Supplementary Information

Identifying New Isatin Derivatives with GSK-3β Inhibition Capacity through Molecular Docking and Bioassays

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[#]Both authors contributed equally to this work.

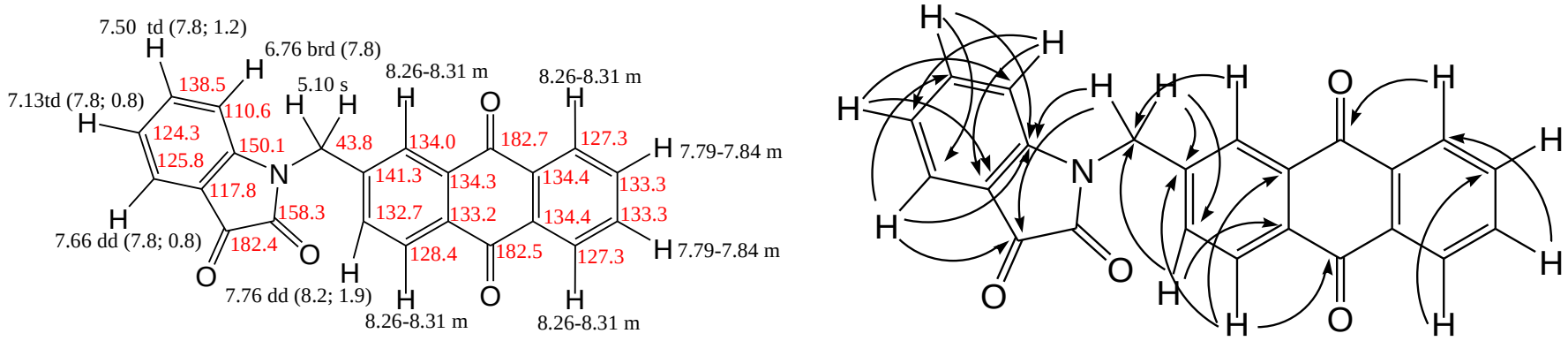


Figure S1. ^{1}H (black) and ^{13}C (red) NMR chemical shifts (ppm) and main heteronuclear multiple bond correlation (HMBC) correlation observed in the NMR data of the compound **2a**. *J* in Hz.

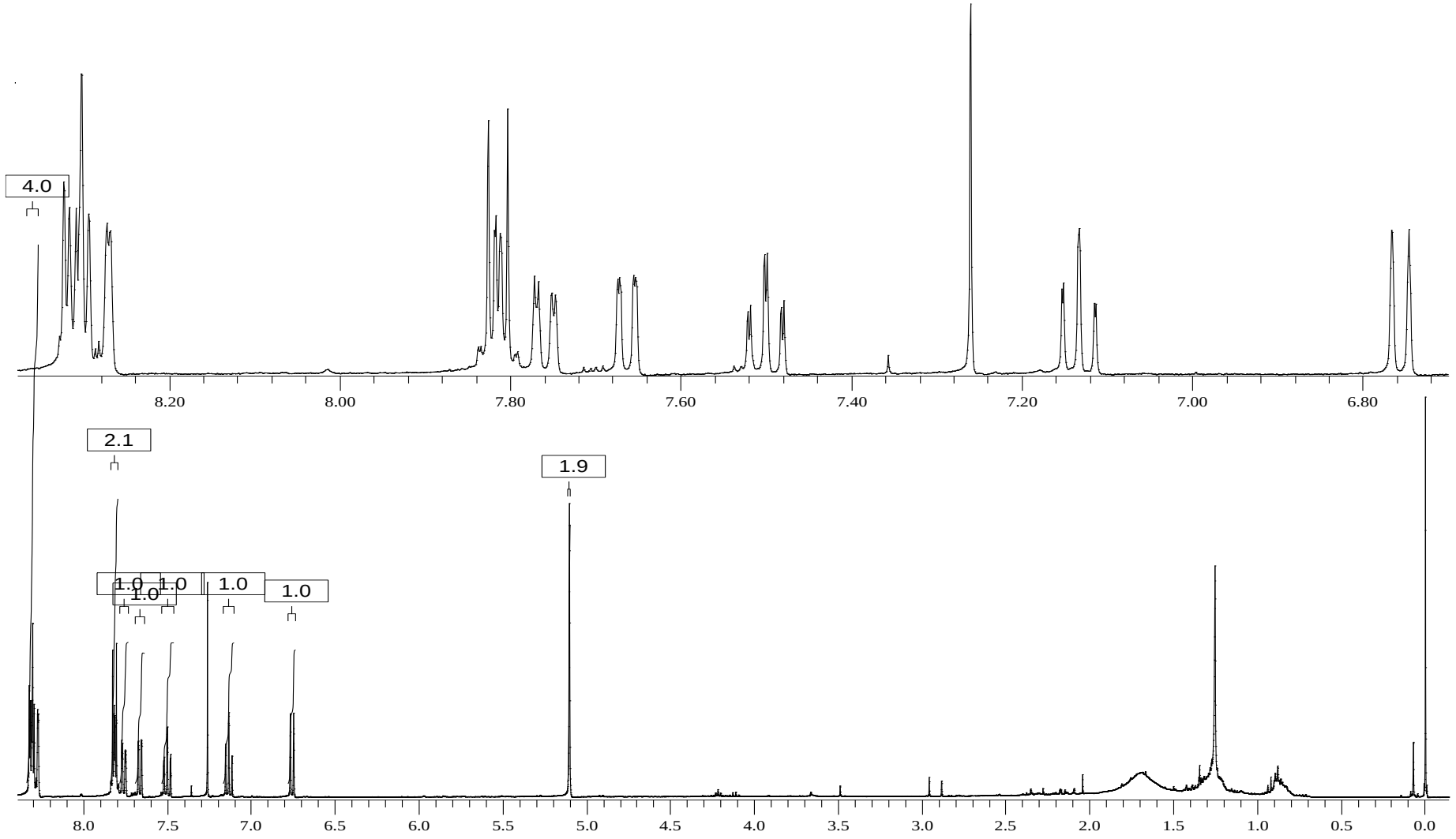


Figure S2. ^{1}H nuclear magnetic resonance (NMR) spectrum (400 MHz, $CDCl_3$) of compound **2a**.

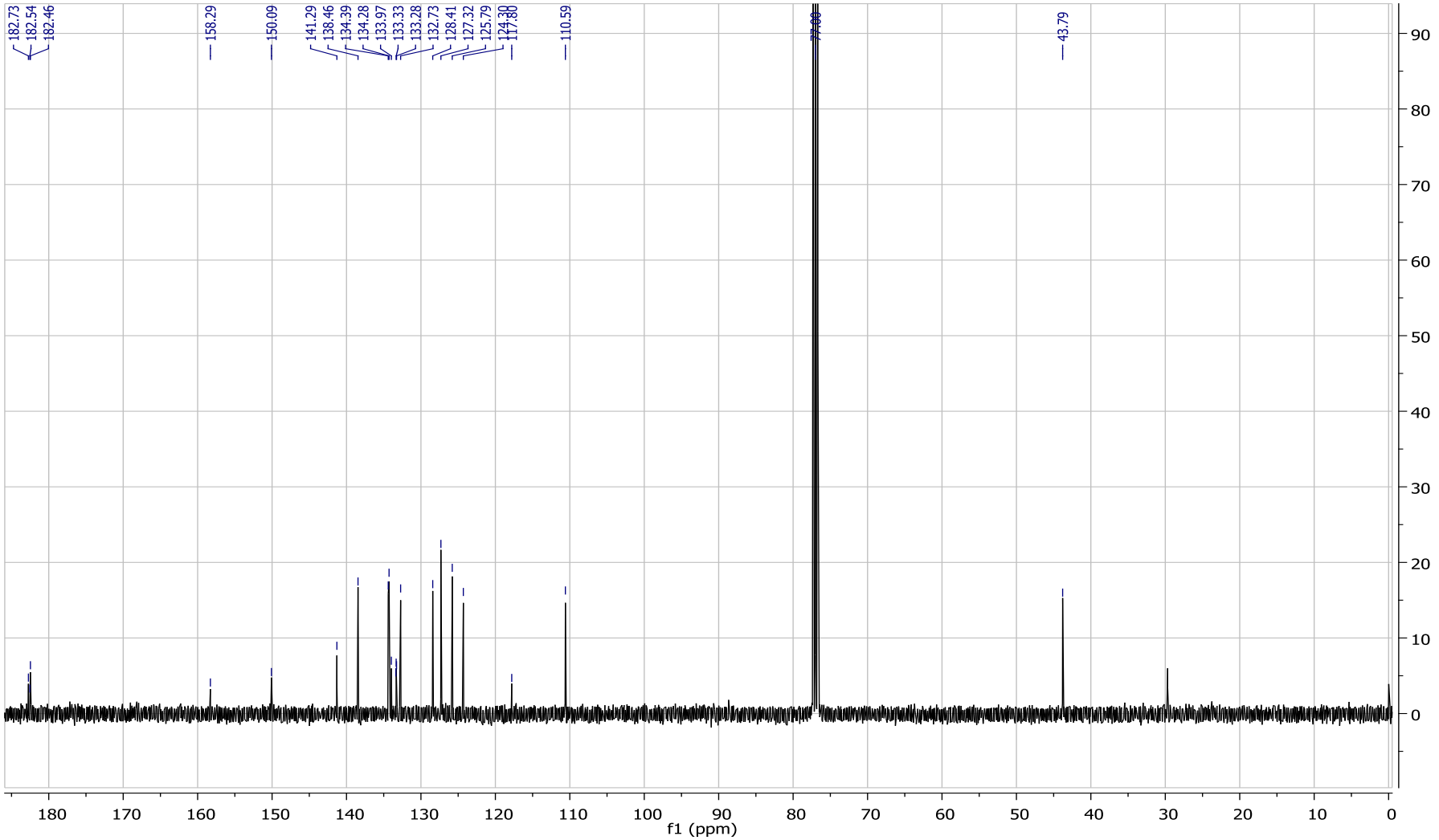


Figure S3. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **2a**.

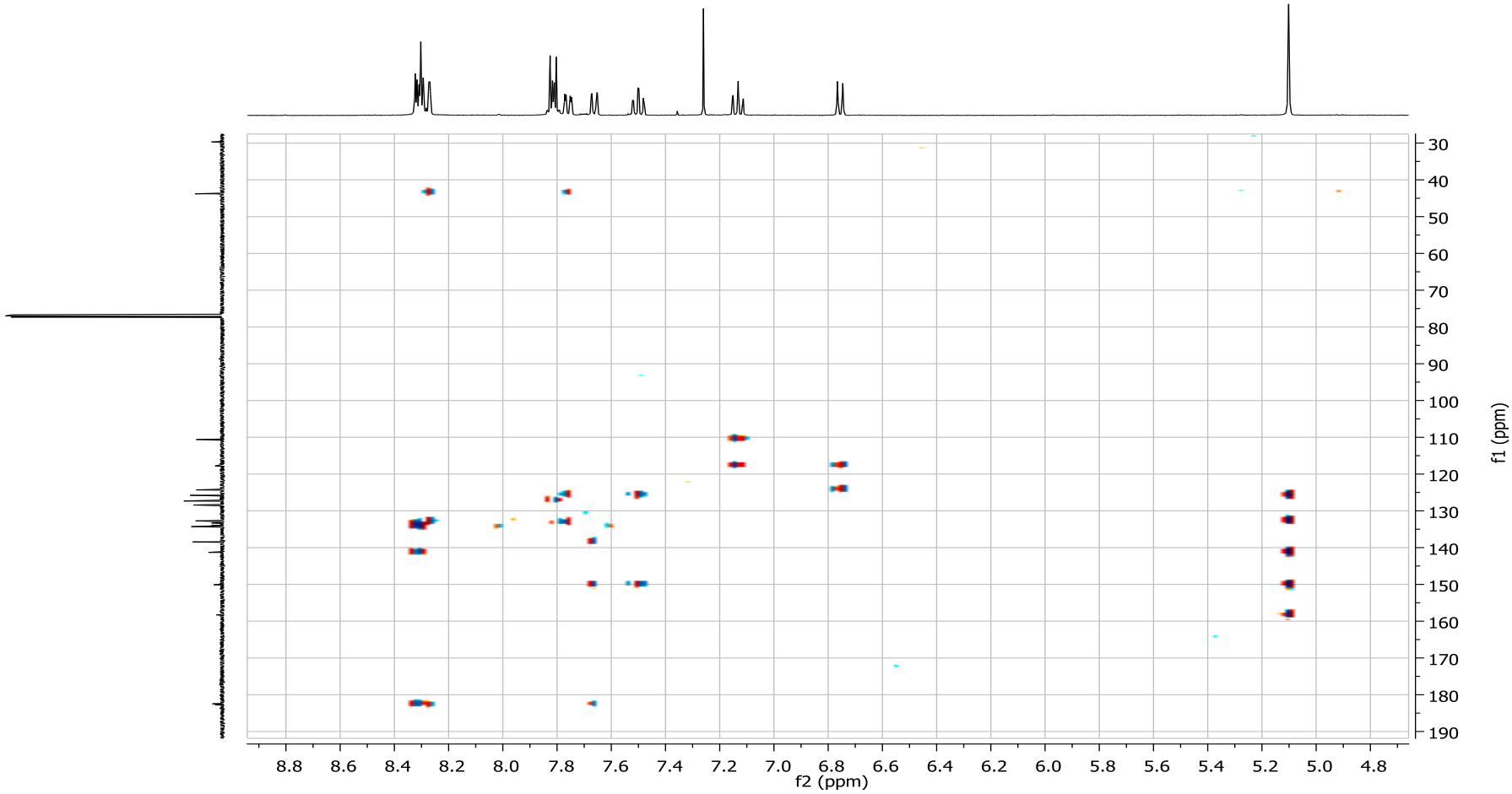


Figure S4. HMBC spectrum (400 MHz, $CDCl_3$) of compound **2a**.

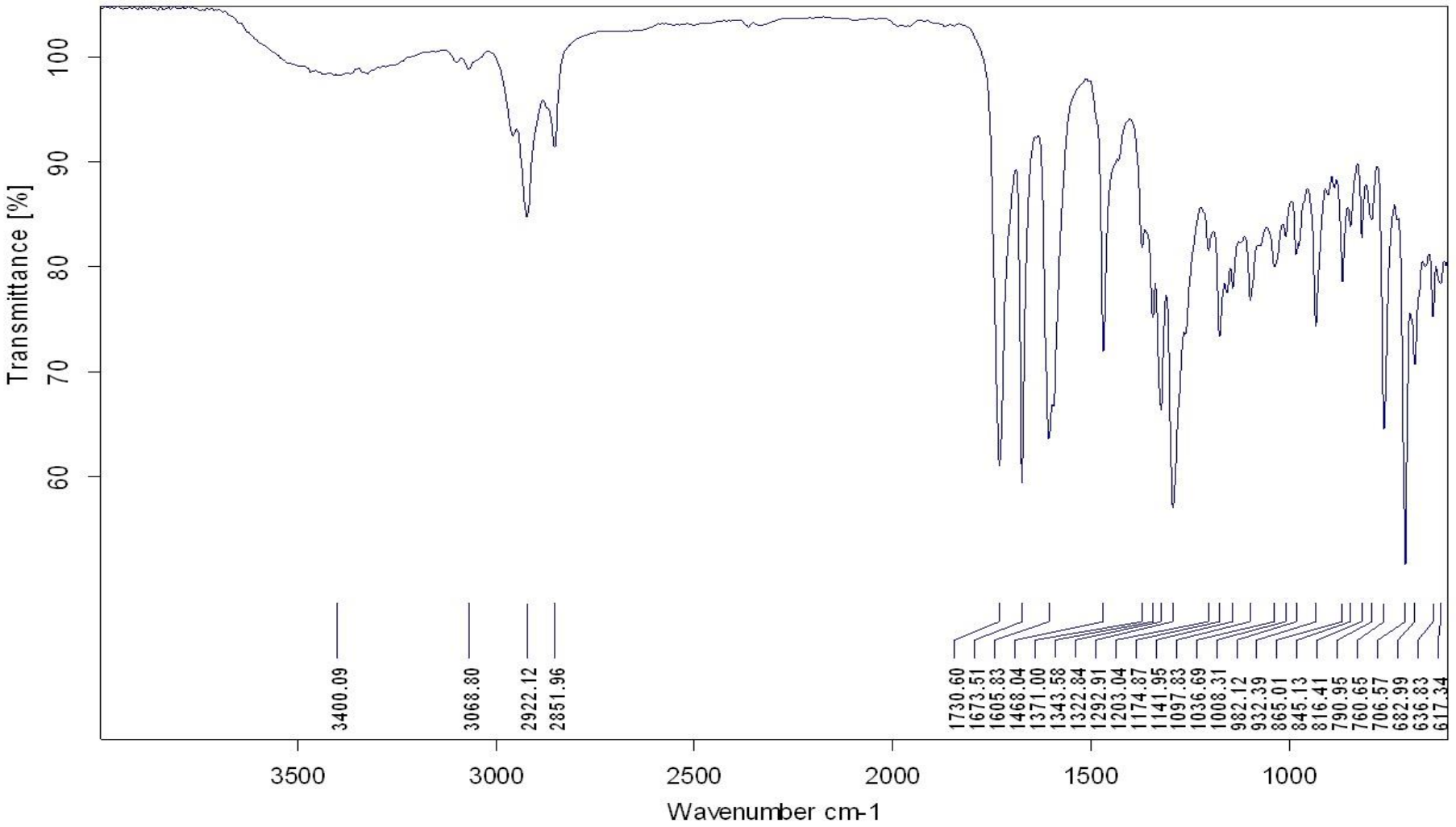


Figure S5. Attenuated total reflection infrared (ATR-IR) spectrum of compound **2a**.

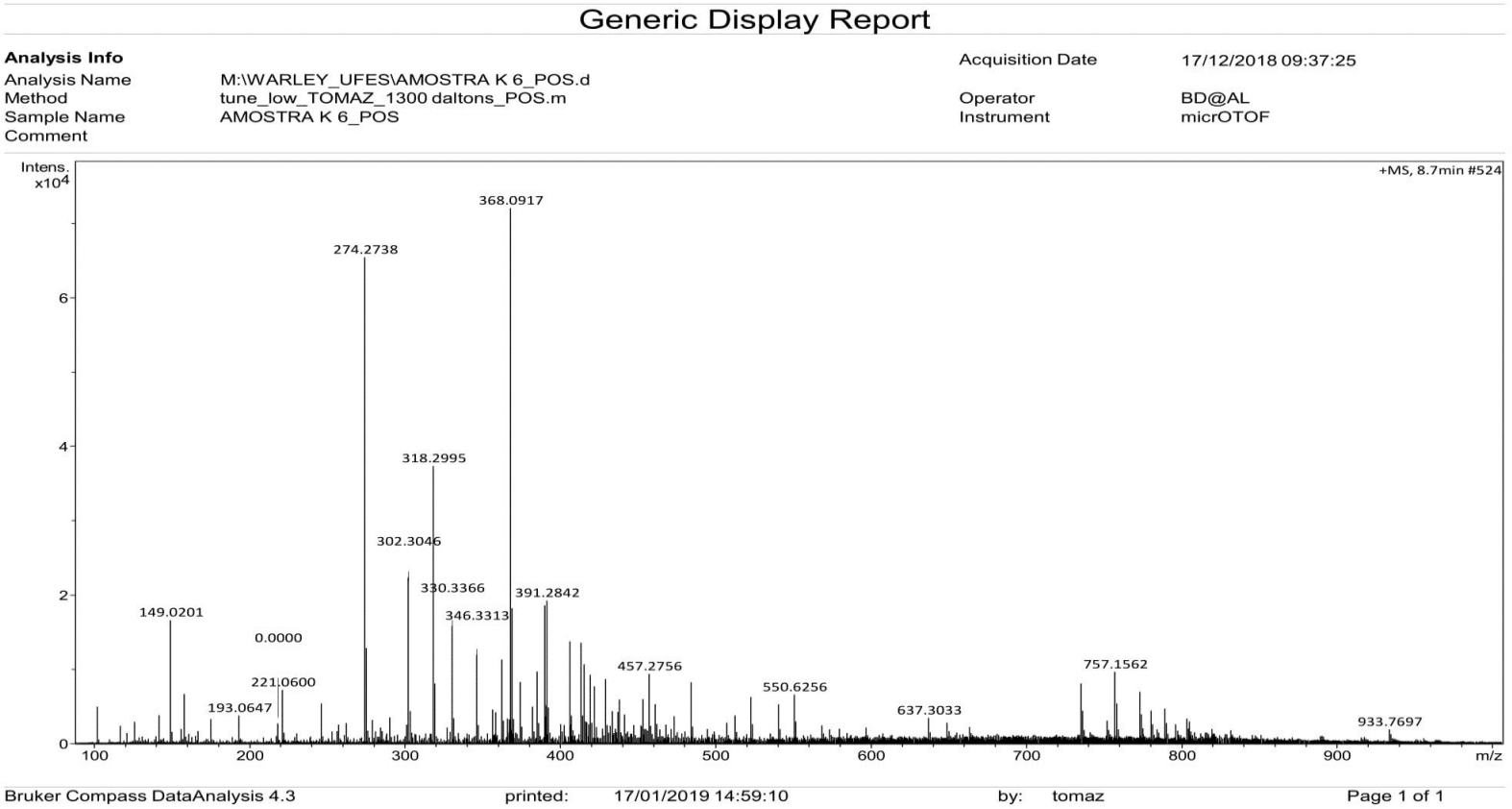


Figure S6. HRMS spectrum of compound **2a**.

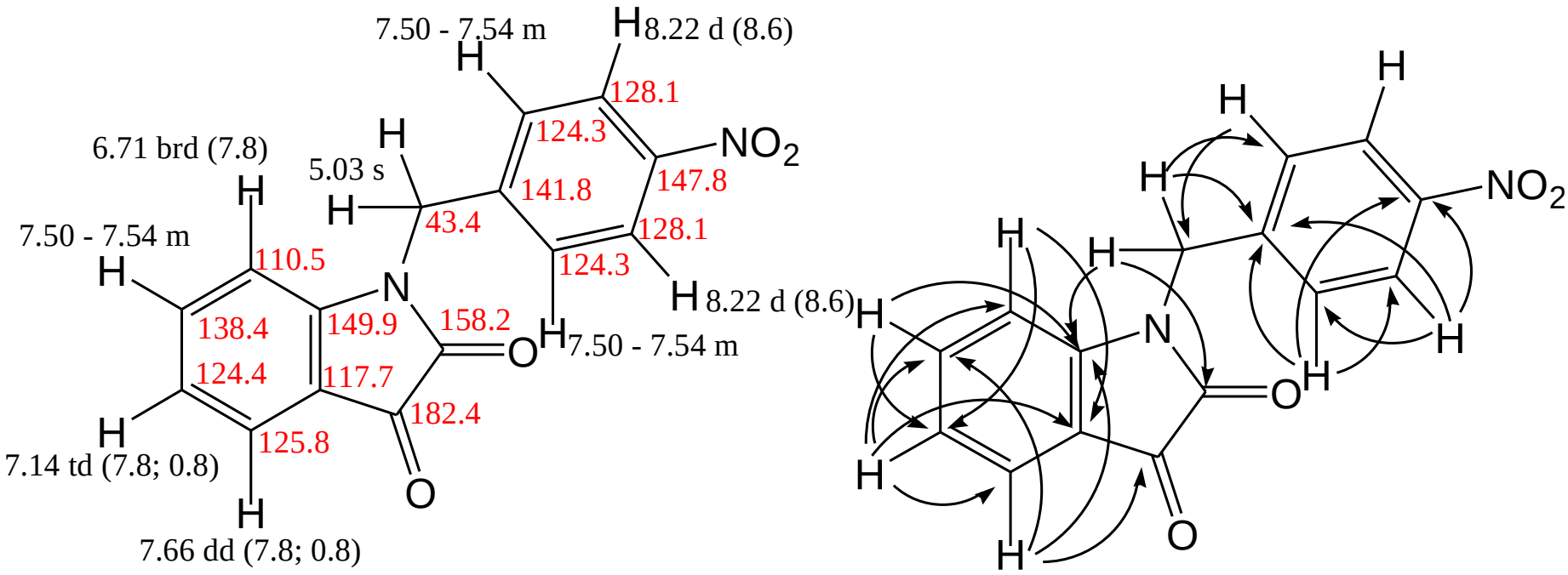


Figure S7. ^{1}H (black) and ^{13}C (red) NMR chemical shifts (ppm) and main HMBC correlation observed in the NMR data of the compound **2b**. *J* in Hz.

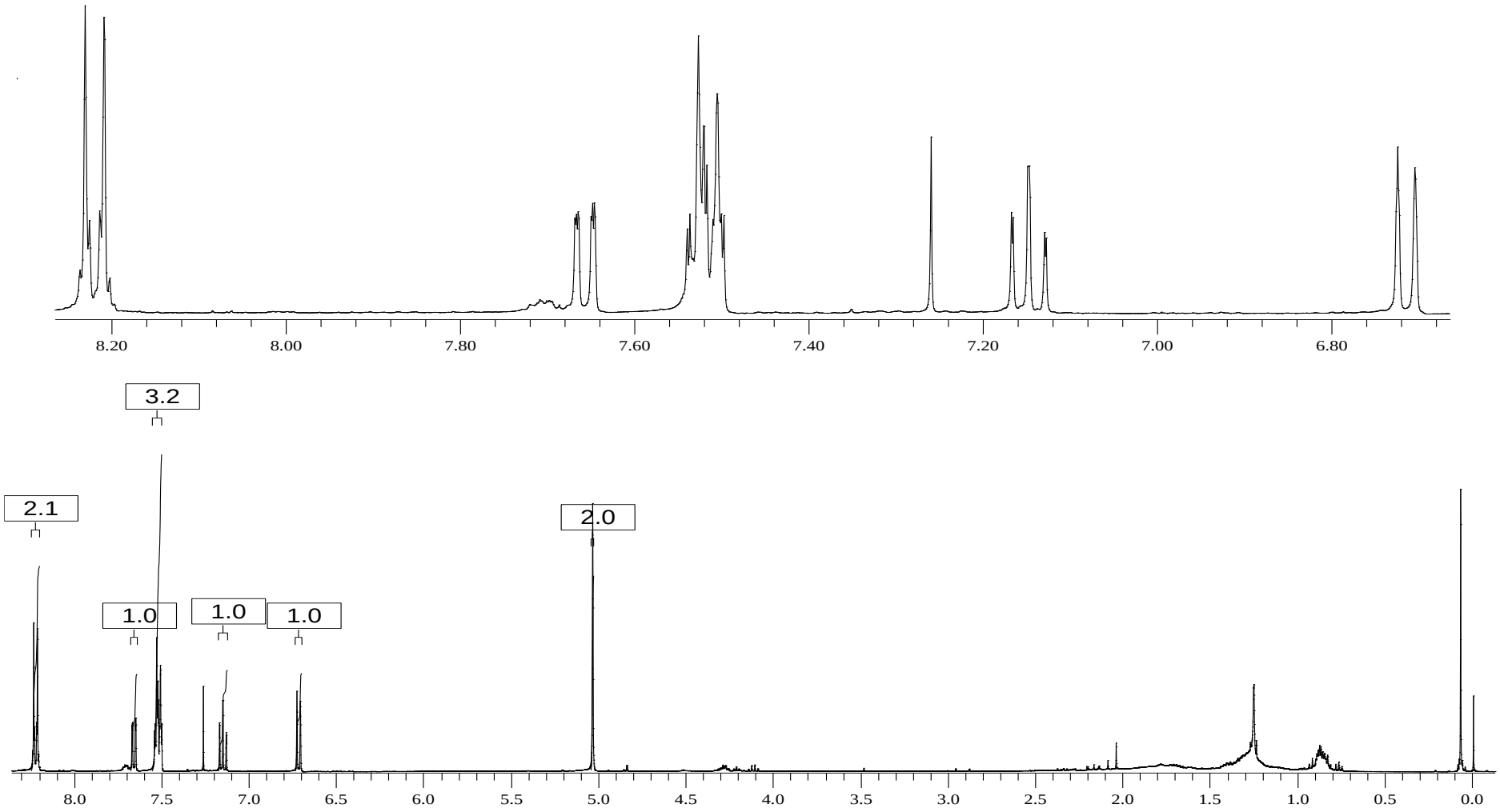


Figure S8. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **2b**.

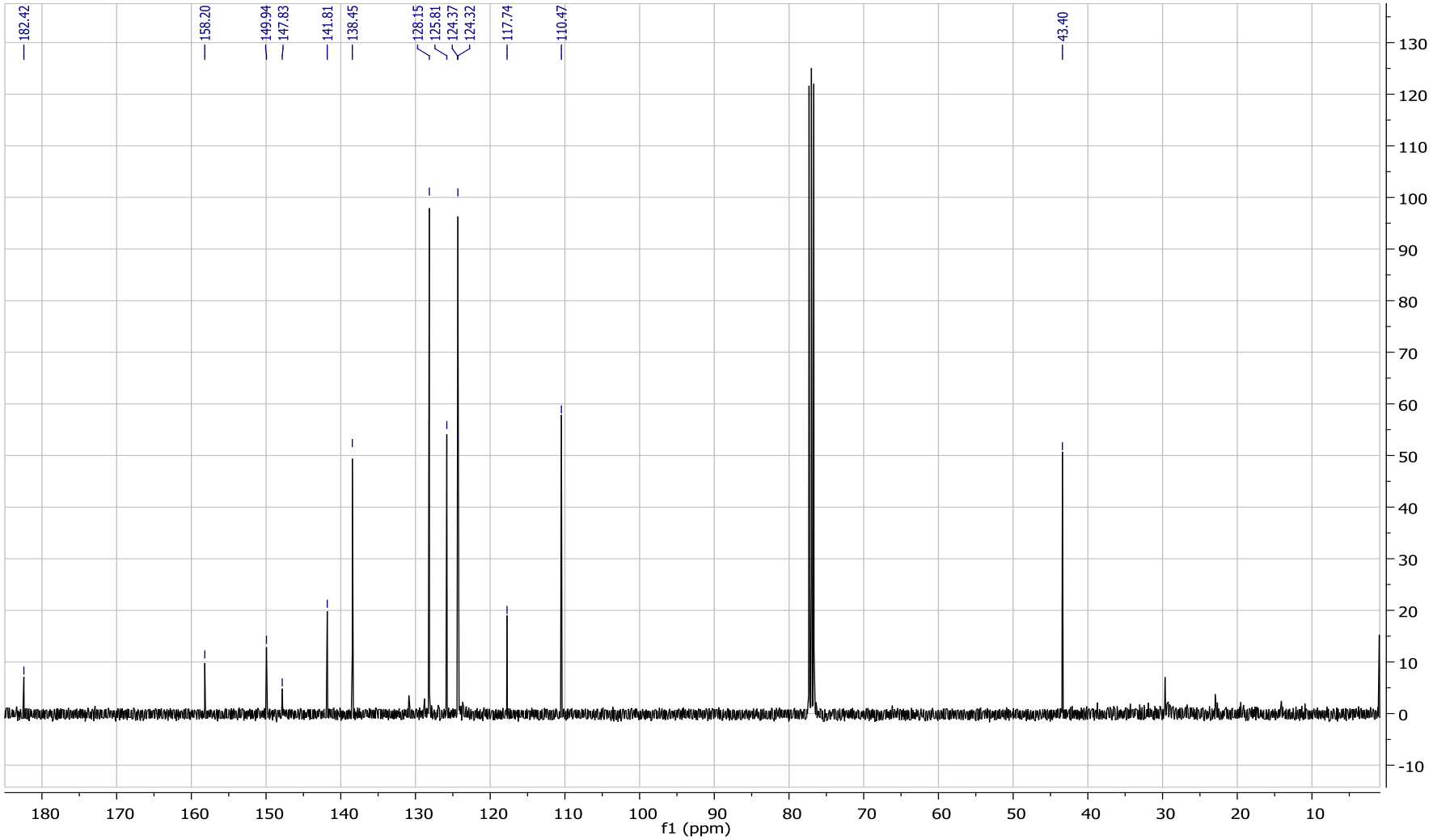


Figure S9. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **2b**.

