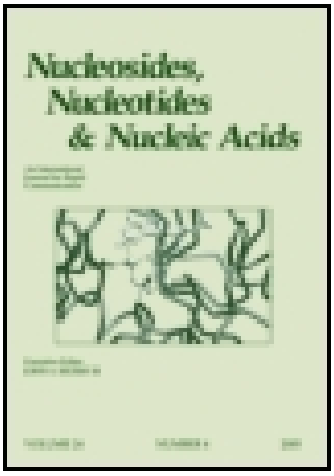

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Synthesis and Biological Evaluation of Pyrimidine and Purine α-L-2′,3′-Dideoxy Nucleosides

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SYNTHESIS AND BIOLOGICAL EVALUATION OF PYRIMIDINE AND PURINE α-L-2',3'-DIDEOXY NUCLEOSIDES

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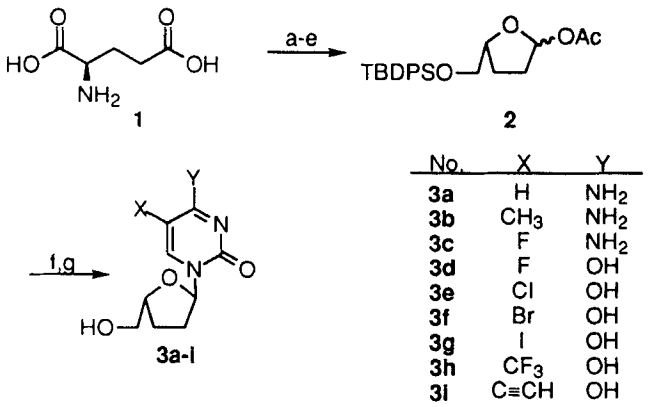
Abstract. A series of α-L-2',3'-dideoxy nucleosides was prepared as potential antiviral agents. The pyrimidine nucleosides were prepared by standard Vorbrüggen coupling reactions. The purine analogues were prepared by enzymatic transfer of the dideoxy sugar from a pyrimidine to a purine base. These compounds were inactive against HIV-1, HBV, HSV-1 and -2, VZV, and HCMV.

Introduction. The search for novel nucleoside structures for use as antiviral agents has led to an explosion of synthetic effort in the field.[1] While it was long believed that only the "natural" stereoisomeric nucleoside structures (those possessing the β-D-configuration) would have any antiviral activity, recently there have been examples of "unnatural", enantiomeric (β-L) nucleosides with activity against human immunodeficiency virus (HIV),[2] hepatitis B virus (HBV),[3] and herpes simplex virus (HSV-1).[4] Examples of α-D-nucleosides are plentiful in the literature, usually arising from an unselective coupling of a 2-deoxy sugar to a pyrimidine or purine heterocycle.[5] In all cases reported, these compounds were devoid of antiviral activity. Our interest in α-L-nucleosides lay in the supposition that the lack of antiviral activity of the α-D-isomers may be due to the unnatural configuration of the 1'-carbon. The α-L-nucleosides maintain the natural configuration of $C_{1'}$ but have inverted configuration at $C_{4'}$. Exploration of this series could give greater insight into the stereochemical requirements for antiviral activity. This report presents the preparation and evaluation of a series of novel pyrimidine and purine α-L-2',3'-dideoxynucleosides.

Chemistry. The synthesis of pyrimidine α-L-2',3'-dideoxy nucleosides was effected in a straightforward manner from D-glutamic acid, **1** (scheme 1). The same general synthetic method has been applied to the preparation of β-D-dideoxy nucleosides using L-glutamic acid as the starting material.[6] Our synthesis followed these literature procedures but yielded the products with the opposite configuration. A straightforward, five-step



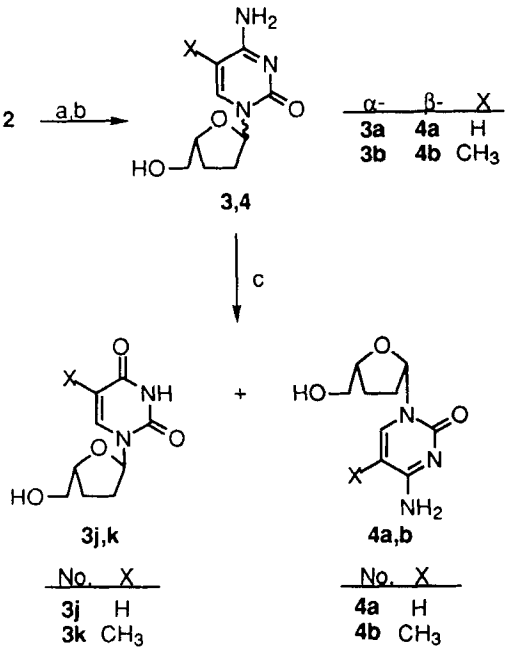
No.	X	Y
3a	H	NH_2
3b	CH_3	NH_2
3c	F	NH_2
3d	F	OH
3e	Cl	OH
3f	Br	OH
3g	I	OH
3h	CF_3	OH
3i	C≡CH	OH

a. $NaNO_2/H^+$; b. $BH_3{\cdot}(CH_3)_2S$; c. TBDPSCl/Imidazole;
d. DIBAL; e. Ac_2O/pyridine; f. pyrimidine base/BSA; TMSOTf;
g. Et_4NF

Scheme 1

process from **1** gave acetoxy dideoxy sugar **2**.[7] This scheme is known to yield enantiomerically pure products when applied to the preparation of the D-isomers since all manipulations at the ultimate 4'-carbon proceed stereospecifically.[8] Coupling of **2** under standard Vorbrüggen[9] conditions followed by deprotection using tetraethylammonium fluoride gave the target nucleosides as α,β-anomeric mixtures.

