Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Cytidine Deaminase Can Deaminate Fused Pyrimidine Ribonucleosides

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Supplementary Information

1. Materials and Methods

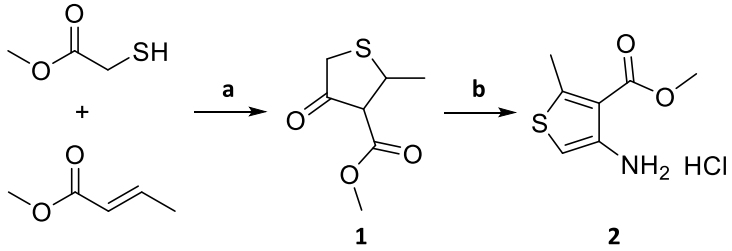
Reagents were purchased from Sigma-Aldrich, TCI, and Acros and were used without further purification unless otherwise specified. Solvents were purchased from Sigma-Aldrich and Fisher Scientific, and dried by standard techniques. NMR solvents were purchased from Cambridge Isotope Laboratories (Andover, MA). All reactions were monitored with analytical TLC (Merck Kieselgel 60 F_{254}). Column chromatography separations were carried out with Teledyne ISCO Combiflash Rf with silica gel particle size 40–63 um. NMR spectra were obtained on Varian Mercury 300 MHz and Varian VX 500 MHz spectrometers. Mass spectra were obtained on an Agilent 6230 HR-ESI-TOF MS at the Mass Spectrometry Facility at the UC San Diego Chemistry and Biochemistry Department.

1.1 Abbreviations

CDA: Cytidine Deaminase; DMSO: Dimethyl sulfoxide; DTT: Dithiothreitol; EDTA: Ethylenediaminetetraacetic acid; THF: Tetrahydrofuran; MeOH: Methanol; OEt_2: Diethyl ether; DCM: Dichloromethane; EtOAc: Ethyl acetate; ACN: Acetonitrile; TMS Triflate: Trimethylsilyl trifluoromethanesulfonate.

2. Synthetic Procedures

^{tz}C and **thC** were synthesized based on previously published procedures[S1–S3] and cytidine was purchased from Sigma-Aldrich.



1 **2**

Scheme S1. Synthetic pathway to key intermediate **2**. Reagents and Conditions: (a) i) Piperidine, 50°C, 2 h; ii) NaH (60% in mineral oil), THF, 70°C, overnight, 19% over two steps. (b) i) **1**, $BaCO_3$, hydroxylamine hydrochloride, MeOH, 70°C, overnight; ii) 2M HCl in OEt_2, OEt_2, MeOH, RT, 24 h, 78% over two steps.

Methyl 2-methyl-4-oxotetrahydrothiophene-3-carboxylate (1)

Piperidine (0.31 g, 0.36 mL, 3.7 mmol) was added to a mixture of methyl thioglycolate (19.3 g, 16.2 mL, 182 mmol) and methyl crotonate (20.0 g, 21.2 mL, 200 mmol) in a round bottom flask while stirring at room temperature. The solution was heated to 50°C and stirred for 2 hours. The solution was allowed to cool and the piperidine and excess reagent evaporated off to afford a yellow liquid.

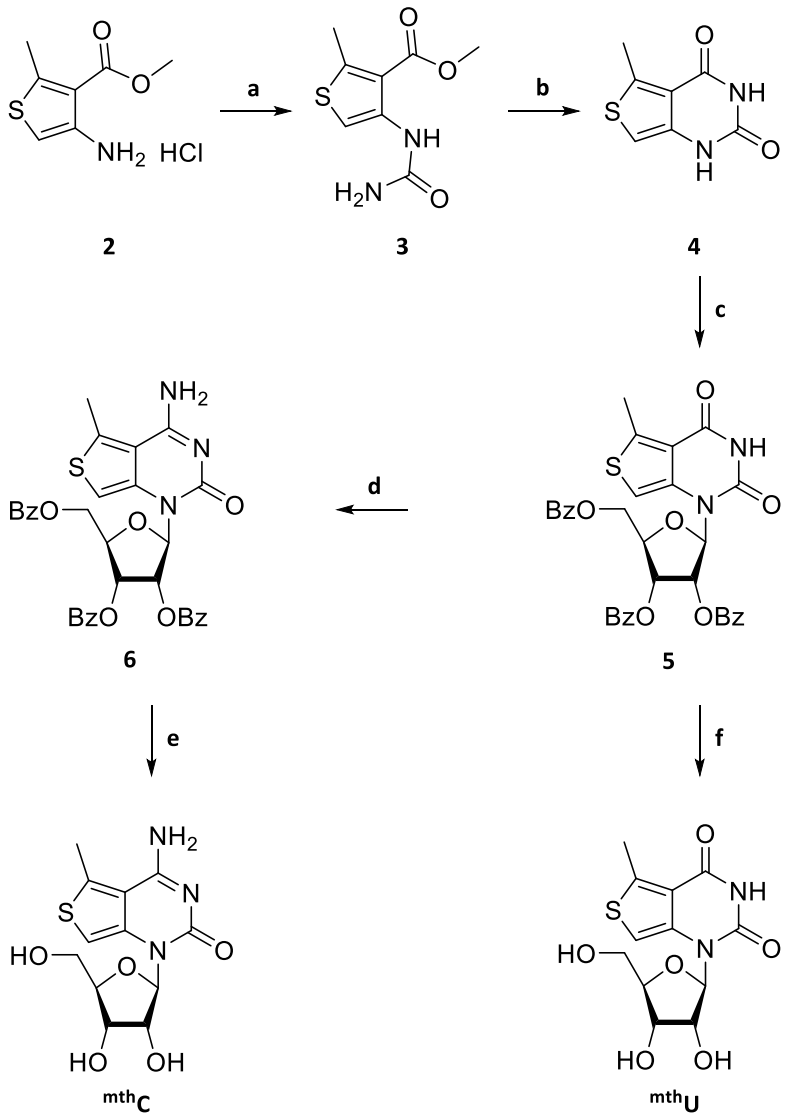
In a separate round bottom flask, sodium hydride (60 wt% in mineral oil, 8.75 g, 219 mmol) was added to dry THF (540 mL) under argon while stirring at room temperature. To the mixture was slowly added the yellow liquid obtained above over four hours while stirring at room temperature. Excess pressure was released through a needle. The resulting mixture was brought to 70°C and stirred overnight. The solution was brought to room temperature and quenched with water (200 mL). The pH was adjusted to 1 with concentrated HCl and the solution extracted with DCM three times. The organic layer was dried with sodium sulfate and evaporated to a residue. The resulting residue was loaded on a

column and eluted with a gradient of 0 to 20% EtOAc in hexanes. The desired fractions were combined and evaporated yielding **1** as a clear, yellow liquid (6.6 g, 19%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.12 (s), 3.91–3.15 (m), 1.55 –1.40 (m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 205.75, 172.43, 168.12, 104.71, 63.91, 52.83, 51.62, 42.78, 40.38, 39.75, 35.29, 24.84, 19.51. ESI-HRMS calculated for $C_7H_{10}O_3S$ $[M+Na]^+$ 197.0243, found 197.0246.

Methyl 4-amino-2-methylthiophene-3-carboxylate hydrochloride (2)

To a solution of **1** (6.60 g, 38 mmol) in methanol (280 mL) was added barium carbonate (17.2 g, 87.1 mmol) and hydroxylamine hydrochloride (6.06 g, 87.1 mmol) while stirring at room temperature. The solution was refluxed overnight at 70^{o}C. The solution was then cooled to room temperature and then filtered. The filtrate was dried to a white solid, suspended in water, and the suspension extracted three times with ethyl acetate. The organic phase was dried with sodium sulfate and evaporated to a clear, yellow residue.

The yellow residue was dissolved in ether (70 mL) and methanol (20 mL) under argon while stirring at room temperature. To the solution was added 2 M HCl in ether (45 mL) and stirring continued at room temperature for 24 hours. The resulting pink precipitate, **2**, (6.1 g, 78%) was filtered, rinsed with cold ether, and collected without further purification. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.03 (br s, 2H), 7.41 (s, 1H), 3.80 (s, 3H), 2.64 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.97, 150.60, 132.24, 121.64, 113.29, 52.24, 16.70. ESI-HRMS calculated for $C_7H_{10}NO_2S$ $[M+H]^+$ 172.0427, found 172.0426.



Scheme S2. Synthetic pathway to ^{mth}**U** and ^{mth}**C**. Reagents and Conditions: (a) Potassium cyanate, acetic acid (30%), RT, overnight, 94%. (b) Sodium methoxide, MeOH, RT, 15 h, 91%. (c) *N,O*-bis(trimethylsilyl)acetamide, β-D-ribofuranose 1-acetate 2,4,5-tribenzoate, TMS Triflate, ACN, 85°C, 3 h, 85%. (d) i) Phosphoryl (V) Chloride, 1,2,4-triazole, pyridine, RT, 1 h; ii) Saturated ammonium hydroxide, RT, 3 h, 43% over two steps. (e) Ammonia saturated MeOH, 65°C, overnight, 53%. (f) Ammonia saturated MeOH, 65°C, overnight, 85%.

Methyl 2-methyl-4-ureidothiophene-3-carboxylate (3)

Solid **2** (1.0 g, 4.8 mmol) and potassium cyanate (670 mg, 8.2 mmol) were dissolved in a mixture of water (8.2 mL) and acetic acid (8.2 mL). The resulting solution was stirred at room temperature overnight. The precipitate formed was filtered, rinsed with water, dried and collected to yield **3** (0.97 g, 94%) as a brownish pink powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.44 (br s, 1H), 7.47 (s, 1H), 4.58 (br s, 2H), 3.92 (s, 3H), 2.67 (s, 3H). ^{13}C NMR (125 MHz, CD_3OD): δ 165.02, 157.54, 148.04, 137.54, 117.81, 102.74, 50.67, 15.81. ESI-HRMS calculated for $C_8H_{11}N_2O_3S$ $[M+H]^+$ 215.0485, found 215.0483.

