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"Synthesis of 4-aryl-6-indolylpyridine-3-carbonitriles and Evaluation of Their Antiproliferative Activity" data files

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Supplementary Data

Synthesis of 4-aryl-6-indolylpyridine-3-carbonitriles and evaluation of their antiproliferative activity

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Experimental

Chemical protocols

All reagents and solvents used were purchased from common commercial of analytical grad, melting points are uncorrected and were measured using an electro-thermal IA 9100 apparatus (Shimadzu, Japan). IR spectra were carried out on JASCO FT/IR 6100 Japan spectrometer (National Research Center Cairo, Egypt) using KBr disc. ^{1}H-NMR spectra were determined using JEOL ECA-500 run for ^{1}H-NMR at 500 MHz and run for ^{13}C-NMR at 125 MHz spectrometer (National Research Center, Cairo, Egypt). Chemical shifts were expressed in part per million δ (ppm) against tetramethylsilane (TMS) as an internal standard. The coupling constant J is expressed in Hz. The Mass spectra were recorded on GCMS Finnigan mat SSQ 7000 spectrometer (National Research Centre, Cairo, Egypt). Reactions under microwave irradiation were performed using CEM Discover mode l908005 using closed vessel with magnetic stirring. All reactions were followed up by thin layer chromatography (TLC) using aluminum sheets recoated with UV fluorescent silica gel (Merck Kieselgel 60 F_{245}), UV lamp, iodine vapor, and ninhydrin reagent.

Synthesis of 6-(1H-indol-3-yl)-2-oxo-4-substituted-1,2-dihydropyridine-3-carbonitrile (**13a-e**).

Method (A). A mixture of ethyl cyanoacetate (1.13 g, 10 mmol), 3-indolylmethyl ketone **1** (1.59 g, 10 mmol), aromatic aldehydes (2-thiophenaldehyde (1.12 g, 10 mmol), 4-methoxybenzaldehyde (1.36 g, 10 mmol), 4-fluorobenzaldehyde (1.24 g, 10 mmol), 4-chlorobenzaldehyde (1.40 g, 10 mmol), or 4-bromobenzaldehyde (1.85 g, 10 mmol)), ammonium acetate (4.62 g, 6 mmol) in anhydrous ethylene glycol (3 mL) and pipredine (1 mL) as a catalyst

were placed in a (100 mL) round flask and heated under reflux for given time 10-17 h. The reaction progress was monitored by TLC. Then it was cooled to room temperature after completion. The crude solid product was filtrated off, dried, and recrystallized from methanol/DMF (12 mL, 3:1 v/v) giving the corresponding derivatives **13a-e** as yellow crystals.

Method (B). The procedure was similar to that described in method A except that the solvent used is ethylene glycol (1 mL) and the mixture was placed in closed (10 mL) vessels and irradiated in a microwave reactor at 250 W and 150 ^{o}C for a given time. The reaction progress was monitored by TLC. The reaction mixture was cooled to room temperature after completion, then the crude material boiled in ethanol (20 mL) where a yellow solid product was separated, filtrated off, dried, and recrystallized from methanol/DMF (12 mL 3:1 v/v) to give derivatives **13a-e** as yellow crystals.

6-(1H-Indol-3-yl)-2-oxo-4-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile (**13a**)**.** Yield (77%); mp: 259-261 ^{o}C; IR (KBr) cm^{-1}: 3275 (NH indole), 3116 (NH pyridone), 2209 (CN), and 1661 (CO amide). ^{1}H-NMR (DMSO-d$_6$) σ 6.67 (s, 1H, H-5 pyridone), 7.10 (m, 2H, indole); 7.30 (d, *J* = 7.5 Hz, 1H, indolyl); 7.43 (t, *J* = 7.3 Hz, 1H, thiophenyl ring), 7.67 (d, *J* = 6.4 Hz, 1H, thiophenyl ring), 7.95 (d, *J* = 6.2 Hz ,1H, thiophenyl ring), 8.02 (d, *J* = 7.5 Hz, 1H, indolyl), 8.33 (d, 1H, indolyl), 11.62 (s,1H, NH pyridone, D_2O exchangeable); 11.81 (broad, 1H, NH indole, D_2O exchangeable). ^{13}C NMR (DMSO-d$_6$), σ 102.91 (CN), 108.20, 113.35, 118.06, 119.88, 122.10, 123.37, 124.56, 129.29, 130.08, 130.98, 131.65, 137.58, 137.86, 147.01, 148.23, 151.05

(aromatic carbons), 162.84 (CO amide). MS (m/z, %): calcd. for $C_{18}H_{11}N_3OS$: 317.06; found 317.00 [M^+, 100%].

6-(1H-Indol-3-yl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**13b**). Yield (79%); mp: 283-286 ^oC; IR (KBr) cm^{-1}: 3448 (NH indolyl ring), 3262 (NH pyridone), 2210 (CN), and 1644 (CO amide). ^{1}H-NMR (DMSO-d_6) σ 3.8 (s, 3H, OC*H*$_3$), 6.57 (s, 1H, H-5 pyridone), 7.08 (d, *J* = 8.4 Hz, 2H, aromatic), 7.18 (m, 2H, indolyl), 7.49 (d, *J* = 8.5 Hz, 1H, indolyl), 7.69 (d, *J* = 8.4 Hz, 2H, aromatic), 7.86 (d, *J* = 8.5 Hz, 1H, indolyl), 8.27 (d, 1H, indolyl), 12.06 (s, 1H, NH pyridone, D_2O exchangeable), 12.35 (1H, d, NH indole, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6), σ 108.82 (CN), 113.28, 114.81, 117.96, 120.09, 121.99, 123.33, 124.56, 129.13, 129.71, 129.88, 130,32, 137.35, 137.51, 147.59, 159.9, 161.47 (aromatic carbons), 162.43 (CO amide). MS (m/z, %) calcd. for $C_{21}H_{15}N_3O_3$: 341.12; found 341.00 [M^+, 100].

6-(1H-Indol-3-yl)-(4-florophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**13c**). Yield (87%); mp > 310 ^oC; IR (KBr) cm^{-1}: 3356 (NH indolyl), 3109 (NH pyridone), 2210 (CN), and 1635 (CO amide). ^{1}H-NMR (DMSO-d_6) σ 6.69 (s, 1H, H5-pyridone), 7.24 (m, 2H, indolyl), 7.44 (d, *J* = 8.7 Hz, 2H, aromatic); 7.51 (d, *J* = 7.71Hz, 1H, indolyl), 7.82 (d, *J* = 8.5 Hz, 2H, aromatic), 7.92 (d, *J* = 8.2 Hz, 1H, indolyl), 8.31 (d, 1H, indolyl), 12.11 (s, 1H, NH pyridone, D_2O exchangeable), 12.42 (d, 1H, NH indolyl, D_2O exchangeable). ^{13}C-NMR, (DMSO-d_6) σ 104. 48 (CN), 112.25, 113.21, 115.19, 115.34, 116.31, 116.45, 120.76, 121.98, 122.44, 123.33,

124.57, 128.53, 130.06, 131.12, 137.53, 159.52 (aromatic carbons), 162.69 (CO amide). MS (m/z, %) calcd. for $C_{20}H_{12}FN_3O$: 329.10; found 329.00 [M^+, 100].

6-(1H-Indol-3-yl)-4-(4-chlorophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**13d**)**.** Yield (82%); mp > 310 ^oC; IR (KBr) cm^{-1}: 3418 (NH indolyl), 3128 (NH pyridone), 2219 (CN), and 1636 (CO amide). ^1H-NMR (DMSO-d_6) σ 6.76 (s, 1H, H-5 pyridone), 7.17-.22 (m, 2H, indolyl), 7.52 (d, *J* = 7.7 Hz,1H, indolyl), 7.65 (d, *J* = 8.1 Hz, 2H, aromatic), 7.77 (d, *J* = 8.2 Hz, 2H, aromatic), 7.93 (1H, *J* = 8.76 Hz, d, indolyl), 8.32 (d, 1H, indolyl), 12.12 (s, 1H, NH pyridone, D_2O exchangeable); 12.41 (d,1H, NH indole, D_2O exchangeable). ^{13}C-NMR, (DMSO-d_6) σ 108.40 (CN), 108.46, 109.90, 113.23, 117.51, 120.23, 122.01, 123.35, 124.56, 129.40, 130.14, 130.53, 135.58, 135.90, 137.53, 148.53, 159.05 (aromatic carbons), 162.60 (, CO amide). MS (m/z, %) calcd. for $C_{20}H_{12}ClN_3O$: 345.07; found 345.00 [M^+, 15].

6-(1H-Indol-3-yl)-4-(4-bromophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**13e**)**.** Yield (83%); mp > 310 ^oC; IR (KBr) cm^{-1}: 3259 (NH indolyl), 3114 (NH pyridone), 2210 (CN), and 1638 (CO amide). ^1H-NMR (DMSO-d_6) σ 6.69 (s, 1H, H-5 pyridone); 7.17-7.23 (m, 2H, indolyl), 7.47 (d, *J* = 6.7 Hz, 1H, indolyl), 7.65 (d, *J* = 8.4 Hz, 1H, aromatic), 7.76 (2H, *J* = 8.4 Hz, d, aromatic), 7.87 (d, *J* = 6.7 Hz, 1H, indolyl), 8.28 (d, 1H, indolyl), 12.11 (s, 1H, NH pyridone, D_2O exchangeable); 12.54 (d, 1H, NH indolyl, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6) σ 104.32 (CN), 113.21, 117.50, 120.24, 122.02, 123.37, 124.35, 124.56, 130.16, 130.76, 132.35, 136.29, 137.52, 159.17 (aromatic carbons), 162.60 (CO amide), MS m/z calcd. for $C_{20}H_{12}FN_3O$: 389.02; found 389.00 [M^+, 100].

2. 2-Chloro-6-(1H-indol-3-yl)-4 substituted nicotinonitrile (**14a-e**)**.** A mixture of compound **2** (0.01 mmol) and phosphoryl chloride (10 mL) was subjected to gentle heating under reflux for 18-24 h. Then, it was poured onto crushed ice and neutralized by a saturated solution of $NaHCO_3$. Whereby, a yellow solid product was separated, filtered off, washed with water for several times, dried, and crystallized from ethanol (25 mL) affording pale yellow crystals of compounds **14a-e**.

2-Chloro-6-(1H-indol-3-yl)-4-(thiophen-2-yl)nicotinonitrile (14a). Yield (95%); mp: 274-277 ^{o}C; IR (KBr) cm^{-1}: 3305 (NH indolyl), and 2224 (CN). ^{1}H-NMR (DMSO-d_6) σ 7.23 (t, *J* = 6.2 Hz, 2H, indolyl ring), 7.30 (t, *J* = 9.2 Hz, 1H, thiophene), 7.52 (d, *J* = 9.2 Hz, 1H, indolyl), 7.85 (d, *J* = 9.9 Hz, 1H, thiophenyl), 7.93 (d, *J* = 9.9 Hz, 1H, thiophenyl), 8.03(s, 1H, pyridine), 8.46 (d, *J* = 9.2 Hz, 1H, indolyl), 8.50 (d, 1H, indolyl), 12.10 (broad,1H, NH indolyl, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6) σ 100.02 (CN), 112.82, 113.49, 116.45, 116.45, 121.79, 122.13, 123.19, 125.52, 129.04, 130.75, 131.24, 131.33, 136.83, 137.74, 146.54, 153.71, 158.87 (aromatic carbons). MS (m/z, %) calcd. for $C_{18}H_{10}ClN_3S$: 335.03; found 335.00 [M^+, 100].

2-Chloro-6-(1H-indol-3-yl)-4-(4-methoxyphenyl)nicotinonitrile (14b). Yield (98%); mp: 255-257 ^{o}C; IR (KBr) cm^{-1}: 3319 (NH indolyl), and 2219(CN). ^{1}H-NMR (DMSO-d_6): σ 3.83 (s, 3H, OCH_3), 7.12 (d, *J* = 6.9 Hz, 2H, aromatic), 7.20 (t, *J* = 6.2 Hz, 2H, indolyl), 7.45 (d, *J* = 6.1 Hz, 1H, indolyl), 7.70 (d, *J* = 6.9 Hz, 2H, aromatic), 7.99 (s,1H, H-5 pyridine), 8.41 (d, *J* = 6.1 Hz, 1H, indolyl), 8.53 (d, 1H, indolyl), 12.03 (broad, 1H, NH indolyl, D_2O exchangeable). ^{13}C-NMR

(DMSO-d*6*): σ 55.95 (OC*H₃*); 102.09 (CN), 112.83, 113.64, 114.80, 116.74, 117.88, 121.79, 122.15, 123.21, 125.55, 128.21, 130.77, 131.13, 137.71, 153.00, 154.59, 158.83, 161.35 (aromatic carbons). MS (m/z, %) calcd. for $C_{21}H_{14}ClN_3O$: 358.08; found 358.00 [M^+, 100].

2-Chloro-4-(4-fluorophenyl)-6-(1H-indol-3-yl)nicotinonitrile (14c). Yield (95%); mp: 290-293 ^oC; IR (KBr) cm^{-1}: 3227 (NH indolyl) and 2217 (CN). ^1H-NMR (DMSO-d_6): σ 7.19 (d, *J* = 8.4 Hz, 2H, aromatic), 7.34-7.41 (m, 2H, indolyl), 7.47 (d, *J* = 7.6 Hz, 1H, indolyl), 7.75 (2H, *J* = 8.4 Hz, d, aromatic), 7.96 (s, 1H, H-5 pyridine), 8.38 (d, *J* = 7.6 Hz,1H, indolyl), 8.45 (d,1H, indolyl), 11.27 (broad, 1H, NH indolyl, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): σ 102.44 (CN), 112.84, 113.61, 116.29, 116.65, 118.29, 121.86, 122.19, 123.26, 125.55, 131.43, 131.64, 131.71, 137.74, 152.85, 153.84, 159.05, 162.72, 164.69 (aromatic carbons). MS (m/z) calcd. for $C_{20}H_{11}ClFN_3$: 347.06; found 347 [M^+].

2-Chloro-4-(4-chlorophenyl)-6-(1H-indol-3-yl)nicotinonitrile (14d). Yield (98%); mp: 270-273 ^oC; IR (KBr) cm^{-1}: 3290 (NH indolyl) and 2224 (CN). ^1H-NMR (DMSO-d6): σ 7.21-7.25 (m, 2H, indolyl), 7.52 (d, *J* = 7.7 Hz, 1H, indolyl), 7.60 (d, *J* = 8.4 Hz, 2H, aromatic), 7.78 (d, *J* = 8.4 Hz, 2H, aromatic), 7.99 (s, 1H, H-5 pyridine), 8.44 (d, *J* = 7.7 Hz, 1H, indolyl), 8.53 (d, 1H, indolyl), 11.27 (broad,1H, NH indolyl, D_2O exchangeable). ^{13}C (DMSO-d_6) σ 102 (CN), 112.87, 113.59, 116.36, 118.27, 121.91, 122.19, 123.31, 125.54, 129.43, 131.13, 131.53, 135.00, 135.63, 137.75, 152.86, 153.66, 159.12 (aromatic carbons). MS (m/z, %) calcd. for $C_{20}H_{11}Cl_2N_3$: 363.03; found 363.00 [M^+, 100].

2-Chloro-4-(4-bromophenyl)-6-(1H-indol-3-yl)nicotinonitrile (14e). Yield (97%); mp: 272-275 ^{o}C; IR (KBr) cm^{-1}: 3288 (NH indolyl), and 2220 (CN). ^{1}H NMR (DMSO-d$_6$): σ 7.19-7.20 (t, *J* = 7.7 Hz, 2H, indolyl), 7.45 (d, *J* = 6.9 Hz, 1H, indolyl), 7.67 (d, *J* = 8.4 Hz, 2H, aromatic), 7.79 (d, *J* = 8.4 Hz, 2H, aromatic), 8.05 (s, 1H, H-5 pyridine), 8.43 (1H, *J* = 6.9 Hz, d, indolyl), 8.54 (d, 1H, indolyl), 12.07 (broad, 1H, NH indolyl, D_2O exchangeable). ^{13}C (DMSO-d$_6$): σ 102.30 (CN); 112.86, 113.61, 116.31, 118.21, 121.91, 122.21, 123.29, 124.38, 125.55, 131.34, 131.55, 132.35, 135.37, 137.75, 152.87, 152.87, 153.7, 159.13 (aromatic carbons). MS (m/z) calcd. for $C_{20}H_{11}BrClN_3$: 428.98; found 428.00 [M^+ + Na].

3. 2-((2-Aminoethyl)amino)-6-(1H-indol-3-yl)-4-substitued nicotinonitrile (15a-e). A mixture of compound **14a-e** (10 mmol) and ethylendiamine (15 mmol) in ethanol (10 mL) was refluxed. The progress of the reaction was monitored by TLC using eluent system (DCM: Methanol 2:1 v/v) for 48 h. Then the reaction mixture was poured onto crushed ice and neutralized by HCl (1 M), filtered off, washed with water, dried and crude products crystallized from methanol (25 mL) pale yellow crystals of compounds **15a-e**.

2-((2-Aminoethyl)amino)-6-(1H-indol-3-yl)-4-(thiophen-2yl)nicotinonitrile (15a). Yield (91%); mp: 214-217 ^{o}C; IR (KBr) cm^{-1}: 3361 (NH), 3307 (NH_2), and 2191(CN). ^{1}H-NMR (DMSO-d$_6$): σ 2.94 (t, *J* = 6.1Hz, H, C*H*$_2$), 3.47 (broad, 2H, NH_2, D_2O exchangeable), 3.69 (m, 2H, C*H*$_2$), 4.09 (broad, 1H, NH, D_2O exchangeable), 7.18 (t, *J* = 6.9 Hz, 2H, indolyl), 7.30 (t, *J* = 8.8 Hz, 1H, thiophenyl), 7.46 (d, *J* = 7.2 Hz, 1H, thiophenyl), 7.47 (d, *J* = 8.6 Hz, 1H, thiophenyl), 7.80 (d, *J* = 8.5 Hz, 1H, indolyl), 7.83 (1H, *J* = 8.6 Hz, d, indolyl), 8.09 (s, 1H, H-5

pyridine), 8.41 (d, 1H, indolyl), 11.82 (1H, d, NH indolyl, D_2O exchangeable). ^{13}C-(DMSO-d_6) σ 50.67 (*C*H_2), 54.53 (*C*H_2); 107.42 (CN), 112.76, 115.03, 121.68, 121.98, 122.94, 125.50, 128.98, 129.14, 129.55, 129.74, 131.28, 133.31, 137.53, 138.47, 145.80, 153.42, 160.22 (aromatic carbons). MS (m/z, %) calcd. for $C_{20}H_{17}N_5S$: 359.12; found 359.00 [M^+, 100].

2-((2-Aminoethyl)amino)-6-(1H-indol-3-yl)-4-(4-methoxyphenyl)nicotinonitrile (**15b**). Yield (96%); mp: 205-209 ^{o}C; IR (KBr) cm^{-1}: 3366 (NH & NH_2), 2201 (CN). ^{1}H-NMR (DMSO-d_6): σ 2.86 (t, *J* = 6.1 Hz, 2H, C_{H2}); 3.31 (broad, 1H, NH, D_2O exchangeable), 3.66 (m, 2H, C*H*$_2$), 3.82 (s, 3H, OC*H*$_3$), 4.09 (broad, 1H, N*H*); 7.07 (d, *J* = 8.4 Hz, 2H, aromatic), 7.14 (t, *J* = 77 Hz, 2H, indolyl), 7.33 (d, *J* = 6.1 Hz, 1H, indolyl), 7.41 (d, *J* = 6.1 Hz,1H, indoly), 7.60 (d, *J* = 8.4 Hz, 2H, aromatic), 8.31 (s, 1H, H-5 pyridine), 8.47 (d, 1H, indolyl), 11.73 (d, 1H, NH indolyl, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6) σ 40.16 (*C*H_2), 40.33 (*C*H_2), 55.87 (O*C*H_3), 108.11 (CN); 112.51, 114.63, 121.17, 122.39, 122.55, 125.97, 128.86, 129.39, 130.24, 137.62, 153.44, 158.18, 158.44, 160.16, 160.70 (aromatic carbons). MS (m/z, %) calcd. for $C_{23}H_{21}N_5O$: 3830.17; found 383.00 [M^+, 10].

