–17 °C. The solution contained 9.9 mCi (50%) of a product that had an ultraviolet spectrum nearly identical with that of **1c**. Radiochemical purity was 92–95% with use of either of the TLC solvents above. Specific activity of 44 Ci/mmol was calculated by dividing the amount of ^3H in an aliquot of the solution by the concentration of [^3H]-**1c** in the solution. Concentration was determined from the UV absorbance of an aliquot of the solution at 309 nm with ϵ 18.1 mM^{-1} cm^{-1} and from its EC_{50} determined with known amounts of unlabeled **1c** in the presence of estrogen receptor.

Radioreceptor Assays. Affinities of **1a–e** for estrogen receptor prepared from immature rat uteri were determined as described.[17] Incubation mixtures contained one of the following: [^3H]-**1c** (2.3 nM), [^3H]-4-hydroxytamoxifen (3.0 nM), or [^3H]estradiol (3.5 nM) and increasing concentrations (0, 1, 3, 10, 30, 100 nM) of competing ligand. Nonspecific binding was determined from incubations containing the ^3H-labeled ligand accompanied by an excess (1000 nM) of its radioinert counterpart.

Distribution of [^3H]-1c in Vivo. A. Animals and Dosing. Female Sprague–Dawley rats, 20–25 days old, were obtained from our breeding colony. Each treatment group had four animals. For each group, a solution of 48 μCi (1.1 nmol) of [^3H]-**1c** in 0.1 mL of ethanol was mixed with 0.8 mL of 1.15% aqueous KCl just prior to use. Each animal received 0.2 mL (0.26 nmol, 11.2 μCi) of this ip or iv (tail vein). Animals were sacrificed at 1, 4, or 8 h after dosing, and blood, uteri, and sections of liver and small intestine (contents) were removed and weighed. To study the effects of estradiol on distribution of [^3H]-**1c**, a solution of 8 μg (30 nmol/0.2 mL) was prepared as described above and given ip, in turn, to each of four animals. A second group received 0.2 mL of 1.15% aqueous KCl, ip. Each animal then received, ip, 0.26 nmol of [^3H]-**1c** as described above. After 1 h, all eight animals were sacrificed, and blood and organ samples were removed.

B. Tissue Processing. Pooled blood was centrifuged at 500*g* for 10 min, and 0.5-mL aliquots were counted. Uterine and liver tissues and intestinal contents were separately pooled and homogenized in 9 volumes of methanol. Homogenates were centrifuged at 500*g* for 10 min, and 1.0-mL aliquots were counted.

C. Treatment of Extracts with β-Glucuronidase. Freeze-dried plasma, or methanol extract concentrate, containing 150–200 nCi of ^3H, was redissolved in 1.0 mL of 50 mM phosphate buffer, pH 7.4. To 0.5 mL of the resulting solution was added 100 units of β-glucuronidase in 0.1 mL of water; to the remaining 0.5 mL was added 0.1 mL of water. The solutions were capped and allowed to stand at room temperature for 24 h. Then the solutions were freeze-dried. Each residue was extracted with two 0.8-mL portions of methanol. Respective extracts were combined and concentrated. Each residue was redissolved in 100 mL of methanol and subjected to TLC with **1c** as an internal standard. Developed plates were cut into ca. 1 cm wide segments by using the position of **1c** as a guide. ^3H in each segment was determined after elution in 8 mL of liquid scintillation fluid for at least 16 h.

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Registry No. **1c**, 114221-52-2; [^3H]**1C**, 114221-53-3; diiodo **1c**, 114221-54-4; **1d**, 114221-55-5; **1e**, 114221-56-6; **2**, 114221-57-7; **3** (X = Br), 114221-58-8; **3** (X = F), 114221-59-9; **4**, 114221-60-2; 2-phenyl-1,1-bis(4-hydroxyphenyl)ethylene, 66422-18-2; 2-chloro-2-phenyl-1,1-bis(4-hydroxyphenyl)ethylene, 106692-29-9; 4,4′-dihydroxybenzophenone, 611-99-4; *p*-bromobenzyl bromide, 589-15-1; *p*-fluorobenzyl bromide, 459-46-1.

1-(2,3-Anhydro-β-D-lyxofuranosyl)cytosine Derivatives as Potential Inhibitors of the Human Immunodeficiency Virus

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We report here that 1-(2,3-anhydro-β-D-lyxofuranosyl)cytosine has activity against the human immunodeficiency virus in vitro. A number of 2′,3′-anhydro-β-D-lyxofuranosyl nucleoside derivatives were prepared, but none had the activity of the title compound. New efficient procedures were developed for the synthesis of 3′-deoxy-3′-alkyl- and 3′-deoxy-β-D-arabinosylpyrimidine derivatives.

Of the agents currently being evaluated as potential therapeutic agents for the treatment of the acquired immunodeficiency syndrome[1] (AIDS), the best (in vitro) appear to be nucleoside analogues such as 3′-deoxy-3′-azidothymidine[2] (AZT) and other 2′,3′-dideoxynucleosides[3,4] (ddN). AZT and ddN appear to be converted into the corresponding 5′-triphosphates by cellular enzymes. These triphosphates selectively inhibit the synthesis of DNA by the human immunodeficiency virus (HIV) reverse transcriptase.[4,5] The precise mechanism of action has not been elucidated, but appears that compounds such as ddN-5′-triphosphate are better substrates for retroviral reverse transcriptase (RT) than for the cellular DNA polymerases such as polymerase α (Pol α).[6] When incorporated into a growing viral DNA chain, these analogues halt further DNA synthesis since they lack a 3′-hydroxyl group.