5-methylthieno[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4)

Solid **3** (970 mg, 4.5 mmol) was dissolved in 0.5 M sodium methoxide in methanol (20 mL, 10 mmol) under argon and stirred at room temperature overnight. The precipitate formed was filtered and collected to yield **4** (750 mg, 91%) as an off-white powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.66 (br s, 2H), 6.44 (s, 1H), 2.73 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.52, 151.79, 146.98, 139.17, 117.73, 97.60, 15.25. ESI-HRMS calculated for $C_7H_5N_2O_2S$ $[M-H]^-$ 181.0077, found 181.0078.

(2*R*,5*R*)-2-((benzoyloxy)methyl)-5-(5-methyl-2,4-dioxo-3,4-dihydrothieno[3,4-*d*]pyrimidin-1(2*H*)-yl)tetrahydrofuran-3,4-diyl dibenzoate (5)

Solid **4** (710 mg, 3.9 mmol) was dried under vacuum (P_2O_5) overnight and was dissolved in ACN (20 mL, dried on 4 Å molecular sieves) under argon. *N,O*-bis(trimethylsilyl)acetamide (2.38 g, 11.7 mmol) was added and the solution stirred at room temperature for 2 hours. β-D-ribofuranose 1-acetate 2,4,5-tribenzoate (2.16 g, 4.29 mmol), dried under vacuum (P_2O_5) overnight, and TMS triflate (1.1 mL, 6.2 mmol) were added and the resulting brown solution stirred at room temperature for 2 hours, then raised to 70^{o}C and stirred for 1.5 hours. The solution was allowed to cool to room temperature and evaporated to a yellow foam. The foam was loaded on a column and eluted with a gradient of 0 to 40% EtOAc in Hexanes. The desired fractions were combined and evaporated to yield **5** (2.1 g, 85%) as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.38 (s, 1H), 8.01–7.98 (m, 2H), 7.90–7.84 (m, 4H), 7.68–7.60 (m, 3H), 7.52–7.48 (m, 2H), 7.45–7.40 (m, 4H), 7.17 (s, 1H), 6.38 (d, *J* = 3.4 Hz, 1H), 6.12 (dd, *J* = 6.9, 3.5 Hz, 1H), 6.03 (t, *J* = 7.1 Hz, 1H), 4.77–4.67 (m, 2H), 4.61 (dd, *J* = 13.0, 6.0 Hz, 1H), 2.75 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 165.91, 165.18, 165.14, 159.12, 150.40, 148.71, 137.37, 134.32, 134.28, 134.02, 129.79, 129.76, 129.63, 129.26, 129.14, 129.06, 128.99, 118.06, 100.54, 89.52, 78.41, 72.51, 70.38, 63.85, 15.47. ESI-HRMS calculated for $C_{33}H_{27}N_2O_9S$ $[M+H]^+$ 627.1432, found 627.1426.

(2*R*,5*R*)-2-(4-amino-5-methyl-2-oxothieno[3,4-*d*]pyrimidin-1(2*H*)-yl)-5-((benzoyloxy)methyl)tetrahydrofuran-3,4-diyl dibenzoate (6)

To pyridine (24 mL, dried on 4 Å molecular sieves) was added 1,2,4-triazole (1.92 g, 27.7 mmol) while stirring. The solution was placed in an ice bath and $POCl_3$ (0.85 mL, 9.1 mmol) was added slowly. After stirring on ice for 40 minutes, the solution was filtered. To solid **5** (285 mg, 0.46 mmol), dried under vacuum (P_2O_5) overnight, was added the yellow filtrate under argon while stirring at room temperature. After stirring for 1 hour, the solution was placed in an ice bath and concentrated ammonium hydroxide (5 mL) was added slowly. After 15 minutes, the solution was brought to room temperature and stirred for 1 hour. The solution was then co-evaporated with methanol (3 x 20 mL) and DCM (3 x 20 mL). The resulting solid was suspended in DCM (50 mL), filtered, and the filtrate extracted with water (3 x 50 mL). The organic layers were combined, dried with sodium sulfate, and evaporated to a residue. The residue was loaded onto a column and eluted with a gradient of 0 to 100% EtOAc in Hexanes. The desired fractions were combined and evaporated to yield **6** (122 mg, 43%) as a yellow film. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.20 (s, 2H), 8.11–8.05 (m, 2H), 7.96–7.89 (m, 4H), 7.59–7.29 (m, 9H), 6.62 (s, 1H), 6.55 (d, *J* = 4.6 Hz, 1H), 6.25 (dd, *J* = 6.7, 4.9 Hz, 1H), 6.13 (t, *J* = 6.7 Hz, 1H), 4.86 (dd, *J* = 12.1, 3.0 Hz, 1H), 4.73 (dd, *J* = 12.1, 4.3 Hz, 1H), 4.69–4.63(m, 1H), 2.78 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.19, 165.44, 165.33, 159.90, 155.96, 140.93, 139.59, 133.48, 133.45, 133.32, 129.86, 129.78, 129.57, 128.85, 128.57, 128.38, 114.77, 97.86, 88.79, 79.52, 71.49, 70.64, 64.00, 16.23. ESI-HRMS calculated for $C_{33}H_{28}N_3O_8S$ $[M+H]^+$ 626.1592, found 626.1587.

1-((2*R*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylthieno[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (^{mth}U)

A solution of **5** (500 mg, 0.80 mmol) in methanolic ammonia (12 mL) was refluxed at 65^{o}C overnight in a sealed tube. The solution was placed in an ice bath, cap removed, and then evaporated to a residue. The residue was loaded onto a column and eluted with a gradient of 0 to 100% EtOAc in Hexanes. The desired fractions were combined and evaporated to afford ^{mth}**U** (210 mg, 85%) as an off-white powder. ^{1}H NMR (300 MHz, CD_3OD): δ 7.16 (s, 1H), 6.09 (d, *J* = 6.1 Hz, 1H), 4.72 (t, *J* = 6.3 Hz, 1H), 4.30 (dd, *J* = 6.4, 4.7 Hz, 1H), 3.98–3.95 (m, 1H), 3.86, 3.78 (dABq, *J* = 6.0, 2.6 Hz, 2H), 2.82 (s, 3H). ^{13}C NMR (125 MHz, CD_3OD): δ 159.16, 151.01, 147.34, 135.87, 118.59, 102.03, 88.82, 85.31, 69.53, 68.42, 61.57, 15.41. ESI-HRMS calculated for $C_{12}H_{15}N_2O_6S$ $[M+H]^+$ 315.0645, found 315.0646.

4-amino-1-((2*R*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylthieno[3,4-*d*]pyrimidin-2(1*H*)-one (^{mth}C)

A solution of **6** (68 mg, 0.11 mmol) in methanolic ammonia (5 mL) was heated at 45^{o}C for 20 hours in a sealed tube. The solution was cooled in an iced bath before opening and then evaporated to a residue. The residue was suspended in isopropanol and filtered. The precipitate was washed with DCM several times. The filtrate was dried and loaded on a reverse phase silica gel column and eluted with 2% ACN in water. The desired fractions were combined with the precipitate and dried to yield ^{mth}**C** (18 mg, 53%) as an off-white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 7.06 (s, 1H), 6.09 (d, *J* = 5.5 Hz, 1H), 4.76 (t, *J* = 5.8 Hz, 1H), 4.31 (t, *J* = 4.6 Hz, 1H), 3.97 (s, 1H), 3.85, 3.77 (ABq, *J* = 7.5 Hz, 2H), 2.83 (s, 3H). ^{13}C NMR (125 MHz, CD_3OD): δ 159.51, 156.35, 140.93, 139.30, 115.19, 99.08, 89.63, 84.91, 69.68, 68.66, 61.71, 16.26. ESI-HRMS calculated for $C_{12}H_{16}N_3O_5S$ $[M+H]^+$ 314.0805, found 314.0800.

3. Absorption and Emission Spectroscopy

3.1 General

Spectroscopic grade DMSO and dioxane were obtained from Sigma Aldrich and aqueous solutions were prepared with MilliQ water. All measurements were carried out in a 3 mL, 1 cm four-sided quartz cuvette (extinction coefficient measurements) or a 125 µL, 1 cm four-sided quartz cuvette (quantum yield measurements) from Helma.

Absorption spectra were taken on a Shimadzu UV-2450 spectrophotometer setting the slit width to 1 nm and resolution to 0.5 nm. All spectra were corrected for the blank. Steady state emission spectra were taken on a Horiba Fluoromax-4 equipped with a cuvette holder with a stirring system setting the excitation slit width to 2 nm and the emission slit width to 3 nm, the resolution to 1 nm, and the integration time to 0.1 s. Steady state fluorescence spectra used for quantum yield determinations were taken upon excitation at the corresponding molecules maximum wavelength of absorption. All spectra were corrected for the blank and instrument response.

Both instruments were equipped with a thermostat-controlled ethylene glycol-water bath fitted to specially designed cuvette holder and the temperature was kept at 25.0 ± 0.1 ^{o}C.

Nucleosides were dissolved in DMSO to prepare highly concentrated stock solutions: ^{mth}**C** (9.53 mM), ^{mth}**U** (11.9 mM).

Table S1. Photophysical Properties of ^{mth}**C** and ^{mth}**U**.

	Solvent	$\lambda_{abs}(\varepsilon)^a$	$\lambda_{em}(\phi)^a$	Φε	Stokes Shift[a]
^{mth}**C**	Water	323 (3.35 ± 0.02)	455 (0.24 ± 0.009)	804	8930 ± 50
	Dioxane	325 (3.15 ± 0.04)	445 (0.03 ± 0.003)	95	8300 ± 110
^{mth}**U**	Water	306 (2.55 ± 0.01)	427 (0.30 ± 0.003)	765	9260 ± 330
	Dioxane	305 (2.72 ± 0.01)	387 (0.12 ± 0.01)	353	6950 ± 150

[a]λ_{abs}, ε, λ_{em}, and Stokes shift are reported in units of nm, M^{-1} cm^{-1}, nm, and cm^{-1} respectively.

