ELECTRONIC SUPPLEMENTARY MATERIAL

Anti-HIV and Antimicrobial Activity of 7-Hydrazino-8-hydroxyquinoline-Based Aromatic Hydrazones

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Figure S1. HPLC-HRMS analysis of the compound **I**.

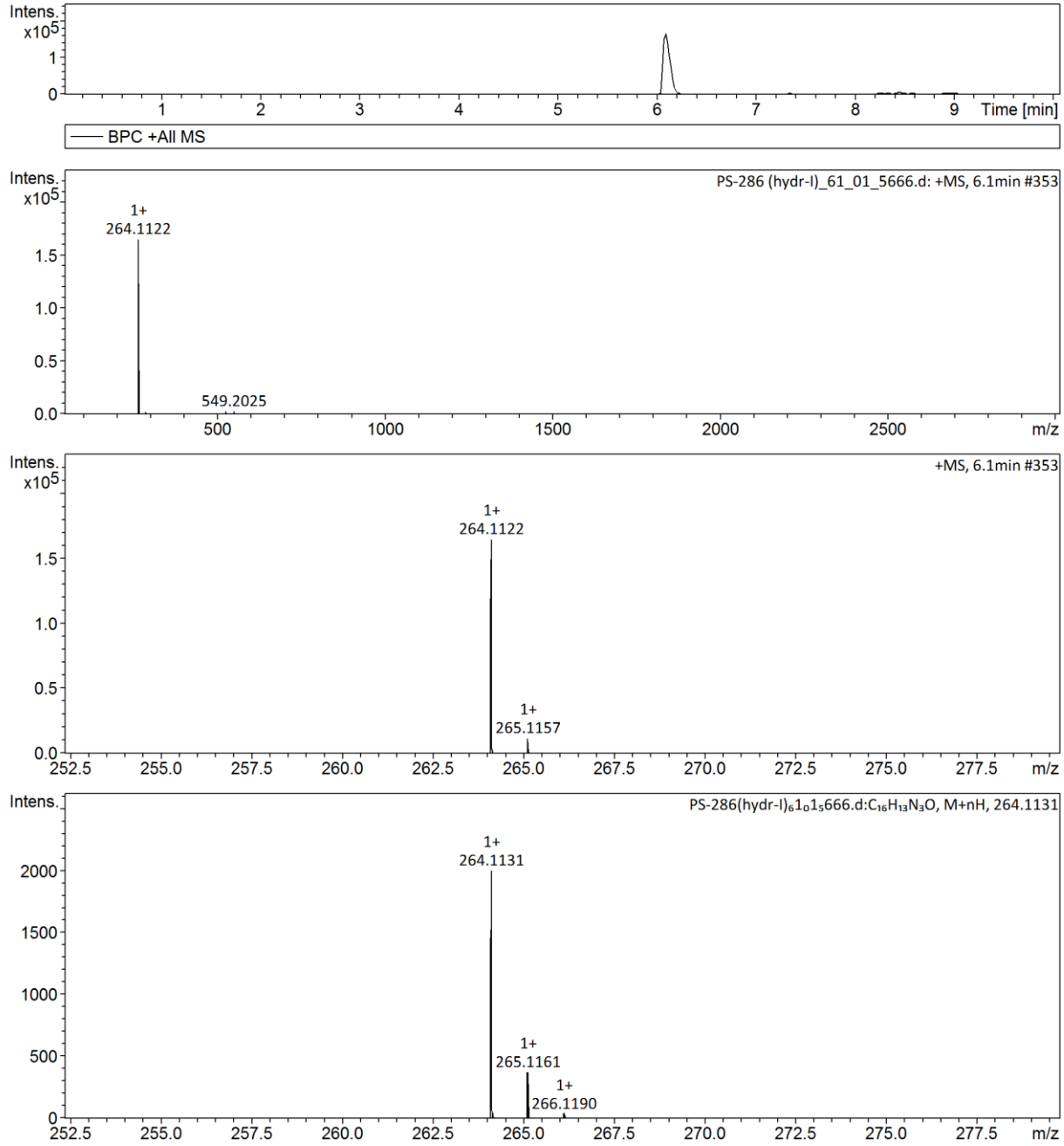
Display Report

Analysis Info

Analysis Name	D:\Data\PS-286 (hydr-I)_61_01_5666.d	Acquisition Date	8/8/2025 7:24:04 PM
Method	la_2.2_small2-nonfrg.m	Operator	BDAL@DE
Sample Name	PS-286 (hydr-I)	Instrument	compact 8255754.20088
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4300 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



PS-286 (hydr-I)_61_01_5666.d

Figure S2. HPLC-HRMS analysis of the compound **II**.

Display Report

Analysis Info

Analysis Name	D:\Data\PS-287 (hydr-II)_62_01_5667.d	Acquisition Date	8/8/2025 7:35:36 PM
Method	la_2.2_small2-nonfrg.m	Operator	BDAL@DE
Sample Name	PS-287 (hydr-II)	Instrument	compact 8255754.20088
Comment			

Acquisition Parameter

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Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
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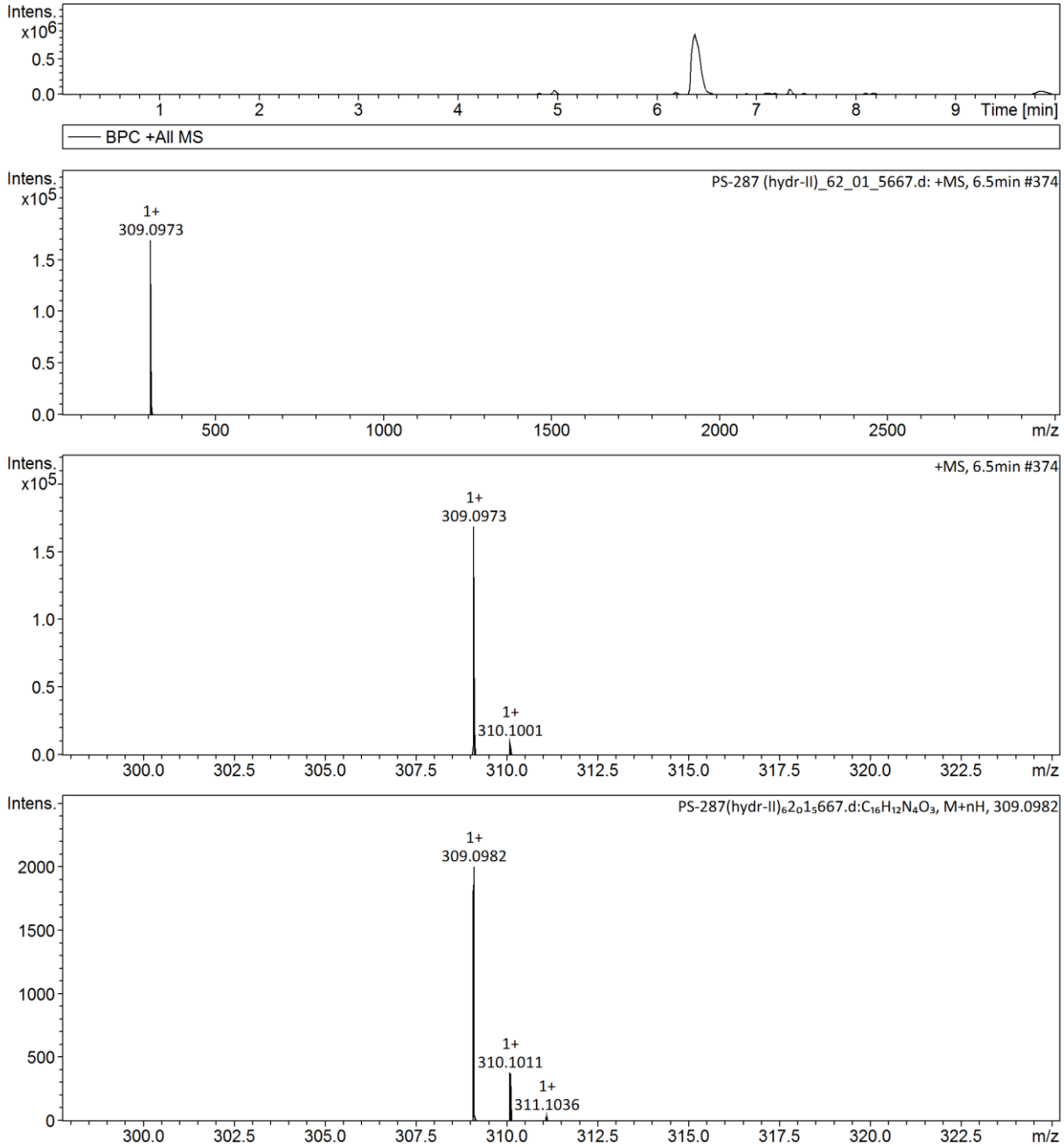

PS-287 (hydr-II)_62_01_5667.d

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Figure S3. HPLC-HRMS analysis of the compound **III**.

Display Report

Analysis Info

Analysis Name	D:\Data\PS-288 (hydr-III)_63_01_5668.d	Acquisition Date	8/8/2025 7:47:11 PM
Method	la_2.2_small2-nonfrg.m	Operator	BDAL@DE
Sample Name	PS-288 (hydr-III)	Instrument	compact 8255754.20088
Comment			

Acquisition Parameter

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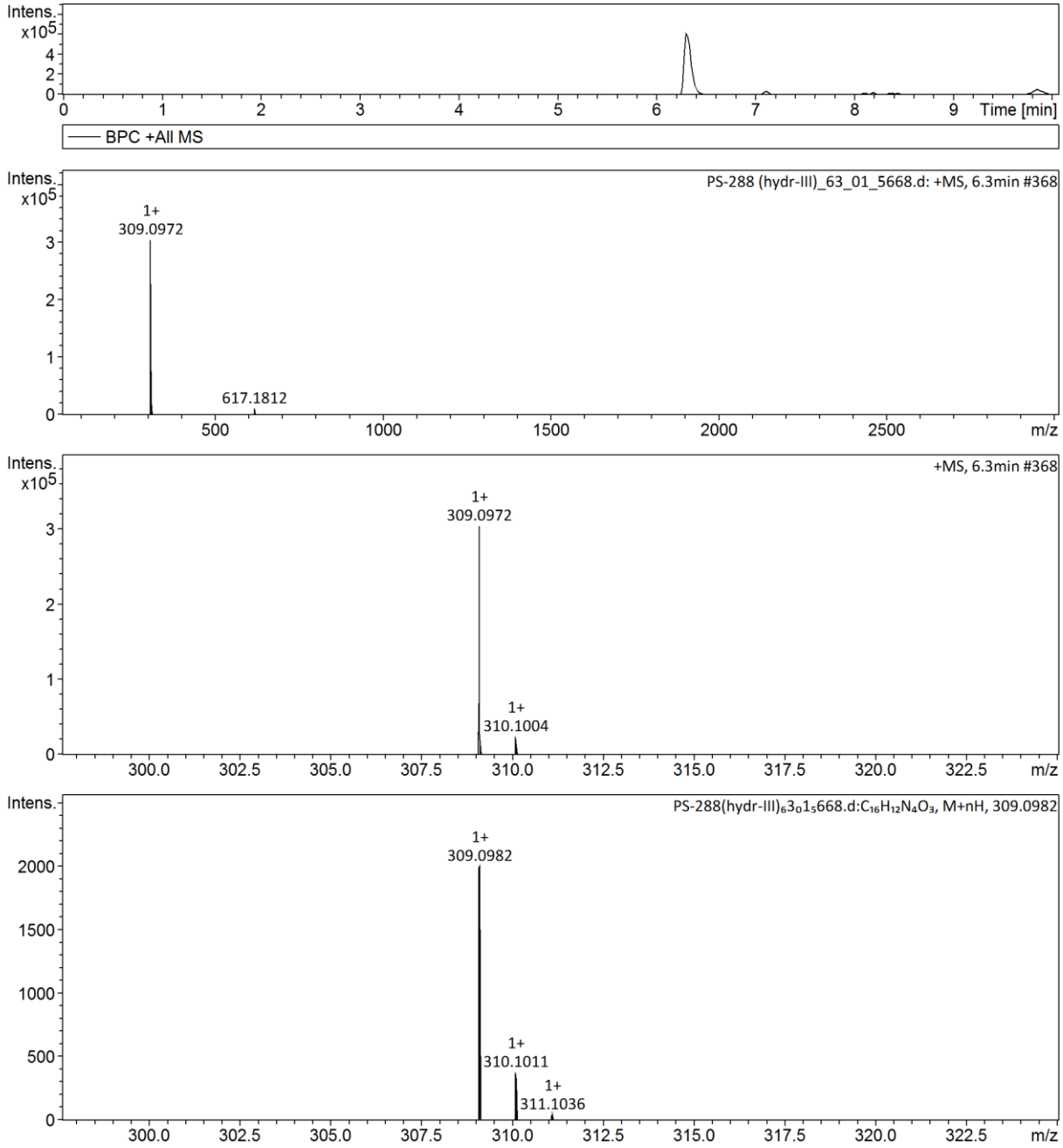
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



PS-288 (hydr-III)_63_01_5668.d

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Figure S4. HPLC-HRMS analysis of the compound **IV**.

