# Nucleoside 4′,5′-Enol Acetates. Synthesis and Chemistry of a Unique Uridine $O^2$,4′-Anhydronucleoside[1]

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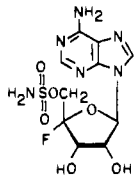
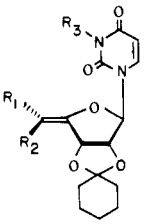
**Abstract:** The synthesis of the uridine 4′,5′-enol acetate **2a** and its conversion to the unique anhydronucleoside **5a** are described. The chemistry of **5a** with regard to a variety of nucleophiles is examined. For example, treatment of **5a** with methanol, ethanol, or ethylene glycol and $AgNO_3$ produced epimeric 4′-alkoxy substituted nucleosides, isolated as the 5′-hemiacetal (**16a, 16d, 17a, 17d, 18**). These hemiacetals were readily reduced with $NaBH_4$ to the corresponding alcohols (**16c, 16f, 16g, 17c, 17f, 17g**). Selective removal of the cyclohexylidene protecting group in the presence of the newly generated ketal at $C_4'$ was possible under carefully controlled conditions utilizing aqueous trifluoroacetic acid.

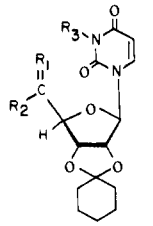
The development of synthetic methods for substituent incorporation at $C_4'$ of nucleosides was prompted by the discovery and structure elucidation of the antibacterial agent nucleocidin (**1**), which has a fluorine attached to $C_4'$.[2-5] Strategies based on a 4′,5′-*exo*-methylene precursor have allowed introduction of fluorine[6-8] and methoxyl,[9-12] though difficulties are sometimes encountered in the reintroduction of oxygen functionality at $C_5'$. An aldol-Cannizzaro sequence on several nucleoside 5′-aldehydes led to the incorporation of a hydroxymethyl group at $C_4'$.[13-15] Our approach has been to utilize 4′,5′ unsaturation while maintaining oxygen functionality (or its equivalent) at $C_5'$. Thus far, we have directed our efforts toward the synthesis of nucleoside 4′,5′-enol acetates[1b] and nucleoside 4′,5′-enamines[16] and their utilization in the preparation of 4′-substituted nucleosides. We have found that various nucleoside 4′,5′-enol acetates (from uridine, adenosine, thymidine, and cytidine) are readily available from the corresponding nucleoside 5′-aldehydes and are generally stable, and we have reported one reaction leading to 4′-substituent incorporation.[1b] Also available in quantitative yield from the 5′-aldehyde is 1-(5-deoxy-5-pyrrolidino-2,3-*O*-cyclohexylidene-β-D-*erythro*-pent-4-enofuranosyl)uracil (**2d**).[16] Alkylation of the enamine with allylic bromides on nitrogen, followed by a Claisen-type rearrangement, allows introduction of an allyl side chain at $C_4'$. The double bond then provides a handle for further manipulation.



**1**



**2a**, $R_1$ = OAc; $R_2$ = $R_3$ = H
**b**, $R_1$ = H; $R_2$ = OAc, $R_3$ = H
**c**, $R_1$ = OAc; $R_2$ = H, $R_3$ = Ac
**d**, $R_1$, $R_2$ = H, –N⟨ ⟩; $R_3$ = H



**3a**, $R_1$ = OH, OH; $R_2$ = $R_3$ = H
**b**, $R_1$ = OAc, OAc; $R_2$ = $R_3$ = H
**c**, $R_1$ = O; $R_2$ = $OCH_3$; $R_3$ = H
**d**, $R_1$ = O; $R_2$ = OH; $R_3$ = H
**e**, $R_1$ = O; $R_2$ = $OCH_3$; $R_3$ = $CH_3$
**f**, $R_1$ = H, H; $R_2$ = OH; $R_3$ = H
**g**, $R_1$ = $CH_3$, $CH_3$; $R_2$ = OH; $R_3$ = H
**h**, $R_1$ = D, D; $R_2$ = OH; $R_3$ = H

Our work with nucleoside enol acetates has initially focused on 1-(5-*O*-acetyl-2,3-*O*-cyclohexylidene-β-D-*erythro*-pent-4-enofuranosyl)uracil (**2a**). The purposes of this paper are to describe the conversion of **2a** to a unique $O^2$,4′-anhydronucleoside, and to discuss the chemistry of this anhydronucleoside, which has led, among other things, to a versatile new synthesis of 4′-alkoxynucleosides.

## Results and Discussion

The precursor to **2a** is the known 2′,3′-*O*-cyclohexylideneuridine 5′-aldehyde monohydrate (**3a**), which is available from uridine[17-19] by suitable modification (see Experimental Section) of literature procedures in overall yields of ca. 60%. Literature procedures for the conversion of aldehydes to enol acetates generally involve treatment of the aldehyde with either isopropenyl acetate and a catalytic amount of *p*-toluenesulfonic acid[20] or with acetic anhydride (reactant and solvent) and KOAc.[21] The former method gave a complex mixture of products with **3a**, while the latter method afforded a 1:1 mixture of enol acetate **2a** and 5′-diacetate **3b**. A study of various bases ($KHCO_3$, $K_2CO_3$, pyridine) as substitutes for KOAc demonstrated $K_2CO_3$ to be the most effective at reducing reaction times, and, if the reaction temperature was held at 80 °C, the amount of **2a** was maximized. Higher temperatures caused some decomposition as well as the production of the N-acetylated enol acetate **2c**. These optimized conditions afforded 85–90% isolated yields of **2a**, still contaminated with minor amounts of **3b** and **2c**. If, however, the reaction is carried out in acetonitrile with 2.2 equiv of acetic anhydride and $K_2CO_3$, only a trace of **3b** is produced (<2%), no **2c** is produced, and isolated yields of **2a** are 92% or higher. More recently, we have found that it is possible to obtain a 78% yield of **2a** by stirring **3a** in acetic anhydride with triethylamine and a small amount of the potent acylation catalyst 4-dimethylaminopyridine at room temperature. This procedure may have promise as a very mild method for enol acetate formation in general.

With regard to the mechanism of enol acetate formation, it is quite clear that diacetate **3b** and enol acetate **2a** are formed by different mechanisms. When diacetate **3b** is isolated and then subjected to the conditions for enol acetate formation, no enol acetate is produced. Since in our systems diacetate is formed at the lower temperatures, and it is only of the β-D-ribo configuration, it may be produced by direct acetylation of the aldehyde hydrate. The enol acetate presumably is formed via the enolate, since the small amount of aldehyde remaining in the acetic anhydride/acetonitrile system is a mixture of β-D-ribo and α-L-lyxo configurations.

In all cases only one of the two possible enol acetates is formed from **3a** as indicated by the sharp singlet for $H_5'$. The disposition of the double bond was clarified by a nuclear Overhauser experiment. If the geometry was as depicted for **2a**, then H-3′ and H-5′ would be, according to models, approximately 3 Å apart, and a significant enhancement should occur. Irradiation of H-3′ in a thoroughly degassed sample of **2a** resulted in a 10% enhancement in the integrated area of H-5′. This corresponds to an internuclear distance of about 2.9 Å.[22] In the one report on enol acetates from carbohydrate al-

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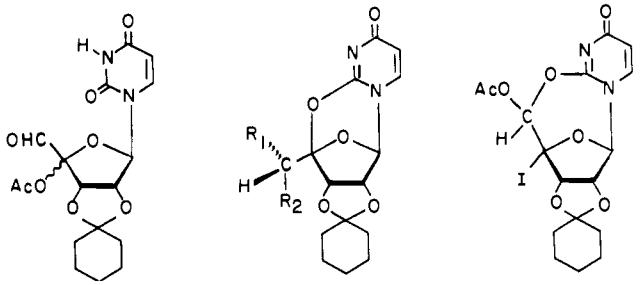
dehydes, only one of the two possible isomers was formed, and in one instance a geometry analogous to that of **2a** was confirmed by X-ray crystallography.[23] In the carbohydrate study photoisomerization of the initially produced *Z* isomer to the *E* isomer was possible; however, in our case similar conditions resulted in no isomerization. We have been able to produce the *E* isomer by experiments to be detailed later.

Though **2a** has oxygens attached to both ends of the double bond, analysis of the $^{13}C$ NMR chemical shifts allows the prediction that **2a** will react as a normal vinyl ether in its direction of addition of unsymmetrical reagents. The shifts for $C_4'$ and $C_5'$ are $\delta$ 144.8 and 115.1 (see Table III), respectively, indicating that $C_4'$ is considerably more electron deficient than $C_5'$ and thus that the same regiospecificity as observed in the *exo*-methylene nucleosides should be witnessed.[6,7,9] The already reported[1b] epoxidation rearrangement sequence in **2a** to give intially aldehydes **4** demonstrated that the chemistry proceeded as expected from these data.

Treatment of **2a** with *N*-iodosuccinimide (NIS)[24] and sodium acetate in acetonitrile produced a more polar compound over several hours in 75–85% yields. This same compound was formed rapidly by treatment of **2a** with silver acetate and iodine in dichloromethane at room temperature. Detailed investigation has demonstrated that this compound is the unique $O^2$,4′-anhydronucleoside **5a,** with both iodo and acetoxy



**4** **5** **6**

**5a**, $R_1$ = OAc, $R_2$ = I
**b**, $R_1$ = I, $R_2$ = OAc
**c**, $R_1$ = OAc, $R_2$ = Br
**d**, $R_1$ = Br, $R_2$ = OAc
**e**, $R_1$, $R_2$ = OH, $OCH_3$

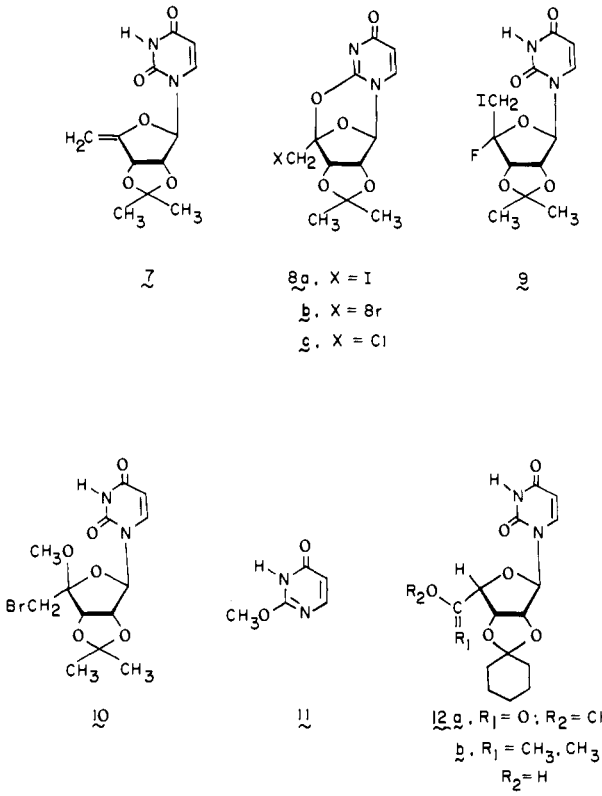
functions on the same carbon. Precedent for this general skeleton is well established,[7,10–12] and, in fact, stable $O^2$,4′-anhydronucleosides have been isolated,[10,12] though little chemistry has been reported on them. When **2a** is treated with NIS in acetonitrile in the absence of sodium acetate both **5a** and **5b** are formed in a 3:1 ratio, with **5a** predominating. The silver acetate–iodine reaction also produces a mixture (9:1, **5a**/**5b**).

