Electronic Supplementary Information for

Optimization of the anti-cancer activity of phosphatidylinositol-3 kinase pathway inhibitor PITENIN-1: switching a thiourea with 1,2,3-triazole

Yadagiri Kommagalla[a], Sinziana Cornea[b], Robert Riehle[c], Vladimir Torchilin[c], Alexei Degterev[b], C.V. Ramana[a]

[a] Division of organic Chemistry, CSIR-National Chemical Laboratory, Pune, India 411008

[b] Department of Developmental, Molecular and Chemical Biology, Tufts University, 136 Harrison Avenue, Boston, MA 02111

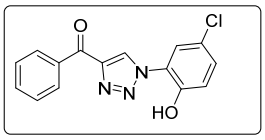
[c]Department of Pharmaceutical Sciences, Bouve College of Health Sciences, Northeastern University, 140 The Fenway, Boston, MA 02115

Table of Contents:

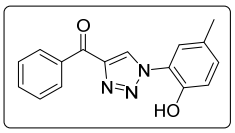
General information: Reactions were carried out in anhydrous solvents under an atmosphere of argon in oven-dried glassware. ^{1}H NMR spectra were recorded on JEOL AL-400 (400 MHz), Bruker AC 200 MHz, Bruker DRX 400 MHz and Bruker DRX 500 MHz spectrometers, and TMS was used as an internal standard of spectrometers. The chemical shifts were reported in parts per million (δ) relative to internal standard TMS (0 ppm), for $CDCl_3$ (7.27 ppm), MeOH (D_4) (3.35 ppm) and DMSO (D_6) (2.50 ppm). The peak patterns are indicated as follows: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet; q, quartet. The coupling constants, *J*, are reported in Hertz (Hz). ^{13}C NMR spectra were obtained by JEOL AL-400 (100 MHz), (125 MHz), (100 MHz) and (50 MHz) spectrometers and referenced to the internal solvent signals (central peak is 77.0 ppm in $CDCl_3$, 48.0 ppm in MeOH (D_4) and 39.5 ppm in DMSO (D_6)). $CDCl_3$, DMSO (D_6) and MeOH (D_4) were used as a NMR solvents. Mass spectroscopy was carried out on PI QStar Pulsar (Hybrid Quadrupole-TOF LC/MS/MS) and High-resolution mass spectra (HRMS) were recorded on a Thermo Scientific Q-Exactive, Accela 1250 pump, and IR spectra were recorded on FT-IR PerkinElmer spectrometer by neat for oil sample and a CH_3Cl solution for solid samples. Column chromatography was performed over silica gel 100-200 mesh. All reagents were weighed and handled in air and backfilled under argon at room temperature. Unless otherwise noted, all reactions were performed under an argon atmosphere. All reagents were purchased from Aldrich and Alfa Easer and used without further purification.

General experimental procedure: To a solution of azide **4** (1.0 eq.) and alkyne **3** (1.1 eq.) in ^{t}BuOH:H_2O (3:1) at rt, sodium ascorbate (0.2 eq.) and $CuSO_4.5H_2O$ (0.2 eq.) were added and the resulting brick reddish mixture was stirred vigorously for 10 min. The reaction mixture was diluted and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and the solvents were evaporated under reduced pressure. The product was purified by column chromatography.

(1-(5-Chloro-2-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)(phenyl)methanone (1aa): Isolated by column chromatography (pet.ether/AcOEt = 8:2, R_f = 0.2). The title compound was determined as colourless solid (87%). mp: 210–211 °C; ^{1}H NMR (200 MHz, $CDCl_3$ + DMSO (D_6)): δ 7.04 (d, J = 8.8 Hz, 1H), 7.18 (dd, J = 2.3, 8.7 Hz, 1H), 7.42-7.58 (m, 3H), 7.79 (s, 1H), 8.29 (d, J = 6.4 Hz, 2H), 8.96 (s, 1H), 10.49 (br s, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO (D_6)): δ 117.9 (d), 123.0 (d), 123.4 (s), 123.7 (s), 127.6 (d, 2C), 129.1 (d), 129.3 (d), 129.5 (d, 2C), 132.4 (d), 135.9 (s), 146.4 (s), 147.1 (s), 184.7 (s) ppm.; IR(cm^{-1}):ν 3070, 3010, 2774, 1622, 1596, 1425, 1222, 1095, 902, 721, 681; HRMS(ESI) calcd for $C_{15}H_{10}O_2N_3ClNa$ [M+Na] $^+$: 322.0354; found: 322.0357.

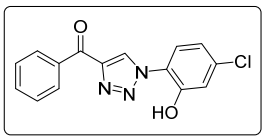


(1-(2-Hydroxy-5-methylphenyl)-1H-1,2,3-triazol-4-yl)(phenyl)methanone (1ab): Isolated by column chromatography (pet.ether/AcOEt = 8:2, R_f = 0.3). The title compound was determined as colourless solid (91%). Mp: 200–202 °C; ^{1}H NMR (200 MHz, $CDCl_3$ + MeOH (D_4)) δ 2.27 (s, 3H), 6.90 (d, J = 8.3, 1H), 7.05 (dd, J = 1.6, 8.3, 1H), 7.42–7.61 (m, 4H), 8.28–8.32 (m, 2H), 8.90 (s, 1H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$ + MeOH (D_4)) δ 19.6 (q), 116.5 (d), 123.1 (s), 124.0 (d), 128.0 (d), 129.3 (s, 2C), 129.9 (d), 130.7 (d, 4C), 133.0 (d), 136.4 (s), 146.3 (s), 173.1 (s) ppm; IR(cm^{-1}):ν 3176, 3148, 2956, 2921, 1649, 1521, 1448, 1225, 1180, 1050, 907, 816, 722, 685; calcd for $C_{16}H_{13}O_2N_3$ (M + Na$^+$): 302.0900; found: 302.0889.

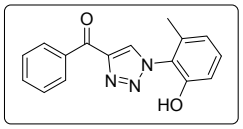


(1-(4-Chloro-2-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)(phenyl)methanone (1ac): Isolated by column chromatography (pet.ether/AcOEt = 8:2, R_f = 0.2). The title compound was determined as colourless solid (87%). Mp: 238–239 °C; ^{1}H NMR (500 MHz, $CDCl_3$ + MeOH (D_4)): δ 7.55 (d, J = 8.6 Hz, 1H), 7.13 (s, 1H), 7.54–7.59 (m, 2H), 7.68 (t, J = 7.3 Hz, 1H), 7.81 (d, J = 7.5 Hz,

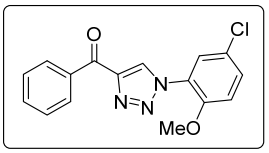
1H), 8.33 (d, J = 7.3 Hz, 2H), 9.01 (s, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$ + MeOH (D_4)): δ 116.7 (d), 119.7 (d), 122.5 (s), 124.9 (d), 128.0 (d, 3C), 129.8 (d, 2C), 133.0 (d), 135.3 (s), 136.3 (s), 146.4 (s), 149.5 (s), 186.1 (s) ppm; IR (cm^{-1}):ν 2954, 2913, 2846, 1510, 1453, 1419, 1243, 1160, 890, 854, 725, 682; HRMS(ESI) calcd for $C_{15}H_{10}O_2N_3ClNa$ (M^++Na): 322.0354; found: 322.0355.



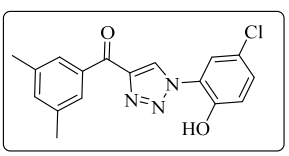
(1-(2-Hydroxy-6-methylphenyl)-1H-1,2,3-triazol-4-yl)(phenyl)methanone (1ae): Isolated by column chromatography (pet.ether/AcOEt = 8:2, R_f = 0.3). The title compound was determined as colourless solid (84%). Mp: 182–184 °C; ^{1}H NMR (200 MHz, $CDCl_3$ + MeOH (D_4)): δ 2.35 (s, 3H), 6.83 (dd, J = 1.1, 8.2 Hz, 1H), 6.90 (s, 1H), 7.50-7.62 (m, 3H), 7.67 (d, J = 8.2 Hz, 1H), 8.31–8.37 (m, 2H), 8.97 (s, 1H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$ + MeOH (D_4)) δ 20.6 (q), 117.2 (d), 120.5 (d), 121.2 (s), 123.5 (d), 128.1 (d, 2C), 129.8 (d), 129.9 (d, 2C), 133.0 (d), 136.4 (s), 140.8 (s), 146.3 (s), 148.5 (s), 186.3 (s) ppm; IR (cm^{-1}):ν 2993, 2415, 1601, 1569, 1515, 1421, 1260, 1158, 981, 897, 723, 683; HRMS(ESI) calcd for $C_{16}H_{13}O_2N_3Na$ (M^++Na): 302.0900; found: 302.0896.



(1-(5-Chloro-2-methoxyphenyl)-1H-1,2,3-triazol-4-yl)(phenyl)methanone (1af): Isolated by column chromatography (pet.ether/AcOEt = 8:2, R_f = 0.2). The title compound was determined as colourless solid (43%). Mp: 135–137 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.94 (s, 3H), 7.04 (d, J = 9.0 Hz, 1H), 7.44 (dd, J = 2.7, 9.0 Hz, 1H), 7.51–7.64 (m, 3H), 7.95 (d, J = 2.7 Hz, 1H), 8.43–8.48 (m, 2H), 8.87 (bs, 1H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): δ 56.4 (q), 113.5 (d), 125.1 (d), 126.4 (s, 2C), 128.4 (d, 2C), 130.3 (d), 130.6 (d, 3C), 133.3 (d), 149.5 (s, 2C), 175.7 (s), 197.8 (s) ppm; IR(cm^{-1}):ν 3020, 1647, 1498, 1239, 1132, 1014, 986, 894, 813, 719, 640; HRMS(ESI) calcd for $C_{16}H_{12}O_2N_3ClNa$ (M^++Na): 336.0510; found: 336.0510.

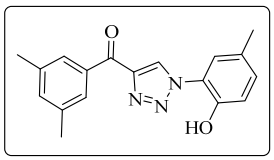


(1-(5-Chloro-2-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)(3,5-dimethylphenyl)methanone (1ba): Isolated by column chromatography (pet.ether/AcOEt = 8:2, R_f = 0.2). The title compound was determined as colourless solid (83%). Mp: 232–234 °C; ^{1}H NMR (200 MHz, $CDCl_3$ + DMSO (D_6)): δ 2.40 (s, 6H), 7.01 (d, J = 8.7 Hz, 1H), 7.22–7.29 (m, 2H), 7.84 (d, J = 2.5 Hz, 1H), 7.95 (s, 2H), 8.97 (s, 1H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$ + DMSO (D_6)): δ 21.2 (q, 2C), 118.7 (d), 123.2 (d, 2C), 128.16

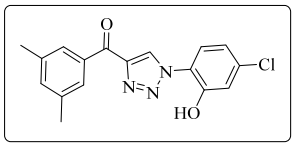


(d, 3C), 129.20 (s), 130.02 (d), 135.20 (s, 2C), 138.07 (s, 2C), 147.50 (s), 151.82 (s), 175.3 (s) ppm; IR(cm^{-1}):*v* 3174, 2958, 2918, 2114, 1623, 1589, 1496, 1295, 1258, 1212, 1021, 801, 731, 651; HRMS(ESI) calcd for $C_{17}H_{15}O_2N_3Cl$ (M^++H): 328.0847; found: 328.0847.

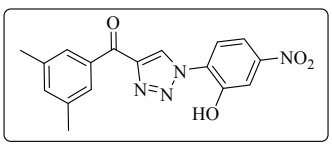
(3,5-Dimethylphenyl)(1-(2-hydroxy-5-methylphenyl)-1H-1,2,3-triazol-4-yl)methanone (1bb): Isolated by column chromatography (pet.ether/AcOEt = 8:2, R_f = 0.3). The title compound was determined as colourless solid (85%). Mp: 188–190 °C; ^{1}H NMR (200 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)) δ 2.34 (s, 3H), 2.40 (s, 6H), 6.99 (d, *J* = 8.34 Hz, 1H), 7.09–7.20 (m, 1H), 7.29 (s, 1H), 7.56–7.63 (m, 1H), 7.88 (s, 2H), 8.94 (s, 1H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)): δ 18.7 (q), 19.6 (q, 2C), 115.9 (d), 122.6 (s), 123.6 (d), 126.8 (d, 2C), 128.5 (s), 129.3 (d), 130.0 (d), 133.8 (d), 136.0 (s), 137.1 (s, 2C), 145.7 (s), 145.9 (s), 185.4 (s) ppm; IR(cm^{-1}):*v* 3182, 2956, 2921, 2859, 1616, 1593, 1521, 1298, 1253, 1208, 1151, 1019, 805, 767, 696; HRMS(ESI) calcd for $C_{18}H_{18}O_2N_3$ (M^++H): 308.1394; found: 308.1387.



(1-(4-Chloro-2-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)(3,5-dimethylphenyl)methanone (**1bc**): Isolated by column chromatography (pet.ether/AcOEt = 8:2, R_f = 0.2). The title compound was determined as colourless solid (91%). Mp: 149–151 °C; ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO (D_6)) δ 2.35 (s, 6H), 6.95 (dd, *J* = 2.0, 8.6 Hz, 1H), 7.12 (d, *J* = 2.0 Hz, 1H), 7.22 (bs, 1H), 7.70 (d, *J* = 8.6 Hz, 1H), 7.85 (s, 2H), 8.91 (s, 1H) 11.0 (bs, 1H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$ + DMSO (D_6)) δ 19.7 (q, 2C), 115.8 (d), 118.2 (d), 121.4 (s), 124.03 (d), 126.3 (d, 2C), 128.6 (d), 133.2 (d), 133.4 (s), 135.3 (s), 136.2 (s, 2C), 145.4 (s), 148.8 (s), 184.0 (s) ppm; IR(cm^{-1}):*v* 3067, 2950, 2400, 1587, 1499, 1424, 1297, 1228, 1022, 854, 797, 765; HRMS(ESI) calcd for $C_{17}H_{15}O_2N_3Cl$ (M^++H): 328.0847; found: 328.0833.

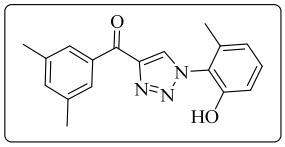


(3,5-Dimethylphenyl)(1-(2-hydroxy-4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methanone (1bd): Isolated by column chromatography (pet.ether/AcOEt = 7:3, R_f = 0.2). The title compound was determined as yellow solid (86%). Mp: 237–239 °C; ^{1}H NMR (200 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)): δ 2.38 (s, 6H), 6.21 (d, *J* = 8.1 Hz, 1H), 7.18 (s, 1H), 7.28 (d, *J* = 8.8 Hz, 1H), 7.55 (s, 2H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.73–7.88 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$ + MeOH

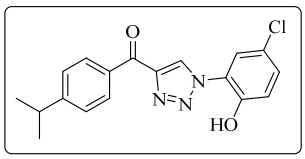


(D_4) + DMSO (D_6)): δ 20.3 (q, 2C), 109.3 (d), 110.9 (d), 115.9 (d), 124.6 (d, 2C), 133.0 (d), 134.6 (s), 137.4 (s, 2C), 138.2 (s), 141.1 (d), 141.2 (s), 141.7 (s), 145.0 (s), 191.4 (s) ppm; IR(cm^{-1}):*v* 3377, 3311, 3091, 2885, 2198, 1929, 1627, 1594, 1521, 1428, 1262, 1182, 1081, 948, 870, 742, 643.

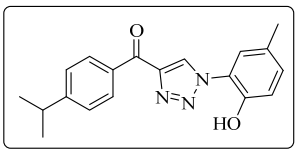
(3,5-Dimethylphenyl)(1-(2-hydroxy-6-methylphenyl)-1H-1,2,3-triazol-4-yl)methanone (**1be**): Isolated by column chromatography (pet.ether/AcOEt = 8:2, R_f = 0.3). The title compound was determined as colourless solid (91%). Mp: 188–190 °C; ^{1}H NMR (200 MHz, $CDCl_3$ + MeOH (D_4)): δ 2.37 (s, 3H), 2.42 (s, 6H), 6.85 (d, *J* = 8.2 Hz, 1H), 6.91 (s, 1H), 7.31 (s, 1H), 7.65 (d, *J* = 8.1 Hz, 1H), 7.88 (s, 2H), 8.92 (s, 1H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$ + MeOH (D_4)): δ 19.6 (q, 3C), 116.3 (d), 119.7 (d), 120.7 (s), 123.3 (d), 126.8 (d, 2C), 129.3 (d), 133.8 (d), 136.0 (s), 137.1 (s, 2C), 140.1 (s), 145.7 (s), 148.1 (s), 185.4 (s) ppm; IR(cm^{-1}):*v* 3402, 2918, 2254, 2128, 1626, 1595, 1521, 1430, 1234, 1022, 996, 824, 761; HRMS(ESI) calcd for $C_{18}H_{18}O_2N_3$ (M^++H): 308.1394; found: 308.1381.



(1-(5-Chloro-2-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)(4-isopropylphenyl)methanone (1ca): Isolated by column chromatography (pet.ether/AcOEt = 8:2, R_f = 0.2). The title compound was determined as colourless solid (86%). Mp: 187–189 °C; ^{1}H NMR (400 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)): δ 1.23 (d, *J* = 6.9 Hz, 6H), 2.95 (spt, *J* = 6.9 Hz, 1H), 7.05 (d, *J* = 8.8 Hz, 1H), 7.29 (dd, *J* = 2.5, 8.8 Hz, 1H), 7.36 (d, *J* = 8.31 Hz, 2H), 7.75 (d, *J* = 2.7 Hz, 1H), 8.20 (d, *J* = 8.1 Hz, 2H), 8.97 (s, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)): δ 21.9 (q, 2C), 33.0 (d), 117.2 (d), 122.9 (s), 123.0 (d), 123.5 (s), 125.3 (d, 2C), 129.0 (d), 129.2 (d), 129.3 (d, 2C), 133.4 (s), 145.8 (s), 147.2 (s), 153.8 (s), 184.1 (s) ppm; IR(cm^{-1}):*v* 3459, 2989, 1621, 1575, 1515, 1286, 1250, 1196, 1027, 785, 762.

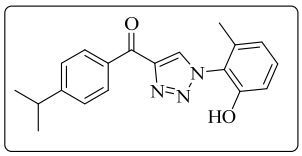


(1-(2-Hydroxy-5-methylphenyl)-1H-1,2,3-triazol-4-yl)(4-isopropylphenyl)methanone (1cb): Isolated by column chromatography (pet.ether/AcOEt = 8:2, R_f = 0.3). The title compound was determined as colourless solid (83%). Mp: 131–133 °C; ^{1}H NMR (500 MHz, MeOH (D_4)): δ 1.48 (d, *J* = 6.8 Hz, 6H), 2.52 (s, 3H), 3.20 (spt, *J* = 6.9 Hz, 1H), 7.20–7.35 (m, 2H), 7.59 (d, *J* = 8.3 Hz, 2H), 7.78 (s,

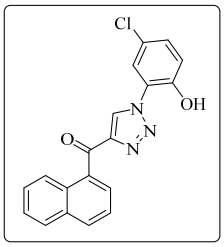


1H), 8.50 (d, J = 8.3 Hz, 2H), 9.18 (s, 1H) ppm; ^{13}C NMR (125 MHz, MeOH (D_4)): δ 20.6 (q), 24.2 (q, 2C), 35.7 (d), 118.1 (d), 125.1 (s), 126.0 (d), 127.8 (d, 2C), 131.0 (s), 131.7 (d), 131.8 (d, 2C), 132.4 (d), 136.1 (s), 148.1 (s), 148.5 (s), 156.5 (s), 187.1 (s) ppm; IR(cm^{-1}):v 3177, 2961, 2925, 2869, 1622, 1600, 1523, 1416, 1348, 1274, 1187, 1047, 907, 814, 773; HRMS(ESI) calcd for $C_{19}H_{20}O_2N_3$ (M^++H): 322.1550; found: 322.1548.

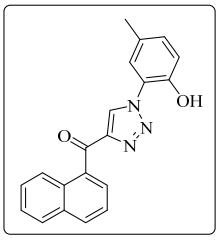
(1-(2-Hydroxy-6-methylphenyl)-1H-1,2,3-triazol-4-yl)(4-isopropylphenyl)methanone (1ce): Isolated by column chromatography (pet.ether/AcOEt = 8:2, R_f = 0.3). The title compound was determined as colourless solid (82%). Mp: 172–174 °C; ^{1}H NMR (500 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)): δ 1.26 (s, 3H), 1.27 (s, 3H), 2.33 (s, 3H), 2.98 (spt, J = 6.87 Hz, 1H), 6.83 (d, J = 7.3 Hz, 1H), 6.99 (s, 1H), 7.44 (d, J = 8.2 Hz, 2H), 7.60 (d, J = 8.2 Hz, 1H), 8.29 (d, J=8.2 Hz, 2H), 9.01 (s, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)): δ 21.3 (q), 23.9 (q, 2C), 34.5 (d), 118.1 (d), 121.1 (d, 2C), 122.4 (s), 125.3 (d), 127.1 (d, 2C), 131.1 (d, 2C), 135.2 (s), 141.6 (s), 147.4 (s), 150.1 (s), 155.2 (s), 185.5 (s) ppm; IR(cm^{-1}):v 3176, 2960, 1629, 1604, 1520, 1504, 1425, 1267, 1159, 1049, 907, 820, 770; HRMS(ESI) calcd for $C_{19}H_{20}O_2N_3$ (M^++H): 322.1550; found: 322.1550.



(1-(5-Chloro-2-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)(naphthalen-1-yl)methanone (1da): Isolated by column chromatography (pet.ether/AcOEt = 8:2, R_f = 0.2). The title compound was determined as colourless solid (87%). Mp: 255–257 °C; ^{1}H NMR (500 MHz, MeOH (D_4) + DMSO (D_6)): δ 7.12 (d, J = 8.9 Hz, 1H), 7.38 (dd, J = 2.4, 8.5 Hz, 1H), 7.56 (dd, J = 3.4, 6.4 Hz, 2H), 7.61(t, J = 7.9 Hz, 1H),), 7.74 (d, J = 2.4 Hz, 1H), 8.01 (d, J = 7.3 Hz, 2H), 8.13 (d, J = 8.2 Hz, 1H), 7.54 (dd, J = 3.1, 5.8 Hz, 1H), 9.07 (s, 1H); ^{13}C NMR (125 MHz, MeOH (D_4) + DMSO (D_6)): δ 119.2 (d), 123.9 (s), 125.2 (d), 125.3 (s), 125.4 (d), 125.7 (d), 127.1 (d), 128.2 (d), 129.2 (d), 130.1 (d), 130.9 (s), 131.1 (d), 131.6 (d), 132.8 (d), 134.3 (s), 135.5 (s), 148.1 (s), 149.5 (s), 188.8 (s) ppm; IR(cm^{-1}):v 3067, 2950, 1942, 1736, 1645, 1598, 1437, 1303, 1233, 1158, 880, 748, 624; HRMS(ESI) calcd for $C_{19}H_{12}O_2N_3ClNa$ (M^++Na): 372.0510; found: 372.0507.

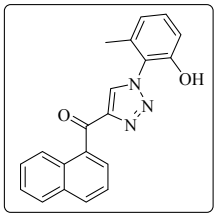


(1-(2-Hydroxy-5-methylphenyl)-1H-1,2,3-triazol-4-yl)(naphthalen-1-yl)methanone (1db): Isolated by column chromatography (pet.ether/AcOEt = 8:2, R_f = 0.3). The

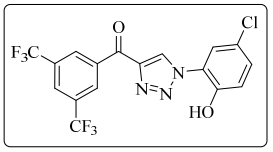


title compound was determined as colourless solid (91%). Mp: 222–223 °C; ^{1}H NMR (400 MHz, $CDCl_3$ + MeOH (D_4)): δ 2.36 (s, 3H), 6.98 (d, J = 8.3 Hz, 1H), 7.14–7.16 (m, 1H), 7.56–7.65 (m, 5H), 7.96–7.98 (m, 1H), 8.04 (dd, J = 1.0, 7.1 Hz, 1H), 8.11 (d, J = 8.1 Hz, 1H), 8.35–8.41 (m, 1H), 8.96 (s, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$ + MeOH (D_4)): δ 19.5 (q), 116.4 (d), 123.0 (s), 123.9 (d), 124.0 (d), 124.7 (d), 126.0 (d), 127.1 (d), 128.0 (d), 129.0 (d), 129.2 (s), 130.0 (d), 130.2 (s), 130.7 (d), 132.2 (d), 133.5 (s), 134.3 (s), 146.3 (s), 146.9 (s), 188.6 (s) ppm; IR(cm^{-1}): v 3070, 2920, 1627, 1522, 1457, 1368, 1286, 1256, 1164, 1031, 903, 786; HRMS(ESI) calcd for $C_{20}H_{16}O_2N_3$ (M^++H): 330.1237; found: 330.1243.

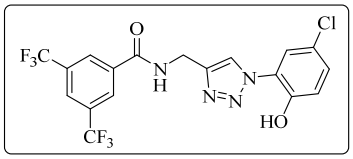
(1-(2-Hydroxy-6-methylphenyl)-1H-1,2,3-triazol-4-yl)(naphthalen-1-yl)methanone (1de): Isolated by column chromatography (pet.ether/AcOEt = 8:2, R_f = 0.3). The title compound was determined as colourless solid (84%). Mp: 221–223 °C; ^{1}H NMR (500 MHz, DMSO (D_6)): δ 2.27 (s, 3H), 6.72 (d, J = 8.2 Hz, 1H), 6.87 (s, 1H), 7.47–7.51 (m, 2H), 7.53 (d, J = 8.2 Hz, 1H), 7.58 (d, J = 8.2 Hz, 1H), 7.86–7.88 (m, 1H), 7.99 (t, J = 7.5 Hz, 2H), 8.27–8.29 (m, 1H), 8.85 (s, 1H), 10.21 (bs, 1H) ppm; ^{13}C NMR (125 MHz, DMSO (D_6)): δ 20.3 (q), 116.8 (d), 119.7 (d), 120.7 (s), 123.0 (d), 123.5 (d), 124.3 (d), 125.5 (d), 126.5 (d), 127.5 (d), 128.5 (d), 129.1 (d), 129.6 (s), 131.3 (d), 132.7 (s), 133.9 (s), 139.7 (s), 146.5 (s), 147.9 (s), 187.5 (s) ppm; IR(cm^{-1}): v 3161, 2921, 1628, 1608, 1522, 1436, 1283, 1254, 1032, 902, 786, 764; HRMS(ESI) calcd for $C_{20}H_{16}O_2N_3$ (M^++H): 330.1237; found: 330.1221.



(3,5-Bis(trifluoromethyl)phenyl)(1-(5-chloro-2-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)methanone (1ea): Isolated by column chromatography (pet.ether/AcOEt = 8:2, R_f = 0.2). The title compound was determined as colourless solid (82%). Mp: 210–212 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.08 (d, J = 8.9 Hz, 1H), 7.32 (dd, J = 2.4, 8.9 Hz, 1H), 7.94 (d, J = 2.4 Hz, 1H), 8.17 (bs, 1H), 8.99 (bs, 2H), 9.22 (s, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 118.0 (d), 121.6 (s), 123.6 (d, 2C), 123.8 (s), 124.0 (s), 124.4 (s), 126.0 (d, t, J = 3.6 Hz), 130.1 (d), 130.4 (d, J = 2.7 Hz), 130.5 (d), 131.4 (s, d, J = 33.6 Hz), 131.9 (s, d, J = 34.5 Hz), 137.8 (s), 146.1 (s), 147.5 (s), 182.5 (s) ppm; IR(cm^{-1}):v 3187, 2959, 1640, 1527, 1419, 1280, 1134, 910, 819, 768; HRMS(ESI) calcd for $C_{17}H_9O_2N_3ClF_6$ (M^++H): 436.0282; found: 436.0289..



***N*-((1-(5-chloro-2-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)-3,5-bis(trifluoromethyl)benzamide (1fa):** Isolated by column chromatography (pet.ether/AcOEt = 9:1, R_f = 0.5). The title compound was determined as colourless solid (85%). Mp: 204–206 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.43 (s, 2H), 6.69 (d, J = 8.8 Hz, 1H), 6.93 (dd, J = 2.7, 8.7 Hz, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.71 (bs, 1H), 8.12 (s, 1H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): δ 34.6 (t), 117.6 (d), 119.8 (d), 123.4 (d), 123.8 (s), 124.3 (d), 124.5 (d), 125.2 (s), 127.4 (d, J = 2.9 Hz), 129.1 (d), 130.6 (s), 130.7 (s, d, J = 33.7 Hz), 131.6 (s, d, J = 34.0 Hz), 135.6 (s), 143.5 (s), 147.4 (s, 2C), 164.8 (s) ppm; IR(cm^{-1}):ν 3085, 2926, 1645, 1597, 1460, 1376, 1280, 1176, 1132, 906, 773, 689; HRMS(ESI) calcd for $C_{18}H_{12}O_2N_4ClF_6$ (M^++H): 465.0547; found: 465.0533.



Biology

Cells. Human ovarian carcinoma A2780, malignant glioblastoma U87MG and breast carcinoma T47D cells were obtained from ATCC. Cells were maintained in DMEM media (Fisher) supplemented with 10% fetal bovine serum (Sigma) and 1% antibiotic-antimicotic mix (Invitrogen).

Lipid overlay assay. Assay was performed using 1 μg/ml recombinant Akt PH domain protein as described previously.[1]

Cell viability. Cells were seeded into white clear bottom tissue culture treated 96 well plates at the density of $10*10^3$ cells per well. After 24 hr, cells were treated with the inhibitors in DMSO (final DMSO concentration was maintained at 0.5% in all wells). Cell viability relative to the control, DMSO treated wells was determined using CellTiter-Glo viability assay (Promega).

Western blotting. Cells were treated with indicated concentrations of compounds in 6 well plates ($6*10^5$ cells per well) for 7 hr. Cells were lysed in 1XRIPA buffer (Cell Signaling). Protein concentrations were normalized using 660 nm protein assay reagent (Pierce). Equal amounts of protein were loaded on SDS-PAGE. Western blotting was performed using standard protocols using S6 and phospho-Ser235/236-S6 antibodies (Cell Signaling).

Wounds healing assay. Assay was performed using monolayers of A2780 cells as previously described.[2]

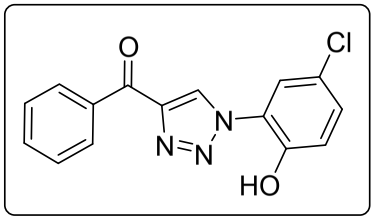
Metabolic stability measurements. Mouse microsomal stability assays were performed by Cyprotex. Pharmacokinetcs analysis was performed by PharmaLegacy.

