A Direct Comparison of Azide and Nitrile Vibrational Probes

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PCCP

Supporting Information

1. Synthetic Chemistry

General Experimental

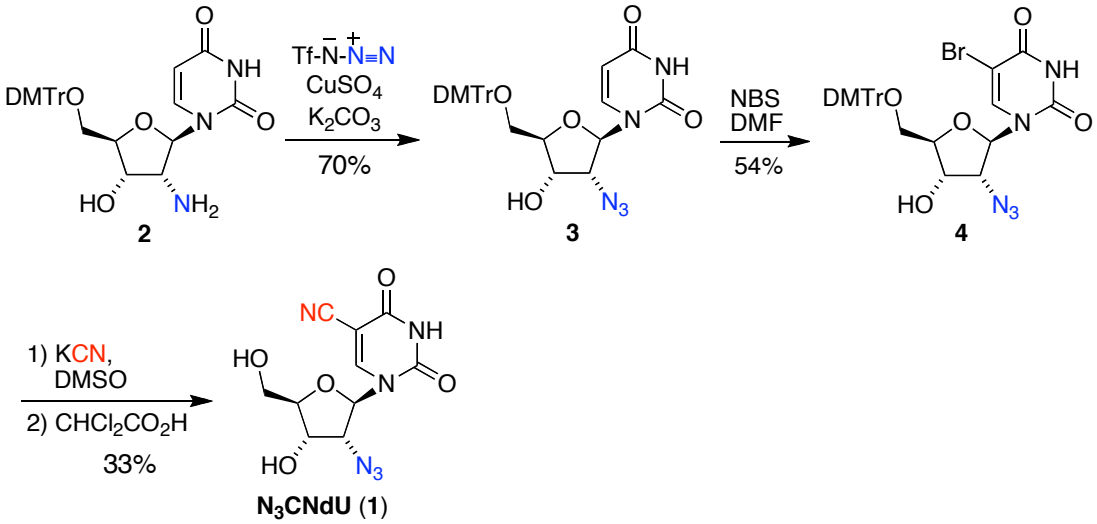
All reagents were ACS reagent quality and used without further purification unless otherwise noted. The following compounds were prepared according to literature procedures: 5'-*O*-(4,4'-dimethoxytrityl)-2'-amino-2'-deoxyuridine (2) by the method of McGee,[1] trifluoromethanesulfonyl azide was prepared by the method of Yan et. al. [2] Synthetic methods for 5'-*O*-(4,4'-dimethoxytrityl)-2'-azido-2'-deoxyuridine (3), are known[3, 4] but an alternate synthesis and additional characterization data are provided.

All reactions were stirred with a magnetic stir bar and conducted under a dry argon atmosphere. Analytical thin layer chromatography (TLC) was performed on 0.2 mm silica plastic coated sheets with F254 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). Chemical shifts are reported in parts per million (ppm) and coupling constants are reported in hertz (Hz). ^{1}H NMR spectra in $CDCl_3$ were referenced to tetramethylsilane (TMS = 0.0 ppm) as an internal standard. ^{1}H NMR spectra in methanol-d_4 were referenced to the residual water peak at 4.87 ppm. ^{13}C NMR spectra taken in $CDCl_3$ were referenced to the solvent peak at 77.0 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); DMF (*N,N*-dimethylformamide); DMSO (Dimethyl sulfoxide); FC (flash column chromatography using silica gel); MeOH (methanol); EtOAc (ethyl acetate); H_2O (deionized water); NBS (N-Bromosuccinimide); NEt_3 (triethylamine).

