A vibrational probe for local nucleic acid environments: 5-Cyano-2′-deoxyuridine

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Supporting Information

EXPERIMENTAL

Synthetic Chemistry

General

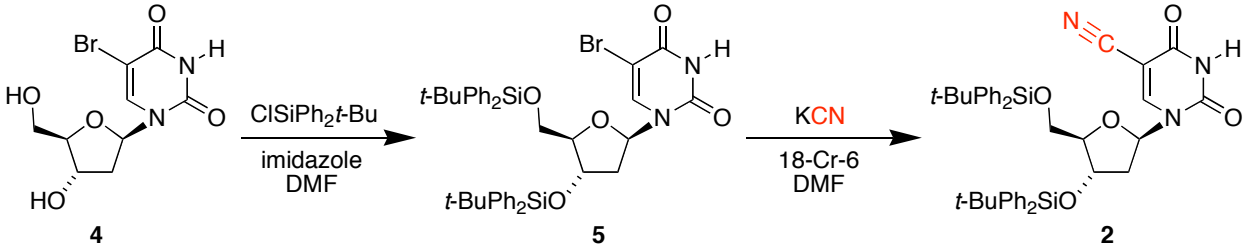
All reagents were ACS reagent quality and used without further purification unless otherwise noted. 5-Bromo-2′-deoxyuridine was purchased from Chem-Impex International, 5-trifluoromethyl-2′-deoxyuridine was purchased from ChemicalLand21 or General Intermediates of Canada. The following compounds were prepared according to literature procedures: 5-Cyano-2′-deoxyuridine (**1**) by the method of Markley et al.[1] and 2,6-diheptanamidopyridine (**3a**) by the method of van Doorn et al. [2] 3′,5′-Bis-*O-(tert*-butyldiphenylsilyl)-5-Cyano-2′-deoxyuridine (**2**) is known,[3] but an alternate synthesis is provided below.

All reactions were stirred with a magnetic stir bar and conducted under a dry nitrogen or argon atmosphere. Analytical thin layer chromatography (TLC) was performed on 0.2 mm silica plastic coated sheets (Selecto Scientific) with F_{254} indicator. Preparative TLC was performed on 1.0 mm silica coated glass plates or 0.3-1.7 mm silica Tapered plates with F_{254} indicator. Flash column chromatography was performed on 230-400 mesh silica gel.

NMR spectra were obtained at the following frequencies: ^{1}H (500 MHz) and ^{13}C (125 MHz). Spectra were obtained in chloroform-*d* ($CDCl_3$) unless otherwise noted. Chemical shifts are reported in parts per million (ppm) and coupling constants are reported in hertz (Hz). ^{1}H spectra in $CDCl_3$ were referenced to tetramethylsilane (TMS = 0.0 ppm) as an internal standard. ^{13}C NMR spectra in $CDCl_3$ were referenced to the solvent peak at 77.0 ppm. ^{1}H NMR spectra in methanol-*d4* were referenced to the residual protiosolvent peak at 3.31 ppm. IR spectra for characterization purposes were obtained as ATR spectra of a thin film and the absorptions are reported in cm^{-1}. Melting points were measured on a Mel–Temp melting point apparatus and are uncorrected.

Abbreviations: ATR (attenuated total reflectance); DMAP (4-dimethylaminopyridine); EtOAc (ethyl acetate); FC (flash column chromatography using silica gel); PE (low boiling petroleum ether); MeOH (methanol); H_2O (deionized water).

