Electronic Supplementary Material (ESI) for MedChemComm.

Supplementary Information for-

Amiloride as a new RNA-binding scaffold with activity against HIV-1 TAR

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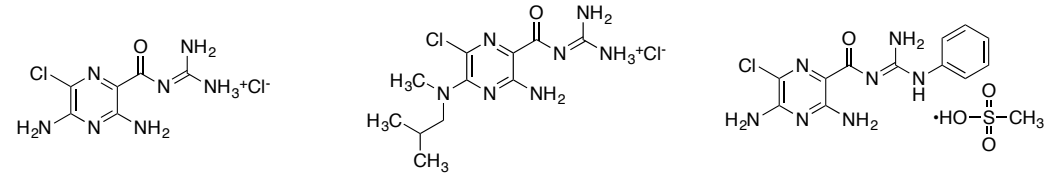
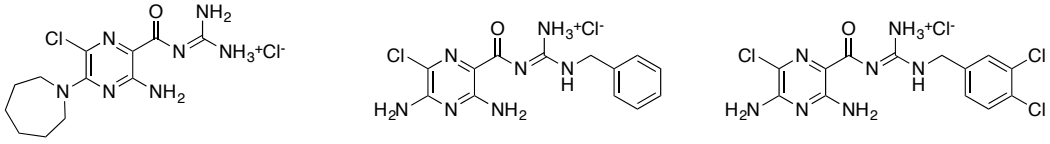
Section A: Chemistry

1. Structures of commercially available amiloride derivatives used in displacement assay and SOFAST NMR experiments:



S1 **S2** **S3**



S4 **S5** **S6**

Figure S1: Structures of commercially available amiloride derivatives tested in assays.

2. Synthetic Procedures and Characterization Data:

General Procedures: All reactions were conducted in oven-dried glassware under an inert atmosphere of nitrogen or argon using magnetic stirring. Dry solvents were obtained using the PureSolv™ solvent purification system prior to use. All other chemical reagents were purchased from commercial sources and were used without further purification. Thin layer chromatography (TLC) was performed either on aluminum backed silica gel or aluminum oxide (neutral) plates purchased from Merck. Column chromatography was performed on flash grade silica gel (40-63 μm) purchased from Silicycle Inc. ^{1}H NMR spectra were recorded on a Varian Unity 500 MHz or Varian Inova 400 MHz spectrometers; the corresponding ^{13}C NMR resonant frequencies were 126 and 101 MHz, respectively. Chemical shifts are reported in ppm from tetramethylsilane (TMS) with the solvent resonance as an internal standard (Ex: $CDCl_3$: 7.26 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (Hz), and integration. Broad singlet peaks, speculated to arise from NH protons, are also noted. In case of ^{13}C NMR, chemical shifts are reported in ppm with the solvent resonance as the internal standard ($CDCl_3$: 77.16 ppm). Extra peaks from solvents and other known impurities are marked as X on the spectra and not integrated in ^{1}H NMR or picked in ^{13}C-NMR (**SI-3**). High-resolution mass spectroscopy (HRMS) was performed on an Agilent LCMS time-of-flight (TOF) mass spectrometer using either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). HPLC analyses were performed on Shimadzu LC system using a Phenomex-C_{18} reverse phase column using water (with 0.1% v/v of TFA) and acetonitrile as eluents. All compounds tested in binding or activity assays are >95% pure by ^{1}H NMR and HPLC analyses unless otherwise noted.

General Procedure A: Nucleophilic Aromatic Substitution on Pyrazine with amines.

To a solution of 5,6 dichloropyrazine methyl ester (0.050 g, **2)** in dimethyl formamide (DMF, 0.1 M total reaction concentration) in a round bottom flask under inert atmosphere of nitrogen was added diisopropyl ethylamine (5 eq) and the mixture was allowed to stir for 5 min. Appropriate amine (1.1 eq) was added to this solution, portion-wise with stirring under nitrogen atmosphere. The resulting dark reddish brown solution was stirred at room temperature until thin layer chromatography showed no presence of starting material **2** (18 - 24h). Upon completion, the reaction flask was transferred to a rotary evaporator and the volatiles were removed to dryness. The resulting brown residue was partitioned between water and ethyl acetate. The organic layer was

separated and washed with water (3X 10 mL) and brine (1X 10 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting yellow oil was purified by flash chromatography on silica gel to yield the C(5) functionalized derivatives **3a-n, 5, 12, 15**. TLC retention factors (Rf), purification conditions, ^{1}H and ^{13}C NMR, and HRMS data are supplied under each individual intermediate or derivative.

General Procedure B: Guanidinylation reaction to convert methyl ester intermediates final acyl guanidines.

To a solution of appropriate methyl ester intermediates **3a-3n, 6-8,** and **13, 16, 21a-f, 23** in tetrahydrofuran (THF, 0.1 M total reaction concentration) or DMF, was added a 2M solution of guanidine in methanol (5 eq). The resulting white cloudy or pale yellow clear reaction mixture was heated to 60 ºC on a oil bath and allowed to stir for 24h, or until maximum conversion to the product was observed. After completion, the reaction mixture was concentrated under reduced pressure. The resulting pale yellow/white residue was purified using flash column chromatography on silica gel, to yield the final acyl guanidines **4a-n, 9-11, 14, 17, 22a-f, 24** as off white or yellow solids. These solids were then treated with a 2M solution of HCl in diethyl ether at 0 ºC in a ice water bath for 10 min, triturated with diethyl ether (3 X 10 mL) and dried under high vacuum overnight. TLC retention factors (Rf), reaction yields, ^{1}H and ^{13}C NMR, HRMS data, and HPLC purities are supplied under each individual intermediate or derivative.

General Procedure C: Sonogashira cross-coupling reactions.

To a solution of methyl 3-amino-6-chloro-5-(dimethylamino) pyrazine-2-carboxylate **15** or amiloride **18** in DMF (0.1 M total reaction concentration) was added Phenylacetylene (2.0 equiv), triethylamine (5 equiv). The resulting yellowish orange solution was then degassed by bubbling argon gas through it for 30 min. CuI (0.01 equiv) and $Pd(PPh_3)_2Cl_2$ (0.025 equiv) was then added to this solution upon which the color of the solution changes to orange-brown. This solution is then heated to 85 ºC and stirred for 18h or until thin layer chromatography indicated complete consumption of the starting compound **15** or **18**. The reaction mixture was then separated between equal volumes of ethyl acetate and brine 50 mL, and the phases were separated. The organic phases were then combined and washed with water (3 X 25 mL) and brine (1 X 25 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. Resulting brown oil was then purified by flash chromatography to yield product **16** and **19** as yellow oils that solidified upon standing. TLC retention

factors (Rf), reaction yields, ^{1}H and ^{13}C NMR, HRMS data, and HPLC purities are supplied under each individual intermediate or derivative.

General Procedure D: Suzuki cross-coupling reactions.

To a solution of methyl 3-amino-6-chloro-5-(dimethylamino) pyrazine-2-carboxylate **15** in a 1:1 mixture of THF and water (0.1 M total reaction concentration) was added appropriate boronic acid (1.2 equiv), Na_2CO_3 (5 equiv). The resulting clear or yellowish solution was then degassed by bubbling argon gas through it for 30 min. $Pd(PPh_3)_4$ catalyst (0.05 equiv) was then added to this solution upon which the color of the solution changes to deep yellow. This solution is then heated to 65 ºC on an oil bath and stirred for 18h or until thin layer chromatography indicated complete consumption of the starting compound **15**. The reaction mixture was then separated between equal volumes of ethyl acetate and brine 50 mL, and the phases were separated. The organic phases were then washed with water (3 X 25 mL) and brine (1 X 25 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. Resulting yellow solid residue was then purified by flash chromatography to yield products **21a-f** and **23** as yellow solids. TLC retention factors (Rf), reaction yields, ^{1}H and ^{13}C NMR, HRMS data, are supplied under each individual intermediate or derivative.

Characterization Data for Amilorides and Intermediates -

Methyl 3-amino-6-chloro-5-(pyrrolidin-1-yl)pyrazine-2-carboxylate (3a)[1]: Synthesized by General Procedure A, using 5,6 dichloropyrazine methyl ester **2** and pyrrolidine. 80% yield, Rf: 0.35 (5:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.9 (s, 3H), 3.7 (m, 4H), 1.9 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.4, 154.2, 151.3, 120.3, 110.5, 51.9, 50.1, 25.5. HRMS (ESI+): Calculated for $C_{10}H_{13}ClN_4NaO_2$ [M+Na]: 279.0619, Found: 279.0618 (± 0.525 ppm).

Methyl 3-amino-6-chloro-5-(piperidin-1-yl) pyrazine-2-carboxylate (3b)[2]: Synthesized by General Procedure A, using 5,6 dichloropyrazine methyl ester **2** and piperidine. 83% yield, Rf 0.3 (5:1- Hexanes: EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.9 (s, 3H), 3.5 (m, 4H), 1.6 (m, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.3, 154.9, 153.9, 123.8, 112.6, 52.1, 49.2, 25.7, 24.3. HRMS (ESI+): Calculated for $C_{11}H_{16}ClN_4O_2$ [M+H]: 271.0956, Found: 271.0962 (± 1.9 ppm).

Methyl 3-amino-6-chloro-5-(phenethylamino)pyrazine-2-carboxylate (3c)[1]: Synthesized by General Procedure A, using 5,6 dichloropyrazine methyl ester **2** and phenethylamine. 97% yield, Rf: 0.25 (5:1 -

Hexanes: EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.4-7.2 (m, 5H), 5.6 (bs, 1H, NH), 3.9 (s, 3H), 3.74 (q, J = 7.0 Hz, 2H), 2.95 (t, J = 7.1 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.8, 155.8, 151.7, 138.7, 129.0, 127.0, 121.8, 110.6, 52.3, 42.8, 35.4. Overlap of peaks observed in the aromatic region. HRMS (ESI+): Calculated for $C_{14}H_{16}ClN_4O_2$ [M+H]: 307.0956, Found: 307.0957 (± 1.4 ppm).

Methyl 3-amino-6-chloro-5-((4-fluorophenethyl)amino)pyrazine-2-carboxylate (3d): Synthesized by General Procedure A, using 5,6 dichloropyrazine methyl ester **2** and 2-(4-fluorophenyl)ethan-1-amine. 88% yield, Rf: 0.35 (5:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.2-7.1 (m, 2H), 7.0-6.9 (m, 2H), 5.6 (bs, 1H, NH), 3.9 (s, 3H), 3.6 (m, 2H), 2.9 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.8, 162.9, 161.0, 155.8, 151.6, 134.4, 130.4 (d, $J_{C\text{-}F}$ = 21 Hz), 121.7, 115.8 (d, $J_{C\text{-}F}$ = 21 Hz), 110.7, 52.3, 42.8, 34.6. One extra peak in the aromatic region, due to strongly split fluorine substituted carbon in aromatic ring. The 1 bond C-F coupling pattern for this peak is indiscernible in this spectrum.; HRMS (ESI+): Calculated for $C_{14}H_{15}ClFN_4O_2$ [M+H]: 325.0862, Found: 325.0868 (± 1.7 ppm).

Methyl 3-amino-5-(benzylamino)-6-chloropyrazine-2-carboxylate (3e)[1, 2]: Synthesized by General Procedure A, using 5,6 dichloropyrazine methyl ester **2** and benzylamine. 91% yield, Rf: 0.35 (5:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.5-7.2 (m, 5H), 5.9 (bs, 1H, NH), 4.7 (m, 2H), 3.9 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.8, 155.8, 151.6, 137.8, 129.1, 128.1, 128.0, 121.6, 111.0, 52.3, 45.5. HRMS (ESI+): Calculated for $C_{13}H_{14}ClN_4O_2$ [M+H]: 293.0800, Found: 293.0807 (± 2.6 ppm).

Methyl 5-((3-(1*H*-imidazol-1-yl)propyl)amino)-3-amino-6-chloropyrazine-2-carboxylate (3f): Synthesized by General Procedure A, using 5,6 dichloropyrazine methyl ester **2** and 3-(1*H*-imidazol-1-yl)propan-1-amine. 86% yield, Rf: 0.3 (90:9:1 – DCM: MeOH: NH_4OH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.5 (s, 1H), 7.1 (s, 1H), 6.9 (s, 1H), 5.8 (bs, 1H, NH), 4.0 (m, 2H), 3.9 (s, 3H), 3.5 (m, 2H), 2.1 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.7, 155.6, 151.7, 137.5, 129.9, 121.4, 119.1, 110.9, 52.3, 44.6, 38.5, 30.7; HRMS (ESI+): Calculated for $C_{12}H_{16}ClN_6O_2$ [M+H]: 311.1018, Found: 311.1025 (± 2.1 ppm).

Methyl 3-amino-6-chloro-5-((3-(2-oxopyrrolidin-1-yl)propyl)amino)pyrazine-2-carboxylate (3g): Synthesized by General Procedure A, using 5,6 dichloropyrazine methyl ester **2** and 1-(3-aminopropyl)pyrrolidin-2-one. 91% yield. Rf: 0.3 (90:9:1 – DCM: MeOH: NH_4OH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.8 (bs, 1H, NH), 3.9 (s, 3H), 3.5-3.3 (m, 6H), 2.4 (t, J = 8.1 Hz, 2H), 2.1 (p, J = 7.5 Hz, 2H), 1.8 (p, J = 6.2 Hz,

2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.3, 166.8, 155.8, 151.8, 122.2, 110.0, 52.1, 47.6, 40.0, 37.7, 31.0, 26.3, 18.2.; HRMS (ESI+): Calculated for $C_{13}H_{19}ClN_5O_3$ [M+H]: 328.1171, Found: 328.1172 (± 0.8 ppm).

Methyl 3-amino-6-chloro-5-((2-(piperazin-1-yl)ethyl)amino)pyrazine-2-carboxylate (3h): Synthesized by General Procedure A, using 5,6 dichloropyrazine methyl ester **2** and 2-(piperazin-1-yl)ethan-1-amine. 88% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.9-4.0 (s, 3H), 3.75-3.55 (m, 4H), 3.5-3.3 (m, 6H), 3.0-2.7 (m, 2H), 2.65-2.5 (m, 4H), 2.44 (t, J = 6.1 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.5, 154.8, 154.1, 123.9, 113.4, 61.1, 53.2, 52.5, 48.2, 38.9; HRMS (ESI+): Calculated for $C_{12}H_{20}ClN_6O_2$ [M+H]: 315.1331, Found: 315.1333 (± 1.3 ppm)

Methyl 3-amino-6-chloro-5-((4-sulfamoylphenethyl)amino)pyrazine-2-carboxylate (3i): Synthesized by General Procedure A, using 5,6 dichloropyrazine methyl ester **2** and 4-(2-aminoethyl)benzenesulfonamide. 29% yield, Rf: 0.22 (90:9:1 – DCM: MeOH: NH_4OH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.4-7.2 (m, 2H), 7.1-6.9 (m, 2H), 3.7 (s, 3H), 3.65-3.5 (m, 2H), 2.95-2.8 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.6, 156.3, 151.9, 144.2, 142.8, 129.9, 126.4, 120.4, 109.2, 51.9, 42.7, 34.7; HRMS (ESI+): Calculated for $C_{14}H_{17}ClN_5O_4S$ [M+H]: 386.0684, Found: 386.0682 (± 0.5 ppm)

Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-chloropyrazine-2-carboxylate (3j): Synthesized by General Procedure A, using 5,6 dichloropyrazine methyl ester **2** and tryptamine. 73% yield. Rf: 0.15 (90:9:1 – DCM: MeOH: NH_4OH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45-8.2 (bs, 1H, NH), 7.7-7.6 (m, 1H), 7.5-7.3 (m, 1H), 7.3-7.1(m, 2H), 7.1-6.95(m, 1H), 5.7 (bs, 1H, NH), 3.9 (s, 3H), 3.85-3.65 (m, 2H), 3.2-2.95 (m, 2H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.8, 155.9, 151.8, 136.8, 127.4, 122.5, 122.5, 121.9, 119.8, 118.9, 112.7, 111.7, 110.4, 52.3, 41.7, 25.2.; HRMS (ESI+): Calculated for $C_{16}H_{17}ClN_5O_2$ [M+H]: 346.1065, Found: 346.1071 (± 1.6 ppm)

Methyl 3-amino-6-chloro-5-((3,4-difluorobenzyl)amino)pyrazine-2-carboxylate (3k): Synthesized by General Procedure A, using 5,6 dichloropyrazine methyl ester 2 and (3,4-difluorophenyl)methanamine. 72% yield, Rf: 0.4 (5:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24-7.10 (m, 2H), 7.10-7.0 (m, 1H), 5.9 (bs, 1H, NH), 4.7-4.55 (m, 2H), 3.95-3.85 (s, 3H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.7, 155.6, 151.4, 149.0, 134.9, 123.8, 121.4, 117.8(d, $J_{C\text{-}F}$ = 17.4 Hz), 116.9 (d, $J_{C\text{-}F}$ = 17.6 Hz), 111.4, 52.4, 44.4. Peak overlap seen in aromatic region, which leads to one less peak than expected. The 1 bond C-F coupling patterns are indiscernible in this spectrum ; HRMS (ESI+): Calculated for $C_{13}H_{12}ClF_2N_4O_2$ [M+H]: 329.0611, Found: 329.0613 (± 0.4 ppm)

Methyl 5-((2-(1*H*-benzo[*d*]imidazol-2-yl)ethyl)amino)-3-amino-6-chloropyrazine-2-carboxylate (3l): Synthesized by General Procedure A, using 5,6 dichloropyrazine methyl ester **2** and 2-(1*H*-benzo[*d*]imidazol-2-yl)ethan-1-amine. 72% yield, Rf: 0.2 (90:9:1 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, $CDCl_3$) δ 7.62-7.5 (m, 2H), 7.2 (m, 2H), 6.5 (bs, 1H, NH), 4.0-3.9 (m, 2H), 3.8 (s, 3H), 3.3-3.1 (m, 2H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.0, 155.6, 152.5, 151.7, 122.8, 122.8, 121.9, 115.1, 110.5, 52.2, 39.7, 28.7.; HRMS (ESI+): Calculated for $C_{15}H_{16}ClN_6O_2$ [M+H]: 347.1018, Found: 347.1020 (± 0.6 ppm)

Methyl 3-amino-6-chloro-5-(((tetrahydrofuran-2-yl)methyl)amino)pyrazine-2-carboxylate (3m): Synthesized by General Procedure A, using 5,6 dichloropyrazine methyl ester **2** and racemic-(tetrahydrofuran-2-yl)methanamine. 86% yield, Rf: 0.4 (5:1 - Hexanes: EtOAc); ^1H NMR (500 MHz, $CDCl_3$) δ 5.9 (bs, 1H, NH), 4.11 (qt, J = 6.4, 3.1 Hz, 1H), 3.98 - 3.88 (m, 4H), 3.82 (dt, J = 8.4, 6.8 Hz, 1H), 3.72 (ddd, J = 13.7, 6.1, 3.5 Hz, 1H), 3.47 -3.39 (m, 1H), 2.05 (ddt, J = 13.9, 11.2, 6.3 Hz, 1H), 2.00 -1.91 (m, 2H), 1.68 -1.56 (m, 1H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.5, 155.5, 151.6, 121.6, 110.3, 77.0 (overlapping with $CDCl_3$ solvent peak), 68.2, 52.0, 44.9, 28.8, 25.9.; HRMS (ESI+): Calculated for $C_{11}H_{16}ClN_4O_3$[M+H]: 287.0911, Found: 287.0907 (± 1.4 ppm)

Methyl 3-amino-6-chloro-5-((2-(4-methylpiperazin-1-yl)ethyl)amino)pyrazine-2-carboxylate (3n): Synthesized by General Procedure A, using 5,6 dichloropyrazine methyl ester **2** and 2-(4-methylpiperazin-1-yl)ethan-1-amine. 56% yield. Rf: 0.2 (90:9:1 – DCM: MeOH: NH_4OH)**;** ^1H NMR (500 MHz, $CDCl_3$) δ – 6.4 (bs, 1H, NH), 3.9-3.7 (s, 3H), 3.41 (tdd, J = 6.1, 4.5, 0.9 Hz, 2H), 2.9-1.9 (m, 13H) – Broad peak observed for 8H in piperazine ring which overlaps with one methylene peak (2H), and N-CH_3 (3H) peak.; ^{13}C NMR (126 MHz, $CDCl_3$) δ166.8, 155.9, 151.7, 121.8, 110.1, 55.7, 55.4, 52.8, 52.2, 46.2, 37.8.; HRMS (ESI+): Calculated for $C_{13}H_{22}ClN_6O_2$ [M+H]: 329.1487, Found: 329.1490 (± 0.8 ppm)

3-amino-6-chloro-*N*-(diaminomethylene)-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide (4a)[1, 3]: Synthesized by General Procedure B from starting material **3a**. 67% yield. Rf: 0.2 (85:13:2 – DCM: MeOH: NH_4OH)**;** ^1H NMR (500 MHz, CD_3OD) δ 3.88-3.56 (m, 4H), 1.86 (dq, J = 10.6, 6.6, 4.9 Hz, 4H).; ^{13}C NMR (126 MHz, CD_3OD) δ 165.5, 155.8, 154.5, 151.6, 120.0, 109.0, 50.8, 25.3.; HRMS (ESI+): Calculated for $C_{10}H_{15}ClN_7O$ [M+H] – 284.1021, Found: 284.1023 (± 0.57 ppm). HPLC analysis: Ret. time – 7.458 min, Purity – 97%.

3-amino-6-chloro-*N*-(diaminomethylene)-5-(piperidin-1-yl)pyrazine-2-carboxamide (4b)[2, 3]: Synthesized by General Procedure B from starting material **3b**. 69% yield, Rf: 0.3 (85:13:2 – DCM: MeOH: NH_4OH)**;** ^1H

NMR (500 MHz, CD_3OD) δ 3.71 (q, J = 12.8, 9.2 Hz, 4H), 1.89-1.52 (m, 6H).; ^{13}C NMR (126 MHz, CD_3OD) δ 166.0, 156.0, 155.6, 154.8, 122.3, 111.3, 49.2, 25.9, 24.2.; HRMS (ESI+): Calculated for $C_{11}H_{17}ClN_7O$ – 298.1178, Found: 298.1178 (± 0.2 ppm). HPLC analysis: Ret. time – 7.923 min, Purity – 98%

3-amino-6-chloro-*N*-(diaminomethylene)-5-(phenethylamino)pyrazine-2-carboxamide (4c)[3, 4]: Synthesized by General Procedure B from starting material **3c**. 88% yield, Rf: 0.30 (85:13:2 – DCM: MeOH: NH_4OH)**;** ^1H NMR (500 MHz, CD_3OD) δ 7.38 - 7.10 (m, 5H), 3.70 (dd, J = 8.6, 6.5 Hz, 2H), 2.93 (t, J = 7.6 Hz, 2H). ^{13}C NMR (126 MHz, CD_3OD) δ 165.7, 156.3, 155.8, 152.8, 139.2, 128.8, 128.3, 126.2, 121.2, 108.4, 42.8, 34.9.; HRMS (ESI+): Calculated for $C_{14}H_{17}ClN_7O$ - 334.1178, Found – 334.1176 (± 0.8 ppm); HPLC analysis: Ret. time – 8.229 min, Purity – 99%

3-amino-6-chloro-*N*-(diaminomethylene)-5-((4-fluorophenethyl)amino)pyrazine-2-carboxamide (4d)[3]: Synthesized by General Procedure B from starting material 3d. 86% yield, Rf: 0.35 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 7.36 - 7.21 (m, 2H), 7.09 - 6.96 (m, 2H), 3.70 (dd, J = 8.2, 6.5 Hz, 2H), 2.93 (t, J = 7.4 Hz, 2H).; ^{13}C NMR (126 MHz, CD_3OD) δ 172.7, 165.7, 162.8, 156.4, 155.9, 152.9, 135.2, 130.45 (d, $J_{C\text{-}F}$ = 6.7 Hz), 121.2, 114.85 d, ($J_{C\text{-}F}$ = 21.4 Hz), 108.5, 42.7, 34.0.; One extra peak in the aromatic region, due to strongly split fluorine substituted carbon in aromatic ring. The 1 bond C-F coupling pattern for this peak is indiscernible in this spectrum.; HRMS (ESI+): Calculated for $C_{14}H_{16}ClFN_7O$ – 352.1083, Found – 352.1081 (± 0.5 ppm); HPLC analysis: Ret. time – 8.392 min, Purity – 99%.

3-amino-5-(benzylamino)-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (4e)[1, 2, 4]: Synthesized by General Procedure B from starting material **3e**. 58% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH)**;** ^1H NMR (500 MHz, CD_3OD) δ 7.57 - 7.12 (m, 5H), 4.71 (d, J = 9.3 Hz, 2H).; ^{13}C NMR (126 MHz, CD_3OD) δ 165.7, 156.4, 155.9, 153.0, 138.6, 128.1, 127.3, 126.9, 121.1, 108.8, 44.3.; HRMS (ESI+): Calculated for $C_{13}H_{15}ClN_7O$ – 320.1021, Found: 320.1023 (± 0.6 ppm).; HPLC analysis: Ret. time – 7.940 min, Purity – 95%.

5-((3-(1*H*-imidazol-1-yl)propyl)amino)-3-amino-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (4f): Synthesized by General Procedure B from starting material **3f**. 89% yield, Rf: 0.12 (85:13:2 – DCM: MeOH: NH_4OH)**;** ^1H NMR (500 MHz, CD_3OD) δ 9.04 (d, J = 1.8 Hz, 1H), 7.86 - 7.68 (m, 1H), 7.66 - 7.52 (m, 1H), 4.39 (t, J = 7.0 Hz, 2H), 3.62 (t, J = 6.4 Hz, 2H), 2.42 - 2.21 (m, 2H).; ^{13}C NMR (126 MHz, CD_3OD) δ 165.8, 156.2, 155.9, 153.1, 135.4, 122.2, 121.2, 120.0, 108.8, 47.3 (overlapping with CD_3OD peak), 38.0,

29.3.; HRMS (ESI+): Calculated for $C_{12}H_{17}ClN_9O$ – 338.1239, Found – 338.1239 (± 0.2 ppm); HPLC analysis: Ret. time – 5.437 min, Purity – 98%

3-amino-6-chloro-*N*-(diaminomethylene)-5-((3-(2-oxopyrrolidin-1-yl)propyl)amino)pyrazine-2-carboxamide (4g): Synthesized by General Procedure B from starting material **3g**. 70% yield, Rf: 0.25 (85:13:2 – DCM: MeOH: NH_4OH)**;** ^{1}H NMR (500 MHz, CD_3OD) δ 3.62 - 3.57 (m, 2H), 3.54 (t, J = 6.7 Hz, 2H), 3.44 (t, J = 6.9 Hz, 2H), 2.54 (t, J = 8.1 Hz, 2H), 2.14 (p, J = 7.7 Hz, 2H), 1.93 (p, J = 6.7 Hz, 2H).; ^{13}C NMR (126 MHz, CD_3OD) δ 177.4, 165.7, 156.2, 155.8, 152.8, 121.3, 108.5, 48.2 (overlapping with CD_3OD peak), 40.5, 38.2, 30.8, 25.9, 17.6.; HRMS (ESI+): Calculated for $C_{13}H_{20}ClN_8O_2$ – 355.1392, Found – 355.1392 (± 0.33 ppm). HPLC analysis: Ret. time – 6.317 min, Purity – 94%

3-amino-6-chloro-*N*-(diaminomethylene)-5-((2-(piperazin-1-yl) ethyl)amino)pyrazine-2-carboxamide(4h): Synthesized by General Procedure B from starting material **3h**. 27% yield, Rf <0.1 (85:13:2 – DCM: MeOH: NH_4OH)**;**^{1}H NMR (500 MHz, D_2O) δ 3.75 (d, J = 0.9 Hz, 2H), 3.51 (m, 8H), 3.35 (d, J = 1.0 Hz, 2H).; ^{13}CNMR (126 MHz, CD_3OD) δ 166.9, 156.8, 156.3, 155.7, 123.8, 114.3, 54.7, 53.2, 46.2, 35.1.; HRMS (ESI+): Calculated for $C_{12}H_{21}ClN_9O$ – 342.1552, Found – 342.1554 (± 0.7 ppm).; HPLC analysis: Ret. time – 4.926 min, Purity – 95%

3-amino-6-chloro-*N*-(diaminomethylene)-5-((4-sulfamoylphenethyl)amino)pyrazine-2-carboxamide (4i): Synthesized by General Procedure B from starting material **3i**. 19% yield, Rf <0.1 (85:13:2 – DCM: MeOH: NH_4OH)**;** ^{1}H NMR (500 MHz, CD_3OD) δ 7.37 - 7.23 (m, 2H), 7.10 - 6.94 (m, 2H), 3.71 (dd, J = 8.2, 6.5 Hz, 2H), 2.94 (t, J = 7.4 Hz, 2H), 2.23 (s, 2H); ^{13}CNMR (126 MHz, DMSO-d_6) δ 166.0, 156.4, 156.0, 152.8, 139.8, 129.4, 129.0, 126.9, 120.8, 109.0, 43.3, 34.9.; HRMS (ESI+): Calculated for $C_{14}H_{18}ClN_8O_3S$ – 413.0906, Found – 413.0907 (± 0.82 ppm); HPLC analysis: Ret. time – 6.897 min, Purity – 96%

5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (4j): Synthesized by General Procedure B from starting material **3j**. 39% yield, Rf: 0.2 (85:13:2 – DCM: MeOH: NH_4OH)**;** ^{1}H NMR (500 MHz, CD_3OD) δ 7.61 (d, J = 7.8 Hz, 1H), 7.35 (d, J = 8.1 Hz, 1H), 7.18 - 6.92 (m, 3H), 3.92 - 3.67 (m, 2H), 3.10 (t, J = 7.3 Hz, 2H).; ^{13}C NMR (126 MHz, CD_3OD) δ 165.7, 156.5, 155.9, 153.0, 137.0, 127.6, 122.4, 121.3, 121.2, 118.5, 118.2, 111.9, 111.1, 108.4, 42.1, 24.6.; HRMS (ESI+): Calculated for $C_{16}H_{18}ClN_8O$ – 373.1287, Found – 373.1285 (± 0.6 ppm).; HPLC analysis: Ret. time – 8.181 min, Purity – 99%

3-amino-6-chloro-*N*-(diaminomethylene)-5-((3,4-difluorobenzyl)amino)pyrazine-2-carboxamide (4k): Synthesized by General Procedure B from starting material **3k**. 72% yield, Rf: 0.3 (85:13:2 – DCM: MeOH: NH_4OH)**;** ^1H NMR (500 MHz, CD_3OD) δ 7.36 - 7.28 (m, 1H), 7.21 (tt, J = 5.7, 2.7 Hz, 2H), 4.66 (s, 2H).; ^{13}C NMR (126 MHz, CD_3OD) δ 165.7, 156.2, 155.8, 152.8, 150.9 (dd, J_{C-F} = 82.7, 12.6 Hz), 148.9 (dd, J_{C-F} = 81.8, 12.6 Hz), 136.3, 124.2, 121.1, 117.0 (d, J_{C-F} = 17.4 Hz), 116.7 (d, J_{C-F} = 17.6 Hz), 109.0, 43.4.; HRMS (ESI+): Calculated for $C_{13}H_{13}ClF_2N_7O$ – 356.0833, Found – 356.0831 (± 0.9 ppm); HPLC analysis: Ret. time – 8.126 min, Purity – 98% .

5-((2-(1*H*-benzo[*d*]imidazol-2-yl)ethyl)amino)-3-amino-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (4l): Synthesized by General Procedure B from starting material **3l**. 56% yield, Rf: 0.1 (85:13:2 – DCM: MeOH: NH_4OH)**;** ^1H NMR (500 MHz, CD_3OD) δ 7.77 (dt, J = 6.7, 3.3 Hz, 2H), 7.58 (dd, J = 6.2, 3.1 Hz, 2H), 4.05 (t, J = 6.1 Hz, 2H), 3.56 (t, J = 6.2 Hz, 2H), 3.37 (s, 1H, NH).; ^{13}C NMR (126 MHz, CD_3OD) δ 165.7, 155.9, 155.8, 153.0, 152.0, 131.2, 126.3, 121.0, 113.6, 109.2, 39.2, 26.8.; HRMS (ESI+): Calculated for $C_{15}H_{17}ClN_9O$ – 374.1239, Found – 374.1236 (± 0.75 ppm); HPLC analysis: Ret. time – 5.874 min, Purity – 99%

3-amino-6-chloro-*N*-(diaminomethylene)-5-(((tetrahydrofuran-2-yl)methyl)amino)pyrazine-2-carboxamide (4m): Synthesized by General Procedure B from starting material **3m**. 44% yield, Rf: 0.3 (85:13:2 – DCM: MeOH: NH_4OH)**;** ^1H NMR (500 MHz, CD_3OD) δ 4.20 (qd, J = 6.9, 4.4 Hz, 1H), 3.92 (dt, J = 8.3, 6.5 Hz, 1H), 3.79 (q, J = 7.4 Hz, 1H), 3.67 - 3.48 (m, 2H), 2.11 -1.88 (m, 3H), 1.68 (ddd, J = 11.6, 8.3, 5.9 Hz, 1H). ^{13}C NMR (126 MHz, CD_3OD) δ 165.7, 156.3, 155.8, 153.0, 121.1, 108.7, 77.2, 67.9, 44.9, 28.7, 25.4.; HRMS (ESI+): Calculated for $C_{11}H_{17}ClN_7O_2$ – 314.1127, Found – 314.1128 (± 0.42 ppm); HPLC analysis: Ret. time – 6.746 min, Purity – 95%

3-amino-6-chloro-*N*-(diaminomethylene)-5-((2-(4-methylpiperazin-1-yl)ethyl)amino)pyrazine-2-carboxamide (4n): Synthesized by General Procedure B from starting material **3o**. 88% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH)**;** ^1H NMR analysis performed at 50 ºC to enable sharpening of peaks and resolve overlapping peaks. ^1H NMR (500 MHz, CD_3OD, 50 ºC) δ 3.94 (t, J = 5.8 Hz, 2H), 3.88 - 3.53 (m, 8H), 3.48 (t, J = 5.3 Hz, 2H), 3.00 (s, 3H).; ^{13}C NMR (126 MHz, CD_3OD) δ 165.8, 156.1, 155.8, 153.4, 121.5, 109.4, 55.9, 50.2, 49.3, 42.4, 35.8.; HRMS (ESI+): Calculated for $C_{13}H_{23}ClN_9O$ – 356.1709, Found – 356.1706 (± 0.66 ppm); HPLC analysis: Ret. time – 4.995 min, Purity – 98%

Methyl 3-amino-5-((2-((*tert*-butoxycarbonyl)amino)ethyl)amino)-6-chloropyrazine-2-carboxylate (5): Synthesized by General Procedure A, using 5,6 dichloropyrazine methyl ester **2** and *tert*-butyl (2-aminoethyl)carbamate. 80% yield, Rf: 0.35 (1:1 Hexanes: EtOAc)**;** ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.4-6.3 (bs, 1H, NH), 5.05-4.92 (bs, 1H, NH), 3.9 (s, 3H), 3.59-3.47 (m, 2H), 3.46-3.35 (m, 2H), 1.46 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.8, 157.2, 155.7, 152.1, 121.9, 110.5, 80.3, 52.3, 43.1, 39.9, 28.6. HRMS (ESI+): Calculated for $C_{13}H_{21}ClN_5O_4$ – 346.1277, Found – 346.1279 (± 0.8 ppm).

Methyl 3-amino-5-((2-aminoethyl)amino)-6-chloropyrazine-2-carboxylate (6): To a solution of Boc protected derivative **5** in CH_2Cl_2 (1 mL) was added trifluoroacetic acid (TFA, 1 mL), and resulting yellow solution was stirred at room temperature for 20 h or until thin layer chromatography indicated complete consumption of starting material. The volatiles were removed under reduced pressure and the resulting yellow-brown oil was triturated thrice with Et_2O to yield a yellow amorphous solid, which was thoroughly dried under high vacuum before being used in further reactions.; 91% yield.; Rf <0.1 (85:13:2 – DCM: MeOH: NH_4OH).; Analytical sample prepared by recrystallization from hot ethanol. ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.7 (s, 3H), 3.65-3.55 (m, 2H), 3.04-3.03 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.5, 156.1, 152.3, 120.7, 109.5, 51.9, 39.4 (partially overlapping with DMSO-d_6 peak), 38.9.; HRMS (ESI+): Calculated for $C_8H_{13}ClN_5O_2$ – 246.0751, Found – 246.0751 (± 0.4 ppm).

Methyl 3-amino-6-chloro-5-((2-(dibenzylamino)ethyl)amino)pyrazine-2-carboxylate (7): To a solution of amine **6** (0.050 g, 0.2 mmol, 1 equiv) in DMF (2 mL) was added DIEA (0.177 mL, 1.2 mmol, 6 equiv) and resulting yellow solution was stirred for 10 min at room temperature. Benzyl bromide (0.050 mL, 0.42 mmol, 2.05 equiv) was then added and the resulting solution was stirred for 48h at room temperature. The reaction mixture was then partitioned between equal volume of EtOAc and water (1:1 - 25 mL: 25 mL) in a separatory funnel. The phases were separated and the organics were washed with water (3 X 25 mL) and brine (1 X 25 mL), dried over sodium sulfate and concentrated under reduced pressure to yield a yellow brown oil, which was purified by flash chromatography over silica gel using 5:1 EtOAc: Hexanes as the eluent (Rf = 0.25). To yield dibenzyl product **7** in 54% yield.; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 - 7.31 (m, 8H), 7.30 - 7.24 (m, 2H), 6.24 (bs, 1H, NH), 3.92 (s, 3H), 3.65 (s, 4H), 3.44 (q, J = 5.3 Hz, 2H), 2.72 (t, J = 5.9 Hz, 2H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.9, 155.9, 151.7, 139.1, 129.1, 128.8, 127.5, 121.9, 110.2, 58.7, 52.2, 51.3, 38.6.; HRMS (ESI+): Calculated for $C_{22}H_{25}ClN_5O_2$ [M+H] – 426.1691, Found – 426.1682 (± 2.2 ppm).

Methyl 3-amino-6-chloro-5-((2-((4-methylphenyl)sulfonamido)ethyl)amino)pyrazine-2-carboxylate (8): To a solution of amine **6** (0.050 g, 0.2 mmol) in THF (2 mL) was added DIEA (0.177 mL, 1.2 mmol, 6 equiv) and the resulting yellow solution was stirred for 10 min at room temperature. A solution of tosyl chloride (0.0465 g, 0.25 mmol, 1.2 equiv) in THF 1 mL was added and the resulting solution was stirred for 24h at rt. The reaction mixture was then partitioned between equal volume of EtOAc and water (1:1 50 mL: 50 mL) in a separatory funnel. The phases were separated and the organics were washed with water (3 X 25 mL) and brine (1 X 25 mL), dried over sodium sulfate and concentrated under reduced pressure to yield a clear oil, which was purified by flash chromatography over silica gel using 3:2 EtOAc: Hexanes as the eluent. (Rf = 0.32). To yield dibenzyl product **8** in 90% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.70 - 7.47 (m, 2H) partially overlaps with NH peak, 7.30 (q, J = 12.1, 11.0 Hz, 2H) partially overlaps with NH peak, 3.74 (s, 3H), 3.48 - 3.26 (m, 2H), 2.98 (t, J = 6.3 Hz, 2H), 2.31 (s, 3H).; ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.6, 156.1, 151.8, 143.2, 137.9, 130.2, 127.2, 120.5, 109.3, 51.9, 41.5, 41.0 (peaks partially overlapping with DMSO-d_6 peak), 21.6.; Calculated for $C_{15}H_{19}ClN_5O_4S$ [M+H] – 400.0841, Found – 400.0840 (± 0.55 ppm).

3-amino-5-((2-aminoethyl)amino)-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (9): Synthesized by General Procedure B from starting material **6**. Rf = 0.1 (DCM: MeOH: NH_4OH – 85: 13: 2). 21% yield.; ^{1}H NMR (500 MHz, D_2O) δ 3.76 (dd, J = 6.7, 4.8 Hz, 2H), 3.73 (bs, 1H, NH), 3.33 (bs, 1H, NH), 3.26 (t, J = 5.9 Hz, 2H).; ^{13}C NMR (126 MHz, D_2O) [Chemical shifts referenced to assumed guanidine carbon at 165.5] δ 165.5, 155.4, 154.9, 153.1, 121.6, 109.3, 39.4, 38.5.; HRMS (ESI+): Calculated for $C_8H_{14}ClN_8O$ [M+H] – 273.094, Found – 273.0974 (± 0.3 ppm); HPLC analysis: Ret. time – 4.751 min, Purity – 98%

3-amino-6-chloro-*N*-(diaminomethylene)-5-((2-(dibenzylamino)ethyl)amino)pyrazine-2-carboxamide (10): Synthesized by General Procedure B from starting material **7.** Rf = 0.3 (DCM: MeOH: NH_4OH – 85: 13: 2). 51% yield.; ^{1}H NMR (400 MHz, CD_3OD) δ 7.57 (dd, J = 6.9, 2.4 Hz, 4H), 7.45 - 7.35 (m, 6H), 4.43 (s, 4H), 3.71 (t, J = 6.0 Hz, 2H), 3.37 (s, 2H).; ^{13}C NMR (126 MHz, CD_3OD) δ 165.8, 155.8, 155.7, 152.5, 131.6, 130.0, 129.3, 129.2, 121.2, 109.4, 58.6, 50.0, 36.6.; HRMS (ESI+): Calculated for $C_{22}H_{26}ClN_8O$ [M+H] – 453.1913, Found – 453.1914 (± 0.5 ppm).; HPLC analysis: Ret. time – 8.491 min, Purity – 99%

3-amino-6-chloro-*N*-(diaminomethylene)-5-((2-((4-methylphenyl)sulfonamido)ethyl)amino)pyrazine-2-carboxamide (11): Synthesized by General Procedure B from starting material **8.** Rf = 0.3 (DCM: MeOH: NH_4OH – 85: 13: 2). 83% yield.;^{1}H NMR (500 MHz, CD_3OD) δ 7.69 (d, J = 7.8 Hz, 2H), 7.29 (d, J = 7.9 Hz,

2H), 3.54 (t, J = 6.0 Hz, 2H), 3.17 (t, J = 6.0 Hz, 2H), 2.37 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 165.7, 156.2, 152.9, 143.4, 137.5, 129.6, 129.3, 126.7, 121.2, 108.7, 41.2, 40.9, 20.4.; HRMS (ESI+): Calculated for $C_{15}H_{20}ClN_8O_3S$ [M+H] – 427.1062, Found – 427.1058 (± 1.1 ppm); HPLC analysis: Ret. time – 7.498 min, Purity – 99%.

Methyl 3-amino-6-chloro-5-(prop-2-yn-1-ylamino)pyrazine-2-carboxylate (12): Synthesized by General Procedure A, using 5,6 dichloropyrazine methyl ester **2** and propargyl amine. 85% yield, Rf: 0.3 (5:1 - Hexanes: EtOAc)**;** ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.78 - 5.59 (bs, 1H, NH), 4.21 (ddd, J = 5.4, 2.7, 1.7 Hz, 2H), 3.87 (s, 3H), 2.27 (t, J = 2.6 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.7, 155.5, 150.9, 121.4, 111.5, 79.3, 72.3, 52.4, 31.2.; HRMS (ESI+): Calculated for $C_9H_{10}ClN_4O_2$ [M+H] – 241.0487, Found – 241.0489 (± 1.0 ppm)

Methyl 3-amino-5-(((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)amino)-6-chloropyrazine-2-carboxylate (13): Adapted from a literature procedure.[5] To a solution of benzyl bromide (0.068 mL, 0.58 mmol, 1 equiv) in water + acetone (1:1, 5 mL) was added sodium azide (0.038 g, 0.58 mmol, 1 equiv). The resulting clear solution was stirred at room temperature for 18h. After 18h the reaction mixture was partitioned between ethyl acetate and water (1:1, 25 mL: 25 mL) in a separatory funnel and the phases were separated. The organic phase was washed with sat. $NaHCO_3$ solution (1 X 20 mL) and brine (1 X 20 mL). The organic phase was collected, dried over anhydrous Na_2SO_4 and concentrated to 20% of the final volume (~ 7 mL final volume left) on a rotary evaporator and was used in the next reaction as is. (***Caution:*** *Due to potentially explosive nature of resultant benzyl azide,[6] this mixture is not concentrated to dryness*) To this ethyl acetate solution, was added alkyne **12** (0.140 g, 0.58 mmol, 1 equiv) as a solution in isopropanol (2 mL), sodium ascorbate (0.052 g, 0.26 mmol, 0.45 equiv), copper sulfate (0.022 g, 0.09 mmol, 0.15 equiv), dichloromethane (1 mL) and water (2 mL). The resulting solution turns bluish green and is stirred for 18h at room temperature. The reaction mixture was then partitioned between water and ethyl acetate (1:1 – 25 mL: 25 mL) and the phases were separated. The organics were washed with water (1 X 25 mL), sat $NaHCO_3$ (1 X 25 mL), and brine (1 X 25 mL). The aqueous layers were combined and back extracted with EtOAc (1 X 25 mL). The organic phases are then combined and dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting residue was purified by flash chromatography over silica gel. Rf – 0.2 (95:5 – DCM: MeOH). 75% yield.; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 - 7.22 (m, 6H), 6.14 (bs, 1H, NH), 5.54 (s, 2H), 4.73 (d, J = 5.6 Hz, 2H), 3.91 (s, 3H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.7, 155.6, 151.3, 134.6, 129.4, 129.1, 128.3, 121.7, 111.0, 54.7, 52.3, 36.9. Peak

overlap observed in aromatic region burying the triazole carbon peaks; HRMS (ESI+): Calculated for $C_{16}H_{17}ClN_7O_2$ [M+H] – 374.1127, Found – 374.1124 (± 0.74 ppm)

3-amino-5-(((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)amino)-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (14): Synthesized by General Procedure B from starting material **13.** Rf = 0.3 (DCM: MeOH: NH_4OH – 85: 13: 2). 68% yield.; ^{1}H NMR (500 MHz, CD_3OD) δ 8.00 (d, J = 1.7 Hz, 1H), 7.51 - 7.14 (m, 5H), 5.56 (d, J = 1.7 Hz, 2H), 4.70 (d, J = 1.7 Hz, 2H).; ^{13}C NMR (126 MHz, CD_3OD) δ 165.8, 156.2, 155.8, 152.7, 144.3, 134.9, 128.9, 128.6, 128.1, 124.6, 121.1, 109.2, 54.5, 35.7. HRMS (ESI+): Calculated for $C_{16}H_{18}ClN_{10}O$ [M+H] – 401.1348, Found – 401.1347 (± 0.4 ppm).; HPLC analysis: Ret. time – 7.364 min, Purity – 93%

Methyl 3-amino-6-chloro-5-(dimethylamino)pyrazine-2-carboxylate (15)[1, 7]: Synthesized by General Procedure A, using 5,6 dichloropyrazine methyl ester **2** and dimethyl amine. 80% yield, Rf: 0.3 (4:1 - Hexanes: EtOAc)**;** ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (s, 4H), 3.15 (s, 6H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.6, 154.6, 154.0, 121.9, 112.2, 52.3, 41.2.; HRMS (ESI+): Calculated for $C_8H_{12}ClN_4O_2$ [M+H] - 231.0649, Found – 231.0653 (± 0.5 ppm).

Methyl 3-amino-5-(dimethylamino)-6-(phenylethynyl)pyrazine-2-carboxylate (16): Synthesized by General Procedure C using starting material **15** and phenyl acetylene. 83% yield, Rf = 0.3 (3:2 – Hexanes: EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 - 7.39 (m, 2H), 7.35 - 7.16 (m, 3H), 3.87 (s, 3H), 3.30 (s, 6H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ166.5, 156.7, 153.6, 131.1, 128.3, 123.1, 114.6, 113.7, 91.7, 88.6, 52.1, 40.2. Peak overlap observed in the aromatic region.; HRMS (ESI+): Calculated for $C_{16}H_{17}N_4O_2$ [M+H] – 297.1352, Found – 297.1351 (± 0.2 ppm).

3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-(phenylethynyl)pyrazine-2-carboxamide (17): Synthesized by General Procedure B using starting material **16**. 57% yield, Rf = 0.1 (85:13:2 – DCM: MeOH: NH_4OH).; ^{1}H NMR (500 MHz, CD_3OD) δ 7.62 - 7.50 (m, 2H), 7.43 (tt, J = 3.0, 1.7 Hz, 3H), 3.46 (s, 6H).; ^{13}C NMR (126 MHz, CD_3OD) δ 165.7, 157.1, 155.7, 154.2, 130.9, 128.9, 128.6, 122.7, 114.0, 111.9, 91.8, 87.9, 39.8. HRMS (ESI+): Calculated for $C_{16}H_{18}N_7O$ [M+H] – 324.1567, Found – 324.1566 (± 0.37 ppm).; HPLC analysis: Ret. time – 8.560 min, Purity – 97%

3,5-diamino-*N*-(diaminomethylene)-6-(phenylethynyl)pyrazine-2-carboxamide (19)[4, 8]: Synthesized by General Procedure C using commercial amiloride hydrochloride **18** and phenyl acetylene. 61% yield, Rf < 0.1 (85:13:2 – DCM: MeOH: NH_4OH).;^{1}H NMR (500 MHz, CD_3OD) δ 7.75 - 7.58 (m, 2H), 7.50 - 7.37 (m, 3H).; ^{13}C

NMR (126 MHz, CD_3OD) δ 165.9, 158.2, 156.2, 155.8, 131.6, 129.0, 128.5, 122.3, 114.5, 111.9, 92.9, 83.5.; HRMS (ESI+): Calculated for $C_{14}H_{14}N_7O$ [M+H] – 296.1254, Found – 296.1255 (± 0.35 ppm); HPLC analysis: Ret. time – 7.454 min, Purity – 95%

3,5-diamino-*N*-(diaminomethylene)pyrazine-2-carboxamide (20)[1, 4]: To a suspension of 10% palladium on carbon (0.048 g, 0.038 mmol, 0.1 equiv) in methanol under a nitrogen atmosphere was added a solution of amiloride hydrochloride **18** (0.100 g, 0.38 mmol, 1 equiv). A hydrogen filled balloon was attached to the reaction flask and the solution was stirred for 48h at room temperature and monitored by HPLC analysis. When the HPLC analysis showed that the starting material peak at 5.743 min was significantly diminished, the reaction was worked up by filtering off the catalyst on a bed of Celite. The solvent was then removed under reduced pressure and the resulting white residue was re-crystallized from a mixture of ethanol+ water (1:1) and dried under high vacuum to yield **20** as a white solid. 88% yield.; ^1H NMR (500 MHz, D_2O) δ 7.33 (s, 1H), 3.71 (bs, 0.5H, NH), 3.31 (bs, 0.5H, NH).; ^{13}C NMR (126 MHz, D_2O) [Chemical shifts referenced to assumed guanidine carbon at 165.5] δ 165.5, 154.5, 153.8, 152.2, 123.2, 110.4.; HRMS (ESI+): Calculated for $C_6H_{10}N_7O$[M+H] – 196.0941, Found – 196.0943 (± 0.8 ppm).; HPLC analysis: Ret. time – 3.921 min, Purity – 91%.

Methyl 3-amino-5-(dimethylamino)-6-phenylpyrazine-2-carboxylate (21a): Synthesized by General Procedure D using starting material **15** and benzeneboronic acid. 51% yield.; Rf = 0.25 (4:1 Hexanes: EtOAc); ^1H NMR (500 MHz, $CDCl_3$) δ 7.58 (d, J = 7.6 Hz, 2H), 7.39 (t, J = 7.3 Hz, 2H), 7.29 (t, J = 7.3 Hz, 1H), 3.90 (s, 3H), 2.83 (s, 6H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.7, 156.1, 153.9, 140.4, 132.5, 128.7, 128.0, 127.6, 113.2, 52.2, 41.0. HRMS (ESI+): Calculated for $C_{14}H_{17}N_4O_2$ [M+H] – 273.1346, Found - 273.1347 (± 0.55 ppm).

Methyl 6-([1,1'-biphenyl]-4-yl)-3-amino-5-(dimethylamino)pyrazine-2-carboxylate (21b): Synthesized by General Procedure D using starting material **15** and [1,1'-biphenyl]-4-ylboronic acid. 92% yield.; Rf = 0.3 (3:1 Hexanes: EtOAc); ^1H NMR (500 MHz, $CDCl_3$) δ 7.73 - 7.52 (m, 6H), 7.47 - 7.39 (m, 2H), 7.37 - 7.29 (m, 1H), 3.89 (s, 3H), 2.86 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.4, 155.8, 153.6, 140.8, 140.1, 139.1, 131.8, 128.8, 128.1, 127.3, 127.2, 127.0, 113.1, 52.0, 40.8. Peak overlap observed in aromatic region of the spectrum.; HRMS (ESI+): Calculated for $C_{20}H_{21}N_4O_2$ [M+H] – 349.1659, Found – 349.1660 (± 1.0 ppm).

Methyl 3-amino-6-(4-cyanophenyl)-5-(dimethylamino)pyrazine-2-carboxylate (21c): Synthesized by General Procedure D using starting material **15** and (4-cyanophenyl)boronic acid. 86% yield.; Rf = 0.2 (3:2

Hexanes: EtOAc)**.;** ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 - 7.69 (m, 2H), 7.68 - 7.64 (m, 2H), 3.88 (s, 3H), 2.82 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.4, 156.1, 154.1, 144.9, 132.7, 129.6, 128.5, 119.3, 114.3, 110.8, 52.5, 41.3. HRMS (ESI+): Calculated for $C_{15}H_{16}N_5O_2$ [M+H] – 298.1299, Found – 298.1298 (± 0.52 ppm).

Methyl 3-amino-5-(dimethylamino)-6-(naphthalen-2-yl)pyrazine-2-carboxylate (21d): Synthesized by General Procedure D using starting material **15** and naphthalen-2-ylboronic acid. 88% yield.; Rf = 0.3 (4:1 Hexanes: EtOAc)**.;** ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (d, J = 1.9 Hz, 1H), 7.90-7.75 (m, 3H), 7.72 (dd, J = 8.4, 1.8 Hz, 1H), 7.53 - 7.35 (m, 2H), 3.92 (s, 3H), 2.84 (s, 6H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.7, 156.3, 153.9, 137.9, 133.8, 133.0, 132.3, 128.4, 128.4, 127.9, 126.8, 126.4, 126.2, 126.1, 113.4, 52.3, 41.0.; HRMS (ESI+): Calculated for $C_{18}H_{19}N_4O_2$ [M+H] – 323.1503, Found – 323.1501 (± 0.45 ppm).

Methyl 3-amino-5-(dimethylamino)-6-(*p*-tolyl)pyrazine-2-carboxylate (21e): Synthesized by General Procedure D using starting material **15** and *p*-tolylboronic acid. 94% yield.; Rf = 0.35 (4:1 Hexanes: EtOAc)**.;** ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 - 7.39 (m, 2H), 7.23 - 7.10 (m, 2H), 3.91 (s, 3H), 2.84 (s, 6H), 2.37 (s, 3H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.8, 156.1, 153.8, 137.6, 137.4, 132.8, 129.4, 127.9, 113.1, 52.2, 40.9, 21.5; HRMS (ESI+): Calculated for $C_{15}H_{19}N_4O_2$ [M+H] – 287.1503, Found – 287.1505 (± 0.78 ppm).

Methyl 3-amino-5-(dimethylamino)-6-(4-fluorophenyl)pyrazine-2-carboxylate (21f): Synthesized by General Procedure D using starting material 15 and (4-fluorophenyl)boronic acid. 63% yield.; Rf = 0.3 (4:1 Hexanes: EtOAc).; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (ddq, J = 7.8, 4.5, 1.1 Hz, 2H), 7.11 - 7.02 (m, 2H), 3.88 (s, 3H), 2.81 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.3, 163.4, 160.9, 155.9, 153.6, 136.2, 131.3, 129.45 (d, $J_{C\text{-}F}$ = 8.4 Hz), 115.4 (d, $J_{C\text{-}F}$ = 21.6 Hz), 113.0, 52.0, 40.7. One extra peak in the aromatic region, due to strongly split fluorine substituted carbon in aromatic ring. 1 bond C-F coupling pattern for this peak is indiscernible in this spectrum. ; HRMS (ESI+): Calculated for $C_{14}H_{16}FN_4O_2$ [M+H] – 291.1252, Found – 291.1253 (± 0.78 ppm).

3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-phenylpyrazine-2-carboxamide (22a) [4, 9]: Synthesized by General Procedure B using starting material **22a**. 70% yield. Rf = 0.2 (85:13:2 – DCM:MeOH:NH_4OH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.62 -7.56 (m, 2H), 7.50 (dd, J = 8.4, 6.9 Hz, 2H), 7.45 - 7.40 (m, 1H), 2.95 (s, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 166.4, 155.7, 155.5, 153.6, 139.5, 132.4, 128.5, 128.0, 110.6, 40.5. Peak overlap in the aromatic region of spectrum. HRMS (ESI+): Calculated for $C_{14}H_{18}N_7O$ [M+H] – 300.1567, Found – 300.1566 (± 0.49 ppm); HPLC analysis: Ret. time – 8.261 min, Purity – 96%

6-([1,1'-biphenyl]-4-yl)-3-amino-*N*-(diaminomethylene)-5-(dimethylamino)pyrazine-2-carboxamide (22b): Synthesized by General Procedure B using starting material **22b**. 51% yield. Rf = 0.3 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 7.79 - 7.72 (m, 2H), 7.71 - 7.65 (m, 4H), 7.48 (dd, J = 8.5, 6.9 Hz, 2H), 7.41 - 7.35 (m, 1H), 2.95 (s, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 167.5, 158.0, 156.7, 155.7, 141.7, 141.5, 139.8, 132.8, 129.8, 129.4, 128.5, 127.9, 127.7, 111.7, 41.1.; HRMS (ESI+): Calculated for $C_{20}H_{22}N_7O$ [M+H] – 376.1880, Found – 376.1878 (± 0.68 ppm); HPLC analysis: Ret. time – 9.358 min, Purity – 97%

3-amino-6-(4-cyanophenyl)-*N*-(diaminomethylene)-5-(dimethylamino)pyrazine-2-carboxamide (22c): Synthesized by General Procedure B using starting material **22c**. 54% yield. Rf <0.2 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 7.87 - 7.83 (m, 2H), 7.82 - 7.79 (m, 2H), 2.92 (s, 6H).; ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.4, 163.2, 161.2, 156.6, 155.2, 154.1, 136.1, 130.6, 116.0, 115.8, 111.0, 41.2. HRMS (ESI+): Calculated for $C_{15}H_{17}N_8O$ [M+H] – 325.1520, Found – 325.1519 (± 0.37 ppm); HPLC analysis: Ret. time – 7.818 min, Purity – 99%

3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-(naphthalen-2-yl)pyrazine-2-carboxamide (22d): Synthesized by General Procedure B using starting material **22d**. 66% yield. Rf = 0.2 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 8.08 - 7.89 (m, 4H), 7.75 (dd, J = 8.5, 1.8 Hz, 1H), 7.59 - 7.51 (m, 2H), 2.92 (s, 6H).; ^{13}C NMR (126 MHz, CD_3OD) δ 167.6, 158.0, 156.8, 155.6, 138.1, 134.6, 134.2, 133.2, 129.3, 129.2, 128.7, 127.7, 127.6, 127.5, 126.9, 111.9, 41.3; HRMS (ESI+): Calculated for $C_{18}H_{20}N_7O$ [M+H] – 350.1724, Found – 350.1729 (± 1.37 ppm); HPLC analysis: Ret. time – 8.725 min, Purity – 99%

3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-(*p*-tolyl)pyrazine-2-carboxamide (22e): Synthesized by General Procedure B using starting material **22e**. 31% yield. Rf = 0.3 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 7.50 - 7.44 (m, 2H), 7.31 (dt, J = 6.8, 1.0 Hz, 2H), 2.93 (s, 6H), 2.42 (s, 3H).; ^{13}C NMR (126 MHz, CD_3OD) δ 166.5, 156.3, 155.8, 154.1, 138.0, 136.9, 132.6, 129.1, 127.9, 110.4, 40.2, 20.2.; HRMS (ESI+): Calculated for $C_{15}H_{20}N_7O$ [M+H] – 314.1724, Found: 314.1724 (± 0.45 ppm); HPLC analysis: Ret. time – 8.378 min, Purity – 97%

3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-(4-fluorophenyl)pyrazine-2-carboxamide (22f): Synthesized by General Procedure B using starting material 22f. 55% yield. Rf <0.2 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 7.75 - 7.46 (m, 2H), 7.22 (t, J = 8.8 Hz, 2H), 2.91 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.4, 163.2, 161.2, 156.6, 155.2, 154.4, 136.1, 130.6 (d, $J_{C\text{-}F}$ = 6.6 Hz), 116.0(d, $J_{C\text{-}F}$ =

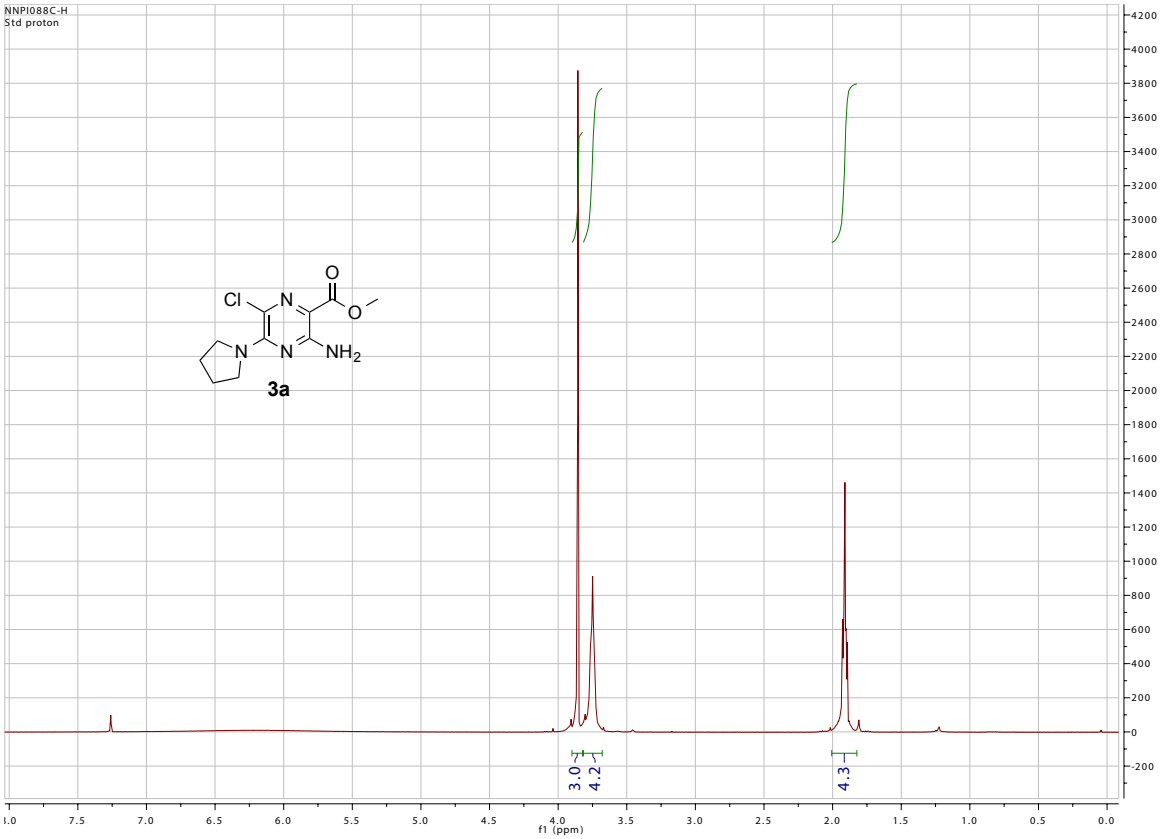
21.2 Hz), 111.0, 41.2. 1 bond C-F coupling pattern is indiscernible in this spectrum.; HRMS (ESI+): Calculated for $C_{14}H_{17}FN_7O$ [M+H] – 318.1473, Found – 318.1473 (± 0.37 ppm); HPLC analysis: Ret. time – 8.127 min, Purity – 99%

Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-phenylpyrazine-2-carboxylate (23): Synthesized by General Procedure D using starting material **3j** and phenylboronic acid. 89% yield.; Rf = 0.35 (1:1 Hexanes: EtOAc).; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (bs, 1H, NH), 7.61 (d, J = 7.8 Hz, 1H), 7.45 - 7.27 (m, 6H), 7.26 - 7.19 (m, 1H), 7.18 - 7.09 (m, 1H), 6.88 (d, J = 2.0 Hz, 1H), 5.39 (bs, 1H, NH), 3.91 (s, 3H), 3.76 (q, J = 6.4 Hz, 2H), 3.06 (t, J = 6.6 Hz, 2H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.0, 156.0, 153.4, 136.7, 132.5, 129.2, 128.7, 128.6, 127.4, 122.5, 122.3, 119.8, 118.9, 113.1, 111.5, 52.2, 41.5, 25.1. Peak overlap observed in aromatic region of the spectrum.; HRMS (ESI+): Calculated for $C_{22}H_{22}N_5O_2$ [M+H] – 388.1768, Found – 388.1768 (± 0.575 ppm).

