Selective Inhibition of Collagen Prolyl 4-Hydroxylase in Human Cells

James D. Vasta,[†] Kristen A. Andersen,[‡] Kathryn M. Deck,[§] Christopher P. Nizzi,[§] Richard S. Eisenstein,[§] and Ronald T. Raines*[,†,∥]

[†]Department of Biochemistry, University of Wisconsin–Madison, 433 Babcock Drive, Madison, Wisconsin 53706, United States

[‡]Graduate Program in Molecular and Cellular Pharmacology, University of Wisconsin–Madison, 1300 University Avenue, Madison, Wisconsin 53706, United States

[§]Department of Nutritional Sciences, University of Wisconsin–Madison, 1415 Linden Drive, Madison, Wisconsin 53706, United States

[∥]Department of Chemistry, University of Wisconsin–Madison, 1101 University Avenue, Madison, Wisconsin 53706, United States

*E-mail: rtraines@wisc.edu

EXPERIMENTAL PROCEDURES

I. General

2,4-Pyridinedicarboxylic acid (24PDC), 2,5-pyridinedicarboxylic acid (25PDC), 2,2′-bipyridine (bipy), 2-(1*H*-imidazol-2-yl)pyridine (pyim), and 2,2′-bipyridine-5,5′-dicarboxylic acid (bipy55′DC) were from Sigma–Aldrich (St. Louis, MO). 2-(1*H*-Pyrazol-3-yl)pyridine was from Combi-Blocks (San Diego, CA). Phosphine ligands and phosphonium salts were from either Sigma–Aldrich or Strem (Newberryport, MA), stored in a dessicator, and used without further purification. $Pd(OAc)_2$ was from Sigma–Aldrich, stored in a dessicator, and used without further purification. Deferroxamine mesylate was from Santa Cruz Biotechnology (Dallas, TX). Ethyl dihydroxybenzoate (EDHB) was from Combi-Blocks and was recrystallized from EtOAc before use in cell culture experiments. All other reagent chemicals were obtained from commercial sources (Sigma–Aldrich, Acros, Combi-Blocks, Oakwood Products, Enamine, Bachem, or Novabiochem) and used without further purification. HIF-1α peptide$_{556\text{-}575}$ was from AnaSpec (Fremont, CA) and used without further purification.

All glassware was flame- or oven-dried, and reactions were performed under N_2(g) unless indicated otherwise. DCM and toluene were dried over a column of alumina. Dimethylformamide was dried over alumina and further purified through an isocyanate scrubbing column. Other anhydrous solvents were obtained in septum-sealed bottles. Flash chromatography was performed with columns of 40–63 Å silica gel, 230–400 mesh from Silicycle (Québec City, Canada). Thin-layer chromatography (TLC) was performed on plates of EMD 250 μm silica 60-F_{254} with visualization by UV light or staining with $KMnO_4$.

The phrase “concentrated under reduced pressure” refers to the removal of solvents and other volatile materials using a rotary evaporator at water aspirator pressure (<20 torr) while maintaining water-bath temperature below 40 °C. Residual solvent was removed from samples at high vacuum (<0.1 torr). The term “high vacuum” refers to vacuum achieved by a mechanical belt-drive oil pump. All reported yields are unoptimized. All procedures were performed at ambient temperature unless indicated otherwise.

II. Instrumentation

NMR spectra were acquired at ambient temperature with a DMX-400 Avance spectrometer or an Avance 500i spectrometer from Bruker (Billerica, MA) at the National Magnetic Resonance Facility at Madison (NMRFAM) and were referenced to TMS or a residual protic solvent. Some compounds exist as either mixtures of rotomers or tautomers that do not interconvert on the NMR timescale at ambient temperature and therefore exhibit multiple sets of NMR signals (as indicated). Electrospray ionization (ESI) and electron ionization (EI) mass spectrometry were performed with a Micromass LCT® or Micromass AutoSpec® instruments, respectively, from Waters (Milford, MA) at the Mass Spectrometry Facility in the Department of Chemistry at the University of Wisconsin–Madison. The progress of reactions catalyzed by prolyl 4-hydroxylases was determined by analytical HPLC (Waters system equipped with a Waters 996 photodiode array detector, Empower 2 software). Preparative HPLC was performed using a Prominence HPLC instrument from Shimadzu (Kyoto, Japan) equipped with two LC-20AP pumps, an SPD-M20A photodiode array detector, and a CTO-20A column oven. Iron complexes with biheteroaryl ligands were analyzed by spectrophotometry using a Cary 60 UV–Vis

spectrophotometer from Agilent Technologies (Santa Clara, CA). Protein concentrations were calculated from their absorbance at 280 nm as measured with a NanoVue Plus spectrophotometer from GE Healthcare using an extinction coefficient[1] of 290,000 $M^{-1}cm^{-1}$ for human CP4H[1] and 36,9000 $M^{-1}cm^{-1}$ for human PHD2.[2] Values of IC_{50}, EC_{50}, and LD_{50} were calculated from experimental data with Prism version 6.0 software from GraphPad Software (La Jolla, CA).

III. Production of Recombinant Human CP4H1

Human CP4H containing the α(I) isoform was produced heterologously in Origami B(DE3) *Escherichia coli* cells and purified as described previously.[1]

IV. Assay of Human CP4H1 Activity in the Presence of Inhibitors

The catalytic activity of human CP4H1 was assayed as described previously.[1] Briefly, activity assays were carried out at 30 °C in 100 μL Tris–HCl buffer, pH 7.8, containing human CP4H1 (100 nM), inhibitor (0–500 μM), substrate (dansyl-Gly-Pro-Pro-Gly-OEt, 500 μM), $FeSO_4$ (50 μM), BSA (1 mg/mL), catalase (0.1 mg/mL), sodium ascorbate (2 mM), DTT (100 μM), and α-ketoglutarate (100 μM). Reactions were pre-incubated with or without inhibitor for 2 min at 30 °C, after which the reaction was initiated by the addition of α-ketoglutarate. After 15 min, reactions were quenched by boiling for 45 s and centrifuged at 10,000*g*. The supernatant (20–50 μL) was injected into a Nucleodur® C18 Gravity reversed-phase column (4.6 × 250 mm, 5-μm particle size) from Macherey–Nagel (Bethlehem, PA). The column was eluted at 1 mL/min with a linear gradient (20 min) of aqueous acetonitrile (20–45% v/v) containing TFA (0.1% v/v). The absorbance of the eluent was monitored at 289 nm. All assays were performed in triplicate. Data are reported as catalytic activity relative to control reactions lacking inhibitor, where catalytic activity is determined from the percent conversion of substrate to product. Dose–response curves were generated for each inhibitor by plotting the relative activity versus the log of the inhibitor concentration. IC_{50}-values for each inhibitor were interpolated from the data by non-linear regression to the sigmoidal dose–response equation in Prism software.

V. Production of Recombinant Human PHD2

A cDNA encoding human $PHD2_{181-426}$ possessing an N-terminal hexahistidine (His_6) tag ($NHis_6$-$PHD2_{181-426}$) was cloned using the Gibson strategy,[3] and the encoded protein was produced and purified as described previously.[2]

VI. Assay of Human PHD2 Activity in the Presence of Inhibitors

The catalytic activity of human PHD2 was assayed as described previously.[2] Briefly, activity assays were carried out at 30 °C in 100 μL Tris–HCl buffer, pH 7.8, containing human $NHis_6$-$PHD2_{181–426}$ (5 μM), inhibitor (0–50 μM), substrate (HIF-1α $peptide_{556–574}$, 50 μM), $FeSO_4$ (50 μM), BSA (1 mg/mL), catalase (0.3 mg/mL), sodium ascorbate (2 mM), DTT (1 mM), and α-ketoglutarate (35 μM). Reaction mixtures were pre-incubated with or without inhibitor for 2 min at 30 °C, after which the reaction was initiated by the addition of α-ketoglutarate. After 10 min, reactions were quenched by boiling for 60 s and centrifuged at 10,000*g*. The supernatant (50 μL) was injected into a Nucleodur® C18 Gravity reversed-phase column (4.6 × 250 mm,

5-μm particle size) from Macherey–Nagel. The column was eluted at 1 mL/min with a gradient (34 min) of aqueous acetonitrile (5–56% v/v) containing TFA (0.1% v/v). The absorbance of the eluent was monitored at 218 nm. All assays were performed in triplicate. Data is reported as activity relative to control reactions lacking inhibitor, where activity is determined from the percent conversion of substrate to product.

VII. Estimation of the pK_a Values of Biheteroaryl Ligands

The pK_a value of biheteroaryl compounds was estimated with potentiometric titration. Compounds (5–10 mM) were dissolved in water and the pH was adjusted to ~12 by adding 10 M NaOH. The pH of the solution was recorded with an Accumet™ XL500 pH meter equipped with an Orion 912600® pH electrode from Thermo Fisher Scientific while titrating the solution with HCl (1 M or 100 μM, as necessary) until a pH value of ~1.75 was achieved. Titration curves were prepared by plotting the solution pH versus the volume of 1 M HCl added, and pK_a values were estimated from derivative plots of the titration curves using Prism software.

VIII. Assay of Fe(II)-Affinity for Biheteroaryl Ligands

The affinity of biheteroaryl ligands for Fe(II) was determined comparatively by measuring the half-maximal concentration (EC_{50}) required for binding 20 μM Fe(II) (Fe_{20}-EC_{50}) in sodium phosphate buffer at pH 7.0. Stock solutions of ligands were prepared in either water for high affinity ligands (typically, Fe_{20}-EC_{50} <1 mM) or DMSO for low affinity ligands (typically, Fe_{20}-EC_{50} >1 mM). Stock solutions of $FeSO_4$ were prepared in H_2O and used within 3 h of preparation. Ligand solutions (3 μM–18 mM, depending on affinity) were prepared in 10 mM sodium phosphate buffer, pH 7.0, after which Fe(II) stock solution was added to initiate complex formation. For high-affinity ligands, solutions were allowed to equilibrate for 15 min, after which the absorbance was recorded at the λ_{max} for the complex under study. For most low affinity ligands, the corresponding Fe(II) complexes were unstable and observed to dissociate over time. Therefore, the absorbance value was determined within 30 s of mixing, where the absorbance was measured at the λ_{max} for the complex under study. Complexes with pyox were not observed under these conditions. Absorbance values were corrected by subtracting the absorbance value in the absence of ligand. Dose–response curves were generated for each ligand by plotting the absorbance *versus* the log of the ligand concentration. Values of Fe_{20}-EC_{50} for each ligand were interpolated from the dose–response curves by non-linear regression using the sigmoidal dose–response function in Prism software. All experiments were performed in triplicate. Experiments to study pyimDC were performed as described above, except that phosphate buffer was excluded from the assay solution, bipy was added last to a final concentration of 300 μM, and the absorbance of the $Fe(bipy)_3^{2+}$ complex was measured at 523 nm.

IX. Determination of Fe(II) Complex Stoichiometry

The stoichiometry of biheteroaryl complexes with Fe(II) was estimated with Job's method. Briefly, reactions were prepared such that the total moles of Fe(II) and ligand were kept constant, but the mole fraction of the ligand was varied from 0 to 1. The total concentration of ligand and Fe(II) used for each individual ligand was based upon the iron affinity and extinction coefficient,

and ranged from 0.4 mM to 2 mM. Stock solutions of ligands were prepared in water. Stock solutions of $FeSO_4$ were prepared in water and used within 3 h of preparation. Reaction mixtures were prepared in 10 mM sodium phosphate buffer, pH 7.0, and complex formation was initiated by the addition of Fe(II) solution. For high-affinity ligands (typically, Fe_{20}-EC_{50} <1 mM), solutions were allowed to equilibrate for 15 min, after which the absorbance was recorded at the λ_{max} for the complex under study. For most low-affinity ligands (typically, Fe_{20}-EC_{50} >1 mM), the corresponding Fe(II) complexes were unstable and observed to dissociate over time. Therefore, the absorbance value was determined within 30 s of mixing, where the absorbance was measured at the λ_{max} for the complex under study. Complexes with pyox were not observed under these conditions. Absorbance values were corrected by subtracting the absorbance value of a blank solution in absence of $FeSO_4$, after which the values were normalized relative to the reaction with the highest absorbance value. All experiments were performed in at least duplicate. Job's plots were constructed by plotting the normalized absorbance versus the mole fraction of biheteroaryl ligand, after which the stoichiometry of the complex was estimated from the mole fraction of the reaction with the highest absorbance value. If necessary, blank titrations using only Fe(II) were used to correct the Job's plots.

X. General Mammalian Cell Culture

The HEK293T line of human embryonic kidney cells was from American Type Culture Collection (ATCC, Manassas, VA), and the MDA-MB-231 line of human breast cancer cells was a generous gift from Dr. Beth A. Weaver (University of Wisconsin–Madison). Cell lines were maintained according to the procedures recommended by the ATCC. Cells were grown in a cell culture incubator at 37 °C under CO_2 (5% v/v) in flat-bottomed culture flasks. The culture medium was DMEM supplemented with GIBCO fetal bovine serum (FBS) (10% v/v), penicillin (100 units/mL), streptomycin (100 μg/mL) and L-glutamine (2 mM). Cells were counted by hemocytometry with Trypan Blue prior to use in assays.

XI. Cytotoxicity Assays

The toxicity of esterified biheteroaryl compounds for human breast cancer cells was evaluated with the CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) from Promega (Madison, WI). Briefly, MDA-MB-231 cells grown as described in Section X were plated at a concentration of 5,000 cells/well in a clear 96-well plate. The cells were allowed to adhere for 4 h, after which the medium was removed and discarded. Fresh medium was added and the cells were treated with varying concentrations of the test compound at 37 °C for 24 h. The medium was removed, and cells were washed with Dulbecco's PBS. The MTS reagent was added at a ratio of 1:5, and the cells were incubated at 37 °C for 2 h before measuring the absorbance at 490 nm. The average absorbance was measured in triplicate for each concentration tested, and the entire experiment was repeated in duplicate. The percentage of viable cells was determined by normalizing to a PBS control (100% viable), and a H_2O_2 control (0% viable). For all of the compounds tested, the LD_{50}-value could not be measured due to its being higher than the aqueous solubility limit: diethyl bipy55′DC, LD_{50} >100 μM; diethyl pyimDC, LD_{50} >1000 μM; diethyl pythiDC, LD_{50} >500 μM.

XII. Effect of CP4H Inhibitors on Iron Metabolism and P4HA1 Levels in Human Cells

MDA-MB-231 or HEK293T cells were plated at 50,000 cells/well into 12-well plates and grown to confluence (~24 h) as described in Section X. The cells were washed, and the medium was replaced with 1.0 mL of serum-free medium. Stock solutions of all test compounds were prepared at 100× in DMSO and added to a final concentration of 1× (0.1–0.5 mM). After addition of the test compound or DMSO vehicle, cells were incubated for 24 h. The cells were then washed with 100 μL MPER solution from Thermo Fisher Scientific (Waltham, MA) and collected. Protein concentrations were determined by BCA assay (Thermo Fisher Scientific), and samples corresponding to 40 μg total protein were analyzed with an immunoblot.

XIII. Immunoblotting

For all immunoblot analyses, protein samples where boiled in SDS–PAGE buffer, separated by electrophoresis through a Tris–glycine SDS–PAGE gel from Bio-Rad Laboratories (Hercules, CA), and subsequently transferred to a PVDF membrane for immunoblotting. All primary antibodies were used at the working dilution specified by the manufacturer. Primary antibodies were visualized using a complementary secondary antibody fused to horseradish peroxidase (HRP), with detection of HRP by chemiluminescence, imaging using an ImageQuant LAS 4000 (GE Healthcare), and quantification by densitometry (ImageJ software). The anti-rabbit antibody from Promega and the anti-mouse antibody from Abbiotec (San Diego, CA) were used at the working dilution specified by the manufacturer. Statistical comparisons were performed using Student's *t*-test or one-way ANOVA functions available in Prism.

For investigations of iron metabolism, protein samples (40 μg) were separated by SDS–PAGE through a 12% w/v acrylamide gel, and blots were probed with primary antibodies to human ferritin (rabbit monoclonal) from Abcam (Cambridge, MA), human transferrin receptor (mouse monoclonal) from Invitrogen, human HIF-1α (mouse monoclonal) from BD Biosciences, and human β-actin (rabbit monoclonal) from Cell Signaling Technology (Danvers, MA). Quantifications were normalized to the signal from β-actin, after which treated samples were compared to vehicle-treated controls.

For investigations of P4HA1 levels, protein samples were treated and quantified as described above for iron metabolism except that the blots were probed with primary antibodies to human P4HA1 (rabbit polyclonal) from Thermo Fisher Scientific and human β-actin.

For investigations of collagen secretion, protein samples were separated by SDS–PAGE through a 7.5% w/v acrylamide gel. Blots were first stained for total protein using Ponceau S, after which they were probed with a primary antibody to human type-I collagen (α1) (rabbit polyclonal) from Novus Biologicals (Littleton, CO). Quantifications were normalized to the total protein signal obtained from Ponceau S staining, after which treated samples were compared to vehicle-treated controls.

ph. Effect of CP4H Inhibitors on the Binding of the Iron-Responsive Element by IRPs

MDA-MB-231 cells were plated at 3×10^6 cells/dish into 100 mm × 20 mm dishes and grown to confluence (~24 h) as described in Section X. The cells were washed, and the medium was replaced with 10 mL of serum-free medium. Stock solutions of all test compounds were prepared at 100× in DMSO and added to a final concentration of 1× (as denoted). After addition of the test

compound or DMSO vehicle, cells were incubated for 24 h, after which they were harvested by trypsinization and centrifugation. The resultant pellets (~50 μL) were washed with PBS and then lysed using a protocol described previously.[4] Briefly, cell pellets were resuspended in 200 μL of cell lysis buffer, which was 20 mM Hepes–HCl buffer, pH 7.4, containing sodium pyrophosphate (10 mM), sodium fluoride (50 mM), β-glycerophosphate (50 mM), EDTA (5 mM), GTP (1 mM), sodium orthovanadate (1 mM), benzamidine hydrochloride (2 mM), Nonidet NP-40 (0.5% v/v), *p*-nitrophenyl *p′*-guanidinobenzoate·HCl (25 μg/mL), DTT (1 mM), leupeptin (40 μg/mL), pepstatin (4 μg/mL), SBTI (100 μg/mL), MG132 (10 μM), PMSF (200 μM), and BHT (5 μg/mL) by vortexing and lysed on ice by vortexing every few min. After 15 min, the lysates were centrifuged at 14,000 rpm and the supernatants aliquoted and stored at −80 °C until analysis by an electrophoretic mobility shift assay (EMSA).

XV. Electrophoretic Mobility Shift Assay for IRE-Binding by IRPs

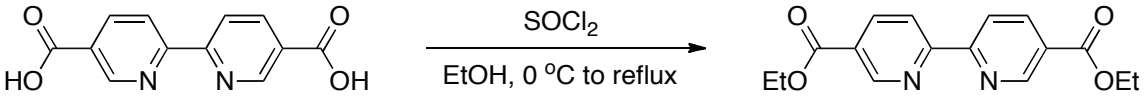
EMSAs were performed and quantified essentially as described previously.[5] Briefly [^{32}P]-L-ferritin IRE (1 nM RNA, specific radioactivity ~7,000 dpm/fmol) was incubated with lysate protein (2.5 μg as determined by the Bradford assay) in binding buffer for 10 min on ice. Heparin was added (to 0.5 mg/mL), and the reaction mixture was incubated for an additional 5 min on ice before separating bound and free RNA on a nondenaturing polyacrylamide gel at ambient temperature.

XVI. Effect of CP4H Inhibitors on Collagen Production in Mammalian Cells

MDA-MB-231 cells were plated at 50,000 cells/well into 12-well plates and grown to confluence (~24 h) as described in Section X. The cells were washed, and the medium was replaced with 1.5 mL of serum-free medium containing sodium ascorbate (50 μg/mL). Stock solutions of all test compounds were prepared at 100× in DMSO and added to a final concentration of 1× (as denoted). After the addition of a test compound or vehicle (DMSO), cells were incubated for 48 h, after which 1.0 mL of conditioned medium was removed and added to 4.0 mL of chilled acetone (–20 °C). The resultant mixtures were incubated for 3 h at –20 °C, after which the precipitated protein was pelleted by centrifugation at 10,000*g* for 30 min. The supernatants were discarded and the pellets dried briefly in a fume hood. Pellets were stored at –20 °C prior to analysis with an immunoblot.

XVII. Synthetic Procedures

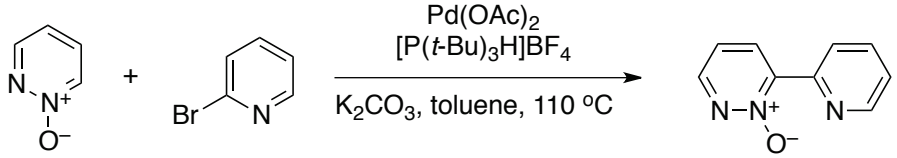
Diethyl 2,2′-Bipyridine-5,5′-dicarboxylate (diethyl bipy55′DC)



Bipy55′DC (200 mg, 0.82 mmoles) and EtOH (13 mL) were added to a dried flask, and the resulting solution was stirred on ice. Thionyl chloride (1.3 mL) was added dropwise on ice, after which the flask was fitted with a reflux condenser and heated at reflux. After 24 h, the reaction mixture was cooled on ice and quenched by the dropwise addition of saturated aqueous Na_2CO_3 (20 mL). The aqueous layer was extracted with CH_2Cl_2 (4 × 20 mL), and the combined organic

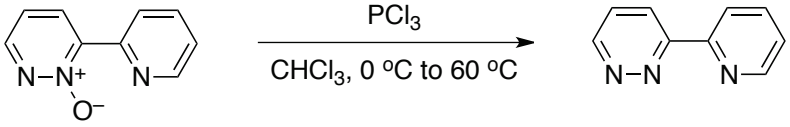
extracts were dried over Na_2SO_4(s) and concentrated under reduced pressure. The crude product was purified by chromatography on silica (3% v/v acetone in 1:1 DCM/hexanes) to afford the title compound (190 mg, 77%) as a white solid. **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 9.32 (dd, J = 0.5, 2.0 Hz, 1 H), 8.59 (dd, J = 0.5, 8.5 Hz, 1 H), 8.46 (dd, J = 2.0, 8.5 Hz, 1 H), 4.47 (q, J = 7.5 Hz, 2 H), 1.46 (t, J = 7.5 Hz, 3 H); **^{13}C NMR** (125 MHz, $CDCl_3$, δ): 165.2, 158.3, 150.6, 138.1, 126.6, 121.3, 61.6, 14.3; **HRMS** (ESI) m/z 301.1193 [calc'd for $C_{16}H_{17}N_2O_4$ $(M + H)^+$ 301.1183].

6-(Pyridin-2-yl)pyridazine-1-oxide



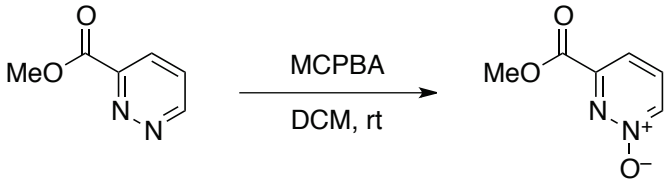
$Pd(OAc)_2$ (29.0 mg, 0.13 mmoles), $[P(t\text{-}Bu)_3H]BF_4$ (113 mg, 0.39 mmoles), pyridazine-1-oxide (500 mg, 5.2 mmoles), and K_2CO_3 (719 mg, 5.2 mmoles) were added to a dried flask. The flask was fitted with a reflux condenser capped with a septum, evacuated, and purged with N_2(g) (~5 times). A degassed solution of 2-bromopyridine (411 mg, 2.6 mmoles) in dry toluene (13 mL) was added via syringe, and the reaction mixture was stirred at 110 °C for 18 h. The reaction mixture was then cooled and filtered through Celite®, and the filtrate was concentrated under reduced pressure. The crude product was purified by chromatography on silica (35% v/v acetone in hexanes) to afford the title compound (206 mg, 46%) as a grey solid. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 8.90 (d, J = 8.0 Hz, 1H), 8.70 (d, J = 4.0 Hz, 1 H), 8.63 (dd, J = 1.6, 8.0 Hz, 1 H), 8.47 (s, 1 H), 7.84 (t, J = 7.6 Hz, 1 H), 7.36 (dd, J = 5.2, 7.2 Hz, 1 H), 7.19 (dd, J = 5.2, 8.0 Hz, 1 H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 149.8, 149.5, 148.2, 142.5, 136.7, 135.6, 125.0, 124.7, 116.5; **HRMS** (ESI) m/z 174.0658 [calc'd for $C_9H_8N_3O$ $(M + H)^+$ 174.0662].

2-(Pyridin-2-yl)pyridazine (pypyrid)



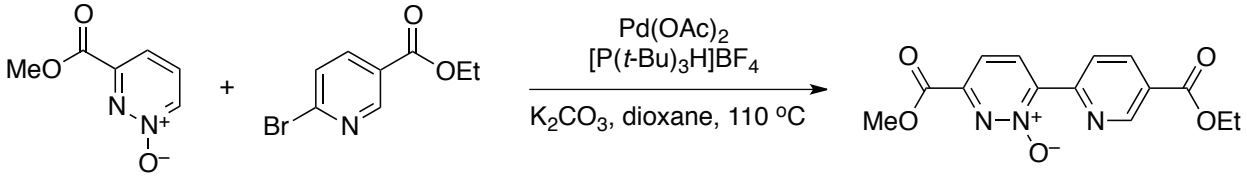
6-(Pyridin-2-yl)pyridazine-1-oxide (150 mg, 0.87 mmoles) was dissolved in dry $CHCl_3$ (4.3 mL) and cooled to 0 °C in an ice bath, after which PCl_3 (230 μL, 2.6 mmoles) was added dropwise with stirring. The reaction mixture was stirred at 60 °C until the starting material was consumed completely, as judged by TLC. The reaction mixture was quenched by the dropwise addition of saturated aqueous Na_2CO_3 (10 mL) while stirring on ice. The product was extracted with $CHCl_3$ (4 × 10 mL), and the combined organic extracts were dried over Na_2SO_4(s) and concentrated under reduced pressure. The crude product was purified by chromatography on silica (40% v/v EtOAc in hexanes) to afford the title compound (50 mg, 37%) as a brown solid. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 9.19 (dd, J = 1.6, 5.2 Hz, 1H), 8.70–8.67 (m, 2 H), 8.55 (dd, J = 1.6, 8.8 Hz, 1 H), 7.87 (td, J = 1.6, 8.0 Hz, 1 H), 7.59 (dd, J = 4.8, 8.8 Hz, 1 H), 7.38 (ddd, J = 1.2, 4.8, 7.6 Hz, 1 H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 158.6, 153.5, 151.2, 149.4, 137.2, 127.0, 124.7, 124.4, 121.6; **HRMS** (ESI) m/z 158.0714 [calc'd for $C_9H_8N_3$ $(M + H)^+$ 158.0713].

Methyl Pyridazine-3-carboxylate-1-oxide



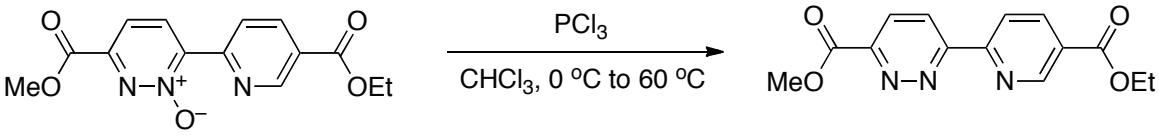
Methyl pyridazine-3-carboxylate (2.0 g, 14.5 mmoles) and *m*-chloroperoxybenzoic acid (3.9 g, 17.4 mmoles) were dissolved in dry DCM (29 mL) in a flame-dried flask. The reaction mixture was stirred at ambient temperature for 5 h. The solvent was evaporated under reduced pressure, and the resulting residue was purified by chromatography on silica (30% v/v acetone in hexanes) to afford the title compound as an off-white solid (1.97 g, 88%). **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 8.28 (dd, J = 2.4, 5.2 Hz, 1 H), 7.79–7.75 (m, 2 H), 4.03 (s, 3 H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 162.2, 150.2, 136.9, 134.4, 116.8, 53.6; **HRMS** (ESI) m/z 155.0452 [calc'd for $C_6H_7N_2O_3$ $(M + H)^+$ 155.0452].

Methyl 6-(5-Methoxycarbonylpyridin-2-yl)pyridazine-3-carboxylate-1-oxide



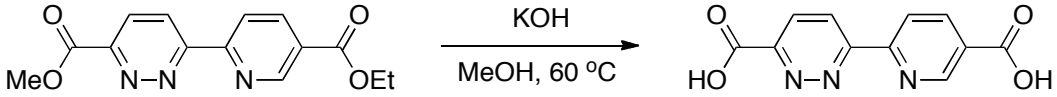
$Pd(OAc)_2$ (7.3 mg, 0.033 mmoles), $[P(t\text{-}Bu)_3H]BF_4$ (28 mg, 0.098 mmoles), K_2CO_3 (180 mg, 1.3 mmoles), ethyl 6-bromonicotinate (150 mg, 0.65 mmoles), and methyl pyridazine-3-carboxylate-1-oxide (200 mg, 1.3 mmoles) were added to a dried flask. The flask was fitted with a reflux condenser capped with a septum, evacuated, and purged with N_2(g) (~5 times). Dry dioxane (4 mL) was added via syringe, and the reaction mixture was stirred at 110 °C for 18 h. The reaction mixture was then cooled and filtered through Celite®, and the filtrate was concentrated under reduced pressure. The crude product was purified by chromatography on silica (40% v/v EtOAc in hexanes) to afford the title compound (82 mg) as a pale orange solid. Due to the presence of minor contaminants that were difficult to remove by chromatography or recrystallization, the slightly crude product was used directly in the next reaction before further purification and characterization.**^{1}H NMR** (500 MHz, $CDCl_3$, δ): 9.26 (d, J = 2.0 Hz, 1 H), 9.06 (d, J = 8.5, Hz, 1 H), 8.85 (d, J = 8.5 Hz, 1 H), 8.42 (dd, J = 2.0, 8.5 Hz, 1 H), 7.85 (dd, J = 8.5 Hz, 1 H), 4.41 (q, J = 7.0 Hz, 2 H), 4.00 (s, 3 H), 1.40 (t, J = 7.0 Hz, 3 H); **HRMS** (ESI) m/z 304.0931 [calc'd for $C_{14}H_{14}N_3O_5$ $(M + H)^+$ 304.0928].

Methyl 6-(5-Ethoxycarbonylpyridin-2-yl)pyridazine-3-carboxylate (ethylmethyl pyrpyridDC)



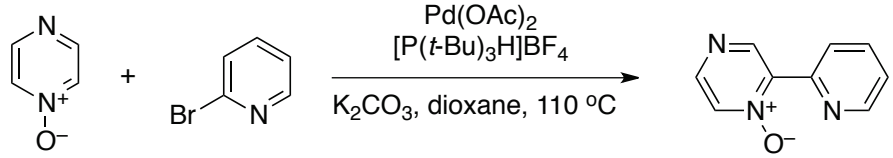
Methyl 6-(5-methoxycarbonylpyridin-2-yl)pyridazine-3-carboxylate-1-oxide (80 mg, 0.28 mmoles) was dissolved in dry $CHCl_3$ (1.3 mL) and cooled to 0 °C in an ice bath, after which PCl_3 (370 μL, 3.7 mmoles) was added dropwise with stirring. The reaction mixture was stirred at 60 °C until the starting material was consumed completely, as judged by TLC. The reaction was quenched by the dropwise addition of saturated aqueous Na_2CO_3 (10 mL) while stirring on ice. The product was extracted with $CHCl_3$ (4 × 10 mL), and the combined organic extracts were dried over Na_2SO_4(s) and concentrated under reduced pressure. The crude product was purified by chromatography on silica (35% v/v EtOAc in hexanes) to afford the title compound (37 mg, 20% over two steps) as a pale yellow solid. **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 9.12 (d, J = 1.5 Hz, 1 H), 8.66 (d, J = 8.0 Hz, 1 H), 8.57 (d, J = 8.5 Hz, 1 H), 8.30 (dd, J = 2.0, 8.0 Hz, 1 H), 8.14 (d, J = 8.5 Hz, 1 H), 4.26 (q, J = 7.0 Hz, 2H), 3.92 (s, 3H), 1.25 (t, J = 7.0 Hz, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$, δ): 164.7, 164.3, 158.9, 155.5, 151.1, 150.6, 138.2, 128.2, 127.2, 125.3, 121.6, 61.5, 53.3, 14.1; **HRMS** (ESI) m/z 288.0982 [calc'd for $C_{14}H_{14}N_3O_4$ $(M + H)^+$ 288.0979].

6-(5-Carboxypyridin-2-yl)pyridazine-3-carboxylic Acid (pypyridDC)



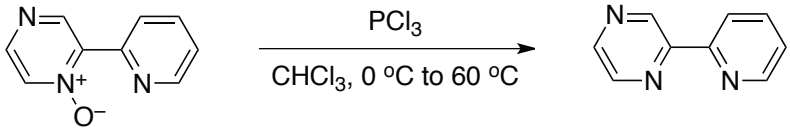
Methyl 6-(5-ethoxycarbonylpyridin-2-yl)pyridazine-3-carboxylate (37 mg, 0.13 mmoles) and KOH (34 mg, 0.52 mmoles) were added to a vial. MeOH (1.3 mL) was added to the vial, and the reaction mixture was heated to 60 °C with stirring until the starting material was consumed completely, as judged by TLC. The reaction mixture was cooled and concentrated under reduced pressure. The crude product was dissolved in water (2 mL). The aqueous layer was washed with EtOAc (1 × 2 mL), after which the product was precipitated from the aqueous layer by adjusting to pH 3–4 with 1 M HCl. After cooling to 4 °C, the product was filtered, washed with water (3 × 1 mL), and dried under high vacuum to afford the title compound (23 mg, 73%) as a pale yellow solid. **^{1}H NMR** (500 MHz, DMSO-d_6, δ): 13.73 (bs, 2H), 9.11 (dd, J = 1.0, 2.0 Hz, 1 H), 8.63 (dd, J = 1.0, 8.5 Hz, 1 H), 8.60 (d, J = 9.0 Hz, 1 H), 8.40 (dd, J = 2.0, 8.5 Hz, 1 H), 8.24 (d, J = 9.0 Hz, 1 H); **^{13}C NMR** (125 MHz, DMSO-d_6, δ): 166.2, 165.3, 158.8, 155.7, 152.5, 150.9, 139.1, 129.2, 128.2, 126.0, 121.9; **HRMS** (ESI) m/z 246.0517 [calc'd for $C_{11}H_8N_3O_4$ $(M + H)^+$ 246.0510].

2-(Pyridin-2-yl)pyrazine-1-oxide



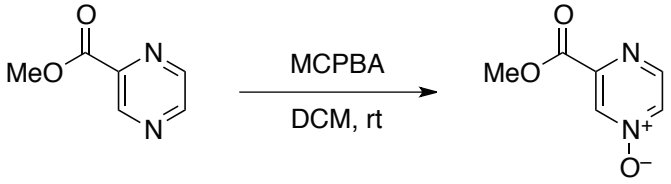
$Pd(OAc)_2$ (39.0 mg, 0.17 mmoles), $[P(t\text{-}Bu)_3H]BF_4$ (151 mg, 0.52 mmoles), pyrazine-1-oxide (1.0 g, 10.4 mmoles), and K_2CO_3 (959 mg, 6.9 mmoles) were added to a dried flask. The flask was fitted with a reflux condenser capped with a septum, evacuated, and purged with N_2(g) (~5 times). A degassed solution of 2-bromopyridine (548 mg, 3.5 mmoles) in dry dioxane (17 mL) was added via syringe, and the reaction mixture was stirred at 110 °C for 18 h. The reaction mixture was then cooled and filtered through Celite®, and the filtrate was concentrated under reduced pressure. The crude product was purified by chromatography on silica (30% v/v acetone in hexanes) to afford the title compound (312 mg, 52%) as a pale yellow solid. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 9.35 (s, 1H), 8.75–8.72 (m, 2 H), 8.38 (d, J = 4.0 Hz, 1 H), 8.15 (dd, J = 0.4, 4.0 Hz, 1 H), 7.81 (ddd, J = 1.6, 2.0, 8.0 Hz, 1 H), 7.25 (ddd, J = 0.8, 4.8, 8.0 Hz, 1 H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 149.9, 149.7, 147.3, 145.8, 142.2, 136.5, 134.5, 125.4, 124.8; **HRMS** (ESI) m/z 174.0662 [calc'd for $C_9H_8N_3O$ $(M + H)^+$ 174.0662].

2-(Pyridin-2-yl)pyrazine (pypyraz)



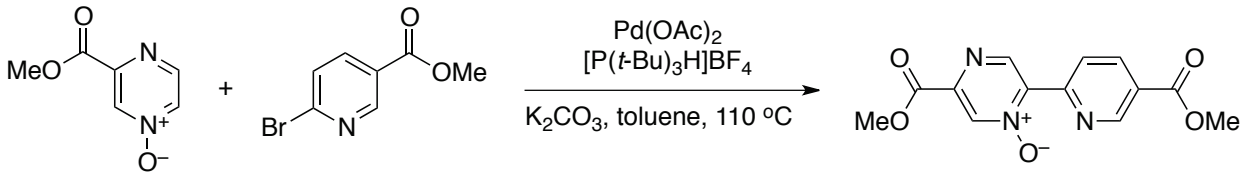
2-(Pyridin-2-yl)pyrazine-1-oxide (200 mg, 1.2 mmoles) was dissolved in dry $CHCl_3$ (23 mL) and cooled to 0 °C in an ice bath, after which PCl_3 (360 μL, 4.2 mmoles) was added dropwise with stirring. The reaction mixture was stirred at 60 °C until the starting material was consumed completely, as judged by TLC. The reaction mixture was quenched by the dropwise addition of saturated aqueous Na_2CO_3 (20 mL) while stirring on ice. The product was extracted with $CHCl_3$ (4 × 20 mL), and the combined organic extracts were dried over Na_2SO_4(s) and concentrated under reduced pressure. The crude product was purified by chromatography on silica (30% v/v EtOAc in hexanes) to afford the title compound (103 mg, 57%) as a pale yellow solid. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 9.63 (d, J = 1.2 Hz, 1H), 8.71–8.69 (m, 1 H), 8.60–8.58 (m, 2 H), 8.34 (d, J = 8.0 Hz, 1 H), 7.82 (ddd, J = 1.6, 2.0, 8.0 Hz, 1 H), 7.34 (ddd, J = 1.2, 4.8, 7.6 Hz, 1 H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 154.2, 151.1, 149.4, 144.4, 143.5, 143.3, 137.0, 124.4, 121.4; **HRMS** (ESI) m/z 158.0708 [calc'd for $C_9H_8N_3$ $(M + H)^+$ 158.0713].

Methyl Pyrazine-3-carboxylate-1-oxide



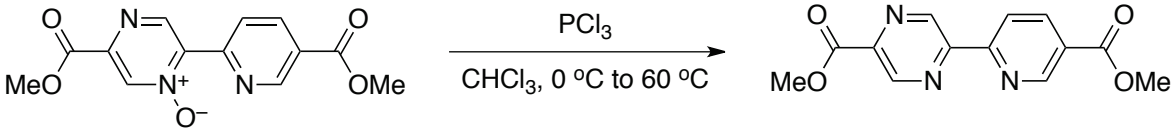
Methyl pyrazine-2-carboxylate (3.0 g, 21.7 mmoles) and *m*-chloroperoxybenzoic acid (7.3 g, 32.6 mmoles) were dissolved in dry DCM (43 mL) in a flame-dried flask. The reaction mixture was stirred at ambient temperature for 48 h. The solvent was evaporated under reduced pressure, and the resulting residue was purified by chromatography on silica (40–50% v/v acetone in hexanes followed by 80–100% v/v EtOAc in hexanes) to afford the title compound as a white solid (751 mg, 22%). **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 8.76 (dd, J = 0.8, 1.6 Hz, 1 H), 8.57 (dd, J = 0.4, 4.0 Hz, 1 H), 8.22 (dd, J = 2.0, 4.0 Hz, 1 H), 4.05 (s, 3 H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 162.6, 147.6, 147.3, 136.0, 135.9, 53.7; **HRMS** (ESI) *m*/*z* 155.0451 [calc'd for $C_6H_7N_2O_3$ $(M + H)^+$ 155.0452].

Methyl 2-(5-Methoxycarbonylpyridin-2-yl)pyrazine-5-carboxylate-1-oxide



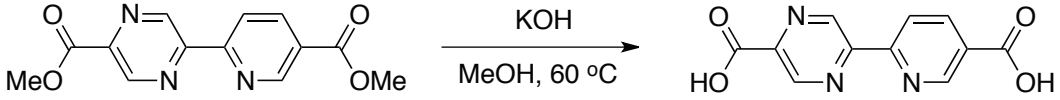
$Pd(OAc)_2$ (21 mg, 0.09 mmoles), $[P(t\text{-}Bu)_3H]BF_4$ (81 mg, 0.28 mmoles), K_2CO_3 (511 mg, 3.7 mmoles), methyl 6-bromonicotinate (400 mg, 1.9 mmoles), and methyl pyrazine-3-carboxylate-1-oxide (571 mg, 3.7 mmoles) were added to a dried flask. The flask was fitted with a reflux condenser capped with a septum, evacuated, and purged with N_2(g) (~5 times). Dry toluene (9 mL) was added via syringe, and the reaction mixture was stirred at 110 °C for 24 h. The reaction mixture was then cooled and filtered through Celite®, and the filtrate was concentrated under reduced pressure. The crude product was purified by chromatography on silica (45% v/v EtOAc in hexanes) to afford the title compound (100 mg) as a pale orange solid. Due to the presence of minor contaminants that were difficult to remove by chromatography or recrystallization, the slightly crude product was used directly in the next reaction before further purification and characterization. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 9.63 (s, 1 H), 9.38 (dd, J = 0.4, 2.0, Hz, 1 H), 9.06 (dd, J = 0.8, 8.4 Hz, 1 H), 8.89 (d, J = 0.4 Hz, 1 H), 8.48 (dd, J = 2.0, 8.4 Hz, 1 H), 4.10 (s, 3 H), 4.03 (s, 3 H); **HRMS** (ESI) *m*/*z* 290.0770 [calc'd for $C_{13}H_{12}N_3O_5$ $(M + H)^+$ 290.0772].

Methyl 2-(5-Methoxycarbonylpyridin-2-yl)pyrazine-5-carboxylate (dimethyl pypyrazDC)



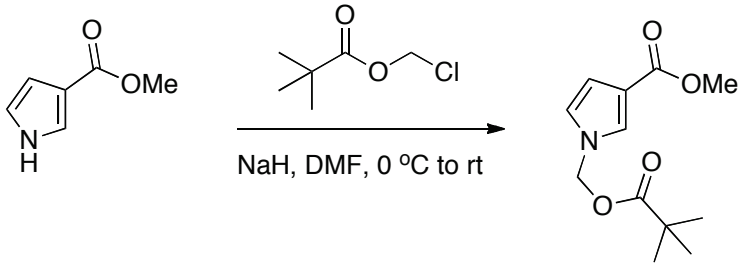
Methyl 2-(5-methoxycarbonylpyridin-2-yl)pyrazine-5-carboxylate-1-oxide (100 mg, 0.35 mmoles) was dissolved in dry $CHCl_3$ (1.5 mL) and cooled to 0 °C in an ice bath, after which PCl_3 (100 μL, 1.0 mmoles) was added dropwise with stirring. The reaction mixture was stirred at 60 °C until the starting material was consumed completely, as judged by TLC. The reaction was quenched by the dropwise addition of saturated aqueous Na_2CO_3 (10 mL) while stirring on ice. The product was extracted with $CHCl_3$ (3 × 10 mL), and the combined organic extracts were dried over Na_2SO_4(s) and concentrated under reduced pressure. The crude product was recrystallized from $CHCl_3$ and then EtOAc to afford the title compound (28 mg, 6% over two steps) as a pale yellow solid. **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 9.84 (d, J = 1.5 Hz, 1 H), 9.38 (d, J = 1.0 Hz, 1 H), 9.36 (dd, J = 0.5, 2.0 Hz, 1 H), 8.58 (d, J = 0.5, 8.0 Hz, 1 H), 8.50 (dd, J = 2.0, 8.5 Hz, 1 H), 4.11 (s, 3H), 4.03 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$, δ): 165.4, 164.4, 156.4, 152.4, 150.8, 145.2, 143.3, 143.0, 138.4, 126.9, 121.9, 53.3, 52.7; **HRMS** (ESI) m/z 274.0824 [calc'd for $C_{13}H_{12}N_3O_4$ $(M + H)^+$ 274.0823].

2-(5-Carboxypyridin-2-yl)pyrazine-5-carboxylic Acid (pypyrazDC)



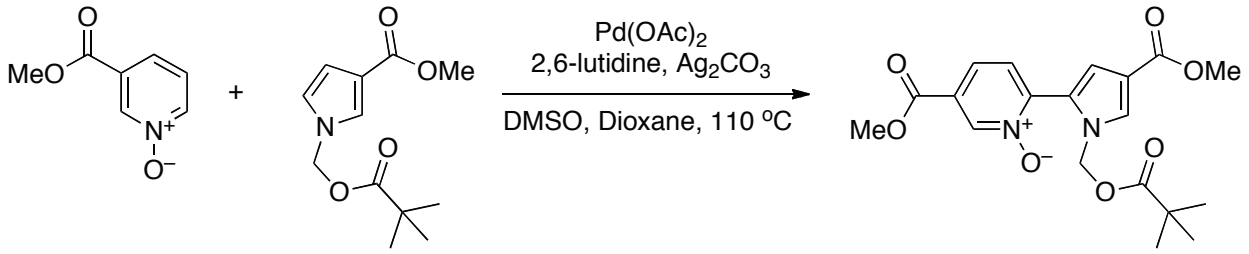
Methyl 2-(5-methoxycarbonylpyridin-2-yl)pyrazine-5-carboxylate (28 mg, 0.10 mmoles) and KOH (26 mg, 0.41 mmoles) were added to a vial. MeOH (3 mL) was added to the vial, and the reaction mixture was heated to 60 °C with stirring until the starting material was consumed completely, as judged by TLC. The reaction mixture was cooled and concentrated under reduced pressure. The crude product was dissolved in water (2 mL). The aqueous layer was washed with EtOAc (1 × 2 mL), after which the product was precipitated from the aqueous layer by adjusting to pH 3–4 with 1 M HCl. After cooling to 4 °C, the product was filtered, washed with water (3 × 1 mL), and dried under high vacuum to afford the title compound (17 mg, 68%) as a light brown solid. **^{1}H NMR** (500 MHz, DMSO-d_6, δ): 13.84 (bs, 2H), 9.69 (d, J = 1.5 Hz, 1 H), 9.30 (d, J = 1.0 Hz, 1 H), 9.25 (dd, J = 0.5, 2.0 Hz, 1 H), 8.54 (d, J = 7.5 Hz, 1 H), 8.50 (dd, J = 2.0, 8.0 Hz, 1 H); **^{13}C NMR** (125 MHz, DMSO-d_6, δ): 168.8, 165.9, 156.8, 152.1, 151.4, 146.0, 145.3, 143.3, 139.7, 128.7, 122.7; **HRMS** (ESI) m/z 246.0505 [calc'd for $C_{11}H_8N_3O_4$ $(M + H)^+$ 246.0510].

Methyl 1-Pivaloyloxymethyl-1*H*-pyrrole-3-carboxylate



NaH (60% w/v in mineral oil, 267 mg, 6.7 mmoles) was added to a dry flask. The flask was evacuated and purged with N_2(g) (~5 times). Dry DMF (3 mL) was added, and the flask was cooled on ice. A degassed solution of methyl 1*H*-pyrrole-3-carboxylate (836 mg, 6.7 mmoles) in dry DMF (4 mL) was added dropwise over 5 min with stirring. The reaction mixture was stirred on ice for 45 min until gas evolution was complete, after which chloromethyl pivalate (0.97 mL, 6.7 mmoles) was added dropwise. The reaction mixture was allowed to come to ambient temperature overnight, after which the reaction mixture was concentrated under reduced pressure to a crude oil. The oil was taken up in H_2O (25 mL), and the aqueous layer extracted with DCM (5 × 25 mL). The combined organic extracts were dried over Na_2SO_4(s) and concentrated under reduced pressure, after which the crude product was purified by chromatography on silica (25% v/v EtOAc in hexanes) to afford the title compound (1.20 g, 75%) as a golden oil. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 7.46 (dd, J = 0.4, 2.0 Hz, 1 H), 6.79 (dd, J = 0.4, 2.4 Hz, 1 H), 6.60 (dd, J = 1.2, 1.6 Hz, 1 H), 5.79 (s, 2 H), 3.81, (s, 3 H), 1.18 (s, 9 H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 177.7, 164.9, 126.9, 122.6, 117.4, 111.0, 70.8, 51.2, 38.8, 26.8; **HRMS** (ESI) m/z 240.1233 [calc'd for $C_{12}H_{18}NO_4$ $(M + H)^+$ 240.1231].

