

Supporting Information

A Scaffold Hopping Strategy to Generate New Aryl-2-Aminopyrimidine MRSA Biofilm Inhibitors

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Methods and Materials

General Biological Experimental

Bacterial strains, media, and antibiotics *A. baumannii* strains 4106, was obtained from Walter Reed Army Institute for Research (WRAIR). *K. pneumoniae* strains B9, A5 and F2210219^{mcr-1} were obtained from Professor Robert Ernst at The University of Maryland, Baltimore. MRSA strains 43300, BAA-44, 33591 and *S. aureus* strain 6538 were obtained from the ATCC. Stock cultures were stored in 25% glycerol and maintained at -80 °C. Prior to use, colonies were grown on LB (Lennox) agar (*A. baumannii* and *K. pneumoniae*) or tryptic soy agar (*S. aureus*). Cation adjusted mueller hinton broth (CAMHB) (cat# 212322) and Mueller-Hinton broth (MHB) (cat# 211443) were purchased from BD Diagnostics. Tryptic soy broth (TSB) was purchased from Sigma-Aldrich (cat# 22092). Glucose was purchased from Acros (cat# 41095-5000). Colistin sulfate salt was purchased from Sigma (cat# C4461). All assays were run in duplicate and repeated at least two separate times. **Broth microdilution method for the determination of minimum inhibitory concentrations (*A. baumannii*, *K. pneumoniae*):** Bacteria were cultured for 4 to 6 hours in CAMHB and subcultured to 5×10^5 CFU/mL in fresh CAMHB. To aliquots (1 mL) was added compound from 100 mM stock solutions in DMSO, such that the compound concentration equaled the highest concentration tested. Samples were then dispensed (200 μ L) into the first row of a 96-well microtiter plate in which all but the final row of subsequent wells were prefilled with 100 μ L of the untreated bacterial subculture. The final row was filled with media to act as a sterility control and blank. Row one wells were mixed 6-7 times, then, 100 μ L was withdrawn and transferred to row two. Row two wells were mixed 6-7 times followed by a 100 μ L transfer from row two to row three. This procedure was used to serially dilute the rest of the rows of the microtiter plate, excluding the last prefilled row, which was used to measure growth in the absence

of compound. Plates were then sealed with GLAD Press'n Seal and incubated under stationary conditions at 37 °C. After 16 hours, the plates were removed, and MIC values were measured by recording the OD₆₀₀ of each well. MIC values were determined as the minimum concentration required to achieve 90% growth inhibition compared to growth in untreated wells

Broth microdilution method for measurement of colistin potentiation: Bacteria were cultured for 4 to 6 hours in CAMHB and diluted to 5×10^5 CFU/mL in fresh CAMHB. To aliquots (4 mL) was added compound from 100 mM stock solutions in DMSO. One aliquot was not dosed to allow measurement of the colistin MIC in the absence of compound. A 1 mL aliquot of each sample was dosed with colistin, and from this 200 μ L was dispensed into the first row of a 96-well microtiter plate in which all but the final row of subsequent wells was prefilled with 100 μ L of the corresponding compound dosed bacterial suspension. The final row was filled with media to act as a sterility control and blank. Row one wells were mixed 6-7 times, then, 100 μ L was withdrawn and transferred to row two. Row two wells were mixed 6-7 times followed by a 100 μ L transfer from row two to row three. This procedure was used to serially dilute the rest of the rows of the microtiter plate, excluding the last prefilled row, which was used to measure growth in the presence of compound alone. Plates were then sealed with GLAD Press'n Seal and incubated under stationary conditions at 37 °C. After 16 hours, the plates were removed, and MIC values were measured by recording the OD₆₀₀ of each well. MIC values were determined as the minimum concentration required to achieve 90% growth inhibition compared to growth in untreated wells.

Inhibition of 43300 Biofilm Formation: Inhibition assays were performed by taking an overnight culture of bacterial strain and subculturing it at an OD₆₀₀ of 0.01 into tryptic soy broth (Sigma-Aldrich) with a 2.0% (w/v) glucose supplement (TSBG). Stock solutions of predetermined concentrations of the test compound were then made in TSBG. These stock solutions were

aliquoted (100 μ L) into the wells of the 96-well PVC microtiter plate. Sample plates were then wrapped in GLAD Press n' Seal® followed by an incubation under stationary conditions for 24 h at 37 °C. After incubation, the medium was discarded from the wells and the plates were washed thoroughly with water. Plates were then stained with 110 μ L of 0.1% solution of crystal violet (CV) and then incubated at ambient temperature for 30 min. Plates were washed with water again and the remaining stain was solubilized with 200 μ L of 200 proof ethanol. A sample of 125 μ L of solubilized CV stain from each well was transferred to the corresponding wells of a polystyrene microtiter dish. Biofilm inhibition was quantitated by measuring the OD₅₄₀ of each well in which a negative control lane wherein no biofilm was formed served as a background and was subtracted out.

Growth curves for compounds in biofilm conditions: MRSA ATCC# 43300 was grown overnight in TSBG, and this culture was used to inoculate fresh TSBG (OD₆₀₀=0.01). Inoculated medium was aliquoted (3 mL) into culture tubes, and compound was added, with untreated inoculated medium serving as the control. Tubes were incubated at 37 °C with shaking. Samples were taken at 2, 4, 6, 8, and 24 h time points, serially diluted in fresh TSBG, and plated on nutrient agar. Plates were incubated at 37 °C overnight in stationary conditions, and the number of colonies was enumerated. The total number of bacterial colonies on each plate was determined using a SphereFlash colony counter (NEUTECH Group Inc.)

Procedure to Determine the Dispersal Effect of Test Compounds on MRSA ATCC# 43300

Preformed Biofilms: Dispersion assays were performed by taking an overnight culture of MRSA ATCC 43300 in tryptic soy broth (Sigma-Aldrich) with a 2.0% glucose supplement (TSBG) and subculturing it at an OD₆₀₀ of 0.01 into TSBG. The resulting bacterial suspension was aliquoted (100 μ L) into the wells of a 96-well PVC microtiter plate. Plates were then wrapped in GLAD

Press n' Seal® followed by an incubation under stationary conditions at 37 °C to establish the biofilms. After 24 h, the medium was discarded from the wells and the plates were washed thoroughly with water. Stock solutions of predetermined concentrations of the test compound were then made in the necessary medium. These stock solutions were aliquoted (100 µL) into the wells of the 96-well PVC microtiter plate with the established biofilms. Medium alone was added to a subset of the wells to serve as a control. Sample plates were then incubated for 24 h at 37 °C. After incubation, the medium was discarded from the wells and the plates were washed thoroughly with water. Plates were then stained with 110 µL of 0.1% solution of crystal violet (CV) and then incubated at ambient temperature for 30 min. Plates were washed with water again and the remaining stain was solubilized with 200 µL of 200 proof ethanol. A sample of 125 µL of solubilized CV stain from each well was transferred to the corresponding wells of a polystyrene microtiter dish. Biofilm dispersion was quantitated by measuring the OD₅₄₀ of each well in which a negative control lane wherein no biofilm was formed served as a background and was subtracted out.

Hemolysis Assay: Hemolysis assays were performed on mechanically defibrinated sheep blood (Hemostat Laboratories: DSB100). Defibrinated blood (1.5 mL) was placed into an Eppendorf tube and centrifuged for 10 min at 10,000 rpm. The supernatant was removed and then the cells were resuspended in 1 mL of phosphate-buffered saline (PBS). The suspension was centrifuged as before, the supernatant removed, and the cells were resuspended two more times. The final cell suspension was diluted 10-fold. Compound was added from a DMSO stock solution to aliquots of the 10-fold suspension dilution of blood to give the desired concentrations to be tested. Triton X (1%) was used as a positive control (100% lysis). PBS was used as a negative control (zero hemolysis). Samples were placed in an incubator at 37 °C with shaking at 200 rpm for 1 h. After

1 h, the samples were centrifuged for 10 min at 10,000 rpm. The resulting supernatant was diluted by a factor of 40 in distilled water. The absorbance of the supernatant was then measured with a UV spectrometer at 540 nm.

Checkerboard Assay for measurement of synergy with colistin: CAMHB was inoculated with *K. pneumoniae* (5×10^5 CFU/mL) and 100 μ L aliquots were distributed to all wells of a 96-well plate except for well 1A. Inoculated CAMHB (200 μ L) containing the selected compound (at a concentration for 2x the highest concentration being tested) was added to well 1a, and 100 μ L of the same sample was added to wells 2A-12A. Row A cells were mixed 6-8 times, and then 100 μ L was withdrawn and transferred to row B. This process was repeated up to row G (row H was not mixed to determine the MIC of the antibiotic alone). Inoculated media (100 mL) containing colistin at 2x the highest concentration being tested was placed in wells 1A- 1H and serially diluted, all the way until column 11 (column 12 was not mixed to determine the MIC of the compound alone). The plates were covered and sealed with Glad Press'n Seal, and incubated under stationary conditions at 37 °C for 16 h. After 16 h the MIC values of both compound and antibiotic were recorded, as well as combination. The Σ FIC values were calculated as follows: Σ FIC = FIC (compound) + FIC (antibiotic), where FIC (compound) is the MIC of the compound in the combination/MIC of the compound alone and FIC (antibiotic) is the MIC of the antibiotic in the combination/MIC of the antibiotic. The combination is considered synergistic when the Σ FIC is ≤ 0.5 , indifferent when the Σ FIC is between 0.5 and 2, and antagonistic when the Σ FIC is ≥ 2 .

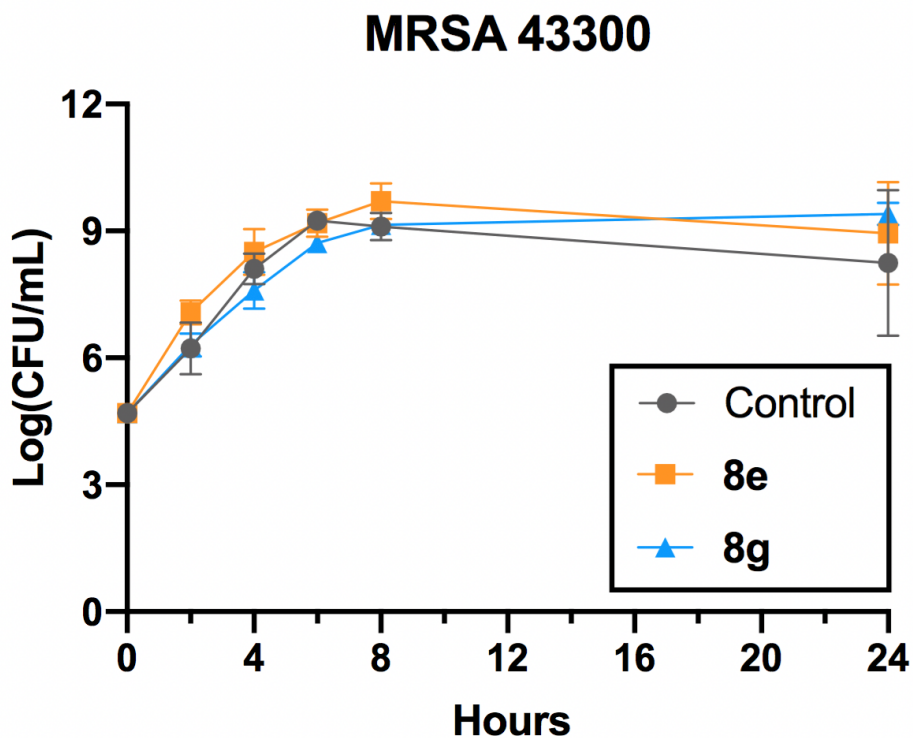


Figure S1: Time kill curve of control (grey line represents bacteria without compound) compared to compound **8e** dosed at its IC₅₀ concentration of 26.4 μM (orange), and **8g** dosed at its IC₅₀ concentration of 17.4 μM (blue).

Table S1. MIC of compound and potentiation of colistin in Gram-negative bacteria, *A. baumannii* 4106 and *K. pneumoniae* B9

Compound	AB 4106		KP B9	
	Compound MIC (μM)	Colistin MIC (μg/mL) + Compound (at 60 μM)	Compound MIC (μM)	Colistin MIC (μg/mL) + Compound (at 60 μM)

-	-	1024	-	512
8c	>200	>64	>200	>64
8b	>200	>64	>200	>64
8a	>200	>64	>200	>64
13b	>200	>64	>200	>64
15a	>200	>64	>200	>64
13a	>200	>64	>200	>64
14	>200	>64	>200	>64
8e	>200	>64	>200	>64
8d	>200	>64	>200	>64
13c	>200	>64	>200	>64
15c	>200	>64	>200	>64
15b	>200	>64	>200	>64
8h	>200	>64	>200	>64
8g	>200	>64	>200	16
8f	>200	>64	>200	>64
13e	>200	>64	>200	>64
13d	>200	>64	>200	>64
15d	>200	>64	>200	64

Table S2. Hemolysis of sheep's blood of compounds **8e** and **8g** dosed at 200 μ M

Compound	% lysis at 200 μ M
8e	5
8g	3.4

Table S3. Σ FIC values for the *K. pneumoniae* strains to show synergy with colistin

<i>K. pneumoniae</i> Strain	Σ FIC
KP B9	≤ 0.09
KP A5	≤ 0.31
F 2210291 ^{mcr-1}	≤ 0.19

General Chemistry Experimental All reactions were carried out under an atmosphere of nitrogen using anhydrous solvents unless otherwise specified. All chemical reagents for synthesis were used without further purification. Analytical thin layer chromatography (TLC) was performed using 250 μ m Silica Gel 60 F254 pre-coated plates (EMD Chemicals Inc.). Flash column chromatography was performed using 230–400 Mesh 60Å Silica Gel from Sorbent Technologies. NMR spectra were recorded using broadband probes on a Bruker AVANCE III HD Nanobay (400 MHz for ^1H and 100 MHz for ^{13}C) All Spectra are presented using MestReNova (Mnova) software and ^1H NMR are typically displayed from 12 to -0.7 ppm without the use of the signal suppression function. Spectra were obtained in the following solvents (reference peaks also included for the ^1H and ^{13}C NMRs): *d*₆-DMSO (^1H NMR: 2.50 ppm; ^{13}C NMR: 39.52 ppm) and *d*₄-MeOD (^1H NMR: 3.31 ppm; ^{13}C NMR: 49.00 ppm). All NMR experiments were performed at room temperature. Chemical shift values (δ) are reported in parts per million (ppm) for all ^1H and ^{13}C spectra. ^1H NMR multiplicities are reported as: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br = broad. High-resolution mass spectra were obtained for all new compounds from the mass spectrometry and proteomics facility at university of Notre Dame performed on a Bruker-TOF-ESI spectrometer in positive module using direct infusion in 9:1 acetonitrile: water. IR spectra were recorded on Bruker Alpha IIFTIR spectrometer. UV data was taken using a Thermo Scientific, Genesys 10 UV scanning spectrometer

General procedure of cyclization on nitro acetophenones

To a flame dried flask was added nitro-acetophenone (1.00 g, 6.06 mmol) in dry DMF (15 mL). Dimethylformamide dimethylacetal (0.722 g, 6.06 mmol) in the same solvent was added (804 μ L). The resulting solution was heated at 110 °C for 4 h under argon. After cooling, the solution was poured into H₂O (150 mL) and then extracted with EtOAc (3x15 mL). The combined organic layers were dried using Na₂SO₄ and concentrated to dryness under reduced pressure. Guanidine hydrochloride (0.868 g, 9.08 mmol), and anhydrous K₂CO₃ (1.76 g, 12.7 mmol) were then added and the mixture was dissolved in 2-methoxyethanol (20 mL). The solution was then heated at reflux temperature for 24 h under argon. After cooling, 100 mL of H₂O was added it was extracted with EtOAc (3x30 mL). The combined organic layers were washed with brine (2x15 mL), dried with Na₂SO₄ and concentrated to dryness under reduced pressure. The remaining residue was purified via silica gel column chromatography

General procedure for Suzuki coupling

To a flame dried round bottom flask, 2-amino-5-iodopyrimidine (1 eq.), PdCl₂(PPh₃) (0.05 eq), K₂CO₃ (4 eq), and nitro-boronic acid (2 eq.) were added. The flask was evacuated then placed under argon then dissolved in 20 mL of THF. Argon was subsequently bubbled through the solvent for 30 minutes then the reaction was heated to reflux and allowed to stir until completion via TLC analysis. The reaction was allowed to cool and ethyl acetate (100 mL) was added. The reaction mixture washed then with Sodium bicarbonate (3x30 mL) followed by water (3x30 mL) and then brine (2x30 mL). The organic layer was then dried using anhydrous sodium sulfate, evaporated and purified via silica gel column chromatography.

General Procedure for reduction of nitro intermediate

To a flame dried round bottom flask, the nitro-intermediate was added along with 0.1 equivalents of Pd/C. The round bottom flask was evacuated, and the solids were placed under argon. 10 mL of anhydrous methanol was then added to dissolve the nitro-intermediate and suspend the Pd/C. The reaction mixture was then allowed to stir with hydrogen gas bubbling through the solvent being pulled with a vacuum for 30 minutes after which the flask was taken off vacuum and hydrogen was allowed to bubble through the solvent while stirring in the hood while vented by an open needle. If further time was required, the needle was removed and the reaction was allowed to stir overnight under hydrogen. The reaction was allowed to stir until completion via TLC analysis. Upon completion, the reaction was filtered through a pad of celite which was washed with methanol. The solution was then evaporated under vacuum to yield in most cases a pure product which was then used without further purification in subsequent synthetic steps.

General procedure for acylation of aniline intermediate and HCl salt formation

To an oven dried vial was added crude aniline-intermediate residue (1 eq) and K_3PO_4 (1.25 eq unless otherwise stated). Anhydrous THF (10 mL) was added under argon gas and the solution was cooled to 0 °C and allowed to stir for 20 minutes. The desired acid-chloride (1 eq unless otherwise stated) was then added dropwise and the reaction stirred overnight at room temperature. The reaction was quenched with water/ethyl acetate. The organic material was extracted with ethyl acetate (3 x 20 mL). The organic fractions were combined and dried over Na_2SO_4 and then concentrated under reduced pressure. In many cases the product was recrystallized in ethyl acetate to afford the pure product, otherwise, the residue was purified by silica gel chromatography. The pure product was then dissolved in methanol and 12 N HCl was added to form the HCl salt.

Previously Reported Compounds

4-(2-nitrophenyl)pyrimidin-2-amine (6a): Compound was synthesized using previously reported methods.¹ Spectral data were consistent with previous reports.¹

4-(3-nitrophenyl)pyrimidin-2-amine (6b): Compound was synthesized using previously reported methods.² Spectral data were consistent with previous reports.²

4-(4-nitrophenyl)pyrimidin-2-amine (6c): Compound was synthesized using previously reported methods.³ Spectral data were consistent with previous reports.³

5-(3-nitrophenyl)pyrimidin-2-amine (11b): Compound was synthesized using previously reported methods.⁴ Spectral data were consistent with previous reports.⁴

5-(4-nitrophenyl)pyrimidin-2-amine (11c): Compound was synthesized using previously reported methods.⁵ Spectral data were consistent with previous reports.⁵

Novel Compound Characterization

5-(2-nitrophenyl)pyrimidin-2-amine (11a): Following the general procedure for Suzuki coupling, 2-amino-5-bromopyrimidine (592.6 mg, 3.4 mmol, 0.95 eq), (2-nitrophenyl)boronic acid (1.2 g, 7.2 mmol, 2 eq), K₂CO₃ (2 g, 14 mmol, 4 eq), and PdCl₂(PPh₃)₂ (130 mg, 0.18 mmol, .05 eq) afforded compound **11a** as an orange solid. Yield 22 % (170 mg, 0.79 mmol). ¹H NMR (400 MHz, Methanol-*d*₄) δ 8.26 (s, 2H), 8.02 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.76 (td, *J* = 7.6, 1.3 Hz, 1H), 7.62 (td, *J* = 7.8, 1.5 Hz, 1H), 7.53 (dd, *J* = 7.7, 1.4 Hz, 1H). ¹³C NMR (126 MHz, MeOD) δ 164.08, 158.29, 150.29, 134.22, 133.15, 131.44, 130.20, 125.69, 122.17. UV (λ_{max} nm): 208; IR_vmax (cm⁻¹): 182; HRMS (ESI): calcd for C₁₀H₉N₄O₂ [M+H]⁺: 217.0720, found: 217.0727

***N*-(2-(2-aminopyrimidin-4-yl)phenyl)-3,5-difluorobenzamide hydrochloride (8a):** Following the general procedure for aniline intermediate acylation, crude residue of compound **7a** (75 mg, 0.40 mmol, 1 eq), 3,5-difluorobenzoyl chloride (0.06 mL, 0.44 mmol, 1.1 eq), and potassium phosphate tribasic (85 mg, 0.40 mmol, 1 eq) afforded compound **8a** as a white solid. Yield 70 % (92 mg, 0.28 mmol). ¹H NMR (500 MHz, Methanol-*d*₄) δ 8.35 (d, *J* = 6.3 Hz, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 7.7 Hz, 1H), 7.65 (t, *J* = 7.9 Hz, 1H), 7.59 – 7.53 (m, 2H), 7.41 (t, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 5.9 Hz, 1H), 7.24 (tt, *J* = 8.8, 2.4 Hz, 1H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 162.7 (d, *J* = 12.2 Hz), 162.3 (dd, *J* = 247.7, 12.6 Hz), 158.3, 153.2, 137.8 (d, *J* = 7.5 Hz), 136.2 (d, *J* = 16.8 Hz), 131.7, 130.0, 127.4 (d, *J* = 12.3 Hz), 125.2, 123.7 (d, *J* = 14.7 Hz), 111.0 (dd, *J* = 20.0, 6.6 Hz), 108.7, 107.3 (t, *J* = 26.0 Hz), 104.2. UV (λ_{max} nm): 204; Melting point (°C): decays > 241; HRMS (ESI): calcd for C₁₇H₁₃F₂N₄O [M+H]⁺: 327.1052, found: 327.1054.

***N*-(3-(2-aminopyrimidin-4-yl)phenyl)-3,5-difluorobenzamide hydrochloride (8b):** Following the general procedure for aniline intermediate acylation, crude residue of compound **7b** (50 mg, 0.27 mmol, 1 eq), 3,5-difluorobenzoyl chloride (0.03 mL, 0.27 mmol, 1 eq), and potassium

phosphate tribasic (71 mg, 0.34 mmol, 1.25 eq) afforded compound **8b** as a white solid. Yield 53% (46 mg, 0.14 mmol). ¹H NMR (400 MHz, Methanol-*d*₄) δ 8.73 (t, *J* = 2.0 Hz, 1H), 8.34 (d, *J* = 6.6 Hz, 1H), 8.04 (dt, *J* = 8.0, 1.3 Hz, 1H), 7.88 (ddd, *J* = 8.1, 2.2, 1.0 Hz, 1H), 7.65 – 7.56 (m, 3H), 7.51 (d, *J* = 6.6 Hz, 1H), 7.25 (tt, *J* = 8.9, 2.4 Hz, 1H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 168.7, 163.0 (t, *J* = 2.8 Hz), 162.2 (dd, *J* = 247.3, 12.8 Hz), 157.6, 150.7, 139.4, 137.9, 135.5, 129.5, 124.7, 123.7, 119.9, 111.3 (dd, *J* = 19.5, 6.7 Hz), 107.3 (t, *J* = 25.1 Hz), 106.1. UV (λ_{max} nm): 204; Melting point (°C): decays > 225; HRMS (ESI): calcd for C₁₇H₁₃F₂N₄O [M+H]⁺: 327.1052, found: 327.1059.