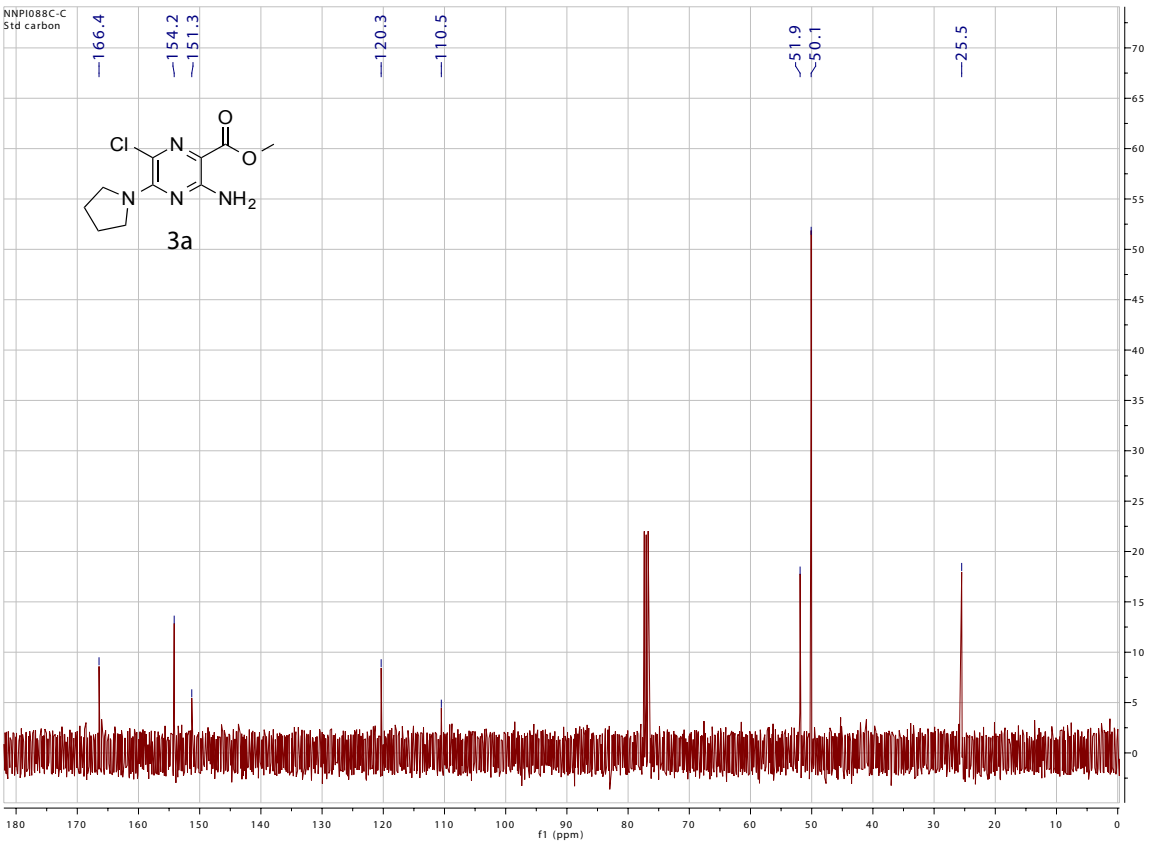
5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-(diaminomethylene)-6-phenylpyrazine-2-carboxamide (24): Synthesized by General Procedure B using starting material **23**. 62% yield.; Rf <0.2 ((85:13:2 – DCM: MeOH: NH_4OH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.54 (dt, J = 8.0, 1.0 Hz, 1H), 7.43 - 7.29 (m, 6H), 7.12 - 7.08 (m, 2H), 7.01 (ddd, J = 8.0, 7.0, 1.0 Hz, 1H), 3.82 (t, J = 6.9 Hz, 2H), 3.11 (t, J = 6.9 Hz, 2H).; ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.7, 156.4, 155.3, 154.4, 136.7, 136.1, 132.2, 129.5, 129.3, 127.8, 123.4, 121.7, 119.1, 112.2, 112.1, 109.4, 42.1, 24.7. Peak overlap observed in aromatic region of the spectrum.; HRMS (ESI+): Calculated for $C_{22}H_{23}N_8O$ [M+H] – 415.1989, Found – 415.1939 (± 0.45 ppm); HPLC analysis: Ret. time – 8.901 min, Purity – >99%

3. ^{1}H-NMR and ^{13}C-NMR spectra of compounds **3a - 24**

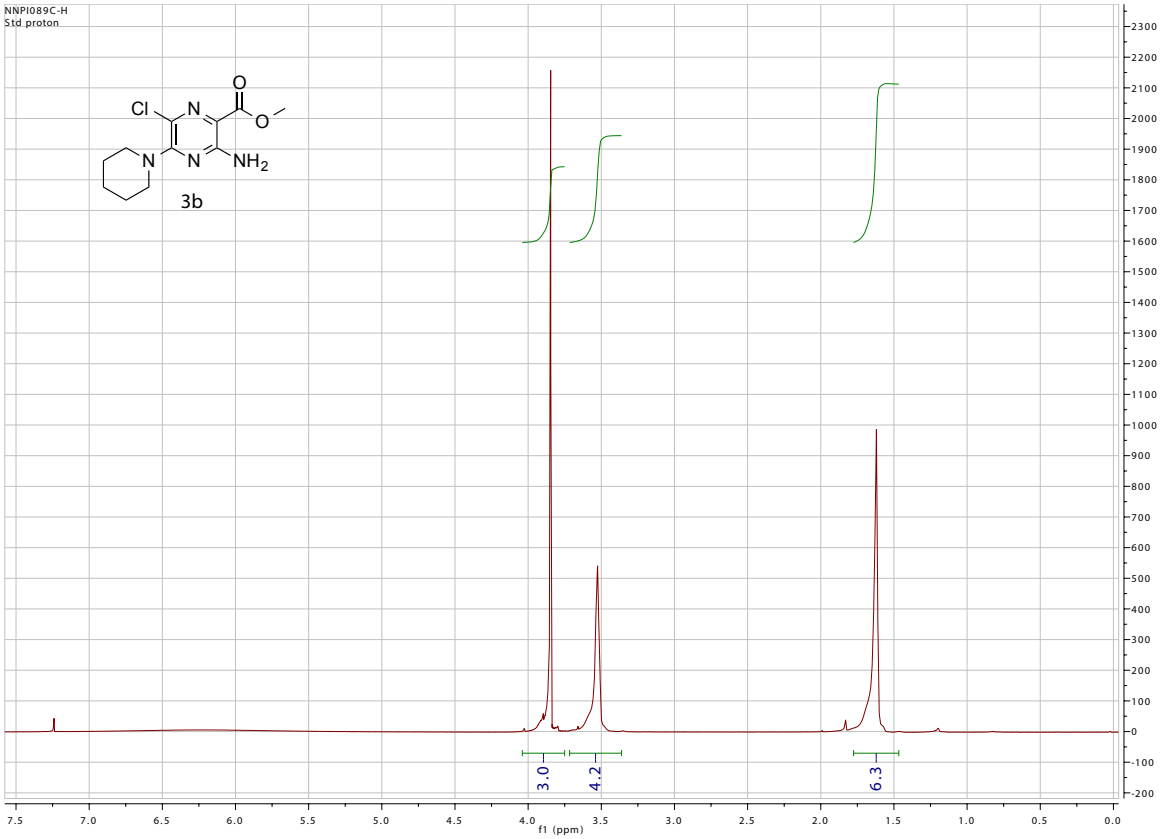
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-(pyrrolidin-1-yl)pyrazine-2-carboxylate (3a):



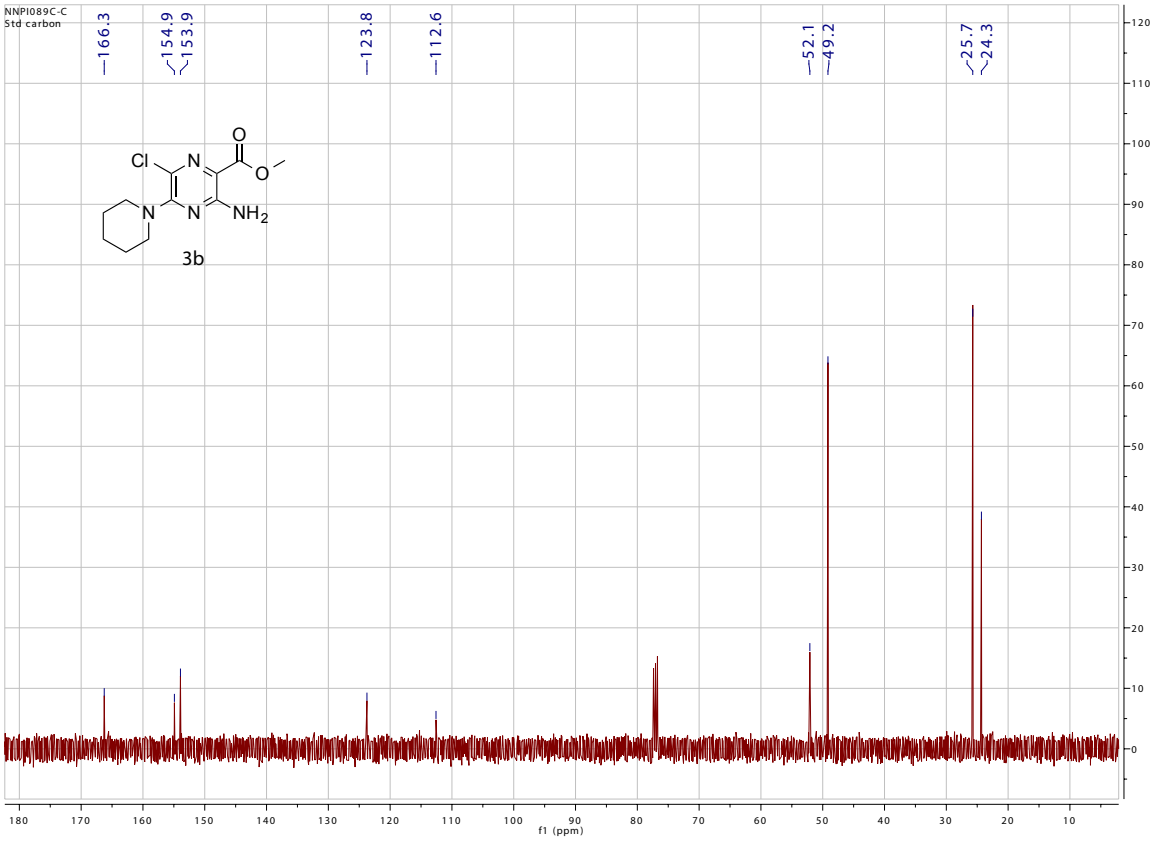
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-(pyrrolidin-1-yl)pyrazine-2-carboxylate (3a):



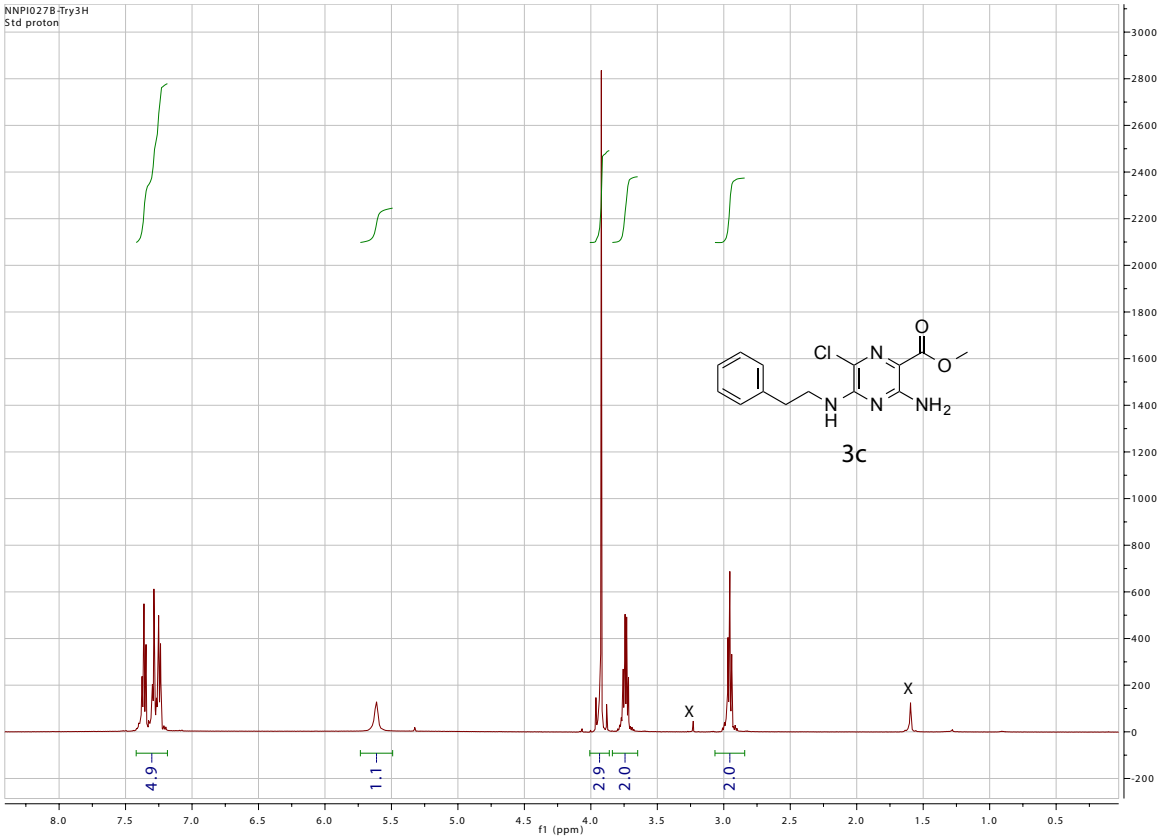
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-(piperidin-1-yl) pyrazine-2-carboxylate (3b):



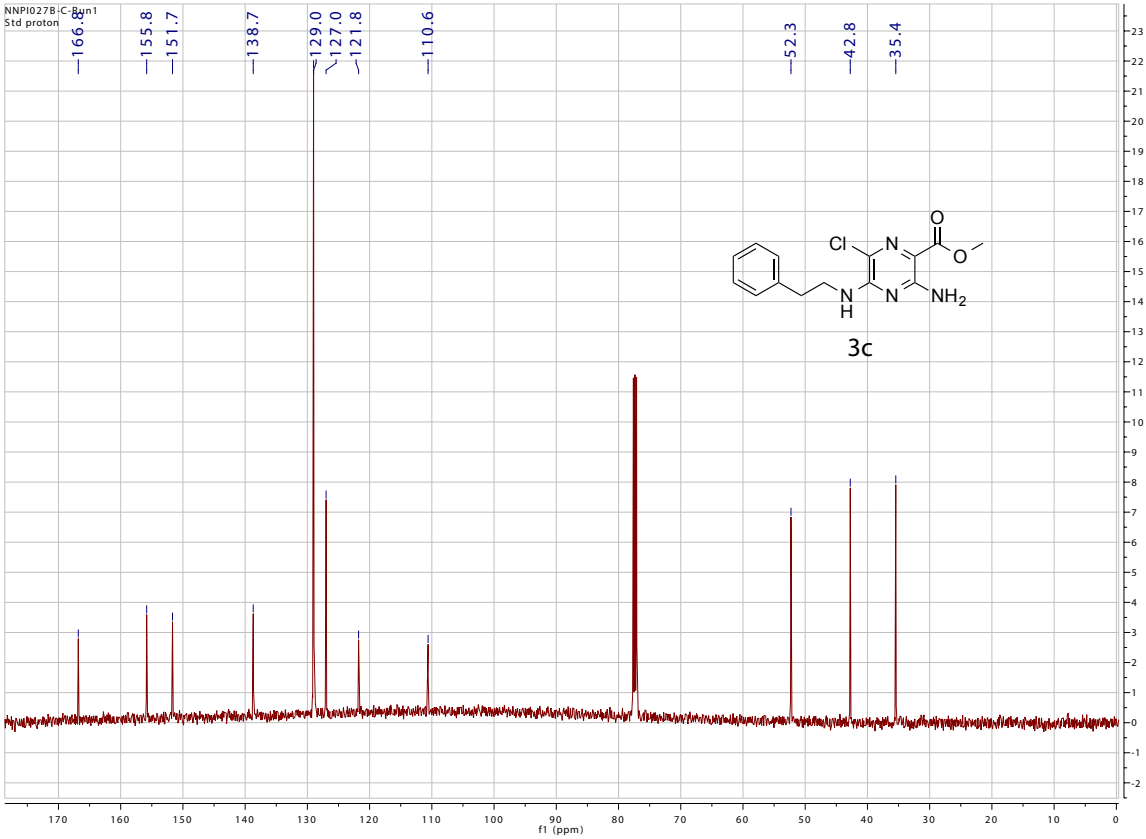
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-(piperidin-1-yl) pyrazine-2-carboxylate (3b):



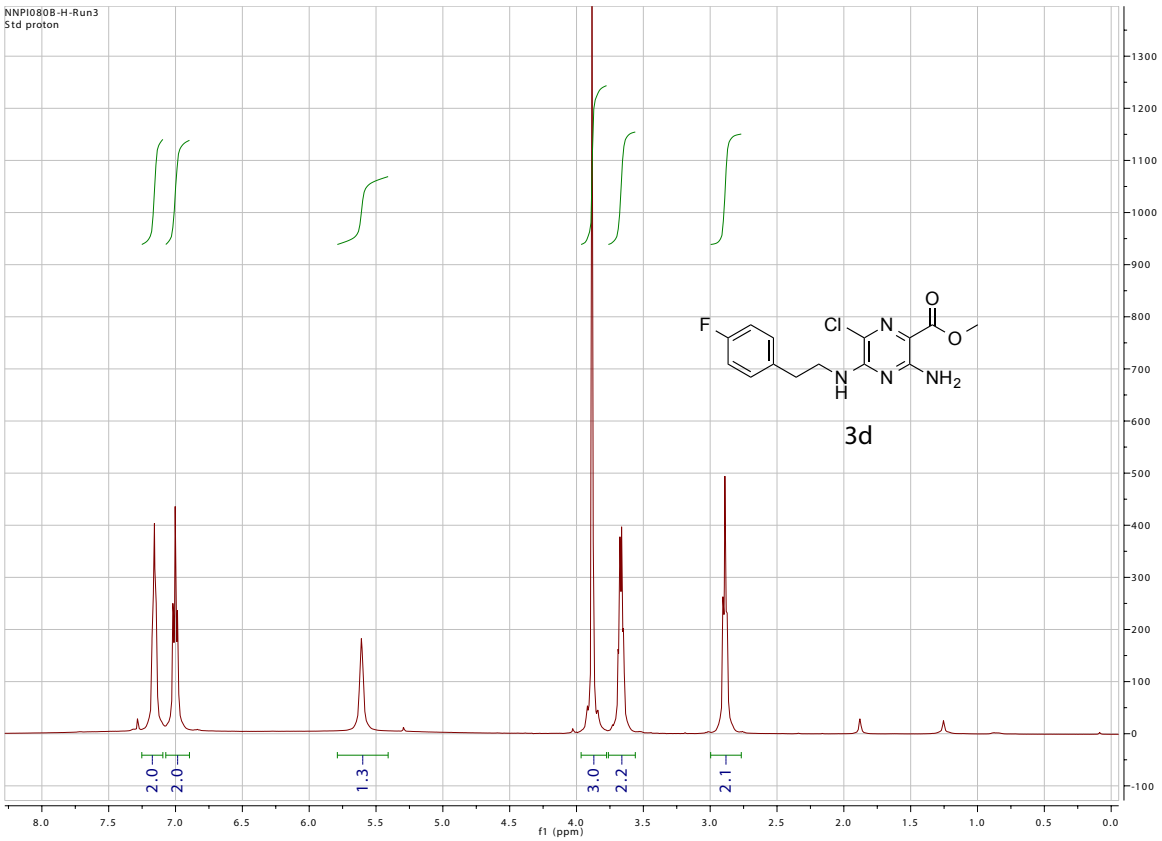
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-(phenethylamino)pyrazine-2-carboxylate (3c):



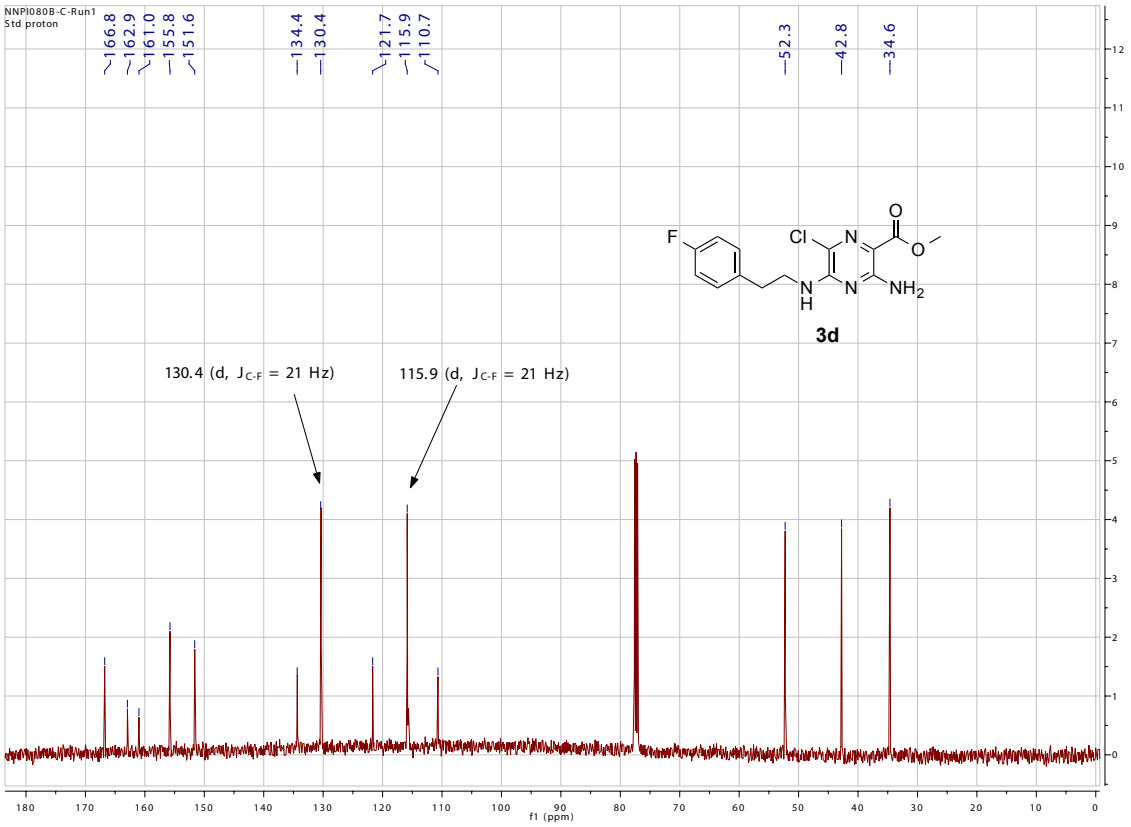
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-(phenethylamino)pyrazine-2-carboxylate (3c): Overlap of peaks observed in the aromatic region.



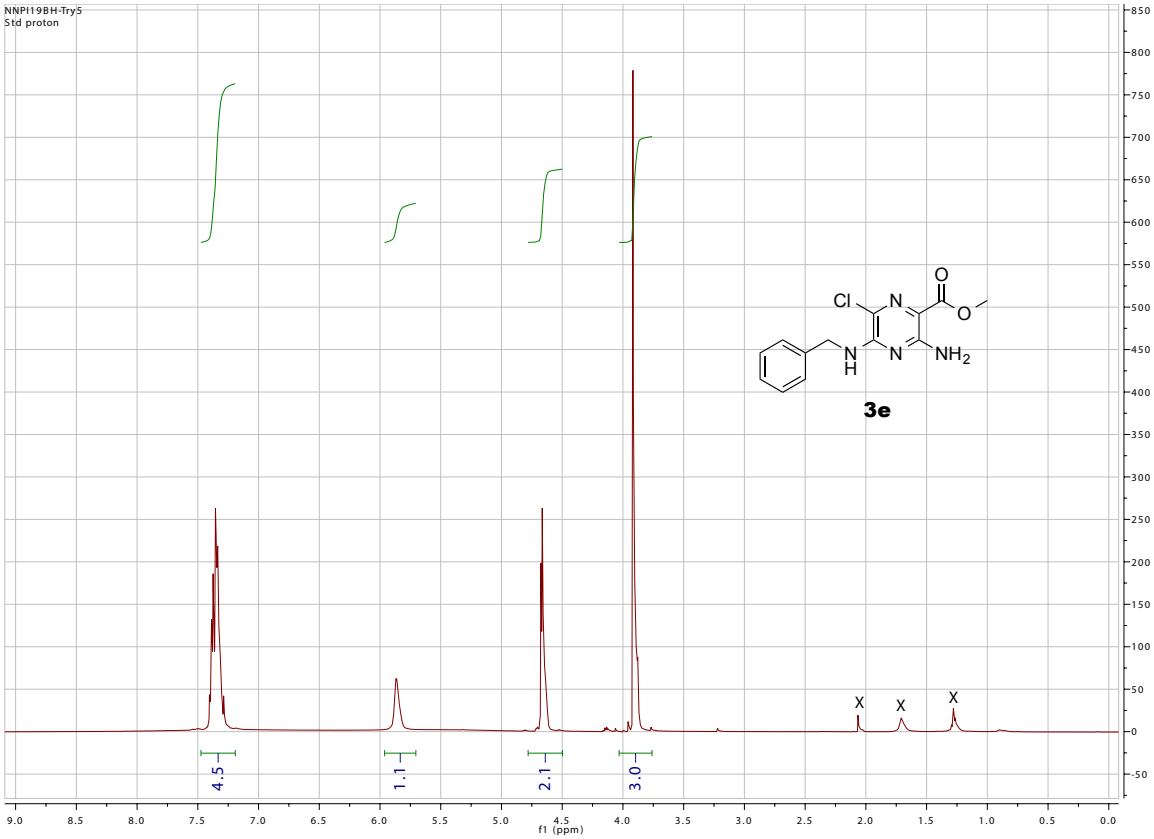
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-((4-fluorophenethyl)amino)pyrazine-2-carboxylate (3d):



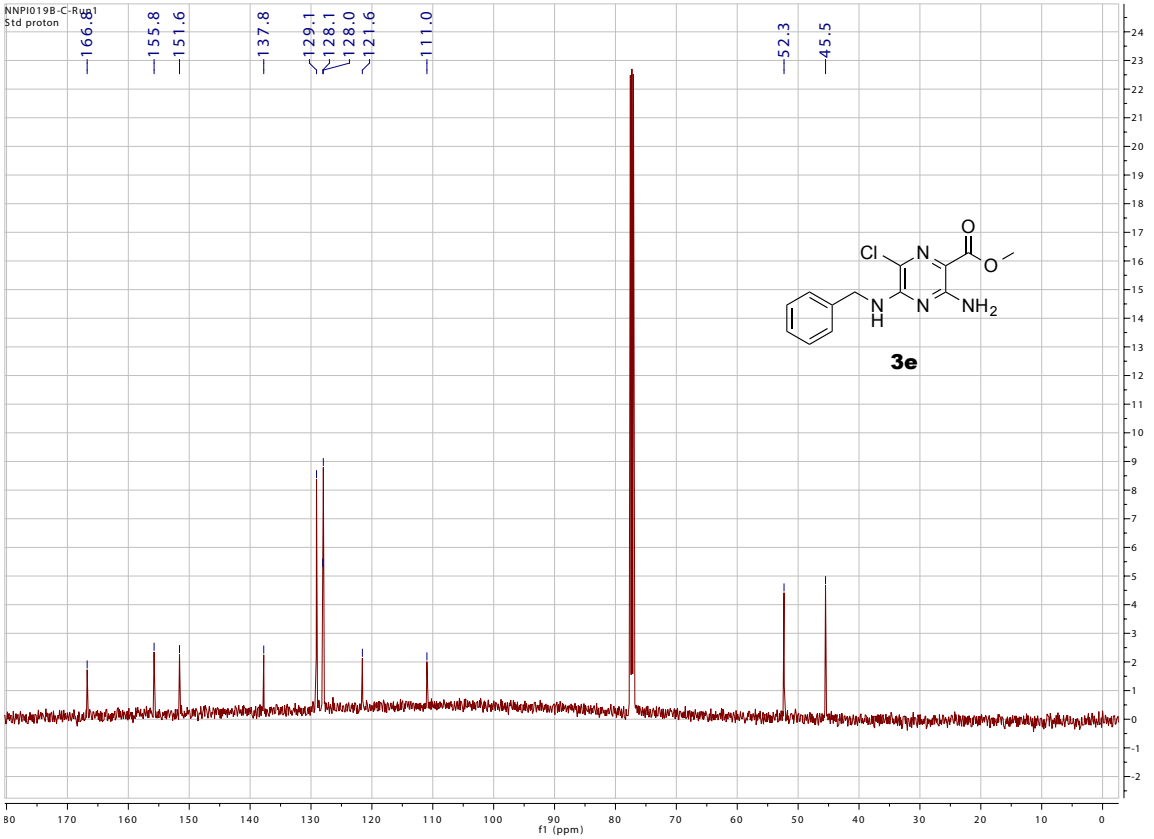
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-((4-fluorophenethyl)amino)pyrazine-2-carboxylate (3d): Splitting of peaks corresponding to C-F coupling seen at 130.4 ppm (d, J = 21Hz) and 115.9 ppm (d, J = 21 Hz). One extra peak seen in the aromatic region, due to strongly split fluorine substituted carbon in aromatic ring. The 1 bond C-F coupling pattern for this peak is indiscernible in this spectrum.



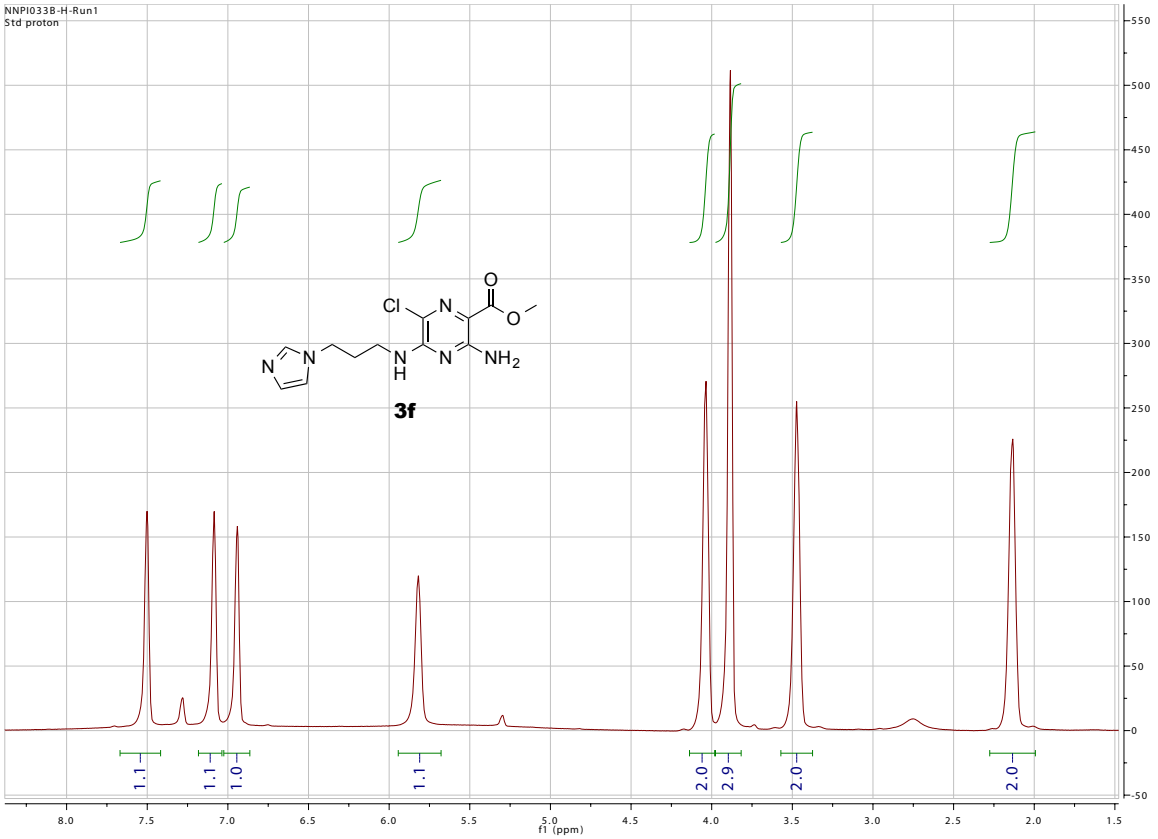
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-5-(benzylamino)-6-chloropyrazine-2-carboxylate (3e):



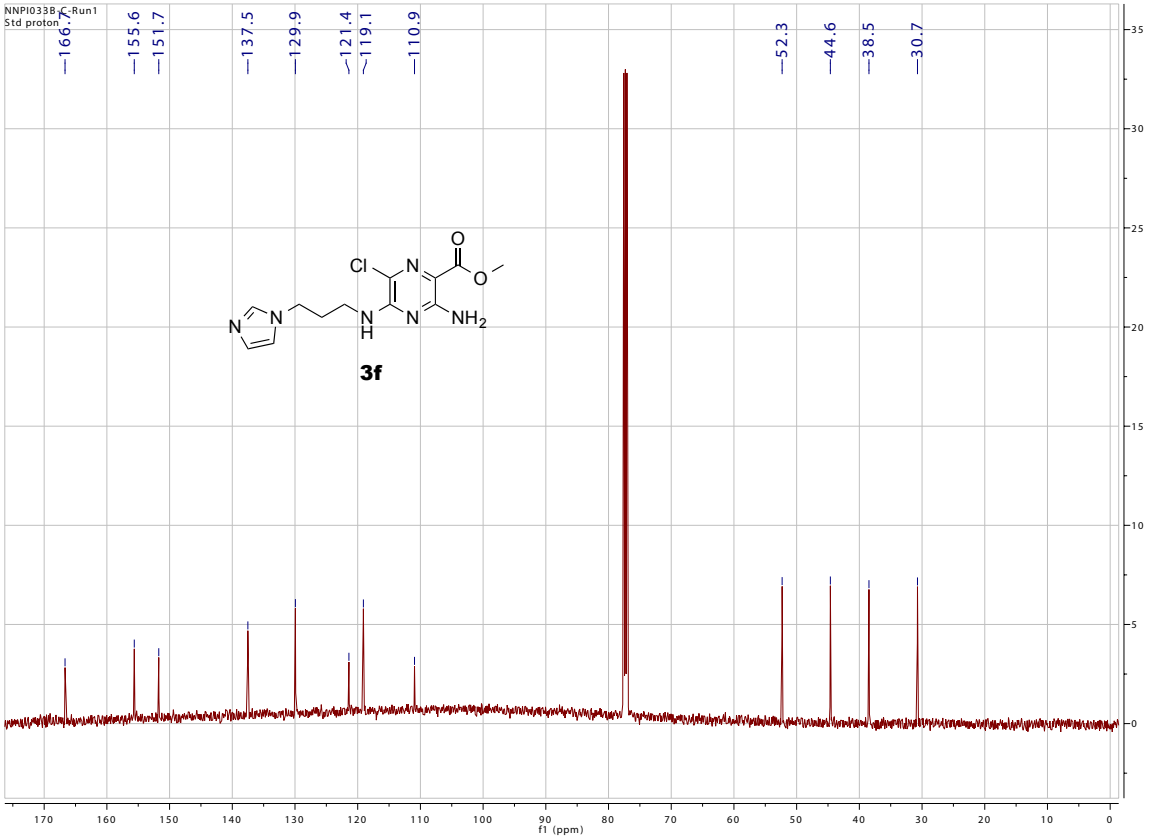
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-5-(benzylamino)-6-chloropyrazine-2-carboxylate (3e): Peak partial overlap seen at 128 ppm.



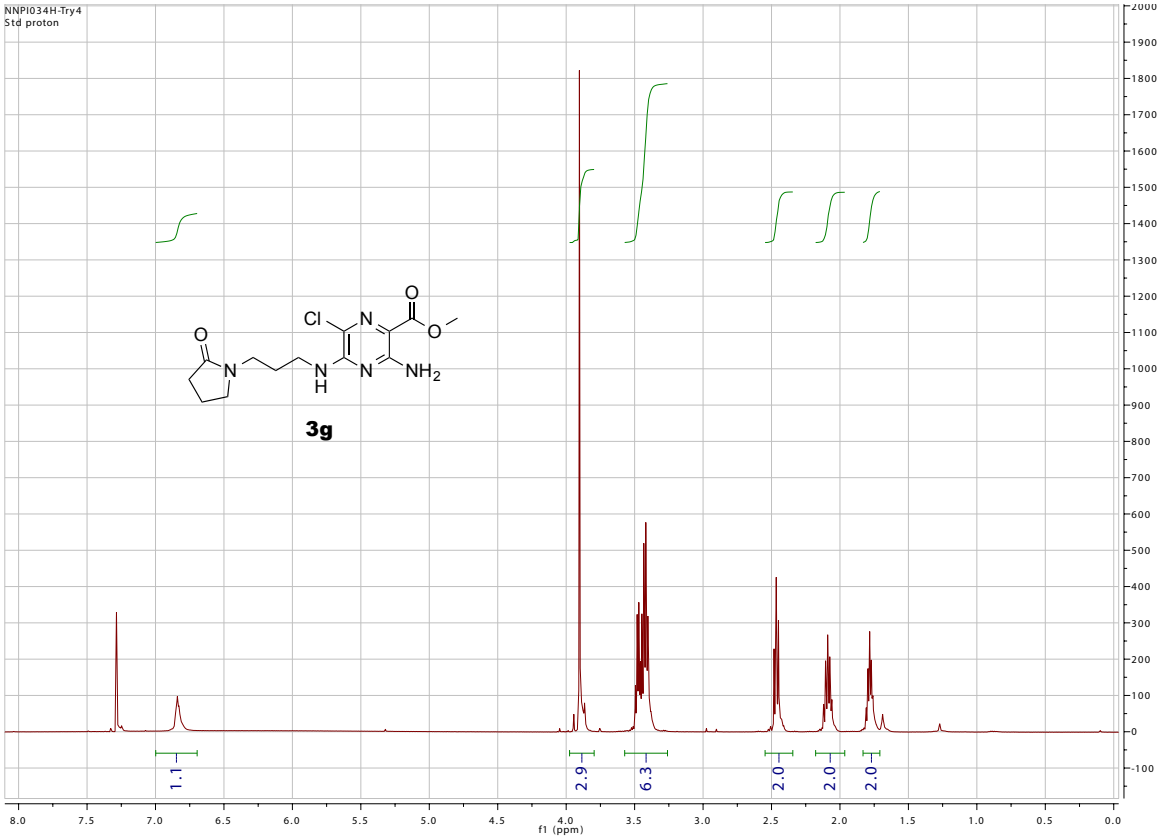
^{1}H NMR ($CDCl_3$) of Methyl 5-((3-(1*H*-imidazol-1-yl)propyl)amino)-3-amino-6-chloropyrazine-2-carboxylate (3f):



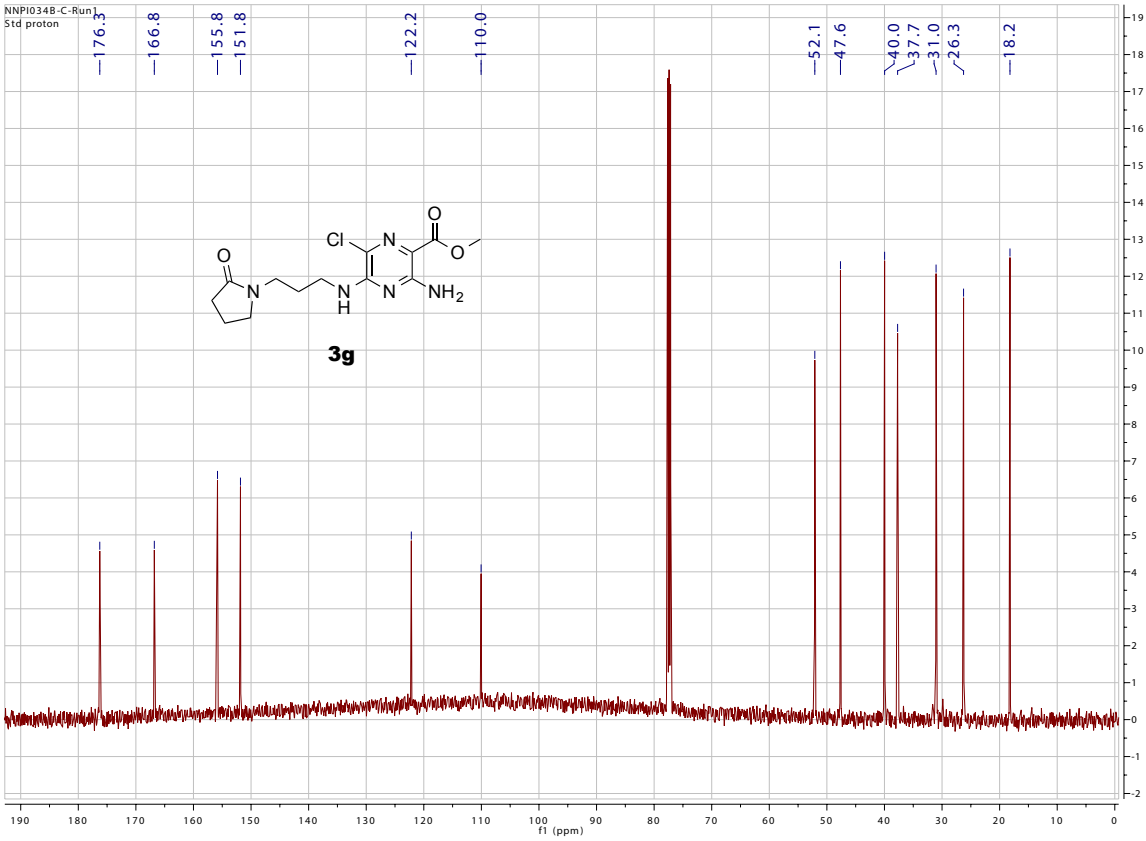
^{13}C NMR ($CDCl_3$) of Methyl 5-((3-(1*H*-imidazol-1-yl)propyl)amino)-3-amino-6-chloropyrazine-2-carboxylate (3f):



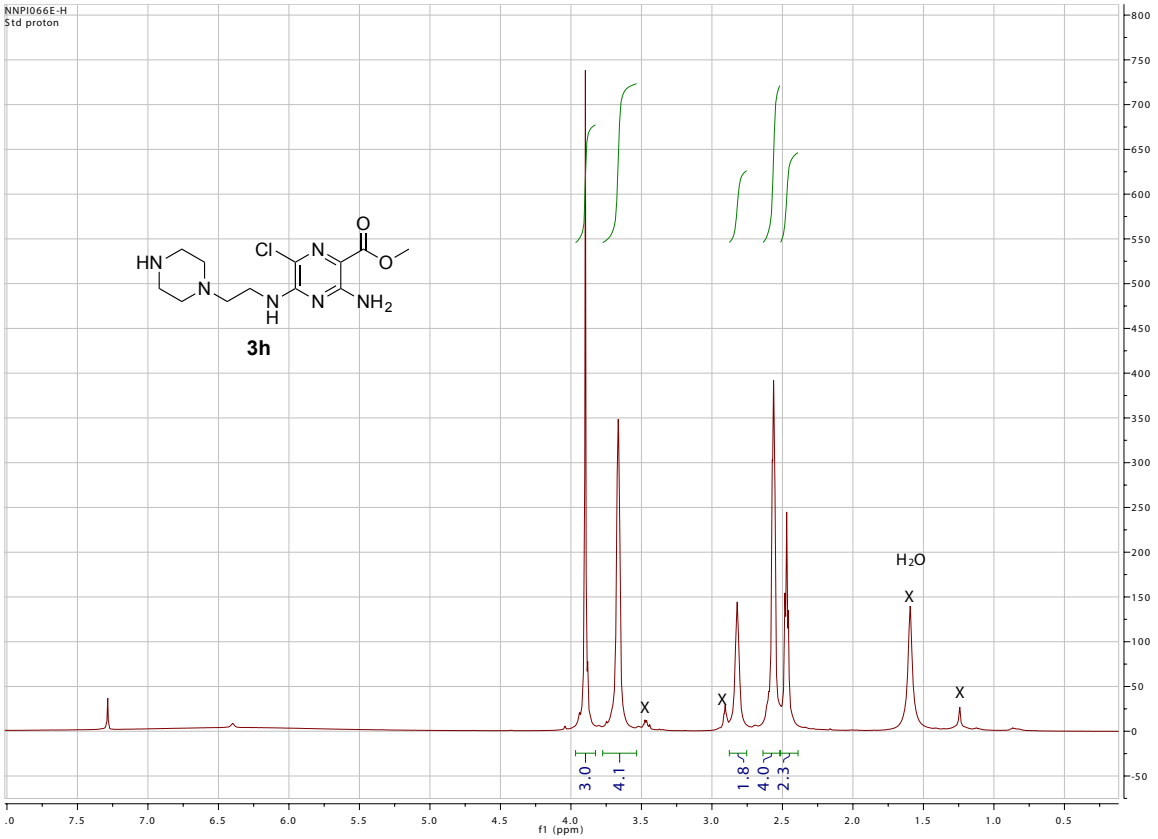
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-((3-(2-oxopyrrolidin-1-yl)propyl)amino)pyrazine-2-carboxylate (3g):



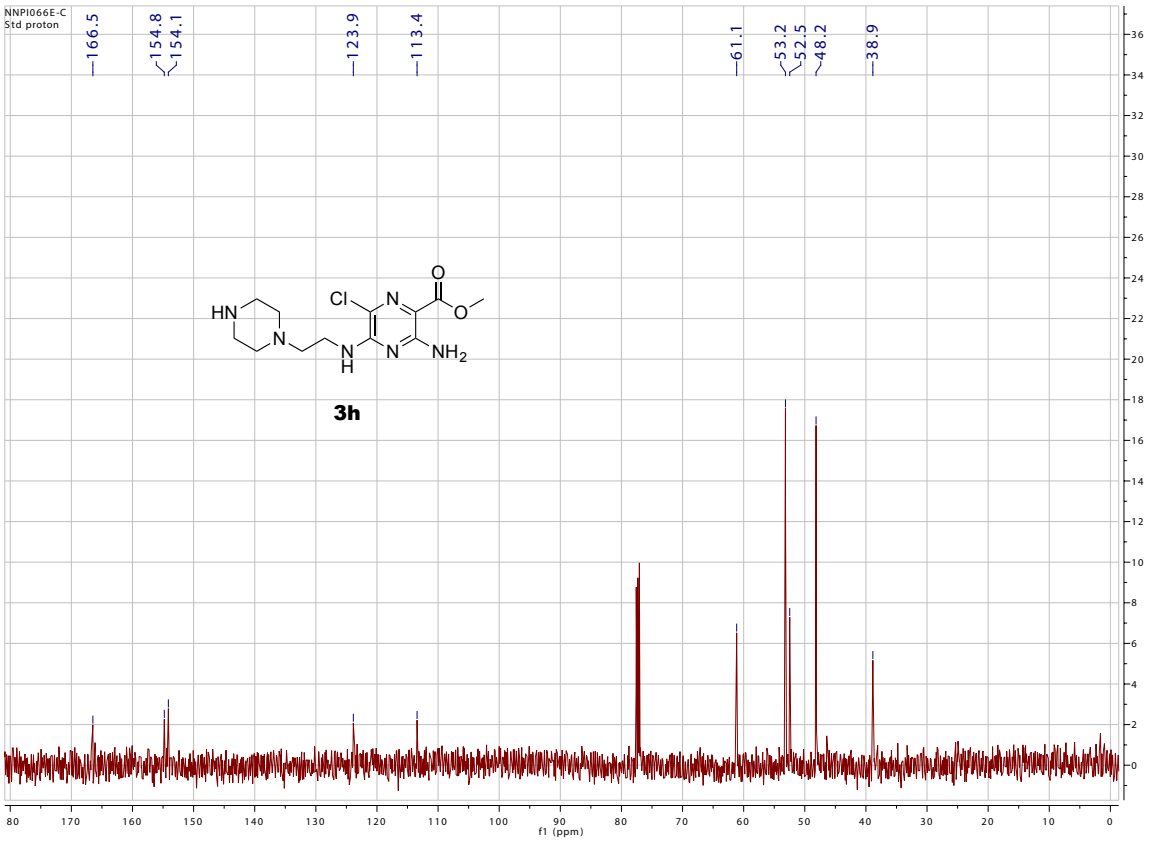
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-((3-(2-oxopyrrolidin-1-yl)propyl)amino)pyrazine-2-carboxylate (3g):



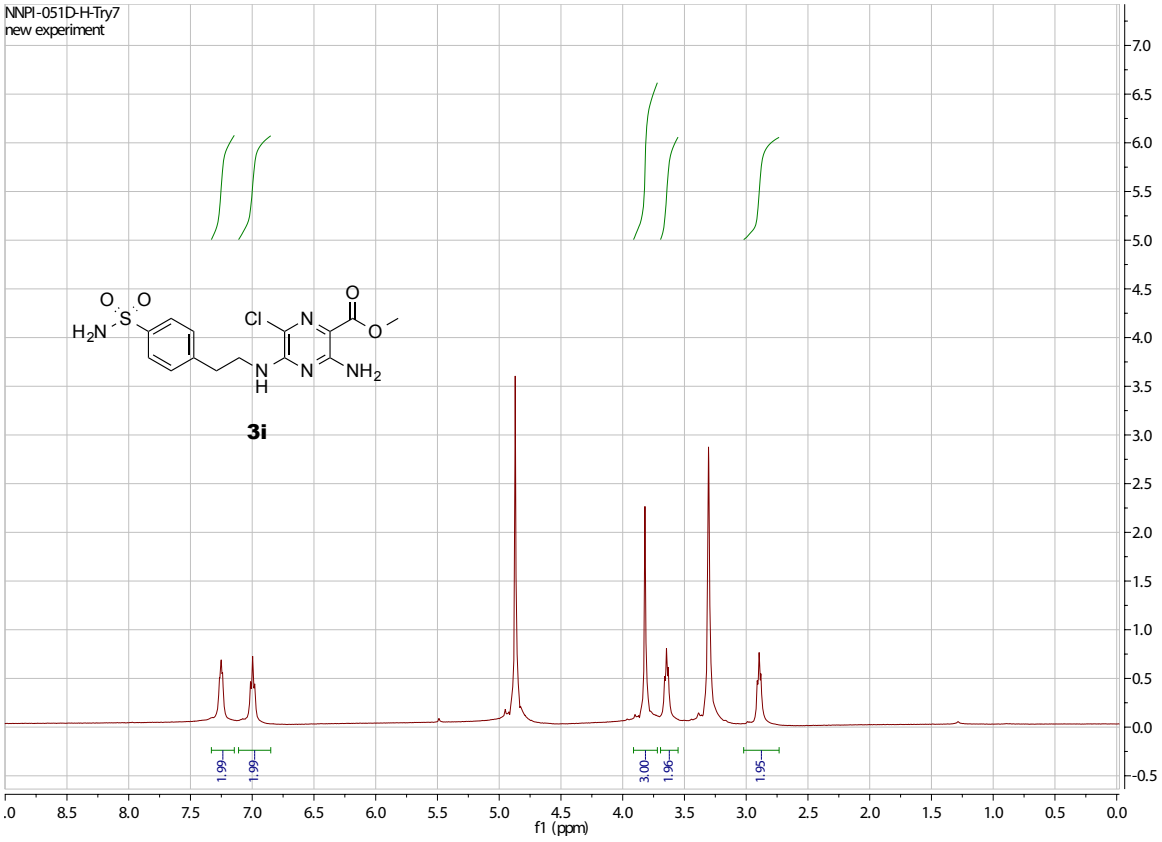
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-((2-(piperazin-1-yl)ethyl)amino)pyrazine-2-carboxylate (3h):



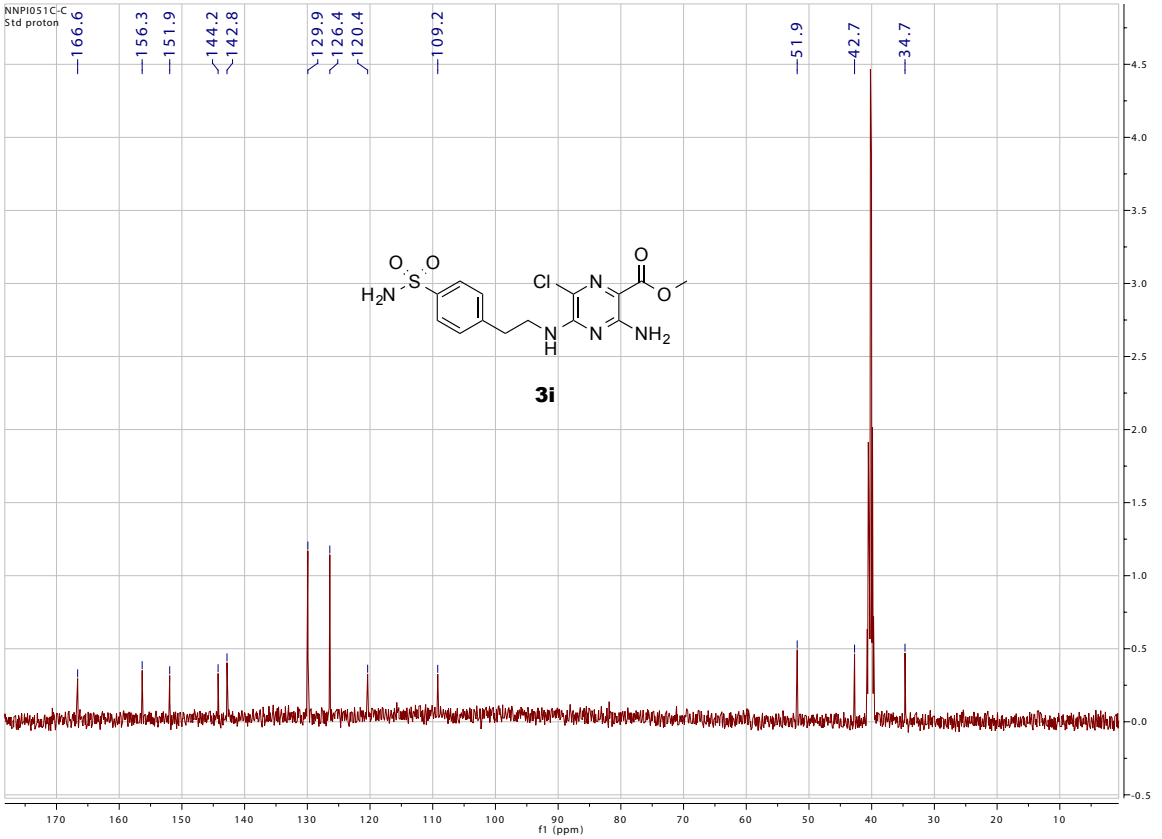
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-((2-(piperazin-1-yl)ethyl)amino)pyrazine-2-carboxylate (3h):



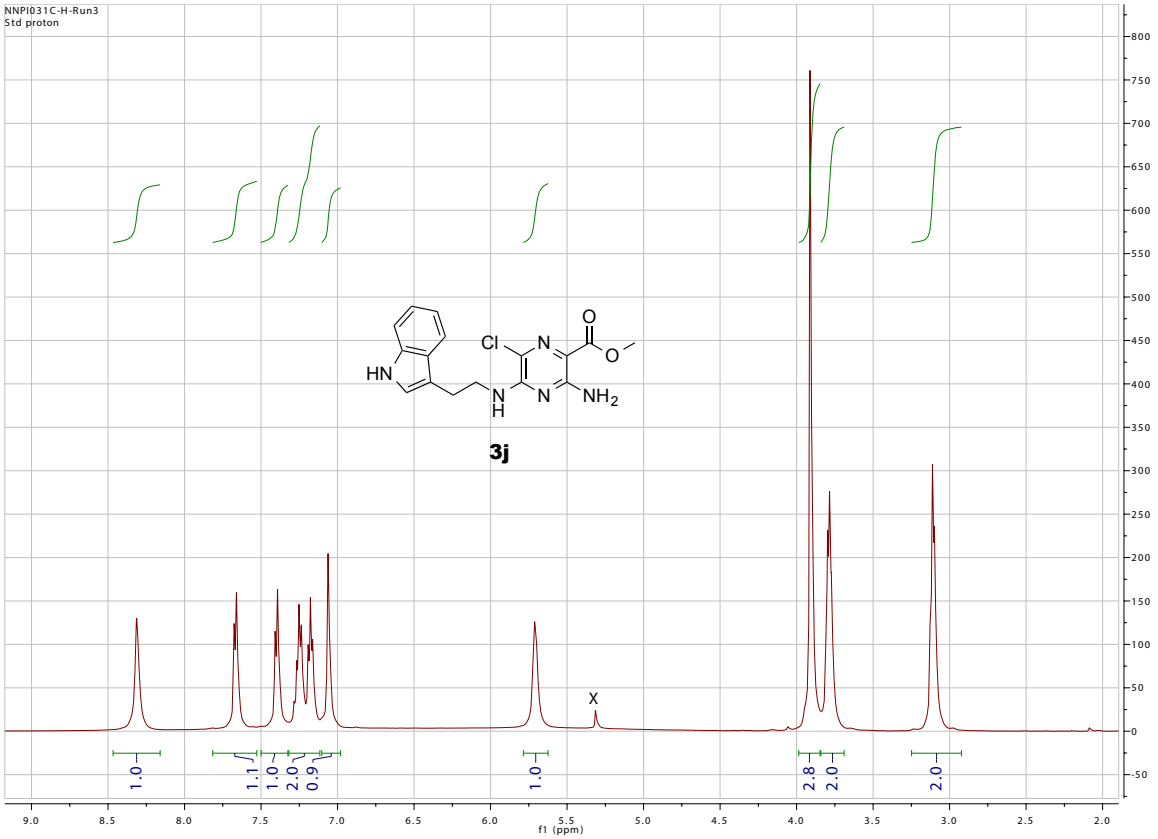
^{1}H NMR (CD_3OD) of Methyl 3-amino-6-chloro-5-((4-sulfamoylphenethyl)amino)pyrazine-2-carboxylate (3i):



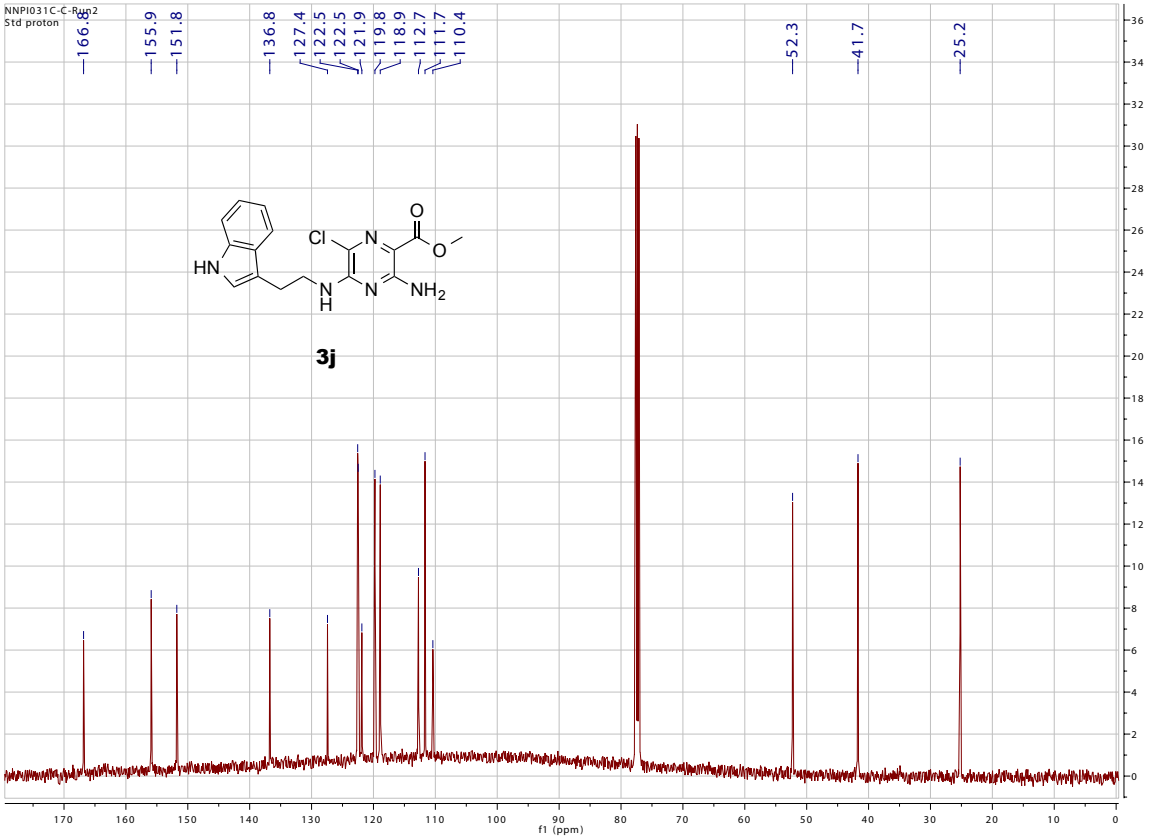
^{13}C NMR (DMSO-d_6) of Methyl 3-amino-6-chloro-5-((4-sulfamoylphenethyl)amino)pyrazine-2-carboxylate (3i):



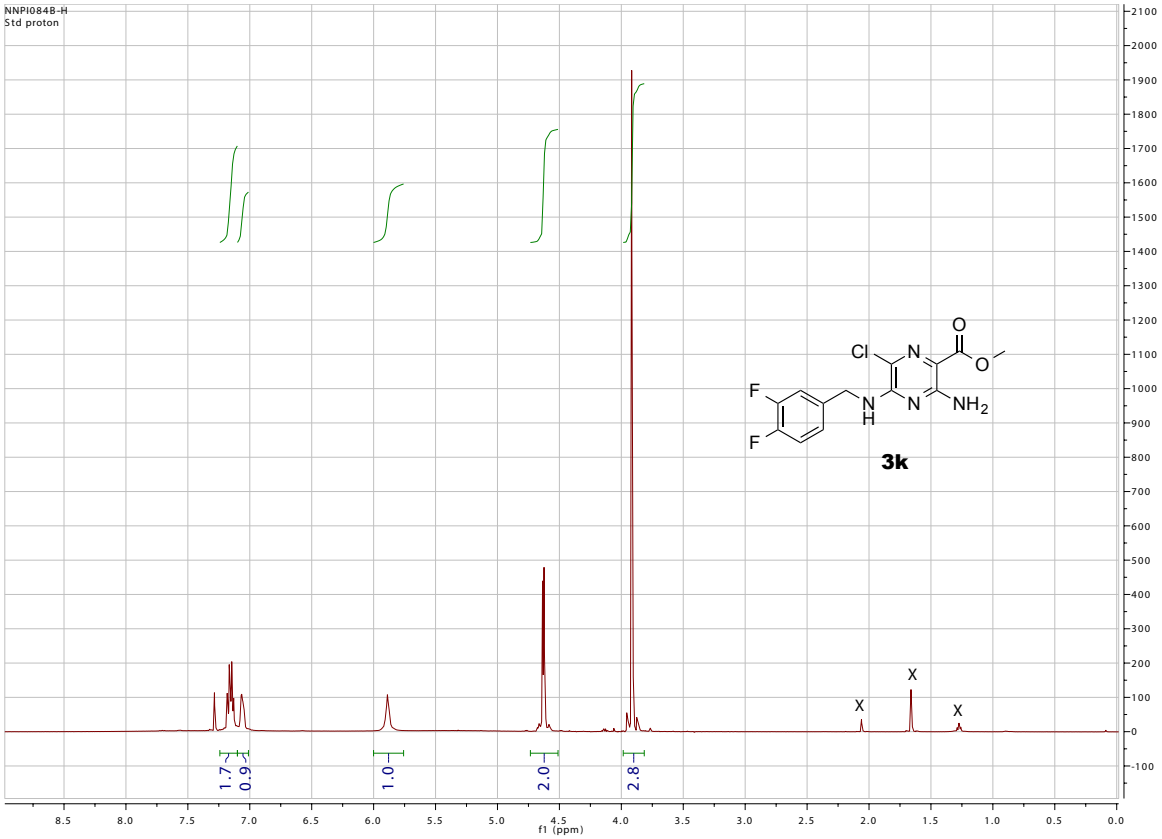
^{1}H NMR ($CDCl_3$) of Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-chloropyrazine-2-carboxylate (3j):



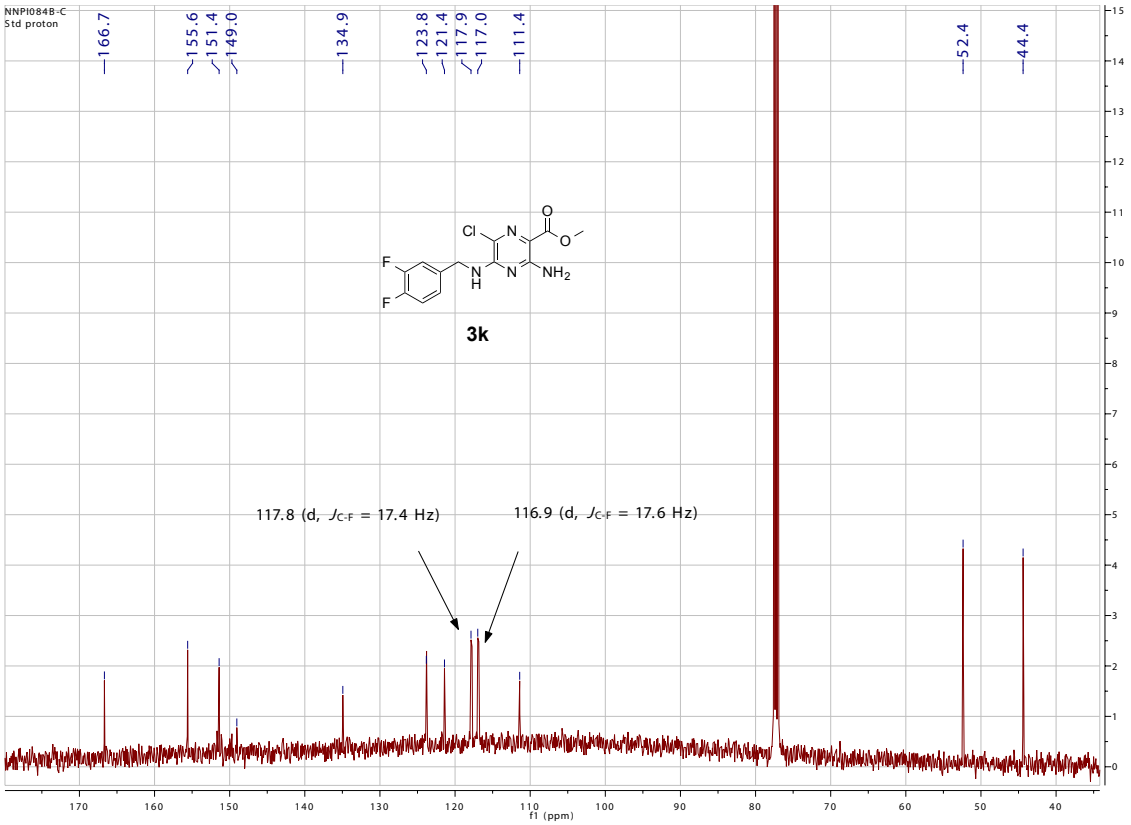
^{13}C NMR ($CDCl_3$) of Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-chloropyrazine-2-carboxylate (3j):



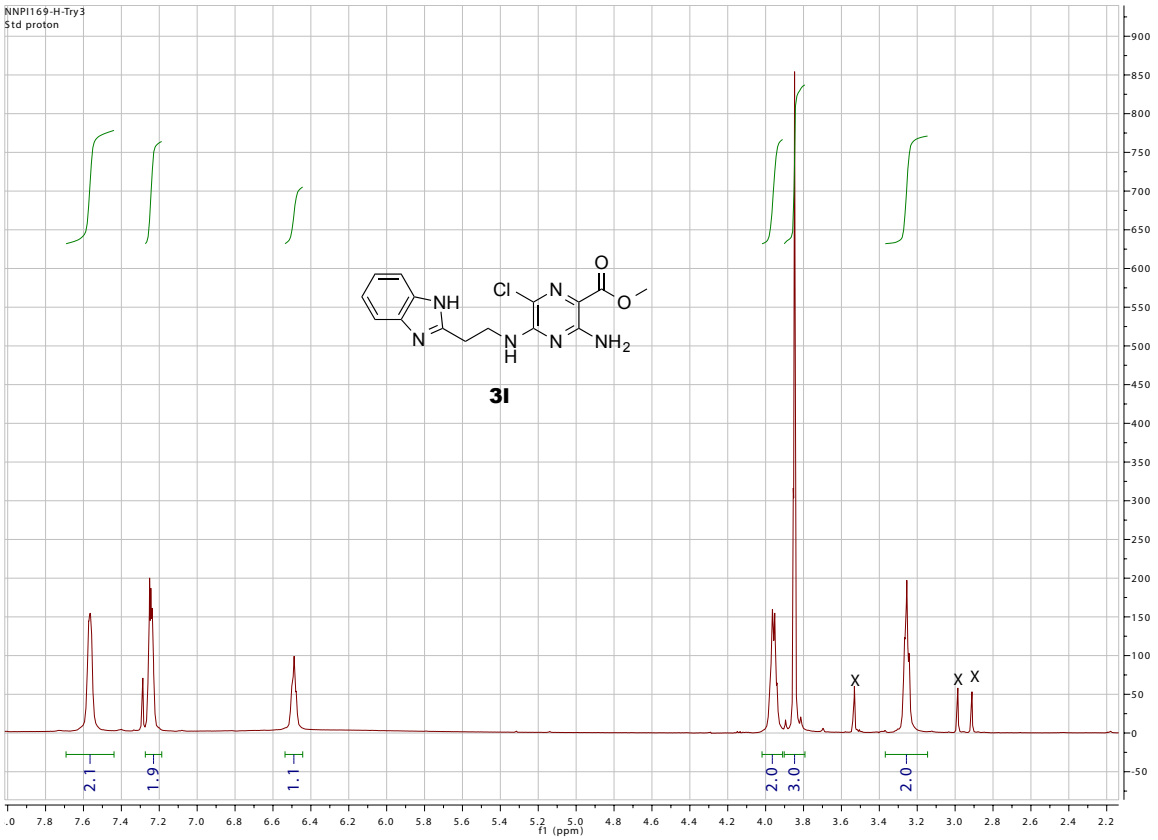
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-((3,4-difluorobenzyl)amino)pyrazine-2-carboxylate (3k):



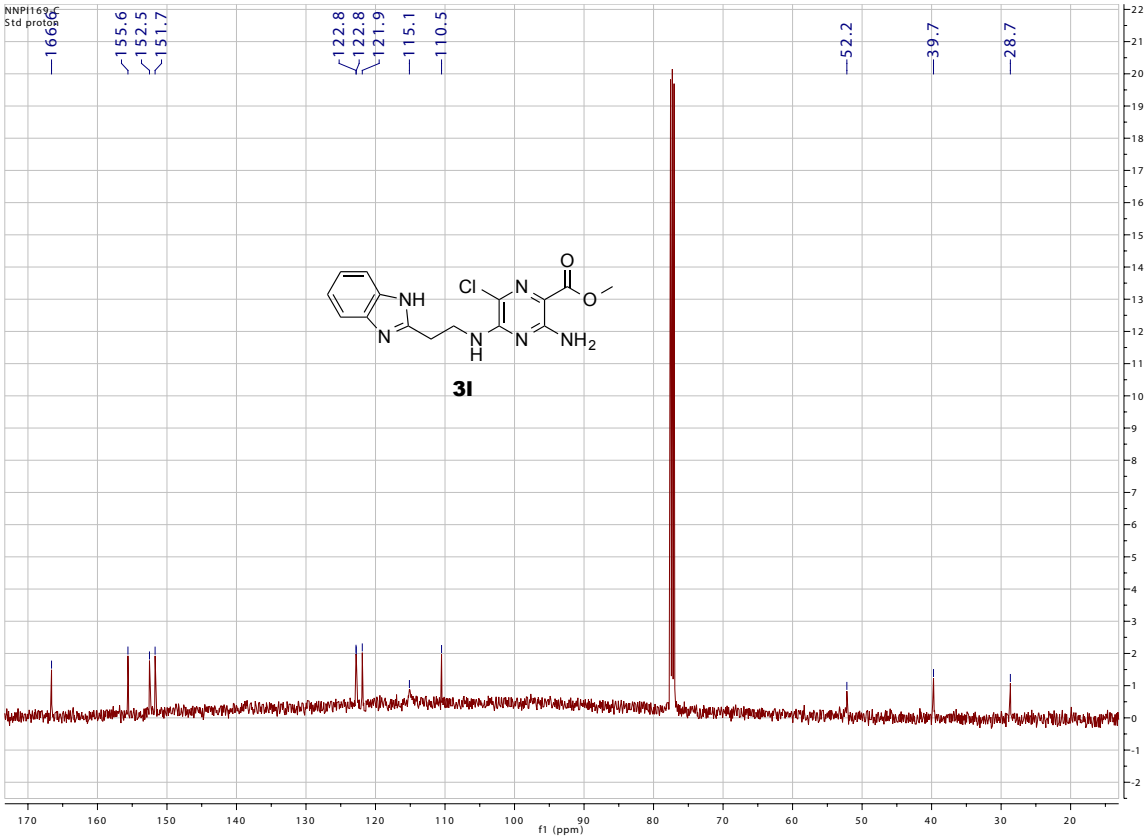
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-((3,4-difluorobenzyl)amino)pyrazine-2-carboxylate (3k): Peak splitting corresponding to C-F coupling seen at 117.9 ppm (J = 17.4 Hz) and 117.0 ppm (J = 17.6 Hz). Peak overlap seen in aromatic region, which leads to one less peak than expected. The 1 bond C-F coupling patterns are indiscernible in this spectrum.



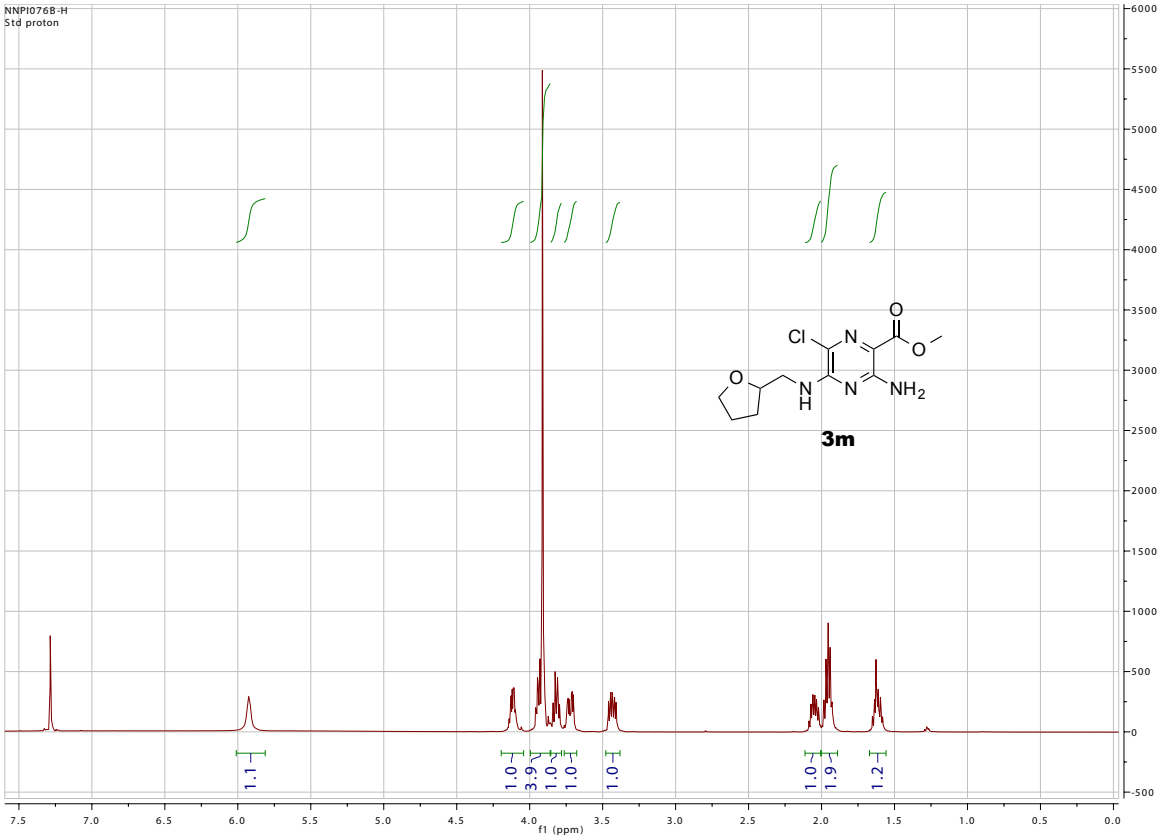
^{1}H NMR ($CDCl_3$) of Methyl 5-((2-(1*H*-benzo[*d*]imidazol-2-yl)ethyl)amino)-3-amino-6-chloropyrazine-2-carboxylate (3l):



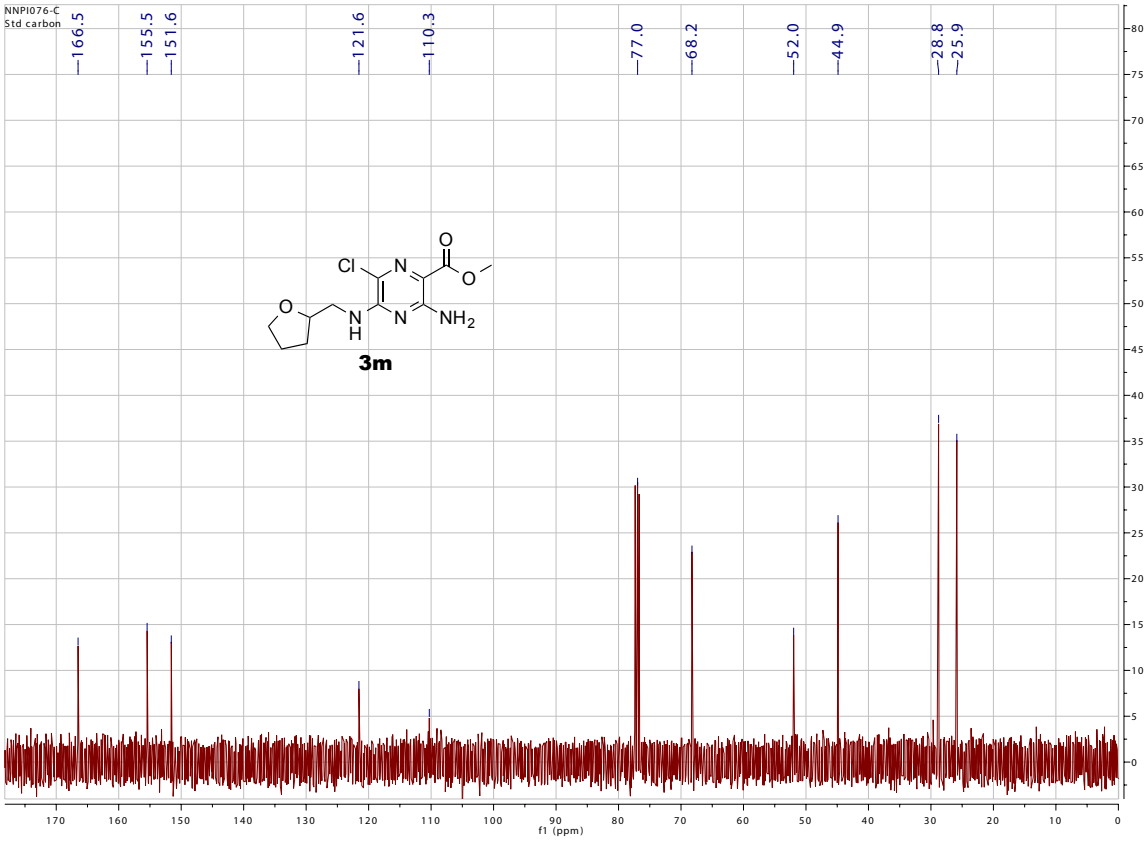
^{13}C NMR ($CDCl_3$) of Methyl 5-((2-(1*H*-benzo[*d*]imidazol-2-yl)ethyl)amino)-3-amino-6-chloropyrazine-2-carboxylate (3l):



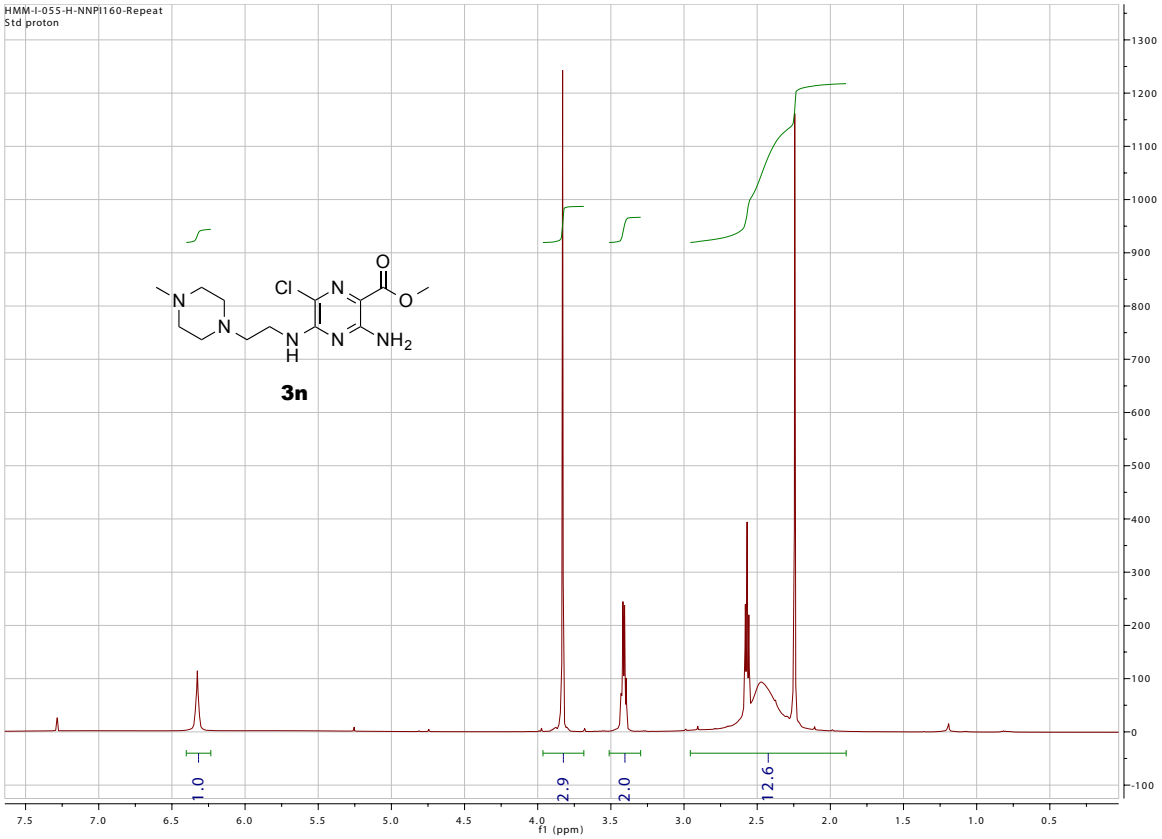
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-(((tetrahydrofuran-2-yl)methyl)amino)pyrazine-2-carboxylate (3m):



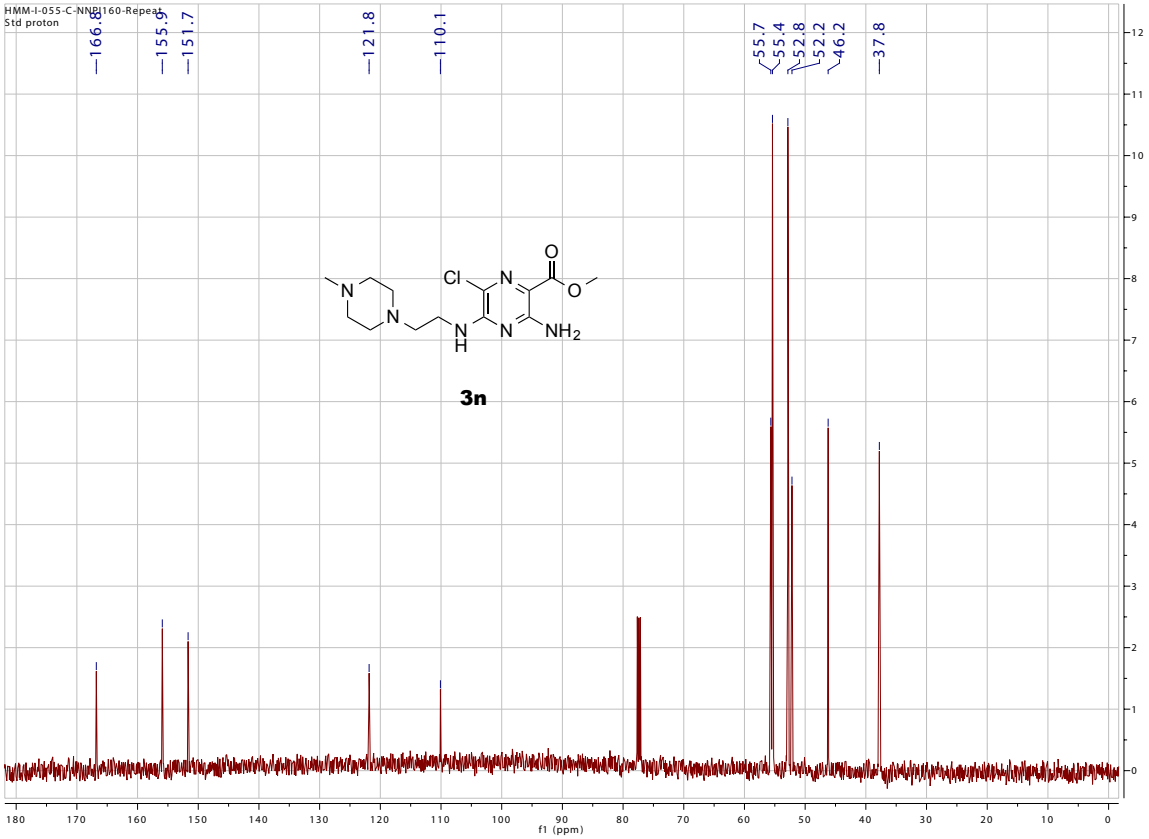
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-(((tetrahydrofuran-2-yl)methyl)amino)pyrazine-2-carboxylate (3m):



