Supporting Information

Bioisosteric modification of known fucosidase inhibitors to discover a novel inhibitor of α-L-fucosidase

Chandramohan Bathula[1, §], Shreemoyee Ghosh[2, §], Santanu Hati[1], Sayantan Tripathi[3], Shailja Singh[3], Saikat Chakrabarti[2], Subhabrata Sen[1, *]

[1]*Department of Chemistry, School of Natural Sciences, Shiv Nadar University, Tehsil Dadri, Chithera,Uttar Pradesh 201314, India*

[2]*Structural Biology and Bio-Informatics Division, CSIR-Indian Institute of Chemical Biology, 4, Raja SC Mullick Road, Kolkata, West Bengal 700032, India*

[1]*Department of Life Sciences, School of Natural Sciences, Shiv Nadar University, Tehsil Dadri, Chithera,Uttar Pradesh 201314, India*

Contents

Experimental

General

Unless otherwise noted, all reactions were carried out in flame-dried glassware under a static nitrogen atmosphere with anhydrous solvent. All reagents were purchased from Sigma Aldrich, Acros or Alfa Aesar. Solvents were treated with 4 Å molecular sieves or sodium and distilled prior to use. Purifications of reaction products by washing with diethyl ether. ^{1}H NMR and ^{13}C NMR spectra were recorded with tetramethylsilane (TMS) as internal standard at ambient temperature unless otherwise indicated Bruker 500 and 400 MHz 120 and 100 MHz for ^{13}C NMR. Chemical shifts are reported in parts per million (ppm) and coupling constants are reported as Hertz (Hz). Splitting patterns are designated as singlet (s), broad singlet (bs), doublet (d), triplet (t). Splitting patterns that could not be interpreted or easily visualized are designated as multiple (m). The Mass Spectrometry analysis was done on the 6540 UHD Accurate-Mass Q-TOF LC/MS system (Agilent Technologies) equipped with Agilent 1290 LC system obtained by the Dept. of Chemistry, School of Natural Sciences, Shiv Nadar University, Uttar Pradesh 203207, India

General procedure for the knoevenagel condensation 6-methylfuro[3,4-c]pyridine-3,4(1H,5H)-dione and appropriate aldehydes.

To a stirred solution of 6-methylfuro[3,4-c]pyridine-3,4(1H, 5H)-dione (0.2 g, 1.0 eq.) in ethanol (5 mL) added piperidine (0.1eq.), followed by corresponding aldehyde (1.1 eq.) under N_2 atmosphere pre-tared vial at room temperature, then the reaction mixture stirred at 80° C, once TLC indicated complete consumption of the starting material (about 6 hours) allowed to cool to room temperature the resulting precipitate filter and washed with diethyl ether (2 × 10 mL) to get substituted 6-methylfuro[3,4-c]pyridine-3,4(1H,5H)-diones.

General procedure for the synthesis of c-4/c5 substituted thiophene 2 aldehydes.

To a stirred solution of 4-bromothiophene-2-carbaldehydes/5-bromothiophene-2-carbaldehydes (1 eq.) in 1, 4-dioxane (10 mL), added corresponding arylboronic acids (1.2 eq.) followed by aqueous (2 M) K_2CO_3 (3 eq.) then purged with argon gas for 15 min and then bis(triphenylphosphine) palladium(II) dichloride (0.05 eq.) was added. The reaction mixture was heated at 100 °C, once TLC indicated complete

consumption of the starting material (about 14 hours), the reaction mixture was allowed to cool to room temperature filtered through celite, quenched with 20 volumes of brine solution, extracted with ethyl acetate (2 ×10 mL), dried over anhydrous Na_2SO_4, filtered evaporated to dryness to give the crude c-4/c5 substituted thiophene 2 aldehydes which were used for synthesis of 4d, 4e, 4f and 5e.

(Z)-6-methyl-1-((1-methyl-1H-indol-3-yl)methylene)furo[3,4-c]pyridine-3,4(1H,5H)-dione (4a)

Following the general protocol 1-methyl-1H-indole-3-carbaldehyde (212 mg, 1.1 eq.) afforded **4a** in 363 mg (yield 98%).^{1}H NMR (500 MHz; DMSO-d6): δ 11.97 (br. s, 1H), 8.04 (s, 1H), 8.03 (d, *J* = 8 Hz, 1H), 7.55 (d, *J* = 8 Hz, 1H), 7.35 (s, 1H), 7.31-7.28 (t, *J* = 7.5 Hz, 1H), 7.26-7.23 (t, *J* = 7.5 Hz, 1H), 6.84 (s, 1H), 3.93 (s, 3H), 2.33 (s, 3H), ^{13}C NMR (125 MHz; DMSO-d6): δ 164.35, 158.08, 155.28, 153.91, 138.86, 136.49, 133.84, 127.01, 122.71, 120.83, 118.89, 110.63, 108.19, 105.93, 103.12, 96.48, 33.10, 19.57. HRMS (ESI): $[M + H]^+$ calculated for ($C_{18}H_{15}N_2O_3$) 307.1004, found 307.1003.

(Z)-1-(benzo[b]thiophen-3-ylmethylene)-6-methylfuro[3,4-c]pyridine-3,4(1H,5H)-dione (4b)

Following the general protocol benzo[b]thiophene-3-carbaldehyde (168 mg, 1.1 eq.) afforded **4b** in 359 mg (yield 96%).^{1}H NMR (500 MHz; DMSO-d6): δ 9.54 (s, 1H), 9.22 (s, 1H), 8.97 (s, 1H), 8.76 (d, *J* = 8.4 Hz, 1H), 7.98 (s, 1H), 7.79 (s, 1H), 7.60 (s, 1H), 3.89 (s, 3H), ^{13}C NMR (125 MHz; DMSO-d6): δ 164.10, 158.29, 156.52, 155.90, 144.10, 139.34, 138.41, 131.73, 128.24, 125.68, 125.35, 123.61, 122.39, 106.02, 103.16, 99.74, 20.22. HRMS (ESI): $[M + H]^+$ calculated for ($C_{17}H_{12}NO_3S$) 310.0460, found 310.0443.

(Z)-1-(3,5-difluorobenzylidene)-6-methylfuro[3,4-c]pyridine-3,4(1H,5H)-dione (4c)

Following the general protocol 3,5-difluorobenzaldehyde (189 mg, 1.1 eq.) afforded **4c** in 262 mg (yield 75%).^{1}H NMR (500 MHz; DMSO-d6): δ 12.36 (br. s, 1H), 7.44 (d, *J* = 10 Hz, 2H), 7.35-7.31 (m, 1H), 6.95 (s, 1H), 6.69 (s, 1H), 2.35 (s, 3H), ^{13}C NMR (125 MHz; DMSO-d6): δ 168.21, 158.00, 156.47, 156.26, 143.69, 130.88,

128.11, 118.99, 118.76, 109.32, 106.00, 97.23, 20.07 HRMS (ESI): $[M + H]^+$ calculated for ($C_{15}H_{10}F_2NO_3$) 290.0550, found 290.0551.

(Z)-6-methyl-1-((5-(pyridin-3-yl)thiophen-2-yl)methylene)furo[3,4c]pyridine3,4(1H,5H)-dione (4d)

Following the general protocol 5-(pyridin-3-yl)thiophene-2-carbaldehyde (252 mg, 1.1 eq.) afforded **4d** in 317 mg (yield 78%). ^{1}H NMR (500 MHz; DMSO-d6): δ 12.22 (s, 1H), 8.99 (s, 1H), 8.55 (d, *J* = 8 Hz, 1H), 8.14 (d, *J* = 8 Hz, 1H), 7.75 (d, *J* = 8 Hz, 1H), 7.52 (s, 1H), 7.51-7.47 (t, *J* = 11 Hz, 1H), 7.38 (s, 1H), 6.73 (s, 1H), 2.34 (s, 3H), ^{13}C NMR (125 MHz; DMSO-d6): δ 185.00, 168.95, 167.24, 158.40, 154.97, 144.57, 143.98, 141.39, 140.83, 139.22, 128.85, 127.25, 108.96, 100.07, 22.29. HRMS (ESI): $[M + H]^+$ calculated for ($C_{18}H_{13}N_2O_3S$) 337.0647, found 337.0641.

(Z)-1-((5-(6-methoxypyridin-3-yl)thiophen-2-yl)methylene)-6-methylfuro[3,4-c]pyridine-3,4

(1H, 5H)-dione (4e)

Following the general protocol 5-(6-methoxypyridin-3-yl)thiophene-2-carbaldehyde (292 mg, 1.1 eq.) afforded **4e** in 364 mg (yield 82%).^{1}H NMR (500 MHz; DMSO-d6): δ 10.65 (br. s, 1H), 8.56 (s, 1H), 8.05 (d, *J* = 5 Hz, 1H), 7.56 (s, 1H), 7.46 (s, 1H), 7.32 (s, 1H), 6.90 (d, *J* = 10 Hz, 1H), 6.69 (s, 1H), 3.89 (s, 3H), 2.31 (s, 3H), ^{13}C NMR (125 MHz; DMSO-d6): δ 163.60, 163.46, 157.75, 156.47, 155.26, 145.28, 143.71, 140.51, 136.57, 134.83, 133.49, 124.48, 123.23, 111.06, 106.08, 105.40, 96.70, 53.52, 19.64. HRMS (ESI): $[M + H]^+$ calculated for ($C_{19}H_{15}N_2O_4S$) 367.3974, found 367.0976.

(Z)-6-methyl-1-((4-phenylthiophen-2-yl)methylene)furo[3,4-c]pyridine-3,4(1H,5H)-dione (4f)

Following the general protocol 4-phenyl-thiophene-2-carbaldehyde (248 mg, 1.1 eq.) afforded **4f** in 324 mg (yield 80%). ^{1}H NMR (500 MHz; DMSO-d6): δ 12.17 (br. s, 1H), 8.14 (s, 1H) 7.80 (s, 1H), 7.69 (d, *J* = 10 Hz, 2H), 7.41 (t, *J* = 5 Hz, 2H), 7.29 (d, *J* = 10 Hz, 2H), 6.68 (s, 1H), 2.34 (s, 3H). ^{13}C NMR (125 MHz; DMSO-d6): δ 164.11, 158.23, 156.01, 155.72, 142.27, 141.61, 137.00, 134.80, 130.56, 129.58, 128.14, 127.55, 126.55, 106.31, 106.15, 97.24, 20.15. HRMS (ESI) m/z: [M+ H]+ calculated for ($C_{19}H_{14}NO_3S$) 336.0989, found 336.0980.

General procedure for the knoevenagel condensation 2-thioxoimidazolidin-4-one and appropriate aldehydes

To a stirred solution of 2-thioxoimidazolidin-4-one (200 mg, 1.0 eq.) in ethanol (5 mL) added piperidine (0.1eq.), followed by corresponding aldehyde (1.1 eq.) under N_2 atmosphere in a pre-tared vial at room temperature, then the reaction mixture stirred at 80° C, reaction monitored by TLC, once TLC indicated complete consumption of the starting material allowed to cool to room temperature the resulting precipitate filter and washed with diethyl ether (2 × 10 mL) to get substituted 2-thioxoimidazolidin-4-ones.

(Z)-2-thioxo-5-((1-tosyl-1H-indol-3-yl)methylene)imidazolidin-4-one (5a)

Following the general protocol 1-tosyl-1H-indole-3-carbaldehyde (556 mg, 1.1 eq.) afforded **5a** in 583 mg (yield 85%).^{1}H NMR (400 MHz; DMSO-d6): δ 12.40 (br. s, 2H), 8.82 (s, 1H), 7.99-7.95 (m, 3H), 7.86 (d, *J* = 8 Hz, 1H), 7.44-7.33 (m, 4H), 6.62 (s, 1H), 2.20 (s, 3H), ^{13}C NMR (100 MHz; DMSO-d6): δ 179.25, 165.85, 146.32, 134.25, 134.14, 130.83, 129.92, 128.15, 127.49, 127.40, 126.14, 124.54, 120.01, 114.79, 113.62, 100.53, 21.51. HRMS (ESI): $[M + H]^+$ calculated for ($C_{19}H_{16}N_3O3S2$) 399.0628, found 399.0624.

(Z)-5-(3,5-difluorobenzylidene)-2-thioxoimidazolidin-4-one (5b)

Following the general protocol 3,5-difluorobenzaldehyde (269 mg, 1.1 eq.) afforded **5b** in 380 mg (yield 92%). ^{1}H NMR (400 MHz; DMSO-d6): δ 7.75-7.72 (m, 2H), 6.99-6.93 (m, 1H), 5.96 (s, 1H), ^{13}C NMR (100 MHz; DMSO-d6): δ 184.18, 172.14. 163.61, 163.47, 161.19, 161.05, 144.15, 140.13, 111.86, 111.79, 111.67, 111.60, 104.09, 101.53, 101.27, 101.01. HRMS (ESI): $[M + H]^+$ calculated for ($C_{10}H_7F_2N_2OS$) 241.0242, found 241.0239.

(Z)-5-((4-bromothiophen-2-yl)methylene)-2-thioxoimidazolidin-4-one (5c)

Following the general protocol 4-bromothiophene-2-carbaldehyde (359 mg, 1.1 eq.) afforded **5c** in 446 mg (yield 90%).^{1}H NMR (500 MHz; DMSO-d6): δ 9.38 (br. s, 2H), 7.98 (d, *J* = 11.5 Hz, 1H), 7.78 (s, 1H), 7.59 (s, 1H). ^{13}C NMR (125 MHz; DMSO-d6): δ 179.84, 174.27, 140.30, 133.98, 129.24, 127.69, 120.80, 110.61. HRMS (ESI): $[M + H]^+$ calculated for ($C_8H_6BrN_2OS_2$) 288.9027, found 288.9025.

(Z)-5-benzylidene-2-thioxoimidazolidin-4-one (5d)

Following the general protocol benzaldehyde (201 mg, 1.1 eq.) afforded **5d** in 344 mg (yield 98%). ^{1}H NMR (400 MHz; DMSO-d6): δ 6.68 (d, *J* = 8 Hz, 2H), 6.41-6.34 (m, 3H), 5.45 (s, 1H). ^{13}C NMR (100 MHz; DMSO-d6): δ 179.54, 166.12, 132.57, 130.36, 129.54, 129.04, 128.06, 111.93. HRMS (ESI) m/z: [M + H]+ calculated for ($C_{10}H_9N_2OS$) 205.0430, found 205.0432.

(Z)-5-((5-(pyridin-3-yl)thiophen-2-yl)methylene)-2-thioxoimidazolidin-4-one (5e)

Following the general protocol 5-(pyridin-3-yl)thiophene-2-carbaldehyde (359 mg, 1.1 eq.) afforded **5e** in 420 mg (yield 85%). ^{1}H NMR (500 MHz; DMSO-d6): δ 9.34 (br. s, 2H), 8.96 (d, *J* = 11 Hz, 1H), 8.55 (d, *J* = 3.5 Hz, 1H), 8.10 (d, *J* = 8 Hz, 1H), 7.83 (d, *J* = 12 Hz, 1H), 7.79 (d, *J* = 4 Hz, 1H), 7.65-7.62 (m, 1H), 7.49-7.46 (m, 1H), ^{13}C NMR (125 MHz; DMSO-d6): 179.93, 174.31, 149.24, 146.24, 143.56, 139.28, 134.22, 132.85, 129.02, 128.36, 126.62, 124.20, 121.78. HRMS (ESI): $[M + H]^+$ calculated for ($C_{13}H_{10}N_3O_3S_2$) 288.0187, found 288.0188.

(Z)-5-(3, 4-dimethoxybenzylidene)-2-thioxoimidazolidin-4-one (5f)

Following the general protocol 3,4-dimethoxybenzaldehyde (315 mg, 1.1 eq.) afforded **5f** in 432 mg (yield 95%). ^{1}H NMR (400 MHz; DMSO-d6): 9.35 (br. s, 1H), 9.08 (br. s, 1H), 7.55 (s, 1H), 7.17-7.09 (m, 3H), 3.81 (s, 6H), ^{13}C NMR (500 MHz; DMSO-d6): δ 150.54, 149.38, 129.96, 127.20, 127.09, 123.33, 113.21, 112.55, 100.00, 56.15, 55.94. HRMS (ESI): $[M + H]^+$ calculated for ($C_{12}H_{13}N_2O_3S$) 265.0641, found 265.0643.

General procedure for the knoevenagel condensation between imidazolidine-2, 4-dione andappropriate aldehydes

To a stirred solution of imidazolidine-2, 4-dione (200 mg, 1.0 eq.) in ethanol (5 mL) added piperidine (0.1eq.), followed by corresponding aldehyde (1.1 eq.) under N_2 atmosphere in a pre-tared vial at room temperature, then the reaction mixture stirred at 80° C, reaction monitored by TLC, once TLC indicated complete consumption of the starting material allowed to cool to room temperature the resulting precipitate

filter and washed with diethyl ether (2 × 10 mL) to get substituted imidazolidine-2, 4-diones.

(Z)-5-(4-nitrobenzylidene)imidazolidine-2,4-dione (6a)

Following the general protocol 4-nitrobenzaldehyde (332 mg, 1.1 eq.) afforded **6a** in 346 mg (yield 75%). ^{1}H NMR (400 MHz; DMSO-d6): δ 11.16 (br. s, 2H), 8.19 (d, *J* = 8 Hz, 2H), 7.84 (d, *J* = 8 Hz, 2H), 6.48 (s, 1H), ^{13}C NMR (100 MHz; DMSO-d6): δ 165.71, 156.23, 146.65, 140.47, 131.32, 130.57, 124.15, 105.60. HRMS (ESI) m/z: [M - H] calculated for ($C_{10}H_6N_3O_4$) 232.0437, found 232.0435.

(Z)-5-(pyridin-4-ylmethylene)imidazolidine-2,4-dione (6b)

Following the general protocol isonicotinaldehyde (235 mg, 1.1 eq.) afforded **6b** in 299 mg (yield 80%). ^{1}H NMR (500 MHz; DMSO-d6): δ 8.51 (d, *J* = 10 Hz, 2H), 7.57 (d, *J* = 5 Hz, 2H), 6.16 (s, 1H), ^{13}C NMR (125 MHz; DMSO-d6): δ 167.83, 158.81, 149.77, 149.25, 141.45, 123.29, 122.99, 102.33. HRMS (ESI) m/z: [M - H] calculated for ($C_9H_6N_3O_2$) 188.0538, found 188.0534.