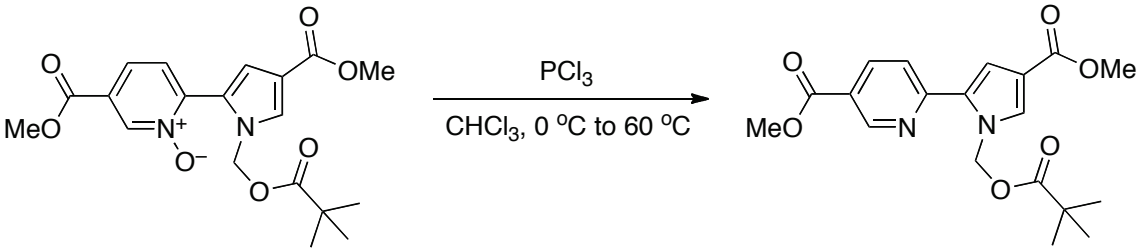
Methyl 2-(4-Methoxycarbonyl-1-pivaloyloxymethyl-1*H*-pyrrol-2-yl)pyridine-5-carboxylate-1-oxide



$Pd(OAc)_2$ (9.2 mg, 0.041 mmoles), Ag_2CO_3 (450 mg, 1.6 mmoles), and 3-methoxycarbonylpyridine-1-oxide (125 mg, 0.82 mmoles) were added to a dried flask. The flask was fitted with a reflux condenser capped with a septum, evacuated, and purged with N_2(g) (~5 times). A degassed solution of methyl 1-pivaloyloxymethyl-1*H*-pyrrole-3-carboxylate (215 mg, 0.90 mmoles), 2,6-lutidine (29 μL, 0.25 mmoles), and DMSO (275 μL, 5% [v/v]) in dry dioxane (5.2 mL) was added via syringe, and the reaction mixture was stirred at 110 °C for 48 h. The reaction mixture was then cooled and filtered through Celite®, and the filtrate was concentrated under reduced pressure. The crude product was purified by chromatography on silica (60–100% v/v EtOAC in hexanes) to afford the title compound (30 mg, 9.4%) as a yellow solid. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 8.88 (d, J = 1.2 Hz, 1 H), 7.87 (dd, J = 1.2, 8.0 Hz, 1 H),

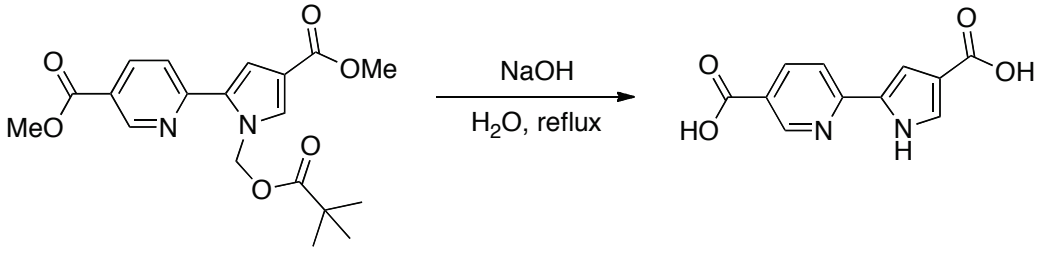
7.67 (d, *J* = 2.0 Hz, 1 H), 7.50 (d, *J* = 8.0 Hz, 1 H), 6.87 (d, *J* = 1.6 Hz, 1 H), 6.03 (s, 2 H), 3.99 (s, 3 H), 3.83 (s, 3 H), 1.03 (s, 9 H); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ*): 177.1, 164.1, 163.2, 145.5, 141.2, 130.6, 128.9, 128.4, 125.9, 125.4, 117.4, 115.8, 71.5, 53.1, 51.4, 38.6, 26.7; **HRMS** (ESI) *m*/*z* 391.15041 [calc'd for $C_{19}H_{23}N_2O_7$ $(M + H)^+$ 391.1500].

Methyl 2-(4-Methoxycarbonyl-1-pivaloyloxymethyl-1*H*-pyrrol-2-yl)pyridine-5-carboxylate



Methyl 2-(4-methoxycarbonyl-1-pivaloyloxymethyl-1*H*-pyrrol-2-yl)pyridine-5-carboxylate-1-oxide (30 mg, 0.077 mmoles) was dissolved in dry $CHCl_3$ (800 μL) and cooled to 0 °C in an ice bath, after which PCl_3 (8.1 μL, 0.092 mmoles) was added. The reaction mixture was stirred at 60 °C until starting material was consumed completely, as judged by TLC. The reaction was quenched by the dropwise addition of saturated aqueous Na_2CO_3 (3 mL) while stirring on ice. The product was extracted with DCM (4 × 3 mL), and the combined organic extracts were dried over Na_2SO_4(s) and concentrated under reduced pressure to afford the title compound (28 mg, 97%) as a brown solid. **^{1}H NMR** (400 MHz, $CDCl_3$, *δ*): 9.14 (d, *J* = 0.8, 2.0 Hz, 1 H), 8.28 (dd, *J* = 2.4, 8.4 Hz, 1 H), 7.66 (dd, *J* = 0.8, 8.4 Hz, 1 H), 7.63 (d, *J* = 2.0 Hz, 1 H), 7.19 (d, *J* = 1.6 Hz, 1 H), 6.49 (s, 2 H), 3.97 (s, 3 H), 3.86 (s, 3 H), 1.08 (s, 9 H); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ*): 177.5, 165.7, 164.4, 154.2, 150.2, 137.5, 132.1, 131.6, 123.2, 120.3, 116.6, 114.3, 71.5, 52.3, 51.4, 38.7, 26.8; **HRMS** (ESI) *m*/*z* 375.1550 [calc'd for $C_{19}H_{23}N_2O_6$ $(M + H)^+$ 375.1551].

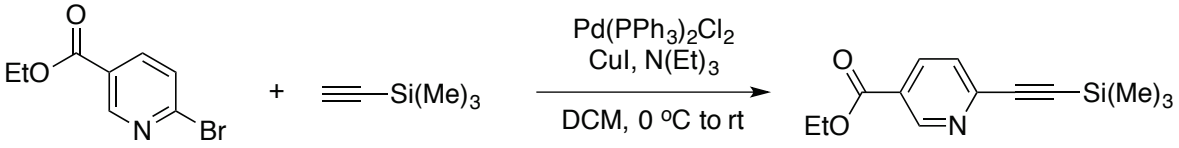
2-(4-Carboxy-1*H*-pyrrol-2-yl)pyridine-5-carboxylic Acid (pypyrroleDC)



Methyl 2-(4-methoxycarbonyl-1-pivaloyloxymethyl-1*H*-pyrrol-2-yl)pyridine-5-carboxylate (28 mg, 0.075 mmoles) and 2 M NaOH (1.0 mL) were added to a vial. The reaction mixture was heated to reflux with stirring until the starting material was consumed completely, as judged by TLC. The reaction mixture was cooled and concentrated under reduced pressure. The crude product was dissolved in water (2 mL). The aqueous layer was washed with EtOAc (1 × 2 mL), after which the product was precipitated from the aqueous layer by adjusting to pH 3–4 with 1 M HCl. After cooling to 4 °C, the product was filtered, washed with water (3 × 1 mL), and dried under high vacuum to afford the title compound (20 mg, 78%) as a grey solid. **^{1}H NMR**

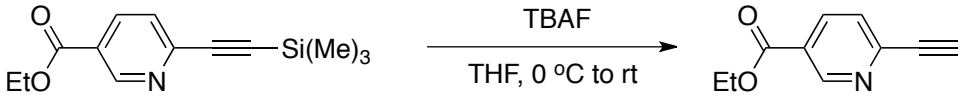
(500 MHz, DMSO-d_6, δ): 13.23 (bs, 1 H), 12.30 (s, 1 H), 12.13, (bs, 1 H), 9.00 (dd, J = 1.0, 2.5 Hz, 1 H), 8.21 (dd, J = 2.5, 8.5 Hz, 1 H), 7.91 (d, J = 8.5 Hz, 1 H), 7.46 (dd, J = 2.5, 3.5 Hz, 1 H), 7.26 (dd, J = 1.5, 2.5 Hz, 1 H); **^{13}C NMR** (125 MHz, DMSO-d_6, δ): 169.3, 168.4, 156.1, 153.3, 140.8, 134.7, 129.9, 126.8, 121.1 (2 signals), 114.0; **HRMS** (EI) m/z 232.0474 [calc'd for $C_{11}H_8N_2O_4$ $(M)^+$ 232.0479].

Ethyl 2-(2-Trimethylsilylethynyl)pyridine-5-carboxylate



$Pd(PPh_3)_2Cl_2$ (21 mg, 0.030 mmoles), CuI (142 mg, 0.089 mmoles), and ethyl 2-bromonicotinate (532 mg, 2.3 mmoles) were added to a dried flask on ice. The flask was evacuated and purged with N_2(g) (~5 times). A degassed solution of trimethylsilylacetylene (420 μL, 3.0 mmoles) in DCM (5 mL) was added to the flask with stirring on ice. Triethylamine (1.4 mL, 10.0 mmoles) was added to the flask with stirring, after which the flask was allowed to come to ambient temperature overnight. The reaction mixture was diluted with hexanes (5 mL) and filtered through Celite®, and the filtrate was washed with H_2O (50 mL) and brine (50 mL). The organic layer was dried over Na_2SO_4(s) and concentrated under reduced pressure to afford a solid, which was purified by chromatography on silica (5% v/v EtOAc in hexanes) to afford the title compound (544 mg, 95%) as a pale yellow solid. **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 9.17 (d, J = 1.6 Hz, 1 H), 8.26 (dd, J = 2.0, 8.0 Hz, 1 H), 7.54 (d, J = 7.6 Hz, 1 H), 4.43 (q, J = 7.2 Hz, 2 H), 1.43 (t, J = 7.2 Hz, 3 H), 0.30 (s, 9 H); **^{13}C NMR** (125 MHz, $CDCl_3$, δ): 167.5, 153.7, 149.2, 139.8, 129.4, 127.8, 105.8, 101.0, 64.3, 17.0, 2.3; **HRMS** (ESI) m/z 248.1102 [calc'd for $C_{13}H_{18}NO_2Si$ $(M + H)^+$ 248.1102].

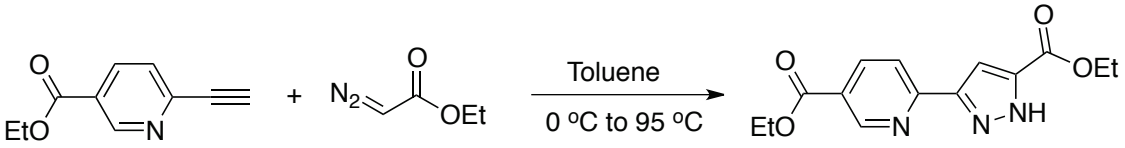
Ethyl 2-Ethynylpyridine-5-carboxylate



Ethyl 2-(2-trimethylsilylethynyl)pyridine-5-carboxylate (400 mg, 1.6 mmoles) was added to a flame-dried flask. The flask was evacuated and purged with N_2(g), after which THF (1.6 mL) was added on ice. 1.0 M TBAF in THF (1.9 mL) was added dropwise with stirring on ice, after which the flask was allowed to come to ambient temperature with stirring. After 20 min, the reaction mixture was concentrated under reduced pressure, and the crude residue was partitioned between EtOAc (10 mL) and 10% w/v $NaHCO_3$ (10 mL). The organic layer was collected, and the aqueous layer was extracted with EtOAc (10 mL). The combined organic extracts were dried over Na_2SO_4(s) and concentrated under reduced pressure to afford a solid, which was purified by chromatography on silica (20% v/v EtOAc in hexanes) to afford the title compound (144 mg, 51%) as a pale yellow solid. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 9.19 (d, J = 1.6 Hz, 1 H), 8.29 (dd, J = 2.0, 8.0 Hz, 1 H), 7.56 (d, J = 8.4 Hz, 1 H), 4.43 (q, J = 7.2 Hz, 2 H), 3.33 (s, 1 H), 1.43 (t, J

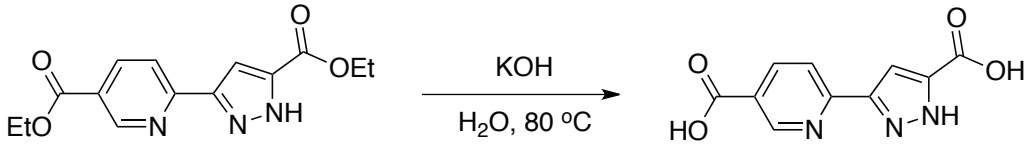
= 7.2 Hz, 3 H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 164.6, 151.0, 145.7, 137.2, 126.9, 125.6, 82.3, 79.8, 61.7, 14.2; **HRMS** (ESI) *m/z* 176.0708 [calc'd for $C_{10}H_{10}NO_2$ $(M + H)^+$ 176.0707].

Ethyl 2-(5-Ethoxycarbonyl-1*H*-pyrazol-3-yl)pyridine-5-carboxylate (diethyl pypyrDC)



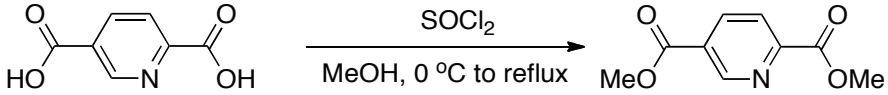
Ethyl 2-ethynylpyridine-5-carboxylate (91 mg, 0.52 mmoles) was dissolved in toluene (5 mL) in a glass vial, and the resulting solution was chilled on ice with stirring. A 73% w/w solution of ethyl diazoacetate in DCM (162 mg, 1.04 mmoles) was added dropwise, after which the vial was purged with N_2(g) and stirred at 95 °C. After 24 h, the reaction mixture was chilled on ice, and a second portion of ethyl diazoacetate (162 mg, 1.04 mmoles) was added. The reaction mixture was stirred at 95 °C for an addition 24 h, after which the reaction was concentrated under reduced pressure to afford a solid. The crude product was purified by chromatography on silica (30% EtOAc in hexanes) to afford the title compound (125 mg, 83%) as a pale yellow solid. **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 12.06 (bs, 1 H), 9.29 (dd, J = 0.5, 2.0 Hz, 1 H), 8.43 (dd, J = 2.0, 8.5 Hz, 1 H), 7.91 (d, J = 8.5 Hz, 1 H), 7.46 (s, 1 H), 4.483 (q, J = 7.0 Hz, 2 H), 4.480 (q, J = 7.0 Hz, 2 H), 1.47 (t, J = 7.0 Hz, 6 H); **^{13}C NMR** (125 MHz, $CDCl_3$, δ): 167.6, 164.0, 154.4, 153.6, 148.4 (br), 144.8 (br), 141.1, 128.3, 122.5, 110.2, 64.3, 64.1, 17.0 (2 signals); **HRMS** (ESI) *m/z* 312.0952 [calc'd for $C_{14}H_{15}N_3O_4Na$ $(M + Na)^+$ 312.0955].

2-(5-Carboxy-1*H*-pyrazol-3-yl)pyridine-5-carboxylic Acid (pypyrDC)



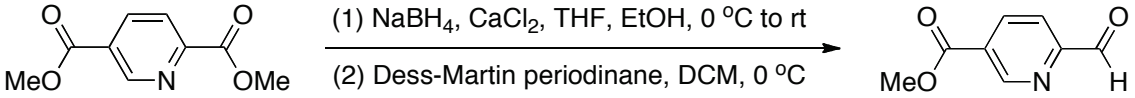
Ethyl 2-(5-ethoxycarbonyl-1*H*-pyrazol-3-yl)pyridine-5-carboxylate (38 mg, 0.13 mmoles) and KOH (34 mg, 0.525 mmoles) were added to a vial. H_2O (1.75 mL) was added to the vial and the reaction mixture was heated to 80 °C and stirred for 2 hr. The reaction mixture was cooled and washed with EtOAc (1 × 3 mL), after which the product was precipitated from the aqueous layer by adjusting to pH 3–4 with 1M HCl. After cooling to 4 °C, the product was filtered, washed with water (3 × 2 mL), and dried in vacuo to afford the title compound (24 mg, 78%) as a white solid. **^{1}H NMR** (500 MHz, DMSO-d_6, δ): 14.16–13.60 (m, 2 H), 9.10 (dd, J = 1.0, 2.5 Hz, 1 H), 8.33 (dd, J = 2.0, 8.0 Hz, 1 H), 8.10 (d, J = 8.5 Hz, 1 H), 7.37 (s, 1 H); **^{13}C NMR** (125 MHz, DMSO-d_6, δ): 169.2, 164.6 (br), 161.4 (q, J = 37 Hz, TFA), 160.0 (br), 153.4, 150.4 (br), 143.0 (br), 141.3, 128.6, 122.7, 118.4 (q, J = 287 Hz, TFA), 110.5; **HRMS** (ESI) *m/z* 235.0509 [calc'd for $C_{10}H_8N_3O_4$ $(M+H)^+$ 234.0509]. (Note: A small drop of TFA was added to the sample prior to recording the ^{13}C NMR spectrum in order to sharpen the peaks corresponding to the pyrazole C3, C4, C5, and carbonyl carbons, as is common with many free NH pyrazoles.[6])

Dimethyl 2,5-Pyridinedicarboxylate



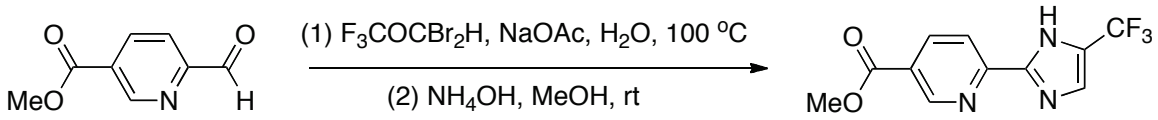
Thionyl chloride (2.2 mL) was added dropwise to MeOH (14 mL) while stirring on ice. 2,5-Pyridinedicarboxylic acid (1.0 g, 6.0 mmoles) was added, and the reaction mixture was heated at reflux for 3 h. The reaction mixture was cooled, and the solvent removed under reduced pressure. The resulting residue was dissolved in DCM (15 mL), after which saturated aqueous Na_2CO_3 (15 mL) was added while stirring on ice. The aqueous layer was extracted with DCM (3 × 15 mL), and the combined organic extracts were washed with saturated aqueous Na_2CO_3 (2 × 40 mL), dried over Na_2SO_4(s), and concentrated under reduced pressure to afford the title compound (915 mg, 78%) as a pale yellow solid. **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 9.32 (dd, J = 0.5, 2.0 Hz, 1 H), 8.47 (dd, J = 2.0, 8.0 Hz, 1 H), 8.23 (dd, J = 0.5, 8.0 Hz, 1 H), 4.06 (s, 3 H), 4.01 (s, 3 H); **^{13}C NMR** (125 MHz, $CDCl_3$, δ); 165.0, 164.9, 150.8, 150.8, 138.4, 128.6, 124.7, 53.3, 52.8; **HRMS** (ESI) m/z 196.0600 [calc'd for $C_9H_{10}NO_4$ $(M + H)^+$ 196.0605].

Methyl 6-Formylnicotinate



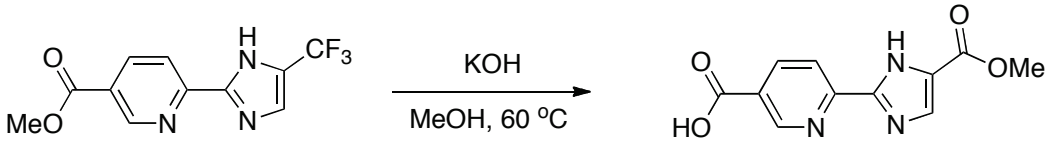
Dimethyl-2,5-pyridinedicarboxylate (960 mg, 4.9 mmoles) and $CaCl_2$ (2.198 g, 19.7 mmoles) were added to a dried flask. THF (11 mL) and EtOH (12 mL) were then added, and the resulting suspension was stirred on ice for 30 min. $NaBH_4$ (465 mg, 12.3 mmoles) was added portion-wise with stirring. The reaction mixture was allowed to come to ambient temperature overnight. After 18 h, the reaction was quenched by the dropwise addition of an aqueous solution of NH_4Cl (20 mL of saturated aqueous NH_4Cl plus 40 mL of H_2O) while stirring on ice. The aqueous layer was extracted with DCM (4 × 30 mL), and the combined organic extracts were dried over Na_2SO_4(s) and concentrated under reduced pressure to afford crude product (678 mg) as a pale yellow solid. The crude product was dissolved in dry DCM (45 mL), after which Dess–Martin periodinane (2.6 g, 6.1 mmoles) was added portion-wise with stirring on ice. After 6 h, the reaction was quenched by the dropwise addition of a solution of 5% w/v $Na_2S_2O_3$ in half-saturated $NaHCO_3$ (80 mL). The aqueous layer was extracted with DCM (3 × 40 mL), and the combined organic extracts were dried over Na_2SO_4(s) and concentrated under reduced pressure to afford the title compound (504 mg, 62%) as a pale yellow solid. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 10.16 (s, 1 H), 9.38 (dd, J = 0.5, 2.0 Hz, 1 H), 8.49 (dd, J = 2.0, 8.0 Hz, 1 H), 8.05 (dd, J = 0.5, 8.0 Hz, 1 H), 4.02 (s, 1 H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 192.6, 164.8, 154.9, 151.2, 138.3, 121.1, 52.9; **HRMS** (EI) m/z 165.0415 [calc'd for $C_8H_7NO_3$ (M^+) 165.0421]

Methyl 2-(5-Trifluoromethyl-1*H*-imidazol-2-yl)pyridine-5-carboxylate



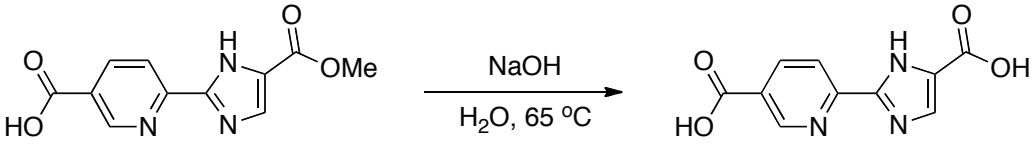
NaOAc (277 mg, 3.4 mmoles) and 2,2-dibromo-1,1,1-trifluoroacetone (454 mg, 1.7 mmoles) were dissolved in H_2O (770 μL) and stirred at 100 °C for 30 min. The reaction mixture was cooled to ambient temperature, after which a solution of methyl 6-formylnicotinate (250 mg, 1.5 mmoles) and concentrated NH_4OH (1.5 mL) in MeOH (4.6 mL), was added dropwise while stirring. The reaction mixture was stirred overnight, after which the solvent was removed under reduced pressure. $CHCl_3$ (25 mL) and 10% v/v $NaHCO_3$ (25 mL) were added to the residue. The aqueous layer was extracted with $CHCl_3$ (2 × 25 mL), and the combined organic extracts were dried over Na_2SO_4(s) and concentrated under reduced pressure. The crude product was purified by chromatography on silica (25% v/v EtOAc in hexanes) and recrystallized from 1:1 hexanes/EtOAc to afford the title compound (128 mg, 47%) as a pale yellow crystalline solid. **^{1}H NMR** (500 MHz, CD_3OD, δ): 9.13 (dd, J = 1.0, 2.0 Hz, 1 H), 8.38 (dd, J = 2.0, 8.5 Hz, 1 H), 8.15 (dd, J = 1.0, 8.5 Hz, 1 H), 7.72 (d, J = 1.0 Hz, 1 H), 3.96 (s, 3 H); **^{13}C NMR** (125 MHz, CD_3OD, δ): 165.2, 150.5, 150.2, 146.7, 137.9, 132.6 (q, J = 39 Hz), 125.7, 121.7 (q, J = 265 Hz), 119.3, 119.1, 51.6; **HRMS** (ESI) m/z 272.0630 [calc'd for $C_{11}H_9F_3N_3O_2$ $(M + H)^+$ 272.0642].

2-(5-Methoxycarbonyl-1-*H*-imidazol-2-yl)pyridine-5-carboxylic Acid



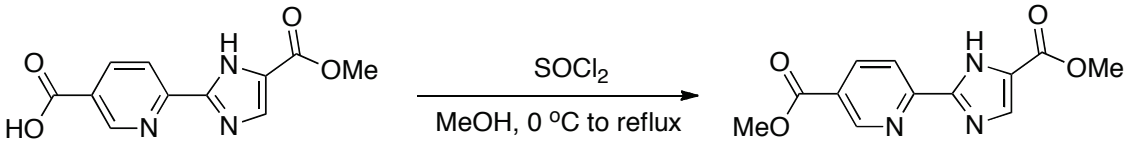
Methyl 2-(5-trifluoromethyl-1*H*-imidazol-2-yl)pyridine-5-carboxylate (103 mg, 0.38 mmoles) and KOH (147 mg, 2.28 mmoles) were added to a vial. MeOH (11 mL) was added to the vial, and the reaction mixture was heated to 60 °C until the starting material was consumed completely, as judged by TLC. The reaction mixture was cooled, and concentrated under reduced pressure. The crude product was dissolved in water (3 mL). The aqueous layer was washed with EtOAc (1 × 3 mL), after which the product was precipitated from the aqueous layer by adjusting to pH 3–4 with 1 M HCl. After cooling to 4 °C, the product was filtered, washed with water (3 × 1 mL), and dried under high vacuum to afford the title compound (84 mg, 94%) as a white solid. **^{1}H NMR** (500 MHz, DMSO-d_6, δ); 13.74 (s, 1 H), 13.56 (s, 1 H), 9.12 (d, J = 2.0 Hz, 1 H), 8.40 (dd, J = 2.0, 8.0 Hz, 1 H), 8.22 (d, J = 8.0 Hz, 1 H), 7.99 (d, J = 2.5 Hz, 1 H), 3.81 (s, 3 H); **^{13}C NMR** (125 MHz, DMSO-d_6, δ): 166.4, 163.1, 151.4, 150.5, 146.4, 138.9, 134.2, 126.6 (2 signals), 120.3, 51.7; **HRMS** (ESI) m/z 248.0671 [calc'd for $C_{11}H_{10}N_3O_4$ $(M + H)^+$ 248.0666].

2-(5-Carboxy-1-*H*-imidazol-2-yl)pyridine-5-carboxylic Acid (pyimDC)



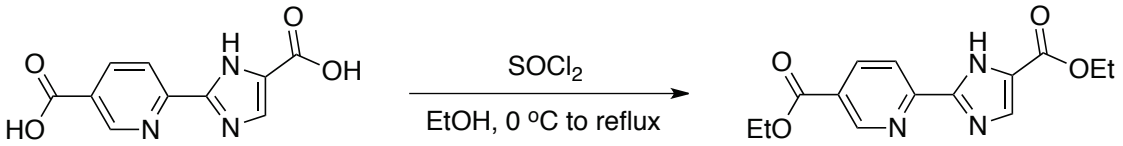
2-(5-Methoxycarbonyl-1*H*-imidazol-2-yl)pyridine-5-carboxylic acid (50 mg, 0.20 mmoles), 1 M NaOH (400 μL), and water (3.6 mL) were added to a vial. The reaction mixture was heated to 65 °C with stirring. After 24 h, the reaction mixture was cooled and washed with EtOAc (1 × 3 mL), after which the product was precipitated from the aqueous layer by adjusting to pH 3–4 with 1 M HCl. After cooling to 4 °C, the product was filtered, washed with water (3 × 1 mL), and dried under high vacuum to afford the title compound (44 mg, 93%) as a pale yellow solid. **^{1}H NMR** (500 MHz, DMSO-d_6, δ): 13.62 (s, 1 H), 13.30 (bs, 1 H), 12.74 (bs, 1 H), 9.11 (dd, J = 0.5, 1.0 Hz, 1 H), 8.39 (dd, J = 2.0, 8.5 Hz, 1 H), 8.20 (d, J = 8.0 Hz, 1 H), 7.88 (d, J = 2.5 Hz, 1 H); **^{13}C NMR** (125 MHz, DMSO-d_6, δ): 166.4, 164.0, 151.5, 150.5, 146.1, 138.9, 135.3, 126.5, 126.2, 120.2; **HRMS** (EI) m/z 233.0432 [calc'd for $C_{10}H_7N_3O_4$ $(M)^+$ 233.18].

Methyl 2-(5-Methoxycarbonyl-1*H*-imidazol-2-yl)pyridine-5-carboxylate (dimethyl pyimDC)



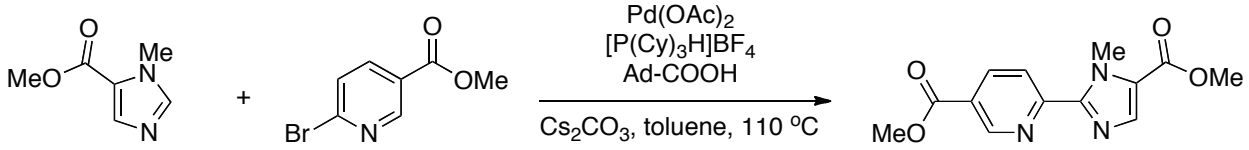
2-(5-Methoxycarbonyl-1*H*-imidazol-2-yl)pyridine-5-carboxylic acid (100 mg, 0.41 mmoles) and MeOH (7.0 mL) was added to a round-bottom flask and stirred on ice. Thionyl chloride (750 μL) was added dropwise on ice, after which the flask was fitted with a reflux condenser and heated at reflux. After 24 h, the reaction mixture was cooled on ice and quenched by the dropwise addition of saturated aqueous Na_2CO_3 (20 mL). The aqueous layer was extracted with CH_2Cl_2 (5 × 20 mL) and the combined organic extracts were dried over Na_2SO_4(s), and concentrated under reduced pressure. The crude product was purified by chromatography on silica (50% v/v EtOAc in hexanes), to afford a pale yellow solid. The solid was dissolved in minimal DCM, precipitated by the dropwise addition of cold hexanes, and filtered to afford the title compound (50 mg, 48%) as a white solid. **^{1}H NMR** (500 MHz, $CDCl_3$, ~5:2 ratio of 2 tautomers, δ); 11.01 (bs, 1.4 H), 9.18–9.15 (m, 1.4 H), 8.44–8.38 (m, 2.4 H), 8.29 (d, J = 8 Hz, 0.4 H), 7.91 (s, 1 H), 7.84 (s, 0.4 H), 3.99–3.94 (m, 8.4 H); **^{13}C NMR** (125 MHz, $CDCl_3$, ~5:2 ratio of 2 tautomers, δ): 165.3 (2 signals), 163.2, 160.2, 150.7, 150.4 (2 signals), 150.3, 147.8, 146.2, 138.4 (2 signals), 136.7, 135.4, 126.1, 125.9, 124.3 (2 signals), 120.2, 120.0, 52.6 (2 signals), 52.1 (2 signals); **HRMS** (ESI) m/z 262.0829 [calc'd for $C_{12}H_{12}N_3O_4$ $(M + H)^+$ 262.0823].

Ethyl 2-(5-Ethoxycarbonyl-1*H*-imidazol-2-yl)pyridine-5-carboxylate (diethyl pyimDC)



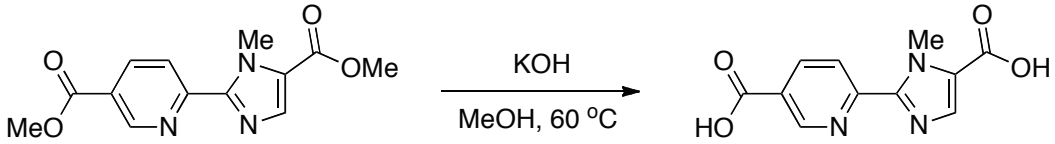
2-(5-Carboxy-1*H*-imidazol-2-yl)pyridine-5-carboxylic acid (85 mg, 0.36 mmoles) and EtOH (6.3 mL) were added to a round-bottom flask and stirred on ice. Thionyl chloride (710 μL) was added dropwise on ice, after which the flask was fitted with a reflux condenser and heated at reflux. After 24 h, the reaction mixture was cooled on ice and quenched by the dropwise addition of saturated aqueous Na_2CO_3 (10 mL). The aqueous layer was extracted with CH_2Cl_2 (6 × 10 mL) and the combined organic extracts were dried over Na_2SO_4(s) and concentrated under reduced pressure. The crude product was purified by chromatography on silica (2% v/v MeOH in 1:1 DCM/hexanes) to afford the title compound (95 mg, 90 %) as a pale yellow solid. **^{1}H NMR** (500 MHz, $CDCl_3$, ~5:4 ratio of two tautomers, *δ*): 11.16 (bs, 1 H), 11.02 (bs, 0.8 H), 9.19 (dd, *J* = 1.0, 2.0 Hz, 0.8 H); 9.15–9.14 (m, 1 H), 8.44–8.38 (m, 2.8 H), 8.28 (dd, *J* = 1.0, 8.0 Hz, 0.8 H), 7.90–7.86 (m, 1.8 H), 4.48–4.38 (m, 7.2 H), 1.46–1.39 (m, 10.8 H); **^{13}C NMR** (125 MHz, $CDCl_3$, *δ*): 164.9, 164.8 162.8, 159.8, 150.7, 150.4 (2 signals), 150.3, 147.8, 146.3, 138.4, 138.3, 136.5, 135.7, 126.3, 126.1, 124.7, 124.1, 120.1, 120.0, 61.7 (2 signals), 61.3, 61.0, 14.5, 14.4, 14.3 (2 signals); **HRMS** (ESI) *m*/*z* 290.1136 [calc'd for $C_{14}H_{16}N_3O_4$ $(M + H)^+$ 290.1132].

Methyl 2-(5-Methoxycarbonyl-1-methyl-1*H*-imidazol-2-yl)pyridine-5-carboxylate (dimethyl NMe-pyimDC)



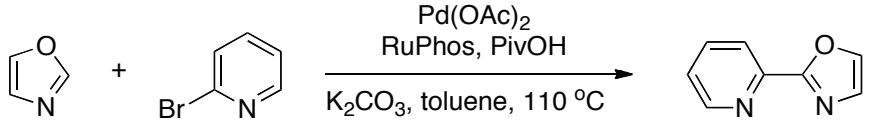
$Pd(OAc)_2$ (32 mg, 0.14 mmoles), $[P(Cy)_3H]BF_4$ (158 mg, 0.43 mmoles), 1-adamantanecarboxylic acid (258 mg, 1.4 mmoles), Cs_2CO_3 (1.9 g, 5.7 mmoles), methyl 6-bromonicotinate (618 mg, 2.9 mmoles) and methyl 1-methyl-1*H*-imidazole-5-carboxylate (400 mg, 2.9 mmoles) were added to a dried flask. The flask was fitted with a reflux condenser capped with a septum, evacuated, and purged with N_2(g) (~5 times). Dry toluene (4.8 mL) was added via syringe, and the reaction mixture was stirred at 110 °C for 48 h. The reaction mixture was then cooled and filtered through Celite®, and the filtrate was concentrated under reduced pressure. The crude product was purified by chromatography on silica (30% v/v EtOAc in hexanes) to afford the title compound (40 mg, 5%) as a pale yellow solid. **^{1}H NMR** (400 MHz, $CDCl_3$, *δ*): 9.23 (d, *J* = 1.2 Hz, 1 H), 8.38 (dd, *J* = 2.0, 8.4 Hz, 1 H), 8.28 (d, *J* = 8.4 Hz, 1 H), 7.84 (s, 1 H), 4.43 (s, 3 H), 3.98 (s, 3 H), 3.89 (s, 3 H); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ*): 165.4, 160.8, 153.2, 149.8, 148.2, 137.6, 137.3, 125.6, 125.1, 123.8, 52.5, 51.6, 35.0; **HRMS** (ESI) *m*/*z* 298.0796 [calc'd for $C_{13}H_{13}N_3O_4Na$ $(M + Na)^+$ 298.0799].

2-(5-Carboxy-1-methyl-1*H*-imidazol-2-yl)pyridine-5-carboxylic Acid (NMe-pyimDC)



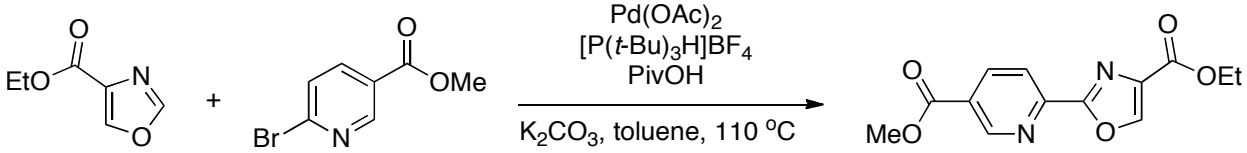
Methyl 2-(5-methoxycarbonyl-1-methyl-1*H*-imidazol-2-yl)pyridine-5-carboxylate (30 mg, 0.11 mmoles) and KOH (28 mg, 0.44 mmoles) were added to a vial. MeOH (3 mL) was added to the vial and the reaction mixture was heated to 60 °C with stirring until the starting material was consumed completely, as judged by TLC. The reaction mixture was cooled and concentrated under reduced pressure, after which the crude product was dissolved in water (2 mL). The aqueous layer was washed with EtOAc (1 × 2 mL), after which the product was precipitated from the aqueous layer by adjusting to pH 3–4 with 1 M HCl. After cooling to 4 °C, the product was filtered, washed with water (3 × 1 mL), and dried under high vacuum to afford the title compound (16 mg, 58%) as a pale yellow solid. **^{1}H NMR** (500 MHz, DMSO-d_6, δ): 13.49 (bs, 1 H), 13.2 (bs, 1 H), 9.16 (d, J = 2.0 Hz, 1 H), 8.41 (dd, J = 2.0, 8.5 Hz, 1 H), 8.25 (d, J = 8.5 Hz, 1 H), 7.79 (s, 1 H), 4.33 (s, 3 H); **^{13}C NMR** (125 MHz, DMSO-d_6, δ): 166.4, 161.7, 153.1, 149.9, 148.0, 138.5, 137.2, 126.7, 126.3, 124.3, 35.1; **HRMS** (EI) m/z 247.0583 [calc'd for $C_{11}H_9N_3O_4$ $(M)^+$ 247.0588].

2-(Oxazol-2-yl)pyridine (pyox)



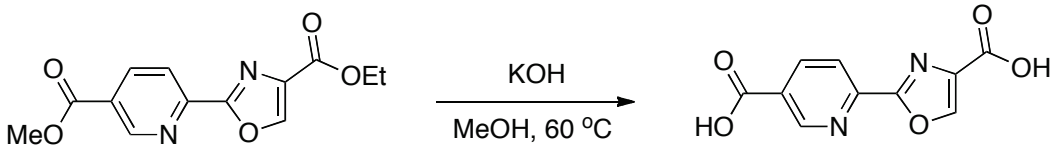
$Pd(OAc)_2$ (33 mg, 0.15 mmoles), RuPhos (135 mg, 0.29 mmoles), pivalic acid (119 mg, 1.2 mmoles) and K_2CO_3 (1.2 g, 8.7 mmoles) were added to a dried flask. The flask was fitted with a reflux condenser capped with a septum, evacuated, and purged with N_2(g) (~5 times). A degassed solution of oxazole (400 mg, 5.8 mmoles) and 2-bromopyridine (457 mg, 2.9 mmoles) in dry toluene (14.5 mL) was added via syringe, and the reaction mixture was stirred at 110 °C for 24 h. The reaction mixture was then cooled and filtered through Celite®, and the filtrate was concentrated under reduced pressure. The crude product was purified by chromatography on silica (20–75% v/v EtOAc in hexanes followed by 10% v/v acetone in 1:1 DCM/hexanes) to afford the title compound (62 mg, 7%) as a golden oil. **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 8.67 (d, J = 5.0 Hz, 1 H), 8.08 (d, J = 8.0 Hz, 1 H), 7.78–7.74 (m, 2 H), 7.30 (ddd, J = 1.0, 5.0, 8.0 Hz, 1 H), 7.25 (s, 1 H); **^{13}C NMR** (125 MHz, $CDCl_3$, δ): 160.7, 149.9, 146.0, 139.8, 137.0, 128.8, 124.7, 122.0; **HRMS** (EI) m/z 146.0472 [calc'd for $C_8H_7N_2O$ $(M)^+$ 146.0475].

Methyl 2-(4-Ethoxycarbonyloxazol-2-yl)pyridine-5-carboxylate (methylethyl pyoxDC*)



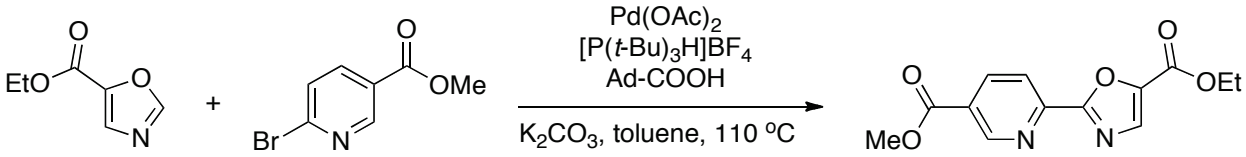
$Pd(OAc)_2$ (15.9 mg, 0.071 mmoles), $[P(t\text{-}Bu)_3H]BF_4$ (61.8 mg, 0.21 mmoles), pivalic acid (29.0 mg, 0.28 mmoles), K_2CO_3 (391 mg, 2.8 mmoles), ethyl oxazole-4-carboxylate (200 mg, 1.4 mmoles), and methyl 6-bromonicotinate (612 mg, 2.8 mmoles) were added to a dried flask. The flask was fitted with a reflux condenser capped with a septum, evacuated, and purged with N_2(g) (~5 times). Dry toluene (7 mL) was added via syringe, and the reaction mixture was stirred at 110 °C for 24 h. The reaction mixture was then cooled and filtered through Celite®, and the filtrate was concentrated under reduced pressure. The crude product was purified via chromatography on silica (2% v/v acetone in DCM followed by 10% v/v acetone in 1:1 DCM/hexanes) to afford the title compound (113 mg, 29%) as a white solid. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 9.31 (dd, J = 0.8, 2.0 Hz, 1 H), 8.46 (dd, J = 2.0, 8.4 Hz, 1 H), 8.42 (s, 1 H), 8.38 (dd, J = 0.8, 8.4 Hz, 1 H), 4.45 (q, J = 7.2 Hz, 2 H), 4.00 (s, 3 H), 1.42 (t, J = 7.2 Hz, 3 H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 164.9, 160.8, 160.2, 151.1, 148.0, 145.2, 138.3, 135.3, 127.1, 122.3, 61.6, 52.7, 14.3; **HRMS** (ESI) m/z 277.0815 [calc'd for $C_{13}H_{13}N_2O_5$ $(M + H)^+$ 277.0823].

2-(4-Carboxyoxazol-2-yl)pyridine-5-carboxylic Acid (pyoxDC*)



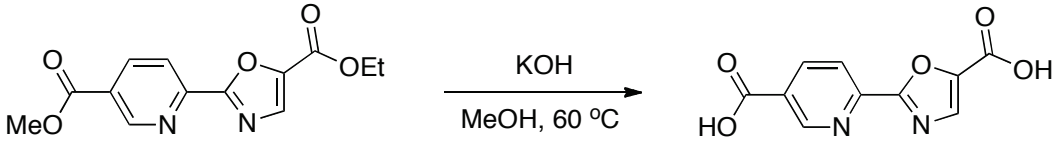
Methyl 2-(4-ethoxycarbonyloxazol-2-yl)-pyridine-5-carboxylate (50 mg, 0.18 mmoles) and KOH (46.7 mg, 0.72 mmoles) were added to a vial. MeOH (5.0 mL) was added to the vial, and the reaction mixture was heated to 60 °C with stirring until the starting material was consumed completely, as judged by TLC. The reaction mixture was cooled and concentrated under reduced pressure, after which the crude product was dissolved in water (2 mL). The aqueous layer was washed with EtOAc (1 × 2 mL), after which the product was precipitated from the aqueous layer by adjusting to pH 3–4 with 1 M HCl. After cooling to 4 °C, the product was filtered, washed with water (3 × 1 mL), and dried under high vacuum to afford the title compound (30 mg, 71%) as a white solid. **^{1}H NMR** (500 MHz, DMSO-d_6, δ): 13.72 (bs, 1 H), 13.40 (bs, 1 H), 9.19 (dd, J = 0.5, 2.0 Hz, 1 H), 9.02 (s, 1 H), 8.47 (dd J = 2.0, 8.5 Hz, 1 H), 8.28 (dd, J = 0.5, 8.5 Hz, 1 H); **^{13}C NMR** (125 MHz, DMSO-d_6, δ): 166.1, 162.2, 160.1, 151.1, 148.0, 147.4, 139.1, 135.4, 128.2, 122.8; **HRMS** (ESI) m/z 233.0195 [calc'd for $C_{10}H_5N_2O_5$ $(M - H)^-$ 233.0203].

Methyl 2-(5-Ethoxycarbonyloxazol-2-yl)pyridine-5-carboxylate (ethylmethyl pyoxDC)



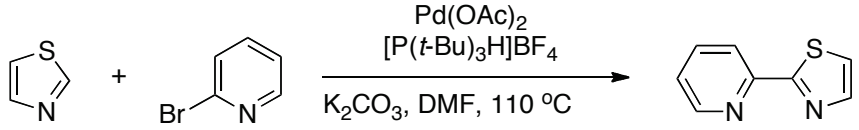
$Pd(OAc)_2$ (31.8 mg, 0.14 mmoles), $[P(t\text{-}Bu)_3H]BF_4$ (123.2 mg, 0.42 mmoles), 1-adamantanecarboxylic acid (153 mg, 0.85 mmoles), K_2CO_3 (782 mg, 5.7 mmoles), and methyl 6-bromonicotinate (918 mg, 4.3 mmoles) were added to a dried flask. The flask was fitted with a reflux condenser capped with a septum, evacuated, and purged with N_2(g) (~5 times). A degassed solution of ethyl oxazole-5-carboxylate (400 mg, 2.8 mmoles) in dry toluene (7 mL) was added via syringe, and the reaction mixture was stirred at 110 °C for 8 h. The reaction mixture was then cooled and filtered through Celite®, and the filtrate was concentrated under reduced pressure. The crude product was purified by chromatography on silica (30% EtOAc in hexanes followed by 2% v/v acetone in 1:1 DCM/hexanes) to afford the title compound (183 mg, 23%) as a white solid. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 9.37 (dd, J = 0.8, 2.0 Hz, 1 H), 8.47 (dd, J = 2.0, 8.0 Hz, 1 H), 8.30 (dd, J = 0.8, 8 Hz, 1 H), 7.96 (s, 1 H), 4.46 (q, J = 7.2 Hz, 2 H), 4.00 (s, 3 H), 1.43 (t, J = 7.2 Hz, 3 H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 164.9, 161.6, 157.5, 151.5, 148.0, 143.8, 138.3, 135.5, 127.3, 122.5, 61.9, 52.7, 14.3; **HRMS** (ESI) m/z 277.0823 [calc'd for $C_{13}H_{13}N_2O_5$ $(M + H)^+$ 279.0819].

2-(5-Carboxyoxazol-2-yl)pyridine-5-carboxylic Acid (pyoxDC)



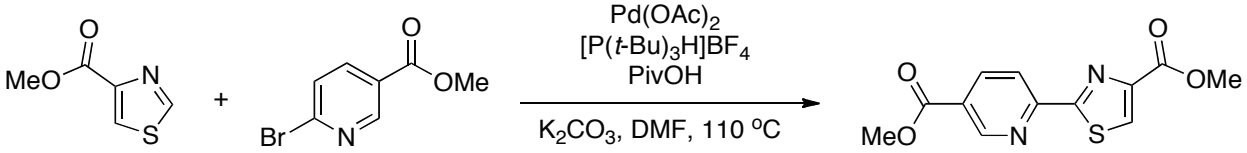
Methyl 2-(5-ethoxycarbonyloxazol-2-yl)-pyridine-5-carboxylate (45 mg, 0.16 mmoles) and KOH (42 mg, 0.65 mmoles). MeOH (4.7 mL) was added to the vial, and the reaction mixture was heated to 60 °C with stirring until the starting material was consumed completely, as judged by TLC. The reaction mixture was cooled and concentrated under reduced pressure. The crude product was dissolved in water (2 mL). The aqueous layer was washed with EtOAc (1 × 2 mL), after which the product was precipitated from the aqueous layer by adjusting to pH 3–4 with 1 M HCl. After cooling to 4 °C, the product was filtered, washed with water (3 × 1 mL), and dried under high vacuum to afford the title compound (24.6 mg, 64%) as a light green solid. **^{1}H NMR** (500 MHz, DMSO-d_6, δ): 13.96 (bs, 1 H), 13.78 (bs, 1 H), 9.22 (dd, J = 1.0, 2.0 Hz, 1 H), 8.48 (dd, J = 2.0, 8.5 Hz, 1 H), 8.23 (dd, J = 1.0, 8.5 Hz, 1 H), 8.16 (s, 1 H); **^{13}C NMR** (125 MHz, DMSO-d_6, δ): 166.1, 161.6, 158.9, 151.2, 147.9, 144.6, 139.1, 135.7, 128.4, 123.3; **HRMS** (ESI) m/z 233.0203 [calc'd for $C_{10}H_5N_2O_5$ $(M - H)^-$ 233.0203].

2-(Thiazol-2-yl)pyridine (pythi)



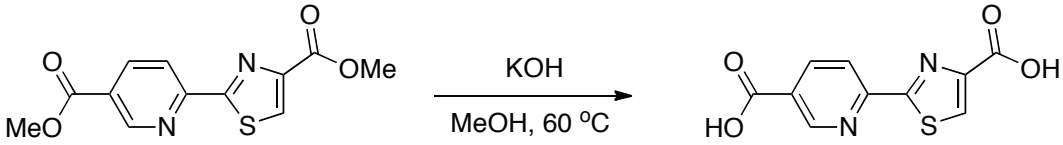
$Pd(OAc)_2$ (35.5 mg, 0.16 mmoles), $[P(t\text{-}Bu)_3H]BF_4$ (137.5 mg, 0.47 mmoles), and K_2CO_3 (874 mg, 6.3 mmoles) were added to a dried flask. The flask was fitted with a reflux condenser capped with a septum, evacuated, and purged with N_2(g) (~5 times). A degassed solution of thiazole (1.08 g, 12.7 mmoles) and 2-bromopyridine (500 mg, 3.2 mmoles) in dry DMF (16 mL) was added via syringe, and the reaction mixture was stirred at 110 °C for 24 h. The reaction mixture was cooled, H_2O (60 mL) was added, and the aqueous layer was extracted with ether (4 × 60 mL). The combined organic extracts were washed with H_2O (1 × 100 mL) and brine (1 × 100 mL), dried over Na_2SO_4(s), and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (20–40% v/v EtOAc in hexanes followed by 2% v/v acetone in 1:1 DCM/hexanes) to afford the title compound (142 mg, 28%) as a white solid. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 8.58 (d, J = 4.8 Hz, 1 H), 8.16 (d, J = 8.0 Hz, 1 H), 7.89 (d, J = 3.2 Hz, 1 H), 7.75 (td, J = 1.6, 8.0 Hz, 1 H), 7.41 (d, J = 3.2 Hz, 1 H), 7.26 (ddd, J = 1.2, 4.8, 8.0 Hz, 1 H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 169.3, 151.3, 149.4, 144.0, 137.0, 124.4, 121.4, 119.6; **HRMS** (EI) m/z 162.0247 [calc'd for $C_8H_7N_2S$ $(M)^+$162.0247].

