Supporting Information for: Late-Stage Diversification of Biologically Active Molecules via Chemoenzymatic C-H Functionalization

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Materials

Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. HPLC analyses were performed using HPLC grade acetonitrile (Fisher Scientific), 18 MΩ water from a Milli-Q purification system (model No. QGARD00D2), and trifluoroacetic acid (Oakwood Chemicals). RebH and MBP-RebF were expressed and purified according to published procedures.[1] Water and isopropanol used in Suzuki-Miyaura reactions were deoxygenated by sparging with N_2 for 30 minutes. Anhydrous dioxane was purchased from Acros in an AcroSeal bottle and used as received. Toluene was obtained from an Innovative Technologies solvent purification system (solvent deoxygenated by sparging with N_2 and dried over alumina). $[(allyl)PdCl]_2$ was prepared according to the literature.[2] Thenalidine was obtained from Novartis through the CCHF. Glucose dehydrogenase (GDH, product No. GDH-105), and NAD (product No. NAD-004626) were purchased from Codexis (Redwood City, CA). FAD (product No. 00151) was purchased from Chem-Impex International (Wood Dale, IL). Catalase (product No. C1345-1G) was purchased from Aldrich. Biotage reversed-phase columns were purchased from Biotage (FSUL-0401-0012). AeraSeal film was purchased from Research Products International (product No. 202504). Representative batches of proteins expressed and purified (3-SS, 4-V, and MBP-RebF) were analyzed by SDS-PAGE and estimated to be >95% pure with regards to the desired protein.[3]

General Comments

Reversed-phase chromatography was carried out using a Biotage Isolera One. NMR spectra (^1H, and ^{13}C) were obtained using a Bruker Ultrashield 500 Plus 500 MHz spectrometer at room temperature. Chemical shifts are reported in ppm and referenced to residual solvent peaks.[4] Coupling constants are reported in Hz. In some cases the carbon atoms in the trifluoroacetate ion

cannot be observed in a reasonable number of scans.[5] Mass spectra were obtained from the University of Chicago mass spectrometry facility using an Agilent Technologies 6224 TOF LC/MS.

General Procedure for 10 mg Scale Bioconversions

Substrate (10.0 mg), NaBr/NaCl (20 equiv., 10 mM final concentration), glucose (40 equiv., 20 mM final concentration), glucose dehydrogenase (9 U/mL final concentration GDH), and catalase (35 U/mL final concentration) were added as solids to a crystallization dish (100 x 50 mm) containing a magnetic stir bar. The resulting mixture was diluted to an appropriate volume with phosphate buffer (25 mM K_2HPO_4, pH 8.0) and isopropanol (3.5% v/v) was added until a concentration of 0.5 mM with respect to substrate is reached (typically 80-120 mL final volume). 10 mM aqueous solutions of FAD and NAD were prepared and added to the reaction mixture (0.20 equiv., 0.10 mM final concentration each). Stock solutions of RebH and RebF, stored in a HEPES/glycerol buffer (25 mM HEPES, pH 7.4, 10% glycerol v/v) following purification, were thawed in an ice water bath. RebH (0.01-0.05 equiv., 5-25 μM final concentration) and MBP-RebF (0.0005 equiv. 0.25 μM final concentration) were added to the reaction mixture as solutions to give the indicated final enzyme concentrations. The reaction vessel was sealed with an AeraSeal film and gently stirred at room temperature for 16 hours. The reactions were monitored by UPLC (Agilent 1200 UPLC with an Agilent Eclipse Plus C18 4.6 x 150 mm column, 3.5 μm particle size; solvent A = H_2O/0.1% TFA, solvent B = MeCN) until maximum conversion was observed. The following method was used for all substrates: 0-10 min, B = 15%; 10-20 min, B = 15-100%; 20-24 min, B = 100%. The bioconversion were quenched with HCl (5 M, until pH<2) and saturated with solid NaCl. Precipitated protein was filtered out through a pad of Celite and the filtrate was brought to pH>12 through addition of NaOH (5M).

The filtrate was extracted 3x with CH_2Cl_2, and combined organic extracts were concentrated using a rotary evaporator.

General Procedure for Suzuki-Miyaura Coupling on Crude Extract of Bioconversion

The crude extracts from an enzymatic halogenation were transferred to a 50 mL round bottomed flask, and Ar-$B(OH)_2$ (1.5 equiv.), $Pd(OAc)_2$ (0.05 equiv.), sodium 2′-dicyclohexylphosphino-2,6-dimethoxy-1,1′-biphenyl-3-sulfonate hydrate (water soluble Sphos, 0.05 equiv.), and a magnetic stir bar were added. The flask was equipped with a reflux condenser and capped with a rubber septum. The apparatus was purged by three cycles of evacuation and N_2 refill. A deoxygenated 1:1 mixture (20 mL) of isopropanol and phosphate buffer (170 mM K_2HPO_4, pH 8.5) was added via syringe and the mixture was allowed to stir under reflux in an oil bath. After 1 hour, the reaction vessel was allowed to cool to room temperature, and the isopropanol was removed by rotary evaporation. The aqueous solution was extracted with CH_2Cl_2 (3x, 10 mL each), and the combined organic extracts were dried over Na_2SO_4 and concentrated by rotary evaporation. The crude mixture was purified by reversed-phase chromatography (Biotage SNAP-KP-C18-HS, gradient from pure H_2O to 60% MeCN/H_2O) and isolated as the trifluoroacetate (TFA) salt.

General Procedure for Buchwald-Hartwig Amination on Crude Extract of Bioconversion

The crude extracts from an enzymatic halogenation were transferred to a 20 mL round scintillation vial, and $ArNH_2$ (3 equiv.), $Pd(OAc)_2$ (0.03 equiv.), BrettPhos (0.03 equiv.), NaO*t*-Bu (6 equiv.) and a magnetic stir bar were added. The vial was transferred to an inert atmosphere dry box, dioxane (1 mL) was added, and the vial was sealed with a Teflon lined cap. The vial was removed from the dry box and the mixture was allowed to stir in a 100 °C oil bath.

After 14 hours, the reaction vessel was allowed to cool to room temperature, and the contents filtered over silica, eluting with 150 mL 4:1 CH_2Cl_2/MeOH. The filtrate was collected and concentrated by rotary evaporation. The crude mixture was purified by reversed-phase chromatography (Biotage SNAP-KP-C18-HS, gradient from pure H_2O to 60% MeCN/H_2O) and isolated as the trifluoroacetate (TFA) salt.

Preparation of Compounds

Preparation of 6-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**1a)**

1a was prepared from tryptoline (10 mg, 0.058 mmol) and 4-methoxyphenylboronic acid following the general procedure for enzymatic bromination with 3-SS and Suzuki-Miyaura Coupling outlined above in >99% yield (23.17 mg of **1a**·TFA, 0.059 mmol). ^{1}H NMR (500 MHz, MeOD) δ 7.64 (s, 1H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.39 (s, 2H), 6.98 (d, *J* = 8.5 Hz, 2H), 4.46 (s, 2H), 3.83 (s, 3H), 3.61 (t, *J* = 5.9 Hz, 2H), 3.13 (t, *J* = 5.6 Hz, 2H). ^{13}C NMR (126 MHz, MeOD) δ 160.08, 137.43, 136.34, 129.31, 129.04, 128.09, 127.06, 122.81, 121.35, 116.72, 115.16, 112.49, 55.76, 43.77, 42.30, 19.50. HRMS calculated for $C_{18}H_{19}N_2O$ $[M + H]^+$: 279.1498, found: 279.1515.

Preparation of 6-(benzo[*d*][1,3]dioxol-5-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**1b)**

1b was prepared from tryptoline (10 mg, 0.058 mmol) and 3,4-(methylenedioxy)phenylboronic acid following the general procedure for enzymatic bromination with 3-SS and Suzuki-Miyaura Coupling outlined above in 64% yield (15.1 mg **1b**·TFA, 0.037 mmol). ^{1}H NMR (500 MHz, MeOD) δ 7.62 (s, 1H), 7.41 – 7.31 (m, 2H), 7.09 (m, 2H), 6.87 (d, *J* = 8.0 Hz, 1H), 5.96 (s, 2H), 4.45 (s, 2H), 3.60 (t, *J* = 6.0 Hz, 2H), 3.12 (t, *J* = 5.7 Hz, 2H). ^{13}C NMR (126 MHz, MeOD) δ 149.52, 147.84, 138.30, 137.55, 134.26, 128.04, 127.16, 122.96, 121.45, 117.00, 112.50, 109.35, 108.60, 107.39, 102.31, 43.77, 42.30 19.49. HRMS calculated for $C_{18}H_{17}N_2O_2$ $[M + H]^+$: 293.1290, found: 293.1291.

Preparation of methyl 4-(2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-6-yl)benzoate (**1c**)

1c was prepared from tryptoline (10 mg, 0.058 mmol) and 4-methoxycarbonylphenylboronic acid following the general procedure for enzymatic bromination with 3-SS and Suzuki-Miyaura Coupling outlined above in 55% yield (13.5 mg **1c**·TFA, 0.032 mmol). ^{1}H NMR (500 MHz, MeOD) δ 8.07 (d, *J* = 8.1 Hz, 2H), 7.91 – 7.64 (m, 3H), 7.48 (m, *J* = 27.9, 8.5 Hz, 2H), 4.47 (s, 2H), 3.92 (s, 3H), 3.62 (t, *J* = 5.7 Hz, 2H), 3.15 (br, 2H). ^{13}C NMR (126 MHz, MeOD) δ 181.67, 168.72, 148.56, 138.32, 132.89, 131.02, 129.08, 128.01, 127.98, 127.60, 123.00, 117.80, 112.87, 107.73, 52.53, 43.76, 42.29, 19.48. HRMS calculated for $C_{19}H_{19}N_2O_2$ $[M + H]^+$: 307.1447, found: 307.1446.

Preparation of 4-(2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-6-yl)benzonitrile (**1d**)

1d was prepared from tryptoline (10 mg, 0.058 mmol) and 4-cyanophenylboronic acid following the general procedure for enzymatic bromination with 3-SS and Suzuki-Miyaura Coupling outlined above in 73% yield (16.5 mg **1d**·TFA, 0.043 mmol). ^{1}H NMR (500 MHz, MeOD) δ 7.83 (m, 3H), 7.76 (m, 2H), 7.49 (m, 3H), 4.47 (s, 2H), 3.62 (t, *J* = 6.0 Hz, 2H), 3.15 (t, *J* = 6.0 Hz, 2H). ^{13}C NMR (126 MHz, MeOD) δ 148.49, 138.48, 133.64, 132.09, 128.77, 128.24, 127.86, 122.85, 120.08, 117.95, 113.03, 110.65, 107.81, 43.71, 42.23, 19.46. HRMS calculated for $C_{18}H_{16}N_3$ $[M + H]^+$: 274.1344, found: 274.1345.

Preparation of *N*-(4-(2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-6-yl)phenyl)acetamide (**1e**)

1e was prepared from tryptoline (10 mg, 0.058 mmol) and (4-acetamido)phenylboronic acid following the general procedure for enzymatic bromination with 3-SS and Suzuki-Miyaura Coupling outlined above in 58% yield (14.1 mg **1e**·TFA, 0.034 mmol). ^{1}H NMR (500 MHz, MeOD) δ 7.70 (s, 1H), 7.60 (s, 4H), 7.45 – 7.39 (m, 2H), 4.47 (s, 2H), 3.62 (t, *J* = 6.0 Hz, 2H), 3.14 (t, *J* = 5.8 Hz, 2H), 2.15 (s, 3H). ^{13}C NMR (126 MHz, MeOD) δ 170.03, 152.37, 138.37, 133.82, 131.77, 128.54, 128.27, 122.84, 121.64, 121.56, 116.98, 113.95, 112.59, 43.79, 42.32, 23.79, 19.50. HRMS calculated for $C_{19}H_{20}N_3O$ $[M + H]^+$: 306.1606, found: 306.1614.

Preparation of 4-(2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-6-yl)phenol (**1f**)

1f was prepared from tryptoline (10 mg, 0.058 mmol) and 4-hyroxyphenylboronic acid following the general procedure for enzymatic bromination with 3-SS and Suzuki-Miyaura Coupling outlined above in 97% yield in roughly 90% purity (21.2 mg **1f**·TFA, 0.056 mmol). ^{1}H NMR (500 MHz, MeOD) δ 7.61 (s, 4H), 7.45 (d, *J* = 8.4 Hz, 8H), 7.37 (s, 6H), 6.84 (d, *J* = 8.4 Hz, 8H), 4.45 (s, 8H), 3.68 – 3.48 (m, 10H), 3.12 (t, *J* = 5.7 Hz, 7H). ^{13}C NMR (126 MHz, MeOD) δ 137.32, 135.29, 134.47, 129.09, 128.07, 126.95, 126.09, 122.81, 121.63, 113.96, 112.43, 107.28, 43.79, 42.32, 19.51. HRMS calculated for $C_{17}H_{17}N_2O$ $[M + H]^+$: 265.1341, found: 265.1356.

Preparation of V 6-(6-fluoropyridin-3-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**1g**)

1g was prepared from tryptoline (10 mg, 0.058 mmol) and 6-fluoro-3-pyridinylboronic acid following the general procedure for enzymatic bromination with 3-SS and Suzuki-Miyaura Coupling outlined above in 86% yield (19.0 mg **1g**·TFA, 0.050 mmol). ^{1}H NMR (500 MHz, MeOD) δ 8.44 (d, *J* = 2.2 Hz, 1H), 8.20 (m, 1H), 7.75 (s, 1H), 7.61 – 7.25 (m, 2H), 7.13 (dd, *J* = 8.4, 2.4 Hz, 1H), 4.48 (s, 2H), 3.62 (t, *J* = 6.0 Hz, 2H), 3.15 (t, *J* = 5.8 Hz, 2H). ^{13}C NMR (126

MHz, MeOD) δ 146.12 (d, $J_{C\text{-}F}$ = 13.8 Hz), 141.72 (d, $J_{C\text{-}F}$ = 8.0 Hz), 138.17, 137.86 (d, J = 4.3 Hz), 129.38, 128.26, 127.80, 122.69, 117.66, 113.10, 110.56, 110.27, 107.64, 43.72, 42.24, 19.46. ^{19}F NMR (470 MHz, MeOD) δ -75.51 (s), -76.97 (s) (TFA). HRMS calculated for $C_{16}H_{15}FN_3$ $[M + H]^+$: 268.1250, found: 268.1265.

Preparation of *N*-(4-(trifluoromethyl)pyridin-2-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-6-amine (**1h**)

1h was prepared from tryptoline (10 mg, 0.058 mmol) and 2-amino-4-trifluromethylpyridine following the general procedure for enzymatic bromination with 3-SS and Buchwald-Hartwig amination outlined above in 87% yield (22.4 mg **1h**·TFA, 0.050 mmol). ^{1}H NMR (500 MHz, MeOD) δ 7.96 (d, J = 6.6 Hz, 1H), 7.62 – 7.51 (m, 2H), 7.39 (s, 1H), 7.17 (dd, J = 8.5, 1.7 Hz, 1H), 7.13 (d, J = 6.7 Hz, 1H), 4.49 (s, 2H), 3.60 (t, J = 5.8 Hz, 2H), 3.11 (br 2H). ^{13}C NMR (126 MHz, MeOD) δ 160.75, 139.54, 137.93, 129.09, 128.69, 123.38, 120.71, 120.53, 118.89, 116.54, 114.47, 112.28, 109.35, 107.86, 43.60, 42.12, 19.36. ^{19}F NMR (470 MHz, MeOD) δ -68.18, -77.67 (TFA). HRMS calculated for $C_{17}H_{16}F_3N_4$ $[M + H]^+$: 333.1327, found: 333.1327.

Preparation of *N*-(6-methylpyridin-2-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-6-amine (**1i**)

1i was prepared from tryptoline (10 mg, 0.058 mmol) and 2-amino-6-methylpyridine following the general procedure for enzymatic bromination with 3-SS and Buchwald-Hartwig amination outlined above in 63% yield (14.3 mg **1i**·TFA, 0.036 mmol). ^{1}H NMR (500 MHz, MeOD) δ 7.81 (dd, J = 17.4, 9.8 Hz, 2H), 7.51 (s, 1H), 7.14 (dd, J = 8.5, 1.6 Hz, 1H), 6.84 (d, J = 8.9 Hz, 1H), 6.80 (d, J = 7.0 Hz, 1H), 4.49 (s, 2H), 3.60 (t, J = 6.0 Hz, 2H), 3.09 (t, J = 5.7 Hz, 2H). ^{13}C NMR (126 MHz, MeOD) δ 149.46, 145.92, 137.41, 128.97, 128.70, 128.50, 121.07, 116.37,

114.25, 113.94, 113.23, 110.36, 107.64, 43.62, 42.13, 19.36, 19.07. HRMS calculated for $C_{17}H_{19}N_4$ $[M + H]^+$: 279.1609, found: 279.1621.

Preparation of *N*-(quinolin-3-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-6-amine (**1j**)

1j was prepared from tryptoline (10 mg 0.058 mmol) and 3-aminoquinoline following the general procedure for enzymatic bromination with 3-SS and Buchwald-Hartwig amination outlined above in 51% yield (12.7 mg **1j**·TFA, 0.030 mmol). ^{1}H NMR (500 MHz, MeOD) δ 8.70 (s, *N*-H, 1H), 8.04 (s, 1H), 7.94 (d, *J* = 7.7 Hz, 1H), 7.82 (d, *J* = 8.3 Hz, 1H), 7.63 (m, 2H), 7.42 (m, *J* = 8.5 Hz, 2H), 7.12 (d, *J* = 8.6 Hz, 1H), 4.44 (s, 2H), 3.57 (br, 3H), 3.05 (br, 3H). ^{13}C NMR (126 MHz, MeOD) δ 143.91, 137.94, 136.13, 133.41, 131.69, 130.48, 130.03, 128.51, 128.10, 128.02, 123.35, 122.66, 120.98, 119.69, 113.69, 113.30, 107.25, 43.73, 42.26, 19.45. HRMS calculated for $C_{20}H_{19}N_4$ $[M + H]^+$: 315.1609, found: 315.1619.