2-((2-Aminoethyl)amino)-4-(4-fluorophenyl)-6-(1H-indol-3-yl)nicotinonitrile (**15c**). Yield (89%); mp: 193-196 $^\circ$C; IR (KBr) cm^{-1}: 3381 (NH), 3049 (NH_2), and 2195 (CN). ^{1}H-NMR (DMSO-d_6): σ 2.98 (t, *J* = 6.2 Hz, 2H, CH_2), 3.13 (broad, 1H, NH, D_2O exchangeable), 3.42. (m, 2H, CH_2), 4.10 (broad, 1H, NH, D_2O exchangeable), 7.19 (t, *J* = 7.6 Hz, 2H, indole), 7.25 (d, *J* = 7.1 Hz, 1H, indolyl), 7.13 (d, *J* = 8.5 Hz, 2H, aromatic), 7.34 (d, *J* = 8.5 Hz, 2H, aromatic), 8.01 (d, *J* =7.3 Hz, 1H, indolyl), 8.21 (s, 1H, H-5 pyridine), 8.31 (d, 1H, indolyl), 11.59 (d, 1H, NH

indolyl, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6) σ 40.88 (*C*H_2), 43.87 (*C*H_2), 107.76 (CN), 112.36, 112.67, 115.66, 119.24, 121.29, 122.12, 122.62, 124.64, 125.87, 128.88, 129.79, 137.68, 150.45, 154.30, 157.72, 160.31, 162.82 (aromatic carbons). MS (m/z, %) calcd. for $C_{22}H_{18}FN_5$ 371.15; found 371.00 [M^+, 100].

2-((2-Aminoethyl)amino)-4-(4-chlorophenyl)-6-(1H-indol-3-yl)nicotinonitrile (15d). Yield (93%); mp: 204-207 °C; IR (KBr) cm^{-1}: 3381, 3049 (NH and NH_2), and 2195 (CN). ^{1}H-NMR (DMSO-d_6) σ 2.91 (t, *J* = 6.4 Hz, 2H, C*H*$_2$), 3.42 (broad, 2H, NH_2, D_2O exchangeable), 3.62 (m, 2H, C*H*$_2$); 4.09 (broad, 1H, NH, D_2O exchangeable), 7.19 (t, *J* = 7.7 Hz, 2H, indolyl), 7.22 (d, *J* = 6.1 Hz, 1H, indolyl), 7.46 (d, *J* = 6.1 Hz, 1H, indolyl); 7.60 (d, *J* = 8.4 Hz, 2H, aromatic); 7.65 (2H, *J* = 8.4 Hz, d, aromatic); 8.36 (s,1H, H-5 pyridine); 8.48 (d, 1H, indolyl), 11.79 (1H, d, NH indolyl, D_2O exchangeable). ^{13}C (DMSO-d_6) σ 39.99 (*C*H_2), 40.16 (*C*H_2), 108.20 (CN), 112.36, 112.66, 115.66, 119.23, 121.29, 122.10, 122.62, 124.65, 125.87, 128.88, 129.79, 137.68, 150.45, 154.30, 157.71, 160.31, 162.82 (aromatic carbons). MS (m/z, %) calcd. for $C_{22}H_{18}ClN_5$: 387.13; found 387.00 [M^+, 9].

2-((2-Aminoethyl)amino)-4-(4-bromophenyl)-6-(1H-indol-3-yl)nicotinonitrile (15e). Yield (93%); mp: 198-201 ^{o}C; IR (KBr) cm^{-1}: 3377 (NH), 3059 (NH_2), and 2202 (CN).^{1}H NMR (DMSO-d_6): σ 2 .69 (2H, *J* = 6.1 Hz, C*H*$_2$), 3.35 (2H, broad, NH_2, D_2O exchangeable), 3.59 (m, 2H, C*H*$_2$), 4.07 (broad, 1H, NH, D_2O exchangeable), 7.14 (t, *J* = 6.9 Hz, 2H, indolyl), 7.18 (d, *J* = 9.9 Hz, 1H, indolyl), 7.42 (1H, *J* = 8.5 Hz, d, indolyl), 7.58 (2H, *J* = 8.5 Hz, d, aromatic), 7.73 (d, *J* = 8.5 Hz, 2H, aromatic), 8.31(s, 1H, H-5 pyridine), 8.48 (d, 1H, indolyl), 11.84 (d, 1H, NH

indolyl, D_2O exchangeable). ^{13}C (DMSO-d_6): σ 40.48 (*C*H_2), 40.74 (*C*H_2), 108.71 (CN), 112.50, 115.32, 115.45, 117.91, 122.00, 122.51, 123.52, 125.73, 129.85, 129.85, 130.55, 132.25, 137.00, 137.55, 152.56, 158.34, 159.77 (aromatic carbons). MS (m/z, %) calcd. for $C_{22}H_{18}BrN_5$: 432.07; found 432.00 [M^+, 55].

Cell culture

Cell culture. Human cervix adenocarcinoma HeLa (ATCC no. CCL-2), human ovarian adenocarcinoma cell line SK-OV-3 (ATCC no. HTB-77), and human breast adenocarcinoma MCF-7 (ATCC no. HTB-22) were obtained from American Type Culture Collection. Cells were grown on 75 cm^2 cell culture flasks with EMEM (Eagle's minimum essential medium), supplemented with 10% fetal bovine serum, and 1% penicillin/streptomycin solution (10,000 units of penicillin and 10 mg of streptomycin in 0.9% NaCl) in a humidified atmosphere of 5% CO_2, 95% air at 37 ºC.

Cell proliferation assay. Cell proliferation assay was carried out using CellTiter 96 aqueous one solution cell proliferation assay kit (Promega, USA). Briefly, upon reaching about 75-80% confluency, 5000 cells/well were plated in 96-well microplate in 100 µL media. After seeding for 24 h, the cells were treated with compounds (50 µM) in triplicate. Doxorubicin (10 µM) was used as the positive control. At the end of the sample exposure period (72 h), 20 µL CellTiter 96 aqueous solution was added. The plate was returned to the incubator for 1 h in a humidified atmosphere at 37 °C. The absorbance of the formazan product was measured at 490 nm using microplate reader. The blank control was recorded by measuring the absorbance at 490 nm with

wells containing medium mixed with CellTiter 96 aqueous solution but no cells. Results were expressed as the percentage of the control (without compound set at 100%).

Partition Coefficient: Calculated partition coefficient values of all the compounds were determined by using ChemDraw 10.0 software from CambridgeSoft (Table S1).

Table S1. Calculated partition coefficient of synthesized compounds.

Compound No,	Log P[a]
13a	2.43
13b	2.32
13c	2.61
13d	3.01
13e	3.28
14a	4.93
14b	4.82
14c	5.10
14d	5.50
14e	5.77
15a	3.34
15b	3.23
15c	3.52
15d	3.92
15e	4.19
Dox	-1.34

[a]Calculated partition coefficient determined by using ChemDraw 10.0 software from CambridgeSoft.

IC_{50} determination assay. IC_{50} determination assay was carried out using CellTiter 96 aqueous one solution cell proliferation assay kit (Promega, USA). Briefly, SK-OV-3, HeLa, and MCF-7 cells (5000 cells/well) were plated in 96-well microplate in 100 µL media. After seeding for 24 h, the cells were treated with different concentrations of compounds (1 µM- 50 µM) in triplicate. After 72 h of incubation, 20 µL CellTiter 96 aqueous solution was added. The plate was returned to the incubator for 1 h in a humidified atmosphere at 37 $^{\circ}$C. The absorbance of the formazan product was measured at 490 nm using microplate reader. The blank control was recorded by measuring the absorbance at 490 nm with wells containing medium mixed with CellTiter 96 aqueous solution but no cells. The IC_{50} values were extrapolated from concentration–effect curves using linear regression analysis in GraphPad Prism®, version 5.03.

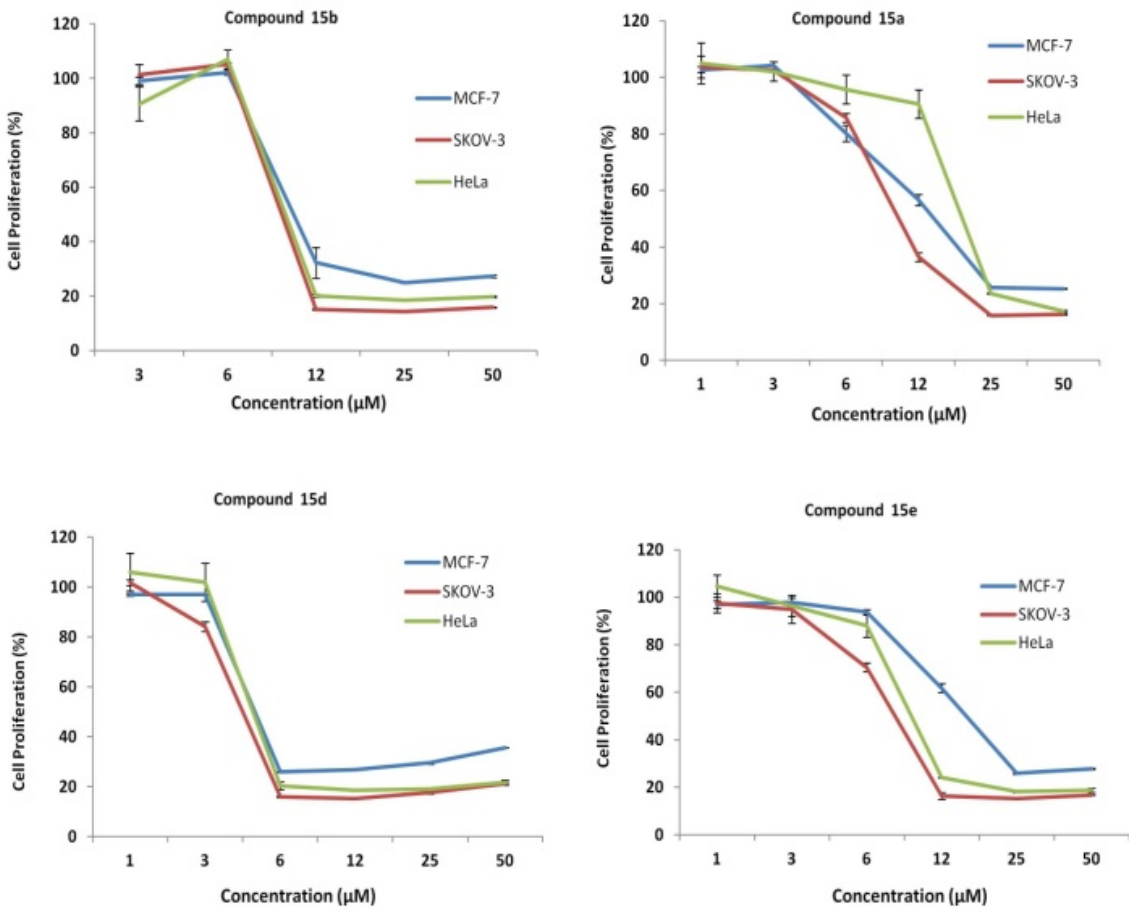
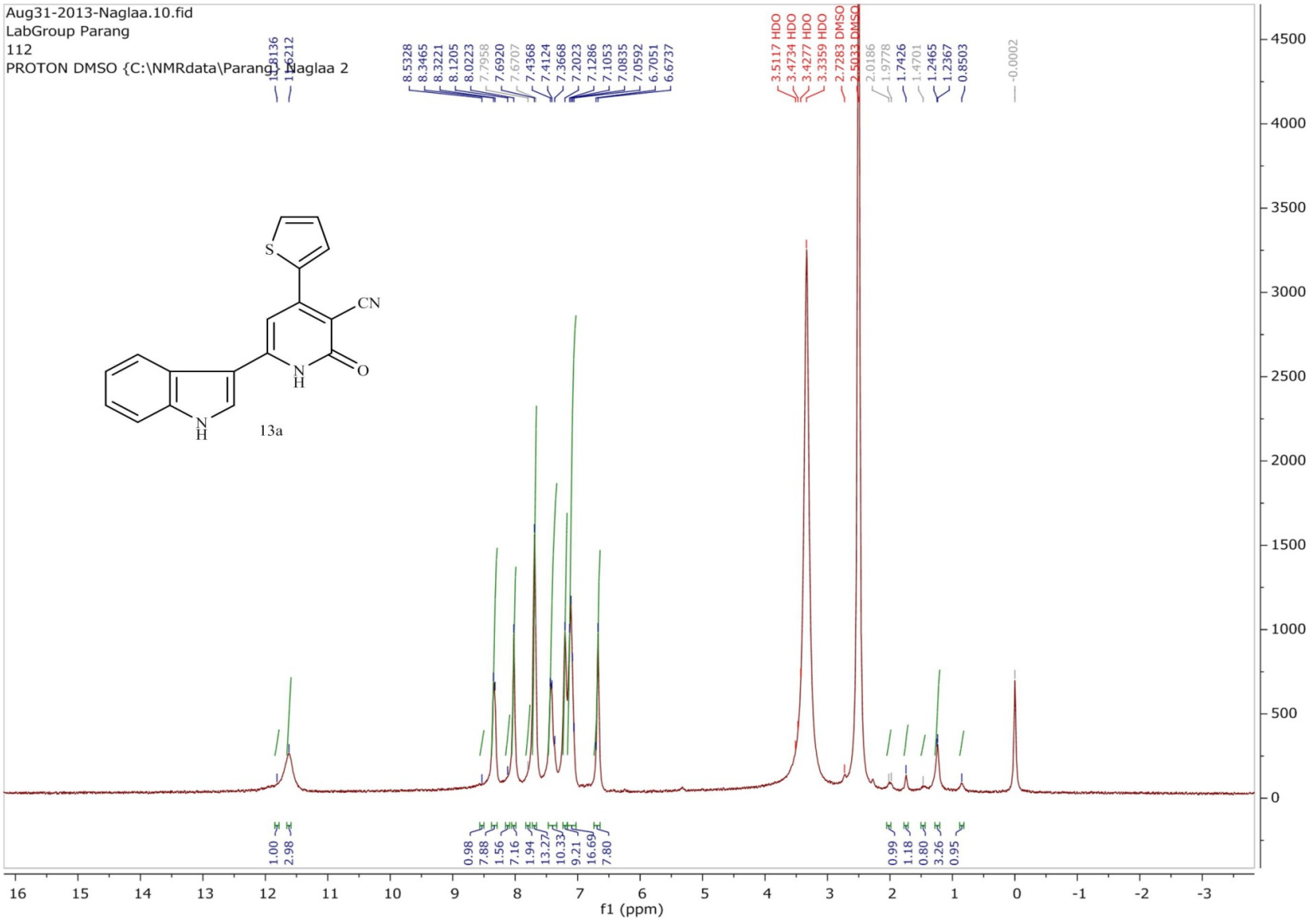


Figure S1. IC_{50} determination for compounds **15a**, **15b**, **15d**, and **15e** against SKOV-3, MCF-7 and Hela cells.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LabGroup Parang
112
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12.6212
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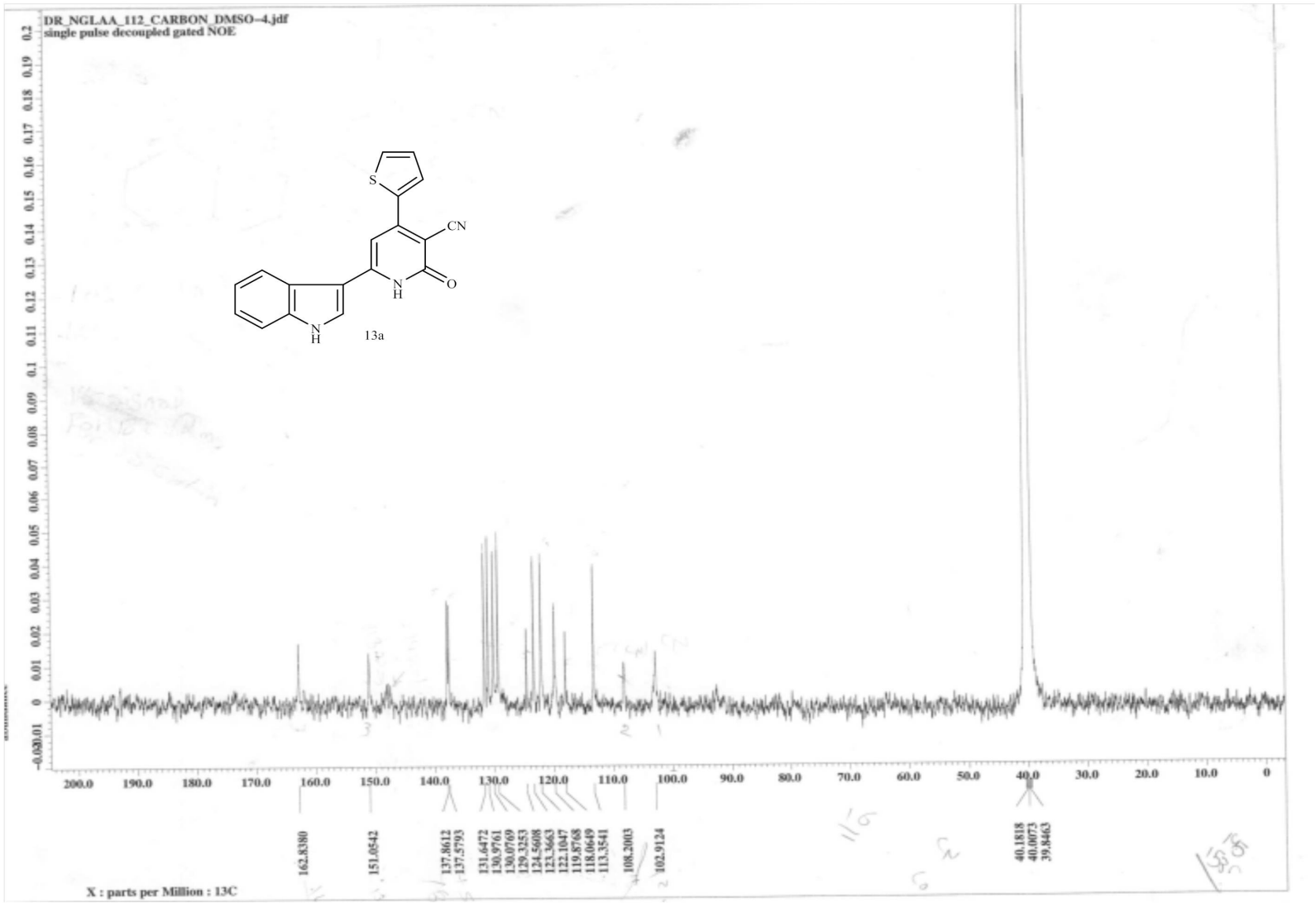
8.3465
8.3221
8.1205
8.0223
7.7958
7.6920
7.6707
7.4368
7.4124
7.3668
7.2023
7.1286
7.1053
7.0835
7.0592
6.7051
6.6737
3.5117 HDO
3.4734 HDO
3.4277 HDO
3.3359 HDO
2.7283 DMSO
2.5033 DMSO
2.0186
1.9778
1.7426
1.4701
1.2465
1.2367
0.8503
-0.0002
S
CN
N
H
O
N
H
13a
1.00
2.98
0.98
7.88
1.56
7.16
1.94
13.27
10.33
9.21
16.69
7.80
0.99
1.18
0.80
3.26
0.95
f1 (ppm)


