Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Experimental Supporting Information

Synthesis and identification of heteroaromatic N-benzyl sulfonamides as potential anticancer agents

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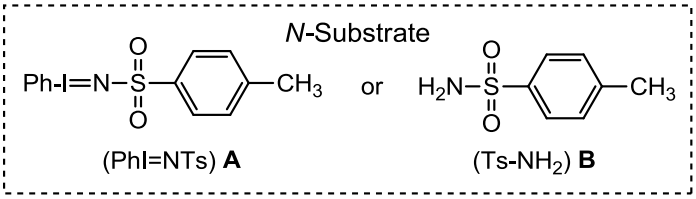

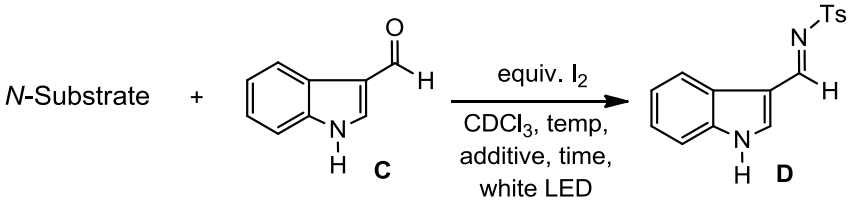

Entry	equiv. **C**	*N*-substrate (equiv.)	Additive (equiv.)	equiv. I_2	Time (h)	Temp. (°C)	% **D**[a]
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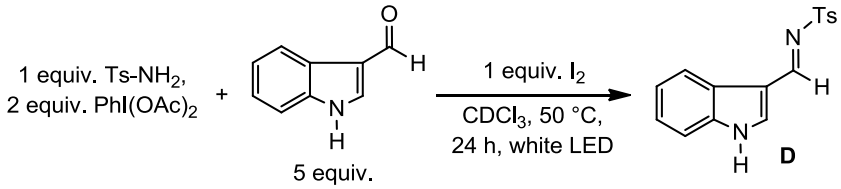
2	5	**A** (1)	None	1	10	50	24
3	5	**A** (1)	None	1	24	50	33
4	5	**A** (1)	None	1	48	50	34
5	1	**A** (1)	None	1	24	50	28
6	2	**A** (1)	None	1	24	50	30
7	0.5	**A** (1)	None	1	24	50	4
8	5	**A** (1)	None	2	24	50	24
9	1	**B** (1)	$PhI(OAc)_2$ (2)	1	24	50	3
10	2	**B** (1)	$PhI(OAc)_2$ (2)	1	24	50	26
11	3	**B** (1)	$PhI(OAc)_2$ (2)	1	24	50	74
12	5	**B** (1)	$PhI(OAc)_2$ (2)	1	24	50	80

a) All yields were calculated using ^{1}H NMR and internal standard.

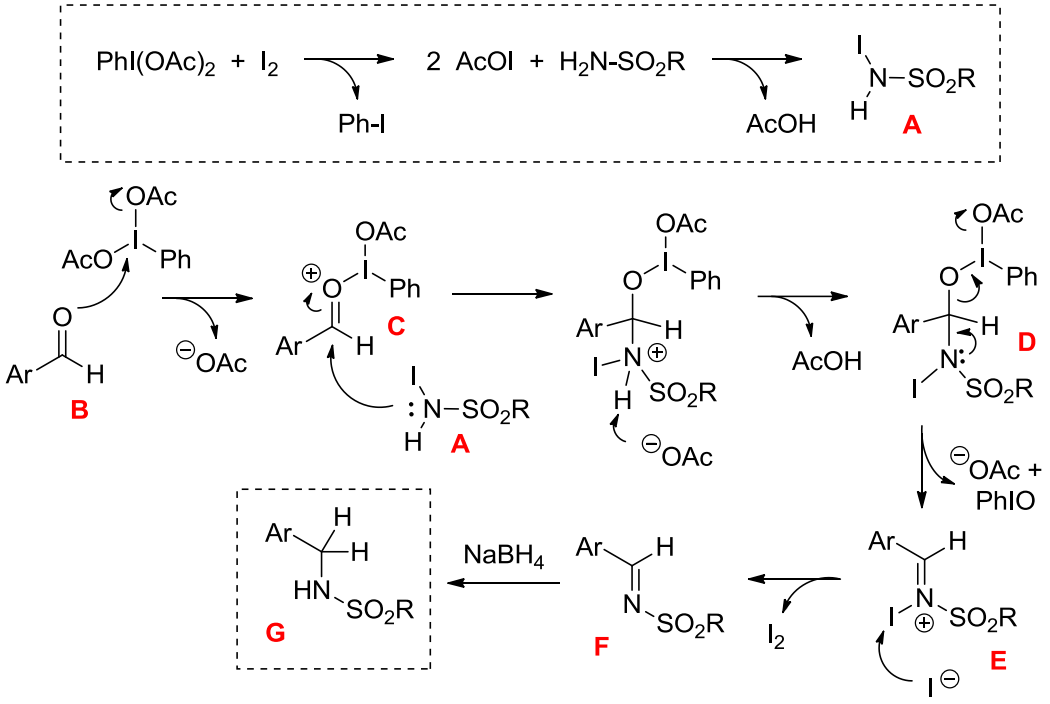
Table S1. Optimization of reaction conditions for formation of intermediate N-sulfonyl imine **D.**



Entry	Deviation from standard conditions	% **D**[a]
1	No deviation	80
2	0 equiv. I_2	12
3	0.2 equiv. I_2	87
4[b]	Reaction performed in the dark	83
5[b]	Dark, 48 h, 20 °C	53

a) All yields were calculated using ^{1}H NMR and internal standard. b) Reaction vessel was wrapped in aluminum foil and reaction was performed in a fume hood with all laboratory lights turned off.

Table S2. Control reactions and mechanistic experiments.



Scheme S1. Plausible mechanism.

Though the exact nature of the reaction mechanism is unknown at this time, a plausible mechanism for the formation of *N*-benzyl sulfonamide from $PhI(OAc)_2$, I_2, and sulfonamide is proposed in Scheme S1 on the basis of previous reports with related systems[1-5] and the following experimental observations: 1) an enhanced product yield when using an excess (2-5 equivalents) of aldehyde substrate, and 2) the requirement of either a stoichiometric or catalytic amount of iodine. The formation of AcOI, a source of electrophilic "I^+", is known to occur from the combination of $PhI(OAc)_2$ and I_2.[6-7] In the presence of sulfonamide, AcOI generates an *N*-iodosulfonamide **A**. The addition of iodine serves to reduce the nucleophilic strength of the sulfonamide nitrogen. This masking of the sulfonamide, along with the use of an excess of aldehyde substrate, allows for an aldehydic oxygen atom **B** to coordinate the electrophilic center of $PhI(OAc)_2$ similar to a previously reported mechanism.[5] The sulfonamide **A** then attacks the electron-deficient carbonyl carbon **C** leading to intermediate **D**. Loss of PhIO and acetate to produce intermediate **E** would occur with complete retention of the aldehydic C-H bond, which has been observed in the formation of *N*-sulfonyl imines from benzaldehydes under similar conditions.[3] Molecular iodine is regenerated in the formation of imine **F**, which accounts for the ability to perform the reaction using a catalytic amount of I_2. The resulting *N*-sulfonyl imine **F** is then reduced with $NaBH_4$ to form *N*-benzyl sulfonamide product **G**. Further investigation into the precise nature of the mechanism is currently underway.

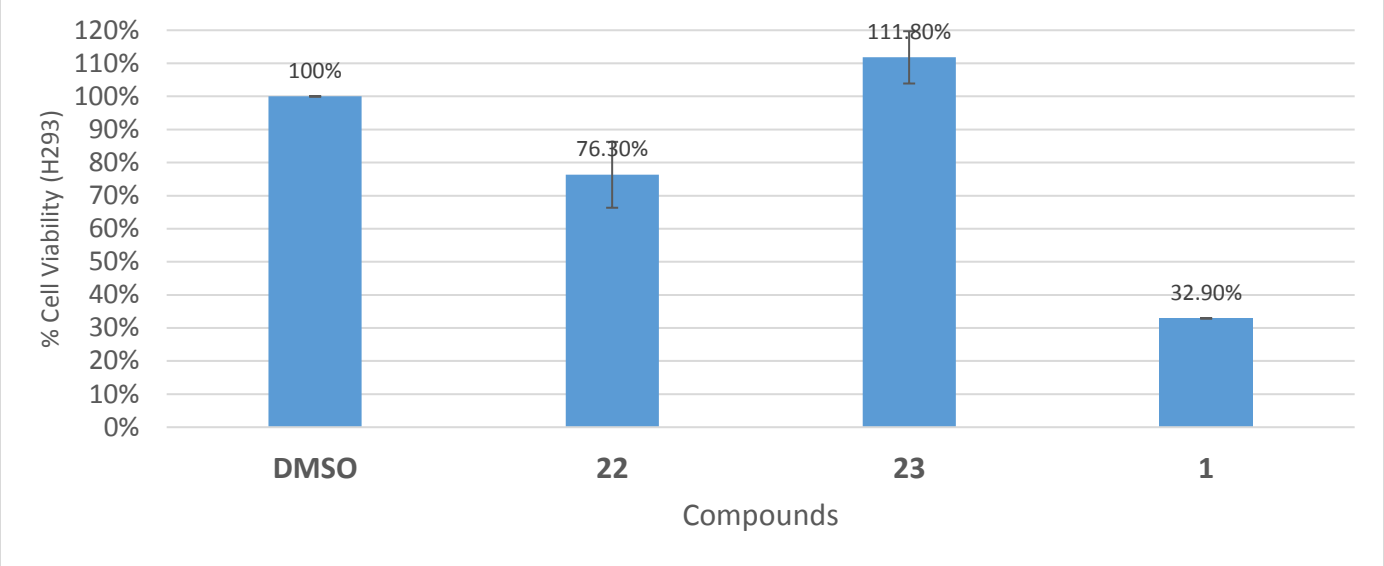


Table S3. Viability assay of protected indoles performed at 100 μM using Cell Titer Blue method.

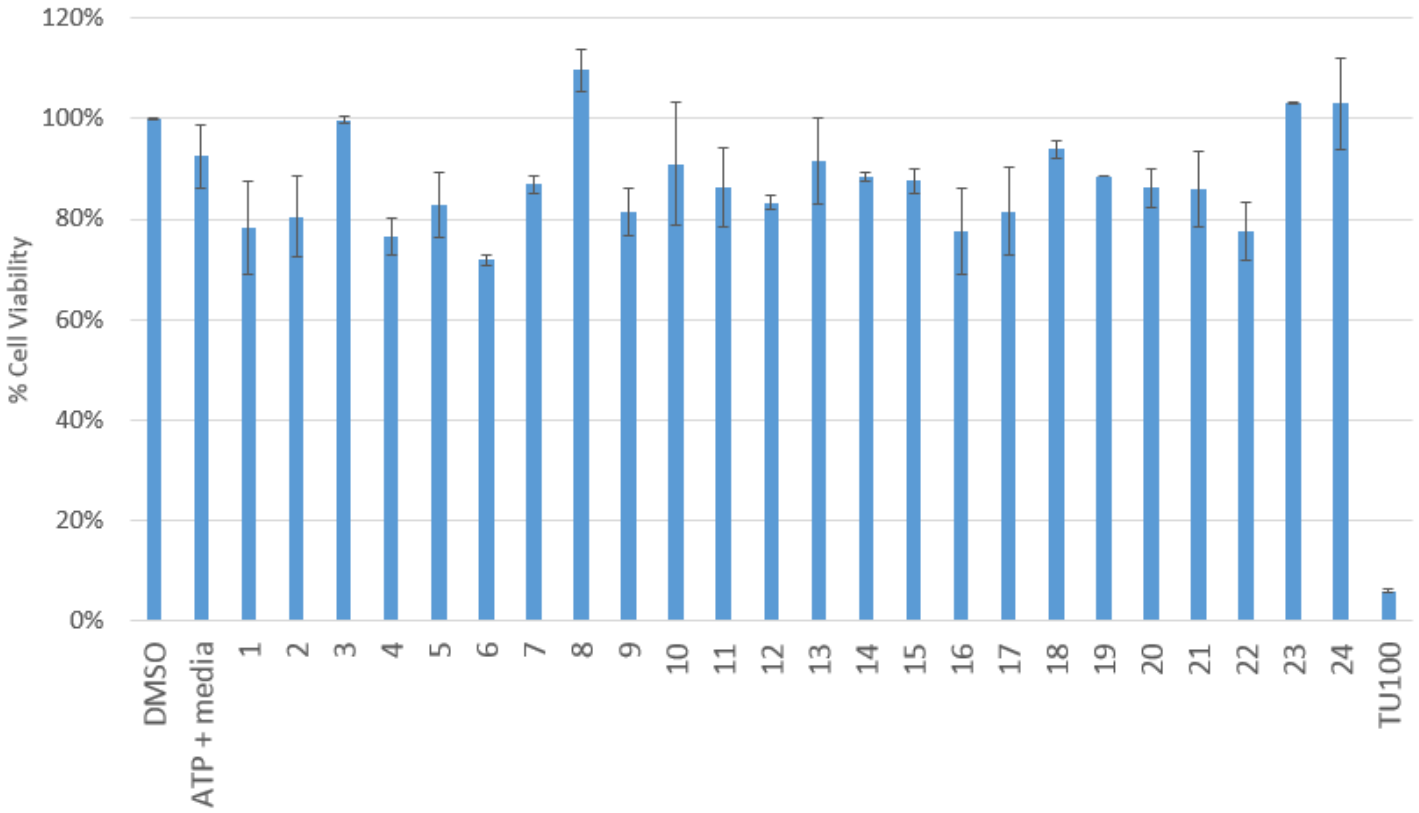


Table S4. Control experiments performed at 5 h exposure of 100 μM of compounds using Cell Titer Glo assay with exogenous ATP added. No inhibition of the luciferase-producing assay itself was observed by the compounds (**1-24**). TU100, a known inhibitor of the luciferase assay,[8] was used as a positive control.

Materials and Instrumentation (Synthesis):

All reagents and solvents were purchased from commercial sources and used without further purification. Heteroaryl aldehydes were purchased from Synthonix. I_2 was purchased from Alfa Aesar in 99.99+% purity (metals basis). ABT-751 was purchased from Ambeed. Indisulam was purchased from Sigma-Aldrich. Iminoiodinanes were prepared according to literature precedent[4,9] from $PhI(OAc)_2$, sulfonamide, and KOH, and the purity of the iminoiodinane was verified by decomposition point (decomposition is followed immediately by a dark red coloration: PhI=NNs = 123 °C; PhI=NTs = 102-105 °C; PhI=NCs = 116-118 °C; PhI=NBs = 118 °C). All synthesized iminoiodinanes were stored under an argon atmosphere at -4 °C (freezer) and used within one month. A description of the construction of our light bath (LED) photochamber is provided in Figure S1. ^1H and ^{13}C NMR spectra were recorded on a Varian 400/100 (400 MHz) spectrometer in deuterated chloroform ($CDCl_3$) with the solvent residual peak as internal reference unless otherwise stated ($CDCl_3$: ^1H = 7.26 ppm, ^{13}C = 77.02 ppm). Data are reported in the following order: Chemical shifts (δ) are reported in ppm, and spin-spin coupling constants (*J*) are reported in Hz, while multiplicities are abbreviated by s (singlet), bs (broad singlet), d (doublet), bd (broad doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets), t (triplet), bt (broad triplet), dt (double of triplets), td (triplet of doublets), m (multiplet). Infrared spectra were recorded on a Nicolet iS50 FT-IR spectrometer, and peaks are reported in reciprocal centimeters (cm^{-1}). Melting points were recorded on a Mel-Temp II (Laboratory Devices, USA) and were uncorrected. MS (EI) were obtained using a Shimadzu GC-2010 Plus with GCMS-QP2010. Accurate mass spectrum (HRMS-High Resolution Mass Spectrometry) was performed using a Thermo Scientific Exactive spectrometer (Waltham, MA, USA) operating in positive ion electrospray mode (ESI-electrospray ionization). Additional HRMS (ESI+) accurate mass spectra were obtained at The University of Oklahoma with the assistance of Dr. Steven Foster.

General procedure for the preparation of N-sulfonyl imine D (from Table S1):

^1H NMR integration yield – Method A.

To an oven-dried reaction tube was added aldehyde (5 equiv., 0.100 g), iminoiodinane (1 equiv.), I_2 (1 equiv.), and $CDCl_3$ (1.5-2 mL). The mixture was stirred at 50 °C under argon for 24 hours. After 24 hours, the reaction was cooled to room temperature and internal standard was added (1,4-dimethoxybenzene, 1 equiv.), and the mixture was stirred for an additional 5-10 minutes. The percent yield was calculated against internal standard via ^1H NMR integrations.

^1H NMR integration yields - Method B.

To an oven-dried reaction tube was added aldehyde (5 equiv. 0.100 g), sulfonamide (1 equiv.), $PhI(OAc)_2$ (2 equiv.), I_2 (1 equiv.), and $CDCl_3$ (1.5-2 mL). The mixture was stirred at 50 °C under argon for 24 hours. After 24 hours, the reaction was cooled to room temperature and internal standard was added (1,4-dimethoxybenzene, 1 equiv.), and the mixture was stirred for an additional 5-10 minutes. The percent yield was calculated against internal standard via ^1H NMR integrations.

General procedure for the preparation of N-benzyl sulfonamides:

To an oven-dried reaction tube was added aldehyde (0.375-0.625 mmol, 3-5 equiv), sulfonamide (0.125 mmol, 1 equiv.), iodobenzene diacetate (0.25 mmol, 2 equiv.), I_2 (0.125 mmol, 1 equiv.) in $CHCl_3$ (1.5 mL). The mixture was stirred at 50 °C under argon for 24 hours. After 24 hours, the reaction was cooled to room temperature and the solvent was removed. The crude was dissolved in 1.5 mL MeOH and 1.5 mL DCM and placed in an ice bath to cool to 0 °C. $NaBH_4$ (0.0780g, ~2 mmol) was added in small portions with stirring. After addition, the mixture was removed from the ice bath and stirred at room temperature for an additional 45 minutes. After 45 minutes, the reaction was quenched with 3 mL H_2O and then extracted with EtOAc (2 x 5 mL). The combined organic layers were washed with brine (10 mL), dried (Na_2SO_4), and the solvent removed under vacuum. The crude amine was purified by flash chromatography using a hexanes:EtOAc eluent.

Materials and Methods (Bioactivity Assays):

Cell-based Glo kits, such as Cell Titer Blue (to measure cell viability via conversion of resazurin to the fluorescent resorufin) and BacTiter-Glo were obtained from Promega (Madison, WI, USA). All cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). All other materials and supplies were purchased from commercial sources and used without additional purification. Cell cultures were maintained in DMEM (Fisher Scientific) supplemented with 10% fetal bovine serum and Penn/Strep. Cultures were maintained in a 37 °C water-jacketed incubator with 5% CO_2. For experiments in 96-well plates, proliferating cells were removed from the stock plate using PBS plus 2.5 mM EDTA. The desired number of cells (~20,000) were distributed in a 96-well plate containing 100 μL DMEM plus 10% FBS and allowed to attach overnight. After 24 hours, cells were treated with the indicated library compound **1-24** or DMSO control (5%). After 24 hours, cell viability was determined by adding 10 μL Cell Titer Blue reagent (resazurin) for 1-4 hours. Fluorescence was measured on a TECAN Safire plate reader (ex^{560}/em^{590}). IC_{50} values were determined using non-linear regression analysis in Graph-Prism software from library compound doses of 0 μM, 6.25 μM, 12.5 μM, 25 μM, 50 μM, and 100 μM (Cell Titer Blue assay).

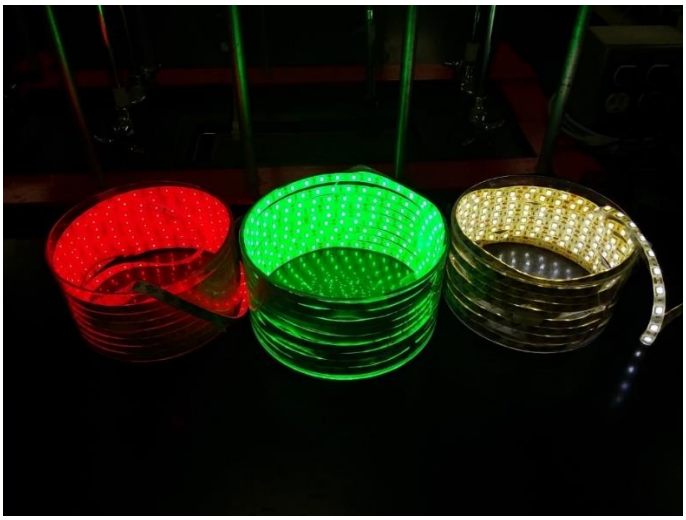


Figure S1. On the left – A red LED strip light bath reaction vessel; In the middle – A green LED strip light bath reaction vessel; On the right – A white LED strip (cool white) light bath reaction vessel.

Photocatalytic reactions were set up in a light bath which was constructed in our laboratory by coiling LED strips around an evaporating dish according to our previous reports:[1-3, 10-11]

Waterproof 5050 LED strips (12V with power adapter, 18 LEDs/foot, approximately 0.24 Watt per LED – 72 Watt per strip) are coiled around the interior of evaporating dish (170mm x 90mm) using the adhesive backing of the LED strip. A Petri dish (150 x 20 mm) is placed upside down at the bottom of the dish to serve as an elevated glass "floor" to ensure that a round-bottom flask receives maximum light exposure. The ambient temperature inside the dish is monitored and is generally maintained (air-cooled) between 19-22 °C (the temperature has not been observed above 25 °C).