Preparation of 1-(2-(2-methoxyphenoxy)ethylamino)-3-(1-(4-methoxyphenyl)-9*H*-carbazol-4-yloxy)propan-2-ol (**2a**)

2a was prepared from carvedilol (10 mg, 0.025 mmol) and 4-methoxyphenylboronic acid following the general procedure for enzymatic chlorination with 4-V. The crude extracts from the bioconversion were transferred to a 20 mL round scintillation vial, and $Pd(OAc)_2$ (0.28 mg, 0.0012 mmol, 0.05 equiv.), Sphos (0.50 mg, 0.0012 mmol, 0.05 equiv.), 4-MeO-C_6H_4-$B(OH)_2$ (5.61 mg, 0.037 mmol, 1.5 equiv), K_3PO_4 (10.4 mg, 0.043 mmol, 2 equiv.), and a magnetic stir bar were added. The vial was transferred to an inert atmosphere dry box, dioxane (0.8 mL) and water (0.2 mL) were added, and the vial was sealed with a teflon lined cap. The vial was removed from the dry box and the mixture was allowed to stir in a 100 °C oil bath. After 12 hours, the reaction vessel was allowed to cool to room temperature, and the contents filtered over

silica, eluting with 150 mL 4:1 CH_2Cl_2/MeOH. The filtrate was collected and concentrated by rotary evaporation. The crude mixture was purified by reversed-phase chromatography (Biotage SNAP-KP-C18-HS, gradient from pure H_2O to 60% MeCN/H_2O) and isolated in 93% yield (14.3 mg **2a**·TFA, 0.023 mmol). ^{1}H NMR (500 MHz, MeOD) δ 8.28 (d, *J* = 7.8 Hz, 1H), 7.56 (dd, *J* = 6.6, 4.8 Hz, 2H), 7.46 (d, *J* = 8.1 Hz, 1H), 7.32 – 7.25 (m, 2H), 7.08 (d, *J* = 8.6 Hz, 2H), 7.05 – 6.95 (m, 4H), 6.93 – 6.88 (m, 1H), 6.81 (d, *J* = 8.2 Hz, 1H), 4.56 (dt, *J* = 9.5, 5.8 Hz, 1H), 4.42 (dd, *J* = 9.9, 4.6 Hz, 1H), 4.31 (dt, *J* = 13.5, 6.7 Hz, 3H), 3.87 (s, 3H), 3.78 (s, 3H), 3.67 (dd, *J* = 12.7, 2.9 Hz, 1H), 3.60 – 3.55 (m, 2H), 3.50 (dd, *J* = 12.6, 9.9 Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 160.36, 155.19, 151.14, 148.43, 141.07, 140.21, 132.82, 130.53, 127.21, 125.84, 124.25, 123.69, 123.56, 122.35, 120.47, 120.04, 116.69, 115.46, 113.97, 113.38, 111.75, 102.35, 71.05, 66.83, 66.23, 56.42, 55.84, 51.76, 48.38. HRMS calculated for $C_{31}H_{32}N_2O_5$ $[M + H]^+$: 513.2389, found: 513.2400.

Preparation of 1-(isopropylamino)-3-(7-(4-methoxyphenyl)-1*H*-indol-4-yloxy)propan-2-ol (**3a**)

3a was prepared from pindolol (10 mg, 0.040) and 4-methoxyphenylboronic acid following the general procedure for enzymatic bromination with 4-V and Suzuki-Miyaura Coupling outlined above in >99% yield (19.4 mg **3a**·TFA, 0.041 mmol). ^{1}H NMR (500 MHz, MeOD) δ 7.50 (d, *J* = 8.7 Hz, 2H), 7.23 – 7.12 (m, 1H), 7.10 – 6.95 (m, 3), 6.68 – 6.56 (m, 2H), 4.38 – 4.29 (m, 1H), 4.25 (dd, *J* = 9.9, 4.9 Hz, 1H), 4.15 (dd, *J* = 9.9, 6.0 Hz, 1H), 3.48 (dt, *J* = 13.1, 6.5 Hz, 1H), 3.37 (dd, *J* = 12.6, 2.9 Hz, 1H), 3.23 (dd, *J* = 12.6, 9.6 Hz, 1H), 1.38 (dd, *J* = 6.5, 4.5 Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ 160.22, 152.36, 133.10, 130.22, 124.99, 124.83, 122.76, 121.42, 120.62, 115.36, 102.08, 99.94, 71.07, 67.08, 55.79, 52.10, 19.33, 18.81. HRMS calculated for $C_{21}H_{27}N_2O_3$ $[M + H]^+$: 355.2021, found: 355.2036.

Preparation of *N*-(4'-methoxybiphenyl-4-yl)-1-methyl-*N*-(thiophen-2-ylmethyl)piperidin-4-amine (**4a**)

4a was prepared from thenalidine (10 mg, 0.035 mmol) and 4-methoxyphenylboronic acid following the general procedure for enzymatic bromination with 4-V and Suzuki-Miyaura Coupling outlined above in 74% yield (13.1 mg **4a**·TFA, 0.026 mmol). ^{1}H NMR (500 MHz, MeOD) δ 7.44 (dd, *J* = 15.2, 8.5 Hz, 4H), 7.23 (d, *J* = 4.8 Hz, 1H), 7.06 – 6.88 (m, 5H), 4.65 (s, 2H), 4.06 (t, *J* = 11.5 Hz, 1H), 3.80 (s, 3H), 3.58 (d, *J* = 11.2 Hz, 2H), 3.18 (t, *J* = 11.9 Hz, 2H), 2.87 (s, 3H), 2.27 – 2.09 (m, 2H), 2.00 (d, *J* = 12.0 Hz, 2H). ^{13}C NMR (126 MHz, MeOD) δ 128.36, 128.25 , 128.11, 128.04, 127.76, 125.51, 125.15, 118.01, 115.21, 115.16, 55.74, 55.49, 47.04, 43.82, 31.11, 28.46 (Some aryl carbons cannot be distinguished due to overlap). HRMS calculated for $C_{24}H_{29}N_2OS$ $[M + H]^+$: 393.2000, found: 393.2000.

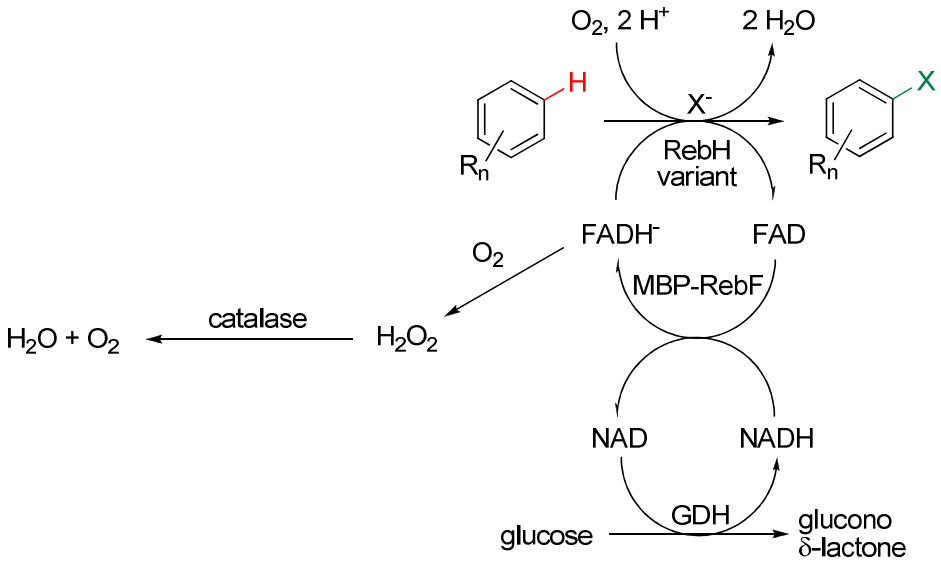
Preparation of 1-methyl-*N*-(thiophen-2-ylmethyl)-*N*-(4-(2,2,2-trifluoroethoxy)phenyl)piperidin-4-amine (**4b**)

4b was prepared from thenalidine (10 mg, 0.035 mmol) following the general procedure for enzymatic bromination with 4-V. The crude extracts from the bioconversion were transferred to a 20 mL round scintillation vial, and $[(allyl)PdCl]_2$ (0.06 mg, $1.74x10^{-4}$ mmol, 0.005 equiv.), RockPhos (0.24 mg, $5.22x10^{-4}$ mmol, 0.015 equiv.), Cs_2CO_3 (20.6 mg, 0.070 mmol, 2 equiv.), and a magnetic stir bar were added. The vial was transferred to an inert atmosphere dry box, toluene (1 mL) and trifluoroethanol (5.3 μL, 0.070 mmol, 2 equiv.) were added, and the vial was sealed with a teflon lined cap. The vial was removed from the dry box and the mixture was allowed to stir in a 90 °C oil bath. After 14 hours, the reaction vessel was allowed to cool to

room temperature, and the contents filtered over silica, eluting with 150 mL 4:1 CH_2Cl_2/MeOH. The filtrate was collected and concentrated by rotary evaporation. The crude mixture was purified by reversed-phase chromatography (Biotage SNAP-KP-C18-HS, gradient from pure H_2O to 60% MeCN/H_2O) and isolated in 33% yield (5.6 mg **4b**·TFA, 0.011 mmol). ^{1}H NMR (500 MHz, MeOD) δ 7.24 (dd, *J* = 5.1, 1.1 Hz, 1H), 7.06 (m, 2H), 6.94 (m, 3H), 6.91 – 6.87 (m, 1H), 4.63 (s, 2H), 4.45 (dd, *J* = 17.0, 8.5 Hz, 2H), 3.80 (t, *J* = 11.7 Hz, 1H), 3.56 (d, *J* = 12.2 Hz, 2H),3.11 (t, *J* = 11.7 Hz, 2H), 2.85 (s, 3H), 2.21 (d, *J* = 13.6 Hz, 2H), 1.89 (m, *J* = 23.9, 11.2 Hz, 2H). ^{13}C NMR (126 MHz, MeOD) δ 161.32, 127.59, 126.52, 125.84, 124.10, 123.30, 120.17, 117.73, 116.83, 67.38, 67.10, 55.26, 49.85, 43.72, 28.59. ^{19}F NMR (470 MHz, MeOD) δ -75.89, -77.13 (TFA). HRMS calculated for $C_{19}H_{24}F_3N_2OS$ $[M + H]^+$: 385.1561, found: 385.1574.

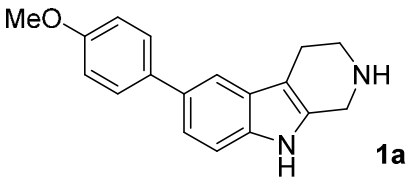
Scheme Showing Cofactor Regeneration System

Figure S1:

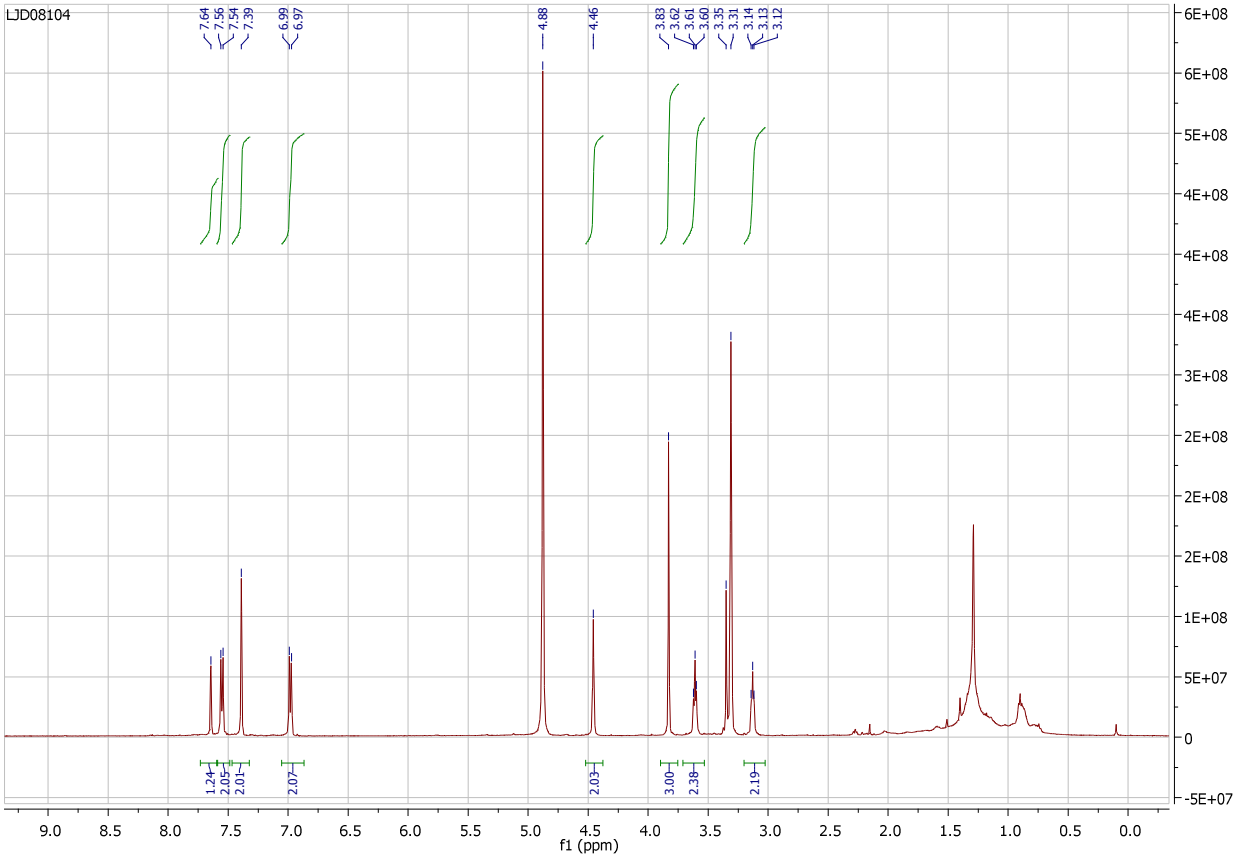


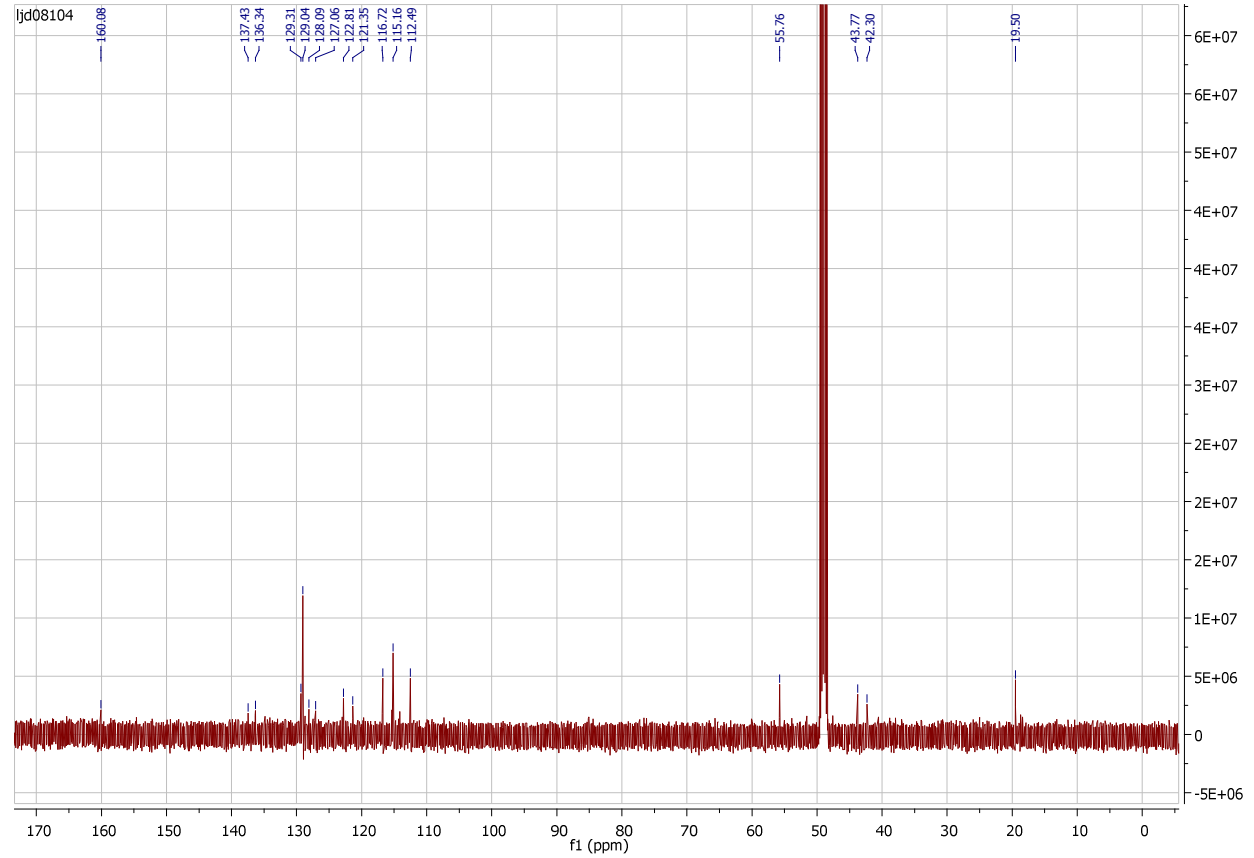
In this system, glucose dehydrogenase (GDH) uses glucose to reduce NAD, generating glucono δ-lactone as a byproduct. MBP-RebF then reduces FAD using the NADH generated by GDH, but given that MBP-RebF and the RebH variants employed are not perfectly coupled, the reduced FAD can then react with O_2 to generate H_2O_2. Catalase is thus added to remove this unwanted H_2O_2, converting it to H_2O and O_2. Reduced FAD that reacts as desired is able to reduce O_2 in the RebH active site in order to ultimately effect halogenation.[6] H_2O is generated as a byproduct of this reaction.