Evidence for the structural assignment of **5a** comes from many sources. Its UV spectrum, with $\lambda_{max}$(EtOH) 232 nm, is very characteristic of an anhydronucleoside.[12] The $^1H$ NMR spectrum exhibited all the expected resonances, and, since $H_3'$ was a sharp doublet, $C_4'$ was still devoid of a proton. The two likely structural candidates are **5a** and an $O^2$,5′-anhydronucleoside with an iodine at $C_4'$ (**6**) which would result if the direction of addition were reversed. A clear distinction between these two possibilities was derived from the $^{13}C$ NMR spectrum (see Table III). Using standard structural assignments based on the literature and other compounds from this work, it was possible to readily assign all resonances but $C_4'$ and $C_5'$ ($\delta$ 110.1 and 47.4). The well-known dramatic upfield shift caused by an attached iodine[25] must mean that the $\delta$ 47.4 resonance belongs to the carbon with iodine. Off-resonance decoupling had no effect on the $\delta$ 110.1 resonance but caused the higher field resonance to split into a doublet. Thus, the carbon attached to iodine also must have a proton and hence must be $C_5'$ in **5a**. The $\delta$ 110.1 resonance is perfectly consistent with a carbon attached to two oxygens ($C_4'$ in **5a**). The NMR signals are all sharp resonances with no doubling of peaks, and it is quite clear that **5a** is a single stereoisomer at $C_5'$. The evidence for its configuration being *S*, as shown, will be presented shortly. As further evidence for the structure of **5a,** preparation of the analogue of **5a** utilizing *N*-bromosuccinimide in the identical reaction was carried out. Since this product was considerably less stable than **5a,** all spectral data were recorded on freshly prepared samples. Interestingly, in this reaction both stereoisomers about $C_5'$ were obtained (**5c** and **5d**). In the $^{13}C$ NMR spectrum, substitution of bromine for an iodine should result in a signal shift of about 20 ppm downfield.[25] Confirming that prediction, $C_4'$ of **5c**/**5d** occurs at $\delta$ 110.5 (both signals overlap) and $C_5'$ at $\delta$ 67.5 and 69.9. Once again off-resonance decoupling causes the $C_4'$ signals to remain as singlets, while the $C_5'$ resonance split into doublets. All the other resonances in **5c**/**5d** correspond quite closely to those in **5a**.

Turning to the various reactions leading to **5a–d,** a number of experiments were conducted to gain some insight into the chemical processes occurring. As will be discussed in the next section, treatment of **5a** with acid causes reversion back to starting enol acetate as an *E*/*Z* mixture. Thus, the NIS reaction without NaOAc as well as the silver acetate–iodine reaction may give both isomers owing to an acid-catalyzed process converting **5a** to **2a** and **2b,** which are then reconverted to **5a** and **5b**. In the latter case, of course, acid is produced as the reaction proceeds, and 1.1 equiv of $I_2$ is employed. In the former case, the excess NIS present for maximization of yields would also allow this sequence to occur. In fact, if the NIS reaction of **2a** (without NaOAc) was allowed to proceed for longer periods of time, once all of the **2a** was converted to **5a**/**5b,** the anhydronucleosides gradually went back partially to enol acetate (as the excess NIS is consumed). Spectral examination of this enol acetate showed it to be pure *Z* isomer **2a,** with no **2b** present. To determine the reason for this behavior, reasonably pure **2b** was necessary. This was obtained by treatment of **5a** with tributyltin hydride in benzene with a catalytic amount of iodine to afford an 87% yield of enol acetates favoring the *E* isomer **2b** (3:2, *E*/*Z*). Partial chromatographic separation provided enol acetate with a 6:1 *E*/*Z* ratio, and this material was suitable for our purposes.[26] When a 6:1 **2b**/**2a** mixture was treated with NIS and NaOAc in acetonitrile, anhydronucleoside was obtained in a 6:1 **5b**/**5a** ratio, as expected. When the 3:2 *E*/*Z* mixture was treated with NIS and NaOAc and the reaction stopped with about 25–30% of the starting material still remaining, the anhydronucleoside isolated was a 3:1 (*R*/*S*) mixture, and the remaining enol acetate was pure *Z* isomer **2a**. Thus, the *E* isomer reacts much faster than the *Z* isomer with NIS, and this would account for a gradual funneling of enol acetate to the *Z* isomer in the acid-catalyzed reversion of anhydronucleoside to enol acetate. That the NBS reaction gave a 1:1 *R*/*S* mixture with or without NaOAc while the NIS gave the aforementioned results might be explained by looking at the specific mechanism of formation of **5a**/**5b** and **5c**/**5d**. In the NIS case, the mechanism probably involves stereospecific anti opening of the iodonium ion by $O^2$, and this can only occur when the iodonium ion is formed from the cyclohexylidene side of the double bond. Iodonium ion formed from the uracil side simply returns to *Z* enol acetate. Thus *R* isomer **5b** could only be formed via acid-catalyzed conversion of the anhydronucleoside back to the mixture of *E* and *Z* enol acetates followed by formation of the correct iodonium ion from the *E* isomer. With NBS, however, after initial formation of the bromonium ion from either face of the molecule, considerable literature precedent exists to indicate that the bromonium ion could open to the relatively stabilized 4′-carbonium ion which would then continue on to **5c**/**5d**.[28] Thus either *Z* or *E* enol acetate could go to both anhydronu-

cleosides, even without acid catalysis, simply depending upon which face of the double bond was attacked. Since positive bromine is more reactive (less selective) than positive iodine, an increase in attack on the apparently more hindered underside would be expected in any case. No double bond isomerization was observed when **2a** was treated with NBS (0.5 equiv) and reisolated.

Anhydronucleoside **5a** clearly represented a potentially valuable intermediate for substituent incorporation at $C_4'$. Attack by nucleophiles at $C_4'$ with opening of the anhydro linkage would provide 4′-substituted nucleosides stereospecifically of the β-D-ribo configuration. The sequence of $O^2$,4′-anhydronucleoside formation and attack with ring opening at $C_4'$ has been proposed as an explanation for exclusive formation of **9** from **7** via suggested intermediate **8a**




with silver fluoride and iodine.[7] The bromo compound **8b** has actually been isolated and opened with methanol to yield the α-L-lyxo isomer **10**,[10] presumably by equilibration from the β-D-ribo isomer.[9] The rather unusual stability of **5a** with reference to $C_5'$ and its geminal iodo and acetoxy groups appeared to be due to the steric inaccessibility of the site, with the furanose and cyclohexylidene moieties blocking attack from one side and the iodo and acetoxy groups screening it from the other side. Thus $C_4'$, further buried in the molecule, is even more inaccessible on steric grounds. The remainder of this paper is devoted to an examination of the chemistry of the anhydronucleoside **5a**.

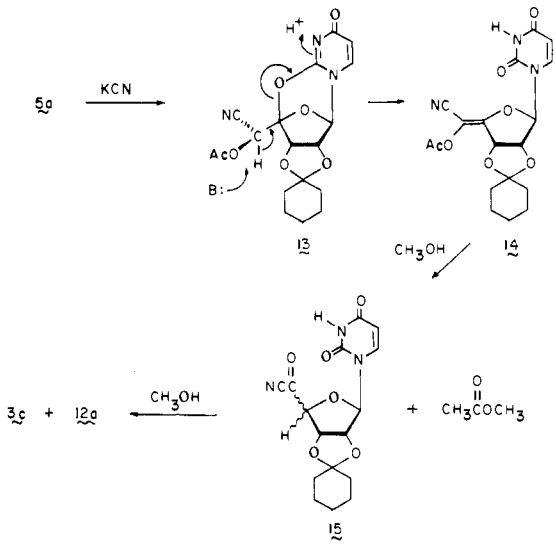
While reasonably stable in the solid state below 0 °C, **5a** releases iodine over a period of days at room temperature. In both acetonitrile and methanol over a period of hours **5a** decomposes to iodine, a mixture of the *Z* and *E* enol acetates **2a** and **2b** in about a 3:1 ratio, and several unstable aldehydes of undetermined structure. In tetrahydrofuran and particularly dichloromethane **5a** is stable for days up to more than 1 week. If **5a** was stirred in $CH_3CN$ containing some solid $NaHCO_3$, it was stable indefinitely. This led us to believe that the decomposition was brought about by traces of acid. Treatment of **5a** with either a catalytic amount or 1 equiv of anhydrous HI resulted in the rapid production of a mixture consisting of about 80% enol acetates (4:1, *Z*/*E* ratio) and 20% aldehydes.

Among the many possible sites for nucleophilic attack on **5a**, our explorations have revealed chemistry exemplifying attack at $C_2$, $C_4'$, $C_5'$, the iodine atom, and the acetate carbonyl. Treatment of **5a** with various bases in methanol resulted in several modes of attack. When **5a** was stirred in methanol with $NaHCO_3$ at room temperature it was slowly converted to a less stable anhydronucleoside, the hemiacetal **5e**, formed by attack at the acetate carbonyl and displacement of iodine ion by methanol.[29] Treatment of **5a** with sodium methoxide in methanol at room temperature resulted in a rapid reaction with the formation of 2-methoxy-4(3*H*)-pyrimidinone (**11**). A logical mechanism for the formation of **11** would involve first formation of **5e** followed by methoxide attack at $C_2$ with opening and cleavage of the glycosidic linkage. Similar behavior was noted for anhydronucleoside **8c** with ethoxide.[12] Stirring **5a** with $K_2CO_3$ in methanol also produced **11**, presumably by formation in situ of methoxide.

When treated with most nucleophiles, **5a** was converted back to starting enol acetate **2a** by attack on the iodine atom initiating a stereospecific elimination to open the anhydronucleoside. Benzyl mercaptan, potassium thiophenoxide, potassium isothiocyanate, potassium isocyanate, and potassium iodide all followed this pattern, forming iodine as a byproduct in each case. The formation of iodine from the sulfur and oxygen nucleophiles is readily explained, using thiophenoxide as a typical example, by homolytic cleavage and appropriate recombination of the phenylsulfenyl iodide formed in the initial attack. The sodium salt of nitromethane also reacted to re-form starting enol acetate but no iodine. Presumably iodonitromethane is the other product in this case. Tributyltin hydride, as mentioned earlier, causes reversion to enol acetate but is not stereospecific. The nitromethane anion reaction, the cleanest and highest yielding of these reactions, was used as evidence of the *S* configuration of **5a**. If a trans elimination of **5a** is assumed, and, of course, a trans addition to form **5a** from **2a**, then the re-formation of **2a** from **5a** requires the *S* configuration. In the corollary experiment, treatment of a 6:1 mixture of **5b** and **5a**, respectively, with nitromethane anion gave a 6:1 mixture of **2b** and **2a** as expected.

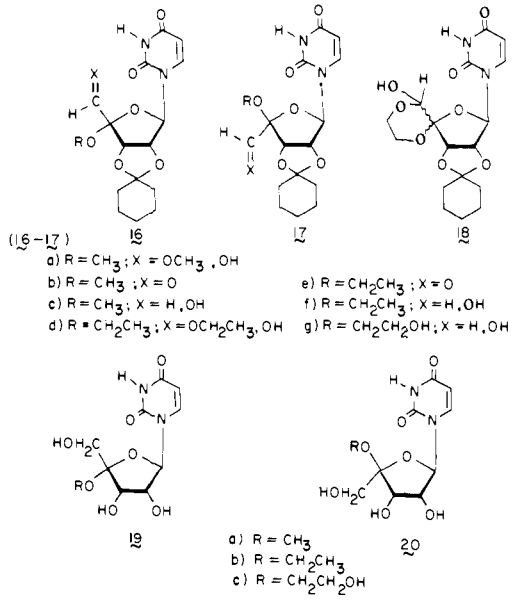
A fourth mode of attack was seen when **5a** was treated with a catalytic or equivalent amount of potassium cyanide in methanol. Two products were formed in a ca. 2:1 ratio in 65% yield and were identified as the isomeric esters **3c** and **12a**. Chemical characterization was achieved by treatment of the mixture of the two isomers with methylmagnesium iodide, which resulted in the formation of the 5′-dimethyl compounds **3g** and **12b**. To clearly establish that **3c** had the β-D-ribo configuration, an independent synthesis of it has been developed. Treatment of **3a** with excess *m*-chloroperoxybenzoic acid in $CH_2Cl_2$ produced the acid **3d**,[30] which was esterified with diazomethane to an ester identical in all respects with **3c** plus a small amount of the N-methylated ester **3e**. Treatment of the synthetic ester with methylmagnesium iodide produced only **3g**. Interestingly, when the mixture of esters produced from **5a** is treated with $NaBH_4$ at room temperature, the sole product (with loss of both esters) is 2′,3′-*O*-cyclohexylideneuridine (**3f**). When the reduction is carried out at 0 °C, only **3c** is reduced, and **12a** can be recovered unchanged. Since facile reduction of esters with $NaBH_4$ is not common without activation by conversion to an anhydride,[31] it is attractive to postulate that under the reaction conditions the uracil ring is participating through $O^2$ to form a cyclic anhydride which is readily reduced with $NaBH_4$. At room temperature under basic conditions the α-L-lyxo isomer is epimerizing to the β-D-ribo isomer and is then reduced by this mechanism. At lower temperatures this equilibration is slow and thus **12a** can be reisolated from the reaction. This transformation opens the

Scheme I



way for facile incorporation of deuterium (or tritium) at $C_5'$ of uridine, and indeed $NaBD_4$ reduction of **3c** readily affords the 5′,5′-dideuterio derivative **3h**. In principle, then, other nucleoside 5′-esters capable of mixed anhydride formation should be readily labelable at $C_5'$.