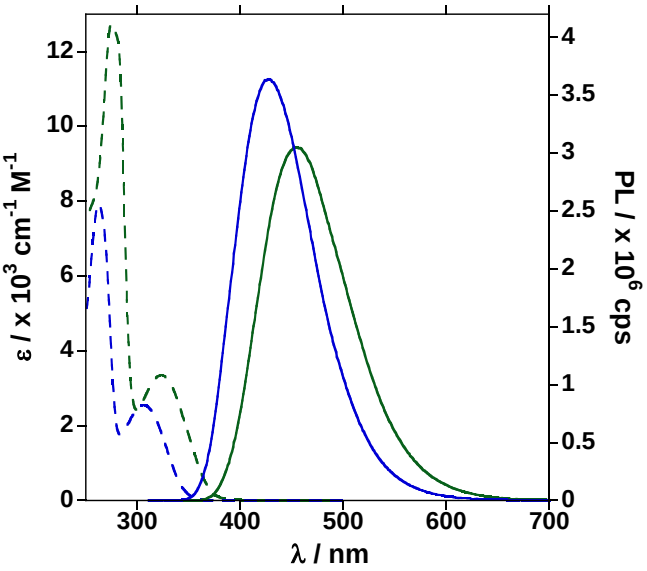


Figure S1. Absorption (dashed lines) and emission (solid lines) spectra of ^{mth}**C** (green) and ^{mth}**U** (blue) in deionized water.

3.2 Fluorescence Quantum Yield Determination

The samples concentrations were adjusted to have an optical density lower than 0.07 at the excitation wavelength (λ_{ex}). The fluorescence quantum yields (ϕ) were evaluated based on an external standard, 2-aminopurine (0.68 in water), by using the following equation:

$$\Phi = \Phi_{STD} \frac{I}{I_{STD}} \frac{OD_{STD}}{OD} \frac{n^2}{n_{STD}^2}$$

where ϕ_{STD} is the fluorescence quantum yield of the standard, I and I_{STD} are the integrated area of the emission band of the sample and the standard respectively, OD and OD_{STD} are the optical density at the excitation wavelength for the sample and the standard respectively, and n and n_{STD} are the solvent refractive index of the sample and the standard solutions respectively.

4. Analysis of CDA Kinetics

4.1 General Methods

Recombinant human CDA variant Q27/A70 was obtained from BioVision (EC Number 7363-100) and stored in at –80°C. The commercial solution [0.5 mg mL^{-1} in 20 mM Tris-HCl buffer (pH 8.0), 100 mM NaCl, 1 mM DTT, 2 mM EDTA, 40% glycerol] was diluted to 0.01 mg mL^{-1} by dissolving an aliquot (1

µL) in the same storage buffer (49 µL). The enzyme stock solution was freshly prepared, stored at –20°C, and used for at most 2 hours of experimentation.

Concentrated stock solutions in DMSO were prepared for cytidine (4.47 mM), ^{tz}**C** (3.46 mM), th**C** (3.21 mM), and ^{mth}**C** (4.66 mM). A working solution (1 mM) was made the day of experimentation by dissolving an appropriate aliquot in MilliQ water for a total volume of (50 µL).

The CDA-mediated enzymatic conversion of cytidine (and its analogues) was followed by absorbance and emission (for the emissive analogues ^{tz}**C**, th**C**, and ^{mth}**C**) spectroscopy by monitoring the intensity variation as a function of time. The real-time conversion of cytidine (and its analogues) to uridine (and the corresponding analogues) was performed on a Shimadzu UV-2450 spectrophotometer (slit width: 1nm, resolution 0.5 nm) and Horiba Fluoromax-4 (slit widths: 3 nm, resolution: 1 nm). Each instrument was equipped with a thermostat-controlled ethylene glycol/water bath fitted to a specially designed cuvette holder and the temperature was kept at 25.0 ± 0.1 °C.

All measurements were carried out in a 125 µL, 1 cm four-sided quartz cuvette from Helma. Tris-HCl buffer (100 mM, pH 7.5) was freshly prepared. Buffer, cytidine or cytidine analogue, and CDA were diluted in water to final concentrations of 50 mM Tris-HCl, pH 7.5, 10 µM, and 0.16 µg mL^{-1} respectively.

4.2 Monitoring of Enzymatic Conversion of C and C Analogues to Corresponding U and U Analogues by Absorption Spectroscopy

The absorbance of cytidine, ^{tz}**C**, th**C**, and ^{mth}**C** was monitored at 260, 340, 330, and 330 nm respectively. MilliQ water, buffer, and cytidine analogue were added to the cuvette, thoroughly mixed, and an initial measurement at the respective wavelength was taken. An aliquot of enzyme stock solution was taken straight from the –20°C freezer and added to the reaction cuvette. The reaction solution was mixed with a pipette several times and measurements were continued every 60 seconds. Cytidine was monitored for 3600 seconds. Cytidine analogues were monitored for 1560 seconds. All experiments were run in triplicate.

All absorption data were plotted against time. A pseudo-first order curve (Eqs. 1–2) was fit to each resulting time plot yielding k_{app} values and $t_{1/2}$ values. Averages and standard deviations of the k_{app} and $t_{1/2}$ values were calculated from cytidine and each cytidine analogues set of time plots. In addition, a set of ordinary differential equations (ODEs) (Eqs. 3–6) consistent with Michaelis-Menten kinetics was solved using the Runge-Kutta method with a variable time step in MatLab (function ode45). Initial concentrations used for each reaction were given above. The resulting fitted curves for each species were optimized by iteratively testing k values (Table 1) that maximized R^2.

4.3 Monitoring of Enzymatic Conversion of C and C Analogues to Corresponding U and U Analogues by Fluorescence Spectroscopy

The emission intensity of ^{tz}**C**, th**C**, and ^{mth}**C** was monitored at 408, 400, and 427 nm upon excitation at 293, 292, and 305 nm, respectively. MilliQ water, buffer, and cytidine analogue were added to the cuvette, thoroughly mixed, and an initial measurement at the respective wavelength was taken. An aliquot of enzyme stock solution (kept at –20°C) was added to the reaction cuvette. The reaction solution was mixed with a pipette several times and measurements were continued every 60 seconds for 1560 seconds. All experiments were run in triplicate.

All emission data were plotted against time. A pseudo-first order curve (Eqs. 1–2) was fit to each resulting time plot yielding k_{app} values and $t_{1/2}$ values. Averages and standard deviations of the k_{app} and $t_{1/2}$ values were calculated from each cytidine analogues set of time plots. In addition, a set of ordinary differential equations (ODEs) (Eqs. 3–6) consistent with Michaelis-Menten kinetics was solved using the Runge-Kutta method with a variable time step in MatLab (function ode45). Initial concentrations used for each reaction were given above. The resulting fitted curves for each species were optimized by iteratively testing k values (Table 1) that maximized R^2.

4.4 Screening of CDA Inhibitors via Fluorescence Spectroscopy

The same procedure as described in section 4.3 was used for all measurements except for the addition of inhibition. Reactions were also only monitored for 600 seconds. Concentrated stock solutions of each inhibitor were prepared in MilliQ water (10 mM). An aliquot of the desired inhibitor was added to the reaction solution after the cytidine analogue and before mixing. Inhibitors were tested at concentrations of 0, 0.1, 1, 10, 100, and 1000 µM. All experiments were run in triplicate.

All emission data for a give experiment were converted to a percent change relative to the initial time point. For a given cytidine analogue, percent change signal at one time point was divided by the percent change signal of the reaction without inhibitor present at the same time point to yield the percent activity. Percent activity was subtracted from 1 to yield percent inhibition. Percent inhibition was plotted against the corresponding inhibitor concentration. A Hill curve was fit to the resulting plot and the IC_{50} value was calculated from the obtained constants. The K_I was then calculated from the IC_{50} and K_M of the cytidine analogue used to monitor enzyme activity (Eq. 7).

4.5. HPLC End Point Analysis of CDA Activity On C, ^{tz}C, thC, and ^{mth}C

To corroborate the experiments above, CDA-mediated deamination of cytidine, **^{tz}C**, **thC**, and **^{mth}C** was monitored by chromatography. HPLC analysis was carried out with an Agilent 1200 series system with a Sepak Bio C18 analytical column (250 x 4.6 mm, 5 µm particle size). 0.1% formic acid stock solutions were prepared by dissolving 1 mL of formic acid (Acros, 99%) in 999 mL MilliQ water or HPLC grade acetonitrile (Sigma) and filtered using Millipore type GNWP 0.2 µM filters before use. Each injection (10 µL) was subjected to a gradient (20 minutes, from 0.5 to 20% acetonitrile 0.1% formic acid in water 0.1% formic acid) followed by a flush (10 minutes). A flow rate of 1 mL min^{-1} was used and the run was carried out at 25.0 ± 0.1 ^{o}C. Each run was monitored at 260 nm and 320 nm with calibrated references at 650 nm and slit set at 4 nm.

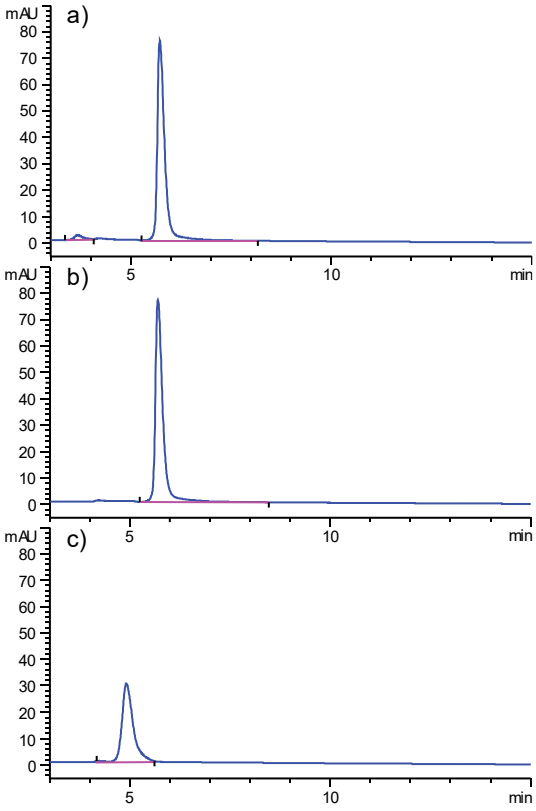


Figure S2. HPLC traces of the reaction of CDA with **C** after 60 minutes (a), **U** (b), and **C** (c) monitored by absorption at 260 nm.

