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The 5′,6-Oxomethylene Transglycosidic Tether for Conformational Restriction of Pyrimidine Ribonucleosides. Investigation of 6-Formyl- and 6-(Hydroxymethyl)uridine 5′-Carboxaldehydes

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Abstract—In an effort to develop a new motif for the transglycosidic tethering of the pyrimidine nucleoside framework, the 2′,3′-*O*-isopropylidenated and unprotected versions of 6-formyl- and 6-(hydroxymethyl)uridine 5′-carboxaldehyde were prepared and these were examined for their ability to adopt 5′,6-oxomethylene tethered solution structures. In aqueous solution, the 2′,3′-*O*-isopropylidenated nucleosides readily generated spiro-dihydrouridines via proximity-induced transglycosidic intramolecular reactions. In stark contrast, their unprotected counterparts existed mainly as the untethered aldehyde hydrates. Based on these findings, the 5′,6-oxomethylene transglycosidic tether appears to constitute a useful conformational restriction motif for the pyrimidine ribonucleoside framework, but only when the 5′-OH group is functionalized.

Introduction

Conformational restriction is a powerful means of selecting for a subset of biologically active rotamers at the expense of inactive ones to increase the effectiveness of a biochemical probe or medicinal agent. In the case of the nucleosides, of which uridine and its nucleotides constitute a biologically important subgroup,[1] there are just two freely rotating bonds which determine much of the overall molecular topography. These are the C4′–C5′ bond and the glycosidic one (C1′–N1 for pyrimidines and C1′–N9 for purines). Transglycosidic tethers that simultaneously restrict both of these have been developed in the past, but efforts to date have afforded compounds that are not very biomimetic and therefore are of limited utility as biochemical probes.[2] The desired features of a truly useful transglycosidic nucleoside tethering motif include (1) a restriction of the glycosidic bond rotation in its most often biologically relevant *anti* conformation, (2) a preservation of all of the natural, biorecognition-critical hydrogen bonding capabilities of the heterocyclic aglycon, (3) a retention of all of the natural, bio-functionalizable carbohydrate hydroxyl groups, and (4) a design that is readily applicable to both the pyrimidine and the purine nucleoside frameworks. We have been examining new tethering motifs that could meet all of these criteria.[3] In the present work, we focused attention on the 5′,6-oxomethylene tethering of pyrimidine ribonucleosides and specifically sought to determine whether this tether was compatible with an unprotected 5′-hydroxyl functionality.

Our previous investigations into the unusual structural properties of the 6-formyluracil-based glycofuranosides[4–6] led to the discovery that their formyl group is so highly electrophilic that it imbues these nucleosides with certain carbohydrate-like properties. In specific, several new transglycosidically tethered cyclic hemiacetals were found to be dominant structural forms in solution as well as in the solid state. For example, the ‘free’ aldehyde form of the parent uridine-6-carboxaldehyde accounts for only 10% of the material in $(CD_3)_2SO$ solution—the remainder is a single 5′-cyclic hemiacetal diastereomer. In D_2O, the aldehyde cannot be detected at all by ^1H NMR. Instead, in this solvent this nucleoside exists as a 2:1 mixture of its hydrate and the same 5′-cyclic hemiacetal diastereomer that predominates in $(CD_3)_2SO$. Finally, 6-formyluridine crystallizes out of water exclusively as a 5′-cyclic hemiacetal diastereomer.[5]

Keywords: nucleosides; uridines; aldehydes; cyclisation; acylals conformation; transglycosidic; tethered.
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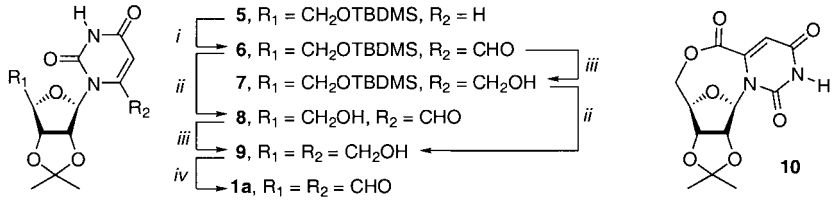
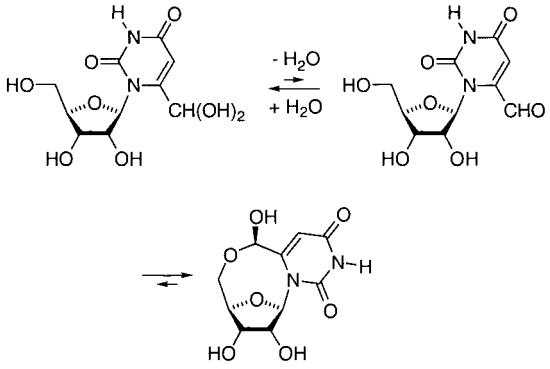
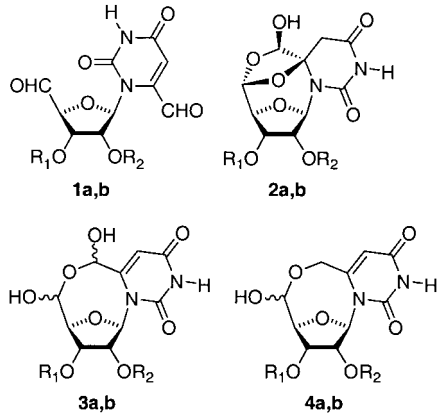


Scheme 1. *Reagents and conditions*: (i) 2.5 equiv. LDA, −78°C, then HCO_2Et, −78°C; (ii) TBAF, THF; (iii) $NaBH_4$, EtOH; (iv) Dess–Martin periodinane.



The preponderance of transglycosidically tethered structures displayed by the 6-formyluridines reveals that the 5′,6-oxomethylene tether is energetically favored when attached to a uridine nucleoside platform. With this as our starting point, we began to explore variations on this tether motif that could lead to more biochemically relevant structures. In particular, we were interested in regaining the 5′-OH group that had been rendered 'absent' during the facile 5′-cyclic hemiacetal formations encountered in our earlier studies. Interestingly, the same facile covalent hydrate formation that is endemic to the 6-formyluridines has long been known to be a property of the nucleoside 5′-carboxaldehydes as well,[7] albeit to a lesser degree. We reasoned that if *both* of these hydration-prone aldehydes were present in the same molecule, then a single bridging hydration might occur to produce a new transglycosidically tethered structure possessing the desired 5′-OH group (i.e. **3**, shown below). To explore this possibility, we chose to prepare the 6-formyluridine 5′-carboxaldehydes **1a**,**b**. It was essential that the 2′,3′-*O*-isopropylidene-equipped **1a** be studied in addition to its unprotected counterpart **1b**, since 2′,3′-*O*-methylidene-based protecting groups are known to enhance transglycosidic reactivity in nucleosides



a: R_1—R_2 = CMe_2; **b**: R_1 = R_2 = H

by inducing a proximity effect.[8] Indeed, we encountered the unusual spiro-fused dihydrouridine **2a** very early on in our investigation of **1a**,[9] and can now with certainty attribute its ready formation to the presence of the 2′,3′-*O*-isopropylidene group.

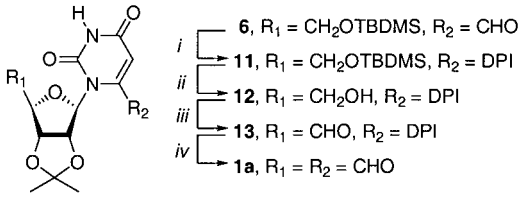
The objectives of the present investigation, then, were to develop a reasonable synthesis of the carbohydrate protected 6-formyluridine 5′-carboxaldehyde **1a**, to deprotect it to **1b**, and to characterize the solution structures displayed by these two dialdehydes—whether they be the spiro-dihydrouridines **2a**,**b**, the transglycosidically tethered monohydrates **3a**,**b**, or simply the untethered dihydrates. An additional objective was to prepare and study the analogously protected and unprotected versions of 6-(hydroxymethyl)uridine 5′-carboxaldehyde, again searching for 5′-hydroxyl-containing structural forms (i.e. hemiacetals **4a**,**b**) among the solution structures.