A proposed mechanism for the formation of **3c** and **12a** from **5a** is shown in Scheme I. Attack by cyanide ion at $C_5'$ to displace iodide ion would afford **13**. The now fairly acidic 5′ proton[32] is removed by base to initiate eliminative ring opening to generate the unsaturated nucleoside **14**. Methanolysis of **14** would first give the epimeric cyano ketones **15** and then the esters **3c** and **12a**.[33] Some support for this hypothesis was obtained by treatment of **5a** with 1.1 equiv of KCN in acetonitrile, forming the relatively reactive **14** which produced the mixture of esters **3c** and **12a** when treated with methanol. Compound **14** may prove to be a valuable intermediate for the production of a variety of $C_4'$- and $C_5'$-modified nucleosides. In addition, treatment of anhydronucleoside **5e** with KCN in $CH_3CN$ also afforded the mixture of esters **3c** and **12a**, the hemiacetal serving as its own source of methanol. An alternate mechanistic possibility might be attack of cyanide on the iodine to generate enol acetate and ICN, which could in some fashion react to produce **3c** and **12a**. However, the enol acetate was found to be unreactive toward freshly prepared ICN.



A fifth mode of attack by nucleophiles on **5a** was realized when **5a** was added to a stirred mixture of silver nitrate and methanol. Precipitation of silver iodide was immediate, and two major products, identified as hemiacetals **16a** and **17a**, each a mixture at $C_5'$, were formed. Treatment of **16a** with Dowex 50($H^+$) ion exchange resin in aqueous THF resulted in the formation of a 2/1 mixture of 5′-aldehyde **16b** and its monohydrate. The α-L-lyxo hemiacetal **17a** under the same conditions gave only the aldehyde **17b**.

Reduction of **16b** and **17b** with $NaBH_4$ gave the alcohols **16c** and **17c**, which were used for configurational assignments about $C_4'$. The well-established fact that a sterically compressed carbon will be at higher field than a comparable carbon in an unhindered environment formed the basis for our assignments, as it did in previous work on 4′-methoxynucleosides.[9,34] Thus, we expect $C_5'$ in the α-L-lyxo isomer **17c** to be at higher field than $C_5'$ in **16c**. In pyridine-$d_5$, $C_5'$ of **17c** is at 57.8 ppm while $C_5'$ of **16c** is at 62.7 ppm (see Table III for complete data). This observation held for all compounds discussed in this paper. In addition, the free nucleoside **19a** derived from **16c** was identical with that material prepared elsewhere.[9] Another characteristic of the series of cyclohexylidene protected compounds is exemplified by **16c** and **17c**, in which $C_1'H$ occurred at δ 5.81 and 6.38, respectively (in $CDCl_3$, see Table I). It proved more convenient from a preparative standpoint to directly reduce the crude hemiacetal mixture with $NaBH_4$ and then separate isomers via LC. In this manner a 66% overall yield of **16c**:**17c** from **5a** was obtained (**16c**:**17c**, 54:46). Two other primary alcohols, ethanol and ethylene glycol (the only others examined), served as satisfactory replacements for methanol. For ethanol, chemistry analogous to that for methanol was seen, with initial formation of the hemiacetals **16d** and **17d**, which could be hydrolyzed similarly to the aldehydes **16e** and **17e** and reduced to the alcohols **16f** and **17f**. Once again it proved most efficient to directly reduce the hemiacetals affording **16f** and **17f** in 46% overall yield (**16f**:**17f**, 57:43). With ethylene glycol, the experimental procedure was modified slightly to maximize yields of the mixture of internal hemiacetals **18**. No change in spectral characteristics was observed when **18** was treated with Dowex 50 ($H^+$) and thus the six-membered ring internal hemiacetal must be highly favored over the aldehyde in this system (no aldehyde signal was observed). Reduction of the hemiacetals to the alcohols **16g** and **17g** occurred smoothly with $NaBH_4$. When a secondary alcohol, 2-propanol, was substituted, it was several minutes before AgI began to precipitate, and a complex mixture of products was formed. Thus the sequence appears synthetically useful only for primary alcohols, with steric hindrance as the likely limiting factor.

The available evidence concerning the mechanism of these transformations is (1) the products are the hemiacetals, AgI, and nitric acid; (2) substitution of silver acetate or silver benzoate for silver nitrate results in very slow production of **5e**, with only small amounts of hemiacetals **16–18** formed, and the vast majority of starting material is untouched. Since both β-D-ribo and α-L-lyxo products are formed, the anhydronucleoside $O^2$–$C_4'$ bond must be cleaved prior to attack of alcohol at $C_4'$. It is attractive to surmise that the reaction is acid catalyzed, with the acid assisting in rapid opening of the anhydronucleoside to the oxygen-stabilized 4′-carbonium ion, which can be attacked by external nucleophiles or the 2-oxygen of uracil. Thus, silver ion would serve to precipitate AgI (thus removing iodide, which would reconvert **5a** to **2a**) and thereby produce the required nitric acid. Formation of the hemiacetal at $C_5'$ might occur by several mechanisms once the iodine atom is removed. As further confirmation of this general mechanistic scheme, when anhydronucleoside **5e** is treated with an equivalent amount of nitric acid in methanol, it is converted in good yield to the mixture of hemiacetals **16a** and **17a**.

Table I. 60-MHz NMR Chemical Shifts (ppm)

| compd | solvent[a] | $C_{1'}$ H | $C_{2'}$ H | $C_{3'}$ H | $C_{4'}$ H | $C_{5'}$ H | $C_5$ H | $C_6$ H | cyclohexylidene | other |
|---|---|---|---|---|---|---|---|---|---|---|
| **2a** | C | 5.82 (s) | 5.12 (d) | 5.43 (d) | | 6.90 (s) | 5.77 (d) | 7.32 (d) | 1.60 (m) | 2.15 (OAc) |
| **2b** | C | 5.63 (s) | 5.05 (d) | 5.60 (dd) | | 7.15 (d) | 5.70 (d) | 7.15 (d) | 1.60 (m) | 2.17 (OAc) |
| **2c** | C | 5.77 (d) | 5.03 (dd) | 5.33 (d) | | 6.87 (s) | 5.75 (d) | 7.27 (d) | 1.57 (m) | 2.15 (OAc) 2.52 (NAc) |
| **3b** | C | 5.67 (s) | 5.02 (m) | 5.02 (m) | 4.28 (q) | 7.00 (d) | 5.73 (d) | 7.27 (d) | 1.57, 1.73 (m) | 2.06, 2.12 (OAc) |
| **3c** | C | 5.58 (s) | 5.15 (d) | 5.38 (dd) | 4.73 (d) | | 5.73 (d) | 7.37 (d) | 1.58, 1.70 (m) | 3.73 ($OCH_3$) |
| **3d** | P | 6.25 (s) | 5.65 (d) | 5.79 (dd) | 5.17 (d) | | 5.81 (d) | 8.00 (d) | 1.60, 1.80 (m) | 9.00 (NH) |
| **3e** | C | 5.48 (s) | 5.08 (d) | 5.33 (dd) | 4.67 (d) | | 5.70 (d) | 7.20 (d) | 1.57, 1.70 (m) | 3.18 ($NCH_3$), 3.68 ($OCH_3$) |
| **3g** | C | 5.70 (s) | 4.95 (d) | 4.95 (d) | 3.88 (d) | | 5.77 (d) | 7.47 (d) | 1.57, 1.73 (m) | 1.30 (s, $2CH_3$) |
| **3h** | C | 5.67 (s) | 4.97 (d) | 4.97 (d) | 4.27 (br s) | | 5.73 (d) | 7.48 (d) | 1.58, 1.70 (m) | |
| **5a** | C | 5.92 (s) | 5.16 (d) | 5.50 (d) | | 7.25 (s) | 6.02 (d) | 7.48 (d) | 1.60 (m) | 2.18 (OAc) |
| **5b** | C | 5.72 (s) | 5.25 (d) | 5.25 (d) | | 7.13 (s) | 5.97 (d) | 7.22 (d) | 1.60 (m) | 2.12 (OAc) |
| **5c/5d** | C | 5.95 (s) | 5.23 (d) | 5.44 (d) | | 6.90, 6.93 (2s) | 5.98 (d) | 7.32 (d) | 1.57 (m) | 2.17, 2.23 (OAc) |
| **11** | C | | | | | | 6.13 (d) | 7.77 (d) | | 4.02 ($OCH_3$) |
| **12a** | C | 5.52 (s) | 5.15 (d) | 5.32 (d) | 5.28 (s) | | 5.72 (d) | 7.22 (d) | 1.55 (m) | 3.78 ($OCH_3$) |
| **12b** | C | 5.45 (s) | 5.34 (d) | 5.10 (dd) | 4.27 (d) | | 5.73 (d) | 7.23 (d) | 1.57, 1.73 (m) | 1.32, 1.40 (2 s, $2CH_3$) |
| **16b** | C | 5.73 (s) | 4.92 (d) | 5.07 (d) | | 9.48 (s) | 5.77 (d) | 7.37 (d) | 1.53 (m) | 3.43 ($OCH_3$) |
| **16c** | C | 5.81 (d) | 5.03 (m) | 5.03 (m) | | 3.75, 3.85 (2 d) | 5.75 (d) | 7.41 (d) | 1.57, 1.80 (m) | 3.47 ($OCH_3$) |
| **16c** | P | 6.57 (d) | 5.12 (dd) | 5.42 (d) | | 4.05 (s) | 5.75 (d) | 7.90 (d) | 1.62, 1.98 (m) | 3.47 ($OCH_3$) |
| **16e** | C | 5.77 (s) | 5.00 (d) | 5.13 (d) | | 9.48 (s) | 5.78 (d) | 7.33 (d) | 1.57 (m) | 1.25 (t, $CH_3$), 3.58 (m, $OCH_2$) |
| **16f** | C | 5.83 (s) | 5.03 (d) | 5.03 (d) | | 3.93 (br s) | 5.77 (d) | 7.42 (d) | 1.55, 1.82 (m) | 1.23 (t, $CH_3$), 3.93 (m, $OCH_2$) |
| **16f** | P | 6.60 (d) | 5.13 (dd) | 5.43 (dd) | | 4.07 (s) | 5.78 (d) | 7.93 (d) | 1.63, 1.97 (m) | 1.18 (t, $CH_3$), 3.85 (m, $OCH_2$) |
| **16g** | P | 6.53 (d) | 5.08 (dd) | 5.37 (d) | | 3.95 (br d) | 5.73 (d) | 7.88 (d) | 1.50 (m) | 3.95 (br d) |
| **17b** | C | 6.48 (s) | 4.90 (d) | 5.07 (d) | | 9.40 (s) | 5.77 (d) | 7.27 (d) | 1.50 (m) | 3.17 ($OCH_3$) |
| **17c** | C | 6.38 (d) | 5.03 (dd) | 4.77 (d) | | 4.06 (s) | 5.77 (d) | 7.47 (d) | 1.55, 1.73 (m) | 3.28 ($OCH_3$) |
| **17c** | P | 6.70 (d) | 5.33 (dd) | 5.02 (d) | | 4.20 (s) | 5.90 (d) | 7.70 (d) | 1.55, 1.70 (m) | 3.35 ($OCH_3$) |
| **17e** | C | 6.53 (s) | 4.95 (d) | 5.12 (d) | | 9.50 (s) | 5.80 (d) | 7.38 (d) | 1.53 (m) | 1.15 (t, $CH_3$), 3.44 (q, $OCH_2$) |
| **17f** | C | 6.38 (d) | 5.00 (d) | 4.76 (d) | | 3.90 (br s) | 5.78 (d) | 7.53 (d) | 1.55, 1.73 (m) | 1.16 (t, $CH_3$), 3.54 (m, $OCH_2$) |
| **17f** | P | 6.67 (d) | 5.30 (dd) | 5.00 (d) | | 4.20 (s) | 5.93 (d) | 7.75 (d) | 1.57 (m) | 1.17 (t, $CH_3$), 3.75 (m, $OCH_2$) |
| **17g** | C | 6.20 (s) | 5.05 (d) | 4.78 (d) | | 3.73 (br s) | 5.73 (d) | 7.50 (d) | 1.57, 1.70 (m) | 3.73 (br s, $2CH_2$) |
| **17g** | P | 6.58 (s) | 5.17 (d) | 4.92 (d) | | 4.10 (s) | 5.83 (d) | 7.83 (d) | 1.50 (m) | 3.87 (br s, $2CH_2$) |
| **19b** | P | 6.77 (d) | 4.78 (dd) | 5.02 (d) | | 4.10, 4.25 (2 d) | 5.77 (d) | 8.20 (d) | | 1.17 (t, $CH_3$), 3.93 (m, $OCH_2$) |
| **19c** | P | 6.78 (d) | 4.75 (dd) | 5.03 (d) | | 4.20, 4.30 (2 d) | 5.78 (d) | 8.28 (d) | | 3.92 (m, $2CH_2$) |
| **20b** | P | 7.07 (d) | 5.15 (dd) | 4.73 (d) | | 4.25, 4.61 (2 d) | 5.98 (d) | 7.85 (d) | | 1.23 (t, $CH_3$), 4.02 (m, $OCH_2$) |
| **20c** | P | 7.12 (d) | 5.18 (dd) | 4.83 (d) | | 4.28, 4.63 (2 d) | 6.00 (d) | 8.43 (d) | | 4.10 (m, $2CH_2$) |

[a] Solvents: C, $CDCl_3$; P, pyridine-$d_5$.