(Z)-5-(3, 5-difluorobenzylidene)imidazolidine-2,4-dione (6c)

Following the general protocol 3, 5-difluorobenzaldehyde (300 mg, 1.1 eq.) afforded **6c** in 333 mg (yield 75%).^{1}H NMR (400 MHz; DMSO-d6): δ 11.38 (s, 1H), 10.74 (s, 1H), 7.41-7.38 (m, 2H), 7.21-7.15 (m, 1H), 6.41 (s, 1H), ^{13}C NMR (125 MHz; DMSO-d6): δ 179.93, 149.24, 146.24, 143.56, 139.29, 134.22, 132.85, 129.03, 128.36, 126.62, 124.21, 121.78. HRMS (ESI) m/z: [M - H] calculated for ($C_{10}H_5F_2N_2O_2$) 223.0397, found 223.0392.

(Z)-5-(4-(dimethylamino)benzylidene)imidazolidine-2,4-dione (6d)

Following the general protocol 4-(dimethylamino)benzaldehyde (382 mg, 1.1 eq.) afforded **6d** in 361 mg (yield 79%).^{1}H NMR (500 MHz; DMSO-d6): δ 11.05 (s, 1H), 10.27 (s, 1H), 7.48 (d, *J* = 10 Hz, 2H), 6.69 (d, *J* = 10 Hz, 2H), 6.35 (s, 1H), 2.95 (s, 6H), ^{13}C NMR (125 MHz; DMSO- d6): δ 165.71, 155.57, 150.22, 131.00, 123.87, 120.26, 111.94, 111.08, 110.34. HRMS (ESI-TOF) m/z: [M - H] calculated for ($C_{12}H_{12}N_3O_2$) 230.1008, found 230.1005.

(Z)-5-benzylideneimidazolidine-2,4-dione (6e)

Following the general protocol benzaldehyde (232 mg, 1.1 eq.) afforded **6e** in 290 mg (yield 78%).^{1}H NMR (500 MHz; DMSO-d6): δ 11.26 (br. s, 1H), 10.56 (br. s, 1H), 7.61 (d, *J* = 10 Hz, 2H), 7.41-7.38 (m, 2H), 7.34-7.30 (m, 1H), 6.42 (s, 1H), ^{13}C NMR (125 MHz; DMSO-d6): δ 165.62, 155.77, 133.01, 129.43, 128.82, 128.42, 128.00, 108.35. HRMS (ESI-TOF) m/z: [M - H] calculated for ($C_{10}H_7N_2O_2$) 187.0572, found 187.0570.

(Z)-5-((1-tosyl-1H-indol-3-yl)methylene)imidazolidine-2,4-dione (6f)

Following the general protocol 1-tosyl-1H-indole-3-carbaldehyde (650 mg, 1.1 eq.) afforded **6f** in 424 mg (yield 76%).^{1}H NMR (400 MHz; CD_3OD): δ 8.40 (s, 1H), 7.89 (d, *J* = 8 Hz, 2H), 7.82 (d, *J* = 8 Hz, 2H), 7.63 (d, *J* = 8 Hz, 1H), 7.30-7.20 (m, 4H), 6.58 (s, 1H), 2.23 (s, 3H), ^{13}C NMR (100 MHz; DMSO-d6): δ 179.26, 165.86, 146.34, 134.25, 134.14, 130.85, 129.92, 128.14, 127.50, 127.42, 126.15, 124.55, 120.02, 114.79, 113.62, 100.54, 21.51. HRMS (ESI-TOF) m/z: [M - H] calculated for ($C_{19}H_{14}N_3O_4S$) 380.0711, found 380.0714.

Figure S1:

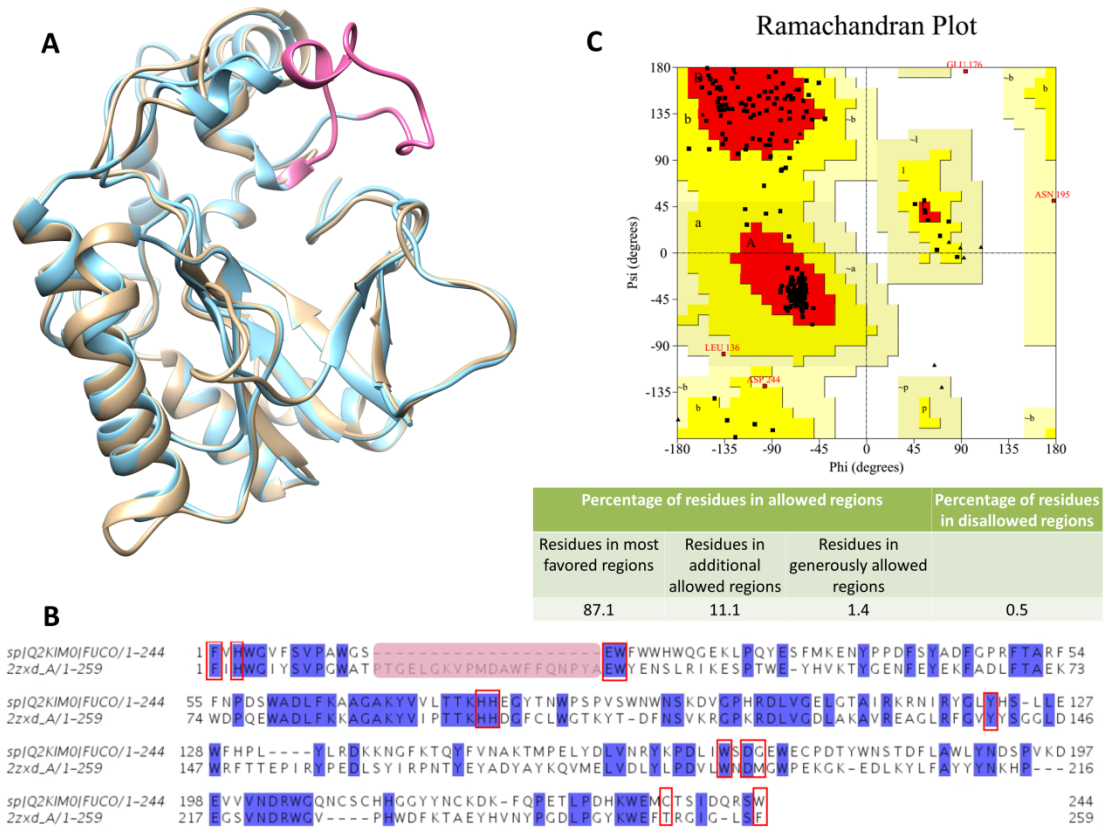


Figure S1. Stereo-chemical properties of the three dimensional (3D) homology model of *Bos taurus* α-L-fucosidase catalytic N-terminal region (residues 60-311). Panel A shows the *Bos taurus* α-L-fucosidase catalytic N-terminal model (colour: golden yellow) superimposed with *Thermotoga maritima* α-L-fucosidase template (colour: cyan) with a RMSD of 0.483Å. Panel B shows the alignment between the N-terminal sequences of *Bos taurus* α-L-fucosidase and *Thermotoga maritima* α-L-fucosidase. The loop marked in pink in panel A corresponds to the pink region in panel B which is missing in the *Bos taurus* α-L-fucosidase catalytic N-terminal model. Conserved residues (sequence identity: 35.02%) are marked in blue boxes whereas the active site residues are shown in red boxes. Panel C shows the Ramachandran plot statistics, which indicate that the model has 99.6% residues in allowed regions.

Figure S2:

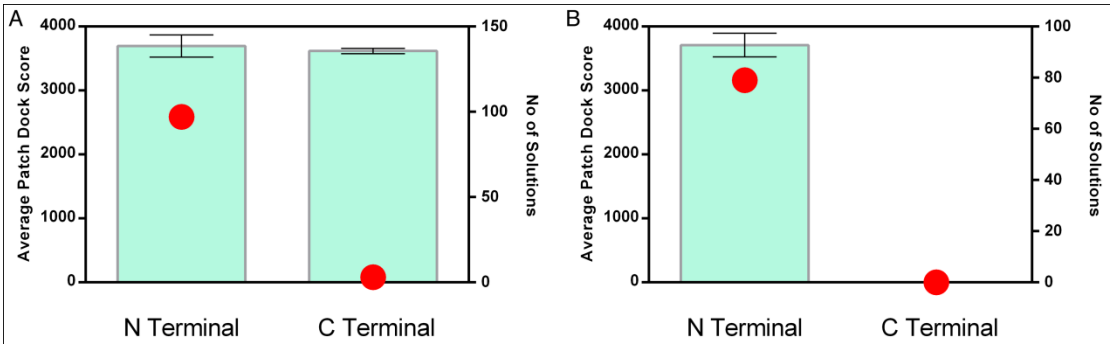


Figure S2. Preference of binding at the N terminal domain of bacterial α-L-fucosidase (PDB ID: 2ZX5). The bar represents the average PatchDock score while the red dot represents the number of solutions docked at N and C terminal domains, respectively. Panel A shows the number of solutions and average PatchDock score for N and C terminal domains with regard to all PatchDock solutions. Among all PatchDock solutions, 97 solutions were observed to bind at the N-terminal with an average PatchDock score of 3695.5 while 3 solutions were seen to bind at the C-terminal with an average PatchDock score of 3617.3. Panel B, on the other hand, shows the number of solutions and average PatchDock score for solutions docked to N and C terminal domains when only solutions from the top three clusters were considered. The top 3 clusters contain the largest number of solutions with highest average PatchDock score. Considering the top three clusters only, all 79 solutions were seen to bind at the N terminal domain with an average score of 3708.5 while none were found to bind at the C terminal.

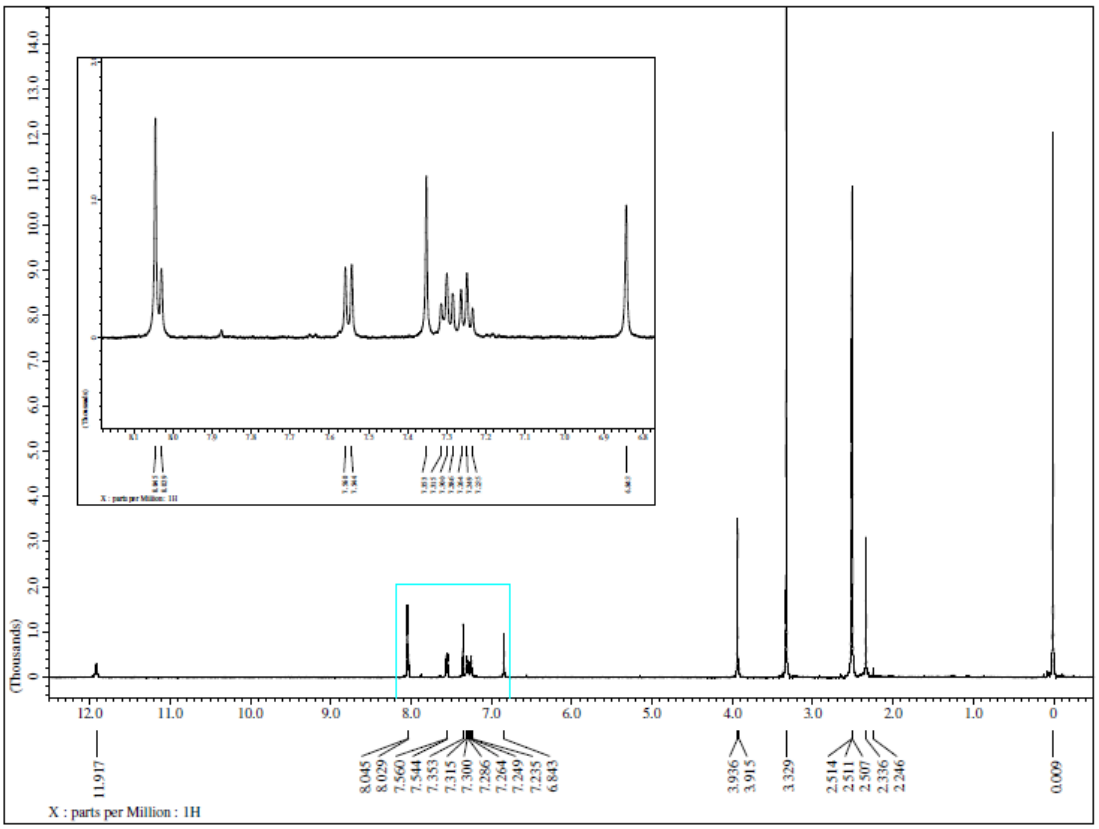
Table S1: COS cell cytotoxicity studies

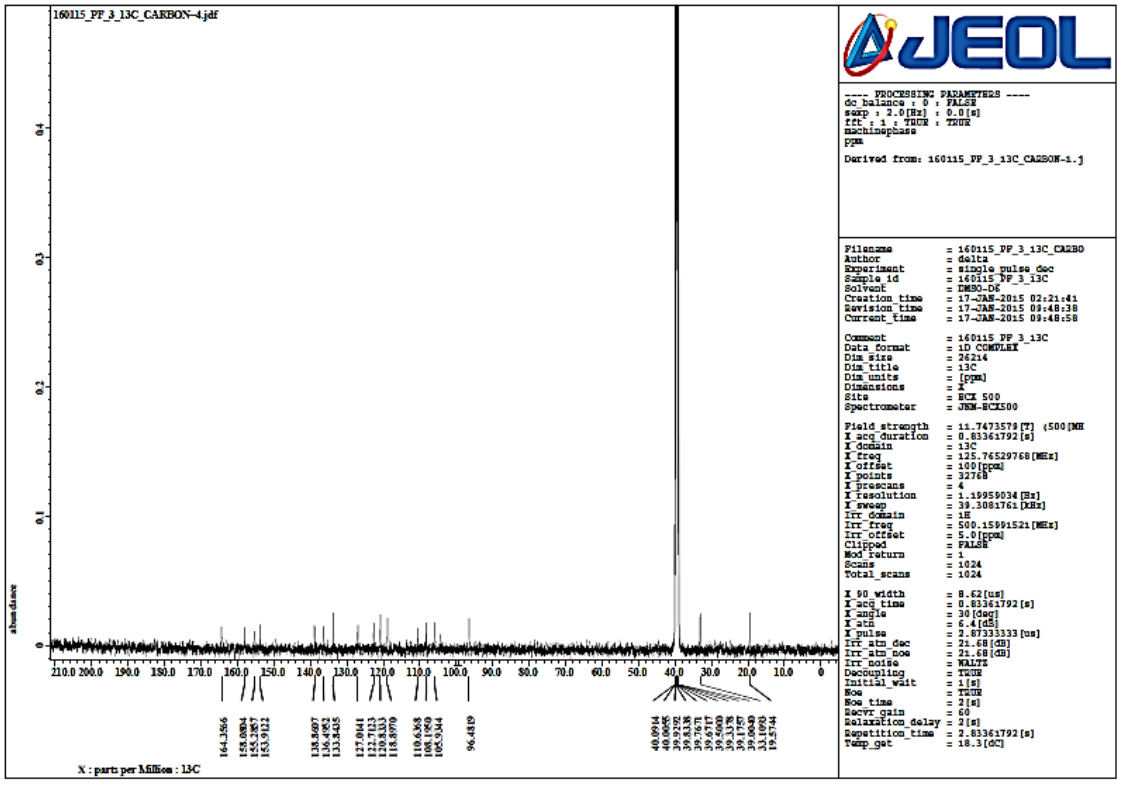
Compound 4d	Concentration (μM)	O.D 1	O.D 2	avg	ctrl-avg	/ctrl	*100 or % inhibition
	control	0.745	0.713	0.729	0	0	0
	50	0.727	0.784	0.7555	-0.0265	-0.03635	-3.635116598
	25	0.731	0.763	0.747	-0.018	-0.02383	-2.382528127
	12.5	0.708	0.714	0.711	0.018	0.024096	2.409638554
	6.25	0.779	0.788	0.7835	-0.0545	-0.07665	-7.665260197
	3.125	0.764	0.741	0.7525	-0.0235	-0.02999	-2.999361838
	1.56	0.905	0.873	0.889	-0.16	-0.21262	-21.26245847
	0.78	0.688	0.652	0.67	0.059	0.066367	6.636670416
	0.39	0.74	0.679	0.7095	0.0195	0.029104	2.910447761
	0.195	0.89	0.773	0.8315	-0.1025	-0.14447	-14.44679352
Compound 4e		**O.D 1**	**O.D 2**	**avg**	**ctrl-avg**	**/ctrl**	***100 or % inhibition**
	control	1.15	1.321	1.2355	0	0	0
	50	1.11	1.22	1.165	0.0705	0.057062	5.706191825
	25	1.191	1.321	1.256	-0.0205	-0.01659	-1.659247268
	12.5	1.223	1.334	1.2785	-0.043	-0.0348	-3.480372319
	6.25	1.102	1.213	1.1575	0.078	0.063132	6.313233509
	3.125	1.534	1.423	1.4785	-0.243	-0.19668	-19.66815055
	1.56	1.089	1.039	1.064	0.1715	0.13881	13.88101983
	0.78	1.05	1.071	1.0605	0.175	0.141643	14.16430595
	0.39	1.068	1.088	1.078	0.1575	0.127479	12.74787535
	0.195	1.121	1.342	1.2315	0.004	0.003238	0.323755565
Compound 4f		**O.D 1**	**O.D 2**	**avg**	**ctrl-avg**	**/ctrl**	***100 or % inhibition**
	control	1.039	1.259	1.149	0	0	0
	50	0.437	0.391	0.414	0.735	0.639687	63.96866841
	25	0.651	0.602	0.6265	0.5225	0.454743	45.4743255
	12.5	0.701	0.718	0.7095	0.4395	0.382507	38.25065274
	6.25	0.679	0.654	0.6665	0.4825	0.41993	41.99303742
	3.125	0.598	0.612	0.605	0.544	0.473455	47.34551784
	1.56	0.811	0.795	0.803	0.346	0.301131	30.11314186
	0.78	0.914	0.879	0.8965	0.2525	0.219756	21.97563098
	0.39	0.899	0.876	0.8875	0.2615	0.227589	22.7589208
	0.195	0.78	0.896	0.838	0.311	0.27067	27.0670148

