Supplementary Data

Targeting the lateral interactions of transmembrane domain 5 of Epstein–Barr virus latent membrane protein 1

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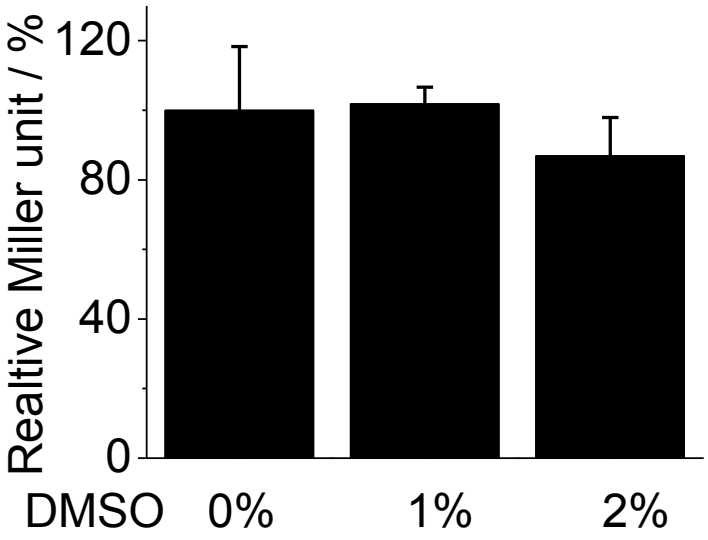
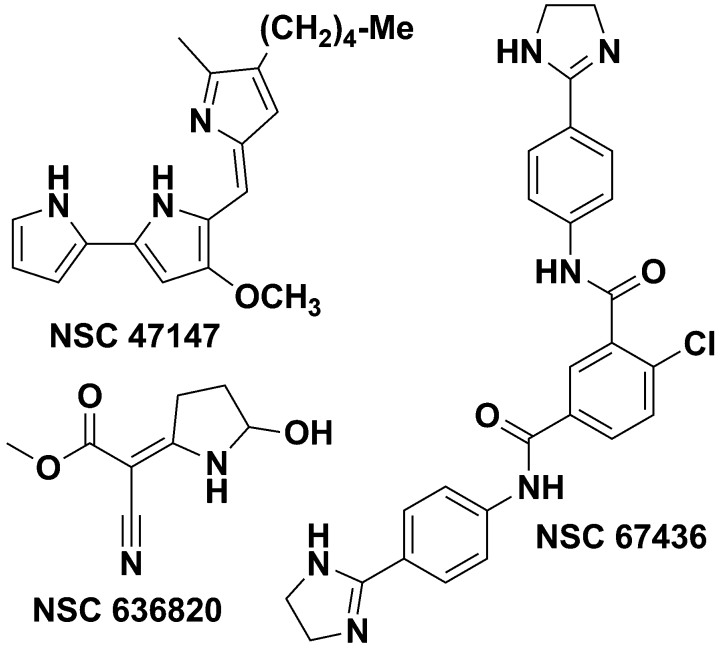
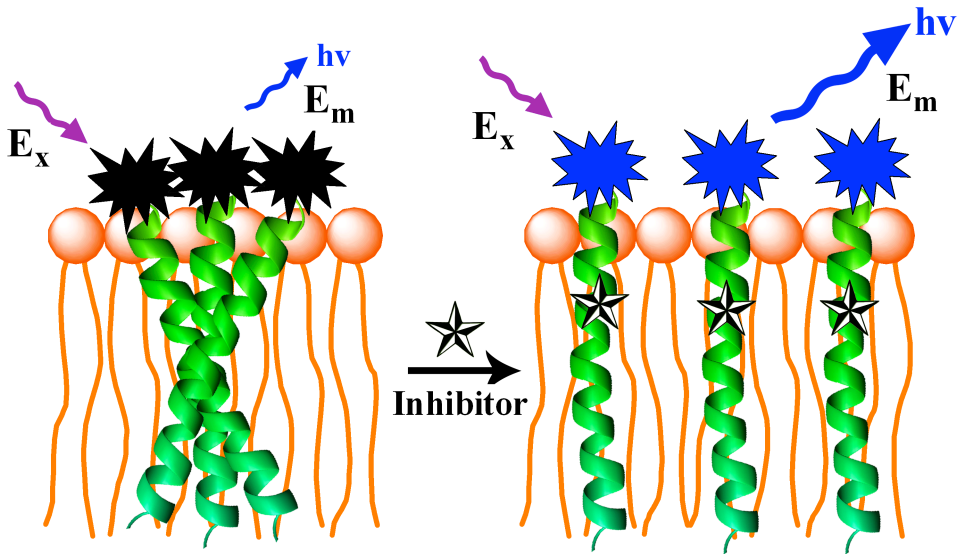
Supplementary Data



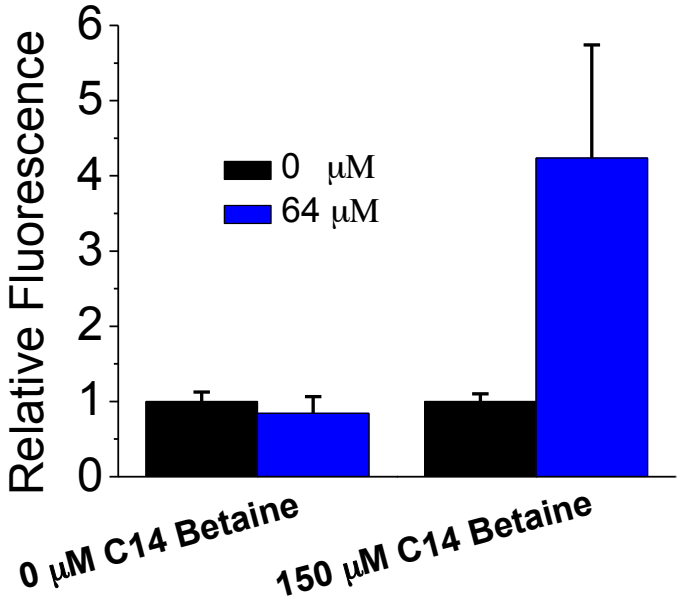
Supplementary Figure S1. Effects of DMSO on LMP-1 TMD-5 oligomerization measured by ToxR assay. TMD-5 oligomerization activity in the absence of DMSO was set as 100%. The result shows even at 2% DMSO, DMSO does not have significant effect on TMD-5 oligomerization.



Supplementary Figure S2. Chemical structures of NSC 47147, 636820 and 67436.



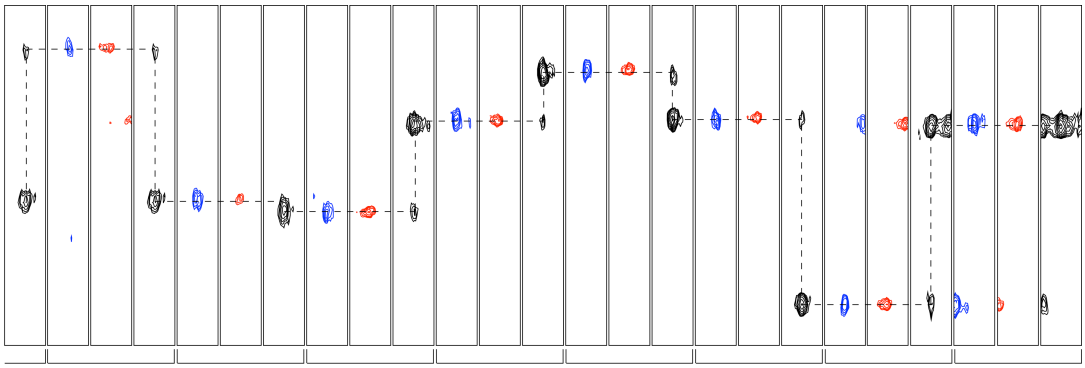
Supplementary Figure S3. Schematic representation of the coumarin fluorescence enhancement assay. Coumarin labeled TMD-5 peptide forms homo-trimer in the micelle and coumarin fluorescence is self-quenched. Upon disruption of the TMD-5 oligomerization, coumarin fluorescence would enhance due to dequenching.



Supplementary Figure S4. Effects of zwitterionic C14 betaine on the inhibitory effects of compound 1. TMD-5 disruptor compound 1 (64 μM) was added to 100 nM of coumarin labeled TMD-5 solution (50 mM HEPES, pH = 7.4) with and without 150 μM of C14 betaine.

Residue	Cα	Cβ	CO	H	Hε1	Hε21	Hε22	N	Nε1	Nε2
W138	59.83	-	178.5	8.138	10.52	-	-	120.3	130.3	-
Q139	59.99	-	178.3	8.221	-	7.157	6.76	119.5	-	109.5
L140	58.04	41.8	179.1	7.956	-	-	-	118.6	-	-
L141	58.49	-	178.7	7.916	-	-	-	118.4	-	-
A142	56.05	-	-	8.48	-	-	-	120.3	-	-
F143	61.76	38.68	177.5	-	-	-	-	-	-	-
F144	62.12	39.12	178	8.511	-	-	-	117.6	-	-
L145	58.47	41.74	178.5	8.736	-	-	-	118.6	-	-
A146	55.63	18.16	178.9	8.308	-	-	-	120.2	-	-
F147	61.12	-	177.2	8.343	-	-	-	117	-	-
F148	61.61	39.08	177.8	8.523	-	-	-	117.8	-	-
L149	58.27	41.25	178.4	8.464	-	-	-	118.3	-	-
D150	56.45	36.8	176.8	8.174	-	-	-	116.9	-	-
L151	58.2	41.56	178.5	8.009	-	-	-	119.1	-	-
I152	65.02	-	177.9	7.887	-	-	-	117.7	-	-
L153	58.41	41.31	178.6	8.208	-	-	-	118.6	-	-
L154	58.37	41.98	178.4	8.097	-	-	-	118.5	-	-
I155	65.72	37.55	178.2	8.116	-	-	-	118	-	-
I156	66.11	37.7	177.8	8.291	-	-	-	118.9	-	-
A157	55.75	18.2	180.1	8.472	-	-	-	121	-	-
L158	57.42	42.05	178.9	8.257	-	-	-	116.3	-	-
Y159	61.11	39.37	177.1	8.219	-	-	-	118.2	-	-
L160	55.95	42.97	178.3	8.244	-	-	-	116.3	-	-

Supplementary Table 1. Isotropic chemical shifts for TMD-5 in bicelles. Assignments are proposed based on the set of 2D ^{1}H-^{15}N HSQC, 3D HNCA, HN(CO)CACB, and HN(CO)CA experiments. 2D ^{1}H-^{15}N HSQC spectrum of selectively ^{15}N-GLA labeled TMD5 facilitated the identification of Gly, Leu, and Ala residues.