Scheme 1



Synthetic Procedures

5'-*O*-(4,4'-dimethoxy)trityl-2'-azido-2'-deoxyuridine (3). To a solution of 2 (751.0 mg, 1.37 mmol) in $CH_3CN/CH_2Cl_2/H_2O$ (5 mL/1 mL/1 mL) was added K_2CO_3 (380.2 mg, 2.75 mmol) and $CuSO_4 \bullet 5H_2O$ (17.1 mg, 0.0685 mmol). The mixture was cooled in an ice-water bath and trifluoromethanesulfonyl azide (282 mg, 1.61 mmol) in CH_3CN (1.5 mL) was added dropwise. The mixture was stirred at ice-bath temperature and allowed to warm to ambient temperature and stir for 29 h. A drop of NEt_3 was added and the reaction mixture was coevaporated with silica gel and purified by FC (4% $MeOH/CHCl_3$ with 1% NEt_3 then 4% $MeOH/CHCl_3$) to give 546.5 mg (70%) of 3 as a pale yellow foam: IR ν 3366.4, 3057.6, 2972.3, 2836.4, 2112.8, 1686.0, 1607.6, 1508.4, 1460.8, 1249.9, 1176.3, 1101.0, 1365.6, 1033.0, 828.4, 735.4, 703; ^{1}H NMR ($CDCl_3$) δ 7.89 (d, *J* = 8.2, 1H), 7.37-7.15 (m, 9H), 6.84 (d, *J* = 9.0, 4H), 5.97 (d, *J* = 3.2, 1H), 5.37 (d, *J* = 8.2, 1H), 4.48 (t, *J* = 6.1, 1H), 4.16 (dd, *J* = 5.5, *J* = 2.7, 1H, 4.03 (m, 1H), 3.79 (s, 6H), 3.59 (dd, *J* = 11.2, *J* = 2.7, 1H), 3.48 (dd, *J* = 11.2, *J* = 2.7, 1H).

5'-*O*-(4,4'-dimethoxy)trityl-2'-azido-5-bromo-2'-deoxyuridine (4). To a solution of 3 (1.24 g, 2.17 mmol) in DMF (20 mL) was added NBS (438.5 mg, 2.50 mmol). The mixture was stirred at 45 °C for 24 h then at ambient temperature for 43 h. The reaction mixture was partitioned between H_2O and EtOAc. The aqueous layer was back-extracted with EtOAc (3×). The combined organic layers were washed with H_2O (2×), dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by FC (4% $MeOH/CHCl_3$ with 1% NEt_3) to give 762.2 mg (54%) of 4 as a pale yellow foam: IR ν 3052.6, 2929.5, 2835.7, 2114.5, 1673.3, 1607.8, 1508.7, 1445.7, 1298.8, 1250.0, 1176.2, 1099.1, 1031.9, 827.9, 584.0; ^{1}H NMR ($CDCl_3$) δ 8.07 (s, 1H), 7.43-7.14 (m, 9H), 6.85 (d, *J* = 8.3, 4H), 6.01 (d, *J* = 4.4, 1H), 4.49 (t, *J* = 5.5, 1H), 4.21 (dd, *J* = 5.5, *J* = 4.5, 1H), 4.09 (m, 1H), 3.79 (s, 6H), 3.50 (dd, *J* = 11.2, *J* = 2.6, 1H), 3.45 (dd, *J* = 11.2, *J* = 2.6, 1H); ^{13}C NMR ($CDCl_3$) δ 158.74, 158.72, 149.55, 144.10, 138.37, 135.25, 135.07, 130.00, 129.98, 129.02, 128.21, 128.17, 127.93, 127.17, 125.28, 113.43, 97.77, 87.70, 87.30, 83.87, 70.60, 66.95, 91.96, 55.27; MS (ESI) 672.2 ($M+Na^+$, 38), 401.3 (96), 239.3 (100).

2'-azido-5-cyano-2'-deoxyuridine (N_3CNdU, 1). To a solution of 4 (752.2 mg, 1.16 mmol) in DMSO (12.5 mL) was added potassium cyanide (155.8 mg, 1.97 mmol). The mixture was stirred at 110 °C for 1 h. The reaction mixture was diluted with EtOAc and washed with H_2O. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was dissolved in 2.5% $Cl_2CHCOOH$ in CH_2Cl_2 (20 mL) and stirred at ambient temperature for 5 min. Silica gel was added and the mixture was concentrated under reduced pressure. Purification by FC (4% $MeOH/CHCl_3$) gave 111.2 mg (33%, two steps) of 1 as a pale yellow foam: mp = 163-165 °C (dec.); IR ν 3376.0, 2242.4, 2115.6, 1697.1, 1630.7, 1458.6, 1261.0, 1097.4; ^{1}H NMR (CD_3OD) δ 9.01 (s, 1H), 5.84 (d, *J* = 2.7, 1H), 4.47 (t, *J* = 5.9, 1H), 4.20 (dd, *J* = 5.9, *J* = 2.4, 1H), 4.04 (m, 1H), 3.98 (dd, *J* = 12.4, *J* = 2.4, 1H), 3.79 (dd, *J* = 12.1, *J* = 2.4, 1H); MS (ESI) 317.2 ($M+Na^+$).

