Cytosine Derivatives Form Hemiprotonated Dimers in Solution and the Gas Phase

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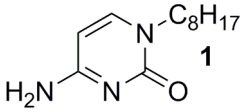
Supporting Information

1. General Information

^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova 400 or Varian Inova 500 spectrometer. 2D NOESY spectra were recorded on a Varian Inova 500 spectrometer. Proton (^{1}H) chemical shifts are reported in parts per million (δ) with respect to tetramethylsilane (TMS, δ=0), and referenced internally with respect to the protio solvent impurity. Deuterated NMR solvents were obtained from Cambridge Isotope Laboratories, Inc., Andover, MA, and used without further purification. Mass spectrometric experiments were carried out on a Waters GCT Premier TOF instrument using direct injection of samples into an electrospray source. Molecules and reagents were obtained from Aldrich Chemical Company, St. Louis, MO and were used as received. Solvents were dried through a commercial solvent purification system (SG Water, Inc.). Density functional theory calculations on the various proton-bound dimers and their monomers were done using the Gaussian03 suite of programs. Geometries were optimized using the B3LYP/6-311++G** basis set.[1]

2. Synthesis of New Compounds



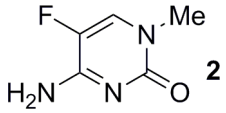
1

Synthesis of 4-Amino-1-octylpyrimidin-2(1H)-one 1:[2]

A dry 50-mL 2-neck round bottomed flask equipped with a magnetic stir bar was charged with N^4-acetylcytosine (2.80g, 18.2 mmol) and K_2CO_3 (3.80g, 27.5 mmol, 1.5 eq) and placed under nitrogen. Anhydrous dimethylformamide (15 mL) was added to the flask via syringe, and the mixture was stirred for 10 minutes (only partial dissolution was observed). 1-iodooctane (4.9 mL, 27.1 mmol, 1.5 eq) was added dropwise via syringe over 10 minutes and heated at 70°C for 5 hours. After cooling to room temperature, the crude product was precipitated by pouring the reaction mixture over 100 mL of water and collected by filtration. The product was washed with 3x20 mL portions of water and dried to yield crude *N*-(2-oxo-1,2-dihydropyrimidin-4-yl)acetamide (1.68 g) as an off-white solid, which was used in the next reaction without further purification.

The crude N^4-acetyloctylcytosine (1.68 g) was placed in a 100 mL round bottomed flask equipped with a magnetic stir bar and treated with 30 mL of methanolic ammonium hydroxide (7M), then stirred for

48 hours at 23ºC to yield a suspension containing a white precipitate. The precipitate was collected by filtration and recrystallized in 95% ethanol to obtain the target 4-amino-1-octylpyrimidin-2(1H)-one **1** as a white solid (1.10 g, 27% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 (d, J = 7.0 Hz, 1H), 5.63 (d, J = 7.0 Hz, 1H), 5.41 (br, 2H), 3.75 (t, J = 7.3 Hz, 2H), 1.70 (m, 2H), 1.28 (m, 9H), 0.87 (t, J = 6.8 Hz, 3H), ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.84, 155.74, 145.97, 92.93, 48.56, 31.18, 28.65, 28.60, 25.95, 22.05, 13.89 (one carbon resonance is obscured by the solvent). MS (ESI): **1H**$^+$ observed *m/z* 224.1766 (expected *m/z* 224.1757).

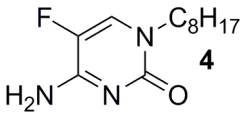


2

Synthesis of 5-fluoro-1-methylcytosine 2:[3]

5-Fluorocytosine (0.129g, 1.00 mmol), *N,N*-dimethylformamide dimethyl acetal (0.833g, 7.00 mmol), and anhydrous dimethylformamide (5 mL) were added to a 25 mL round bottomed flask equipped with a drying tube and condenser and heated at 90^{o}C under nitrogen for 24 hours. The reaction mixture was cooled to room temperature and evaporated to dryness under reduced pressure. The crude 5-fluoro-1-ethyl-N^4-[(dimethylamino)methylene]-cytosine (0.190g, 96% yield) recovered was used in the next step without purification.

Crude 5-fluoro-1-methyl-N^4-[(dimethylamino)methylene]-cytosine (0.190g, 0.96mmol) was dissolved in 10mL concentrated ammonium hydroxide solution and stirred at room temperature for 16 hours in a 25 mL round bottomed flask. The flask was then heated in a steam bath to drive off excess ammonia and then evaporated to dryness under reduced pressure. The resulting solid was recrystallized from methanol, giving 5-fluoro-1-methylcytosine **2** as a white solid (0.093g, 68% yield). ^{1}H NMR (300 MHz, DMSO-d_6, 298 K) δ 7.93 (d, *J* = 6.8 Hz, 1H), 3.31 (s, 3H). ^{19}F NMR (300 MHz, DMSO-d_6, 298 K) δ -170.52 (d, *J*= 6.1 Hz). APCI-ESI MS: **2H**$^+$: observed *m/z* 144.0581 (expected *m/z* 144.0568), **[2•H$^+$•2]**: observed m/z 287.1102 (expected m/z 287.1063).



4

Synthesis of 4-amino-5-fluoro-1-octylpyrimidin-2(1H)-one 4:

In a dry 15 mL 2-neck round bottomed flask, 5-fluorocytosine (0.500g, 3.88 mmol) and K_2CO_3 (0.805g, 5.80 mmol) were placed under nitrogen and stirred in anhydrous dimethylformamide (5 mL) for 10 minutes. Once both had partially dissolved, 1-iodooctane (1.05 mL, 5.8 mmol) was added, and the solution was heated to 70^{o}C for 12 hours in the dark. After cooling to room temperature, the product was precipitated by pouring the reaction mixture into water (20 mL) and then cooling on ice to aid precipitation. The precipitate was collected by vacuum filtration and then triturated in hexanes to remove excess 1-iodooctane. Recrystallization of the product from ethyl acetate resulted in the formation of bright white crystalline product (0.168g, 18%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.24 (d, J = 5.6 Hz, 1H), 3.73 (t, J = 7.0 Hz, 2H), 1.71 (qn, J = 7.0 Hz, 2H), 1.31 (m, 10H), 0.88 (t, J = 7.0 Hz, 3H).

