Supporting Information

An Anthrone-Based Kv7.2 Channel Blocker with Improved Properties for the Potential Treatment of Psychiatric and Neurodegenerative Disorders

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1. Assay Protocols

a) Electrophysiology assay protocol

Electrophysiological patch-clamp recordings were all performed at room temperature (~21–23 ºC) using an Axopatch 200B amplifier coupled with an Axon Digidata 1550B data acquisition board (Molecular Devices Electrophysiology). Voltage responses were recorded using the whole-cell patch-clamp configuration in voltage clamp mode. To isolate KCNQ2/3 tail currents, voltage was held at –60 mV, before stepping to –20 mV for 500 ms, then returning to –60 mV. Tail currents were normalized to the initial values, and maximal channel block was determined by adding XE991 (20 μM) to the patch (taupe shaded boxes in Figure 2). KCNQ2/3 tail currents were quantified as the current 5 ms after step back to –60 mV, relative to the current at the end of the pulse. Patch pipettes had a resistance of 2 – 6 MΩ. Currents were sampled at 2 KHz with cell capacitance cancelled out and series resistances of < 10 MΩ compensated by 60%. The bath solution (Ringer's solution) contained 150 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 8 mM glucose, adjusted to pH 7.4 with NaOH. The internal solution used to fill the patch pipettes contained 175 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, 0.1 mM K_4BAPTA, 3 mM Na_2ATP, and 0.1 mM Na_3GTP, adjusted to pH 7.2 with KOH. The bath solution was perfused at 2 mL/minute, permitting solution exchange surrounding the recording cell with a time constant of 4 s.

b) Thallium flux assay protocol

For thallium flux assays of KCNQ2 and hERG, HEK-293 cells stably expressing either KCNQ2 or hERG were seeded in 384-well PureCoat amine plates at a density of 20,000 cells/well in 20 μL α-MEM containing 10% (v/v) Thermo fetal bovine serum and Corning glutagro. Cells were incubated overnight in a humidified 5% (v/v) CO_2 incubator at 37 ºC. On the day of the assay, test compounds were serially diluted over 10, 3-fold steps in DMSO and then further diluted to 2-fold over their final assay concentration (1:500) in Hanks Balanced Salt Solution, 20 mM HEPES pH 7.3. For loading of the thallium-sensitive fluorescent dye, the cell culture medium in the cell plate was replaced with 20 μL/well of a solution containing Hanks Balanced Salt Solution, 20 mM HEPES pH 7.3, 0.03% (w/v) Pluronic F-127, 0.5% (v/v DMSO) and 1.3 μg/mL ION Biosciences Thallos AM. After a 1 hour incubation at 22 ºC, the dye-loaded cell plate was transferred to a WaveFront Panoptic kinetic imaging plate reader. Ten seconds of baseline data were collected at 1 Hz followed by addition 20 μL/well of test compounds prepared as described above. After 5 minutes of data collection at 1 Hz, 10 μL/well of a solution containing 142 mM potassium gluconate, 22.5 mM Tl_2SO_4, 1.3 mM $CaSO_4$, 0.9 mM $MgSO_4$, 5 mM glucose, and 20 mM HEPES pH 7.3 was added followed by an additional 2 minutes of data collection at 1 Hz. After data collection, fluorescence values were normalized (F/F_0) on a per well basis. Following normalization, values from 16 vehicle control wells were averaged and the resultant vehicle control wave was subtracted from each of the wells on the plate. Next, the initial slopes of the change in fluorescence were calculated for each of the wells. Potency values were obtained from fits of the slope values to a four-parameter logistic equation using IDBS XLfit add-in with Microsoft Excel. All data were obtained from triplicate wells on each day of assay and reported potency values are the average of three independent experiments.

c) Acid stability assay protocol

Compounds DMP-543 (**3**), **6**, and JDP-107 (**18**) were added to separate 20 mL reaction vials. 1:1 (v/v) CH_3CN/0.2N aq. HCl was added to dilute the reactions to 3.0 mM. As an internal standard, furan-2-carboxylic acid (1.0 eq.) was added to each vial, and the reactions were placed in an oil bath heated at 60 °C for 36 h. At the desired time points, the reactions were directly analyzed by LC-MS. Degradation was measured using UV peak area of the original compounds at 254 nm, in comparison to furan-2-carboxylic acid as an internal standard.

d) Kinetic solubility assay protocol

Calibration curves were generated by dissolving compounds in a range of 100 μM to 3.2 μM in 40% ethanol, 59% water and 1 % DMSO. This was accomplished by a serial dilution of compounds in DMSO and the addition of 3 μL of compound solution in DMSO to 297 μL of 40% ethanol. 3 x 80 μL of each solution was dispensed into a UV-star 384 well plate (Greiner 781801), and the absorbance spectra from 230 nm to 450 nm was recorded for each well. Next, 3 μL of a 10 mM compound solution in DMSO was added to 297 μL of water and stirring was continued for 18 h. The mixture was centrifuged at 10,000 rcf for 10 min., and the 180 uL of supernatant was combined with 120 μL of ethanol. 3 x 80 μL of each solution was dispensed into a UV-star 384 well plate (Greiner 781801) and the absorbance spectra from 230 nm to 450 nm was

recorded for each well. Based on the data for each compound, a wavelength was identified that resulted in an absorbance reading between 0.3 and 0.6. The calibration curve for this wavelength was used to determine the compound concentration using a dilution factor of 1.67. We used 280 nm for DMP 543, 266 nm for **6**, and 280 nm for JDP-107.

e) CYP450 assay protocol

Compounds were investigated with a Vivid CYP3A4 Green screening assay (Life Technologies, Carlsbad, CA). Briefly, a master solution was prepared using 273 µL PBS (pH 7.2), 18 µL regeneration solution, and 9 µL CYP3A4 enzyme solution. The P450 enzyme substrate solution contained 89 µL PBS, 1 µL substrate (Vivid DBOMF), and 10 µL NADP+ solution. The compound solution was prepared by diluting a 20 mM solution of compound in DMSO with PBS to achieve 25 µM solution. Using a black 384 well plate (NUNC 262260), 25 µL of master solution was combined with 5 µL of substrate solution and 20 µL of compound solution. The final concentrations were: 5 nM CYP3A4, 2 µM DBOMF substrate, 100 µM NADP+ and 10 µM compound. Positive control compound was ketoconazole at 10 µM. Each compound was investigated in four separate wells with identical conditions. Fluorescence readings at 490 nm excitation and 520 nm emission were carried out each minute for 6 minutes. The data was analyzed with GraphPad Prism by linear regression and slopes were determined. The % inhibition = (1- ((slope of compound – slope of ketoconazole)/(slope of vehicle - slope of ketoconazole)))*100.

f) Liver microsome stability assay protocol

In three separate vials, 4 µL of 1 mM test compound (at a final concentration of 10 µM) in DMSO was pre-incubated at 37 °C for 5 min. on a digital heating/shaking dry bath (Fisher Scientific) in a mixture containing 282 µL of water, 80 µL of phosphate buffer (0.5 M, pH 7.4), 20 µL of NADPH Regenerating System Solution A (BD Bioscience, San Jose, CA) and 4 µL of NADPH Regenerating System Solution B (BD Bioscience, San Jose, CA) in a total volume of 391.2 µl. Following preincubation, the reaction was initiated by addition of 8.8 µL of either human liver microsomes (BD Gentest, San Jose, CA) or mouse liver microsomes (Life technologies, Rockford, IL), at a protein concentration of 0.5 mg/mL. Three aliquots of 50 µL were taken from each vial after one hour and quenched with 100 µL of cold acetonitrile solution containing 3 µM 4,5-diphenylimidazole as internal standard. Each solution was sonicated for 10 s, and centrifuged at 10,000 rpm for 5 min. 100 µL of each supernatant was transferred into Spin-X HPLC filter tubes (Corning Incorporated, NY) and centrifuged at 13,000 rpm for 5 min. The filtrates were diluted 100-fold and subsequently analyzed by LC-MS/MS with a Shimadzu LCMS 8040, (Shimadzu Scientific Instruments, Columbia, MD). The ratio of the peak areas of the internal standard and test compound was calculated for each sample and the percent remaining after 1 h was determined.

g) Cytotoxicity assay protocol

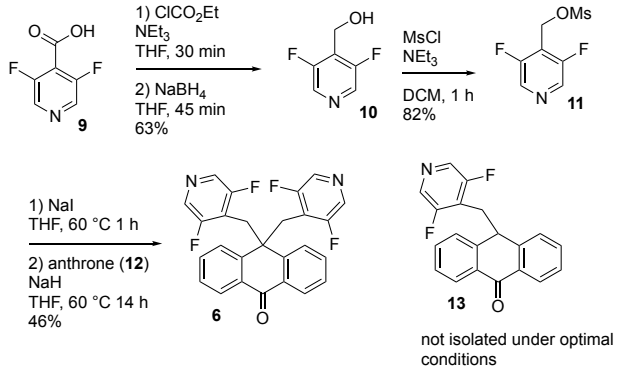
HEPG2 and HEK293T cells were cultured at 37 °C and 5% CO_2 in DMEM/High glucose with L-glutamine and sodium pyruvate (Hyclone, SH3024301), supplemented with non-essential amino acids (Hyclone, #SH30238.01), 10 mM HEPES (Hyclone, #SH302237.01), 5 x 10^6 units of penicillin, streptomycin (Hyclone, #SV30010), and 10% of heat inactivated fetal bovine serum (Gibco, #10082147). HEK293T culture flask and assay plates were coated with Matrigel (BD Bioscience, #354234) prior to cell culture. 1.5×10^4 cells per well were plated in 384-well white optical bottom plates (Corning 3765) and incubated for 4 h before the addition of 100 nL of compound at different concentrations in DMSO using a Freedom EVO liquid handling system (Tecan, Maennedorf, Switzerland) equipped with a pin tool (V&P Scientific, San Diego, CA). Assay controls were vehicle and 200 µM 3-dibutylamino-1-(4-hexyl-phenyl)-propan-1-one. Assay plates were incubated for 18 h, followed by the addition of 25 µL CellTiter-Glo™ reagent (Promega, Madison, WI) and incubation for 30 min. before reading luminescence. Four independent experiments were performed in quadruplicate and CC_{50}s were determined using GraphPad Prism with nonlinear regression with variable slope.

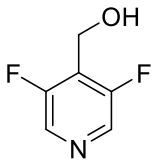
2. Synthetic protocols

General Information

All reagents and solvents were purchased from commercial vendors and used as received. NMR spectra were recorded on Varian 300 MHz or 400 MHz spectrometers as indicated. Proton and carbon chemical shifts are reported in parts per million (ppm; δ) relative to tetramethylsilane, $CDCl_3$ solvent, or CD_3OD (^1H δ 0, ^{13}C δ 77.16, or ^{13}C δ 49.00, respectively). NMR data are reported as follows: chemical shifts, multiplicity (obs = obscured, app = apparent, br = broad, s = singlet, d = doublet, t = triplet, q = quartet, sxt = sextet, m = multiplet, comp = complex overlapping signals); coupling constant(s) in Hz; integration. Unless otherwise indicated, NMR data were collected at 25 °C. Flash chromatography was performed using Biotage SNAP cartridges filled with 40–60 μm silica gel, or C18 reverse phase columns (Biotage® SNAP Ultra C18 or Isco Redisep® Gold C18Aq) on Biotage Isolera systems, with photodiode array UV detectors. Analytical thin layer chromatography (TLC) was performed on Agela Technologies 0.25 mm glass plates with 0.25 mm silica gel. Visualization was accomplished with UV light (254 nm) and aqueous potassium permanganate ($KMnO_4$) stain followed by heating, unless otherwise noted. Tandem liquid chromatography/mass spectrometry (LC-MS) was performed on a Shimadzu LCMS-2020 with autosampler, photodiode array detector, and single-quadrupole MS with ESI and APCI dual ionization, using a Peak Scientific nitrogen generator. Unless otherwise noted, a standard LC-MS method was used to analyze reactions and reaction products: Phenomenex Gemini C18 column (100 x 4.6 mm, 3 μm particle size, 110 A pore size); column temperature 40 °C; 5 μL of sample in MeOH or CH_3CN at a nominal concentration of 1 mg/mL was injected, and peaks were eluted with a gradient of 25−95% CH_3CN/H_2O (both with 0.1% formic acid) over 5 min., then 95% CH_3CN/H_2O for 2 min. Purity was measured by UV absorbance at 210 or 254 nm. High-resolution mass spectra were obtained at the University of Wisconsin-Milwaukee Mass Spectrometry Laboratory with a Shimadzu LCMS-IT-TOF with ESI and APCI ionization or from the University of Cincinnati Environmental Analysis Service Center with an Agilent 6540 LCMS with accurate mass Q-TOF. IR spectra were obtained as a thin film on NaCl or KBr plates using a Thermo Scientific Nicolet iS5 spectrometer.

