Nucleosides. 134. 3′-Amino-3′-deoxy-β-D-hexopyranosyl Nucleosides. 8. Synthesis of 2,3′-Imino Nucleosides by Solvolysis of 2′-O-Mesylglucosyluracil Derivatives[1]

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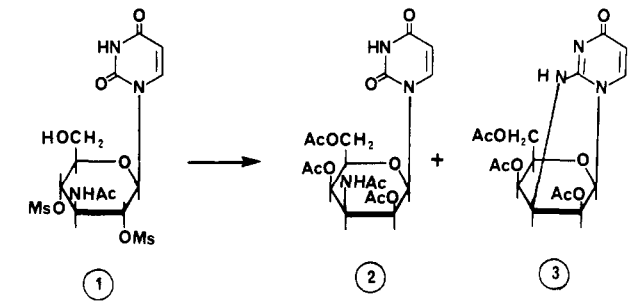
Treatment of 1-(3′-acetamido-3′-deoxy-2′,4′-di-O-mesyl-β-D-glucopyranosyl)uracil (1) with sodium acetate in aqueous methylcellosolve afforded 1-(3′-amino-3′-deoxy-β-D-talopyranosyl)uracil and 2,3′-imino-1-(3′-amino-3′-deoxy-β-D-talopyranosyl)isocytosine in almost equal amounts. These products were separated and characterized as their corresponding peracetyl derivatives **2** and **3**. The manno isomer **10** and the 6′-deoxy analogue **19** of **3** were also synthesized by solvolysis of 1-(3′-amino-3′-deoxy-2′-O-mesyl-β-D-glucopyranosyl)uracil trifluoroacetic acid salt (**8**) and 1-(3′-acetamido-3′,6′-dideoxy-2′,4′-di-O-mesyl-β-D-glucopyranosyl)uracil (**17**), respectively. A plausible mechanism for the formation of the 2,3′-imino linkage is discussed.

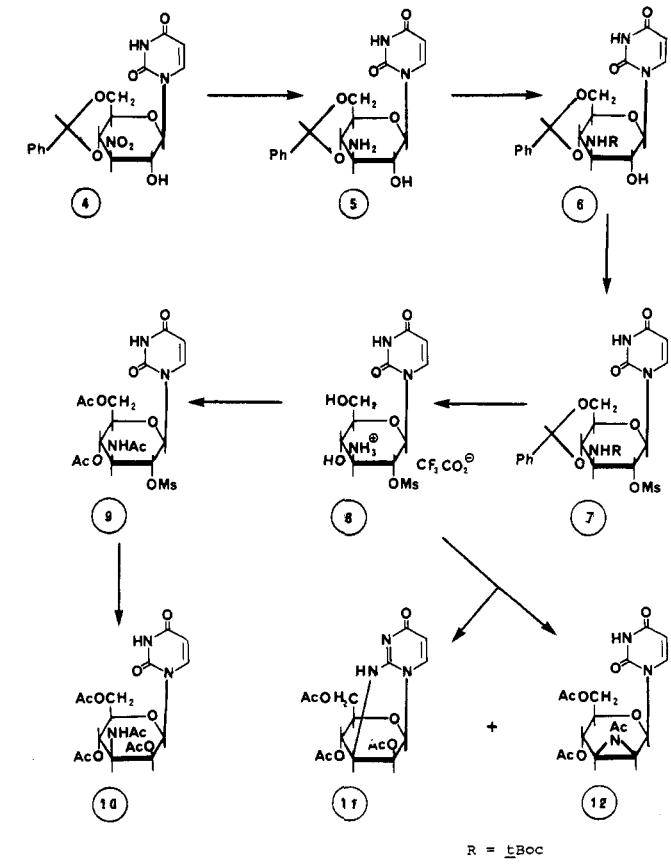
The intramolecular nucleophilic displacement reaction is an intriguing subject to investigate, particularly in derivatives of carbohydrates and nucleosides where there are multiple leaving groups and nucleophilic centers.[2-6] Since the development by Baker and Schaub[7] of a method to convert a trans vic amino alcohol to a cis configuration by solvolysis of the *N*-acetyl-*O*-sulfonyl derivative, a number of new amino sugars have been synthesized.[8] It was shown that the 4-*O*-mesyl group is much more susceptible than the 2-*O*-mesyl function to intramolecular displacement in poly-*O*-mesyl-D-glycopyranosides.[7,9,10] In the nucleoside area we reported previously[6] that 1-(3′-acetamido-3′-deoxy-2′,4′,6′-tri-*O*-mesyl-β-D-glucopyranosyl)uracil afforded the 2,2′-anhydronucleoside as the sole initial product which was converted into the 2′,6′-anhydro-mannosyluracil apparently via hydrolysis of the anhydro linkage in the initial product followed by attack of the 2′-alkoxide on the C-6′ position to form the 2,5-dioxabicyclo[2.2.2]octane system. The 2′,6′-anhydromannosyluracil was eventually transformed into the 2′,6′-anhydrotalo nucleoside. These results established that the nucleophilicity of the 2-carbonyl group is much greater than that of the 3′-acetamido function and that the ease with which the internal displacement occurred in the all-trans (β-gluco) system was C-2′ > C-6′ or C-4′.