The 4′-alkoxy-substituted nucleosides were not directly available from the enol acetate **2a**. In our hands, all attempts, including, among other things, treatment of **2a** with NIS or NBS in methanol, led only to trace amounts of hemiacetals **16a** and **17a** at best, with most starting material unreacted. It thus appears that in this series proceeding via the anhydronucleoside **5a** is the most effective method for 4′-alkoxy introduction.

For this unique synthesis to have any practical value, unmasking of the cyclohexylidene group without disturbing the second ketal (at $C_4'$) is required. Exploration of a variety of conditions indicated that the use of aqueous trifluoroacetic acid under carefully controlled conditions would serve well for this purpose. It was quickly determined that the β-D-ribo isomers are deprotected considerably faster than α-L-lyxo isomers,[6] and hence different conditions were required for maximum yields. When the α-L-lyxo isomers **17c,e,g** were treated with

1:1 TFA-$H_2O$ over 2 h, quite good yields of the free nucleosides **20a–c** were obtained. A minor amount of uracil (≤5%) caused no separation difficulties. However, treatment of the β-D-ribo isomers **16c,f,g** with 1:1 TFA-$H_2O$ not only rapidly (ca. 20 min) freed the nucleosides **19a–c** but also resulted in the formation of considerable quantities of uracil. This problem was resolved by dramatically reducing the amount of water in the aqueous TFA. Thus, treatment of the protected nucleosides **16c,f,g** with 98:2 TFA-$H_2O$ for short periods of time allowed conversion to the free nucleosides **24a–c** in reasonable yields with no uracil contamination. A problem consistently encountered was the tendency of the liberated cyclohexanone to recombine with the free nucleoside during evaporative removal of the TFA. Attempts to remove the cyclohexanone as its 2,4-dinitrophenylhydrazone during the reaction were successful but in these systems unfortunately generated an inseparable byproduct. Recycling of any re-formed protected nucleoside was possible and no losses were sustained. If this deblocking procedure was used on the 5′-aldehydes **16b** and **17b,** loss of uracil was reduced considerably, but purification problems after $NaBH_4$ reduction were encountered. The commonly employed 9:1 TFA-$H_2O$ resulted in formation of mainly uracil, even with workup after 1 min.

Complete $^1H$ NMR and $^{13}C$ NMR data are presented in Tables I–III, and several trends can be seen. In all cases spectra were run in $CDCl_3$ and/or pyridine-$d_5$. Aside from the $^{13}C$ data used to assign configuration about $C_4'$, the chemical shifts of $C_4'$ itself were distinctive, occurring at ca. 108 ppm in the β-D-ribo series and at ca. 113 ppm for the α-L-lyxo series. An interesting inversion of the positions of $H_2'$ and $H_3'$ occurs in pyridine-$d_5$ depending upon the configuration about $C_4'$. In the β-D-ribo series $H_2'$ (readily identified by its coupling to $H_1'$) is upfield from $H_3'$ while in the α-L-lyxo compounds $H_3'$ is upfield from $H_2'$.

Table II. First-Order Coupling Constants (Hz)

| compd | solvent[a] | $J_{1',3'}$[b] | $J_{2',3'}$ | $J_{5,6}$ | other |
|---|---|---|---|---|---|
| **2a** | C | | 6 | 8 | $J_{3',5'} = 0.9$[d] |
| **2b** | C | | 6 | 8 | $J_{3',5'} = 1.2$[d] |
| **2c** | C | 1 | 6 | 8 | |
| **3b** | C | | | 8 | $J_{3',4'} = 2, J_{4',5'} = 6$ |
| **3c** | C | | 5 | 8 | |
| **3d** | P | | 7 | 8 | |
| **3e** | C | | 6 | 8 | $J_{3',4'} = 1$ |
| **3g** | C | | c | 8 | $J_{3',4'} = 1$ |
| **3h** | C | | 6 | 8 | |
| **5a** | C | | 5.5 | 7.5 | |
| **5b** | C | | 6 | 8 | |
| **5c/5d** | C | | 5.5 | 7.5 | |
| **11** | C | | | 7 | |
| **12a** | C | | 5 | 8 | |
| **12b** | C | | 5 | 8 | $J_{3',4'} = 3$ |
| **16b** | C | | 6 | 8 | |
| **16c** | C | 1.5 | 6 | 8 | $J_{5a',5b'} = 6$ |
| **16c** | P | 2.5 | 6 | 8 | |
| **16e** | C | | 6 | 8 | |
| **16f** | C | | 6 | 8 | |
| **16f** | P | 3 | 7 | 8 | |
| **16g** | P | 2 | 6 | 8 | |
| **17b** | C | | 6 | 8 | |
| **17c** | C | 1 | 5 | 8 | |
| **17c** | P | 1.5 | 5 | 8 | |
| **17e** | C | | 6 | 8 | |
| **17f** | C | 1.5 | 6 | 8 | |
| **17f** | P | 1 | 6 | 8 | |
| **17g** | C | | 5 | 8 | |
| **17g** | P | | 5 | 8 | |
| **19b** | P | 3.5 | 6 | 8 | $J_{5a',5b'} = 6$ |
| **19b** | P | 7 | 4.5 | 8 | $J_{5a',5b'} = 11$ |
| **20c** | P | 1.5 | 6 | 8 | $J_{5a',5b'} = 5$ |
| **20c** | P | 7 | 4.5 | 8 | $J_{5a',5b'} = 12$ |

[a] Solvents: C, $CDCl_3$; P, pyridine-$d_5$. [b] Coupling constants of ≤0.5 Hz were not detectable. [c] Unresolved. [d] These coupling constants were measured on a Bruker HX-90.

## Summary

The anhydronucleoside **5a** is a uniquely stable representative of an interesting class of compounds. It demonstrated a spectrum of reactivity with various reagents. Particularly interesting was its reaction with silver nitrate and several primary alcohols yielding 4′-alkoxynucleosides of the β-D-ribo and α-L-lyxo configurations. The anhydronucleoside **5e** may provide access to other 4′-substituted nucleosides by making $C_4'$ more susceptible to nucleophilic attack.

## Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting-point apparatus and are corrected. Infrared spectra were recorded on a Perkin-Elmer 467 grating infrared spectrophotometer. $^1H$ NMR spectra were measured with a Varian EM-360 instrument and $^{13}C$ NMR spectra with a Bruker WP-80; chemical shifts are expressed in parts per million downfield from internal tetramethylsilane. The nuclear Overhauser effect experiment was carried out on a carefully degassed, sealed sample of **2a** in $CDCl_3$, with spectra measured on a Bruker HX-90 spectrometer. Ultraviolet absorption spectra were recorded on a Cary 15 ultraviolet-visible spectrophotometer. Mass spectra were recorded with an AEI-MS9 spectrometer at 70 eV. Microanalyses were done by Galbraith Laboratories, Inc., and Mr. William Rond, Department of Chemistry, The Ohio State University.

Acetonitrile, tetrahydrofuran, and dimethyl sulfoxide were purified by distillation from $CaH_2$ and stored over molecular sieves. Methanol was distilled and stored over sodium sulfate. In all cases Dowex 50W-X8 ($H^+$ form, 50–100 mesh) cation exchange resin was employed.

Thin layer chromatography was carried out on precoated glass TLC plates (silica gel F-254, 0.25 mm thickness) from EM Laboratories, Inc., using the following systems: A, 95:5 $CHCl_3$-$CH_3OH$; B, 9:1 $CHCl_3$-$CH_3OH$; C, 4:1 $CHCl_3$-$CH_3OH$.

LC separations were carried out with four 6 ft × ⅜ in. stainless steel columns connected in series, packed with silica gel, utilizing a Milroyal Model DC-1-60R pump. A less polar solvent mixture was used in all cases relative to the TLC systems.

**2′,3′-*O*-Cyclohexylideneuridine-5′-aldehyde Monohydrate (3a).**[17–19,35a] To a stirred solution of uridine (50.0 g, 0.205 mol), cyclohexanone (30.4 g, 0.31 mol), and triethyl orthoformate (45.8 g, 0.31 mol) in 600 mL of DMF was added 15 mL of THF saturated with HCl gas. After 4 days at room temperature the solution was stirred with 600 mL of 1:1 $NH_4OH$ (concentrated)-$H_2O$ and passed through a column of 150 g of Amberlite IR-45 [$OH^-$], eluting first with 400 mL of 2:1:1 $H_2O$-$NH_4OH$-$CH_3OH$, then 400 mL of 1:1:1 $H_2O$-$NH_4OH$-$CH_3OH$. Evaporation of solvents at reduced pressure gave a dark orange, viscous syrup which was immediately dissolved in hot $CHCl_3$ and filtered of any insoluble material. The $CHCl_3$ was allowed to evaporate from the filtrate overnight and, upon seeding, the residue crystallized. The crude product was triturated with cold anhydrous ether, and the solid was collected by suction filtration and washed well with cold ether. After drying, a quantitative crude yield of 2′,3′-*O*-cyclohexylideneuridine was obtained as a buff-colored solid.

A stirred, water-cooled solution of the crude product and DCC (120.3 g, 0.58 mol) in 375 mL of dry $Me_2SO$ was treated with dichloroacetic acid (8 mL, 0.096 mol) in 30 mL of $Me_2SO$. After 3 h at room temperature the mixture was transferred to a 2-L beaker and a solution of oxalic acid dihydrate (49.0 g, 0.39 mol) in 200 mL of $CH_3OH$ was added very slowly (foaming). The mixture was stirred for 30 min at room temperature and the dicyclohexylurea was filtered from solution and washed with a minimum amount of cold $CH_3OH$. Dianilinoethane (41.3 g, 0.19 mol) was added to the filtrate and the mixture stirred overnight at room temperature. The crystalline 5′-deoxy-2′,3′-*O*-cyclohexylidene-5,5′-(*N,N*′-diphenylethylenediamino)uridine was filtered off and washed well with cold $CH_3OH$-ether. To the filtrate was added 25 mL of $H_2O$ and a second crop