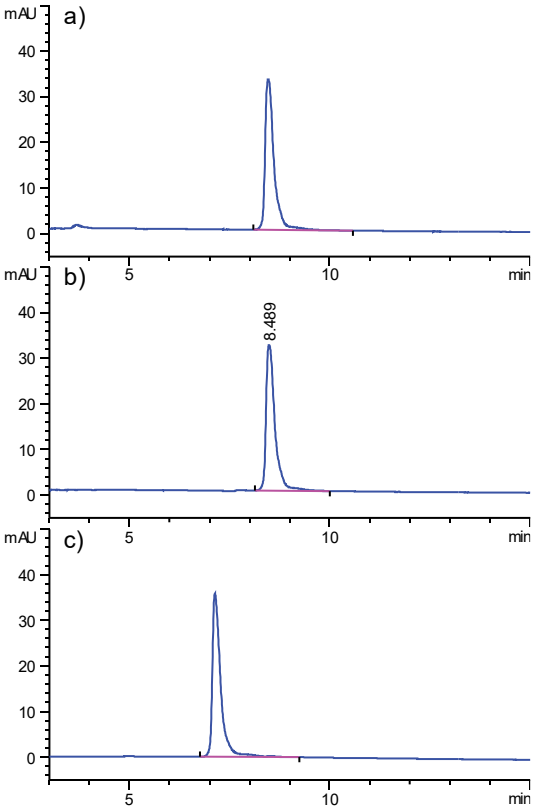


Figure S3. HPLC traces of the reaction of CDA with ^{tz}**C** after 60 minutes (a), ^{tz}**U** (b), and ^{tz}**C** (c) monitored by absorption at 320 nm.

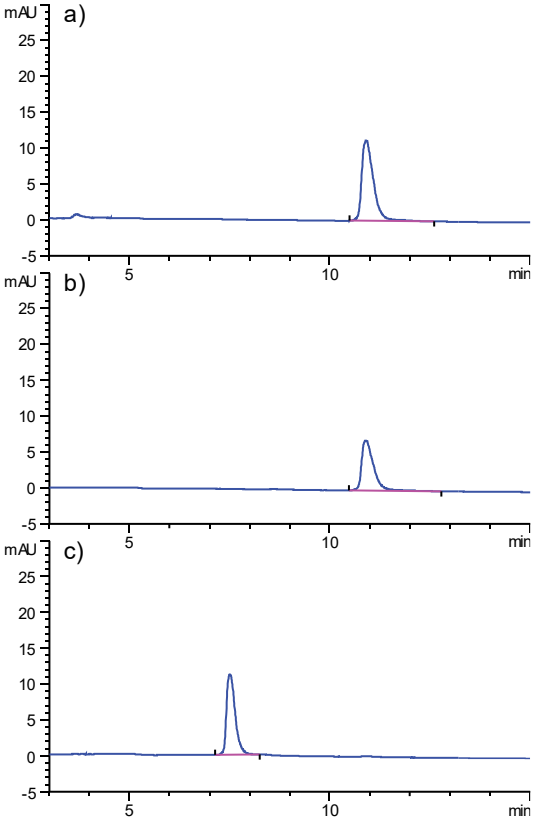


Figure S4. HPLC traces of the reaction of CDA with th**C** after 60 minutes (a), th**U** (b), and th**C** (c) monitored by absorption at 320 nm.

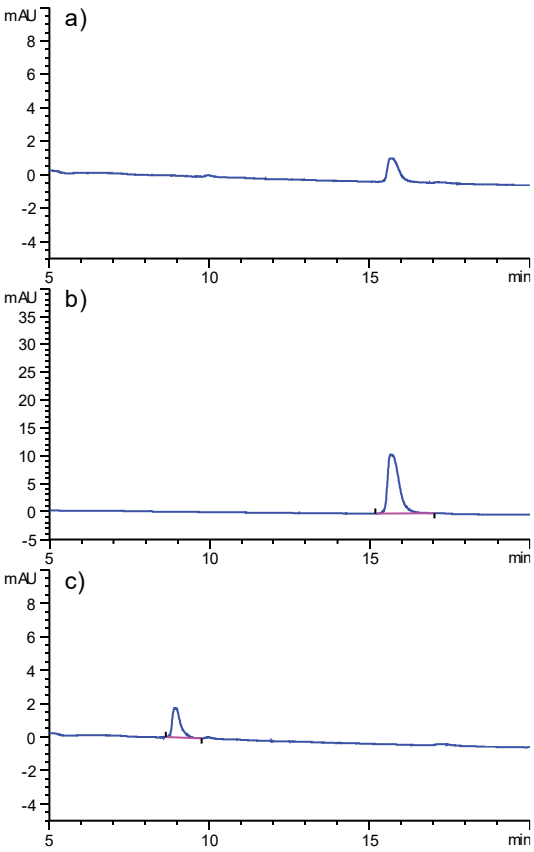


Figure S5. HPLC traces of the reaction of CDA with ^{mth}**C** after 60 minutes (a), ^{mth}**U** (b), and ^{mth}**C** (c) monitored by absorption at 320 nm.

5. ^{1}H and ^{13}C NMR Spectra

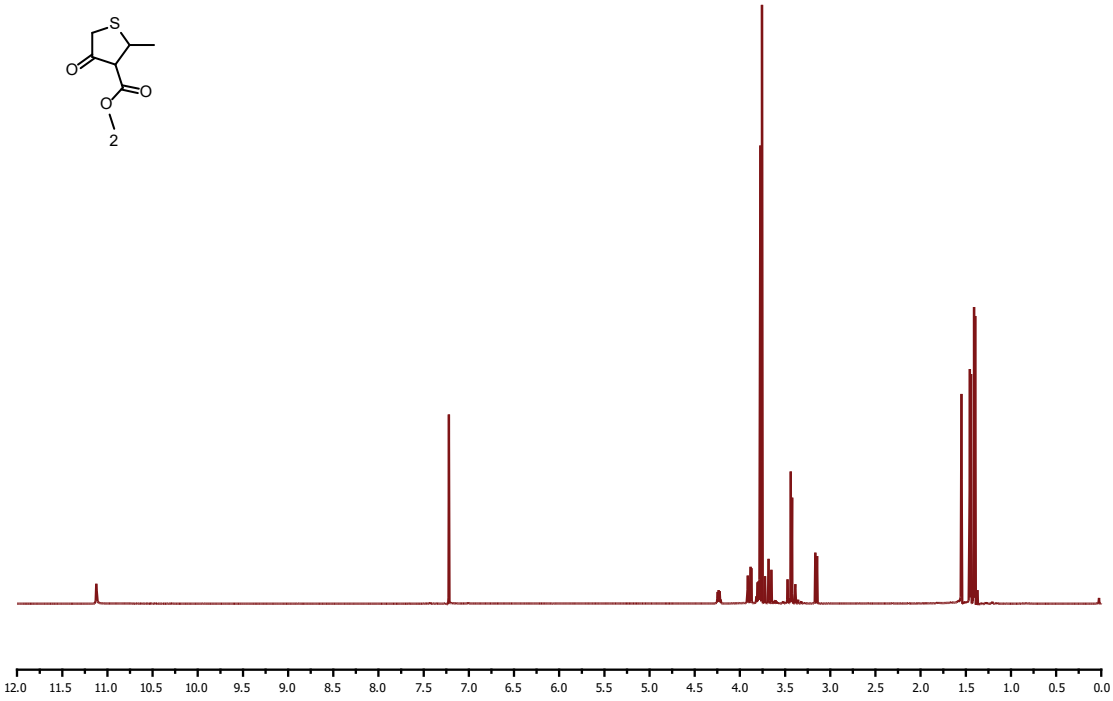


Figure S6. ^{1}H NMR spectra of **1**.

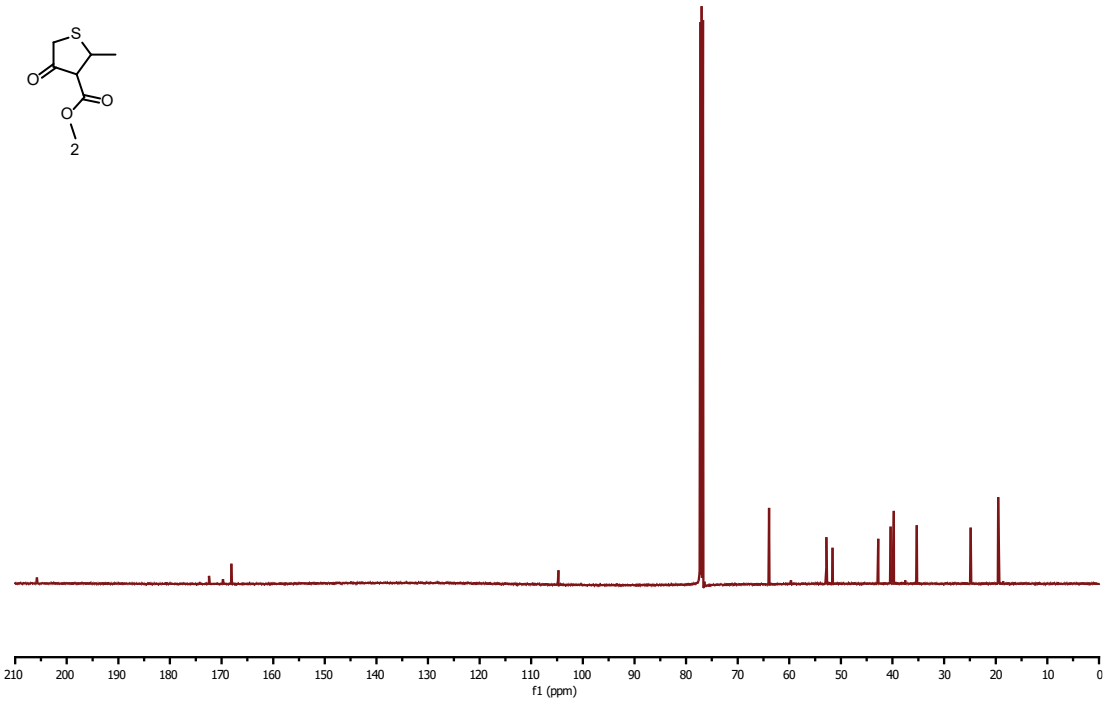


Figure S7. ^{13}C NMR spectra of **1**.