Methyl 2-(4-Methoxycarbonylthiazol-2-yl)pyridine-5-carboxylate (dimethyl pythiDC*)



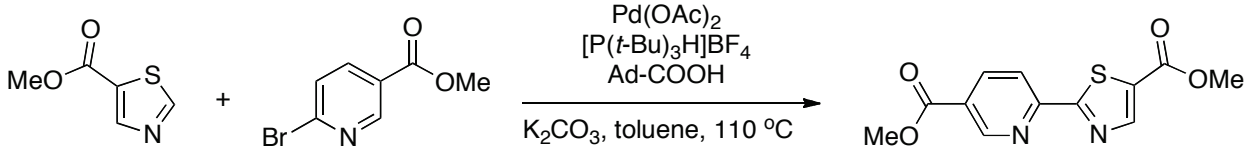
$Pd(OAc)_2$ (15.7 mg, 0.070 mmoles), $[P(t\text{-}Bu)_3H]BF_4$ (60.7 mg, 0.21 mmoles), pivalic acid (28.5 mg, 0.28 mmoles), K_2CO_3 (386 mg, 2.8 mmoles), methyl thiazole-4-carboxylate (200 mg, 1.4 mmoles), and methyl 6-bromonicotinate (603 mg, 2.8 mmoles) were added to a dried flask. The flask was fitted with a reflux condenser capped with a septum, evacuated, and purged with N_2(g) (~5 times). Dry DMF (7 mL) was added via syringe, and the reaction mixture was stirred at 110 °C for 6 h. The reaction mixture was then cooled and filtered through Celite®, and the filtrate was concentrated under reduced pressure. The crude product was purified by chromatography on silica (1% v/v acetone in DCM) to afford the title compound (39 mg, 10%) as a white solid. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 9.18 (dd, J = 0.8, 2.0 Hz, 1 H), 8.41 (dd, J = 2.0, 8.4 Hz, 1 H), 8.38 (dd, J = 0.8, 8.4 Hz, 1 H), 8.33 (s, 1 H), 3.99 (s, 3 H), 3.98 (s, 3 H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 168.7, 165.1, 161.7, 153.3, 150.7, 148.3, 138.3, 130.7, 126.9, 119.6, 52.6, 52.6; **HRMS** (ESI) m/z 279.0423 [calc'd for $C_{12}H_{11}N_2O_4S$ $(M + H)^+$ 279.0435].

2-(4-Carboxythiazol-2-yl)pyridine-5-carboxylic Acid (pythiDC*)



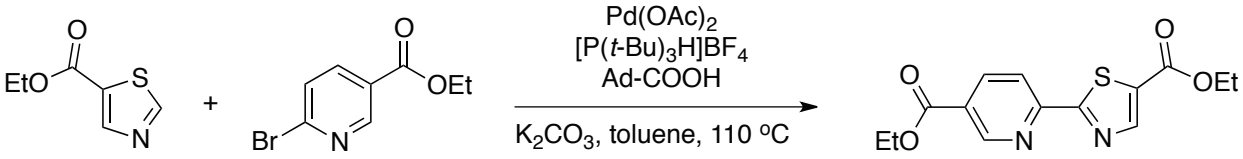
Methyl 2-(4-methoxycarbonylthiazol-2-yl)-pyridine-5-carboxylate (30 mg, 0.11 mmoles) and KOH (27.8 mg, 0.43 mmoles) were added to a vial. MeOH (3.1 mL) was added to the vial, and the reaction mixture was heated to 60 °C with stirring until the starting material was consumed completely, judged by TLC. The reaction mixture was cooled and concentrated under reduced pressure. The crude product was dissolved in water (2 mL). The aqueous layer was washed with EtOAc (1 × 2 mL), after which the product was precipitated from the aqueous layer by adjusting to pH 3–4 with 1 M HCl. After cooling to 4 °C, the product was filtered, washed with water (3 × 1 mL), and dried under high vacuum to afford the title compound (21 mg, 78%) as a white solid. **^{1}H NMR** (500 MHz, DMSO-d_6, δ): 13.51 (bs, 2 H), 9.13 (dd, J = 0.5, 2.0 Hz, 1 H), 8.67 (s, 1 H), 8.47 (dd, J = 2.0, 8.5 Hz, 1 H), 8.28 (dd, J = 0.5, 8.5 Hz, 1 H); **^{13}C NMR** (125 MHz, DMSO-d_6, δ): 168.0, 166.2, 162.5, 153.0, 151.1, 149.4, 139.4, 132.2, 128.6, 119.8; **HRMS** (ESI) m/z 248.9975 [calc'd for $C_{10}H_5N_2O_4S$ $(M - H)^-$ 248.9975].

Methyl 2-(5-Methoxycarbonylthiazol-2-yl)pyridine-5-carboxylate (dimethyl pythiDC)



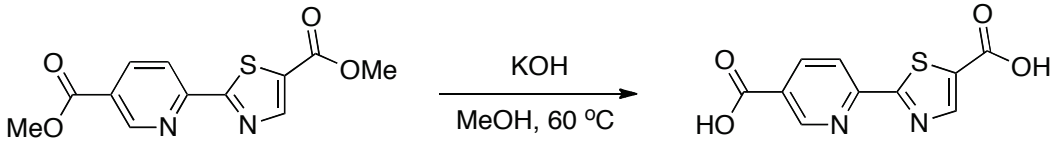
$Pd(OAc)_2$ (3.9 mg, 0.017 mmoles), $[P(t\text{-}Bu)_3H]BF_4$ (10.0 mg, 0.035 mmoles), 1-adamantanecarboxylic acid (18.4 mg, 0.10 mmoles), K_2CO_3 (96.5 mg, 0.70 mmoles), methyl thiazole-5-carboxylate (50 mg, 0.35 mmoles), and methyl 6-bromonicotinate (113 mg, 0.52 mmoles) were added to a dried flask. The flask was fitted with a reflux condenser capped with a septum, evacuated, and purged with N_2(g) (~5 times). Dry toluene (1.7 mL) was added via syringe, and the reaction mixture was stirred at 110 °C for 48 h. The reaction mixture was then cooled and filtered through Celite®, and the filtrate was concentrated under reduced pressure. The crude product was purified by chromatography on silica (3% v/v acetone in 1:1 DCM/hexanes) to afford the title compound (28 mg, 29%) as a white solid. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 9.22 (s, 1 H), 8.51 (s, 1 H), 8.43 (dd, J = 2.0, 8.0 Hz, 1 H), 8.29 (d, J = 8.0 Hz, 1 H), 4.00 (s, 3 H), 3.96 (s, 3 H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 172.7, 165.1, 161.8, 153.6, 150.9, 149.7, 138.4, 131.7, 127.1, 119.6, 52.6, 52.6; **HRMS** (ESI) m/z 279.0439 [calc'd for $C_{12}H_{11}N_2O_4S$ $(M + H)^+$ 279.0435].

Ethyl 2-(5-Ethoxycarbonylthiazol-2-yl)pyridine-5-carboxylate (diethyl pythiDC)



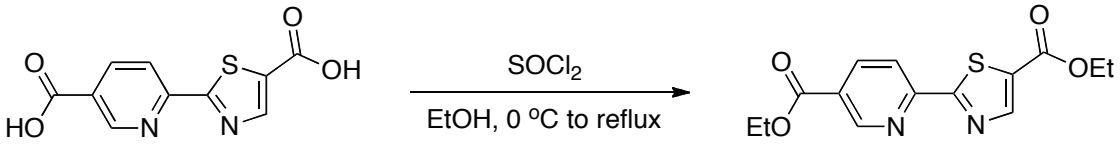
$Pd(OAc)_2$ (150 mg, 0.67 mmoles), $[P(t\text{-}Bu)_3H]BF_4$ (388 mg, 1.34 mmoles), 1-adamantanecarboxylic acid (723 mg, 4.01 mmoles), K_2CO_3 (3.7 g, 27 mmoles), and ethyl 6-bromonicotinate (4.6 g, 20 mmoles) were added to a dried flask. The flask was fitted with a reflux condenser capped with a septum, evacuated, and purged with N_2(g) (~5 times). A solution of ethyl thiazole-5-carboxylate (2.1 g, 13 mmoles) in dry toluene (65 mL) was added, and the resulting solution was stirred at 110 °C for 72 h. The reaction mixture was cooled and filtered through Celite®, and the filtrate was concentrated under reduced pressure. The crude product was purified by chromatography on silica (25% EtOAc/hexanes followed by 3% v/v acetone in 1:1 DCM/hexanes) and recrystallized from 1:1 EtOAc/hexanes to afford the title compound (1.48 g, 40%) as a white crystalline solid. **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 9.22 (dd, J = 0.8, 2.0 Hz, 1 H), 8.5 (s, 1 H), 8.43 (dd, J = 2.0, 8.4 Hz, 1 H), 8.29 (dd, J = 0.8, 8.4 Hz, 1 H), 4.48–4.39 (m, 4 H), 1.46–1.40 (m, 6 H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 172.6, 164.6, 161.3, 153.6, 150.9, 149.5, 138.3, 132.1, 127.4, 119.5, 61.8, 61.7, 14.27 (2 signals); **HRMS** (ESI) m/z 307.0752 [calc'd for $C_{14}H_{15}N_2O_4S$ $(M + H)^+$ 307.0748].

2-(5-Carboxythiazol-2-yl)pyridine-5-carboxylic Acid (pythiDC)



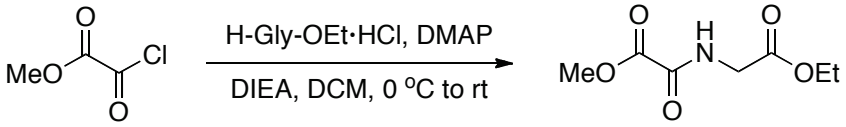
Methyl 2-(5-methoxycarbonylthiazol-2-yl)-pyridine-5-carboxylate (70 mg, 0.25 mmoles) and KOH (63.1 mg, 1.0 mmoles) were added to a vial. MeOH (7.0 mL) was added to the vial, and the reaction mixture was heated to 60 °C with stirring until the starting material was consumed completely, as judged by TLC. The reaction mixture was cooled and concentrated under reduced pressure. The crude product was dissolved in water (4 mL). The aqueous layer was washed with EtOAc (1 × 4 mL), after which the product was precipitated from the aqueous layer by adjusting to pH 2 with 1 M HCl. After cooling to 4 °C, the product was filtered, washed with water (3 × 1 mL), and dried under high vacuum to afford the title compound (38 mg, 61%) as a pale yellow solid. **^{1}H NMR** (500 MHz, DMSO-d_6, δ): 13.80 (bs, 1 H), 13.73 (bs, 1 H), 9.15 (dd, J = 0.5, 2.0 Hz, 1 H), 8.56 (s, 1 H), 8.48 (dd, J = 2.0, 8.0 Hz, 1 H), 8.31 (dd, J = 0.5, 8.0 Hz, 1 H); **^{13}C NMR** (125 MHz, DMSO-d_6, δ): 172.1, 166.1, 162.6, 153.1, 151.2, 149.9, 139.5, 133.6, 128.6, 120.1; **HRMS** (ESI) m/z 248.9977 [calc'd for $C_{10}H_5N_2O_4S$ $(M - H)^-$ 248.9975].

Ethyl 2-(5-Ethoxycarbonylthiazol-2-yl)pyridine-5-carboxylate (diethyl pythiDC)



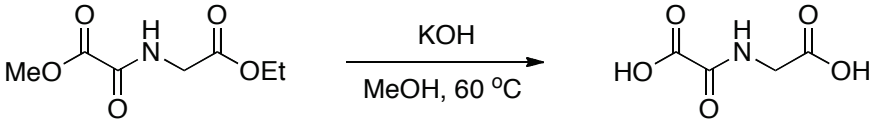
2-(5-Carboxythiazol-2-yl)pyridine-5-carboxylic acid (38 mg, 0.15 mmoles) and EtOH (7 mL) were added to a dried flask and stirred on ice. Thionyl chloride (300 μL) was added dropwise on ice, after which the flask was fitted with a reflux condenser and heated at reflux. After 24 h, the reaction mixture was cooled on ice and quenched by the dropwise addition of saturated aqueous Na_2CO_3 (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL) and the combined organic extracts were dried over Na_2SO_4(s) and concentrated under reduced pressure. The crude product was purified by chromatography on silica (2% v/v acetone in 1:1 DCM/hexanes) to afford the title compound (40 mg, 86 %) as a pale yellow solid. The spectral data for diethyl pythiDC synthesized by this method matched that reported above for the same compound synthesized by the direct cross coupling described above.

***N*-(Methoxyoxalyl)glycine Ethyl Ester**



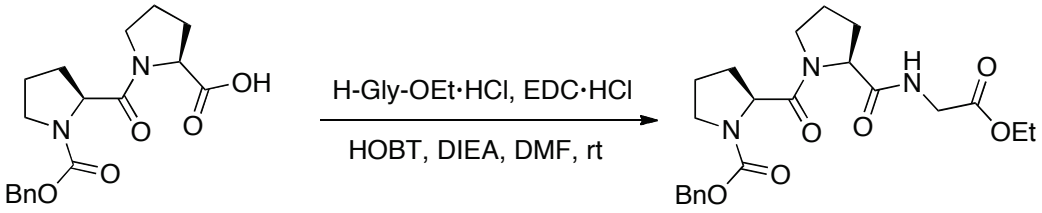
N-(Methoxyoxalyl)glycine ethyl ester was synthesized as reported previously.[7] The spectral data and yield matched that reported previously.

***N*-Oxalylglycine (NOG)**



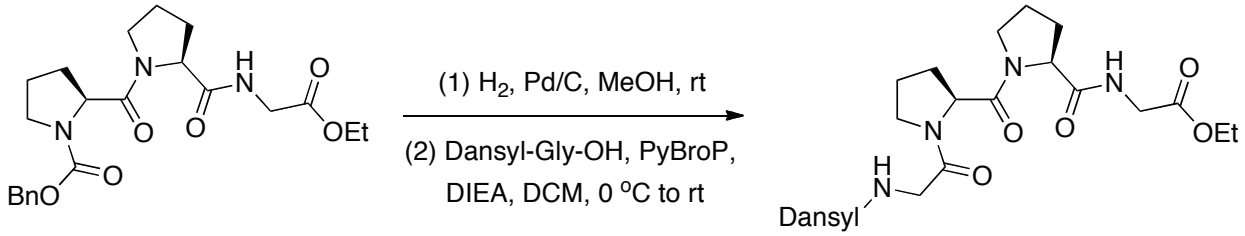
N-Oxalylglycine was synthesized as described previously.[7] The spectral data and yield matched that reported previously.

***N*-Benzyloxycarbonyl-(2*S*)-prolyl-(2*S*)-prolylglycine Ethyl Ester (CbzProProGlyOEt)**



CBbzProProGlyOEt was synthesized as described previously.[7] The spectral data and yield matched that reported previously.

***N*-Dansylglycyl-(2*S*)-prolyl-(2*S*)-prolylglycine Ethyl Ester (dansylGlyProProGlyOEt):**



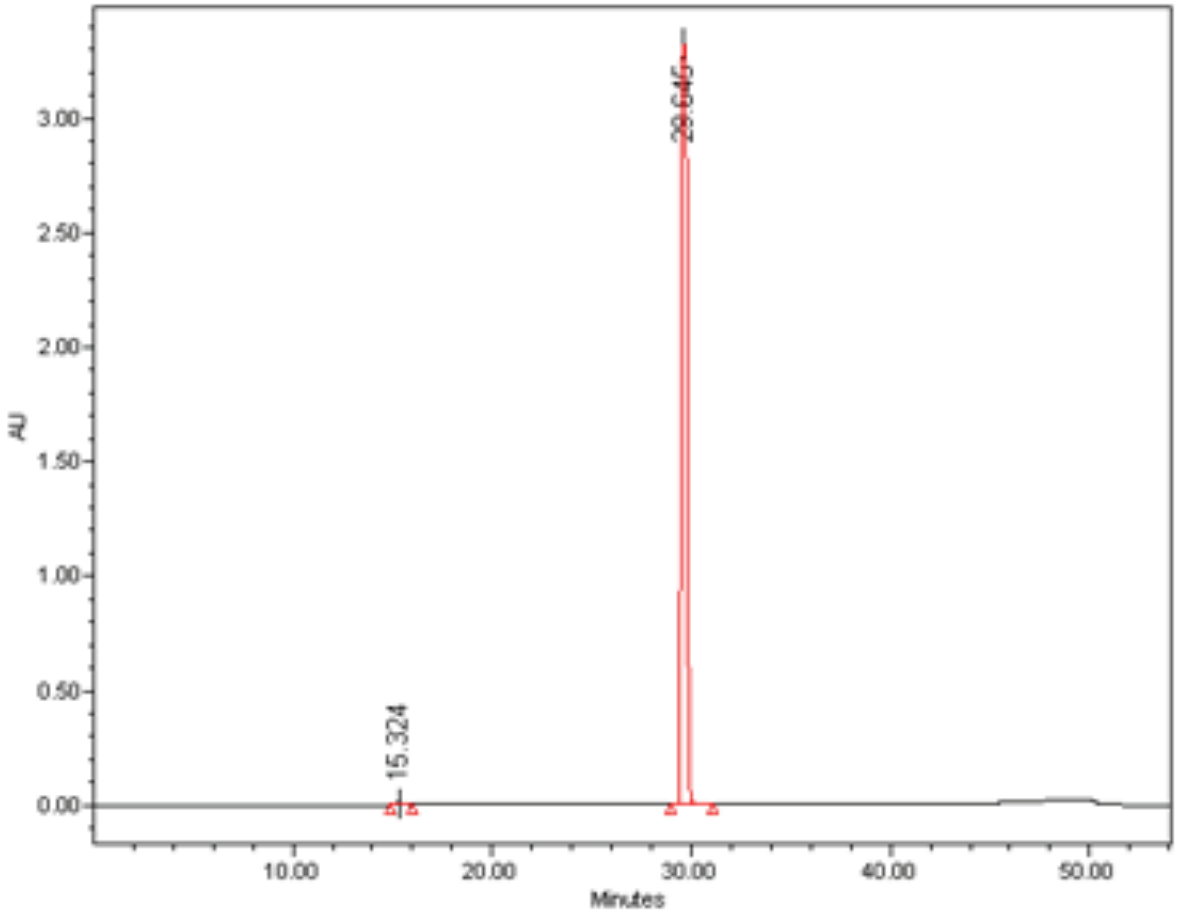
DansylGlyProProGlyOEt was synthesized as described previously.[7] The spectral data and yield matched that reported previously.

XVIII. Determination of Compound Purity

Compounds were analyzed by HPLC to assess their purity. Analyses were performed with a Waters HPLC system equipped with a Nucleodur® C18 Gravity reversed-phase column (4.6 × 250 mm, 5-μm particle size) from Macherey–Nagel. Compounds dissolved in 50 μL of H_2O were injected onto the column and eluted at 1 mL/min over 20 min with a gradient of aqueous acetonitrile (5–95% v/v containing 0.1% v/v TFA). The maximal absorbance in the range of 210–400 nm was used as the detection wavelength.

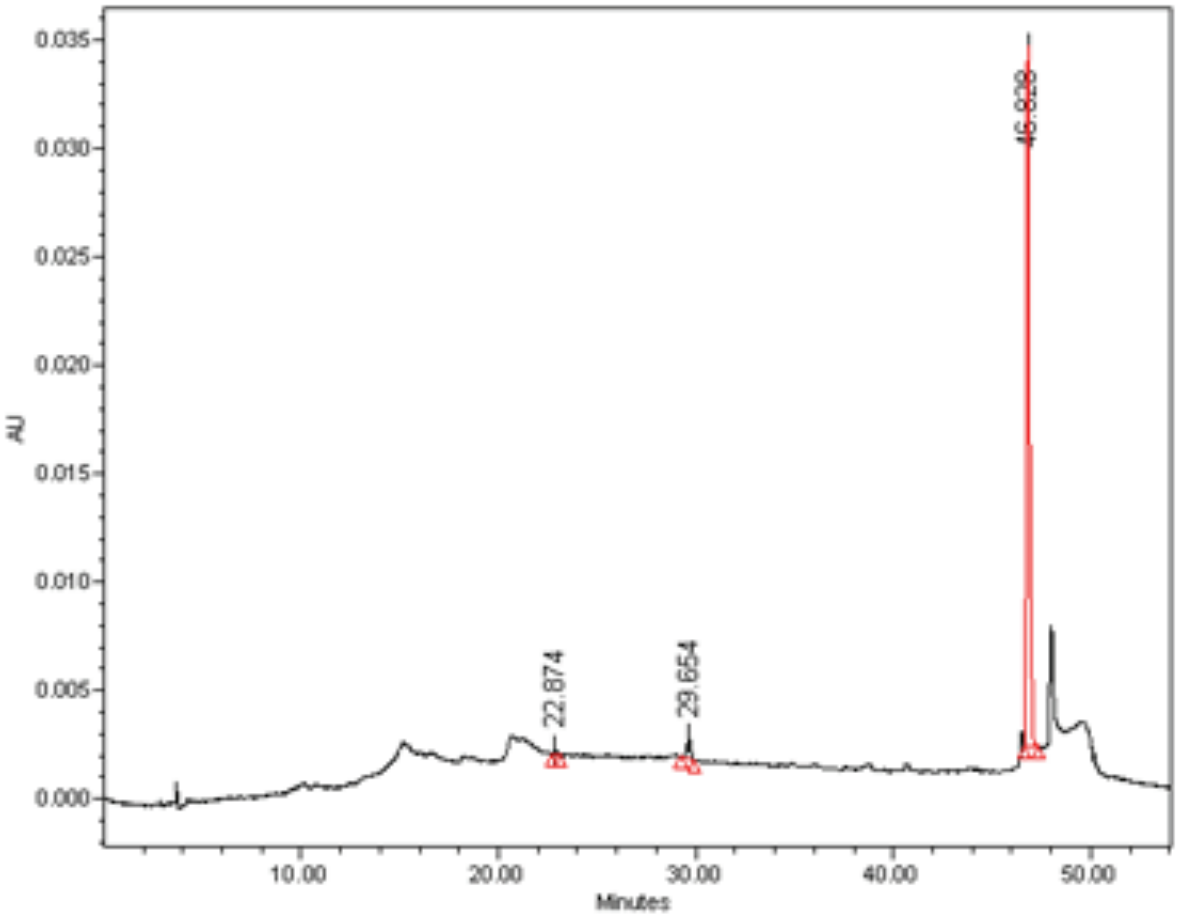
XIX. HPLC Chromatograms

EDHB



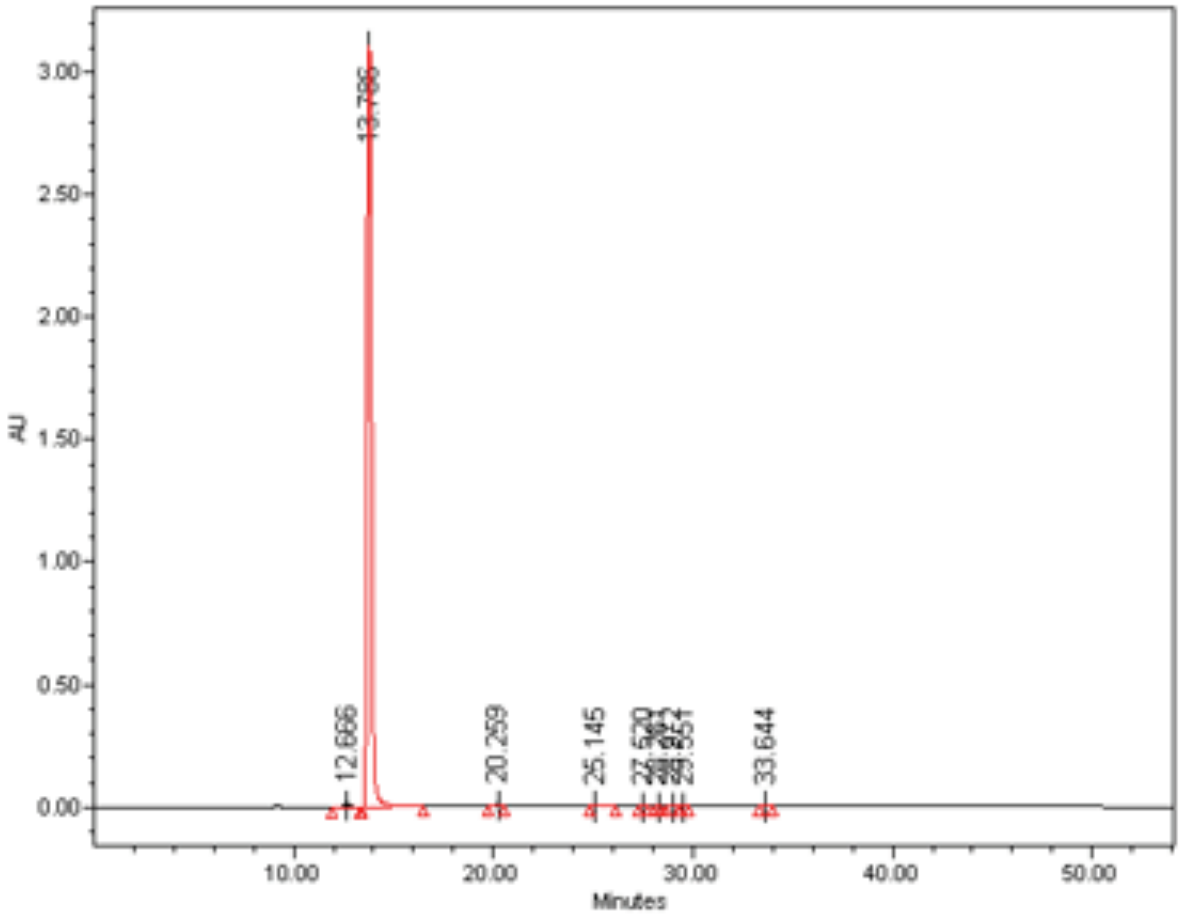
Peak	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)
1	15.324	150595	0.22	10840
2	**29.645**	**66918147**	**99.78**	**3314943**

Diethyl Bipy55′DC



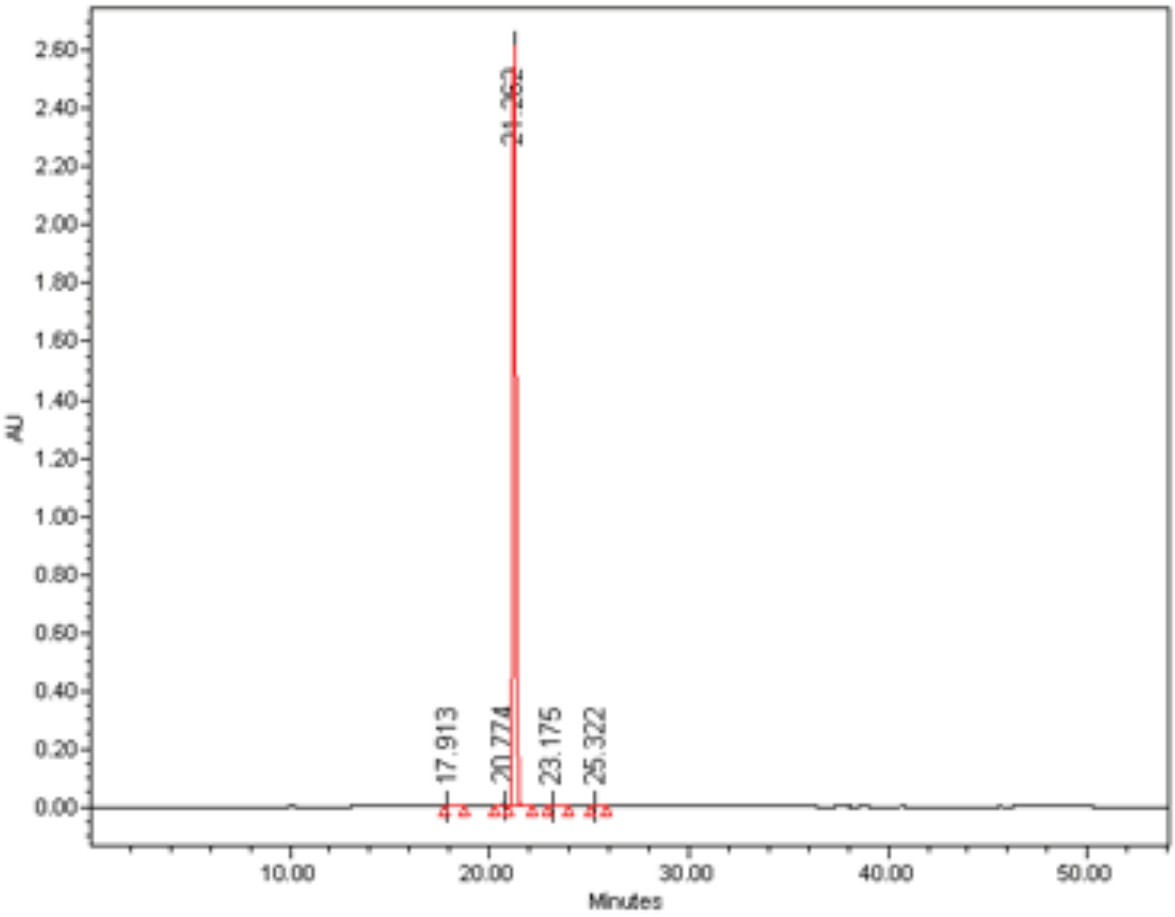
Peak	Retention Time (min)	Area (μV*sec)	% Area	Height (μV)
1	22.874	2381	0.71	244
2	29.654	12873	3.84	945
3	**46.828**	**320086**	**95.45**	**32270**

Pypyrid



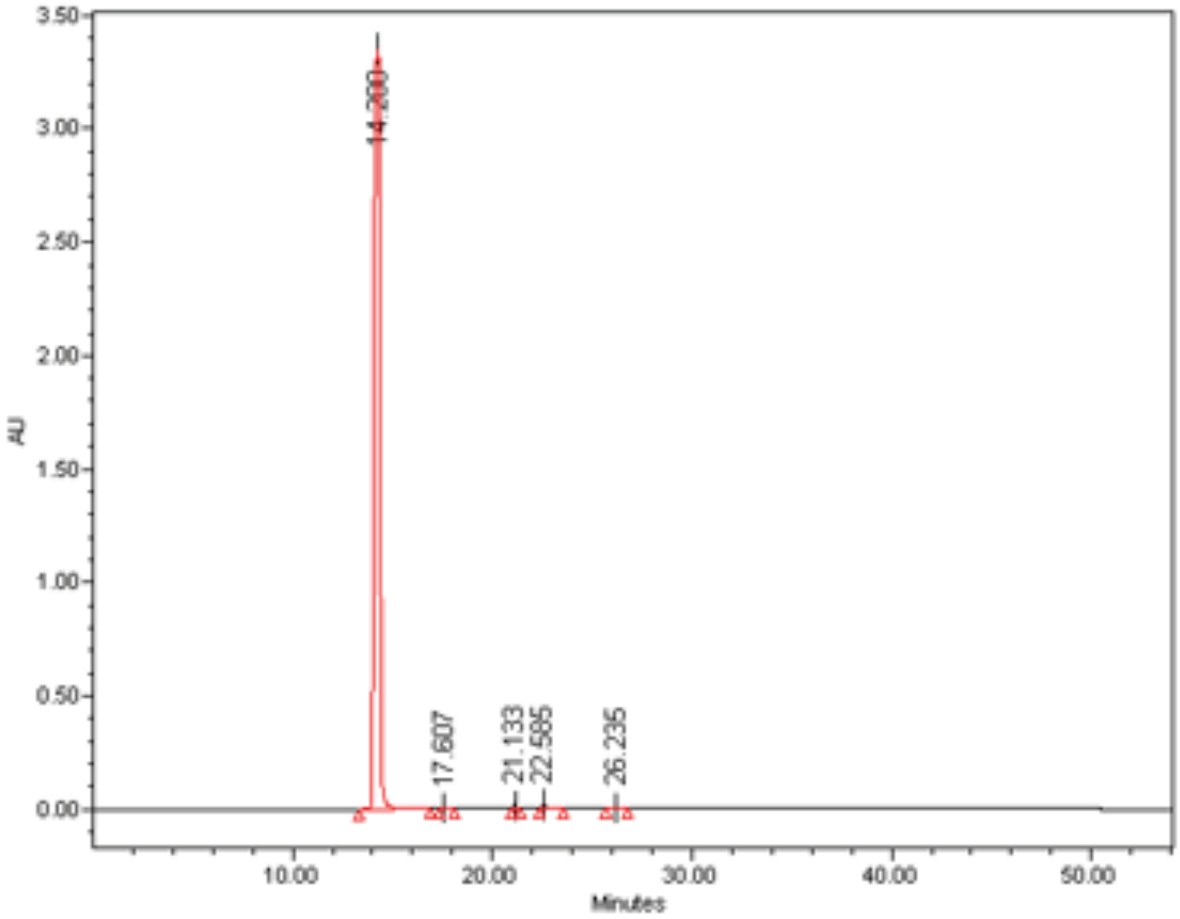
Peak	Retention Time (min)	Area (μV*sec)	% Area	Height (μV)
1	12.666	182786	0.38	11247
2	**13.786**	**47247296**	**99.05**	**3107888**
3	20.259	110278	0.23	6186
4	25.145	99102	0.21	3873
5	27.520	6972	0.01	535
6	28.261	10478	0.02	1022
7	28.972	10369	0.02	785
8	29.551	7661	0.02	641
9	33.644	23147	0.05	1836

PypyridDC



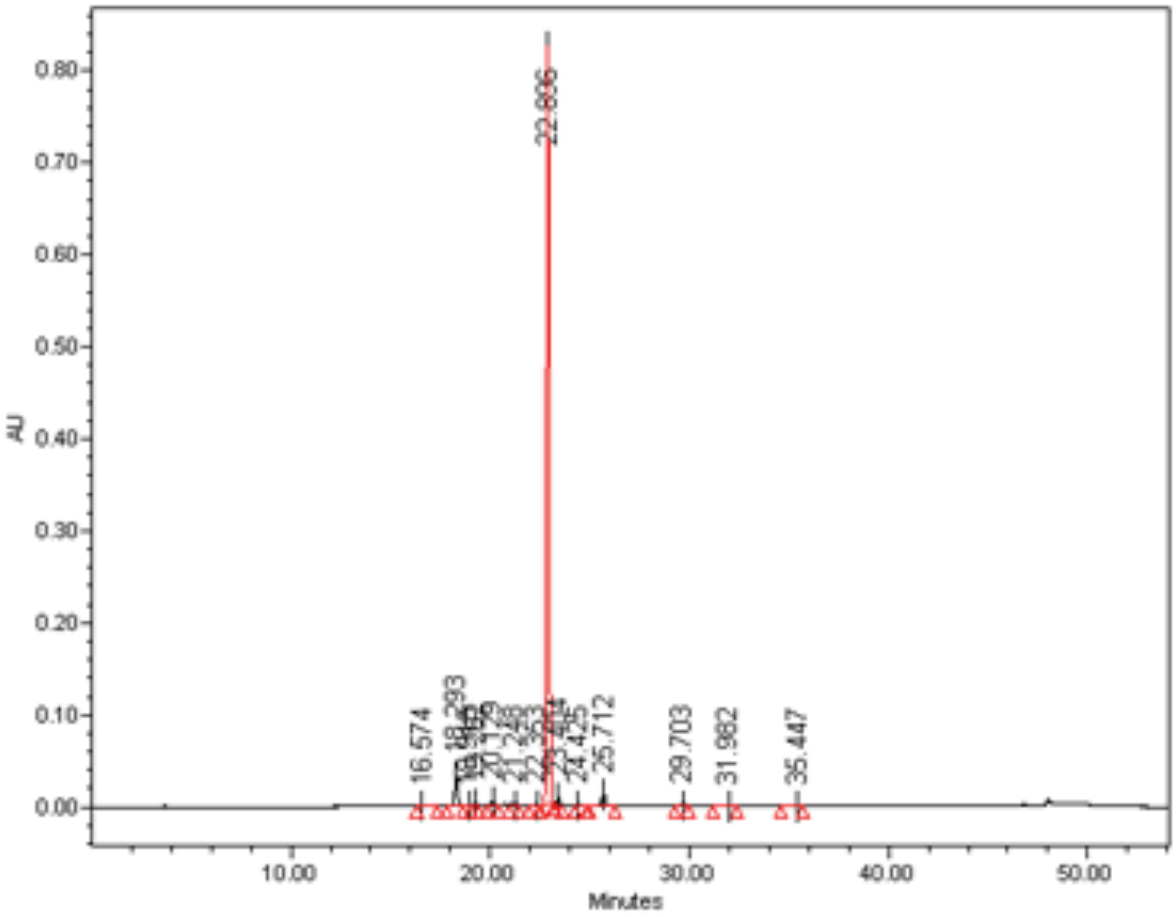
Peak	Retention Time (min)	Area (μV*sec)	% Area	Height (μV)
1	17.913	30262	0.11	2810
2	20.774	28341	0.10	2501
3	**21.262**	**27550533**	**99.58**	**2615842**
4	23.175	38610	0.14	1904
5	25.322	19995	0.07	1672

Pypyraz



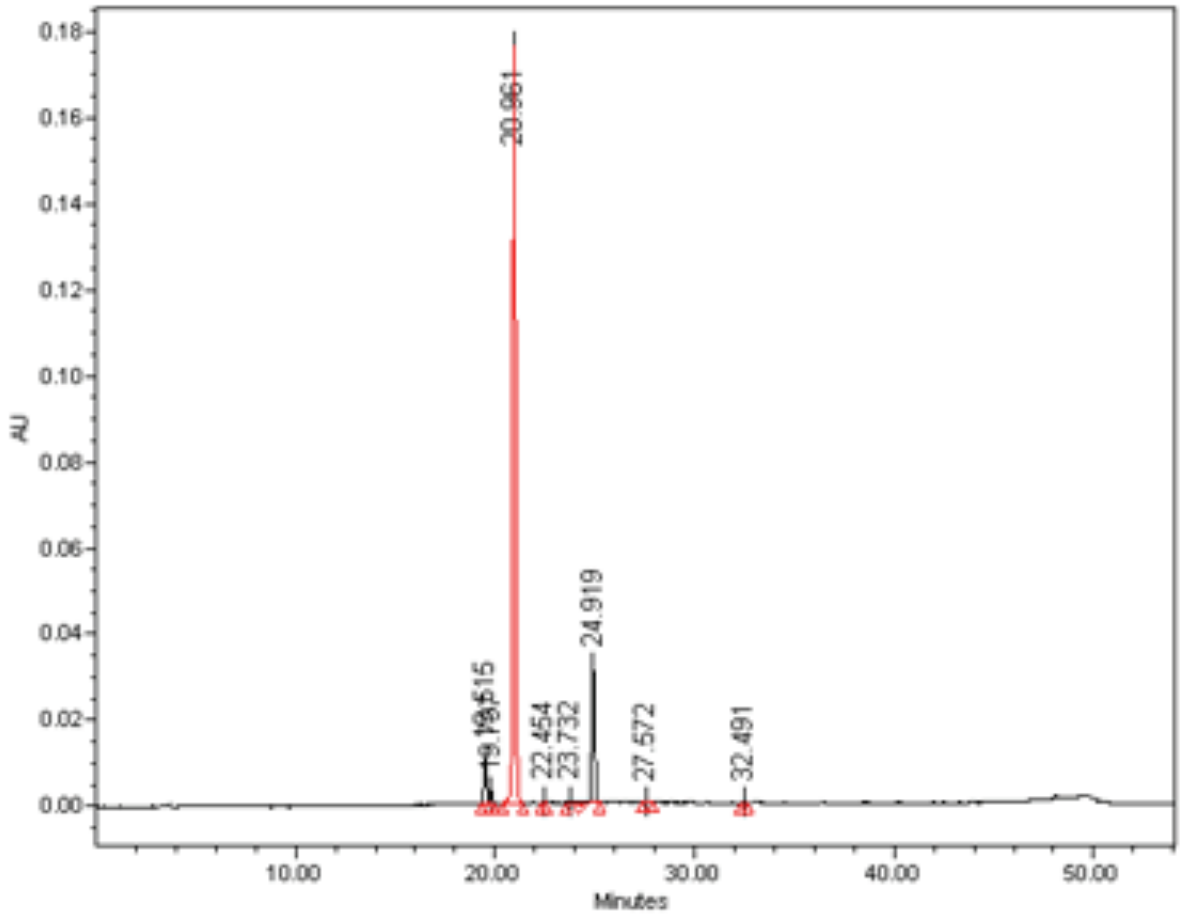
Peak	Retention Time (min)	Area (μV*sec)	% Area	Height (μV)
1	**14.280**	**67339739**	**99.13**	**3343388**
2	17.607	72606	0.11	6741
3	21.133	164524	0.24	14808
4	22.585	271119	0.4	19513
5	26.235	79762	0.12	6994

PypyrazDC



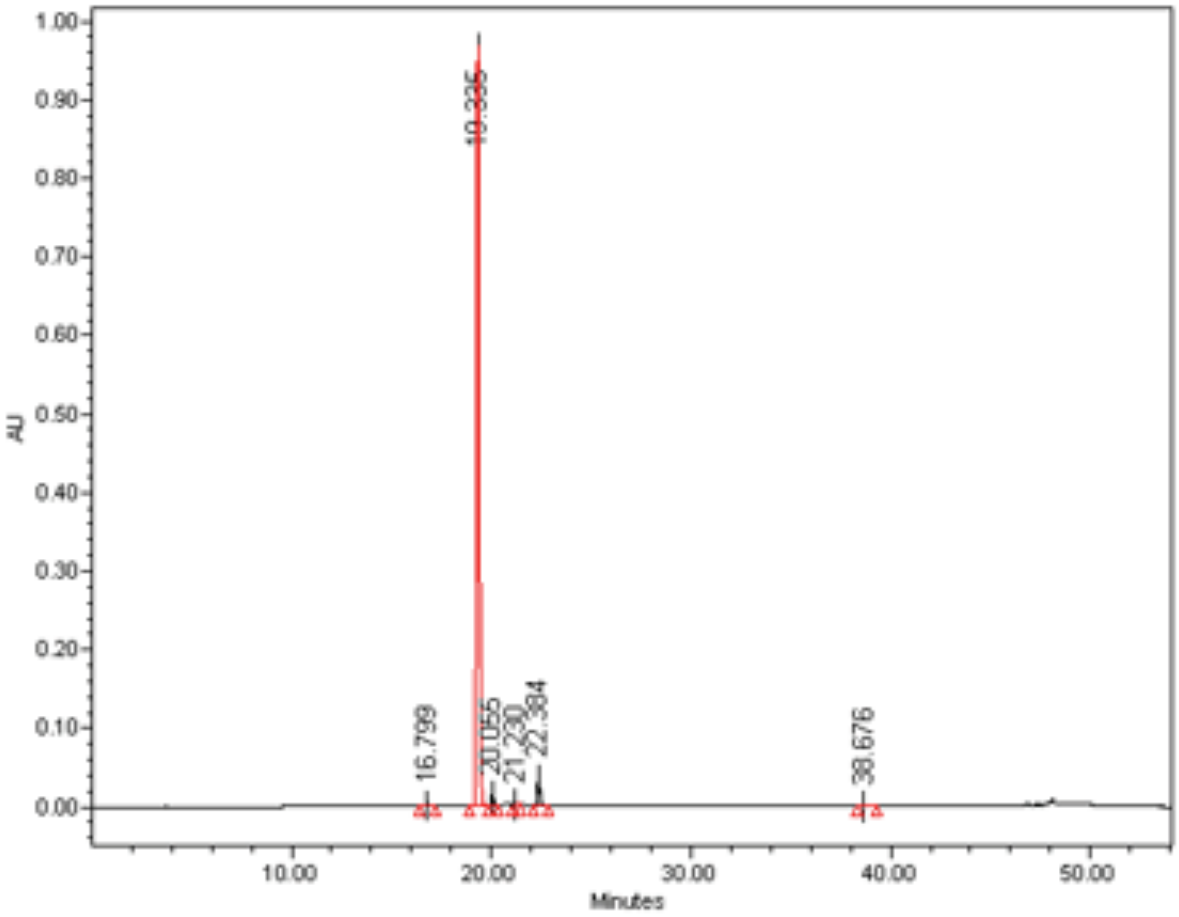
Peak	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)
1	16.574	15613	0.18	525
2	18.293	335430	3.85	34147
3	18.915	4923	0.06	446
4	19.282	32690	0.37	3363
5	20.129	57338	0.66	4989
6	21.248	11891	0.14	1009
7	22.353	9429	0.11	1039
8	**22.896**	**7967853**	**91.37**	**828483**
9	23.404	83288	0.96	8722
10	24.425	9131	0.10	858
11	25.712	162359	1.86	13071
12	29.703	14965	0.17	1262
13	31.982	7611	0.09	263
14	35.447	7857	0.09	370

PypyrroleDC



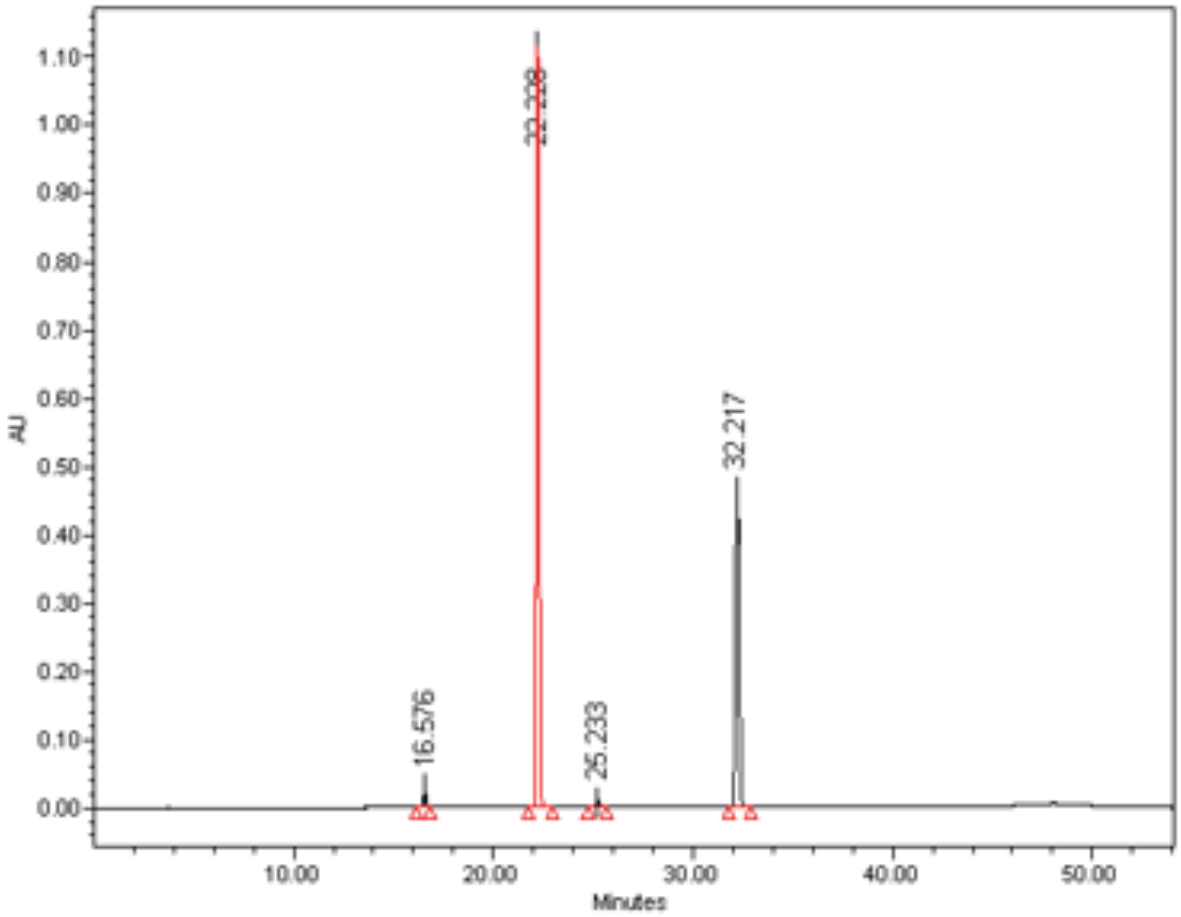
Peak	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)
1	**19.515**	99453	4.40	10429
2	19.797	28183	1.25	2870
3	**20.961**	**1789909**	**79.25**	**176621**
4	22.454	4889	0.22	588
5	22.732	5495	0.24	482
6	24.919	323630	14.33	31386
7	27.572	2766	0.12	259
8	32.491	4252	0.19	385

PypyrDC



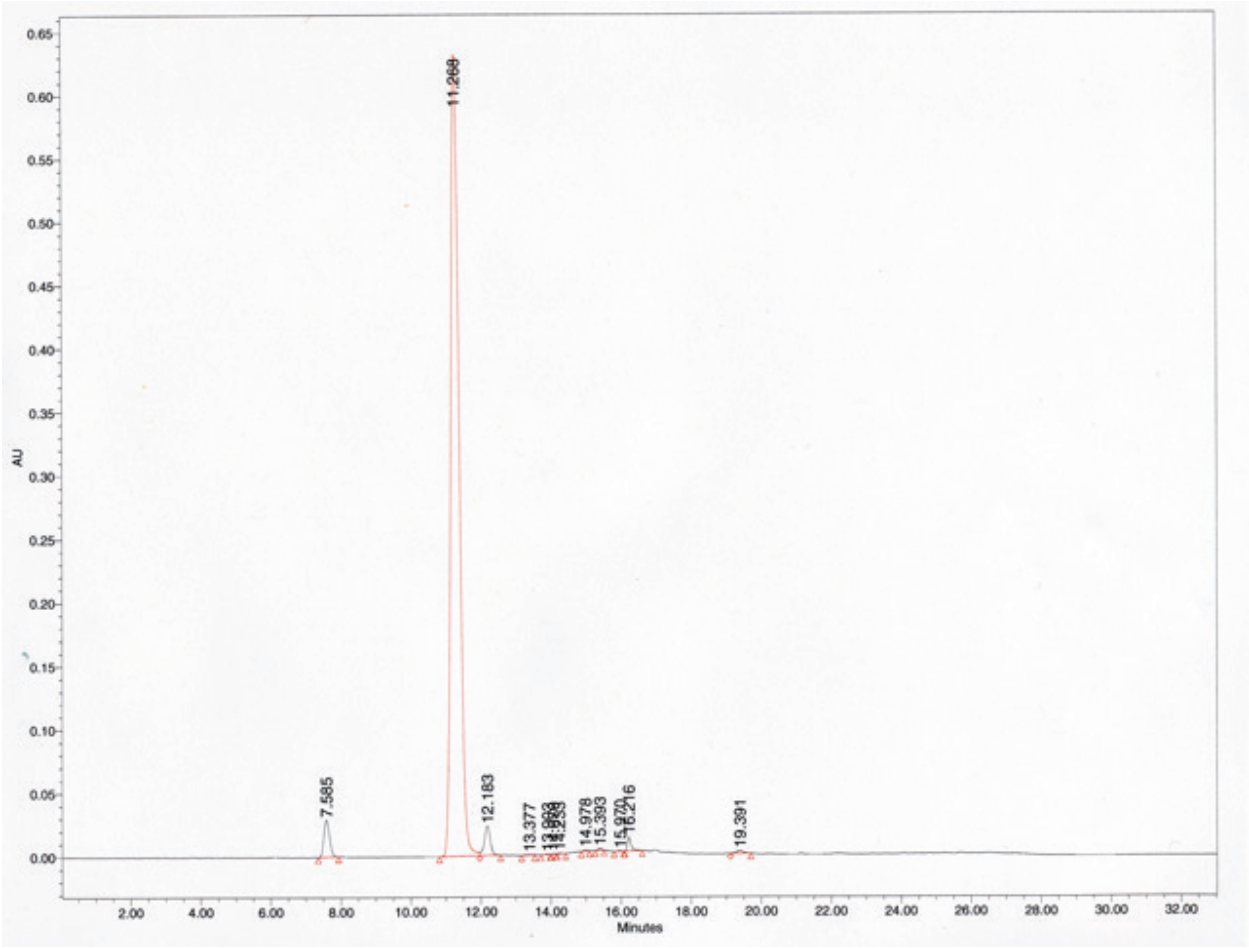
Peak	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)
1	16.799	14556	0.15	1315
2	**19.335**	**9322099**	**95.10**	**966494**
3	20.055	123856	1.26	12778
4	21.230	14138	0.14	1516
5	22.384	319544	3.26	34363
6	38.676	8026	0.08	386