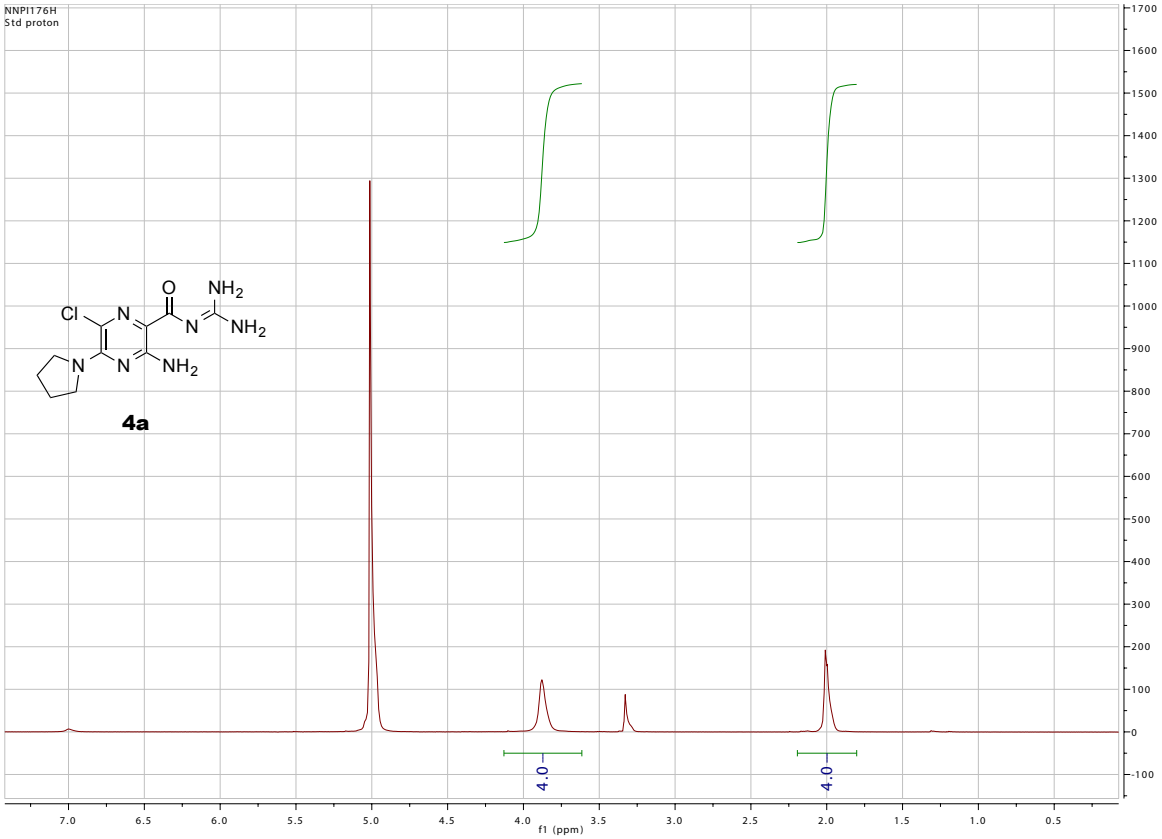
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-((2-(4-methylpiperazin-1-yl)ethyl)amino)pyrazine-2-carboxylate (3n):



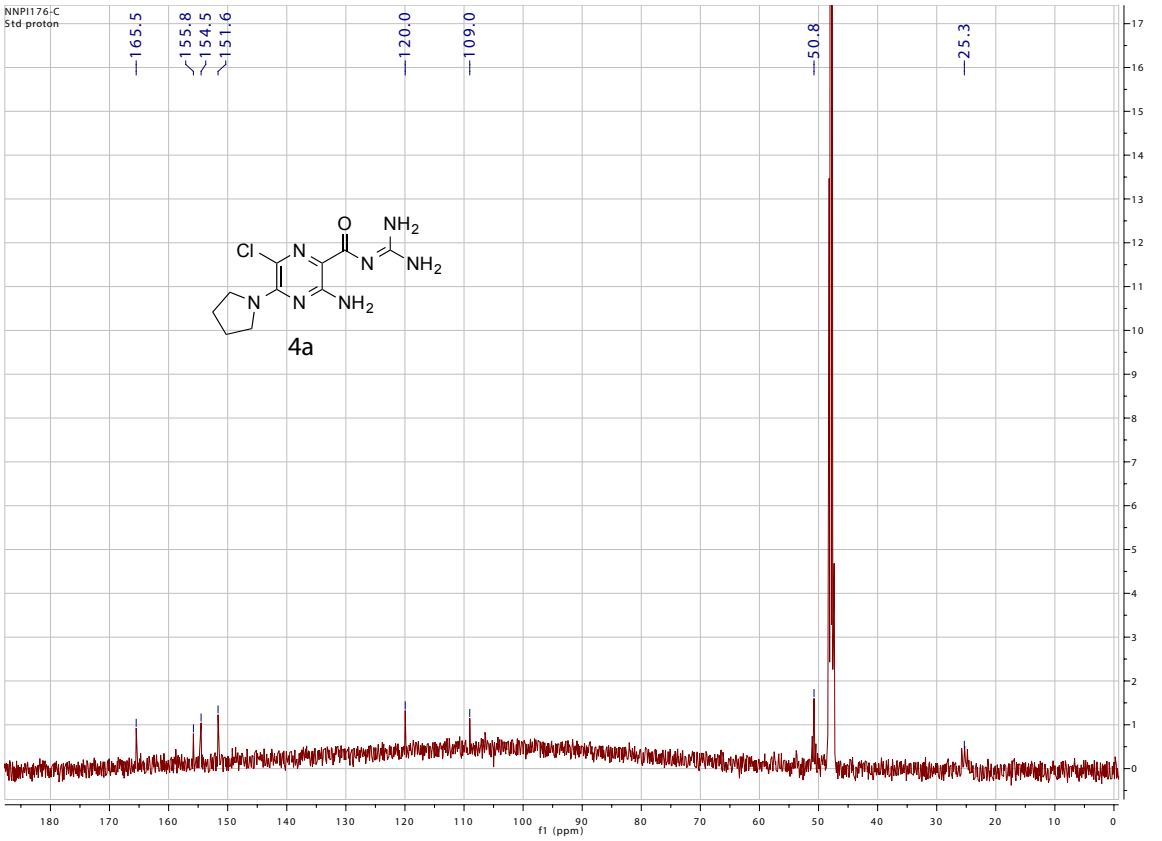
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-((2-(4-methylpiperazin-1-yl)ethyl)amino)pyrazine-2-carboxylate (3n):



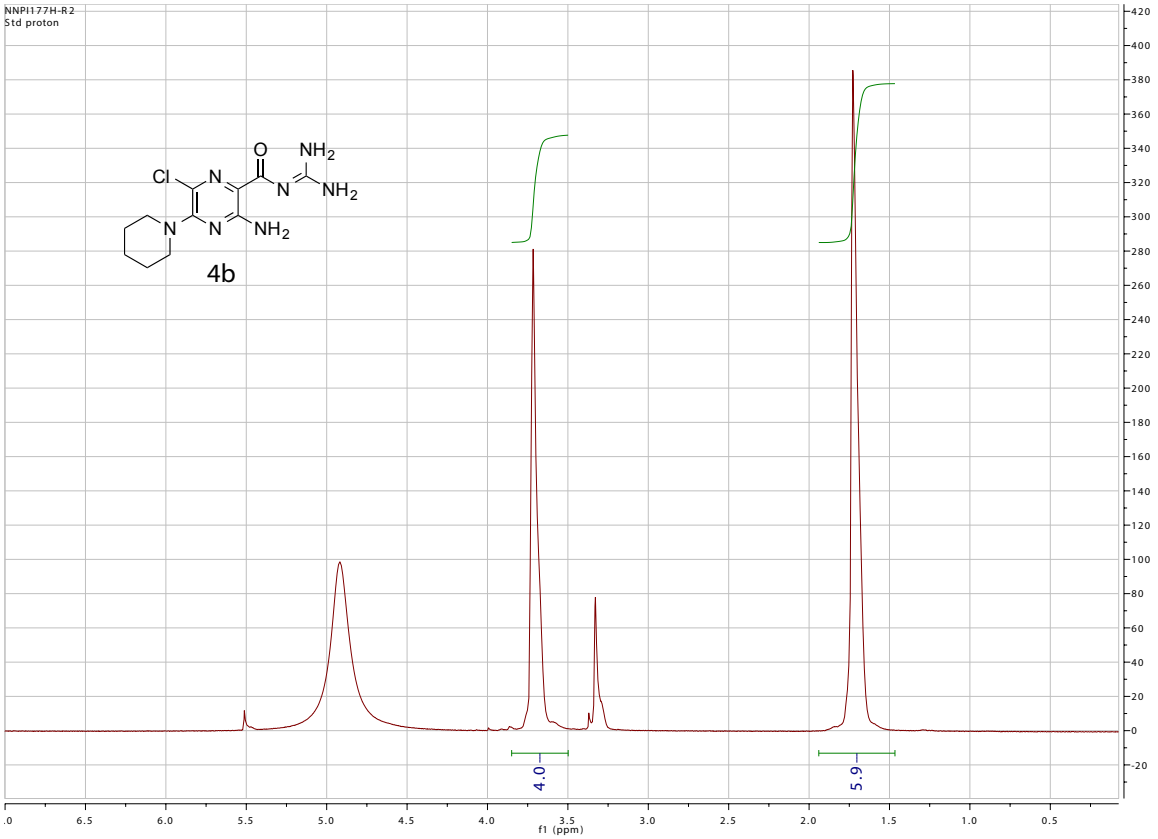
^{1}H NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide (4a):



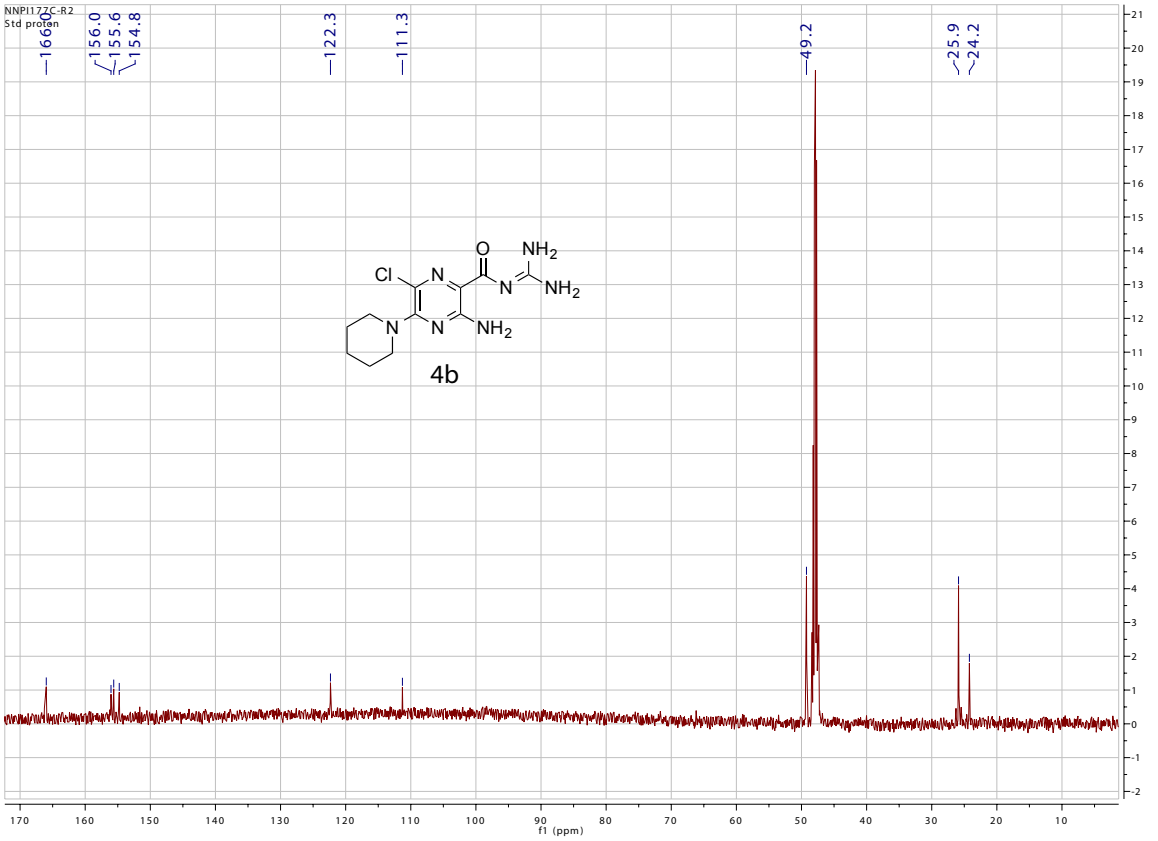
^{13}C NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide (4a):



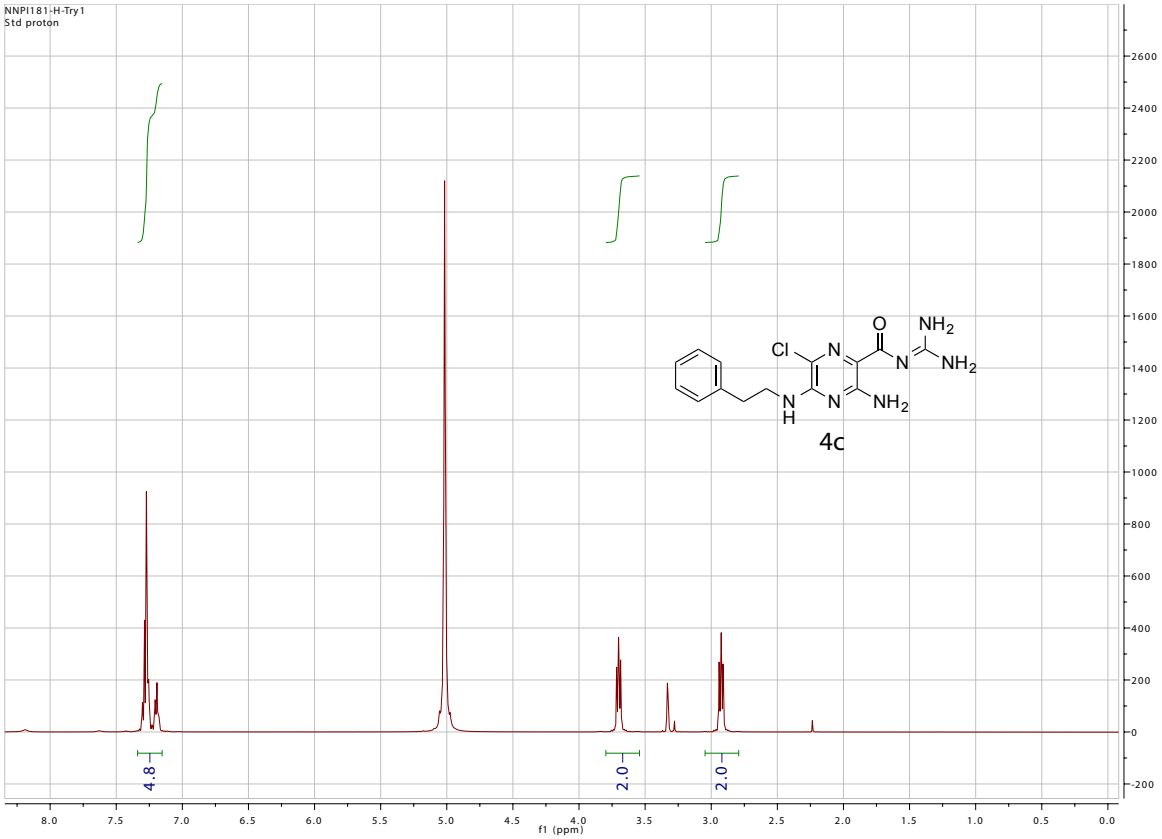
^{1}H NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-(piperidin-1-yl)pyrazine-2-carboxamide (4b):



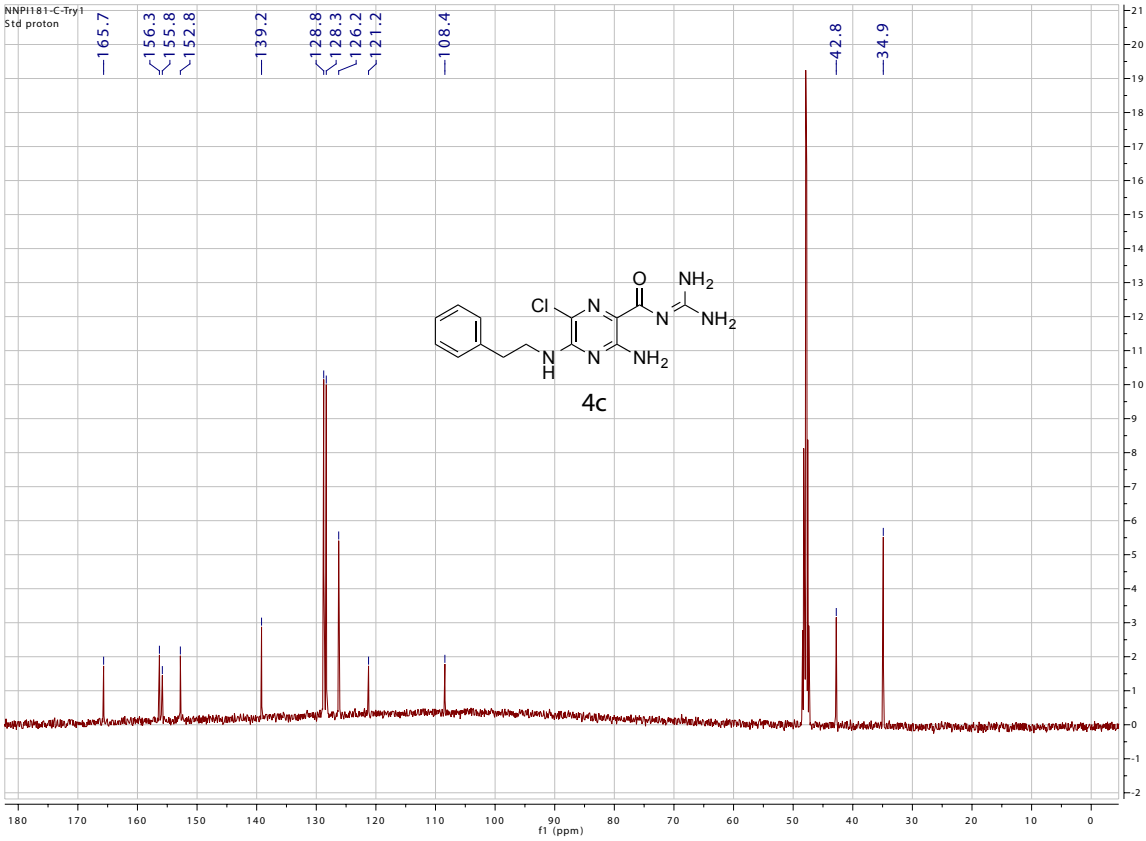
^{13}C NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-(piperidin-1-yl)pyrazine-2-carboxamide (4b):



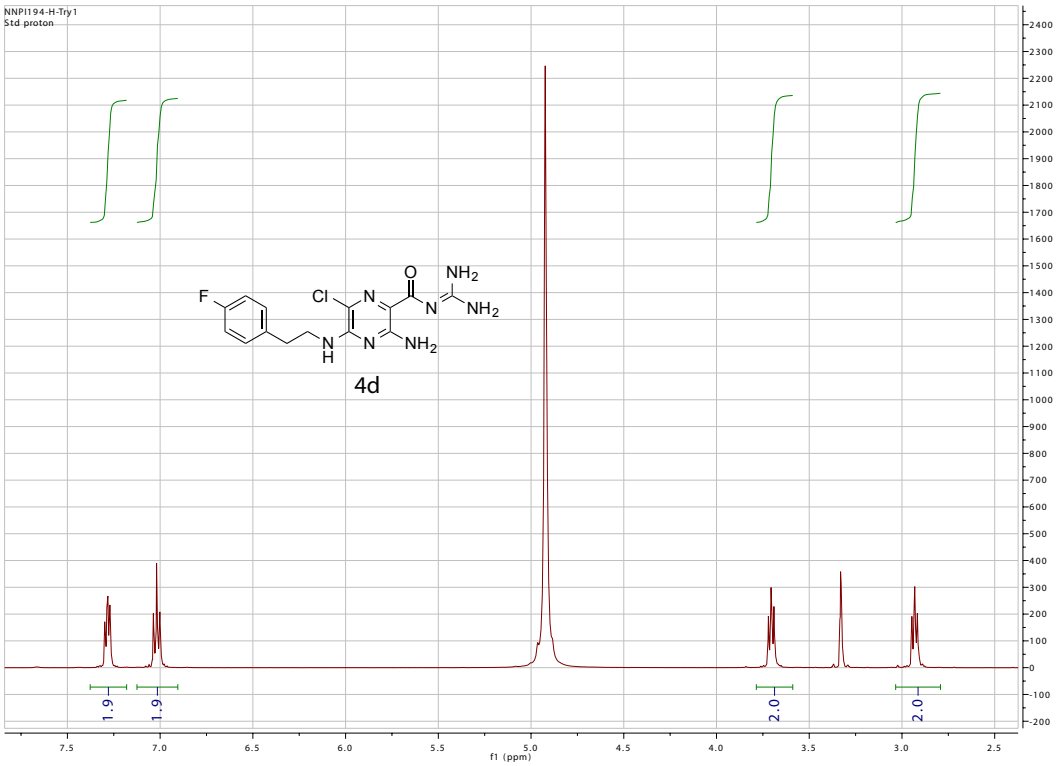
^{1}H NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-(phenethylamino)pyrazine-2-carboxamide (4c):



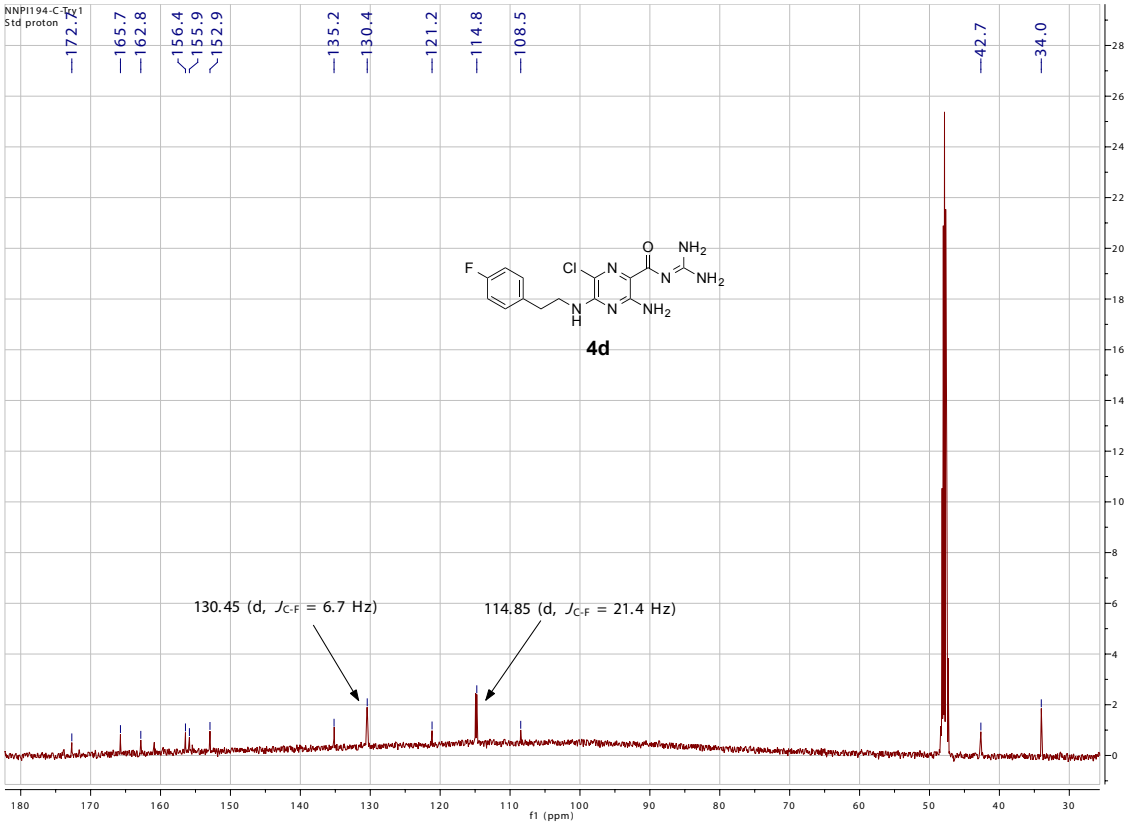
^{13}C NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-(phenethylamino)pyrazine-2-carboxamide (4c):



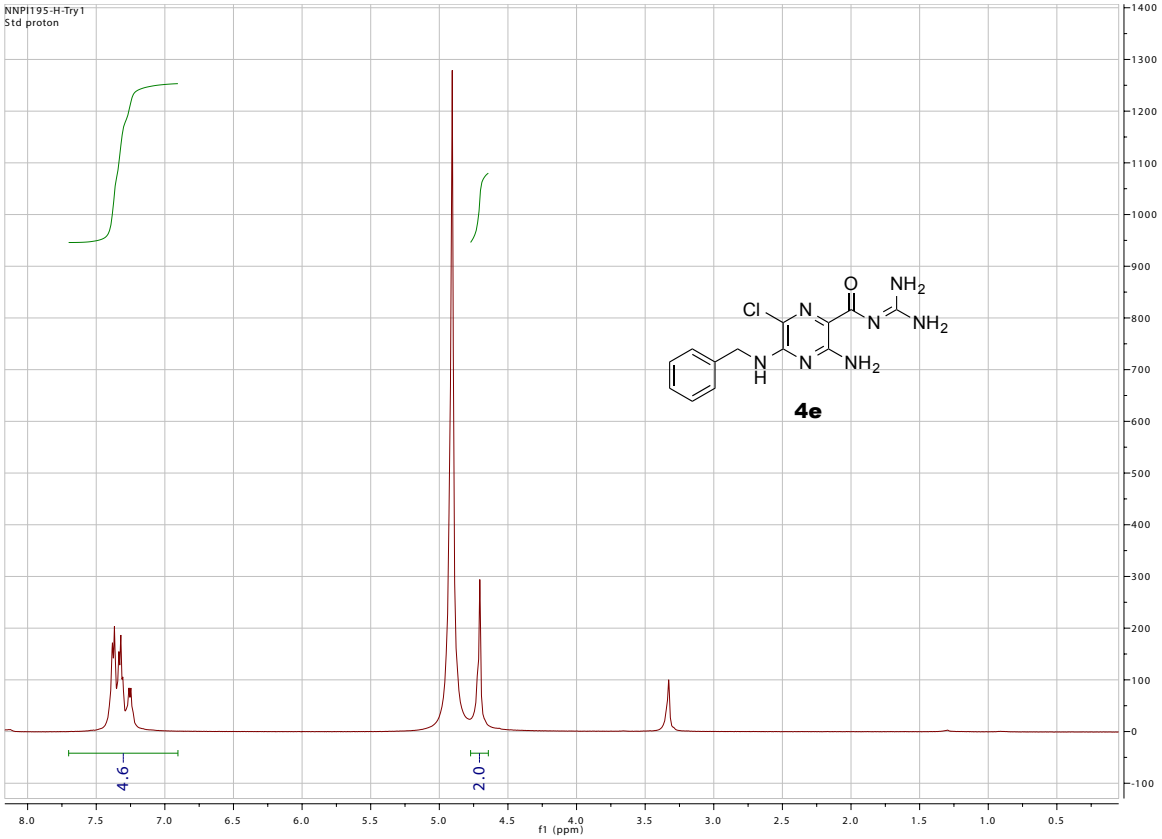
^{1}H NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-((4-fluorophenethyl)amino)pyrazine-2-carboxamide (4d):



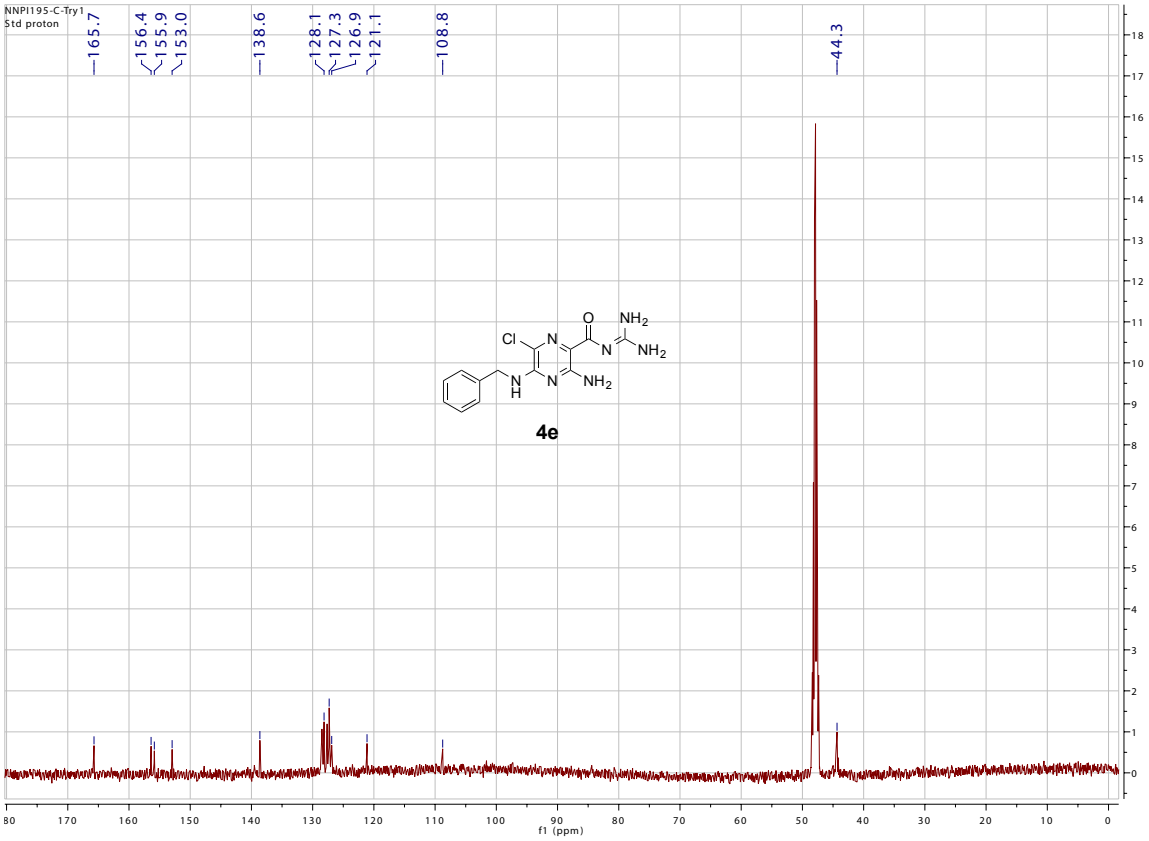
^{13}C NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-((4-fluorophenethyl)amino)pyrazine-2-carboxamide (4d): Doublets due to C-F coupling seen at 130.45 (d, $J_{C\text{-}F}$ = 6.7 Hz) and 114.85 (d, $J_{C\text{-}F}$ = 21.4 Hz). One extra peak in the aromatic region, due to strongly split fluorine substituted carbon in aromatic ring. 1 bond C-F coupling pattern for this peak is indiscernible in this spectrum.



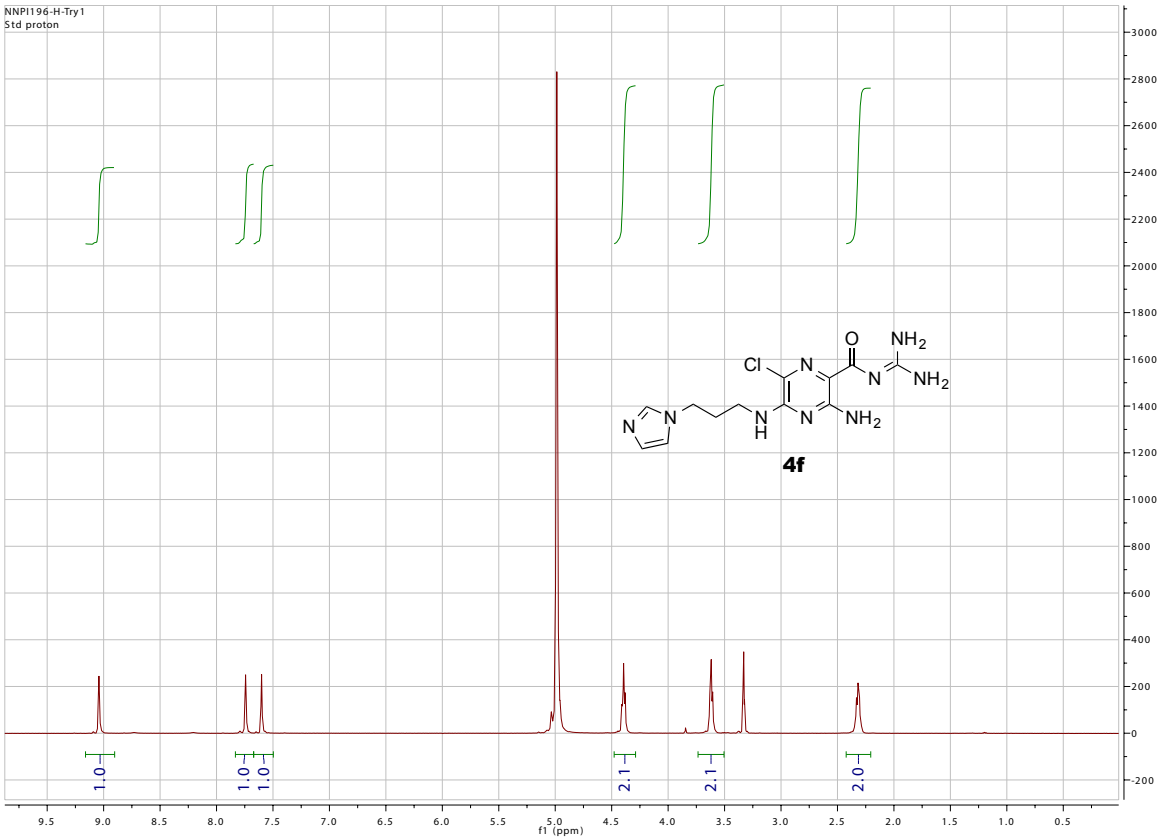
^{1}H NMR (CD_3OD) of 3-amino-5-(benzylamino)-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (4e):



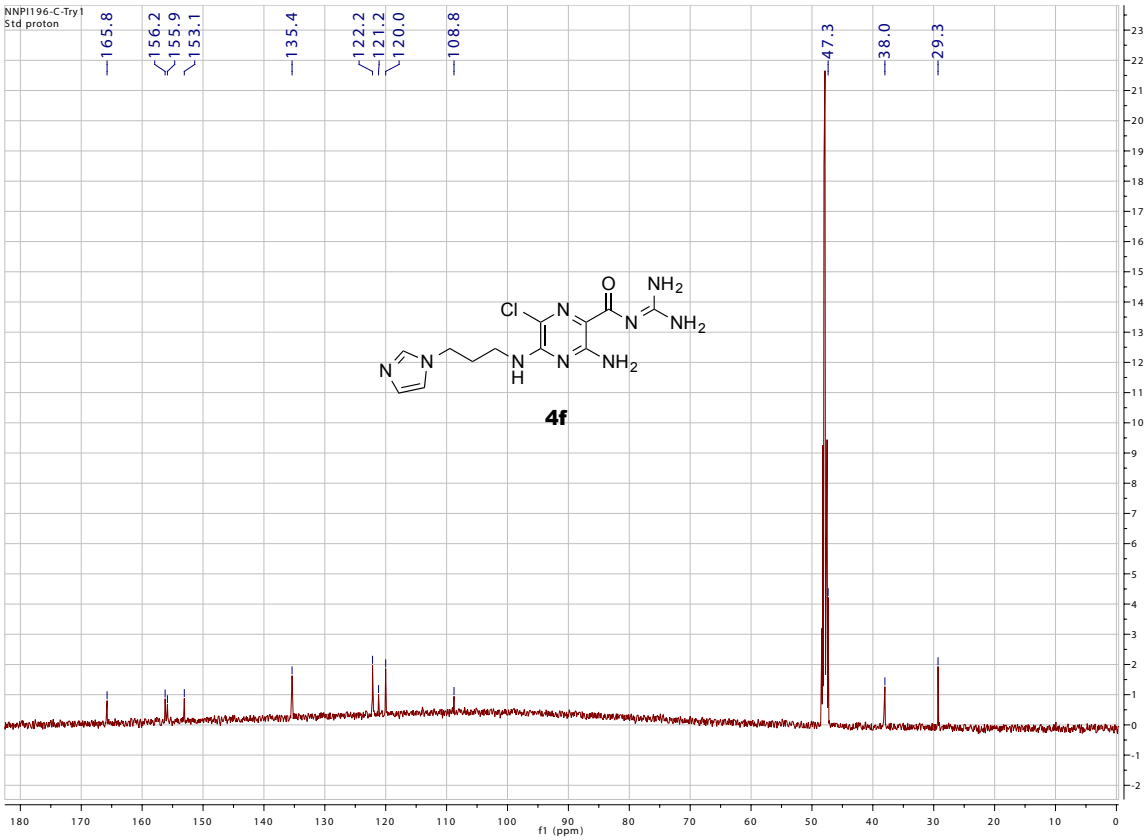
^{13}C NMR (CD_3OD) of 3-amino-5-(benzylamino)-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (4e):



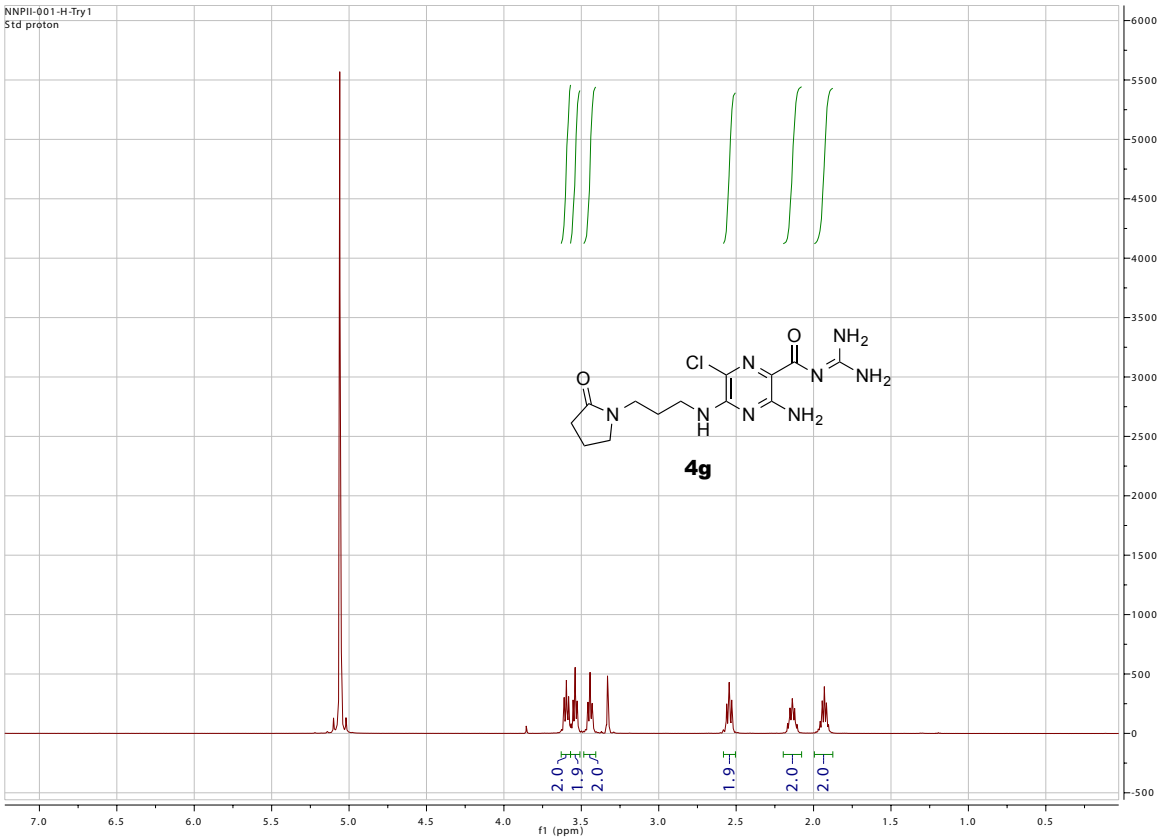
^{1}H NMR (CD_3OD) of 5-((3-(1*H*-imidazol-1-yl)propyl)amino)-3-amino-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (4f):



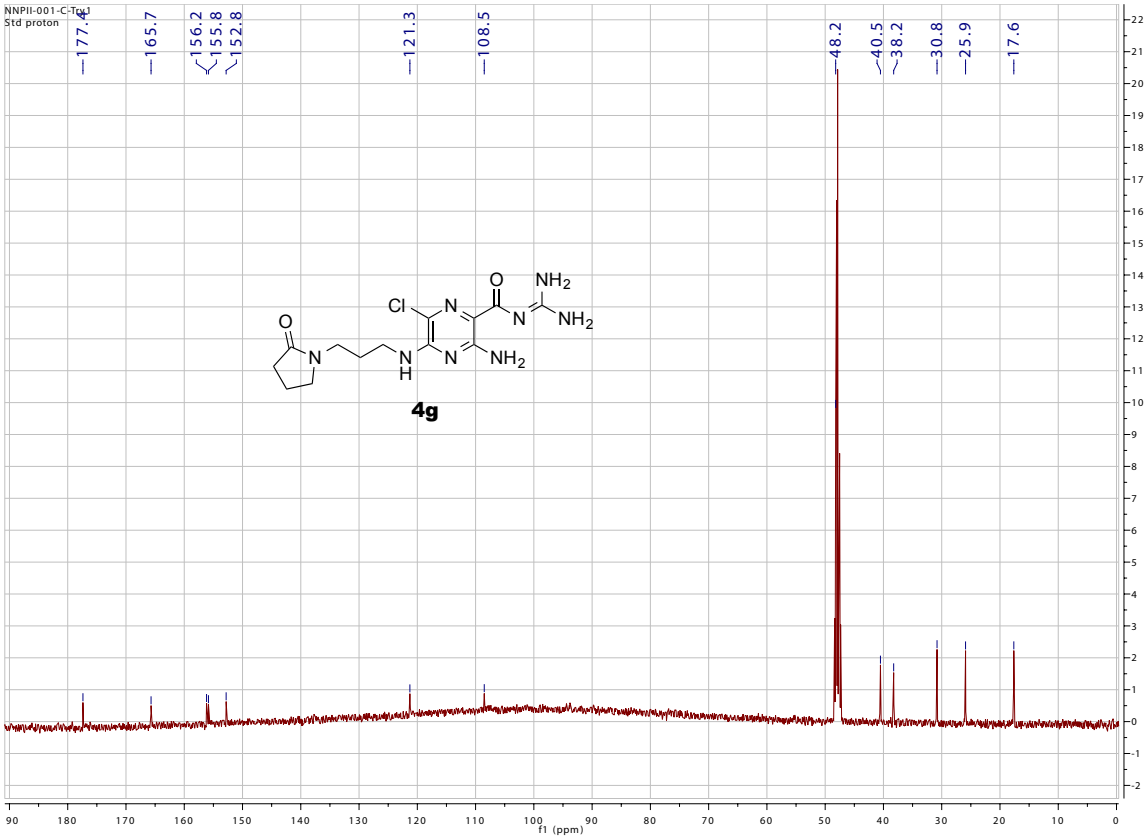
^{13}C NMR (CD_3OD) of 5-((3-(1*H*-imidazol-1-yl)propyl)amino)-3-amino-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (4f):



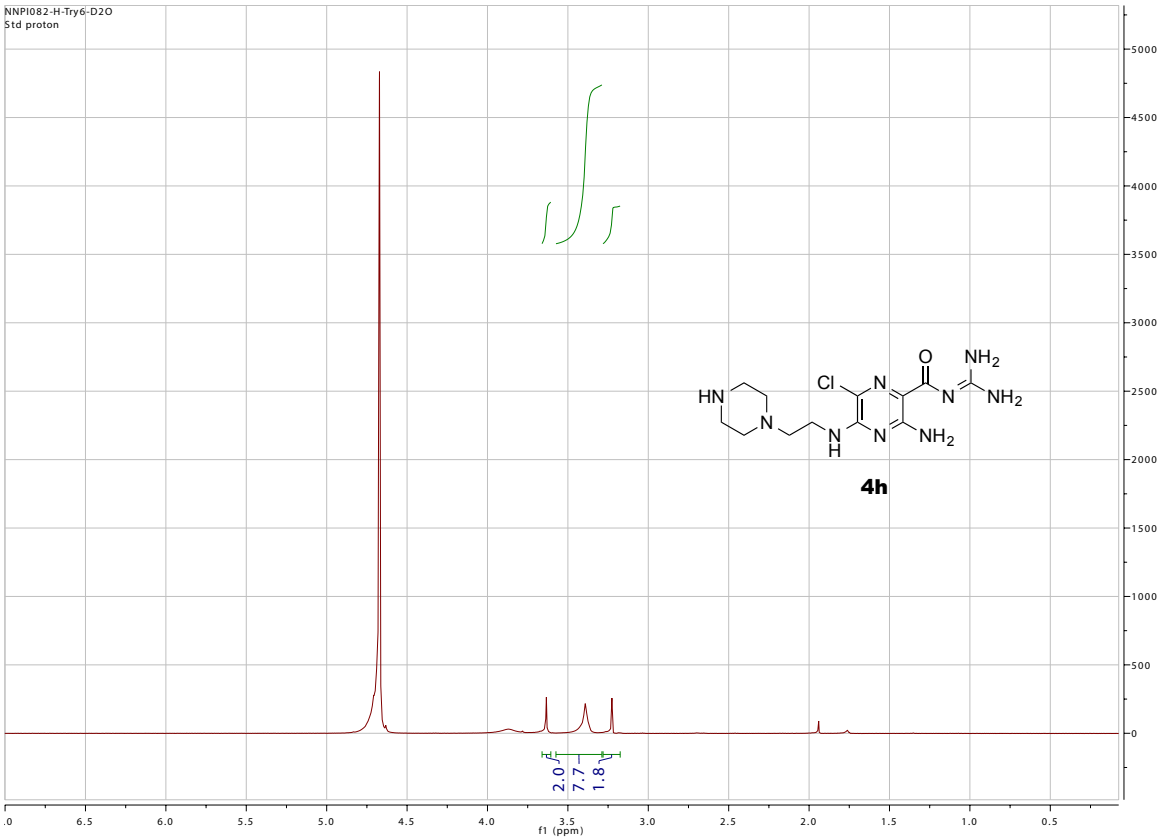
^{1}H NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-((3-(2-oxopyrrolidin-1-yl)propyl)amino)pyrazine-2-carboxamide (4g):



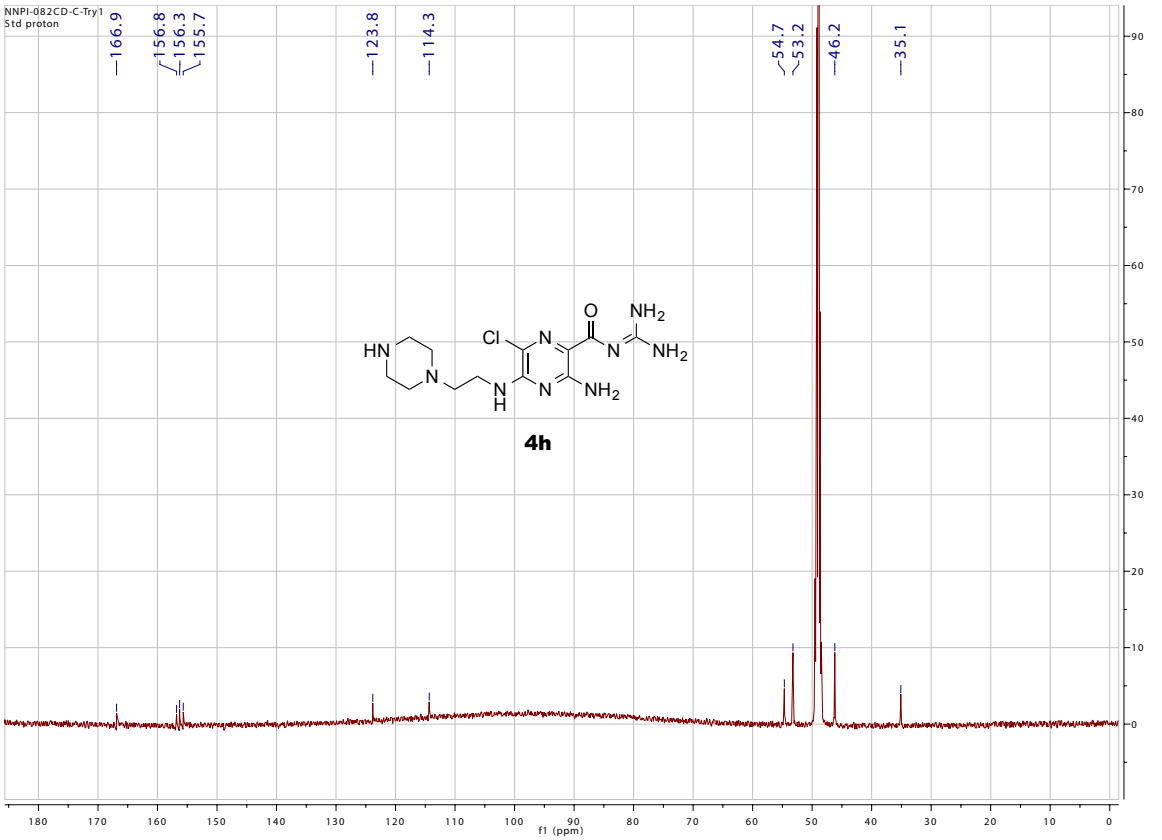
^{13}C NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-((3-(2-oxopyrrolidin-1-yl)propyl)amino)pyrazine-2-carboxamide (4g): One alkyl peak overlapped by solvent at 48.2



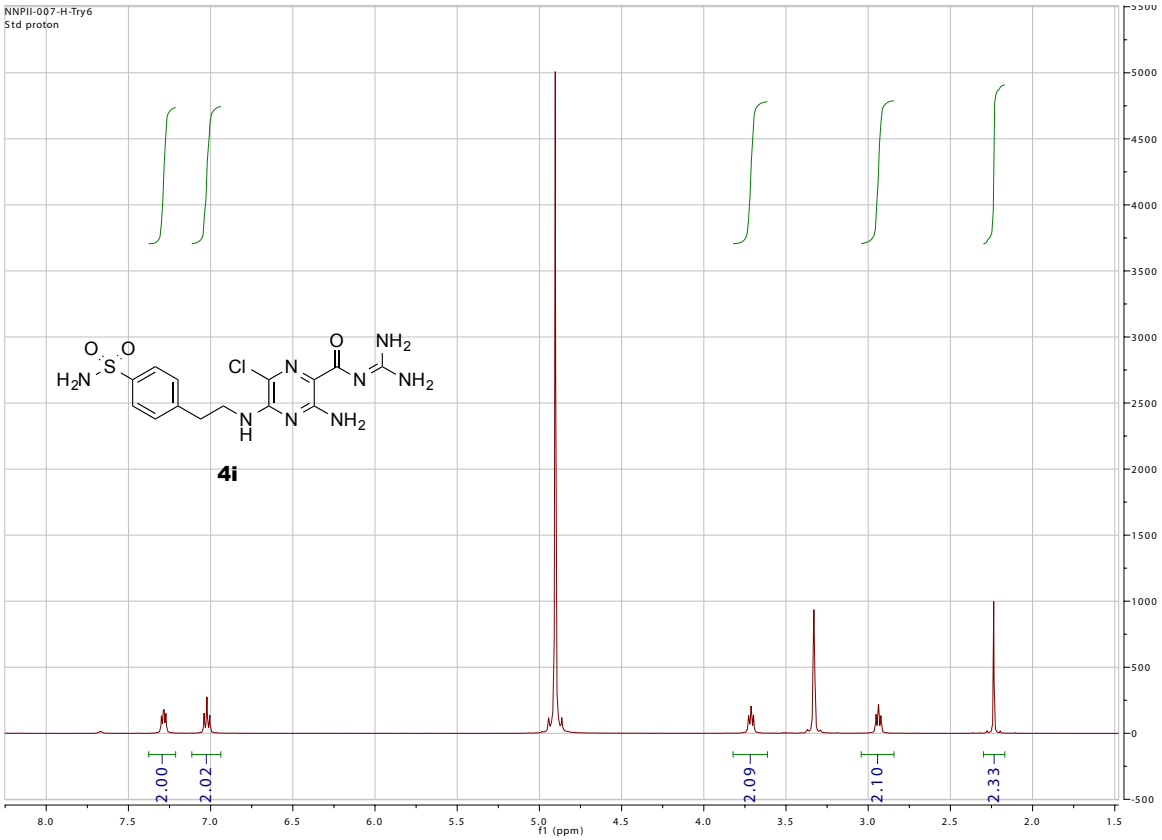
^{1}H NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-((2-(piperazin-1-yl) ethyl)amino)pyrazine-2-carboxamide(4h):



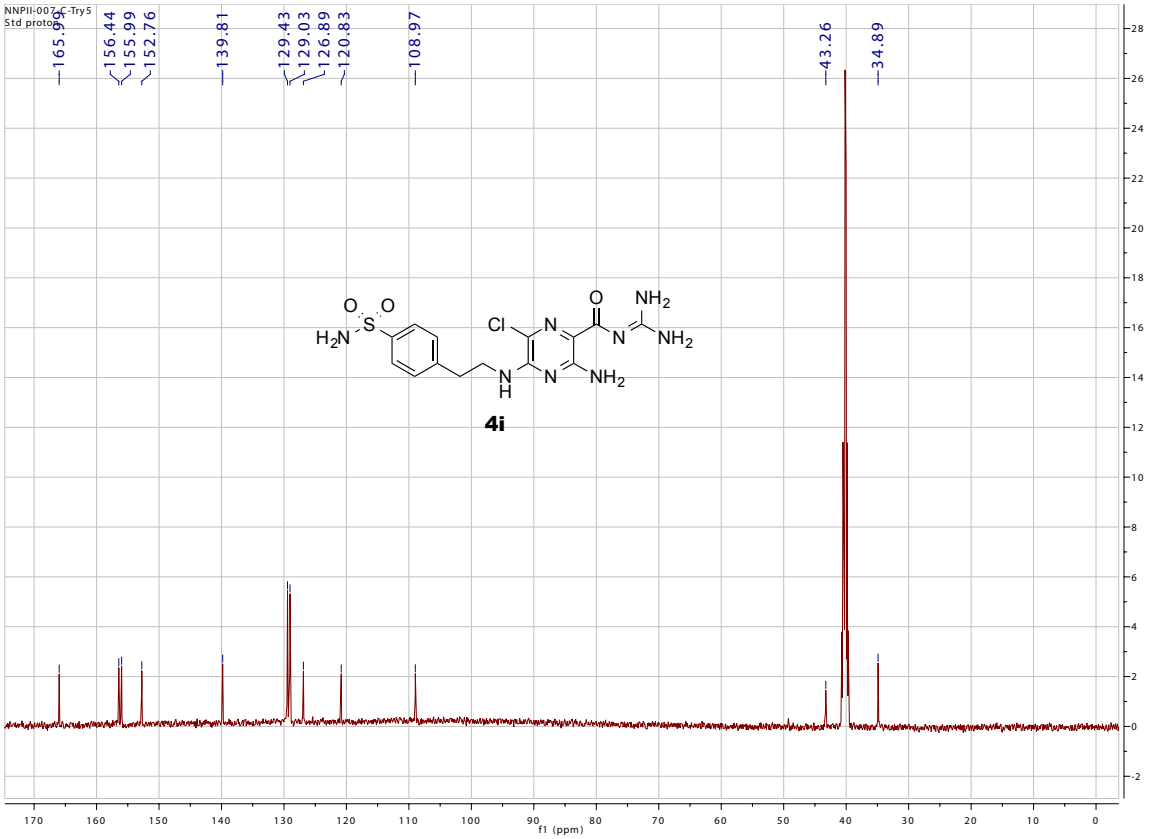
^{13}C NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-((2-(piperazin-1-yl) ethyl)amino)pyrazine-2-carboxamide(4h):



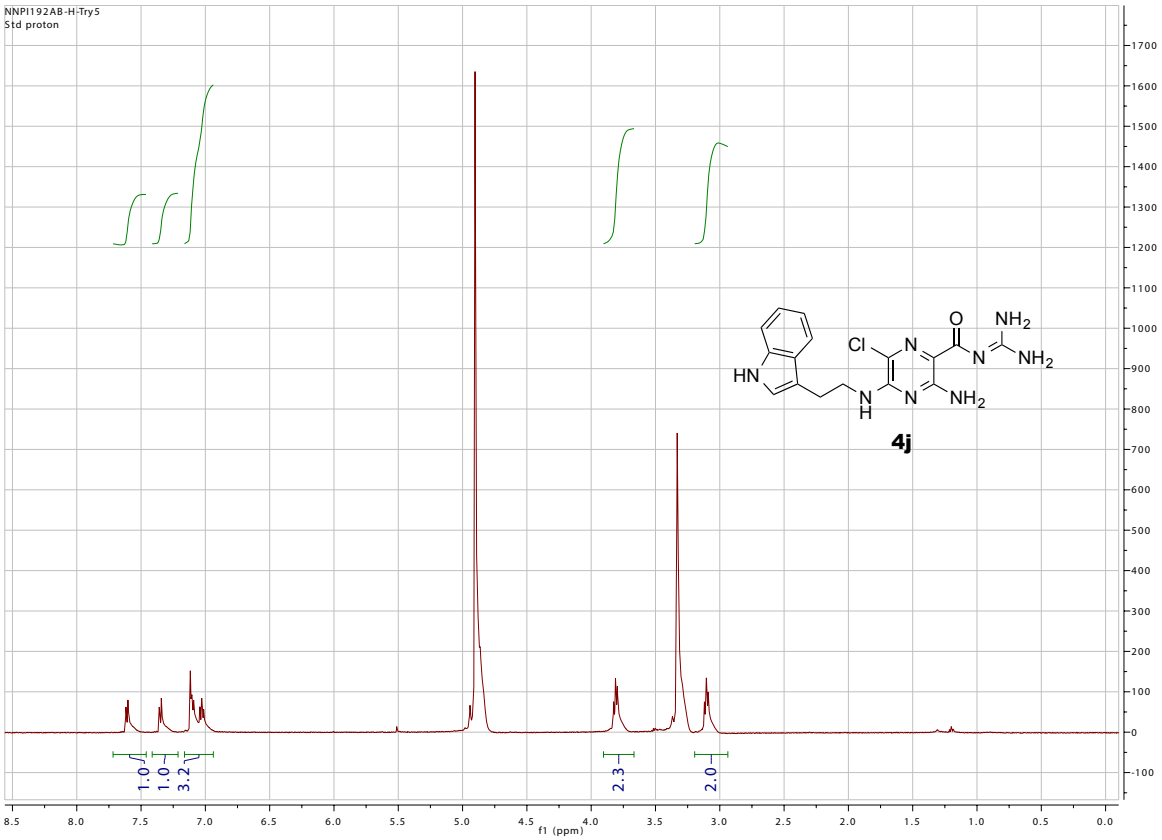
^{1}H NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-((4-sulfamoylphenethyl)amino)pyrazine-2-carboxamide (4i):



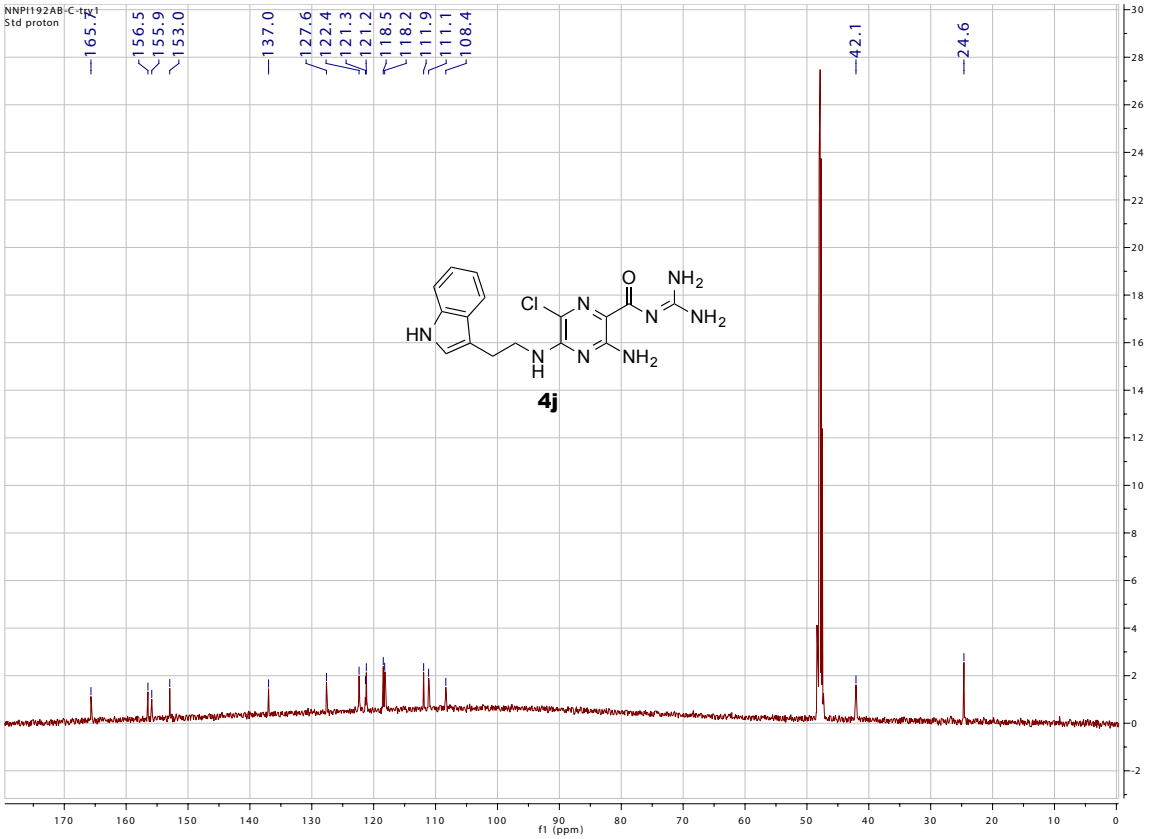
^{13}C NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-((4-sulfamoylphenethyl)amino)pyrazine-2-carboxamide (4i):



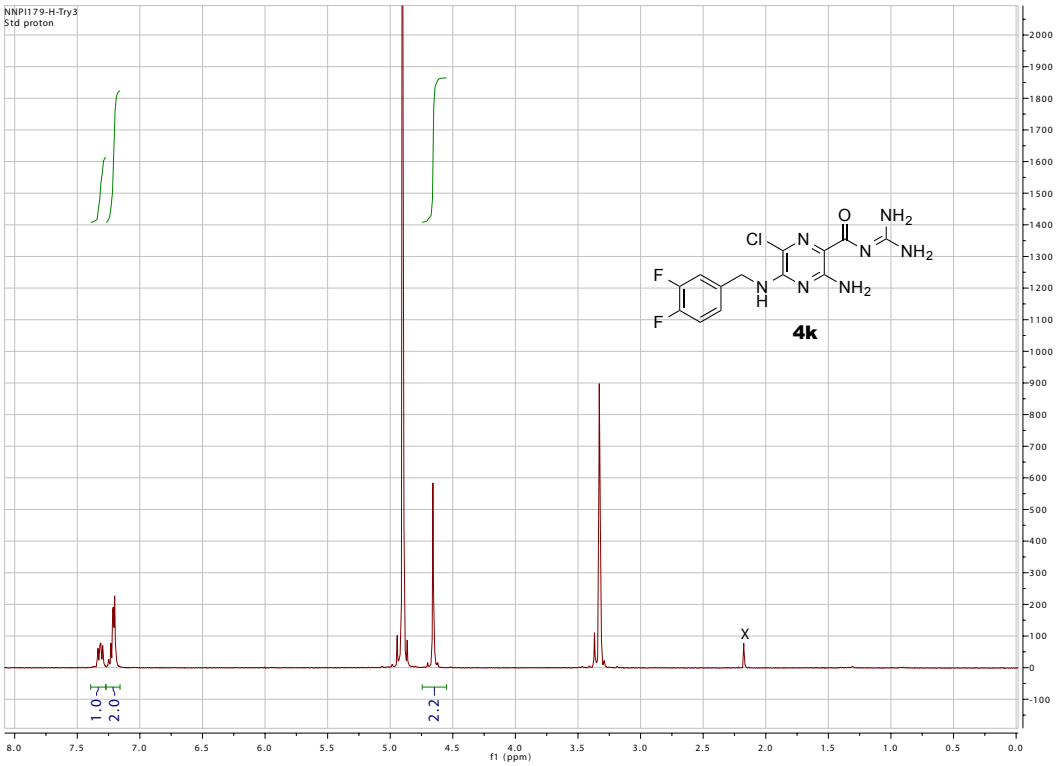
^{1}H NMR (CD_3OD) of 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (4j):



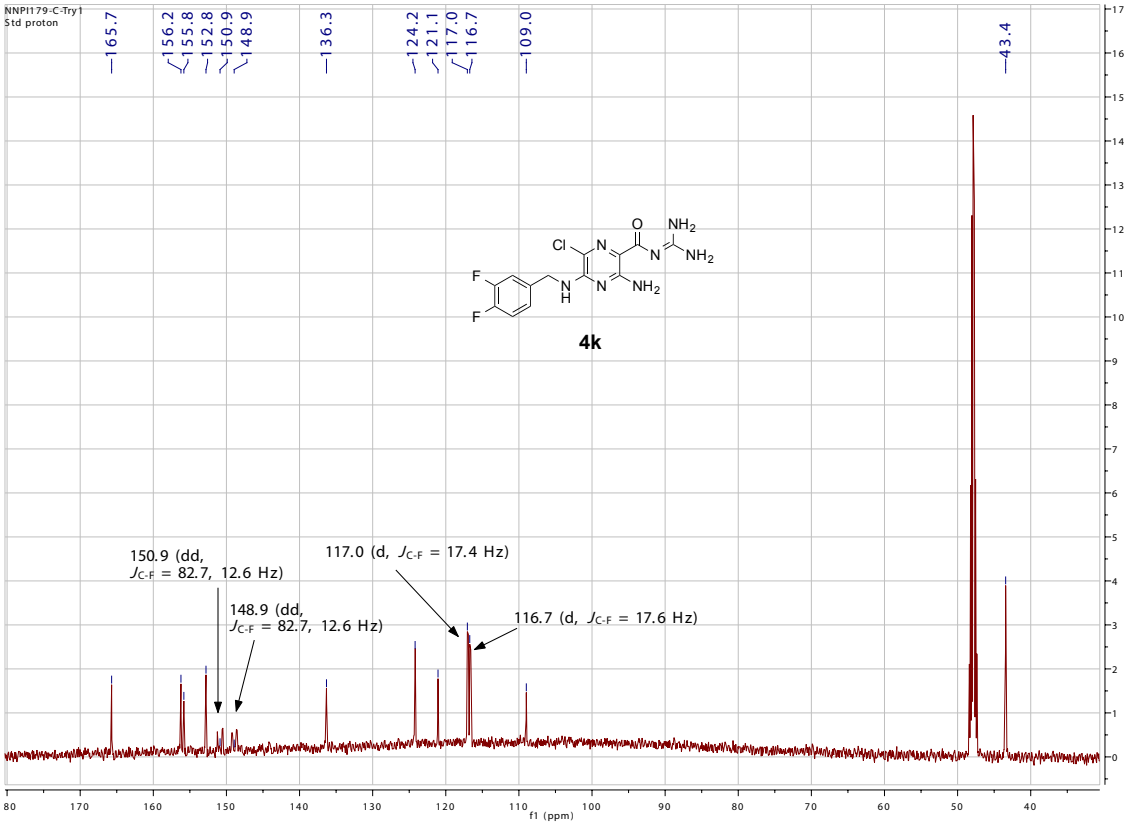
^{13}C NMR (CD_3OD) of 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (4j):



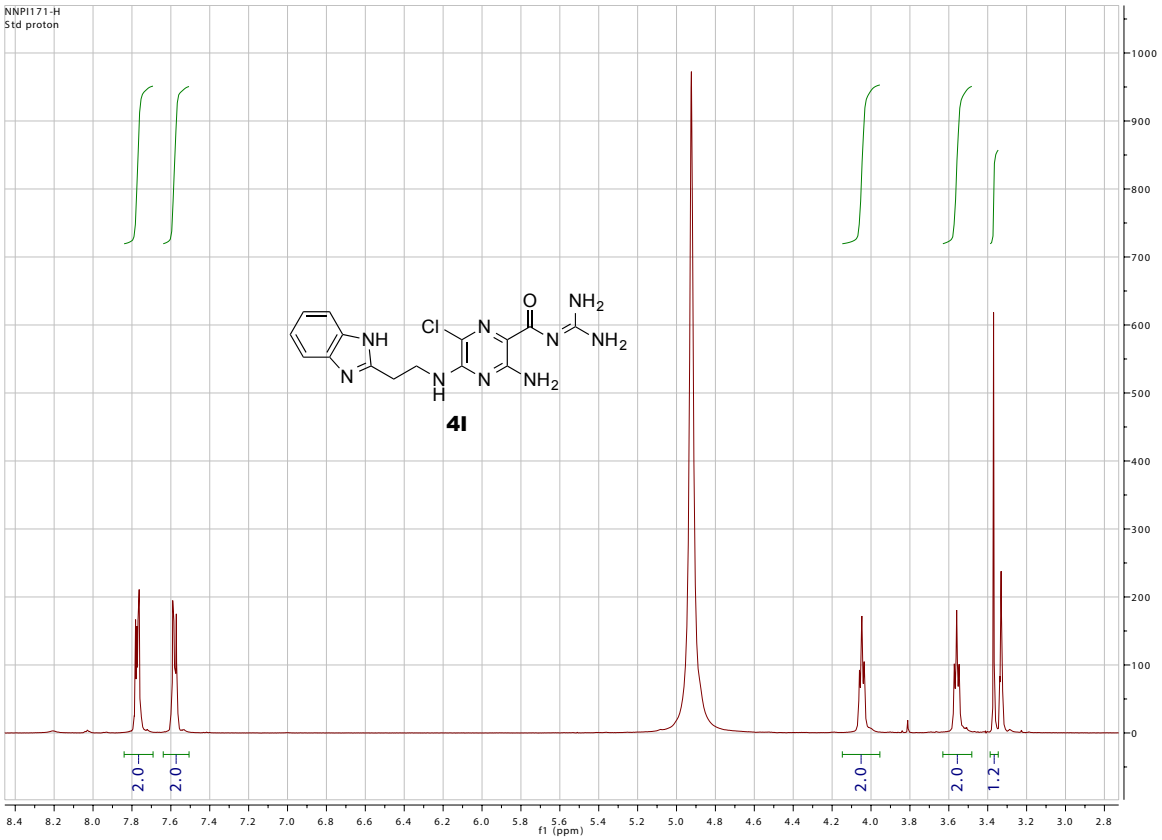
^{1}H NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-((3,4-difluorobenzyl)amino)pyrazine-2-carboxamide (4k):



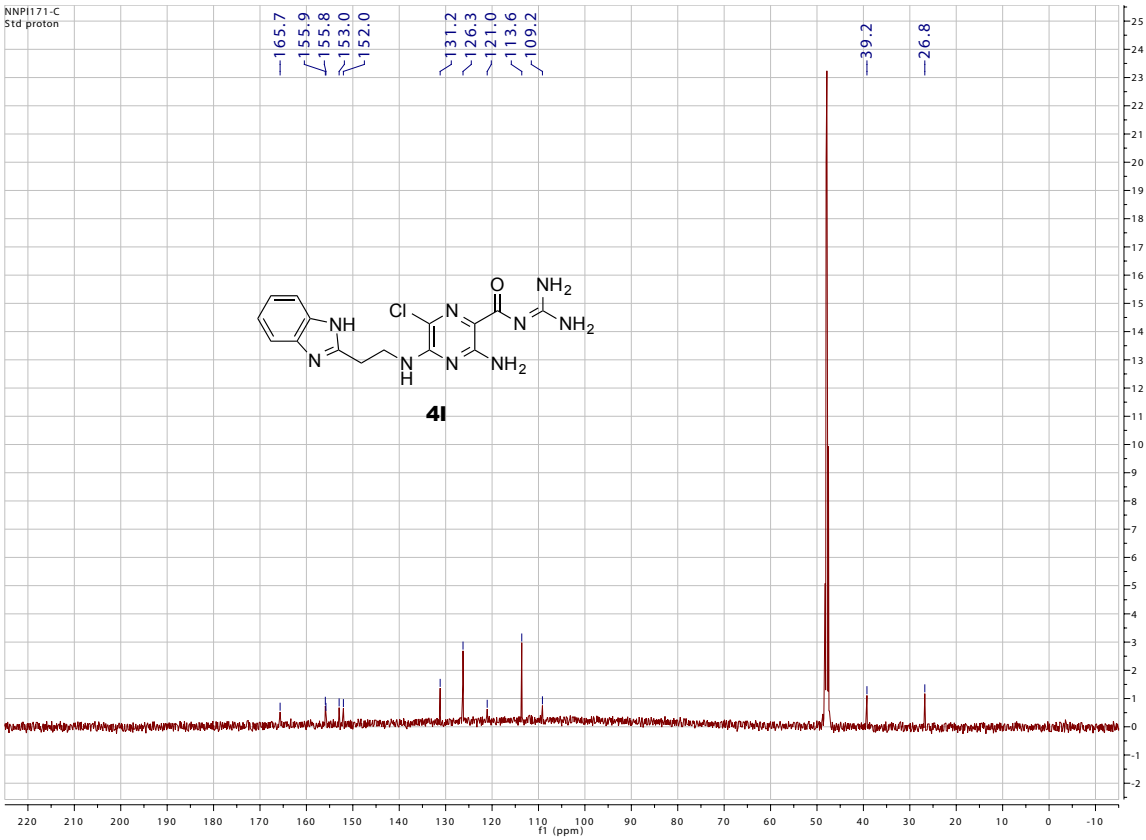
^{13}C NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-((3,4-difluorobenzyl)amino)pyrazine-2-carboxamide (4k): Peak splitting corresponding to C-F coupling seen at 150.9 (dd, $J_{C\text{-}F}$ = 82.7, 12.6 Hz), 148.9 (dd, $J_{C\text{-}F}$ = 81.8, 12.6 Hz), 117.0 (d, $J_{C\text{-}F}$ = 17.4 Hz), 116.7 (d, $J_{C\text{-}F}$ = 17.6 Hz).



