Synthesis and Biological Evaluation of 3,3-Difluoropyridine-2,4(1*H*,3*H*)-dione and 3-Deaza-3-fluorouracil Base and Nucleoside Derivatives

Morris J. Robins,*[,†] Hong Yang,[†,§] Karl Miranda,[†,||] Matt A. Peterson,*[,†] Erik De Clercq,[‡] and Jan Balzarini[‡]

Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602-5700, Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

Received February 17, 2009

New 3-deaza-3-halouracil nucleosides including 3-deaza-3-fluorouridine and its 2′-deoxy and arabino analogues have been prepared by fluorination of protected precursors. The resulting 3,3-difluoropyridine-2,4(1*H*,3*H*)-dione derivatives underwent palladium-catalyzed hydrogenolysis of one C−F bond at atmospheric pressure, and deprotection gave the 3-deaza-3-fluorouracil compounds. Selective reaction of a stabilized Wittig reagent at C4 of the 3,3-difluoro-2,4-dione intermediates gave exocyclic alkenes that underwent hydrogenation accompanied by spontaneous elimination of hydrogen fluoride. Ammonolysis of the exocyclic carbethoxymethyl substituent and ester protecting groups gave 4-(carboxamidomethyl)-3-deaza-3-fluorouridine and its analogues. Grignard additions at C4 of the ribo and 2′-deoxy 3,3-difluoro-2,4-dione intermediates followed by deprotection gave the 3-deaza-3,3-difluoro-4-hydroxy-4-(substituted)uracil nucleosides. The cytostatic activity of 3-fluoro-3-deazauridine (CC_{50} = 4.4−9.6 μM) in three cancer cell lines paralleled that of 3-deazauridine, whereas no significant inhibitory activity was observed with a variety of virus-infected cell cultures.

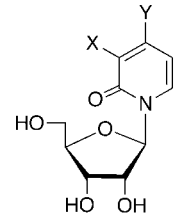
Downloaded by Jonathan Berry on September 11, 2009 | http://pubs.acs.org
Publication Date (Web): April 20, 2009 | doi: 10.1021/jm900203h

Introduction

Robins[1] and Currie reported the synthesis and antibacterial activity of 3-deazauridine (**1**) (Figure 1) in 1968.[2] Synthesis[3] of a number of related 3-deazapyrimidine nucleosides and testing[4] against several microbial and tumor cell systems also were described. Antileukemic activity[4] was reported with **1** and 3-deazacytidine (**2**) in cell culture and murine models as well as inhibition of replication of RNA animal viruses and Gross leukemia virus.[5] Human clinical trials with **1** as a single agent showed minor antileukemic effects,[6] and the in vivo as well as in vitro antitumor and antiviral activities of several pyrimidine nucleoside drugs were enhanced by coadministration of 3-deazauridine.[7] Cytidine triphosphate synthetase is obligatory for the biosynthetic transformation of UTP into CTP, and the 5′-triphosphate of **1** is a competitive inhibitor of this crucial enzyme.[8]

We[9] had effected bromination (and deuterium labeling) of 3-deazauridine at C5, and the Parke Davis group[10] prepared 3-deaza-3-halopyrimidine nucleoside derivatives **3**−**7**. Their 3-deaza-3-fluorocytidine (**3**) showed activity against implanted P388 leukemia in mice and against rhinovirus type 34. Compound **3** as well as 3-bromo-3-deazauridine (**6**) and 3-chloro-3-deazauridine (**7**) showed activity against murine L1210 leukemia in vitro, but their attempts to synthesize 3-deaza-3-fluorouridine were unsuccessful.[10]

The marked alteration of chemical stability, enhancement of small molecule association with macromolecules, and generation of biological activity upon substitution of hydrogen by fluorine are well-known.[11] Introduction of fluorine adjacent to a carbonyl group enhances the electrophilicity of the carbonyl carbon,



1 X = H, Y = OH
2 X = H, Y = NH_2
3 X = F, Y = NH_2
4 X = Br, Y = NH_2
5 X = Cl, Y = NH_2
6 X = Br, Y = OH
7 X = Cl, Y = OH

Figure 1. Reported compounds.

