^{15}N NMR Studies of a Nitrile-Modified Nucleoside

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Nitrile-modified molecules have proven to be excellent probes of local environments in biomolecules via both vibrational and fluorescence spectroscopy. The utility of the nitrile group as a spectroscopic probe has been expanded here to ^{15}N NMR spectroscopy by selective ^{15}N incorporation. The ^{15}N NMR chemical shift ($\delta_{^{15}N}$) of the ^{15}N-labeled 5-cyano-2′-deoxyuridine (C^{15}NdU, **1a**) was found to change from 153.47 to 143.80 ppm in going from THF-d_8 to D_2O. A 0.81 ppm downfield shift was measured upon formation of a hydrogen-bond-mediated heterodimer between 2,6-diheptanamidopyridine and a silyl ether analogue of **1a** in chloroform, and the small intrinsic temperature dependence of $\delta_{^{15}N}$ of C^{15}NdU was measured as a 0.38 ppm downfield shift from 298 to 338 K. The experiments were complemented with density functional theory calculations exploring the effect of solvation on the ^{15}N NMR chemical shift.

Introduction

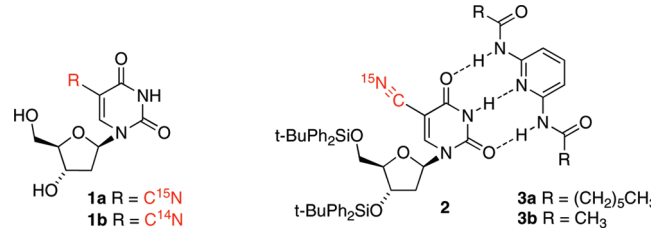
Nitriles (R-CN) have been utilized as a sensitive, stable, and minimally perturbative vibrational probe of proteins and nucleic acids.[1−6] The nitrile moiety is an excellent IR probe because of the sensitivity of the nitrile stretching frequency to local environment, the relatively strong extinction coefficient of this vibrational mode, and the position of the stretching frequency, which is in a relatively clear region of the infrared spectrum. Additionally, the small size and intermediate polarity of the nitrile group permits incorporation of this probe with minimal structural perturbation of the biomolecule of interest.[3,7−10]

The nitrile group has been introduced into the side chains of a number of amino acids, including phenylalanine and alanine.[3,11−13] The nitrile-modified phenylalanine residue (*p*-cyanophenylalanine, pCNPhe) exhibits a significantly larger nitrile extinction coefficient than the alanine derivative and the nitrile symmetric stretch of pCNPhe is sensitive to local environment.[3] Standard FMOC peptide synthetic methods have been used to incorporate pCNPhe into short peptides, while an engineered aminoacyl-tRNA synthetase/tRNA pair has been used to incorporate pCNPhe site-specifically into proteins.[9,10,14,15]

The utility of pCNPhe as a probe of protein structure was extended with fluorescence spectroscopy in addition to IR spectroscopy. This unnatural amino acid (UAA) forms a Förster resonance energy transfer (FRET) pair with the natural amino acid tryptophan (Trp) with a Förster distance of 16 Å.[14] pCNPhe and Trp form an effective FRET pair since pCNPhe can be selectively excited at ∼240 nm in the presence of natural aromatic amino acids, the emission spectrum of pCNPhe overlaps with the electronic absorbance spectrum of Trp, and the emission of pCNPhe can be selectively monitored. This FRET pair has been successfully utilized to probe protein conformational changes associated with protein unfolding in a number of peptide and protein systems.[9,10,14,16]

The nitrile spectroscopic probe has also been utilized to study nucleic acid structure.[4−6] Previously, we showed that the nitrile stretching frequency of 5-cyano-2′-deoxyuridine (C^{14}NdU, **1b**; Chart 1) was sensitive to solvent, blue-shifting 9.2 cm^{-1} in going from THF to water due primarily to hydrogen bonding between the nitrile group and water. The nitrile vibrational frequency was additionally found to be sensitive to the formation of a hydrogen-bond-mediated base-pair mimic heterodimer, while having a small intrinsic temperature dependence.[6] The nitrile symmetric stretch of **1** will serve as an IR probe of the major groove when incorporated into duplex DNA.