Display Report

Analysis Info

Analysis Name	D:\Data\PS-289 (hydr-IV)_64_01_5669.d	Acquisition Date	8/8/2025 7:58:43 PM
Method	la_2.2_small2-nonfrg.m	Operator	BDAL@DE
Sample Name	PS-289 (hydr-IV)	Instrument	compact 8255754.20088
Comment			

Acquisition Parameter

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Focus	Active	Set Capillary	4300 V	Set Dry Heater	180 °C
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Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
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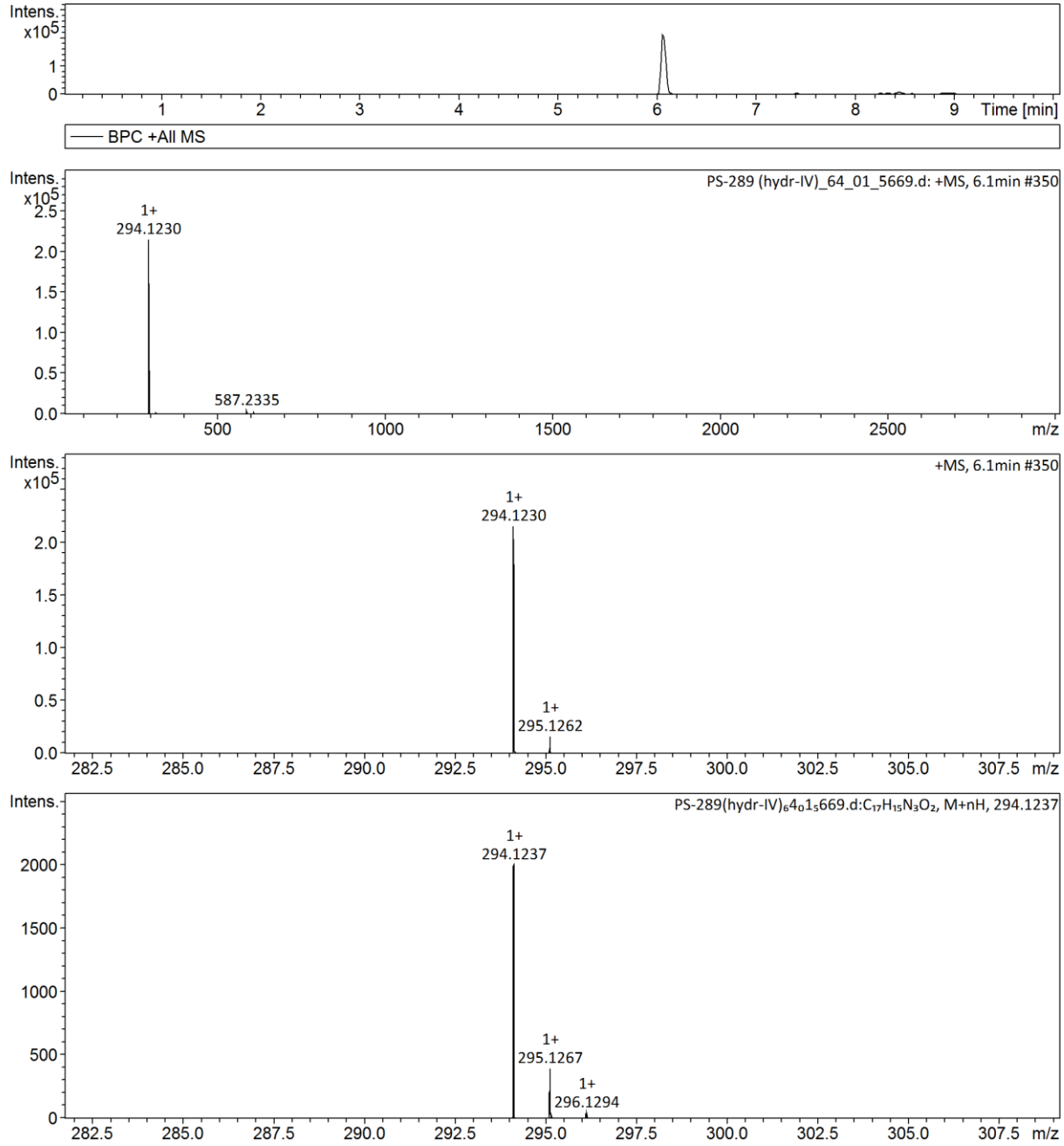

PS-289 (hydr-IV)_64_01_5669.d

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Figure S5. HPLC-HRMS analysis of the compound **V**.

Display Report

Analysis Info

Analysis Name	D:\Data\PS-290 (hydr-V)_65_01_5670.d	Acquisition Date	8/8/2025 8:10:21 PM
Method	la_2.2_small2-nonfrg.m	Operator	BDAL@DE
Sample Name	PS-290 (hydr-V)	Instrument	compact 8255754.20088
Comment			

Acquisition Parameter

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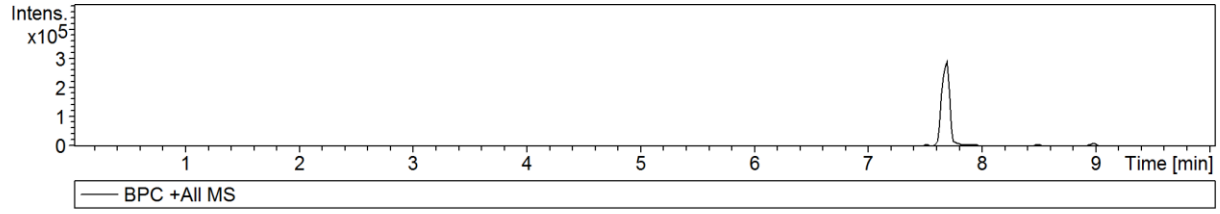
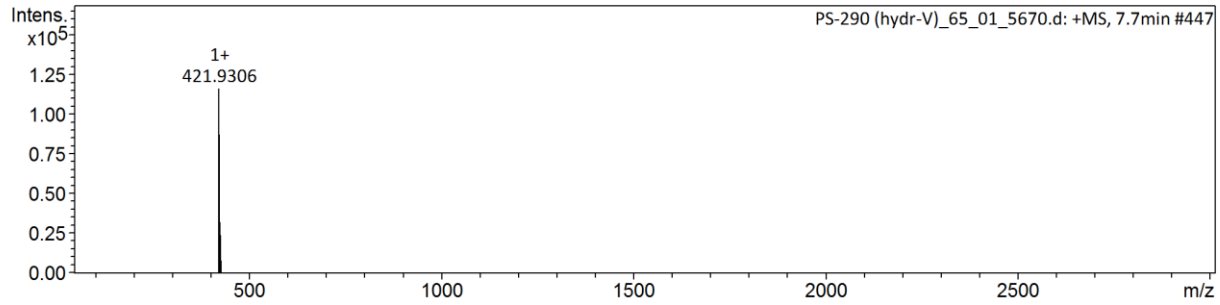
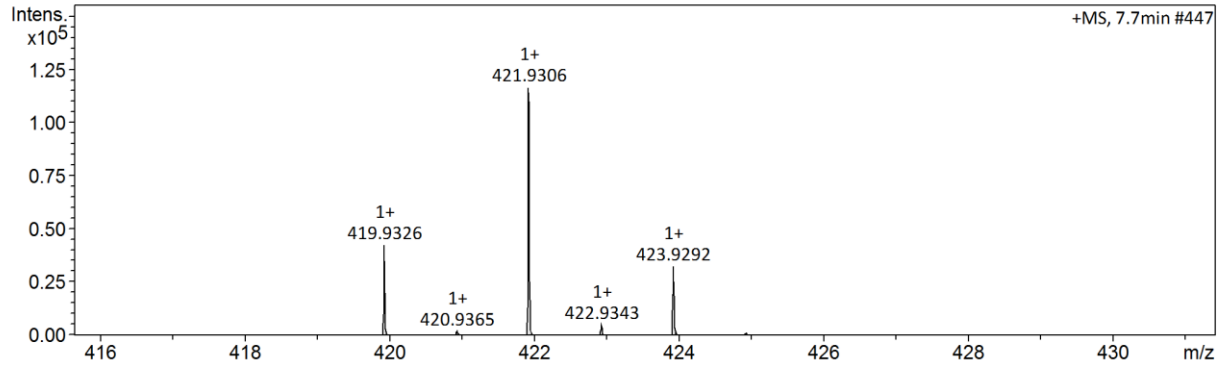
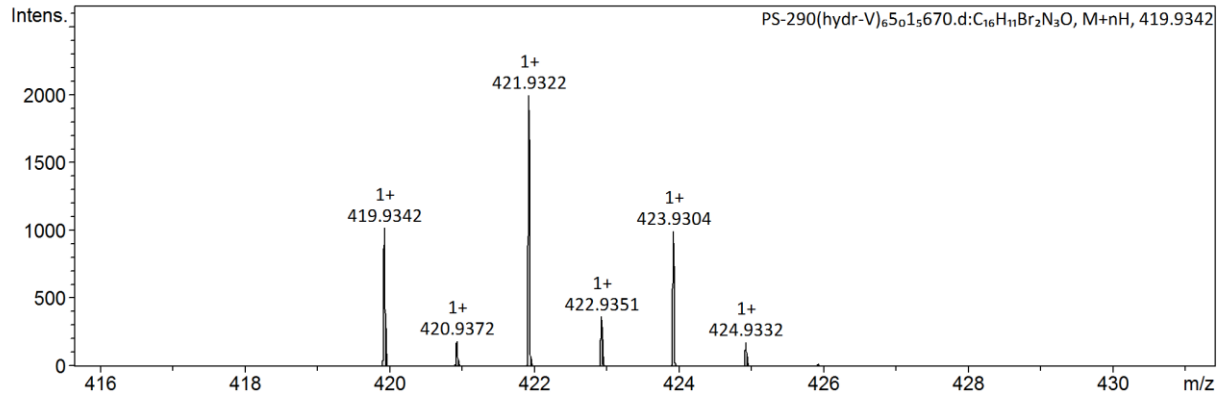
Focus	Active	Set Capillary	4300 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C









PS-290 (hydr-V)_65_01_5670.d

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Figure S6. HPLC-HRMS analysis of the compound **VI**.

Display Report

Analysis Info

Analysis Name	D:\Data\PS-291 (hydr-VI)_66_01_5671.d	Acquisition Date	8/8/2025 8:21:56 PM
Method	la_2.2_small2-nonfrg.m	Operator	BDAL@DE
Sample Name	PS-291 (hydr-VI)	Instrument	compact 8255754.20088
Comment			

Acquisition Parameter

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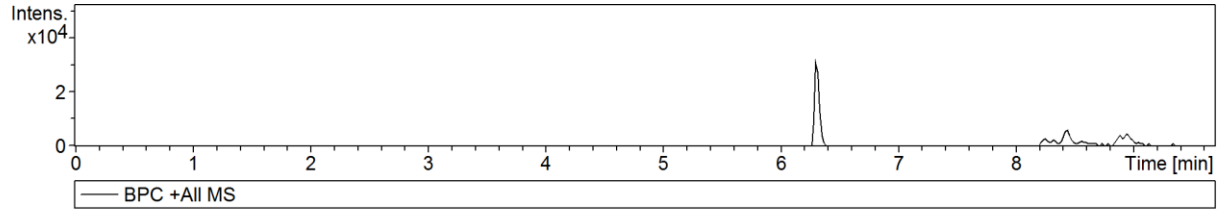
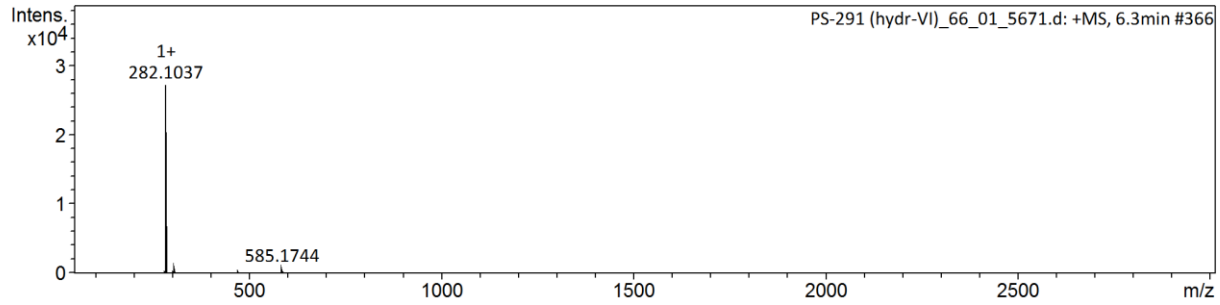
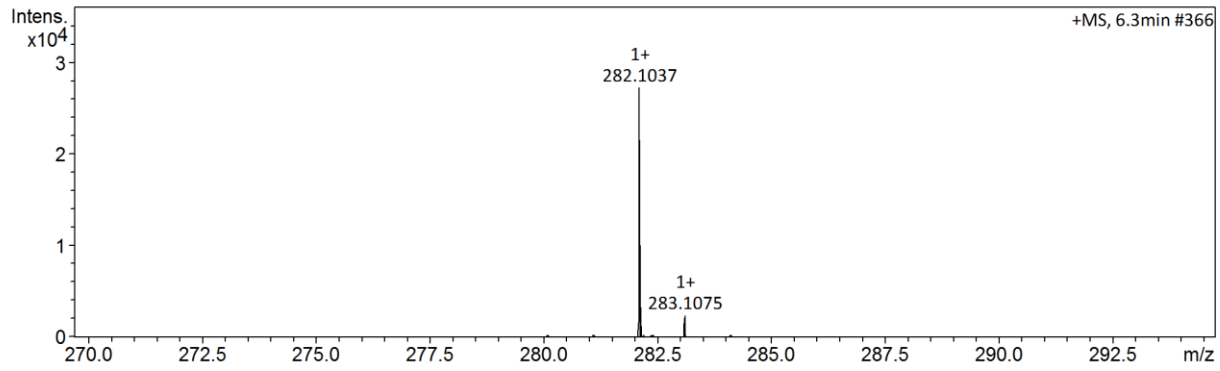
		Set Corona	0 nA	Set APCI Heater	0 °C







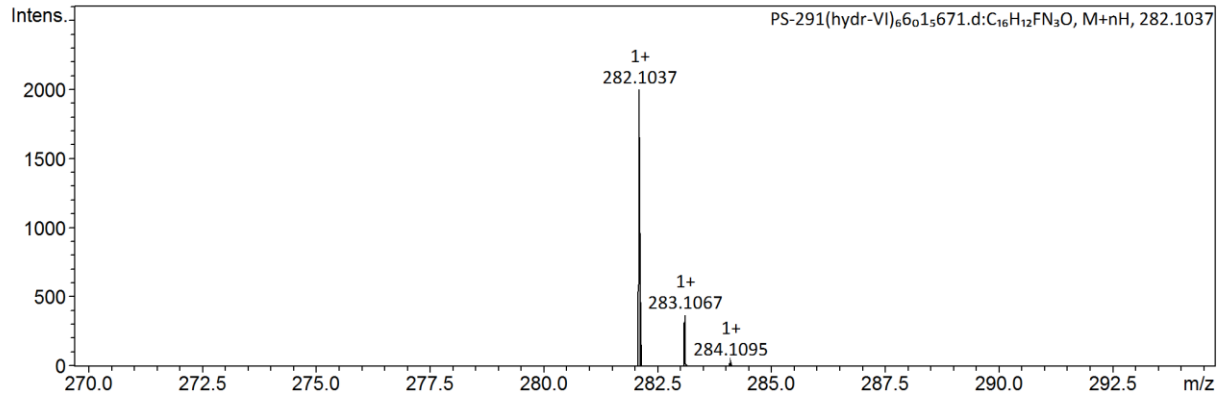


Figure S7. ^{1}H NMR spectrum of the compound **I** (*anti*-isomer + ~20% *syn*-isomer).