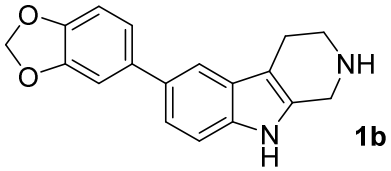
NMR Spectra



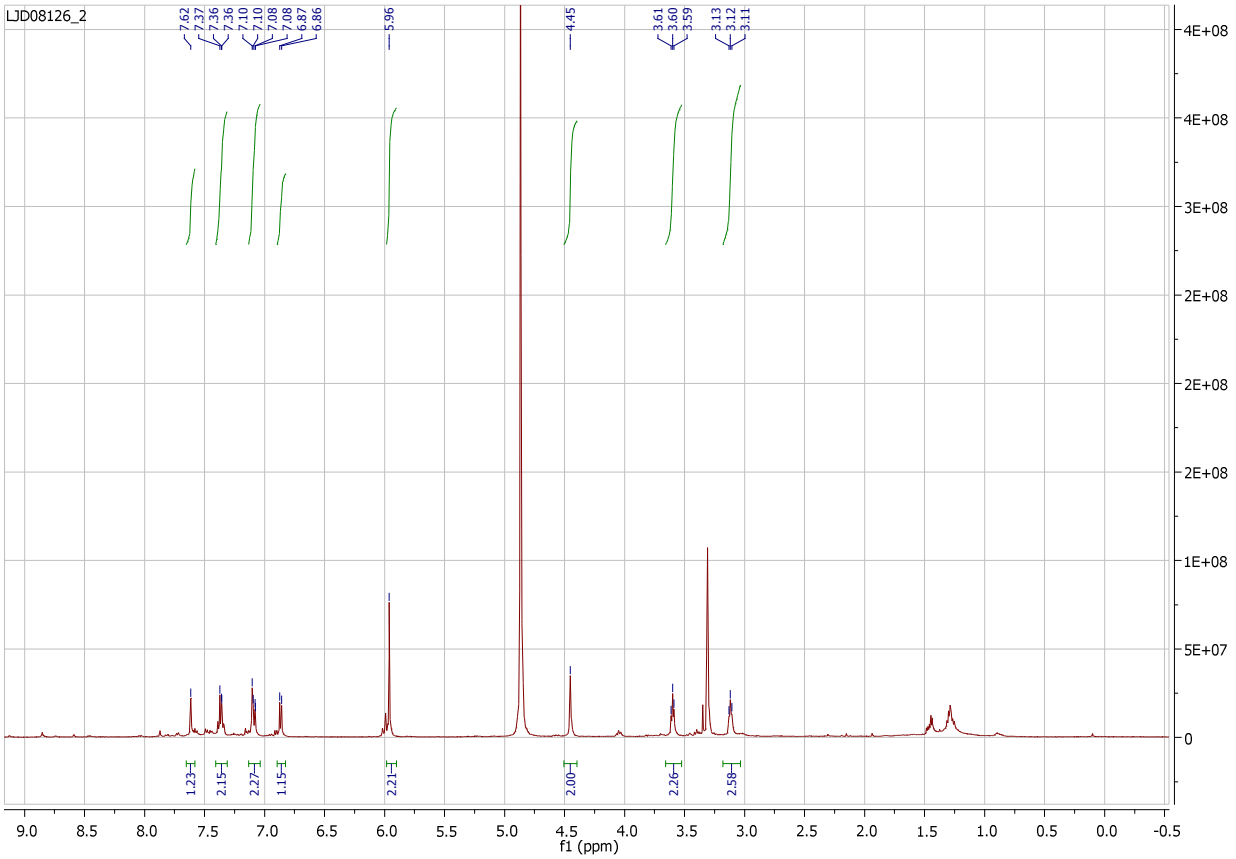
1a

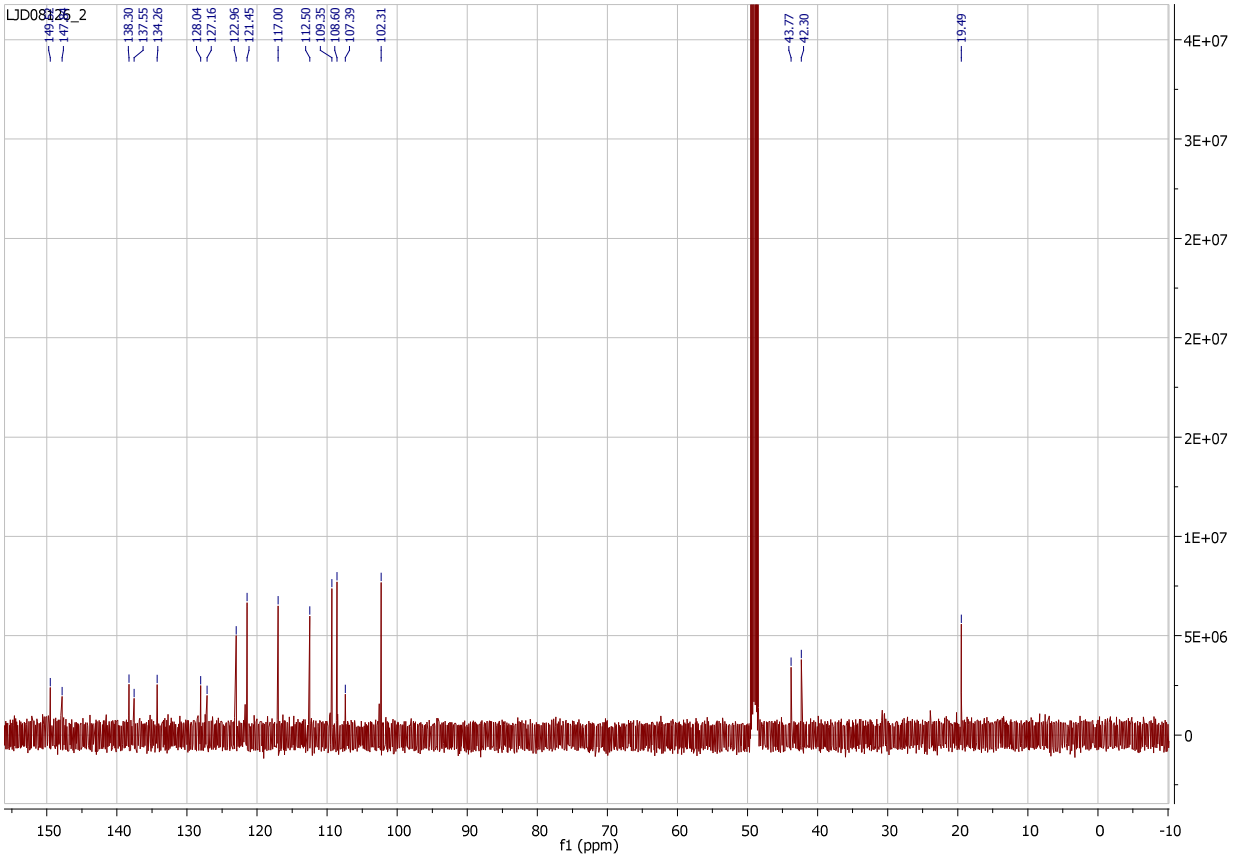


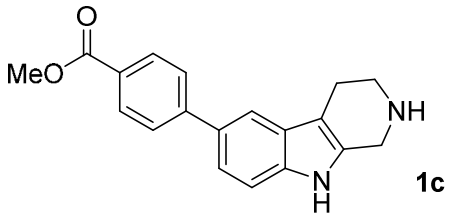

ljd08104
160.08
137.43
136.34
129.31
129.04
128.09
127.06
122.81
121.35
116.72
115.16
112.49
55.76
43.77
42.30
19.50
6E+07
6E+07
5E+07
4E+07
4E+07
4E+07
3E+07
2E+07
2E+07
2E+07
1E+07
5E+06
0
-5E+06
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


O
O
NH
N
H

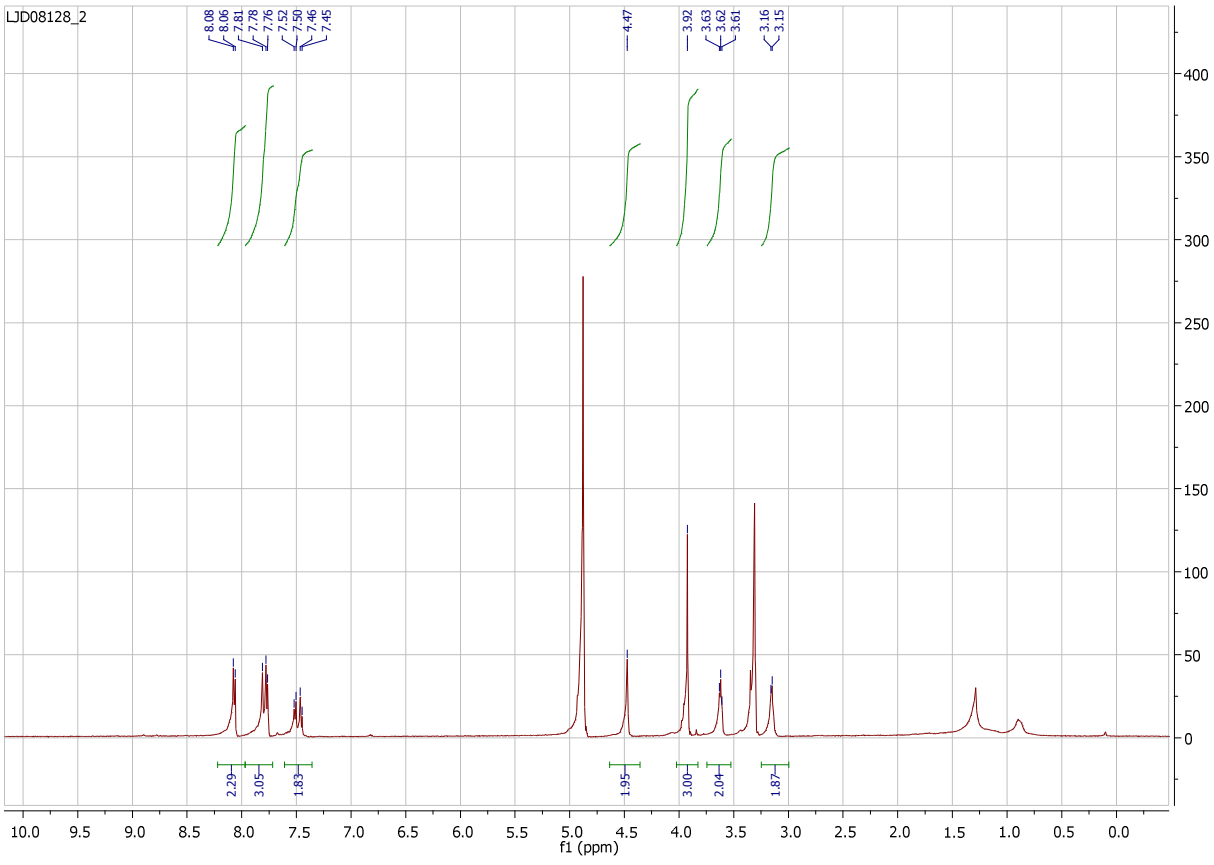
1b

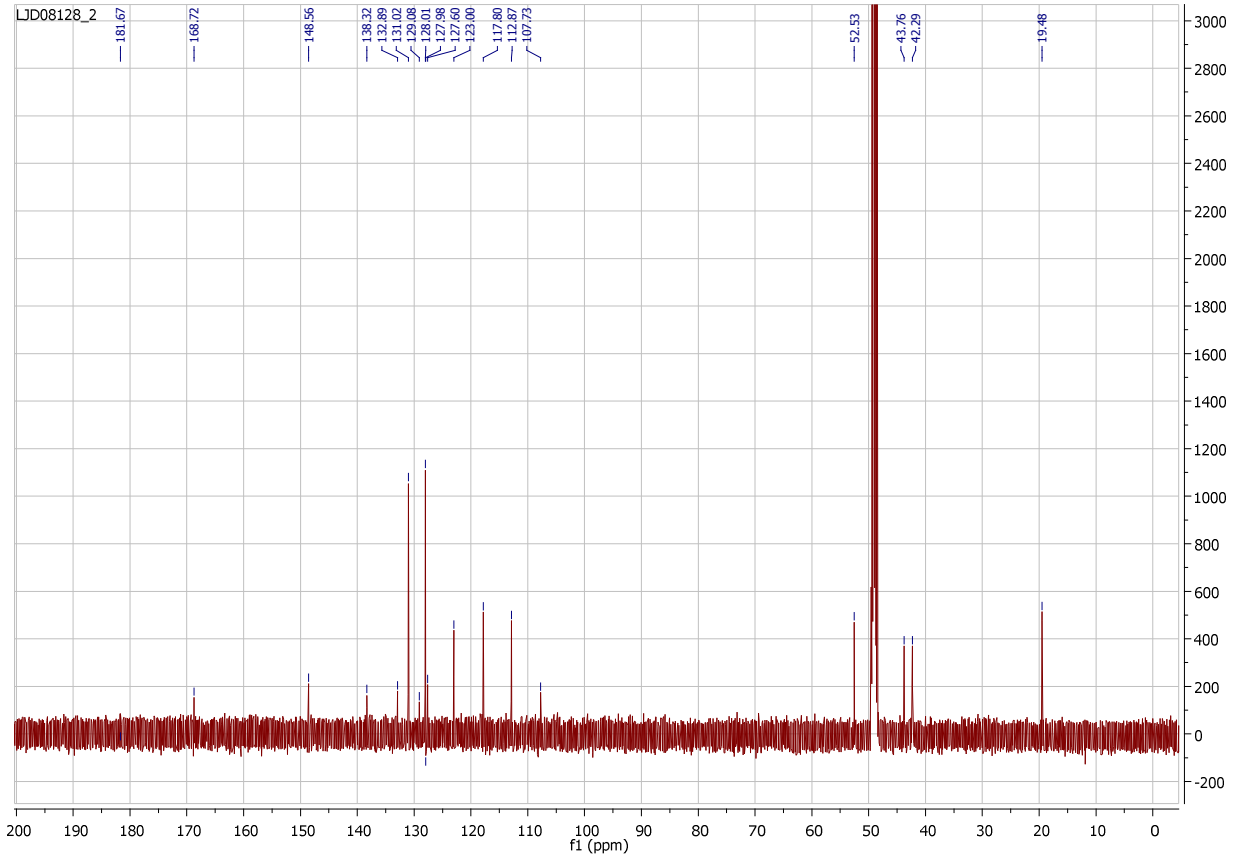

LJD08126_2
7.62
7.37
7.36
7.36
7.10
7.10
7.08
7.08
6.87
6.86
5.96
4.45
3.61
3.60
3.59
3.13
3.12
3.11
1.23
2.15
2.27
1.15
2.21
2.00
2.26
2.58
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

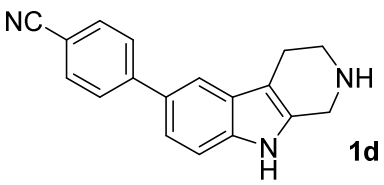

LJD083125_2
149.25
147.26
138.30
137.55
134.26
128.04
127.16
122.96
121.45
117.00
112.50
109.35
108.60
107.39
102.31
43.77
42.30
19.49
4E+07
3E+07
2E+07
2E+07
2E+07
1E+07
5E+06
0
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


O
MeO
NH
N
H

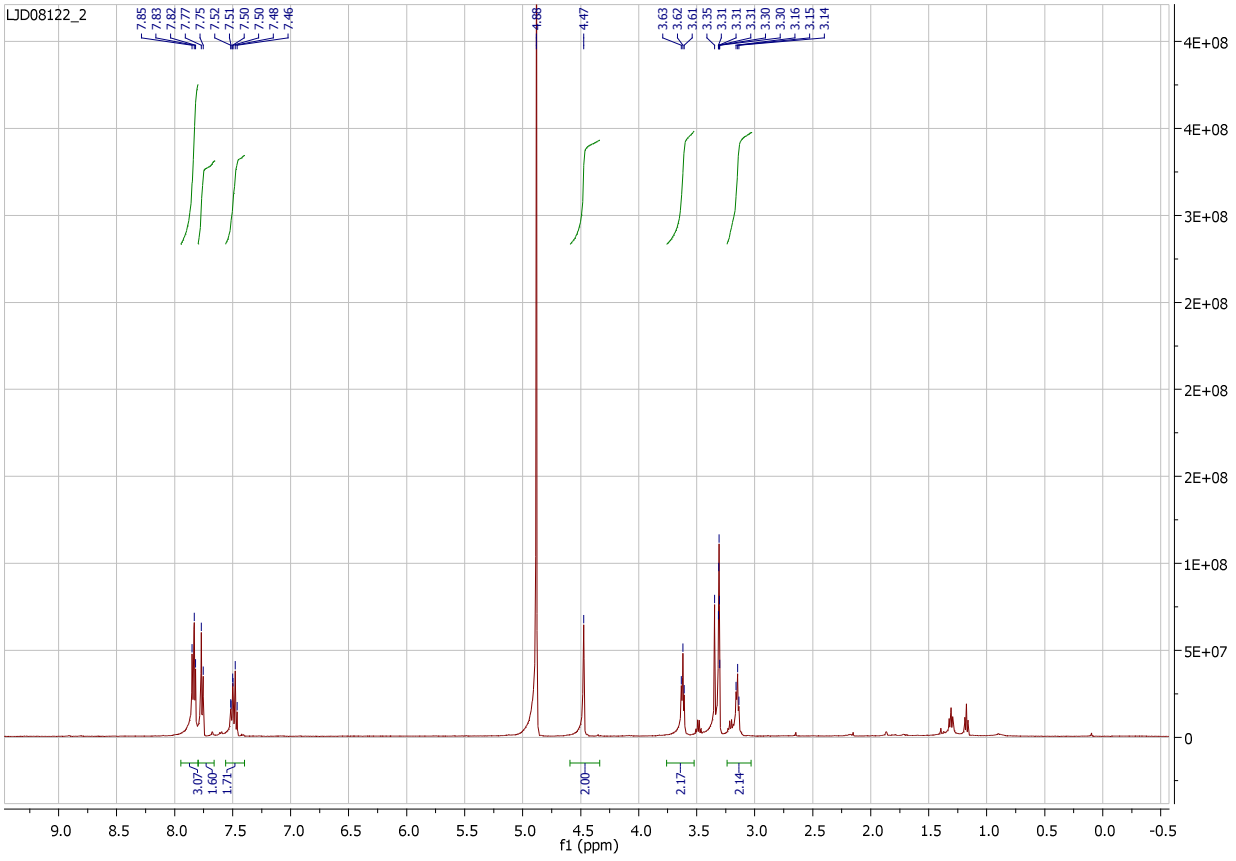
1c

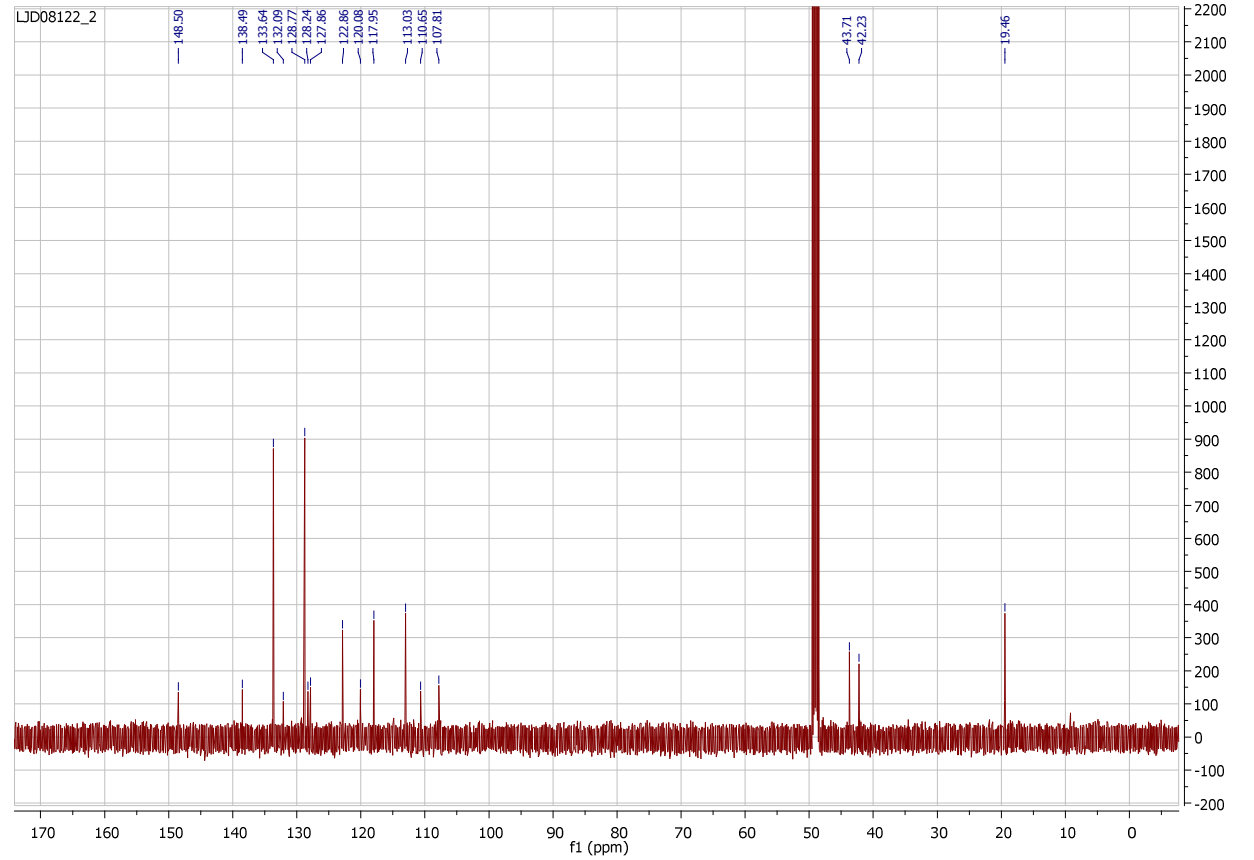

LJD08128_2
8.08
8.06
7.81
7.78
7.76
7.52
7.50
7.46
7.45
4.47
3.92
3.63
3.62
3.61
3.16
3.15
2.29
3.05
1.83
1.95
3.00
2.04
1.87
f1 (ppm)