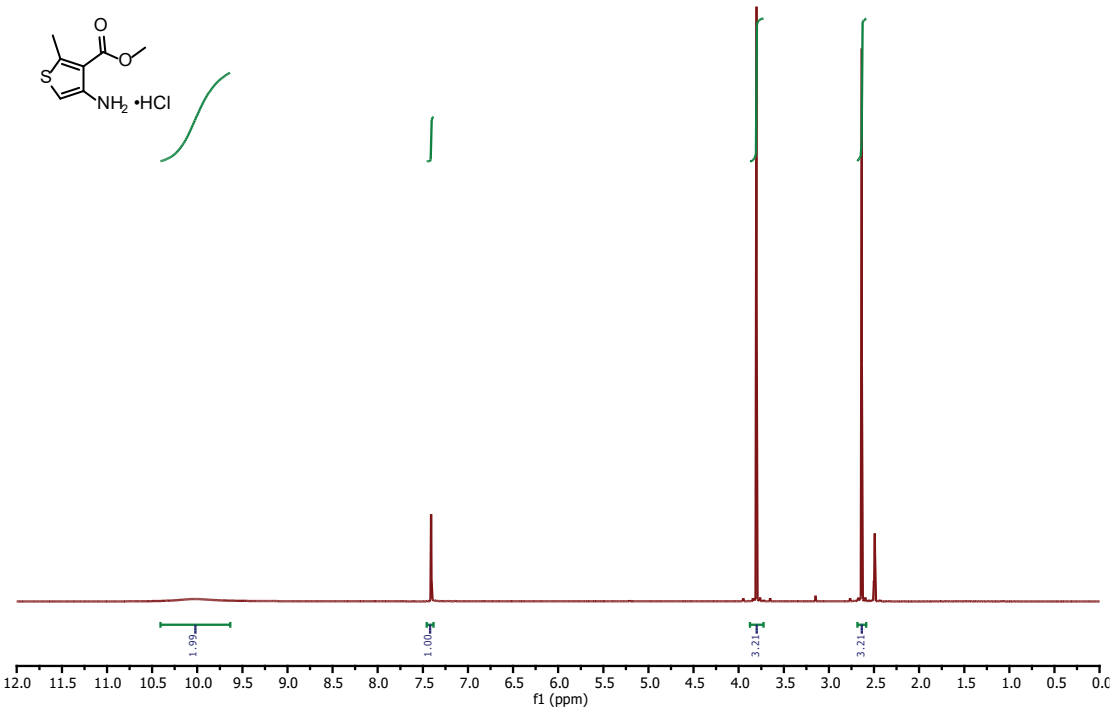


Figure S8. ^{1}H NMR spectra of **2**.

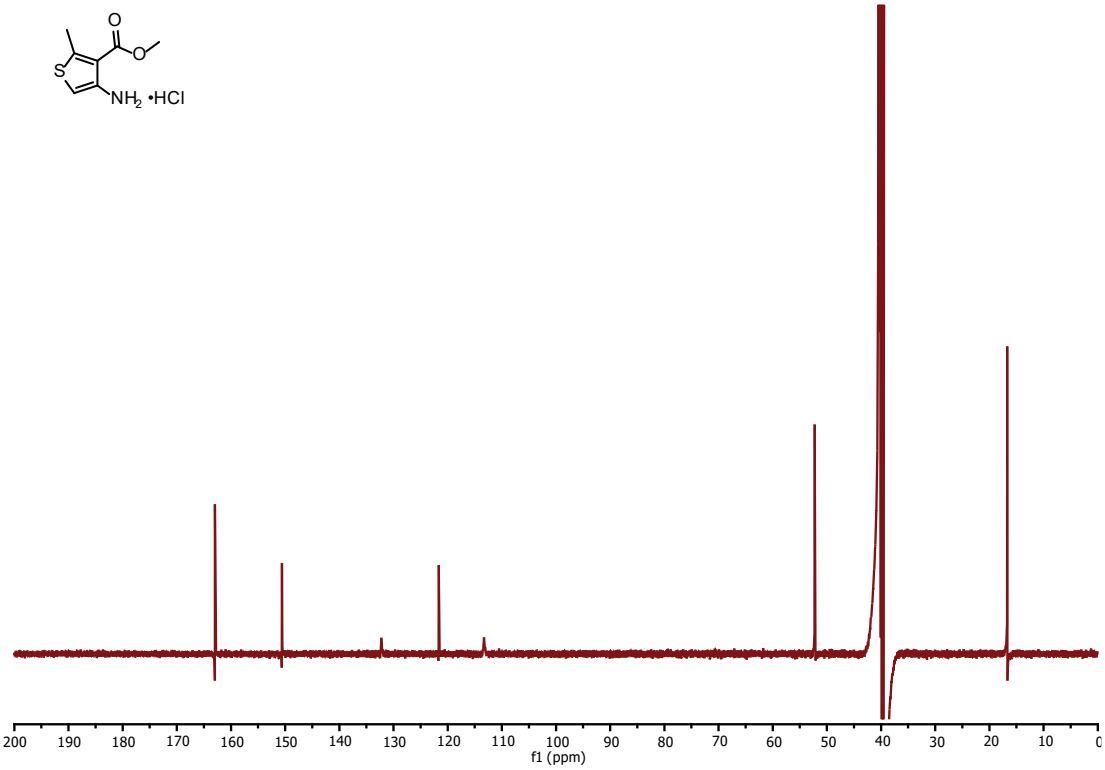


Figure S9. ^{13}C NMR spectra of **2**.

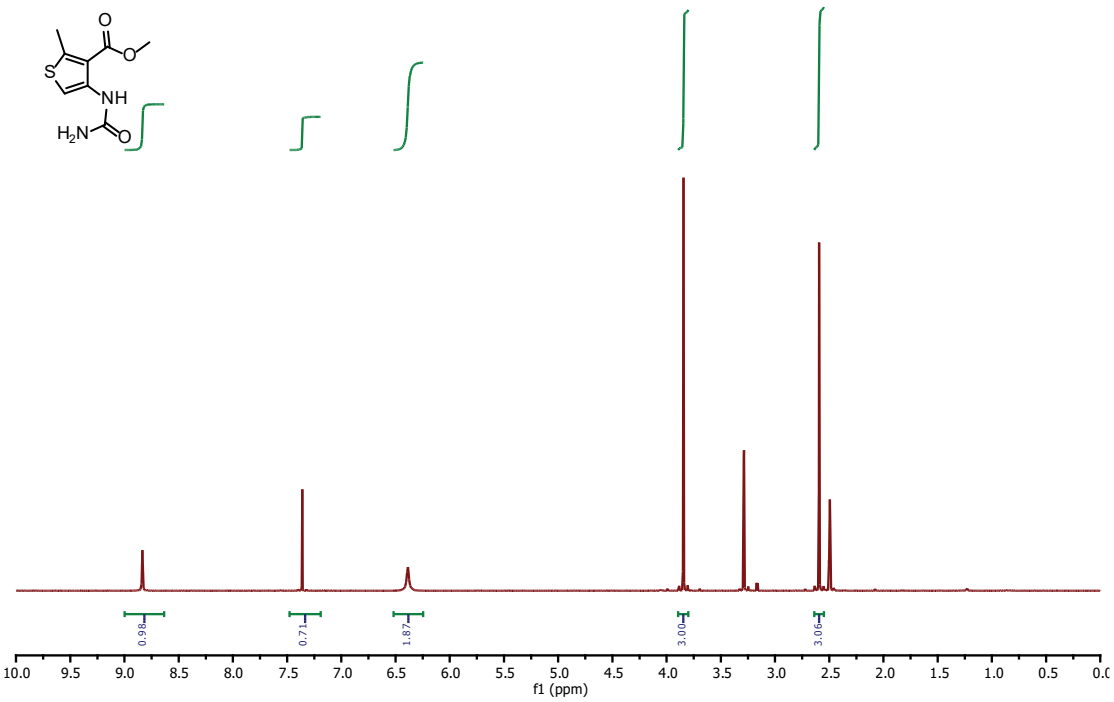


Figure S10. ^{1}H NMR spectra of **3**.