We now describe a novel and simple method for the synthesis of 2,3′-iminohexosyluracil nucleosides by solvolysis of 3′-acetamido-3′-*O*-mesyl-β-D-glucopyranosyluracils. Solvolysis of 1-(3′-acetamido-3′-deoxy-2′,4′-di-*O*-mesyl-β-D-glucopyranosyl)uracil (**1**)[8] under the Baker–Schaub conditions [NaOAc, methylcellosolve–H_2O (9:1)] afforded two products in nearly equal amounts. After peracetylation, these products were separated on a silica gel column. One of the products isolated was identical with 1-(3′-acetamido-2′,4′,6′-tri-*O*-acetyl-3′-deoxy-β-D-talopyranosyl)uracil (**2**) (Scheme I).[6] The other product obtained had UV spectral characteristics very similar to those of 2,3′-imino-1-(3′-amino-2′,3′-dideoxy-β-D-threo-pentofuranosyl)-5-methylisocytosine,[11] suggesting a 2,3′-imino

Scheme I



Scheme II



(1) This investigation was supported in part by funds from the National Cancer Institute, U.S. Department of Health and Human Services (Grants CA-07848 and CA-18601).

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linkage (e.g., **3**) for this product. The ^1H NMR spectrum (Me_2SO-d_6) showed that the compound contained three acetyl groups and one dissociable (NH) proton. The small coupling constants between the neighboring sugar ring protons ($J_{1',2'} = J_{2',3'} = 3.1$ and $J_{3',4'} = 3.7$ Hz) with the exception of $J_{4',5'}$ (7.5 Hz) are consistent with that of the 2,3′-imino(2′,4′,6′-tri-*O*-acetyl-3′-amino-3′-deoxy-β-D-talopyranosyl)isocytosine structure (**3**) or its manno isomer **11** (Scheme II) in a conformation close to $^0S_2$.[12,13]

In order to establish the identity of **3**, we synthesized the 2,3′-imino manno nucleoside **11** by the procedure shown in Scheme II. The known and readily available 1-(4′,6′-*O*-benzylidene-3′-deoxy-3′-nitro-β-D-glucoyrnosyl)uracil (**4**)[14] was reduced with Raney nickel in methanol to the 3′-amino nucleoside **5**. After selective protection of the amino group with *tert*-butoxycarbonyl group, the product **6** was mesylated to give 1-(4′,6′-*O*-benzylidene-3′-(*tert*-butoxycarbonyl)amino-3′-deoxy-2′-*O*-mesyl-β-D-glucopyranosyl)uracil (**7**). Treatment of **7** with 90% trifluoroacetic acid afforded 1-(3′-amino-3′-deoxy-2′-*O*-mesyl-β-D-glucopyranosyl)uracil as the crystalline trifluoroacetic acid salt (**8**). Peracetylation of **8** afforded the triacetate **9** which, upon solvolysis under the Baker–Schaub conditions, afforded only the known[15] 1-(3′-acetamido-2′,4′,6′-tri-*O*-acetyl-3′-deoxy-β-D-mannoyranosyl)-uracil (**10**). Similar treatment of **8** gave two products. The major product (isolated in 79% yield) had similar UV spectral characteristics to those of the 2,3′-imino nucleoside,[11] and the ^1H NMR parameters as well as combustion data of the peracetylated product were consistent with the structure of 2,3′-imino-1-(2′,4′,6′-tri-*O*-acetyl-3′-amino-3′-deoxy-β-D-mannopyranosyl)isocytosine (**11**). The synthesis of **11** also established the structure of the 2,3′-imino nucleoside derived from the 2′,4′-dimesylate **1** by solvolysis as possessing the *talo* configuration **3**. The minor product which was isolated after peracetylation (13%) showed the UV spectral characteristics similar to those of uridine.[16] The ^1H NMR spectrum of this compound showed the presence of three acetyl groups, one NH proton, and protons for H-2′ and H-3′ as doublets ($J_{2',3'} = 8.5$, $J_{1',2'} = J_{3',4'} = 0$ Hz). These spectral data together with combustion analyses are consistent with the epimino structure **12**.

The formation of 2,3′-imino linkage by solvolysis is unprecedented and unexpected. This formation might be explained by attack of 6′-OH on the 2,2′-anhydro linkage to form a 2,6′-anhydro manno (or talo) intermediate which was converted to the 2,3′-imino-bridged nucleoside (**3** or **11** after acetylation) by intramolecular attack of the 3′-amido nitrogen on the 2,6′-anhydro linkage. Many examples of intramolecular anhydronucleoside interconversions have been reported[3,5] since the first discovery in our laboratory.[17] There are precedents for intramolecular involvement of exocyclic hydroxymethyl functions in the sugar moiety in anhydronucleoside reactions. For example, treatment of 2,3′-anhydro-1-(β-D-xylofuranosyl)uracil with hydrogen halides[18] or LiN_3[19] afforded the 5′-substituted-5′-deoxy xylosyluracils. A plausible mechanism proposed[20] for these reactions was attack of the 5′-OH group on the 2,3′-anhydro linkage to form the 2,5′-anhydro xylo nucleoside intermediate which was further attacked by nucleophiles giving rise to the 5′-substituted-5′-deoxy xylo nucleosides.

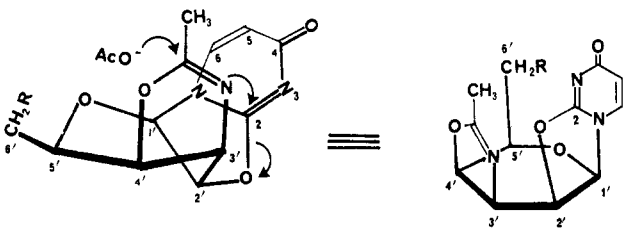
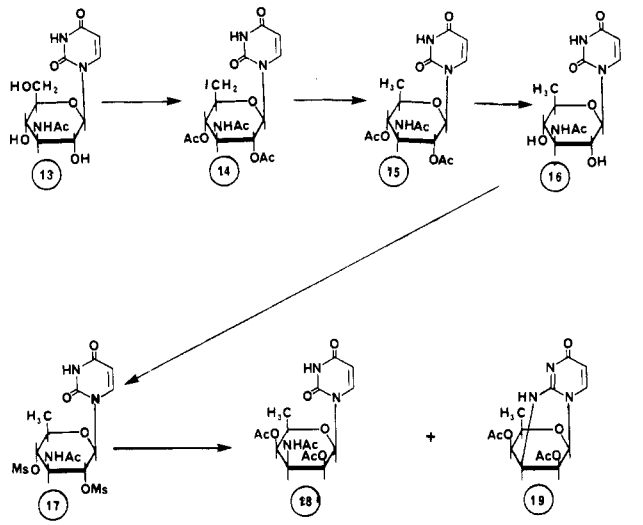


Figure 1. Structure of 2,2′-anhydro-3′,4′-oxazoline intermediate. **20**, R = OH; **21**, R = H.

