-d_6) spectra of compound **3z**

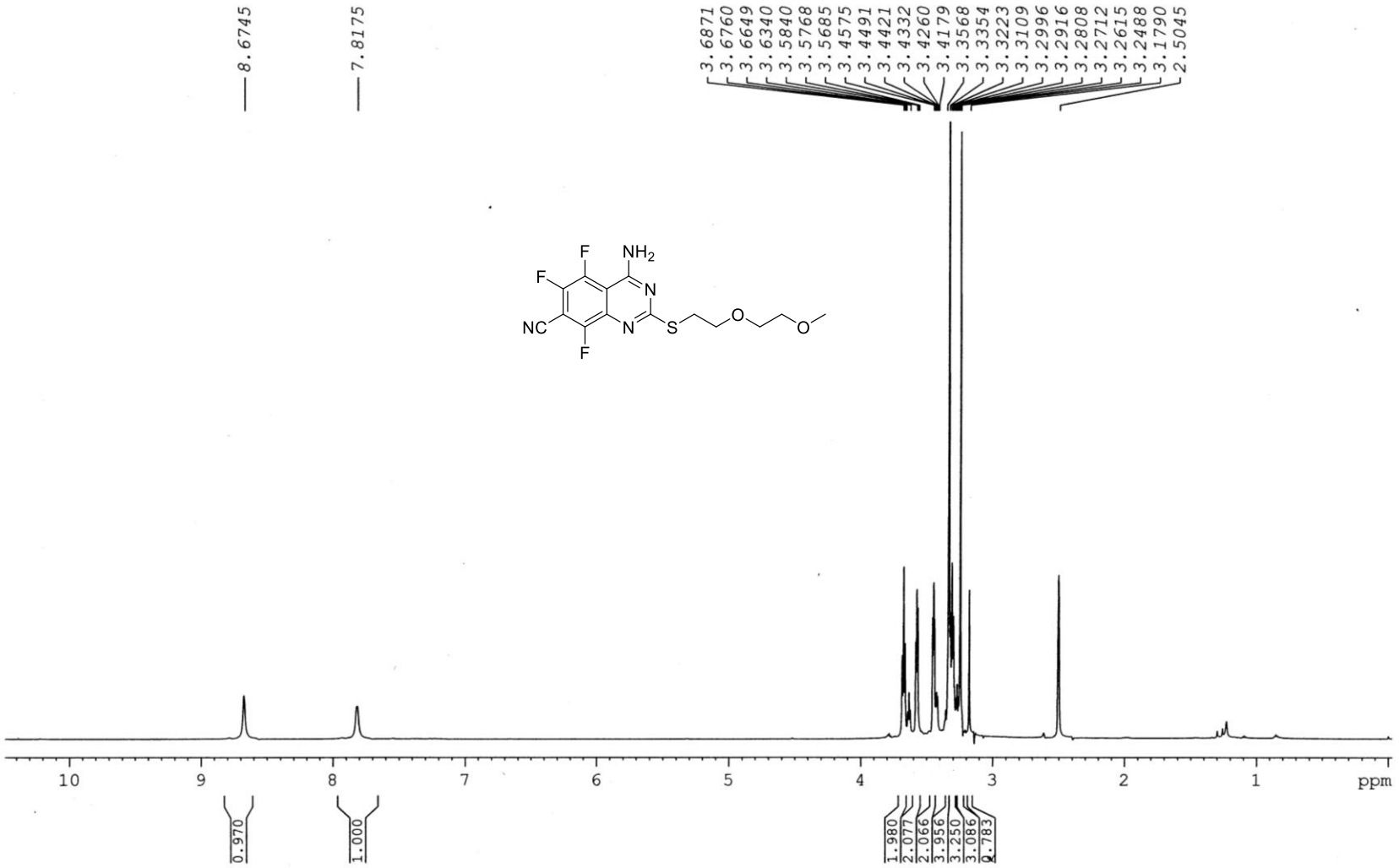

Figure S56. ^{1}H NMR (600 MHz, DMSO-d_6) spectra