Electronic Supplementary Information

Template-guided selection of RNA ligands using imine-based dynamic combinatorial chemistry

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1. General chemistry methods and synthesis procedures

1.1 General methods

Reagents were purchased from commercial sources and were used without further purification. All solvents were ACS-grade or better and were used without further purification. All reactions except reductive amination using Hantzsch ester were conducted under an inert atmosphere of nitrogen or argon using magnetic stirring. Reductive amination with Hantzsch ester was conducted under air with magnetic stirring. Thin layer chromatography was performed on aluminum-backed silica gel plates (F_{254}, Sigma). All purifications were performed by flash column chromatography using ultra-pure silica gel (230-400 mesh, 60 Å, Silicycle). All synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and high-resolution mass spectrometry.^{1}H and ^{13}C NMR spectra were recorded using a 500 MHz Varian or Bruker spectrometer. NMR spectra were analyzed using the MNova software. Chemical shifts (δ) are listed in ppm and were referenced to that of the solvent. Coupling constants (*J*) are listed in Hertz (Hz); multiplicity: s, singlet; d, doublet; m, multiplet. High resolution mass spectrometry (HRMS) was performed on an Agilent 6224 LCMS time-of-flight (TOF) mass spectrometer using electrospray ionization (ESI). Final compounds were further characterized by HPLC and were all found to be >93% pure at 254 nm.

1.2 Testing amine reactivity

A typical reaction was carried out using 5 mM 3-trifluoromethylaniline with 1 mM 4-hydroxybenzaldehyde in the presence of 0.25 mM 2-(4-biphenylyloxy)propionic acid as an internal standard (Figure S1A). The following stock solutions were prepared in DMSO: stock A containing 40 mM 4-hydroxybenzaldehyde (**4**) and 10 mM 2-(4-biphenylyloxy)propionic acid and stock B containing 200 mM aniline. 20 µL of stock A and 20 µL of stock B were mixed in a 1.5-mL Eppendorf tube. The solution was immediately diluted with buffer to a final volume of 800 µL, vortexed and allowed to sit at room temperature. This reaction set up was used for amines in Figure S1B. Reactive amines from this series were later confirmed to react with the amiloride aldehyde (compound **1**). Amines in Figure S1C were directly tested with the amiloride aldehyde (**1**) as they were expected to be reactive based on their HOMO energies (Table S1). After equilibration for 1.5 h, a 180-µL aliquot of the reaction was transferred to a clean Eppendorf tube. Twenty microliters of a freshly prepared 0.1 M solution of $NaBH_3CN$ in 18 MΩ water were added. After vortexing, reduction was allowed to proceed at room temperature for 24 hours. 200 µL of HPLC-grade methanol were added to the mixture. The sample was filtered into an HPLC vial using either 0.2- or 0.45 µm GHP-membrane Acrodisc® syringe filters (Life Sciences). HPLC sample analysis was performed on a Shimadzu LC system using a Phenomenex reverse phase column (Luna 5 µm C18(2) 100 Å, New Column 150 x 4.6 mm). Eluents: acetonitrile and water containing 0.1% trifluoroacetic acid. Chromatographic method: 0-1 min: 10% MeCN, 1-10 min: linear gradient to 90% MeCN, 10-14 min: hold at 90% MeCN, 14-18 min: linear gradient to 10% MeCN. Flow rate = 1 mL/mim. Chromatograms at 254 nm were integrated and the peak area corresponding to the aldehyde was normalized to the area of 2-(4-biphenylyloxy)propionic acid (internal standard) to evaluate extent of aldehyde conversion to secondary amine (see Figure S2D-E for example chromatograms). Secondary amine peaks were confirmed by comparison of the retention time and UV absorption profile to that of a synthesized standard, or by analysis of the reaction sample by LCMS on an Agilent 1100 Series instrument. Table S1 and Figure S2 show the reactivity of all amines along with the predicted HOMO energies and pK_a values. The reactivity of the amines was binary, with some amines consuming >90% of the aldehyde while others showed no reactivity.

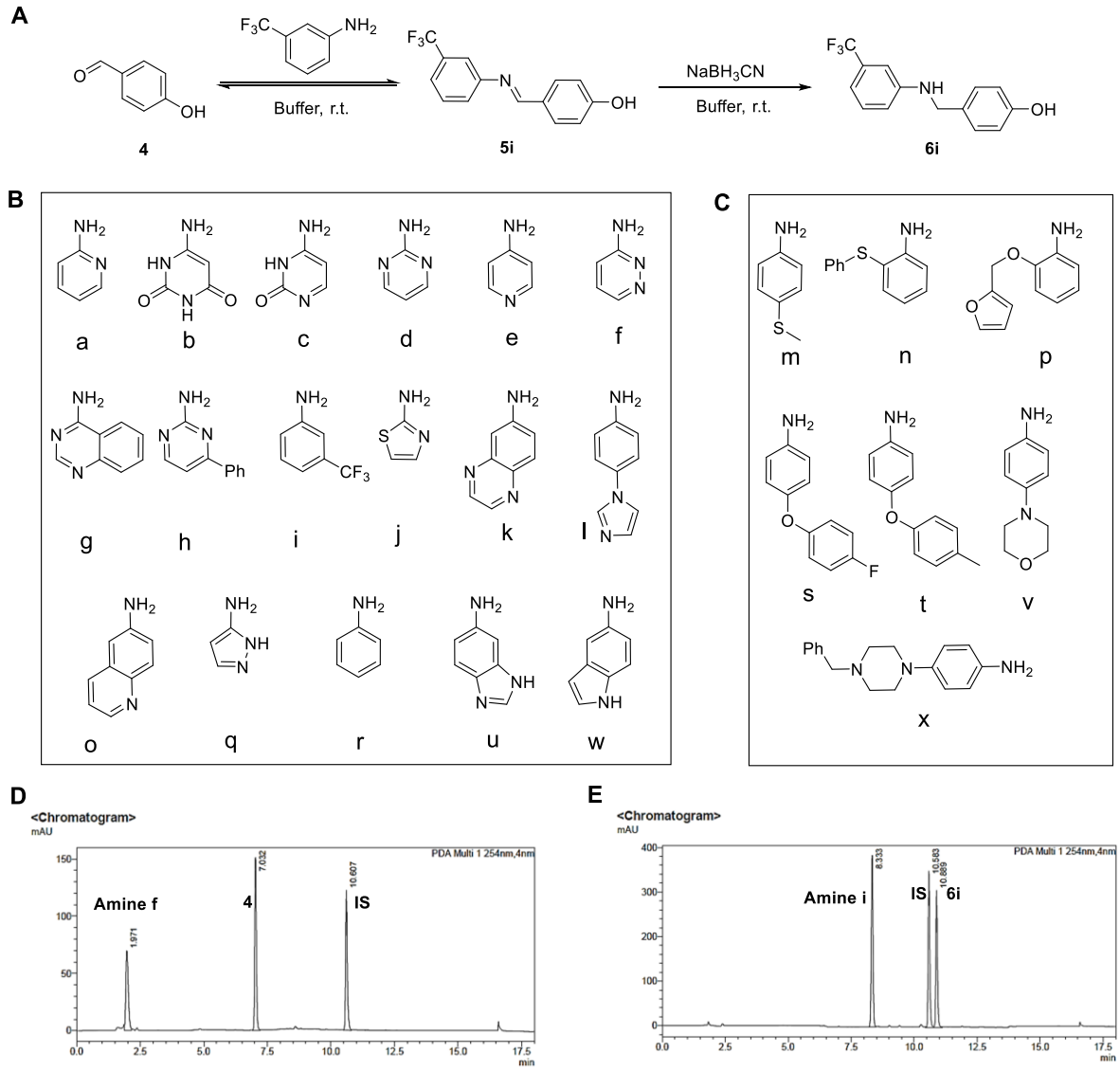


Figure S1. Testing amine reactivity. (A) Example reaction. (B) Amines tested with aldehyde **4** (Buffer: 20 mM BisTris, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 6.3). (C) Amines tested with amiloride aldehyde **1 (**Buffer: 20 mM BisTris, 25 mM NaCl, 1 mM $MgCl_2$, 0.1 mM EDTA, pH 6.3). The buffer with lower magnesium was used for these amines as it had been selected for DCC experiments to reduce potential competition for RNA binding between the positively charged library members and magnesium ions. (D) HPLC chromatogram for an unreactive amine. No secondary amine was observed. (E) HPLC chromatogram for a reactive amine. The secondary amine was observed and all the aldehyde was consumed. IS: internal standard (2-(4-biphenylyloxy)propionic acid).

Table S1. Predicted amine HOMO energies and pK_a values. HOMO energies were calculated using DFT-B3LYP 6-31G* in Spartan 18 V1.4.1 (equilibrium geometry at the ground state in the gas phase; total charge: neutral). pK_a values were calculated in the ChemAxon Marvin Suite V19.24.0 (mode: macro; temperature: 298K; tautomerization/resonance consideration: enabled).

Amine Name	Amine ID	HOMO energy (eV)	pK_a	Reactivity
2-Aminopyridine	a	-6.68	6.84	None
6-Aminouracil	b	-6.26	1.53	None
Cytosine	c	-6.18	4.76	None
2-Aminopyrimidine	d	-6.17	3.62	None
4-Aminopyridine	e	-6.11	4.95	None
Pyridazin-3-amine	f	-6.1	4.41	None

Quinazolin-4-amine	g	-6.08	4.99	None
4-Phenylpyrimidine-2-amine	h	-6.02	4.14	None
3-Trifluoromethylaniline	i	-5.84	3.23	High
Thiazol-2-amine	j	-5.71	5.09	None
6-Aminoquinoxaline	k	-5.71	3.62	High
4-(1H-imidazol-1-yl)aniline	l	-5.59	6.08	High
4-(Methylthio)aniline	m	-5.58	4.55	High
2-(Phenylthio)aniline	n	-5.54	3.32	High
6-Aminoquinoline	o	-5.46	5.15	High
2-(2-Furylmethoxy)aniline	p	-5.46	4.09	High
3-Aminopyrazole	q	-5.44	4.1	High
Aniline	r	-5.39	4.64	High
4-(4-Fluorophenoxy)aniline	s	-5.36	4.11	High
4-(4-Methylphenoxy)aniline	t	-5.29	4.32	High
5-aminobenzimidazole	u	-5.1	6.55	High
1H-indol-5-amine	v	-4.83	3.79	High
4-Morpholinoaniline	w	-4.82	5.86	High
4-(4-Benzylpiperazino)aniline	x	-4.72	8.2	High

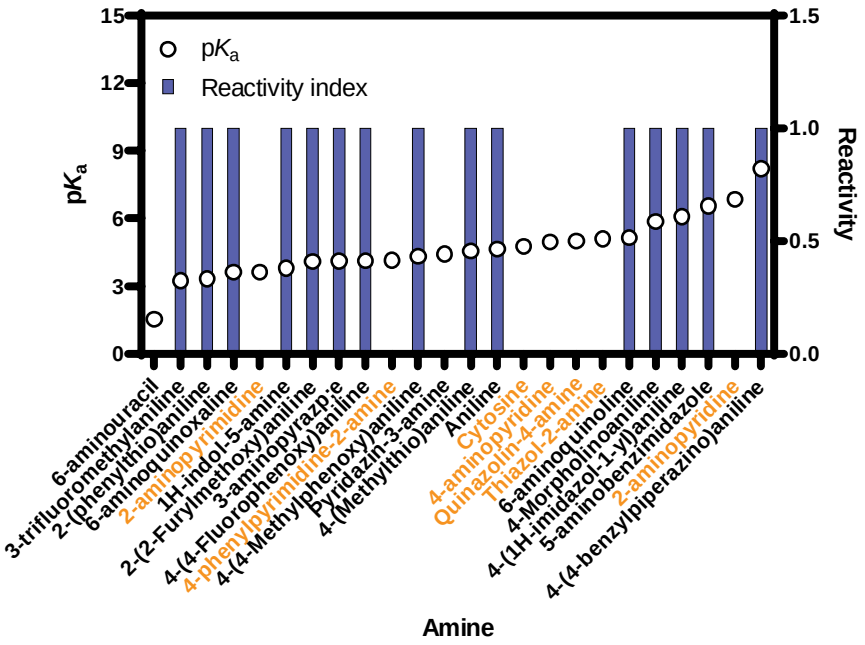


Figure S2. Graphical comparison of amine reactivity with amine pK_a values. The pKa values are not predictive of amine reactivity.

1.3 Selection of final amines for use in DCC

To select the final set of amines for DCC, we constructed a virtual amiloride library based on the 15 reactive amines (Figure S3A). We then calculated the logD of this library using ChemAxon Chemical Terms Evaluator (Marvin 16.4.11.0, 2016, http://www.chemaxon.com)[1] as proxy for expected solubility in buffer (Figure S3B). The calculations were conducted without using SMILES codes corresponding to the structures shown in Figure 3A without tautomer or protonation correction. Amines **q** – **r** were chosen based on their high predicted solubility (low logD), while amines w and o were excluded based on their high similarity **u** and **k**, respectively.

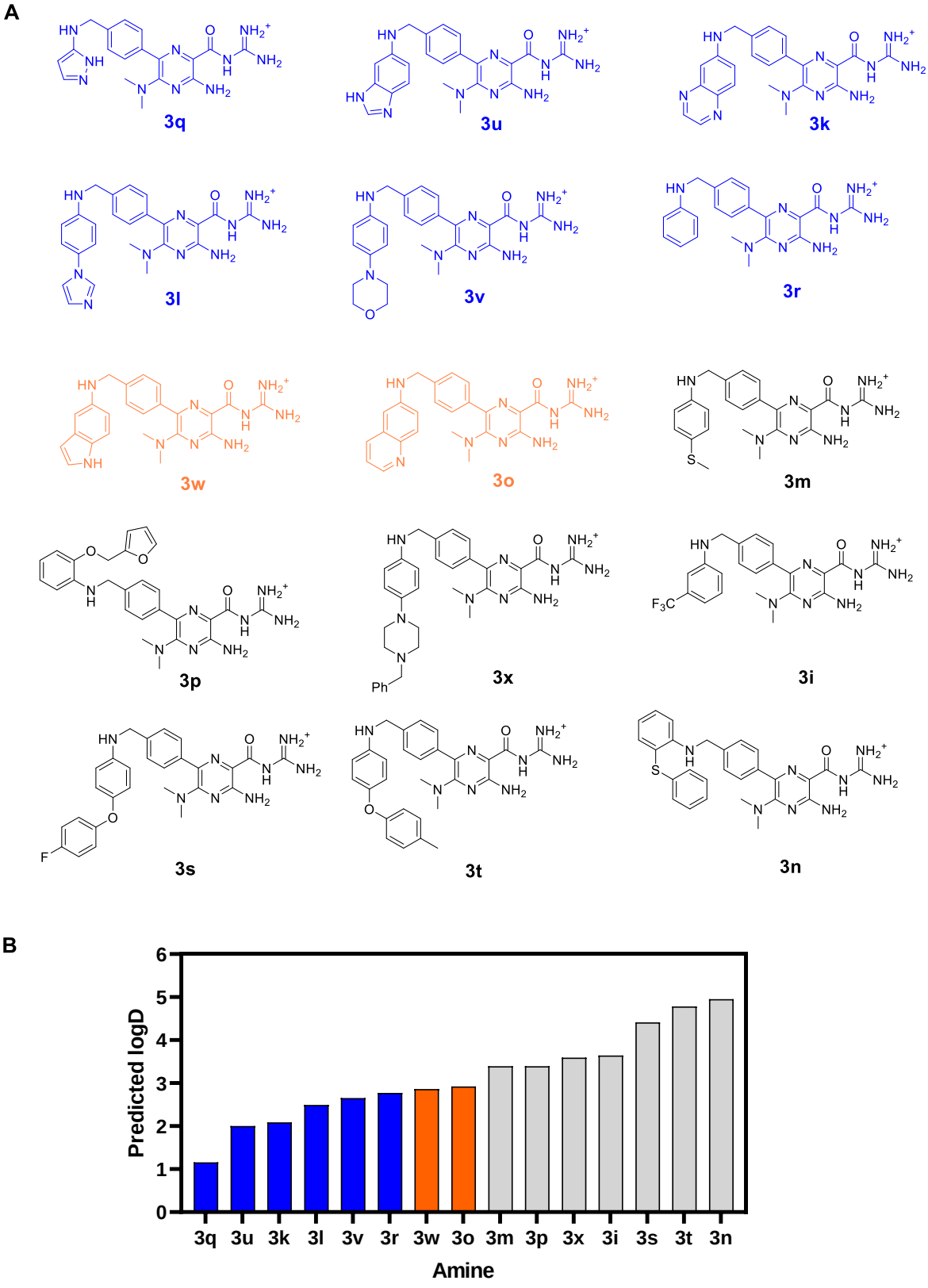


Figure S3. Selection of final amines for DCC. (A) Virtual amiloride library based on reactive amines; (B) Predicted logD of the amiloride library.

1.4 Effect of pH on reductive amination

In the course of testing amine reactivity, we also tested secondary amine formation at different pH values for a representative reactive amine (Figure S4) using the same procedure. 5 mM 3-trifluoromethylaniline was incubated with 1mM 4-hydroxybenzaldehyde (**4**) for 3 hours at three pH values (7.4, 6.9 and 6.3). The reactions mixtures were reduced with 10 mM $NaBH_3CN$ for 24 hours and samples analyzed by HPLC. The peak area of the secondary amine product (6i) was observed to be significantly higher at pH 6.3 compared to other pH values (Figure S4B). We therefore chose to use this pH as it resulted in higher secondary amine yields with short incubation periods.

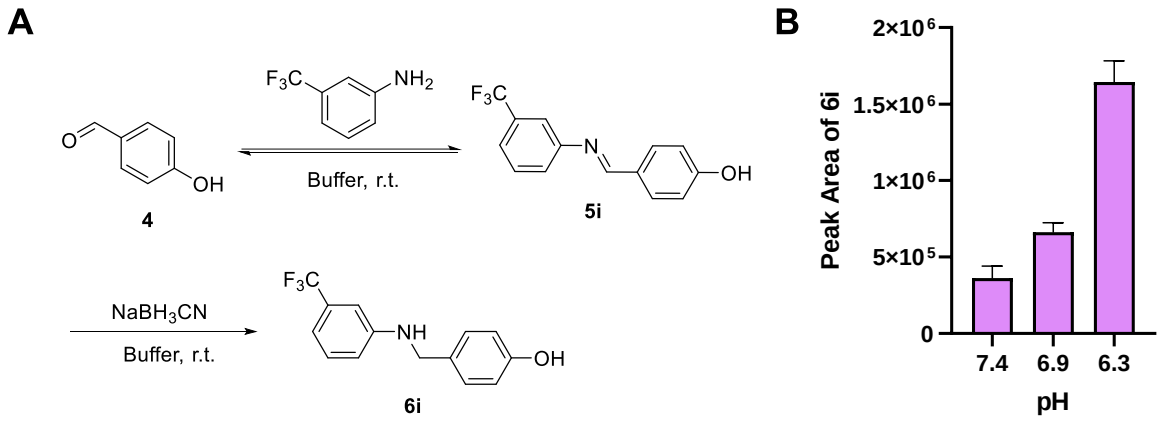
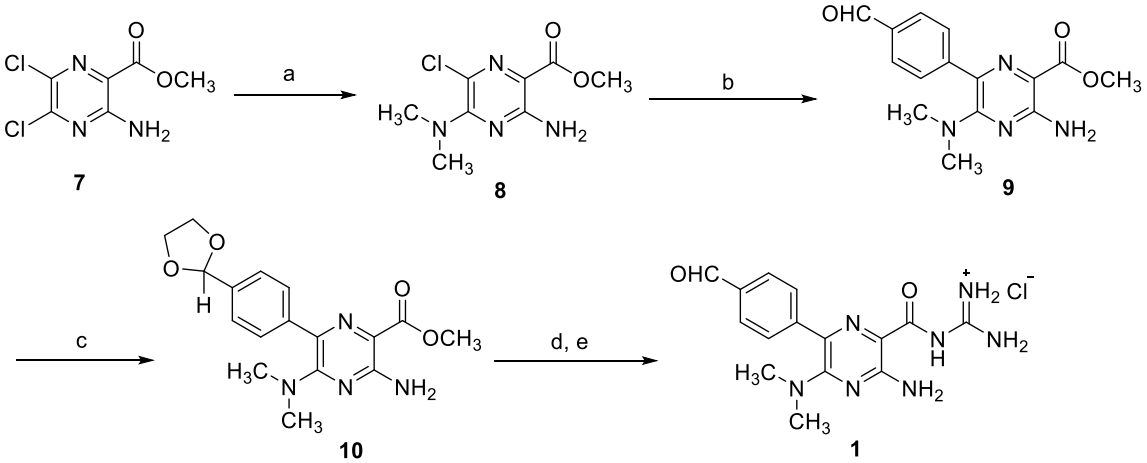


Figure S4**.** Testing effect of pH on reductive amination. (A) Reaction scheme (Buffer: 20 mM BisTris, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA); (B) HPLC peak area of the secondary amine product (average ± standard deviation of three reactions).

1.5 Synthesis of the amiloride aldehyde

The aldehyde analogue of the amiloride scaffold was synthesized in 4 steps from the commercially available methyl ester **7** (Scheme S1).



Scheme S1: Synthesis amiloride aldehyde analog. Reagents: a: Dimethylamine (2M in tetrahydrofuran, 2 equiv.), diisopropylethylamine (5 equiv.), dimethylformamide, r.t., 16-24 hrs; b: (4-Formylphenyl)boronic acid (1.05 equiv.), Na_2CO_3 (3 equiv.), $Pd(PPh_3)_4$ (0.05 equiv.), THF:H_2O (1:1), 75°C, 18-20 hrs; c: Ethylene glycol (5 equiv.), pyridinium *p*-toluenesulfonic acid (0.2 equiv.), toluene, 110°C, 15-21 hrs; d: Guanidine (1.5M solution in MeOH, 3 equiv.), tetrahydrofuran, 60°C, 18-24 hrs; d: 1N HCl (aq.), acetone, 12 hrs.

Methyl 3-amino-6-chloro-5-(dimethylamino)pyrazine-2-carboxylate (8): Methyl 3-amino-5,6-dichloropyrazine-2-carboxylate (700 mg, 3.15 mmol) was added to a dry round bottom flask, followed by dimethylformamide (10 mL), diisopropylethylamine (2.7 mL) and 3.2 mL of a 2M solution of dimethylamine in

tetrahydrofuran (6.30 mol). The purple mixture was stirred at room temperature under a nitrogen atmosphere for 16 hours. The liquids were evaporated under reduced pressure. The residue was re-dissolved in ethyl acetate and water and the mixture transferred into a separatory funnel. After discarding the aqueous layer, the organic layer was washed with water twice and then with brine. The organic layer was dried over anhydrous Na_2SO_4 for at least 15 minutes. Concentration of the organic layer afforded a pale brown solid. The solid was purified by flash column chromatography (75:25 hexanes:EtOAc) to yield a beige solid (600 mg; 83%). R_f = 0.4 (75:25 hexanes:EtOAc). ^{1}H NMR (500 MHz, methyl chloride-D2) δ 3.85 (s, 3H), 3.18 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.26, 154.23, 153.71, 121.46, 111.83, 51.99, 40.84. HRMS (ESI+): Calculated for $C_8H_{11}ClN_4O_2$ $[M+H]^+$: 231.0643 ($[M+H]^+$); Found = 231.0649 (± 2.5 ppm).

Methyl 3-amino-5-(dimethylamino)-6-(4-formylphenyl)pyrazine-2-carboxylate (9): Compound **8** (186.2 mg, 0.81 mmol), 4-formylboronic acid (127.2 mg, 0.85 mmol), Na_2CO_3 (258.0 mg, 2.44 mmol), water (8 mL) and THF (8 mL) were added to a round-bottom flask. Argon was bubbled through the mixture for at least 30 minutes using a balloon equipped with a long needle. $Pd(PPh_3)_4$ (47.0 mg, 0.04 mmol) was then added and the mixture was refluxed at 75 °C for 20 hours under a nitrogen atmosphere. The yellow mixture was cooled to room temperature, diluted with ethyl acetate, washed with water three times and then with brine. The organic layer was dried over anhydrous Na_2SO_4 for at least 15 minutes. The dried organic layer was concentrated under reduced pressure to yield a yellow-brown residue. The residue was purified via flash chromatography (75:25 hexanes:EtOAc) to yield a yellow oily substance (176 mg, 73%) that solidifies upon further drying under high reduced pressure. R_f = 0.20 (75:25 hexanes:EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.02 (s, 1H), 7.91 (d, *J* = 8.2 Hz, 2H), 7.77 (d, *J* = 8.3 Hz, 2H), 3.91 (s, 3H), 2.85 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.89, 167.26, 155.94, 153.85, 146.27, 135.15, 130.14, 130.09, 128.23, 113.92, 52.18, 41.02. HRMS (ESI+): Calculated for $C_{15}H_{16}N_4O_3$ $[M+H]^+$: 301.1295; Found: 301.1297 (± 0.7 ppm).

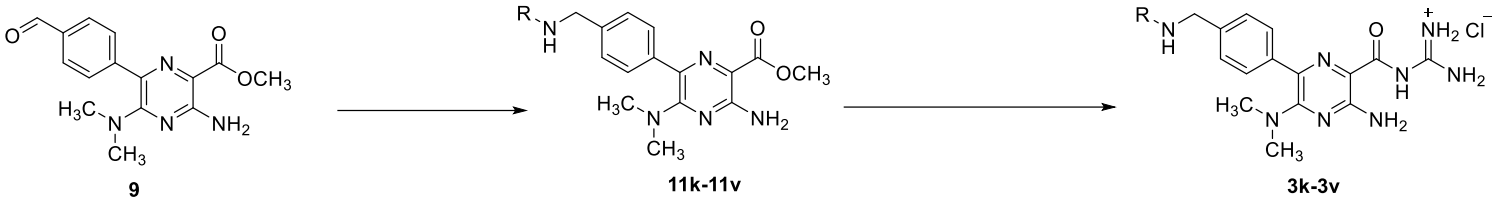
Methyl 6-(4-(1,3-dioxolan-2-yl)phenyl)-3-amino-5-(dimethylamino)pyrazine-2-carboxylate (10): Aldehyde **9** (176.6 mg, 0.59 mmol), ethylene glycol (164 µL, 2.94 mmol), pyridinium *p*-toluenesulfonate (29.8 mg, 0.12 mmol) and toluene (3 mL) were combined in an dry round-bottom flask. The flask was equipped with a Dean Stark apparatus and the mixture was heated at 110 °C for 18 hours after which only a minimal amount of 3 remained unconverted. The mixture was allowed to cool to room temperature and liquids were evaporated under vacuum. The brown residue was diluted in ethyl acetate, washed with water three times, then with brine. The organic layer was dried over anhydrous Na_2SO_4 for at least 15 minutes. The solvent was evaporated under reduced pressure. The product was purified via flash column chromatography (75:25 hexanes:EtOAc) to yield a beige solid (112.8 mg, 56%). Partial hydrolysis of the acetal on silica gel was observed. R_f = 0.19 (70:30 hexanes:EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (d, *J* = 8.2 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 2H), 5.80 (s, 1H), 4.15 – 4.06 (m, 2H), 4.04 – 3.99 (m, 2H), 3.86 (s, 3H), 2.79 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.45, 155.82, 153.68, 141.12, 137.04, 131.72, 127.73, 126.75, 113.11, 103.61, 65.30, 52.03, 40.89. HRMS (ESI+): Calculated for $C_{17}H_{20}N_4O_4$ $[M+H]^+$: 345.1557; Found: 345.1557 (± 0.1 ppm).

3-amino-N-(diaminomethylene)-5-(dimethylamino)-6-(4-formylphenyl)pyrazine-2-carboxamide (1): Methyl ester **10** (320 mg, 0.93 mmol) was dissolved in tetrahydrofuran (7.4 mL) in a round-bottom flask. 1.9 mL of a 1.5 M guanidine solution in methanol (2.8 mmol) was added and the mixture was heated at 60 °C for 18 hours. The reaction was allowed to cool to room temperature and liquids were evaporated under reduced pressure. Attempts to purify the residue by flash column chromatography on silica gel (80:6.7:6.7:6.7 EtOAc:MeOH:H_2O:MeCN) resulted in an inseparable mixture of guanidinylated acetal and compound **9** due to silica-induced acetal hydrolysis. To complete acetal deprotection, the obtained compound mixture was dissolved in acetone (1.3 mL) and treated with 3 M HCl (0.7 mL) overnight. Liquids were evaporated under reduced pressure to afford a yellow solid. NMR spectroscopy revealed the solid to be the hydrate form of compound 9. Purification of the hydrate by flash column chromatography on silica gel (80:6.7:6.7:6.7 EtOAc:MeOH:H_2O:MeCN) afforded conversion of the hydrate to compound 5 (116 mg, 38%). R_f = 0.14 (80:6.7:6.7:6.7 EtOAc:MeOH:H_2O:MeCN). ^{1}H NMR (500 MHz, CD_3OD) δ 10.04 (s, 1H), 8.01 (d, *J* = 7.9 Hz, 2H), 7.81 (d, *J* = 7.9 Hz, 2H), 7.57 (s, 1H), 2.91 (s, 6H), 2.88 (s, 1H). ^{13}C NMR (126 MHz, CD_3OD) δ 193.59, 167.63, 158.08, 156.92, 155.96, 146.89, 136.94, 131.37, 130.94, 129.60, 112.61, 41.32. HRMS (ESI+): Calculated for $C_{15}H_{17}N_7O_2$ $[M+H]^+$: 328.1517; Found = 328.1517 (± 0.1 ppm). HPLC purity: 99%.

1.6 Synthesis of DCC library members

1.6.1 Synthesis procedure of secondary amines

DCC library members were synthesized from aldehyde methyl ester **9** through reductive amination followed by conversion of the methyl ester to an acyl guanidine hydrochloride (Scheme S2).



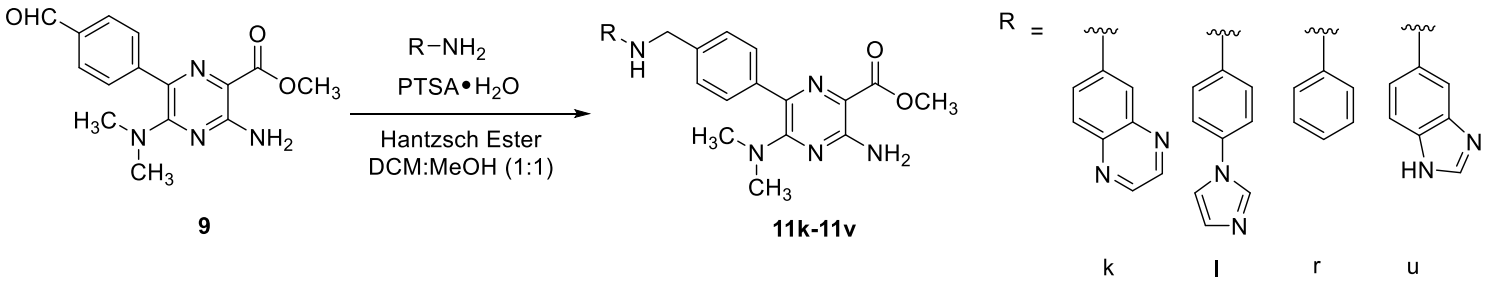
Scheme S2: General scheme for the synthesis of DCC library members.