Scheme III



In order to ascertain if the 6′-OH was involved in the conversion of **1** to **3** and **8** to **11**, we synthesized the 6′-deoxy analogue **16** as shown in Scheme III. Treatment of 1-(3′-acetamido-3′-deoxy-β-D-glucopyranosyl)uracil (**13**)[14] with *N*-iodosuccinimide and triphenylphosphine in *N*,*N*-dimethylformamide (DMF) followed by acetylation of the product afforded the crystalline 6′-iodide **14** which was reduced catalytically to the 6′-deoxy nucleoside **15**. Methanolysis of **15** to **16** followed by mesylation gave 1-(3′-acetamido-3′,6′-dideoxy-2′,4′-di-*O*-mesyl-β-D-glucopyranosyl)uracil (**17**). Solvolysis of **17** gave a mixture which (after peracetylation) afforded 1-(3′-amino-2′,4′-di-*O*-acetyl-3′,6′-dideoxy-β-D-talopyranosyl)uracil (**18**) and 2,3′-imino-1-(3′-acetamido-2′,4′-di-*O*-acetyl-3′,6′-dideoxy-β-D-talopyranosyl)isocytosine (**19**) isolated in 42% and 47% yield, respectively. The UV and ^1H NMR spectral characteristics as well as combustion analyses of these products are consistent with structures **18** and **19**, respectively.

The above results indicate that the hydroxyl group at C-6′ is not involved mechanistically in the 2,3′-imino linkage formation. The fact that the 2′-monomesylate **9**

(11) Doerr, I. L.; Cushley, R. J.; Fox, J. J. *J. Org. Chem.* **1968**, *33*, 1592.
(12) The proton dihedral angles 1′,2′, 2′,3′, 3′,4′, and 4′,5′ are 49°, 87°, 33°, and 33°, respectively, for the talo isomer and 49°, 87°, 87°, and 153°, respectively, for the manno derivative. These values were calculated arithmatically from the angles quoted by Angyal and Hoskinson (Angyal, S.; Hoskinson, R. M. *J. Chem. Soc.* **1962**, 2991) with the assumption that the pyranoid ring in **7** and **14** has geometry similar to that of an idealized cyclohexane ring. The actual geometry, of course, deviates from the theoretical one, and, consequently, ^1H NMR data do not match perfectly.
(13) Studies with molecular models showed that the skew conformation in **7** and **14** is not so flexible due to the 2,6-dioxa-8-azabicyclo-[3.3.1]nonane system.
(14) Matsuda, A.; Chun, M. W.; Watanabe, K. A. *Nucleosides Nucleotides* **1982**, *1*, 173.
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(20) Kikugawa, K.; Ichino, M.; Ukita, T. *Chem. Pharm. Bull.* **1969**, *17*, 785.

was converted almost quantitatively to the mannosyluracil **10** whereas the 2′,4′-dimesylates **1** and **17** gave the corresponding 2,3′-imino products **3** and **19** strongly suggests the formation of 2,2′-anhydro-3′,4′-oxazolines as intermediates (**20** and **21**, Figure 1). Studies with molecular models show that the most favorable conformation for **20** and **21** is $^0S_2$ in which the oxazoline nitrogen (3′-N) is close enought to C-2 to form the imino bridge[21] by the assitance of nucleophilic attack of the acetate ion on the oxazoline ring as shown in Figure 1.[22] The formation of the imino-bridged nuclsodie **11** as the major product by solvolysis of the 3′-amino-2′-mesylate **8** may be due to greater nucleophilicity of the amino in **8** than the acetamido group in **9** under the solvolytic conditions (NaOAc in aqueous methylcellosolve).[23] It should be noted that treatment of **1** with aqueous sodium hydroxide followed by acetylation of the product afforded only the talosyluracil **2**.[6] This may be due to the rapid hydrolysis of the 2,2′-anhydro linkage in **20** in aqueous alkaline medium. It is also interesting to note that in a trans 2′-"down"-(sulfonyloxy)-3′-"up"-hydroxy system, base treatment always gives nucleoside 2′,3′-"up"-epoxide, and this system has been utilized effectively in the synthesis of 2′,3′-dideoxy nucleosides including the natural products cytosinine[24] and pentopyranine A.[25] It is, therefore, surprising that the amino mesylate **8** was converted predominantly into the 2,3′-imino isocytosine nucleoside **11** and not into the 2′,3′-aziridine isomer **12**.

Experimental Section

General. Melting points were determined on a Thomas-Hoover capillary apparatus and are corrected. Column chromatography was performed on silica gel G60 (70–230 mesh, ASTM, Merck). Elemental analyses were performed by Galbraith Laboratories, Inc. ^{1}H NMR spectra were recorded on a JEOL PFT-100 spectrometer using Me_2SO-d_6 as the solvent and Me_4Si as the internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), dd (double doublet), dt (doublet triplet), m (multiplet), br s (broad singlet), br d (broad doublet). Values given for coupling constants are first order. IR spectra were recorded on a Perkin-Elmer Infracord using pressed KBr pellets. UV spectra were measured on a UNICAM SP-800 spectrometer or on a Cary Model 15 recording spectrometer. HPLC was performed on a μBondapak C_{18} column (7.8 × 300 mm, 10 μm) using a Waters Model 6000A solvent delivery system, equipped with a Waters U6K injector and a Series 440 absorbance detector.