Procedures



3',5'-Bis-O-(tert-butyldiphenylsilyl)-5-bromo-2'-deoxyuridine (5). 5-bromo-2'-deoxyuridine (**4**) (501 mg, 1.63 mmol) was dissolved in DMF (2.0 mL) and *tert*-butyldiphenylchlorosilane (1.1 mL, 4.23 mmol) and imidazole (504 mg, 7.43 mmol) were added. The mixture was stirred at ambient temperature for 18 h. The reaction mixture was diluted with water and extracted with diethyl ether. The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by FC (1% MeOH/CH_2Cl_2) to give 969 mg (76%) of **5** as a white solid: mp 70-71 °C; IR 3391.0, 2930.5, 2857.4, 1686.3, 1427.0, 1271.1, 1103.2, 1024.6, 965.7, 821.5, 739.2, 698.4; ^{1}H NMR δ 8.69 (s, 1H), 7.99 (s, 1H), 7.61-7.25 (m, 20H), 6.43 (dd, *J* = 8.8, *J* = 5.1, 1H), 4.49 (d, *J* = 5.1, 1H), 3.98 (s, 1H), 3.70 (m, 1H), 3.27 (dd, *J* = 11.7, *J* = 2.7, 1H), 2.41 (dd, *J* = 12.9, *J* = 5.1, 1H), 1.96 (m, 1H), 1.07 (s, 9H), 0.95 (s, 9H); ^{13}C NMR δ 158.79, 149.34, 139.18, 135.65, 135.58, 135.52, 135.37, 133.05, 132.94, 132.40, 132.08, 130.06, 130.01, 129.99, 129.98, 127.89, 127.87, 127.86, 96.97, 88.28, 85.93, 74.10, 63.91, 41.89, 26.94, 26.83, 19.17, 18.93.

3',5'-Bis-*O*-(*tert*-butyldiphenylsilyl)-5-Cyano-2'-deoxyuridine (2). A mixture of **5** (640 mg, 0.082 mmol), potassium cyanide (CAUTION: toxic) (424 mg, 5.35 mmol), 18-crown-6 (21.8 mg, 0.082 mmol) and DMF (7 mL). The reaction mixture was heated under argon at 70 °C for 24.5 h. The reaction mixture was diluted with water (15 mL) and extracted with diethyl ether (2 × 10 mL). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The crude product was purified by FC (2% MeOH/CH_2Cl_2) to give 247 mg (42%) of **2** as a white powder. The spectral data of the product matched the literature values.[3]

Infrared Spectroscopy and Analysis

Materials. Tetrahydrofuran (Acros, 99.9%), chloroform-D (Cambridge Isotope Labs, 99.8%) and deuterium oxide (Cambridge Isotope Labs, 99.9%) were used without further purification. 18 MΩ·cm water was used to prepare all aqueous solutions. Chloroform (Aldrich, 99.9%) was purified by elution through a column of basic alumina.

Equilibrium FTIR Measurements. Equilibrium FTIR absorbance spectra were recorded on a Bruker Vertex 70 FTIR spectrometer equipped with a globar source, KBr beamsplitter and a liquid nitrogen cooled mercury cadmium telluride (MCT) detector. The spectra were the result of 256 scans recorded at a resolution of either 0.5 or 1.0 cm^{-1}. The transmission measurements were recorded using a temperature-controlled transmission cell consisting of calcium fluoride windows with a path length of ~100 μm. The temperature of the IR cell was controlled by a water bath and the sample temperature was measured by a thermocouple attached to the cell. The FTIR absorbance spectra were baseline corrected. The concentration of all nitrile-containing molecules was 50 mM unless otherwise noted and were recorded at 293 K except for

the variable temperature spectra in water of **1**. The mixed solvent systems of THF and water were prepared by volume.

Global Line Shape Fitting. Global line shape analysis was used to simultaneously model the nitrile IR absorbance band of compound **1** in different water-THF solvent mixtures. The nitrile IR absorbance band was modeled by three line shape functions. Each line shape function was the weighted sum of a Gaussian and Lorenztian function both having the same band position. The band widths of the two functions were allowed to optimize independently. Initially, the nitrile IR absorbance band in pure water and pure THF were fit to a combined Lorenztian and Gaussian line shape function (see Figure S6). These parameters were then used in the line shape analysis of the IR spectra corresponding to the various water-THF mixtures. The Lorenztian contribution to the overall line shape functions ranged from 20 – 60%. The line shape analysis was performed using the solver function in MS Excel.