of compound **3a'**

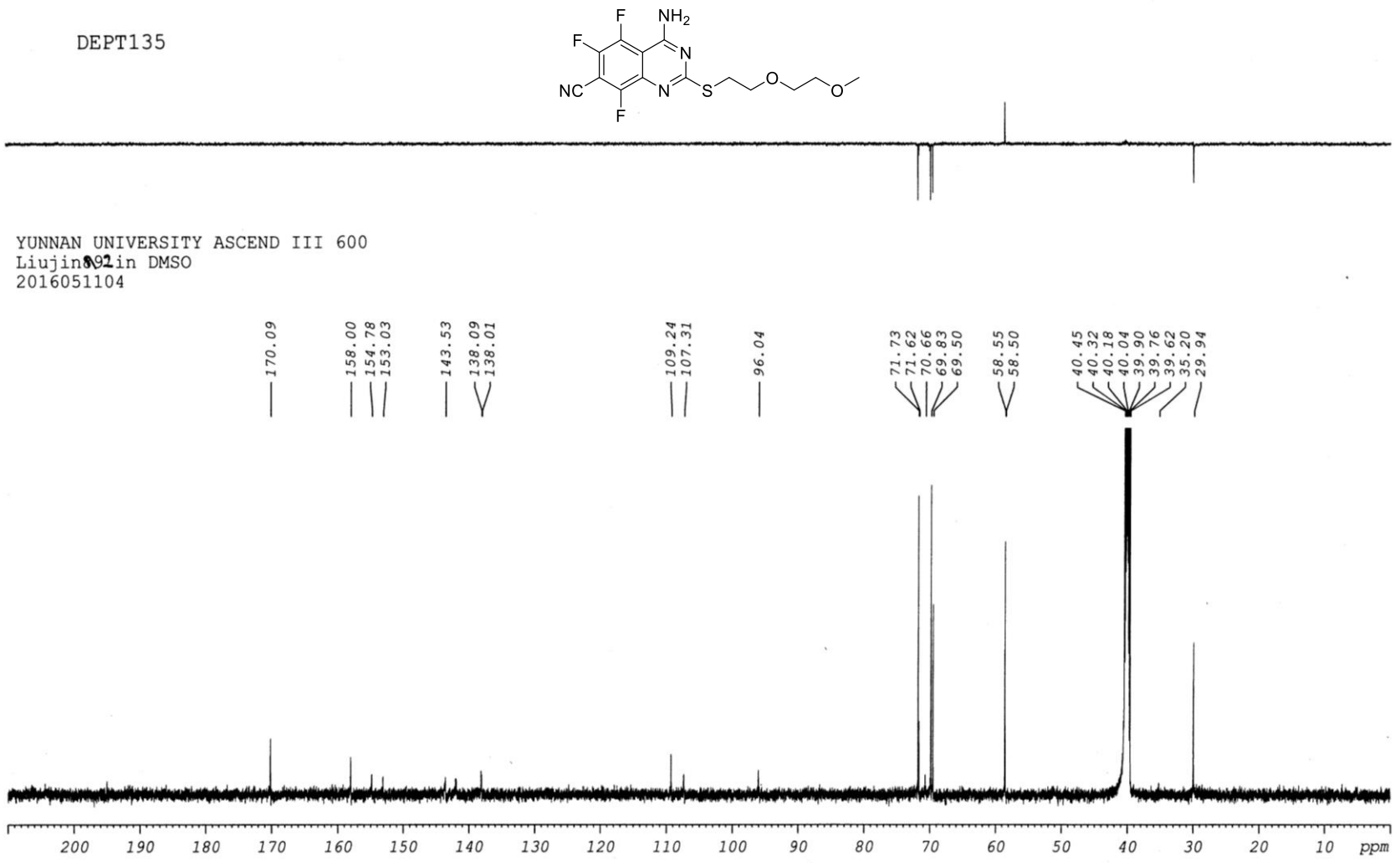

Figure S57. ^{13}C NMR (150 MHz, DMSO-d_6) spectra of compound **3a'**