CHART 1



The ^{15}N labeling of the nitrogen atom of the nitrile group can extend the utility of this vibrational probe. For instance, Krummel and Zanni demonstrated that the distance-dependent coupling between C^{14}N and C^{15}N oscillators could be utilized to measure the distance between these groups in a DNA oligomer labeled with C^{14}NdU and C^{15}NdU.[5] The ^{15}N labeling also permits ^{15}N NMR spectroscopy to be utilized to probe the local environment of the ^{15}N nuclei of the nitrile group,[17] which is the subject of this work.

^{15}N NMR spectroscopy has been successfully utilized to probe DNA, RNA, and protein biomolecules.[18−20] ^{15}N NMR spectroscopy of biomolecules typically requires ^{15}N enrichment due to the low natural abundance (0.37%) and the relatively low sensitivity of the ^{15}N nucleus, which is 1.04×10^{-3} times that of a proton.[18] ^{15}N NMR spectroscopy is commonly used to probe amide (^{15}N−H) groups of protein backbones, where the peptide backbone of the protein has been uniformly ^{15}N labeled, through standard 2D measurements.[20] In nucleic acids, single nitrogen atoms in the nucleoside bases have been replaced with ^{15}N atoms in order to use 1D direct detect ^{15}N NMR measurements to probe local structure.[21,22] For instance, Poulter and Livingston used benzoyluridine with a ^{15}N label in the 3-position to detect hydrogen bonding in A-U base pairs,[23] while Jones and co-

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workers have published an extensive series of studies using ^{15}N as a local structural monitor in nucleic acids.[24−28] Selective ^{15}N enrichment of nucleic acids has also been used to investigate direct N−metal bonds in nucleic acids[25−27] and to compare the binding potential of different metal binding sites.[29]

Here, we have introduced a ^{15}N isotopic label in the nitrile group of 5-cyano-2′-deoxyuridine (**1a**) in order to use ^{15}N NMR to probe the local environment around this nucleus. The potential of this experimental methodology for the study of nucleic acid structure was explored by measuring the dependence of the ^{15}N NMR chemical shift of C^{15}NdU (**1a**) on solvent, heterodimer formation, and temperature. The experiments were complemented with density functional theory calculations exploring the effect of solvation on the ^{15}N NMR chemical shift.

Materials and Methods

General Materials. All reagents were ACS reagent quality and used without further purification unless otherwise noted. 5-Trifluoromethyl-2′-deoxyuridine was purchased from ChemicalLand21 or General Intermediates of Canada. ^{15}N-labeled (99+%) ammonium chloride was purchased from Icon Isotopes. 2,6-Diheptanamidopyridine (**3a**) was synthesized according to the literature procedure.[30]

NMR Spectroscopy. NMR spectra were obtained with a Varian INOVA 500 multinuclear Fourier transform NMR spectrometer at the following frequencies: ^1H (499.7 MHz), and ^{15}N (50.5 MHz). Spectra were obtained in chloroform-*d* ($CDCl_3$), methanol-d_4, THF-d_8, and D_2O at 293 K unless otherwise noted. Chemical shifts are reported in parts per million (ppm) and coupling constants are reported in hertz (Hz). ^1H spectra in $CDCl_3$ were referenced to tetramethylsilane (TMS = 0.0 ppm) as an internal standard. ^1H NMR spectra in methanol-d_4 were referenced to the residual protiosolvent peak at 3.31 ppm. ^{15}N spectra were referenced to a 100 mM solution of ^{15}N-labeled formamide in DMSO ($HCO^{15}NH_2$ = 0.0 ppm). The formamide scale can be converted to the ammonia scale by adding 112 ppm.[31]

Synthetic Methods. 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 (Selecto Scientific) with F_{254} indicator. Flash column chromatography was performed on 230−400 mesh silica gel.

^{15}N-Labeled 5-Cyano-2′-deoxyuridine (1a). [32] A mixture of $^{15}NH_4Cl$ (276.2 mg, 5.07 mmol), potassium hydroxide (206.4 mg, 3.68 mmol), triethylamine (0.48 mL), H_2O (2 mL), and acetonitrile (2.4 mL) was stirred for 10 min at 40 °C. Then 5-trifluoromethyl-2′-deoxyuridine (198.6 mg, 0.671 mmol) was added and the stirring was continued for 30 h at 45 °C. Volatiles were removed under reduced pressure, yielding a glassy white solid. The residue was preloaded onto silica gel using methanol and purified by FC (1:10:10 MeOH/CH_2Cl_2/EtOAc) to give 153 mg (90%) of **1a** as a white flaky solid: IR (ATR, cm^{-1}) 3436.0, 3069.7, 2930.5, 2210.6, 1726.9, 1673.8, 1614.8, 1469.7, 1273.2, 1093.8, 754.9; ^1H NMR (CD_3OD) δ 8.90 (s, 1H), 6.17 (t, J = 6.1, 1H), 4.40 (m, 1H), 3.97 (m, 1H), 3.86 (dd, J = 12.1, J = 3.0, 1H), 3.75 (dd, J = 12.1, J = 3.0, 1H), 2.39 (m, 1H), 2.27 (m, 1H).