***N*-(4-(2-aminopyrimidin-4-yl)phenyl)-3,5-difluorobenzamide hydrochloride (8c)**: Following the general procedure for aniline intermediate acylation, crude residue of compound **7c** (75 mg, 0.40 mmol, 1 eq), 3,5-difluorobenzoyl chloride (0.06 mL, 0.44 mmol, 1.1 eq), and potassium phosphate tribasic (85 mg, 0.40 mmol, 1 eq) afforded compound **8c** as a light tan solid. Yield 31% (40 mg, 0.12 mmol). ¹H NMR (500 MHz, Methanol-*d*₄) δ 8.33 – 8.30 (m, 2H), 8.28 (d, *J* = 6.6 Hz, 1H), 8.01 – 7.98 (m, 2H), 7.64 – 7.58 (m, 2H), 7.56 (d, *J* = 6.6 Hz, 1H), 7.25 (tt, *J* = 8.9, 2.3 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 168.3, 162.2 (dd, *J* = 247.3, 12.5 Hz), 156.8, 149.3, 143.2, 137.8 (t, *J* = 8.6 Hz), 129.6, 129.0, 120.3, 120.2, 111.5 (dd, *J* = 18.0, 7.4 Hz), 107.4 (t, *J* = 25.9 Hz), 105.5. UV (λ_{max} nm): 226; Melting point (°C): >260; HRMS (ESI): calcd for C₁₇H₁₃F₂N₄O [M+H]⁺: 327.1052, found: 327.1058.

***N*-(2-(2-aminopyrimidin-4-yl)phenyl)-3,5-dichlorobenzamide hydrochloride (8d)**: Following the general procedure for aniline intermediate acylation, crude residue of compound **7a** (75 mg, 0.40 mmol, 1 eq), 3,5-dichlorobenzoyl chloride (84 mg, 0.4 mmol, 1 eq), and potassium phosphate tribasic (110 mg, 0.50 mmol, 1.25 eq) afforded compound **8d** as a tan solid. Yield 73 % (106 mg, 0.295 mmol). ¹H NMR (400 MHz, Methanol-*d*₄) δ 8.35 (d, *J* = 6.6 Hz, 1H), 8.18 (dd, *J* = 8.2, 1.1 Hz, 1H), 7.91 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.90 (d, *J* = 1.9 Hz, 2H), 7.71 (t, *J* = 1.9 Hz, 1H), 7.67 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.43 (td, *J* = 7.7, 1.2 Hz, 1H), 7.36 (d, *J* = 6.6 Hz, 1H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 165.5, 162.2, 161.7, 158.7, 137.7, 136.6, 134.6, 131.2, 130.9, 129.6, 126.6, 126.2, 124.7, 122.7, 108.2. UV (λ_{max} nm): 206; Melting point (°C): decay > 253; HRMS (ESI): calcd for C₁₇H₁₃Cl₂N₄O [M+H]⁺: 359.0461, found: 359.0462.

***N*-(3-(2-aminopyrimidin-4-yl)phenyl)-3,5-dichlorobenzamide hydrochloride (8e)**: Following the general procedure for aniline intermediate acylation, crude residue of compound **7b** (50 mg, 0.27 mmol, 1 eq), 3,5-dichlorobenzoyl chloride (56 mg, 0.27 mmol, 1.1 eq), and potassium phosphate tribasic (71 mg, 0.34 mmol, 1.25 eq) afforded compound **8e** as a 206 solid. Yield 27 % (26 mg, 0.072 mmol). ¹H NMR (400 MHz, Methanol-*d*₄) δ 8.76 (t, *J* = 2.0 Hz, 1H), 8.35 (d, *J* = 6.7 Hz, 1H), 8.05 (dt, *J* = 8.0, 1.2 Hz, 1H), 7.95 (d, *J* = 1.9 Hz, 2H), 7.88 (ddd, *J* = 8.1, 2.2, 1.0 Hz, 1H), 7.72 (t, *J* = 1.9 Hz, 1H), 7.60 (t, *J* = 8.0 Hz, 1H), 7.54 (d, *J* = 6.7 Hz, 1H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 162.9, 158.1, 151.4, 142.8, 139.4, 137.7, 135.6, 134.4, 131.1, 129.5, 126.6, 124.4, 123.7, 119.8, 106.1. UV (λ_{max} nm): 204; Melting point (°C): decay > 225; HRMS (ESI): calcd for C₁₇H₁₃Cl₂N₄O [M+H]⁺: 359.0461, found: 359.0464.

***N*-(2-(2-aminopyrimidin-4-yl)phenyl)-3,5-dibromobenzamide hydrochloride (8f)**: Following the general procedure for aniline intermediate acylation, crude residue of compound **7a** (75 mg, 0.40 mmol, 1 eq), 3,5-dibromobenzoyl chloride (130 mg, 0.44 mmol, 1.1 eq), and potassium phosphate tribasic (85 mg, 0.40 mmol, 1 eq) afforded compound **8f** as a white solid. Yield 37 %

(67 mg, 0.15 mmol). ¹H NMR (500 MHz, DMSO-*d*₆) δ 12.25 (s, 1H), 8.39 (d, *J* = 5.4 Hz, 1H), 8.29 (dd, *J* = 8.2, 1.2 Hz, 1H), 8.11 (t, *J* = 1.7 Hz, 1H), 8.05 (d, *J* = 1.8 Hz, 2H), 7.81 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.55 (ddd, *J* = 8.5, 7.4, 1.6 Hz, 1H), 7.32 (td, *J* = 7.6, 1.2 Hz, 1H), 7.17 (s, 2H), 7.06 (d, *J* = 5.4 Hz, 1H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 165.8, 162.2, 161.4, 158.2, 138.1, 136.6, 136.6, 131.0, 129.6, 129.4, 126.8, 124.8, 122.9, 122.9, 108.3. UV (λ_{max} nm): 206; Melting point (°C): decay > 242; HRMS (ESI): calcd for C₁₇H₁₃Br₂N₄O [M+H]⁺: 446.9451, found: 446.9432. *N*-

(3-(2-aminopyrimidin-4-yl)phenyl)-3,5-dibromobenzamide hydrochloride (8g): Following the general procedure for aniline intermediate acylation, crude residue of compound **7b** (50 mg, 0.27 mmol, 1 eq), 3,5-dibromobenzoyl chloride (80 mg, 0.27 mmol, 1 eq), and potassium phosphate tribasic (71 mg, 0.34 mmol, 1.25 eq) afforded compound **8g** as a white solid. Yield 18 % (22 mg, 0.049 mmol). ¹H NMR (400 MHz, Methanol-*d*₄) δ 8.76 (t, *J* = 2.0 Hz, 1H), 8.35 (d, *J* = 6.6 Hz, 1H), 8.13 (d, *J* = 1.8 Hz, 2H), 8.05 (dt, *J* = 8.0, 1.2 Hz, 1H), 7.99 (t, *J* = 1.8 Hz, 1H), 7.88 (ddd, *J* = 8.1, 2.2, 1.0 Hz, 1H), 7.60 (t, *J* = 8.0 Hz, 1H), 7.55 (d, *J* = 6.7 Hz, 1H). ¹³C NMR (126 MHz, MeOD) δ 173.2, 165.5, 157.5, 148.1, 140.7, 139.5, 138.2, 136.4, 130.8, 130.8, 127.0, 125.8, 124.2, 122.0, 107.5. UV (λ_{max} nm): 208; Melting point (°C): decay > 167 HRMS (ESI): calcd for C₁₇H₁₃Br₂N₄O [M+H]⁺: 446.9451, found: 446.9426.

***N*-(4-(2-aminopyrimidin-4-yl)phenyl)-3,5-dibromobenzamide hydrochloride (8h):** Following the general procedure for aniline intermediate acylation, compound **7c** (75 mg, 0.40 mmol, 1 eq), 3,5-dibromobenzoyl chloride (130 mg, 0.44 mmol, 1.1 eq), and potassium phosphate tribasic (85 mg, 0.40 mmol, 1 eq) afforded compound **8h** as a yellow solid. Yield 55 % (108 mg, 223 mmol). ¹H NMR (400 MHz, Methanol-*d*₄) δ 8.33 – 8.29 (m, 2H), 8.27 (d, *J* = 6.8 Hz, 1H), 8.13 (d, *J* = 1.8 Hz, 2H), 8.00 (d, *J* = 2.0 Hz, 2H), 7.99 (d, *J* = 5.4 Hz, 1H), 7.55 (d, *J* = 6.8 Hz, 1H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 167.5, 162.9, 162.8, 157.7, 150.5, 142.85, 142.7, 137.9, 137.9, 136.5, 136.5, 129.9, 129.8, 128.7, 122.6, 120.2, 120.1, 105.4. UV (λ_{max} nm): 206; Melting point (°C): decay > 215; HRMS (ESI): calcd for C₁₇H₁₃Br₂N₄O [M+H]⁺: 446.9451, found: 446.9432.

***N*-(2-(2-aminopyrimidin-5-yl)phenyl)-3,5-difluorobenzamide hydrochloride (13a):** Following the general procedure for the reduction of nitro intermediate, compound **11a** (100 mg, 0.46 mmol, 1 eq) was dissolved in methanol (20 mL) and reduced using palladium on carbon (4.9 mg, 0.046 mmol, 0.1 eq) under hydrogen atmosphere for 16 h. Upon reaction completion via TLC analysis, the reaction was filtered through a pad of celite and rinsed with MeOH until all product was collected. MeOH was evaporated under vacuum to afford product residue. The residue was dissolved in anhydrous THF (10 mL) and used as is in following the general procedure for aniline intermediate acylation. Upon addition of 3,5-difluorobenzoyl chloride (150 μL, 1.21 mmol, 2.6 eq), and potassium phosphate tribasic (150 mg, 0.707 mmol, 1.5 eq), compound **13a** was afforded as a white solid in 11 % yield (16 mg, 0.049 mmol). ¹H NMR (500 MHz, Methanol-*d*₄) δ 8.32 (s, 2H), 7.49 – 7.41 (m, 6H), 7.19 (tt, *J* = 8.8, 2.3 Hz, 1H); ¹³C NMR (126 MHz, Methanol-*d*₄) δ 166.4, 164.4 (dd, *J* = 248.8, 12.3 Hz), 163.6, 158.8, 138.9 (t, *J* = 8.5 Hz), 135.7, 134.4, 131.2, 129.8, 129.1, 129.0, 123.7, 111.8 (dd, *J* = 20.6, 6.4 Hz), 108.1 (t, *J* = 25.8 Hz). ; Melting point (°C): decay > 135; HRMS (ESI): calcd for C₁₇H₁₃F₂N₄O [M+H]⁺: 327.1052, found: 327.1052.

***N*-(4-(2-aminopyrimidin-5-yl)phenyl)-3,5-difluorobenzamide hydrochloride (13b):** Following the general procedure for aniline intermediate acylation, crude residue of compound **12c** (66 mg, 0.35 mmol, 1 eq), 3,5-difluorobenzoyl chloride (0.05 mL, 0.39 mmol, 1.1 eq), and

potassium phosphate tribasic (75 mg, 0.35 mmol, 1 eq) afforded compound **13b** as a light tan solid. Yield 63 % (73 mg, 0.22 mmol). ¹H NMR (500 MHz, Methanol-*d*₄) δ 8.77 (s, 2H), 7.89 – 7.86 (m, 2H), 7.67 – 7.64 (m, 2H), 7.59 (ddd, *J* = 6.9, 2.3, 1.2 Hz, 2H), 7.24 (tt, *J* = 8.9, 2.4 Hz, 1H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 162.9 (t, *J* = 2.7 Hz), 162.2 (dd, *J* = 247.2, 12.6 Hz), 158.6, 154.9, 138.4, 138.1, 129.0, 125.9, 121.7, 121.0, 111.3 (dd, *J* = 20.2, 6.6 Hz), 107.1 (t, *J* = 26.2 Hz). UV (λ_{max} nm): 204; Melting point (°C): decays >203; HRMS (ESI): calcd for C₁₇H₁₃F₂N₄O [M+H]⁺: 327.1052, found: 327.1062.

***N*-(4-(2-aminopyrimidin-5-yl)phenyl)-3,5-dichlorobenzamide hydrochloride (13c):** Following the general procedure for aniline intermediate acylation, crude residue of compound **12c** (75 mg, 0.40 mmol, 1 eq), 3,5-dichlorobenzoyl chloride (93 mg, 0.44 mmol, 1.1 eq), and potassium phosphate tribasic (85 mg, 0.40 mmol, 1 eq) afforded compound **13c** as a light yellow solid. Yield 84 % (121 mg, 0.337 mmol). ¹H NMR (500 MHz, Methanol-*d*₄) δ 8.86 (s, 2H), 7.93 (d, *J* = 1.9 Hz, 2H), 7.89 (d, *J* = 8.7 Hz, 2H), 7.70 (t, *J* = 1.9 Hz, 1H), 7.68 (d, *J* = 8.7 Hz, 2H). ¹³C NMR (126 MHz, DMSO) δ 162.7, 156.5, 154.4, 138.8, 137.8, 134.3, 131.0, 128.1, 126.6, 126.1, 121.7, 120.9. UV (λ_{max} nm): 208; Melting point (°C): decay > 235; HRMS (ESI): calcd for C₁₇H₁₃Cl₂N₄O [M+H]⁺: 359.0461, found: 359.0462.

***N*-(3-(2-aminopyrimidin-5-yl)phenyl)-3,5-dibromobenzamide hydrochloride (13d):** Following the general procedure for aniline intermediate acylation, crude residue of compound **12b** (75 mg, 0.40 mmol, 1 eq), 3,5-dibromobenzoyl chloride (130 mg, 0.44 mmol, 1.1 eq), and potassium phosphate tribasic (85 mg, 0.40 mmol, 1 eq) afforded compound **13d** as a tan solid. Yield 30 % (58 mg, 0.12 mmol). ¹H NMR (500 MHz, Methanol-*d*₄) δ 8.87 (s, 2H), 8.13 (d, *J* = 1.7 Hz, 2H), 8.11 (t, *J* = 1.9 Hz, 1H), 7.99 (t, *J* = 1.8 Hz, 1H), 7.68 (ddd, *J* = 8.1, 2.1, 1.0 Hz, 1H), 7.53 (t, *J* = 8.0 Hz, 1H), 7.46 (ddd, *J* = 7.7, 1.8, 1.1 Hz, 1H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 162.6, 156.9, 154.8, 139.4, 138.1, 136.4, 133.1, 129.7, 129.6, 122.6, 122.0, 121.7, 120.3, 117.9. UV (λ_{max} nm): 266; Melting point (°C): decay > 250; HRMS (ESI): calcd for C₁₇H₁₃Br₂N₄O [M+H]⁺: 446.9451, found: 446.9429.

***N*-(4-(2-aminopyrimidin-5-yl)phenyl)-3,5-dibromobenzamide hydrochloride (13e):** Following the general procedure for aniline intermediate acylation, crude residue of compound **12c** (66 mg, 0.35 mmol, 1 eq), 3,5-dibromobenzoyl chloride (120 mg, 0.39 mmol, 1.1 eq), and potassium phosphate tribasic (75 mg, 0.35 mmol, 1 eq) afforded compound **13e** as a yellow solid. Yield 54 % (86 mg, 0.19 mmol). ¹H NMR (400 MHz, Methanol-*d*₄) δ 8.88 (s, 2H), 8.11 (d, *J* = 1.8 Hz, 2H), 7.98 (t, *J* = 1.7 Hz, 1H), 7.90 (d, *J* = 8.6 Hz, 2H), 7.68 (d, *J* = 8.7 Hz, 2H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 163.0, 157.0, 154.9, 139.2, 138.6, 136.8, 130.2, 128.6, 126.6, 123.1, 122.2, 121.3. UV (λ_{max} nm): 210; Melting point (°C): decay > 215; HRMS (ESI): calcd for C₁₇H₁₃Br₂N₄O [M+H]⁺: 446.9451, found: 446.9424.

***N*-(4-(2-aminopyrimidin-4-yl)phenyl)-3,5-dichlorobenzamide hydrochloride (14):** To a round bottom flask was added compound **6c** (1.125 g, 5.20 mmol, 1 eq) dissolved in DCM (30 mL). Di-*tert*-butyl dicarbonate (3.41 g, 15.6 mmol, 3 eq) and DMAP (31.79 mg, 0.26 mmol, .05 eq) were added to the round bottom, and the reaction was allowed to stir for 16 h to afford di-*tert*-butyl (4-(4-nitrophenyl)pyrimidin-2-yl)carbamate as a white solid. Next, di-*tert*-butyl (4-(4-nitrophenyl)pyrimidin-2-yl)carbamate (248 mg, 0.60 mmol, 1 eq) was reduced using the general procedure for reduction of nitro intermediates with palladium on carbon (mg, 0.097 mmol, 0.1 eq)

to yield the crude residue of di-*tert*-butyl (4-(4-aminophenyl)pyrimidin-2-yl)carbamate as a yellow residue. Following the general procedure for acylation of aniline intermediates, di-*tert*-butyl (4-(4-aminophenyl)pyrimidin-2-yl)carbamate (137 mg, 0.35 mmol, 1 eq), 3,5-dichlorobenzoyl chloride (73.2 mg, 0.35 mmol, 1 eq), and potassium phosphate tribasic (92.7 mg, 0.44 mmol, 1.25 eq) were reacted to afford di-*tert*-butyl (4-(4-(3,5-dichlorobenzamido)phenyl)pyrimidin-2-yl)carbamate as a white solid in 34 % yield (67 mg, 0.12 mmol). Then, di-*tert*-butyl (4-(4-(3,5-dichlorobenzamido)phenyl)pyrimidin-2-yl)carbamate was dissolved in DCM (3 mL) and TFA (3 mL) was added slowly to deprotect the exocyclic nitrogen on the pyrimidine. Upon reaction completion via TLC analysis, DCM and TFA were removed *in vacuo*. The remaining white solid was dissolved in MeOH (10 mL) and 12 N HCl (1 mL) was added dropwise to form the corresponding HCl salt. The final HCl salt product was washed with cold hexanes to afford compound **14** as a white solid in 84 % yield (40 mg, 0.10 mmol). ¹H NMR (500 MHz, Methanol-*d*₄) δ 8.31 – 8.25 (m, 3H), 7.97 (d, *J* = 8.8 Hz, 2H), 7.94 (d, *J* = 1.9 Hz, 2H), 7.71 (t, *J* = 1.9 Hz, 1H), 7.50 (d, *J* = 6.5 Hz, 1H); ¹³C NMR (126 MHz, Methanol-*d*₄) δ 171.7, 165.8, 158.3, 149.0, 144.7, 139.2, 136.6, 132.7, 131.5, 130.5, 127.6, 121.7, 107.0. UV (λ_{max} nm): 206 Melting point (°C): 206; HRMS (ESI): calcd for C₁₇H₁₃Cl₂N₄O [M+H]⁺: 359.0461, found: 359.0460.

***N*-(3-(2-aminopyrimidin-5-yl)phenyl)-3,5-difluorobenzamide hydrochloride (15a)**: A mixture of 2-amino-5-bromopyrimidine (11.22 g, 64.48 mmol, 1 eq) and di-*tert*-butyl dicarbonate (84.44 g, 386.9 mmol, 6 eq), was dissolved in pyridine (32 mL, 400 mmol, 6.2 eq). The reaction vessel was placed under an argon atmosphere, heated to 70 °C, and allowed to stir at that temperature for 16 h. The reaction solution was then cooled to room temperature and diluted with ethyl acetate (100 mL). The diluted reaction mixture was then washed with water (30 mL x 2), followed by brine (30 mL x 2 of times done). The aqueous layers were combined and back extracted with ethyl acetate (100 mL x 2). The organic layers were combined and dried with Na₂SO₄, then concentrated *in vacuo*. The residue was purified using flash chromatography with silica gel, to yield di-*tert*-butyl 5-bromopyrimidin-2-ylcarbamate as an orange solid in 83 % yield (20 g, 53 mmol). Following the general procedure for Suzuki coupling 5-bromopyrimidin-2-ylcarbamate (1.5 g, 4 mmol, 1 eq), (3-nitrophenyl)boronic acid (1.3 g, 8 mmol, 2 eq), K₂CO₃ (2.2 g, 16 mmol, 4 eq), and PdCl₂(PPh₃)₂ (140 mg, 0.2 mmol, .05 eq) were reacted to afford *tert*-butyl (5-(3-nitrophenyl)pyrimidin-2-yl)carbamate as a yellow solid. in 30 % yield (379 mg, 1.20 mmol). *tert*-butyl (5-(3-nitrophenyl)pyrimidin-2-yl)carbamate (308 mg, 0.97 mmol, 1 eq) was then reduced using the general procedure for reduction of nitro intermediates with palladium on carbon (10.4 mg, 0.097 mmol, 0.1 eq) to yield the crude residue of *tert*-butyl (5-(3-aminophenyl)pyrimidin-2-yl)carbamate as a yellow residue in 72 % yield (200 mg, 0.70 mmol). Following the general procedure for acylation of aniline intermediates, *tert*-butyl (5-(3-aminophenyl)pyrimidin-2-yl)carbamate (100 mg, 0.35 mmol, 1 eq), 3,5-difluorobenzoyl chloride (65 μL, 0.52 mmol, 1.5 eq), and potassium phosphate tribasic (74.1 mg, 0.35 mmol, 1 eq) were dissolved in DCM (10 mL) to afford *tert*-butyl (5-(3-(3,5-difluorobenzamido)phenyl)pyrimidin-2-yl)carbamate as a white solid in 36 % yield (54 mg, 0.13 mmol). Next, *tert*-butyl (5-(3-(3,5-difluorobenzamido)phenyl)pyrimidin-2-yl)carbamate was dissolved in DCM (3 mL) and TFA (3 mL) was added dropwise to deprotect the exocyclic nitrogen on the pyrimidine. Upon reaction completion via TLC analysis, DCM and TFA were removed *in vacuo*. The white solid residue was dissolved in MeOH (# mL) and 12 N HCl (1 mL) was added dropwise to yield the corresponding HCl salt product of (list compound name). The HCl salt product was washed with cold hexanes (3 mL x 10 of times) to afford compound **15a** as a tan solid in 94 % yield (39 mg, 0.12 mmol). ¹H

NMR (500 MHz, Methanol-*d*₄) δ 8.78 (s, 2H), 8.06 (t, *J* = 1.9 Hz, 1H), 7.69 (ddd, *J* = 8.1, 2.1, 1.0 Hz, 1H), 7.61 (ddd, *J* = 6.8, 2.3, 1.2 Hz, 2H), 7.54 – 7.49 (m, 1H), 7.44 (ddd, *J* = 7.8, 1.8, 1.0 Hz, 1H), 7.24 (tt, *J* = 8.9, 2.3 Hz, 1H); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 162.90 (dd, *J* = 246.9, 13.0 Hz), 156.30, 139.97, 138.90 (d, *J* = 8.4 Hz), 135.72, 130.32, 130.19, 122.61, 122.02, 120.08, 118.14, 111.94, 111.72, 107.85 (t, *J* = 26.0 Hz). UV (λ_{max} nm): 266; Melting point (°C): 154; HRMS (ESI): calcd for C₁₇H₁₃F₂N₄O [M+H]⁺: 327.1052, found: 327.1053.