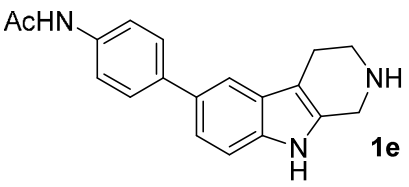

LJD08128_2
181.67
168.72
148.56
138.32
132.89
131.02
129.08
128.01
127.98
127.60
123.00
117.80
112.87
107.73
52.53
43.76
42.29
19.48
f1 (ppm)


NC
NH
N
H

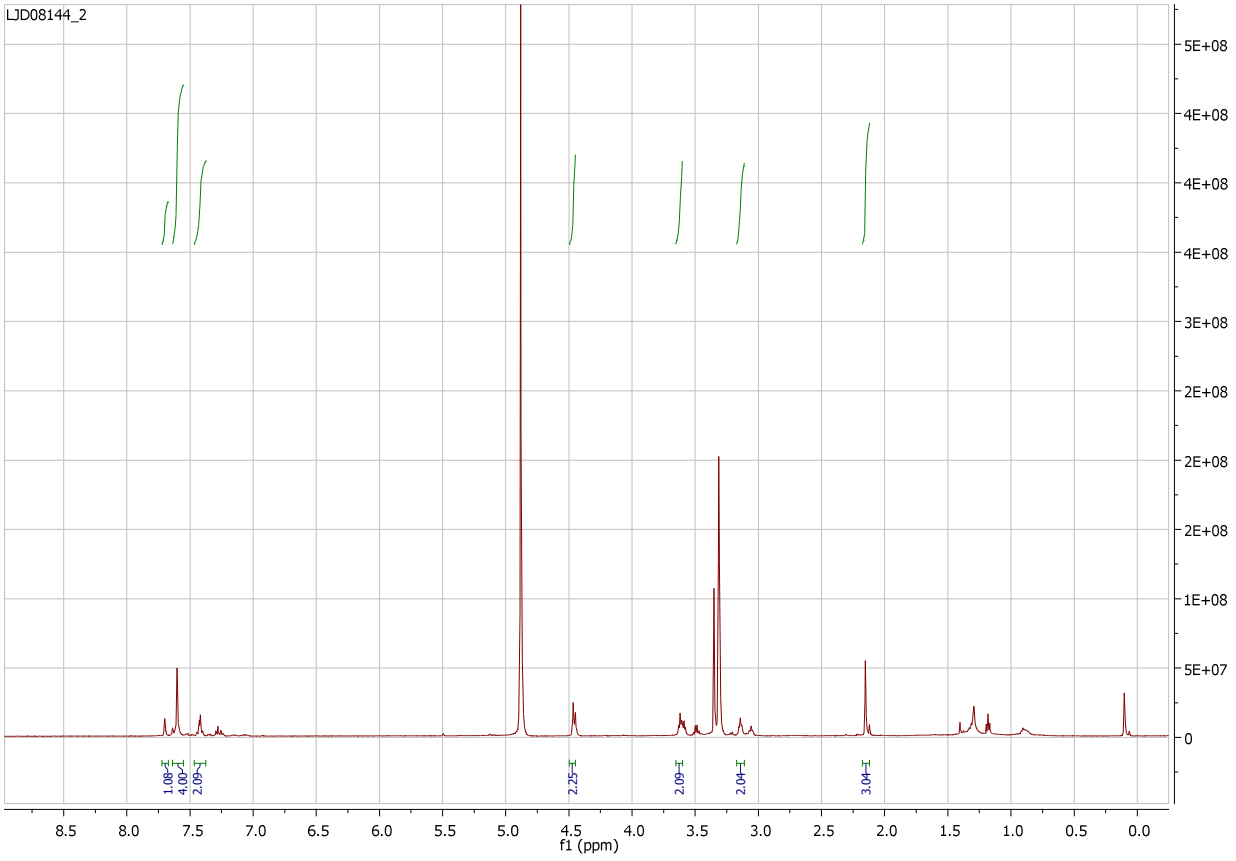
1d

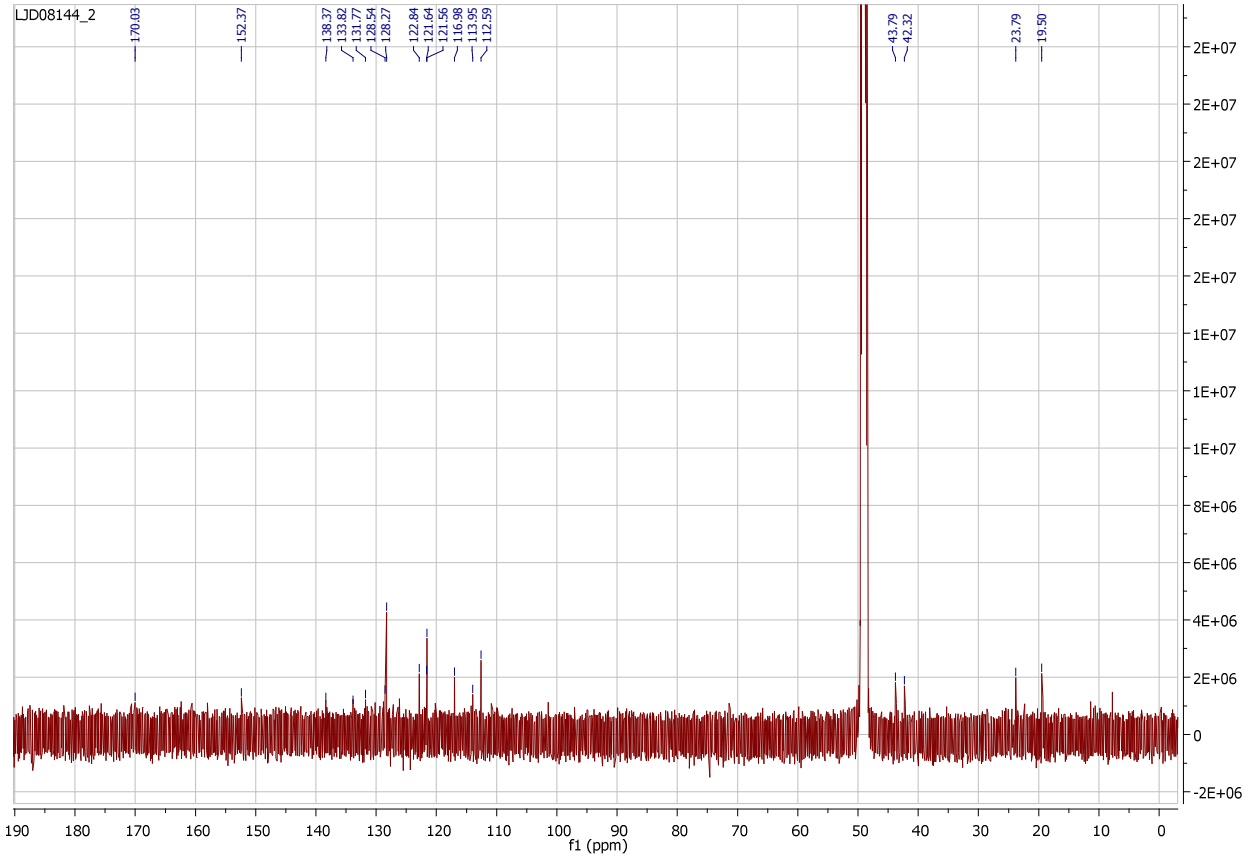

LJD08122_2
7.85
7.83
7.82
7.77
7.75
7.52
7.51
7.50
7.50
7.48
7.46
4.48
4.47
3.63
3.62
3.61
3.35
3.31
3.31
3.31
3.30
3.30
3.16
3.15
3.14
3.07
1.60
1.71
2.00
2.17
2.14
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

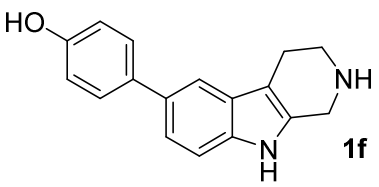

LJD08122_2
148.50
138.49
133.64
132.09
128.77
128.24
127.86
122.86
120.08
117.95
113.03
110.65
107.81
43.71
42.23
19.46
f1 (ppm)


AcHN
NH
N
H

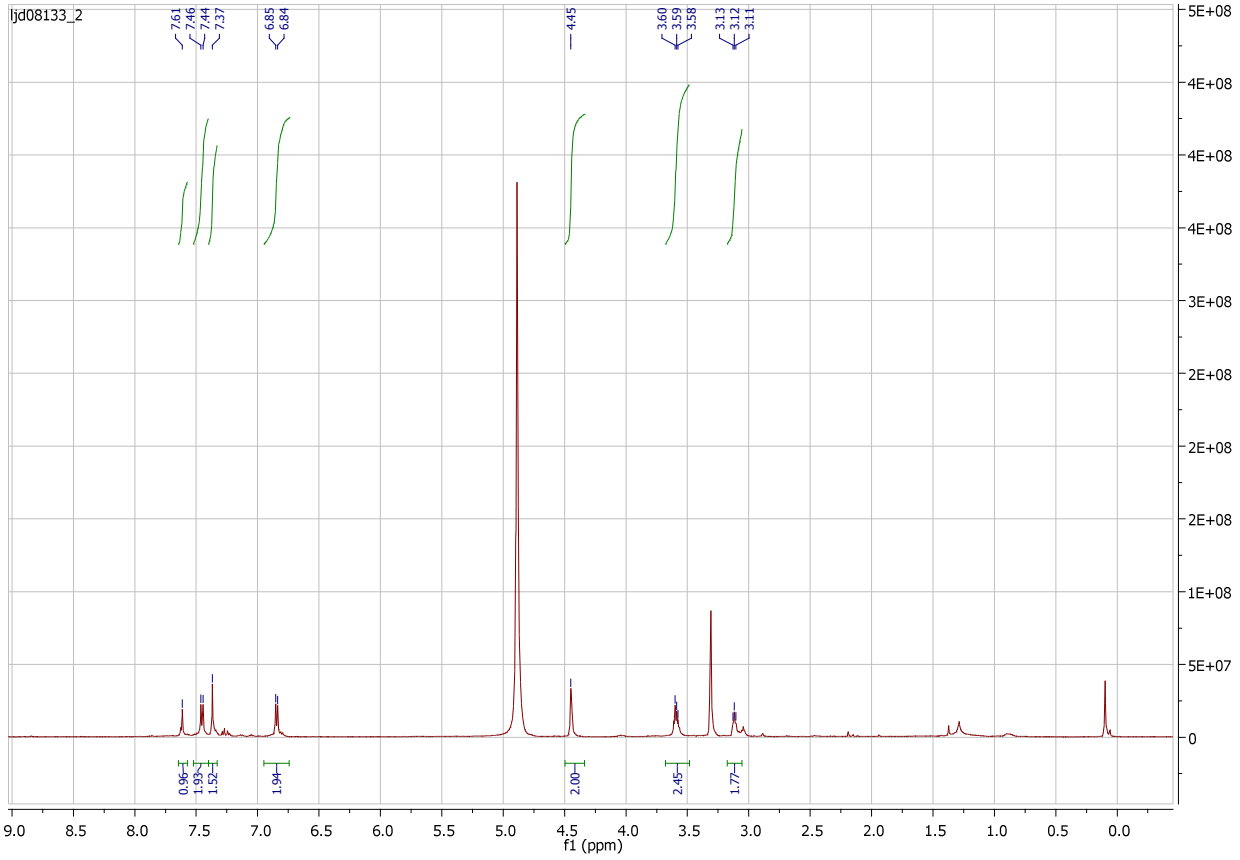
1e

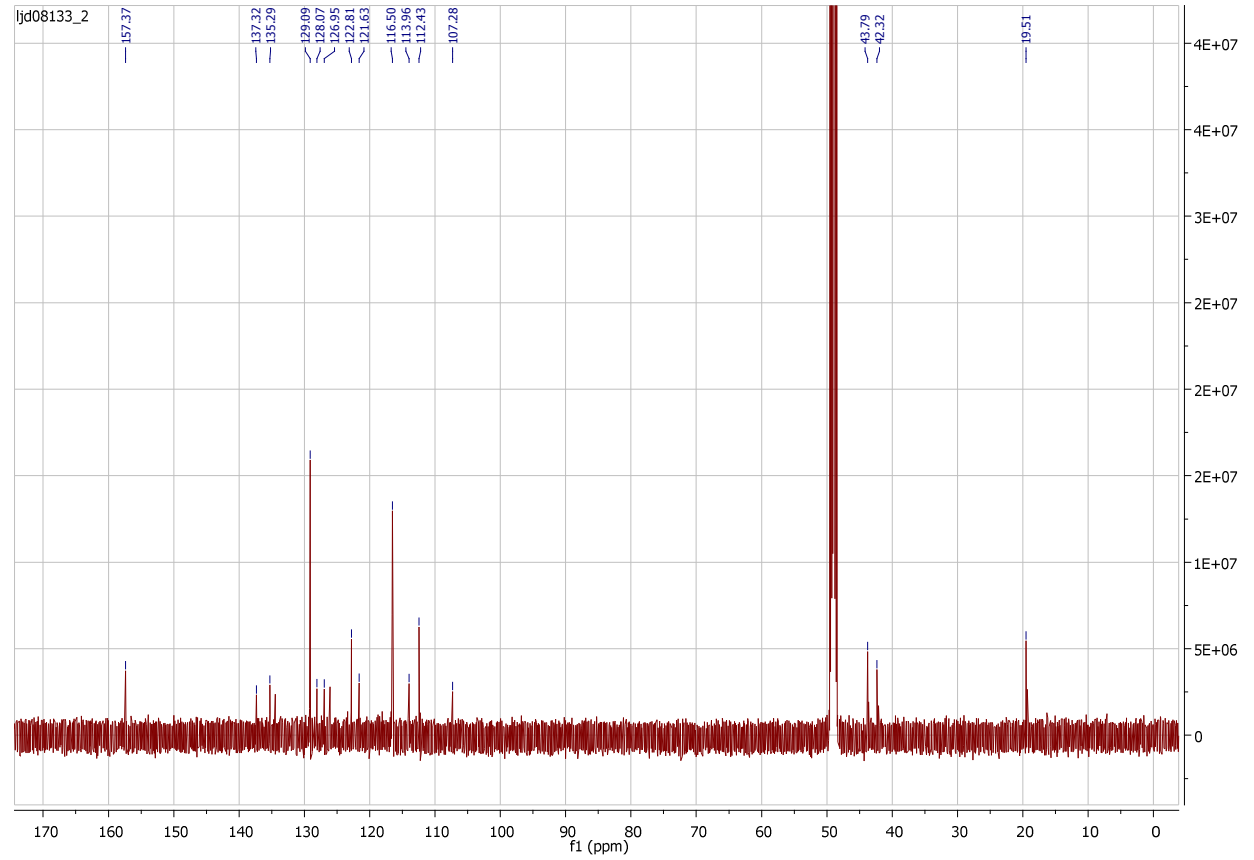

LJD08144_2
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
1.08
4.00
2.09
2.25
2.09
2.04
3.04
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

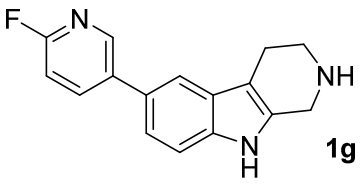

LJD08144_2
170.03
152.37
138.37
133.82
131.77
128.54
128.27
122.84
121.64
121.56
116.98
113.95
112.59
43.79
42.32
23.79
19.50
2E+07
2E+07
2E+07
2E+07
2E+07
1E+07
1E+07
1E+07
8E+06
6E+06
4E+06
2E+06
0
-2E+06
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