Table III. $^{13}C$ Chemical Shifts (ppm)

| compd | solvent[a] | $C_{1'}$ | $C_{2'}$ | | $C_{3'}$ | $C_{4'}$ | $C_{5'}$ | $C_2$ | $C_4$ | $C_5$ | $C_6$ | O-C-O | other |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **2a** | C | 97.8 | 78.7 | | 82.5 | 144.8 | 115.1 | 150.2 | 163.7 | 102.8 | 143.0 | 115.0 | 167.1 (OAc) |
| **3c** | C | 98.2 | 84.0 | or | 84.1 | 88.0 | 170.1 | 150.8 | 163.7 | 102.8 | 144.0 | 114.5 | 52.5 ($OCH_3$) |
| **3d** | C | 92.3 | 83.3 | or | 83.8 | 86.8 | *b* | 150.6 | 163.3 | 101.2 | 142.9 | 112.9 | |
| **3g** | C | 91.7 | 79.2 | or | 82.7 | 70.4 | 94.9 | 150.5 | 163.3 | 102.8 | 142.9 | 115.5 | 34.8, 37.3 ($2CH_3$) |
| **5a** | C | 90.2 | 81.1 | | 83.5 | 110.1 | 47.4 | 151.5 | 170.3 | 110.7 | 137.5 | 116.0 | 167.3 (OAc) |
| **5c/5d** | C | 92.0 | | 82.2 | | 110.5 | 67.5 | 152.2 | 171.2 | 109.4 | 138.5 | 116.2 | 167.2, 167.8 |
| | | 91.0 | | 82.9 | | | 69.9 | | | 109.7 | | | (OAc) |
| | | | | 83.3 | | | | | | | | | |
| **12a** | C | 99.0 | 82.3 | or | 84.4 | 85.5 | 168.3 | 151.0 | 163.9 | 102.6 | 144.6 | 114.8 | 52.2 ($OCH_3$) |
| **16c** | P | 90.3 | 84.4 | | 81.8 | 107.8 | 62.7 | 151.7 | 164.1 | 103.2 | 141.6 | 116.2 | 50.3 ($OCH_3$) |
| **16f** | C | 93.5 | 83.5 | | 80.9 | 107.5 | 63.4 | 150.4 | 163.5 | 103.0 | 142.7 | 116.7 | 15.8, 58.7 ($OCH_2CH_3$) |
| **16f** | P | 90.3 | 84.5 | | 81.7 | 107.9 | 63.3 | 151.7 | 164.1 | 103.2 | 141.6 | 116.2 | 16.1, 58.6 ($OCH_2CH_3$) |
| **16g** | C | 93.4 | 83.3 | | 81.3 | 107.3 | 64.2 | 150.4 | 163.5 | 103.1 | 142.9 | 116.6 | 61.9, 63.0 ($OCH_2CH_2O$) |
| **16g** | P | 90.6 | 84.4 | | 81.9 | 107.9 | 65.2 | 151.6 | 164.2 | 103.2 | 141.7 | 116.3 | 62.0, 63.5 ($OCH_2CH_2O$) |
| **17c** | C | 91.8 | 84.7 | | 83.5 | 112.1 | 58.6 | 150.8 | 163.1 | 103.1 | 140.8 | 115.2 | 49.2 ($OCH_3$) |
| **17c** | P | 91.9 | 85.0 | | 83.8 | 112.9 | 57.8 | 152.3 | 164.1 | 103.1 | 141.5 | 114.4 | 48.8 ($OCH_3$) |
| **17f** | C | 91.8 | 84.9 | | 83.7 | 112.1 | 57.7 | 150.7 | 163.2 | 102.8 | 141.2 | 115.1 | 15.1, 59.4 ($OCH_2CH_3$) |
| **17f** | P | 91.9 | 85.2 | | 84.0 | 112.8 | 57.4 | 152.2 | 164.1 | 102.9 | 141.9 | 114.3 | 15.2, 58.5 ($OCH_2CH_3$) |
| **17g** | C | 92.4 | 84.7 | | 83.3 | 112.4 | 58.7 | 150.7 | 163.3 | 103.1 | 141.6 | 114.4 | 61.5, 63.5 ($OCH_2CH_2O$) |
| **17g** | P | 92.0 | 85.0 | | 84.0 | 112.7 | 58.3 | 152.2 | 164.1 | 103.4 | 142.0 | 115.1 | 61.3, 64.2 ($OCH_2CH_2O$) |
| **19a** | P | 91.1 | 75.0 | | 71.9 | 107.9 | 61.8 | 152.0 | 164.2 | 102.9 | 141.0 | | 50.1 ($OCH_3$) |
| **19b** | P | 91.2 | 75.1 | | 71.8 | 108.1 | 62.3 | 152.1 | 164.2 | 102.9 | 142.0 | | 16.3, 58.4 ($OCH_2CH_3$) |
| **19c** | P | 91.5 | 75.3 | | 71.5 | 108.0 | 64.4 | 151.9 | 164.3 | 102.6 | 140.9 | | 61.7 ($OCH_2CH_2O$) |
| **20a** | P | 89.5 | 75.2 | or | 75.4 | 111.5 | 57.8 | 152.6 | 164.1 | 103.7 | 140.6 | | 49.3 ($OCH_3$) |
| **20b** | P | 89.7 | 75.4 | | 75.4 | 111.4 | 57.5 | 152.6 | 164.2 | 103.5 | 140.6 | | 15.6, 59.3 ($OCH_2CH_3$) |
| **20c** | P | 89.6 | 75.4 | or | 75.5 | 111.4 | 58.5 | 152.7 | 164.3 | 103.7 | 142.0 | | 61.5, 64.6 ($OCH_2CH_2O$) |

[a] Solvents: C, $CDCl_3$; P, pyridine-$d_5$. [b] It was not possible to see this carbon, presumably owing to its relaxation time.

obtained. A third crop was obtained in the same manner to give a combined yield of 46.1 g. Addition of $H_2O$ to the filtrate and evaporation of $CH_3OH$ gave an additional 13.5 g for a total crude yield of 59.6 g (64.3%).

A mixture of the crude imidazolidine derivative (14.0 g, 27.1 mmol) and 24.7 g of dried Dowex 50 [$H^+$] in 310 mL of 1:1 THF-$H_2O$ was stirred at room temperature for 4 h. The resin was removed by suction filtration and washed well with THF (6 × 25 mL). The THF was evaporated at reduced pressure and the aqueous solution filtered of insolubles. Removal of the water at reduced pressure followed by vacuum drying gave 8.30 g (90%) of **3a** as a white solid. Similar batch runs gave **3a** in 88–93% yields. This material was suitable for use in all subsequent reactions.

**(*Z*)-1-(5-*O*-Acetyl-2,3-*O*-cyclohexylidene-β-D-*erythro*-pent-4-enofuranosyl)uracil (2a). Method A.** A stirred mixture of **3a** (1.36 g, 4 mmol), anhydrous $K_2CO_3$ (1.11 g, 8 mmol), and 10 mL of acetic anhydride was heated at 80 °C for 45 min ($CO_2$ evolved). Excess acetic anhydride was evaporated at reduced pressure and the residue stirred with 50 mL of $CHCl_3$ and filtered, washing the collected solid well with $CHCl_3$. The filtrate was concentrated and the residue purified by column chromatography (silica gel, 2 × 20 cm column, elution with 100 mL of $CHCl_3$, then 300 mL of 99.5:0.5 $CHCl_3$-$CH_3OH$) to afford 1.25 g of **2a** (86%, $R_f$ 0.60, A) and 118 mg of **2c** (7%, $R_f$ 0.75, A) as light yellow foams and 89 mg of **3b**[35b] (5%, $R_f$ 0.55, A) as a colorless syrup.

**2a:** NMR values are in Tables I–III; UV $\lambda_{max}$ (95% EtOH) 259 nm; exact mass *m/e* 364.1277 (calcd, *m/e* 364.1270). **2a** was accompanied by varying amounts of $CHCl_3$ after purification, which made analysis difficult. Attempts to remove all of the $CHCl_3$ resulted in decomposition.

**2c:** NMR values are in Tables I and II; UV $\lambda_{max}$ (95% EtOH) 261 nm; exact mass *m/e* 406.1383 (calcd, *m/e* 406.1376).

**3b:** NMR values are in Tables I and II; exact mass *m/e* 424.1493 (calcd, *m/e* 424.1481).

**Method B.** A stirred mixture of **3a** (3.00 g, 8.81 mmol), anhydrous $K_2CO_3$ (2.56 g, 18.5 mmol), acetic anhydride (1.89 g, 18.5 mmol), and 40 mL of dry $CH_3CN$ was warmed at 60 °C for 45 min. The cooled mixture was filtered and the collected solid washed well with $CHCl_3$. The solvent was evaporated at reduced pressure and the residue eluted through a short column (silica gel, 2 × 8 cm column, elution with 150 mL of 98.5:1.5 $CHCl_3$-$CH_3OH$) to afford 3.16 g (98%) of **2a** as a colorless foam.

**Method C.**[36] A mixture of **3a** (200 mg, 0.588 mmol), acetic anhydride (240 mg, 2.35 mmol), 4-dimethylaminopyridine (7 mg, 0.06 mmol), triethylamine (125 mg, 1.23 mmol), and 2 mL of THF was stirred at room temperature for 2 h. The solvent and excess liquid reagents were evaporated at reduced pressure and the residue was purified by preparative TLC using 95:5 $CHCl_3$-$CH_3OH$. Elution of the major band gave 167 mg (78%) of **2a** as a colorless foam. A $^1H$ NMR revealed no trace of diacetate **3b**.

**4′(*S*), 5′(*R* and/or *S*)-*O*², 4′-Anhydro-1-(5-*O*-acetyl-2,3-*O*-cyclohexylidene-5-*C*-iodo-β-D-*erythro*-pentodialdofuranosyl-4-ulose)uracil (5a/5b). Method A.** A mixture of **2a** (2.0 g, 5.5 mmol), *N*-iodosuccinimide (2.47 g, 11 mmol), and sodium acetate (0.9 g, 11 mmol) in 20 mL of dry $CH_3CN$ was stirred at room temperature for 3 h. The mixture was filtered, washing well with $CHCl_3$, and the solvent evaporated from the filtrate at reduced pressure to give a dark orange foam. Purification by column chromatography (silica gel, 2 × 23 cm column, elutions with 100 mL of $CHCl_3$, 200 mL of 98:2 $CHCl_3$-$CH_3OH$, 200 mL of 95:5 $CHCl_3$-$CH_3OH$, and 100 mL of 9:1 $CHCl_3$-$CH_3OH$) gave 1.90 g (71%) of **5a** as a yellow solid which was conveniently isolated by trituration with $Et_2O$ with no noticeable loss in yield. Analysis by $^1H$ and $^{13}C$ NMR revealed only the presence of **5a**. When a 6:1 mixture of **2b**/**2a** was treated in this manner a 6:1 ratio

of **5b/5a** was obtained. Crystallization from $CH_2Cl_2$–$Et_2O$ provided analytically pure material as white needles: mp 153–156 °C dec; NMR values are in Tables I–III; UV $\lambda_{max}$ (absolute EtOH) 232 nm.

Anal. Calcd for $C_{17}H_{19}IN_2O_7$ (490.28): C, 41.65; H, 3.91; N, 5.71. Found: C, 41.55; H, 4.01; N, 5.71.

**Method B.** A mixture of **2a** (364 mg, 1 mmol) and *N*-iodosuccinimide (450 mg, 2 mmol) in 5 mL of dry $CH_3CN$ was stirred at room temperature for 3 h. The solvent was evaporated at reduced pressure and the dark brown residue purified by column chromatography (silica gel, 1.5 × 18 cm column, elution with 50 mL of $CHCl_3$, 100 mL of 98:2 $CHCl_3$–$CH_3OH$, 100 mL of 95:5 $CHCl_3$–$CH_3OH$, and 50 mL of 9:1 $CHCl_3$–$CH_3OH$) to afford 369 mg (75%, $R_f$ 0.46, B) of **5a/5b** as a yellow foam. NMR analysis showed the product to be a 3:1 mixture of *S* (**5a**) and *R* (**5b**) isomers, respectively.

**Method C.** To a stirred solution of **2a** (200 mg, 0.55 mmol) in 2 mL of $CH_2Cl_2$ was added ~40 mg of silver acetate. A portion of a solution of iodine (208 mg, 0.82 mmol) in 8 mL of $CH_2Cl_2$ was added dropwise until the $I_2$ color persisted. Alternating 40-mg portions of silver acetate and iodine solution were added until all starting material was consumed (TLC). The total amount of silver acetate required was 187 mg (1.12 mmol) and 6 mL (0.62 mmol) of $I_2$ solution was used.[37] The mixture was filtered through Celite, washing the collected AgI well with $CHCl_3$. The solvent was evaporated from the filtrate at reduced pressure and the residue purified by preparative TLC using 9:1 $CHCl_3$–$CH_3OH$. Elution of the major band afforded 149 mg (55%) as an orange foam which, by $^1H$ NMR analysis, displayed an approximate *S*/*R* ratio of 9:1.