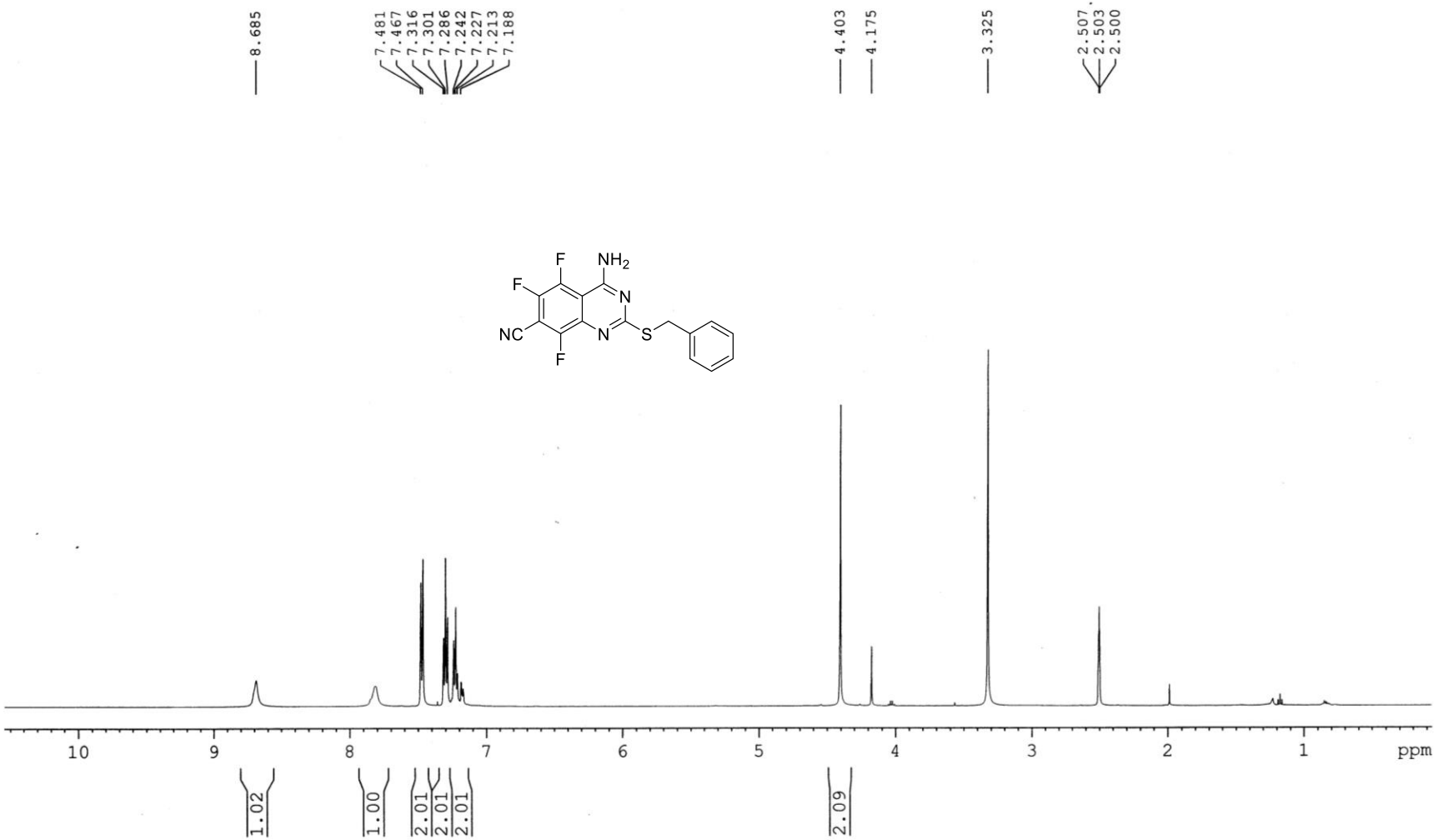

Figure S58. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3b'**