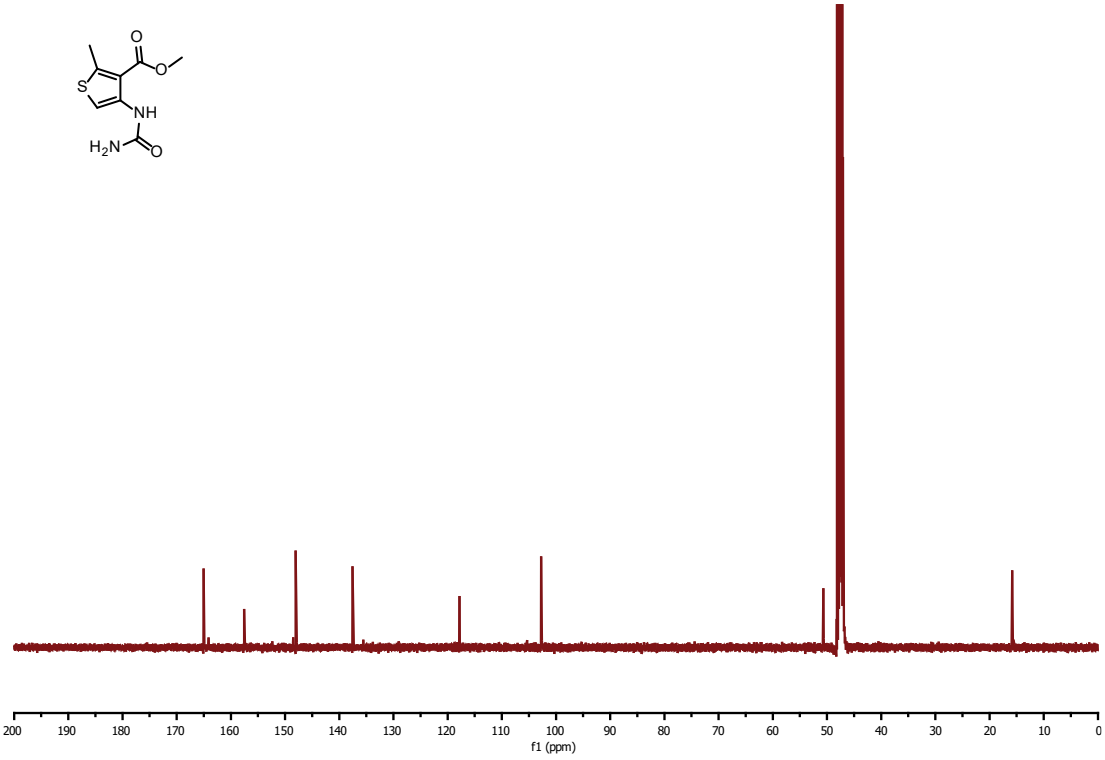


Figure S11. ^{13}C NMR spectra of **3**.

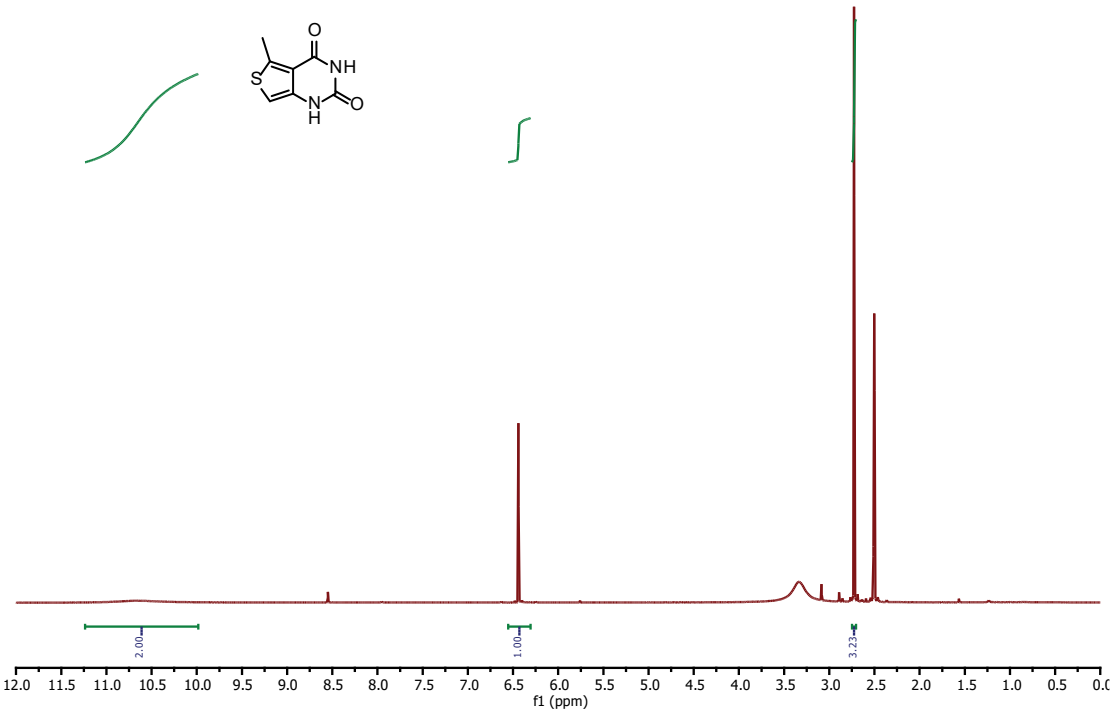


Figure S12. ^{1}H NMR spectra of **4**.

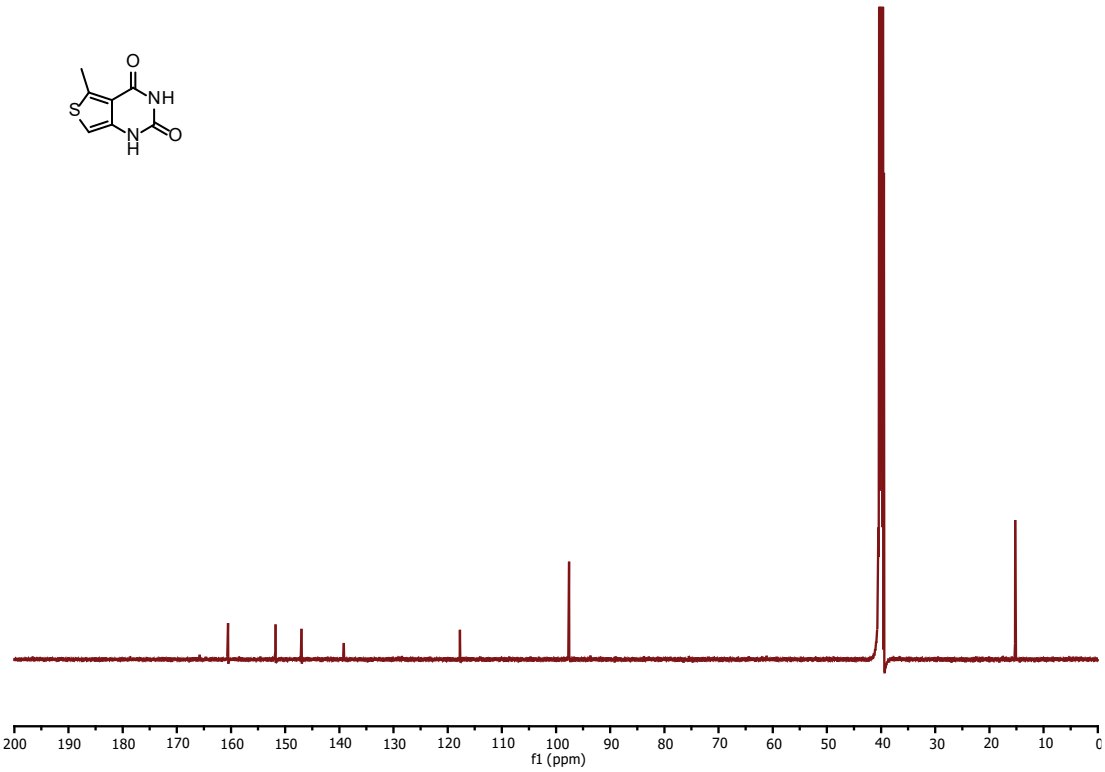


Figure S13. ^{13}C NMR spectra of **4**.

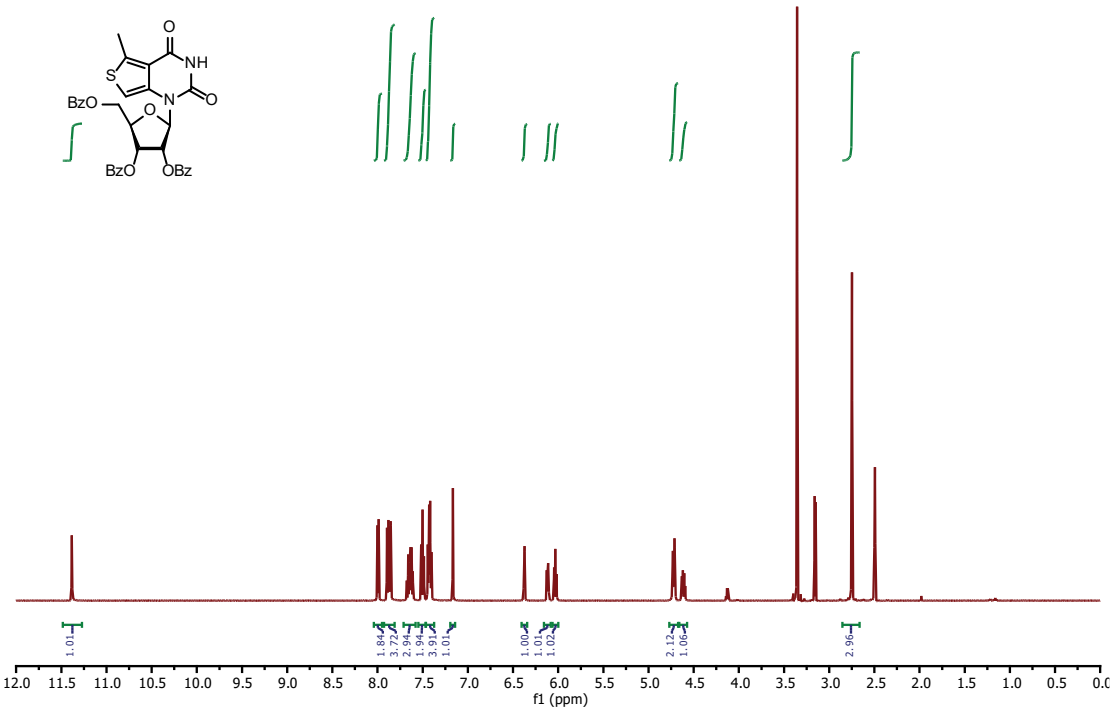


Figure S14. ^{1}H NMR spectra of **5**.