^{19}F NMR (300 MHz, $CDCl_3$): δ -170.116 (d, J = 5.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.26, 154.93, 137.55, 135.22, 129.32, 50.47, 31.92, 29.31, 26.71, 22.79, 14.24. MS (ESI): **[4•H$^+$•4]** observed *m/z* 483.3349 (expected *m/z* 483.3254).

3. NMR Spectra

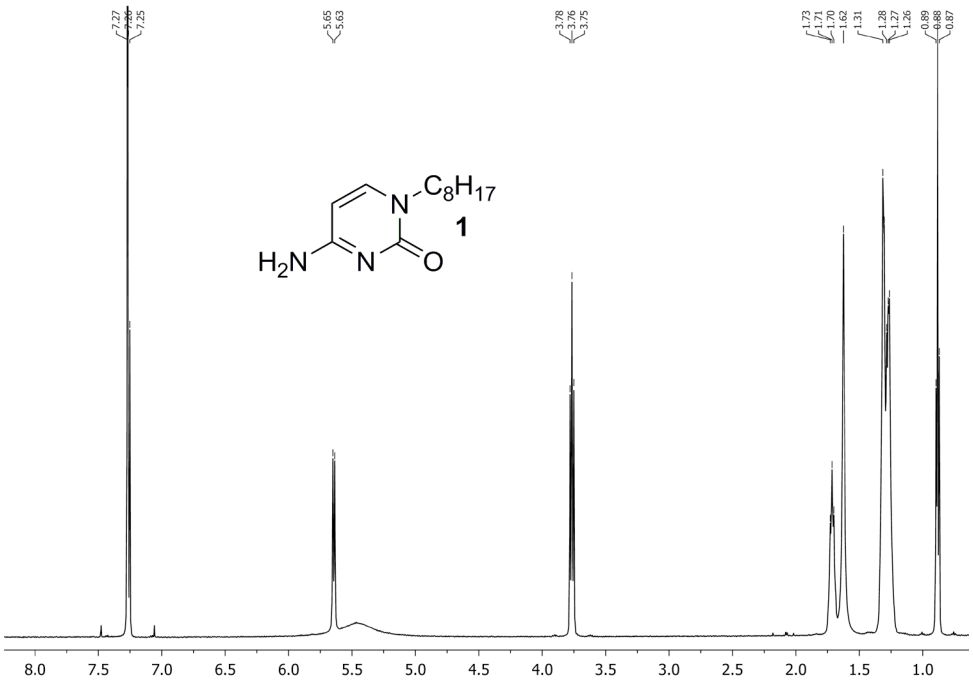


Figure *S*-1. Full scale ^{1}H NMR spectrum of 1-octylcytosine **1** (298K, $CDCl_3$, 400 MHz).

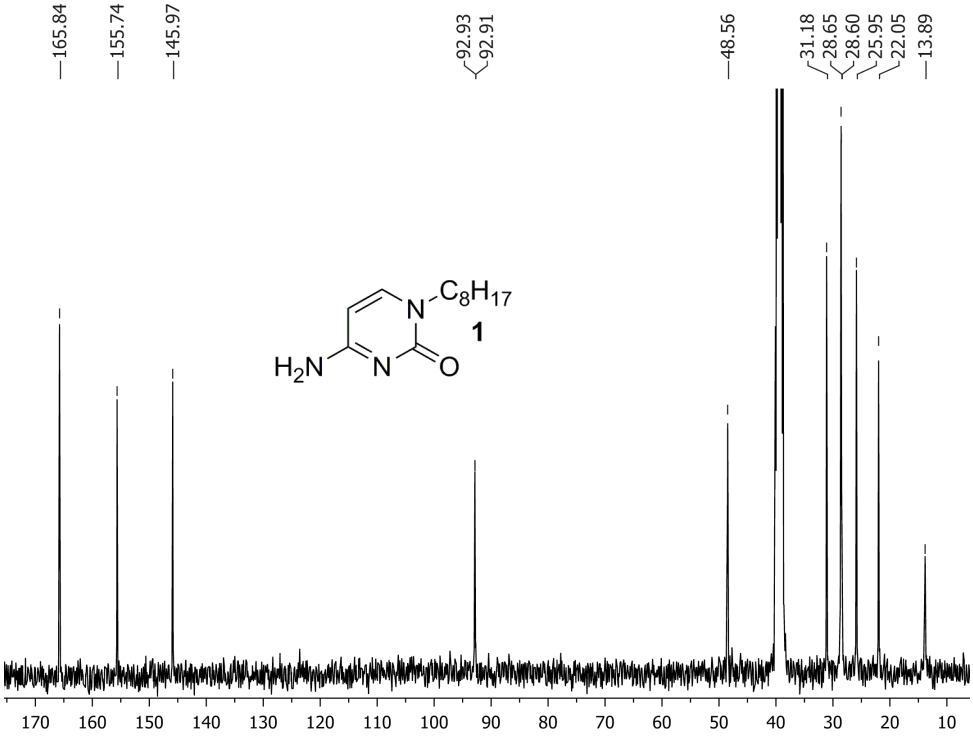


Figure *S*-2. ^{13}C NMR spectrum of 1-octylcytosine **1** (298K, DMSO-d_6, 100 MHz).