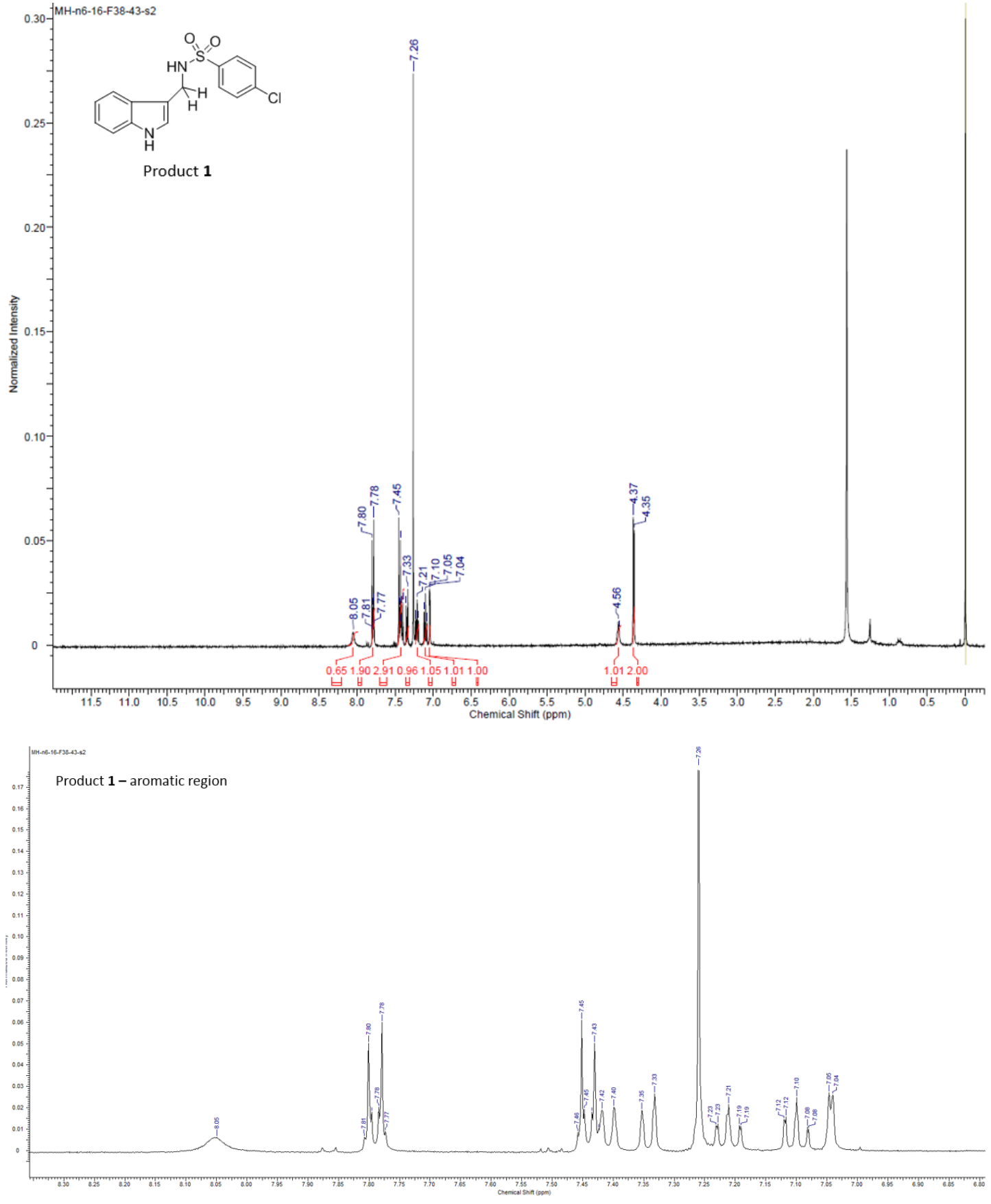


Figure S2. ^{1}H NMR of Product **1**.

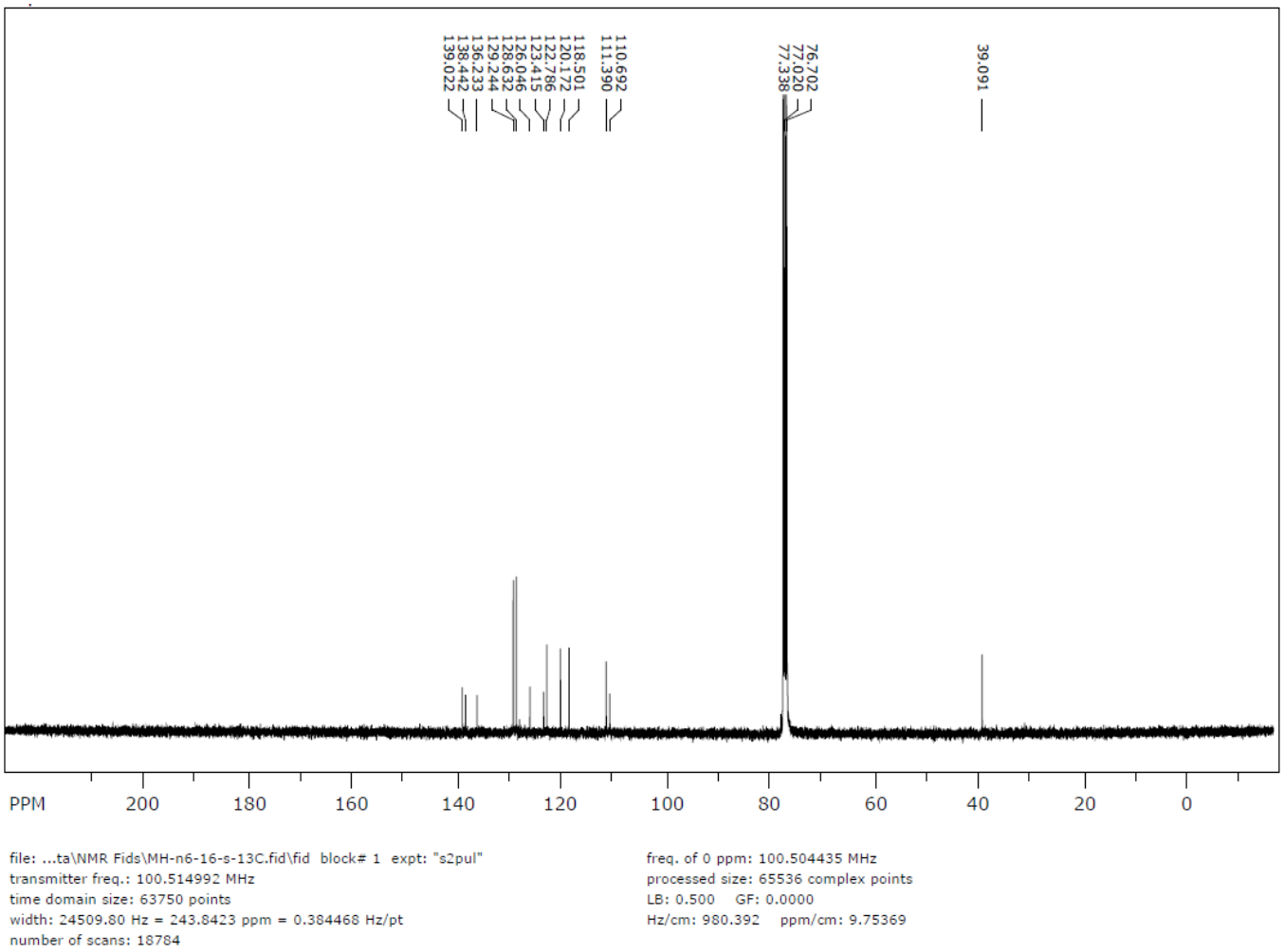


Figure S3. ^{13}C NMR of Product **1**.

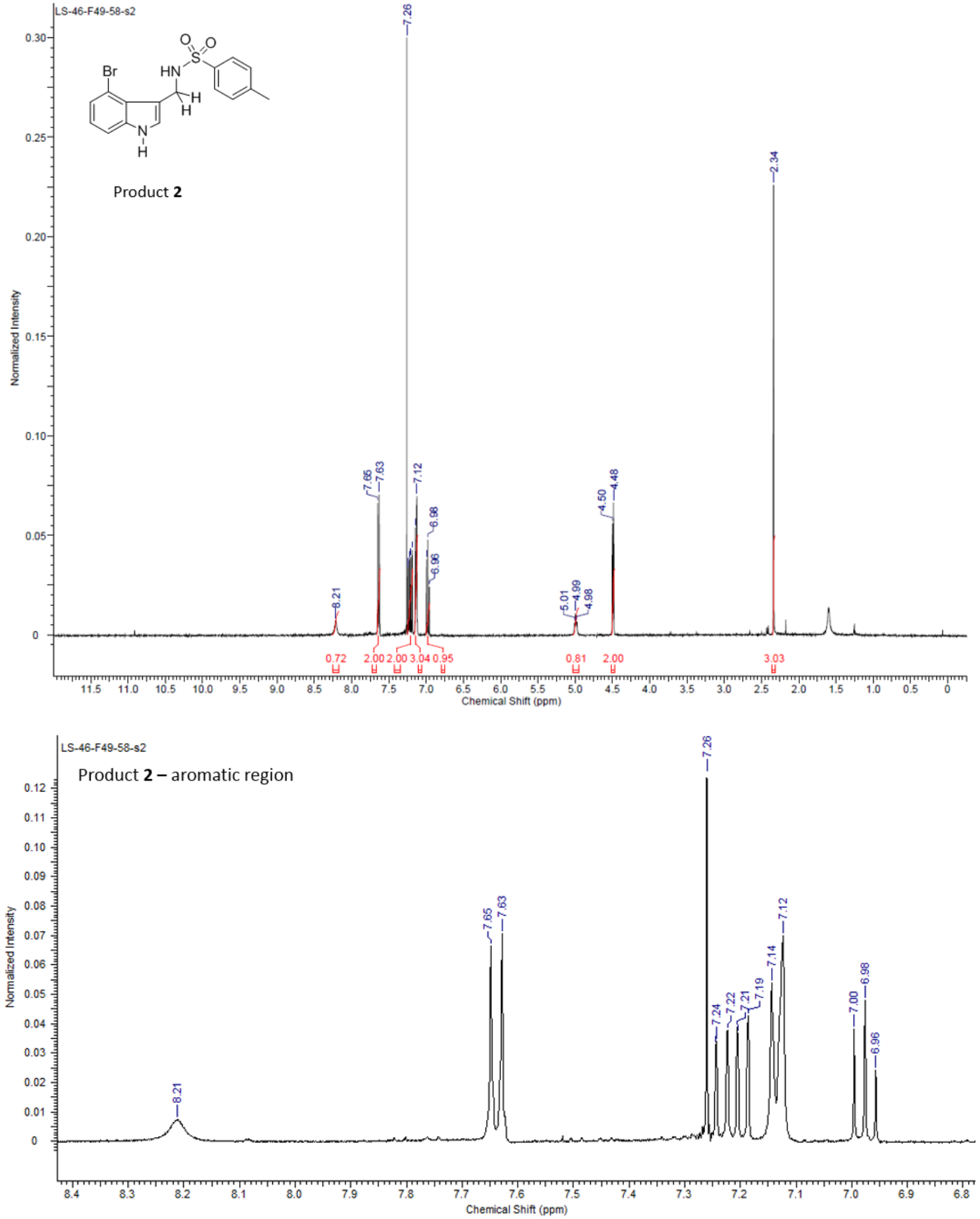
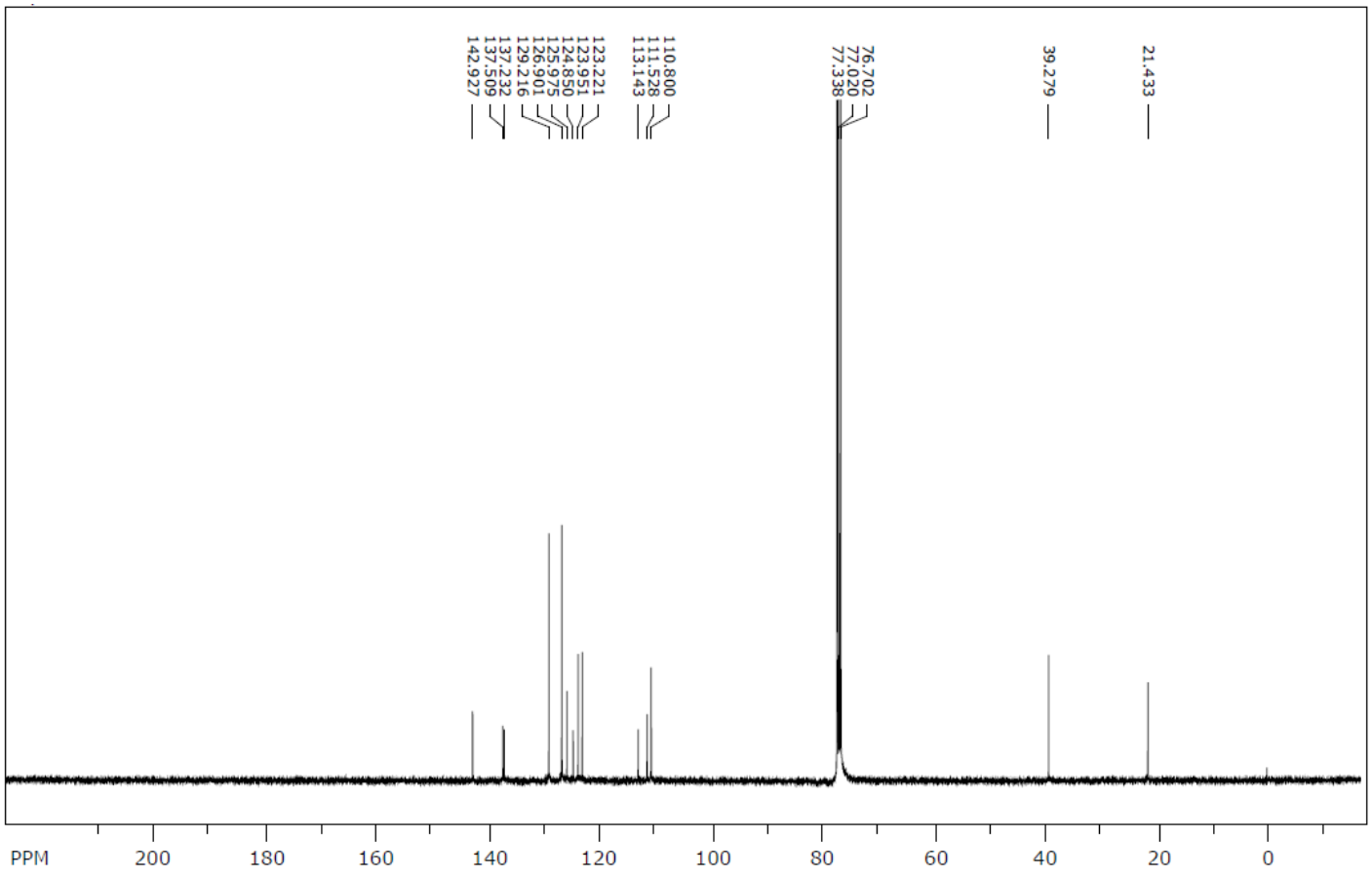


Figure S4. ^{1}H NMR of Product **2**.



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Hz/cm: 980.392 ppm/cm: 9.75369

Figure S5. ^{13}C NMR of Product **2**.

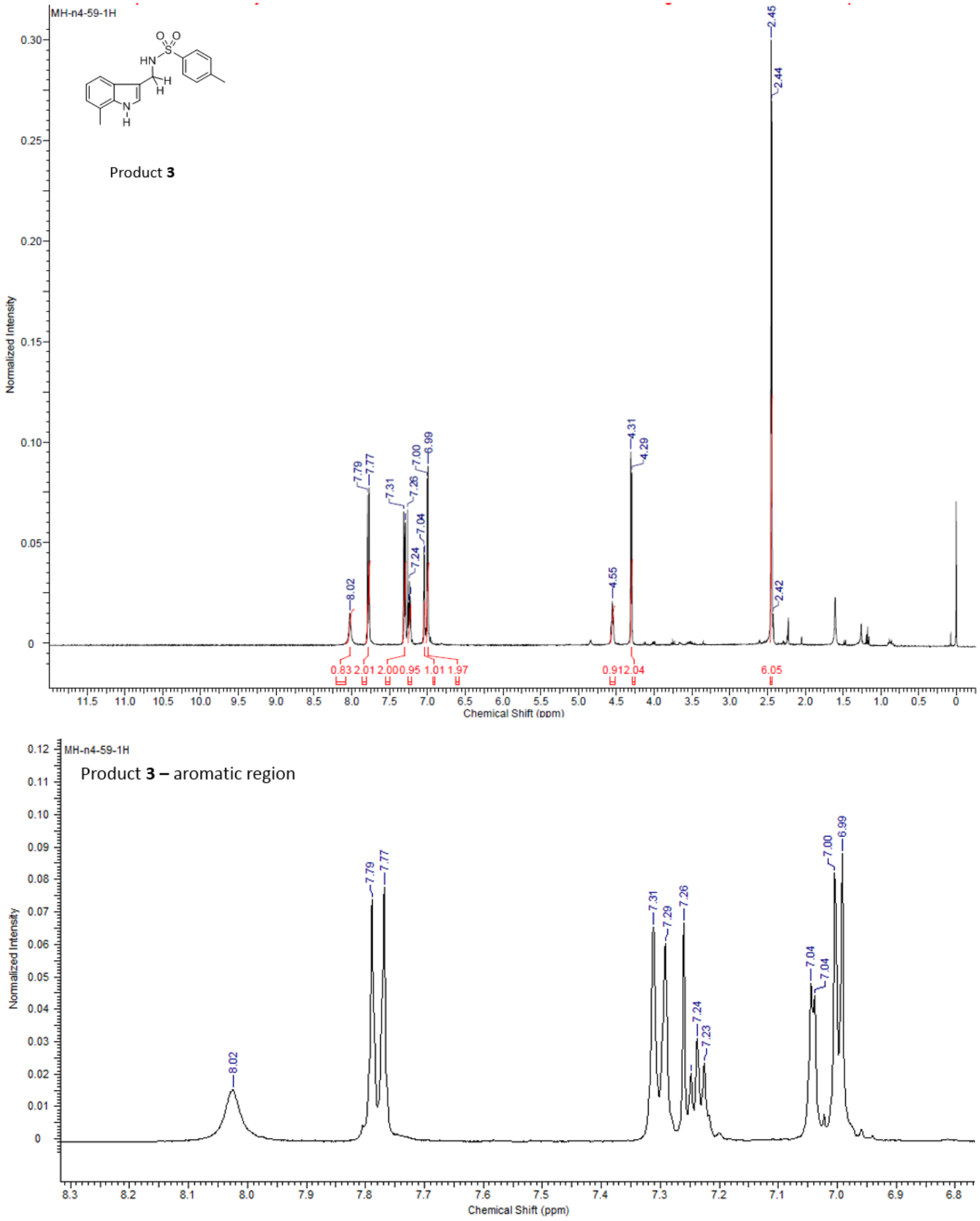

Figure S6. ^{1}H NMR of Product **3**.

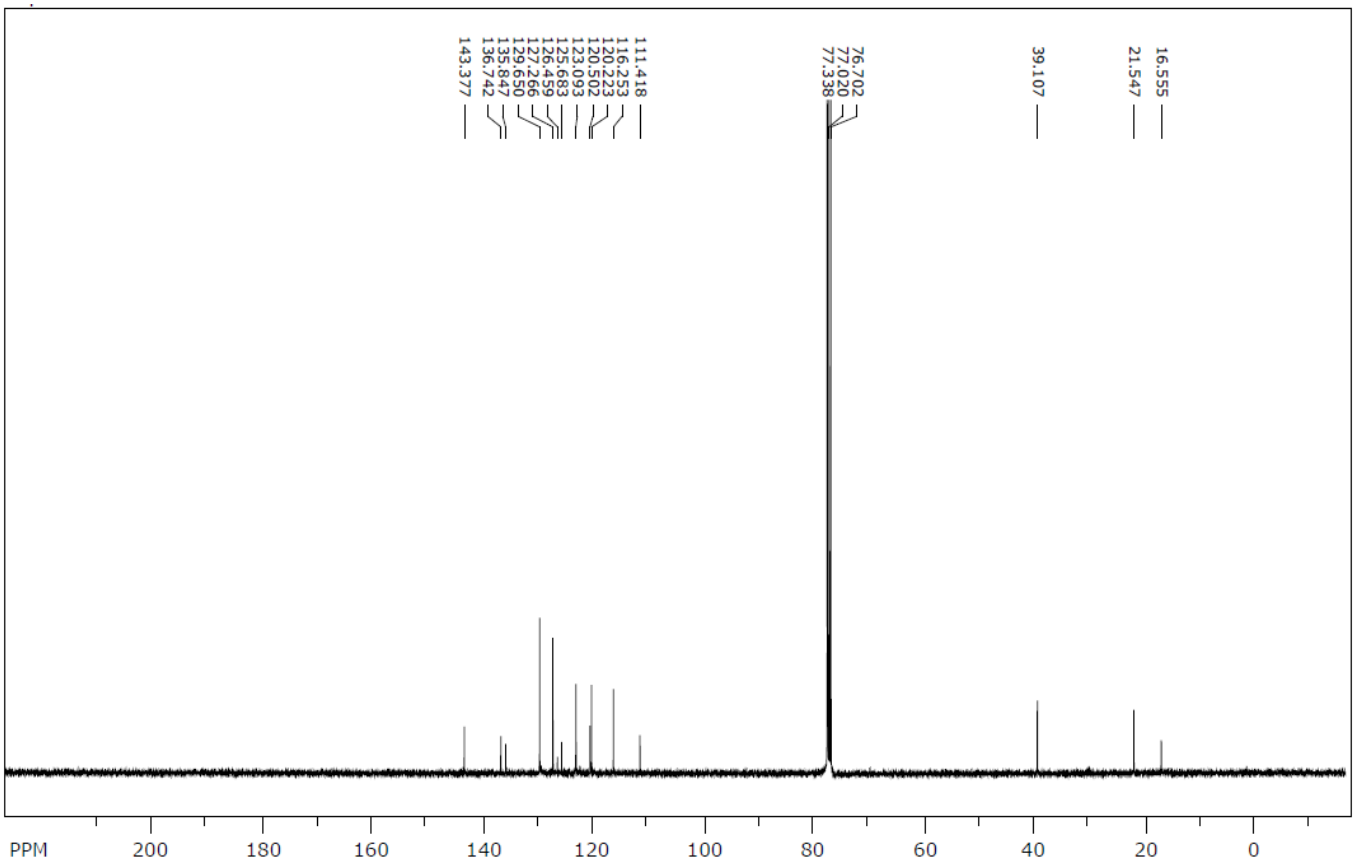

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Figure S7. ^{13}C NMR of Product **3**.

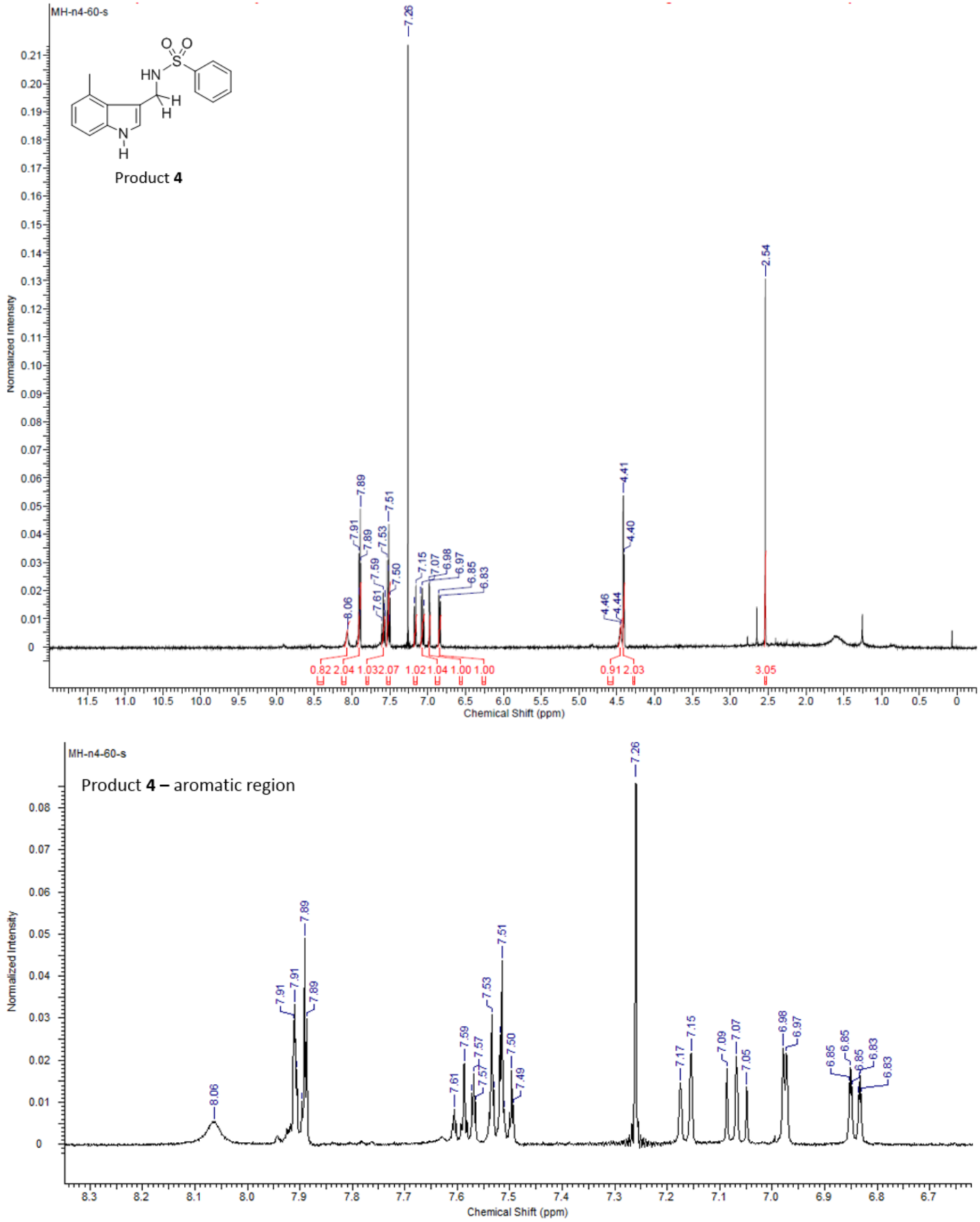

Figure S8. ^{1}H NMR of Product **4**.