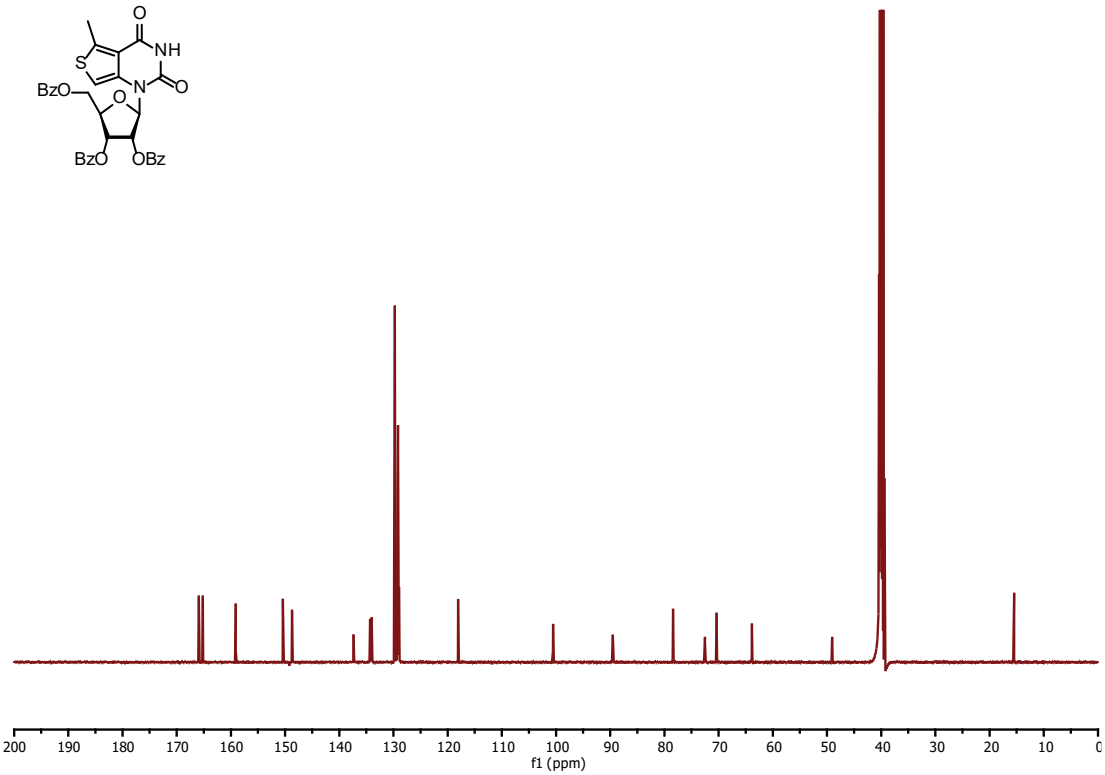


Figure S15. ^{13}C NMR spectra of **5**.

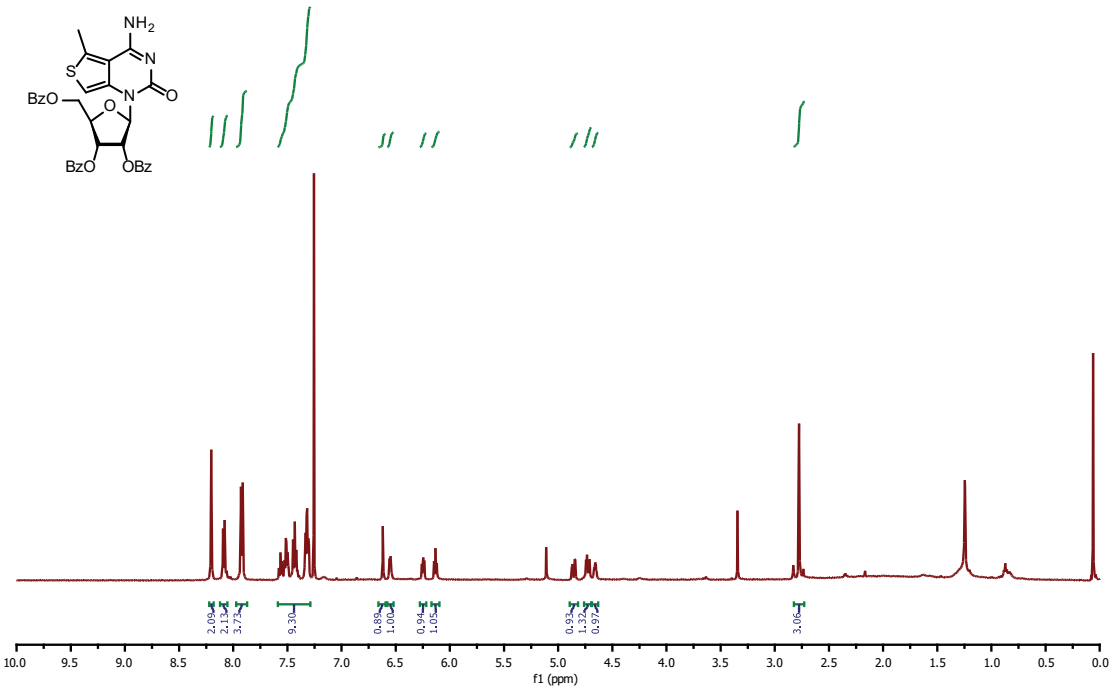


Figure S16. ^{1}H NMR spectra of **6**.

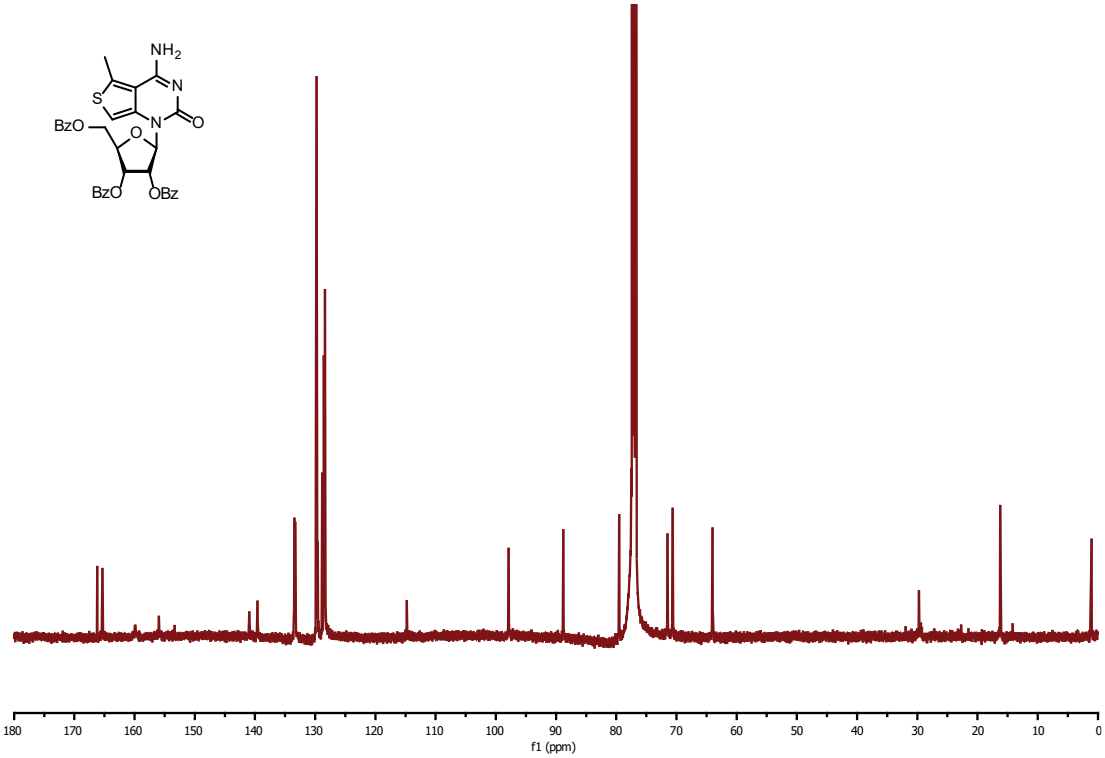


Figure S17. ^{13}C NMR spectra of **6**.

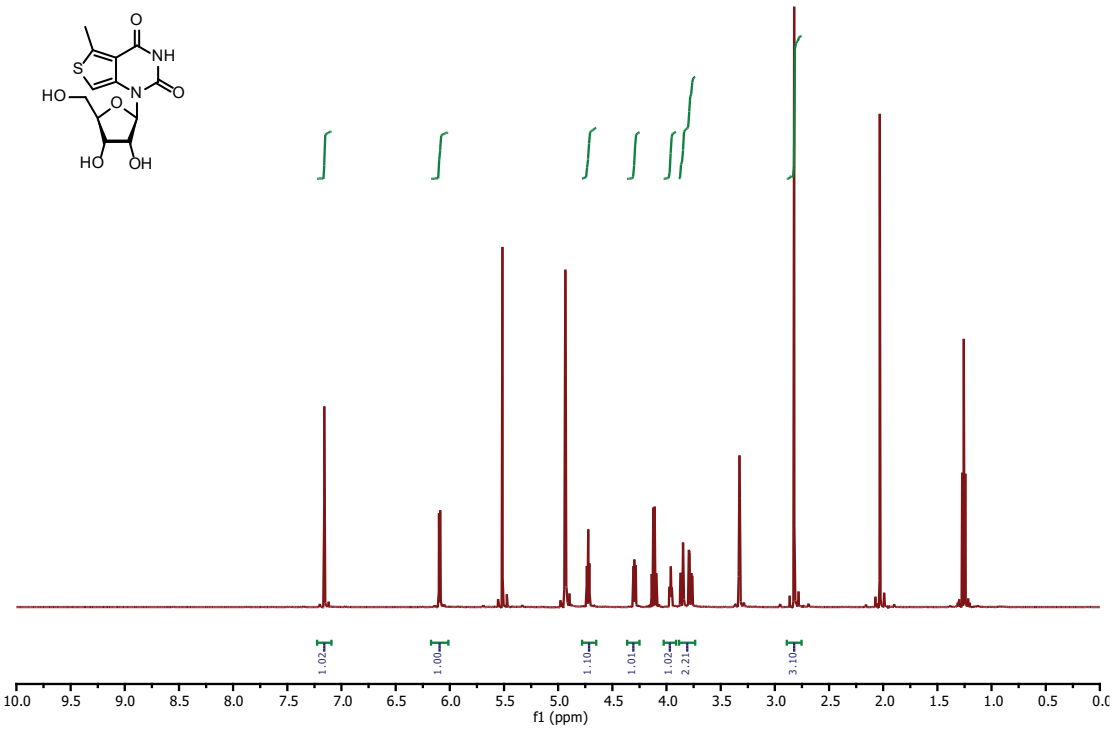


Figure S18. ^{1}H NMR spectra of **^{mth}U**.