The α,β-anomeric mixtures were separated either by preparative HPLC, flash chromatography, or by enzymatic resolution as shown in scheme 2. Preparative HPLC separation of the cytosine analogues gave compounds **3a-c**. The anomers of the uracil analogues were easily resolved by flash chromatography to give compounds **3d-3i.** Because chromatographic separations of the uracil and thymine analogues **3j and 3k,** respectively, were impractical or not possible, a different synthetic method was used which avoided the formation of the β-anomers. Preparation of α,β-L-dideoxycytidine (**3a, 4a**) and α,β-L-5-methyl-2',3'-dideoxycytidine (**3b, 4b**) was accomplished as described above. Cytidine deaminase deaminated the α-anomer of these compounds much more rapidly than the β-anomer. By taking advantage of this enzymatic selectivity, pure α–L-2',3'-dideoxyuridine (**3j**) and α–L-3'-deoxythymidine (**3k**) were prepared (Scheme 2). These compounds were easily separated from the unreacted β-cytidine analogues by flash chromatography.



a. cytosine or 5-methylcytosine/BSA/TMSOTf; b. Et_4NF;
c. *E. coli* cytidine deaminase, pH 7.5

Scheme 2

The assignment of the anomeric configuration of the separated products was determined by ^1H NOE experiments. Enhancement of the $H4'$ signals upon irradiation of $H6$ in compounds **3a-k** was consistent with the α-stereochemistry (Figure 1).

The purine dideoxynucleosides (**5a-d**) were far more difficult to prepare using Vorbrüggen-type conditions. Coupling reactions using purine bases were plagued by low yields and formation of complex mixtures of products (α, β; N-7 and N-9 isomers). As an alternative synthetic approach, a phosphorylase-catalyzed enzymatic trans-ribosylation was explored.[10] There is some literature precedent for α-nucleosides being substrates for nucleoside phosphorylases. In 1977, Doskoch and Hôly reported that 9-(α–L-lyxofuranosyl)adenine was cleaved by *E. coli* purine nucleoside phosphorylase (PNP).[11] In the same paper, however, they showed that 9-(α–L-arabinofuranosyl)adenine was not a substrate for PNP. To our knowledge, there is no literature precedent for the enzymatic transfer of α–L-dideoxynucleosides.

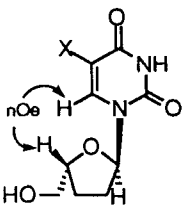
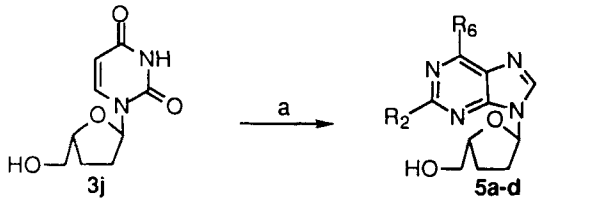


FIG 1: NOE enhancement of the $H4'$ signal upon irradiation of $H6$.



No.	R_2	R_6
5a	H	NH_2
5b	NH_2	NH_2
5c	H	OCH_3
5d	NH_2	OCH_3

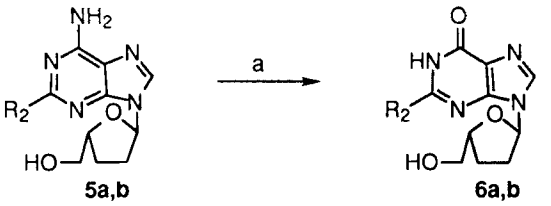
a. purine base/PNP/TPase/$K_xH_yPO_4$

Scheme 3

Under conditions modeled after those described previously for the thymidine phosphorylase (TPase) and PNP-catalyzed transfer of sugars from pyrimidine nucleosides to purine bases,[10] the pentosyl moiety of α–L-dideoxyuridine (**3j**) was efficiently transferred to various purine bases with retention of the α–L-configuration. The reactions were complete within 4-48 h, depending on the amount of enzyme used, and proceeded to 60-80% conversion based on HPLC analysis of the crude reaction mixtures. Four analogues, **5a-d**, were prepared by this method (Scheme 3).

Finally, the hypoxanthine and guanine analogues, **6a** and **6b**, were prepared by treatment of the corresponding adenine (**5a**) and diaminopurine (**5b**) analogues with adenosine deaminase (Scheme 4).