Table S2: MCF 7 screening of compounds 4a, **b**, **e** and **f**

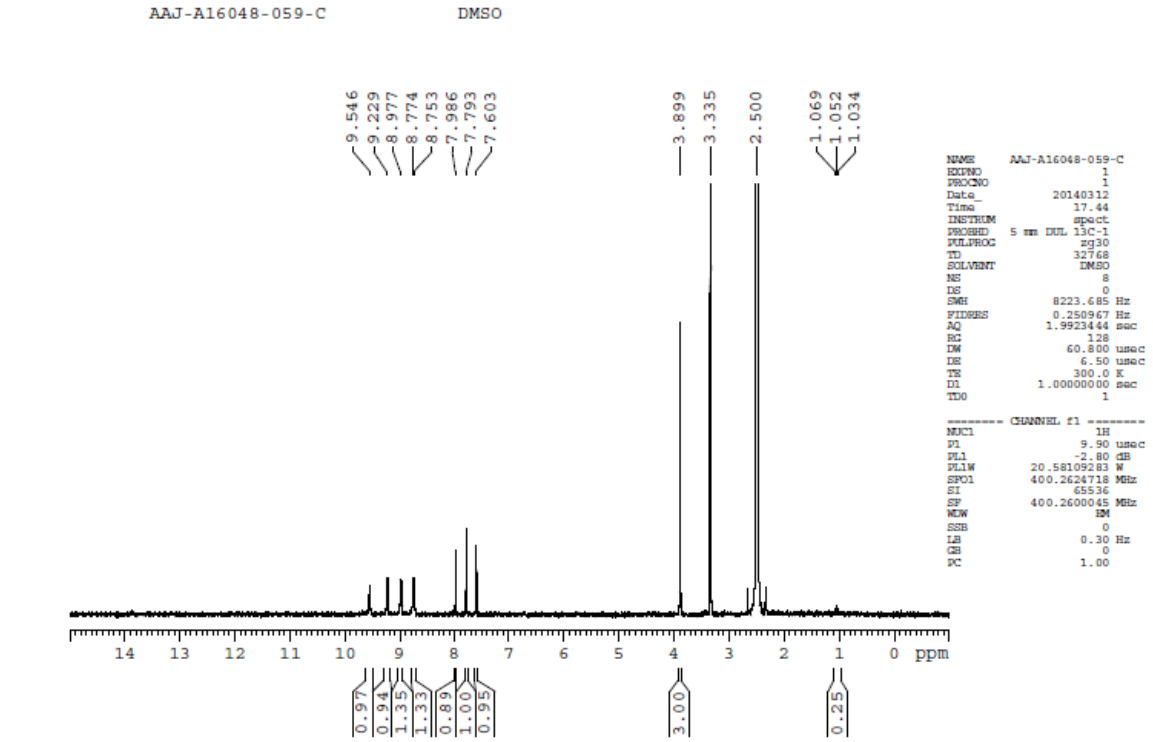
	O.D1	O.D2	O.D3	AVG	CTRL-TEST	CTRL-TEST/CTRL	*100
CONTROL	1.093	1.012	1.21	1.105	0	0	
eto	0.107	0.24	0.312	0.1735	0.9315	0.842986425	84.29864
4a	0.555	0.558	0.558	0.558	0.547	0.495022624	49.50226
4b	0.449	0.425	0.439	0.437667	0.667333333	0.603921569	60.39216
4e	0.457	0.44	0.362	0.419667	0.685333333	0.620211161	62.02112
4f	0.216	0.183	0.101	0.166667	0.938333333	0.849170437	84.91704

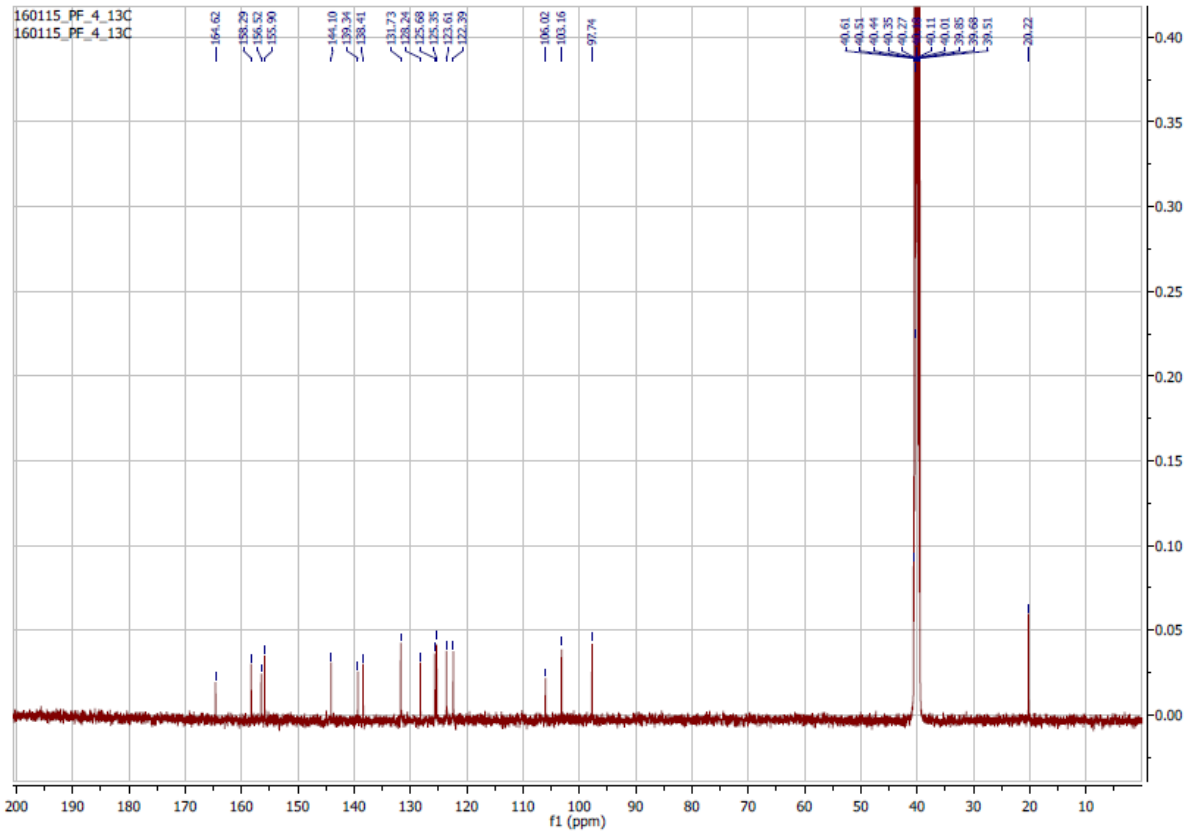
Compound **4a**


(Thousands)
X : parts per Million : 1H
11.917
8.045
8.029
7.560
7.544
7.353
7.315
7.300
7.286
7.264
7.249
7.235
6.843
3.936
3.915
3.329
2.514
2.511
2.507
2.336
2.246
0.009

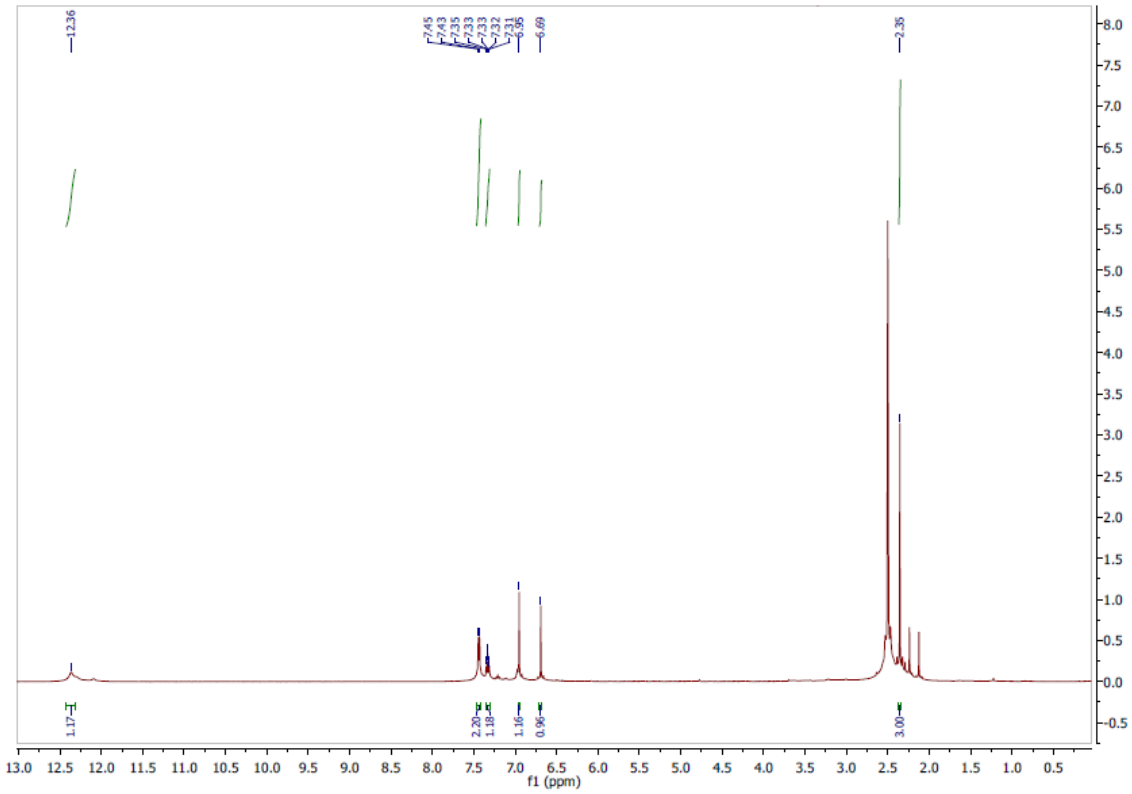

160115_PF_3_13C_CARBON-4.jdf
X : parts per Million : 13C
----- PROCESSING PARAMETERS -----
Filename = 160115_PF_3_13C_CARBO
Author = delta
Experiment = single_pulse_dec
Sample_id = 160115_PF_3_13C
Solvent = DMSO-D6
Creation_time = 17-JAN-2015 02:21:41
Revision_time = 17-JAN-2015 09:48:38
Current_time = 17-JAN-2015 09:48:58
Comment = 160115_PF_3_13C
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = RCX 500
Spectrometer = JNM-RCX500
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 1024
Total_scans = 1024
X_90_width = 8.62[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 6.4[dB]
X_pulse = 2.87333333[us]
Irr_atn_dec = 21.68[dB]
Irr_atn_noe = 21.68[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Recvr_gain = 60
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_get = 18.3[dC]

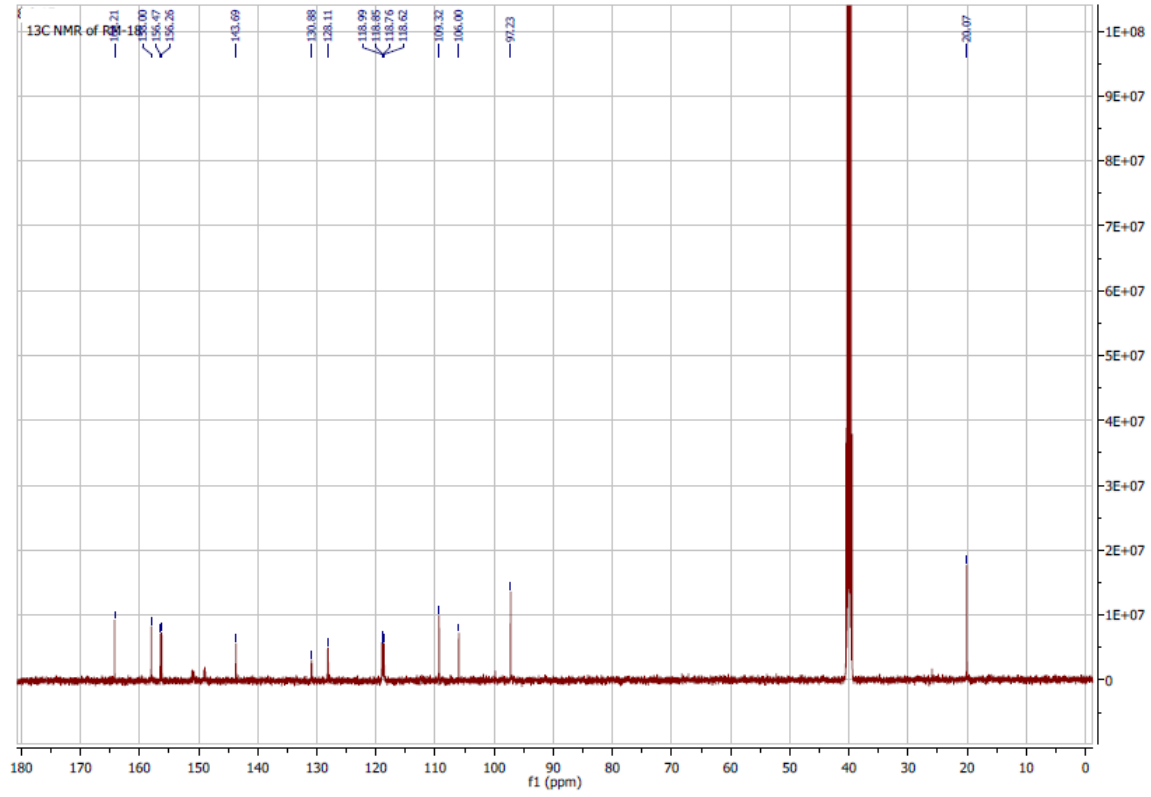
Compound **4b**


AAJ-A16048-059-C
DMSO
9.546
9.229
8.977
8.774
8.753
7.986
7.793
7.603
3.899
3.335
2.500
1.069
1.052
1.034
0.97
0.94
1.35
1.33
0.89
1.00
0.95
3.00
0.25
ppm

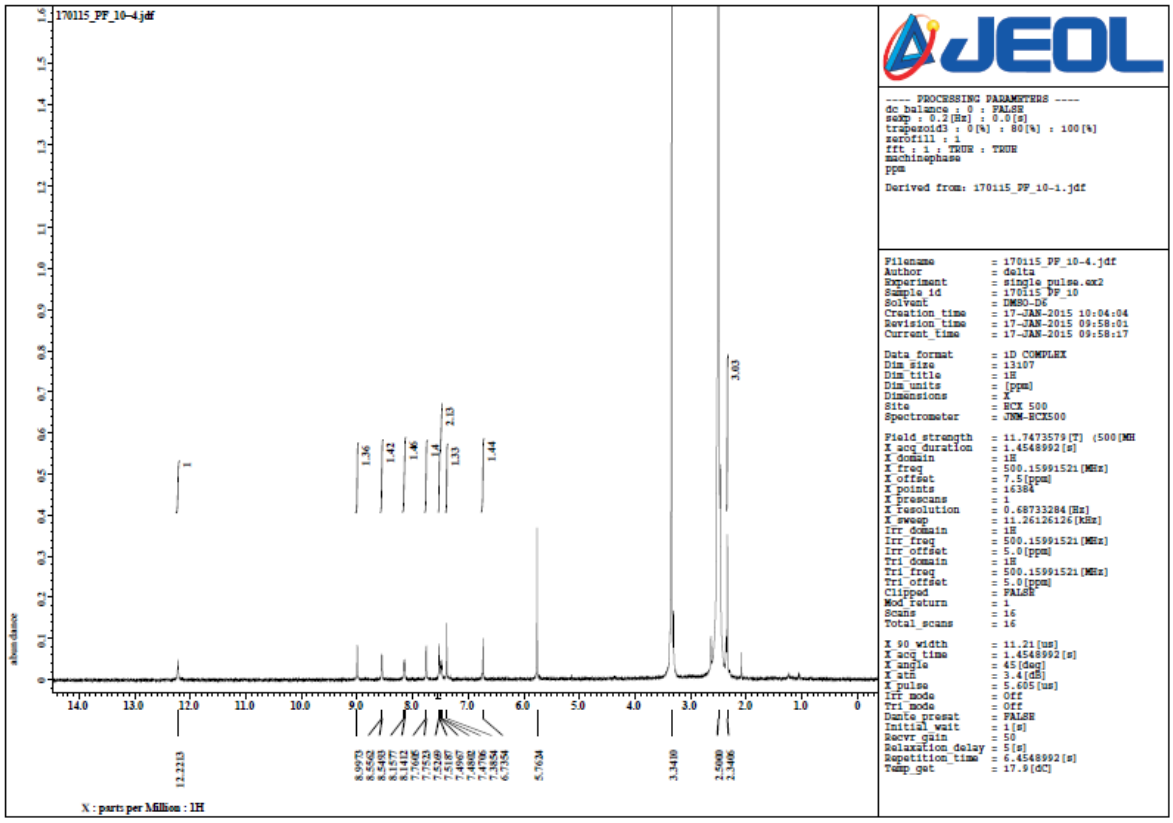

160115_PF_4_13C
160115_PF_4_13C
164.62
158.29
156.52
155.90
144.10
139.34
138.41
131.73
128.24
125.68
125.35
123.61
122.39
106.02
103.16
97.74
40.61
40.51
40.44
40.35
40.27
40.11
40.01
39.85
39.68
39.51
20.22
f1 (ppm)

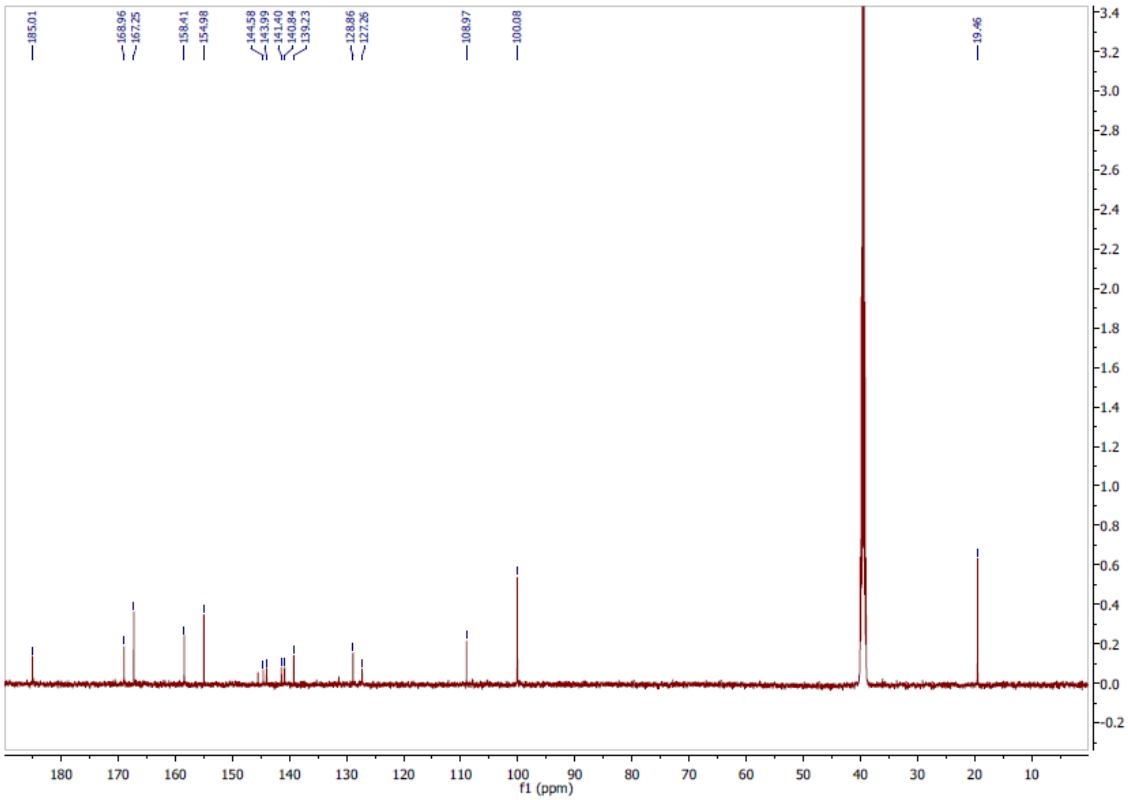
Compound **4c**


12.36
7.45
7.43
7.35
7.33
7.33
7.32
7.31
6.95
6.69
2.35
1.17
2.20
1.18
1.16
0.96
3.00
f1 (ppm)