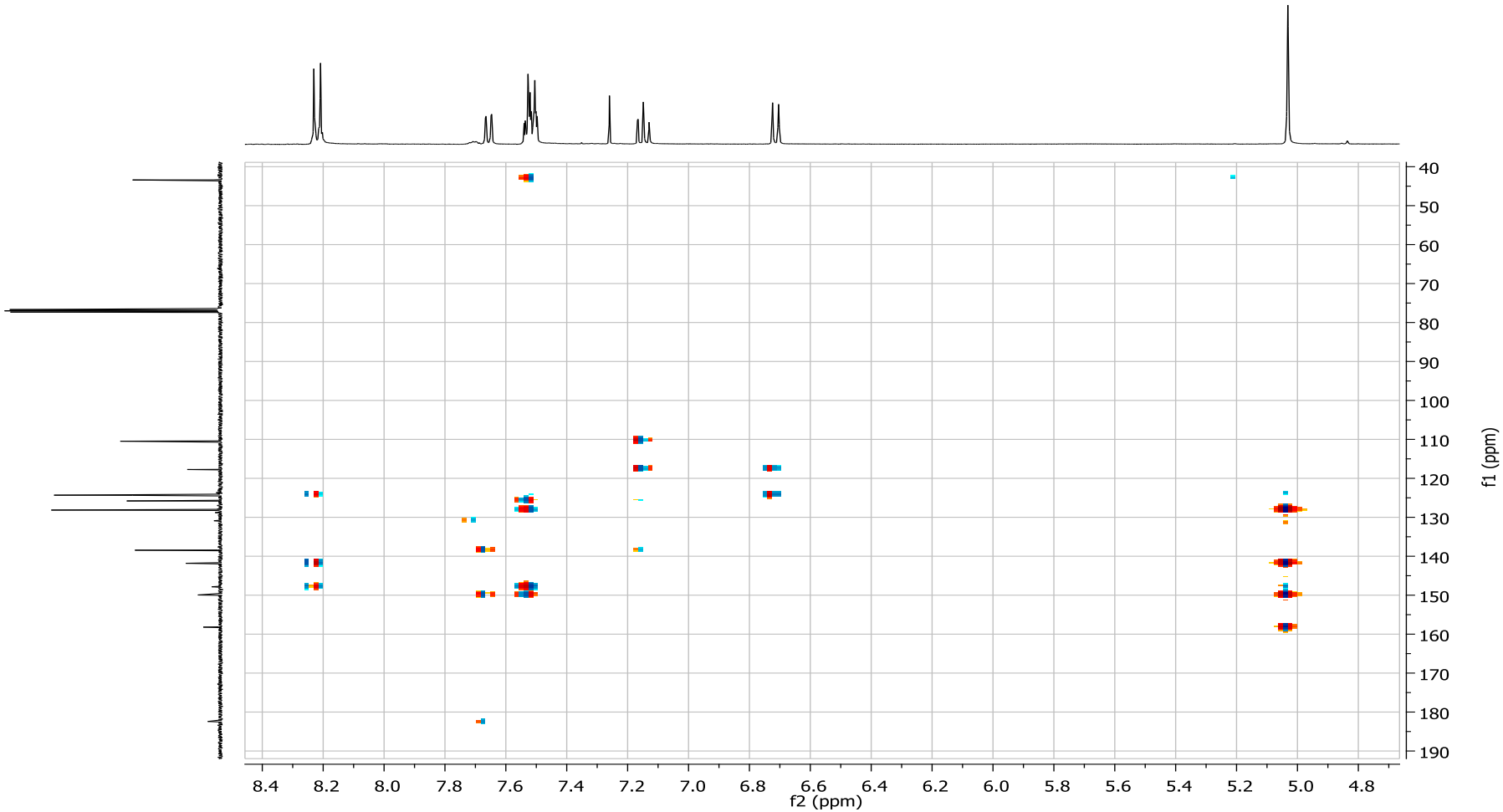


Figure S10. HMBC spectrum (400 MHz, $CDCl_3$) of compound **2b**.

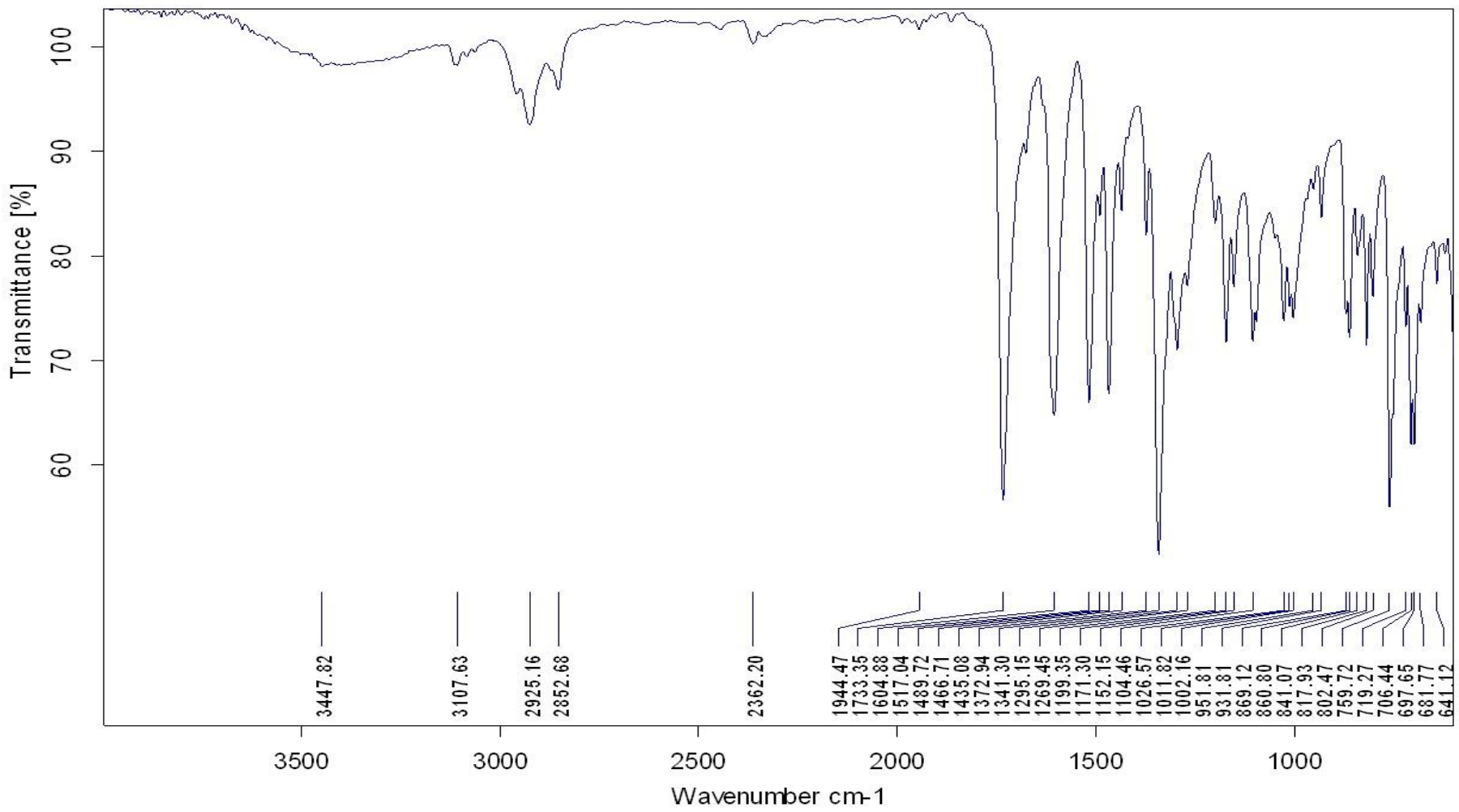


Figure S11. ATR-IR spectrum of compound **2b**.

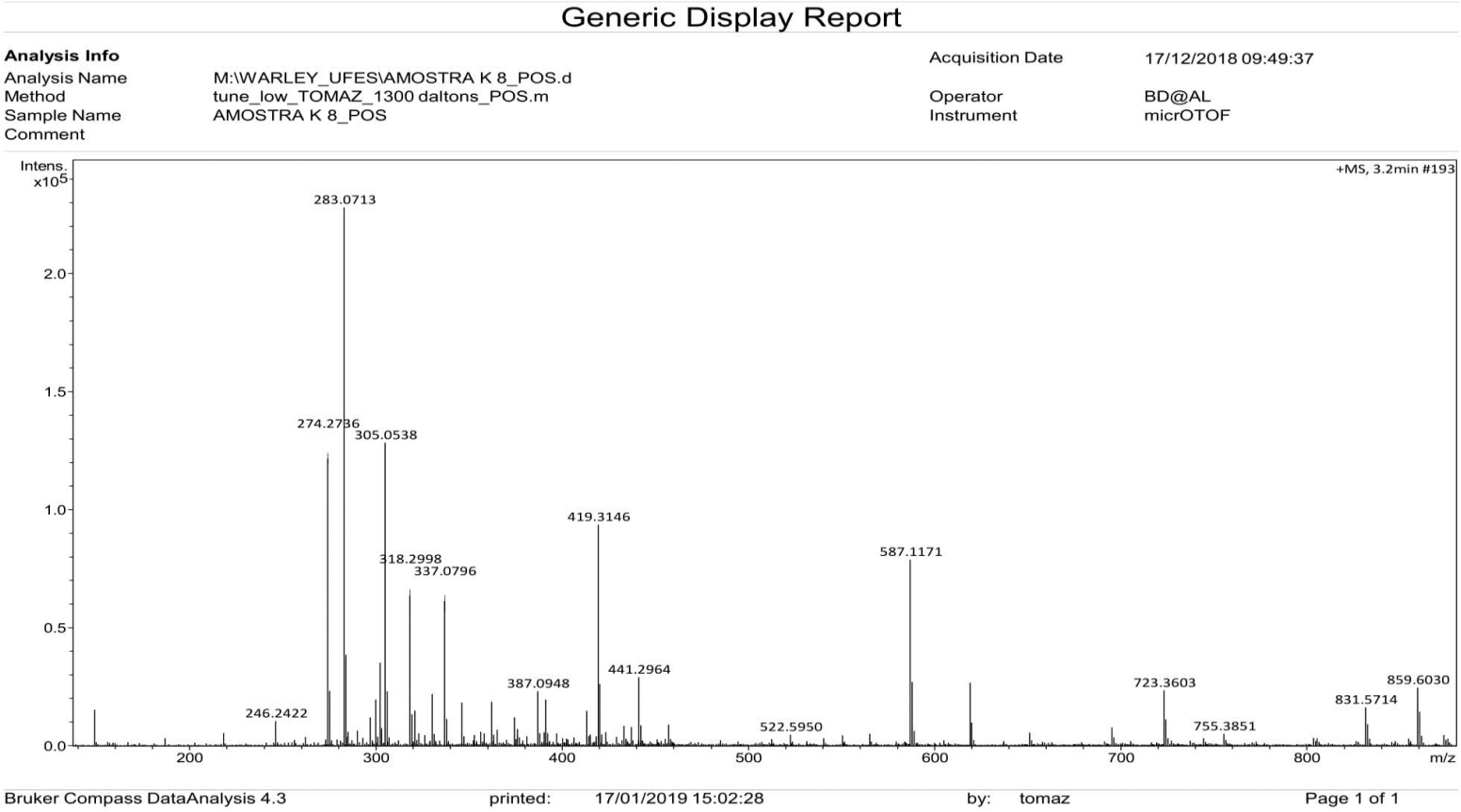


Figure S12. HRMS spectrum of compound **2b**.

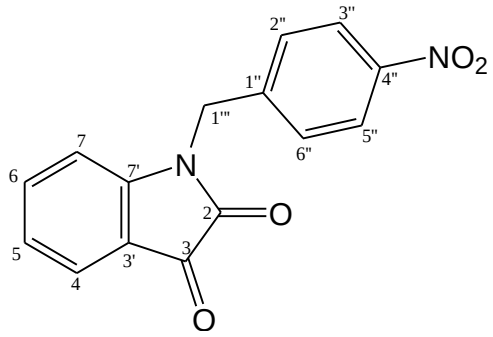


Table S1. NMR data of **2b** and comparison with literature (*J* in Hz)

Position	**2b** (400 MHz, $CDCl_3$)		Furdas *et al.*[1] (400 MHz, DMSO-d_6)		Makhija *et al.*[2] (500 MHz, $CDCl_3$)	Gui *et al.*[3] (300 MHz, DMSO-d_6)	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{1}H	^{13}C
2		158.2		158.9			158.5
3		182.4		183.1			182.7
3’		117.7		118.4			117.9
4	7.66 dd (7.8; 0.8)	125.8	7.61-7.56 m	124.9	7.67 d	7.54-7.60 m	124.5
5	7.14 td (7.8; 0.8)	124.4	7.14 t (7.3)	123.9	7.15 t	7.14 t (7.5)	123.7
6	7.50-7.54 m	138.4	7.61-7.56 m	138.3	7.53 t	7.54-7.60 m	137.9
7	6.71 brd (7.8)	110.5	6.95 dd (7.9; 0.4)	111.3	6.72 d	6.94 d (7.8)	110.9
7’		149.9		147.4			149.9
1’’		141.8		150.4			143.5
2’’; 6’’	7.50-7.54 m	124.3	7.74 d (8.4)	128.9	7.53 m	7.73 d (8.7)	123.5
3’’; 5’’	8.22 d (8.6)	128.1	8.20 d (8.4)	124.1	8.23 d	8.19 d (8.7)	128.5
4’’		147.8		143.9			146.9
1’’’ (*Bn*–CH_2)	5.03 s	43.4	5.70 s	42.9	5.10 s		42.5

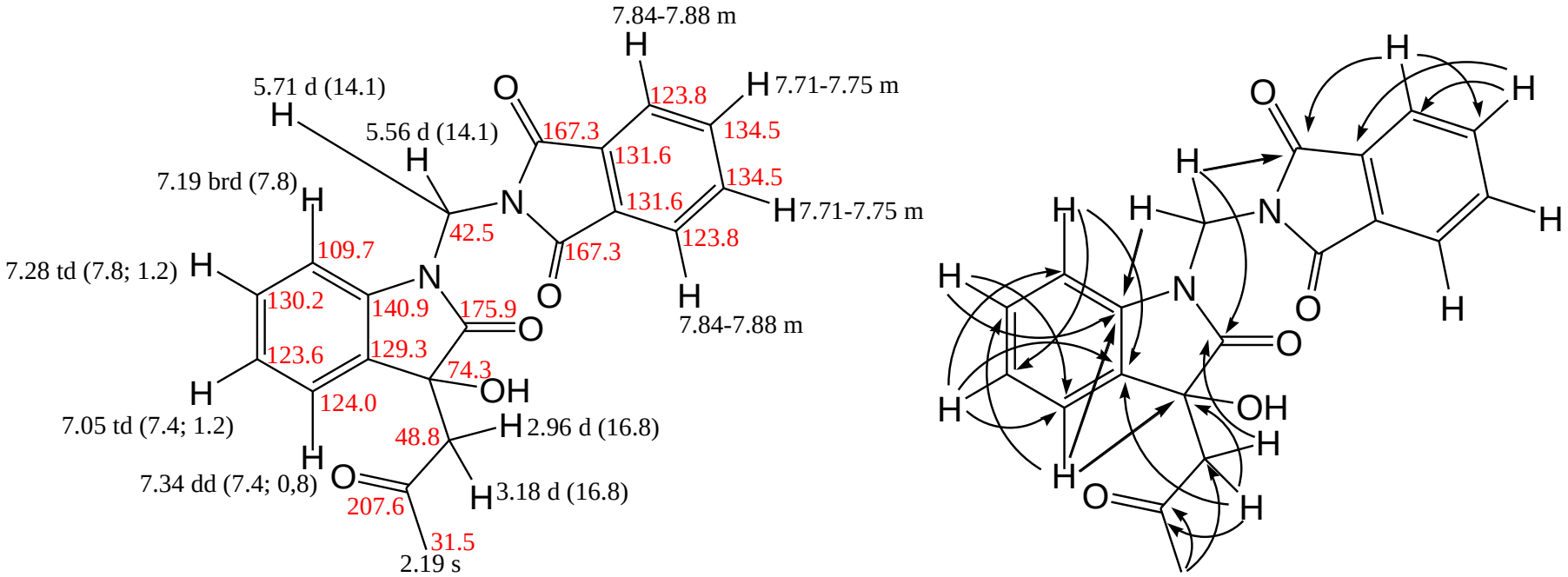


Figure S13. ^{1}H (black) and ^{13}C (red) NMR chemical shifts (ppm) and main HMBC correlation observed in the NMR data of the compound **2c**. *J* in Hz.

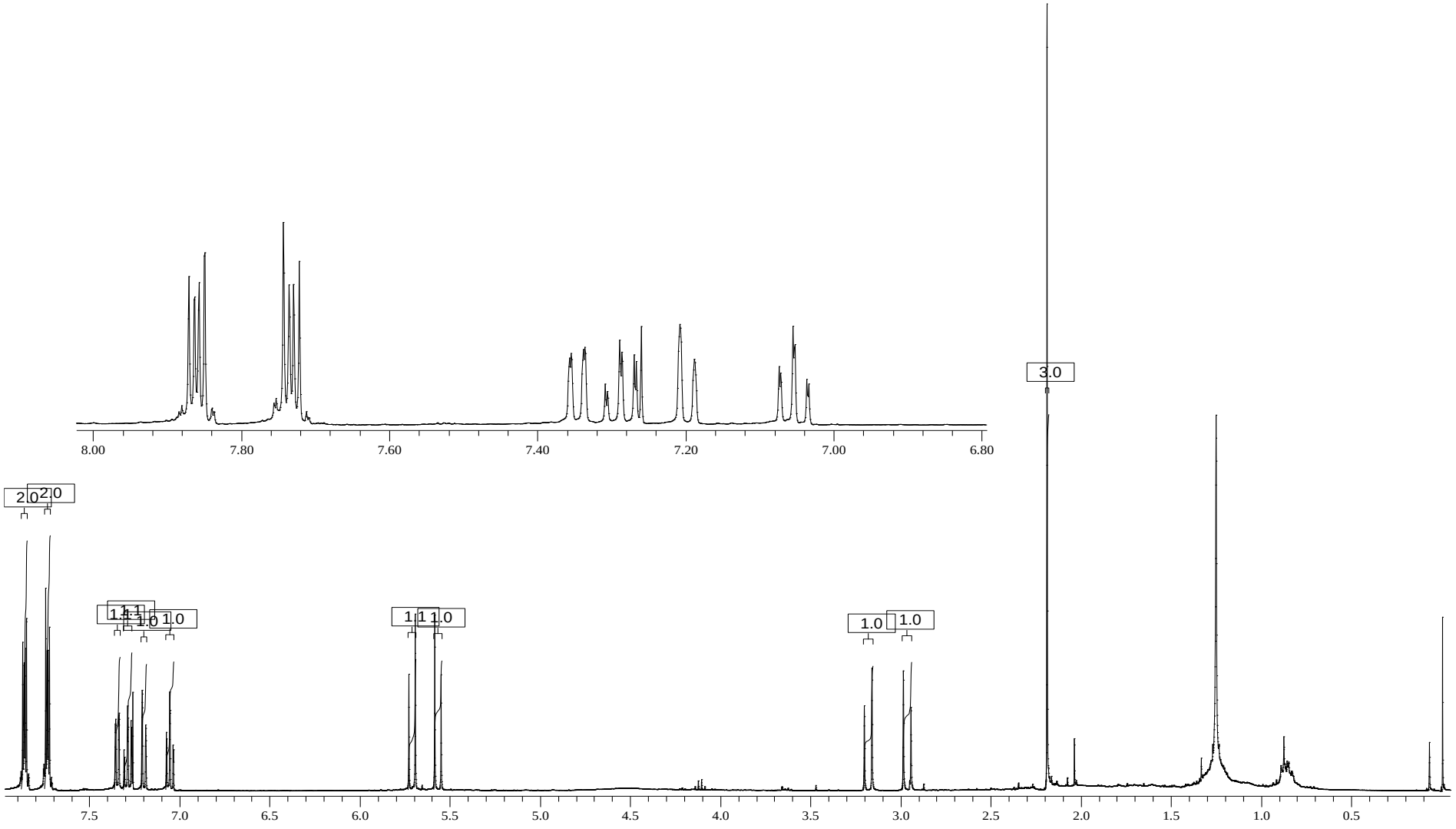


Figure S14. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **2c**.

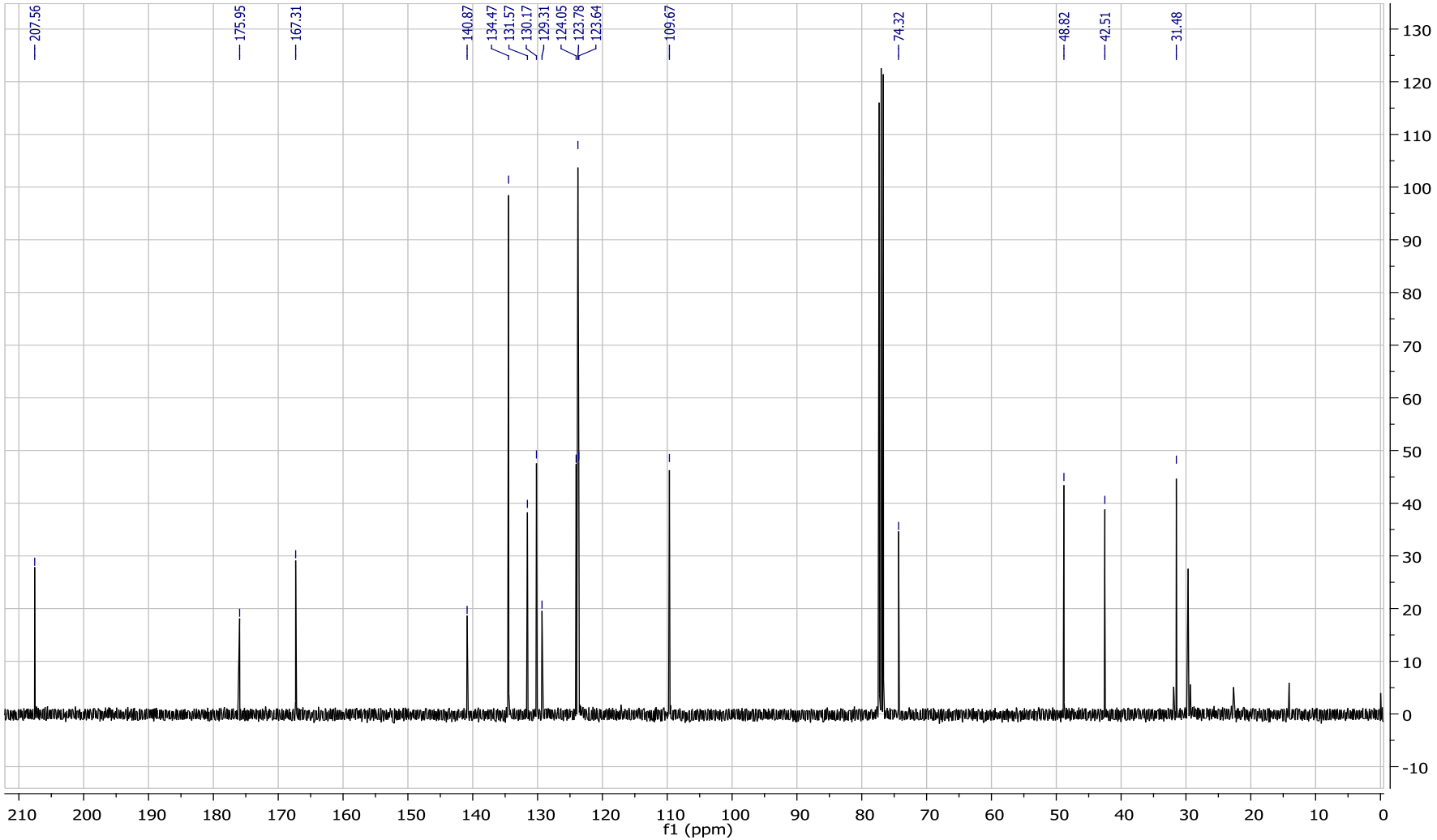


Figure S15. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **2c**.

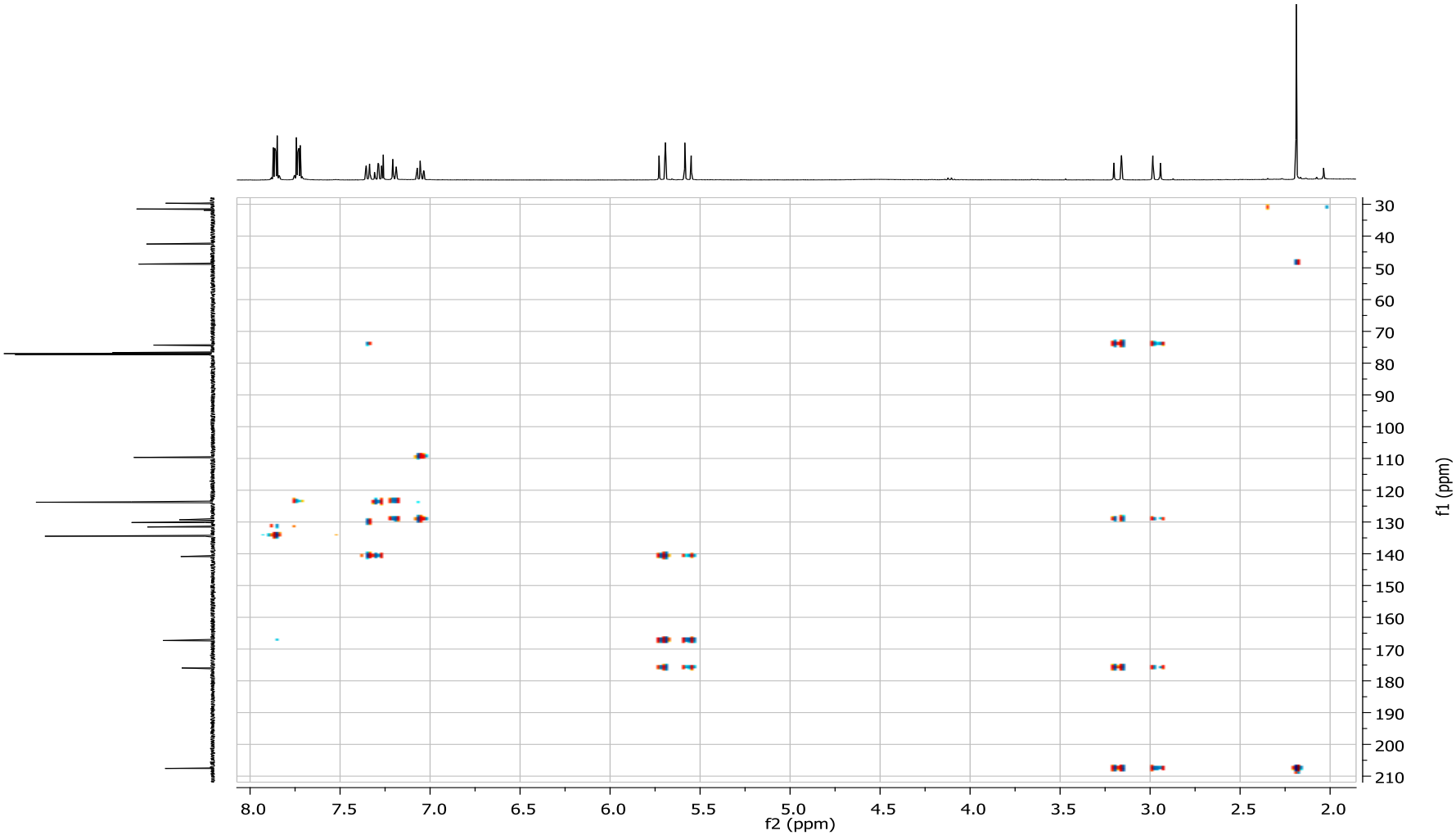


Figure S16. HMBC spectrum (400 MHz, $CDCl_3$) of compound **2c**.

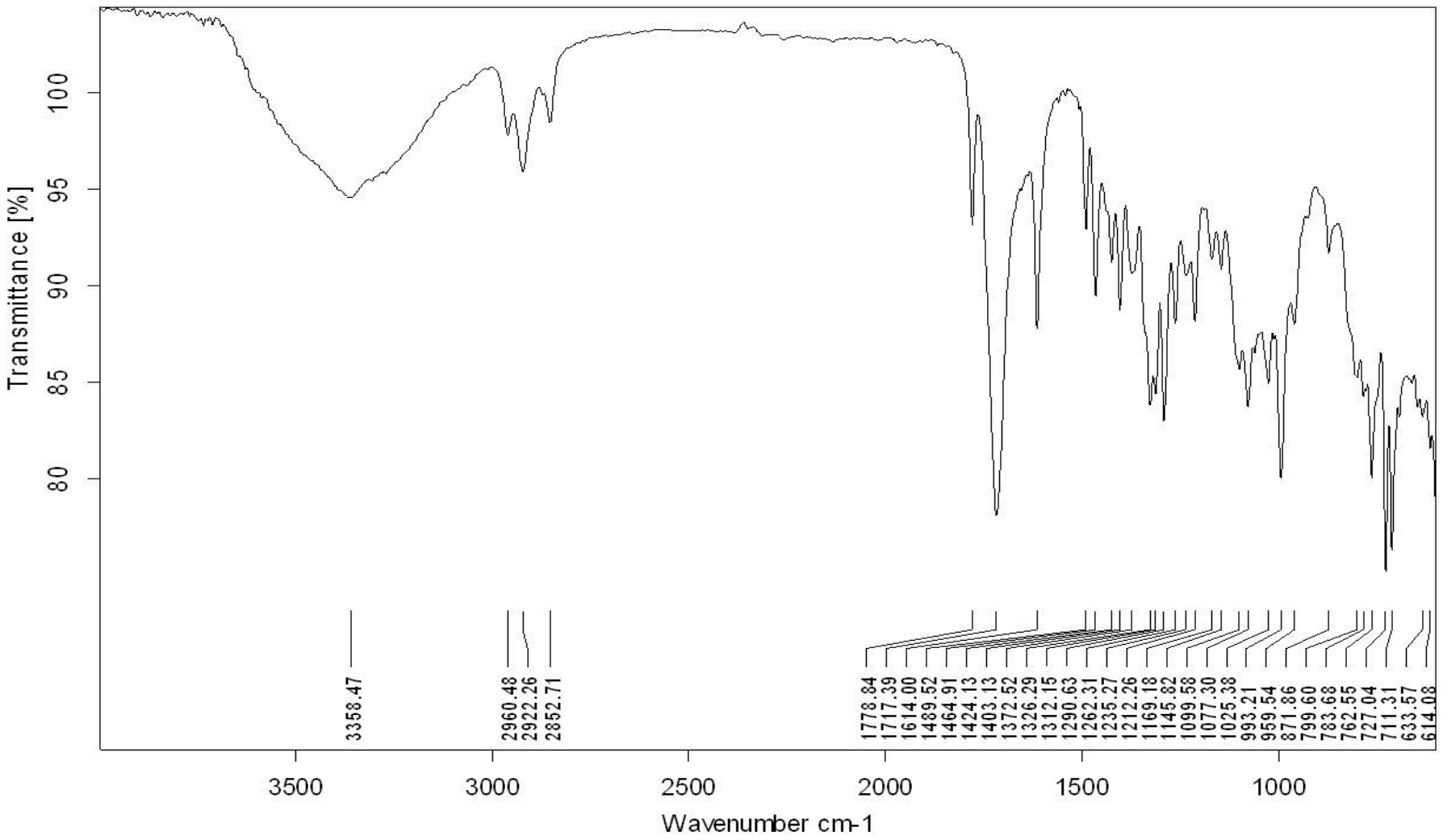


Figure S17. ATR-IR spectrum of compound **2c**.

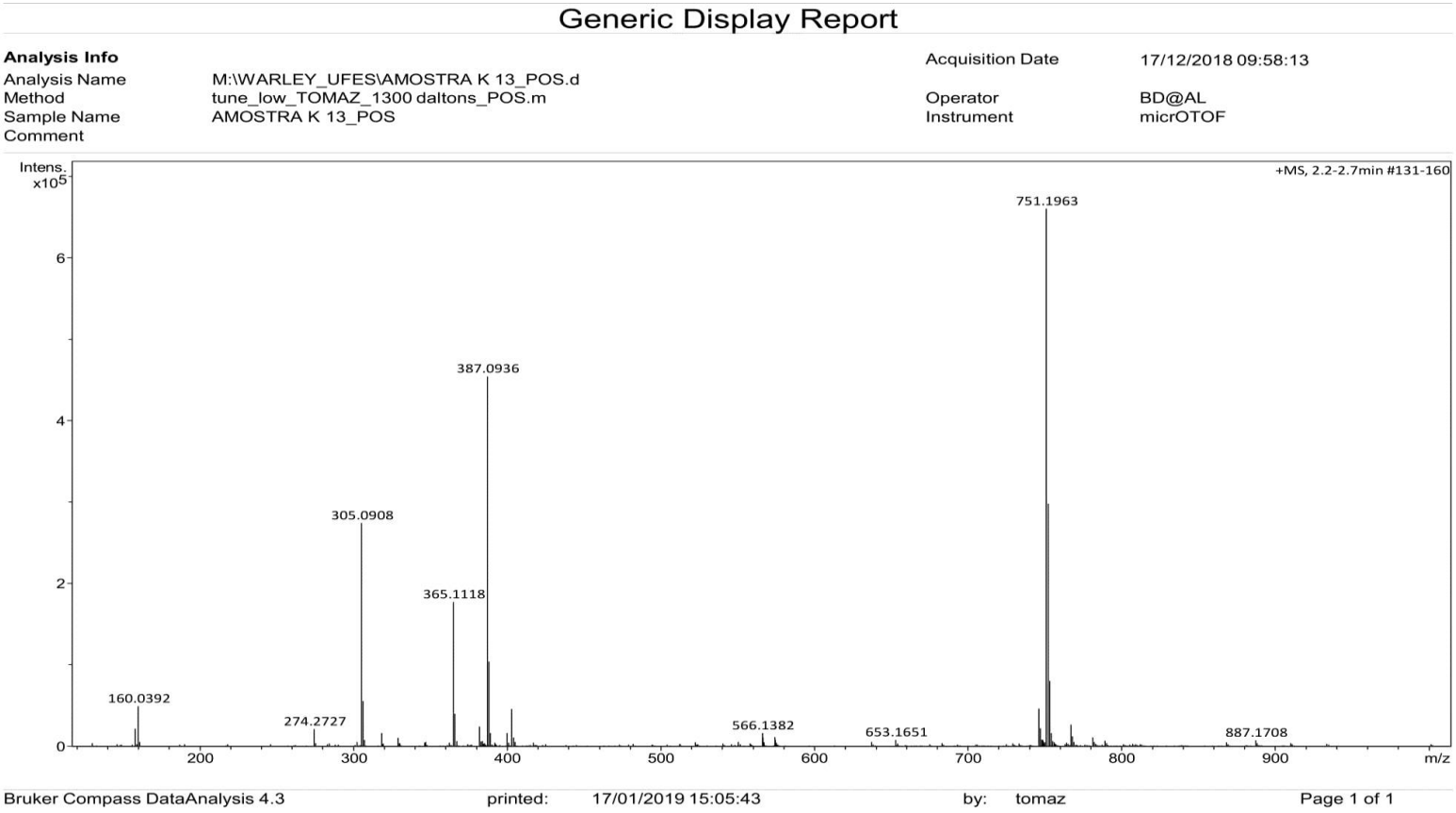


Figure S18. HRMS spectrum of compound **2c**.