Biological Evaluation. Compounds **3a-k**, **5a-d**, and **6a-b** were evaluated in a variety of antiviral screens, including human immunodeficiency virus (HIV-1), herpes simplex virus (HSV-1 and HSV-2), varicella zoster virus (VZV), and cytomegalovirus (CMV). None of the compounds exhibited any significant antiviral activity ($IC_{50} > 40$ μM). None



5a,b 6a,b

a. adenosine deaminase

No.	R_2
6a	H
6b	NH_2

Scheme 4

of the compounds showed any significant cytotoxicity at 100 μM in IM-9, CEM, and Molt-4 cells. In addition, compounds **3a**, **3d**, **3f**, **3j**, **3k**, **5a**, and **6b** were evaluated against hepatitis B virus (HBV) and had IC_{50} values greater than 40 μM. From this lack of biological activity, we conclude that the relative configuration of $C_{1'}$ and $C_{4'}$ is critical to activity in the dideoxy series. Presumably, the trans configuration of the α-anomers prohibits recognition of these compounds by the cellular enzymes responsible for phosphorylation and/or incorporation and thereby renders them inactive.

The biological activity of the β-anomers described herein will be presented elsewhere.

Experimental Section

General. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. All reactions involving organometallic reagents were conducted under a N_2 atmosphere. Melting points (Pyrex capillary) are uncorrected. Concentrations for rotation data are given as g/100 mL of solvent. ^1H NMR spectra were recorded on a Varian XL-300 spectrometer. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane; coupling constants are expressed in hertz. UV data are reported as nm (ε ($M^{-1}cm^{-1}$)). Flash chromatography refers to the procedure of Still, Kahn, and Mitra.[12] Thymidine phosphorylase (EC 2.4.2.4, TPase) and purine nucleoside phosphorylase (EC 2.4.2.1, PNP) were purified from *E. coli*.[10] One unit of enzyme catalyzed the formation of 1 μmol of product per minute under the defined assay conditions. Cytidine deaminase (CDase) was purified from *E. coli*.[13] Calf intestinal adenosine deaminase (EC 3.5.4.4, ADA) was purchased from Boehringer Mannheim. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA.

HPLC Methods. *Buffer Preparation.* Buffer A: To 1800 mL of H_2O was added triethylamine (2.0 mL). To the stirring solution was added trifluoroacetic acid until the pH = 2.35 (approximately 1.5 mL). The solution was diluted to 2 L with H_2O. The pH of the final solution was 2.40. The solution was filtered through a 0.22 μm GS Millipore filter and stored at 4 °C.

Buffer B: To 1200 mL of CH_3CN was added H_2O (600 mL) and triethylamine (2.0 mL). To the stirring solution was added sufficient trifluoroacetic acid to give an apparent pH = 2.35 (approximately 1.5 mL). The mixture was diluted with H_2O to 2 L. The apparent pH of the final solution was 2.40. The solution was filtered through a 0.22 μm GS Millipore filter and stored at 4 °C.

Method 1: Analysis was performed on a Microsorb C_{18} reverse-phase HPLC column (4.6 mm x 15 cm, 5 μM particles) at a flow rate of 1 mL/min according to the gradient below. The effluent was monitored at 270 nm.

Stage	Equil Time (sec)	Buffer A	Buffer B	Gradient
1	60	95	5	
2	1200	90	10	linear
3	300	75	25	step

Method 2: Analysis was performed on a Microsorb C_{18} HPLC column (4.6 mm x 15 cm, 5 μM particles) at a flow rate of 1.5 mL/min according to the gradient below. The effluent was monitored at 265 nm.

Stage	Equil Time (sec)	50 mM NH_4Ac pH = 5.5	CH_3CN	Gradient
1	600	100	0	
2	1200	80	20	linear
3	120	0	100	linear

General procedure for the preparation of pyrimidine α-L-2',3'-dideoxynucleosides (3a-i). A mixture of an appropriately substituted pyrimidine base (3.8 mmol), *N,O*-bis(trimethylsilyl)acetamide (BSA, 2.8 mL, 11.3 mmol), and toluene (7.5 mL) was heated at 90 °C for 2-12 h, until the solution was homogeneous. The solution was cooled to 0 °C and a mixture of (2*RS*,5*S*)-2-acetoxy-5-((*tert*-butyldiphenylsilyl)oxymethyl)tetrahydrofuran[7] (**2**, 1.0 g, 2.5 mmol) in CH_3CN (4.0 mL) was added followed by trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.74 mL, 3.8

mmol). When no starting sugar remained as evidenced by TLC (30% ether/petroleum ether), the reaction was quenched by the addition of saturated $NaHCO_3$ solution (20 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 x 15 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and the solvent was removed by rotary evaporation to give the protected nucleosides as mixtures of α- and β-anomers. These intermediate products were purified by flash chromatography using an appropriate mixture of CH_2Cl_2:CH_3OH (generally, 99:1 to 95:5).

To a solution of silyl-protected nucleoside (2.5 mmol) in THF (15 mL) was added tetraethylammonium fluoride (TEAF, 0.55 g, 3.8 mmol, 1.5 eq). The reaction was monitored by TLC (95:5 CH_2Cl_2:CH_3OH) until no starting material could be detected. In most cases, the product was insoluble in THF and formed a thick gum on the walls of the flask. If the reaction was still incomplete after 12 h, additional TEAF was added in 0.5 eq portions at 30 min intervals until the reaction was complete. In some cases, a total of 5 eq of TEAF were required. Methanol was added to the reaction mixture until a homogeneous solution resulted. Silica gel (10-15 mL) was added and the solvents removed with a rotary evaporator. The resulting dry powder was applied to the top of a 5 x 20 cm flash chromatography column which had been packed with CH_2Cl_2:CH_3OH. The products were eluted with an appropriate gradient of CH_2Cl_2:CH_3OH. The fractions containing product were concentrated with a rotary evaporator to yield the separated α- and β-anomers as white powders, thick oils, or glasses. The residue was dissolved in a minimum amount of H_2O and lyophilized.