2-(5-Methoxycarbonyl-1H-imidazol-2-yl)pyridine-5-carboxylic Acid



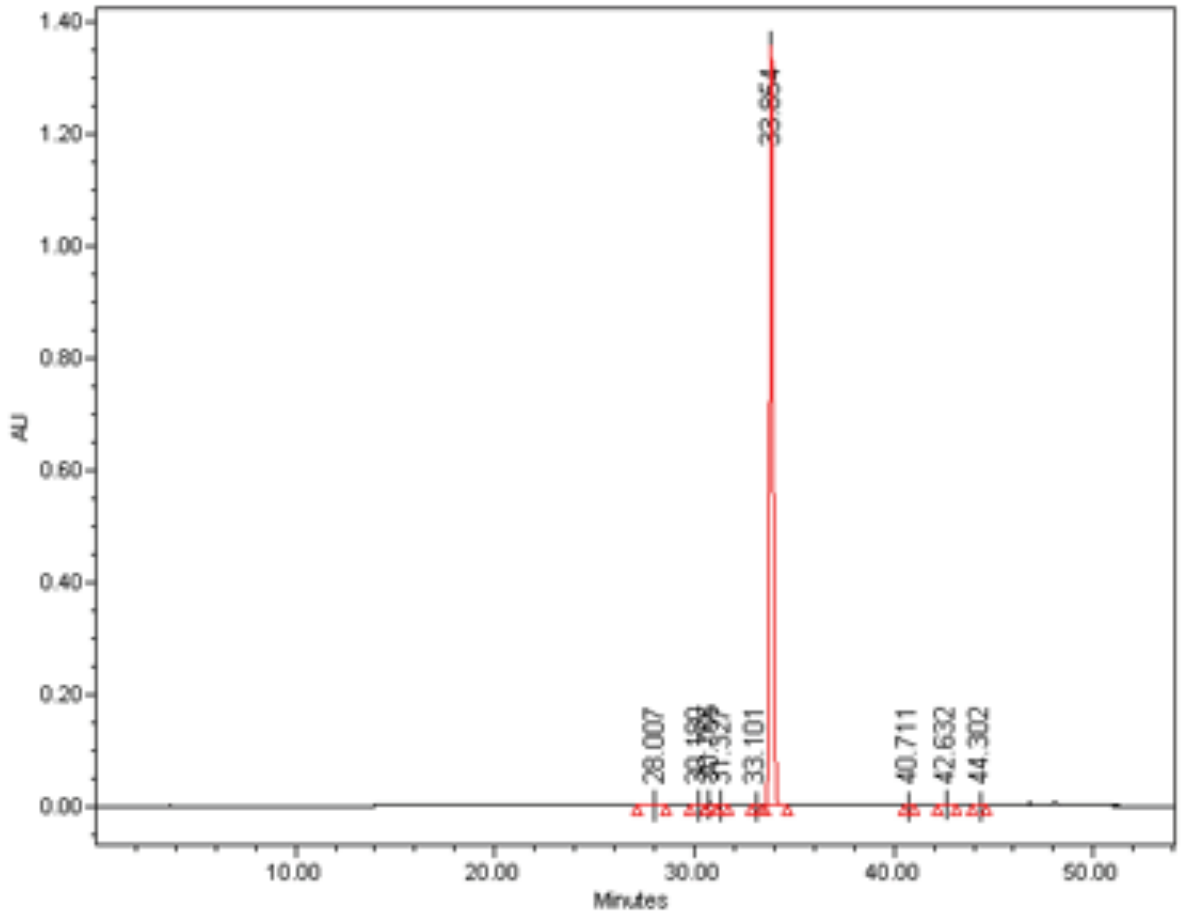
Peak	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)
1	16.576	239579	1.47	27336
2	**22.228**	**10474669**	**64.07**	**1113188**
3	25.233	86460	0.53	8268
4	32.217	5549123	33.94	466031

PyimDC



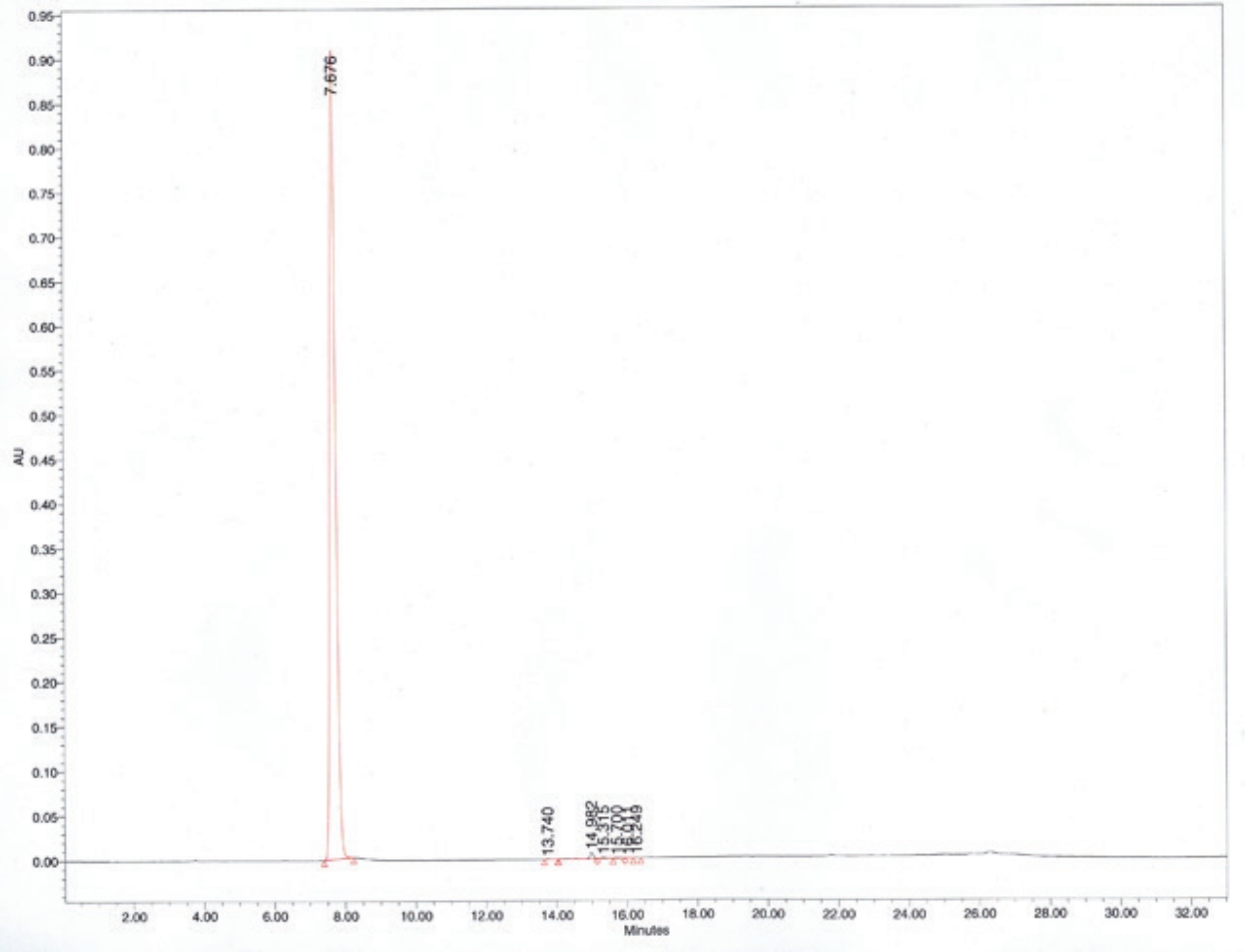
Peak	Retention Time (min)	Area (μV*sec)	% Area	Height (μV)
1	7.585	327025	2.95	29352
2	**11.268**	**10327125**	**93.26**	**632576**
3	12.183	271399	2.45	23307
4	13.377	5513	0.05	431
5	13.903	2916	0.03	396
6	14.070	2029	0.02	413
7	14.233	2007	0.02	323
8	14.978	6827	0.06	1163
9	15.393	11308	0.10	1505
10	15.970	2620	0.02	339
11	16.216	82209	0.74	11228
12	19.391	32836	0.30	2188

Diethyl PyimDC



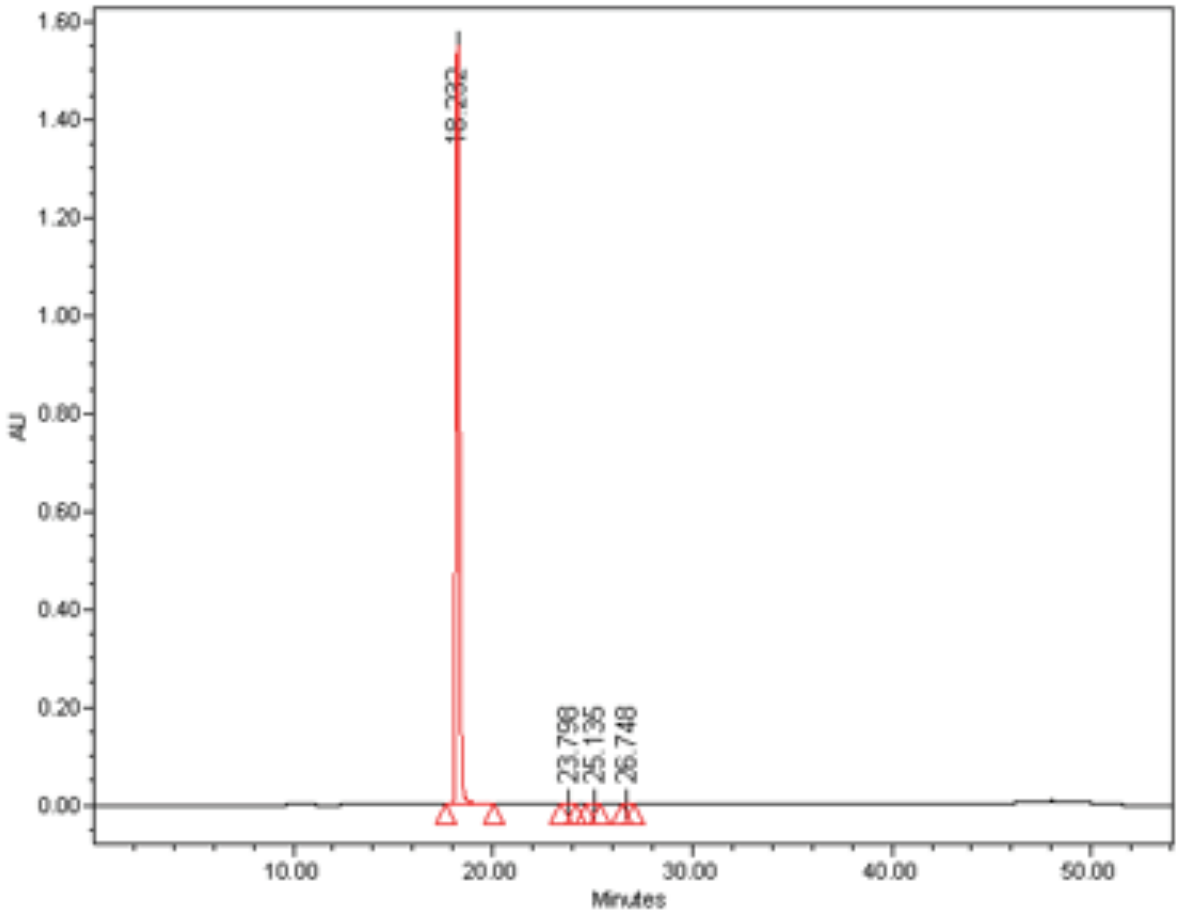
Peak	Retention Time (min)	Area (μV*sec)	% Area	Height (μV)
1	28.007	19751	0.12	560
2	30.180	9650	0.06	865
3	30.755	46934	0,28	4158
4	31.327	17379	0.11	1513
5	33.101	5461	0.03	448
6	**33.854**	**16407846**	**99.14**	**1356438**
7	40.711	3385	0.02	240
8	42.632	32365	0.20	2394
9	44.302	7665	0.05	517

NmePyimDC



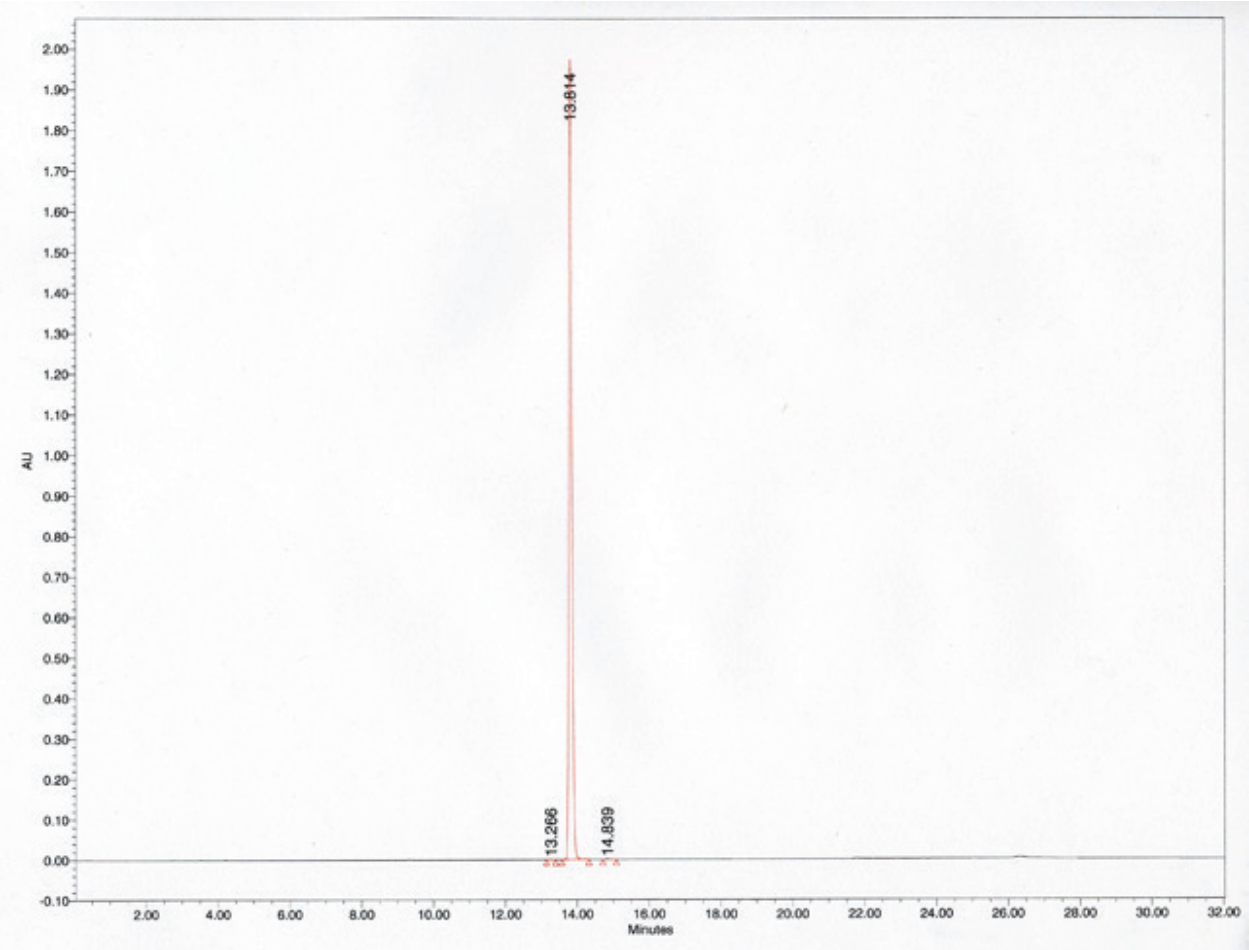
Peak	Retention Time (min)	Area (μV*sec)	% Area	Height (μV)
1	7.676	10301024	99.36	908220
2	13.74	1890	0.02	232
3	14.982	46920	0.45	6863
4	15.315	8943	0.09	1305
5	15.7	4949	0.05	626
6	16.011	1809	0.02	272
7	18.249	2222	0.02	407

Pyox



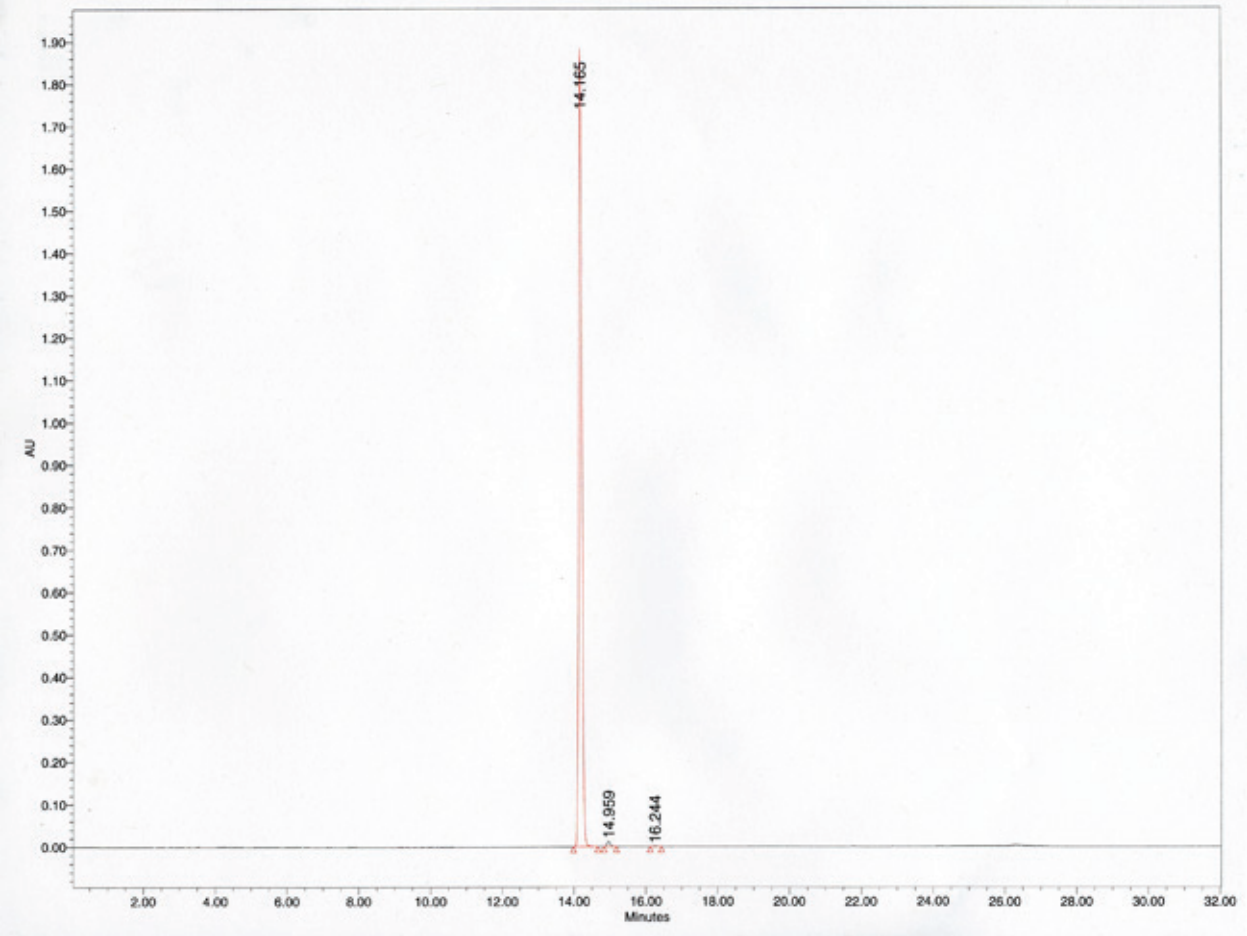
Peak	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)
1	**18.232**	**17914865**	**99.86**	**1547767**
2	23.798	2997	0.02	287
3	25.135	17964	0.10	1625
4	26.748	4629	0.03	396

PyoxDC*



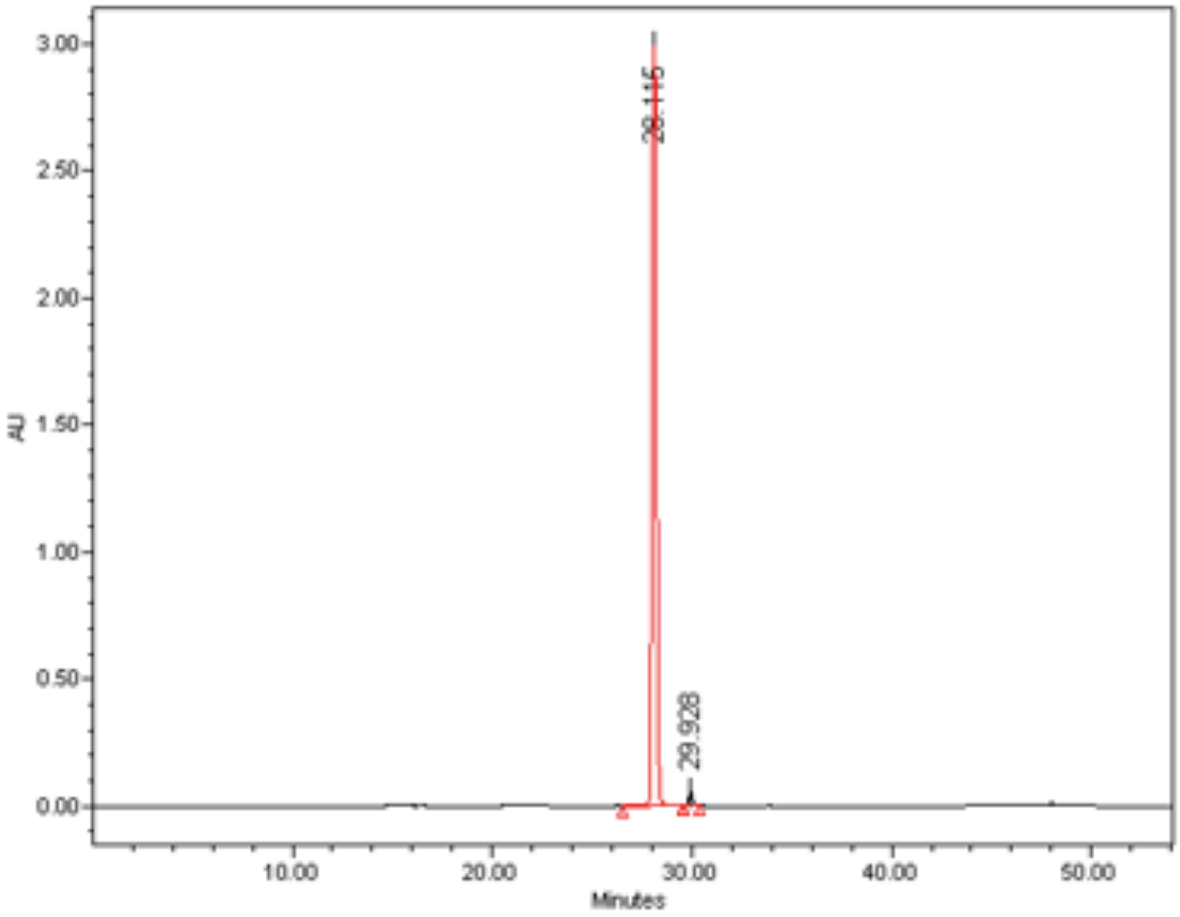
Peak	Retention Time (min)	Area (μV*sec)	% Area	Height (μV)
1	13.266	1757	0.01	278
2	**13.814**	**11844742**	**99.89**	**1974317**
3	14.839	10808	0.09	1590

PyoxDC



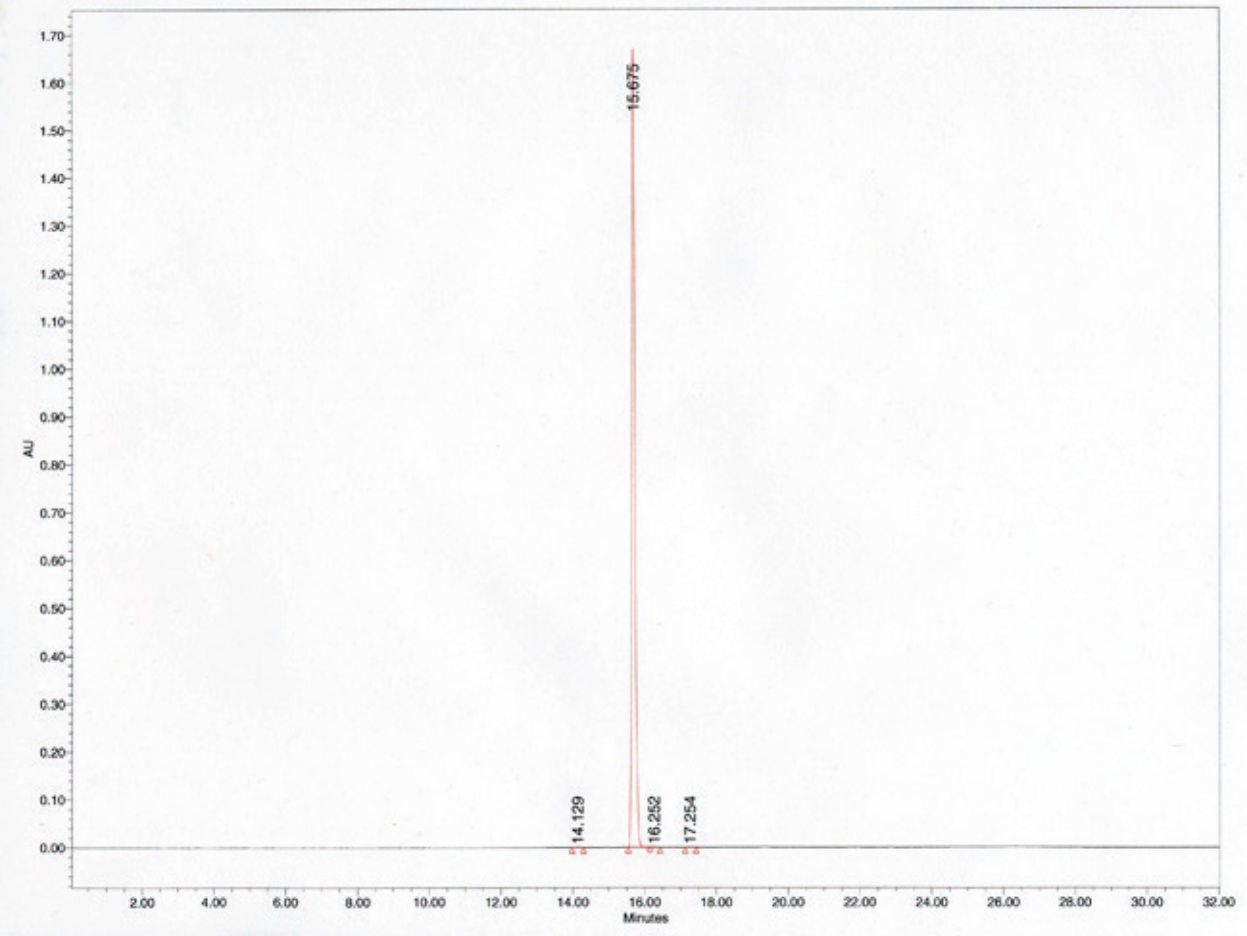
Peak	Retention Time (min)	Area (μV*sec)	% Area	Height (μV)
1	14.165	11469567	99.25	1882775
2	14.959	79794	0.69	12911
3	16.244	7183	0.06	1120

Pythi



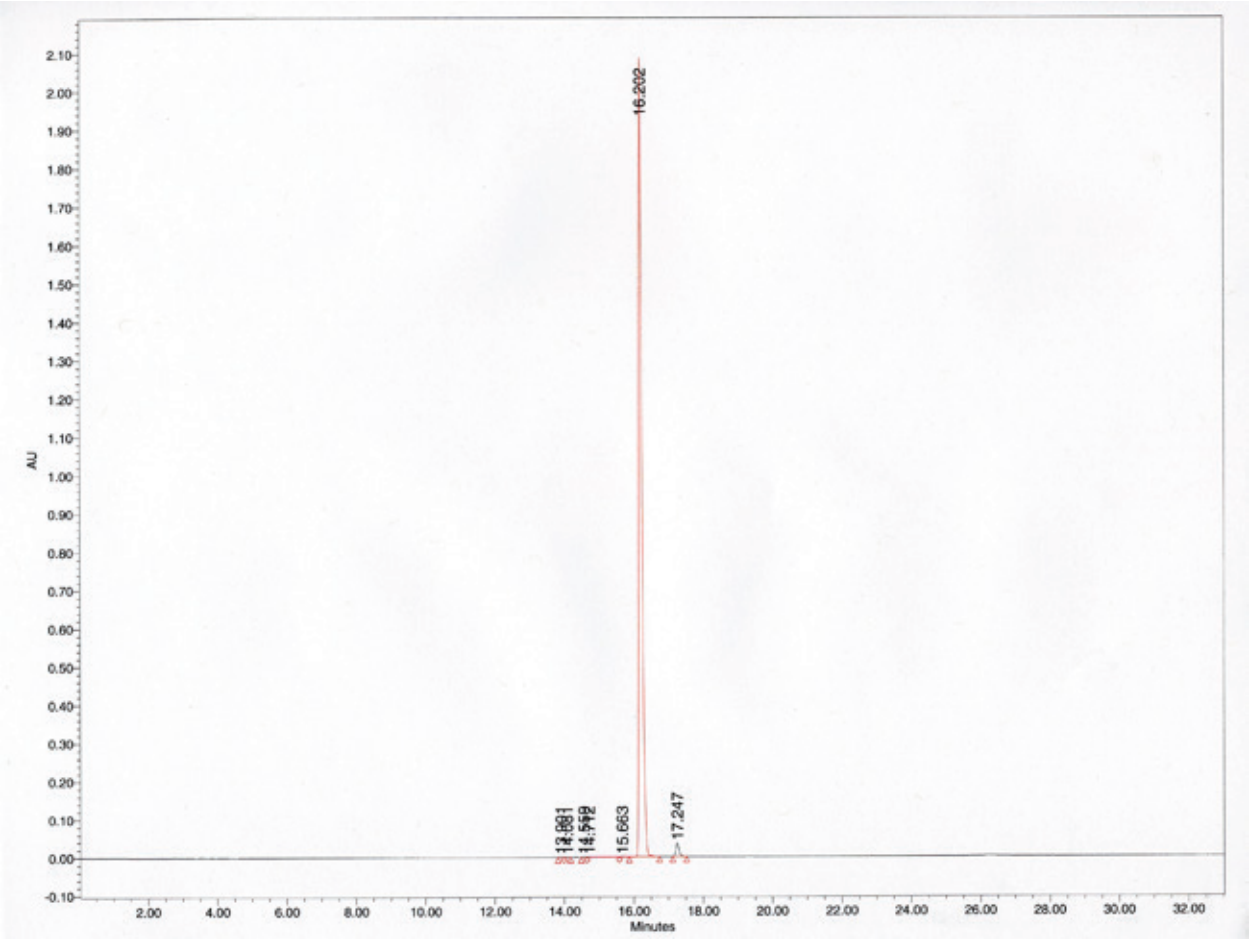
Peak	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)
1	28.115	45177691	98.36	2990871
2	29.928	753307	1.64	57240

PythiDC*



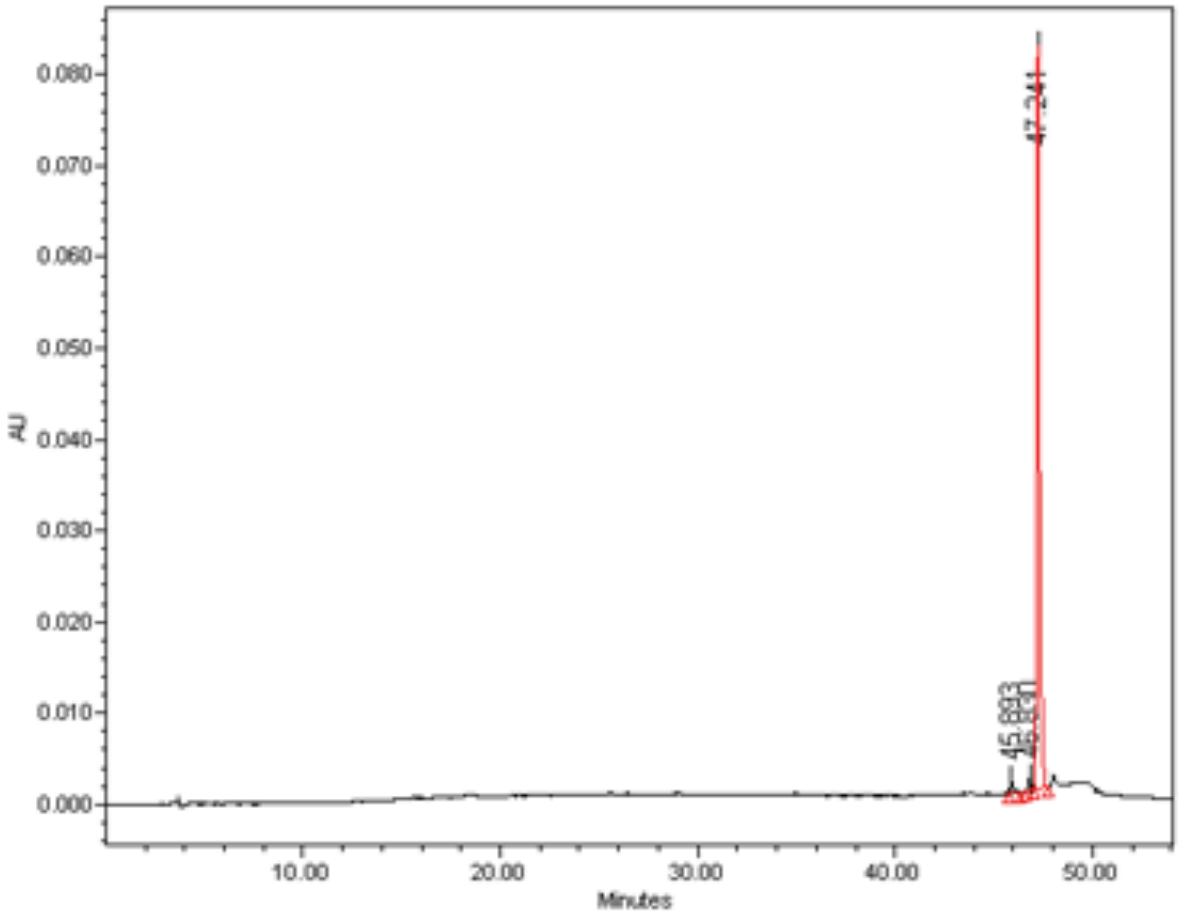
Peak	Retention Time (min)	Area (μV*sec)	% Area	Height (μV)
1	14.129	3477	0.03	577
2	**15.675**	**9952801**	**99.87**	**1658904**
3	16.252	5422	0.05	669
4	17.254	4409	0.04	720

PythiDC



Peak	Retention Time (min)	Area (μV*sec)	% Area	Height (μV)
1	13.901	2126	0.02	378
2	14.081	1788	0.01	325
3	14.559	1253	0.01	228
4	14.712	3091	0.02	361
5	15.663	1782	0.01	301
6	**16.202**	**13342462**	**98.22**	**2100713**
7	17.247	231648	1.71	35634

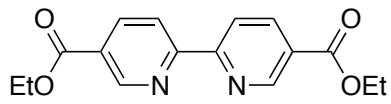
Diethyl PythiDC

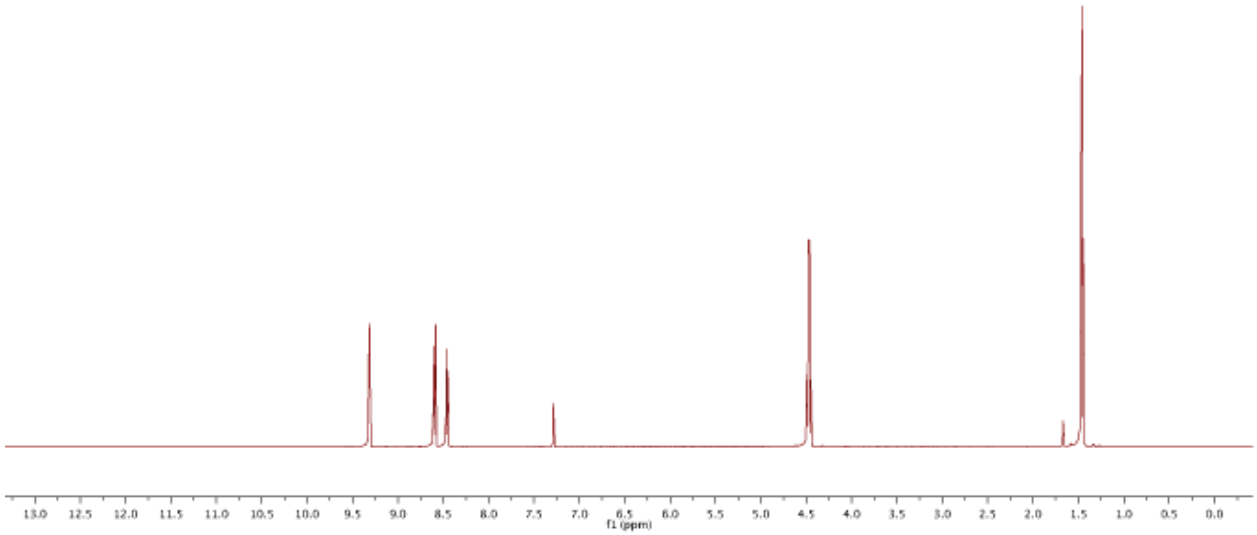


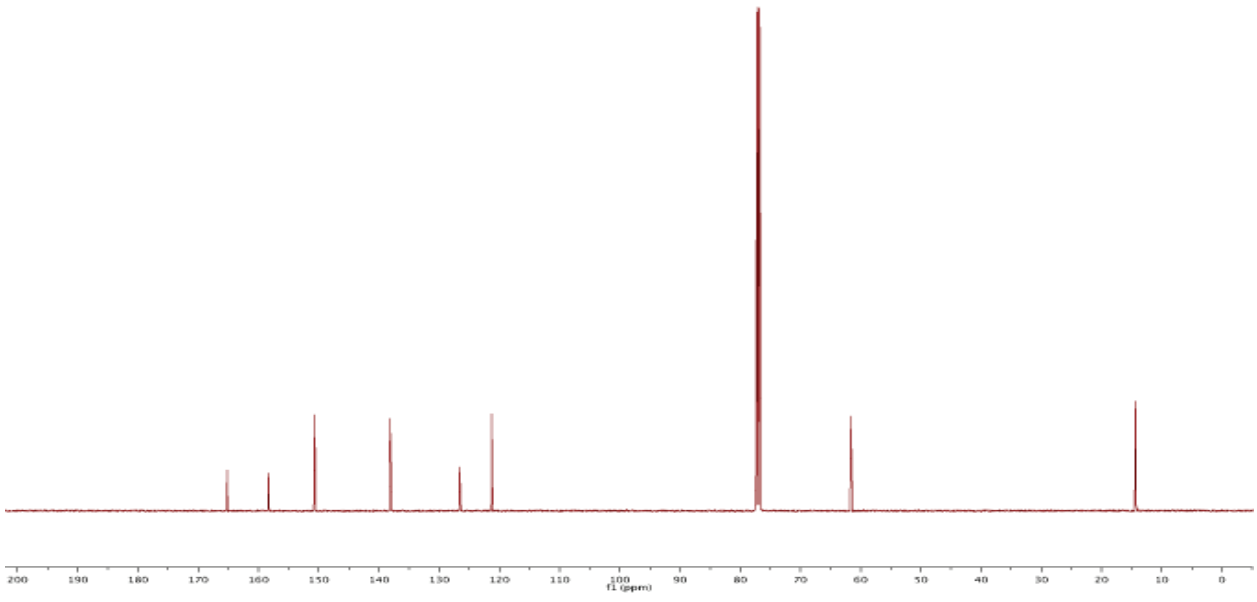
Peak	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)
1	45.893	12615	1.5	1276
2	46.830	11096	1.32	1220
3	**47.241**	**815559**	**97.17**	**81319**

XX. NMR Spectra

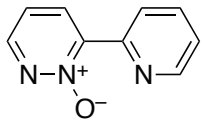
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Diethyl Bipy55′DC

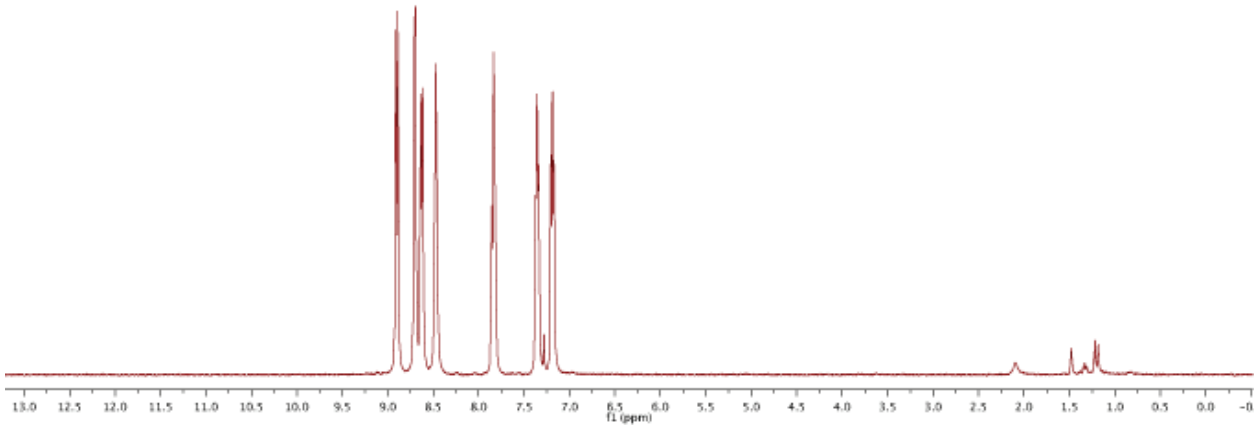


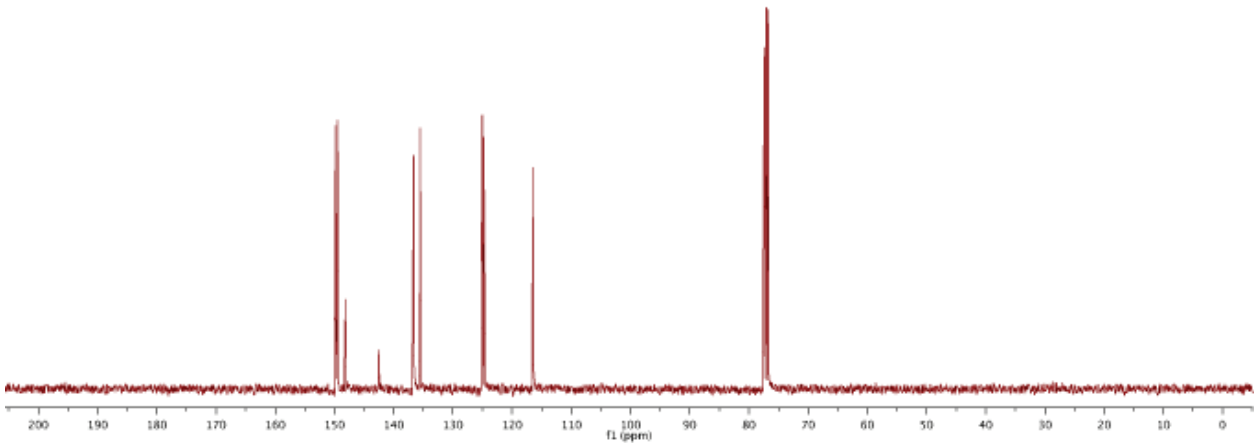




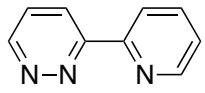
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of 6-(Pyridin-2-yl)pyridazine-1-oxide

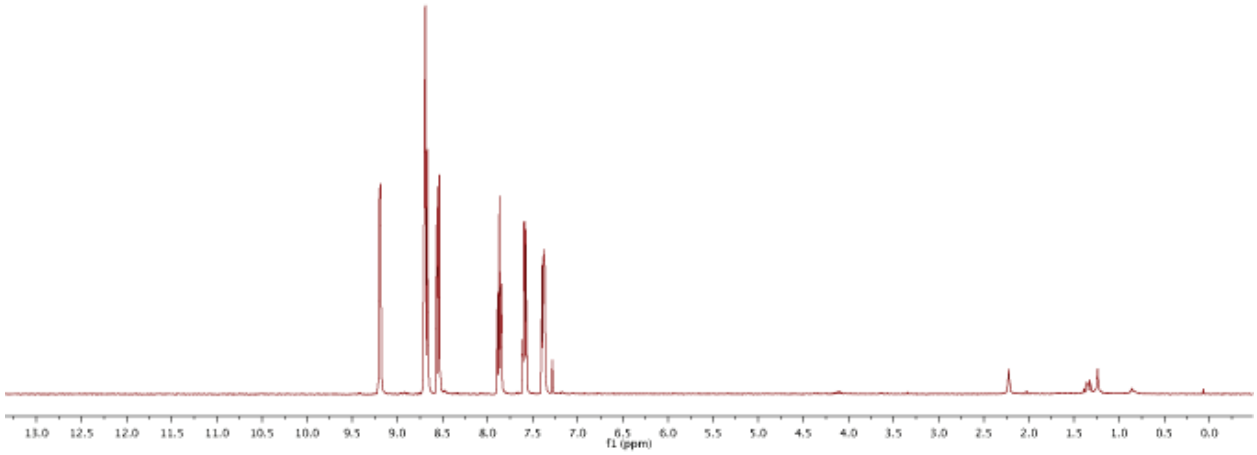


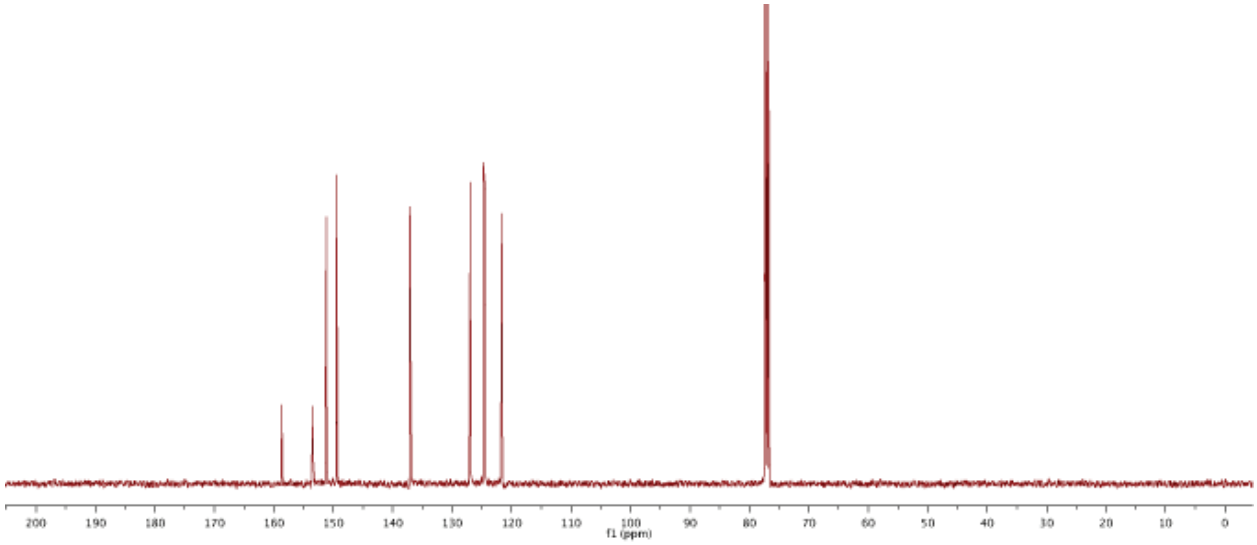




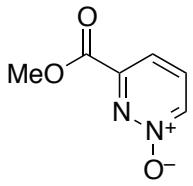
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of 3-(Pyridin-2-yl)pyridazine (pypyrid)