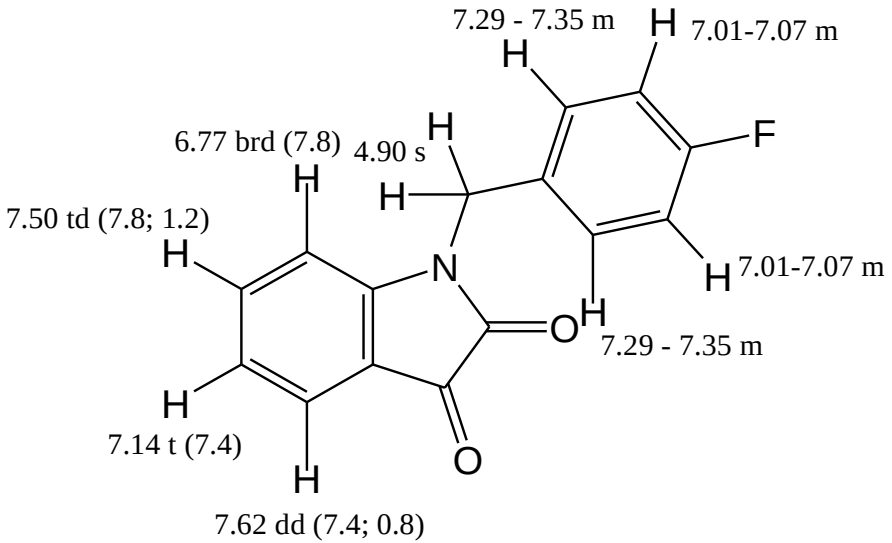


Figure S19. ^{1}H (black) NMR chemical shifts (ppm) data of the compound **2d**. *J* in Hz.

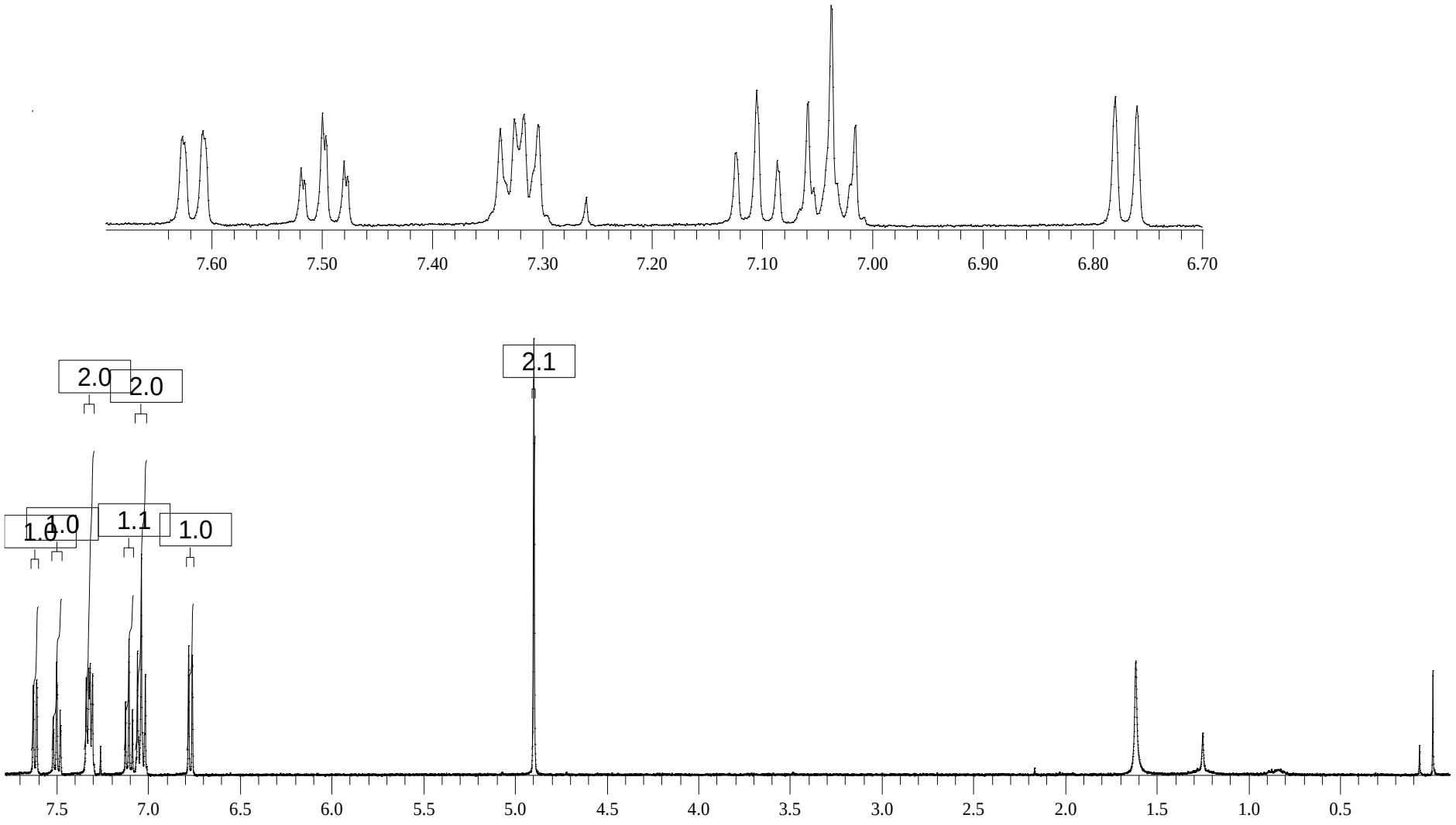


Figure S20. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **2d**.

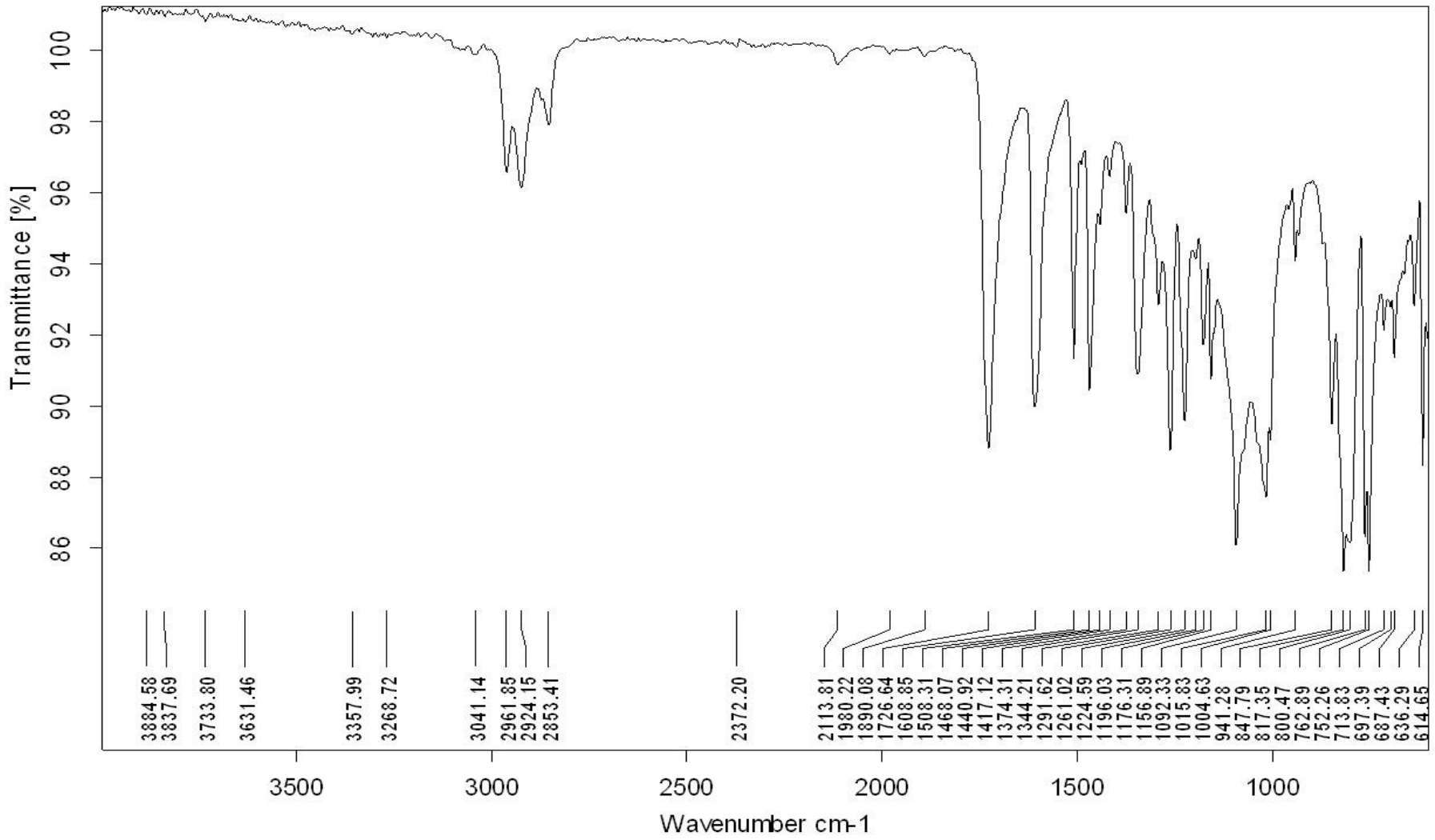


Figure S21. ATR-IR spectrum of compound **2d**.

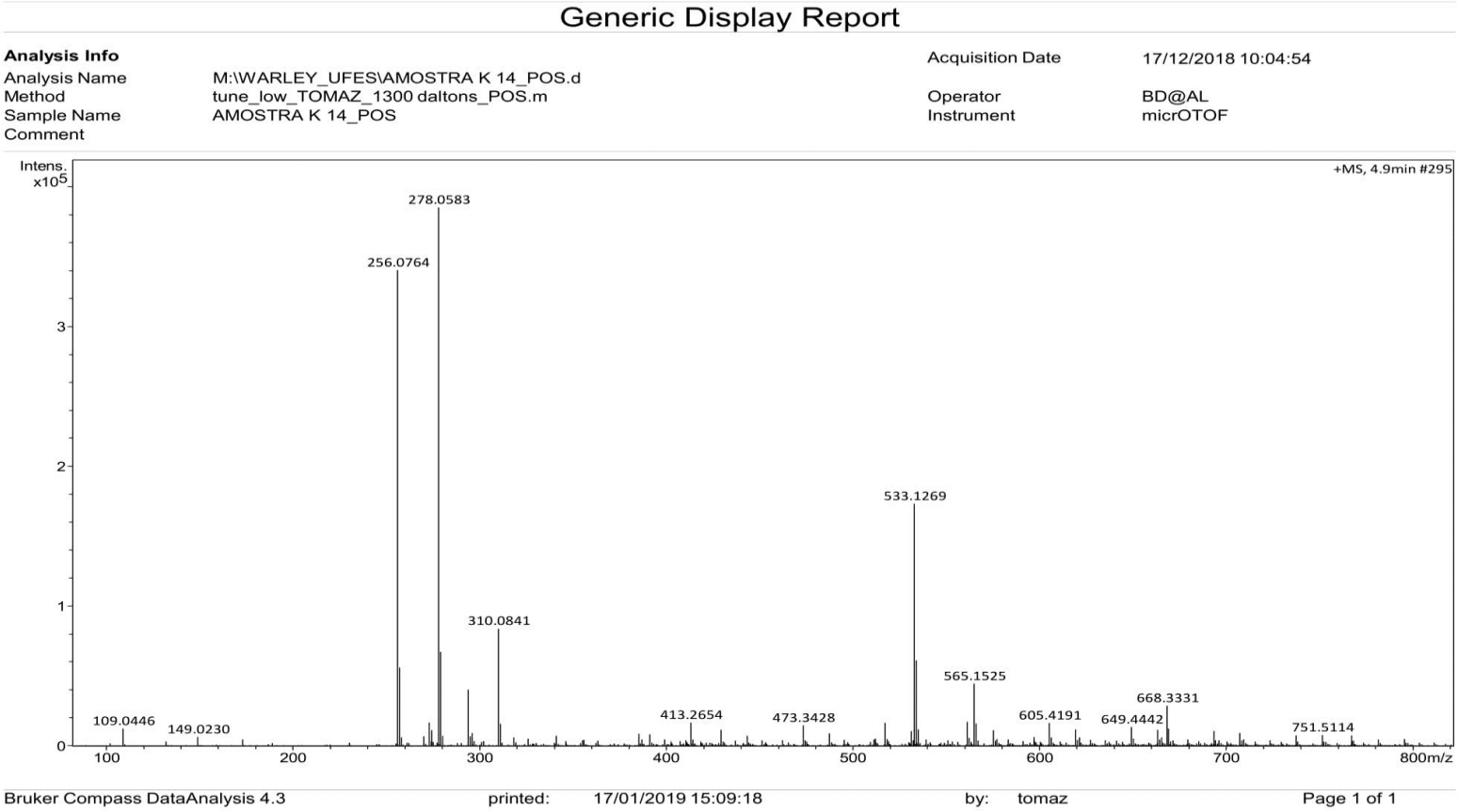


Figure S22. HRMS spectrum of compound **2d**.

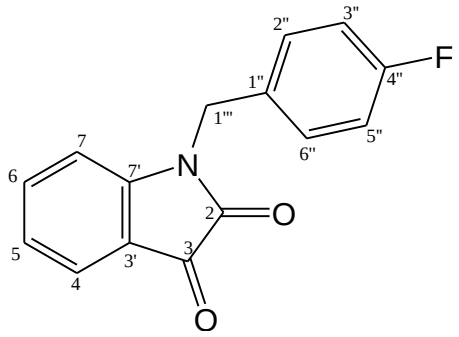


Table S2. NMR data of **2d** and comparison with literature (*J* in Hz)

Position	**2d** (400 MHz, $CDCl_3$)	Shi *et al.*[4] (400 MHz, $CDCl_3$)	Tehrani *et al.*[5] (400 MHz, $CDCl_3$)
	^1H	^1H	^1H
4	7.62 dd (7.4; 0.8)	7.59-7.66 m	7.65 d (6.0)
5	7.14 t (7.4)	7.11 td (7.6; 0.8)	7.14 dt (6.8; 0.8)
6	7.50 td (7.8; 1.2)	7.50 td (7.8; 1.4)	7.53 t (6.6)
7	6.77 brd (7.8)	6.77 d (8.0)	6.80 d (8.0)
2’’; 6’’	7.29-7.35 m	7.29-7.36 m	7.33-7.37 m
3’’; 5’’	7.01-7.07 m	7.00-7.08 m	7.04-7.10 m
1’’’ (*Bn*–CH_2)	4.90 s	4.90 s	4.93 s

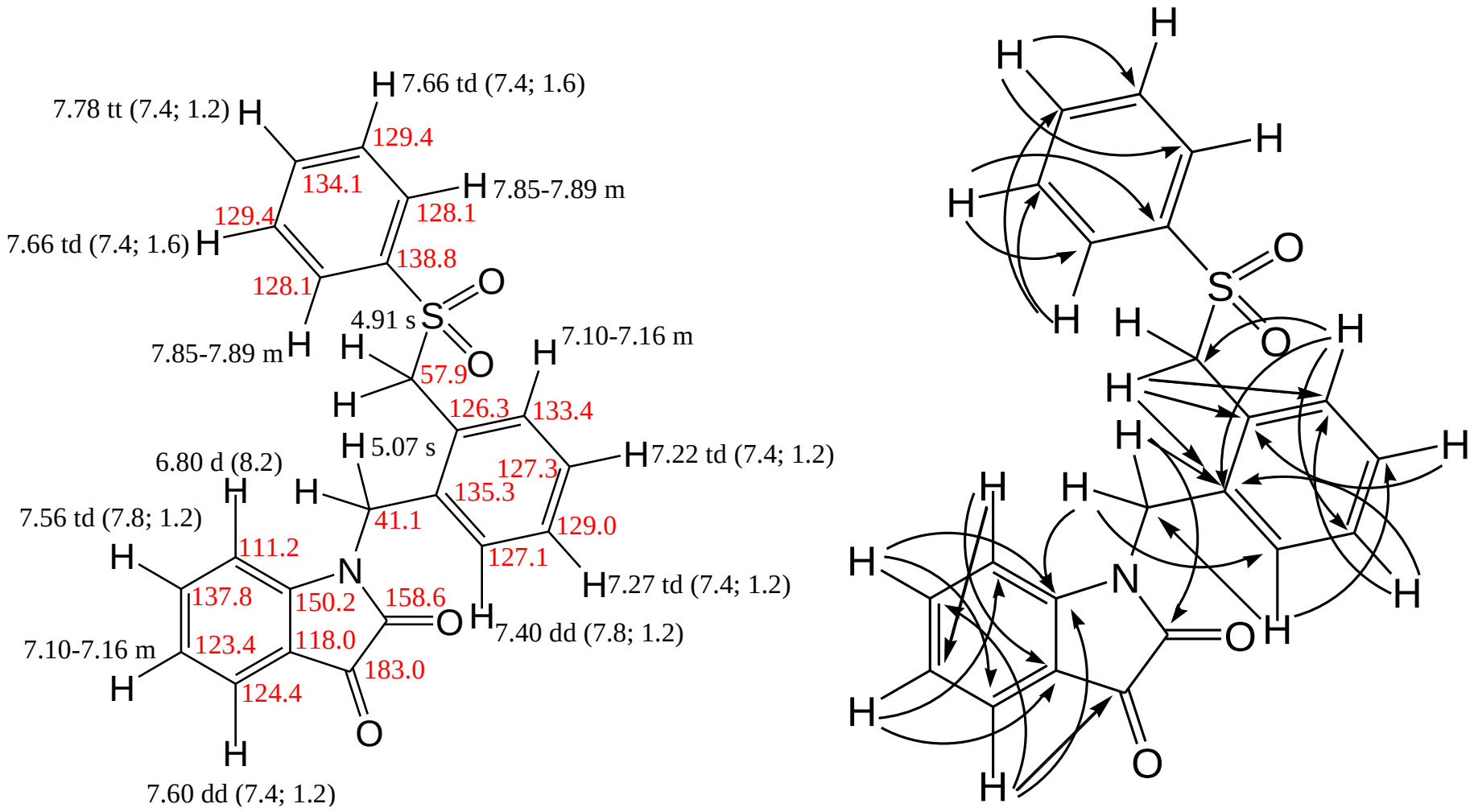


Figure S23. ^{1}H (black) and ^{13}C (red) NMR chemical shifts (ppm) and main HMBC correlation observed in the NMR data of the compound **2e**. *J* in Hz.

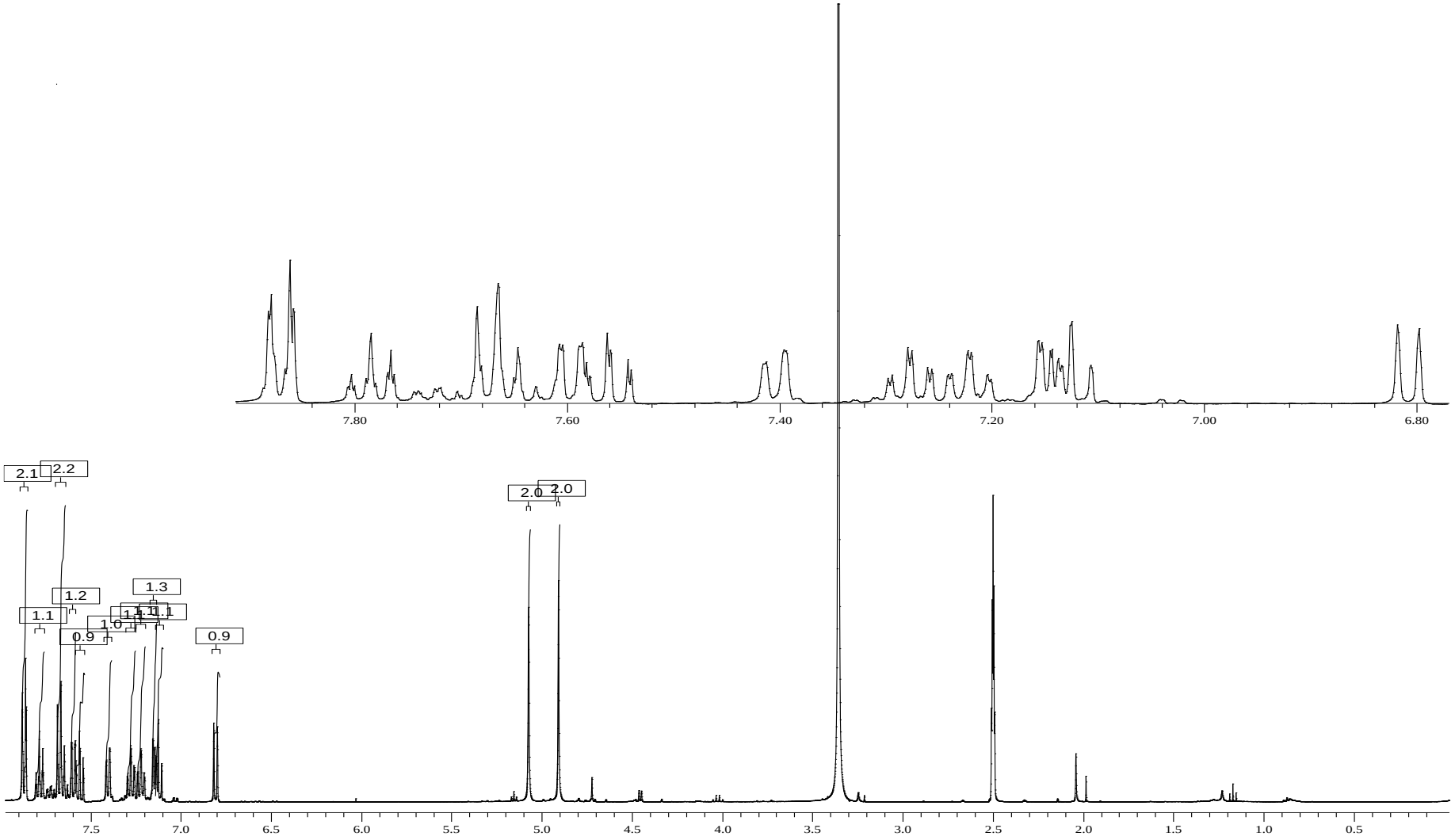


Figure S24. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **2e**.

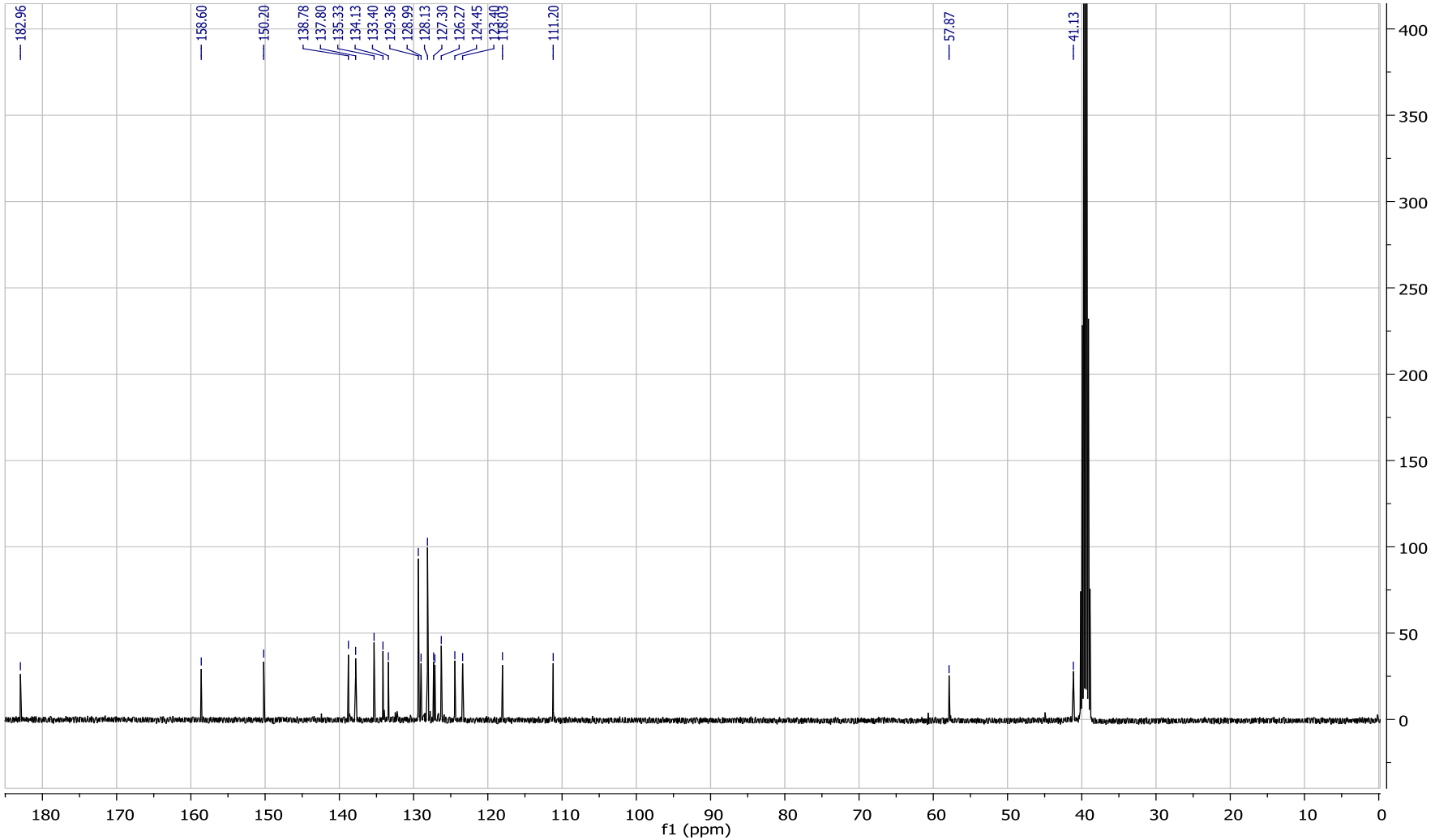


Figure S25. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **2e**.

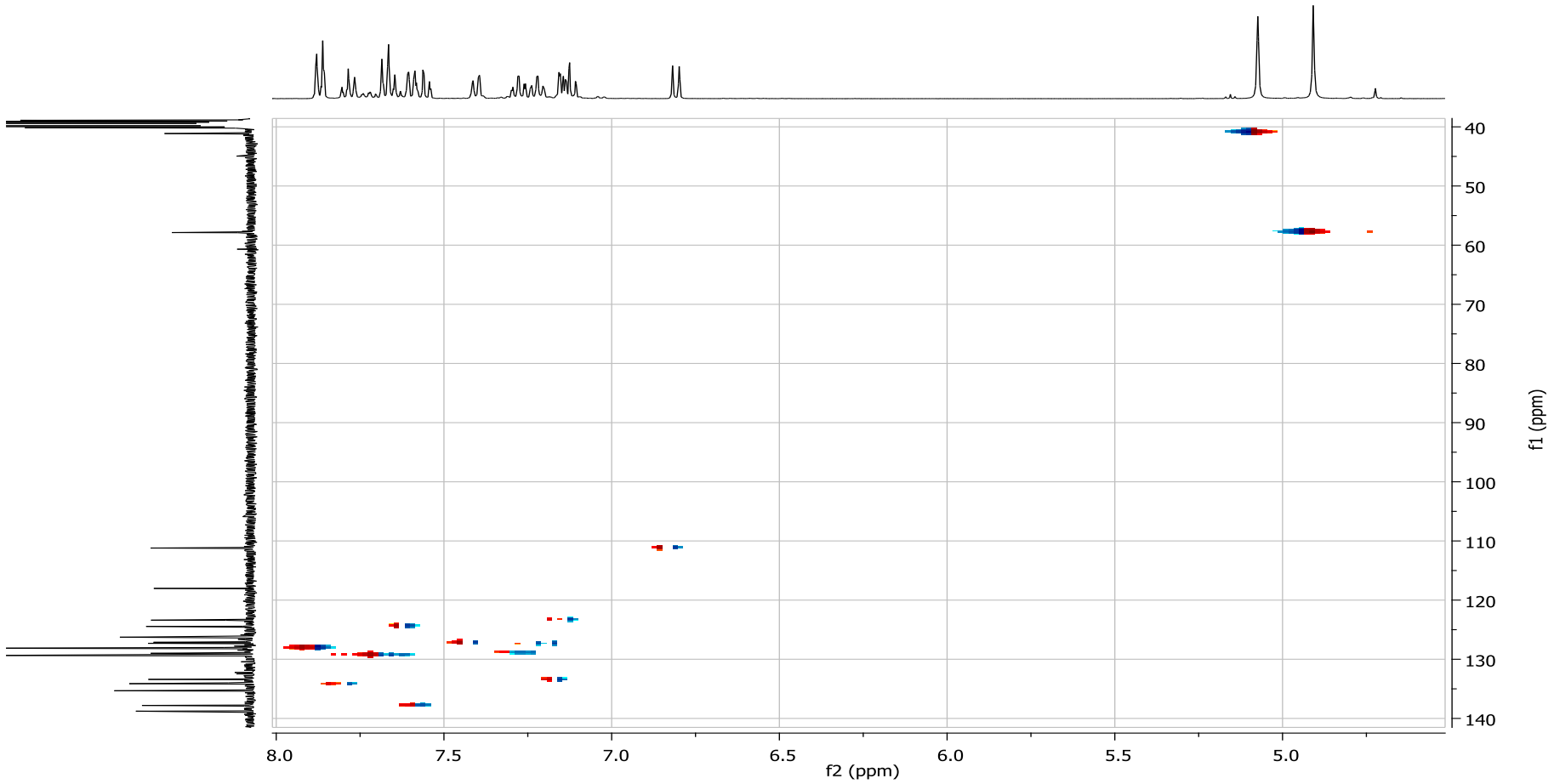


Figure S26. Heteronuclear single quantum coherence spectroscopy (HSQC) spectrum (400 MHz, DMSO-d_6) of compound **2e**.