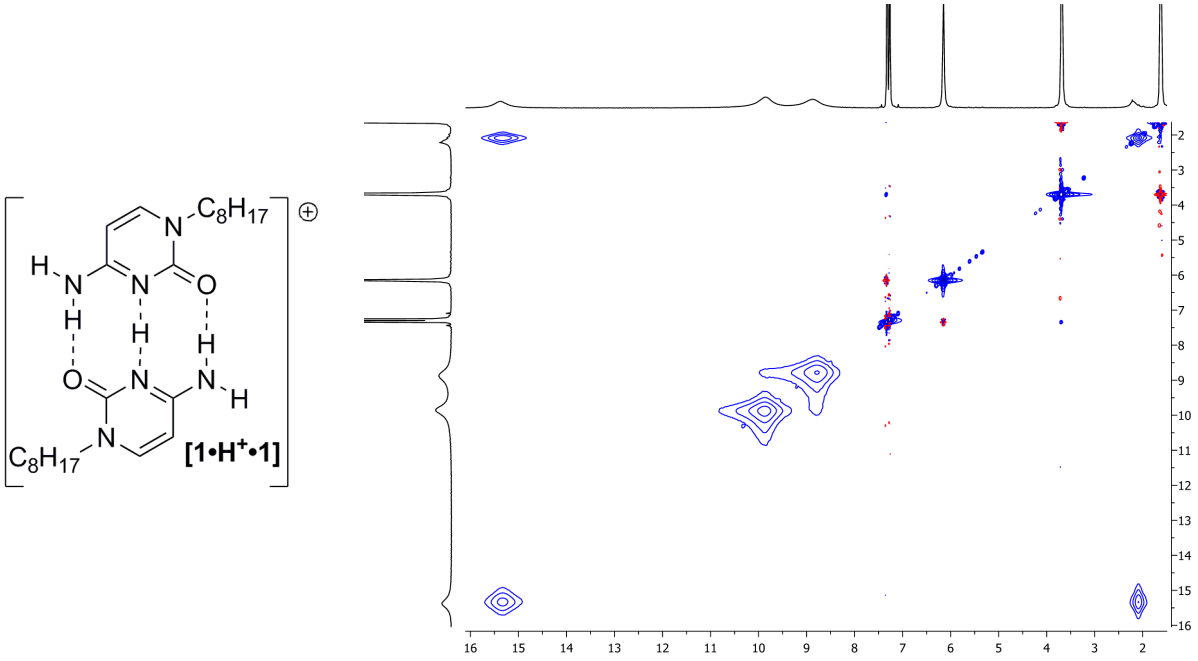


Figure *S*-3. 2D NOESY spectrum of **[1•H$^+$•1]** at 10 ms mixing time (243K, $CDCl_3$, 500 MHz).

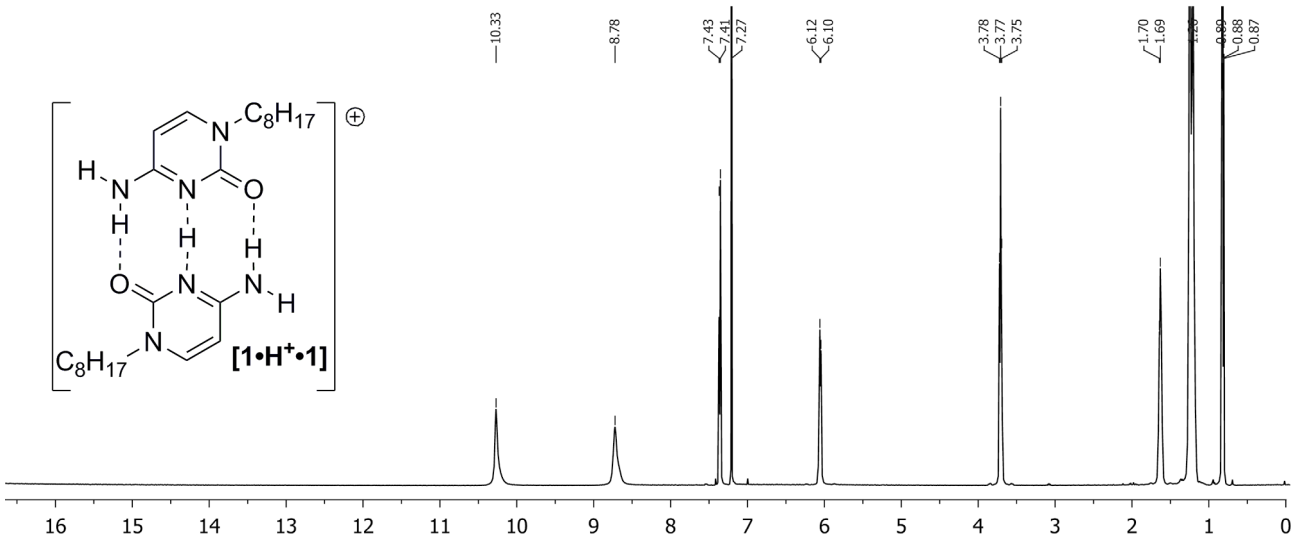


Figure *S*-4. ^{1}H NMR spectrum of the **[1•H$^+$•1]** homodimer (0.7 mol-eq. CF_3CO_2H, 298K, $CDCl_3$, 500 MHz).