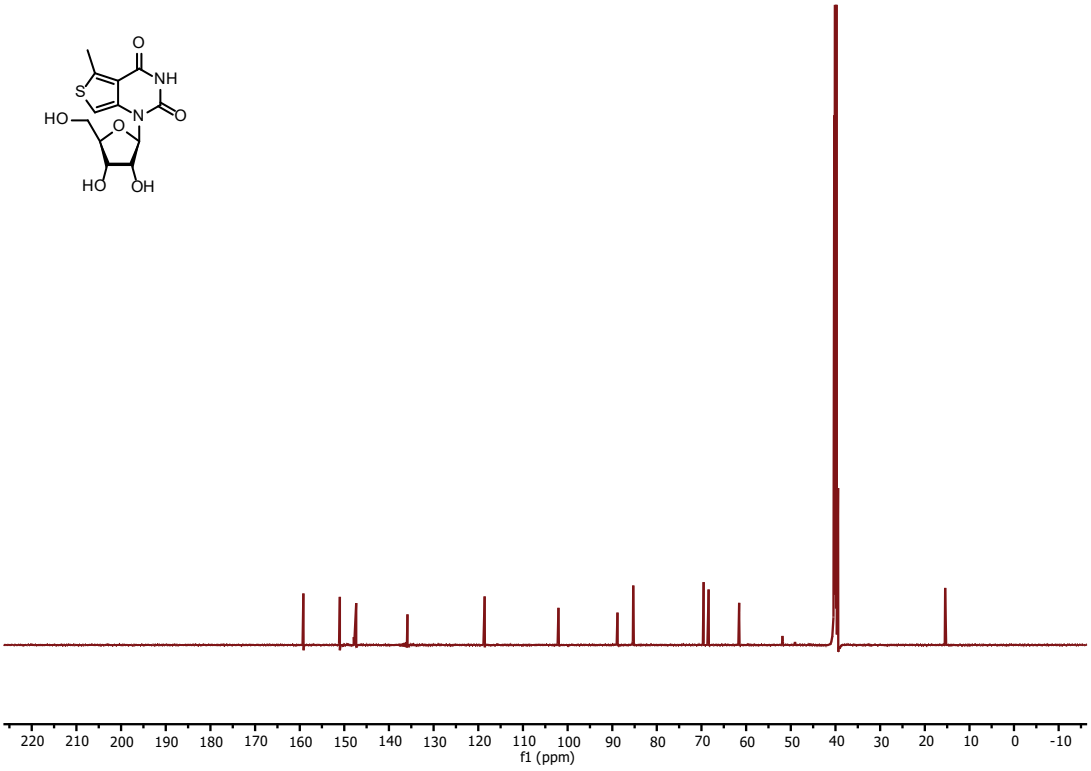


Figure S19. ^{13}C NMR spectra of **^{mth}U**.

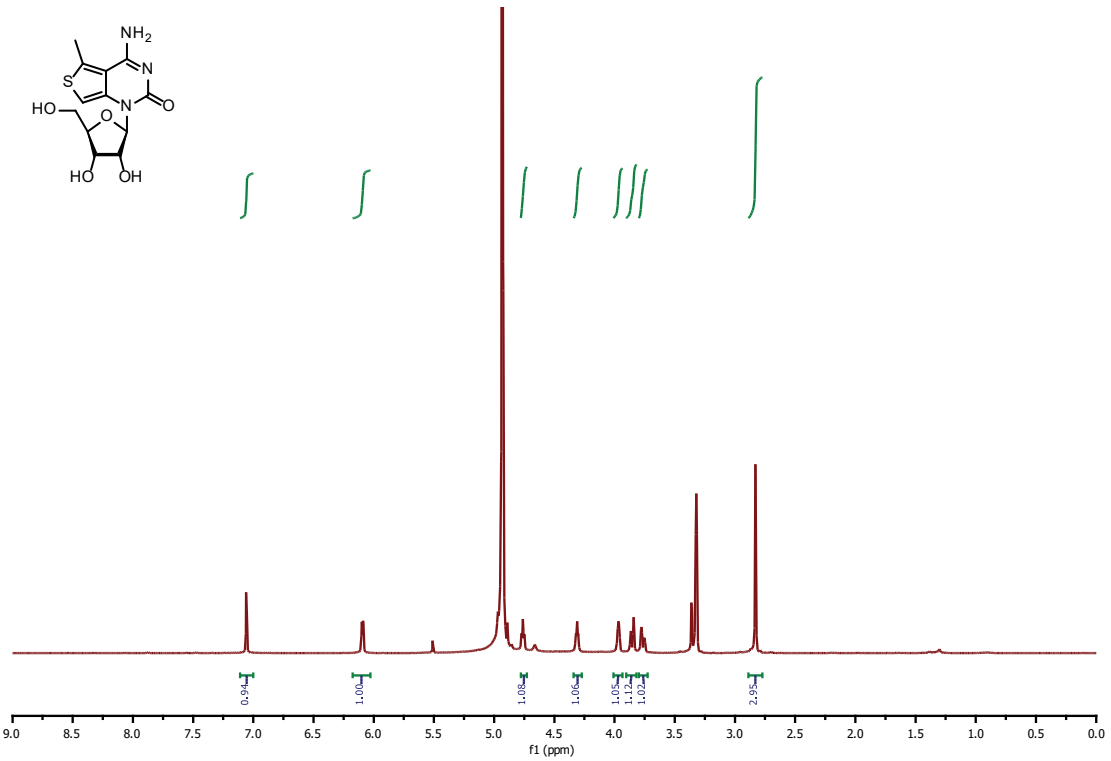


Figure S20. ^{1}H NMR spectra of **^{mth}C**.

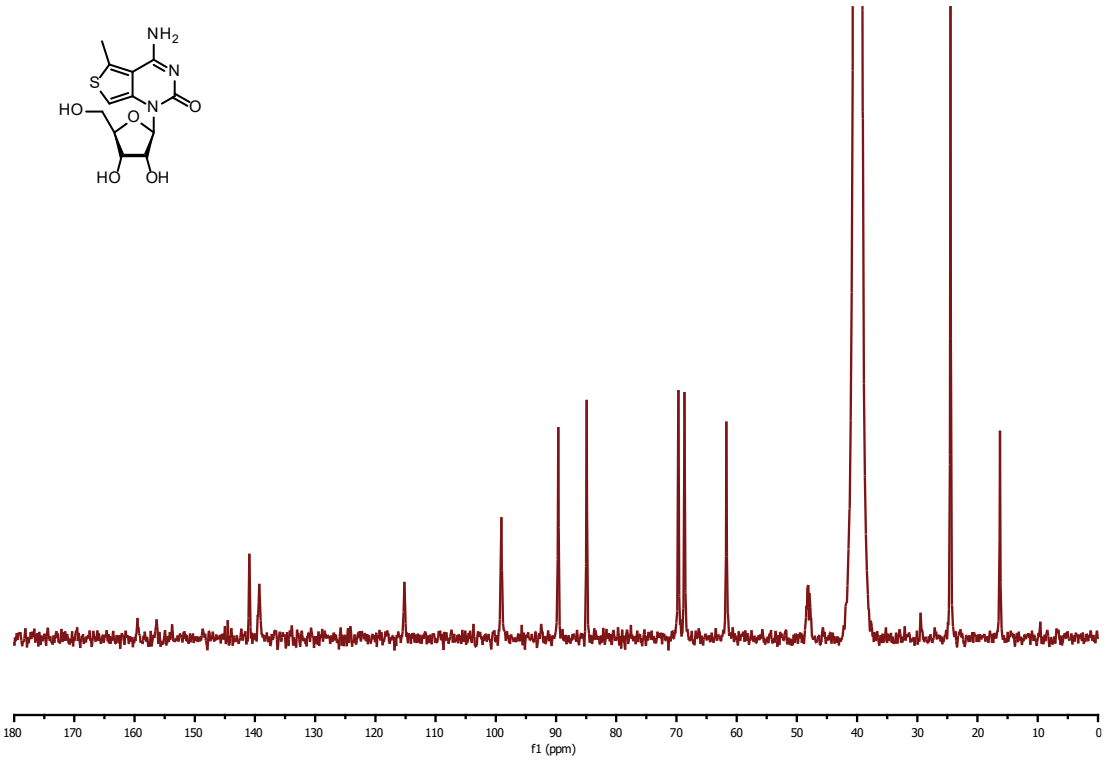


Figure S21. ^{13}C NMR spectra of **^{mth}C**.

6. Supplementary References

(S1) D. Shin; R. W. Sinkeldam; Y. Tor. *J. Am. Chem. Soc.* 2011, **133** (38), 14912–14915.

(S2) A. R. Rovira; A. Fin; Y. Tor. *J. Am. Chem. Soc.* 2015, **137** (46), 14602–14605.

(S3) A. R. Rovira; A. Fin; Y. Tor. *Chem. Sci.* 2017, **8**, 2983–2993.