Due to differing properties of the amines, three procedures were employed for different amine classes in the reductive amination step (Schemes S3-S5). For all compound, the same procedure was used for guanidinylation and conversion of the product to the hydrochloride form (Scheme S6).

A. Reductive amination procedures

Reductive amination using Hantzsch ester as reducing agent: Synthesis of 11k, 11l, 11r, 11u

For a fast and selective procedure that does not result in aldehyde reduction, we adopted work by Ghafuri and Hashemi, where the dihydropyridine Hantzsch ester was used as reducing agent in water in the presence of *p*-toluenesulfonic acid monohydrate as catalyst.[2] We substituted water for a 1:1 mixture of DCM and MeOH to maintain aldehyde and amine solubility (Scheme S3).

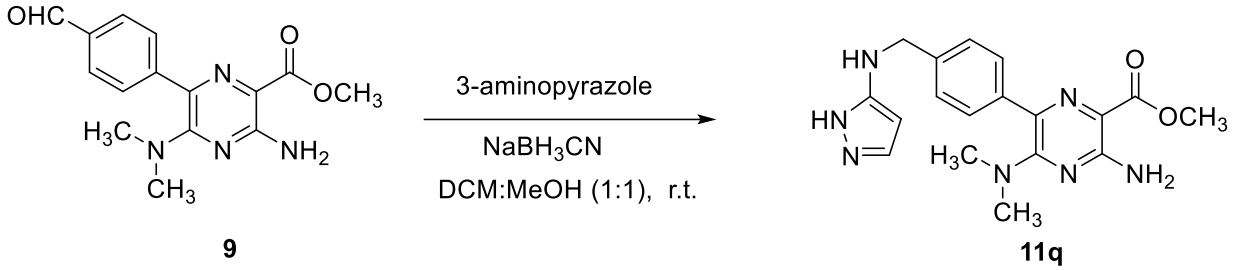


Scheme S3**:** Reductive amination with Hantzsch ester (diethyl 1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate). PTSA: *p*-toluenesulfonic acid.

Example procedure (**11r**): Aldehyde methyl ester **9** (35 mg, 0.117 mmol), Hantzsch ester (29.4 mg, 0.116 mmol), aniline (11 mg, 0.118 mmol), p-toluene sulfonic acid hydrate (2.5 mg, 0.013 mmol) and 1:1 DCM:MeOH (0.2 mL) were added to a 5-mL round-bottom flask. The mixture was stirred at ambient temperature under air for two hours, after which proton NMR showed complete conversion to the product. The mixture was then dissolved in DCM and purified by flash chromatography in 70:30 Hexanes:EtOAc to yield a yellow solid (43.5 mg, 98%). *Notes*: For compounds **11k**, **11l**, **11u**, longer reaction times or increasing the Hantzsch ester equivalents to 1.5 may be necessary to effect complete reduction of the imine intermediate. With 1 equivalent of Hantzsch ester and a 2-hour reaction time, leftover imine intermediate can be reduced by treatment of the isolated mixture with $NaBH_4$ in 1:1 DCM:MeOH. Compound **11k** was purified in 100% EtOAc while **11l** and **11u** were purified in 90:3.3:3.3:3.3 EtOAc:MeCN:MeOH:H_2O.

Reductive amination using $NaBH_3CN$ as reducing agent: Synthesis of **11q**

The Hantzsch procedure (Scheme S3) was unsuccessful with 3-aminopyrazole because the presence of acid induces reaction at C4 of the amine instead of at the NH_2 group as previously observed.[3] We therefore employed reductive amination with $NaBH_3CN$ (Scheme S4) despite the associated aldehyde reduction side-reaction.

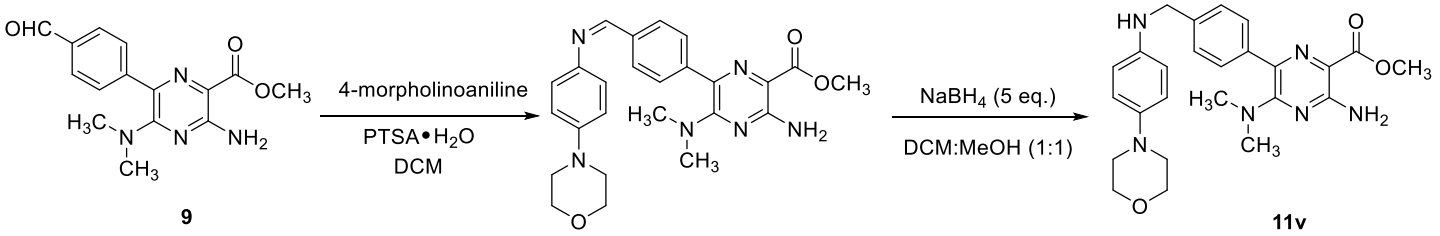


Scheme S4: Synthesis of **11q** via $NaBH_3CN$-mediated reductive amination

Procedure: Aldehyde methyl ester **9** (100.6 mg, 0.335 mmol) and 3-aminopyrazole (56 mg, 0.674 mmol) were dissolved in 1:1 DCM:MeOH (1.2 mL) in an oven-dried round-bottom flask. $NaBH_3CN$ (65 mg, 1.034 mmol) was added along with 4Å molecular sieves. The mixture was stirred at ambient temperature under nitrogen for 16 hours. The mixture was diluted in ethyl acetate, washed with water (30 mL), then washed with brine (30 mL) twice. The organic layer was dried over sodium sulfate. Solvent was evaporated under reduced pressure to afford a yellow mixture of the product **11q** and the alcohol byproduct. The product was isolated by flash chromatography in 95% EtOAc, 5% (1:1:1) MeCN:MeOH:H_2O as a pale yellow solid (21.5 mg, 17.5%).

Two-step reductive amination: Synthesis of **11v**

With 4-morpholinoaniline, the Hantzsch procedure (Scheme S3) stopped at the imine formation intermediate with no noticeable reduction to the corresponding secondary amine. This was probably due to high stability of the imine to Hantzsch ester as a reducing agent. As a result, we utilized a modified two-step procedure where PTSA monohydrate is used to catalyzed imine formation and $NaBH_4$ is used to reduce the imine (Scheme S5).

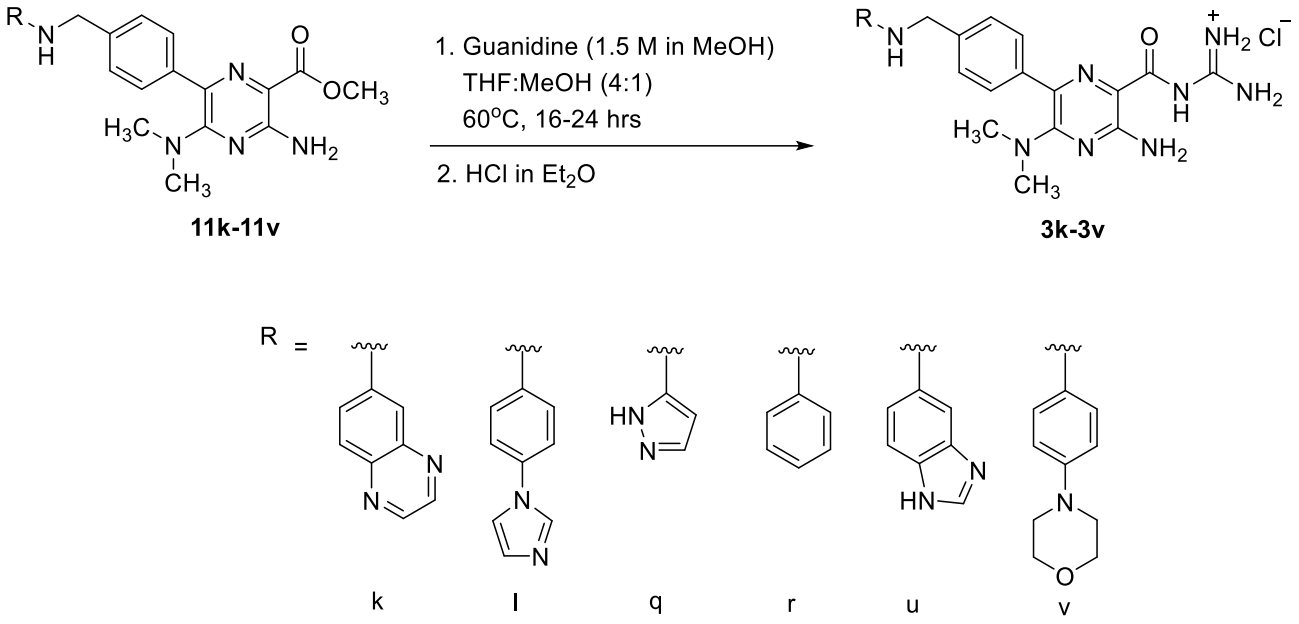


Scheme S5: Synthesis of **11v** in two steps. PTSA: *p*-toluenesulfonic acid.

Procedure: Aldehyde methyl ester **9** (82.9mg, 0.276 mmol), 4-morpholinoaniline (49.6 mg, 0.278 mmol) and p-toluenesolfonic acid monohydrate (5.6 mg, 0.0294 mmol) were dissolved in DCM (0.46 mL) in glass vial. The mixture was stirred at ambient temperature under air. After 3 hours, the mixture had thickened to a brown goo. The imine intermediate was separated from any leftover aldehyde by flash chromatography in 20:79:1 EtOAc:DCM:Et_3N. The imine was then dissolved in 2.5 mL 1:1 DCM:MeOH and treated with 52.3 mg of $NaBH_4$ for 2 hours under air. After reduction, the mixture was diluted in ethyl acetate and washed with brine (30 mL) twice. The organic layer was dried over sodium sulfate and concentrated under reduced pressure to afford a pale yellow solid (27.8 mg, 22%).

B. Conversion of methyl esters **11k-11v** to final library members **12k-12v**

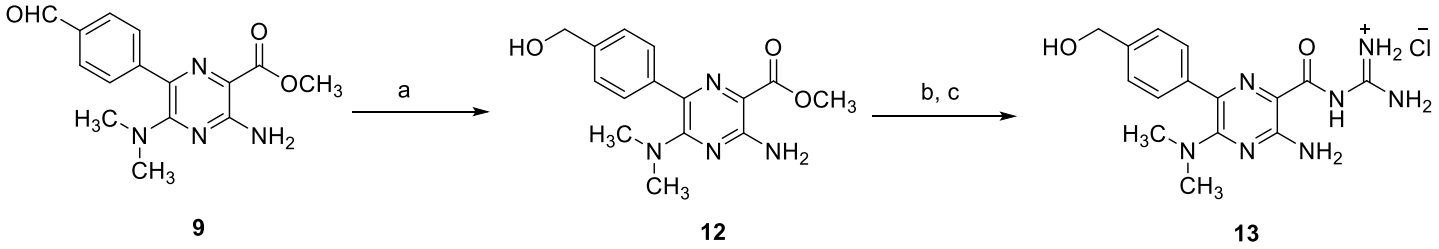
Conversion of methyl esters to acyl guanidines was conducted following a previously published procedure (Scheme S6).[4]



Scheme S6: Synthesis of final library members.

Example procedure: Methyl ester **11v** (27.8 mg, 0.060 mmol) was dissolved in tetrahydrofuran (0.6 mL) in a round-bottom flask. 1.5 M guanidine solution (0.12 mL, 0.180 mmol) was added and the mixture was heated for 16 hours at 60°C in an oil bath under nitrogen. The solvents were evaporated under reduced pressure, and the residue was purified by flash chromatography in 68:10:10:10:2 EtOAc:MeCN:MeOH:H_2O:NH_4OH. The solid was dissolved in MeOH, 10 mL of ice-cold diethyl ether added, followed by 1 mL of 2 M HCl solution in diethyl ether. The hydrochloride form of the compound precipitated as a yellow solid. The solvents were decanted off and the solid was washed with 10 mL of cold diethyl ether. Evaporation of residual solvent yielded 14.4 mg of compound **3v** (45.5% yield).

1.6.2 Synthesis procedure of alcohol derivative



Scheme S7: Synthesis of amiloride alcohol. Reagents: a: $NaBH_4$ (5 equiv.), DCM:MeOH (9:1), r.t., 20 min; b: Guanidine (1.5M solution in MeOH, 3 equiv.), tetrahydrofuran, 60°C, 16 hrs; c: HCl in Et_2O.

Procedure: Aldehyde methyl ester **9** (66.6 mg, 0.222 mmol) was dissolved in 9:1 DCM:MeOH (2.2 mL) in a glass vial. $NaBH_4$ (43 mg, 1.137 mmol) was added and the mixture stirred at ambient temperature for 20 min. The reaction was quenched with water and organic compounds extracted with EtOAc. The EtOAc layer was washed with water (30 mL), then with brine (30 mL). The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography in 1:1 DCM:EtOAc to yield 41.5 mg of product **12** (62% yield). Methyl ester **12** was converted to acyl guanidine hydrochloride **13** following the procedure in Scheme S6 (obtained 37.9 mg, 75% yield).

1.6.3 Characterization data for intermediates and final compounds

Methyl ester intermediates (11k–11v and 12)

Methyl 3-amino-5-(dimethylamino)-6-(4-((phenylamino)methyl)phenyl)pyrazine-2-carboxylate (11r): 98% yield, Rf: 0.41 (70:30 Hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (d, *J* = 8.0 Hz, 2H), 7.39 (d, *J* = 7.9 Hz, 2H), 7.16 – 7.19 (m, 2H), 6.73 (t, *J* = 7.3 Hz, 1H), 6.65 (d, *J* = 8.0 Hz, 2H), 4.34 (s, 2H), 3.89 (s, 3H), 2.83 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.55, 155.97, 153.72, 148.00, 139.35, 138.64, 132.15, 129.36, 128.12, 127.74, 117.86, 113.19, 113.13, 52.07, 48.35, 40.86. HRMS (ESI+): Calculated for $C_{21}H_{23}N_5O_2$ $[M+H]^+$: 378.19245 ; Found = 378.1930 (± 1.5 ppm).

Methyl 6-(4-(((4-(1H-imidazol-1-yl)phenyl)amino)methyl)phenyl)-3-amino-5-(dimethylamino)pyrazine-2-carboxylate (11l): 28%, Rf: 0.32; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.24 – 7.03 (m, 4H), 6.66 (d, *J* = 8.7 Hz, 2H), 4.37 (s, 2H), 3.88 (s, 3H), 2.83 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.50, 155.99, 153.74, 147.76, 139.58, 138.04, 132.25, 131.97, 129.29, 128.24, 127.89, 127.56, 123.50, 119.30, 113.47, 113.18, 52.10, 48.13, 40.88. HRMS (ESI+): Calculated for $C_{24}H_{25}N_7O_2$ $[M+H]^+$: 444.2143; Found = 444.2144 (± 0.4 ppm).

Methyl 6-(4-(((1H-benzo[d]imidazol-5-yl)amino)methyl)phenyl)-3-amino-5-(dimethylamino)pyrazine-2-carboxylate (11u): 40% yield, Rf: 0.3 (90:3.3:3.3:3.3 EtOAc:MeCN:MeOH:H_2O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.45 – 7.48 (m, 3H), 7.32 (d, *J* = 8.1 Hz, 2H), 6.66 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.51 (s, 1H), 4.31 (s, 2H), 3.82 (s, 3H), 2.80 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.32, 156.01, 153.92, 144.87, 139.30, 139.22, 138.79, 136.64, 133.37, 132.37, 128.21, 127.31, 118.00, 112.54, 111.93, 95.33, 51.99, 48.93, 40.83. HRMS (ESI+): Calculated for $C_{22}H_{23}N_7O_2$ $[M+H]^+$: 418.1986; Found = 418.1986 (± 0.0 ppm).

Methyl 6-(4-(((1H-pyrazol-5-yl)amino)methyl)phenyl)-3-amino-5-(dimethylamino)pyrazine-2-carboxylate (11q): 17.5% yield, Rf: 0.36 (95% EtOAc, 5% (1:1:1) MeCN:MeOH:H_2O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.0 Hz, 2H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 2.1 Hz, 1H), 5.60 (d, *J* = 2.1 Hz, 1H), 4.36 (s, 2H), 3.88 (s, 3H), 2.82 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.57, 156.60, 156.00, 153.75, 139.27, 139.04, 132.23, 130.70, 128.05, 127.82, 113.08, 91.40, 52.09, 49.18, 40.88. HRMS (ESI+): Calculated for $C_{21}H_{23}N_5O_2$ $[M+H]^+$: 378.1925; Found = 378.1930 (± 0.0 ppm).

Methyl 3-amino-5-(dimethylamino)-6-(4-((quinoxalin-6-ylamino)methyl)phenyl)pyrazine-2-carboxylate (11k): 40.5%; Rf: 0.4 (100% EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.61 (d, *J* = 1.8 Hz, 1H), 8.51 (d, *J* = 1.8 Hz, 1H), 7.84 (d, *J* = 9.1 Hz, 1H), 7.56 (d, *J* = 7.9 Hz, 2H), 7.40 (d, *J* = 7.9 Hz, 2H), 7.19 (dd, *J* = 9.1, 2.4 Hz, 1H), 7.01 (d, *J* = 2.2 Hz, 1H), 4.46 (s, 2H), 3.87 (s, 4H), 2.82 (s, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 167.50, 155.95, 153.74, 149.24, 145.14, 144.51, 140.45, 139.77, 138.38, 137.26, 131.89, 130.28, 128.31, 127.85, 122.47, 113.18, 103.86, 52.07, 47.92, 40.91. HRMS (ESI+): Calculated for $C_{23}H_{23}N_7O_2$ $[M+H]^+$: 430.1986; Found = 430.1988 (± 0.4 ppm).

Methyl 3-amino-5-(dimethylamino)-6-(4-(((4-morpholinophenyl)amino)methyl)phenyl)pyrazine-2-carboxylate (11v): 22%, Rf: 0.2 (20:79:1 EtOAc:DCM:Et_3N); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (d, *J* = 7.8 Hz, 2H), 7.38 (d, *J* = 7.9 Hz, 2H), 6.82 (d, *J* = 8.5 Hz, 2H), 6.62 (d, *J* = 8.4 Hz, 2H), 4.30 (s, 2H), 3.89 (s, 3H), 3.87 – 3.82 (m, 4H), 3.03 – 2.83 (m, 4H), 2.83 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.58, 156.01, 153.74, 143.84, 142.85, 139.28, 139.06, 132.23, 128.10, 127.75, 118.49, 114.15, 113.15, 67.26, 52.10, 51.38, 48.97, 40.89. HRMS (ESI+): Calculated for $C_{25}H_{30}N_6O_3$ $[M+H]^+$: 463.2452; Found = 463.2453 (± 0.2 ppm).

Methyl 3-amino-5-(dimethylamino)-6-(4-(hydroxymethyl)phenyl)pyrazine-2-carboxylate (12): 62%, Rf: 0.45 (1:1 DCM:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (d, *J* = 8.2 Hz, 2H), 7.36 (d, *J* = 8.3 Hz, 2H), 4.68 (s, 2H), 3.88 (s, 3H), 2.82 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.54, 155.92, 153.71, 140.21, 139.67, 132.08, 128.03, 127.22, 113.13, 65.19, 52.08, 40.90. HRMS (ESI+): Calculated for $C_{15}H_{18}N_4O_3$ $[M+H]^+$: 303.1452; Found = 303.1457 (± 1.9 ppm).

Final guanidinylated compounds (3k–3v and 13)

3-Amino-N-carbamimidoyl-5-(dimethylamino)-6-(4-((phenylamino)methyl)phenyl)pyrazine-2-carboxamide hydrochloride hydrochloride (3r): 24%, Rf: 0.3 (68:10:10:10:2 EtOAc:MeCN:MeOH:H_2O:NH_4OH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.65 (d, *J* = 7.9 Hz, 2H), 7.57 – 7.45 (m, 7H), 4.70 (s, 2H), 2.89 (s, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 167.51, 156.84, 154.87, 141.89, 135.87, 132.22, 131.99, 131.30, 131.24, 131.07, 129.72, 124.60, 112.18, 56.43, 41.66. HRMS (ESI+): Calculated for $C_{21}H_{24}N_8O$ $[M+H]^+$: 405.2146; Found = 405.21486 (± 0.7 ppm). HPLC purity: 93%.

6-(4-(((4-(1H-imidazol-1-yl)phenyl)amino)methyl)phenyl)-3-amino-N-carbamimidoyl-5-(dimethylamino)pyrazine-2-carboxamide hydrochloride (3l): 14%, Rf: 0.33 (68:10:10:10:2 EtOAc:MeCN:MeOH:H_2O:NH_4OH); ^{1}H NMR (500 MHz, CD_3OD) δ 9.01 (s, 1H), 7.79 (s, 1H), 7.59 (s, 1H), 7.55 (d, *J* = 7.9 Hz, 2H), 7.49 (d, *J* = 7.9 Hz, 2H), 7.36 (d, *J* = 8.7 Hz, 2H), 6.77 (d, *J* = 8.6 Hz, 2H), 4.46 (s, 2H), 2.88 (s, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 167.71, 158.25, 156.94, 155.92, 151.32, 140.78, 139.85, 135.21, 133.30, 129.24, 128.42, 125.85, 124.42, 122.72, 122.49, 114.19, 111.76, 47.84, 41.17. HRMS (ESI+): Calculated for $C_{24}H_{26}N_{10}O$ $[M+H]^+$: 471.2364; Found = 471.2366 (± 0.5 ppm). HPLC purity: 94%.

6-(4-(((1H-benzo[d]imidazol-5-yl)amino)methyl)phenyl)-3-amino-N-carbamimidoyl-5-(dimethylamino)pyrazine-2-carboxamide hydrochloride (3u): 63%, Rf: 0.32 (68:10:10:10:2 EtOAc:MeCN:MeOH:H_2O:NH_4OH); ^{1}H NMR (500 MHz, CD_3OD) δ 9.52 (s, 1H), 7.99 (d, *J* = 9.8 Hz, 2H), 7.71 (d, *J* = 8.8 Hz, 1H), 7.66 (d, *J* = 7.7 Hz, 2H), 7.61 (d, *J* = 7.8 Hz, 2H), 4.78 (s, 2H), 2.89 (s, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 167.54, 157.07, 156.96, 156.89, 155.03, 142.99, 141.73, 136.70, 132.32, 132.30, 131.60, 131.16, 129.72, 122.62, 117.27, 112.18, 109.45, 55.91, 41.58. HRMS (ESI+): Calculated for $C_{22}H_{24}N_{10}O$ $[M+H]^+$: 445.2207; Found = 445.2208 (± 0.1 ppm). HPLC purity: 94%.

6-(4-(((1H-pyrazol-5-yl)amino)methyl)phenyl)-3-amino-N-carbamimidoyl-5-(dimethylamino)pyrazine-2-carboxamide hydrochloride (3q): 32%, Rf: 0.32 (68:10:10:10:2 EtOAc:MeCN:MeOH:H_2O:NH_4OH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.88 (d, *J* = 2.0 Hz, 1H), 7.62 (d, *J* = 7.9 Hz, 2H), 7.50 (d, *J* = 7.9 Hz, 2H), 5.90 (d, *J* = 2.8 Hz, 1H), 4.51 (s, 2H), 2.90 (s, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 167.62, 157.51, 156.92, 155.33, 153.54, 140.34, 138.55, 136.87, 132.98, 129.51, 128.71, 111.93, 91.94, 41.41. HRMS (ESI+): Calculated for $C_{18}H_{22}N_{10}O$ $[M+H]^+$: 395.2051; Found = 395.2050 (± 0.3 ppm). HPLC purity: 96%.

3-Amino-N-carbamimidoyl-5-(dimethylamino)-6-(4-((quinoxalin-6-ylamino)methyl)phenyl)pyrazine-2-carboxamide hydrochloride (3k): 15.6%, Rf: 0.24 (68:10:10:10:2 EtOAc:MeCN:MeOH:H_2O:NH_4OH); ^{1}H NMR (500 MHz, CD_3OD) δ 8.78 (d, *J* = 3.1 Hz, 1H), 8.57 (d, *J* = 3.1 Hz, 1H), 8.02 (d, *J* = 9.5 Hz, 1H), 7.72 (dd, *J* = 9.5, 2.4 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* = 7.9 Hz, 2H), 6.86 (d, *J* = 2.6 Hz, 1H), 4.67 (s, 2H), 2.88 (s, 6H).^{13}C NMR (126 MHz, CD_3OD) δ 167.65, 158.03, 156.90, 155.89, 155.75, 143.95, 140.44, 138.66, 138.06, 137.12, 134.34, 132.89, 132.13, 129.56, 128.83, 128.37, 111.90, 93.59, 47.82, 41.26. HRMS (ESI+): Calculated for $C_{23}H_{24}N_{10}O$ $[M+H]^+$: 457.2207; Found = 457.2209 (± 0.4 ppm). HPLC purity: 98%.

3-Amino-N-carbamimidoyl-5-(dimethylamino)-6-(4-(((4-morpholinophenyl)amino)methyl)phenyl)pyrazine-2-carboxamide hydrochloride (3v): 45.5%, Rf: 0.31 (68:10:10:10:2 EtOAc:MeCN:MeOH:H_2O:NH_4OH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.62 (d, *J* = 7.7 Hz, 2H), 7.55 – 7.46 (m, 4H), 7.22 (d, *J* = 7.8 Hz, 2H), 4.58 (s, 2H), 4.06 – 4.00 (m, 4H), 3.56 – 3.50 (m, 4H), 2.89 (s, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 167.66, 157.84, 156.93, 155.64, 141.07, 140.44, 135.63, 132.68, 130.28, 129.49, 128.08, 121.13, 120.37, 112.00, 66.19, 54.26, 52.36, 41.35. HRMS (ESI+): Calculated for $C_{25}H_{31}N_9O_2$ $[M+H]^+$: 490.2674; Found = 490.26812 (± 1.6 ppm). HPLC purity: 97%.

3-Amino-N-carbamimidoyl-5-(dimethylamino)-6-(4-(hydroxymethyl)phenyl)pyrazine-2-carboxamide hydrochloride (13): 75%, Rf: 0.18 (68:10:10:10:2 EtOAc:MeCN:MeOH:H_2O:NH_4OH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.58 (d, *J* = 7.7 Hz, 2H), 7.49 (d, *J* = 7.6 Hz, 2H), 4.68 (s, 2H), 3.00 (s, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 167.22, 156.77, 154.55, 152.89, 143.31, 138.82, 133.65, 129.32, 128.15, 111.80, 64.73, 42.34. HRMS (ESI+): Calculated for $C_{15}H_{19}N_7O_2$ $[M+H]^+$: 330.1673; Found = 330.1679 (± 1.8 ppm). HPLC purity: 99%.

2. Preparation of RNA for DCC and binding assays

2.1 RNA synthesis and purification

All RNAs (Table S2) were synthesized by *in vitro* transcription (IVT). Sense and antisense DNA templates carrying the T7 promoter (Table S3) were purchased as 100 μM solutions from Integrated DNA Technologies. Ribonucleotides were purchased (IDT) either individually at 100 mM or as a cocktail containing 25 mM each. All buffers and solutions were prepared using nuclease-free water (Ambion). The following steps were followed for RNA synthesis:

(1) DNA annealing: 45 μL of the sense strand, 45 μL of the antisense strand, 54 μL of 0.01 M $MgCl_2$ and 36 μL nuclease free water were combined in a 1.5-mL microcentrifuge tube. The tube was incubated at 95°C for 10 min in an Eppendorf ThermoMixer, then snap-cooled on ice for at least one hour. DNA annealing was confirmed by running the annealed sample on a Novex® DNA retardation gel.

(2) In vitro transcription: IVT reactions contained 0.091 M Tris (pH = 7.4), 0.023 M MgCl2, 0.027 M dithiothreitol, 0.002 M Spermidine, 2.7 mM rATP, 2.7 mM rCTP, 2.7 mM rGTP, 2.7 mM rUTP, 0.4 μM annealed double-stranded DNA template and 2.7 U/μL T7 RNA polymerase (custom preparation). Reagents were combined in a 15-mL falcon tube, incubated at 37°C in an Eppendorf ThermoMixer for 14 to 24 hours.

HIV-1 TAR and HIV-2 TAR were purified by gel electrophoresis (20% polyacrylamide containing 8M urea) in 0.1 M Tris, 0.1 M boric acid, 2 mM EDTA. RNA was eluted by crush-and-soak in 50 mM Tris, 300 mM NaOAc, 10 mM EDTA, pH 7.4. The RNA was concentrated using a 9 KDa molecular weight cutoff Amicon® filtration membrane, washed extensively with nuclease-free water, then isolated by ethanol precipitation.

RRE-IIB was purified using the ZymoResearch Clean & Concentrator™ kit following the manufacturer's protocol, except that three wash steps were performed instead of two. Before purification, the crude IVT mixture was treated with DNase I to remove the DNA template. Procedure for DNase treatment: To a 1-mL IVT reaction were added 180 μL of 10X DNase I buffer (New England Biolabs) and 37.5 μL of DNase I (2000 U/mL, New England Biolabs). After incubation for 30 min at 37 °C, another 37.5 μL of DNase I was added and the mixture incubated again for 30 min.