Results and Discussion

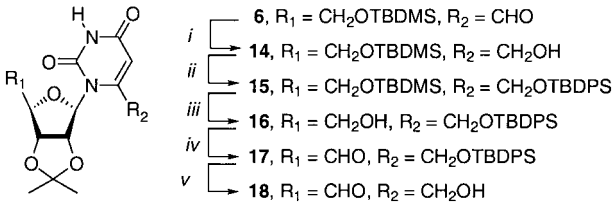
Our first synthesis of dialdehyde **1a** was short but not inefficient. The previously reported[7,10] 2′,3′-*O*-isopropylideneuridine 5′-carboxaldehyde was converted to its DPI (1,3-diphenylimidazolidin-2-yl) derivative, and this was then subjected to Miyasaka's conditions for uridin-3,6-diyl dilithium generation.[11] Perhaps due to the bulk of the DPI group, this dianion formation was problematic and a quench with HCO_2Et gave the 6-carboxaldehyde only in a very poor yield. We did manage to obtain **1a** by subsequent DPI group removal, but we immediately sought other routes.

Our second route to **1a** (Scheme 1) was based on the expectation that each of the hydroxymethyl groups in 6-(hydroxymethyl)-2′,3′-*O*-isopropylideneuridine (**9**) could be oxidized to aldehydes in a single operation. When an attempt to formylate the purported[12] trianion of 2′,3′-*O*-isopropylideneuridine failed to provide **8**, we prepared it instead by desilylating (49%) 6-formyl-5′-*O*-TBDMS-2′,3′-*O*-isopropylideneuridine (**6**),[12] itself obtained from the known[6] protected uridine **5** by a Miyasaka dianion-based formylation. The hydroxy-aldehyde **8**, accessed by others along a different route,[13] is carbohydrate-like in that it exists almost exclusively in cyclic hemiacetal form in $(CD_3)_2SO$. Thus, not surprisingly, our attempts to oxidize **8** directly to **1a** were unsuccessful. Aldehyde **8** gave the lactone **10** (52%) under Moffatt conditions,[14] but it was found to be inert to the Dess–Martin periodinane oxidant.[15]

Therefore, we reduced nucleoside **8** to the diol **9**, which we subsequently found could also be prepared by reducing



Scheme 2. *Reagents and conditions*: (i) $(PhNHCH_2)_2$, cat. AcOH; (ii) TBAF, THF; (iii) Dess–Martin periodinane; (iv) TsOH, Me_2CO/CH_2Cl_2.



Scheme 3. *Reagents and conditions*: (i) $NaBH_4$, EtOH; (ii) TBDPS-Cl, imidazole, DMF; (iii) PPTS, EtOH; (iv) Dess–Martin periodinane, then $Na_2S_2O_3$; (v) TBAF, THF.

aldehyde **6** and then desilylating the product alcohol **7** (70% overall yield). Diol **9** was oxidized with excess Dess–Martin periodinane, and the reaction mixture was immediately separated by a silica gel chromatography employing an alcohol-free eluent (Me_2CO/CH_2Cl_2) to protect the assured alcohol-reactive **1a**. This gave a chromatographically homogeneous ternary mixture of **1a**, **8** (as the hemiacetal), and an unknown periodinane-functionalized nucleoside, by ^1H NMR. Exposure of this mixture to $Na_2S_2O_3$ in saturated aqueous $NaHCO_3$[9,10] removed the latter component, and another chromatographic separation afforded a mixture of **1a** and **8** (18% combined yield) and the same C6 spiro-fused nucleoside **2a** (7%) later discovered to spontaneously arise from **1a** (see below).

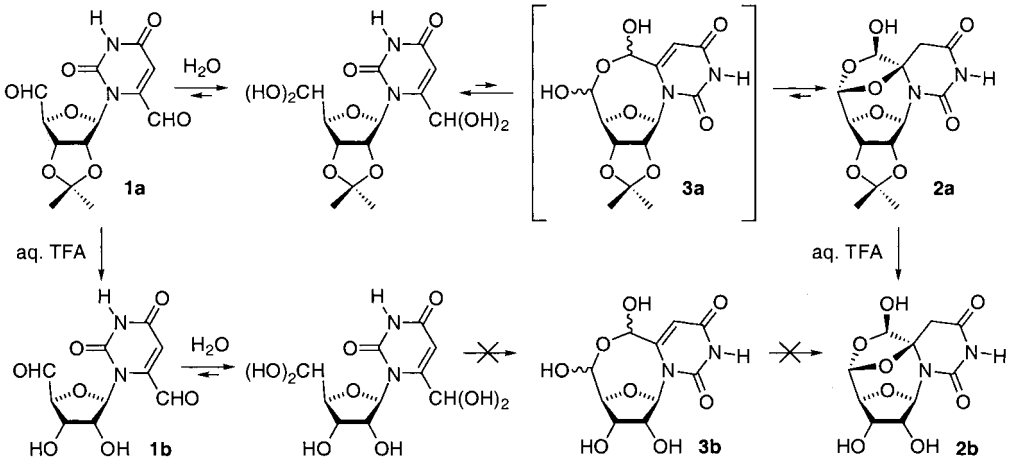
Scheme 2 shows our third and best route to **1a**. The aldehyde **6** from above was converted to its DPI derivative (73%) and the resulting protected nucleoside **11** was desilylated to the alcohol **12** (94%). Oxidation of **12** with the Dess–Martin periodinane gave the aldehyde **13** (94%), which was deprotected to **1a** (60%).

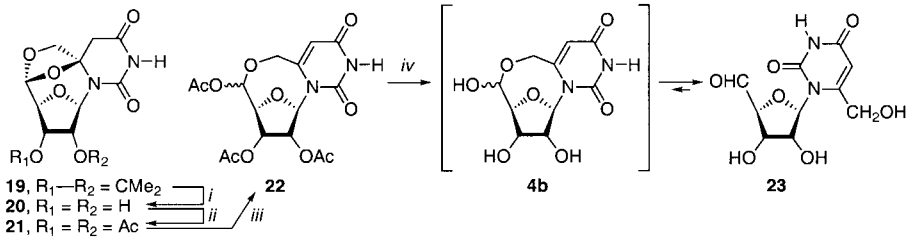
Dialdehyde **1a** immediately and exclusively forms a dihydrate when dissolved water, by ^1H NMR. By both ^1H NMR and UV, this dihydrate gives rise to the structurally-unusual C6 spiro-fused dihydrouridine **2a**[9] in a moderately facile transformation ($t_{1/2}$~2 h at 23°C) that most likely proceeds through the bridged hydrate **3a** as a transient intermediate. Deprotection of **1a** with aqueous TFA gave **1b**, but this too failed to form a bridged monohydrate structure (**3b**). Interestingly, it also did not undergo transformation to the unprotected spiro-fused nucleoside **2b**, which we obtained separately by deprotecting **2a**. The striking difference in reactivity between **1a** and **1b** clearly shows that the 2′,3′-*O*-isopropylidene group in **1a** is essential for the trans-glycosidic reactions leading to **2a**. Likely, it facilitates these by rendering the C1′–N1 and C4′–C5′ bonds closer to coparallel from within an O4′-*exo* ($_4$E) ribosyl conformational form.[16]

The fact that dialdehyde **1b** does not exist even partly in bridged monohydrate form (**3b**) is in stark contrast to the behavior of uridine-6-carboxaldehyde itself, which readily adopts a 5′-cyclic hemiacetal form in both solution and in the solid state.[4–6] We suspect that it is the 5′-hemiacetal portion of **3b** which is particularly labile.

Turning to the 6-(hydroxymethyl)uridine 5′-carboxaldehydes that might possibly exist as the 5′-OH-equipped cyclic hemiacetals **4a**,**b**, we synthesized 6-(hydroxymethyl)-2′,3′-*O*-isopropylideneuridine 5′-carboxaldehyde (**18**) by the route shown in Scheme 3. Aldehyde **6** was reduced with $NaBH_4$ to the alcohol **14** (76%), and this was then protected with a TBDPS group (86%) to give **15**, which in turn was regioselectively deprotected (97%) to the alcohol **16** for a Dess–Martin periodinane-mediated oxidation (80%) to aldehyde **17**. Deprotection of **17** with TBAF gave the 2,4-DNP-positive aldehyde **18** by TLC, but this proved to have a fleeting existence.

Because the TBAF deprotection conditions had produced the alcohol as its alkoxide, **18** underwent a very rapid spiro dioxolane ring assembly ($t_{1/2}$~a few minutes). The spiro-fused product **19**, the 7-deoxy congener of **2a**, was isolated in a 55% yield, and this was deprotected (aq. TFA, 62%) to give the stable spiro dihydrouridine **20** (Scheme 4). Under mild acetylating conditions (Ac_2O, pyridine, 0°C for 15 min, 25°C for 1.5 h), **20** gave the diacetate **21**, and under slightly more forcing conditions





Scheme 4. *Reagents and conditions*: (i) aq. TFA; (ii) Ac_2O, pyridine, 23°C; (iii) Ac_2O, pyridine, 65°C; (iv) MeOH, cat. KCN, 23°C.