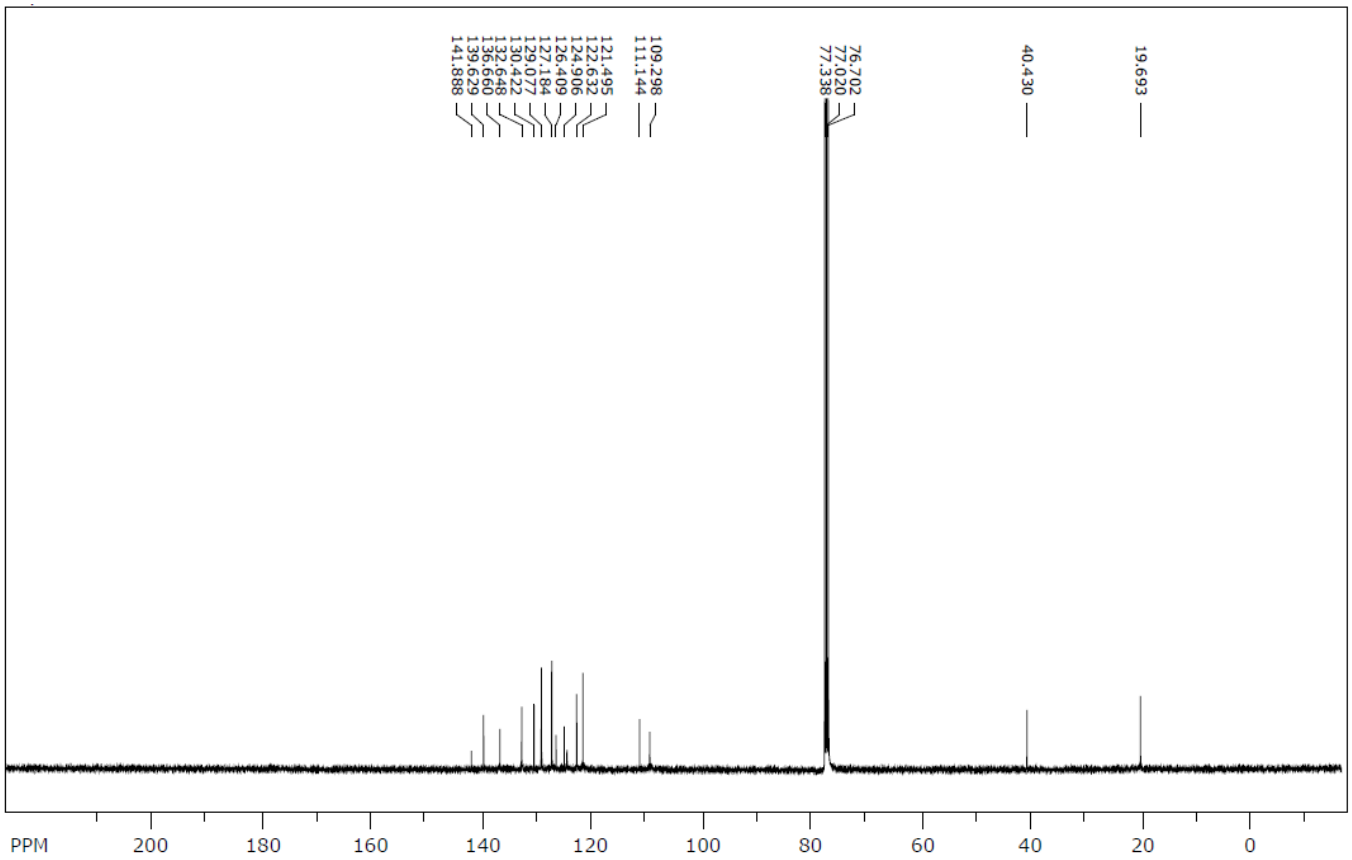

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Figure S9. ^{13}C NMR of Product **4**.

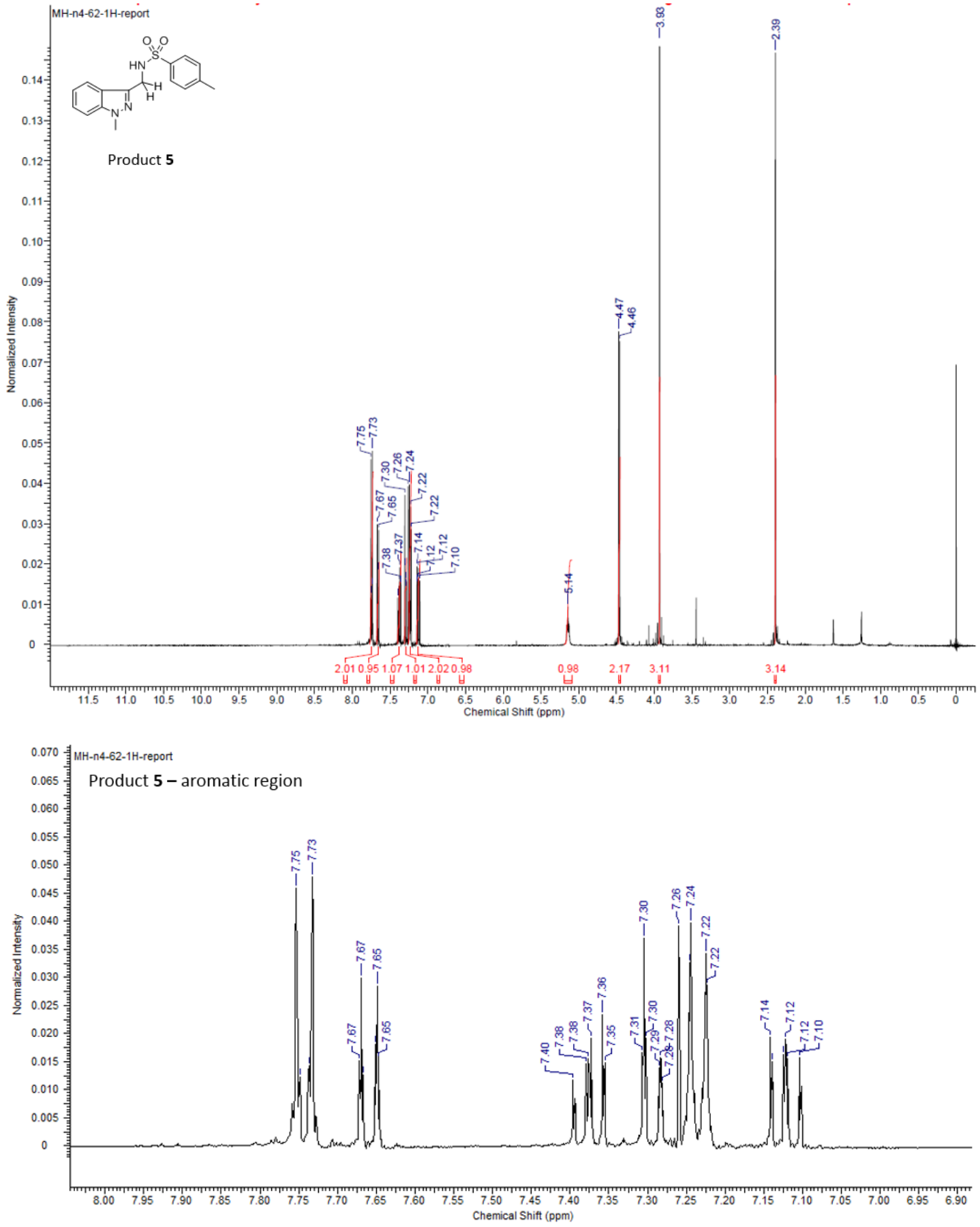

Figure S10. ^{1}H NMR of Product **5**.

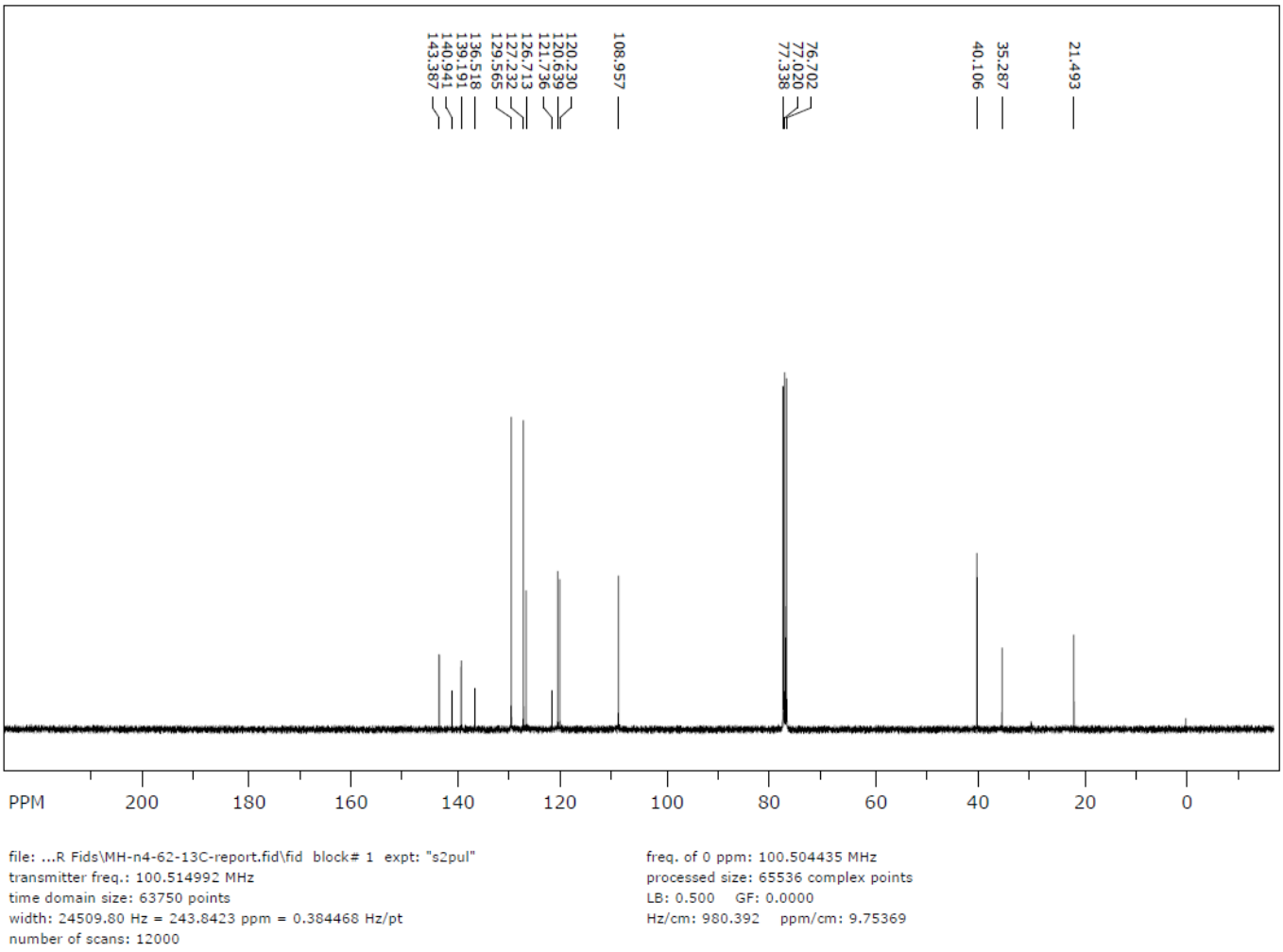

Figure S11. ^{13}C NMR of Product **5**.

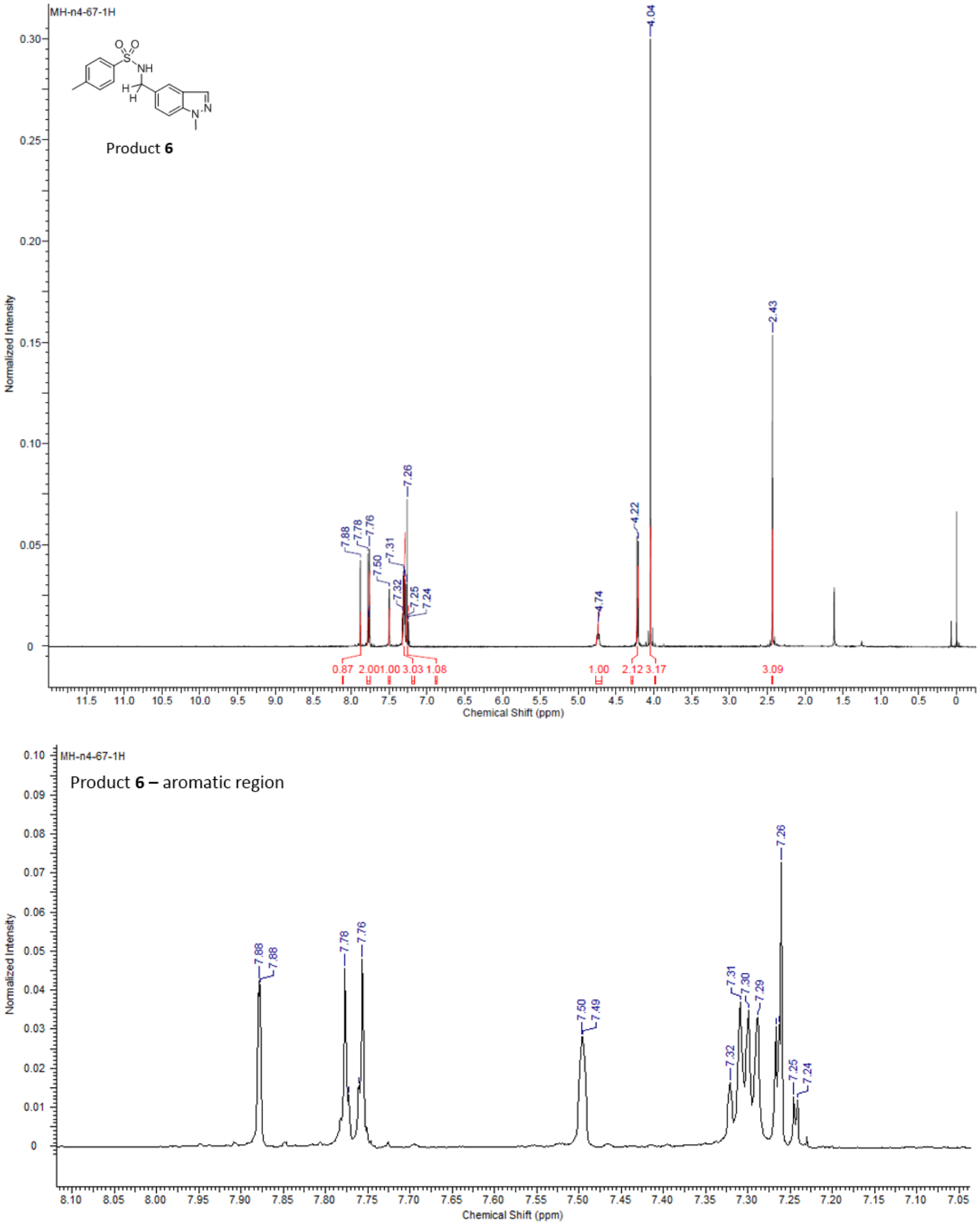

Figure S12. ^{1}H NMR of Product **6**.

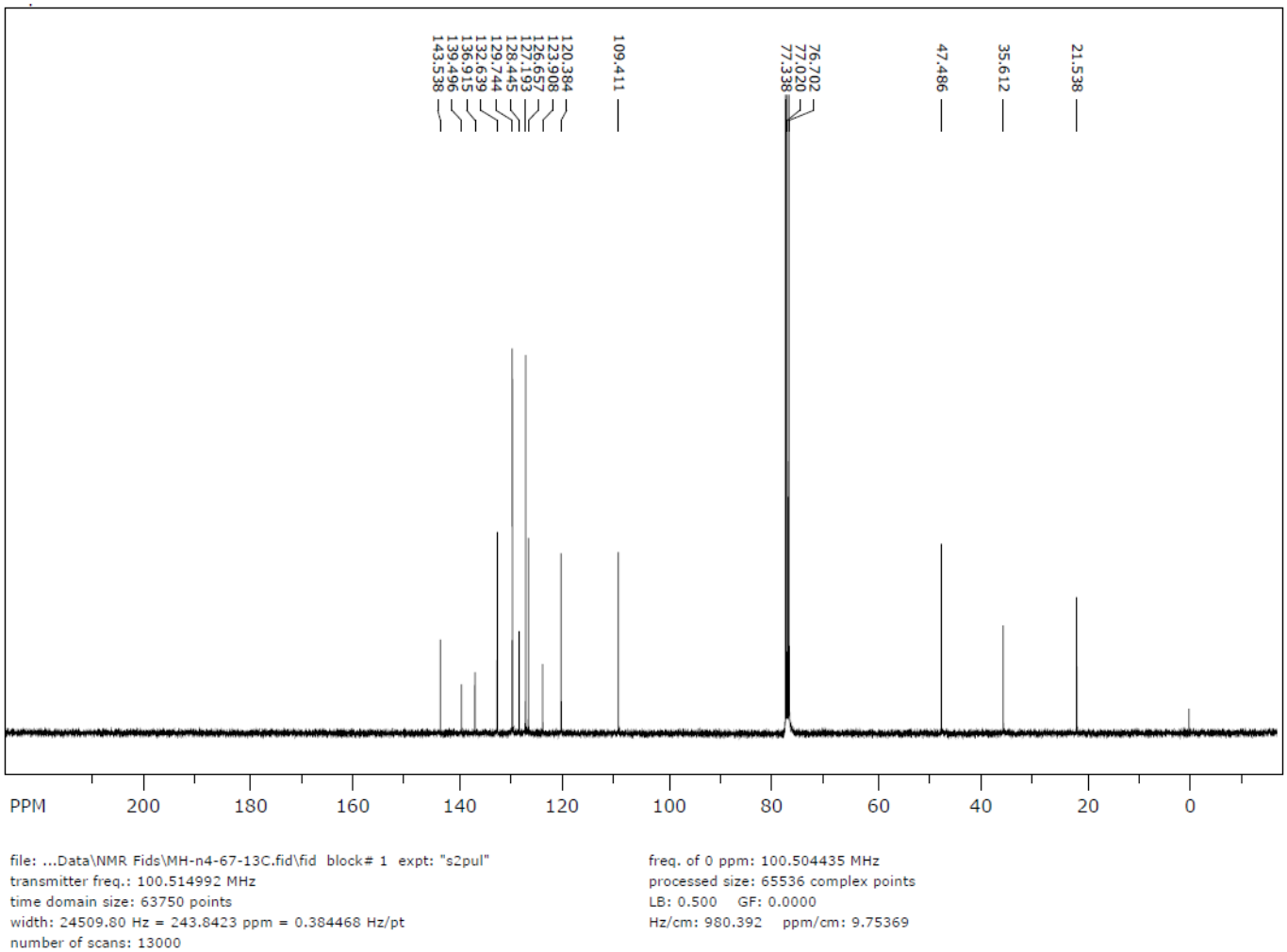

Figure S13. ^{13}C NMR of Product **6**.

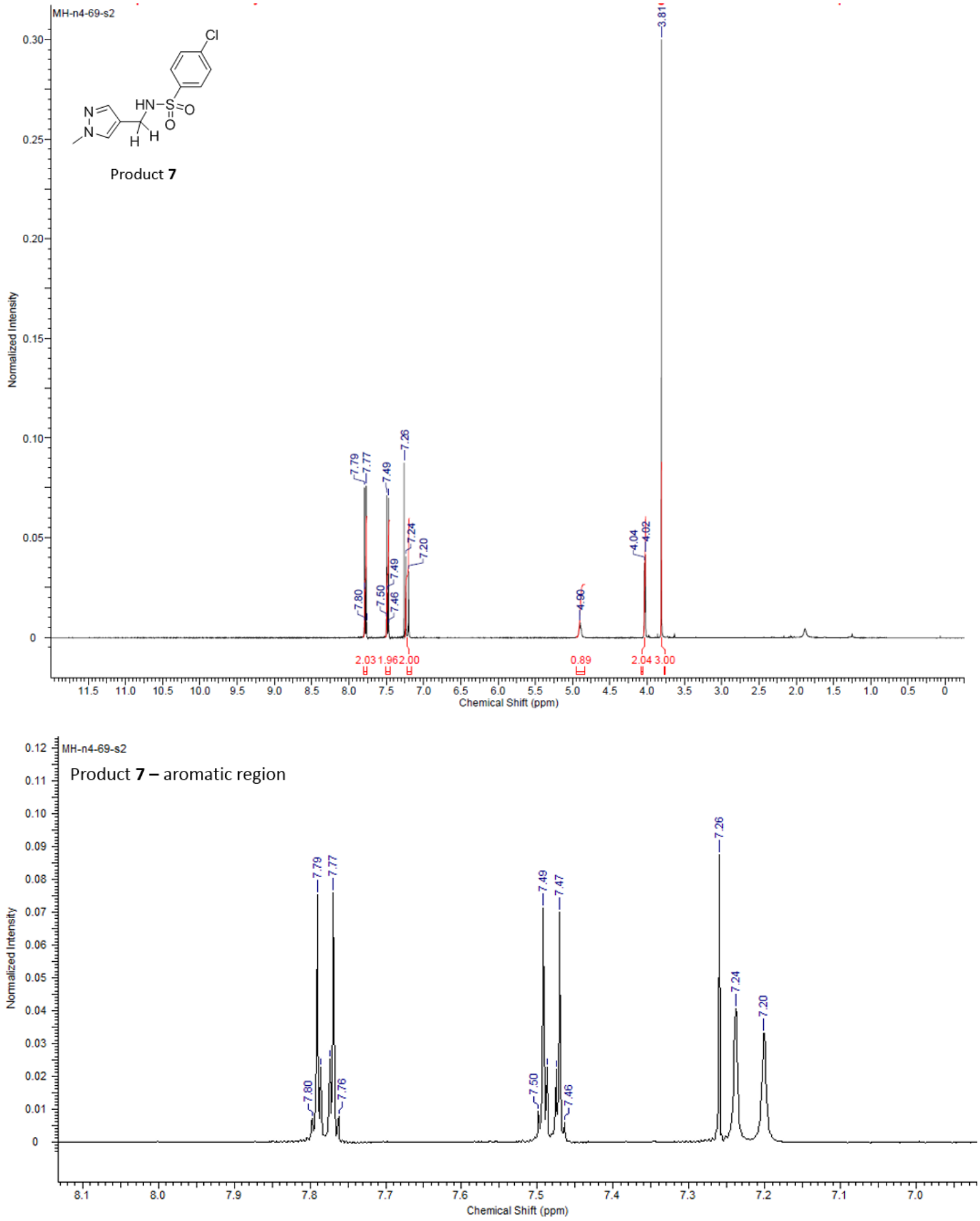

Figure S14. ^{1}H NMR of Product **7**.