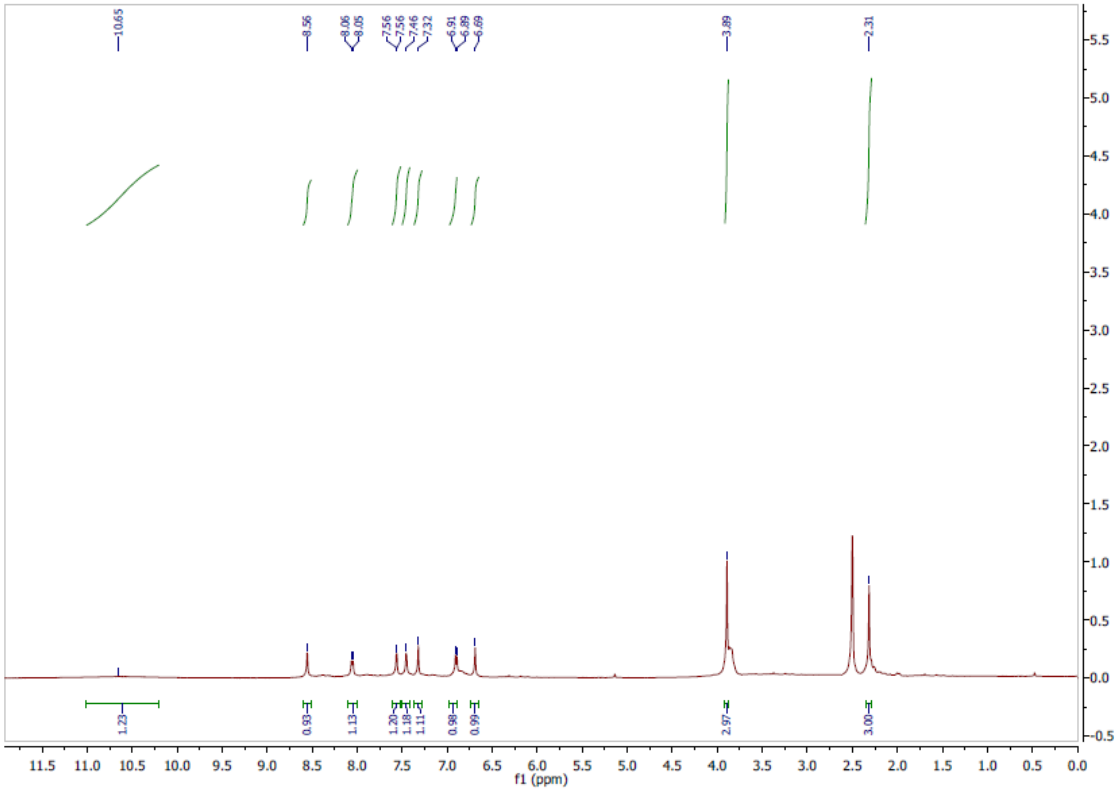

13C NMR of RM-18
143.69
130.88
128.11
118.99
118.85
118.76
118.62
109.32
106.00
97.23
20.07
f1 (ppm)

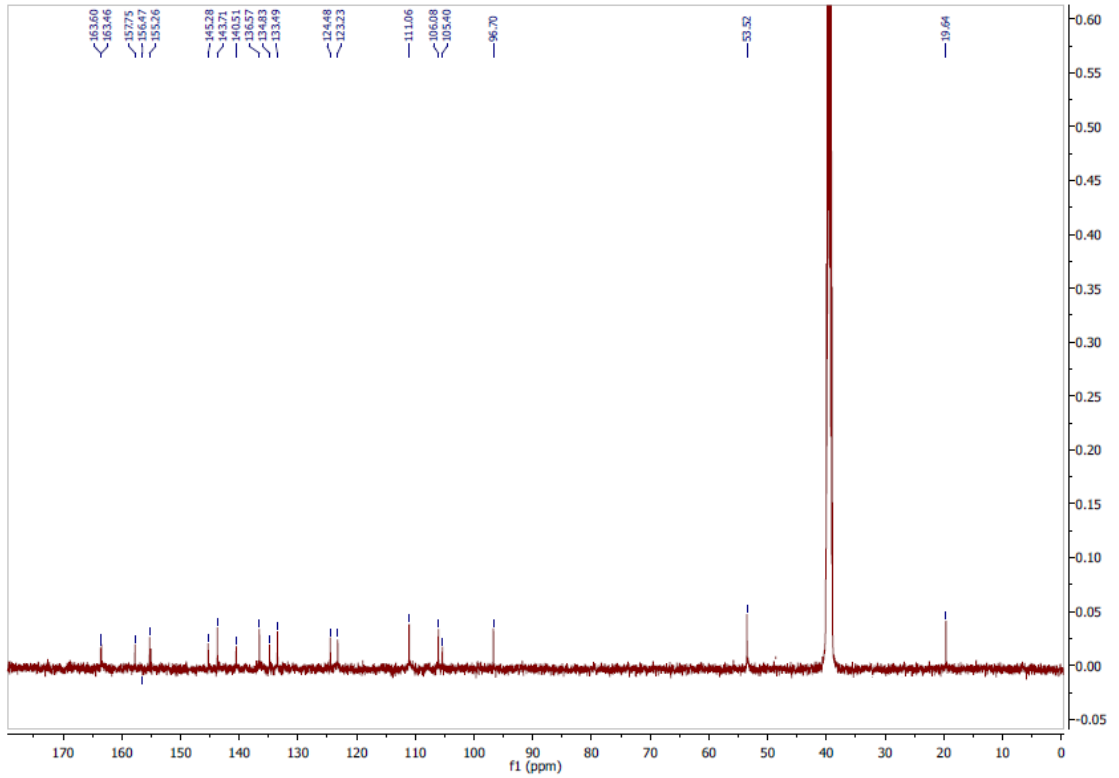
Compound **4d**


170115_PF_10-4.jdf
JEOL
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
trapezoid3 : 0[%] : 80[%] : 100[%]
zerofill : 1
fft : 1 : TRUE : TRUE
machinephase
ppm
Derived from: 170115_PF_10-1.jdf
Filename = 170115_PF_10-4.jdf
Author = delta
Experiment = single_pulse.ex2
Sample_id = 170115_PF_10
Solvent = DMSO-D6
Creation_time = 17-JAN-2015 10:04:04
Revision_time = 17-JAN-2015 09:58:01
Current_time = 17-JAN-2015 09:58:17
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = NCX 500
Spectrometer = JNM-ECX500
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 1.4548992[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 7.5[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.68733284[Hz]
X_sweep = 11.26126126[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 11.21[us]
X_acq_time = 1.4548992[s]
X_angle = 45[deg]
X_atn = 3.4[dB]
X_pulse = 5.605[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 50
Relaxation_delay = 5[s]
Repetition_time = 6.4548992[s]
Temp_get = 17.9[dC]
abundance
X : parts per Million : 1H

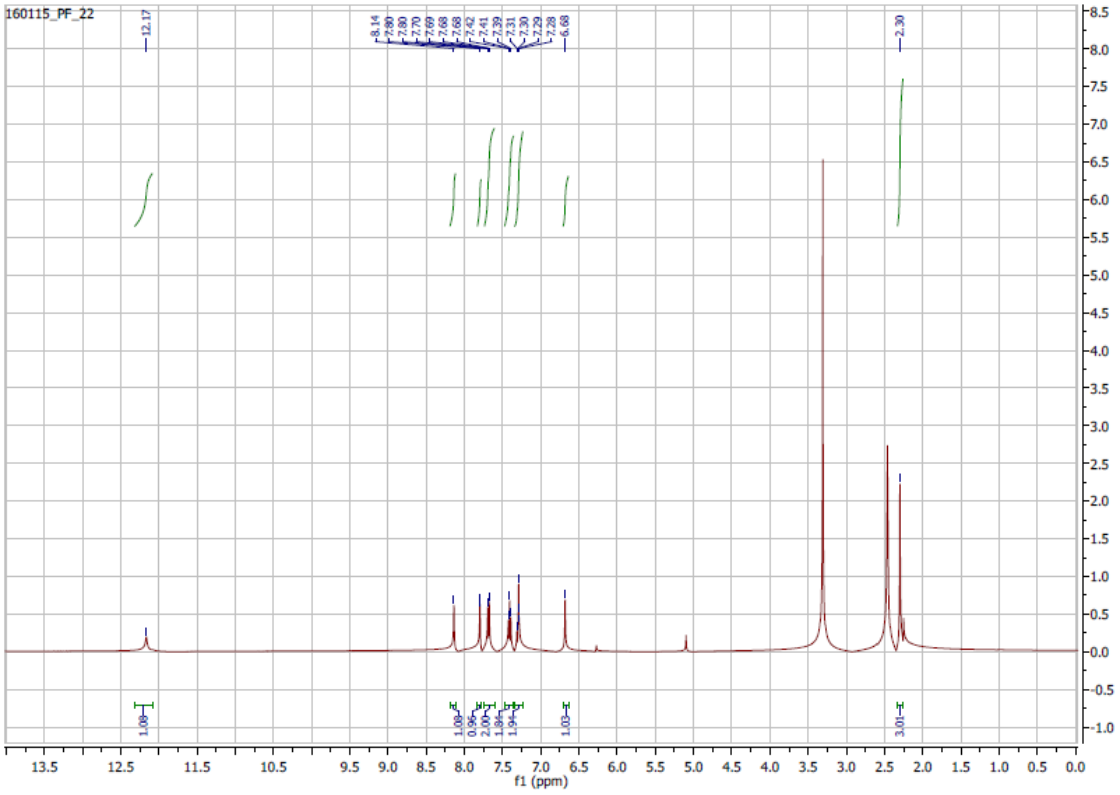

185.01
168.96
167.25
158.41
154.98
144.58
143.99
141.40
140.84
139.23
128.86
127.26
108.97
100.08
19.46
f1 (ppm)

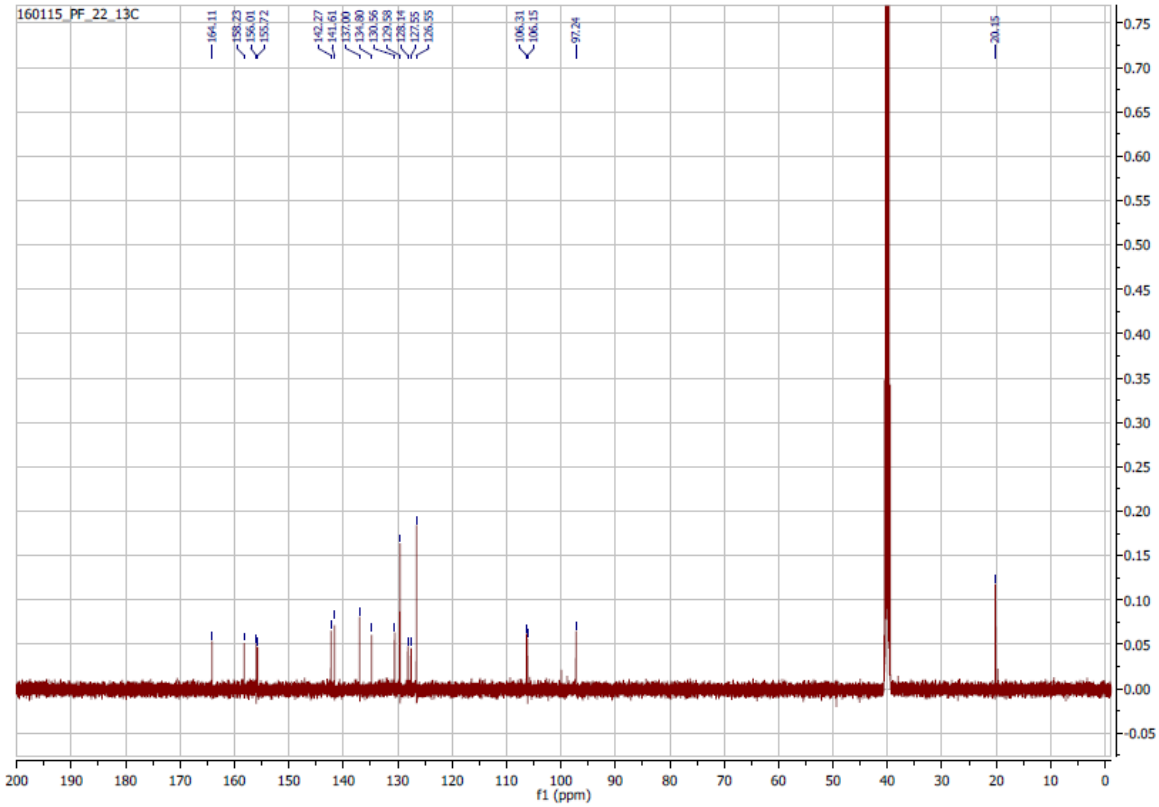
Compound **4e**


10.65
8.56
8.06
8.05
7.56
7.56
7.46
7.32
6.91
6.89
6.69
3.89
2.31
1.23
0.93
1.13
1.20
1.18
1.11
0.98
0.99
2.97
3.00
f1 (ppm)

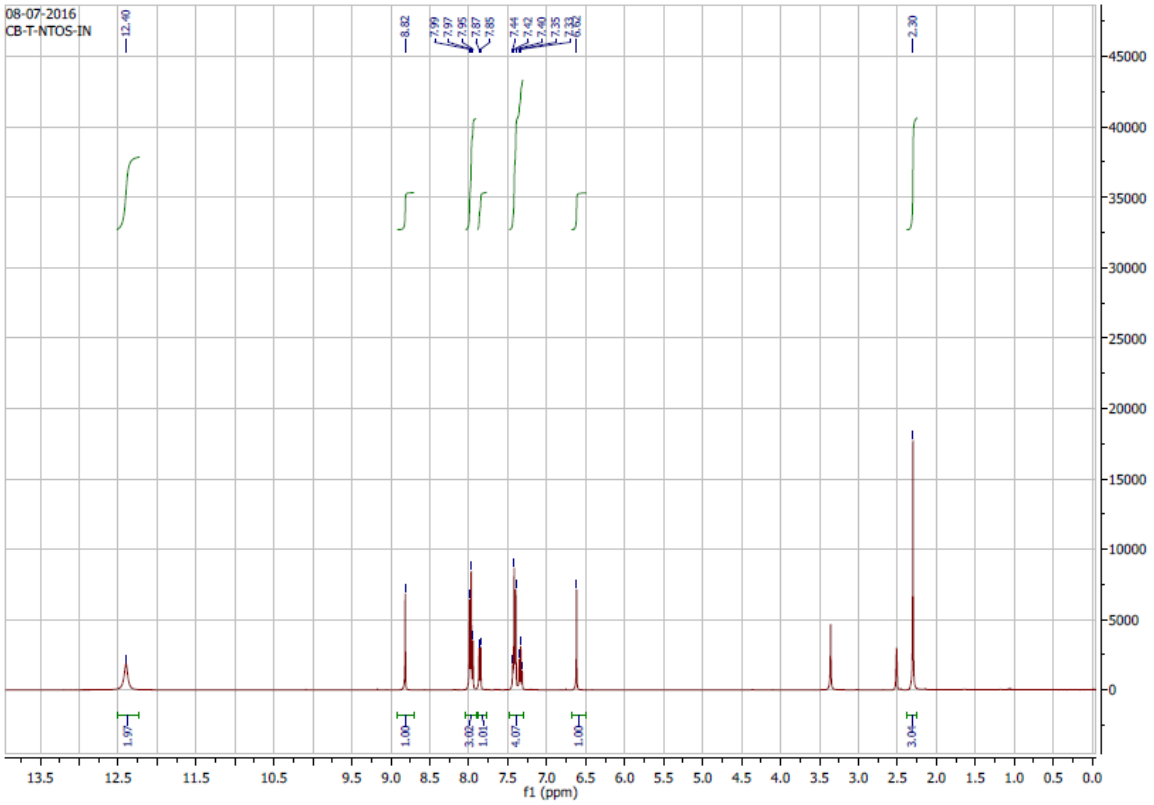

163.60
163.46
157.75
156.47
155.26
145.28
143.71
140.51
136.57
134.83
133.49
124.48
123.23
111.06
106.08
105.40
96.70
53.52
19.64
f1 (ppm)