(65°C, 6.5 h) **21** in turn underwent an acylative ring opening to give the stable triacetate **22**. Nucleoside **22**, the 5′,6-oxomethylene tethered version of 2′,3′,5′-tri-*O*-acetyluridine, was isolated as a 7:3 mixture of diastereomers (90% yield from **20**).

Applying the mild conditions developed by Nudelman's group for the deacylation of carbohydrates (abs. MeOH, cat. KCN),[17] triacetate **22** was smoothly deprotected (88%) within minutes directly to the aldehyde **23**, which was generated under these conditions in its 5′-methyl hemiacetal form. Just as with **1b**, **23** exhibited no tendency whatsoever toward existing in cyclic hemiacetal form. Only the aldehyde hydrate was detected in D_2O solution, by ^1H NMR.

Conclusions

Our results reveal some of the consequences of attaching a 5′,6-oxomethylene transglycosidic tether to the pyrimidine ribonucleoside framework. When the 5′-hydroxyl group is functionalized as it is in **22**, this type of tether can be used to create stable conformationally restricted uridines. Once an unprotected 5′-OH functionality is revealed, though, the formation of a spirodihydrouridine is energetically favored if a 2′,3′-*O*-methylidene-based protecting group is present, as was seen in the facile transformations **1a→2a** and **18→19**. Finally, without such a 2′,3′-*O*-cyclic protecting group to facilitate spirodihydrouridine formation, it is simply the untethered, 'open' hydroxy-aldehyde that becomes favored, as was seen with **1b** and **23**. Thus, while an inherent lability renders the 5′,6-oxomethylene tethered uridine ribonucleo*side* derivatives of little practical value, the corresponding 5′-ribonucleo*tide* derivatives should be nicely stable. Work along these lines is in progress.

Experimental

General procedures

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, and TLC analyses were performed on Analtech 250 μm silica gel GF Uniplates. 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 Varian VXR-500 (500 and 125 MHz) instrument. These spectra were recorded with $(CH_3)_4Si$ or 2,2-dimethyl-2-silapentane-5-sulfonic acid, sodium salt (DSS) (δ=0.0 for ^1H), and $CDCl_3$ (δ=77.0 for ^{13}C), $(CD_3)_2SO$ (δ=39.5 for ^{13}C), or 1,4-dioxane (δ=66.5 for ^{13}C in D_2O) as internal reference. ^1H and ^{13}C NMR spectral assignments were made with the assistance of ^1H–^1H homonuclear shift correlation (COSY) and ^1H–^{13}C heteronuclear shift correlation (HETCOR) 2D NMR spectroscopic analyses. Except where noted, the purity of compounds was shown to be >95% by TLC and high-field ^1H NMR. UV spectra were recorded on a Shimadzu UV-160U spectrophotometer. BuLi, iPr_2NH, HCO_2Et, $(PhNHCH_2)_2$, 2-iodobenzoic acid, $KBrO_3$, TsOH, $NaBH_4$, 2,2′-biquinoline, anhydrous *i*BuOH, and 1 M TBAF in THF solution were purchased from the Aldrich Chemical Co. TBDMS-Cl and TBDPS-Cl were obtained from Hüls America, Inc. The BuLi was titrated by the modified Watson-Eastham procedure.[18] THF and Et_2O were dried by distillation from Na-benzophenone ketyl under argon. Pyridine and iPr_2NH were dried by distillation from CaH_2 under argon. The HCO_2Et was dried by distillation from P_2O_5 under argon. Dowex-50 (H^+ form) was obtained from the Sigma Chemical Co., and before use was washed with 1N HCl and then rinsed with distilled water until pH neutral. The Dess–Martin periodinane reagent was prepared according to the literature procedure.[15] Elemental microanalyses and mass spectral analyses were obtained from the University of Illinois.

5′-*O*-(*tert*-Butyldimethylsilyl)-6-(hydroxymethyl)-2′,3′-*O*-isopropylideneuridine (7). A solution of **6**[6] (3.04 g, 7.13 mmol) in 90 mL of THF was added in portions to a stirred suspension of $NaBH_4$ in 75 mL of EtOH at 23°C. The reaction mixture was stirred for 40 min, and then it was concentrated in vacuo to a residue that was separated by column chromatography (5% MeOH/CH_2Cl_2 as eluent) to give 2.29 g (76%) of **7** as a foam: mp 91–93°C. ^1H NMR ($CDCl_3$) δ 10.2 (bs, 1), 5.82 (s, 1), 5.80 (s, 1), 5.19 (d, 1), 4.80 (dd, 1), 4.53 (bs, 2), 4.29 (bs, 1), 4.15 (m, 1), 3.87 (m, 2), 1.55 (s, 3), 1.34 (s, 3), 0.90 (s, 9), 0.08 (s, 6). ^{13}C NMR ($CDCl_3$) δ 164.1, 155.5, 150.4, 113.9, 101.2, 91.1, 89.4, 84.2, 81.6, 64.2, 60.5, 27.1, 25.2, 25.9, 18.4. Low-resolution EI-mass spectrum, *m/e* 413.2 (M^+-Me), 371.2 [100%, (M^+-CMe_3)]. Low-resolution CI-mass spectrum, *m/e* 430.2 (M^++2), 413.2 (M^+-Me), 371.2 [95%, (M^+-CMe_3)].

6-Formyl-2′,3′-*O*-isopropylideneuridine (8). A solution of **7** (427 mg, 1.00 mmol) in 5 mL of anhydrous THF was treated dropwise with 1.1 mL of a 1.0 M solution of TBAF

in THF and the reaction mixture was stirred at 23°C for 24 h. The white precipitate produced exhibited a ^{1}H NMR spectrum consistent with that expected for the O5′-tetrabutylammonium salt of **8**, it was collected by filtration and was rinsed with fresh anhydrous THF and CH_2Cl_2. The combined filtrate and washings were rotary evaporated at 40–50°C in vacuo to an oil. This oil was combined with the solid from above and the whole was dissolved in a small amount of 5% MeOH/CH_2Cl_2. Radial chromatographic separation (same solvent system as eluent) gave 153 mg (49%) of a material that was found to exist as a mixture of (7*R*)-7-hydroxy-2′,3′-*O*-isopropylidene-O5′,6-methanouridine [**8**, 7(*R*),O5′-hemiacetal], its (7*S*)-diastereomer counterpart, and aldehyde **8** by ^{1}H NMR analysis of a freshly prepared $(CD_3)_2SO$ solution. After 3 d, the minor (7*S*)-diastereomer was absent, by ^{1}H NMR. 7(*R*),O5′-Hemiacetal of **8**: ^{1}H NMR [$(CD_3)_2SO$] δ 11.4 (bs, 1H, NH), 7.32 (d, J=6.6 Hz, 1H, 7-OH), 6.28 (d, J=1.2 Hz, 1H, H1′), 5.82 (s, 1H, H5), 5.74 (d, J=6.6 Hz, 1H, H7), 4.74 (d, J=6.3 Hz, 1H, H3′), 4.68 (dd, J=6.3, 1.2 Hz, 1H, H2′), 4.60 (d, J=3.0 Hz, 1H, H4′), 3.99 (d, J=12.9 Hz, 1H, H5′R), 3.83 (dd, J=12.9, 3.0 Hz, 1H, H5′S), 1.45 and 1.27 (each s, each 3H, each Me). 7(*S*),O5′-Hemiacetal of **8**: ^{1}H NMR ($CDCl_3$) δ 7.65 (d, 1H, 7-OH), 6.36 (d, 1H, H1′). Aldehyde **8**: ^{1}H NMR ($CDCl_3$) δ 9.52 (s, 1H, CHO).

6-(Hydroxymethyl)-2′,3′-*O*-isopropylideneuridine (9). A solution of **7** (171 mg, 0.40 mmol) in 2 mL of THF was treated with 0.40 mL of a 1.0 M solution of TBAF (0.40 mmol) in THF. The reaction mixture was stirred at 23°C for 6 h and then was concentrated in vacuo. Radial chromatographic separation of the residue (10% MeOH/CH_2Cl_2) afforded 112 mg (89%) of **9** as a hygroscopic foam identical by ^{1}H NMR to that obtained in the following procedure.