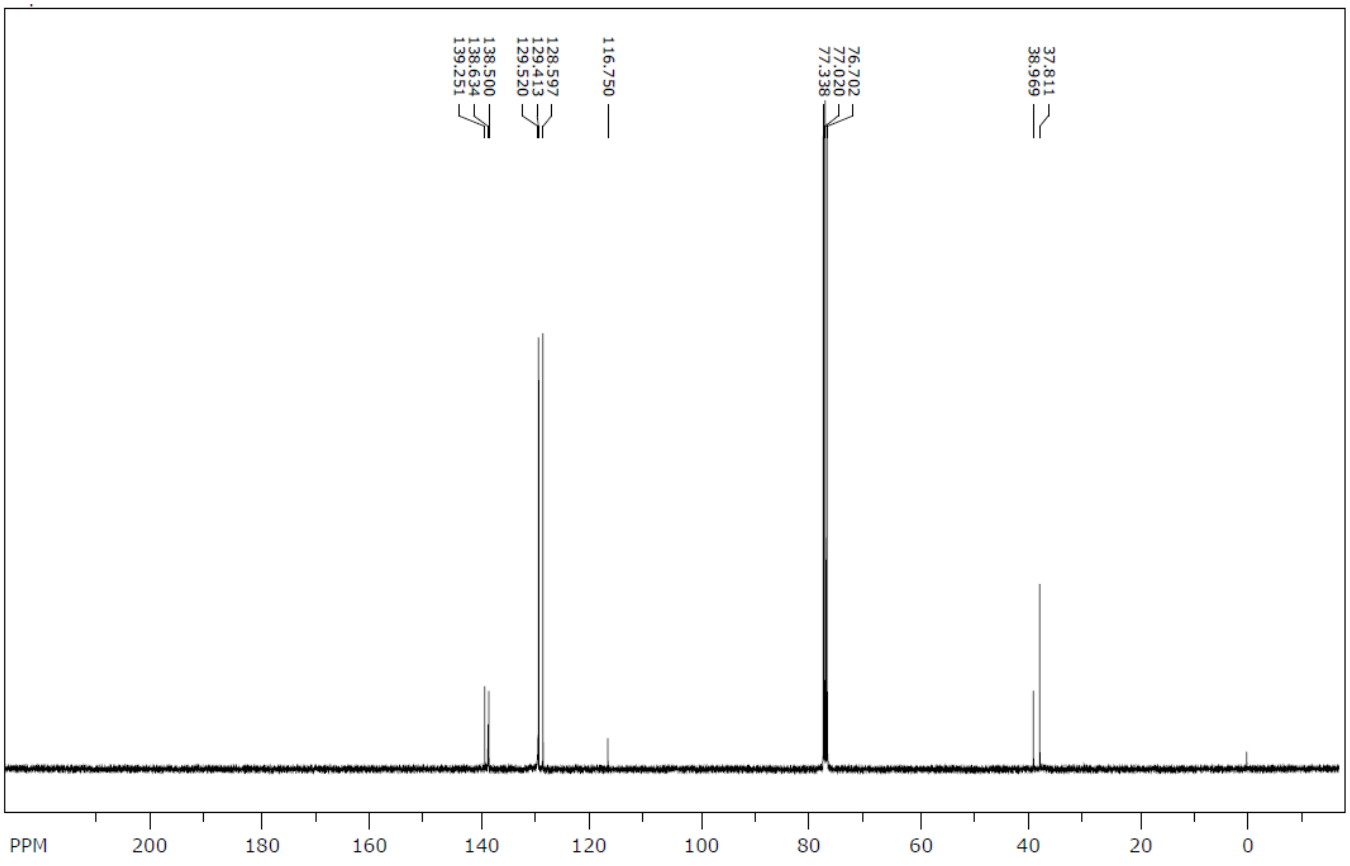

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Figure S15. ^{13}C NMR of Product **7**.

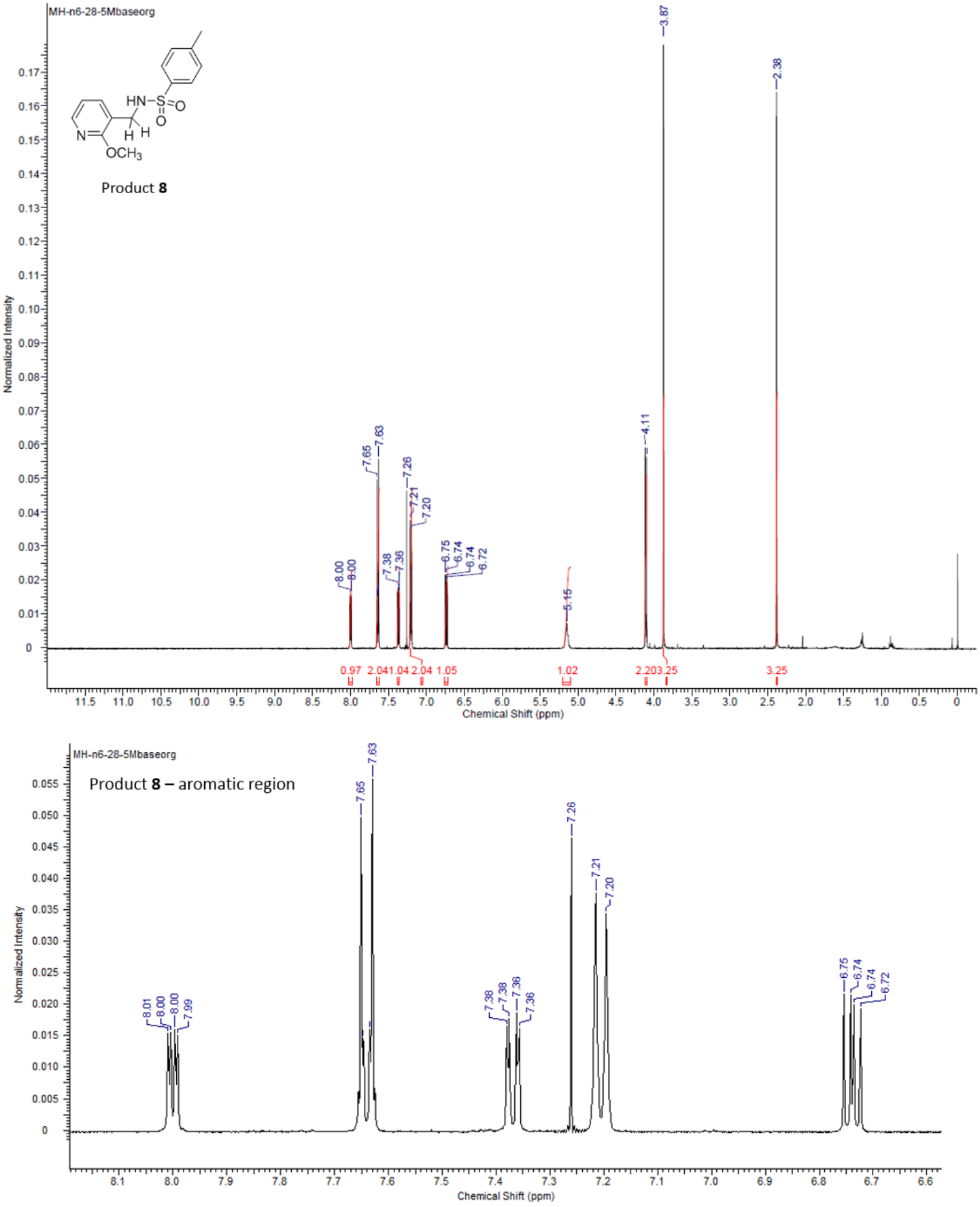

Figure S16. ^{1}H NMR of Product **8**.

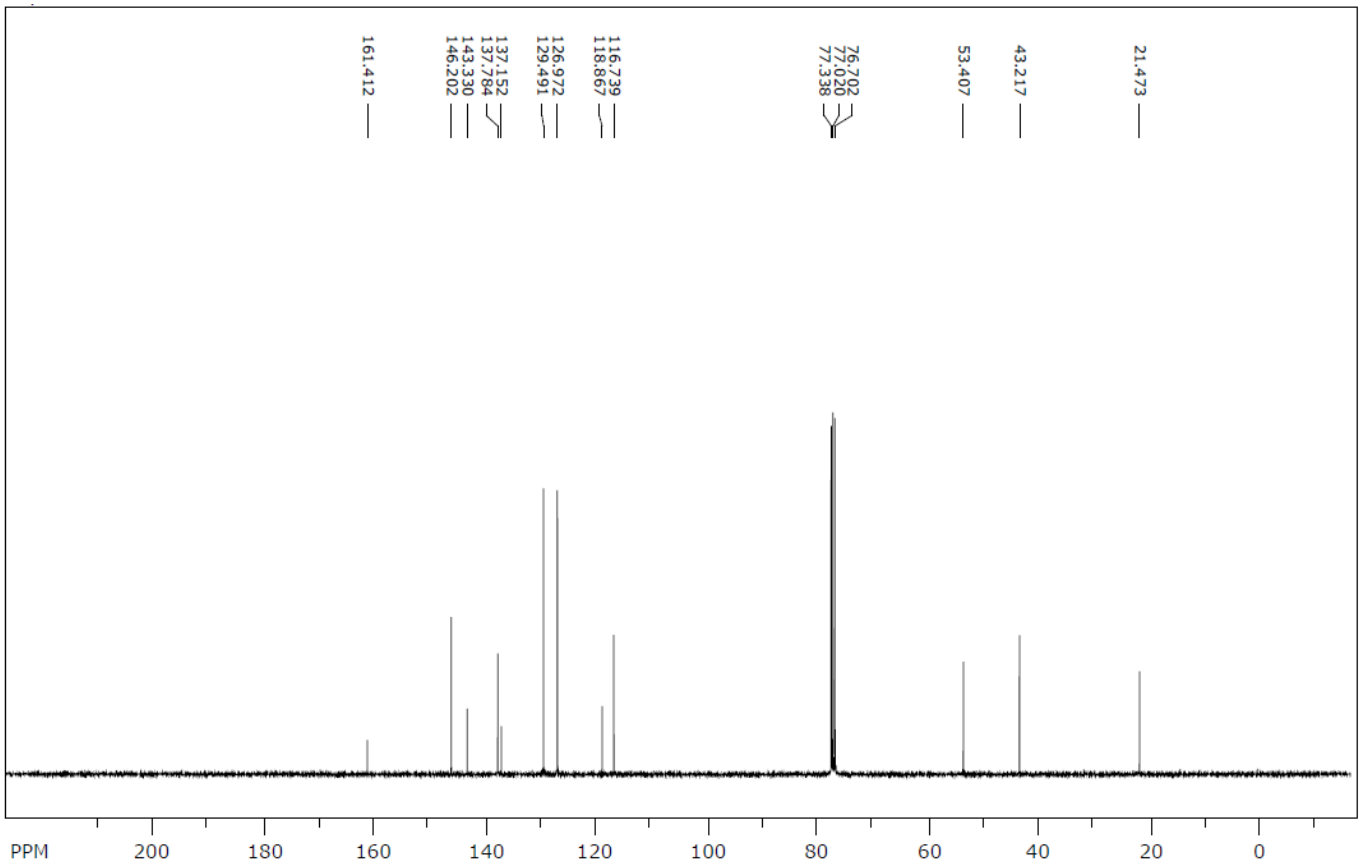

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Figure S17. ^{13}C NMR of Product **8**.

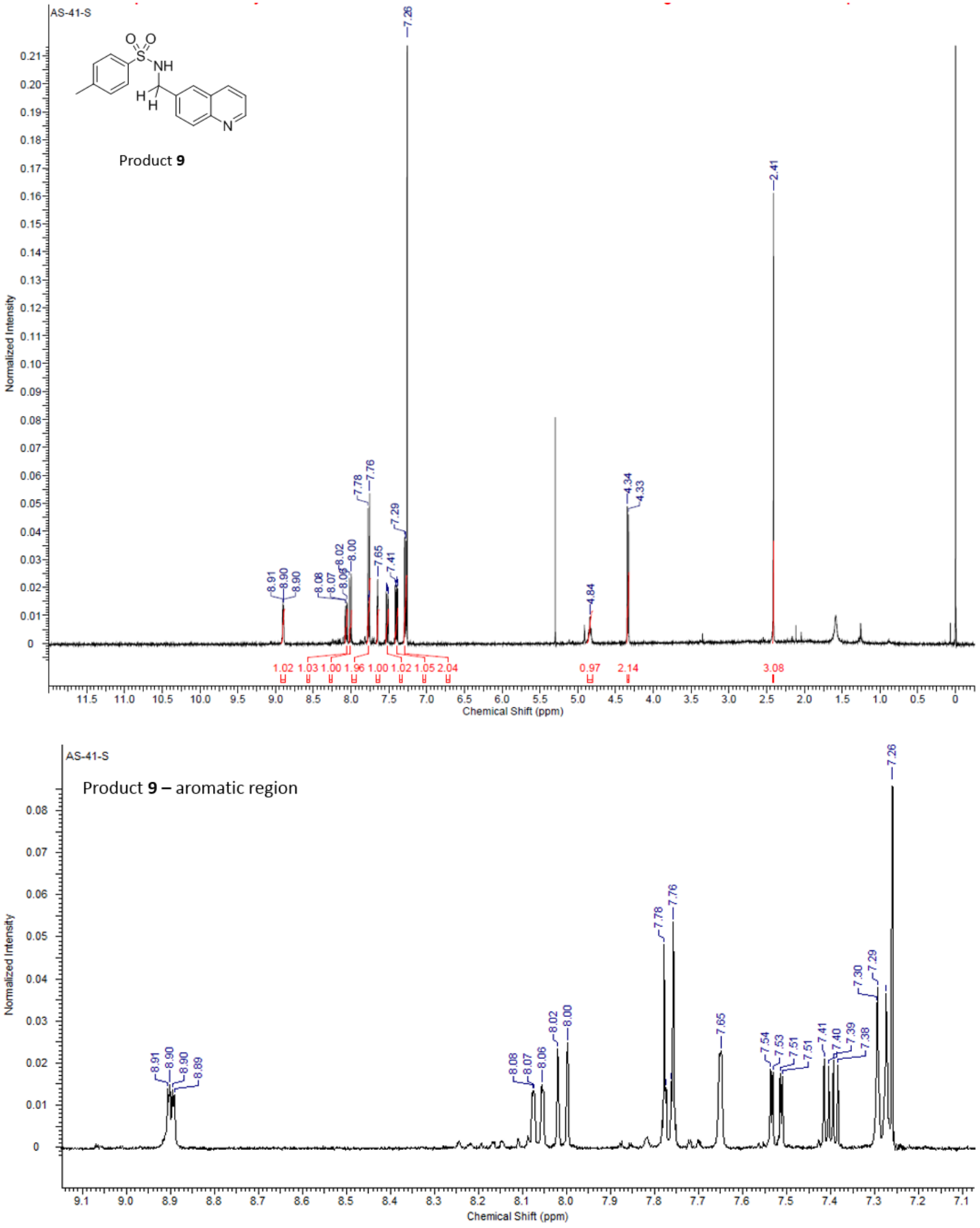

Figure S18. ^{1}H NMR of Product **9**.

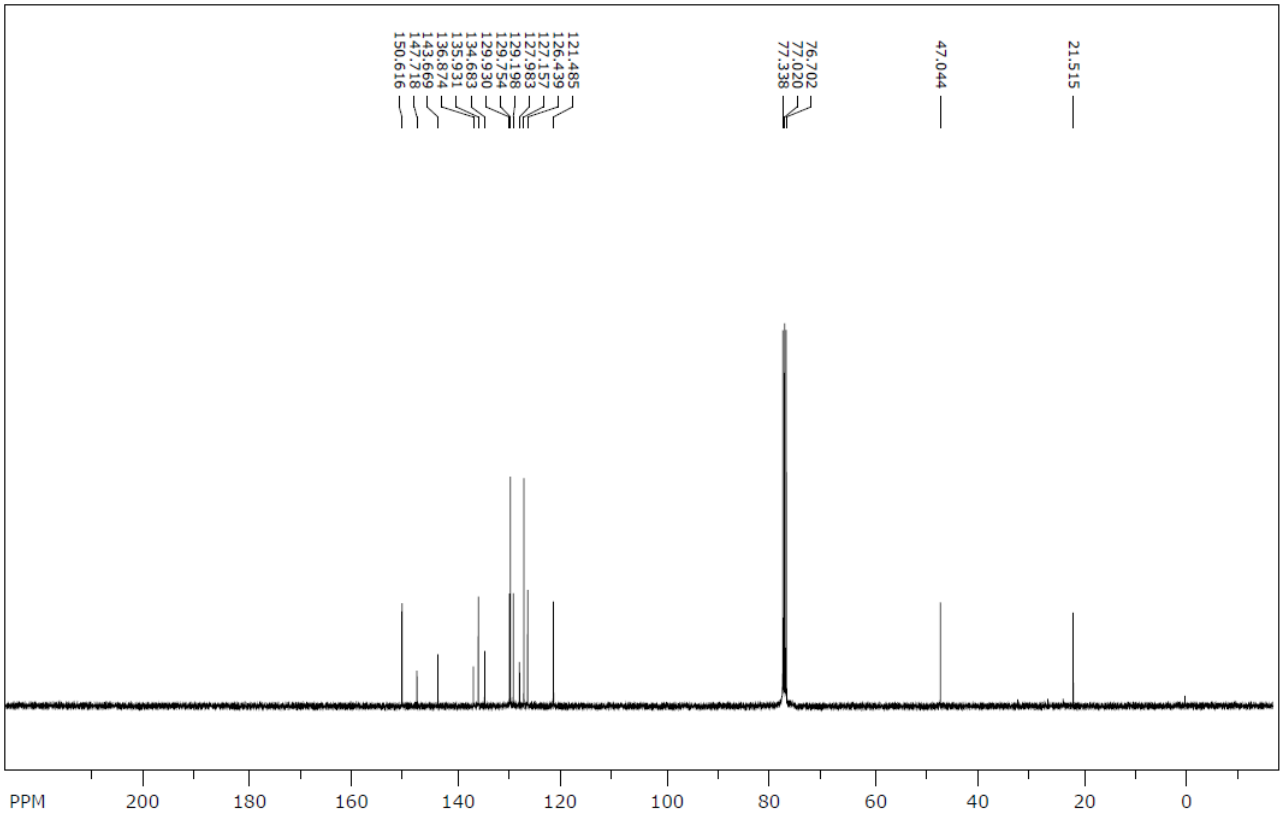

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Figure S19. ^{13}C NMR of Product **9**.

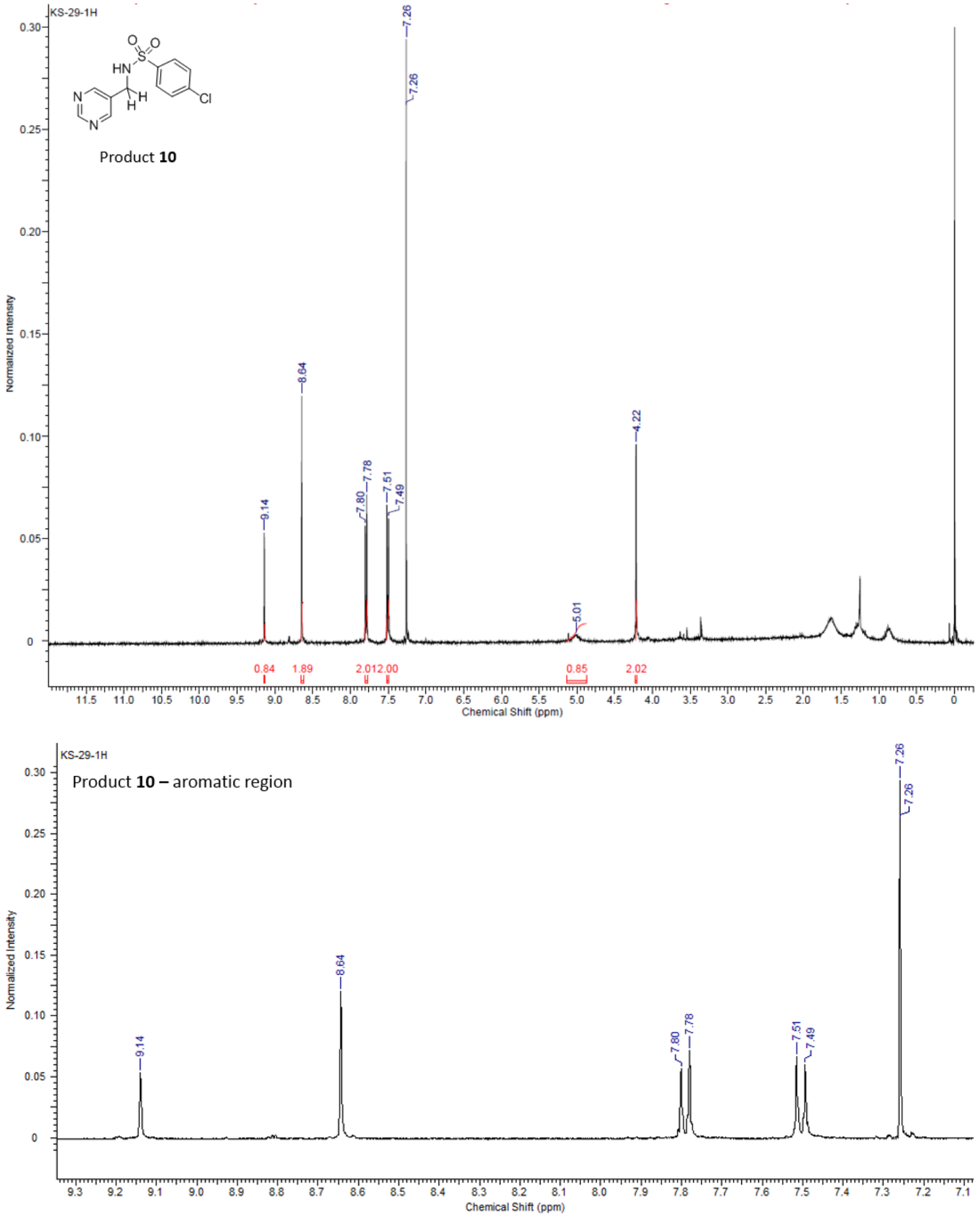


Figure S20. ^{1}H NMR of Product **10**.