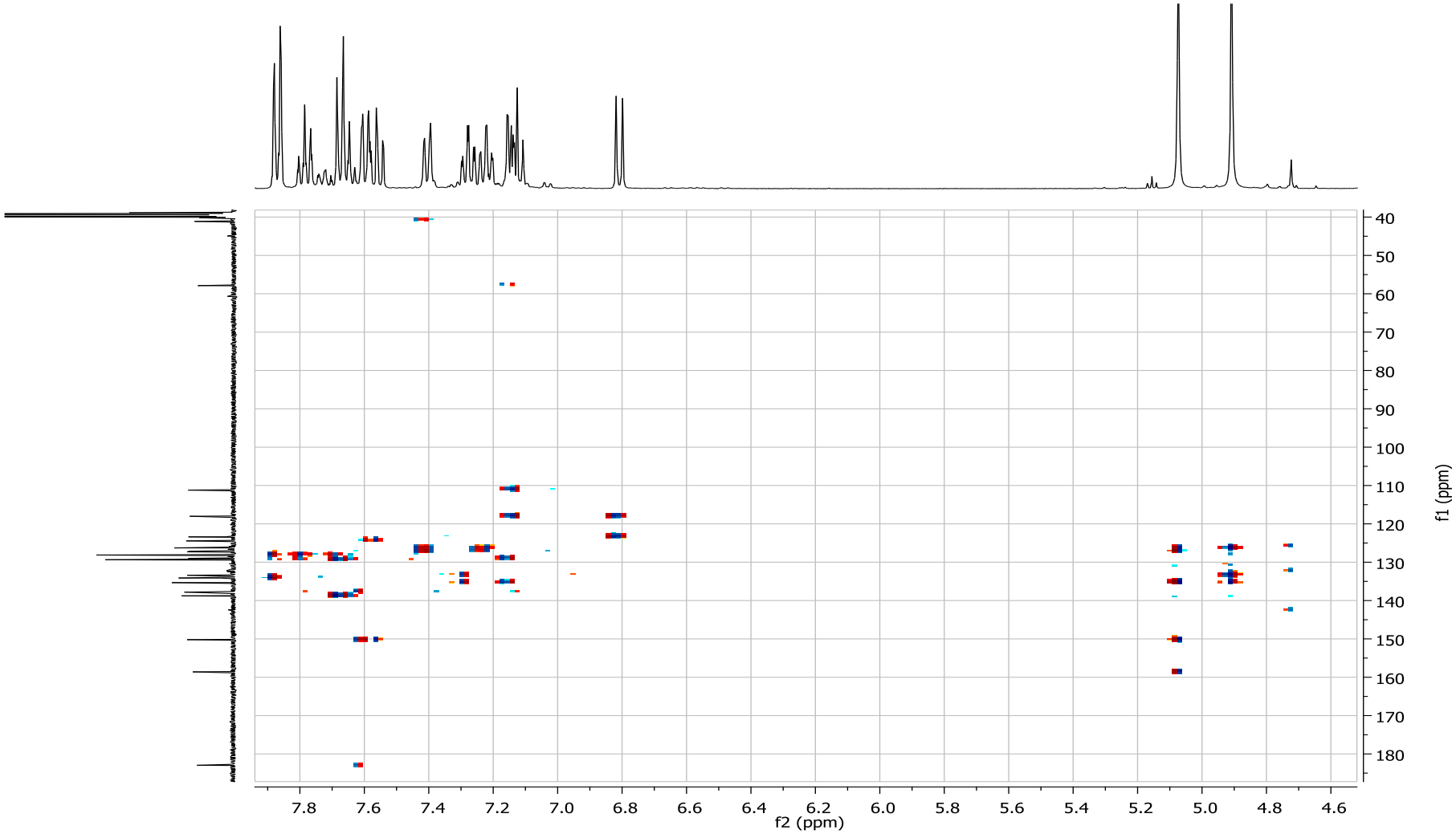


Figure S27. HMBC spectrum (400 MHz, DMSO-d_6) of compound **2e**.

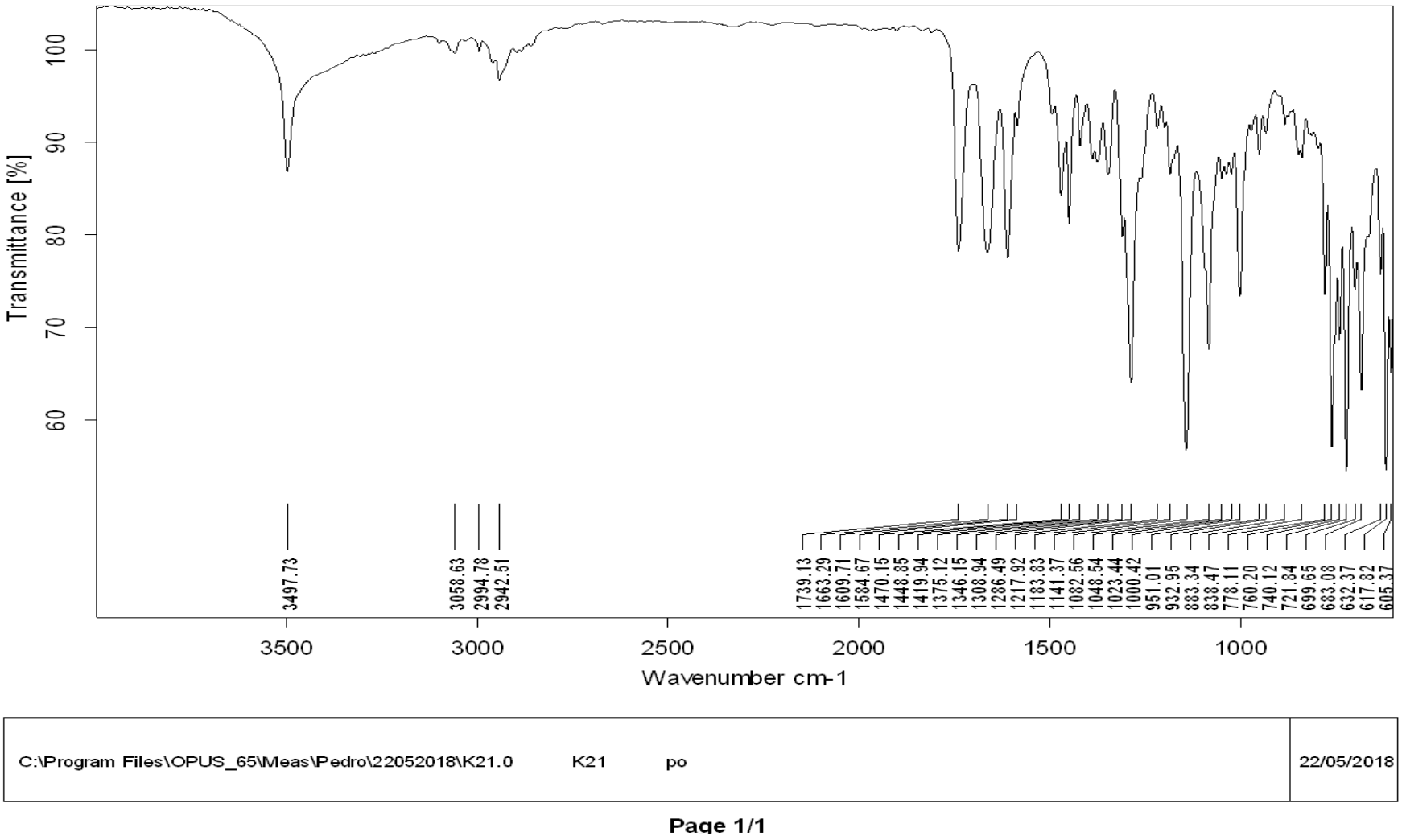


Figure S28. ATR-IR spectrum of compound **2e**.

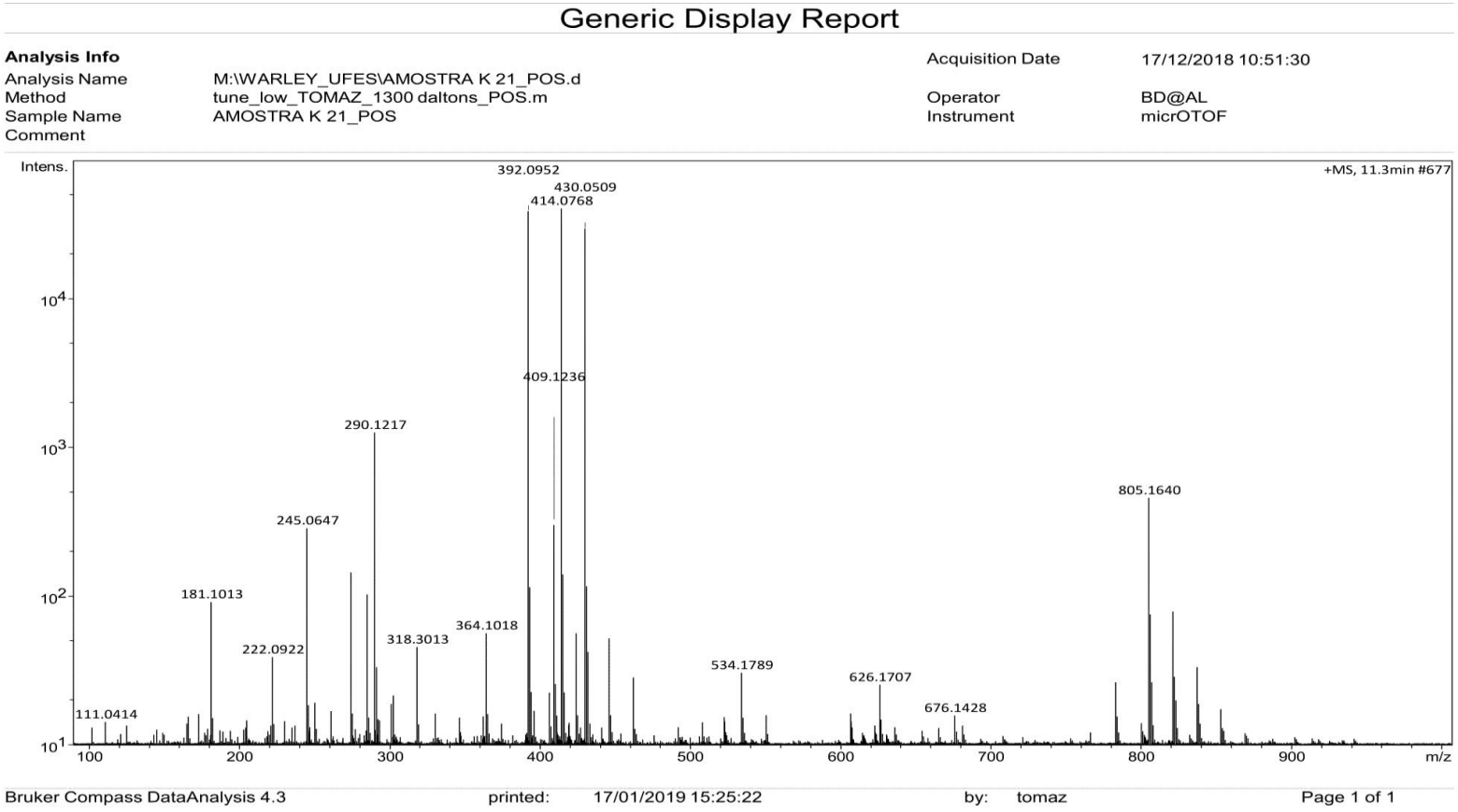


Figure S29. HRMS spectrum of compound **2e**.

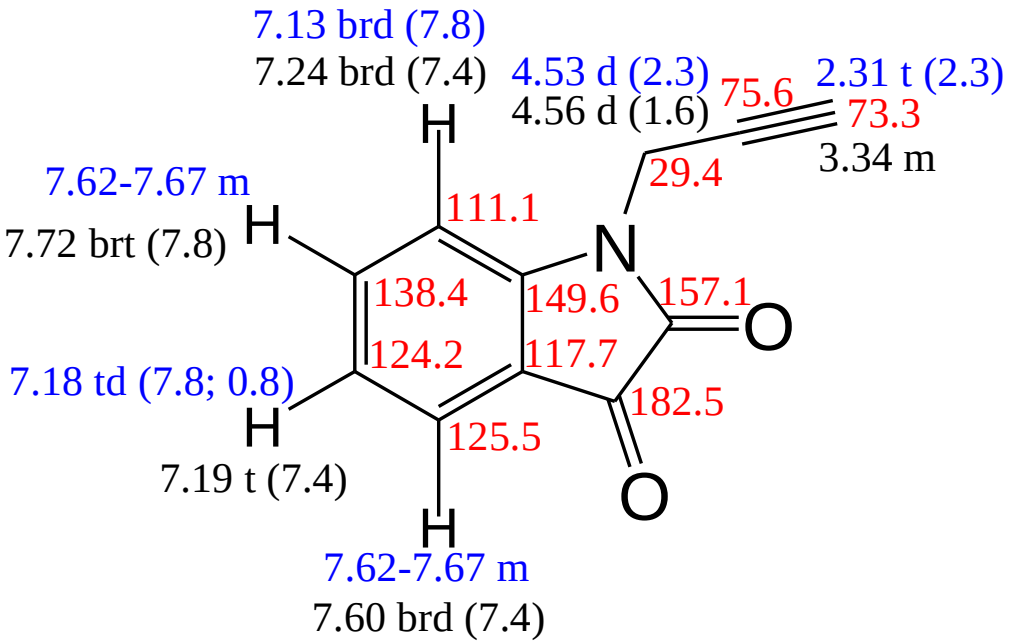


Figure S30. ^{1}H (DMSO-D_6 in black and $CDCl_3$ in blue) and ^{13}C (red) NMR chemical shifts (ppm) data of the compound **2f**. *J* in Hz.

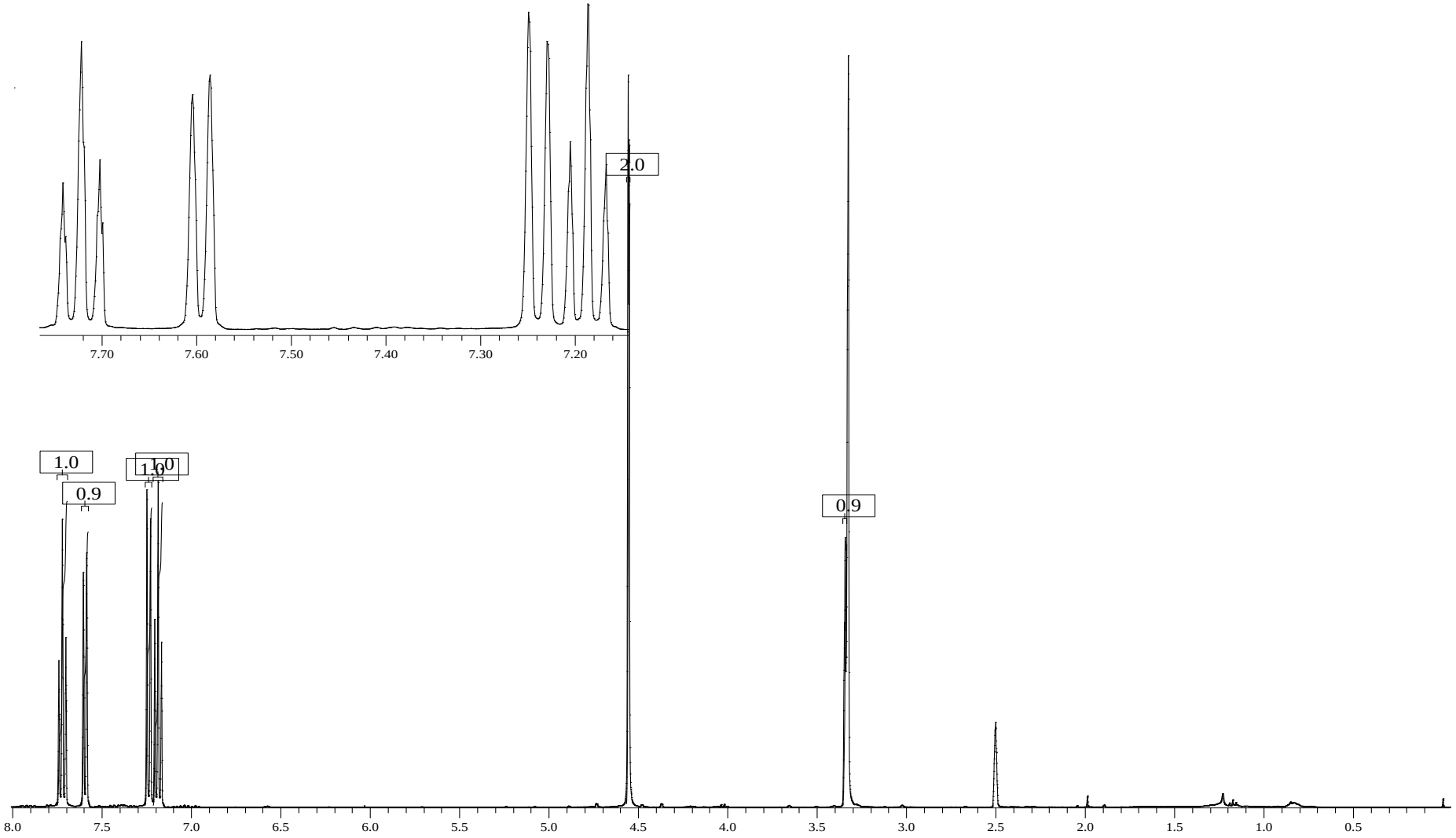


Figure S31. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **2f**.

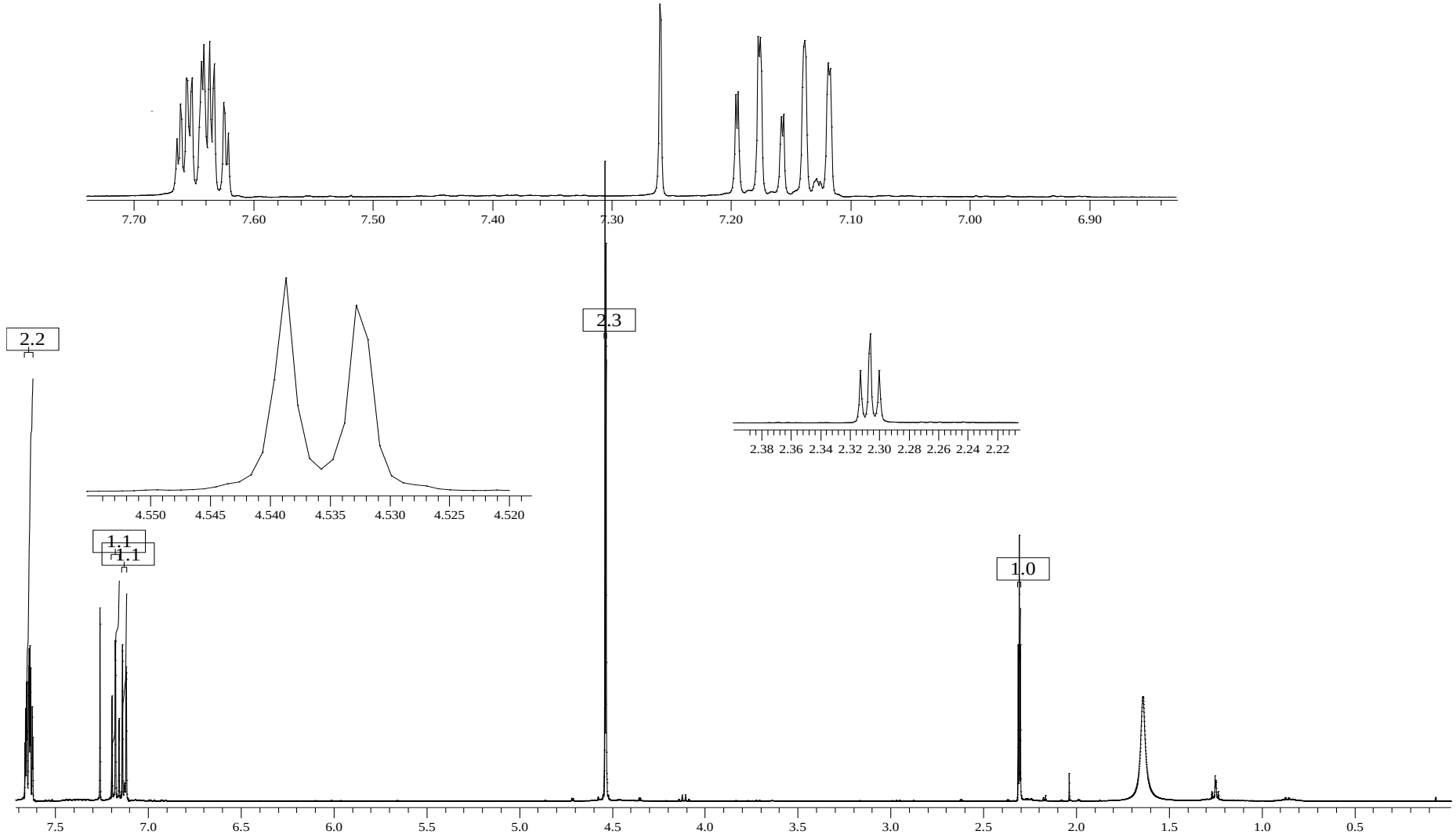


Figure S32. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **2f**.

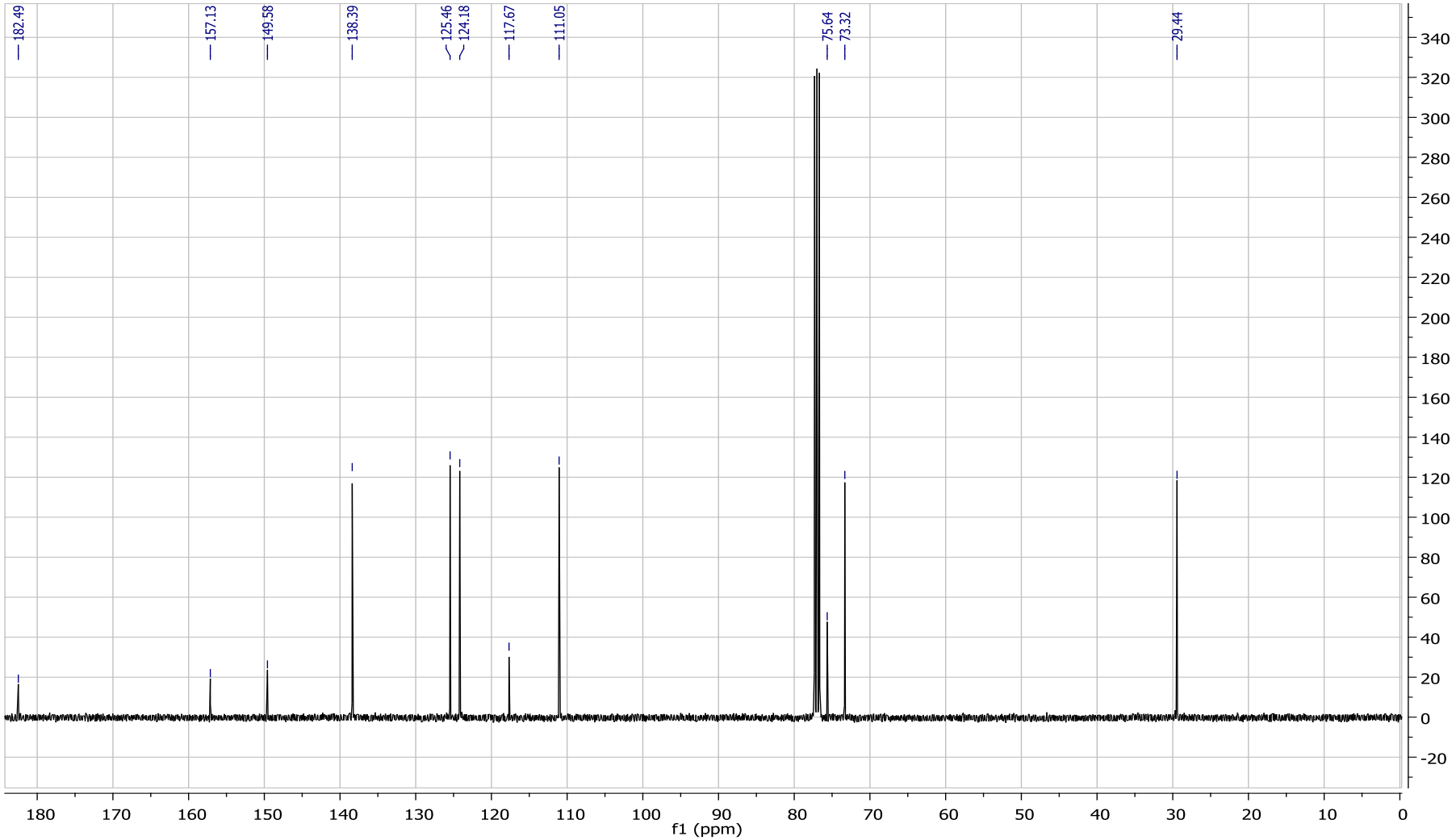


Figure S33. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **2f**.

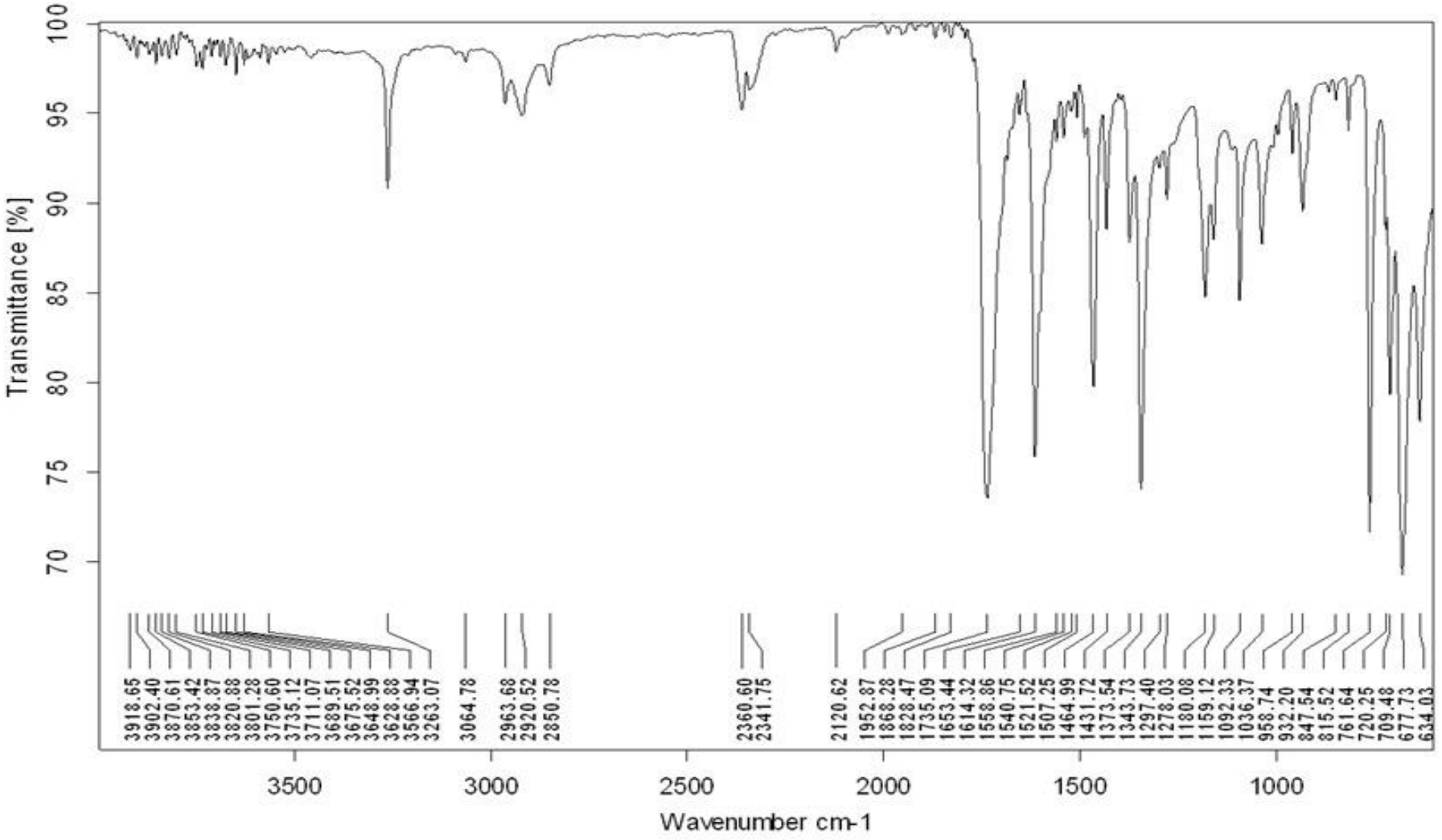


Figure S34. ATR-IR spectrum of compound **2f**.

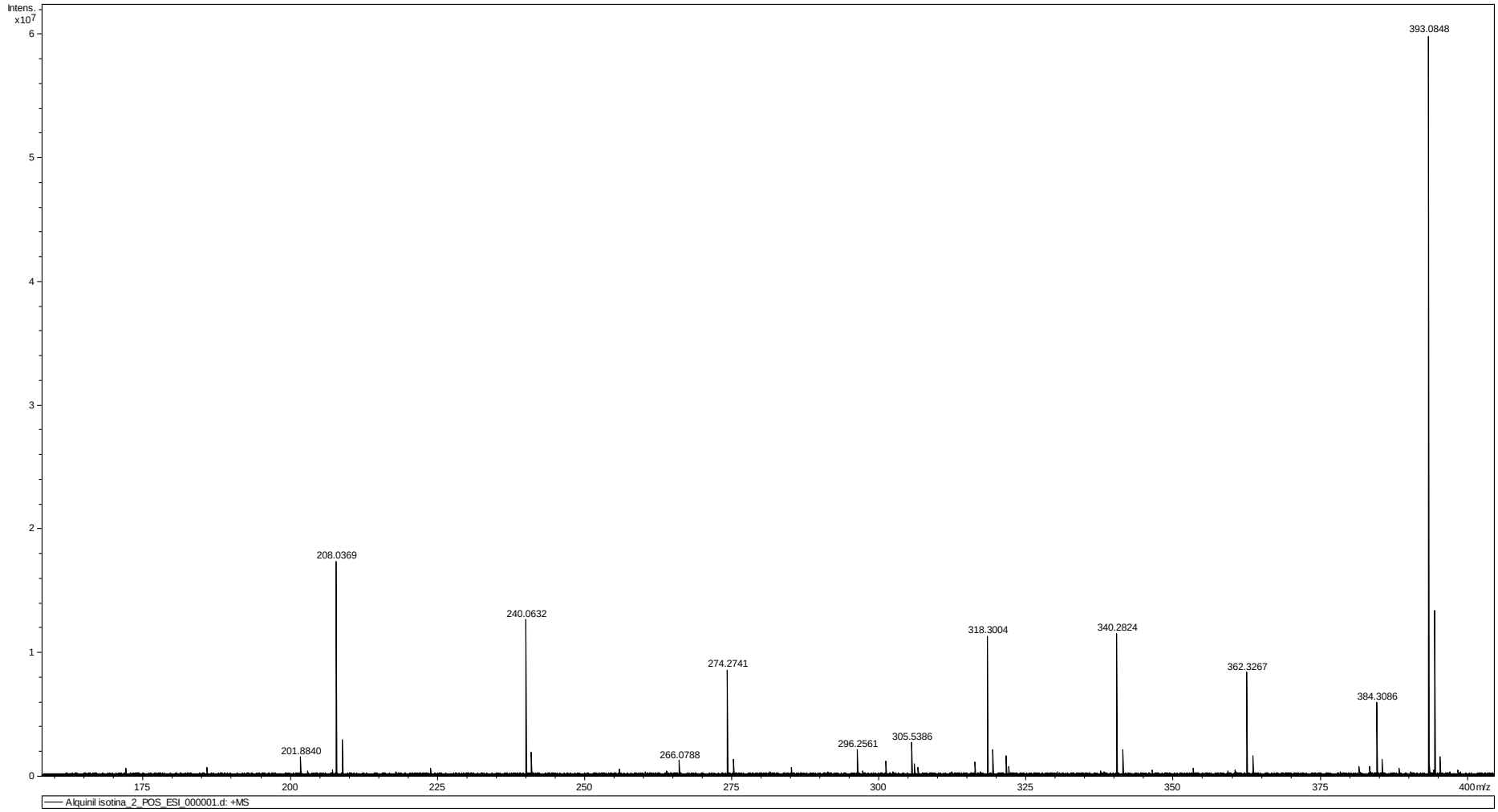


Figure S35. HRMS spectrum of compound **2f**.