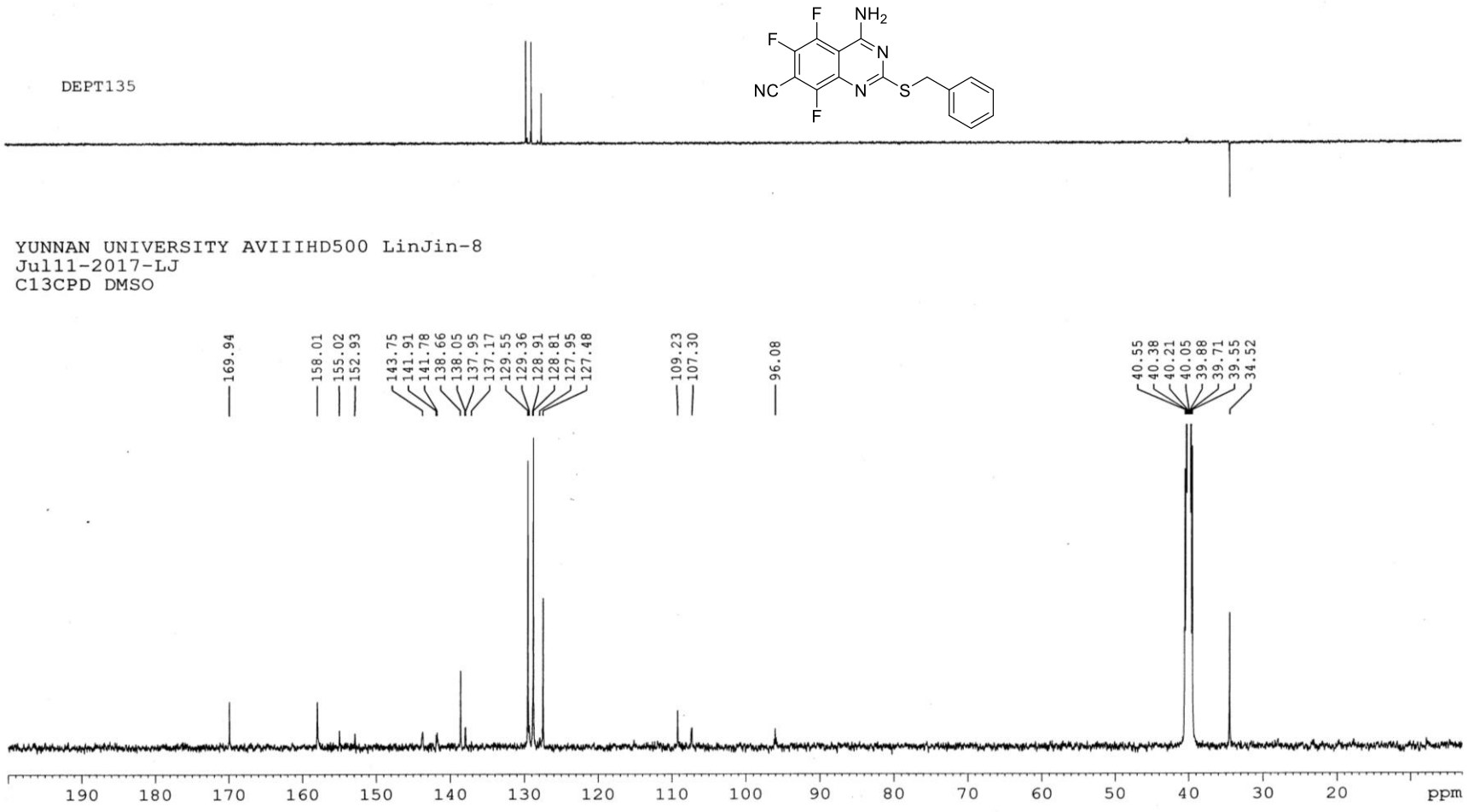

Figure S59. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3b'**