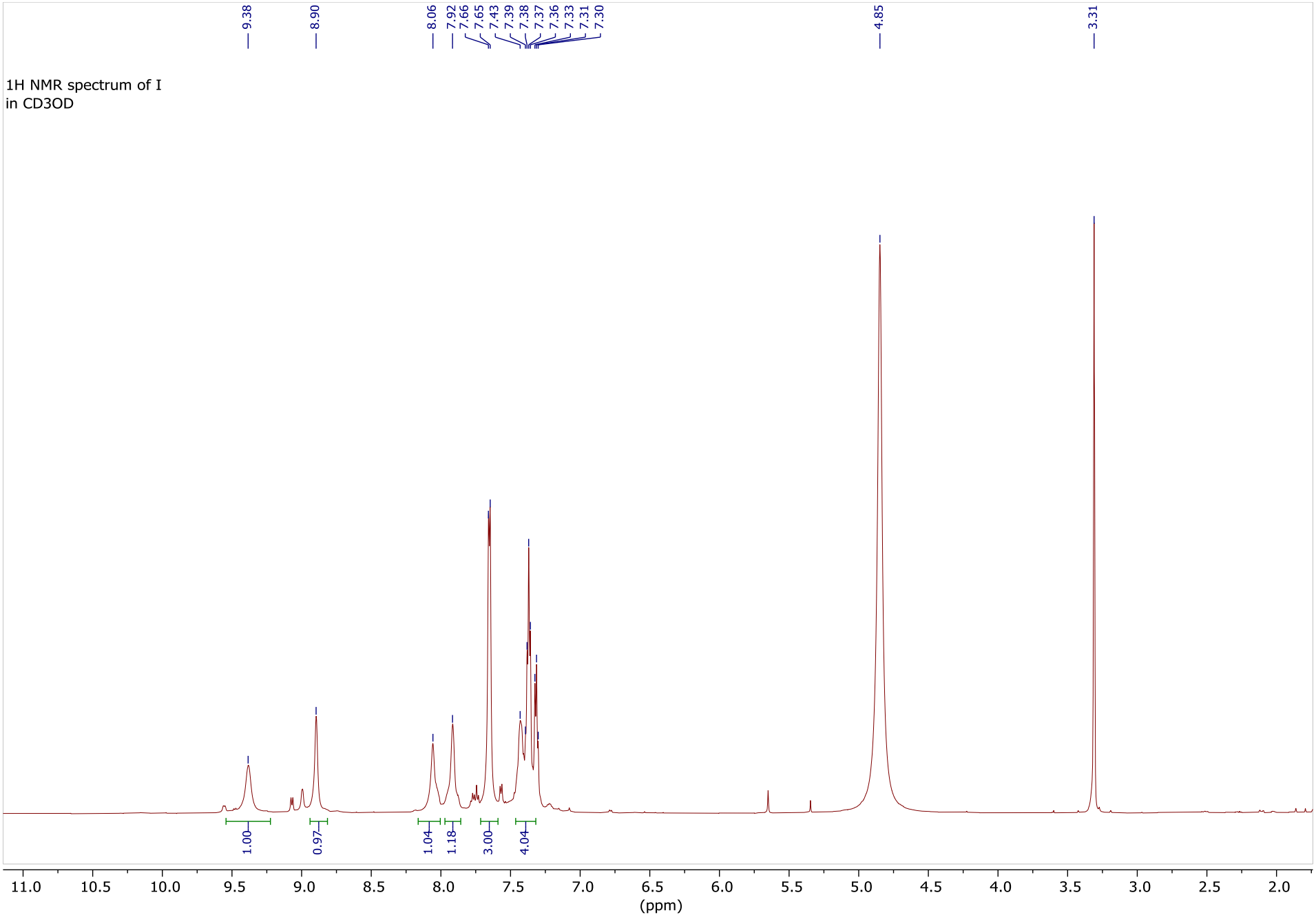


Figure S8. ^{13}C NMR spectrum of the compound **I** (*anti*-isomer + ~20% *syn*-isomer).

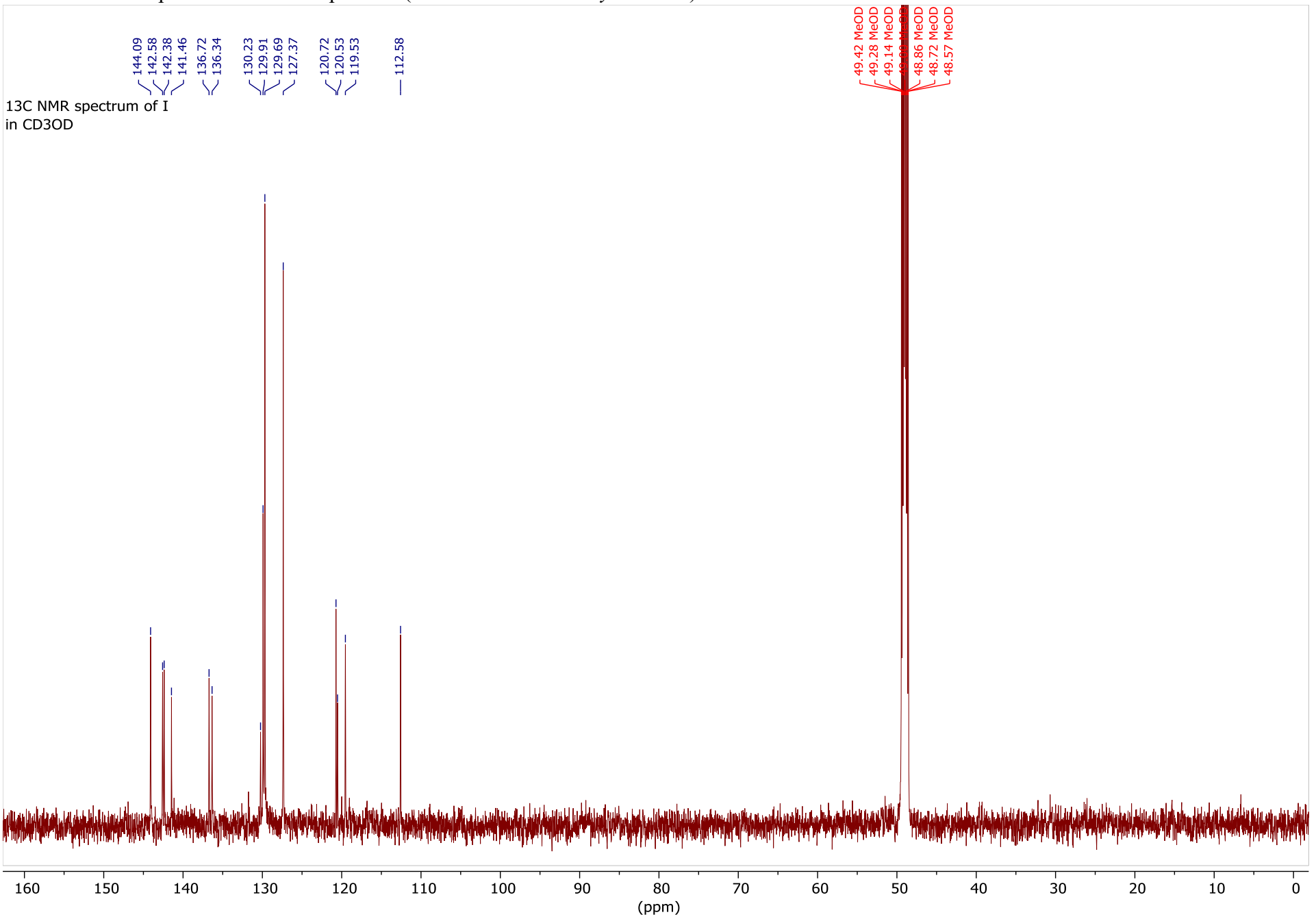


Figure S9. ^{1}H-^{13}C HSQC NMR spectrum of the compound **I** (*anti*-isomer + ~20% *syn*-isomer).

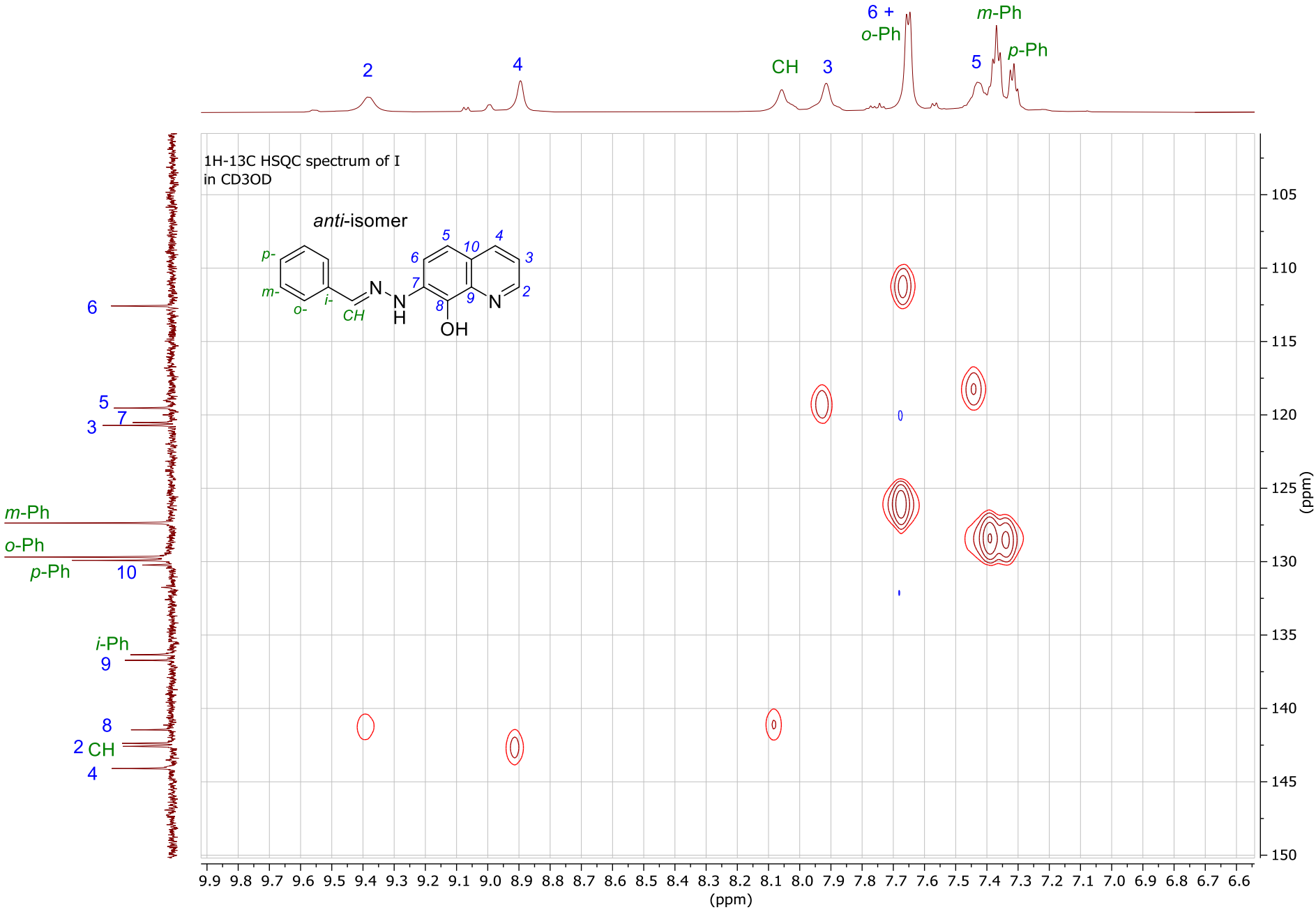


Figure S10. ^{1}H NMR spectrum of the compound **II** (*anti*-isomer + 40% *syn*-isomer).