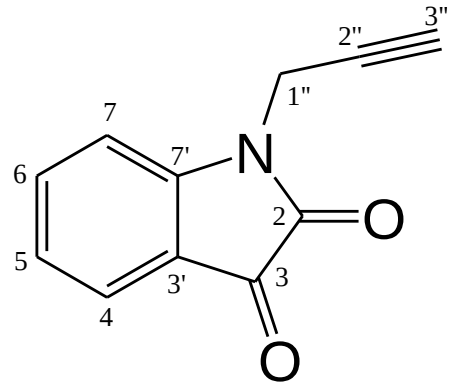


Table S3. NMR data of **2f** and comparison with literature (*J* in Hz)

Position	**2f** (400 MHz, DMSO-d_6)	**2f** (400 MHz, $CDCl_3$)		Bouhfid *et al.*[6] (300 MHz, $CDCl_3$)		Macpherson *et al.*[7] (400 MHz, CD_3OD)
	^1H	^1H	^{13}C	^1H	^{13}C	^1H
2	–	–	157.1	–	158.6	–
3	–	–	182.5	–	182.7	–
3’	–	–	117.7	–	117.4	–
4	7.60 brd (7.4)	7.62-7.67 m	125.5	7.22-7.63 m	126.2	7.26 m
5	7.19 t (7.4)	7.18 td (7.8; 0.8)	124.2	7.22-7.63 m	125.2	7.17 m
6	7.72 brt (7.8)	7.62-7.67 m	138.4	7.22-7.63 m	139.3	7.43 d
7	7.24 brd (7.4)	7.13 brd (7.8)	111.1	7.22-7.63 m	111.7	7.17 m
7’	–	–	149.6	–	149.4	–
1’’	4.56 d (2.3)	4.53 d (2.3)	29.4	4.52 d (2.4)	36.8	4.56 m
2’’	–	–	75.6	–	–	–
3’’	3.34 m	2.31 t (2.3)	73.3	2.35 t (2.4)	73.9	2.68 t

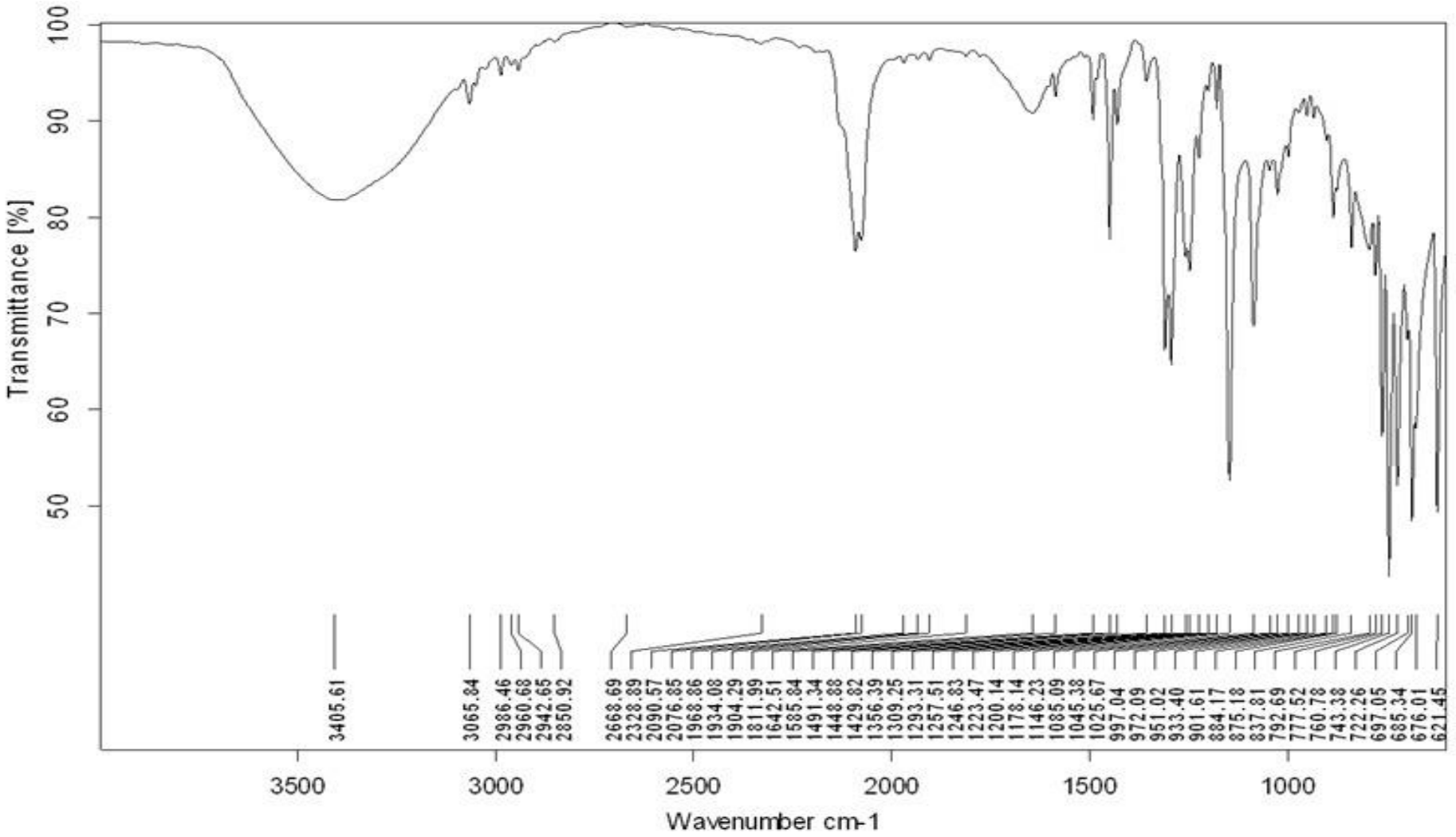


Figure S36. ATR-IR spectrum of compound **k**.

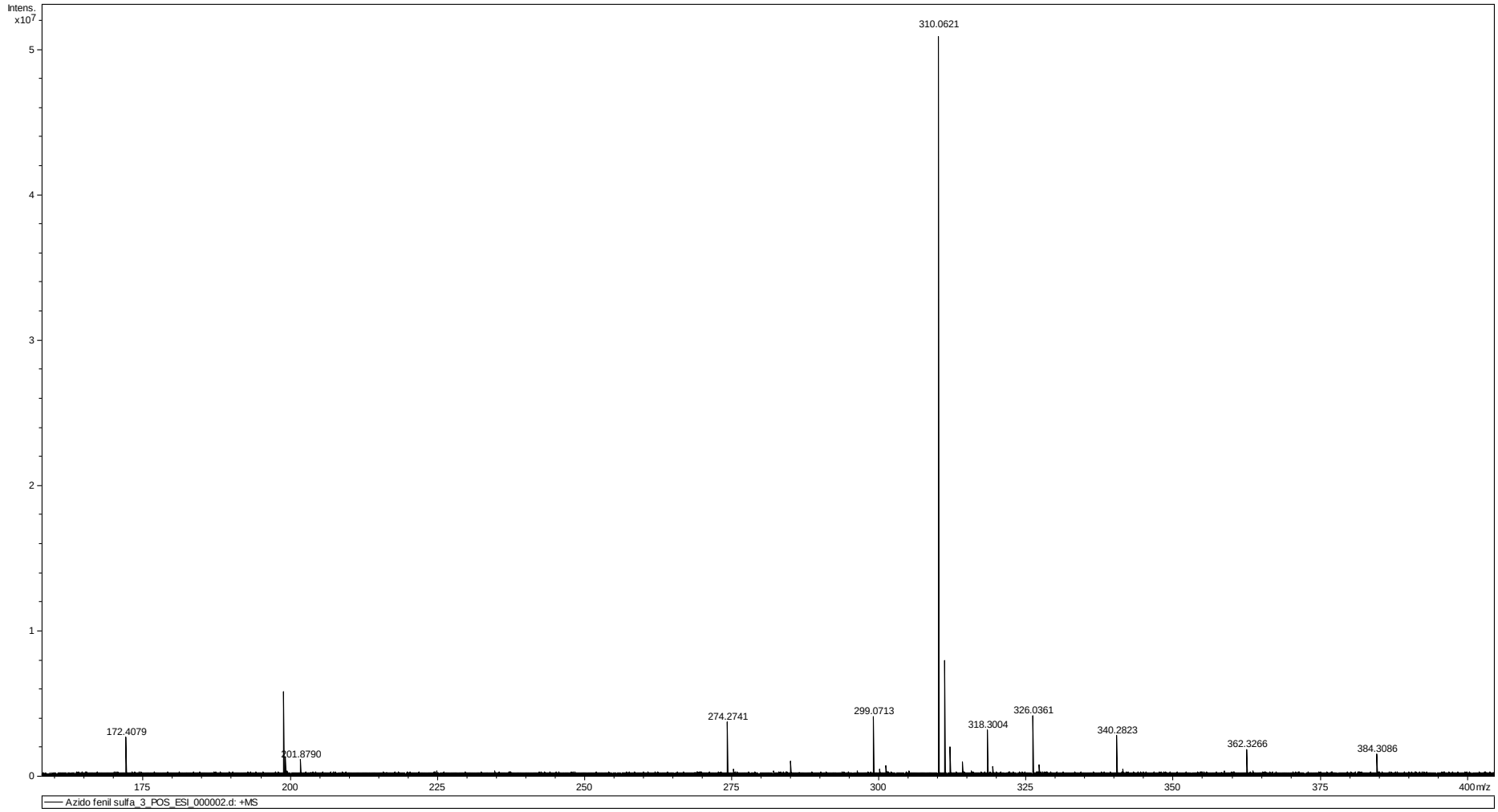


Figure S37. HRMS spectrum of compound **k**.

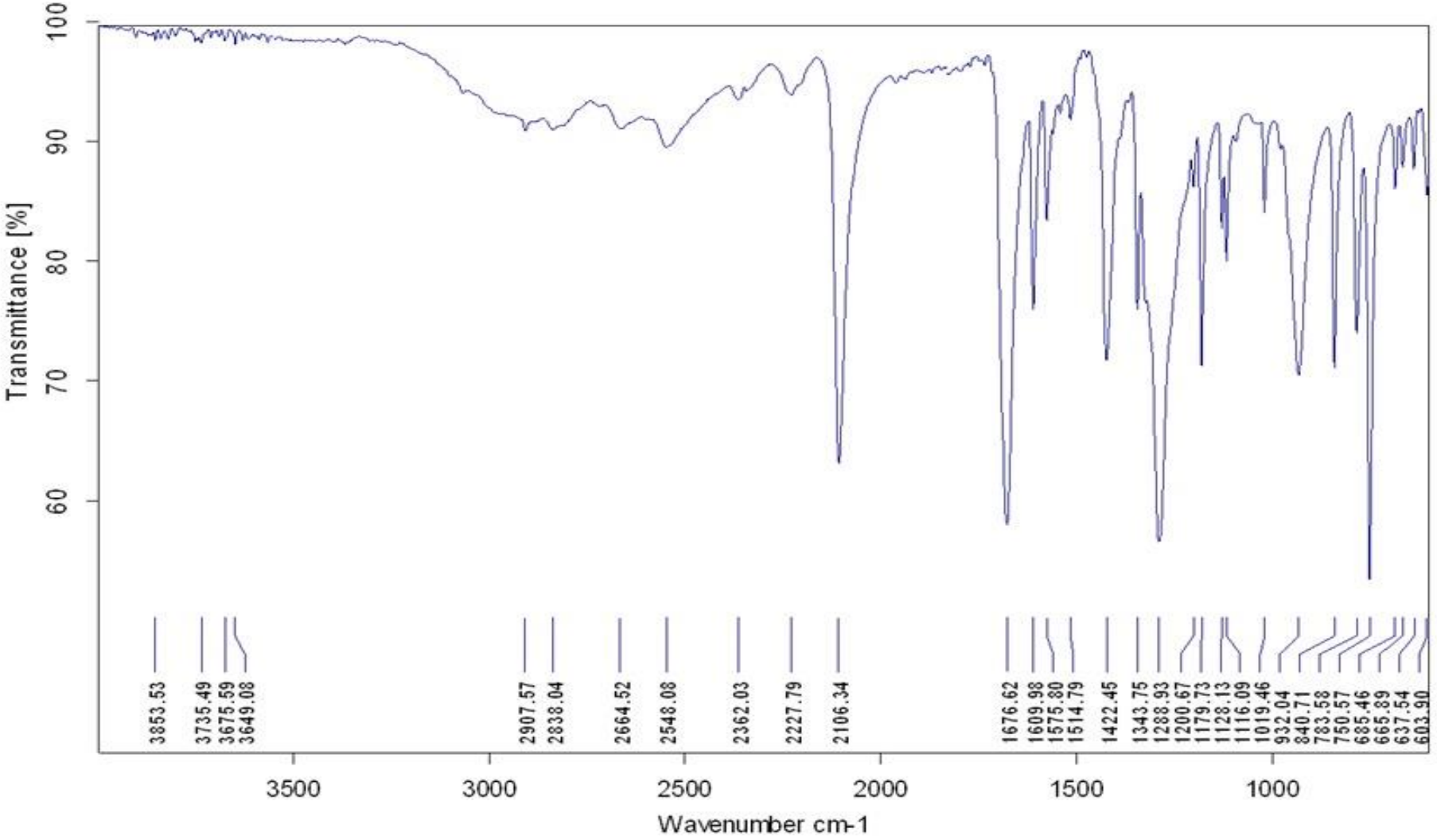


Figure S38. ATR-IR spectrum of compound **l**.

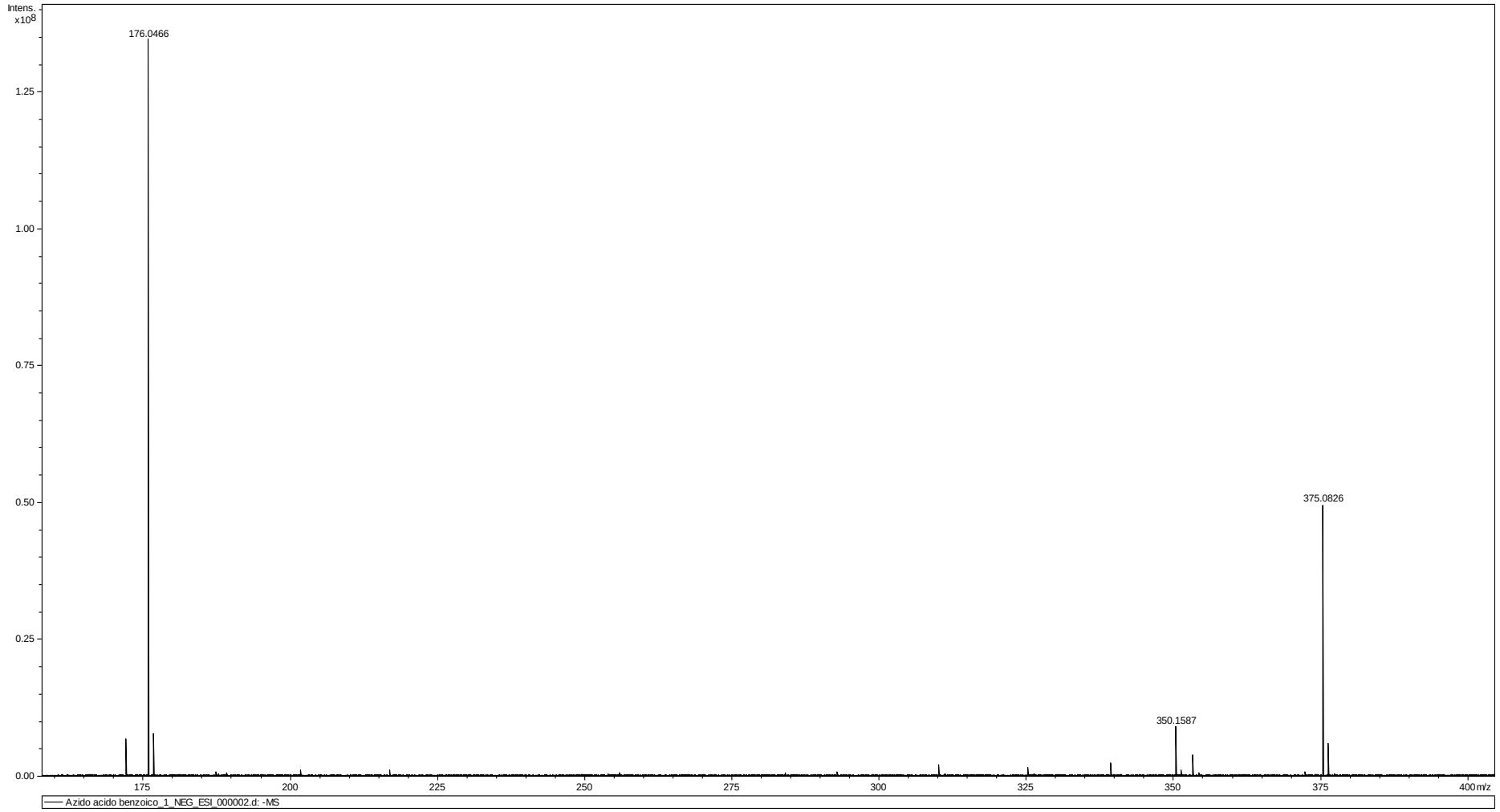


Figure S39. HRMS spectrum of compound **l**.

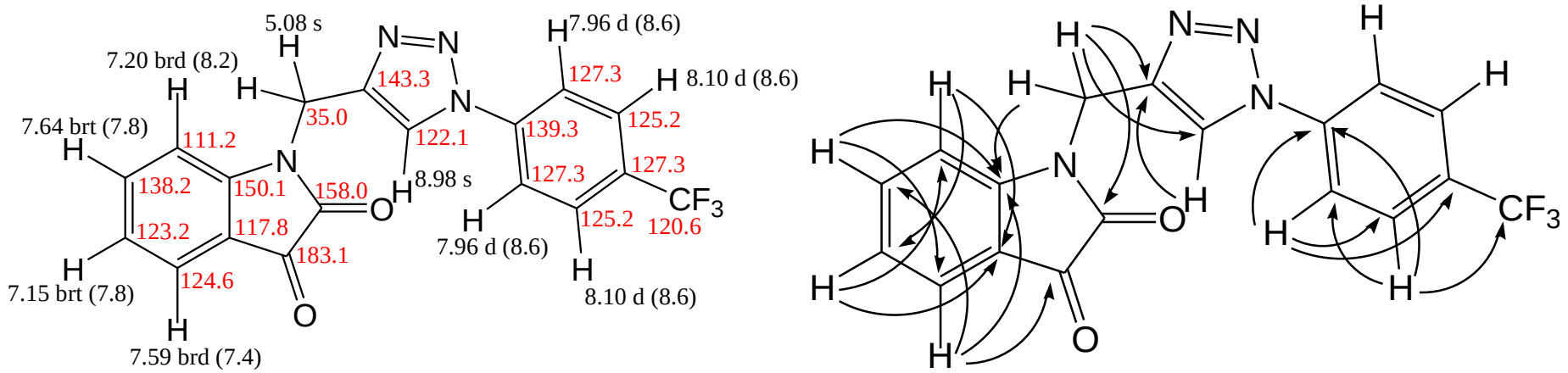


Figure S40. ^{1}H (black) and ^{13}C (red) NMR chemical shifts (ppm) and main HMBC correlation observed in the NMR data of the compound **4h**. *J* in Hz.

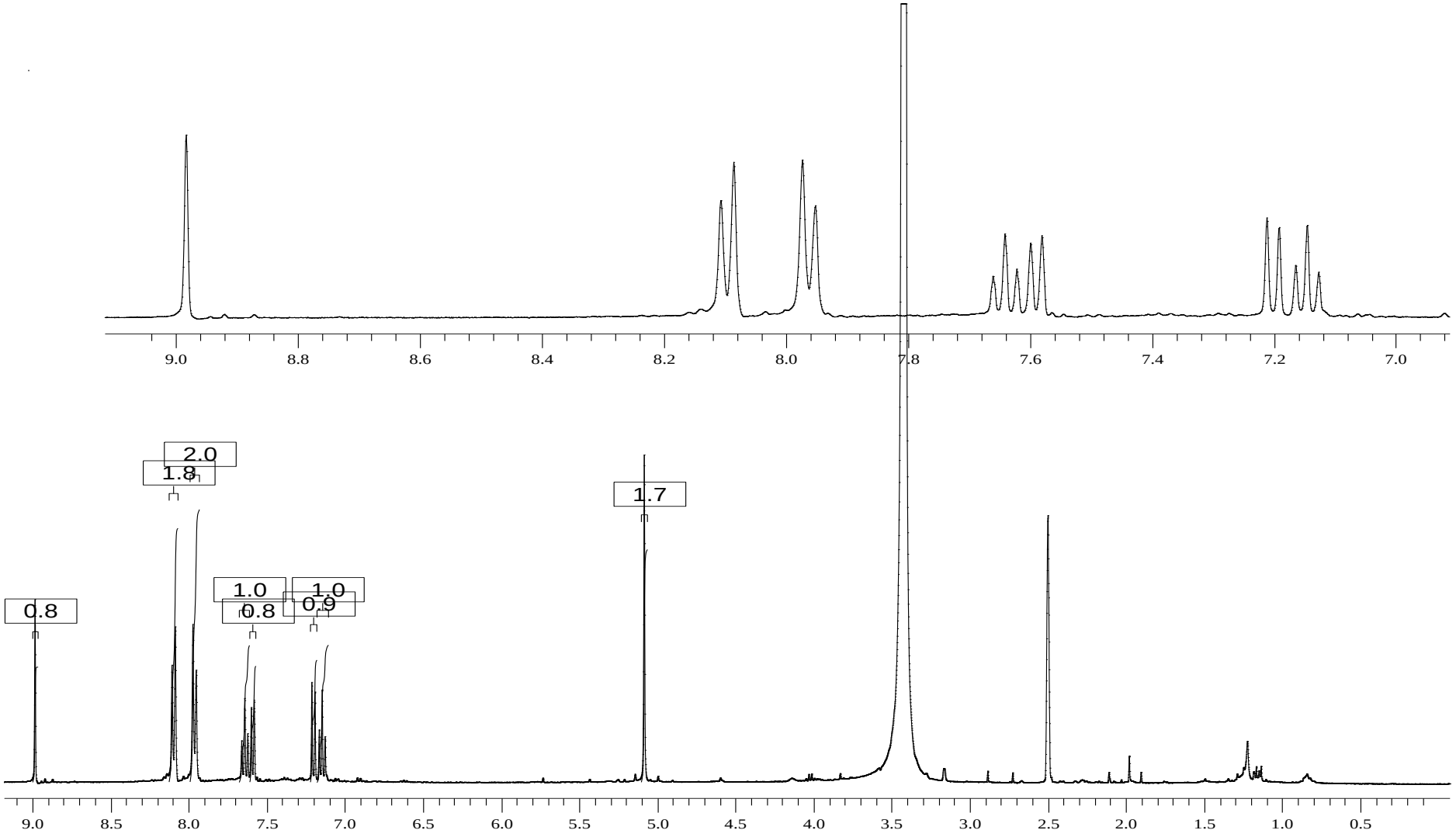


Figure S41. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **4h**.

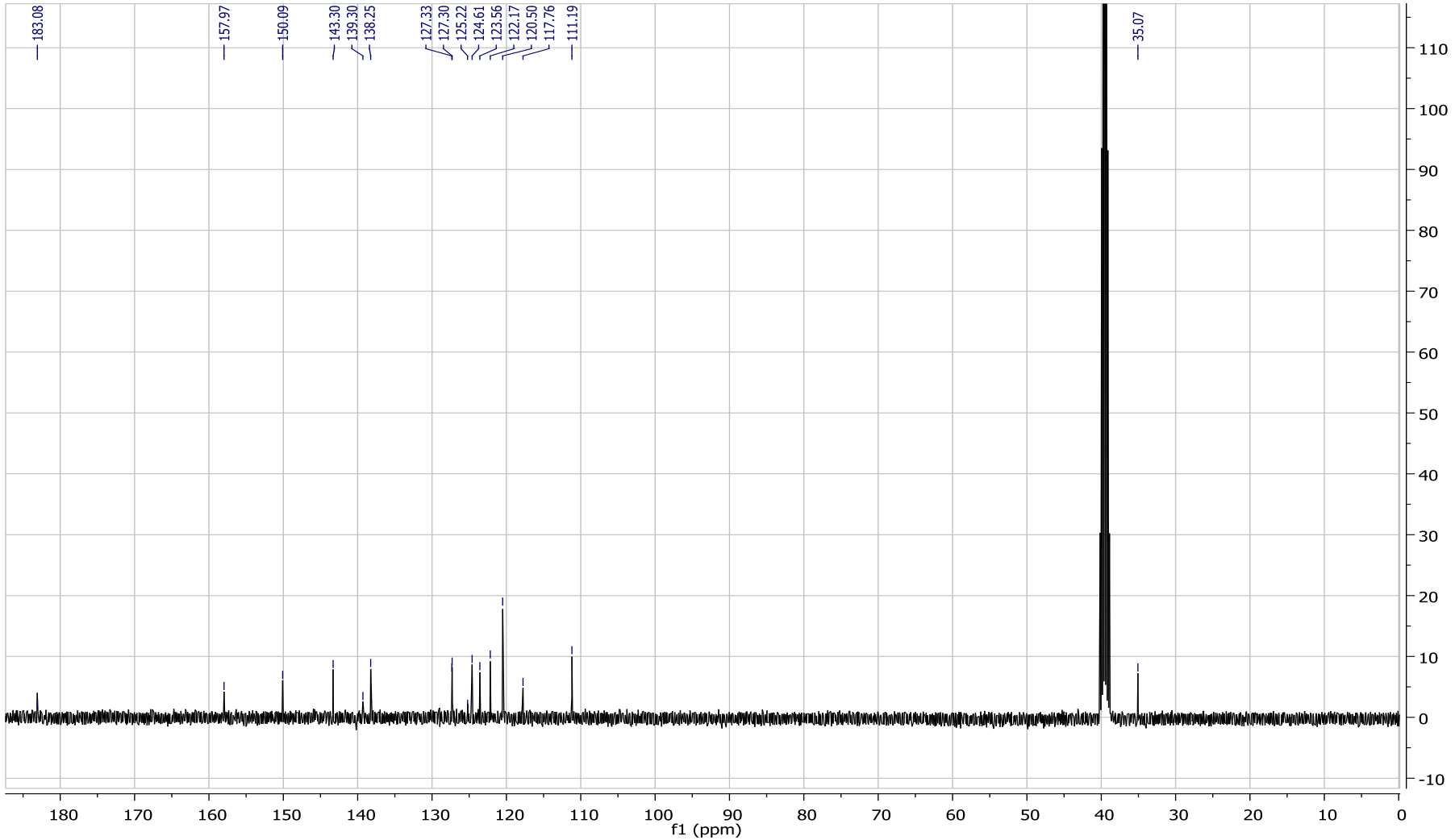


Figure S42. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **4h**.

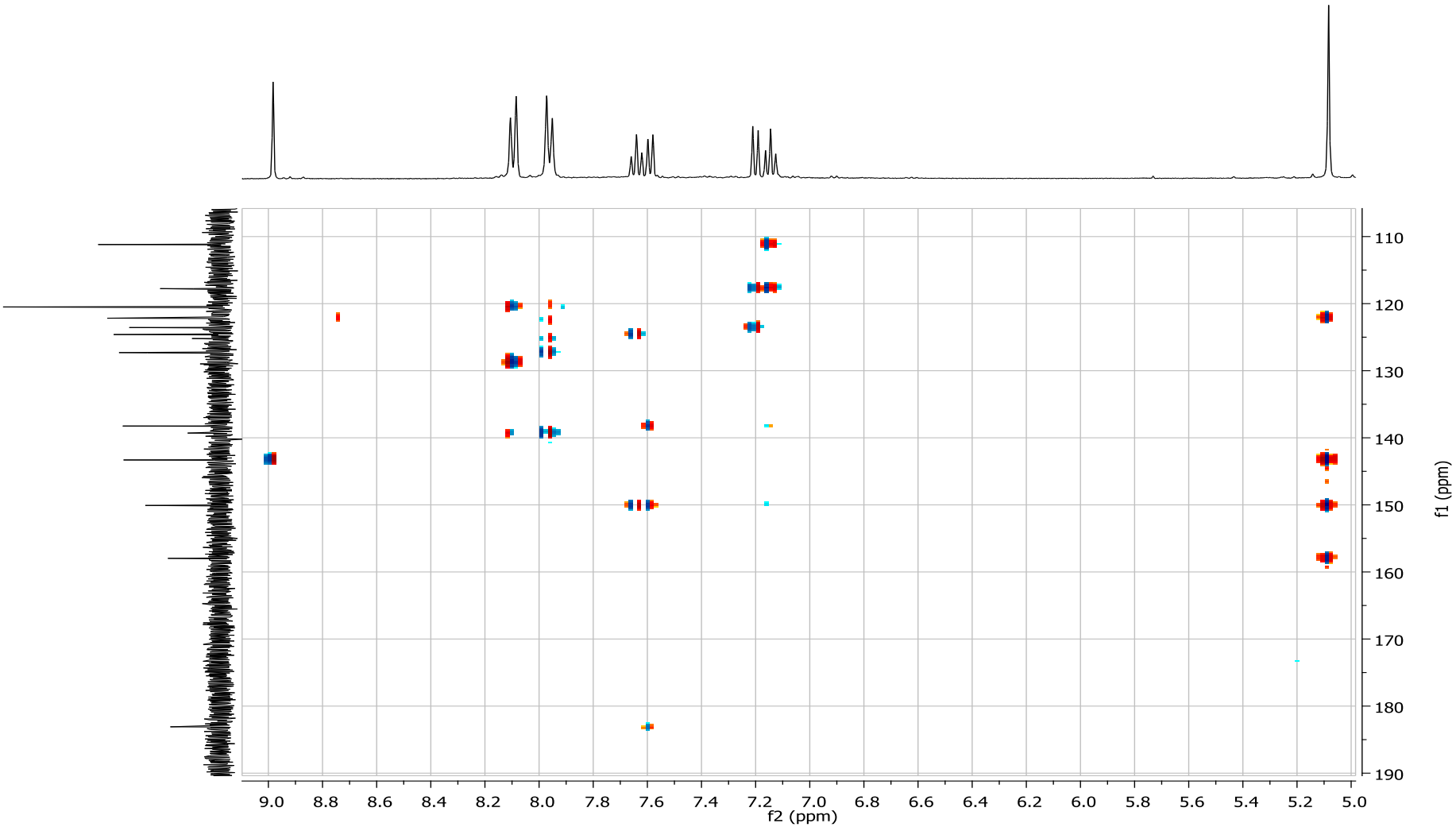


Figure S43. HMBC spectrum (400 MHz, DMSO-d_6) of compound **4h**.

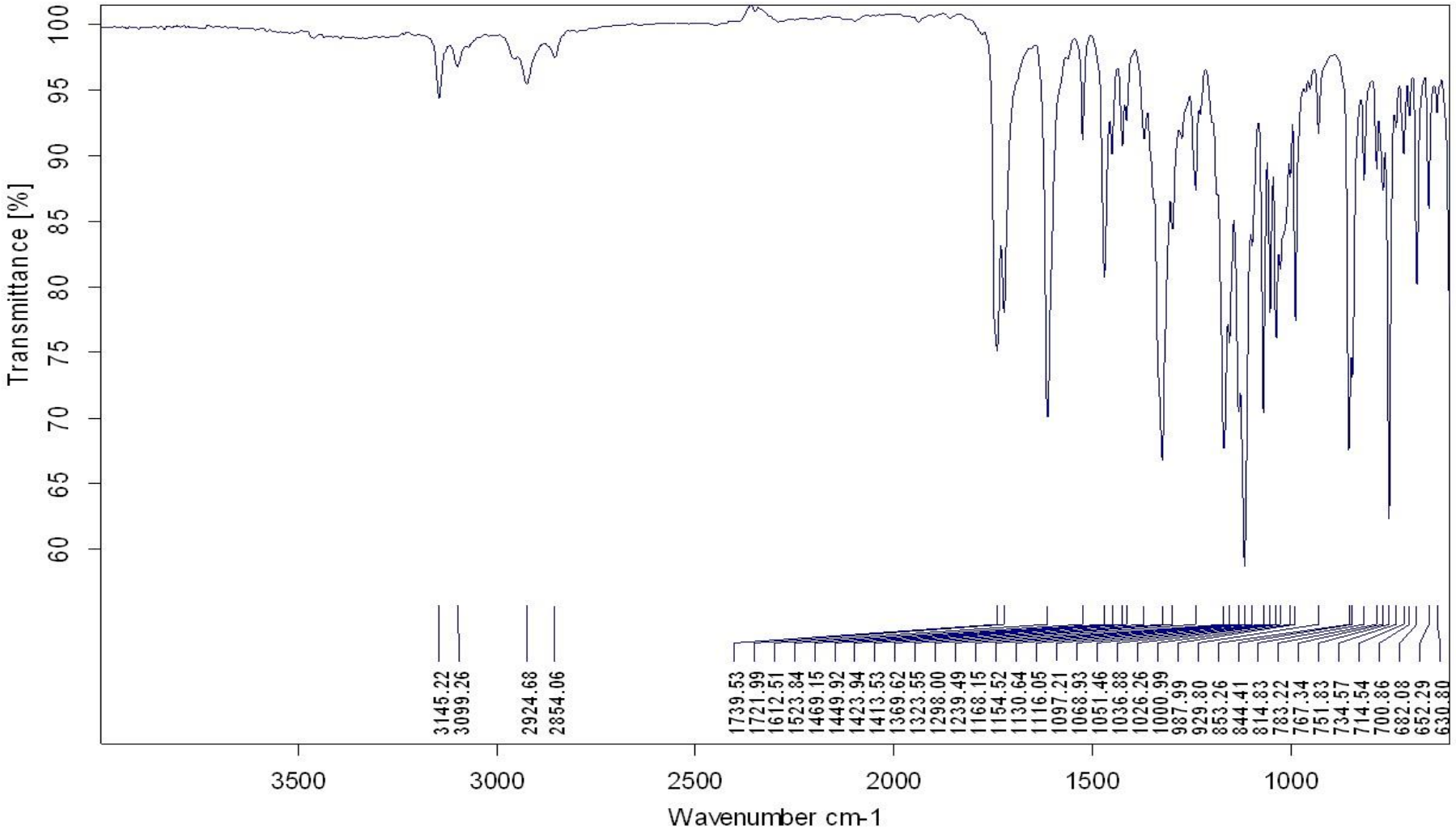


Figure S44. ATR-IR spectrum of compound **4h**.