***N*-(2-(2-aminopyrimidin-5-yl)phenyl)-3,5-dichlorobenzamide hydrochloride (15b):** A mixture of 2-amino-5-bromopyrimidine (11.22 g, 64.48 mmol, 1 eq) and di-*tert*-butyl dicarbonate (84.44 g, 386.9 mmol, 6 eq), was dissolved in pyridine (32 mL, 400 mmol, 6.2 eq). The reaction vessel was placed under an argon atmosphere, heated to 70 °C, and allowed to stir at that temperature for 16 h. The reaction solution was then cooled to room temperature and diluted with ethyl acetate (100 mL). The diluted reaction mixture was then washed with water (30 mL x 2), followed by brine (30 mL x 2 of times done). The aqueous layers were combined and back extracted with ethyl acetate (100 mL x 2). The organic layers were combined and dried with Na₂SO₄, then concentrated *in vacuo*. The residue was purified using flash chromatography with silica gel, to yield di-*tert*-butyl 5-bromopyrimidin-2-ylcarbamate as an orange solid in 83 % yield (20 g, 53 mmol). Following the general procedure for Suzuki coupling 5-bromopyrimidin-2-ylcarbamate (4.804 g, 12.84 mmol, 1 eq), (2-nitrophenyl)boronic acid (4.29 g, 25.68 mmol, 2 eq), K₂CO₃ (7.097 g, 51.35 mmol, 4 eq), and PdCl₂(PPh₃)₂ (450 mg, 0.64 mmol, .05 eq) were reacted to afford di-*tert*-butyl (5-(3-nitrophenyl)pyrimidin-2-yl)carbamate as a yellow solid. in 33 % yield (1.781 mg, 4.28 mmol). Di-*tert*-butyl (5-(2-nitrophenyl)pyrimidin-2-yl)carbamate (1.781 g, 4.28 mmol, 1 eq) was then reduced using the general procedure for reduction of nitro intermediates with palladium on carbon (45.5 mg, 0.43 mmol, 0.1 eq) to yield the crude residue of di-*tert*-butyl (5-(2-aminophenyl)pyrimidin-2-yl)carbamate as a yellow residue in 41 % yield (675 mg, 1.75 mmol). Following the general procedure for acylation of aniline intermediates, di-*tert*-butyl (5-(2-aminophenyl)pyrimidin-2-yl)carbamate (135 mg, 0.35 mmol, 1 eq),), 3,5-dichlorobenzoyl chloride (74.2 mg, 0.35 mmol, 1 eq), and potassium phosphate tribasic (92.7 mg, 0.44 mmol, 1.25 eq) afforded di-*tert*-butyl (5-(2-(3,5-dichlorobenzamido)phenyl)pyrimidin-2-yl)carbamate as a white solid in 15 % yield (30 mg, 0.054 mmol). Next, di-*tert*-butyl (5-(2-(3,5-dichlorobenzamido)phenyl)pyrimidin-2-yl)carbamate was dissolved in DCM (3 mL) and TFA (3 mL) was added dropwise to deprotect the exocyclic nitrogen on the pyrimidine. Upon reaction completion via TLC analysis, DCM and TFA were removed *in vacuo*. The white solid residue was dissolved in MeOH (# mL) and 12 N HCl (1 mL) was added dropwise to yield the corresponding HCl salt product of (list compound name). The HCl salt product was washed with cold hexanes (2 mL x 10 of times) to afford compound **15b** as a brown solid in 96 % yield (20 mg, 0.051 mmol). ¹H NMR (500 MHz, Methanol-*d*₄) δ 8.55 (s, 2H), 7.80 (d, *J* = 1.9 Hz, 2H), 7.69 (t, *J* = 1.9 Hz, 1H), 7.57 – 7.41 (m, 4H). ¹³C NMR (126 MHz, Methanol-*d*₄) δ 156.5, 149.3, 148.1, 128.8, 127.2, 126.4, 123.3, 122.5, 121.8, 121.4, 119.57, 119.3, 117.9, 114.4. UV (λ_{max} nm): 206; Melting point (°C): decay > 160; HRMS (ESI): calcd for C₁₇H₁₃Cl₂N₄O [M+H]⁺: 359.0461, found: 359.0457.

***N*-(3-(2-aminopyrimidin-5-yl)phenyl)-3,5-dichlorobenzamide hydrochloride (15c):** A mixture of 2-amino-5-bromopyrimidine (11.22 g, 64.48 mmol, 1 eq) and di-*tert*-butyl dicarbonate (84.44 g, 386.9 mmol, 6 eq), was dissolved in pyridine (32 mL, 400 mmol, 6.2 eq). The reaction vessel

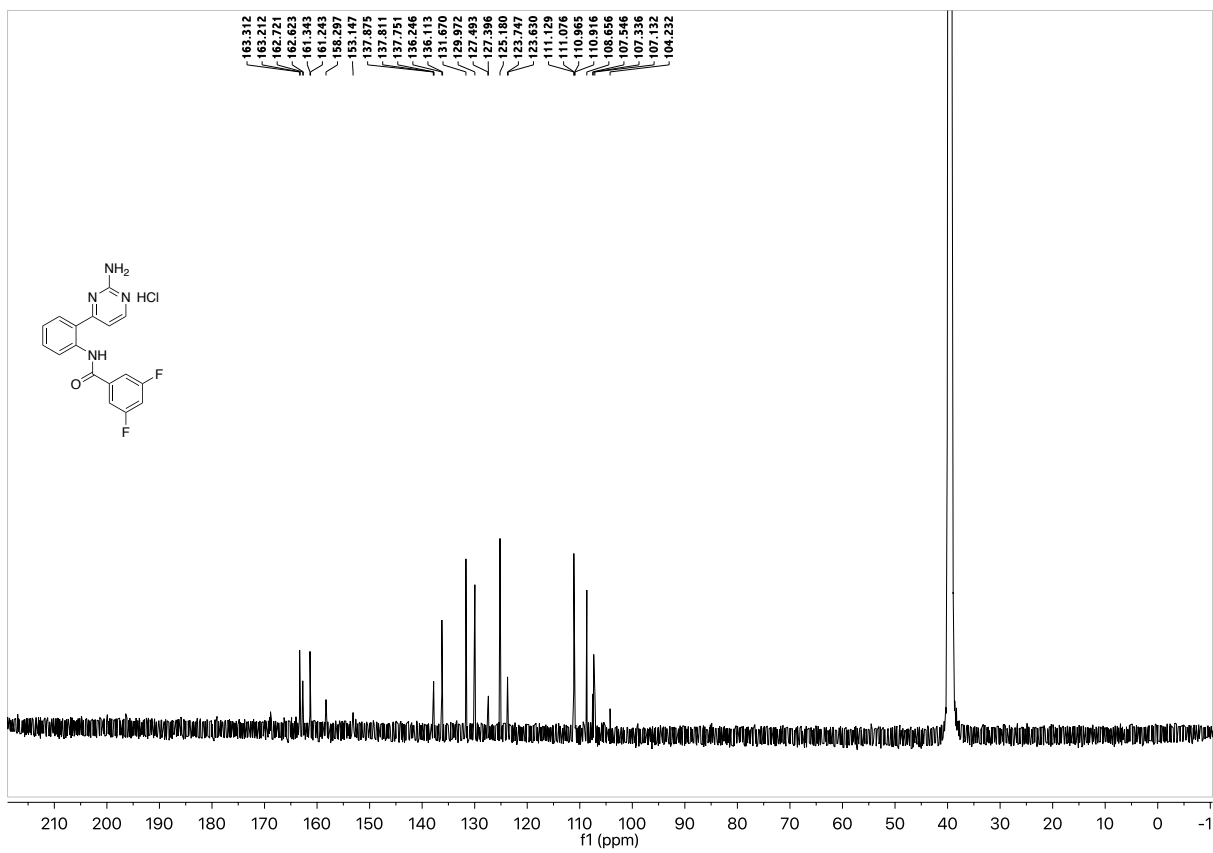
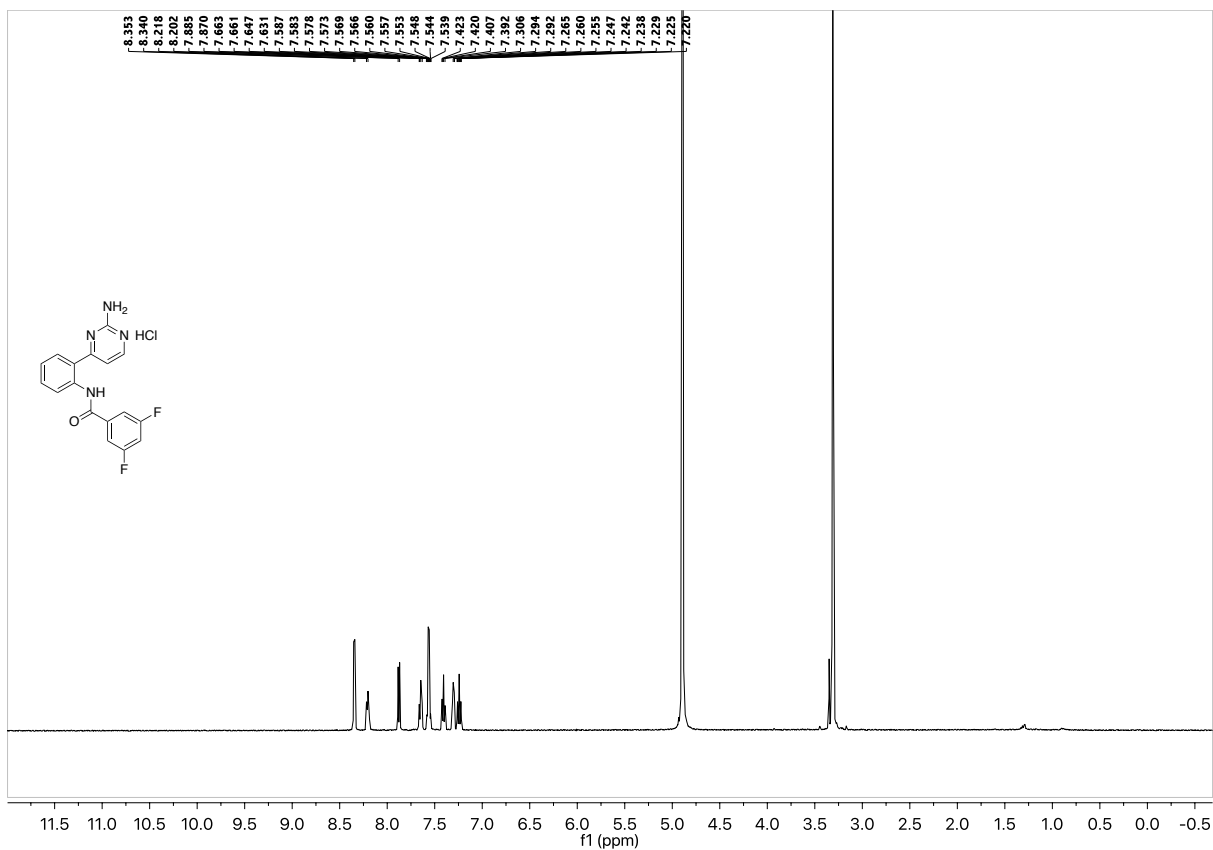
was placed under an argon atmosphere, heated to 70 °C, and allowed to stir at that temperature for 16 h. The reaction solution was then cooled to room temperature and diluted with ethyl acetate (100 mL). The diluted reaction mixture was then washed with water (30 mL x 2), followed by brine (30 mL x 2 of times done). The aqueous layers were combined and back extracted with ethyl acetate (100 mL x 2). The organic layers were combined and dried with Na₂SO₄, then concentrated *in vacuo*. The residue was purified using flash chromatography with silica gel, to yield di-*tert*-butyl 5-bromopyrimidin-2-ylcarbamate as an orange solid in 83 % yield (20 g, 53 mmol). Following the general procedure for Suzuki coupling 5-bromopyrimidin-2-ylcarbamate (1.5 g, 4 mmol, 1 eq), (3-nitrophenyl)boronic acid (1.3 g, 8 mmol, 2 eq), K₂CO₃ (2.2 g, 16 mmol, 4 eq), and PdCl₂(PPh₃)₂ (140 mg, 0.2 mmol, .05 eq) were reacted to afford *tert*-butyl (5-(3-nitrophenyl)pyrimidin-2-yl)carbamate as a yellow solid. in 30 % yield (379 mg, 1.20 mmol). *tert*-butyl (5-(3-nitrophenyl)pyrimidin-2-yl)carbamate (308 mg, 0.97 mmol, 1 eq) was then reduced using the general procedure for reduction of nitro intermediates with palladium on carbon (10.4 mg, 0.097 mmol, 0.1 eq) to yield the crude residue of *tert*-butyl (5-(3-aminophenyl)pyrimidin-2-yl)carbamate as a yellow residue in 72 % yield (200 mg, 0.70 mmol). Following the general procedure for acylation of aniline intermediates, *tert*-butyl (5-(3-aminophenyl)pyrimidin-2-yl)carbamate (100 mg, 0.35 mmol, 1 eq), 3,5-dichlorobenzoyl chloride (110 mg, 0.52 mmol, 1.5 eq), and potassium phosphate tribasic (74.1 mg, 0.35 mmol, 1 eq) were dissolved in DCM (10 mL) to afford *tert*-butyl (5-(3-(3,5-difluorobenzamido)phenyl)pyrimidin-2-yl)carbamate as a white solid in 54 % yield (87 mg, 0.19 mmol). Next, *tert*-butyl (5-(3-(3,5-dichlorobenzamido)phenyl)pyrimidin-2-yl)carbamate was dissolved in DCM (3 mL) and TFA (3 mL) was added dropwise to deprotect the exocyclic nitrogen on the pyrimidine. Upon reaction completion via TLC analysis, DCM and TFA were removed *in vacuo*. The white solid residue was dissolved in MeOH (# mL) and 12 N HCl (1 mL) was added dropwise to yield the corresponding HCl salt product of (list compound name). The HCl salt product was washed with cold hexanes (2 mL x 10 of times) to afford compound **15c** as a white solid in 98% yield (65 mg, 0.16 mmol). ¹H NMR (500 MHz, Methanol-*d*₄) δ 8.87 (s, 2H), 8.12 (t, *J* = 1.9 Hz, 1H), 7.95 (d, *J* = 1.8 Hz, 2H), 7.71 (t, *J* = 1.9 Hz, 1H), 7.69 (ddd, *J* = 8.1, 2.1, 1.0 Hz, 1H), 7.53 (t, *J* = 7.9 Hz, 1H), 7.46 (ddd, *J* = 7.8, 1.9, 1.1 Hz, 1H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 162.7, 157.2, 154.8, 139.4, 137.7, 134.3, 133.2, 131.0, 129.5, 126.6, 122.0, 121.7, 120.2, 117.9. UV (λ_{max} nm): 264; Melting point (°C): decay > 205; HRMS (ESI): calcd for C₁₇H₁₃Cl₂N₄O [M+H]⁺: 359.0461, found: 359.0465.

***N*-(2-(2-aminopyrimidin-5-yl)phenyl)-3,5-dibromobenzamide hydrochloride (15d):** A mixture of 2-amino-5-bromopyrimidine (11.22 g, 64.48 mmol, 1 eq) and di-*tert*-butyl dicarbonate (84.44 g, 386.9 mmol, 6 eq), was dissolved in pyridine (32 mL, 400 mmol, 6.2 eq). The reaction vessel was placed under an argon atmosphere, heated to 70 °C, and allowed to stir at that temperature for 16 h. The reaction solution was then cooled to room temperature and diluted with ethyl acetate (100 mL). The diluted reaction mixture was then washed with water (30 mL x 2), followed by brine (30 mL x 2 of times done). The aqueous layers were combined and back extracted with ethyl acetate (100 mL x 2). The organic layers were combined and dried with Na₂SO₄, then concentrated *in vacuo*. The residue was purified using flash chromatography with silica gel, to yield di-*tert*-butyl 5-bromopyrimidin-2-ylcarbamate as an orange solid in 83 % yield (20 g, 53 mmol). Following the general procedure for Suzuki coupling 5-bromopyrimidin-2-ylcarbamate (4.804 g, 12.84 mmol, 1 eq), (2-nitrophenyl)boronic acid (4.29 g, 25.68 mmol, 2 eq), K₂CO₃ (7.097 g, 51.35 mmol, 4 eq), and PdCl₂(PPh₃)₂ (450 mg, 0.64 mmol, .05 eq) were reacted to afford di-*tert*-butyl (5-(3-nitrophenyl)pyrimidin-2-yl)carbamate as a yellow solid. in 33 % yield

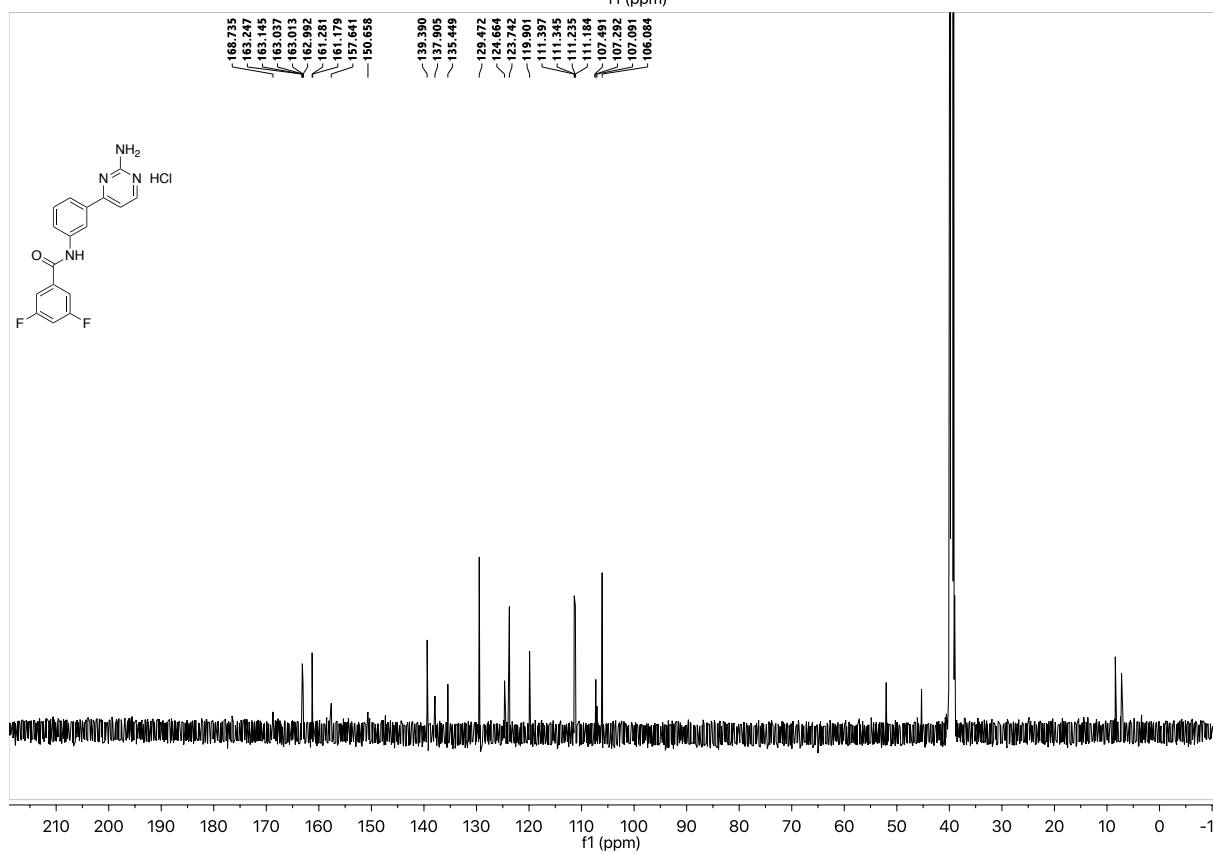
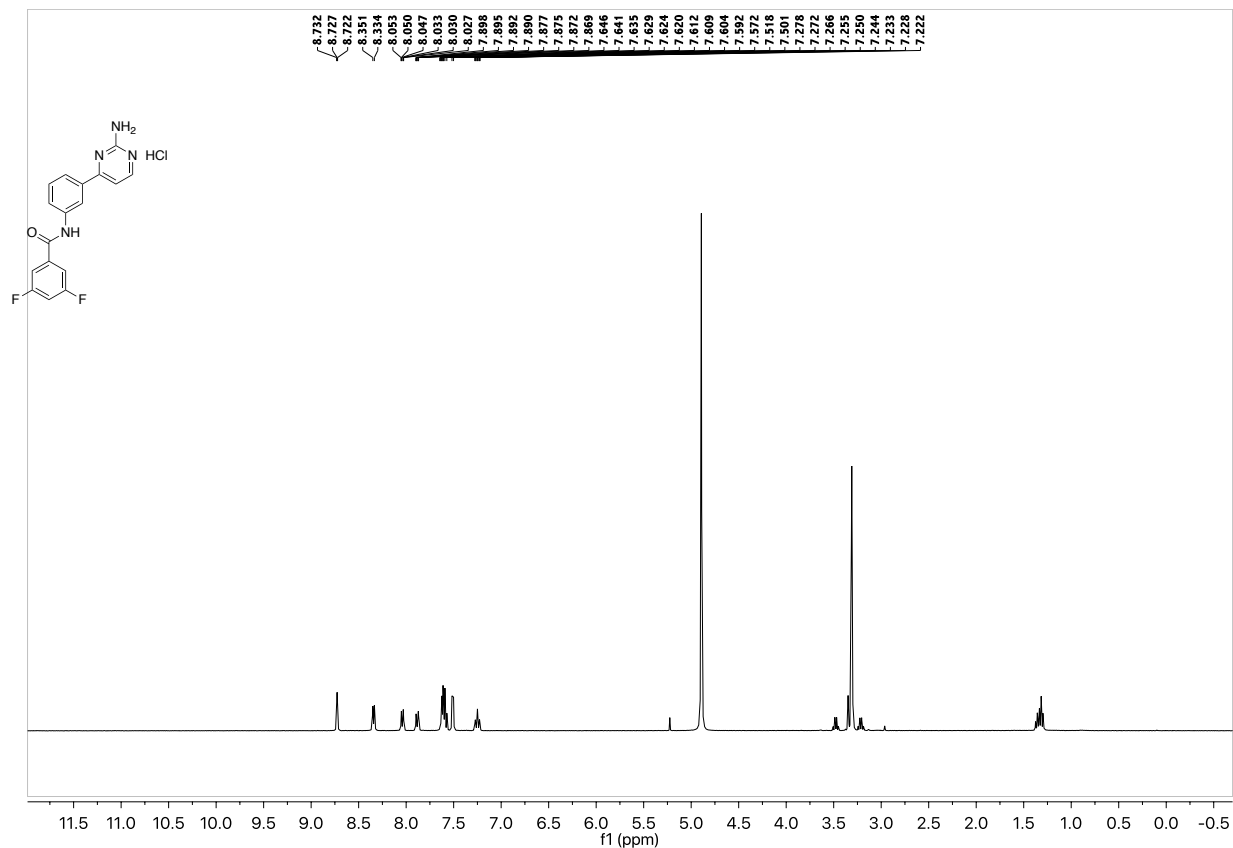
(1.781 mg, 4.28 mmol). Di-*tert*-butyl (5-(2-nitrophenyl)pyrimidin-2-yl)carbamate (1.781 g, 4.28 mmol, 1 eq) was then reduced using the general procedure for reduction of nitro intermediates with palladium on carbon (45.5 mg, 0.43 mmol, 0.1 eq) to yield the crude residue of di-*tert*-butyl (5-(2-aminophenyl)pyrimidin-2-yl)carbamate as a yellow residue in 41 % yield (675 mg, 1.75 mmol). Following the general procedure for acylation of aniline intermediates, di-*tert*-butyl (5-(2-aminophenyl)pyrimidin-2-yl)carbamate (135 mg, 0.35 mmol, 1 eq), 3,5-dibromobenzoyl chloride (104 mg, 0.35 mmol, 1 eq), and potassium phosphate tribasic (92.7 mg, 0.44 mmol, 1.25 eq) afforded di-*tert*-butyl (5-(2-(3,5-dibromobenzamido)phenyl)pyrimidin-2-yl)carbamate as a white solid in 17 % yield (39 mg, 0.06 mmol). Next, di-*tert*-butyl (5-(2-(3,5-dibromobenzamido)phenyl)pyrimidin-2-yl)carbamate was dissolved in DCM (3 mL) and TFA (3 mL) was added dropwise to deprotect the exocyclic nitrogen on the pyrimidine. Upon reaction completion via TLC analysis, DCM and TFA were removed *in vacuo*. The white solid residue was dissolved in MeOH (10 mL) and 12 N HCl (1 mL) was added dropwise to yield the corresponding HCl salt product of *N*-(2-(2-aminopyrimidin-5-yl)phenyl)-3,5-dibromobenzamide hydrochloride. The HCl salt product was washed with cold hexanes (2 mL x 10 of times) to afford compound **15d** as a white solid in 99 % yield (29 mg, 0.06 mmol). ¹H NMR (500 MHz, Methanol-*d*₄) δ 8.54 (s, 2H), 7.99 (d, *J* = 1.7 Hz, 2H), 7.97 (t, *J* = 1.8 Hz, 1H), 7.56 – 7.45 (m, 4H). ¹³C NMR (126 MHz, Methanol-*d*₄) δ 156.3, 149.3, 148.1, 129.1, 128.9, 126.4, 122.5, 121.8, 121.4, 121.2, 119.6, 119.3, 114.8, 114.4. UV (λ_{max} nm): 216; Melting point (°C): decay > 142; HRMS (ESI): calcd for C₁₇H₁₃Br₂N₄O [M+H]⁺: 446.9451, found: 446.9427.