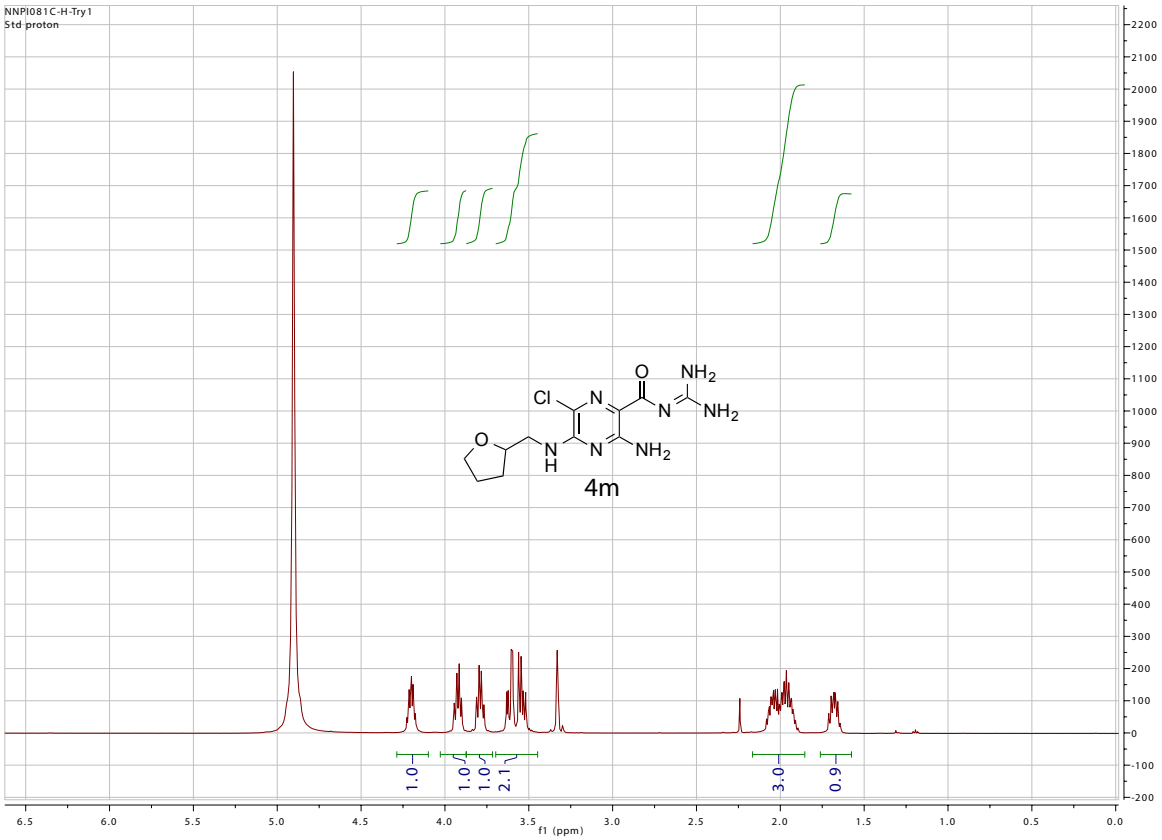
^{1}H NMR (CD_3OD) of 5-((2-(1*H*-benzo[*d*]imidazol-2-yl)ethyl)amino)-3-amino-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (4l):



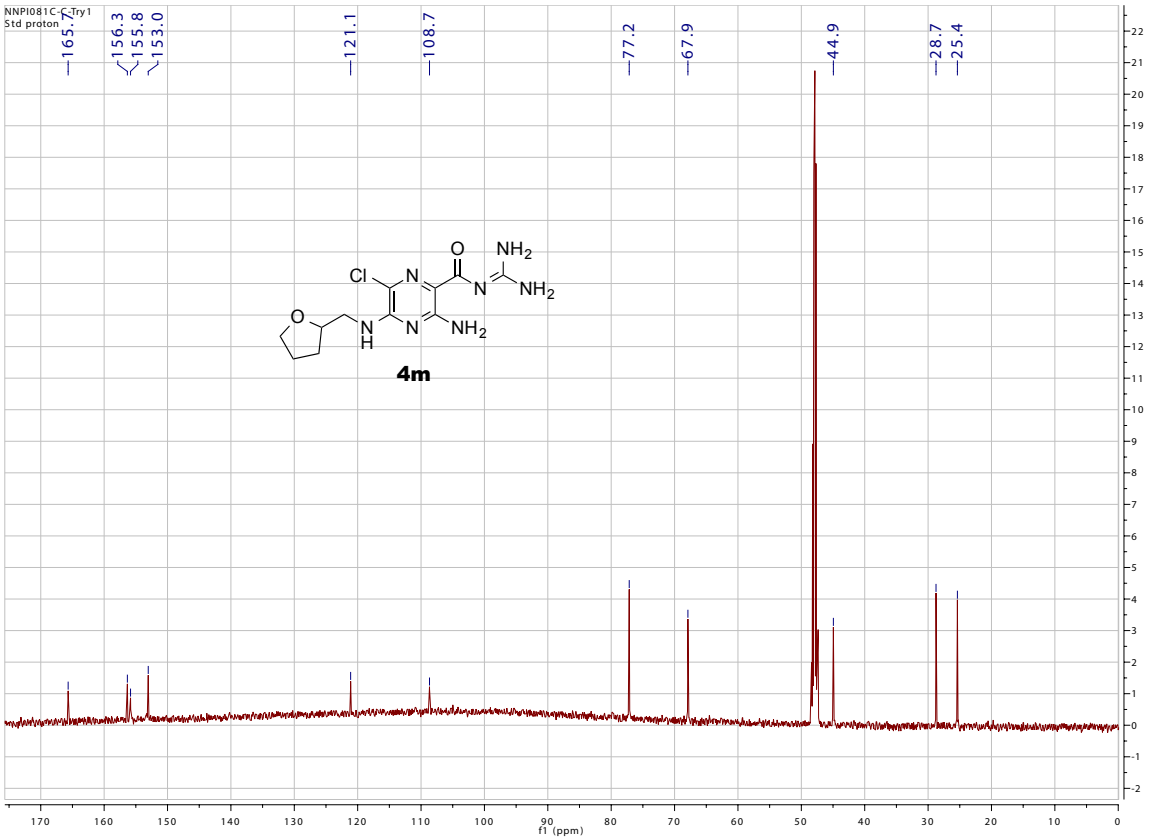
^{13}C NMR (CD_3OD) of 5-((2-(1*H*-benzo[*d*]imidazol-2-yl)ethyl)amino)-3-amino-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (4l):



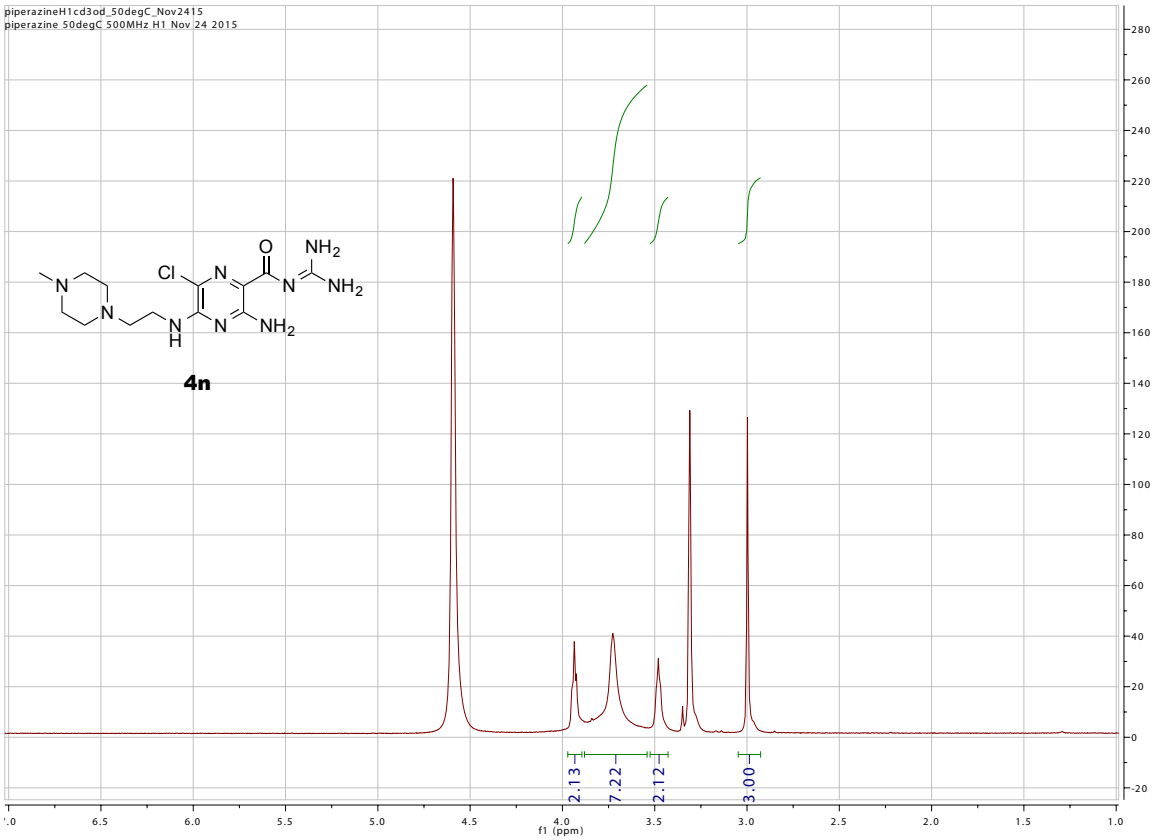
^{1}H NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-(((tetrahydrofuran-2-yl)methyl)amino)pyrazine-2-carboxamide (4m):



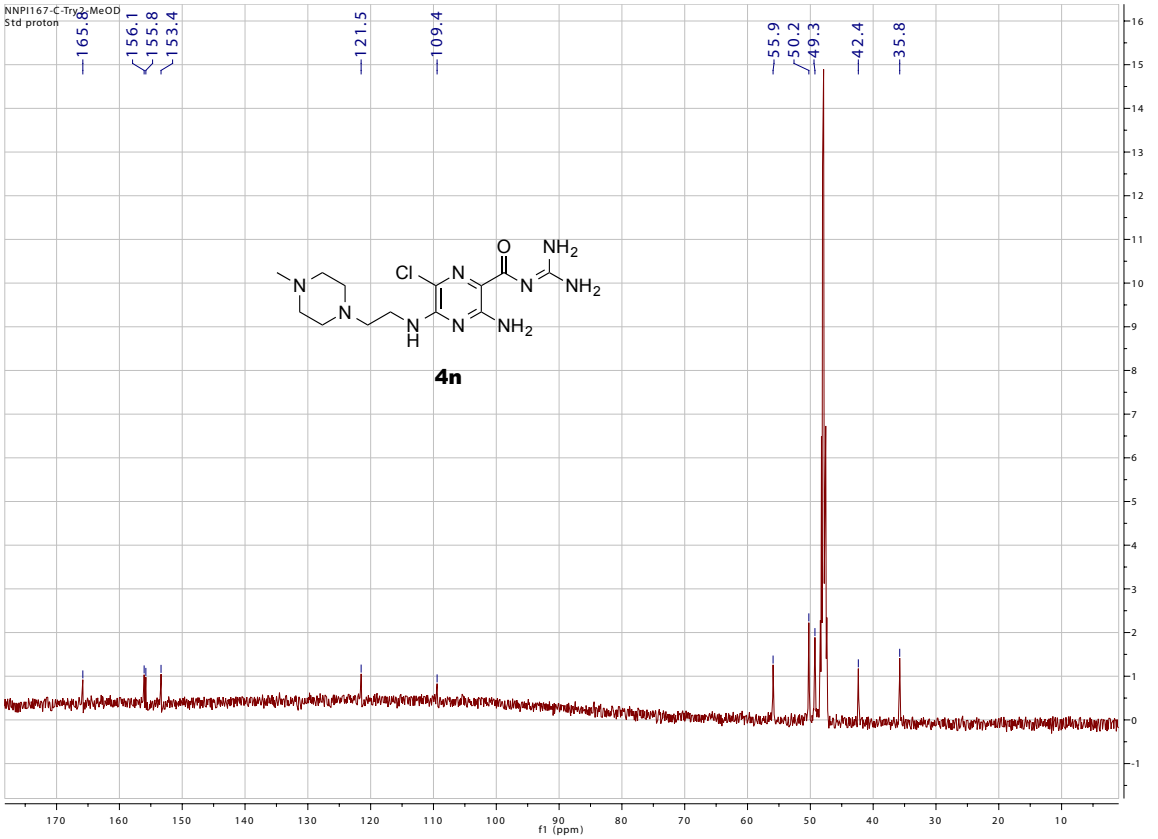
^{13}C NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-(((tetrahydrofuran-2-yl)methyl)amino)pyrazine-2-carboxamide (4m):



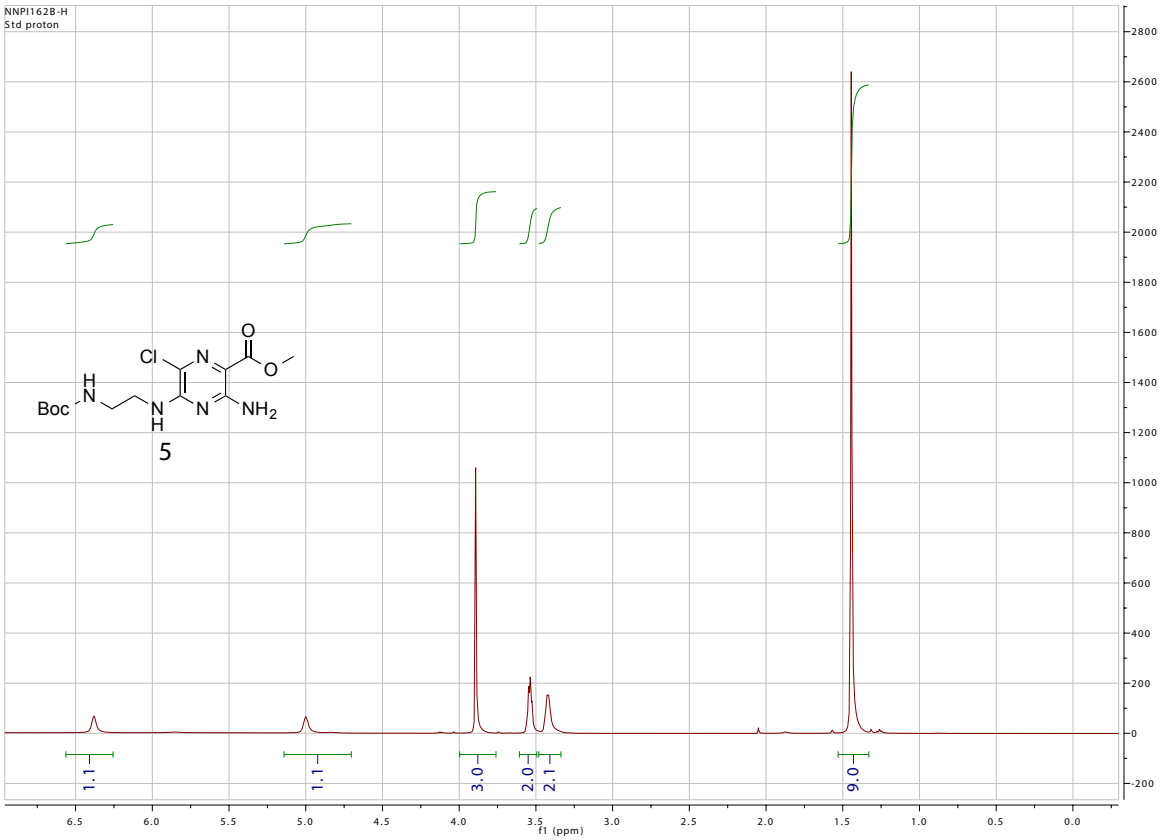
^{1}H NMR (CD_3OD, 50 ºC) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-((2-(4-methylpiperazin-1-yl)ethyl)amino)pyrazine-2-carboxamide (4n):



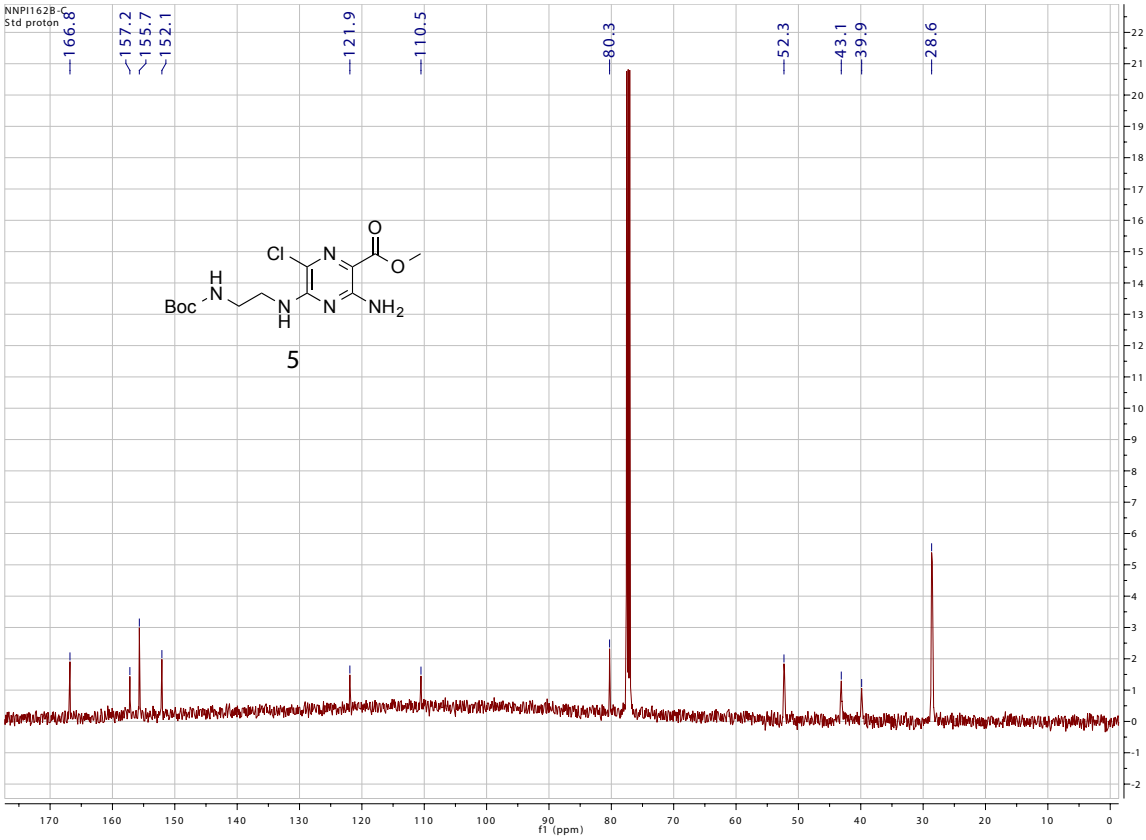
^{13}C NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-((2-(4-methylpiperazin-1-yl)ethyl)amino)pyrazine-2-carboxamide (4n):



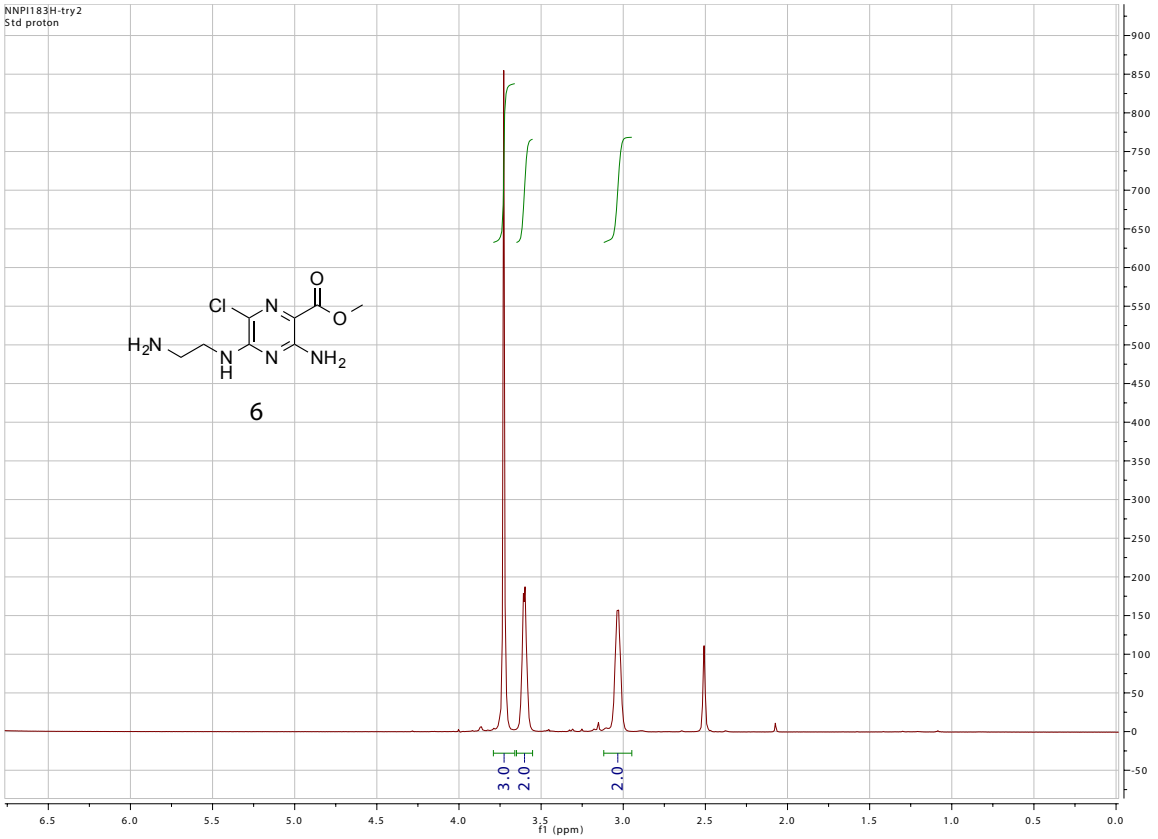
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-5-((2-((*tert*-butoxycarbonyl)amino)ethyl)amino)-6-chloropyrazine-2-carboxylate (5):



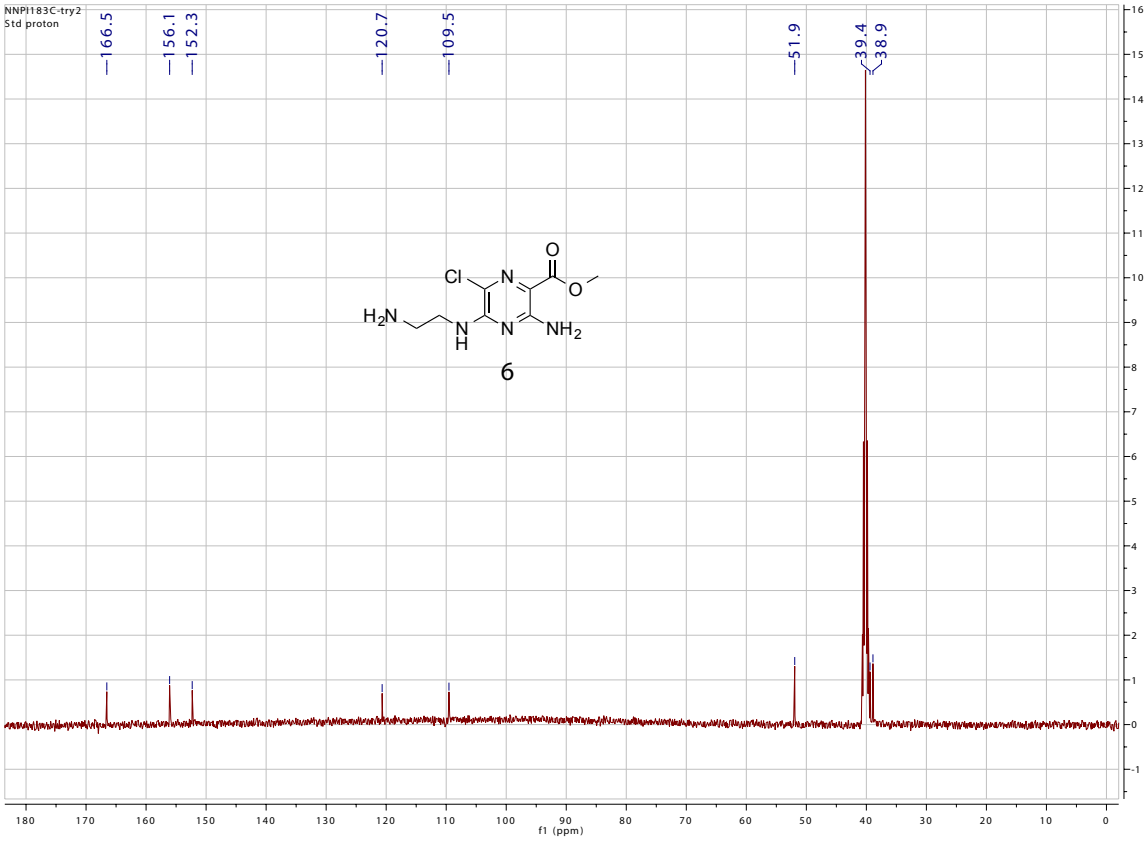
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-5-((2-((*tert*-butoxycarbonyl)amino)ethyl)amino)-6-chloropyrazine-2-carboxylate (5):



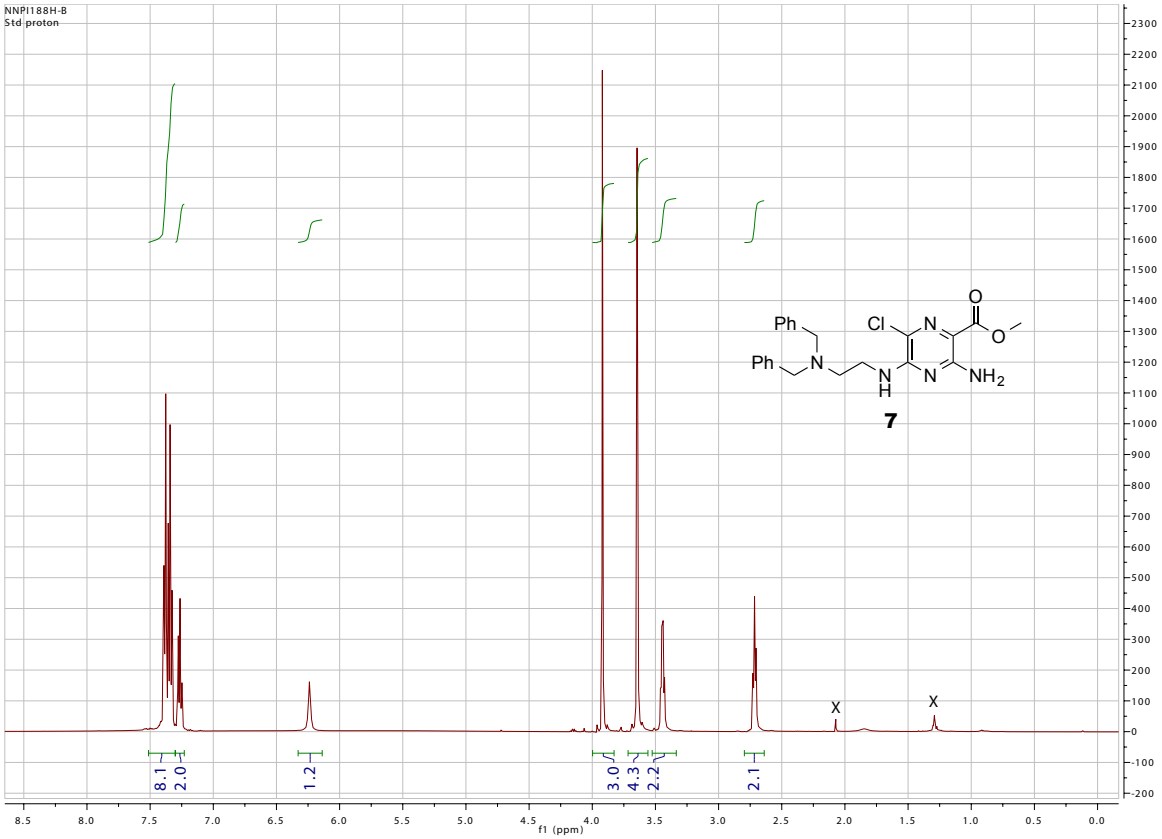
^{1}H NMR (DMSO-d_6) of Methyl 3-amino-5-((2-aminoethyl)amino)-6-chloropyrazine-2-carboxylate (6):



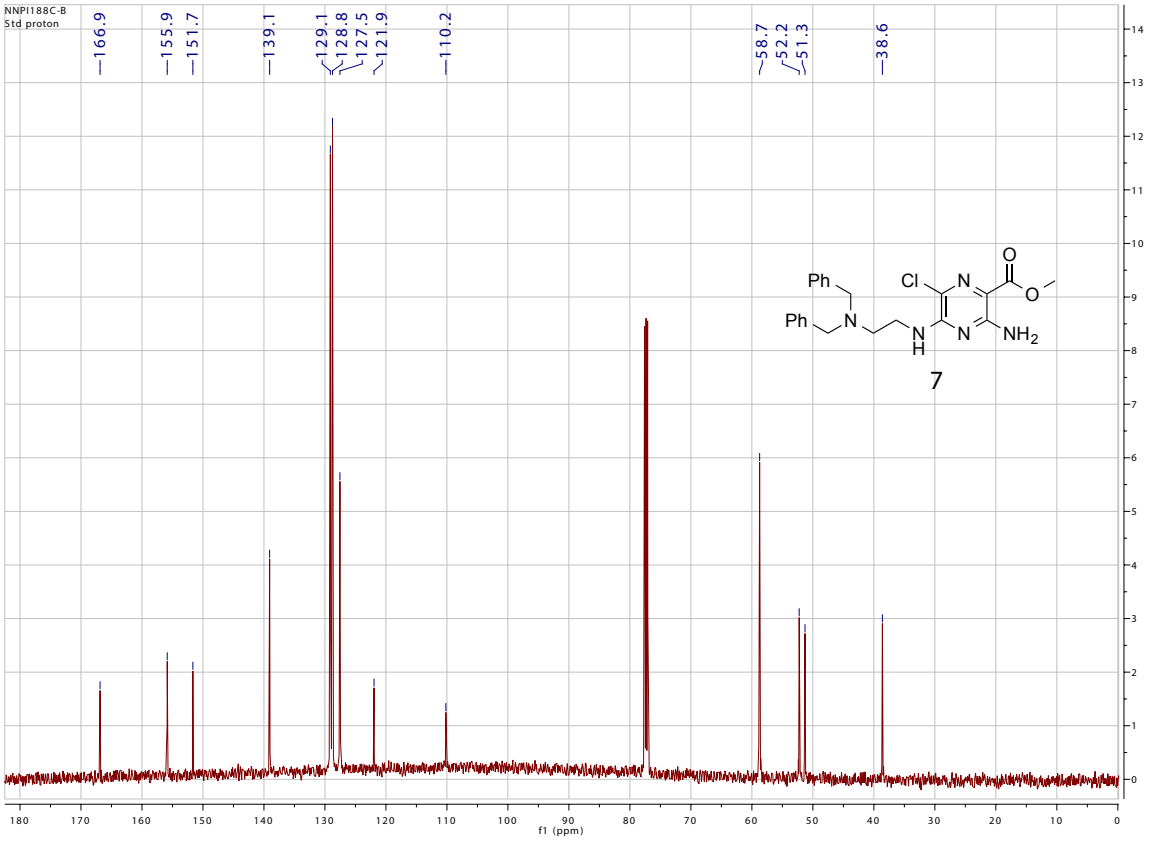
^{13}C NMR (DMSO-d_6) of Methyl 3-amino-5-((2-aminoethyl)amino)-6-chloropyrazine-2-carboxylate (6):



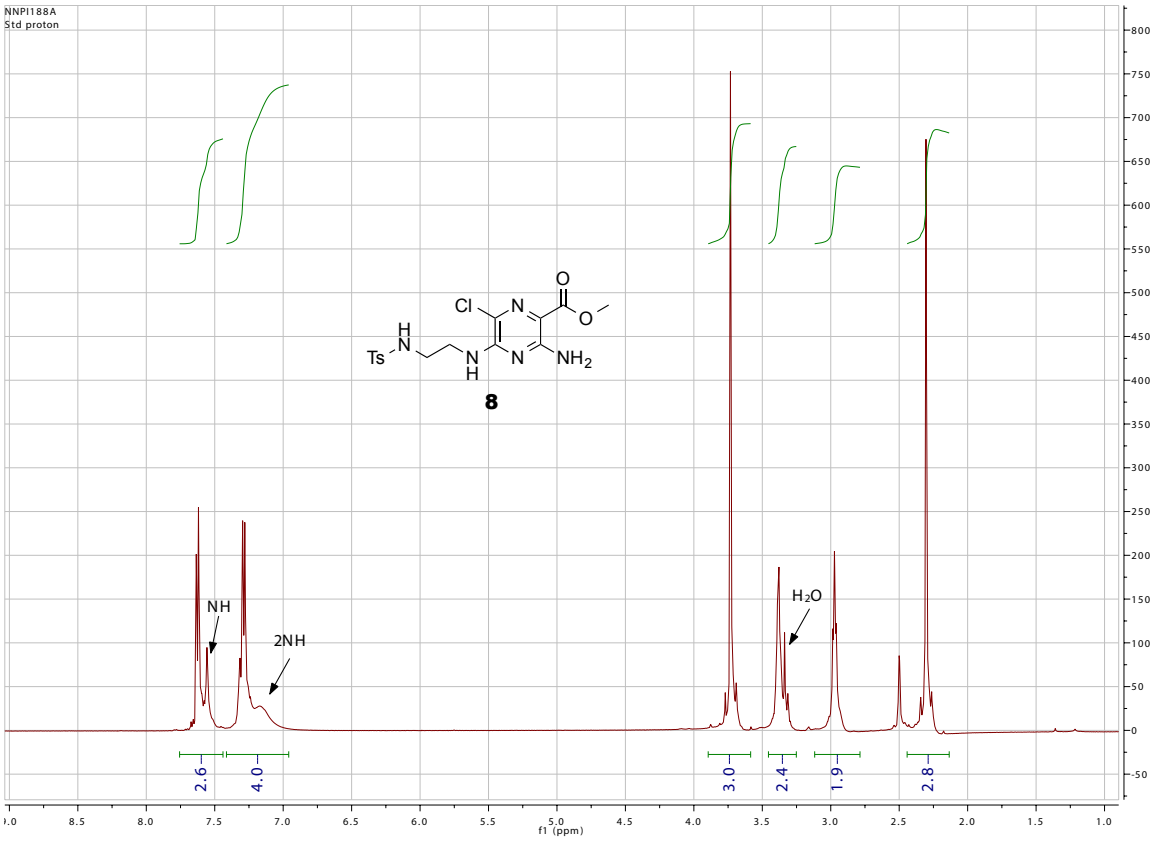
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-((2-(dibenzylamino)ethyl)amino)pyrazine-2-carboxylate (7):



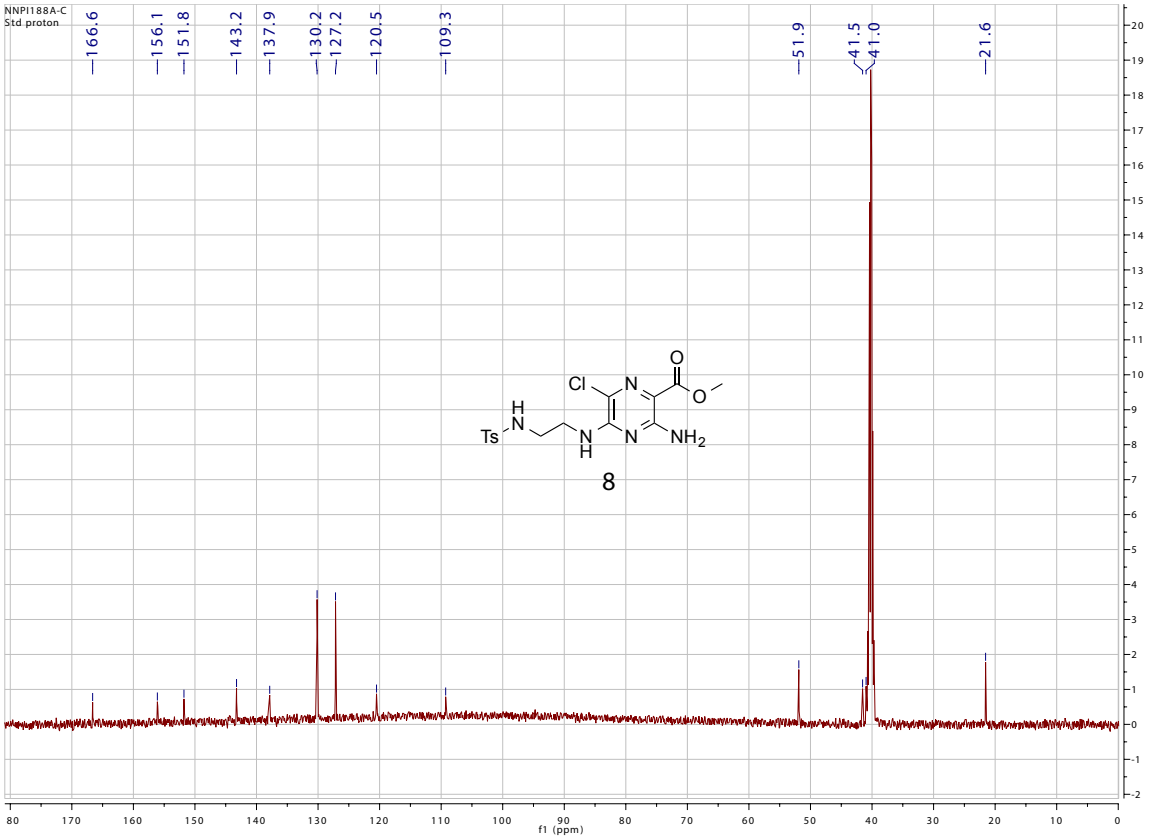
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-((2-(dibenzylamino)ethyl)amino)pyrazine-2-carboxylate (7):



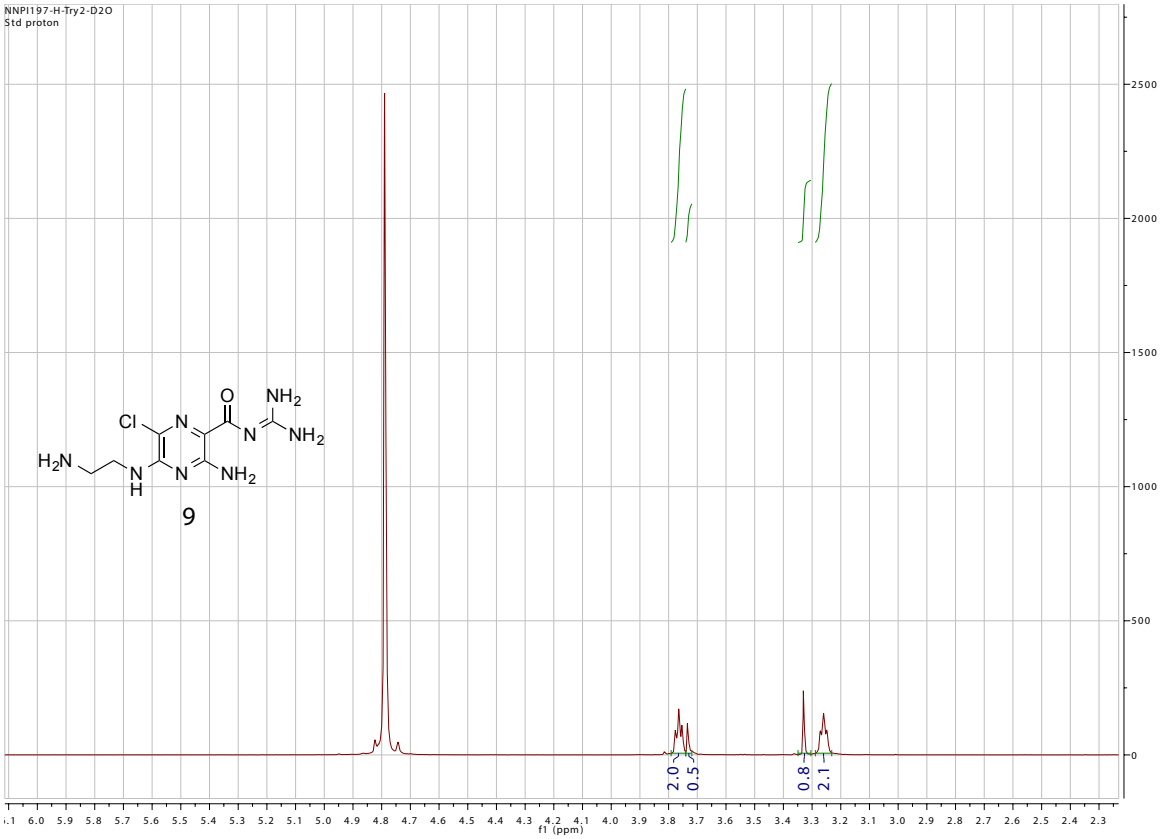
^{1}H NMR (DMSO-d_6) of Methyl 3-amino-6-chloro-5-((2-((4-methylphenyl)sulfonamido)ethyl)amino)pyrazine-2-carboxylate (8): Overlapping peaks indicated



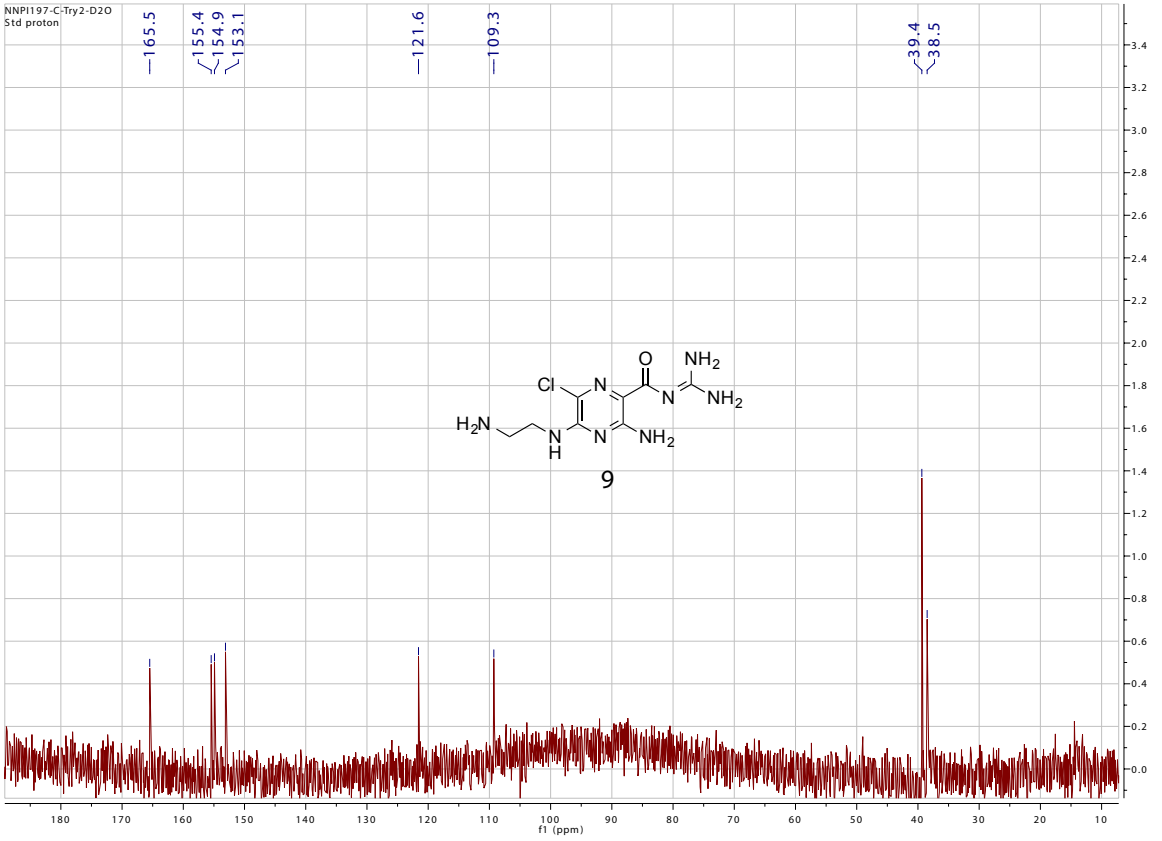
^{13}C NMR (DMSO-d_6) of Methyl 3-amino-6-chloro-5-((2-((4-methylphenyl)sulfonamido)ethyl)amino)pyrazine-2-carboxylate (8):



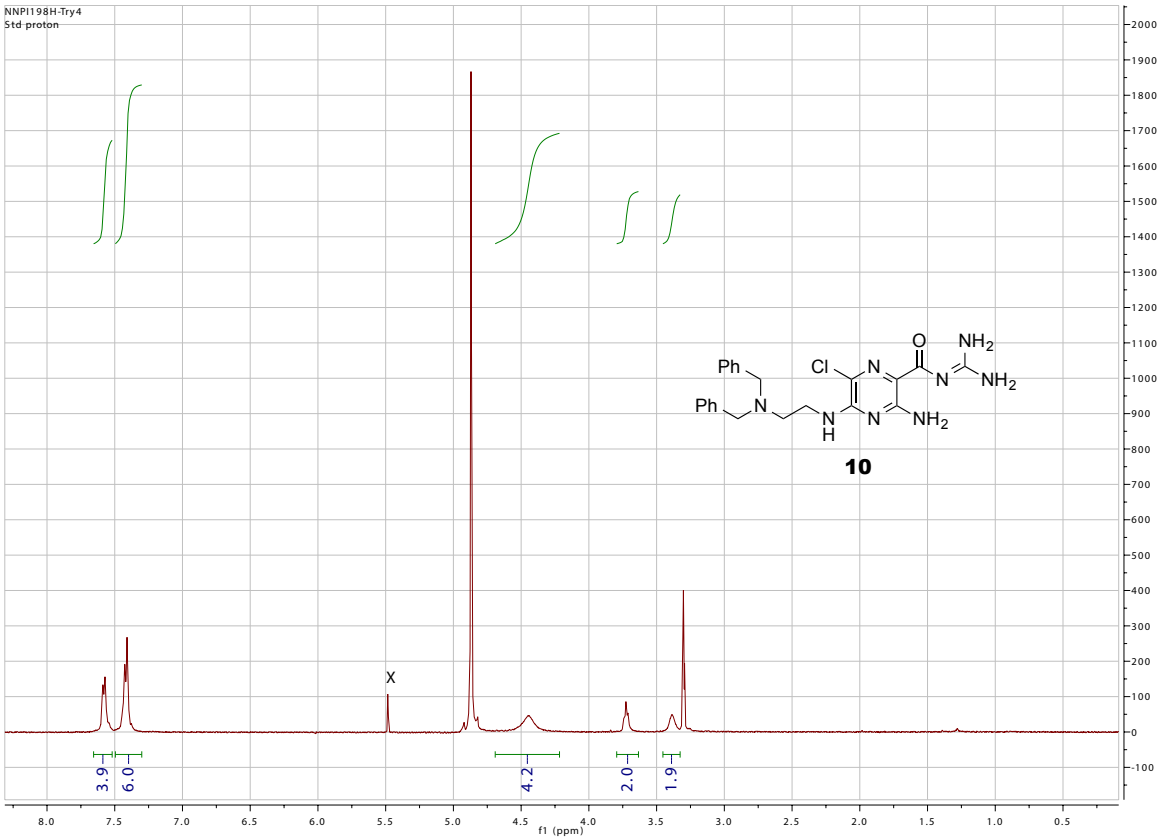
^{1}H NMR (D_2O) of 3-amino-5-((2-aminoethyl)amino)-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (9):



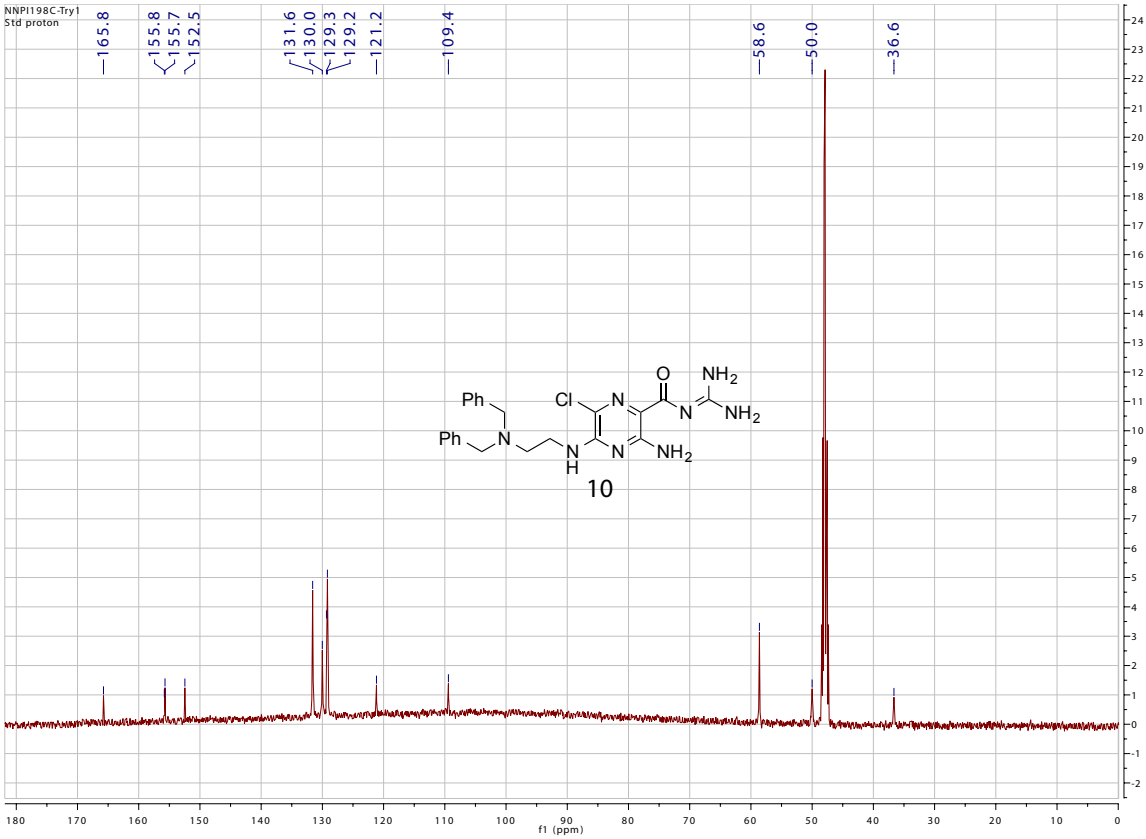
^{13}C NMR (D_2O) of 3-amino-5-((2-aminoethyl)amino)-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (9): Chemical shifts referenced to assumed guanidine carbon at 165.5 ppm



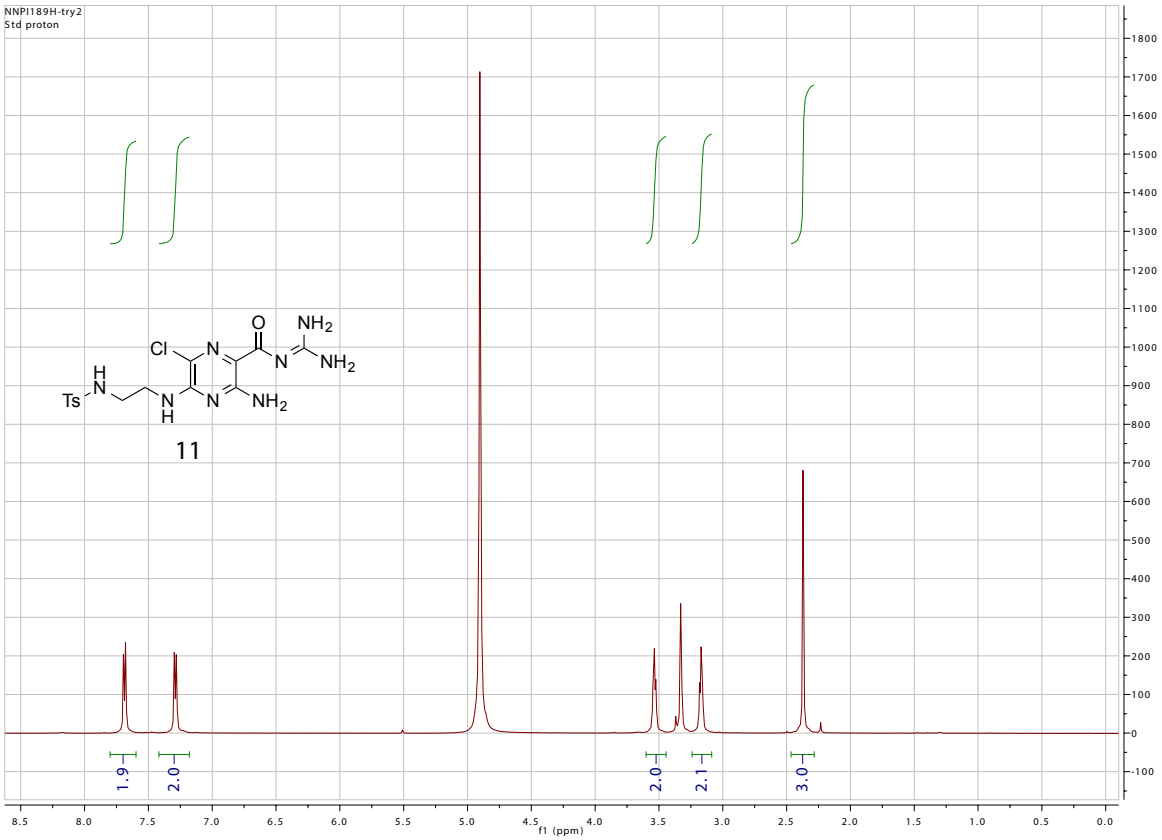
^{1}H NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-((2-(dibenzylamino)ethyl)amino)pyrazine-2-carboxamide (10):



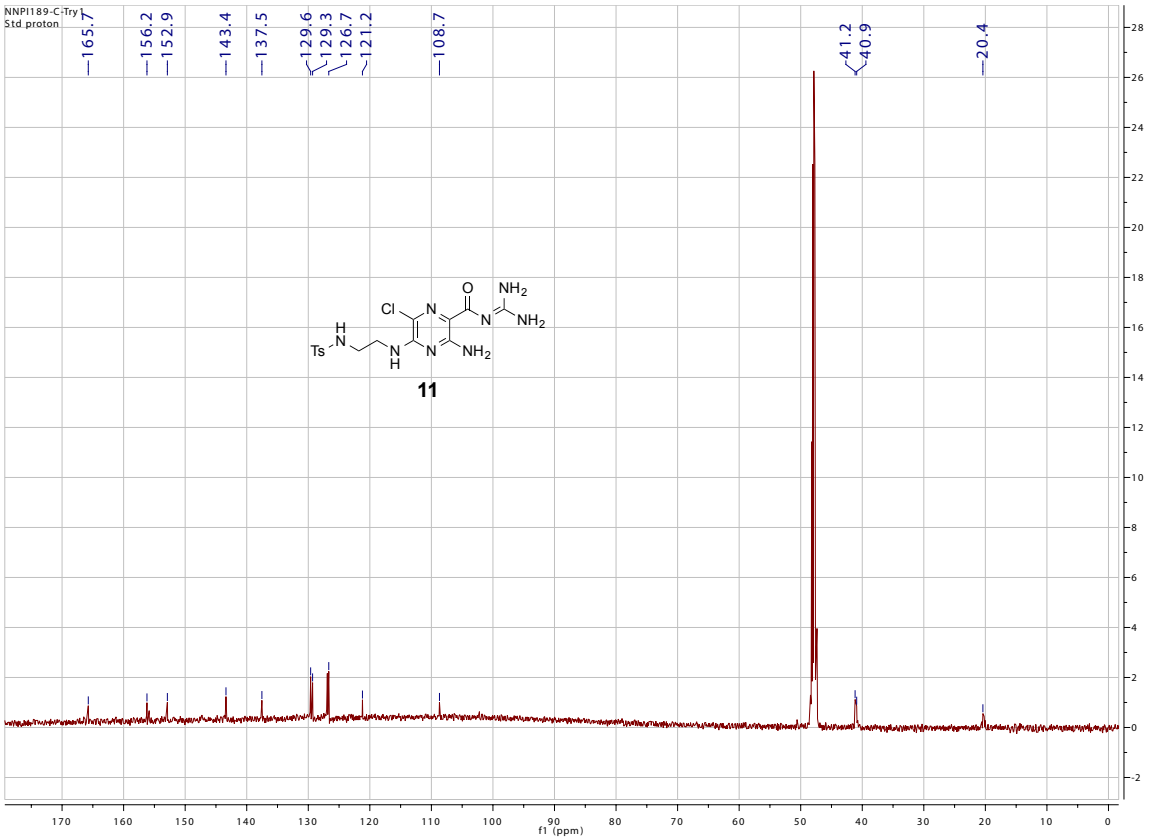
^{13}C NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-((2-(dibenzylamino)ethyl)amino)pyrazine-2-carboxamide (10):



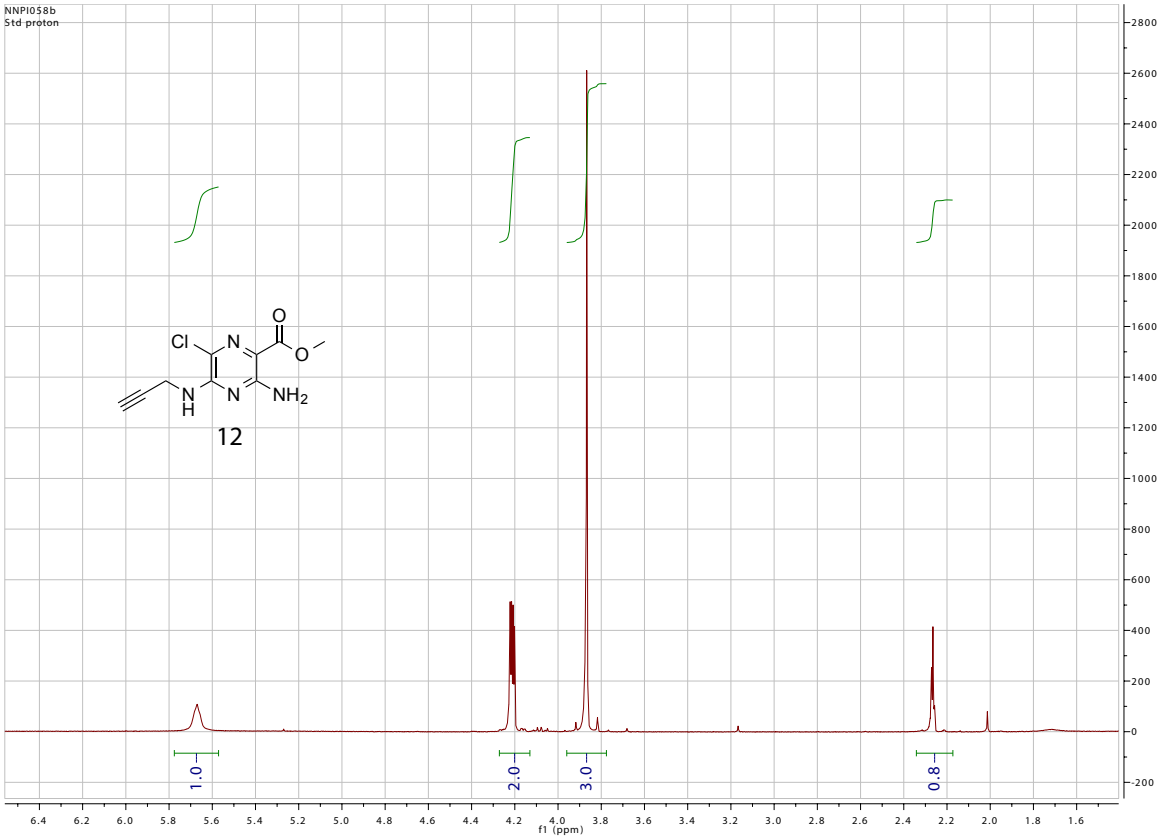
^{1}H NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-((2-((4-methylphenyl)sulfonamido)ethyl)amino)pyrazine-2-carboxamide (11):



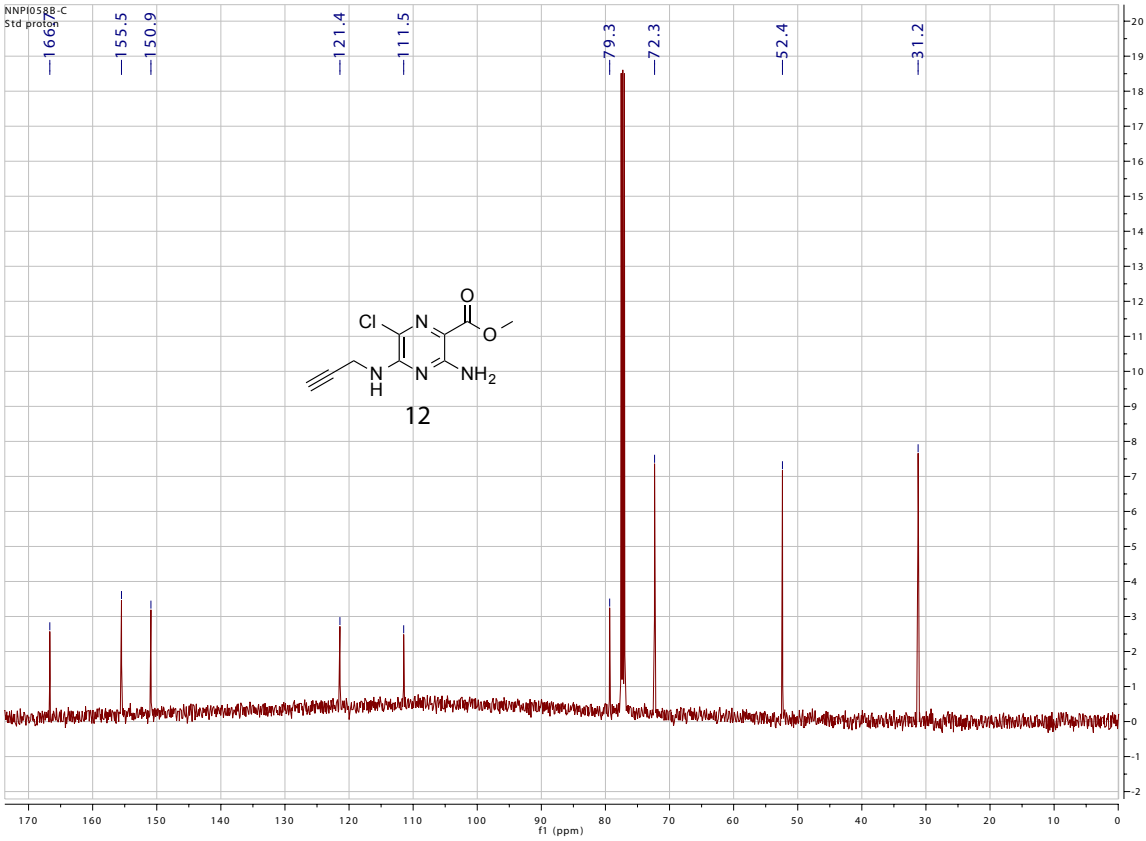
^{13}C NMR (CD_3OD) of 3-amino-6-chloro-*N*-(diaminomethylene)-5-((2-((4-methylphenyl)sulfonamido)ethyl)amino)pyrazine-2-carboxamide (11):



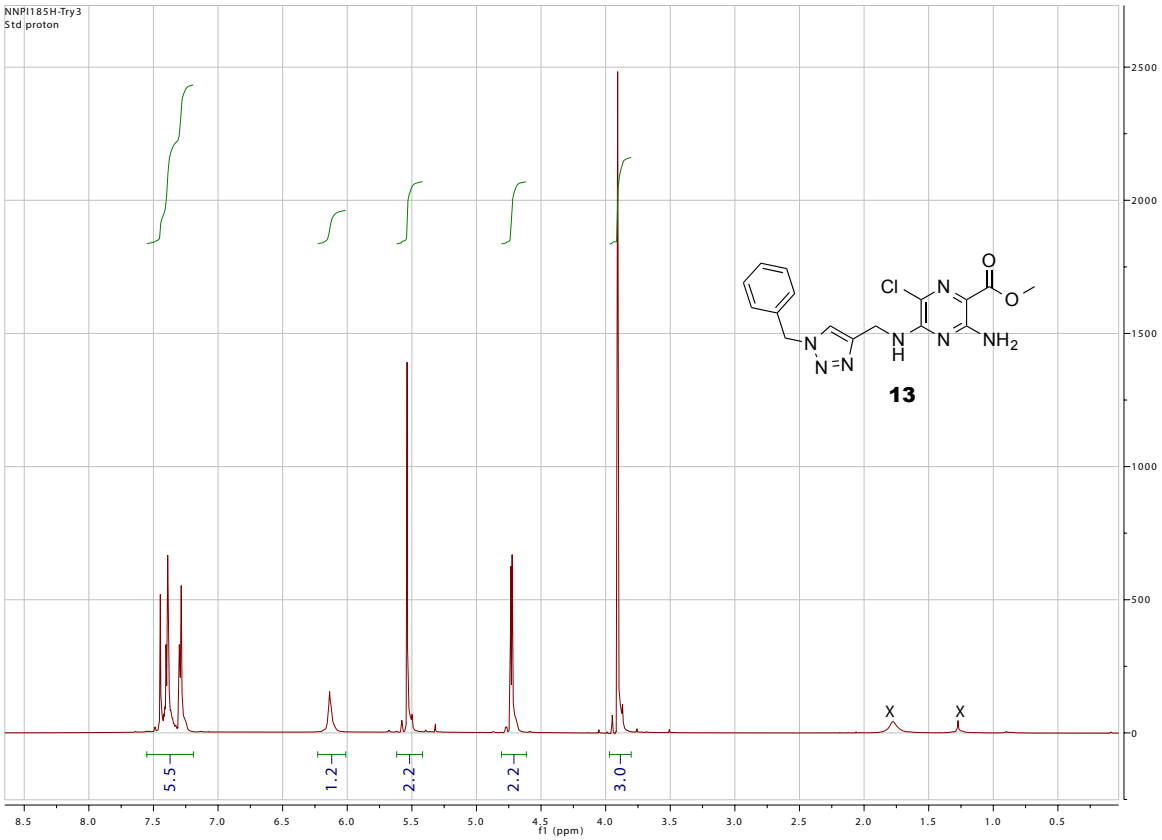
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-(prop-2-yn-1-ylamino)pyrazine-2-carboxylate (12):



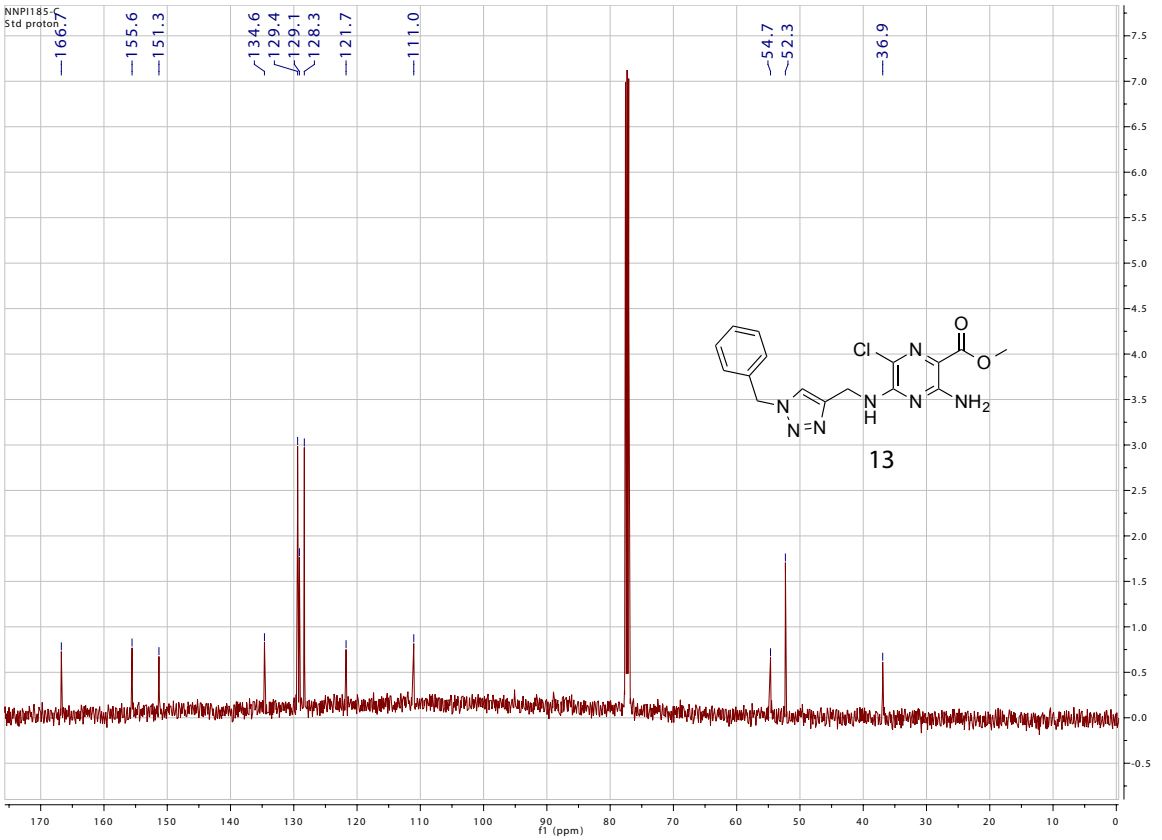
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-(prop-2-yn-1-ylamino)pyrazine-2-carboxylate (12):



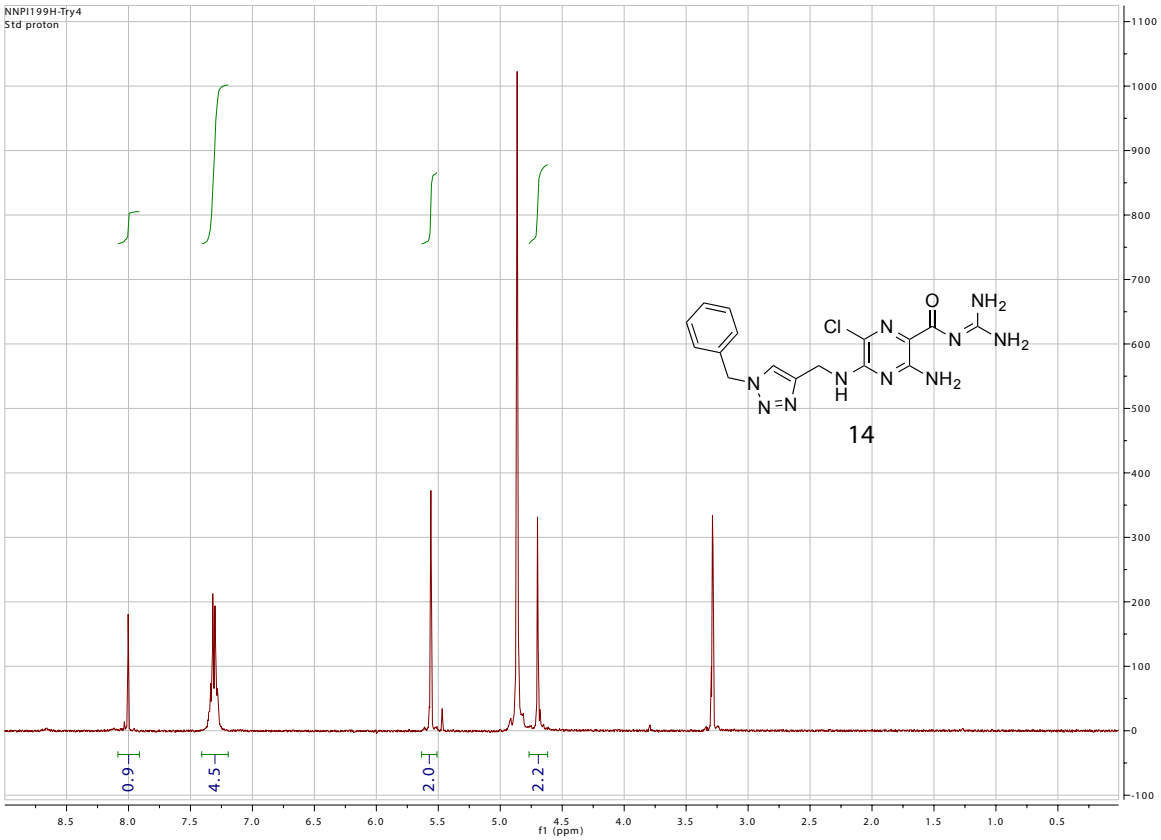
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-5-(((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)amino)-6-chloropyrazine-2-carboxylate (13):



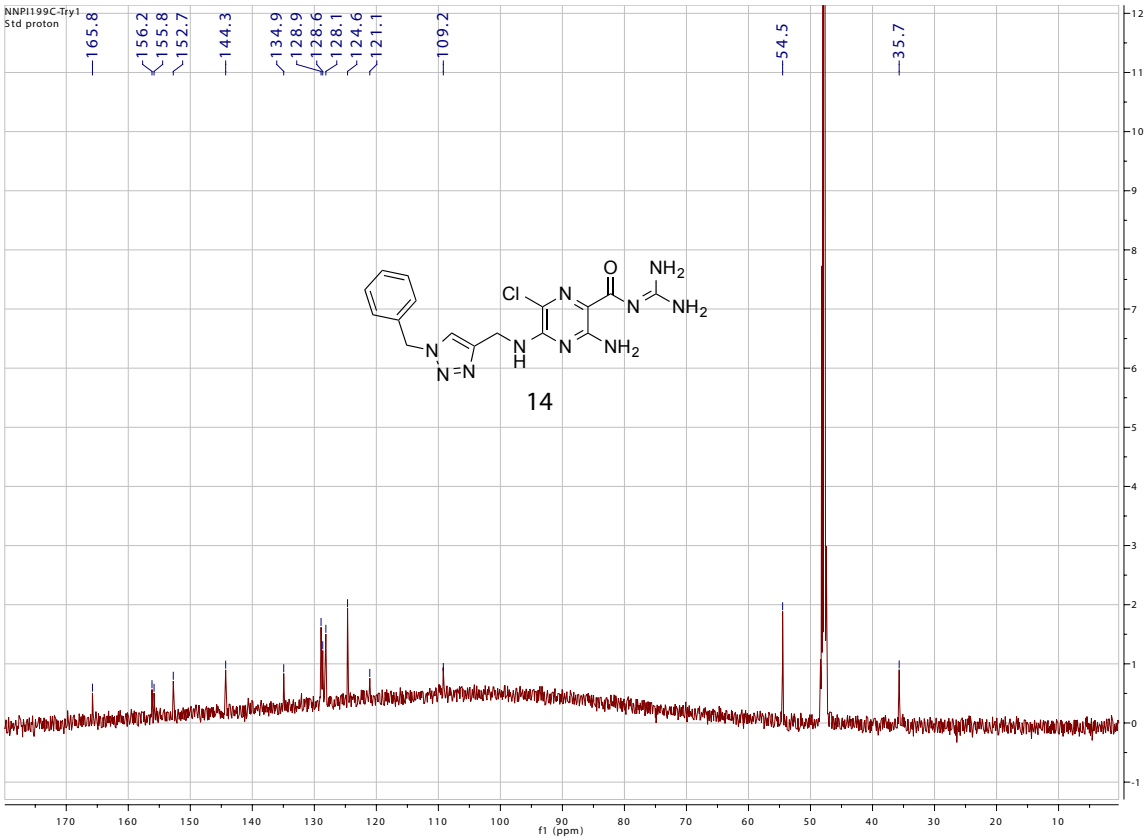
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-5-(((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)amino)-6-chloropyrazine-2-carboxylate (13): Peak overlap observed in aromatic region burying the triazole carbon peaks.