3′,5′-Bis-O-(tert-butyldiphenylsilyl)-5-cyano(C^{15}N)-2′-deoxyuridine (2). [4] **1a** (75.6 mg, 0.30 mmol) was dissolved in DMF (1.2 mL) and *tert*-butyldiphenylchlorosilane (0.31 mL, 1.19 mmol) and imidazole (102.8 mg, 1.51 mmol) were added. The mixture was stirred at ambient temperature for 18 h. Volatiles were removed under reduced pressure, and the glassy white solid was triturated with PE and dried under high vacuum. The residue was preloaded onto silica gel and purified by FC (2:5 EtOAc/hexane) to give 210.2 mg (96%) of **2** as a white solid. The spectral data of the product matched the literature values.

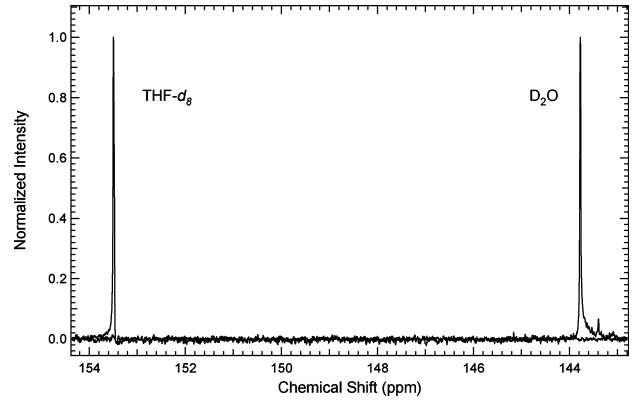

Figure 1. Intensity-normalized ^{15}N NMR spectra of C^{15}NdU in THF-d_8 or D_2O recorded at 293 K and referenced to formamide (0 ppm).

Determination of the Association Constant. The association constant K_a for the interaction between compounds **2** and **3a** (**2** + **3a** ⇌ dimer) was determined by measuring the ^{15}N NMR chemical shift of **2** as a function of the concentration of **3a** at a constant concentration of **2** in the noncompetitive solvent $CDCl_3$. The observed chemical shift dependence on heterodimer formation was modeled using eq 1[33,34]

$$\delta_{obs} = \frac{\left([\mathbf{2}]_t + [\mathbf{3a}]_t + \frac{1}{K_a}\right) - \left(\left([\mathbf{2}]_t + [\mathbf{3a}]_t + \frac{1}{K_a}\right)^2 - 4[\mathbf{2}]_t[\mathbf{3a}]_t\right)^{1/2}}{2[\mathbf{2}]_t} \times (\delta_{dimer} - \delta_{\mathbf{2}}) + \delta_{\mathbf{2}} \quad (1)$$

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

Density Functional Theory Calculations. Geometry optimizations, single-point energy calculations, NMR, and vibrational analyses were carried out on model systems using the quantum chemical software package, Gaussian 03, on a multiprocessor Mac Pro computer.[35] The calculations were performed at the density functional theory (DFT) level using the B3PW91 density functional[36,37] with a 6-31++G(d,p) basis set.[38,39] The ^{15}N isotropic chemical shifts were calculated using the gauge-independent atomic orbital (GIAO) method.[40] The calculations were performed in the gas phase with or without one or five explicit water molecules to simulate different interactions between the nitrile group and the solvent (water). The model structures were constructed using the graphical user interface, GaussView 4. ^{15}N-labeled 5-cyanouracil was used as a model of compounds **1**a and **2**, while compound **3b** was used to model compound **3a**.