DR_NGLAA_112_CARBON_DMSO--4.jdf
single pulse decoupled gated NOE
S
CN
O
N
H
N
H
13a
X : parts per Million : 13C
162.8380
151.0542
137.8612
137.5793
131.6472
130.9761
130.0769
129.3253
124.5608
123.3663
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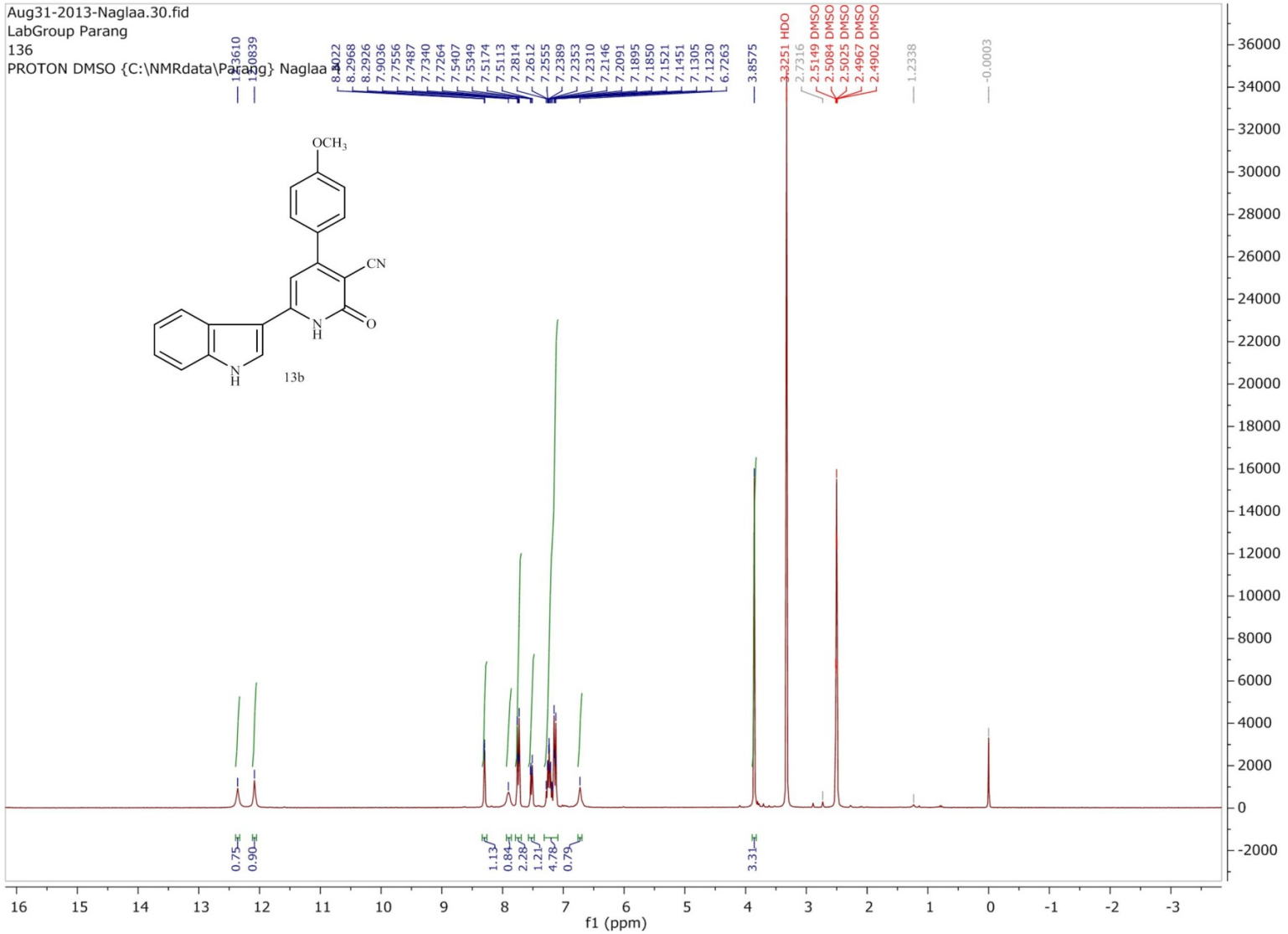
40.1818
40.0073
39.8463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LabGroup Parang
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8.2926
7.9036
7.7556
7.7487
7.7340
7.7264
7.5407
7.5349
7.5174
7.5113
7.2814
7.2612
7.2555
7.2389
7.2353
7.2310
7.2146
7.2091
7.1895
7.1850
7.1521
7.1451
7.1305
7.1230
6.7263
3.8575
3.3251 HDO
2.7316
2.5149 DMSO
2.5084 DMSO
2.5025 DMSO
2.4967 DMSO
2.4902 DMSO
1.2338
-0.0003
OCH3
CN
O
N
H
N
H
13b
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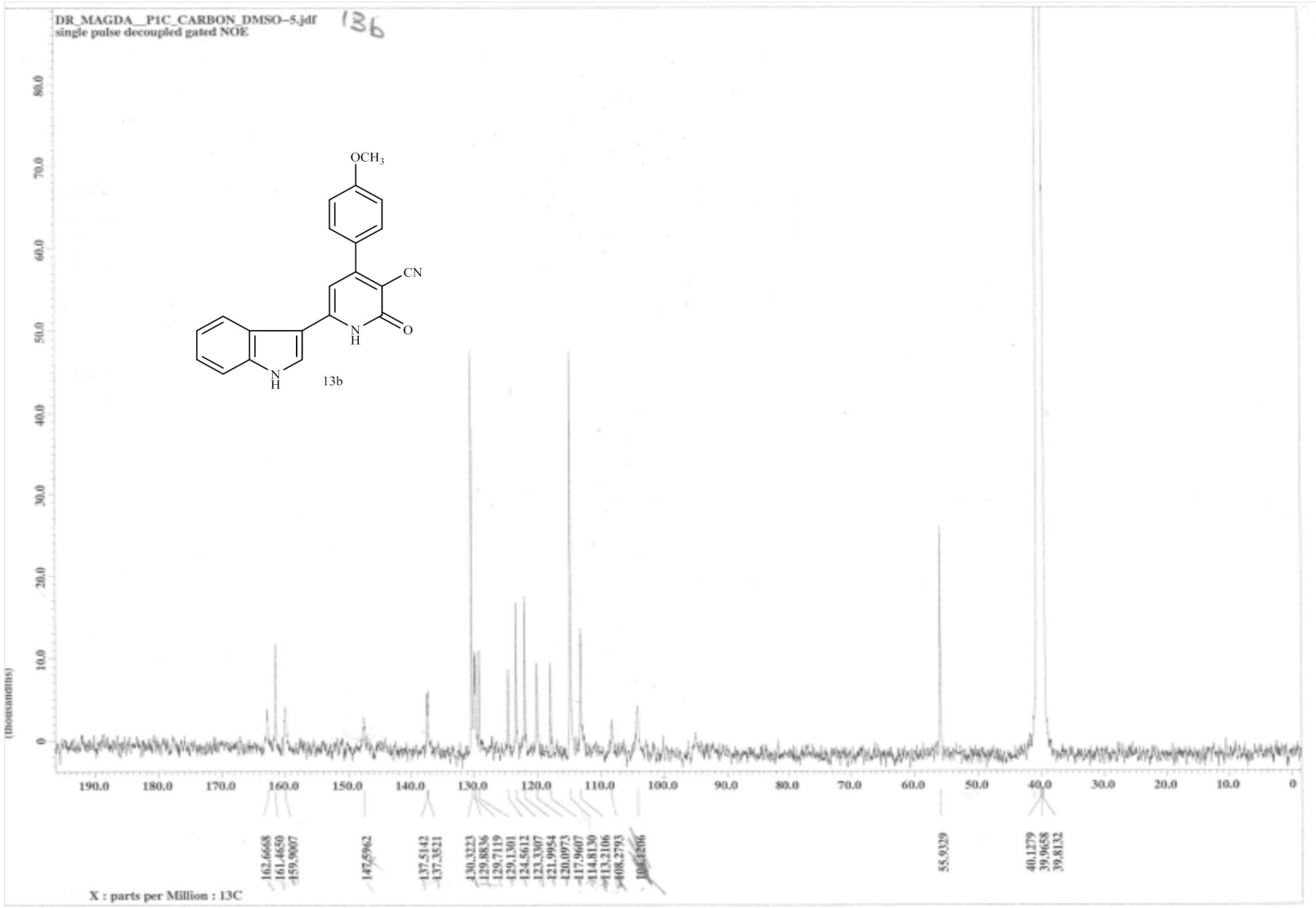
0.90
1.13
0.84
2.28
1.21
4.78
0.79
3.31
f1 (ppm)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single pulse decoupled gated NOE
13b
OCH3
CN
O
N
H
N
H
13b
(thousands)
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
162.6668
161.4650
159.9007
147.5962
137.5142
137.3521
130.3223
129.8836
129.7119
129.1301
124.5612
123.3307
121.9954
120.0973
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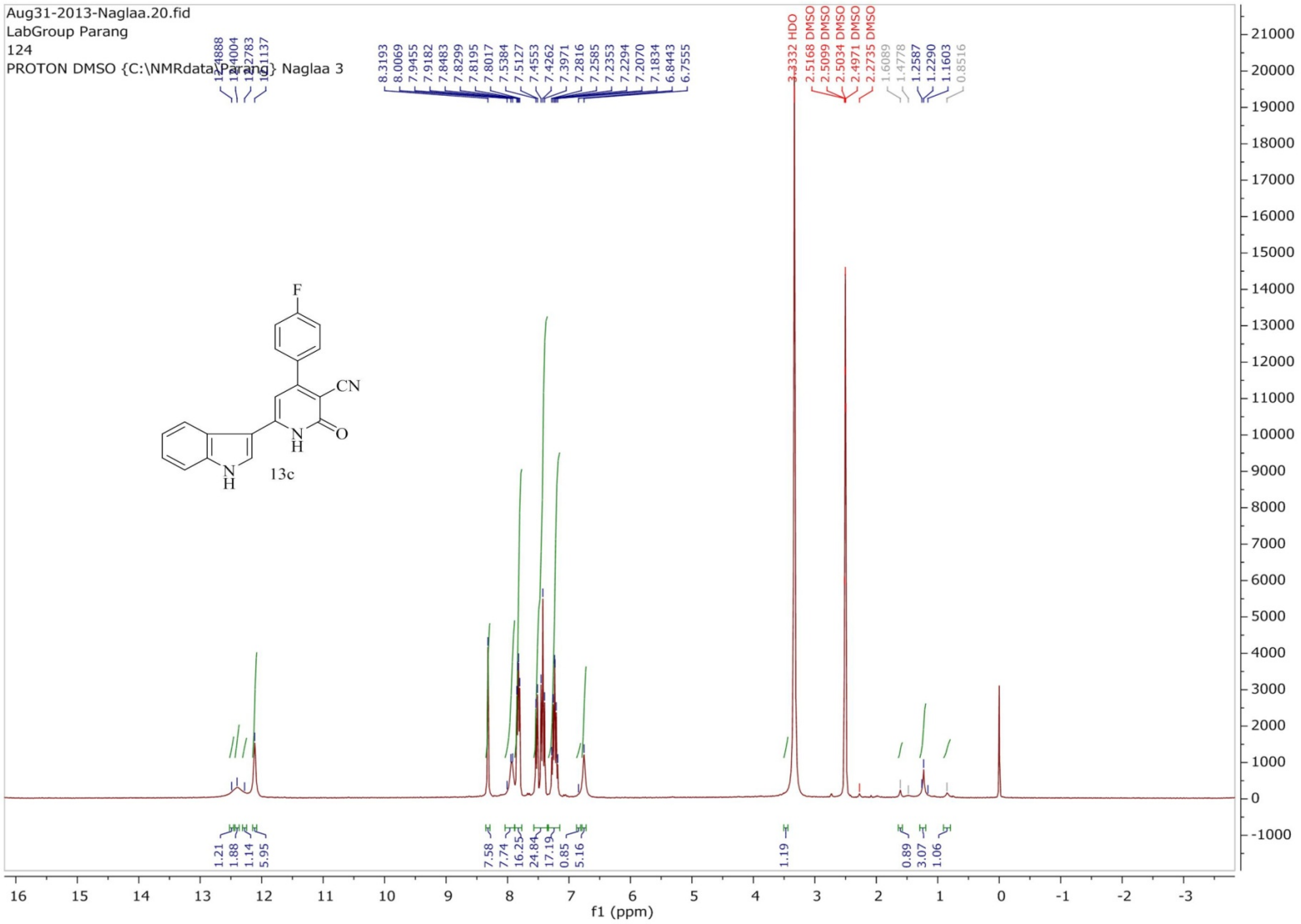
39.9658
39.8132
X : parts per Million : 13C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LabGroup Parang
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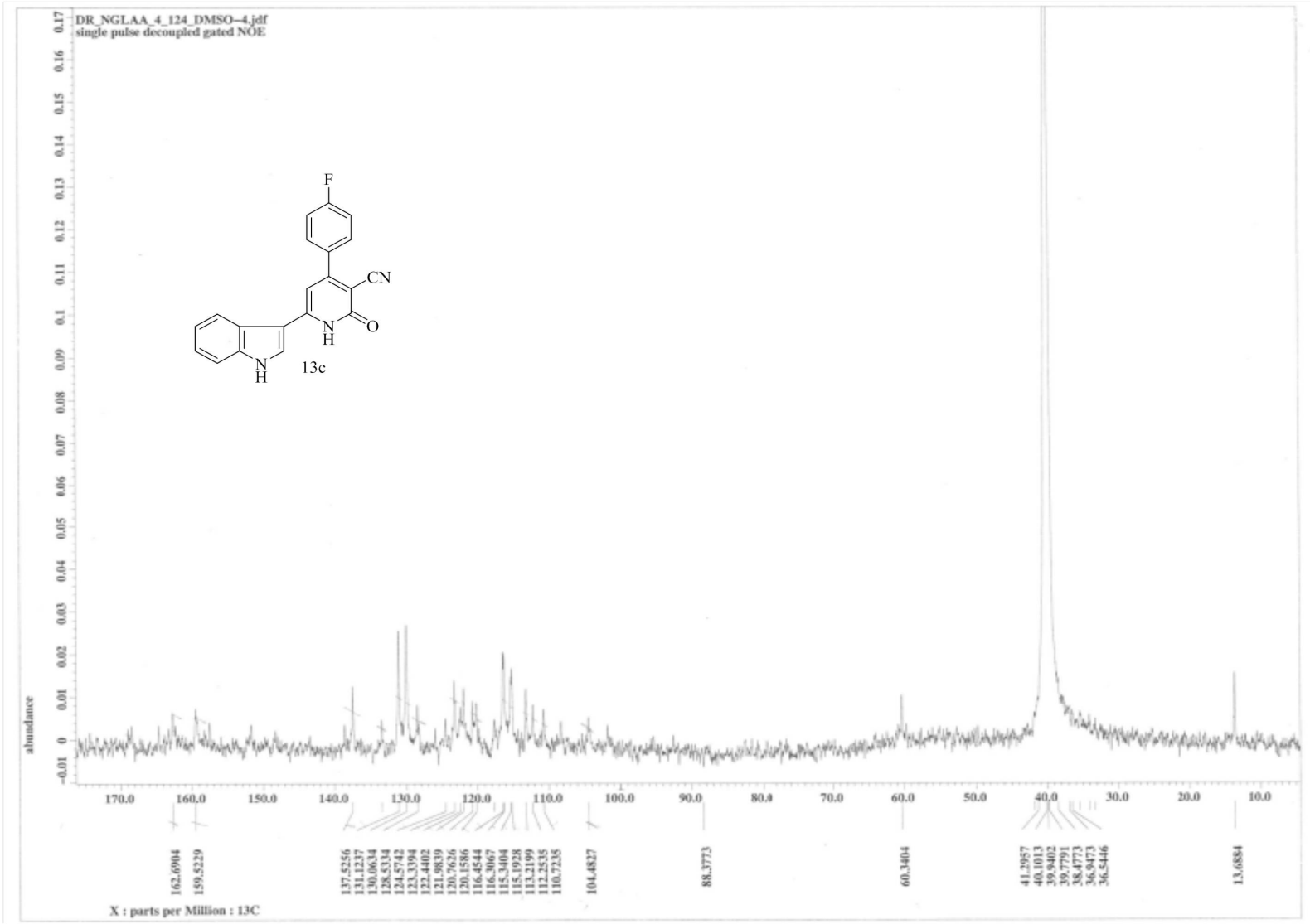
13c
f1 (ppm)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single pulse decoupled gated NOE
F
CN
N
H
O
N
H
13c
abundance
X : parts per Million : 13C
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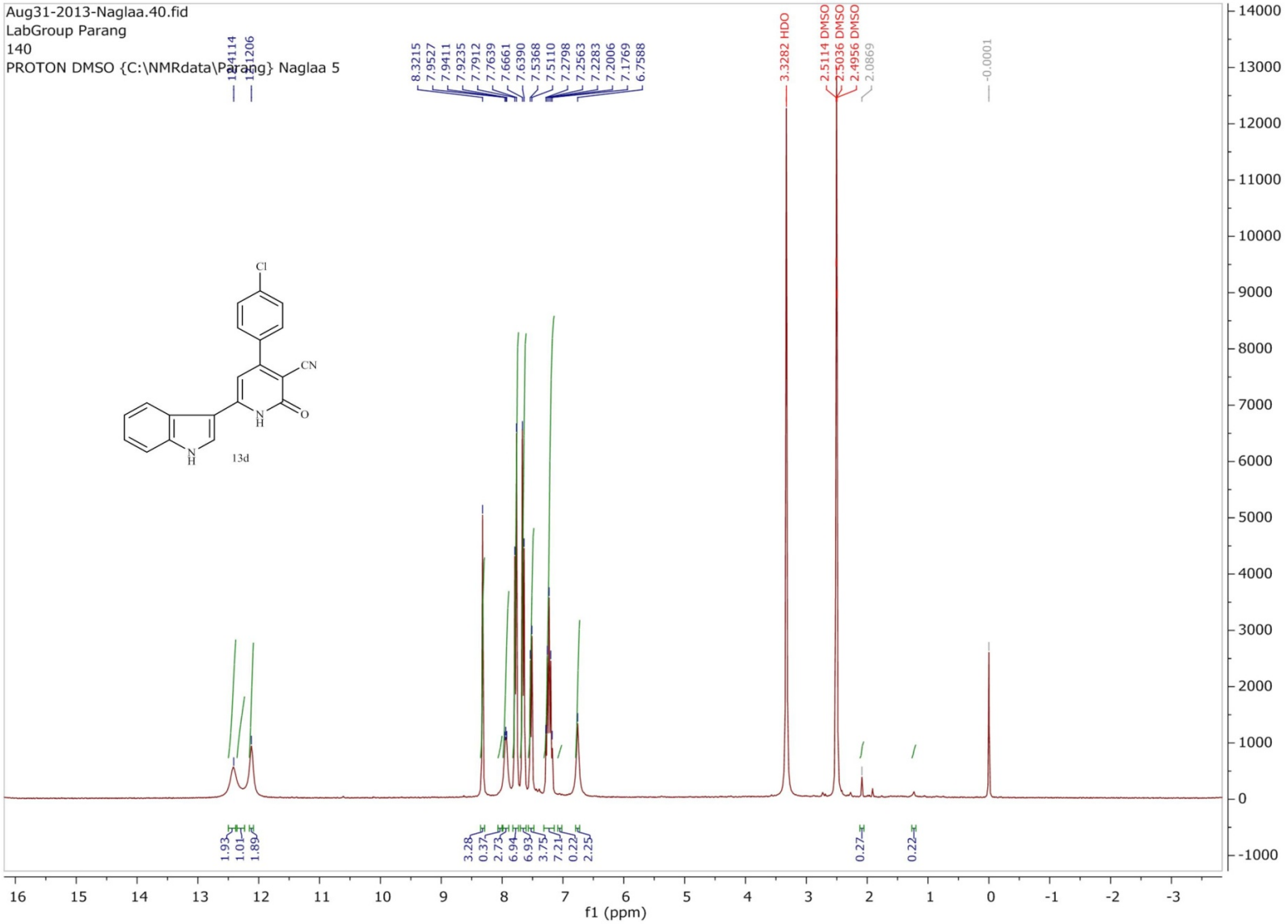
159.5229
137.5256
131.1237
130.0634
128.5334
124.5742
123.3394
122.4402
121.9839
120.7626
120.1586
116.4544
116.3067
115.3404
115.1928
113.2199
112.2535
110.7235
104.4827
88.3773
60.3404
41.2957
40.1013
39.9402
39.7791
38.4773
36.9473
36.5446
13.6884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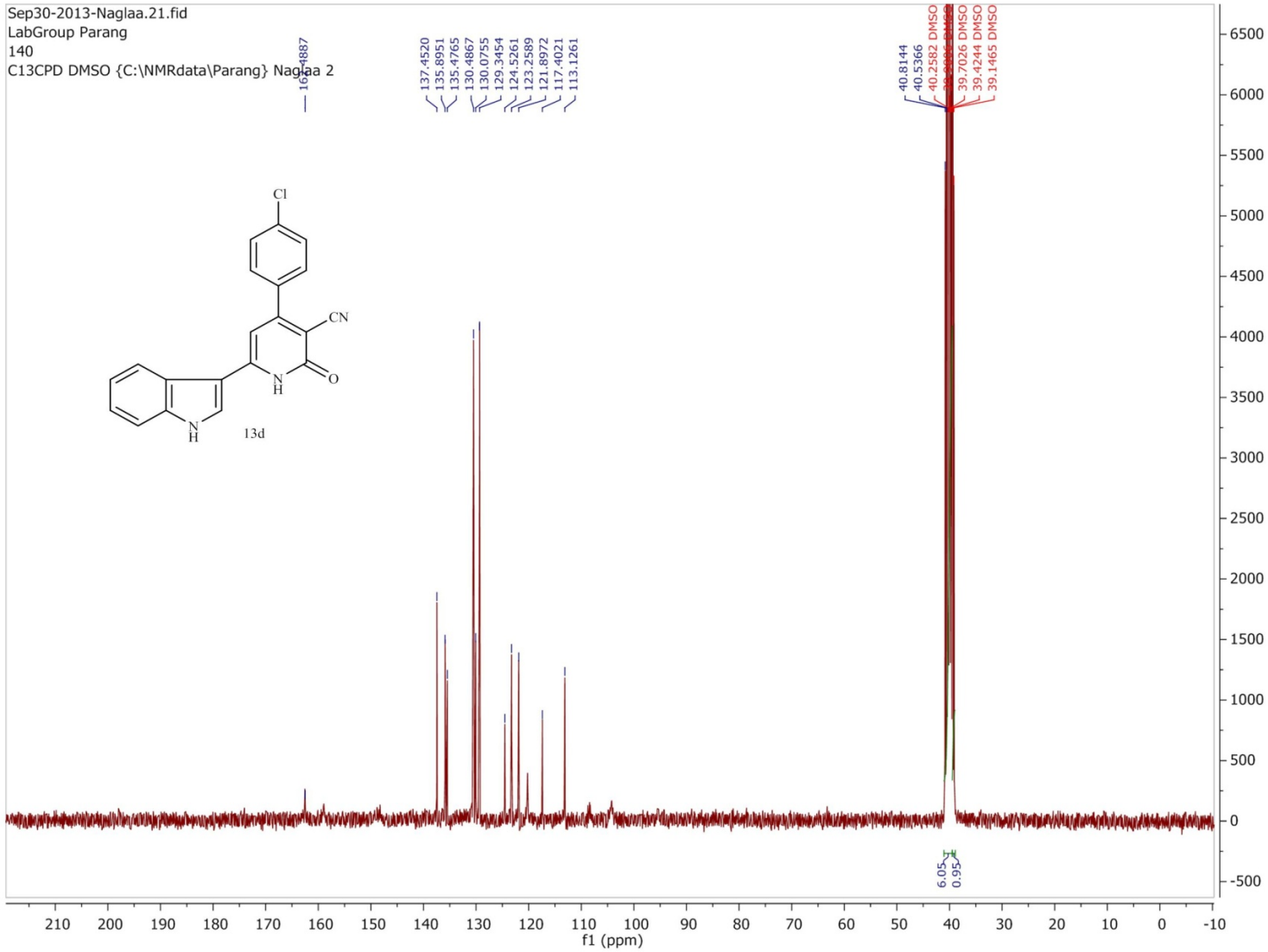
7.9527
7.9411
7.9235
7.7912
7.7639
7.6661
7.6390
7.5368
7.5110
7.2798
7.2563
7.2283
7.2006
7.1769
6.7588
3.3282 HDO
2.5114 DMSO
2.5036 DMSO
2.4956 DMSO
2.0869
-0.0001
Cl
CN
O
N
H
N
H
13d
1.93
1.01
1.89
3.28
0.37
2.73
6.94
6.93
3.75
7.21
0.22
2.25
0.27
0.22
f1 (ppm)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LabGroup Parang
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C13CPD DMSO {C:\NMRdata\Parang} Naglaa 2
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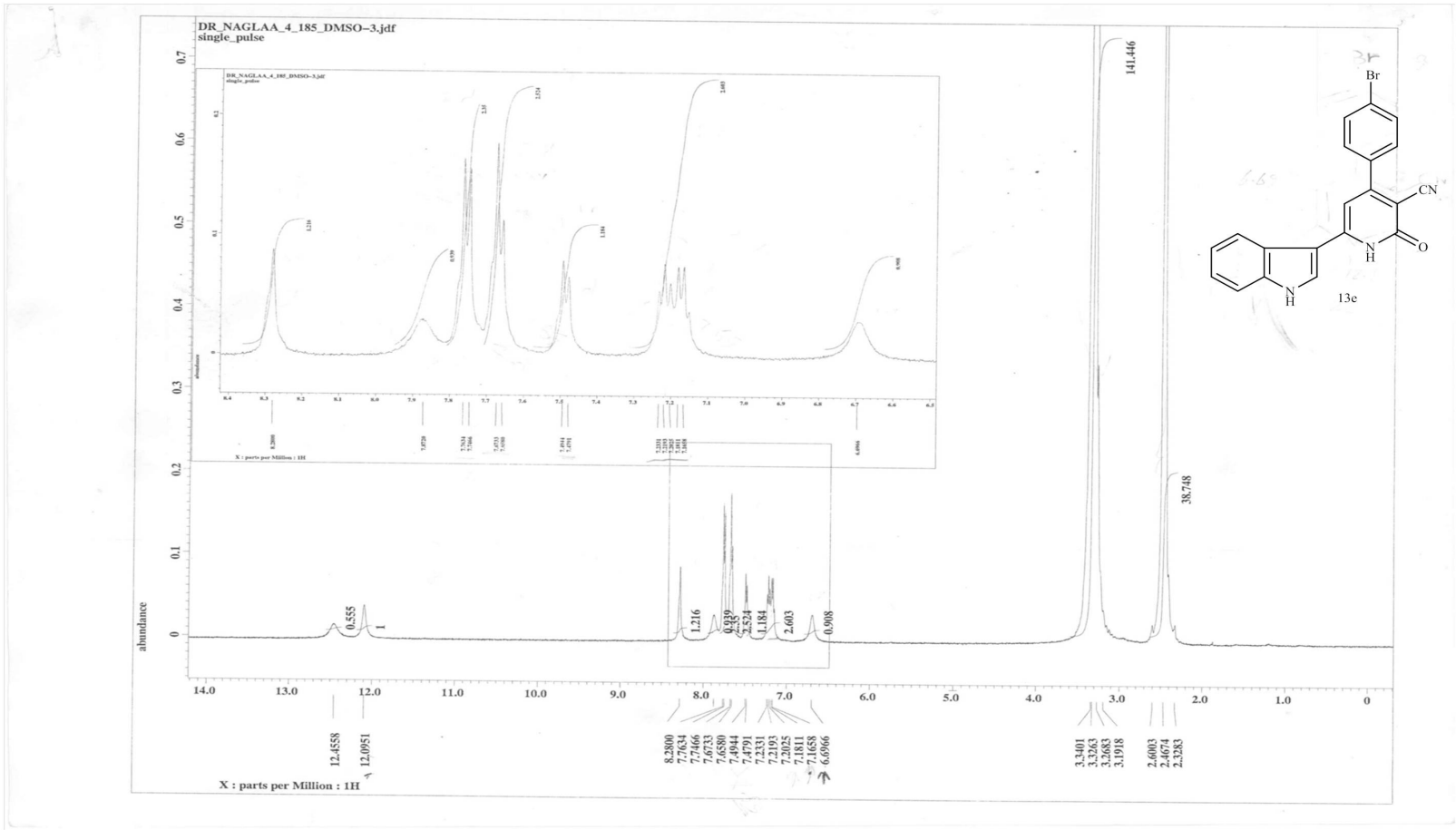
135.4765
130.4867
130.0755
129.3454
124.5261
123.2589
121.8972
117.4021
113.1261
40.8144
40.5366
40.2582 DMSO
39.7026 DMSO
39.4244 DMSO
39.1465 DMSO
Cl
CN
O
N
H
N
H
13d
6.05
0.95
f1 (ppm)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single_pulse
abundance
X : parts per Million : 1H
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1
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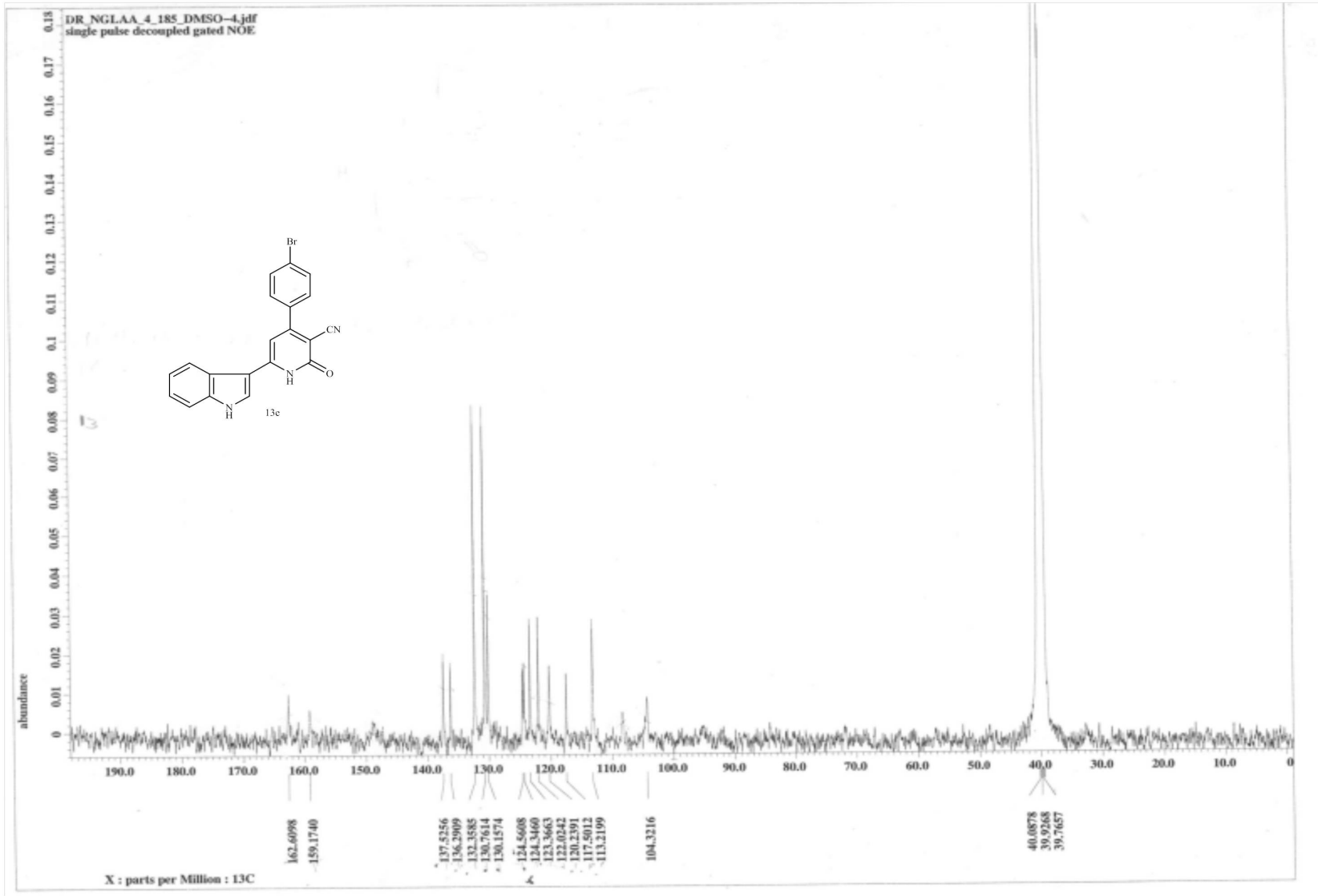
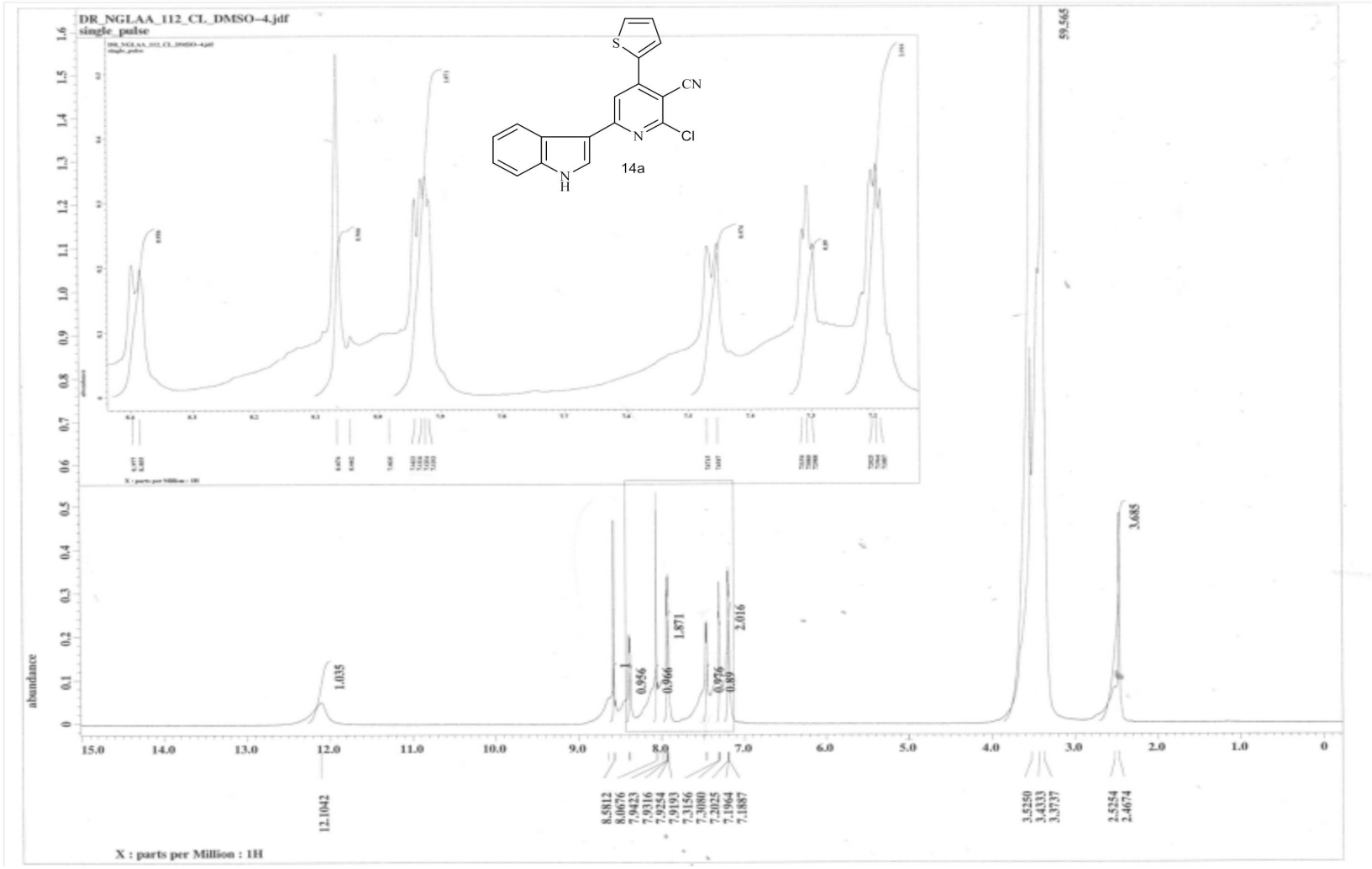
2.524
1.184
2.603
0.908
12.4558
12.0951
8.2800
7.7634
7.7466
7.6733
7.6580
7.4944
7.4791
7.2331
7.2193
7.2025
7.1811
7.1658
6.6966
3.3401
3.3263
3.2683
3.1918
2.6003
2.4674
2.3283
Br
CN
O
N
H
N
H
13e