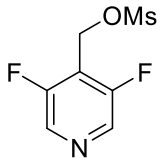
Scheme 1. Synthesis of 3,5-difluoropyridine analog **6**



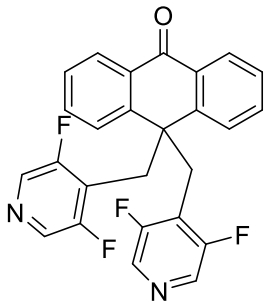


(3,5-difluoropyridin-4-yl)methanol (10). 3,5-Difluoropyridine-4-carboxylic acid (0.510 g, 3.14 mmol) was added to an oven dried 250 mL round bottom flask. The flask was sealed with a septum, purged with nitrogen, and anhydrous THF (50 mL) was added. Next, triethylamine (0.542 mL, 3.77 mmol) was added via syringe. The solution was sonicated until it became homogeneous, then ethyl chloroformate (0.372 mL, 3.77 mmol) was added. The reaction was stirred for 30 min. before being filtered through a short pad of Celite® into a separate oven dried 250 mL round bottom flask. $NaBH_4$ (0.261 g, 6.91 mmol) was added followed by MeOH (5 mL). The reaction was stirred for 45 min. at room temperature. A sample aliquot was taken from the reaction, dissolved in HPLC grade MeCN (1 mL), and analyzed with LC-MS to confirm the completion of the reaction. The reaction was quenched with sat. ammonium chloride and diluted with EtOAc (150 mL). The organic layer was washed with DI water (50 mL) and brine, then dried over sodium sulfate and condensed to give a

yellow oil. The oil was dissolved in DCM and purified by flash chromatography (5 g SiO_2 cartridge, 0–12% MeOH/DCM gradient) to give the title compound as a clear semisolid (0.285 g, 63%). This compound has been previously reported and characterized (CAS# 924649-16-1). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 4.81 (s, 2H), 8.28 (s, 2H) 8.30 (s, 2 H).

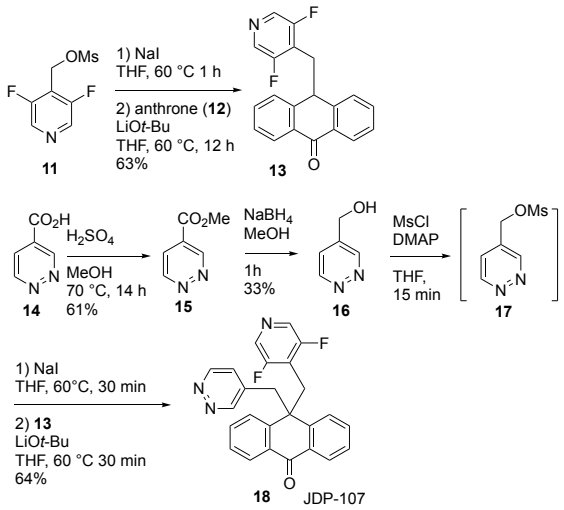


(3,5-difluoropyridin-4-yl)methyl methanesulfonate (11). Alcohol **10** (0.420 g, 2.89 mmol) was added to an oven dried 250 mL round bottom flask, followed by DCM (80 mL). Mesyl chloride (0.365 g, 3.18 mmol) was then added followed by triethylamine (0.444 mL, 3.18 mmol). The reaction stirred for 4 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was diluted with EtOAc (50 mL) and washed with water (3 x 30 mL). The aqueous layer was saturated with NaCl and extracted with EtOAc (3 x 40 mL) to give a light brown oil (0.475 g, 82%). The crude material was advanced without further purification or analysis.

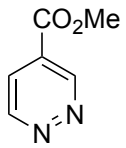


10,10-bis[(3,5-difluoropyridin-4-yl)methyl]-9,10-dihydroanthracen-9-one (6). The mesylate **11** (0.010 g, 0.045 mmol) was added to an oven dried 8 mL reaction vial followed by NaI (0.003 g, 0.023 mmol). The vial was capped and purged with nitrogen before anhydrous THF (2.0 mL) was added. The solution was placed in an oil bath and heated to 60 °C for 1 h. Anthrone (0.004 g, 0.022 mmol) was added to a separate oven dried 4 mL reaction vial, followed by NaH (0.002 g, 0.054 mmol). The vial was capped and purged with nitrogen before anhydrous THF (2.0 mL) was added. After 1 h, the resulting anthrone anion solution was added to the mesylate/iodide solution under nitrogen dropwise over 2 min at 60 °C, and the reaction was stirred for 14 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The oil was dissolved in DCM and purified by flash chromatography (5 g SiO_2 cartridge, 0–12% MeOH/DCM gradient) to give the title compound as a light brown oil (0.005 g, 46 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.90 (s, 4H) 7.45 (t, *J* = 7.6 Hz, 2H) 7.66 (t, *J* = 7.6 Hz, 2H) 7.86 (d, *J* = 7.8 Hz, 2H) 7.93 (s, 4H) 8.19 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ = 30.8, 38.7, 123.3 (t, *J*= 16.7 Hz), 127.5, 128.9, 130.1 (t, *J*=1.8 Hz), 132.5, 134.5,134.6 (dd, *J*=27.2, 3.8), 145.3, 184.3; ^{19}F NMR (376 MHz, $CDCl_3$) δ = –126.8; HRMS (ESI^+) calcd for $C_{26}H_{17}F_4N_2O$ $[M+H]^+$ 449.1272, found 449.1257.

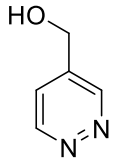
Scheme 2. Synthesis of pyridine-pyridazine JDP-107 (**18**)



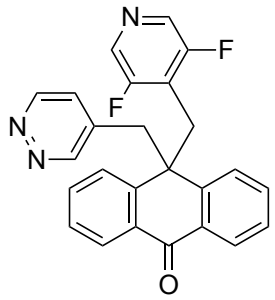
10-[(3,5-difluoropyridin-4-yl)methyl]-9,10-dihydroanthracen-9-one (13). The mesylate **11** (0.099 g, 0.445 mmol) was added to an oven dried 50 mL round bottom flask containing NaI (0.051 g, 0.334 mmol). The flask was capped with a septum and purged with nitrogen before anhydrous THF (10 mL) was added via syringe. The flask was placed in an oil bath heated to 60 °C for 1 h. Meanwhile, anthrone (0.072 g, 0.371 mmol) and Li*O*t-Bu were added to an oven dried 25 mL round bottom flask. The flask was sealed with a septum and purged with nitrogen before anhydrous THF (10 mL) was added via syringe. After 30 min., the resulting anion was added by syringe to the mesylate solution at 60 °C under nitrogen, and the reaction was stirred for 12 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction.
The reaction was quenched with brine and diluted with EtOAc (200 mL), and the aqueous layer was re-extracted with EtOAc (50 mL). The combined organic layers were washed with brine, dried over sodium sulfate, and condensed to give a brown oil. The oil was dissolved in DCM and purified by flash chromatography (5 g SiO_2 cartridge, 0–50% EtOAc/hexanes gradient) to give the title compound as a tan semisolid (0.076 g, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.13 (d, *J* = 7.0 Hz, 2H) 4.48 (t, *J* = 7.0 Hz, 1H) 7.16 - 7.24 (m, 2H) 7.50 (comp, 4 H) 8.20 (s, 2H) 8.26 (dd, *J* = 7.6, 1.4 Hz, 2H); ^{13}C NMR (75 MHz, (400 MHz, $CDCl_3$) δ = 36.1, 43.3, 122.7 (t, *J* = 16.8 Hz), 128.0, 128.2, 132.2, 133.0, 134.0 (dd, *J*=25.3, 3.5 Hz), 143.2,156.5 (d, *J* = 3.46 Hz), 159.9 (d, *J* = 3.46 Hz,) 184.4; ^{19}F NMR (376 MHz, $CDCl_3$) δ = –125.2; HRMS (ESI$^+$) calcd for $C_{20}H_{14}F_2NO$ $[M+H]^+$ 322.1038, found 322.1045.



methyl pyridazine-4-carboxylate (15). Pyridazine-4-carboxylic acid 0.470 g, 3.79 mmol) was added to an oven dried 15 mL pressure flask, followed by anhydrous methanol (10 mL) and concentrated sulfuric acid (0.244 mL, 4.36 mmol). The flask was sealed and heated at 70 °C for 14 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was diluted with EtOAc (150 mL), then washed with half sat. aq. sodium bicarbonate solution (15 mL). The aqueous layer was then re-extracted with EtOAc (50 mL). The combined organic layers were washed with brine, dried over sodium sulfate, and condensed to give the desired product as an off-white semi-solid (0.262 g, 52%). The crude product was advanced without further purification. This compound has been previously reported and characterized (CAS# 34231-77-1). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 3.98 (s, 3H), 7.95 (dd, *J* = 5.1, 2.2 Hz, 1H), 9.40 (d, *J* = 5.0 Hz, 1H), 9.63 (s, 1H)

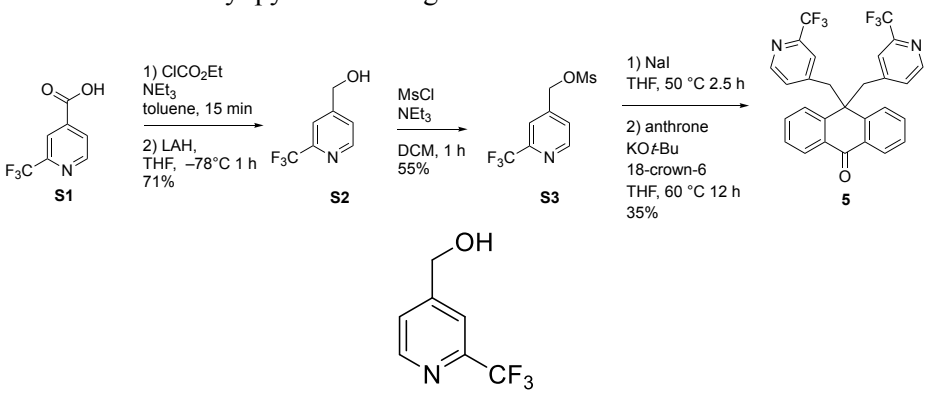


(pyridazine-4-yl)methanol (16). The methyl ester **15** (0.133 g, 0.963 mmol) was added to an oven dried 20 mL reaction vial followed by methanol (12 mL). Sodium borohydride (0.073 g, 1.93 mmol) was added and the reaction stirred for 1 h. A sample aliquot was taken from the reaction, quenched with sat. ammonium chloride, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was quenched with sat. ammonium chloride and condensed to dryness to give a white solid. After condensing, DCM (100 mL) was added to the flask and the white solids were broken up and sonicated for 10 min, then vacuum filtered through a paper filter. The mother liquor was concentrated to give a yellow oil. The oil was dissolved with DCM and purified by flash chromatography (5 g SiO_2 cartridge; 0–12% MeOH/DCM gradient) to give the title compound as a yellow oil (0.035 g, 33%). This compound has been previously reported and characterized (CAS# 50901-43-4). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 4.85 (s, 2H), 7.60 (dd, J = 5.3, 2.4 Hz, 1H), 9.06 (d, J = 5.3 Hz, 1H), 9.15 (s, 1H).