Results and Discussion

The sensitivity of the ^{15}N NMR chemical shift of ^{15}N-labeled 5-cyano-2′-deoxyuridine (**1a**, C^{15}NdU) to local environments was explored in deuterated THF (THF-d_8), deuterium oxide (D_2O), and mixtures of these two solvents. These solvents and their mixtures present a wide range of local environments to the nitrile group of C^{15}NdU including a varying solvent dielectric constant and potential for H-bonding with D_2O. Figure 1 shows

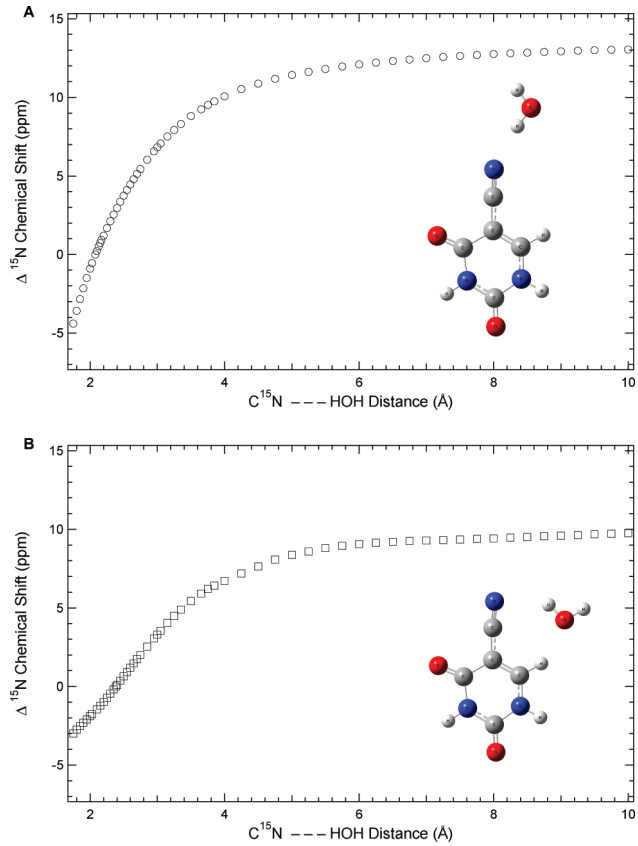

Figure 2. Calculated ^{15}N isotropic chemical shift dependence of ^{15}N-labeled 5-cyanouracil on the distance between the C^{15}N group of 5-cyanouracil and a water molecule interacting in two different hydrogen-bonding configurations. The calculated ^{15}N isotropic chemical shift of the geometry-optimized structure was subtracted from the subsequent distance-dependent calculations for each configuration. Inset: geometry-optimized structures of 5-cyanouracil hydrogen bonded to a water molecule.

the ^{15}N NMR spectra of **1a** in THF-d_8 and D_2O. The ^{15}N NMR chemical shift ($\delta_{^{15}N}$) of C^{15}NdU was found to be 153.47 ppm in THF-d_8 and 143.80 ppm in D_2O. The 9.67 ppm upfield shift of $\delta_{^{15}N}$ in going from the aprotic solvent, THF-d_8, to the protic solvent, D_2O, is the result of differences in the dielectric environments presented by each solvent and specific solute−solvent interactions such as H-bonding between D_2O and the nitrile group of C^{15}NdU.

The molecular basis for the experimentally observed solvent-induced ^{15}N NMR chemical shift of C^{15}NdU was explored through DFT calculations at the B3PW91/6-31++G(d,p) level. Specifically, the nature and impact of solute−solvent interactions between C^{15}NdU and D_2O were modeled with ^{15}N-labeled 5-cyanouracil with one or five explicit water molecules. The insets in Figure 2 show two optimized configurations of hydrogen bonding between a water molecule and the nitrile group of 5-cyanouracil. The geometry of the H-bond in the Figure 2A inset is characterized by a C≡^{15}N···H angle of 153° between the nitrile group and the water molecule. This σ-H-bond between the lone pair of the ^{15}N atom and the water molecule induces an upfield shift of the ^{15}N NMR isotropic chemical shift of 14.1 ppm relative to 5-cyanouracil in the absence of a water molecule. The second optimized configuration shown in the Figure 2B inset reveals a bent configuration between 5-cyanouracil and the water molecule characterized by a C≡^{15}N···H angle of 90°. This configuration results in a H-bond between the hydrogen atom of the water molecule and the π-orbital of the C^{15}N group and is thus referred to as a π-H-bond. This π-H-bond results in a 10.8 ppm upfield shift of the ^{15}N isotropic chemical shift of 5-cyanouracil relative to 5-cyanouracil in the absence of water. The calculated upfield shift induced by H-bonding (in either geometry) between the nitirile group and the water molecule is in qualitative agreement with the experimentally observed upfield shift of the ^{15}N chemical shift of C^{15}NdU in going from THF-d_8 to D_2O. The agreement is not quantitative due to limitations of the basis set.[41−45] These calculations do, however, provide molecular insight into the nature and impact of H-bonding between the nitrile group and water molecules on the ^{15}N NMR chemical shift.