HO
NH
N
H

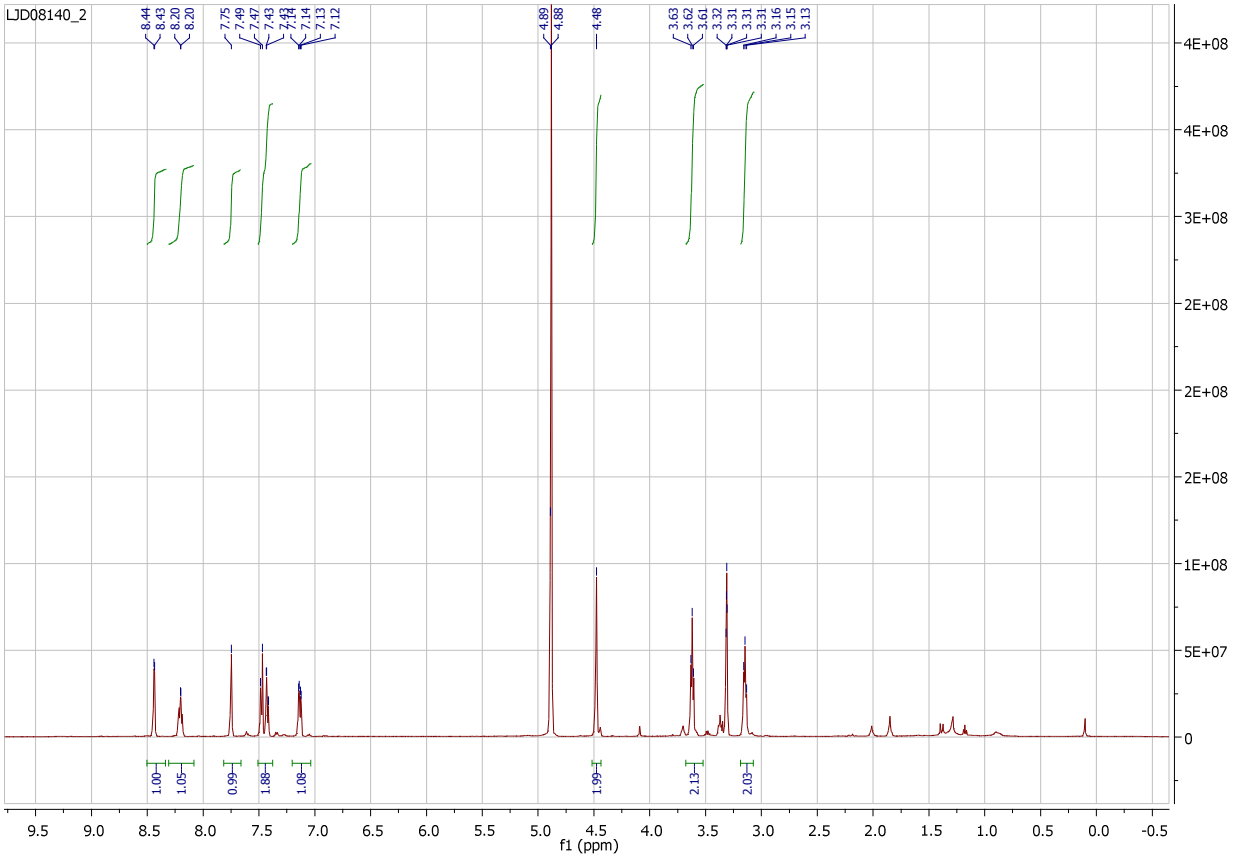
1f

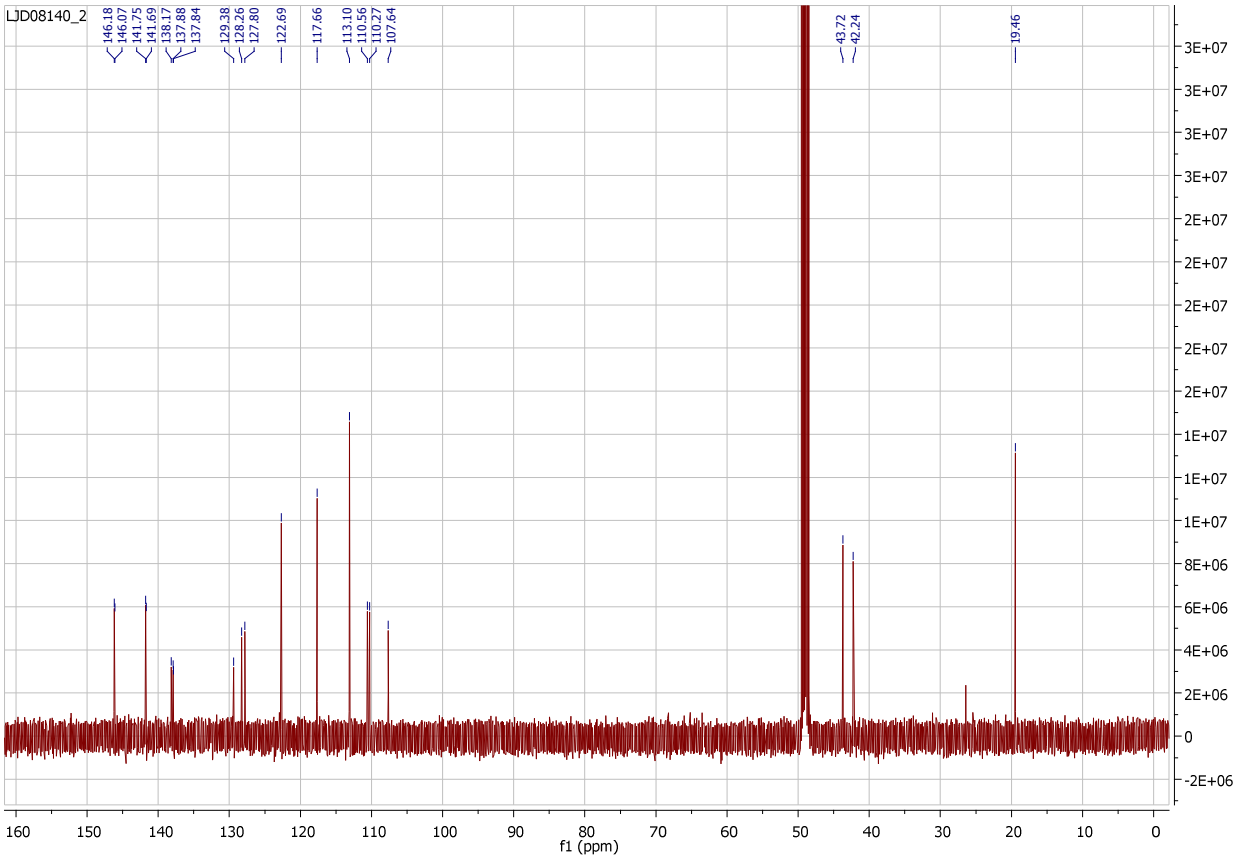

ljd08133_2
7.61
7.46
7.44
7.37
6.85
6.84
4.45
3.60
3.59
3.58
3.13
3.12
3.11
0.96
1.93
1.52
1.94
2.00
2.45
1.77
f1 (ppm)

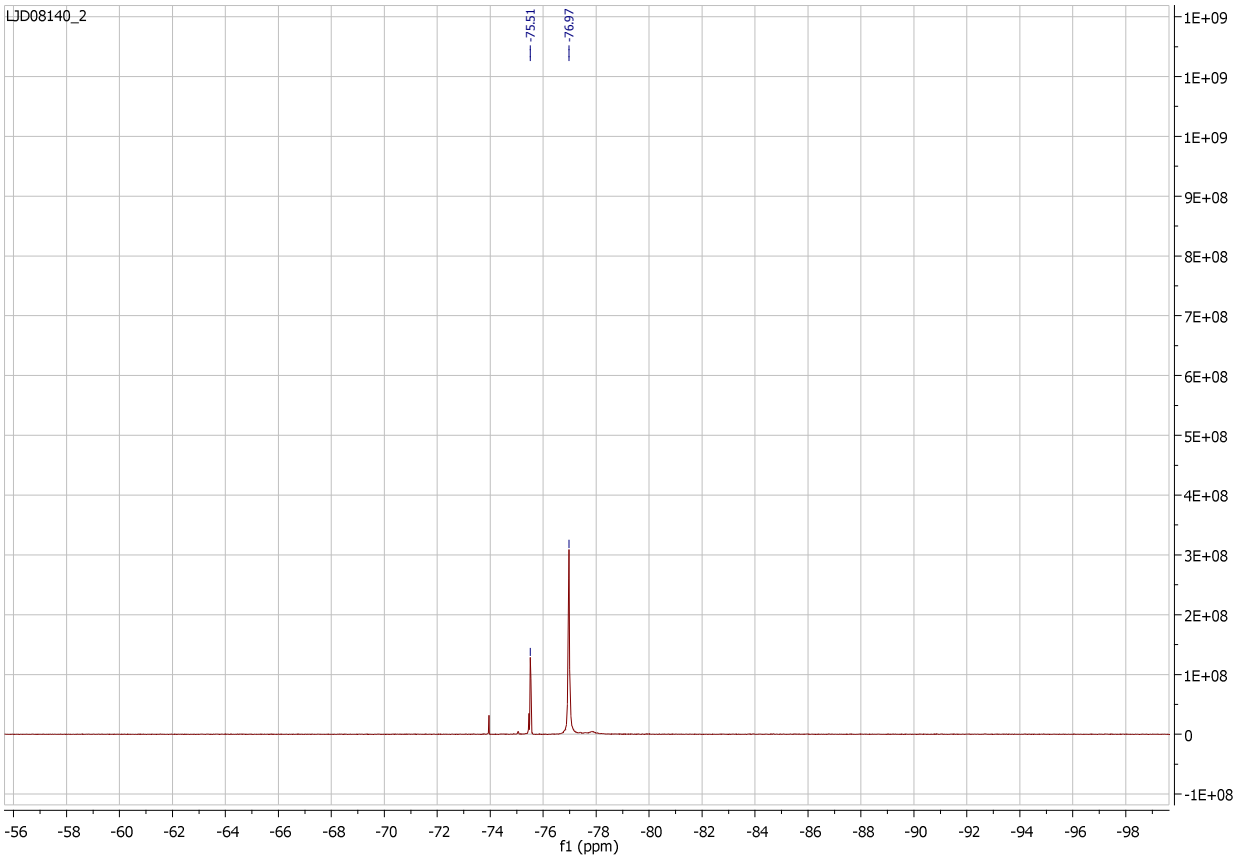

ljd08133_2
157.37
137.32
135.29
129.09
128.07
126.95
122.81
121.63
116.50
113.96
112.43
107.28
43.79
42.32
19.51
4E+07
4E+07
3E+07
2E+07
2E+07
2E+07
1E+07
5E+06
0
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

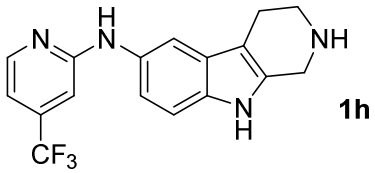

F
N
NH
N
H

1g

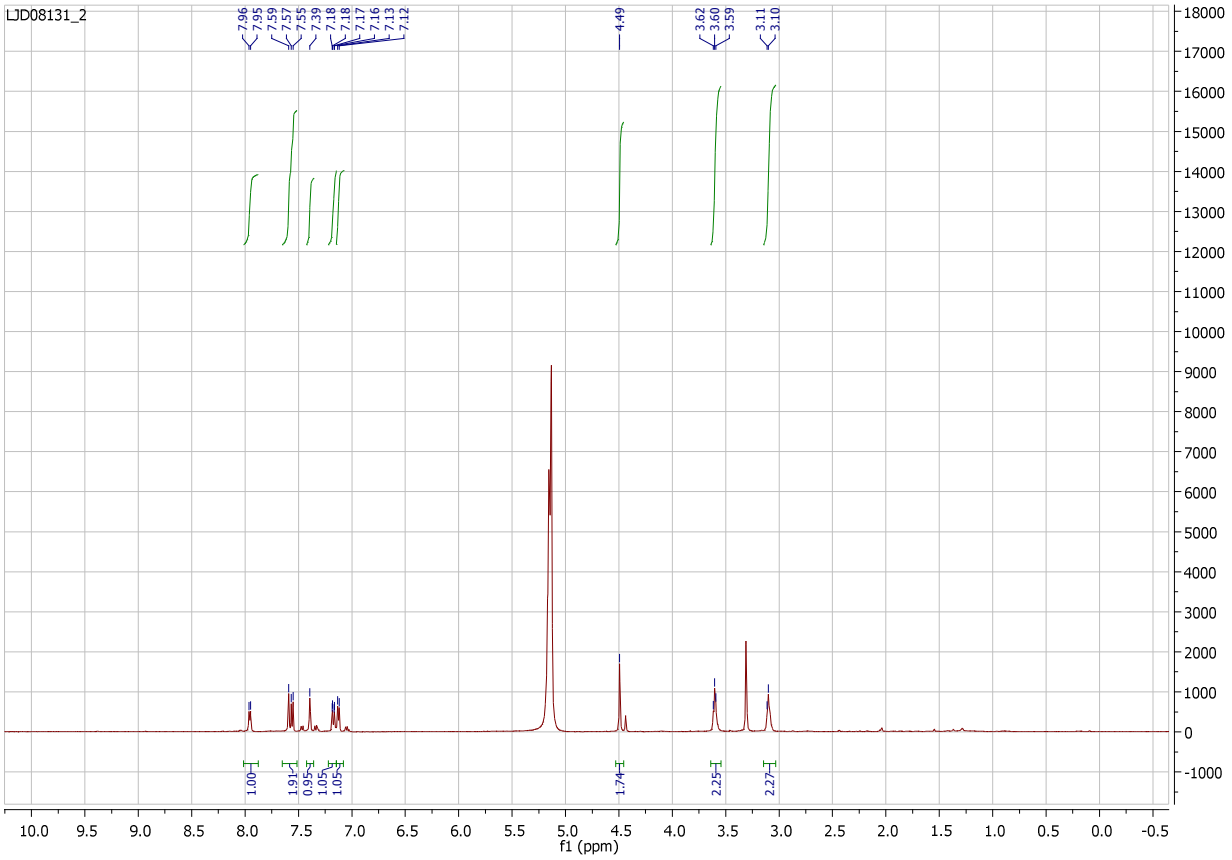

LJD08140_2
8.44
8.43
8.20
8.20
7.75
7.49
7.47
7.43
7.43
7.14
7.13
7.12
4.89
4.88
4.48
3.63
3.62
3.61
3.32
3.31
3.31
3.31
3.16
3.15
3.13
1.00
1.05
0.99
1.88
1.08
1.99
2.13
2.03
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

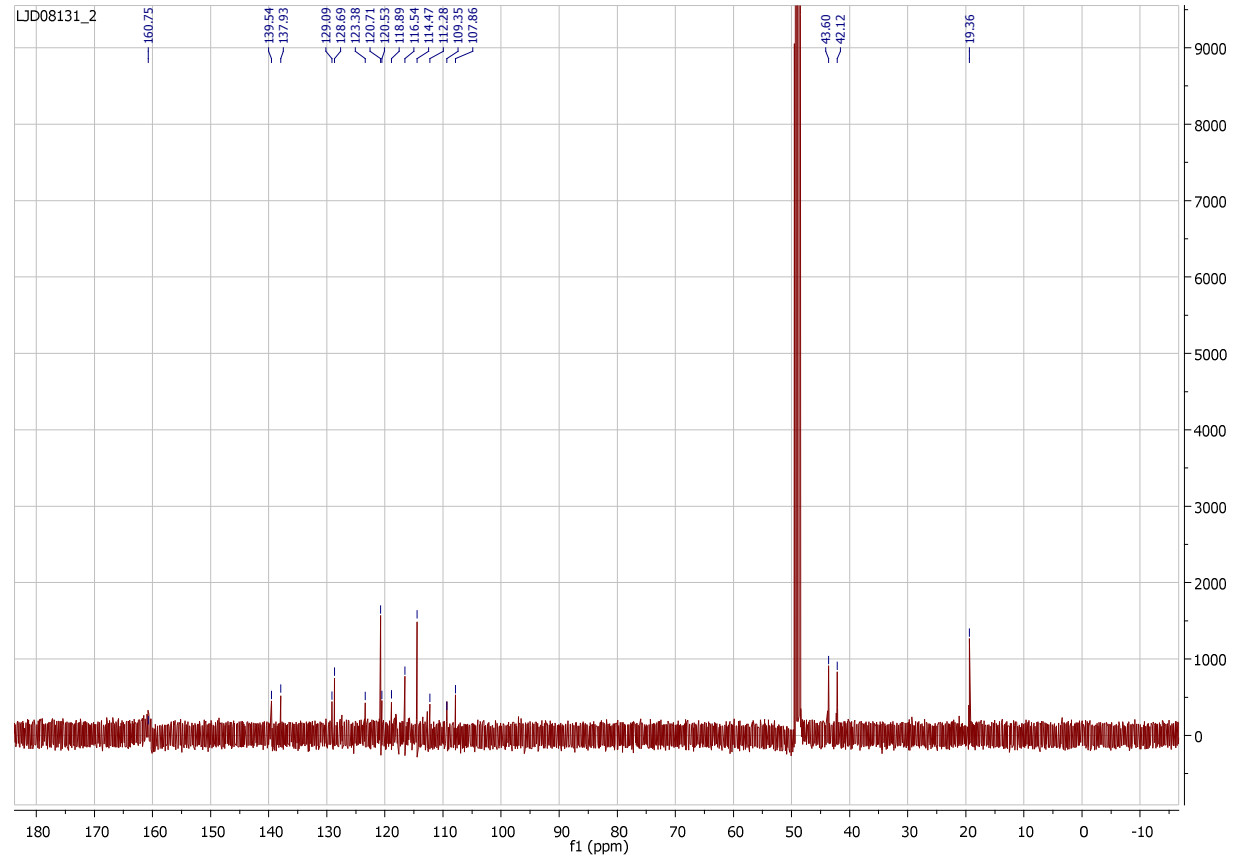

LJD08140_2
146.18
146.07
141.75
141.69
138.17
137.88
137.84
129.38
128.26
127.80
122.69
117.66
113.10
110.56
110.27
107.64
43.72
42.24
19.46
3E+07
3E+07
3E+07
3E+07
2E+07
2E+07
2E+07
2E+07
2E+07
2E+07
1E+07
1E+07
1E+07
8E+06
6E+06
4E+06
2E+06
0
-2E+06
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