Determination of the Association Constant. The association constant, K_a for the interaction between compound **2** and **3a** $\left(\mathrm{cmpd}\ \mathbf{2} + \mathrm{cmpd}\ \mathbf{3a} \rightleftharpoons \mathrm{dimer}\right)$ was determined by measuring the nitrile stretching frequency of **2** as a function of the concentration of **3a** at a constant concentration of **2**. The observed nitrile stretching frequency $\left(\tilde{\nu}_{obs}\right)$ dependence on dimer formation was modeled using Equation 1:

$$\tilde{\nu}_{obs} = \frac{\left(\left[cmpd\ \mathbf{2}\right]_t + \left[cmpd\ \mathbf{3a}\right]_t + \frac{1}{K_a}\right) - \left(\left(\left[cmpd\ \mathbf{2}\right]_t + \left[cmpd\ \mathbf{3a}\right]_t + \frac{1}{K_a}\right)^2 - 4\left[cmpd\ \mathbf{2}\right]_t\left[cmpd\ \mathbf{3a}\right]_t\right)^{1/2}}{2\left[cmpd\ \mathbf{2}\right]_t}\left(\tilde{\nu}_{\mathrm{dimer}} - \tilde{\nu}_{cmpd\ \mathbf{2}}\right) + \tilde{\nu}_{cmpd\ \mathbf{2}} \qquad (1)$$

where the subscript t refers to the total concentration of the given compound. The fit was performed using the solver function in MS Excel.

Singular Value Decomposition. Singular value decomposition (SVD) [4-9] was utilized to determine the number of spectral components required to describe the solvent-dependence of the nitrile stretching frequency and line shape in water-THF mixtures. The solvent-dependent difference FTIR spectra were used to construct the data matrix, $A\left(\tilde{\nu},T\right)$ where each column represents the difference FTIR spectra for a specific solvent system. The SVD analysis of this data matrix results in three matrices, $A = USV^T$ where the U, S and V^T matrices contain the basis spectra, the singular values and the evolution of the basis spectra over the different water-THF mixtures, respectively. These matrices were truncated retaining the components containing signal, while removing components containing only noise. The SVD analysis was performed in IGOR Pro (WaveMetrics, Inc.).

DFT Calculations

Density Functional Theory Calculations. Geometry optimizations, single-point energy calculations and vibrational analyses were carried out on model systems using the quantum chemical software package, Gaussian 03 on a multi-processor Mac Pro computer.[10] The calculations were performed at the density function theory (DFT) level using the B3PW91 density functional[11,12] with a 6-31++G(d,p) basis set. [13,14] The calculations were performed in the gas phase with or without one explicit water molecule to simulate hydrogen-bonding between the nitrile group and the solvent (water). The model structures were constructed and the normal modes of vibrations were visualized using the graphical user interface, GaussView 4. 5-cyanouracil was used as a model of compounds **1** and **2** while compound **3b** was used to model compound **3a**.

Figures

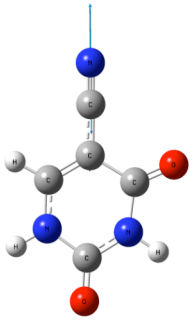


Figure S1. Eigenvector projection for the 2356.1 cm^{-1} vibrational mode of 5-cyanouracil comprised primarily of a C≡N stretch.

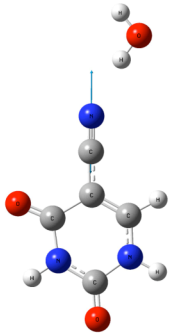


Figure S2. Eigenvector projection for the 2362.3 cm^{-1} vibrational mode of 5-cyanouracil with one explicit water molecule comprised primarily of a C≡N stretch.

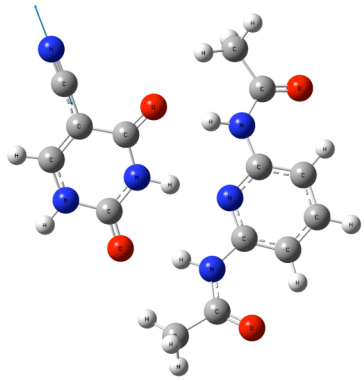


Figure S3. Eigenvector projection for the 2358.7 cm^{-1} vibrational mode of 5-cyanouracil forming a dimer with compound **3b** comprised primarily of a C≡N stretch.