The impact of both types of hydrogen-bonding geometries between 5-cyanouracil and water on the ^{15}N NMR isotropic chemical shift was further explored by calculating the dependence of this spectroscopic observable on the H-bond distance. Figure 2A shows the change in the calculated ^{15}N NMR chemical shift on the distance between the ^{15}N atom of the nitrile group of 5-cyanouracil and the H atom of the water molecule involved in the σ-H-bond. The plot shows that the ^{15}N NMR chemical shift decreases rapidly as the σ-H-bond distance is decreased and increases rapidly as the distance is increased in the region near the optimized σ-H-bond length (∼1.8−4.0 Å). The magnitude of the change in the ^{15}N NMR chemical shift becomes essentially constant at relatively large distances (>6 Å). Figure 2B shows that the calculated ^{15}N NMR chemical follows a similar profile as a function of the π-H-bond distance where the ^{15}N NMR chemical shift increases with increasing distance reaching a maximum change at large distances (>6 Å). Both of these plots illustrate the sensitivity of the ^{15}N NMR chemical shift of the ^{15}N atom of the nitrile group to small variations in the H-bond distance with water. Consequently, the ^{15}N NMR chemical shift is dependent upon both the H-bond distance and the angle between the nitrile group and the water molecule involved in the H-bond interactions, similar to previous NMR calculated properties of hydrogen-bonded systems.[46,47]

Given the sensitivity of the ^{15}N NMR chemical shift of the nitrile group on the geometry of direct H-bond interaction with water, four additional water molecules were added around the ring of 5-cyanouracil to further explore the ^{15}N NMR chemical shift dependence on the solvation of 5-cyanouracil. A water molecule was initially added in close proximity to each C═O and N−H group of the ring in addition to the C≡^{15}N group. These positions were selected since each of these groups could participate in H-bonds with water. The geometry-optimized structures of 5-cyanouracil interacting with five water molecules are given in the Supporting Information (see Figures S2 and S3). Two optimized configurations are shown where the principal difference is the C≡^{15}N···H angle. The first configuration has a C≡^{15}N···H angle of 167° and represents a σ-H-bond between the water molecule the nitrile group, while the second configuration has an angle of 90° resulting in a π-H-bond between the water molecule and the nitrile group. The C≡^{15}N···H angle for the σ-H-bond for the five water model increased slightly (14°), becoming more linear compared to the single water system, while the C≡^{15}N···H angle for the π-H-bond remained the same.

The σ-H-bond and π-H-bond interactions for the five water models resulted in a 16.9 and 12.8 ppm upfield shift of the ^{15}N NMR isotropic chemical shift of 5-cyanouracil relative to isolated 5-cyanouracil, respectively. The direction of the shift is in qualitative agreement with the single water molecule configurations with 5-cyanouracil, although the magnitude of the shift for the five water molecule models is slightly larger.