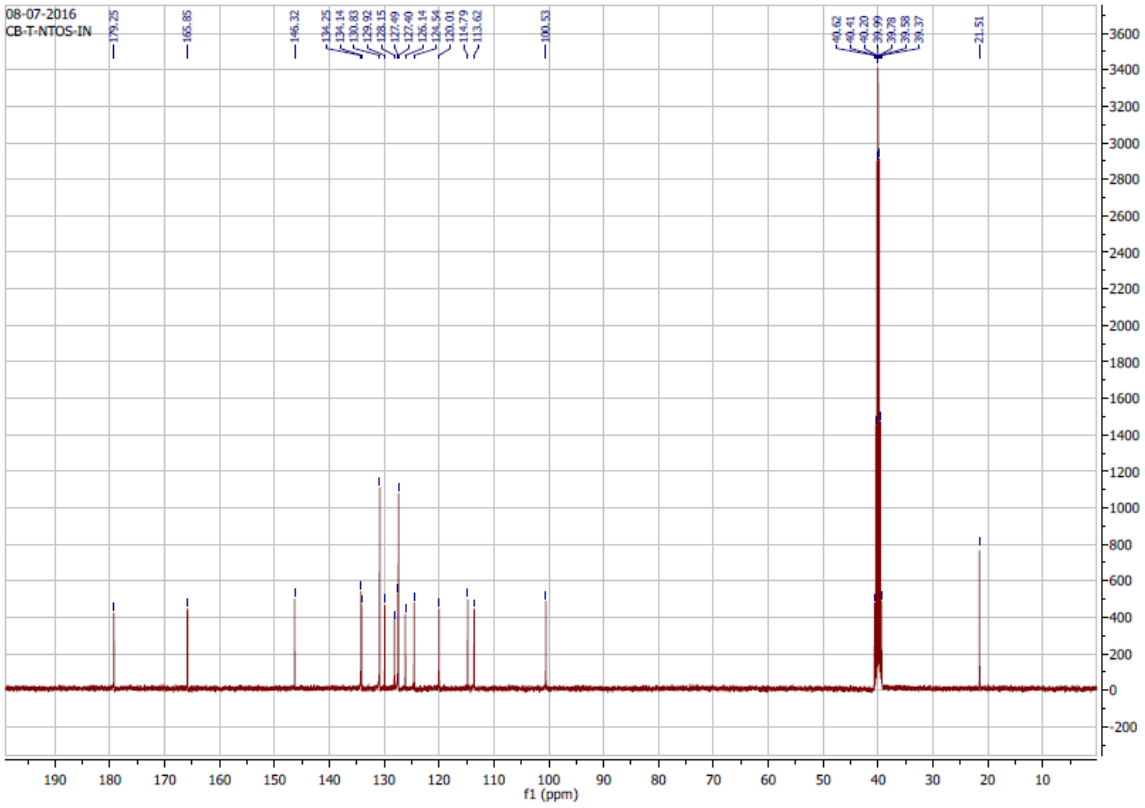
Compound **4f**


160115_PF_22
12.17
8.14
7.80
7.80
7.79
7.69
7.68
7.42
7.41
7.39
7.31
7.30
7.29
7.28
6.68
2.30
1.09
1.09
0.96
2.04
1.94
1.03
3.01
f1 (ppm)

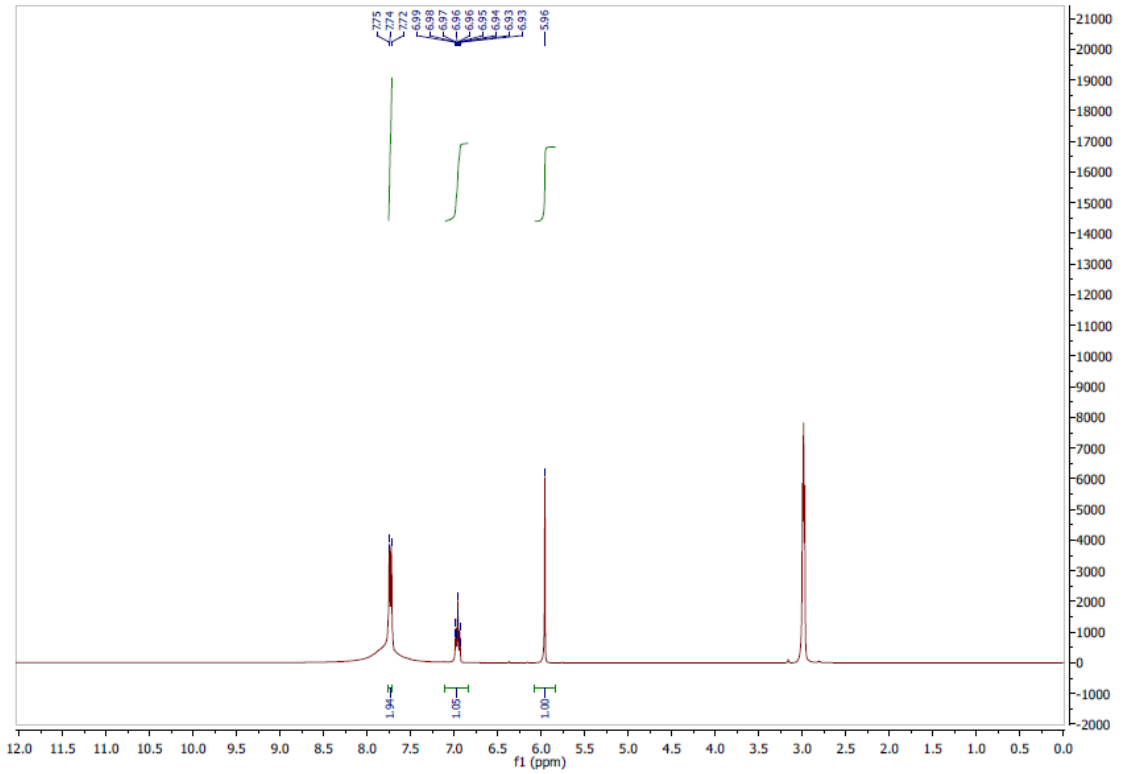

160115_PF_22_13C
164.11
158.23
156.01
155.72
142.27
141.61
137.00
134.80
130.56
129.58
128.14
127.55
126.55
106.31
106.15
97.24
20.45
f1 (ppm)

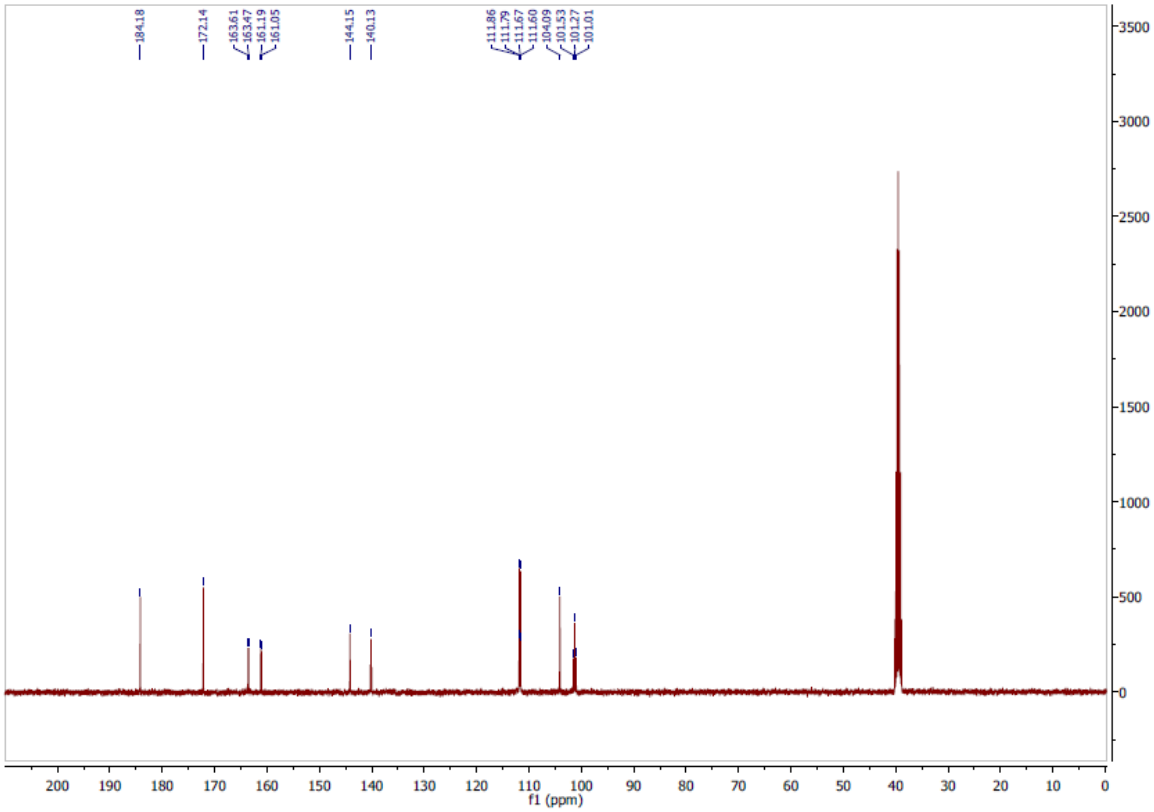
Compound **5a**


08-07-2016
CB-T-NTOS-IN
12.40
8.82
7.99
7.97
7.95
7.87
7.85
7.44
7.42
7.40
7.35
7.33
7.32
2.30
1.97
1.00
3.02
1.01
4.07
1.00
3.04
f1 (ppm)

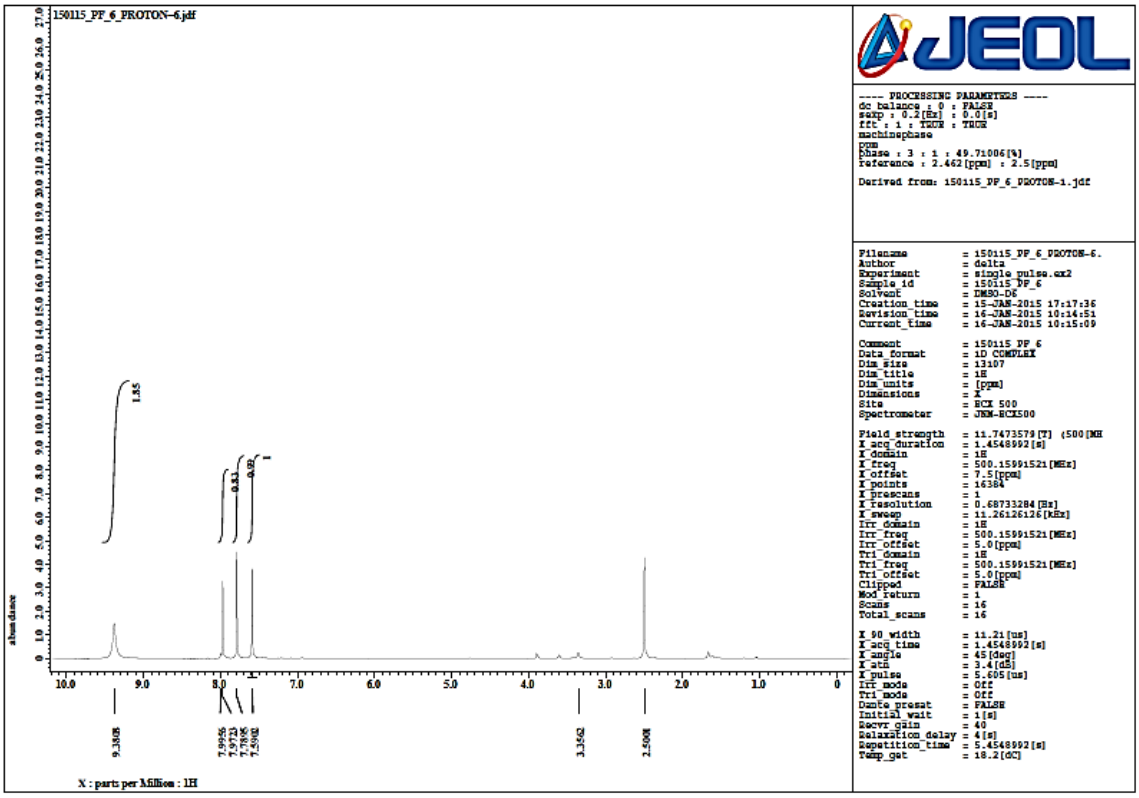

08-07-2016
CB-T-NTOS-IN
179.25
165.85
146.32
134.25
134.14
130.83
129.92
128.15
127.69
127.40
126.14
124.54
120.01
114.79
113.62
100.53
40.62
40.41
40.20
39.99
39.78
39.58
39.37
21.51
f1 (ppm)

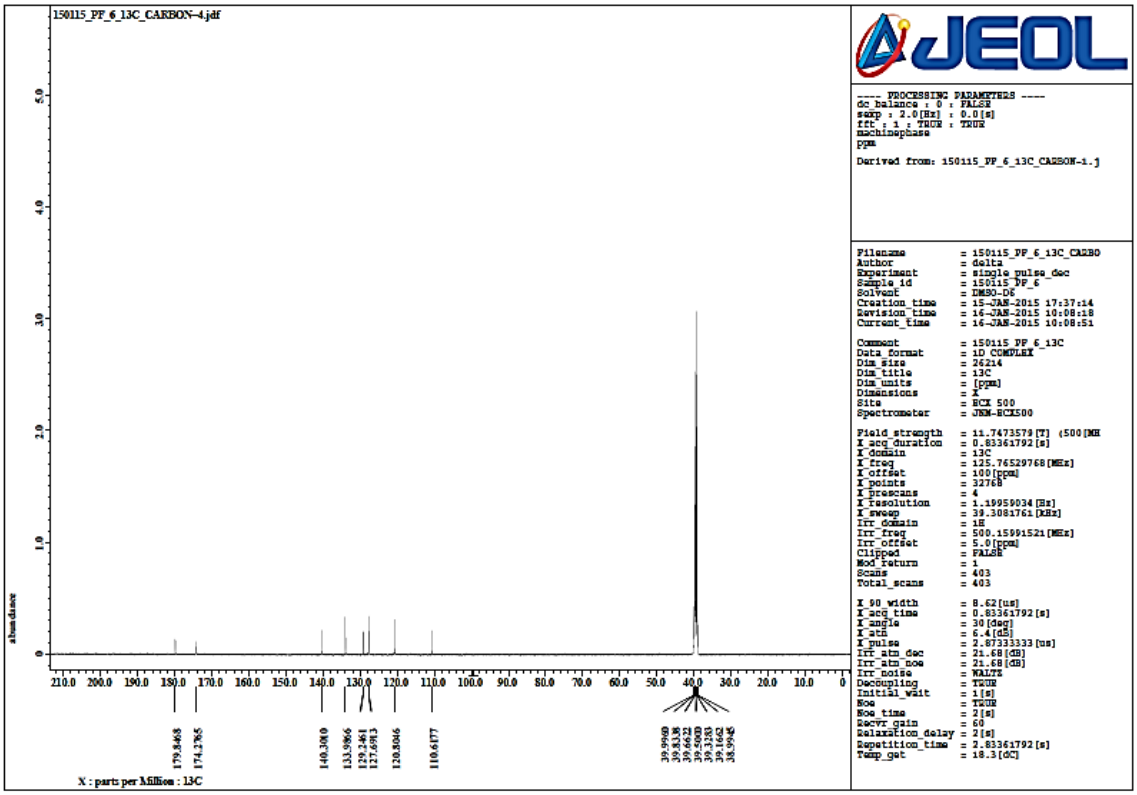
Compound **5b**


7.75
7.74
7.72
7.72
6.99
6.98
6.97
6.96
6.96
6.95
6.94
6.93
6.93
5.96
1.94
1.05
1.00
f1 (ppm)

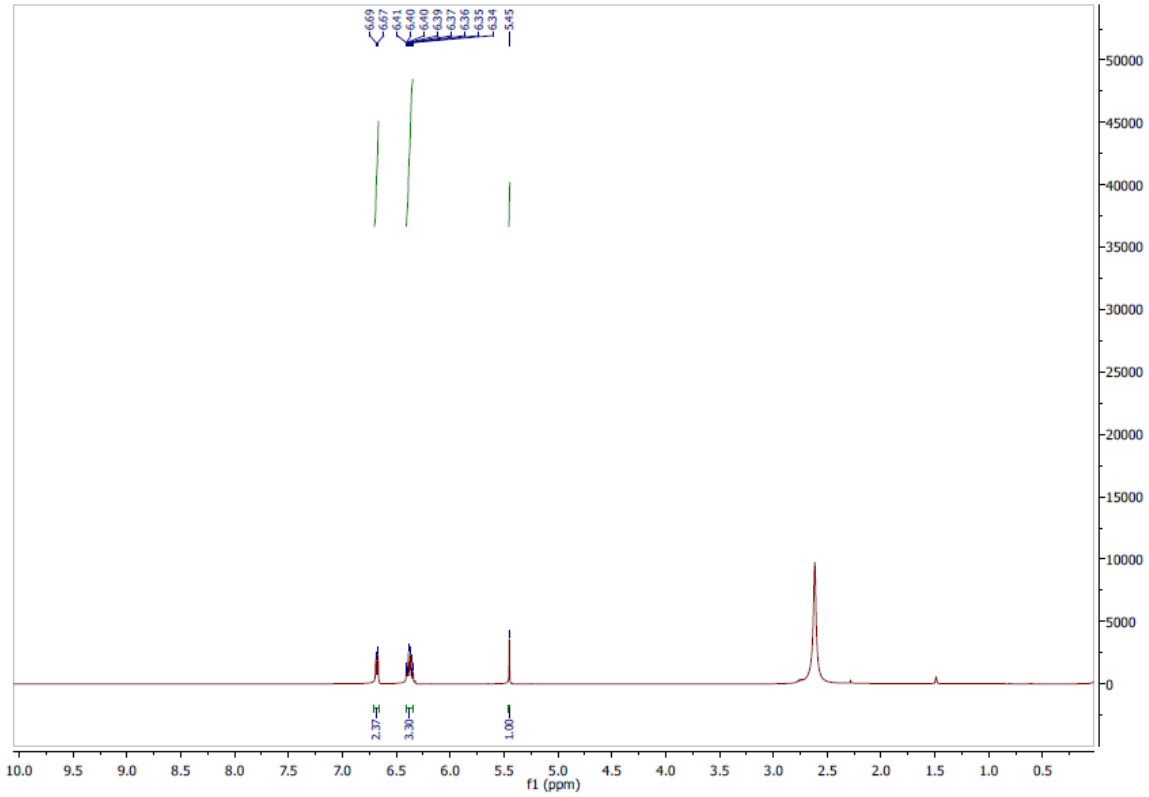

184.18
172.14
163.61
163.47
161.19
161.05
144.15
140.13
111.86
111.79
111.67
111.60
104.09
101.53
101.27
101.01
f1 (ppm)