**4′(*S*),5′(*R* and *S*)-$O^2$,4′-Anhydro-1-(5-*O*-acetyl-2,3-*O*-cyclohexylidene-5-*C*-bromo-β-D-*erythro*-pentodialdofuranosyl-4-ulose)uracil (5c/5d).** A stirred, water-cooled mixture of **2a** (227 mg, 0.62 mmol) and sodium acetate (77 mg, 0.94 mmol) in 2 mL of dry $CH_3CN$ was treated with NBS (166 mg, 0.93 mmol). After 15 min at room temperature the solvent was removed under reduced pressure and the residue purified by preparative TLC using 9:1 $CHCl_3$–$CH_3OH$ to give 190 mg (69%) of **5c/5d** as a colorless foam. This material slowly decomposed even when stored at 0 °C and all spectra were therefore obtained on freshly prepared samples. The compound had precisely the same $R_f$ value as **5a** and was composed of a 50:50 mixture of *R* and *S* isomers: NMR values are in Tables I–III; UV $\lambda_{max}$ 230 nm, inflection 245 nm (absolute EtOH). Product instability precluded elemental or mass spectral analysis. Conducting the reaction in the presence of a radical inhibitor (2,6-di-*tert*-butyl-4-methylphenol) had no effect on the **5c/5d** ratio.

**4′(*S*)-$O^2$,4′-Anhydro-1-(2,3-*O*-cyclohexylidene-5-aldehydo-β-D-*erythro*-pentodialdofuranosyl-4-ulose)uracil 5′-(Methyl Hemiacetal) (5e).** A mixture of **5a** (300 mg, 0.612 mmol) and $NaHCO_3$ (300 mg, 3.57 mmol) in 30 mL of $CH_3OH$ was stirred at room temperature for 7 h. The mixture was filtered, washing well with $CH_3OH$, and the solvent evaporated from the filtrate at reduced pressure. The residue was triturated with 50 mL of 9:1 $CHCl_3$–$CH_3OH$ and the insolubles were filtered from solution. The filtrate was concentrated at reduced pressure and the resulting gel filtered, washing with 1:1 $CHCl_3$–$Et_2O$ and then $Et_2O$ to afford 150 mg (70%) of **5e** as a light yellow solid. This material was not stable to chromatographic purifications and decomposed during attempts at crystallization: $^1H$ NMR (pyridine-$d_5$) δ 1.53 (m, cyclohexylidene), 3.57 (s, $OCH_3$), 5.33 (m, $C_2'$ H and $C_3'$ H), 6.17 (d, $C_5$ H), 6.87 (br s, $C_1'$ H), 8.20 (d, $C_6$ H); UV $\lambda_{max}$ (absolute EtOH) 230 nm, inflection 250 nm. Product instability precluded satisfactory elemental or mass spectral analysis.

**Methanolysis of 5a. Formation of 3,4-Dihydro-4-keto-2-methoxypyrimidine (11).** A solution of **5a** (70 mg, 0.14 mmol) in 4 mL of $CH_3OH$ was treated with 1 mL of 0.43 M sodium methoxide in $CH_3OH$ (23 mg, 0.43 mmol) and the mixture allowed to stir for 8 min at room temperature. Neutralization of the resulting yellow solution with glacial acetic acid followed by evaporation of the solvent at reduced pressure gave a yellow foam having only one UV-active component ($R_f$ 0.44, B). Purification by preparative TLC using 9:1 $CHCl_3$–$CH_3OH$ gave 15 mg (85%) of 3,4-dihydro-4-keto-2-methoxypyrimidine (**11**) as a white solid. A sample crystallized from ethyl acetate gave white needles: mp 163–164 °C (lit. mp[38] 167–168 °C); NMR values are in Tables I and II; UV $\lambda_{max}$ (95% EtOH) 268 nm; exact mass *m/e* 126.0432 (calcd, *m/e* 126.0429).

**Decomposition of 5a in Acetonitrile. A.** A solution of **5a** (180 mg, 0.367 mmol) in 10 mL of dry $CH_3CN$ was allowed to stir at room temperature. After 30 min the solution was dark brown and a TLC showed no trace of **5a**, the major component matching the $R_f$ of **2a/2b**. This decomposition was not gradual. Once the $I_2$ color began to appear the reaction rapidly went to completion (ca. 5 min). The solvent was evaporated at reduced pressure and the residue dissolved in 5 mL of $CHCl_3$. The solution was washed with 10% aqueous $NaHCO_3$ (1 × 3 mL), 10% aqueous sodium thiosulfate (1 × 3 mL), and water (1 × 3 mL) and the organic layer dried over anhydrous $Na_2SO_4$. Evaporation of the solvent at reduced pressure afforded 155 mg of a white foam. $^1H$ NMR analysis showed this material to contain ~60 mol % of **2a/2b** in a *Z*/*E* ratio of 4:1 and ~40 mol % of an unidentified aldehyde, possibly an epimeric mixture, which displayed a major (9.40 ppm) and minor (9.48 ppm) singlet in a ratio of 6:1. The intensity of the major aldehyde singlet decreased markedly upon storage for 4 days at 0 °C.

In a separate experiment, run simultaneously with the above, **5a** (10 mg, 0.26 mmol) and $NaHCO_3$ (5 mg, 0.06 mmol) in 1 mL of dry $CH_3CN$ were allowed to stir at room temperature. No detectable decomposition took place over the 4-h time of observation.

**B.** To a stirred, ice-cooled solution of **5a** (100 mg, 0.204 mmol) in 3 mL of $CH_3CN$ was added HI (13 mg, 0.102 mmol theoretical maximum), generated in a separate flask by dropping 26 mg of 50% aqueous HI onto $P_2O_5$ and flushed through the solution with $N_2$. Darkening immediately commenced and a TLC after 5 min confirmed complete reaction. Processing as described above provided 67 mg of a mixture containing 75% **2a/2b** (*Z*/*E* ratio unchanged) and 25% of the aldehyde components (major/minor ratio unchanged). An ice-cooled solution of **5a** in $CH_3CN$ did not decompose during the duration of this experiment. Repetition of this experiment with ca. 1.5 equiv of anhydrous HI gave identical results.

**Reaction of 5a with ($n$-Bu)$_3$SnH.** To a stirred suspension of **5a** (70 mg, 0.14 mmol) in benzene under $N_2$ was added ($n$-Bu)$_3$SnH in $Et_2O$ (0.7 mL of 0.3 M) and the mixture warmed at 65 °C. Introduction of a small crystal of $I_2$ resulted in rapid disappearance of **5a** over 5 min, **2a/2b** being the only visible UV-active component by TLC. Four additional runs were made on this scale, the product combined, and the solvent removed at reduced pressure. Purification by preparative TLC using 95:5 $CHCl_3$–$CH_3OH$ gave 227 mg (87%) of **2a/2b** found by $^1H$ NMR analysis to be a 60:40 (*E*/*Z*) mixture. Partial resolution of the mixture was accomplished by LC using 99:1 $CHCl_3$–$CH_3OH$ in that four portions were collected containing *E*/*Z* ratios of 1:3 (50 mg, 22%), 55:45 (70 mg, 31%), 6:1 (61 mg, 27%), and ~100% *E* (7 mg, 3%): NMR values for the *E* isomer are in Table I; exact mass *m/e* 364.1277 (calcd, *m/e* 364.1270).

**Reaction of 5a and 5b with $NaCH_2NO_2$.** To a stirred mixture of NaH (7 mg, 50% dispersion, 0.15 mmol) in 5 mL of $CH_3NO_2$ was added **5a** (37 mg, 0.075 mmol). After 3.5 h at room temperature the reaction was complete (TLC) and the mixture was filtered through Celite, washing with $CH_2Cl_2$. Evaporation of the solvent at reduced pressure gave 25 mg (91%) of crude **2a** as a yellow foam. $^1H$ NMR analysis verified the presence of **2a** exclusively. When an 86:14 mixture of **5b/5a** was treated identically, the *E*/*Z* ratio was ca. 86:14.

**Reaction of 5a with KCN. Formation of 1-[Methyl(2,3-*O*-cyclohexylidene-β-D-ribofuranosyl)uronate]uracil (3c) and 1-[Methyl(2,3-*O*-cyclohexylidene-α-L-lyxofuranosyl)uronate]uracil (12a). Method A.** A solution of **5a** (268 mg, 0.547 mmol) and KCN (39 mg, 0.60 mmol) in 9 mL of $CH_3OH$ was stirred at room temperature for 1.5 h. The solvent was evaporated at reduced pressure and the residue purified by preparative TLC using 95:5 $CHCl_3$–$CH_3OH$ to give 120 mg (63%) of a mixture containing 67% **3c** and 33% of **12a**: NMR values are in Tables I–III (obtained on the separated isomers described in the Experimental Section below); UV $\lambda_{max}$ 260 nm (95% EtOH); exact mass *m/e* 352.1276 (calcd, *m/e* 352.1270).

**Method B.** A solution of **5a** (200 mg, 0.408 mmol) and KCN (29 mg, 0.45 mmol) in 7 mL of $CH_3CN$ was stirred at room temperature for 2.5 h. The solvent was removed at reduced pressure and the residue stirred with 2 mL of $CH_2Cl_2$ and then filtered through Celite, washing with 2 mL of $CH_2Cl_2$. The solvent was evaporated at reduced pressure to give 154 mg (97%) of crude 1-(5-*O*-acetyl-5-cyano-2,3-*O*-cyclohexylidene-β-D-*erythro*-pent-4-enofuranosyl)uracil (**14**) as an orange foam. A $^1H$ NMR spectrum was not well resolved but two closely spaced acetoxy signals were visible: IR (neat) 2216 $cm^{-1}$; exact mass *m/e* 389.1231 (calcd, *m/e* 389.1223).

Treatment of **14** with methanol resulted in rapid production of **3c** and **12a** in a ratio of 2:1.

**Reduction of 3c and 12a with $NaBH_4$ and $NaBD_4$. A.** A stirred solution of **3c** and **12a** obtained directly from **5a** (100 mg, 0.204 mmol)

as described above in 2 mL of THF was treated with $NaBH_4$ (77 mg, 2.04 mmol) in 1 mL of $H_2O$. After 30 min at room temperature the reduction was complete (TLC) and the solution was neutralized with glacial acetic acid. Evaporation of the solvent was followed by a plug filtration through silica gel, washing with 30 mL of 9:1 $CHCl_3$-$CH_3OH$. The solvent was removed at reduced pressure to give 100 mg of crude product which was purified by preparative TLC using 9:1 $CHCl_3$-$CH_3OH$. Elution of the major band provided 28 mg (42% overall from **5a**) of 2′,3′-*O*-cyclohexylideneuridine (**3f**) as a colorless foam which was chromatographically and spectroscopically identical with an authentic sample.

**B.** An ice-cooled, stirred solution of purified **3c** and **12a** (100 mg, 0.28 mmol) in 3.5 mL of THF was treated with $NaBD_4$ (61 mg, 1.45 mmol) in 1 mL of $CH_3OH$. After 1.5 h the mixture was neutralized with 20% aqueous AcOH and the solvent evaporated at reduced pressure. The residue was filtered through a plug of silica gel, washing with 30 mL of 9:1 $CHCl_3$-$CH_3OH$. Evaporation of the solvent, then purification and separation by preparative TLC using 9:1 $CHCl_3$-$CH_3OH$, afforded 56 mg (61%) of 2′,3′-*O*-cyclohexylidene-5′,5′-dideuteriouridine (**3h**) ($R_f$ 0.3) and 17 mg (19%) of **12a** ($R_f$ 0.5) as colorless foams.

**3h**: NMR values are in Tables I and II; exact mass 326.1455 (calcd, *m/e* 326.1447).

**12a**: NMR values are in Tables I–III; IR (neat) 1760 $cm^{-1}$. Ester **12a** was chromatographically and spectroscopically identical with the minor component of the epimeric mixture **3c** and **12a** described above.

**Reaction of 3c and 12a with $CH_3MgI$.** A stirred mixture of 2:1 **3c**/**12a** (84 mg, 0.24 mmol) in 9 mL of THF was cooled to −78 °C (under $N_2$) and a solution of $CH_3MgI$ in $Et_2O$ (5 mL of 1 M) added over a 1-min period. After 11 h (−78 → 0 °C) the mixture was poured into 20 mL of saturated aqueous $NH_4Cl$ and this mixture extracted with $CH_2Cl_2$ (2 × 25 mL), washed with $H_2O$, and dried over $Na_2SO_4$. Solvent removal at a reduced pressure gave 83 mg of a crude mixture of **3g** and **12b**. Purification by preparative TLC using 95:5 $CHCl_3$-$CH_3OH$ (two developments) provided 46 mg (55%) of 2′,3′-*O*-cyclohexylidene-5′,5′-dimethyluridine (**3g**) ($R_f$ 0.40, A) and 24 mg (29%) of 1-(2,3-*O*-cyclohexylidene-5,5-dimethyl-α-L-lyxofuranosyl)uracil (**12b**) ($R_f$ 0.45, A) as colorless foams.