Solvolysis of 1-(3′-Acetamido-3′-deoxy-2′,4′-di-*O*-mesyl-β-D-glucopyranosyl)uracil (1). Synthesis of 1-(3′-Acetamido-2′,4′,6′-tri-*O*-acetyl-3′-deoxy-β-D-talopyranosyl)uracil (2) and 2,3′-Imino-1-(3′-amino-2′,4′,6′-tri-*O*-acetyl-3′-deoxy-β-D-talopyranosyl)isocytosine (3). A mixture of **1**[6] (490 mg, 1.04 mmol) and NaOAc (2.0 g) in a 9:1 mixture of methylcellosolve and water (100 mL) was heated under reflux for 24 h and then concentrated in vacuo to dryness. The residue, after being dried further by several azeotropic distillations with toluene, was treated with pyridine (20 mL) and Ac_2O (2.0 mL) overnight at room temperature. The mixture was concentratted in vacuo, and the residue was thoroughly extracted with acetone. The combined acetone extracts were evaporated in vacuo, and the residue was chromatographed over a silica gel column (2.2 × 35 cm) using 8% (v/v) EtOH/$CHCl_3$ as the eluent. Compound **3** was eluted first from the column followed by **2**. The first nucleosidic fraction was concentrated and the residue recrystallized from acetone to afford **3** (182 mg, 46%): mp 156–159 °C; UV λ_{max} (H_2O) 212 nm, 262 (sh), (ϵ 34000, 2800), λ_{max} (0.1 N HCl), 253 (5900), λ_{min} (0.1 N HCl) 241 (6,110); ^{1}H NMR δ 1.96 (3 H, s, Ac), 2.04 (3 H, s, Ac), 2.06 (3 H, s, Ac), 3.71 (1 H, dd, H-6′, $J_{5',6'}$ = 7.5, $J_{6',6''}$ = 12.0 Hz), 4.04 (1 H, dd, H-3′, $J_{2',3'}$ = 3.1, $J_{3',4'}$ = 3.7 Hz), 4.06 (1 H, dd, H-6″, $J_{5',6'}$ = 5.2, $J_{6',6''}$ = 12.0 Hz), 4.40 (1 H, dt, H-5′, $J_{4',5'}$ = $J_{5',6'}$ = 7.5, $J_{5',6''}$ = 5.2 Hz), 5.23 (1 H, t, H-2′, $J_{1',2'}$ = $J_{2',3'}$ = 3.1 Hz), 5.30 (1 H, dd, H-4′, $J_{3',4'}$ = 3.7, $J_{4',5'}$ = 7.5 Hz), 5.64 (2 H, d, H-5 and H-1′, $J_{5,6}$ = 7.5 Hz), 7.53 (1 H, d, H-6, $J_{5,6}$ = 7.5 Hz), 8.66 (1 H, br s, NH).

Anal. Calcd for $C_{16}H_{19}N_3O_8$: C, 50.39; H, 5.02; N, 11.02. Found: C, 50.20; H, 5.20; N, 10.92.

The second nucleosidic fraction contained **2** which was recrystallized from MeOH. Yield of **2** was 180 mg (42%), mp 187–189 °C. The ^{1}H NMR and IR spectra of this sample were identical with those of an authentic sample.[6]

1-(3′-Amino-4′,6′-*O*-benzylidene-3′-deoxy-β-D-glucopyranosyl)uracil (5). To a solution of **4**[14] (4.2 g, 10.7 mmol) in 15% aqueous MeOH (230 mL) was added active Raney Ni (10 g, wet weight), and the mixture was shaken in an H_2 atmosphere for 3 h at an initial pressure of 45 psi. The catalyst was removed by filtration and washed with EtOH. The combined filtrate and washings were concentrated in vacuo, and the residue was recrystallized from EtOH to give 3.2 g (83%) of **5**: mp 234–240 °C; UV (EtOH) λ_{max} 258 nm (ϵ 8000), λ_{min} 228 (2800); ^{1}H NMR δ 5.55 (1 H, d, H-1′, $J_{1',2'}$ = 8.9 Hz), 5.59 (1 H, s, PhC*H*), 5.66 (1 H, d, H-5, $J_{5,6}$ = 8.2 Hz), 7.40 (5 H, m, Ph), 7.70 (1 H, d, H-6, $J_{5',6'}$ = 8.2 Hz).

Anal. Calcd for $C_{17}H_{19}N_3O_6$: C, 56.51; H, 5.30; N, 11.63. Found: C, 56.35; H, 5.51; N, 11.48.

1-(4′,6′-*O*-Benzylidene-3′-[(*tert*-butoxycarbonyl)amino]-3′-deoxy-β-D-glucopyranosyl)uracil (6). To a refluxing solution of **5** (3.61 g, 10 mmol) in 10% aqueous DMF (50 mL) was added dropwise a solution of *tert*-butoxycarbonyl *N*-hydroxysuccinimide ester (3.6 g, 15 mmol) in THF (50 mL) over a period of 1 h. The reaction mixture was refluxed for further 4 h, and then concentrated in vacuo. The residue was chromatographed over a silica gel column (3.5 × 20 cm) using 8% EtOH in $CHCl_3$ as the eluent. The major UV absorbing fraction was concentrated to give **6** (3.7 g, 79%) as colorless crystals: mp 218–219 °C (eff); UV (EtOH) λ_{max} 258 nm (ϵ 8300), λ_{min} 228 (2890); ^{1}H NMR δ 1.43 (9 H, s, *t*-Bu), 5.53 (1 H, s, PhC*H*), 5.71 (1 h, d, H-5, $J_{5',6'}$ = 8.2 Hz), 7.40 (5 H, m, Ph), 7.48 (1 H, d, H-6, $J_{5',6'}$ = 8.2 Hz).