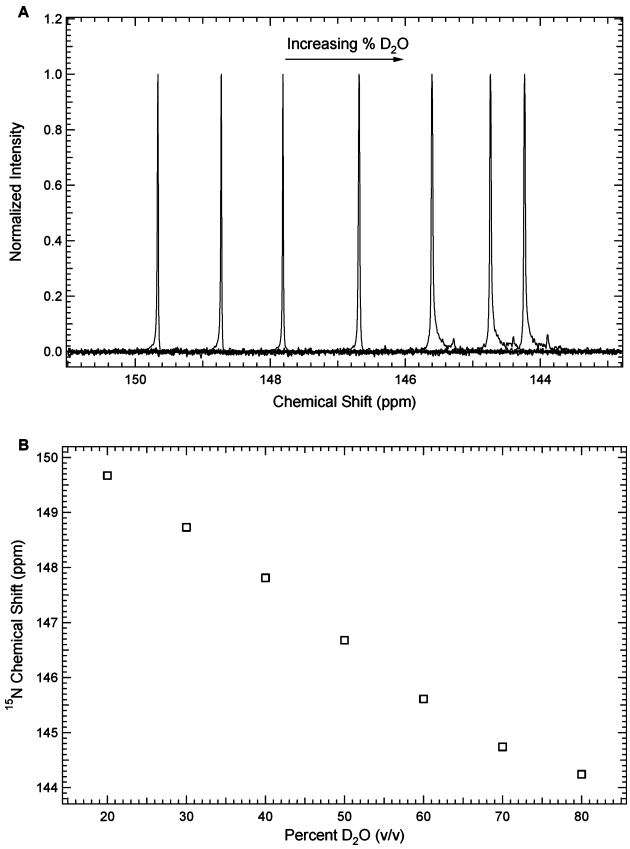

Figure 3. (A)^{15}N NMR spectra of C^{15}NdU in D_2O/THF-d_8 mixtures ranging from 20 to 80% D_2O (v/v) in approximately 10% increments recorded at 293 K. The spectra were intensity normalized and referenced to formamide (0 ppm). (B) ^{15}N NMR chemical shift dependence of C^{15}NdU on the D_2O percentage in the D_2O−THF-d_8 mixtures.

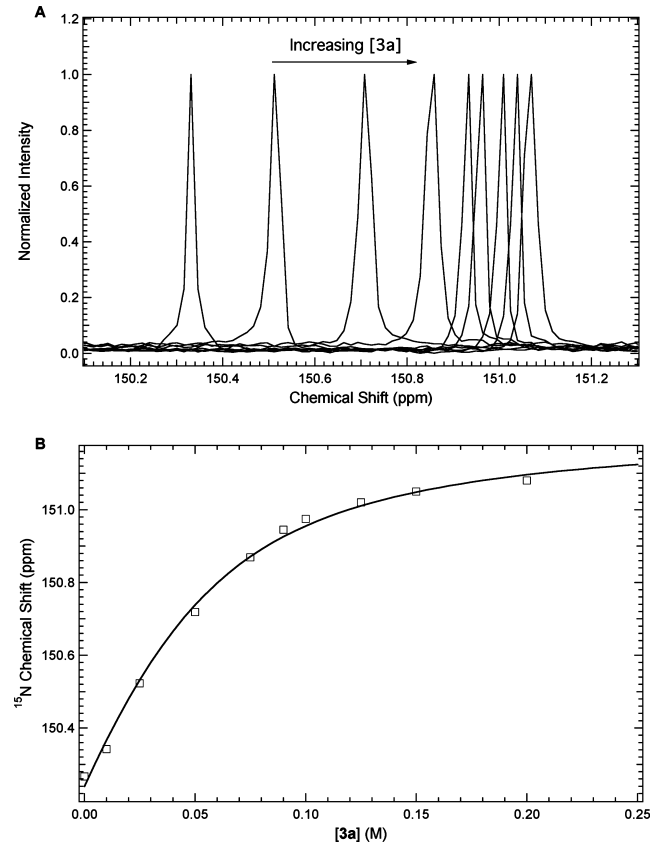

Figure 4. (A) Intensity-normalized ^{15}N NMR spectra of **2** recorded at 293 K as a function of the concentration of the heterodimer partner, **3a**, in $CDCl_3$ referenced to formamide (0 ppm). (B) Dependence of the ^{15}N NMR chemical shift of **2** (open squares) on the concentration of **3a** in $CDCl_3$ fit to eq 1 (solid curve).

The increase in the magnitude of the shift illustrates that the ^{15}N NMR chemical shift of the nitrile group is sensitive to direct H-bond interactions of the ^{15}N atom of the nitrile group with water molecules and to the solvation of the rest of the molecule. A more detailed quantitative description of the H-bond properties of these systems is not possible because of known limitations of the basis set in the determination of such properties.[41−45] However, the key result from these calculations is the sensitivity of the ^{15}N NMR chemical shift to H-bonding with water, which serves as the principal molecular basis for the observed ^{15}N NMR chemical shift of C^{15}NdU between THF-d_8 and D_2O.