2. Experimental IR Data

Equilibrium FTIR Measurements. Equilibrium FTIR absorbance spectra were recorded on a Bruker Tensor 37 FTIR spectrometer equipped with a globar source, KBr beamsplitter and a room temperature deuterated L-alanine-doped triglycine sulfate (RT-DLaTGS) detector. The spectra were the result of 256 scans recorded at a resolution of 1.0 cm^{-1}. The transmission measurements were recorded using a temperature-controlled 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 embedded in the cell. The FTIR absorbance spectra were baseline corrected. The concentration of N_3CNdU was 50 mM for each spectra unless otherwise noted and were recorded at 293 K except for the variable temperature spectra in water. The mixed solvent systems of DMSO and water were prepared by volume.

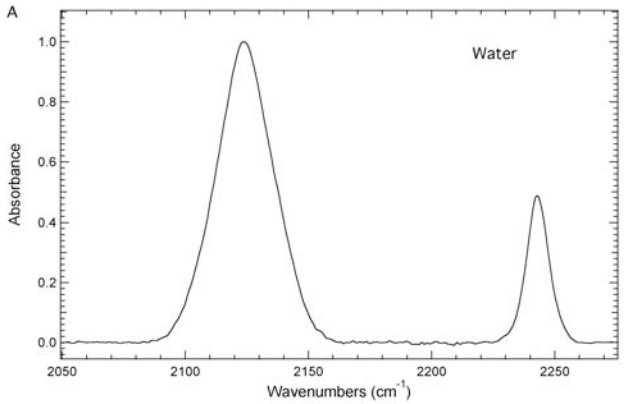
Line Shape Fitting. Line shape analysis was used to model the azide and/or nitrile IR absorbance band of N_3CNdU in THF and methanol. Each absorbance band was modeled by one or more line shape functions. Each line shape function consisted of a linear combination of a Gaussian and Lorentzian function as shown in Equation 1: [5]

$$F(\tilde{\nu}) = (a_o + b_o\tilde{\nu}) + A\left[(1 - m_{Lorentz})\frac{(4\ln 2)^{1/2}}{\pi^{1/2} fwhm} e^{-\frac{(4\ln 2)(\tilde{\nu}-\tilde{\nu}_o)^2}{fwhm^2}} + m_{Lorentz}\frac{2}{\pi}\frac{fwhm}{4(\tilde{\nu}-\tilde{\nu}_o)^2 + fwhm^2}\right] \quad (1)$$

where $m_{Lorentz}$ is the fraction of the Lorentzian line shape, $\tilde{\nu}_o$ is the band position, $fwhm$ is the full-width at half maximum for the line shape, A is the area, and $(a_o + b_o\tilde{\nu})$ is a linear baseline offset. The line shape analysis was performed in Igor Pro (Wavemetrics).

Table 1. Frequencies corresponding to the peak absorbance of the nitrile and azide IR absorbance bands of N_3CNdU in a variety of solvents. The last column is the ratio of the peak extinctions of the azide and nitrile IR absorbance bands.

Solvent	Nitrile Frequency (cm^{-1})	Azide Frequency (cm^{-1})	A_{N_3}/A_{CN}
Water	2242.7	2124.1	2.1
Methanol	2237.4	2113.5	3.3
2-Propanol	2238.4	2113.5	3.4
1-Butanol	2237.4	2113.0	3.5
tert-Butanol	2239.3	2113.5	3.2
Trifluoroethanol (TFE)	2247.5	2123.1	4.2
Tetrahydrofuran (THF)	2233.5	2111.5	2.9
Dimethyl Sulfoxide (DMSO)	2230.2	2108.7	2.3
Acetone	2233.5	2114.0	2.6
Nitromethane	2236.4	2119.7	2.6
Formamide	2236.0	2116.9	3.0
N-Methylformamide (NMF)	2233.5	2113.0	2.7
Dimethylformamide (DMF)	2231.1	2108.2	2.6
Propylene Carbonate (PC)	2233.5	2115.9	2.9