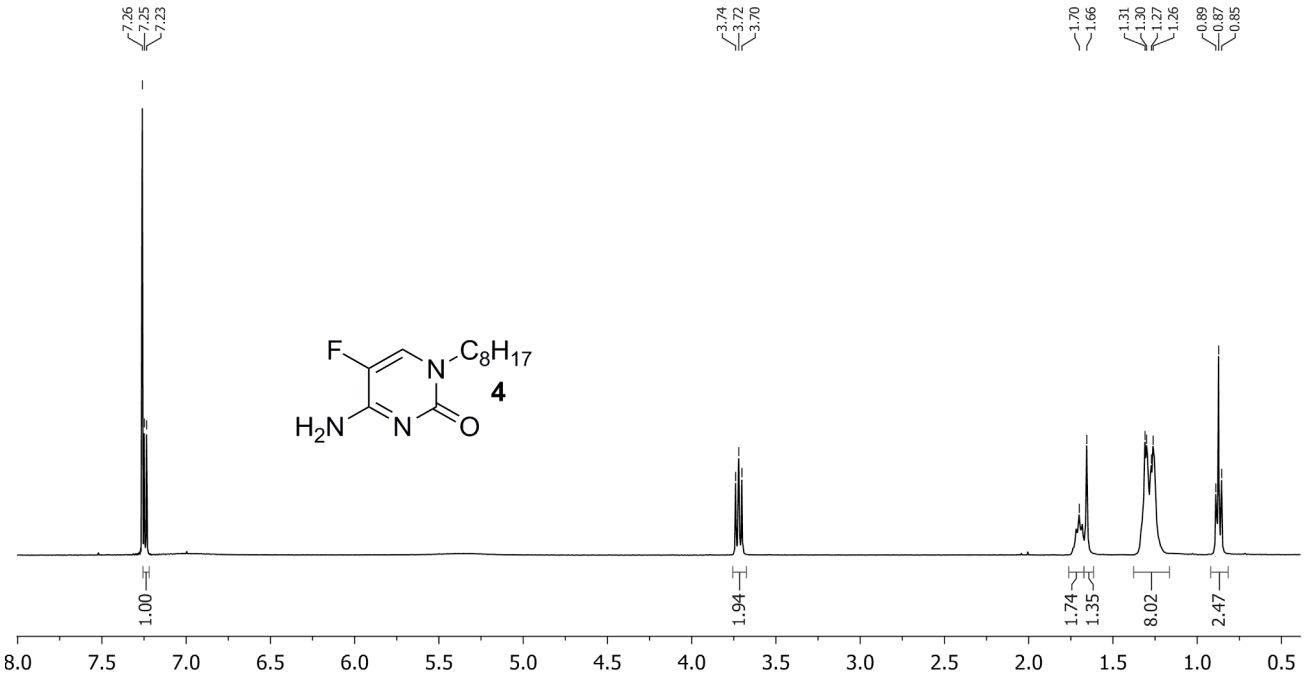


Figure *S*-5. ^{1}H NMR spectrum of 5-fluoro-1-octylcytosine **4** (298K, $CDCl_3$, 400 MHz).

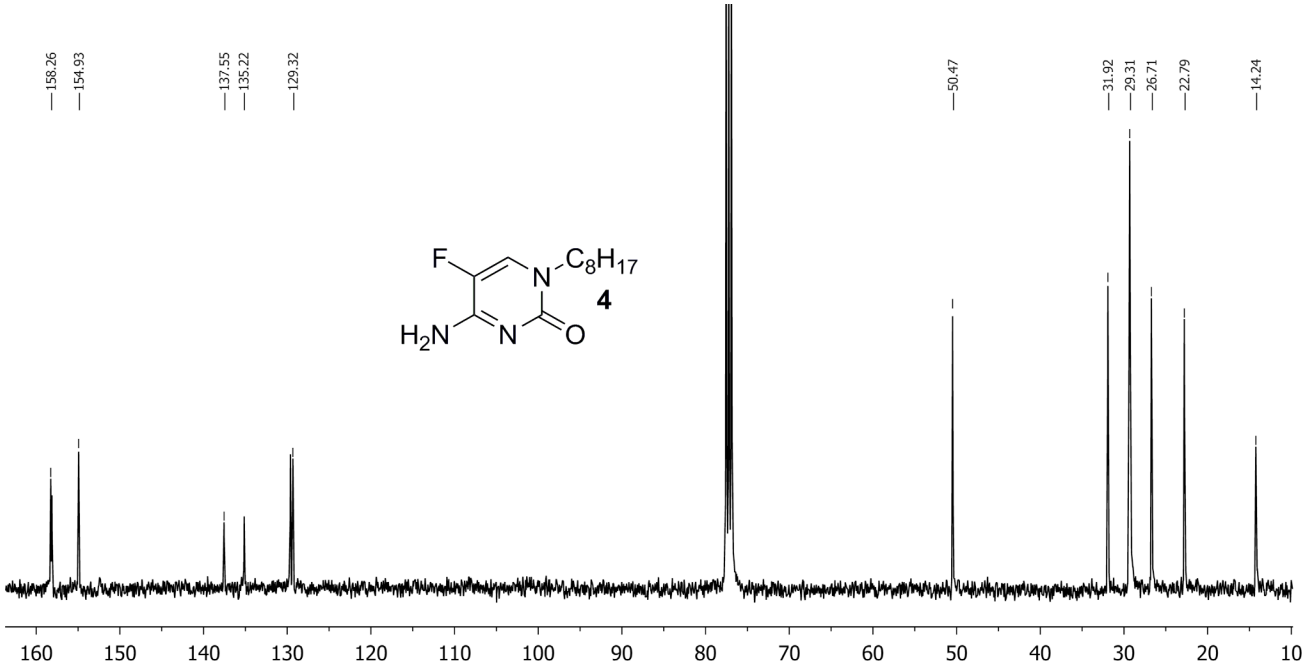


Figure *S*-6. ^{13}C NMR spectrum of 5-fluoro-1-octylcytosine **4** (298K, $CDCl_3$, 100 MHz).