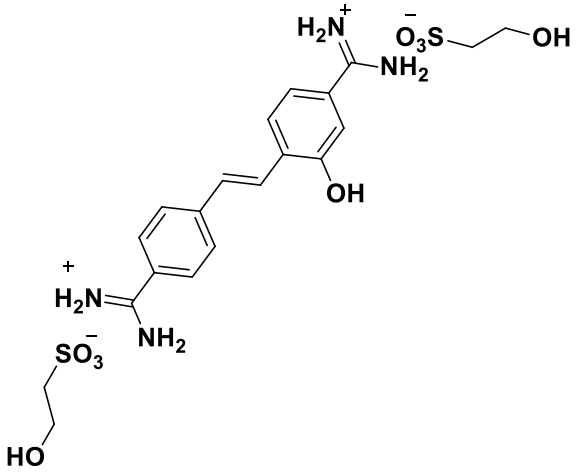


Residue	A146	F147	F148	L149	D150	L151	I152	L153	L154
^{1}H	8.31	8.34	8.52	8.46	8.17	8.01	7.89	8.21	8.10
^{15}N	120.2	117.0	117.8	118.3	116.9	119.0	117.7	118.6	118.5

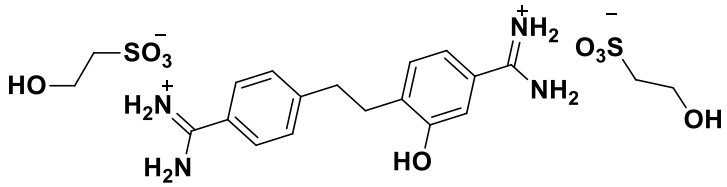
Supplementary Figure S5. 3D strip plots of TMD-5 A146-L154 region. Residues are numbered according their position in the native LMP-1 sequence. Match of backbone Cα resonances in the HNCA (black), HN(CO)CACB (blue), and HN(CO)CA (red) spectra facilitated the backbone assignment. The strip plots were prepared in Sparky (Goddard, T. D. and Kneller, D. G., SPARKY 3, UCSF) by aligning 2D ^{13}C-^{13}C slices of 3D spectra, the corresponding ^{1}H and ^{15}N shift is indicated under the figure.

Chemistry general methods

All reactions were run under an inert atmosphere of either N_2 gas. Reaction solvents were purchased anhydrous and of HPLC quality. All other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. Yields were calculated for material judged homogenous by thin layer chromatography and nuclear magnetic resonance (NMR). Thin layer chromatography (TLC) was performed on Merck Kieselgel 60 F_{254} plates, eluting with the solvent indicated, visualized by a 254 nm UV lamp, and stained with an ethanolic solution of 12-molybdophosphoric acid. Glassware for reactions was oven dried at 125 °C prior to use. Column flash chromatography was performed using Silica Gel Premium R_f, 60 Å, 200 x 400 mesh from Sorbent Technologies. Nuclear magnetic resonance spectra were acquired on a Bruker spectrometer; 300 MHz for ^1H and 75 MHz for ^{13}C. Chemical shifts for ^1H NMR spectra are reported in parts per million (ppm) and referenced to the signal of residual nondeuterated chloroform at 7.26 ppm or methanol at 3.31 ppm. Chemical shifts for ^{13}C NMR and DEPT spectra are reported in parts per million (ppm) and referenced to the center line of the $CDCl_3$ triplet at 77.23 ppm or CD_3OD at 49.00 ppm. Chemical shifts of the unprotonated carbons ('C') for DEPT spectra were obtained by comparison with the ^{13}C NMR spectrum. Mass spectrometry was performed at the mass spectrometer facility of the University of Colorado Boulder, Department of Chemistry & Biochemistry on a ESI-qTOF-MS (electrospray-triple quadrupole-time-of-flight mass spectrometer) from Applied Biosystems, PE SCIEX/ABI API QSTAR Pulsar i Hybrid LC/MS/MS. Compounds with a ppm error less than 4 % from HRMS were deemed pure.

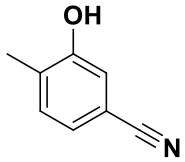


Olefin Isomer Identification of hit NSC 259242 2-hydroxystilbamidine diisethionate (Compound 1) ^1H NMR (300 MHz, MeOD) δ 7.85 – 7.81 (m, 2H), 7.73 (d, *J* = 16.62 Hz, 1H), 7.49 (d, *J* = 16.57 Hz, 1H), 7.26 (dd, *J* = 1.89, 8.00 Hz, 2H), 7.22 (d, *J* = 1.75 Hz, 1H), 4.61 (s, 2H), 3.92 (t, *J* = 7.00, 7.00 Hz, 4H), 3.09 – 2.99 (m, 4H).



Preparation of 4-(4-carbamimidoylphenethyl)-3-hydroxybenzimidamide diisethionate (Compound 2). Into a 5 mL round bottom flask (*E*)-amino(4-(4-(amino(iminio)methyl)-2-hydroxystyryl)phenyl)methaniminium 2-hydroxyethanesulfonate (NCI HTS 259242) (0.002 g, 0.0042 mmol, 1eq) and 10% Pd/C (0.001 g, 30 mol%) were taken up into anhydrous MeOH (0.1 mL, 0.7 M). The atmosphere was evacuated and replaced with N_2 (3 times) then H_2 (balloon). The reaction mixture was allowed to stir at room temperature overnight protected from light.

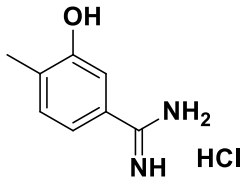
After 19 h, the yellow solution had turned clear. The reaction mixture was filtered through a 1 x 3 cm plug of Celite and washed with MeOH (3 mL) followed by EtOAc (3 mL). The solvent was removed under reduced pressure to yield the desired alkane as a yellow film (0.0021 g, 97%); ^{1}H NMR (300 MHz, MeOD) δ 7.76 – 7.69 (m, 2H), 7.52 – 7.41 (m, 2H), 7.26 – 7.20 (d, *J* = 7.8 Hz, 1H), 7.16 – 7.08 (m, 2H), 3.97 – 3.87 (t, *J* = 7.0 Hz, 4H), 3.06 – 3.01 (m, 8H), 1.90 (s, 1H); ^{13}C NMR (75 MHz, MeOD) δ 163.72 (C, 2), 158.18 (C), 156.17 (C), 150.47 (C), 132.09 (CH), 130.64 (CH), 129.14 (C), 128.99 (C), 128.59 (CH), 124.13 (CH), 115.04 (CH), 58.96 (CH_2), 54.67 (CH_2), 36.32 (CH_2), 31.75 (CH_2); HRMS (ESI^+) = calcd $C_{16}H_{19}N_4O$ ($M+H^+$) = 283.1554, found = 283.1546.



Preparation of 3-hydroxy-4-methylbenzonitrile. Into a 25 mL round bottom flask, (0.200 g, 1.069 mmol, 1 eq) and Cu(I)CN (0.195 g, 2.139 mmol, 2 eq) were taken up in N-methylpyrrolidone (NMP)(1 mL, 1.16 M). The reaction mixture was heated over 1 h to 190 °C and allowed to stir for 2 h at that temperature. Over this time period, a brown black precipitate forms. The mixture was cooled to 80 °C for 1hr than quenched with the dropwise addition of a solution of $FeCl_3$•$6H_2O$ / H_2O / conc. HCl (1.58 g / 2.4 mL / 0.4 mL).

The solution was allowed to stir for an additional 30 min at 80 °C then was cooled to room temperature. The dark brown mixture was diluted with 60 mL of EtOAc and 10 mL of H_2O. The layers were separated and the aqueous layer was back extracted with EtOAc (3 x 50 mL). The organic layers were dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The resulting dark brown-black oil was purified via flash SiO_2 chromatography (2.5 x 6 cm, 150 mL 3%

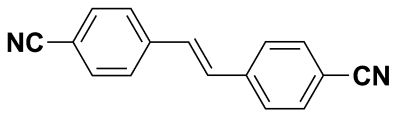
EtOAc / hexanes then 200 mL of 40 : 1 $CHCl_3$ / MeOH). Fractions 24-32 were collected and the solvent was removed under reduced pressure. The resulting orange oil was taken up into EtOAc (50 mL) and washed with H_2O (5 x 100 mL) and brine (1 x 100 mL) to remove remaining NMP. The organic layer was dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure to yield the desired phenol compound as a light orange powder; (0.117g, 83%); R_f = 0.4 (20% EtOAc / hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 – 7.12 (m, 1H), 7.12 – 7.07 (m, 2H), 6.66 (s, 1H), 2.26 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.86 (C), 131.93 (CH), 131.62 (C), 124.55 (CH), 119.17 (C), 117.99 (CH), 109.55 (C), 16.52 (CH_3); HRMS (ESI^+) = calcd C_8H_7NOLi ($M+Li^+$) = 140.0688, found = 140.0690.



Preparation of 3-hydroxy-4-methylbenzimidamide hydrochloride (Compound 3). Into a 5 mL round bottom flask, the nitrile, 3-hydroxy-4-methylbenzonitrile, (0.022 g, 0.30.165 mmol, 1 eq) is taken up into abs. EtOH (4.2 mL, 0.05 M) to this anhydrous HCl gas is bubbled through the reaction for 2 h at 0 °C. (Note HCl gas was prepared in situ by the dropwise addition of concentrated HCl into $CaCl_2$) Reaction mixture was allowed to stir at room temperature overnight.

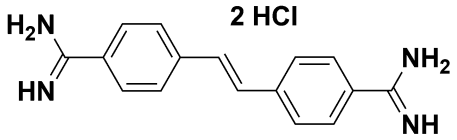
The solvent was removed under reduced pressure to yield the ethyl 3-hydroxy-4-methylbenzimidate hydrochloride as a light yellow powder. This was taken directly up into absolute EtOH (4.2 mL, 0.05 M) and cooled to 0 °C. To this, NH_3 gas was bubbled into the solution for 5 min at which time the solution turned brown orange. The solution was warmed to room temperature an allowed to stir for 16 h. The solvent was removed under reduced pressure yielding the desired amidine free base as a brown film (0.023 g, 93%); ^{1}H NMR (300 MHz, MeOD) δ 7.90 (s, 1H), 7.32 – 7.27 (m, 1H), 7.20 – 7.14 (m, 2H), 2.27 (s, 3H); ^{13}C NMR (75 MHz, MeOD) δ 168.46 (C), 157.41 (C), 133.32 (C), 132.6 (CH), 127.88 (C), 119.72 (CH), 114.32 (CH), 16.45 (CH_3); HRMS (ESI^+) = calcd $C8H11N_2O$ ($M+H^+$) = 151.0865, found = 151.0866.