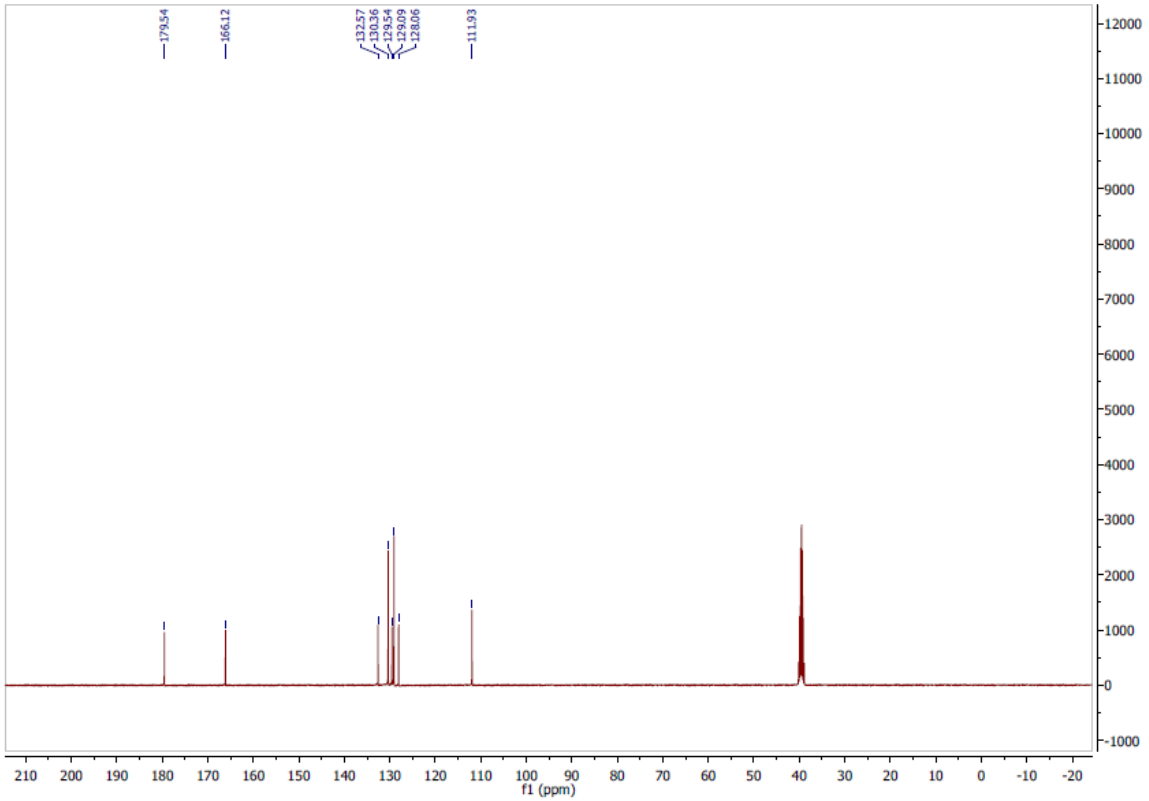
Compound **5c**


150115_PF_6_PROTON-6.jdf
JEOL
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
fft : 1 : TRUE : TRUE
machinephase
ppm
phase : 3 : 1 : 49.71006[%]
reference : 2.462[ppm] : 2.5[ppm]
Derived from: 150115_PF_6_PROTON-1.jdf
Filename = 150115_PF_6_PROTON-6.
Author = delta
Experiment = single_pulse.ex2
Sample_id = 150115_PF_6
Solvent = DMSO-D6
Creation_time = 15-JAN-2015 17:17:36
Revision_time = 16-JAN-2015 10:14:51
Current_time = 16-JAN-2015 10:15:09
Comment = 150115_PF_6
Data_format = 1D COMPLEX
Dim_size = 13107
Dim_title = 1H
Dim_units = [ppm]
Dimensions = F1
Site = RCX 500
Spectrometer = JNM-RCX500
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 1.4548992[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 7.5[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.68733284[Hz]
X_sweep = 11.26126126[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 11.21[us]
X_acq_time = 1.4548992[s]
X_angle = 45[deg]
X_atn = 3.4[dB]
X_pulse = 5.605[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Recvr_gain = 40
Relaxation_delay = 4[s]
Repetition_time = 5.4548992[s]
Temp_get = 18.2[dC]
X : parts per Million : 1H

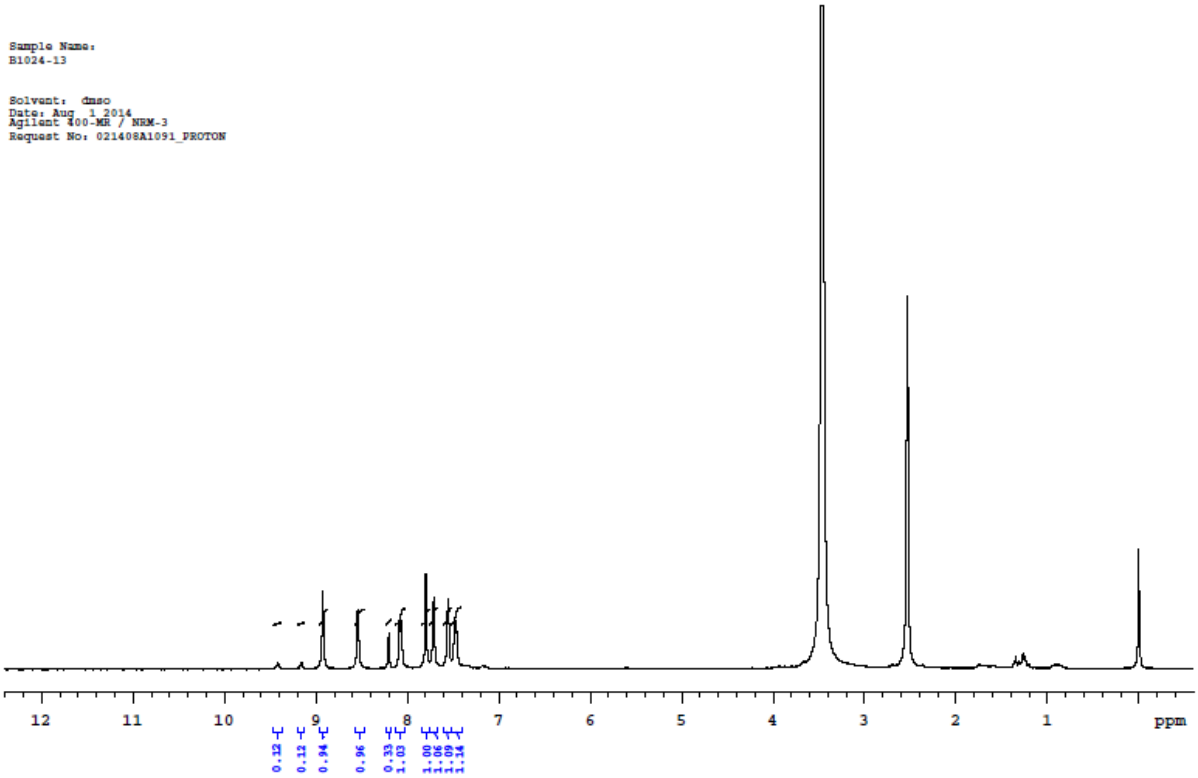

150115_PF_6_13C_CARBON-4.jdf
JEOL
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 2.0[Hz] : 0.0[s]
fft : 1 : TRUE : TRUE
machinephase
ppm
Derived from: 150115_PF_6_13C_CARBON-1.j
Filename = 150115_PF_6_13C_CARBO
Author = delta
Experiment = single_pulse_dec
Sample_id = 150115_PF_6
Solvent = DMSO-D6
Creation_time = 15-JAN-2015 17:37:14
Revision_time = 16-JAN-2015 10:08:18
Current_time = 16-JAN-2015 10:08:51
Comment = 150115_PF_6_13C
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = F1
Site = RCX 500
Spectrometer = JNM-RCX500
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 403
Total_scans = 403
X_90_width = 8.62[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 6.4[dB]
X_pulse = 2.87333333[us]
Irr_atn_dec = 21.68[dB]
Irr_atn_noe = 21.68[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Recvr_gain = 60
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_get = 18.3[dC]
X : parts per Million : 13C

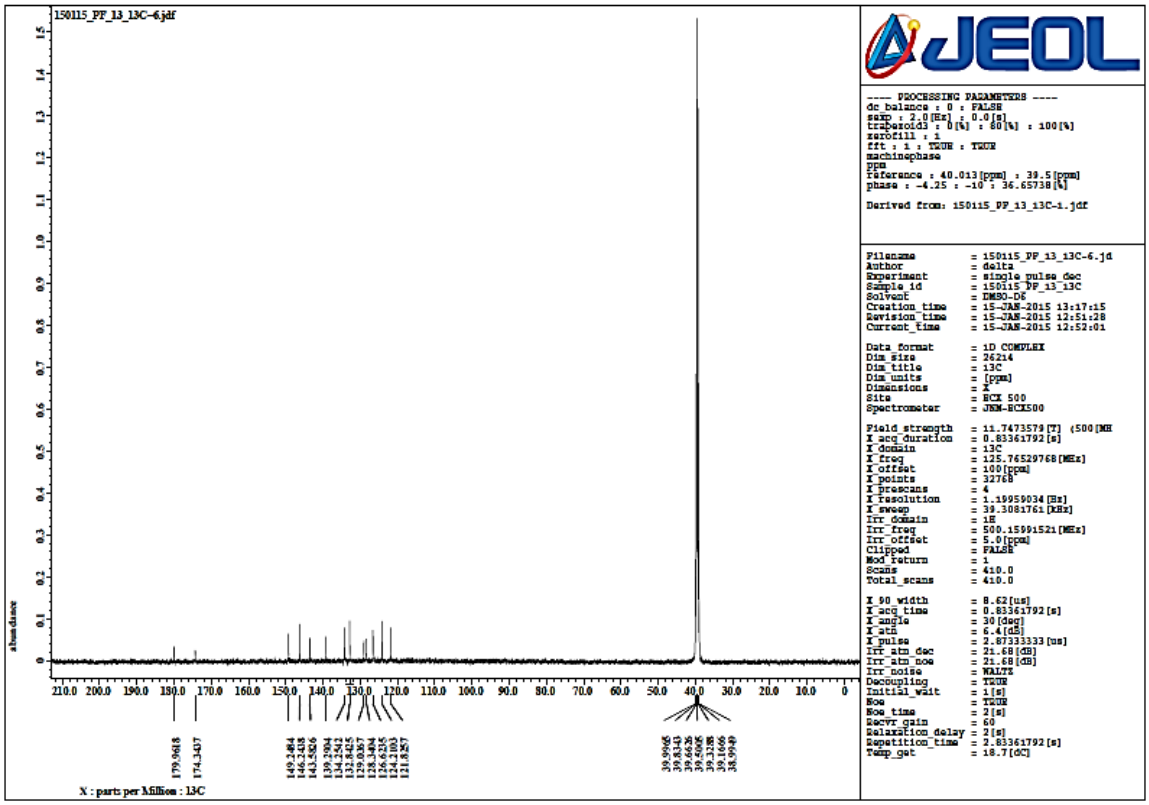
Compound **5d**


6.69
6.67
6.41
6.40
6.40
6.39
6.37
6.36
6.35
6.34
5.45
2.37
3.30
1.00
f1 (ppm)

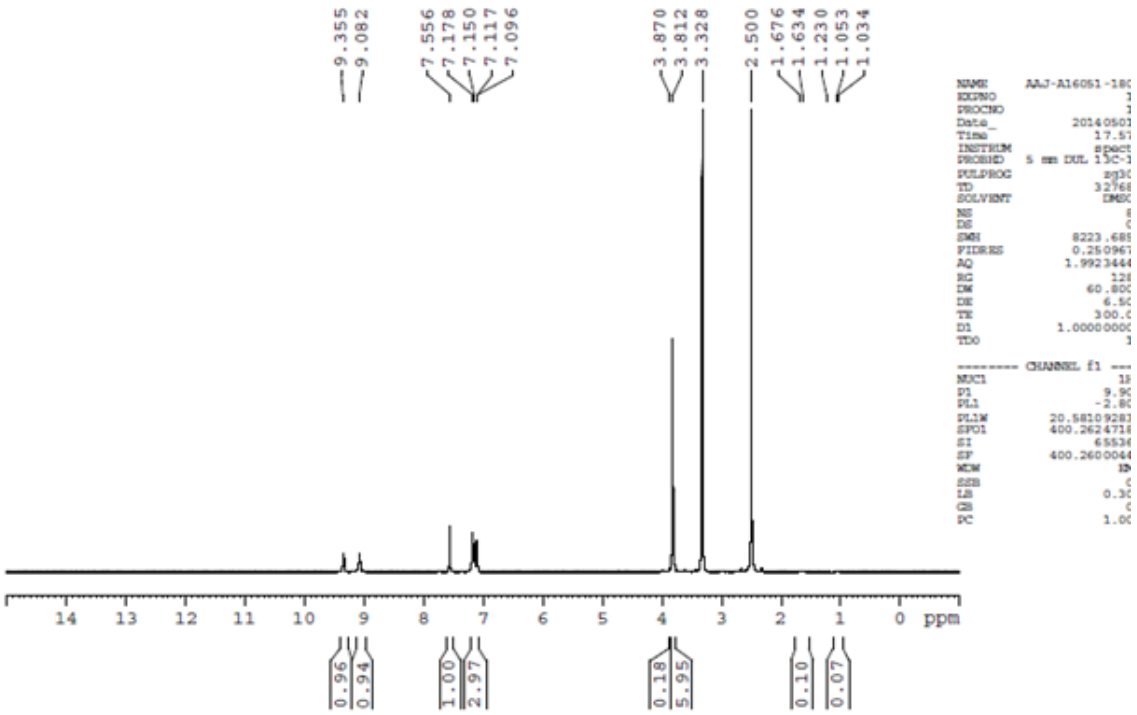

179.54
166.12
132.57
130.36
129.54
129.09
128.06
111.93
f1 (ppm)

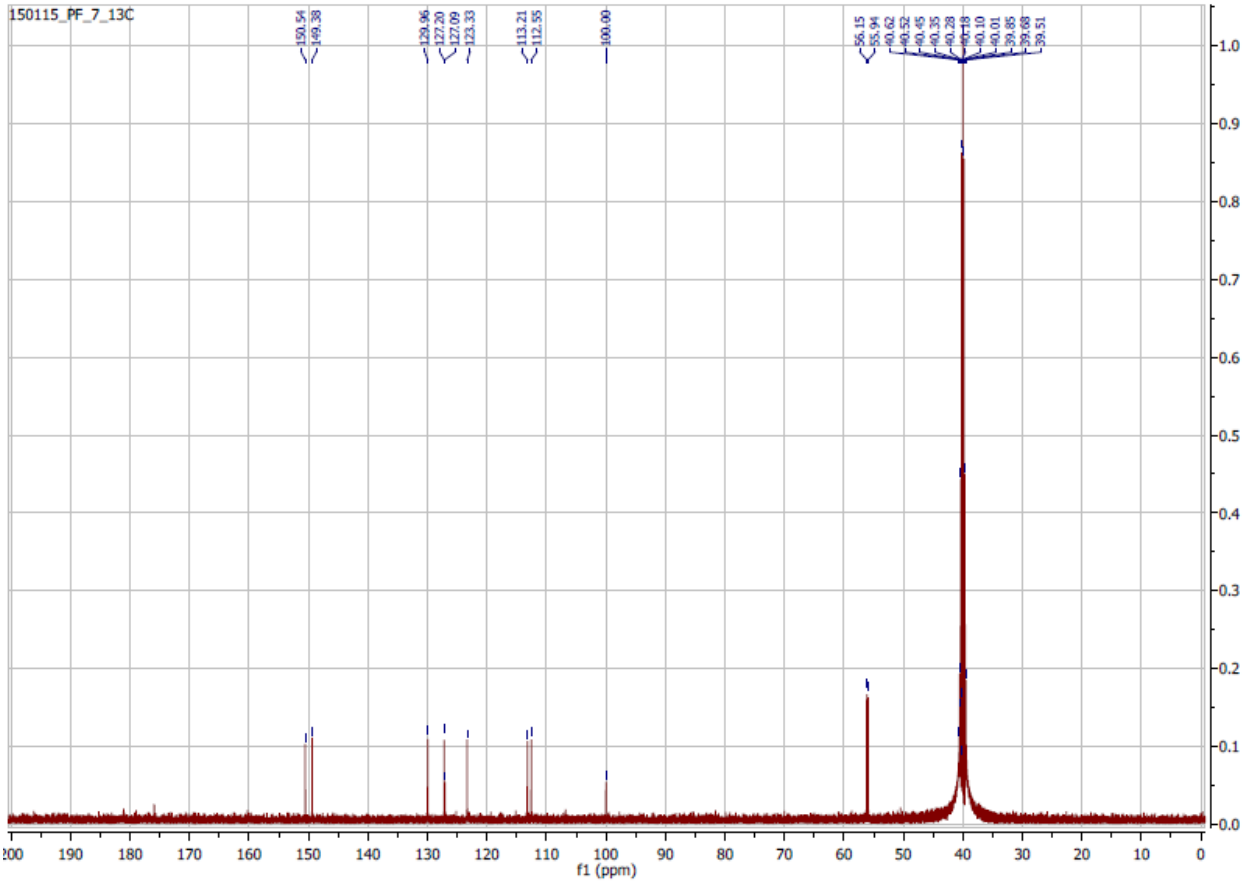
Compound **5e**


Sample Name:
B1024-13
Solvent: dmso
Date: Aug 1 2014
Agilent 400-MR / NRM-3
Request No: 021408A1091_PROTON
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.12
0.12
0.94
0.96
0.33
1.03
1.00
1.06
1.09
1.14