N-(3-(2-aminopyrimidin-4-yl)phenyl)-3-bromo-5-chlorobenzamide hydrochloride (17): A mixture of compound **7b** (50 mg, 0.27 mmol), 3-bromo-5-chlorobenzoic acid (**16**) (95 mg, 0.40 mmol), DMAP (16 mg, 0.13 mmol), and EDC (310 mg, 1.6 mmol) were added to a flame dried round bottom flask and refluxed overnight in DCM (4.5 mL) under argon. The reaction mixture was then diluted with DCM (20 mL) then washed with 1M HCl (10 mL x 3 times) followed by saturated sodium bicarbonate (10 mL x 3 times) and brine (10 mL x 3 times). The organic layer was then dried of sodium sulfate, filtered, concentrated and then purified using silica gel chromatography. The white solid was then dissolved in MeOH (10 mL) and 12 N HCl (1 mL) was added dropwise to yield the corresponding HCl salt product *N*-(3-(2-aminopyrimidin-4-yl)phenyl)-3-bromo-5-chlorobenzamide hydrochloride. The HCl salt product was washed with cold hexanes (2 mL x 10 of times) to afford compound **17** as a yellow solid in 30 % yield (35 mg, 0.08 mmol). ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.59 (s, 1H), 8.44 (s, 1H), 8.34 (d, *J* = 5.1 Hz, 1H), 8.16 (s, 1H), 8.04 (d, *J* = 17.8 Hz, 2H), 7.91 (d, *J* = 8.1 Hz, 1H), 7.81 (d, *J* = 7.9 Hz, 1H), 7.50 (t, *J* = 8.0 Hz, 1H), 7.07 (d, *J* = 5.2 Hz, 1H), 6.69 (s, 2H). ¹³C NMR (126 MHz, MeOD) δ 172.13, 164.42, 156.14, 146.75, 139.46, 138.09, 135.48, 135.12, 134.23, 129.66, 129.20, 126.71, 125.81, 124.68, 122.86, 120.78, 106.41. UV (λ_{max} nm): 208; Melting point (°C): decay > 165; HRMS (ESI): calcd for C₁₇H₁₃BrClN₄O [M+H]⁺: 402.9956 found: 402.9964.

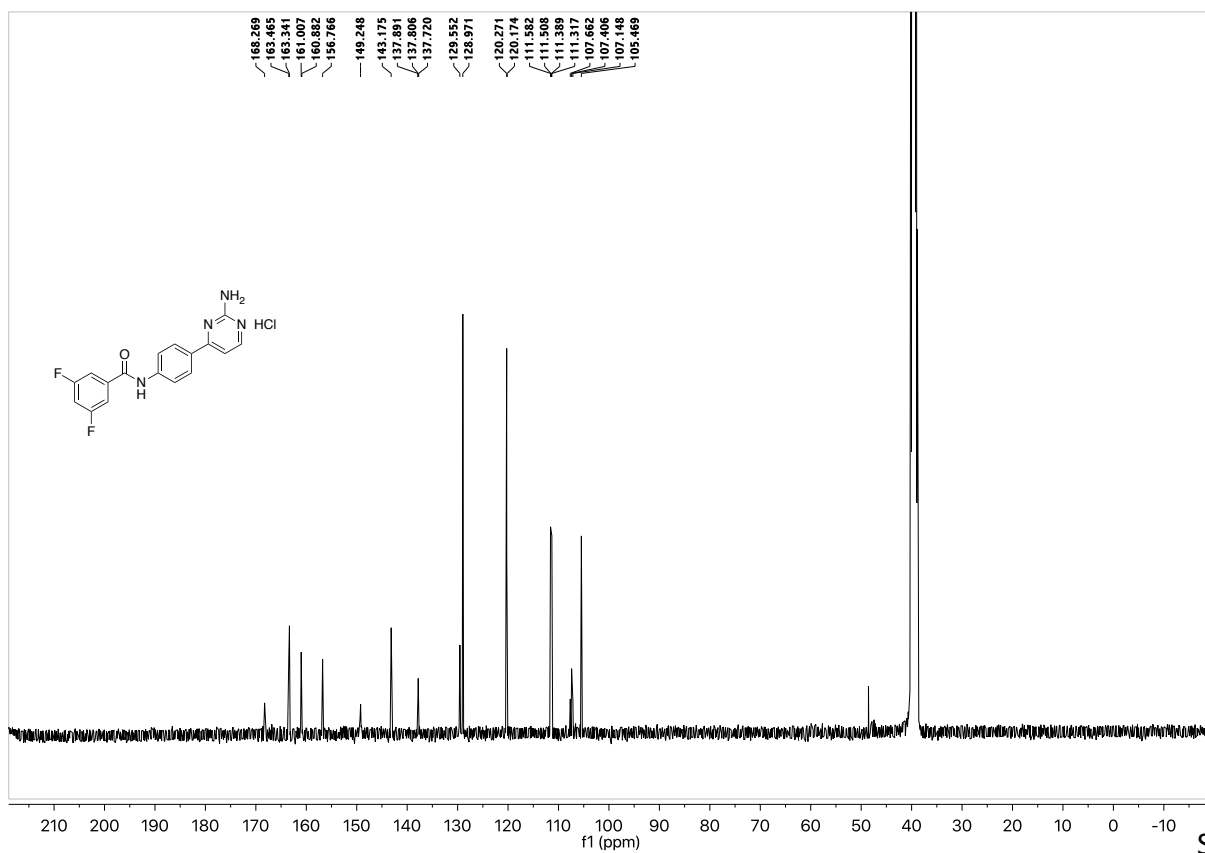
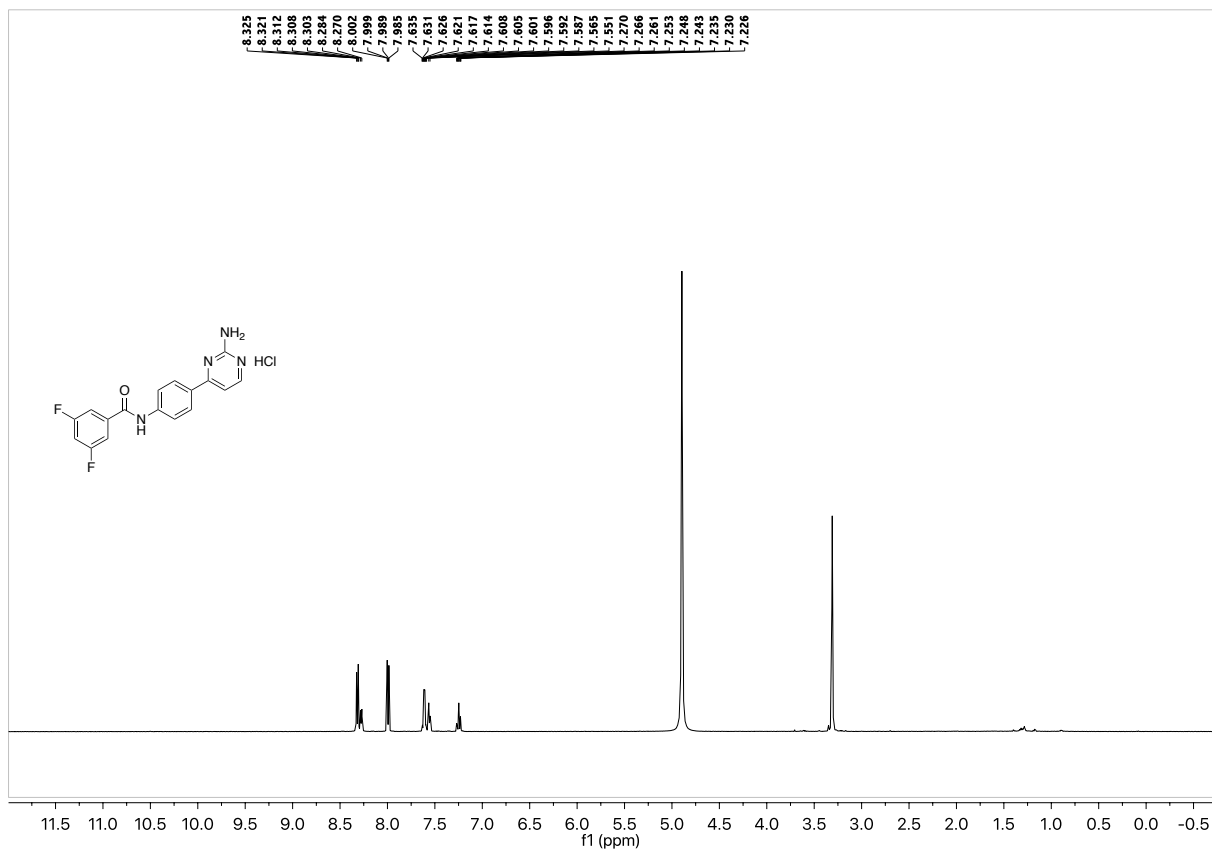
Compound 8a