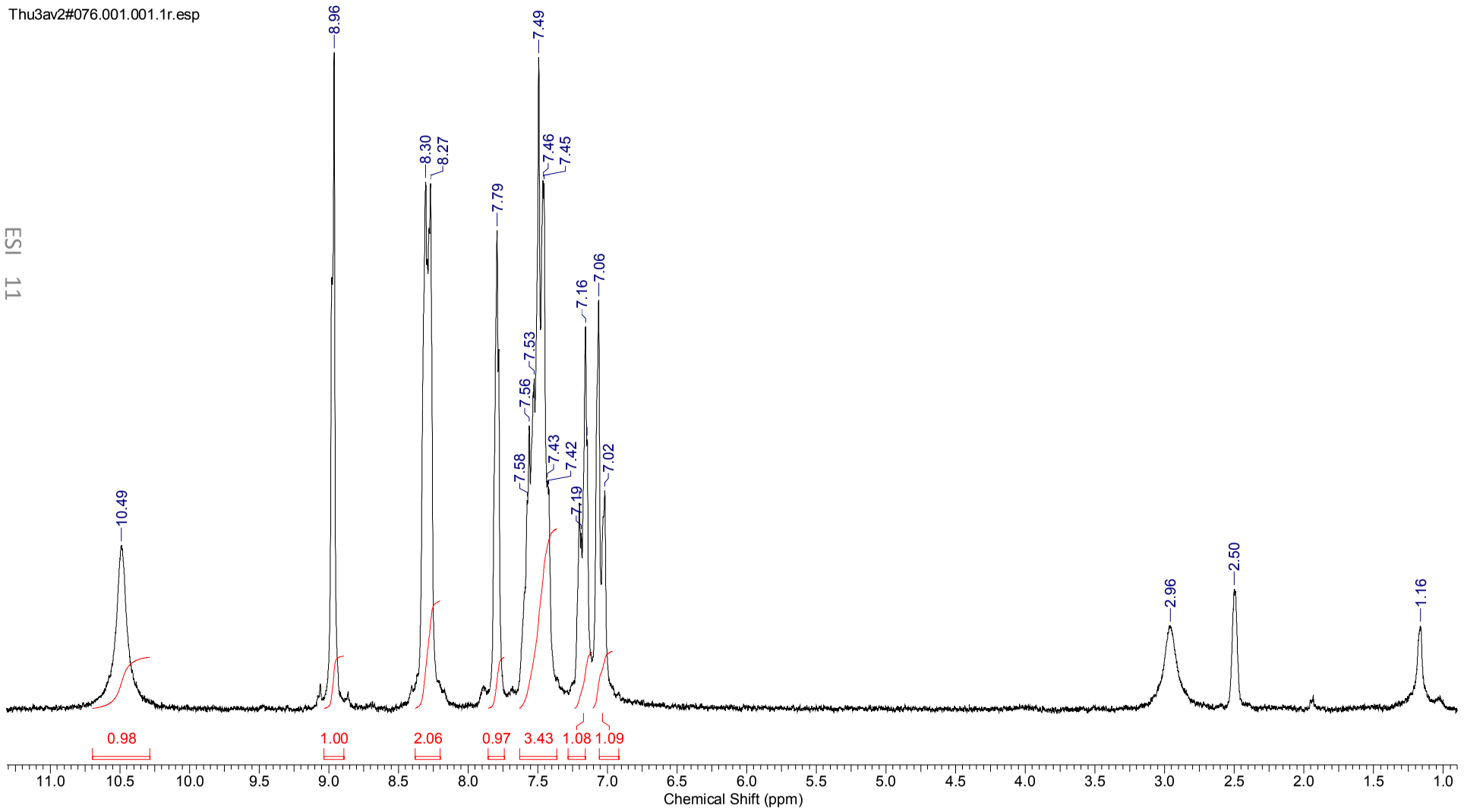
References:

1. B. C. Miao, I. Skidan, J. S. Yang, A. Lugovskoy, M. Reibarkh, K. Long, T. Brazell, K. A. Durugkar, J. Maki, C. V. Ramana, B. Schaffhausen, G. Wagner, V. Torchilin, J. Y. Yuan and A. Degterev, *Proc. Natl. Acad. Sci. U.S.A.*, 2010, **107**, 20126-20131.

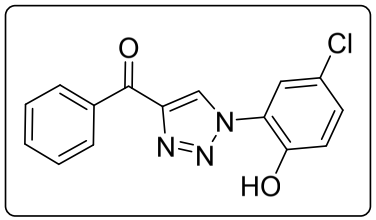
2. B. Miao, I. Skidan, J. Yang, Z. You, X. Fu, M. Famulok, B. Schaffhausen, V. Torchilin, J. Yuan and A. Degterev, *Oncogene*, 2012, **31**, 4317-4332.

1aa, 200 MHz, $CDCl_3$ + DMSO (D_6)



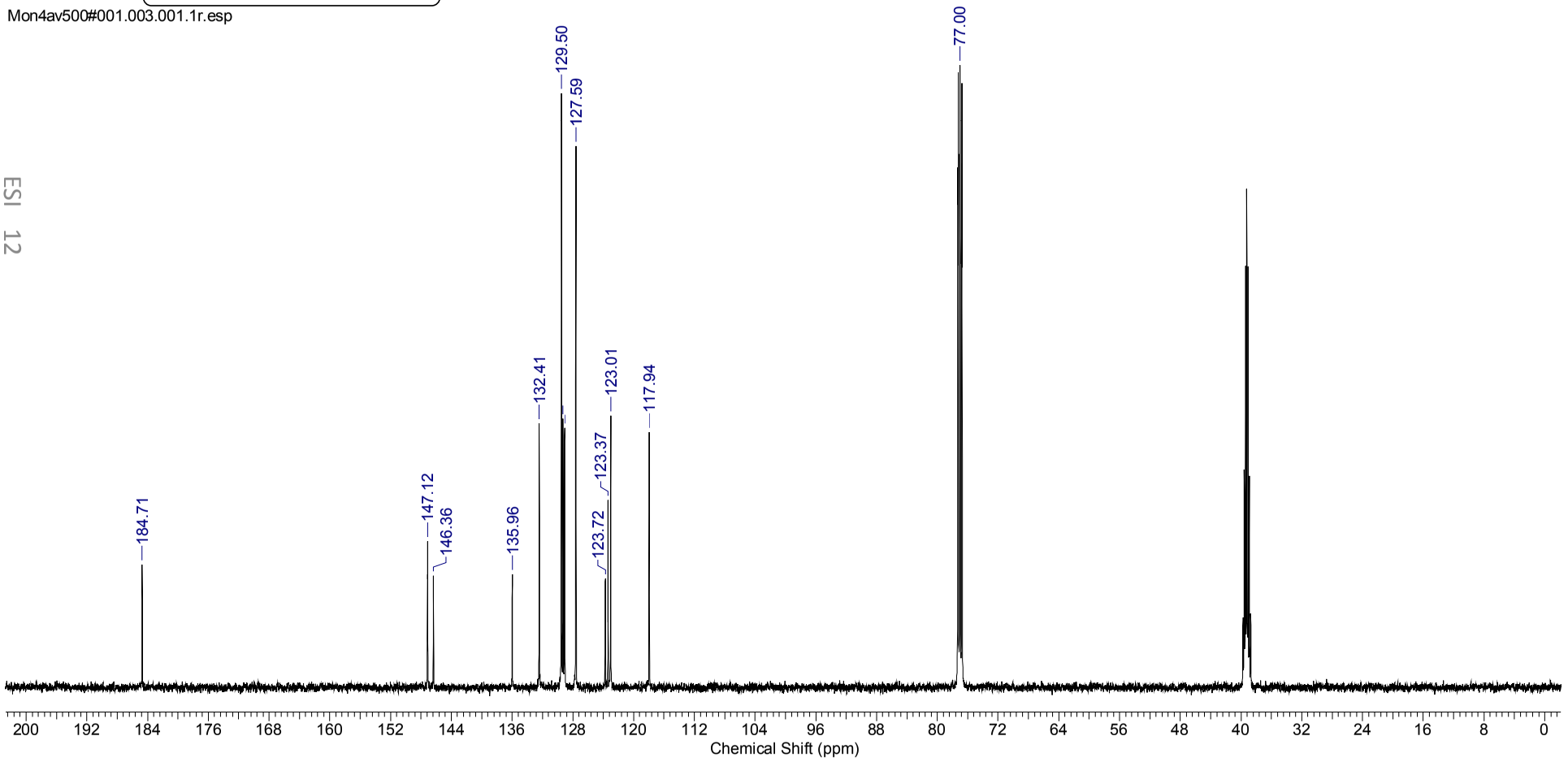


1aa, 125 MHz, $CDCl_3$ + DMSO (D_6)

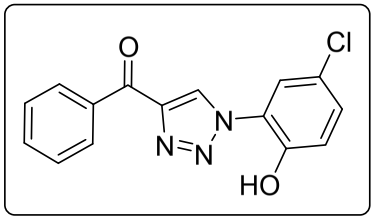


Mon4av500#001.003.001.1r.esp

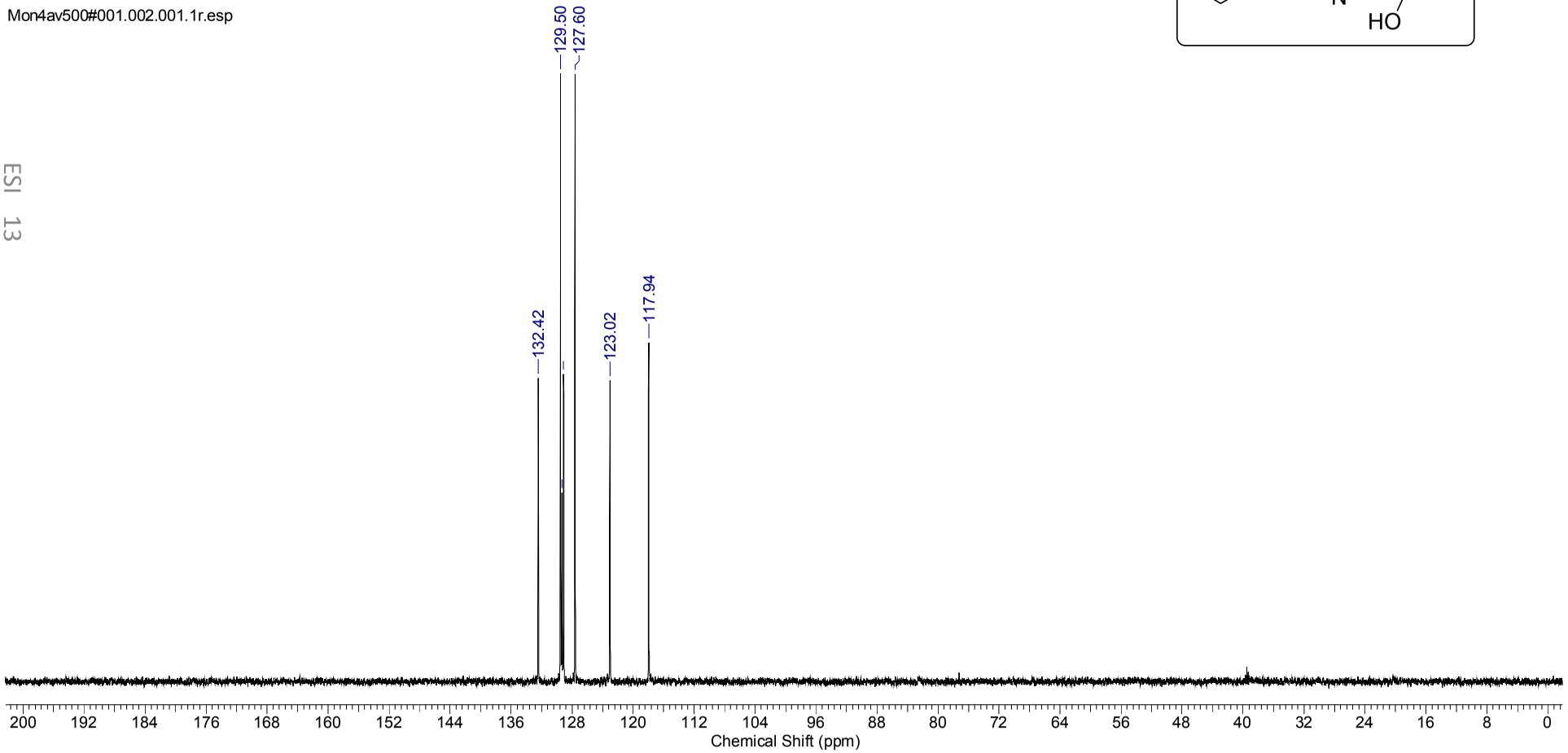
ESI 12



1aa, 125 MHz, $CDCl_3$ + DMSO (D_6)



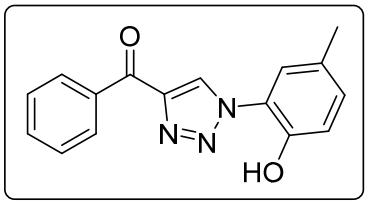
Mon4av500#001.002.001.1r.esp



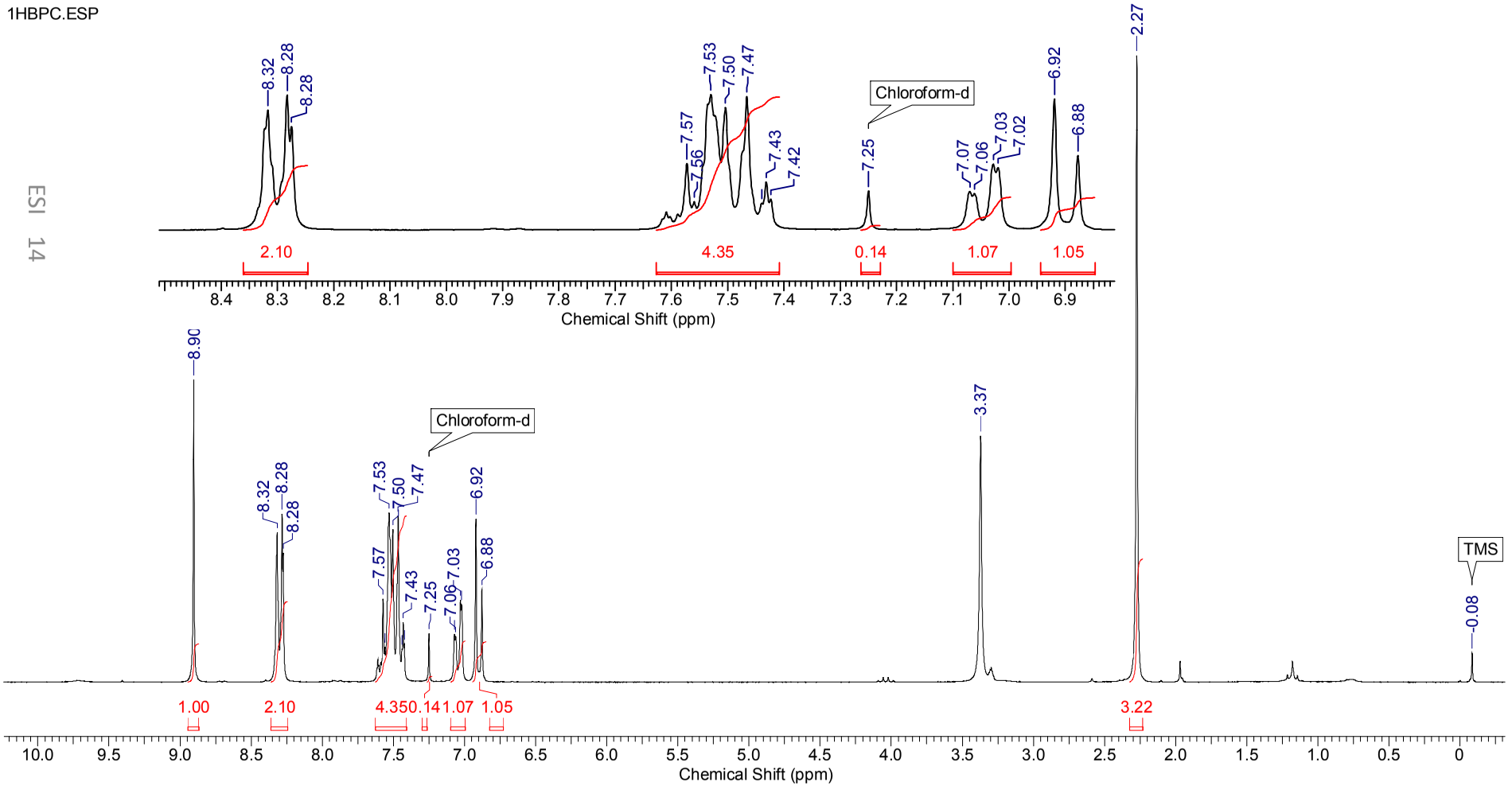
ESI 13

1HBPC.ESP

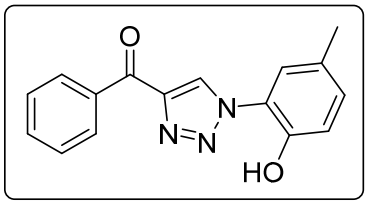
1ab, 200 MHz, $CDCl_3$ + MeOH (D_4)

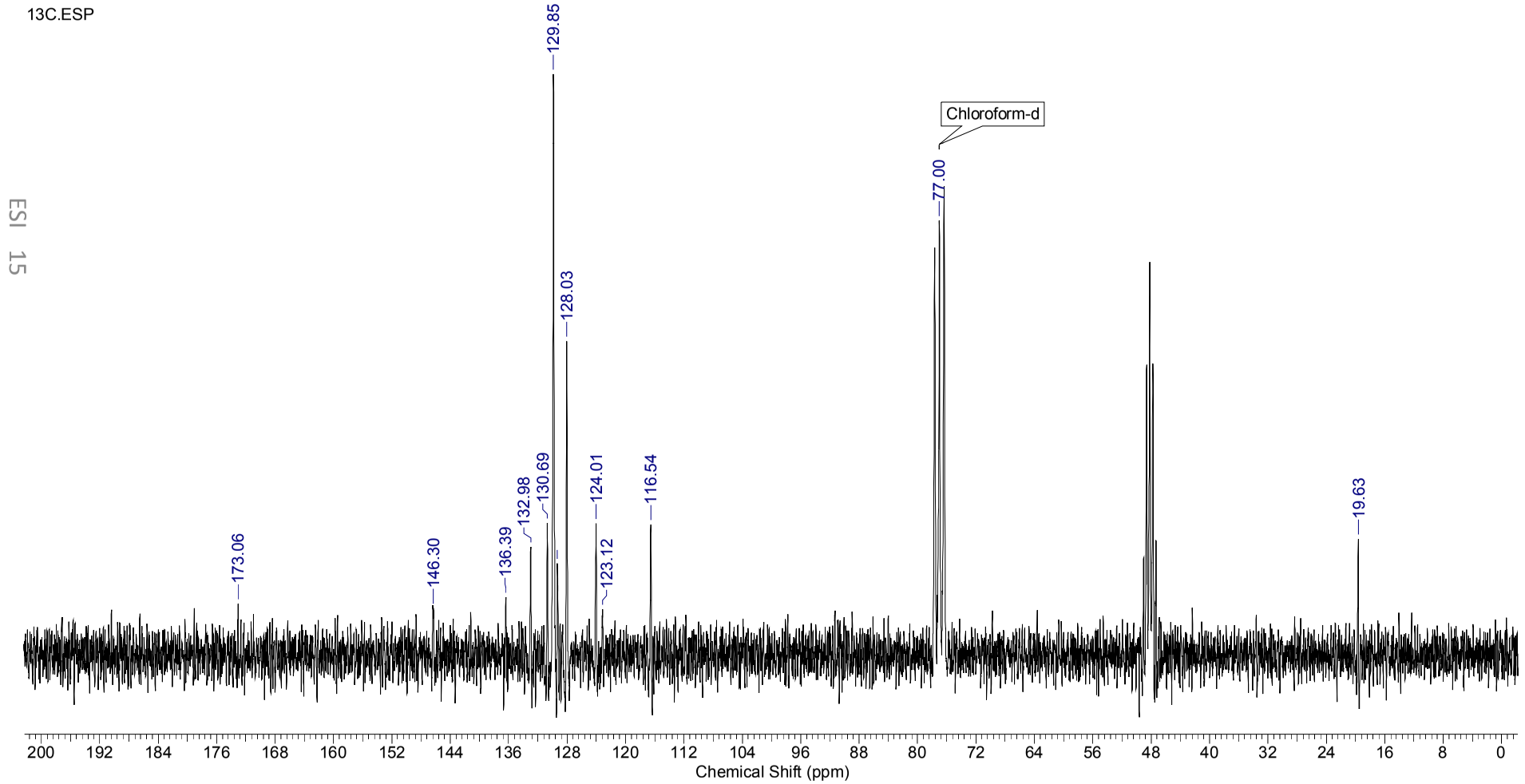


1HBPC.ESP

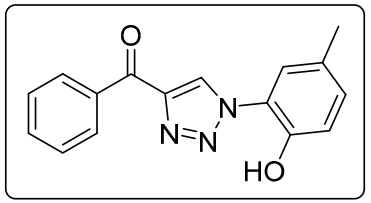


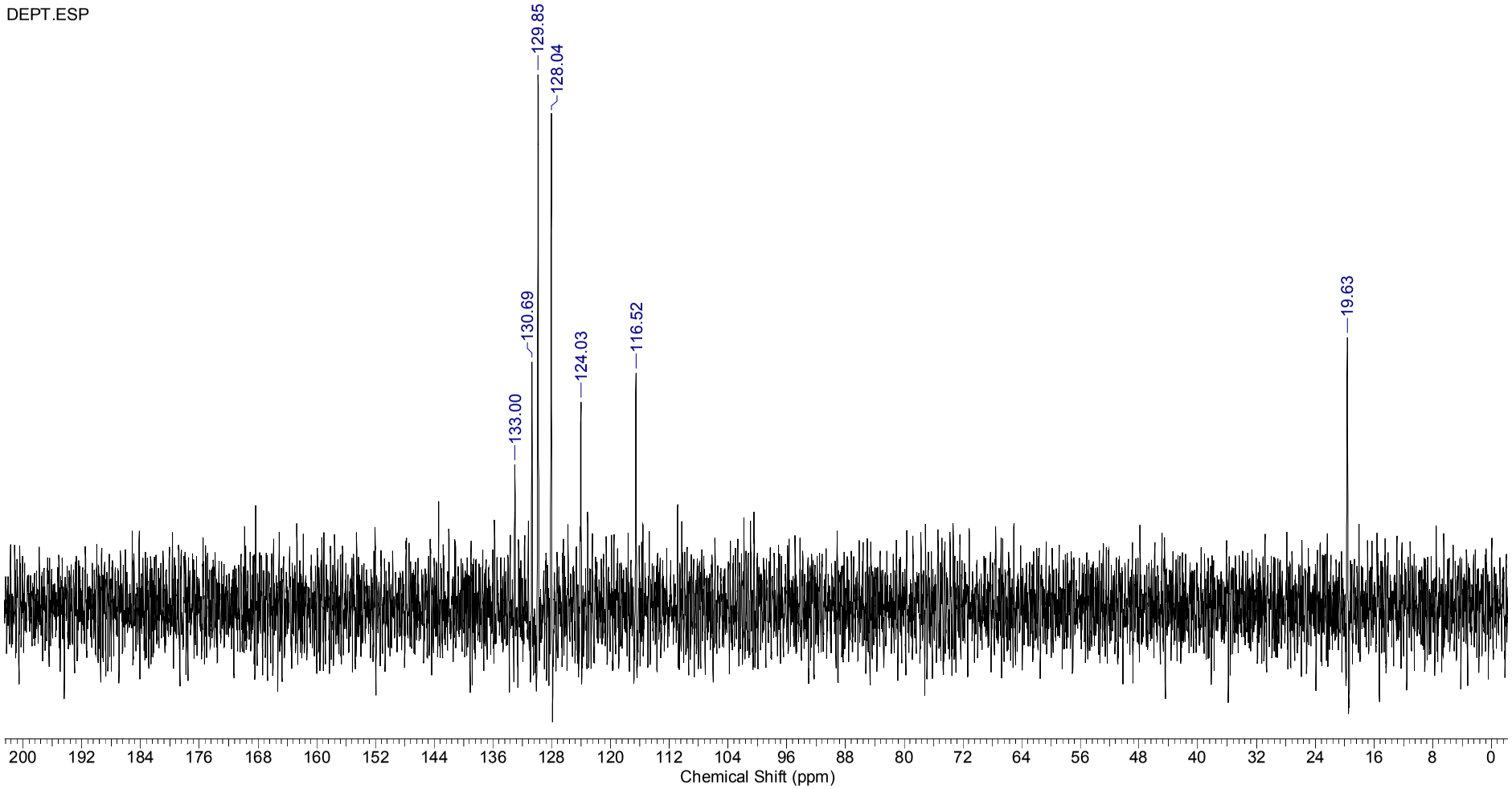
1ab, 50 MHz, $CDCl_3$ + MeOH (D_4)



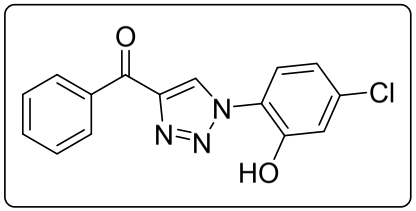


1ab, 50 MHz, $CDCl_3$ + MeOH (D_4)





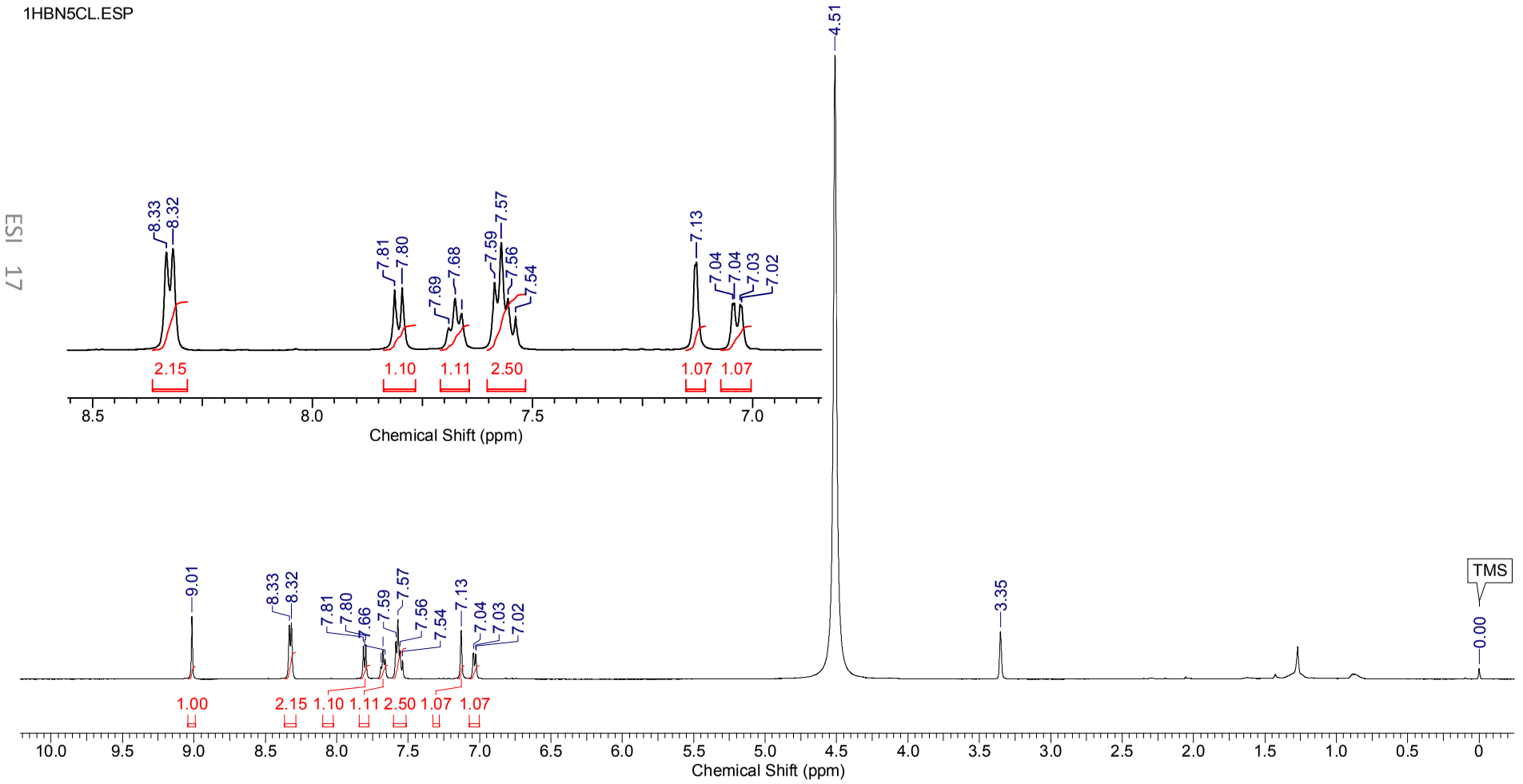
1ac, 500 MHz, $CDCl_3$ + MeOH (D_4)