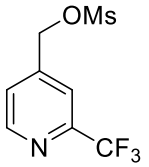


10-[(3,5-difluoropyridin-4-yl)methyl]-10-[(pyridazin-4-yl)methyl]-9,10-hydroanthracen-9-one (18). The alcohol **16** (0.015 g, 0.136 mmol) was added to an oven dried 20 mL reaction vial followed by DMAP (0.013 g, 0.102 mmol). The vial was sealed with a septum cap and anhydrous THF (5.0 mL) was added via syringe followed by NEt_3 (0.019 mL, 0.136 mmol) and MsCl (0.011 mL, 0.059 mmol). The reaction was stirred for 15 min. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The mesylate solution was filtered through a syringe filter (0.22 µm PTFE) under nitrogen into a capped, nitrogen flushed and oven dried 20 mL reaction vial containing NaI (0.010 g 0.065 mmol). The reaction was stirred at 60 °C for 30 min. Meanwhile, the monoalkylated anthrone **13** (0.028 g, 0.087 mmol) and Li*O*t-Bu (0.011 g, 0.131 mmol) were added to a separate oven dried 20 mL reaction vial. The vial was capped and purged with nitrogen before anhydrous THF (5.0 mL) was added via syringe. The solution was sonicated for 2 min. until homogeneous, then kept at room temperature for 30 min. The resulting anion solution was added to the mesylate/iodide solution at 60 °C via syringe over 2 min. The reaction was stirred for 30 min., then a sample aliquot was taken and dissolved in HPLC grade MeCN (1 mL) and analyzed with LC-MS to confirm the completion of the reaction. The reaction was quenched with brine, then diluted with EtOAc (150 mL). The organic layer was washed with brine, dried over sodium sulfate, and condensed to give a brown oil. The oil was dissolved in DCM and purified by flash chromatography (5 g SiO_2 cartridge, 0–12% MeOH/DCM gradient) to give the title compound as a light brown oil (0.023 g, 64 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 3.70 (s, 2H), 3.92 (s, 2H), 6.34 (dd, J = 5.6, 2.4 Hz, 1H), 7.50 (t, J = 7.5 Hz, 2 H), 7.75 (t, J = 7.6 Hz, 2H), 7.84 - 7.92 (m, 2H), 7.98 (s, 2H), 8.20 (dd, J = 7.9, 0.6 Hz, 2H), 8.41 (s, 1H) 8.59 (d, J = 5.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 39.3, 44.5, 47.2, 120.5 (t, J = 16.7 Hz) 126.5, 127.3 (t, J = 1.73 Hz) 128.3, 128.6, 132.1, 133.8, 134.2 (dd, J = 25.5, 4.0 Hz), 135.9, 142.7, 150.5, 152.9, 156.0, 159.5, 182.1; ^{19}F NMR (376 MHz, $CDCl_3$) δ = –126.0; IR (film) 1662, 1601, 1421, 1284, 1060, 695 cm^{-1}; HRMS (ESI$^+$) calcd. for $C_{25}H_{18}F_2N_3O$ $[M+H]^+$ 414.1412, found 414.1423.

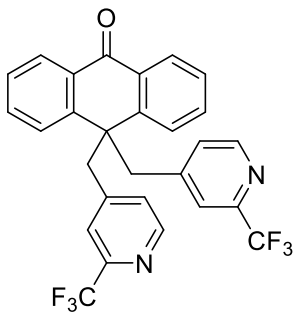
Scheme S1. Synthesis of 2-trifluoromethyl pyridine analog **5**



[2-(trifluoromethyl)pyridin-4-yl]methanol (S2). 2-(Trifluoromethyl)pyridine-4-carboxylic acid (0.206 g, 1.04 mmol) was added to an oven dried, nitrogen purged 4 mL vial containing anhydrous toluene (6.0 mL). Triethylamine (0.152 mL, 1.09 mmol) was added as a solution in anhydrous toluene (6.0 mL). Next, ethyl chloroformate (0.104 mL, 1.088 mmol) was added via syringe and the reaction stirred for 15 min. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the formation of the mixed anhydride. The solution was filtered through syringe filter (0.22 µm PVDF filter) and condensed to an oil. The oil was dissolved in anhydrous THF (6.0 mL) under nitrogen. This solution was added dropwise to a 20 mL reaction vial containing LAH (0.043 g, 1.09 mmol) in anhydrous THF (4.0 mL) cooled to –78 °C using a dry ice in acetone bath, then stirred for 1 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was warmed to 0 °C and quenched with sat. sodium sulfate solution (2 mL). The solution was filtered through a pad of Celite® and condensed to give a yellow oil. The oil was dissolved in DCM and purified by flash chromatography (5 g SiO_2 cartridge; 0–100% EtOAc/hexanes gradient) to yield the title compound as a colorless oil (0.132 g, 71%). This compound has been previously reported and characterized (CAS# 131747-61-0) ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.66 (br. s, 1H), 4.85 (s, 2H), 7.50 (d, *J*=5.1 Hz, 1H), 7.72 (s, 1 H), 8.67 (d, *J* = 5.1 Hz, 1H).

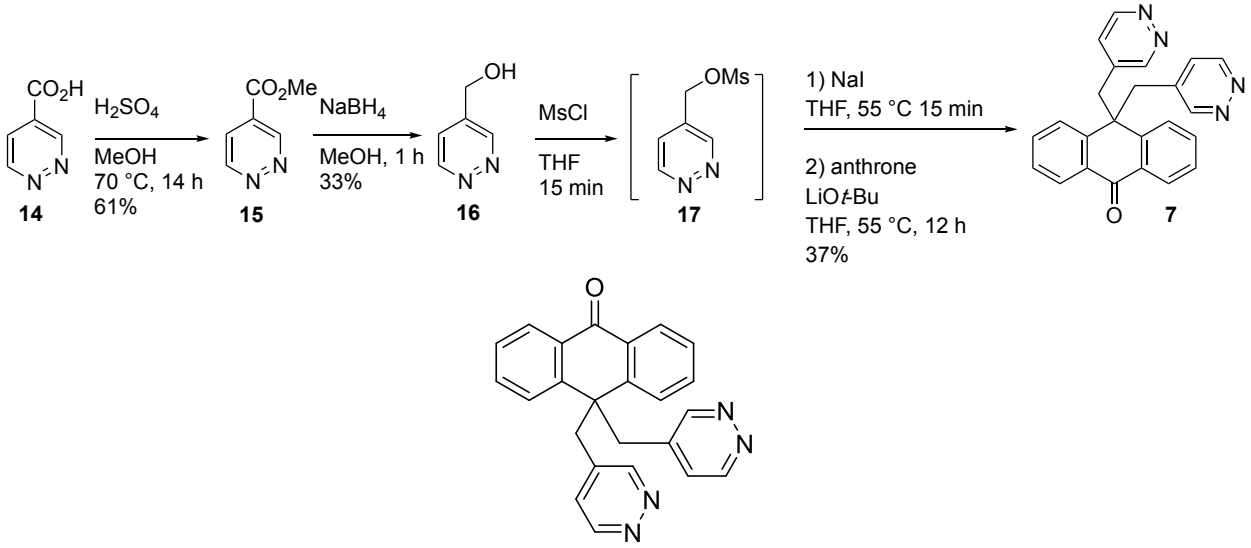


[2-(trifluoromethyl)pyridin-4-yl]methyl methanesulfonate (S3). Alcohol **S2** (0.132 g, 0.745 mmol) was added to an oven dried 20 mL reaction vial, followed by DCM (10 mL). MsCl (0.063 mL, 0.820 mmol) was added via syringe followed by triethylamine (0.114 mL. 0.820 mmol), and the reaction stirred for 1 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was diluted with EtOAc (50 mL) and washed with water (3 x 40 mL). The aqueous layer was saturated with NaCl and re-extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine before being condensed to give the product as a yellow oil (0.130 g, 55%). The crude product was pushed forward without further purification or analysis.



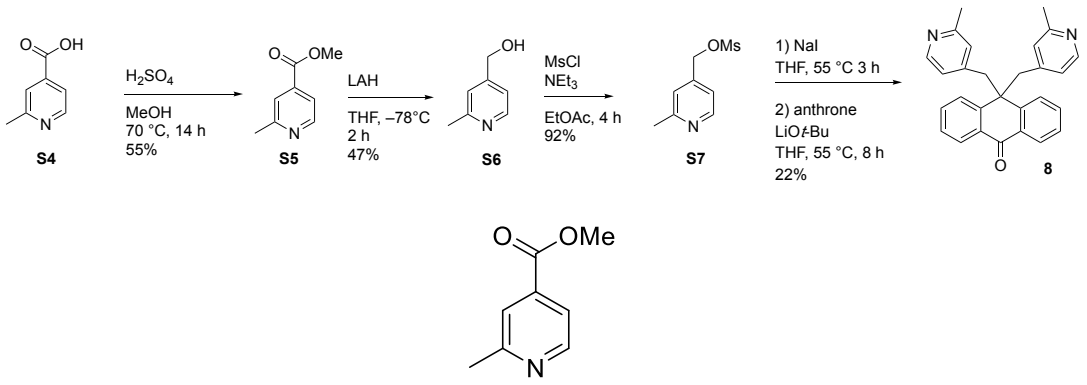
10,10-bis({[2-(trifluoromethyl)pyridin-4-yl]methyl})-9,10-dihydroanthracen-9-one (5). Mesylate **S2** (0.132 g, 0.464 mmol) was placed in an oven dried 20 mL vial followed by sodium iodide (0.034 g, 0.222 mmol). The vial was sealed and purged with nitrogen before anhydrous THF (8.0 mL) was added. The solution was heated to 50 °C for 2.5 h. 18-crown-6 (0.367 g, 1.36 mmol) was dissolved in THF (3.0 mL) in a separate nitrogen purged 4 mL vial containing 4 Å molecular sieves. The solution was stirred for 1 h before being added by syringe to a 20 mL vial containing anthrone (0.040 g, 0.202 mmol) in THF (2.5 mL). Next, potassium *tert*-butoxide (0.070 g, 0.605 mmol) was added as a solution in THF (2.5 mL). The anthrone anion solution was added to the mesylate/iodide solution dropwise over 10 min. at 50 °C, and the reaction was stirred for 12 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was diluted with EtOAc (50 mL) and washed with brine (5 x 20 mL). The organic layer was condensed to give an orange oil, then dissolved with DCM and purified by flash chromatography (5 g SiO_2 cartridge; 0–60% EtOAc/hexanes gradient) to give the title compound as a clear oil (0.036 g, 35%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 3.84 (s, 4H), 6.35 (d, *J* = 5.0 Hz, 2H), 6.59 (s, 2H), 7.51 (t, *J* = 7.6 Hz, 2H), 7.87 (t, *J* = 7.6 Hz, 2H), 8.04 (d, *J* = 8.2 Hz, 2H) 8.09 - 8.18 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 48.2, 49.4, 119.45, 121.5, 121.6, 121.6, 121.6, 123.1, 126.8, 127.1, 128.4, 128.6, 132.7, 134.0, 142.8, 146.7, 147.4, 147.8, 149.3, 181.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ = –69.1; ; IR (film) 1661, 1602, 1326, 1178, 1135, 1117, 1088, 707, 687 cm^{-1}; HRMS (ESI$^+$) calcd for $C_{28}H_{18}F_6N_2O$ $[M+H]^+$ 513.1396, found 513.1383.