The HCl salt was formed by taking up the amidine into MeOH (3 mL). To this 1 mL of 2 M HCl in ether was added dropwise. The solvent was then removed under reduced pressure yielding a brown solid that was then dried under high vacuum overnight. HRMS (ESI^+) = calcd $C_8H_{11}N_2O$ ($M+H^+$) = 151.0867, found = 151.0866.

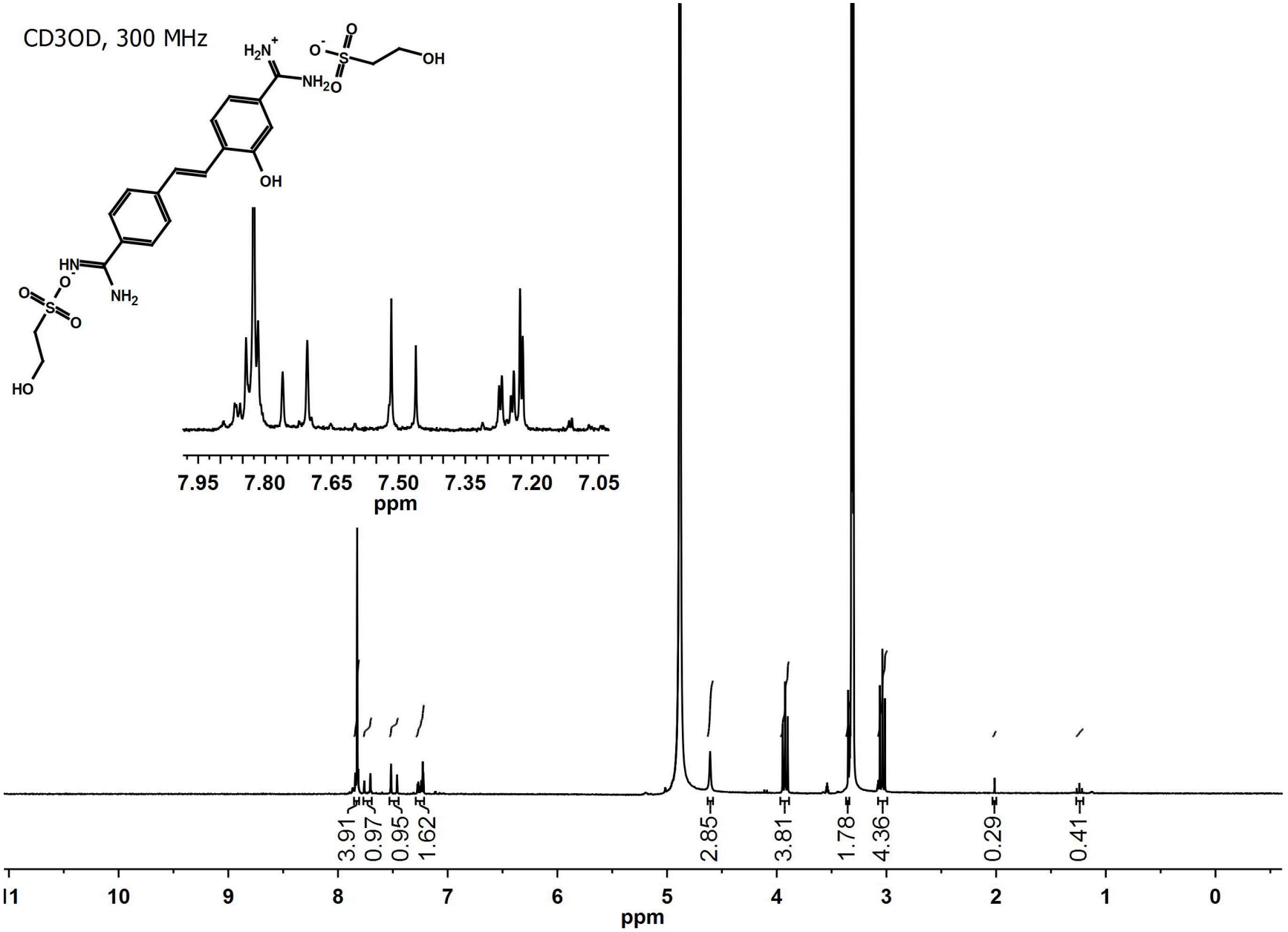


Preparation of (E)-4,4'-(ethene-1,2-diyl)dibenzonitrile (Compound 5). To a 100 mL round bottom flask charged with 4-iodobenzonitrile (0.700 g, 3.06 mmol), and

tetrakis(triphenylphosphine)palladium(0) (0.162 g, 0.140 mmol) in toluene (14 mL) was added *trans*-1,2-bis(tributylstannyl)ethene (0.843 g, 1.39 mmol). The reaction mixture was heated at reflux for 15 hours. After this time the reaction mixture was allowed to cool to room temperature. Aqueous sodium hydroxide (1 N, 15 mL) was added and the biphasic mixture was allowed to vigorously stir for 2.5 hours. The mixture was diluted with water (40 mL), ethyl acetate (40 mL), and the layers were separated. The organic layer was filtered through a plug of silica gel, eluting with ethyl acetate. The solvent was removed under reduced pressure to afford a yellow residue. The residue was crystallized from hot toluene to give compound **5** (0.111 g, 35%) as an off-white solid; ^{1}H NMR (300 MHz, DMSO-d^6) δ 7.90-7.82 (m, 2H), 7.57 (s, 2H); ^{13}C NMR (300 MHz, DMSO-d^6) δ 141.57, 133.17, 130.94, 128.06, 119.35, 110.75; HRMS (ESI$^-$) = calcd $C_{16}H_{10}N_2$ (M+Cl$^-$)= 265.0533, found = 265.0533.