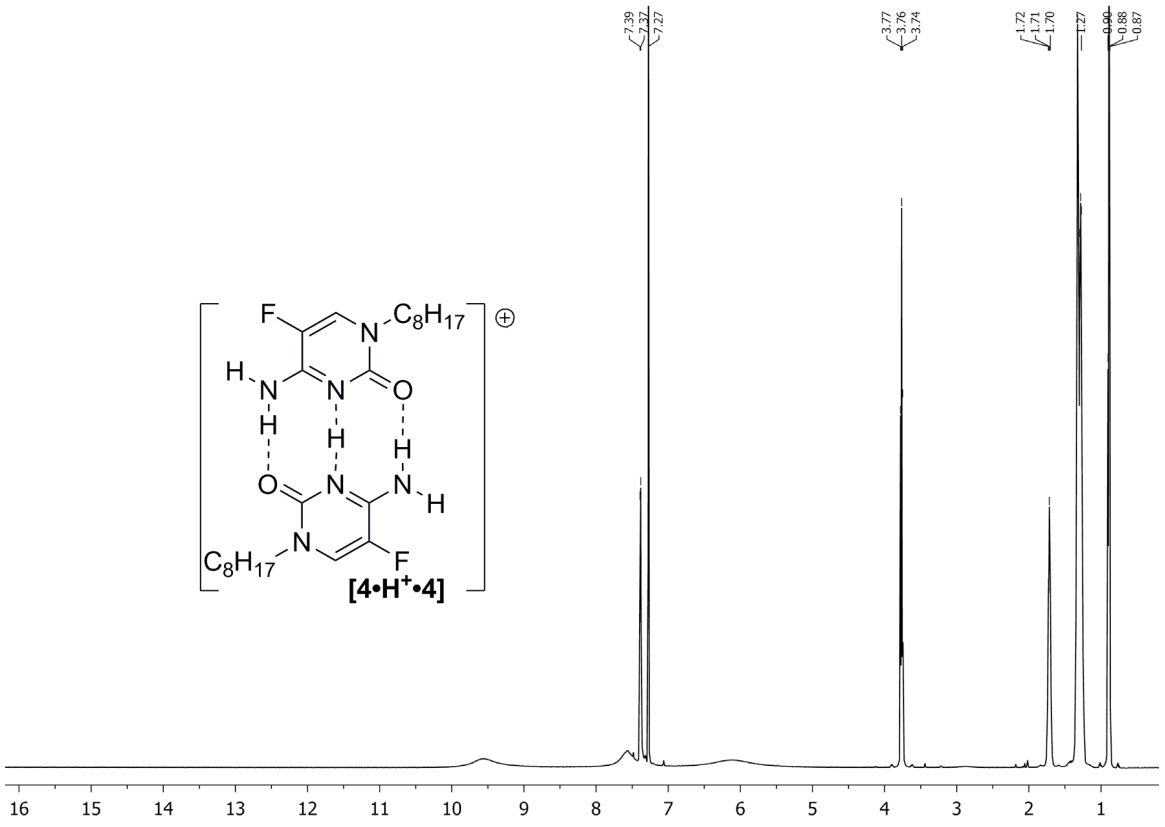


Figure *S*-7. Room temp. ^{1}H NMR spectrum of the **[4•H^{+}•4]** dimer (0.5 mol-eq. CF_3CO_2H, 298K, $CDCl_3$, 500 MHz).

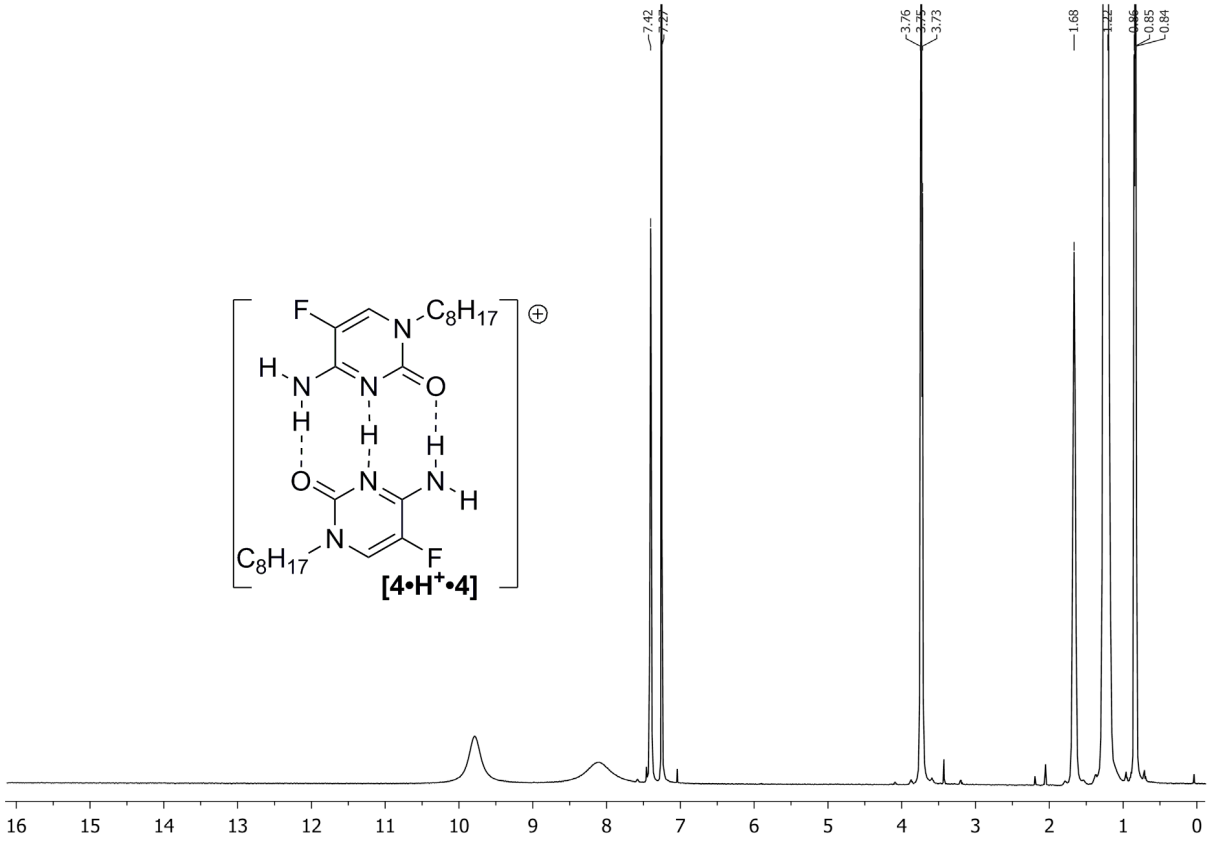


Figure *S*-8. Low temperature ^{1}H NMR spectrum of the **[4•H^{+}•4]** dimer (0.5 mol-eq. CF_3CO_2H, 243K, $CDCl_3$, 500 MHz).