Table S2. Sequences of RNA constructs used in DCC

RNA	Sequence (5'-> 3')
HIV-1 TAR	GGCAGAUCUGAGCCUGGGAGCUCUCUGCC
HIV-2 TAR	GGCAGAUUGAGCCCUGGGAGGUUCUCUGCC
RRE-IIB	GGUCUGGGCGCAGCGCAAGCUGACGGUACAGGCC

Table S3. Sequences of DNA templates used for in vitro transcription of the RNA constructs

DNA template	Strand	Sequence (5' -> 3')
HIV-1 TAR	Sense	GCAGCTAATACGACTCACTATAGGCAGATCTGAGCCTGGGAGCTCTCTGCC
	Antisense	GGCAGAGAGCTCCCAGGCTCAGATCTGCCTATAGTGAGTCGTATTAGCTGC
HIV-2 TAR	Sense	GCAGCTAATACGACTCACTATAGGCAGATTGAGCCCTGGGAGGTTCTCTGCC
	Antisense	GGCAGAGAACCTCCCAGGGCTCAATCTGCCTATAGTGAGTCGTATTAGCTGC
RRE-IIB	Sense	GCAGCTAATACGACTCACTATAGGTCTGGGCGCAGCGCAAGCTGACGGTACAGGCC
	Antisense	GGCCTGTACCGTCAGCTTGCGCTGCGCCCAGACCTATAGTGAGTCGTATTAGCTGC

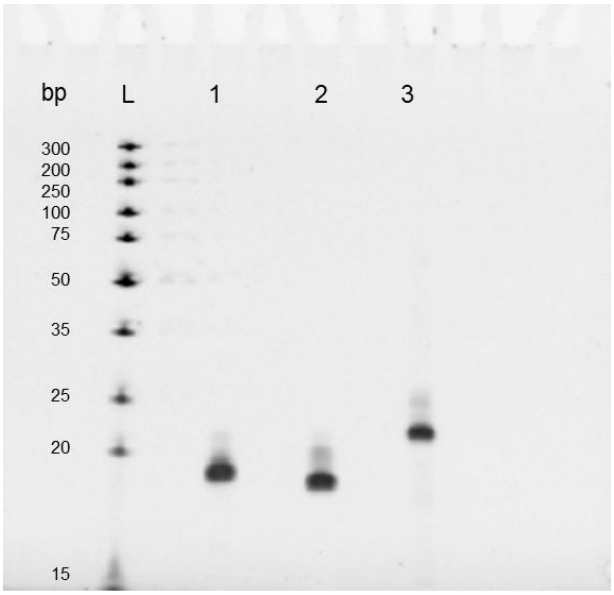


Figure S5. Assessment of RNA purity: 15% polyacrylamide gel electrophoresis run at 180 V for 70 min in TBE buffer (0.1 M tris, 0.1 M boric acid, 2 mM EDTA). The gel was stained with Diamond™ Nucleic Acid Dye in TBE buffer for 30 min. L: O'GeneRuler Ultra Low Range dsDNA ladder; 1: HIV-1 TAR; 2: HIV-2 TAR; 3: RRE-IIB.

2.2 Characterization of RNA by MALDI-TOF mass spectrometry

The matrix was prepared as 9:1 3-Hydroxypicolinic acid (in water):100 mM diammonium citrate buffer. Matrix (1 µL) was dried first on polished steel plate. The samples were first desalted with C-18 ZipTips and then mixed with diammonium citrate buffer in a 1:1 ratio. 1 µL of diluted sample was dried over matrix on the plate. Calibration was performed with insulin prepared in the same matrix. Spectra were acquired in linear (low-resolution) mode with negative polarity. All three RNA constructs had the expected molecular weight (Table S5, Figure S6).

Table S5. RNA characterization by MALDI TOF mass spectrometry

RNA	Calculated for $[M-H]^-$	**Obtained $[M-H]^-$**
HIV-1 TAR pppGGCAGAUCUGAGCCUGGGAGCUCUCUGCC	9529	**9528.866**
HIV-2 TAR pppGGAGAUUGAGCCCUGGGAGGUUCUCUCC	9225	**9224.960**
RRE pppGGUCUGGGCGCAGCGCAAGCUGACGGUACAGGCC	11261	**11260.481**

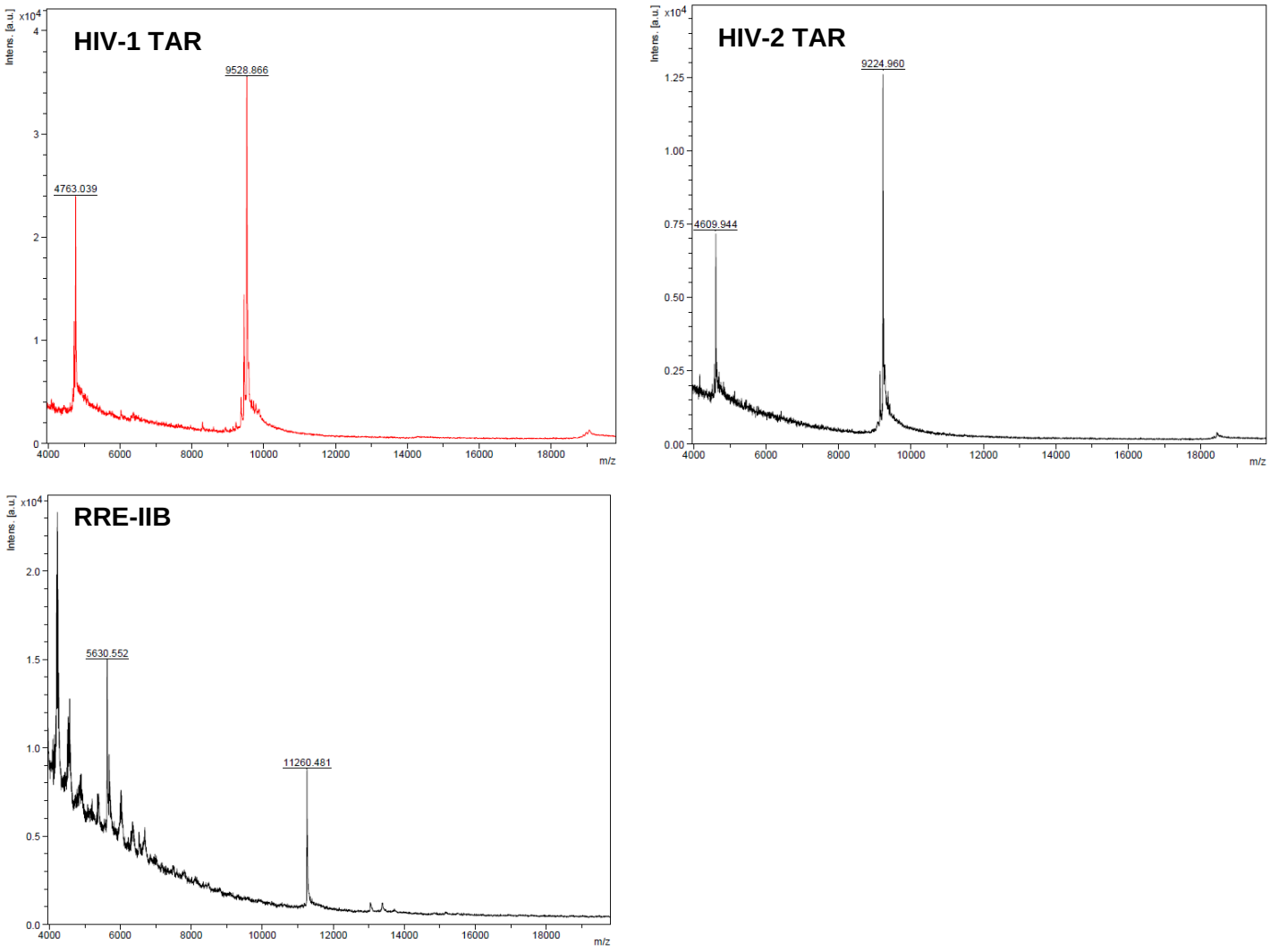


Figure S6: RNA MALDI-TOF spectra.

3. Dynamic combinatorial chemistry procedure

Reaction set-up

A 20 mM solution of aldehyde (**1**) was prepared in DMSO. The solution was sonicated for 10 min at 30°C to maximize solubility. For each of the 6 amines, a 100 mM solution was prepared in DMSO. A cocktail solution containing the 6 amines was then prepared by combining 5 µL each of 3-aminopyrazole (**q**), 4-(1H-imidazol-1-yl)aniline (**l**), aniline (**r**), 5-aminobenzimidazole (**u**), 4-morpholinoaniline (**v**) and 15 µL of 6-aminoquinoxaline (**k**). The total volume was brought to 100 µL by adding 60 µL of DMSO.

Control reactions were set up in 1.5-mL microcentrifuge tubes by combining 2.5 µL of aldehyde stock, 2.5 µL of amine cocktail, 90 µL of buffer (20 mM BisTris, 25 mM NaCl, 1 mM $MgCl_2$, 0.1 mM EDTA, pH = 6.3), and 5 µL of $NaBH_3CN$ freshly prepared in buffer. The $NaBH_3CN$ was added approximately 15 min after mixing of all the other reagents. A higher concentration of 6-aminoquinoxaline was necessary to allow an acceptable signal-to-noise ratio for the resulting library member. Three replicate control reactions were set up in each independent experiment.

RNA-containing reactions were set up the same way, but with 12.5 µM final concentration of RNA. The buffer volume was adjusted to account for the volume of the RNA solution. Before use in DCC reactions, the RNA was

annealed by heating a 40 µM at 95°C for 10 min followed by cooling on ice for at least an hour. For each RNA, three RNA-containing reactions were set up in each independent experiment.

All reactions were incubated at ambient temperature (~21°C) for 20 hours. Table S6 shows the final concentrations of each component in the DCC reactions.

Table S6. Reagent concentrations in DCC reactions.

Component	Concentration
Aldehyde (**1**)	0.5 mM
3-aminopyrazole (**q**)	0.25 mM
4-(1H-imidazol-1-yl)aniline (**l**)	0.25 mM
Aniline (**r**)	0.25 mM
5-aminobenzimidazole (**u**)	0.25 mM
6-aminoquinoxaline (**k**)	0.75 mM
4-morpholinoaniline (**v**)	0.25 mM
RNA	12.5 µM
$NaBH_3CN$	5 mM

Sample analysis and data processing

After incubation, samples were vortexed and prepared for analysis. 100 µL of HPLC-grade water was added, followed by 400 µL of HPLC-grade MeOH. 100 uL of the resulting solution were further diluted with 400 µL of MeOH. An aliquot of this final solution was analyzed by electrospray ionization mass spectroscopy on an Agilent 6460 Triple Quad LC/MS instrument with single ion monitoring in flow injection mode (injection volume: 2 µL; flow rate: 0.5 mL/min; mobile phase: isocratic run of a 1:1 mixure of 100:3:0.3 H_2O:MeCN:Formic Acid and 100:3:0.3 MeCN:H_2O:Formic Acid; total acquisition time: 2 min; dwell time on each ion: 50 ms). Each sample was analyzed at least in triplicate to minimize error from fluctuations in instrument sensitivity. The $[M+H]^+$ species were monitored for each library member, as well as the aldehyde scaffold and the alcohol byproduct (8 ions total). For each compound, a chromatogram was obtained which reports on the relative amount of the corresponding compound that is present in the mixture (Figure S7A). Chromatograms were integrated using the Agilent MassHunter Qualitative Analysis B.07.00 software. Peak areas for each compound in replicate reactions were averaged (Figure S7B), and the following equation was used to calculate the change in compound abundance in the presence of RNA relative to no-RNA control reactions:

$$\%\ \text{Change} = \frac{100 \times (A_{\text{RNA}} - A_{\text{NoRNA}})}{A_{\text{NoRNA}}}$$

where A_{RNA} is the average peak area in the presence of RNA and A_{NoRNA} is the average peak area in the absence of RNA.

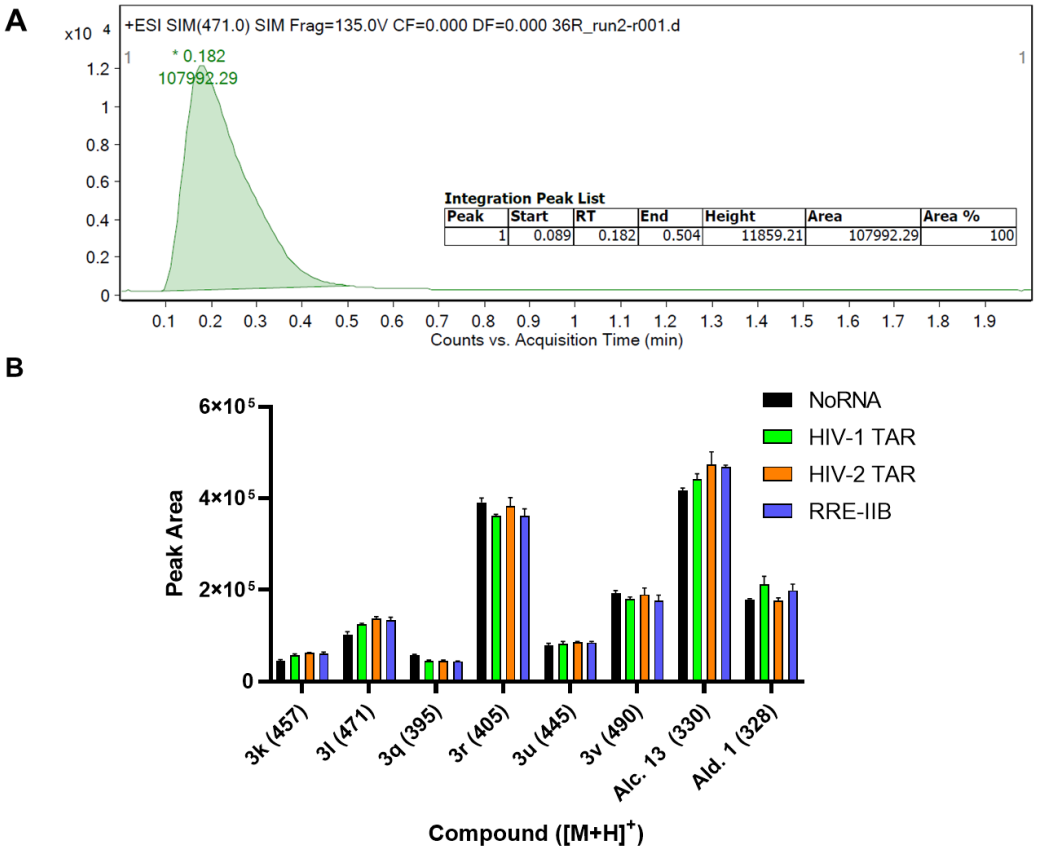


Figure S7. Mass spectrometry analysis of DCC reactions by single ion monitoring. (A) Example chromatogram obtained for each compound (**3l** is shown); (B) Peak areas from a representative experiment (average ± standard deviation of 3 triplicate reactions).

In addition to single-ion monitoring, in each experiment a control reaction and an RNA-containing reaction were also analyzed in scan mode to make sure there are no unexpected byproducts in the reaction mixture. In all cases only the expected ions were observed in both control reactions and RNA-containing reactions, with the exception of three ions (427, 449 and 499) observed in a blank mixture containing only DMSO and buffer (Figure S8).

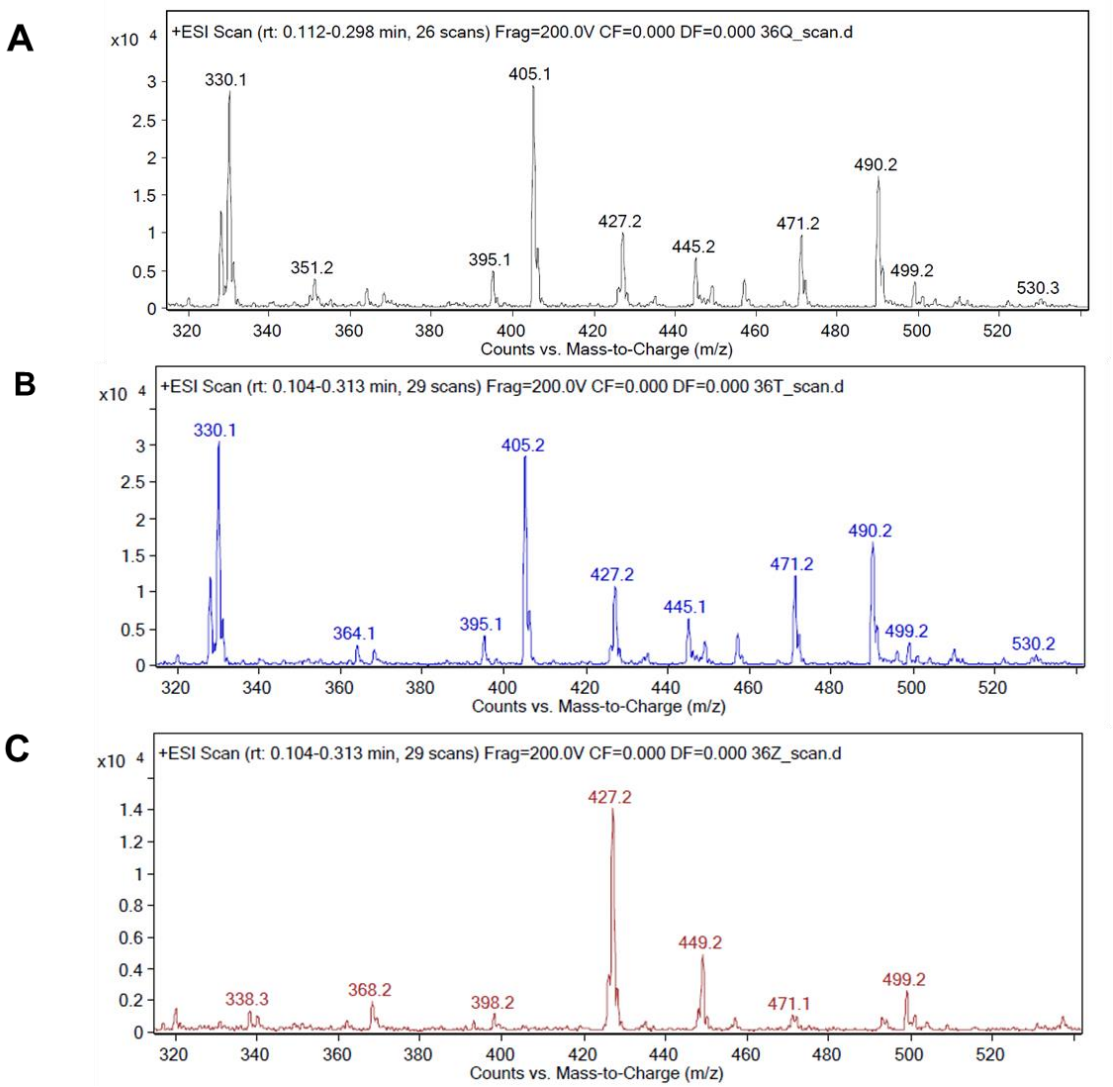


Figure S8. Mass spectrometry analysis of DCC reactions in scan mode. (A) Control reaction without RNA; (B) Reaction containing HIV-1 TAR; (C) Blank sample containing only buffer and 5% DMSO.

4. Evaluation of library member binding to RNA

4.1 TOPRO-1 displacement

Assay

In vitro transcribed RNA was annealed at 40 µM by heating at 95°C and cooling on ice for at least one hour. Compound stock solutions were prepared at 50 mM in DMSO. The DMSO solutions were sonicated for 10 min at 30 degrees. TOPRO-1 dye was purchased from Thermo Fisher Scientific as a 1 mM solution in DMSO.

RNA working solutions were prepared at 1.125 µM in buffer (20 mM BisTris, 25 mM NaCl, 1 mM $MgCl_2$, 0.1 mM EDTA, 0.01% Triton-X-100, pH = 6.3). TOPRO-1 working solutions were prepared at 1.5 µM in buffer. The compounds were serially diluted in buffer containing 15% DMSO at 10 concentrations between 1.7 and 948.7 µM (two serial dilutions with overlapping concentrations were performed). The compound (5 µL), the RNA working solution (5 µL) and the TOPRO-1 working solution (5 µL) were combined in a Corning® low volume 384-well plate (Table S7) shows the final concentration of each component). The plate was shaken for 5 min at 100 rpm on a platform shaker to mix assay components, then centrifuged at 4000 rpm for 1 min and allowed to incubate at ambient temperature for 30min. The plate was analyzed on a BMG ClARIOstar microplate reader at an excitation wavelength of 505 nm (bandwidth: 15 nm) and an emission wavelength of 535 nm (bandwidth: 20

nm). For each RNA-compound pair, the displacement assay was performed in three separate experiments and each replicate contained technical triplicates. Control wells containing only the compound and the RNA at all compound concentrations were included to evaluate whether the compound possessed fluorescence at the assay wavelengths.

Table S7. Concentrations of the TOPRO-1 assay components.

Component	Concentration
RNA	375 nM
TOPRO-1	500 nM
Compound	0.56, 1.00, 1.77, 3.16, 10, 17.8, 31.6, 56.2, 100, 316 µM
DMSO	5 % (v/v)

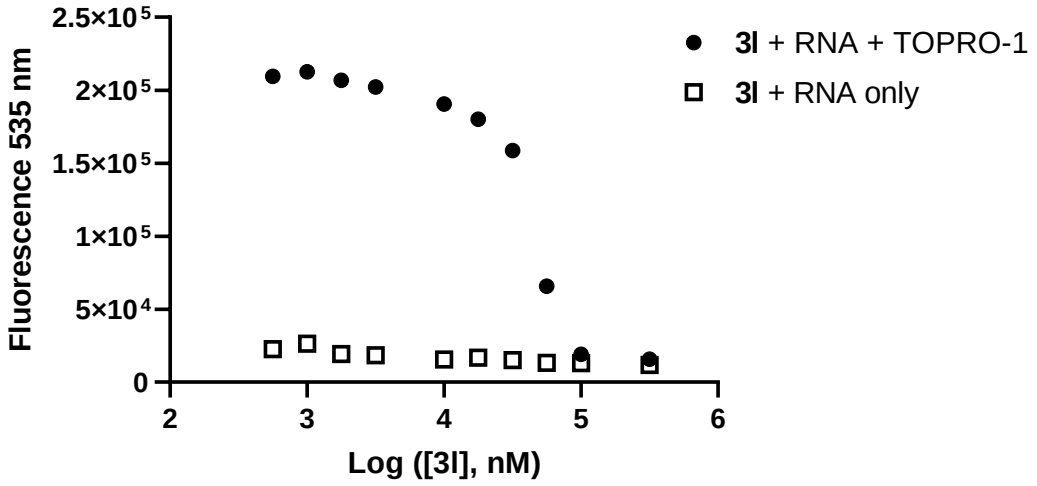


Figure S9. Sample raw data. Assay of compound **3l** with HIV-1 TAR. All compounds exhibited similar curves, with virtually no fluorescence in the absence of TOPRO-1 (compound + RNA only).

Curve fitting

For each independent experiment, the average compound + RNA fluorescence was subtracted from the average compound + RNA + TOPRO-1 fluorescence (Figure S9). The resulting fluorescence values were fit to the log(inhibitor) vs. response – variable slope (four parameters) equation in GraphPad Prism 8.3.0.538. The resulting CD_{50} values are shown in Table S8 and the curves for all compound-RNA pairs are shown in Figures S10-S12.

Table S8. RNA-binding activity of DCC library members (average ± standard deviation of three independent experiments).

	CD_{50} (µM)		
Compound	HIV-1 TAR	HIV-2 TAR	RRE-IIB
3k	34.6 ± 0.3	31.9 ± 3.6	38.2 ± 4.1
3l	44.4 ± 1.3	35.1 ± 8.6	42.3 ± 9.2
3q	82.8 ± 5.7	84.8 ± 13.0	82.8 3.6
3r	91.1 ± 11.1	76.1 ± 2.1	90.4 ± 3.7
3u	40.9 ± 1.2	26.3 ± 1.7	39.1 ± 2.4

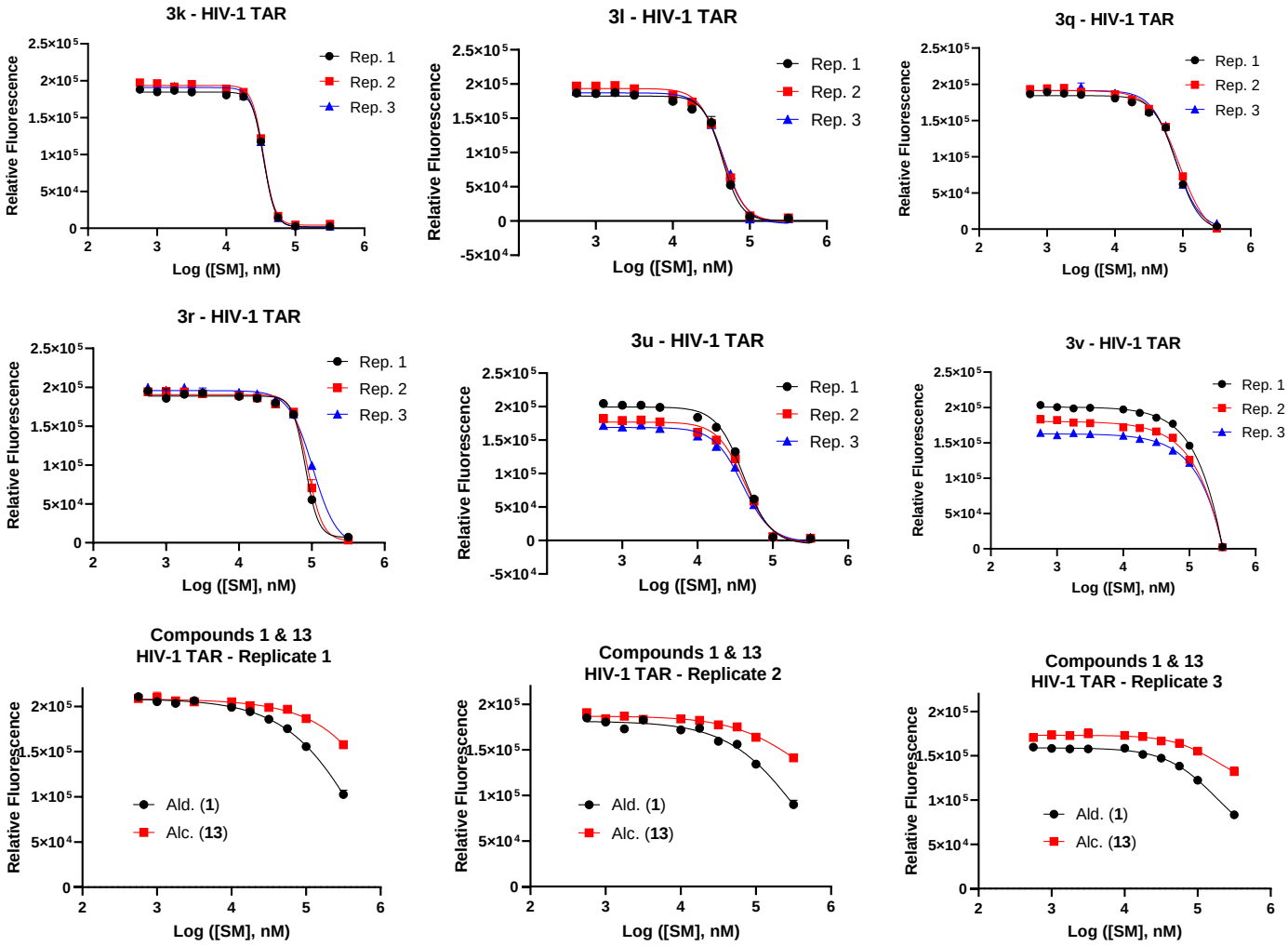


Figure S10. TOPRO-1 assay. Compound binding curves to HIV-1 TAR. Rep.: independent replicate. Each curve depicts one independent experiment (average values ± propagated standard deviation from technical triplicates).

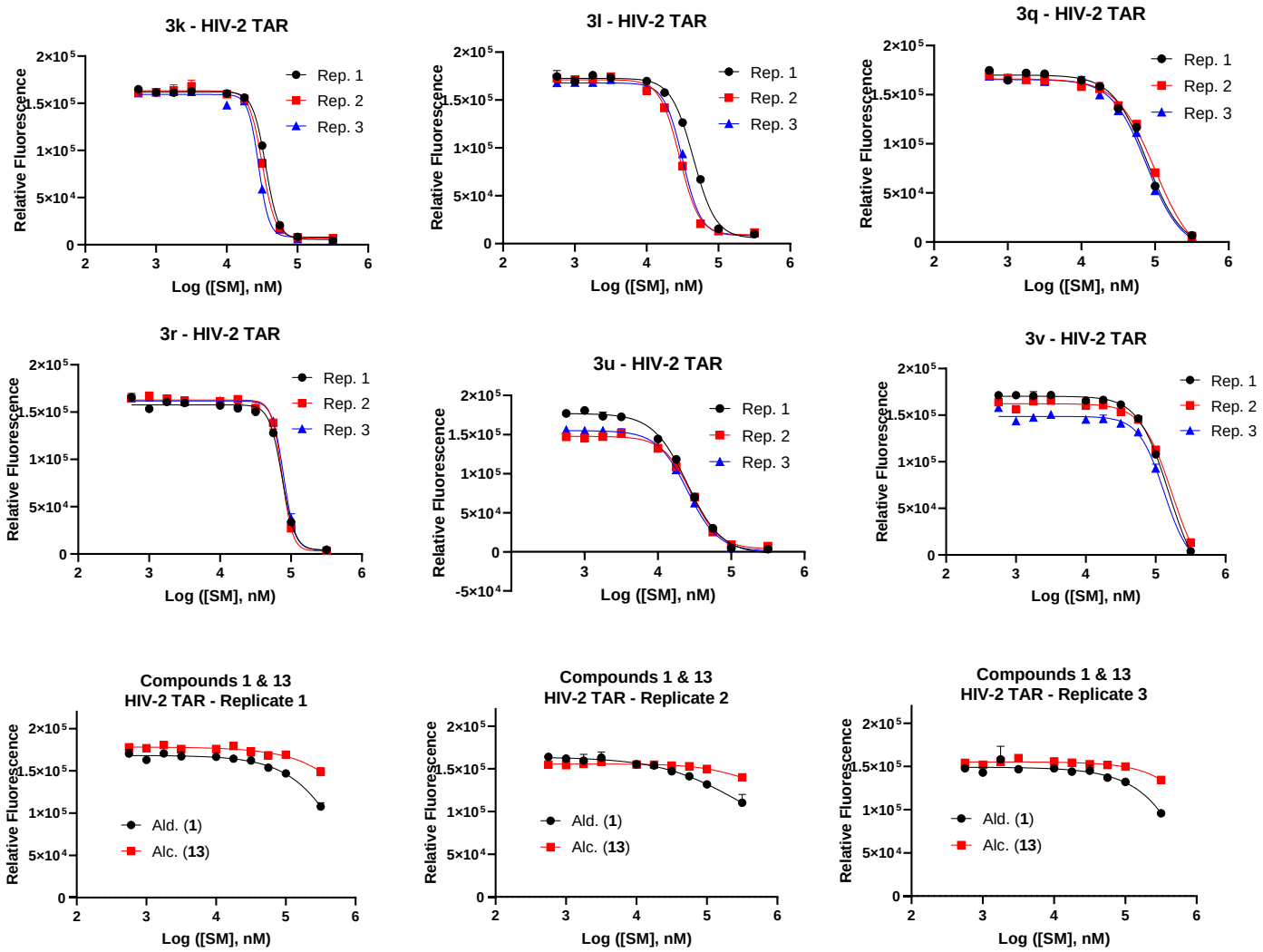


Figure S11. TOPRO-1 assay. Compound binding curves to HIV-2 TAR. Rep.: independent replicate. Each curve depicts one independent experiment (average values ± propagated standard deviation from technical replicates).