DR_NGLAA_4_185_DMSO--4.jdf
single pulse decoupled gated NOE
Br
CN
N
H
O
N
H
13e
abundance
X : parts per Million : 13C
162.6098
159.1740
137.5256
136.2909
132.3585
130.7614
130.1574
124.5608
124.3460
123.3663
122.0242
120.2391
117.5012
113.2199
104.3216
40.0578
39.9268
39.7657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single_pulse
S
CN
N
Cl
N
H
14a
abundance
X : parts per Million : 1H
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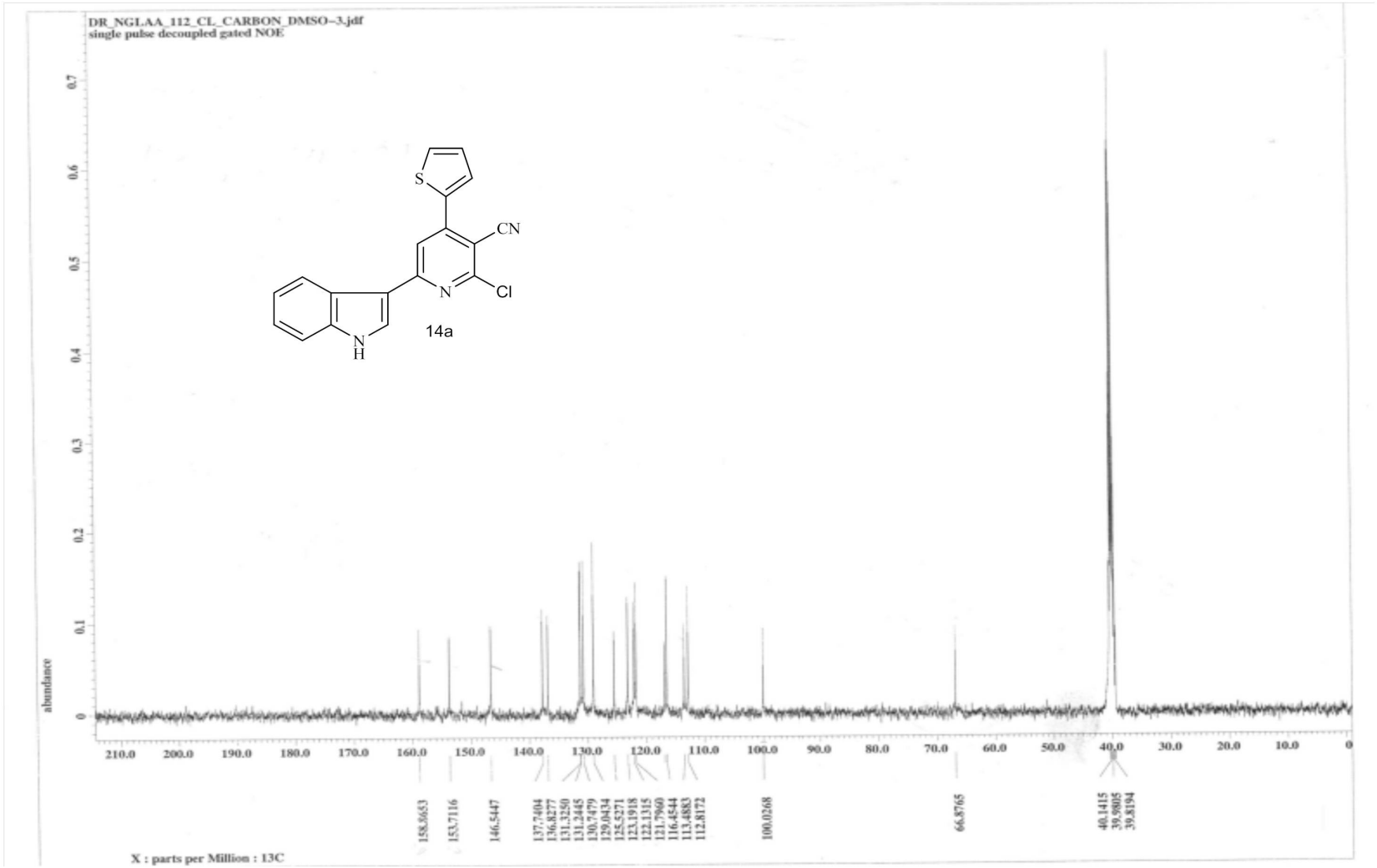
3.685
2.016
1.871
1
0.956
0.966
0.926
0.89
1.035
12.1042
8.5812
8.0676
7.9423
7.9316
7.9254
7.9193
7.3156
7.3080
7.2025
7.1964
7.1887
3.5250
3.4333
3.3737
2.5254
2.4674