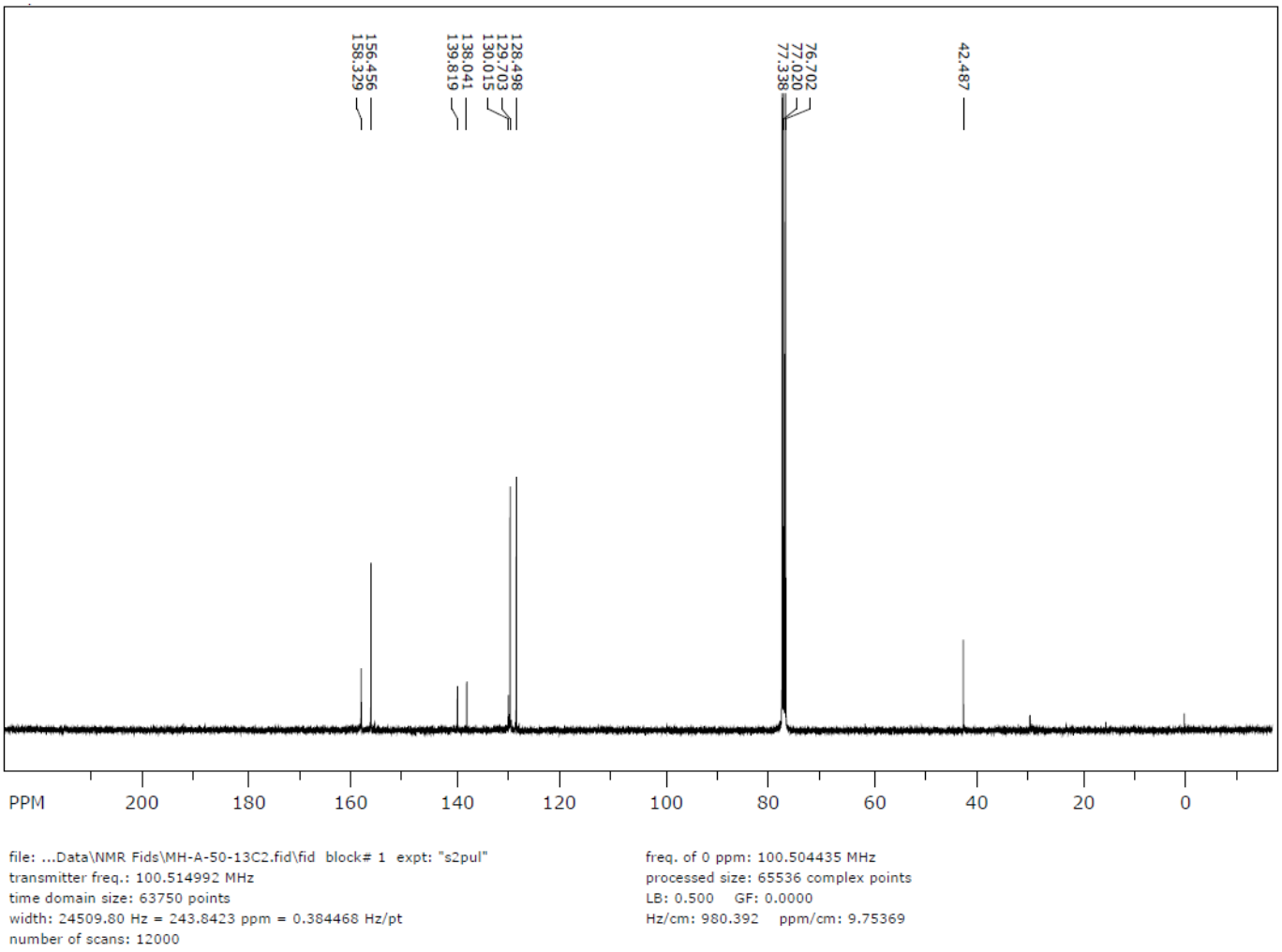


Figure S21. ^{13}C NMR of Product **10**.

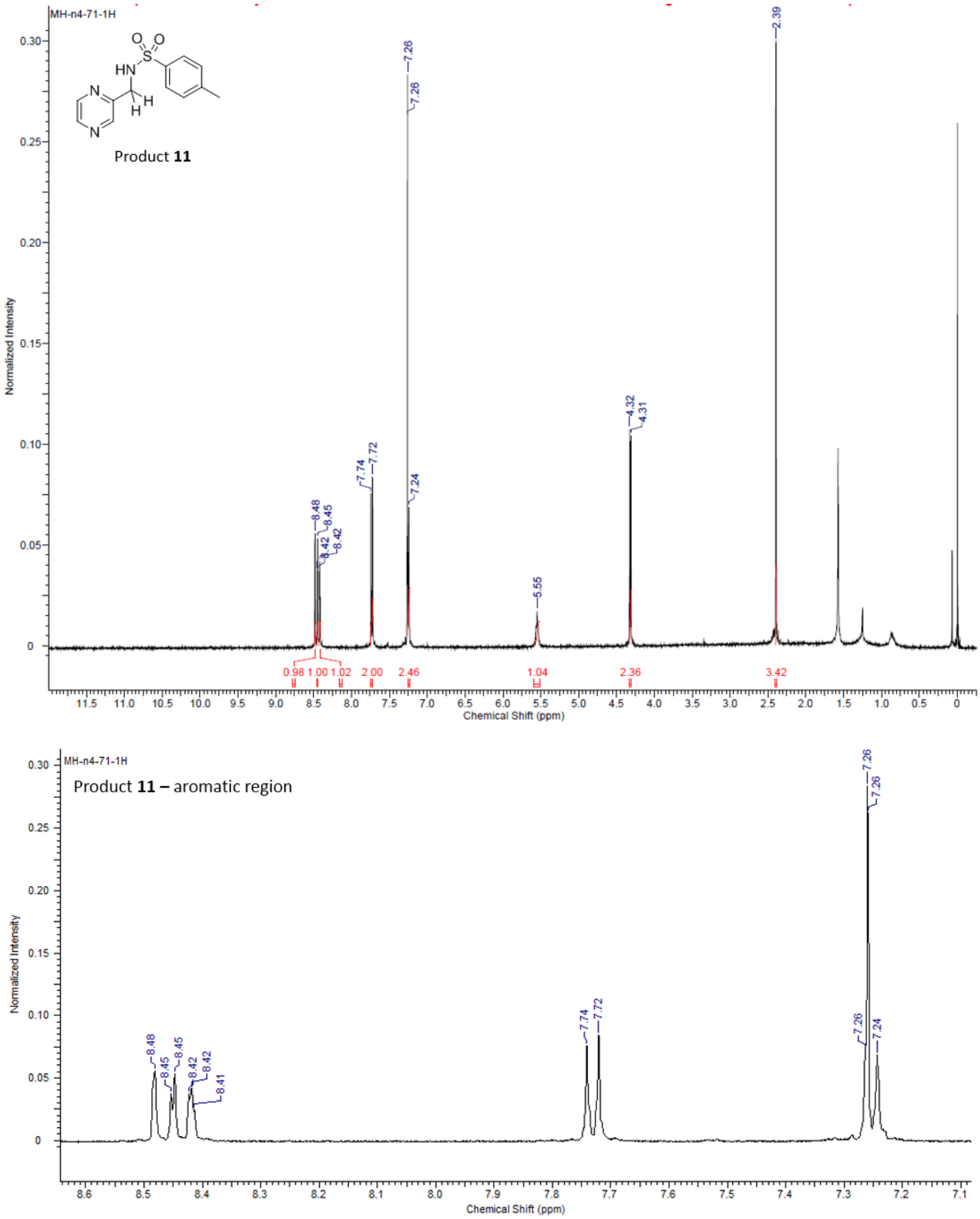
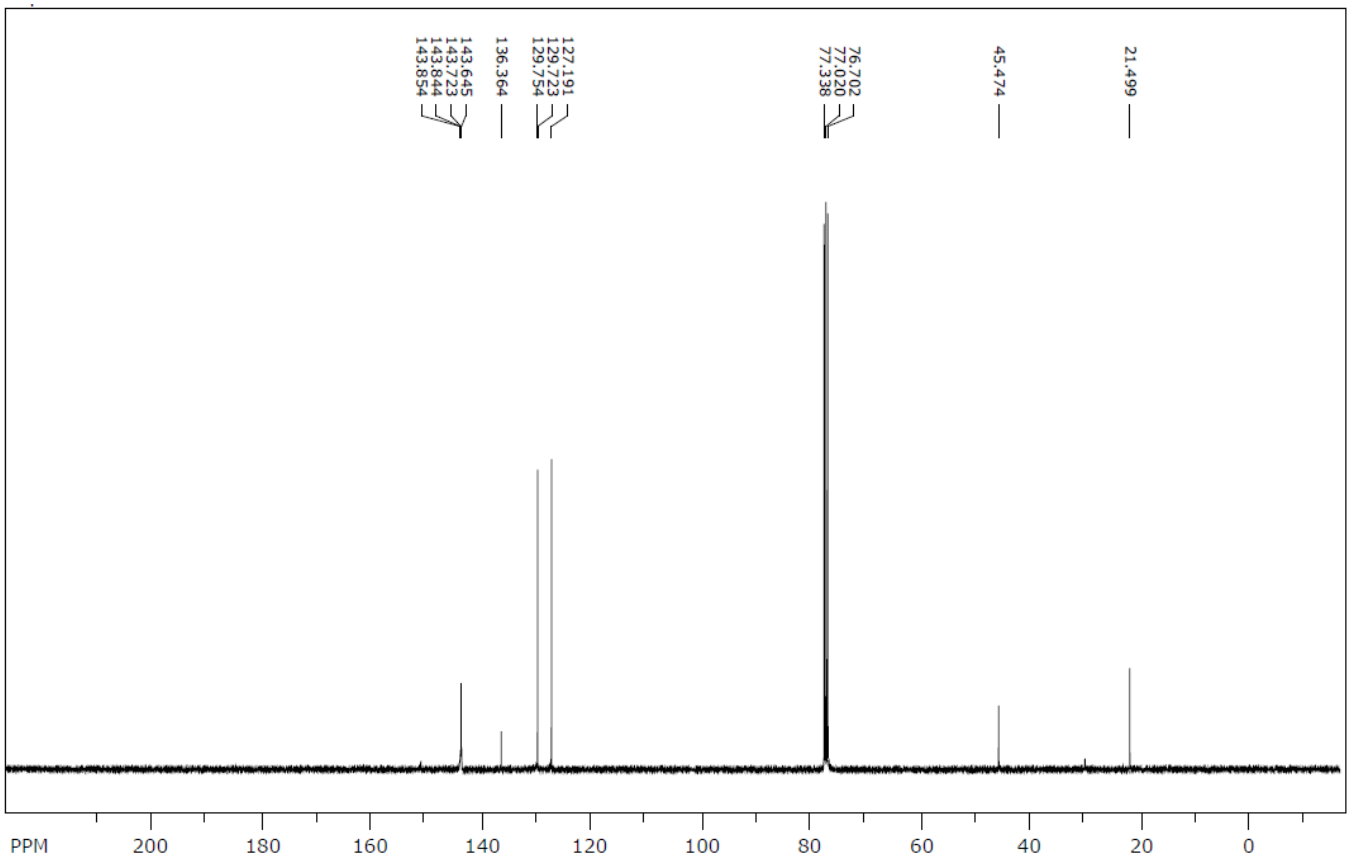


Figure S22. ^{1}H NMR of Product **11**.



file: ... Data\NMR Fids\MH-A-49-13C.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 100.514992 MHz
time domain size: 63750 points
width: 24509.80 Hz = 243.8423 ppm = 0.384468 Hz/pt
number of scans: 11296

freq. of 0 ppm: 100.504436 MHz
processed size: 65536 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 980.392 ppm/cm: 9.75369

Figure S23. ^{13}C NMR of Product **11**.

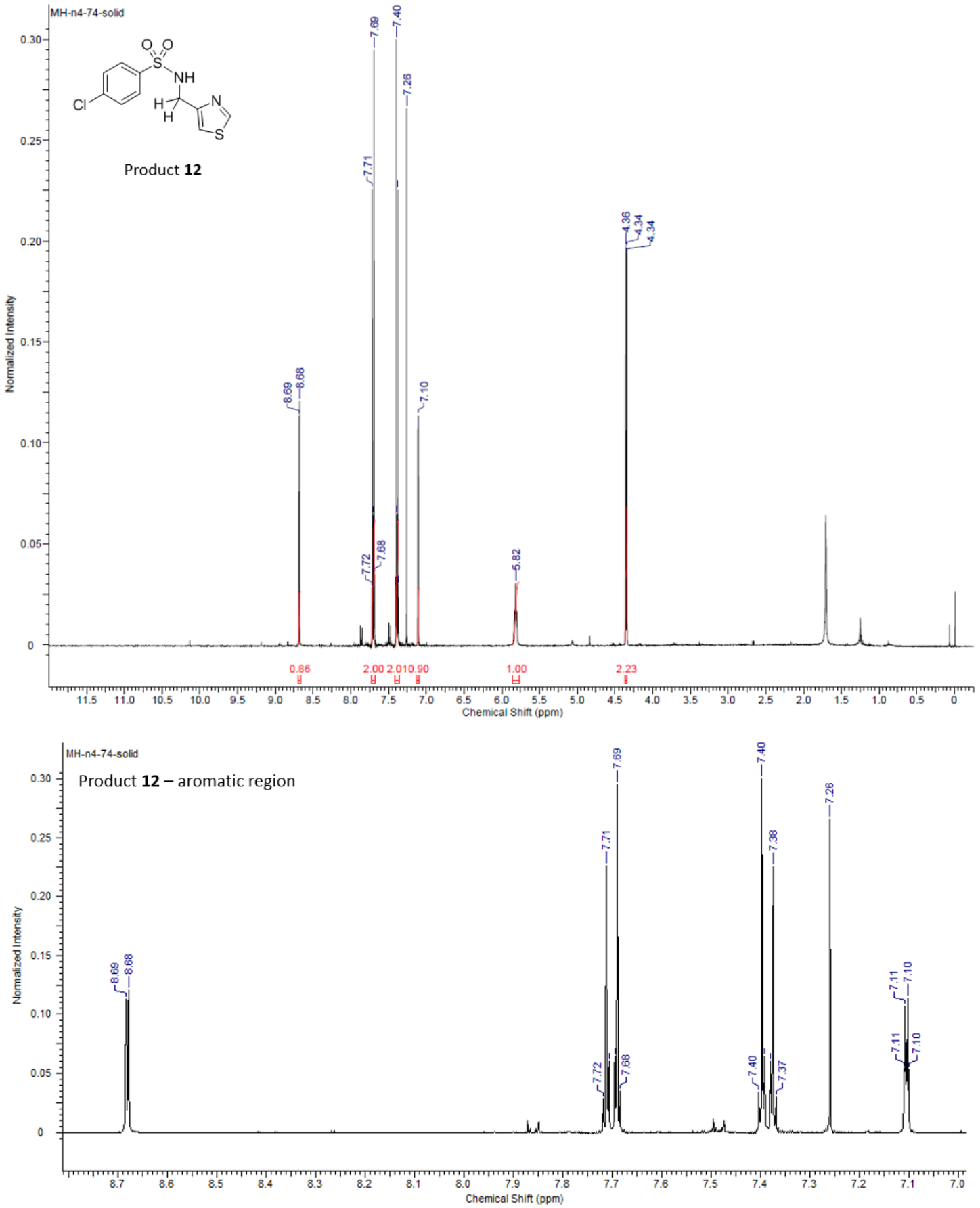
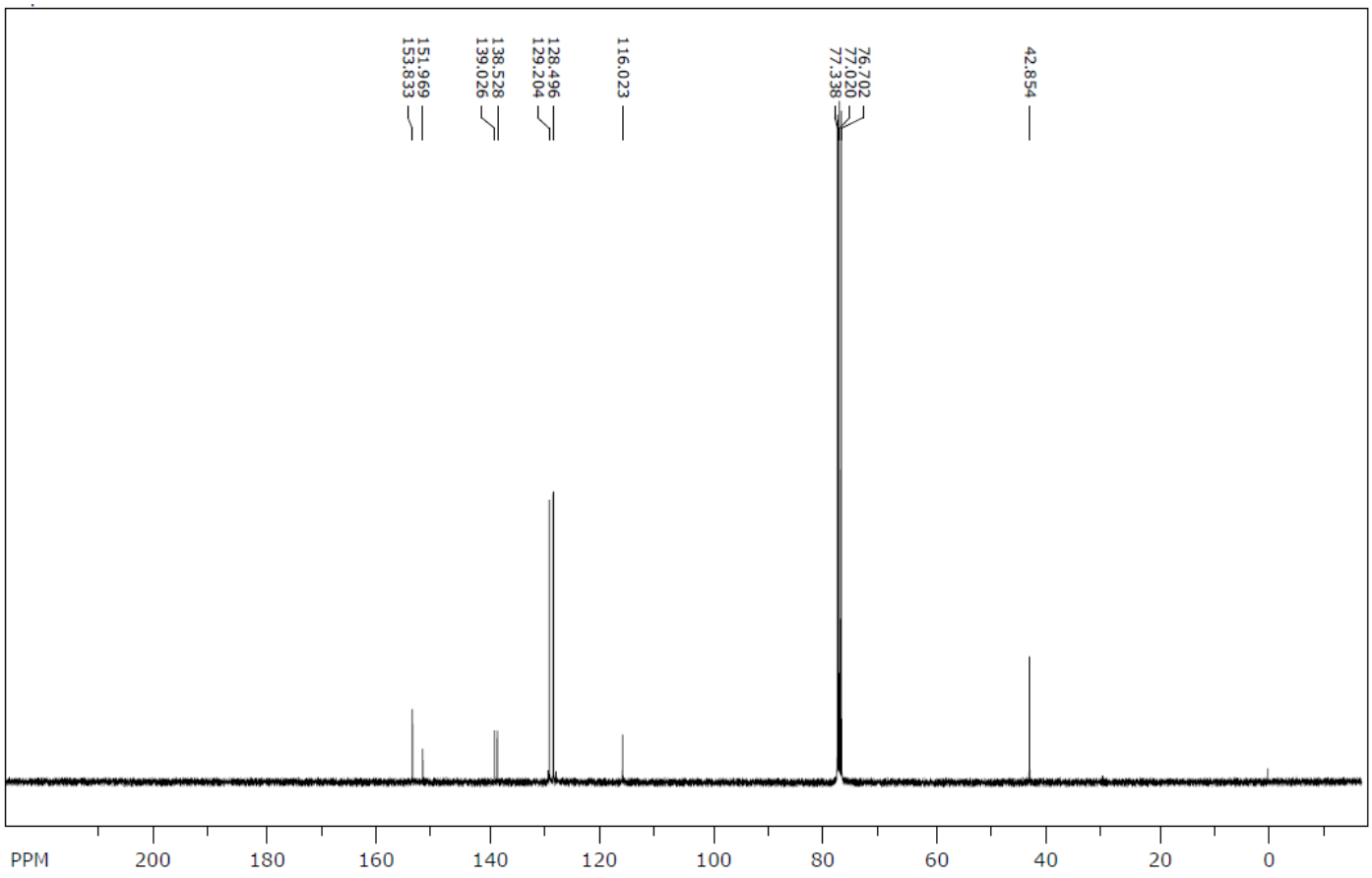


Figure S24. ^{1}H NMR of Product **12**.



file: ...ata\NMR Fids\MH-n4-74-13C2.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 100.514992 MHz
time domain size: 63750 points
width: 24509.80 Hz = 243.8423 ppm = 0.384468 Hz/pt
number of scans: 18000

freq. of 0 ppm: 100.504435 MHz
processed size: 65536 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 980.392 ppm/cm: 9.75369

Figure S25. ^{13}C NMR of Product **12**.

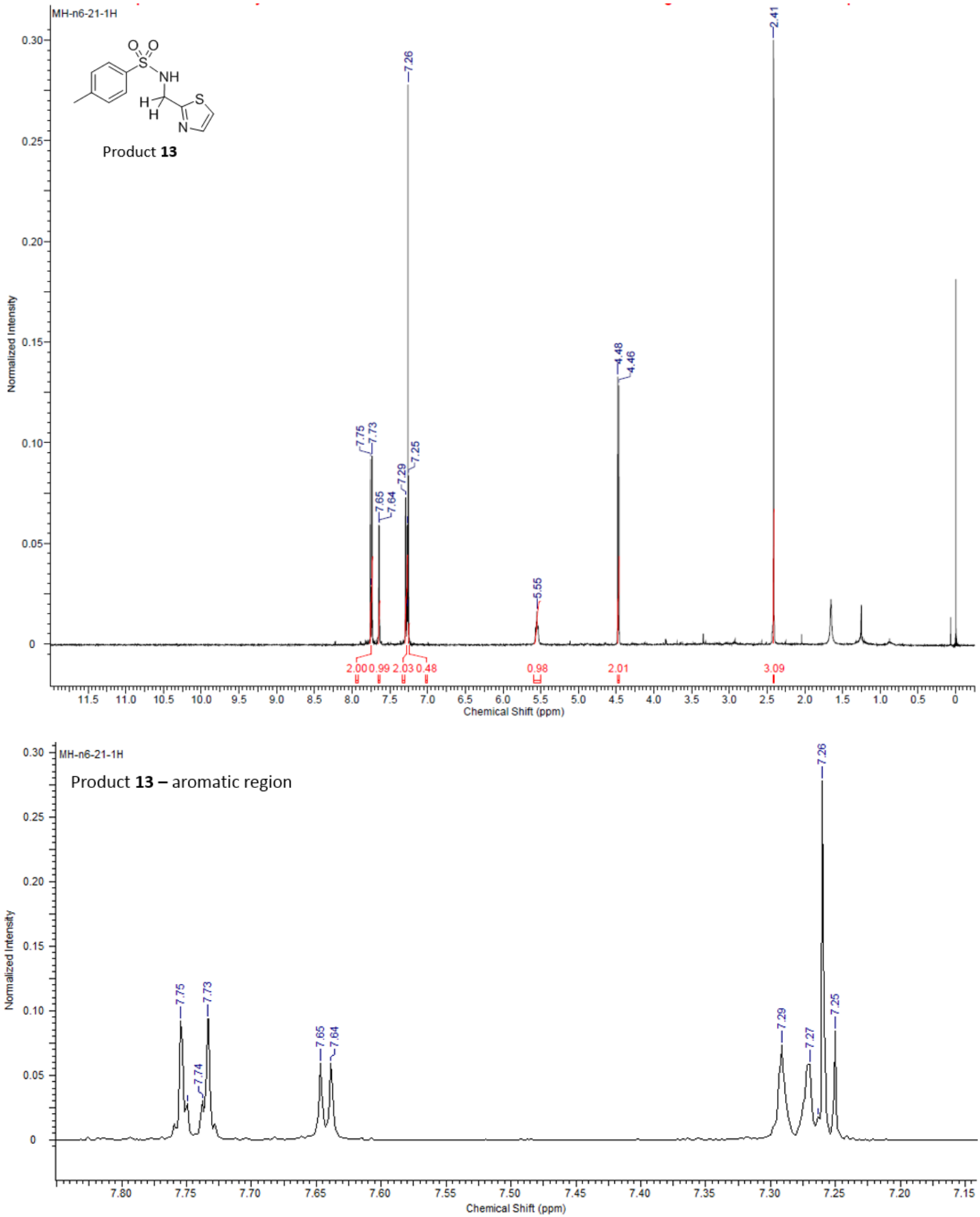


Figure S26. ^{1}H NMR of Product **13**.