A suspension of **8** (94 mg, 0.3 mmol) in 3 mL of anhydrous EtOH was treated with $NaBH_4$ (39 mg, 1 mmol). After a few min, the suspension was replaced by a clear solution. The mixture was then concentrated by rotary evaporation in vacuo, and the residue was purified by radial chromatography (10% MeOH/CH_2Cl_2 as eluent) to give 66 mg (70%) of **9** as a hygroscopic foam: ^{1}H NMR [$(CD_3)_2SO$] δ 11.40 (bs, exchanges upon addition of D_2O, 1H, NH), 5.85 (t, exchanges upon addition of D_2O, 1H, 7-OH), 5.74 (s, 1H, H1′ or H5), 5.69 (s, 1H, H1′ or H5), 5.19 (d, 1H, H2′), 4.81 (t, exchanges upon addition of D_2O, 1H, 5′-OH), 4.74 (dd, 1H, H3′), 4.34 (m, 2H, 6-CH_2OH), 4.94 (dd, 1H, H4′), 3.56–3.33 (m, 2H, 5′-CH_2), 1.48 and 1.25 (each s, each 3H, Me); $^3J_{1'-2'}$=0.0 Hz, $^3J_{2'-3'}$=6.4 Hz, $^3J_{3'-4'}$=0.0 Hz, $^3J_{5'-5'\text{-OH}}$=5.8 Hz, $^3J_{7-7\text{-OH}}$=5.6 Hz.

Oxidation of 9. A solution of **9** (168 mg, 0.53 mmol) in 10 mL of anhydrous MeCN was added to a suspension of Dess–Martin periodinane reagent[15] (359 mg, 1.28 mmol) in 3 mL of the same solvent. The reaction mixture was stirred at 23°C for 3.5 h, after which time the starting material had been consumed, by TLC analysis. The resulting mixture was rotary evaporated to dryness, and the residue purified by radial chromatography (60% Me_2CO/CH_2Cl_2 as eluent) to afford an inseparable mixture consisting of dialdehyde **1a**, hemiacetal **8**, and an unknown periodinane-derived species. When attempts at further purification by repeated chromatography failed, the periodinane-derived species was removed by dissolving the mixture in excess satd. aq. $NaHCO_3$ containing $Na_2S_2O_3$,[15,19] followed by a chromatographic separation as described above. This gave a 30 mg sample of a binary mixture of **1a** and **8**, and a 12 mg sample of a nucleoside subsequently identified as spiro **2a** by ^{1}H, ^{1}H–^{1}H COSY, ^{1}H-coupled and -decoupled ^{13}C, and short-range ^{1}H–^{13}C HETCOR NMR, low and high resolution mass spectral, and X-ray crystallographic analyses.

2′,3′-*O*-Isopropylideneorotidine 5′-lactone (10). A solution of **8** (312 mg, 1.0 mmol) and DCC (0.8 g, 3.9 mmol) in 10 mL of anhydrous DMSO was treated with dry pyridine (0.1 mL) and TFA (0.05 mL), and the resulting mixture was stirred at 23°C for 50 h. Water (1 mL) was then added and the mixture was stirred for additional 0.5 h. The precipitated dicyclohexylurea was removed by suction filtration, and the filtrate was evaporated to dryness at 50°C in vacuo. Radial chromatography (5% MeOH/CH_2Cl_2 as eluent) gave 161 mg (52%) of lactone **10** as a white solid: mp 270–275°C (dec.). ^{1}H NMR ($CDCl_3$) δ 8.32 (bs, 1H, NH), 6.08 (s, 1H, H5), 5.98 (d, 1H, H1′), 5.02 (m, 1H, H5′), 4.97 (dd, 1H, H2′), 4.78 (d, 1H, H3′), 4.72 (m, 1H, H4′), 4.25 (m, 1H, H5′), 1.58 and 1.37 (each s, each 3H, each Me). ^{1}H NMR [$(CD_3)_2SO$] δ 11.74 (bs, 1H, NH), 5.76 (s, 1H, H5), 5.73 (d, 1H, H1′), 5.11 (d, 1H, H5′), 4.68 (t, 1H, H3′), 4.63 (t, 1H, H2′),4.55 (dd, 1H, H4′), 4.17 (t, 1H, H5″), 1.45 (s, 3H, Me), 1.28 (s, 3H, Me). ^{13}C NMR [$(CD_3)_2SO$] δ 165.5 (C7), 162.8 (C4), 148.7 (C2), 143.1 (C6), 112.0 (C5 or *C*Me_2), 103.0 (C5 or *C*Me_2), 95.0, 86.4, 85.4, and 79.7 (each C1′, C2′, C3′, or C4′), 66.2 (C5′), 26.3, and 24.7 (CMe_2). Low-resolution ACE-mass spectrum, *m/e* 310.2 (M^+), 311.2 (MH^+).

5′-*O*-(*tert*-Butyldimethylsilyl)-6-(1,3-diphenylimidazolidin-2-yl)-2′,3′-*O*-isopropylideneuridine (11). A solution of **6** (427 mg, 1.0 mmol) in 10 mL of dry CH_2Cl_2 was treated with a solution of $(PhNHCH_2)_2$ (910 mg, 4.3 mmol) in dry diethyl ether (5 mL) and 0.12 mL of glacial acetic acid. The reaction mixture was stirred at 23°C for 3 d and then was partitioned between saturated aqueous $NaHCO_3$ and CH_2Cl_2. The layers were separated, and the aqueous phase was extracted with fresh CH_2Cl_2. The organic solutions were combined, dried over $MgSO_4$, and then rotary evaporated to dryness. Column chromatography (2.5% MeOH/CH_2Cl_2 as eluent) gave 453 mg (73%, 98% based upon unrecovered starting material) of **11**: ^{1}H NMR ($CDCl_3$) δ 9.82 (bs, 1H, exchanges upon addition of D_2O, NH), 7.31–6.60 (m, 10H, two Ph), 5.86 (d, 1H, H1′), 5.85 (s, 1H, H5 or H7), 5.83 (s, 1H, H5 or H7), 5.27 (dd, 1H, H2′), 5.72 (dd, 1H, H3′), 4.00 (m, 1H, H4′), 3.81 (m, 2H, 5′-CH_2), 3.73–3.44 (m, 4H, NCH_2CH_2N), 1.25 and 1.02 (each s, each 3H, each Me), 0.89 (s, 9H, CMe_3), 0.07 and 0.50 (each s, each 3H, $SiMe_2$); $^3J_{1'-2'}$=1.5 Hz, $^3J_{2'-3'}$=6.6 Hz, $^3J_{3'-4'}$=4.2 Hz, $^3J_{4'-5'}$=6.9 Hz. ^{13}C NMR ($CDCl_3$) δ 163.7 (C4), 153.5, 151.4, 148.6, 145.4, 130.0, 129.8, 123.6, 120.4, 120.0, 114.6, 113.7, 102.4, 92.5, 89.8, 84.2, 82.6, 77.8, 64.7 (C5′), 52.1 and 47.1 (NCH_2CH_2N), 26.9 and 26.3 (CMe_2), 26.3 (CMe_3), 18.9 ($SiMe_2$). LR-EIMS, *m/e* 620.5 (M^+); LR-CIMS, *m/e* 621.4 (MH^+).

6-(1,3-Diphenylimidazolidin-2-yl)-2′,3′-*O*-isopropylideneuridine (12). A solution of **11** (336 mg, 0.54 mmol) in

2 mL of anhydrous THF was treated with 0.6 mL of 1.0 M TBAF in THF solution. The reaction mixture was stirred at 23°C for 36 h, and then the solvents were removed by rotary evaporation in vacuo. The residue was purified by radial chromatography (5% MeOH/CH_2Cl_2 as eluent) to afford 253 mg (94%) of **12** as a foam: ^{1}H NMR ($CDCl_3$) δ 10.38 (bs, exchanges upon addition of D_2O, 1H, NH), 7.22–6.68 (m, 10H, two Ph), 5.87 (s, 1H, H5 or H7), 5.79 (s, 1H, H5 or H7), 5.77 (d, 1H, H1′), 5.28 (dd, 1H, H2′), 4.97 (dd, 1H, H3′), 4.01 (dd, 1H, H4′), 3.92–3.70 (m, 2H, 5′-CH_2), 3.70–3.40 (m, 5H, NCH_2CH_2N and 5′-OH), 1.25 and 1.02 (each s, each 3H, each Me); $^3J_{1'-2'}$=2.4 Hz, $^3J_{2'-3'}$=6.9 Hz, $^3J_{3'-4'}$=3.9 Hz, $^3J_{4'-5'}$=2.7 Hz, $^3J_{5'-5''}$=12.0 Hz. ^{13}C NMR ($CDCl_3$) δ 163.0 (C4), 152.5, 151.8, 146.9, 146.0, 129.5, 129.3, 121.8, 120.8, 117.8, 116.1, 113.8, 102.5, 92.1, 87.4, 83.0, 80.4, 76.2, 62.5 (C5′), 50.1 and 48.1 (NCH_2CH_2N), 26.4 and 25.3 (CMe_2). LR-EIMS, *m/e* 506.3 (M^+); LR-CIMS, *m/e* 507.3 (MH^+).