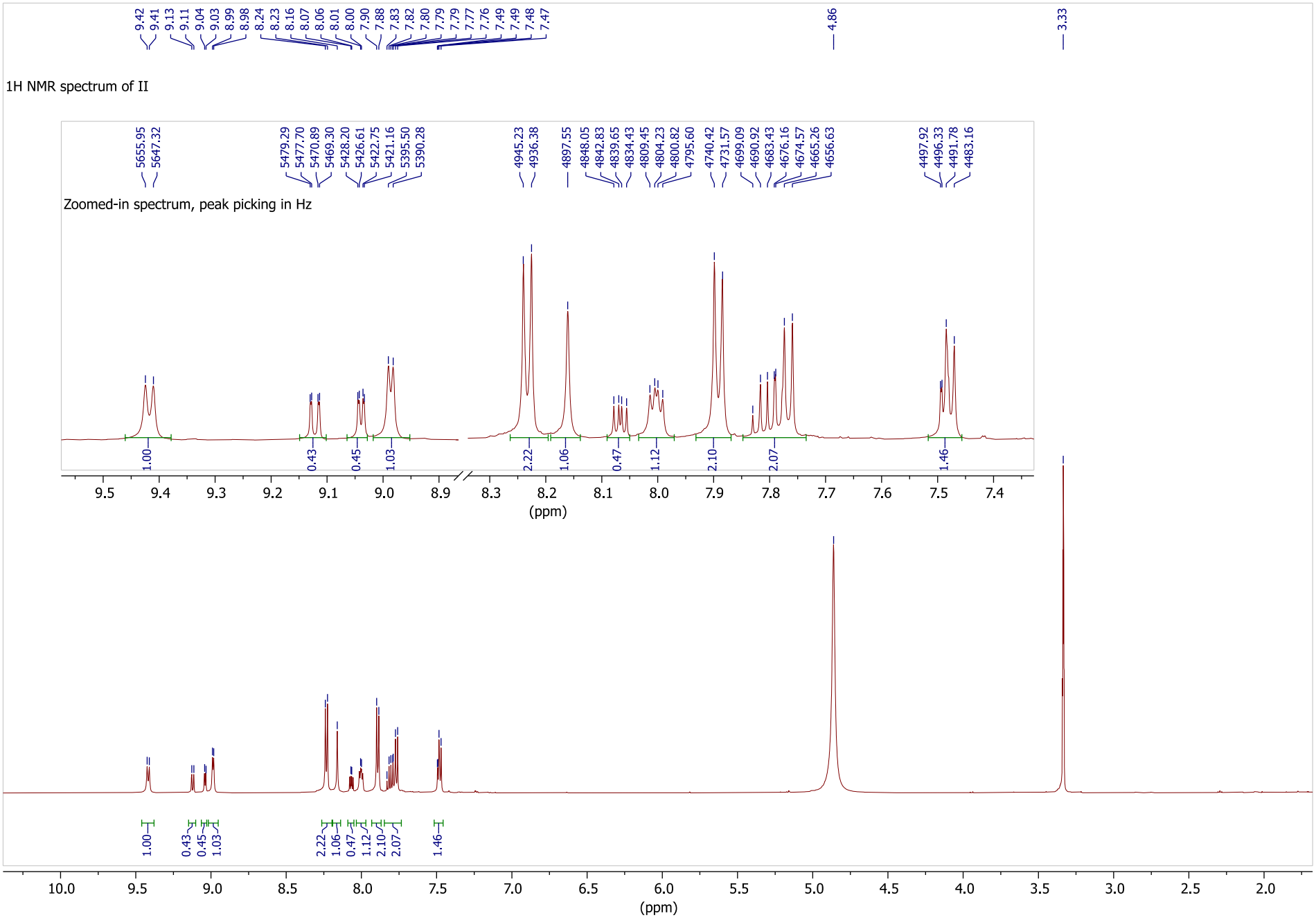


Figure S11. ^{13}C NMR spectrum of the compound **II** (*anti*-isomer + 40% *syn*-isomer).

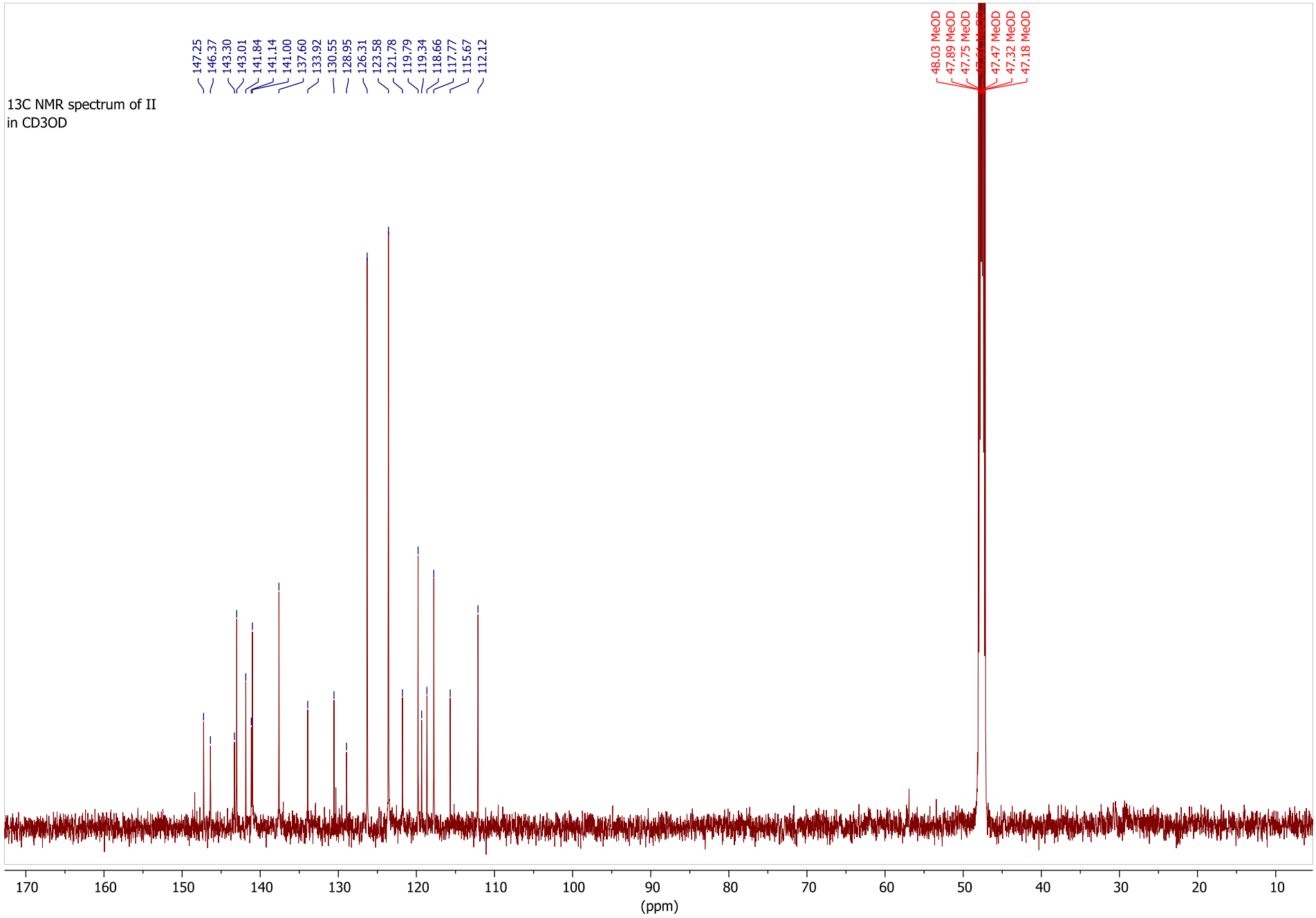


Figure S12. ^{1}H-^{13}C HSQC NMR spectrum of the compound **II** (*anti*-isomer + 40% *syn*-isomer).

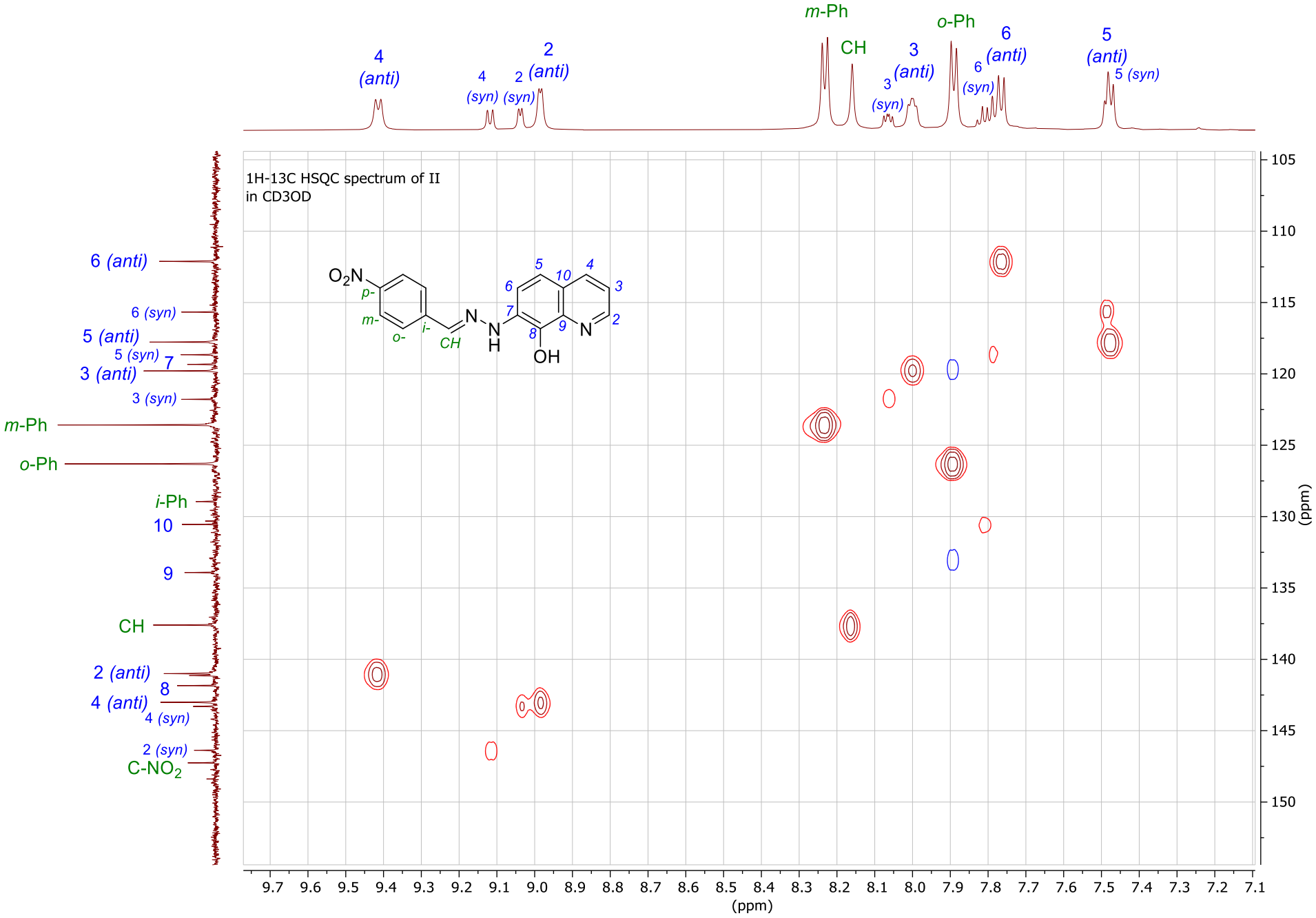


Figure S13. ^{1}H NMR spectrum of the compound **III** (*anti*-isomer + 10% *syn*-isomer).

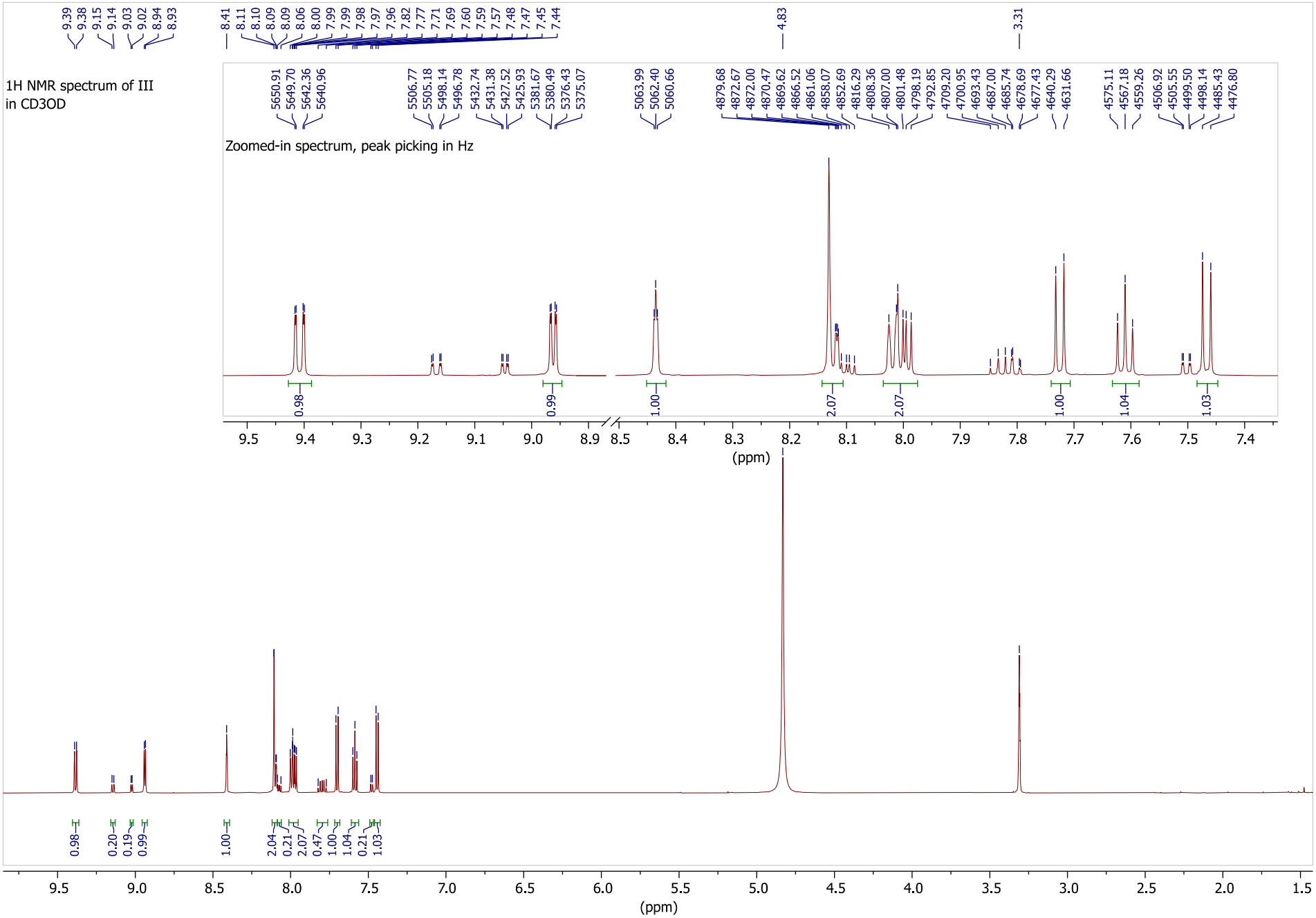


Figure S14. ^{13}C NMR spectrum of the compound **III** (*anti*-isomer + 10% *syn*-isomer).