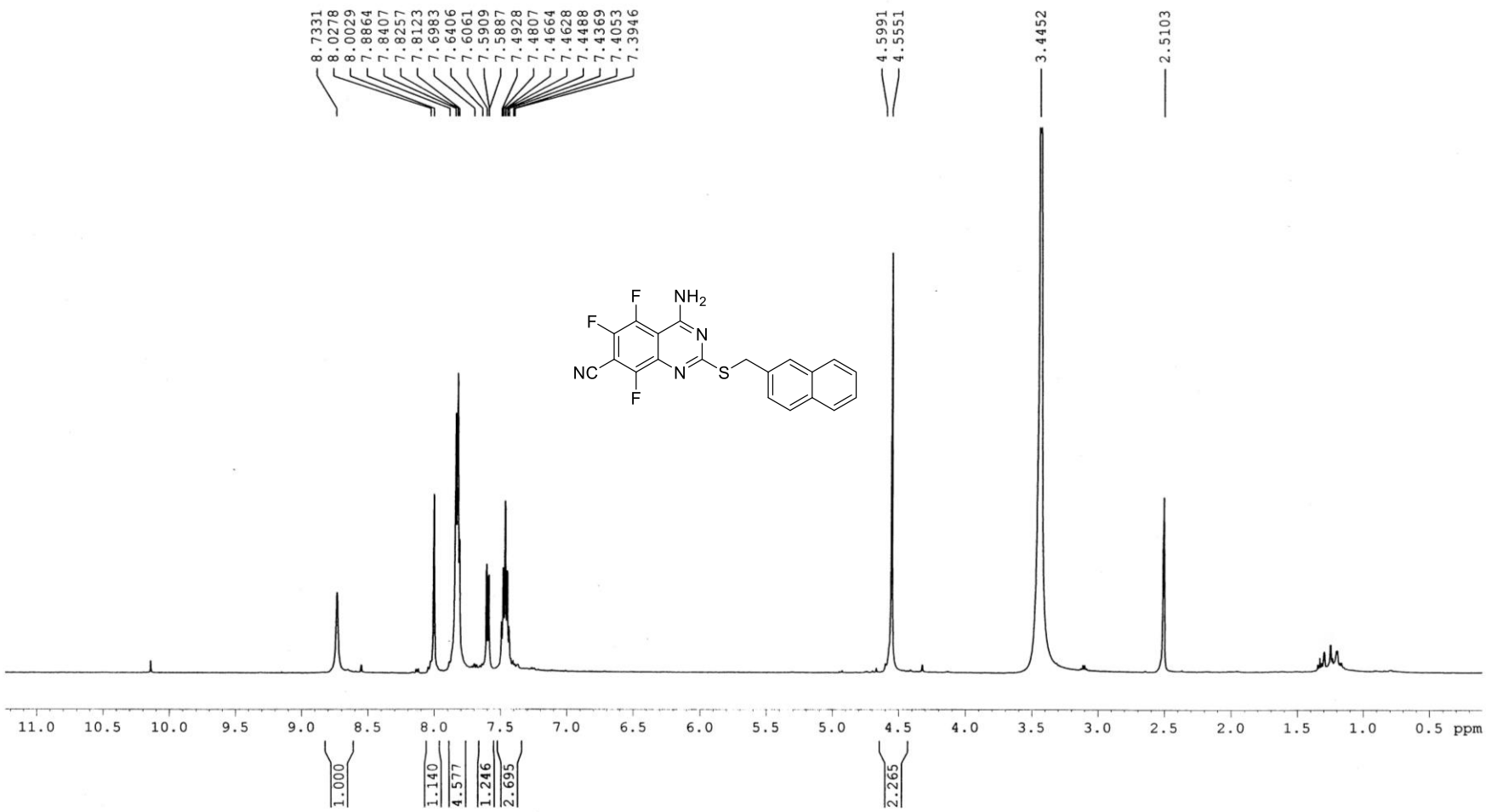

Figure S60. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of compound **3c'**