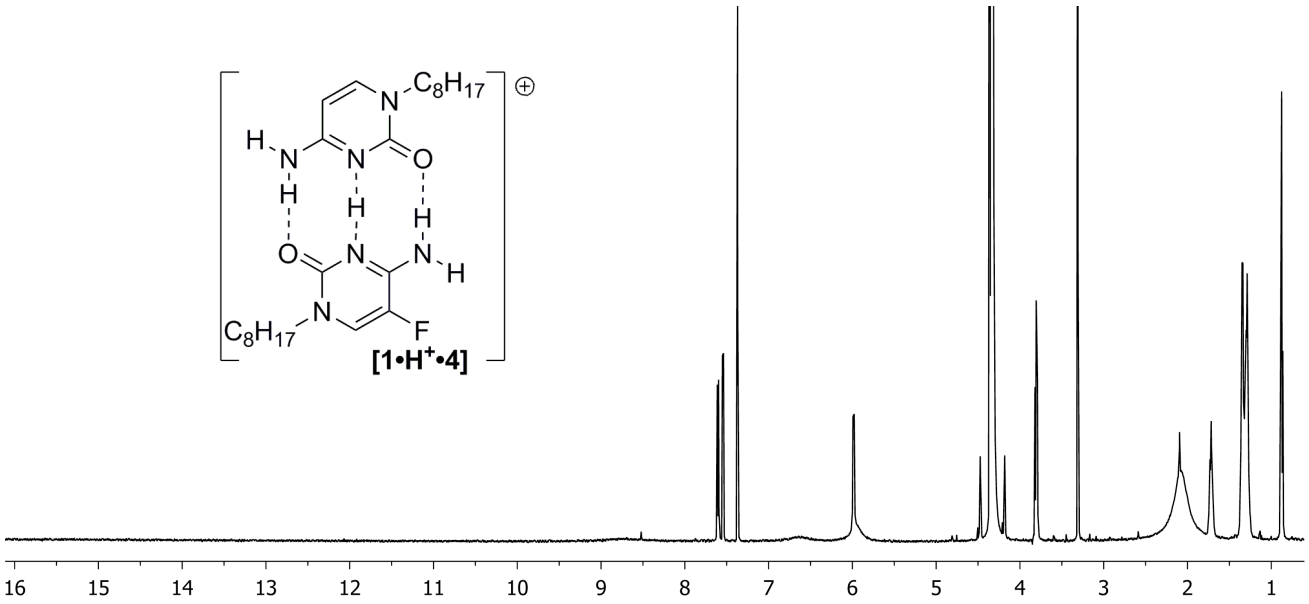


Figure *S*-9. Room temperature ^{1}H NMR spectrum of the **[1•H^{+}•4]** heterodimer (0.5 mol-eq. CF_3CO_2H, 298K, $CDCl_3$, 500 MHz).

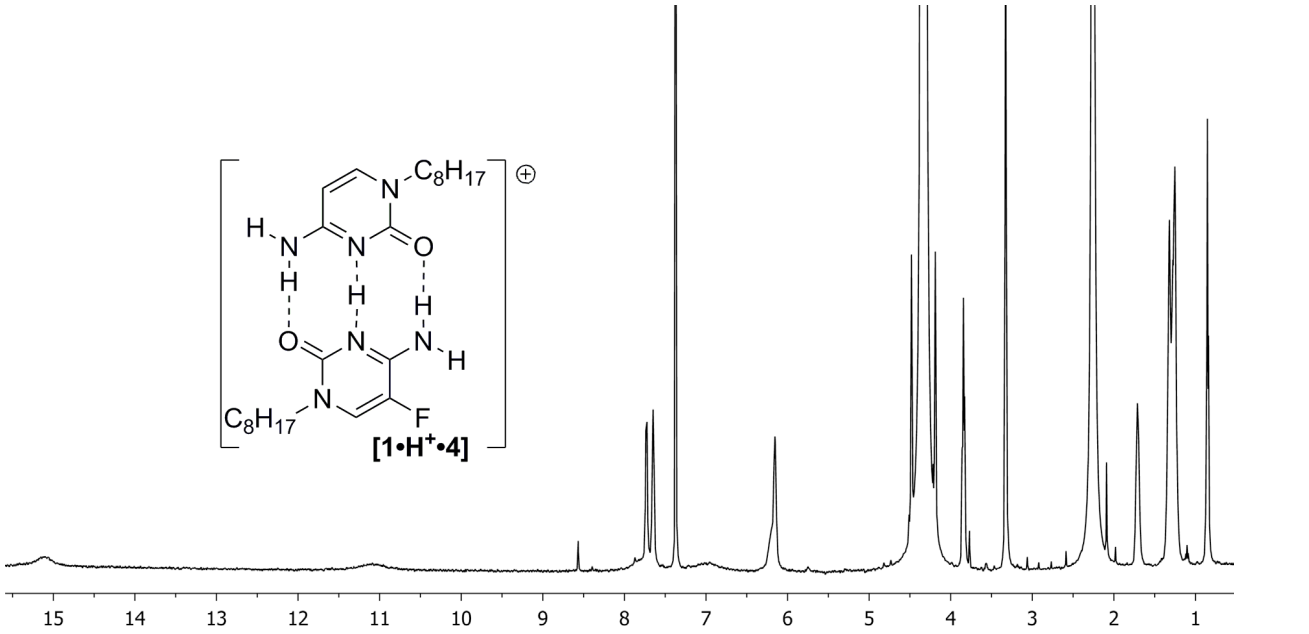


Figure *S*-10. Low temperature ^{1}H NMR spectrum of the **[1•H^{+}•4]** heterodimer (0.5 mol-eq. CF_3CO_2H, 243K, $CDCl_3$, 500 MHz).