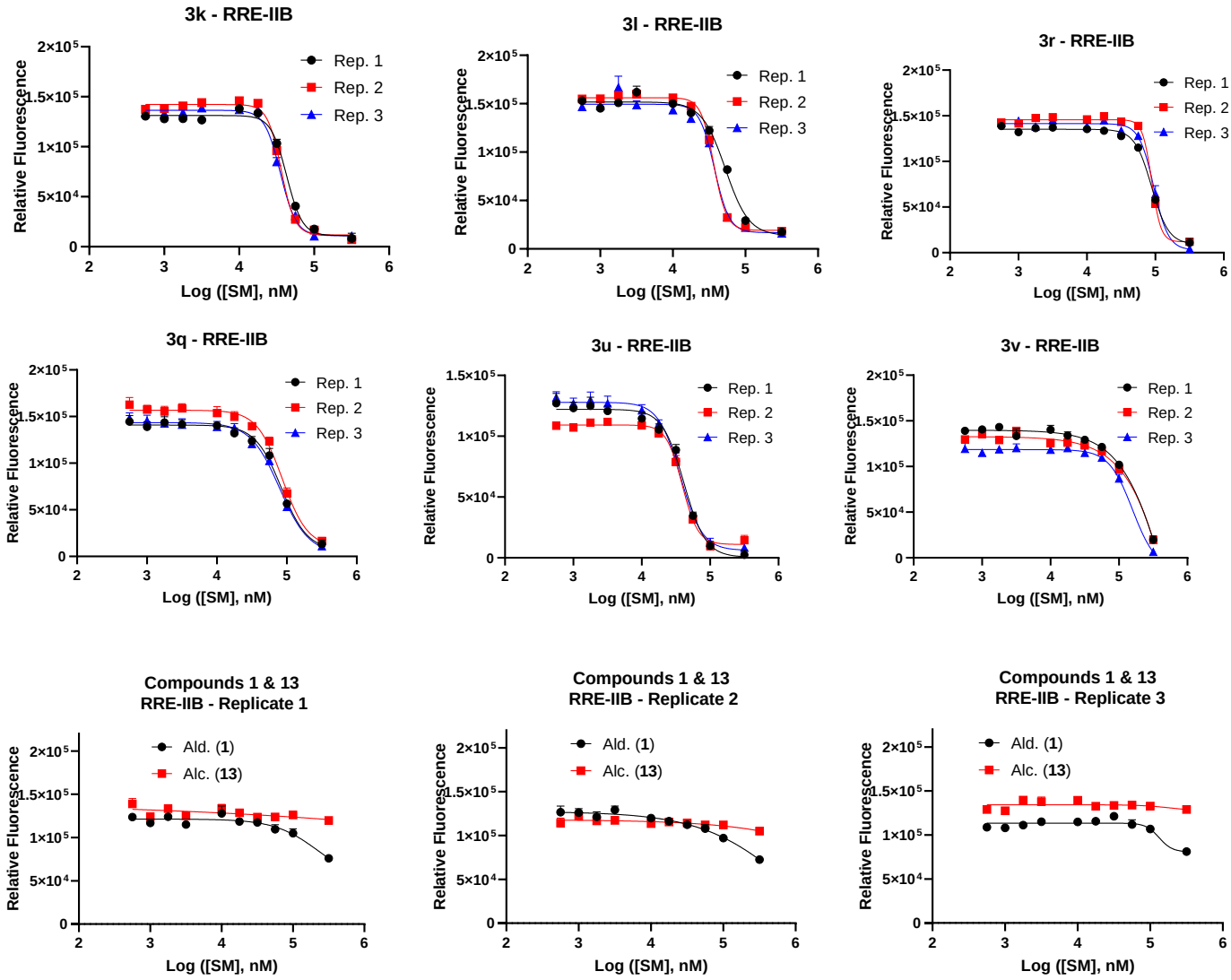


Figure S12. TOPRO-1 assay. Compound binding curves to RRE-IIB. Rep.: independent replicate. Each curve depicts one independent experiment (average values ± propagated standard deviation from technical replicates).

4.2 Surface plasmon resonance

Surface plasmon resonance (SPR) experiments were performed on a BIAcore T200 instrument (GE Healthcare Life Sciences) using a CM5 series S sensor chip. The temperature for the sensor chip modification and binding measurement was set to 25 ℃. The protocol recently published by Wilson and co-workers was followed to perform SPR measurements.[5] Specifically, the steps below were followed::

1. Instrument treatment to deactivate RNases:

After standard desorb procedure, three additional desorb steps using 50% (v/v) RNaseZap (Invitrogen by ThermoFisher Scientific) instead of desorb solution 1 and desorb solution 2 were conducted, then a manual run with 25 µL/min flow rate using Elga water was performed for 4 hrs to eliminate excess RNaseZap in the system.

2. Immobilization of streptavidin:

The CM5 series S sensor chip was docked and the flow was stopped. Then the running buffer was changed to the immobilization buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.005% (v/v) P20, pH = 7.4), and the

system was primed for 3 times to fully wet the surface and make it homogeneous with the running buffer. A manual run was started with 5 µL/min flow rate, before immobilization, a stable baseline was required, namely a curve with <1 ΔRU for 60 s. Then the following solutions were prepared and placed on the rack: 200 µg/ml streptavidin (Sigma-Aldrich) prepared in NaOAc/AcOH buffer (pH=4.5), 1 M Ethanolamine-HCl in water (pH 8.5) as deactivation solution (GE Healthcare Inc.), a mixture of 80 µL 1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) in water (11.5 mg/ml) and 80 µL *N*-hydroxysuccinimide (NHS) in water (75 mg/mL). Because EDC/NHS has a 30-min active window, it should be prepared right before use.

Right after the mixing of EDC and NHS, the activation of the sensor chip surface was started by injecting 50 µL EDC/NHS mixture into the reference and the working cells for 600 s. Then the streptavidin solution was injected at the same flow rate to both cells to reach 5798/5618 RU. After streptavidin immobilization, the deactivation solution was injected into both cells for 600 s to anneal any unreacted ligation sites on the surface.

3. Immobilization of HIV-1 TAR

The CM5 series S sensor chip with immobilized streptavidin was primed with immobilization buffer for 3 times, then a manual run with 1 µL/min flow rate was started. The biotinylated HIV-1 TAR (Integrated DNA Technologies) in a concentration of 34.3 µM was first annealed in nuclease-free water (Invitrogen) by heating for 5 min at 95 ºC followed by cooling on ice for 1 hr. Before immobilization, a stable baseline was achieved when <1 ΔRU was observed in a 120-s window. The flow path was then switched to the working cell only and 25 nM 5'-biotinylated HIV-1 TAR (diluted in pH 4.5 NaOAc/AcOH buffer) was injected to achieve 769.5/702.3 RU density. The system was switched to running buffer (50 mM Tris-HCl, 50 mM KCl, 5% DMSO, 0.01% Triton-X-100, pH 7.4) and primed for 3 times before measurement.

4. Binding measurement

Binding measurements were carried out for each compound by injecting various concentrations of the compound (Table S9) in running buffer (50 mM Tris-HCl, 50 mM KCl, 5% DMSO, 0.01% Triton-X-100, pH 7.4) at 50 µL/min flow rate at 25 ºC. The association process lasted for 60 s followed by a 120-s dissociation process and a 60-s regeneration process using 1 M NaCl aqueous solution. At least five non-zero concentrations were applied for each compound and one of the concentrations was repeated to evaluate internal stability of the instrument. Each sensorgram (Figure S14) was processed by subtracting the reference cell signal and then the zero-concentration injection signal. Binding constants (Table S10) were obtained by global fit using a 1:1 Langmuir binding equation using BIAevaluation software (v2.0).

Table S9. Compound concentrations used in SPR assays.

Compound / Conc (µM)	**3q**	**3l**	**3r**	**3u**	**3k**	**3v**
1st sensor chip (Replicates 1–4)	0	0	0	0	0	0
	1.563	3.125	3.125	3.125	3.125	3.125
	3.125	6.25	6.25	6.25	6.25	6.25
	6.25	12.5	12.5	12.5	12.5	12.5
	12.5	15	15	15	15	25
	15	25	25	25	25	31.25
	6.25	12.5	12.5	12.5	12.5	12.5
2nd sensor chip (Replicates 5–6)	0	0	0	0	0	0
	2	4	5	5	5	10
	4	6	7.5	7.5	10	15
	6	8	10	10	15	17.95
	8	10	12.5	12.5	20	20
	10	12	15	15	25	25
	6	8	10	10	15	20

Table S10. Equilibrium dissociation constants of DCC library members to HIV-1 TAR.

	K_D (μM)							
	Rep. 1[a]	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Rep. 6	Average	Standard deviation
3k	123.0	71.9	-[b]	10.8	13.8	7.6	45.4	50.9
3l	152.0	132.0	94.2	-	45.8	10.3	86.9	58.9
3q	781.0	673.0	786.0	725.0	781.0	386.0	688.7	154.7
3r	276.0	83.6	953.0	641.0	101.0	577.0	438.6	343.9
3u	108.0	127.0	407.0	30.7	4.3	5.3	113.7	152.9
3v	1480.0	114.0	-	891.0	32.6	32.5	510.0	651.7

[a]Rep.: Replicate
[b]Data unavailable. Sensor chip quality had deteriorated to some extent.

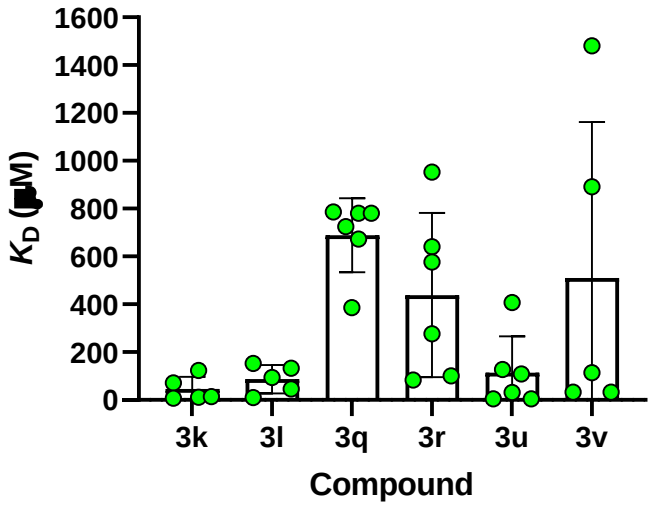


Figure S13. Equilibrium dissociation constants (K_D) of DCC library members with HIV-1 TAR determined by SPR. Bar graph: means of K_D values; error bars: standard deviation. For 3r and 3v, steady-state fitting may be necessary to obtain a more consistent K_D.

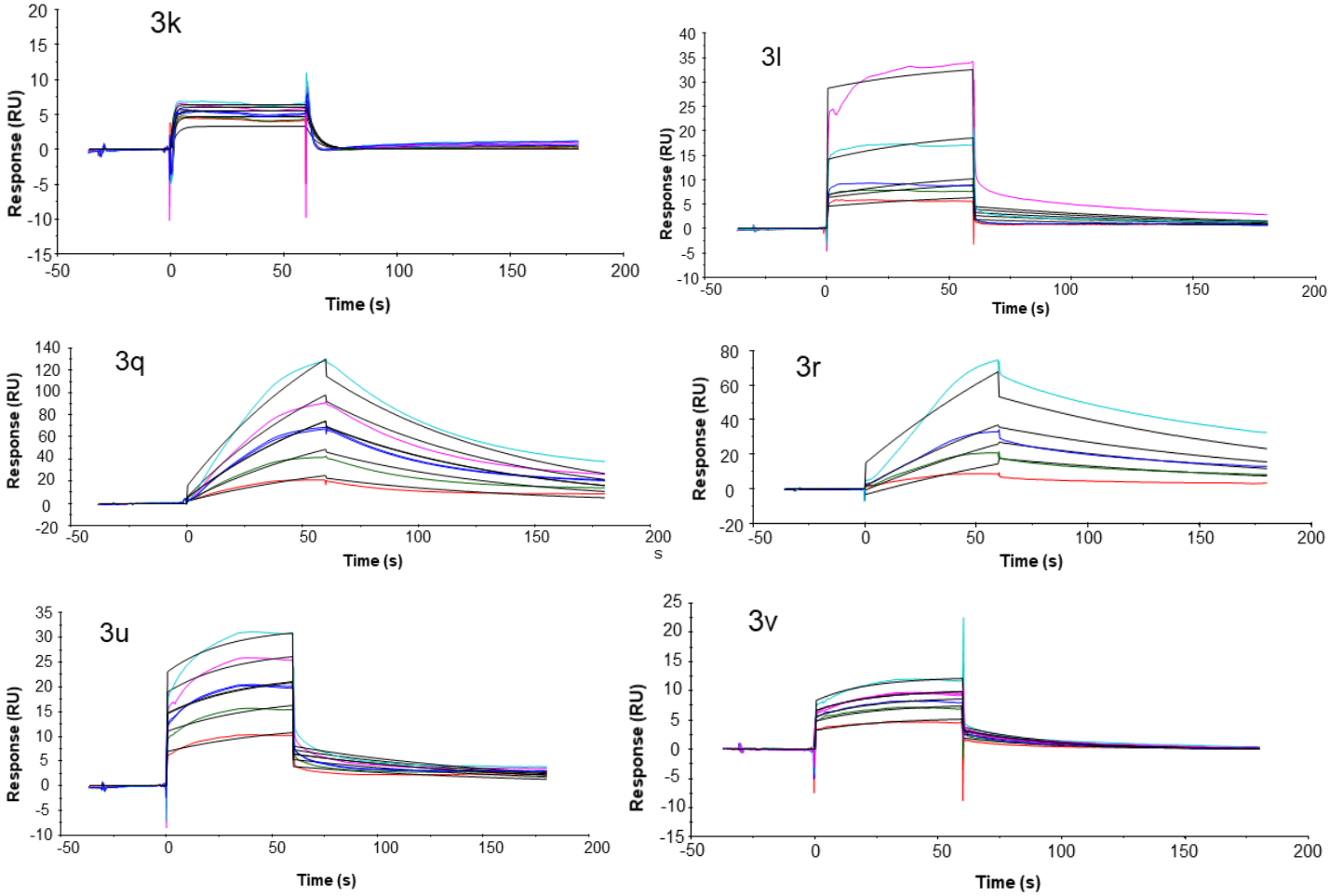
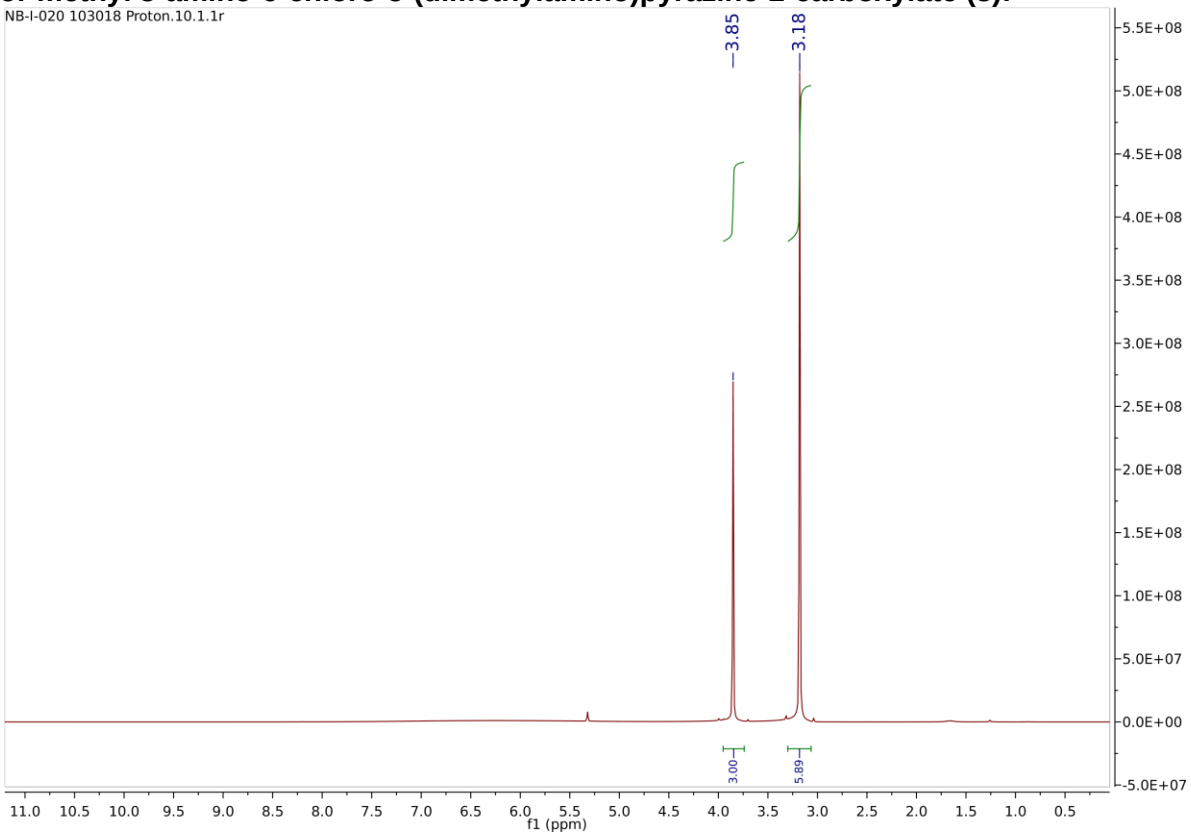


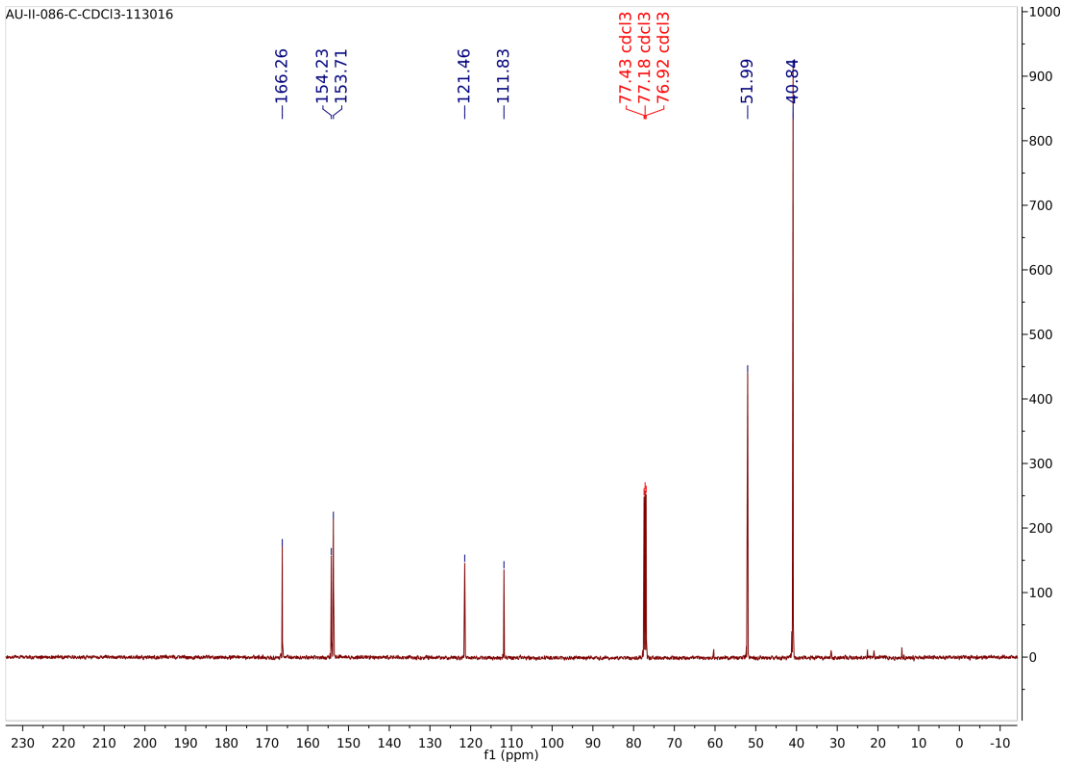
Figure S14. SPR sensorgrams of DCC library members binding to HIV-1 TAR. Color: measured curves. Black: fitted curves.

5. NMR spectra of intermediates and final compounds

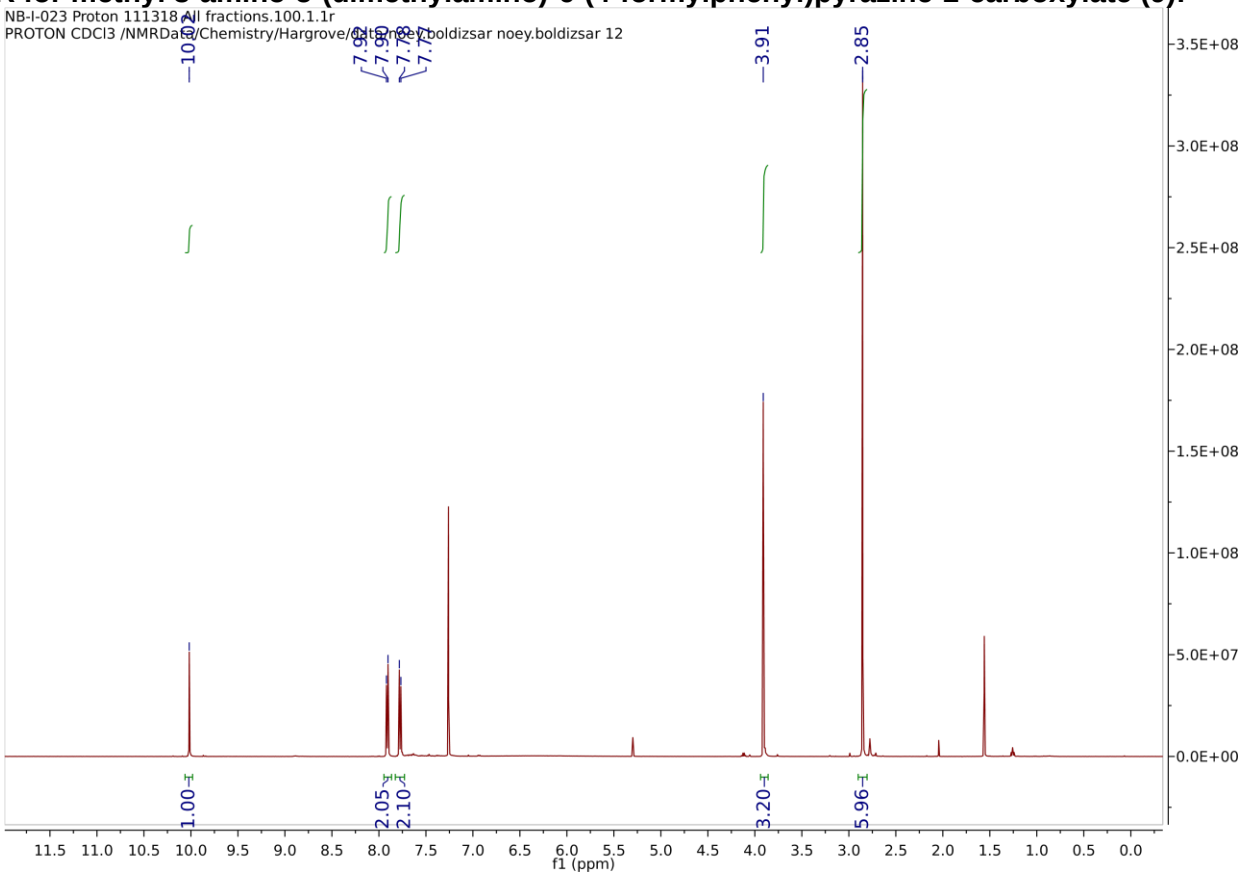
^{1}H NMR for methyl 3-amino-6-chloro-5-(dimethylamino)pyrazine-2-carboxylate (8):



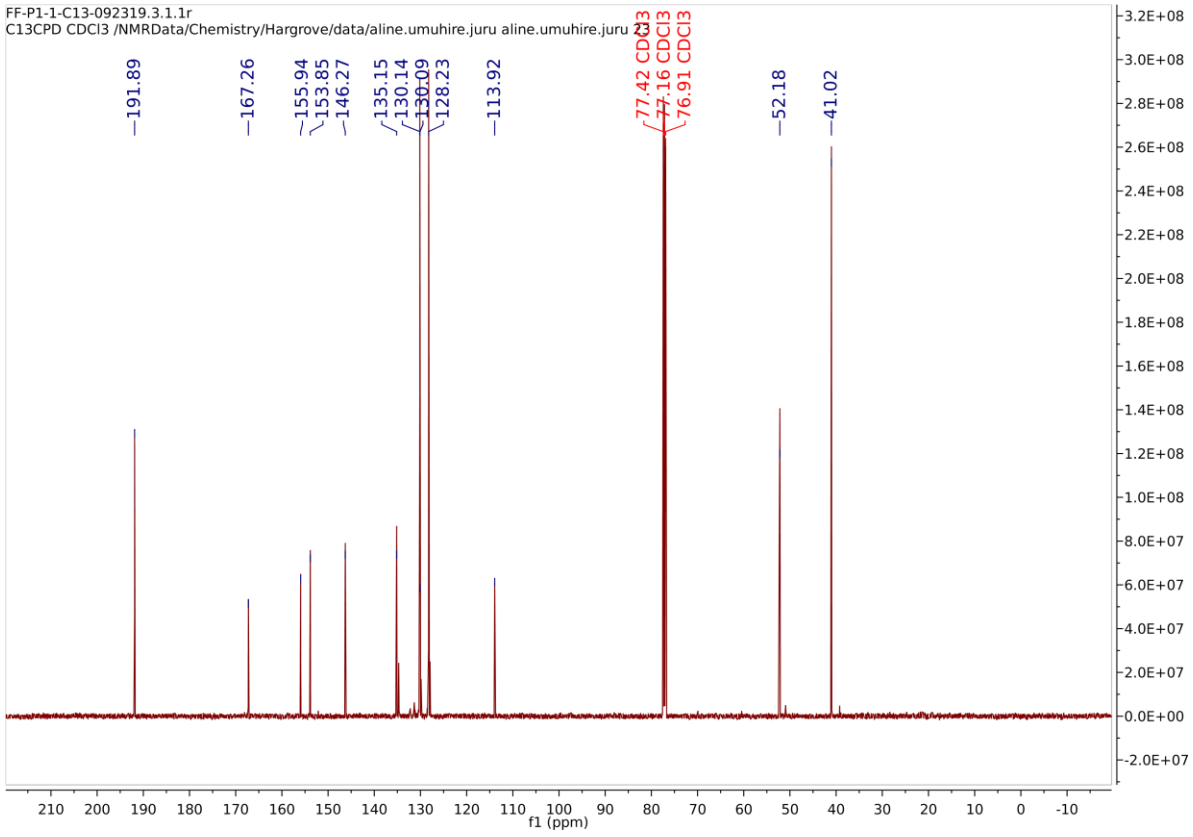
^{13}C NMR for methyl 3-amino-6-chloro-5-(dimethylamino)pyrazine-2-carboxylate (8):



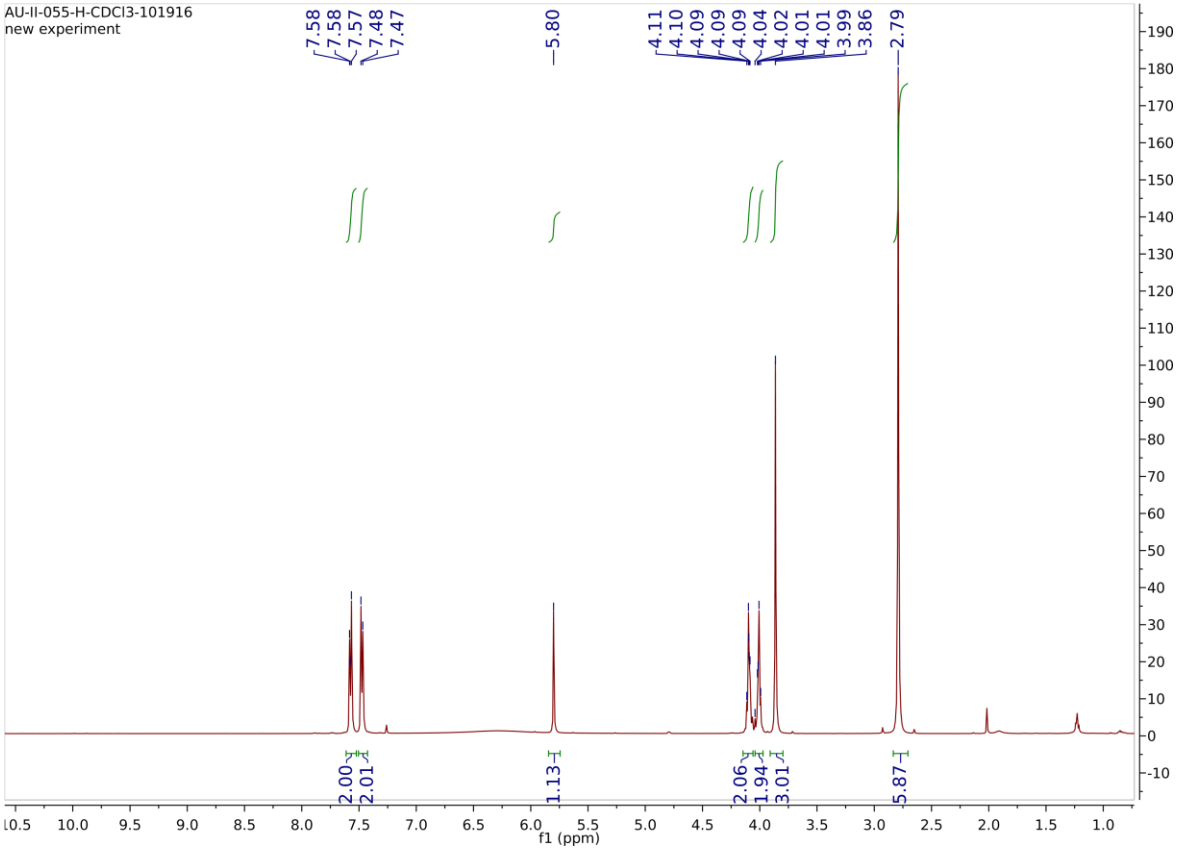
^{1}H NMR for methyl 3-amino-5-(dimethylamino)-6-(4-formylphenyl)pyrazine-2-carboxylate (9):