6-(1,3-Diphenylimidazolidin-2-yl)-2′,3′-*O*-isopropylideneuridine 5′-carboxaldehyde (13). A solution of **12** (251 mg, 0.50 mmol) in 2 mL of CH_2Cl_2 was added to a suspension of the Dess–Martin periodinane (315 mg, 0.75 mmol) in 3 mL of CH_2Cl_2 at 23°C. The mixture was stirred for 1 h, and then diethyl ether (13 mL) and a solution of $Na_2S_2O_3{\cdot}5H_2O$ (1.30 g, 5.22 mmol) in 20 mL of saturated aqueous $NaHCO_3$ were added. The two layers were separated, and the aqueous phase was extracted with fresh CH_2Cl_2. The organic solutions were combined, dried over $MgSO_4$, and then rotary evaporated to dryness. The residue was purified by radial chromatography (2:1 EtOAc/hexanes as eluent) to afford 235 mg (94%) of **13** as a foam: ^{1}H NMR ($CDCl_3$) δ 10.15 (bs, exchanges upon addition of D_2O, 1H, NH), 9.42 (s, 1H, CHO), 7.35–6.65 (m, 10H, two Ph), 6.05, 5.89, and 5.85 (each s, each 1H, each H1′, H5, or H7), 5.10 and 4.16 (each d, each 1H, H2′ and H3′), 4.42 (s, 1H, H4′), 3.75–3.45 (m, 4H, NCH_2CH_2N), 1.27 and 0.99 (each s, each 3H, each Me); $^3J_{1'-2'}$=0.0 Hz, $^3J_{2'-3'}$=6.9 Hz, $^3J_{3'-4'}$=0.0 Hz. ^{13}C NMR ($CDCl_3$) δ 199.7 (CHO), 163.1 (C4), 152.4, 151.8, 147.5, 145.3, 129.5, 129.3, 122.7, 120.1, 119.0, 114.8, 112.8, 102.6, 94.1, 93.8, 84.8, 84.0, 76.1, 50.9 and 47.4 (NCH_2CH_2N), 25.5 and 25.3 (CMe_2). LR-EIMS, *m/e* 504.3 (M^+); LR-CIMS, *m/e* 505.3 (MH^+).

6-Formyl-2′,3′-*O*-isopropylideneuridine 5′-carboxaldehyde (1a). A solution of **13** (285 mg, 0.5 mmol) in 17 mL of CH_2Cl_2 was treated with a solution of $TsOH{\cdot}H_2O$ in 8 mL of Me_2CO, and the reaction mixture was stirred at 23°C for 40 min. $NaHCO_3$ (250 mg, 3.0 mmol) was then added, and the suspension obtained was stirred for 5 min before the mixture was filtered and the solid was rinsed with fresh Me_2CO and CH_2Cl_2. The combined filtrate and washings were then dried ($MgSO_4$) and evaporated to dryness. The residue was dissolved in a small amount of Me_2CO and purified by radial chromatography (3:2 Me_2CO/CH_2Cl_2 as eluent). The product isolated was nearly pure but contained small amounts of hemiacetal and/or hydrate species, by ^{1}H NMR spectral analysis. Abderhalden drying (P_2O_5, 78°C) in vacuo overnight gave 93 mg (60%) of pure dialdehyde **1a**: ^{1}H NMR [$(CD_3)_2SO$] δ 12.0 (bs, exchanges upon addition of D_2O, 1H, NH), 9.53 (s, 1H, CHO), 9.44 (s, 1H, CHO), 6.58 (s, 1H, H1′), 6.53 (s, 1H, H5), 5.06 (d, 1H, H2′ or H3′), 4.97 (d, 1H, H2′ or H3′), 4.50 (s, 1H, H4′), 1.45 and 1.29 (each s, each 3H, each Me); $^3J_{1'-2'}$=0.0 Hz, $^3J_{2'-3'}$=6.0 Hz. ^{1}H NMR ($CDCl_3$) δ 10.5 (bs, exchanges upon addition of D_2O, 1H, NH), 9.60 (s, 1H, CHO), 9.42 (s, 1H, CHO), 9.2 (bs, exchanges upon addition of D_2O, 1H, NH), 6.75 (s, 1H, H5), 6.34 (s, 1H, H1′), 5.22 (dd, 1H, H2′), 5.08 (d, 1H, H3′), 5.55 (d, 1H, H4′), 1.55 and 1.36 (each s, each 3H, each Me); $^3J_{1'-2'}$=1.5 Hz, $^3J_{2'-3'}$=6.3 Hz, $^3J_{3'-4'}$=1.5 Hz. ^{13}C NMR [$(CD_3)_2SO$] δ 201.1 (CHO), 188.0 (CHO), 162.6 (C4), 151.5 (C2 or C6), 146.7 (C2 or C6), 114.4 (C5), 111.9 (CMe_2), 93.3 (C4′), 92.0 (C1′), 85.0 (C2′ or C3′), 84.0 (C2′ or C3′), 26.3 and 24.6 (CMe_2). LR-EIMS, *m/e* 310.1 (M^+); LR-CIMS, *m/e* 311.1 (MH^+). UV λ_{max}, nm ($\epsilon\times10^{-3}$): (H_2O) 261 (8.9), 204 (9.2). Anal. Calcd for $C_{13}H_{14}N_2O_7$: C, 50.33; H, 4.55; N, 9.03. Found: C, 50.20; H, 4.40; N, 8.98. **1a**, 5′-dimethyl acetal: ^{1}H NMR ($CDCl_3$) δ 9.58 (s, 1H, H7), 9.42 (bs, exchanges upon addition of D_2O, 1H, NH), 6.60 (s, 1H, H5 or H1′), 6.25 (s, 1H, H5 or H1′), 5.22 (d, 1H, H2′), 5.00 (dd, 1H, H3′), 4.67 (d, 1H, H5′), 4.25 (dd, 1H, H4′), 3.48 and 3.35 (each s, each 3H, $C(OMe)_2$), 1.58 and 1.38 (each s, each 3H, each Me); $^3J_{1'-2'}$=0.0 Hz, $^3J_{2'-3'}$=6.6 Hz, $^3J_{3'-4'}$=4.3 Hz, $^3J_{4'-5'}$=7.5 Hz. LR-EIMS, *m/e* 341.2 [$(M-Me)^+$]; LR-CIMS, *m/e* 357.2 (MH^+).

A freshly prepared sample of pure **1a** in D_2O solution revealed NMR spectral features consistent with the simple dihydrate: ^{1}H NMR (D_2O) δ 6.32 (s, 1H, H1′, H5, or H7), 6.07 (s, 1H, H1′, H5, or H7), 5.95 (s, 1H, H1′, H5, or H7), 5.37 (d, 1H, H2′), 5.10 (d, 1H, H5′), 5.03 (dd, 1H, H3′), 5.93 (dd, 1H, H4′), 1.60 and 1.75 (each s, each 3H, each Me); $^3J_{1'-2'}$ not well resolved, $^3J_{2'-3'}$=3.9 Hz, $^3J_{3'-4'}$=4.5 Hz, $^3J_{4'-5'}$=7.5 Hz. ^{13}C NMR (D_2O) δ 165.7 (C4), 155.3 (C2), 151.7 (C6), 114.5 (CMe_2), 100.2 (C5), 91.4 (C1′), 89.9 (C4′), 89.7 (C5′), 86.3 (C7), 84.2 (C2′), 81.7 (C3′), 26.0 and 24.3 (CMe_2).

6-Formyluridine 5′-carboxaldehyde (1b). A solution of **1a** (51 mg, 0.17 mmol) in 50% aqueous TFA (1 mL) was stirred at 23°C for 2 h. The resulting solution was evaporated to dryness, and residual TFA was removed from the residue by repetitive azeotropic coevaporation with water. Lyophilization afforded essentially pure **1b** in quantitative yield: 210–220°C (dec.). The NMR spectral features of **1b** in D_2O solution were consistent with a dihydrate structure: ^{1}H NMR (D_2O) δ 6.14 (s, 1H, H5), 5.98 (m, 2H, H1′ and H7), 5.12 (d, 1H, H5′), 4.84 (s, H2′ under HOD), 4.50 (pseudo-t, 1H, H3′), 3.80 (pseudo-t, 1H, H4′); $^3J_{1'-2'}$ not well resolved, $^3J_{2'-3'}$=6.2 Hz, $^3J_{3'-4'}$=5.9 Hz, $^3J_{4'-5'}$=5.7 Hz. ^{13}C NMR (D_2O) δ 165.7 (C4), 156.0 (C2), 151.6 (C6), 100.2 (C5), 92.1 (C1′), 89.9 (C5′), 85.8 (C7), 85.5 (C4′), 71.6 (C2′), 70.3 (C3′). UV λ_{max}, nm ($\epsilon\times10^{-3}$): (H_2O) 261 (8.9), 204 (9.2); (pH 1) 262 (7.8), 209 (7.2). LR-CIMS of 1b, *m/e* 271.1 (MH^+); HR-CIMS, *m/e* calcd for $C_{10}H_{11}N_2O_7$ 271.0566, found 271.0564.