1-(α-L-2',3'-Dideoxyribofuranosyl)cytosine (3a). A 1:1 α:β mixture of L-2',3'-dideoxycytidine (**3a** and **4a**) was prepared as described in the general procedure (0.50 g, 71%). To separate the anomers, reverse-phase preparative HPLC using a Waters prep 2000 with model 486 monitor at 250 nm and two radial-compression column cartridges were employed. The column was equilibrated with 95:5 buffer A:buffer B (see general section for buffer preparation) at 20 mL/min. The product (0.50 g) was dissolved in H_2O (10 mL) and loaded onto the column at 20 mL/min. The flow rate was increased to 40 mL/min and the product eluted within 10 min. The portion of the eluant which was enriched in α-anomer (as determined by analytical HPLC using method 1 described in the general section) was collected and concentrated with a rotary evaporator. The residual product was dissolved in H_2O and re-loaded onto the prep HPLC column which had been equilibrated with 97:3 buffer A:buffer B. The product was applied at 20 mL/min and eluted at 40 mL/min. The portion of the eluant which was solely α-anomer was concentrated. Desalting was accomplished by dissolving the residue in CH_3OH and applying the solution to a 2.5 x 10 cm column of Dowex AG-1 (hydroxide form) resin packed with CH_3OH. The column was flushed with CH_3OH until no UV activity remained in the eluant. The

methanol was removed with a rotary evaporator. The residue was lyophilized from H_2O to give 0.080 g (16% recovery) of the title compound as a white powder. $[\alpha]_D^{20}$ +69.8° (c = 0.50, DMF). mp: 184 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 1.71-1.98 (m, 3), 2.22-2.34 (m, 1), 3.38 (br s, 2), 4.36 (pent, 1, J = 5.0), 4.78 (br t, 1, J = 4.6), 5.70 (d, 1, J = 7.4), 5.96 (dd, 1, J = 3.8, 6.1), 7.07 (br s, 1), 7.08 (br s, 1), 7.54 (d, 1, J = 7.4). UV: pH = 7: λ_{max} 272 (9,000); λ_{min} 249 (5,000). pH = 13: λ_{max} 272 (9,200); λ_{min} 249 (5,500). MS (CI) m/z 212 (M + H). Anal. Calcd for $C_9H_{13}N_3O_3 \cdot 0.15\ H_2O$: C, 50.53; H, 6.27; N, 19.64. Found: C, 50.55; H, 6.20; N, 19.69.

1-(α-L-2',3'-Dideoxyribofuranosyl)-5-methylcytosine (3b). A 1:1 α:β mixture of 5-methyl-L-2',3'-dideoxycytidine (0.25 g, 65%) was prepared according to the general method, and 0.25 g of this material was separated as described for compound **3a**. The prep HPLC column was initially equilibrated with 98:2 buffer A:buffer B and only one run was required. Desalting of the product by Dowex AG-1 (hydroxide) and subsequent lyophilization gave **3b** as a white powder (0.088 g, 35% recovery). $[\alpha]_D^{20}$ +47.8° (c = 0.50, DMF). mp: 89 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 1.72-1.97 (m, 3), 1.86 (d, 1, J = 0.6), 2.24-2.30 (m, 1), 3.34-3.41 (m, 2), 4.38 (m, 1), 4.77 (t, 1, J = 5.3), 5.99 (dd, 1, J = 4.3, 6.2), 6.71 (br s, 1), 7.18 (br s, 1), 7.35 (q, 1, J = 0.6). UV: pH = 7: λ_{max} 278 (8,700); λ_{min} 254 (4,500). pH = 13: λ_{max} 278 (8,700); λ_{min} 254 (4,500). MS (CI) m/z 226 (M + H). Anal. Calcd for $C_{10}H_{15}N_3O_3 \cdot 0.65\ H_2O$: C, 50.69; H, 6.93; N, 17.73. Found: C, 50.74; H, 6.99; N, 17.64.

1-(α-L-2',3'-Dideoxyribofuranosyl)-5-fluorocytosine (3c). A 1:1 α:β mixture of 5-fluoro-L-2',3'-dideoxycytidine (0.28 g, 48%) was prepared according to the general method and separated as described for compound **3a**, except that the prep HPLC column was equilibrated with 97:3 buffer A:buffer B for the first run. Desalting of the product by Dowex AG-1 (hydroxide) resin as above gave **3c** as a white powder (0.11 g, 39% recovery). $[\alpha]_D^{20}$ +72.6° (c = 0.50, DMF). mp: 73-76 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 1.71-1.80 (m, 1), 1.82-1.99 (m, 2), 2.24-2.32 (m, 1), 3.38 (m, 2), 4.41 (m, 1), 4.77 (t, 1, J = 5.6), 5.90-5.94 (m, 1), 7.44-7.49 (br s, 1), 7.62-7.67 (br s, 1), 7.77 (d, 1, J = 6.9). UV: pH = 7: λ_{max} 281 (8,700); λ_{min} 258 (5,000); sh 233 (7,900); pH = 13: λ_{max} 281 (9,000); λ_{min} 258 (5,300); sh 230 (8,300). MS (CI) m/z 230 (M + H). Anal. Calcd for $C_9H_{12}N_3O_3F \cdot 0.47\ H_2O$: C, 45.48; H, 5.49; N, 17.68. Found: C, 45.88; H, 5.50; N, 17.29.