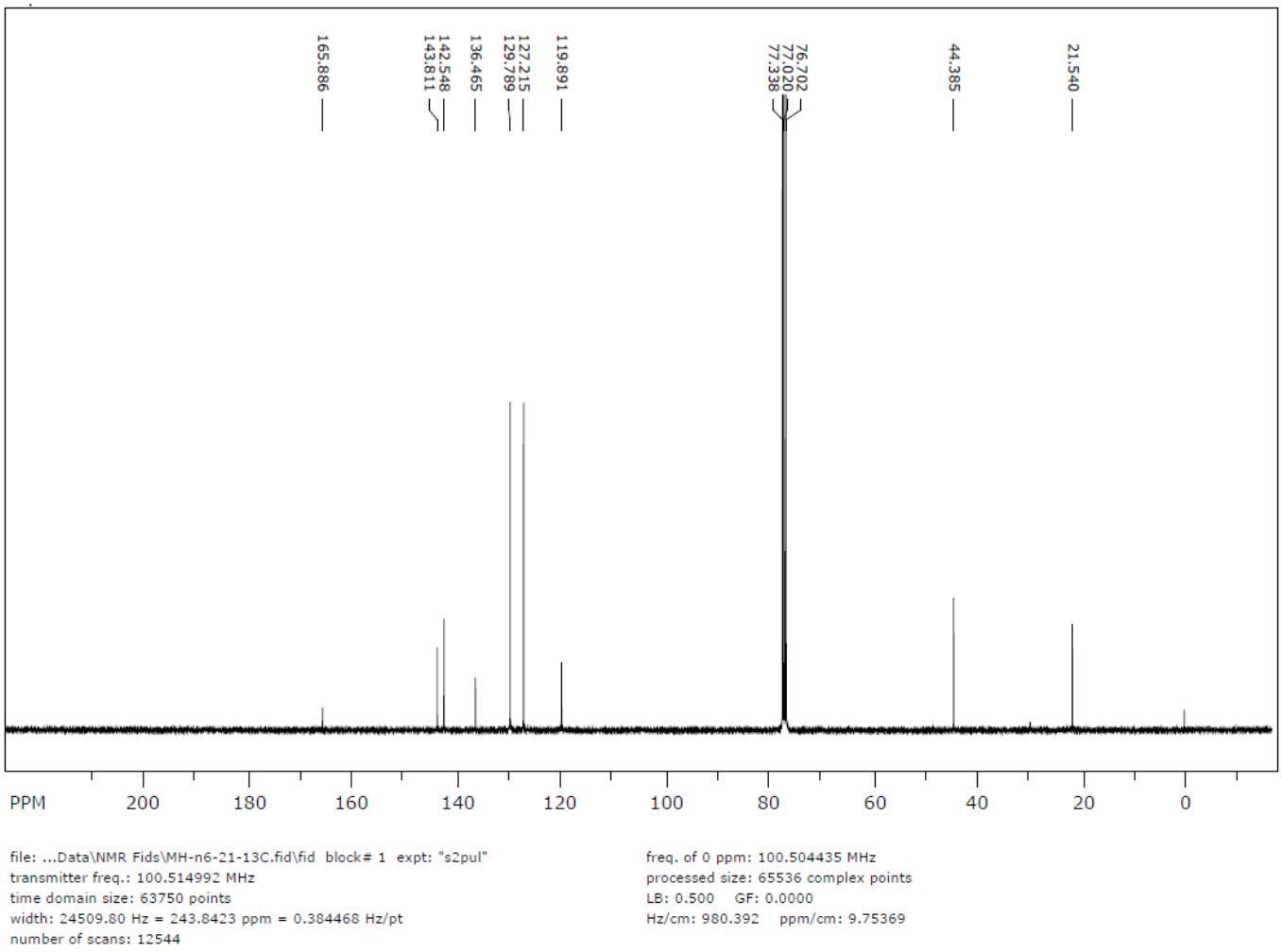


Figure S27. ^{13}C NMR of Product **13**.

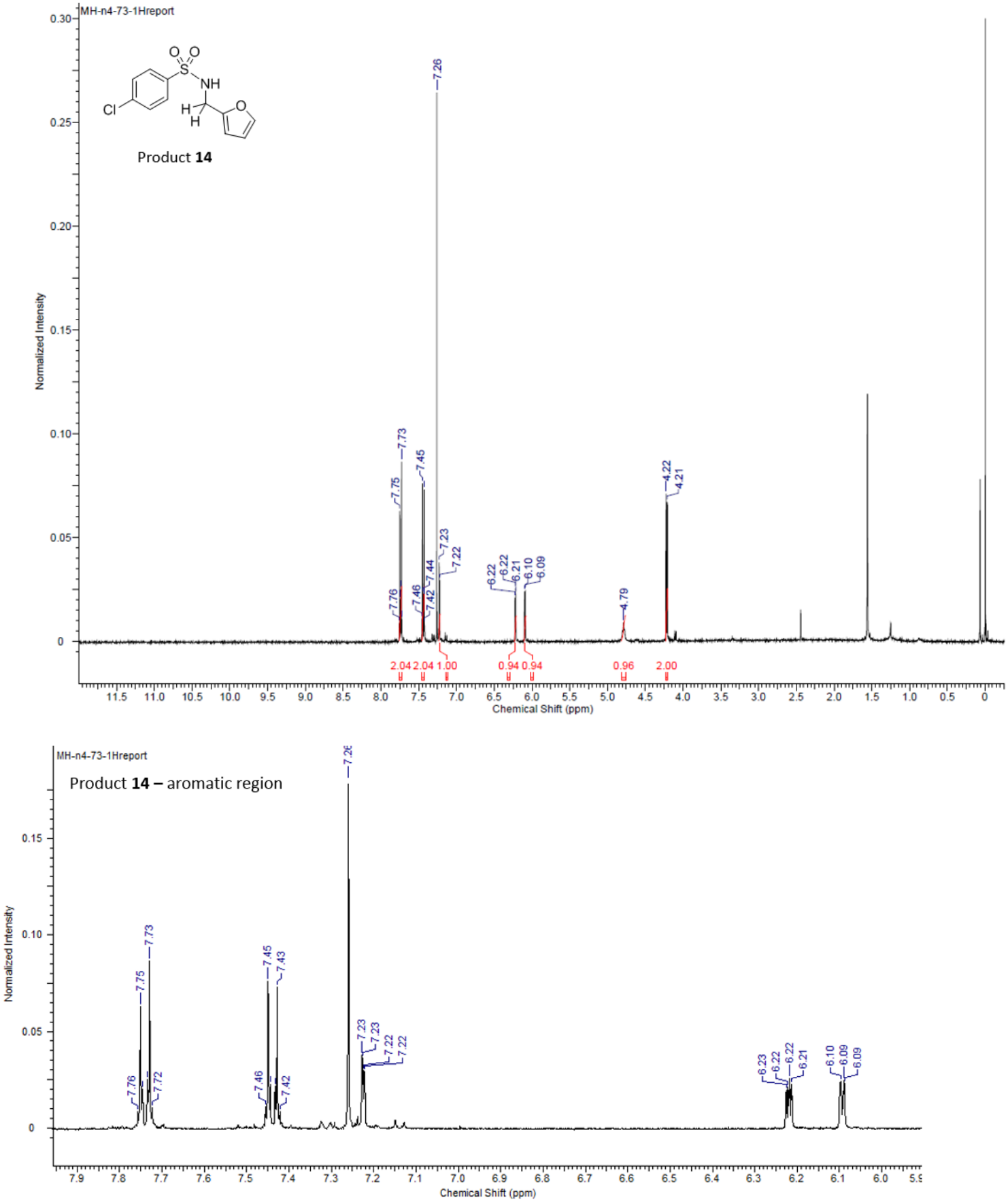


Figure S28. ^{1}H NMR of Product **14**.

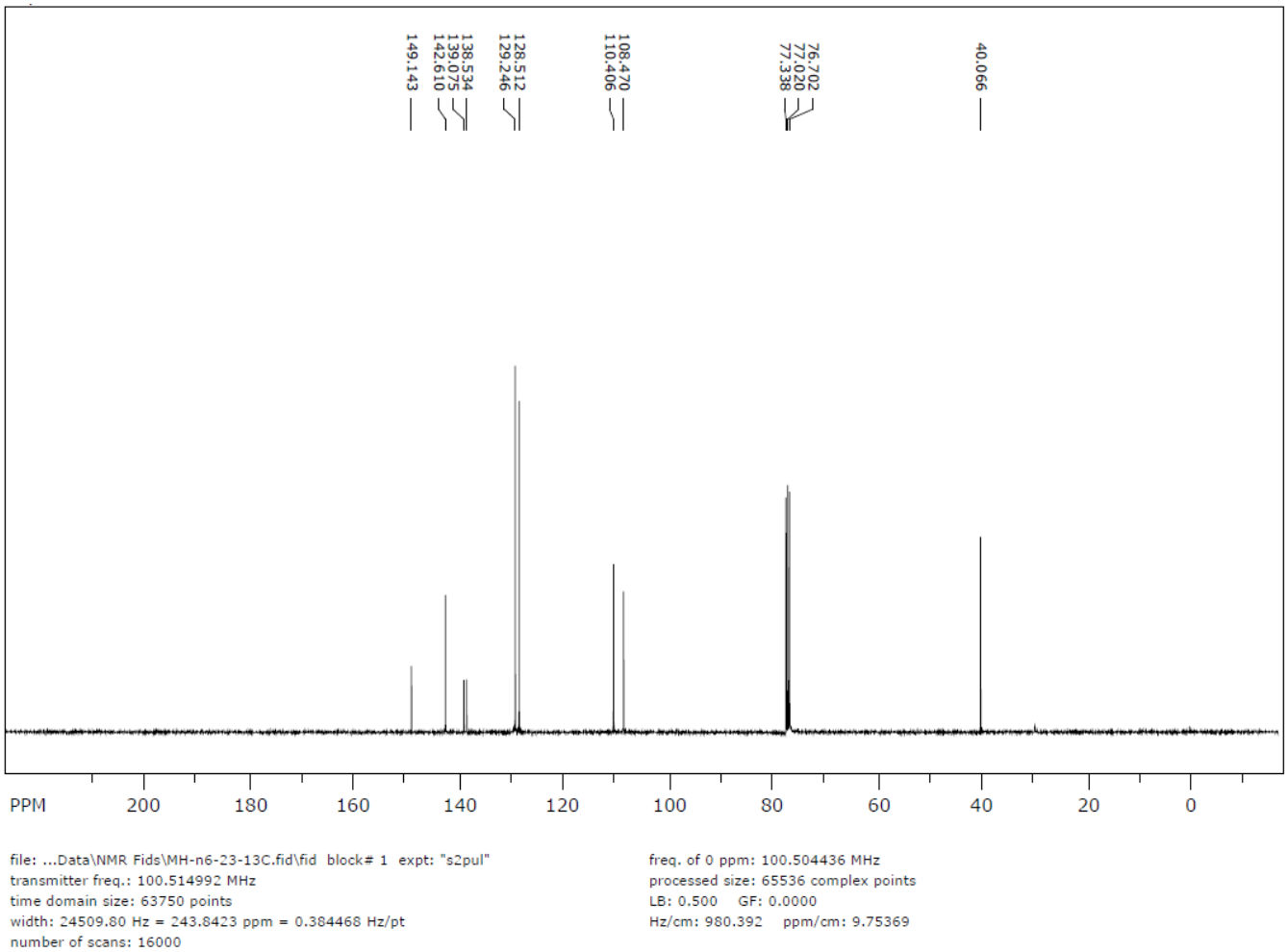
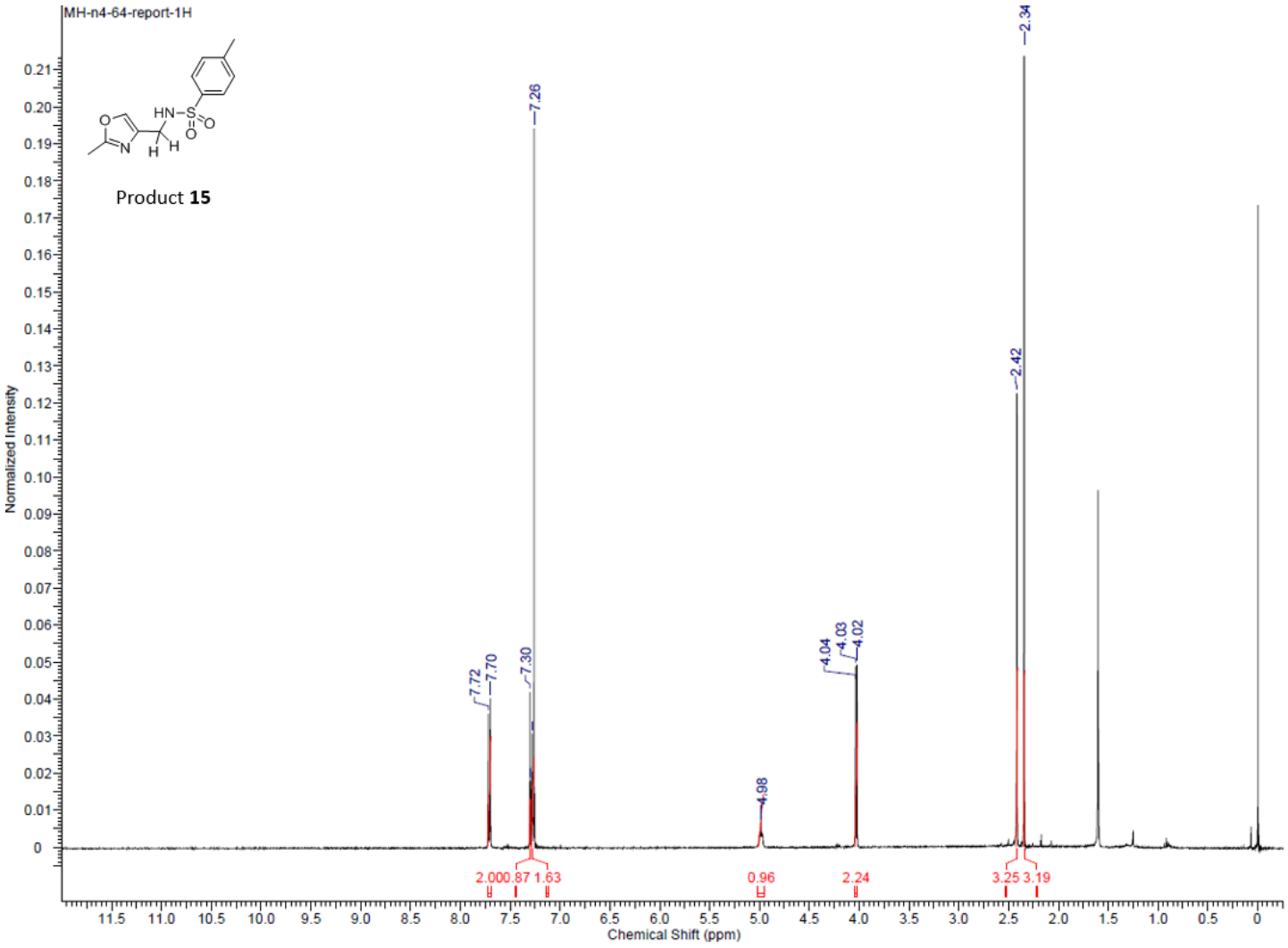


Figure S29. ^{13}C NMR of Product **14**.



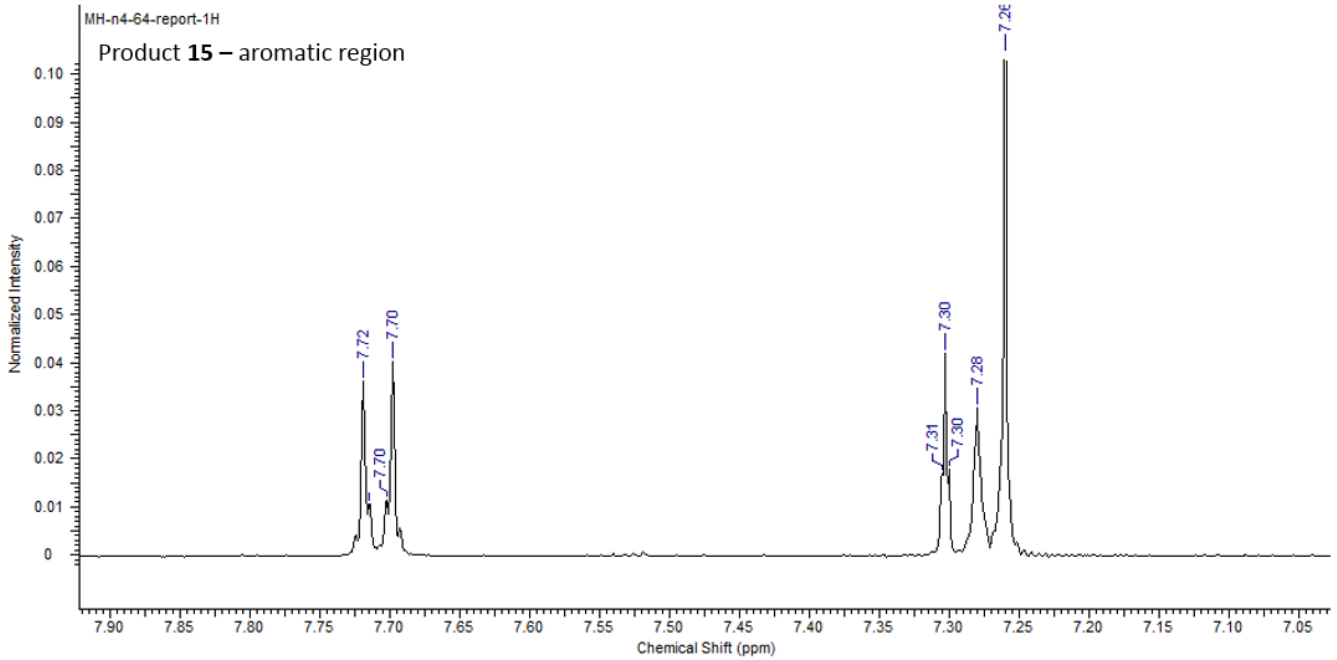
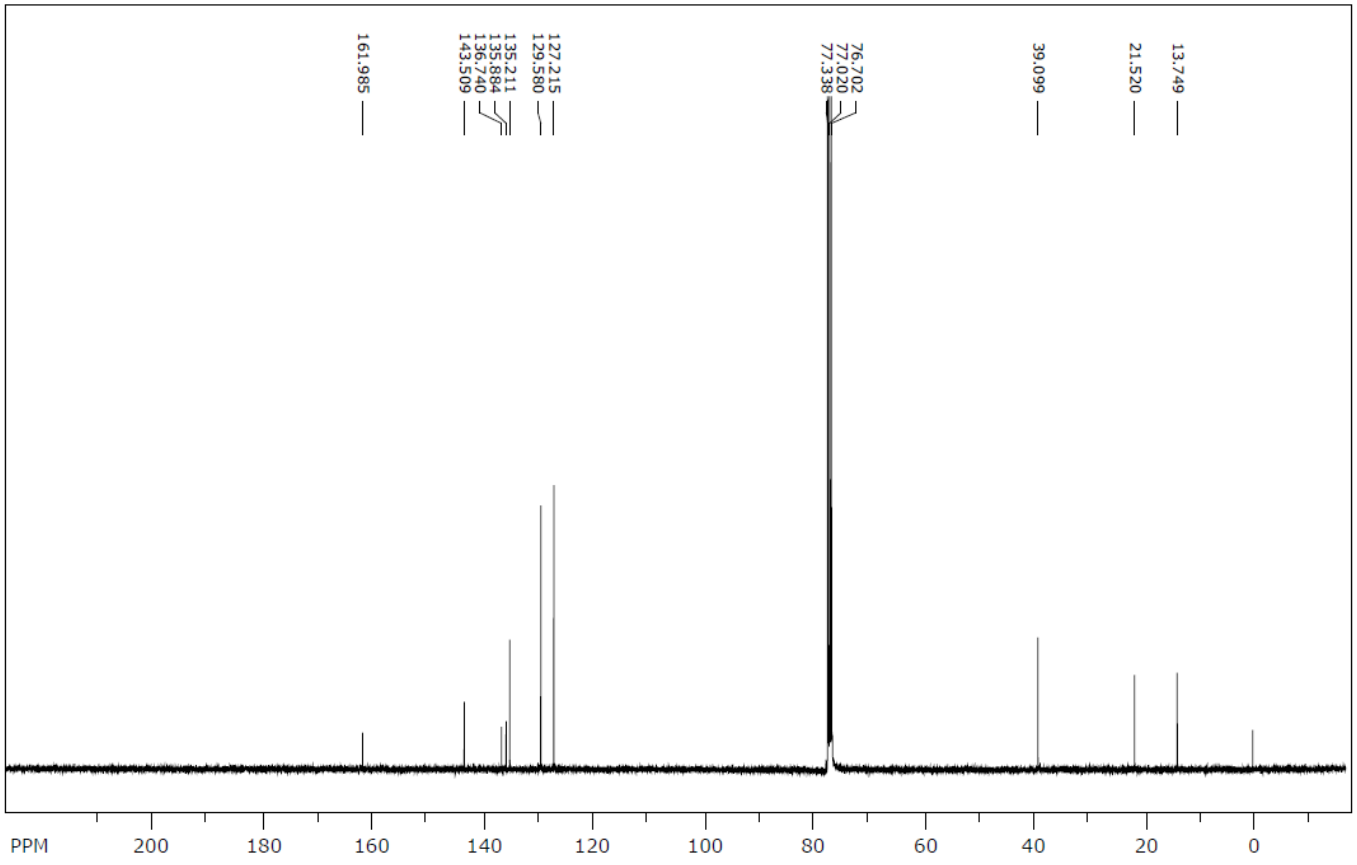


Figure S30. ^{1}H NMR of Product **15**.



file: ...R Fids\MH-n4-64-report-13C.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 100.514992 MHz
time domain size: 63750 points
width: 24509.80 Hz = 243.8423 ppm = 0.384468 Hz/pt
number of scans: 17280

freq. of 0 ppm: 100.504434 MHz
processed size: 65536 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 980.392 ppm/cm: 9.75369

Figure S31. ^{13}C NMR of Product **15**.