(6*R*,7*R*,5′*S*)-6,5′:7,5′-Dianhydro-5,6-dihydro-6,5′-dihydroxy-6-[(dihydroxy)methyl]-2′,3′-*O*-isopropylideneuridine (2a). Slow evaporation of an aqueous solution of **1a** within a NaOH-containing desiccator charged with an argon atmosphere afforded X-ray quality crystals of **2a**: mp 205–210°C (dec.). ^{1}H NMR [$(CD_3)_2SO$] δ 10.7 (bs, exchanges upon addition of D_2O, 1H, NH), 7.60 (d, exchanges upon addition of D_2O, 1H, 7-OH), 6.10 (s, 1H, H1′), 5.83 (d, 1H, H5′), 5.44 (d, 1H, H7), 4.82 (d, 1H, H2′ or

H3′), 4.76 (d, 1H, H2′ or H3′), 4.28 (d, 1H, H4′), 3.16 [d, 1H, H5(S)], 2.77 [d, 1H, H5(R)], 1.40 and 1.26 (each s, each 3H, each Me); $^3J_{1'-2'}$=0.0 Hz, $^3J_{2'-3'}$=6.0 Hz, $^3J_{3'-4'}$=0.0 Hz, $^3J_{4'-5'}$=1.2 Hz, $^3J_{7-7\text{-OH}}$=6.9 Hz, $^2J_{5R-5S}$=15.6 Hz. ^{13}C NMR [$(CD_3)_2SO$] δ 166.9 (C4), 150.8 (C2), 111.6 (CMe_2), 103.8 (C5′), 102.4 (C7), 91.4 (C6), 89.4 (C1′), 86.4 (C2′ or C3′), 78.0 (C2′ or C3′), 85.7 (C4′), 37.3 (C5), 26.1, and 24.3 (CMe_2). LR-CIMS, *m/e* 329.2 (MH^+); HR-CIMS, *m/e* calcd for $C_{13}H_{17}N_2O_8$ 329.0985, found 329.0989. UV λ_{max}, nm ($\epsilon\times10^{-3}$): (H_2O) 262 (1.7), 209 (6.8); (pH 1) 261 (1.2), 210 (6.0); (pH 7) 263 (1.3), 210 (6.1); (pH 11) 271 (3.6), 238 (6.2), 215 (4.8), 206 (4.4).

(6*R*,7*R*,5′*S*)-6,5′:7,5′-Dianhydro-5,6-dihydro-6,5′-dihydroxy-6-[(dihydroxy)methyl]uridine (2b). A solution of **2a** (20 mg, 0.06 mmol) in 0.5 mL of 50% aqueous TFA was stirred at 23°C for 36 h. The solution was evaporated to dryness in vacuo, and any residual TFA was removed azeotropically by repetitive coevaporation with water under reduced pressure. Recrystallization of the resulting crude product from water afforded 13 mg (74%) of **2b** as a white solid: ^{1}H NMR [$(CD_3)_2SO$] δ 10.6 (bs, 1), 7.51 (d, 1), 6.00 (d, 1), 5.78 (d, 1), 5.42 (d, 1), 5.24 (d, 1), 5.08 (d, 1), 4.30 (m, 1), 4.12 (m, 1), 4.05 (d, 1), 3.16 (d, 1), 2.76 (d, 1). ^{13}C NMR [$(CD_3)_2SO$] δ 167.0, 150.9, 104.0, 102.4, 91.6, 91.4, 88.9, 78.0, 69.5, 38.4. UV λ_{max}, nm ($\epsilon\times10^{-3}$): (H_2O) 271 (1.0), 212 (7.9); (pH 1) 261 (2.0), 203 (9.0); (pH 11) 274 (13.1). LR-CIMS, *m/e* 289.1 (100, MH^+), 271.1 (70%, MH^+-H_2O), 253.1 (15%, MH^+-2H_2O); HR-CIMS, *m/e* calcd for $C_{10}H_{13}N_2O_8$ 289.0672, found 329.0670.

5′-*O*-(*tert*-Butyldimethylsilyl)-6-(hydroxymethyl)-2′,3′-*O*-isopropylideneuridine (14). A solution of **6**[6] (3.04 g, 7.13 mmol) in 90 mL of THF was added in portions to a stirred suspension of $NaBH_4$ in 75 mL of EtOH at 23°C. The reaction mixture was stirred for 40 min, and then it was concentrated in vacuo to a residue that was separated by column chromatography (5% MeOH/CH_2Cl_2 as eluent) to give 2.29 g (76%) of **14** as a foam: mp 91–93°C. ^{1}H NMR ($CDCl_3$) δ 10.2 (bs, 1), 5.82 (s, 1), 5.80 (s, 1), 5.19 (d, 1), 4.80 (dd, 1), 4.53 (bs, 2), 4.29 (bs, 1), 4.15 (m, 1), 3.87 (m, 2), 1.55 (s, 3), 1.34 (s, 3), 0.90 (s, 9), 0.08 (s, 6). ^{13}C NMR ($CDCl_3$) δ 164.1, 155.5, 150.4, 113.9, 101.2, 91.1, 89.4, 84.2, 81.6, 64.2, 60.5, 27.1, 25.2, 25.9, 18.4. Low-resolution EI-mass spectrum, *m/e* 413.2 (M^+-Me), 371.2 [100%, (M^+-CMe_3)]. Low-resolution CI-mass spectrum, *m/e* 430.2 (M^++2), 413.2 (M^+-Me), 371.2 [95%, (M^+-CMe_3)].

6-[(*tert*-Butyldiphenylsilanoxy)methyl]-2′,3′-*O*-isopropylidene-5′-*O*-(*tert*-butyldimethylsilyl)uridine (15). A solution of **14** (2.14 g, 5.0 mmol) and imidazole (0.749 g, 11.0 mmol) in 5 mL of anhydrous DMF was treated dropwise with TBDPS-Cl (1.51 g, 1.43 mL, 5.5 mmol). The reaction mixture was stirred at 23°C for 4.5 h and then it was evaporated to dryness in vacuo. The residue was dissolved in CH_2Cl_2 and the mixture was separated by radial chromatography using 5% MeOH/CH_2Cl_2 as eluent. The isolated product was dried in vacuo in an Abderhalden chamber over P_2O_5 at 56°C, giving **15** as a pale yellow glassy solid (3.50 g, 82% purity, 86% calculated yield): mp 55–62°C. This sample contained 0.12 equiv. of DMF, by ^{1}H NMR. ^{1}H NMR ($CDCl_3$) δ 9.7 (bs, 1H, NH), 7.75–7.32 (m, 10H, two Ph), 5.80 (s, 1H, H1′ or H5), 5.71 (d, 1H, H1′ or H5), 5.20 (d, 1H, H2′), 4.80 (dd, 1H, H3′), 4.58 and 4.40 (each d, each 1H, 6-CH_2O), 4.13 (m, 1H, H4′), 3.80 (m, 2H, H5′), 1.50 and 1.32 (each s, each 3H, each Me), 1.08 (s, 9H, Me_3CSiPh_2), 0.88 (s, 9H, Me_3CSiMe_2), 0.04 (s, 6H, $SiMe_2$); $J_{2'-3'}$=6.3 Hz, $J_{3'-4'}$=4.5 Hz, J_{7a-7b}=14.1 Hz, $J_{1'-2'}$, $J_{4'-5'}$ not well resolved. ^{13}C NMR ($CDCl_3$) δ 163.7 (C4), 154.0 (C2), 150.6 (C6), 135.4, 134.7, 131.8, 130.2, 129.5, 128.0, 127.9, and 127.6 (eight C's's's from two Ph), 113.5 (CMe_2), 101.6 (C5), 91.3 (C1′), 89.7 (C4′), 84.3 (C2′), 81.9 (C3′), 64.3 (C5′), 62.0 (C7), 27.2 and 25.3 (CMe_2), 26.5 (Me_3CSiPh_2), 25.9 (Me_3CSiMe_2), 19.1 and 18.4 (Me_2Si). LR-EIMS, m/e 609.3 [25%, (M^+-CMe_3)]. LR-CIMS, *m/e* 667.4 (50%, MH+), 609.3 [70%, (M^+-CMe_3)].