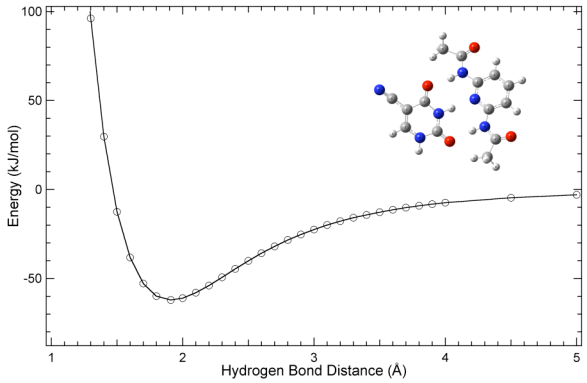


Figure S4. Potential energy surface (PES) corresponding to the energy of interaction between 5-cyanouracil and **3b** formed by modulating the distance between the imino N-H group of 5-cyanouracil and the pyridine N of **3b**. The energy at a hydrogen bond distance of 10 Å was subtracted from all other calculated energies to determine the energy of interaction between the molecules.

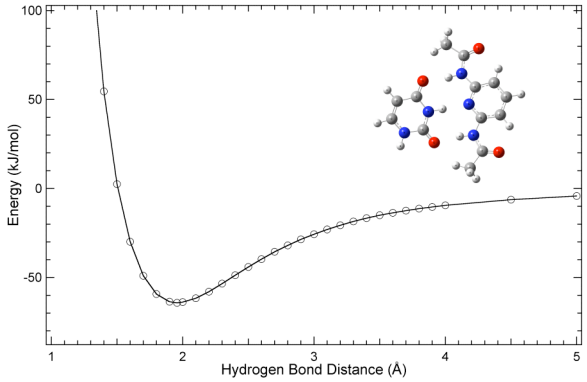


Figure S5. Potential energy surface (PES) corresponding to the energy of interaction between uracil and compound **3b** (see inset for structure) formed by modulating the distance between the imino N-H group of uracil and the pyridine N of compound **3b**. The energy at a hydrogen bond distance of 10 Å was subtracted from all other calculated energies to determine the energy of interaction between the molecules.

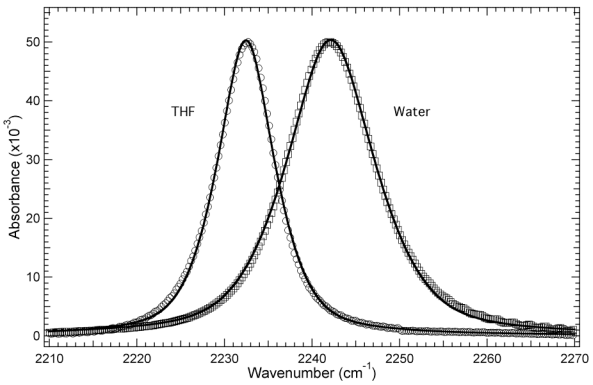


Figure S6. FTIR absorbance spectra of **1** in either pure THF (open circles) or pure water (open squares) fit to a combined Lorenztian and Gaussian line shape function. The spectra were normalized to the same maximum absorbance.

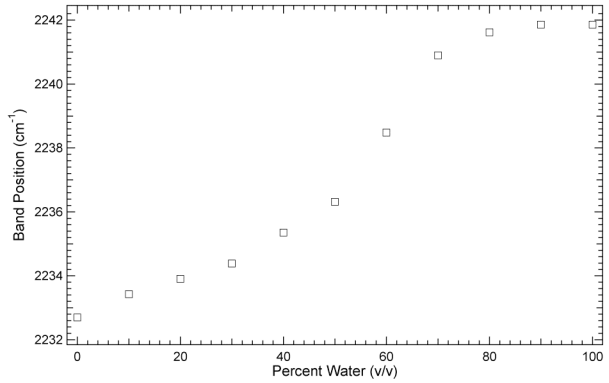


Figure S7. The dependence of the IR absorbance band position of the nitrile stretch of **1** on the amount of water in the water-THF solvent mixtures (open squares).