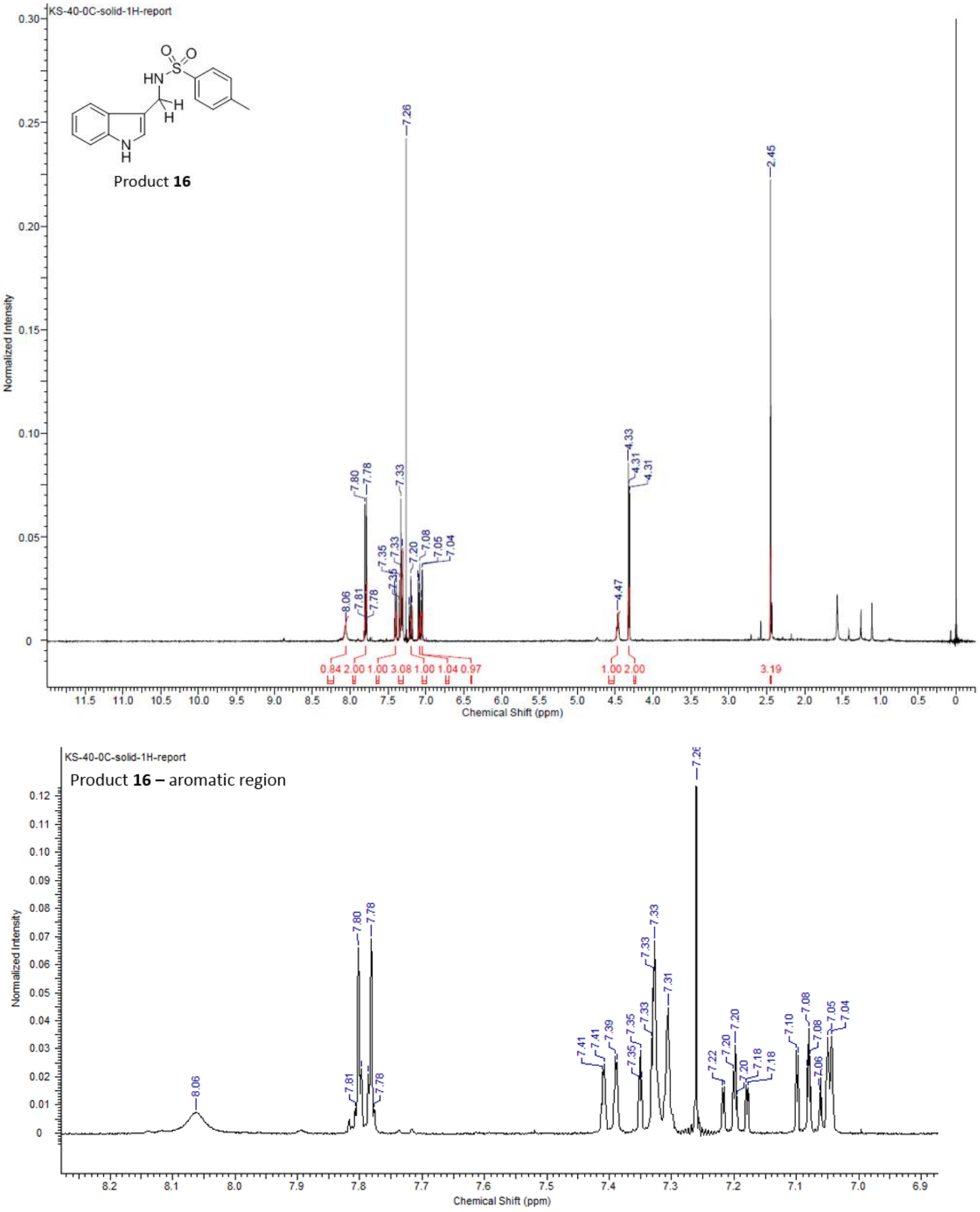


Figure S32. ^{1}H NMR of Product **16**.

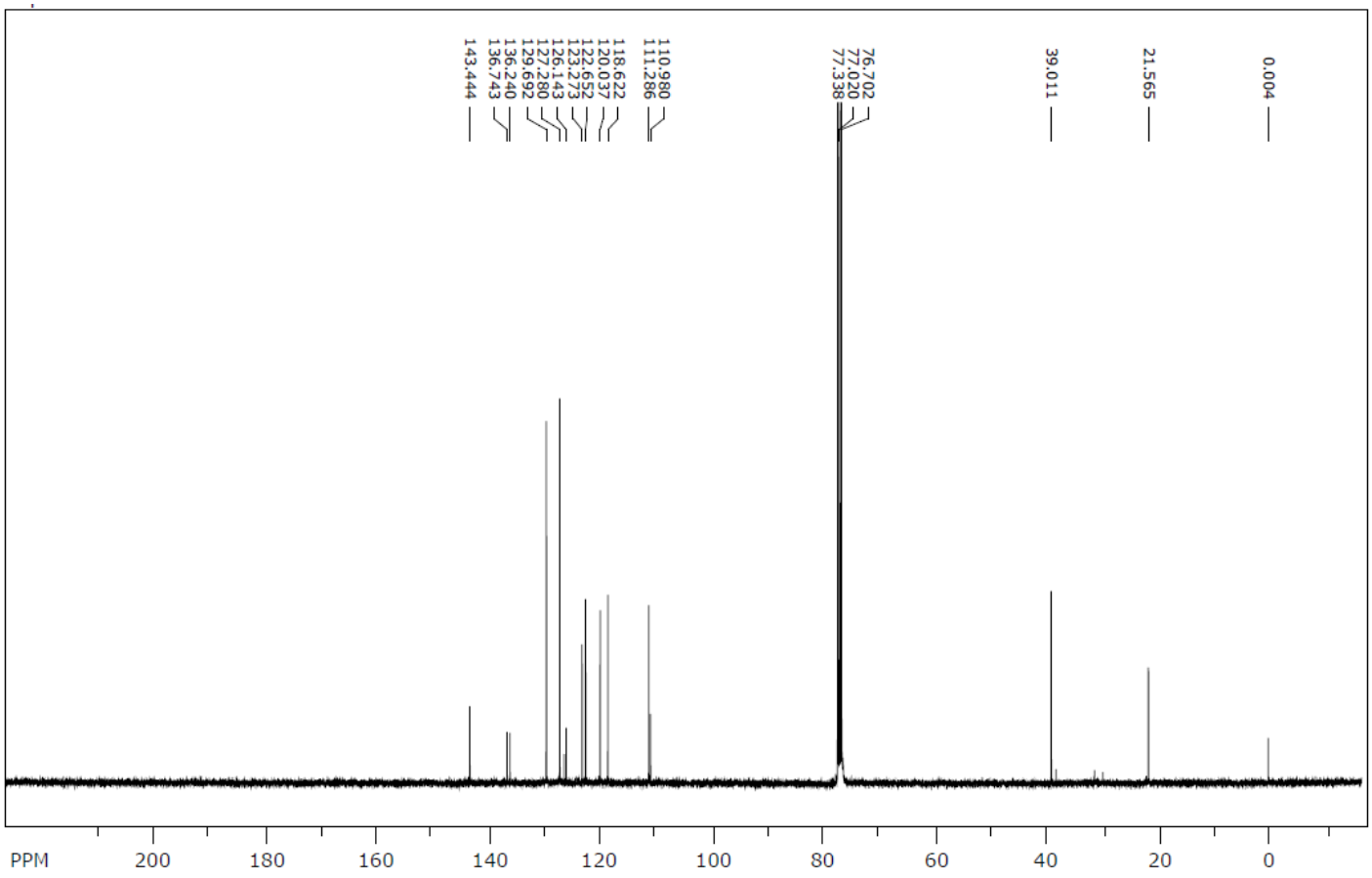


Figure S33. ^{13}C NMR of Product **16**.

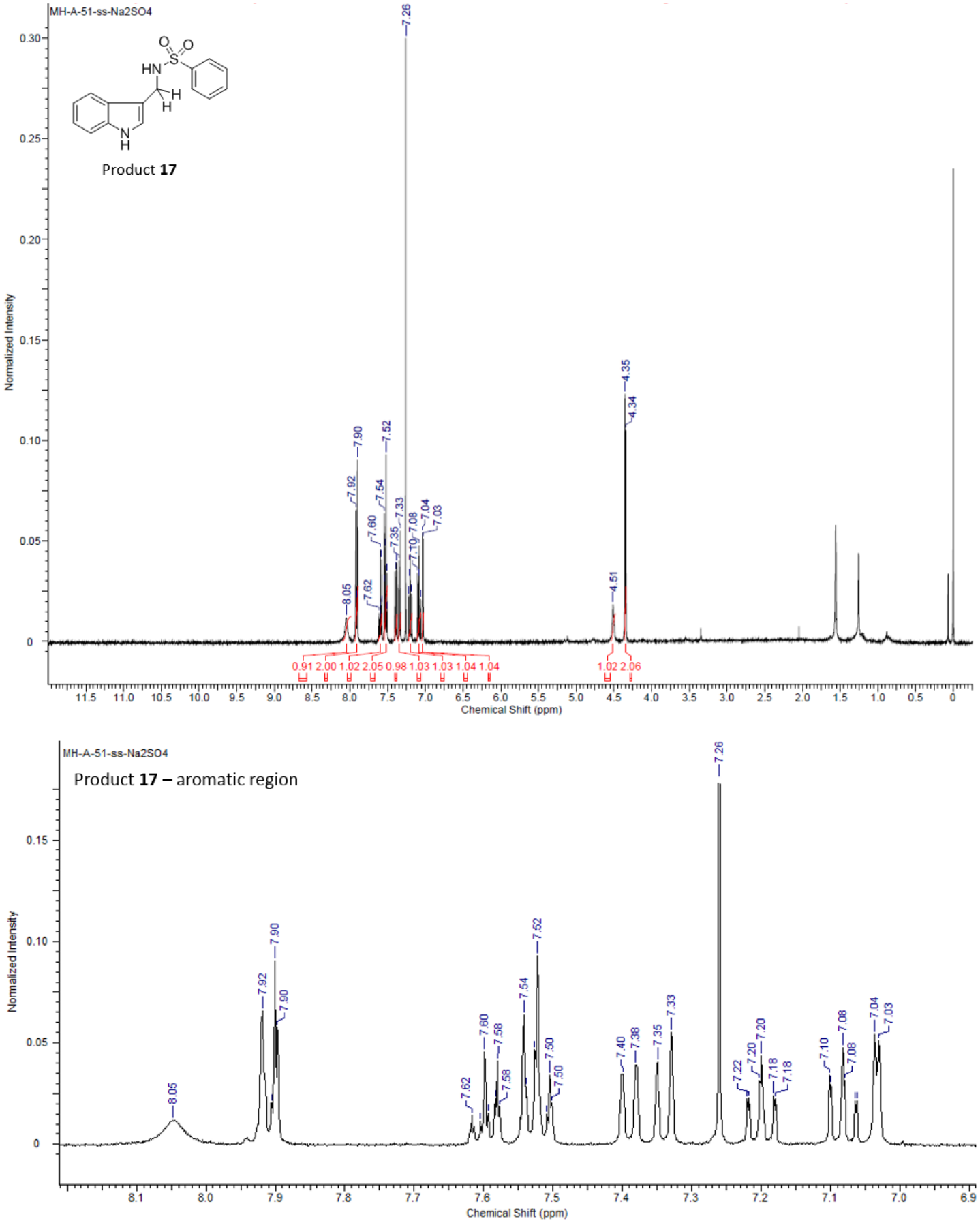
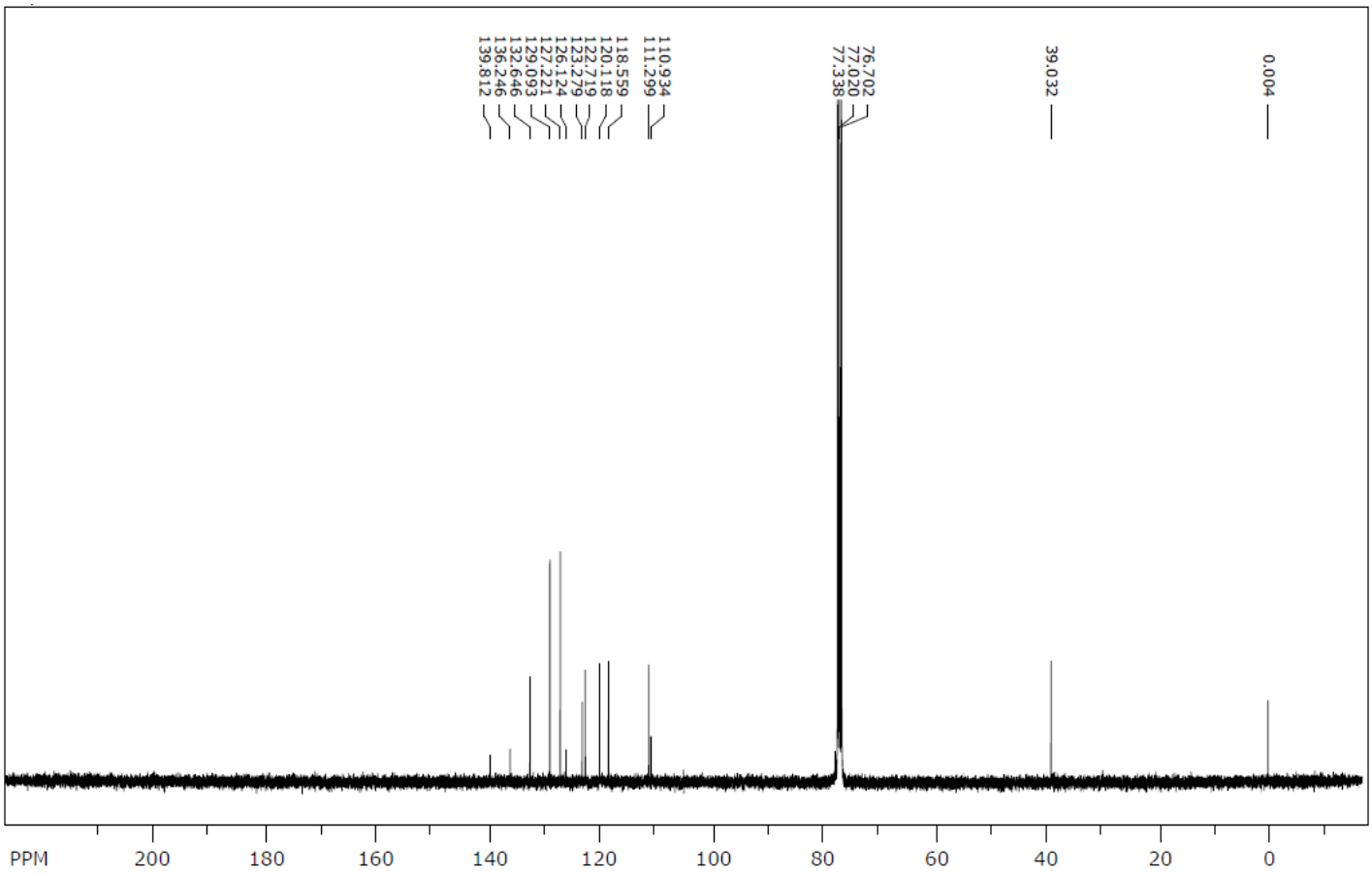


Figure S34. ^{1}H NMR of Product **17**.



file: ...ta\NMR Fids\MH-A-51-ss-13C.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 100.514992 MHz
time domain size: 63750 points
width: 24509.80 Hz = 243.8423 ppm = 0.384468 Hz/pt
number of scans: 16000

freq. of 0 ppm: 100.504434 MHz
processed size: 65536 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 980.392 ppm/cm: 9.75369

Figure S35. ^{13}C NMR of Product **17**.

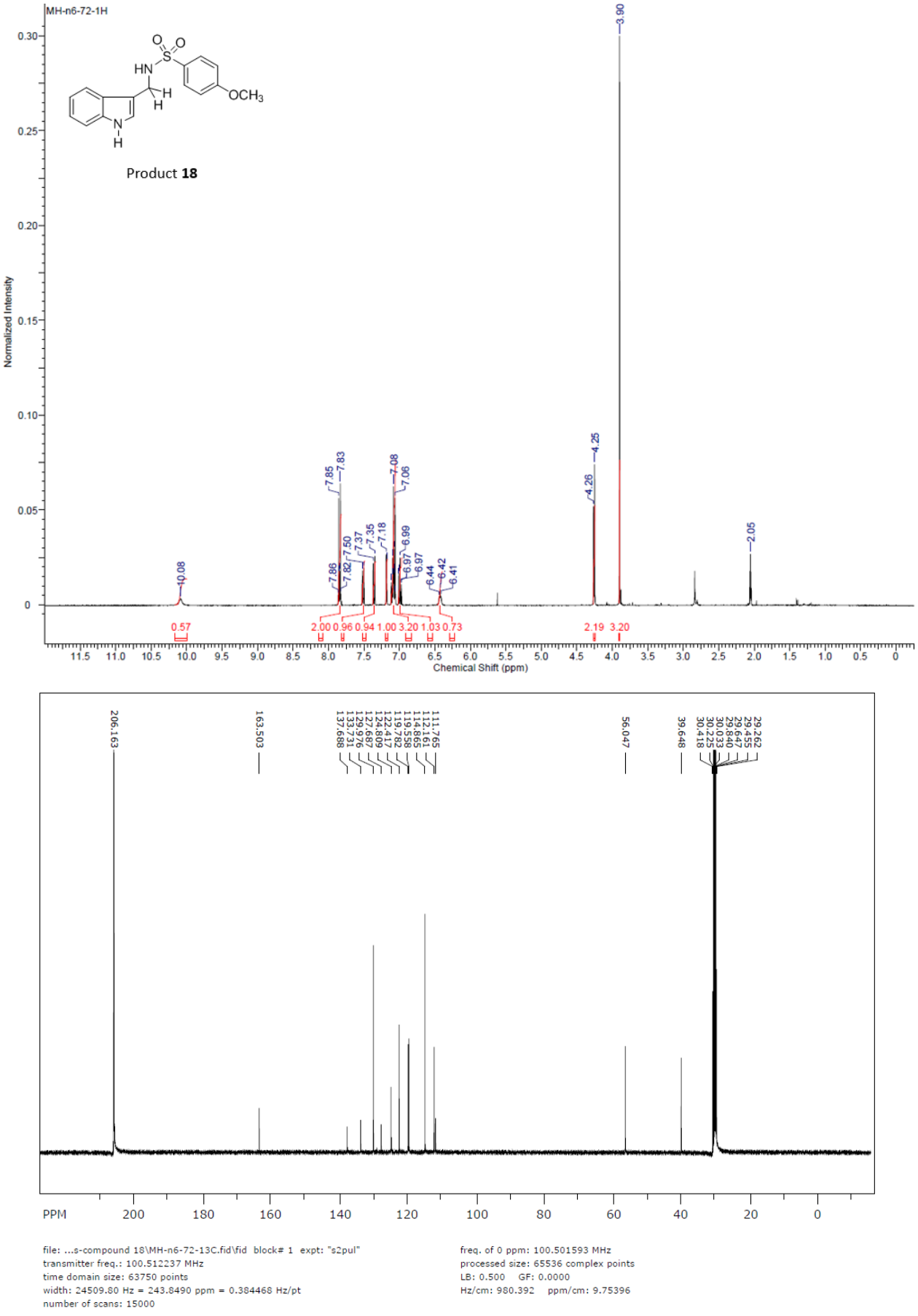


Figure S36. ^{1}H and ^{13}C NMR of Product **18**.

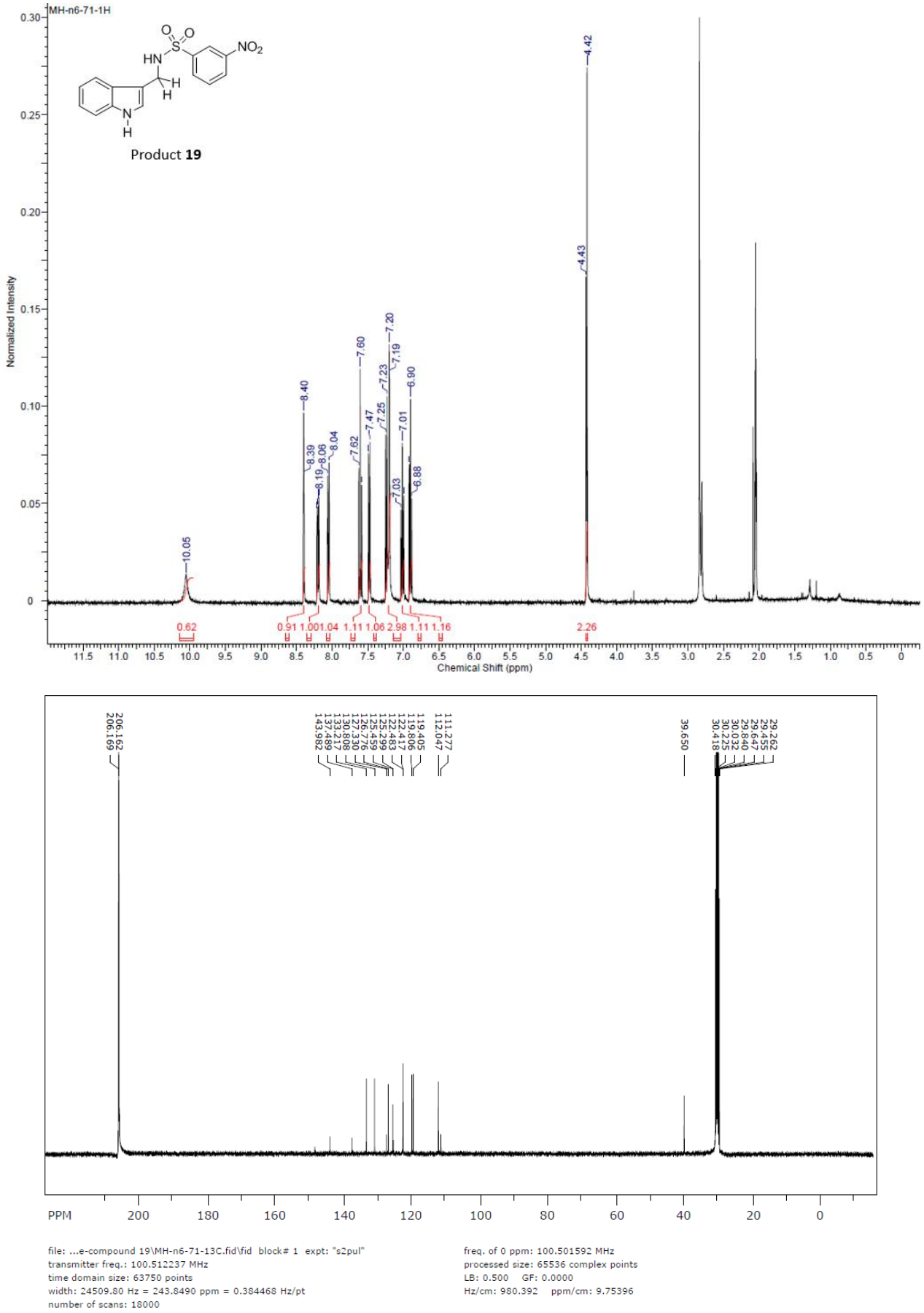


Figure S37. ^{1}H and ^{13}C NMR of Product **19**.