DR_NGLAA_112_CL_CARBON_DMSO--3.jdf
single pulse decoupled gated NOE
S
CN
N
Cl
N
H
14a
abundance
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
210.0
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
158.5653
153.7116
146.5447
137.7404
136.8277
131.3250
131.2445
130.7479
129.0434
125.5271
123.1918
122.1315
121.7960
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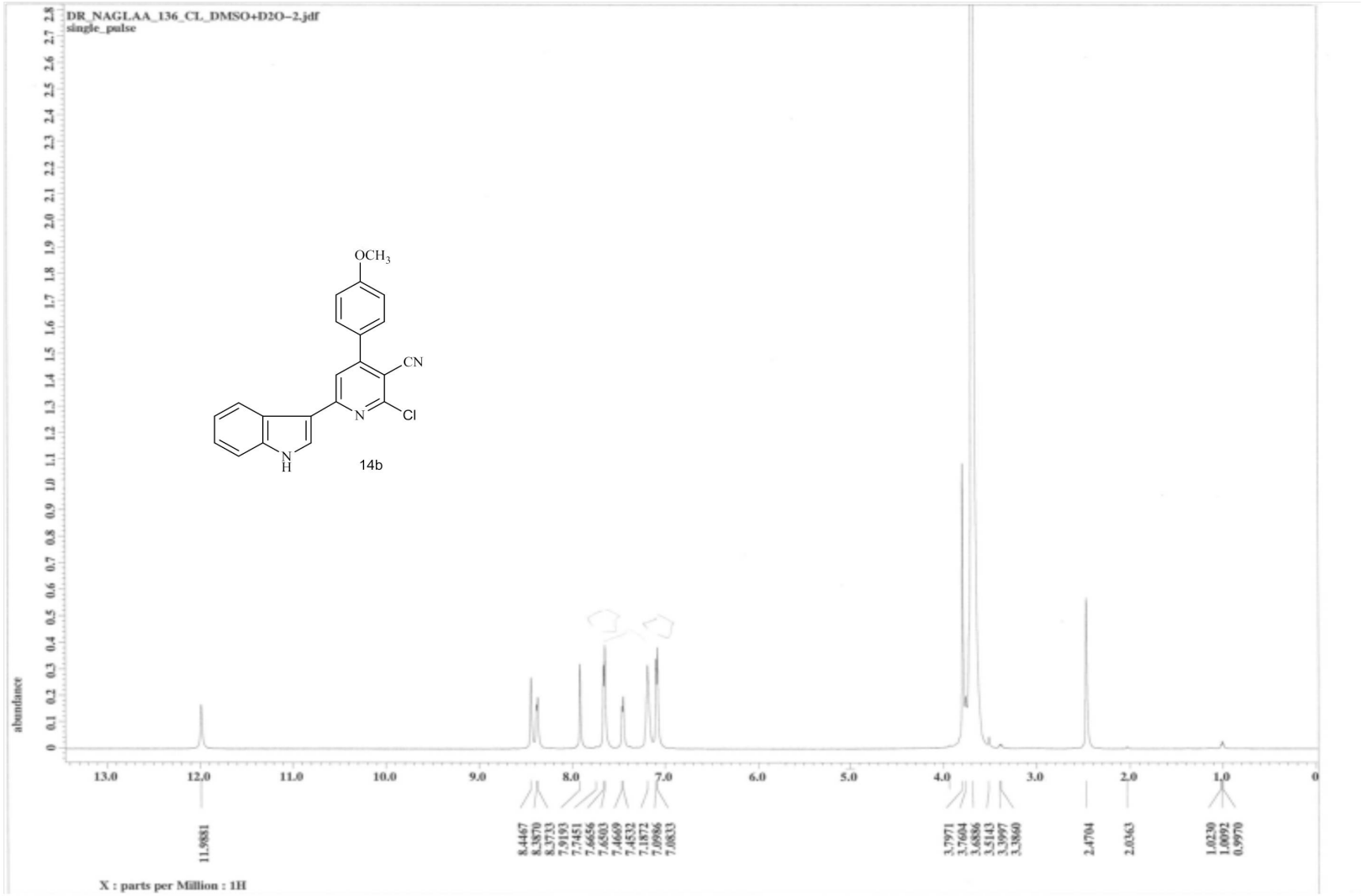
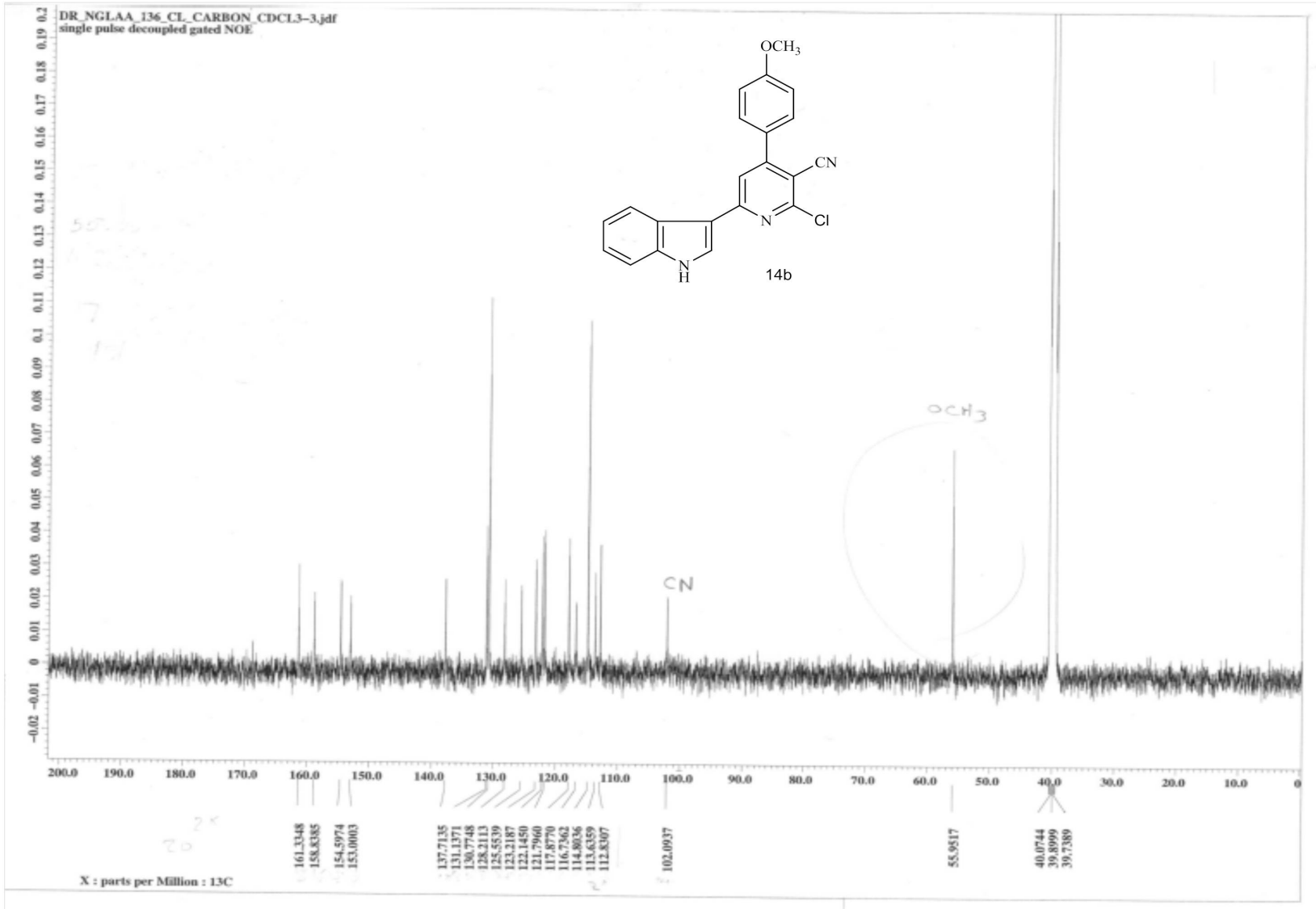
112.8172
100.0268
66.8765
40.1415
39.9805
39.8194
X : parts per Million : 13C


DR_NAGLAA_136_CL_DMSO+D2O--2.jdf
single_pulse
OCH3
CN
Cl
N
N
H
14b
abundance
X : parts per Million : 1H
11.9881
8.4467
8.3870
8.3733
7.9193
7.7451
7.6656
7.6503
7.4669
7.4532
7.1872
7.0986
7.0833
3.7971
3.7604
3.6886
3.5143
3.3997
3.3660
2.4704
2.0363
1.0230
1.0092
0.9970


DR_NGLAA_136_CL_CARBON_CDCL3--3.jdf
single pulse decoupled gated NOE
OCH3
CN
Cl
N
N
H
14b
CN
OCH3
161.3348
158.8385
154.5974
153.0003
137.7135
131.1371
130.7748
128.2113
125.5539
123.2187
122.1450
121.7960
117.8770
116.7362
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112.8307
102.0937
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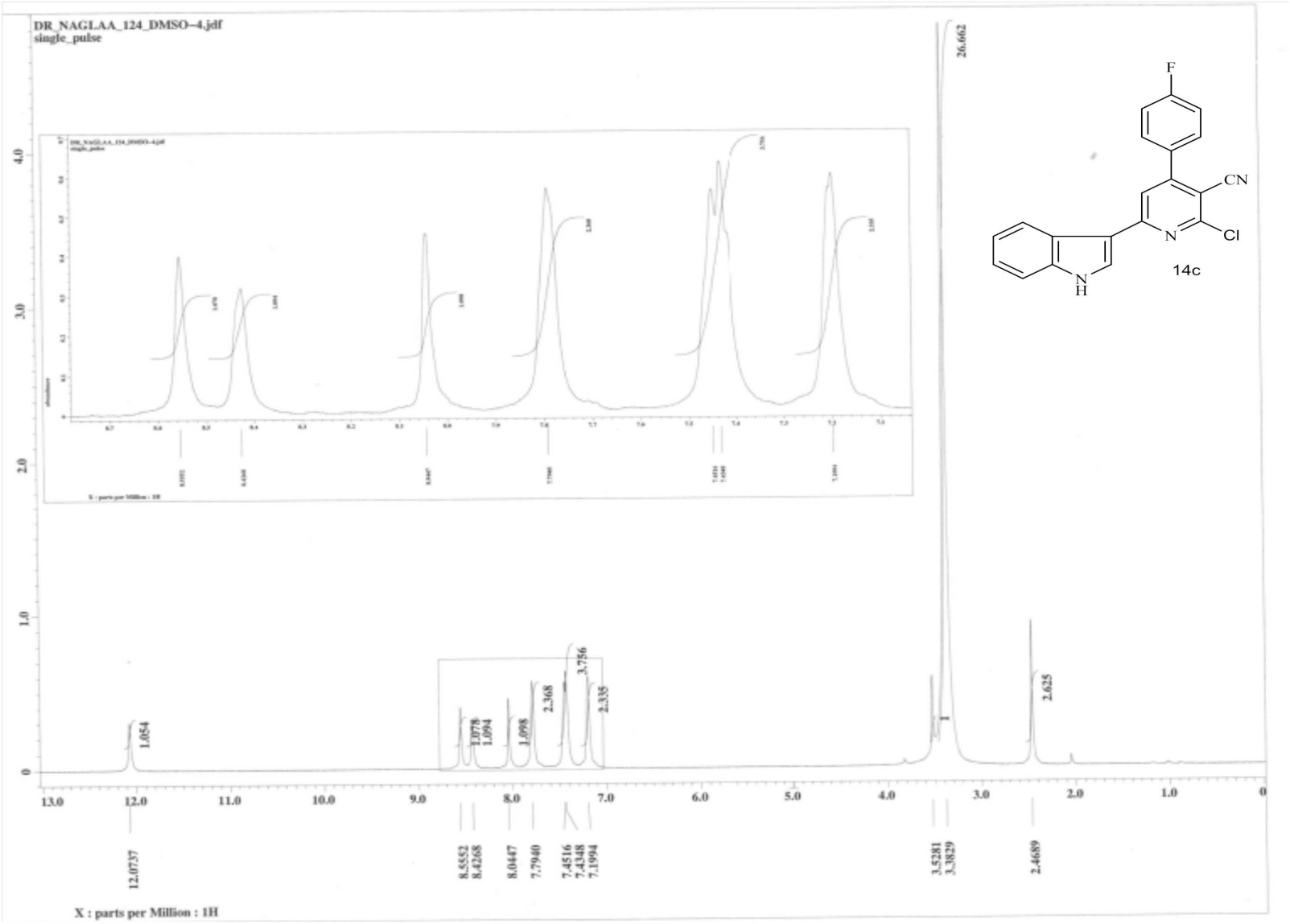
X : parts per Million : 13C


DR_NAGLAA_124_DMSO--4.jdf
single_pulse
F
CN
N
Cl
N
H
14c
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1.078
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12.0737
8.5552
8.4268
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7.4516
7.4348
7.1994
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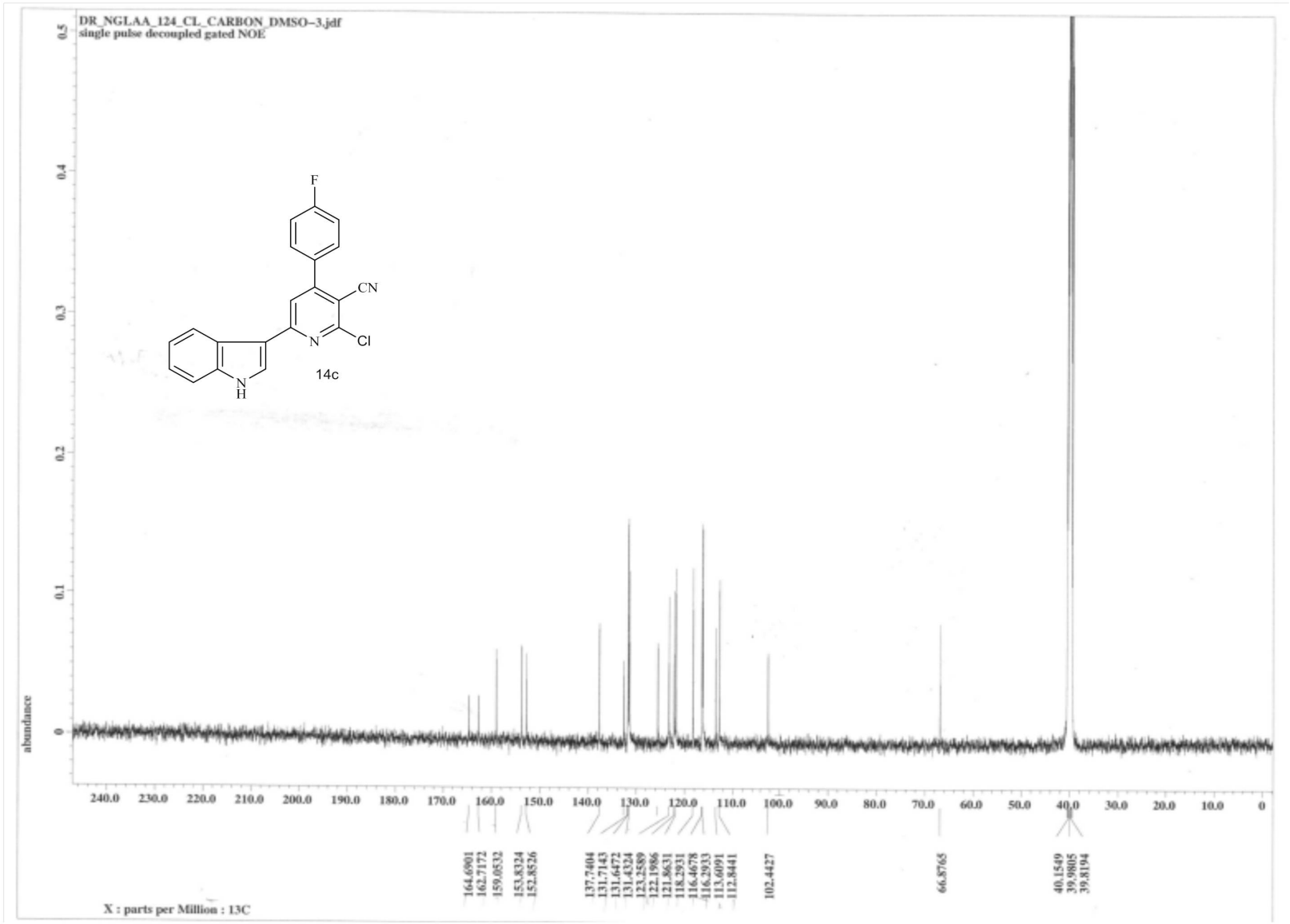
3.3829
2.4689
X : parts per Million : 1H