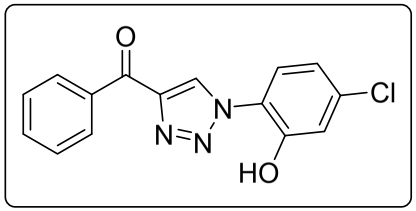
1HBN5CL.ESP

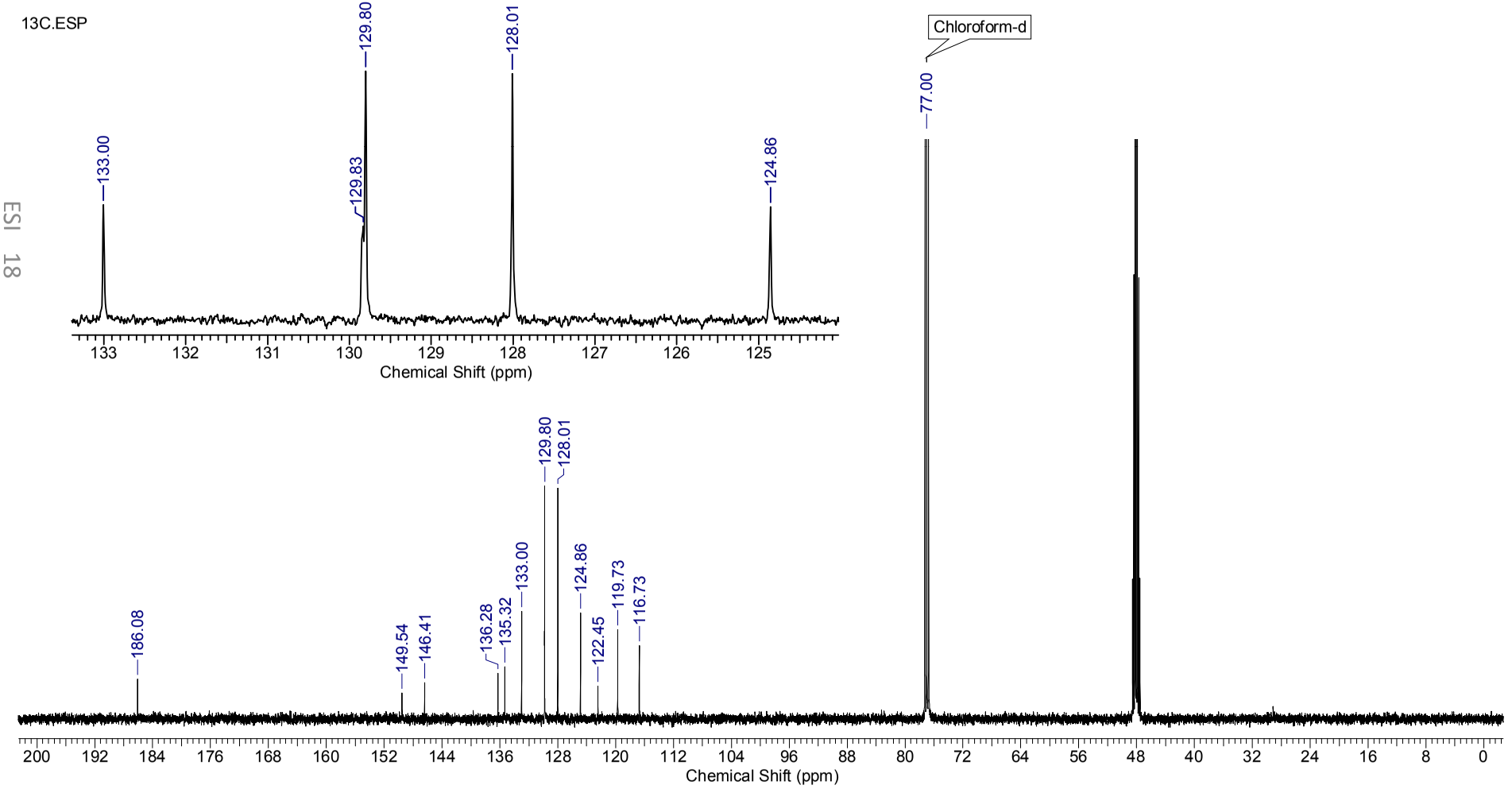
1HBN5CL.ESP

ESI 17

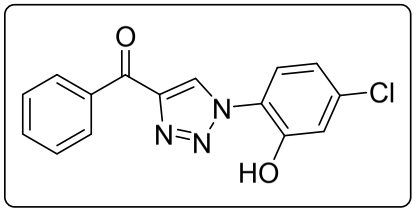


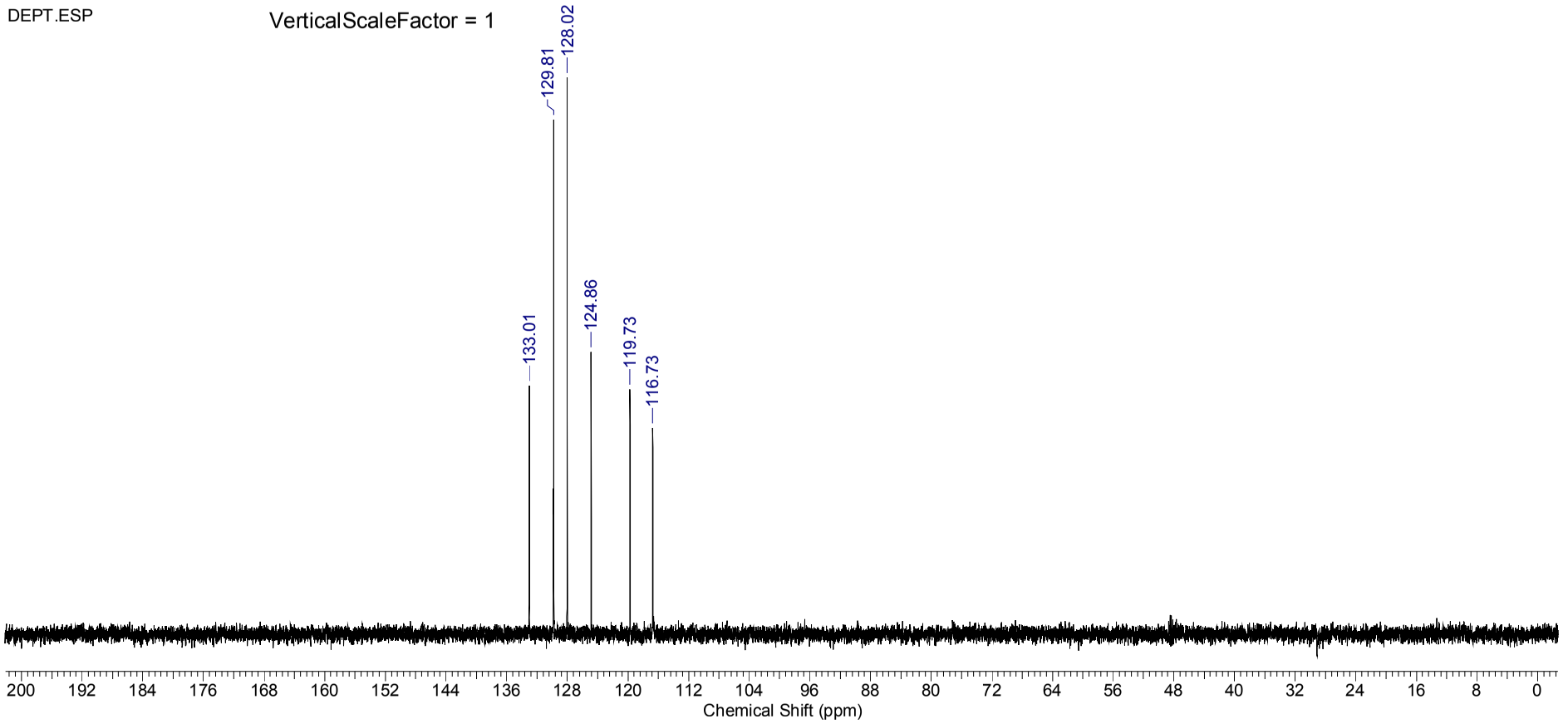
1ac, 125 MHz, $CDCl_3$ + MeOH (D_4)





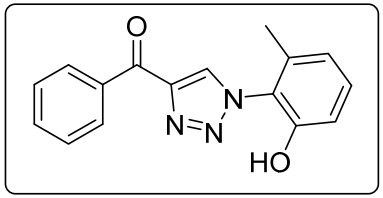
1ac, 125 MHz, $CDCl_3$ + MeOH (D_4)

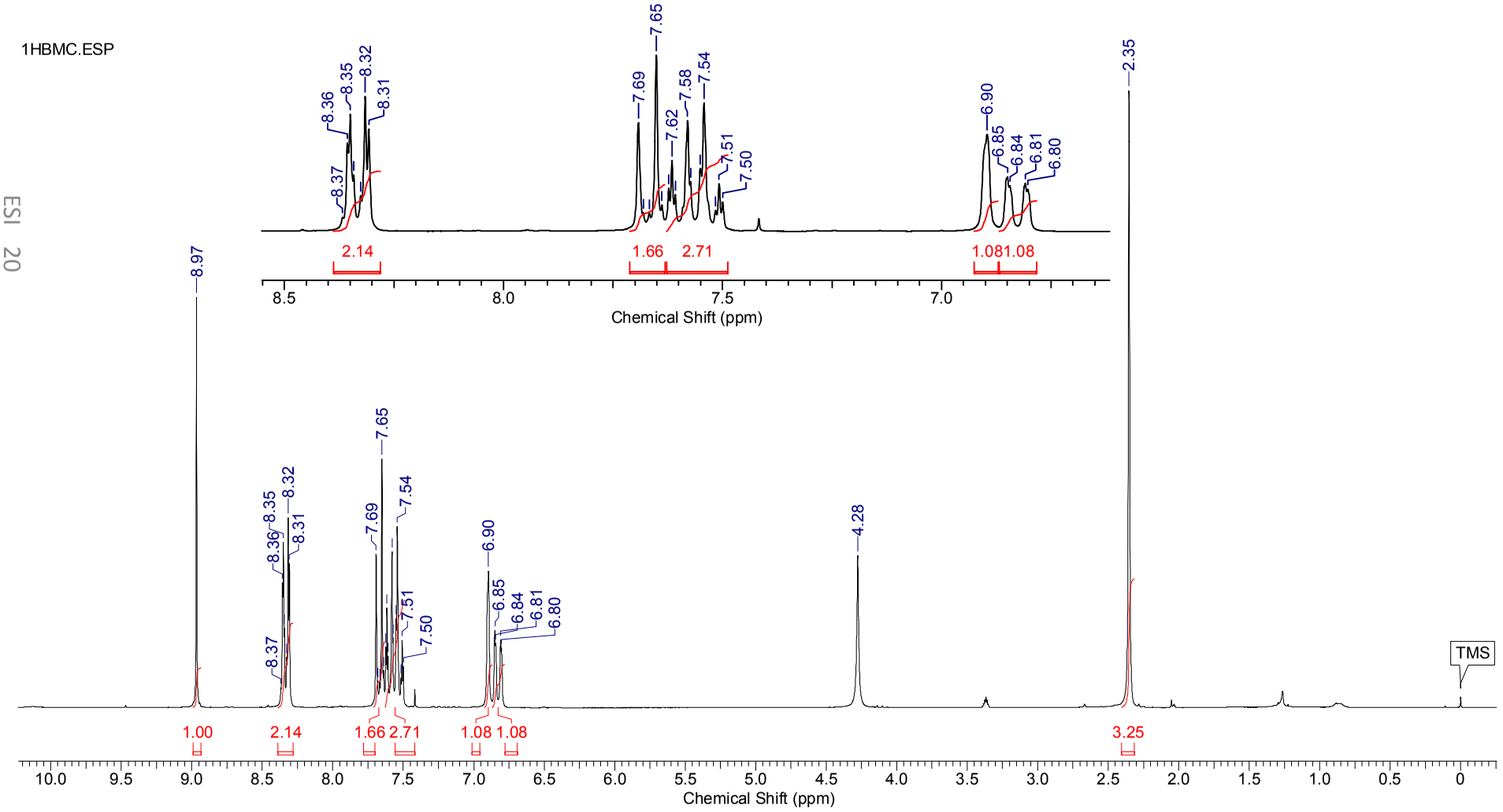


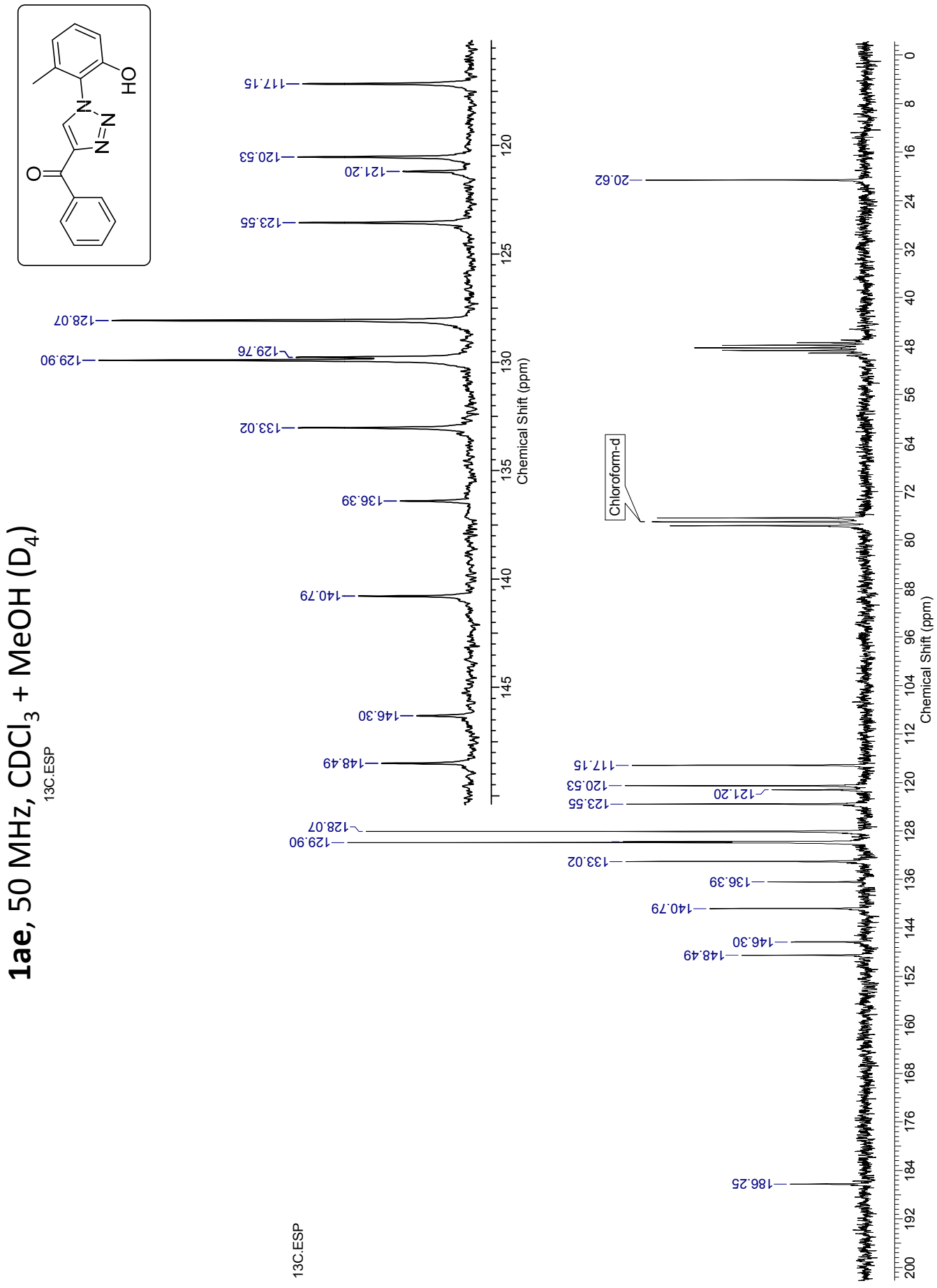


1HBMC.ESP

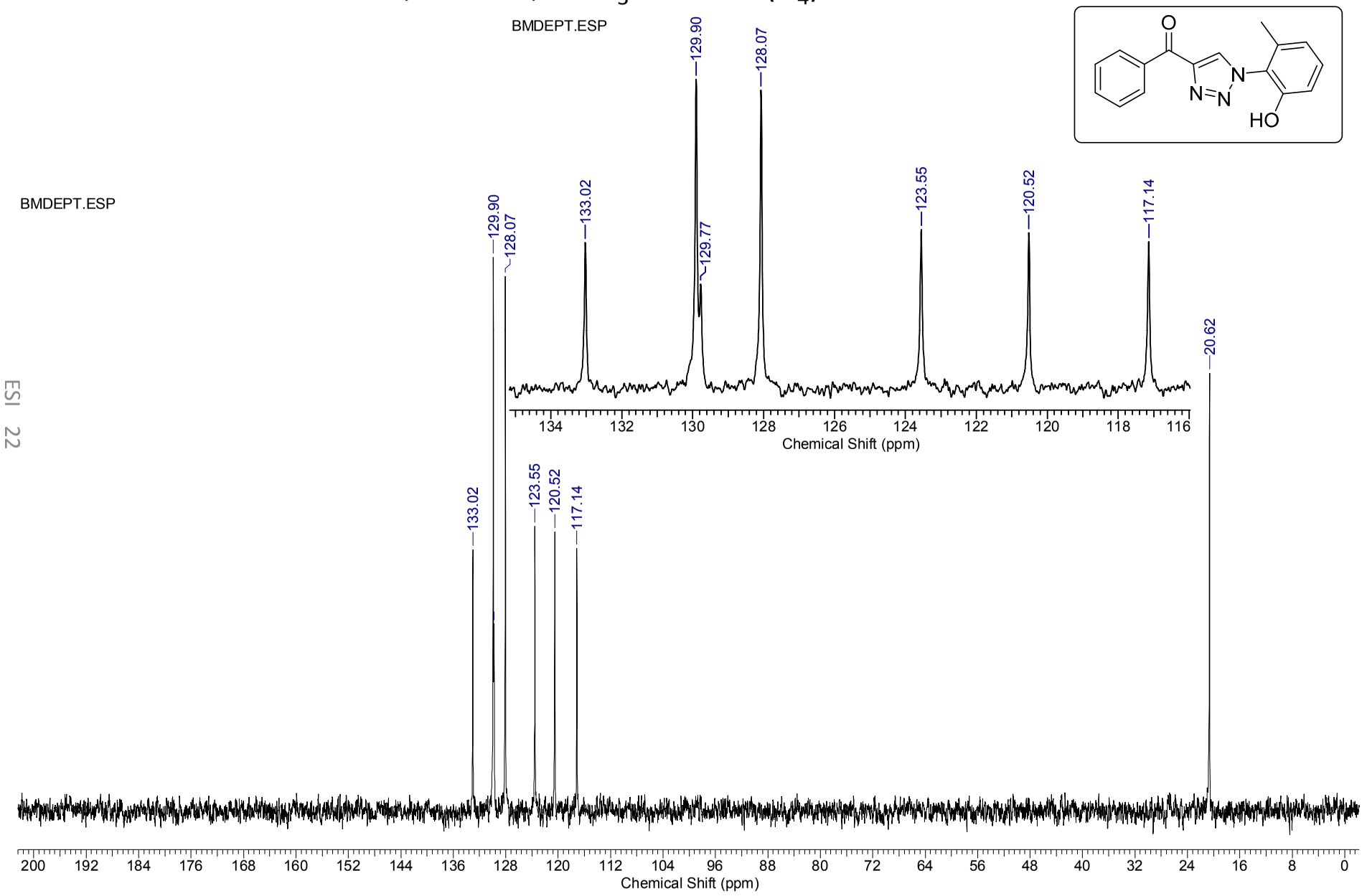
1ae, 200 MHz, $CDCl_3$ + MeOH (D_4)





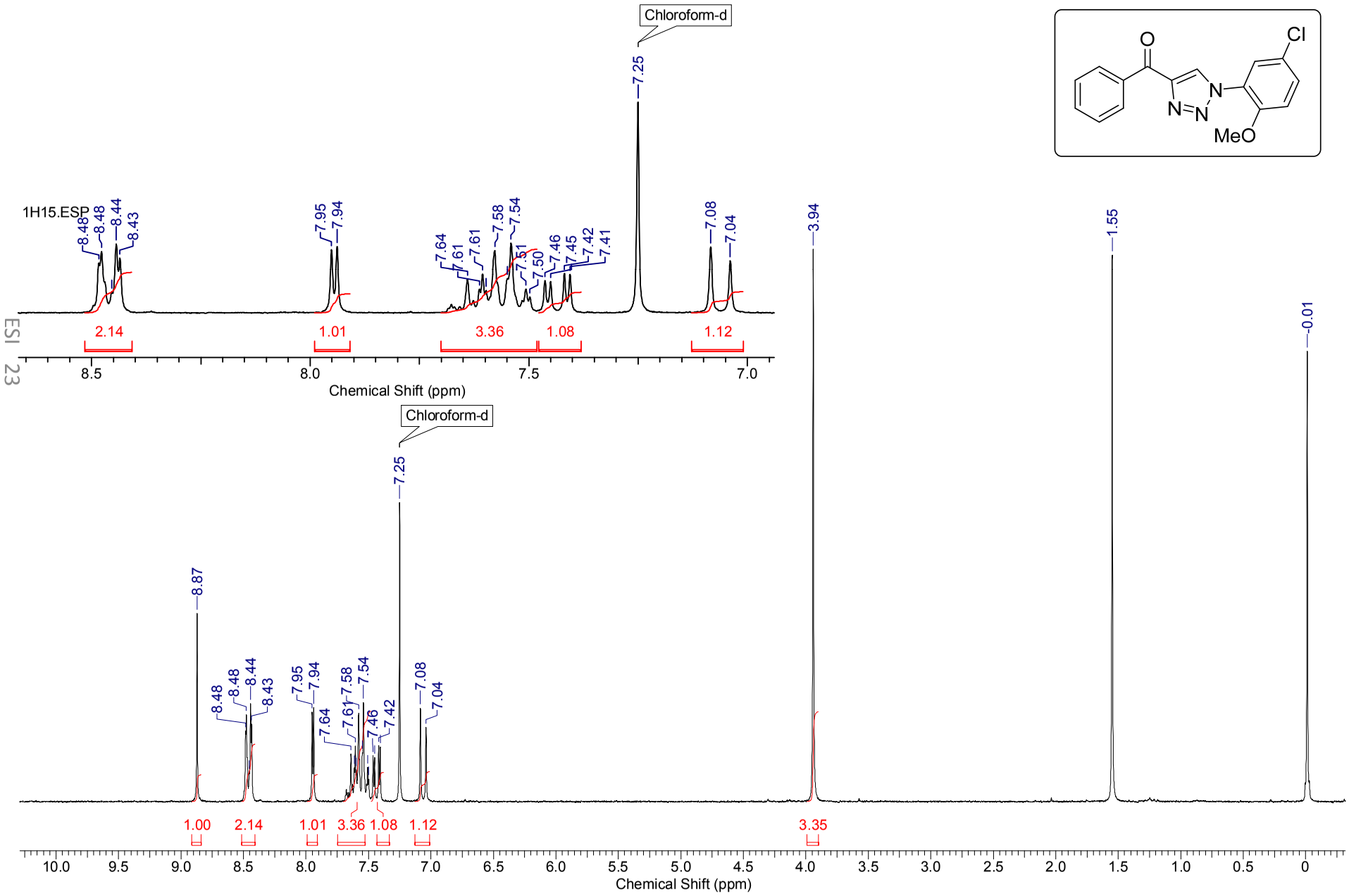

1ae, 50 MHz, CDCl3 + MeOH (D4)
13C.ESP
HO
O
N
N
117.15
120.53
121.20
123.55
128.07
129.76
129.90
133.02
136.39
140.79
146.30
148.49
20.62
Chloroform-d
186.25
Chemical Shift (ppm)
13C.ESP

1ae, 50 MHz, $CDCl_3$ + MeOH (D_4)