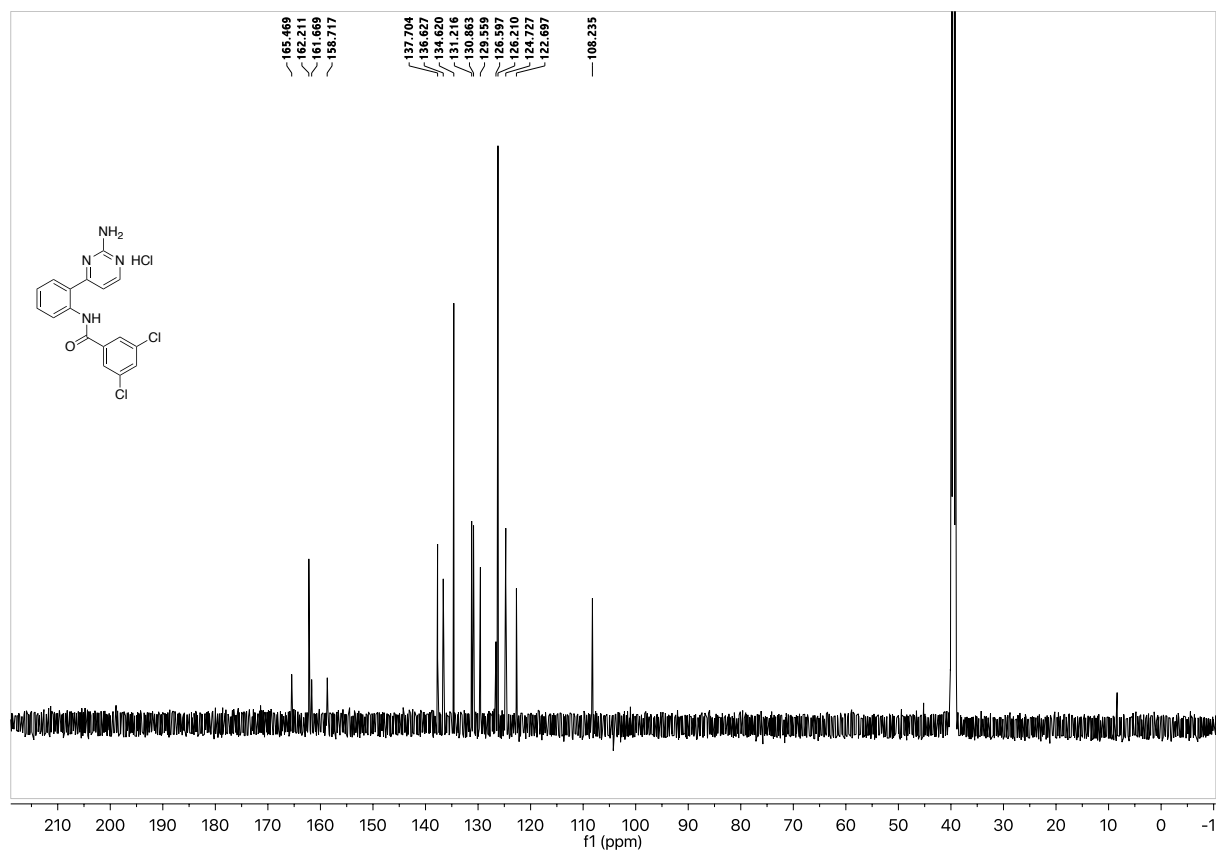
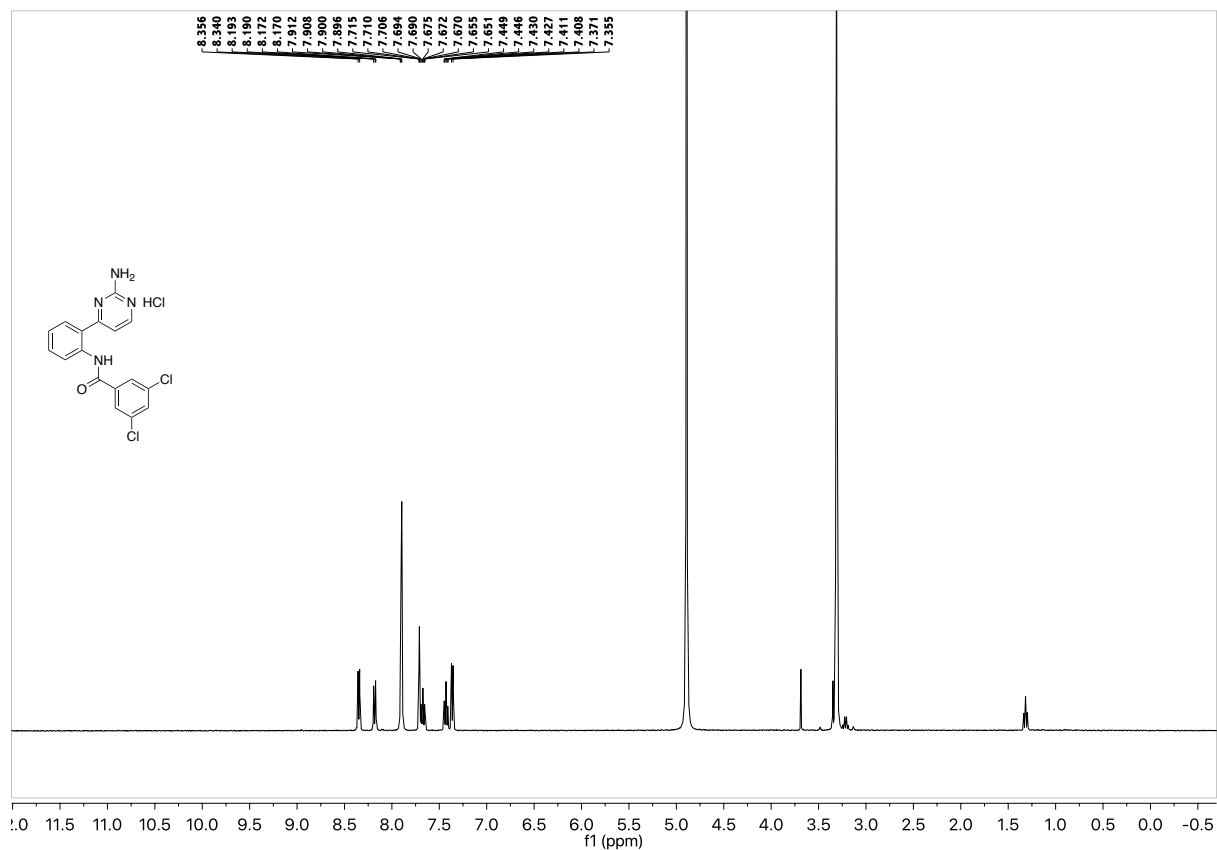
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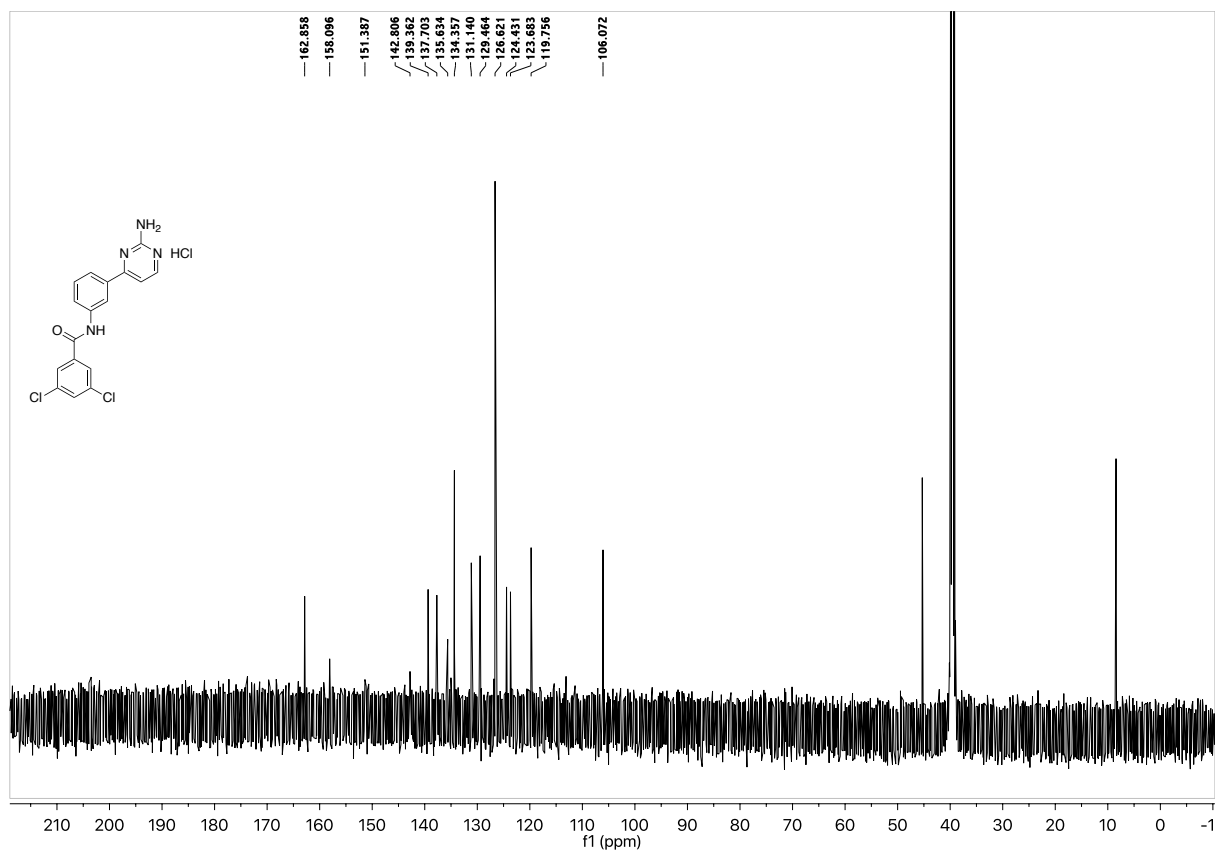
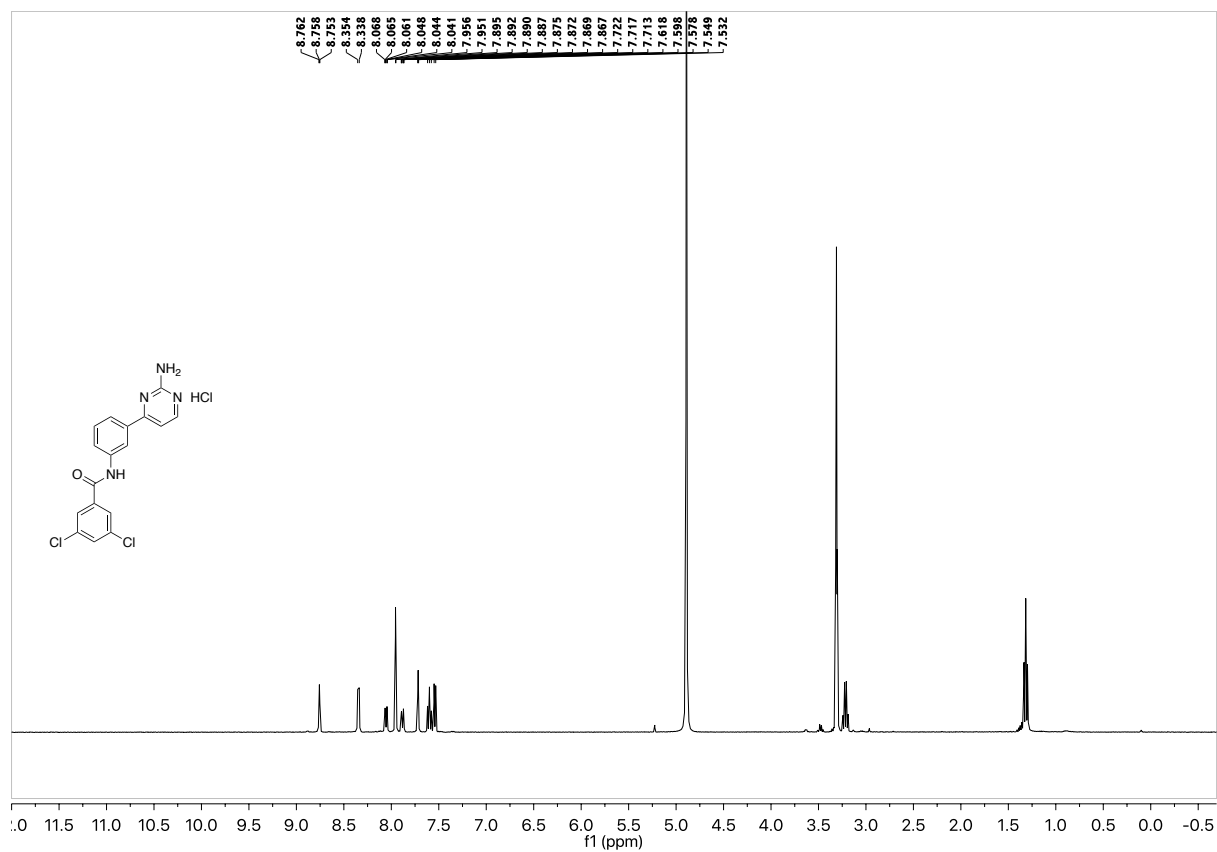
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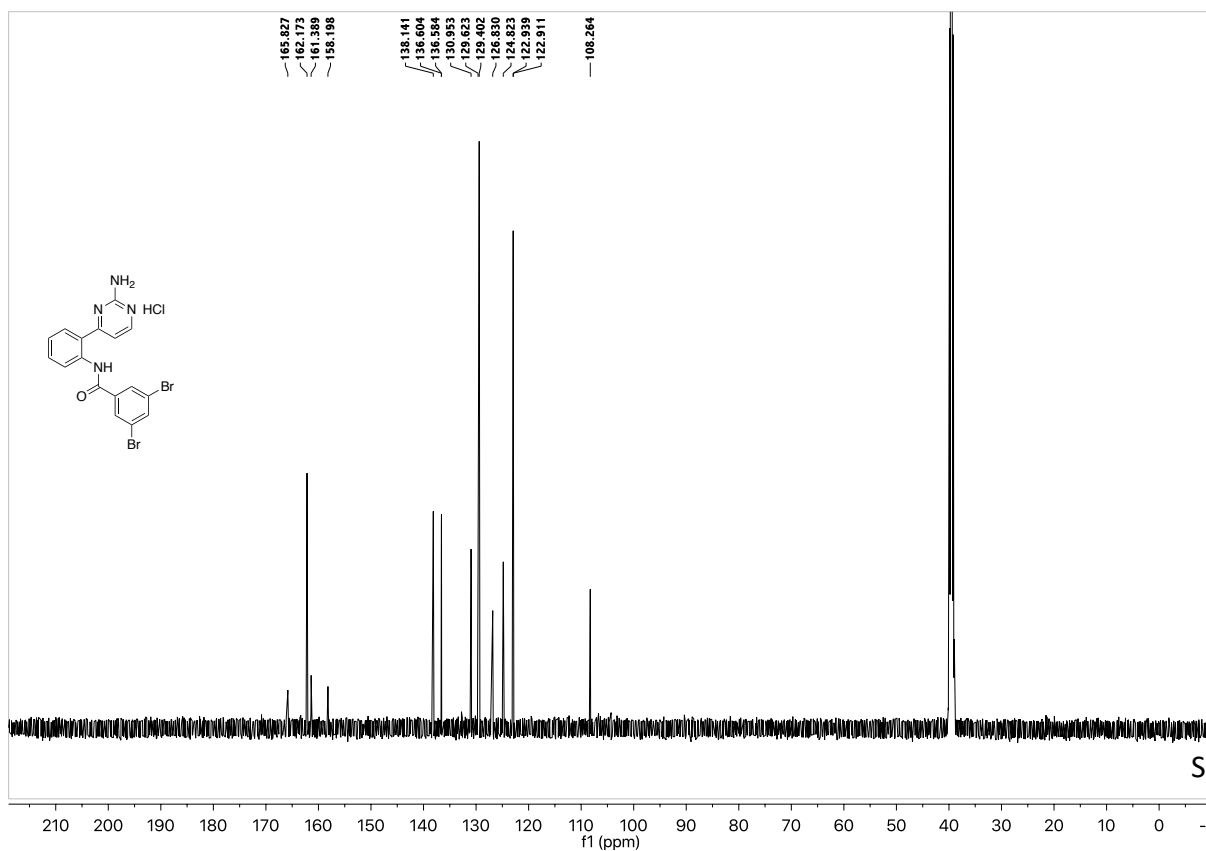
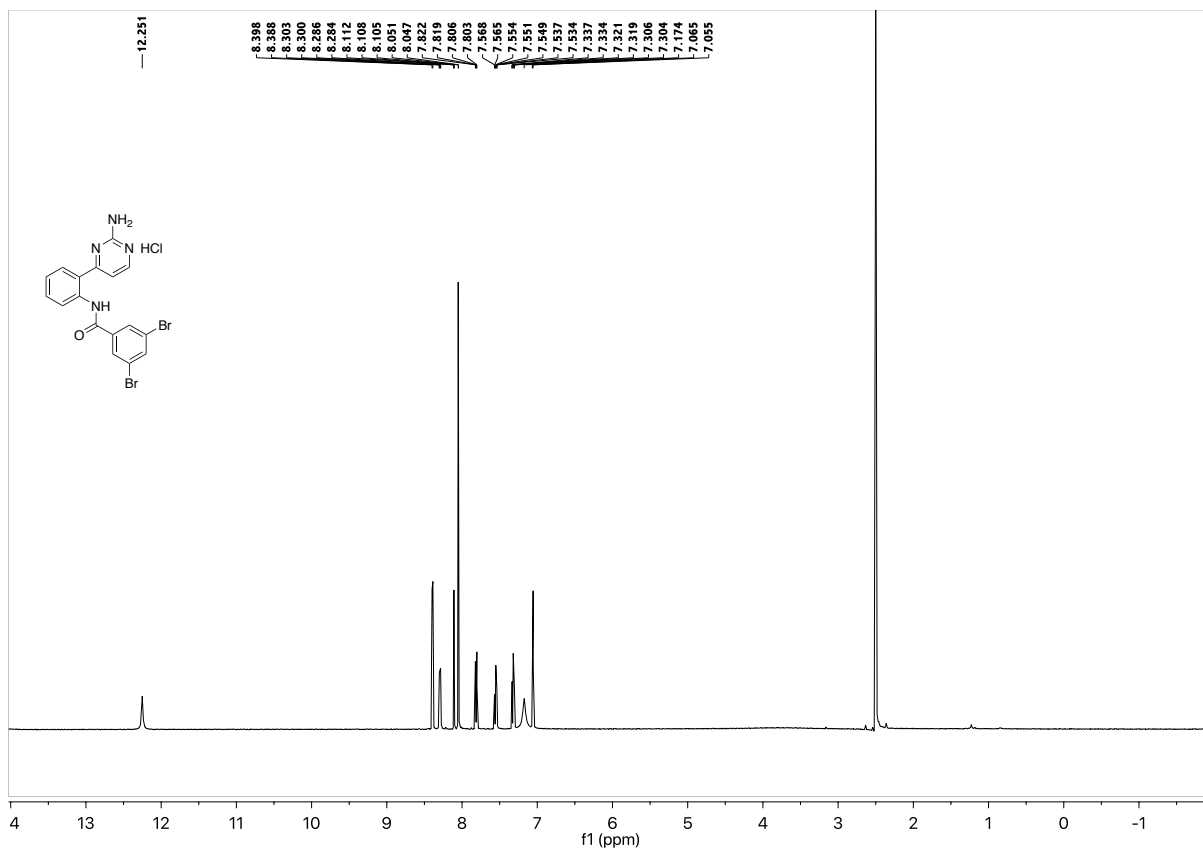
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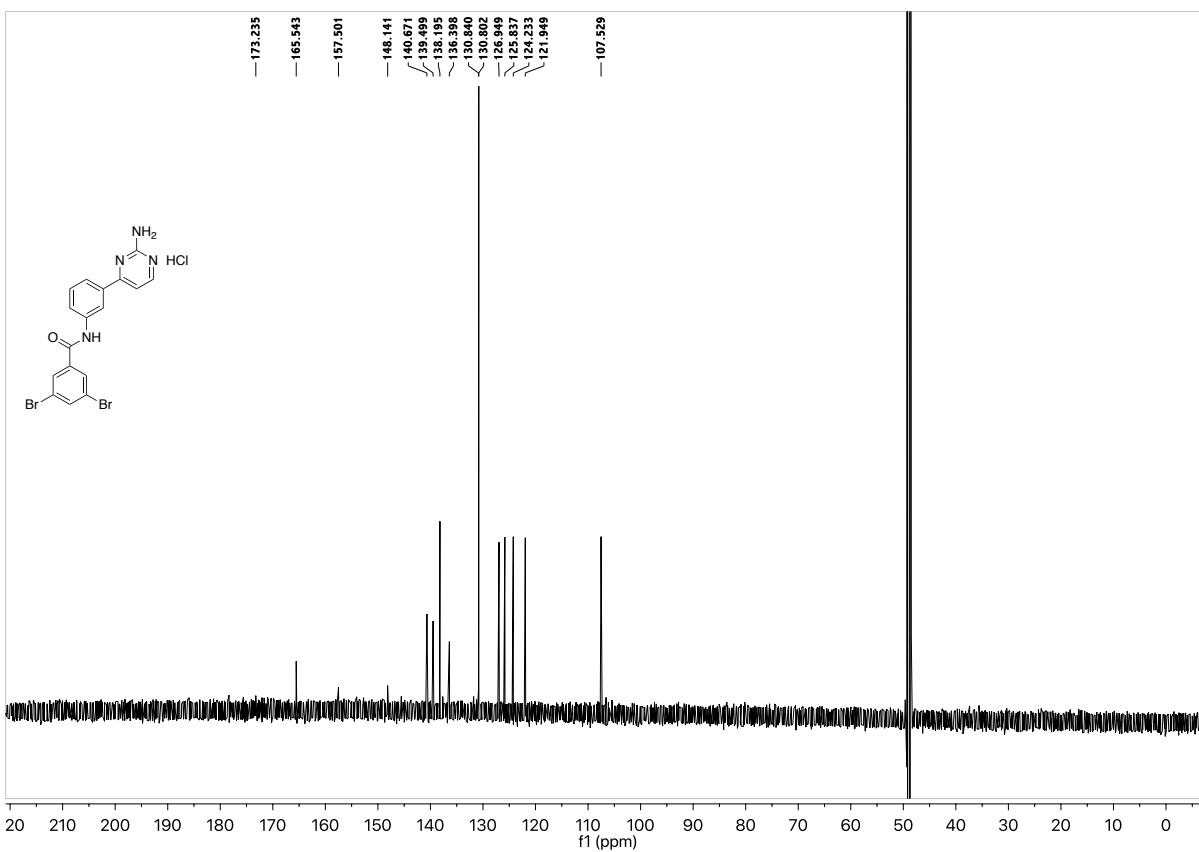
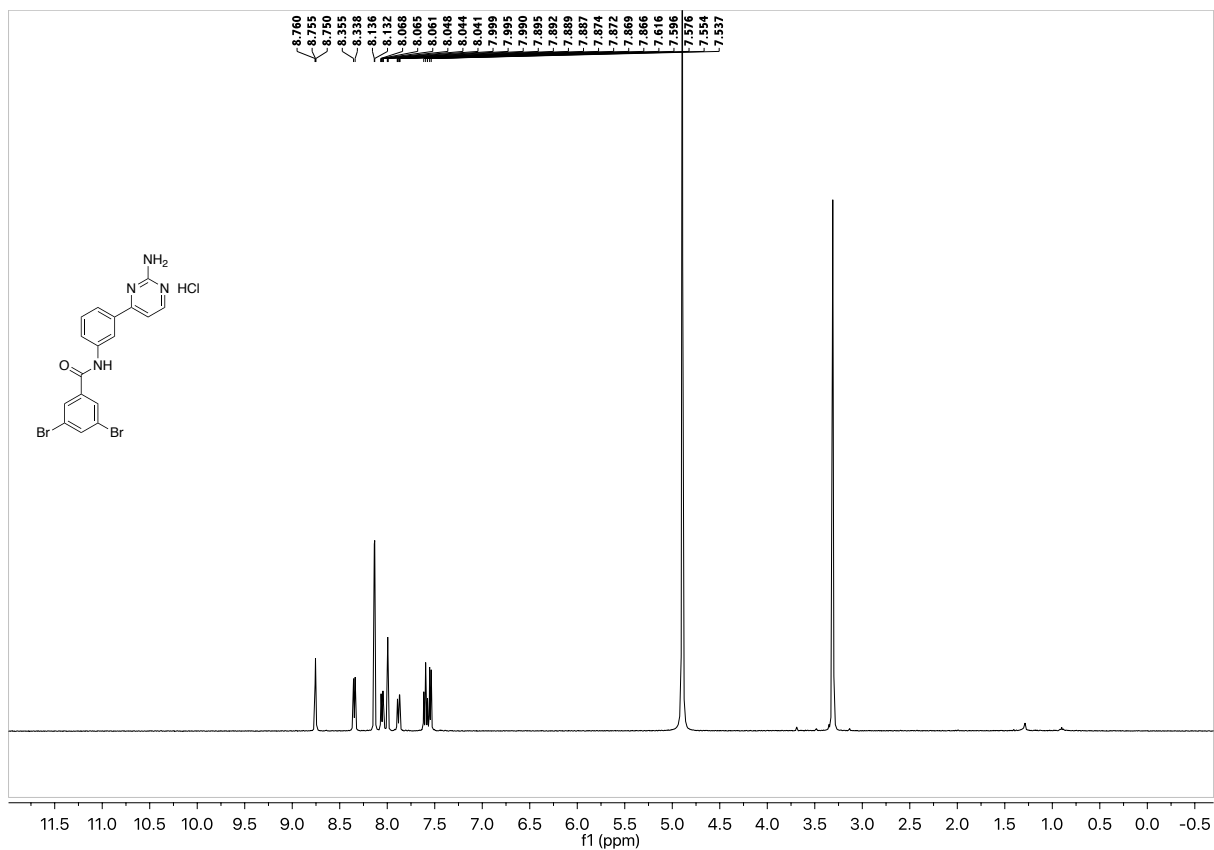
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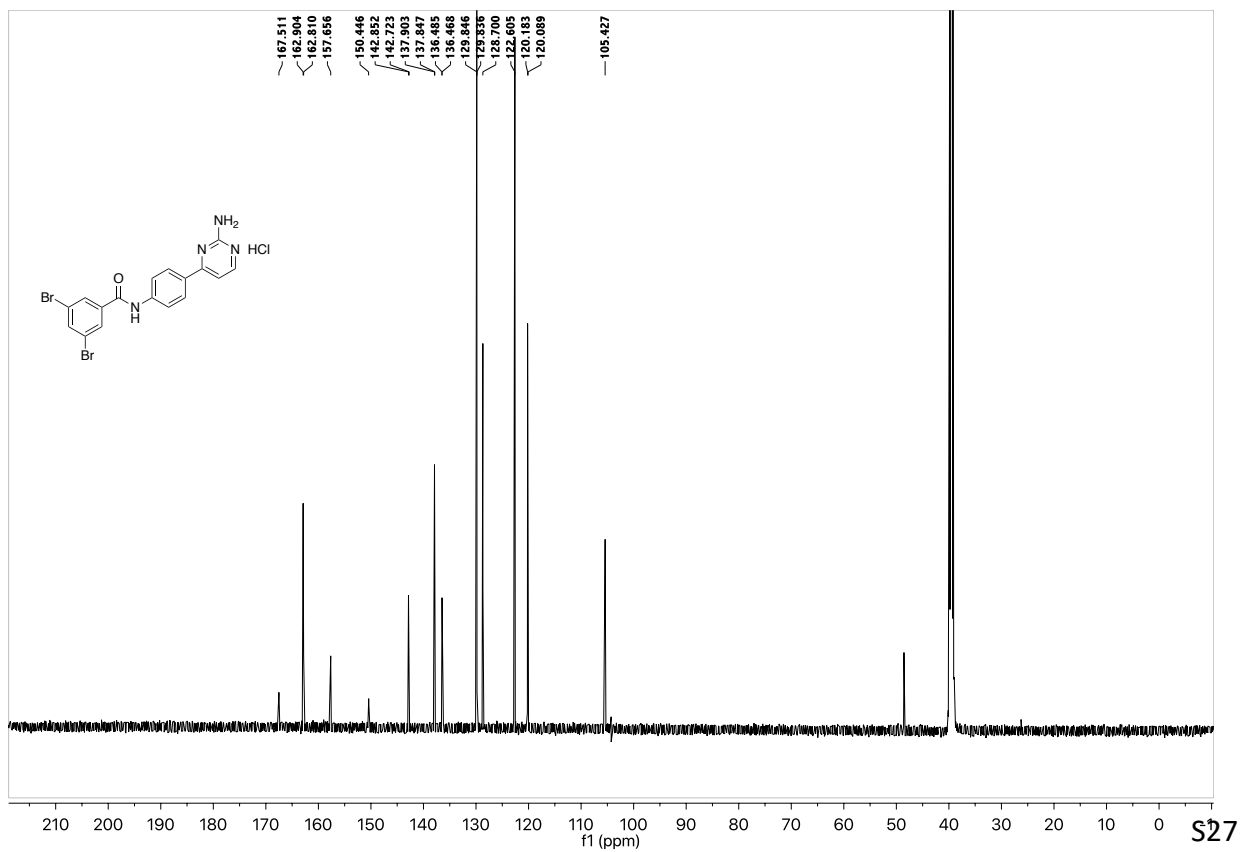
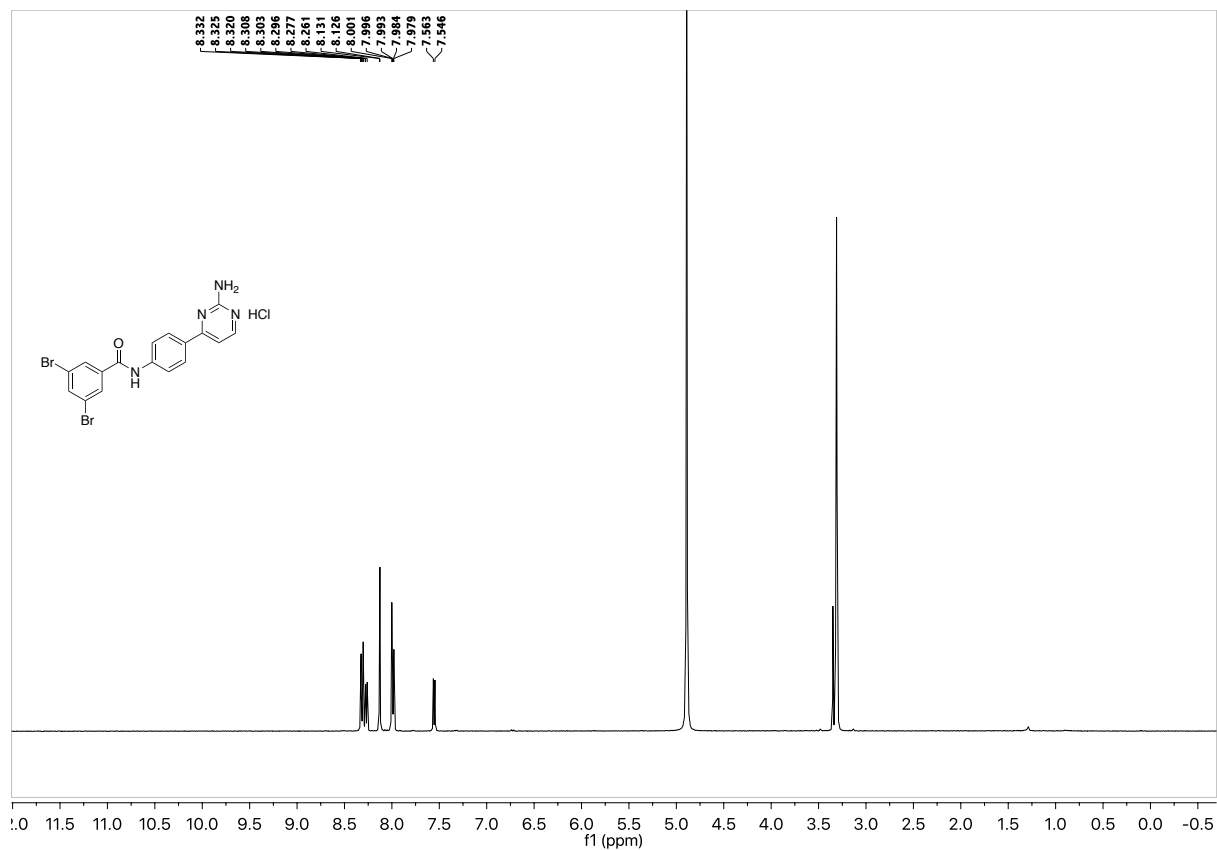
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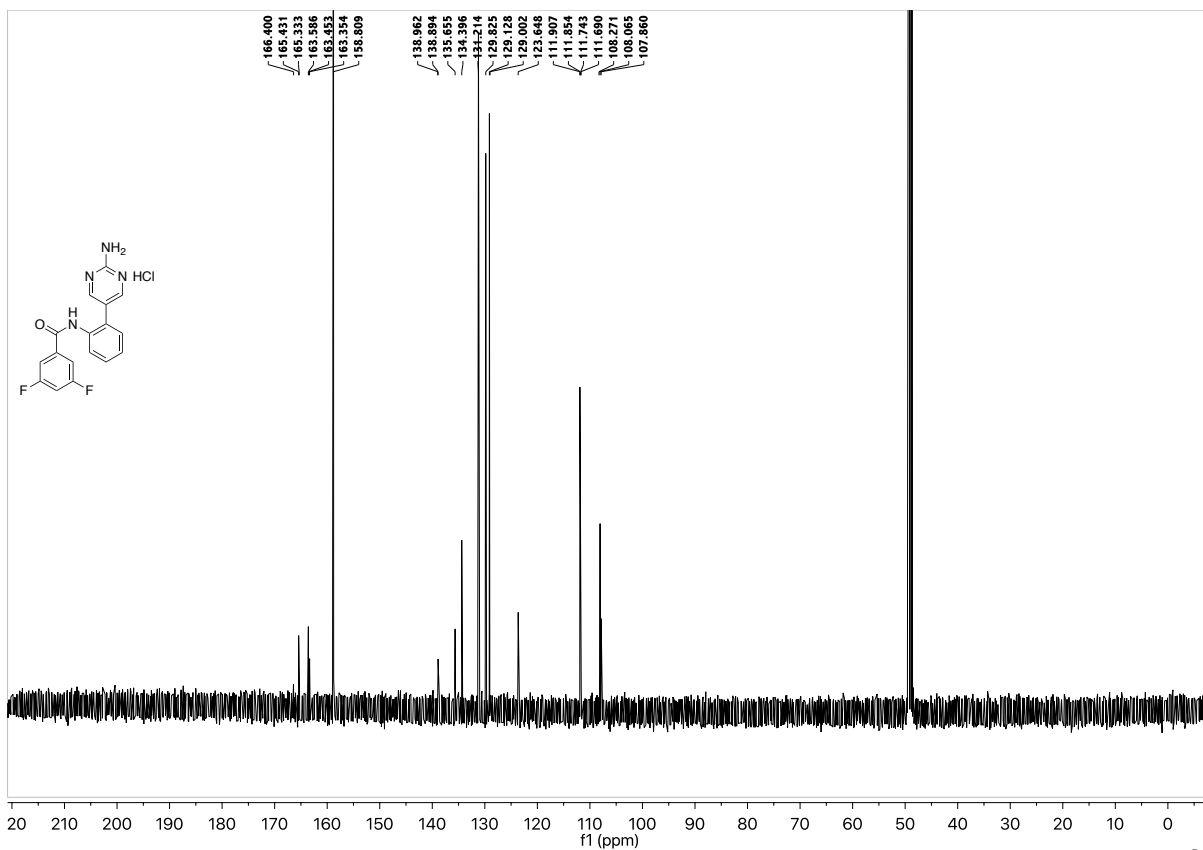
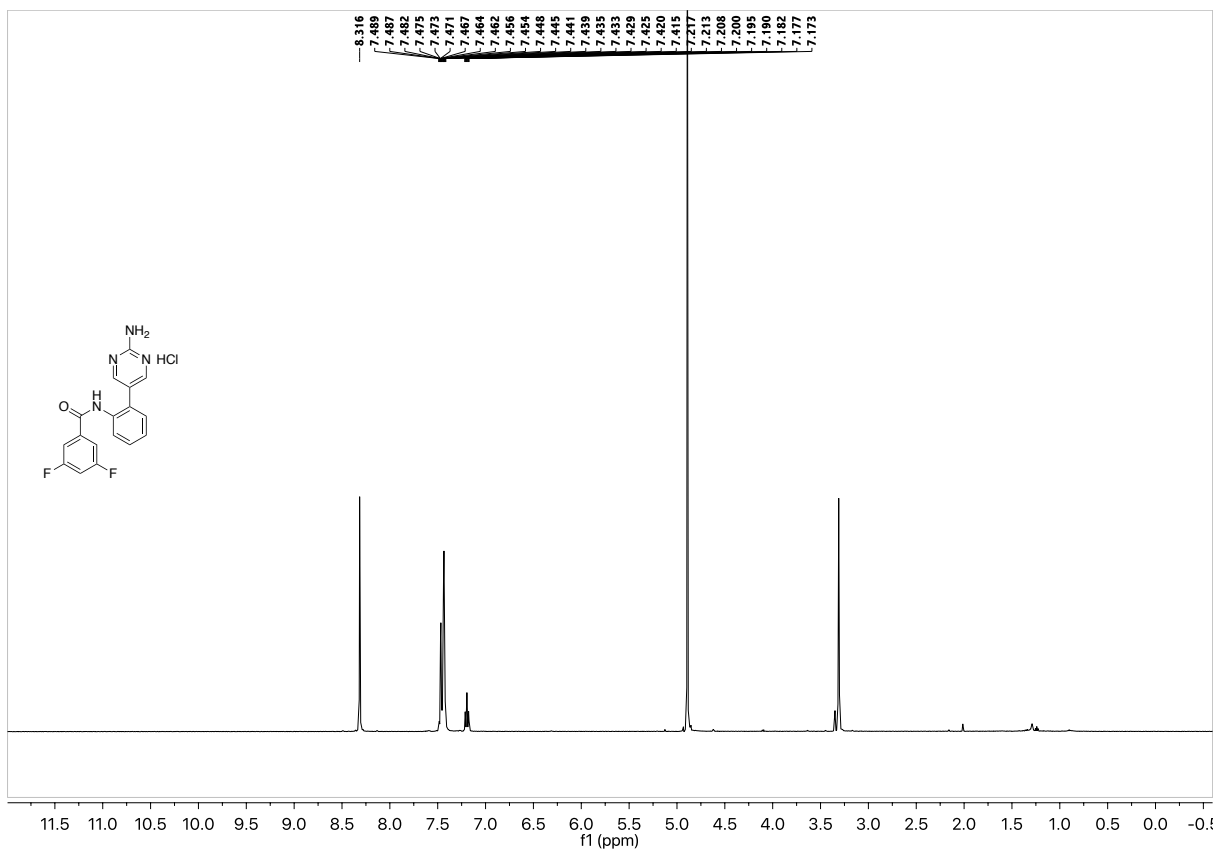
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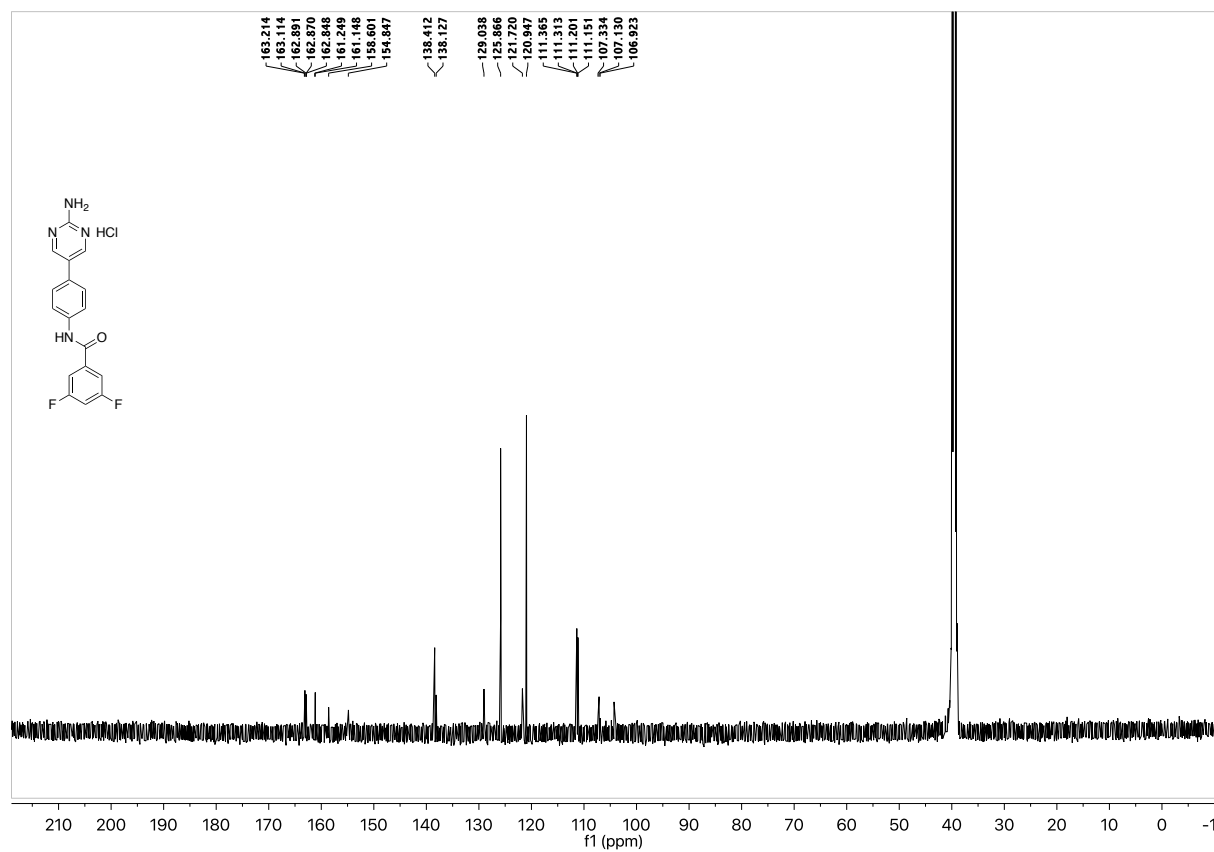
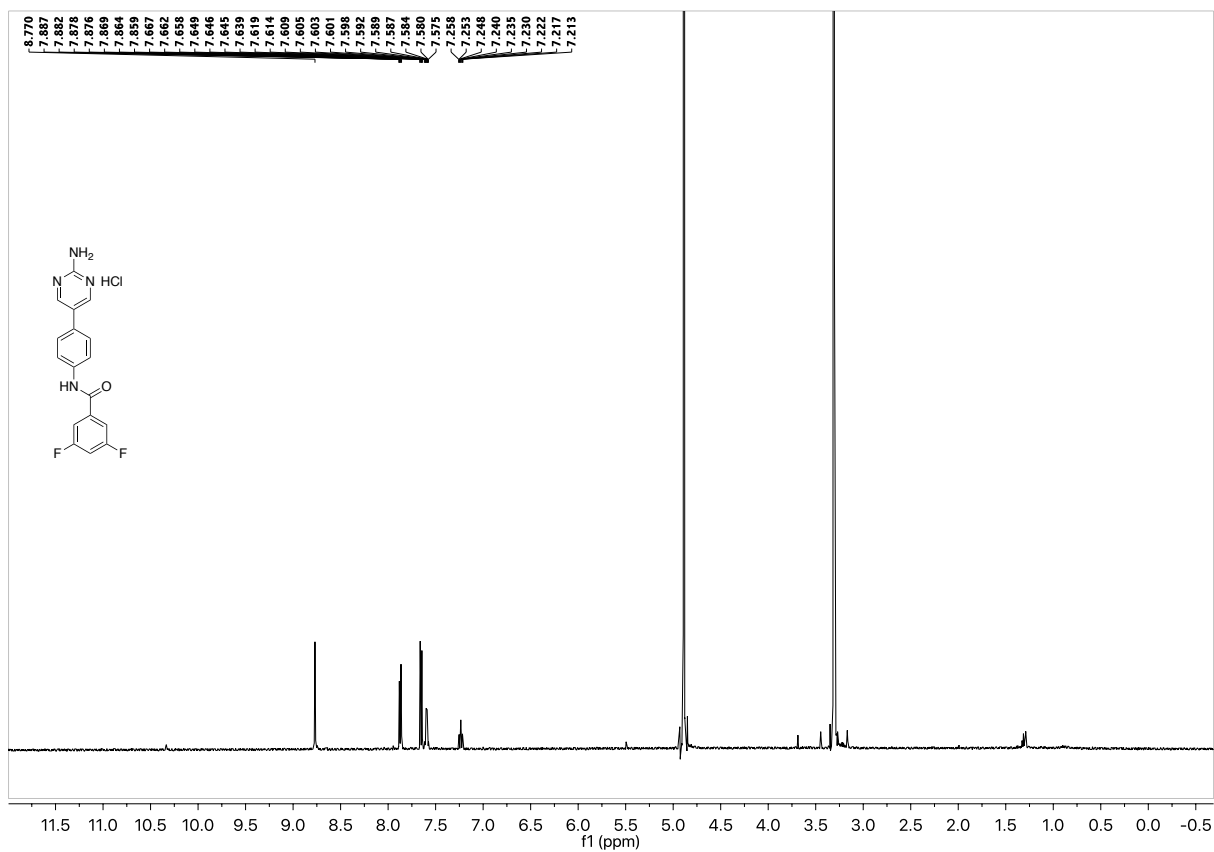
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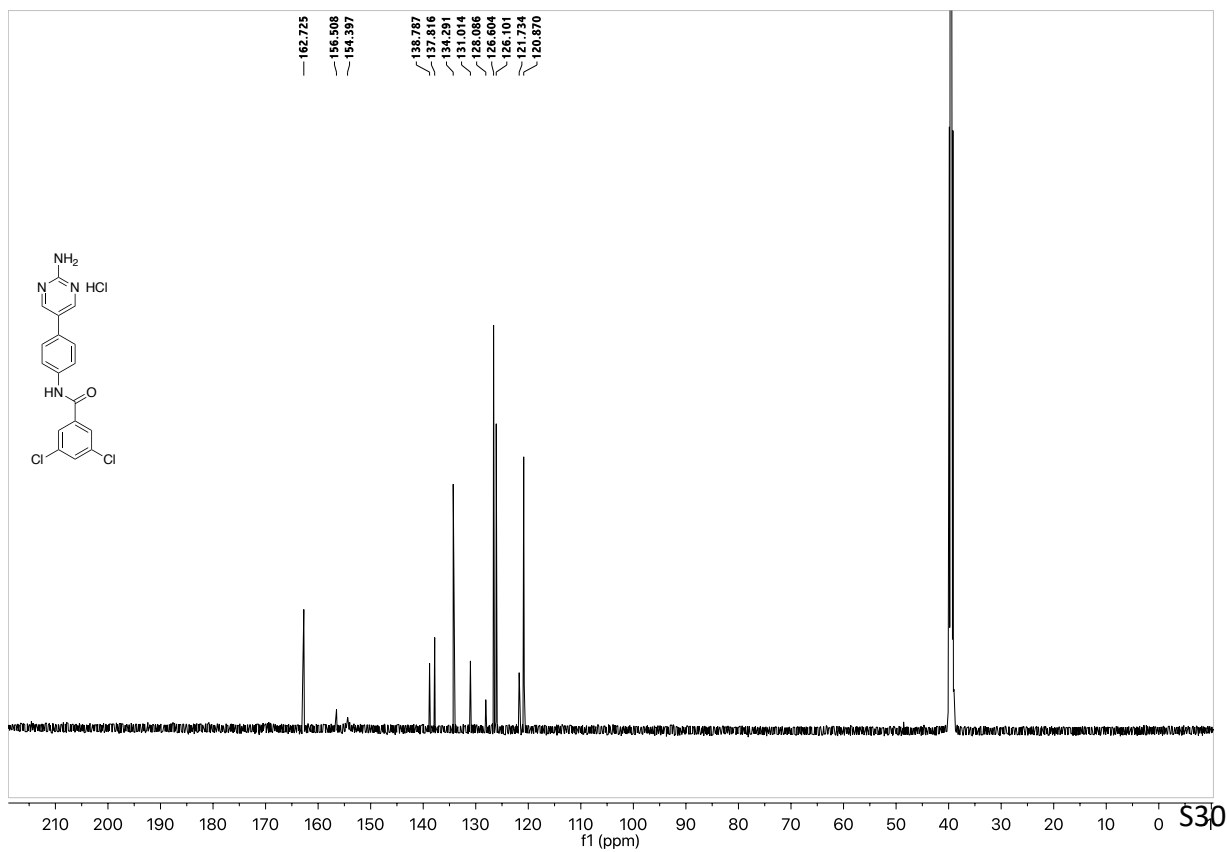
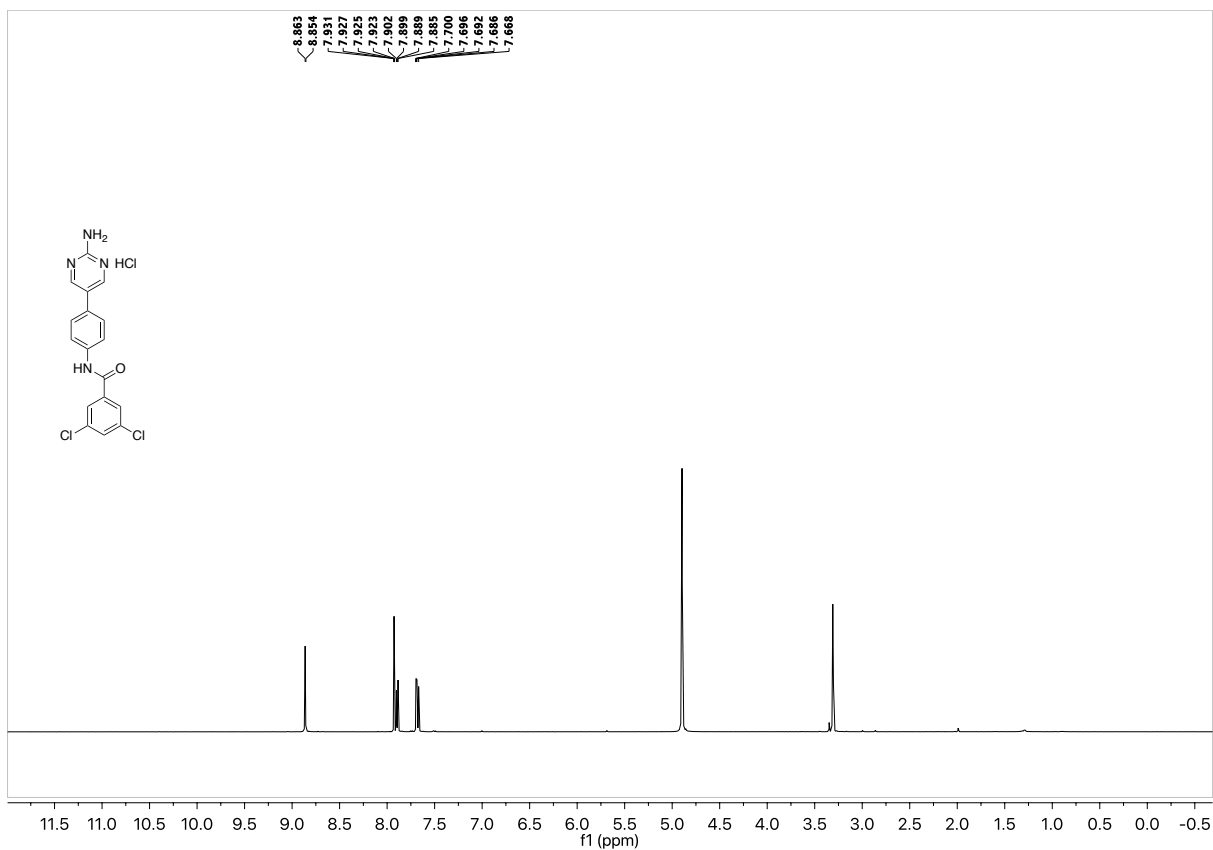
Compound 13a