DR_NGLAA_124_CL_CARBON_DMSO--3.jdf
single pulse decoupled gated NOE
F
CN
N
Cl
N
H
14c
abundance
0.5
0.4
0.3
0.2
0.1
0
240.0 230.0 220.0 210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0
164.6901
162.7172
159.0532
153.8324
152.8526
137.7404
131.7143
131.6472
131.4324
123.2589
122.1986
121.8631
118.2931
116.4678
116.2933
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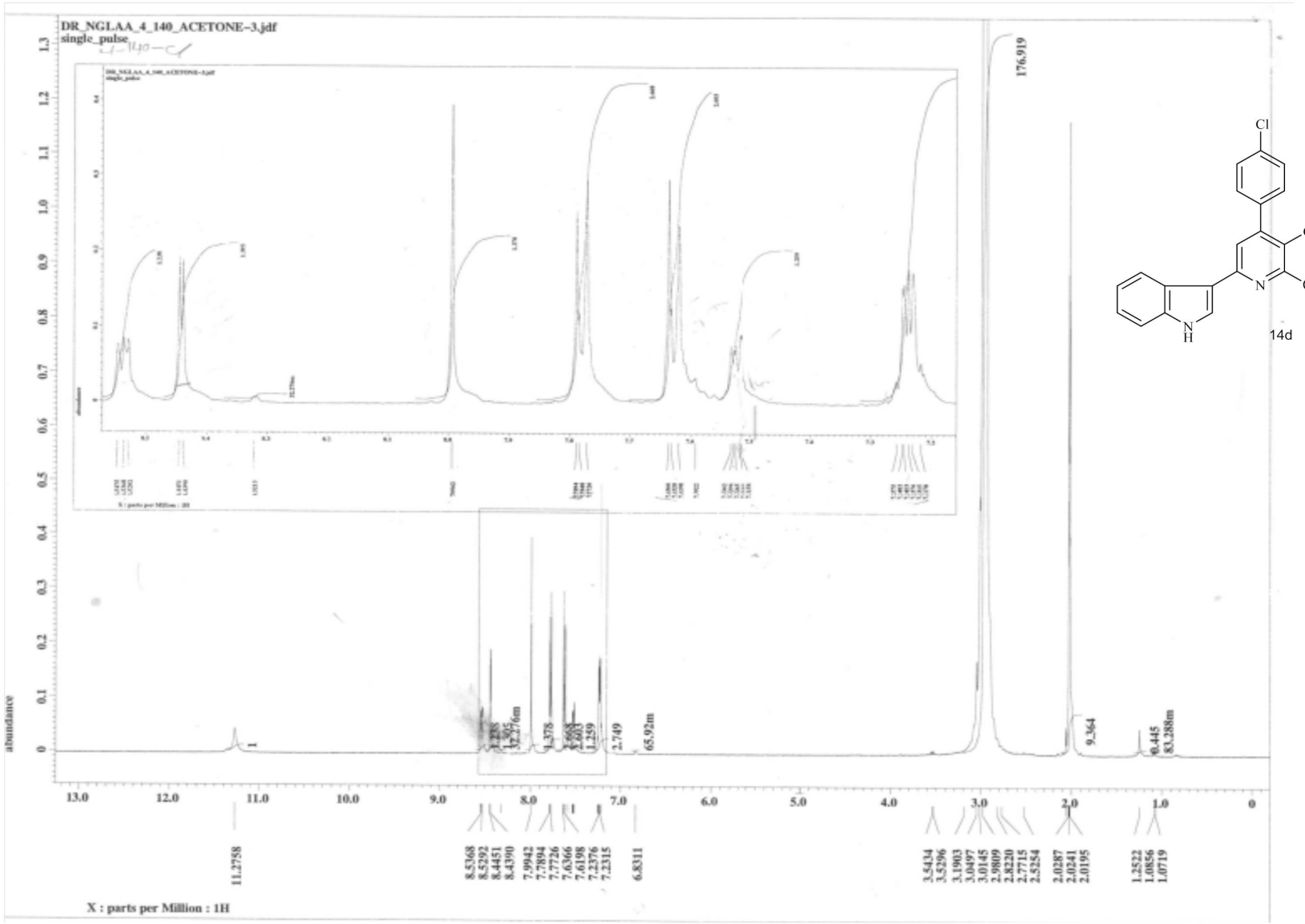
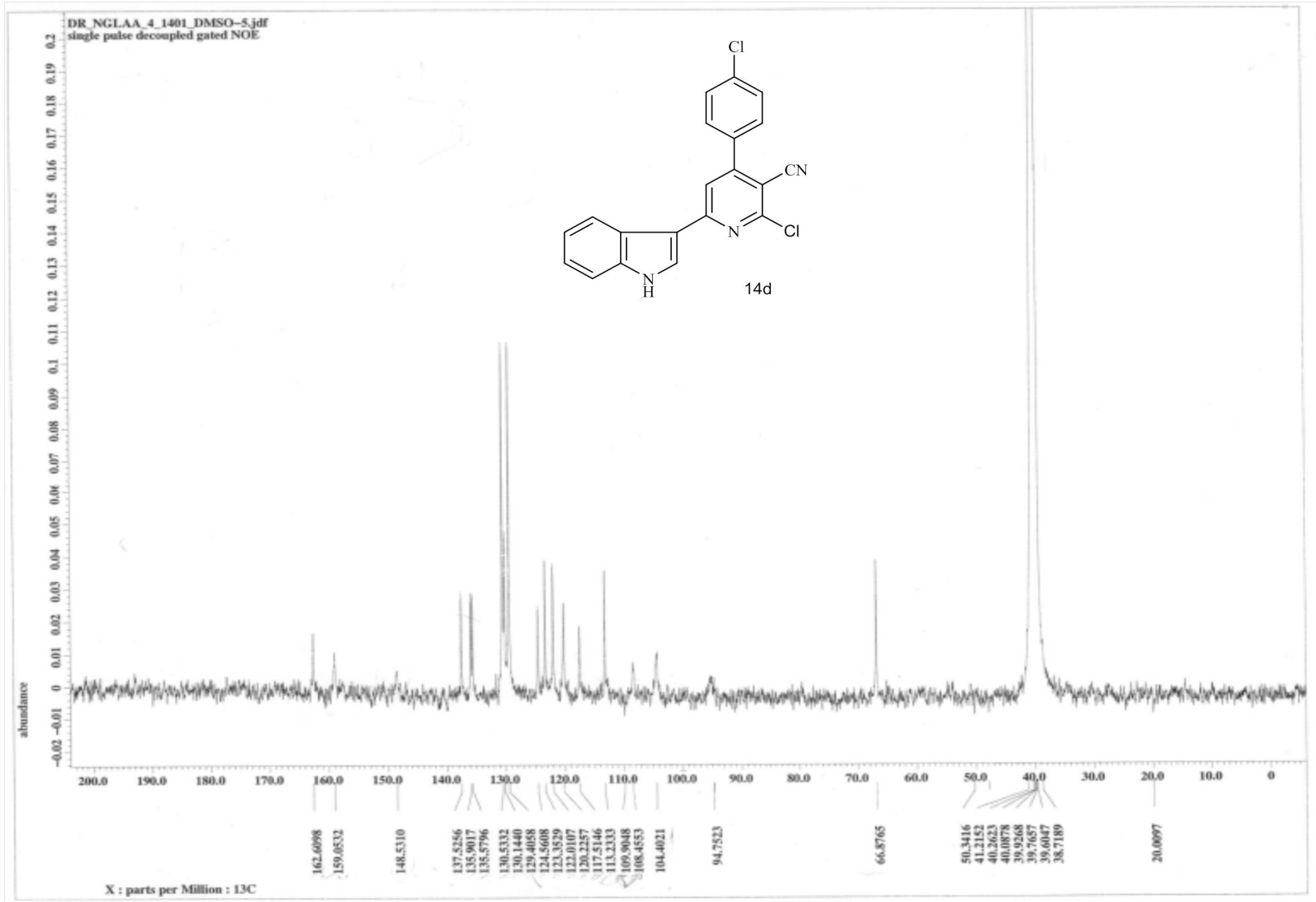
112.8441
102.4427
66.8765
40.1549
39.9805
39.8194
X : parts per Million : 13C


DR_NGLAA_4_140_ACETONE-3.jdf
single_pulse
176.919
Cl
CN
N
Cl
N
H
14d
abundance
X : parts per Million : 1H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single pulse decoupled gated NOE
Cl
CN
N
Cl
N
H
14d
abundance
X : parts per Million : 13C
162.6098
159.0532
148.5310
137.5256
135.9017
135.5796
130.5332
130.1440
129.4058
124.5608
123.3529
122.0107
120.2257
117.5146
113.2333
109.9048
108.4553
104.4021
94.7523
66.8765
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41.2152
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39.7657
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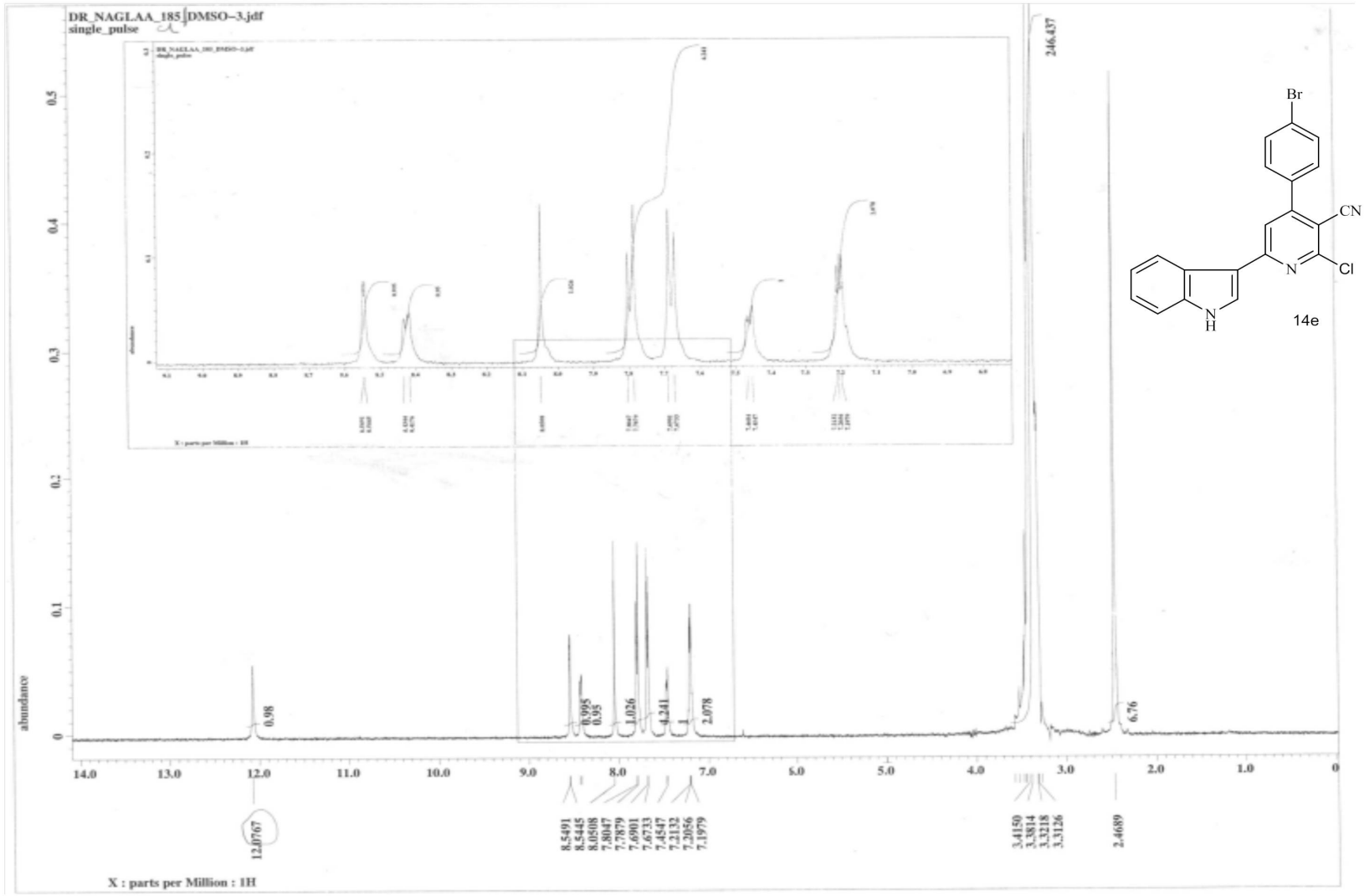
38.7189
20.0097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single_pulse
abundance
X : parts per Million : 1H
14e
Br
CN
Cl
N
N
H
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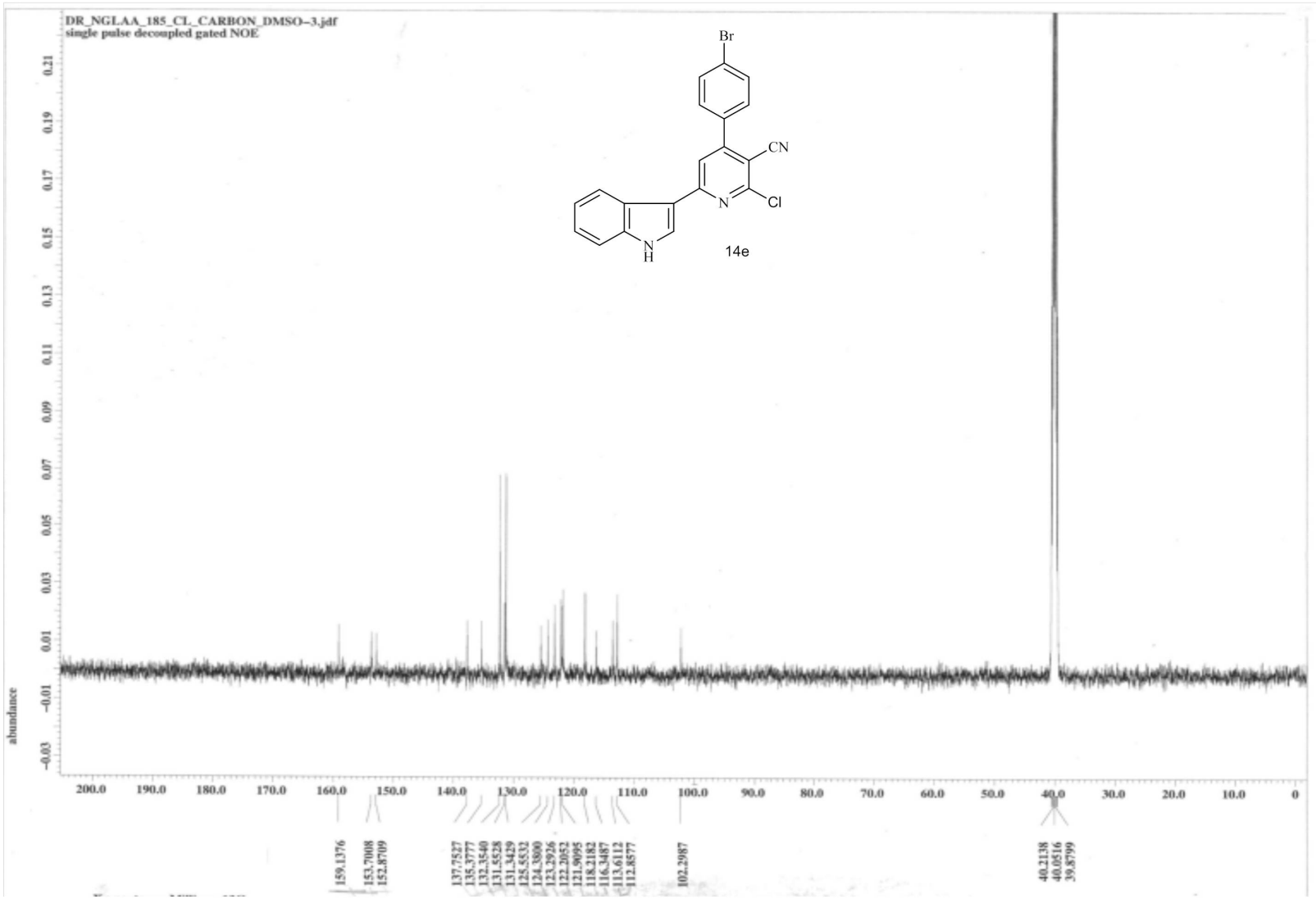
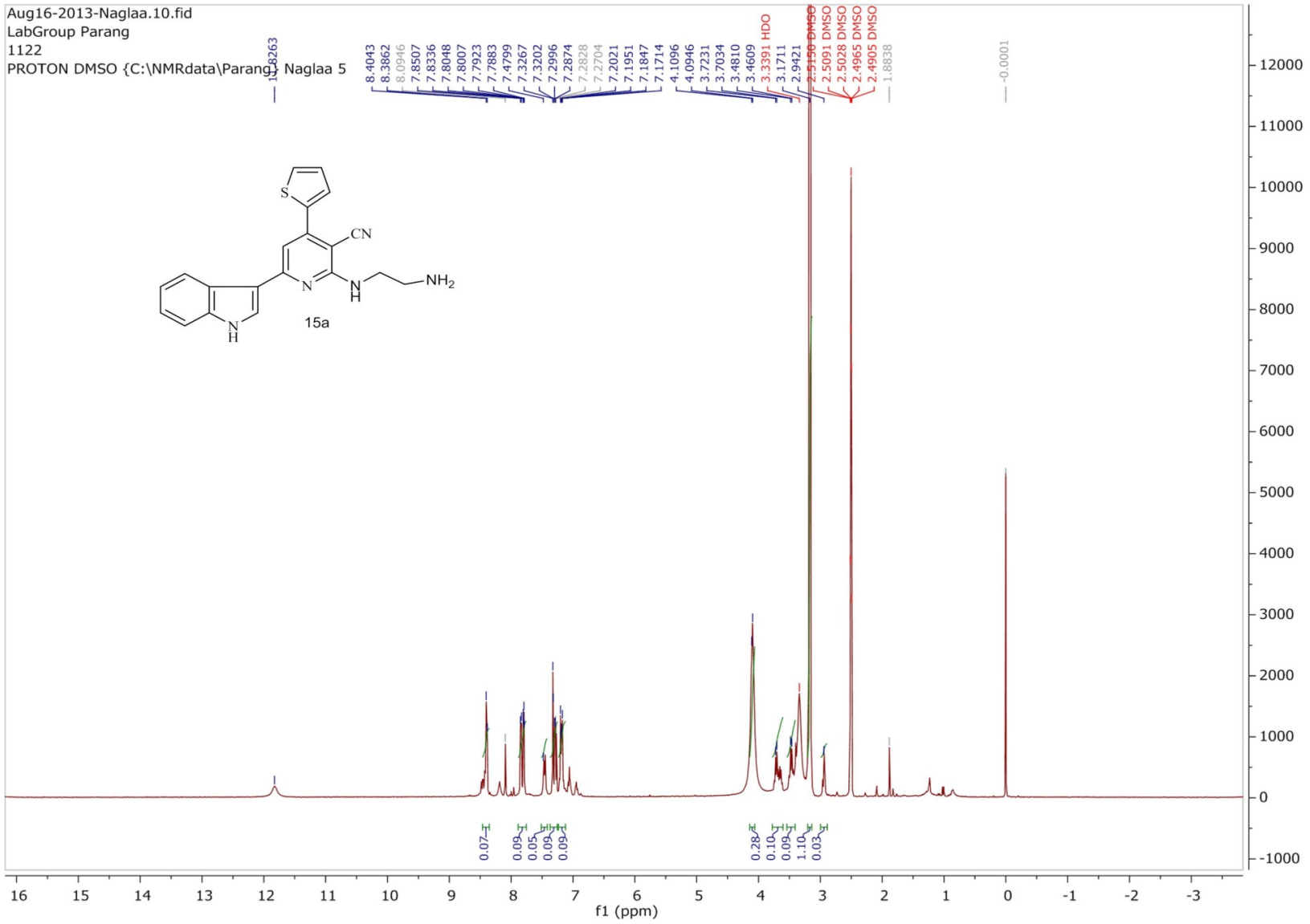
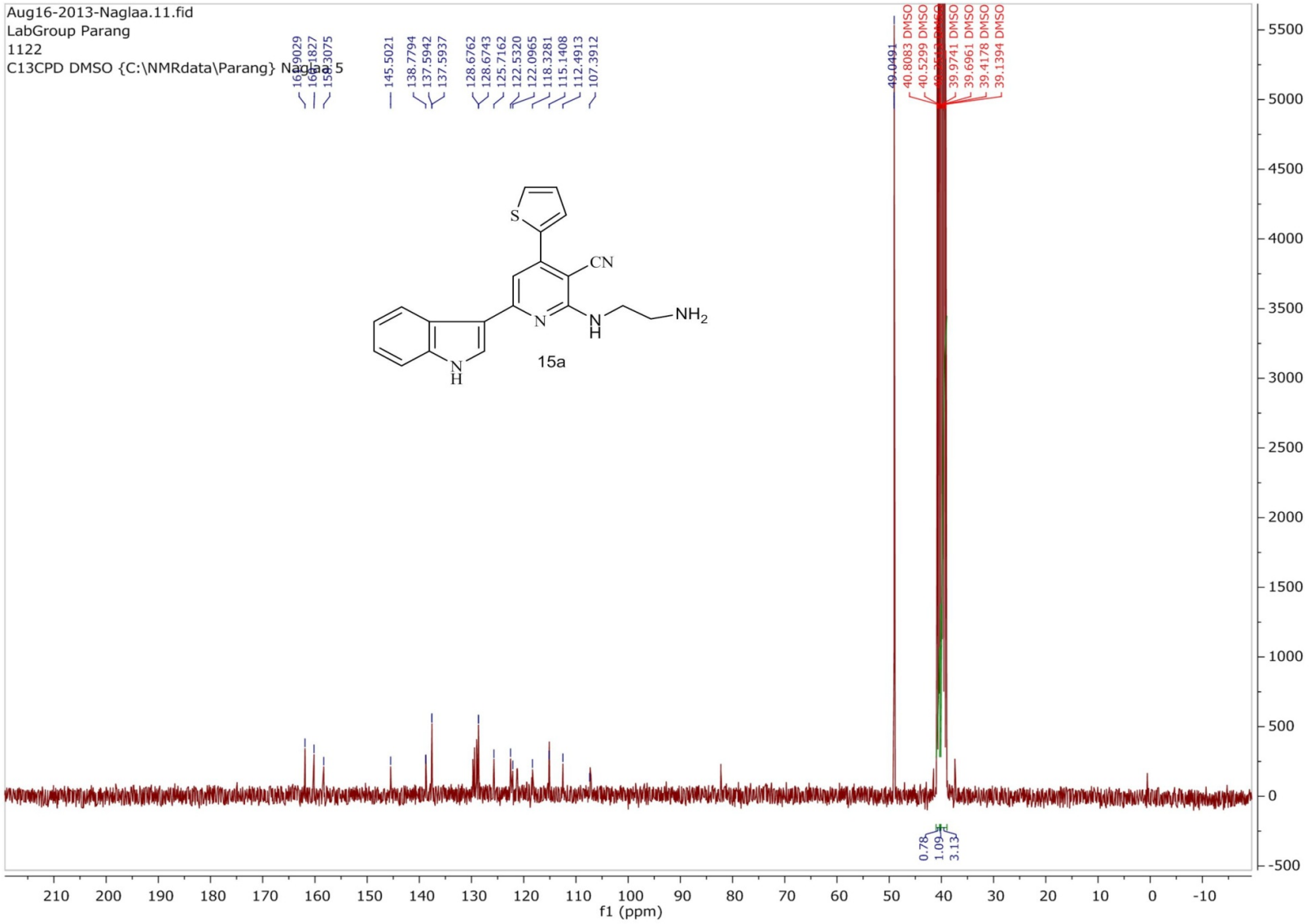
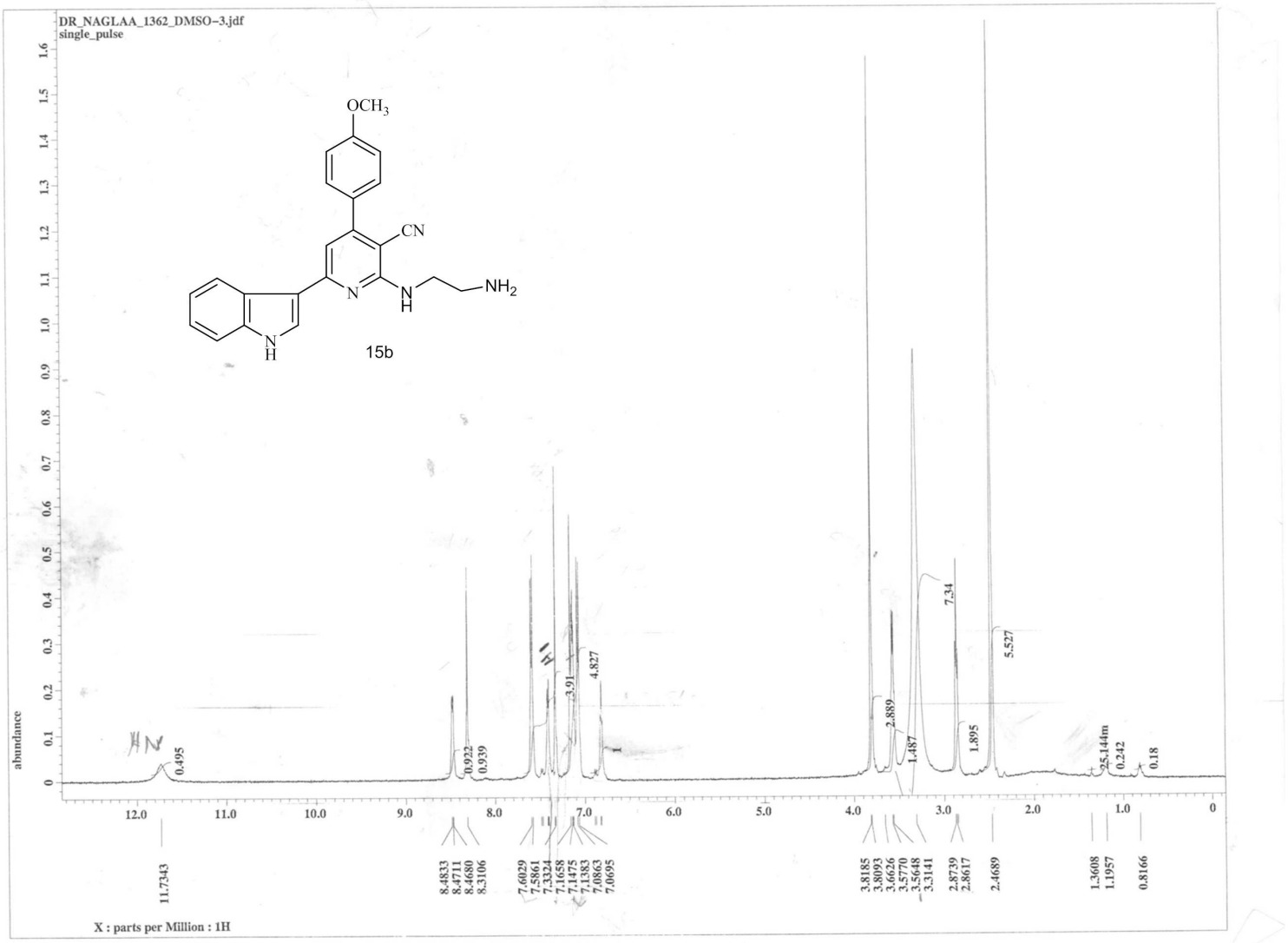
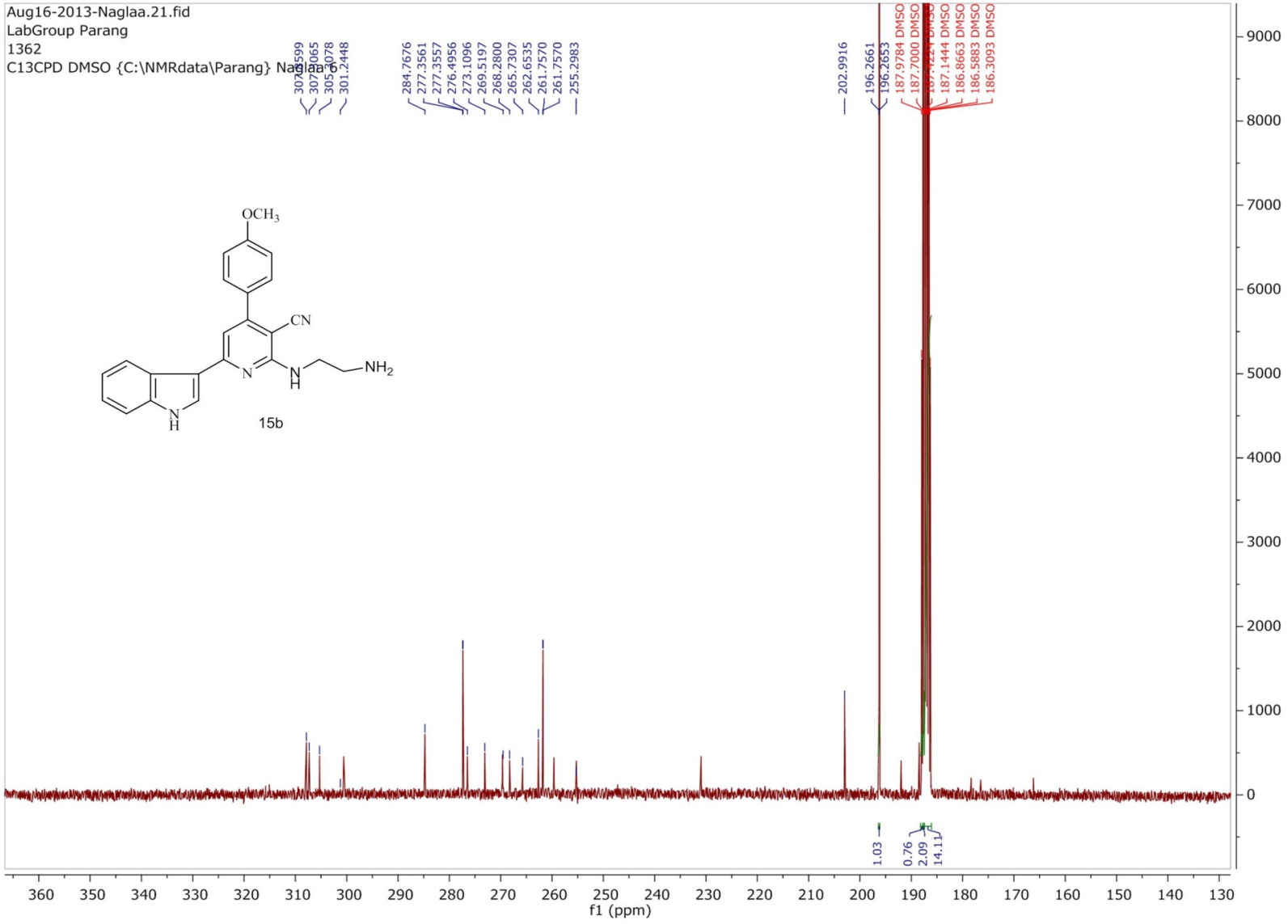
0.98
6.995
0.95
1.026
4.341
1
2.078
6.76
12.0767
8.5491
8.5445
8.0508
7.8047
7.7879
7.6901
7.6733
7.4547
7.2132
7.2056
7.1979
3.4150
3.3814
3.3218
3.3126
2.4689