Anal. Calcd for $C_{22}H_{27}N_3O_8 \cdot 0.5H_2O$: C, 56.16; H, 6.00; N, 8.93. Found: C, 56.46; H, 6.07; N, 8.86.

1-(4′,6′-*O*-Benzylidene-3′-[(*tert*-butoxycarbonyl)amino]-3′-deoxy-2′-*O*-mesyl-β-D-glucopyranosyl)uracil (7). To a solution of **6** (2.3 g, 5 mmol) in dry pyridine (20 mL) was added MsCl (1.2 mL, 15 mmol), and the mixture was stirred for 3 h at room temperature. The solvent was removed in vacuo, and the residue was chromatographed on a silica gel column (2.5 × 20 cm) using 4% EtOH in $CHCl_3$ as the eluent. Upon concentration of the major UV absorbing fraction, 2.61 g (97%) of **7** was obtained as colorless crystals: mp 187–188 °C dec; UV (EtOH) λ_{max} 255 nm (ϵ 10000), λ_{min} 225 (2570); ^{1}H NMR δ 1.37 (9 H, s, *t*-Bu), 3.09 (3 H, s, Ms), 5.58 (1 H, s, PhC*H*), 5.73 (1 H, d, H-5, $J_{5',6'}$ = 8.2 Hz), 7.20 (1 H, d, H-1′, $J_{1',2'}$ = 8.9 Hz), 7.40 (5 H, s, Ph), 7.61 (1 H, d, H-6, $J_{5',6'}$ = 8.2 Hz).

Anal. Calcd for $C_{23}H_{29}N_3O_{10}S$: C, 51.20; H, 5.42; N, 7.79; S, 5.94. Found: C, 51.09; H, 5.53; N, 7.76; S, 5.82.

1-(3′-Amino-3′-deoxy-2′-*O*-mesyl-β-D-glucopyranosyl)uracil Trifluoroacetic Acid Salt (8). Compound **7** (2.16 g, 5 mmol) was treated with 90% aqueous CF_3CO_2H (20 mL) for 1.5 h at room temperature. The mixture was concentrated in vacuo, and the residue was triturated with EtOH to give crystalline **8**, 1.8 g (97%): mp 211–213 °C dec; UV (H_2O) λ_{max} 257 nm (ϵ 7600), λ_{min} 222 (2400); ^{1}H NMR δ 3.31 (3 H, s Ms), 5.72 (1 H, d, H-5, $J_{5',6'}$ = 7.9 Hz), 5.88 (1 H, d, H-1′, $J_{1',2'}$ = 8.0 Hz), 7.58 (1 H, d, H-6, $J_{5',6'}$ = 7.9 Hz), 8.48 (3 H, br s, NH_3^+), 11.37 (1 H, s, NH).

Anal. Calcd for $C_{11}H_{17}N_3O_8S \cdot CF_3CO_2H$: C, 33.55; H, 3.90; N, 9.03; S, 6.89 Found: C, 33.43; H, 3.86; N, 8.89; S, 6.74.

(21) Direct measurement of the distance between the oxazolidine nitrogen and the 2-position of the pyrimidine in a Dreiding molecular model in the $^0S_2$ conformation yields the value of about 3.00–3.13 Å.

(22) Opening of oxazoline in basic media should result in the formation of *N*-acetylaminohydrin[7-9] (not vic-amino alcohol) which, unlike **8**, would not give rise to a 2,3′-imino bridge.

(23) Amine **8** is not protonated during solvolysis due to the presence of a large excess of NaOAc.

(24) Watanabe, K. A.; Wempen, I.; Fox, J. J. *Chem. Pharm. Bull.* **1970**, *18*, 236.

(25) Watanabe, K. A.; Chiu, T. M. K.; Hollenberg, D. H.; Fox, J. J. *J. Org. Chem.* **1974**, *39*, 2487.

1-(3′-Acetamido-4′,6′-di-*O*-acetyl-3′-deoxy-2′-*O*-mesyl-β-D-glucopyranosyl)uracil (9). Compound **8** (400 mg, 0.86 mmol) was treated with Ac_2O (0.6 mL) in pyridine (10 mL) overnight at room temperature. The mixture was concentrated in vacuo, and the residue was chromatographed on a silica gel column (2.2 × 10 cm) using 4% EtOH in $CHCl_3$ as the eluent. The UV absorption fraction was concentrated, and the residue was crystallized from EtOH–Et_2O to give 330 mg (80%) of **9**: mp 140 °C (eff); ^1H NMR δ 1.82 (3 H, s, Ac), 2.01 (6 H, s, Ac), 3.08 (3 H, s, Ms), 4.1–5.0 (6 H, m, H-2′,3′,4′,5′,6′,6″), 5.73 (1 H, d, H-5, $J_{5,6}$ = 8.1 Hz), 6.04 (1 H, br s, H-1′), 7.54 (1 H, d, H-6), 8.03 (1 H, br s, N*H*Ac), 11.41 (1 H, br s 3-NH).

Anal. Calcd for $C_{17}H_{23}N_3O_{11}S$: C, 42.77; H, 4.85; N, 8.80; S, 6.71. Found: C, 42.63; H, 4.96; N, 8.67; S, 6.92.