1-(α-L-2',3'-Dideoxyribofuranosyl)-5-fluorouracil (3d). This compound was prepared according to the general method (powder, 0.17 g, 29%). $[\alpha]_D^{20}$ +28.4° (c = 0.50, DMF). mp: 140-142 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 1.71-1.81 (m, 1), 1.93-2.04 (m, 2), 2.25-2.34 (m, 1), 4.42 (pent, 1, J = 5.4), 4.78 (t, 1, J = 5.6), 5.95-5.98 (m, 1), 7.92 (d, 1, J = 7.0), 11.73-11.82 (br s, 1). UV: pH = 7: λ_{max} 271

(8,400); λ_{min} 237 (2,200); pH = 13: λ_{max} 269 (6,900); λ_{min} 247 (4,300). Anal. Calcd for $C_9H_{11}N_2O_4F$: C, 46.96; H, 4.82; N, 12.17. Found: C, 46.68; H, 4.77; N, 12.07.

1-(α-L-2',3'-Dideoxyribofuranosyl)-5-chlorouracil (3e). This compound was prepared as a glass according to the general method (0.10 g, 16%). ^1H NMR (300 MHz, DMSO-d_6): δ 1.72-1.82 (m, 1), 1.93-2.11 (m, 2), 2.26-2.35 (m, 1), 3.39-3.44 (br m, 2), 4.43 (pent, 1, J = 5.3), 4.79 (br s, 1), 5.95 (dd, 1, J = 4.2, 6.1), 7.92 (s, 1), 11.80 (br s, 1). UV: pH = 7 λ_{max} 278 (9,500); λ_{min} 241 (1,600); pH = 13: λ_{max} 276 (7,200); λ_{min} 248 (3,300). MS (CI) m/z 247 (^{35}Cl, M + H), 249 (^{37}Cl, M + H). Anal. Calcd for $C_9H_{11}N_2O_4Cl \cdot 0.3\ H_2O$: C, 42.89; H, 4.64; N, 11.11; Cl, 14.07. Found: C, 43.03; H, 4.67; N, 10.89; Cl, 14.09.

1-(α-L-2',3'-Dideoxyribofuranosyl)-5-bromouracil (3f). This compound was prepared as an oil according to the general method (0.23 g, 32%). $[\alpha]_D^{20}$ +13.7° (c = 0.51, DMF). ^1H NMR (300 MHz, DMSO-d_6): δ 1.72-1.82 (m, 1), 1.93-2.09 (m, 2), 2.26-2.34 (m, 1), 3.36-3.45 (m, 1), 4.42 (pent, 1, J = 4.5), 4.79 (br s, 1), 5.94 (dd, 1, J = 4.3, 6.3), 7.96 (s, 1), 11.77 (br s, 1). ^{13}C NMR (75 MHz, DMSO-d_6): δ 26.67, 32.37, 64.34, 82.61, 88.02, 96.44, 141.11, 150.66, 160.27. UV: pH = 7: λ_{max} 281 (9,000); λ_{min} 244 (1,500); pH = 13: λ_{max} 278 (7,500); λ_{min} 251 (3,900). MS (CI) m/z 291 (^{79}Br, M + H), 293 (^{81}Br, M + H). Anal. Calcd for $C_9H_{11}N_2O_4Br \cdot 0.3\ H_2O$: C, 36.46; H, 3.94; N, 9.45; Br, 26.95. Found: C, 36.66; H, 3.91; N, 9.29; Br, 26.74.

1-(α-L-2',3'-Dideoxyribofuranosyl)-5-iodouracil (3g). This compound was prepared as a powder according to the general method (0.070 g, 8%). $[\alpha]_D^{20}$ -0.8° (c = 0.50, DMF). mp: 88-90 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 1.75 (m, 1), 1.97-2.03 (m, 2), 2.27-2.31 (m, 1), 3.36-3.42 (m, 2), 4.40 (pent, 1, J = 4.9), 4.79 (t, 1, J = 5.6), 5.94 (dd, 1, J = 4.4, 6.3), 7.92 (s, 1), 11.61 (br s, 1). UV: pH = 7 λ_{max} 290 (7,100); λ_{min} 248 (1,600); pH = 13 λ_{max} 280 (5,200); λ_{min} 251 (2,600). MS (CI) m/z 339 (M + H). Anal. Calcd for $C_9H_{11}N_2O_4I$: C, 31.97; H, 3.28; N, 8.29. Found: C, 31.78; H, 3.26; N, 8.21.