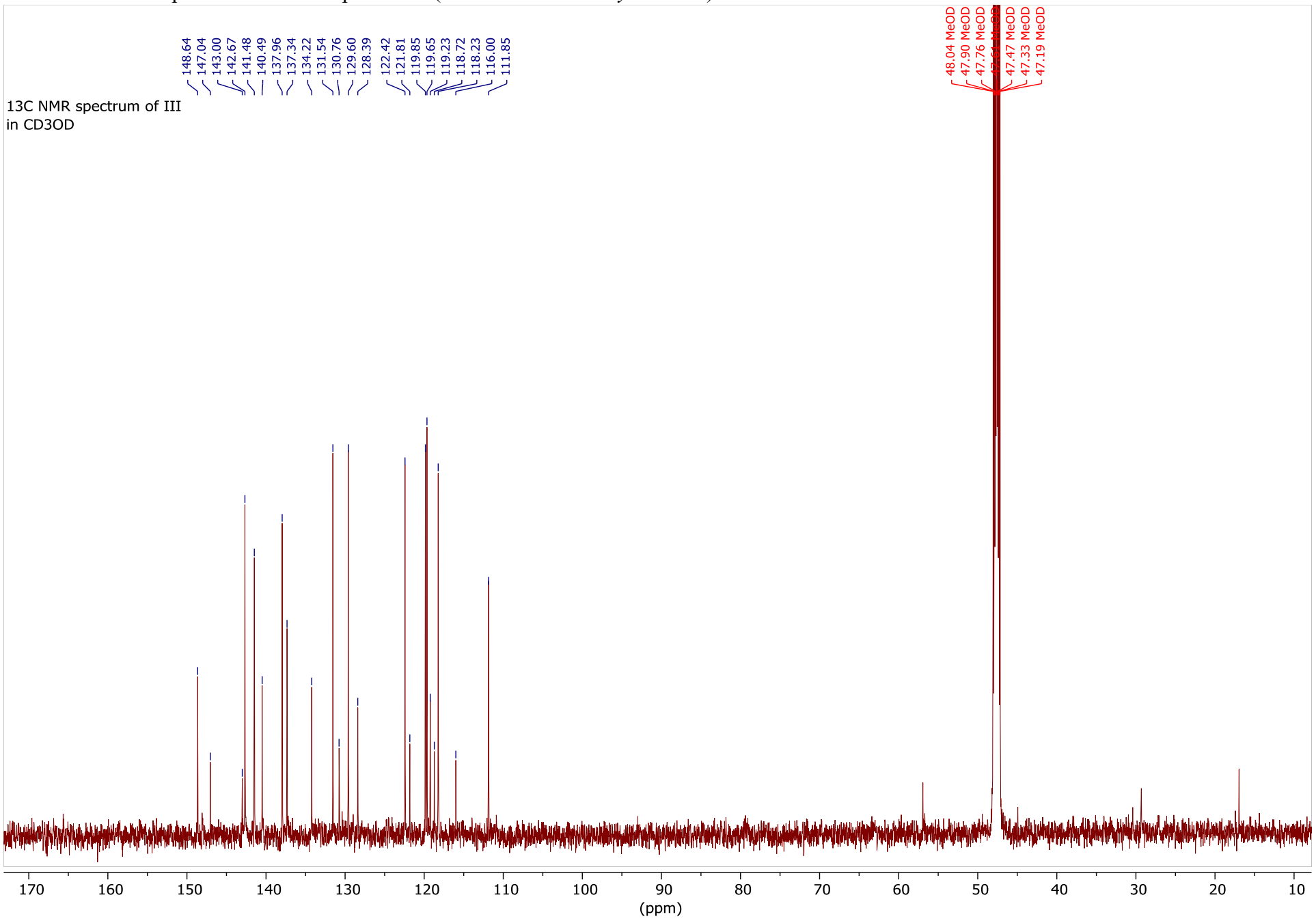


Figure S15. ^{1}H-^{13}C HSQC NMR spectrum of the compound **III** (*anti*-isomer + 10% *syn*-isomer).

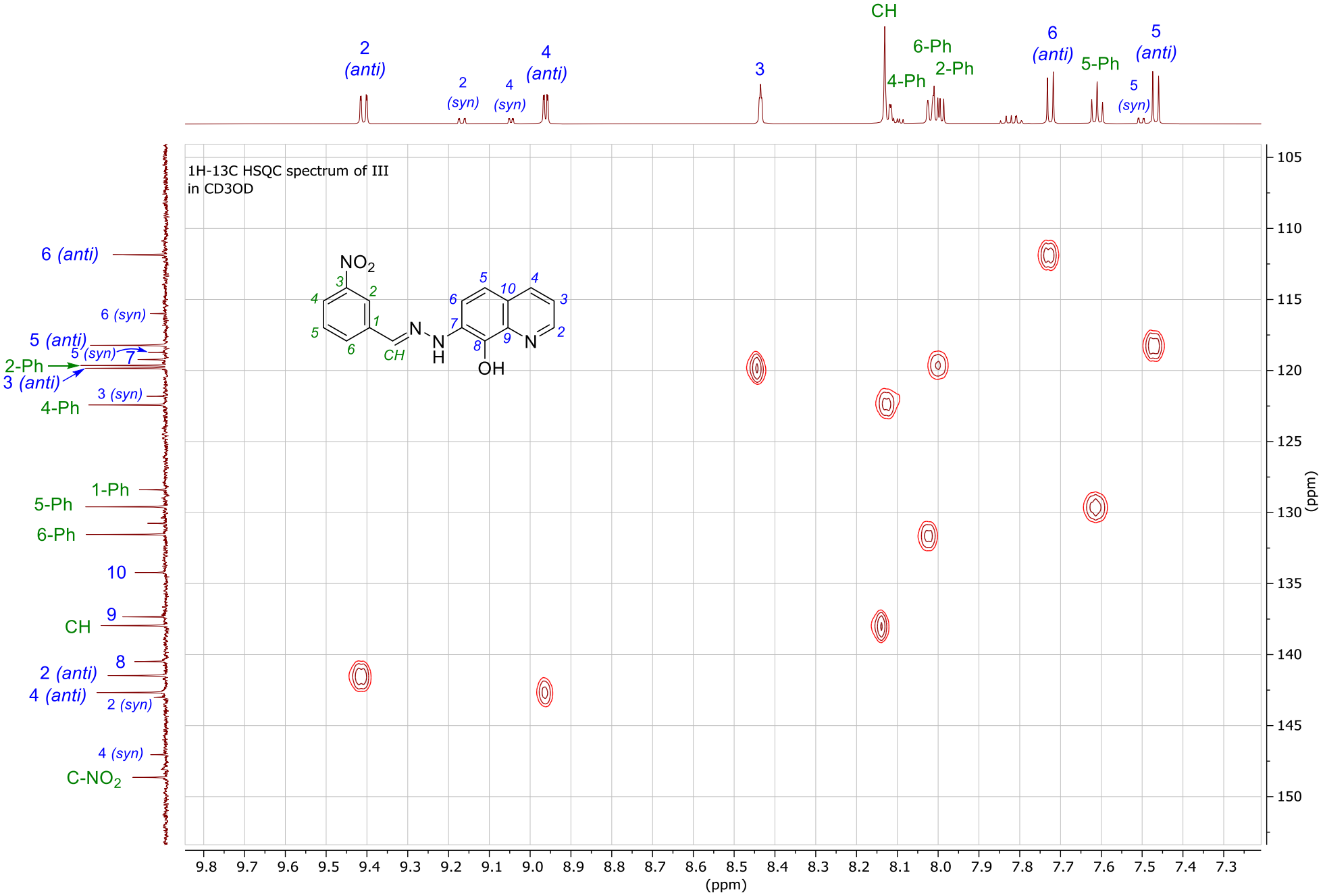


Figure S16. ^{1}H NMR spectrum of the compound **IV** (*anti*-isomer + 10% *syn*-isomer).

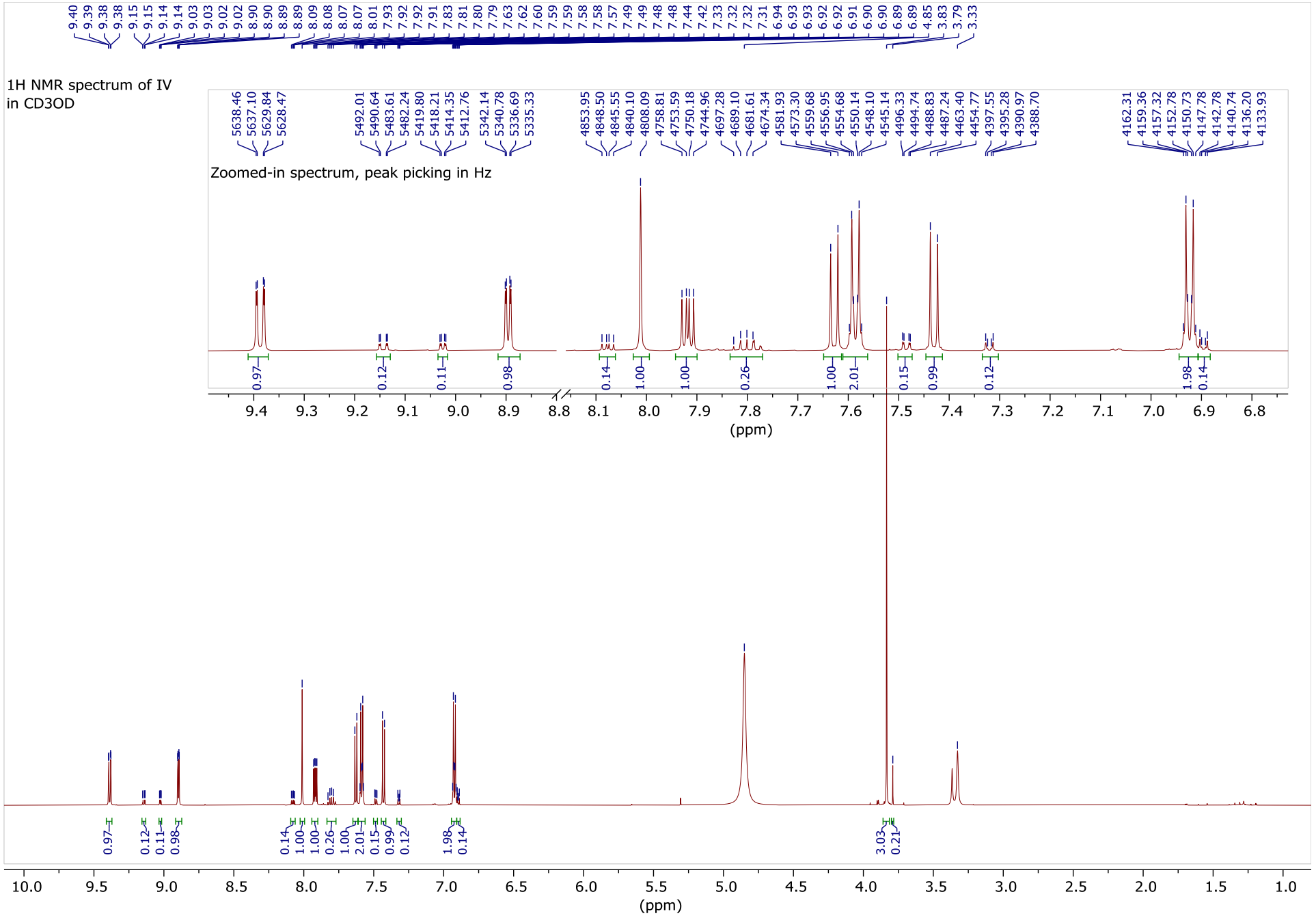


Figure S17. ^{13}C NMR spectrum of the compound **IV** (*anti*-isomer + 10% *syn*-isomer).