Scheme S2. Synthesis of pyridazine analog **7**

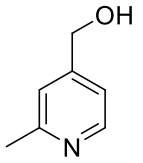


10,10-bis[(pyridazin-4-yl)methyl]-9,10-dihydroanthracen-9-one (7). Alcohol **16** (0.030 g, 0.272 mmol) was added to an oven dried 8 mL vial followed by DMAP (0.017 g, 0.136 mmol). The vial was capped and purged with nitrogen before anhydrous THF (7.0 mL) was added. Next, MsCl (0.021 mL, 0.272 mmol), then NEt_3 (0.038 mL, 0.272 mmol) were added via syringe. The reaction was stirred at room temperature for 15 min. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The mesylate solution was syringed out of the reaction vial under nitrogen and filtered through a syringe filter (0.22 µm PTFE filter) into a separate sealed, nitrogen purged 20 mL vial containing NaI (0.031 g, 0.201 mmol). The vial was sonicated for 2 min., then heated in an oil bath at 55 °C for 15 min. In a separate oven dried 8 mL reaction vial, anthrone (0.023 g, 0.118 mmol) and Li*O*t-Bu (0.024 g, 0.0242 mmol) were added. The vial was sealed and purged with nitrogen, then THF (7.0 mL) was added via syringe. The solution was sonicated until it became homogeneous. The resulting anthrone anion solution was added to the mesylate/iodide solution over 5 min., then stirred for 12 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was cooled to room temperature and quenched with brine, then diluted with EtOAc (100 mL). The organic layer was separated, then washed with brine (3 x 20 mL) ,dried over sodium sulfate, and condensed to give a brown oil. The oil was dissolved in DCM and purified by flash chromatography (5 g SiO_2 cartridge; 0–12% MeOH/DCM gradient) to give the title compound as a brown oil (0.017 g, 37%). ^{1}H NMR (300 MHz, CD_3OD) δ = 4.01 (s, 4H), 6.55 (br s, 2H), 7.57 (t, *J* = 7.5 Hz, 2H), 7.90 - 8.08 (m, 4H), 8.14 (br s, 2H), 8.41 (d, *J* = 8.2 Hz, 2H) 8.51 - 8.66 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ = 45.7, 48.3, 127.3, 128.0, 128.2, 128.5, 132.4, 134.5, 137.8, 143.2, 150.2, 152.5, 181.9; IR (film) 1658, 1567, 1585, 1458, 1380, 1316, 698 cm^{-1}; HRMS (ESI$^+$) calcd. for $C_{24}H_{19}N_4O$ $[M+H]^+$ 379.1553, found 379.1543.

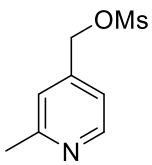
Scheme S3. Synthesis of pyridine analog **8**



methyl 2-methylpyridine-4-carboxylate (S5). 2-Methylpyridine-4-carboxylic acid (0.200 g, 1.46 mmol) was added to an oven dried 20 mL vial. The vial was purged with nitrogen for 10 min. before anhydrous methanol (10 mL) was added. Concentrated H_2SO_4 (0.026 mL, 0.481 mmol) was added to the vial and the reaction was stirred in an oil bath heated to 70 °C for 14 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was diluted with EtOAc (50 mL), and the organic layer was washed with half sat. aq. sodium bicarbonate solution (10 mL). The aqueous layer was saturated with NaCl and extracted with EtOAc (4 x 10 mL). The combined organic layers were washed with brine (10 mL) and dried over sodium sulfate, then condensed down to yield the title compound as a clear oil (0.122 g, 56%). ^{1}H NMR showed the crude compound to be of sufficient purity to advance without further purification. This compound has been previously reported and characterized (CAS# 16830-24-3).^{1}HNMR (300 MHz, $CDCl_3$) δ = 2.59 (s, 3H), 3.90 (s, 3H), 7.59 (d, *J* = 5.0 Hz, 1H), 7.67 (s, 1H), 8.60 (d, *J* = 5.0 Hz, 1 H).

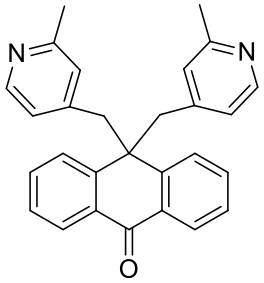


(2-methylpyridin-4-yl)methanol (S6). Ester **S5** (0.120 g, 0.778 mmol) was placed in an oven dried 20 ml vial. The vial was sealed under nitrogen, then anhydrous THF (6.0 mL) was added. LAH (0.031g, 0.817 mmol) was added to a separate oven dried 20 mL reaction vial. The vial was capped and purged with nitrogen before anhydrous THF (10.0 mL) was added. The solution was cooled to –78 °C using a dry ice and acetone bath, then the ester solution was added dropwise via syringe over 5 min. The reaction was stirred at –78 °C for 2 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was warmed to 0 °C, then was diluted with ether before a saturated sodium sulfate solution was added to quench the reaction. The mixture was stirred for 15 min. before being vacuum filtered and condensed to yield the title compound as a light yellow oil (0.041 g, 47%). ^{1}H NMR showed the crude compound to be of sufficient purity to advance without further purification. This compound has been previously reported and characterized (CAS# 105250-16-6). ^{1}H NMR (300 MHz, CD_3OD) δ = 2.51 (s, 3H), 4.63 (s, 3H) 7.21 (d, *J* = 5.3 Hz, 1H) 7.28 (s, 1H) 8.32 (d, *J* = 5.3 Hz, 1H).



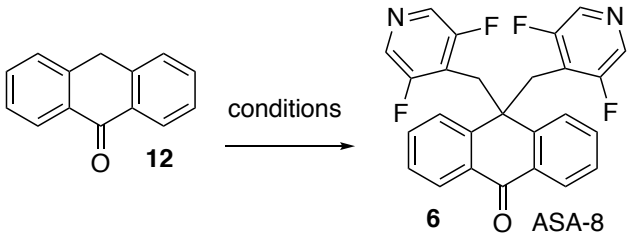
(2-methylpyridin-4-yl)methyl methanesulfonate (S7). Alcohol **S6** (0.040 g, 0.325 mmol) was added to an oven dried 20 mL vial, followed by ethyl acetate (10 mL). Next, mesyl chloride (0.030 mL, 0.390 mmol) was added, followed by NEt_3 (0.054 mL, 0.390 mmol). The reaction was stirred for 4 h, after which time a sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction

was diluted with EtOAc (50 mL), and washed with water (10 mL) and brine before being condensed, yielding the title compound as a light yellow oil. The compound was used directly in the next step of the reaction without further purification or analysis.



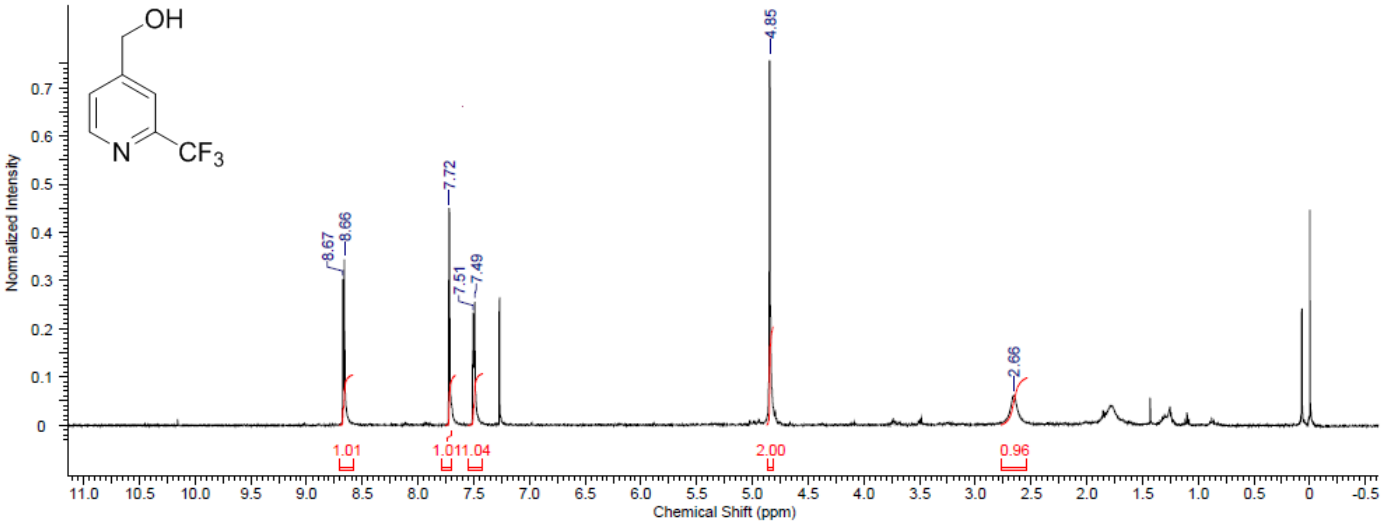
10,10-bis[(2-methylpyridin-4-yl)methyl]-9,10-dihydroanthracen-9-one (8). Mesylate **S7** (0.011 g, 0.026 mmol) was added to an oven dried 20 mL reaction vial followed by NaI (0.005 g, 0.032 mmol). The vial was purged with nitrogen, then anhydrous THF (5.0 mL) was added. The reaction was stirred in an oil bath heated to 55 °C for 3 h. To a separate oven dried 8 mL reaction vial, anthrone (0.005 g, 0.026 mmol) and Li*O*t-Bu (0.008 g, 0.098 mmol) were added. The vial was purged with nitrogen, then anhydrous THF (3.0 mL) was added. The solution was sonicated until homogeneous before being added to the mesylate/iodide solution over 6 h via syringe pump. The reaction was then stirred for an additional 8 h at 55 °C. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was quenched with brine and diluted with EtOAc (75 mL). The organic layer was then washed with additional brine (3 x 50 mL). The organic layer was dried over sodium sulfate and condensed to give a light brown oil. The oil was dissolved in DCM and purified by flash chromatography (5 g SiO_2 cartridge; 0–12% MeOH/DCM gradient) to give the title compound as a yellow semisolid (0.002 g, 22%). ^{1}H NMR (400 MHz, CD_3OD) δ = 2.05 (s, 6H), 3.85 (s, 4H), 6.11 (dd, *J* = 5.3, 1.4 Hz, 2H), 6.19 (s, 2H), 7.45 - 7.52 (m, 2H), 7.72 (d, *J* = 5.1 Hz, 2H), 7.84 - 7.94 (m, 2H), 8.00 (dd, *J* = 7.8, 1.6 Hz, 2H) 8.29 (d, *J* = 7.8 Hz, 2H).