4. Mass Spectrometric Data

0.1 M Stock solutions of 1-methylcytosine and 5-fluoro-1-methylcytosine were prepared by dissolving 0.1 mmol of solid in 1 mL of water. The various mixtures as well as the pure solutions were prepared by adding portions of the stock solutions to a 1.5 mL vial containing 1 mL of methanol. For example,

the 30% 1-methylcytosine 70% 5-fluoro-1-methylcytosine was prepared by delivering 30 μL of 1-methylcytosine and 70 μL of 5-fluoro-1-methylcytosine stock solutions by automatic pipet to the 1.5 mL vial. Mass spectrometric experiments were carried out on a Waters GCT Premier TOF instrument using direct injection of samples into an electrospray source.

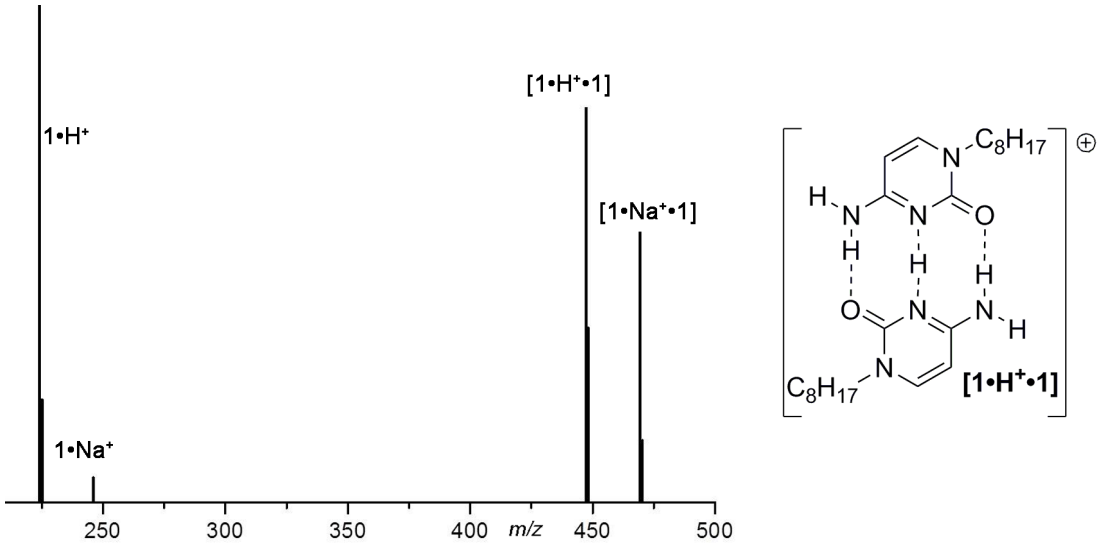


Figure *S*-11. ESI-MS spectrum of **[1•H$^+$•1]**.

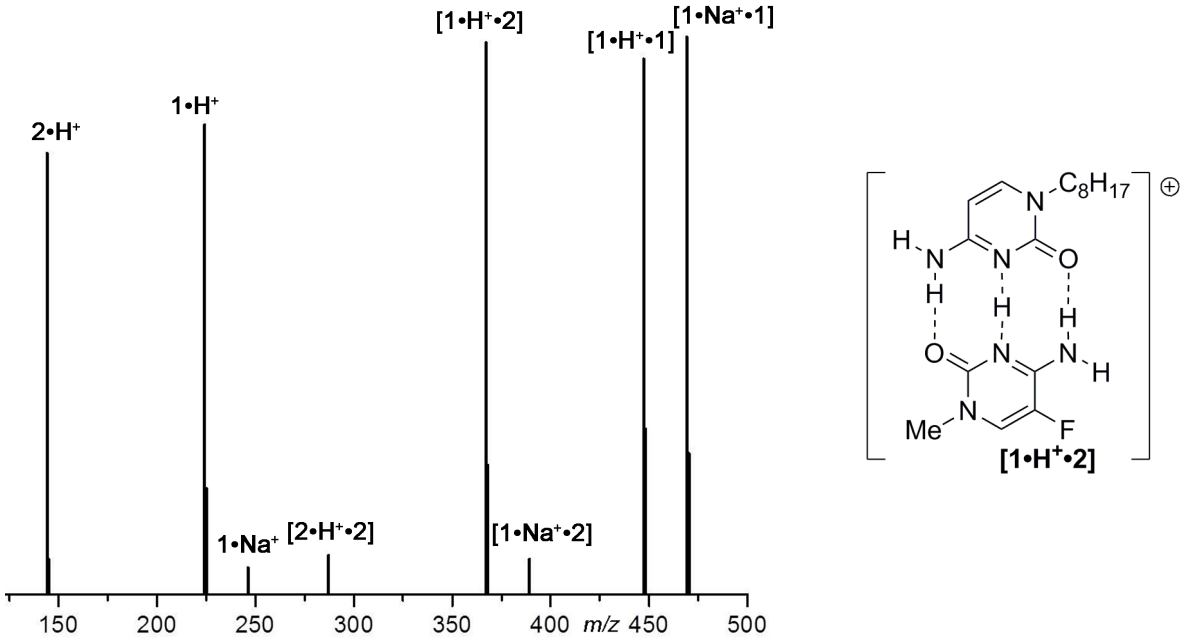


Figure *S*-12. ESI-MS spectrum of **[1•H$^+$•2]**.