It has been shown, in the case of acyclovir, that the primer–template containing this nucleoside analogue at the 3′ terminus is itself a potent inhibitor of Pol α and herpes virus DNA polymerase.[7] Recently it has been demonstrated that (murine or avian) viral RT is not inhibited by synthetic oligodeoxynucleotide primer–template

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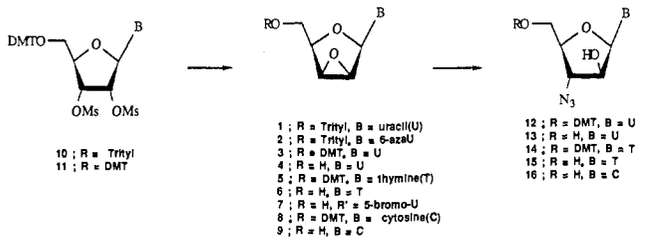
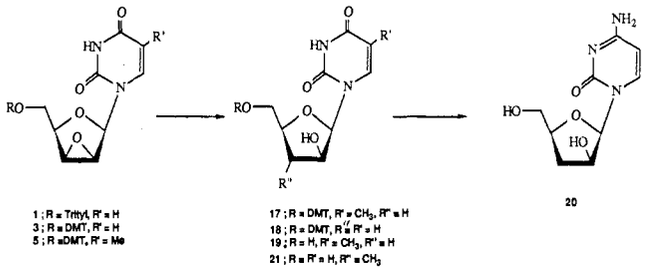
Scheme I



Scheme II



analogues that contain a 3′-terminal nucleoside analogue lacking a correctly oriented 3′-hydroxyl group, though calf thymus Pol α is inhibited by these template–primer analogues.[8] We therefore decided to investigate derivatives that could potentially be reactive when incorporated into a primer–template. From a pragmatic perspective, it is apparent that nucleosides that are analogous to AZT or ddN may have improved in vitro and/or in vivo anti-HIV activity.[9] We therefore also prepared and tested in vitro several compounds analogous to AZT and ddN. We chose as a starting point 1-(2,3-anhydro-β-D-lyxofuranosyl)pyrimidine nucleosides that are reactive and versatile synthetic intermediates and that also possess the desired characteristics (lack of a 2′- and 3′-ribo-oriented hydroxyl group) of a potential anti-HIV nucleoside analogue.

Results and Discussion

The synthesis of (2,3-anhydro-β-D-lyxofuranosyl)pyrimidine derivatives was first reported by Fox and coworkers[10] in the early 1960s. Many subsequent reports have appeared (vide infra), but to the best of our knowledge, no one has ever tested the parent 1-(2,3-anhydro-β-D-lyxofuranosyl)cytosine derivative for antiviral activity. Many investigators have prepared 5′-trityl derivatives such as **1** or **2**,[11,12] which, on acid-promoted detritylation, would be expected to give a mixture of undesired epoxide ring opened product along with the desired deprotected epoxide (such as **4**).[13] We substituted the 4,4′-dimethoxytrityl[14] (DMT) group for the trityl group (Scheme I), since it can be removed under mild conditions that are compatible with the 2′,3′-epoxide ring.

Using this procedure, we prepared several 5-substituted uracil derivatives (**3–7**, Scheme I). The cytosine derivative **8** could be prepared via the triazolide amination procedure from **3** (via **22**, Scheme III),[15] or more directly from cytidine by using a procedure based on the work of Kanai and Ichimo (Scheme II).[16] These workers showed that **10** could be prepared directly from cytidine in very good yield. We found that base treatment of **11** gave **8** in good yield. Removal of the DMT group gave **9** (Scheme II).

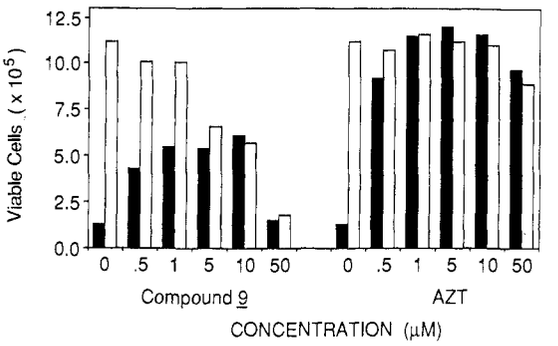


Figure 1. Inhibition of the cytopathic effect of HIV by **9** and AZT against ATH8 cells. See "Experimental" for "Biological Methods".[2-4] The open columns represent cells that were treated with the indicated concentration of drug without virus. The dark columns were treated with compound and 2000 virus (HTLV-III$_B$) particles per cell.