A
Water
Absorbance
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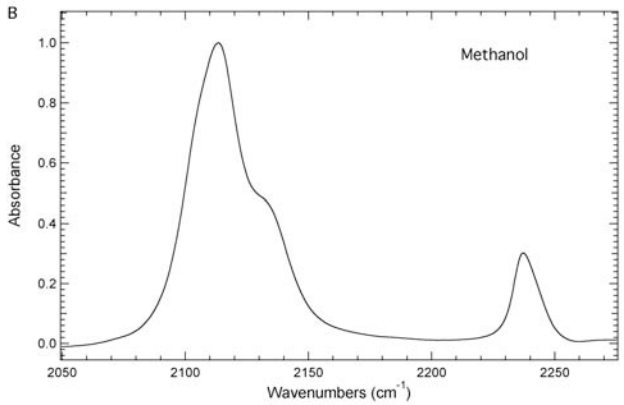
0.4
0.2
0.0
2050
2100
2150
2200
2250
Wavenumbers (cm-1)


B
Methanol
Absorbance
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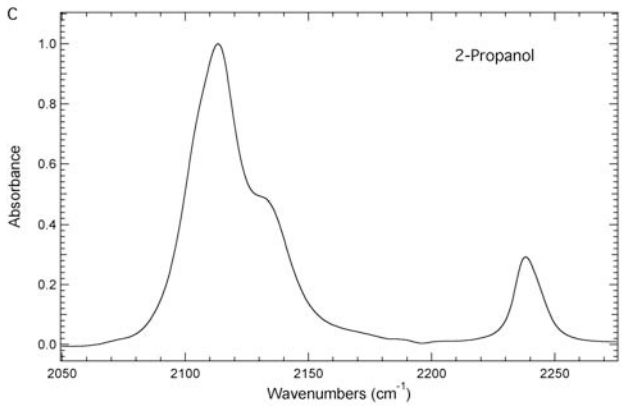
2100
2150
2200
2250
Wavenumbers (cm-1)


C
2-Propanol
Absorbance
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0.4
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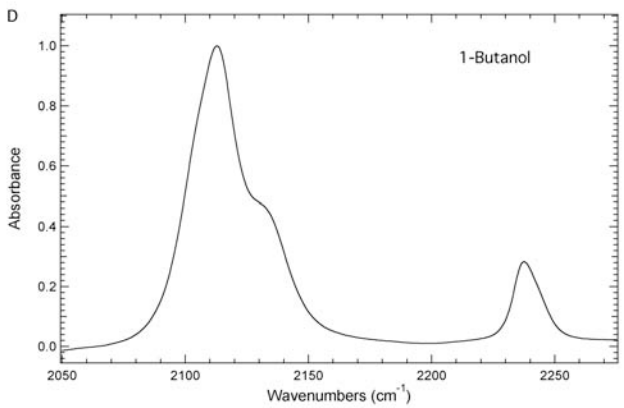
0.0
2050
2100
2150
2200
2250
Wavenumbers (cm-1)


D
1-Butanol
Absorbance
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2150
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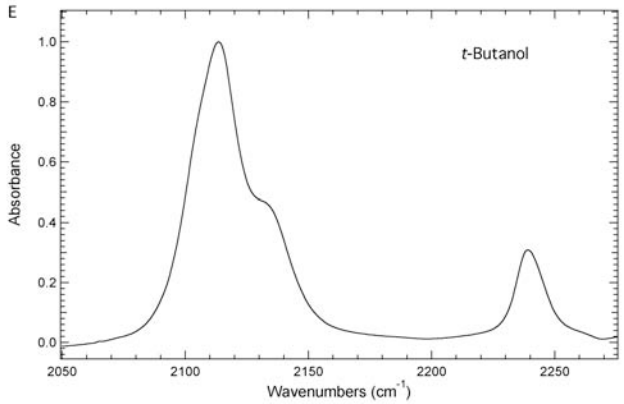
2250
Wavenumbers (cm-1)


E
t-Butanol
Absorbance
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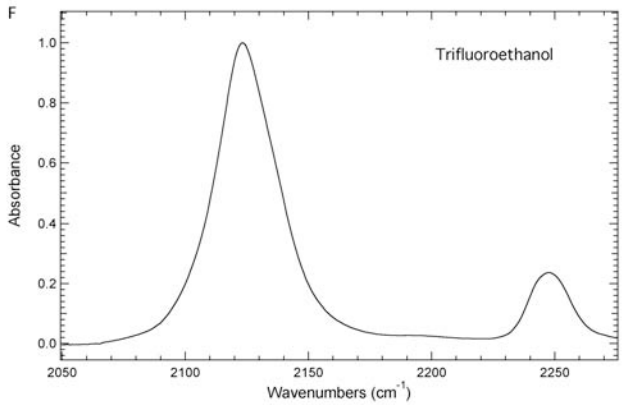
2050
2100
2150
2200
2250
Wavenumbers (cm-1)


