A New Class of Nucleosides Possessing Unusual Physical Properties: Syntheses, Hydration, and Structural Equilibria of 1-(β-D-Glycofuranosyl)uracil-6-carboxaldehydes[1]

Michael P. Groziak,*,† Ali Koohang,†,‡ William C. Stevens,§ and Paul D. Robinson⊥

Department of Chemistry and Biochemistry, Nuclear Magnetic Resonance Facility, and Department of Geology, Southern Illinois University, Carbondale, Illinois 62901-4409

Received January 19, 1993

The 1-β-D-ribofuranosyl, 1-(2′-deoxy-β-D-ribofuranosyl), and 1-β-D-arabinofuranosyl derivatives (**1a–c**) of uracil-6-carboxaldehyde have been prepared for physicochemical analysis. The NMR-determined hydration and structural equilibria of **1a–c** in both D_2O and $(CD_3)_2SO$ solution are compared, and the single-crystal X-ray structure of **1a** is presented. Due to the high degree of electrophilic nature of their aldehyde moiety, all three nucleosides exhibit a strong proclivity toward existing as cyclic hemiacetal structures in solution; one such structure was found to be the sole constituent in the solid state for **1a**. Using a [*formyl*-^{13}C]-labeled derivative of a synthetic precursor to **1a**, the $^1\Delta^{13}C(^{18}O)$ shielding isotope shift effects produced upon hydrate formation were determined at a ±1 ppb level, giving reference values of biomechanistic importance.

Introduction

As the uridine-, thymidine-, and cytidine-based nucleic acids play such a central role in the cellular processes of metabolism and replication,[2] it is not surprising that many synthetic pyrimidine nucleoside analogs have been found to exhibit some degree of antitumor, antiviral, antibacterial, antifungal, or even multiple or "crossover" activity.[3] This broad spectrum of medicinally-important associated bioactivities provides encouragement for the continued development of new classes of these compounds which might also possess useful biological properties. Among the known bioactive nucleoside agents, some possess inherently reactive functionalities which can be exposed or internally-masked by reversible intramolecular interactions. Others have latent reactivities which are revealed only upon activation in specific mechanistic fashion by biomacromolecules.[4]

Interestingly, the 6-formylated versions of the naturally-occurring pyrimidine nucleosides have been overlooked thus far in this search for new bioactive materials, in part due to the fact that investigators have until recently been laboring under certain misconceptions concerning their stability.[5] As an initial research effort in a new program of study seeking to investigate the physicochemical and biological properties of 6-acylpyrimidine nucleosides, we firmly established that a carboxaldehyde moiety attached to the 6-position of a tri-O-protected uridine possessed an unusually high susceptibility toward undergoing 1,2-addition reactions with weakly nucleophilic hydroxylic species (e.g., water and alcohols) and suggested that the previous observations of "instability" reported for these compounds could have arisen as a direct consequence of such a heightened reactivity.[6] This characteristic of "stable" hydrate formation[7] exhibited by the 6-formyluridines can be found in other electrophilic carboxaldehydes such as those with α-halogens like trichloroacetaldehyde (chloral),[8] those with α-carbonyl groups like glyoxal and glyoxylic acid,[9] and those with α-imine groups in heterocyclic rings like pyridine-2-carboxaldehyde.[10]

Our first study culminated in the preparation and a preliminary physicochemical evaluation of the parent compound uridine-6-carboxaldehyde (**1a**), which was found to exhibit a propensity toward existing in 5′-cyclic hemiacetal form in both protic and aprotic solution.

† Department of Chemistry and Biochemistry.
‡ Present address: Graduate Program, Department of Chemistry, University of Illinois at Urbana-Champaign.
§ Nuclear Magnetic Resonance Facility.
⊥ Department of Geology.

(1) Presented in part: (a) Groziak, M. P.; Koohang, A; Robinson, P. D. *Book of Abstracts*, 203rd Meeting of the American Chemical Society, San Francisco, CA, Spring 1992; ORGN 145. (b) Groziak, M. P.; Koohang, A. *Abstracts*, 13th International Congress of Heterocyclic Chemistry, Corvallis, OR, 1991; Abstract G3-27. This article is dedicated to Prof. Leroy B. Townsend, University of Michigan, Ann Arbor, MI, on the occasion of his sixtieth birthday.

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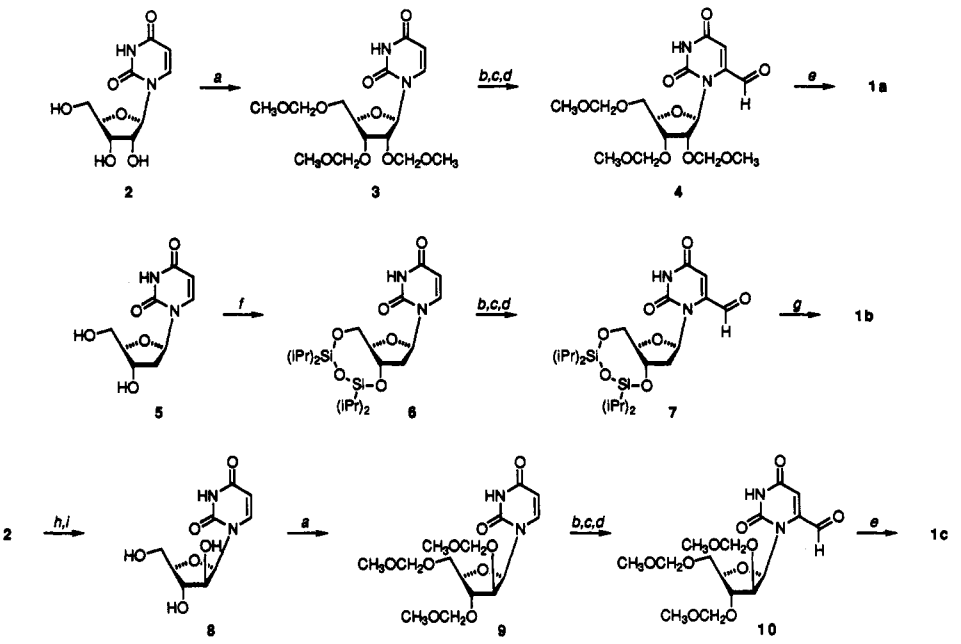
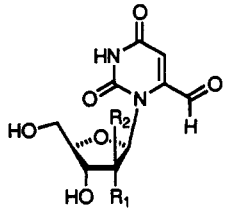
Scheme I. Synthesis of 1a–c[a]



[a] Reagents and conditions: (a) 1:1 $(CH_3O)_2CH_2/CH_2Cl_2$, cat. TfOH, 25 °C; (b) 3 equiv LDA, THF, −78 °C; (c) HCO_2Et, −78 → 25 °C; (d) HOAc; (e) 50% aqueous CF_3CO_2H, 25 °C; (f) $((iPr)_2Si(Cl))_2O$; (g) $(nBu)_4NF$, THF, 25 °C; (h) $(PhO)_2CO$, DMA, 165 °C; (i) 0.1 N NaOH, 25 °C.