These epoxynucleosides, **3**[17] and **5**,[18] when treated with sodium azide in refluxing acetone gave predominately the arabinosyl products **12** and **14** (Scheme I), though small amounts of xylosyl products were detected by thin-layer chromatography (TLC) and ^{1}H NMR, as has been previously demonstrated.[19] The AZT analogues **13** and **15** were obtained pure from **12** and **14**, respectively, by aqueous acetic acid treatment followed by crystallization.[17,18] This procedure was also used to give the cytosine derivative **16**.[16,17]

Since many arabinosyl nucleosides show good antiviral or anticancer activity, we thought it would be interesting to also prepare 3′-deoxy-β-D-arabinosylpyrimidine nucleosides **19** and **20**. We found that **5** could be regiospecifically reduced to **17** by lithium triethylborohydride in tetrahydrofuran (THF), under carefully controlled conditions (Scheme II). The structures of **18–20** are based on UV, ^{1}H NMR, and mass spectral data. The ^{1}H NMR spectrum shows the 1′ proton with the coupling (doublet) and coupling constant ($J_{1',2'}$ = 5.0 Hz) expected for the arabinose configuration of the 2′-hydroxyl.[17,19] The mass spectral data also supports the structures as shown (vide infra). No trace of the corresponding 2′-deoxy-β-D-xylosyl derivative could be detected in this reaction mixture by ^{1}H NMR (no 1′-H triplet). The analogous result was obtained in the conversion of **3** to **18**. Direct reduction of **8** did not lead to the desired product (**20**), so the uracil derivative **18** was treated with (trimethylsilyl)dimethylamine to give the 2′-trimethylsilyl ether, which was converted to the cytosine derivative via the triazolide.[15,20] Aqueous acetic acid treatment of this intermediate 5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(trimethylsilyl)-1-(3-deoxy-β-D-arabinosyl)cytosine gave **20**[21] in good overall yield. The

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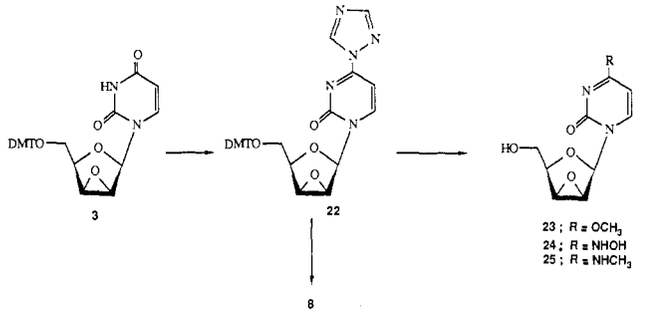
Scheme III



use of lithium triethylborohydride as an agent for the high-yield regiospecific reduction of 2',3'-anhydroadenosine has been reported,[22] but there have been no previous reports, to our knowledge, of the successful use of metal hydride reagents in the synthesis of pyrimidine derivatives such as **17** or **18**.

We were also interested in the synthesis of 1-(3-deoxy-3-alkyl-β-D-arabinosyl)pyrimidine nucleosides. A recent paper has reported a synthesis of **21** by the addition of lithium diathiane to **1**, followed by Raney nickel treatment and detriylation.[10] We decided to investigate a more direct route that has not been reported, in the case of pyrimidine epoxides. Treatment of **3** with excess methylmagnesium chloride and copper cyanide in THF, followed by removal of the DMT group, gave a single addition product tentatively assigned structure **21**, based on literature precedent,[10] in 85% overall yield from **3** (Scheme II).[10,23] Previous use of methylmagnesium iodide and copper iodide to give an analogous addition product from 2',3'-anhydroadenosine in 18% yield has been reported.[23] The conditions reported here for the synthesis of **21** should be useful for the efficient synthesis of other alkyl-substituted nucleosides.

The in vitro activity of **9** against HIV, compared to that of AZT, is shown in Figure 1. As can be seen in Figure 1, **9** shows significant protection of ATH8 cells from the cytopathic effects of HIV at concentrations as low as 1 μM. Similar results are obtained when **9** is compared to ddC in its ability to protect ATH8 cells or normal $CD4^+$ cells in vitro (see Figure 2), though in this assay significant protection is only observed at 5 μM. In these assays the cytotoxicity of **9** appears significant at 5 μM. In an attempt to develop compounds with improved in vitro therapeutic index, compounds **23–25** were prepared (Scheme III).[15] We hypothesized that compounds that are capable of ambiguous base pairing might be better substrates for HIV RT than the host DNA polymerases. Compounds **23–25**, however, did not show any significant in vitro anti-HIV activity at concentrations as high as 100 μM. The nucleoside analogues **4**, **6**, **7**, **13**, **15**, **16**, **19**, **20**, and **21** also had no significant protective effects on ATH8 cells exposed to HIV in vitro, at concentrations as high as 100 μM. These compounds may not be active because they are not converted to the corresponding triphosphates by host enzymes, or because they are not good substrates for HIV RT. It is possible to speculate that the triphosphate of **9** acts as a specific alkylating reagent for HIV RT, but further studies will be needed to address this hypothesis. Alternately, the mechanism of antiviral activity of **9** may be completely analogous to that of AZT or the ddNs.[5]