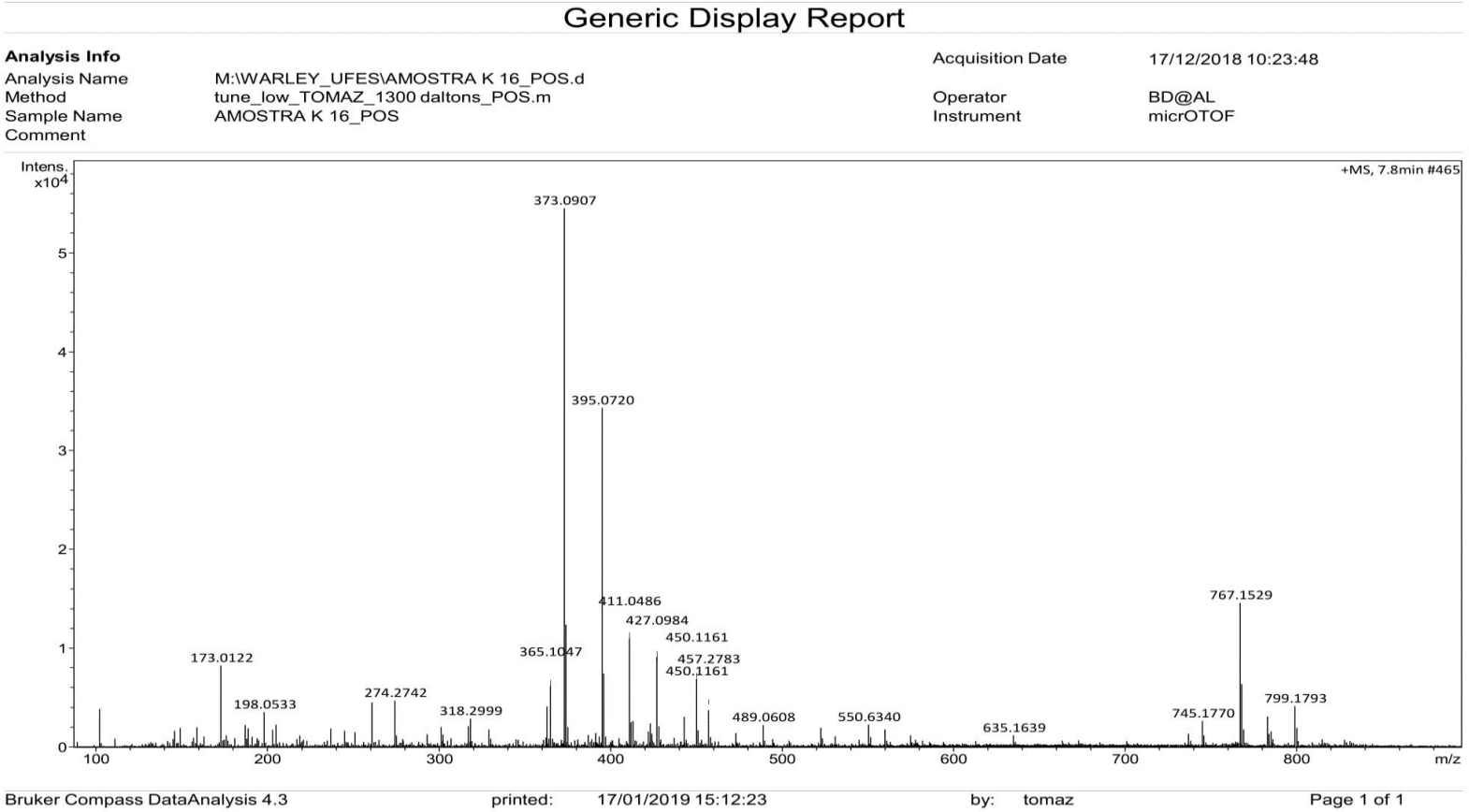


Figure S45. HRMS spectrum of compound **4h**.

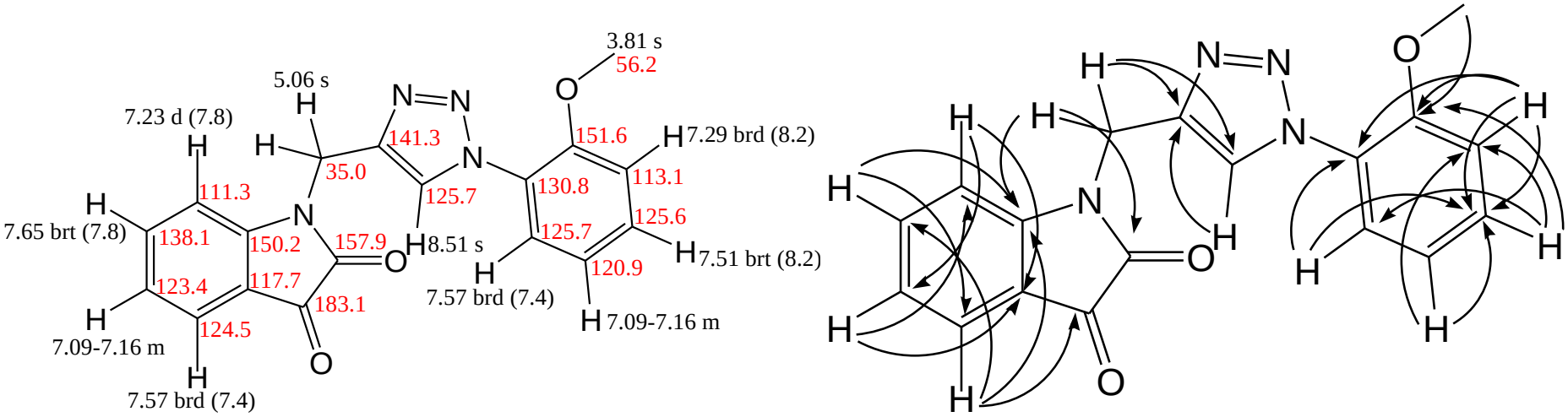


Figure S46. ^{1}H (black) and ^{13}C (red) NMR chemical shifts (ppm) and main HMBC correlation observed in the NMR data of the compound **4i**. *J* in Hz.

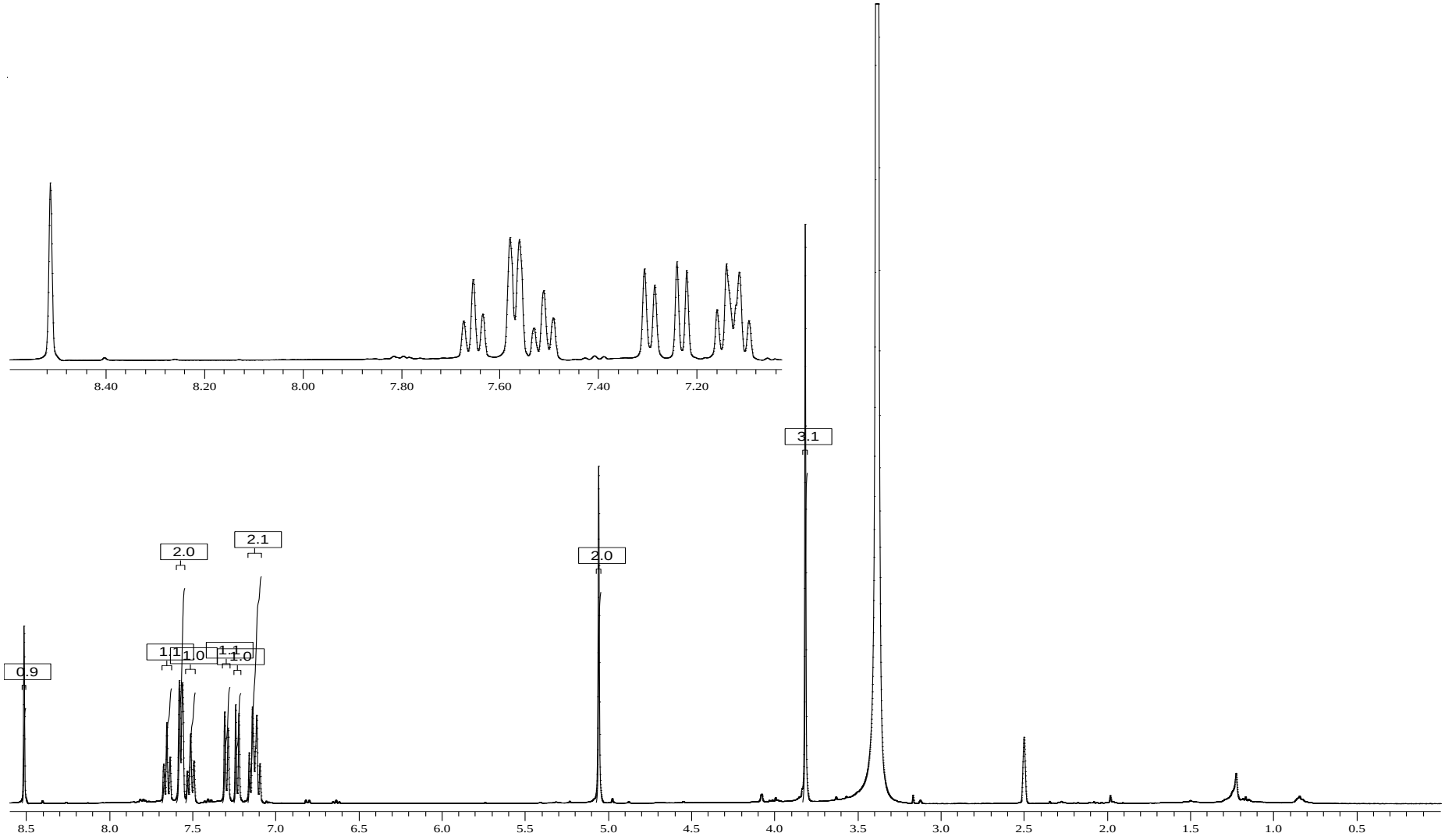


Figure S47. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **4i**.

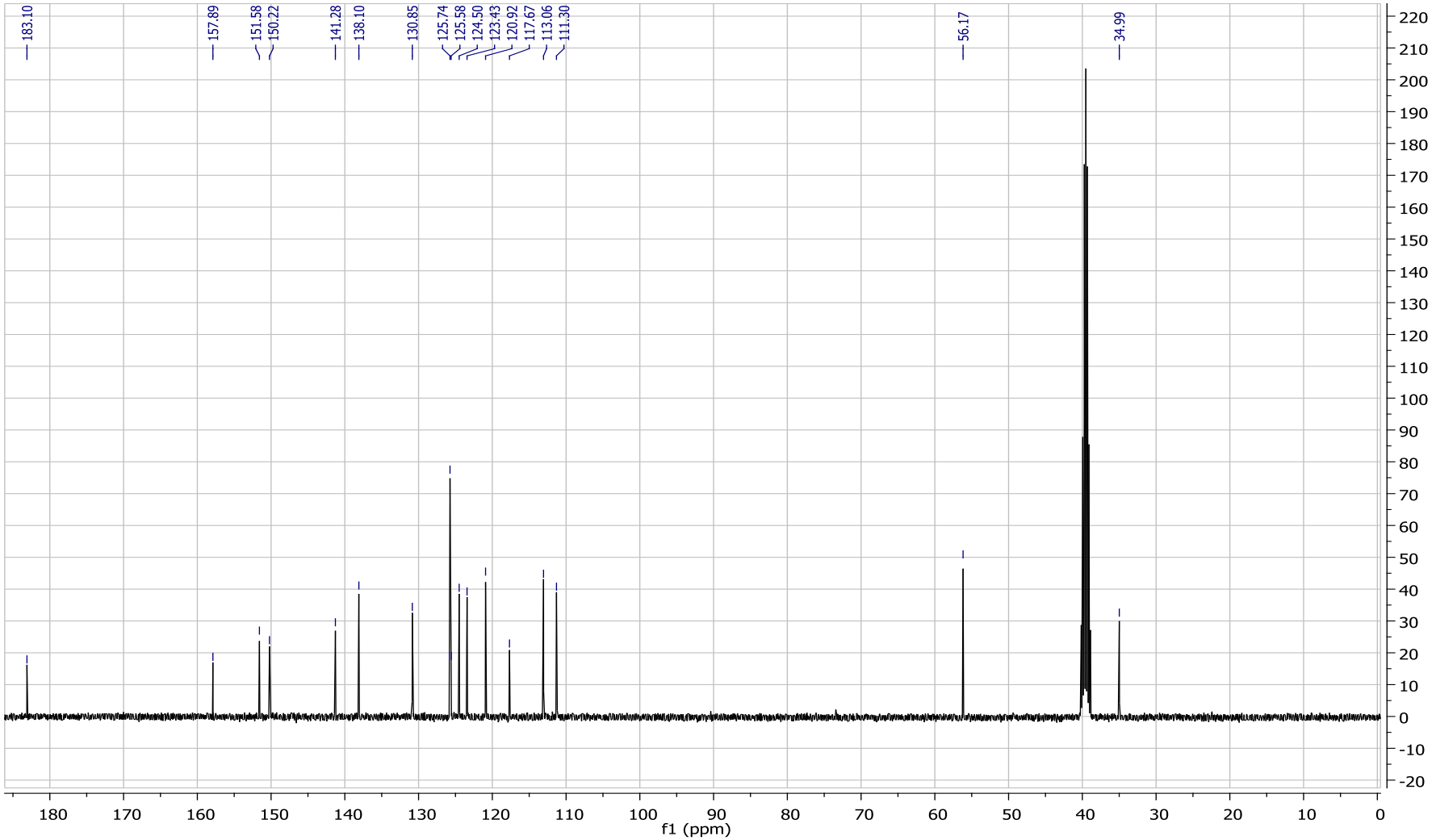


Figure S48. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **4i**.

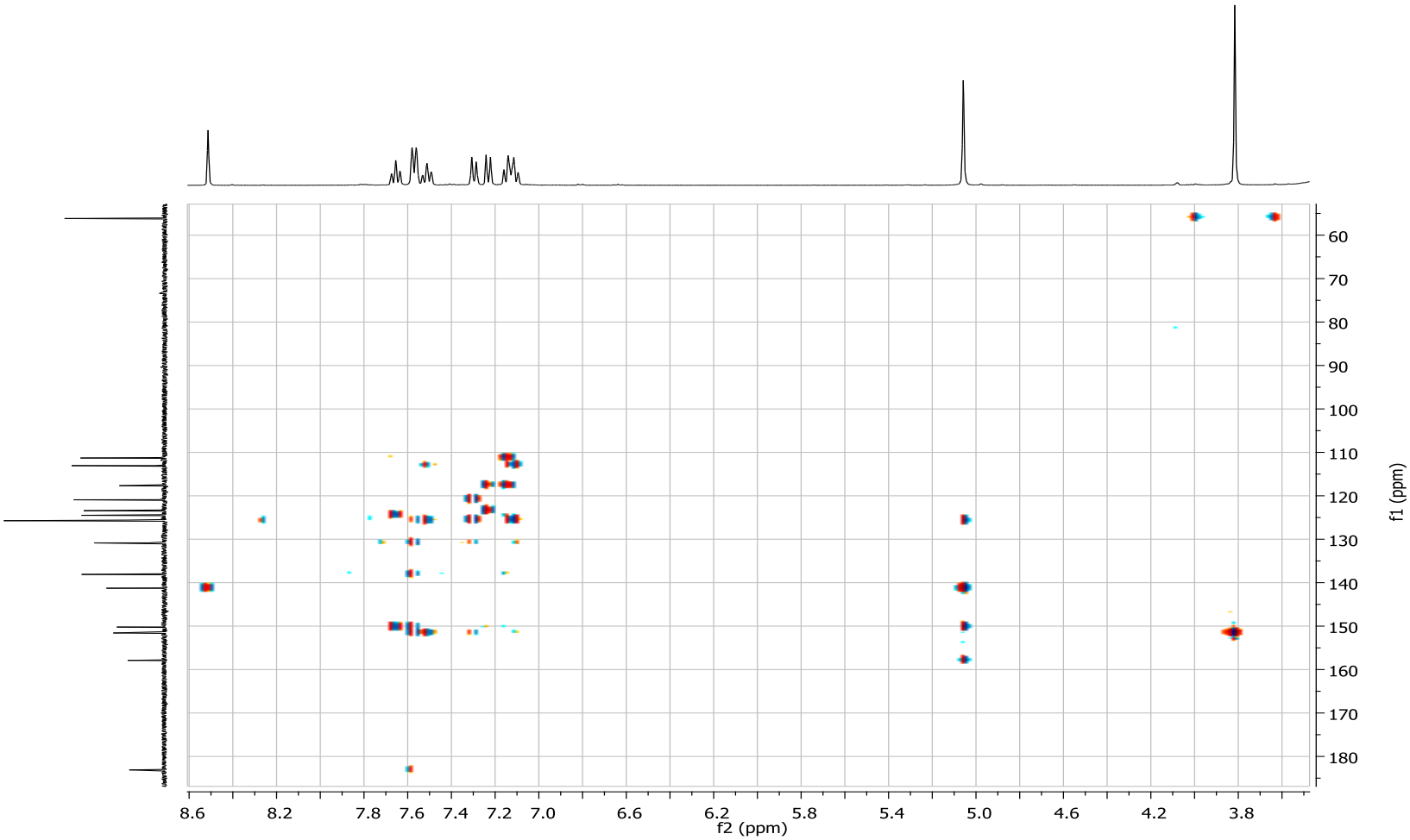


Figure S49. HMBC spectrum (400 MHz, DMSO-d_6) of compound **4i**.

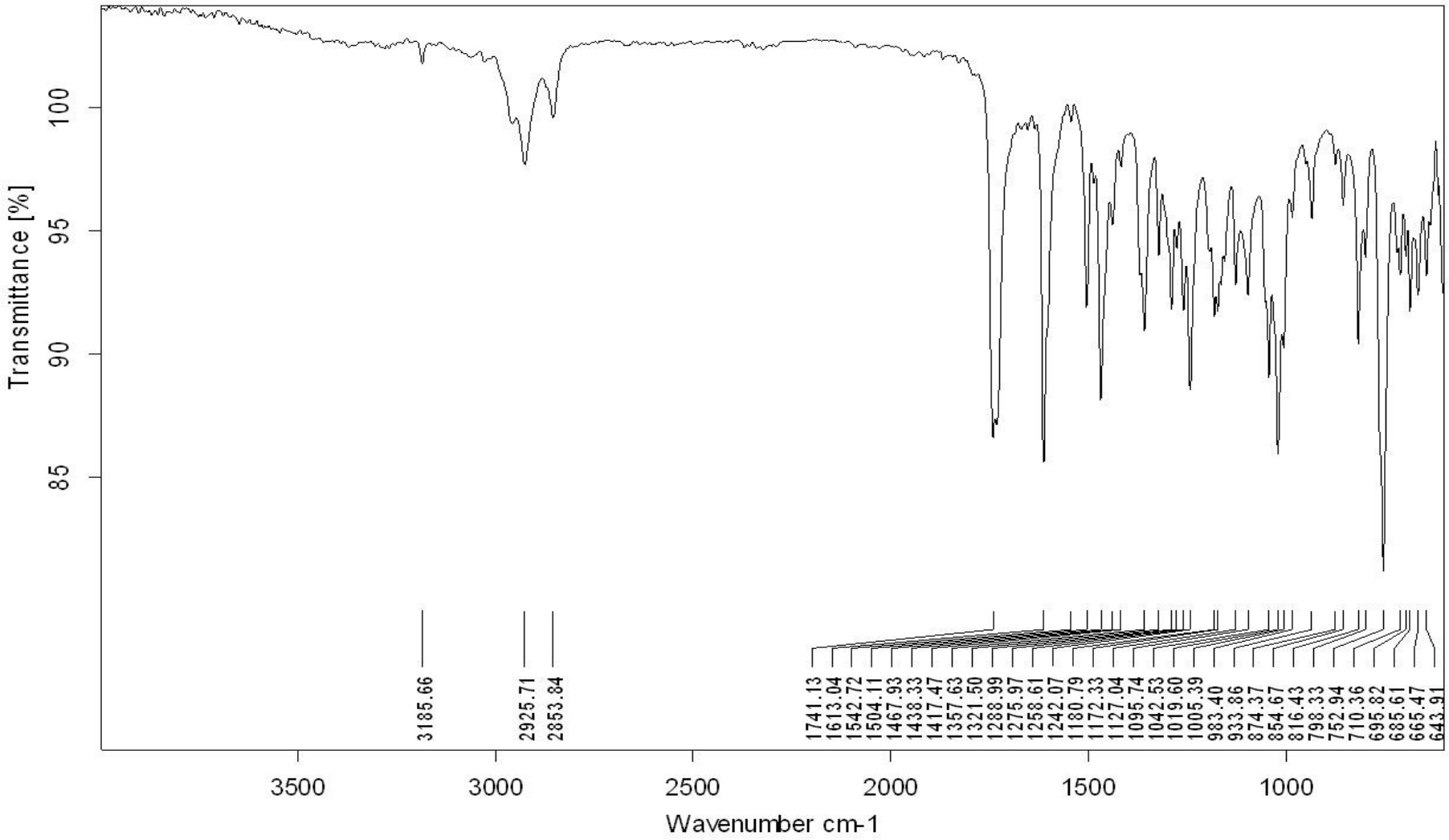


Figure S50. ATR-IR spectrum of compound **4i**.

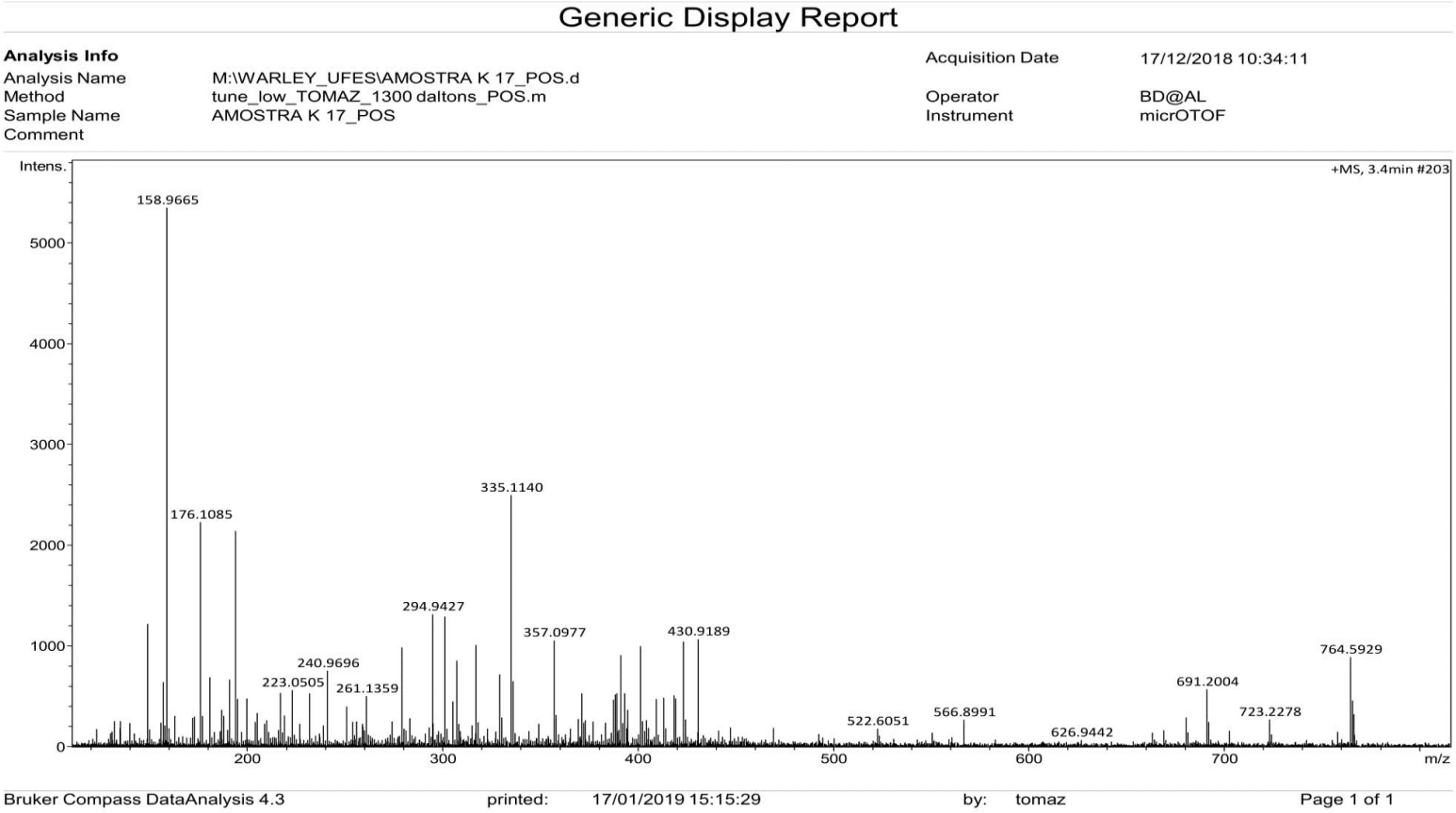


Figure S51. HRMS spectrum of compound **4i**.

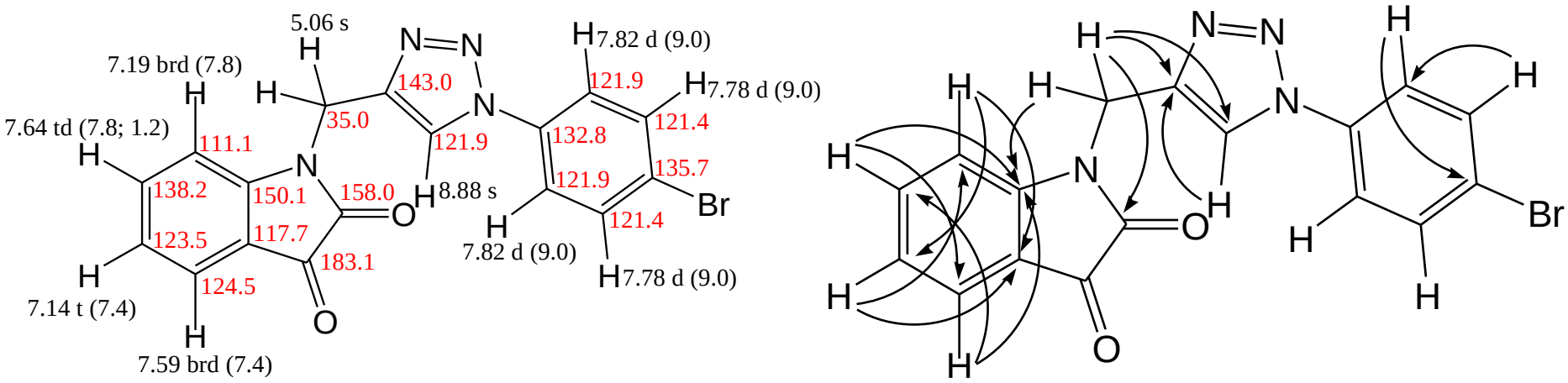


Figure S52. ^{1}H (black) and ^{13}C (red) NMR chemical shifts (ppm) and main HMBC correlation observed in the NMR data of the compound **4j**. *J* in Hz.

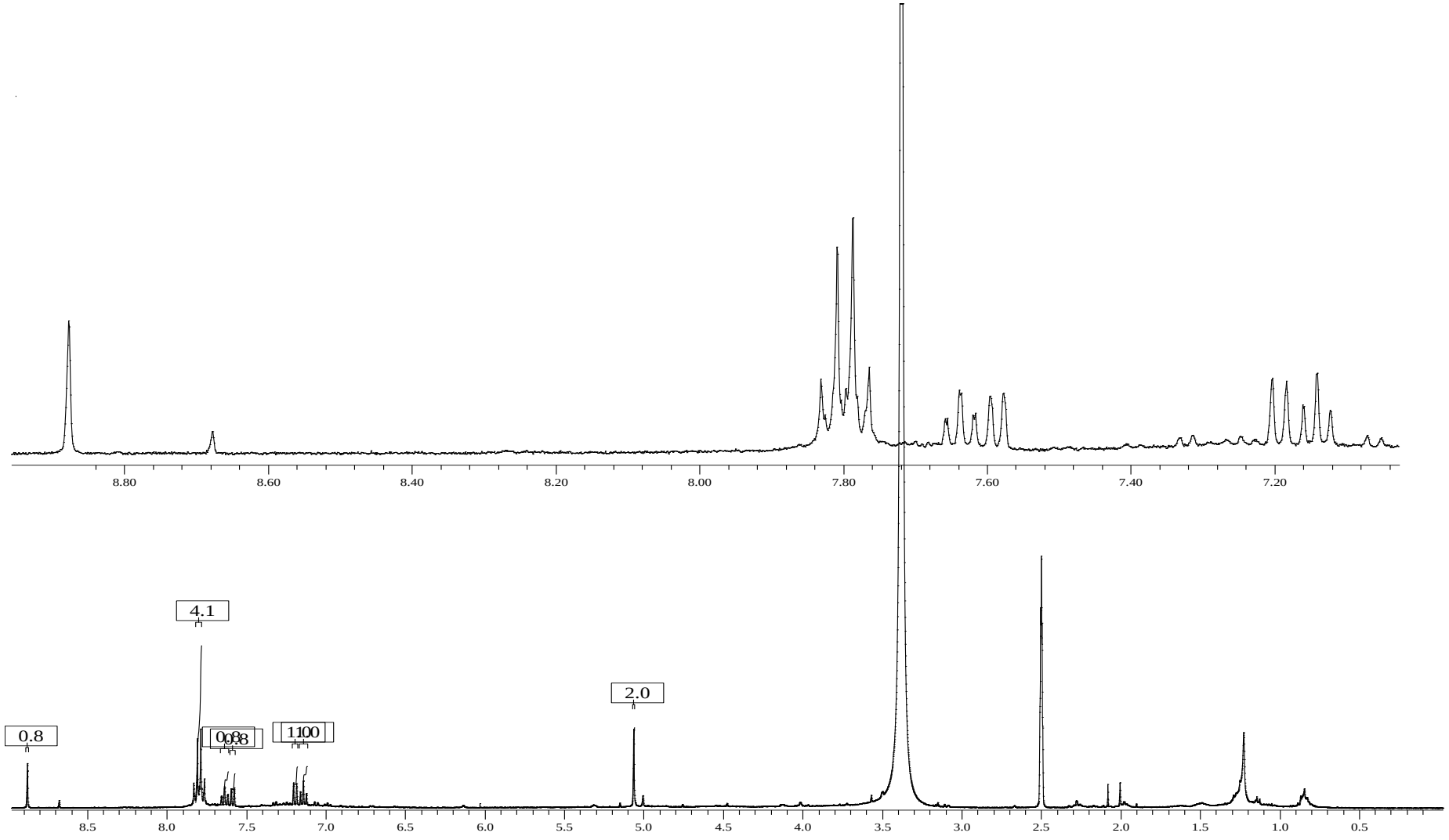


Figure S53. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **4j**.

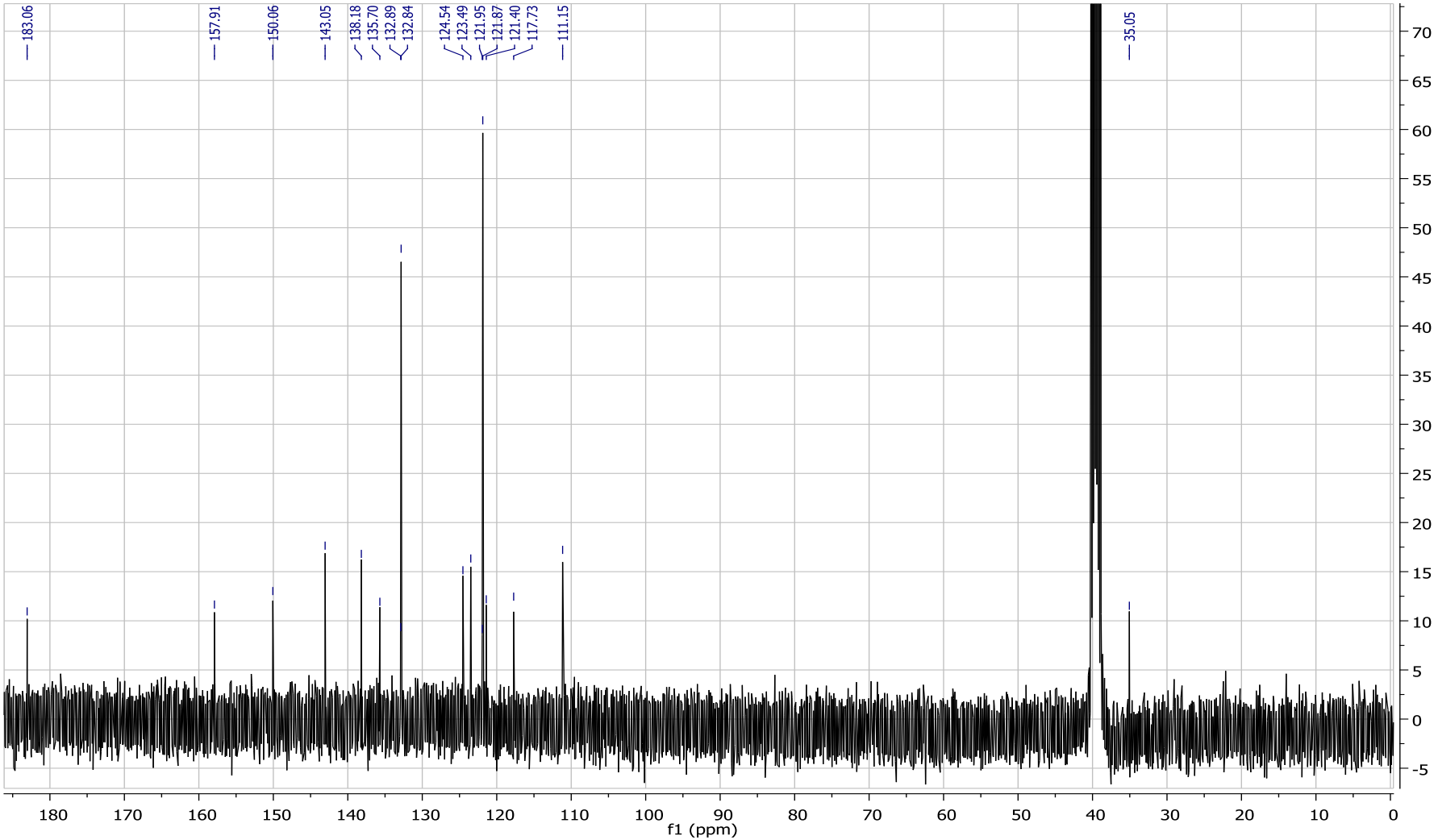


Figure S54. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **4j**.