Preparation of (E)-4,4'-(ethene-1,2-diyl)dibenzimidamide (Compound 4). To a 50 mL round bottom flask charged with compound **3** (0.071 g, 0.31 mmol), and tetrahydrofuran (8 mL), was added lithium hexamethyldisilazide (1 M in tetrahydrofuran/ethylbenzene, 1.6 mL, 1.6 mmol). The reaction mixture was allowed to stir at room temperature for 25 hours. After this time, aqueous hydrochloric acid (2 N, 15 mL) was added to the reaction flask, and was allowed to stir for 20 hours at room temperature. After this time, the suspension was diluted with diethyl ether (20 mL), and the off-white precipitate was collected via filtration to give compound **4** (0.050 g, 48%); ^{1}H NMR (300 MHz, DMSO-d^6)δ 9.40 (bs, 4H), 9.13 (bs, 4H), 7.89 (s, 8H), 7.62 (s, 2H); ^{13}C NMR (300 MHz, DMSO-d^6) δ 165.47, 142.28, 130.68, 129.15, 127.55, 127.44;HRMS (ESI$^+$) = calcd $C_{16}H_{16}N_4$ (M+H$^+$) = 265.1453, found = 265.1452.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7.50
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7.05
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2.85
3.81
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11
10
9
8
7
6
5
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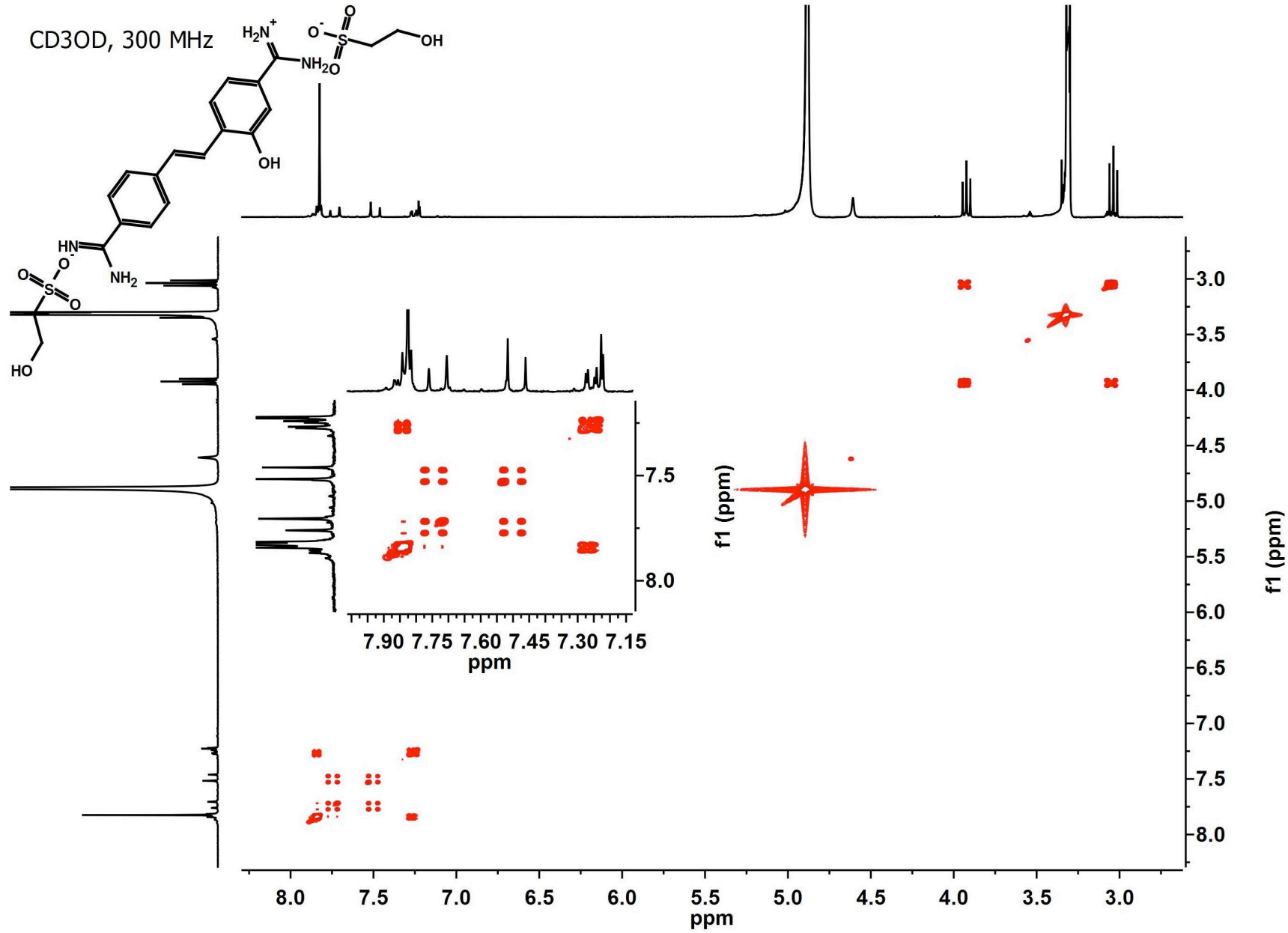
0
ppm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f1 (ppm)
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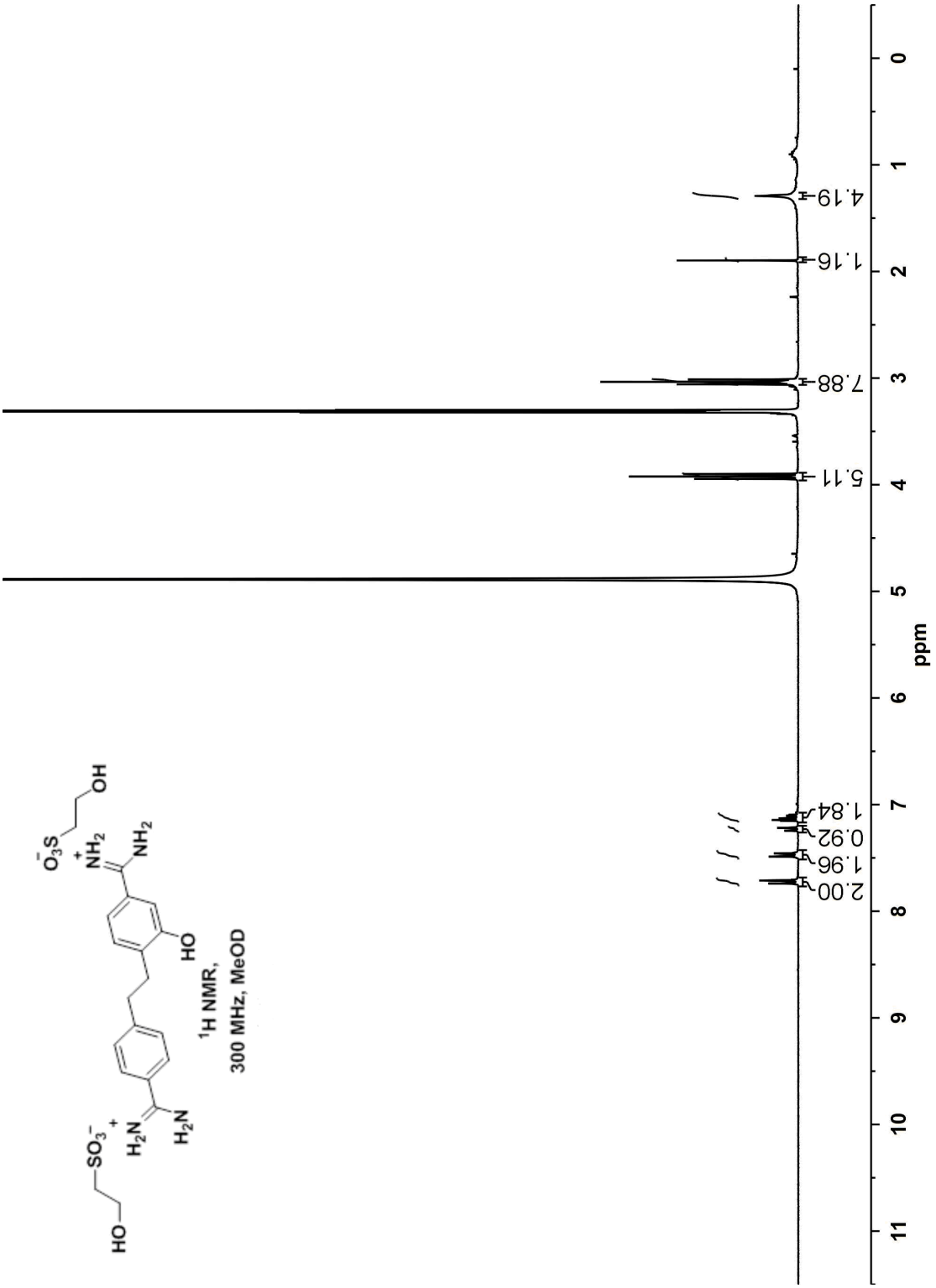
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0.92
1.96
2.00
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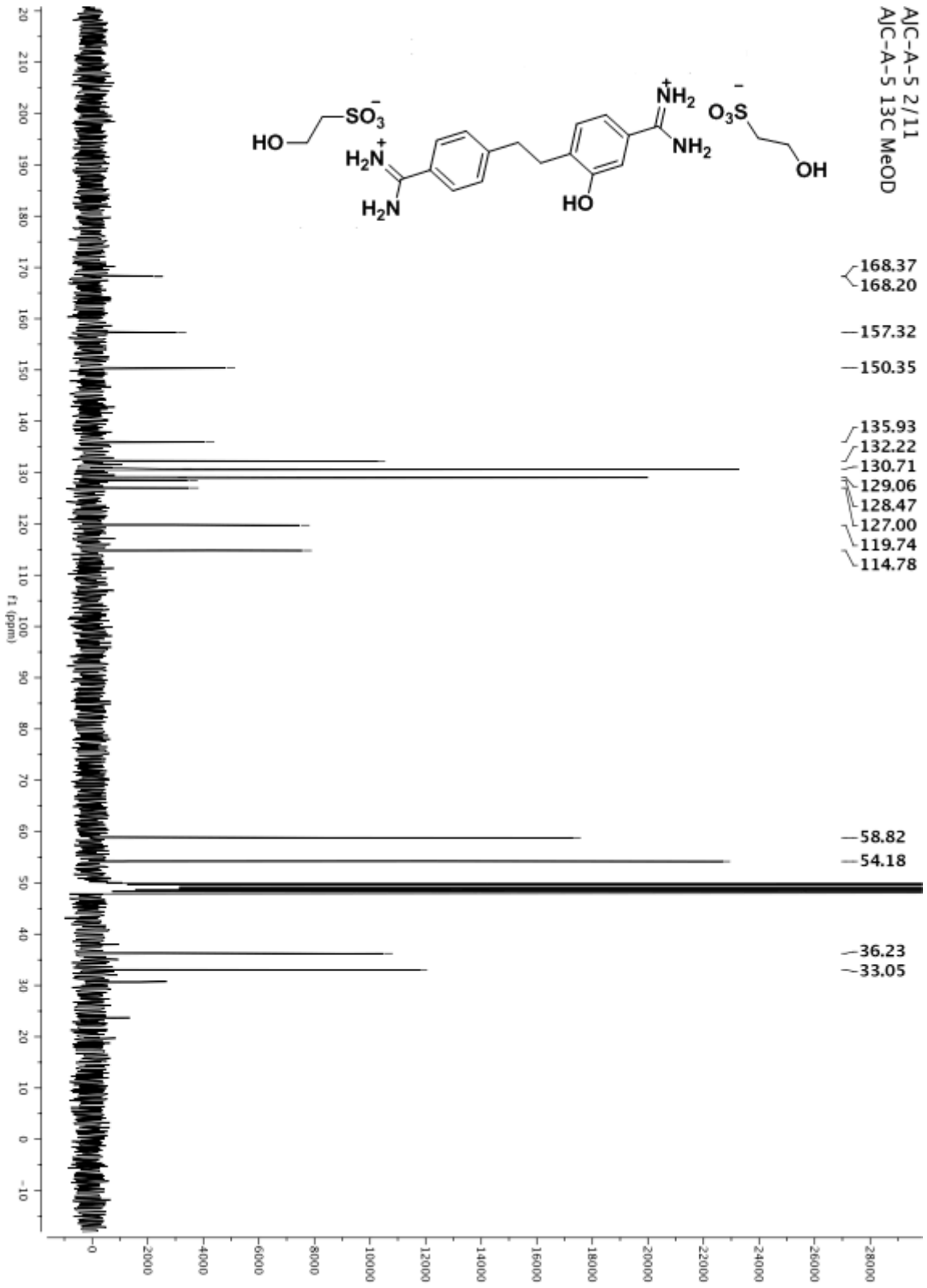
NH2
HO
O3S−
OH
1H NMR,
300 MHz, MeOD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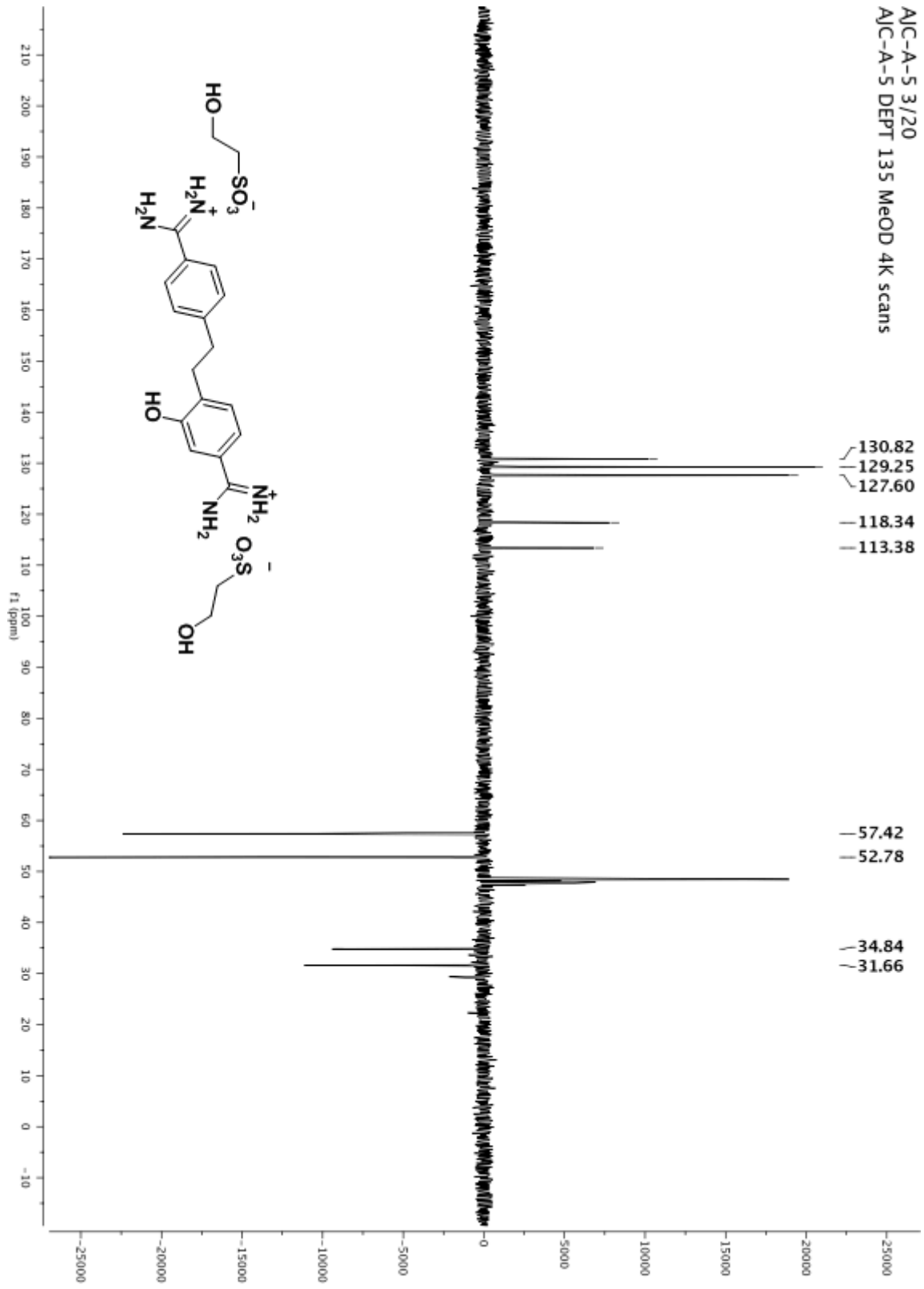
f1 (ppm)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52.78
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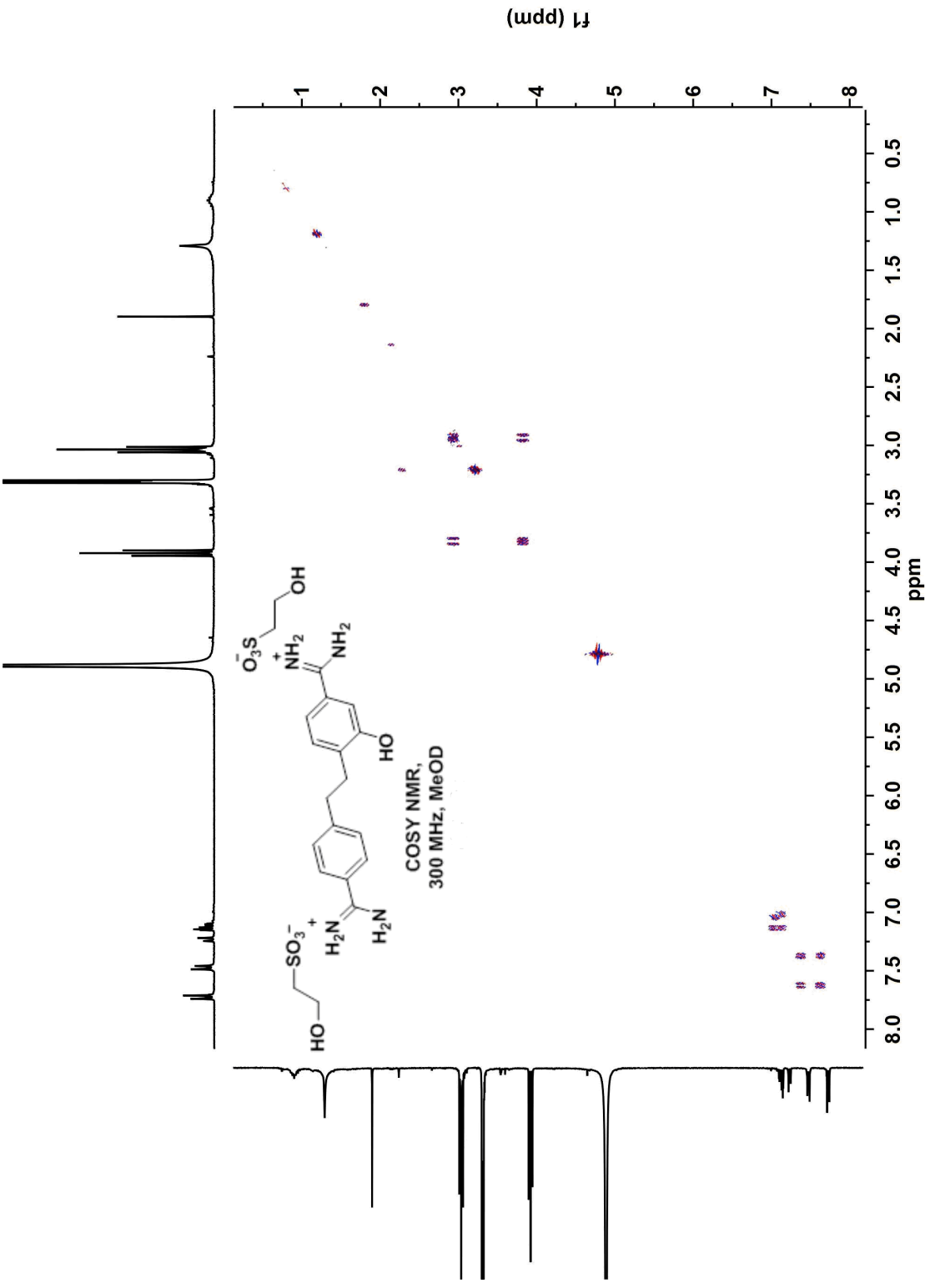
31.66
f1 (ppm)