**3g**: NMR values are in Tables I–III; UV $\lambda_{max}$ (95% EtOH) 259 nm; exact mass *m/e* 352.1640 (calcd, *m/e* 352.1634).

**12b**: NMR values are in Tables I and II; UV $\lambda_{max}$ (95% EtOH) 259 nm; exact mass (same as above).

**1-(2,3-*O*-Cyclohexylidene-β-D-ribofuranosyluronic acid)uracil (3d).** To a solution of **3a** (185 mg, 0.54 mmol) in 6 mL of $CH_2Cl_2$ was added 85% *m*-chloroperoxybenzoic acid (1.09 mmol, 222 mg) and the mixture allowed to stir at room temperature for 20 min. TLC monitoring revealed the reaction to be complete and the solvent was evaporated at reduced pressure. The light brown foam was triturated with cold $Et_2O$ and the resulting white, granular solid collected by suction filtration, washing well with cold $Et_2O$, to give 178 mg (97%) of crude **3d**. Crystallization from acetone gave an analytically pure hemihydrate, mp 193–195 °C; NMR values are in Tables I–III.

Anal. Calcd for $C_{15}H_{18}N_2O_7 \cdot \frac{1}{2}H_2O$ (347.33): C, 51.87; H, 5.51; N, 8.06. Found: C, 51.83; H, 5.45; N, 7.65.

**1-[Methyl(2,3-*O*-cyclohexylidene-β-D-ribofuranosyl)uronate]uracil (3c).** A stirred solution of crude **3d** (125 mg, 0.37 mmol) in 3:2 $CH_3OH$–dioxane was treated with a slight excess of $CH_2N_2$ in ether. Evaporation of the solvent at reduced pressure followed by purification by preparative TLC using 93:7 $CHCl_3$-$CH_3OH$ gave 96 mg (74%) of **3c** ($R_f$ 0.38, A) as a colorless syrup and 30 mg (21%) of a colorless foam identified by $^1H$ NMR as $N^3$-methyl-1-(methyl 2,3-*O*-cyclohexylidene-β-D-ribofuranosyluronate)uracil (**3e**) ($R_f$ 0.60, A).

**3c**: NMR values are in Tables I–III; UV $\lambda_{max}$ (95% EtOH) 260 nm. This material was chromatographically and spectroscopically identical with the major isomer of the **3c** and **12a** mixture described above.

Anal. Calcd for $C_{16}H_{20}N_2O_7 \cdot \frac{1}{4}CHCl_3$ (NMR assay): C, 51.06; H, 5.34; N, 7.33. Found: C, 51.01; H, 5.35; N, 7.10.

**3e**: NMR values are in Tables I and II.

**2′,3′-*O*-Cyclohexylidene-5′,5′-dimethyluridine (3g).** To a stirred solution of **3c** (80 mg, 0.23 mmol) in 8 mL of THF was added $CH_3MgI$ in $Et_2O$ (2.3 mL of 1 M) over a 10-min period at room temperature. After a total of 0.5 h the mixture was poured into 5 mL of saturated aqueous $NH_4Cl$ and extracted with $CH_2Cl_2$ (2 × 15 mL). The organic layer was washed with $H_2O$ and dried over $N_2SO_4$, and the solvent was removed under reduced pressure, leaving 60 mg (75%) of **3g** as a white foam. Purification by preparative TLC (9:1 $CHCl_3$-$CH_3OH$) provided **3g** as an analytically pure methanolate: NMR values are in Tables I–III; UV $\lambda_{max}$ (95% EtOH) 259 nm; exact mass 352.1640 (calcd, *m/e* 352.1634). This material was chromatographically and spectroscopically identical with the major isomer obtained from the reaction of **3c** and **12a** with $CH_3MgI$ above.

Anal. Calcd for $C_{17}H_{24}N_2O_6 \cdot CH_3OH$ (384.43): C, 56.24; H, 7.34; N, 7.29. Found C, 56.04; H, 7.29; N, 7.16.

**4′(*S*)- and 4′(*R*)-1-(2,3-*O*-Cyclohexylidene-4-*O*-methyl-5-aldehydo-β-D-*erythro*-pentodialdofuranosyl-4-ulose)uracil (16b and 17b).** To a stirred mixture of $AgNO_3$ (217 mg, 1.28 mmol) in 18 mL of $CH_3OH$ was added **5a** (500 mg, 1.02 mmol). After 15 min at room temperature, KI (43 mg, 0.26 mmol) was added and the mixture allowed to stir for an additional 10 min at room temperature. The solution was filtered through a plug of silica gel, washing with 40 mL of 3:1 $CHCl_3$-$CH_3OH$. Evaporation of solvent under reduced pressure gave 310 mg of the crude hemiacetal mixture **16a** and **17a** as a foam. Chromatography using preparative TLC (two developments with 95:5 $CHCl_3$-$CH_3OH$) produced two major bands. The slower moving material was eluted with 40 mL of 3:1 $CHCl_3$-$CH_3OH$ to give 106 mg (27%) of **16a** as a colorless foam. Owing to the chiral 5′ center this material exhibited a complex $^1H$ NMR spectrum and appeared as two close-moving spots by TLC. Treatment of **16a** with 1 mL of 1:1 THF-$H_2O$ containing 5 mg of Dowex 50 [$H^+$] ion-exchange resin for 1 h followed by filtration, solvent evaporation, and vacuum drying gave β-D-ribo aldehyde **16b** in quantitative yield as a colorless foam. Similar treatment of the α-L-lyxo hemiacetal **17a**, isolated in the same fashion as **16a** (60 mg, 15%), gave a quantitative yield of **17b** as a homogeneous foam. NMR analysis of **16b** showed it to contain some monohydrate form (2:1 free aldehyde/monohydrate) while **17b** existed entirely as the free aldehyde: NMR values are in Tables I and II; exact mass 352.1276 (calcd, *m/e* 352.1271) (both isomers, in hemiacetal and aldehyde form).

Though it was possible to separate the aldehydes for independent reduction, it was found most convenient to directly reduce the mixture of hemiacetals followed by separation of the isomers at the alcohol stage, as described below.

**16a**: $^1H$ NMR ($CDCl_3$) δ 1.55 and 1.80 (m, cyclohexylidene), 3.45, 3.48, and 3.57 (s, $-OCH_3$), 4.7–5.0 (m, $C_2'$ H and $C_3'$ H), 5.72 (d, $J_{5,6}$ = 8 Hz, $C_5$ H), 6.00 and 6.02 (d, s, $C_1'$ H), 7.42 and 7.53 (d, $C_6$H).

**17a**: $^1H$ NMR ($CDCl_3$) δ 1.53 and 1.75 (m, cyclohexylidene), 3.25, 3.43, and 3.48 (s, $-OCH_3$), 4.6–5.0 (m, $C_2'$ H and $C_3'$ H), 5.72 (d, $J_{5,6}$ = 8 Hz, $C_5$ H), 6.23 and 6.41 (d, $C_1'$ H), 7.32 and 7.33 (d, $C_6$ H).

**4′(*R*)- and 4′(*S*)-1-(2,3-*O*-Cyclohexylidene-4-*O*-methyl-β-D-*erythro*-pentofuranosyl-4-ulose)uracil (16c and 17c).** The crude hemiacetal mixture **16a** and **17a** was taken up in 10 mL of THF and any insolubles were removed by suction filtration. To the stirred solution was added 77 mg (2.04 mmol) of $NaBH_4$ (dissolved in 2 mL of water) dropwise over a period of 5 min, the mixture becoming black owing to precipitation of reduced silver. The solution was allowed to stir for an additional 10 min, then neutralized with dilute aqueous acetic acid and filtered through Celite, washing with 5 mL of $CH_3OH$. The solvent was evaporated under reduced pressure and the residue was taken up in a minimal amount of 85:15 $CHCl_3$-$CH_3OH$, and then filtered through a plug of silica gel, washing with 40 mL of 85:15 $CHCl_3$-$CH_3OH$. Evaporation of solvent left a residue that was triturated with 98:2 $CHCl_3$-$CH_3OH$ and filtered in order to free the crude product of a small amount of uncharacterized polar material. Solvent removal and vacuum drying gave 300 mg of **16c** and **17c** as a yellow foam. Separation of the epimers by LC using 98:2 $CHCl_3$-$CH_3OH$ gave 124 mg (33%) of **16c** and 104 mg (29%) of **17c**, each as a TLC-homogeneous foam, along with 11 mg of unresolved material for a total overall yield of 66% from **5a**.

An analytical sample of β-D-ribo **16c** was prepared by crystallization from $CHCl_3$-$Et_2O$ (mp 114–118 °C): NMR values are in Tables I–III; UV $\lambda_{max}$ (absolute EtOH) 260 nm.

Anal. Calcd for $C_{16}H_{22}N_2O_7$ (354.37): C, 54.30; H, 6.26; N, 7.90. Found: C, 54.21; H, 6.52; N, 7.61.

The α-L-lyxo isomer **17c** failed to crystallize, existing as the hemimethanolate (NMR): NMR values are in Tables I–III; UV $\lambda_{max}$ (absolute EtOH) 260 nm; exact mass *m/e* 354.1434 (calcd, *m/e* 354.1427).

Anal. Calcd for $C_{16}H_{22}N_2O_7 \cdot \frac{1}{2}CH_3OH$ (370.39): C, 53.51; H, 6.53; N, 7.56. Found: C, 53.34; H, 6.40; N, 7.50.

Reduction of **16b** with $NaBH_4$ gave material which was chroma-

tographically and spectroscopically identical with **16c** while **17b** gave **17c** after similar treatment.

**4′(*S*) and 4′(*R*)-1-(2,3-*O*-Cyclohexylidene-4-*O*-ethyl-5-aldehydo-β-D-*erythro*-pentodialdofuranosyl-4-ulose)uracil (16e and 17e).** The ethoxy hemiacetals **16d** and **17d** were prepared and separated analogously to the methoxy derivatives. In this case some variation in the final isomer ratio was found depending upon how long the $AgNO_3$ and ethanol were stirred prior to introduction of **5a** (longer times favored the β-D-ribo isomer). Thus **5a** (300 mg, 0.61 mmol) afforded 80 mg (32%) of **16d** and 54 mg (21%) of **17d** as foams. Hydrolysis of **16d** produced aldehyde **16e** along with some monohydrate (30% of the mixture by NMR) while **17d** afforded the free aldehyde **17e** exclusively. Both isomers were isolated quantitatively as colorless foams: NMR values are in Tables I and II; exact mass (both isomers) 366.1435 (calcd, *m/e* 366.1427).

**16d:** $^1$H NMR ($CDCl_3$) δ 1.23 (m, $-CH_3$), 1.55 (m, cyclohexylidene), 3.3-4.1 (m, $-OCH_2-$), 3.52 (s, $-OCH_3$), 4.7-5.1 (m, $C_2'$ H and $C_3'$ H), 5.78 (d, $J_{5,6}$ = 8 Hz, $C_5$ H), 6.52 (m, $C_1'$ H), 7.37 and 7.48 (d, $C_6$ H).

**17d:** $^1$H NMR ($CDCl_3$) δ 1.23 (m, $-CH_3$), 1.55 (m, cyclohexylidene), 3.50 (s, $-OCH_3$), 3.83 (m, $-OCH_2-$), 4.5-5.2 (m, $C_2'$ H and $C_3'$ H), 5.77 (d, $J_{5,6}$ = 8 Hz, $C_5$ H), 6.08 (m, $C_1'$ H), 7.47 and 7.60 (d, $C_6$ H).

**4′(*R*)- and 4′(*S*)-1-(2,3-*O*-Cyclohexylidene-4-*O*-ethyl-β-D-*erythro*-pentofuranosyl-4-ulose)uracil (16f and 17f).** The crude hemiacetal mixture **16d** and **17d** (460 mg from 1.02 mmol of **5a**) was reduced with $NaBH_4$ (200 mg, 5.3 mmol, 30 min) and the resulting isomers were separated (LC using 97:3 $CHCl_3$-$CH_3OH$) in analogy with the methoxy derivatives to give 94 mg (25%) of **16f** and 71 mg (19%) of **17f** as homogeneous foams along with 8 mg of unresolved material for a total overall yield of 46% from **5a**. A sample of **16f** crystallized from $CHCl_3$-$Et_2O$ gave pure material, mp 110-112 °C.