Solvolysis of 8. Synthesis of 2,3′-Imino-1-(3′-amino-2′,4′,6′-tri-*O*-acetyl-3′-deoxy-β-D-mannopyranosyl)isocytosine (11). A mixture of **8** (465 mg, 1 mmol) and NaOAc (2.0 g) in a 9:1 mixture of methyl cellosolve and water (100 mL) was heated under reflux for 24 h, and then was concentrated in vacuo. The residue was treated with Ac_2O (2 mL) in pyridine (20 mL) overnight at room temperature. The mixture was concentrated in vacuo, and the residue was thoroughly extracted with acetone. The combined extracts were evaporated in vacuo and the residue chromatographed over a silica gel column (2.2 × 30 cm) using 10% EtOH in $CHCl_3$ as the eluent. 1-(*N*-Acetyl-4′,6′-di-*O*-acetyl-2′,3′-dideoxy-2′,3′-epimino-β-D-mannopyranosyl)uracil (**12**) was eluted first from the column. Concentration of the fraction in vacuo followed by recrystallization of the residue from acetone afforded **12** (50 mg, 13%): mp 188–189 °C; ^1H NMR δ 2.01 (3 H, s, Ac), 2.09 (3 H, s, Ac), 2.12 (3 H, s, Ac), 3.05 (1 H, d, H-6′, $J_{6',6''}$ = 6.1 Hz), 3.17 (1 H, d, H-6″, $J_{6',6''}$ = 6.1 Hz), 3.99 (1 H, d, H-3′, $J_{2',3'}$ = 8.5, $J_{3',4'}$ = 0 Hz), 4.07 (2 H, br s H-4′,5′), 4.66 (1 H, d, H-2′, $J_{1',2'}$ = 0, $J_{2',3'}$ = 8.5 Hz), 5.67 (1 H, d, H-5, $J_{5,6}$ = 8.1 Hz), 6.19 (1 H, s, H-1′), 7.66 (1 H, d, H-6, $J_{5,6}$ = 8.1 Hz), 11.52 (1 H, br s, NH).

Anal. Calcd for $C_{16}H_{19}N_3O_8$: C, 50.39; H, 5.02; N, 11.02. Found: C, 50.30; H, 5.15; N, 10.93.

Upon concentration of the second UV absorbing fraction and recrystallization of the residue from acetone, **11** (300 mg, 79%) was obtained as colorless crystals: mp 237–238 °C; UV (H_2O) λ_{max} 212 nm, 262 (sh) (ε 39 000, 3720), λ_{max} (0.1 N HCl) 253 (7550), λ_{min} 241 (6590); ^1H NMR δ 1.98 (3 H, s, Ac), 2.03 (3 H, s, Ac), 2.11 (3 H, s, Ac), 3.80 (1 H, m, H-6′), 3.86 (2 H, m, H-3′,6″), 4.07 (1 H, m, H-5′), 4.99 (1 H, d, H-4′, $J_{3',4'}$ = 0, $J_{4',5'}$ = 3.4 Hz), 5.26 (1 H, t, H-2′, $J_{1',2'}$ = $J_{2',3'}$ = 2.9 Hz), 5.64 (1 H, d, H-5, $J_{5,6}$ = 7.6 Hz), 5.68 (1 H, d, H-1′, $J_{1',2'}$ = 2.9 Hz), 7.55 (1 H, d, H-6, $J_{5,6}$ = 7.6 Hz), 8.82 (1 H, br s N*H*Ac).

Anal. Calcd for $C_{16}H_{19}N_3O_8$: C, 50.39; H, 5.02; N, 11.02. Found: C, 50.30; H, 5.15; N, 10.93.

Solvolysis of 9. Synthesis of 1-(3′-Acetamido-2′,4′,6′-tri-*O*-acetyl-3′-deoxy-β-D-mannopyranosyl)uracil (10). A mixture of **9** (239 mg, 0.5 mmol) and NaOAc (1.0 g) in a 9:1 mixture of methylcellosolve and water (50 mL) was refluxed for 24 h and then concentrated in vacuo. The residue was acetylated and chromatographed as described above for the preparation of **11**. Compound **10** was obtained as a foam (212 mg, 96%). The ^1H NMR spectrum of this sample was identical with that of an authentic sample.[15]

1-(3′-Acetamido-2′,4′-di-*O*-acetyl-3′,6′-dideoxy-6′-iodo-β-D-glucopyranosyl)uracil (14). A mixture of **13**[26] (2.8 g, 8.9 mmol), Ph_3P (7.2 g, 27.5 mmol) and *N*-iodosuccinimide (5.5 g, 24.4 mmol) in DMF (30 mL) was stirred for 3 h at 60–65 °C. EtOH (5 mL) was added, and the mixture was concentrated in vacuo. The residue was partitioned between water and Et_2O. The aqueous layer was concentrated and the residue further dried by azeotropic distillations with EtOH. The residue dissolved in MeOH was mixed with silica gel (50 g). The mixture was dried in vacuo and then placed on the top of a silica gel column (4 × 24 cm). The column was washed with 16% EtOH in $CHCl_3$. The major UV absorbing fraction was concentrated in vacuo, and the residue was suspended in a mixture of 4-(dimethylamino)pyridine (500 mg) and Ac_2O (5 mL) in EtOAc (150 mL). The mixture was stirred at room temperature for 4 h and then concentrated to a half-volume. Compound **14** separated and was collected by filtration and washed with a small amount of EtOAc, 3.11 g (69%): mp 276–277 °C; UV (MeOH) λ_{max} 256 nm (ε 9900), λ_{min} 229 (3540); ^1H NMR δ 1.74 (3 H, s, NAc), 1.89 (3 H, s, OAc), 2.01 (3 H, s, OAc), 3.22 (1 H, dd, H-6′, $J_{5',6'}$ = 5.8, $J_{6',6''}$ = 11.2 Hz), 3.49 (1 H, dd, H-6″, $J_{5',6'}$ = 2.6, $J_{6',6''}$ = 11.2 Hz), 3.89 (1 H, m, H-5′), 4.47 (1 H, t, H-3′, $J_{2',3'}$ = $J_{3',4'}$ = 9.8 Hz), 4.86 (1 H, t, H-4′, $J_{3',4'}$ = $J_{4',5'}$ = 9.8 Hz), 5.04 (1 H, t, H-2′, $J_{1',2'}$ = $J_{2',3'}$ = 9.8 Hz), 5.76 (1 H, d, H-5, $J_{5,6}$ = 8.1 Hz), 5.98 (1 H, d, H-1′, $J_{1',2'}$ = 9.8 Hz), 7.48 (1 H, d, H-6), 7.91 (1 H, br d, N*H*Ac), 11.42 (1 H, br s, NH).