6-[(tert-Butyldiphenylsilanoxy)methyl]-2′,3′-*O*-isopropylideneuridine (16). A solution of **15** (3.08 g, 82% pure, 3.79 mmol) in absolute ethanol (19 mL) was treated with pyridinium *p*-toluenesulfonate (PPTS, 284 mg, 1.13 mmol) and the reaction mixture was stirred at 23°C for 24 h. The solvent was removed in vacuo and the residue was dissolved in CH_2Cl_2. The organic solution was washed with saturated aqueous brine and then dried over $MgSO_4$. The solvent was removed in vacuo and the crude product was purified by radial chromatography using 5% MeOH/CH_2Cl_2 as eluent to give **16** as a white foam (2.03 g, 97%): ^{1}H NMR ($CDCl_3$) δ 10.0 (bs, 1H, NH), 7.70–7.38 (m, 10H, two Ph), 5.74 (d, 1H, H1′), 5.70 (s, 1H, H5), 5.24 (dd, 1H, H2′), 5.02 (dd, 1H, H3′), 4.52 and 4.42 (each d, each 1H, 6-CH_2O), 4.20 (m, 1H, H4′), 3.89–3.78 (m, 2H, H5′), 3.2 (bs, 1H, 5′-OH), 1.47 and 1.32 (each s, each 3H, each Me), 1.08 (9H, s, Me_3CSiPh_2); $J_{1'-2'}$=2.5 Hz, $J_{2'-3'}$=6.5 Hz, $J_{3'-4'}$=4.1 Hz, J_{7a-7b}=14.2 Hz, $J_{4'-5'}$ not well resolved. ^{13}C NMR ($CDCl_3$) δ 162.9 (C4), 153.6 (C2), 151.3 (C6), 135.5, 131.7, 130.3 and 128.0 (four C's's's from two Ph), 114.0 (CMe_2), 102.1 (C5), 91.8 (C1′), 87.5 (C4′), 83.3 (C2′), 80.4 (C3′), 62.7 (C5′), 62.1 (C7), 27.2 and 25.2 (CMe_2), 26.5 (Me_3CSiPh_2), 19.1 (Me_3CSi). LR-EIMS, *m/e* 537.2 (20%, M^+-Me), 495.2 [15%, (M^+-CMe_3)], 437.1 [70%, ($M^+-CMe_3-Me_2CO$)]. LR-CIMS, *m/e* 553.3 (15%, MH^+), 495.2 [20%, (M^+-CMe_3)].

6-[(*tert*-Butyldiphenylsilanoxy)methyl]-2′,3′-*O*-isopropylideneuridine 5′-carboxaldehyde (17). A solution of **16** (1.11 g, 2.0 mmol) in 10 mL of CH_2Cl_2 was added to a suspension of Dess–Martin periodinane (1.70 g, 4.0 mmol) in 20 mL of anhydrous CH_2Cl_2. The reaction mixture was stirred at 23°C for 4 h, at which time the starting material had been consumed, by TLC analysis. The resulting mixture was first treated with $Na_2S_2O_3\cdot5H_2O$ (6.97 g, 28.0 mmol) in saturated aq $NaHCO_3$ (107 mL), and extracted with CH_2Cl_2 (5×100 mL). The combined extracts were dried ($MgSO_4$), concentrated and purified by chromatography (50% EtOAc/hexanes). The expected product **17** was finally isolated as a foam (885 mg, 80%) and was found to be a pure 4′-β epimer. Nucleoside **17** in $CDCl_3$ solution was found to be susceptible to slow air oxidation, giving a 2:1 mixture of **17** and the corresponding orotidine within 10 d. ^{1}H NMR ($CDCl_3$) δ 9.42 (s, 1H, CHO), 8.76 (bs, 1H, NH), 7.72–7.35 (m, 10H, two Ph), 6.09 (s, 1H, H1′), 5.62 (d, 1H, H5), 5.22 (dd, 1H, H2′), 5.12 (d, 1H, H3′), 4.58 and 4.43 (each d, each 1H, 6-C*H*$_2$O), 4.51 (s, 1H, H4′), 1.52 and 1.35 (each s, each

3H, each Me), 1.08 (9H, s, Me_3CSiPh_2); $J_{2'-3'}$=6.3 Hz, $J_{3'-4'}$=0 Hz, $J_{4'-5'}$=0 Hz, J_{7a-7b}=13.8 Hz, $J_{1'-2'}$ not well resolved. ^{13}C NMR ($CDCl_3$) δ 199.6 (C5′), 163.1 (C4), 153.3 (C2), 151.5 (C6), 135.5, 131.7, 130.4 and 128.0 (four C's's's from two Ph), 113.2 (CMe_2), 102.5 (C5), 94.2 (C4′), 94.0 (C1′), 85.3 (C2′ or C3′), 84.1 (C2′ or C3′), 62.2 (C7), 26.5 (Me_3CSiPh_2), 26.4 and 24.7 (CMe_2), 19.2 (Me_3CSi). LR-EIMS, *m/e* 535.2 (10%, M^+-Me), 493.2 [85%, (M^+-CMe_3)], 435.1 [100%, ($M^+-CMe_3-Me_2CO$)]. LR-CIMS, *m/e* 551.2 (100%, MH^+).

(6*R*,5′*S*)-6,5′:7,5′-Dianhydro-5,6-dihydro-6,5′-dihydroxy-6-(hydroxymethyl)-2′,3′-*O*-isopropylideneuridine (19). A solution of 785 mg (1.42 mmol) of **17** in 5 mL of anhydrous THF was treated dropwise with 1.42 mL of a 1.0 M solution of TBAF in THF. By TLC, a 2,4-DNP-positive compound, likely 6-hydroxymethyl-2′,3′-*O*-isopropylideneuridine 5′-carboxaldehyde (**18**), formed rapidly but then gradually diminished. After 30 min, the mixture was concentrated in vacuo and the residue was purified by radial chromatography using 10% MeOH/CH_2Cl_2 as eluent. No trace of **18** was isolated, but instead, the spiro nucleoside **19** was obtained as a white powder (222 mg, 55%) after recrystallization from acetone: mp 205–232°C (dec.). ^{1}H NMR [$(CD_3)_2SO$] δ 10.7 (bs, 1H, NH), 6.13 (s, 1H, H1′), 5.75 (s, 1H, H5′), 4.82 (d, 1H, H2′ or H3′), 4.77 (d, 1H, H2′ or H3′), 4.35 (d, 1H, H4′), 4.18 and 4.03 (each d, each 1H, H7a and H7b), 3.38 and 2.76 (each d, each 1H, H5a and H5b), 1.41 and 1.27 (each s, each 3H, each Me); $J_{2'-3'}$=5.6 Hz, $J_{1'-2'}$=0 Hz, $J_{4'-5'}$=0 Hz, J_{5a-5b}=15.8 Hz, J_{7a-7b}=7.7 Hz, $J_{3'-4'}$ not well resolved. ^{13}C NMR [$(CD_3)_2SO$] δ 166.9 (C4), 150.5 (C2), 134.5 (C6), 111.7 (CMe_2), 104.7 (C5′), 89.8 (C1′), 86.5 (C2′ or C3′), 86.3 (C4′), 79.8 (C2′ or C3′), 78.7 (C7), 41.3 (C5), 26.2 and 24.3 (CMe_2). LR-EIMS, *m/e* 297.1 (60%, M^+-Me), 267.1 [100%, ($M^+-Me-CH_2O$)]. LR-CIMS, *m/e* 313.1 (100%, MH^+). HR-CIMS, *m/e* calcd for $C_{13}H_{17}N_2O_7$ 313.10358, found 313.10360. UV λ_{max}, nm ($\epsilon\times10^{-3}$): (H_2O) 262 (10.1); (pH 1) 260 (15.7), 207 (14.7); (pH 11) 261 (13.7); 227.8 (14.2). Anal. Calcd for $C_{13}H_{16}N_2O_7$: C, 50.00; H, 5.16; N, 8.97. Found: C, 50.30; H, 5.20; N, 9.05.