F
Trifluoroethanol
Absorbance
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0.4
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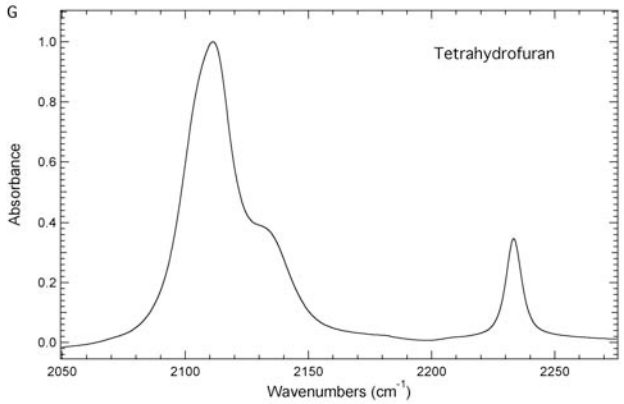
0.0
2050
2100
2150
2200
2250
Wavenumbers (cm-1)


G
Tetrahydrofuran
Absorbance
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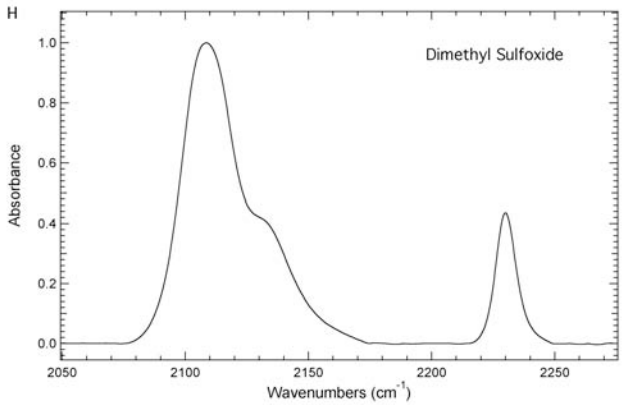
0.6
0.4
0.2
0.0
2050
2100
2150
2200
2250
Wavenumbers (cm-1)


H
Dimethyl Sulfoxide
Absorbance
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0.4
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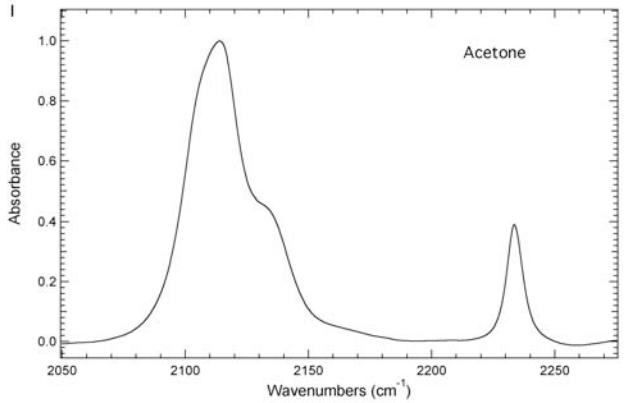
2050
2100
2150
2200
2250
Wavenumbers (cm-1)

I


Acetone
Absorbance
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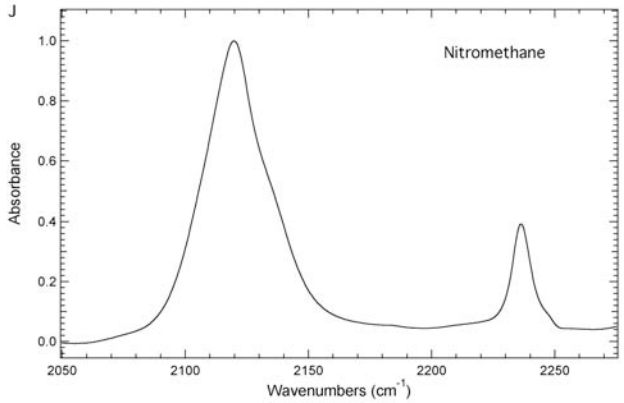
0.6
0.4
0.2
0.0
2050
2100
2150
2200
2250
Wavenumbers (cm-1)