1-(α-L-2',3'-Dideoxyribofuranosyl)-5-trifluoromethyluracil (3h). This compound was prepared according to the general method (powder, 0.35 g, 50%). $[\alpha]_D^{20}$ +57.6° (c = 0.50, DMF). mp: 74-75 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 1.71-1.82 (m, 1), 1.91-2.03 (m, 1), 2.06-2.16 (m, 1), 2.30-2.40 (m, 1), 3.36-3.48 (m, 2), 4.42 (pent, 1, J = 5.3), 4.82 (br s, 1), 5.91 (dd, 1, J = 4.2, 6.2), 7.94 (d, 1, J = 1.0), 11.85 (br s, 1). UV: pH = 7: λ_{max} 263 (9,700); λ_{min} 230 (1,600); pH = 13: λ_{max} 261 (6,800); λ_{min} 237 (3,800). MS (CI) m/z 281 (M + H). Anal. Calcd for $C_{10}H_{11}O_4N_2F_3$: C, 42.87; H, 3.96; N, 10.00. Found: C, 42.73; H, 3.99; N, 9.90.

1-(α-L-2',3'-Dideoxyribofuranosyl)-5-ethynyluracil (3i). This compound was prepared according to the general method (powder, 0.23 g, 39%). $[\alpha]_D^{20}$ +5.8° (c =

0.50, DMF). mp: 133 °C. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 1.72-1.82 (m, 1), 1.95-2.07 (m, 2), 2.28-2.35 (m, 1), 3.36-3.43 (m, 2), 4.12 (s, 1), 4.44 (m, 1), 4.79 (t, 1, J = 5.7), 5.94 (dd, 1, J = 4.1, 6.2), 7.89 (s, 1), 11.60 (br s, 1). UV: pH = 7: λ_{max} 288 (11,400), 229 (10,100); λ_{min} 253 (2,500); pH = 13: λ_{max} 229 (11,600), 285 (9000); λ_{min} 258 (3,800). MS (CI) m/z 237 (m + H). Anal. Calcd for $C_{11}H_{12}N_2O_4$: C, 55.93; H, 5.12; N, 11.86. Found: C, 55.65; H, 5.15; N, 11.78.

General Procedure for the Cytidine Deaminase-Mediated Deamination of Cytosine Analogues. Preparation of 1-(α-L-2',3'-Dideoxyribofuranosyl)-uracil (3j). To a solution of 1:1 α:β **3a:4a** (5.0 g, 23.7 mmol; prepared according to the general procedure) in H_2O (285 mL) was added *E. Coli* cytidine deaminase (2550 u/mL, 180 μL, 460 u). The solution was incubated at 45 °C and the pH of the reaction monitored hourly and adjusted to pH = 7.5 with 0.1 N HCl as necessary. The reaction was allowed to progress until the concentration of α–L-2',3'-dideoxycytidine was <0.5% relative to the β-anomer as evidenced by reverse-phase HPLC using Method 1 described in the general section. The water was removed by lyophilization. The residue was dissolved in CH_3OH and adsorbed onto silica gel. The dry powder was loaded onto a 5 x 20 cm flash column packed with 95:5 CH_2Cl_2:CH_3OH. The column was eluted with the same solvent. The product-containing fractions were concentrated with a rotary evaporator. The residue was lyophilized from H_2O to give **3j** as a thick oil that became a waxy solid on standing (2.1 g (42%)). $[\alpha]_D^{20}$ +24.6° (c = 0.50, DMF). ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 1.76-1.83 (m, 1), 1.92-2.02 (m, 2), 2.28-2.31 (m, 1), 3.36-3.44 (m ,2), 4.35-4.38 (m 1), 4.79 (t, 1, J = 5.8), 5.58 (d, 1, J = 7.9), 5.99 (dd, 1, J = 3.9, 6.4), 7.61 (d, 1, J = 8.1), 11.25 (br s, 1). UV: pH = 7: λ_{max} 264 (4,400); λ_{min} 231 (580); pH = 13: λ_{max} 263 (3,200); λ_{min} 241 (2,000). MS (CI) m/z 213 (M + H). Anal. Calcd for $C_9H_{12}N_2O_4 \cdot 0.20\ H_2O$: C, 50.09; H, 5.79; N, 12.98. Found: C, 50.29; H, 5.87; N, 12.70.

1-(α-L-2',3'-Dideoxyribofuranosyl)thymine (3k). A 1:1 α:β mixture of **3b** and **4b** (0.25 g, 1.1 mmol) was prepared according to the general procedure. The product was treated with *E. coli* cytidine deaminase according to the method described for **3j.** The reaction proceeded at room temperature, and the pH of the solution was not adjusted during the course of the reaction. Compound **3k** was isolated as a white powder (0.12 g, 46%). $[\alpha]_D^{20}$ +2.0° (c = 0.50, DMF). mp: 52-55 °C. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 1.79 (d, 3, J = 0.90), 1.72-1.80 (m, 1), 1.83-2.08 (m, 2), 2.24-2.33 (m, 1), 3.40 (m, 2), 4.31 (m, 1), 4.78 (t, 1, J = 5.7), 6.01 (dd, 1, J = 4.8, 6.5), 7.45 (q, 1, J = 0.9), 11.24 (br s, 1). UV: pH = 7: λ_{max} 269 (10,500); λ_{min} 236 (2,100); pH = 13: λ_{max} 268 (8,000); λ_{min} 245 (4,600). MS (CI) m/z 227 (M + H). Anal. Calcd for $C_{10}H_{14}N_2O_4 \cdot 0.3\ H_2O$: C, 51.85; H, 6.35; N, 12.09. Found: C, 51.77; H, 6.23; N, 12.08.