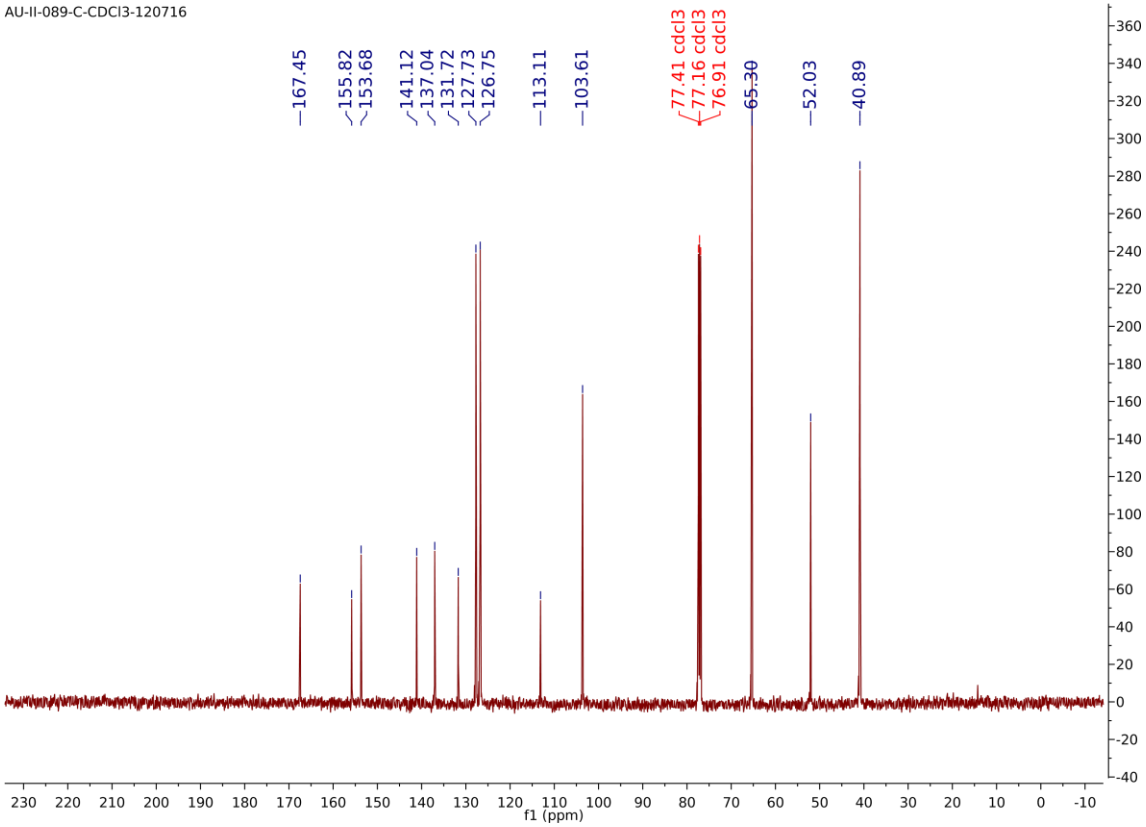
^{13}C NMR for methyl 3-amino-5-(dimethylamino)-6-(4-formylphenyl)pyrazine-2-carboxylate (9):



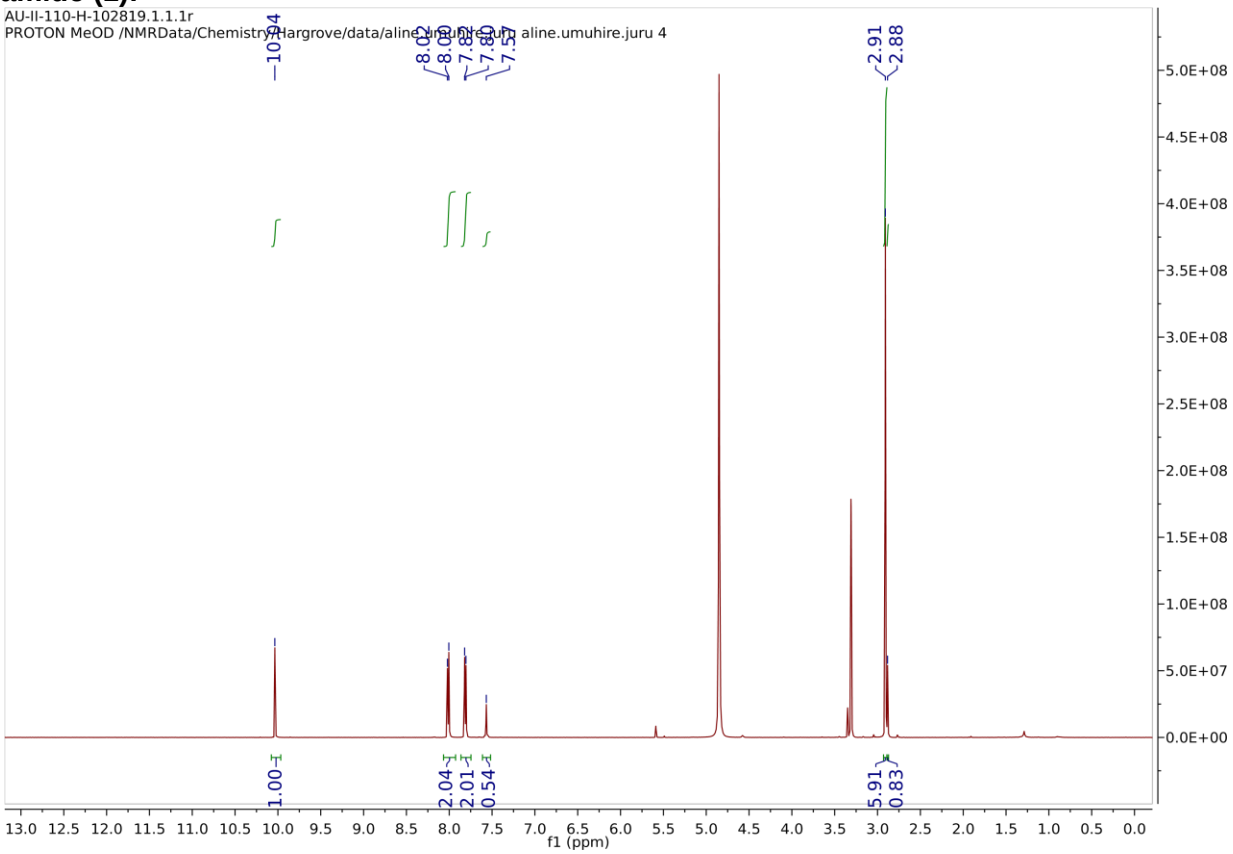
^{1}H NMR for methyl 6-(4-(1,3-dioxolan-2-yl)phenyl)-3-amino-5-(dimethylamino)pyrazine-2-carboxylate (10):



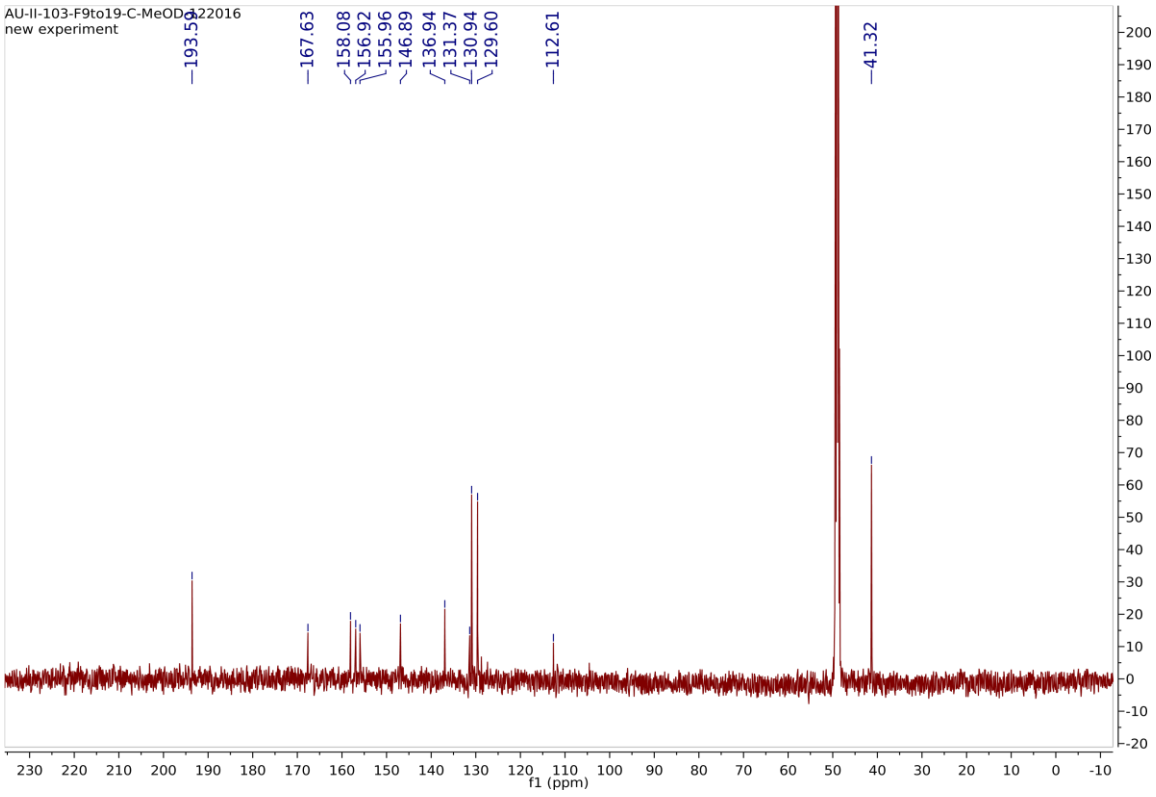
^{13}C NMR for methyl 6-(4-(1,3-dioxolan-2-yl)phenyl)-3-amino-5-(dimethylamino)pyrazine-2-carboxylate (10):



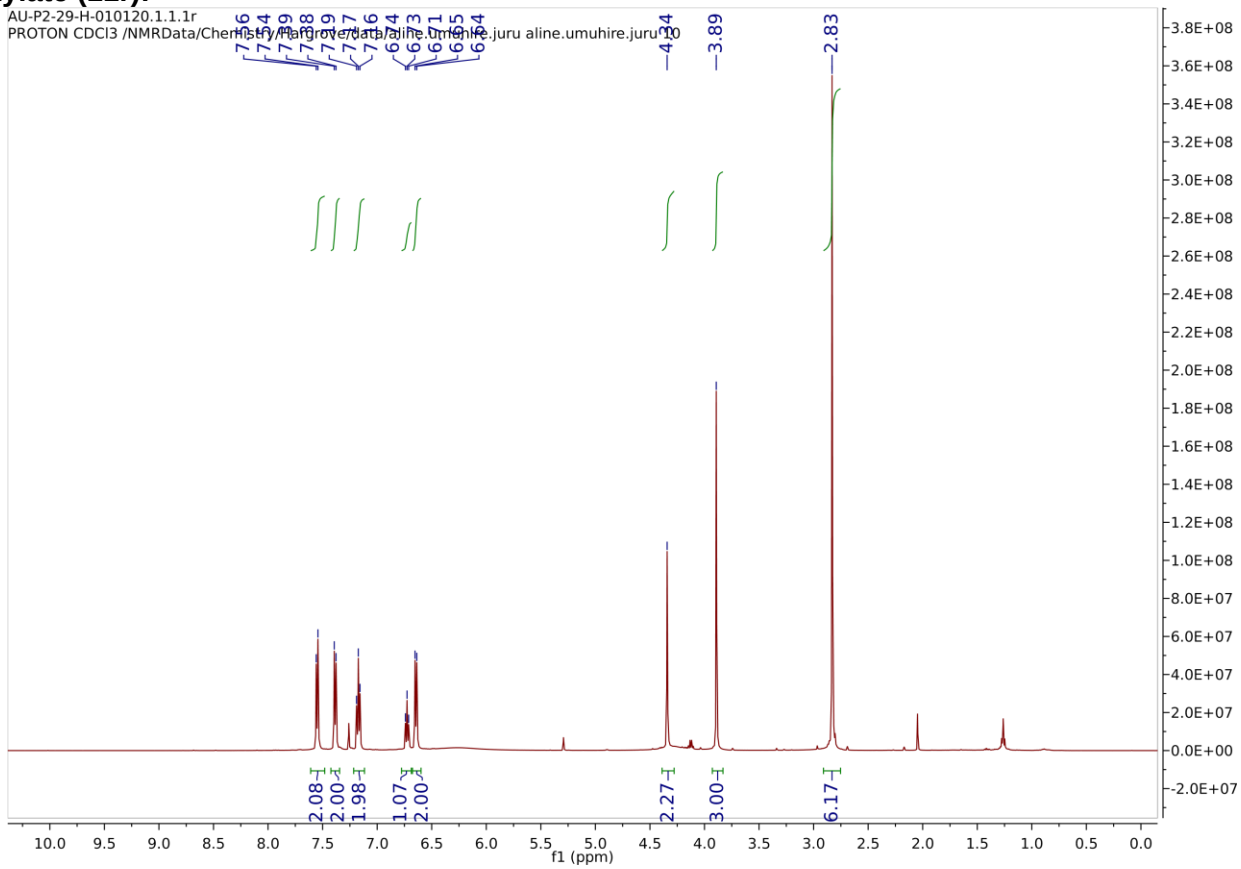
^{1}H NMR for 3-amino-N-(diaminomethylene)-5-(dimethylamino)-6-(4-formylphenyl)pyrazine-2-carboxamide (1):



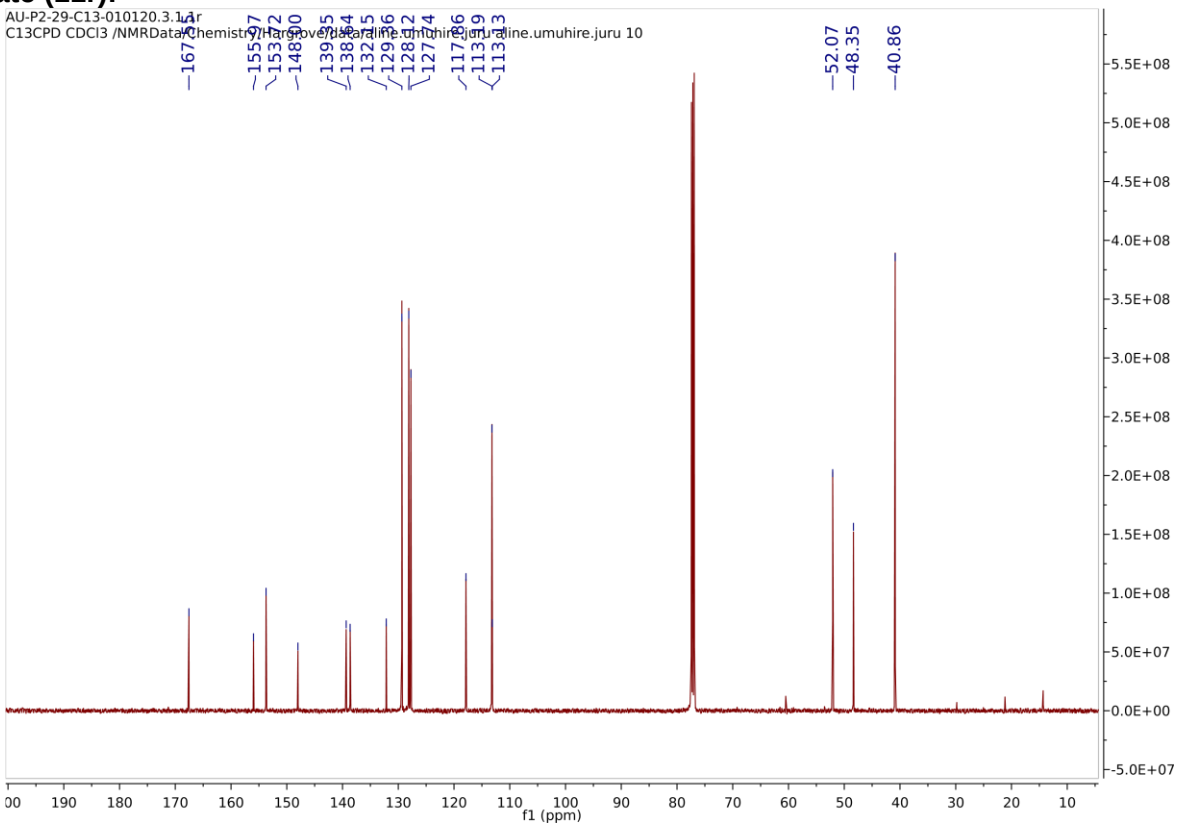
^{13}C NMR for 3-amino-N-(diaminomethylene)-5-(dimethylamino)-6-(4-formylphenyl)pyrazine-2-carboxamide (1):



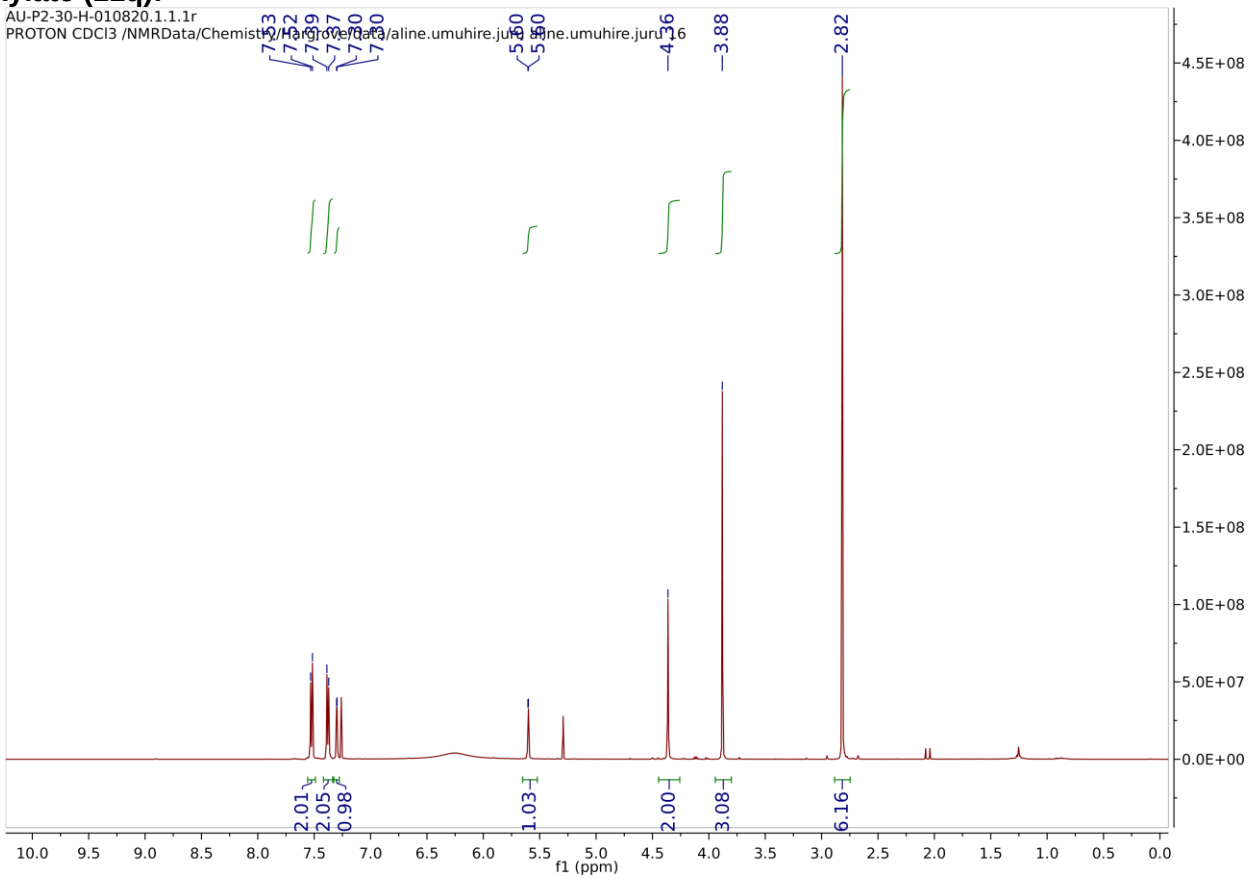
^{1}H NMR for methyl 3-amino-5-(dimethylamino)-6-(4-((phenylamino)methyl)phenyl)pyrazine-2-carboxylate (11r):



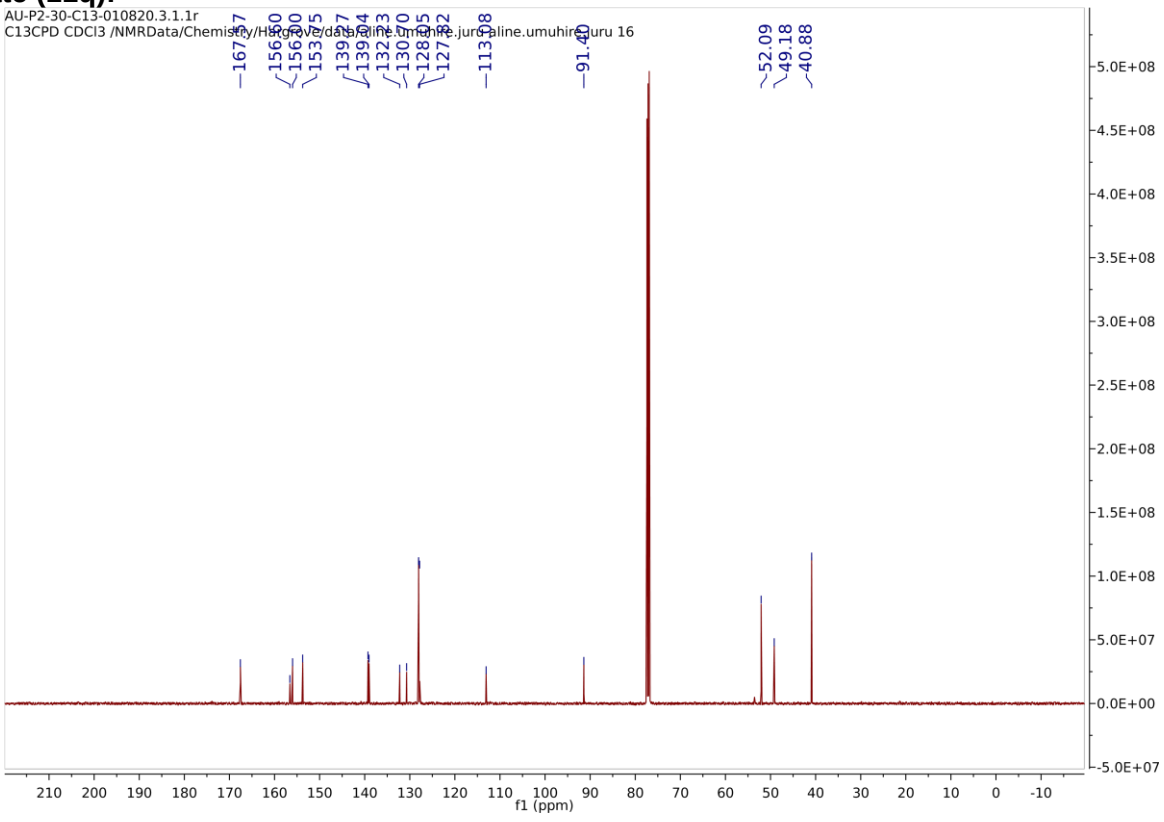
^{13}C NMR for methyl 3-amino-5-(dimethylamino)-6-(4-((phenylamino)methyl)phenyl)pyrazine-2-carboxylate (11r):



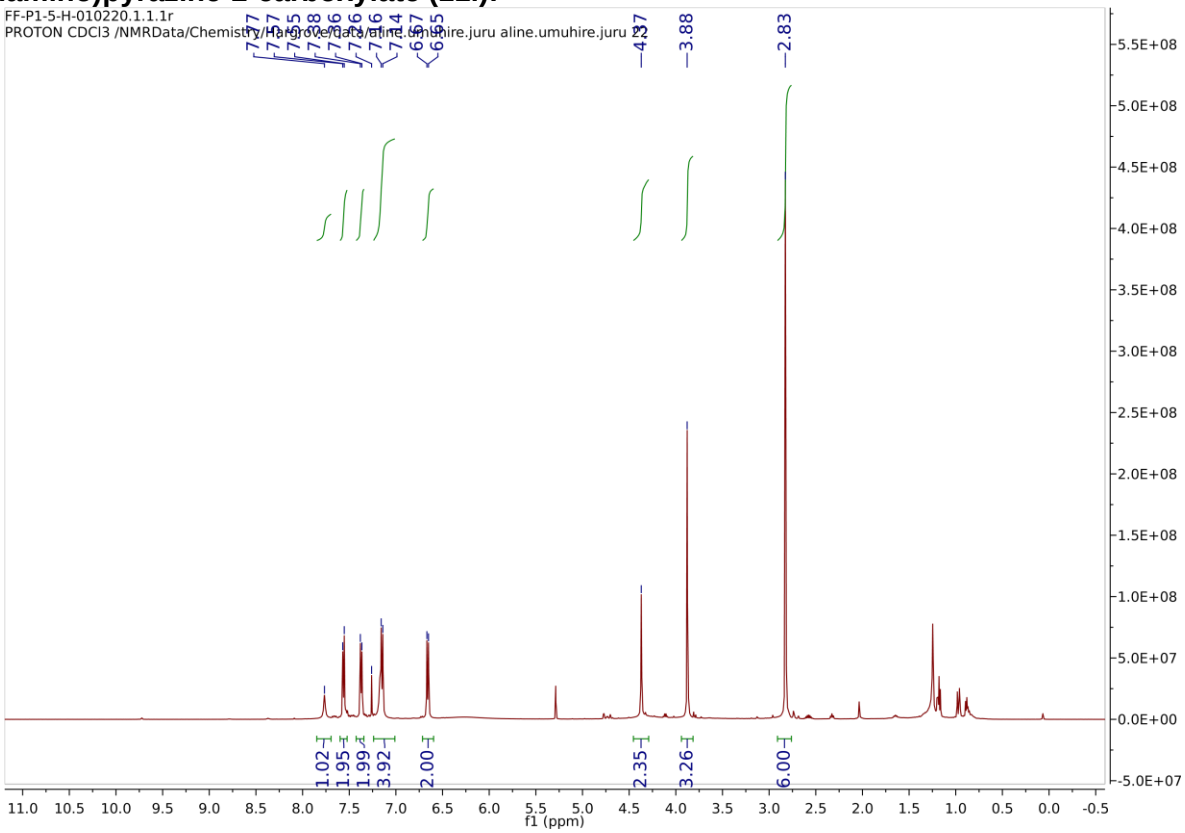
^{1}H NMR for methyl 6-(4-(((1H-pyrazol-5-yl)amino)methyl)phenyl)-3-amino-5-(dimethylamino)pyrazine-2-carboxylate (11q):



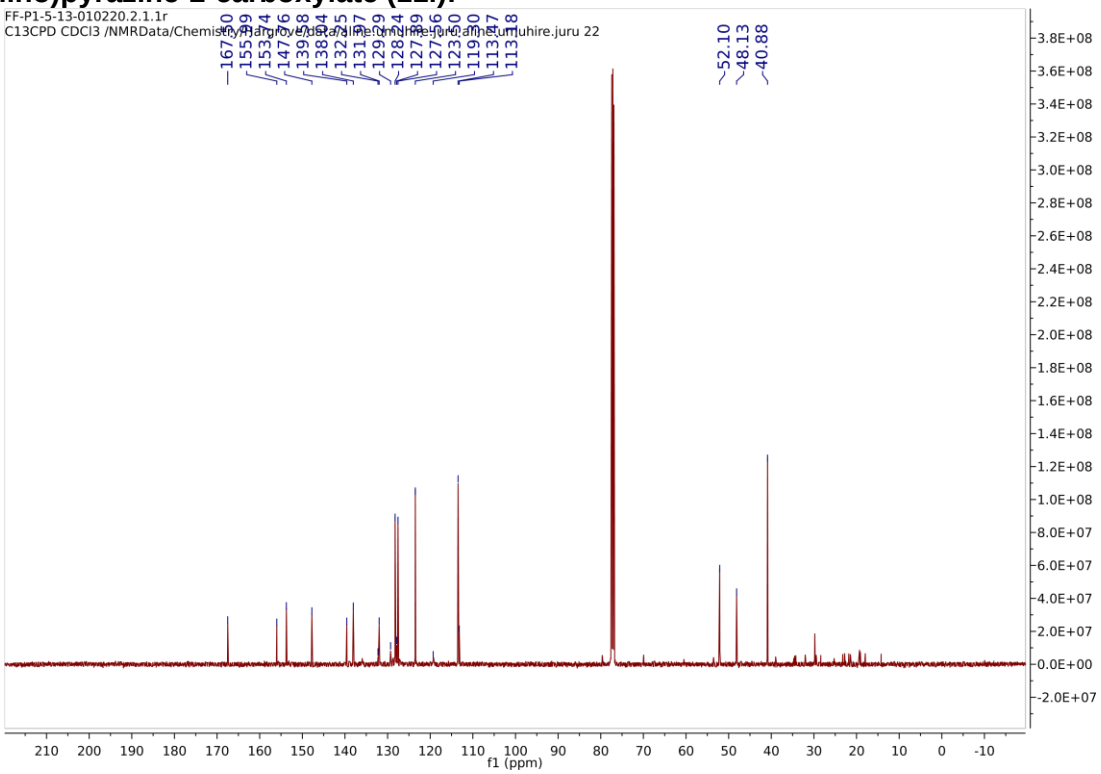
^{13}C NMR for methyl 6-(4-(((1H-pyrazol-5-yl)amino)methyl)phenyl)-3-amino-5-(dimethylamino)pyrazine-2-carboxylate (11q):