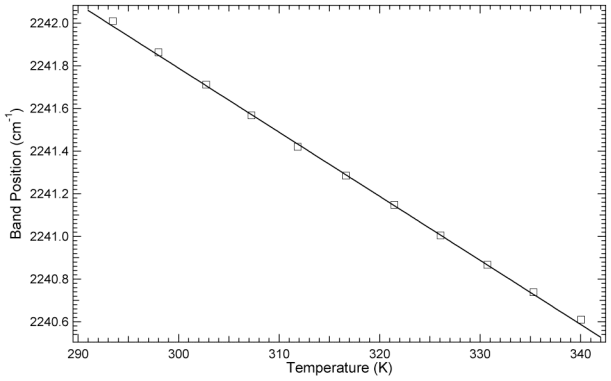


Figure S8. Temperature-dependence of the nitrile IR absorbance band (open squares) of compound of **1** with the corresponding linear fit (solid curve).

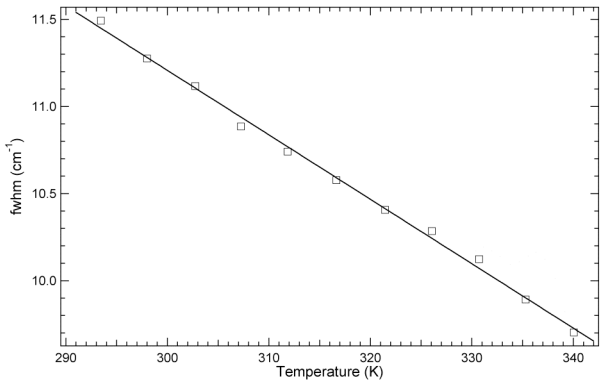


Figure S9. Temperature-dependence of the fwhm (full width at half-maximum) of the nitrile IR absorbance band (open squares) of compound **1** with the corresponding linear fit (solid curve).

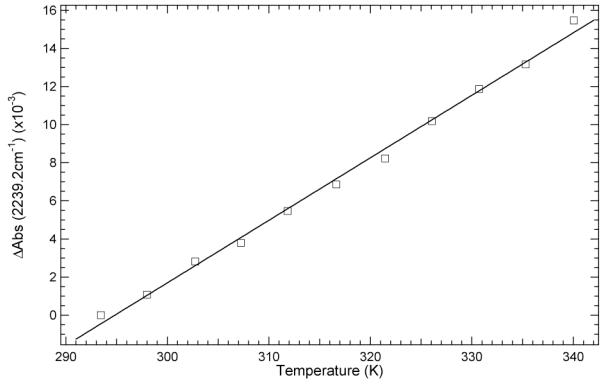


Figure S10. Temperature-dependence of the change in absorbance at 2239.2 cm^{-1} (open squares) of **1** with the corresponding linear fit (solid curve).

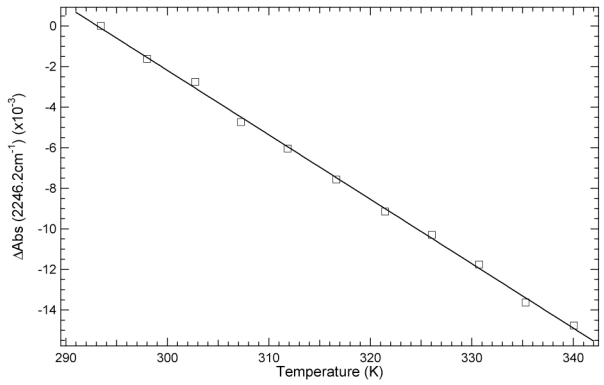


Figure S11. Temperature-dependence of the change in absorbance at 2246.2 cm^{-1} (open squares) of **1** with the corresponding linear fit (solid curve).

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