Anal. Calcd for $C_{16}H_{20}IN_3O_8$: C, 37.74; H, 3.96; I, 24.92; N, 8.25. Found: C, 37.85; H, 4.00; I, 25.16; N, 8.18.

1-(3′-Acetamido-2′,4′-di-*O*-acetyl-3′,6′-dideoxy-β-D-glucopyranosyl)uracil (15). A solution of **14** (2.0 g, 3.9 mmol) in a 1:1 mixture of EtOH and EtOAc (100 mL) was hydrogenated at room temperature in the presence of Et_3N (0.66 mL, 1.2 equiv) and 10% Pd/C (500 mg). After the theoretical amount of H_2 was consumed, the catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residue was suspended in MeOH (50 mL), and silica gel (60 g) was added. The mixture was dried in vacuo, and the residue was placed on the top of a silica gel column (3 × 10 cm) which was washed with 10% EtOH in CH_2Cl_2. The major UV absorbing fraction was concentrated in vacuo, and the residue crystallized from water to give 1.35 g (90%) of **15**: mp 286–287 °C; UV (MeOH) λ_{max} 258 nm (ε 9200), λ_{min} 230 (3030); ^1H NMR δ 1.11 (3 H, d, 5′-Me), 1.75 (3 H, s, NAc), 1.88 (3 H, s, OAc), 2.01 (3 H, s, OAc), 3.96 (1 H, m, H-5′), 4.36 (1 H, t, H-3′, $J_{2',3'}$ = $J_{3',4'}$ = 9.8 Hz), 4.75 (1 H, t, H-4′, $J_{3',4'}$ = $J_{4',5'}$ = 9.8 Hz), 5.07 (1 H, t, H-2′, $J_{1',2'}$ = $J_{2',3'}$ = 9.8 Hz), 5.69 (1 H, d, H-5, $J_{5,6}$ = 8.2 Hz), 5.82 (1 H, d, H-1′, $J_{1',2'}$ = 9.8 Hz), 7.59 (1 H, d, H-6), 7.86 (1 H br d, N*H*Ac), 11.40 (1 H, br s, NH).

Anal. Cacld for $C_{16}H_{21}N_3O_8$: C, 50.13; H, 5.52; N, 10.96. Found: C, 50.12; H, 5.46; N, 10.91.

1-(3′-Acetamido-3′,6′-dideoxy-β-D-glucopyranosyl)uracil (16). Compound **15** (1.3 g, 3.4 mmol) was treated with NH_3/MeOH (100 mL, saturated at 0 °C) for 24 h at room temperature. After concentration of the mixture, the residue was crystallized from EtOH–Et_2O to give 900 mg (89%) of **16**: mp 170–175 °C (eff); UV (MeOH) λ_{max} 258 nm (ε 9300), λ_{min} 229 (2320); ^1H NMR δ 1.17 (3 H, d, 5′-Me), 1.86 (3 H, s, NAc), 3.1–3.6 (4 H, m, H-2′,3′,4′,5′), 5.37 (1 H, d, H-1′, $J_{1',2'}$ = 8.2 Hz), 5.70 (1 H, d, H-5, $J_{5,6}$ = 8.2 Hz), 7.57 (1 H, d, H-6), 7.96 (1 H, br d, N*H*Ac).

Anal. Calcd for $C_{12}H_{17}N_3O_6{\cdot}0.5H_2O$: C, 46.75; H, 5.88; N, 13.63. Found: C, 46.47; H, 5.89; N, 13.79.

1-(3′-Acetamido-3′,6′-dideoxy-2′,4′-di-*O*-mesyl-β-D-glucopyranosyl)uracil (17). To a solution of **16** (502 mg, 1.7 mmol) in dry pyridine (20 mL) was added MsCl (0.52 mL, 2 equiv) at 0 °C. The mixture was stirred at room temperature for 5 h. The reaction was quenched by addition of EtOH (5 mL) and then concentrated in vacuo below 40 °C. The residue was chromatographed on a silica gel column (2 × 15 cm) using 15% EtOH in CH_2Cl_2 as the eluent. The major UV absorbing fraction was concentrated in vacuo, and the residue crystallized from EtOH, mp 180–181 °C (eff). Recrystallization of the product from MeOH–water gave 588 mg (77%) of **17**, mp 188–190 °C (lit.[6] mp 188.5–191 °C). The ^1H NMR spectrum of this sample was identical with that of an authentic sample.[6]

Solvolysis of 17. Preparation of 2,3′-Imino-1-(3′-amino-2′,4′-di-*O*-acetyl-3′,6′-dideoxy-β-D-talopyranosyl)isocytosine (19) and 1-(3′-Acetamido-2′,4′-di-*O*-acetyl-3′,6′-dideoxy-β-D-talopyranosyl)uracil (18). A mixture of **17** (227 mg, 0.5 mmol) and NaOAc (1.0 g) in a 9:1 mixture of methylcellosolve and water (50 mL) was heated at reflux for 24 h, and then concentrated in vacuo. The residue, after being further dried by several azeotropic distillations with toluene, was treated with dry pyridine (10 mL) and Ac_2O (3 mL) overnight at room temperature. The mixture was concentrated in vacuo, and the residue was suspended in acetone. Undissolved materials were removed by filtration. The filtrate was applied on two preparative TLC plates (20 × 20 × 0.1 cm) which were developed in $CHCl_3$–EtOH (15:1). The major band was collected and extracted with 50% EtOH in $CHCl_3$. Upon evaporation of the extracts and crystallization of the residue from EtOH, the 2,3′-imino derivative **19** (36 mg) was obtained as a hydrate: mp 168–171 °C; UV (H_2O) λ_{max} 218 nm, 260 (sh) (ε 23 300, 3300), λ_{max} (0.1 N HCl) 255 (6240), λ_{min} (0.1 N HCl) 240