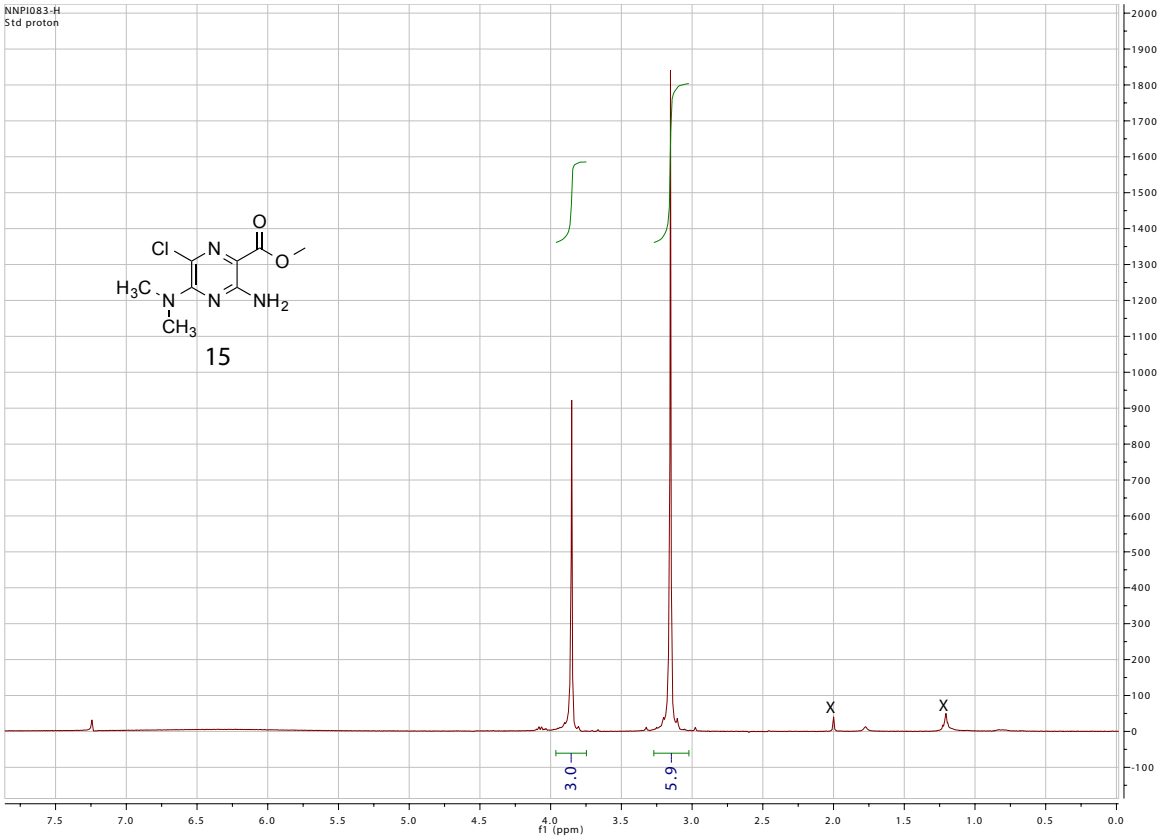
^{1}H NMR (CD_3OD) of 3-amino-5-(((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)amino)-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (14):



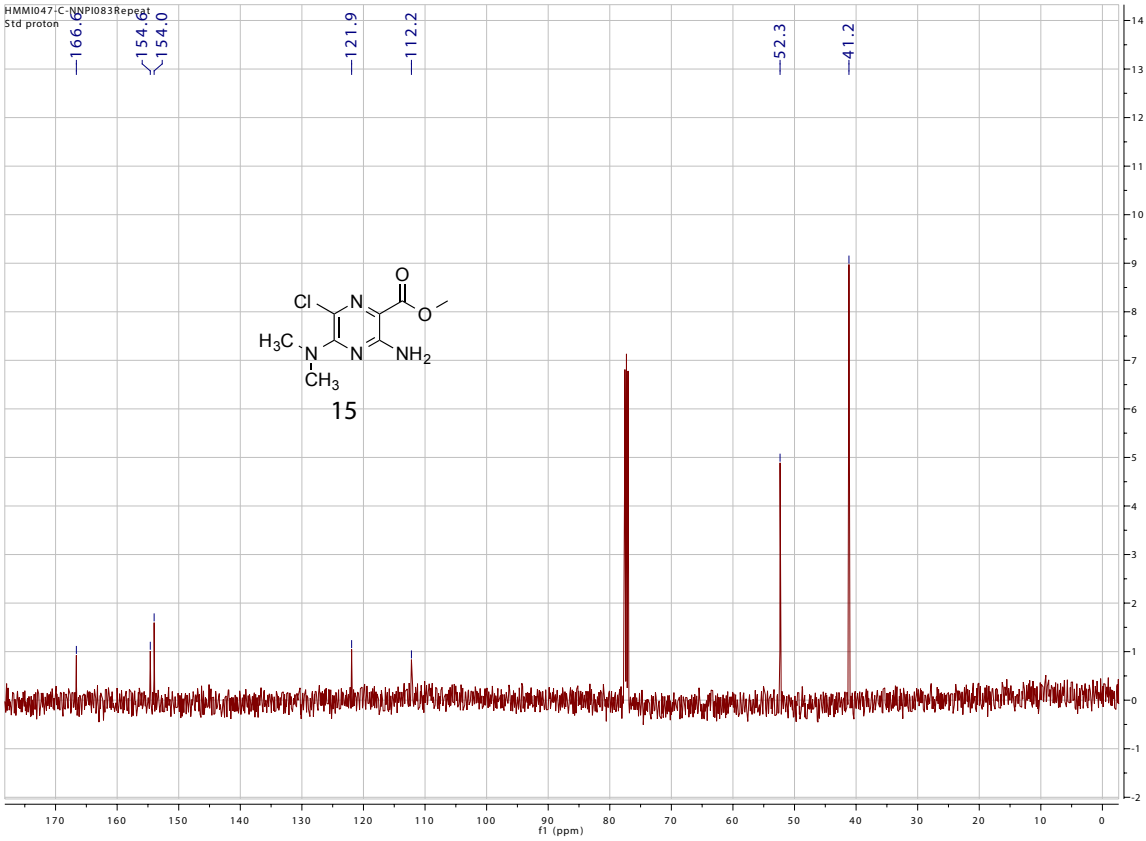
^{13}C NMR (CD_3OD) of 3-amino-5-(((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)amino)-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (14):



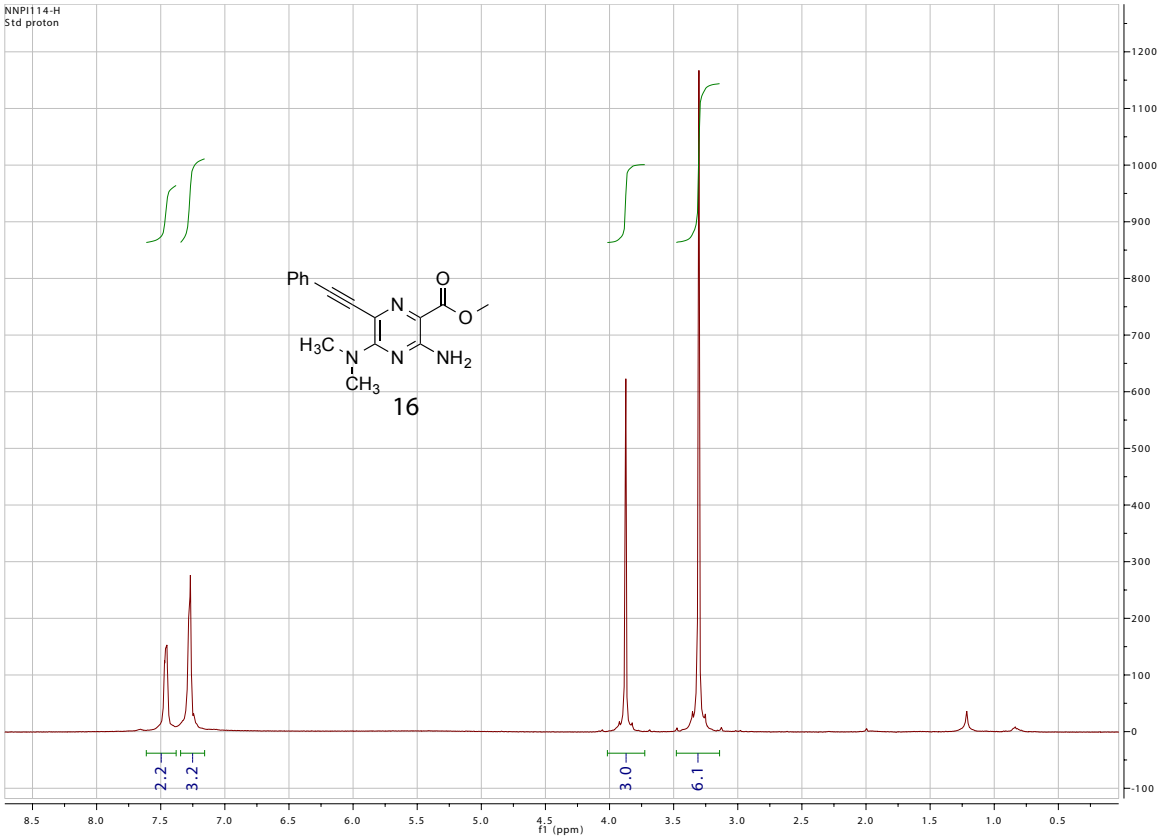
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-(dimethylamino)pyrazine-2-carboxylate (15):



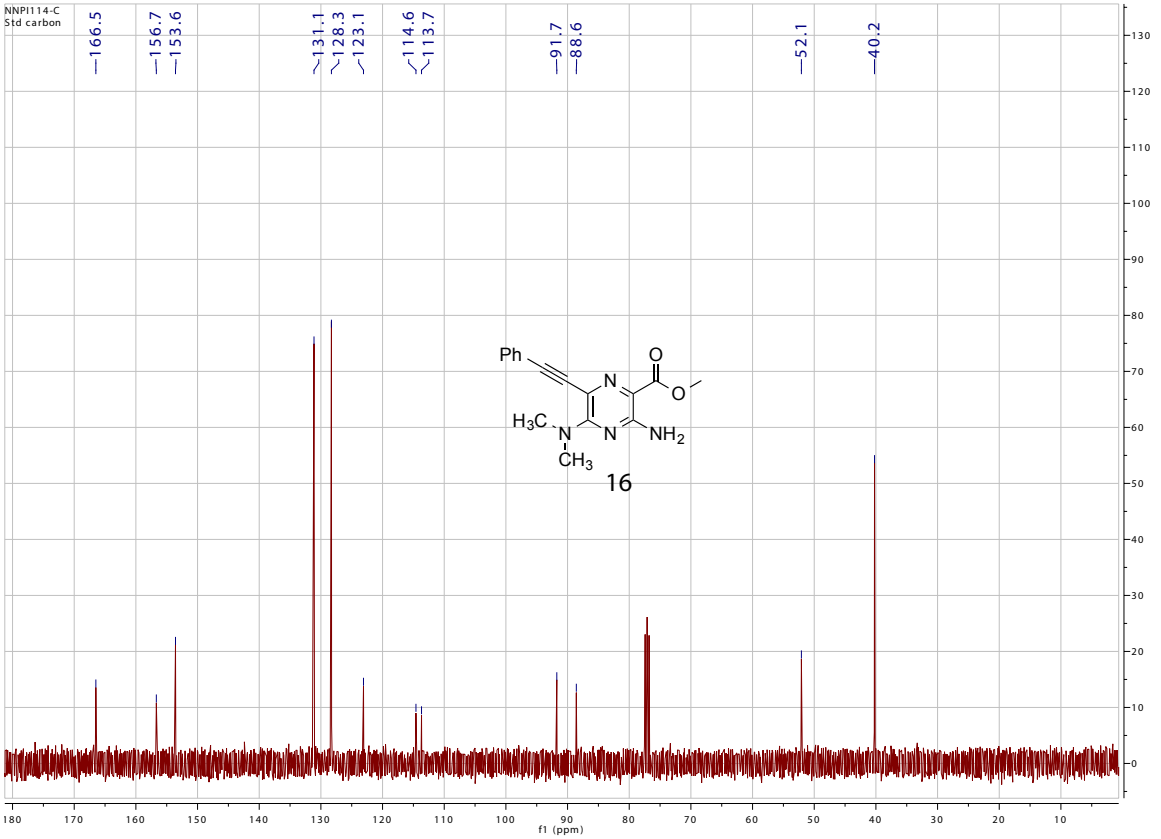
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-6-chloro-5-(dimethylamino)pyrazine-2-carboxylate (15):



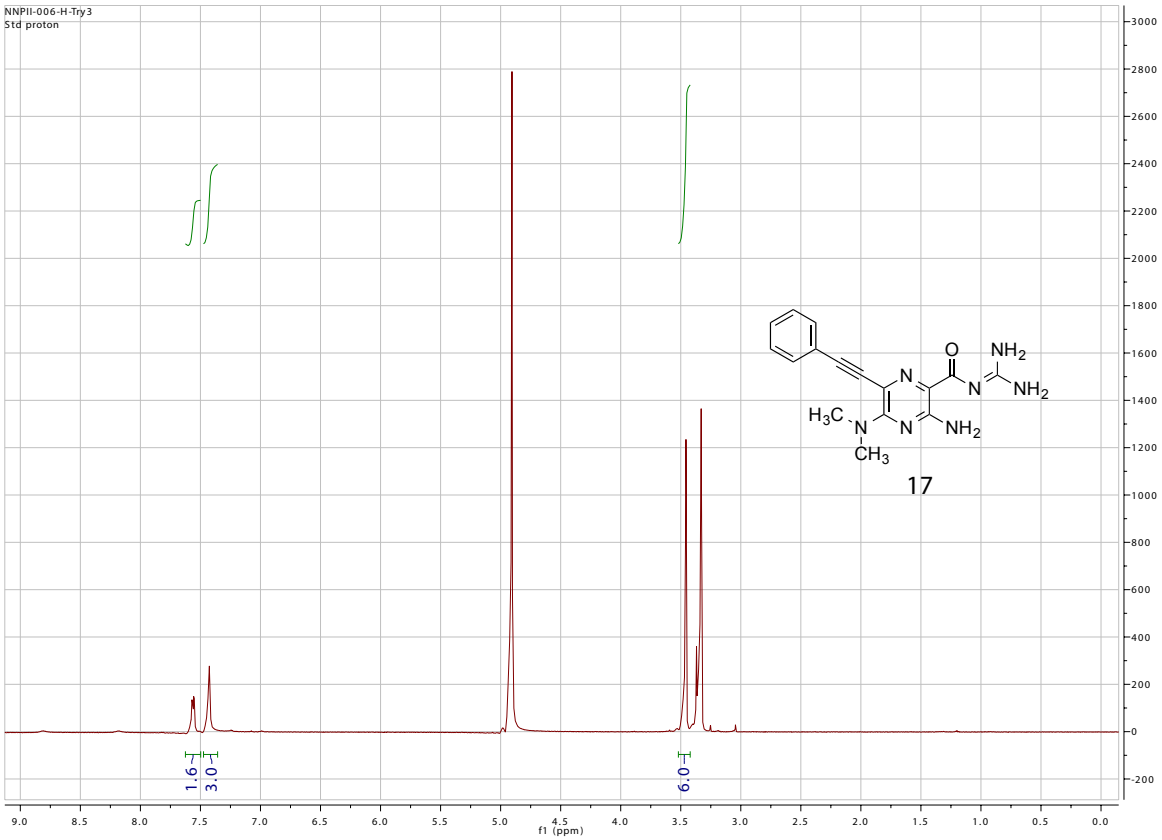
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-5-(dimethylamino)-6-(phenylethynyl)pyrazine-2-carboxylate (16):



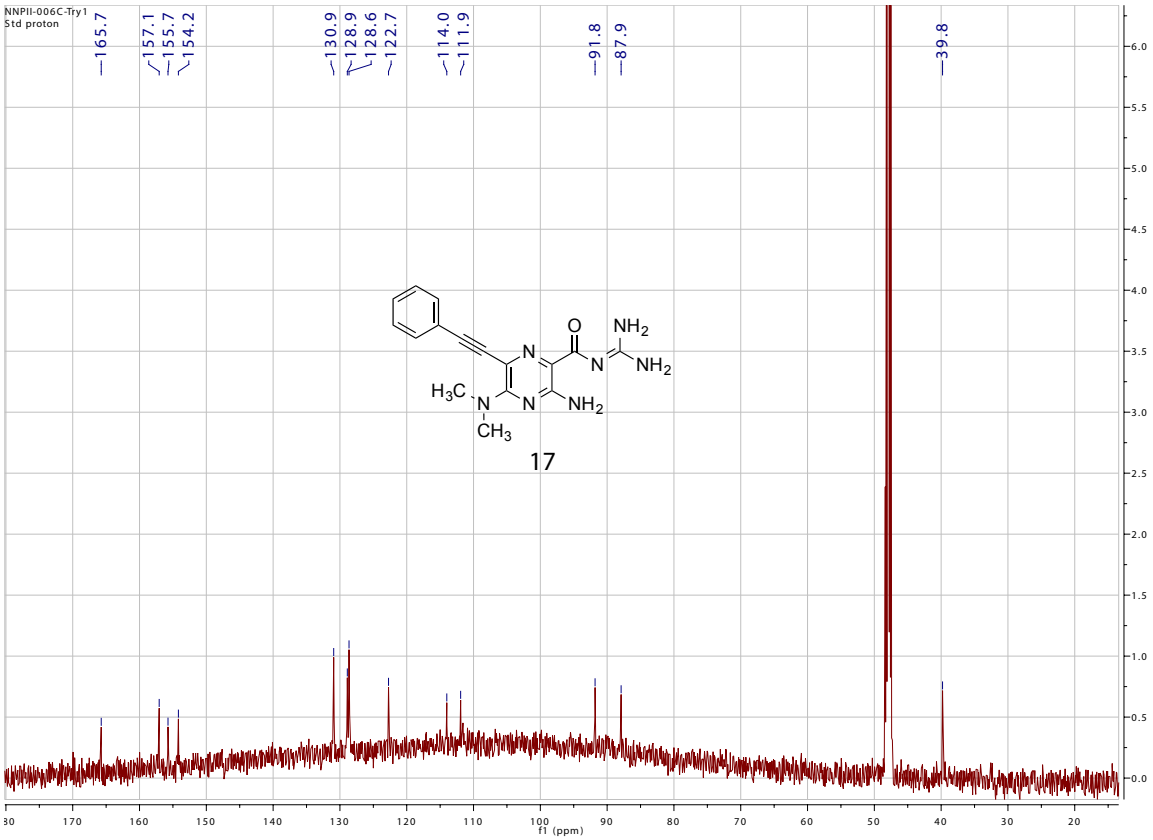
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-5-(dimethylamino)-6-(phenylethynyl)pyrazine-2-carboxylate (16): Peak overlap observed in the aromatic region.



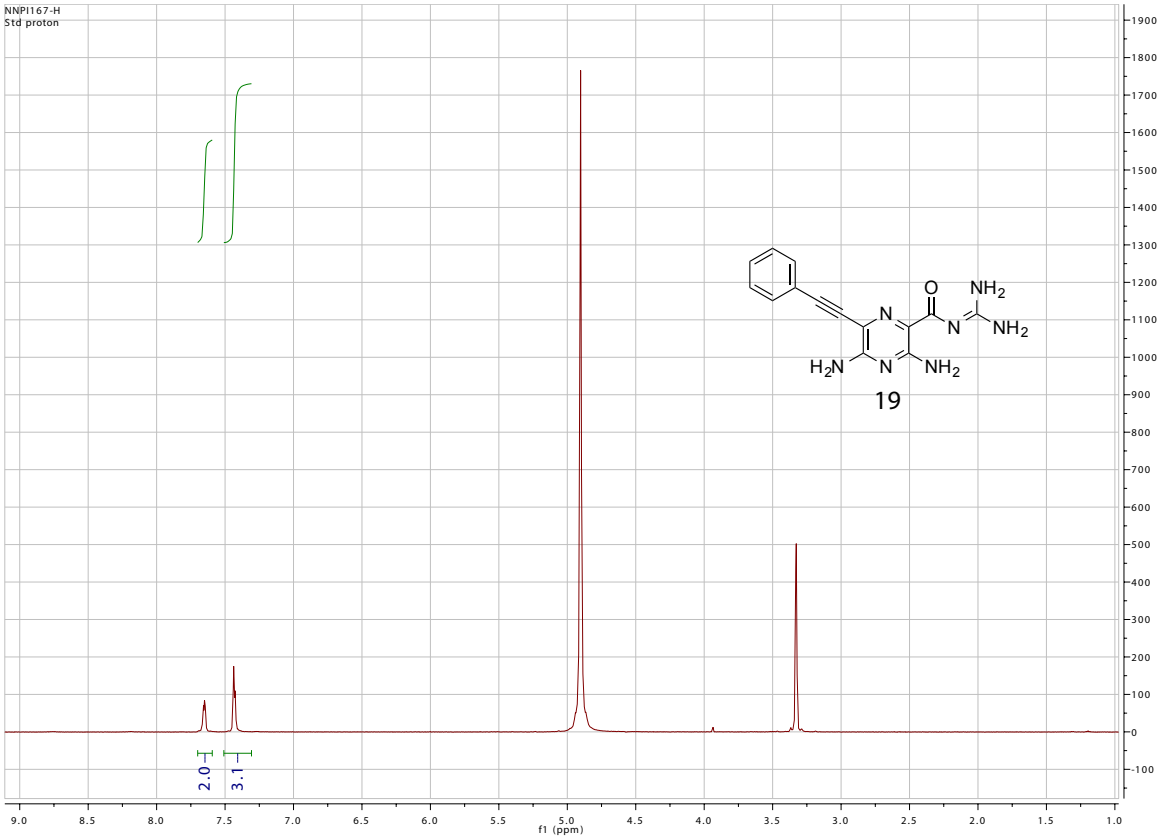
^{1}H NMR (CD_3OD) of 3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-(phenylethynyl)pyrazine-2-carboxamide (17):



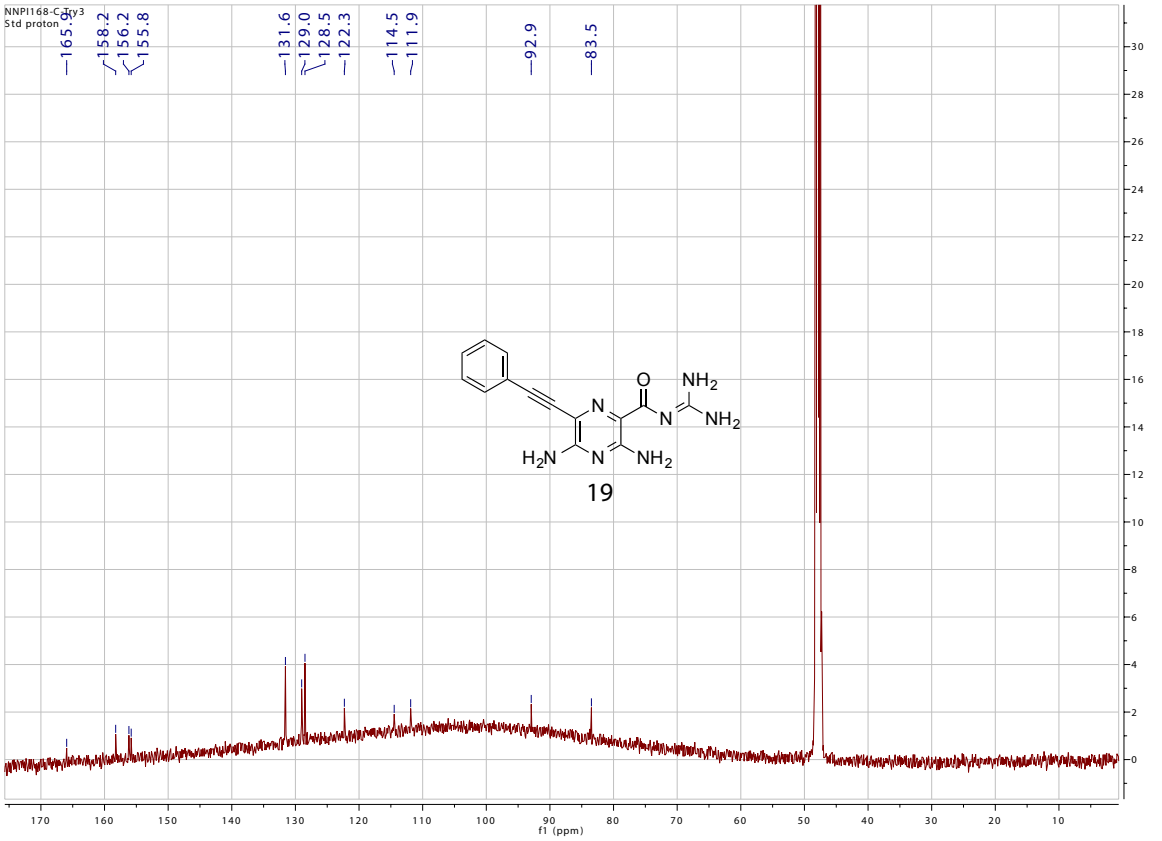
^{13}C NMR (CD_3OD) of 3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-(phenylethynyl)pyrazine-2-carboxamide (17):



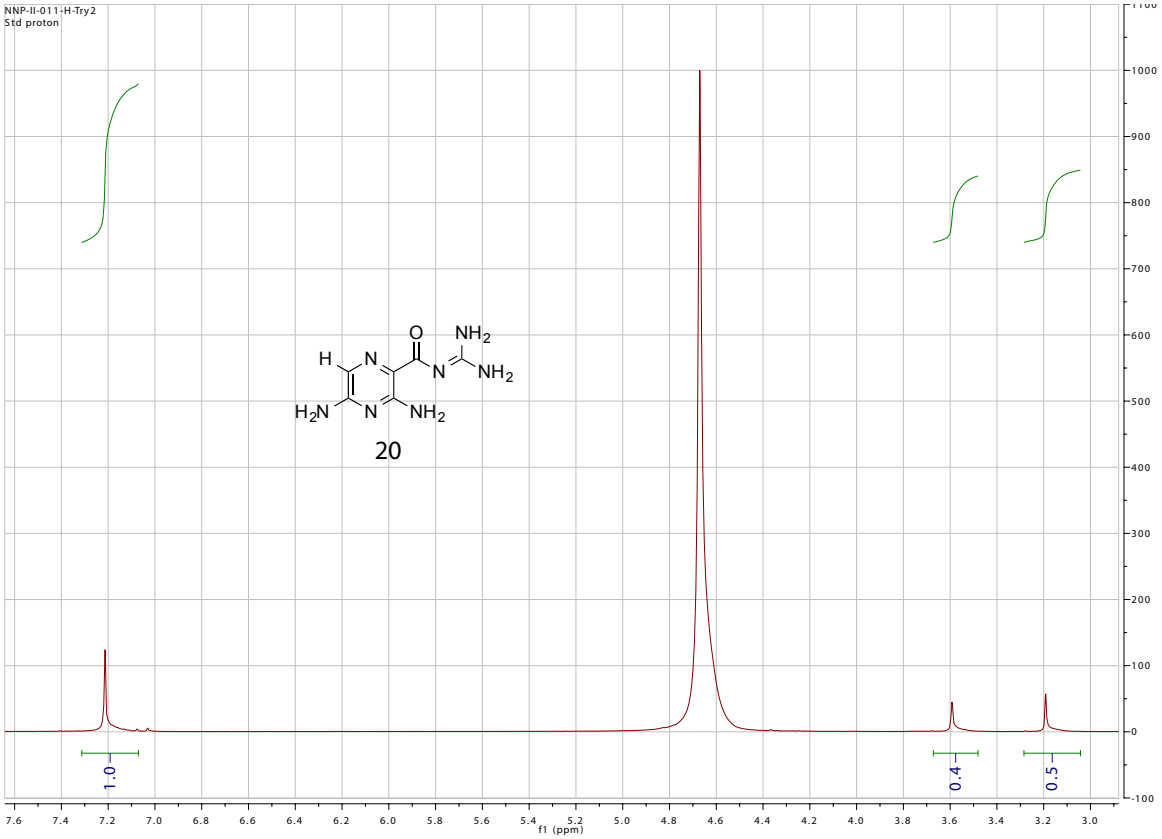
^{1}H NMR (CD_3OD) of 3,5-diamino-*N*-(diaminomethylene)-6-(phenylethynyl)pyrazine-2-carboxamide (19):



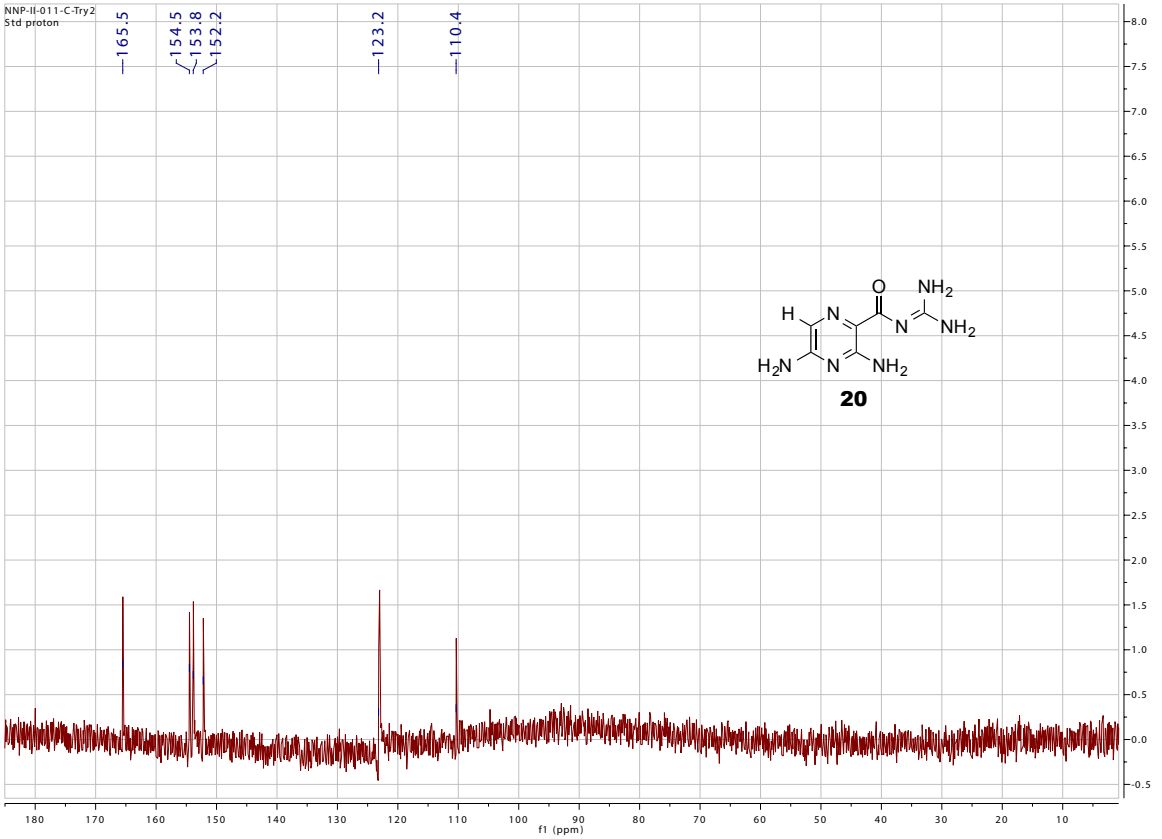
^{13}C NMR (CD_3OD) of 3,5-diamino-*N*-(diaminomethylene)-6-(phenylethynyl)pyrazine-2-carboxamide (19):



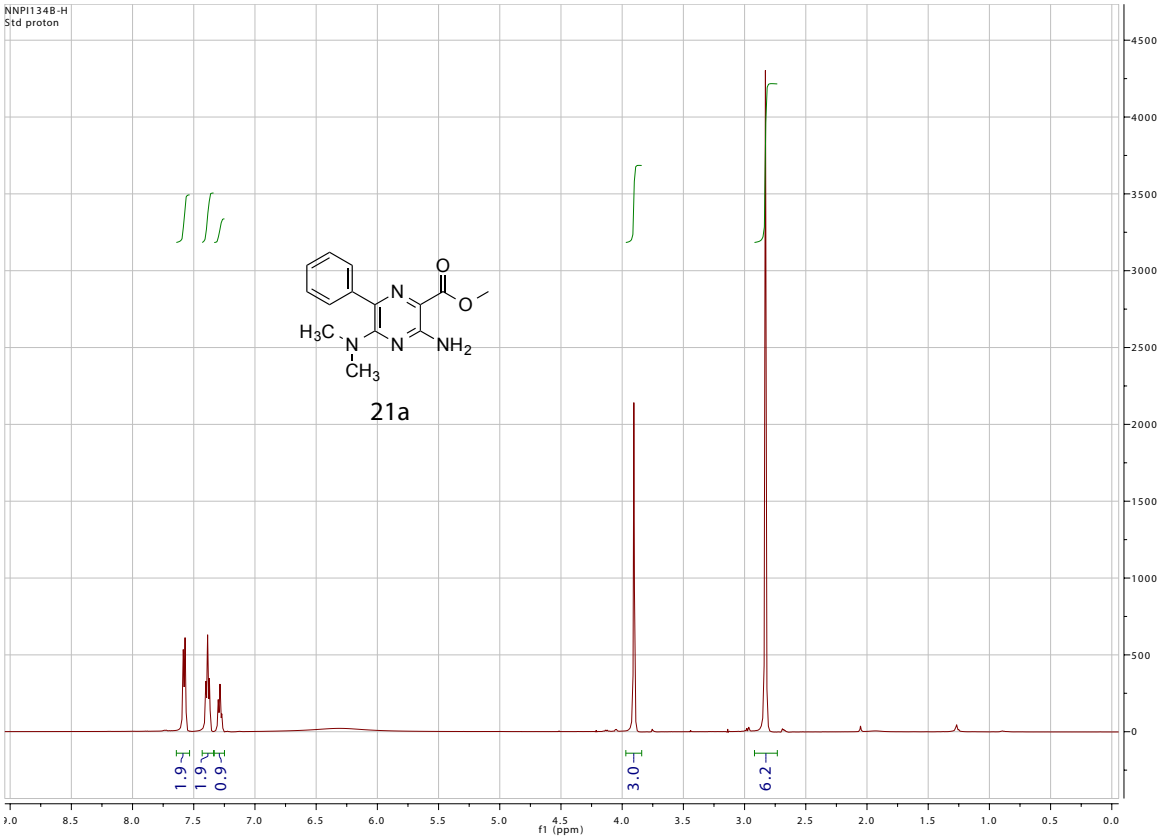
^{1}H NMR (D_2O) of 3,5-diamino-*N*-(diaminomethylene)pyrazine-2-carboxamide (20):



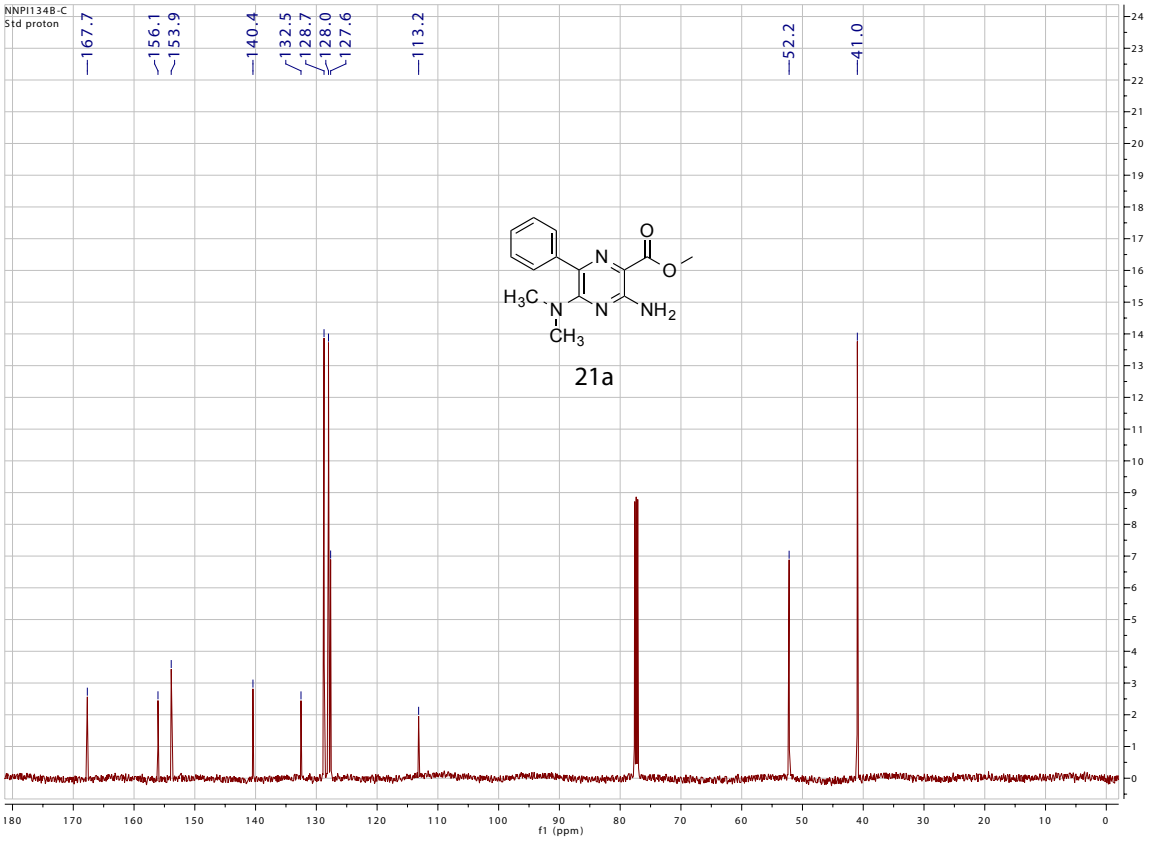
^{13}C NMR (D_2O) of 3,5-diamino-*N*-(diaminomethylene)pyrazine-2-carboxamide (20): Chemical shifts referenced to assumed guanidine peak at 165.5 ppm



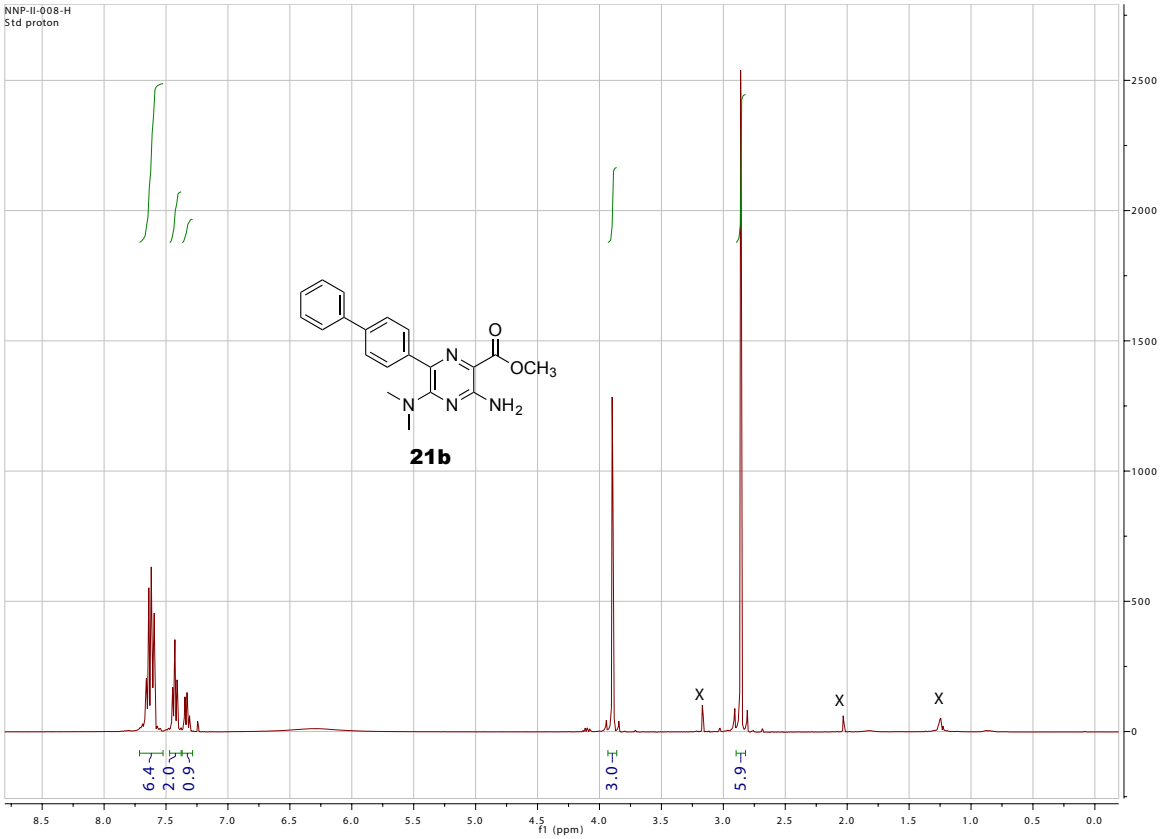
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-5-(dimethylamino)-6-phenylpyrazine-2-carboxylate (21a):



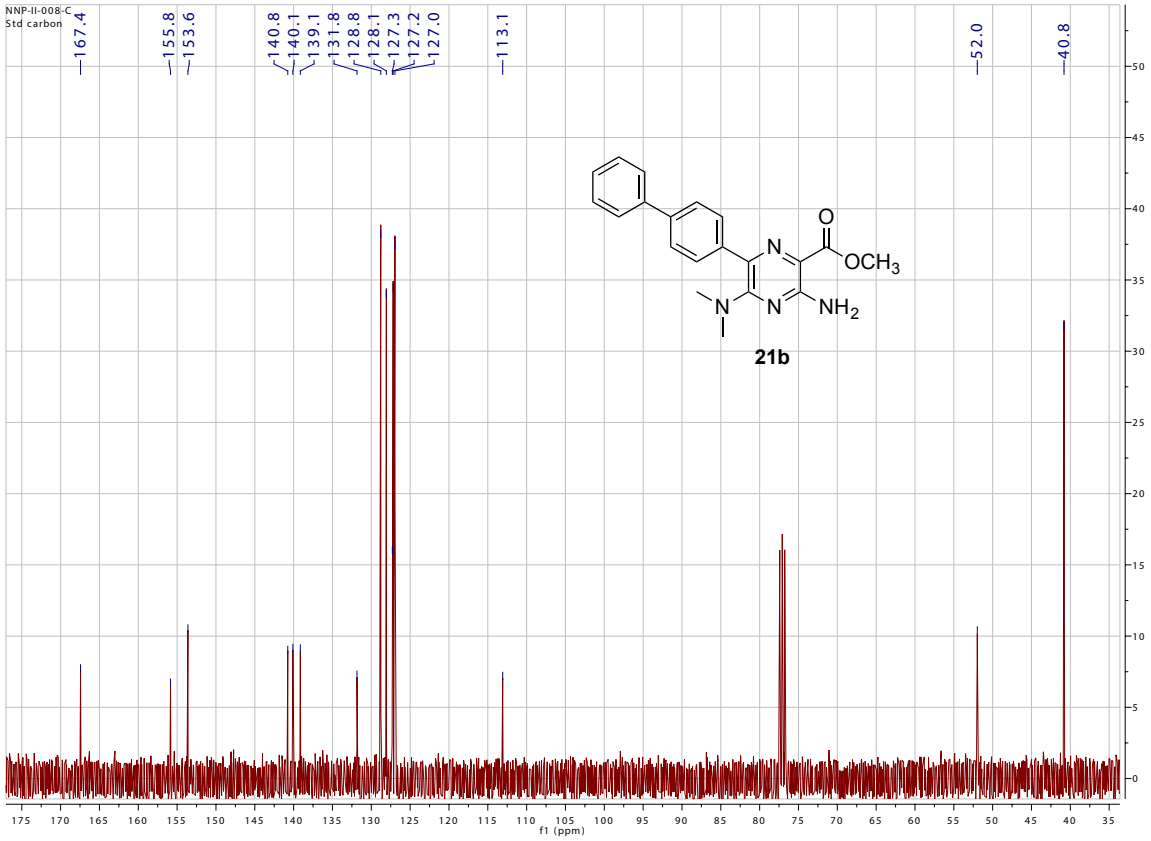
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-5-(dimethylamino)-6-phenylpyrazine-2-carboxylate (21a):



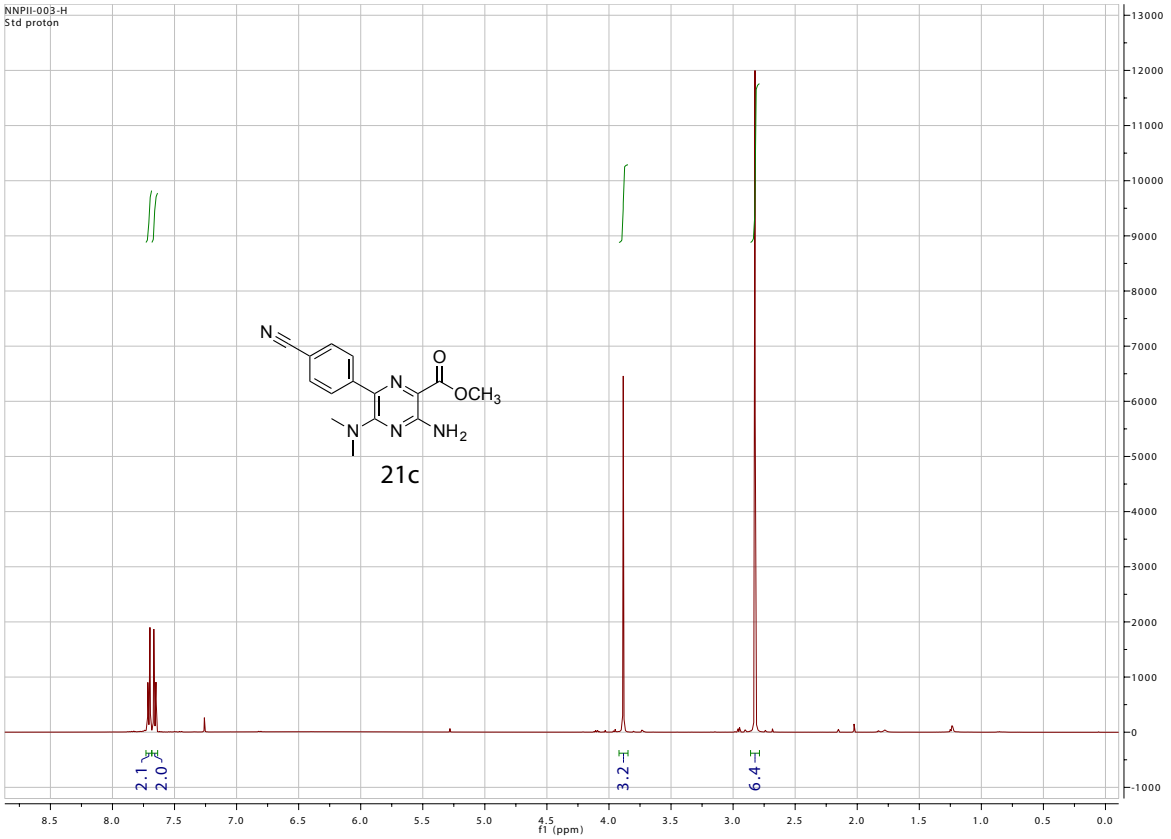
^{1}H NMR ($CDCl_3$) of Methyl 6-([1,1'-biphenyl]-4-yl)-3-amino-5-(dimethylamino)pyrazine-2-carboxylate (21b):



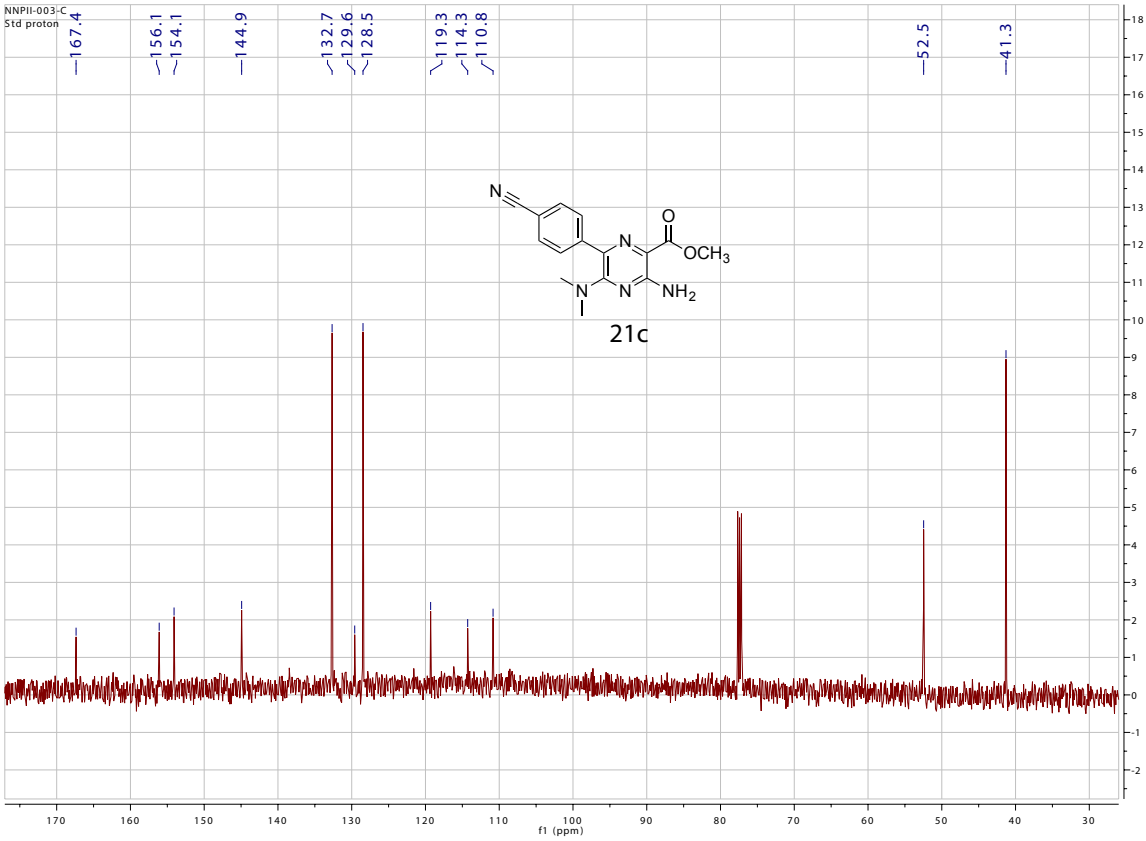
^{13}C NMR ($CDCl_3$) of Methyl 6-([1,1'-biphenyl]-4-yl)-3-amino-5-(dimethylamino)pyrazine-2-carboxylate (21b):



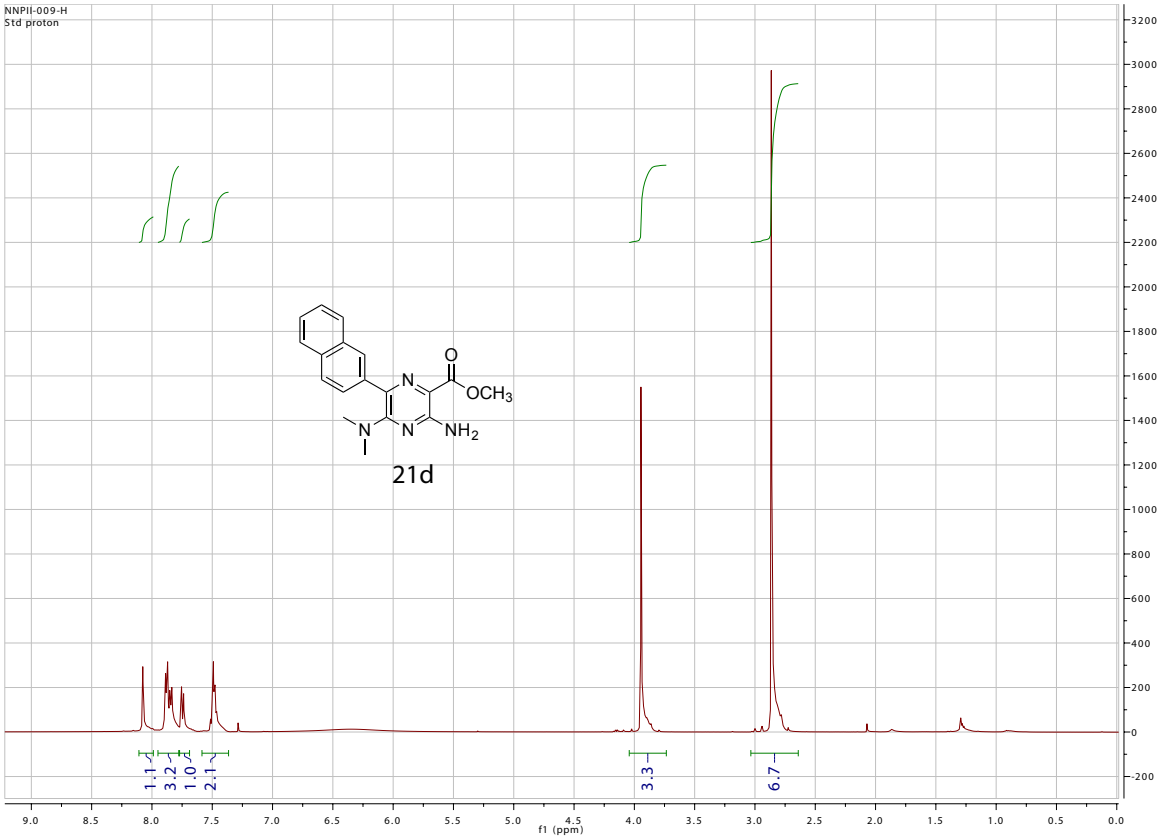
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-6-(4-cyanophenyl)-5-(dimethylamino)pyrazine-2-carboxylate (21c):



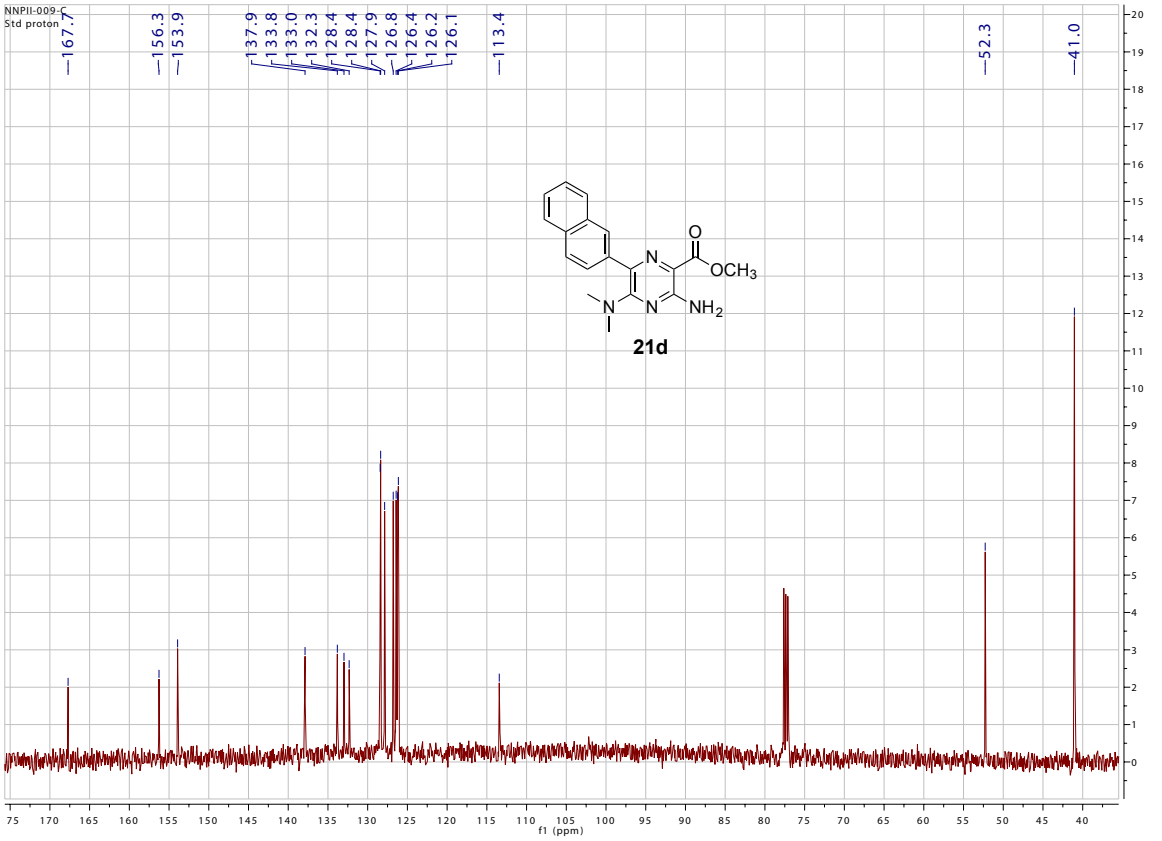
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-6-(4-cyanophenyl)-5-(dimethylamino)pyrazine-2-carboxylate (21c):



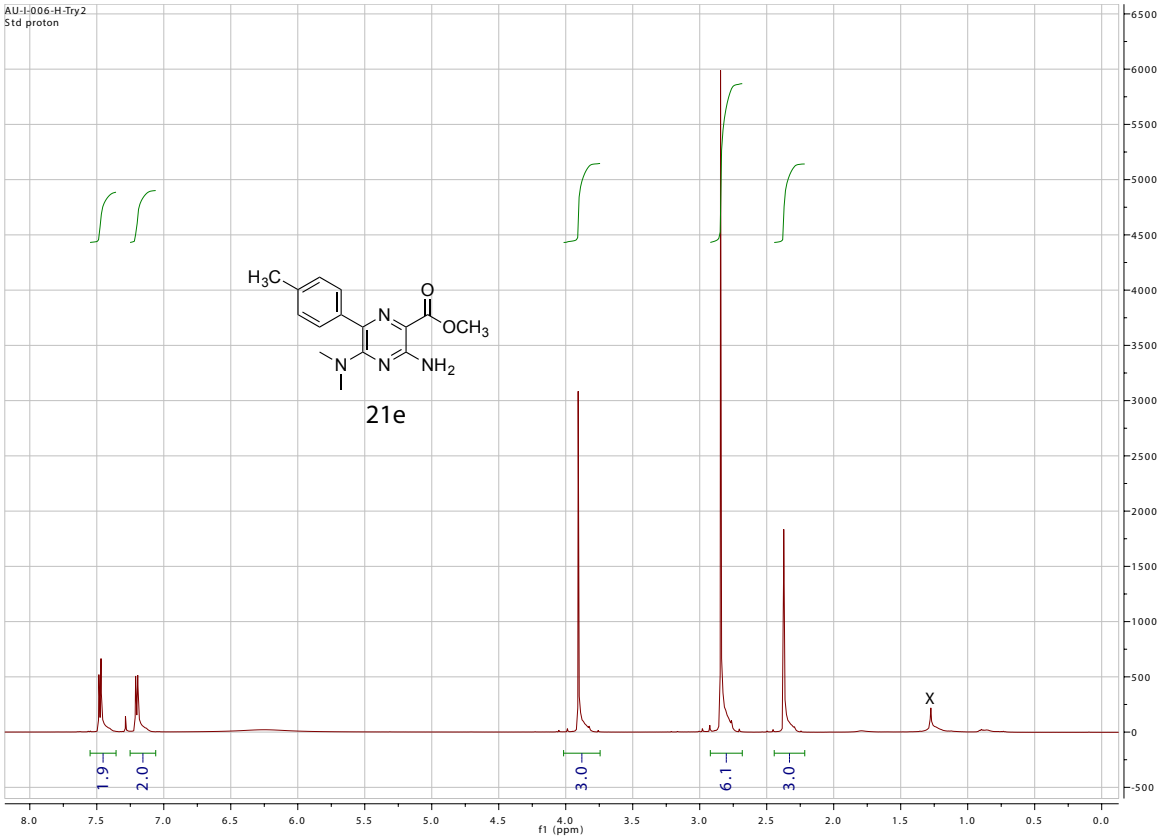
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-5-(dimethylamino)-6-(naphthalen-2-yl)pyrazine-2-carboxylate (21d):



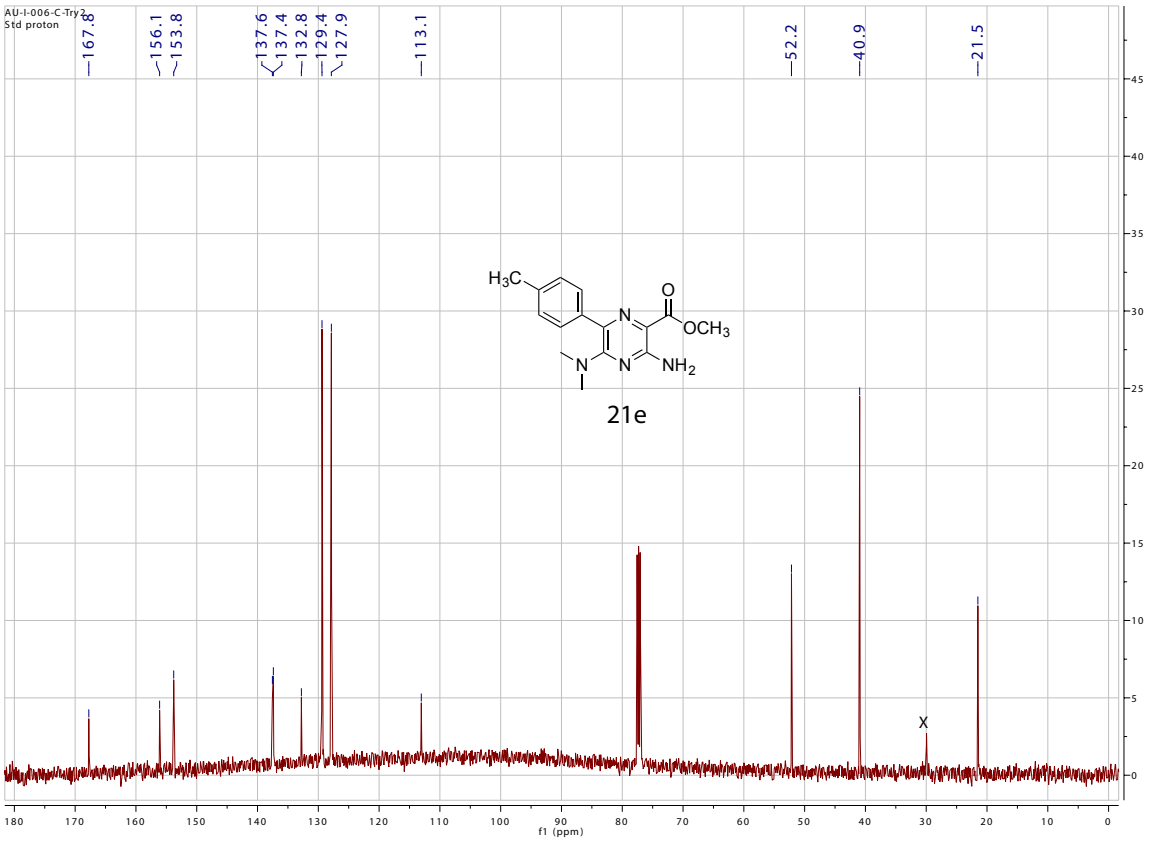
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-5-(dimethylamino)-6-(naphthalen-2-yl)pyrazine-2-carboxylate (21d):



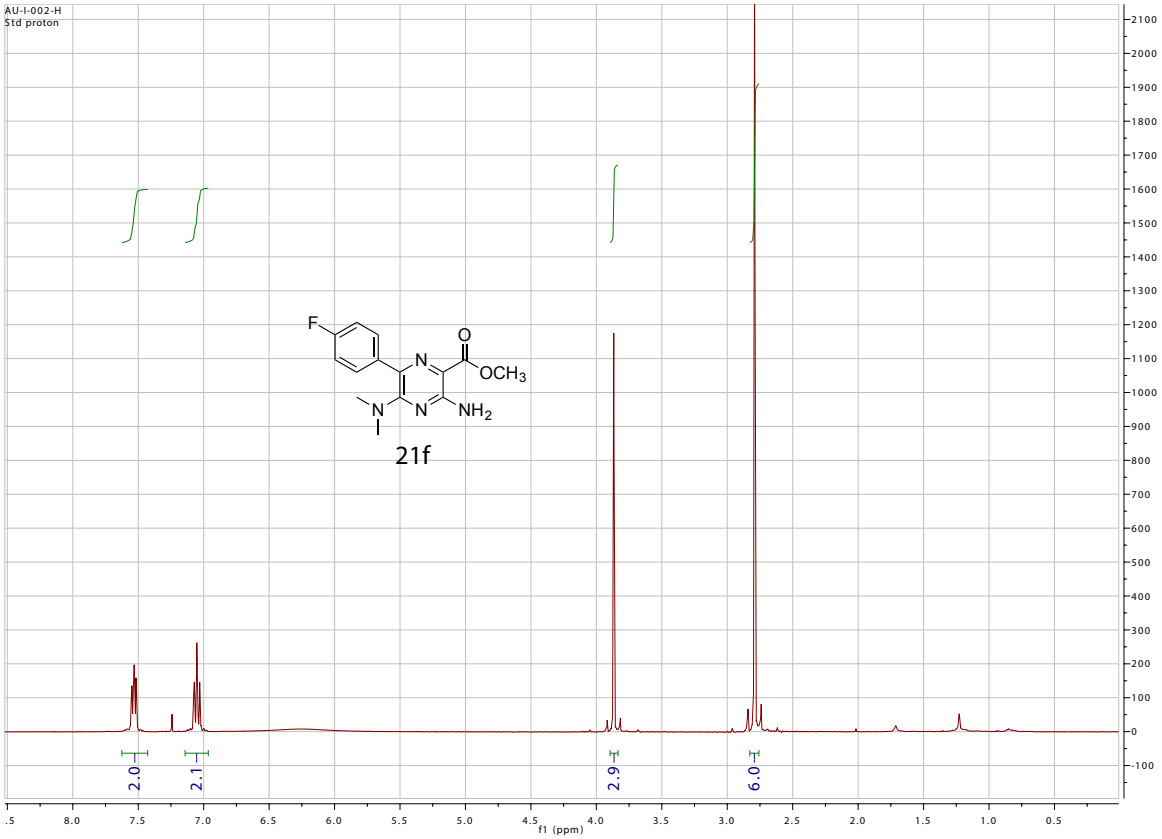
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-5-(dimethylamino)-6-(*p*-tolyl)pyrazine-2-carboxylate (21e):



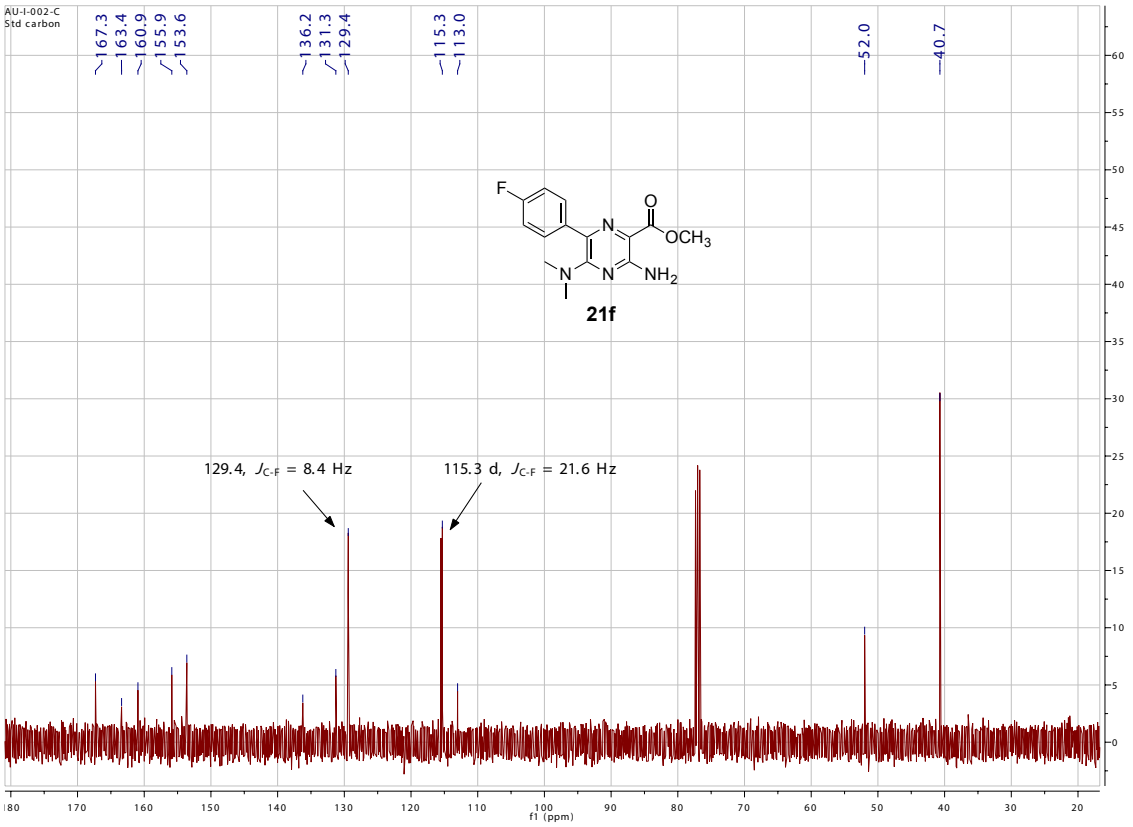
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-5-(dimethylamino)-6-(*p*-tolyl)pyrazine-2-carboxylate (21e): Grease impurity peak observed.



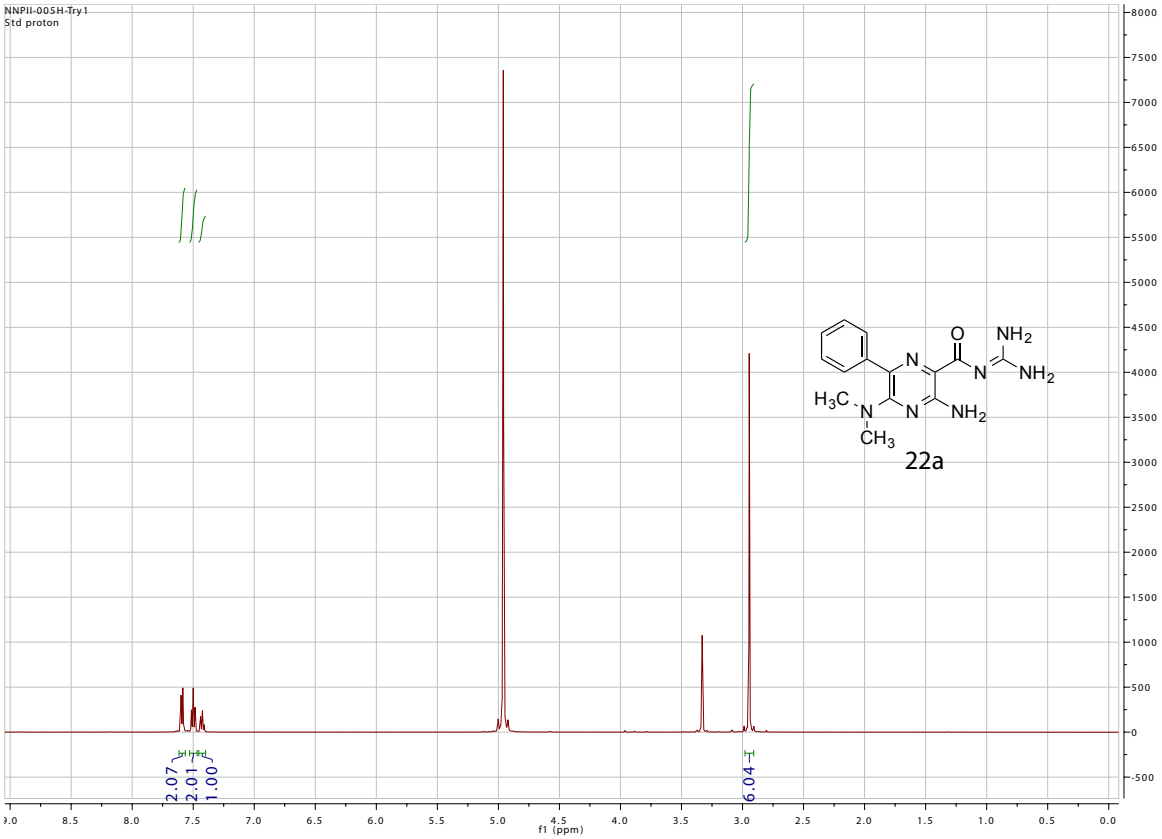
^{1}H NMR ($CDCl_3$) of Methyl 3-amino-5-(dimethylamino)-6-(4-fluorophenyl)pyrazine-2-carboxylate (21f):



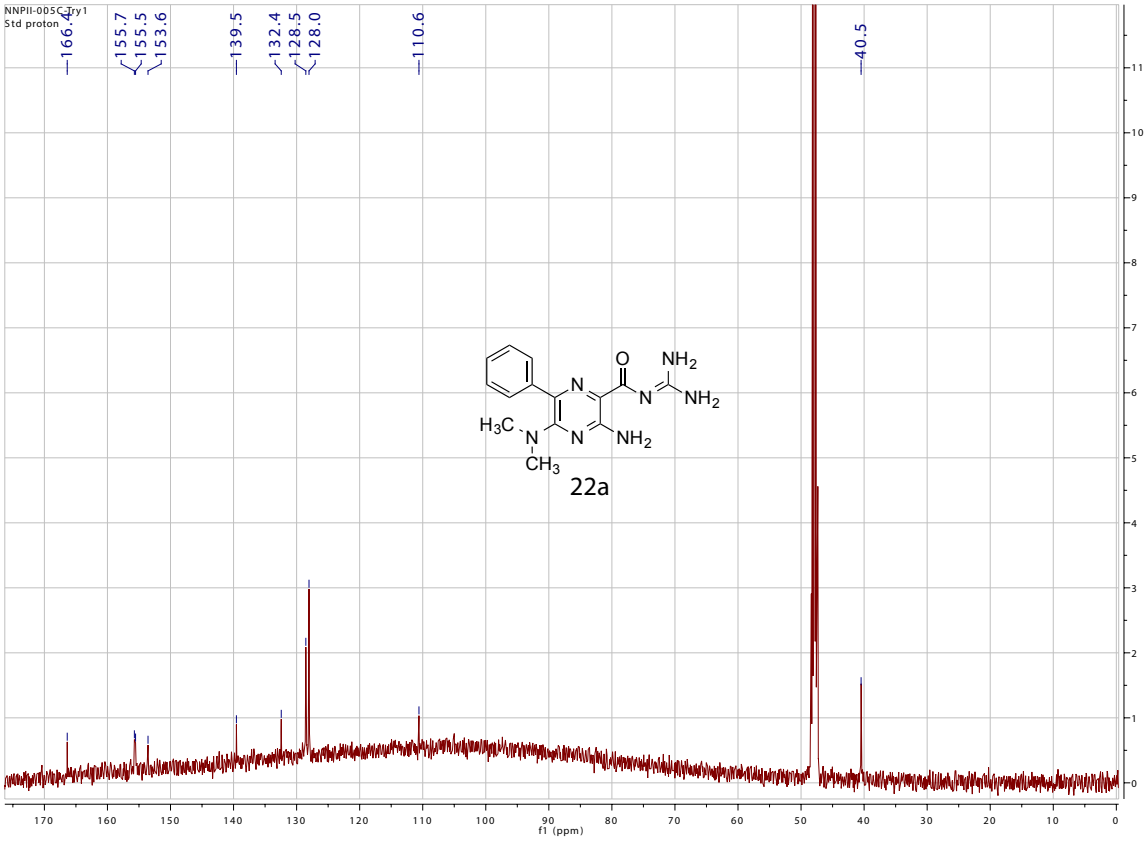
^{13}C NMR ($CDCl_3$) of Methyl 3-amino-5-(dimethylamino)-6-(4-fluorophenyl)pyrazine-2-carboxylate (21f): Split peaks caused by C-F coupling seen at 129.45 (d, $J_{C\text{-}F}$ = 8.4 Hz) and 115.4 (d, $J_{C\text{-}F}$ = 21.6 Hz). One extra peak in the aromatic region, due to strongly split fluorine substituted carbon in aromatic ring. 1 bond C-F coupling pattern for this peak is indiscernible in this spectrum.