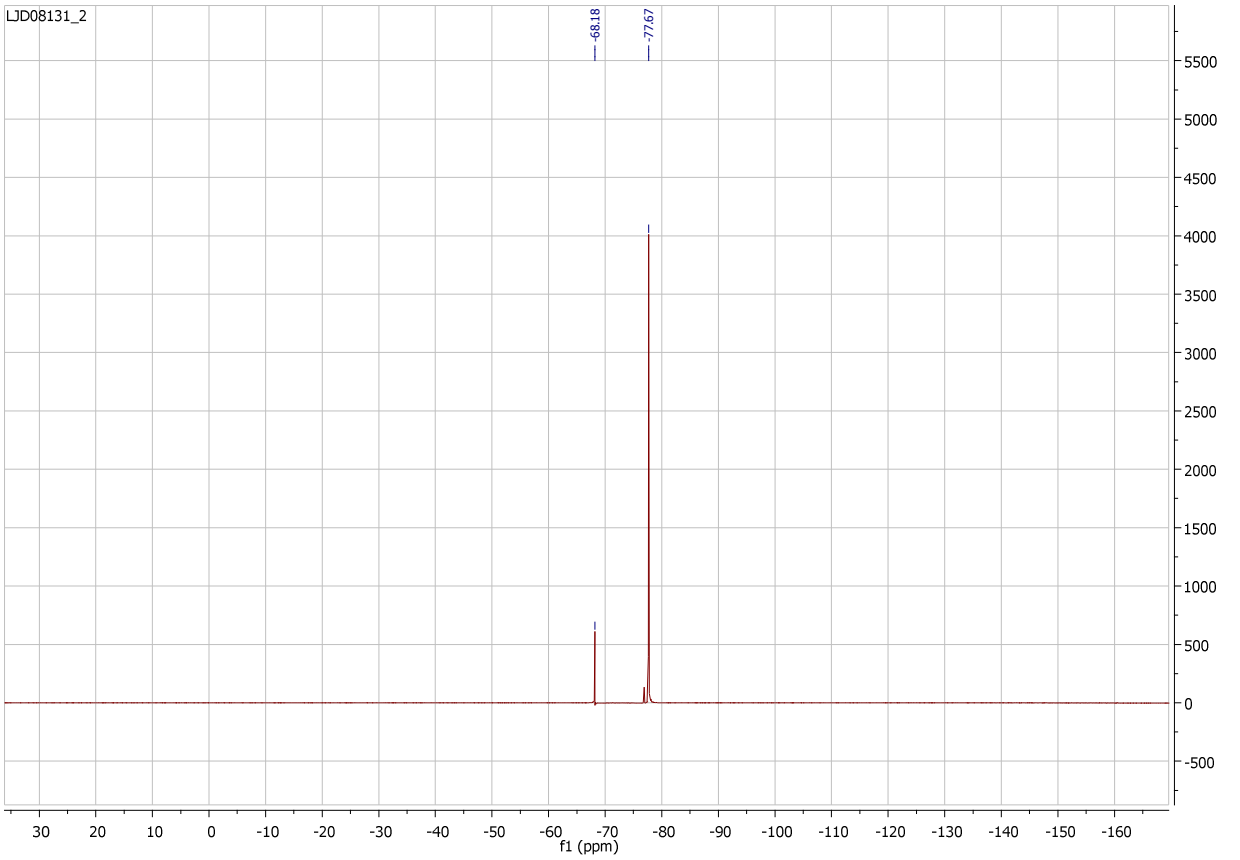

LJD08140_2
-75.51
-76.97
1E+09
1E+09
1E+09
9E+08
8E+08
7E+08
6E+08
5E+08
4E+08
3E+08
2E+08
1E+08
0
-1E+08
-56 -58 -60 -62 -64 -66 -68 -70 -72 -74 -76 -78 -80 -82 -84 -86 -88 -90 -92 -94 -96 -98
f1 (ppm)

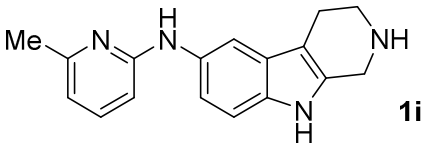

H
N
N
NH
N
H
CF3

1h

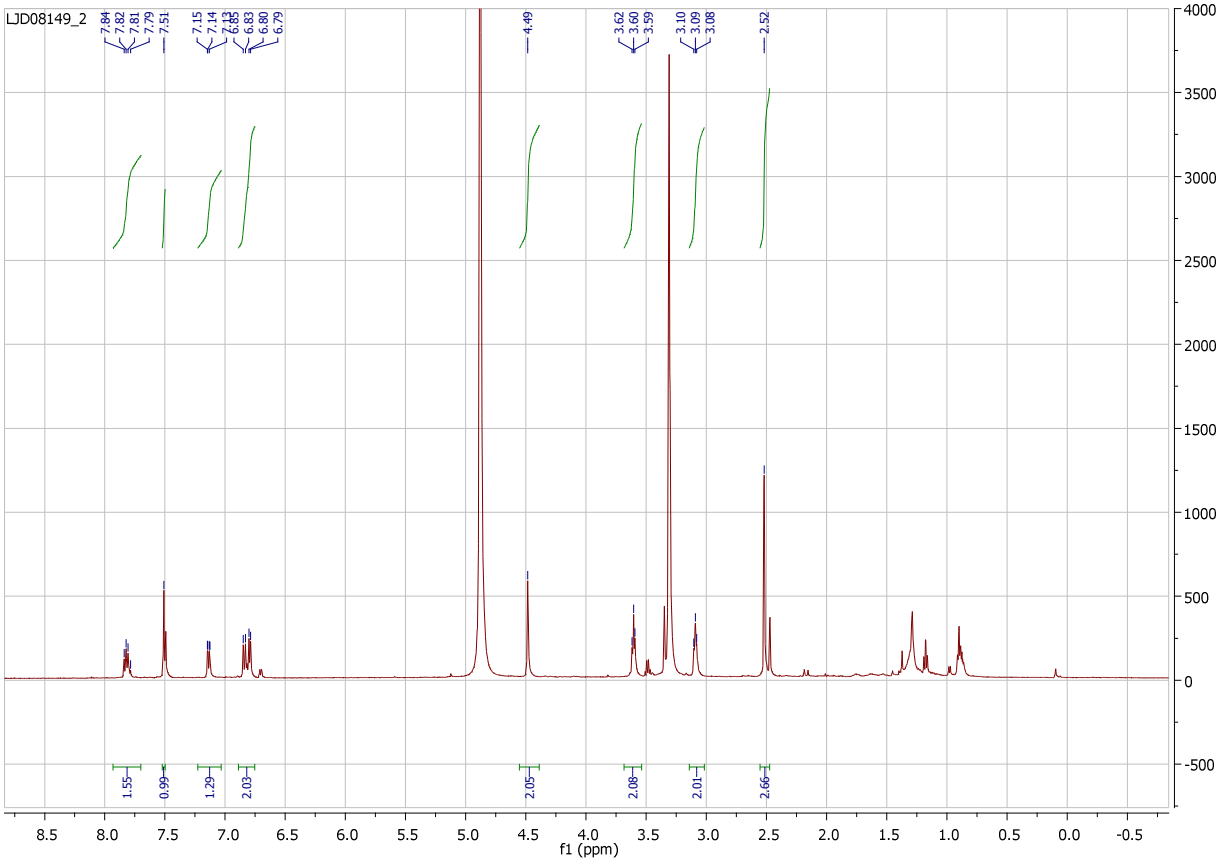

LJD08131_2
7.96
7.95
7.59
7.57
7.55
7.39
7.18
7.18
7.17
7.16
7.13
7.12
4.49
3.62
3.60
3.59
3.11
3.10
1.00
1.91
0.95
1.05
1.05
1.74
2.25
2.27
f1 (ppm)

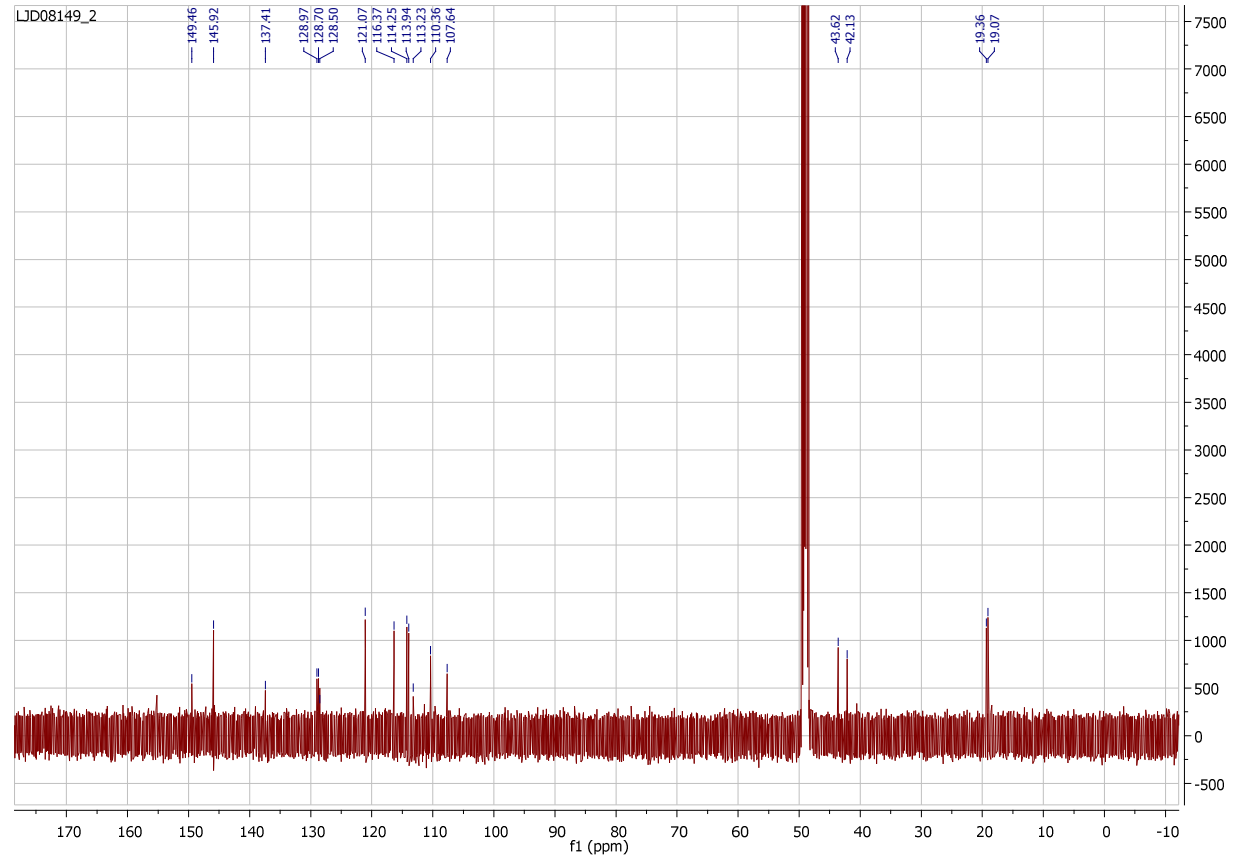

LJD08131_2
160.75
139.54
137.93
129.09
128.69
123.38
120.71
120.53
118.89
116.54
114.47
112.28
109.35
107.86
43.60
42.12
19.36
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

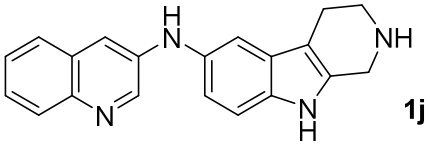

LJD08131_2
-68.18
-77.67
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
f1 (ppm)


Me
N
H
N
NH
N
H

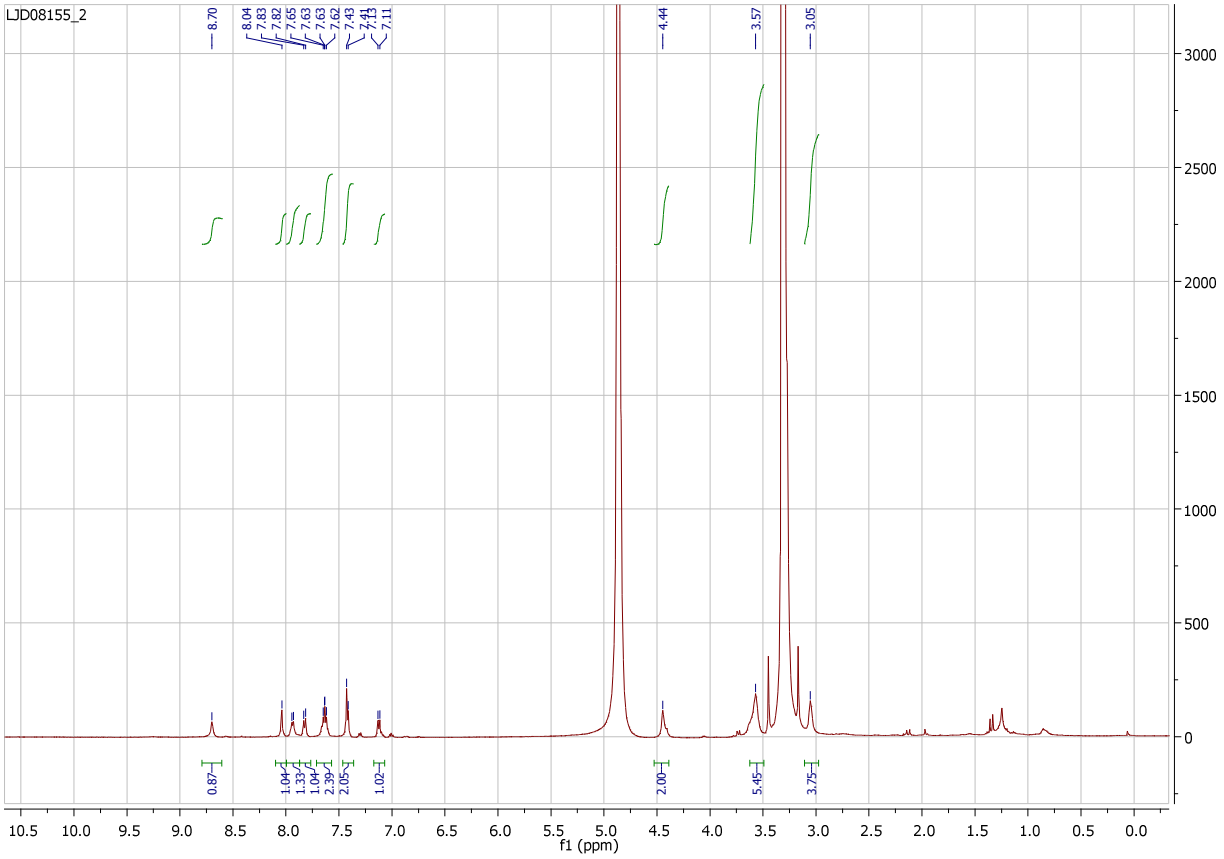
1i

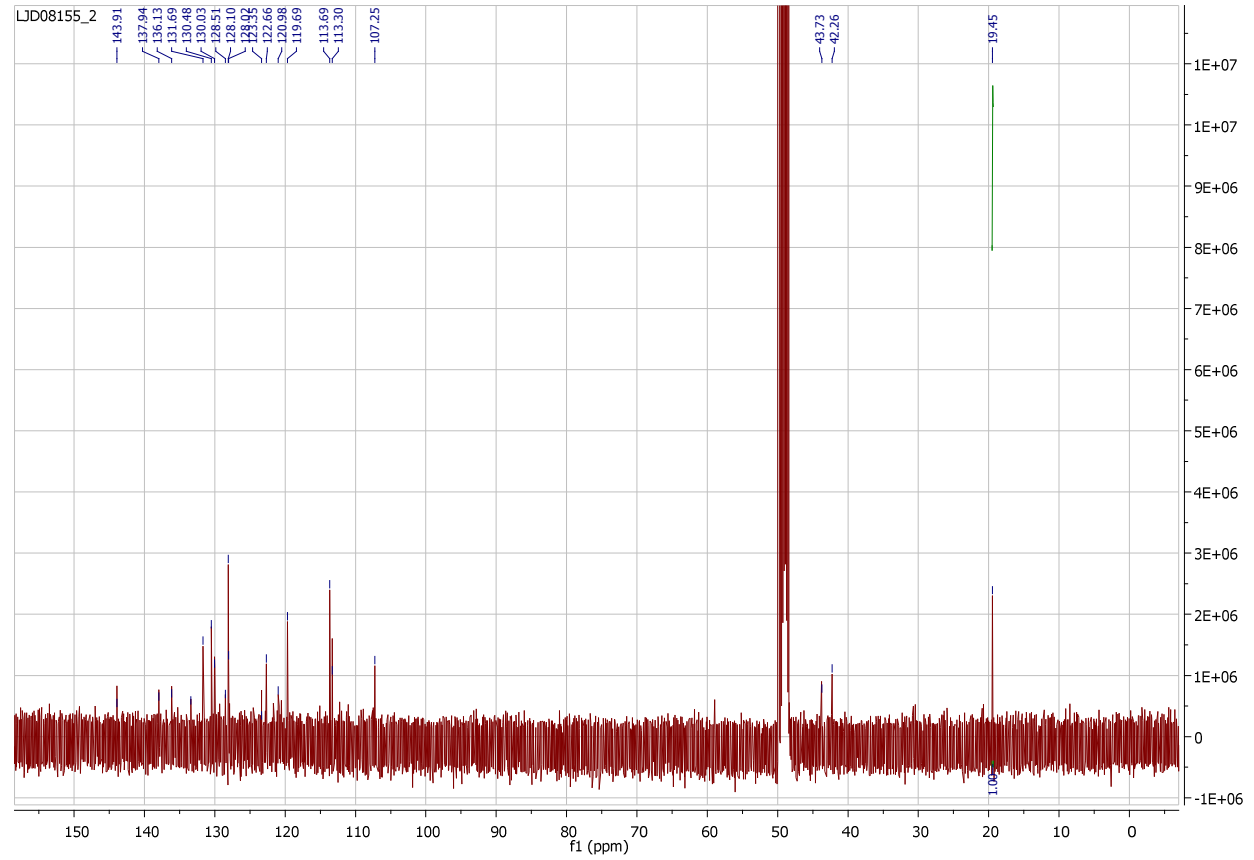

LJD08149_2
7.84
7.82
7.81
7.79
7.51
7.15
7.14
6.83
6.80
6.79
4.49
3.62
3.60
3.59
3.10
3.09
3.08
2.52
1.55
0.99
1.29
2.03
2.05
2.08
2.01
2.66
f1 (ppm)