Herein, we report the results of an expanded investigation designed to compare and contrast the effect that this highly electrophilic 6-carboxaldehyde moiety has on the structural properties of the three members of this new class of pyrimidine nucleosides which contain sugar moieties known to be integral parts of well-established bioactive nucleosides. These are the 1-(β-D-ribofuranosyl)-, 1-(2′-deoxy-β-D-ribofuranosyl)-, and 1-(β-D-arabinofuranosyl)-uracil-6-carboxaldehyde nucleosides **1a–c**. Extensive solution and solid-state data for these structurally unusual nucleosides have been obtained, and an additional benefit



1a, R_1 = OH, R_2 = H
b, R_1 = R_2 = H
c, R_1 = H, R_2 = OH

of this investigation has been realized by utilizing the facile hydration phenomenon associated with these compounds to obtain a highly accurate measurement of a biomechanistically-important $^1\Delta^{13}C(^{18}O)$ isotope shift effect.

Results and Discussion

The synthesis of target nucleosides **1a–c** is shown in Scheme I. In each instance, the 2,4-dioxopyrimidine-3,6-diyl dianion of a suitably-protected nucleoside precursor (compound **3**, **6**, or **9**) was generated upon treatment with excess LDA according to Miyasaka's methodology,[5d-g] and then the 6-carboxaldehyde substituent was introduced by quench with ethyl formate. Subsequent deprotection (of compound **4**, **7**, or **10**) afforded the desired target. In the route to **1a**, the 2′,3′,5′-tri-*O*-methoxymethylated uridine **3** was prepared directly from uridine (**2**) according to our efficient one-pot triprotection method as previously reported.[6] Attempts to employ a similar di-*O*-MOM protection strategy for the synthesis of the 2′-deoxyribonucleoside **1b**, however, were not successful as loss of the C1′–N1 linkage was observed to have occurred (by ^1H NMR spectral analysis) under each of the reaction conditions found to promote removal of the MOM protecting groups from 3′,5′-bis-*O*-(methoxymethyl)-1-(2′-deoxy-β-D-ribofuranosyl)uracil-6-carboxaldehyde. The glycosidic linkage of 2′-deoxyribonucleosides is well known to be more hydrolytically labile than that of their ribonucleoside counterparts under acidic conditions.[11] 1,1,3,3-Tetraisopropyldisiloxanediyl protection[12] of 2′-deoxyuridine (**5**) upon treatment with TIPDS-Cl_2[13] afforded compound **6**[5g] in 93% yield, and this was formylated in the manner described above to give a modest yield (31%) of the protected 2′-deoxyuridine-6-carboxaldehyde **7**. Removal of the TIPDS protecting group from **7** under standard conditions (tetrabutylammonium fluoride in THF at 25 °C) then afforded, after chromatographic purification, target **1b** in 77% yield. For the synthesis of **1c**, starting material 1-β-D-arabinofuranosyluracil (**8**)[14,15] was prepared in slightly improved overall yield from **2** via 2,2′-anhydrouridine[14-16] (not shown in Scheme I). Initial attempts at formylation of the 3′,5′-TIPDS-protected version of this anhydro nucleoside were examined, but these were found instead to promote an LDA-mediated

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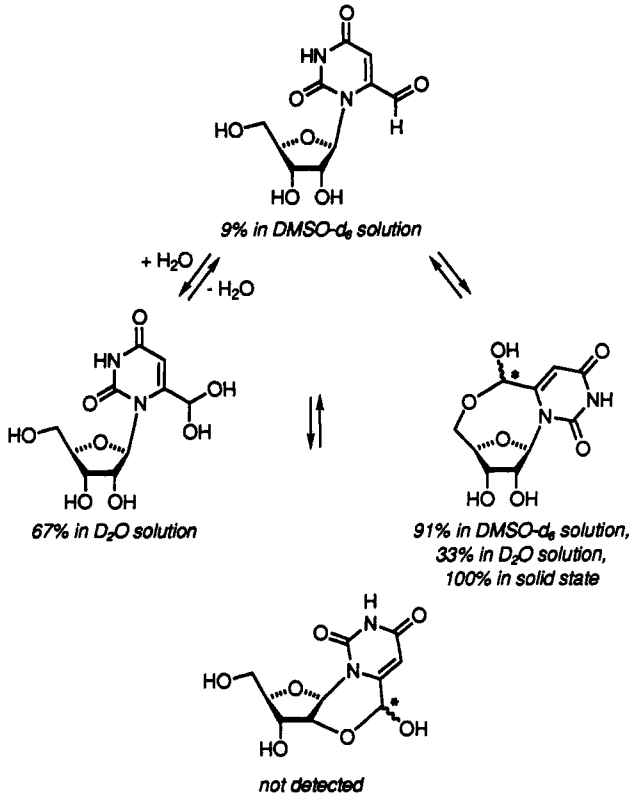
Scheme II



elimination reaction that introduced C1′–C2′ unsaturation, according to ^{1}H NMR spectral analysis. *arabino*-Uridine **8** underwent one-pot tri-*O*-MOM protection to give compound **9** (60% yield), which was formylated as above to give the protected *arabino*-uridine-6-carboxaldehyde **10** (30% yield, based upon unrecovered starting material). Deprotection of compound **10** was accomplished using 50% aqueous TFA at room temperature, and the target **1c** was obtained in 99% yield.

The hydration and structural equilibria determined for ribonucleoside **1a** are shown in Scheme II.[17] We had previously determined that **1a** exists as a 10:1 mixture of a 5′-cyclic hemiacetal diastereomer and the free aldehyde in $(CD_3)_2SO$ solution and as a 2:1 mixture of the hydrate (*gem*-diol) and a 5′-cyclic hemiacetal diastereomer in D_2O solution.[6] We now report that **1a** in the solid state exists exclusively as a single 5′-cyclic hemiacetal diastereomer. In addition, a ribosyl conformational analysis of the 5′-cyclic hemiacetal structure present in $(CD_3)_2SO$ solution by an examination of $^3J_{H-H}$ coupling constant magnitudes has provided evidence consistent with an S-type conformer similar to that described next for the solid-state structure.