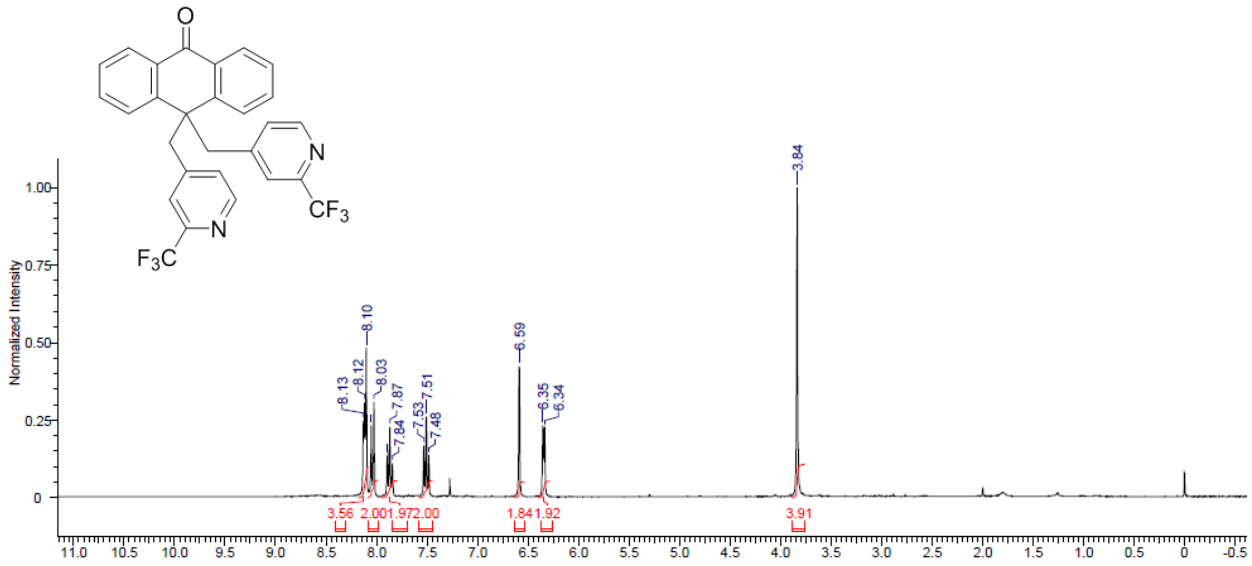
Scheme S4. Optimization of alkylation conditions to synthesize **6**

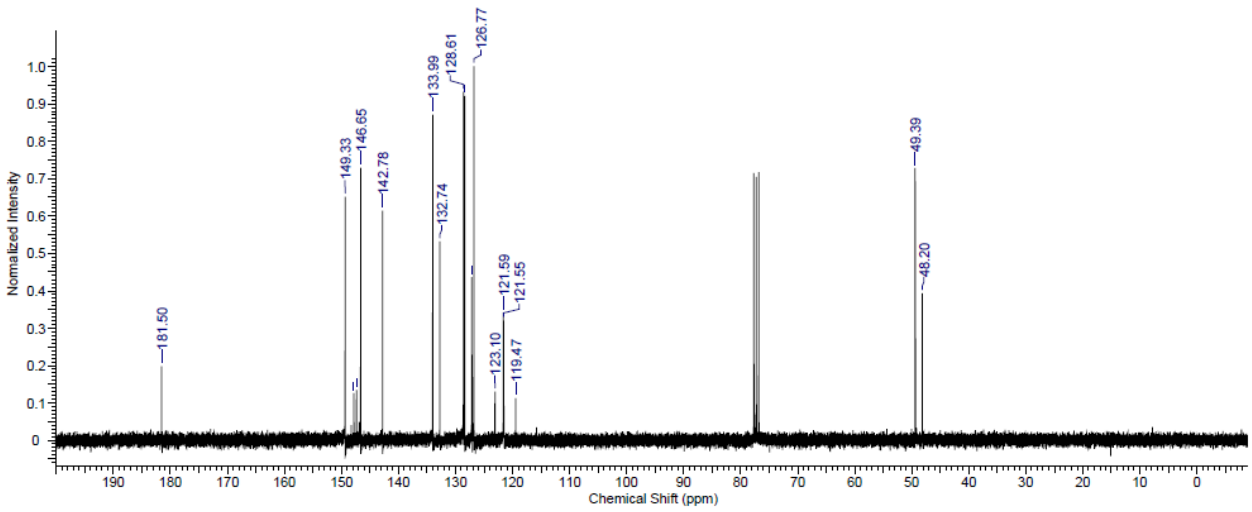


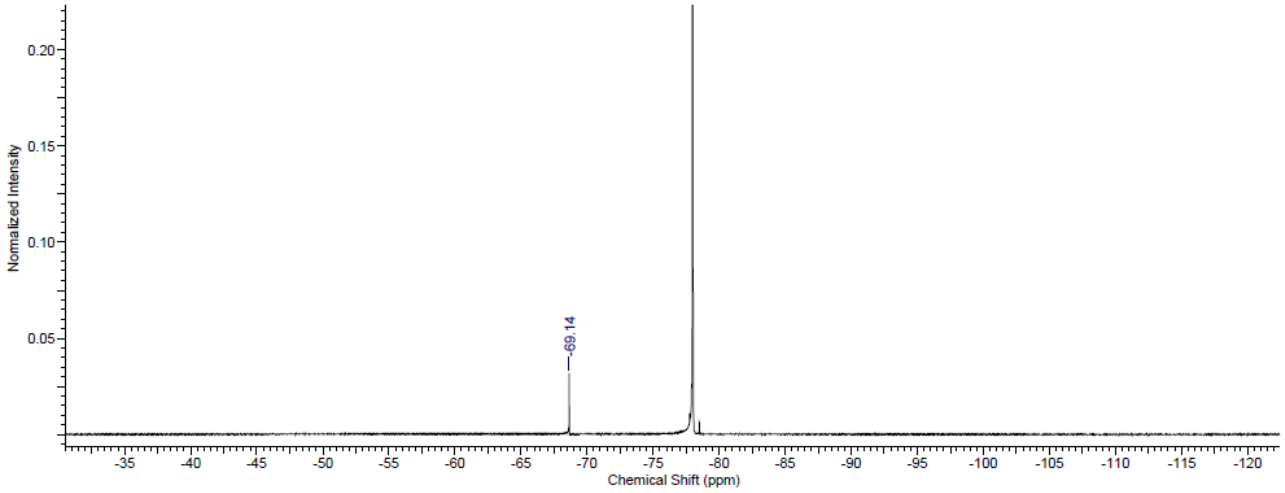
The mesylate **11** (0.025 g, 0.110 mmol) was added to an oven dried 8 mL reaction vial followed by NaI (varying amounts). The vial was capped and purged with nitrogen before anhydrous THF (2.0 mL) was added. The solution was placed in an oil bath heated to 50 °C for 3 h. Anthrone (0.011 g, 0.055 mmol) was added to a separate oven dried 4 mL reaction vial, followed by base (variable amounts). The vial was capped and purged with nitrogen before anhydrous THF (2.0 mL) was added. After 1 h, the anthrone anion solution was added via dropwise over 2 min. via syringe to the mesylate/iodide solution at 50 °C under nitrogen, and the reaction was stirred for 12–16 h. The reaction was quenched with brine, then diluted with EtOAc (50 mL). The organic layer was washed with brine, dried over sodium sulfate, and condensed to give a brown oil. NMR yields were measured in $CDCl_3$ using pentachloroethane as an internal standard. Isolated yields were measured after the crude oil was dissolved in DCM and purified by flash chromatography (5 g SiO_2 cartridge, 0–12% MeOH/DCM gradient) to give the title compound **(6)**.