Figure 3A shows the ^{15}N NMR spectra of C^{15}NdU in a series of D_2O/THF-d_8 mixtures ranging from 20 to 80% D_2O and Figure 3B shows the corresponding dependence of the ^{15}N NMR chemical shift of C^{15}NdU on D_2O composition of the solvent. The $\delta_{^{15}N}$ shifts monotonically from 149.67 to 144.24 ppm over this range, illustrating the sensitivity of this probe over a wide range of environments. This sensitivity is not surprising since the calculated ^{15}N NMR isotropic chemical shift of ^{15}N-labeled 5-cyanouracil was found to be strongly dependent upon the geometry of H-bonding with water. The D_2O/THF-d_8 mixtures will impact the nature of the interactions of C^{15}NdU with D_2O, which will therefore influence the observed ^{15}N NMR chemical shift. Unlike the IR absorbance band corresponding to the nitrile symmetric stretch of C^{14}NdU (**1b**),[6] the ^{15}N NMR peaks of C^{15}NdU (**1a**) are sharp with a full width half-maximum of <0.05 ppm and well resolved from each other in the solvent mixtures. This feature is advantageous in detecting small changes in the local environment of the nitrile manifested in small changes in the ^{15}N NMR chemical shift. The ^{15}N NMR spectra do not have resonances from other nitrogen nuclei in the molecule due to the low natural abundance of ^{15}N. Therefore, the spectra are free from congestion, which will greatly aid the analysis of the ^{15}N NMR spectra of selectively ^{15}N-labeled DNA and RNA oligomers.

The $\delta_{^{15}N}$ of C^{15}NdU is also sensitive to the formation of a hydrogen-bond-mediated base-pair mimic. Figure 4A shows the ^{15}N NMR spectra and Figure 4B shows the corresponding dependence of the ^{15}N NMR chemical shift of **2** on the concentration of the heterodimer partner, **3a**, in the noncompetitive solvent, $CDCl_3$. The $\delta_{^{15}N}$ shifts downfield 0.81 ppm upon formation of the heterodimer. This chemical shift dependence was fit to eq 1 to yield an association constant of ∼40 M^{-1}, which is in agreement with previous IR-monitored heterodimer formation.[6] DFT calculations on ^{15}N-labeled 5-cyanouracil and the heterodimer formed with **3b** predicted a downfield shift of 3.0 ppm upon heterodimer formation, in qualitative agreement with the experimental result. The lack of quantitative agreement is not surprising given the limitation of the basis set in the calculations systems containing cyclic H-bonds, which are present in this system.[43] However, the calculation does provide evidence that the molecular origin of the observed ^{15}N NMR chemical shift resulting from the addition of **3a** to a solution of **2** is heterodimer formation as expected.[6]

Figure 5 shows the intrinsic temperature dependence of the ^{15}N NMR chemical shift of C^{15}NdU in D_2O. The $\delta_{^{15}N}$ shifted monotonically from 143.80 ppm at 298 K to 144.18 ppm at 338 K for C^{15}NdU in D_2O, which is due in part to changes in the H-bonding interactions between C^{15}NdU and D_2O. This relatively small (0.38 ppm) downfield shift is approximately

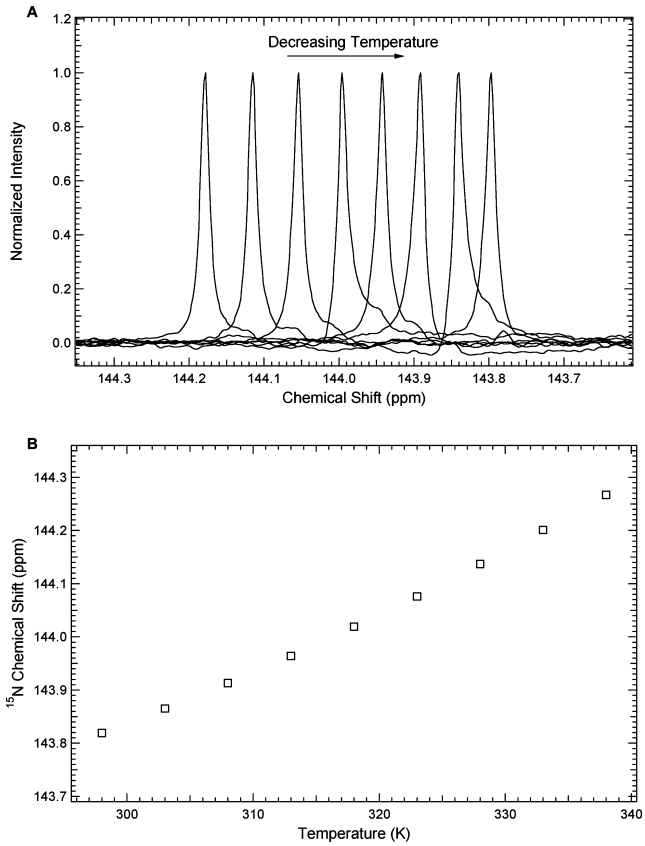

Figure 5. (A) Temperature-dependent ^{15}N NMR spectra of C^{15}NdU in D_2O from 298 to 338 K recorded in approximately 5 K increments. The spectra were intensity normalized and referenced to formamide (0 ppm). (B) ^{15}N NMR chemical shift dependence of C^{15}NdU on temperature in D_2O.