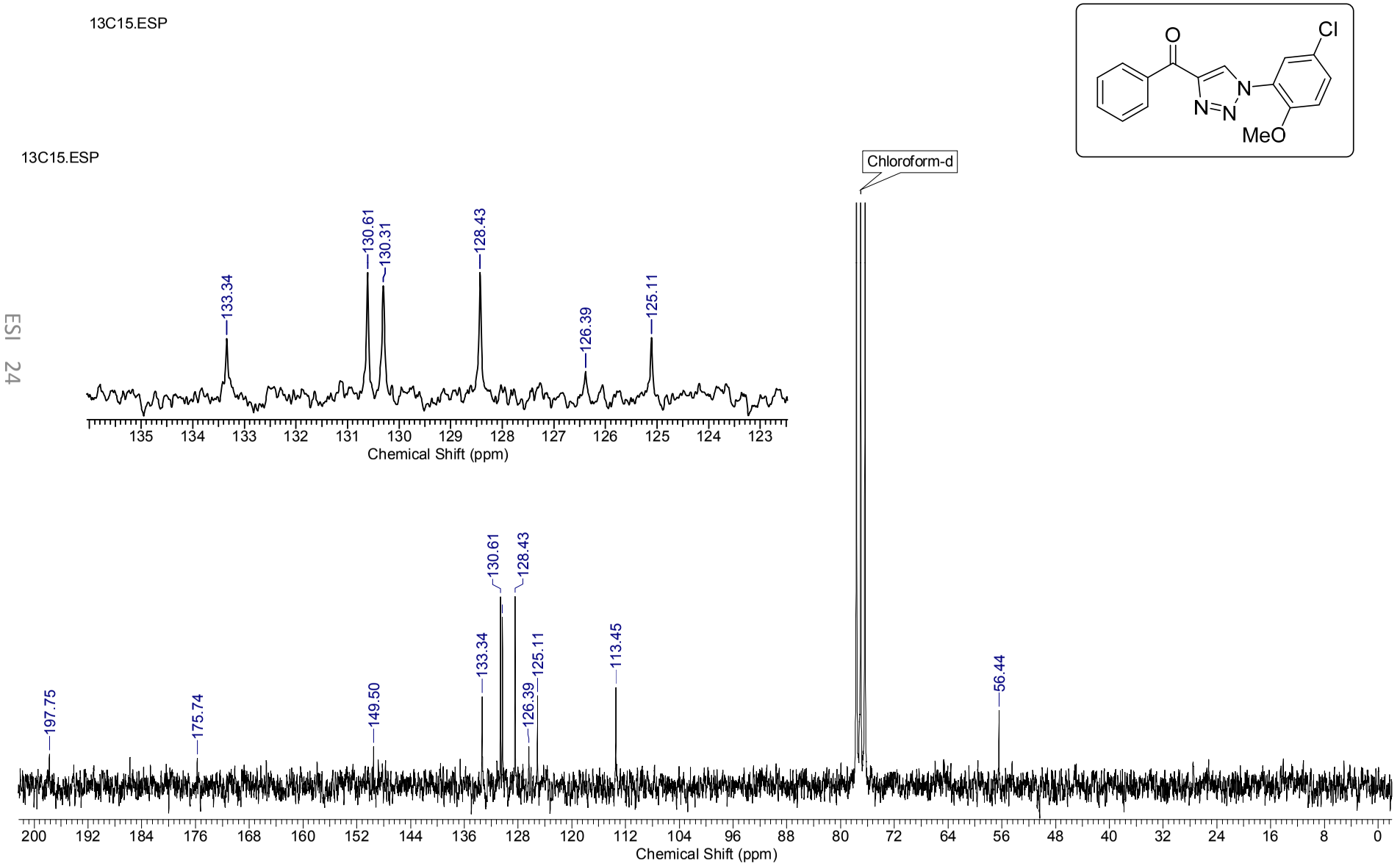


1H15.ESP

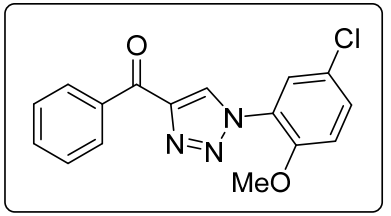
1af, 200 MHz, $CDCl_3$

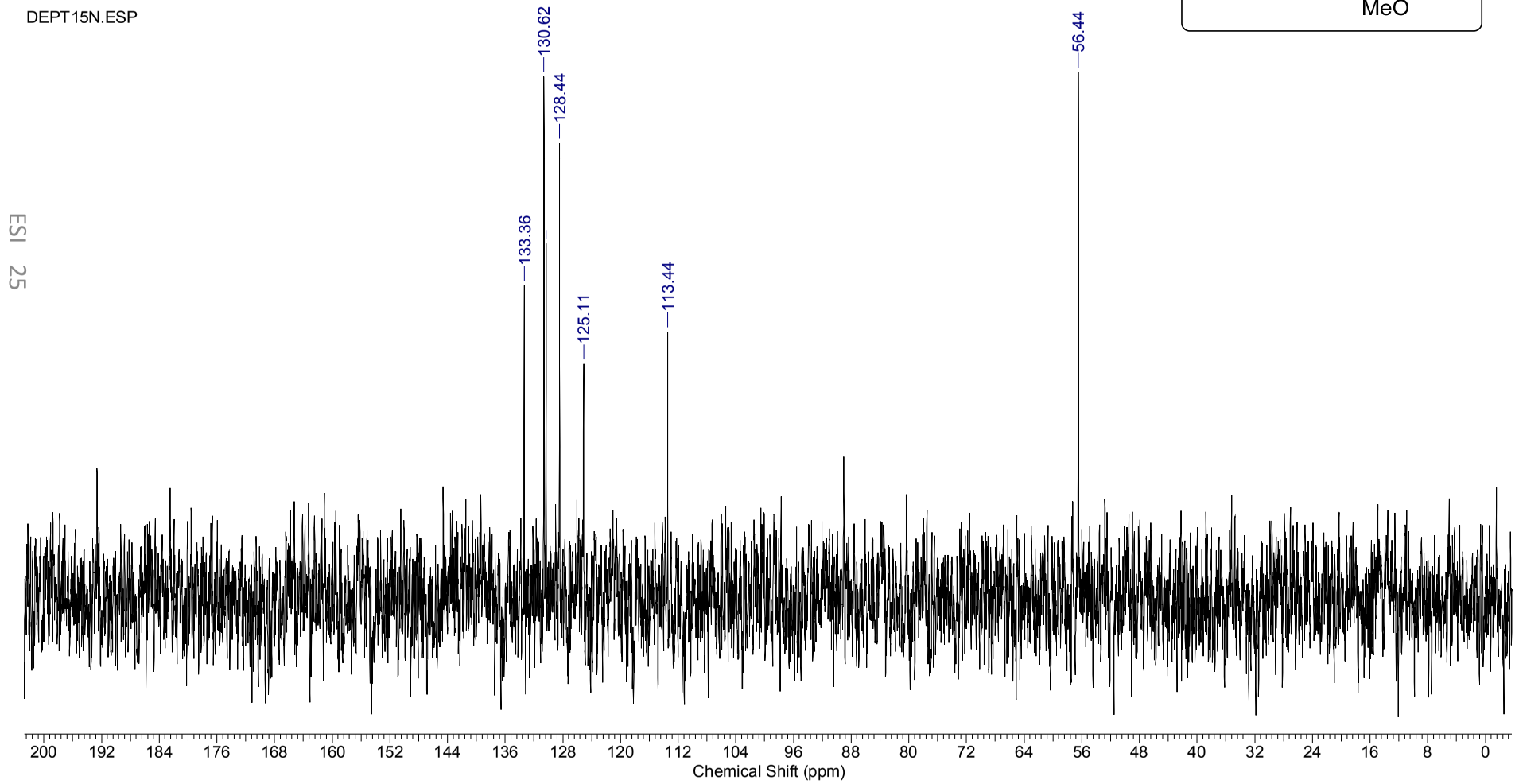


1af, 50 MHz, $CDCl_3$

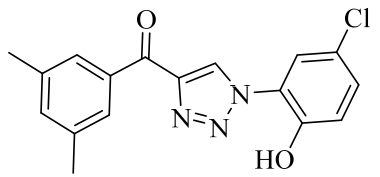


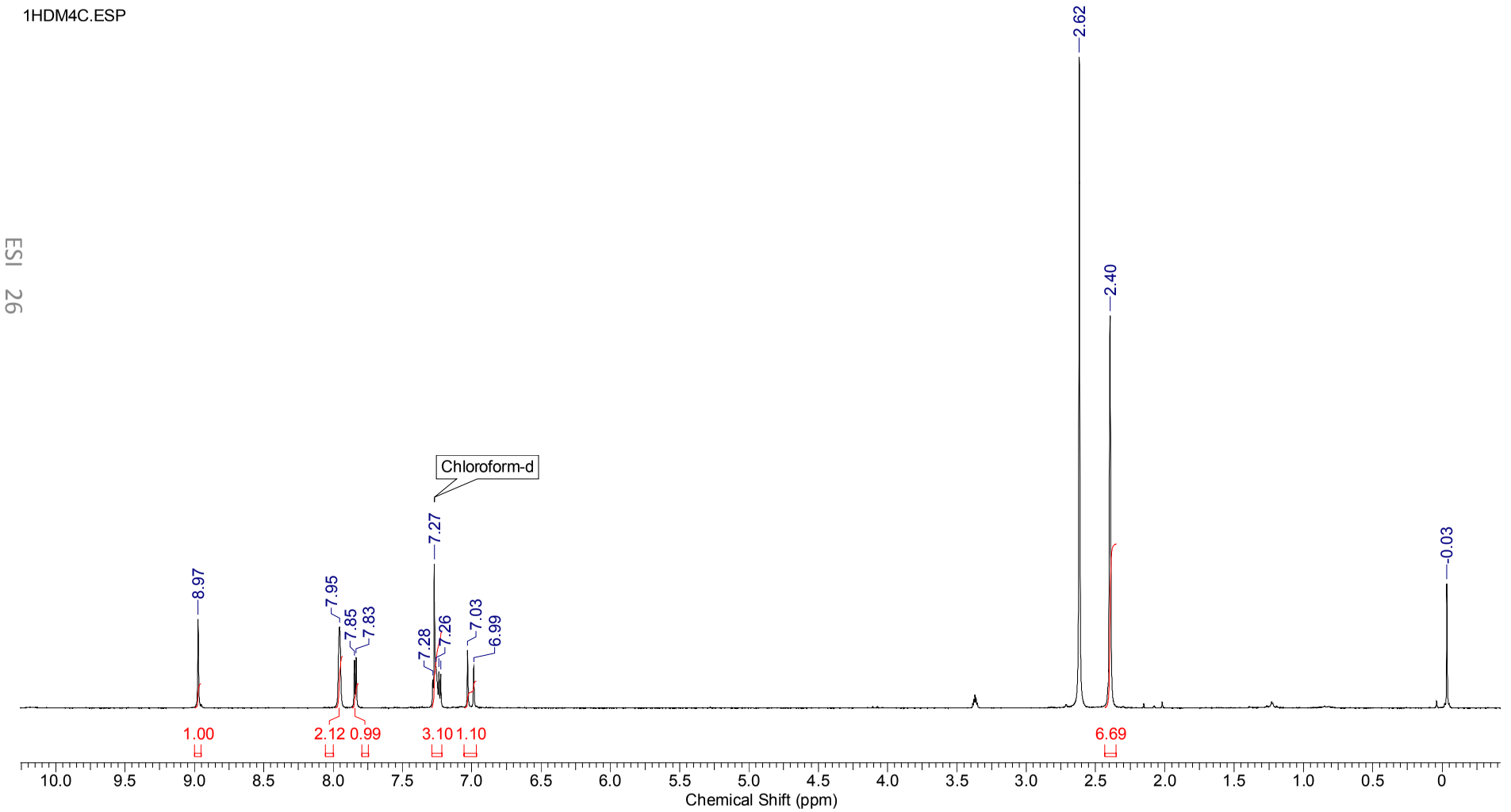
1af, 50 MHz, $CDCl_3$



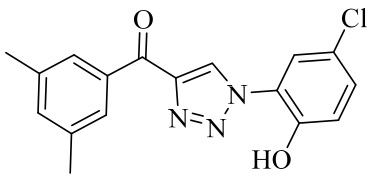


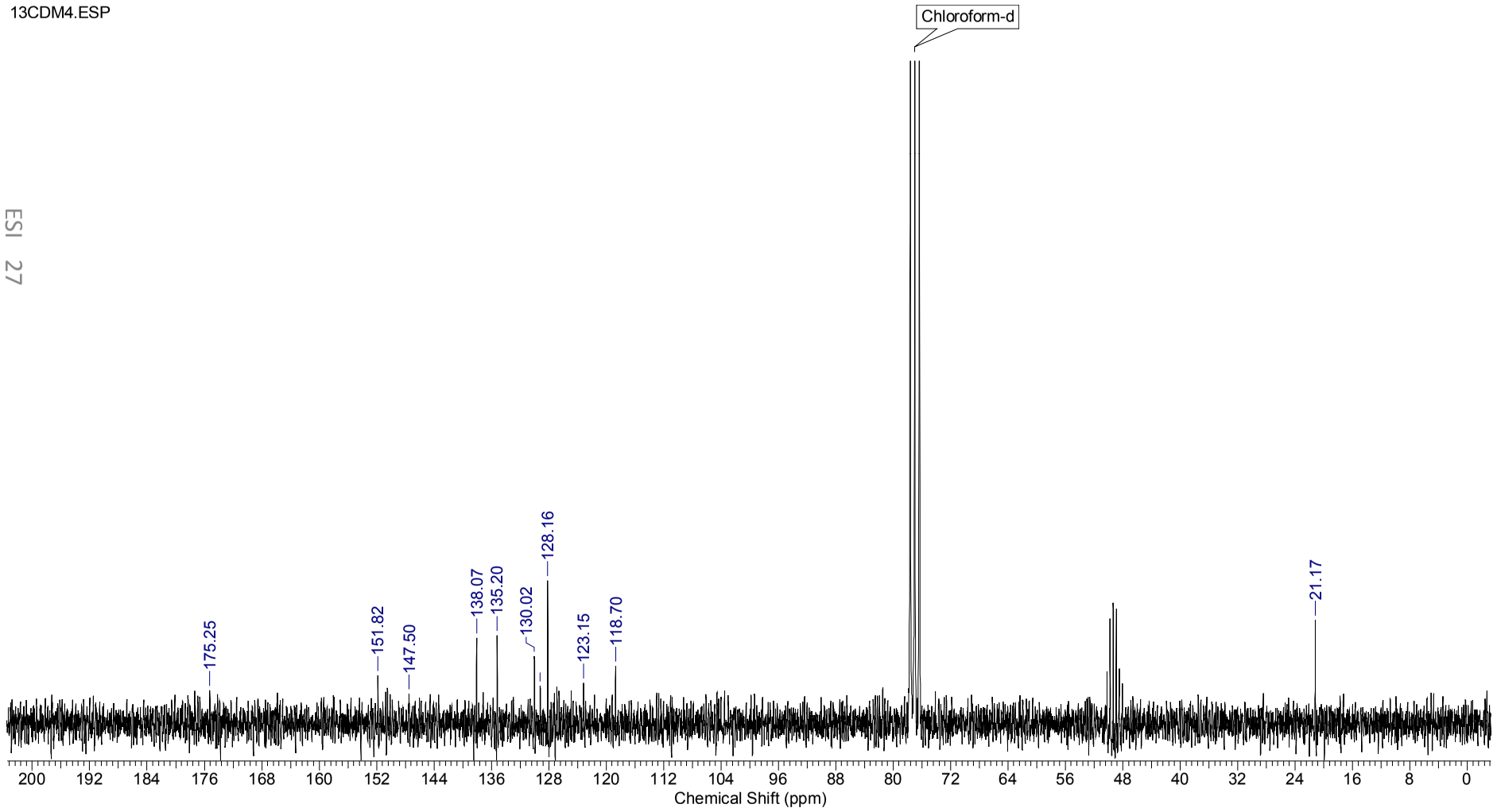
1ba, 200 MHz, $CDCl_3$ + DMSO (D_6)





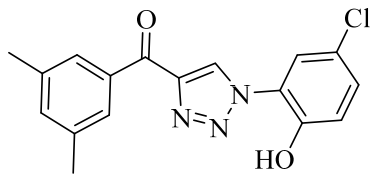
1ba, 50 MHz, $CDCl_3$ + DMSO (D_6)





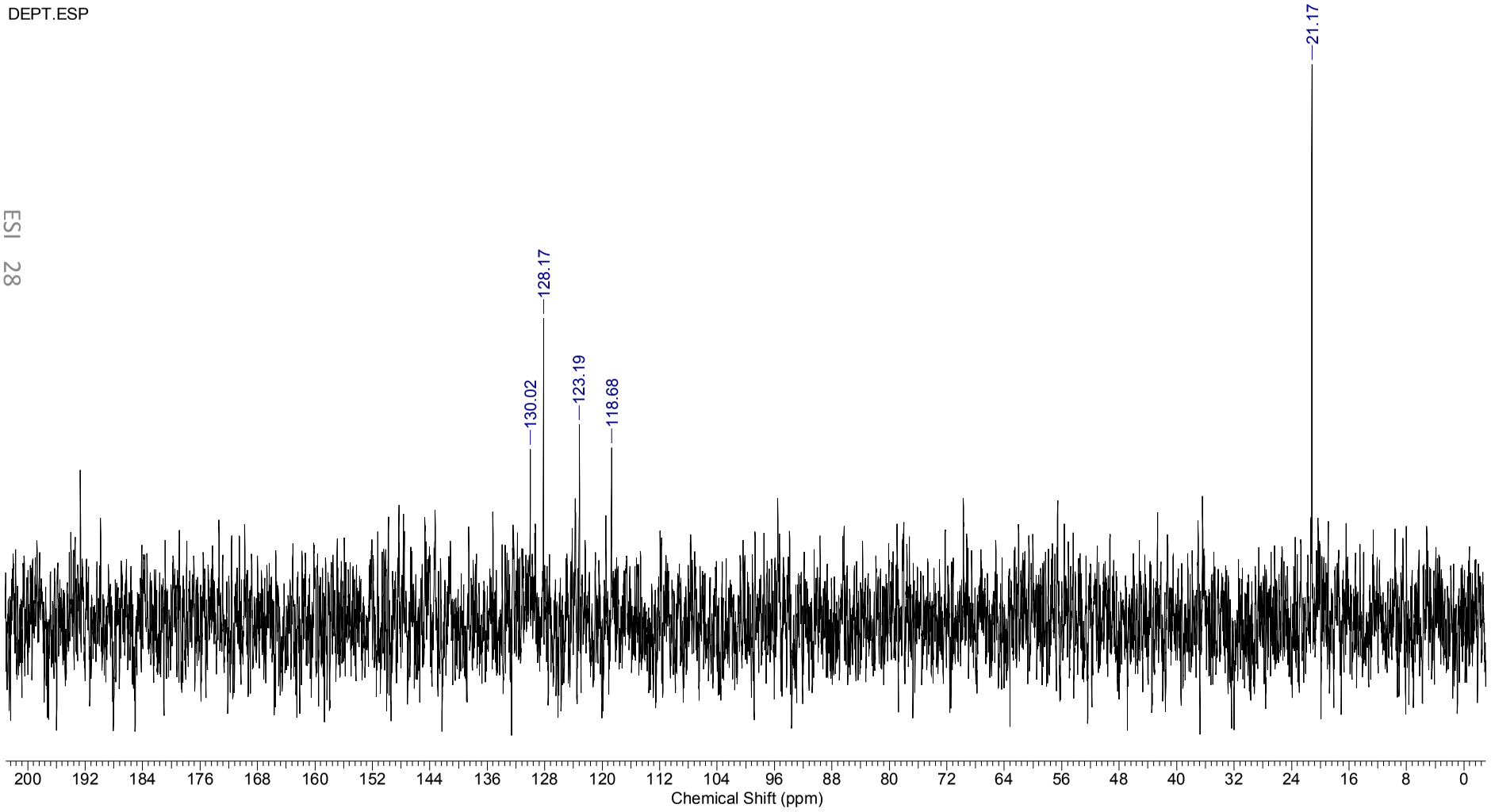
ESI 27

1ba, 50 MHz, $CDCl_3$ + DMSO (D_6)

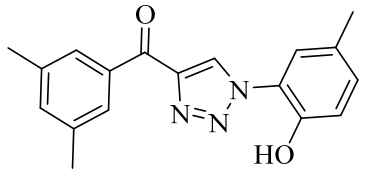


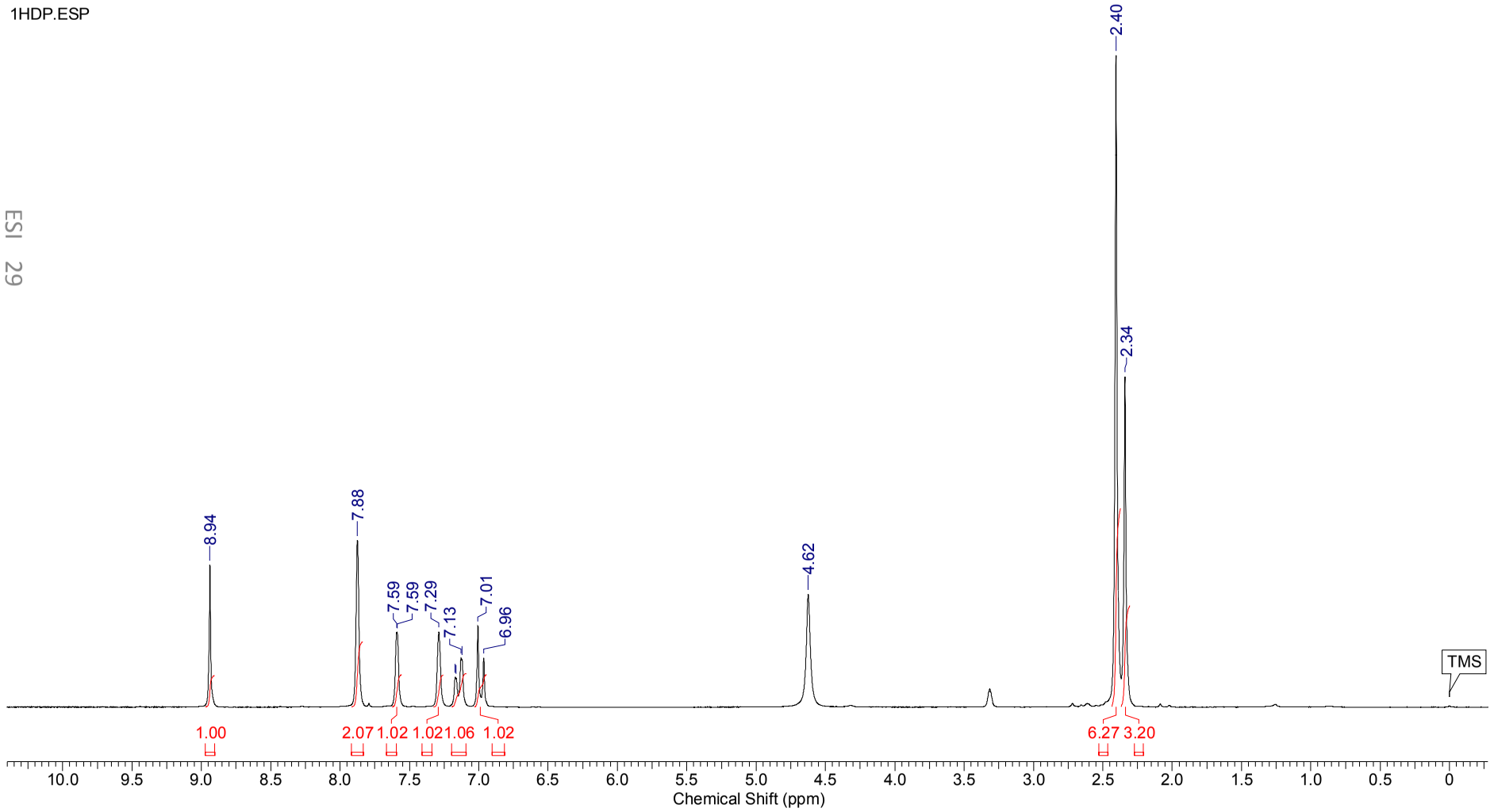
DEPT.ESP

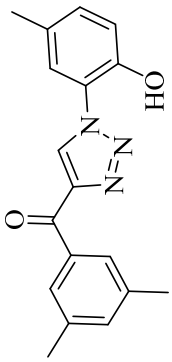
ESI 28



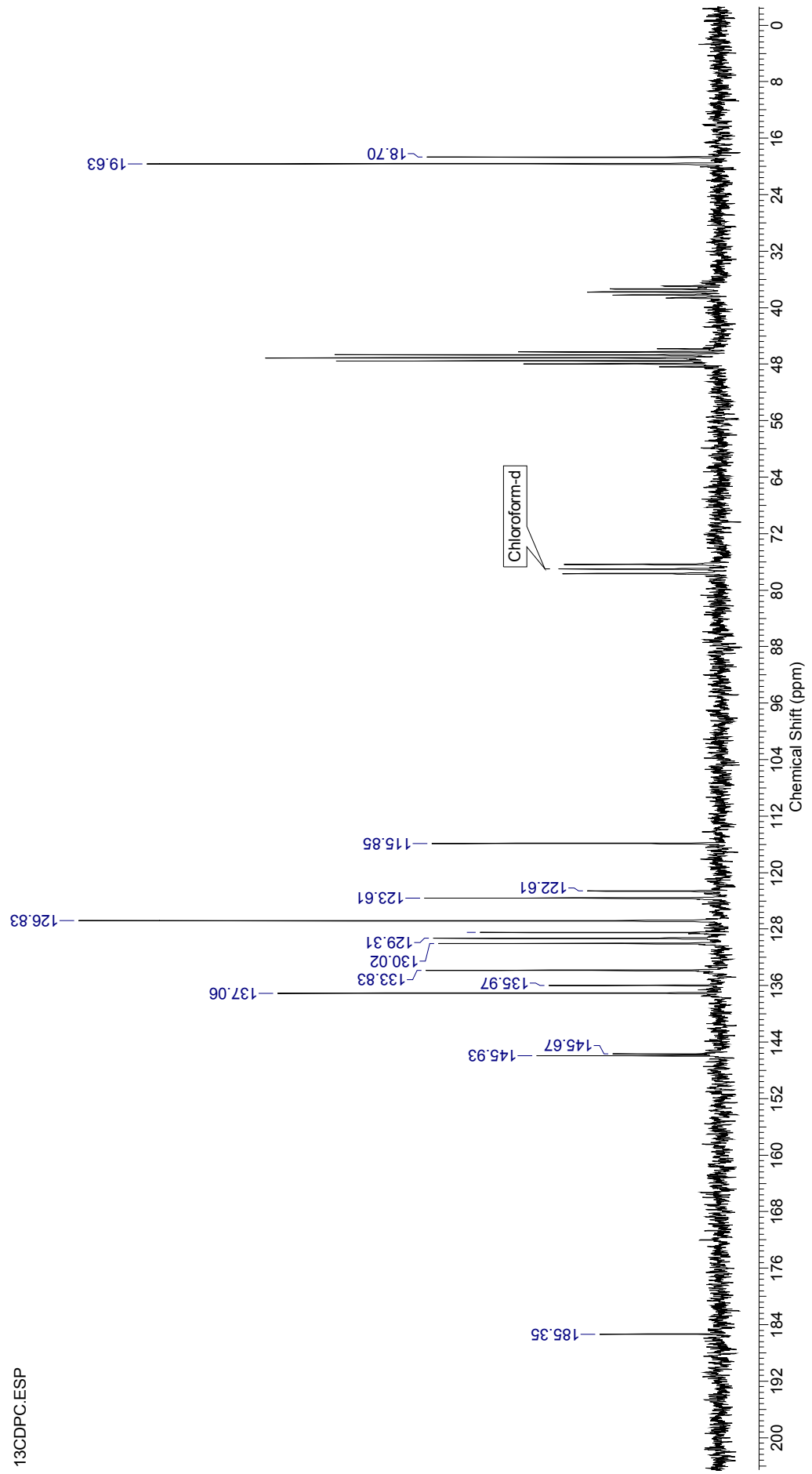
1bb, 200 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)



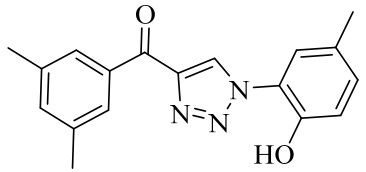


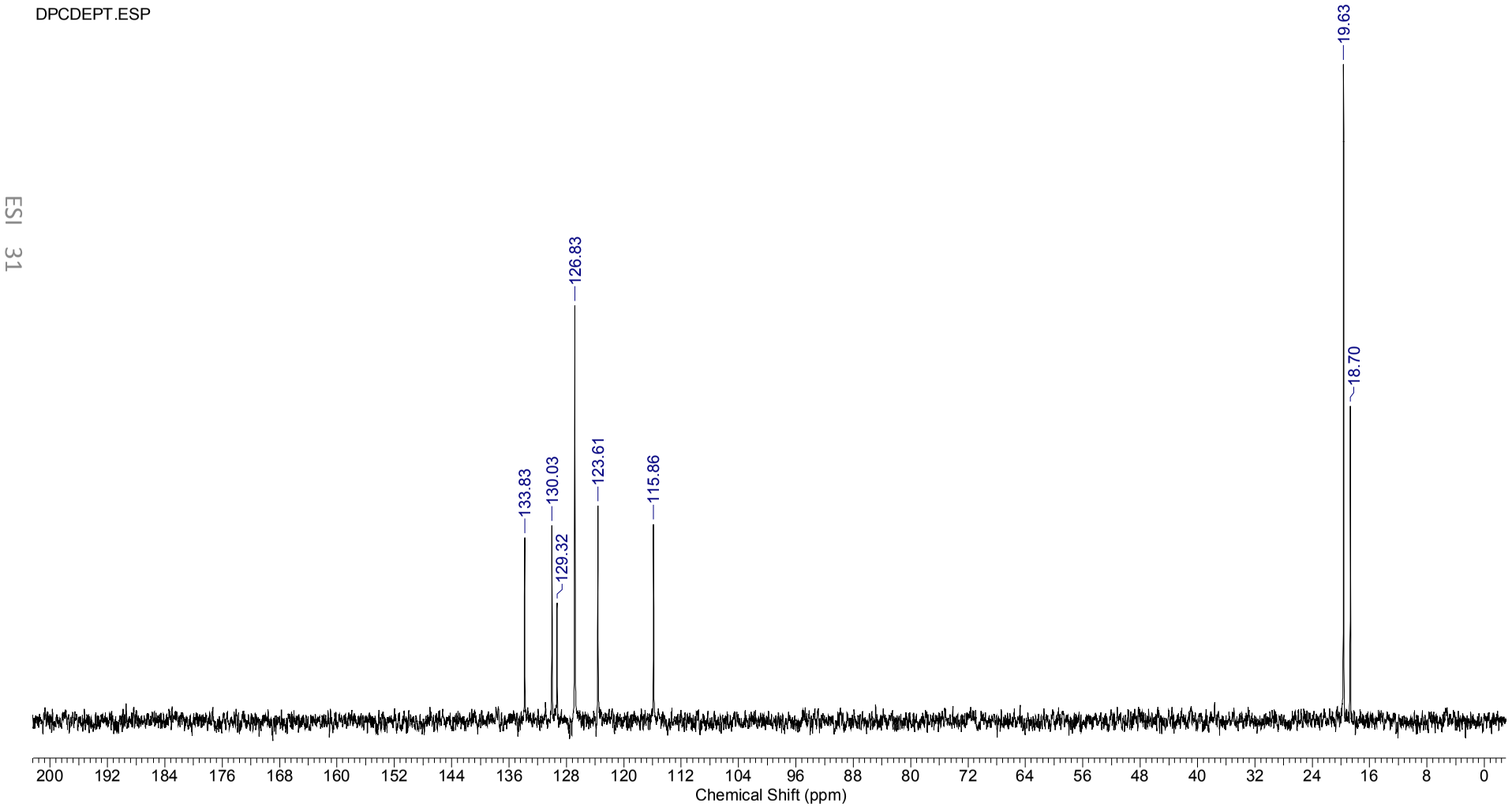


1bb, 50 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)



1bb, 50 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)

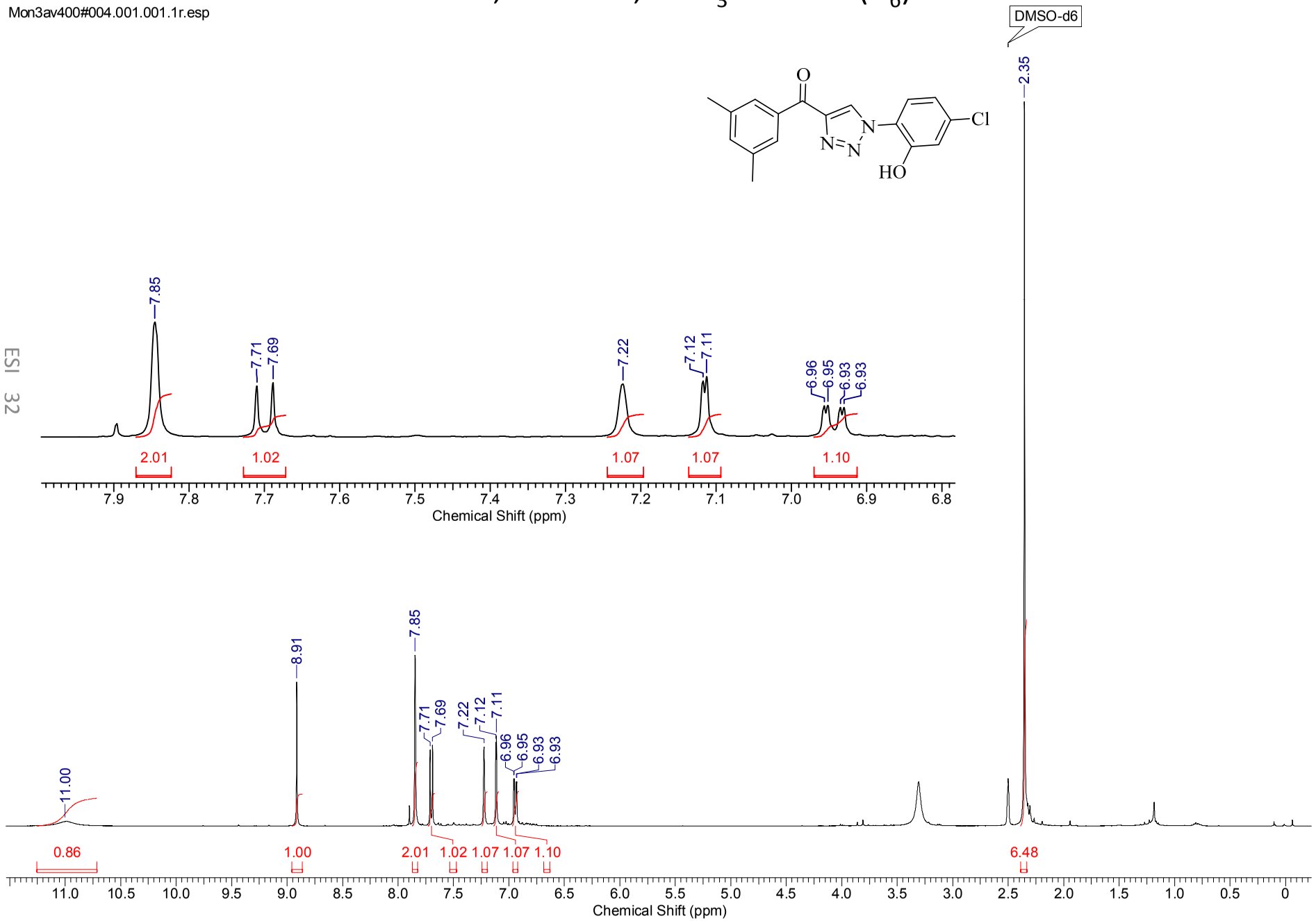




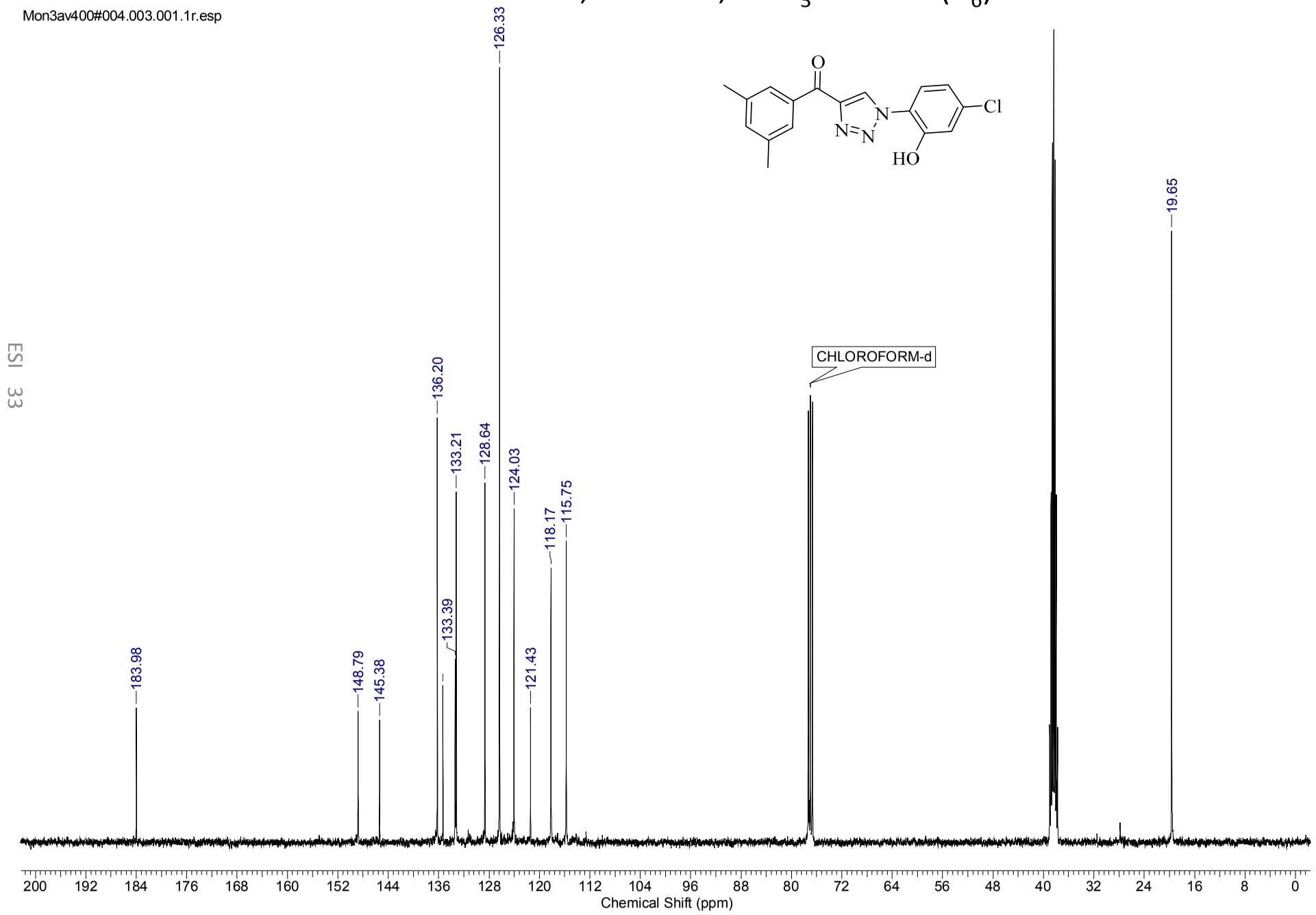
Mon3av400#004.001.001.1r.esp

1bc, 400 MHz, $CDCl_3$ + DMSO (D_6)