(6*R*,5′*S*)-6,5′:7,5′-Dianhydro-5,6-dihydro-6,5′-dihydroxy-6-(hydroxymethyl)uridine (20). A solution 60 mg (0.19 mmol) of **19** in 2.0 mL of 50% aqueous TFA was kept at 23°C for 48 h and then was rotary evaporated to dryness in vacuo. The residual TFA was removed by repetitive azeotropic coevaporation with water in vacuo at 23°C. Recrystallization of the residue from acetone gave 33 mg (62%) of the deprotected spiro nucleoside **20** as a white powder: mp 210–220°C (dec.). ^{1}H NMR [$(CD_3)_2SO$] δ 10.7 (bs, 1H, NH), 6.03 (s, 1H, H1′), 5.71 (s, 1H, H5′), 5.25 (d, 1H, 2′-OH), 5.07 (d, 1H, 3′-OH), 4.32 (t, 1H, H2′), 4.17–4.10 (m, 3H, H7a, H4′, and H3′), 4.00 (d, 1H, H7b), 3.40 and 2.73 (each d, each 1H, H5a and H5b); $J_{1'-2'}$=0 Hz, $J_{2'-2'OH}$=9.3 Hz, $J_{3'-3'OH}$=4.2 Hz, $J_{4'-5'}$=0 Hz, J_{5a-5b}=16.2 Hz, J_{7a-7b}=7.8 Hz; $J_{2'-3'}$ and $J_{3'-4'}$ not well resolved. ^{13}C NMR [$(CD_3)_2SO$] δ 167.0 (C4), 150.6 (C2), 104.7 (C5′), 91.7, 90.0 and 89.4 (C6, C1′ and C4′), 78.7, 78.1 and 69.3 (C2′, C3′, and C7), 41.6 (C5). LR-EIMS, *m/e* 297.1 (60%, M^+-Me), 267.1 (100%, [$M^+-Me-CH_2O$)]. LR-CIMS, *m/e* 313.1 (100%, MH^+). HR-CIMS, *m/e* calcd for $C_{13}H_{17}N_2O_7$ 313.10358, found 313.10360. UV λ_{max}, nm ($\epsilon\times10^{-3}$): (H_2O) 261 (2.1), 210 (5.8); (pH 1) 263 (5.5), 210 (5.6); (pH 11) 264 (9.7); 227 (7.4). Anal. Calcd for $C_{10}H_{12}N_2O_7$: C, 44.10; H, 4.44; N, 10.29. Found: C, 44.01; H, 4.47; N, 10.08.

(6*R*,5′*S*)-6,5′:7,5′-Dianhydro-5,6-dihydro-6,5′-dihydroxy-6-(hydroxymethyl)-2′,3′-di-*O*-acetyluridine (21). A solution of **20** (10 mg) in dry pyridine (0.2 mL) at 0°C under Ar was treated dropwise with Ac_2O (0.1 mL) and was stirred at 0°C for 15 min and then at 23°C for 1.5 h. The solution was evaporated to dryness in vacuo, giving **21** in a quantitative yield: ^{1}H NMR ($CDCl_3$) δ 8.5 (bs, 1H, NH, exchanges with D_2O), 6.51 (s, 1H, H1′), 5.70 (s, 1H, H5′), 5.45 and 5.40 (each s, each 1H, H2′ and H3′), 4.44 and 4.06 (each d, each 1H, H7a and H7b), 4.40 (s, 1H, H4′), 3.28 and 2.85 (each d, each 1H, H5a and H5b), 2.13 and 2.12 (each 3H, each s, each CH_3); $^3J_{7a-7b}$=7.8 Hz, $^3J_{1'-2'}$=0 Hz, $^3J_{2'-3'}$=6.5 Hz, $^3J_{3'-4'}$=0 Hz, $^3J_{5a-5b}$=16.4 Hz.

2′,3′,5′-Tri-*O*-acetyl-5′,6-(oxomethylene)uridine (22). A solution of **21** from above in pyridine (0.2 mL) under Ar was treated with Ac_2O (0.1 mL) was stirred at 60°C for 6.5 h and then was concentrated to dryness in vacuo. The product **22** was isolated (13.2 mg, 90% based on **20**) by successive preparative chromatographic separations on SiO_2 (5% MeOH/CH_2Cl_2 then 2:1 EtOAc/hexanes as eluent). ^{1}H NMR analysis of **22** in $CDCl_3$ revealed that it exists as a mixture (7:3) of 5′-acetal diastereomers. LR-FABMS, *m/e* 399.1 (60%, MH^+), 118.9 (100%). HR-CIMS for $C_{26}H_{30}N_4O_7$ (MH^+): calcd 399.1039, found 399.1038. UV λ_{max}, nm ($\epsilon\times10^{-3}$): (CH_3OH) 265 (8.5), 210 (7.0).

Major diastereomer of 22. ^{1}H NMR ($CDCl_3$) δ 9.02 (bs, 1H, NH, exchanges with D_2O), 6.84 (d, 1H, H1′), 5.75 (d, 1H, H2′) 5.74 (s, 1H, H5′), 5.71 (dd, 1H, H3′), 5.63 (s, 1H, H5), 4.93 and 4.75 (each d, each 1H, each H7), 4.42 (d, 1H, H4′); $^3J_{7a-7b}$=14.4 Hz, $^3J_{1'-2'}$=5.4 Hz, $^3J_{2'-3'}$=5.7 Hz, $^3J_{3'-4'}$=3.3 Hz. ^{13}C NMR ($CDCl_3$) δ 169.8, 169.7, and 168.8 (3×CO), 161.4 (C4), 151.3 and 149.8 (C2/C6), 104.6 (C5), 94.6 (C1′), 90.1 (C5′), 84.3 (C4′), 76.4 and 71.3 (C2′/C3′), 71.5 (C7), 20.8, 20.7, and 20.4 (3×CH_3).

Minor diastereomer of 22. ^{1}H NMR ($CDCl_3$) δ 9.05 (bs, 1H, NH, exchanges with D_2O), 6.61 (d, 1H, H1′), 6.02 (s, 1H, H5′), 5.73 (d, 1H, H2′), 5.65 (s, 1H, H5), 5.53 (d, 1H, H7a), 5.45 (dd, 1H, H3′), 4.44 (d, 1H, H4′), 4.13 (d, 1H, H7b), 2.19, 2.16, and 2.10 (each s, each 3H, each CH_3CO); $^3J_{7a-7b}$=14.0 Hz, $^3J_{1'-2'}$=4.8 Hz, $^3J_{3'-4'}$=1.2 Hz, $^3J_{4'-5'}$=0 Hz. ^{13}C NMR ($CDCl_3$) δ 169.6, 169.5, and 168.4 (3×CO), 161.6 (C4), 151.0 and 149.4 (C2/C6), 105.5 (C5), 90.1 (C1′), 89.6 (C5′), 85.9 (C4′), 75.4 (C2′), 73.2 (C3′), 64.1 (C7), 20.8, 20.6, and 20.4 (3×CH_3).

Deprotection of 22. A solution of **22** (5 mg, 0.013 mmol) in CD_3OD (1.0 mL) at 23°C was treated with KCN (0.4 mg). After 15 min, a 1:1 mixture of methyl hemiacetal diastereomers of **23** had formed, by ^{1}H NMR. The solution was rotary evaporated and the residue was separated by preparative chromatography on SiO_2 (20% MeOH/CH_2Cl_2 as eluent). After Abderhalden drying (P_2O_5, 78°C) in vacuo overnight, 3 mg (88%) of **23** was obtained: ^{1}H NMR (CD_3OD) δ 5.81 and 5.80 (each 1H, each s, each H5), 5.63 ($^3J_{1'-2'}$=4.8 Hz) and 5.59 ($^3J_{1'-2'}$=4.5 Hz) (each 1H,

each d, each H1′), 5.98–4.73 (m, H5′, H2′, and H3′), 4.47 and 4.46 (each s, each 2H, CH_2), 4.07 (dd, 1H, H4′, J=3.3, 3.9 Hz), 4.00 (dd, 1H, H4′, J=4.2, 6.3 Hz). Nucleoside **23** exists only as the hydrate in D_2O: ^{1}H NMR (D_2O) δ 5.97 (s, 1H, H5), 5.58 (d, 1H, H1′), 5.11 (d, 1H, H5′), 4.90 and 4.46 (each m, each 1H, H2′/H3′), 4.59 (s, 2H, CH_2), 3.82 (pseudo-t, 1H, H4′); $^3J_{1'-2'}$=4.2 Hz, $^3J_{4'-5'}$=5.7 Hz. ^{13}C NMR (D_2O) δ 165.9 (C4), 157.0 (C2), 151.8 (C6), 101.2 (C5), 91.6, 89.9, and 85.9 (C5′/C1′/C4′), 74.5 and 70.4 (C2′ and C3′), 59.6 (C7).

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