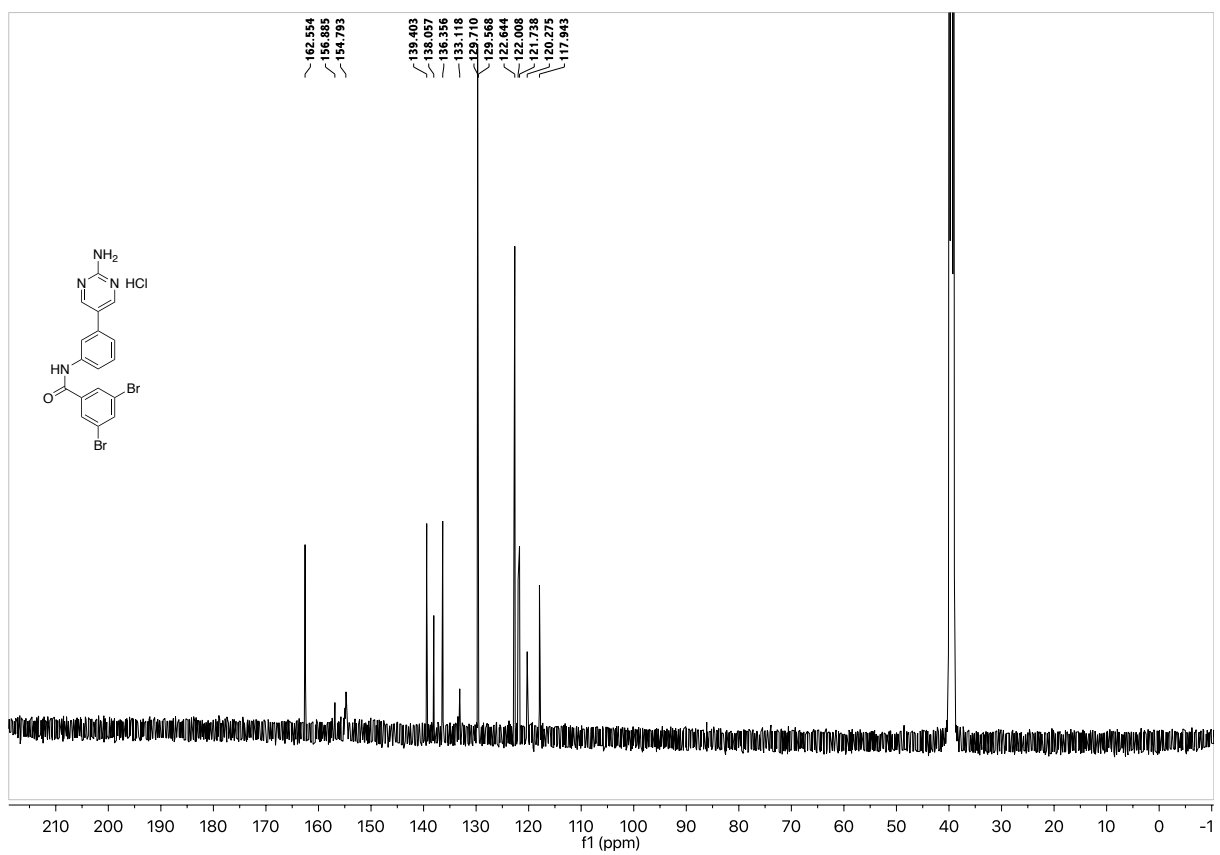
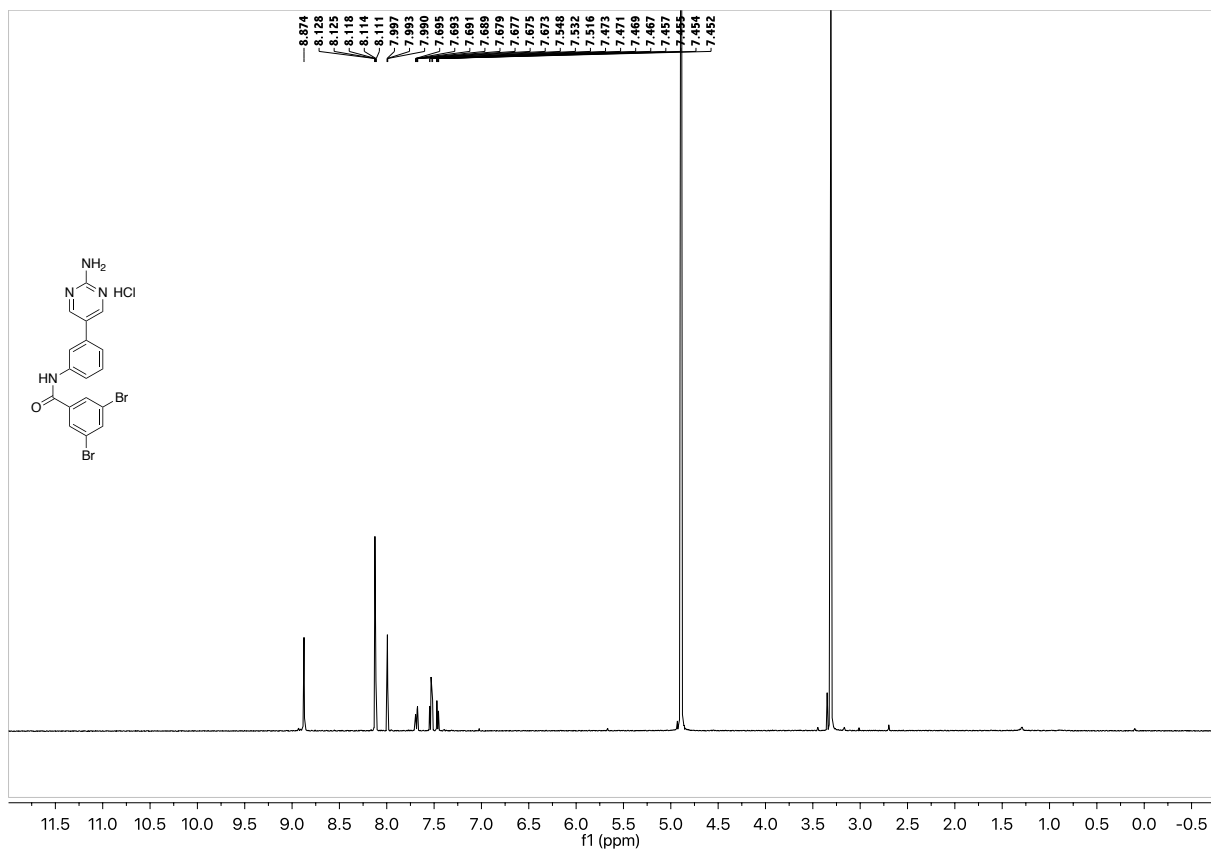
Compound 13b