J


Nitromethane
Absorbance
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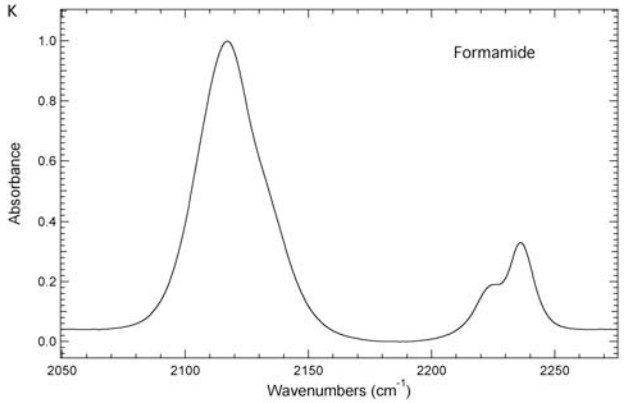
0.4
0.2
0.0
2050
2100
2150
2200
2250
Wavenumbers (cm-1)

K


Formamide
Absorbance
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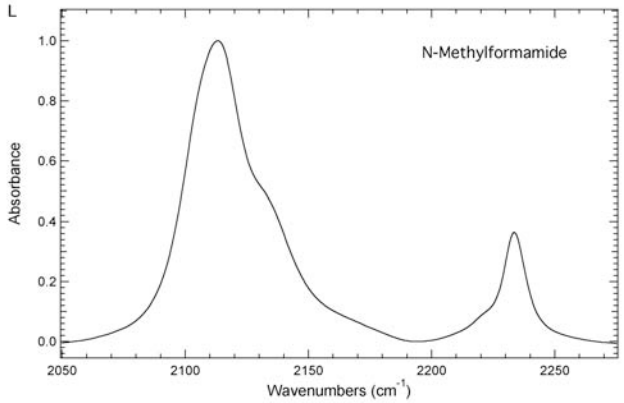
0.8
0.6
0.4
0.2
0.0
2050
2100
2150
2200
2250
Wavenumbers (cm-1)

L


N-Methylformamide
Absorbance
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0.6
0.4
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2050
2100
2150
2200
2250
Wavenumbers (cm-1)

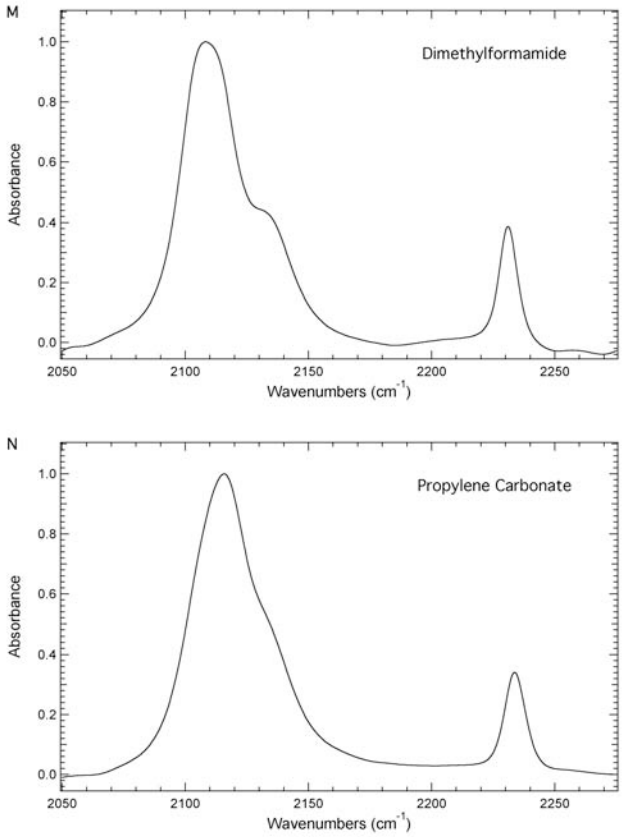

Figure S1. FTIR absorbance spectra of N_3CNdU dissolved in water (A), methanol (B), propanol (C), 1-butanol (D), *t*-butanol (E), trifluoroethanol (F), tetrahydrofuran (G), dimethyl sulfoxide (H), acetone (I), nitromethane (J), formamide (K), N-methylformamide (L), dimethylformamide (M), propylene carbonate (N) recorded at 293 K with a concentration of 50 mM. The maximum absorbance of each spectrum has been normalized to unity.