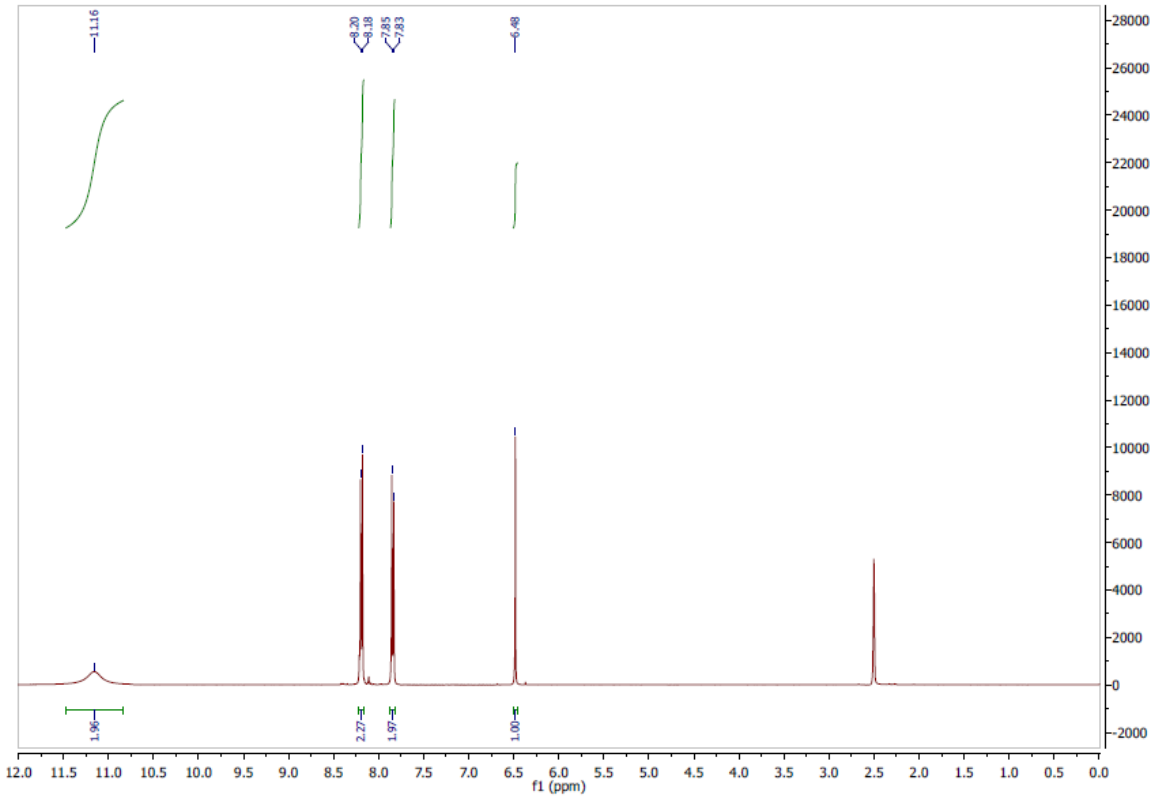

150115_PF_13_13C-6.jdf
JEOL
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 2.0[Hz] : 0.0[s]
trapezoid3 : 0[%] : 60[%] : 100[%]
zerofill : 1
fft : 1 : TRUE : TRUE
machinephase
ppm
reference : 40.013[ppm] : 39.5[ppm]
phase : -4.25 : -10 : 36.65738[%]
Derived from: 150115_PF_13_13C-1.jdf
Filename = 150115_PF_13_13C-6.jd
Author = delta
Experiment = single_pulse_dec
Sample_id = 150115_PF_13_13C
Solvent = DMSO-D6
Creation_time = 15-JAN-2015 13:17:15
Revision_time = 15-JAN-2015 12:51:28
Current_time = 15-JAN-2015 12:52:01
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = RCX 500
Spectrometer = JNM-RCX500
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 410.0
Total_scans = 410.0
X_90_width = 8.62[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 6.4[dB]
X_pulse = 2.87333333[us]
Irr_atn_dec = 21.68[dB]
Irr_atn_noe = 21.68[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Recvr_gain = 60
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_get = 18.7[dC]
abundance
X : parts per Million : 13C

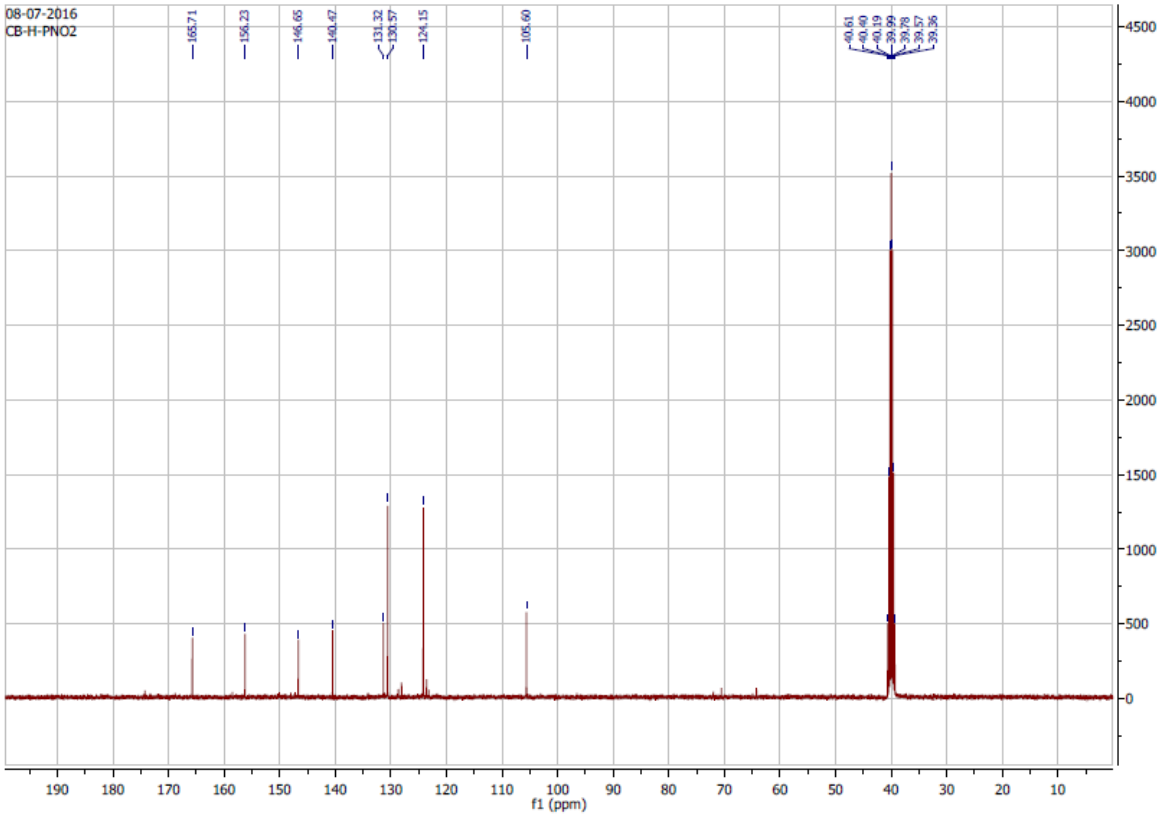
Compound **5f**


9.355
9.082
7.556
7.178
7.150
7.117
7.096
3.870
3.812
3.328
2.500
1.676
1.634
1.230
1.053
1.034
ppm
0.96
0.94
1.00
2.97
0.18
5.95
0.10
0.07

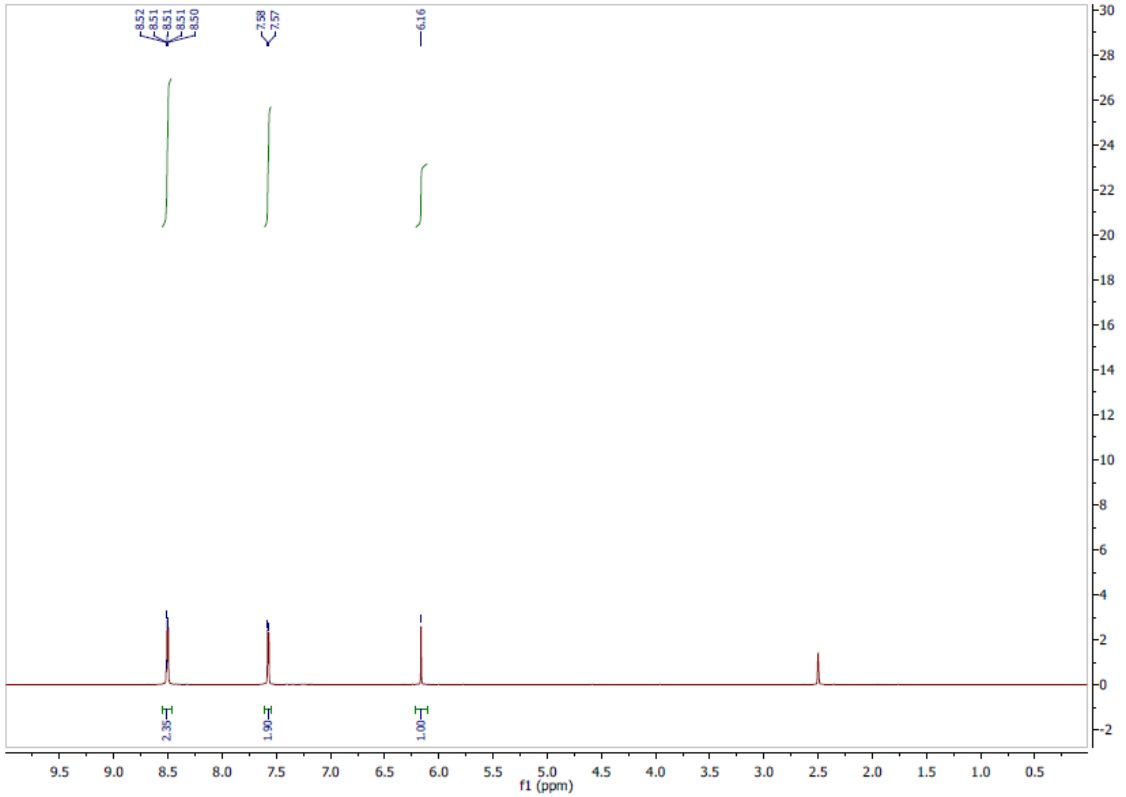

150115_PF_7_13C
150.54
149.38
129.96
127.20
127.09
123.33
113.21
112.55
100.00
56.15
55.94
40.62
40.52
40.45
40.35
40.28
40.18
40.10
40.01
39.85
39.68
39.51
f1 (ppm)

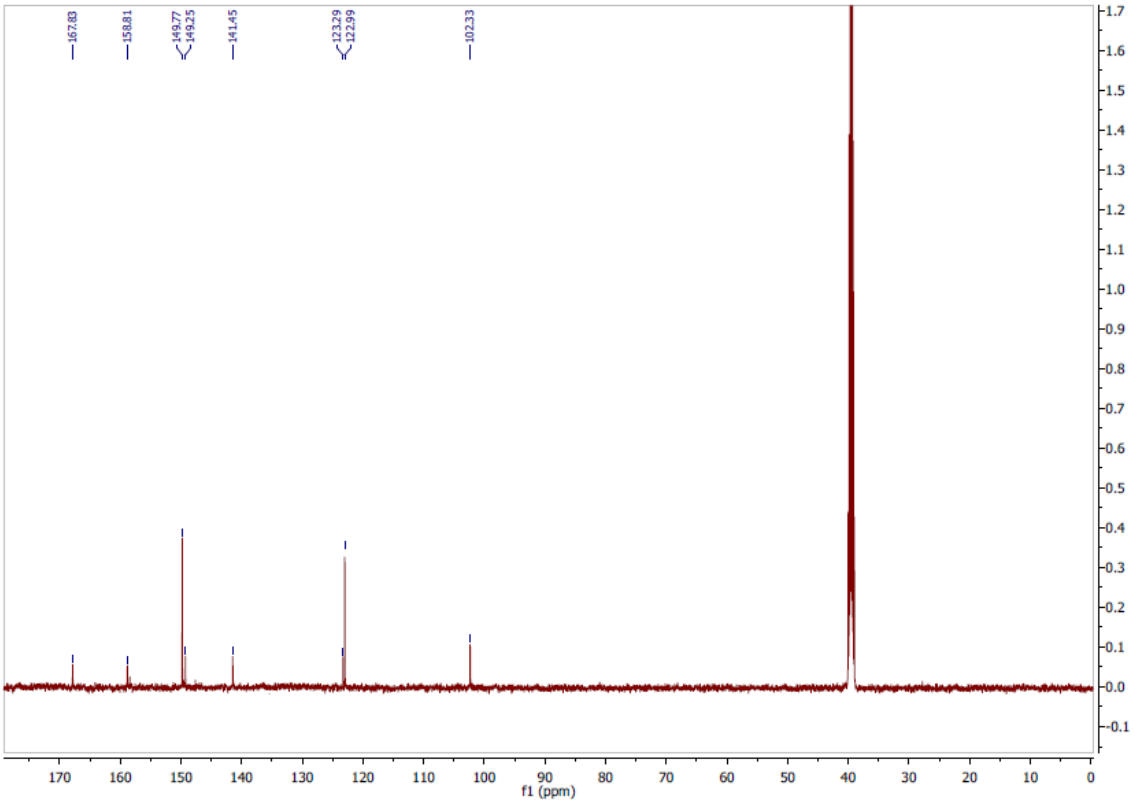
Compound **6a**


11.16
8.20
8.18
7.85
7.83
6.48
1.96
2.27
1.97
1.00
f1 (ppm)

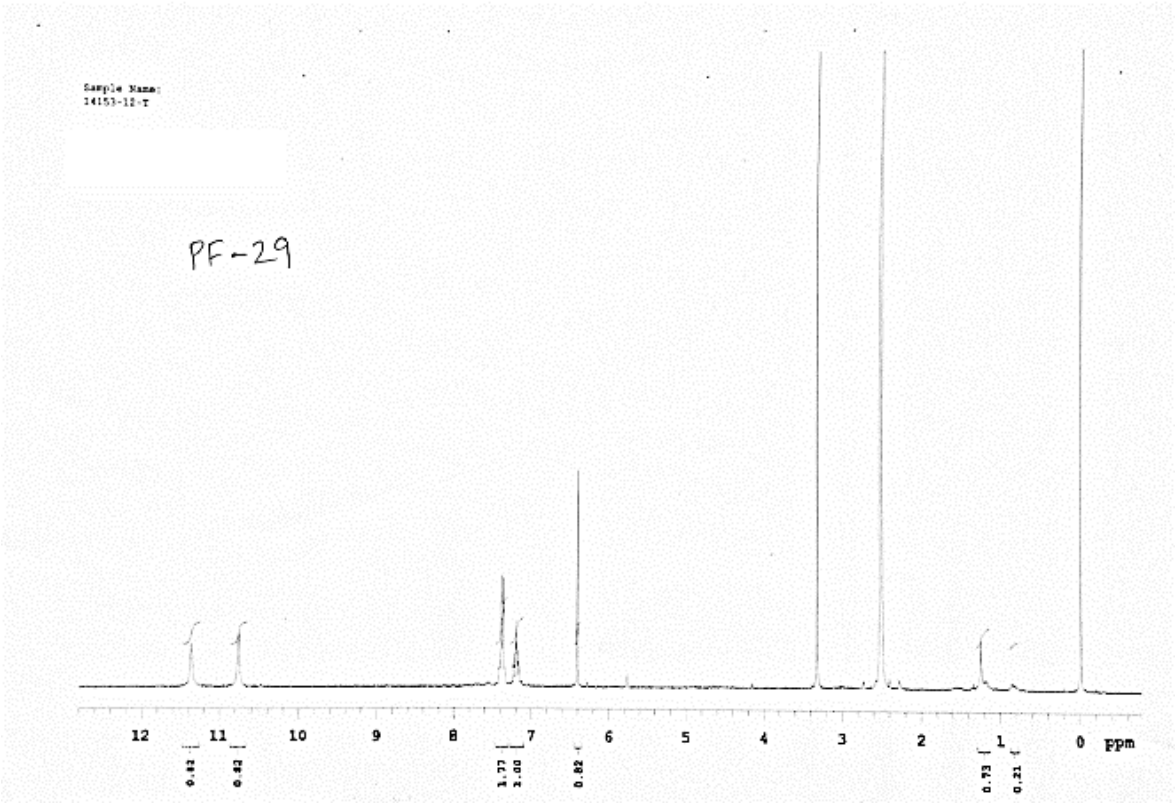

08-07-2016
CB-H-PNO2
165.71
156.23
146.65
140.47
131.32
130.57
124.15
105.60
40.61
40.40
40.19
39.99
39.78
39.57
39.36
f1 (ppm)

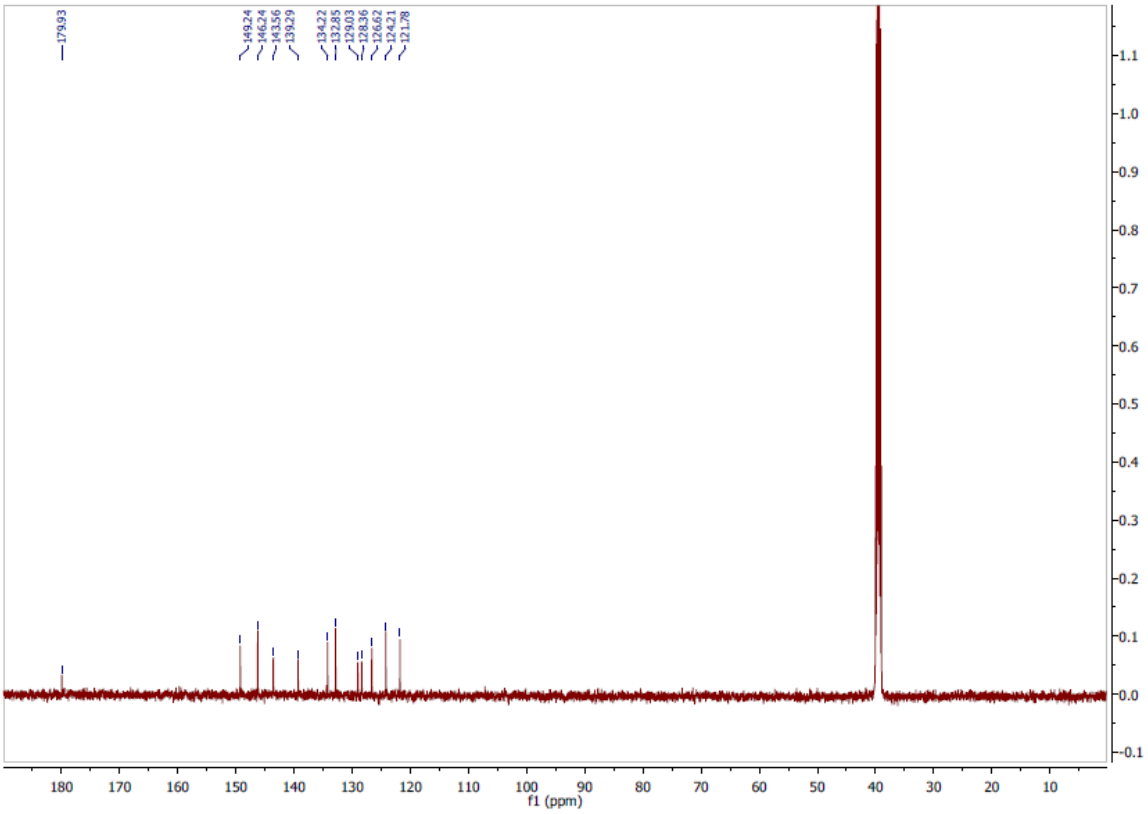
Compound **6b**


8.52
8.51
8.51
8.51
8.50
7.58
7.57
6.16
2.39
1.90
1.00
f1 (ppm)