The single-crystal X-ray structure determination[18] of **1a** reveals several important structural features (Figure 1). Although the suitable crystal of **1a** had been grown from an aqueous solution in which the hydrate structure had been found to predominate (67%), the solid-state structure determination revealed a single diastereomer of a 5′-cyclic hemiacetal structure. Upon concentration, the

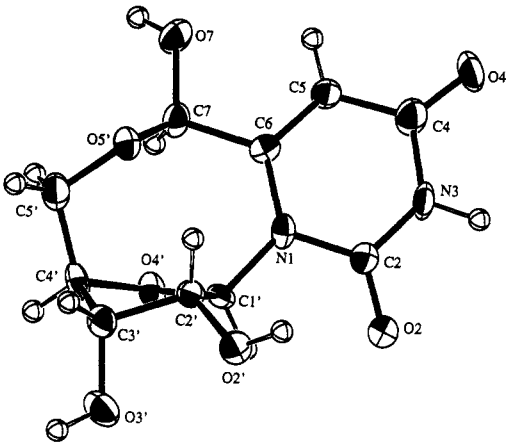


Figure 1. X-ray crystal structure of 1-(β-D-ribofuranosyl)uracil-6-carboxaldehyde (**1a**).

aqueous solution equilibrium between hydrate and 5′-cyclic hemiacetal (with its intervening aldehyde) apparently shifts with concomitant loss of water as the latter is removed by crystal deposition. Many of the available donor and acceptor sites of **1a** were found to be part of an intermolecular hydrogen-bonding network (not shown in Figure 1), a finding connected to the somewhat high calculated crystal density (1.7 g^1 cm^{-3}). The C5′–O5′–C7 glycosyl–aglycon linkage in crystalline **1a** traverses directly over the β-face of the furan ring in a manner virtually identical to that reported recently in the D_2O/DMSO-d_6 solution structure determination of O^5′,6-methanouridine.[19] Finally, it is noted that the small C4′–O4′–C1′–C2′ dihedral angle of −1.5(8)° renders these atoms coplanar to within 0.01 Å. Thus, this sole solid-state structure of **1a** is an S-type ribosyl conformer which, due to its 3′-exo orientation, is described by the notation $_3$E.[20] The pseudorotation angle *P* was calculated to be 197°.

A kinetic process leading to the formation of the observed crystalline diastereomer can be envisaged to begin with rotations about the C4′–C5′ and C1′–N1 bonds of the aldehyde structure which position the 5′-OH group favorably close to the *re* face of the aldehyde moiety. From this putative rotamer, a stereofacially-specific 1,2-addition reaction would produce the (7*R*)-configurational isomer found in the solid-state structure determination.[21] Alternatively, the detection of only one 5′-cyclic hemiacetal diastereomer in the solid state by X-ray analysis as well as in the two different solution media by NMR analysis might be a consequence of the outcome of thermodynamically-controlled processes, in which case there are at least

(17) All solution structures reported herein were determined in part by ^{1}H/^{1}H COSY and short- and long-range ^{1}H/^{13}C HETCOR NMR spectral analyses. For reasons of clarity, structures determined to exist in D_2O solution are not shown in their ^{2}H-labeled form in Schemes II–IV.

(18) The crystallographic data for this structure have been deposited with the Cambridge Crystallographic Data Centre. These data can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

(19) Otter, B. A.; Patil, S. A.; Spada, M. R.; Jelicks, L. A.; Yoshimura, Y.; Matsuda, A.; Klein, R. S. *Nucleosides Nucleotides* **1992**, *11*, 615. These investigators also reported the preparation of the 2′,3′-*O*-isopropylidene derivative of uridine-6-carboxaldehyde, which they found by NMR to exist as a single 5′-cyclic hemiacetal diastereomer in DMSO-d_6 solution. The solution structure of their diastereomer has the same 7*R* configuration (not *S* as stated) as that determined herein for **1a** in the solid state. We thank Dr. Otter for correspondence regarding his group's findings.

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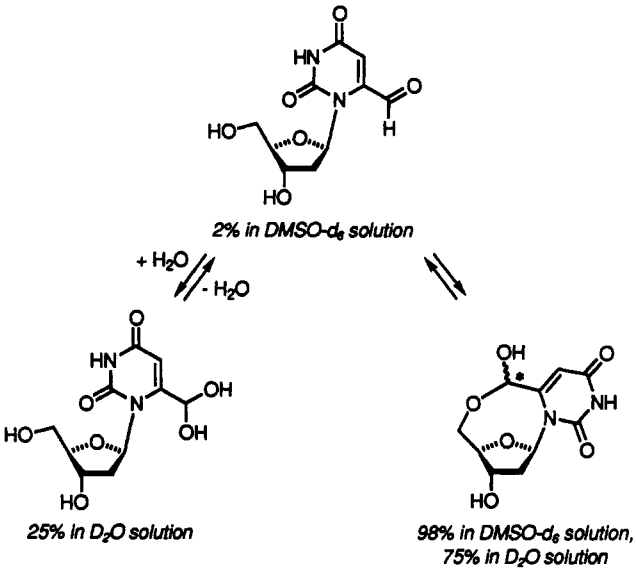
(21) This description assumes a predominance of that C6–C7 rotamer of the *syn* carboxaldehyde structure in which the aldehydic C=O group is coplanar (and thus conjugated) with the heterocyclic enone system and is oriented such that its oxygen atom is positioned away from the ribosyl moiety for steric reasons. Preliminary analysis by molecular mechanics calculations has revealed that such a structure does indeed represent at least a local energy minimum, and further, that rotation about the C1′–N1 bond of this structure is energetically favored when H7 of the aldehyde moiety is positioned directly above O4′ (rather than above C2′), thereby presenting the *re* face to the 5′-OH group above the furanose ring.