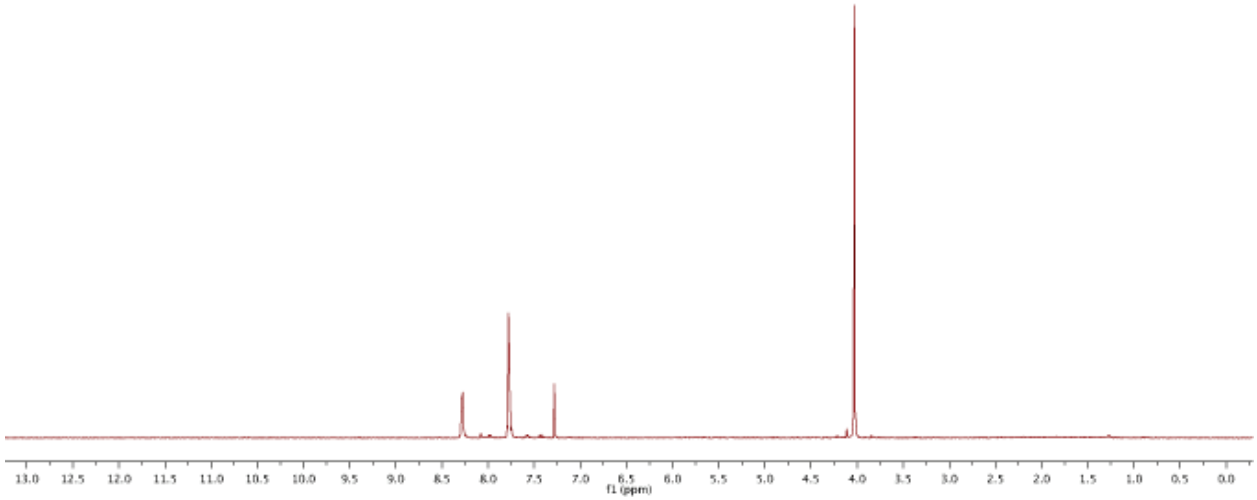


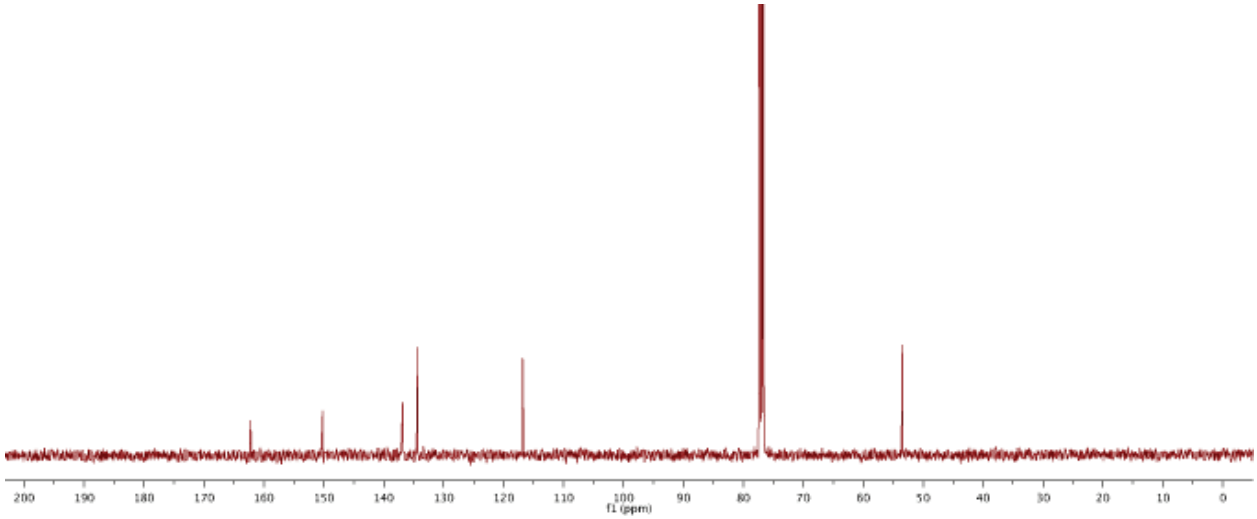




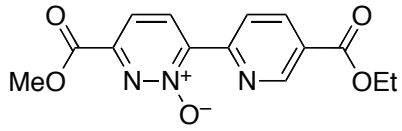
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Methyl Pyridazine-3-carboxylate-1-oxide

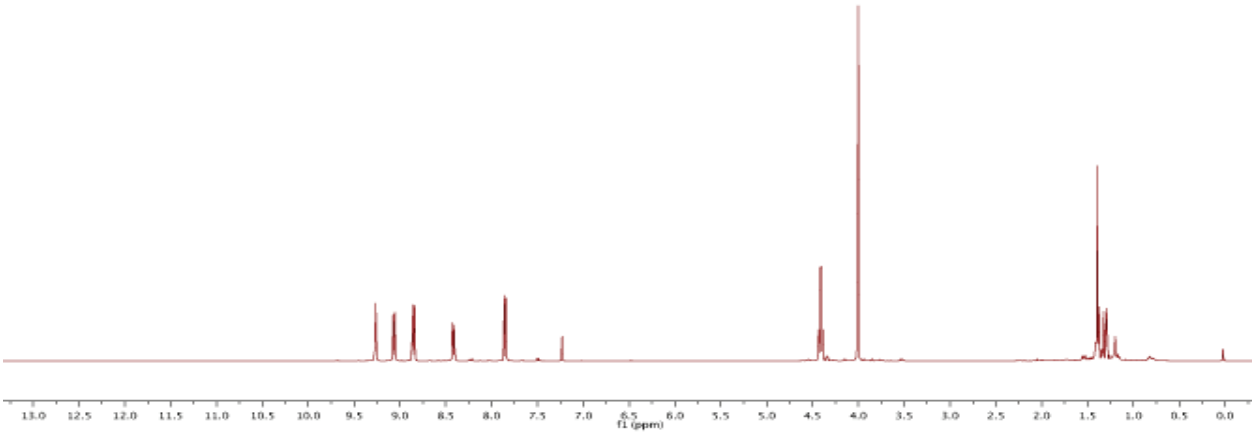




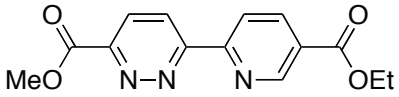


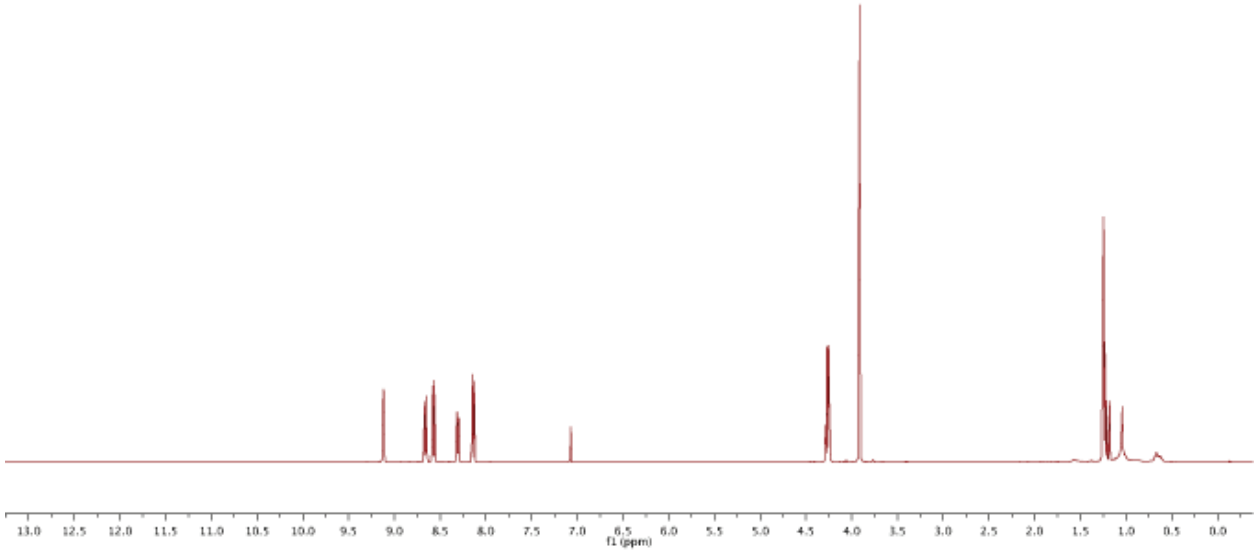
^{1}H NMR ($CDCl_3$) of Crude Methyl 6-(Ethoxycarbonylpyridin-2-yl)pyridazine-3-carboxylate-1-oxide

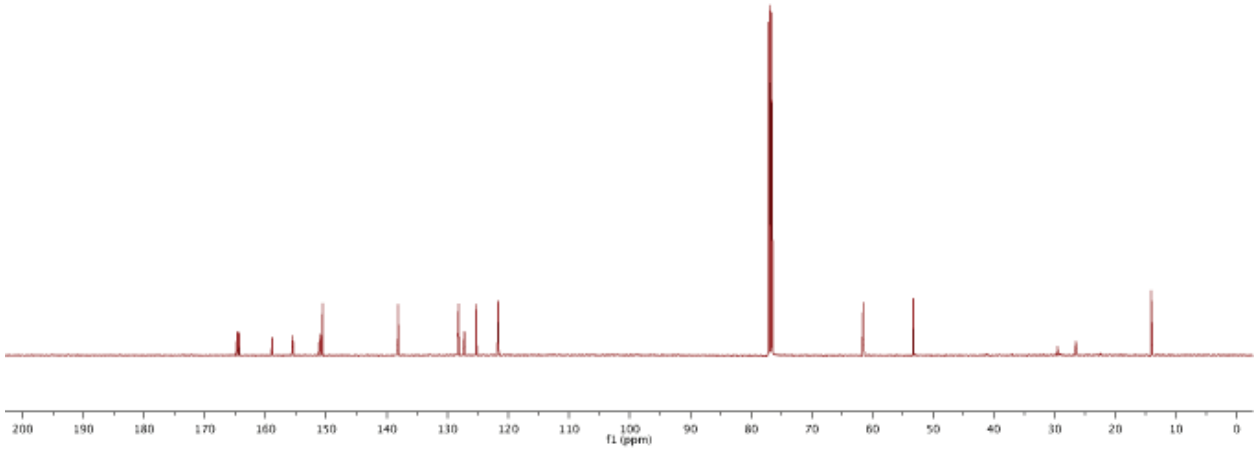




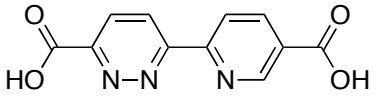
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Methyl 3-(5-Ethoxycarbonylpyridin-2-yl)pyridazine-6-carboxylate (ethylmethyl pyrpyridDC)

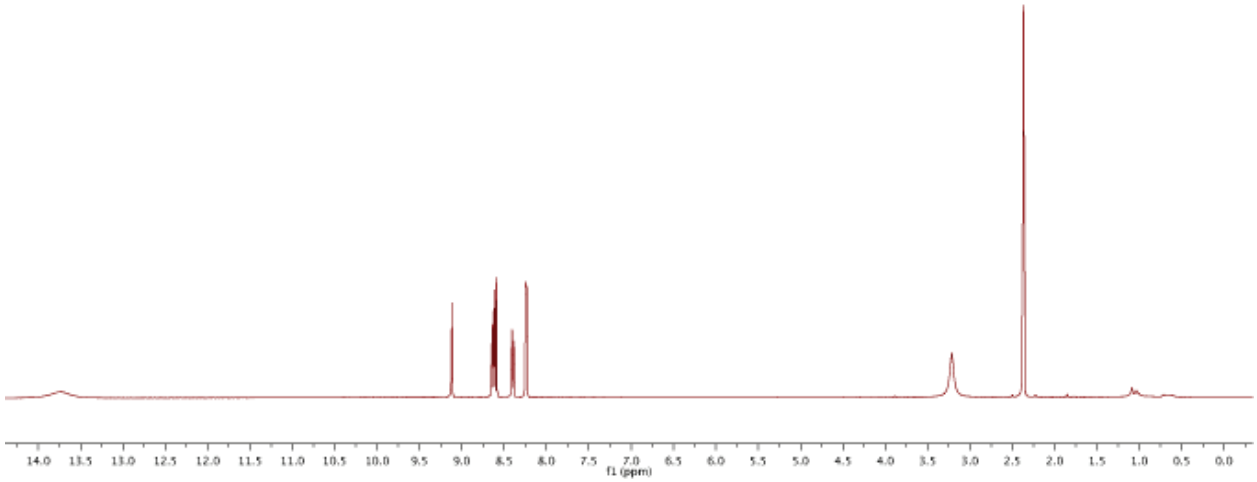


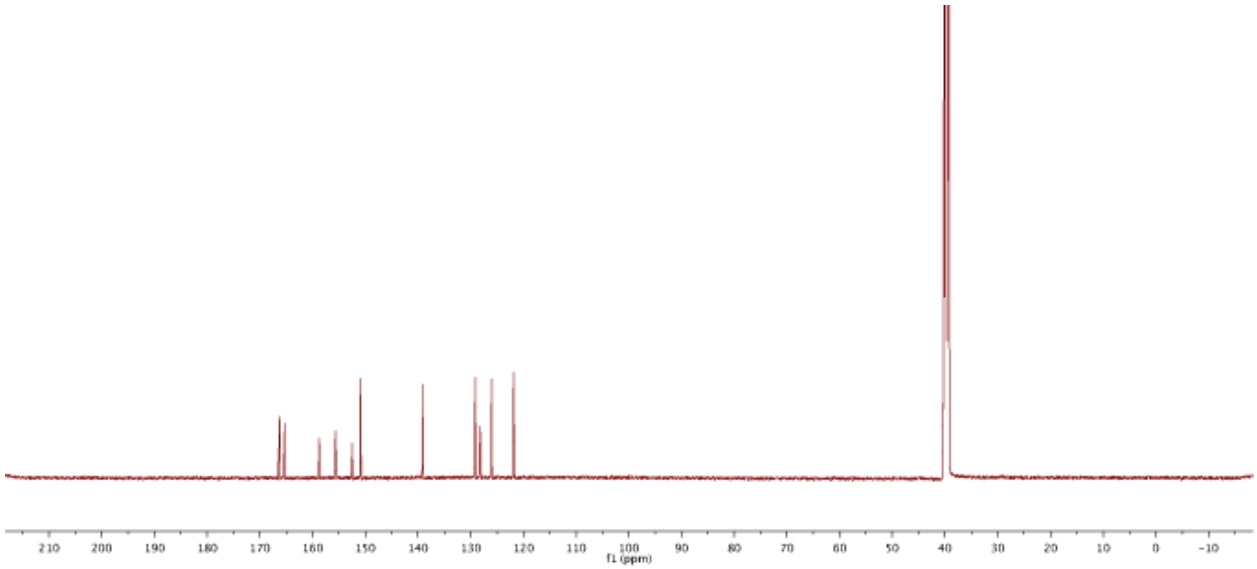




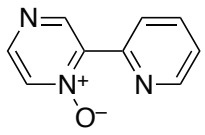
^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of 3-(5-Carboxypyridin-2-yl)pyridazine-6-carboxylic Acid (pypyridDC)

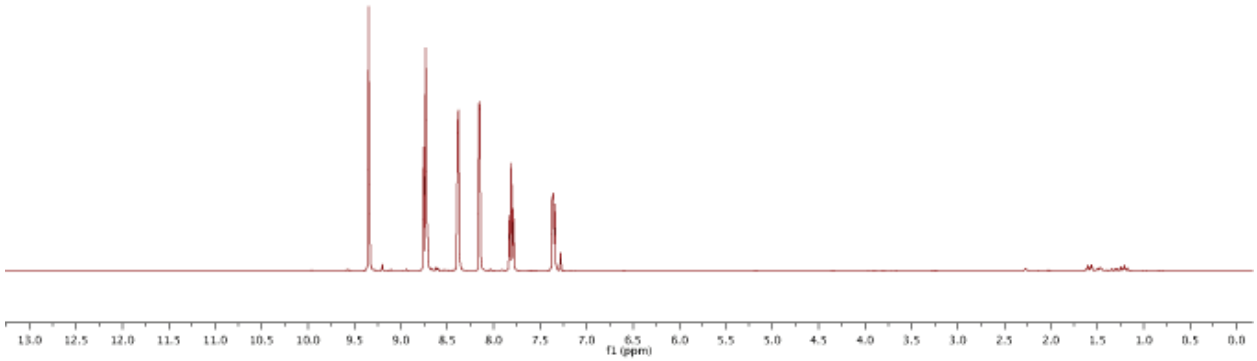


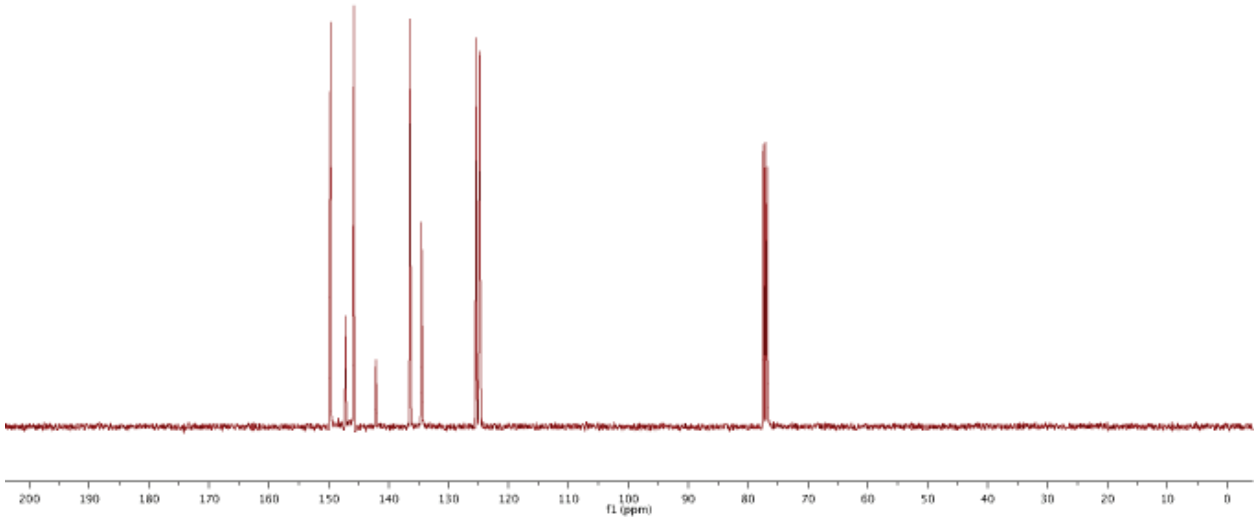




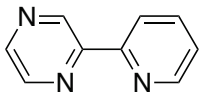
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of 2-(Pyridin-2-yl)pyrazine-1-oxide

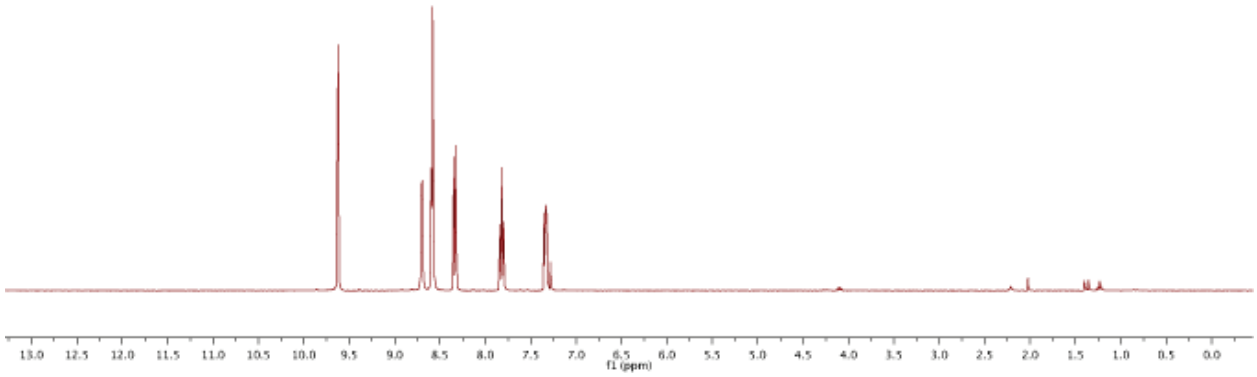


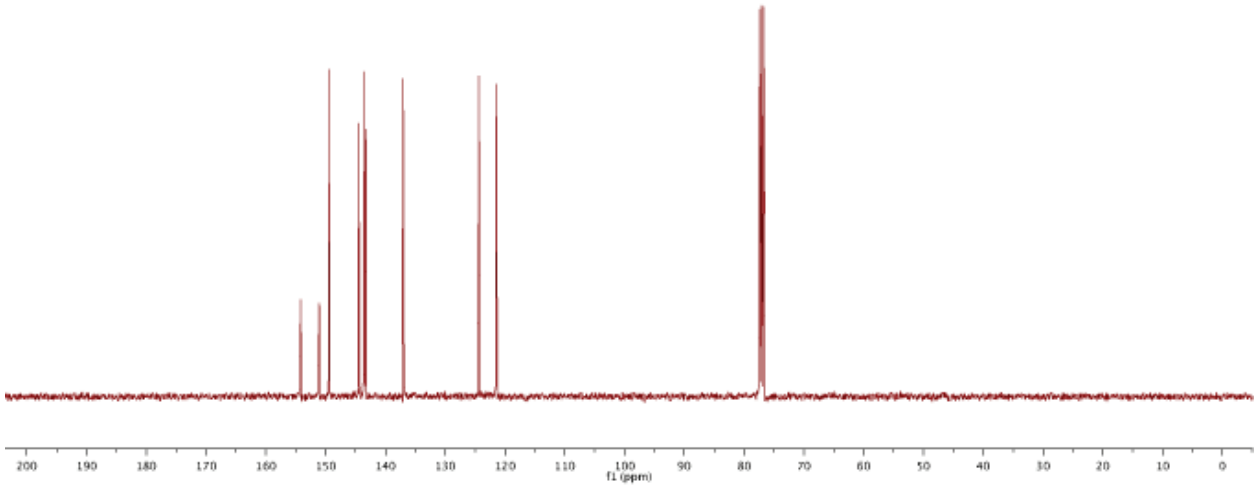




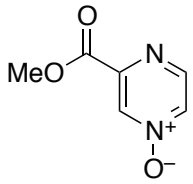
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of 2-(Pyridin-2-yl)pyrazine (pypyraz)

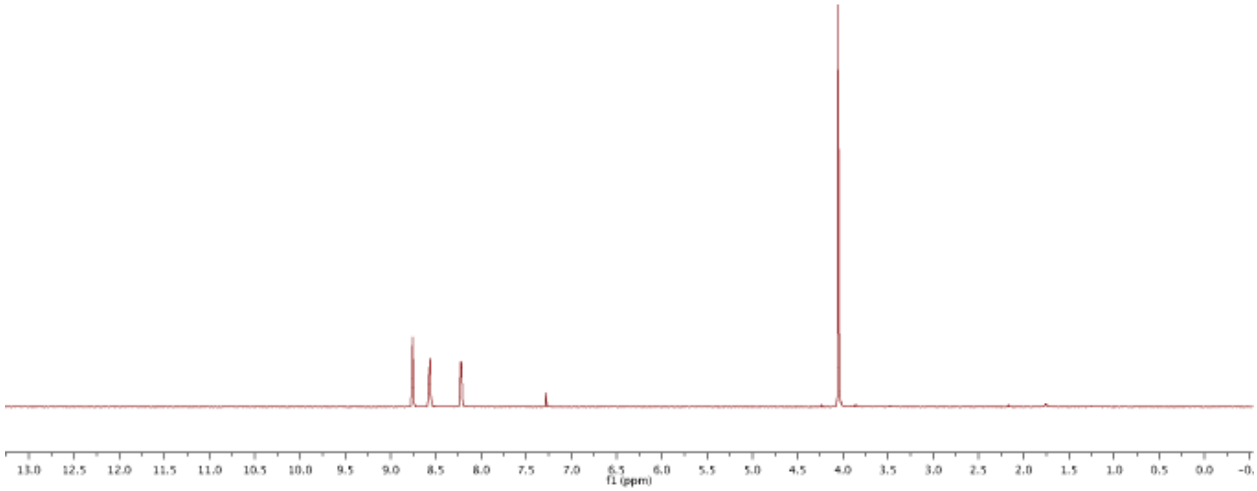


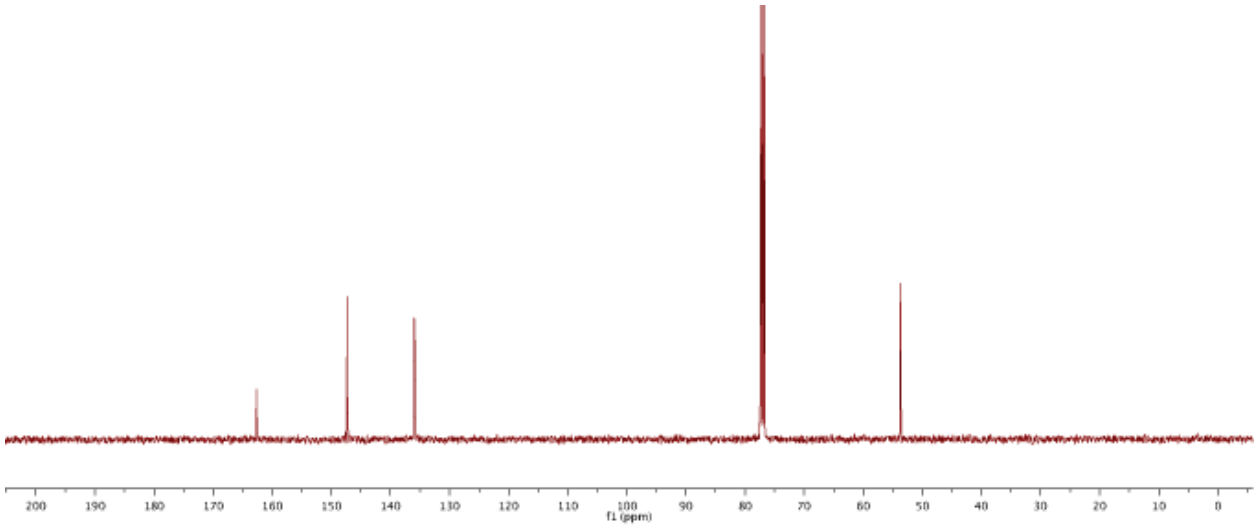




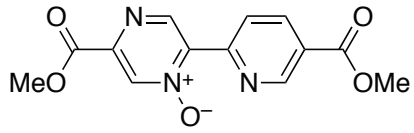
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Methyl Pyrazine-3-carboxylate-1-oxide

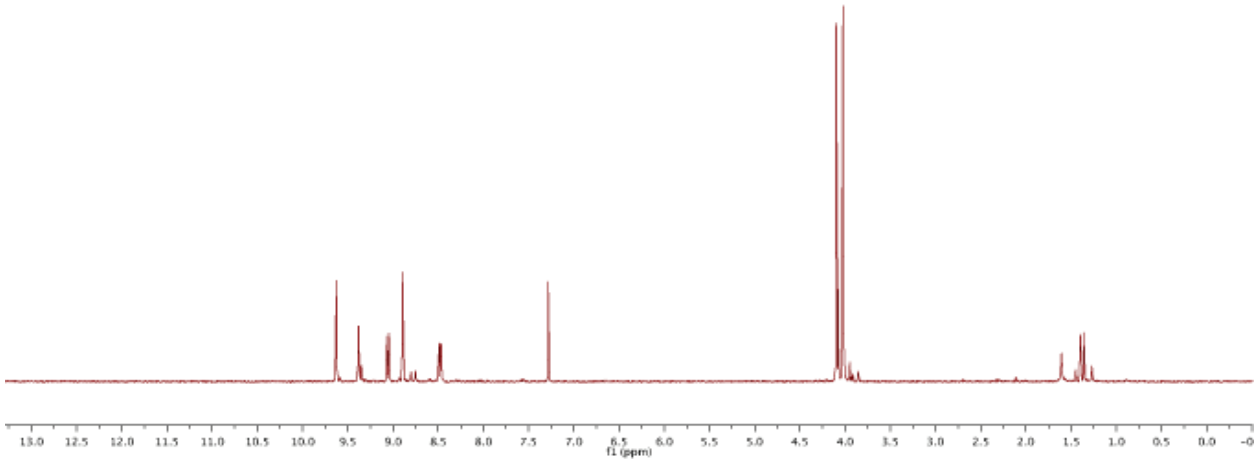




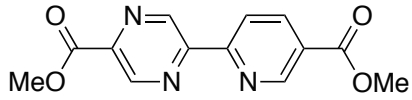


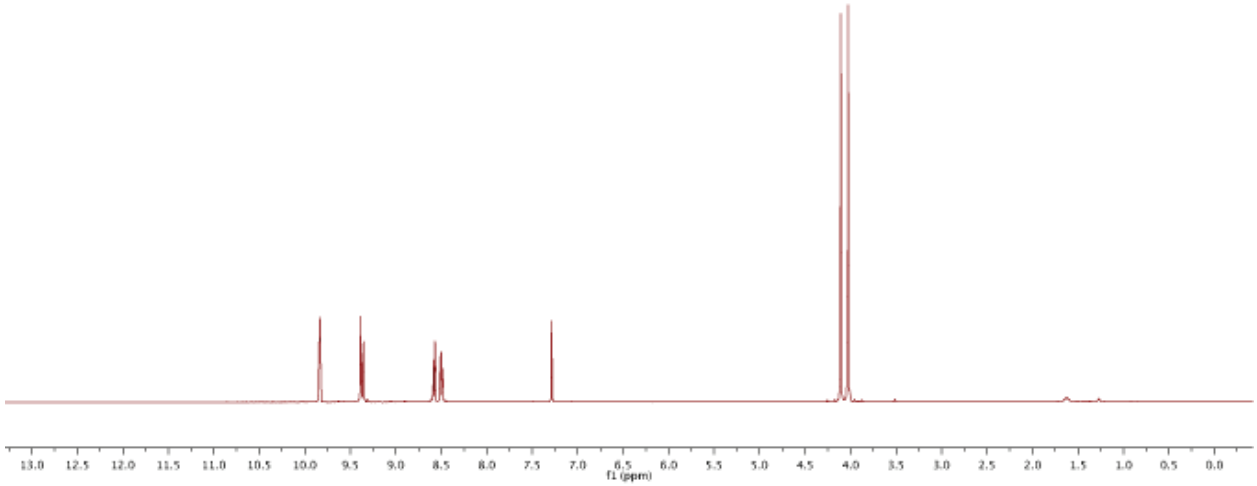
^{1}H NMR ($CDCl_3$) of Crude Methyl 2-(5-Methoxycarbonylpyridin-2-yl)pyrazine-5-carboxylate

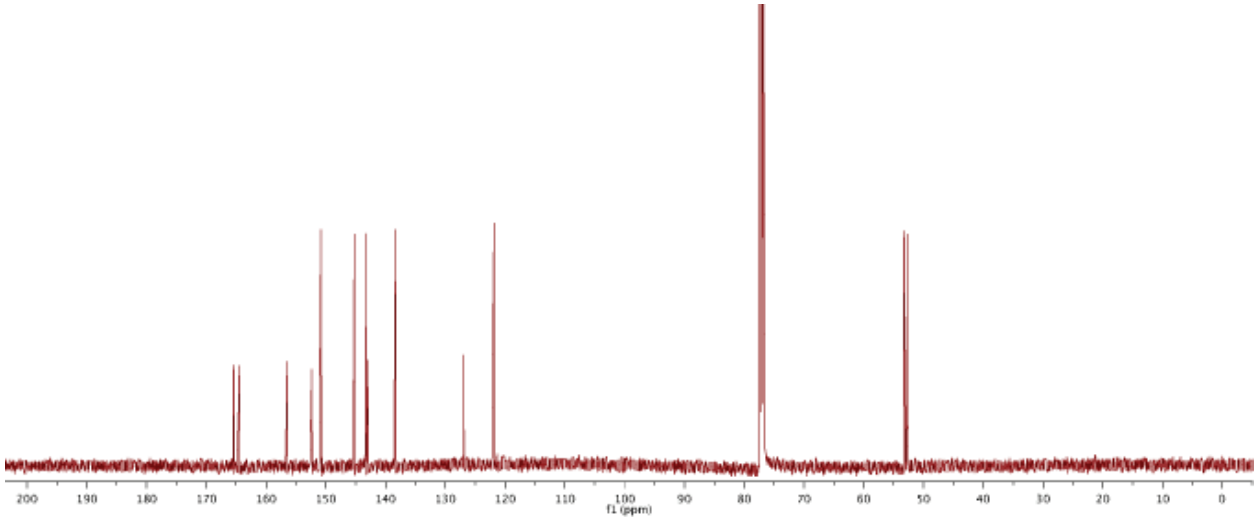




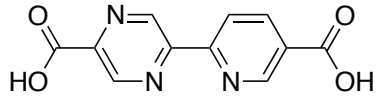
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Methyl 2-(5-Methoxycarbonylpyridin-2-yl)pyrazine-5-carboxylate (dimethyl pypyrazDC)

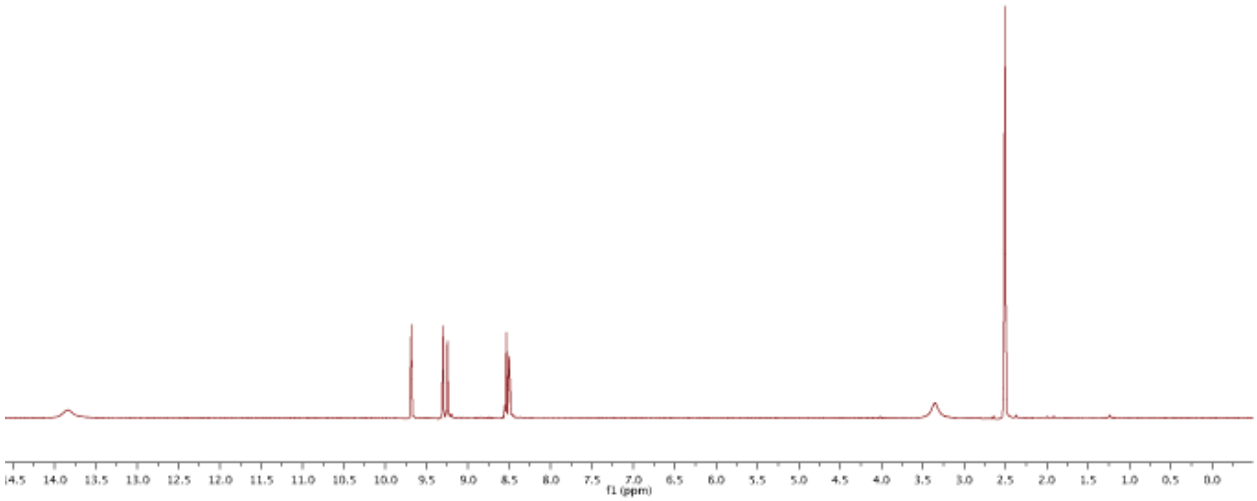


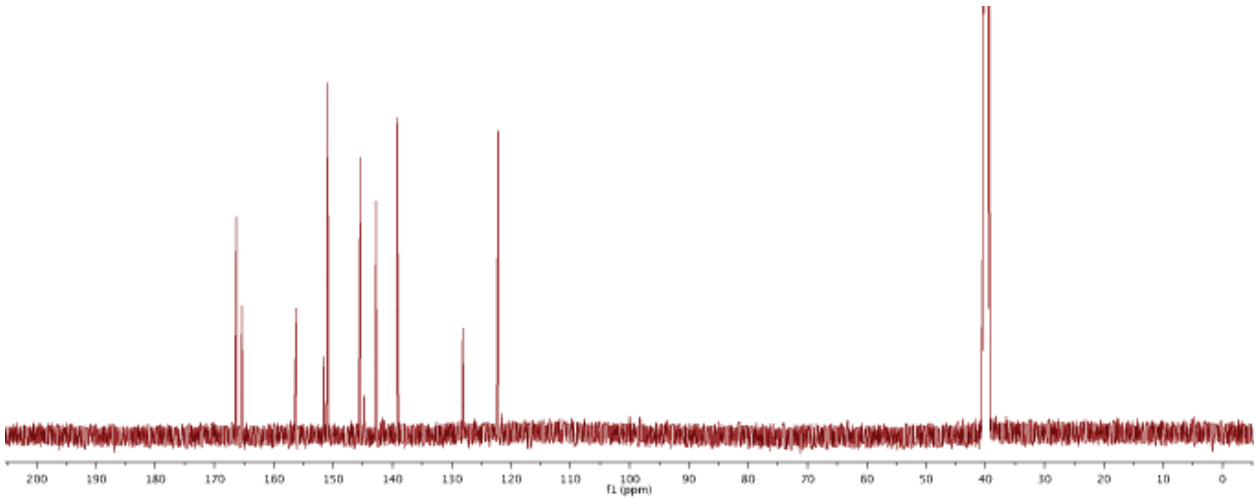




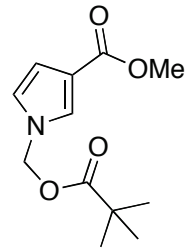
^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of 2-(5-Carboxypyridin-2-yl)pyrazine-5-carboxylic Acid (pypyrazDC)

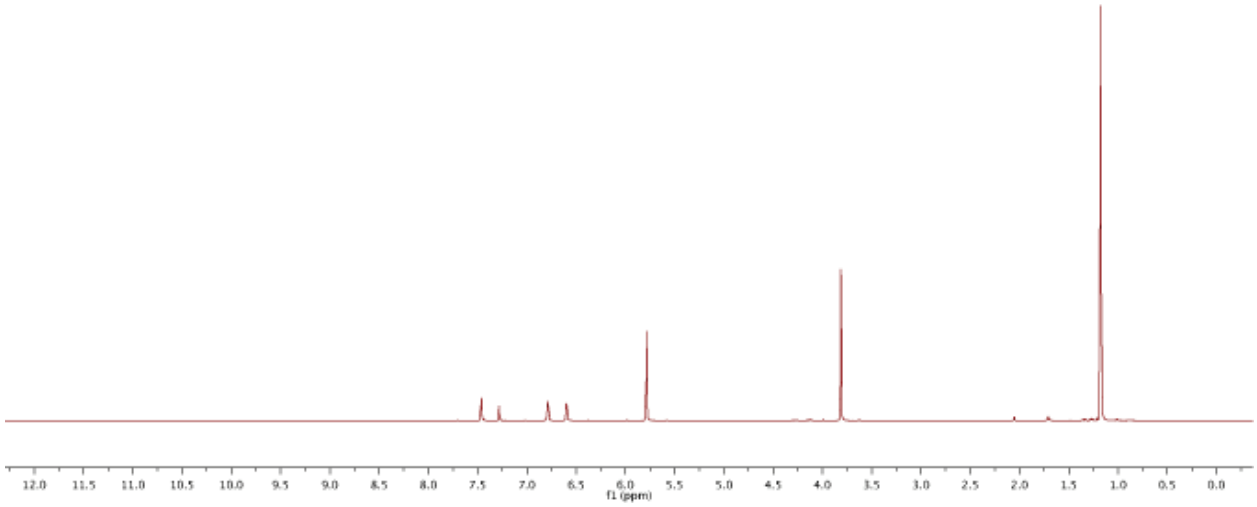


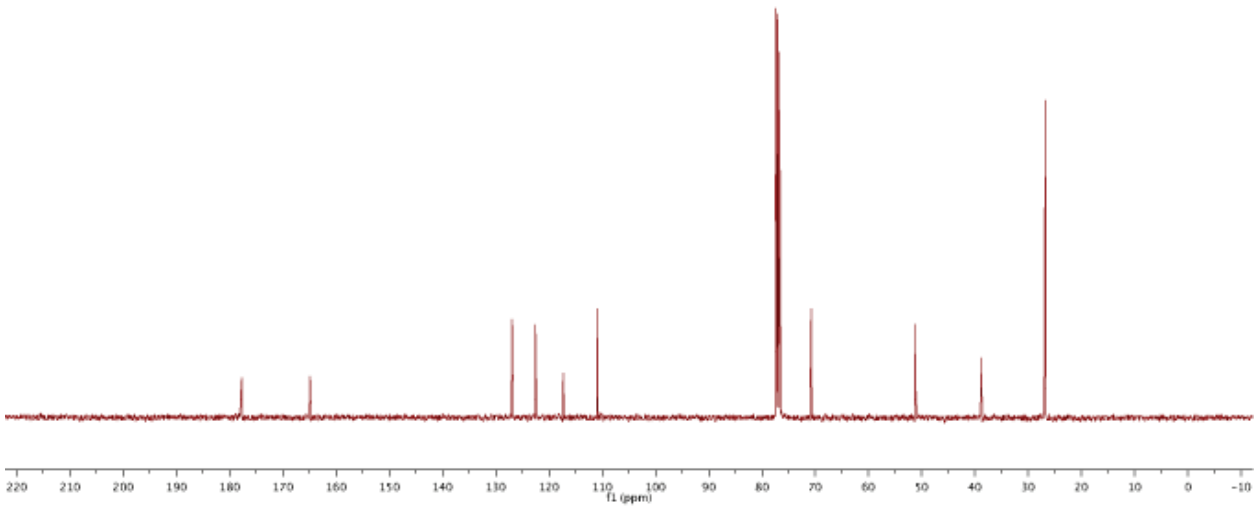




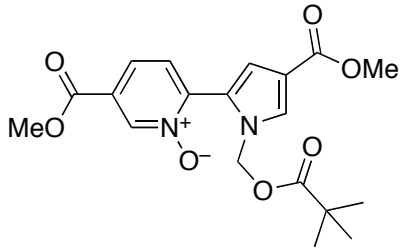
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Methyl 1-Pivaloyloxymethyl-1*H*-pyrrole-3-carboxylate

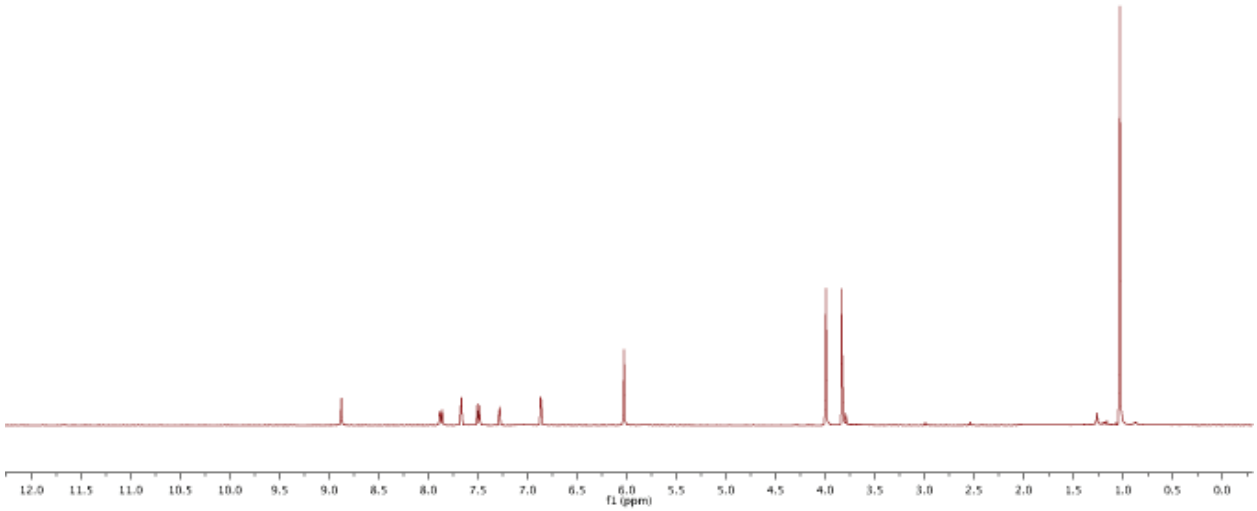


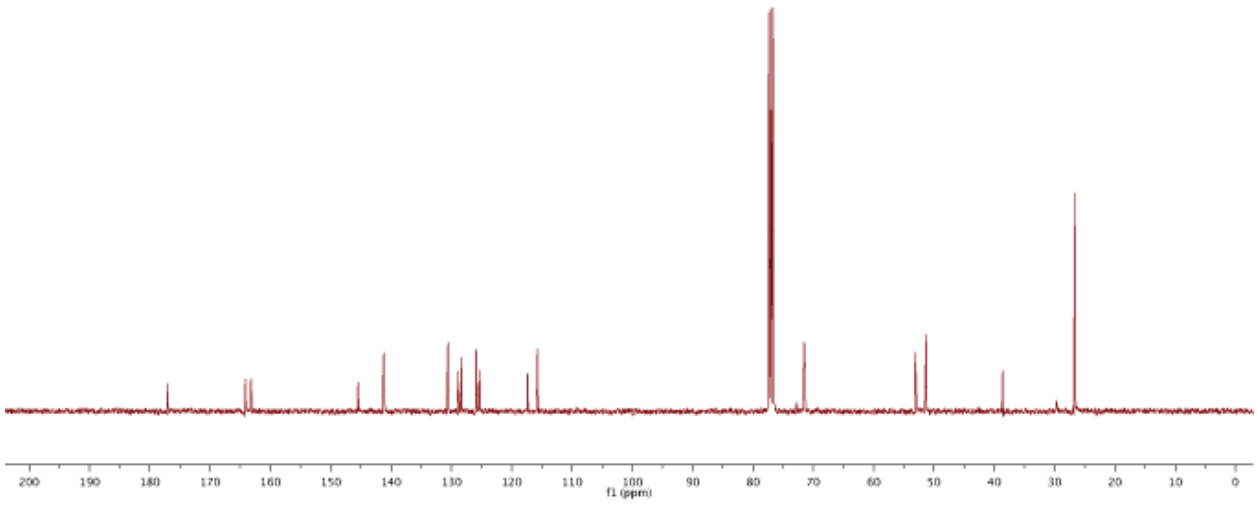




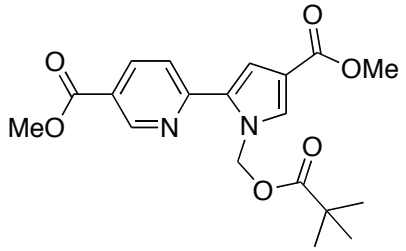
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Methyl 2-(4-Methoxycarbonyl-1-pivaloyloxymethyl-1*H*-pyrrol-2-yl)pyridine-5-carboxylate-1-oxide

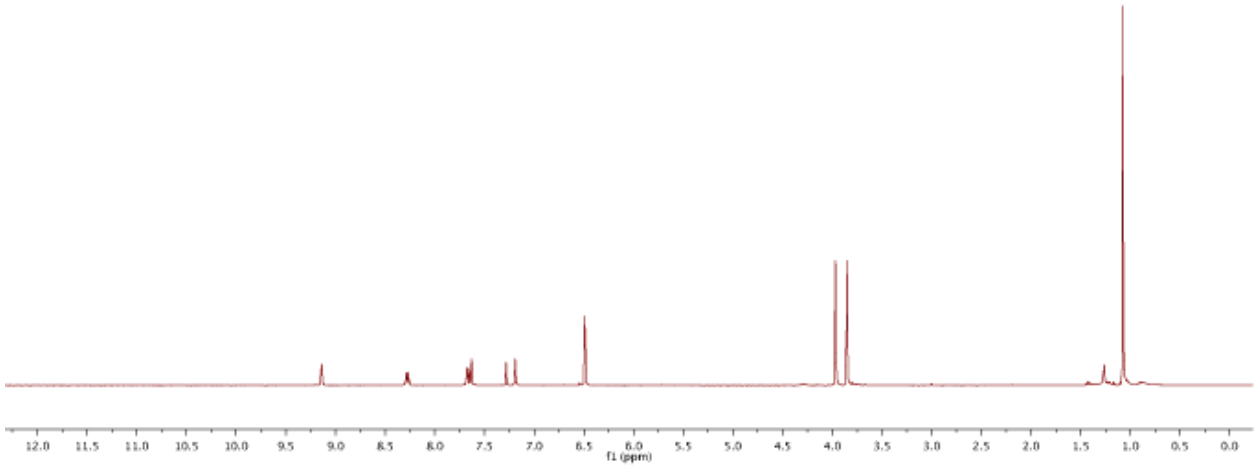


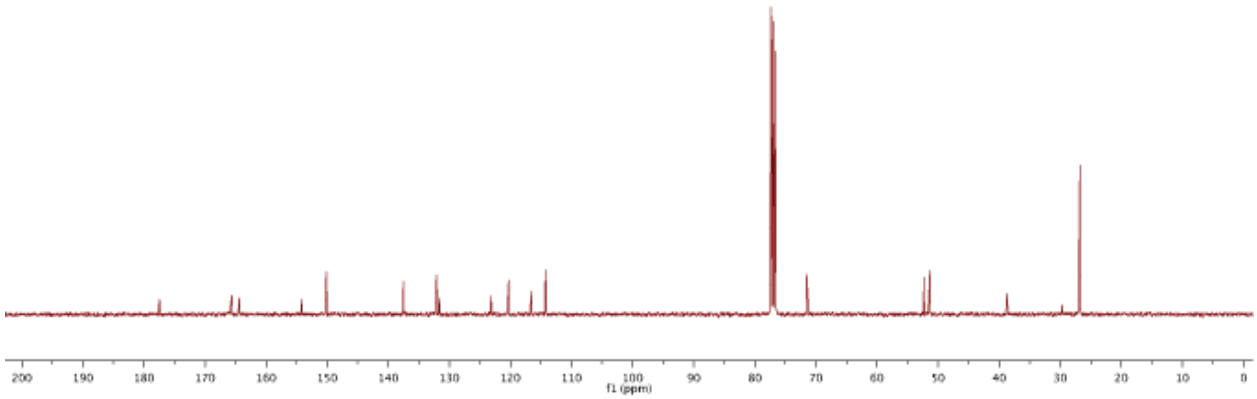




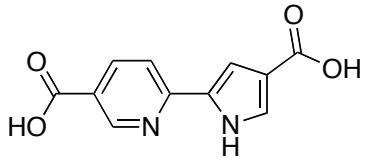
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Methyl 2-(4-Methoxycarbonyl-1-pivaloyloxymethyl-1*H*-pyrrol-2-yl)pyridine-5-carboxylate