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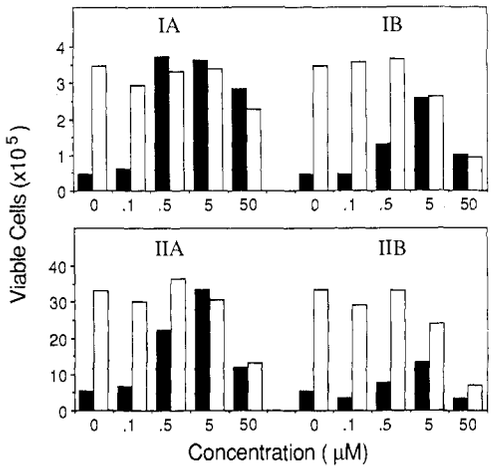

Figure 2. Inhibition of the infectivity and cytopathic effect of HIV by **9** and 2',3'-dideoxycytidine against $CD4^+$ T cells. $CD4^+$ ATH8 cells (2×10^5) (I-A and -B) or normal $CD4^+$ T cells (clone TM11; 2×10^5) (II-A and -B) were exposed to HIV-1 ($HTLV\text{-}III_B$; 2000 virus particles per cell), resuspended in IL-2-containing medium, and cultured in the presence or absence of various concentrations of 2',3'-dideoxycytidine (I-A and II-A) or **9** (I-B and II-B) as previously described.[2a] On day 7 in culture, the viable cells were counted. Normal antigen-specific TM11 cells had been stimulated with antigen (tetanus toxoid) and autologous antigen-resenting cells 5 days prior to this assay. Characteristics of ATH8 cells and TM11 cells have been described elsewhere.[2a,b]

Experimental Section

Biological Methods. HIV cytopathic effect assay was performed with ATH8 cells as previously described.[2a,4] Briefly, 2×10^5 ATH8 cells were exposed to $HTLV\text{-}III_B$ virus (2000 virus particles/cell) for 45 min after treatment with polybrene, resuspended in 2 mL of culture medium containing interleukin-2 in the presence or absence of various concentrations of compounds, and incubated in culture tubes at 37 °C in 5% CO_2/95% air humidified atmosphere. Control cells were treated similarly but were not exposed to the virus. At various time points on days 5–7 in culture, the total viable cells were counted in a hemocytometer by the trypan blue dye exclusion method.

Chemical Methods. 5-Methyluridine was purchased from Sigma Chemical Co. Cytidine and uridine were purchased from Calbiochem; all other reagents were purchased from the Aldrich Chemical Co. ^1H NMR spectra were obtained on an 80-MHz IBM NR/80 spectrometer. ^1H NMR were recorded as ppm (δ) from a TMS internal standard, except that when DMSO-d_6 or D_2O was used as a solvent, an external standard (TMS, 0.0 ppm) was used. Mass spectra were obtained by using the positive-ion fast-atom-bombardment (FAB) technique on a Hewlett-Packard 5985C instrument. Thin-layer chromatography (TLC) was performed with EM DC-Alufolien Kieselgel-60 F_{254} plates. For column chromatography, EM Kieselgel-60 (70–230 mesh) was used. All concentrations were performed under reduced pressure.

General Dedimethoxytritylation Procedure. A general detritylation and purification procedure was used to produce and purify all of the free nucleosides. This procedure consisted of dissolving the compound to be deprotected in a mixture of 80% acetic acid/water to give a ca. 1% solution (w/v). This solution was allowed to react for 30 min at 22 °C. The resulting orange solution was concentrated under vacuum and the residue was dissolved in 10 mL of CH_3OH and then diluted with 40 mL of toluene. The solution was again concentrated under vacuum. This residue was dissolved in 5 mL of CH_3OH and 40 mL of toluene was added. The solution was concentrated to ca. 20 mL and centrifuged. The residue was washed with toluene and then ether and finally dried under vacuum to give a white powder.

5'-*O*-(4,4'-Dimethoxytrityl)-1-(2,3-anhydro-β-D-lyxofuranosyl)uracil (3).[12] A solution of DMTCl (7.2 g, 21 mmol) in 10 mL of dry pyridine was added dropwise to a suspension of uridine (4.88 g, 20 mmol) in 30 mL of dry pyridine over a period of 1 h. This solution was allowed to stir for 1.5 h; 40 mL of CH_2Cl_2 was then added and methanesulfonyl chloride (4 mL, 52 mmol)

was added, while the mixture was cooled in an ice bath. After stirring for 15 min, the mixture was allowed to warm to 20 °C and stirred for an additional 3 h. This solution was diluted with 100 mL of CH_2Cl_2 and stirred with 50 mL of aqueous 5% $NaHCO_3$ for 10 min. The CH_2Cl_2 phase was washed with 100 mL of water, dried ($MgSO_4$), and concentrated. The product was isolated by flash chromatography (SiO_2, 0–4% CH_3OH/CH_2Cl_2). To give 9.0 g of a white foam of 5′-*O*-(4,4′-dimethoxytrityl)-2′,3′-bis-*O*-(methylsulfonyl)uridine, a solution of 1 g of this material was dissolved in a mixture of 50 mL of ethanol and 23 mL of aqueous 1 M NaOH with stirring, and this solution was warmed to 60 °C for 1 h. This reaction could not be followed by TLC since the starting material and product comigrate. The solution was cooled to 20 °C and carefully adjusted to pH 7 with 80% acetic acid/water. This mixture was concentrated under reduced pressure to ca. 20 mL and poured into 50 mL of water. This mixture was extracted with 50 mL of CH_2Cl_2; the CH_2Cl_2 phase was extracted with 100 mL of water, dried ($MgSO_4$), and concentrated. This material (900 mg, 80% based on uridine) was ≥95% pure by ^1H NMR and was used directly in subsequent reactions: ^1H NMR ($CDCl_3$) δ 3.37 (2 H, m, H-5′), 3.74 (6 H, s, CH_3O), 3.85 (2 H, s, H-2′,3′), 4.12 (1 H, t, H-4′), 5.62 (1 H, d, H-5), 6.13 (1 H, s, H-1′), 1.12 (13 H, m, DMT-H), 7.53 (1 H, d, H-6), 8.68 (1 H, brs, N3-H). This product was further characterized by its conversion to known derivatives **4**, **13**, and **9** (vide infra).