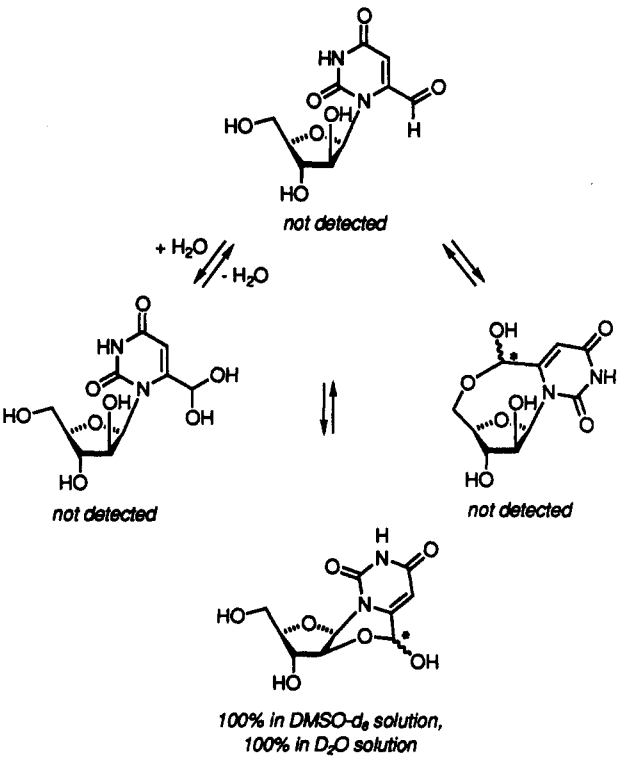
two mechanisms to consider. The ready reversibility of 1,2-addition reactions coupled with a significant difference in product stabilities would provide one thermodynamic explanation for the substantial presence of only one 5′-cyclic hemiacetal. However, such an equilibration argument is dependent upon both *re* and *si* facial attack pathways being energetically accessible, which in turn is dependent upon the inconsequence of any steric hindrance to the directions of rotation about C1′–N1 producing the *anti* conformer necessary for the proximity of the 5′-OH and CHO moieties. It is known that the presence of 6-position steric bulk even as modest as that afforded by a methyl group is sufficient to force uracil-based nucleosides into the *syn* conformational form (with O2 positioned over the furanose ring),[22] and so it is likely that **1a** as its aldehyde structure also exists in such form. From this conformer, both directions of rotation about C1′–N1 are expected to be energetically-demanding, but that which moves the 6-carboxaldehyde group over C2′ would more likely be due to the presence of the β-oriented H2′ atom. If the energy difference between these two rotational pathways is sufficiently large, then the (*7R*)-isomer of **1a** 5′-cyclic hemiacetal may indeed be the thermodynamically more stable product, but one produced along a kinetic pathway. A second thermodynamics-based explanation involves epimerization. The H7 of the 5′-cyclic hemiacetal structures of **1a** could experience the same electron-withdrawing effect that is likely responsible for the activation of the carboxaldehyde moiety toward nucleophilic attack, namely, that due to the C6–C5–C4–O4 enone fragment. Enhanced by partial protonation of O4 during the acidic conditions of *O*-MOM group removal from **4**, this effect could have led to an epimerization at the hemiacetal C7 center, producing the single most stable diastereomer subsequently detected in both solution media and in the solid state. However, ^{1}H NMR spectral analysis of **1a** in D_2O/CF_3CO_2H solution has not provided any evidence of H7 exchange.

The hydration and structural equilibria determined for the 2′-deoxyribonucleoside **1b** and the arabinonucleoside **1c** are shown in Schemes III and IV, respectively. In D_2O solution, a ratio of 1:3 was found for the hydrate and a 5′-cyclic hemiacetal diastereomer structure for **1b**, while in $(CD_3)_2SO$ solution the ratio of free aldehyde to a 5′-cyclic hemiacetal diastereomer for this compound was determined to be 1:49. Thus, **1b** shows an even greater propensity toward existing in 5′-cyclic hemiacetal form than does **1a** in both media. This is undoubtedly a result of the former's lack of a 2′-OH substituent, whose presence in the latter might serve to destabilize the 5′-cyclic hemiacetal form somewhat due to its tendency towards attaining a pseudoaxial site for the stabilizing gauche effect with O4′.[23] For arabinonucleoside **1c**, only 2′-cyclic hemiacetal diastereomeric structures were found in both media. From these determinations, it is evident that the presence of the 2′-β hydroxyl substituent in **1c** provides for the formation of a quite stable 5,6-*cis* fusion of the arabinofuranose and hemiacetal-containing [1,4]oxazine rings. The initial ratio of diastereomers was found to be 3:1 in $(CD_3)_2SO$ solution and 2:1 in D_2O solution, and all ^{1}H and ^{13}C NMR characterization data for **1c** were

Scheme III



Scheme IV



obtained exclusively from solutions which exhibited these ratios. The $(CD_3)_2SO$ solution structures of this compound were examined in greater detail by ^{1}H/^{1}H ROESY 2D NMR spectroscopy.[24] By this method, a through-space interaction between the H2′ and hemiacetal-CH protons of the minor component was revealed. This observation permits us to make the unequivocal stereochemical assignment of *S* to the C7 configuration of the minor (25%) component's structure and, by default, of *R* to that of the major (75%) component's structure. It was noted that the ratio of diastereomers of **1c** in both solutions changed slowly over time to favor the presence of the initially less abundant one, thus identifying it as the thermodynamically more

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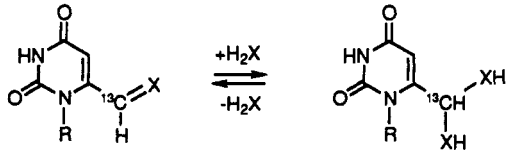
stable structure. Preliminary structural analysis by molecular mechanics calculations has been interpreted to suggest that the hemiacetal OH group of the 7S diastereomer not only is able to assume a pseudoaxial orientation on the [1,4]oxazine ring (energetically favored by the anomeric effect), but also is able to participate in intramolecular hydrogen bonding with the 5′-OH moiety. One or a combination of these effects may be responsible for the greater stability of this diastereomer. We are currently seeking to obtain ROESY-derived diagnostic structural information for **1c** in D_2O solution and of target nucleosides **1a,b** in both deuterated NMR solvents.

Up to now, an accurate determination of the magnitude of the $^1\Delta^{13}C(^{18}O)$ isotope shift effect produced upon hydrate formation of an aldehyde or ketone has not been made.[25] This value is of importance to mechanistic bioorganic chemistry for the differentiation between those hydrolytic enzyme mechanisms that proceed via formation of 1,1-diol intermediates and those that involve the direct 1,2-addition of an enzyme's active-site residue to a substrate's carbonyl group.[26,27] The facility with which uridine-6-carboxaldehydes undergo hydrate formation has enabled us to measure the isotope shift effect in question at a ±1 ppb level. The [*formyl*-^{13}C]-labeled derivative of **4** was prepared from uridine and ethyl [^{13}C]formate according to steps outlined in Scheme I, and a dioxane-d_8 solution of this material was treated with 4 equiv of 1:1 $H_2O/[^{18}O]$-H_2O. After an equilibration interval of $>10t_{1/2}$ for hydrate formation,[6] the hydrate and aldehyde regions



[*formyl*-^{13}C]-**4**

X = ^{16}O, ^{18}O

R = 2,3,5-tris-*O*-(methoxymethyl)-1-β-D-ribofuranosyl

of the ^{13}C NMR spectrum of this solution were examined separately, using acquisition parameters designed to achieve the desired resolution. As shown in Figure 2, the plots of these spectral regions revealed the presence of the expected ^{16}O- and ^{18}O-containing derivatives of both [*formyl*-^{13}C]-**4** hydrate and [*formyl*-^{13}C]-**4** in near-statistical proportions. Accurate $^1\Delta^{13}C(^{18}O)$ shielding isotope shift effects upon the hydrate and aldehyde ^{13}C nuclei of 16 and 44 ppb, respectively, were calculated from these spectral data. Reasonably accurate determinations of the magnitude of the C=O isotope shift effect in vinylogous α-dicarbonyl compounds have been made,[28] but previous investigators have been able to provide only an estimation of a range (20–25 ppb for each C–D bond) for the magnitude of the $C(OH)_2$ isotope shift effect in the hydrates of certain chloromethyl ketones[26] and of keto methylene-containing peptide pseudosubstrates.[27] While the $^1\Delta^{13}C(^{18}O)$ isotope shift effects determined herein for [*formyl*-^{13}C]-**4** hydrate