half of the chemical shift measured for the formation of the base-pair mimic and is in stark contrast to the shape of the binding curve for heterodimer formation (Figure 4B). The chemical shift observed for this base-pair mimic serves as a benchmark for ^{15}N NMR chemical shifts expected upon the thermally induced unfolding of DNA and RNA structures. Consequently, this small intrinsic temperature dependence of the ^{15}N NMR chemical shift should permit the local environments of the ^{15}N nuclei to be probed upon the thermally induced unfolding of C^{15}NdU-labeled DNA or RNA since experimental chemical shifts due to the thermal unfolding of the oligomers will have a different temperature profile and potentially larger chemical shift than the intrinsic temperature dependence of this probe.

Conclusions

The ^{15}N NMR chemical shift of the ^{15}N-labeled 5-cyano-2′-deoxyuridine was found to be sensitive to solvent, shifting from 153.47 to 143.80 ppm in going from THF-d_8 to D_2O. DFT calculations indicate that this sensitivity is partially due to H-bond interactions between C^{15}NdU and D_2O. The $\delta_{^{15}N}$ of C^{15}N was found to be an effective spectroscopic measure of heterodimer formation between **2** and **3a**, while the intrinsic temperature dependence of this spectroscopic handle was small. These qualities coupled with a high spectral resolution illustrate the potential promise of this probe to aid in unraveling the complex local nucleic acid environments present in DNA and RNA structures, including RNA hairpins.

The utility of C^{15}NdU as a spectroscopic probe is not limited to ^{15}N NMR.[5] The ^{15}N isotopic label shifts the nitrile stretching frequency in water of C^{14}NdU from 2241.9 to 2212.0 cm^{-1} in C^{15}NdU (data not shown). The magnitude and direction of the shift are expected due to the change in the reduced mass of the oscillator upon isotopic labeling and DFT calculations of the modified nucleoside. The two spectral bands are clearly resolved from each other in the IR, thus allowing both the bandwidth and position of the C^{14}N and C^{15}N absorbance bands to be used as metrics of local dynamic environments in nucleic acids sequences modified with C^{14}NdU and C^{15}NdU simultaneously.

Recently, we reported an azide-modified nucleoside, 2′-azido-2′-deoxyuridine (N_3dU),[48] designed to probe the sugar phosphate region of nucleic acids to complement C^{15}NdU, which will be a major groove probe when incorporated into duplex DNA. The azide group is an excellent vibrational probe and can be a ^{15}N NMR probe through ^{15}N labeling of the nitrogen atoms similar to the nitrile moiety of CNdU. The azide asymmetric stretch and the ^{15}N NMR chemical shift of the terminally ^{15}N-labeled N_3dU (^{15}NNN-dU) were found to be sensitive to solvent as expected. However, the magnitude of the shifts differed from the corresponding shifts of C^{15}NdU. For instance, the shift of the azide asymmetric stretch was larger (13.5 cm^{-1})[48] than the shift in the nitrile stretching frequency (9.2 cm^{-1})[6] upon going from THF to water, while the ^{15}N NMR chemical shift of terminally ^{15}N-labeled N_3dU (the most sensitive of the three potential ^{15}N-labeled constructs)[48] shifted only 3.8 ppm compared to 9.67 ppm for the ^{15}N-labeled nitrile in going from THF-d_8 to D_2O in ^{15}NNN-dU and C^{15}NdU, respectively. A detailed comparison of these complementary spectroscopic probes will be the subject of a future paper. The development of multiple site-specific probes should significantly aid in understanding nucleic acid solvation and dynamics such as RNA hairpin folding.[49–51]

Abbreviations. DMF (dimethylformamide); FC (flash column chromatography using silica gel); H_2O (deionized water); PE (petroleum ether).

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Supporting Information Available: Determination of the T1 relaxation time of the ^{15}N nuclei of C^{15}NdU, geometry-optimized structures of 5-cyanouracil with five water molecules exhibiting one σ- or π-H-bond between the nitrile group and a water molecule. This material is available free of charge via the Internet at http://pubs.acs.org.

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