Mon3av400#004.001.001.1r.esp

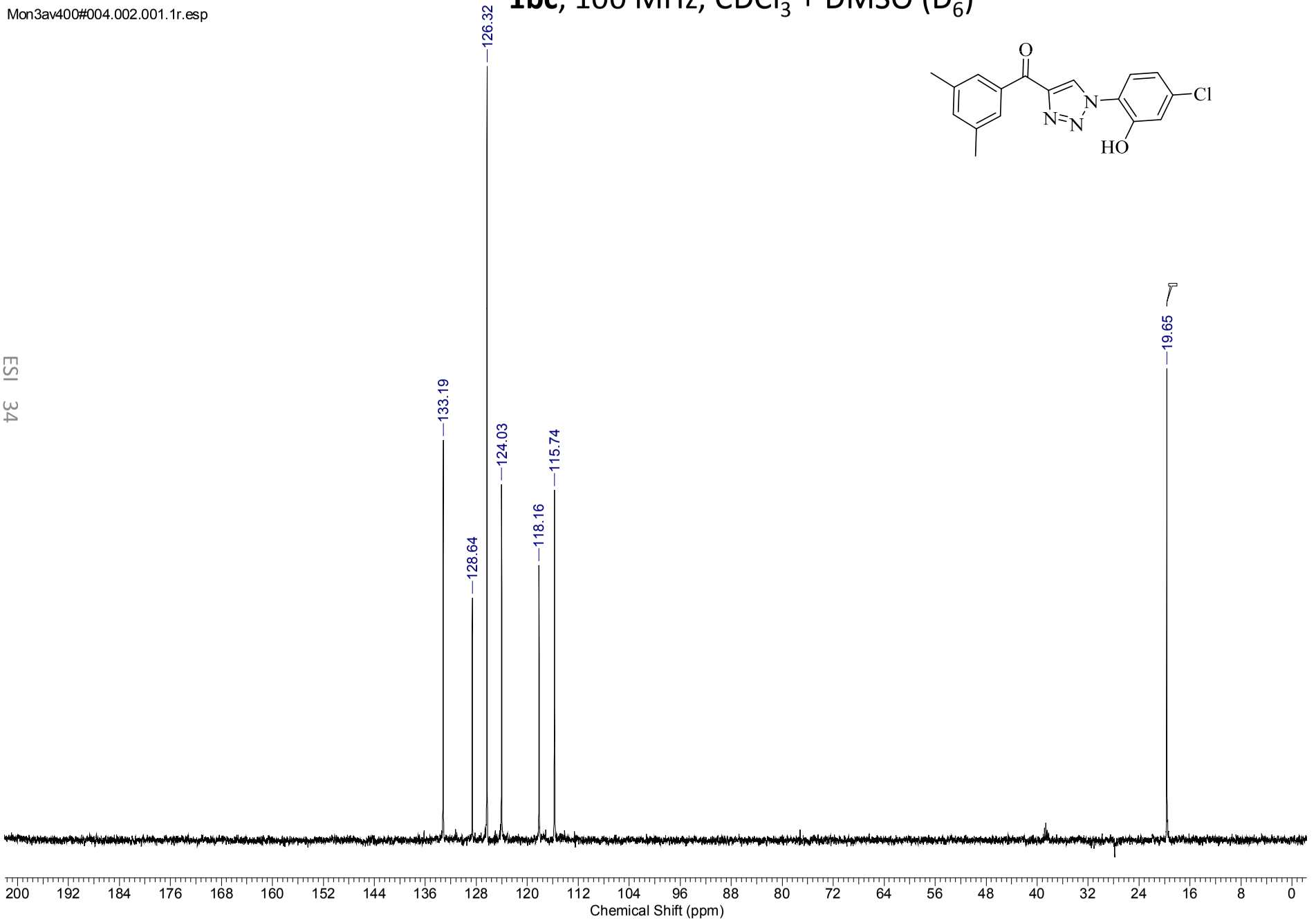


1bc, 100 MHz, $CDCl_3$ + DMSO (D_6)

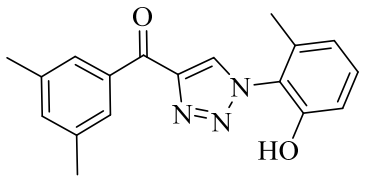


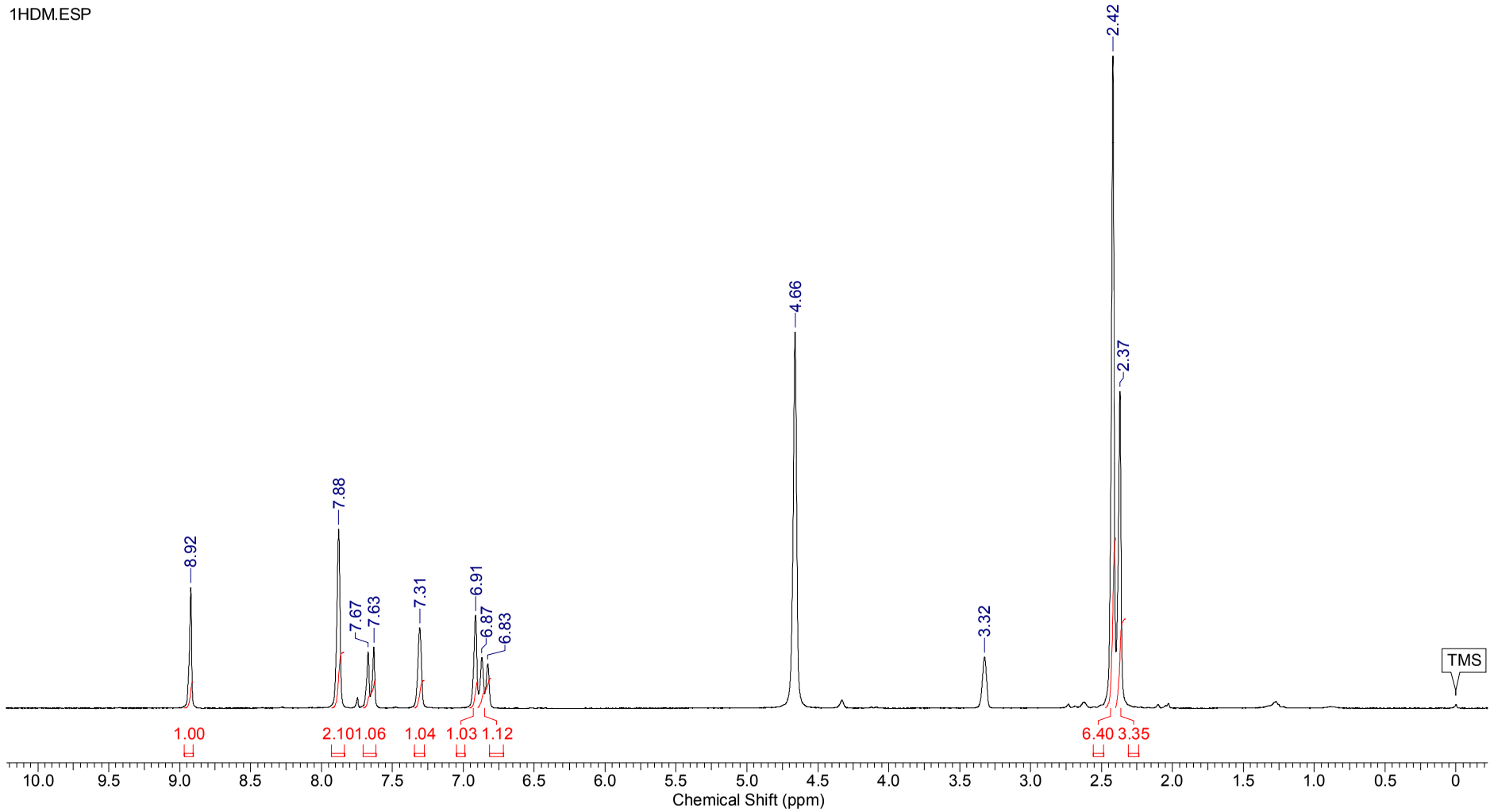
Mon3av400#004.002.001.1r.esp

1bc, 100 MHz, $CDCl_3$ + DMSO (D_6)

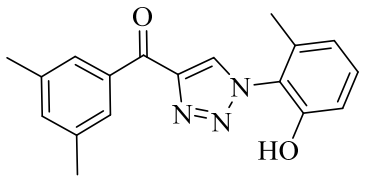


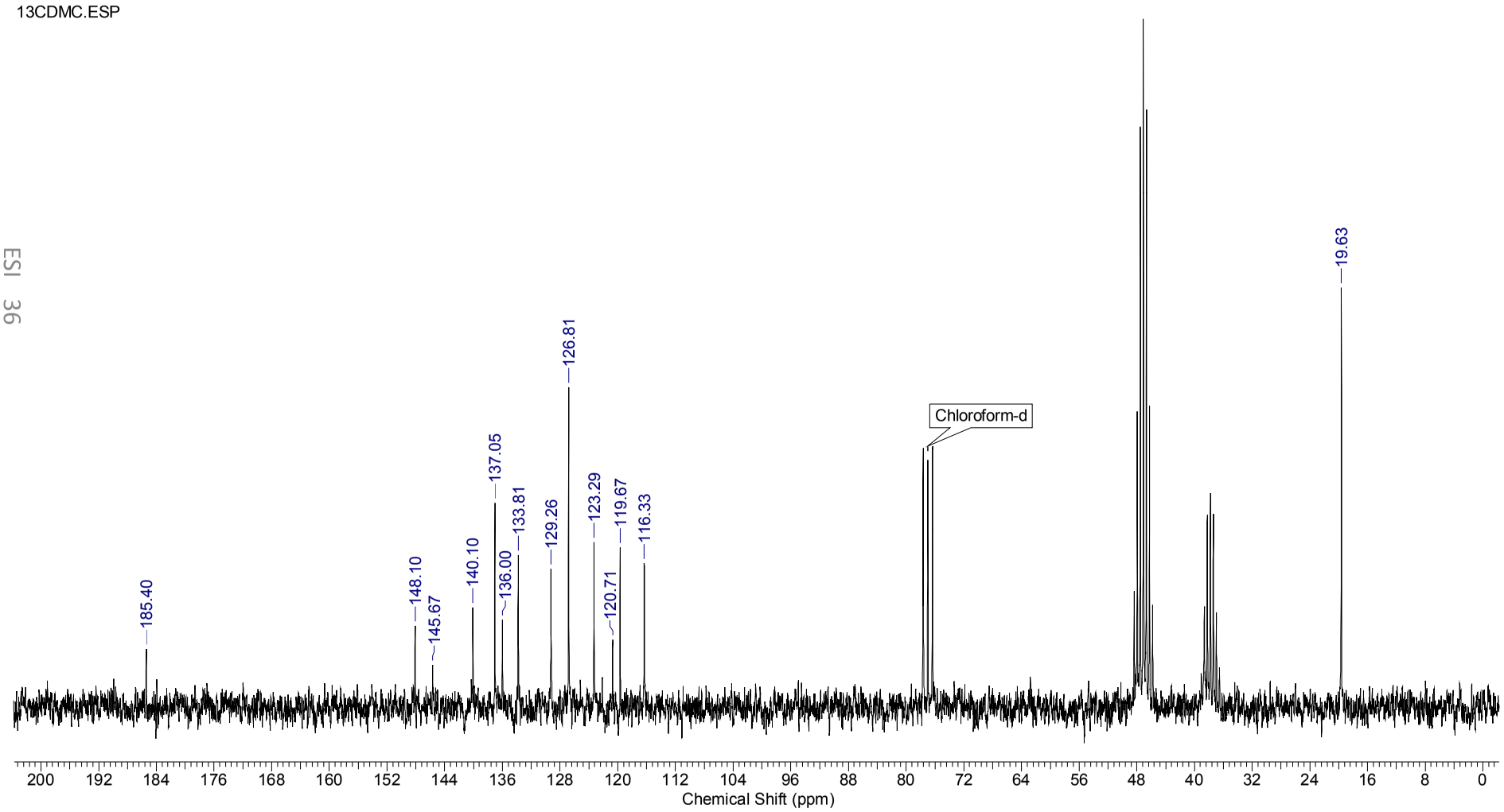
1be, 200 MHz, $CDCl_3$ + MeOH (D_4)



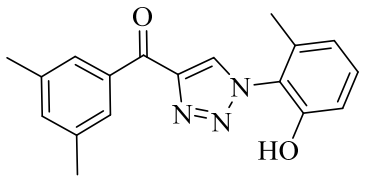


1be, 50 MHz, $CDCl_3$ + MeOH (D_4)

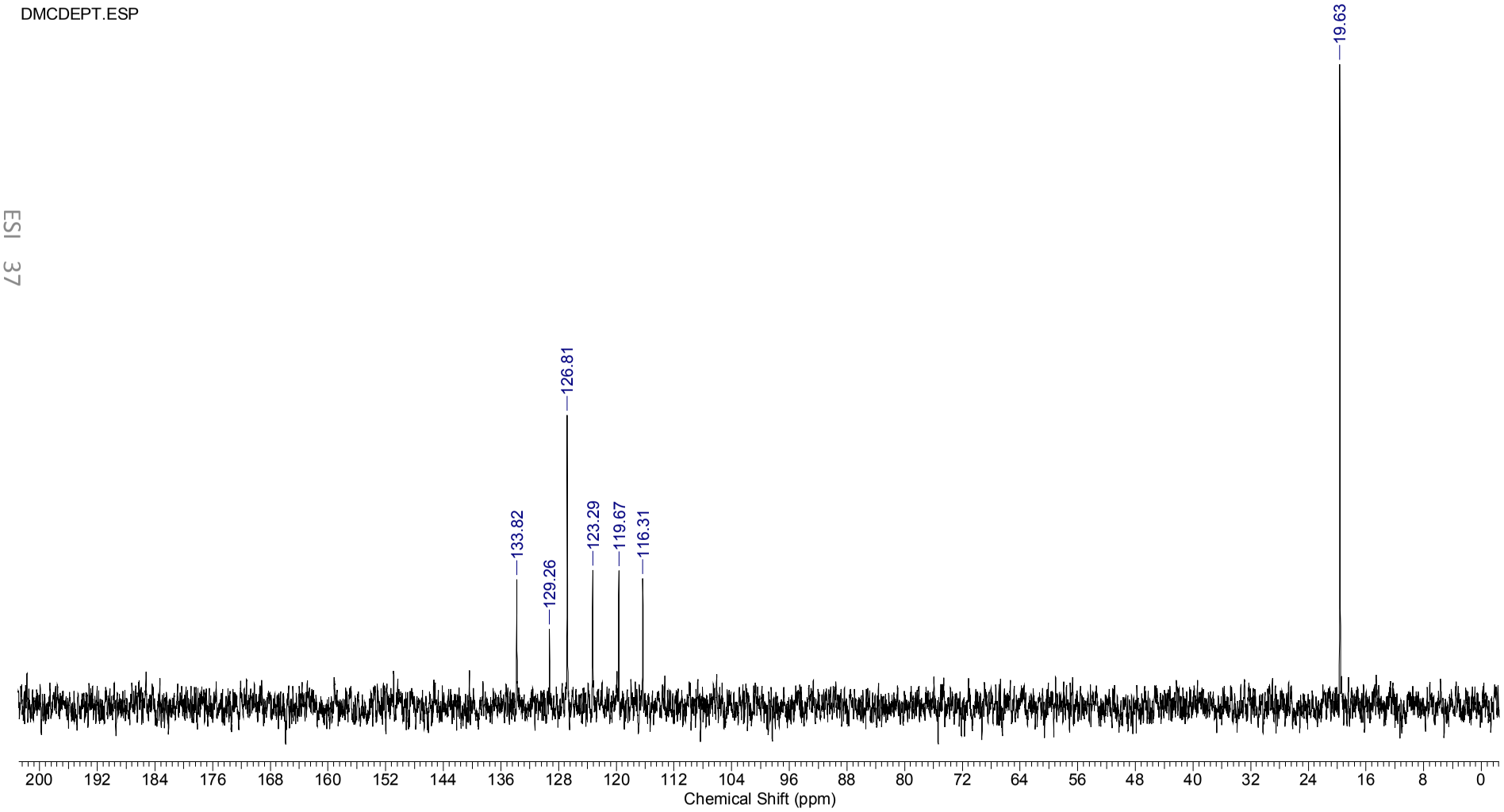




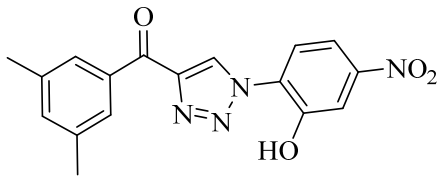
1be, 50 MHz, $CDCl_3$ + MeOH (D_4)

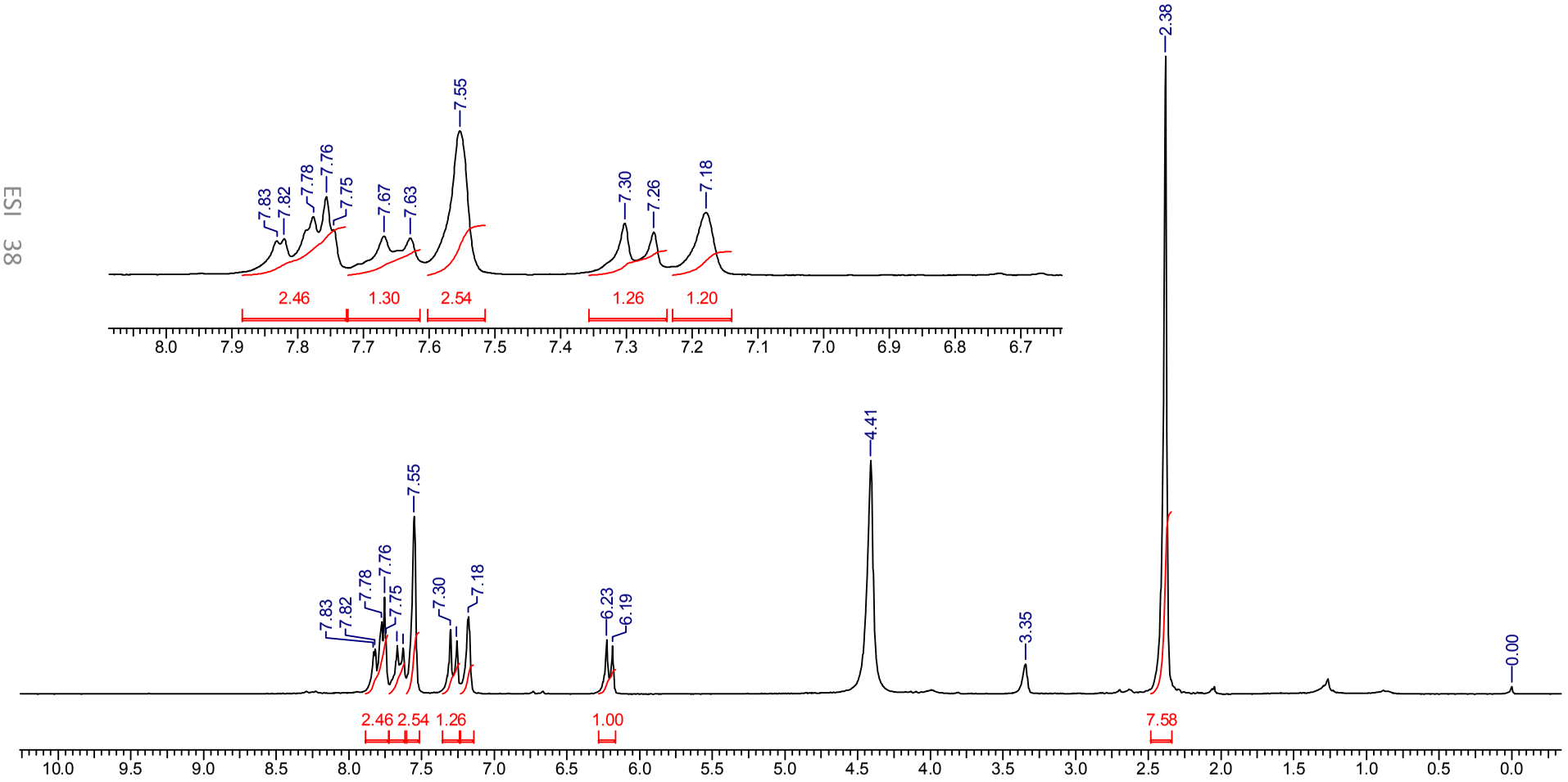


DMCDEPT.ESP

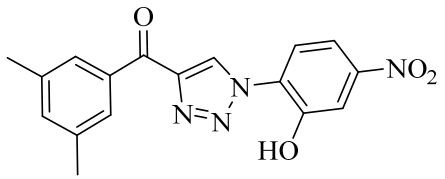


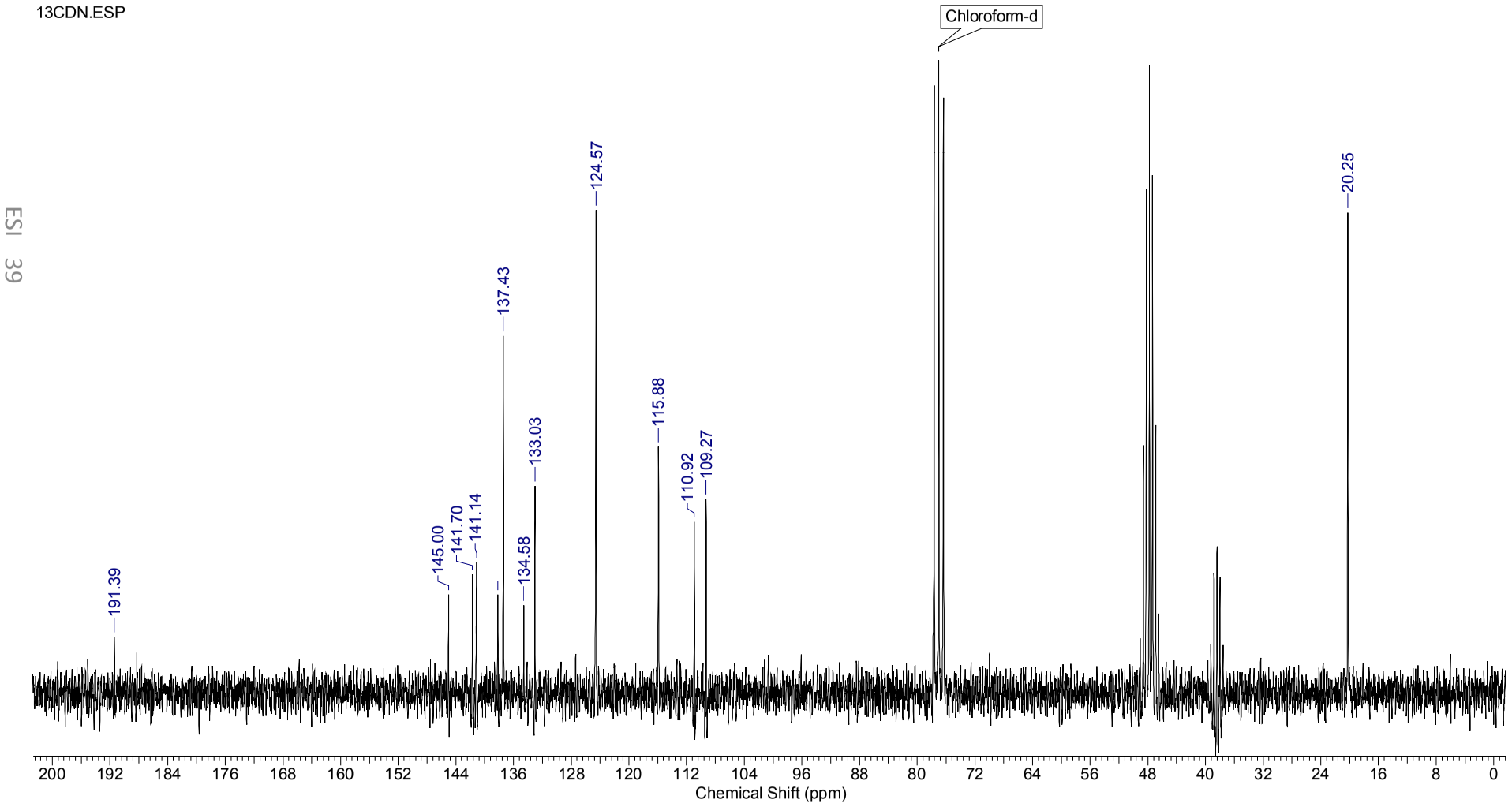
1bd, 200 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)



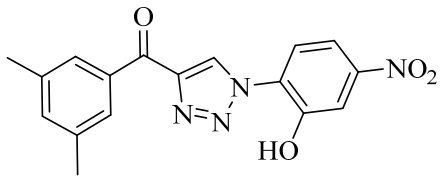


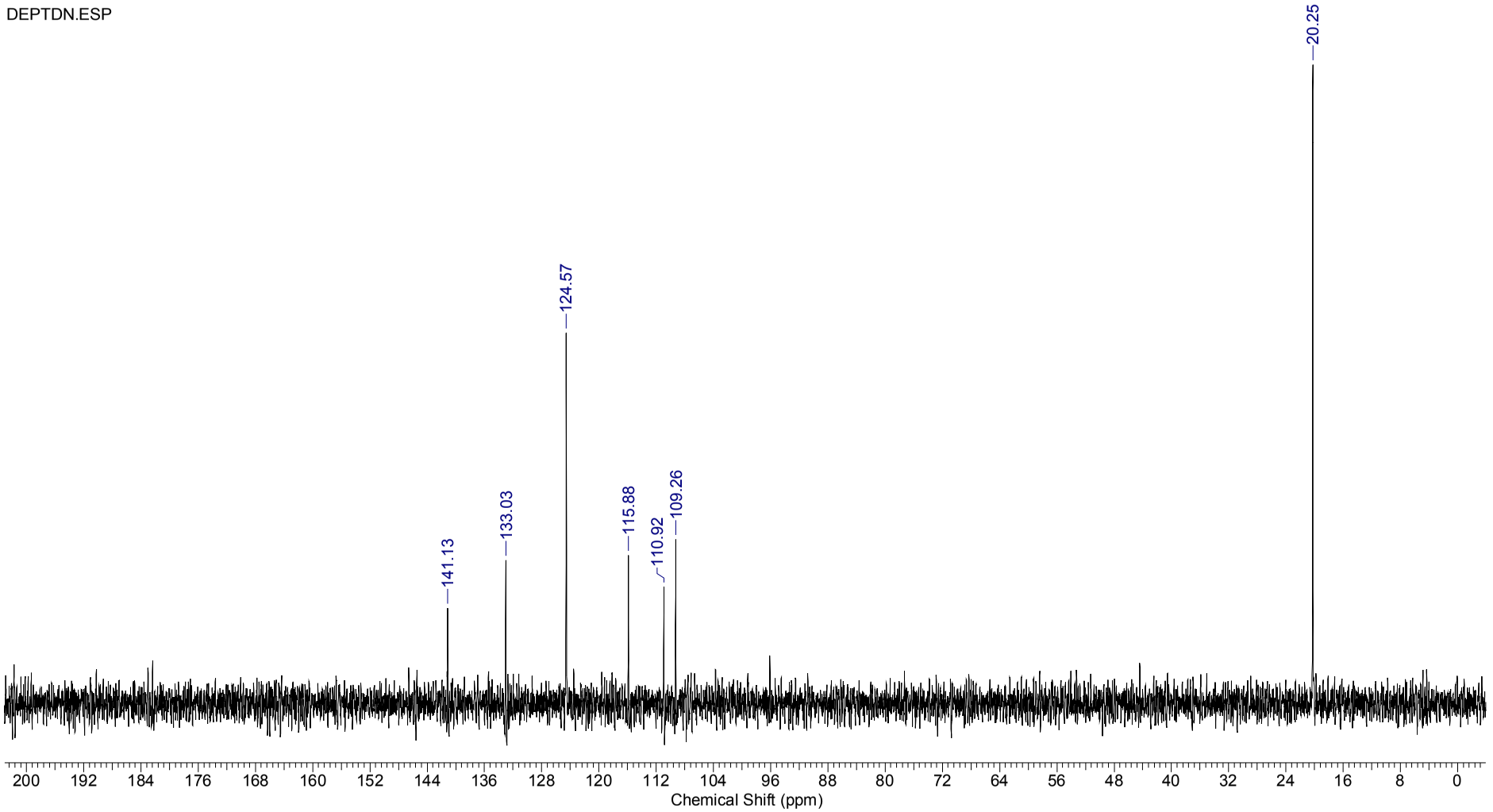
1bd, 50 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)





1bd, 50 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)