f1 (ppm)
ppm
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300 MHz, MeOD
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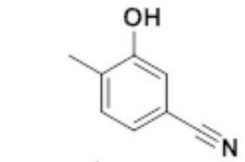
H_2N



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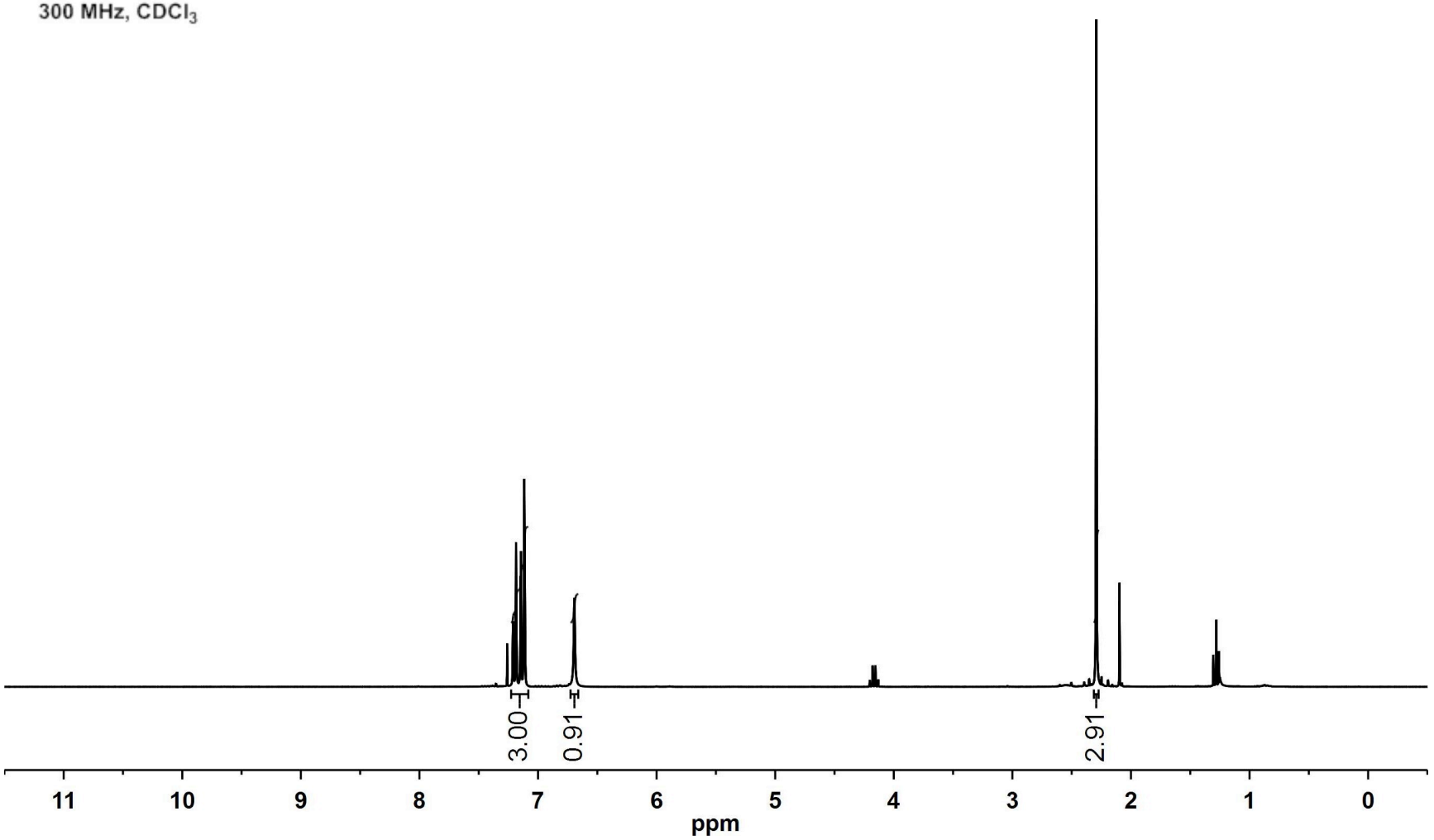