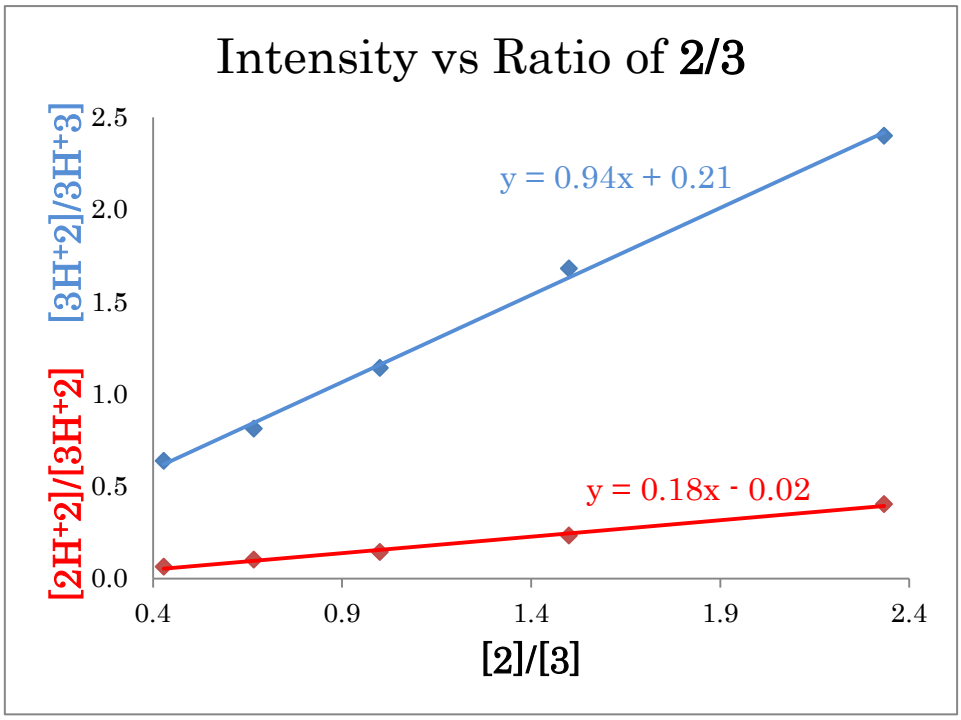
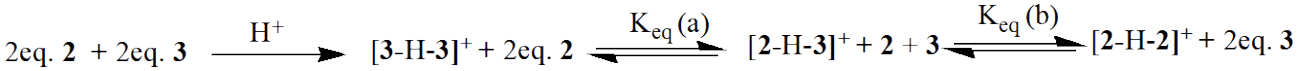


Figure *S*-13. Plot of proton-bound dimer average peak intensity ratios versus the concentration ratio of monomers **3** (1-methylcytosine) and **2** (5-fluoro-1-methylcytosine) in the solution injected into the mass spectrometer.

K_{eq} Description

K_{eq} can be expressed as a ratio of the concentration of the products of a reaction divided by the concentration of its reactants. The equilibrium constant for the transition from a **[3•H$^+$•3]** homodimer to a **[3•H$^+$•2]** heterodimer is shown in Equation 1 below. Equation 1 can be re-arranged to give Equation 2, which is in the form of a linear equation (y = mx + b) where K_{eq} (a) is the slope of the line. (m).



$$K_{eq(a)} = \frac{[2{-}H^{+}{-}3][3][2]}{[3{-}H^{+}{-}3][2]^2} \qquad \textbf{(Eq. 1)}$$

$$\frac{[2{-}H^{+}{-}3]}{[3{-}H^{+}{-}3]} = K_{eq(a)}\frac{[2]}{[3]} \qquad \textbf{(Eq. 2)}$$

The mild nature of electrospray ionization suggests that the concentration of **3** and **2** should not be drastically affected by the addition of H^+ to the system in the course of the ionization process. That is, the ionization of the mixture of the two cytosine analogues will ionize a relatively small percentage of the molecules, leaving the concentration of **3** and **2** essentially unchanged from their prepared ratios. The slope of the trendline (as determined by Microsoft Excel) corresponds to the equilibrium constant for the conversion from one dimer to another when the ratio of the proton-bound dimer peak intensities observed in the mass spectra is plotted against the original concentration ratio of **3** and **2**.

5. Calculations

Binding enthalpies were determined subtracting the sum of the calculated electronic energies of the neutral and protonated monomers from the calculated electronic energy of the proton-bound dimer. Density functional theory calculations on the various proton-bound dimers and their monomers were performed using the Gaussian03 suite of programs. Geometries were optimized using the B3LYP/6-311++G** basis set.[1]

Table *S*-1. Calculated electronic energies (E_{el}) and proton affinities of 5-fluoro-1-methylcytosine (**2**) and 1-methylcytosine (**3**) and their conjugate acids.

	2	**2H$^+$**	**3**	**3H$^+$**
E_{el} (au)	-533.630546	-534.001727	-434.371758	-434.753193
PA (kcal/mol)	-232.92	N/A	-239.35	N/A

Table *S*-2. Calculated electronic energies (E_{el}) and binding enthalpies (BE) of the proton-bound dimers formed between 5-fluoro-1-methylcytosine (**2**) and 1-methylcytosine (**3**).

	3•H$^+$•3	**3•H$^+$•2**	**2•H$^+$•2**
E_{el} (au)	-869.193641	-968.448658	-1067.700109
BE (kcal/mol)	43.1	40.74	42.57

1) Singh, A.; Ganguly, B. *New J. Chem.* **2008**, *32*, 210-213.

2) Procedure adapted from: Lafitte, V. G. H.; Aliev, A. E.; Horton, P. N.; Hursthouse, M.B.; Bala, K.; Golding, P.; Hailes, H. C. *J. Am. Chem. Soc.* **2006**, *128*, 6544-6545.

3) Procedure adapted from: Helfer, D. L.; Hosmane, R. S.; Leonard, N. J. *J. Org. Chem.* **1982**, *46*, 4803-4804.