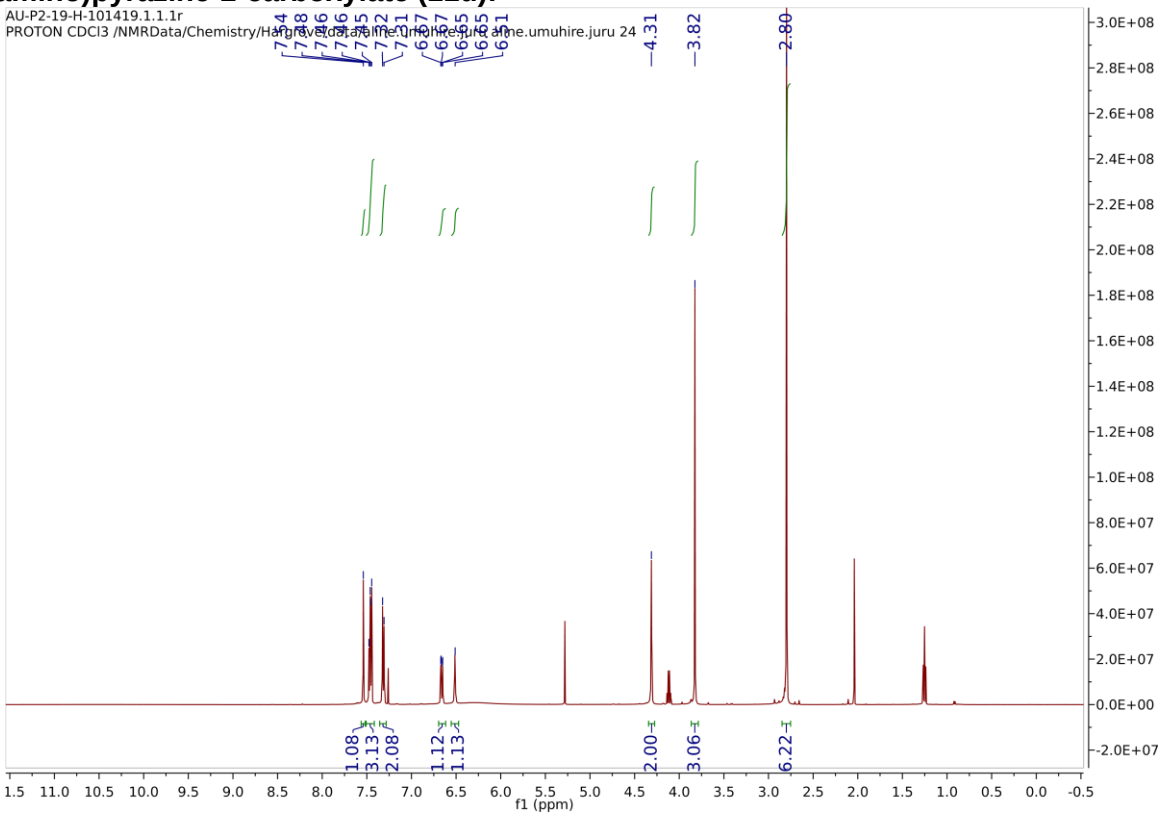
^{1}H NMR for methyl 6-(4-(((4-(1H-imidazol-1-yl)phenyl)amino)methyl)phenyl)-3-amino-5-(dimethylamino)pyrazine-2-carboxylate (11l):



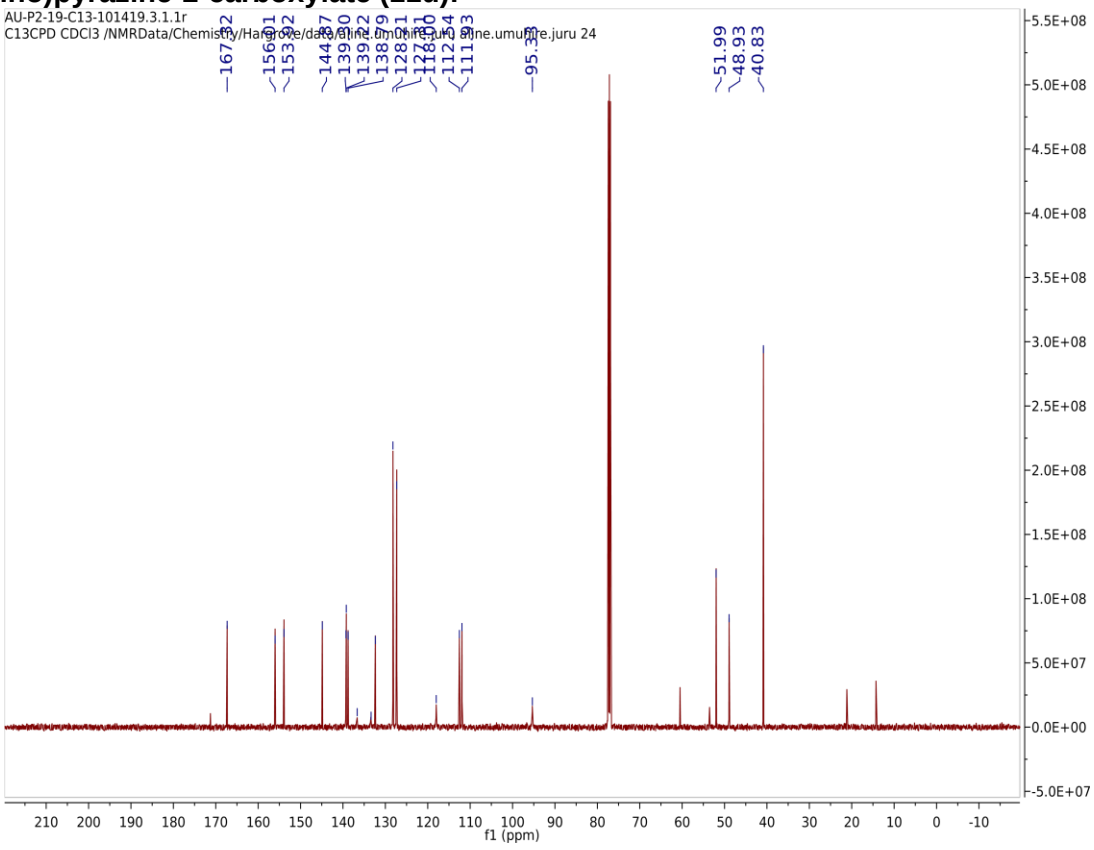
^{13}C NMR for methyl 6-(4-(((4-(1H-imidazol-1-yl)phenyl)amino)methyl)phenyl)-3-amino-5-(dimethylamino)pyrazine-2-carboxylate (11l):



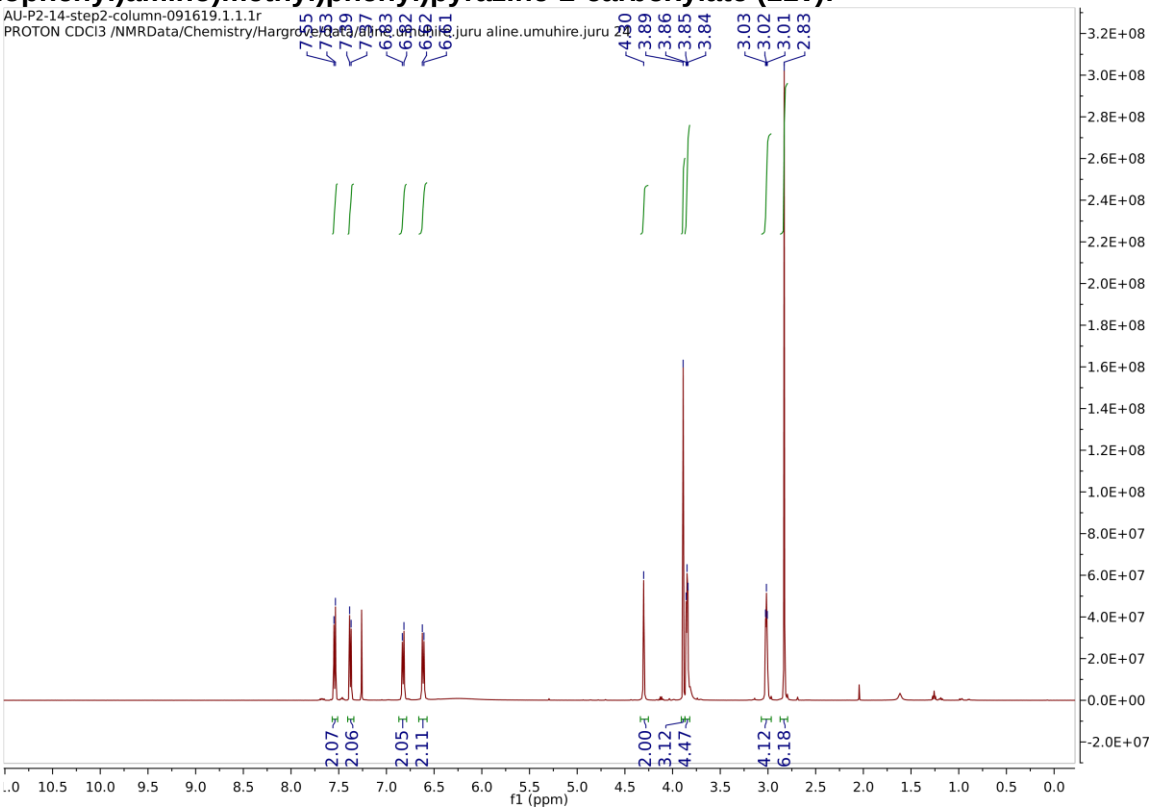
^{1}H NMR for methyl 6-(4-(((1H-benzo[d]imidazol-5-yl)amino)methyl)phenyl)-3-amino-5-(dimethylamino)pyrazine-2-carboxylate (11u):



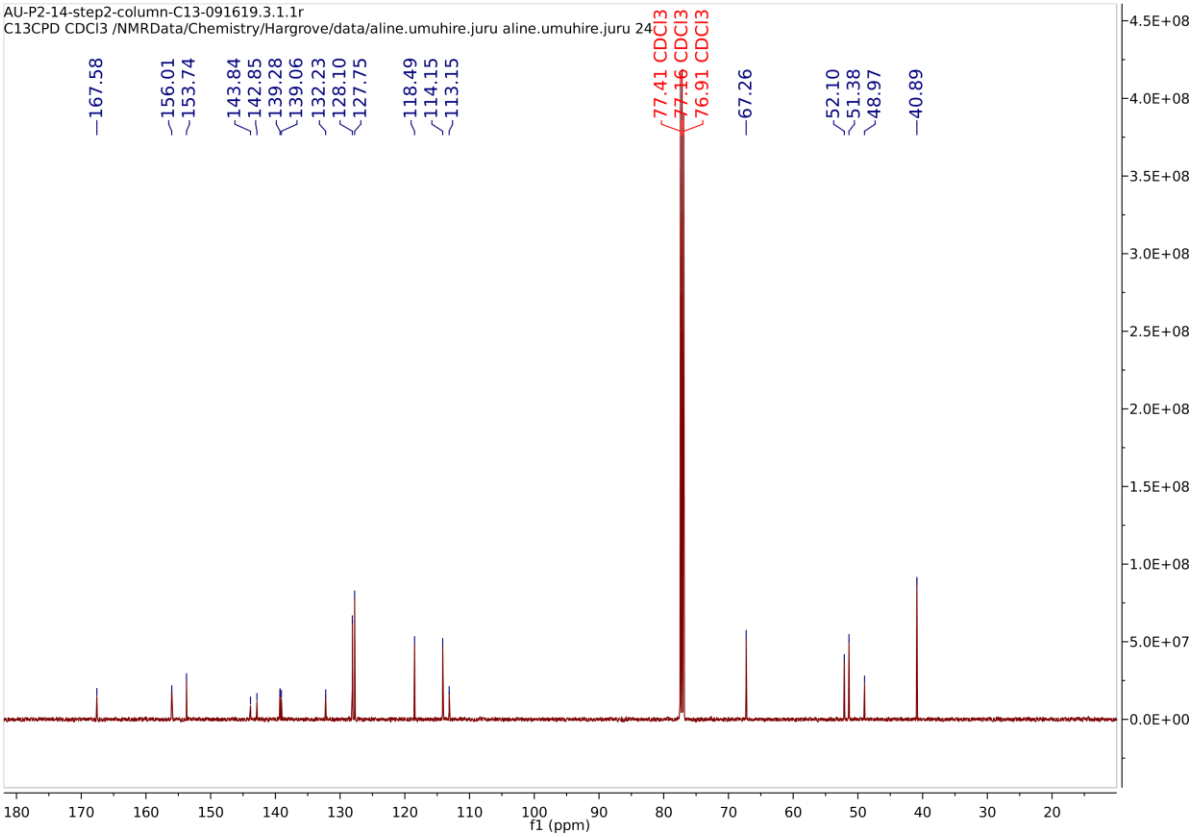
^{13}C NMR for methyl 6-(4-(((1H-benzo[d]imidazol-5-yl)amino)methyl)phenyl)-3-amino-5-(dimethylamino)pyrazine-2-carboxylate (11u):



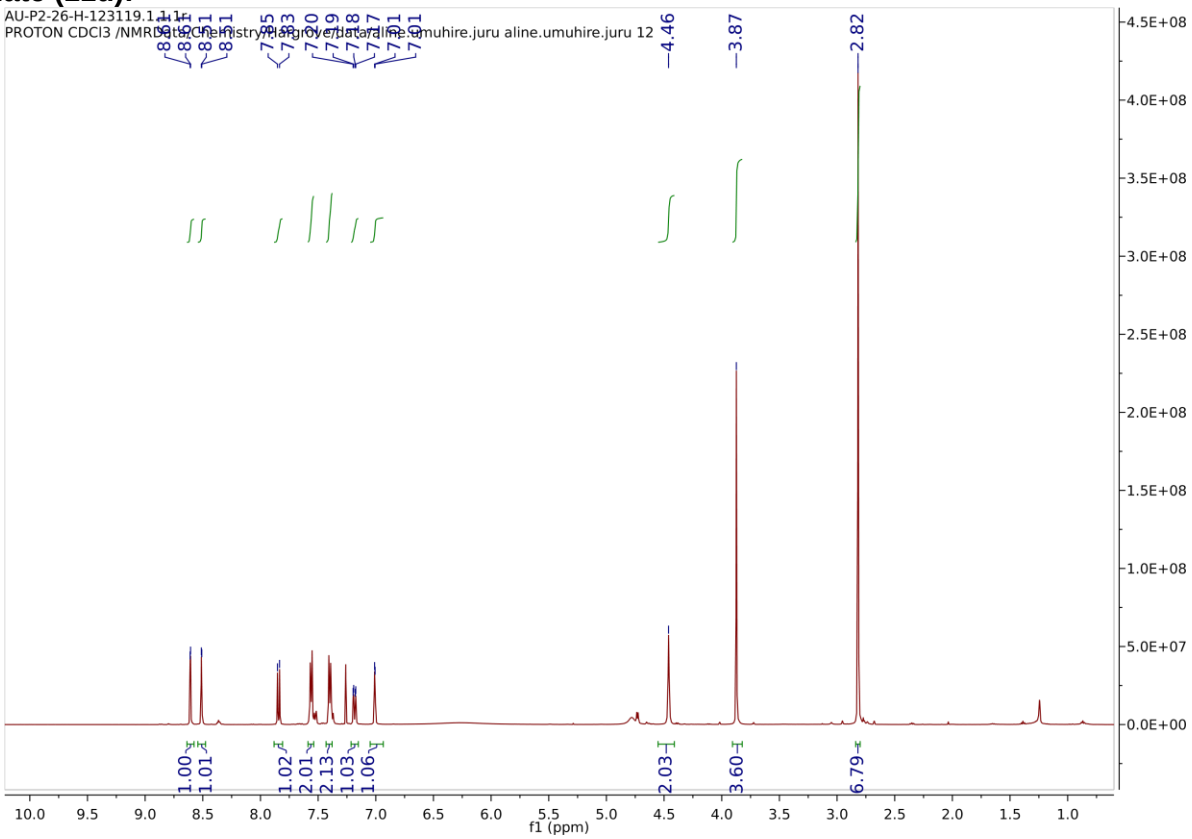
^{1}H NMR for methyl 3-amino-5-(dimethylamino)-6-(4-(((4-morpholinophenyl)amino)methyl)phenyl)pyrazine-2-carboxylate (11v):



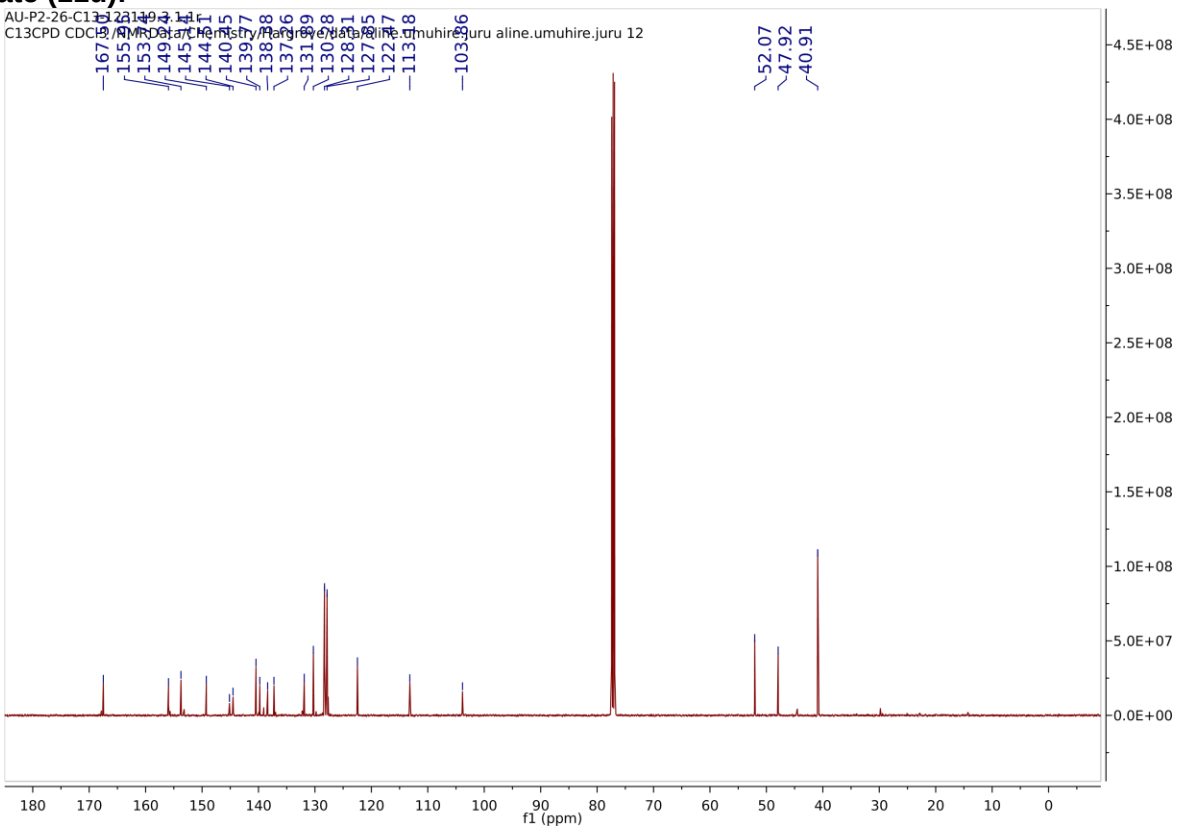
^{13}C NMR for methyl 3-amino-5-(dimethylamino)-6-(4-(((4-morpholinophenyl)amino)methyl)phenyl)pyrazine-2-carboxylate (11v):



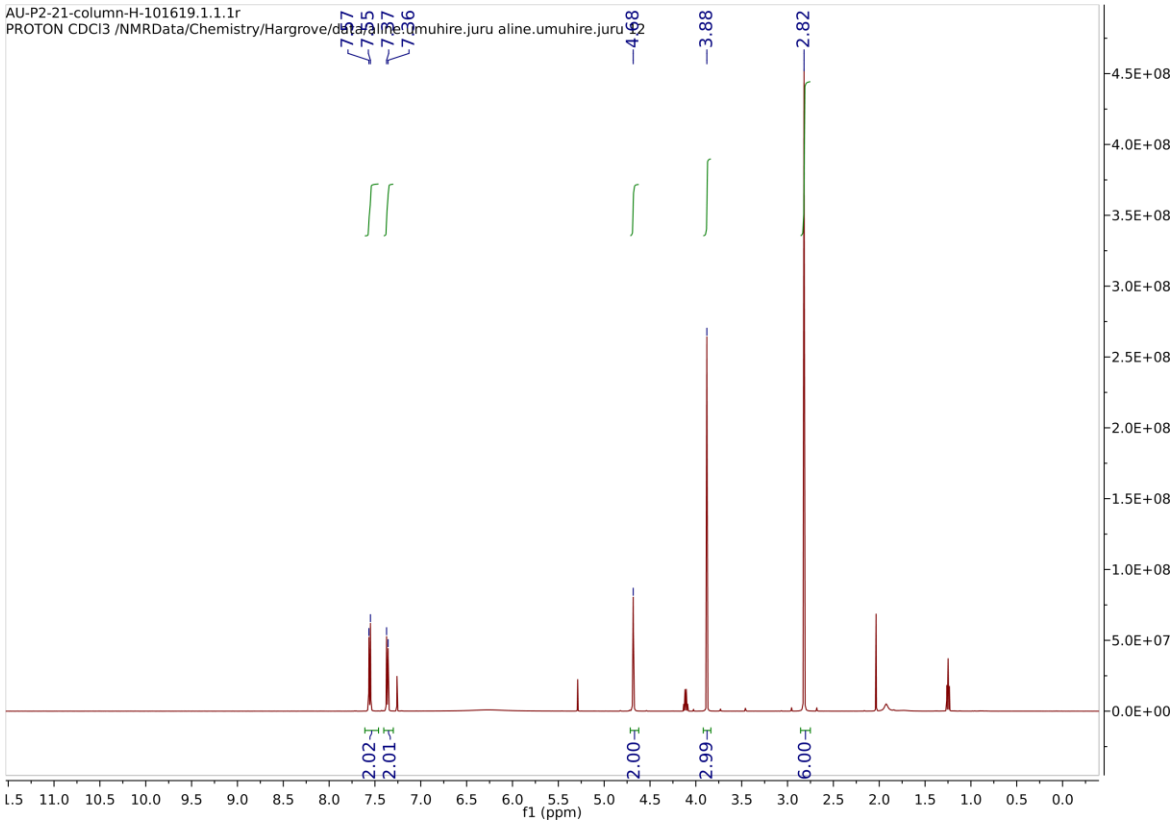
^{1}H NMR for methyl 3-amino-5-(dimethylamino)-6-(4-((quinoxalin-6-ylamino)methyl)phenyl)pyrazine-2-carboxylate (11u):



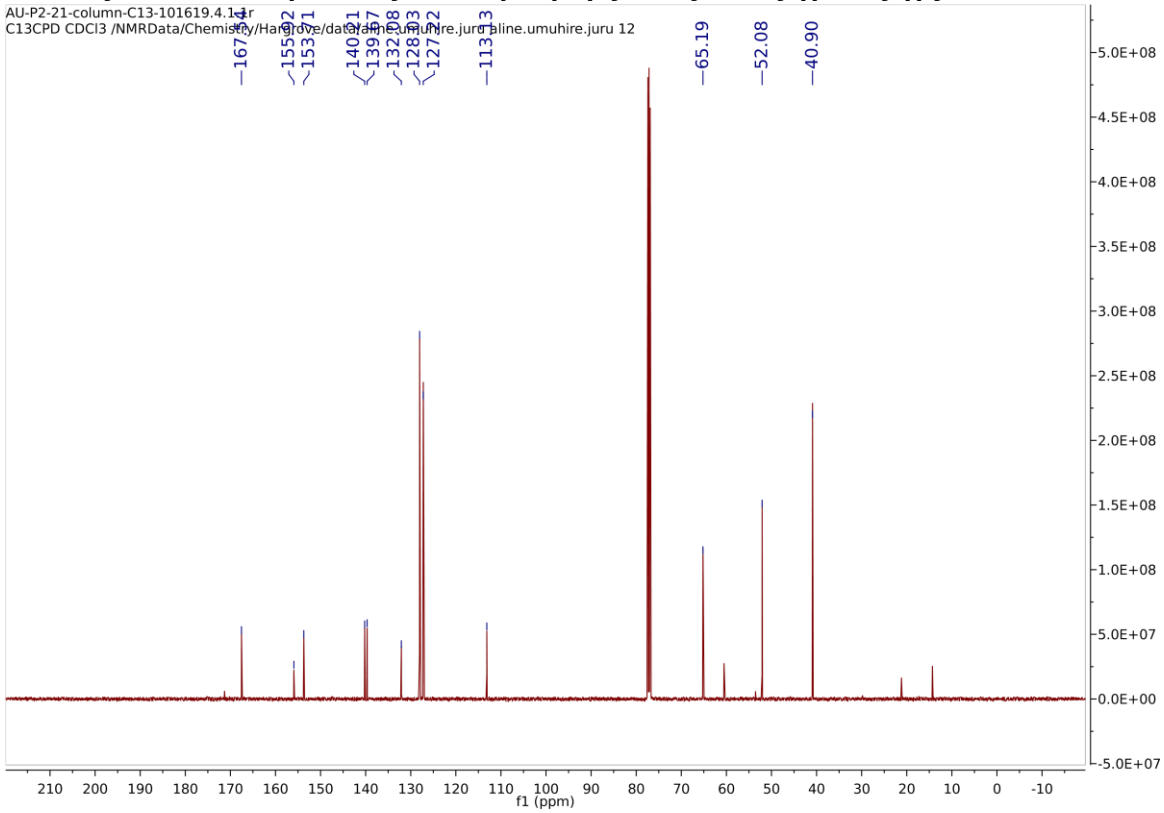
^{13}C NMR for methyl 3-amino-5-(dimethylamino)-6-(4-((quinoxalin-6-ylamino)methyl)phenyl)pyrazine-2-carboxylate (11u):



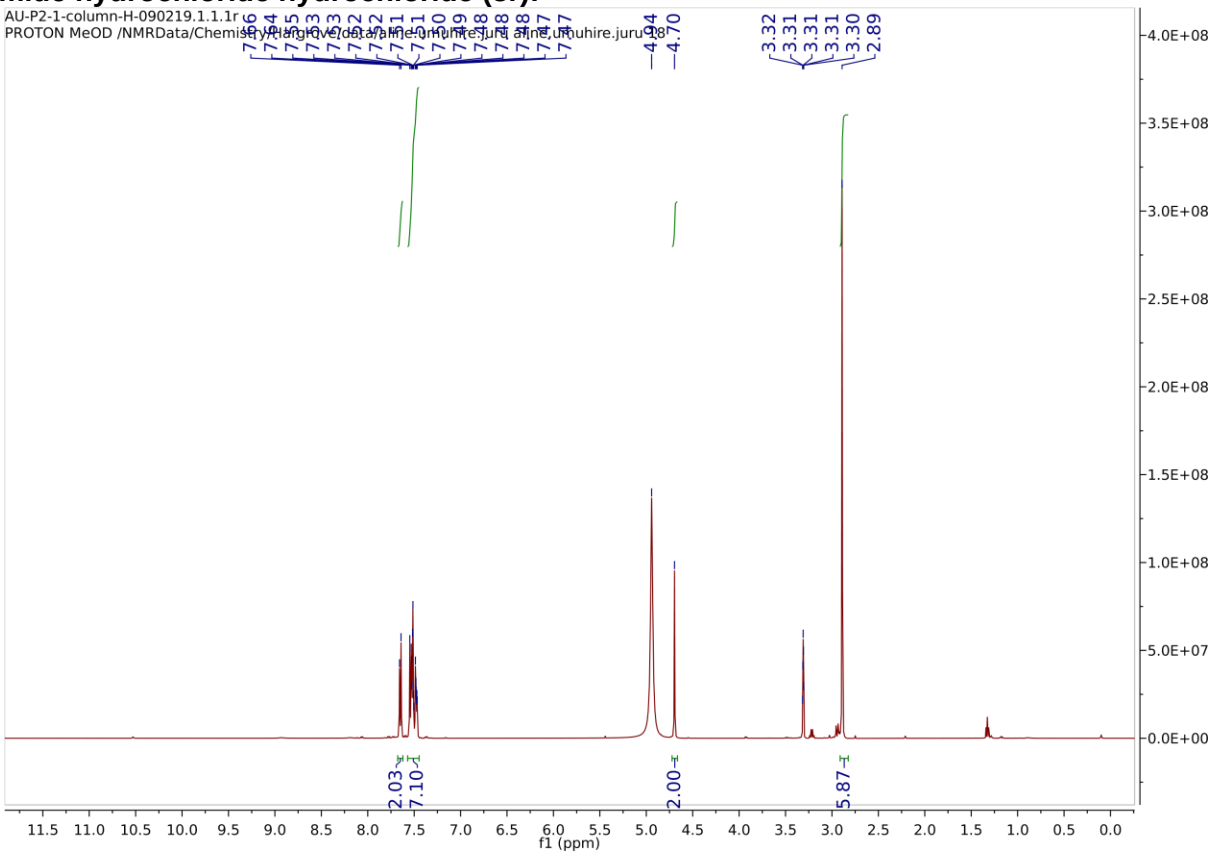
^{1}H NMR for methyl 3-amino-5-(dimethylamino)-6-(4-(hydroxymethyl)phenyl)pyrazine-2-carboxylate (12):



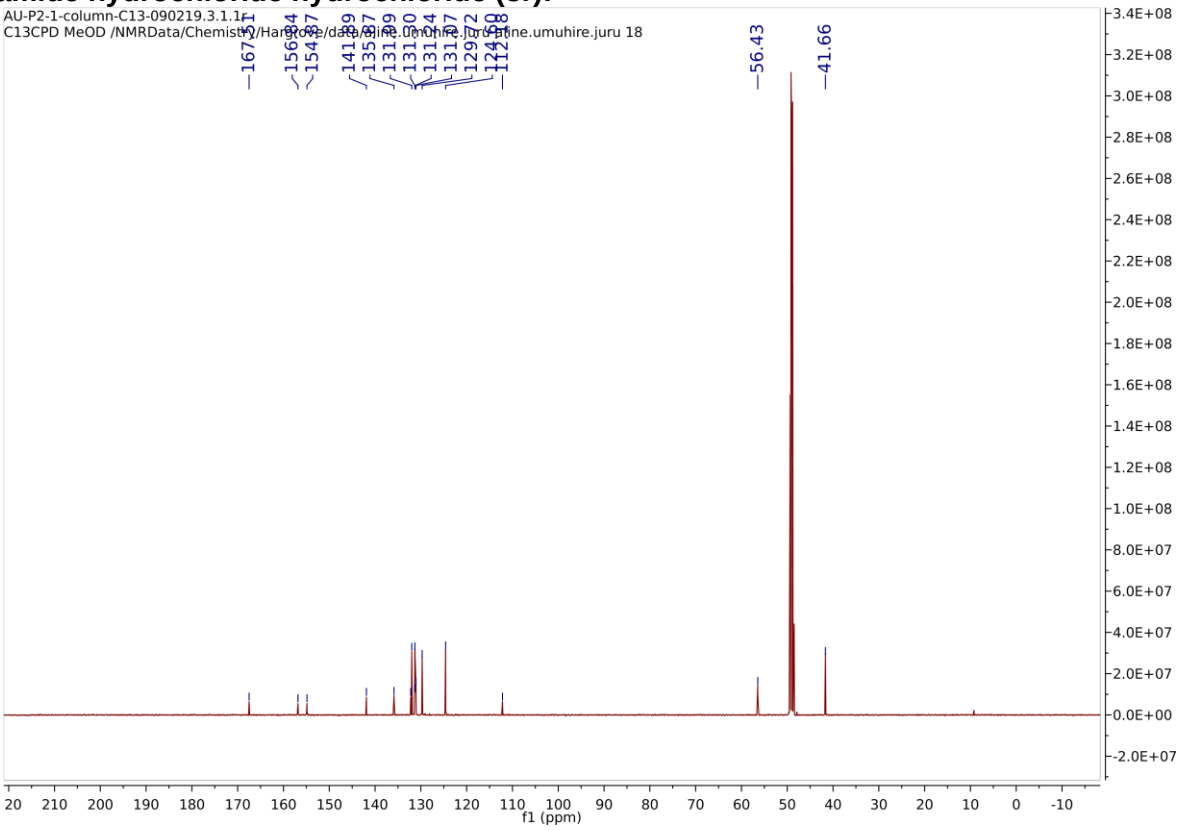
^{13}C NMR for methyl 3-amino-5-(dimethylamino)-6-(4-(hydroxymethyl)phenyl)pyrazine-2-carboxylate (12):