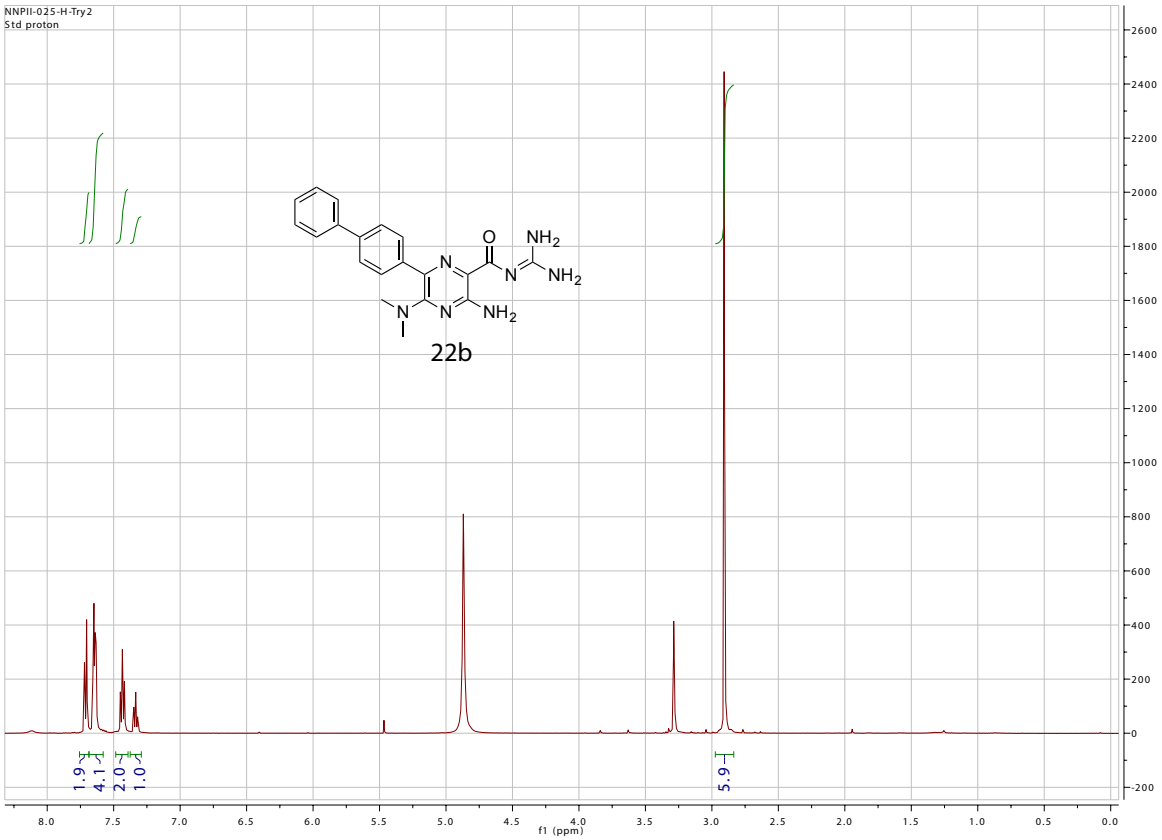
^{1}H NMR (CD_3OD) of 3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-phenylpyrazine-2-carboxamide (22a):



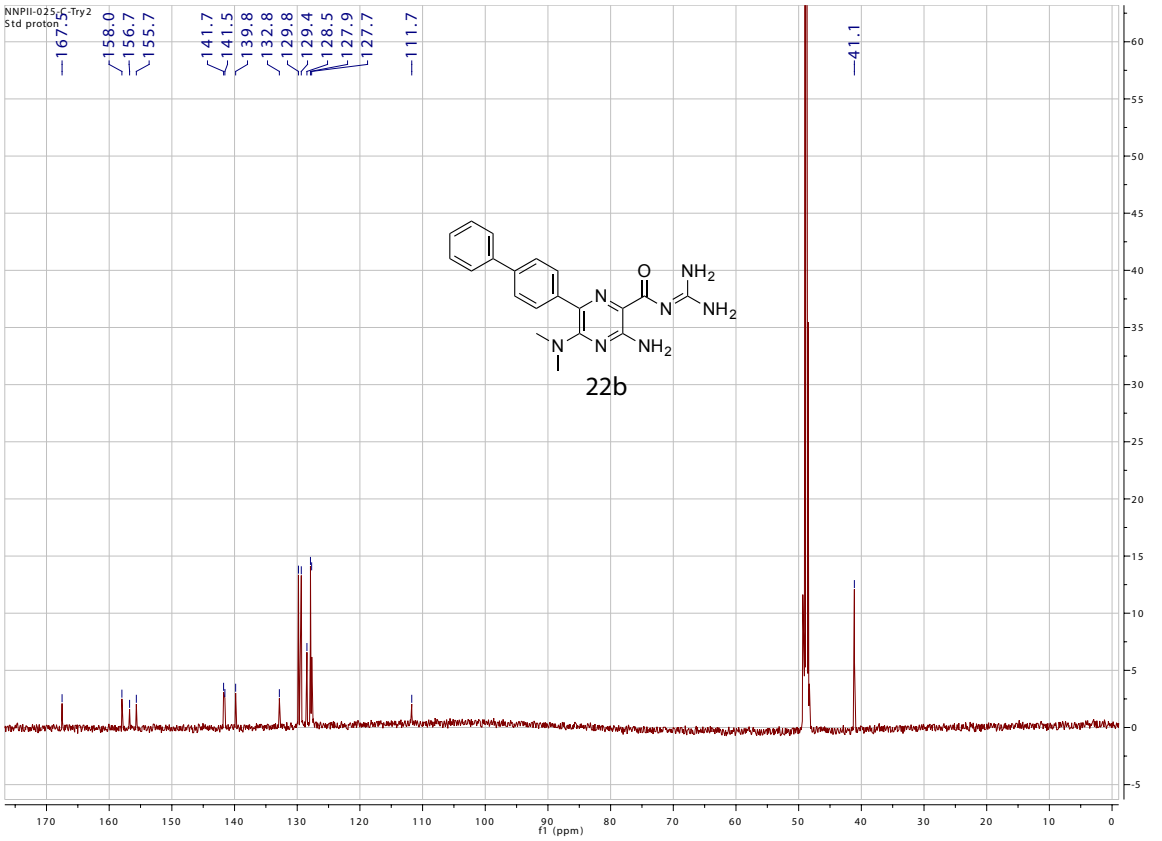
^{13}C NMR (CD_3OD) of 3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-phenylpyrazine-2-carboxamide (22a):



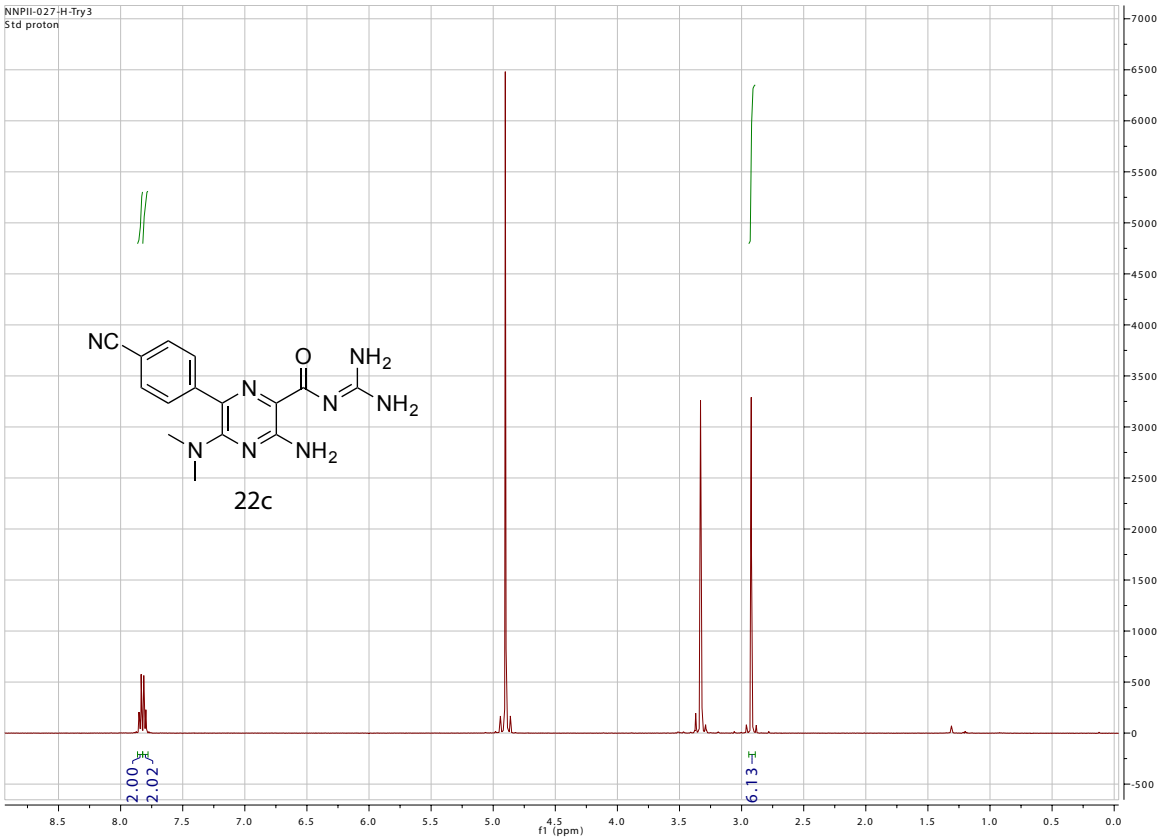
^{1}H NMR (CD_3OD) of 6-([1,1'-biphenyl]-4-yl)-3-amino-*N*-(diaminomethylene)-5-(dimethylamino)pyrazine-2-carboxamide (22b):



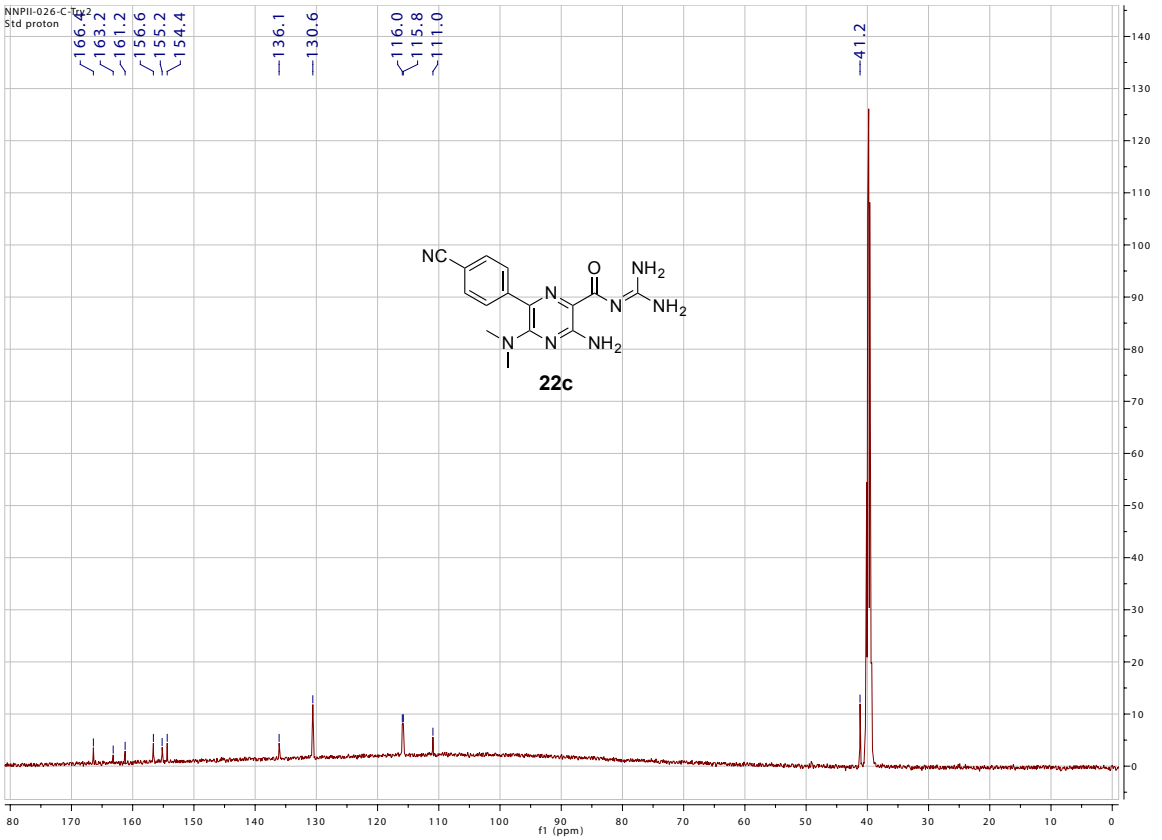
^{13}C NMR (CD_3OD) of 6-([1,1'-biphenyl]-4-yl)-3-amino-*N*-(diaminomethylene)-5-(dimethylamino)pyrazine-2-carboxamide (22b):



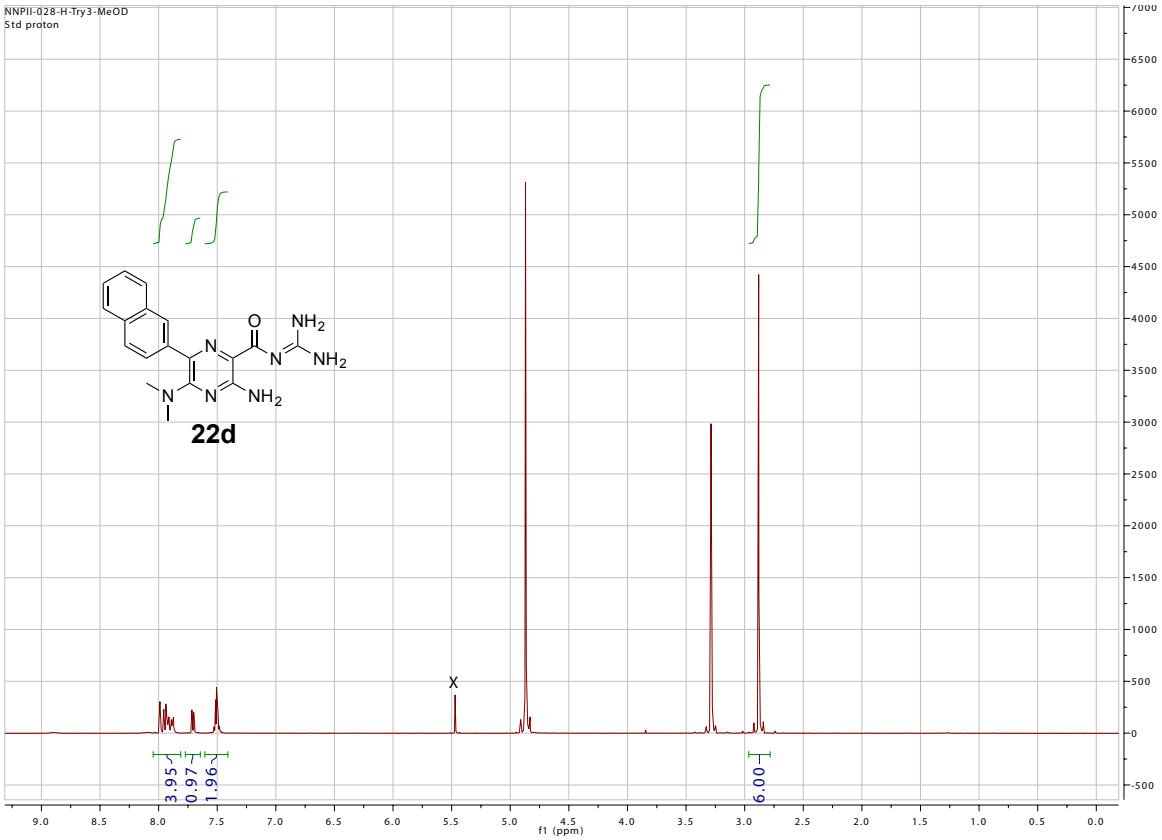
^{1}H NMR (CD_3OD) of 3-amino-6-(4-cyanophenyl)-*N*-(diaminomethylene)-5-(dimethylamino)pyrazine-2-carboxamide (22c):



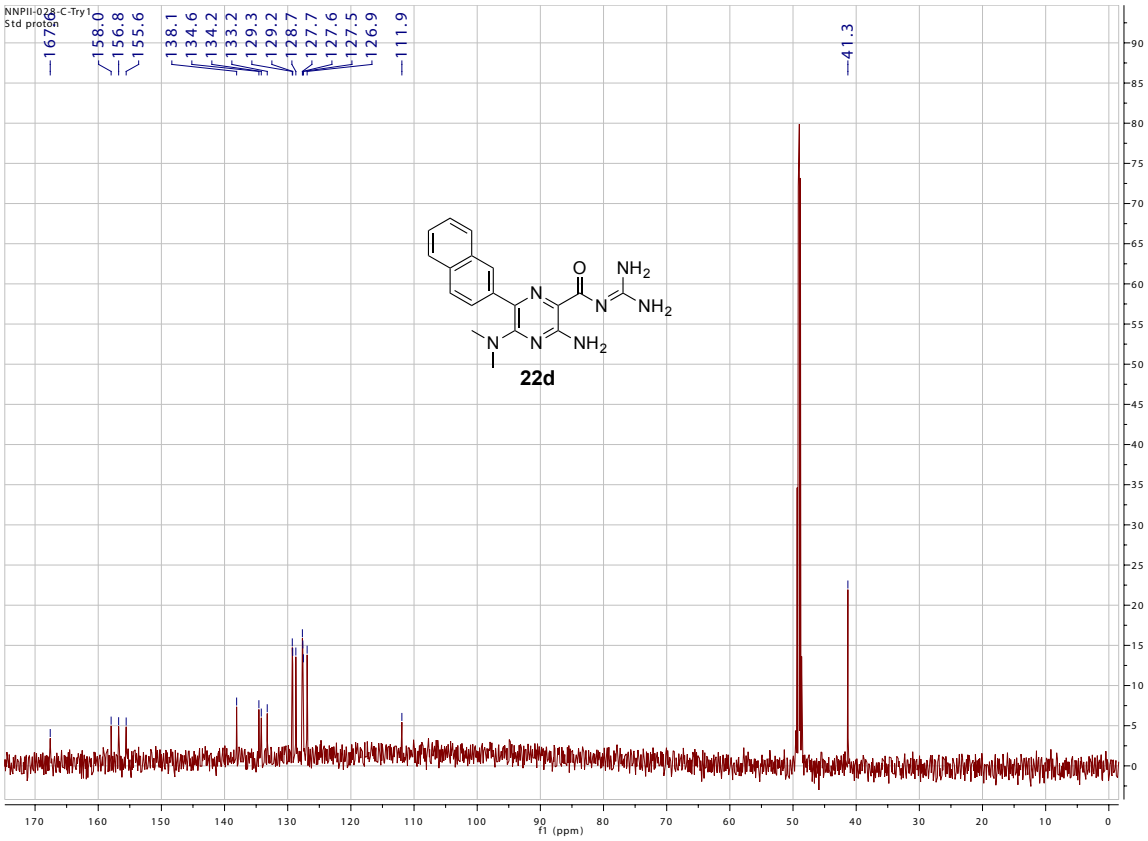
^{13}C NMR (DMSO-d_6) of 3-amino-6-(4-cyanophenyl)-*N*-(diaminomethylene)-5-(dimethylamino)pyrazine-2-carboxamide (22c):



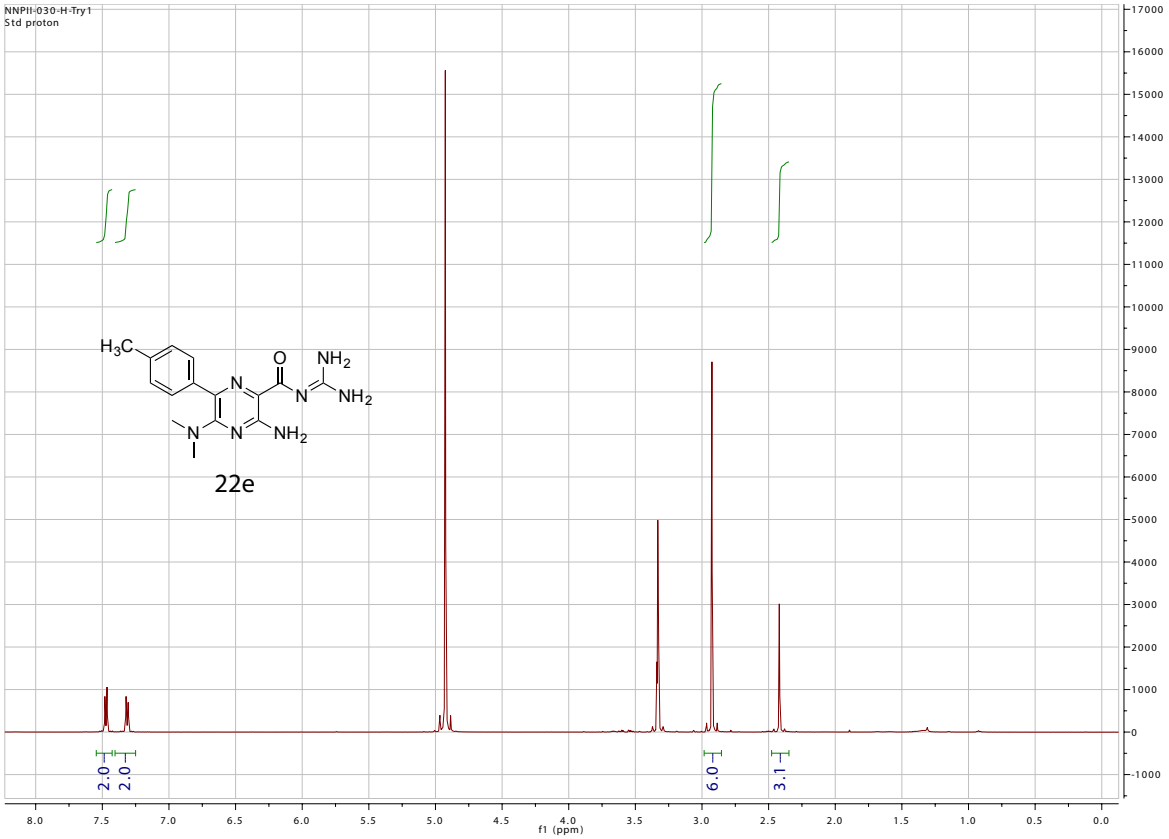
^{1}H NMR (CD_3OD) of 3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-(naphthalen-2-yl)pyrazine-2-carboxamide (22d):



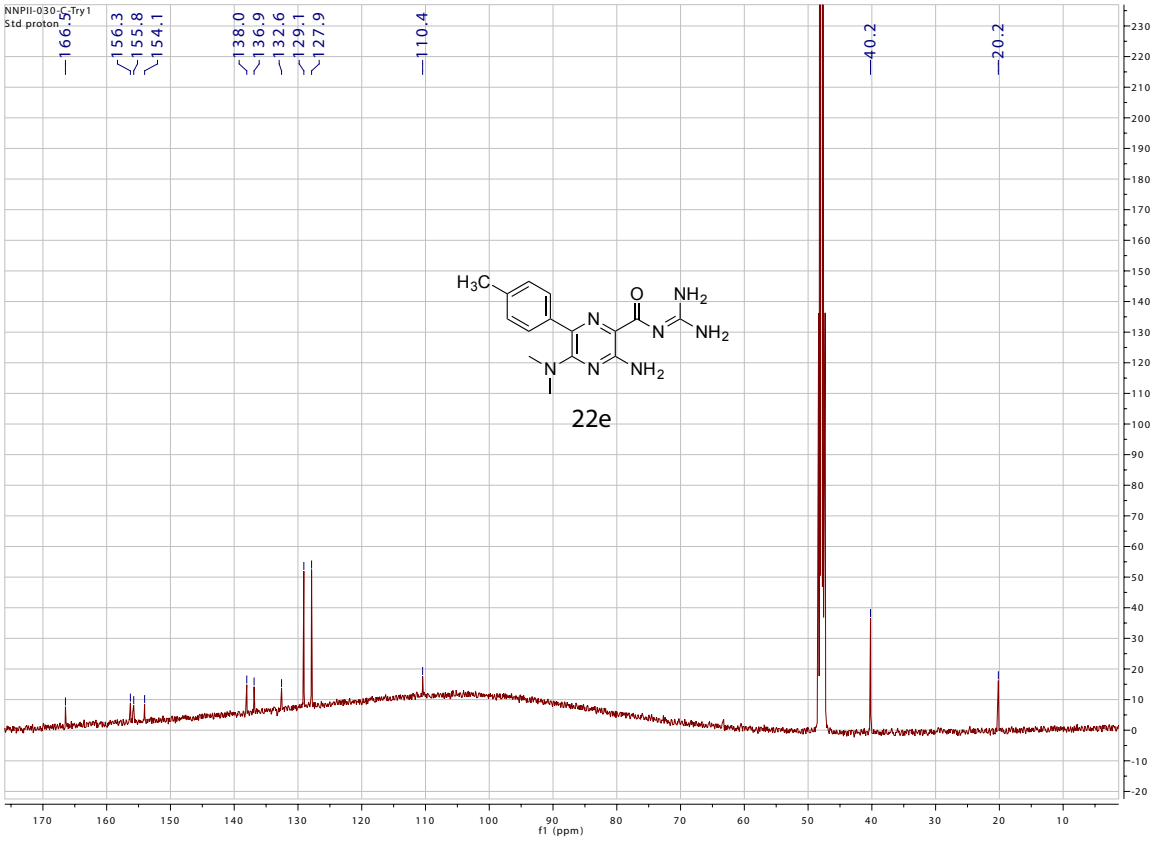
^{13}C NMR (CD_3OD) of 3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-(naphthalen-2-yl)pyrazine-2-carboxamide (22d):



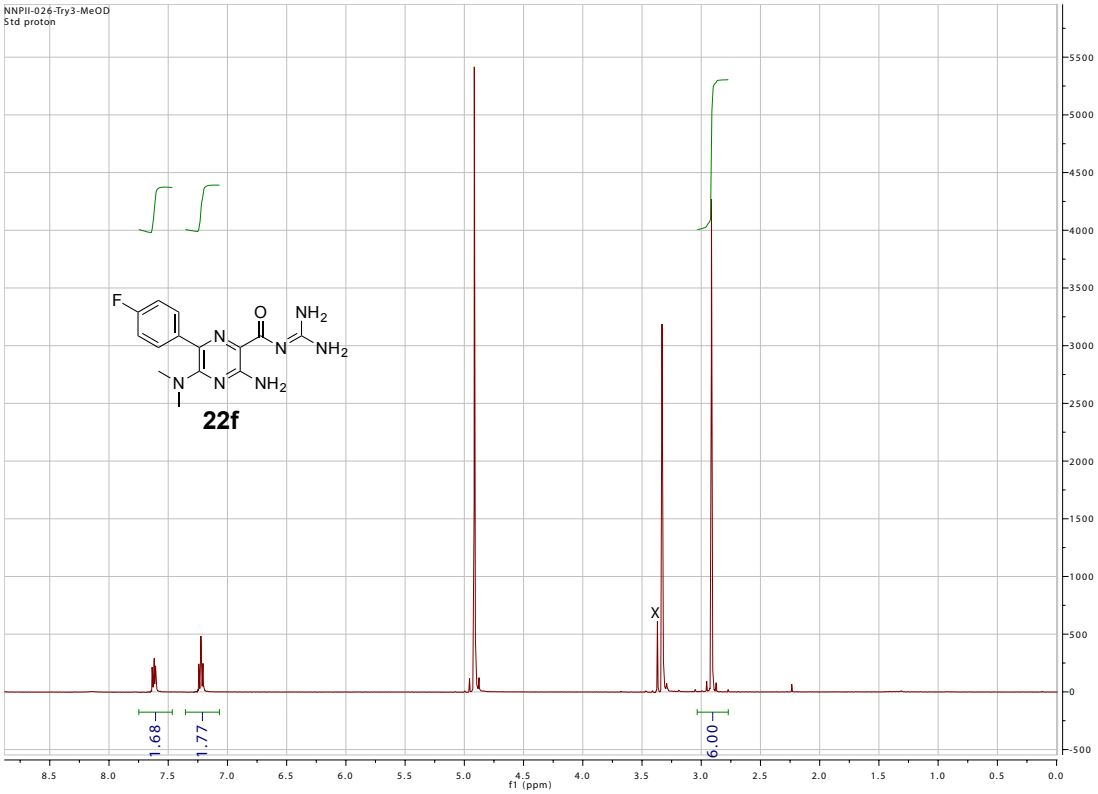
^{1}H NMR (CD_3OD) of 3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-(*p*-tolyl)pyrazine-2-carboxamide (22e):



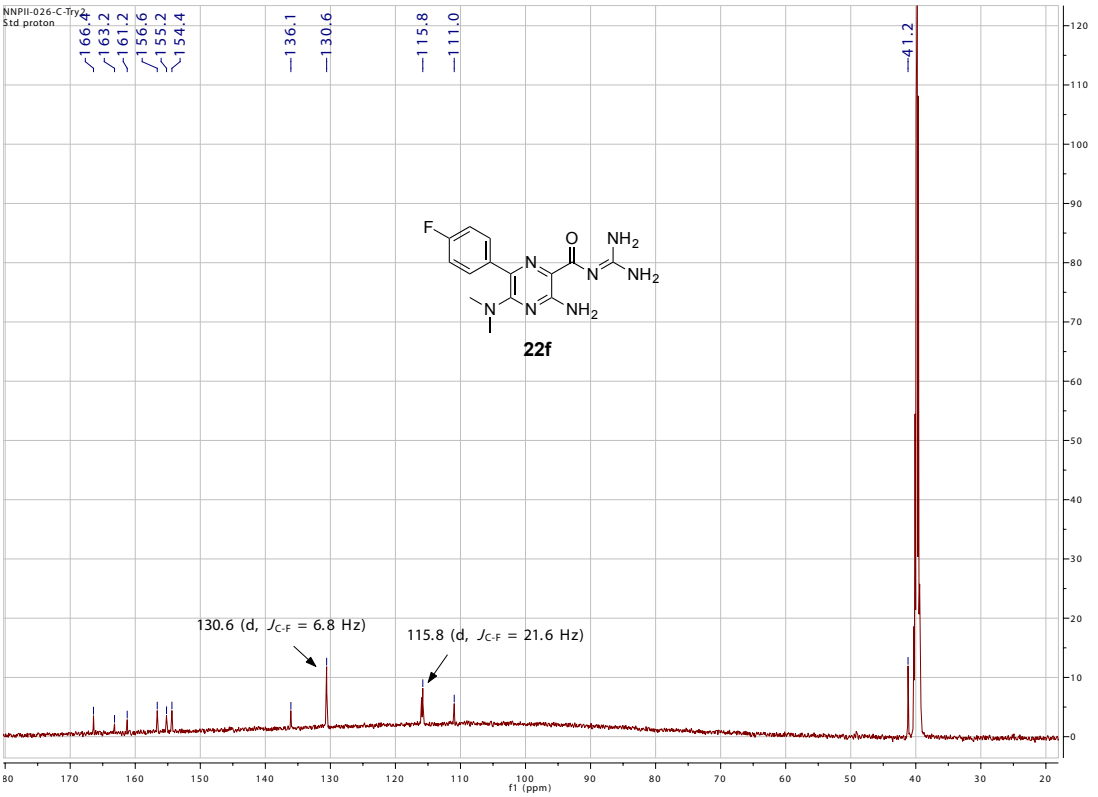
^{13}C NMR (CD_3OD) of 3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-(*p*-tolyl)pyrazine-2-carboxamide (22e):



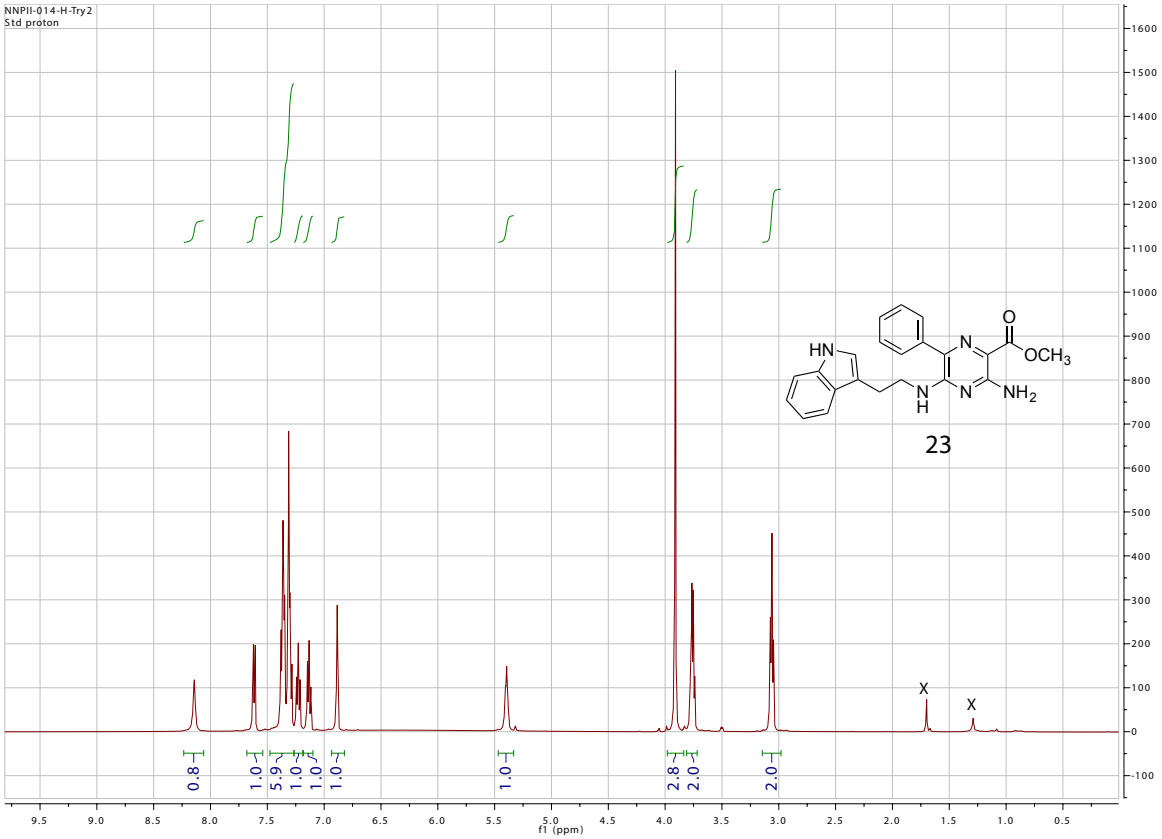
^{1}H NMR (CD_3OD) of 3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-(4-fluorophenyl)pyrazine-2-carboxamide (22f):



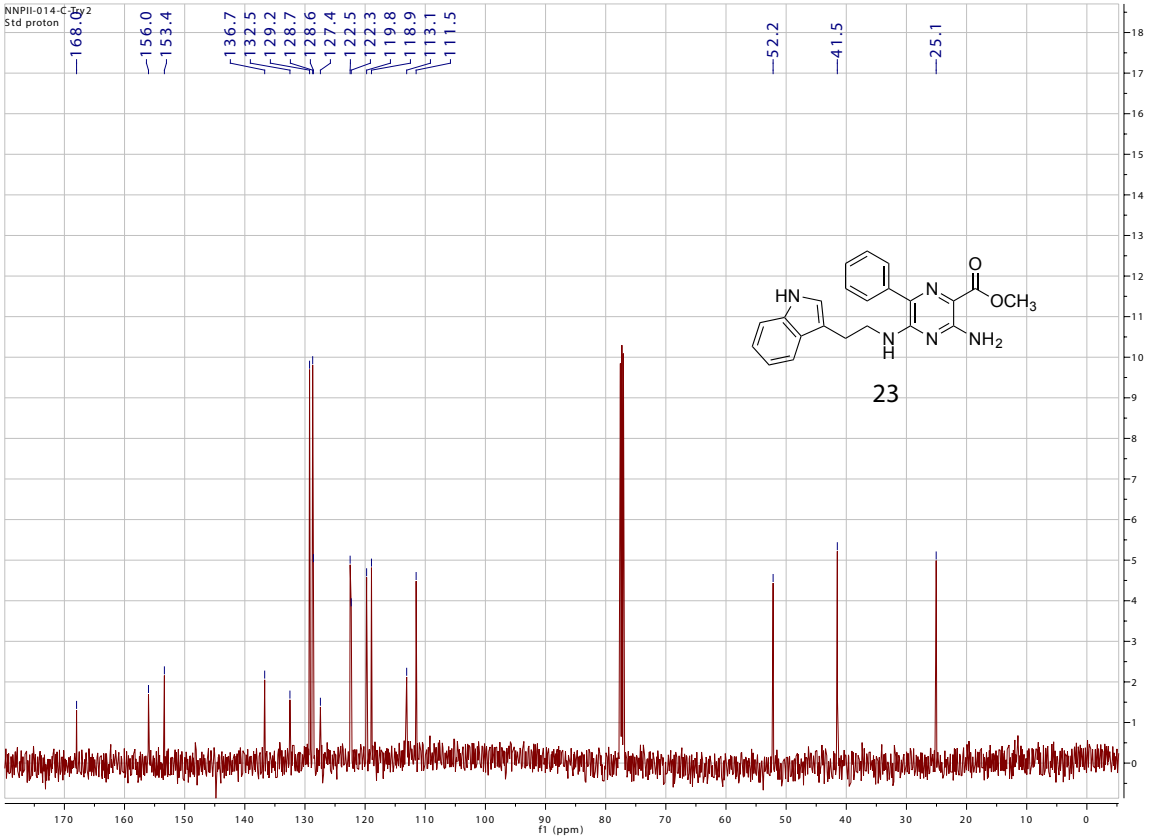
^{13}C NMR (DMSO-d_6) of 3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-(4-fluorophenyl)pyrazine-2-carboxamide (22f): Splitting due to C-F coupling seen at 130.6 (d, $J_{C\text{-}F}$ = 6.6 Hz), 115.8 (d, $J_{C\text{-}F}$ = 21.2 Hz). 1 bond C-F coupling pattern is indiscernible in this spectrum.



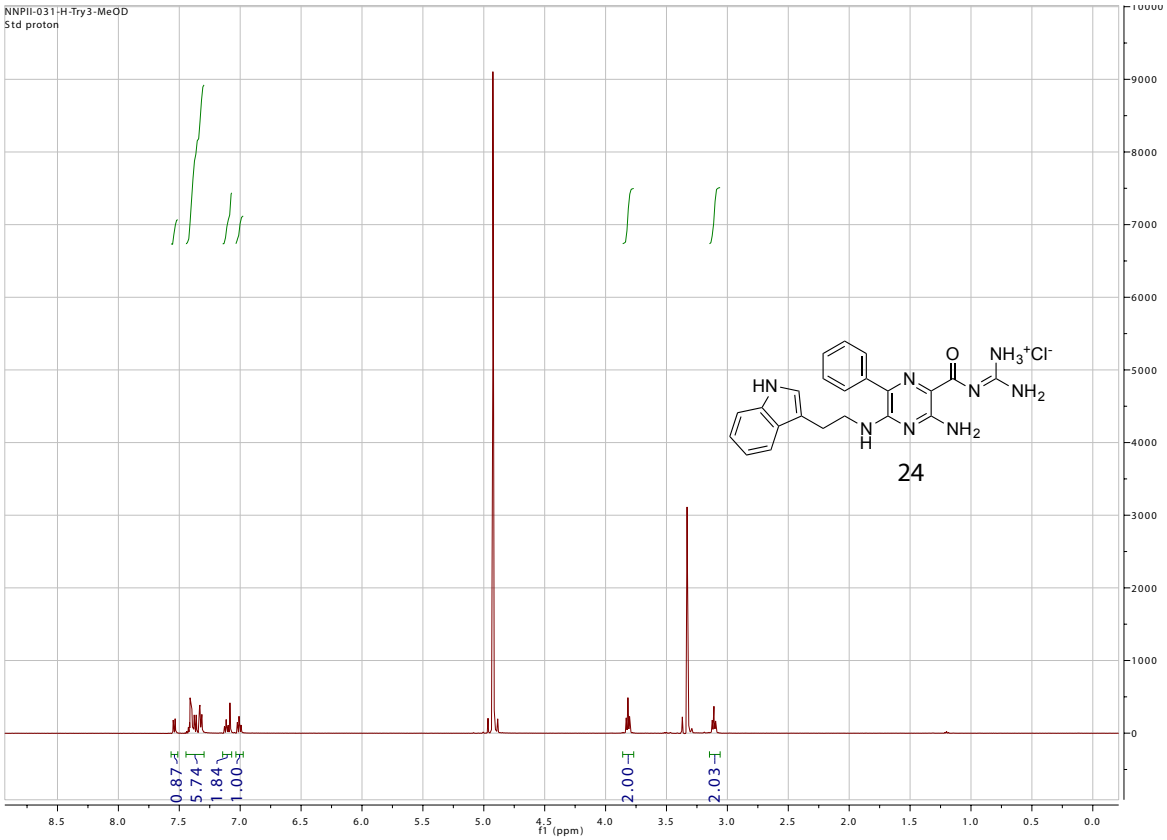
^{1}H NMR ($CDCl_3$) of Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-phenylpyrazine-2-carboxylate (23):



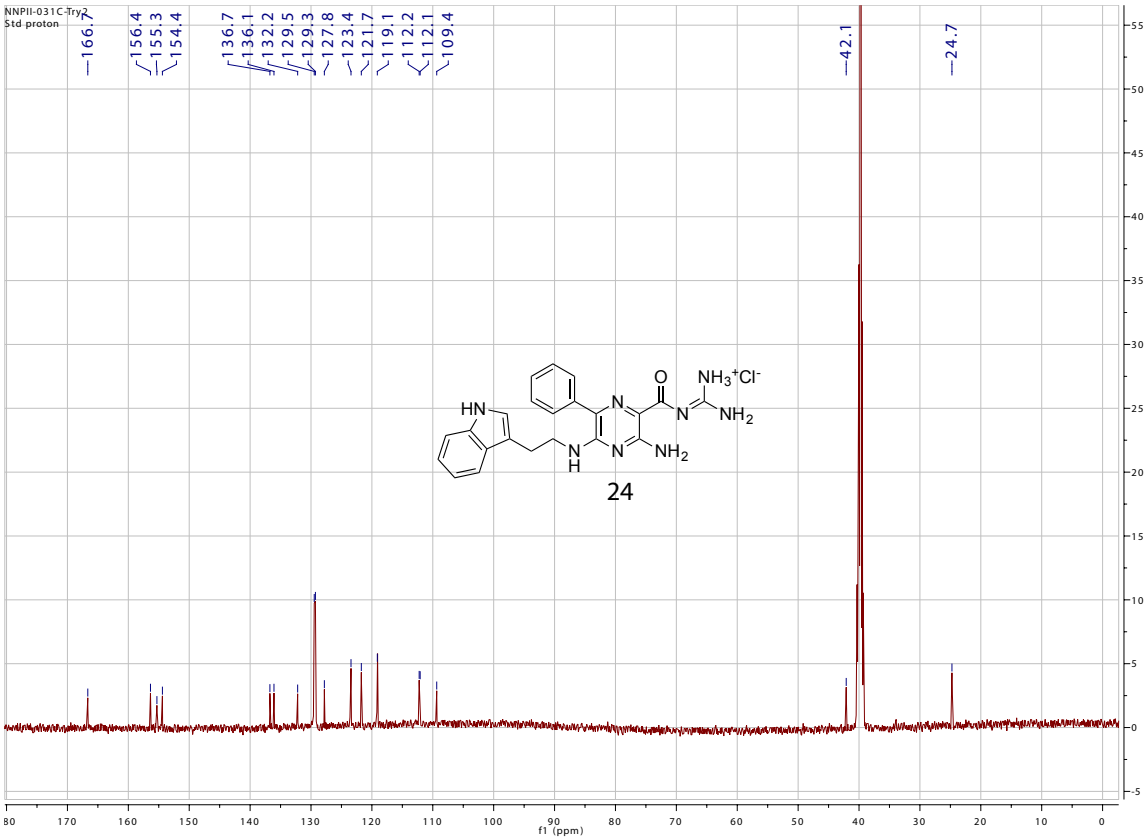
^{13}C NMR ($CDCl_3$) of Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-phenylpyrazine-2-carboxylate (23): Peak overlap observed in aromatic region of the spectrum.



^{1}H NMR (CD_3OD) of 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-(diaminomethylene)-6-phenylpyrazine-2-carboxamide (24):



^{13}C NMR (DMSO-d_6) of 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-(diaminomethylene)-6-phenylpyrazine-2-carboxamide (24): Peak overlap observed in aromatic region of the spectrum.



4. HPLC chromatograms for final amiloride derivatives

HPLC chromatogram of 3-amino-6-chloro-*N*-(diaminomethylene)-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide (4a):

SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI176-R2		
Sample ID	: NNPI176-R2		
Data Filename	: NNPI176-R2.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNPI_08_4_15_R1.lcb		
Vial #	: 1-9	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 8/4/2015 6:54:19 PM	Acquired by	: chemist
Date Processed	: 8/6/2015 5:38:42 PM	Processed by	: chemist

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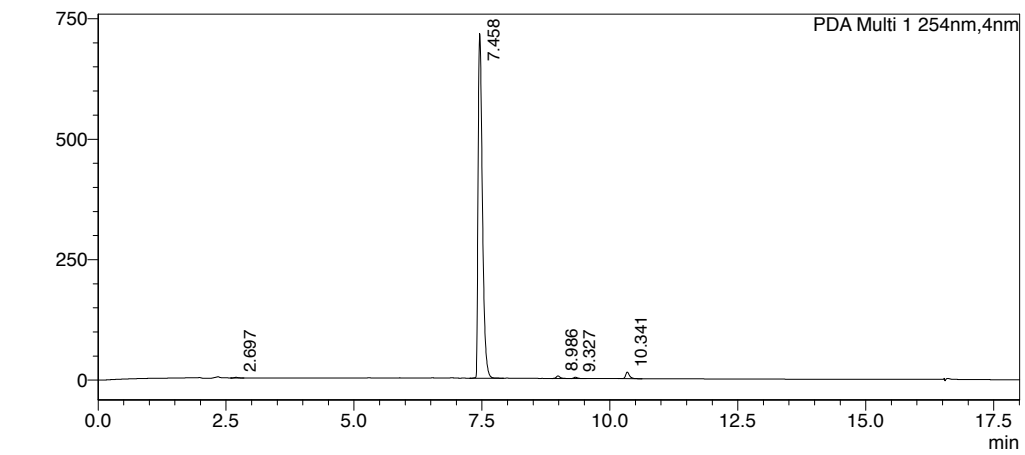
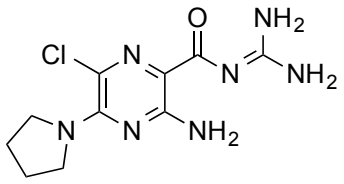


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	2.697	6250	1300	0.146
2	7.458	4173730	697029	97.248
3	8.986	27179	5173	0.633
4	9.327	13242	2621	0.309
5	10.341	71448	13359	1.665
Total		4291850	719482	100.000



4a

C:\LabSolutions\Data\Neeraj\NNPI176-R2.lcd

HPLC chromatogram of 3-amino-6-chloro-*N*-(diaminomethylene)-5-(piperidin-1-yl)pyrazine-2-carboxamide (4b):

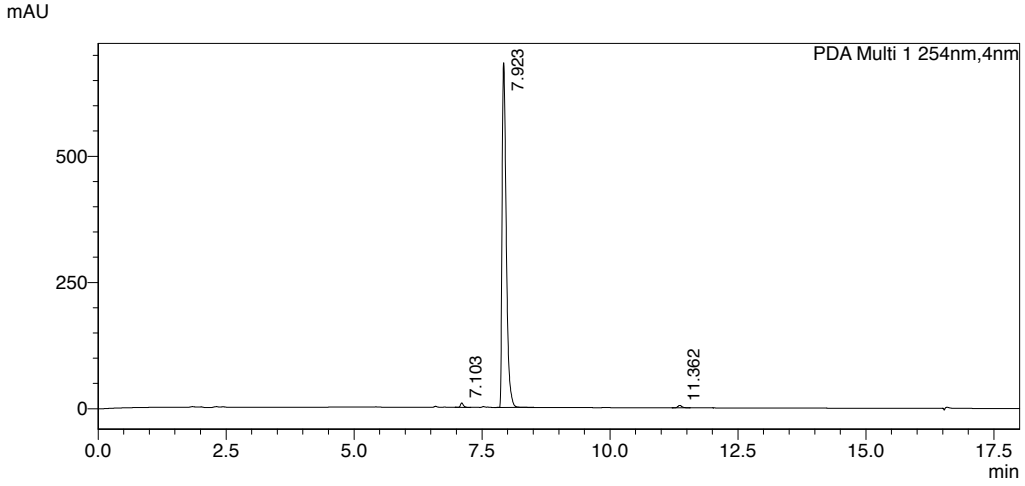
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI177R2		
Sample ID	: NNPI177R2		
Data Filename	: NNPI177R2.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNPI_08_6_15_R1.lcb		
Vial #	: 1-23	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 8/6/2015 5:41:46 PM	Acquired by	: chemist
Date Processed	: 8/6/2015 6:09:15 PM	Processed by	: chemist

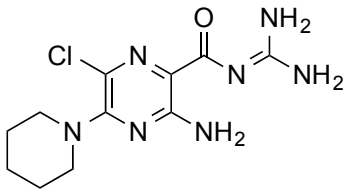
<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	7.103	34874	8435	0.883
2	7.923	3887374	666286	98.432
3	11.362	27048	4721	0.685
Total		3949296	679442	100.000



4b

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HPLC chromatogram of 3-amino-6-chloro-*N*-(diaminomethylene)-5-(phenethylamino)pyrazine-2-carboxamide (4c):

SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI181-R2		
Sample ID	: NNPI181-R2		
Data Filename	: NNPI181-R2.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_09_18_15_run1.lcb		
Vial #	: 1-10	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 9/18/2015 2:32:40 PM	Acquired by	: chemist
Date Processed	: 9/18/2015 3:17:04 PM	Processed by	: chemist

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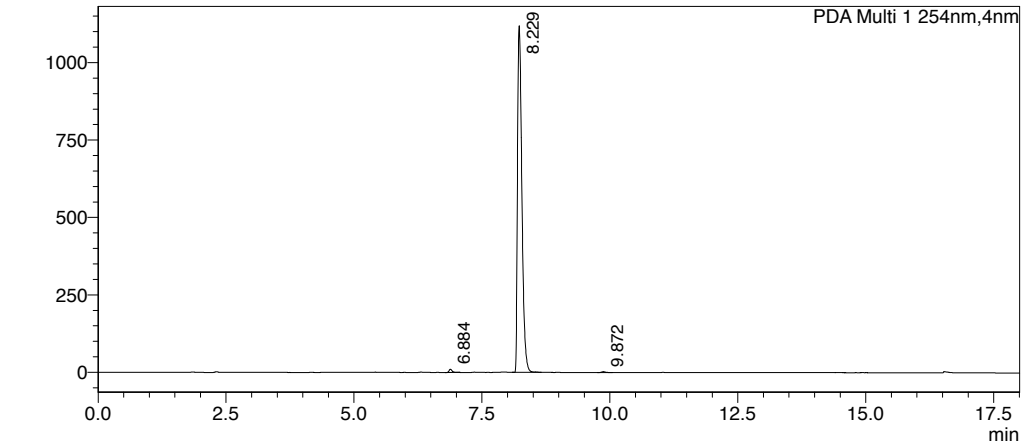
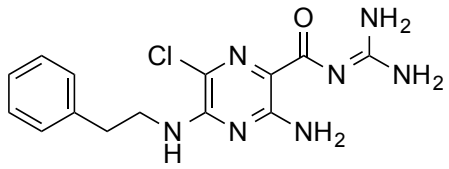


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	6.884	41213	9788	0.609
2	8.229	6711877	1101320	99.144
3	9.872	16722	2888	0.247
Total		6769812	1113996	100.000



4c

C:\LabSolutions\Data\Neeraj\NNPI181-R2.lcd

HPLC chromatogram of 3-amino-6-chloro-*N*-(diaminomethylene)-5-((4-fluorophenethyl)amino)pyrazine-2-carboxamide (4d):

SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI194-R1		
Sample ID	: NNPI194-R1		
Data Filename	: NNPI194-R1.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_10_5_15_run1.lcb		
Vial #	: 1-5	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 10/5/2015 11:57:19 AM	Acquired by	: chemist
Date Processed	: 10/13/2015 2:45:20 PM	Processed by	: chemist

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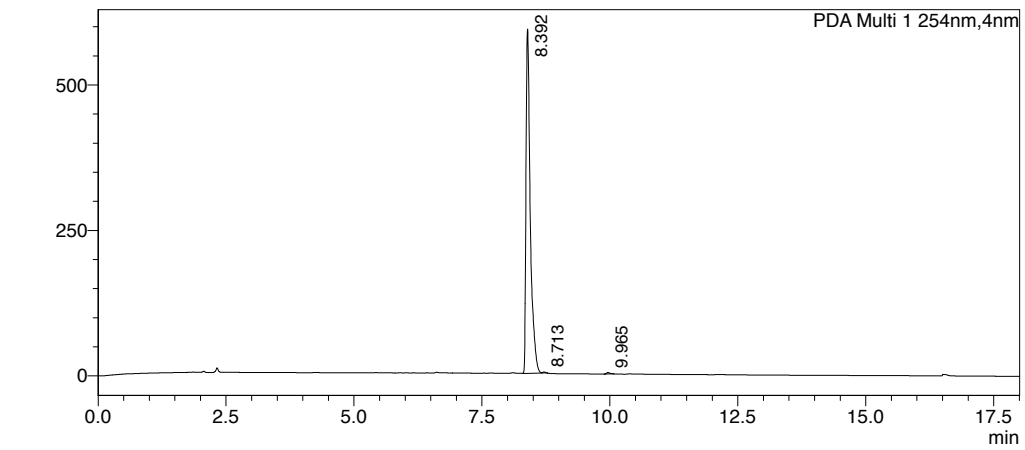
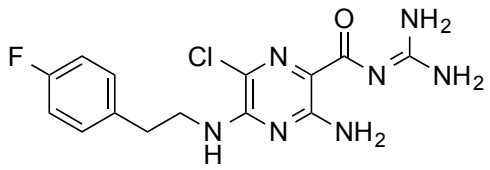


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	8.392	3475971	585499	99.545
2	8.713	4973	1567	0.142
3	9.965	10920	2467	0.313
Total		3491863	589533	100.000



4d

C:\LabSolutions\Data\Neeraj\NNPI194-R1.lcd

3-amino-5-(benzylamino)-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (4e):

SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI195-Run1		
Sample ID	: NNPI195-Run1		
Data Filename	: NNPI195-Run1.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_10_13_15_run1.lcb		
Vial #	: 1-5	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 10/13/2015 2:16:59 PM	Acquired by	: chemist
Date Processed	: 10/13/2015 3:07:28 PM	Processed by	: chemist

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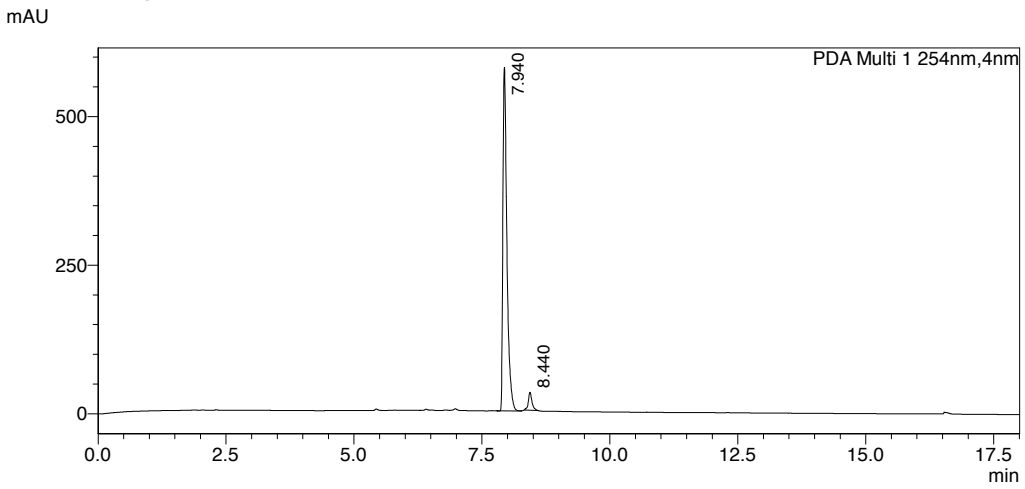
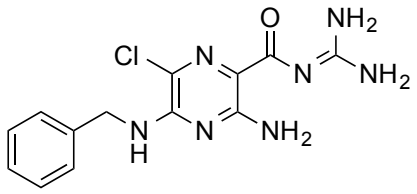


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	7.940	3170167	561405	95.410
2	8.440	152513	29831	4.590
Total		3322681	591236	100.000



4e

C:\LabSolutions\Data\Neeraj\NNPI195-Run1.lcd

5-((3-(1*H*-imidazol-1-yl)propyl)amino)-3-amino-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (4f):

SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI196-Run1		
Sample ID	: NNPI196-Run1		
Data Filename	: NNPI196-Run1.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_10_13_15_run1.lcb		
Vial #	: 1-6	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 10/13/2015 2:35:30 PM	Acquired by	: chemist
Date Processed	: 10/14/2015 11:13:19 AM	Processed by	: chemist

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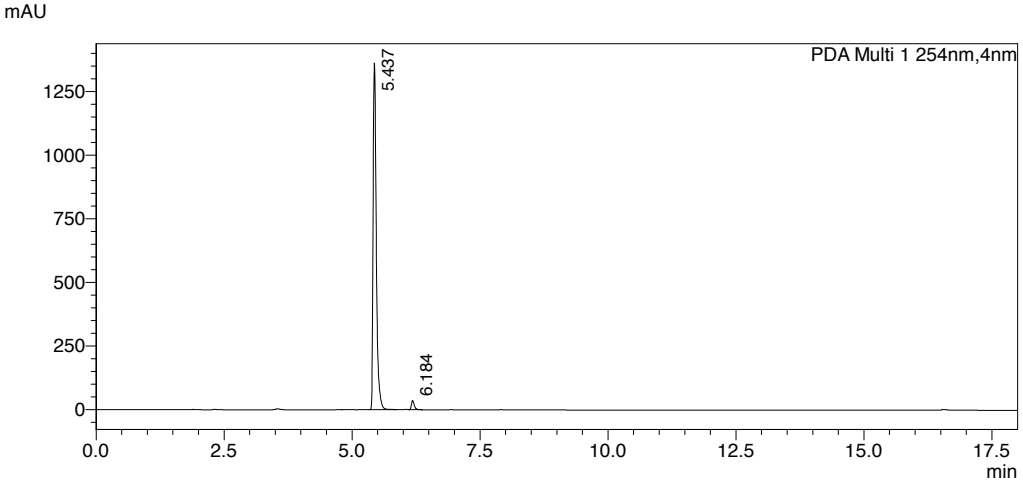
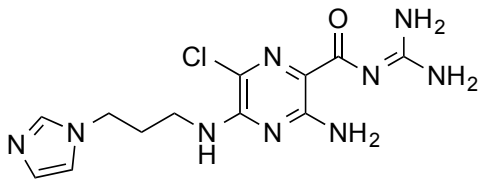


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	5.437	5953633	1340791	97.693
2	6.184	140594	35091	2.307
Total		6094228	1375882	100.000



4f

C:\LabSolutions\Data\Neeraj\NNPI196-Run1.lcd

3-amino-6-chloro-*N*-(diaminomethylene)-5-((3-(2-oxopyrrolidin-1-yl)propyl)amino)pyrazine-2-carboxamide (4g):

SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI053p		
Sample ID	: NNPI053p		
Data Filename	: NNPI053p.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_10_24_2014.lcb		
Vial #	: 1-4	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 10/24/2014 2:29:44 PM	Acquired by	: chemist
Date Processed	: 5/21/2015 12:53:56 PM	Processed by	: chemist

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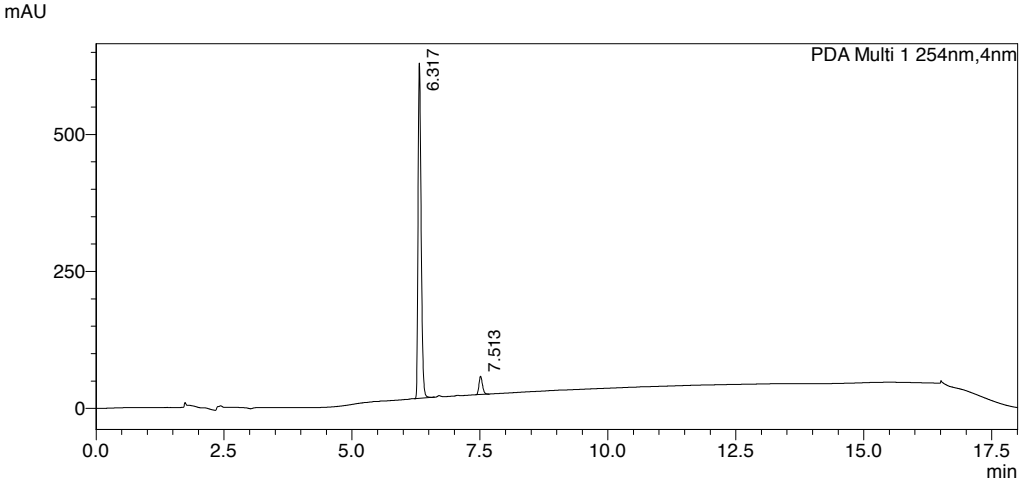
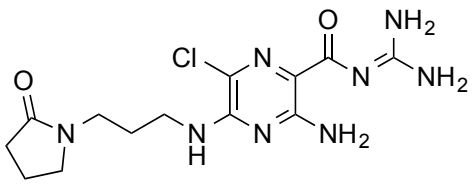


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	6.317	2572050	602113	94.190
2	7.513	158639	32894	5.810
Total		2730689	635007	100.000



4g

C:\LabSolutions\Data\Neeraj\NNPI053p.lcd

3-amino-6-chloro-*N*-(diaminomethylene)-5-((2-(piperazin-1-yl) ethyl)amino)pyrazine-2-carboxamide(4h):

SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI082Bp3		
Sample ID	: NNPI082Bp3		
Data Filename	: NNPI082Bp4.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_05_30_15_82Brepurif.lcb		
Vial #	: 1-9	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/30/2015 1:53:24 PM	Acquired by	: chemist
Date Processed	: 5/30/2015 2:16:24 PM	Processed by	: chemist

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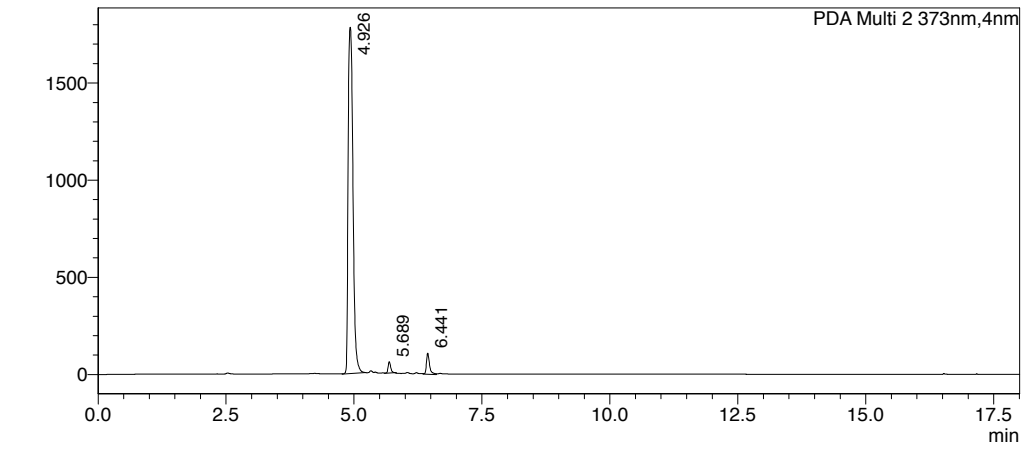
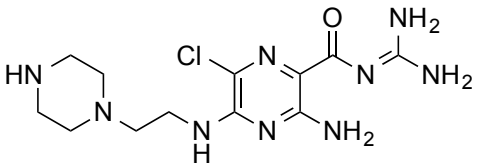


<Peak Table>

PDA Ch2 373nm

Peak#	Ret. Time	Area	Area%
1	4.926	11612487	94.532
2	5.689	205558	1.673
3	6.441	466146	3.795
Total		12284191	100.000



4h

C:\LabSolutions\Data\Neeraj\NNPI082Bp4.lcd

3-amino-6-chloro-*N*-(diaminomethylene)-5-((4-sulfamoylphenethyl)amino)pyrazine-2-carboxamide (4i):

SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI054p		
Sample ID	: NNPI054p		
Data Filename	: NNPI054p.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_10_24_2014.lcb		
Vial #	: 1-5	Sample Type	: Unknown
Injection Volume	: 10 uL		
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Date Processed	: 5/21/2015 12:54:44 PM	Processed by	: chemist

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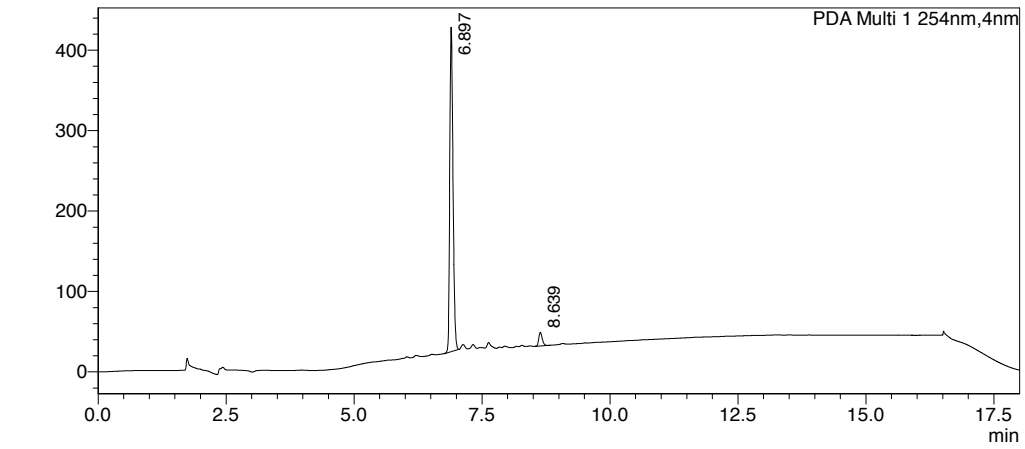
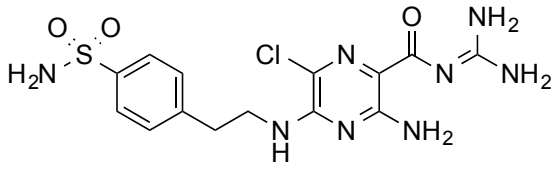


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	6.897	1775853	386341	95.558
2	8.639	82555	16761	4.442
Total		1858408	403102	100.000



4i

C:\LabSolutions\Data\Neeraj\NNPI054p.lcd

5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (4j):

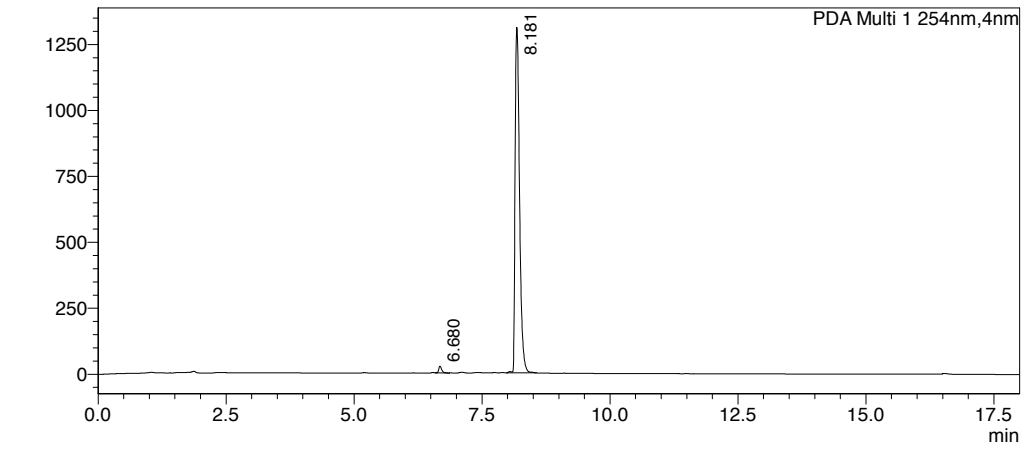
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI192B-R1		
Sample ID	: NNPI192B-R1		
Data Filename	: NNPI192B-R1.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_09_18_15_run1.lcb		
Vial #	: 1-9	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 9/18/2015 2:14:07 PM	Acquired by	: chemist
Date Processed	: 9/18/2015 2:51:22 PM	Processed by	: chemist

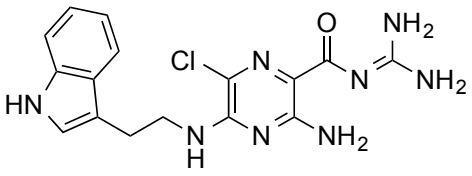
<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	6.680	98247	24725	1.228
2	8.181	7899521	1293363	98.772
Total		7997768	1318087	100.000



4j

C:\LabSolutions\Data\Neeraj\NNPI192B-R1.lcd

3-amino-6-chloro-*N*-(diaminomethylene)-5-((3,4-difluorobenzyl)amino)pyrazine-2-carboxamide (4k):

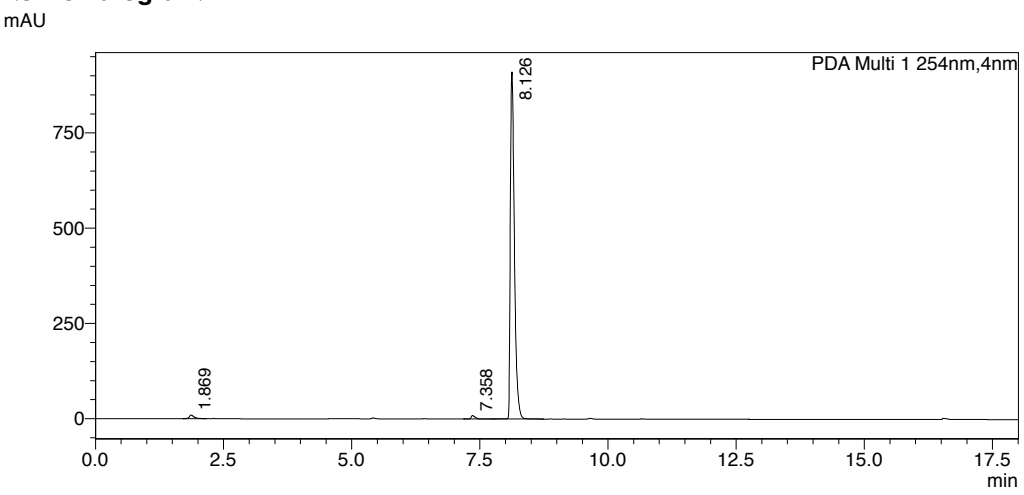
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI179-Run2		
Sample ID	: NNPI179-Run2		
Data Filename	: NNPI179-Run2.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_09_24_15_run1.lcb		
Vial #	: 1-12	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 9/24/2015 4:29:18 PM	Acquired by	: chemist
Date Processed	: 9/24/2015 5:55:24 PM	Processed by	: chemist

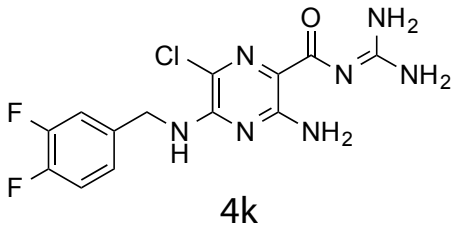
<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	1.869	67242	9567	1.309
2	7.358	54174	9190	1.055
3	8.126	5013931	900086	97.636
Total		5135348	918843	100.000



4k

C:\LabSolutions\Data\Neeraj\NNPI179-Run2.lcd

5-((2-(1*H*-benzo[*d*]imidazol-2-yl)ethyl)amino)-3-amino-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (4l):

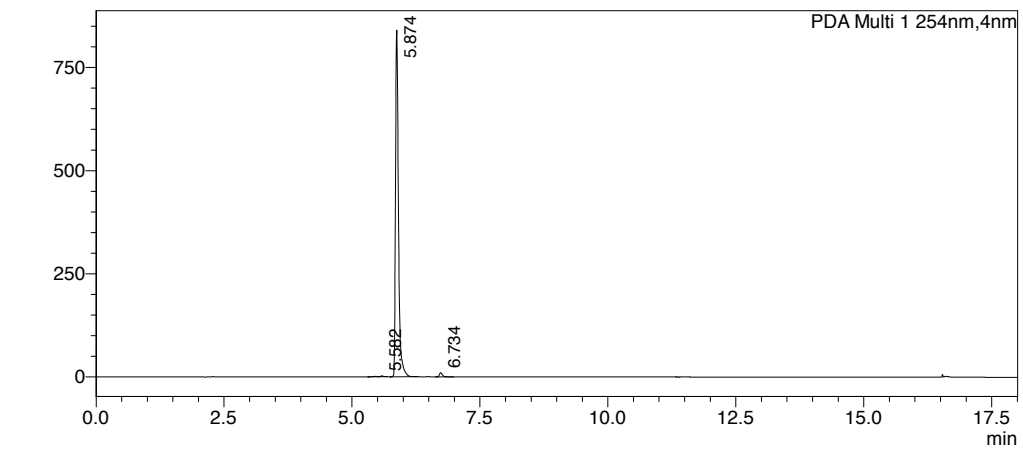
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI172-p2		
Sample ID	: NNPI172-p2		
Data Filename	: NNPI172-p2.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNPI_07_27_15_R2.lcb		
Vial #	: 1-14	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 7/27/2015 10:53:57 AM	Acquired by	: chemist
Date Processed	: 7/27/2015 1:26:57 PM	Processed by	: chemist

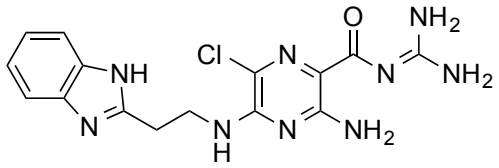
<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	5.582	10915	1927	0.317
2	5.874	3395273	786736	98.506
3	6.734	40585	9761	1.177
Total		3446773	798425	100.000



4l

3-amino-6-chloro-*N*-(diaminomethylene)-5-(((tetrahydrofuran-2-yl)methyl)amino)pyrazine-2-carboxamide (4m):