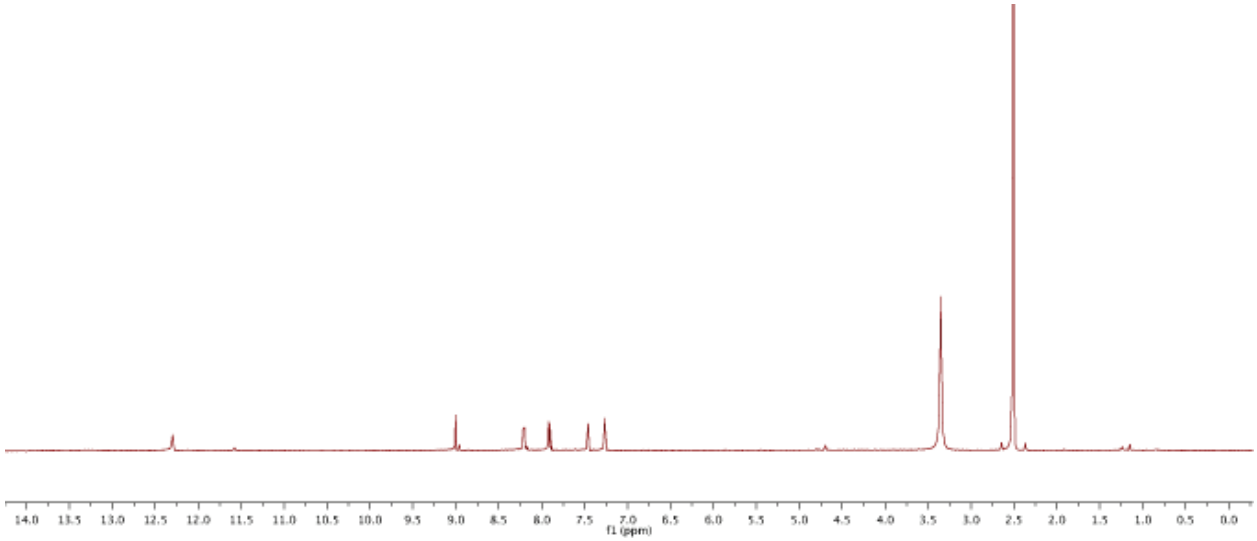


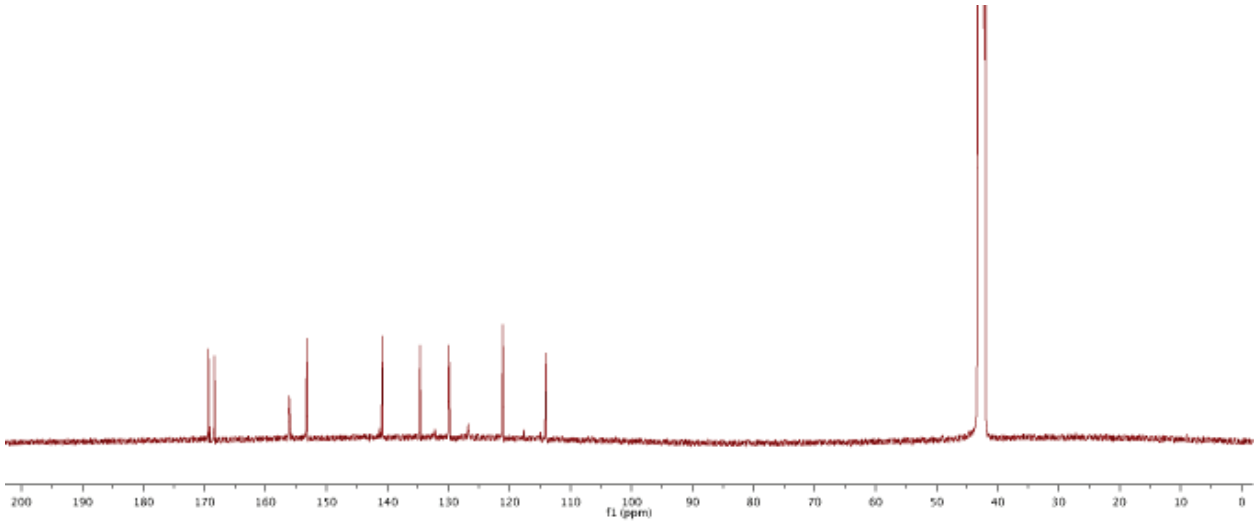




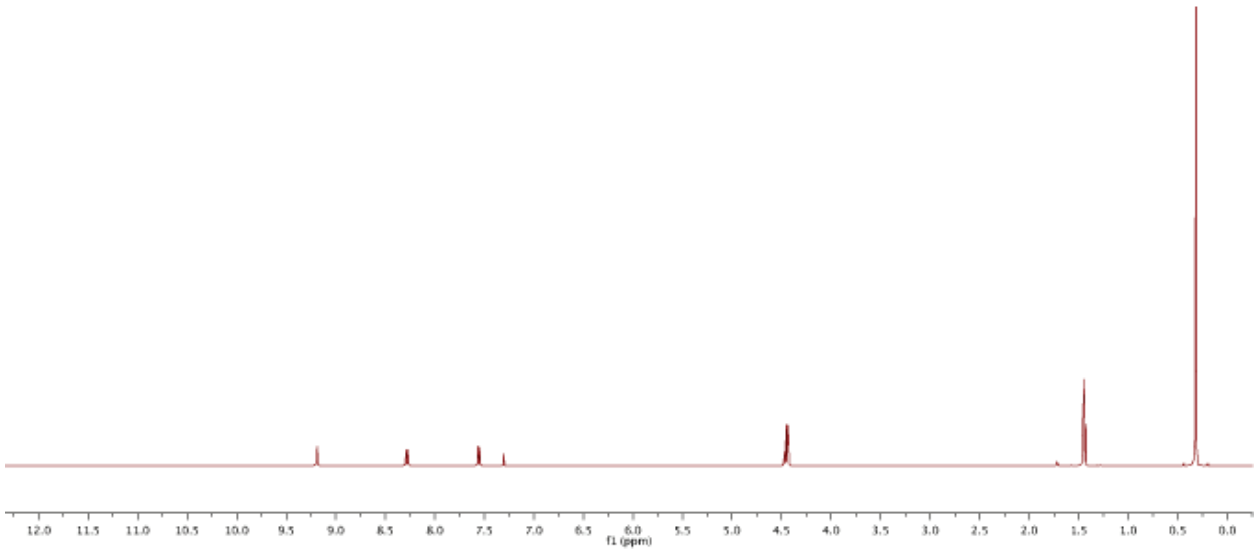
^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of 2-(4-Carboxy-1*H*-pyrrol-2-yl)pyridine-5-carboxylic Acid (pypyrroleDC)

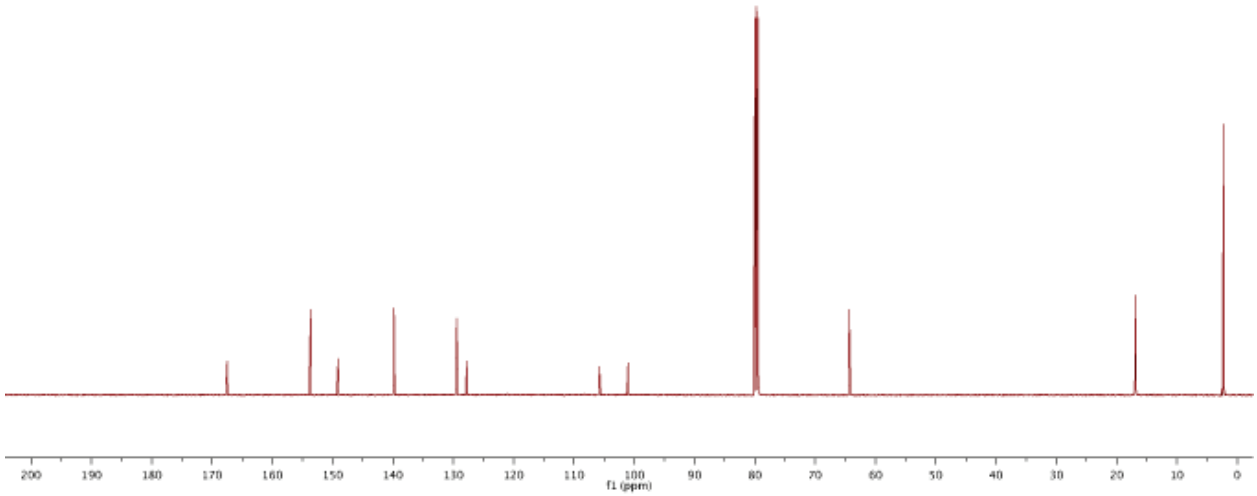




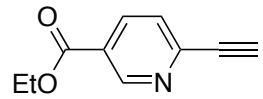


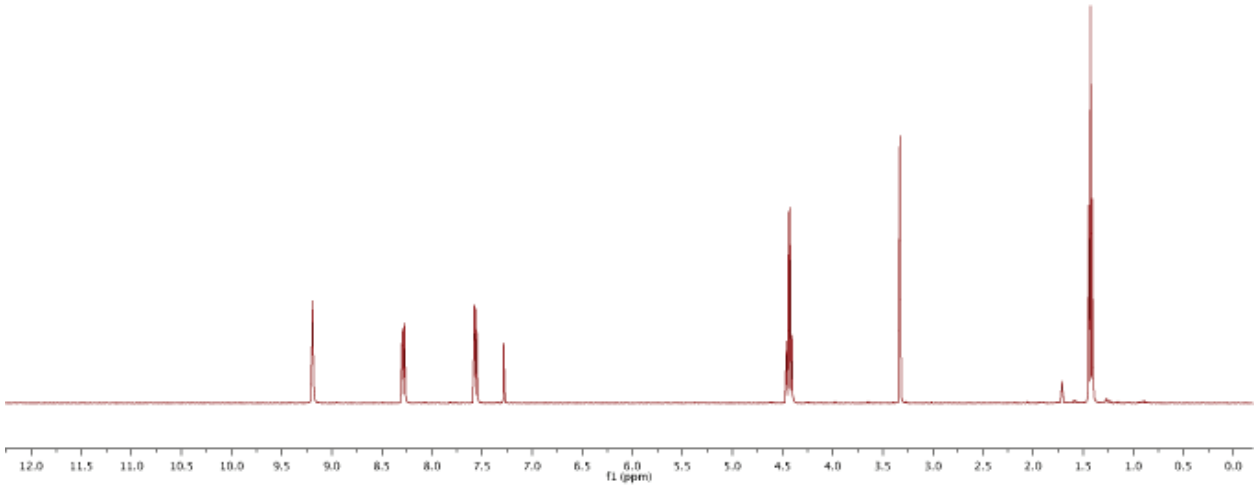
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Ethyl 2-(2-Trimethylsilylethynyl)pyridine-5-carboxylate

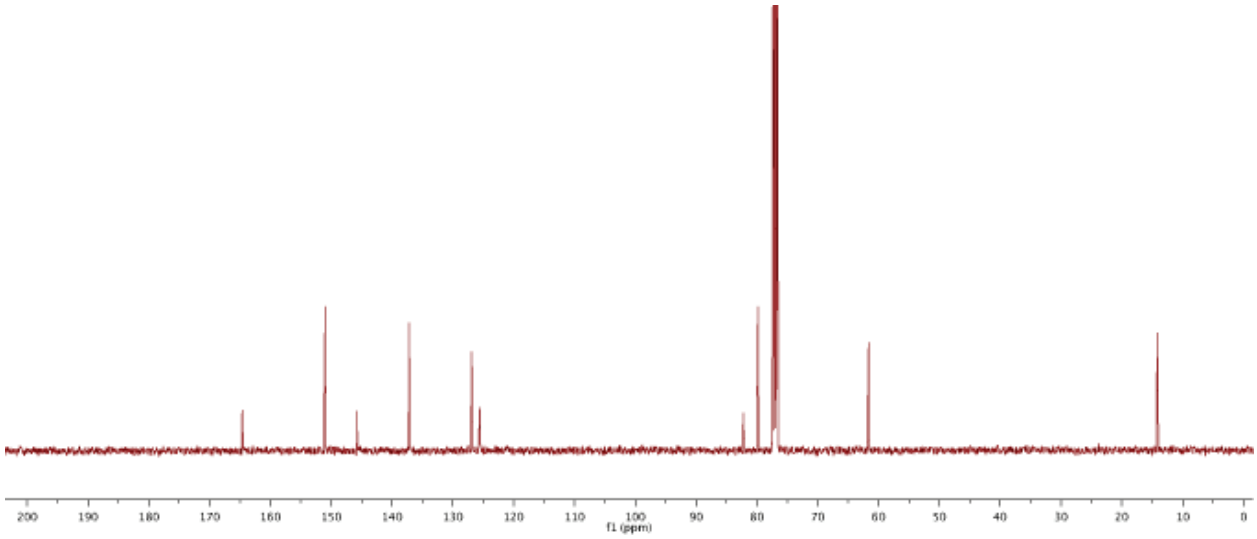




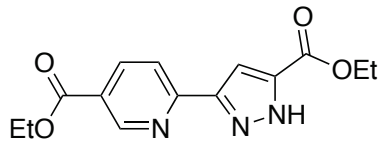
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Ethyl 2-Ethynylpyridine-5-carboxylate

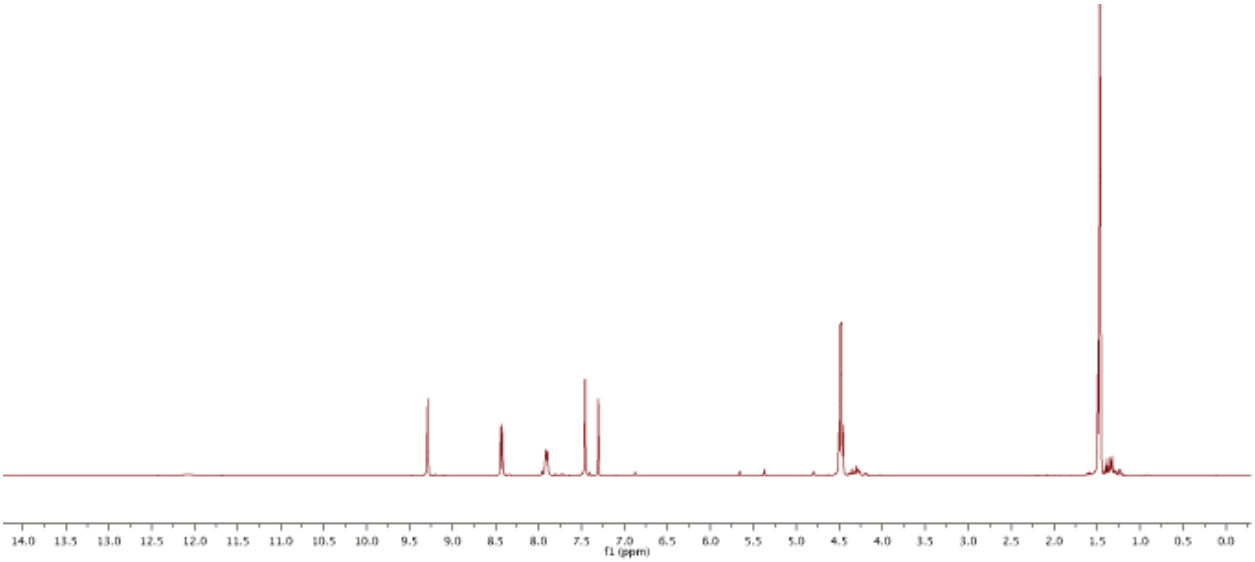


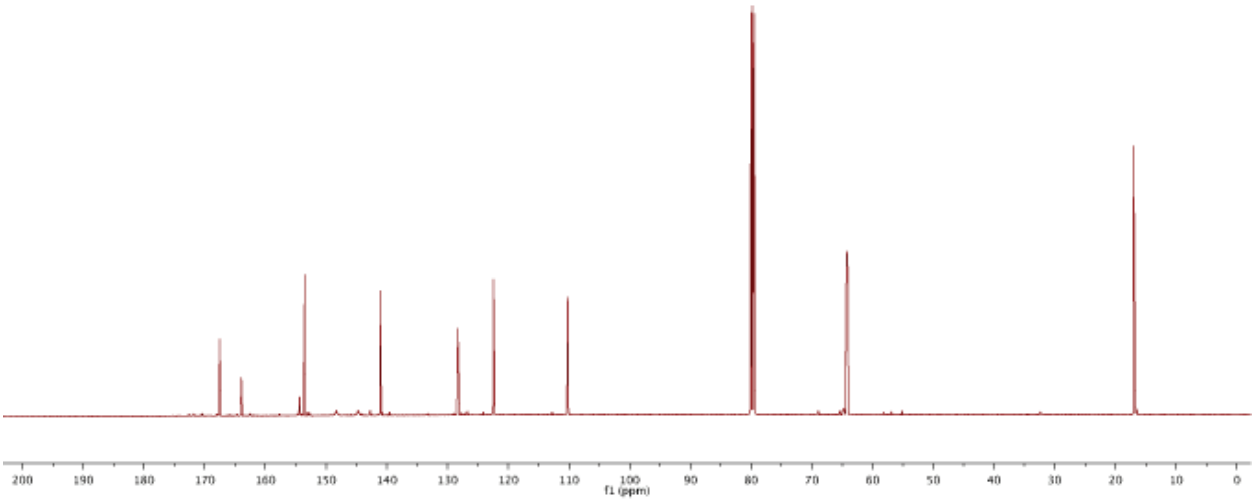




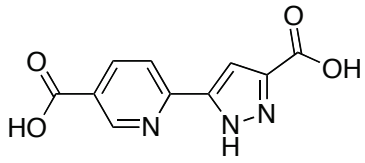
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Ethyl 2-(5-Ethoxycarbonyl-1*H*-pyrazol-3-yl)pyridine-5-carboxylate (diethyl pypyrDC)

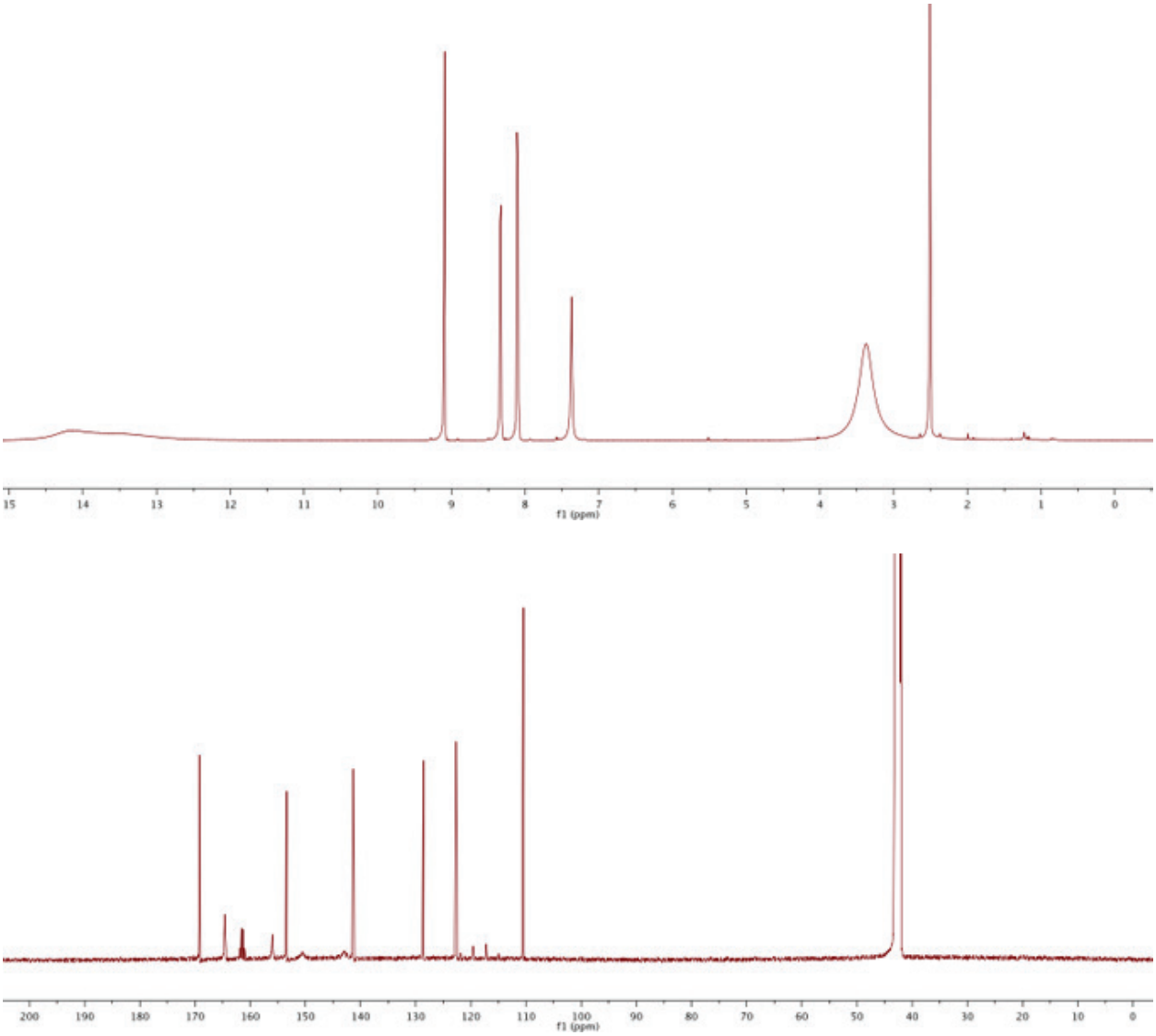




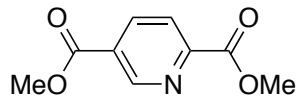


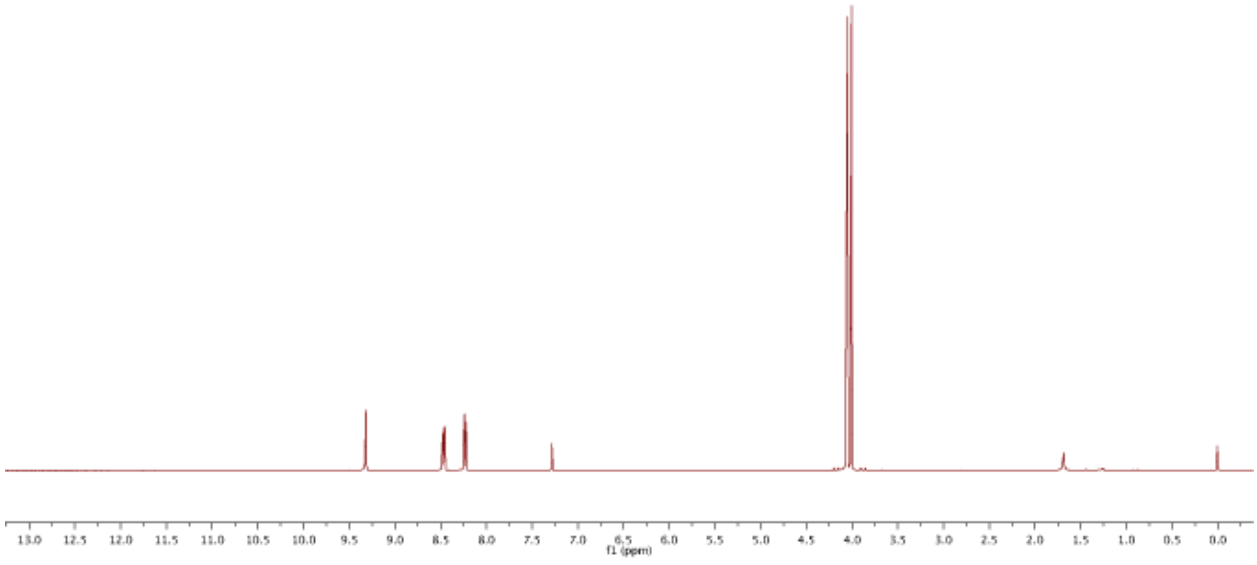
^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of 2-(3-Carboxy-1*H*-pyrazol-5-yl)pyridine-5-carboxylic Acid (pypyrDC)

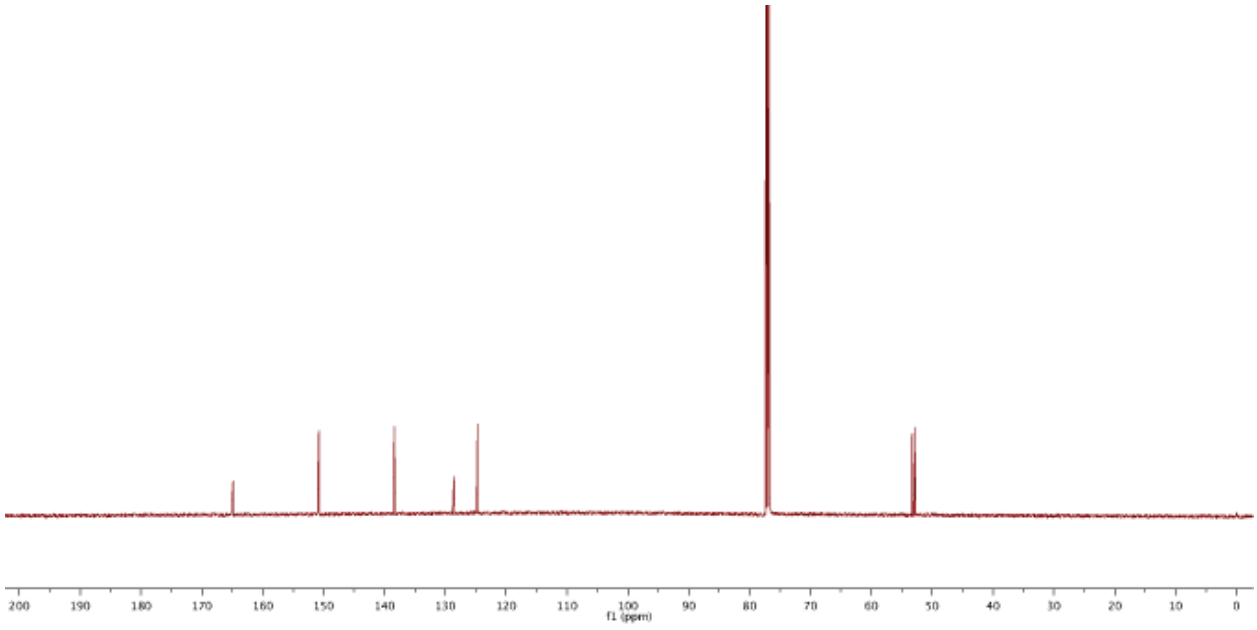




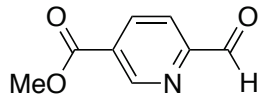
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Dimethyl 25PDC

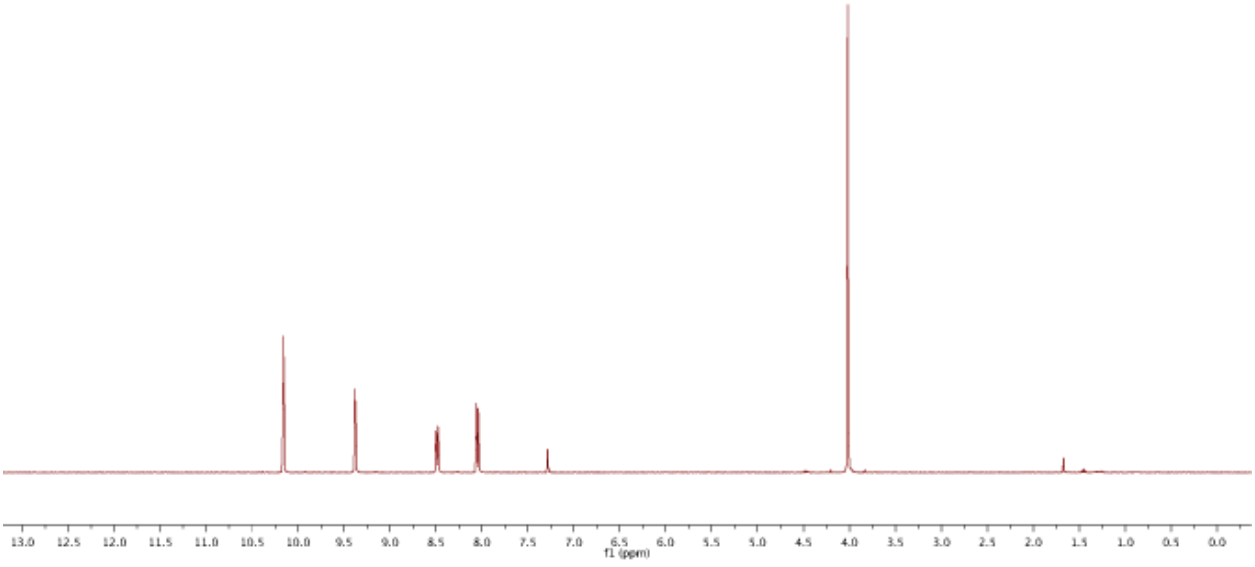


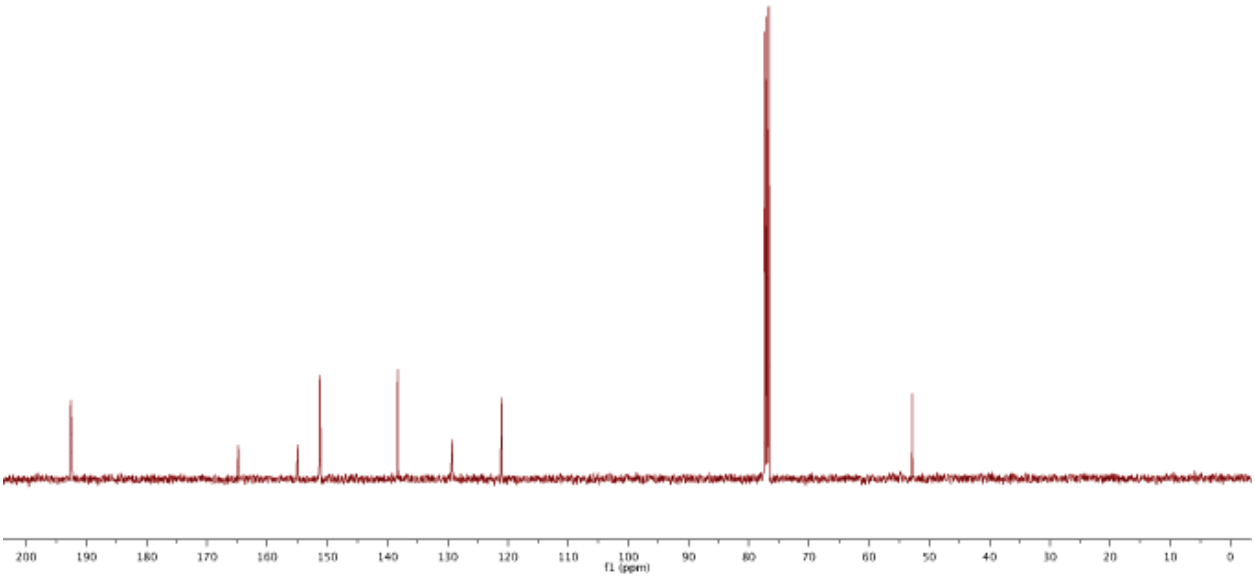




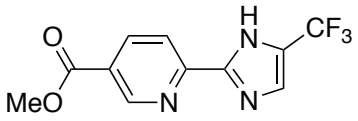
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Methyl 2-Formylnicotinate

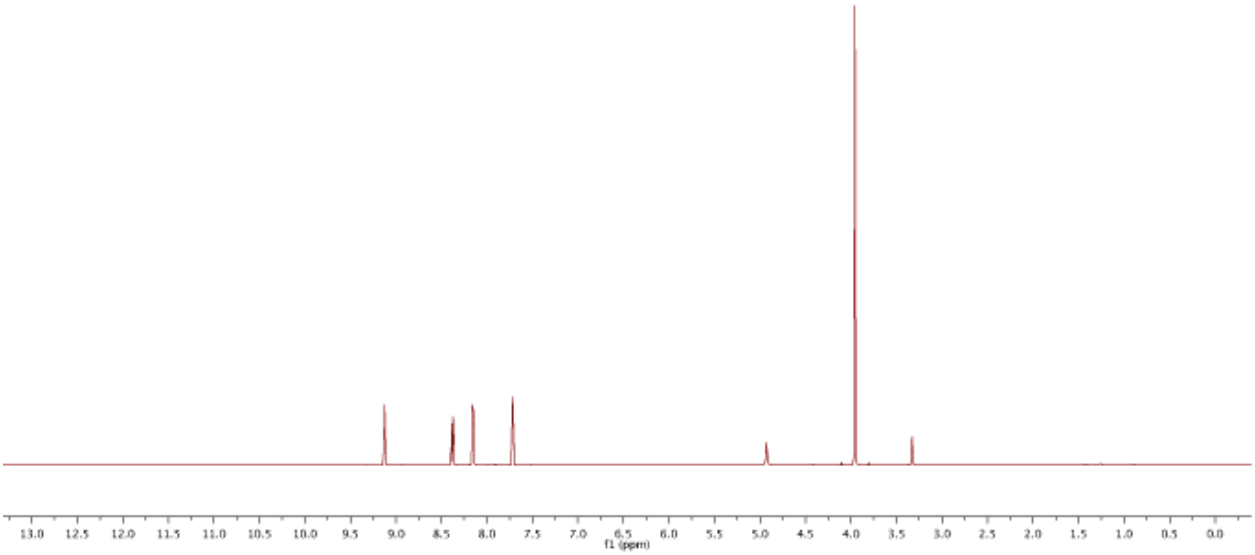


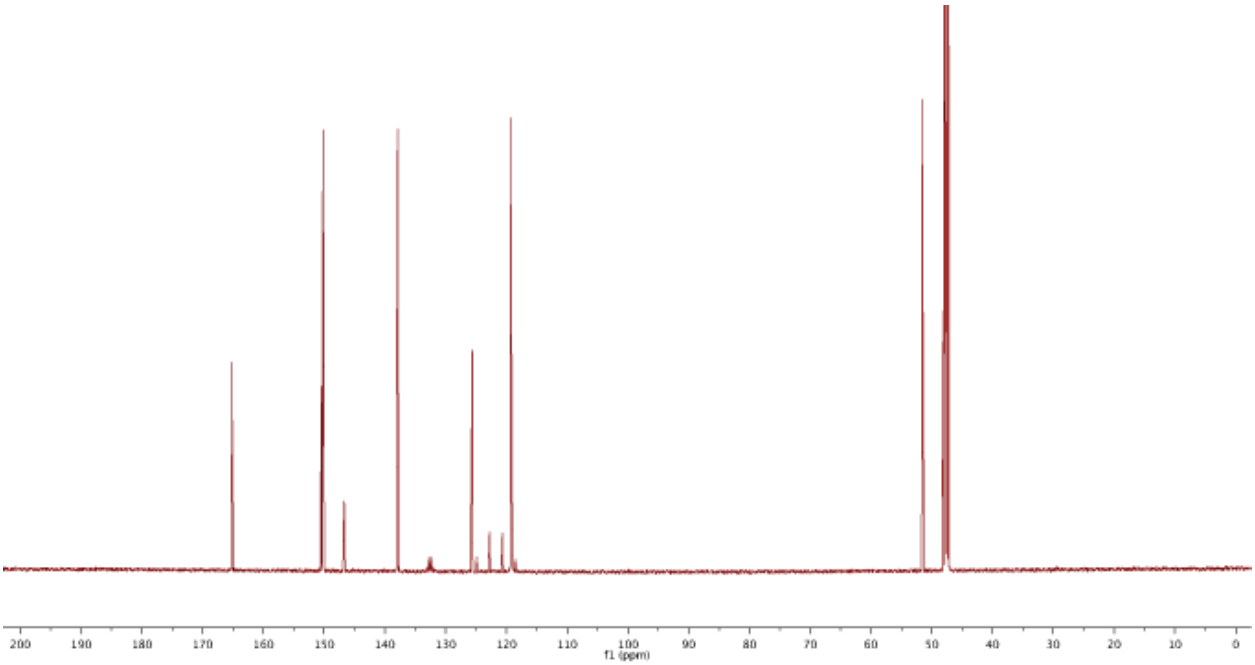




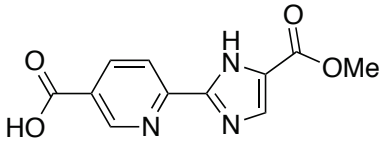
^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) of Methyl 2-(5-Trifluoromethyl-1*H*-imidazol-2-yl)pyridine-5-carboxylate

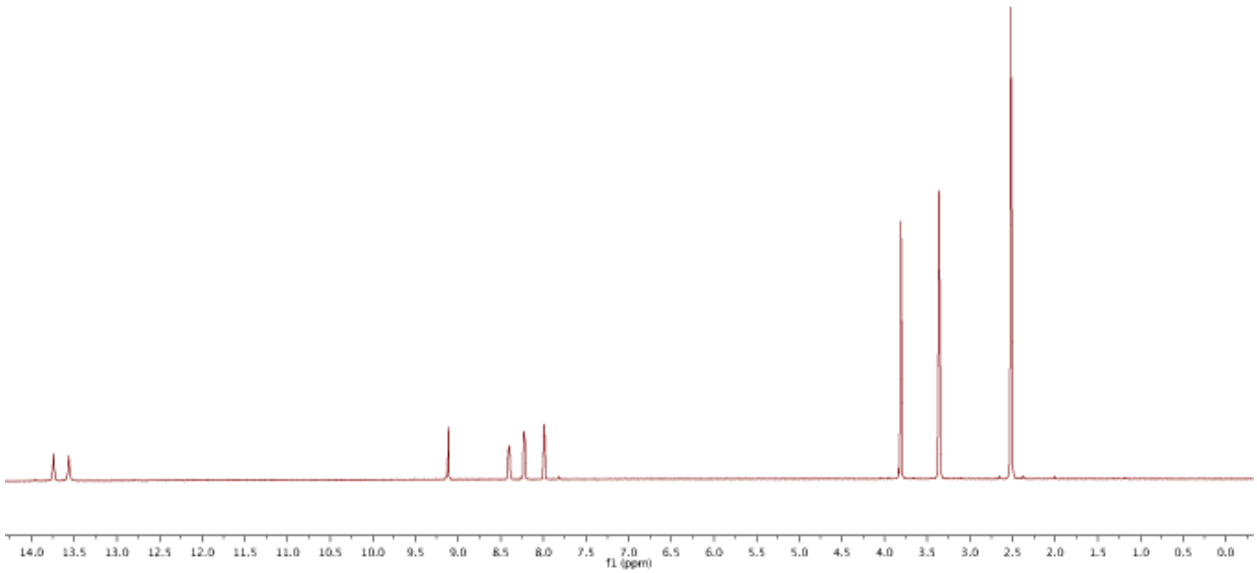


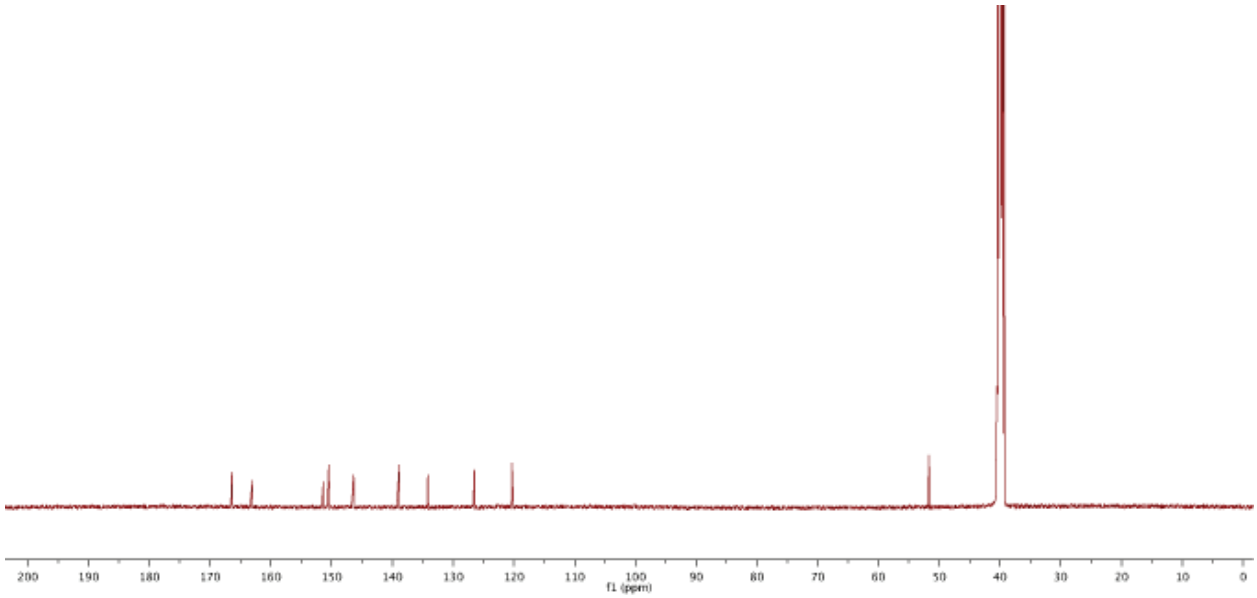




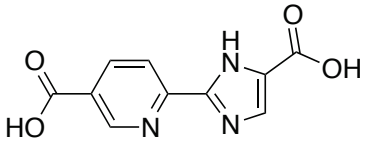
^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of 2-(5-Methoxycarbonyl-1*H*-imidazol-2-yl)pyridine-5-carboxylic Acid

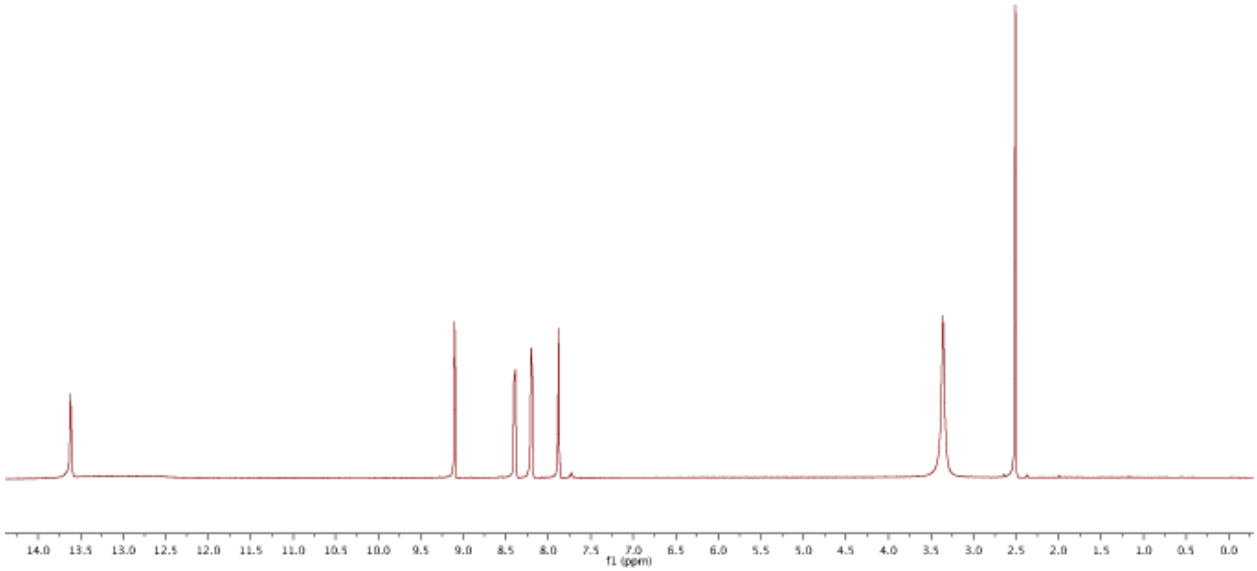


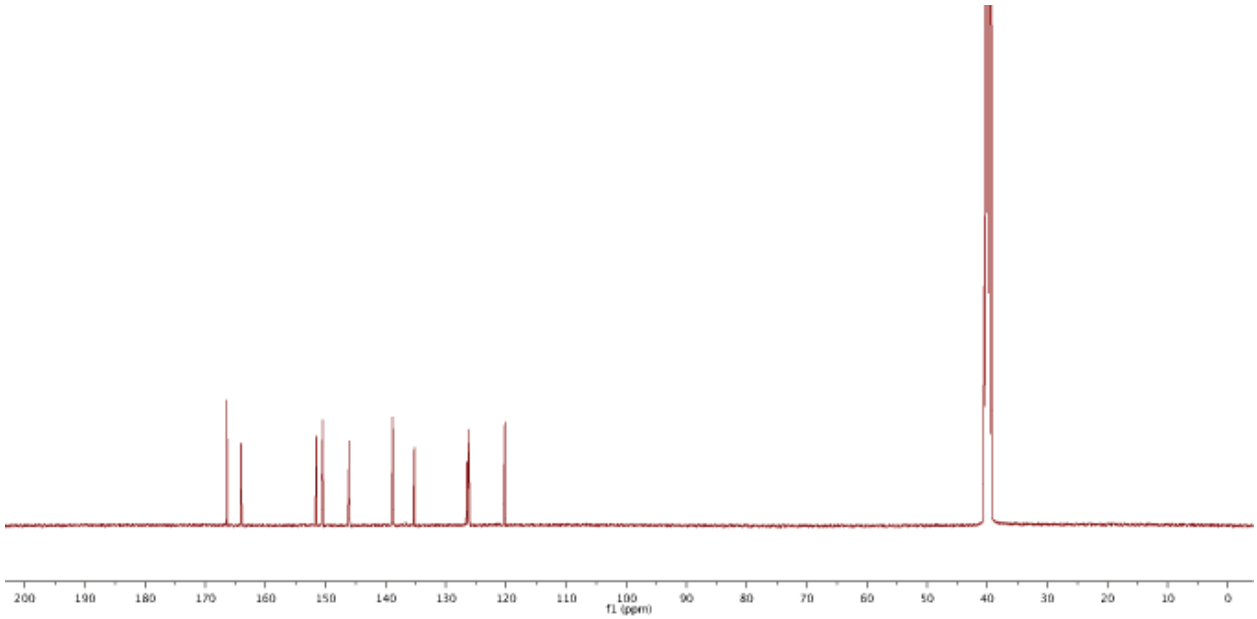




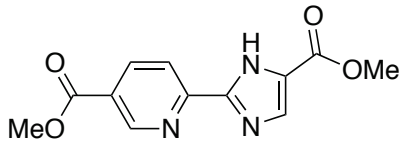
^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of 2-(5-Carboxy-1*H*-imidazol-2-yl)pyridine-5-carboxylic Acid (pyimDC)

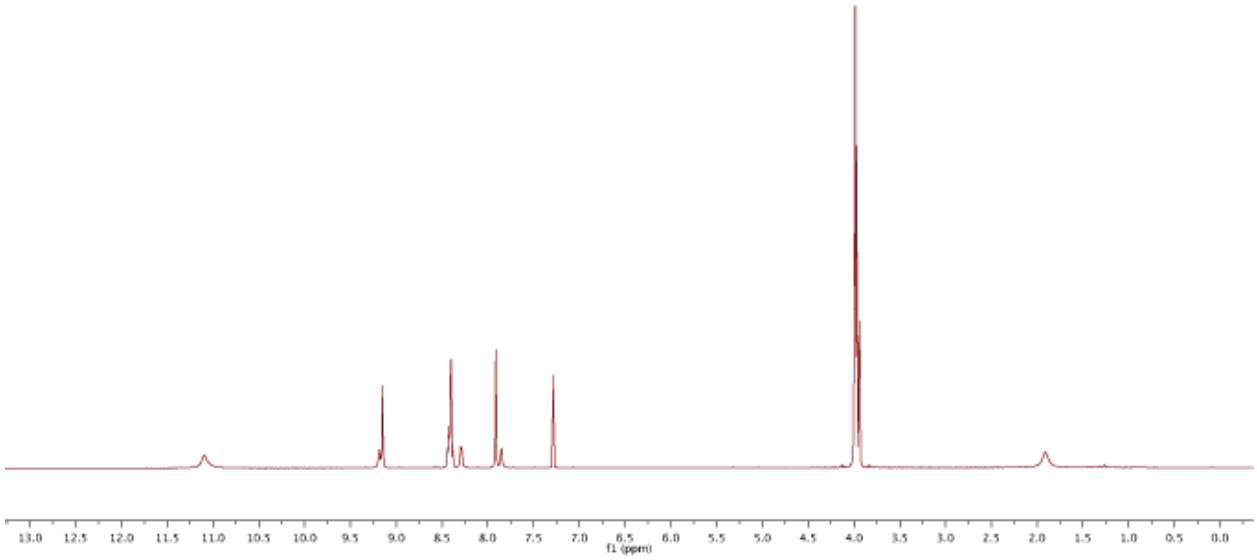


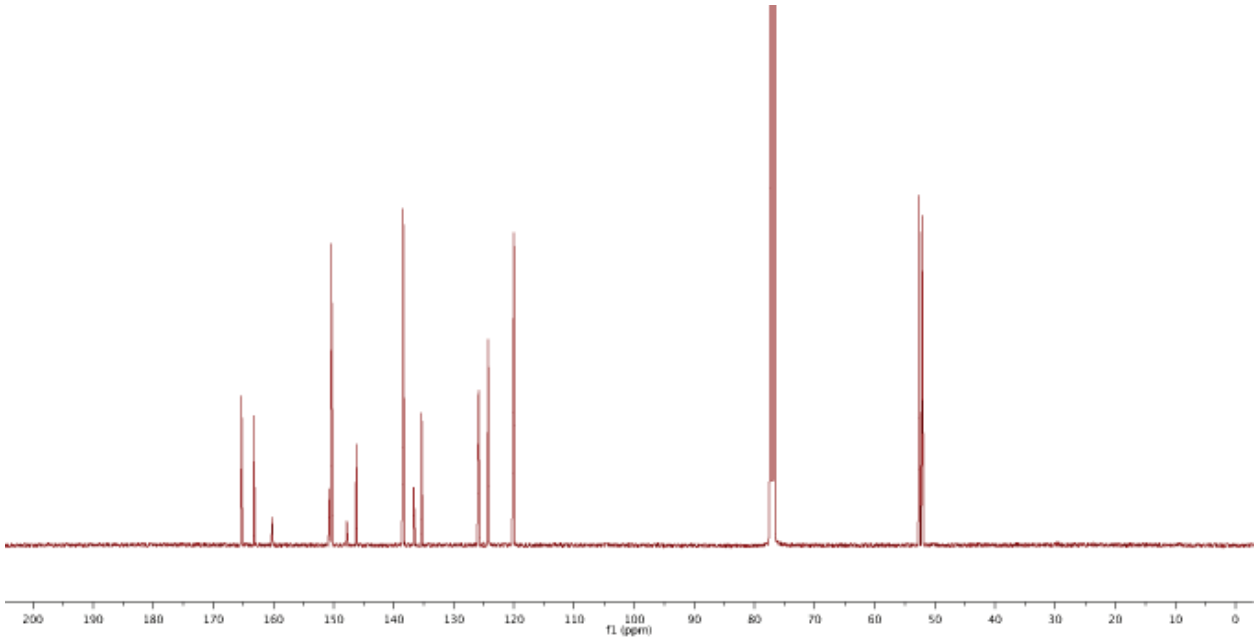




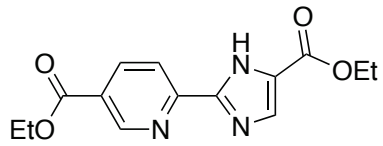
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Methyl 2-(5-Methoxycarbonyl-1*H*-imidazol-2-yl)pyridine-5-carboxylate (dimethyl pyimDC)