General procedure for the preparation of purine α-L-2',3'-dideoxy nucleosides (5a-d). Into a 250-mL triple-baffled flask was weighed α-L-2',3'-dideoxyuridine (**3j**, 0.50 g, 2.4 mmol) and an appropriately-substituted purine base (2.9 mmol). Potassium phosphate buffer (10 mM, pH = 7.0, 100 mL) was added and the mixture was stirred at 45 °C for 15-30 min. The pH of the solution was readjusted to pH = 7.0 with 1N HCl or 1N KOH, if necessary. To the reaction flask was added *E. coli* purine nucleoside phosphorylase (2.1 mL, 11,500 u/mL, sp. activity 92 u/mg) and *E. coli* thymidine phosphorylase (1.45 mL, 16,900 u/mL, sp. activity 226 u/mg). The resulting mixture was stirred slowly at 45 °C. If the reaction was not complete in 24 h as evidenced by reverse-phase HPLC (method 2), an additional 11,500 u of PNP and 24,500 u of TPase were added. The reaction was allowed to continue for an additional 4-8 h, or until no further product formation was apparent by HPLC.

If the reaction mixture was heterogeneous prior to isolation, CH_3OH was added until the mixture was nearly homogeneous. The entire reaction mixture was applied to a 2.5 x 10 cm column of Dowex AG-1 (hydroxide) resin packed in CH_3OH. The column was flushed with CH_3OH until no UV-active substance was present in the eluant. Less than 200 mL of CH_3OH was generally required. The combined eluants were concentrated with a rotary evaporator. The residue was dissolved in CH_3OH and silica gel added. The solvent was removed with a rotary evaporator and the powdery residue applied to a 5 x 20 cm flash column packed with an appropriate mixture of CH_2Cl_2:CH_3OH or CH_2Cl_2:EtOH. Elution of the column with the same solvent and concentration of the product-containing fractions with a rotary evaporator gave the products as solids or thick oils. The residues were lyophilized from H_2O to give the products as powders.

6-Amino-9-(α-L-2',3'-dideoxyribofuranosyl)purine (5a). The product was purified by flash chromatography using 95:5 CH_2Cl_2:CH_3OH as eluant. The product obtained was further purified by preparative reverse-phase C_{18} HPLC with 95:5-90:10 H_2O:CH_3CN as gradient elution. The solvent was removed by lyophilization and **5a** was isolated as a white powder (0.22 g, 41%). $[\alpha]_D^{20}$ -49.0° (c = 0.50, DMF). mp: 165-167 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 1.76-1.88 (m, 1), 2.16-2.27 (m, 1), 2.40-2.54 (m, 2), 3.35-3.48 (m, 2), 4.36 (pent, 1, J = 5.7), 4.76 (t, 1, J = 5.6), 6.26 (t, 1, J = 5.6), 7.21 (s, 2), 8.12 (s, 1), 8.24 (s, 1). UV: pH = 7: λ_{max} 260 (13,400); λ_{min} 227 (1,800); pH = 13: λ_{max} 260 (14,000); λ_{min} 227 (2,300). MS (CI) m/z 236 (M + H). Anal. Calcd for $C_{10}H_{13}N_5O_2 \cdot 0.50\ H_2O$: C, 49.17; H, 5.78; N, 28.67. Found: C, 49.29; H, 5.79; N, 28.76.

2,6-Diamino-9-(α-L-2',3'-dideoxyribofuranosyl)purine (5b). The product was purified by flash chromatography using 9:1 CH_2Cl_2:CH_3OH as eluant. Product **5b** was isolated as a lyophilized powder (0.31 g, 54%). $[\alpha]_D^{20}$ -62.0° (c = 0.50, DMF). mp: 157 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 1.75-1.87 (m, 1), 2.15-2.26 (m, 1), 2.35-

2.42 (m, 2), 3.35-3.48 (m, 2), 4.31 (m, 1), 4.76 (t, 1, J = 5.8), 5.77 (s, 2), 6.09 (t, 1, J = 5.5), 6.60 (s, 2), 7.84 (s, 1). UV: pH = 7: λ_{max} 280 (7,700), 256 (6,900); λ_{min} 265 (5,700), 236 (4, 200); pH = 13: λ_{max} 280 (7,500), 256 (6,700); λ_{min} 265 (5,500), 236 (3,800). MS (CI) m/z 251 (M + H). Anal. Calcd for $C_{10}H_{14}N_6O_2 \cdot 0.75\ H_2O$: C, 45.54; H, 5.92; N, 31.86. Found: C, 45.50; H, 5.67; N, 31.63.