3. Compound Characterization Data





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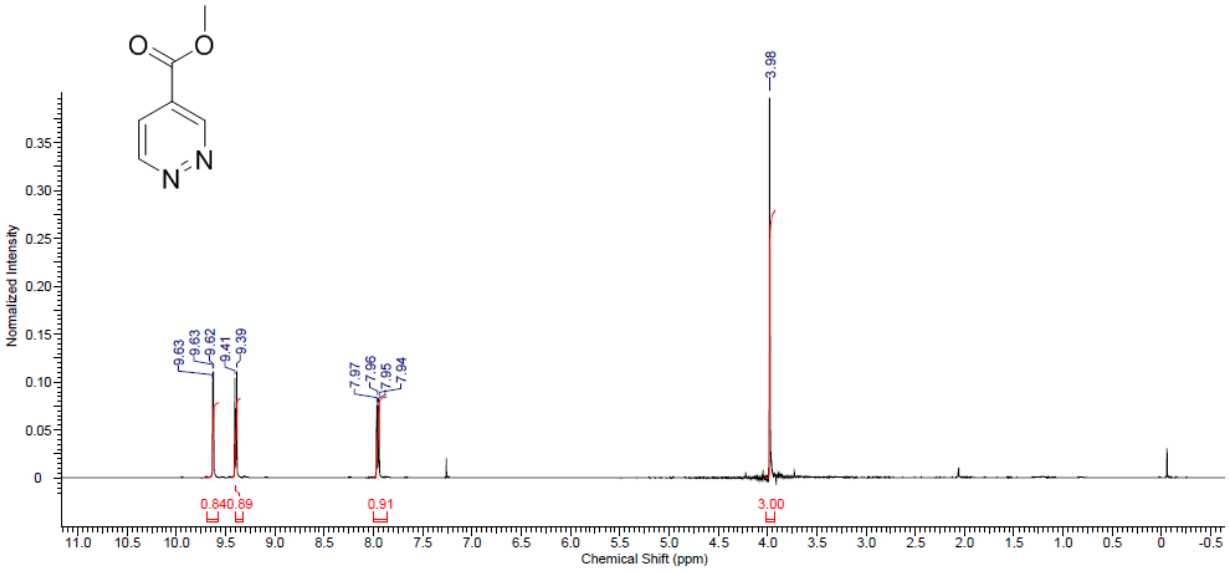
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9.41
9.39
7.97
7.96
7.95
7.94
3.98
0.84 0.89
0.91
3.00
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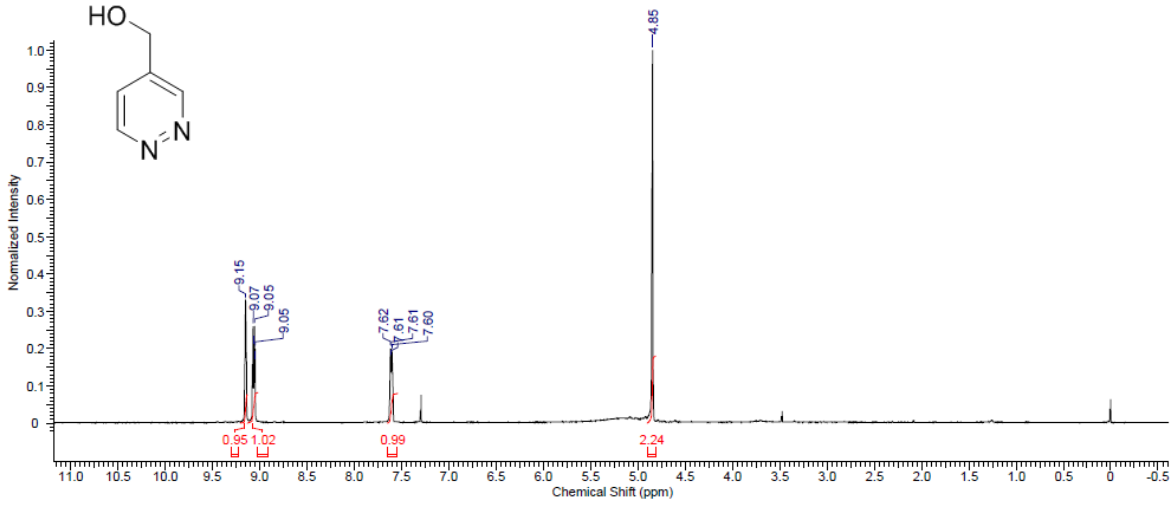
Chemical Shift (ppm)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9.05
9.05
7.62
7.61
7.61
7.60
4.85
0.95 1.02
0.99
2.24
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5
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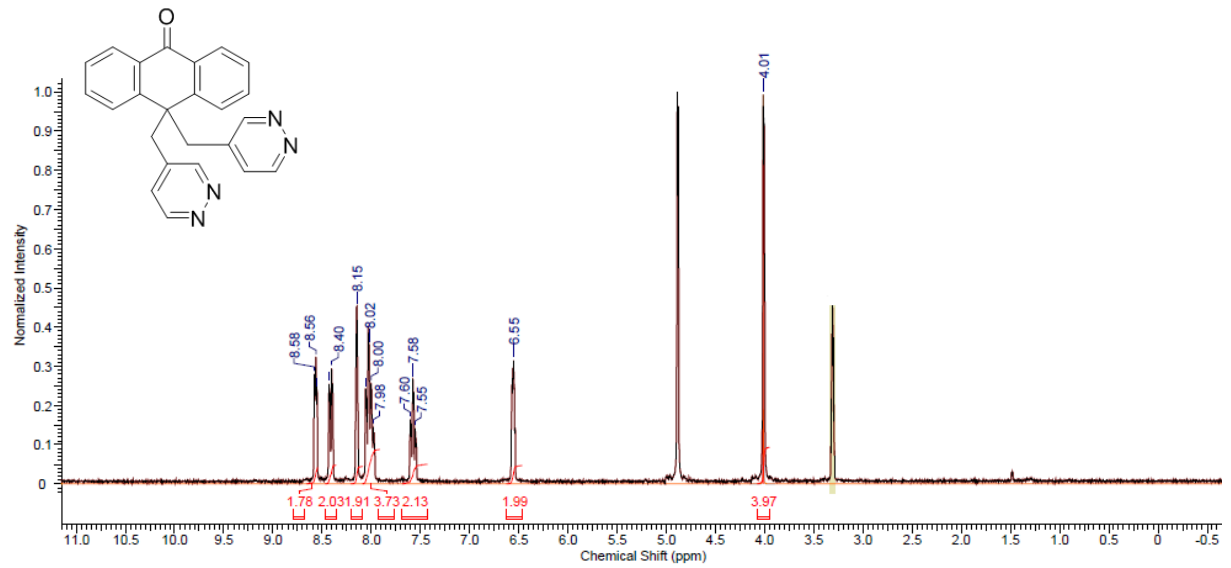
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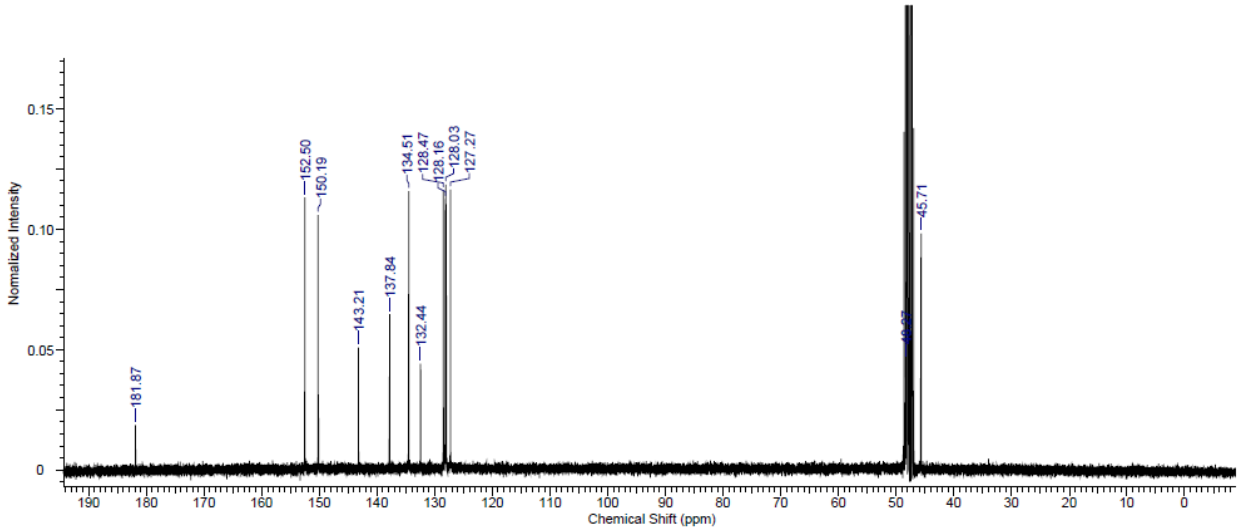
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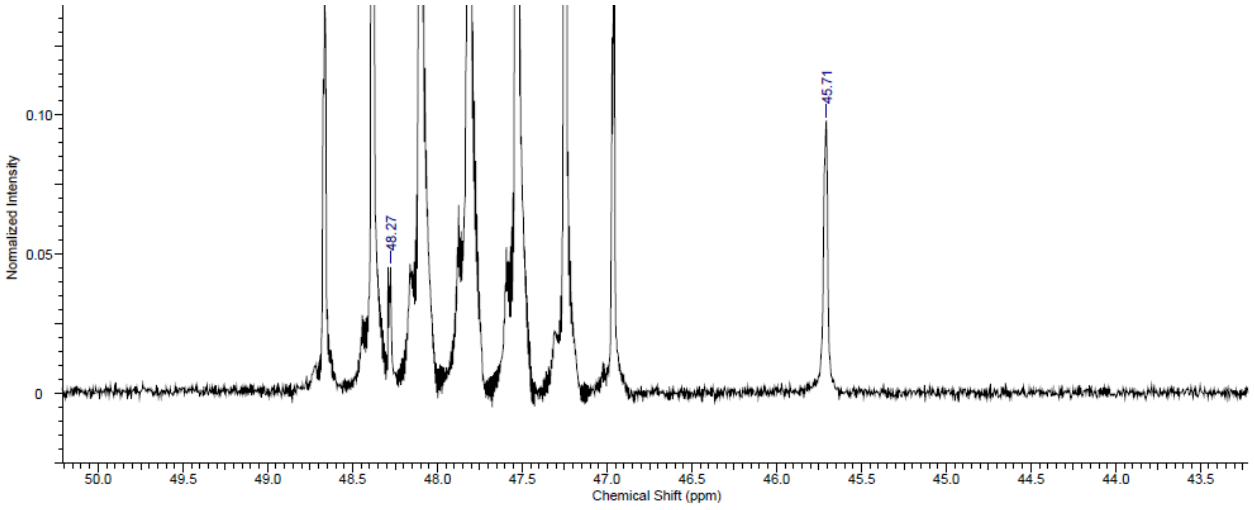
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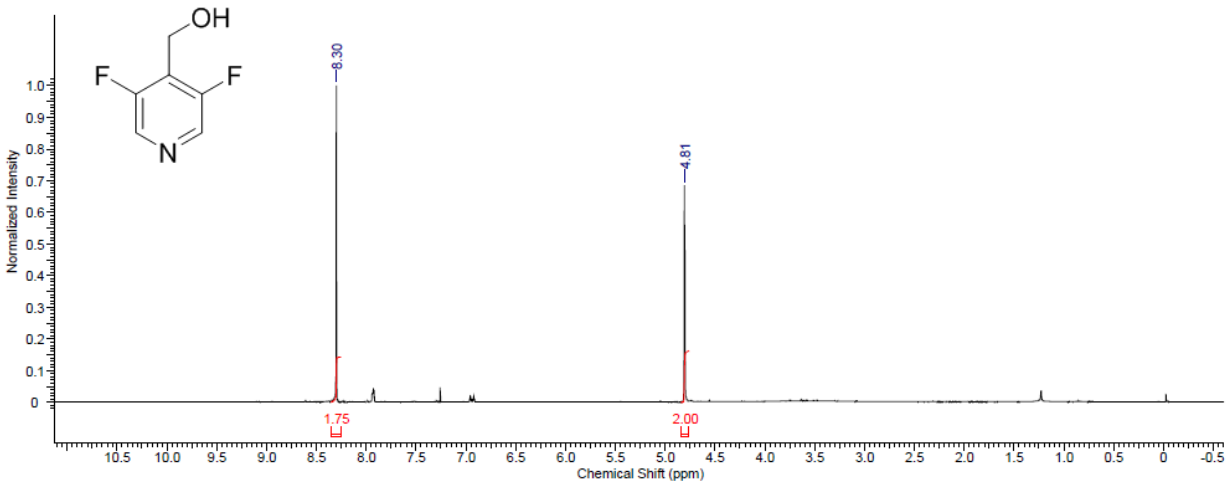