1-(2,3-Anhydro-β-D-lyxofuranosyl)uracil (4).[10] The product (100 mg, 0.19 mmol) from the above reaction (**3**) was dissolved in 50 mL of 80% acetic acid/water and allowed to stir for 0.5 h at 20 °C. This was worked up according to the general detritylation procedure (see above). **4**: yield 35 mg (82%); ^1H NMR (D_2O) δ 3.65 (2 H, m, H-5′), 3.93 (3 H, m, H-2′,3′,4′), 5.68 (1 H, d, J = 8 Hz, H-5), 6.01 (1 H, s, H-1′), 7.68 (1 H, d, J = 5 Hz, H-6); mp 218–220 °C (lit.[10] mp 218–220 °C).

5′-*O*-(4,4′-Dimethoxytrityl)-1-(2,3-anhydro-β-D-lyxofuranosyl)thymine (5). The procedure for the synthesis of **3** was followed, starting from 516 mg (20 mmol) of 5-methyluridine. This gave 495 mg of **5** (46%, based on 5-methyluridine) as a white foam: ^1H NMR ($CDCl_3$) δ 1.85 (3 H, d, J = 1 Hz, 5-CH_3), 3.41 (2 H, m, H-5′), 3.76 (6 H, s, CH_3O), 4.15 (1 H, t, H-4′), 6.18 (1 H, s, H-1′), 7.15 (13 H, m, DMT-H), 8.50 (1 H, brs, N3-H). Anal. ($C_{31}H_{30}N_2O_7 \cdot 0.25H_2O$) C, H, N.

1-(2,3-Anhydro-β-D-lyxofuranosyl)thymine (6).[18] The product from the above reaction (**5**) (60 mg, 0.11 mmol) was converted to **6** and purified by using the general detritylation procedure above. This gave 25 mg (95% from **5**) of pure **6**:[18] ^1H NMR (D_2O) δ 1.88 (3 H, d, J = 1 Hz, CH_3-5), 3.90–4.50 (3 H, m, H-5′, -4′), 6.15 (1 H, s, H-1′), 7.66 (1 H, d, J = 1 Hz, H-6).

1-(2,3-Anhydro-β-D-lyxofuranosyl)-5-bromouracil (7).[24] The procedure for the synthesis of **3** was followed to give 5′-DMT-**7**: ^1H NMR ($CDCl_3$) δ 3.56 (2 H, m, H-5′), 3.80 (6 H, s, 2 CH_3O), 3.90–4.19 (3 H, m, H-2′,3′,4′), 6.15 (1 H, s, H-1′), 6.74–7.59 (13 H, m, DMT-H), 7.88 (1 H, s NH). This material was converted to **7** as for **4** (see the general detritylation procedure above). By this method, 5-bromouridine (1.0 g, 3.1 mmol) gave 417 mg **7** (44%):[24] ^1H NMR (D_2O) δ 3.90–4.50 (4 H, m, H-5′,4′,3′), 6.17 (1 H, s, 1′-H), 8.12 (1 H, s, 6-H); FAB mass spectrum, m/z (relative intensity) 191–193 (5, BH_2^+), 305/307 (100, MH^+).

5′-*O*-(4,4′-Dimethoxytrityl)-1-(2,3-anhydro-β-D-lyxofuranosyl)cytosine (8). Method A. A suspension of 1,2,4-triazole (2.56 g, 37 mmol) in 40 mL of dry acetonitrile was treated with 0.80 mL (8.6 mmol) of $POCl_3$ and stirred at 20 °C.[15] This solution was treated with triethylamine (6.0 mL, 43 mmol), followed by a solution of **3** (1 g, 1.90 mmol) in 4 mL of dry acetonitrile. This mixture was stirred at 20 °C for 1 h and then poured into 100 mL of 5% aqueous $NaHCO_3$. This was extracted with (3 × 50 mL) CH_2Cl_2. The combined CH_2Cl_2 phase was extracted with 100 mL of water, dried ($MgSO_4$), and concentrated. This triazolide [**22**: ^1H NMR ($CDCl_3$) δ 3.50 (2 H, m, H-5′), 3.80 (6 H, s, 2 CH_3O), 3.90–4.20 (3 H, m, H-2′,3′,4′), 6.29 (1 H, s, H-1′), 6.74–7.50 (13 H, m, DMT-H), 8.06 (1 H, d, J = 8 Hz, C6-H), 8.12 (1 H, s, triazole-H), 9.26 (1 H, s, triazole-H). Anal. ($C_{32}H_{29}N_5O_6 \cdot 0.25H_2O$) C, H, N] was dissolved in 15 mL of dry THF and added to 10 mL of anhydrous ammonia and sealed in a Parr reaction vessel. This was allowed to stir for 2 h at 20 °C/60 psi and then vented. The solution was concentrated and purified by column chromatography (SiO_2, 0–8% CH_3OH/CH_2Cl_2) to give 700 mg (70%) of **8**: ^1H NMR ($CDCl_3$) δ 3.41 (2 H, m, H-5′), 3.74 (6 H, s, CH_3O), 4.20 (1 H, t, H-4′), 5.66 (1 H, d, H-5), 6.26 (1 H, s, H-1′), 7.12 (13, m, DMT-H), 7.58 (1 H, d, H-6), 8.18 (2 H, s, NH_2). This material was further characterized by conversion to the known derivative **9** (vide infra).