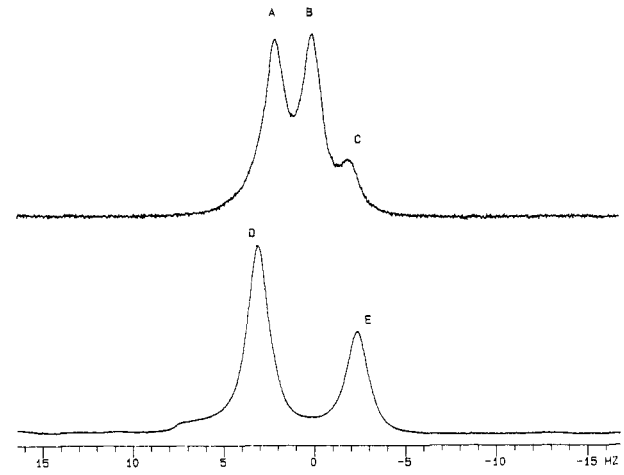


Figure 2. Plots of separate broadband ^1H-decoupled 125-MHz ^{13}C NMR spectral acquisitions of the hydrate (top, ca. 92–82 ppm) and aldehyde (bottom, ca. 192–182 ppm) regions of 0.35 M [*formyl*-^{13}C]-**4** upon equilibration with 4.0 equiv of an equimolar mixture of H_2O and $[^{18}O]$-H_2O in 1,4-dioxane-d_8 solution at rt for 64 h. Peak areas approximate the theoretical equilibrium compositions of 36% [$^{16}O_2$]-, 48% [^{16}O,^{18}O]-, and 16% [$^{18}O_2$]-labeled hydrate (A, B, and C, respectively) and of 60% [^{16}O]- and 40% [^{18}O]-labeled aldehyde species (D and E, respectively). Accurate $^1\Delta^{13}C(^{18}O)$ shielding shift effect magnitudes of Δδ 16 ± 1 ppb for each hydrate hydroxyl moiety and of Δδ 44 ± 1 ppb for the aldehyde moiety were calculated.

do not adversely affect the interpretation of the results of earlier biomechanistic studies, those researchers currently studying the mechanism(s) of enzyme-catalyzed hydrolyses should be aware that the value of the upfield chemical shift experienced by the carbonyl ^{13}C nucleus of an aldehydic or ketonic substrate analog upon formation of a [$^{18}O_2$]-1,1-diol may be as low as 32 (16 × 2) ppb.

Conclusions

The NMR-based solution structure determinations for **1a–c** together with the X-ray-based solid-state one for **1a** have all provided data consistent with the previously established high susceptibility of the 6-carboxaldehyde functionality toward undergoing 1,2-addition reactions with weakly nucleophilic hydroxyl moieties. The striking differences noted for the structural forms exhibited by **1a–c** is a direct result of the availability of hydroxyl substituents, both exogenous and endogenous, for participation in this type of reaction. Interestingly, the degree of electrophilicity of this moiety is sufficiently high so as to render the free aldehyde structure only a minor constituent, at best, among the hydrate, 2′-cyclic hemiacetal, and 5′-cyclic hemiacetal structures which have been revealed for the "carboxaldehydes" of the present study. This intrinsic property has already been utilized to provide an accurate measurement of the $^1\Delta^{13}C(^{18}O)$ isotope shift effects produced upon carboxaldehyde *gem*-diol formation. Continued investigations of members of this class of structurally-unusual 6-acylpyrimidine nucleoside materials are expected to uncover additional utilities of both a physicochemical and biological nature.[29]

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(28) For example, the $^1\Delta^{13}C(^{18}O)$ shielding isotope shift effect upon the exocyclic carbonyl ^{13}C nucleus of 2-(2-oxopropylidene)cyclohexanone in $CDCl_3$ solution was calculated to be 50 ppb: Gingerich, S. B.; Jennings, P. W. *J. Org. Chem.* **1983**, *48*, 2606. A less-than-expected ^{18}O-content in isolated product was attributed to a partial loss of label due to exchange during an aqueous workup procedure.

Experimental Section

Materials and Methods. Melting points were determined on a Thomas-Hoover UniMelt capillary apparatus and are uncorrected. Radial preparative-layer chromatography was performed on a Chromatotron instrument (Harrison Research, Inc., Palo Alto, CA) using Merck silica gel-60 PF254 as the adsorbent. Flash column chromatography was performed using 230-400 mesh ASTM Merck silica gel-60. Lyophilizations were conducted on a Labconco Lypho-Lock 4.5-L bench-top freeze-dryer. ^1H and ^{13}C NMR spectra were recorded on a Varian VXR-300 (300 and 75 MHz) or VXR-500 (500 and 125 MHz) instrument. These spectra were recorded with tetramethylsilane or 3-(trimethylsilyl)propanesulfonic acid, sodium salt (DSS) ($\delta = 0.0$ for ^1H) or $CDCl_3$ ($\delta = 77.0$ for ^{13}C) or $(CD_3)_2SO$ ($\delta = 39.5$ for ^{13}C) as internal reference. Short- and long-range 2D ^1H–^{13}C heteronuclear shift correlation NMR spectra were obtained on the VXR-300 instrument, and the ^{18}O isotope shift effect was conducted on the VXR-500 instrument equipped with a 10-mm broadband tunable probe. 99% [^{18}O]-H_2O and 99% ethyl [^{13}C]formate were obtained from Isotec, Inc. 1-[2,3,5-Tris-*O*-(methoxymethyl)-β-D-ribofuranosyl]uracil-6-[^{13}C]carboxaldehyde ([*formyl*-^{13}C]-4) was prepared as previously reported.[6] 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane was prepared from isopropylmagnesium bromide and trichlorosilane according to a multistep literature procedure.[13] Butyllithium in hexanes, diisopropylamine, *N*,*N*-dimethylacetamide, diphenyl carbonate, ethyl formate, tetrabutylammonium fluoride in THF solution, 99.5% 1,4-dioxane-d_8, and trifluoromethanesulfonic acid were purchased from the Aldrich Chemical Co. The butyllithium was titrated with diphenylacetic acid in anhyd THF solution at rt. Tetrahydrofuran was dried by distillation from sodium under argon, using benzophenone ketyl as indicator. Pyridine was dried by distillation from CaH_2 under argon. Ethyl formate was dried over P_2O_5 and then distilled from the same under argon. Elemental microanalyses were performed by Tom McCarthy and his staff at the University of Illinois, and mass spectral analyses were obtained from Richard Milberg and his staff of the Mass Spectrometry Facility also at the University of Illinois.