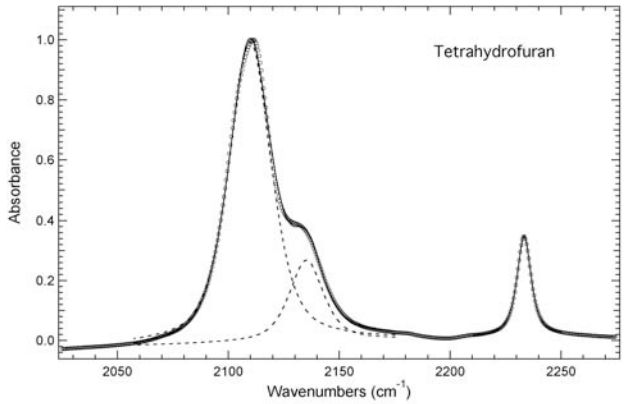

Figure S2. FTIR absorbance spectrum of N_3CNdU in tetrahydrofuran (open circles) recorded at 293 K where the maximum absorbance has been normalized to unity. The azide IR absorbance band was fit (solid curve) to two line shapes (dashed curves) described by Eqn. 1 and the nitrile IR absorbance band was fit (solid curve) to one line shape described by Eqn. 1. The fits represent the minimum number of line shapes required to fit the experimental spectrum.

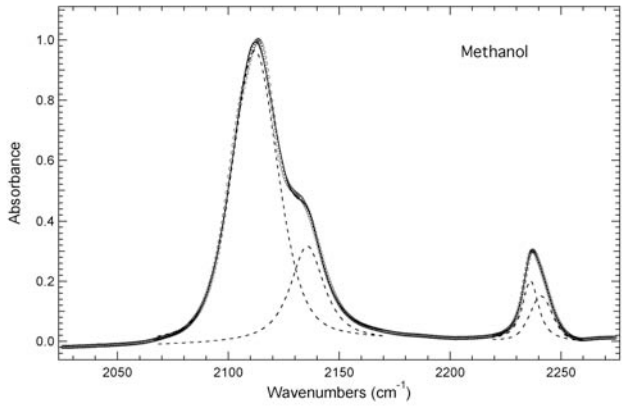

Figure S3. FTIR absorbance spectrum of N_3CNdU in methanol (open circles) recorded at 293 K where the maximum absorbance has been normalized to unity. The azide IR absorbance band was fit (solid curve) to two line shapes (dashed curves) described by Eqn. 1 and the nitrile IR absorbance band was also fit (solid curve) to two line shape described by Eqn. 1. The fits represent the minimum number of line shapes required to fit the experimental spectrum.