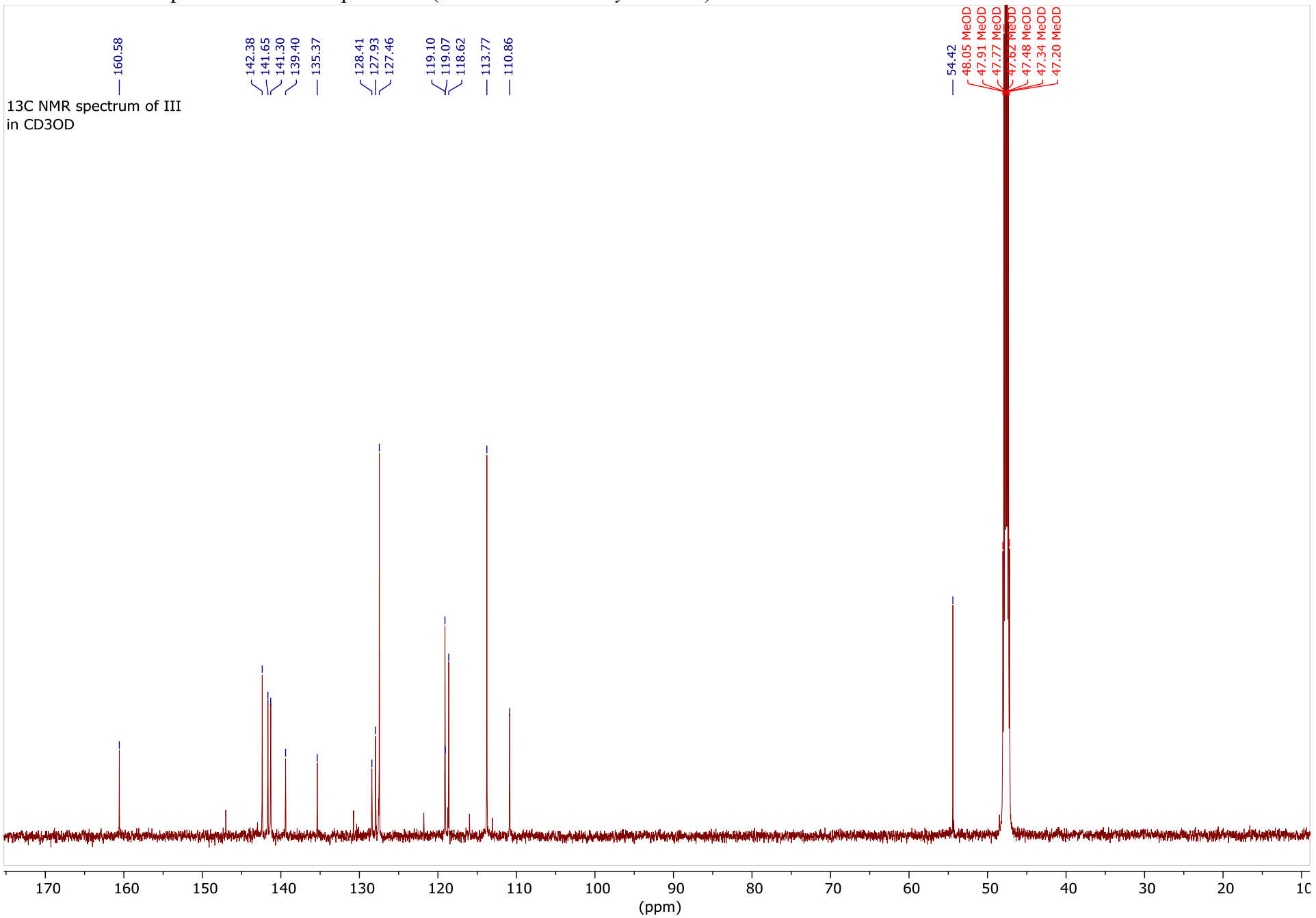


Figure S18. ^{1}H-^{13}C HSQC NMR spectrum of the compound **IV** (*anti*-isomer + 10% *syn*-isomer).

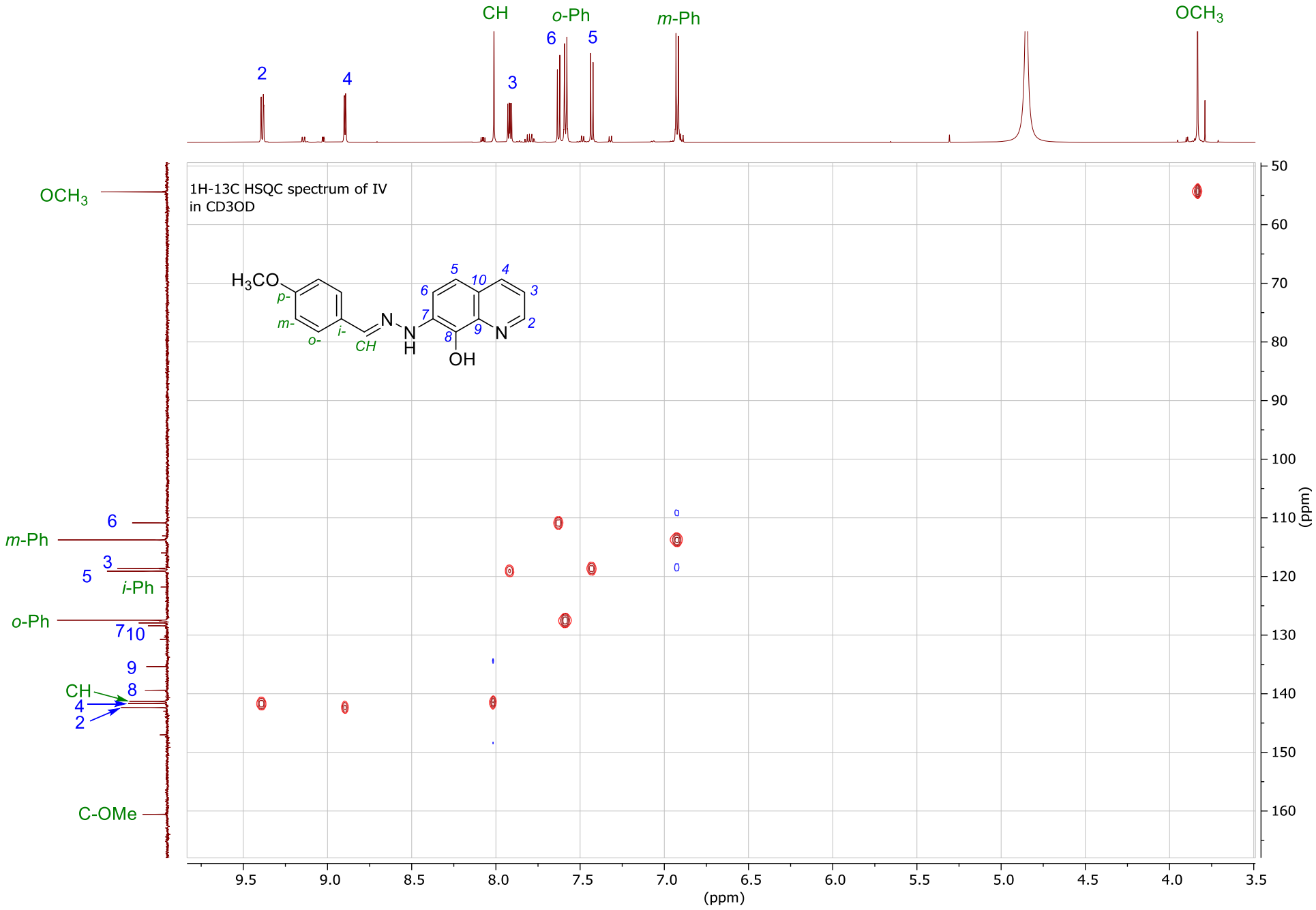


Figure S19. ^{1}H NMR spectrum of the compound **V** (*anti*-isomer + 10% *syn*-isomer).

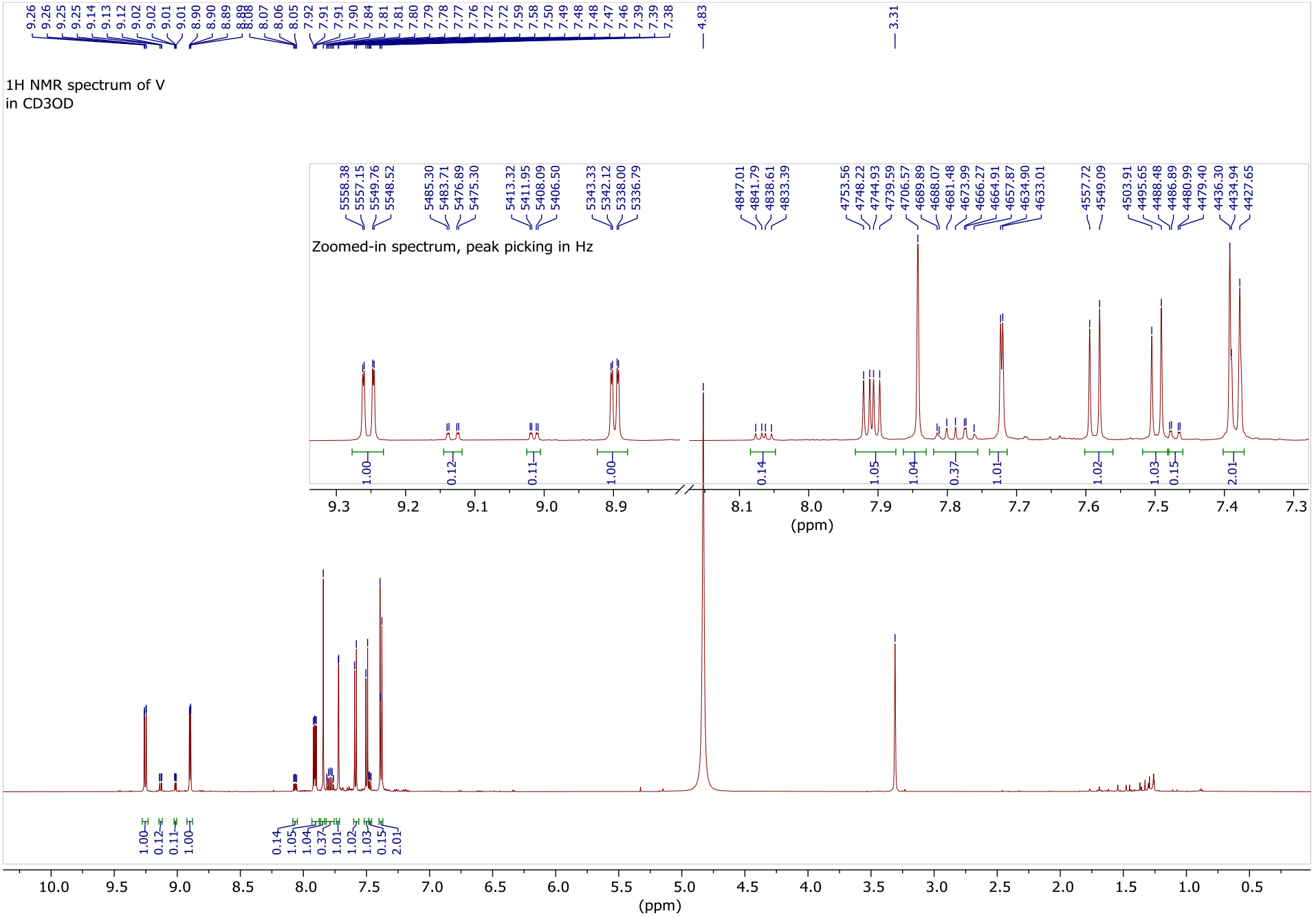


Figure S20. ^{13}C NMR spectrum of the compound **V** (*anti*-isomer + 10% *syn*-isomer)..

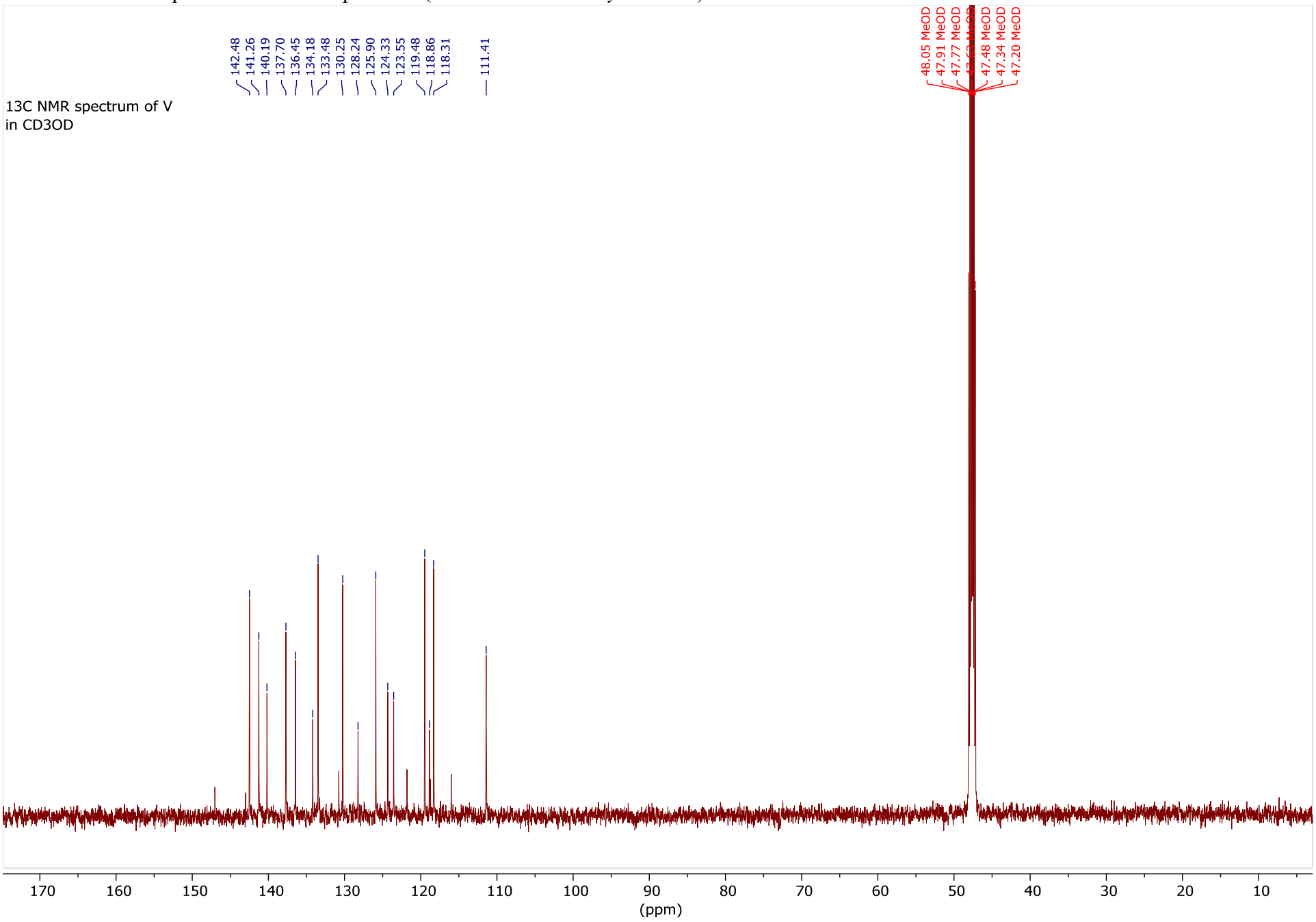


Figure S21. ^{1}H-^{13}C HSQC NMR spectrum of the compound **V** (*anti*-isomer + 10% *syn*-isomer).