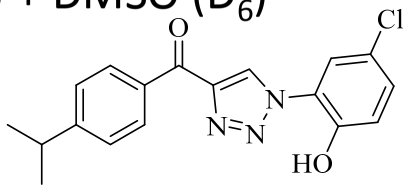


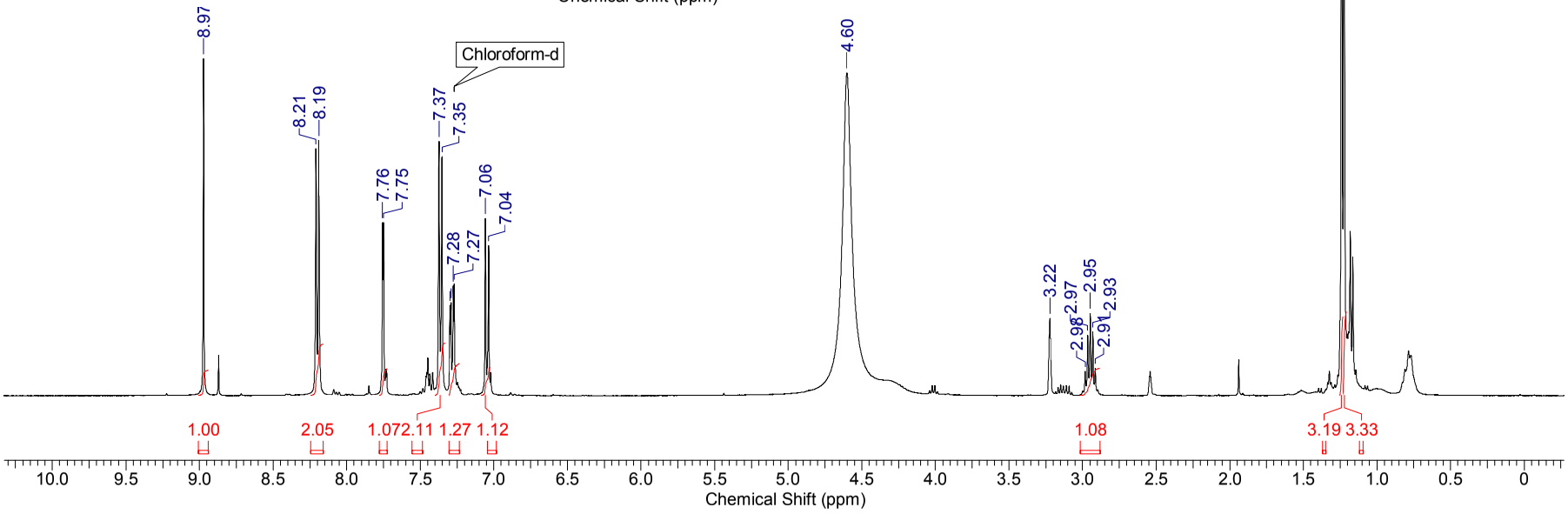


1HNMR.ESP

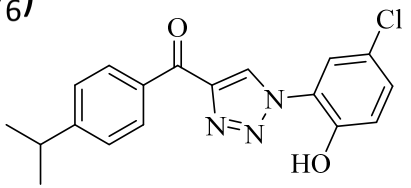
1HNMR.ESP

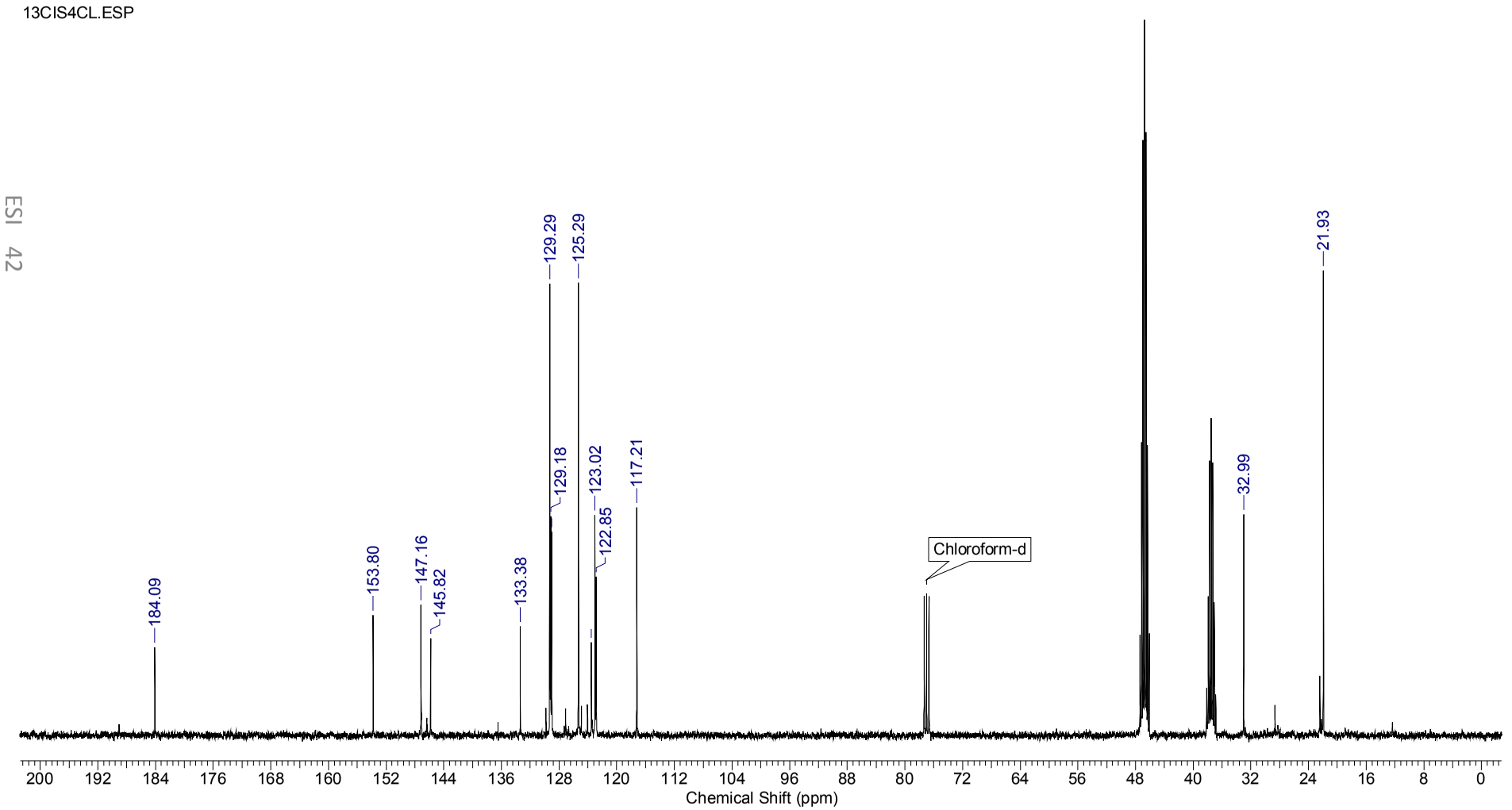
1ca, 400 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)



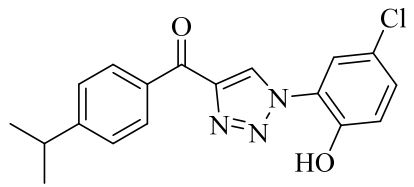


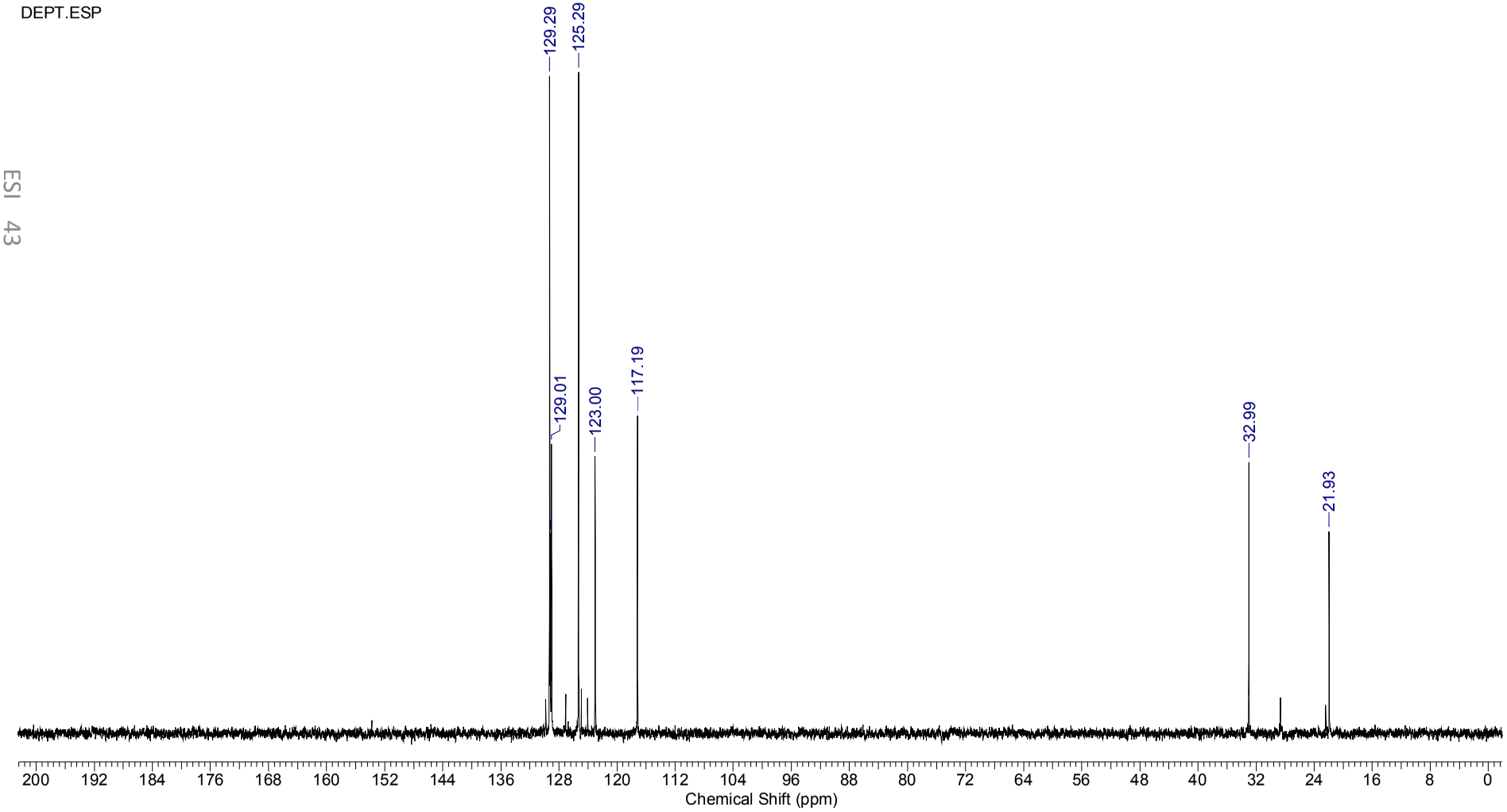
1ca, 100 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)





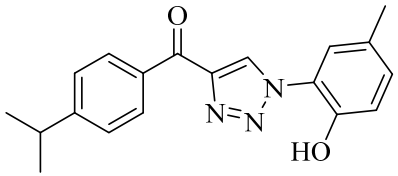
1ca, 100 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)

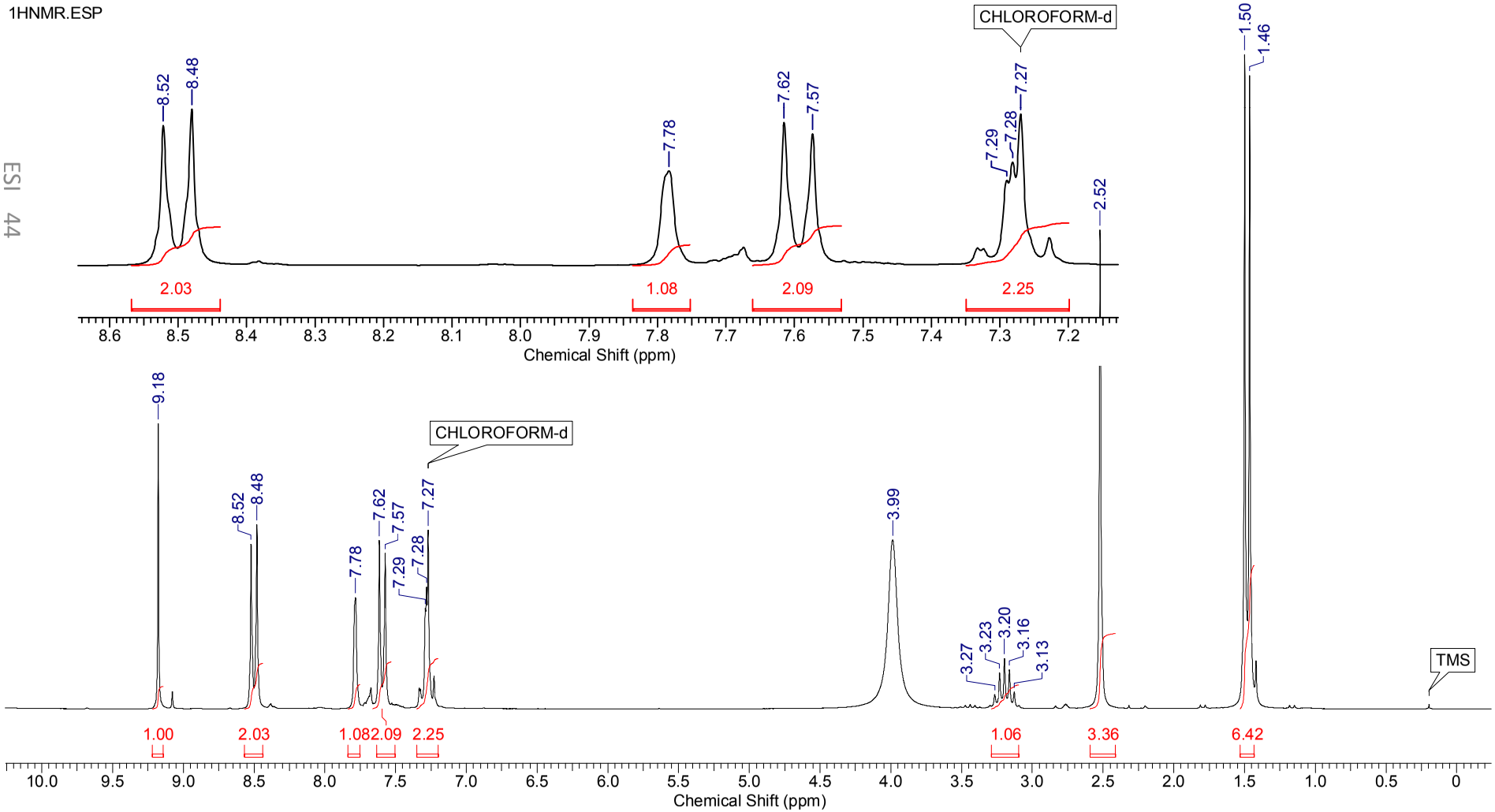




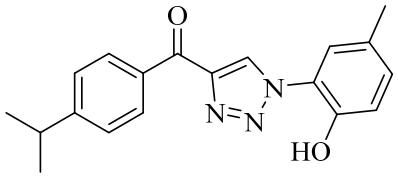
1HNMR.ESP

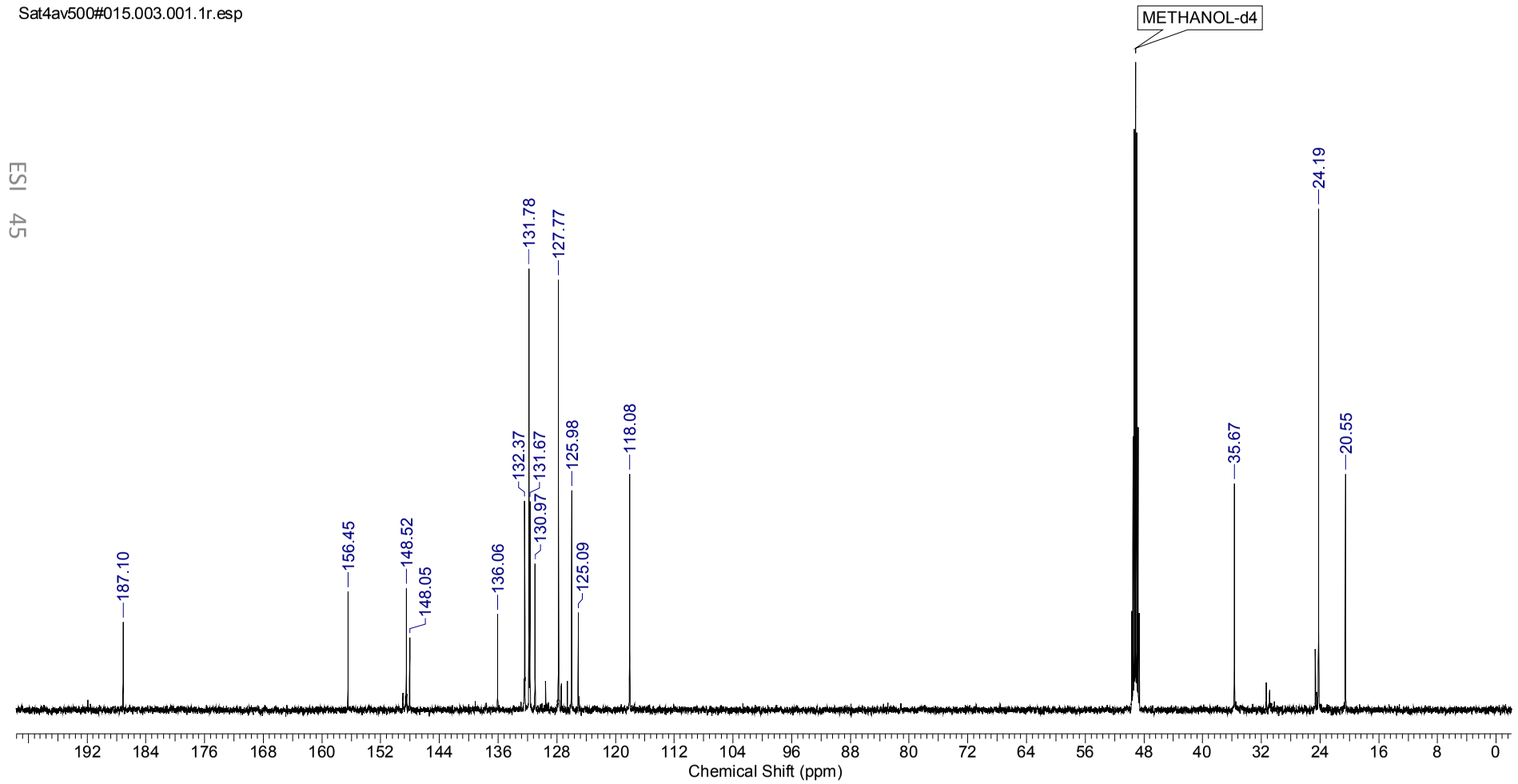
1cb, 500 MHz, MeOH (D_4)



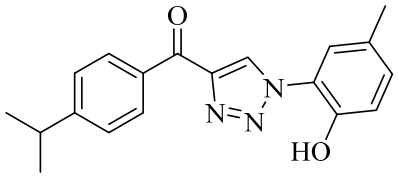


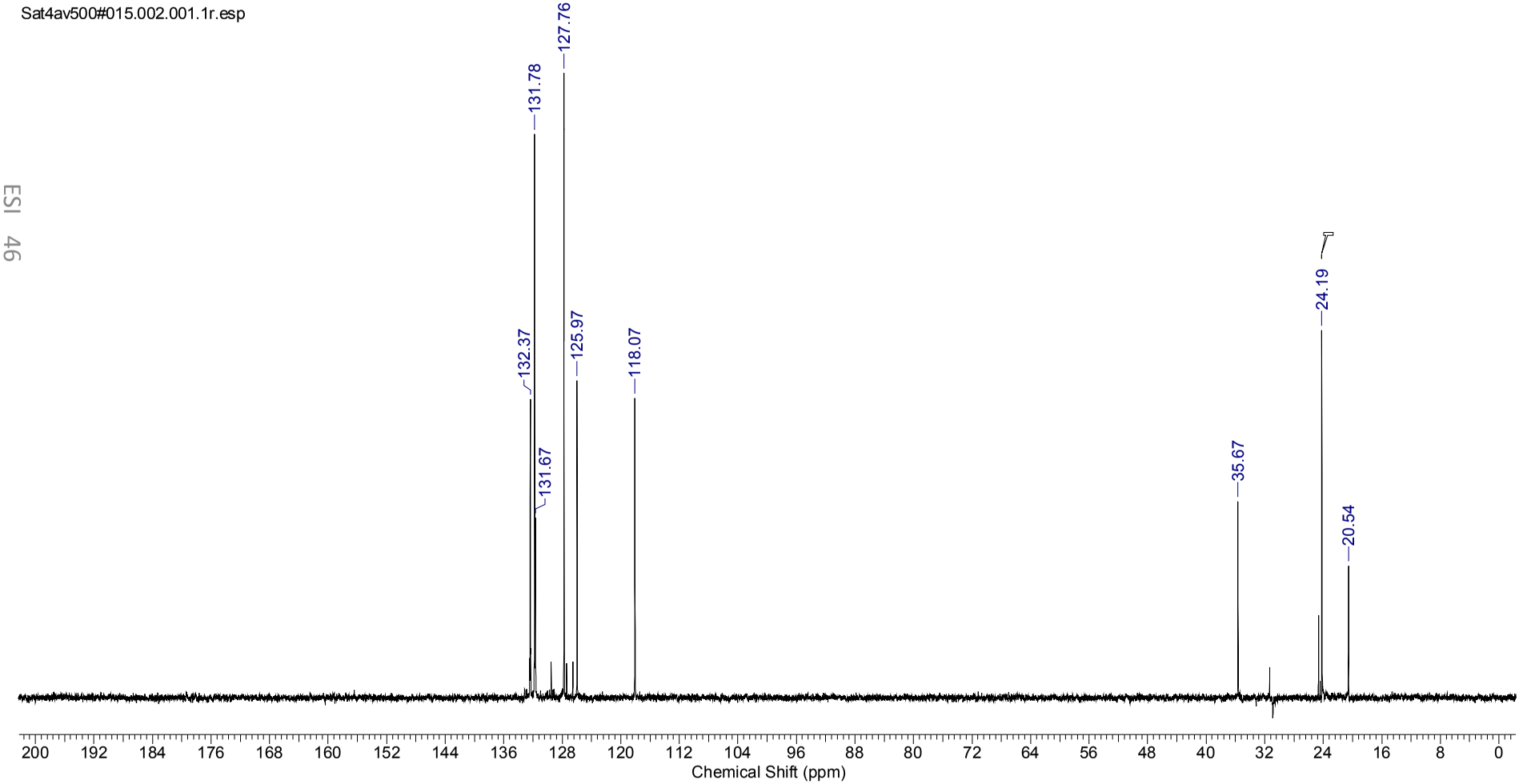
1cb, 125 MHz, MeOH (D_4)





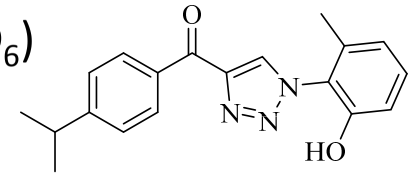
1cb, 125 MHz, MeOH (D_4)

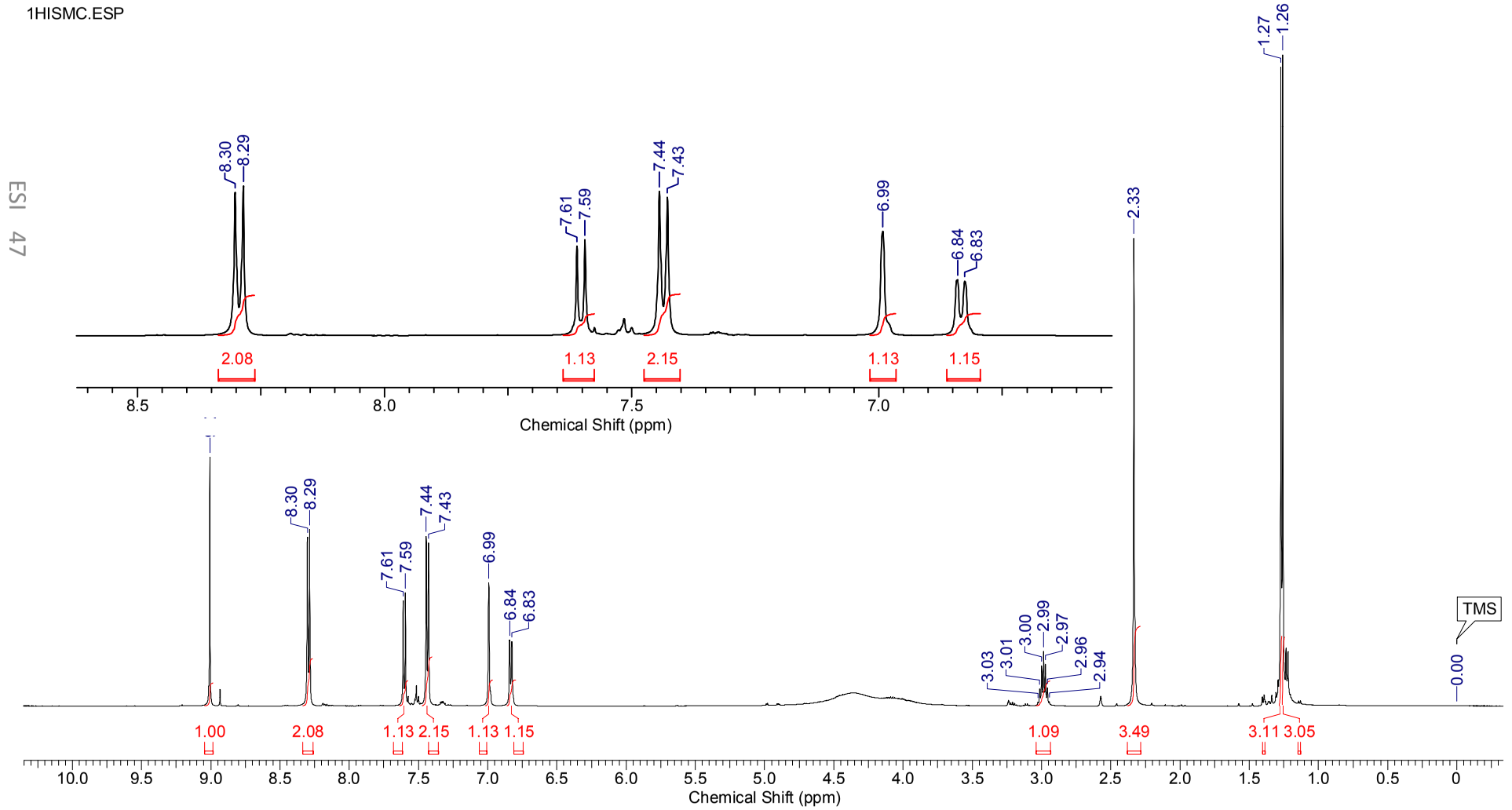




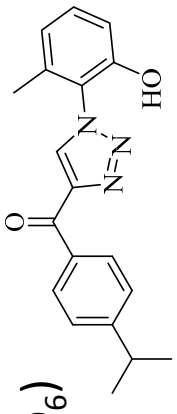
1HISMC.ESP

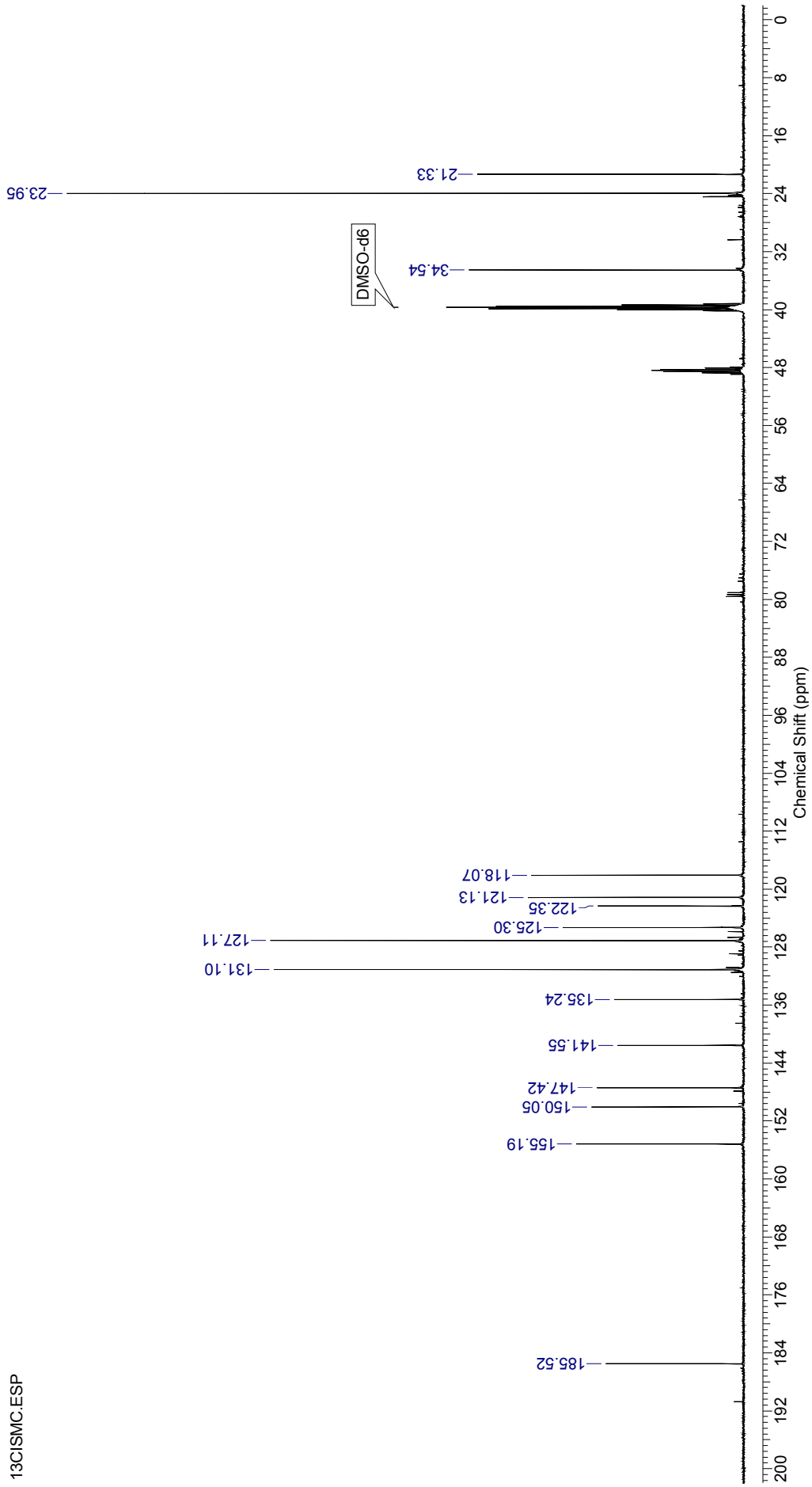
1ce, 500 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)