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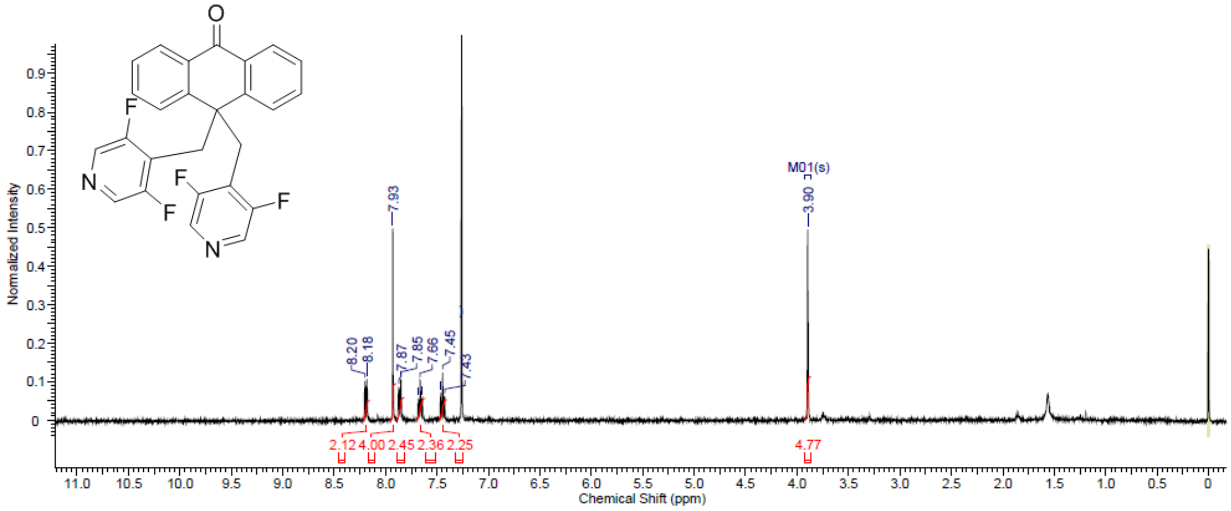
Chemical Shift (ppm)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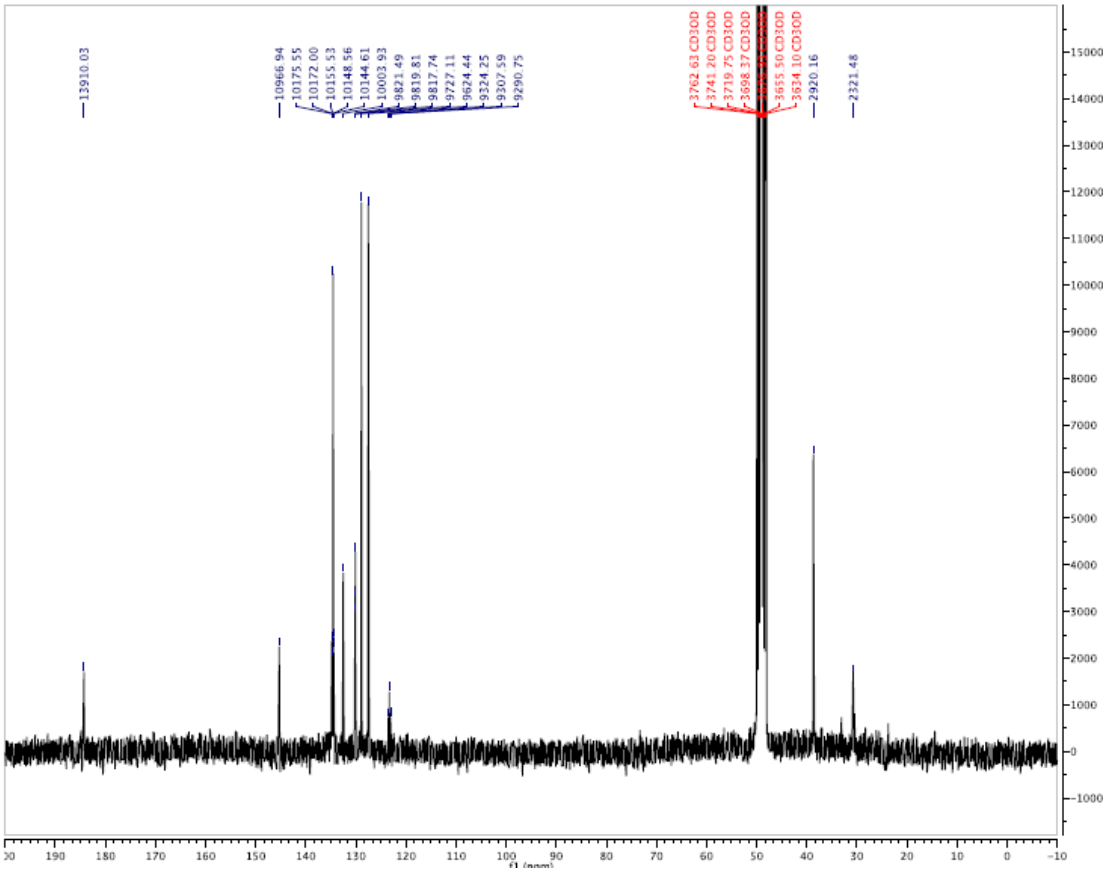
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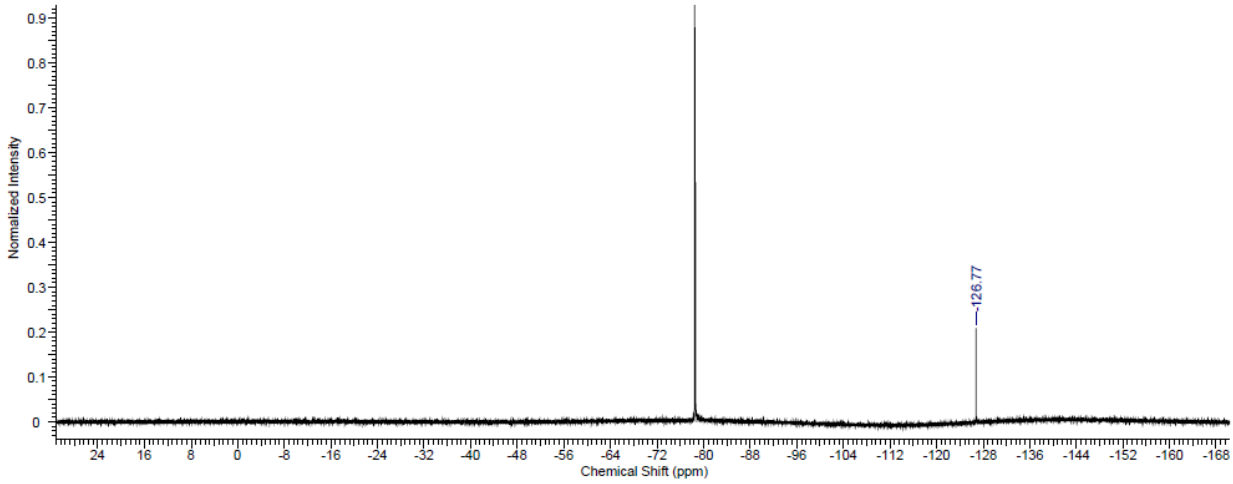
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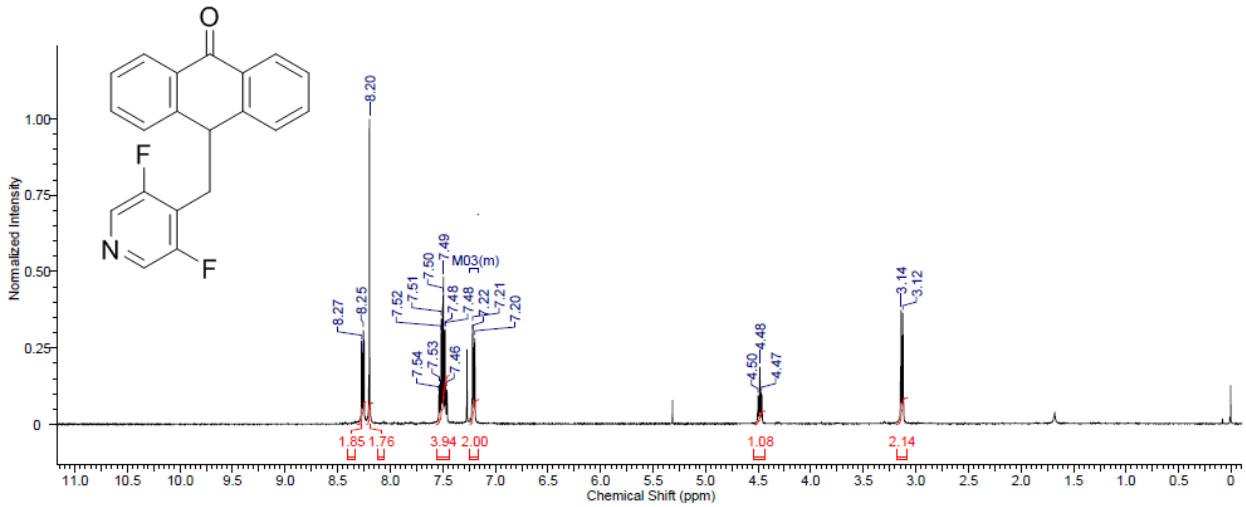
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7.50
7.49
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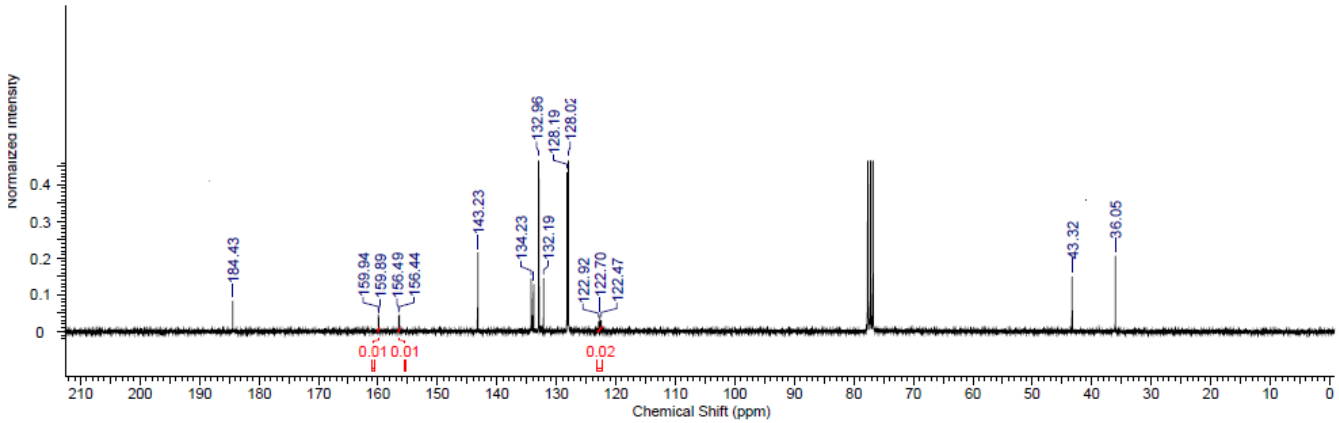
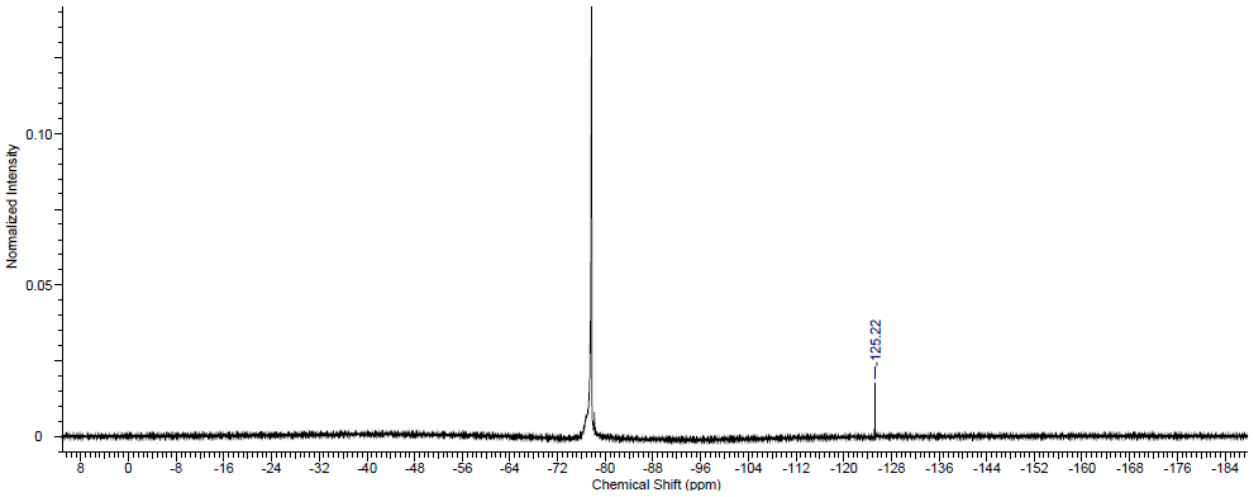
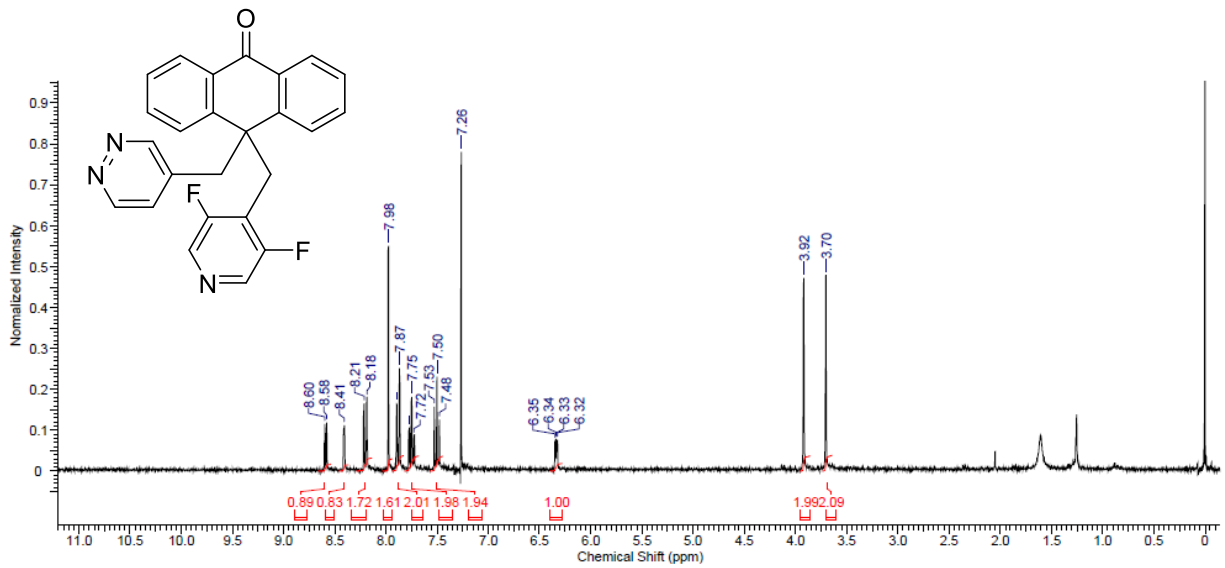
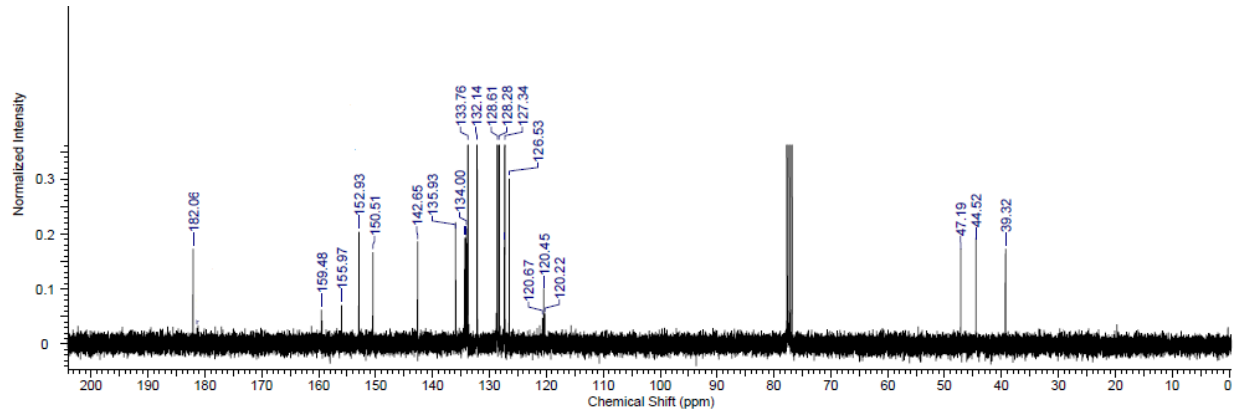
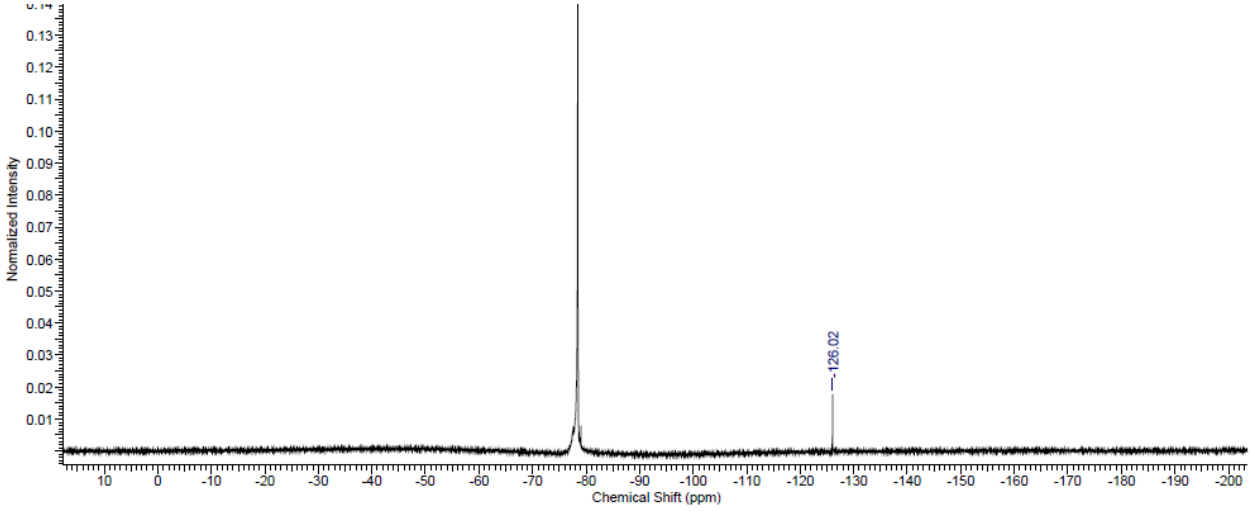
7.48
7.22
7.21
7.20
7.54
7.53
7.46
M03(m)
4.50
4.48
4.47
3.14
3.12
1.85
1.76
3.94
2.00
1.08
2.14
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical Shift (ppm)