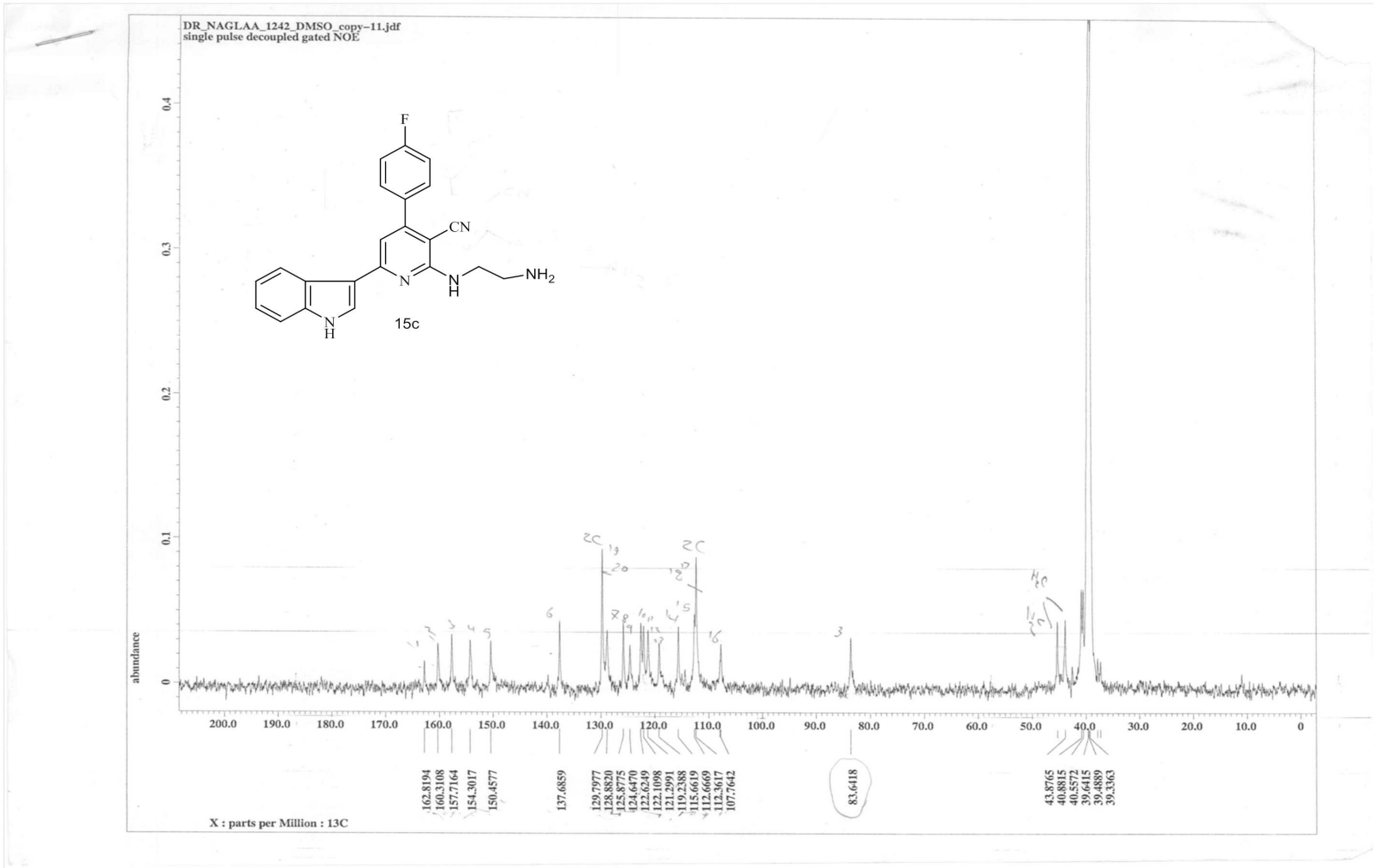

DR_NGLAA_185_CL_CARBON_DMSO--3.jdf
single pulse decoupled gated NOE
Br
CN
N
Cl
N
H
14e
abundance
159.1376
153.7008
152.8709
137.7527
135.3777
132.3540
131.5528
131.3429
125.5532
124.3800
123.2926
122.2052
121.5095
118.2182
116.3487
113.6112
112.8577
102.2987
40.2138
40.0516
39.8799

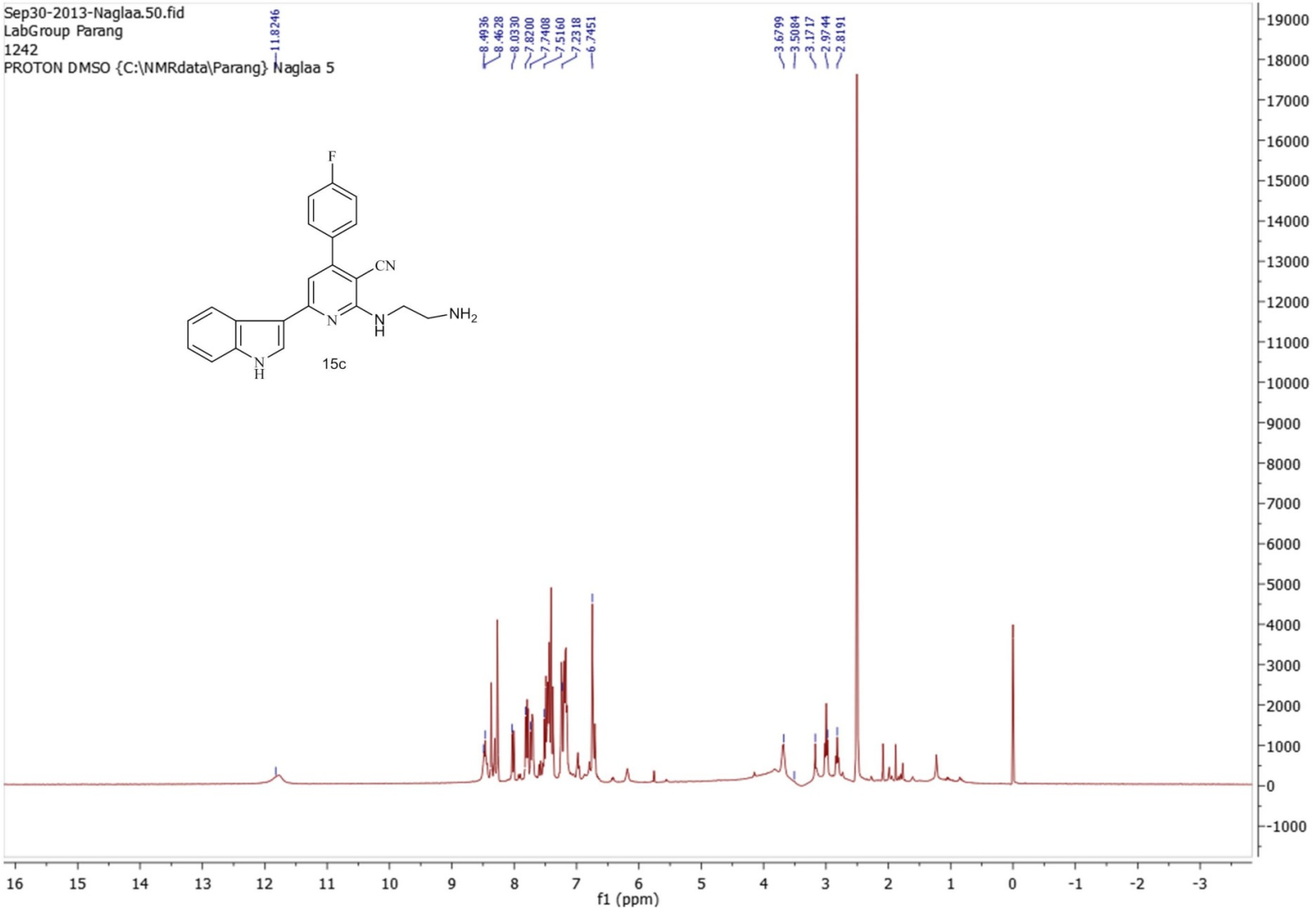

Aug16-2013-Naglaa.10.fid
LabGroup Parang
1122
PROTON DMSO {C:\NMRdata\Parang} Naglaa 5
11.8263
8.4043
8.3862
8.0946
7.8507
7.8336
7.8048
7.8007
7.7923
7.7883
7.4799
7.3267
7.3202
7.2996
7.2874
7.2828
7.2704
7.2021
7.1951
7.1847
7.1714
4.1096
4.0946
3.7231
3.7034
3.4810
3.4609
3.3391 HDO
3.1711
2.9421
2.5150 DMSO
2.5091 DMSO
2.5028 DMSO
2.4965 DMSO
2.4905 DMSO
1.8838
-0.0001
S
CN
N
N
H
NH2
N
H
15a
0.07
0.09
0.05
0.09
0.09
0.28
0.10
0.09
1.10
0.03
f1 (ppm)