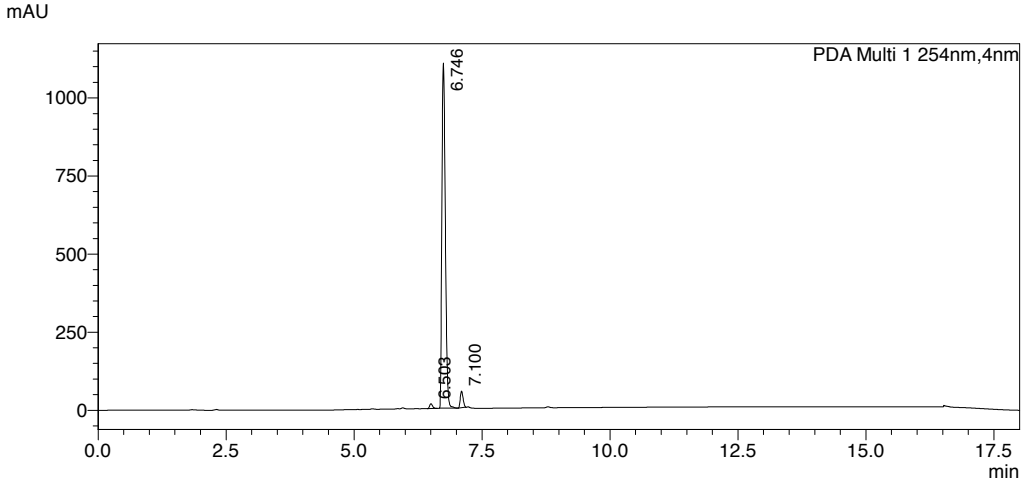
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI081p2		
Sample ID	: NNPI081p2		
Data Filename	: NNPI081p2.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_03_19_15.lcb		
Vial #	: 1-10	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 3/19/2015 2:25:04 PM	Acquired by	: chemist
Date Processed	: 3/19/2015 2:49:35 PM	Processed by	: chemist

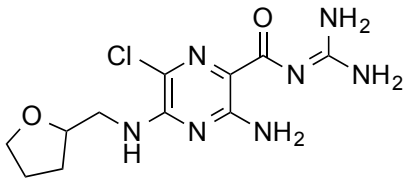
<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	6.503	60455	14845	1.109
2	6.746	5192288	1094081	95.208
3	7.100	200858	51909	3.683
Total		5453601	1160834	100.000



4m

C:\LabSolutions\Data\Neeraj\NNPI081p2.lcd

3-amino-6-chloro-*N*-(diaminomethylene)-5-((2-(4-methylpiperazin-1-yl)ethyl)amino)pyrazine-2-carboxamide (4n):

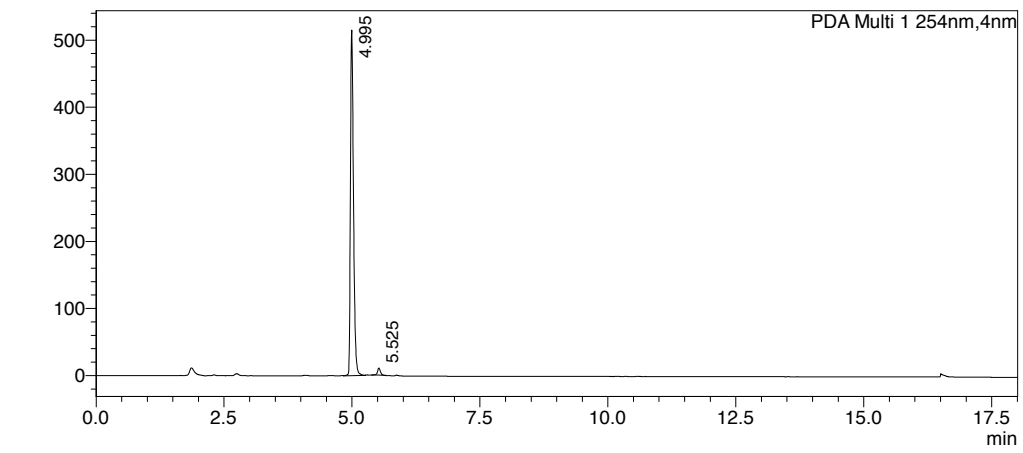
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI167-repurif-R1		
Sample ID	: NNPI167-repurif-R1		
Data Filename	: NNPI167-repurif-R1.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_11_12_15_run1.lcb		
Vial #	: 1-37	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 11/12/2015 11:22:40 AM	Acquired by	: chemist
Date Processed	: 11/12/2015 12:10:26 PM	Processed by	: chemist

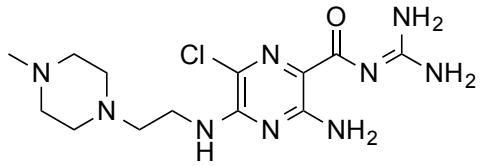
<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	4.995	2154166	489504	98.139
2	5.525	40838	10191	1.861
Total		2195004	499695	100.000



4n

C:\LabSolutions\Data\Neeraj\NNPI167-repurif-R1.lcd

3-amino-5-((2-aminoethyl)amino)-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (9):

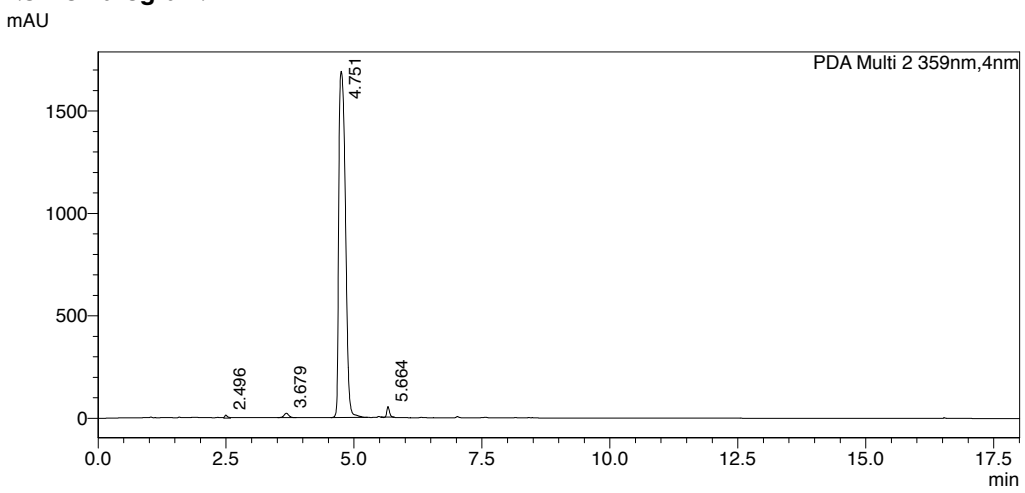
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI197R3		
Sample ID	: NNPI197R3		
Data Filename	: NNPI197R3.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_10_26_15_run1.lcb		
Vial #	: 1-21	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 10/26/2015 5:47:05 PM	Acquired by	: chemist
Date Processed	: 5/4/2016 11:15:11 AM	Processed by	: chemist

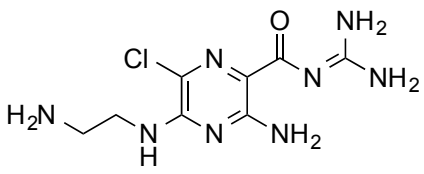
<Chromatogram>



<Peak Table>

PDA Ch2 359nm

Peak#	Ret. Time	Area	Area%
1	2.496	60379	0.386
2	3.679	138640	0.886
3	4.751	15274054	97.562
4	5.664	182735	1.167
Total		15655807	100.000



9

C:\LabSolutions\Data\Neeraj\NNPI197R3.lcd

3-amino-6-chloro-*N*-(diaminomethylene)-5-((2-(dibenzylamino)ethyl)amino)pyrazine-2-carboxamide (10):

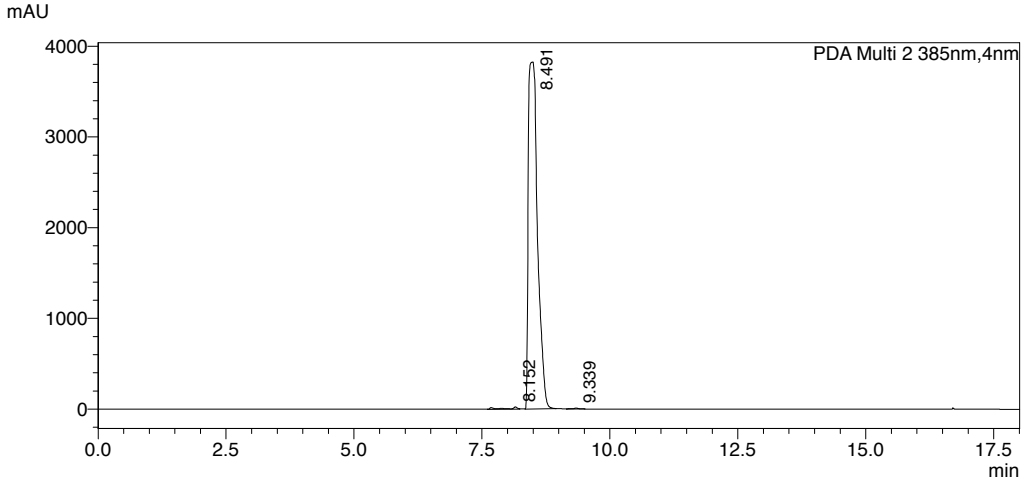
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI198-R6		
Sample ID	: NNPI198-R6		
Data Filename	: NNPI198-R6.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_05_04_16_R2.lcb		
Vial #	: 1-50	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/4/2016 12:36:06 PM	Acquired by	: chemist
Date Processed	: 5/4/2016 12:54:09 PM	Processed by	: chemist

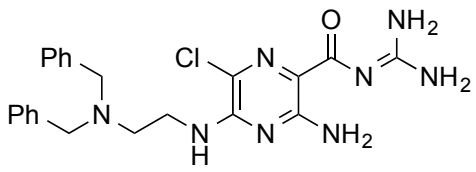
<Chromatogram>



<Peak Table>

PDA Ch2 385nm

Peak#	Ret. Time	Area	Area%
1	8.152	356686	0.736
2	8.491	48027443	99.114
3	9.339	72505	0.150
Total		48456635	100.000



10

C:\LabSolutions\Data\Neeraj\NNPI198-R6.lcd

3-amino-6-chloro-*N*-(diaminomethylene)-5-((2-((4-methylphenyl)sulfonamido)ethyl)amino)pyrazine-2-carboxamide (11):

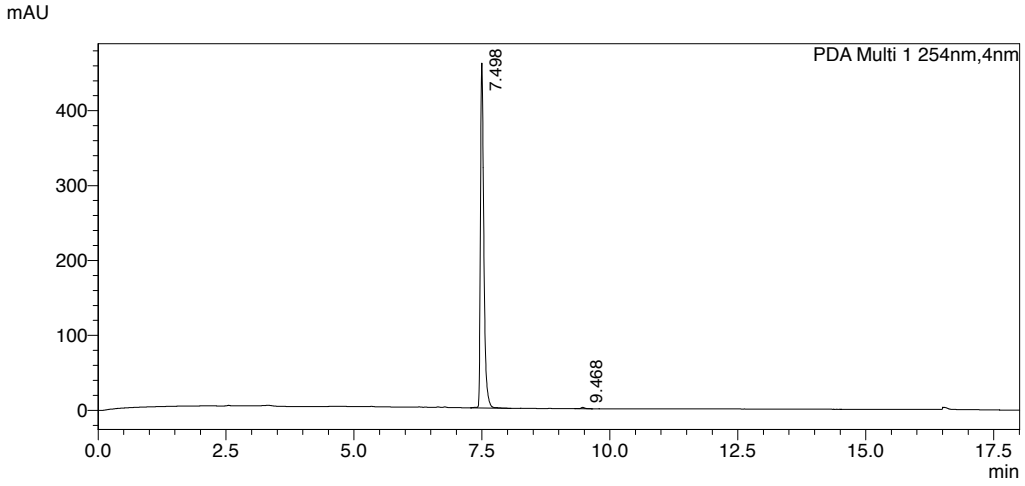
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI189-1		
Sample ID	: NNPI189-1		
Data Filename	: NNPI189-1.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_09_08_15_run3.lcb		
Vial #	: 1-25	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 9/8/2015 1:58:08 PM	Acquired by	: chemist
Date Processed	: 9/8/2015 2:51:57 PM	Processed by	: chemist

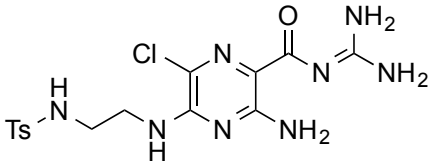
<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	7.498	2115988	454896	99.606
2	9.468	8379	1758	0.394
Total		2124368	456654	100.000



11

C:\LabSolutions\Data\Neeraj\NNPI189-1.lcd

3-amino-5-(((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)amino)-6-chloro-*N*-(diaminomethylene)pyrazine-2-carboxamide (14):

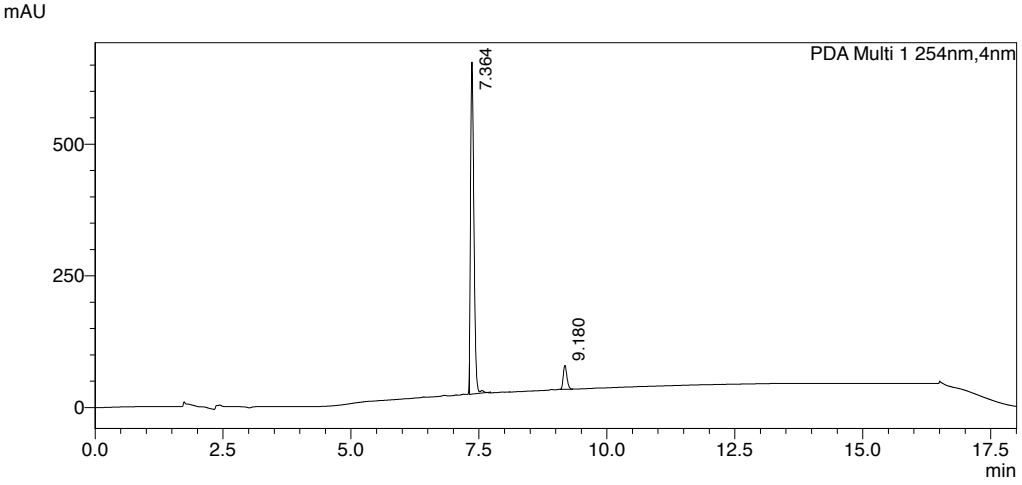
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI063p		
Sample ID	: NNPI063p		
Data Filename	: NNPI063p.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_10_24_2014.lcb		
Vial #	: 1-6	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 10/24/2014 3:06:49 PM	Acquired by	: chemist
Date Processed	: 5/21/2015 12:55:19 PM	Processed by	: chemist

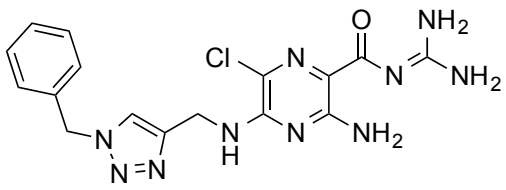
<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	7.364	2898906	609386	92.850
2	9.180	223228	44667	7.150
Total		3122133	654053	100.000



14

C:\LabSolutions\Data\Neeraj\NNPI063p.lcd

3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-(phenylethynyl)pyrazine-2-carboxamide (17):

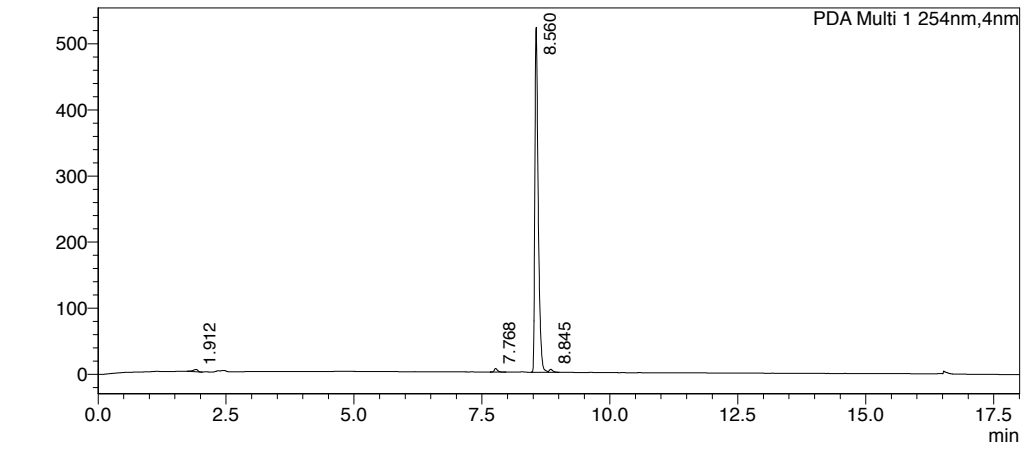
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPII-006repurif-R2		
Sample ID	: NNPII-006repurif-R2		
Data Filename	: NNPII-006repurif-R2.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_12_9_15_run1.lcb		
Vial #	: 1-13	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 12/9/2015 4:47:31 PM	Acquired by	: chemist
Date Processed	: 12/11/2015 2:52:56 PM	Processed by	: chemist

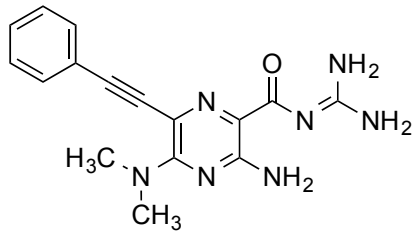
<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	1.912	22559	3505	0.917
2	7.768	23033	5322	0.936
3	8.560	2395394	505184	97.331
4	8.845	20099	4421	0.817
Total		2461085	518432	100.000



17

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3,5-diamino-*N*-(diaminomethylene)-6-(phenylethynyl)pyrazine-2-carboxamide (19):

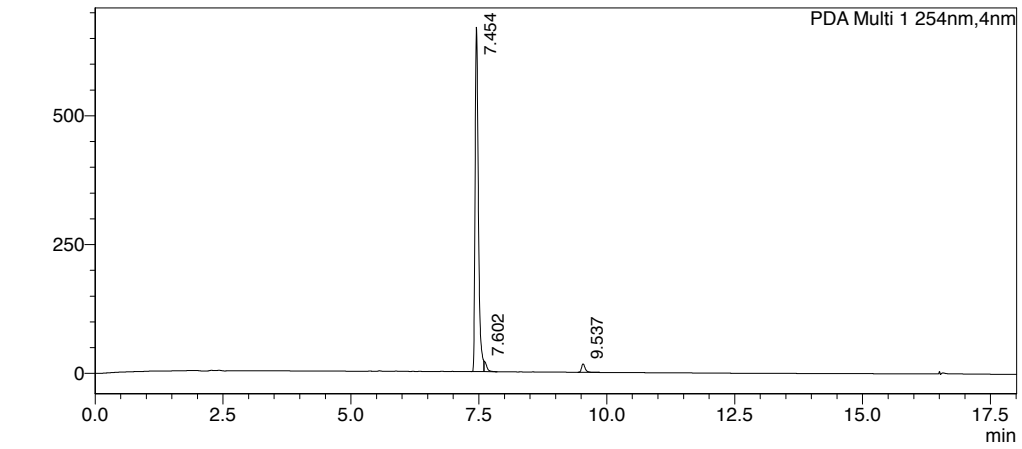
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPI168-r2		
Sample ID	: NNPI168-r2		
Data Filename	: NNPI168-r2.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNPI_07_27_15_R4.lcb		
Vial #	: 1-13	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 7/27/2015 1:06:34 PM	Acquired by	: chemist
Date Processed	: 10/29/2015 2:50:26 PM	Processed by	: chemist

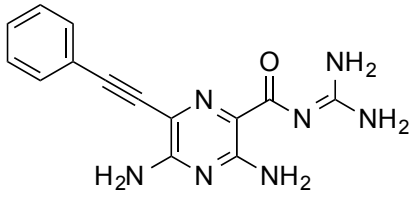
<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	7.454	2979293	656101	95.124
2	7.602	71818	21184	2.293
3	9.537	80882	15932	2.582
Total		3131994	693216	100.000



19

C:\LabSolutions\Data\Neeraj\NNPI168-r2.lcd

3,5-diamino-*N*-(diaminomethylene)pyrazine-2-carboxamide (20):

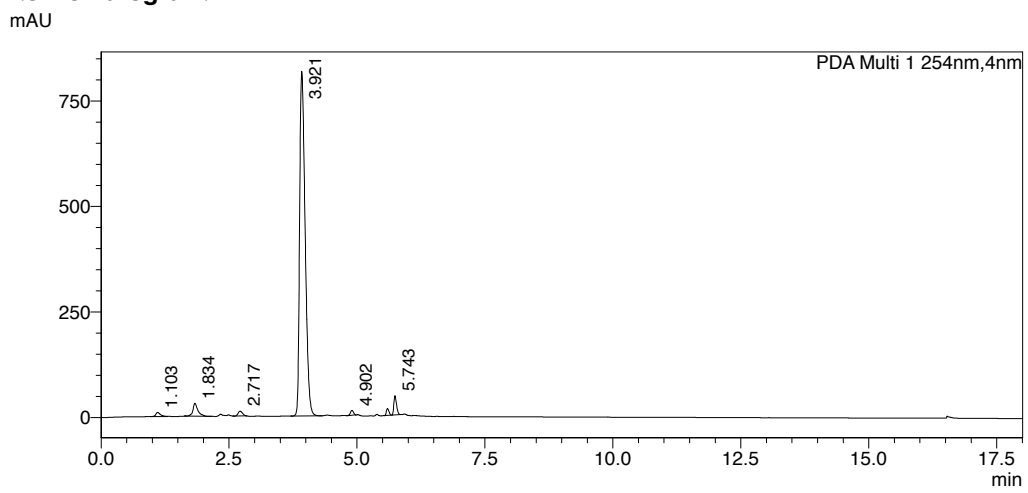
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPII-011-Recryst-R2.lcd		
Sample ID	: NNPII-011-Recryst-R2.lcd		
Data Filename	: NNPII-011-Recryst-R2.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_11_12_15_run1.lcb		
Vial #	: 1-36	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 11/12/2015 12:12:21 PM	Acquired by	: chemist
Date Processed	: 11/12/2015 2:21:57 PM	Processed by	: chemist

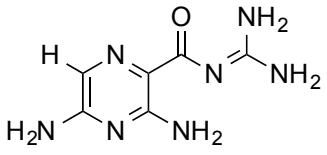
<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	1.103	61662	9462	0.918
2	1.834	207322	30195	3.086
3	2.717	68666	11335	1.022
4	3.921	6109888	805909	90.936
5	4.902	47397	11143	0.705
6	5.743	223935	43976	3.333
Total		6718870	912020	100.000



20

C:\LabSolutions\Data\Neeraj\NNPII-011-Recryst-R2.lcd

3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-phenylpyrazine-2-carboxamide (22a):

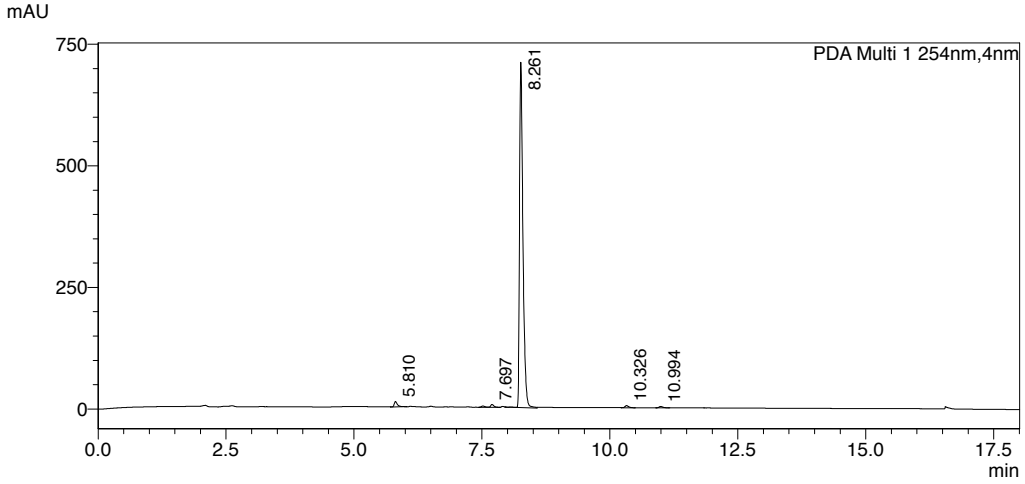
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPII-005-repurif-R2		
Sample ID	: NNPII-005-repurif-R2		
Data Filename	: NNPII-005-repurif-R2.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_12_11_15_run1.lcb		
Vial #	: 1-25	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 12/11/2015 4:01:44 PM	Acquired by	: chemist
Date Processed	: 12/11/2015 6:12:17 PM	Processed by	: chemist

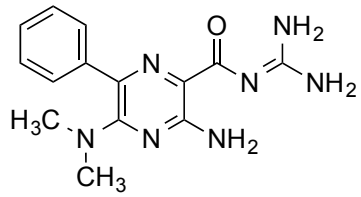
<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	5.810	47748	10384	1.392
2	7.697	37227	5531	1.085
3	8.261	3306101	687579	96.392
4	10.326	23615	4355	0.689
5	10.994	15168	2732	0.442
Total		3429859	710583	100.000



22a

C:\LabSolutions\Data\Neeraj\NNPII-005-repurif-R2.lcd

6-([1,1'-biphenyl]-4-yl)-3-amino-*N*-(diaminomethylene)-5-(dimethylamino)pyrazine-2-carboxamide (22b):

SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPII-025R1		
Sample ID	: NNPII-025R1		
Data Filename	: NNPII-025R1.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_03_04_16.lcb		
Vial #	: 1-7	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 3/4/2016 1:22:42 PM	Acquired by	: chemist
Date Processed	: 3/4/2016 1:59:25 PM	Processed by	: chemist

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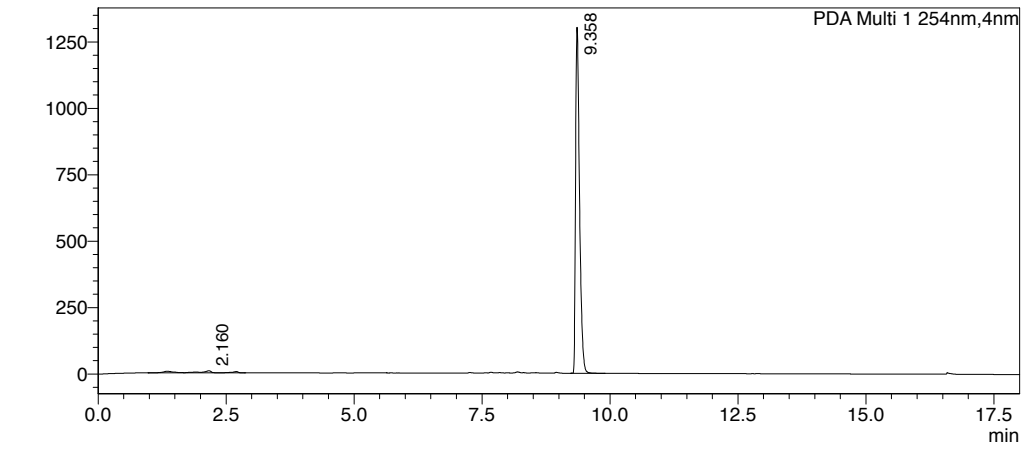
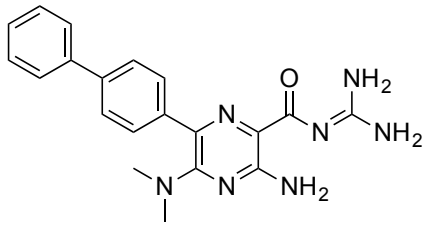


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	2.160	249226	8164	3.417
2	9.358	7045452	1287790	96.583
Total		7294678	1295954	100.000



22b

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3-amino-6-(4-cyanophenyl)-*N*-(diaminomethylene)-5-(dimethylamino)pyrazine-2-carboxamide (22c):

SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPII-027R1		
Sample ID	: NNPII-027R1		
Data Filename	: NNPII-027R1.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_03_04_16.lcb		
Vial #	: 1-9	Sample Type	: Unknown
Injection Volume	: 10 uL		
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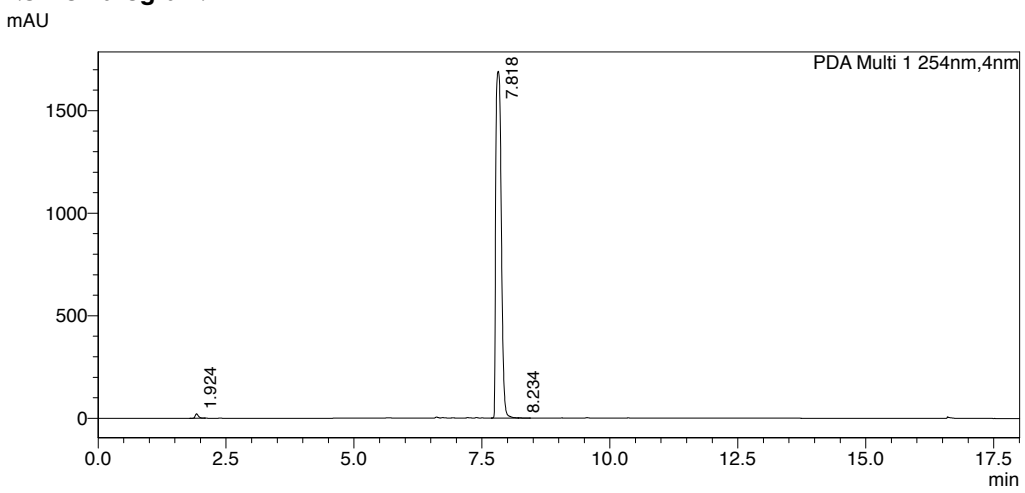
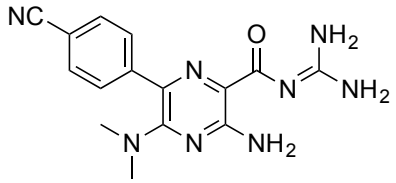


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	1.924	92849	20510	0.729
2	7.818	12633244	1690062	99.214
3	8.234	7276	1272	0.057
Total		12733369	1711845	100.000



22c

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3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-(naphthalen-2-yl)pyrazine-2-carboxamide (22d):

SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPII-028R1		
Sample ID	: NNPII-028R1		
Data Filename	: NNPII-028R1.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_03_04_16.lcb		
Vial #	: 1-10	Sample Type	: Unknown
Injection Volume	: 10 uL		
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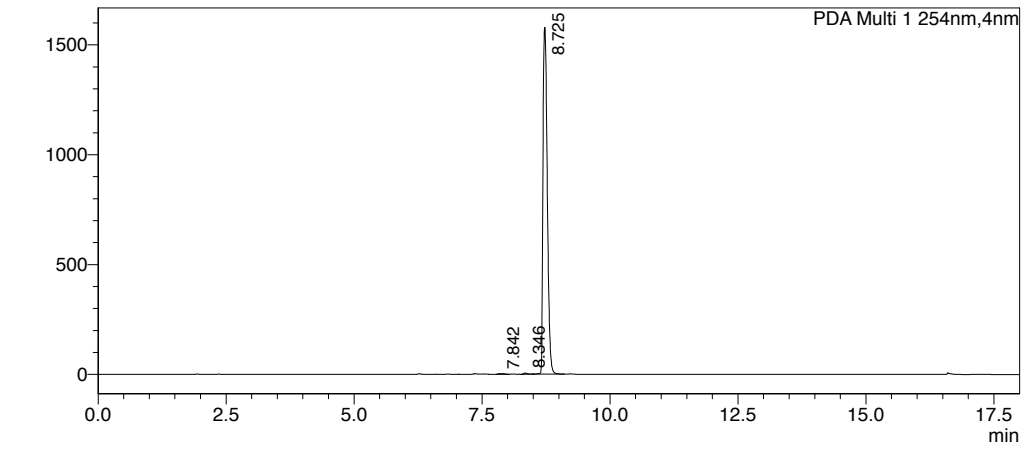
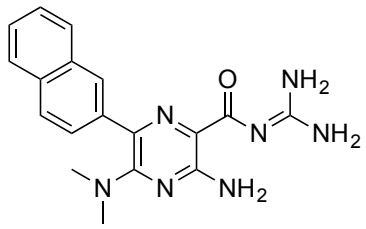


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	7.842	26434	3227	0.283
2	8.346	24540	5057	0.263
3	8.725	9285361	1565160	99.454
Total		9336335	1573444	100.000



22d

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3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-(*p*-tolyl)pyrazine-2-carboxamide (22e):

SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPII-030R1		
Sample ID	: NNPII-030R1		
Data Filename	: NNPII-030R1.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_03_04_16.lcb		
Vial #	: 1-11	Sample Type	: Unknown
Injection Volume	: 10 uL		
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Date Processed	: 3/4/2016 3:13:35 PM	Processed by	: chemist

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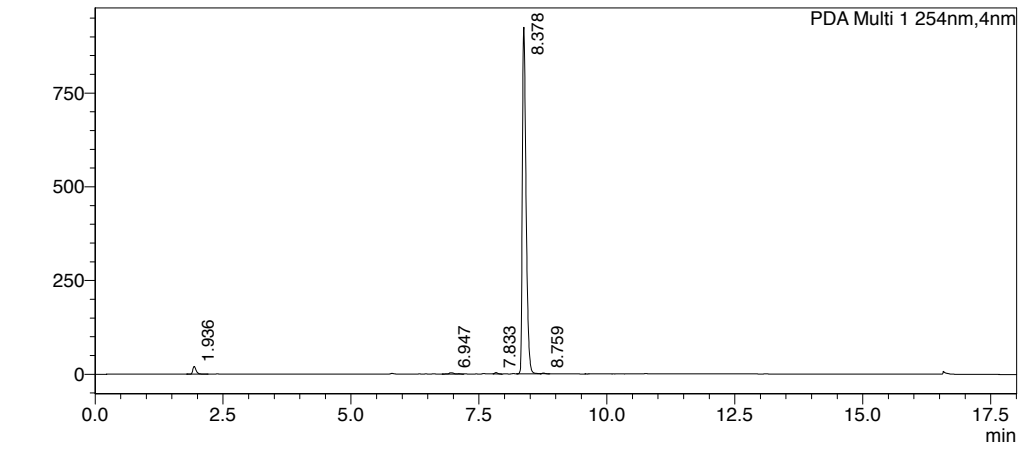
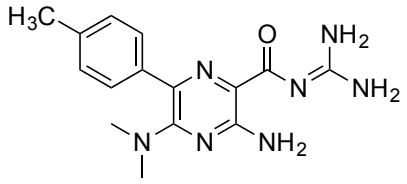


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	1.936	95530	20197	1.973
2	6.947	26861	3414	0.555
3	7.833	14263	3490	0.295
4	8.378	4698292	914705	97.035
5	8.759	6924	1848	0.143
Total		4841870	943655	100.000



22e

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3-amino-*N*-(diaminomethylene)-5-(dimethylamino)-6-(4-fluorophenyl)pyrazine-2-carboxamide (22f):

SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPII-026R1		
Sample ID	: NNPII-026R1		
Data Filename	: NNPII-026R1.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_03_04_16.lcb		
Vial #	: 1-8	Sample Type	: Unknown
Injection Volume	: 10 uL		
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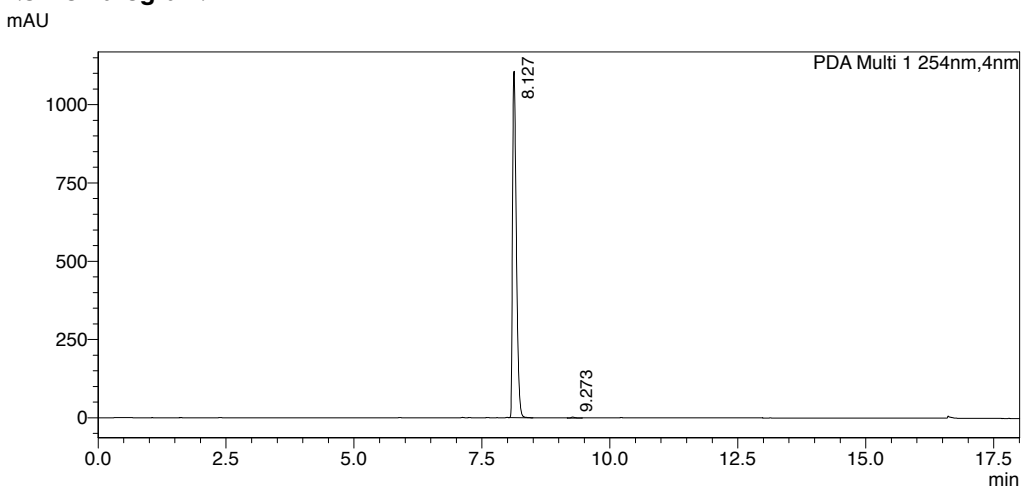
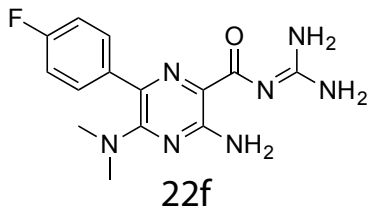


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	8.127	5710163	1086732	99.559
2	9.273	25283	3140	0.441
Total		5735446	1089872	100.000



22f

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5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-(diaminomethylene)-6-phenylpyrazine-2-carboxamide (24):

SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPII-031R1		
Sample ID	: NNPII-031R1		
Data Filename	: NNPII-031R1.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_03_04_16.lcb		
Vial #	: 1-12	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 3/4/2016 2:55:29 PM	Acquired by	: chemist
Date Processed	: 3/4/2016 4:06:54 PM	Processed by	: chemist

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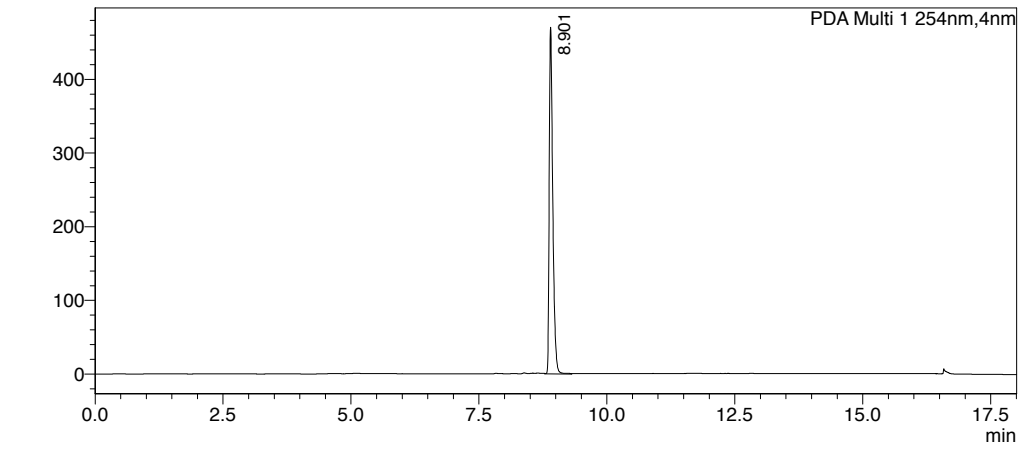
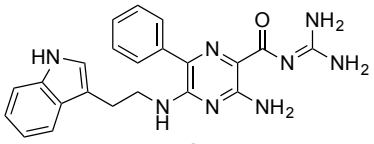


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	8.901	2284909	456864	100.000
Total		2284909	456864	100.000



24

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Section B: Biological Assays:

5. Procedures for Biological Assays:

RNA Preparation

HIV-1 TAR RNA was prepared by *in vitro* transcription. Synthetic template DNA was purchased (Integrated DNA Technologies) containing the T7 promoter and the wtTAR template sequence. DNA was annealed in 3 mM $MgCl_2$ by heating to 95°C for 5 min followed by cooling on ice for 30 min. The transcription reaction ran for ~12 hours at 37°C in optimized conditions [0.1M Tris, 0.03M $MgCl_2$, 0.03M DTT, 2 mM spermine, 0.25 μM DNA template, 3 units/μl T7 RNA polymerase (New England BioLabs) and 3 mM $^{13}C/^{15}N$ labeled nucleotide triphosphates (Cambridge Isotope Laboratories Inc) or unlabeled nucleotide triphosphates]. RNA was purified using 20% (w/v) denaturing polyacrylamide gel electrophoresis (PAGE) with 8M urea and 1X TBE [89 mM Tris base, 89 mM boric acid, 2 mM EDTA]. Purified RNA was extracted by gel electro-elution in 1X TAE [40 mM Tris base, 40 mM acetate 1mM EDTA] buffer and further purified by ethanol precipitation. The purified RNA pellet was dissolved in water to ~ 50 μM RNA, heated to 95°C for 5 min and cooled on ice for 1 hour to anneal.

Displacement Assays

For Tat-displacement assays, TAR RNA was diluted to 150 nM in Tris-HCl assay buffer [50 mM Tris-HCl, 50 mM KCl, 0.01% (v/v) Triton X-100 at pH ~7.4]. The Tat peptide, N-(5-FAM)-AAARKKRRQRRRAAAK(TAMRA)-C (Lifetein, purity > 95%) was dissolved into the same assay buffer to 60 nM. Small molecules were diluted to 3 mM in assay buffer and serially diluted to 0.03 μM. All three components were combined in a 384 well plate (Corning® low volume black round bottom, polystyrene, untreated), such that final assay concentrations were 50 nM TAR, 20 nM Tat peptide and (1 mM-0.01 μM) small molecule. This ratio of TAR to Tat peptide was selected because it gave the highest fluorescence signal, indicating that ~100% Tat peptide was bound by TAR before addition of small molecules, to maximize the signal change during the experiment. TAR was excluded for the Tat-only control and 100-fold excess tRNA (bulk tRNA from baker's yeast) was added for the tRNA control. Similarly, a 100-fold excess of 30-mer (dA-dT) alternating DNA duplex (purchased as a duplex from IDT technologies) was added for the DNA control. Fluorescence intensity (excitation 485 nm, emission 590 nm) was measured on a Clariostar monochromator microplate reader (BMG Labtech). The signal was baseline corrected to the Tat- only control and points with assay interference were excluded from analysis.

CD_{50} values were fit with OriginPro (OrginLab) using dose response curve fitting (Eq. 1 and 2) and the instrumental weighting method.

$$y = A1 + \frac{(A2-A1)}{(1+10^{((Logx0-x)p)})} \quad \text{Eq. 1}$$

$$CD_{50} = 10^{Logx0} \quad \text{Eq. 2}$$

where A1 is the minimum signal, A2 is the maximum signal, p is the hill slope, and Logx0 is the Log of the concentration of half response. Assays were minimally conducted in triplicate and reported error is the standard deviation from the mean. Each replicate contained three technical replicates.

SOFAST NMR

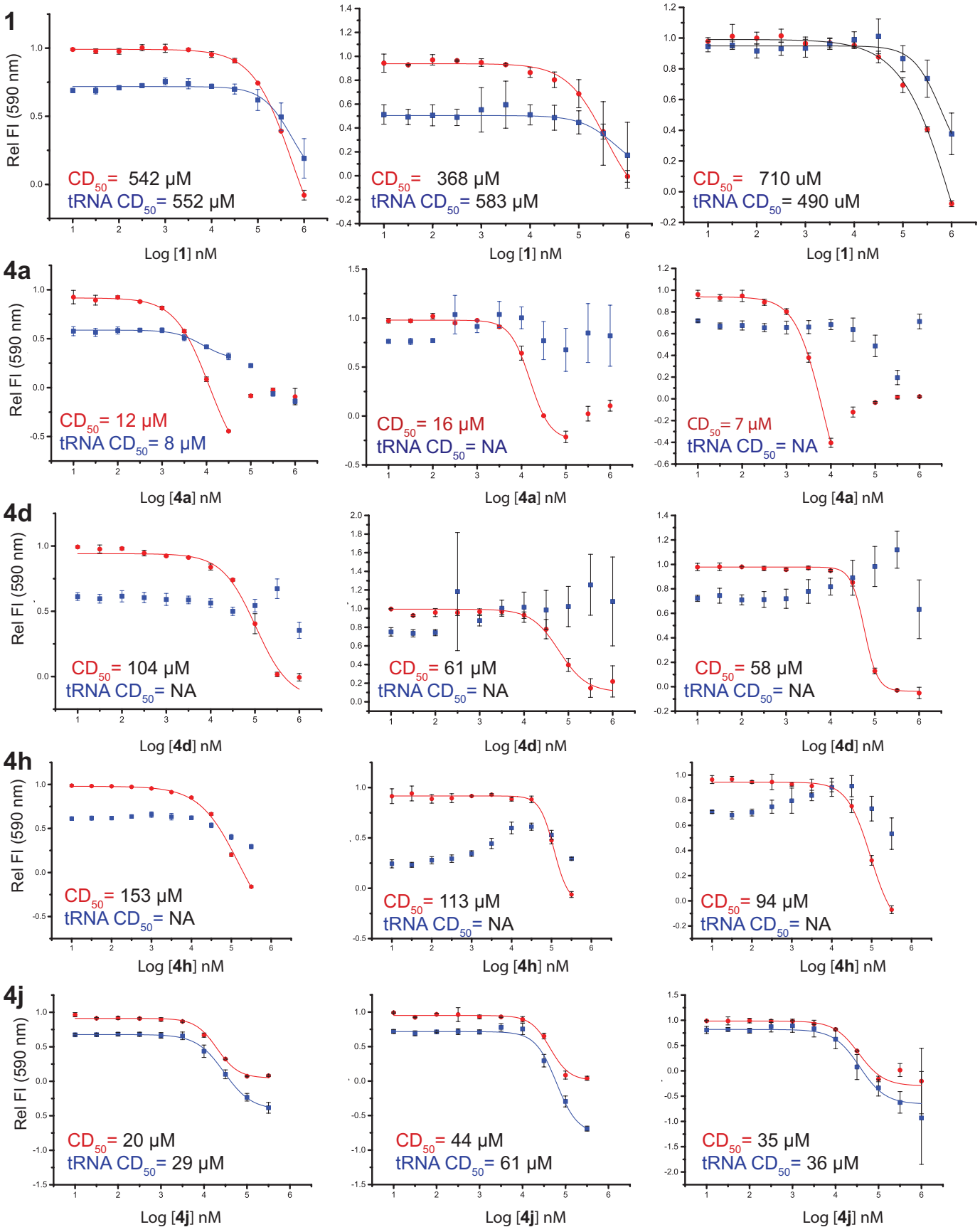
Synthesized $^{13}C/^{15}N$-labeled TAR RNA was exchanged into phosphate NMR buffer [15 mM NaH_2PO_4/Na_2HPO_4, 25 mM NaCl, 0.1 mM EDTA, 10% (v/v) D_2O at pH ~ 6.4] to a concentration of 50 μM RNA. Small molecules were stored in DMSO at 20 mM and added to TAR such that the final amount of DMSO in the NMR sample was 1% (v/v). Equivalent volumes of DMSO were added to the free TAR controls to compensate for minor changes induced by DMSO. Aromatic 2D SOFAST [^1H-^{13}C] HMQC spectra[18] were collected at 25°C (Fig. **1,** Bulgeless and UUCG comparison) or 37°C (all other spectra: **Fig. 3**, **SI-6**) on a 600 MHz Bruker spectrometer equipped with an HCPN cryogenic probe (**4f**, **24**, **4e**, **22d**, **22c** and **22b),** an 800 MHz Agilent spectrometer equipped with an HCN cryogenic probe (**1, 19, 4g, 4j, 4l, 4m, 22a, 17, 4n**) or an 800 MHz Agilent spectrometer equipped with HCN room-temp probe (all other amiloride derivatives). Spectra were processed using nmrPipe[22] and SPARKY.[23] To generate the heat maps shown in **Figure 1, 3, and SI-6**, chemical shift perturbations between free TAR and TAR bound by small molecule were calculated with equation 3.

$$\Delta\delta = \sqrt{(\Delta\delta_H)^2 + \left(\frac{\gamma_H}{\gamma_C}\Delta\delta_C\right)^2} \quad \text{Eq. 3}$$

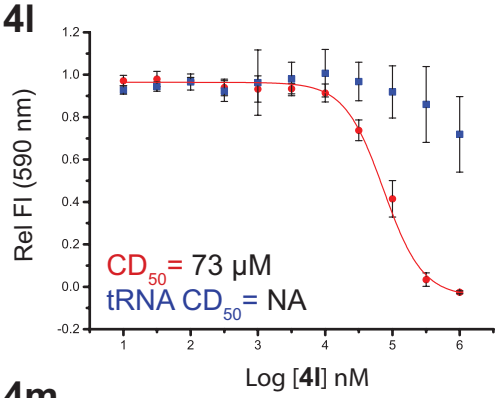
Where $\Delta\delta$ is the change in chemical shift (in ppm) and γ is the gyromagnetic ratio for ^{13}C and ^1H nuclei. Assignments for WT-TAR,[10] UUCG-TAR,[11] and bulgeless-TAR, [12] were previously reported.

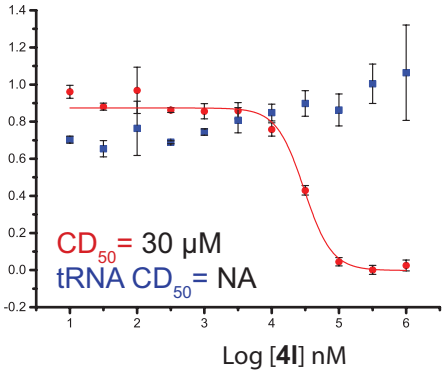
6. Representative set of binding curves obtained from Tat peptide displacement assays

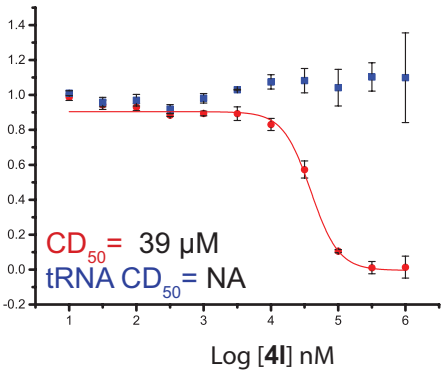
The competitive dose to displace 50% Tat from wtTAR (CD_{50}) was minimally measured in triplicate for each DMA analog with three readings being taken for each replicate. The CD_{50} was also measured in the presence of 100-fold excess tRNA (blue) to test for TAR specificity.



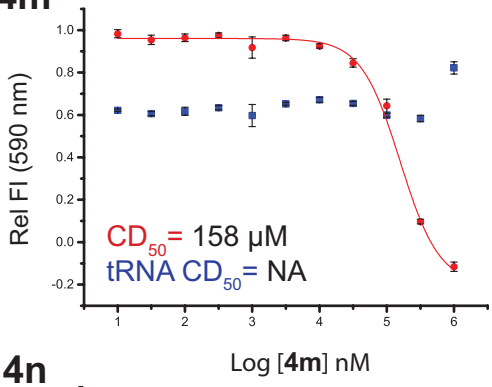
4l

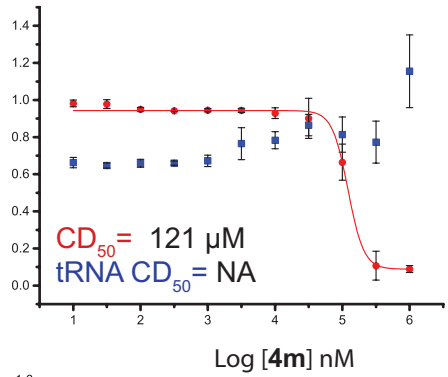

Rel FI (590 nm)
CD50= 73 μM
tRNA CD50= NA
Log [4l] nM

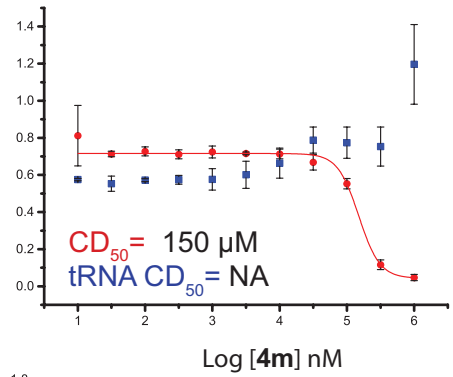

CD50= 30 μM
tRNA CD50= NA
Log [4l] nM


CD50= 39 μM
tRNA CD50= NA
Log [4l] nM

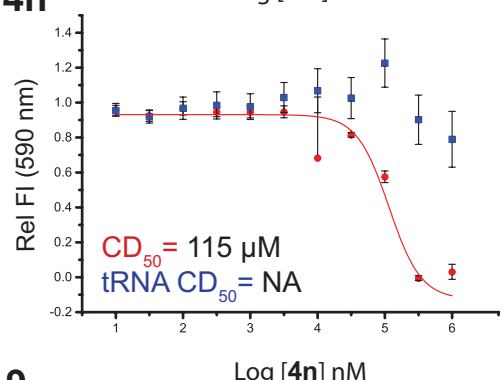
4m

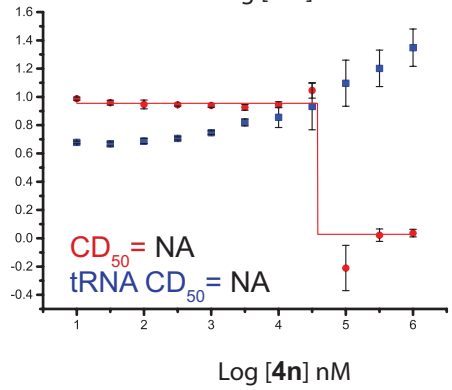

Rel FI (590 nm)
CD50= 158 μM
tRNA CD50= NA
Log [4m] nM

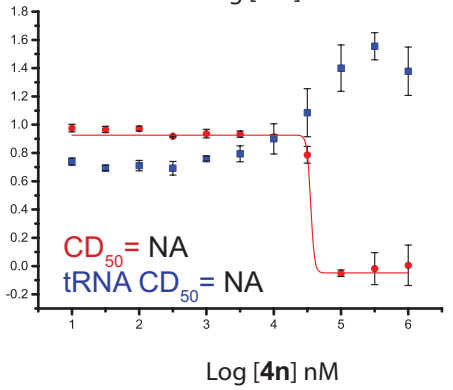

CD50= 121 μM
tRNA CD50= NA
Log [4m] nM


CD50= 150 μM
tRNA CD50= NA
Log [4m] nM

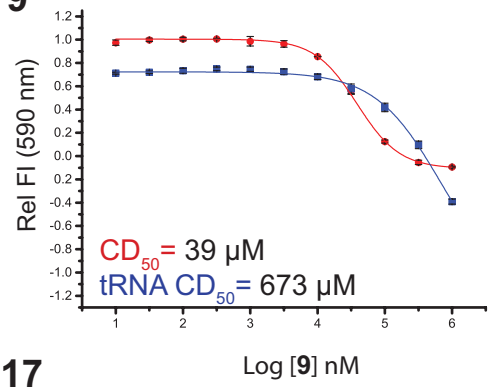
4n

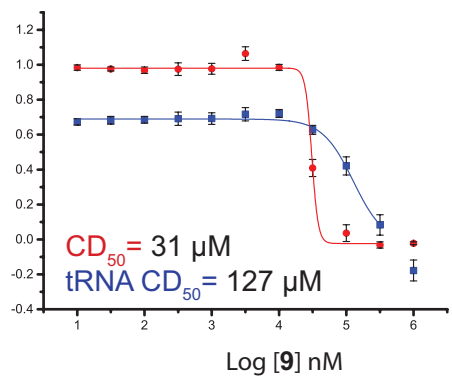

Rel FI (590 nm)
CD50= 115 μM
tRNA CD50= NA
Log [4n] nM

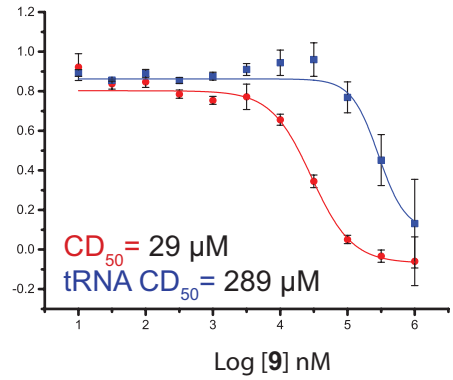

CD50= NA
tRNA CD50= NA
Log [4n] nM


CD50= NA
tRNA CD50= NA
Log [4n] nM

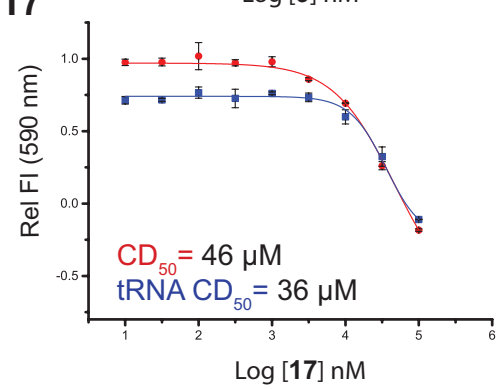
9

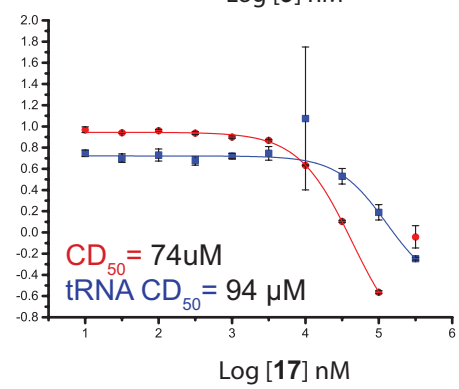

Rel FI (590 nm)
CD50= 39 μM
tRNA CD50= 673 μM
Log [9] nM

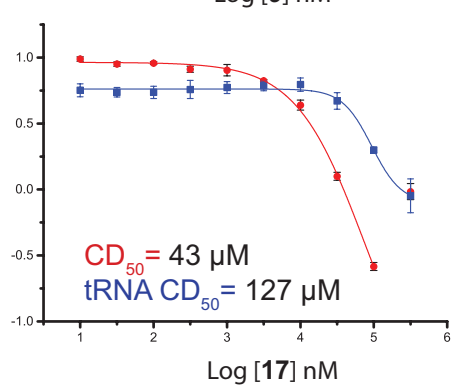

CD50= 31 μM
tRNA CD50= 127 μM
Log [9] nM

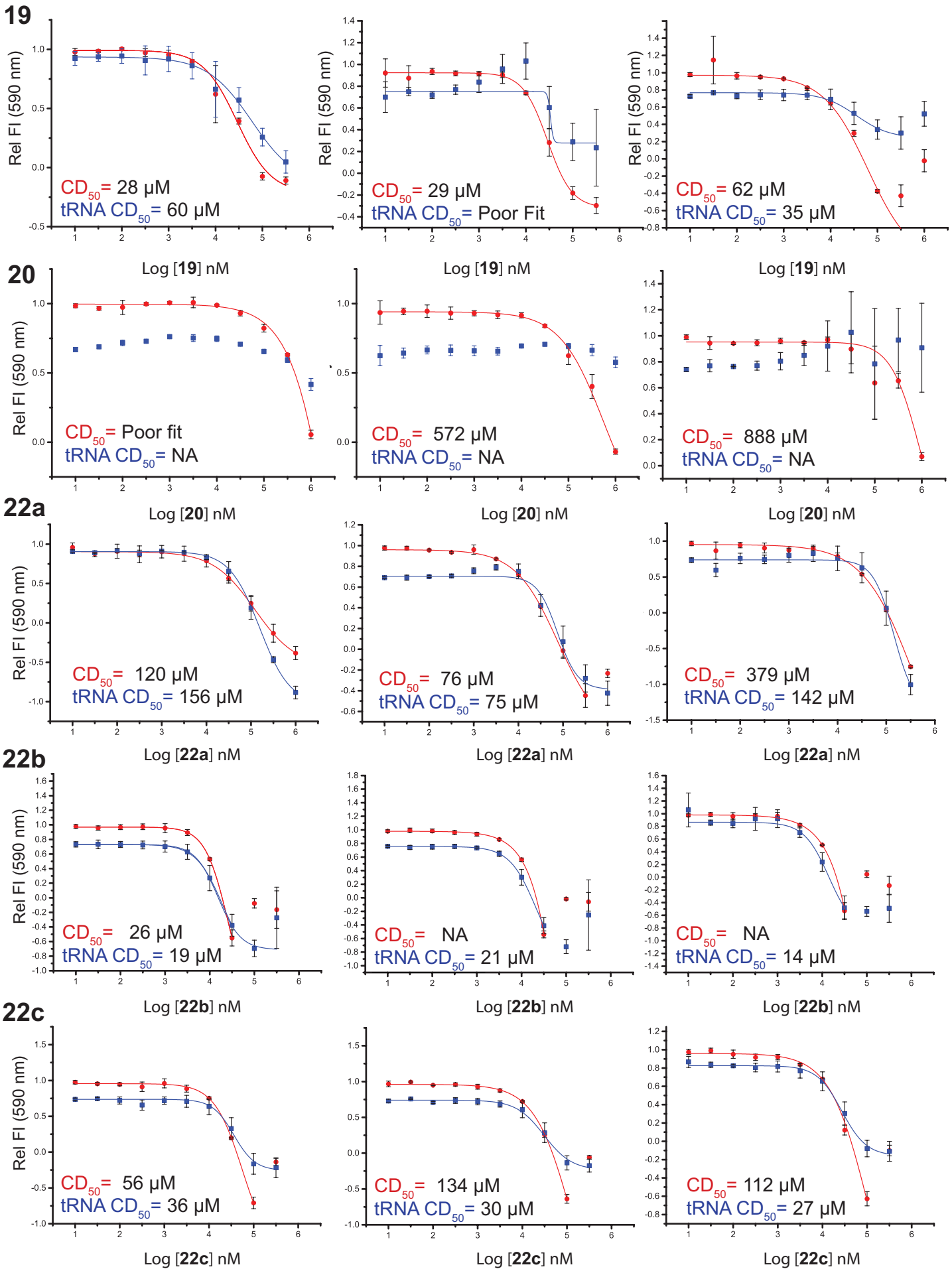

CD50= 29 μM
tRNA CD50= 289 μM
Log [9] nM

17

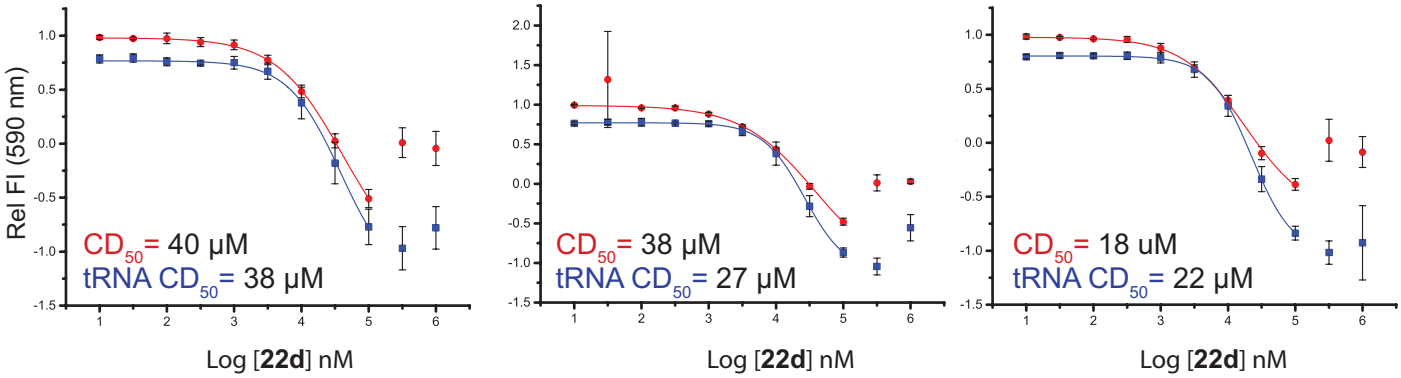

Rel FI (590 nm)
CD50= 46 μM
tRNA CD50= 36 μM
Log [17] nM


CD50= 74uM
tRNA CD50= 94 μM
Log [17] nM

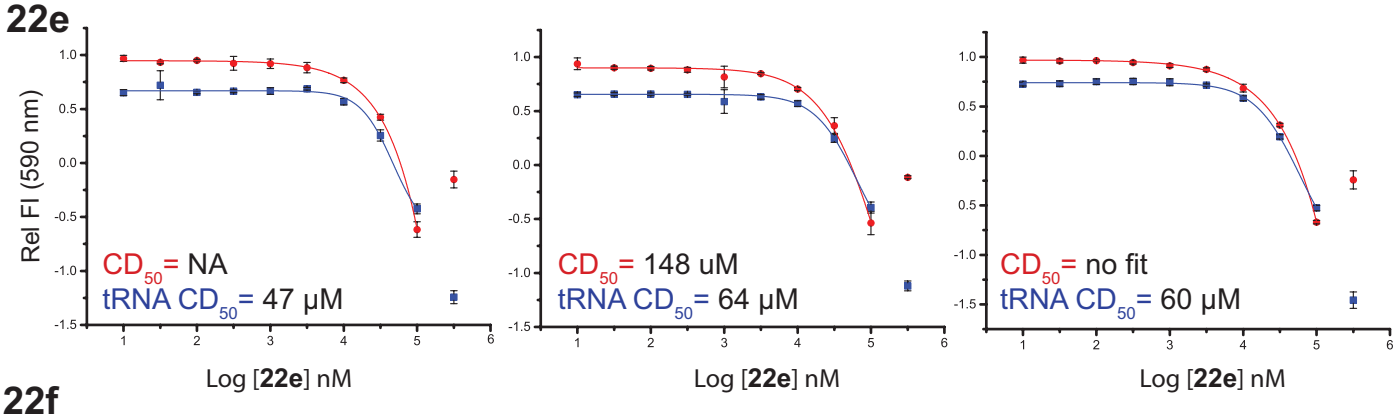

CD50= 43 μM
tRNA CD50= 127 μM
Log [17] nM


19
Rel FI (590 nm)
CD50= 28 μM
tRNA CD50= 60 μM
CD50= 29 μM
tRNA CD50= Poor Fit
CD50= 62 μM
tRNA CD50= 35 μM
Log [19] nM
20
CD50= Poor fit
tRNA CD50= NA
CD50= 572 μM
tRNA CD50= NA
CD50= 888 μM
tRNA CD50= NA
Log [20] nM
22a
CD50= 120 μM
tRNA CD50= 156 μM
CD50= 76 μM
tRNA CD50= 75 μM
CD50= 379 μM
tRNA CD50= 142 μM
Log [22a] nM
22b
CD50= 26 μM
tRNA CD50= 19 μM
CD50= NA
tRNA CD50= 21 μM
CD50= NA
tRNA CD50= 14 μM
Log [22b] nM
22c
CD50= 56 μM
tRNA CD50= 36 μM
CD50= 134 μM
tRNA CD50= 30 μM
CD50= 112 μM
tRNA CD50= 27 μM
Log [22c] nM

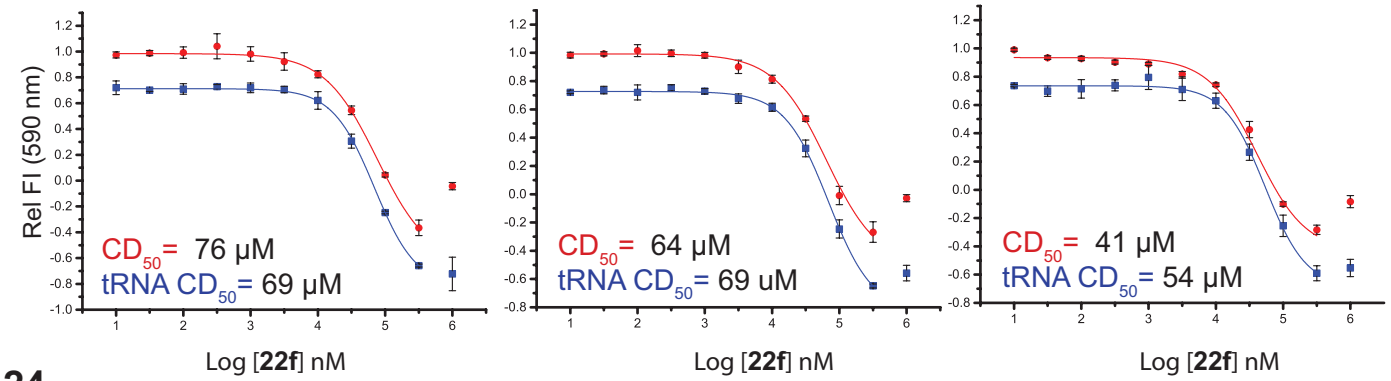
22d



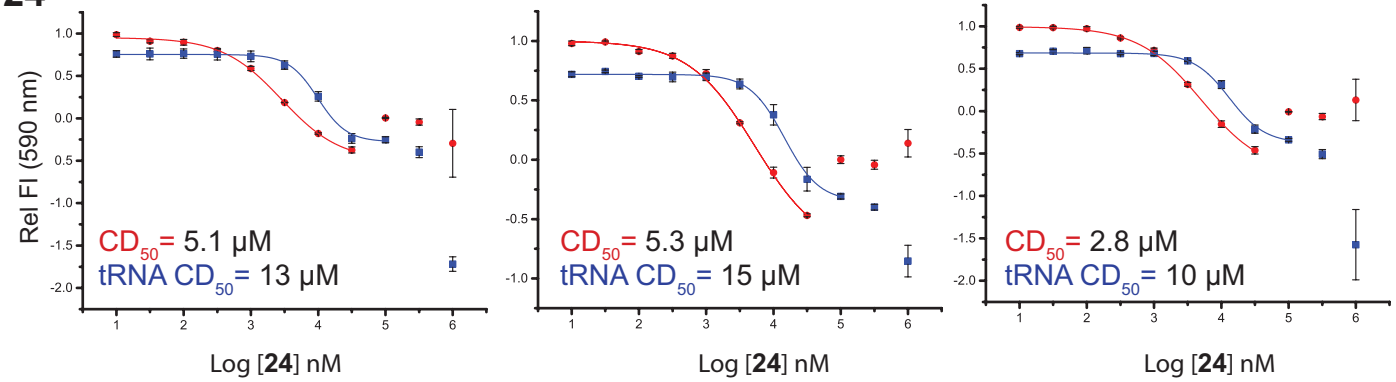
22e



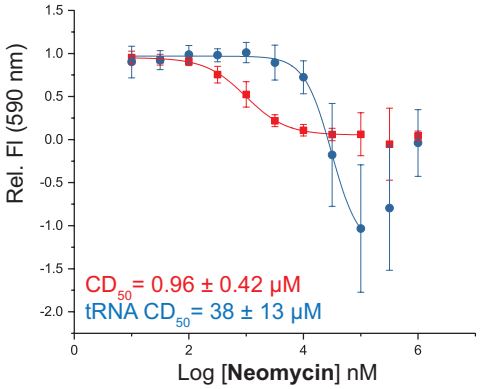
22f



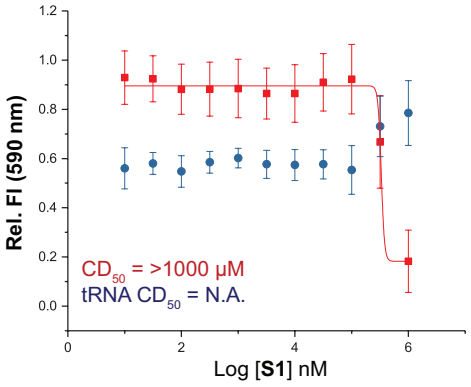
24

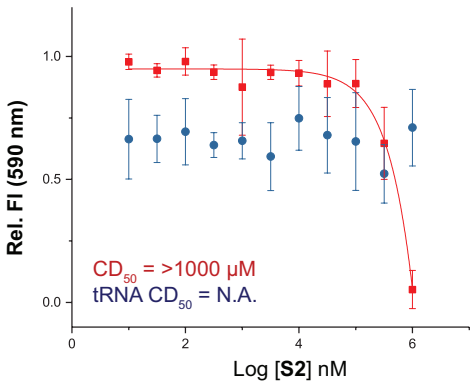


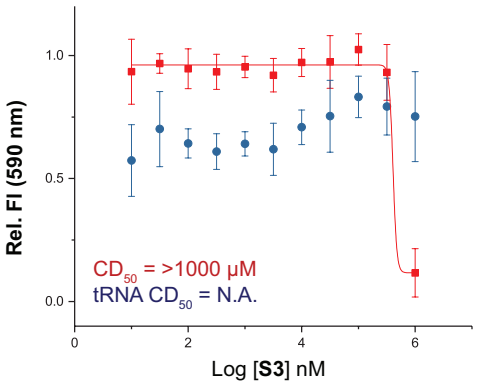
Displacement assay results for control Neomycin B

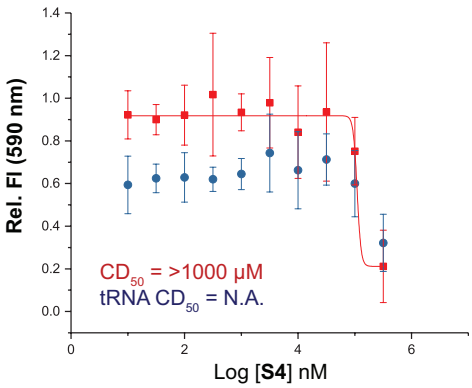


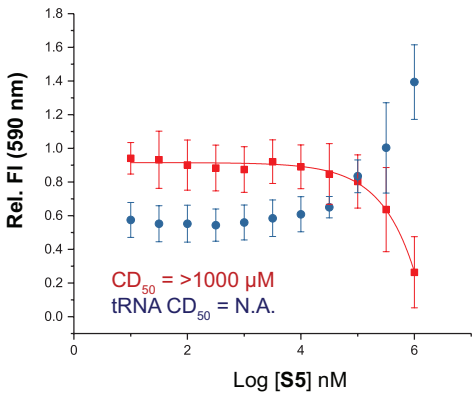
Displacement assay results for commercial amiloride derivatives S1-S6

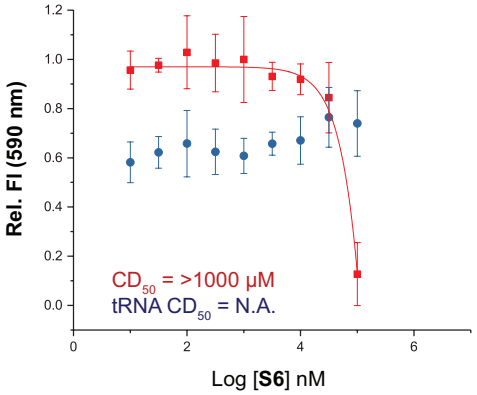




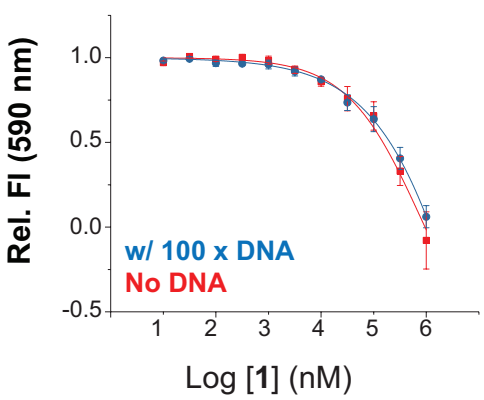


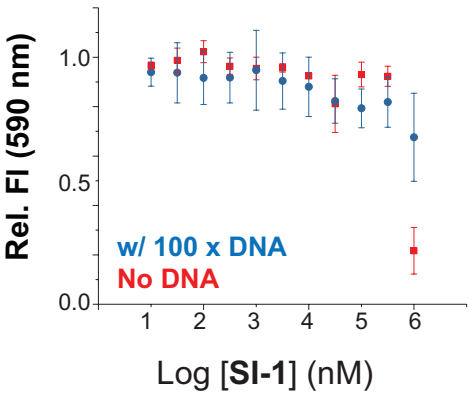


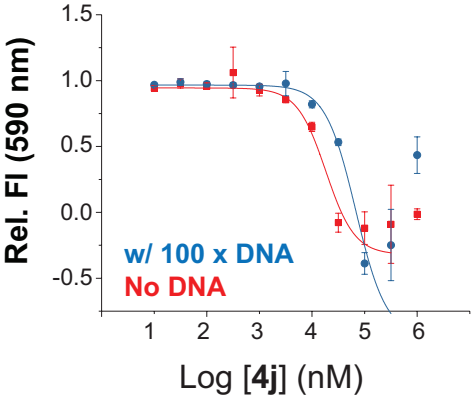


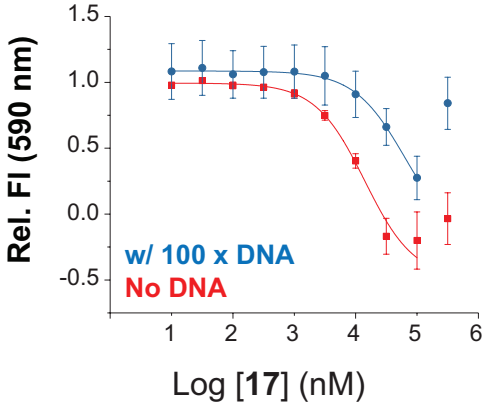


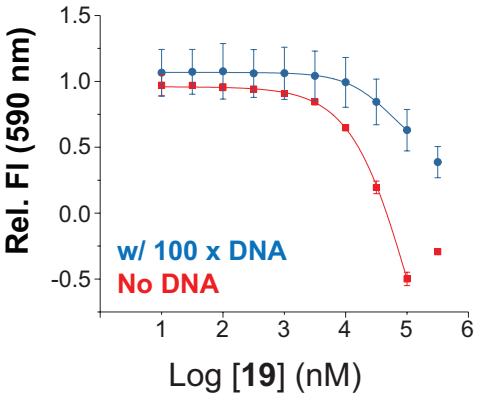
Displacement assay results for selected amiloride derivatives with and without 30-mer (dA-dT) DNA as control.