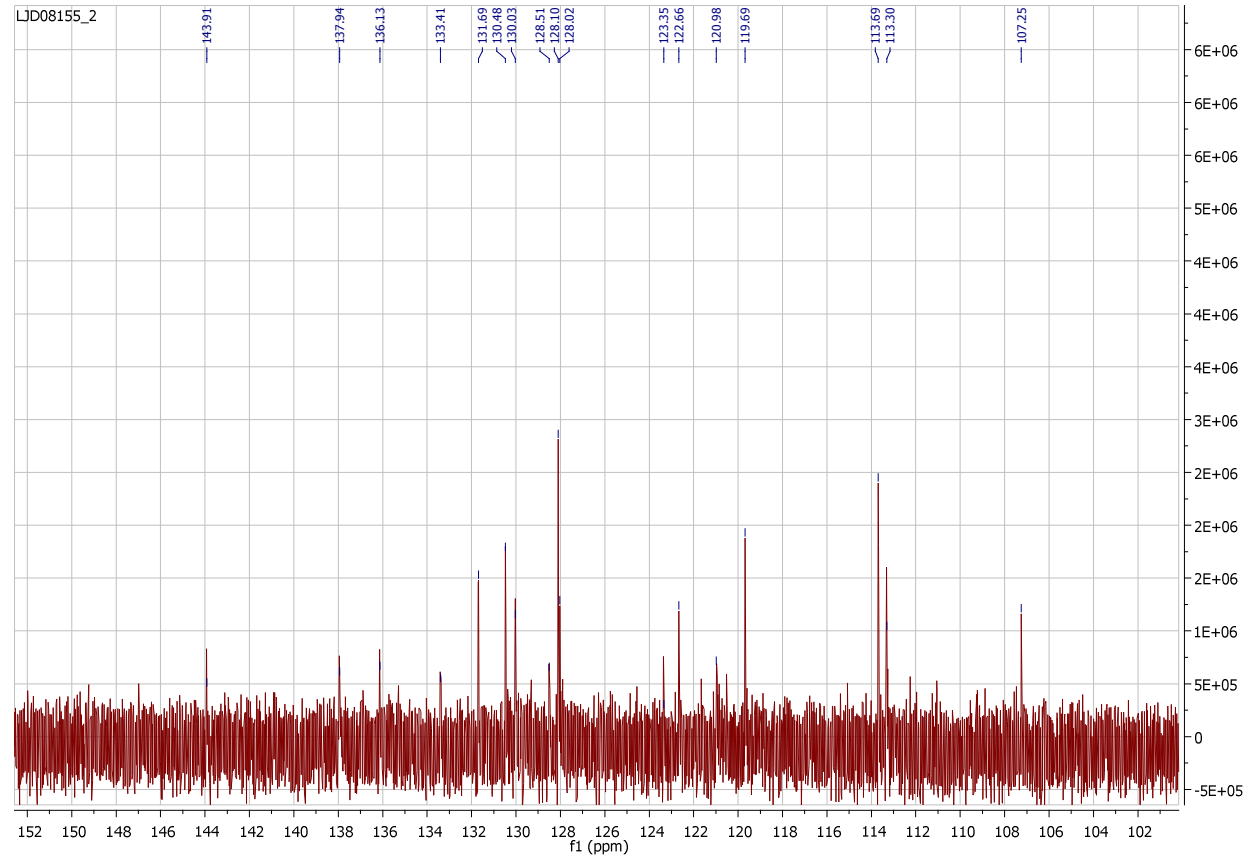

LJD08149_2
149.46
145.92
137.41
128.97
128.70
128.50
121.07
116.37
114.25
113.94
113.23
110.36
107.64
43.62
42.13
19.36
19.07
f1 (ppm)

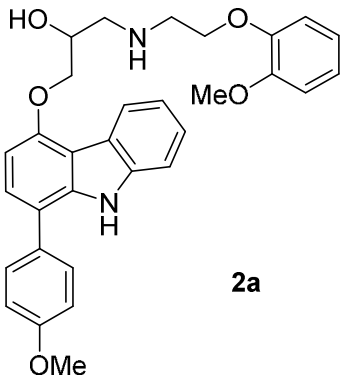

H
N
NH
N
N
H

1j

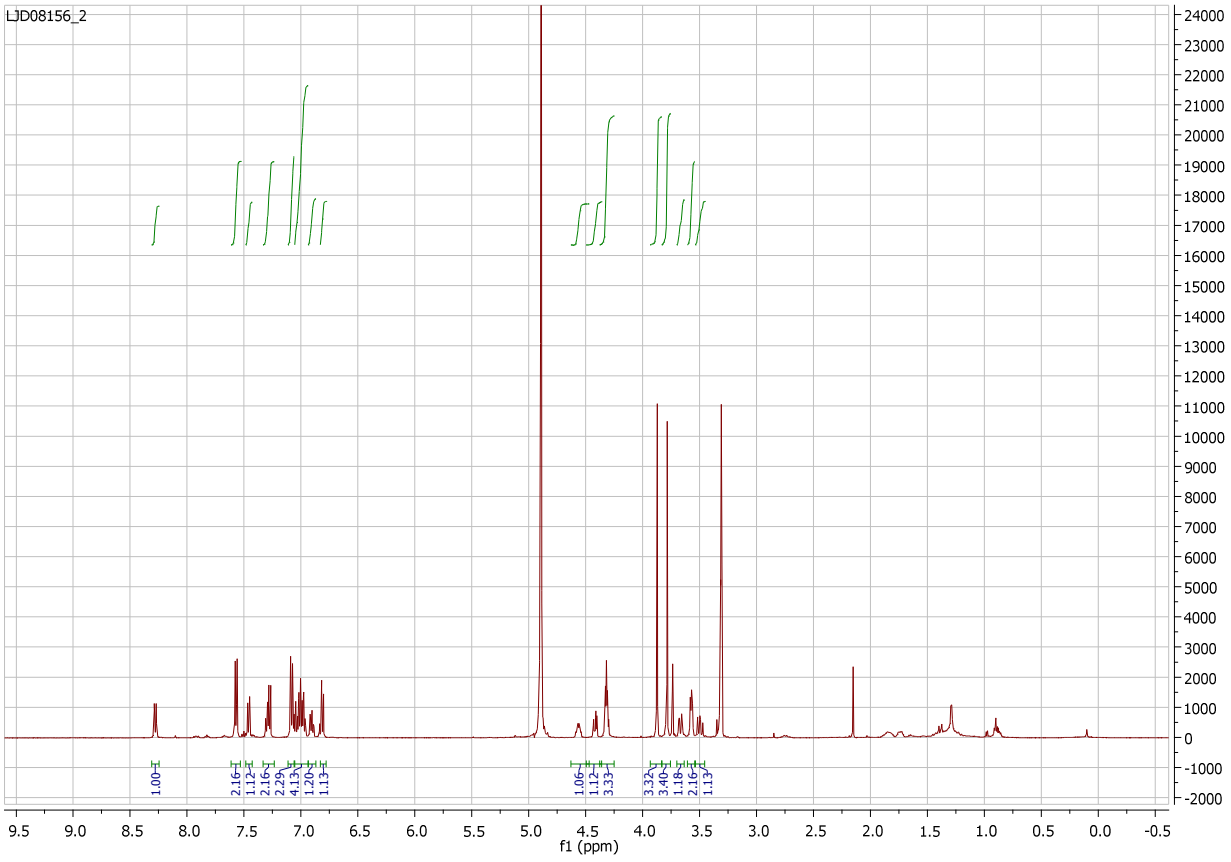

LJD08155_2
8.70
8.04
7.83
7.82
7.65
7.63
7.63
7.62
7.43
7.41
7.13
7.11
4.44
3.57
3.05
0.87
1.04
1.33
1.04
2.39
2.05
1.02
2.00
5.45
3.75
3000
2500
2000
1500
1000
500
0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

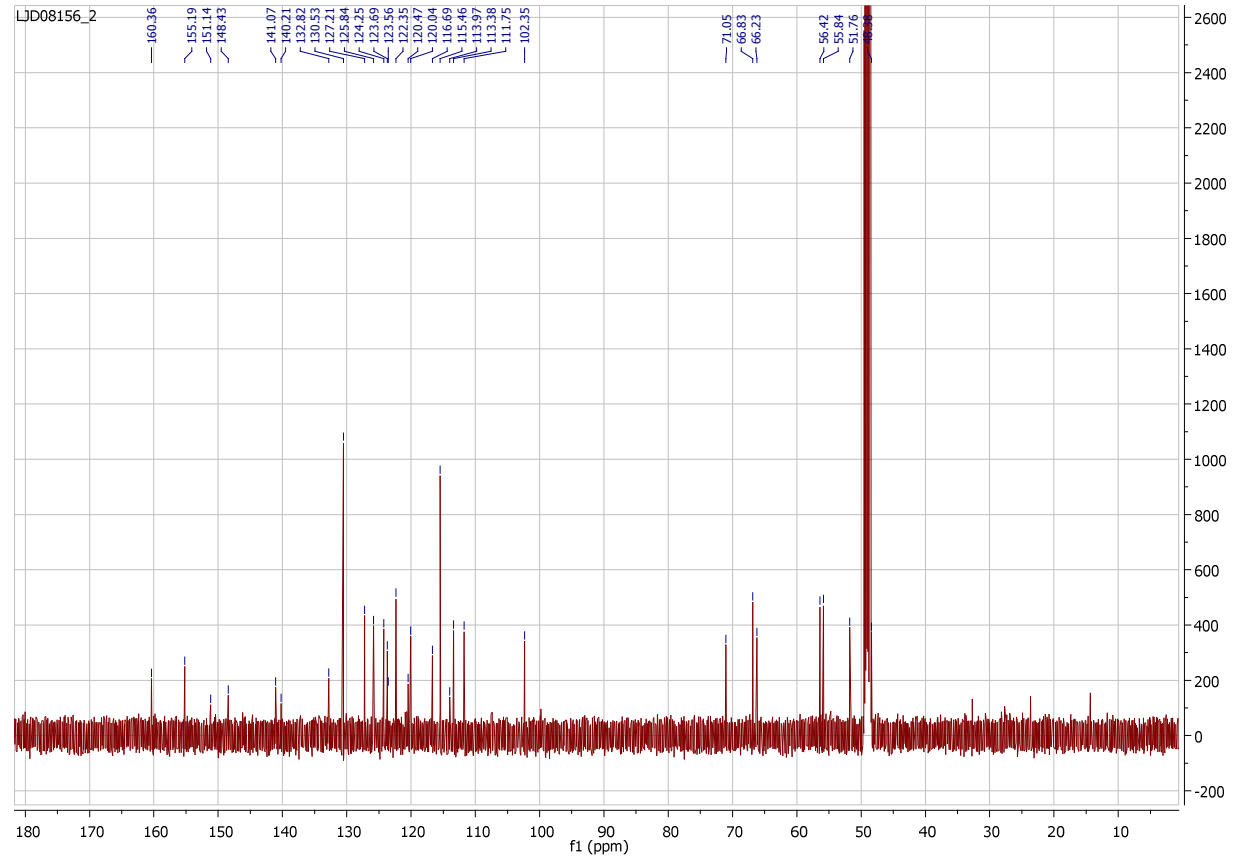

LJD08155_2
143.91
137.94
136.13
131.69
130.48
130.03
128.51
128.10
122.66
120.98
119.69
113.69
113.30
107.25
43.73
42.26
19.45
1.00
1E+07
1E+07
9E+06
8E+06
7E+06
6E+06
5E+06
4E+06
3E+06
2E+06
1E+06
0
-1E+06
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

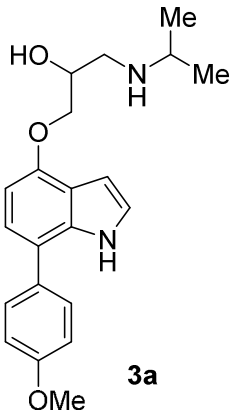

LJD08155_2
143.91
137.94
136.13
133.41
131.69
130.48
130.03
128.51
128.10
128.02
123.35
122.66
120.98
119.69
113.69
113.30
107.25
6E+06
6E+06
6E+06
5E+06
4E+06
4E+06
4E+06
3E+06
2E+06
2E+06
2E+06
1E+06
5E+05
0
-5E+05
152 150 148 146 144 142 140 138 136 134 132 130 128 126 124 122 120 118 116 114 112 110 108 106 104 102
f1 (ppm)



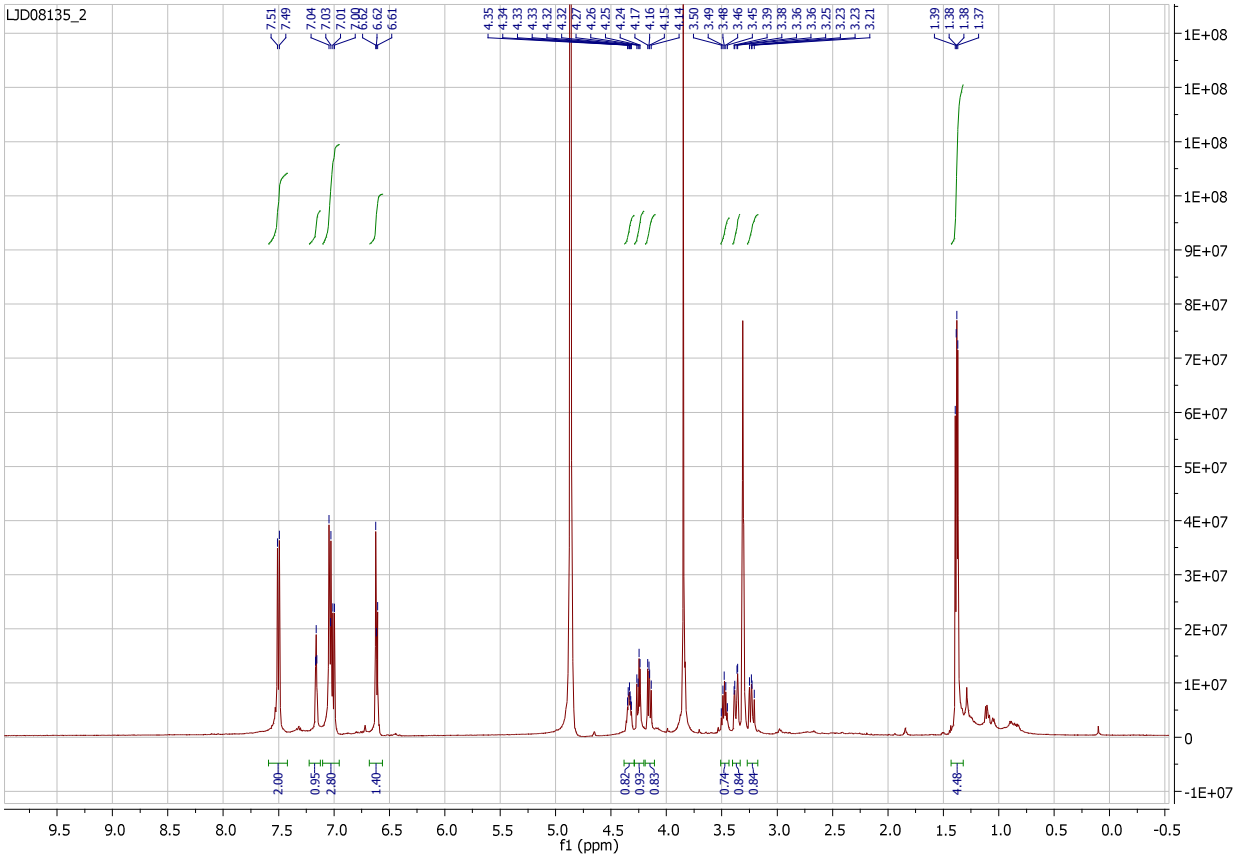
2a

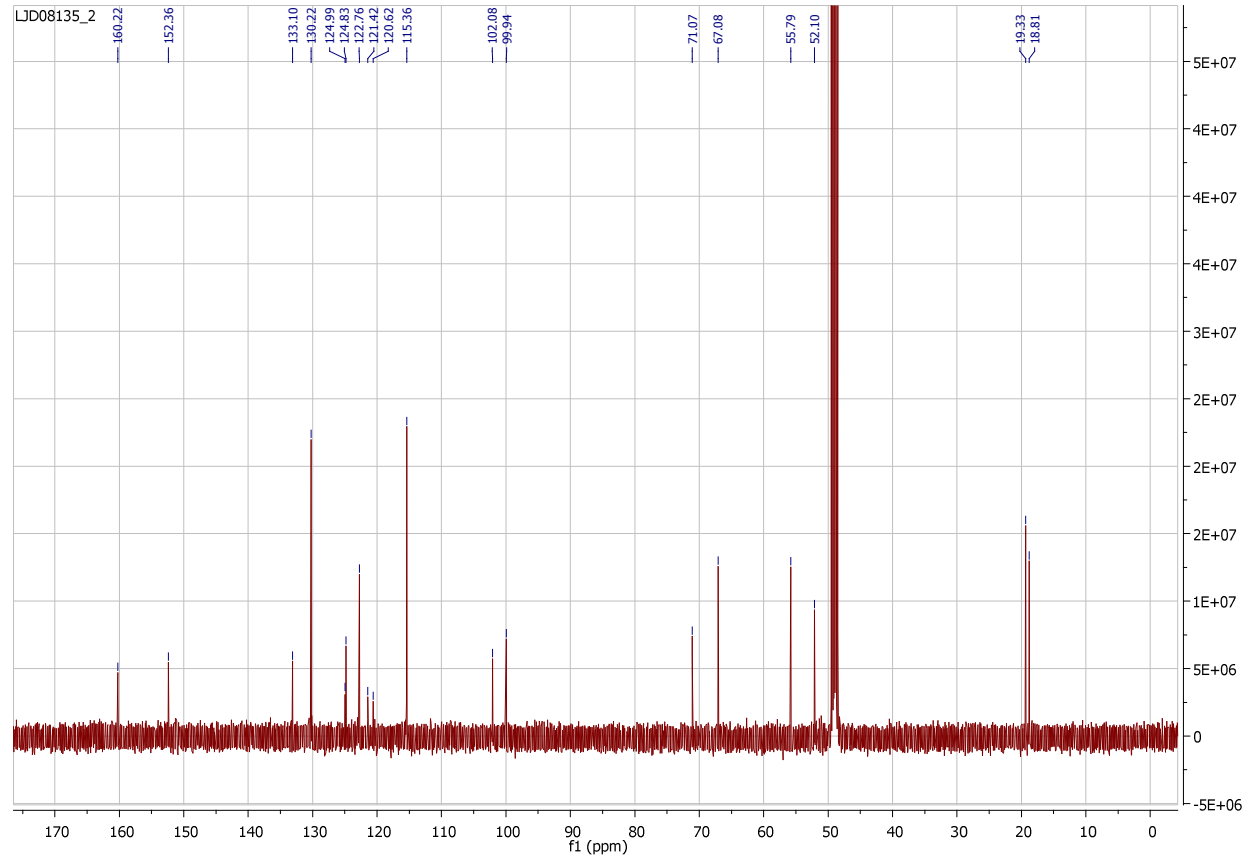


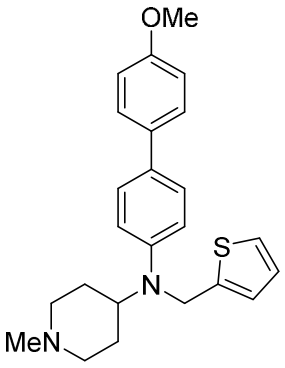

LJD08156_2
160.36
155.19
151.14
148.43
141.07
140.21
132.82
130.53
127.21
125.84
124.25
123.69
123.56
122.35
120.47
120.04
116.69
115.46
113.97
113.38
111.75
102.35
71.05
66.83
66.23
56.42
55.84
51.76
40.00
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)