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(5050); ^{1}H NMR δ 0.95 (3 H, d, 5'-Me), 2.04 (3 H, s, Ac), 2.07 (3 H, s, Ac), 4.03 (1 H, m, H-3'), 4.38 (1 H, m, H-5'), 5.12 (1 H, dd, H-4', $J_{3',4'}$ = 3.7, $J_{4',5'}$ = 7.3 Hz), 5.17 (1 H, t, H-2', $J_{1',2'}$ = $J_{2',3'}$ = 3.2 Hz), 5.57 (1 H, dd, H-1', $J_{1',2'}$ = 3.2, $J_{1',3'}$ = 1.5 Hz), 5.69 (1 H, d, H-5, $J_{5,6}$ = 7.6 Hz), 7.56 (1 H, d, H-6), 8.64 (1 H, br s, NH).

Anal. Calcd for $C_{14}H_{17}N_3O_6 \cdot H_2O$: C, 49.26; H, 5.61; N, 12.31. Found: C, 49.53; H, 5.54; N, 12.44.

The HPLC analysis of the mother liquor of crystallization showed that there were two major components in the solution. Separation of these components was achieved by HPLC on a semipreparative reverse-phase column using H_2O–MeOH (70:30) as the mobile phase. From the first fraction (rV = 20 mL), after concentration in vacuo and crystallization of the residue from acetone, an additional 40 mg of **19** (total yield 47%) was obtained, mp 168–170 °C. The second fraction (rV = 29 mL) was concentrated and the residue crystallized from acetone. Compound **18** (80 mg, 42%) was obtained as colorless crystals: mp 172–174 °C; UV (MeOH) λ_{max} 262 nm (ε 7050), λ_{min} 230 (1520); ^{1}H NMR δ 1.10 (3 H, d, 5'-Me), 1.83 (3 H, s, NAc), 2.03 (3 H, s, OAc), 2.15 (3 H, s, OAc), 4.28 (1 H, m, H-5'), 4.68 (1 H, m, H-3'), 4.89 (1 H, br s, H-4'), 5.00 (1 H, br s, H-2'), 5.63 (1 H, d, H-5, $J_{5,6}$ = 8.1 Hz), 5.92 (1 H, s, H-1', $J_{1',2'}$ = 0 Hz), 7.45 (1 H, d, H-6), 7.76 (1 H, br d, N*H*Ac), 11.39 (1 H, br s, NH).

Anal. Calcd for $C_{16}H_{21}N_3O_8 \cdot {}^1/_3H_2O$: C, 49.35; H, 5.61; N, 10.79. Found: C, 49.43; H, 5.91; N, 10.66.

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Nucleic Acid Related Compounds. 48. Photoaddition of Methanol to 9-(β-D-Ribofuranosyl)purine (Nebularine) To Give Inhibitors of Adenosine Deaminase[1]

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Photolysis of anaerobic solutions of 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (2',3',5'-tri-*O*-acetylnebularine) (**1b**) in methanol at 2537 Å gave a diastereomeric mixture of 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,6-dihydro-6(*R*,*S*)-(hydroxymethyl)purines (**2b**) plus secondary photoproducts. The presence of oxygen resulted in more rapid formation of the 6-(hydroxymethyl)- (**3b**), 1,6-dihydro-6,6-bis(hydroxymethyl)- (**4b**), 6-methyl- (**5b**), and (*R*,*S*)-1,6-dihydro-6-(hydroxymethyl)-6-methyl-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**6b**) byproducts. Reevaluation of recently published claims that photoaddition of methanol to nebularine proceeded with high stereoselectivity is presented based on definitive ^{1}H and ^{13}C NMR spectral data and FAB mass spectrometry.

Adenosine deaminase (adenosine aminohydrolase EC 3.5.4.4) is a crucial catabolic enzyme in the regulation of metabolism of adenosine and 2'-deoxyadenosine.[2] It also effects deamination (and related hydrolytic displacements) of a number of adenine nucleoside analogues and thereby diminishes their effectiveness as drugs.[3] There has been considerable recent interest in the pharmacological evaluation of inhibitors of this enzyme as single agents and as substrate drug/inhibitor combinations.[4]

The naturally occurring 3-(β-D-ribofuranosyl)- (coformycin, Ia)[5] and 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8(*R*)-ol (2'-deoxycoformycin, covidarabine, Ib)[6] nucleosides are the most potent known inhibitors of adenosine deaminase.[7,8] These two compounds are thought to function as "transition-state analogue" inhibitors.[9-11] Photoaddition of methanol to 9-(β-D-ribofuranosyl)purine (nebularine) (**1a**) was reported to give a product, 1,6-dihydro-6(hydroxymethyl)nebularine (**2a**),[12] that is a strong reversible inhibitor of this enzyme.[8,9,11,12] It also is thought to function as a transition-state analogue[9,11-13] with a less complementary fit at the catalytic center than I.

Our continuing interest in substrate binding to adenosine deaminase[3b,14] led us to consider evaluation of related putative tight-binding nucleoside analogues. Some of our prior results were not in harmony with conclusions pub-

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