1-(β-D-Ribofuranosyl)uracil-6-carboxaldehyde (1a). This compound was prepared according to our previously reported procedure.[6] X-ray quality crystals were grown from an aqueous solution by slow evaporation under a stream of argon at rt. Anal. Calcd for $C_{10}H_{12}N_2O_7$: C, 44.12; H, 4.44; N, 10.29. Found: C, 43.93; H, 4.50; N, 10.07. A close examination of a 500-MHz ^1H NMR spectrum of **1a** in $(CD_3)_2SO$ solution revealed the following ribosyl coupling constants for the major (91%, 5′-cyclic hemiacetal) structure: $^3J_{H1'-H2'} = 4.6$ Hz, $^2J_{H2'-2'OH} = 6.5$ Hz, $^3J_{H2'-H3'} = 4.6$ Hz, $^2J_{H3-3'OH} = 4.2$ Hz, $^3J_{H3'-H4'} = 1.7$ Hz.

1-(2′-Deoxy-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl)uracil-6-carboxaldehyde (7). A solution of **6** (4.2 g, 8.9 mmol) in 50 mL of anhyd THF was added dropwise to a −78 °C solution of freshly prepared LDA (27 mmol) in 50 mL of anhyd THF at 78 °C under argon. After 12 h at −78 °C, HCO_2Et (1 mL, 12.4 mmol) was added all at once, and the reaction mixture was allowed to warm to rt over 1 h. The mixture was quenched by the addition of HOAc (1.54 mL, 27 mmol). Analysis by TLC (2,4-DNP visualization) gave evidence of desired product along with starting material. The reaction mixture was rotary evaporated to give a mixture which was purified by column chromatography followed by radial chromatography (each using 4% CH_3OH/CH_2Cl_2 as eluent) to afford 1.4 g (31%) of **7** as a foam: ^1H NMR ($CDCl_3$) δ 9.73 (s, 1H, CHO), 9.30 (s, exchanges with D_2O, 1H, NH), 6.37 (d of d, $J = 5.4$, 1.5 Hz, 1H, H1′), 6.10 (s, 1H, H5), 4.81–4.74 (m, 1H, H3′), 4.01 (m, 1H, H5′a), 3.97 (m, 1H, H5′b), 3.81 (m, 1H, H4′), 2.68 (m, 1H, H2′a), 2.50 (m, 1H, H2′b), 1.13–1.00 (m, 28H, four $CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 184.3 (CHO), 161.9 (C4), 149.5 and 148.4 (each C2/C6), 109.2 (C5), 85.8 and 85.0 (C1′/C4′), 71.8 (C3′), 62.6 (C5′), 40.2 (C2′), 17.5–16.9 (eight $CH(CH_3)_2$), 13.3–12.5 (four $CH(CH_3)_2$); low-resolution FD-mass spectrum, *m*/*z* 498 (M^+), 499 (MH^+); IR (Nujol, NaCl) ν 1700 cm^{-1} (aldehydic C=O stretch). Anal. Calcd for $C_{22}H_{38}N_2O_7Si_2$: C, 52.98; H, 7.68; N, 5.62; Si, 11.26. Found: C, 52.49; H, 7.89; N, 5.43; Si, 10.99.

1-(2′-Deoxy-β-D-ribofuranosyl)uracil-6-carboxaldehyde (1b). Note: All manipulations in this procedure were conducted at room temperature. A solution of **7** (921 mg, 1.85 mmol) in 20 mL of THF was treated dropwise with tetrabutylammonium fluoride (3.7 mL of a 1 M solution in THF). The reaction mixture was stirred for 1 h at rt and rotary evaporated, and the residue was purified by column chromatography followed by radial chromatography (each using 10% $MeOH/CH_2Cl_2$ as eluent) to give 362 mg (77%) of **1b** as a powder. Low-resolution FAB-mass spectrum (using 3-nitrobenzyl alcohol as matrix), *m*/*z* 257.1 (MH^+). High-resolution FAB-mass spectrum (same matrix), 257.0765 ($C_{10}H_{13}N_2O_6$ requires 257.0774). ^1H NMR spectral analysis of this compound showed it to be >98% pure and revealed that it exists as a 49:1 mixture of a single 5′-cyclonucleoside diastereomer and the free aldehyde in $(CD_3)_2SO$ solution (**A** and **B**, respectively, below), and as a 3:1 mixture of a single 5′-cyclonucleoside diastereomer and the free hydrate in D_2O solution (**C** and **D**, respectively, below).

A (98% component in $(CD_3)_2SO$): ^1H NMR ($(CD_3)_2SO$) δ 11.4 (bs, exchanges with D_2O, 1H, NH), 7.22 (d, $J = 7.2$ Hz, exchanges with D_2O, 1H, hemiacetal-OH), 6.50 (pseudo-t, 1H, H1′), 5.82 (m, 2H, H5, and hemiacetal-CH), 5.21 (d, $J = 3.3$ Hz, exchanges with D_2O, 1H, 3′-OH), 4.22 (m, 1H, H4′), 4.19 (m, 1H, H3′), 3.86 (m, 2H, 5′-CH_2), 2.32 (d of d, $J = 13.8$, 7.2 Hz, 1H, H2′a), 2.13 (d of d of d, 1H, H2′b); ^{13}C NMR ($(CD_3)_2SO$) δ 162.2 (C4), 153.4 and 150.9 (each C2/C6), 99.9 (C5), 94.4 (hemiacetal-CH), 89.8 (C1′), 87.3 (C4′), 74.1 (C3′), 69.1 (C5′), 42.6 (C2′).

B (2% component in $(CD_3)_2SO$): ^1H NMR ($(CD_3)_2SO$) δ 9.86 (s, 1H, CHO).

C (75% component in D_2O): ^1H NMR (D_2O) δ 6.62 (pseudo-t, 1H, H1′), 6.17 (s, 1H, H5), 6.10 (s, 1H, hemiacetal-CH), 4.47 (s, 1H, H4′), 4.45 (s, 1H, H3′), 4.10 (d of d, $J = 13.2$, 4.2 Hz, 1H, H5′a), 4.00 (d, $J = 13.2$ Hz, 1H, H5′b), 2.60 (d of d, $J = 15$, 7.2 Hz, 1H, H2′a), 2.38 (d of d of d, 1H, H2′b); ^{13}C NMR (D_2O) δ 168.0 (C4), 157.3 and 154.7 (each C2/C6), 103.2 (C5), 97.3 (hemiacetal-CH), 93.6 (C1′), 90.2 (C4′), 77.6 (C3′), 72.1 (C5′), 44.5 (C2′).

D (25% component in D_2O): ^1H NMR (D_2O) δ 6.36 (pseudo-t, 1H, H1′), 6.08 (s, 1H, H5), 5.99 (s, 1H, hydrate-CH), 4.53 (s, 1H, H3′), 3.93 (s, 1H, H4′), 3.89–3.73 (m, 2H, 5′-CH_2), 2.32–2.21 (m, 2H, 2′-CH_2); ^{13}C NMR (D_2O) δ 168.4 (C4), 158.8 and 154.1 (each C2/C6), 102.7 (C5), 89.1 (C4′), 88.9 (C1′), 88.7 (hydrate-CH), 73.6 (C3′), 64.5 (C5′), 39.6 (C2′).