Method B. A stirred suspension of cytidine (2.43 g, 10 mmol) in 20 mL of dry pyridine was treated with DMTCl (3.72 g, 11 mmol) for 2 h at 20 °C. This solution was cooled to −30 °C and methanesulfonyl chloride (3.0 mL, 39 mmol) was added dropwise, with good stirring over a 0.5-h period. This solution was kept at −20 °C for 16 h and then 3 mL of water was added and the solution was allowed to warm to 20 °C. The solution was concentrated under vacuum (bath temperature ~20 °C) to a sirup, dissolved in 20 mL of CH_3OH and slowly poured into 1 L of ice/water with good stirring. The yellowish solid was collected by filtration and washed with ice–water. The precipitate was dissolved in 100 mL of THF, and 250 mL of ethanol was added, followed by 60 mL of 1 M aqueous NaOH. This solution was warmed to 50 °C, stirred for 45 min, and cooled in an ice bath. This solution was neutralized with 80% aqueous acetic acid and concentrated to a small volume (~60 mL). This residue was partitioned between 100 mL water and 150 mL CH_2Cl_2; the CH_2Cl_2 phase was dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography (SiO_2, 0–6% CH_3OH/CH_2Cl_2) to give 1.6 g (30% from cytidine) of pure **8**.

1-(2,3-Anhydro-β-D-lyxofuranosyl)cytosine (9).[17] Treatment of **8** (110 mg, 0.21 mmol) with aqueous acetic acid and workup employing the general detritylation procedure gave 45 mg (95%) of **9**:[17] ^1H NMR (D_2O) δ 4.11 (2 H, m, 5′-H), 6.26 (1 H, d, 5-H), 6.44 (1 H, s, H-1′), 8.04 (1 H, d, J = 8 Hz, H-6). Conversion to the hydrochloride salt and recrystallization from ethanol gave white crystals; mp 163–165 °C (lit. mp 166–167 °C).[17]

Compounds **13**, **15**, and **16** were prepared by slight modification of the published procedure as follows:[17,18] A solution of 0.1 mmol of the appropriate epoxide (**3**, **5**, or **8**, respectively) in 5 mL of acetone was treated with a solution of 500 mg of NaN_3 in 5 mL of water. This mixture was refluxed for 20 h. The acetone was removed under reduced pressure, and the residue was diluted with 20 mL water and extracted with 50 mL of CH_2Cl_2. The CH_2Cl_2 phase was washed with 50 mL of water, dried ($MgSO_4$), and concentrated. The resulting intermediates **12** and 14 were purified by preparative TLC (8% $MeOH/CH_2Cl_2$). The product from the reaction of **8** with azide was pure by TLC and NMR so it was used directly in the next step. These DMT-protected azido alcohols were treated with 80% acetic acid by using the general detritylation procedure to give **13**,[17] **15**,[18] and **16** with the expected properties after recrystallization according to the literature conditions in 60, 75, and 90% yield from **3**, **5**, and **8** respectively.

5′-*O*-(4,4′-Dimethoxytrityl)-1-(3-deoxy-β-D-arabinofuranosyl)uracil (18). A solution of **3** (810 mg, 1.53 mmol) in 35 mL of a dry THF (under argon) was cooled to −78 °C and treated with 4.6 mL of a 1 M solution of $LiEt_3BH$ with concomitant gas evolution. This solution was stirred for 1 h at −78 °C and then allowed to warm to 20 °C over a 1-h period and worked up as for **19**. The residue was purified by column chromatography (SiO_2, 0–4% $MeOH/CH_2Cl_2$) to give 405 mg (50%) of **18**: ^1H NMR ($CDCl_3$) δ 1.82–2.64 (2 H, m, H-3′), 3.55 (2 H, m, H-5′), 3.76 (3 H, s, CH_3O), 5.50 (1 H, d, J = 8 Hz, H-5), 5.94 (1 H, d, J = 5 Hz, H-1′), 6.70–7.50 (13 H, m, DMT-H), 7.94 (1 H, d, J = 8 Hz, H-6), 8.75 (1 H, brs, NH). Anal. ($C_{30}H_{30}N_2O_m \cdot 0.5H_2O$) C, H, N.