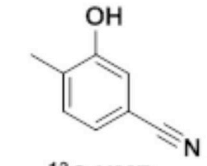

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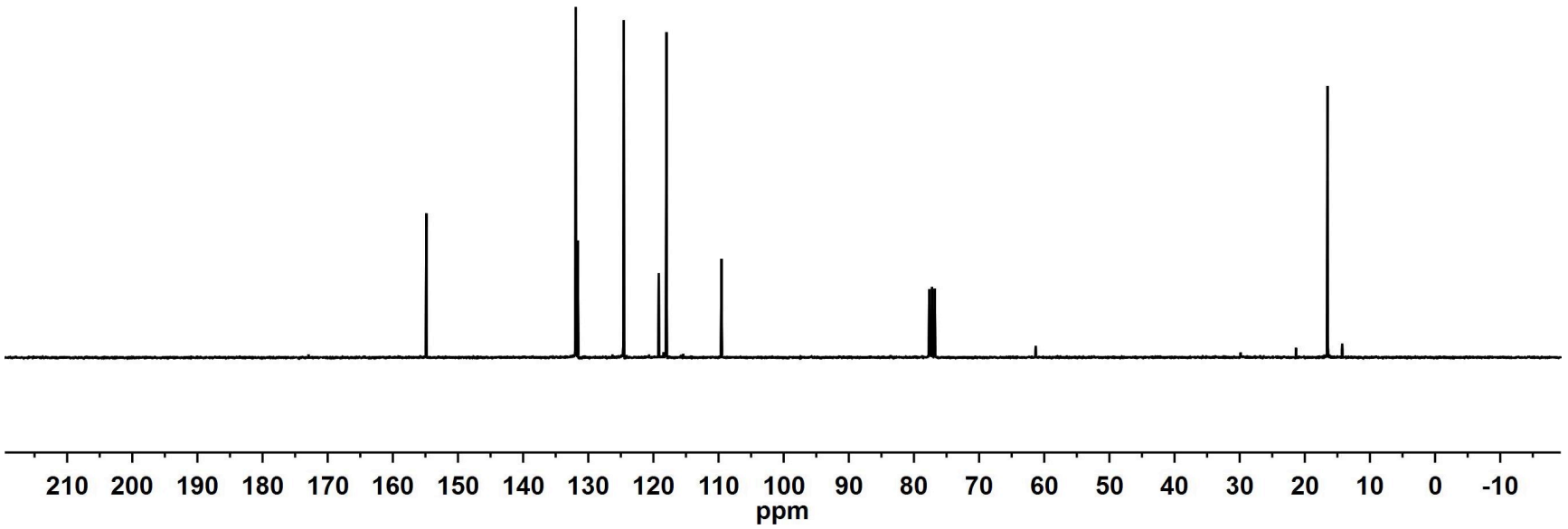
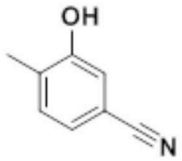
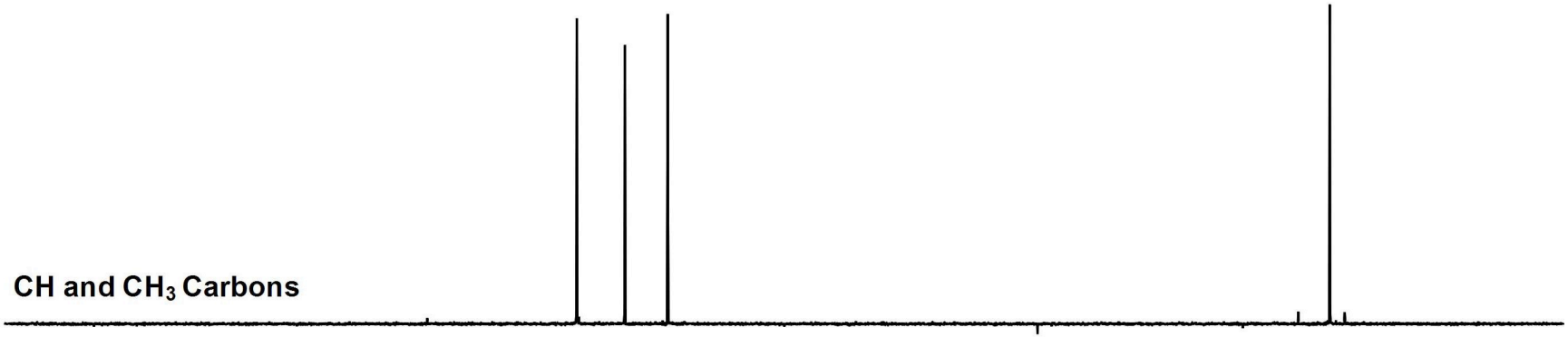
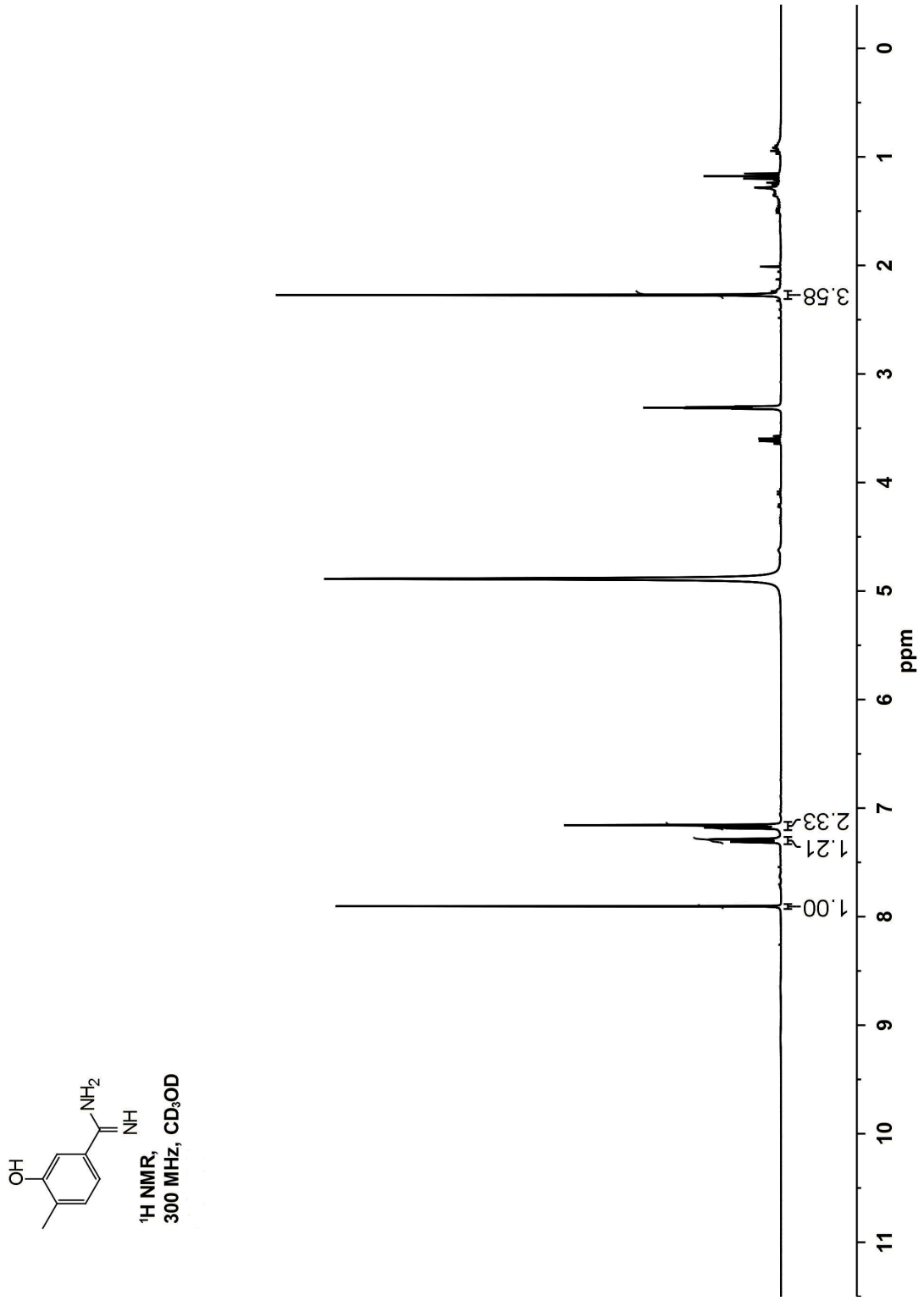
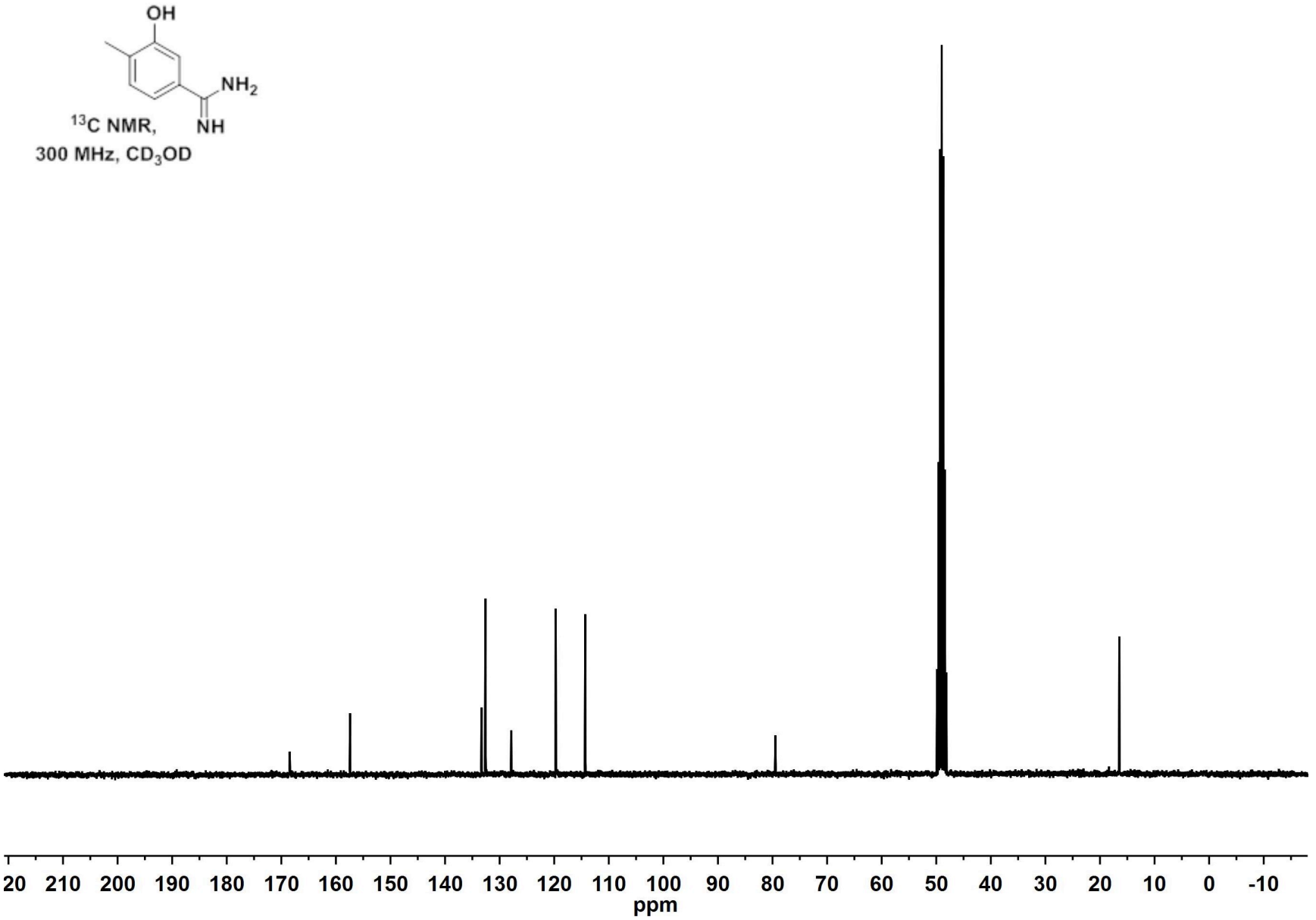