Long-range correlations observed in a 10-Hz-optimized ^1H–^{13}C Hetcor experiment were *H*4′/*C*1′ and 5′CH_2/*C*3′ for **A** and *H*3′/*C*1′ and *H*5′a/*C*3′ for **C**.

2′,3′,5′-Tris-*O*-(methoxymethyl)-1-(β-D-arabinofuranosyl)uracil (9). A suspension of **8** (2.00 g, 8.19 mmol) in 1 L of 50% $CH_2(OCH_3)_2/CH_2Cl_2$ under argon was treated with 20 drops (ca. 1.0 mL, 1.4 equiv) of CF_3SO_3H. The reaction mixture was stirred overnight at rt, and then it was quenched by the addition of 10 mL of concd NH_4OH, dried (Na_2SO_4), and rotary-evaporated to an oil. This oil was purified by column chromatography (4% CH_3OH/CH_2Cl_2 as eluent) to afford 1.83 g (60%) of **9** as an oil: ^1H NMR ($CDCl_3$) δ 9.76 (bs, exchanges with D_2O, 1H, NH), 7.63 (d, $J = 8.2$ Hz, 1H, H6), 6.24 (d, $J = 4.8$ Hz, 1H, H1′), 5.71 (d of d, $J = 8.2$, 2.1 Hz, 1H, H5), 4.77 and 4.70 (each d, each $J = 7.1$ Hz, each 1H, CH_2OCH_3), 4.70 (s, 2H, CH_2OCH_3), 4.62 and 4.56 (each d, each $J = 6.6$ Hz, each 1 H, CH_2OCH_3), 4.37 (d of d, $J = 4.8$, 2.6 Hz, 1H, H2′), 4.17–4.12 (m, 2H, H3′ and H4′), 3.79 (d, $J = 5.1$ Hz, 2H, 5′-CH_2), 3.40 (two s, each 3H, two CH_2OCH_3), 3.28 (s, 3H, CH_2OCH_3); ^{13}C NMR ($CDCl_3$) δ 163.5 (C4), 150.3 (C2), 141.8 (C6), 101.0 (C5), 96.6, 96.3, 95.9 (three CH_2OCH_3), 84.7 (C1′), 81.3 and 80.1 (C2′/C3′), 79.1 (C2′), 66.7 (C5′), 55.9, 55.7, and 55.4 (three CH_2OCH_3); low-resolution ACE (alternating CI/EI)-mass spectrum, CI(CH_4), *m*/*z* 377.3 (MH^+). Anal. Calcd for $C_{15}H_{24}N_2O_9$: C, 47.87; H, 6.43; N, 7.44. Found: C, 48.20; H, 6.50; N, 7.09.

2′,3′,5′-Tris-*O*-(methoxymethyl)-1-(β-D-arabinofuranosyl)uracil-6-carboxaldehyde (10). A solution of 1.83 g (4.86 mmol) of **9** in 15 mL of anhyd THF was added dropwise to a solution of freshly-prepared LDA (14.6 mmol) in 15 mL of anhyd THF at −78 °C under argon. After 11 h at −78 °C, HCO_2Et (1 mL, excess) was added all at once, and the reaction mixture was

(29) In vitro antitumor evaluations of **1a–c** using human tumor cell lines are currently being conducted in collaboration with the laboratories of Dr. Linda L. Wotring, University of Michigan, Ann Arbor, MI.

allowed to warm to rt over 1 h. The reaction mixture was quenched by the addition of HOAc (0.84 mL, 14.6 mmol). Analysis by TLC (4% MeOH/CH_2Cl_2 as eluent, 2,4-DNP visualization) gave evidence of an aldehyde product. The reaction mixture was purified by column chromatography followed by radial chromatography (each using 3% MeOH/CH_2Cl_2 containing 0.5% concd NH_4OH as eluent) to afford 476 mg (25%, 30% based upon unrecovered starting material) of pure **10** as an oil: ^{1}H NMR ($CDCl_3$) δ 10.06 (s, 1H, CHO), 6.42 (d, J = 4.5 Hz, 1H, H1'), 6.13 (s, 1H, H5), 4.78 and 4.61 (each d, each J = 6.8 Hz, each 1H, CH_2OCH_3), 4.70 (s, 2H, CH_2OCH_3), 4.71 and 4.69 (each d, each J = 4.2 Hz, each 1H, CH_2OCH_3), 4.41 (d of d, J = 4.8, 1.2 Hz, 1H, H2'), 4.32 (d of d, J = 5.6, 1.1 Hz, 1H, H3'), 4.01 (d of d, J = 8.7, 4.2 Hz, 1H, H4'), 3.82 and 3.76 (each d, each J = 11.0, 1H, H5'a), 3.81 and 3.75 (each d, each J = 11.0, 1H, H5'b), 3.40 (two s, each 3H, two CH_2OCH_3), 3.28 (s, 3H, CH_2OCH_3); ^{13}C NMR ($CDCl_3$) δ 184.7 (CHO), 162.4 (C4), 150.2 and 149.5 (each C2/C6), 104.1 (C5), 96.7, 96.6, and 96.1 (three CH_2OCH_3), 85.7 (C1'), 81.3, 80.7, and 79.9 (C2'/C3'/C4'), 65.6 (C5'), 56.0, 55.7, and 55.4 (three CH_2OCH_3), low-resolution FD-mass spectrum, m/z 404 (M^+), 405 (MH^+); IR (Nujol, NaCl) ν 1698 cm^{-1} (aldehydic C=O stretch). Anal. Calcd for $C_{16}H_{24}N_2O_{10}$: C, 47.52; H, 5.98; N, 6.93. Found: C, 47.14; H, 6.13; N, 6.77.

1-(β-D-Arabinofuranosyl)uracil-6-carboxaldehyde (1c). A solution of **10** (374.2 mg, 0.925 mmol) in 10 mL of 50% aqueous CF_3CO_2H was stirred at rt for 24 h. The reaction mixture was rotary-evaporated, and traces of CF_3CO_2H were removed by repetitive azeotropic rotary evaporation with water (3 × 50 mL). The residue was purified by radial chromatography (20% CH_3OH/CH_2Cl_2 as eluent), and the product was redissolved in 250 mL of water. Lyophilization for 48 h afforded 250 mg (99% of a nonhydrated structure) of product as an off-white powder. Recrystallization from water gave **1c** as a microcrystalline solid: mp 251–253 °C (dec with gas evolution). Low-resolution FD-mass spectrum, m/z 273 (MH^+). Anal. Calcd for $C_{10}H_{12}N_2O_7$: C, 44.12; H, 4.44; N, 10.29. Found: C, 43.90; H, 4.55; N, 10.09. ^{1}H NMR analysis of this compound revealed that it exists as a 3:1 mixture of 2'-cyclonucleoside diastereomers in $(CD_3)_2SO$ solution (**A** and **B**, respectively, below) and as a 2:1 mixture of 2'-cyclonucleoside diastereomers in D_2O solution (**C** and **D**, respectively, below).