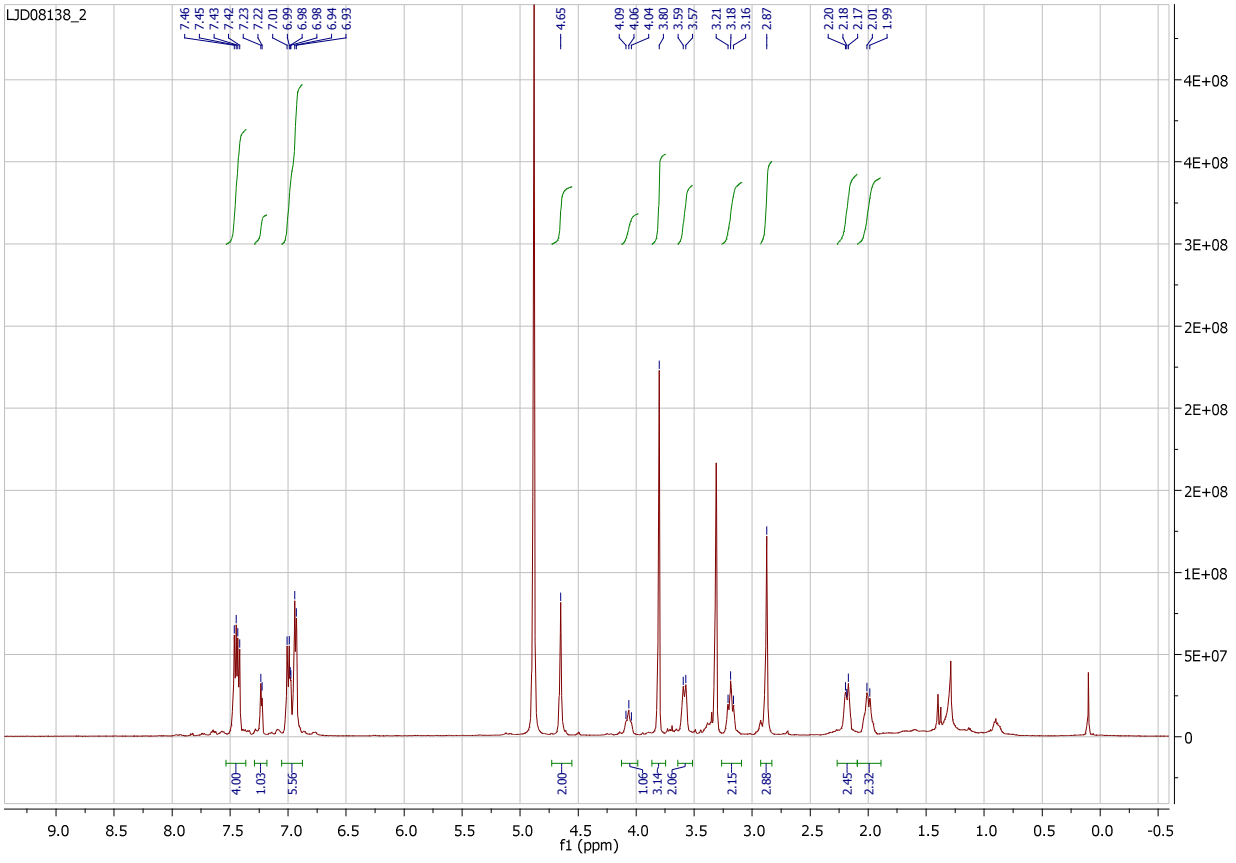
3a

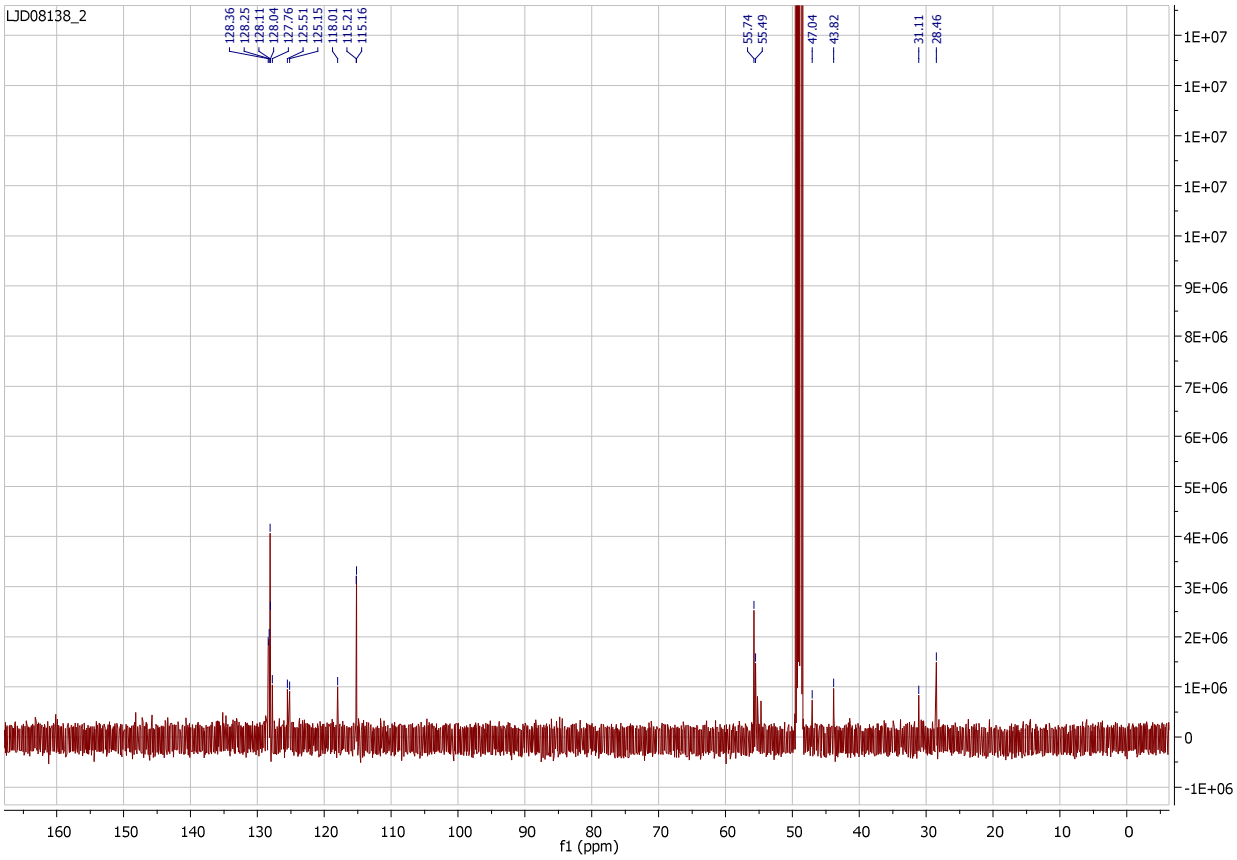


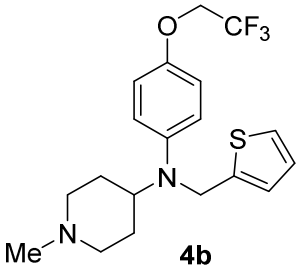

LJD08135_2
160.22
152.36
133.10
130.22
124.99
124.83
122.76
121.42
120.62
115.36
102.08
99.94
71.07
67.08
55.79
52.10
19.33
18.81
5E+07
4E+07
4E+07
4E+07
3E+07
2E+07
2E+07
2E+07
1E+07
5E+06
0
-5E+06
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


OMe
S
N
MeN

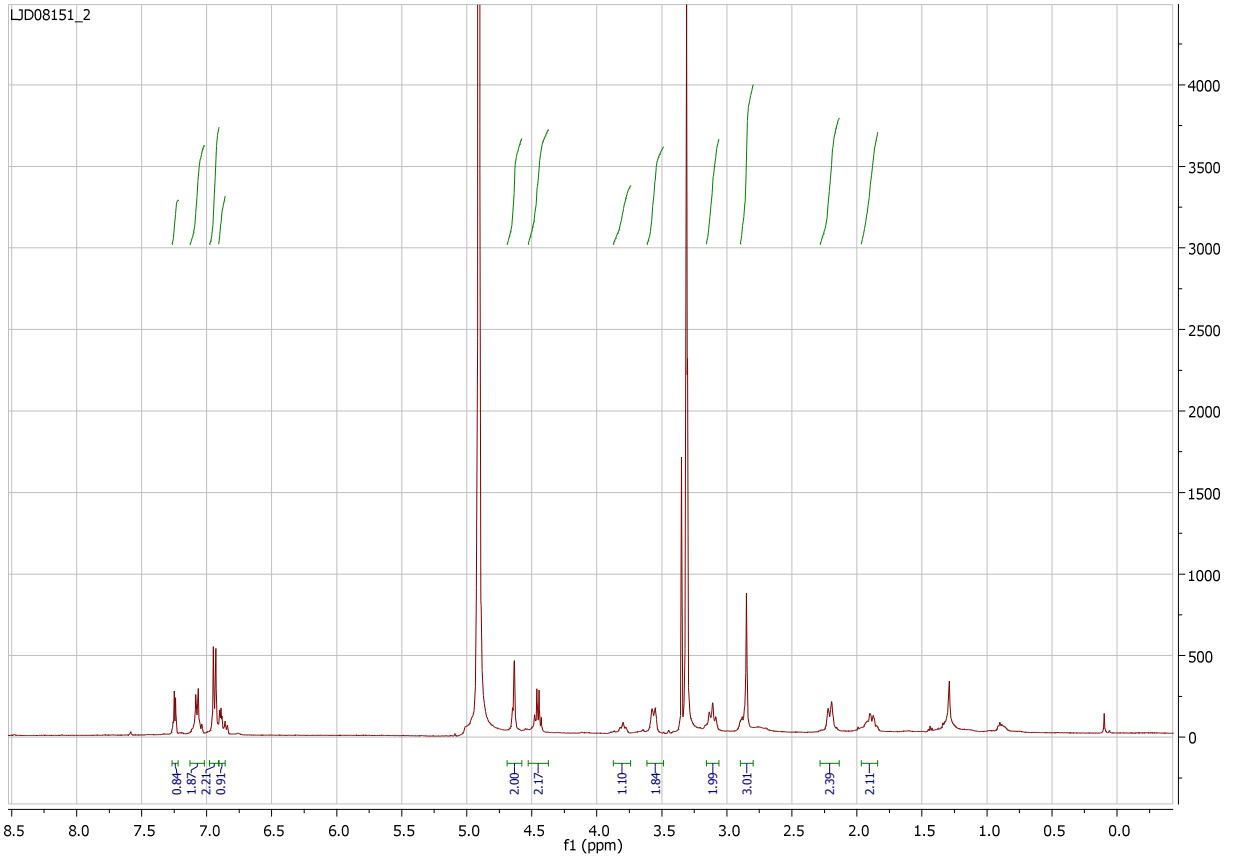
4a

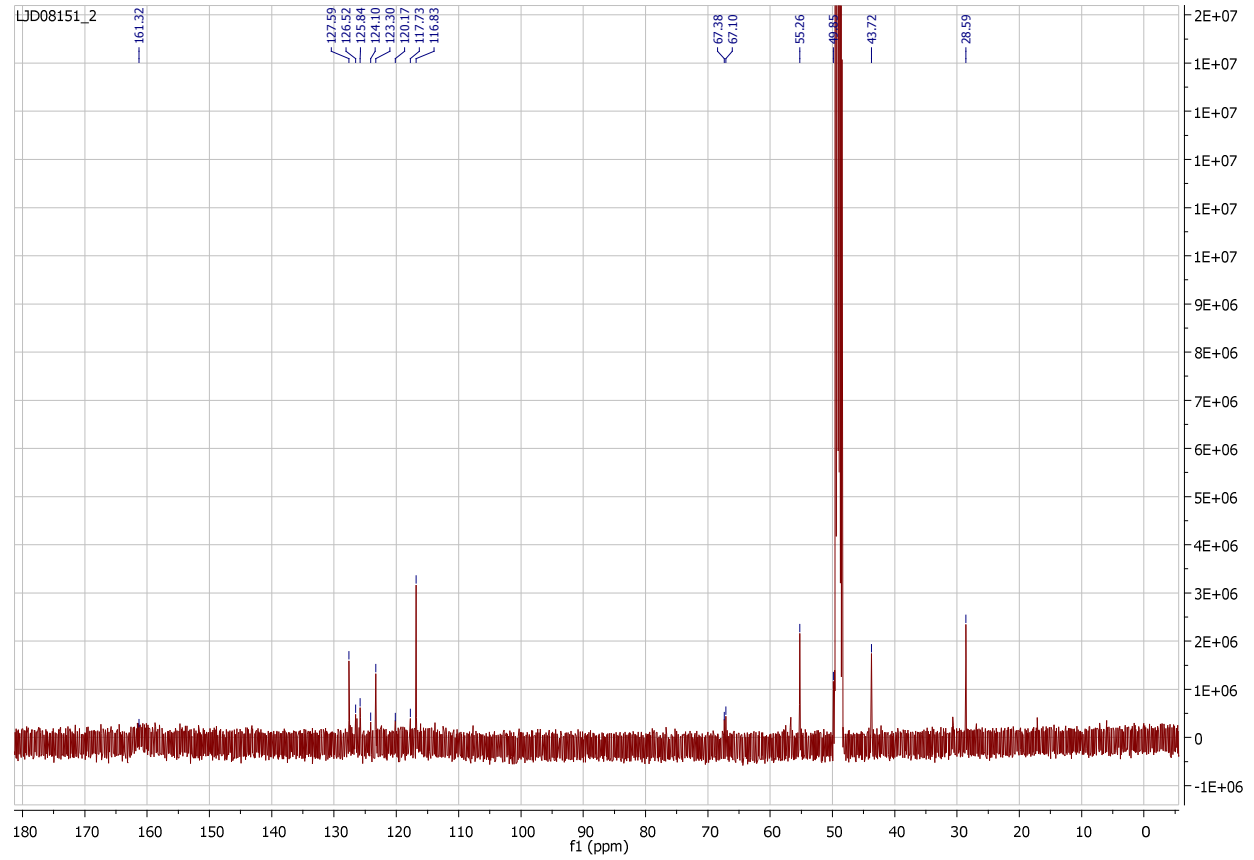

LJD08138_2
7.46
7.45
7.43
7.42
7.23
7.22
7.01
6.99
6.98
6.98
6.94
6.93
4.65
4.09
4.06
4.04
3.80
3.59
3.57
3.21
3.18
3.16
2.87
2.20
2.18
2.17
2.01
1.99
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
4.00
1.03
5.56
2.00
1.06
3.14
2.06
2.15
2.88
2.45
2.32
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

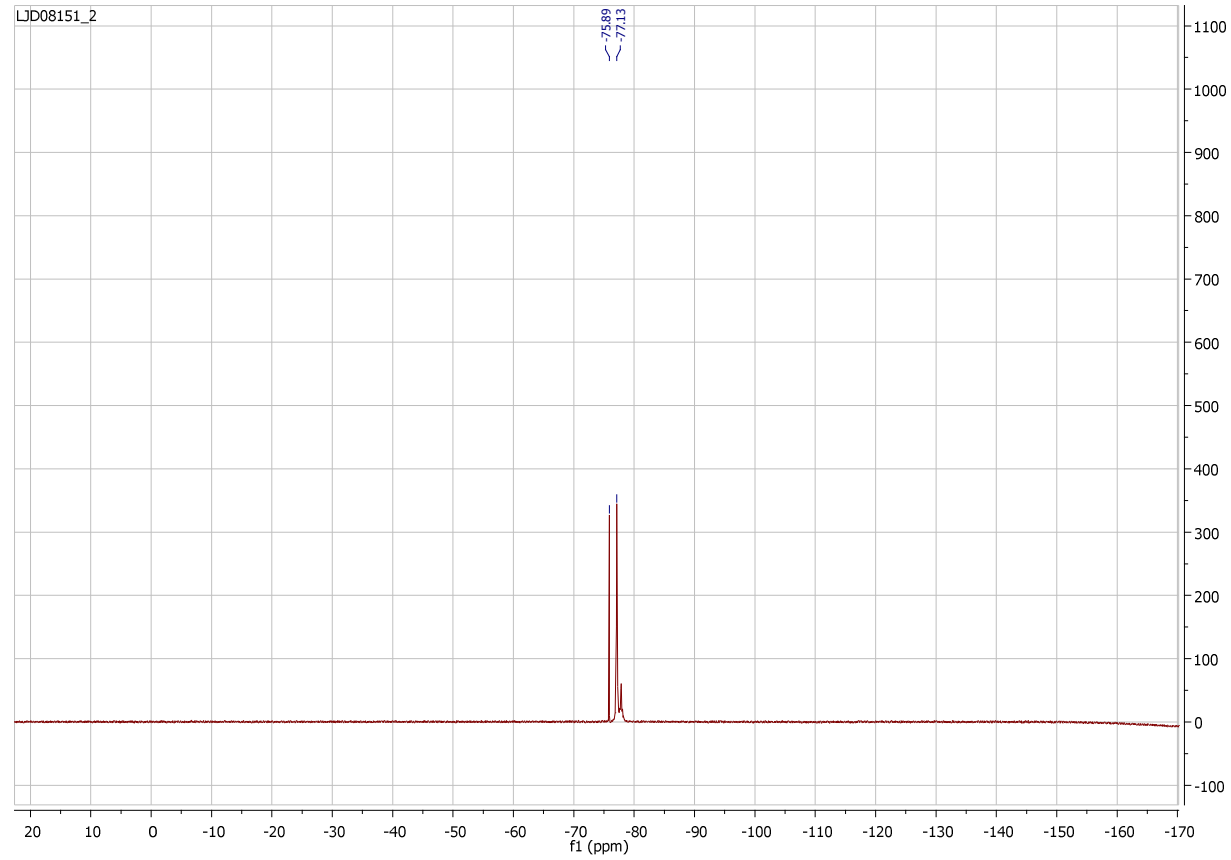

LJD08138_2
128.36
128.25
128.11
128.04
127.76
125.51
125.15
118.01
115.21
115.16
55.74
55.49
47.04
43.82
31.11
28.46
1E+07
9E+06
8E+06
7E+06
6E+06
5E+06
4E+06
3E+06
2E+06
1E+06
0
-1E+06
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


O
CF3
S
N
N
Me

4b


LJD08151_2
4000
3500
3000
2500
2000
1500
1000
500
0
0.84
1.87
2.21
0.91
2.00
2.17
1.10
1.84
1.99
3.01
2.39
2.11
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


LJD08151_2
161.32
127.59
126.52
125.84
124.10
123.30
120.17
117.73
116.83
67.38
67.10
55.26
49.85
43.72
28.59
2E+07
1E+07
1E+07
1E+07
1E+07
1E+07
9E+06
8E+06
7E+06
6E+06
5E+06
4E+06
3E+06
2E+06
1E+06
0
-1E+06
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


LJD08151_2
-75.89
-77.13
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
f1 (ppm)

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