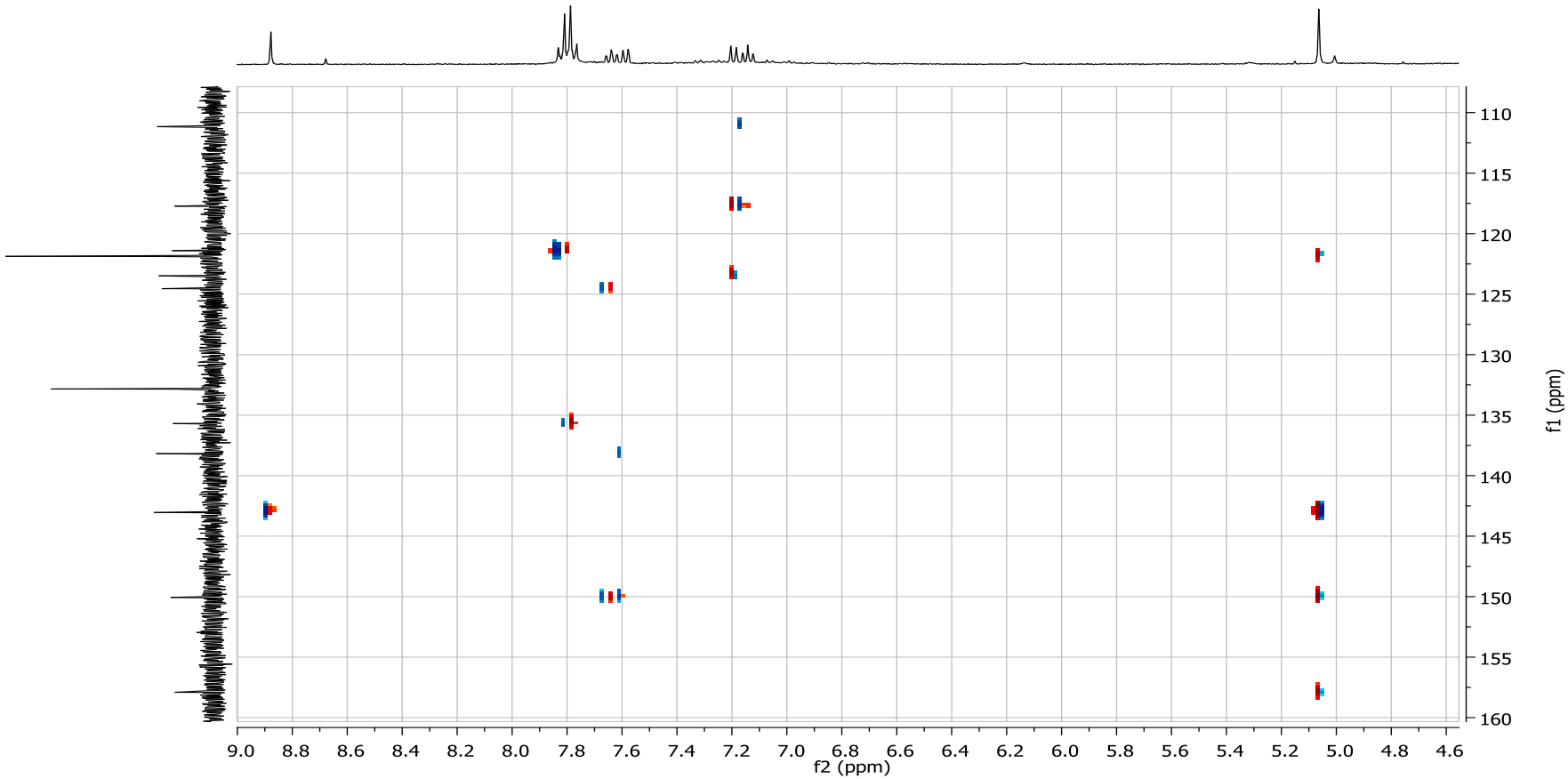


Figure S55. HMBC spectrum (400 MHz, DMSO-d_6) of compound **4j**.

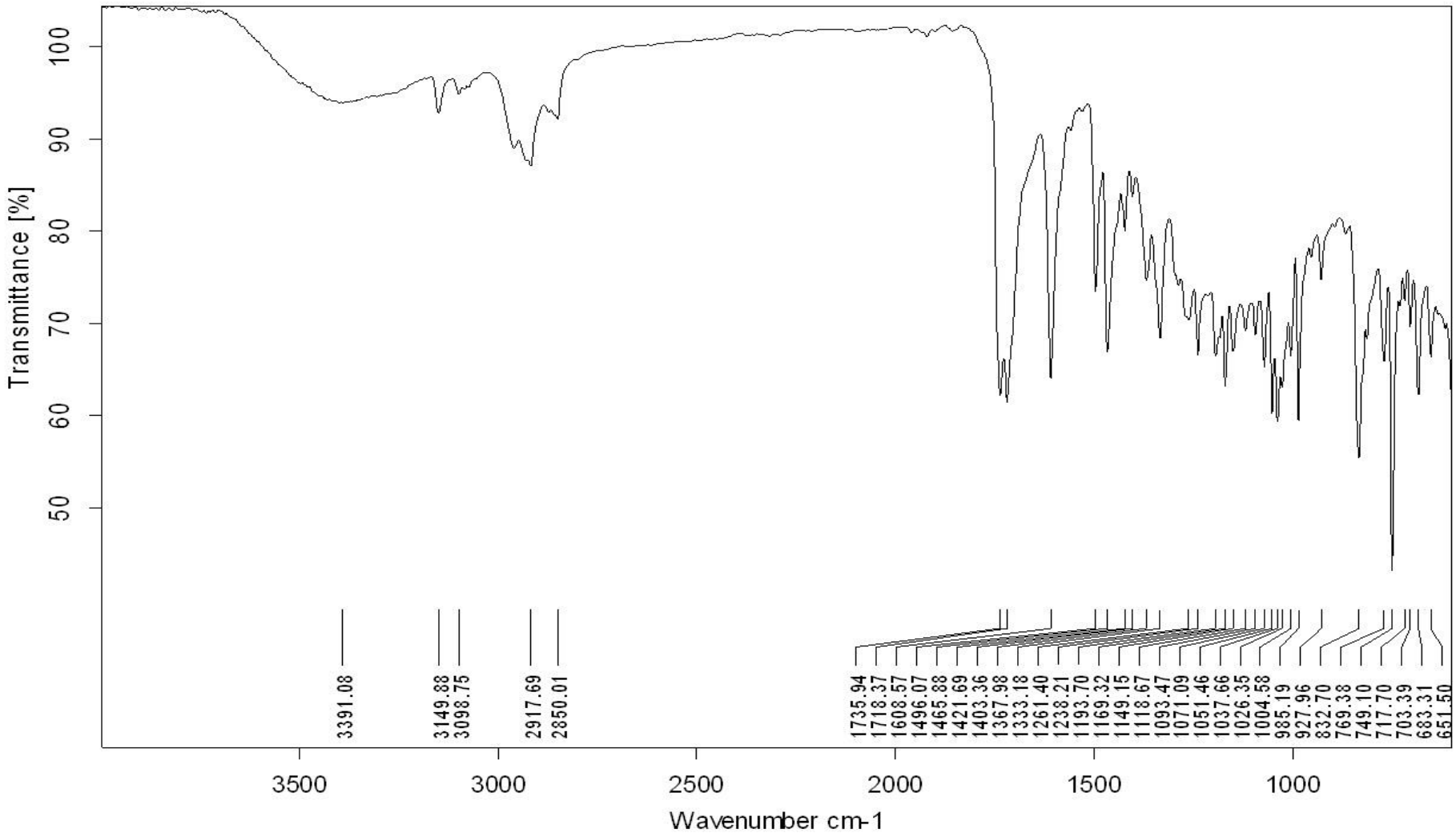


Figure S56. ATR-IR spectrum of compound **4j**.

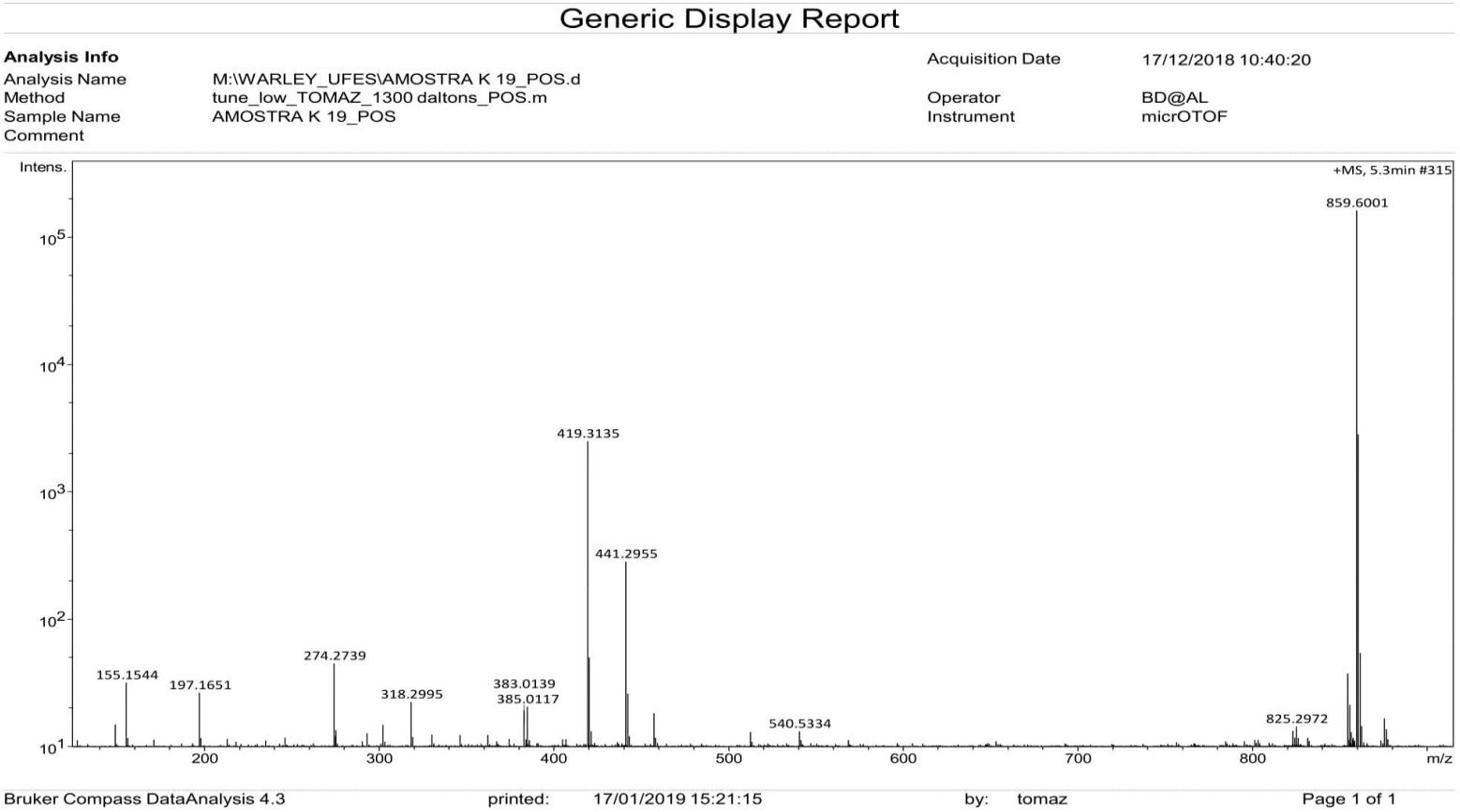


Figure S57. HRMS spectrum of compound **4j**.

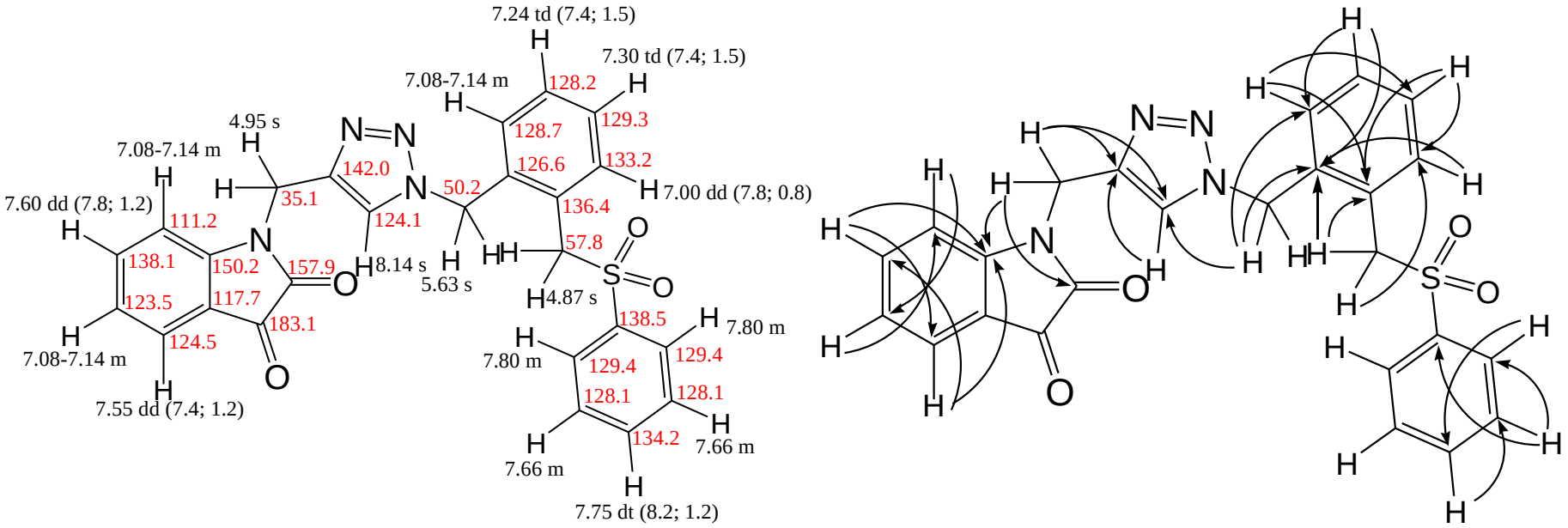


Figure S58. ^{1}H (black) and ^{13}C (red) NMR chemical shifts (ppm) and main HMBC correlation observed in the NMR data of the compound **4k**. *J* in Hz.

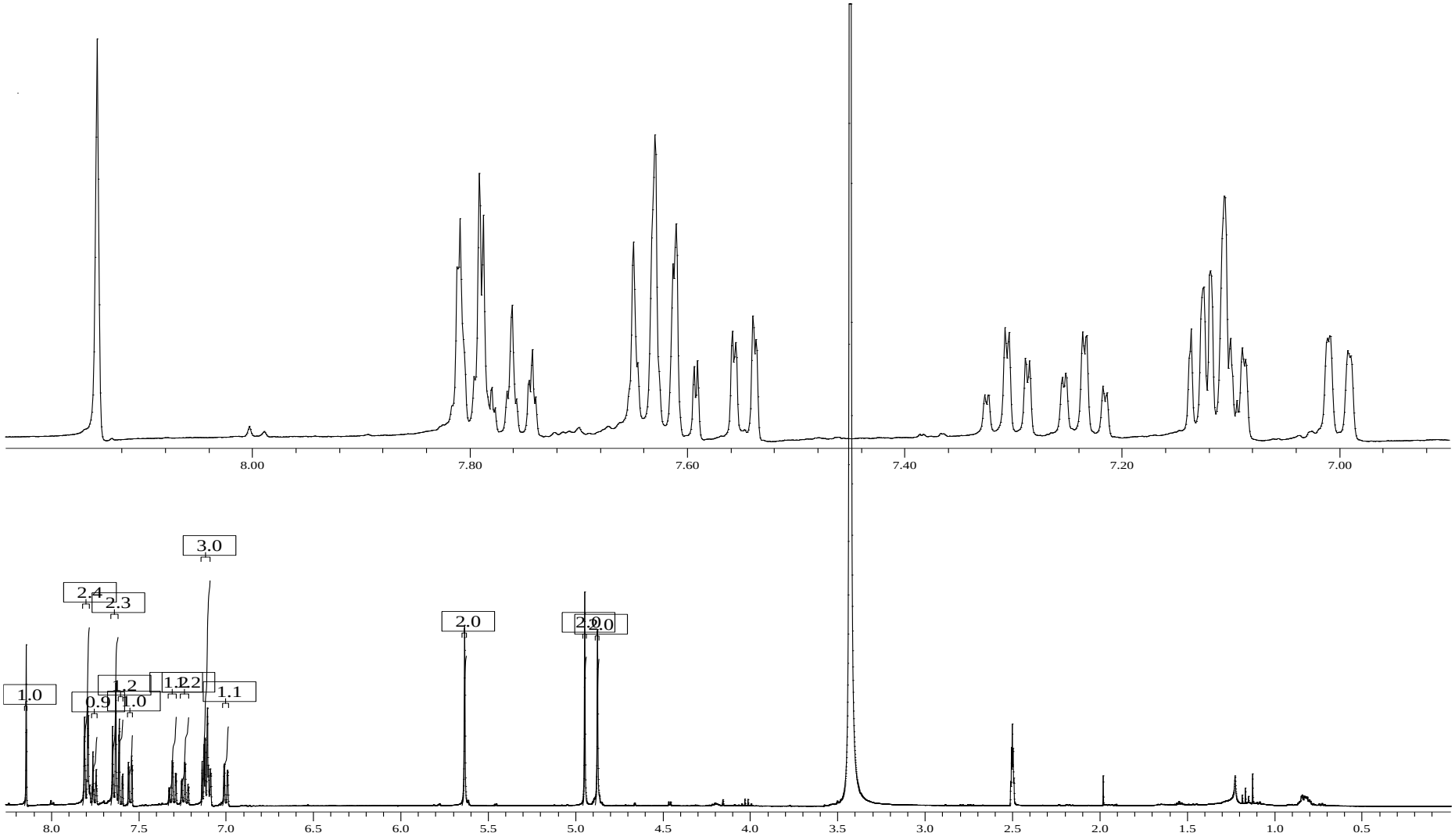


Figure S59. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **4k**.

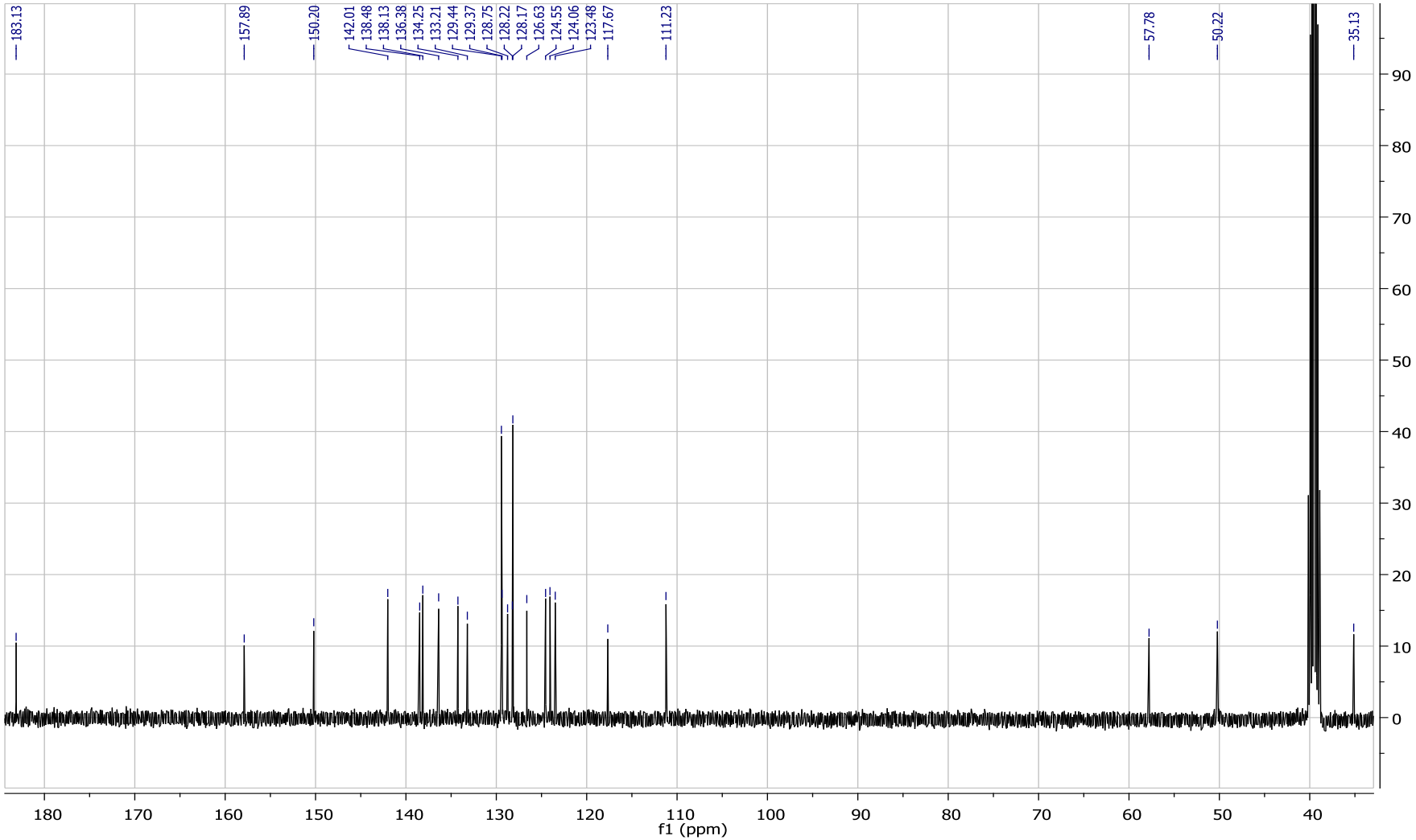


Figure S60. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **4k**.

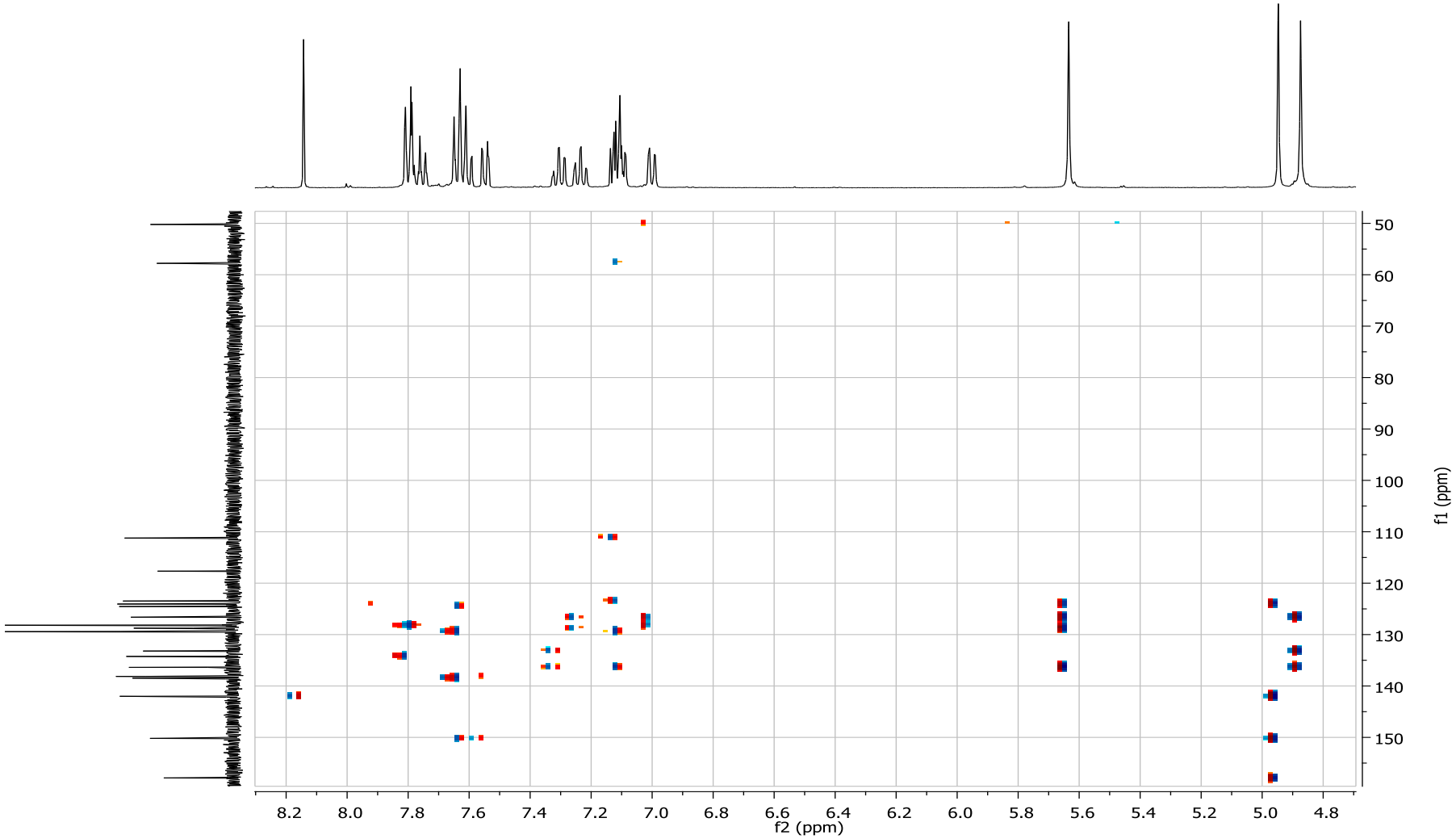


Figure S61. HMBC spectrum (400 MHz, DMSO-d_6) of compound **4k**.

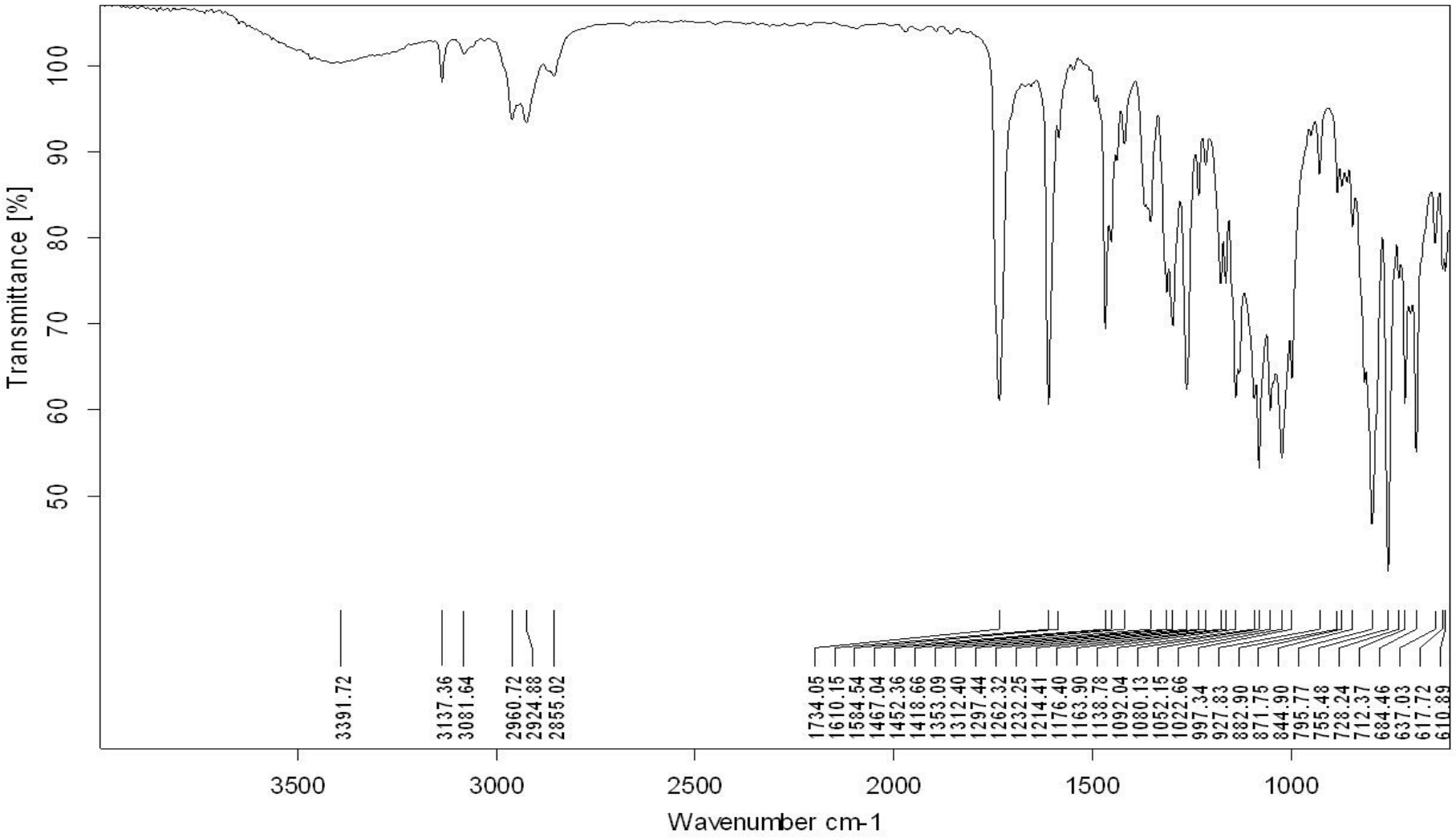


Figure S62. ATR-IR spectrum of compound **4k**.

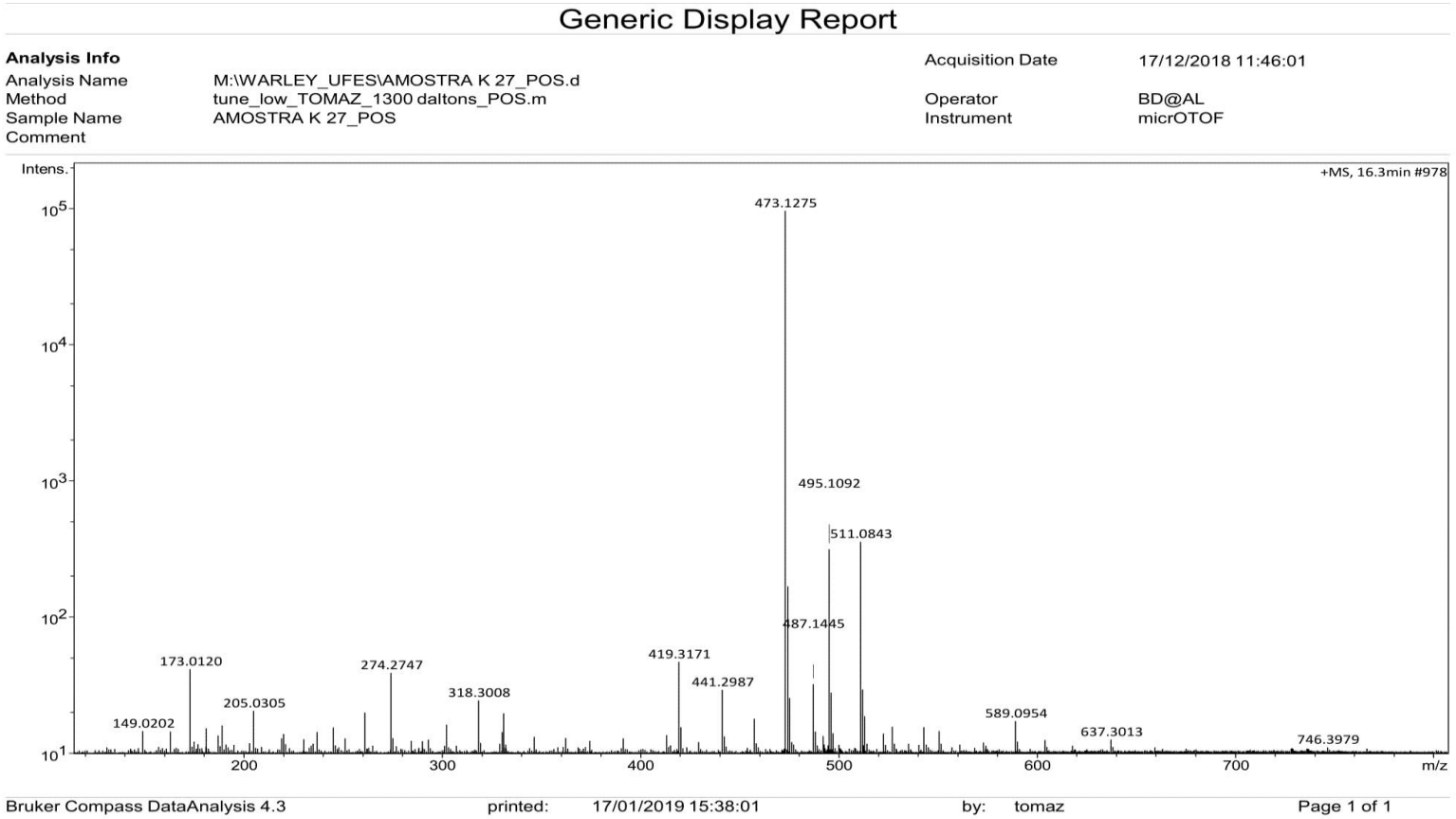


Figure S63. HRMS spectrum of compound **4k**.