A (75% component in $(CD_3)_2SO$): ^{1}H NMR ($(CD_3)_2SO$) δ 11.5 (bs, exchanges with D_2O, 1H, NH) 7.84 (bs, exchanges with D_2O, 1H, hemiacetal-OH), 5.72 (bs, exchanges with D_2O, 1H, 3'-OH), 5.66 (s, 1H, H5), 5.65 (d, J = 3.3 Hz, 1H, H1'), 5.61 (s, 1H, hemiacetal-CH), 4.90 (bs, exchanges with D_2O, 1H, 5'-OH), 4.41 (d, J = 3.3 Hz, 1H, H2'), 4.03 (m, collapses to a d, J = 3.0 Hz, with D_2O, 1H, H3'), 3.66 (m, 1H, H4') 3.46 (m, 2H, 5'-CH_2); ^{13}C NMR ($(CD_3)_2SO$) δ 162.6 (C4), 150.7 and 149.6 (each C2/C6), 99.0 (hemiacetal-CH), 86.9 (C5), 85.2 (C4'), 77.2 (C1'), 75.3 (C2'), 75.1 (C3'), 61.3 (C5').

B (25% component in $(CD_3)_2SO$): ^{1}H NMR ($(CD_3)_2SO$) δ 11.5 (bs, exchanges with D_2O, 1H, NH) 8.08 (bs, exchanges with D_2O, 1H, hemiacetal-OH), 5.72 (bs, exchanges with D_2O, 1H, 3'-OH), 5.61 (s, 1H, H5), 5.49 (d, J = 2.4 Hz, 1H, H1'), 5.46 (s, 1H, hemiacetal-CH), 4.90 (bs, exchanges with D_2O, 1H, 5'-OH), 4.23 (d, J = 2.4 Hz, 1H, H2'), 3.99 (m, collapses to a d, J = 1.8 Hz, with D_2O, 1H, H3'), 3.69 (m, 1H, H4') 3.41 (m, 2H, 5'-CH_2); ^{13}C NMR ($(CD_3)_2SO$) δ 162.5 (C4), 151.1 and 150.8 (each C2/C6), 98.0 (C5), 89.4 (hemiacetal-CH), 86.0 (C4'), 78.4 (C2'), 78.2 (C1'), 75.1 (C3'), 61.9 (C5').

C (67% component in D_2O): ^{1}H NMR (D_2O) δ 5.91 (s, 1H, hemiacetal-CH), 5.89 (s, 1H, H5), 5.84 (d, J = 3.3 Hz, 1H, H1'), 4.68 (d, J = 3.3 Hz, 1H, H2'), 4.29 (m, 1H, H3'), 4.01 (m, 1H, H4') 3.74 (m, 2H, 5'-CH_2); ^{13}C NMR (D_2O) δ 168.1 (C4), 154.6 and 151.9 (each C2/C6), 103.0 (hemiacetal-CH), 89.9 (C5), 87.7 (C4'), 80.9 (C1'), 77.8 (C3'), 77.4 (C2'), 63.9 (C5').

D (33% component in D_2O): ^{1}H NMR (D_2O) δ 6.02 (s, 1H, H5), 5.75 (d, J = 2.7 Hz, 1H, H1'), 5.66 (s, 1H, hemiacetal-CH), 4.48 (d, J = 2.7 Hz, 1H, H2'), 4.31 (m, 1H, H3'), 4.02 (m, 1H, H4') 3.75 (m, 2H, 5'-CH_2). ^{13}C NMR (D_2O) δ 168.0 (C4), 155.1 and 153.7 (each C2/C6), 102.0 (hemiacetal-CH), 92.1 (C5), 88.0 (C4'), 81.6 (C1'), 81.1 (C2'), 78.1 (C3'), 64.3 (C5').

Long-range correlations observed in a 10-Hz-optimized ^{1}H–^{13}C Hetcor experiment were *H*1'/*C*2' for **A** and *H*5/hemiacetal-*C*H for **D**. A 500-MHz ^{1}H/^{1}H ROESY 2D NMR spectrum of **1c** in $(CD_3)_2SO$ solution, obtained in phase-sensitive mode by the method of Kessler et al.[24] revealed the presence of the diagnostic crosspeak at F1/F2 = δ 4.23/5.46 ppm, indicative of an NOE interaction between the H2' and hemiacetal-CH protons for component **B**. For each of the 128 increment fids, 64 transients of 512 data points were collected. The spectral window was 1531.9 Hz, the mixing time was 1 s, and all of the crosspeaks were negative.

^{18}O Isotope Shift Effect Study. A solution of [*formyl*-^{13}*C*]-2',3',5'-tris-*O*-(methoxymethyl)uridine-6-carboxaldehyde[6] (475 mg, 1.17 mmol) in 3.3 mL of 1,4-dioxane-d_8 (0.36 M) was treated with 84 μL of a 1:1 v/v mixture of H_2O and [^{18}*O*]-H_2O (2.33 mmol each; 4.66 mmol, 4.0 equiv total) and was allowed to stand at rt for 64 h. After this period of equilibration, a full-scale (200+ ppm) broadband ^{1}H-decoupled ^{13}C NMR spectrum was recorded at 125 MHz and at 37 ± 5 °C using the 1,4-dioxane-d_8 signal as internal reference (δ = 66.5 ppm). This spectrum revealed two strong signals of ca. 3:1 relative intensity at 186.9 and 87.0 ppm assignable to the aldehyde and hydrate resonances, respectively. Next, separate broadband ^{1}H-decoupled ^{13}C NMR spectra were recorded of the aldehyde and hydrate regions using 64000 data points to describe a spectral window of 1257 Hz width. The raw FIDs, unmodified by any window function, were Fourier transformed. Results are shown in Figure 2. At 0.16 Hz per data point, the error in each measured isotope shift effect was calculated to be ±1 ppb.

Acknowledgment. This work was supported by a grant from the Office of Research Development and Administration at Southern Illinois University. We thank Ms. Colleen M. Lawler for technical assistance.

Supplementary Material Available: Experimental procedures and characterization data for 3',5'-bis-*O*-(methoxymethyl)-2'-deoxyuridine, 3',5'-bis-*O*-(methoxymethyl)-2'-deoxyuridine-6-carboxaldehyde, **6**, 2,2'-anhydrouridine, and **8**; ^{1}H NMR spectral plots of **1b** in $(CD_3)_2SO$ solution (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.