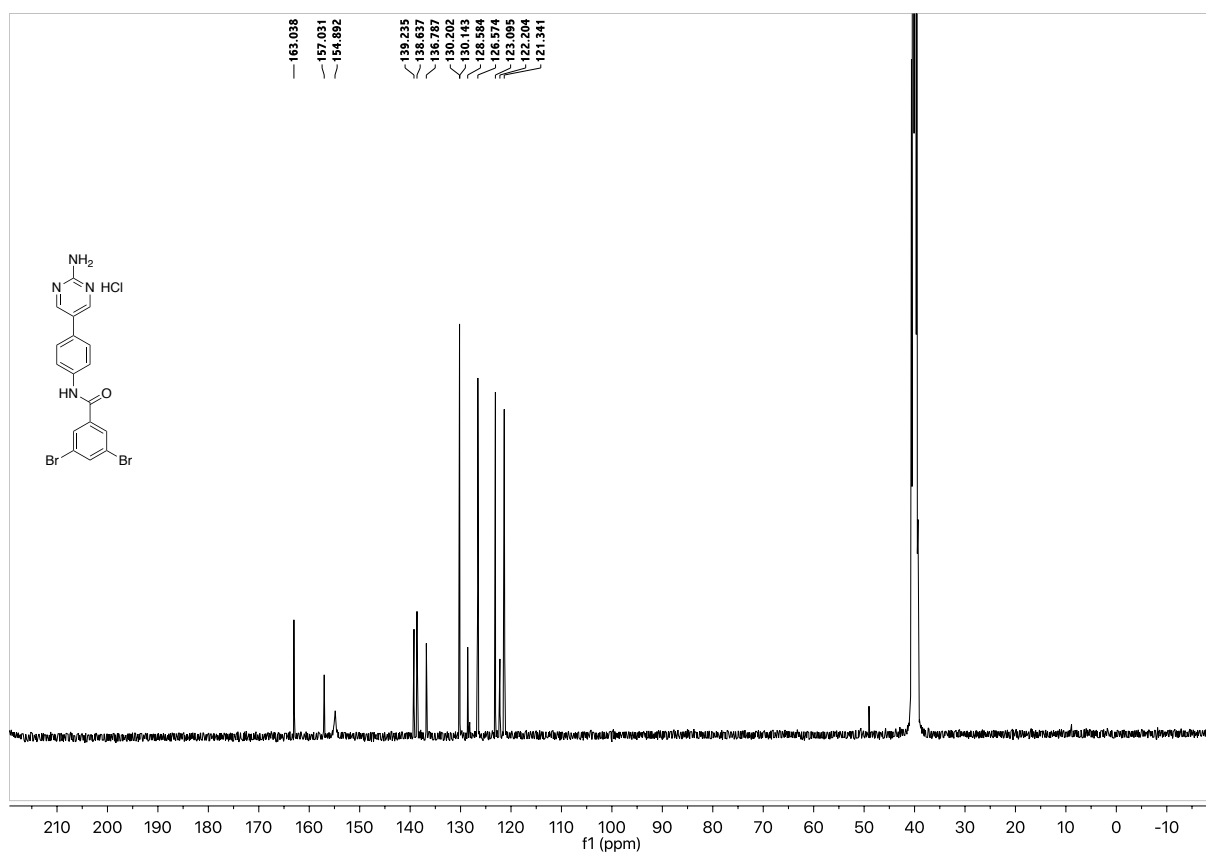
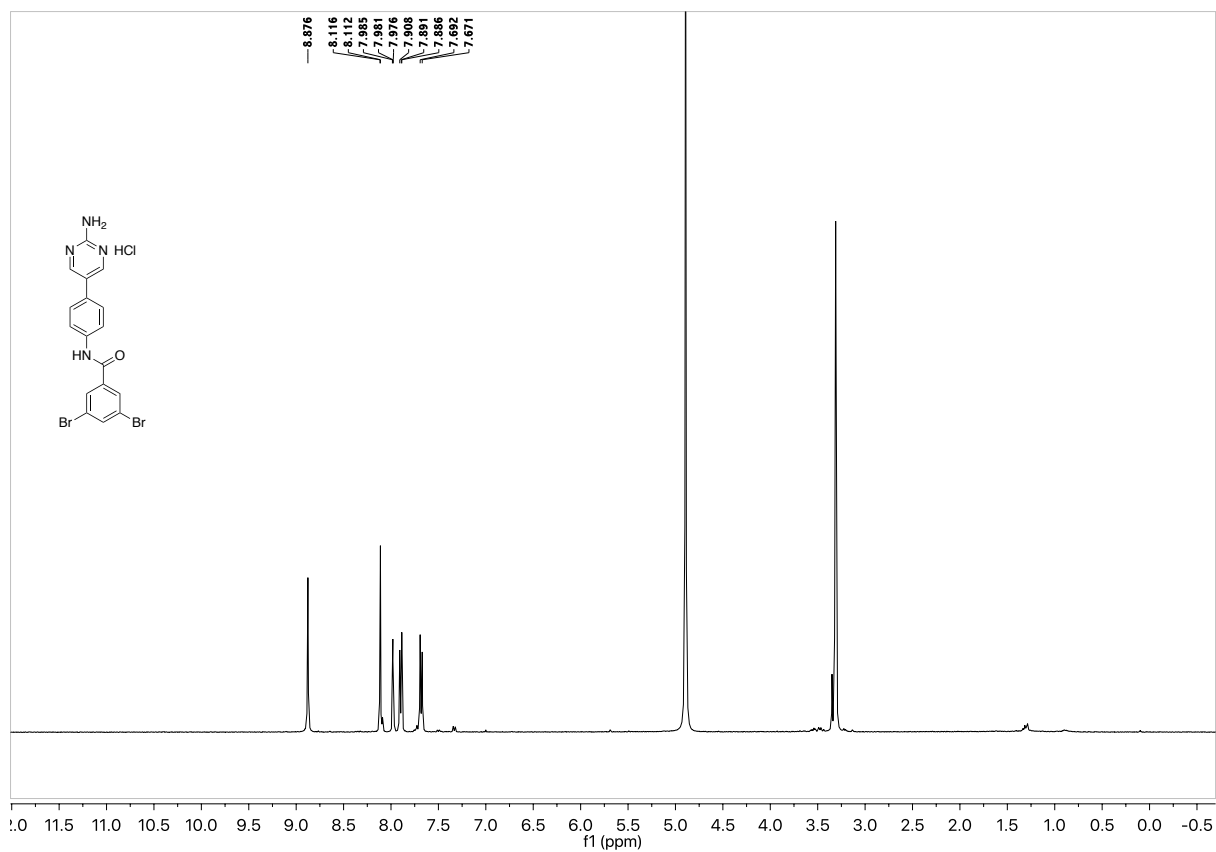
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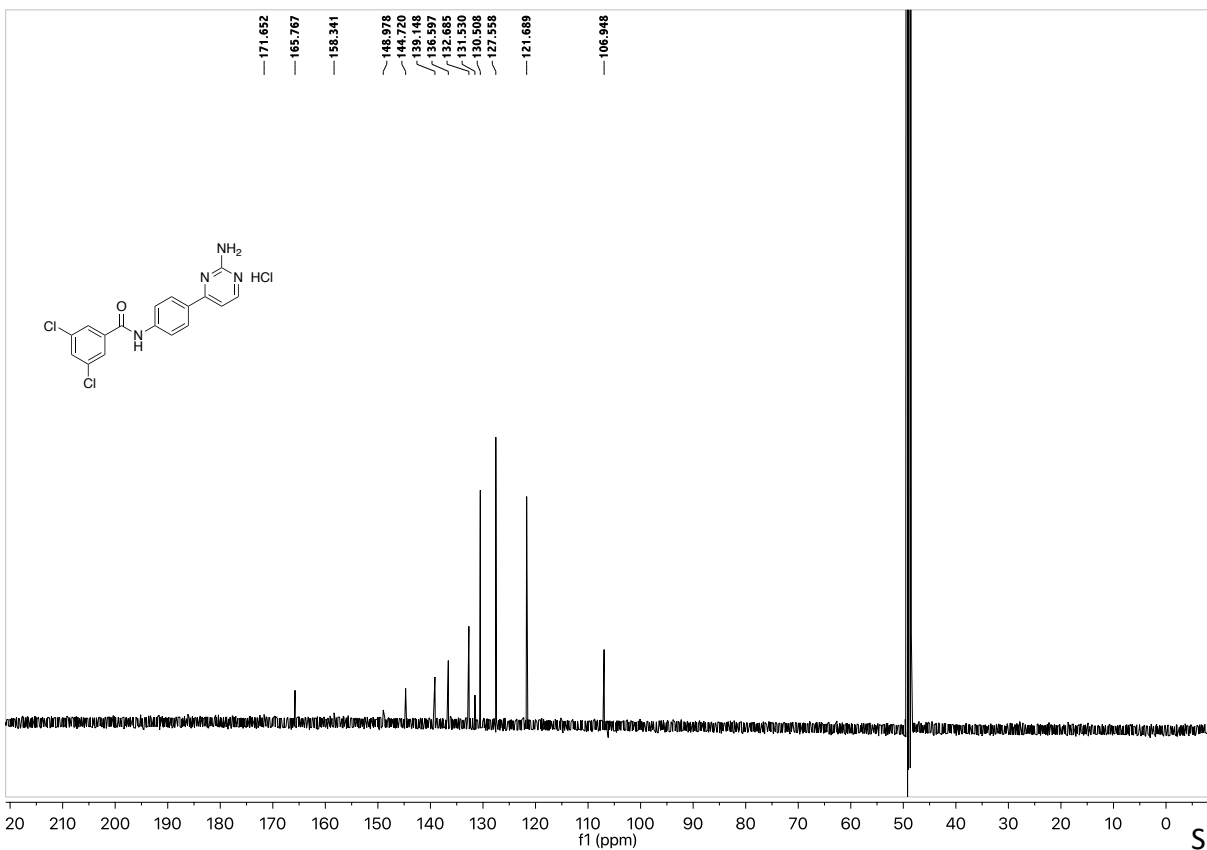
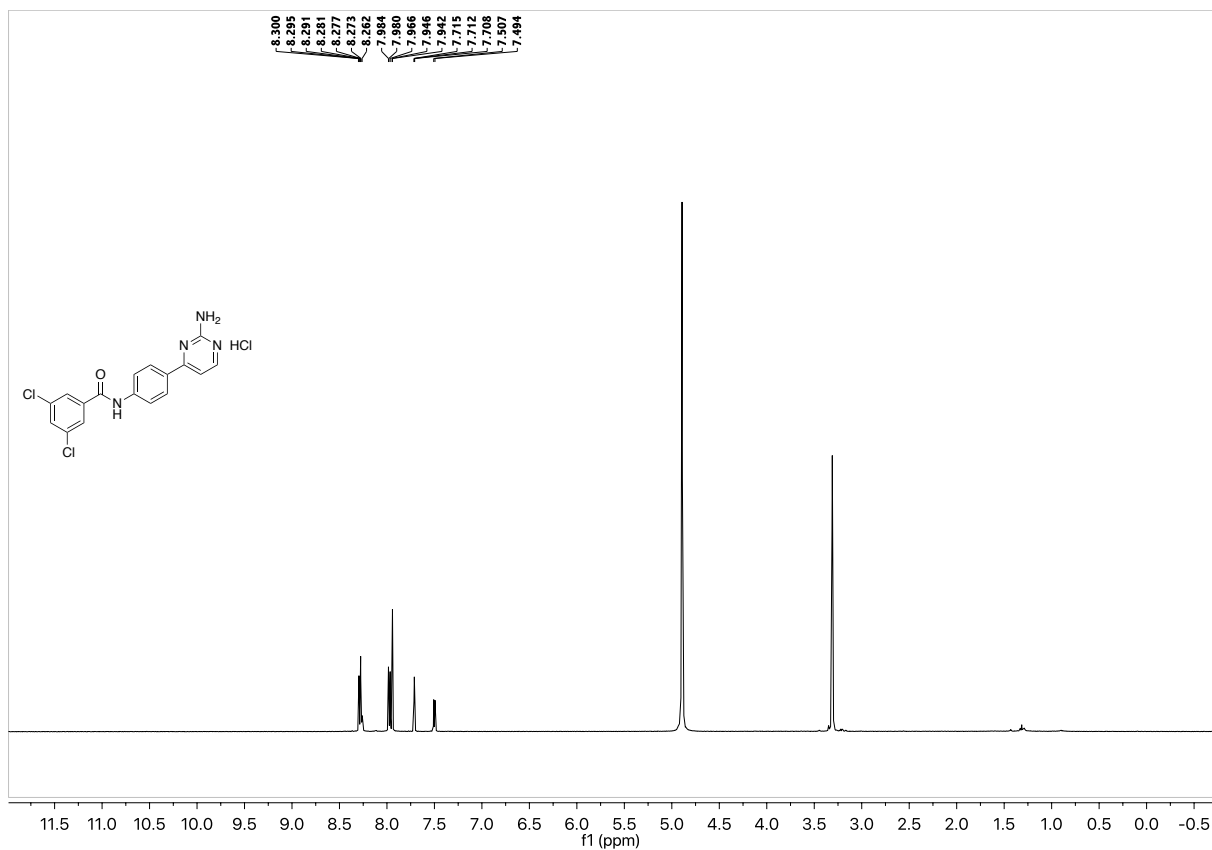
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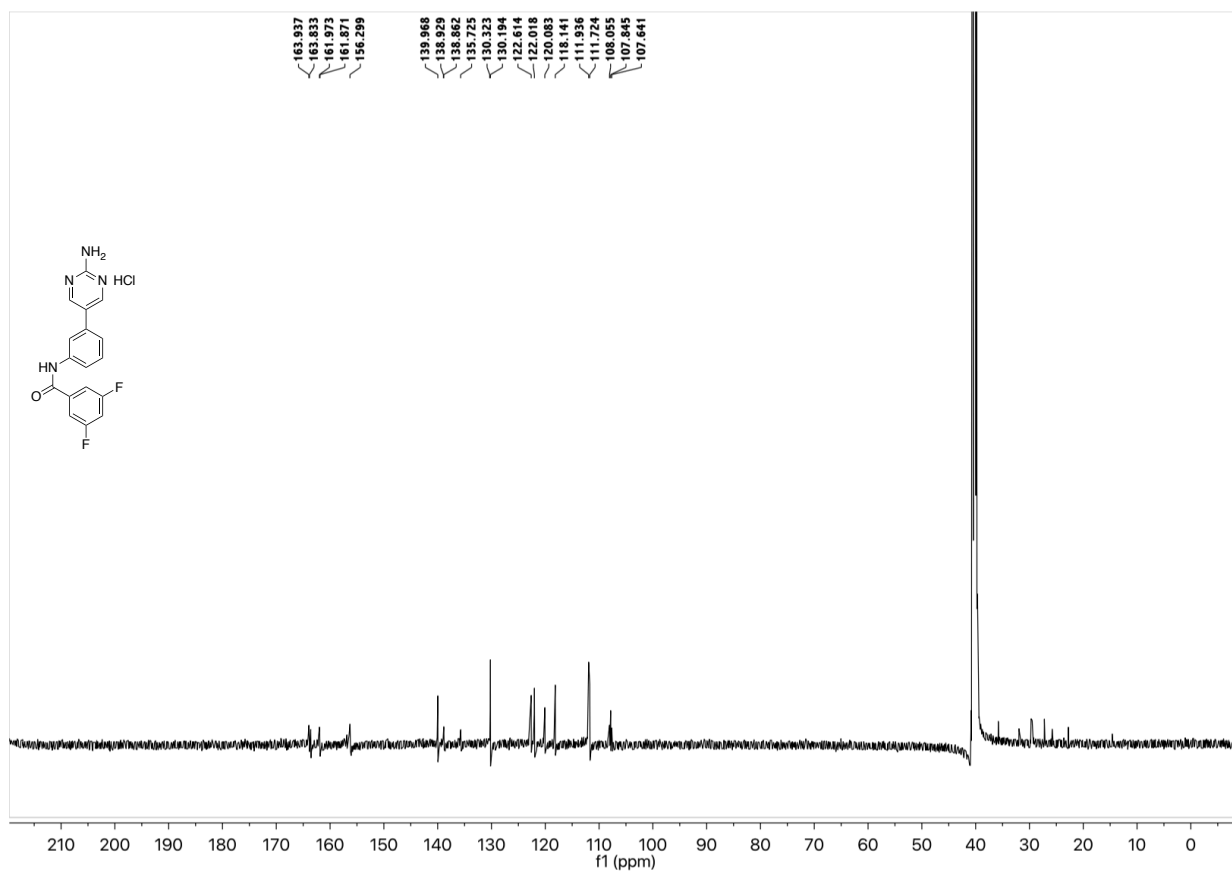
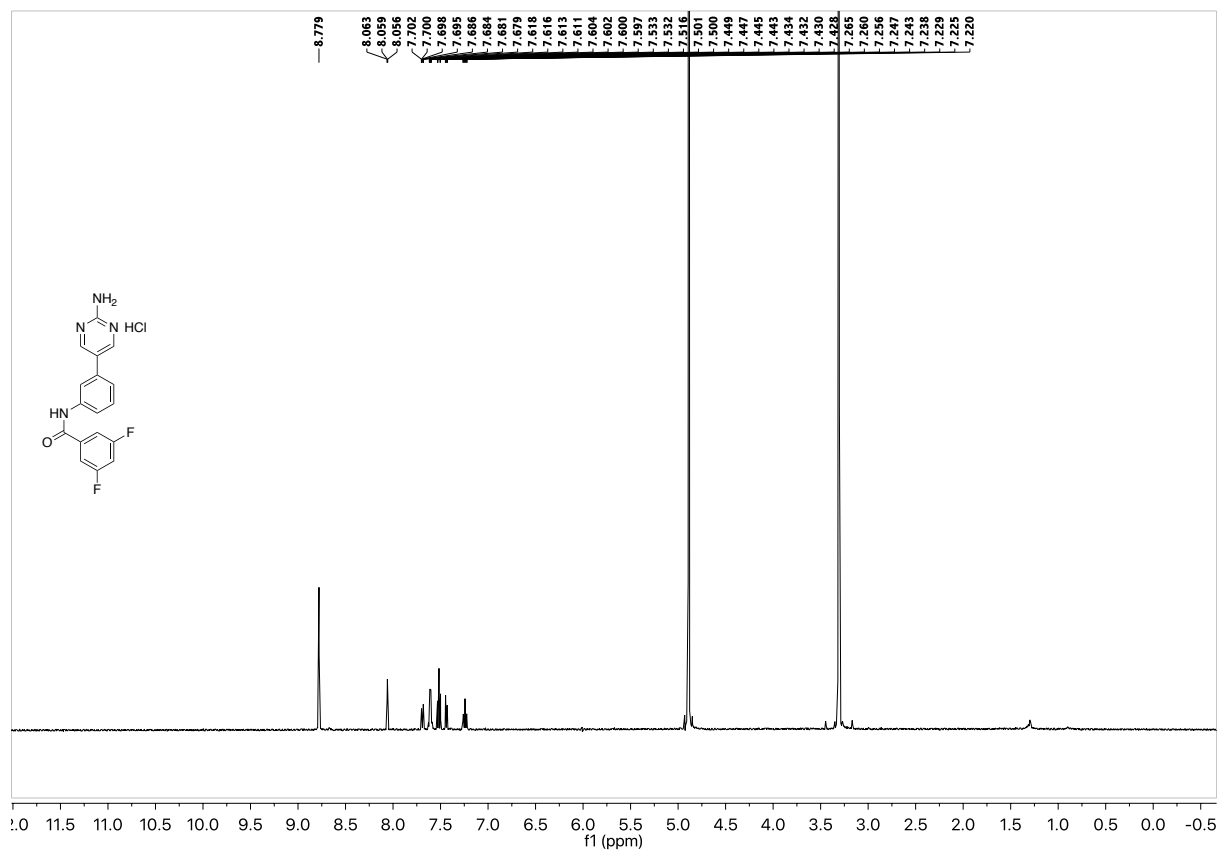
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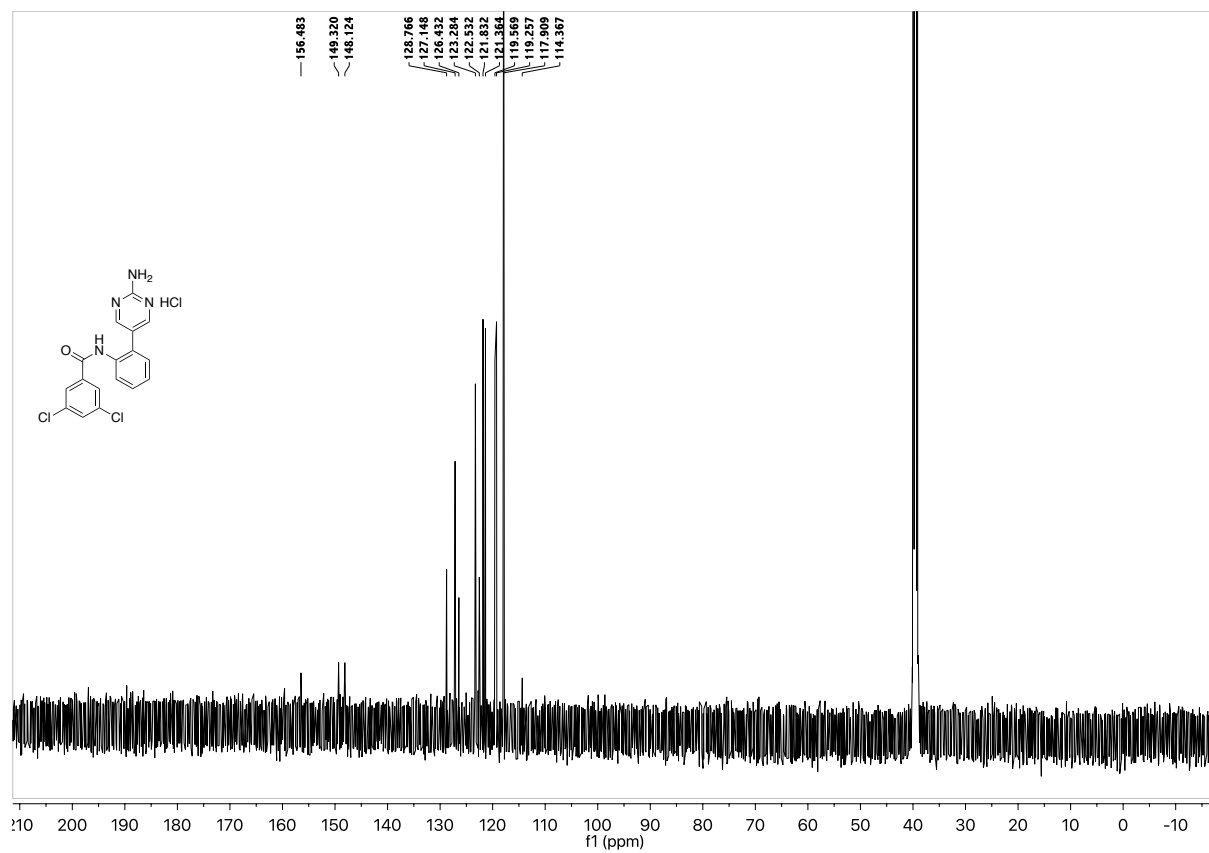
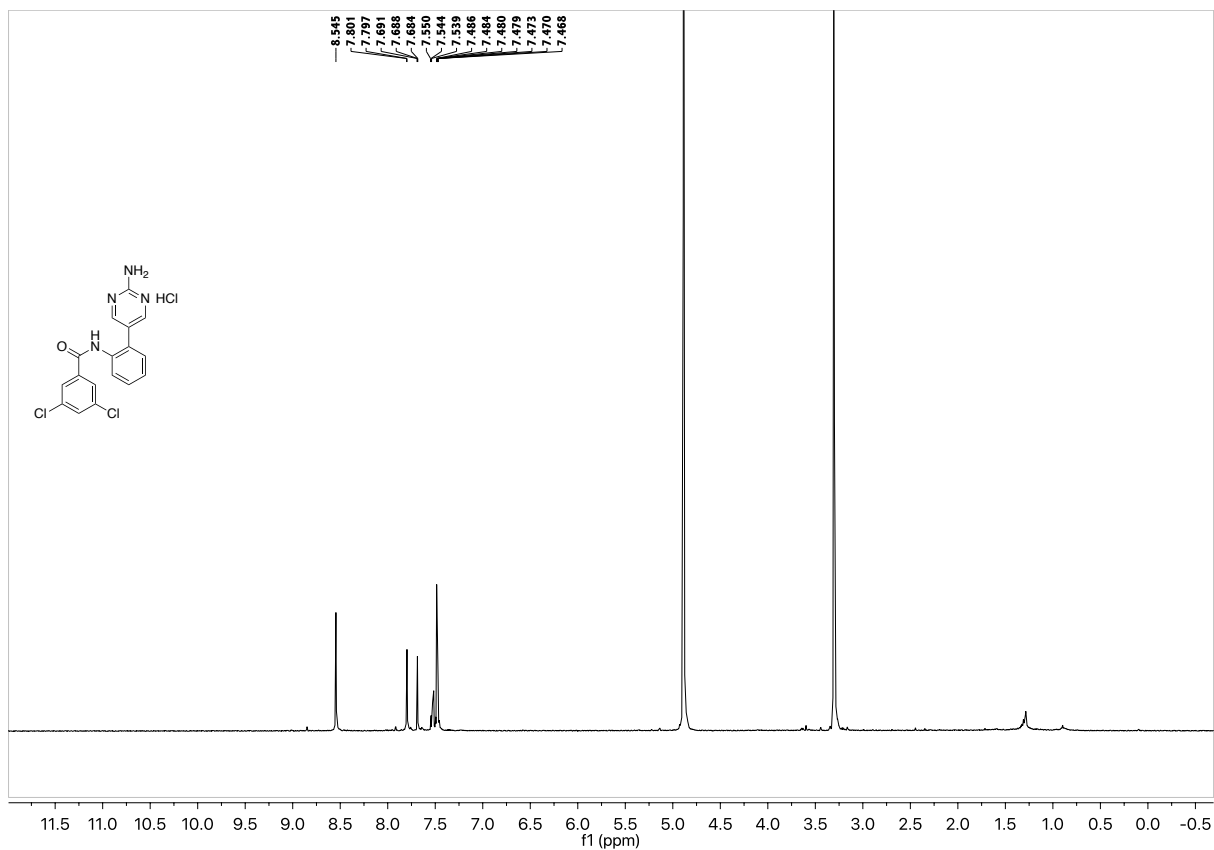
Compound 14