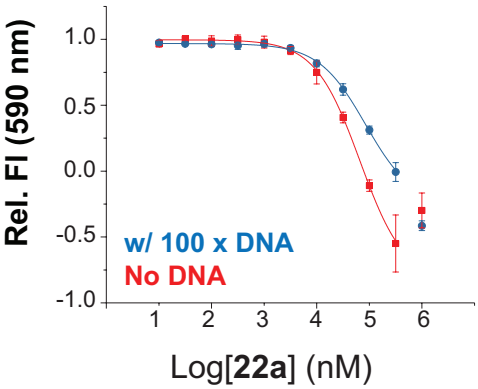


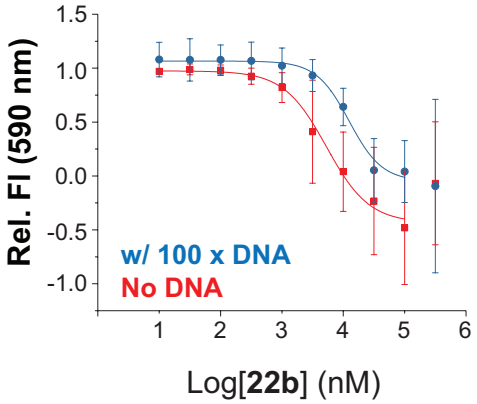


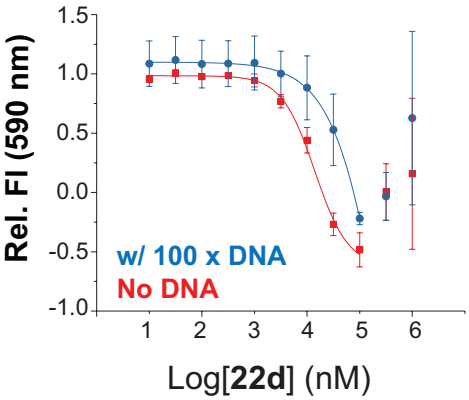


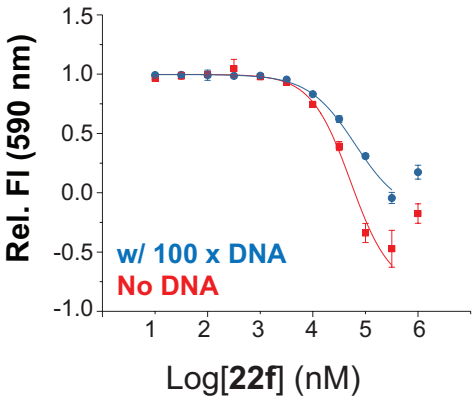












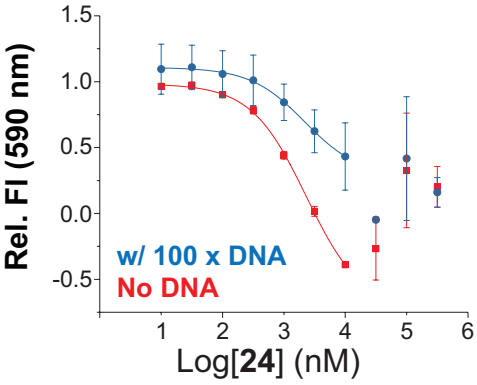
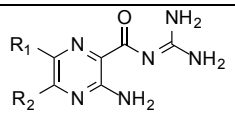
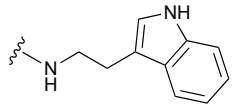
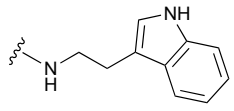
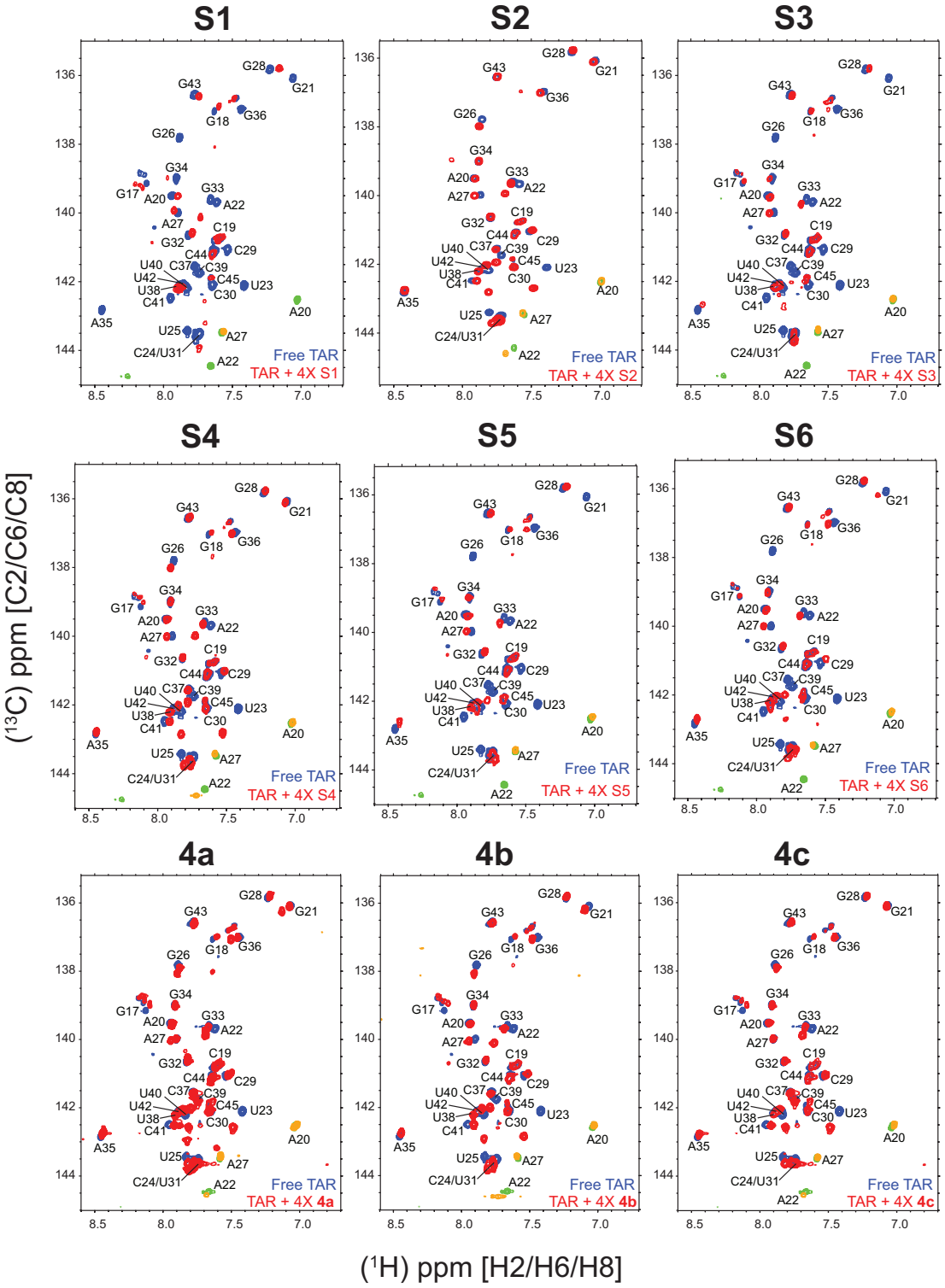


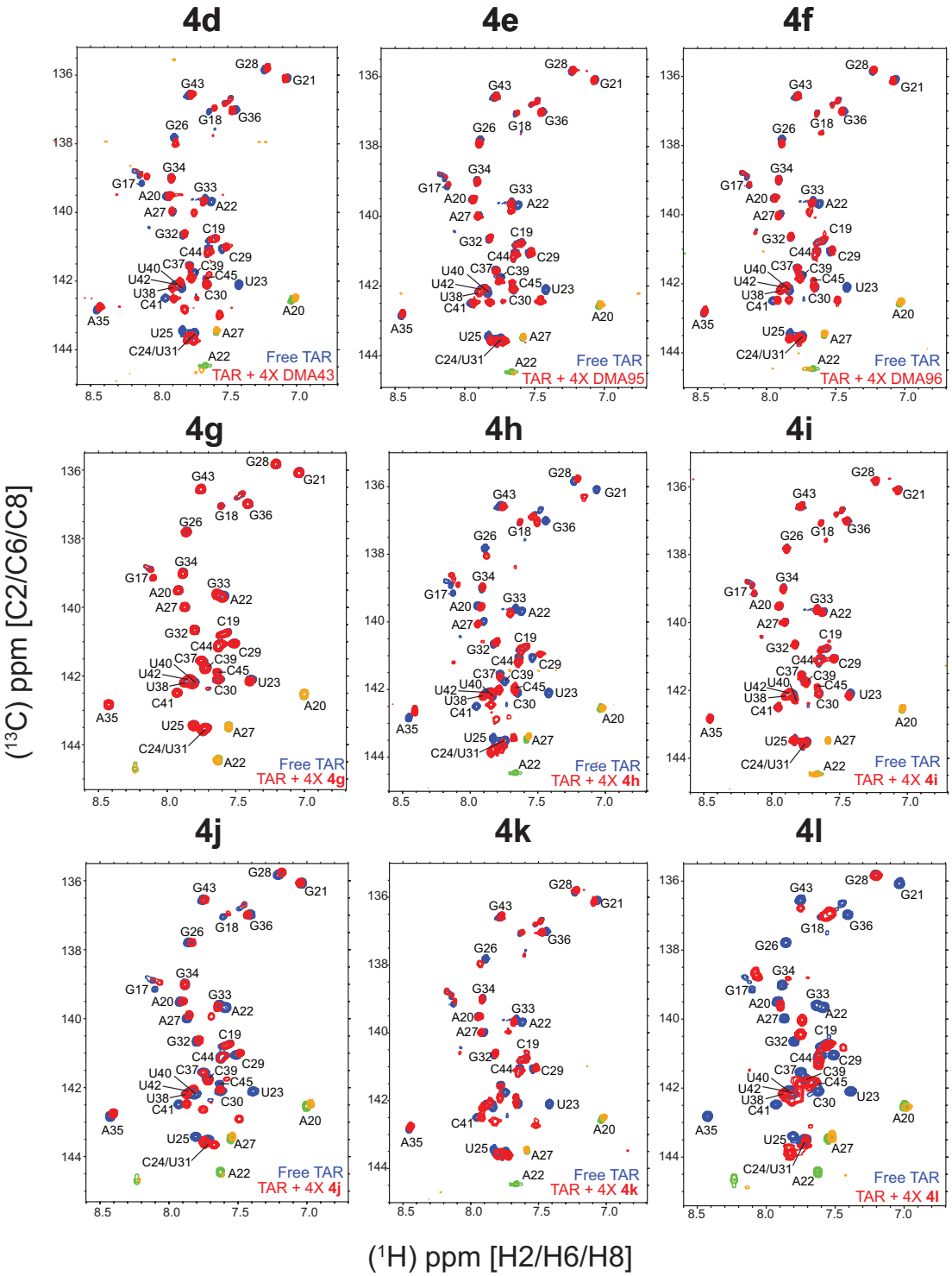
Table S1: Tat peptide displacement screening for select amiloride derivatives with DNA.

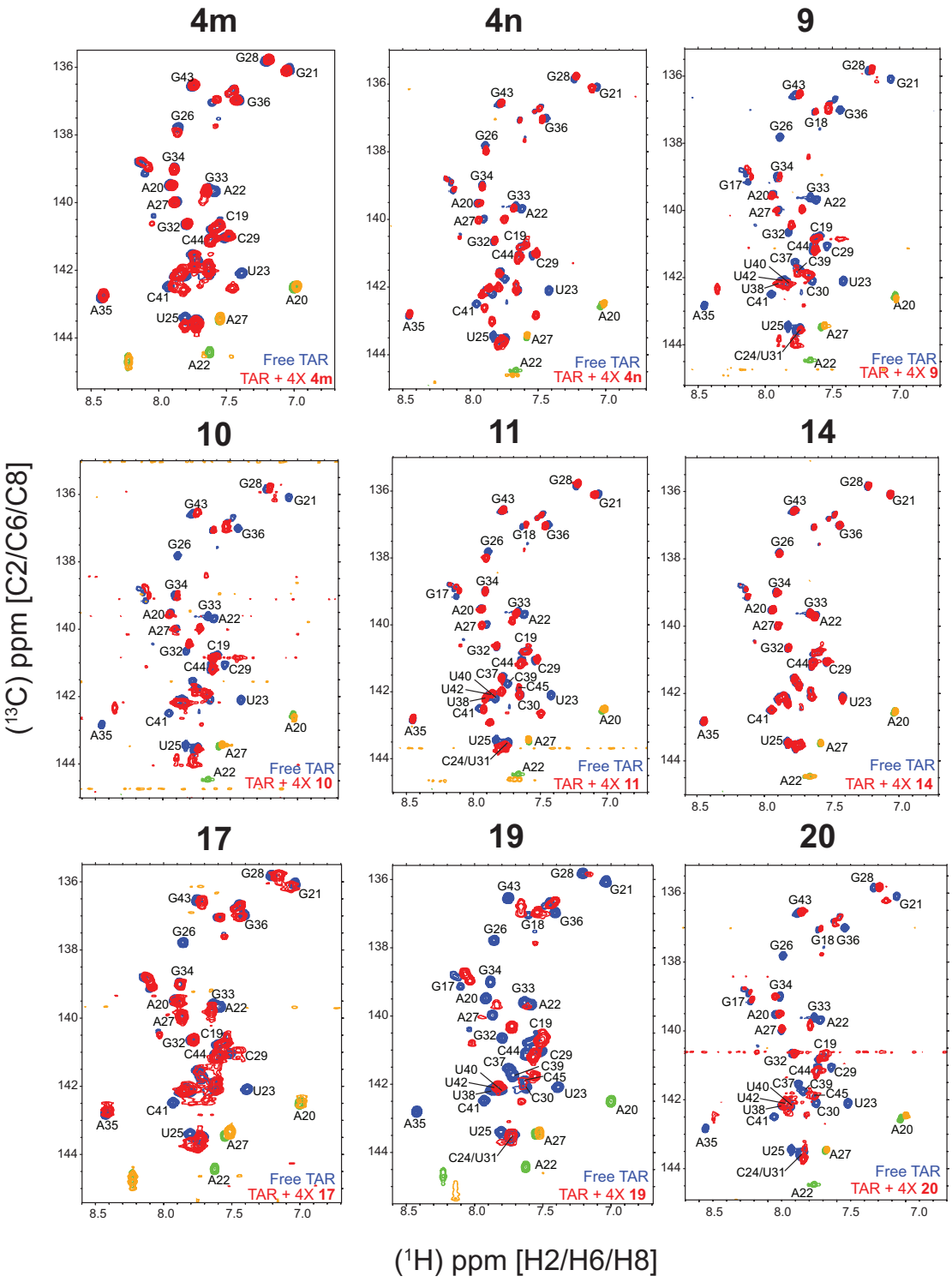
Amiloride		CD_{50} Tat disp. w/ DNA (μM)
C(5) Substitutions (R_1 = Cl)		
1	**R_2 = -$N(CH_3)_2$**	**1300 ± 5900**
S1	**R_2 = -NH_2**	**No Displacement**
4j	R_2 = 	**68 ± 26**
C (6) Substitutions		
17	R_1 = CΞC-Ph R_2 = $N(CH_3)_2$	**64 ± 24**
19	R_1 = CΞC-Ph R_2 = NH_2	**58 ± 11**
22a	R_1 = Ph R_2 = $N(CH_3)_2$	**84 ± 20**
22b	R_1 = (*p*-C_6H_4)-Ph R_2 = $N(CH_3)_2$	**12 ± 1.9**
22d	R_1 = β-napthyl R_2 = $N(CH_3)_2$	**380 ± 540**
22f	R_1 = (*p*-C_6H_4)-F R_2 = $N(CH_3)_2$	**63 ± 21**
C(5) and C(6) Substitutions		
24	R_1 = Ph R_2 = 	**2.2 ± 0.42**

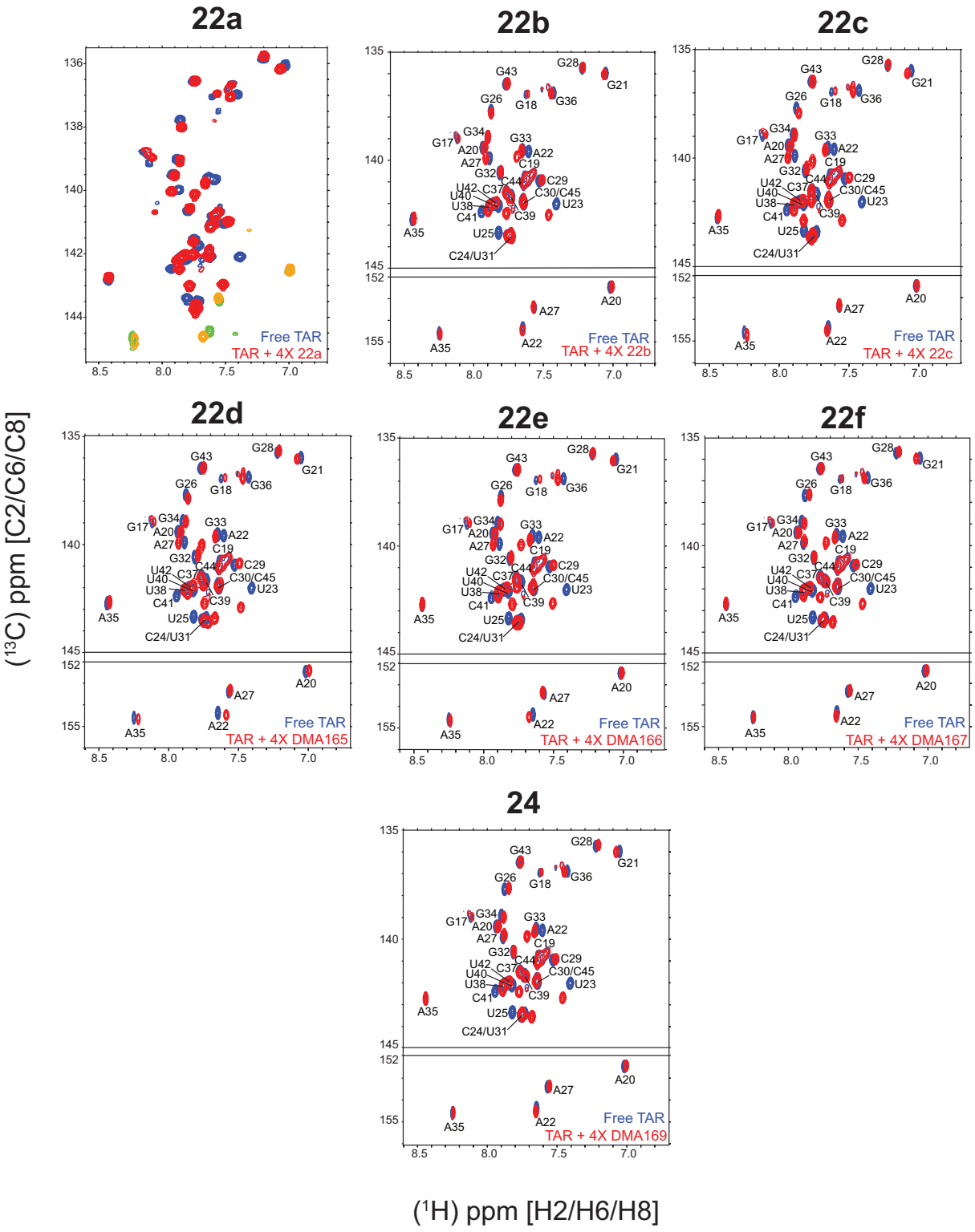
7. 2D SOFAST- [^{1}H-^{13}C] HMQC NMR spectra of amiloride derivatives against TAR:

The effect of each DMA analog on the aromatic spectra of TAR was measured using a 2D SOFAST-HMQC NMR experiment. The free TAR spectra are shown in blue and the spectra of TAR with 4-fold excess small molecule are shown in red. * C2H2 peaks are folded over in the spectral window.




4d
4e
4f
4g
4h
4i
4j
4k
4l
(13C) ppm [C2/C6/C8]
(1H) ppm [H2/H6/H8]
Free TAR
TAR + 4X DMA43
TAR + 4X DMA95
TAR + 4X DMA96
TAR + 4X 4g
TAR + 4X 4h
TAR + 4X 4i
TAR + 4X 4j
TAR + 4X 4k
TAR + 4X 4l


4m
4n
9
10
11
14
17
19
20
Free TAR
TAR + 4X 4m
TAR + 4X 4n
TAR + 4X 9
TAR + 4X 10
TAR + 4X 11
TAR + 4X 14
TAR + 4X 17
TAR + 4X 19
TAR + 4X 20
(^{13}C) ppm [C2/C6/C8]
(^{1}H) ppm [H2/H6/H8]


22a
22b
22c
22d
22e
22f
24
Free TAR
TAR + 4X 22a
TAR + 4X 22b
TAR + 4X 22c
TAR + 4X DMA165
TAR + 4X DMA166
TAR + 4X DMA167
TAR + 4X DMA169
(13C) ppm [C2/C6/C8]
(1H) ppm [H2/H6/H8]

NMR broadening analysis

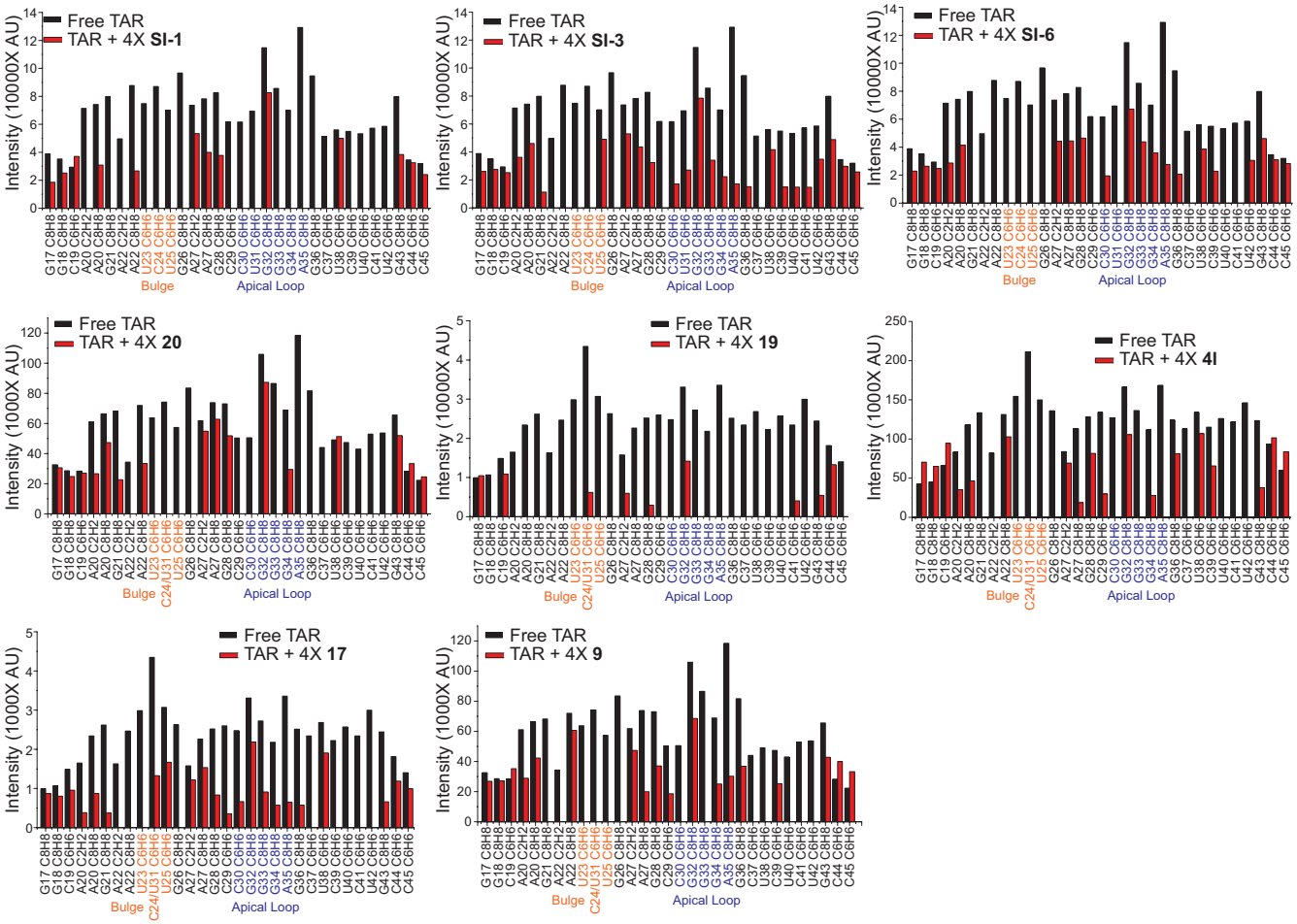


Figure S2: Comparison of peak intensities between Free TAR (black) and TAR in the presence of 4X excess molecule (red) for the eight DMA analogs that induced significant broadening to the TAR spectrum. Resonance intensities were measured in SPARKY (citation in experimental) from the ^{13}C-^{1}H SOFAST-HMQC NMR experiment.

8. Heat maps of perturbations to TAR SOFAST NMR spectra –

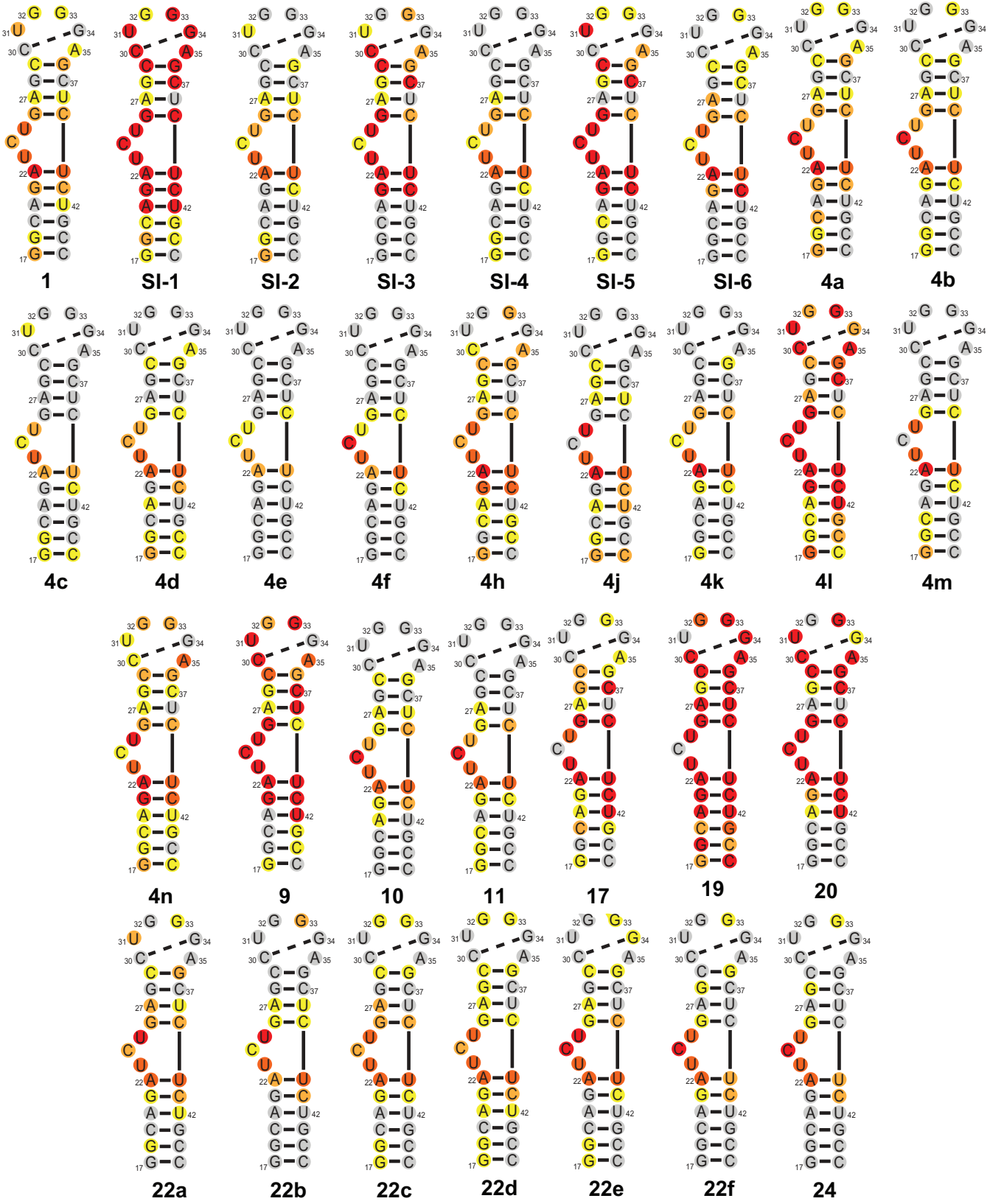


Figure S3: Heat maps showing the magnitude of chemical shift perturbations in the TAR aromatic spectrum between free TAR and DMA-bound TAR for each DMA analog.

9. Vector plots of magnitude and directions of perturbations to select TAR nucleotides by amilorides

Vector plots showing change in magnitude and direction of chemical shift perturbations in the TAR aromatic spectrum between free TAR and DMA-bound TAR for each DMA analog. The peaks listed under “Peak Missing” could not be identified in the DMA-bound spectra due to broadening or too large of a chemical shift change. Perturbations less than 0.25 ppm are considered negligible and are not included in the plot.

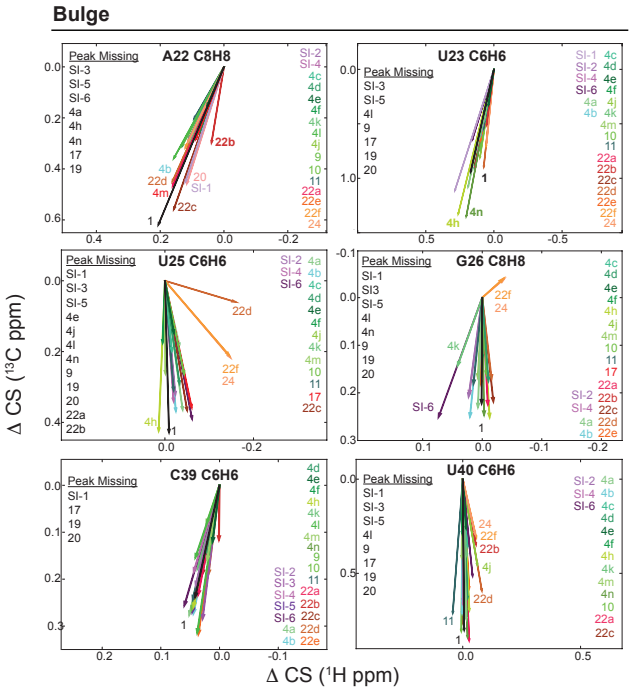


Figure S4: Vector plots for perturbations caused to signals for nucleotide residues in the bulge of HIV-1-TAR.

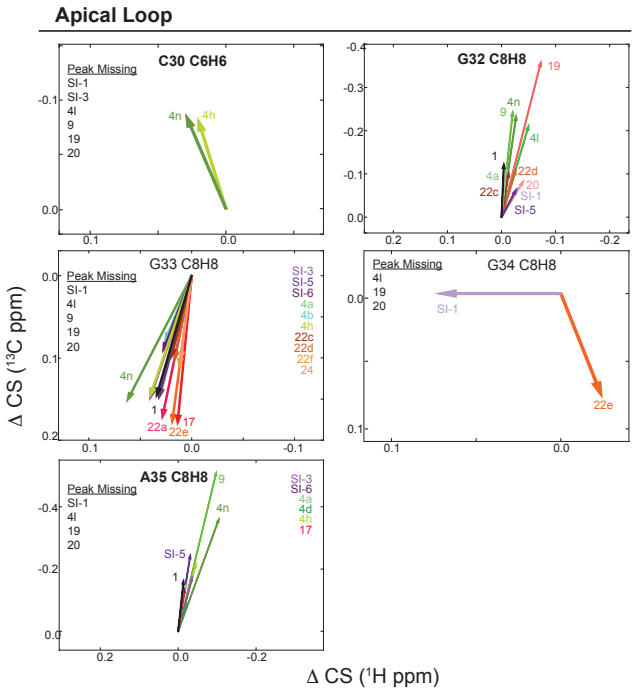


Figure S5: Vector plots for perturbations caused to signals for nucleotide residues in the apical loop of HIV-1-TAR.

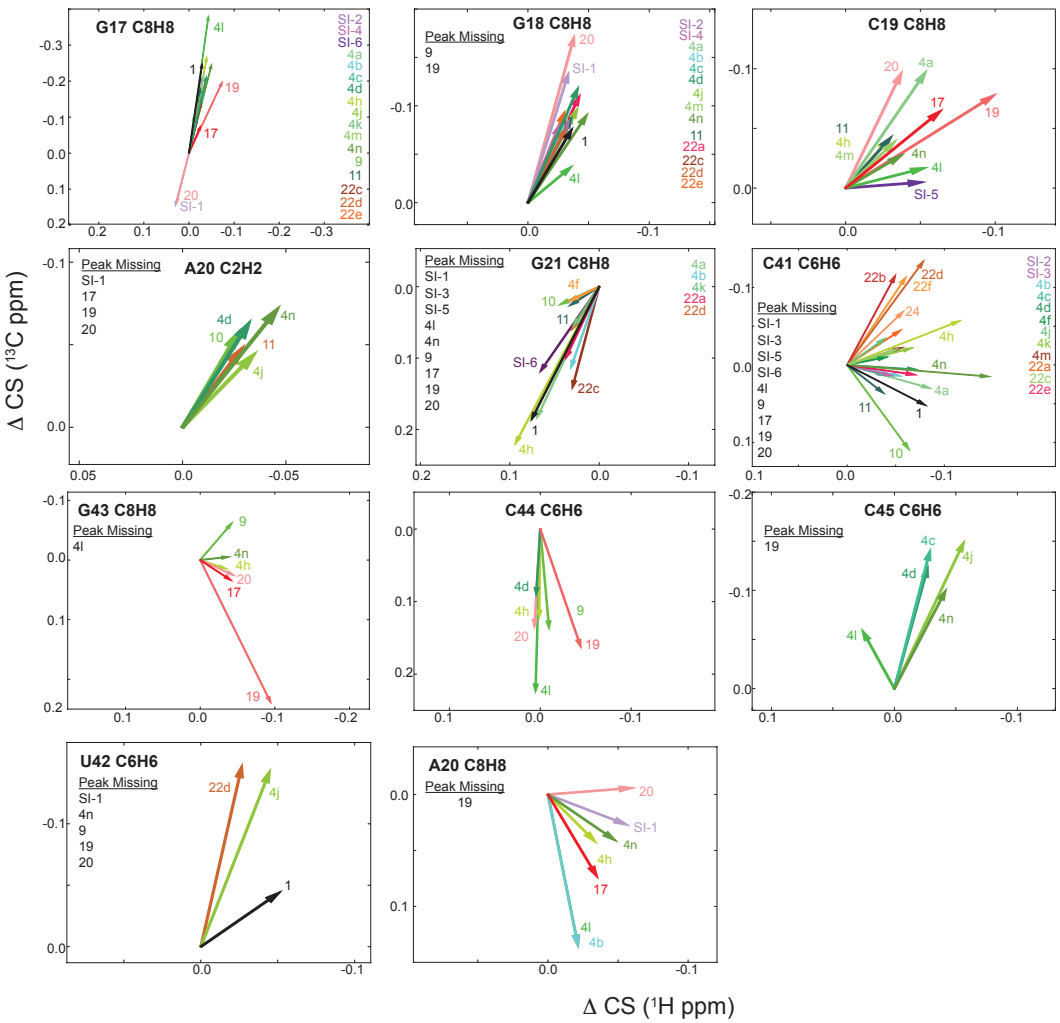


Figure S6: Vector plots for perturbations caused to signals for nucleotide residues in the lower helix of HIV-1-TAR.

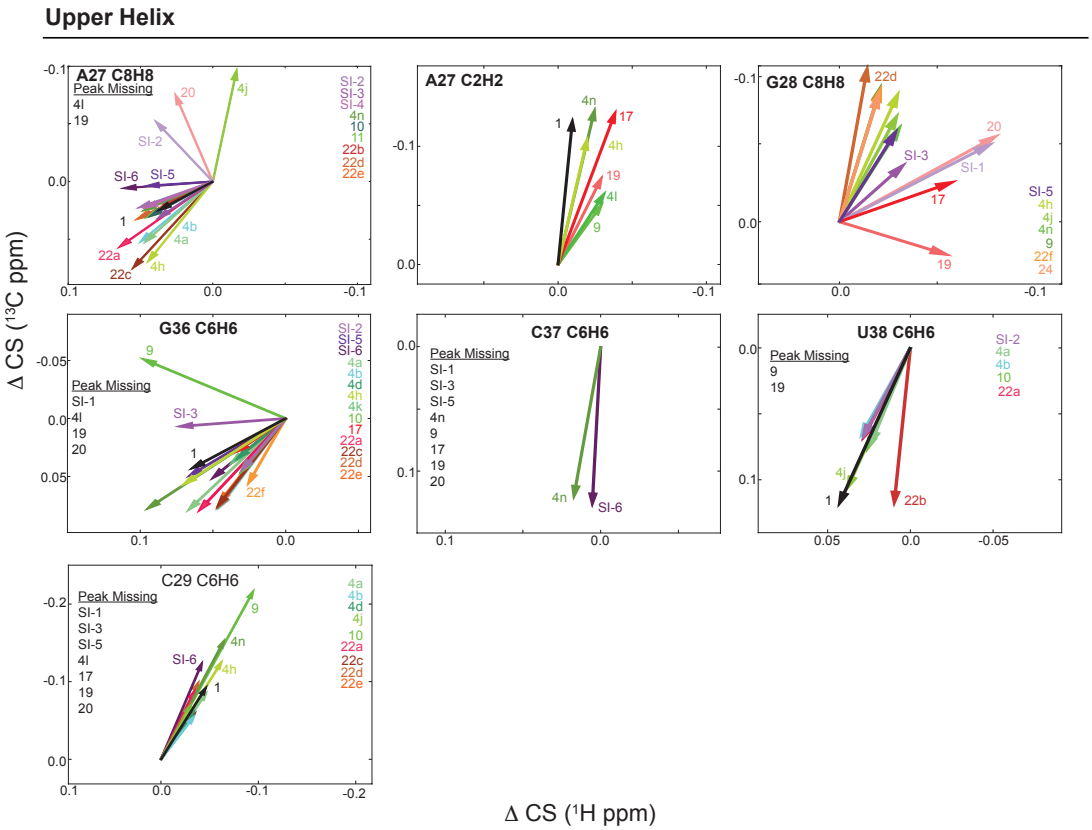


Figure S7: Vector plots for perturbations caused to signals for nucleotide residues in the upper helix of HIV-1-TAR.

10. Dynamic light scattering (DLS) experiments-

To assess aggregation in assay conditions, dynamic light scattering experiments were performed.[13, 14] All measurements were performed on a Wyatt Dynapro Nanostar instrument using a 659 nm/100 mW laser at 100% power and a 90° detection angle at 25 °C. All measurements were performed in a 50 mM phosphate buffer with 4 mM $MgCl_2$ and 0.5 mM EDTA at pH 7.3. Wild type HIV-1 TAR RNA was purchased from Dharmacon as a solid and dissolved in DEPC treated water to yield a 1mM stock solution, which was annealed by heating to 90 ºC and snap cooling on ice for 2 hours. This stock solution was further diluted to make the 75 nM and 50 μM solutions using the above mentioned buffer to be used in the experiments. Stock solutions of amiloride analogs in DMSO (50 mM) were diluted to 1mM concentration also using above buffer. Acquisition times of 10s were collected in 20 replicates, and analyzed using the cumulant fit tool in the Dynamics (6.11.1.3) software with phosphate buffer as the referenced solvent.

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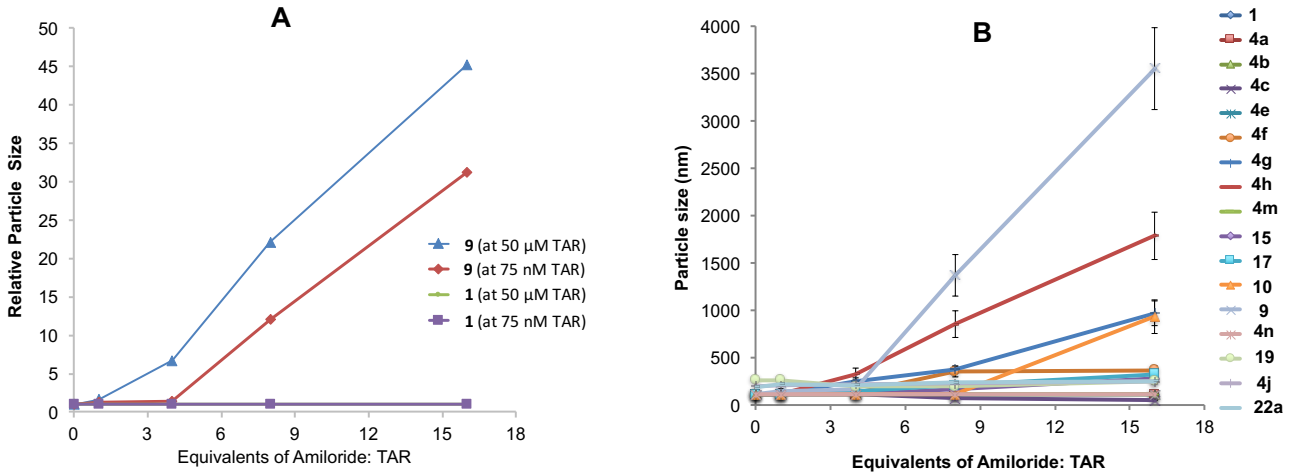

Figure S8: Change in particle sizes upon treatment of TAR with select amiloride derivatives as measured by dynamic light scattering. A. Results for screening of a representative set of amilorides vs TAR - Conditions: 75 nM *TAR,* (1, 4, 8, 16) × DMA; Buffer: 10 mM Na_2HPO_4, 0.1 mM EDTA, 50 mM KCl, pH = 7.3.; B. Results for DLS experiments at two different RNA concentrations (75 nM and 50 μM) with DMA-1 and DMA-148

As seen in figure S8 above, the molecules were first analyzed at concentrations ranging from 75 nM to 800 μM. Only two molecules resulted in detectable scattering signals: **4c**, which displayed no activity in the displacement assay but binding by NMR, and **10**, which displayed interference in the displacement assay. Measurements were then made at four different small molecule:TAR ratios: 01:1, 4:1, 8:1, and 16:1, at a concentration similar to that used in the displacement assay (75 nm). While particles of 80 nM size were observed in TAR-only solutions, most DMA analogs demonstrated no increase in particle size or count. Compound **9**, which displayed broadening by NMR, showed a 30-fold increase in particle size, whereas **4h**, showed similar albeit smaller particle size change, which indicated that the observed large perturbations in the NMR assays, and potent displacement activity of this compound may have resulted from aggregation. Finally, **4g**, which was observed to be inactive in the displacement assay and have weak binding in the NMR and ITC experiments, as well as **10**, which showed interference in the displacement assay and weak perturbations in the NMR experiments, both showed increase in particle size at 1:8 and 1:16 ratios. Furthermore, at the high concentrations of TAR used in the NMR assays (50 μM), most amiloride derivatives showed no increase in particle size whereas **9** showed similar 30-fold increase in particle size. Interestingly, at both the low (75 nM) and high (50 μM) concentrations of TAR, the same particle size of 80 nm was observed, consistent with the formation of discrete aggregates. We are currently in the process of de-convoluting this observation but can conclude that presence of large aggregates is likely not responsible for the NMR signal broadening.

Section C. Docking:

11. Procedure for docking of amilorides to TAR conformers:

Docking runs were conducted by using the program Internal Coordinate Mechanics (ICM, Molsoft) v. ICM-3.8,[15] to evaluate the interaction energies of the set of amiloride derivatives against the ensemble of 20 TAR RNA conformers previously reported.[16] Each TAR conformer was uploaded to ICM in PDB format and converted to an ICM object, including optimizing hydrogens. Binding pockets for each conformer were identified using the PocketFinder module of ICM using a tolerance value of 4.6. All atoms within 5 Å of the predicted binding pockets were used to generate receptor maps and atom occupancy was weighted. The 2D structures of all amiloride derivatives were generated in ChemDraw Professional (15.1.). The guanidine groups were protonated (pKa ~8.7 based on literature evidence as discussed in the text), and basic nitrogens on substituents at C5 positions were also protonated where appropriate. These structures were uploaded to ICM in SDF file format and converted to 3D structures. The ligand is allowed to be fully flexible during docking, while the TAR receptors remain rigid. Docking was run with a thoroughness value of 10, flexible ring sampling level 2, and covalent geometry relaxed. Each ligand: conformer complex was assigned an ICM score that is representative of a binding free energy (kcal/mol). These scores were used to calculate the fractional population of all 20 conformers for each ligand using the Boltzmann distribution, $P = \frac{e^{-\Delta G_i/RT}}{\sum_{i=1}^{i} e^{-\Delta G_i/RT}}$, where P is the population of conformer i, ΔG is the binding free energy given by the docking score, R is the gas constant and T is the temperature (298K). A population-weighted average score was given as the final score for each ligand.

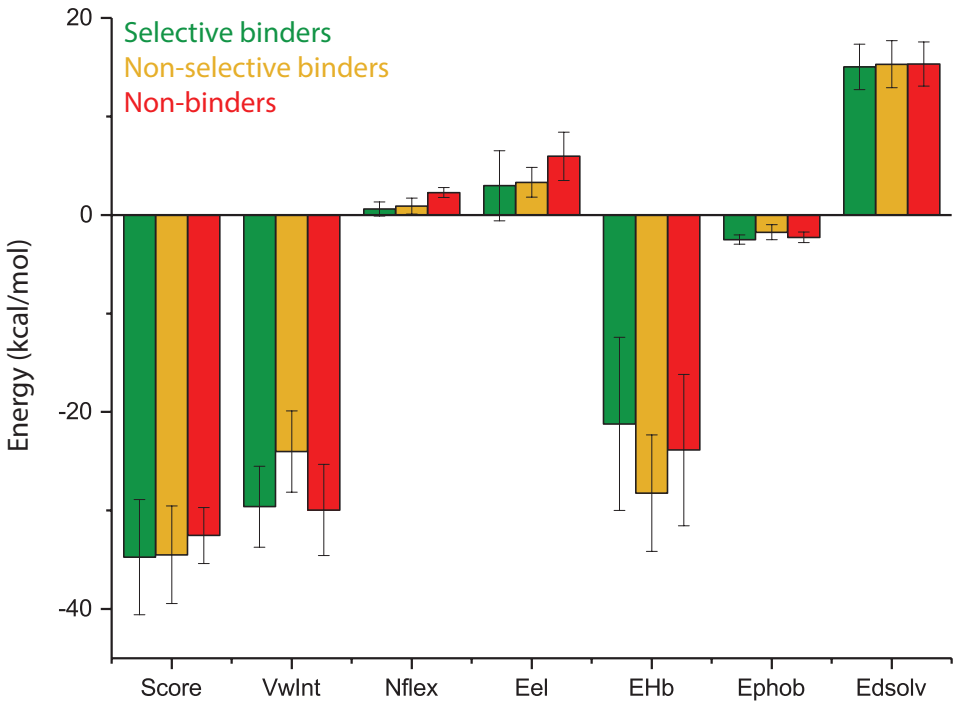

Figure S9: Correlation of components of scoring function with amiloride derivative selectivity performance in Tat displacement assay Average docking predicted energy terms for the preferred poses (>25% populated) of selective TAR binders (group A, green), non-selective TAR binders (group B, yellow) and non-binders (group D, red). The score is equal to the sum of the individual energy terms: van der Waals (VwInt), number of rotatable bonds (Nflex), solvation electrostatics (Eel), hydrogen bonding (EHb), hydrophobic energy of exposing the surface to water (Ephob), and the desolvation of exposed hydrogen bond donors and acceptors (Edsolv). Error bars represent the standard deviation over all ligands in the specified group.

Table S2: ICM Docking scores of amiloride derivatives.

Amiloride Derivative	Boltzmann-weighted binding scores (kcal•mol^{-1})
1	-35.56782313
4a	-27.841998
4b	-31.349704
4c	-29.73672976
4d	-35.97159142
4e	-29.24569534
4f	-29.37329605
4g	-33.62527575
4h	-42.9942
4i	-36.33301053
4j	-38.02843844
4k	-32.43513072
4l	-41.35554419
4m	-35.90501215
4n	-33.6490109
9	-34.1002509
10	-41.32299994
11	-30.54097496
14	-33.09232398
17	-40.17249638
19	-35.52643949
20	-31.99297286
22a	-28.98598285
22b	-36.83502557
22c	-31.76839435
22d	-34.85600707
22e	-31.58446004
22f	-29.12839243
24	-50.5804

Section D: Cheminformatics:

Cheminformatics were determined using the Wenderski-Tan parameters as calculated by IJchem (ChemAxon).[17] Tanimoto coefficients were calculated using Openbabel 2.3.2,[18] while adherence to the Lipinski's rules was predicted for each amiloride derivative using the MolProp TK function of OpenEye. AlogPS v.2.1 was used to determine logP and logS of the neutral compounds. Charges were determined using the pKa prediction tool from MarvinView (ChemAxon), and taking the species predicted to be have the greatest abundance at pH 7.4. PCA and LDA analyses on the cheminformatics parameters was conducted using XlStat, a plugin for Microsoft Excel. Statistical significance was determined using the multiple t-test algorithm in GraphPad Prism 6. (**SI11-14**). None of the 34 derivatives were identified as Pan Assay Interference (PAINS) compounds using the online PAINS analysis tool.[19]

12. Tanimoto coefficients

Tanimoto Coefficients were calculated using the fingerprint method from OpenBabel. To produce the heatmap of Tanimoto coefficients for all vs. all small molecules, the SDF file of each DMA derivative generated during docking (see pages S124-S125) was used as a Tanimoto reference, and the coefficient calculated for every other DMA derivative.

Table S3: Tanimoto coefficients for amiloride derivatives

	1	**4a**	**4b**	**4c**	**11**	**20**	**19**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**	**4k**	**4l**	**4m**	**15**	**22a**	**17**	**10**	**9**	**22b**	**22c**	**4n**	**22d**	**22e**	**22f**	**24**
1	1.000	0.721	0.895	0.776	0.669	0.854	0.523	0.816	0.760	0.782	0.841	0.694	0.669	0.745	0.721	0.707	0.590	0.628	0.591	0.755	0.822	0.607	0.591	0.841	0.623	0.615	0.587	0.474
4a	0.854	1.000	0.963	0.845	0.812	0.663	0.615	0.797	0.840	0.902	0.832	0.758	0.731	0.755	0.786	0.816	0.614	0.564	0.535	0.753	0.868	0.548	0.535	0.832	0.561	0.554	0.559	0.518
4b	0.822	0.963	1.000	0.817	0.645	0.593	0.474	0.771	0.825	0.884	0.804	0.735	0.710	0.742	0.762	0.825	0.607	0.548	0.528	0.730	0.837	0.532	0.520	0.804	0.544	0.538	0.543	0.505
4c	0.776	0.845	0.817	1.000	0.960	0.689	0.559	0.836	0.773	0.781	0.752	0.894	0.861	0.791	0.778	0.744	0.663	0.595	0.565	0.768	0.792	0.578	0.565	0.752	0.592	0.585	0.589	0.624
4d	0.745	0.812	0.786	0.960	1.000	0.667	0.537	0.804	0.746	0.753	0.724	0.861	0.831	0.817	0.751	0.719	0.644	0.575	0.546	0.741	0.761	0.559	0.546	0.724	0.571	0.565	0.614	0.605
11	0.669	0.663	0.645	0.689	0.667	1.000	0.482	0.447	0.716	0.634	0.638	0.713	0.772	0.635	0.667	0.633	0.607	0.580	0.516	0.500	0.739	0.747	0.503	0.713	0.513	0.508	0.498	0.470
20	0.721	0.615	0.593	0.559	0.537	0.482	1.000	0.656	0.588	0.548	0.563	0.606	0.500	0.482	0.635	0.510	0.426	0.640	0.597	0.544	0.645	0.615	0.597	0.606	0.635	0.625	0.593	0.465
19	0.523	0.474	0.469	0.506	0.489	0.447	0.656	1.000	0.518	0.441	0.443	0.468	0.469	0.447	0.481	0.422	0.614	0.910	0.494	0.482	0.595	0.590	0.468	0.610	0.603	0.577	0.477	
4e	0.816	0.797	0.771	0.836	0.804	0.716	0.588	0.518	1.000	0.741	1.000	0.741	0.738	0.746	0.787	0.751	0.746	0.913	0.705	0.754	0.772	0.598	0.514	0.497	0.684	0.788	0.516	0.505	0.748	0.511	0.522	0.511	0.486	
4f	0.760	0.840	0.825	0.773	0.746	0.634	0.548	0.441	0.741	1.000	0.823	0.738	0.787	0.748	0.700	0.677	0.705	0.754	0.772	0.598	0.514	0.497	0.684	0.788	0.516	0.505	0.748	0.511	0.522	0.511	0.486			
4g	0.782	0.902	0.884	0.781	0.753	0.638	0.563	0.443	0.738	0.823	1.000	0.779	0.716	0.683	0.702	0.731	0.749	0.594	0.526	0.500	0.710	0.810	0.511	0.500	0.779	0.523	0.517	0.522	0.488					
4h	0.841	0.832	0.804	0.752	0.724	0.713	0.606	0.468	0.787	0.748	0.779	1.000	0.678	0.656	0.724	0.723	0.690	0.592	0.548	0.529	0.872	0.939	0.532	0.520	1.000	0.545	0.538	0.526	0.469					
4i	0.694	0.758	0.735	0.894	0.861	0.772	0.500	0.469	0.751	0.700	0.678	1.000	0.791	0.717	0.707	0.677	0.619	0.541	0.523	0.696	0.711	0.526	0.515	0.678	0.538	0.532	0.536	0.581						
4j	0.669	0.731	0.710	0.861	0.831	0.635	0.482	0.447	0.746	0.677	0.656	0.791	1.000	0.712	0.749	0.656	0.609	0.524	0.500	0.701	0.686	0.510	0.500	0.656	0.529	0.515	0.520	0.742						
4k	0.745	0.755	0.742	0.791	0.817	0.687	0.537	0.481	0.913	0.705	0.724	0.717	0.712	1.000	0.712	0.672	0.660	0.566	0.538	0.794	0.761	0.559	0.546	0.724	0.563	0.565	0.605	0.521						
4l	0.721	0.786	0.762	0.778	0.751	0.633	0.519	0.453	0.747	0.754	0.731	0.723	0.707	0.749	0.712	1.000	0.690	0.576	0.525	0.508	0.701	0.748	0.527	0.532	0.723	0.522	0.533	0.545						
4m	0.707	0.816	0.825	0.744	0.719	0.607	0.510	0.438	0.703	0.772	0.749	0.690	0.677	0.656	0.690	1.000	0.590	0.500	0.492	0.652	0.724	0.495	0.477	0.690	0.497	0.492	0.497	0.475						
15	0.590	0.614	0.607	0.663	0.644	0.580	0.426	0.640	0.597	0.544	0.645	0.592	0.548	0.529	0.500	0.492	0.652	0.724	0.495	0.465	0.962	0.660	0.457	0.592	0.474	0.470	0.462	0.463						
22a	0.628	0.564	0.548	0.595	0.575	0.516	0.422	0.614	0.910	0.494	0.482	0.595	0.590	0.468	0.610	0.477	0.457	0.933	0.654	0.544	0.545	0.941	1.000	0.520	0.926	0.955	0.868	0.691						
17	0.591	0.535	0.520	0.565	0.546	0.493	0.597	0.590	0.588	0.602	0.516	0.511	0.532	0.526	0.515	0.495	0.465	0.962	0.660	0.556	0.558	1.000	0.941	1.000	0.532	0.954	0.985	0.706						
10	0.755	0.753	0.730	0.768	0.739	0.544	0.494	0.862	0.684	0.710	0.872	0.939	0.872	0.794	0.701	0.652	0.634	0.572	0.552	1.000	0.844	0.556	0.544	0.872	0.569	0.563	0.549	0.512						
9	0.822	0.868	0.837	0.792	0.761	0.747	0.645	0.482	0.831	0.788	0.810	0.939	0.711	0.686	0.761	0.748	0.724	0.608	0.576	0.545	0.844	1.000	0.558	0.545	0.941	1.000	0.532	0.954	0.985	0.893	0.706			
22b	0.607	0.548	0.532	0.578	0.559	0.503	0.615	0.595	0.602	0.516	0.511	0.532	0.526	0.510	0.559	0.527	0.495	0.465	0.962	0.660	0.556	0.558	1.000	0.941	1.000	0.520	0.926	0.955	0.985	0.868	0.691			
22c	0.591	0.535	0.520	0.565	0.546	0.493	0.597	0.590	0.588	0.505	0.500	0.520	0.515	0.500	0.546	0.532	0.477	0.457	0.933	0.654	0.544	0.545	0.941	1.000	0.520	0.926	0.955	0.868	0.691					
4n	0.841	0.832	0.804	0.752	0.724	0.713	0.606	0.468	0.787	0.748	0.779	1.000	0.678	0.656	0.724	0.723	0.690	0.592	0.548	0.529	0.872	0.939	0.532	0.520	1.000	0.545	0.538	0.526	0.469					
22d	0.623	0.561	0.544	0.592	0.571	0.513	0.635	0.610	0.607	0.511	0.523	0.545	0.538	0.529	0.563	0.522	0.497	0.474	0.992	0.677	0.569	0.572	0.954	0.926	0.545	1.000	0.969	0.919	0.733					
22e	0.615	0.554	0.538	0.585	0.565	0.508	0.625	0.603	0.610	0.522	0.517	0.538	0.532	0.515	0.565	0.533	0.492	0.470	0.977	0.669	0.563	0.565	0.985	0.955	0.538	0.969	1.000	0.906	0.714					
22f	0.587	0.559	0.543	0.589	0.614	0.498	0.593	0.577	0.585	0.511	0.522	0.526	0.536	0.520	0.605	0.521	0.497	0.462	0.926	0.640	0.549	0.551	0.893	0.868	0.526	0.919	0.906	1.000	0.715					
24	0.474	0.518	0.505	0.624	0.605	0.470	0.465	0.477	0.540	0.486	0.488	0.469	0.581	0.742	0.521	0.545	0.475	0.463	0.727	0.530	0.512	0.487	0.706	0.691	0.469	0.733	0.714	0.715	1.000					

Table S4: Wanderski-Tan cheminformatic parameters used to classify amiloride derivatives

Comp No.	Class (NMR)	Class (CD50)	MW	logD	HBA	HBD	RotB	tPSA	nStereo	nStereo/MW	Fsp3	relPSA	N	O	Rings	ArRings	RngLg	SysRings	SysRR*	VWSA	logP	logS	Charge
1	Bind	Selective	257.68	0.05	8	3	2	136.51	0	0.00	0.25	0.42	7	1	1	1	6	1	1.00	327.86	-0.02	-2.43	
4a	Bind	Non-selective	283.72	0.45	8	3	2	136.51	0	0.00	0.40	0.38	7	1	2	1	6	2	1.00	361.84	0.8	-2.65	0
4b	Bind	Interference	297.75	0.90	8	3	2	136.51	0	0.00	0.45	0.35	7	1	2	1	6	2	1.00	389.81	1.31	-2.82	0
4c	Bind	Interference	333.78	1.42	8	4	5	145.30	0	0.00	0.14	0.34	7	1	2	2	6	2	1.00	429.23	1.87	-3.6	0
4d	Bind	Non-selective	351.77	1.57	8	4	5	145.30	0	0.00	0.14	0.33	7	1	2	2	6	2	1.00	436.60	1.91	-3.91	0
11	Bind	Interference	426.88	0.56	10	5	6	191.47	0	0.00	0.20	0.35	8	3	2	2	6	2	1.00	545.81	0.77	-3.68	0
20	Bind	Non-selective	195.19	-1.71	8	4	1	159.29	0	0.00	0.00	0.66	7	1	1	1	6	1	1.00	240.25	-1.54	-1.81	0
19	Bind	Selective	295.31	0.79	8	4	3	159.29	0	0.00	0.00	0.43	7	1	2	2	6	2	1.00	366.30	-1.08	-2.21	0
4e	Bind	Interference	319.75	1.14	8	4	4	145.30	0	0.00	0.08	0.37	7	1	2	2	6	2	1.00	397.86	0.68	-3.96	0
4f	Bind	Interference	337.77	-0.69	9	4	6	163.12	0	0.00	0.25	0.38	9	1	2	2	6	2	1.00	431.58	1.53	-3.37	0
4g	No Bind	None	354.80	-1.06	9	4	6	165.61	0	0.00	0.46	0.35	8	2	2	1	6	2	1.00	469.49	0.83	-2.81	0
4h	Bind	Non-selective	342.81	-2.91	9	5	5	165.15	0	0.00	0.50	0.36	9	1	2	1	6	2	1.00	464.55	0.53	-3.15	1
4i	No Bind	None	412.85	0.03	10	5	6	205.46	0	0.00	0.14	0.40	8	3	2	2	6	2	1.00	510.53	-0.16	-2.96	0
4j	Bind	Selective	372.82	1.52	8	5	5	161.09	0	0.00	0.13	0.34	8	1	3	3	6	2	1.50	469.14	1.14	-3.61	0
4k	Bind	Interference	355.73	1.42	8	4	4	145.30	0	0.00	0.08	0.35	7	1	2	2	6	2	1.00	411.74	2.3	-4.29	0
4l	Bind	Non-selective	373.81	0.71	9	5	5	173.98	0	0.00	0.13	0.38	9	1	3	3	6	2	1.50	460.96	1.78	-3.93	0
4m	Bind	Non-selective	313.75	-0.17	9	4	4	154.53	1	0.00	0.45	0.39	7	2	2	1	6	2	1.00	401.09	1.75	-3.94	0
15	No Bind	None	400.83	0.99	10	4	6	176.01	0	0.00	0.13	0.35	10	1	3	3	6	3	1.00	499.16	0.54	-2.95	0
22a	Bind	Selective	299.34	1.26	8	3	3	136.51	0	0.00	0.14	0.33	7	1	2	2	6	2	1.00	419.45	1.56	-3.49	0
17	Bind	Selective	323.36	1.73	8	3	4	136.51	0	0.00	0.13	0.31	7	1	2	2	6	2	1.00	438.02	0.77	-2.96	0
10	Bind	Interference	453.95	2.47	8	5	9	149.74	0	0.00	0.18	0.24	8	1	3	3	6	3	1.00	624.20	1.35	-4.02	1
9	Bind	Non-selective	273.70	-3.36	8	5	4	172.94	0	0.00	0.25	0.50	8	1	1	1	6	1	1.00	342.60	3.05	-4.48	1
22b	Bind	Selective	375.44	2.90	8	3	4	136.51	0	0.00	0.10	0.26	7	1	3	3	6	3	1.00	527.44	-0.62	-2.63	0
22c	Bind	Selective	324.35	1.11	9	3	3	160.30	0	0.00	0.13	0.37	8	1	2	2	6	2	1.00	436.24	2.56	-4.02	0
4n	Bind	Interference	356.84	-1.25	9	5	5	152.98	0	0.00	0.54	0.31	9	1	2	1	6	2	1.00	500.32	0.67	-3.39	1
22d	Bind	Selective	349.40	2.24	8	3	3	136.51	0	0.00	0.11	0.28	7	1	3	3	6	2	1.50	482.01	-1.1	-3.08	0
22e	Bind	Selective	313.37	1.77	8	3	3	136.51	0	0.00	0.20	0.30	7	1	2	2	6	2	1.00	451.67	0.64	-3.21	0
22f	Bind	Selective	317.33	1.40	8	3	3	136.51	0	0.00	0.14	0.32	7	1	2	2	6	2	1.00	426.25	0.92	-3.34	0
24	Bind	Selective	414.47	2.73	8	5	6	161.09	0	0.00	0.09	0.29	8	1	4	4	6	3	1.33	559.04	3.05	-4.79	0

13. Cheminformatic parameters

The Wenderski-Tan cheminformatic parameters were used to classify each of the small molecules. The protonation state of each molecule was determined using Marvinview's pKa plugin; the most common species at pH 7.4 was used to determine cheminformatic parameters. Then an SDF file containing all of the small molecule structures was imported to an Instant Jchem database. 18 cheminformatic parameters were calculated using the IJchem built-in Chemical Terms calculators. The remaining two parameters, logP and logS, were calculated using the webservice AlogPS v. 2.1; since logP and logS concern neutral molecules, these values used the neutral state rather than the charged states used for the 18 IJchem parameters. PCA analysis of cheminformatic parameters was conducted using XLSTAT plugin for Excel.

14. Correlation matrices

Table S5: Results of multiple t-tests between selective and non-selective binding groups

	P value	Mean - Selective	Mean - Non-Selective	Difference	SE of difference	t ratio
logD	0.001462	1.745	-0.774286	2.51929	0.648257	3.88625
ArRings	0.010179	2.5	1.42857	1.07143	0.364682	2.93798
relPSA	0.019623	0.323	0.428571	-0.105571	0.0404172	2.61204
Fsp3	0.027941	0.117	0.267143	-0.150143	0.0617028	2.43332
SysRings	0.044446	2.2	1.71429	0.485714	0.221436	2.19347
VWSA	0.046824	457.556	386.841	70.7146	32.6464	2.16608
HBD	0.068881	3.5	4.28571	-0.785714	0.400934	1.95971
tPSA	0.077518	146.083	158.243	-12.1599	6.41646	1.8951
Rings	0.082215	2.5	1.85714	0.642857	0.345131	1.86264
HBA	0.131099	8.1	8.42857	-0.328571	0.205734	1.59707
MW	0.180574	338.519	304.964	33.5547	23.893	1.40438
nStereo	0.244007	0	0.142857	-0.142857	0.117803	1.21268
O	0.244007	1	1.14286	-0.142857	0.117803	1.21268
N	0.253830	7.3	7.71429	-0.414286	0.349129	1.18663
SysRR	0.556221	1.133	1.07143	0.0615714	0.102293	0.601913
logP	0.580132	0.784	1.18286	-0.398857	0.705388	0.565444
logS	0.851402	-3.334	-3.41	0.0760001	0.398761	0.19059
RotB	0.982565	3.7	3.71429	-0.0142857	0.642899	0.0222208

15. LDA results

LDA plots of cheminformatic data were constructed using the Discriminatory Analysis plugin from XLSTAT.

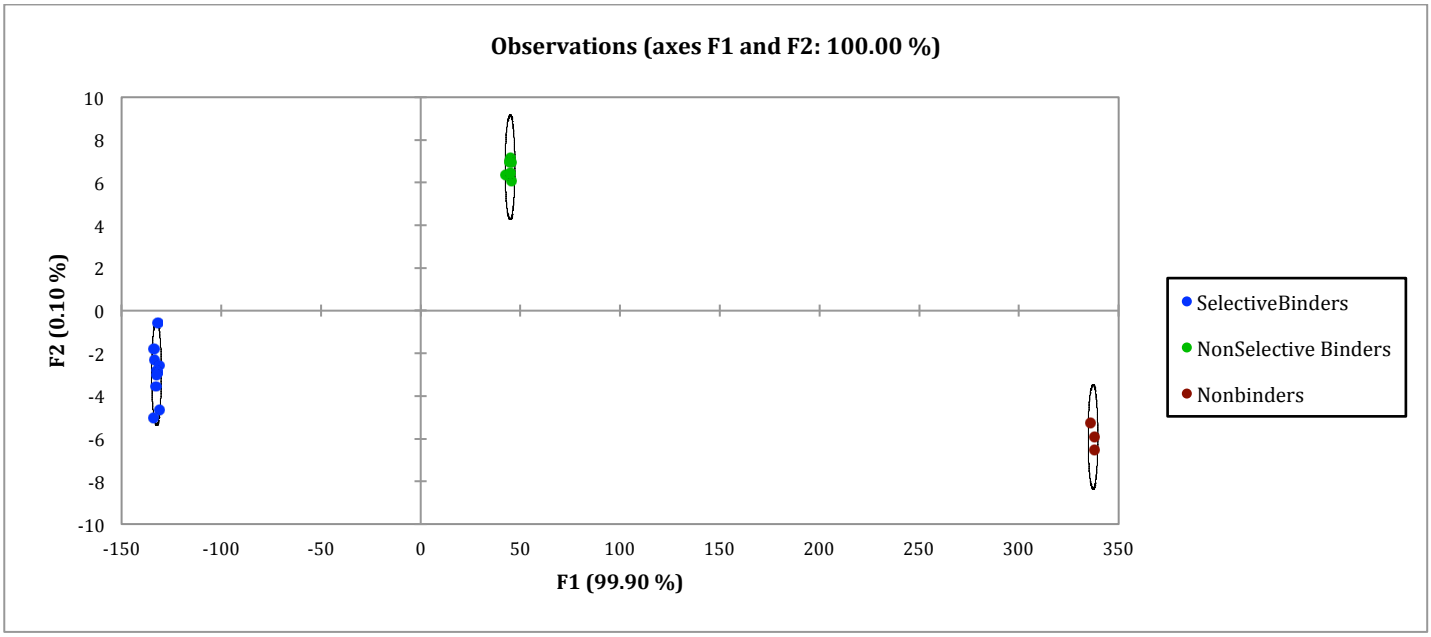

Figure S10: LDA analysis of Non-selective, Selective, and Nonbinding DMA derivatives. The 3 major binding categories show major separation in F1 and minor separation in F2.

Table S6: Confusion matrix for estimated sample

from \ to	1	2	4	Total	% correct
Selective	10	0	0	10	100.00%
NonSelective	0	7	0	7	100.00%
Nonbinding	0	0	3	3	100.00%
Total	10	7	3	20	100.00%

Table S7: Confusion matrix for cross-validation

from \ to	1	2	4	Total	% correct
Selective	7	1	2	10	70.00%
NonSelective	2	4	1	7	57.14%
Nonbinding	1	0	2	3	66.67%
Total	10	5	5	20	65.00%

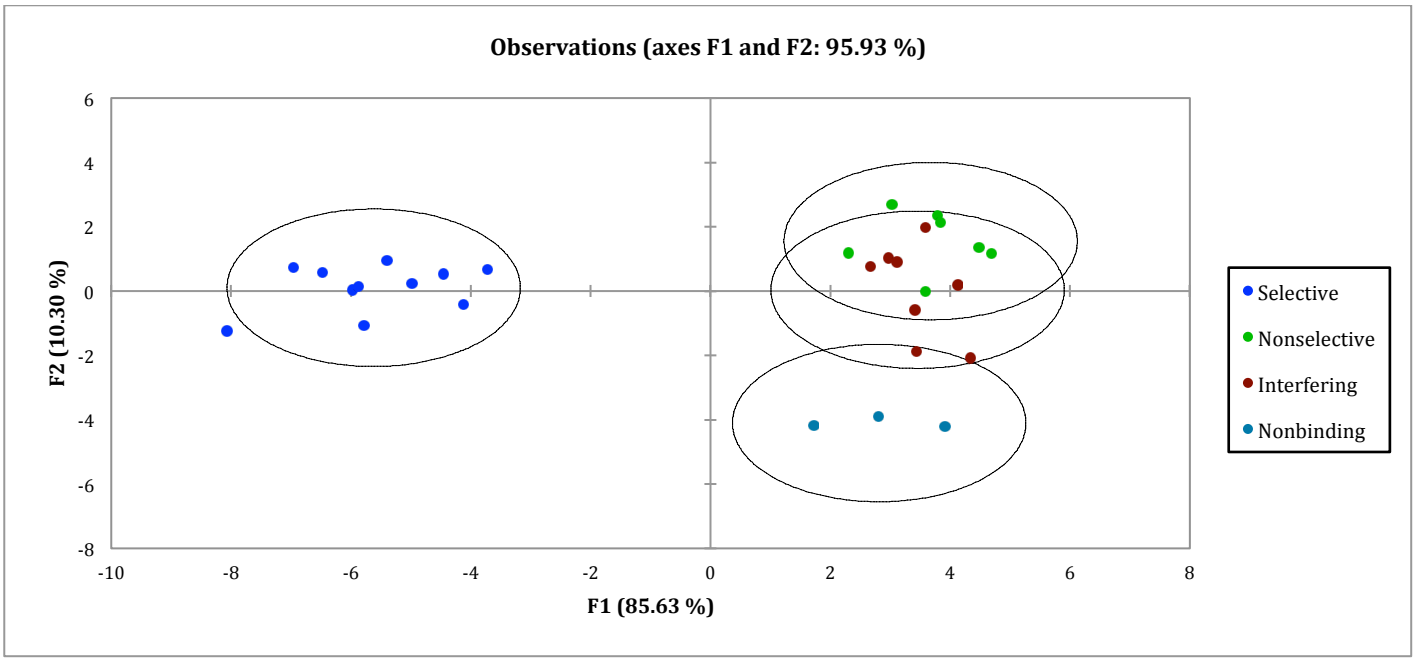

Figure S11: Addition of Interfering data shows significant overlap with the nonselective and nonbinding categories.

Table S8: Confusion matrix for estimated sample, with interfering data included.

from \ to	1	2	3	4	Total	% correct
Selective	11	0	0	0	11	100.00%
NonSelective	0	5	2	0	7	71.43%
Interfering	0	0	8	0	8	100.00%
Nonbinding	0	0	0	3	3	100.00%
Total	11	5	10	3	29	93.10%

Table S9: Confusion matrix for cross-validation, with interfering data included

from \ to	1	2	3	4	Total	% correct
Selective	7	1	2	1	11	63.64%
NonSelective	0	4	3	0	7	57.14%
Interfering	0	4	1	3	8	12.50%
Nonbinding	0	0	3	0	3	0.00%
Total	7	9	9	4	29	41.38%

16. References

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