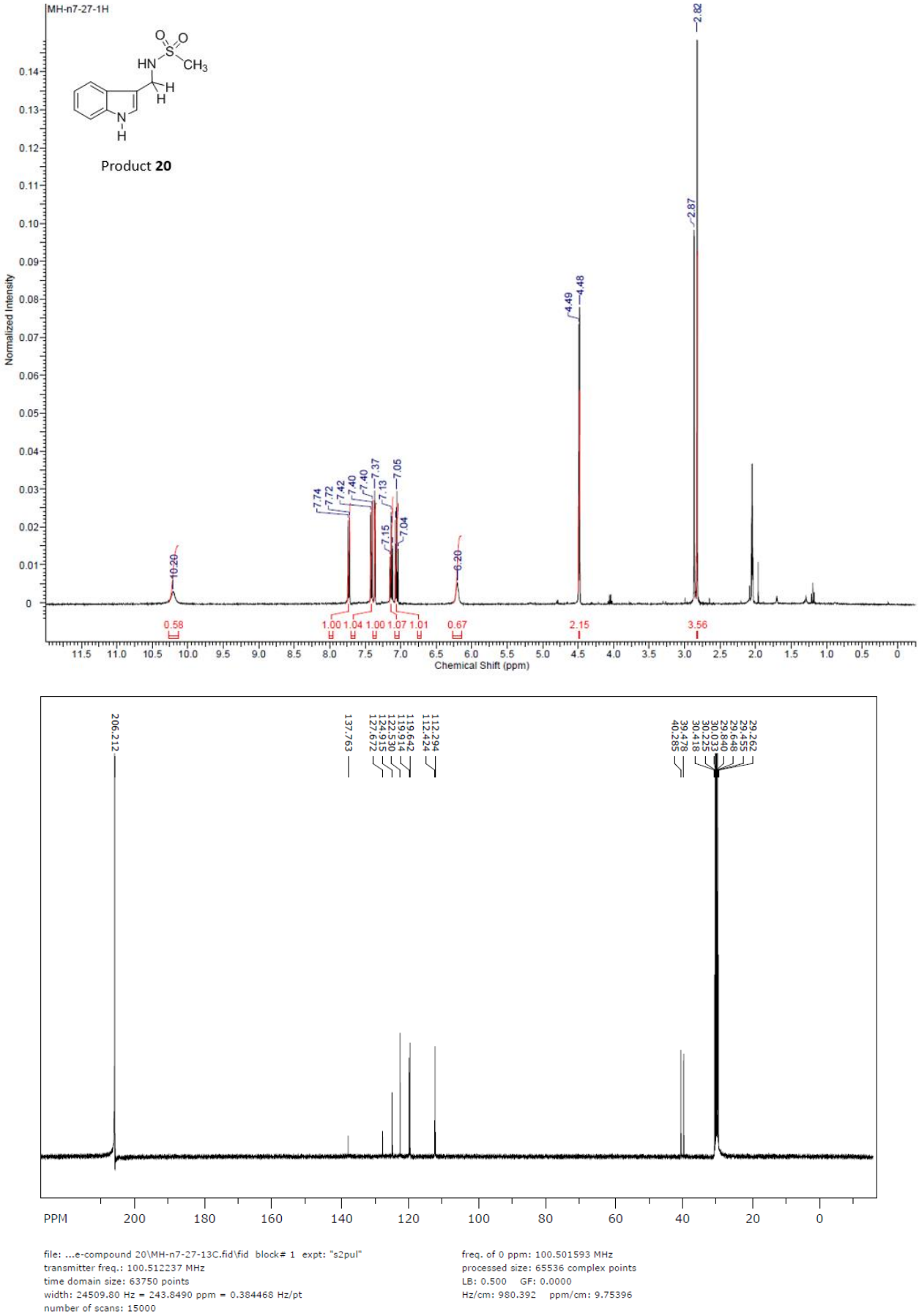


Figure S38. ^{1}H and ^{13}C NMR of Product **20**.

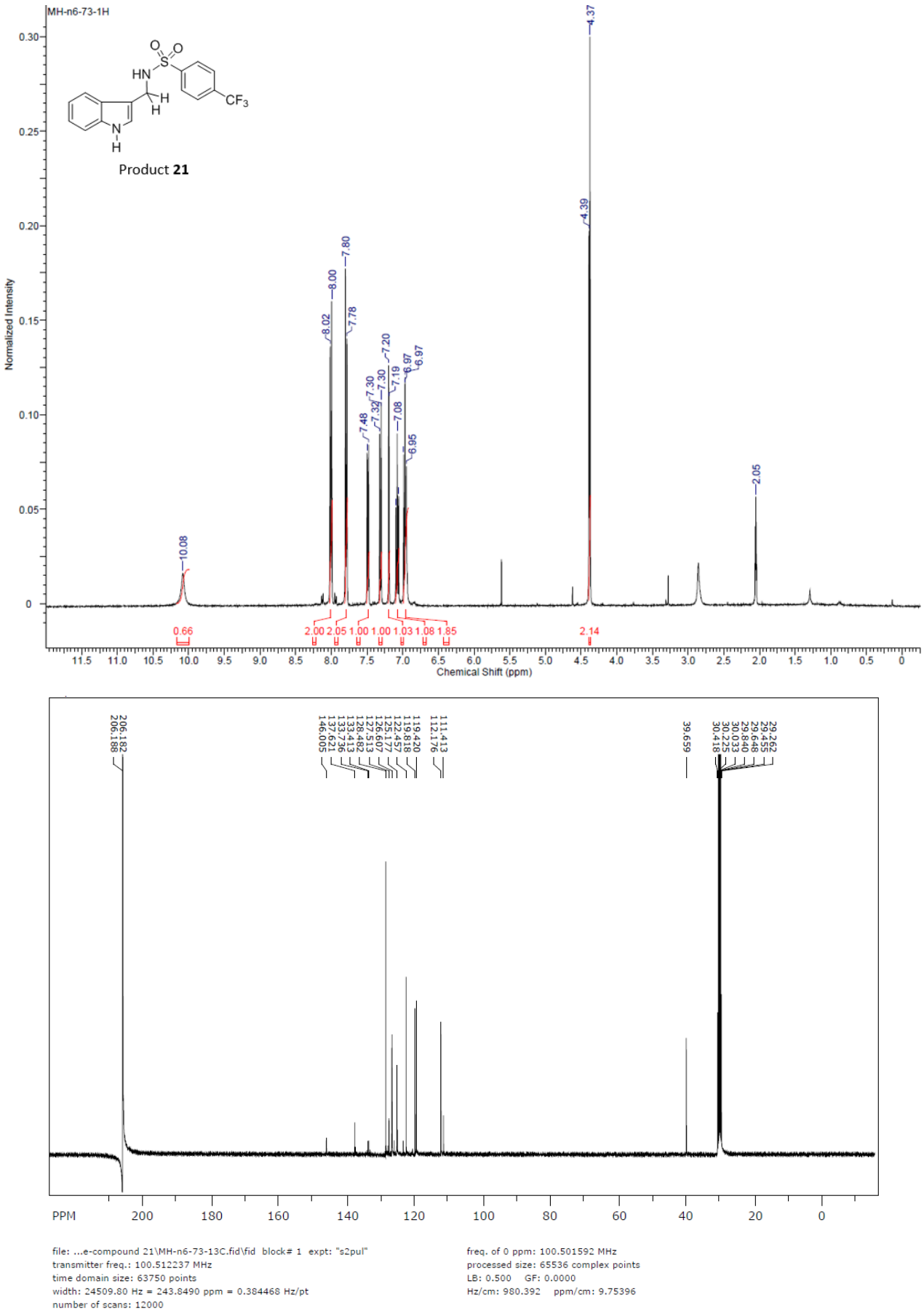


Figure S39. ^{1}H and ^{13}C NMR of Product **21**.

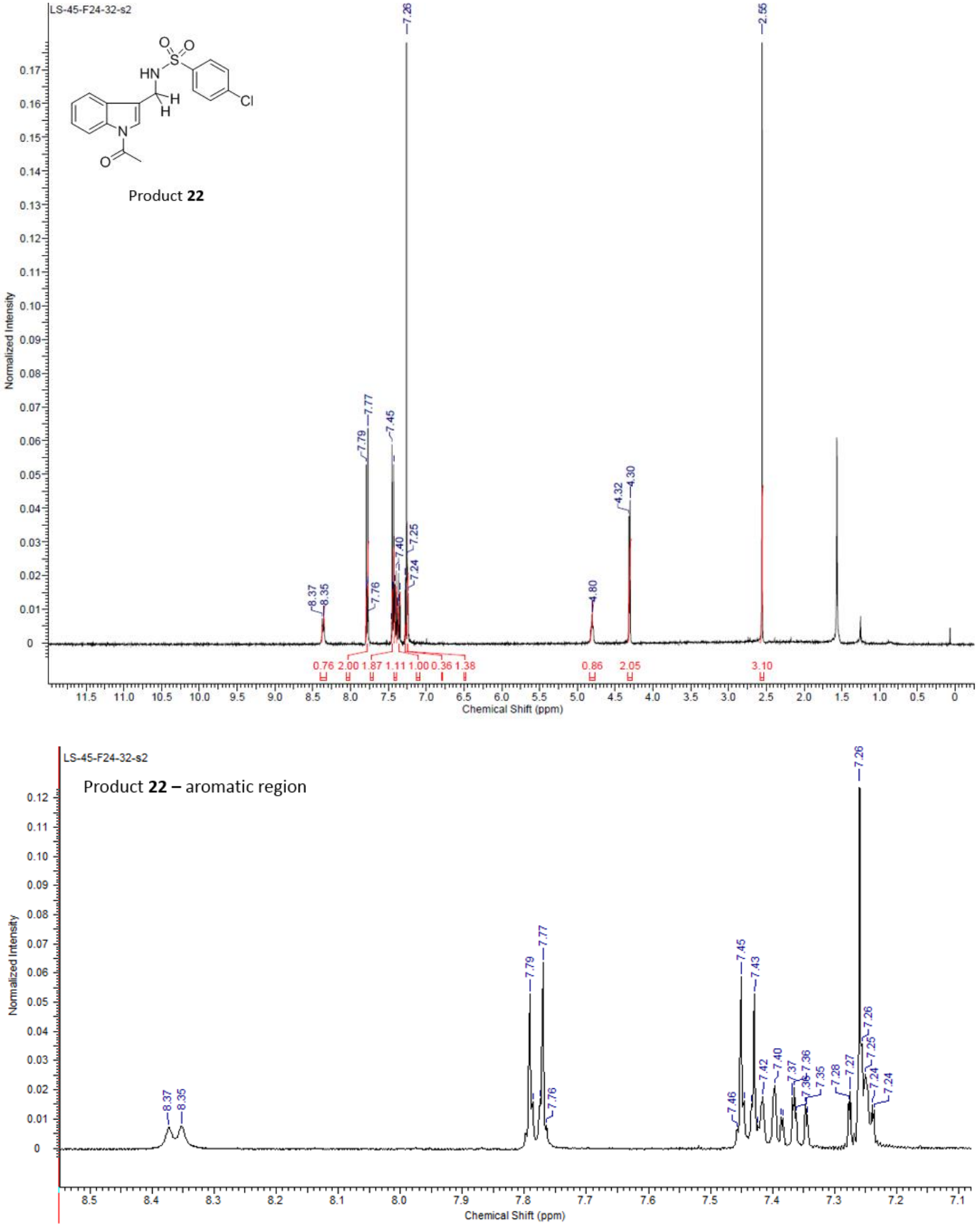
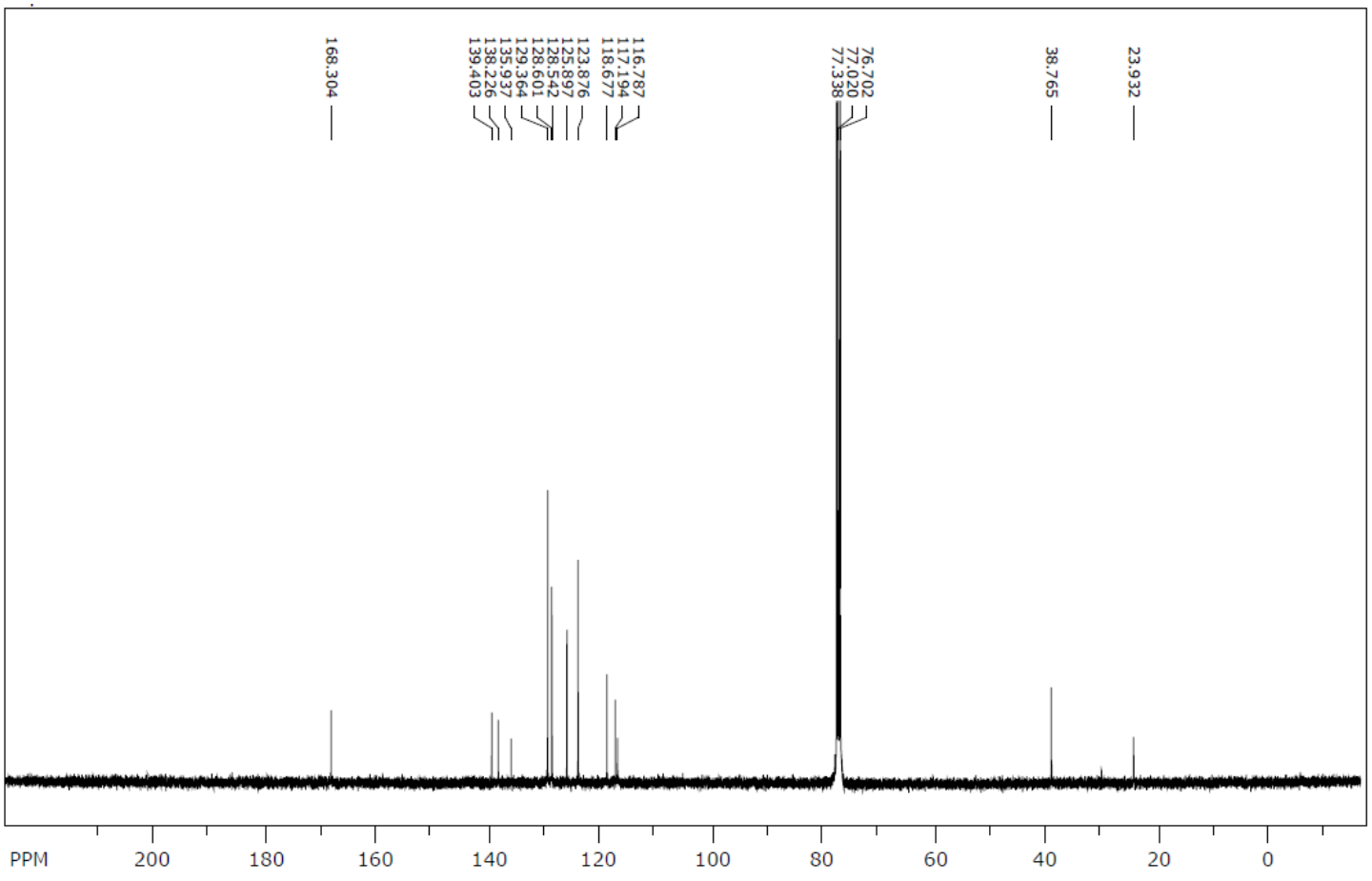


Figure S40. ^{1}H NMR of Product **22**.



file: ...MR Data\NMR Fids\LS-45-13C.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 100.514992 MHz
time domain size: 63750 points
width: 24509.80 Hz = 243.8423 ppm = 0.384468 Hz/pt
number of scans: 16688

freq. of 0 ppm: 100.504434 MHz
processed size: 65536 complex points
LB: 0.500 GF: 0.0000
Hz/cm: 980.392 ppm/cm: 9.75369

Figure S41. ^{13}C NMR of Product **22**.

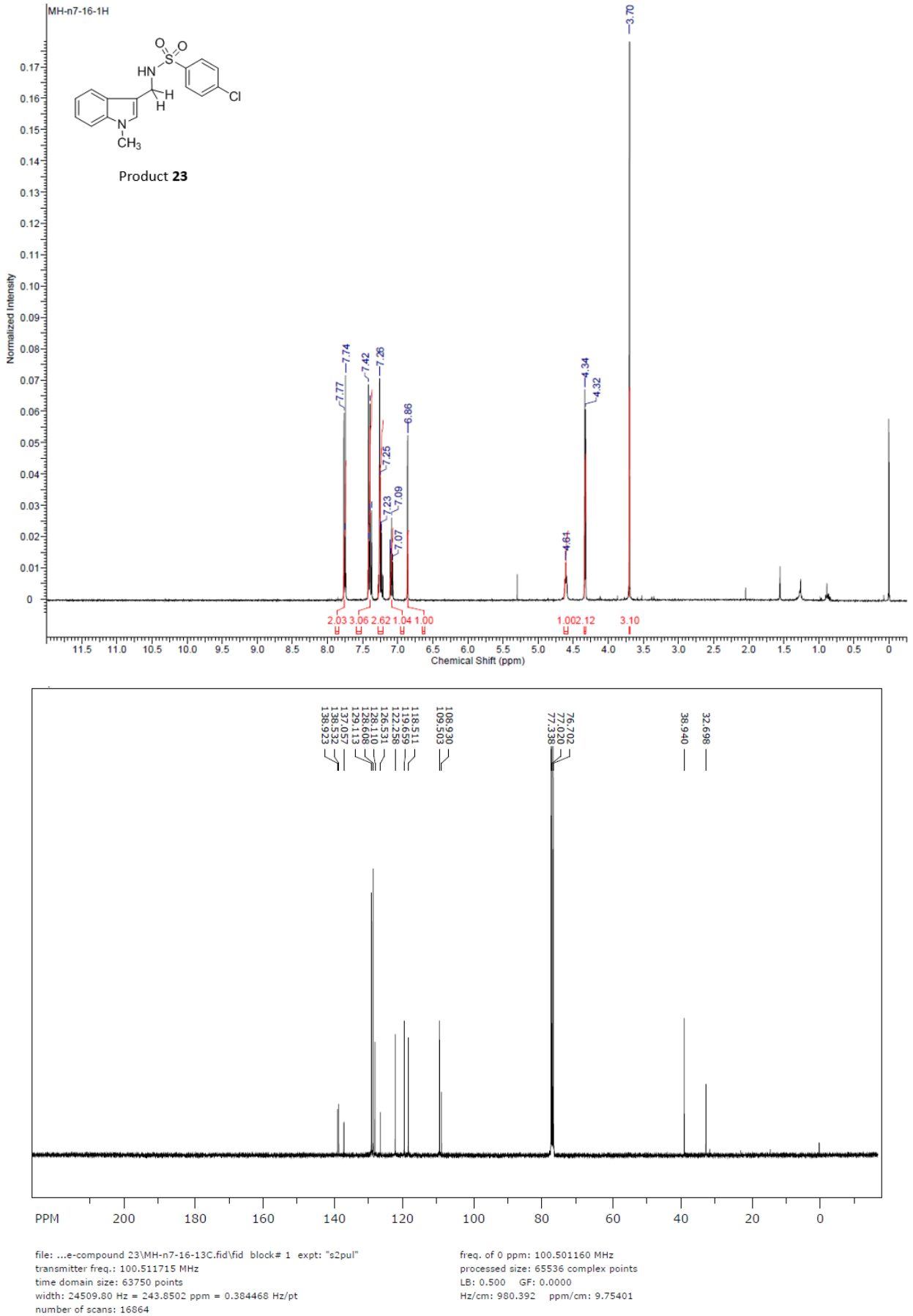


Figure S42. ^{1}H and ^{13}C NMR of Product **23**.

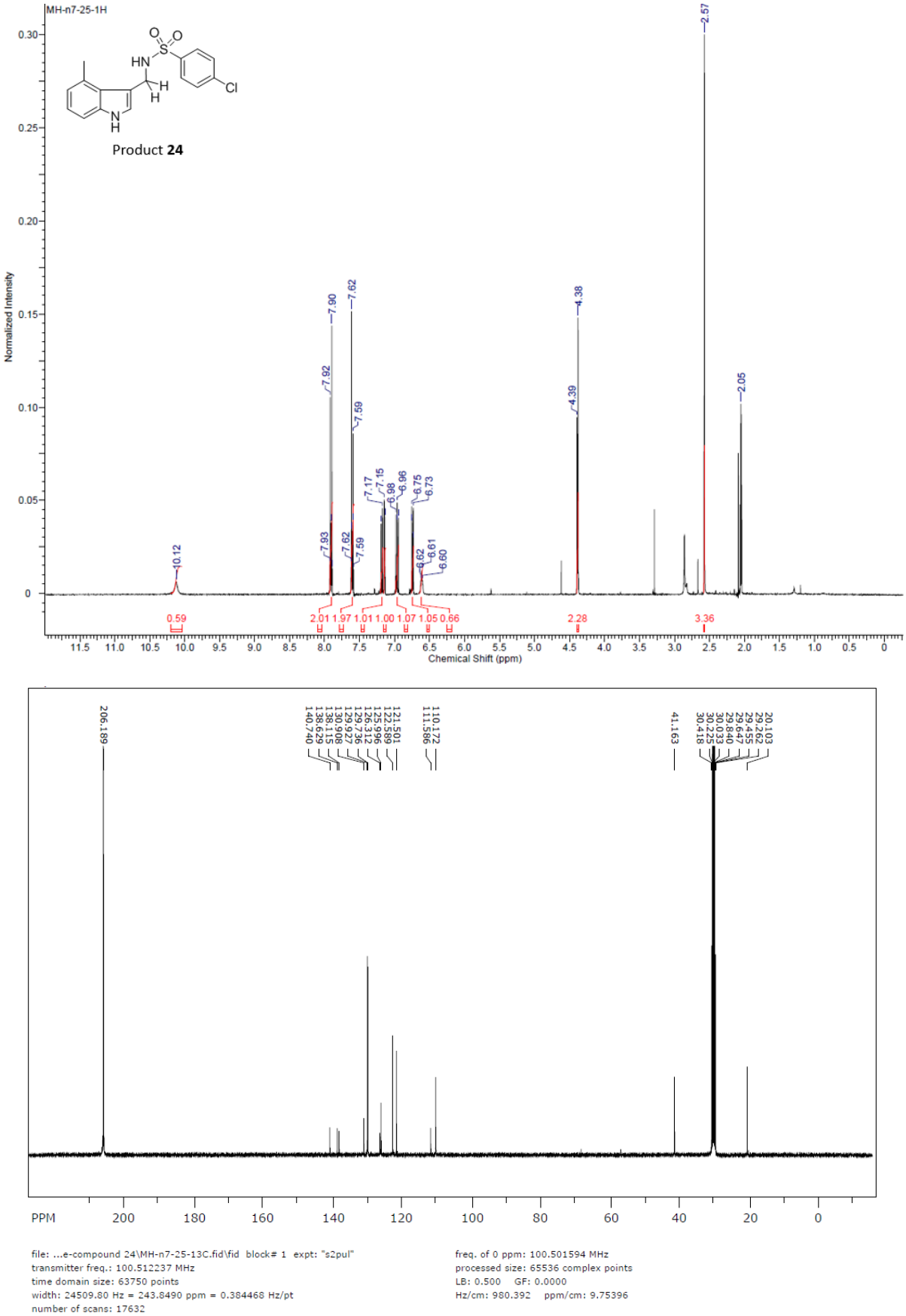


Figure S43. ^{1}H and ^{13}C NMR of Product **24**.

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10. Rogers, D. A.; Brown, R. G.; Brandeburg, Z. C.; Ko, E. Y.; Hopkins, M. D.; LeBlanc, G.; Lamar, A. A., Organic Dye-Catalyzed, Visible-Light Photoredox Bromination of Arenes and Heteroarenes Using N-Bromosuccinimide. *ACS Omega* **2018,** *3* (10), 12868-12877.
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