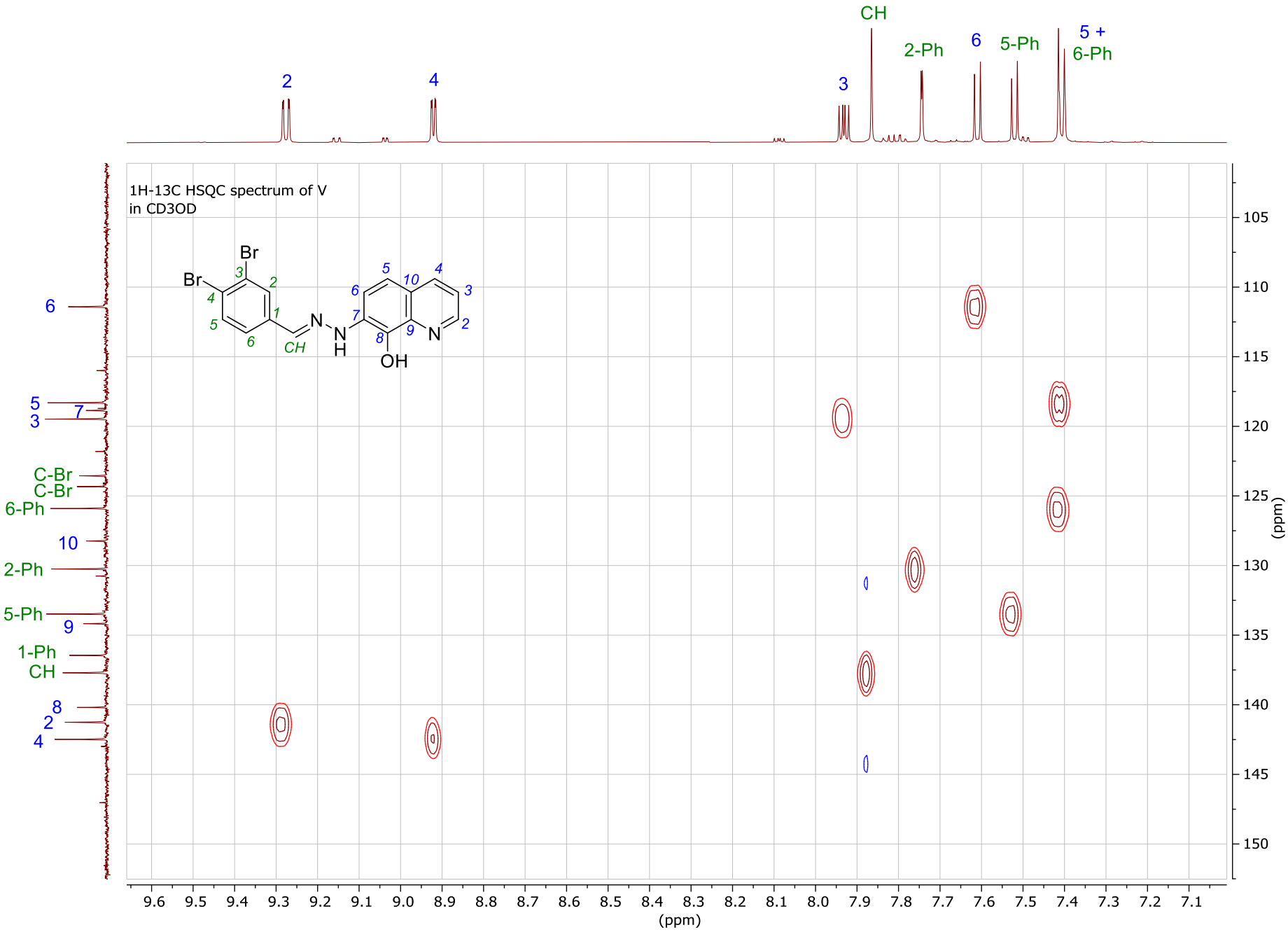


Figure S22. ^{1}H NMR spectrum of the compound **VI** (*anti*-isomer + ~15% *syn*-isomer).

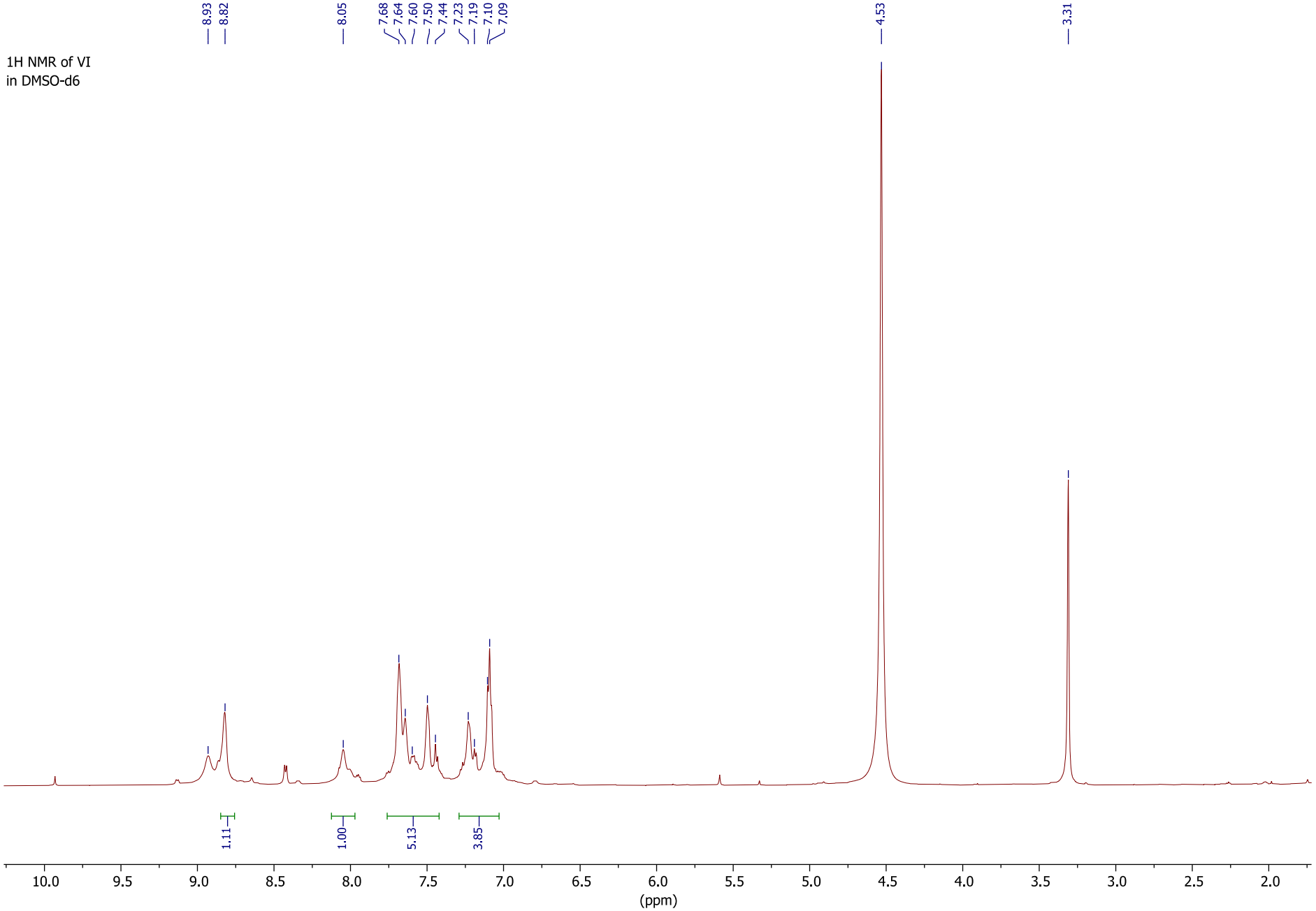


Figure S23. ^{13}C NMR spectrum of the compound **VI** (*anti*-isomer + ~15% *syn*-isomer).

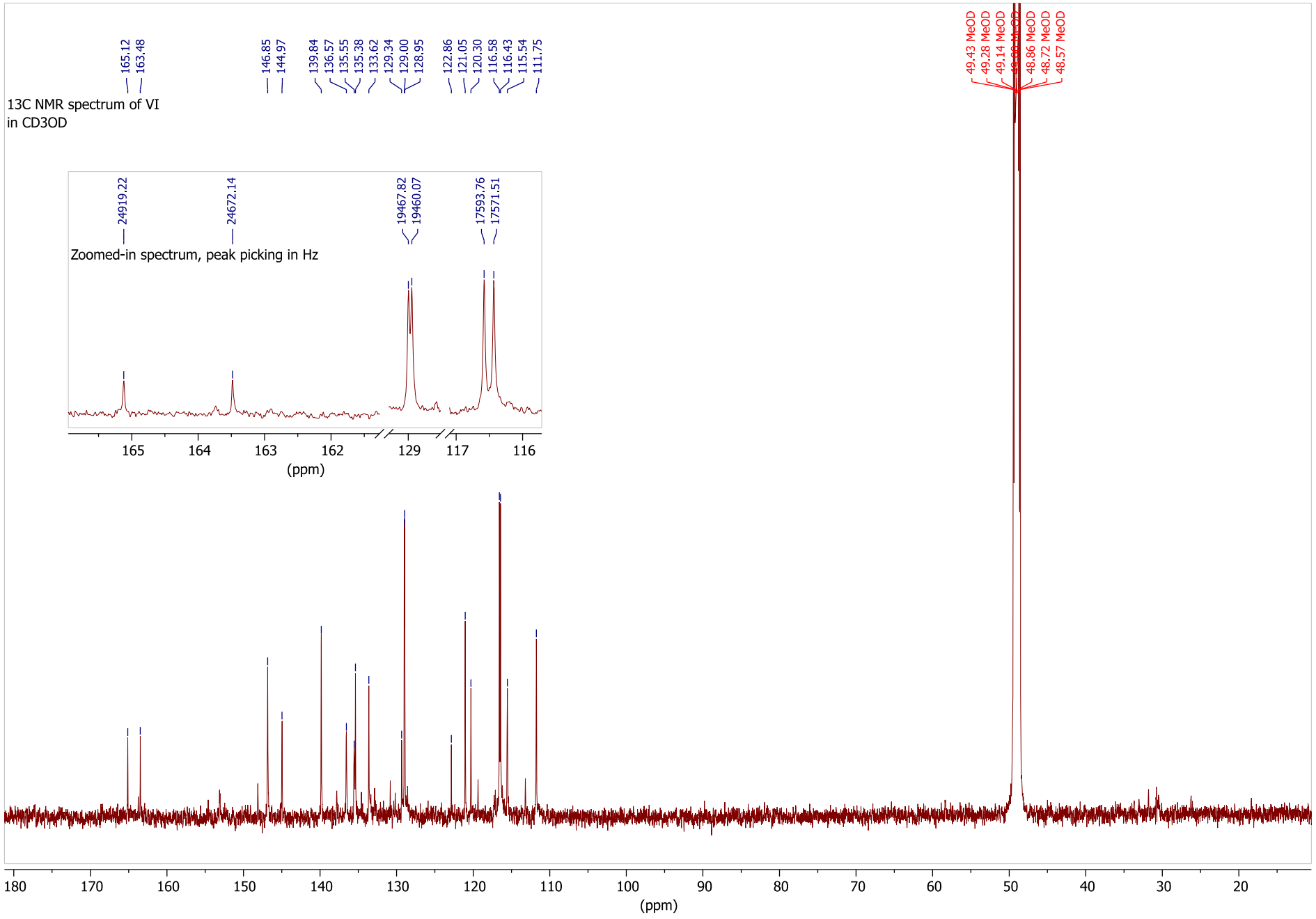
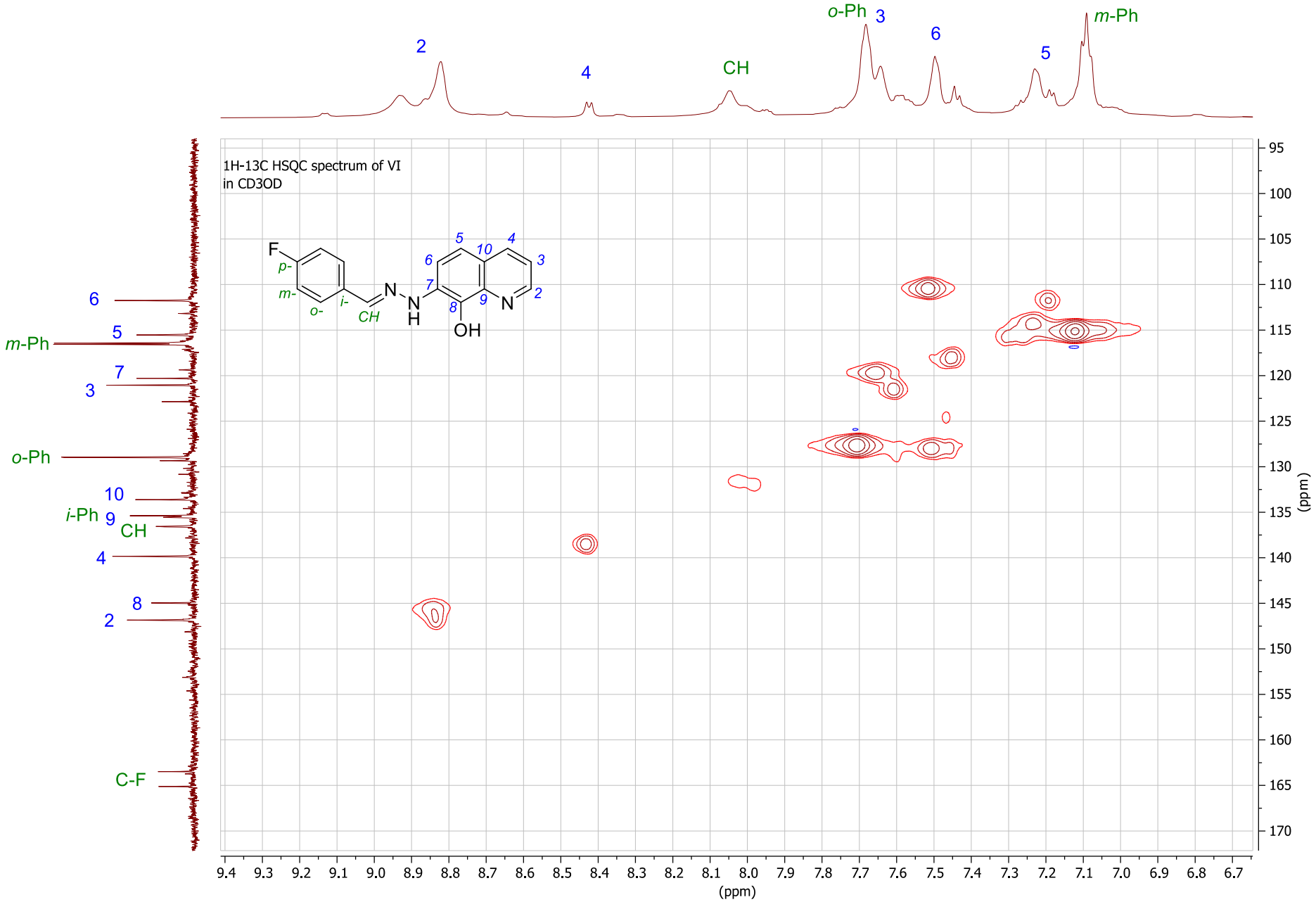


Figure S24. ^{1}H-^{13}C HSQC NMR spectrum of the compound **VI** (*anti*-isomer + ~15% *syn*-isomer).