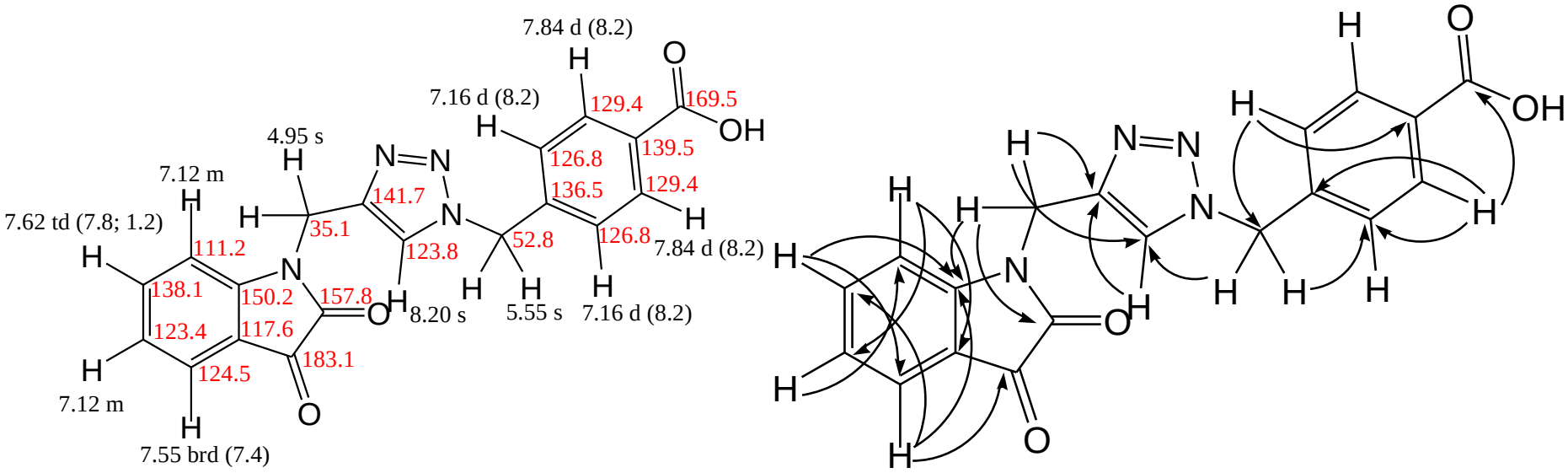


Figure S64. ^{1}H (black) and ^{13}C (red) NMR chemical shifts (ppm) and main HMBC correlation observed in the NMR data of the compound **4l**. *J* in Hz.

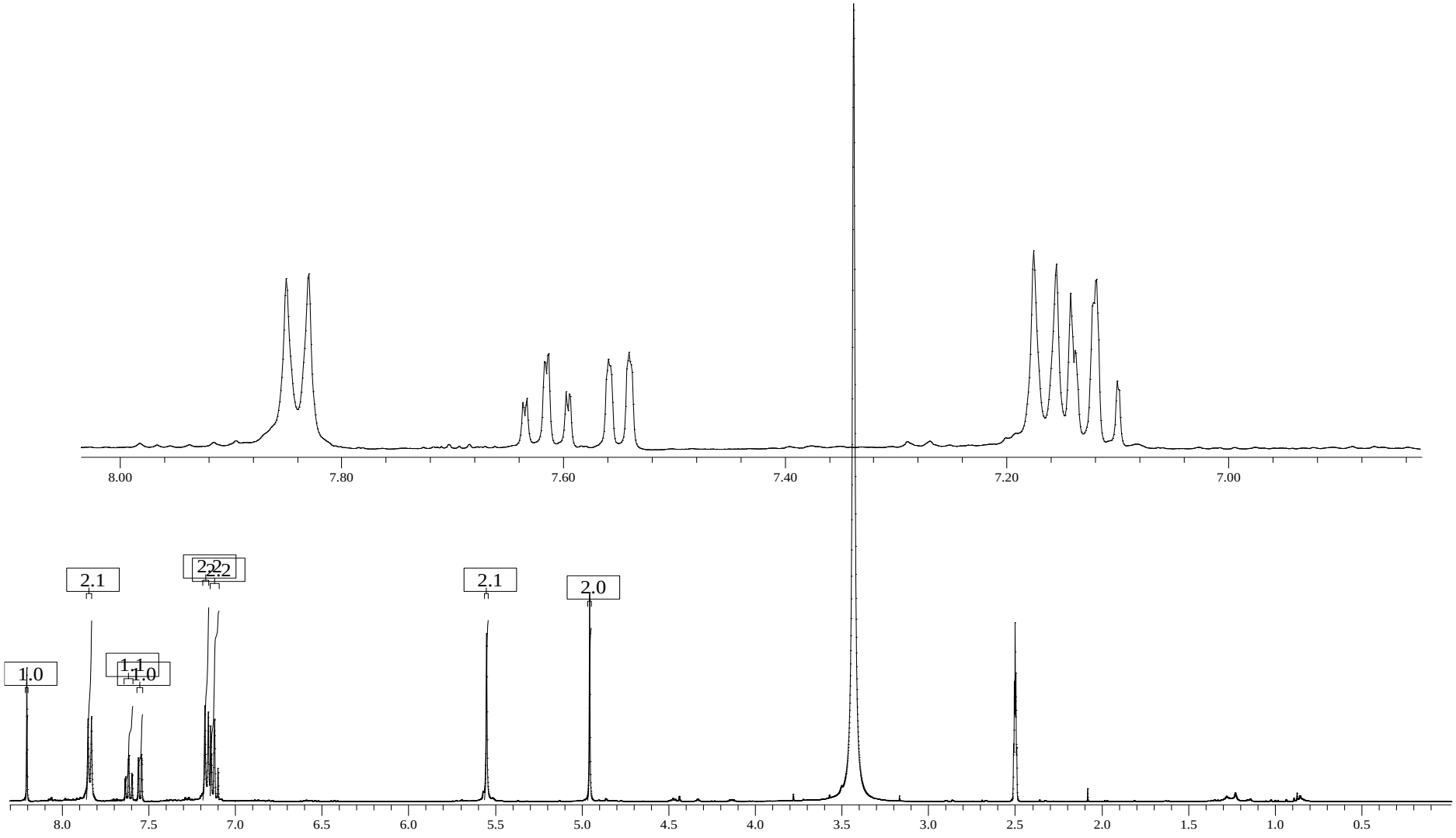


Figure S65. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **4l**.

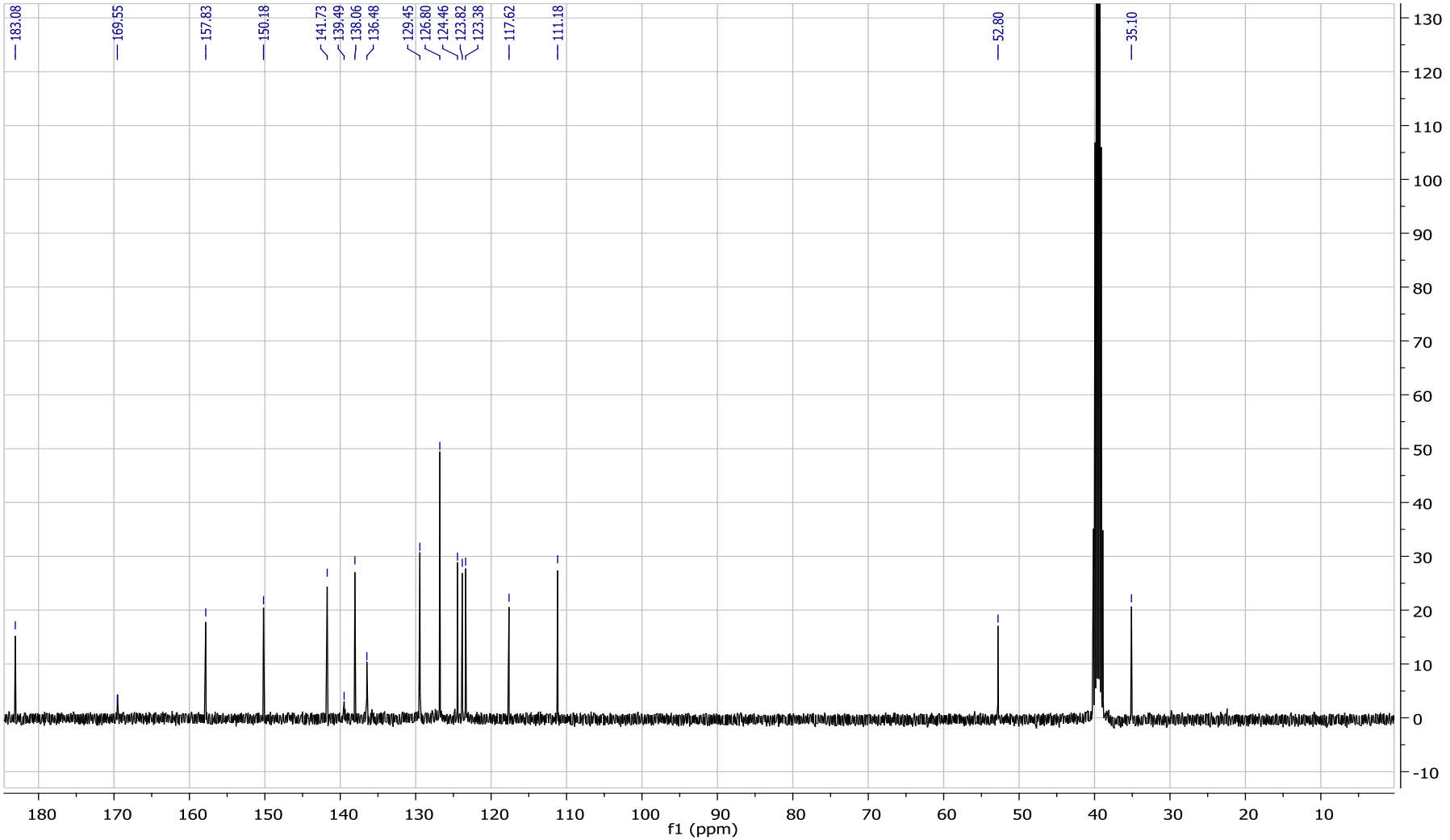


Figure S66. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **4l**.

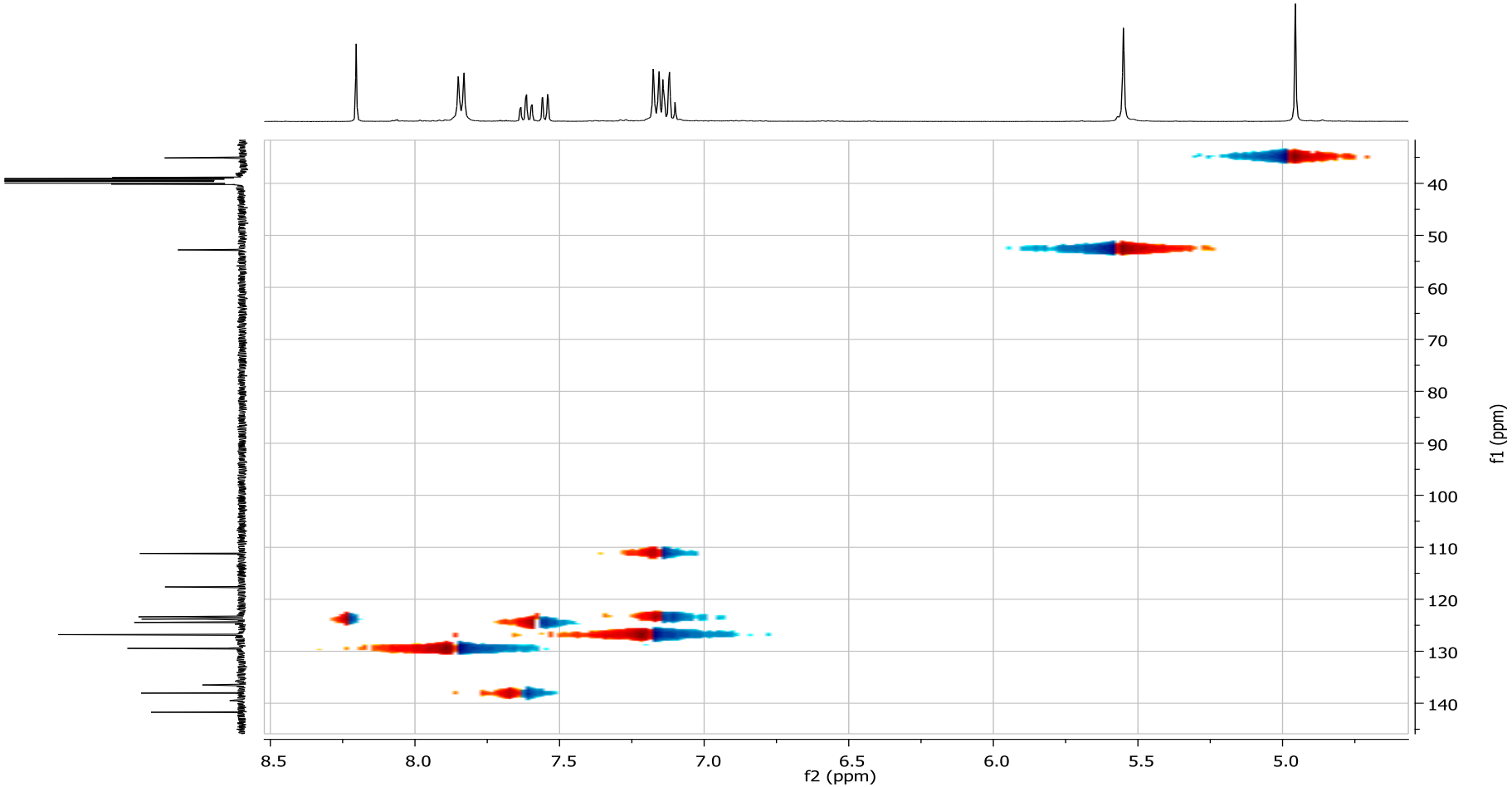


Figure S67. HSQC spectrum (100 MHz, DMSO-d_6) of compound **4l**.

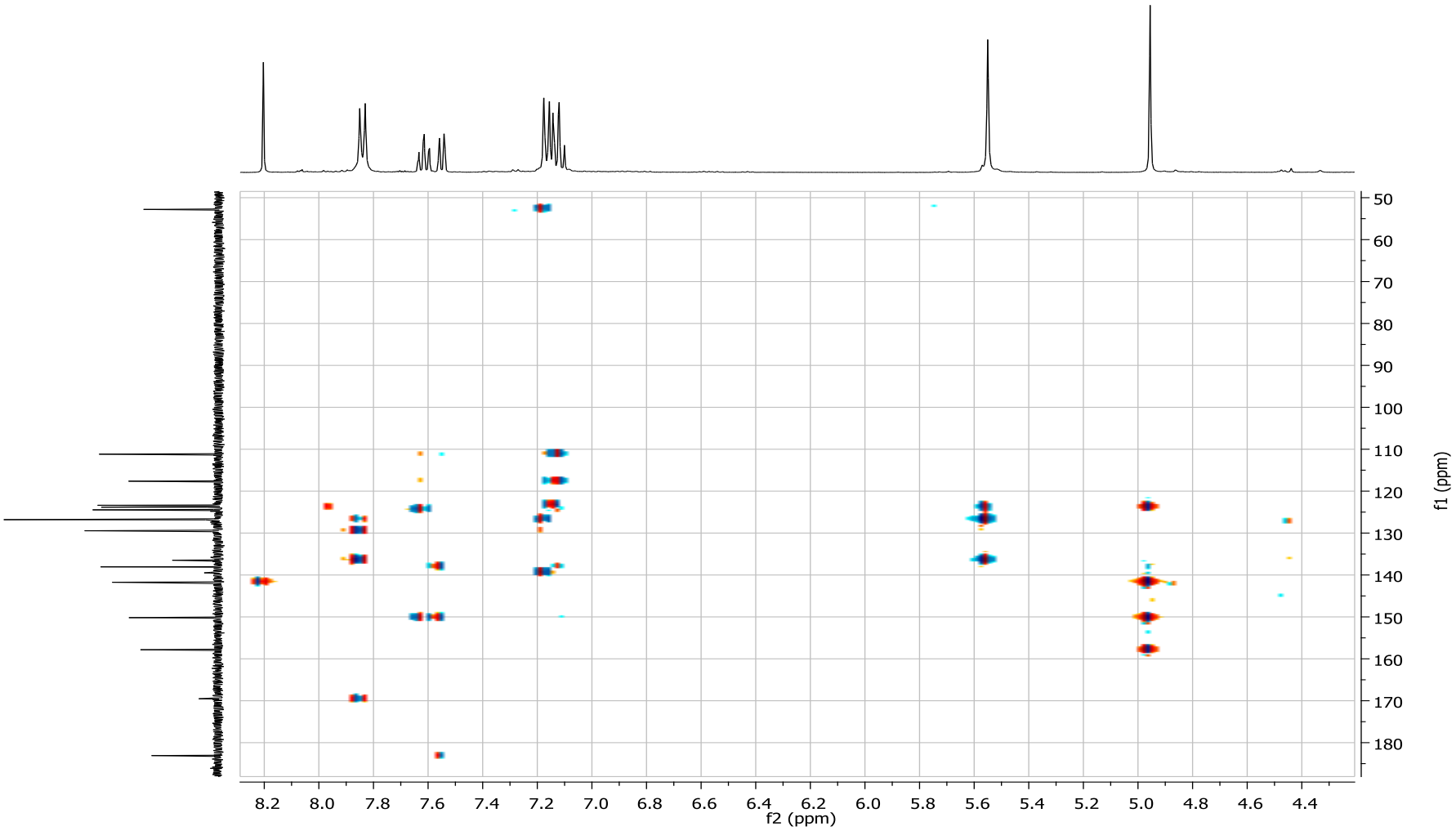


Figure S68. HMBC spectrum (400 MHz, DMSO-d_6) of compound **4l**.

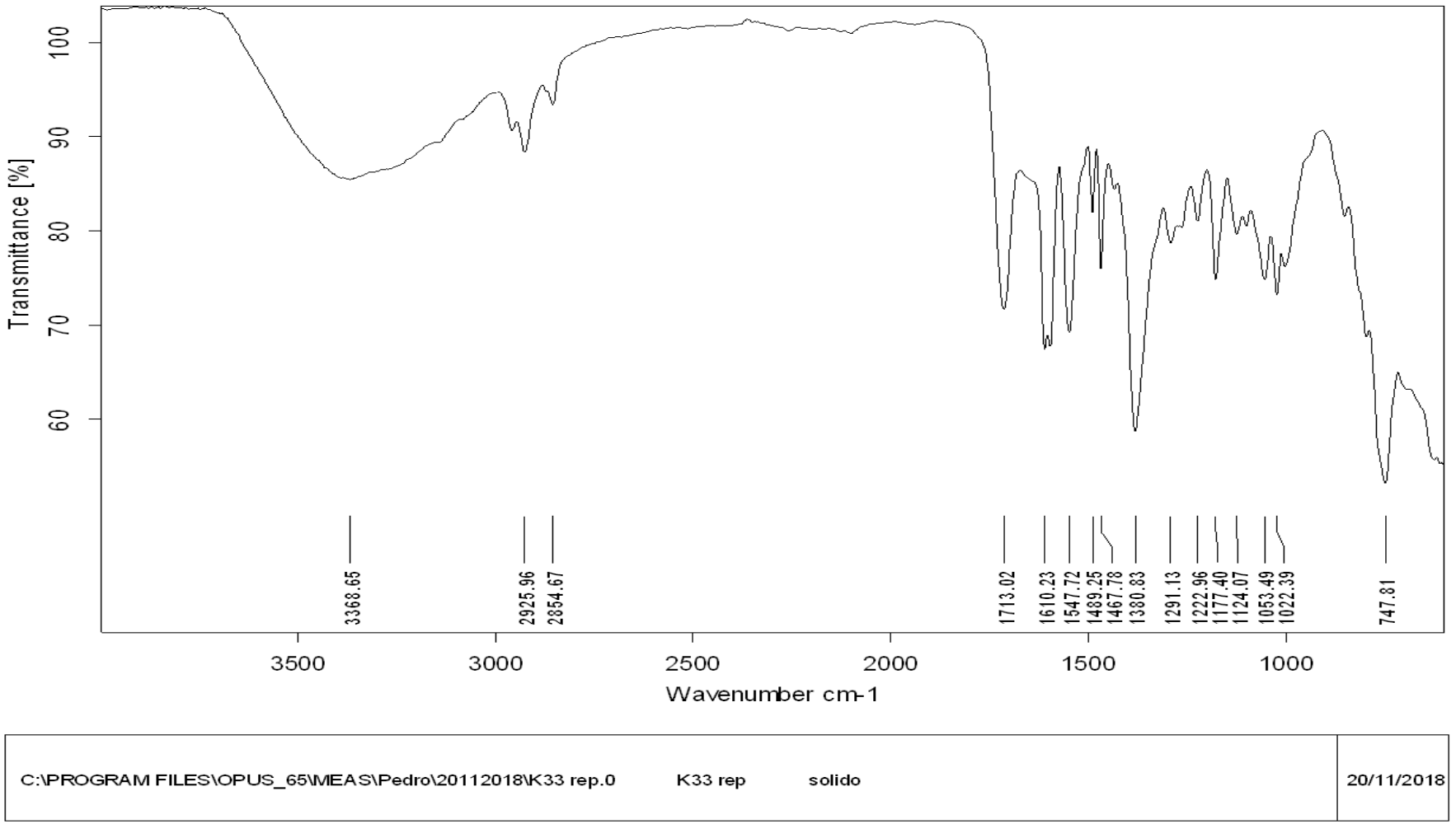


Figure S69. ATR-IR spectrum of compound **4l**.

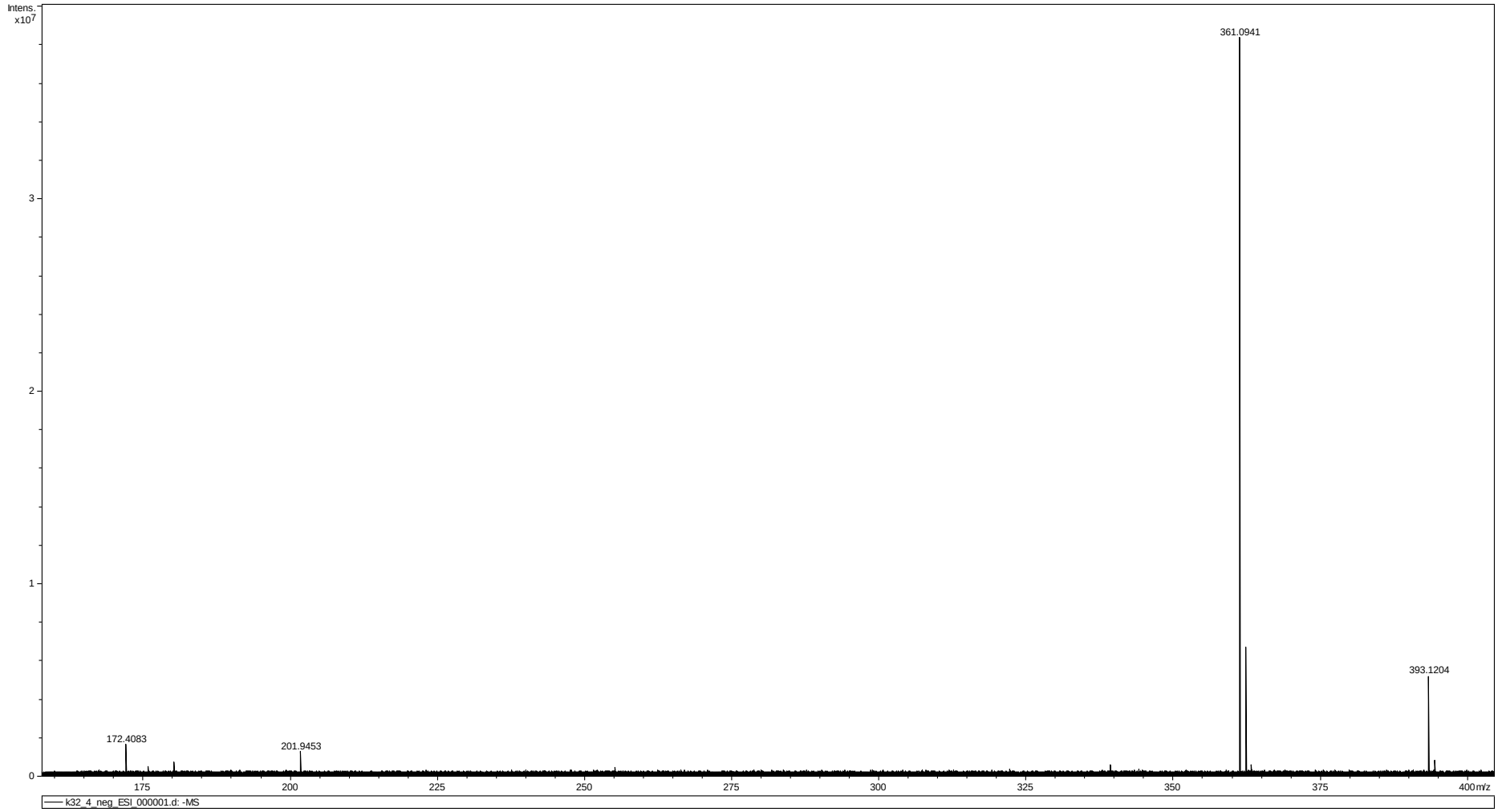
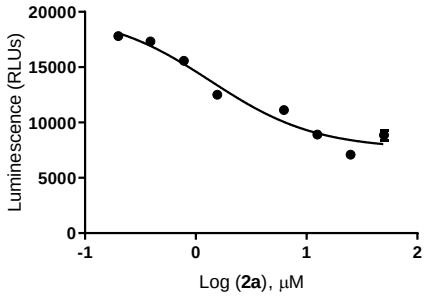
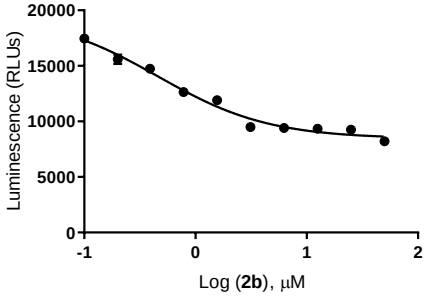


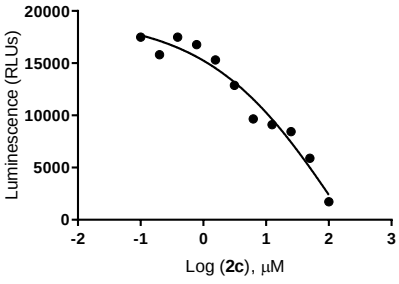
Figure S70. HRMS spectrum of compound **4l**.



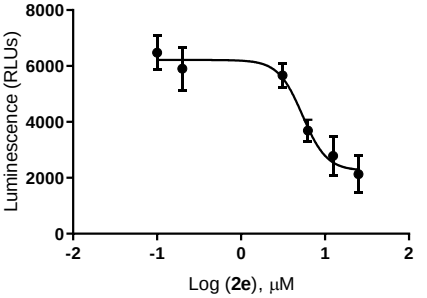
Dose-response curve generated for **2a** derivative



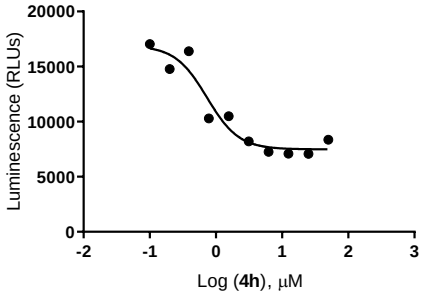
Dose-response curve generated for **2b** derivative



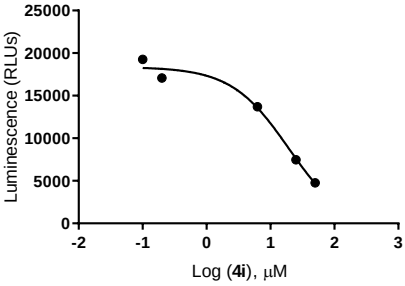
Dose-response curve generated for **2c** derivative



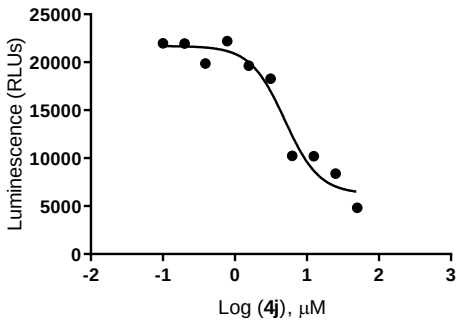
Dose-response curve generated for **2e** derivative



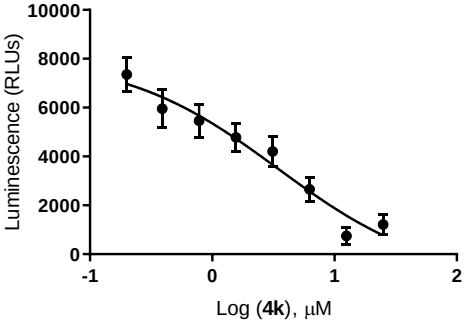
Dose-response curve generated for **4h** derivative



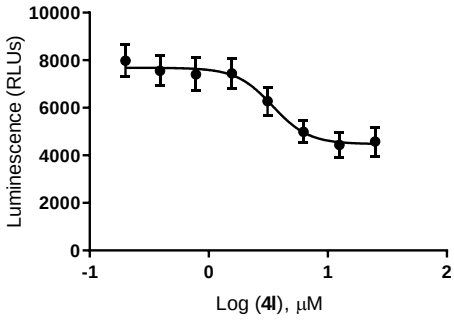
Dose-response curve generated for **4i** derivative



Dose-response curve generated for **4j** derivative



Dose-response curve generated for **4k** derivative



Dose-response curve generated for **4l** derivative

Figure S71. Dose-response curves generated by GraphPad Prism[8] program for compounds **2a-e** and **4h-l** studied against GSK-3β enzyme, used to calculate the IC_{50} values.

References

1. Furdas, S. D.; Shekfeh, S.; Kannan, S.; Sippl, W.; Jung, M.; *MedChemComm* **2012**, *3*, 305.
2. Makhija, M. T.; Kasliwal, R. T.; Kulkarni, V. M.; Neamati, N.; *Bioorg. Med. Chem.* **2004**, *12*, 2317.
3. Gui, J.; Chen, G.; Cao, P.; Liao, J.; *Tetrahedron: Asymmetry* **2002**, *23*, 554.
4. Shi, F.; Tao, Z.-L.; Luo, S.-W.; Tu, S.-J.; Gong, L.-Z.; *Chem. - Eur. J.* **2012**, *18*, 6885.
5. Tehrani, K. H. M. E.; Hashemi, M.; Hassan, M.; Kobarfard, F.; Mohebbi, S.; *Chin. Chem. Lett.* **2016**, *27*, 221.
6. Bouhfid, R.; Joly, N.; Essassi, E. M.; Lequart, V.; Massoui, M.; Martin, P.; *Synth. Commun.* **2011**, *41*, 2096.
7. Macpherson, L. J.; Dubin, A. E.; Evans, M. J.; Marr, F.; Schultz, P. G.; Cravatt, B. F.; Patapoutian, A.; *Nature* **2007**, *445*, 541.
8. *GraphPad Prism*, 6.00 version; GraphPad Software Inc., San Diego, USA, 2018.