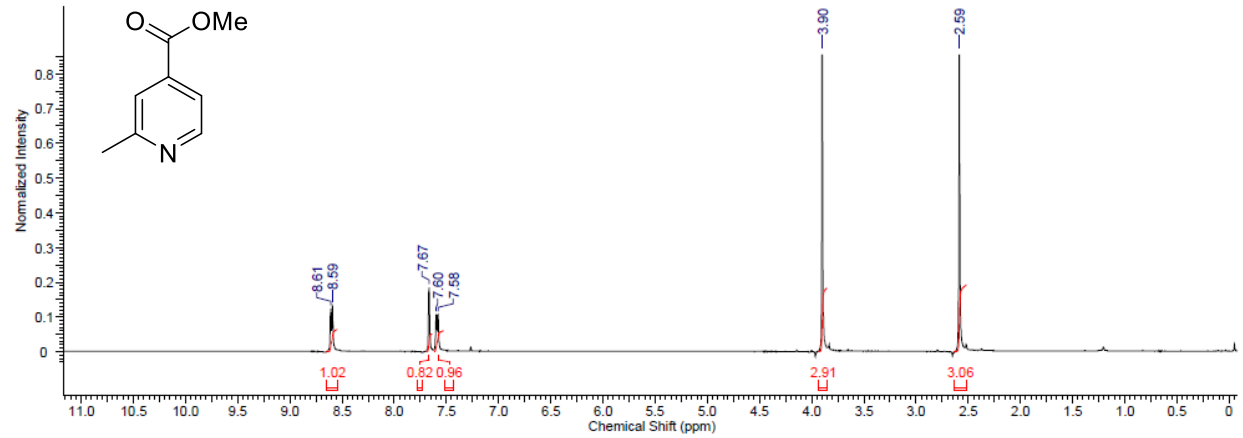

Normalized Intensity
0.4
0.3
0.2
0.1
0
184.43
159.94
159.89
156.49
156.44
143.23
134.23
132.96
132.19
128.19
128.02
122.92
122.70
122.47
43.32
36.05
0.01
0.01
0.02
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Chemical Shift (ppm)

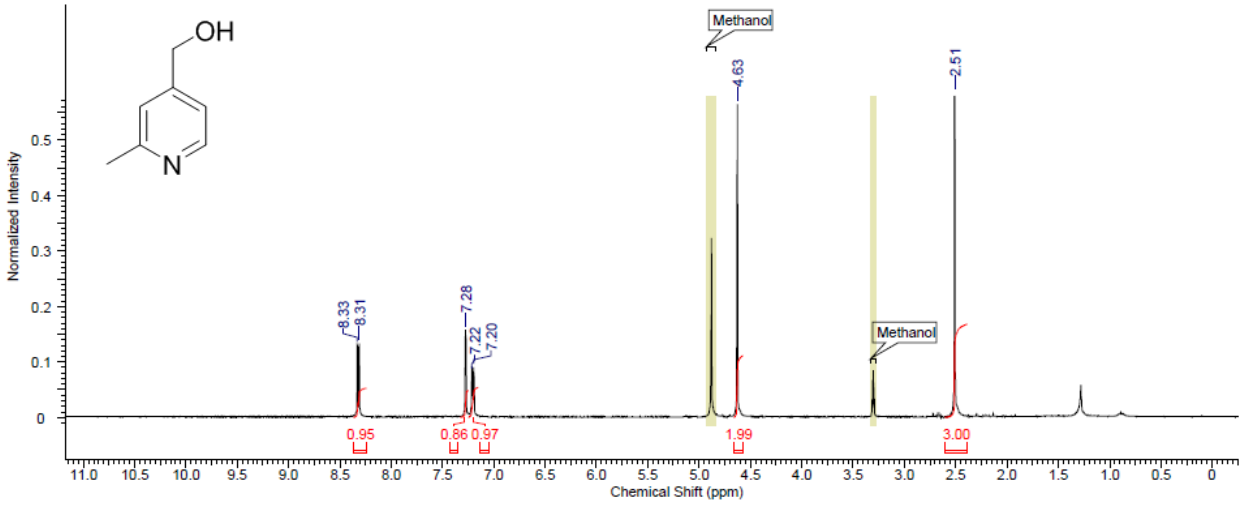

Normalized Intensity
0.10
0.05
0
125.22
8
0
-8
-16
-24
-32
-40
-48
-56
-64
-72
-80
-88
-96
-104
-112
-120
-128
-136
-144
-152
-160
-168
-176
-184
Chemical Shift (ppm)

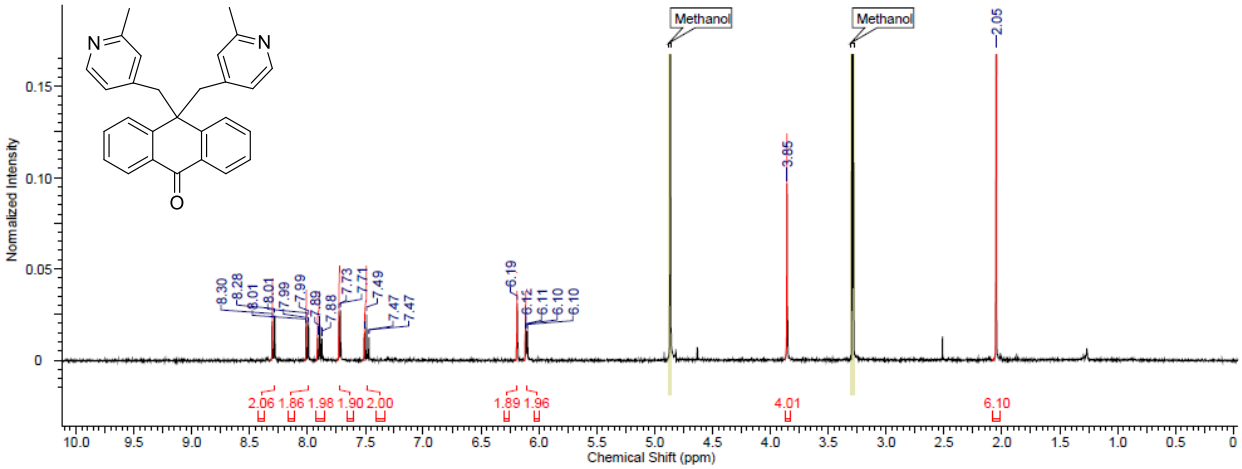

O
N
N
F
F
N
Normalized Intensity
7.26
7.98
3.92
3.70
8.60
8.58
8.41
8.21
8.18
7.87
7.75
7.72
7.53
7.50
7.48
6.35
6.34
6.33
6.32
0.89 0.83 1.72 1.61 2.01 1.98 1.94
1.00
1.99 2.09
Chemical Shift (ppm)


Normalized Intensity
182.06
159.48
155.97
152.93
150.51
142.65
135.93
134.00
133.76
132.14
128.61
128.28
127.34
126.53
120.67
120.45
120.22
47.19
44.52
39.32
Chemical Shift (ppm)


Normalized Intensity
126.02
Chemical Shift (ppm)


O
OMe
N
Normalized Intensity
8.61
8.59
7.67
7.60
7.58
3.90
2.59
1.02
0.82
0.96
2.91
3.06
Chemical Shift (ppm)


OH
N
Methanol
4.63
2.51
Normalized Intensity
8.33
8.31
7.28
7.22
7.20
Methanol
0.95
0.86
0.97
1.99
3.00
Chemical Shift (ppm)


N
N
O
Methanol
Methanol
2.05
3.85
Normalized Intensity
8.30
8.28
8.01
8.01
7.99
7.99
7.89
7.88
7.73
7.71
7.49
7.47
7.47
6.19
6.12
6.11
6.10
6.10
2.06
1.86
1.98
1.90
2.00
1.89
1.96
4.01
6.10
Chemical Shift (ppm)