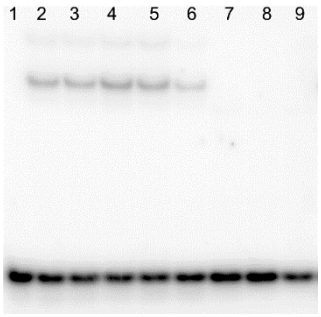


Figure S25. EMSA analysis of the Ku/DNA interaction inhibition by **V**. 1 – negative control in the absence of Ku, 2 – positive control of DNA binding in the absence of inhibitor, 3-9 – binding with the increasing concentration of the inhibitor: 3.9; 7.8; 15.6; 31.3; 62.5; 125 and 250 μM, respectively. Gels were transferred onto a Whatman paper, lyophilized and visualized as a radioautogram (see Materials and Methods section)

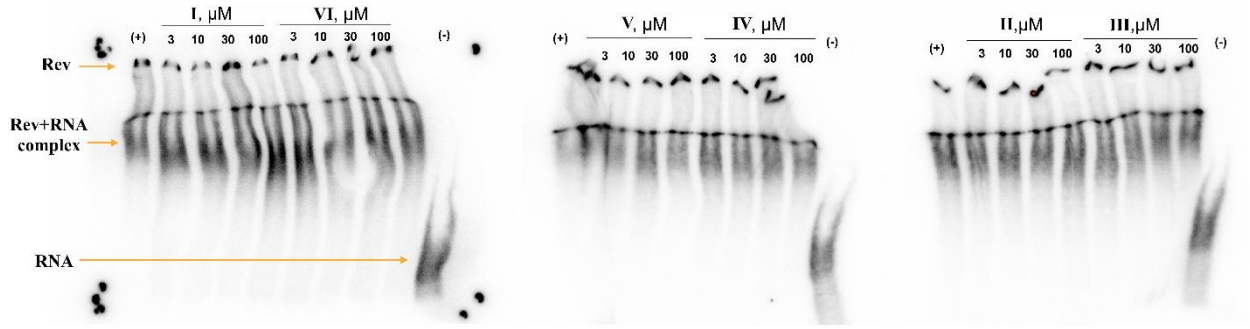


Figure S26. EMSA analysis of the Rev–RNA complex interaction with hydrazones **I-VI**. Radioactive labeled RNA (RNA*) was incubated with the compounds and Rev protein ("+ and – refer to the presence or absence of Rev in the reaction); The protein, RNA and the compounds were incubated at +4°C for 1 h in RBB buffer, then loading buffer was added and the mixture was subjected to native 4% SDS–PAGE. Gels were transferred onto a Whatman paper (images are represented with the slightly warped distortion due to the laborious transfer of the frail gel), lyophilized and visualized as a radioautogram (other details in Materials and Methods section)

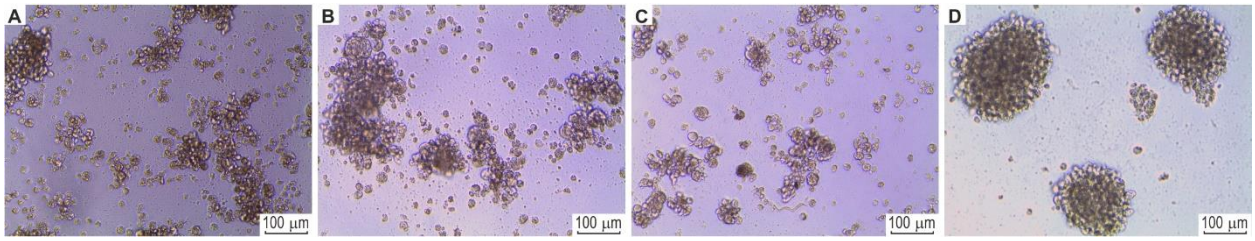


Figure S27. Effects of compound **VI** and EFV on the syncytia formation (in 3 days after infection). (A) infected MT-4 cell culture growing in the presence of **VI** (5 μM); (B) infected MT-4 cell culture growing in the presence of **VI** (1 μM); (C) infected MT-4 cell culture growing in the presence of **VI** (0.5 μM); (D) infected cell culture growing in the presence of EFV (0.5 μM).

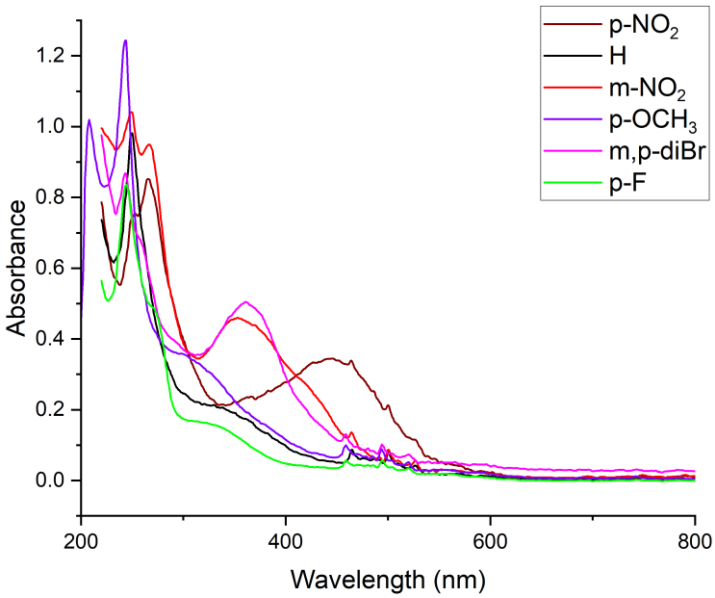


Figure S28. UV-Vis absorption spectra of 7-benzylidenohydrazinyl-8-hydroxyquinolines



Figure S29. 7-Benzylidenohydrazinyl-8-hydroxyquinolines in diluted methanol solutions

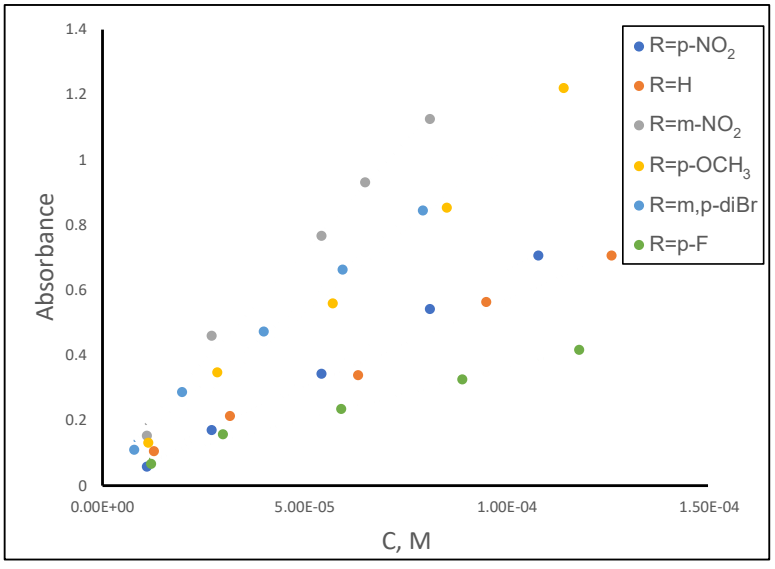


Figure S30. Calibration curves of the compounds at the selected λ_{max}

Table S1. Calculated data for hydrazone-incorporated microgel formation

Compound	λ_{max}, nm	Absorbance exp., Abs	Absorbance theor., Abs	m exp., μg	Load exp., % wt.	Load theor., % wt.
R=*p*-NO_2	450	0.50	0.52	229.80	28.73	33.00
R=H	330	0.43	0.50	228.98	28.61	33.00
R=*m*-NO_2	354	0.83	1.07	209.03	26.12	33.00
R=*p*-OCH_3	335	0.78	0.92	205.63	25.75	33.00
R=*m*,*p*-diBr	375	0.60	0.63	250.19	31.27	33.00
R=*p*-F	333	0.23	0.30	198.45	24.81	33.00

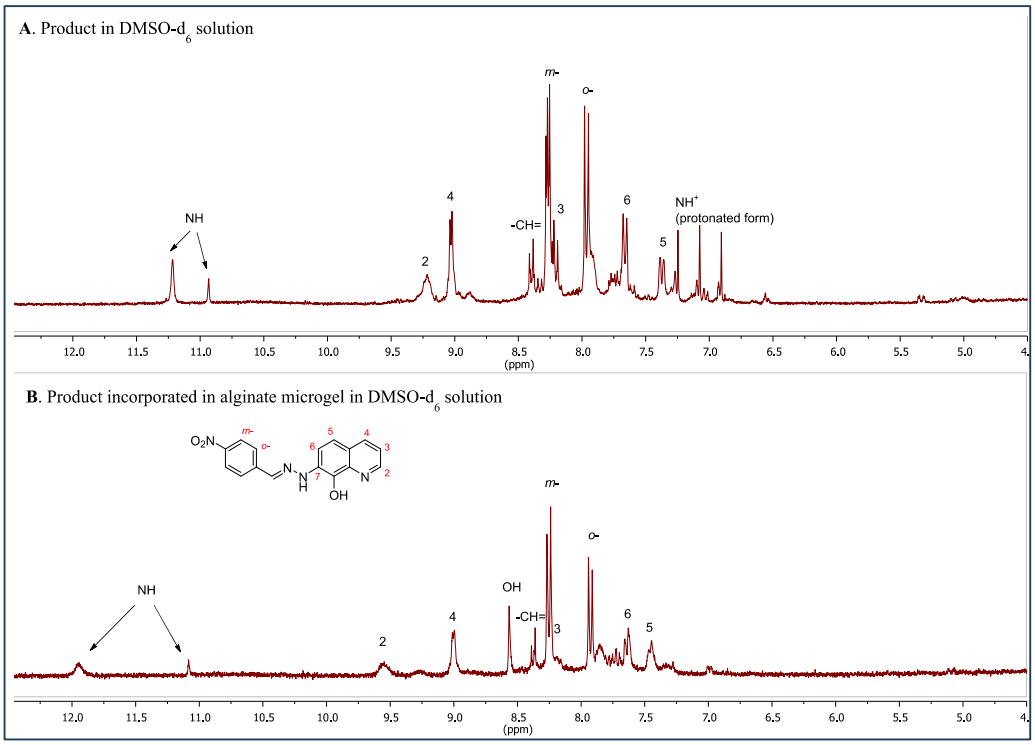


Figure S31. ^{1}H-NMR spectrum of the hydrazone **II** (a mixture of tautomeric forms in DMSO-d_6) (A) and the sample of **II** incorporated into the alginate microgel (B).

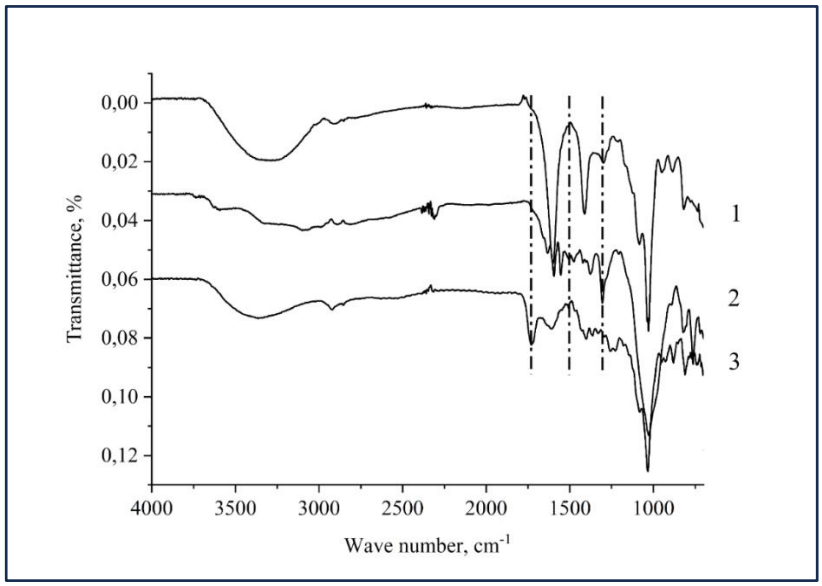


Figure S32. IR spectra of the alginate microgel without the included substance (1), of the hydrazone **II** (R=*p*-NO_2) (2), and of the microgel with the incorporated hydrazone **II** (3).