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# [3-$^{15}$N]-2',3',5'-TRI-O-BENZOYLURIDINE. DETECTION OF HYDROGEN BONDING IN A-U BASE PAIRS BY $^{15}$N NMR.[1]

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Nuclear magnetic resonance has been a particularily useful tool for studying hydrogen bonding in a variety of systems, including the important template interactions between the pyrimidine and purine moieties in nucleic acids.[3] Of the various nuclei located near the sites of hydrogen bonding, $^{1}$H has been used almost exclusively. However, advances in instrumentation and techniques for synthesis of labelled material make it practical to obtain spectra with the less abundant and less sensitive nuclei. Recently, Iwahashi and Kyogoku[4] reported substantial deshielding for the $^{13}$C resonances of C(2) and C(4) in uracil and related derivatives upon hydrogen bonding with 9-ethyladenine. Several experiments suggest that $^{15}$N chemical shifts are also altered by formation of hydrogen bonds, although the direction and magnitude of the shifts depend on the covalent bonding arrangement at nitrogen.[5] However, Hawkes, Randall, and Hull[6] reported that an equimolar mixture of uridine and adenosine showed no effect on the nitrogen chemical shifts and concluded $^{15}$N resonances of the two compounds were not sensitive to base pairing. Unfortunately, those measurements were carried out in dimethylsulfoxide, a solvent which Katz and Penman[7] had previously found unsuitable for studying base pairing between uridine and adenosine by $^{1}$H nmr. In this communication, we report experiments which establish substantial deshielding for N(3) of the uracil moiety in an A-U base pair using chloroform as a solvent.

[3-$^{15}$N]-Uracil was prepared from [$^{15}$N]-potassium cyanate (KOR Isotopes, 99%) using the procedure developed by Roberts and Poulter.[8] The material was converted to the corresponding bis-O-trimethylsilyl derivative which was purified by distillation and condensed with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranoside to yield 2',3',5'-tri-O-benzoyluridine for the nmr experiments.[9] A $^{1}$H nmr spectrum of a 0.5 M solution of the nucleoside in deuteriochloroform showed an one-proton doublet ($J_{^{1}H-^{15}N}$ = 91.3 Hz) at 9.84 ppm and a $^{15}$N spectrum of the same solution gave a doublet at 222.5 ppm.[10]

In order to ascertain the effect of hydrogen bonding with an adenine moiety on the chemical shift of the resonance for N(3) in the blocked uridine, nmr spectra containing varying mole fractions of [3-$^{15}$N]-2',3',5'-tri-O-benzoyluridine and 5'-O-acetyl-2',3'-O-isopropylideneadenosine (Sigma) in deuterio-

chloroform were obtained. To avoid complications that might arise from through base stacking or other concentration effects,[7] the total nucleoside concentration was maintained at 0.5 M while the mole fraction of the adenosine derivative was increased from zero to 0.75. The relative amounts of pyrimidine and purine nucleoside were checked by comparing the integrals of the N(3)-$^1$H resonance in the uridine derivative with those of the isopropylidene methyls or the acetate methyl in the adenosine nucleoside. As shown in Figure 1, the doublet at 222.5 ppm moved downfield to a limiting value of ca. 217.7 ppm ($\Delta\delta$ = 4.73 ppm) in the

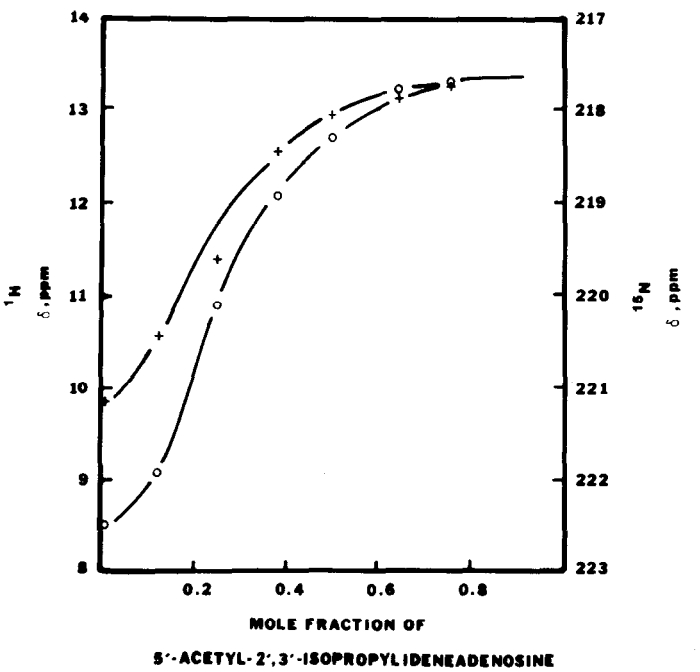


Figure 1. Variations in $^1$H and $^{15}$N chemical shifts in [3-$^{15}$N]-2',3',5'-tri-O-benzoyluridine with added 5'-O-acetyl-2',3'-O-isopropylideneadenosine. Total concentration of nucleosides was maintained at 0.5 M. + - $^1$H shifts. O - $^{15}$N shifts.

presence of a 3-molar excess of the purine nucleoside. In the same samples, the chemical shift of the hydrogen attached to N(3) of the uracil moiety moved downfield from 9.84 ppm to 13.3 ppm ($\Delta\delta$ = 3.46 ppm). The magnitude of the $^1$H deshielding is similar to that found by Scheit[11] for hydrogen bonding between the 5'-O-acetyl-2',3'-O-isopropylidene derivatives of uridine and adenosine.

Although $^{15}N$ chemical shifts are sensitive to changes in solvent, the parallel deshieldings of the $^{1}H$ and $^{15}N$ resonances as a function of added purine nucleoside leave no doubt that the downfield shift of N(3) is due to the formation of an A-U base pair.

We also found that $^{1}J_{^{15}N-^{1}H}$ decreases upon addition of increasing amounts of 5'-O-acetyl-2',3'-O-isopropylideneadenosine. Although it was not possible to measure the coupling constant accurately at intermediate concentrations of the purine nucleoside because of line broadening due to exchange, $^{1}J_{^{15}N-^{1}H}$ decreased from 91.3 Hz to 87.5 Hz ($\Delta^{1}J$ = 3.8 Hz) as the mole fraction of 5'-O-acetyl-2', 3'-O-isopropylideneadenosine in the mixture increased from 0 to 0.75. Variations in $^{1}J_{^{15}N-^{1}H}$ of up to 4 Hz were also reported for a series of substituted anilines and their respective hydrogen bound complexes with dimethylsulfoxide.[5c,12] However, in this case the J's increased upon hydrogen bonding, and the phenomenon was attributed to changes in hybridization of the amino nitrogens. Obviously, the two systems are quite different and further work will be required to determine the reason for the decrease in $^{1}J_{^{15}N-^{1}H}$ we observed in the pyrimidine-purine base pair.

The conclusion of Hawkes, Randall, and Hull[6] that the $^{15}N$ spectra of adenosine and uridine are not sensitive to base pairing is incorrect, at least for the uridine partner. Although $\Delta^{1}J_{^{15}N-^{1}H}$ may be too small to be of any practical value, especially when complications due to line broadening arise, a $\Delta\delta$ for N(3) of ca. 5 ppm is easily measured and can complement the $^{1}H$ and $^{13}C$ methods now in use.

## References


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