6-Methoxy-9-(α-L-2',3'-dideoxyribofuranosyl)purine (5c). The product was purified by flash chromatography using 96:4 CH_2Cl_2:EtOH as eluant. Product **5c** was isolated as a lyophilized powder (0.19 g, 34%). $[\alpha]_D^{20}$ -30.2° (c = 0.50, DMF). mp: 99 °C. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 1.82-1.93 (m, 1), 2.20-2.32 (m, 1), 2.48-2.55 (m, 2), 3.35-3.52 (m, 2), 4.10 (s, 3), 4.37-4.45 (m, 1), 4.80 (t, 1, J = 5.8), 6.39 (t, 1, J = 5.5), 8.53 (s, 1), 8.55 (s, 1). UV: pH = 7: λ_{max} 251 (13,100); λ_{min} 221 (3000); pH = 13: λ_{max} 251 (13,200); λ_{min} 220 (3,100). MS (CI) m/z 251 (M + H). Anal. Calcd for $C_{11}H_{14}N_4O_3 \cdot 0.2\ H_2O$: C, 52.04; H, 5.72; N, 22.07. Found: C, 52.12; H, 5.70; N, 22.06.

2-Amino-6-methoxy-9-(α-L-2',3'-dideoxyribofuranosyl)purine (5d). The product was purified by flash chromatography using 95:5 CH_2Cl_2:CH_3OH as eluant. Product **5d** was isolated as a lyophilized powder (0.25 g, 41%). $[\alpha]_D^{20}$ -63.0° (c = 0.50, DMF). mp: 182 °C. ^{1}H NMR (DMSO-d$_6$): δ 1.80-1.89 (m, 1), 2.18-2.27 (m, 1), 2.37-2.44 (m, 2), 3.35-3.47 (m, 2), 3.95 (s, 3), 4.29-4.35 (m, 1), 4.78 (t, 1, J = 5.7), 6.15 (t, 1, J = 5.5), 6.44 (s, 2), 8.00 (s, 1). UV: pH = 7: λ_{max} 248 (10,400); 281 (10,300); λ_{min} 261 (5,700); pH = 13: λ_{max} 248 (10,600); 280 (10,100); λ_{min} 261 (5,800). MS (CI) m/z 266 (M + H). Anal. Calcd for $C_{11}H_{15}N_5O_3$: C, 49.81; H, 5.70; N, 26.40. Found: C, 49.72; H, 5.73; N, 26.34.

General Procedure for the Adenosine Deaminase-Mediated Deamination of Purine Analogues. Preparation of 9-(α-L-2',3'-dideoxyribofuranosyl)hypoxanthine (6a). To a solution of α–L-2',3'-dideoxyadenosine (**5a**, 0.15 g, 0.64 mmol) in 10 mM potassium phosphate buffer (pH = 7, 50 mL) was added calf intestinal adenosine deaminase (10 mg/mL suspension, 25 μL). The mixture was stirred at room temperature for 15 min and left at room temperature without stirring for 24 h. The reaction was monitored by reverse-phase HPLC (method 2). The H_2O was removed by lyophilization. The white powdery residue was slurried with CH_3OH and filtered. The solid was rinsed thoroughly with CH_3OH until no more UV-absorbing material was present in the filtrate. The product was adsorbed on silica gel and applied to a 2.5 x 20 cm column packed with 9:1 CH_2Cl_2:CH_3OH. Elution with the same solvent and concentration of the product-containing fractions gave a white solid. The product was lyophilized from H_2O to give **6a** as a white powder (0.07 g, 47%). $[\alpha]_D^{20}$ -48.8° (c = 0.50, DMF). mp: 225-228 °C (dec). ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 1.81-1.91 (m,

1), 2.18-2.27 (m, 1), 2.39-2.53 (m, 2), 3.39-3.44 (m, 2), 4.35-4.39 (m, 1), 4.79 (br s, 1), 6.27 (dd, 1, J = 4.2, 6.6), 8.04 (s, 1), 8.23 (s, 1), 12.35 (br s, 1). UV: pH = 7: λ_{max} 249 (11,000); λ_{min} 221 (1,000); pH = 13: λ_{max} 254 (11,400); λ_{min} 223 (1,000). MS (CI) m/z 229 (M + H).Anal Calcd for $C_{10}H_{12}N_4O_3 \cdot 0.30\ H_2O$: C, 49.71; H, 5.26; N, 23.19. Found: C, 49.77; H, 5.18; N, 23.14.

9-(α-L-2',3'-Dideoxyribofuranosyl)guanine (6b). The procedure described above was applied to **5b** (0.098 g, 0.37 mmol). The product was purified by preparative reverse-phase C_{18} HPLC using 95:5 H_2O:CH_3CN as the mobile phase. The solvent was removed by lyophilization to give **6b** as a white powder (0.027 g, 29%). $[\alpha]_D^{20}$ -51.0 ° (c = 0.35, DMF). mp: 250-255 (dec). ^1H NMR (300 MHz, DMSO-d_6): δ 1.76-1.87 (m, 1), 2.12-2.29 (m, 1), 2.30-2.46 (m, 2), 3.34-3.47 (m, 2), 4.31 (m, 1), 4.77 (t, 1, J = 5.7), 6.05 (dd, 1, J = 4.2, 6.5), 6.42 (br s, 2), 7.82 (s, 1), 10.60 (br s, 1). UV: pH = 7: λ_{max} 253 (13,500); λ_{min} 223 (2,700); pH = 13: λ_{max} 266 (11,200); λ_{min} 231 (4,000). MS (CI) m/z 252 (M + H).Anal. Calcd for $C_{10}H_{13}N_5O_3 \cdot H_2O$: C, 44.61; H, 5.61; N, 26.01. Found: C, 44.52; H, 5.63; N, 25.93.

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