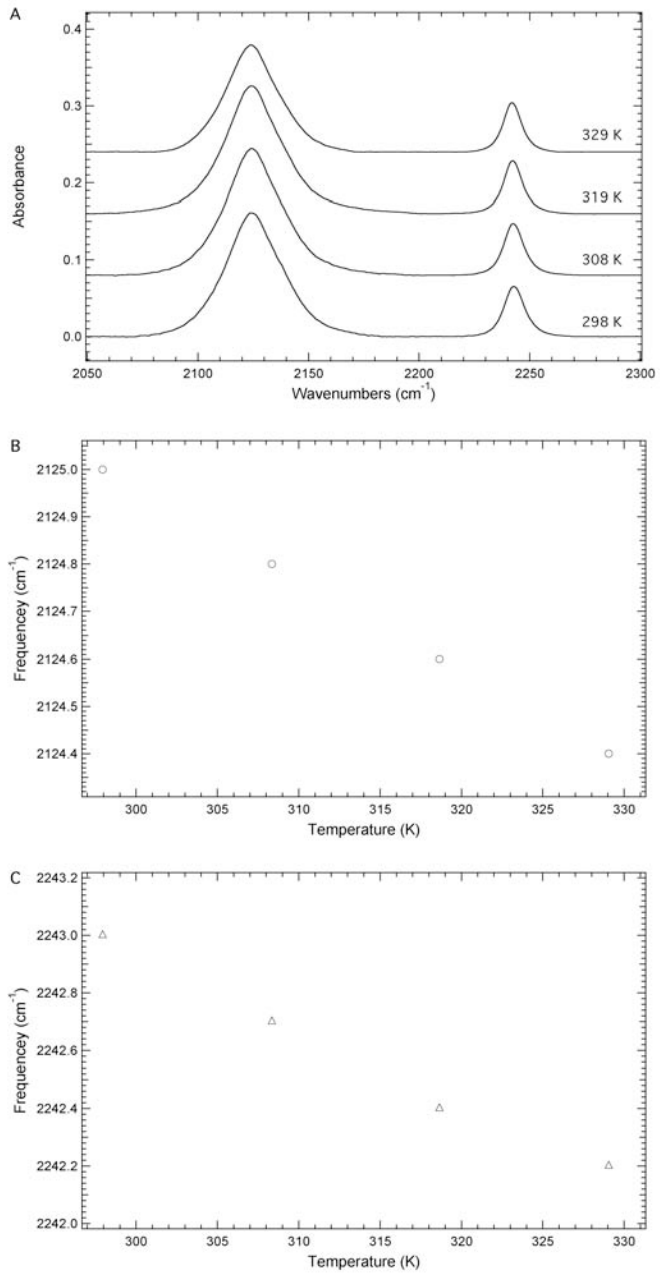

Figure S4. A. Temperature dependent FTIR absorbance spectra of N_3CNdU dissolved in water recorded from 293 to 329 K in ~10 K increments. B. Azide asymmetric stretching frequency dependence on temperature. C. Nitrile symmetric stretching frequency dependence on temperature.

3. DFT Calculations Figures

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. [6] The calculations were performed at the density functional theory (DFT) level using the B3PW91 density functional[7, 8] with a 6-31++G(d,p) basis set. [9, 10] The calculations were performed in the gas phase with or without one explicit water molecules to simulate different H-bond interactions between the nitrile group of 5-cyanouracil or the azide group of 2-azido-1,2-dideoxyribose with water. The model structures were constructed using the graphical user interface, GaussView 4.

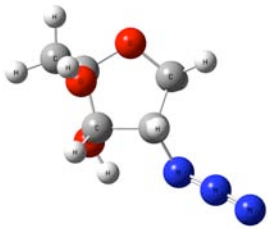

Figure S5. Geometry-optimized structure of 2-azido-1,2-dideoxyribose.

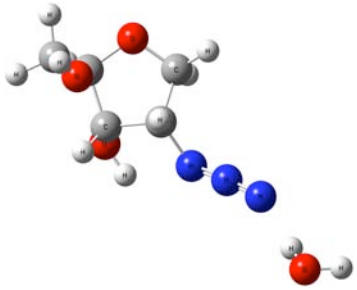

Figure S6. Geometry-optimized structure of 2-azido-1,2-dideoxyribose with one water molecule containing a σ-H-bond between the azide group and the water molecule.

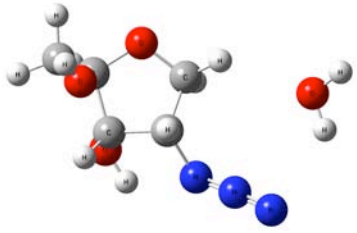

Figure S7. Geometry-optimized structure of 2-azido-1,2-dideoxyribose with one water molecule containing a π-H-bond between the azide group and the water molecule.

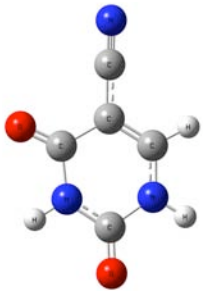

Figure S8. Geometry-optimized structure of 5-cyanouracil.

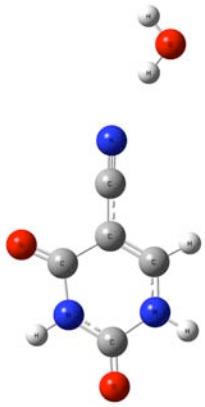

Figure S9. Geometry-optimized structure of 5-cyanouracil with one water molecule containing a σ-H-bond between the nitrile group and the water molecule.

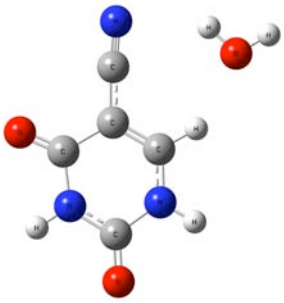

Figure S10. Geometry-optimized structure of 5-cyanouracil with one water molecule containing a π-H-bond between the nitrile group and the water molecule.

4. References

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