A small portion (10 mg) of this material was treated with aqueous acetic acid by using the general detritylation procedure to give an analytical sample of 1-(3-deoxy-β-D-arabinofuranosyl)uracil: ^1H NMR (D_2O) δ 2.0 (2 H, m, H-3′), 3.58 (2 H, m, H-5′), 5.68 (1 H, d, J = 8 Hz, H-5), 5.85 (1 H, d, J = 5 Hz, H-1′), 7.74 (1 H, d, J = 8 Hz, H-6); FAB mass spectrum, m/z (relative intensity) 113 (100, BH_2^+), 229 (90, MH^+); MH^+ calculated for $C_9H_{12}N_2O_5$ 229.0824, found 229.0833 ± 0.002; UV λ_{max} 264 nm (ε 11 000).[25]

5′-*O*-(4,4′-Dimethoxytrityl)-1-(3-deoxy-β-D-arabinofuranosyl)thymine (17). A solution of **5** (100 mg, 0.18 mmol) in 5 mL of dry THF (under argon) was cooled in a dry ice/acetone

(24) Hirata, M.; Kobayashi, T.; Kanao, T. Japanese Patent 6917910, 1969; *Chem. Abstr.* **1970**, *72*, 3725*N*.

bath and 561 μL of a 1 M solution of $LiEt_3BH$ in THF was added dropwise gas evolution was noted during this addition. This solution was allowed to stir for 1 h, then allowed to slowly warm to 20 °C, and then stirred for a 1-h period. This solution was then carefully treated with 100 μL of water, followed by 2 mL of a saturated aqueous solution of NH_4Cl. This was partitioned between 100 mL of water and 100 mL of CH_2Cl_2; the CH_2Cl_2 phase was washed with 100 mL of 5% $NaHCO_3$ solution, dried ($MgSO_4$), and concentrated. The residue was purified by preparative TLC to give 75 mg of **17** (75% yield): ^1H NMR ($CDCl_3$) δ 1.61 (3 H, s, CH_3-5), 3.79 (6 H, s, CH_3O), 5.93 (1 H, d, J = 4 Hz, H-1′), 6.8–7.3 (13 H, m, DMT-H), 7.71 (1 H, d, J = 1 Hz, H-6), 9.03 (1 H, brs, NH). Anal. ($C_{31}H_{32}N_2O_7 \cdot H_2O$) C, H, N.

1-(3-Deoxy-β-D-arabinofuranosyl)thymine (19). A sample of **17** (75 mg, 0.14 mmol) was deprotected and purified according to the general deprotection procedure (see above) to give 25 mg (74% yield) of **19**: ^1H NMR (D_2O δ 1.70 (3 H, d, J = 1 Hz, CH_3-5), 2.28 (2 H, m, H-3′), 3.65 (2 H, m, H-5′), 5.84 (1 H, d, J = 5 Hz, H-1′), 7.59 (1 H, d, J = 1 Hz, H-6); FAB mass spectrum, m/z (relative intensity) 127 (100 BH_2^+), 243 (58, MH^+); MH^+ calculated for $C_{10}H_{14}N_2O_5$ 243.0981, found 243.0997 ± 0.0002; UV (EtOH 268 nm (ε 9600).[25]

1-(3-Deoxy-β-D-arabinofuranosyl)cytosine (20).[21] A solution of 175 mg (0.33 mmol) of **18** in 5 mL of dry CH_3CN was treated with 250 μL (1.56 mmol) of dimethyl(trimethylsilyl)amine and stirred at 20 °C for 1 h. This solution was concentrated to a glass; 10 mL of dry CH_3CN was added and the solution was concentrated again. This residue was dissolved in 1 mL of dry CH_3CN and added to the $POCl_3$/triazole/triethylamine reagent and worked up as for the preparation of **8** (method A: 0.175-fold scale.)[20] The crude product was treated with anhydrous ammonia (10 mL) in THF (10 mL) for 2 h to give 5′-DMT-**20**, which was purified by preparative TLC (10% MeOH/CH_2Cl_2). This material was treated with aqueous acetic acid and purified by using the general detritylation procedure to give 35 mg (46% from **18**) of **20**: ^1H NMR (DMSO-d_6 + D_2O) δ 2.0 (2 H, m, H-3′), 3.56 (2 H, m, H-5′), 5.64 (1 H, d, J = 8 Hz, H-5), 5.88 (1 H, d, J = 5 Hz, H-1′), 7.69 (1 H, d, J = 8 Hz, H-6); FAB mass spectrum, m/z (relative intensity) 112 (100, BH_2^+), 228 (45, MH^+); MH^+ calculated for $C_9H_{13}N_3O_4$ 228.0984, found 228.0989 ± 0.002; UV (EtOH) λ_{max} 274 nm (ε 9600).[25]