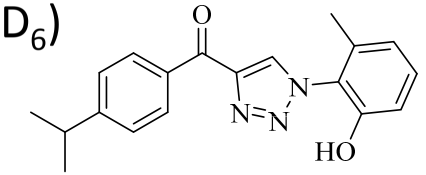


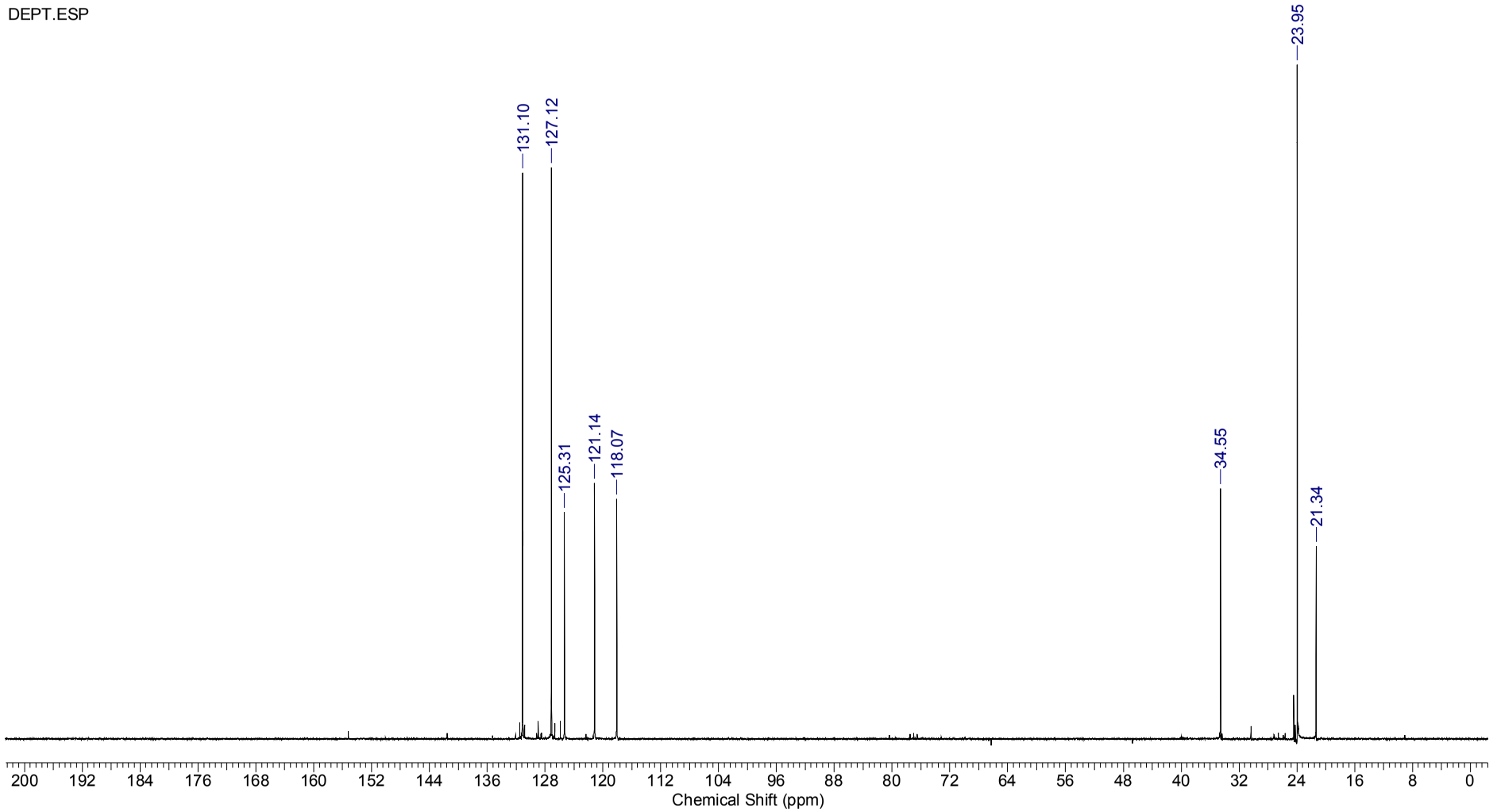
1ce, 125 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)



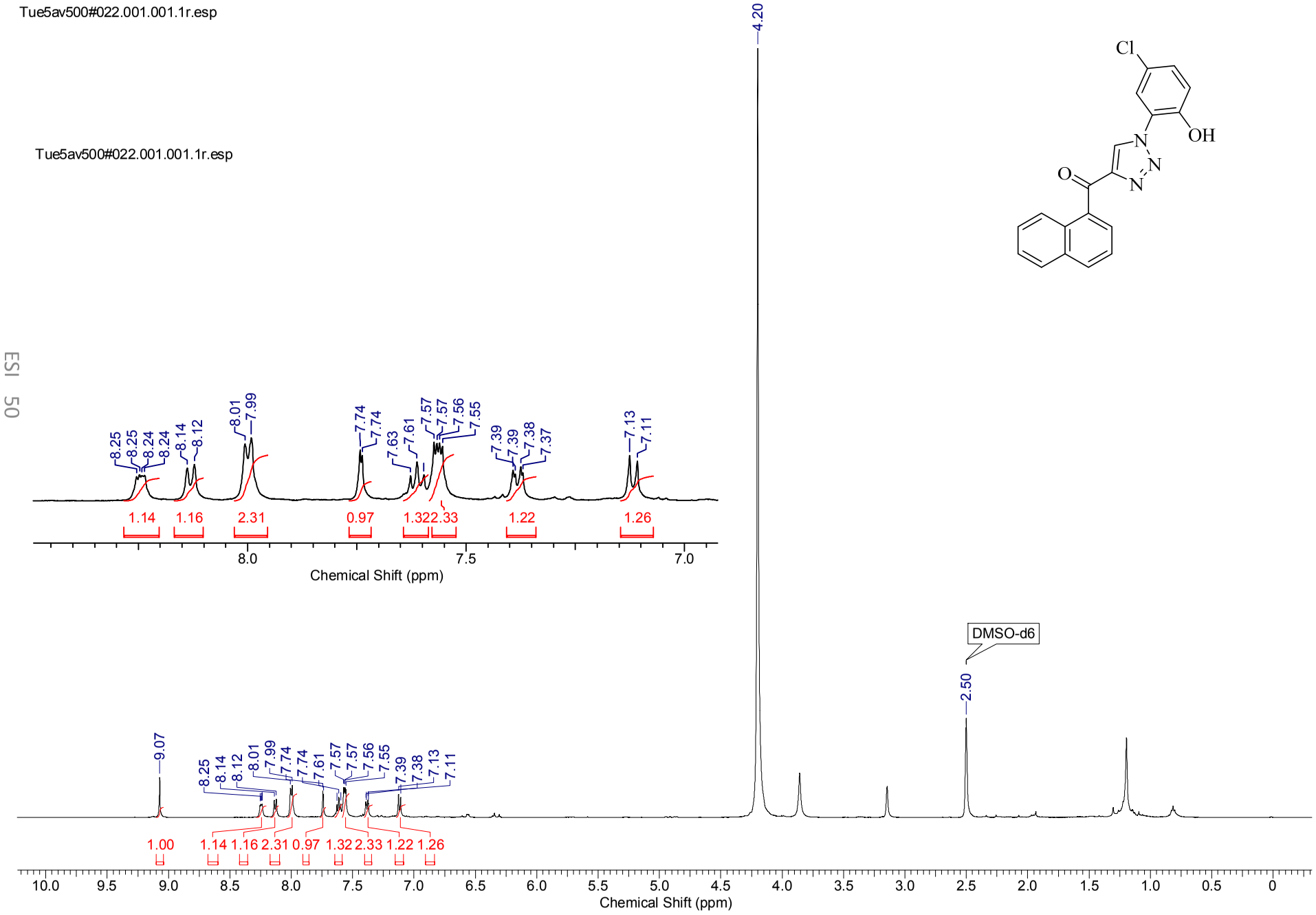


1ce, 125 MHz, $CDCl_3$ + MeOH (D_4) + DMSO (D_6)

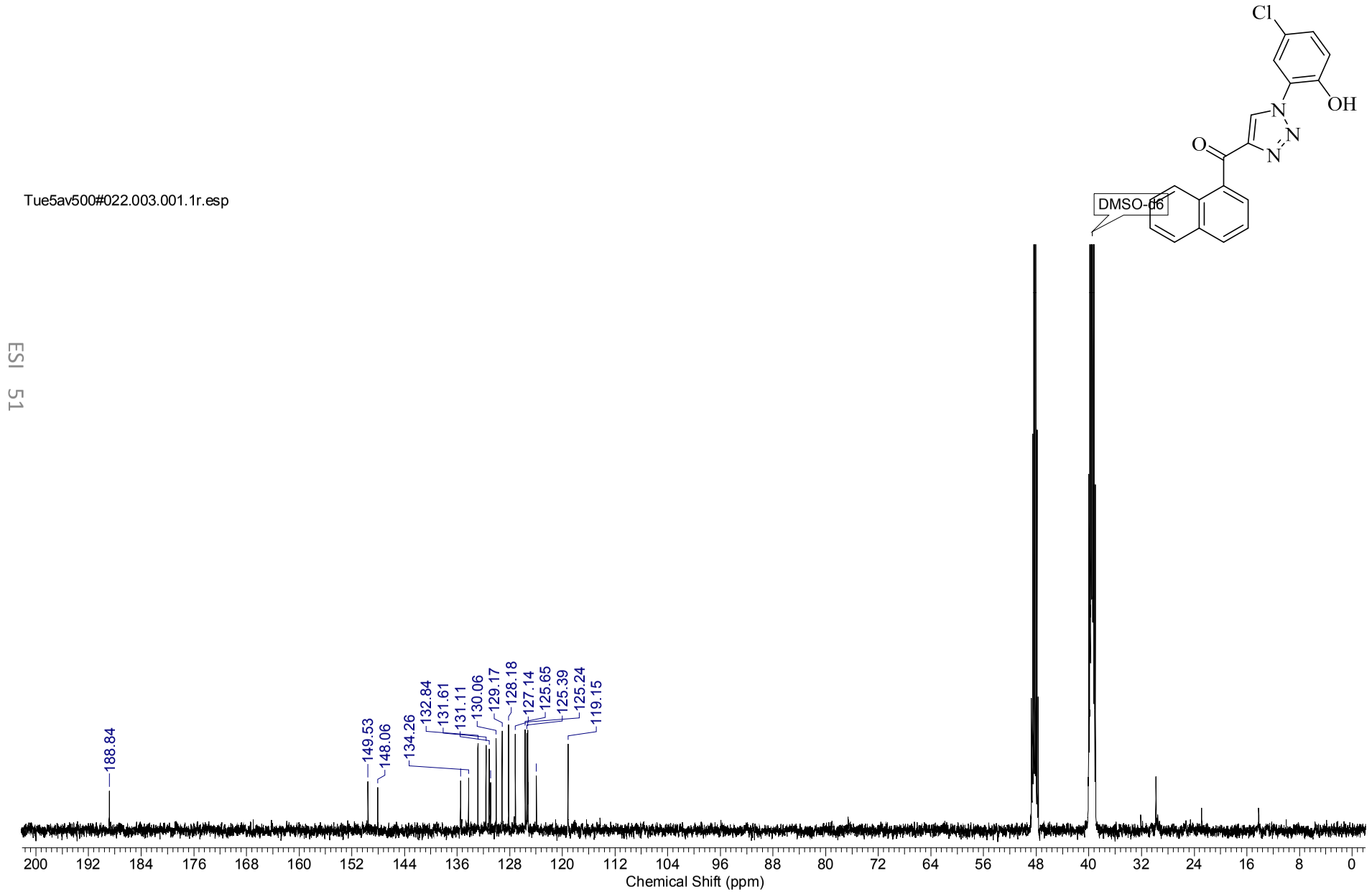




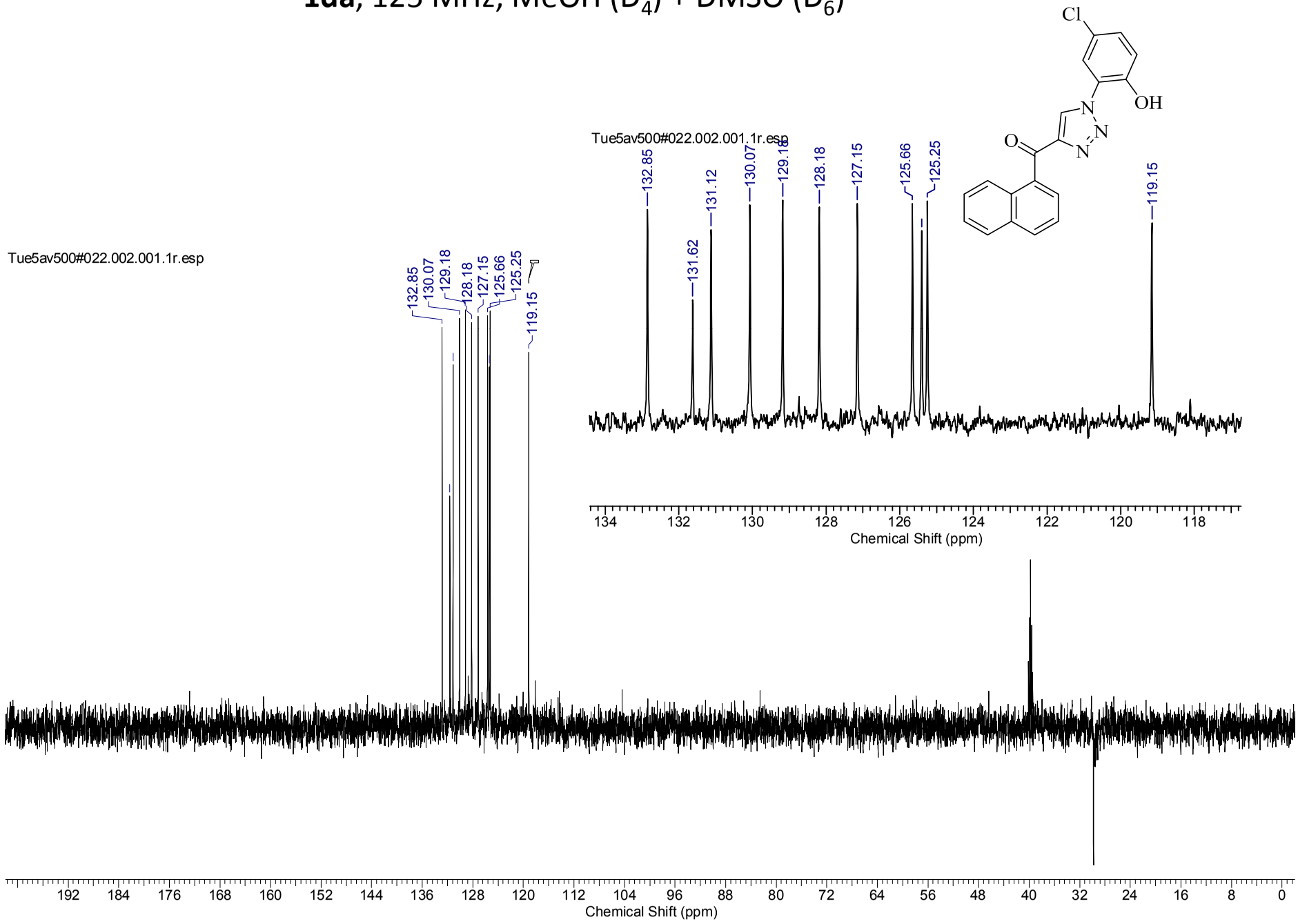
1da, 500 MHz, MeOH (D_4) + DMSO (D_6)



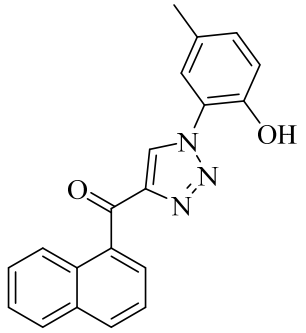
1da, 125 MHz, MeOH (D_4) + DMSO (D_6)



1da, 125 MHz, MeOH (D_4) + DMSO (D_6)



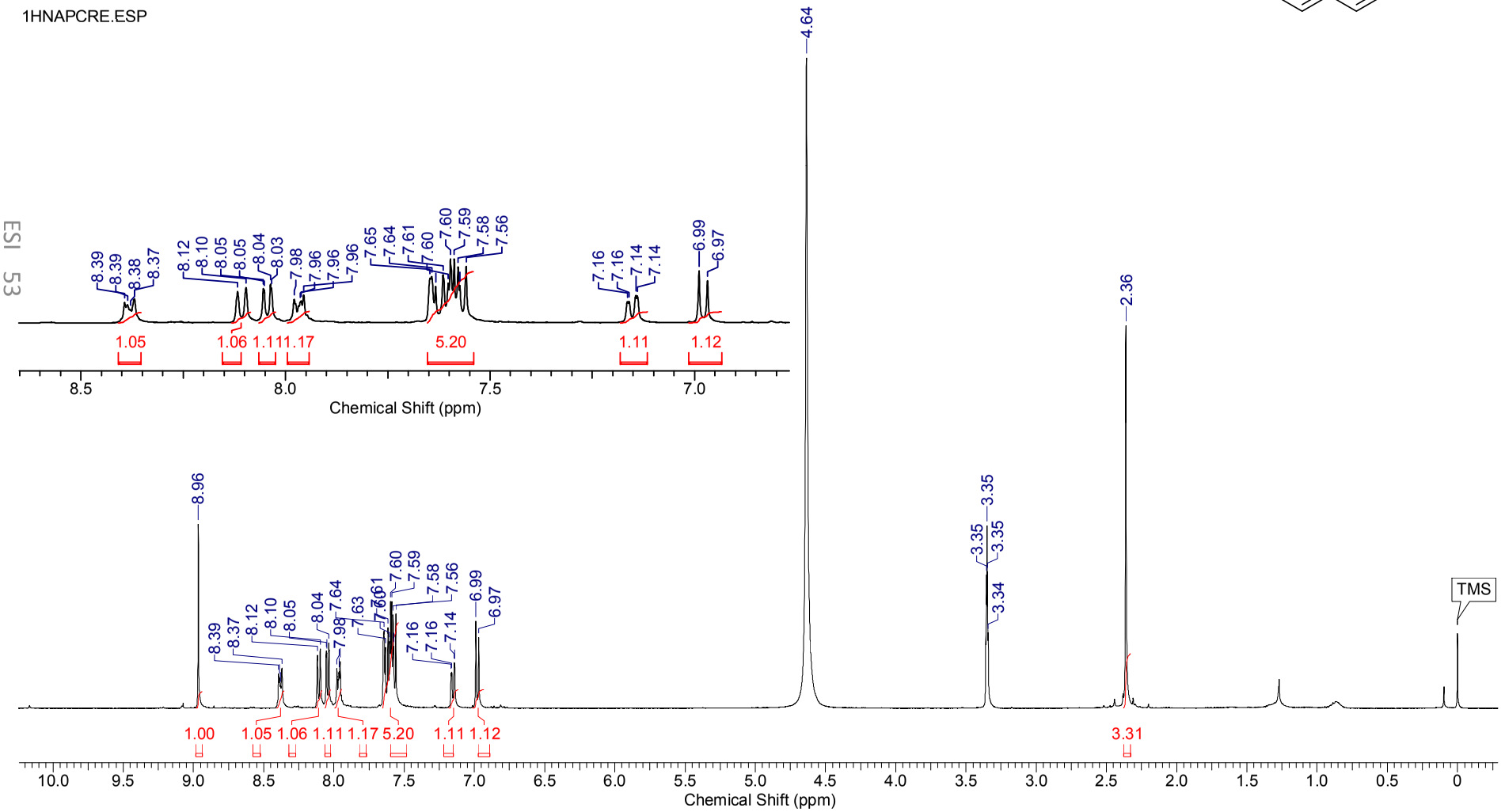
1db, 400 MHz, $CDCl_3$ + MeOH (D_4)



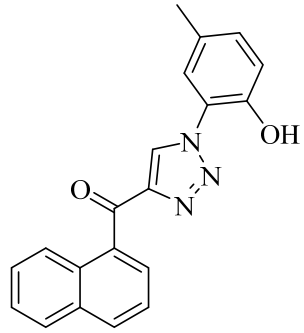
1HNAPCRE.ESP

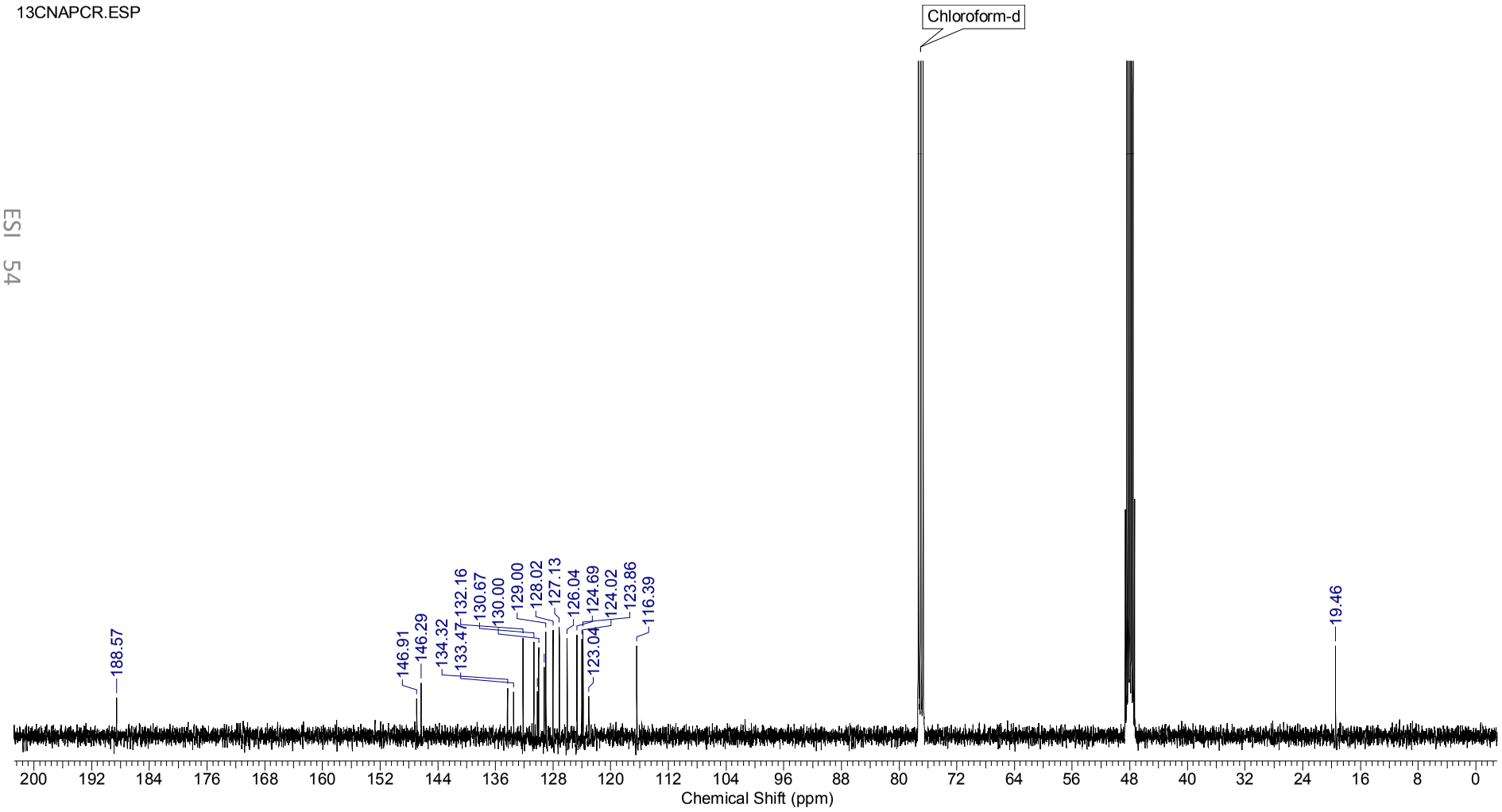
1HNAPCRE.ESP

ESI 53

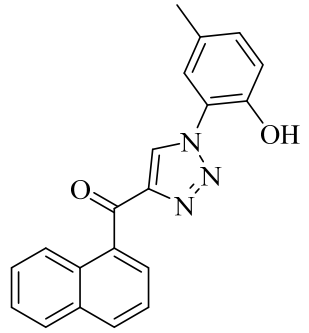


1db, 100 MHz, $CDCl_3$ + MeOH (D_4)

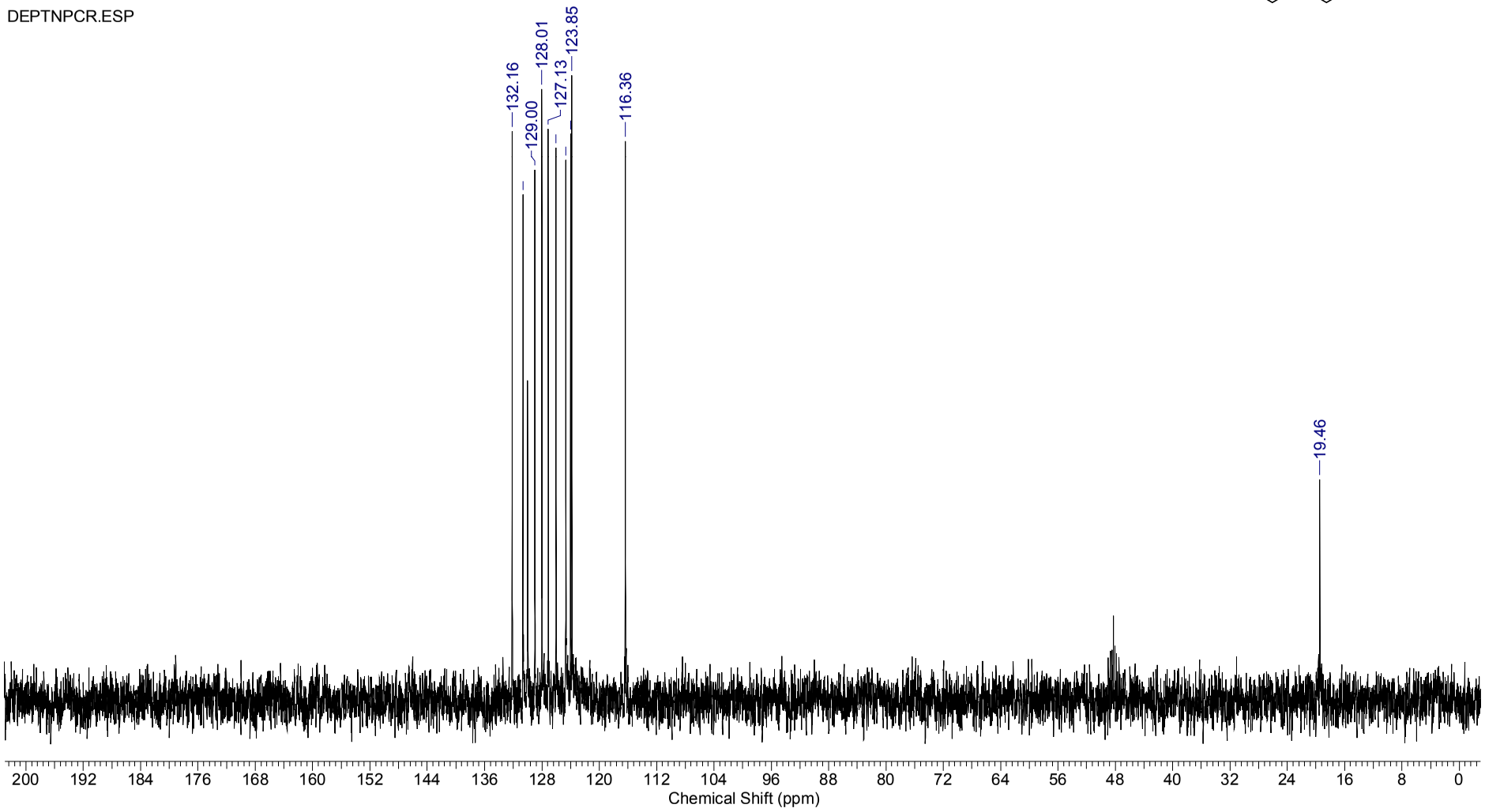




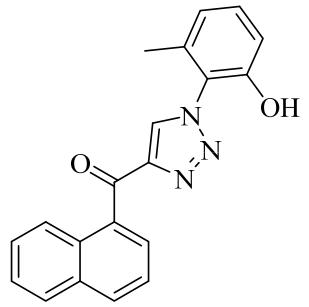
1db, 100 MHz, $CDCl_3$ + MeOH (D_4)



DEPTNPCR.ESP

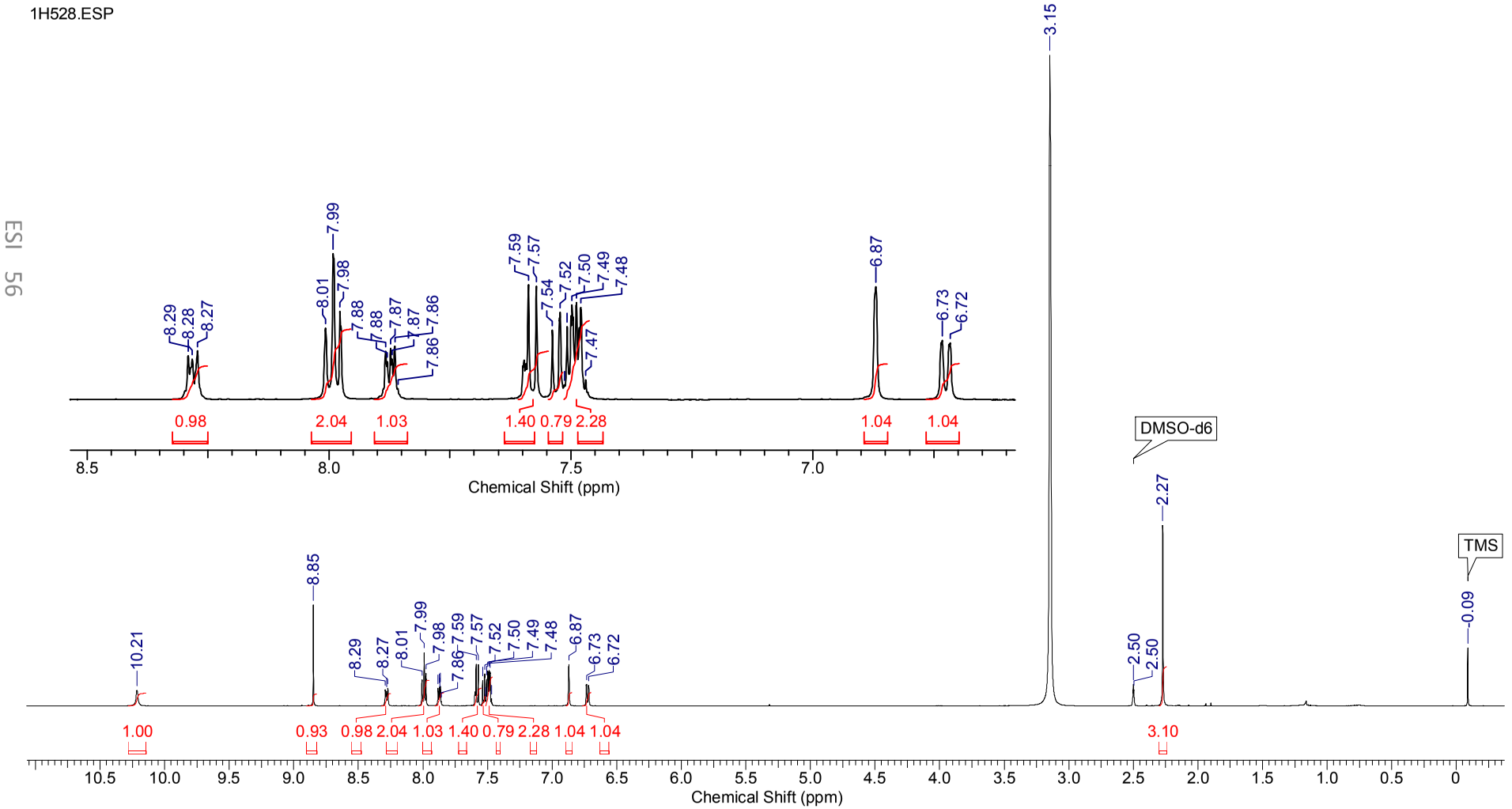


1de, 500 MHz, DMSO (D_6)