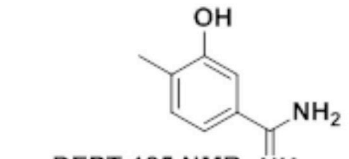


DEPT-135 NMR,
300 MHz, $CDCl_3$

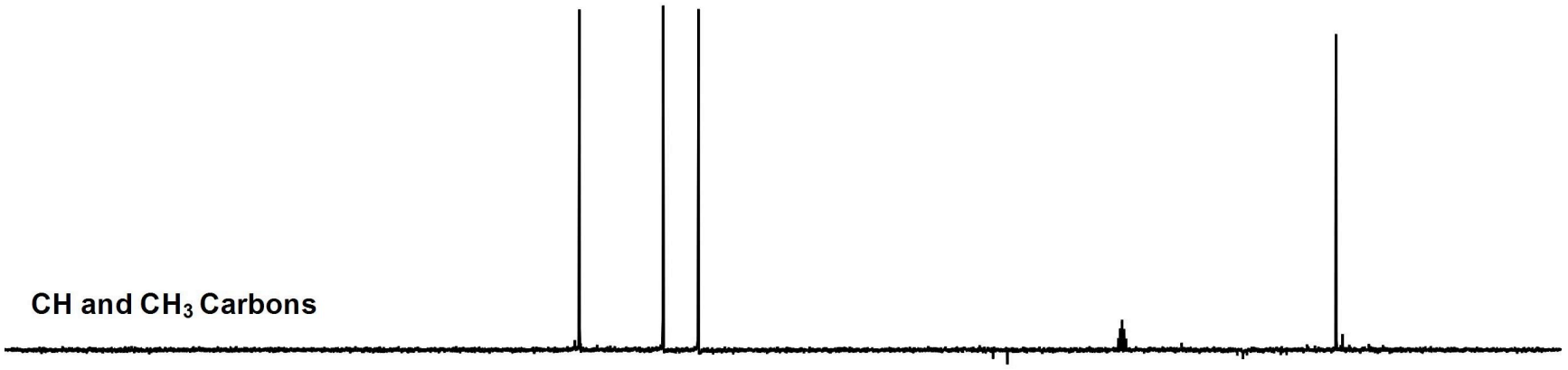


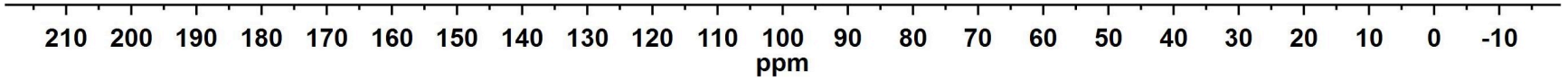

OH
NH2
NH
^{1}H NMR,
300 MHz, CD$_3$OD
3.58
2.33
1.21
1.00
ppm

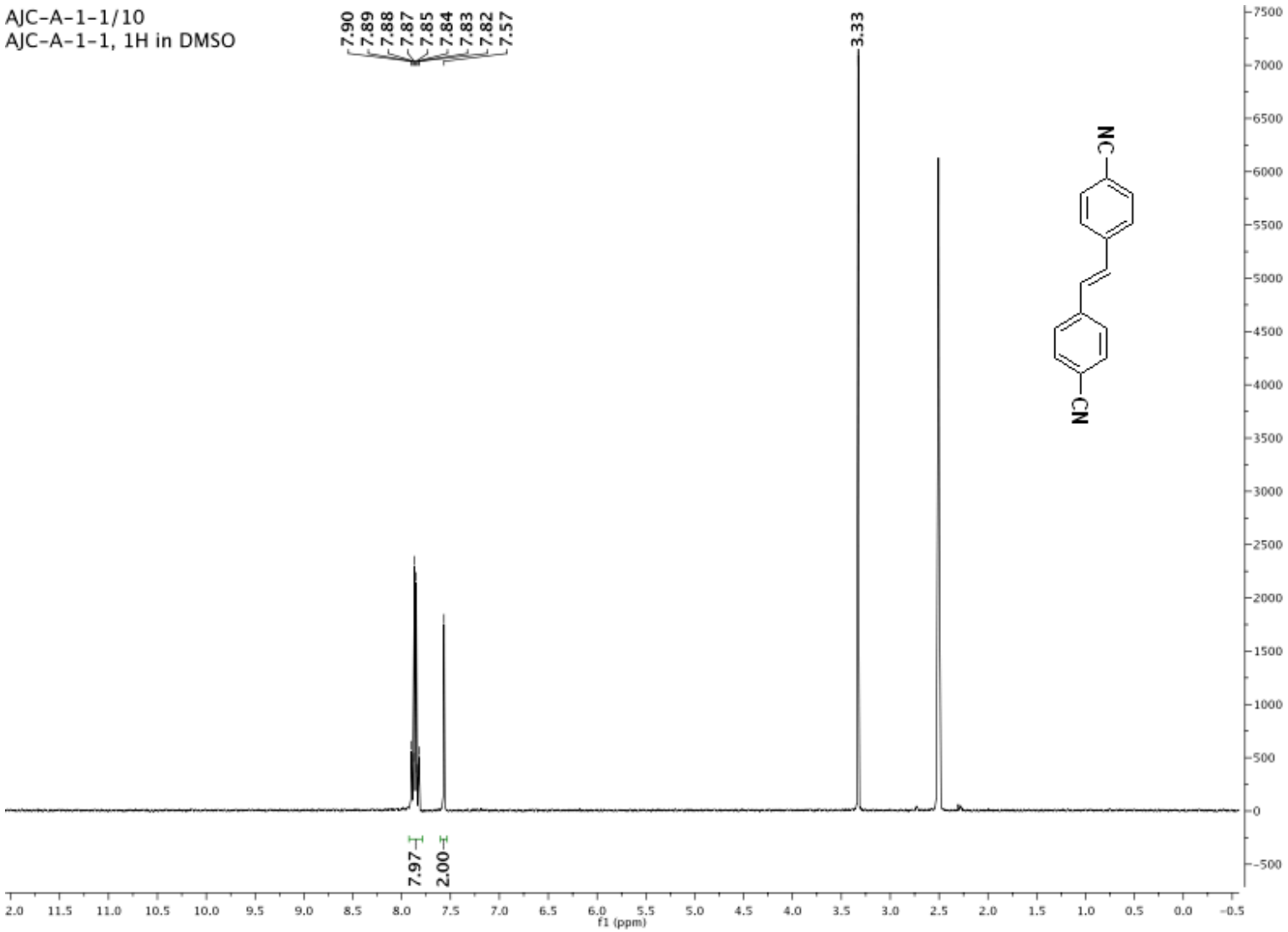

OH
NH2
NH
13C NMR,
300 MHz, CD3OD
20 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
ppm