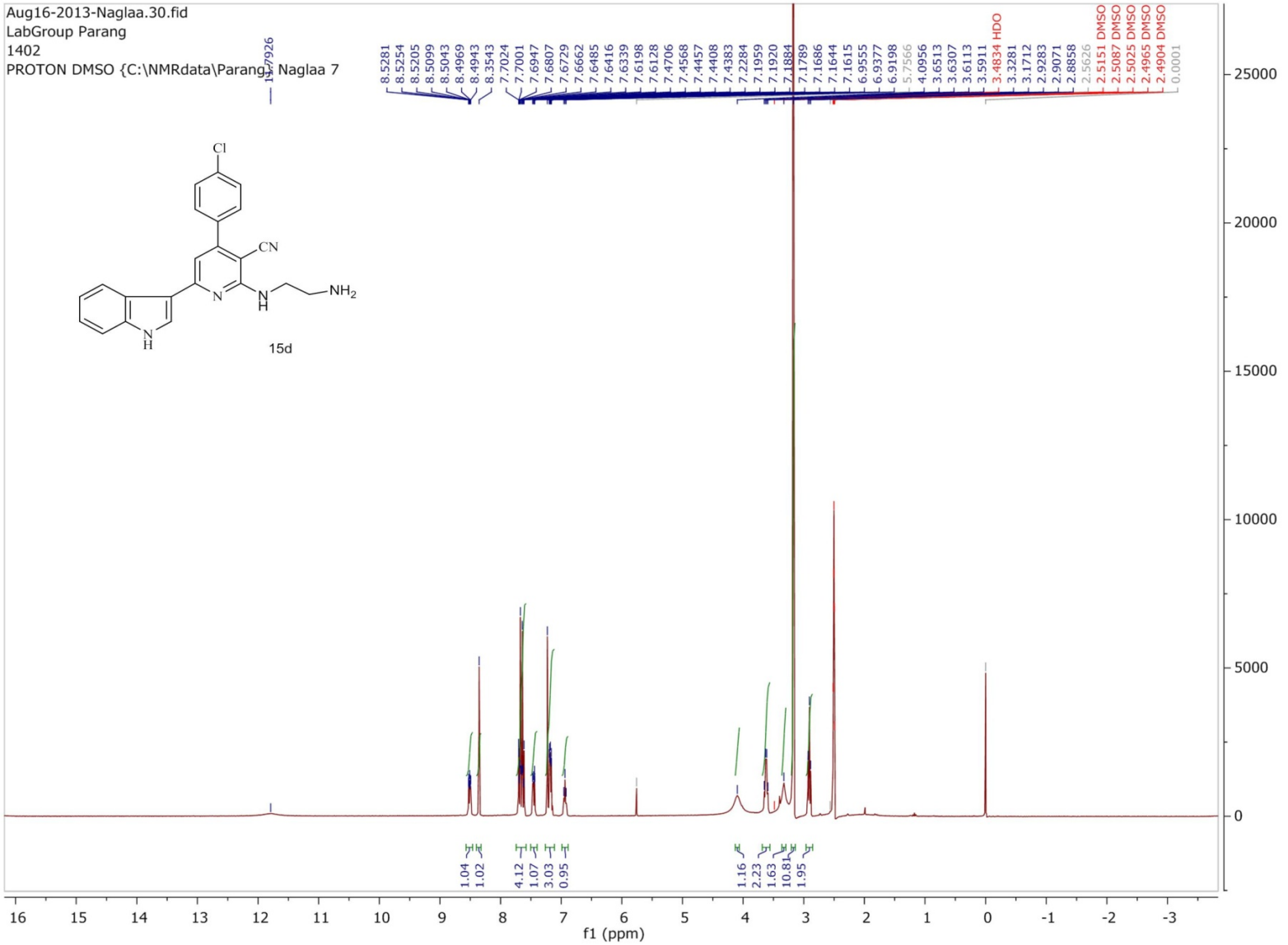

Aug16-2013-Naglaa.11.fid
LabGroup Parang
1122
C13CPD DMSO {C:\NMRdata\Parang} Nagla 5
169.9029
164.1827
158.3075
145.5021
138.7794
137.5942
137.5937
128.6762
128.6743
125.7162
122.5320
122.0965
118.3281
115.1408
112.4913
107.3912
49.0491
40.8083 DMSO
40.5299 DMSO
39.9741 DMSO
39.6961 DMSO
39.4178 DMSO
39.1394 DMSO
S
CN
N
H
NH2
N
H
15a
0.78
1.09
3.13
f1 (ppm)

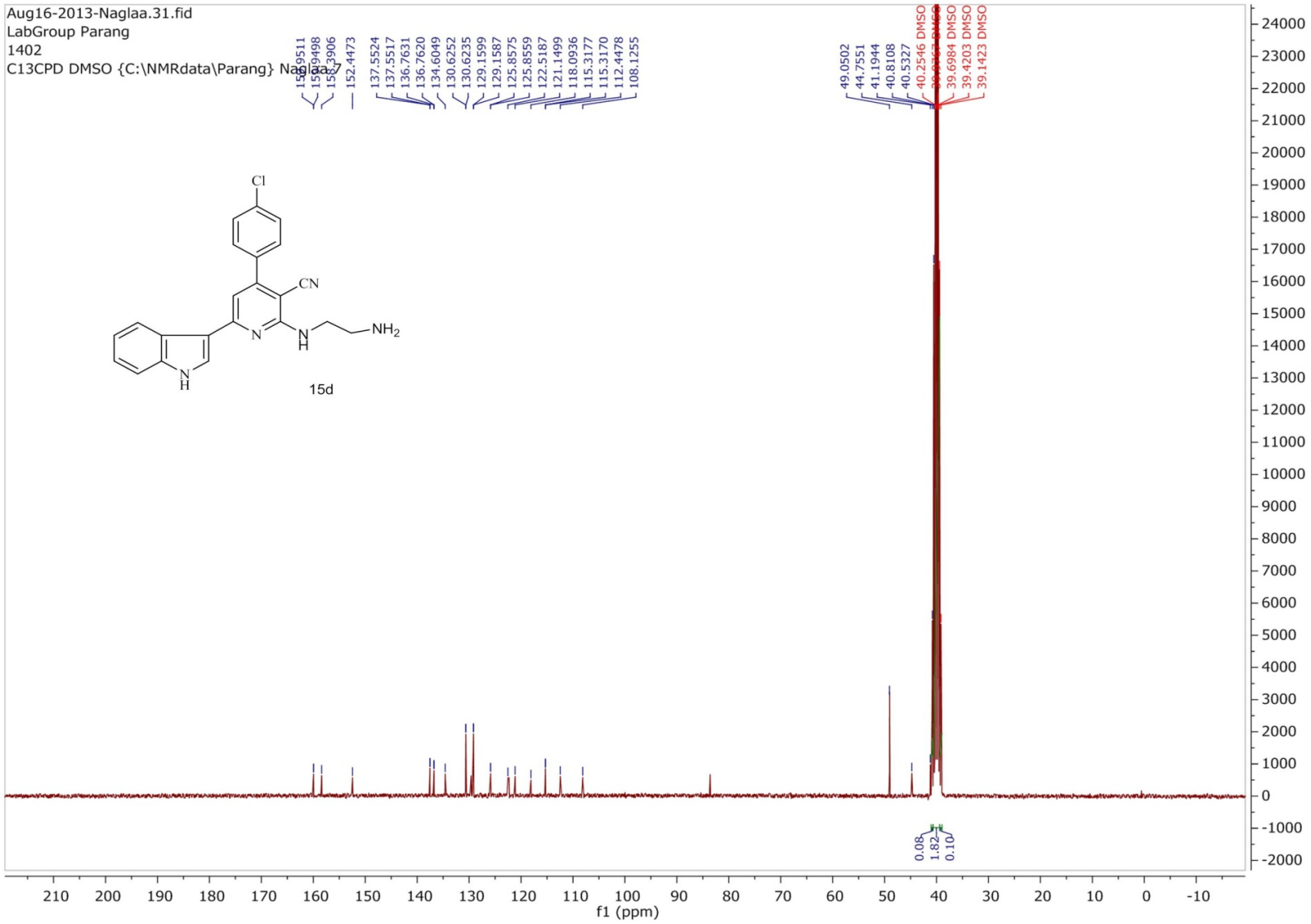

DR_NAGLAA_1362_DMSO-3.jdf
single_pulse
OCH3
CN
N
N
H
NH2
N
H
15b
abundance
0.495
0.922
0.939
3.91
4.827
2.889
1.487
7.34
1.895
5.527
25.144m
0.242
0.18
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
11.7343
8.4833
8.4711
8.4680
8.3106
7.6029
7.5861
7.3324
7.1658
7.1475
7.1383
7.0863
7.0695
3.8185
3.8093
3.6626
3.5770
3.5648
3.3141
2.8739
2.8617
2.4689
1.3608
1.1957
0.8166
X : parts per Million : 1H

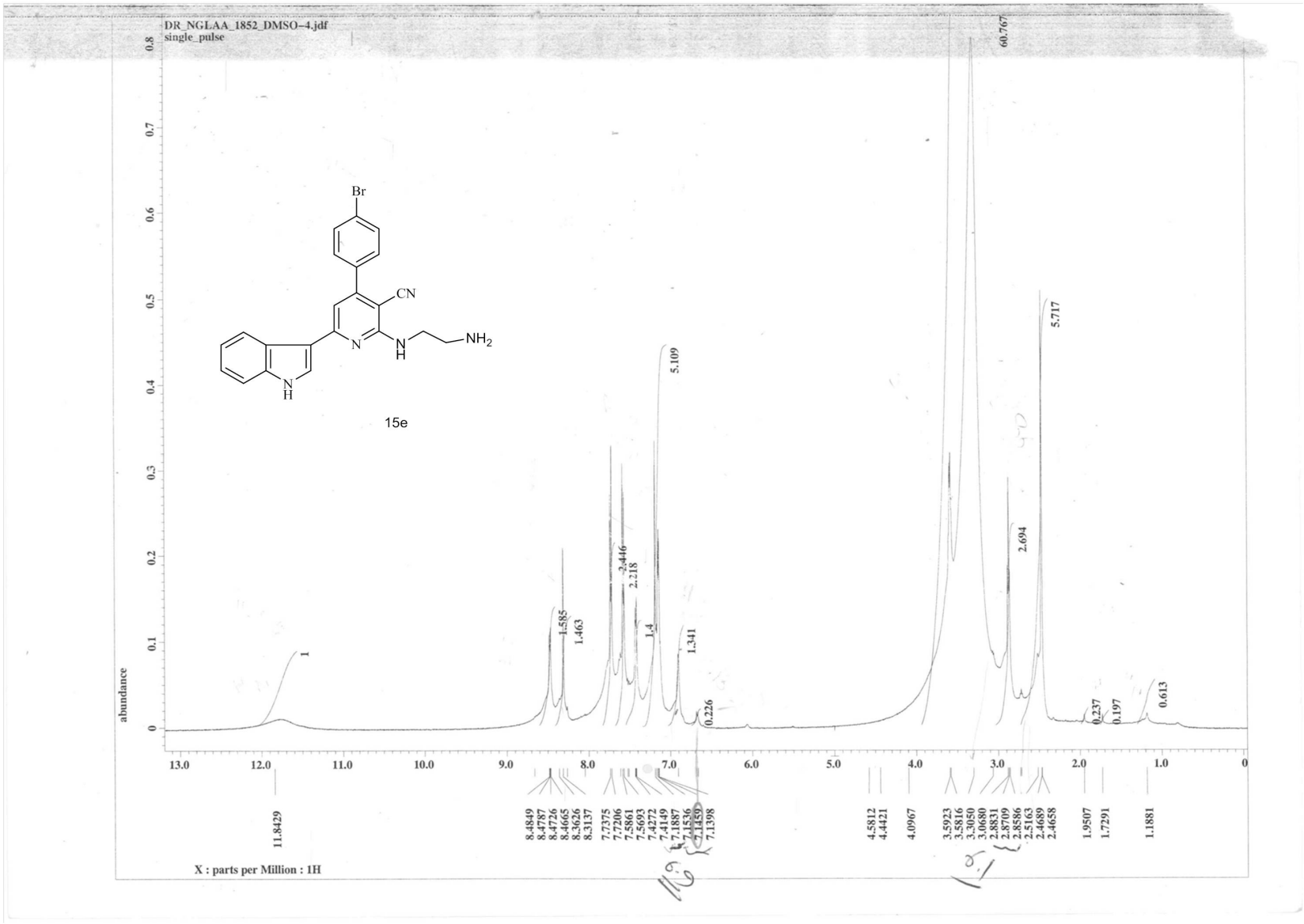

Aug16-2013-Naglaa.21.fid
LabGroup Parang
1362
C13CPD DMSO {C:\NMRdata\Parang} Naglaa 6
307.4599
307.3065
305.3078
301.2448
284.7676
277.3561
277.3557
276.4956
273.1096
269.5197
268.2800
265.7307
262.6535
261.7570
261.7570
255.2983
202.9916
196.2661
196.2653
187.9784 DMSO
187.7000 DMSO
187.1444 DMSO
186.8663 DMSO
186.5883 DMSO
186.3093 DMSO
OCH3
CN
N
N
H
NH2
N
H
15b
1.03
0.76
2.09
14.11
f1 (ppm)

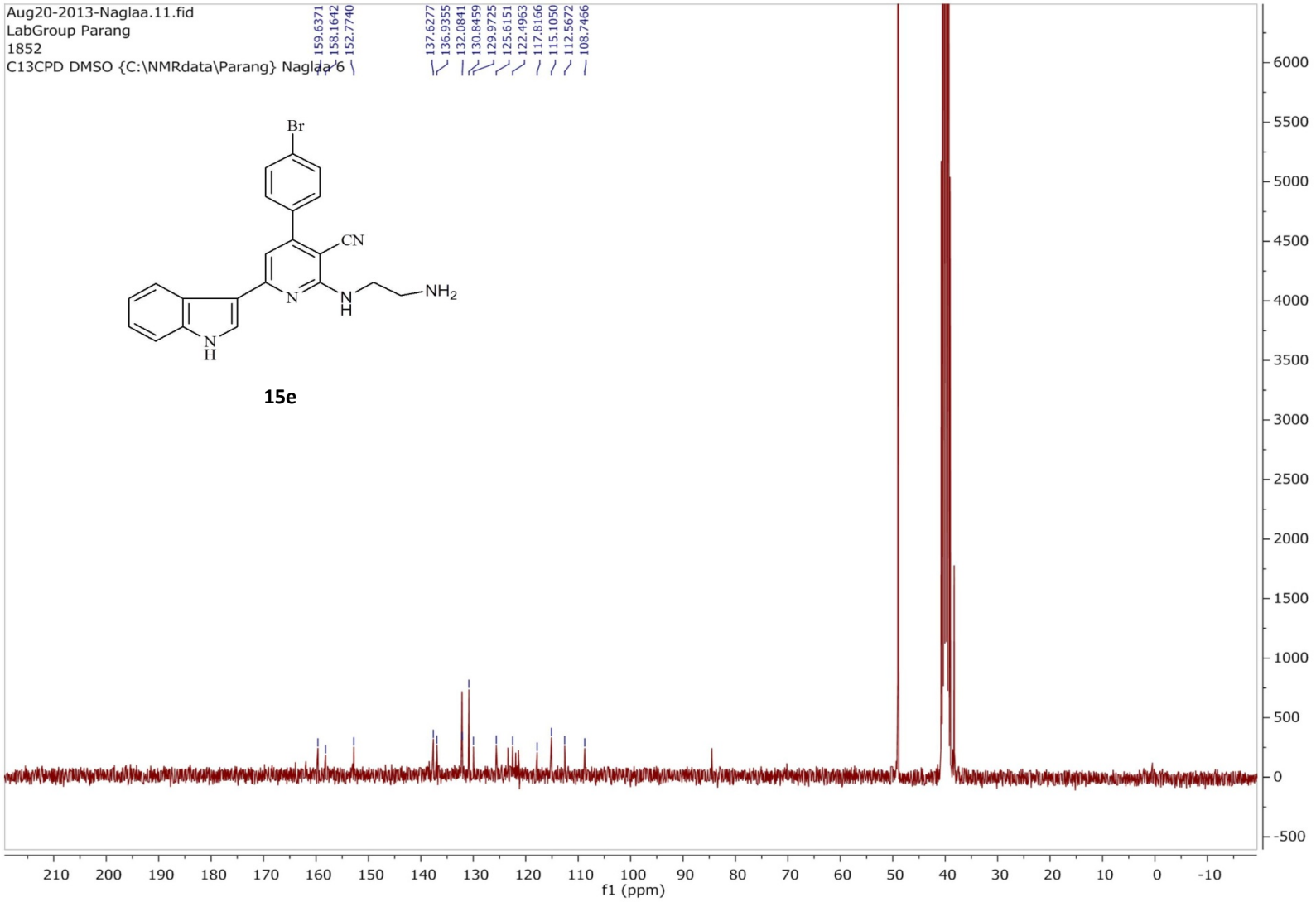

DR_NAGLAA_1242_DMSO_copy-11.jdf
single pulse decoupled gated NOE
F
CN
N
N
H
NH2
N
H
15c
abundance
0.4
0.3
0.2
0.1
0
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
162.8194
160.3108
157.7164
154.3017
150.4577
137.6859
129.7977
128.8820
125.8775
124.6470
122.6249
122.1098
121.2991
119.2388
115.6619
112.6669
112.3617
107.7642
83.6418
43.8765
40.8815
40.5572
39.6415
39.4889
39.3363
X : parts per Million : 13C


Sep30-2013-Naglaa.50.fid
LabGroup Parang
1242
PROTON DMSO {C:\NMRdata\Parang} Naglaa 5
11.8246
8.4936
8.4628
8.0330
7.8200
7.7408
7.5160
7.2318
6.7451
3.6799
3.5084
3.1717
2.9744
2.8191
F
CN
N
N
H
NH2
N
H
15c
f1 (ppm)


Aug16-2013-Naglaa.30.fid
LabGroup Parang
1402
PROTON DMSO {C:\NMRdata\Parang\ Naglaa 7
13.7926
Cl
CN
N
N
H
NH2
N
H
15d
8.5281
8.5254
8.5205
8.5099
8.5043
8.4969
8.4943
8.3543
7.7024
7.7001
7.6947
7.6807
7.6729
7.6662
7.6485
7.6416
7.6339
7.6198
7.6128
7.4706
7.4568
7.4457
7.4408
7.4383
7.2284
7.1959
7.1920
7.1884
7.1789
7.1686
7.1644
7.1615
6.9555
6.9377
6.9198
5.7566
4.0956
3.6513
3.6307
3.6113
3.5911
3.4834 HDO
3.3281
3.1712
2.9283
2.9071
2.8858
2.5626
2.5151 DMSO
2.5087 DMSO
2.5025 DMSO
2.4965 DMSO
2.4904 DMSO
0.0001
1.04
1.02
4.12
1.07
3.03
0.95
1.16
2.23
1.63
10.81
1.95
25000
20000
15000
10000
5000
0
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
f1 (ppm)


Aug16-2013-Naglaa.31.fid
LabGroup Parang
1402
C13CPD DMSO {C:\NMRdata\Parang} Naglaa 7
159.9511
159.9498
153.3906
152.4473
137.5524
137.5517
136.7631
136.7620
134.6049
130.6252
130.6235
129.1599
129.1587
125.8575
125.8559
122.5187
121.1499
118.0936
115.3177
115.3170
112.4478
108.1255
49.0502
44.7551
41.1944
40.8108
40.5327
40.2546 DMSO
39.6984 DMSO
39.4203 DMSO
39.1423 DMSO
Cl
CN
N
N
H
NH2
N
H
15d
0.08
1.82
0.10
f1 (ppm)


DR_NGLAA_1852_DMSO-4.jdf
single_pulse
Br
CN
N
N
H
NH2
N
H
15e
abundance
X : parts per Million : 1H
60.767
5.717
5.109
2.694
2.446
2.218
1.585
1.463
1.4
1.341
0.226
0.613
0.237
0.197
11.8429
8.4849
8.4787
8.4726
8.4665
8.3626
8.3137
7.7375
7.7206
7.5861
7.5693
7.4272
7.4149
7.1887
7.1536
7.1459
7.1398
4.5812
4.4421
4.0967
3.5923
3.5816
3.3050
3.0680
2.8831
2.8709
2.8586
2.5163
2.4689
2.4658
1.9507
1.7291
1.1881


Aug20-2013-Naglaa.11.fid
LabGroup Parang
1852
C13CPD DMSO {C:\NMRdata\Parang} Naglaa 6
159.6371
158.1642
152.7740
137.6277
136.9355
132.0841
130.8459
129.9725
125.6151
122.4963
117.8166
115.1050
112.5672
108.7466
Br
CN
N
N
H
NH2
N
H
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

15e