Because **16f** initially resisted crystallization, an analysis was obtained on the foam which existed as the methanolate (NMR): NMR values are in Tables I-III; exact mass 368.1590 (calcd, *m/e* 368.1583).

Anal. Calcd for $C_{17}H_{24}N_2O_7 \cdot CH_3OH$ (400.43): C, 53.99; H, 7.05; N, 7.00. Found: C, 54.26; H, 6.73; N, 6.99.

A sample of **17f** free of the $CHCl_3$ used in workup could not be obtained (drying at elevated temperatures resulted in decomposition) nor could a sample be obtained in crystalline form. Therefore analysis was performed on material previously determined (NMR) to contain 25 mol % $CHCl_3$: NMR values are in Tables I-III; exact mass, same as for **16f**.

Anal. Calcd for $C_{17}H_{24}N_2O_7 \cdot \frac{1}{4}CHCl_3$ (398.23): C, 52.03; H, 6.14; N, 7.03. Found: C, 51.80; H, 6.30; N, 6.71.

**4′(*S*)- and 4′(*R*)-1-[2,3-*O*-Cyclohexylidene-4-*O*-(2-hydroxyethyl)-β-D-*erythro*-pentodialdofuranosyl-4-ulose]uracil 2″,5′-Hemiacetal (18).** To a stirred mixture of $AgNO_3$ (217 mg, 1.28 mmol) in 0.75 mL (830 mg, 13.4 mmol) of dry ethylene glycol was added **5a** (500 mg, 1.02 mmol) dropwise as a slurry in 6 mL of hot THF. Residual **5a** was dissolved in 4 mL of hot THF and added to the reaction mixture. After the mixture was stirred for 1 h at room temperature, KI (43 mg, 0.26 mmol) was added and the mixture allowed to stir for an additional 10 min. The solution was filtered through a plug of silica gel, washing with 40 mL of 2:1 $CHCl_3$-$CH_3OH$. The solvent was evaporated at reduced pressure and the residue (containing ethylene glycol) purified by preparative TLC using multiple developments with 9:1 $CHCl_3$-$CH_3OH$ to move the product to the top of the plate, elution of which gave 366 mg (94%) of crude **18**. No signals were detectable in the aldehyde region of the spectrum. Treatment of **18** with Dowex 50 ($H^+$) in aqueous THF resulted in recovery of **18** unchanged: exact mass 382.1382 (calcd, *m/e* 382.1376); $^1$H NMR ($CDCl_3$) δ 1.58 (m, cyclohexylidene), 3.2-4.3 (m, $-OCH_2CH_2O-$), 4.5-5.2 (m, $C_2'$ H and $C_3'$ H), 5.77 (d, $C_5$ H), 6.42 (s, $C_1'$ H), 7.50 (m, $C_6$ H).

**4′(*R*)- and 4′(*S*)-1-[2,3-*O*-Cyclohexylidene-4-*O*-(2-hydroxyethyl)-β-D-*erythro*-pentofuranosyl-4-ulose]uracil (16g and 17g).** The crude hemiacetal **18** obtained above was reduced and the resulting isomers were separated (LC using 96:4 $CHCl_3$-$CH_3OH$) in analogy with the ethoxy derivatives to give 44 mg (11%) of crystalline **16g** and 60 mg (15%) of **17g** as a colorless foam along with 84 mg (21%) of unresolved material for an overall yield of 47% from **5a**. The mixture of **16g** and **17g** was determined to contain 80% **16g** by $^1$H NMR. Fractional crystallization of this material from $CHCl_3$-$Et_2O$ gave an additional 36 mg of **16g**.

**16g:** mp 178-180 °C; NMR values are in Tables I-III.

Anal. Calcd for $C_{17}H_{24}N_2O_8$ (384.39): C, 53.14; H, 6.30; N, 7.29. Found: C, 53.34; H, 6.49; N, 7.18.

**17g:** A sample of this material could not be obtained in crystalline form and existed as the methanolate: exact mass 384.1540 (calcd, *m/e* 384.1532); NMR values are in Tables I-III.

Anal. Calcd for $C_{17}H_{24}N_2O_8 \cdot CH_3OH$ (416.43): C, 51.92; H, 6.78; N, 6.73. Found: C, 52.12; H, 6.66; N, 6.67.

**General Procedure for Removal of the Cyclohexylidene Protecting Group.** The protected β-D-ribo nucleosides (usually the combined product of several small runs) were treated with 98:2 TFA-$H_2O$ for 6 min (appropriate details are listed with each compound). The α-L-lyxo isomers were treated with 1:1 TFA-$H_2O$ for 2 h at room temperature. Upon completion of the reaction, all liquid was rapidly evaporated in vacuo (1-2 min total distillation time with a dry ice-acetone cooled receiver placed close to the stillhead) and the residue coevaporated several times with absolute EtOH. Purification was accomplished by column chromatography on silica gel unless otherwise noted.

**4′(*R*)-1-(4-*O*-Methyl-β-D-*erythro*-pentofuranosyl-4-ulose)uracil (4′-Methoxyuridine) (19a).** **16c** (104 mg, 0.29 mmol) and 1 mL of 98:2 TFA-$H_2O$ reacted for 6 min. Chromatography (1 × 15 cm column, elution with 25 mL of 95:5 $CHCl_3$-$CH_3OH$ and 80 mL of 9:1 $CHCl_3$-$CH_3OH$) afforded 13 mg (13%) of **16c** ($R_f$ 0.85, C) and 70 mg (87%) of **19a** ($R_f$ 0.40, C) as colorless foams for a yield of 99% based on recovered starting material. This material was spectroscopically identical with the reported values for 4′-methoxyuridine.[9]

**4′(*S*)-1-(4-*O*-Methyl-β-D-*erythro*-pentofuranosyl-4-ulose)uracil (20a).** **17c** (204 mg, 0.58 mmol) and 2 mL of 1:1 TFA-$H_2O$ reacted for 2 h. Chromatography (1 × 15 cm column, elution with 25 mL of 95:5 $CHCl_3$-$CH_3OH$ and 65 mL of 9:1 $CHCl_3$-$CH_3OH$) gave 101 mg (65%) of **20a** ($R_f$ 0.55, C) and 22 mg (6%) of **17c** ($R_f$ 0.86, C) as colorless foams for a yield of 73% based on recovered starting material. The product values were spectroscopically identical with the reported values for **20a**.[9]

**4′(*R*)-1-(4-*O*-Ethyl-β-D-*erythro*-pentofuranosyl-4-ulose)uracil (4′-Ethoxyuridine) (19b).** **16f** (204 mg, 0.554 mmol) and 1.9 mL of 98:2 TFA-$H_2O$ reacted for 6 min. Chromatography (1 × 15 cm column, elution with 20 mL of 95:5 $CHCl_3$-$CH_3OH$ and 70 mL of 9:1 $CHCl_3$-$CH_3OH$) afforded 48 mg (24%) of **16f** ($R_f$ 0.85, C) and 101 mg (63%) of **19b** ($R_f$ 0.56, C) as colorless foams for a yield of 83% based on recovered starting material. Analysis was obtained on **19b** as the hydrate; NMR values are in Tables I-III.

Anal. Calcd for $C_{11}H_{16}N_2O_7 \cdot H_2O$ (306.28): C, 43.13; H, 5.92; N, 9.14. Found: C, 42.75; H, 5.61; N, 8.72.

**4′(*S*)-1-(4-*O*-Ethyl-β-D-*erythro*-pentofuranosyl-4-ulose)uracil (20b).** **17f** (83 mg, 0.225 mmol) and 1 mL of 1:1 TFA-$H_2O$ reacted for 2 h. Chromatography (1 × 15 cm column, elution with 25 mL of 95:5 $CHCl_3$-$CH_3OH$ and 60 mL of 9:1 $CHCl_3$-$CH_3OH$) gave 45 mg (69%) of **20b** ($R_f$ 0.59, C) and 10 mg (12%) of **17f** ($R_f$ 0.90, C) as colorless foams for a yield of 79% based on recovered starting material. While chromatographically homogeneous, **20b** failed to give a satisfactory elemental analysis:[39,40] NMR values are in Tables I-III; MS (70 eV) 257, 239, 193, 177, 155, 130, 113, 112, 101, 73, 69; FDMS 289 (M + 1, 100), 257 (M − $CH_2OH$, 56), 177 (sugar + 1, 40), 112 (uracil, 41).

**4′(*R*)-1-[4-*O*-(2-Hydroxyethyl)-β-D-*erythro*-pentofuranosyl-4-ulose]uracil (4′-hydroxyethoxyuridine) (19c).** **16g** (168 mg, 0.44 mmol) and 1.6 mL of 98:2 TFA-$H_2O$ reacted for 6 min. Chromatography (1 × 16 cm column, elution with 25 mL of 95:5 $CHCl_3$-$CH_3OH$ and 50 mL of 85:15 $CHCl_3$-$CH_3OH$) afforded 51 mg (30%) of **16g** ($R_f$ 0.85, C) and 83 mg (62%) of **19c** ($R_f$ 0.30, C) as colorless foams for a yield of 90% based on recovered starting material. A sample of **19c** lyophilized from $H_2O$ gave an analytically pure hemihydrate; NMR values are in Tables I-III.

Anal. Calcd for $C_{11}H_{16}N_2O_8 \cdot \frac{1}{2}H_2O$ (313.27): C, 42.17; H, 5.47; N, 8.94. Found: C, 41.98; H, 5.69; N, 8.73.

**4′(*S*)-1-[4-*O*-(2-Hydroxyethyl)-β-D-*erythro*-pentofuranosyl-4-ulose]uracil (20c).** **17g** (151 mg, 0.393 mmol) and 2 mL of 1:1 TFA-$H_2O$ reacted for 2 h. Chromatography by preparative TLC using 75:25 $CHCl_3$-$CH_3OH$ provided 91 mg (76%) of **20c** as a colorless foam. While chromatographically and spectroscopically homogeneous, **20c** did not give a satisfactory elemental analysis:[39,40] NMR values are in Tables I-III; FDMS 327 (M + Na, 21), 305 (M + 1, 41), 273 (M − $CH_2OH$, 59), 112 (uracil, 100).

**Acknowledgment**. Partial support for this research was provided by the Department of the Army, U.S. Army Medical Research and Development Command, Contract DAMD17-78-C-8021. We are grateful to Dr. John Occolowitz of the Lilly Research Laboratories, Indianapolis, Ind., for the field desorption mass spectra, and to Dr. Lan Wong of The Ohio State University Department of Pharmacology for the low-resolution electron impact spectrum of **20b**. Assistance with some of the nomenclature was kindly provided by Professor Derek Horton.

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(40) Field desorption mass spectra (FDMS) were recorded on a Varian-MAT Model 731 mass spectrometer utilizing carbon dendrite emitters. The numbers in parentheses are relative peak intensities. The low-resolution electron impact spectrum of **20b** was recorded on a Hewlett-Packard Model 5985A GC–MS system.

# *lin*-Benzoadenine Nucleotides. Inter- and Intramolecular Interactions in Aqueous Solutions as Observed by Proton Magnetic Resonance

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**Abstract:** The inter- and intramolecular interactions of *lin*-benzoadenine nucleotides have been examined by proton magnetic resonance. When the base is unprotonated, *lin*-benzoadenine nucleotides strongly stack in aqueous solution, with association constants of at least one order of magnitude greater than those of the corresponding adenine nucleotides. Some head-to-tail orientations of stacked *lin*-benzoadenine nucleotides were indicated by the deuterium substitution effect on relaxation times (DESERT). The relative positions of the heteroaromatic proton chemical shifts at infinite dilution (pD 8.5) and under acidic conditions (pD ~4.0) indicated the conformations of the nucleotides (anti and syn, respectively) and the site of ring protonation (the pyrimidine ring).

We have previously reported the interaction of *lin*-benzoadenine nucleotides (**1**) with enzymes and their sensitivity to the environment.[1-9] In order to understand more fully the observed properties of these adenine analogues, we have examined their inter- and intramolecular interactions by proton magnetic resonance. The accumulated data provide detailed information concerning the self-association of these compounds in aqueous solution. In addition, the relative positions of the

0002-7863/79/1501-1564$01.00/0 