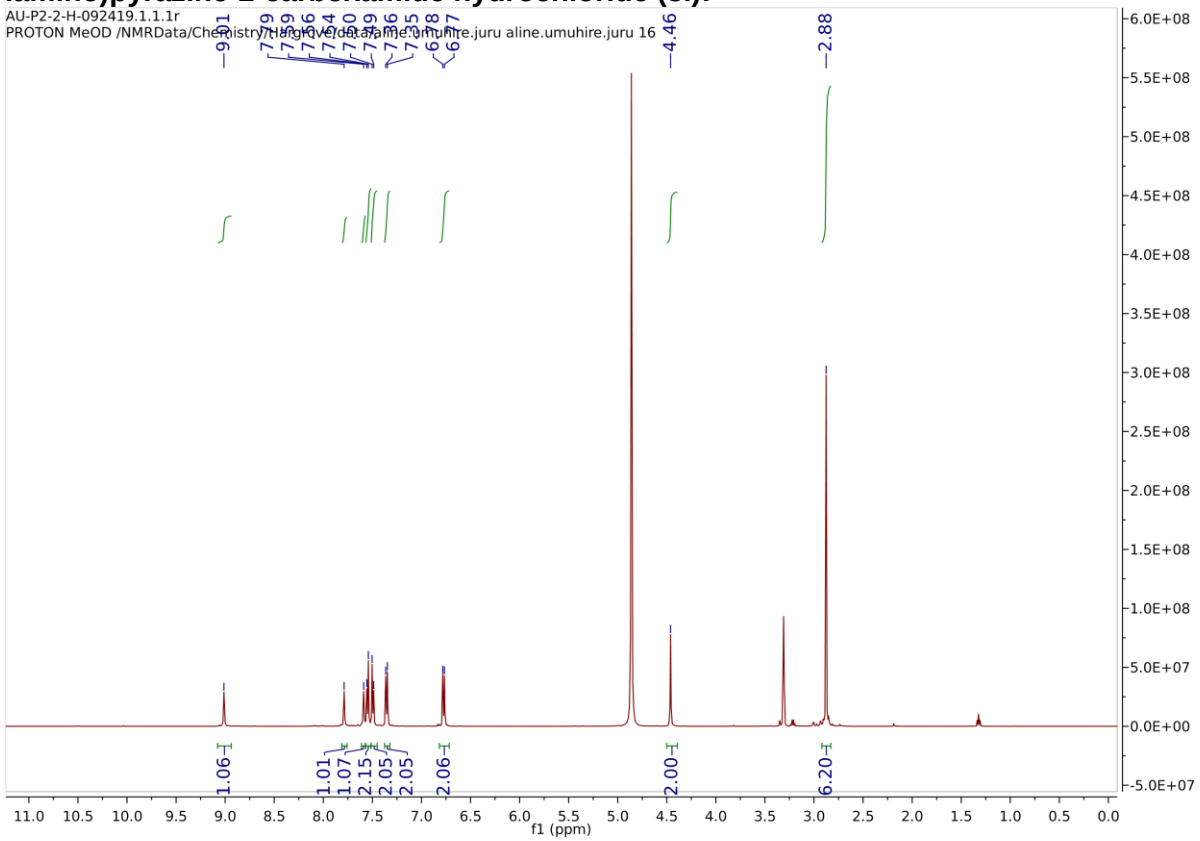
^{1}H NMR for 3-amino-N-carbamimidoyl-5-(dimethylamino)-6-(4-((phenylamino)methyl)phenyl)pyrazine-2-carboxamide hydrochloride hydrochloride (3r):



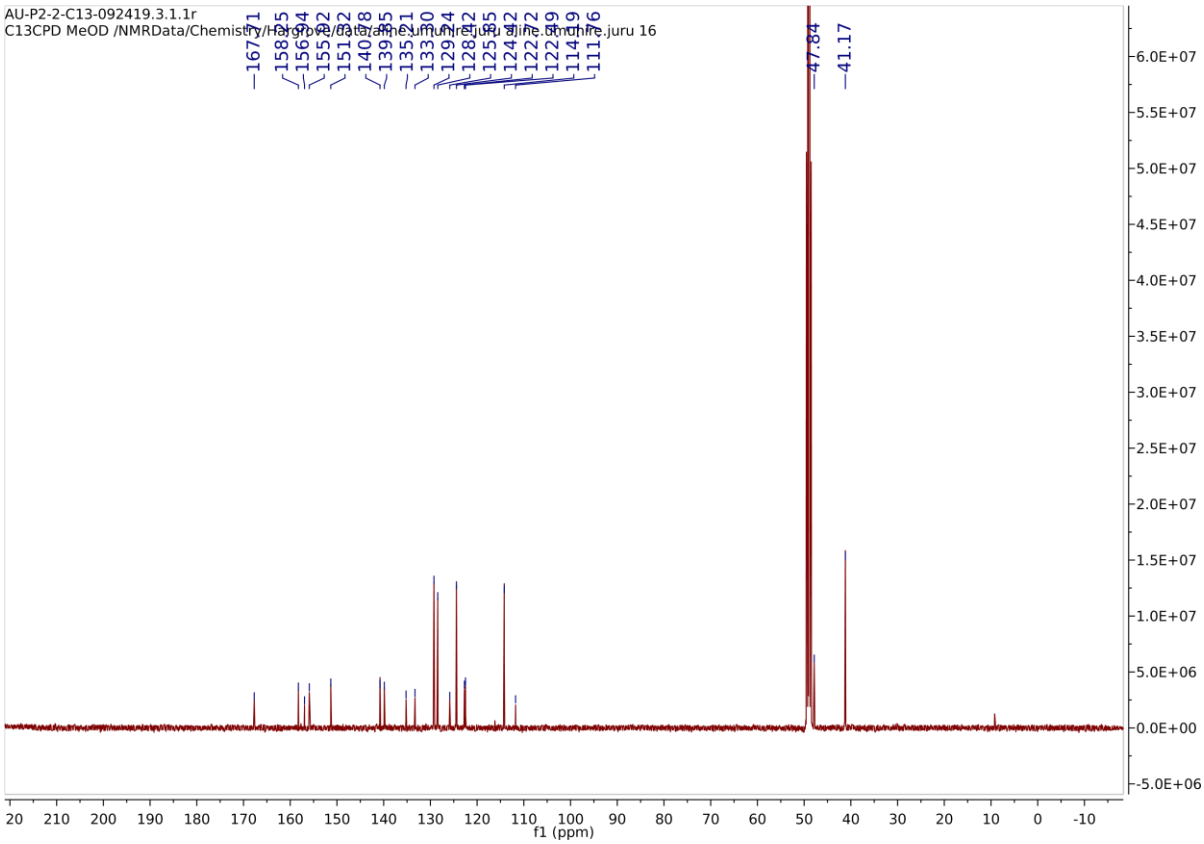
^{13}C NMR for 3-amino-N-carbamimidoyl-5-(dimethylamino)-6-(4-((phenylamino)methyl)phenyl)pyrazine-2-carboxamide hydrochloride hydrochloride (3r):



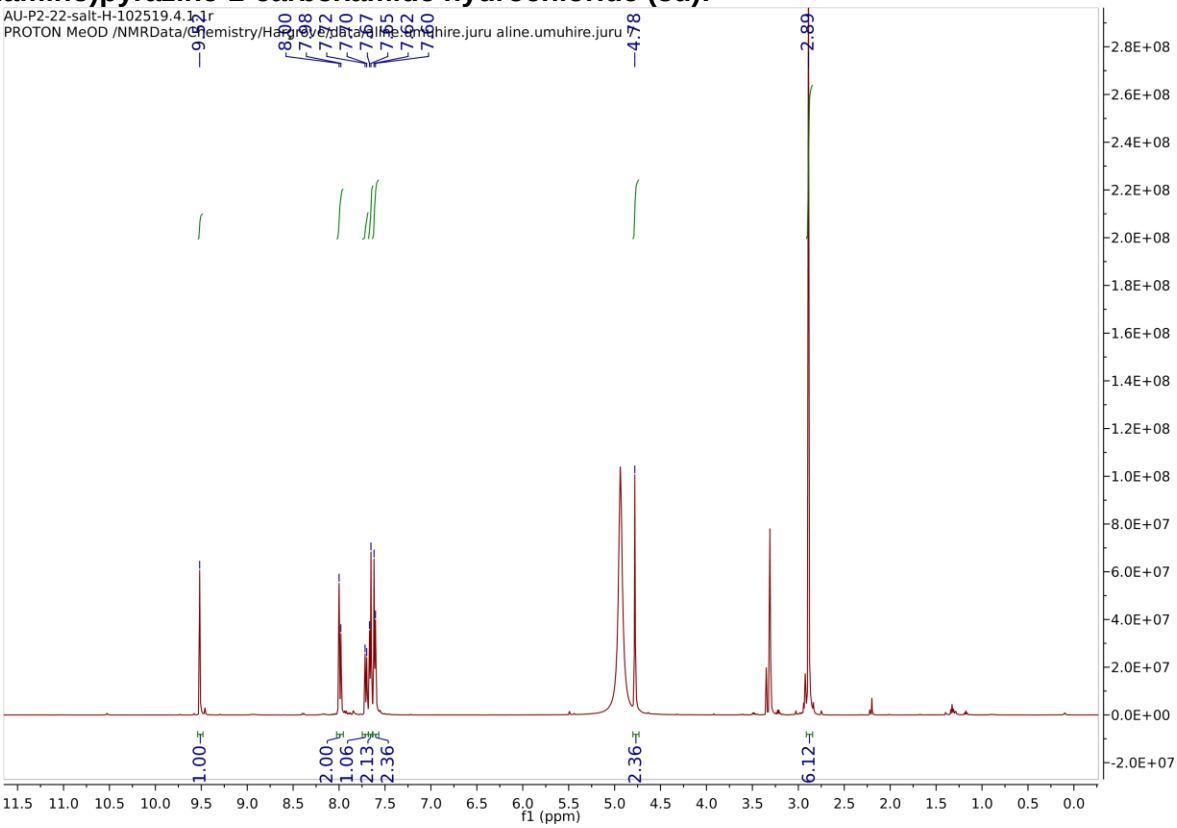
^{1}H NMR of 6-(4-(((4-(1H-imidazol-1-yl)phenyl)amino)methyl)phenyl)-3-amino-N-carbamimidoyl-5-(dimethylamino)pyrazine-2-carboxamide hydrochloride (3l):



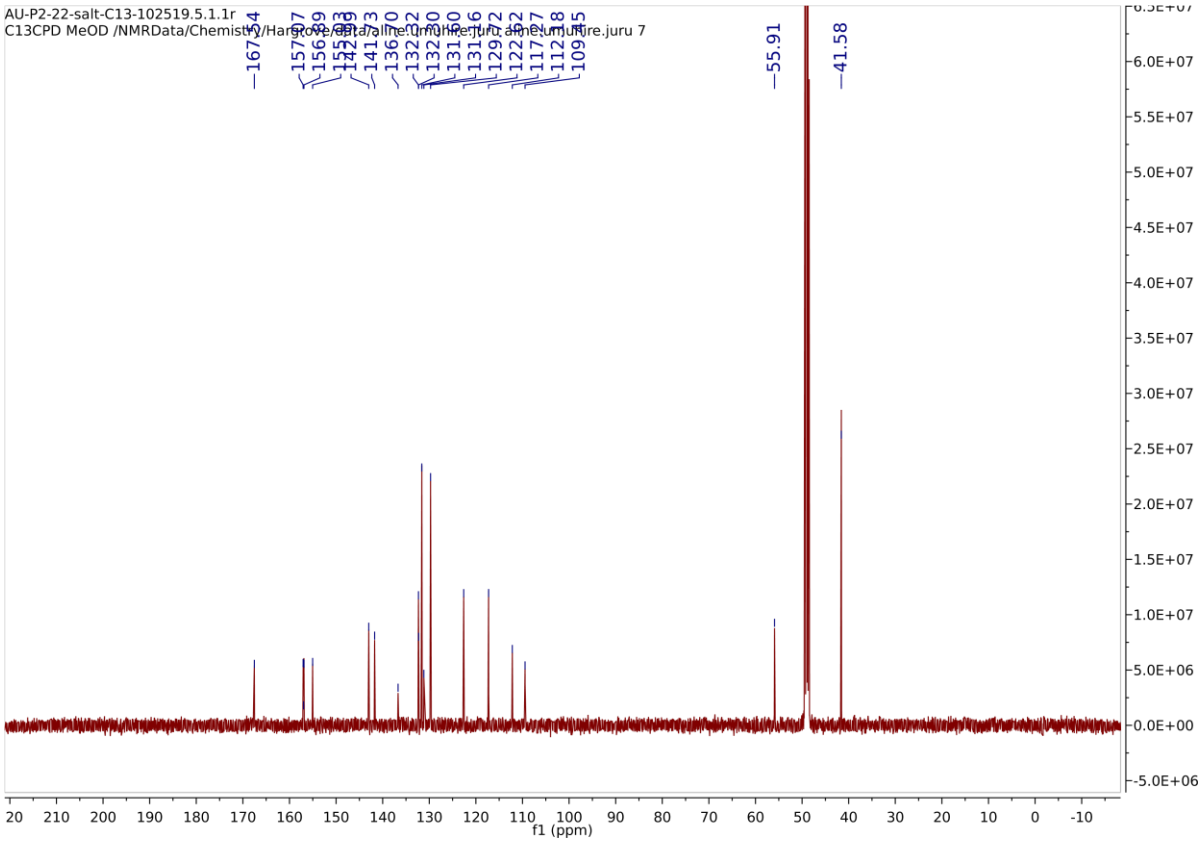
^{13}C NMR of 6-(4-(((4-(1H-imidazol-1-yl)phenyl)amino)methyl)phenyl)-3-amino-N-carbamimidoyl-5-(dimethylamino)pyrazine-2-carboxamide hydrochloride (3l):



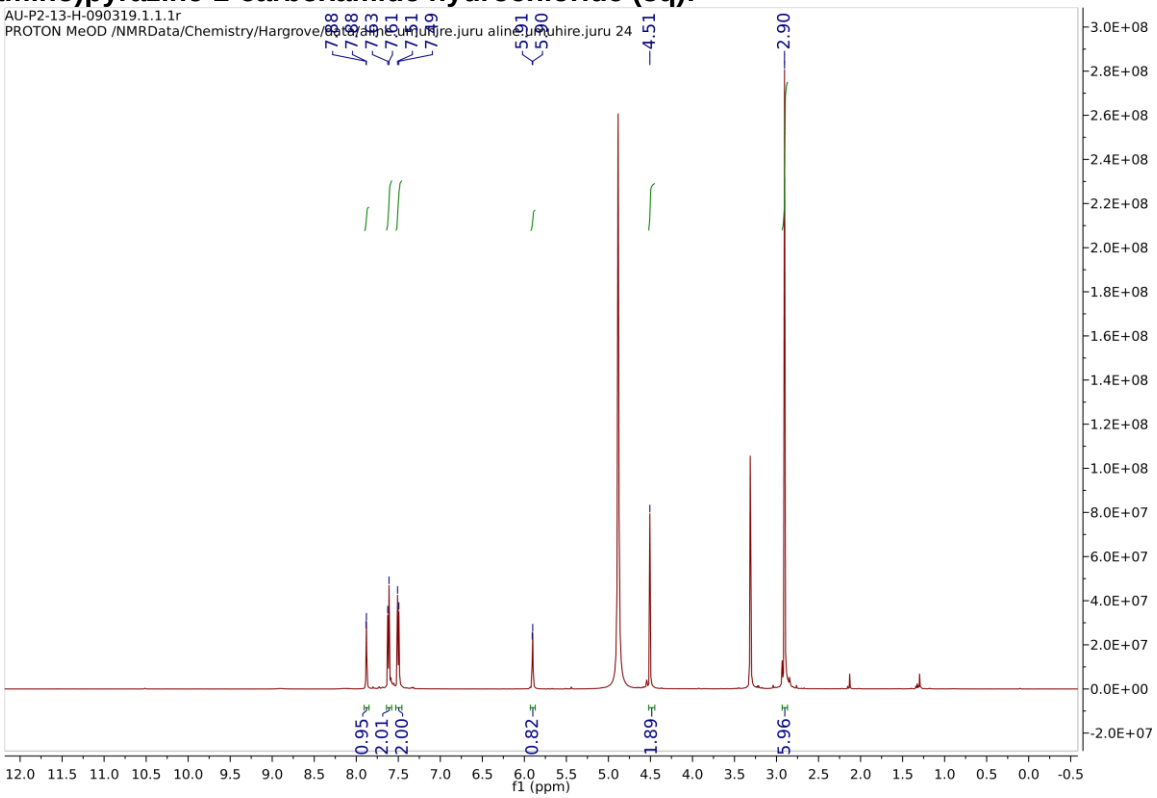
^{1}H NMR for 6-(4-(((1H-benzo[d]imidazol-5-yl)amino)methyl)phenyl)-3-amino-N-carbamimidoyl-5-(dimethylamino)pyrazine-2-carboxamide hydrochloride (3u):



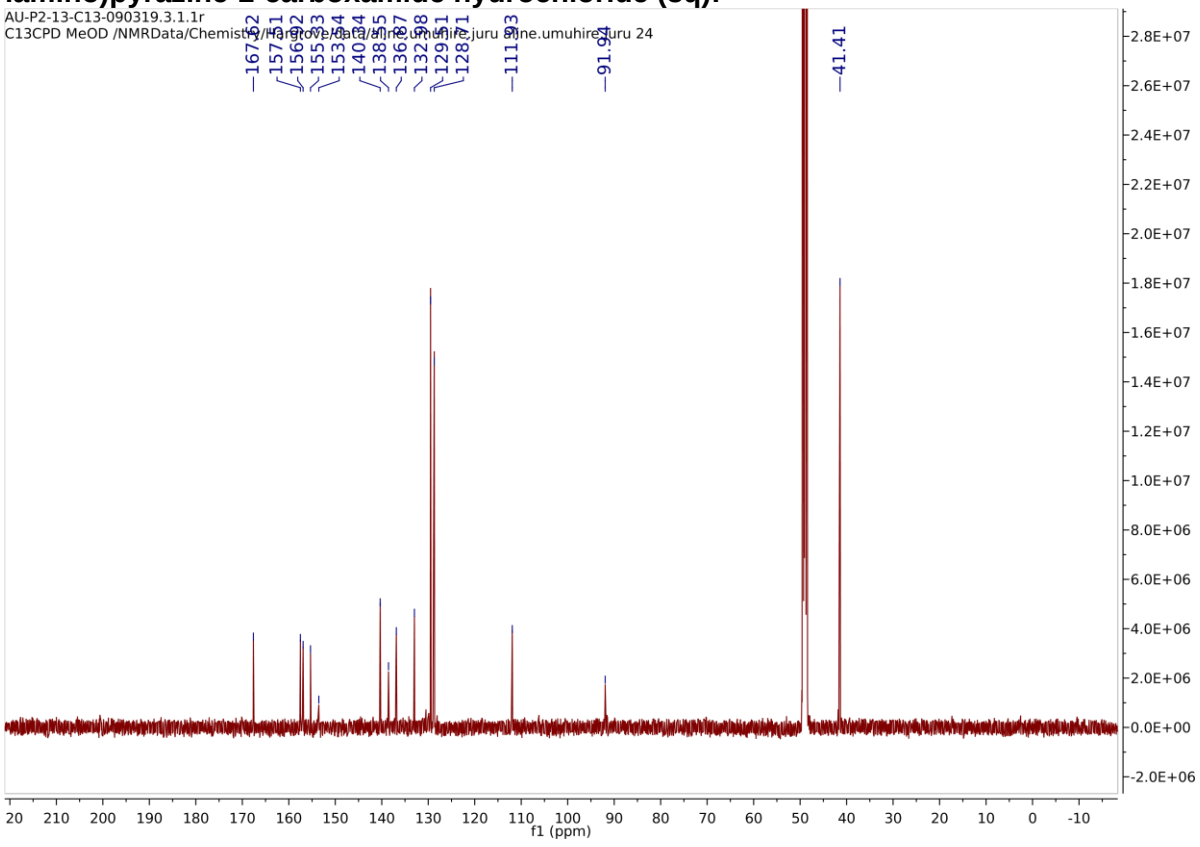
^{13}C NMR for 6-(4-(((1H-benzo[d]imidazol-5-yl)amino)methyl)phenyl)-3-amino-N-carbamimidoyl-5-(dimethylamino)pyrazine-2-carboxamide hydrochloride (3u):



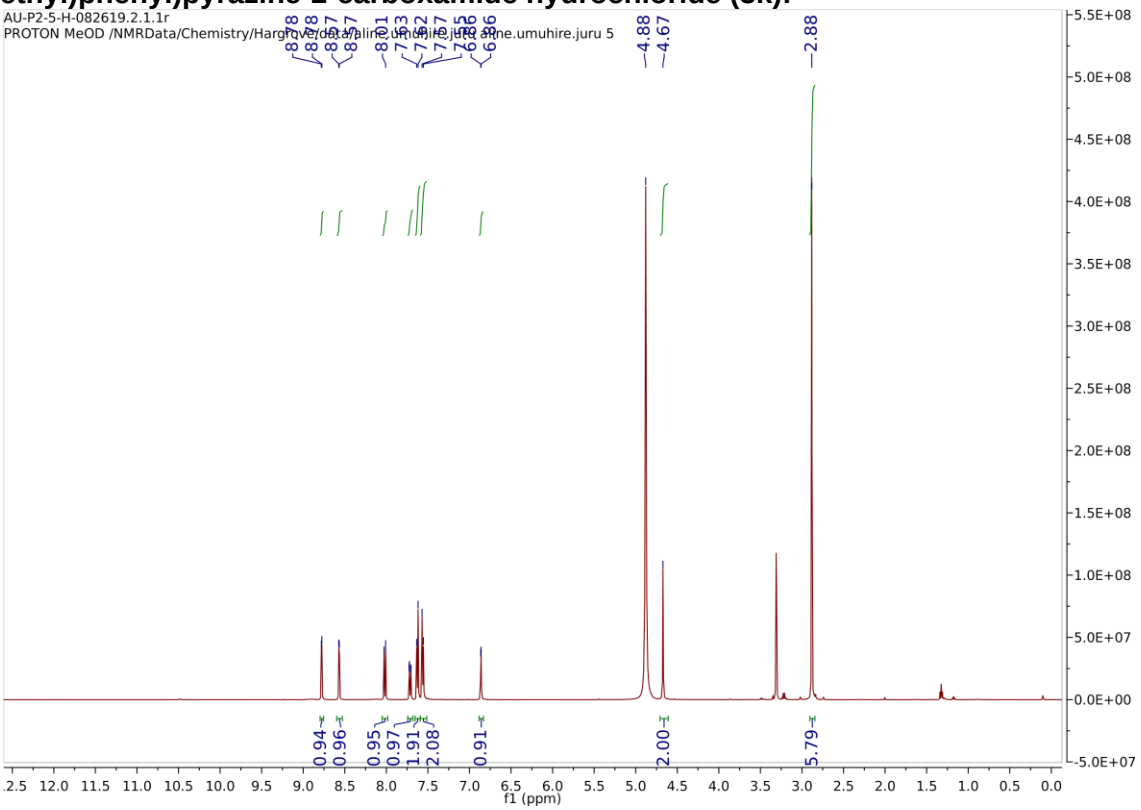
^{1}H NMR for 6-(4-(((1H-pyrazol-5-yl)amino)methyl)phenyl)-3-amino-N-carbamimidoyl-5-(dimethylamino)pyrazine-2-carboxamide hydrochloride (3q):



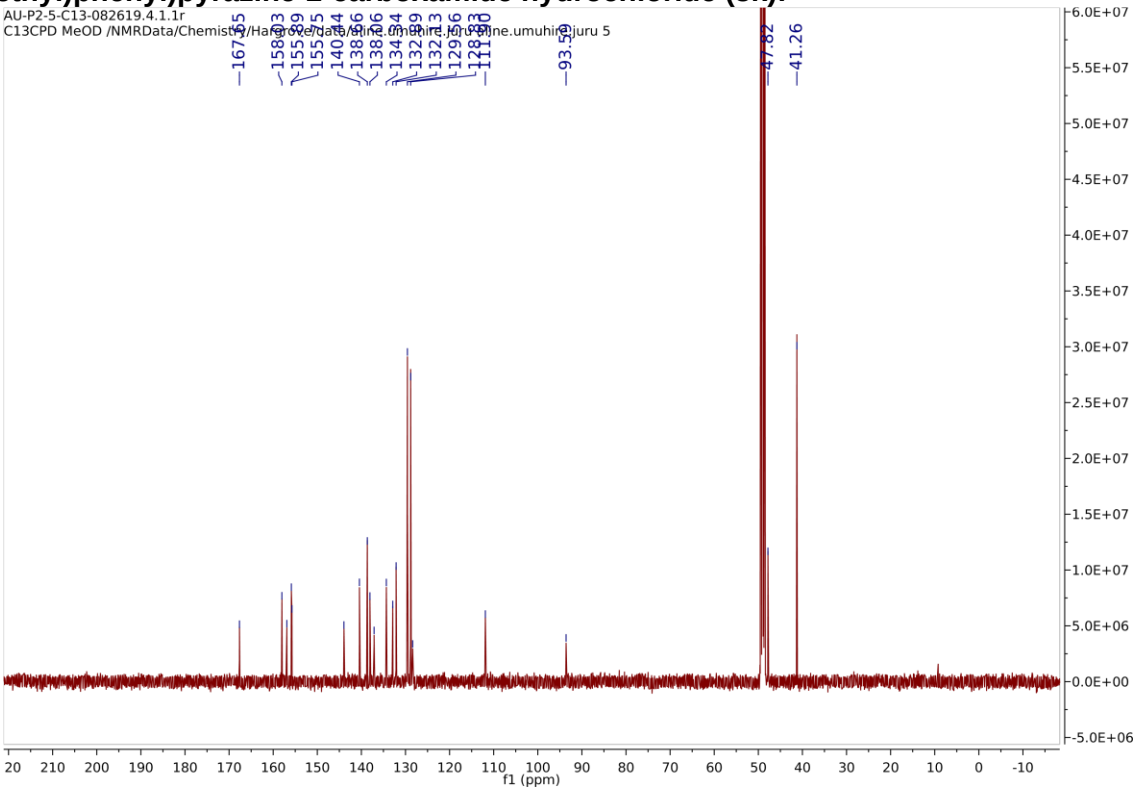
^{13}C NMR for 6-(4-(((1H-pyrazol-5-yl)amino)methyl)phenyl)-3-amino-N-carbamimidoyl-5-(dimethylamino)pyrazine-2-carboxamide hydrochloride (3q):



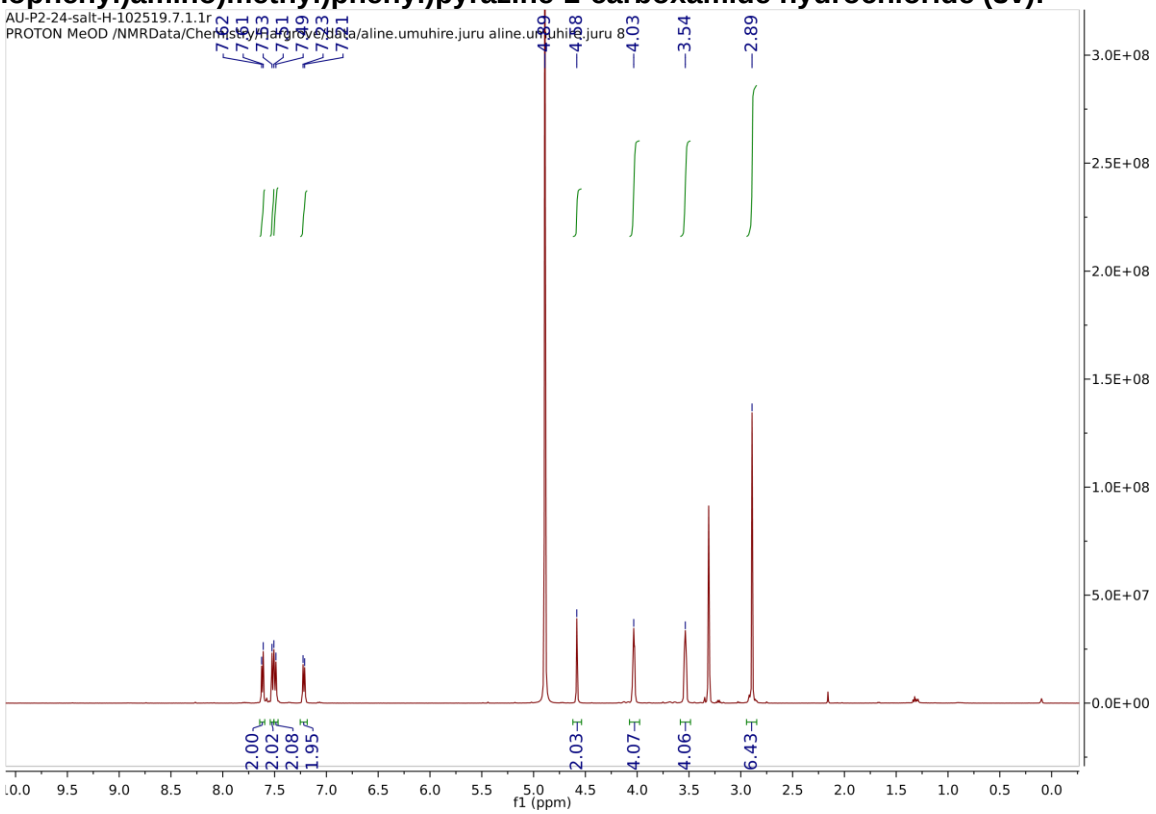
^{1}H NMR for 3-Amino-N-carbamimidoyl-5-(dimethylamino)-6-(4-((quinoxalin-6-ylamino)methyl)phenyl)pyrazine-2-carboxamide hydrochloride (3k):