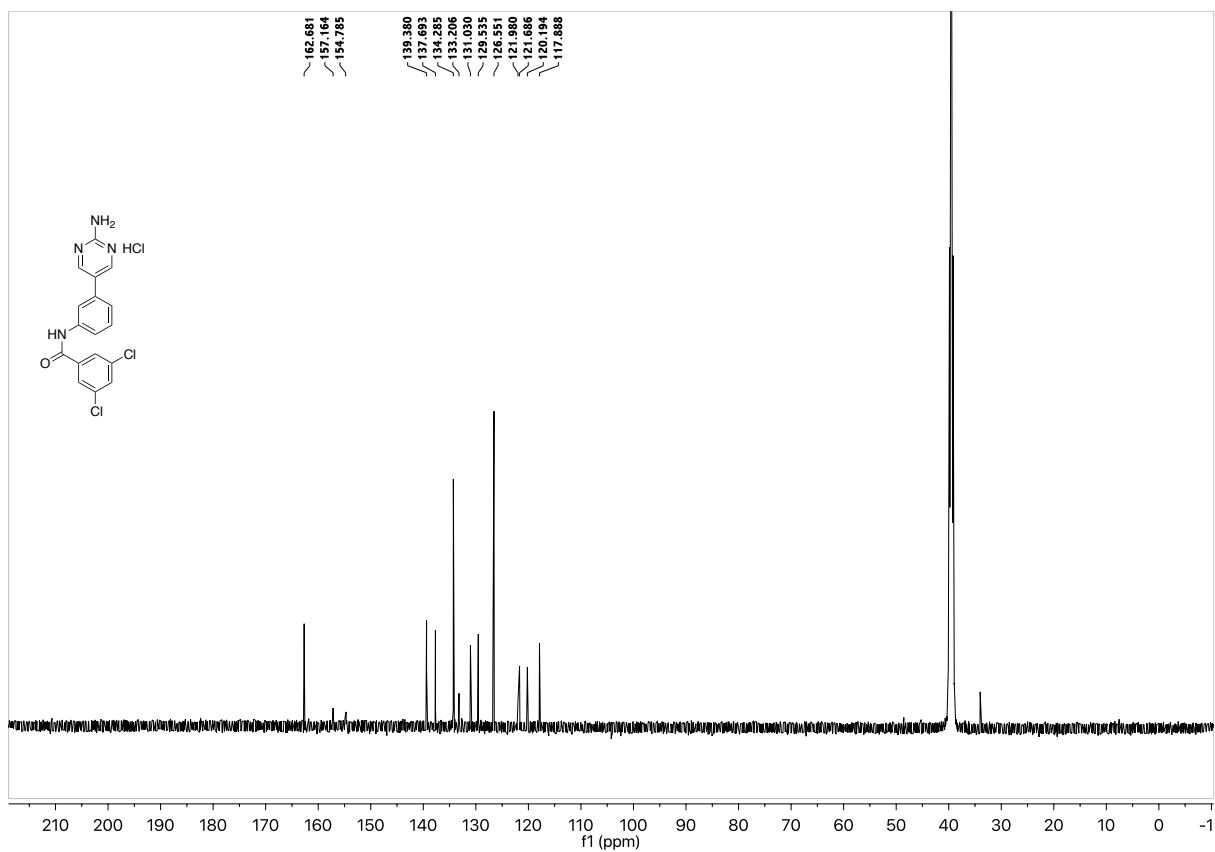
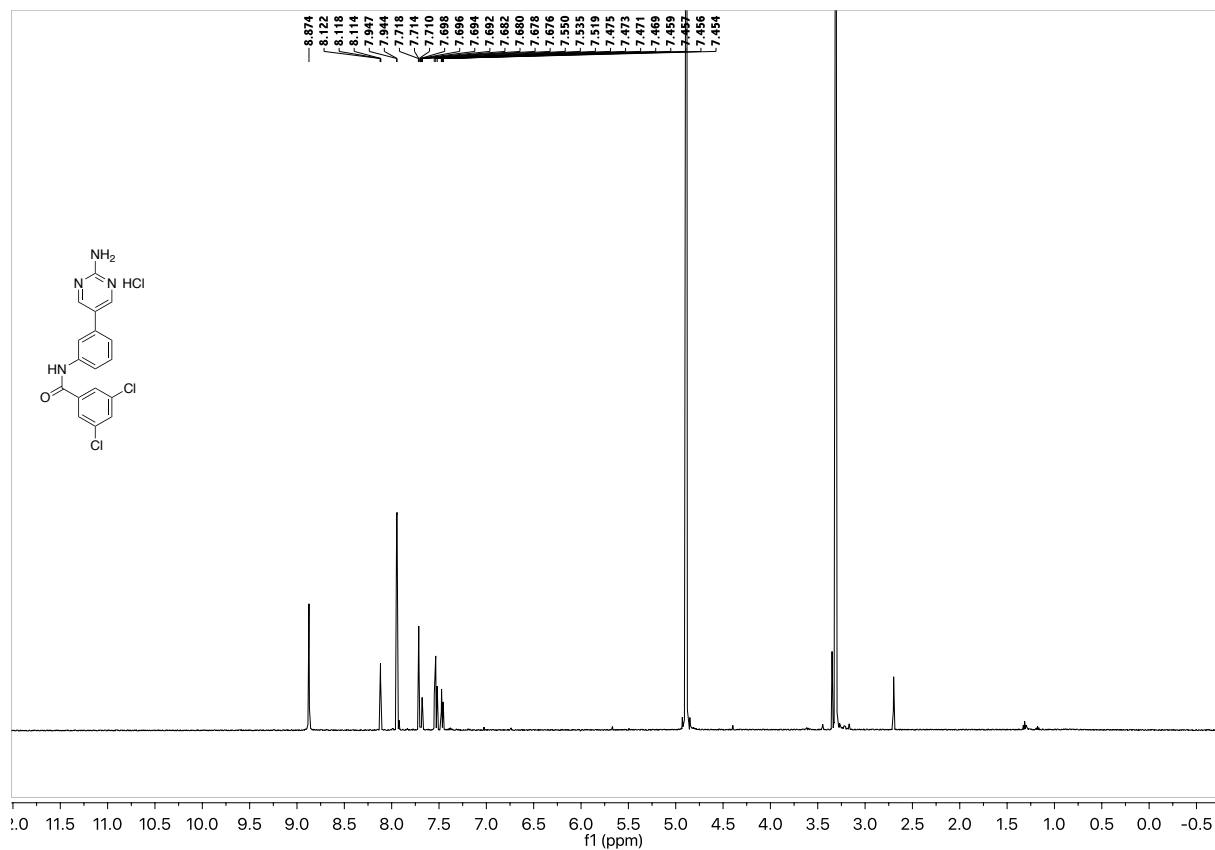
Compound 15a



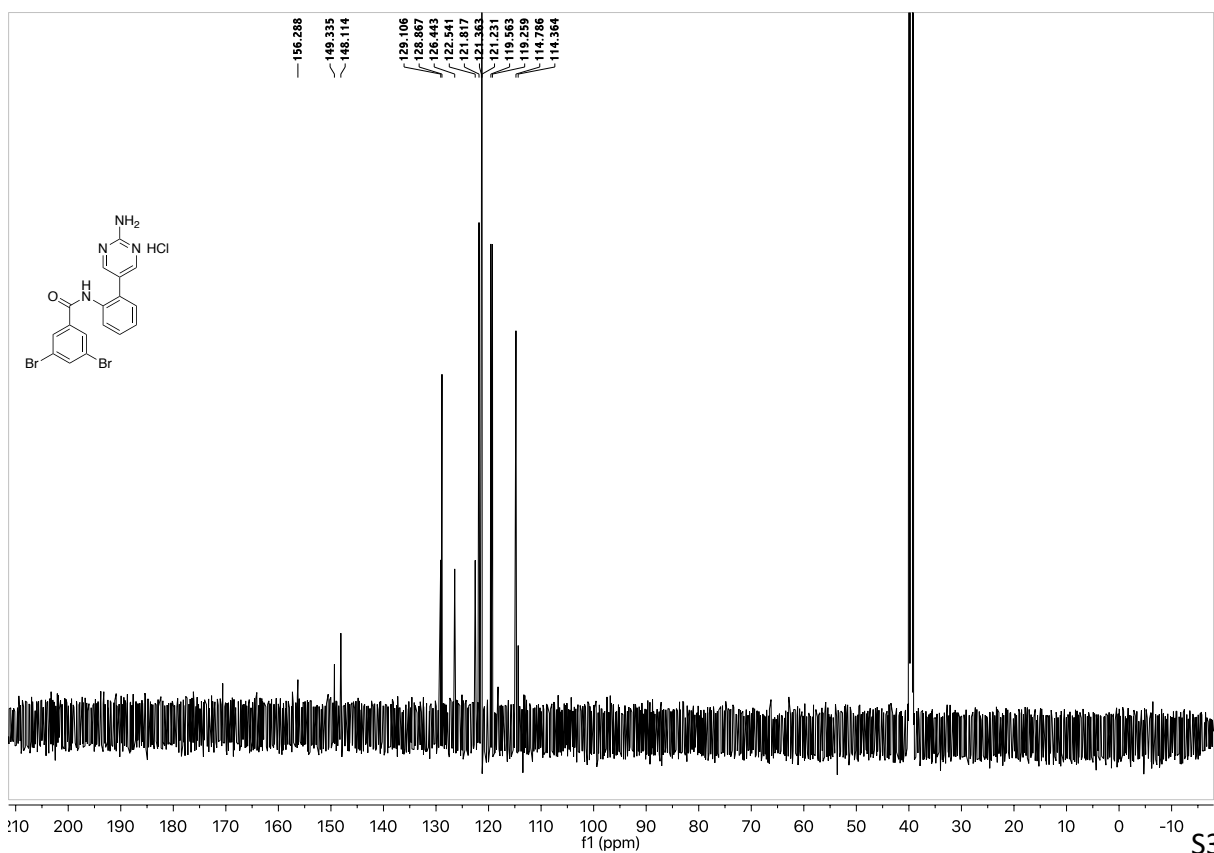
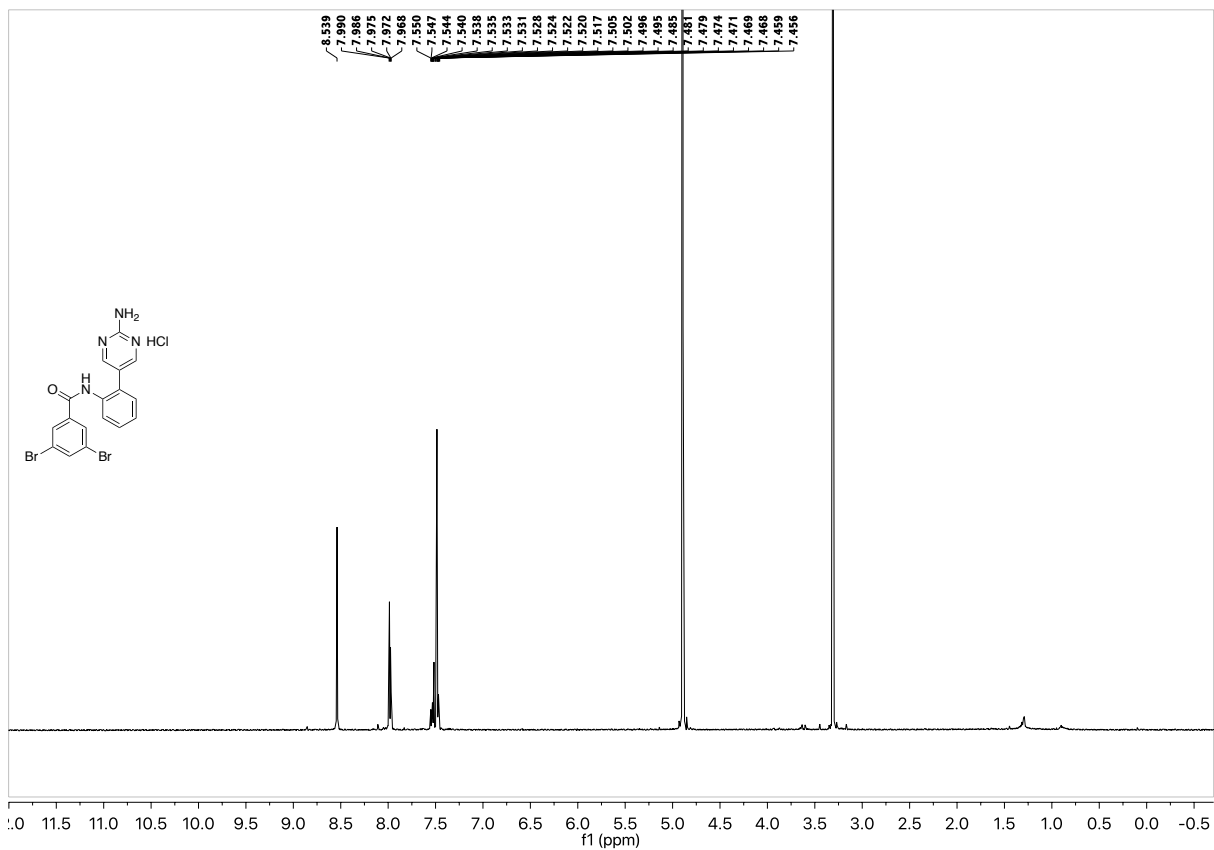
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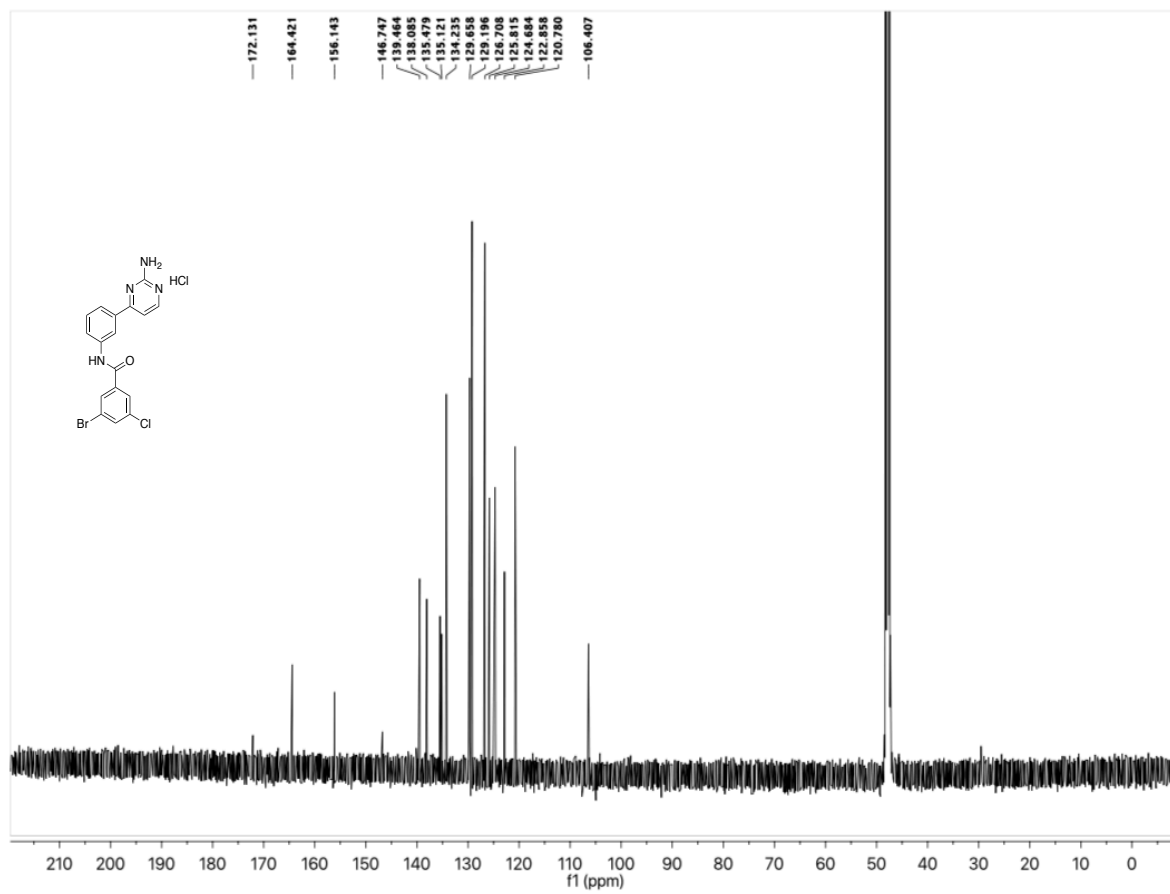
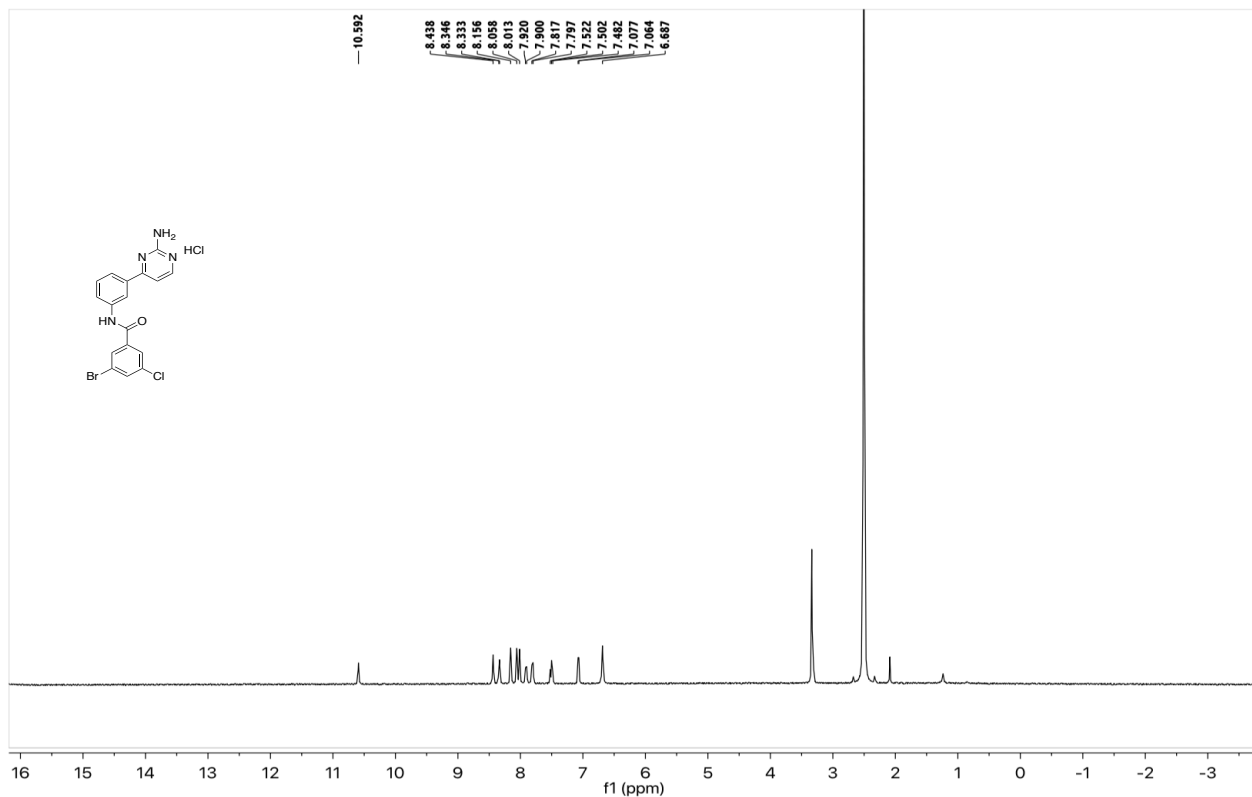
Compound 15c



Compound 15d



Compound 17



References

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