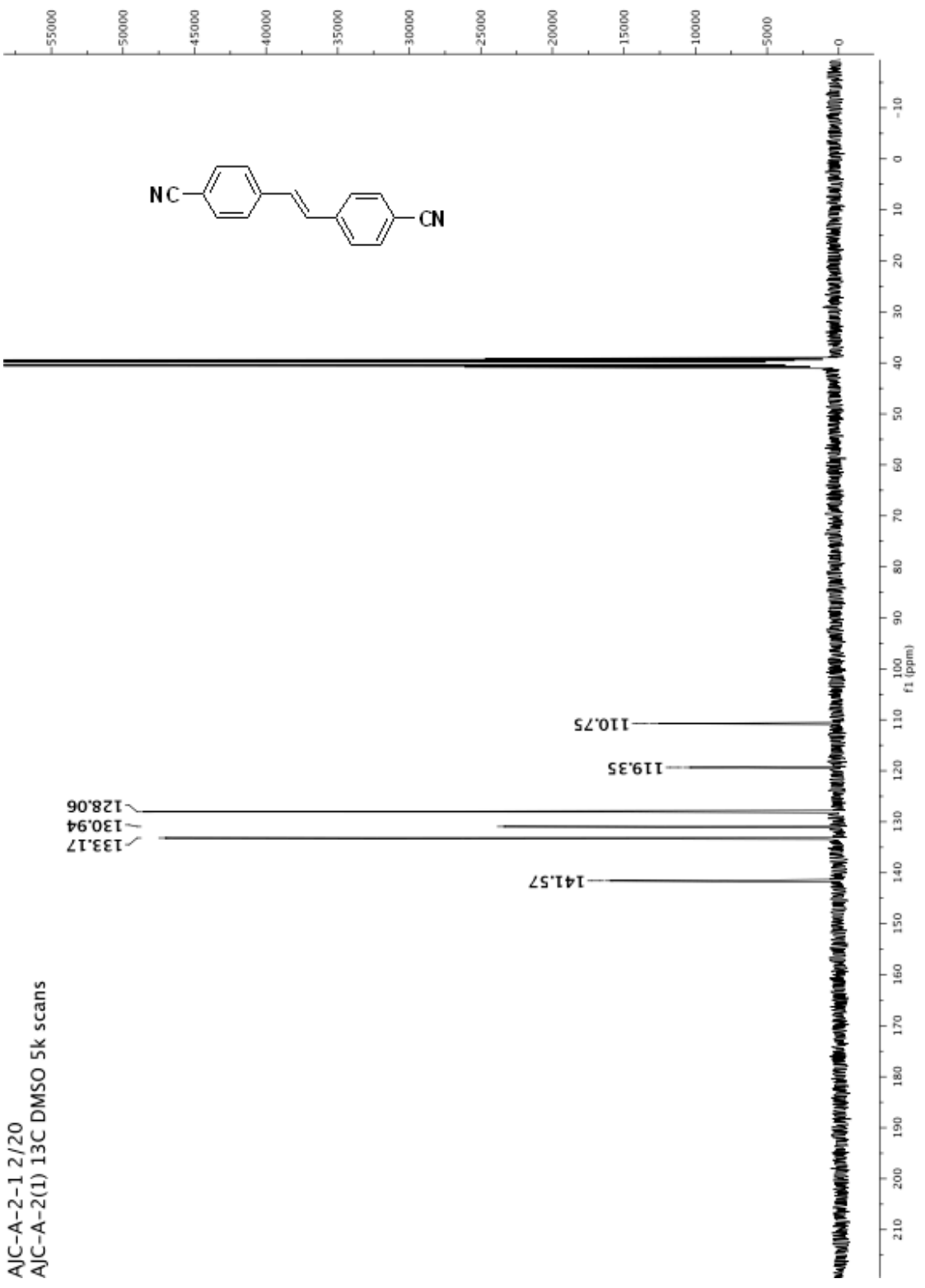


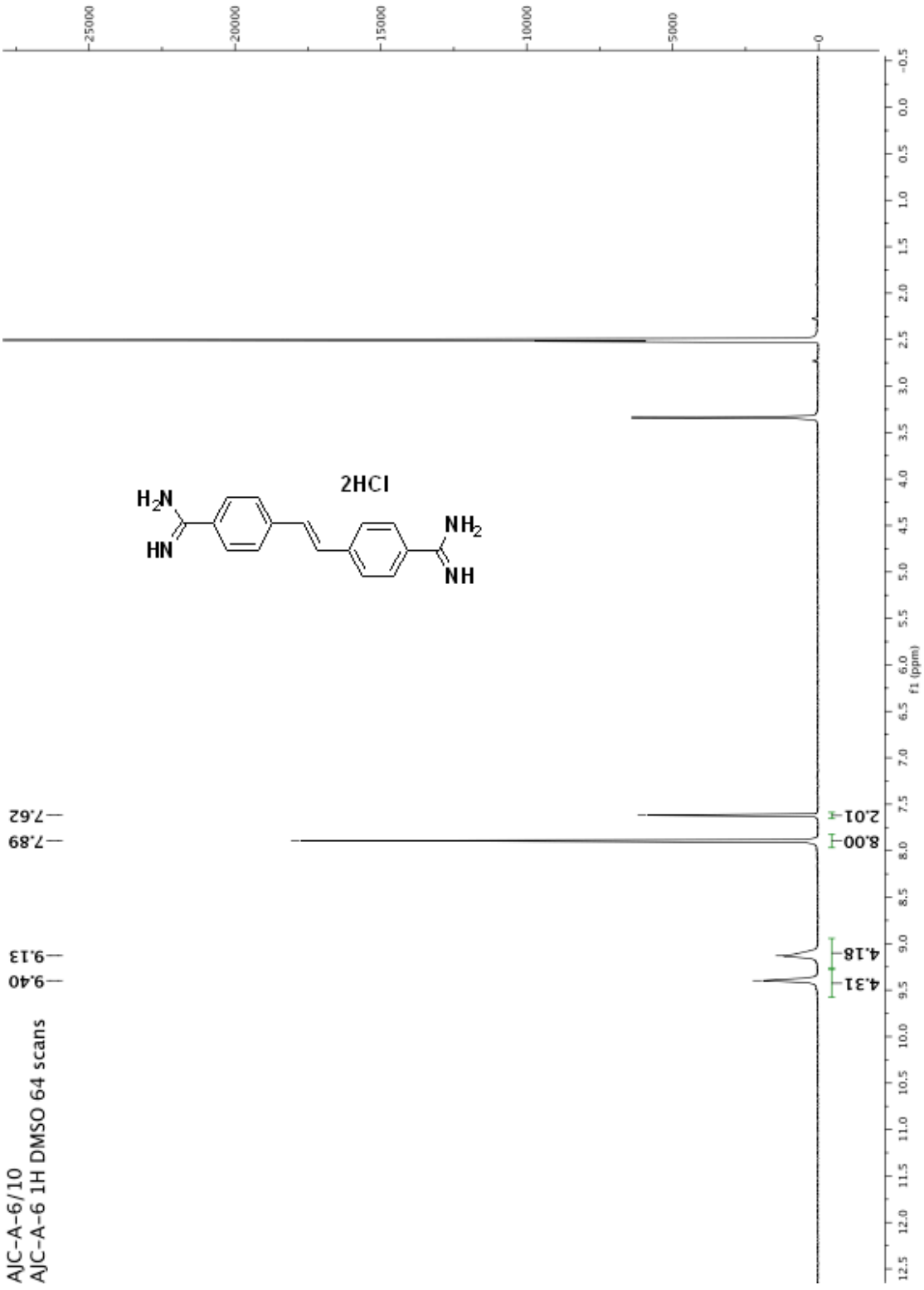
DEPT-135 NMR,
300 MHz, CD_3OD

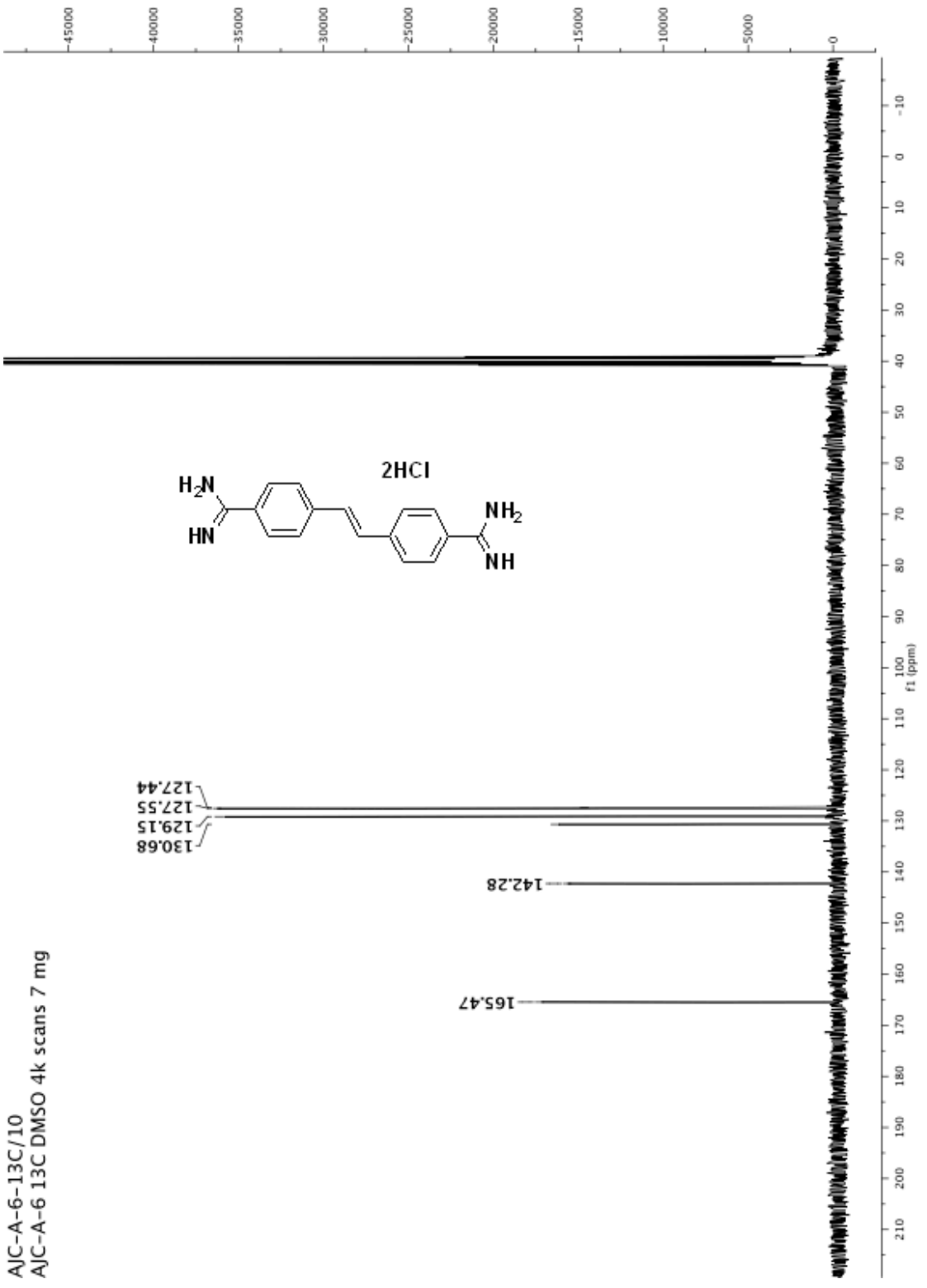





AJC-A-1-1/10
AJC-A-1-1, 1H in DMSO
7.90
7.89
7.88
7.87
7.85
7.84
7.83
7.82
7.57
3.33
7.97
2.00
NC
CN
f1 (ppm)


AJC-A-2-1 2/20
AJC-A-2(1) 13C DMSO 5k scans
NC
CN
141.57
133.17
130.94
128.06
119.35
110.75
f1 (ppm)


AJC-A-6/10
AJC-A-6 1H DMSO 64 scans
9.40
9.13
7.89
7.62
4.31
4.18
8.00
2.01
2HCl
f1 (ppm)


AJC-A-6-13C/10
AJC-A-6 13C DMSO 4k scans 7 mg
2HCl
H2N
HN
NH2
NH
165.47
142.28
130.68
129.15
127.55
127.44
f1 (ppm)