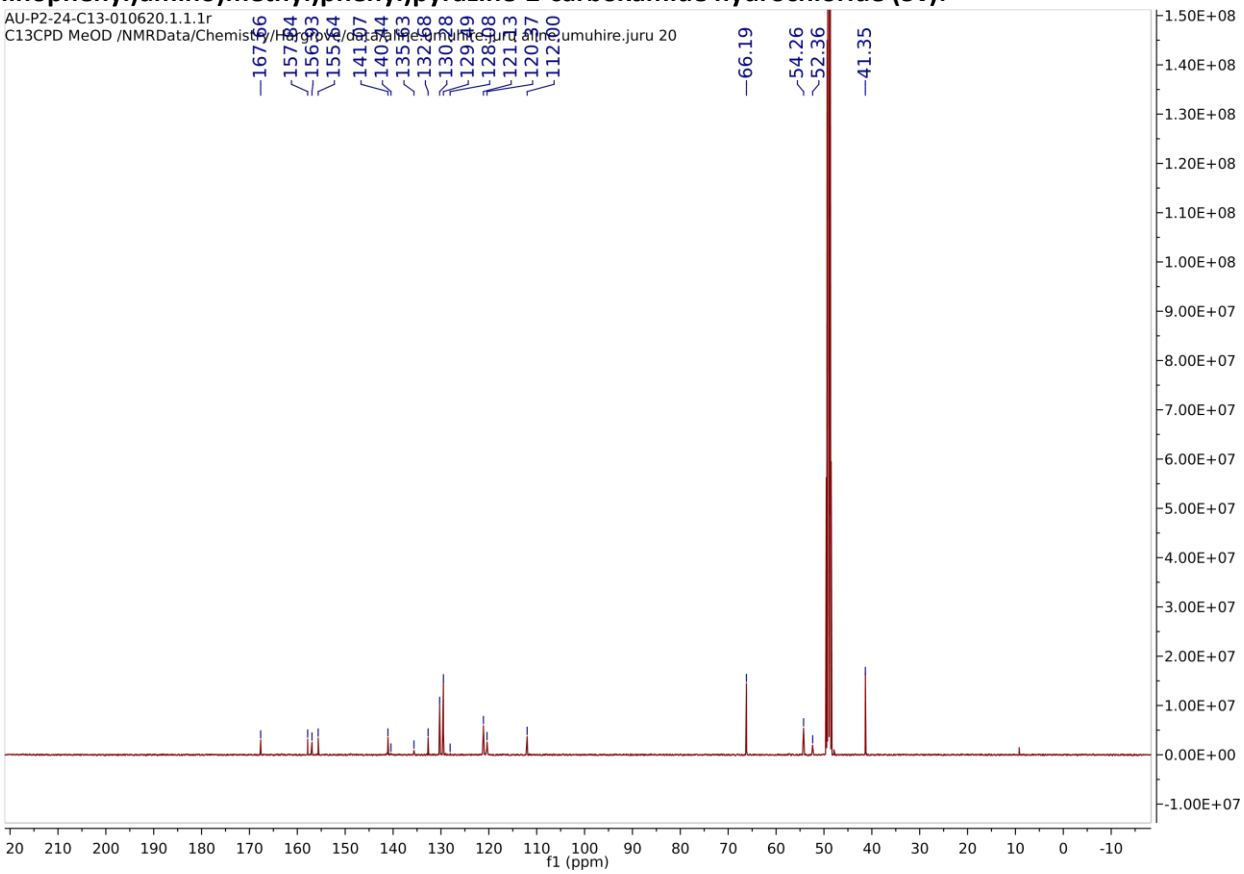
^{13}C NMR for 3-Amino-N-carbamimidoyl-5-(dimethylamino)-6-(4-((quinoxalin-6-ylamino)methyl)phenyl)pyrazine-2-carboxamide hydrochloride (3k):



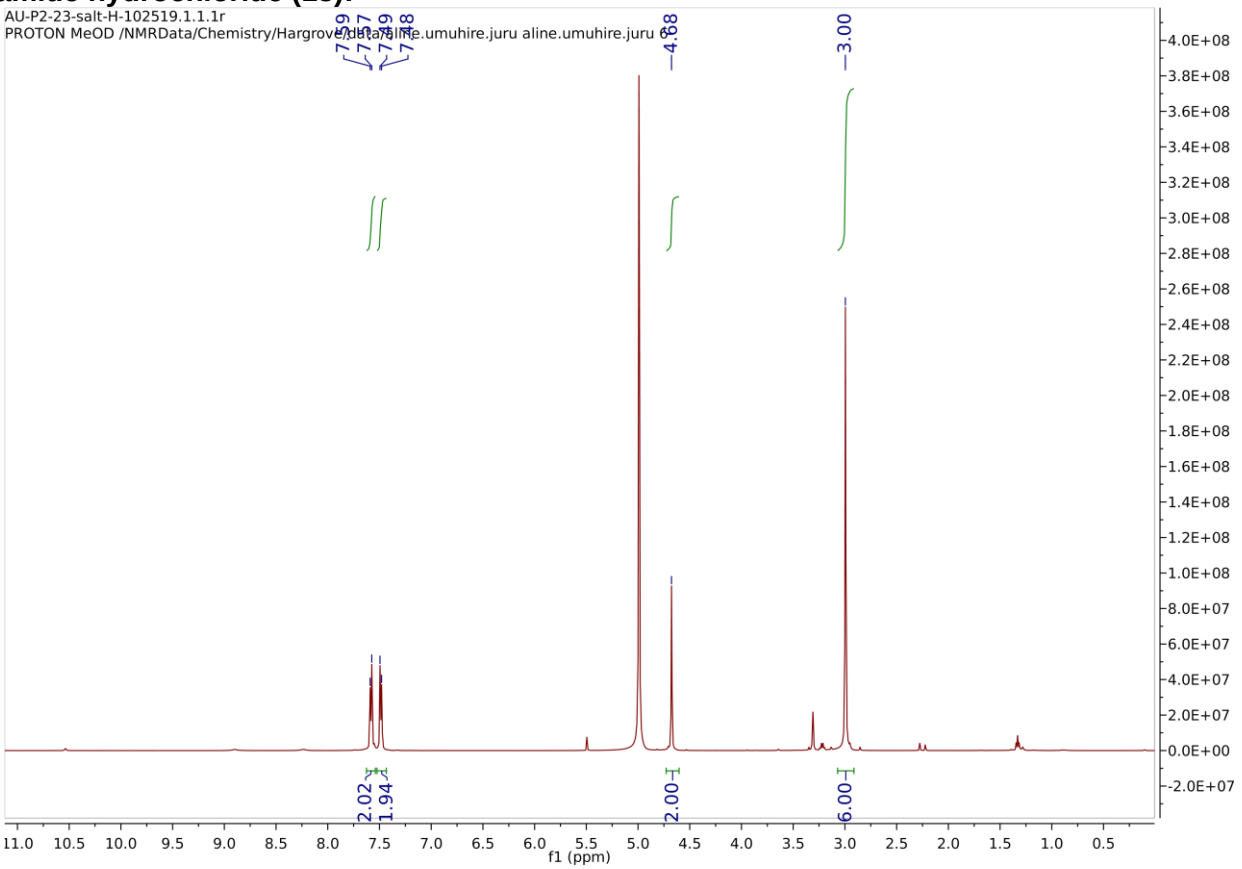
^{1}H NMR for 3-Amino-N-carbamimidoyl-5-(dimethylamino)-6-(4-(((4-morpholinophenyl)amino)methyl)phenyl)pyrazine-2-carboxamide hydrochloride (3v):



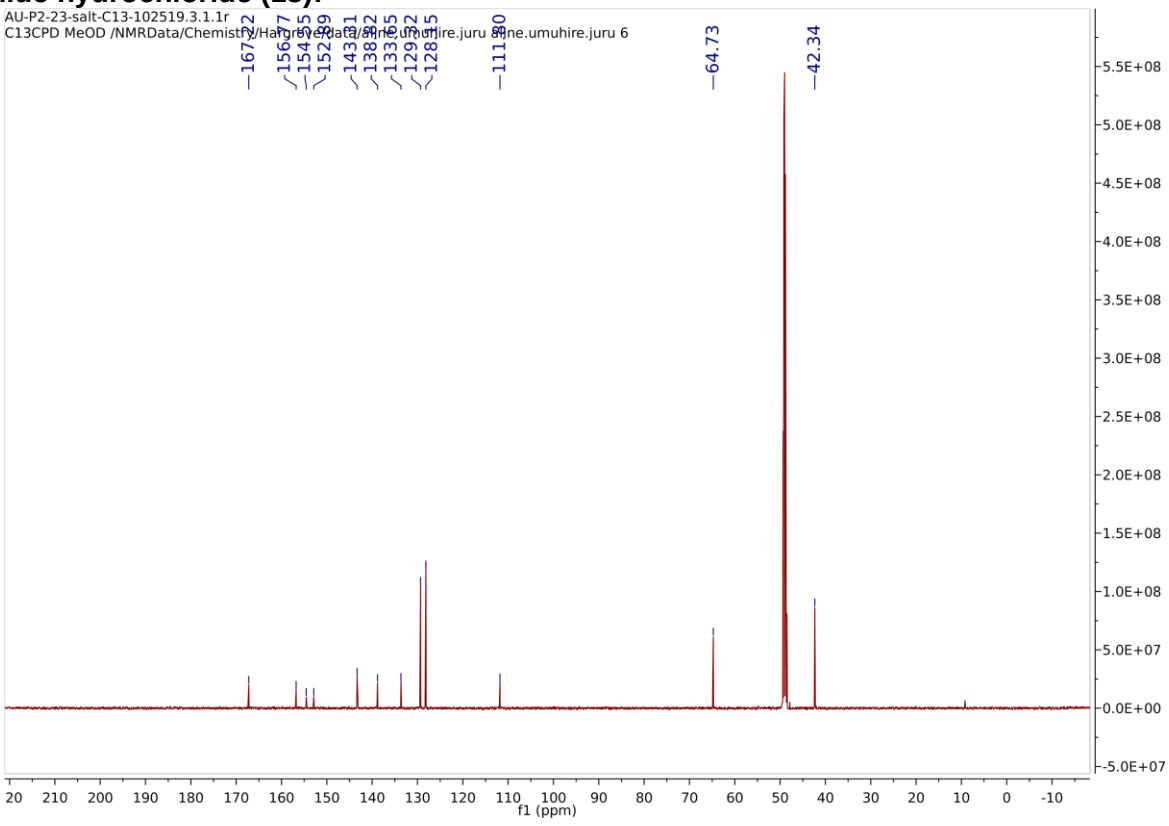
^{13}C NMR for 3-Amino-N-carbamimidoyl-5-(dimethylamino)-6-(4-(((4-morpholinophenyl)amino)methyl)phenyl)pyrazine-2-carboxamide hydrochloride (3v):



^{1}H NMR for 3-amino-N-carbamimidoyl-5-(dimethylamino)-6-(4-(hydroxymethyl)phenyl)pyrazine-2-carboxamide hydrochloride (13):



^{13}C NMR for 3-amino-N-carbamimidoyl-5-(dimethylamino)-6-(4-(hydroxymethyl)phenyl)pyrazine-2-carboxamide hydrochloride (13):



6. HPLC chromatograms of final library members

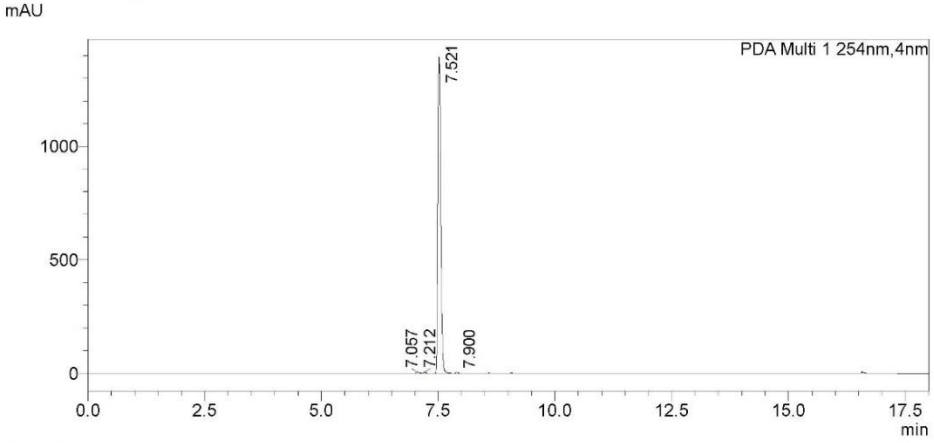
HPLC chromatogram for 3-amino-N-(diaminomethylene)-5-(dimethylamino)-6-(4-formylphenyl)pyrazine-2-carboxamide (1):

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: AU-II-103-F9to19-H2O-121716		
Sample ID	: AU-II-103-F9to19-H2O-121716		
Data Filename	: AU-II-103-F9to19-H2O-121716.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: AU121716.lcb		
Vial #	: 1-101	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 12/17/2016 2:59:32 PM	Acquired by	: chemist
Date Processed	: 12/17/2016 3:17:34 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

RF-20A Ex:350nm,Em:450nm

Peak#	Ret. Time	Area	Height	Area%
Total				

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	7.057	30101	6572	0.475
2	7.212	21487	5566	0.339
3	7.521	6265484	1358646	98.842
4	7.900	21818	4190	0.344
Total		6338890	1374974	100.000

HPLC chromatogram for 3-amino-N-carbamimidoyl-5-(dimethylamino)-6-(4-((phenylamino)methyl)phenyl)pyrazine-2-carboxamide hydrochloride hydrochloride (3r):

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: AU-P2-1-column-072919		
Sample ID	: AU-P2-1-column-072919		
Data Filename	: AU-P2-1-column-072919.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: AU072919.lcb		
Vial #	: 1-47	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 7/29/2019 5:37:43 PM	Acquired by	: chemist
Date Processed	: 7/29/2019 5:55:46 PM	Processed by	: chemist

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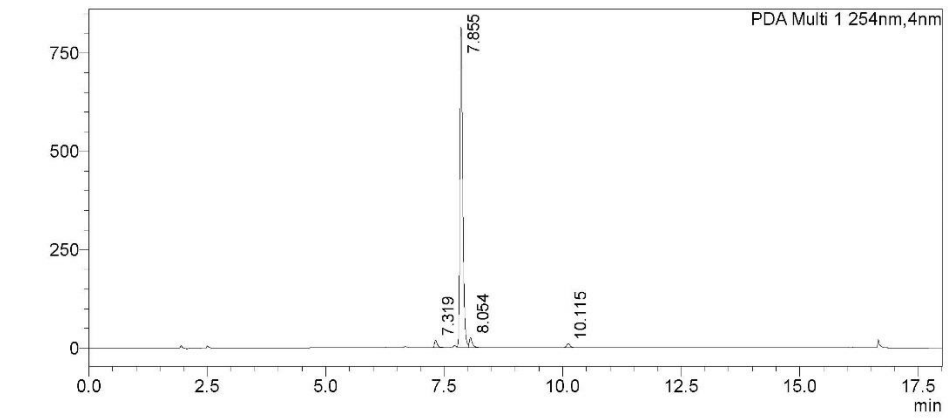

<Peak Table>

RF-20A Ex:350nm,Em:450nm

Peak#	Ret. Time	Area	Height	Area%
Total				

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	7.319	80674	18912	2.197
2	7.855	3430980	787075	93.453
3	8.054	108343	24040	2.951
4	10.115	51335	9882	1.398
Total		3671333	839909	100.000

HPLC chromatogram for 6-(4-(((4-(1H-imidazol-1-yl)phenyl)amino)methyl)phenyl)-3-amino-N-carbamimidoyl-5-(dimethylamino)pyrazine-2-carboxamide hydrochloride (3l):

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: AU-P2-2-072919		
Sample ID	: AU-P2-2-072919		
Data Filename	: AU-P2-2-072919.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: AU072919.lcb		
Vial #	: 1-46	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 7/29/2019 5:00:39 PM	Acquired by	: chemist
Date Processed	: 7/29/2019 5:18:42 PM	Processed by	: chemist

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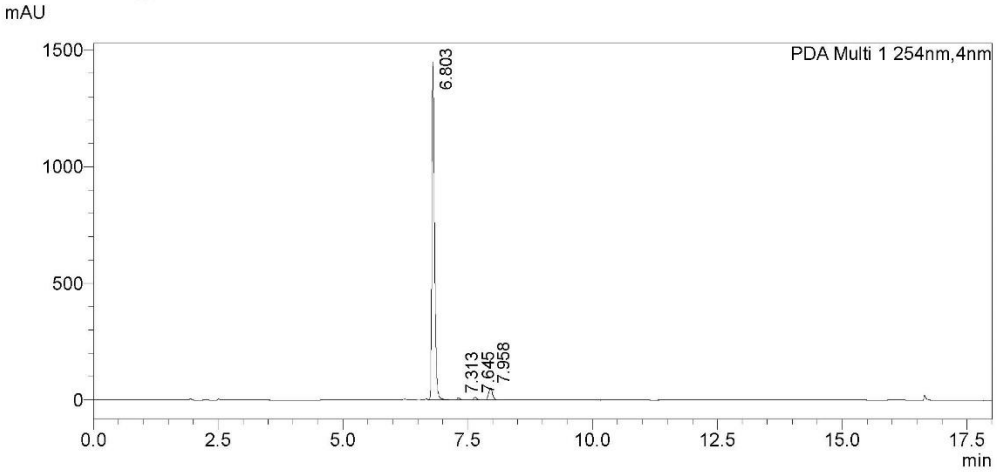


<Peak Table>

RF-20A Ex:350nm,Em:450nm

Peak#	Ret. Time	Area	Height	Area%
Total				

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	6.803	5480817	1345010	93.792
2	7.313	40404	9163	0.691
3	7.645	48381	11519	0.828
4	7.958	273965	50352	4.688
Total		5843566	1416044	100.000

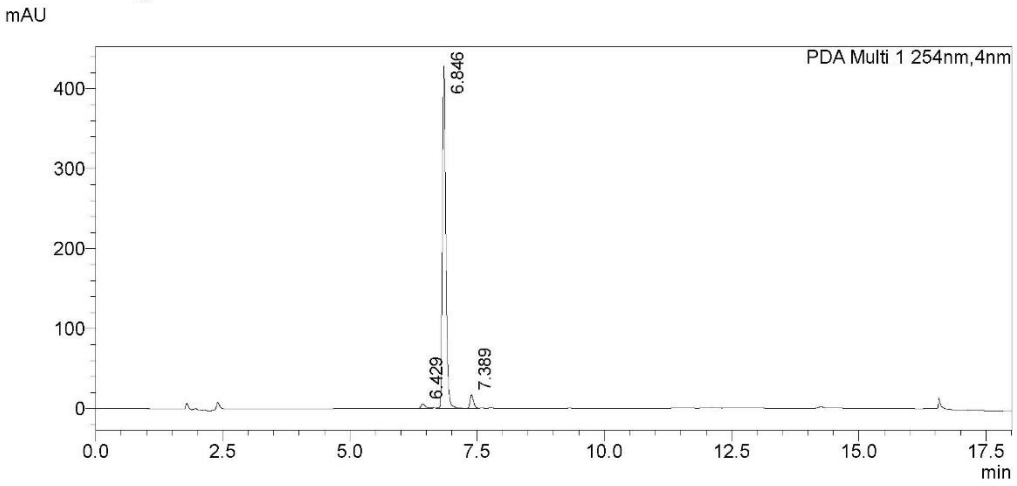
HPLC chromatogram for 6-(4-(((1H-benzo[d]imidazol-5-yl)amino)methyl)phenyl)-3-amino-N-carbamimidoyl-5-(dimethylamino)pyrazine-2-carboxamide hydrochloride (3u):

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: AU-P2-22-column2-101719		
Sample ID	: AU-P2-22-column2-101719		
Data Filename	: AU-P2-22-column2-101719.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: AU101719.lcb		
Vial #	: 1-77	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 10/17/2019 11:31:27 AM	Acquired by	: chemist
Date Processed	: 10/17/2019 11:49:29 AM	Processed by	: chemist

<Chromatogram>



<Peak Table>

RF-20A Ex:350nm,Em:450nm

Peak#	Ret. Time	Area	Height	Area%
Total				

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	6.429	37923	5844	1.766
2	6.846	2023574	421203	94.216
3	7.389	86303	17035	4.018
Total		2147800	444083	100.000

HPLC chromatogram for 6-(4-(((1H-pyrazol-5-yl)amino)methyl)phenyl)-3-amino-N-carbamimidoyl-5-(dimethylamino)pyrazine-2-carboxamide hydrochloride (3q):

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: AU-P2-13-column2-F16-23-082019		
Sample ID	: AU-P2-13-column2-F16-23-082019		
Data Filename	: AU-P2-13-column2-F16-23-082019.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: AU0820193.lcb		
Vial #	: 1-77	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 8/20/2019 10:51:53 AM	Acquired by	: chemist
Date Processed	: 8/20/2019 11:09:56 AM	Processed by	: chemist

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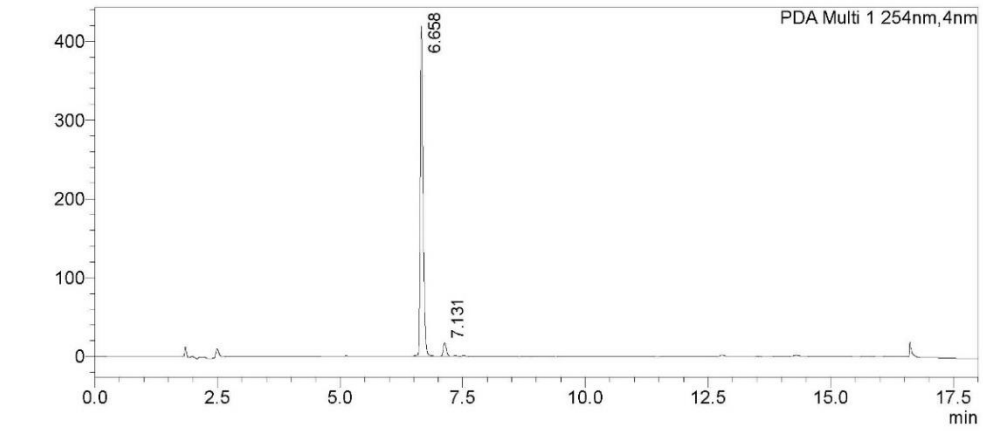


<Peak Table>

RF-20A Ex:350nm,Em:450nm

Peak#	Ret. Time	Area	Height	Area%
Total				

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	6.658	1686614	394427	95.878
2	7.131	72516	16975	4.122
Total		1759130	411402	100.000

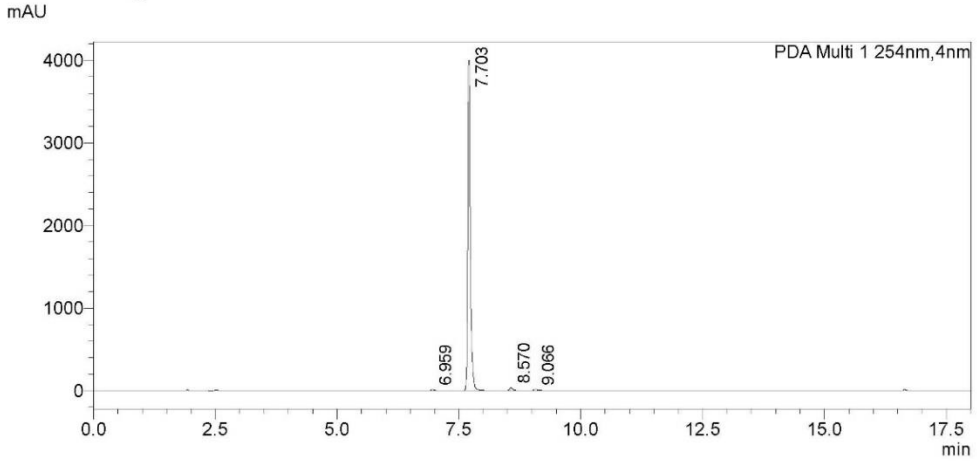
HPLC chromatogram for 3-Amino-N-carbamimidoyl-5-(dimethylamino)-6-(4-((quinoxalin-6-ylamino)methyl)phenyl)pyrazine-2-carboxamide hydrochloride (3k):

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: AU-IV-165-F23-25-071619		
Sample ID	: AU-IV-165-F23-25-071619		
Data Filename	: AU-IV-165-F23-25-071619.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: AU071619.lcb		
Vial #	: 1-34	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 7/16/2019 1:00:47 PM	Acquired by	: chemist
Date Processed	: 7/16/2019 1:18:49 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

RF-20A Ex:350nm,Em:450nm

Peak#	Ret. Time	Area	Height	Area%
Total				

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	6.959	51392	10402	0.332
2	7.703	15187374	3998449	97.987
3	8.570	157582	32487	1.017
4	9.066	103069	13783	0.665
Total		15499416	4055120	100.000

HPLC chromatogram for 3-Amino-N-carbamimidoyl-5-(dimethylamino)-6-(4-(((4-morpholinophenyl)amino)methyl)phenyl)pyrazine-2-carboxamide hydrochloride (3v):

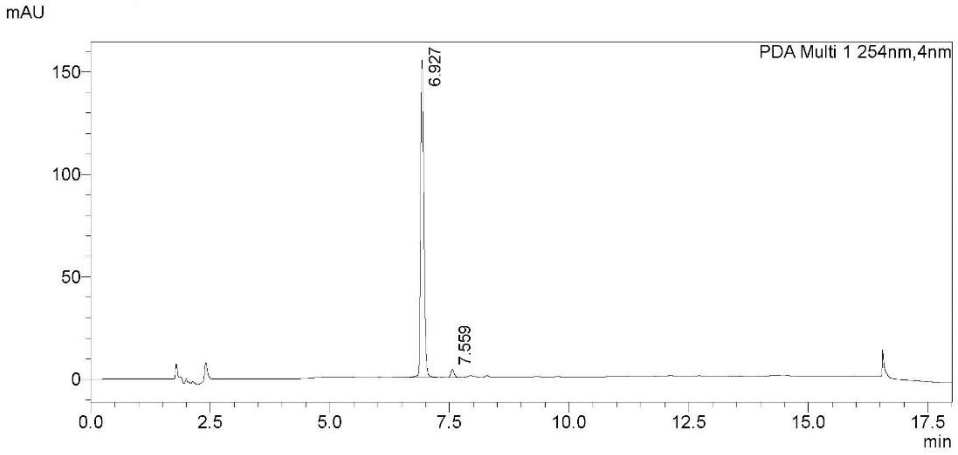
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: AU-P2-24-column-102219		
Sample ID	: AU-P2-24-column-102219		
Data Filename	: AU-P2-24-column-102219.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: AU102219.lcb		
Vial #	: 1-77	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 10/22/2019 12:04:12 PM	Acquired by	: chemist
Date Processed	: 10/22/2019 12:22:14 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

RF-20A Ex:350nm,Em:450nm

Peak#	Ret. Time	Area	Height	Area%
Total				

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	6.927	721645	151892	97.253
2	7.559	20387	3961	2.747
Total		742032	155853	100.000

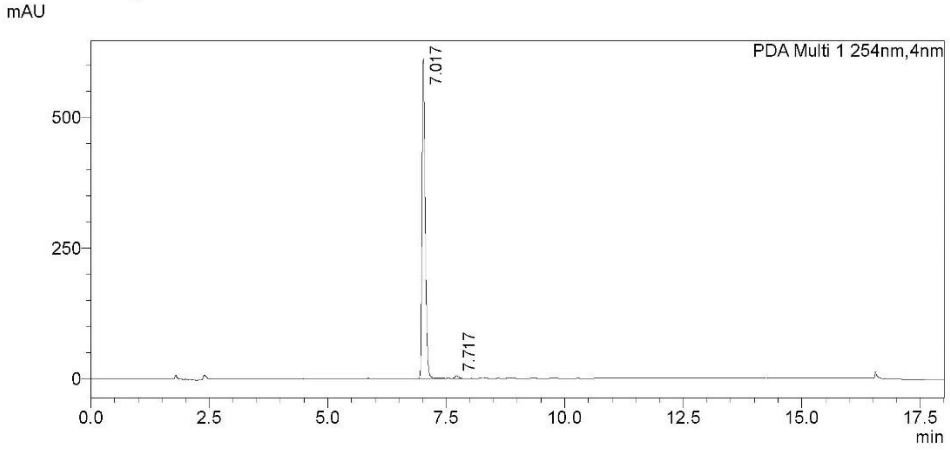
HPLC chromatogram for 3-Amino-N-carbamimidoyl-5-(dimethylamino)-6-(4-(hydroxymethyl)phenyl)pyrazine-2-carboxamide hydrochloride (13):

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: AU-P2-23-column-101919		
Sample ID	: AU-P2-23-column-101919		
Data Filename	: AU-P2-23-column-101919.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: AU101919.lcb		
Vial #	: 1-77	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 10/19/2019 12:12:00 PM	Acquired by	: chemist
Date Processed	: 10/19/2019 12:30:02 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

RF-20A Ex:350nm,Em:450nm

Peak#	Ret. Time	Area	Height	Area%
Total				

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	7.017	2910527	603196	98.930
2	7.717	31485	5193	1.070
Total		2942012	608389	100.000

7. References

1. B. S. Morgan, J. E. Forte, R. N. Culver, Y. Zhang and A. E. Hargrove, *Angew. Chem., Int. Ed.*, 2017, **56**, 13498-13502.
2. H. Ghafuri and M. M. Hashemi, *Sci. Iran.*, 2012, **19**, 1591-1593.
3. B. M. Ahmed, N. A. Rudell, I. Soto and G. Mezei, *J. Org. Chem.*, 2017, **82**, 10549-10562.
4. N. N. Patwardhan, L. R. Ganser, G. J. Kapral, C. S. Eubanks, J. Lee, B. Sathyamoorthy, H. M. Al-Hashimi and A. E. Hargrove, *MedChemComm*, 2017, **8**, 1022-1036.
5. T. Vo, A. Paul, A. Kumar, D. W. Boykin and W. D. Wilson, *Methods*, 2019, **167**, 15-27.