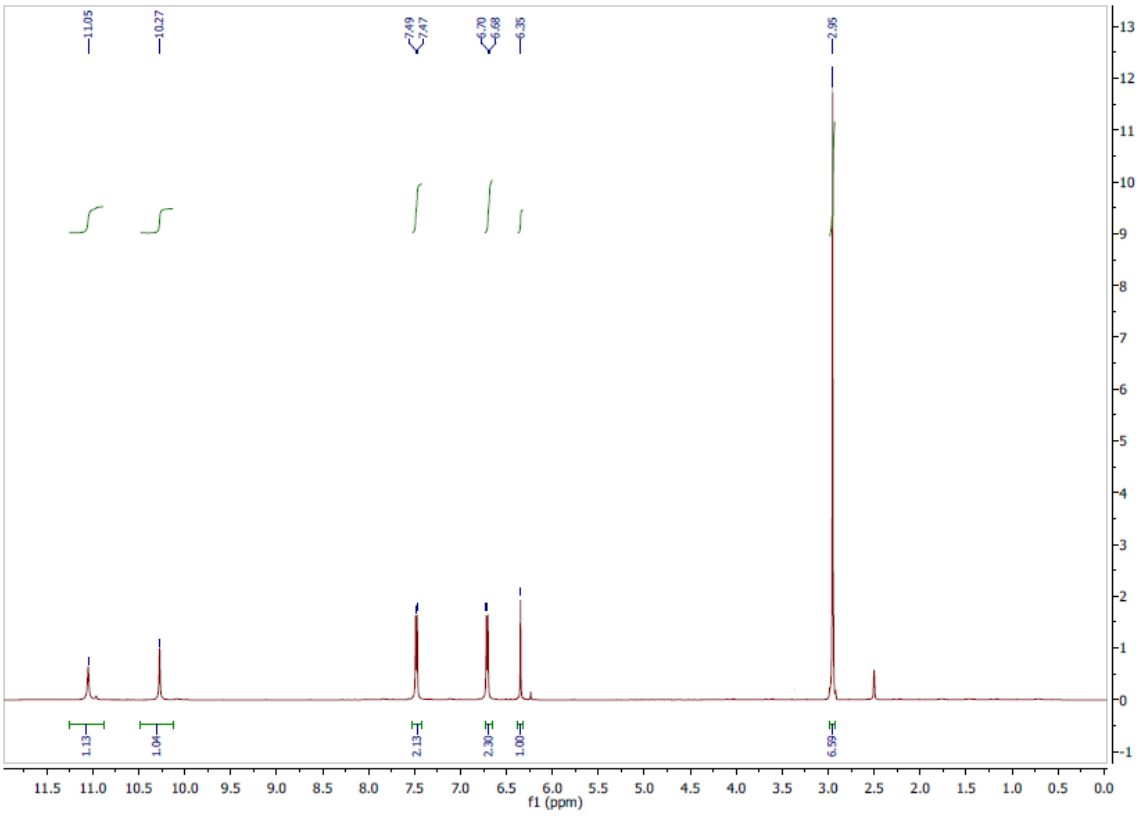

167.83
158.81
149.77
149.25
141.45
123.29
122.99
102.33
f1 (ppm)

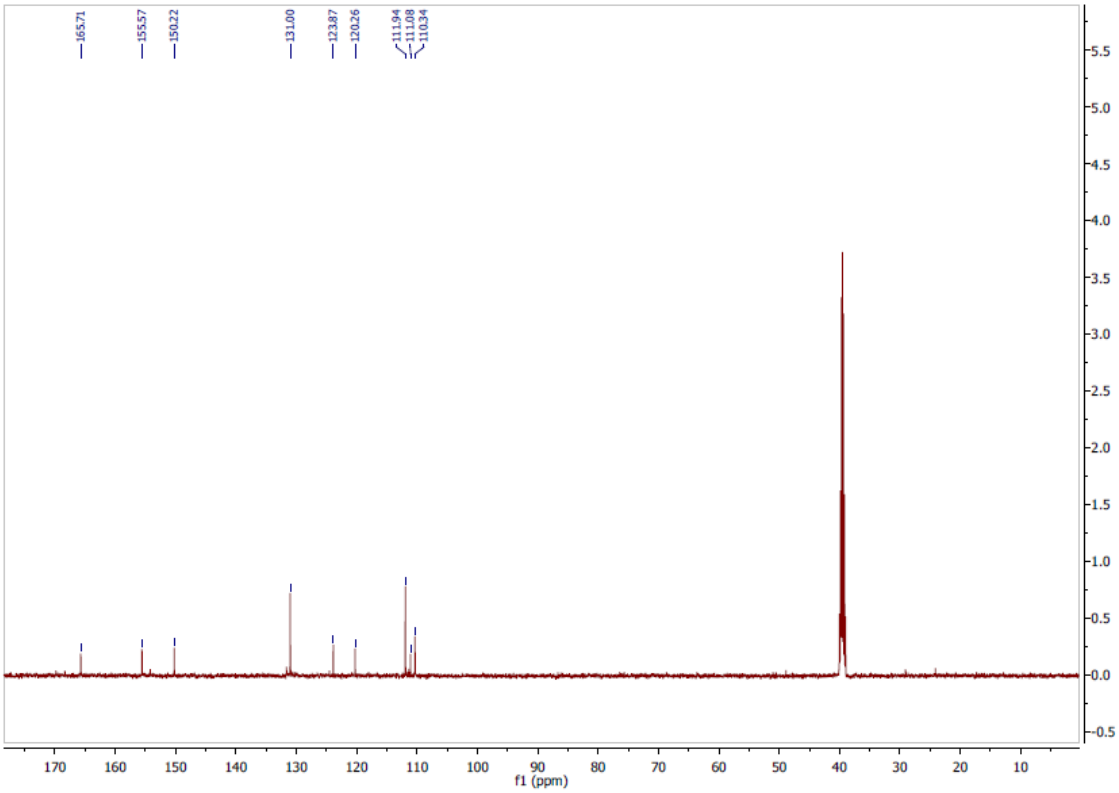
Compound **6c**


Sample Name:
14153-12-1
PF-29
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.42
0.42
1.77
2.00
0.82
0.73
0.21

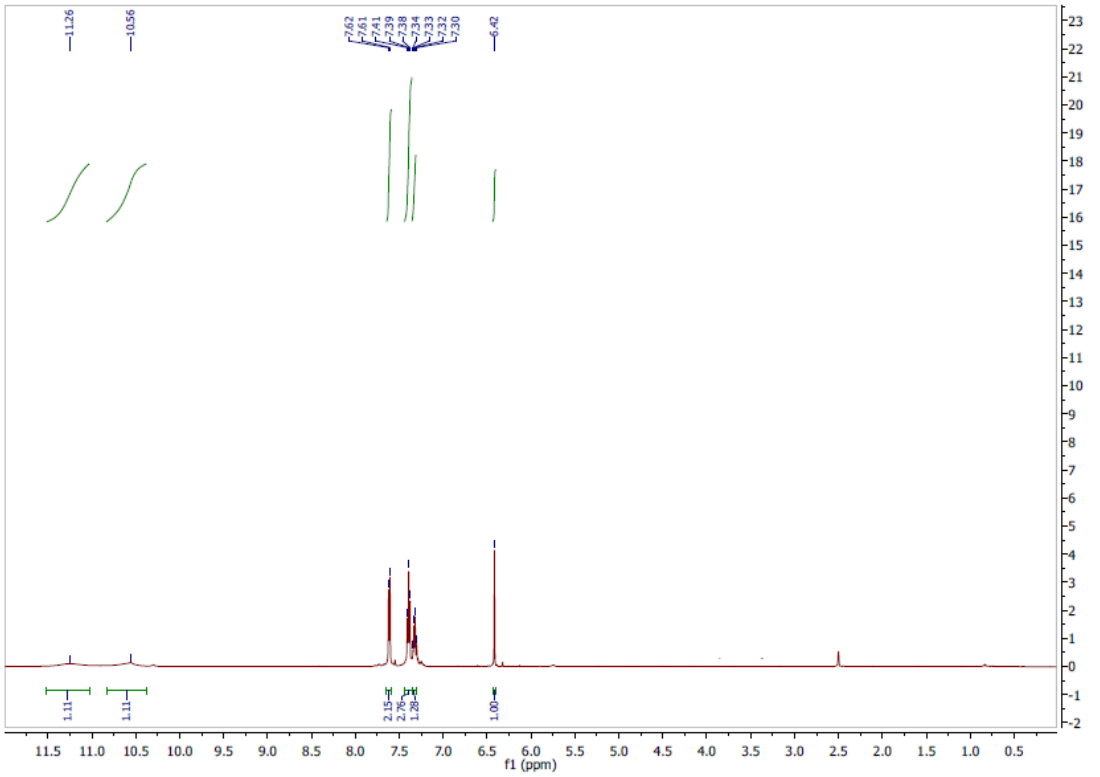

17993
14924
14624
14356
13929
13422
13285
12603
12836
12662
12421
12178
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

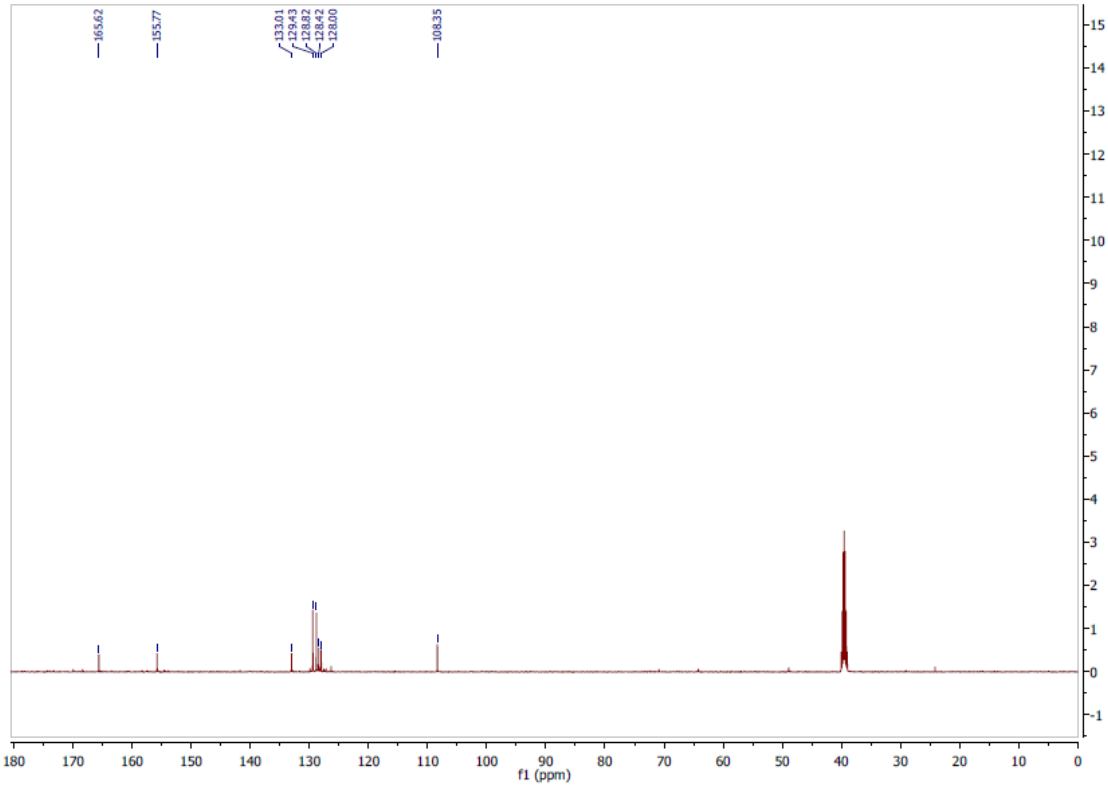
Compound **6d**


11.05
10.27
7.49
7.47
6.70
6.68
6.35
2.96
1.13
1.04
2.13
2.30
1.00
6.59
f1 (ppm)

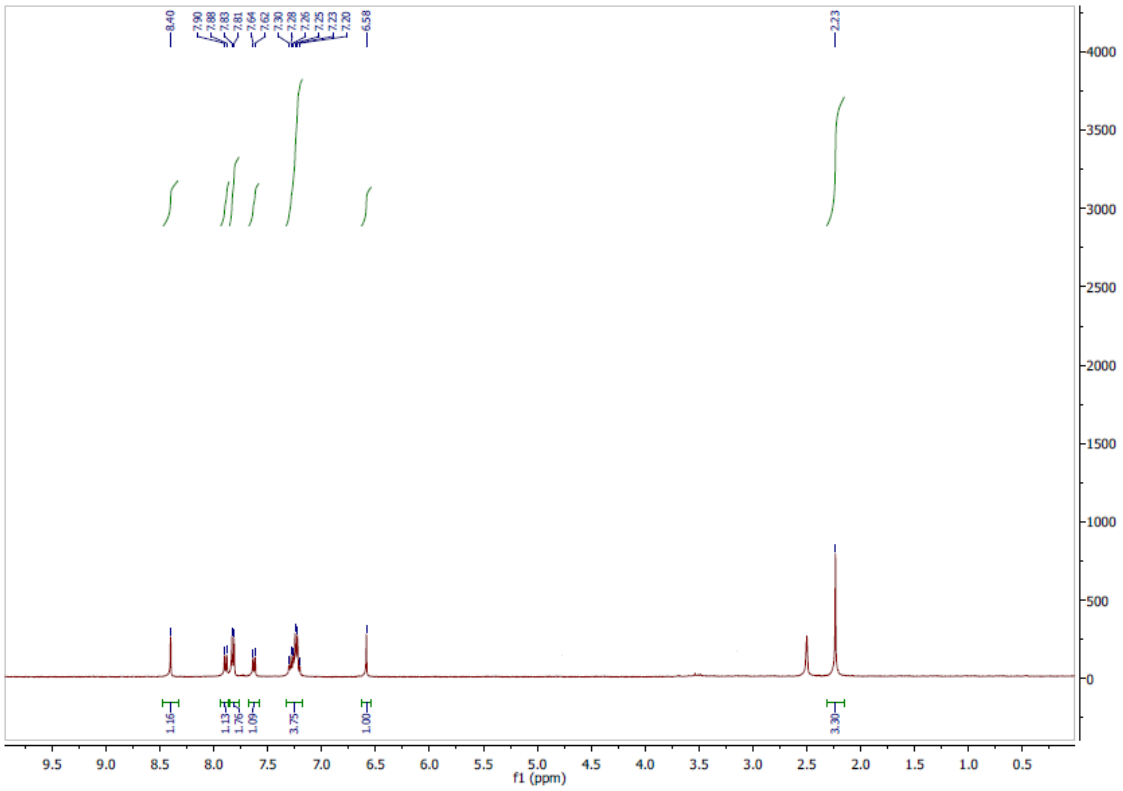

165.71
155.57
150.22
131.00
123.87
120.26
111.94
111.08
110.34
f1 (ppm)

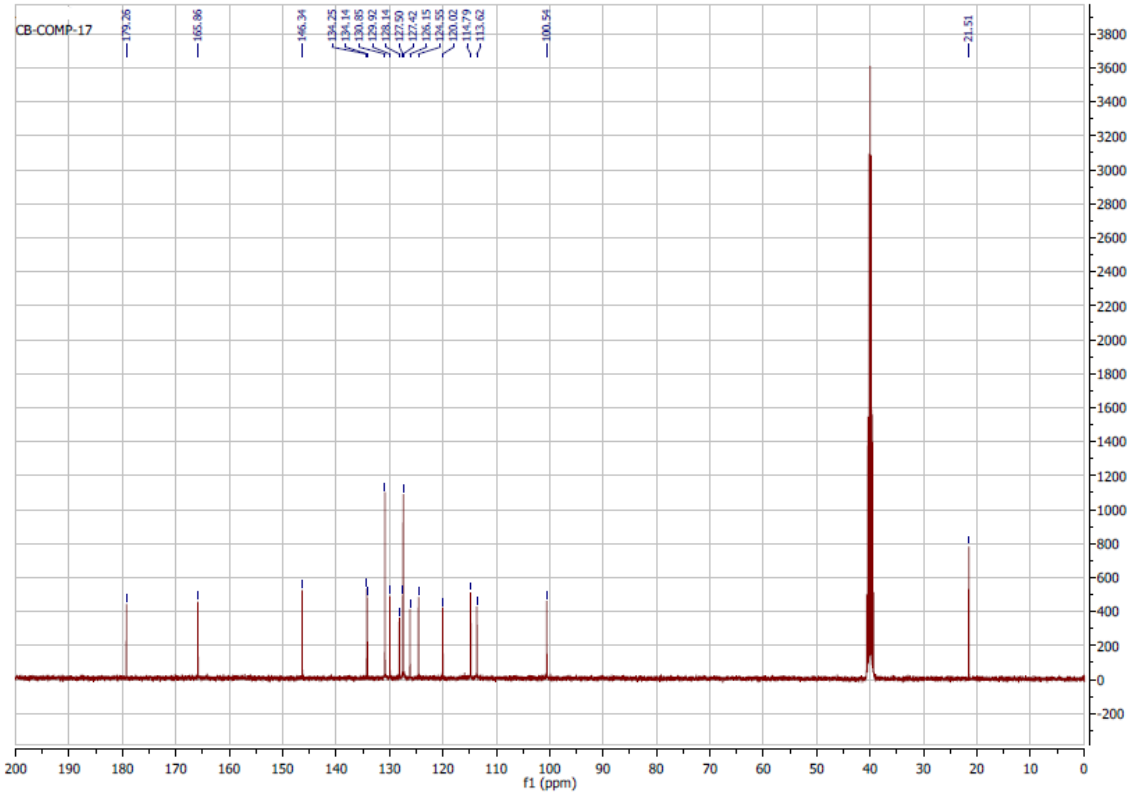
Compound **6e**


11.26
10.56
7.62
7.61
7.41
7.39
7.38
7.34
7.33
7.32
7.30
6.42
1.11
1.11
2.15
2.76
1.28
1.00
f1 (ppm)


165.62
155.77
133.01
129.43
128.82
128.42
128.00
108.35
f1 (ppm)

Compound **6f**


8.40
7.90
7.88
7.83
7.81
7.64
7.62
7.30
7.28
7.26
7.25
7.23
7.20
6.58
2.23
1.16
1.13
1.76
1.09
3.75
1.00
3.30
f1 (ppm)


CB-COMP-17
179.26
165.86
146.34
134.25
134.14
130.85
129.92
128.14
127.50
127.42
126.15
126.55
120.03
114.79
113.62
100.54
21.51
f1 (ppm)