1-(3-Deoxy-3-methyl-β-D-arabinofuranosyl)uracil (21).[12] A solution of 3 M CH_3MgCl (Aldrich) in THF (2 mL, 6 mmol) was added to a stirred suspension of CuCN (50 mg, 0.56 mmol) in 2 mL of dry THF, under argon at 0 °C. After ca. 10 min a clear yellow solution was obtained. This was stirred and a solution of **3** (250 mg, 0.48 mmol) in 2 mL of THF was slowly added, and the evolution of gas (CH_4) was noted during this addition. The solution was warmed to 20 °C and stirred for 1 h, and then 1 mL of 5% water in THF was slowly added at 0 °C, followed by 1 mL of saturated aqueous NH_4Cl. The mixture was partitioned between 50 mL of water and 70 mL of CH_2Cl_2, and the CH_2Cl_2 was washed with 5% aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated. The residue was purified by preparative TLC (60% ethyl acetate/CH_2Cl_2) to give 225 mg of 5′-DMT-**21**. This product was treated with aqueous acetic acid according to the general detritylation procedure and crystallized from methanol/toluene/petroleum ether to give 120 mg of **21** (82% from **3**) as small white needles (**21**): mp 208–210 °C; ^1H NMR (DMSO-d_6 + D_2O) δ 0.96 (3 H, d, J = 9 Hz, 3′-CH_3), 3.56 (2 H, m, H-5′), 5.56 (1 H, d, J = 8 Hz, H-5), 5.94 (1 H, d, J = 6 Hz, H-1′), 7.85 (1 H, d, J = 8 Hz, H-6); FAB mass spectrum, m/z (relative intensity) 113 (100, BH^+), 243 (69, MH^+). Anal. ($C_{10}H_{14}N_2O_5 \cdot 0.25H_2O$) C, H, N.

1-(2,3-Anhydro-β-D-lyxofuranosyl)-4-methoxy-2(1*H*)-pyrimidinone (23). A solution of **22** (100 mg, 0.17 mmol; see above, under the preparation of **8** by method A) in 10 mL of 90% methanol/THF was treated with sodium methoxide (60 mg, 11 mmol) and stirred for 1 h at 20 °C. The solution was then neutralized with pH 5 sodium acetate buffer and concentrated to near dryness. The residue was partitioned between 50 mL of CH_2Cl_2 and 50 mL of water, and the CH_2Cl_2 phase was dried (Na_2SO_4), concentrated, and purified by preparative TLC (8% methanol/CH_2Cl_2). This product was treated with acetic acid and purified by using the general detritylation procedure (see above) to give 30 mg (73% yield) of **23**: ^1H NMR (D_2O) δ 3.61 (2 H, m, H-5′), 3.69 (3 H, s, O4-CH_3), 5.97 (1 H, d, H-5), 5.98 (1 H, s, H-1′), 7.79 (1 H, d, H-6); FAB mass spectrum, m/z (relative intensity) 127 (30, BH_2^+), 241 (100, MH^+); MH^+ calculated for $C_{10}H_{12}N_2O_5$ 241.0824, found 241.0833 ± 0.0002; UV (EtOH) λ_{max} 275 nm (ε 6100).[26]

1-(2,3-Anhydro-β-D-lyxofuranosyl)-4-(hydroxyamino)-2-(1*H*)-pyrimidinone (24). A solution of **22** (150 mg, 0.26 mmol) was added to a solution of $NH_2OH \cdot HCl$ (500 mg, 7.2 mmol) in 50 mL of methanol that had been treated with excess Dowex G-1 × 2 resin and then filtered. This mixture was concentrated to ca. 20 mL and 5 mL of THF was added to give a clear solution, which was allowed to stir for 1 h at 20 °C. After this time, 100 mL of CH_2Cl_2 and 100 mL of water were added, and the CH_2Cl_2 phase was washed with 5% $NaHCO_3$ solution, dired ($MgSO_4$), and concentrated. The residue was purified by preparative TLC (15% methanol/CH_2Cl_2) and then treated with acetic acid and purified by using the general detritylation procedure to give 35 mg (56% yield) of **24**: ^1H NMR (D_2O) δ 3.55 (2 H, m, H-5′), 5.45 (1 H, d, H-5), 5.84 (1 H, s, H-1′), 6.95 (2 H, d, H-6); FAB mass spectrum, m/z (relative intensity) 115 (50, $BH_2^+ - H_2O$), 128 (10, BH_2^+), 242 (100, MH^+); MH^+ calculated for $C_9H_{11}N_3O_5$ 242.0777, found 242.0776 ± 0.0002; UV (pH 7, H_2O) λ_{max} 237 nm (ε 13500).[27]

1-(2,3-Anhydro-β-D-lyxofuranosyl)-4-(methylamino)-2-(1*H*)-pyrimidinone (25). A solution of **22** (90 mg, 0.16 mmol) in 10 mL of 1,4-dioxane (previously saturated with methylamine) and stirred for 1.5 h at 20 °C. This solution was concentrated to dryness and the residue purified by preparative TLC (5% methanol/CH_2Cl_2). This material was treated with aqueous acetic acid and the pure product isolated according to the general detritylation procedure to give 25 mg (65%) of **25**: ^1H NMR (D_2O) δ 2.63 (3 H, s, N4-CH_3) δ 3.62 (2 H, m, H-5′), 5.70 (1 H, d, H-5), 5.92 (1 H, s, H-1′), 7.44 (1 H, d, H-6); FAB mass spectrum, m/z (relative intensity) 126 (45, BH_2^+) 240 (100, MH^+); MH^+ calculated for $C_{10}H_{13}N_3O_4$ 240.0984, found 240.0984 ± 0.002; UV λ_{max} (EtOH) 272 nm (ε 14 200).[25]

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