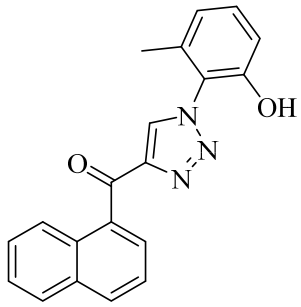


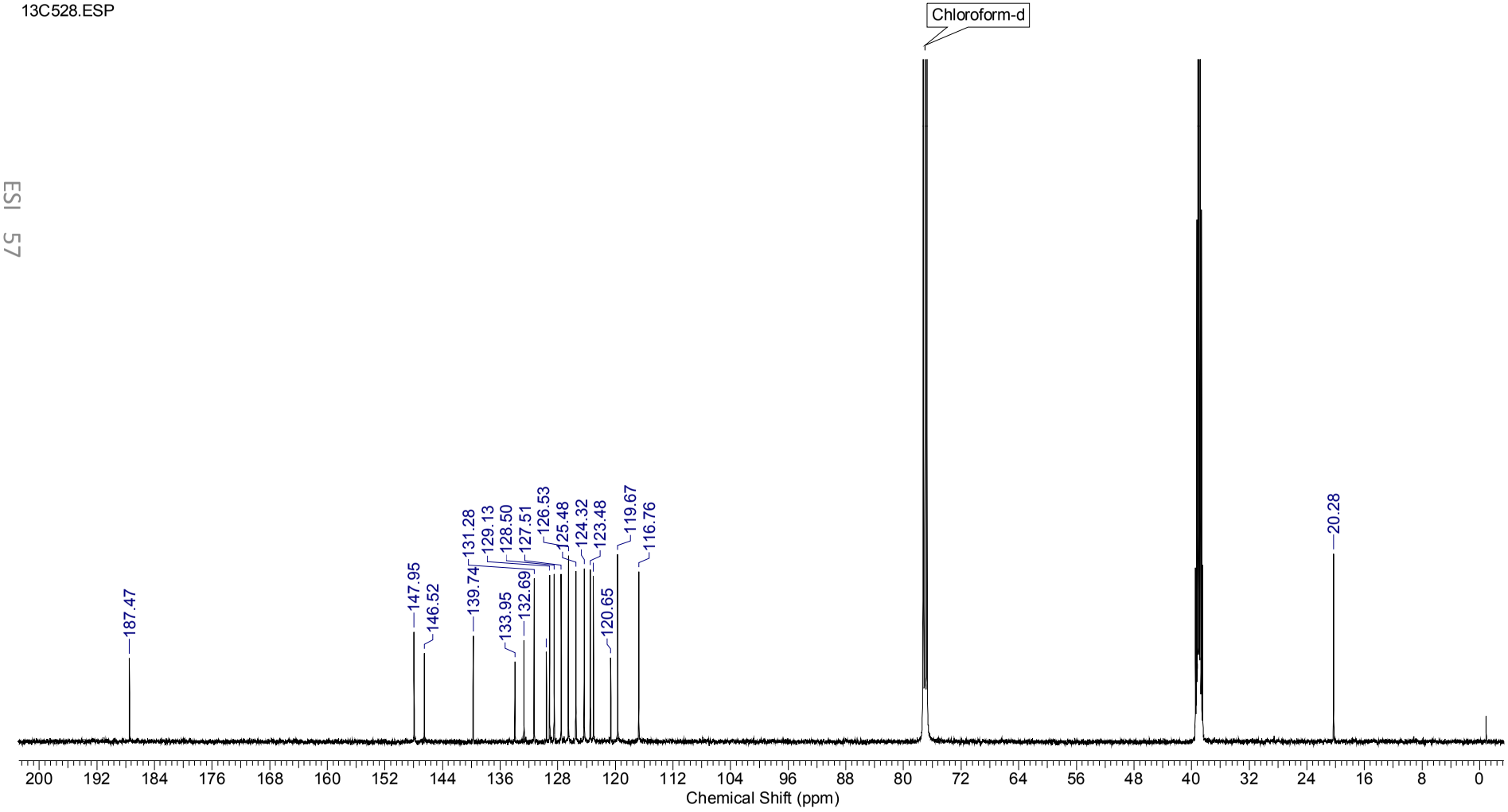
1H528.ESP

1H528.ESP

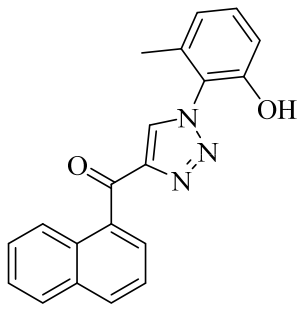


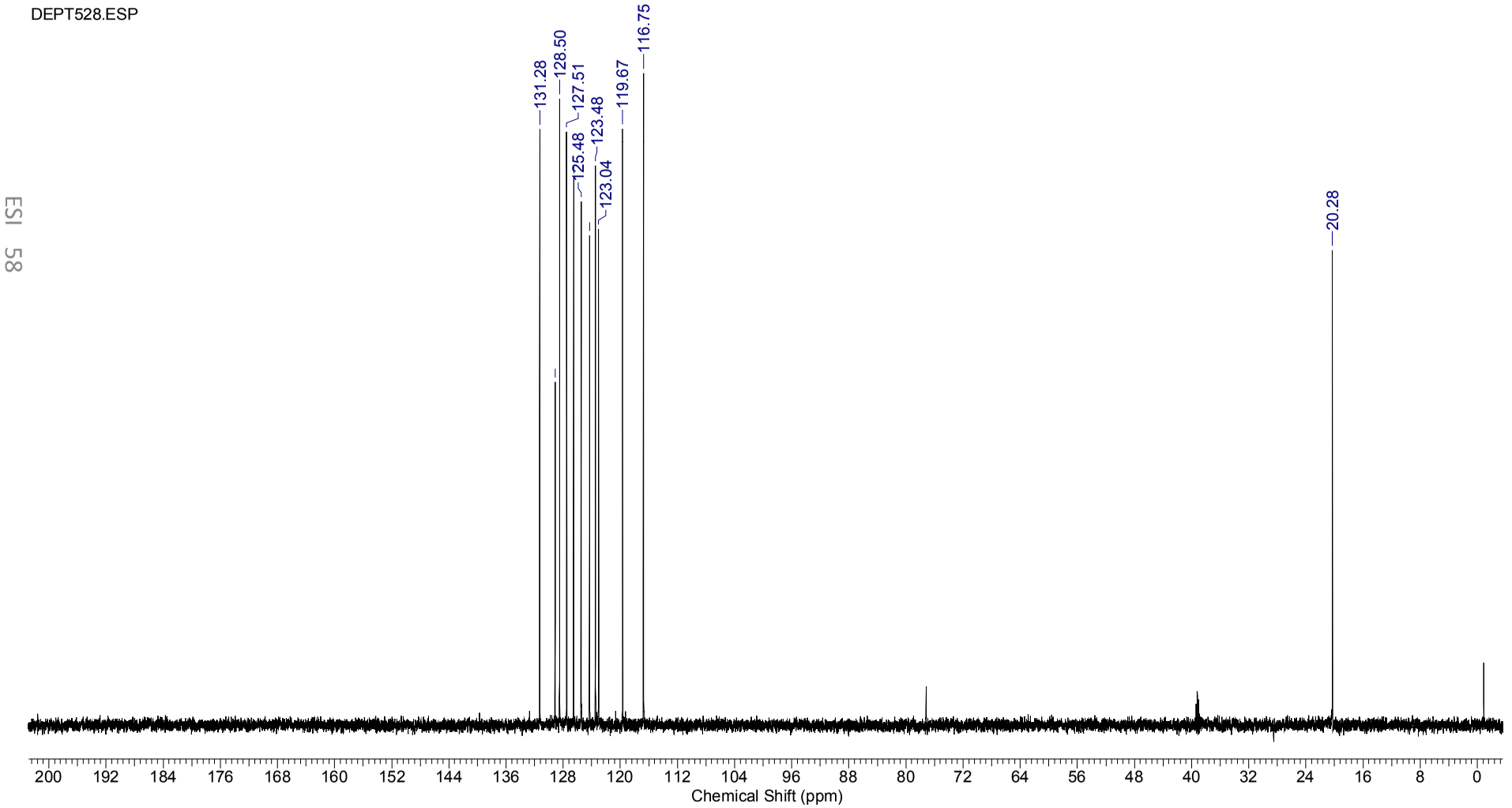
1de, 125 MHz, DMSO (D_6)



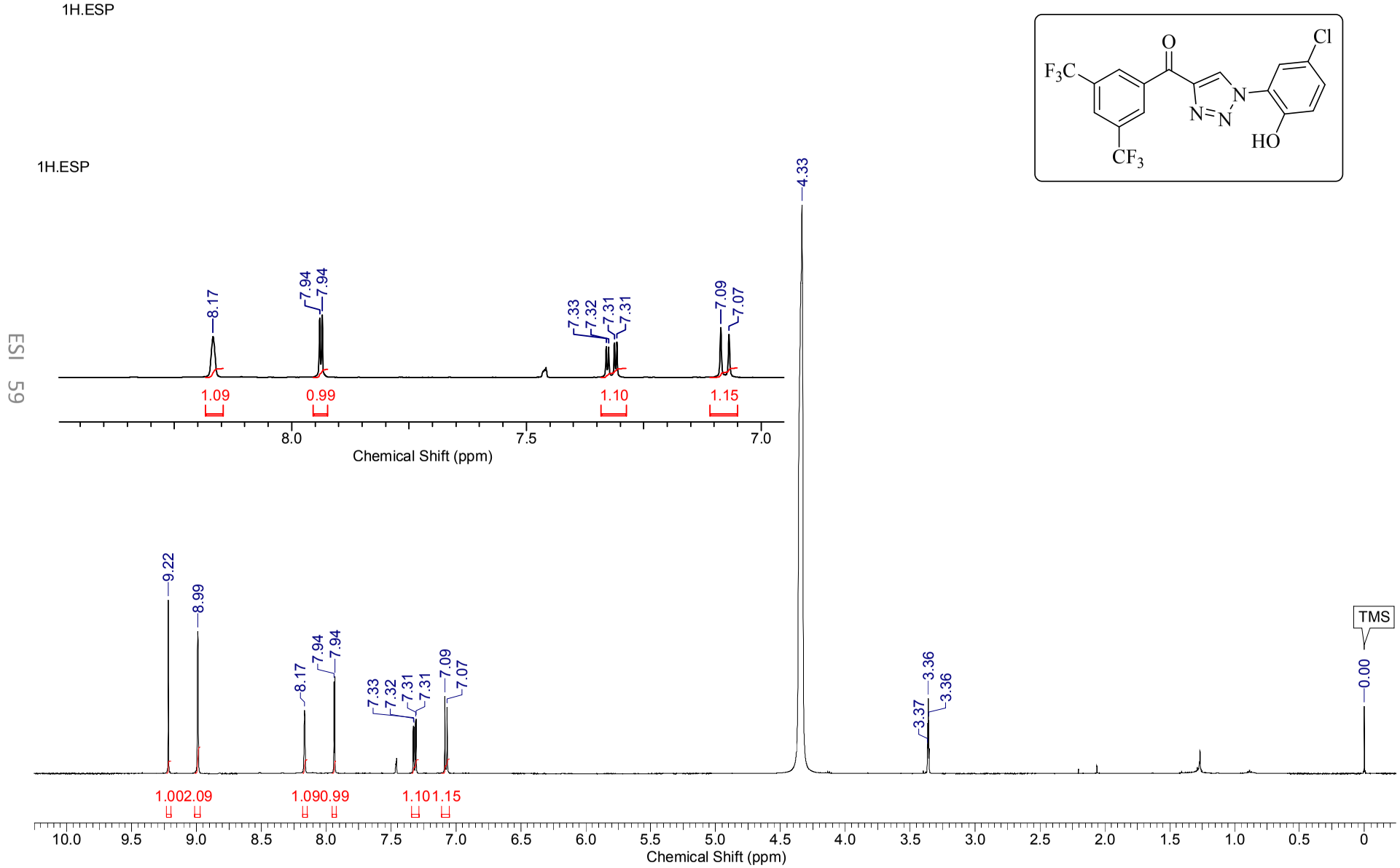


1de, 125 MHz, DMSO (D_6)



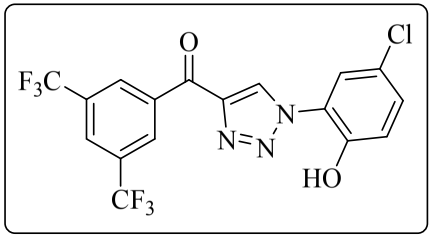


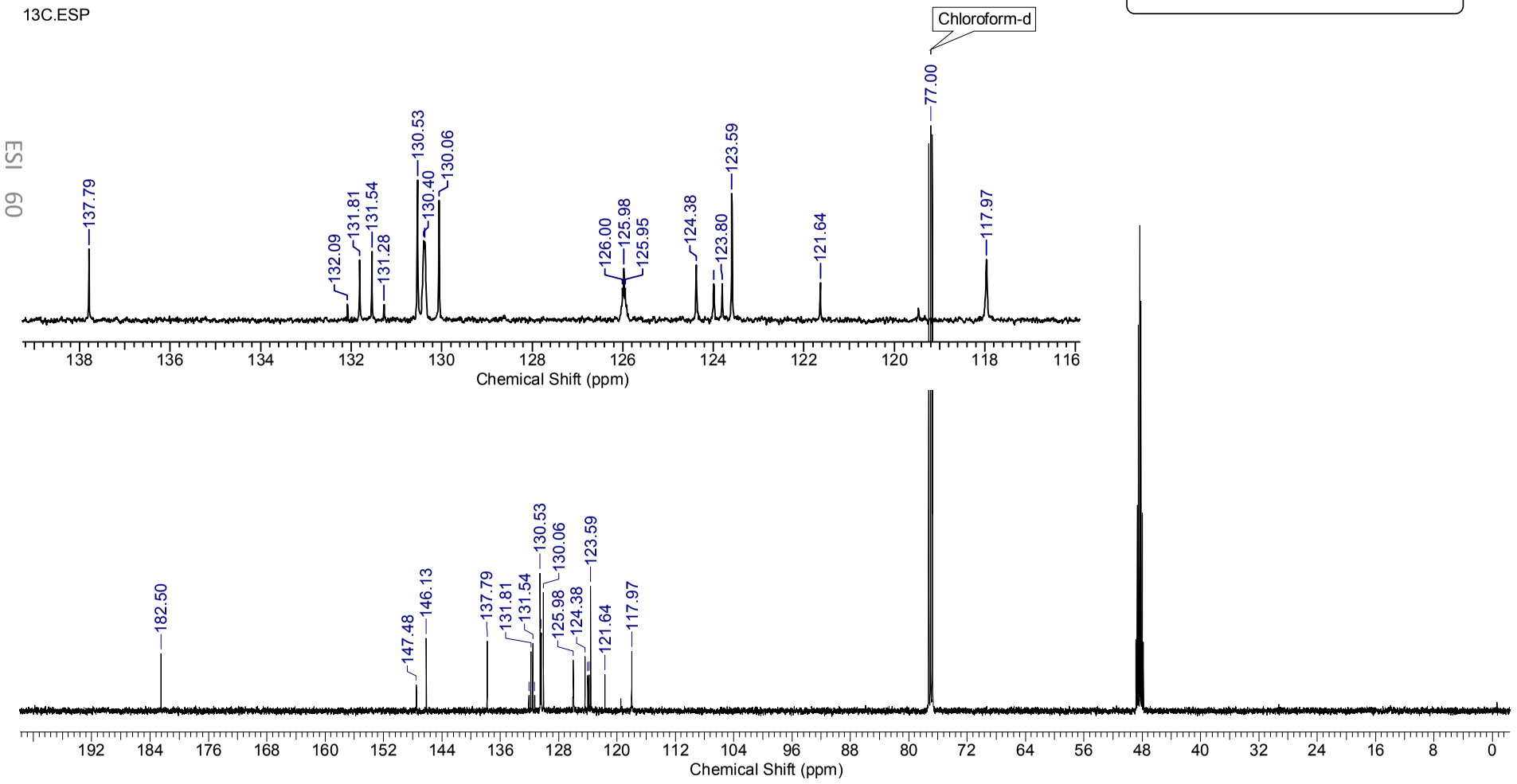
1ea, 500 MHz, $CDCl_3$ + MeOH (D_4)



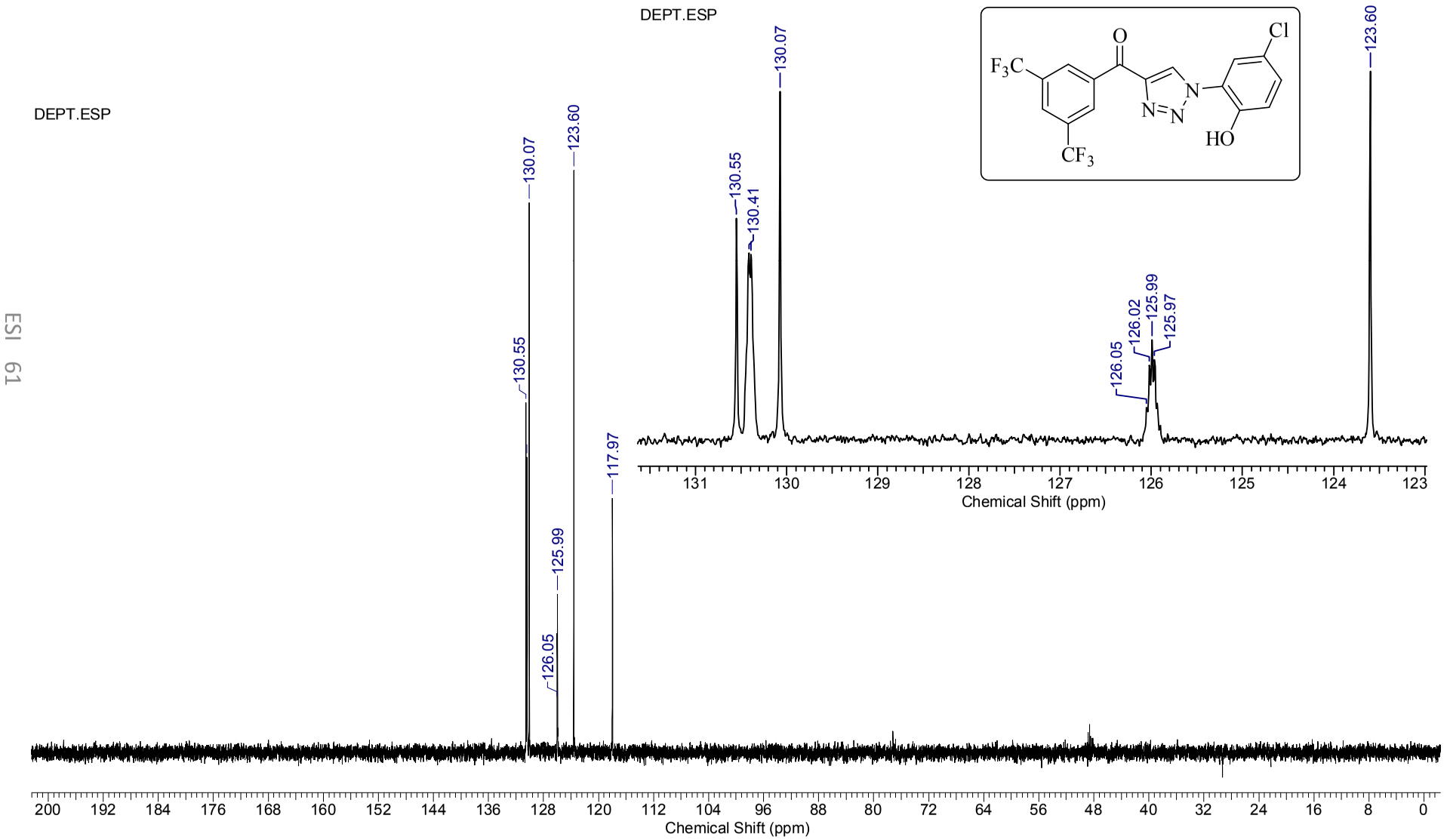
13C.ESP

1ea, 125 MHz, $CDCl_3$ + MeOH (D_4)



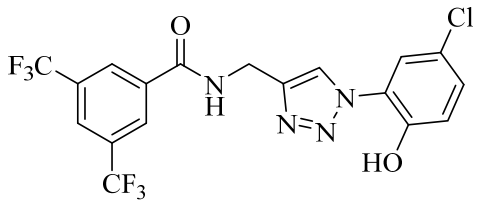


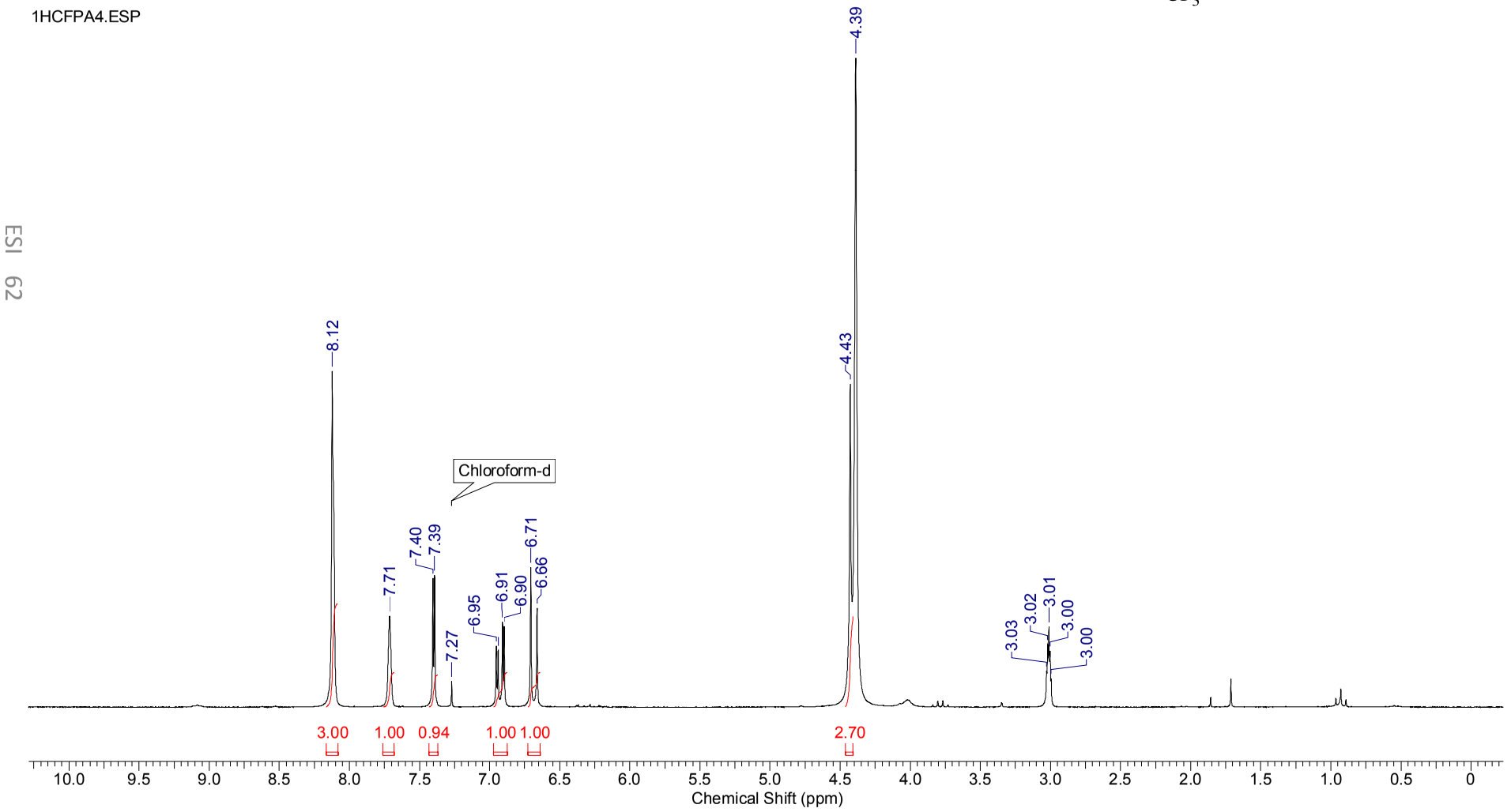
1ea, 125 MHz, $CDCl_3$ + MeOH (D_4)



ESI 61

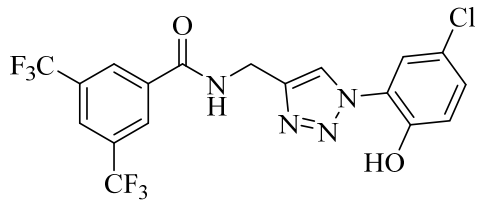
1fa, 200 MHz, $CDCl_3$ + MeOH (D_4)

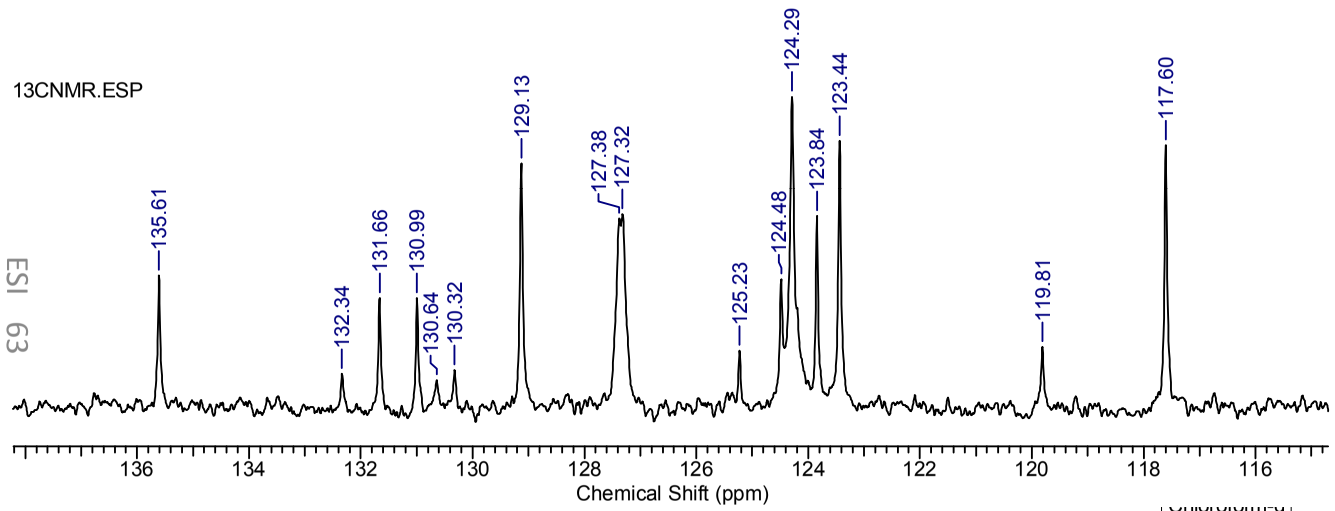


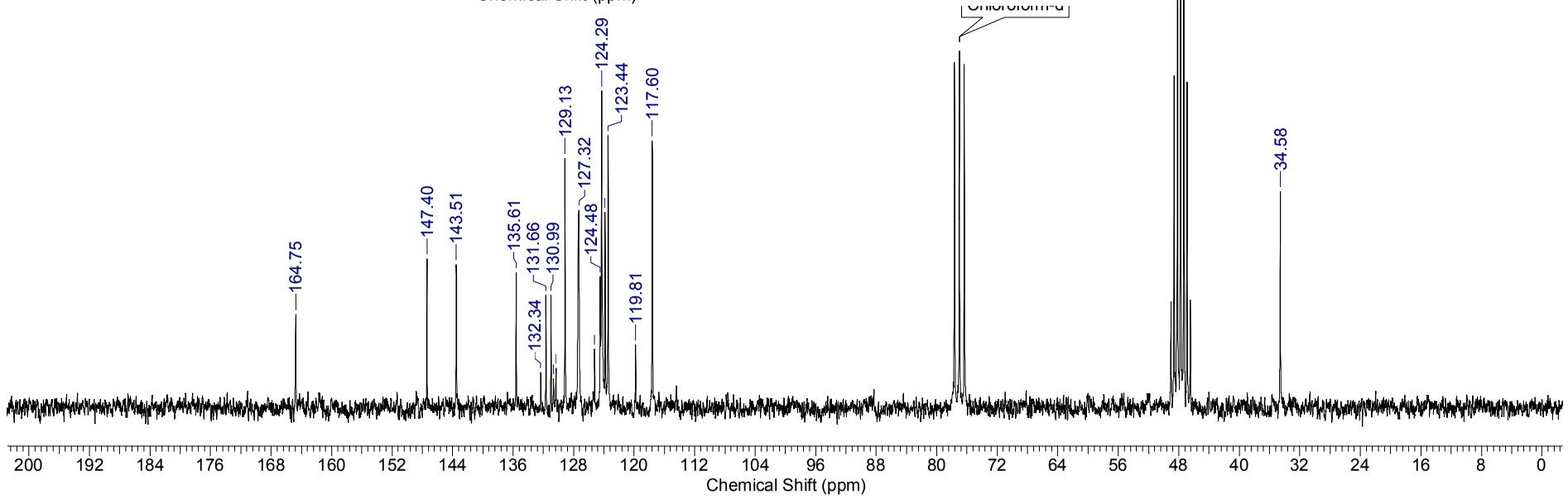


13CNMR.ESP

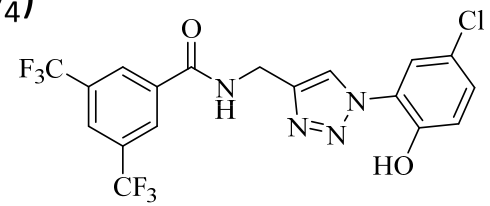
1fa, 50 MHz, $CDCl_3$ + MeOH (D_4)







1fa, 50 MHz, $CDCl_3$ + MeOH (D_4)



DEPT.ESP

ESI 64

34.58
127.34
124.31
123.45
129.14
117.61

200 192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0

Chemical Shift (ppm)