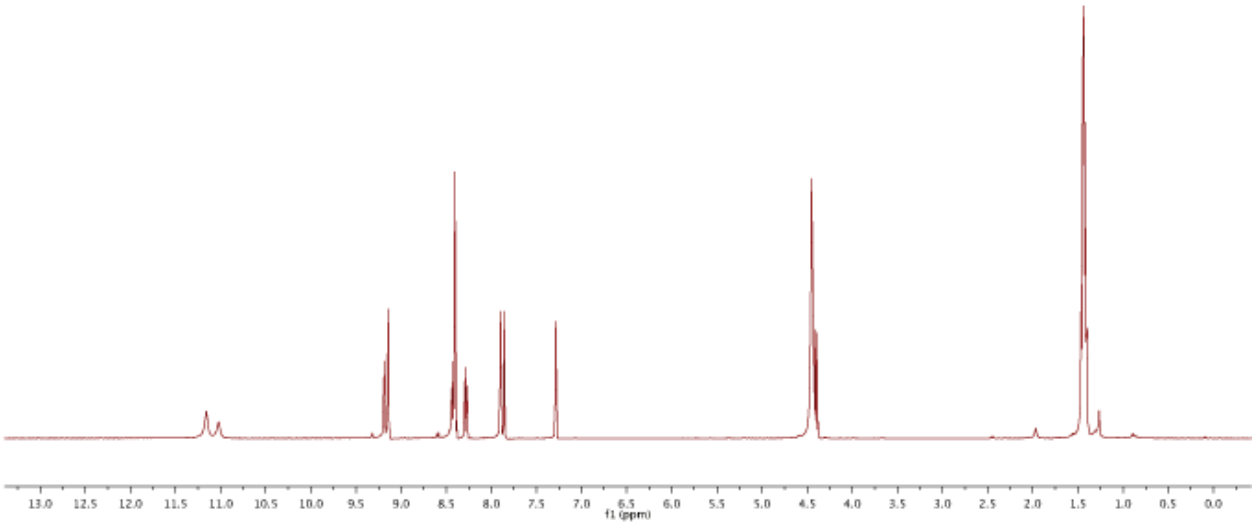


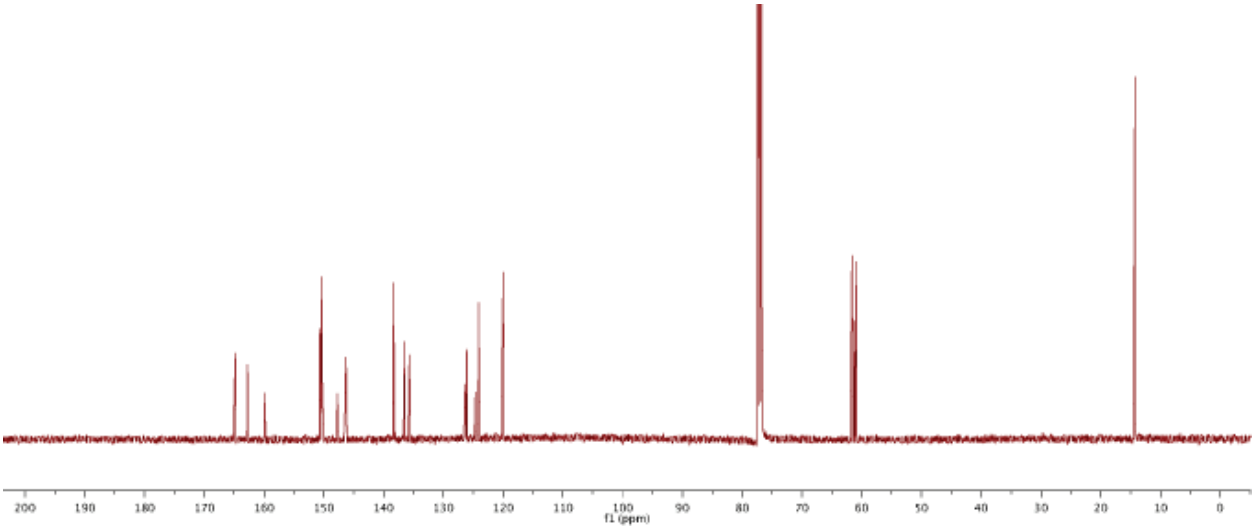




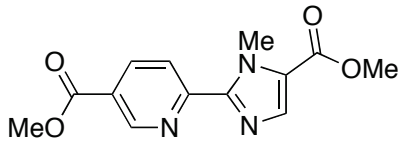
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Ethyl 2-(5-Ethoxycarbonyl-1*H*-imidazol-2-yl)pyridine-5-carboxylate (diethyl pyimDC)

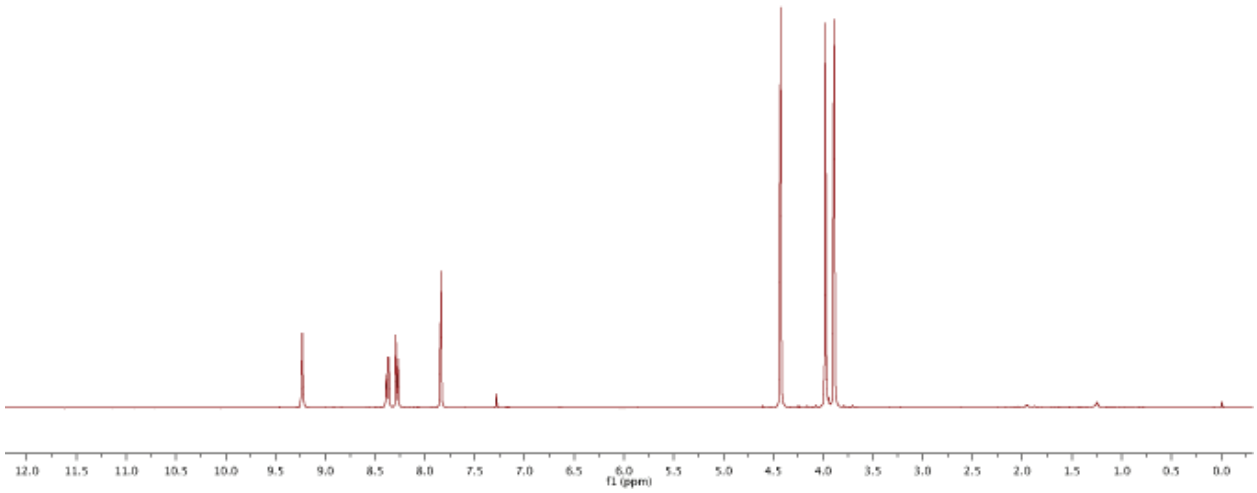


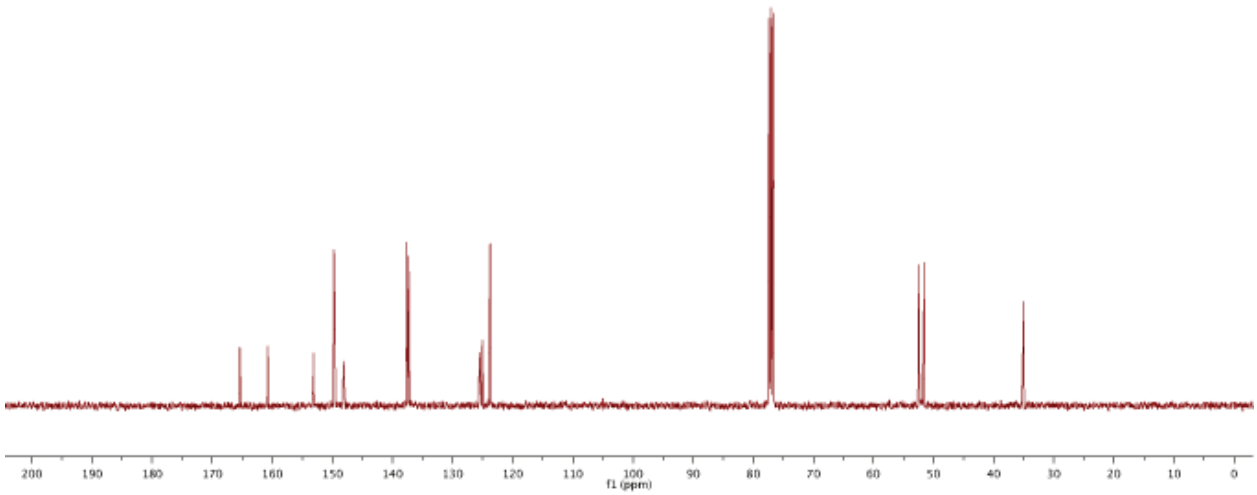




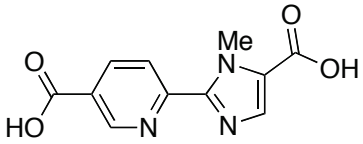
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Methyl 2-(5-Methoxycarbonyl-1-methyl-1*H*-imidazol-2-yl)pyridine-5-carboxylate (dimethyl NMe-pyimDC)

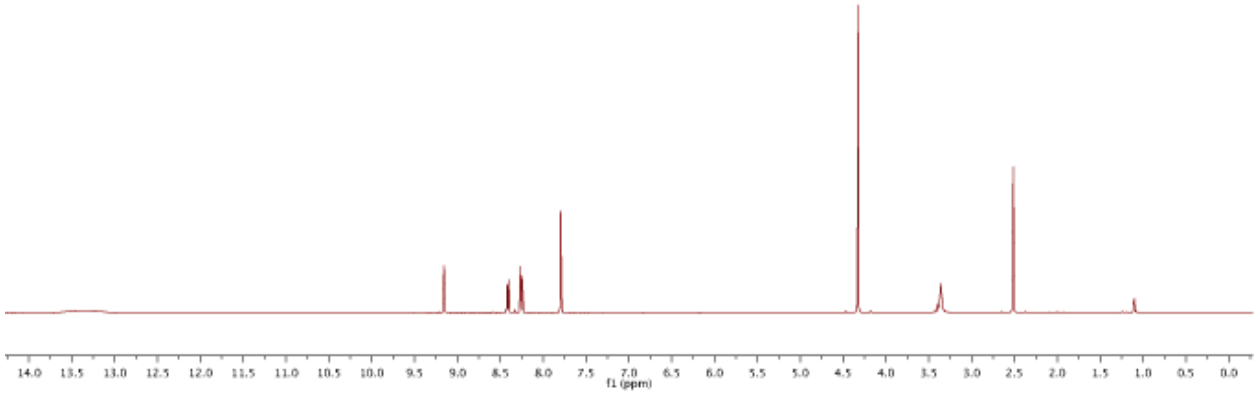


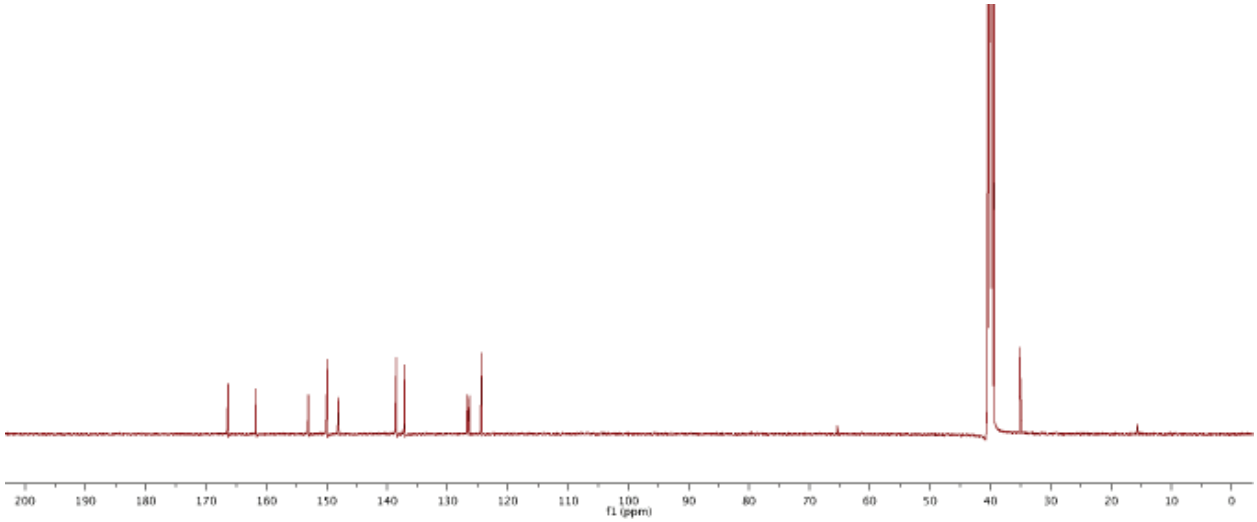




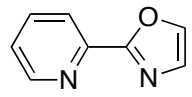
^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of 2-(5-Carboxy-1-methyl-1*H*-imidazol-2-yl)pyridine-5-carboxylic Acid (NMe-pyimDC)

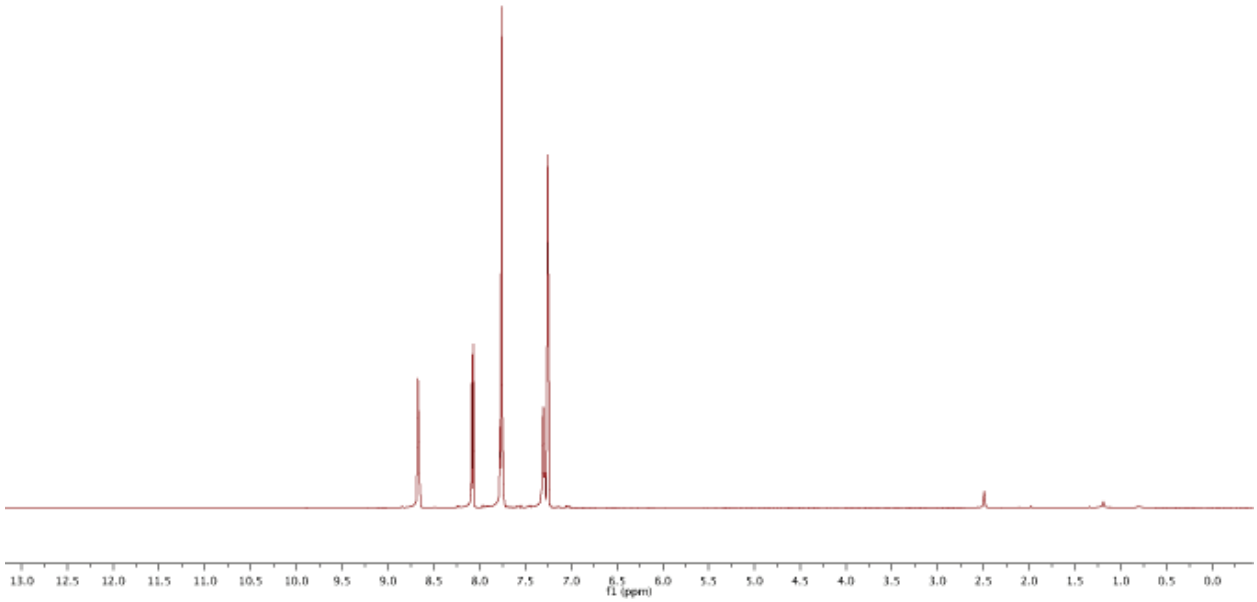


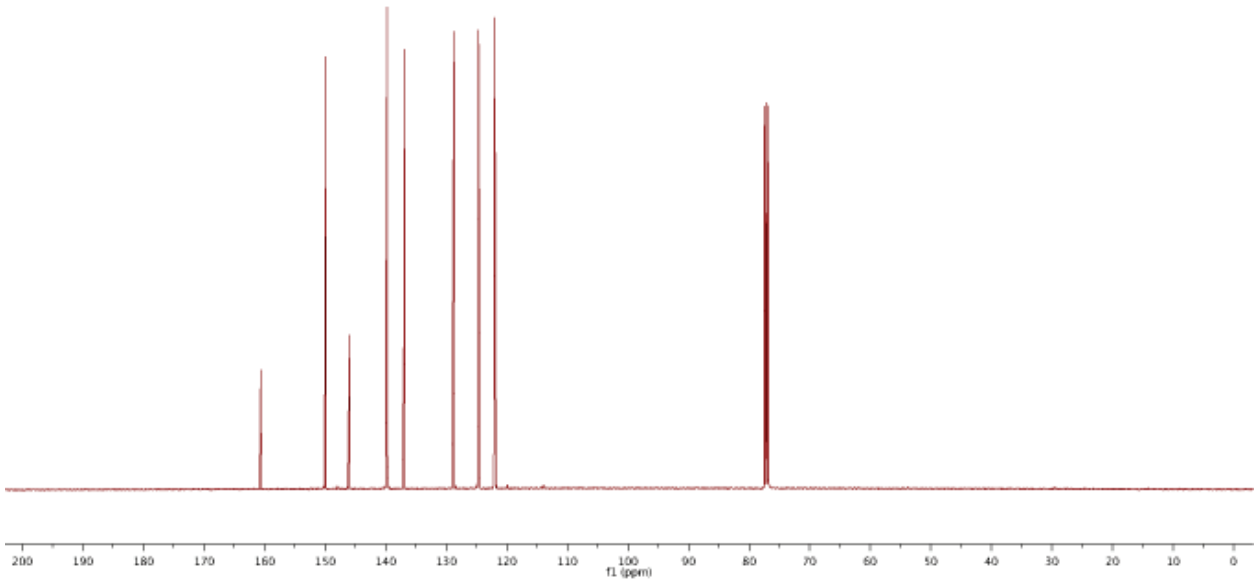




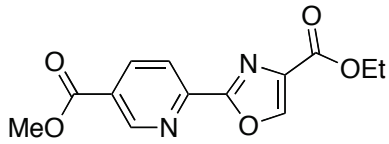
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of 2-(Oxazol-2-yl)pyridine (pyox)

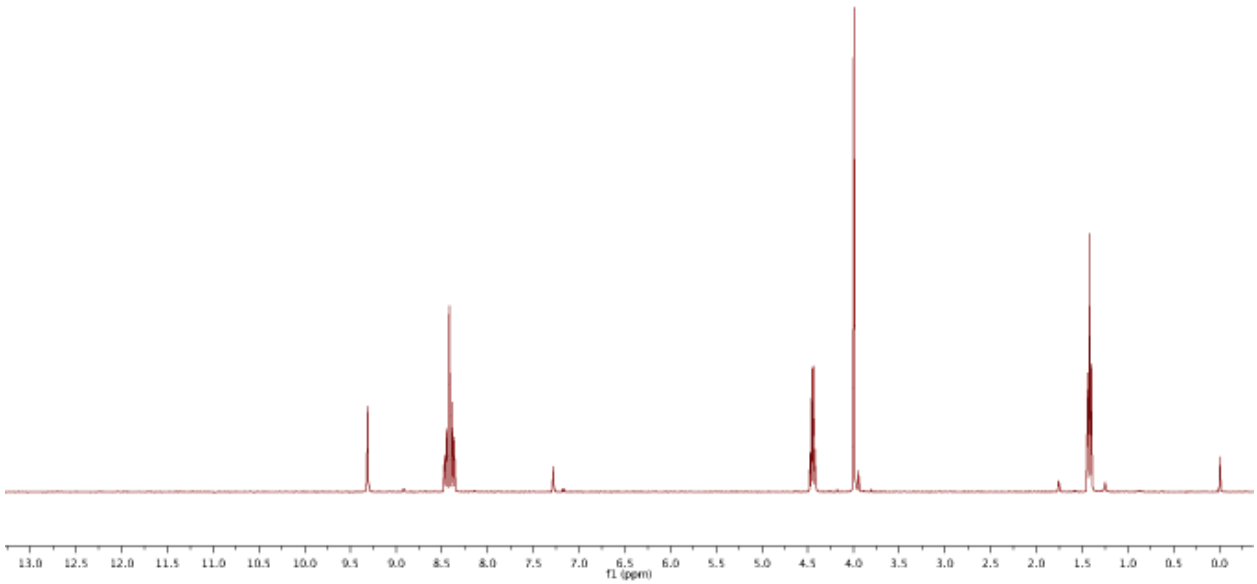


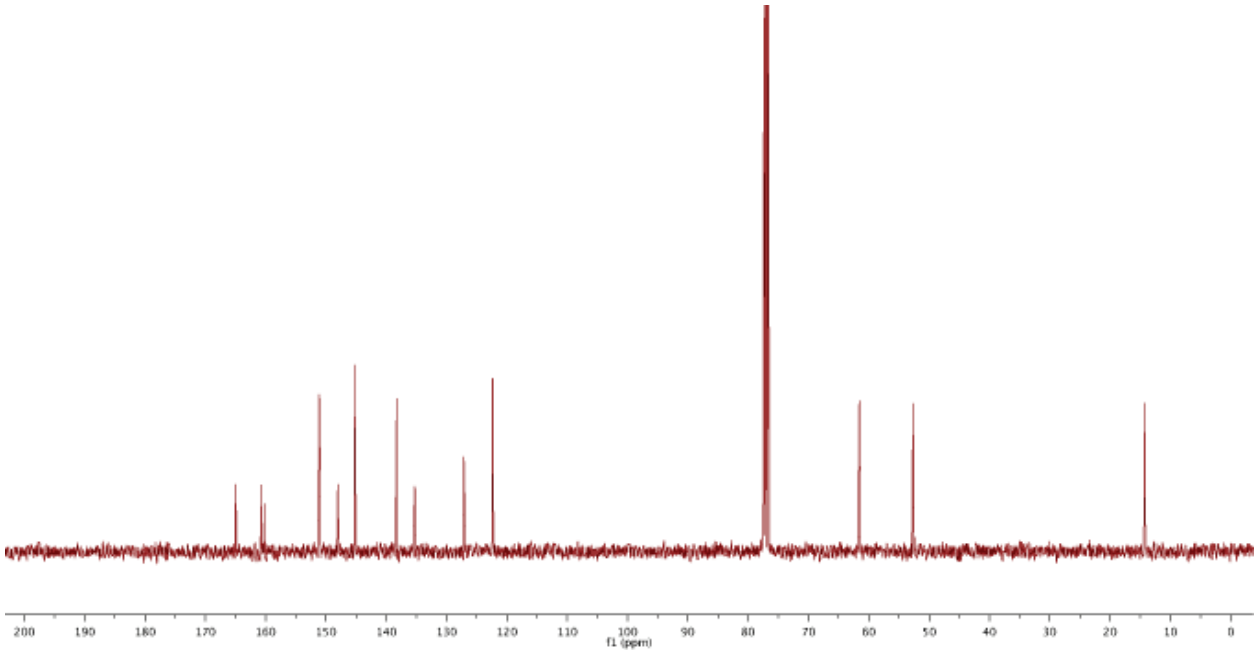




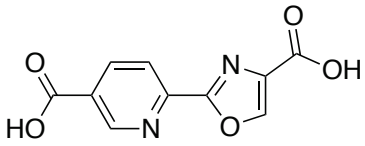
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Methyl 2-(4-Ethoxycarbonyloxazol-2-yl)pyridine-5-carboxylate (ethylmethyl pyoxDC*)

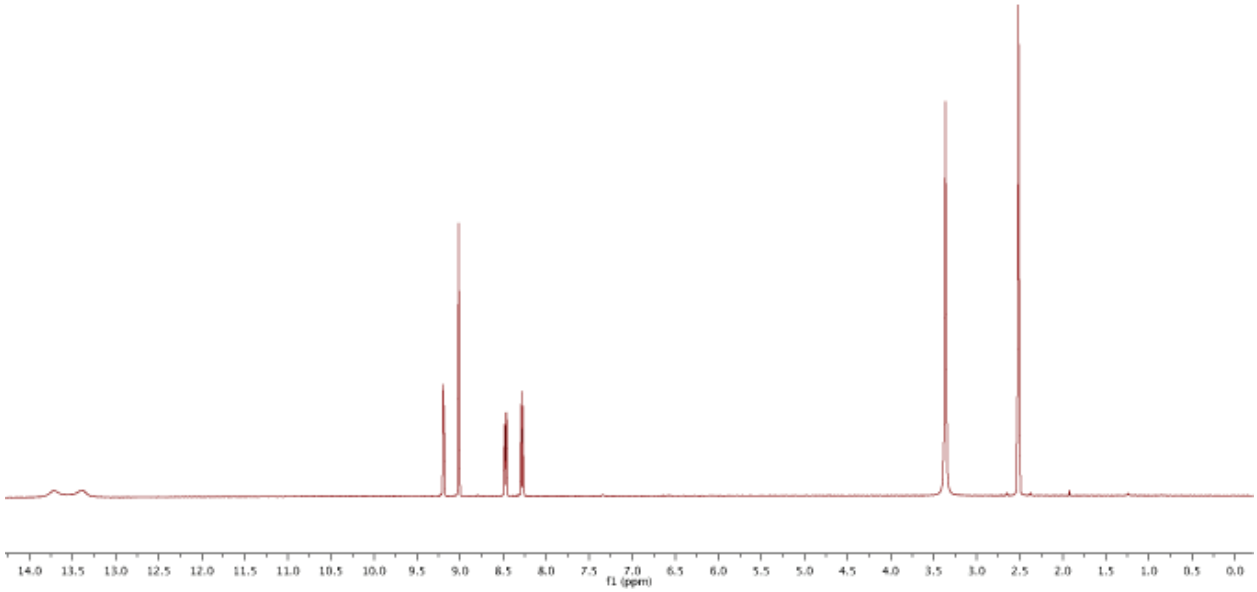


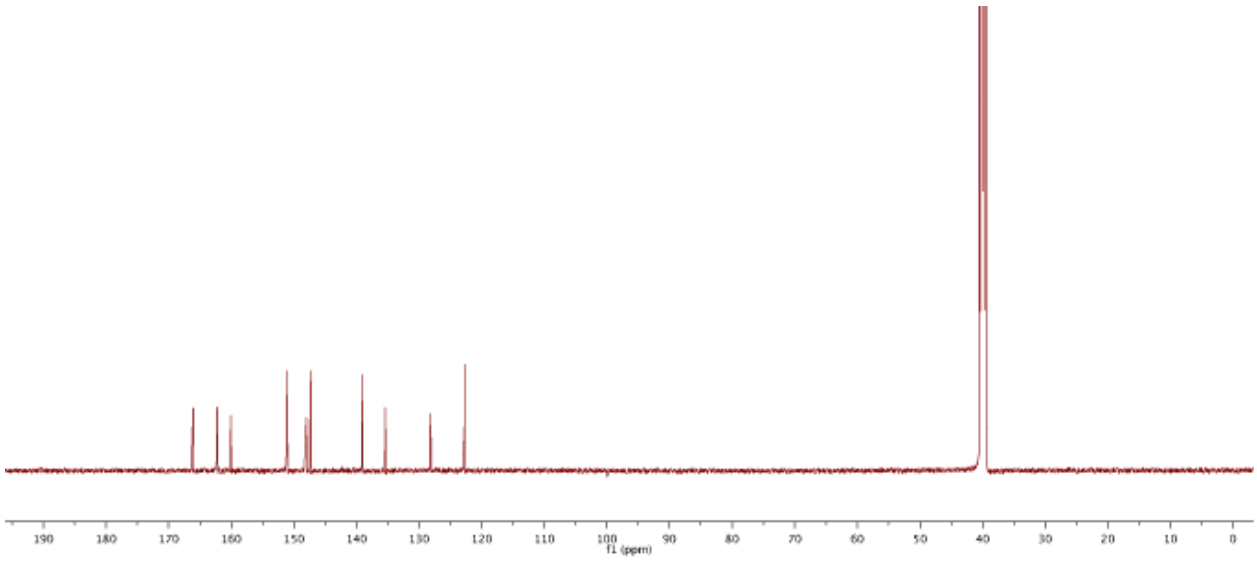




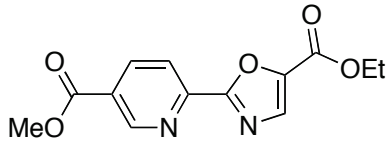
^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of 2-(4-Carboxyoxazol-2-yl)pyridine-5-carboxylic Acid (pyoxDC*)

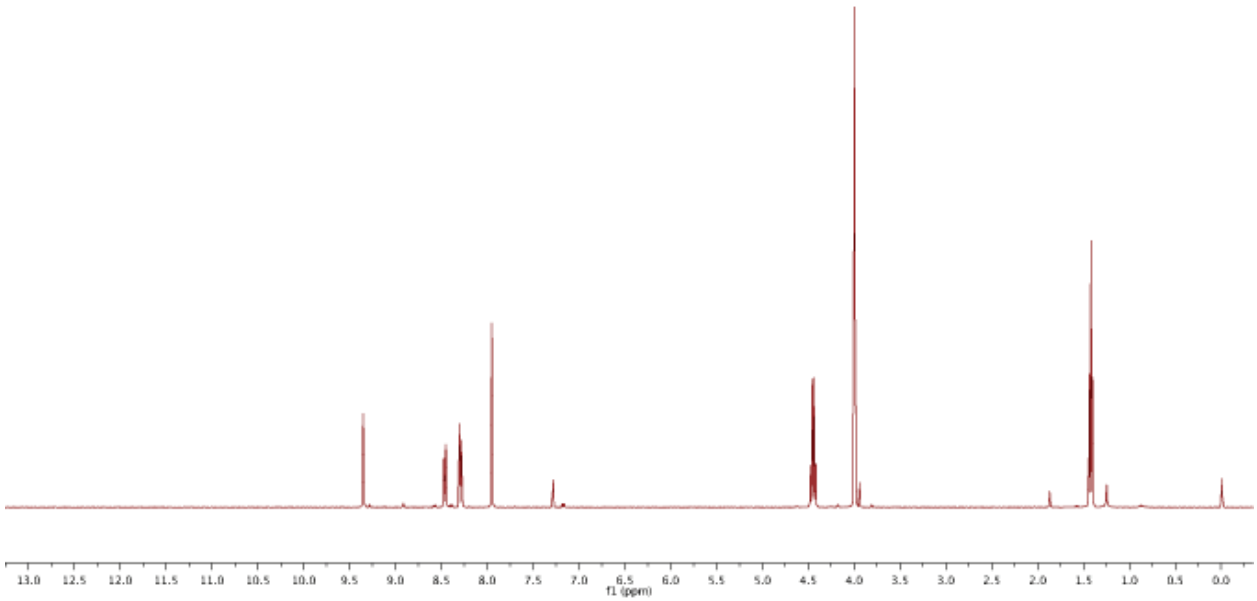


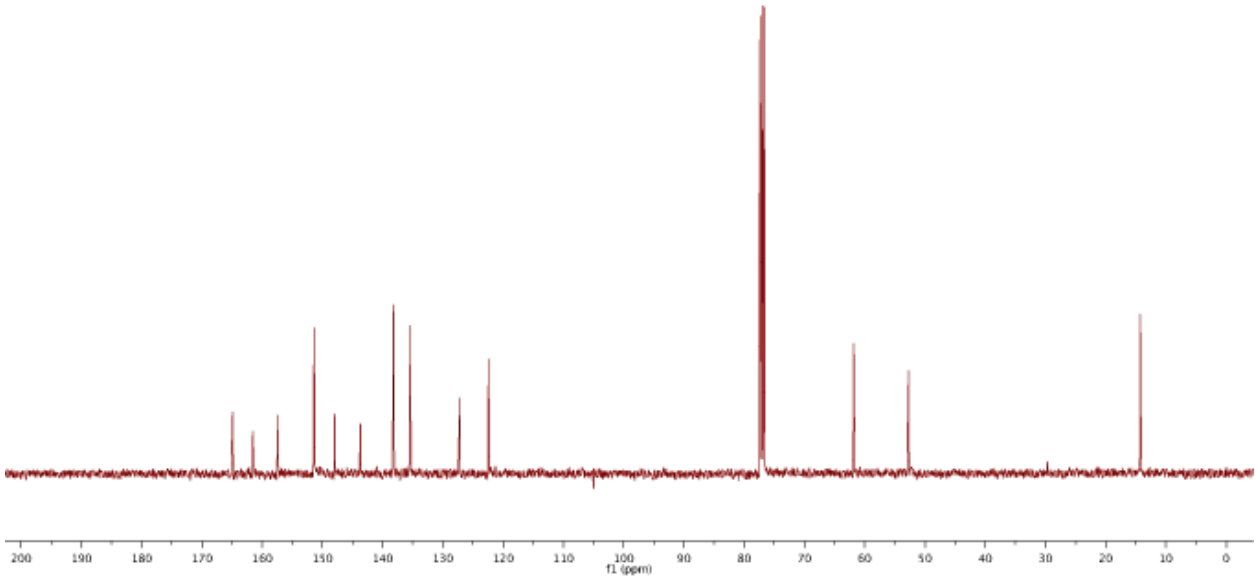




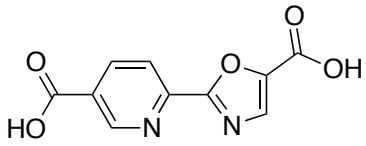
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Methyl 2-(5-Ethoxycarbonyloxazol-2-yl)pyridine-5-carboxylate (ethylmethyl pyoxDC)

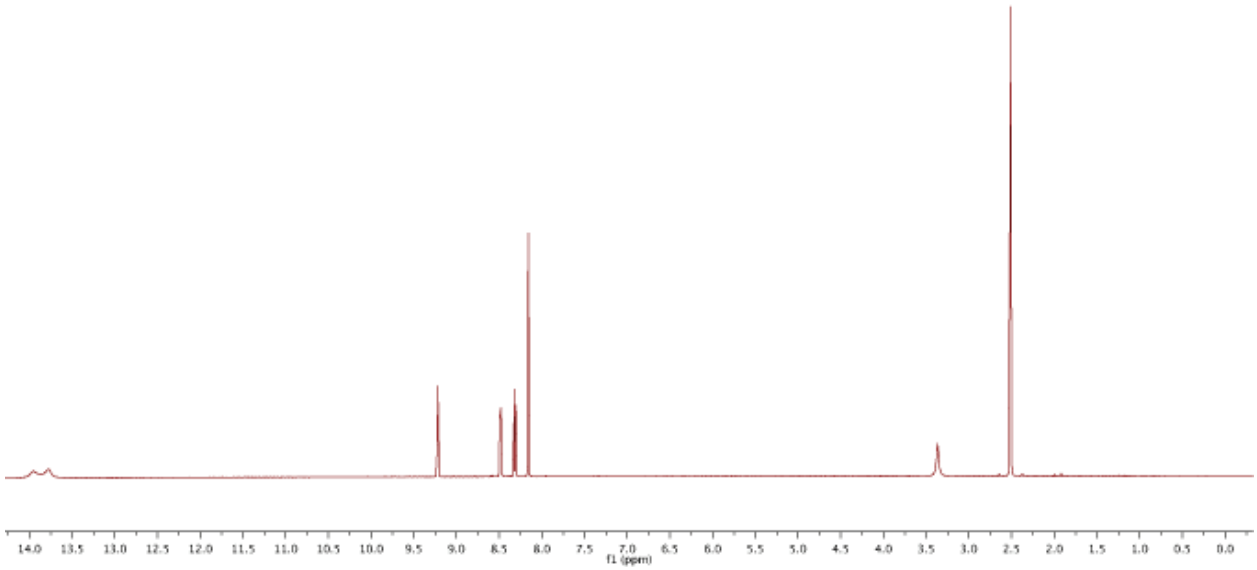


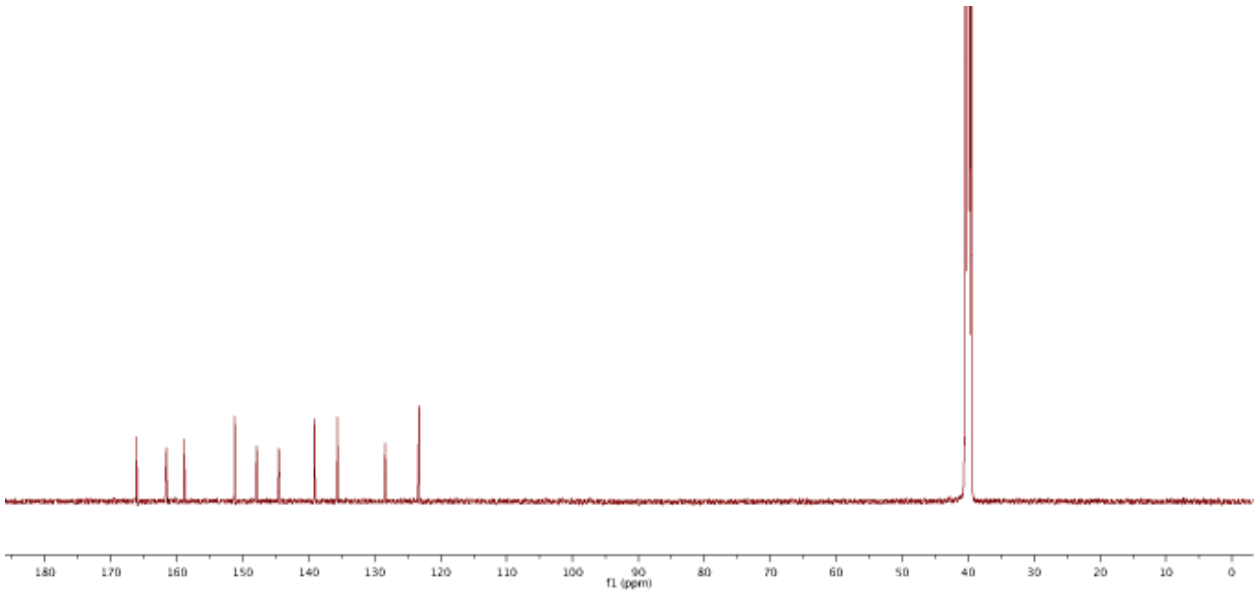




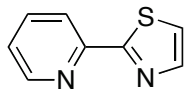
^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of 2-(5-Carboxyoxazol-2-yl)pyridine-5-carboxylic Acid (pyoxDC)

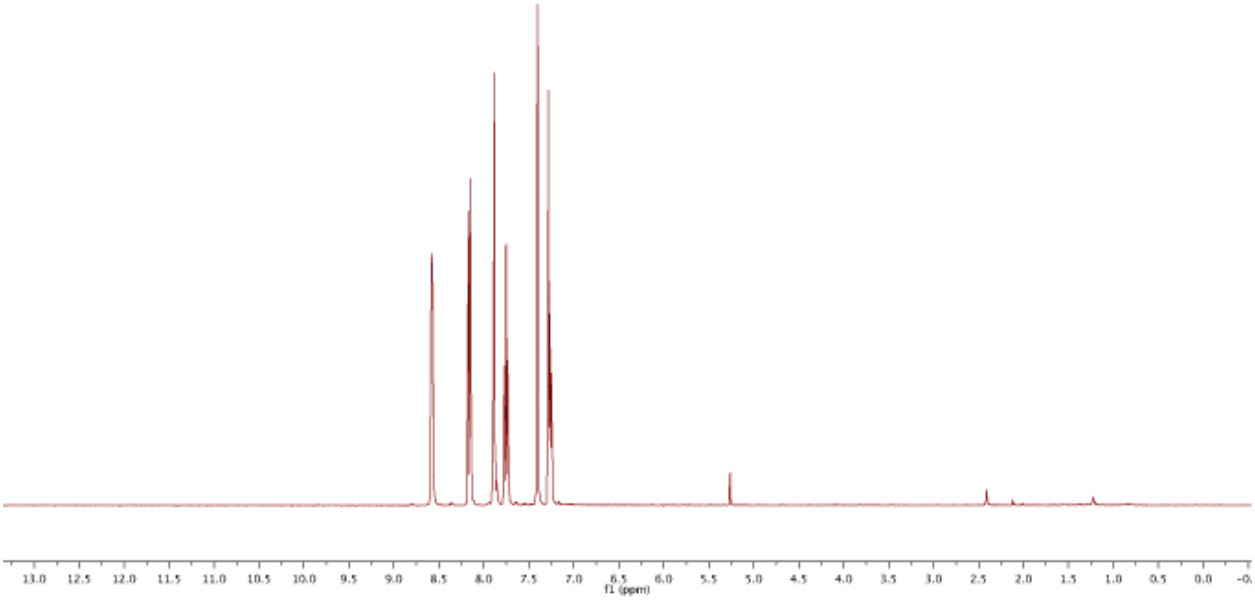


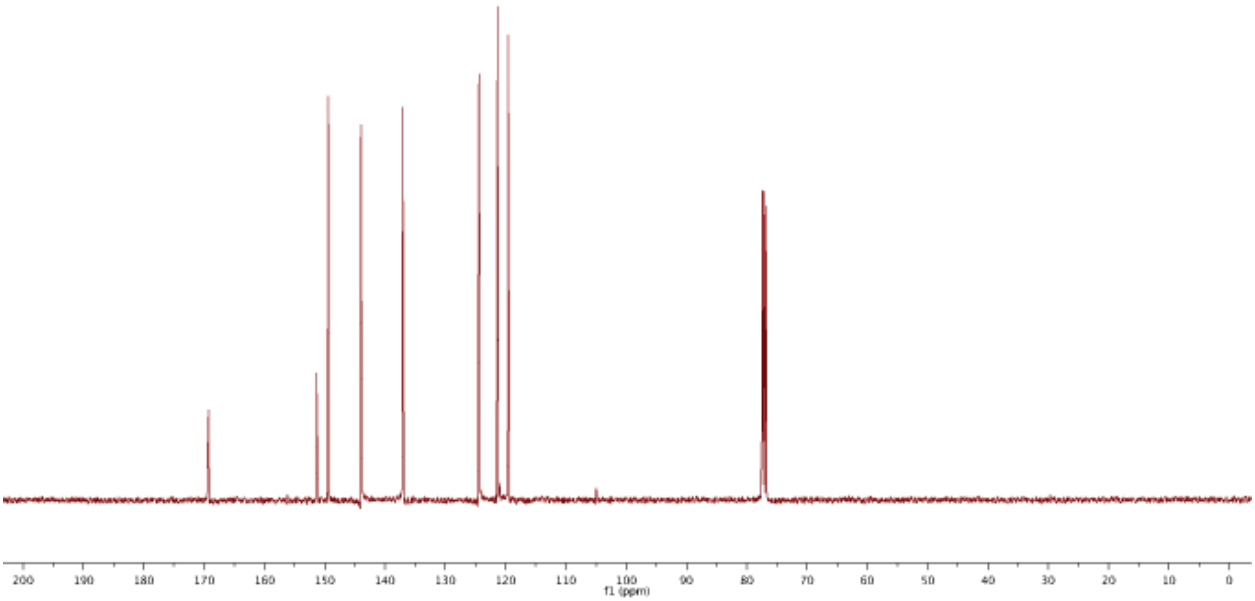




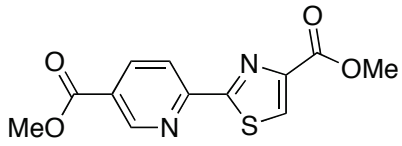
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of 2-(Thiazol-2-yl)pyridine (pythi)

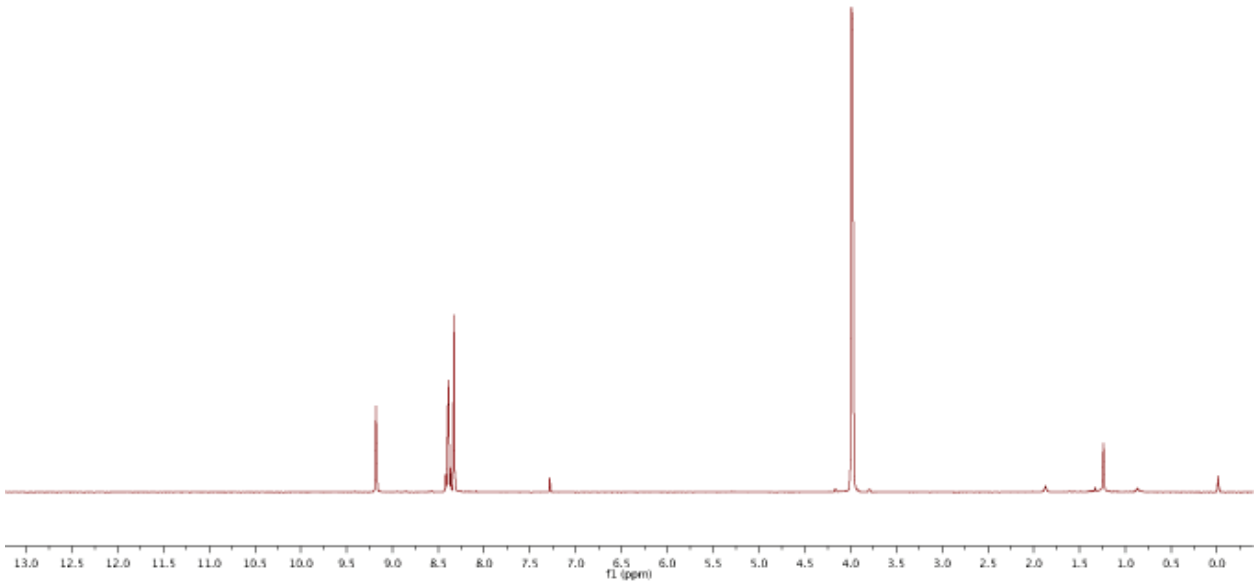


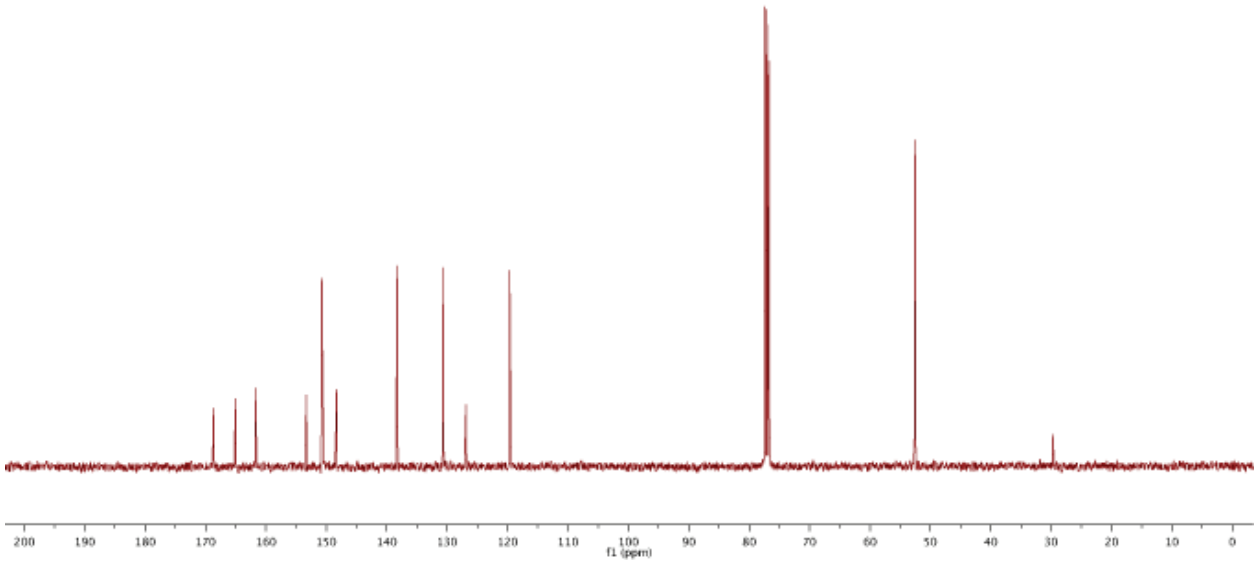




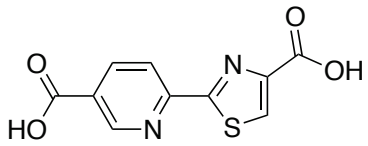
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Methyl 2-(4-Methoxycarbonylthiazol-2-yl)pyridine-5-carboxylate (dimethyl pythiDC*)

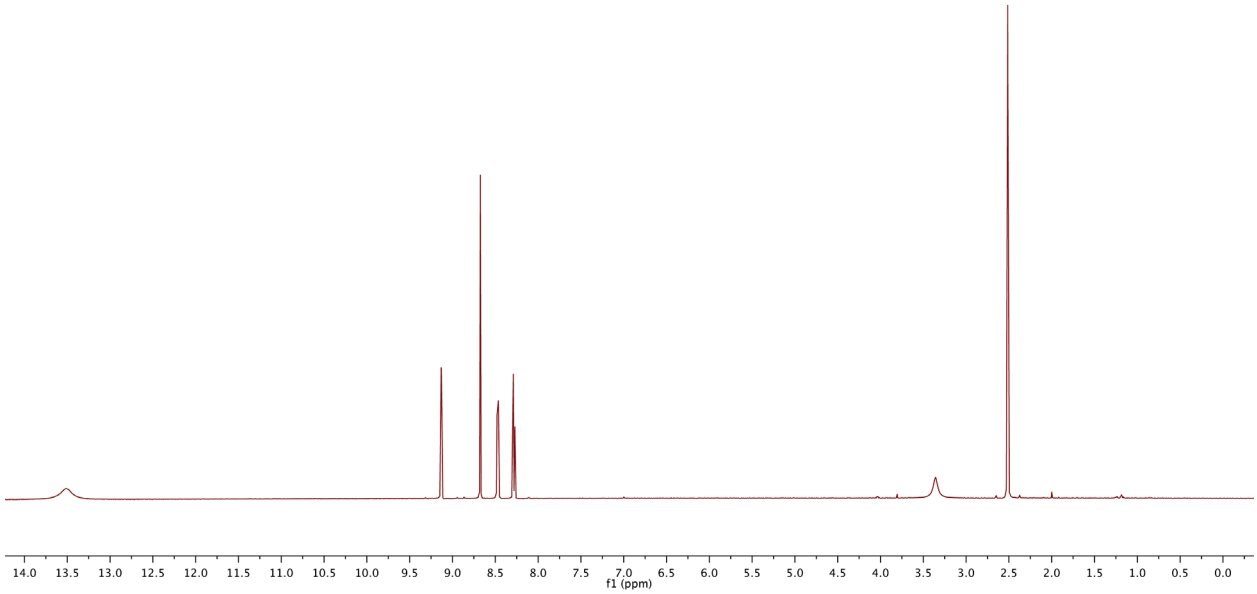


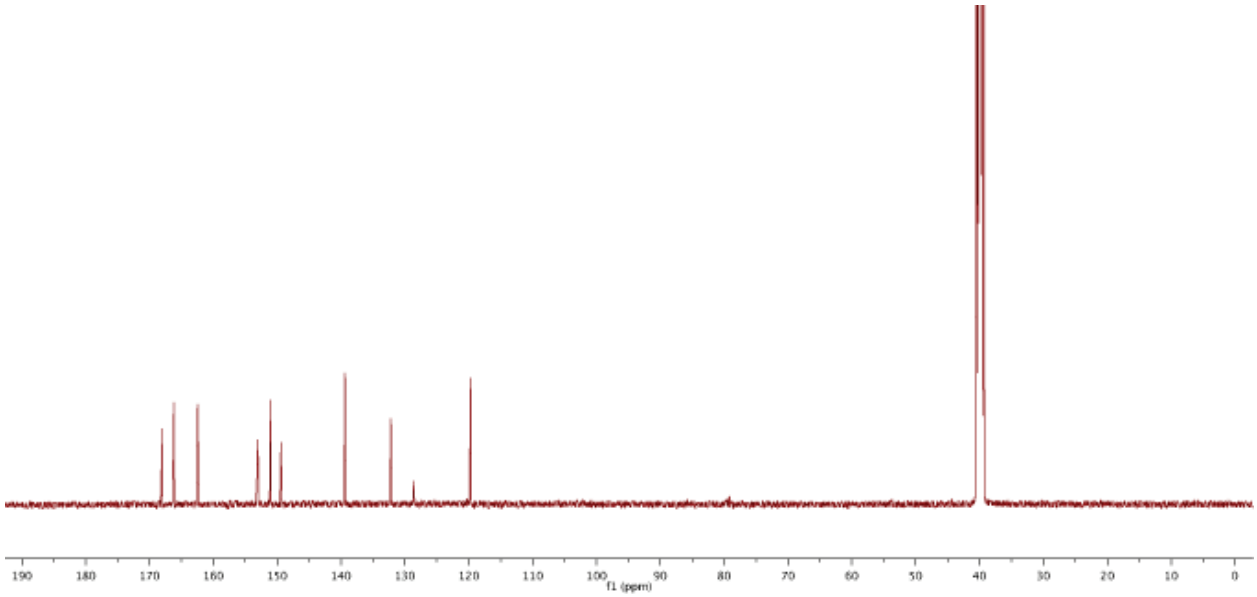




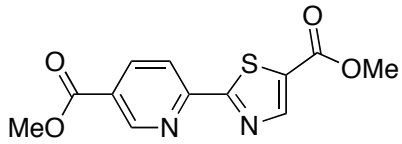
^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of 2-(4-Carboxythiazol-2-yl)pyridine-5-carboxylic Acid (pythiDC*)