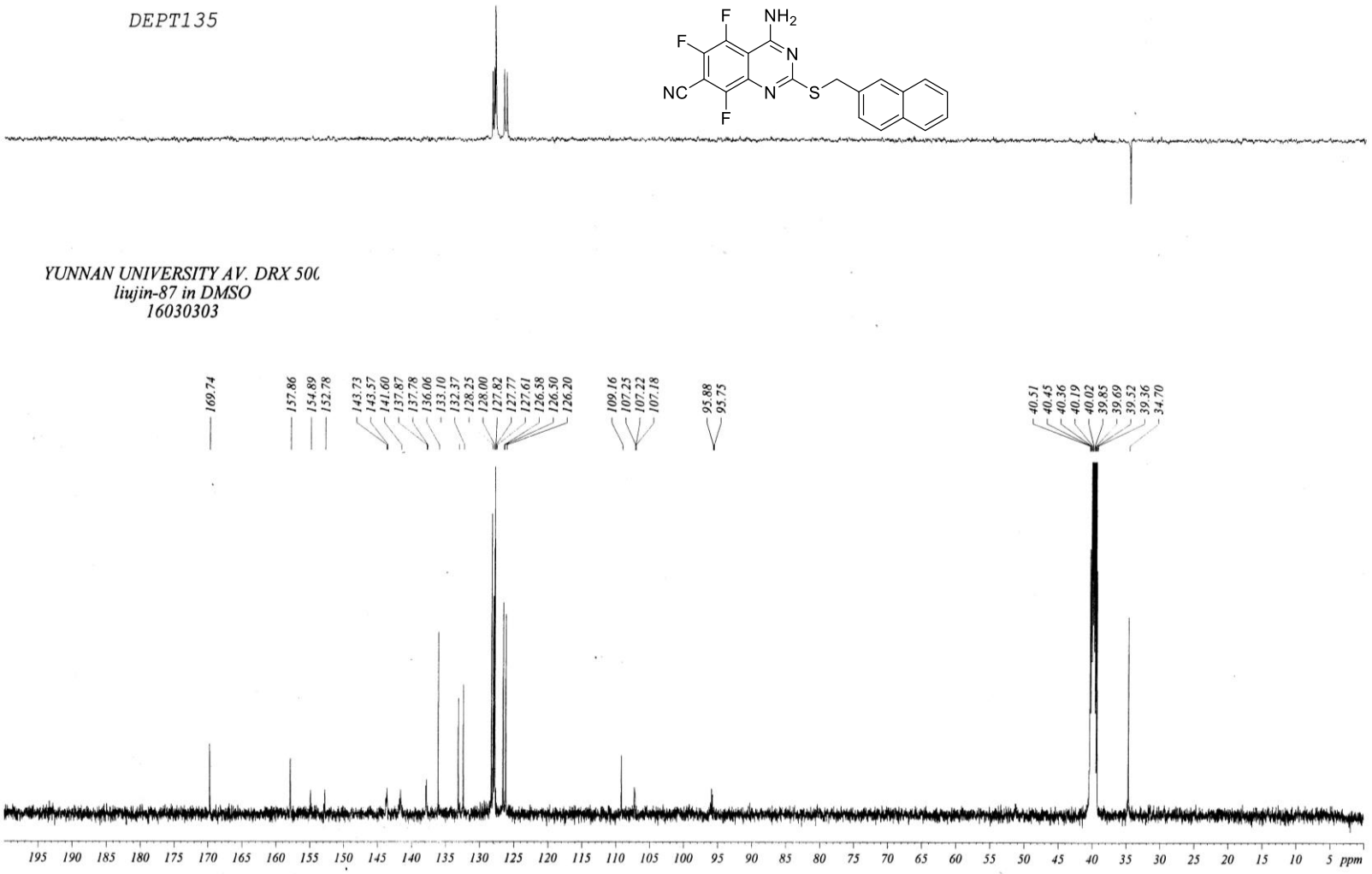

Figure S61. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of compound **3c'**

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