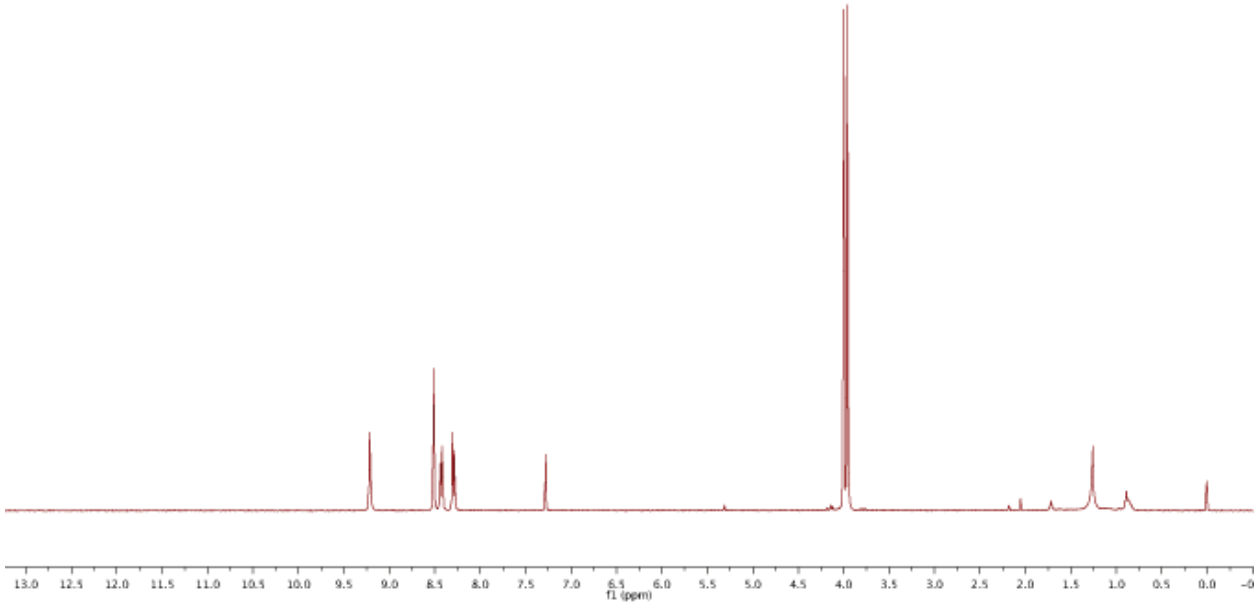


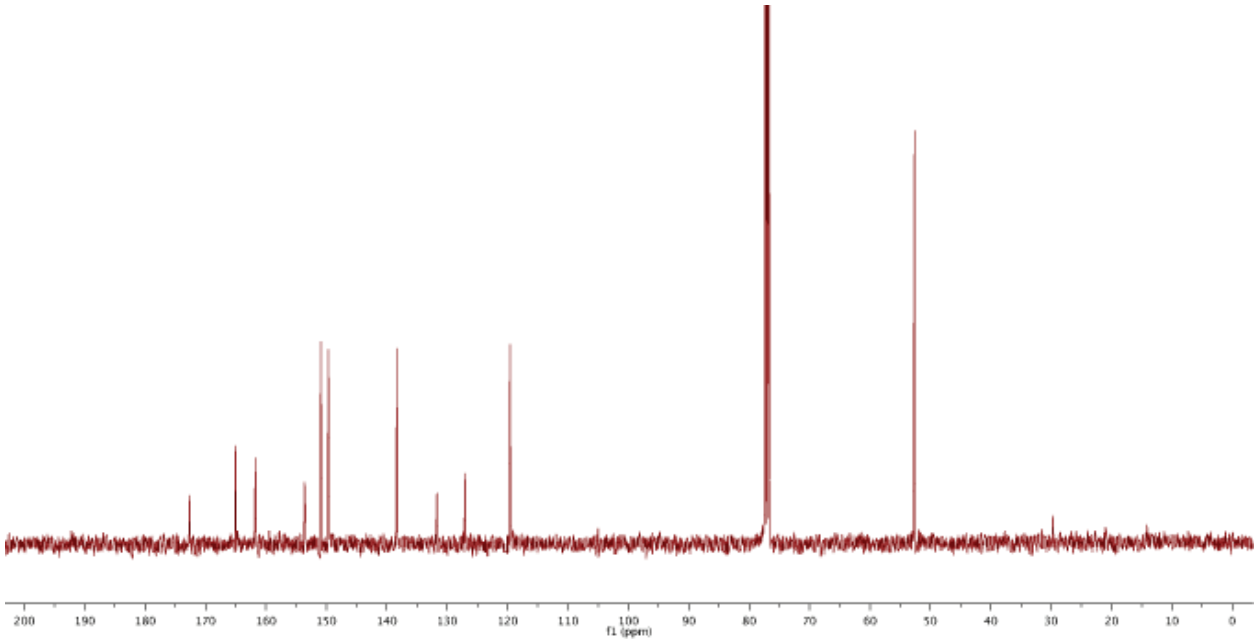




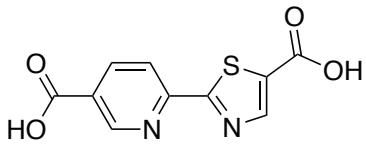
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Methyl 2-(5-Methoxycarbonylthiazol-2-yl)pyridine-5-carboxylate (dimethyl pythiDC)

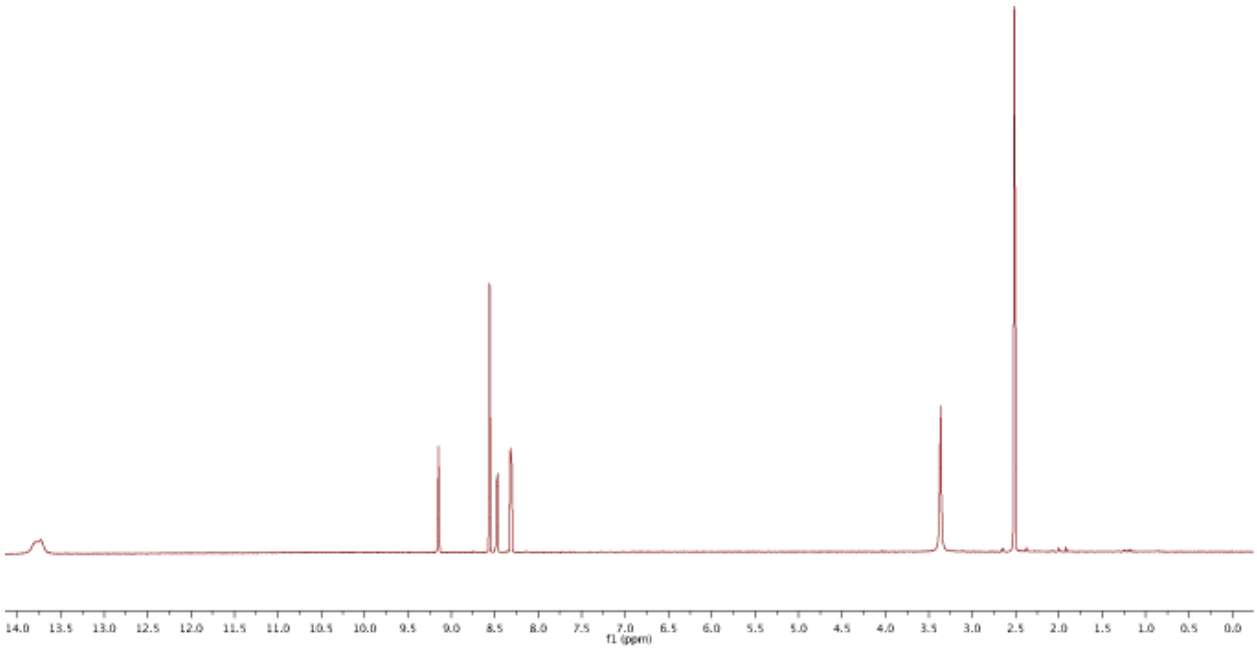


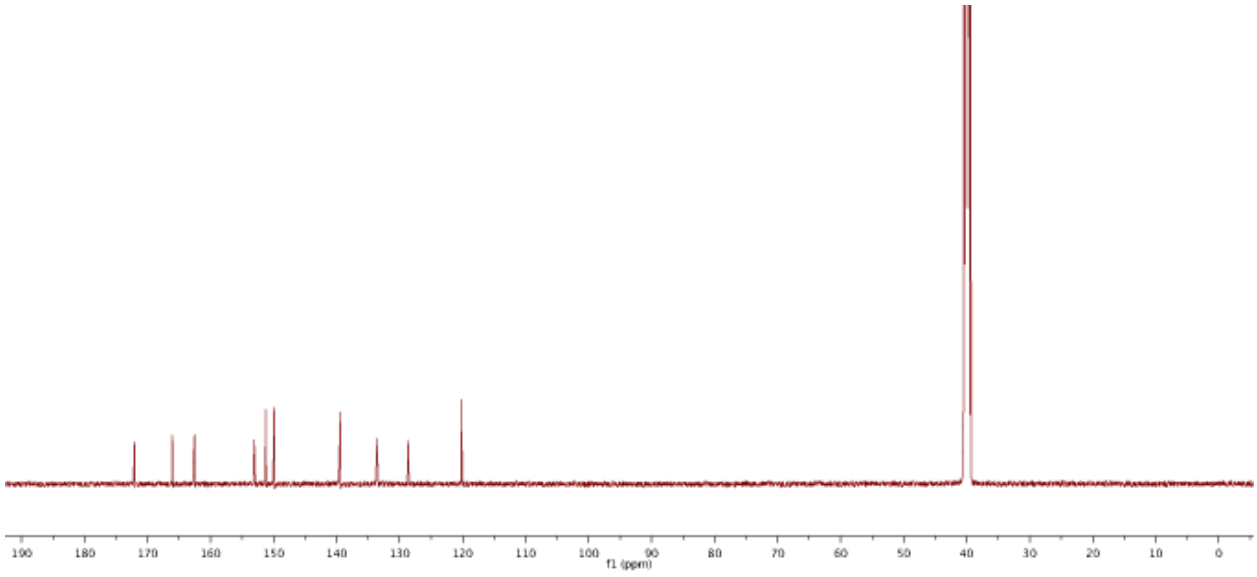




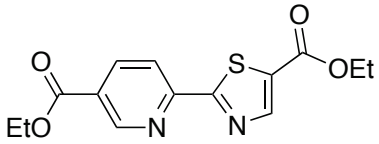
^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of 2-(5-Carboxythiazol-2-yl)pyridine-5-carboxylic Acid (pythiDC)

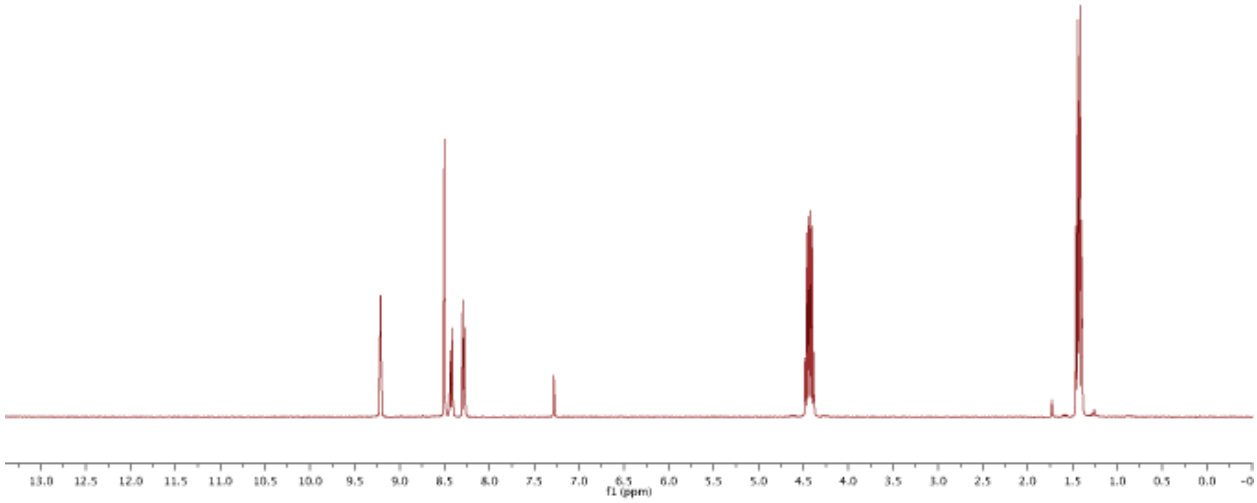


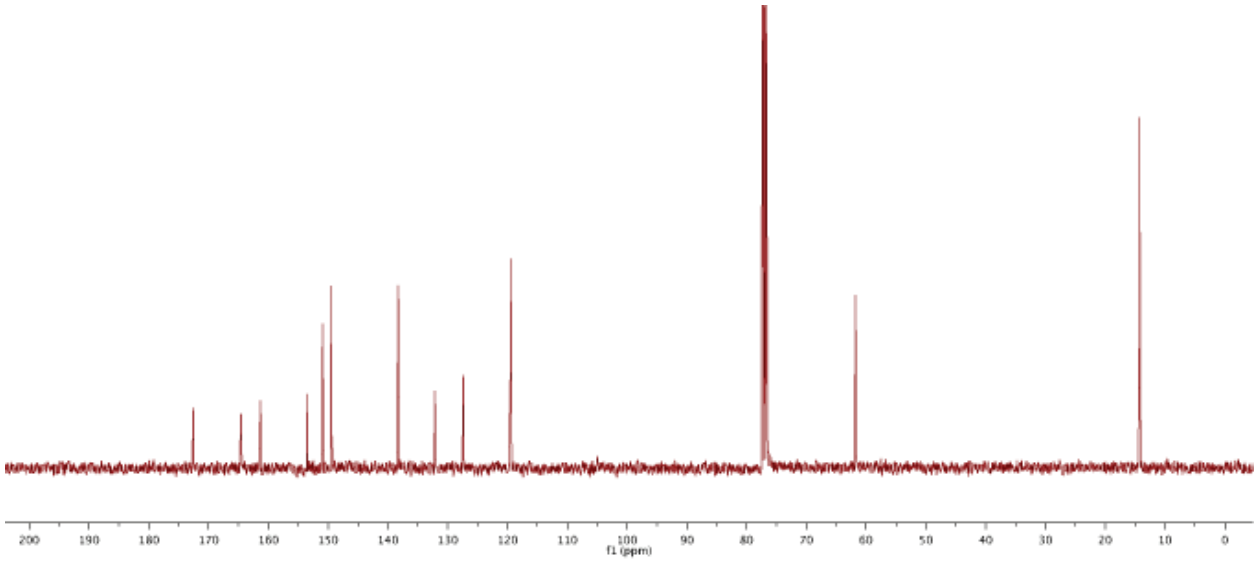




^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of Ethyl 2-(5-Ethoxycarbonylthiazol-2-yl)pyridine-5-carboxylate (diethyl pythiDC)







XXI. Table S1. Estimated Log*P* and Topological Polar Surface Area (TPSA) Values

Table S1. Estimated Log*P* and Topological Polar Surface Area (TPSA) Values

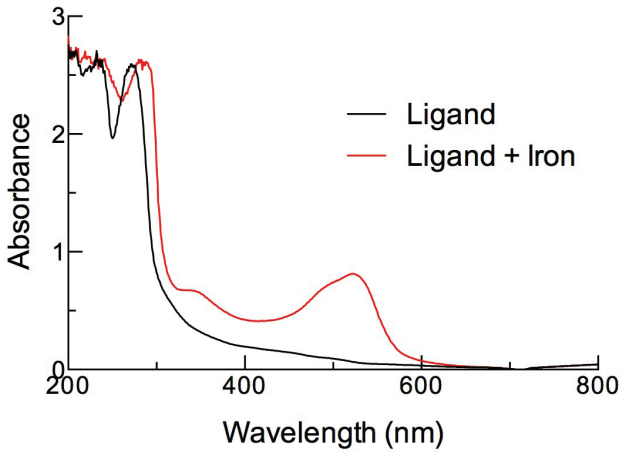
Compound	log*P*[a]	TPSA (Å^2)[a]
bipy	1.44	25.8
bipy55′DC	0.56	100.4
diethyl bipy55′DC	1.87	78.4
pypyrid	0.74	38.7
pypyraz	0.76	38.7
pyim	0.90	41.6
pypyr	0.85	41.6
pythi	1.64	25.8
pyox	1.00	38.9
pyimDC	0.29	116.2
diethyl pyimDC	1.56	94.2
pythiDC	1.03	100.4
diethyl pythiDC	2.30	78.4

[a]Values were estimated with the molecular properties calculator from Molinspiration (http://www.molinspiration.com).

XXII. Absorption Spectra of Ligands and Complexes

Pypyrid and Fe(pypyrid)$_3$

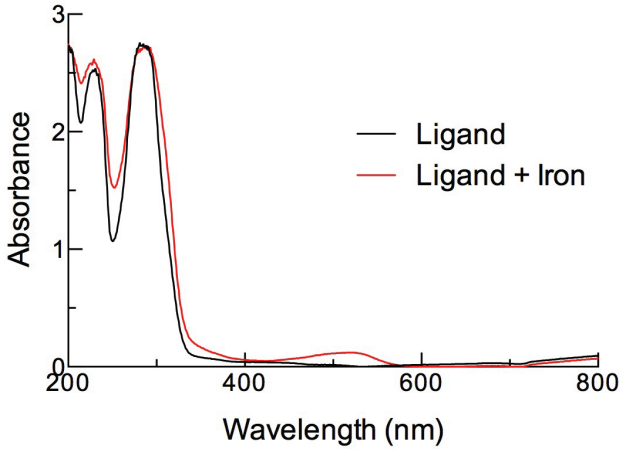
300 μM pypyrid ± 100 μM Fe(II)SO_4 in 10 mM sodium phosphate buffer, pH 7.0



λ_{max} = 522 nm

Pypyraz and Fe(pypyraz)$_3$

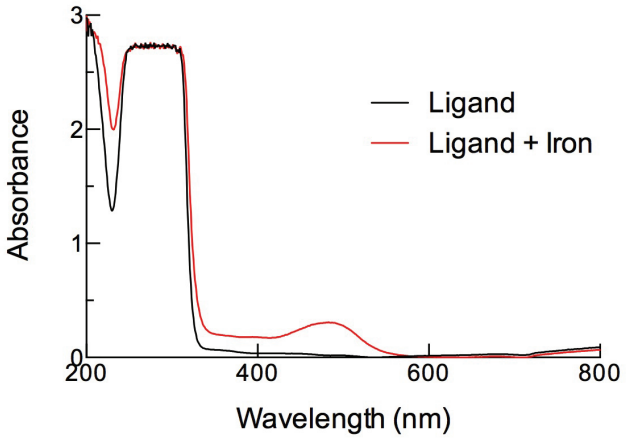
300 μM pypyraz ± 100 μM Fe(II)SO_4 in 10 mM sodium phosphate buffer, pH 7.0



λ_{max} = 523 nm

Pyim and Fe(pyim)$_3$

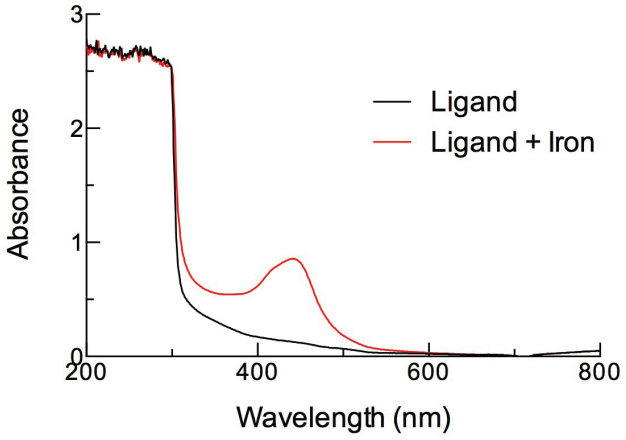
600 μM pyim ± 200 μM Fe(II)SO_4 in 10 mM sodium phosphate buffer, pH 7.0



λ_{max} = 484 nm

Pypyr and Its Complex with Fe(II)

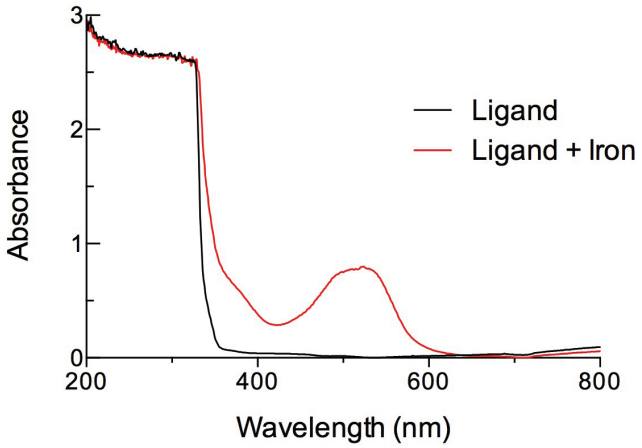
3000 μM pypyr ± 100 μM Fe(II)SO_4 in 10 mM sodium phosphate buffer, pH 7.0



λ_{max} = 441 nm

Pythi and Fe(pythi)$_3$

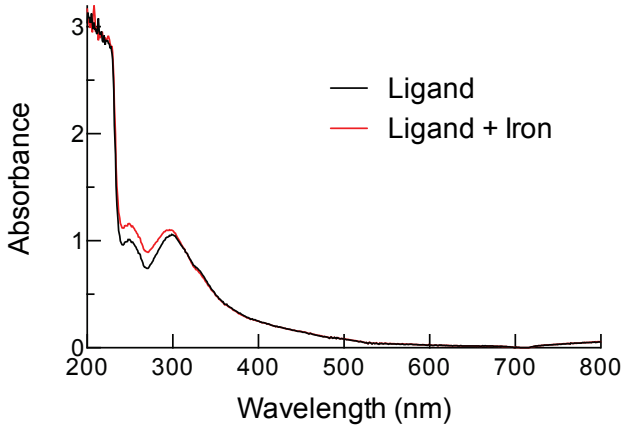
3000 μM pythi ± 1000 μM Fe(II)SO_4 in 10 mM sodium phosphate buffer, pH 7.0



λ_{max} = 524 nm

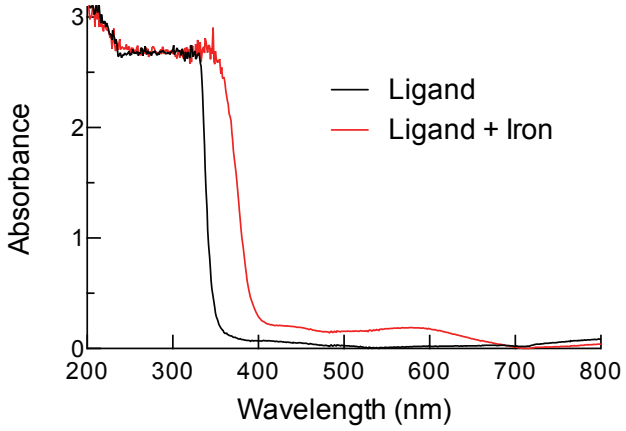
Diethyl Bipy55′DC

100 μM diethyl bipy55′DC ± 20 μM Fe(II)SO_4 in 10 mM sodium phosphate buffer, pH 7.0



Diethyl PyimDC and Its Complex with Fe(II)

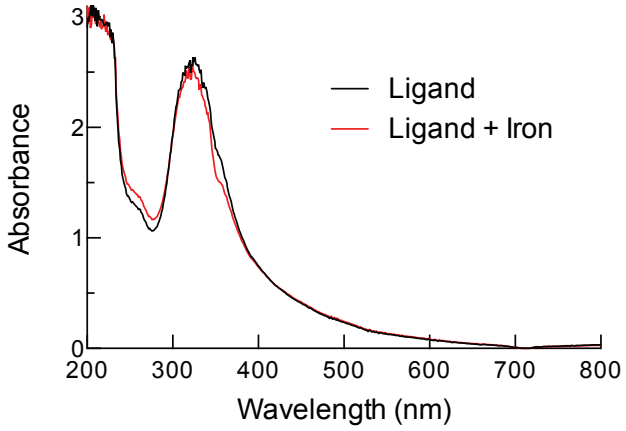
600 μM diethyl pyimDC ± 20 μM Fe(II)SO_4 in 10 mM sodium phosphate buffer, pH 7.0



λ_{max} = 577 nm

Diethyl PythiDC

200 μM diethyl pythiDC ± 20 μM Fe(II)SO_4 in 10 mM sodium phosphate buffer, pH 7.0



XXIII. Figure S1. Ligand Titrations to Form Iron Complexes

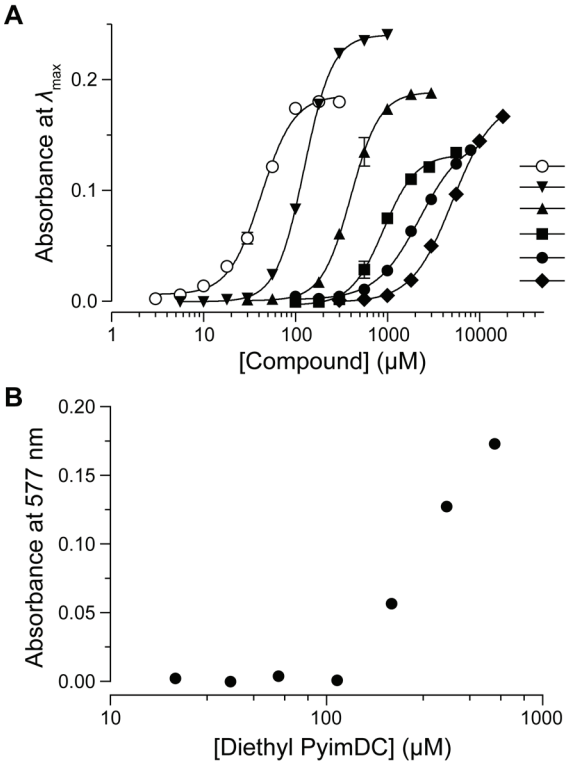
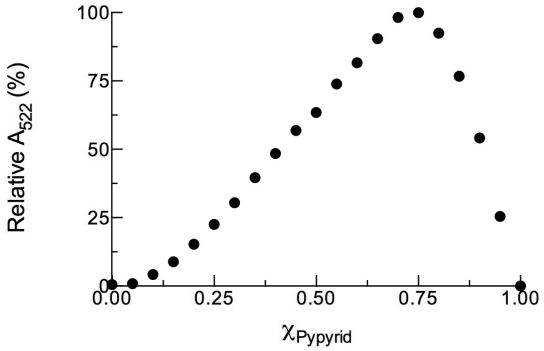


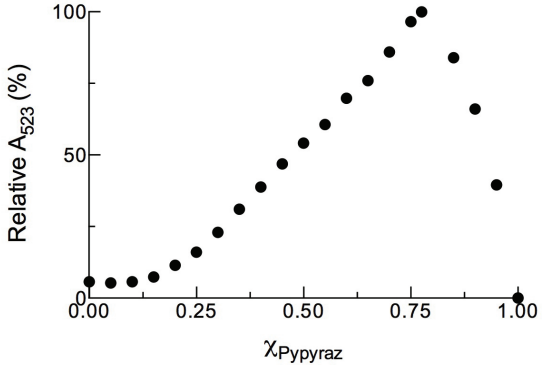
Figure S1. Ligand Titrations to Form Iron Complexes. Data were acquired as described in Section VIII. (A) Biheteroaryl compounds. R^2 > 0.99 for all curve fits. The titration curve for bipy in panel A was reproduced from ref. 7. (B) Diethyl pyimDC. Fe(II) (20 μM) could not be saturated over the range that diethyl pyimDC remained soluble under the assay conditions. Thus, Fe_{20}-EC_{50} >600 μM, which is the highest tested concentration.

XXIV. Job's Plots for Fe(II) Complexes

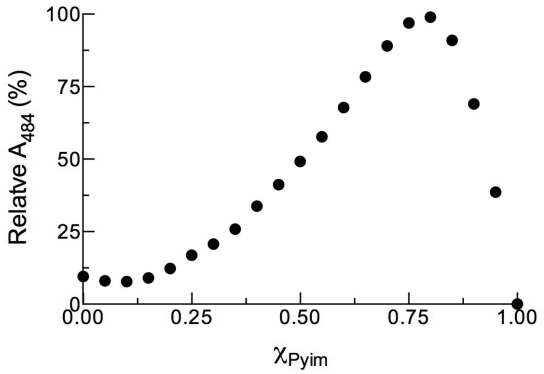
$Fe(pypyrid)_3$



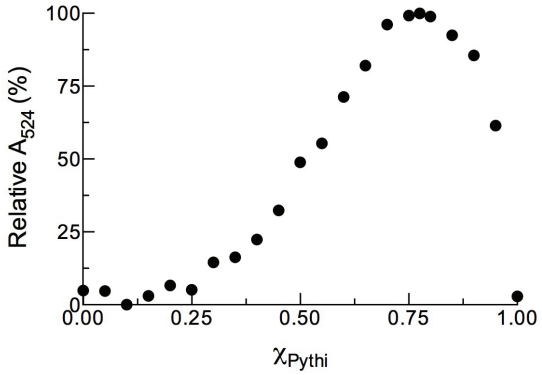
$Fe(pypyraz)_3$



$Fe(pyim)_3$

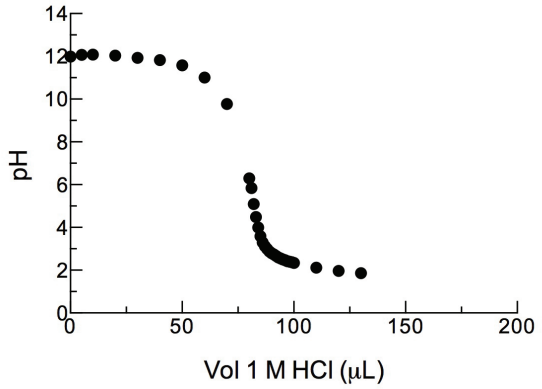


Fe(pythi)$_3$

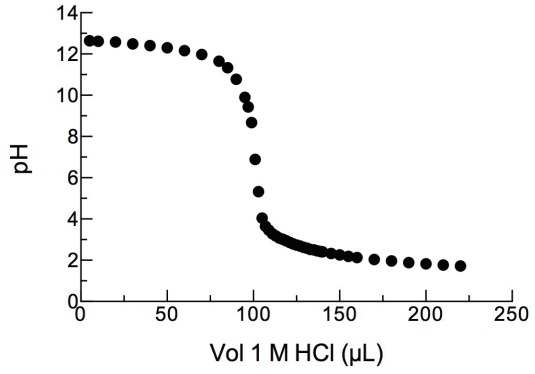


XXV. pH-Titration Curves for Biheteroaryl Ligands

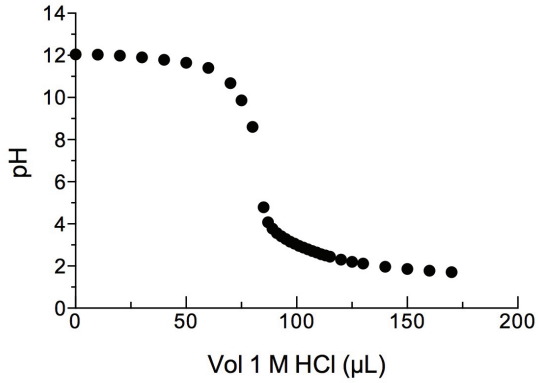
Water Blank



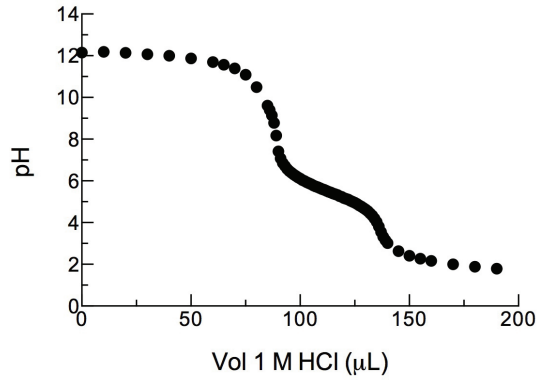
Pypyrid
pK_a <3



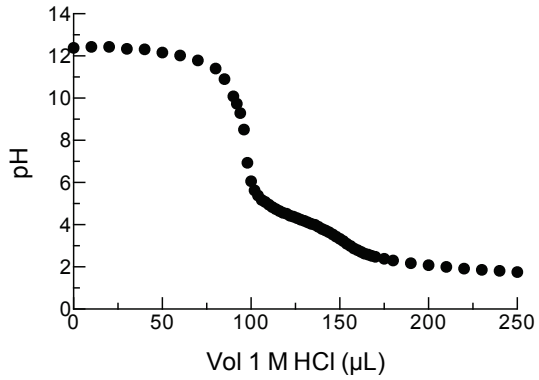
Pypyraz
pK_a <3



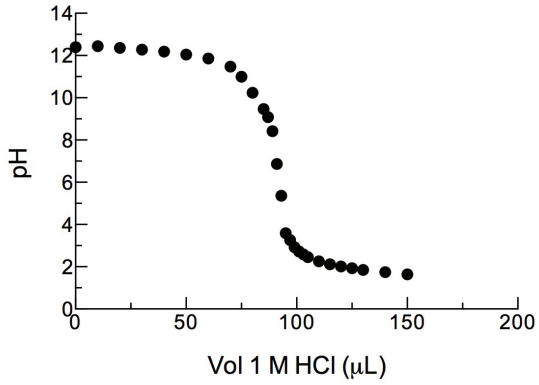
Pyim
pK_a 5.4



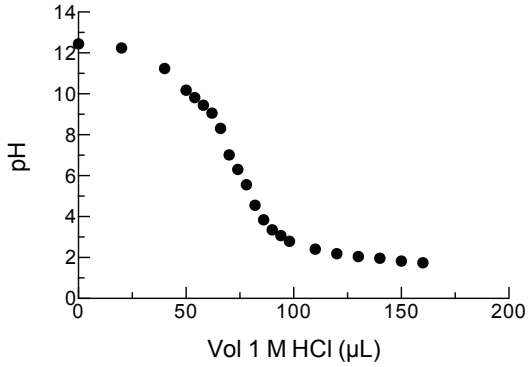
Pypyr
pK_a 4.1



Pythi
pK_a <3



Pyox
pK_a <3



XXVI. Figure S2. Screen for Inhibitors of Human CP4H1

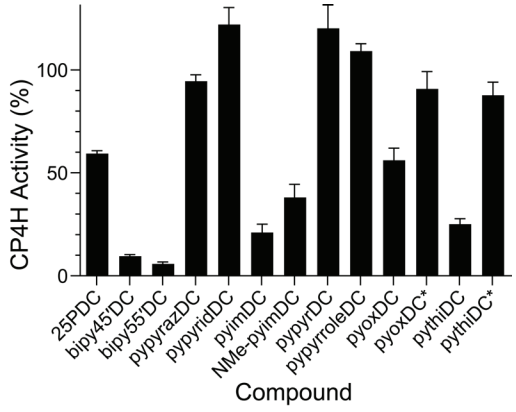


Figure S2. Screen for inhibition of human CP4H1. Compounds (10 μM) were screened for inhibition of the catalytic activity of human CP4H1 as described in Section IV. Relative activity values are the mean (±SD) of three replicates. Data for 25PDC, bipy45′DC, and bipy55′DC are from ref. 7.

XXVII. Figure S3. Competitive Iron-Binding by PyimDC and Ester Derivatives

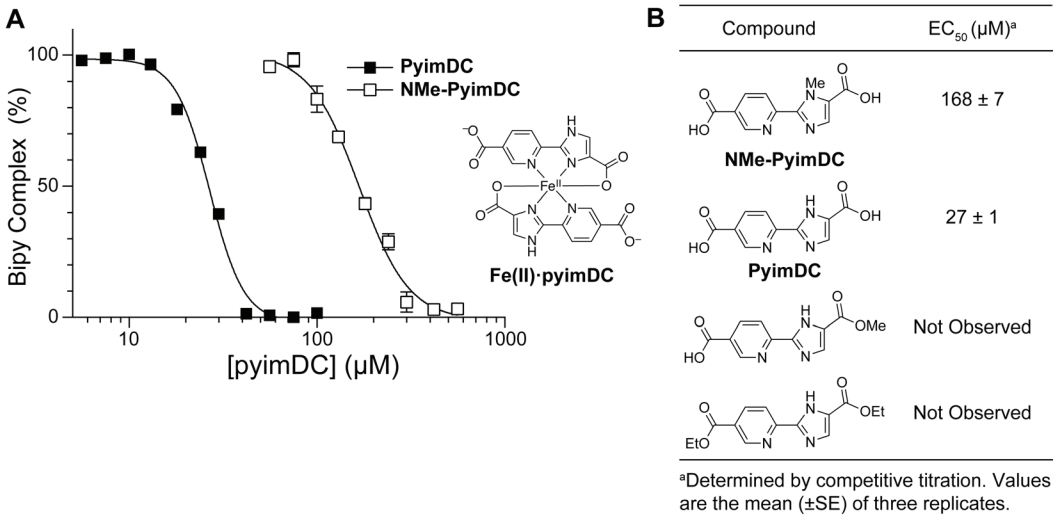


Figure S3. Competitive Iron-Binding by PyimDC and Ester Derivatives. High concentrations of pyimDC can compete with bipy for complexation with Fe(II), as determined with the assay described in Section VIII. This phenomenon was dose-dependent (A) and confined to the diacid (B). Together, these data are consistent with pyimDC forming the depicted 2:1 complex with iron.

XXVIII. Figure S4. Screen for Inhibitors of Human PHD2

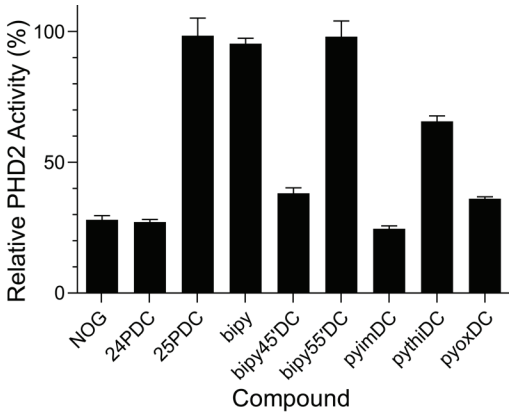


Figure S4. Screen for Inhibition of Human PHD2. Compounds (10 μM) were screened for inhibition of the catalytic activity of human PHD2 as described in Section VI. Relative activity values are the mean (±SD) of three replicates. Data for NOG, 24PDC, 25PDC, bipy, bipy45′DC, and bipy55′DC are from ref. 7.

XXIX. Figure S5. Effect of Biheteroaryl Compounds on Iron Metabolism in Human Breast Cancer Cells

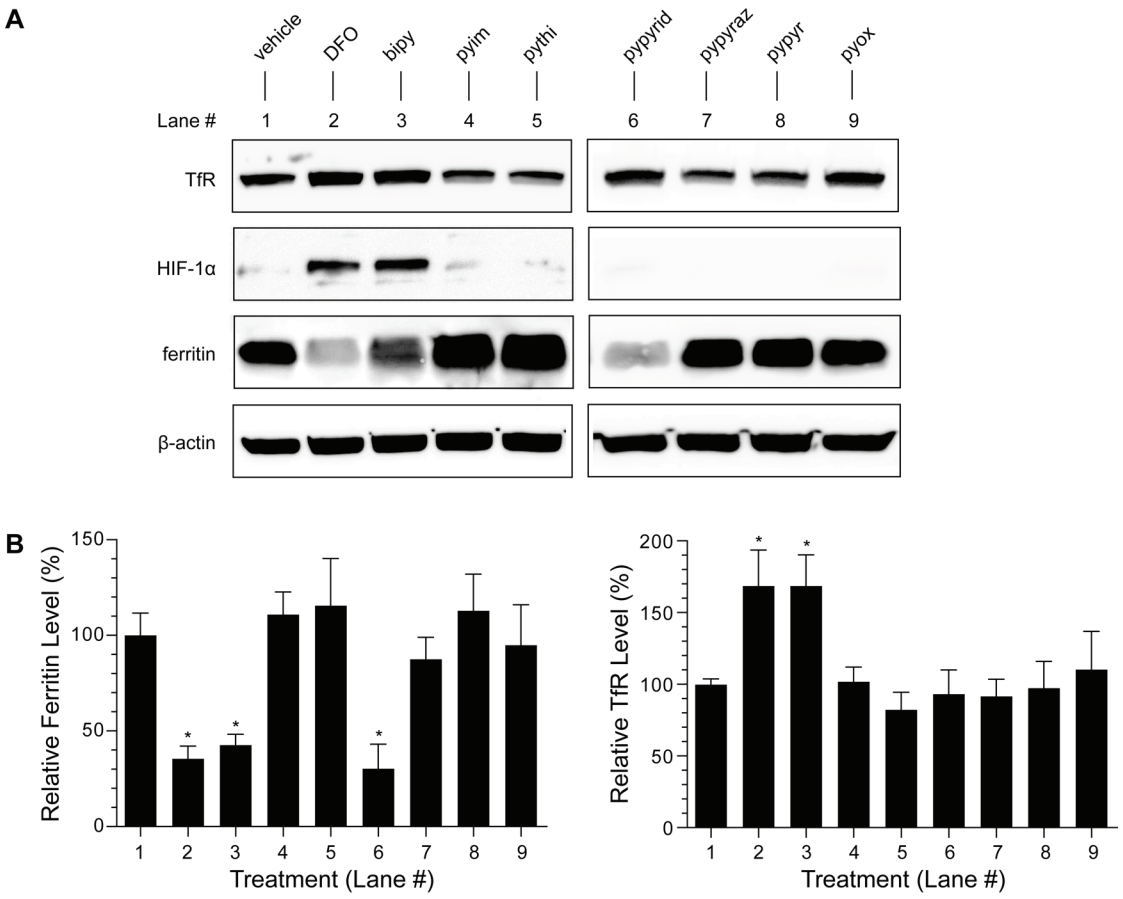


Figure S5. Effect of Biheteroaryl Compounds on Iron Metabolism in Human Breast Cancer Cells. (A) MDA-MB-231 breast cancer cells were treated with deferoxamine (DFO), biheteroaryl compounds, or vehicle (DMSO) as described in Section XII, and analyzed with an immunoblot (*, $p < 0.05$).

XXX. Figure S6. Effect of Biheteroaryl Compounds and Their Diethyl Esters on Iron Metabolism in Human Embryonic Kidney Cells

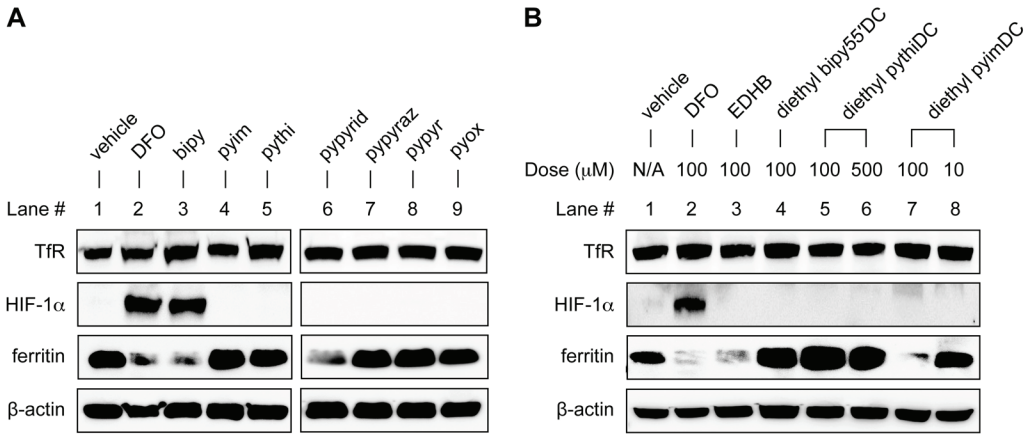


Figure S6. Effect of Biheteroaryl Compounds and Their Diethyl Esters on Iron Metabolism in Human Embryonic Kidney Cells. (A) HEK293T cells were treated with biheteroaryl compounds (100 μM), deferoxamine (DFO, 100 μM), or vehicle (DMSO) as described in Section XII, and analyzed with an immunoblot. (B) HEK293T cells were treated with esterified biheteroaryl compounds, deferoxamine (DFO), ethyl dihydroxybenzoate (EDHB), or vehicle (DMSO) as described in Section XII, and analyzed with an immunoblot.

XXXI. Figure S7. Effect of Esterified Biheteroaryl Compounds on IRE Binding by IRPs in Human Breast Cancer Cells

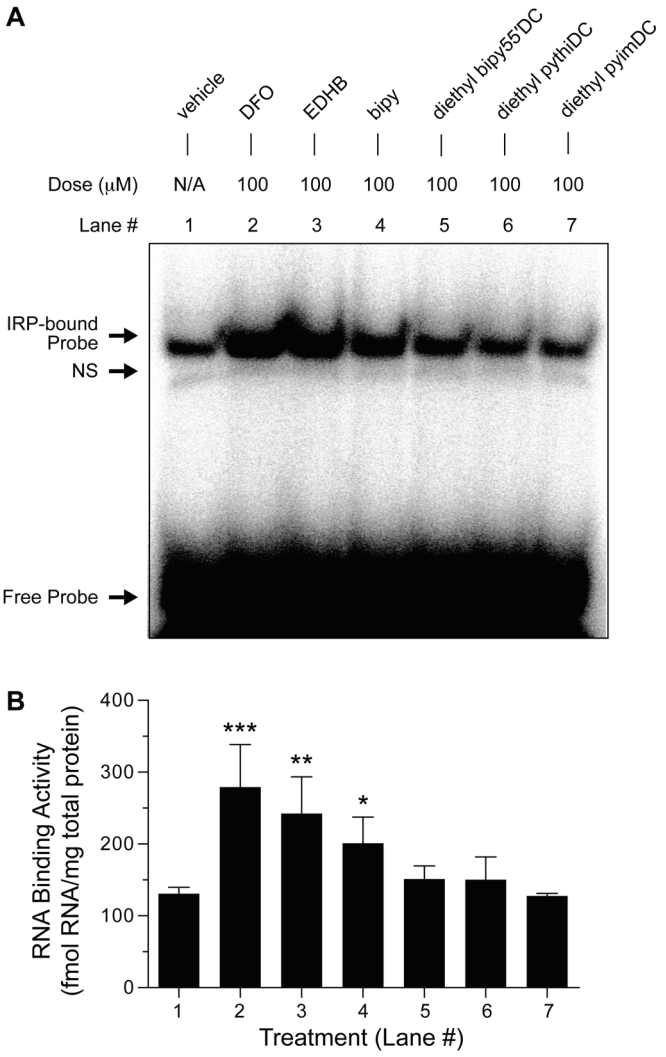


Figure S7. Effect of Esterified Biheteroaryl Compounds on IRE Binding by IRPs in Human Breast Cancer Cells. MDA-MB-231 cells were treated with esterified biheteroaryl compounds, deferoxamine (DFO), ethyl dihydroxybenzoate (EDHB), or vehicle (DMSO) as described in Section XIV, and analyzed by an electrophoretic mobility shift assay (EMSA) using a ^{32}P-labeled RNA ligand for IRPs as described in Section XV. (A) An EMSA representative of 3 replicates. (B) Quantitations of the EMSAs represented in Panel A. Quantitations (n = 3) were obtained by densitometry and derived from the digital light units using a calibration curve obtained by scintillation counting and normalized to total protein and total RNA. Under these conditions, IRP1- and IRP2-bound RNA comigrate in the band labeled "IRP-bound Probe". *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

XXXII. Figure S8. Effect of Esterified Biheteroaryl Compounds on P4HA1 Levels in Human Breast Cancer Cells

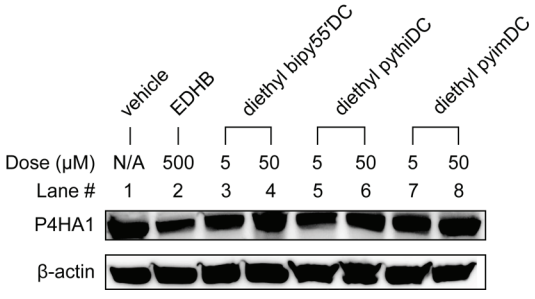


Figure S8. Effect of Esterified Biheteroaryl Compounds on P4HA1 Levels in Human Breast Cancer Cells. MDA-MB-231 cells were treated with esterified biheteroaryl compounds, deferoxamine (DFO), ethyl dihydroxybenzoate (EDHB), or vehicle (DMSO) as described in Section XII, and then analyzed with an immunoblot. Blots are representative of at least 2 replicates.

XXXIII. References

(1) Kersteen, E. A., Higgin, J. J., and Raines, R. T. (2004) Production of human prolyl 4-hydroxylase in *Escherichia coli*. *Protein Expr. Purif. 38*, 279–291.

(2) Hewitson, K. S., Schofield, C. J., and Ratcliffe, P. J. (2007) Hypoxia-inducible factor prolyl-hydroxylase: Purification and assays of PHD2. *Methods Enzymol. 435*, 25–42.

(3) Gibson, D. G. (2011) Enzymatic assembly of overlapping DNA fragments. *Methods Enzymol. 498*, 349–361.

(4) Eisenstein, R. S., Tuazon, P. T., Schalinske, K. L., Anderson, S. A., and Traugh, J. A. (1993) Iron-responsive element-binding protein. Phosphorylation by protein kinase C. *J. Biol. Chem. 268*, 27363–27370.

(5) Goforth, J. B., Anderson, S. A., Nizzi, C. P., and Eisenstein, R. S. (2010) Multiple determinants within iron-responsive elements dictate iron regulatory protein binding and regulatory hierarchy. *RNA 16*, 154–169.

(6) Begtrup, M., Boyer, G., Cabildo, P., Cativiela, C., Claramunt, R. M., Elguero, J., García, J. I., Toiron, C., and Vedsø, P. (1993) ^{13}C NMR of pyrazoles. *Magn. Reson. Chem. 31*, 107–168.

(7) Vasta, J. D., and Raines, R. T. (2015) Selective inhibition of prolyl 4-hydroxylases by bipyridinedicarboxylates. *Bioorg. Med. Chem. 23*, 3081–3090.