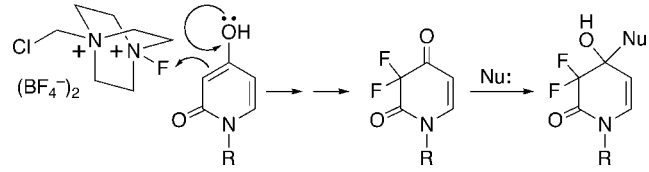


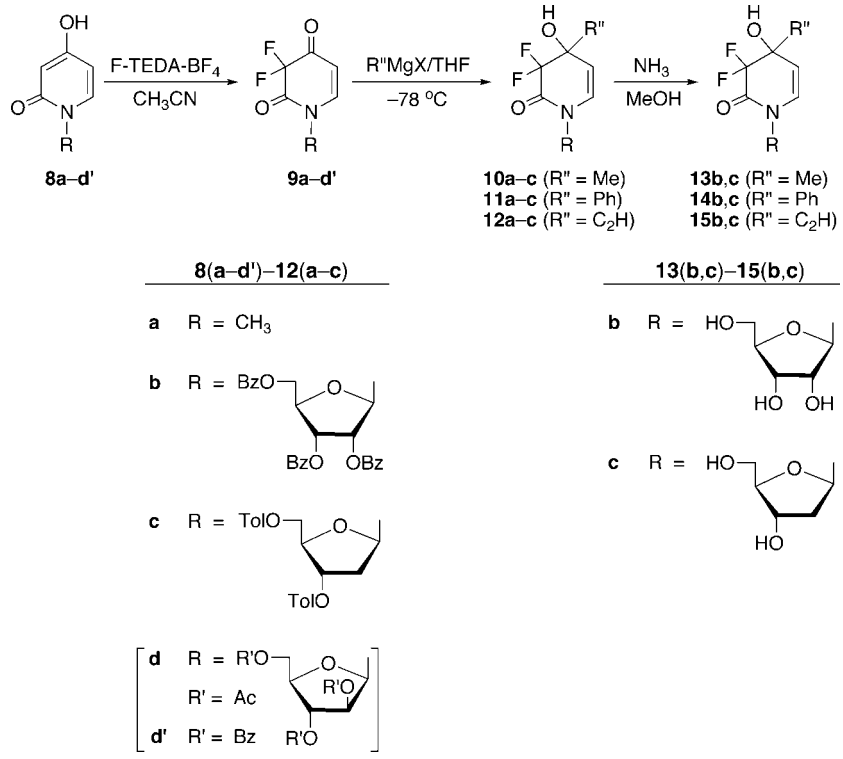
Figure 2. Fluorination of 3-deazauracils and nucleophilic addition at C4.

which facilitates the addition of nucleophiles. Such electrophilic augmentation in α-fluorinated ketones and α,α-difluorinated β-diketones has been suggested to promote enzyme-catalyzed addition of nucleophilic residues at the active sites of a number of enzymes,[11,12] resulting in enhanced inhibition. We reasoned that bis-fluorination at C3 of the β-keto-enol moiety in 3-deazauracils should lock C4 in its keto form, enhance 1,2-addition of nucleophiles at C4, and provide access to new 4-substituted 3,3-difluoro-3,4-dihydro-4-hydroxypyridin-2(1*H*)-ones (Figure 2). The resulting sp^3 center at C4 might mimic tetrahedral intermediates proposed for enzyme-catalyzed transformations in pyrimidine nucleotide biosynthetic pathways including CTP synthetase[13] and cytidine deaminase.[14] Such mimics might show anticancer or antiviral effects as single agents or modulate

* To whom correspondence should be addressed. Phone: 801-422-6272. Fax: 801-422-0153. E-mail: morris_robins@byu.edu.
† Brigham Young University.
‡ Rega Institute for Medical Research, Katholieke Universiteit Leuven.
§ Current address: Gilead Sciences, Foster City, CA.
|| Current address: Novartis Institute for Biomedical Research, Cambridge, MA.

10.1021/jm900203h CCC: $40.75 © 2009 American Chemical Society
Published on Web 04/20/2009

Scheme 1



Downloaded by Jonathan Berry on September 11, 2009 | http://pubs.acs.org
Publication Date (Web): April 20, 2009 | doi: 10.1021/jm900203h

cytosine nucleoside/nucleotide metabolism. The inhibition activities noted for **1**[4] (including clinical trials[6]), **2**,[4] and the 3-deaza-3-halo derivatives **3**, **6**, and **7**[10] provided a compelling incentive. We now report a convenient fluorination route to 3-deaza-3,3-difluorouracil bases and nucleosides, preparation of Grignard and Wittig reaction products, and generation of 3-deaza-3-fluorouracil nucleosides.

Chemistry

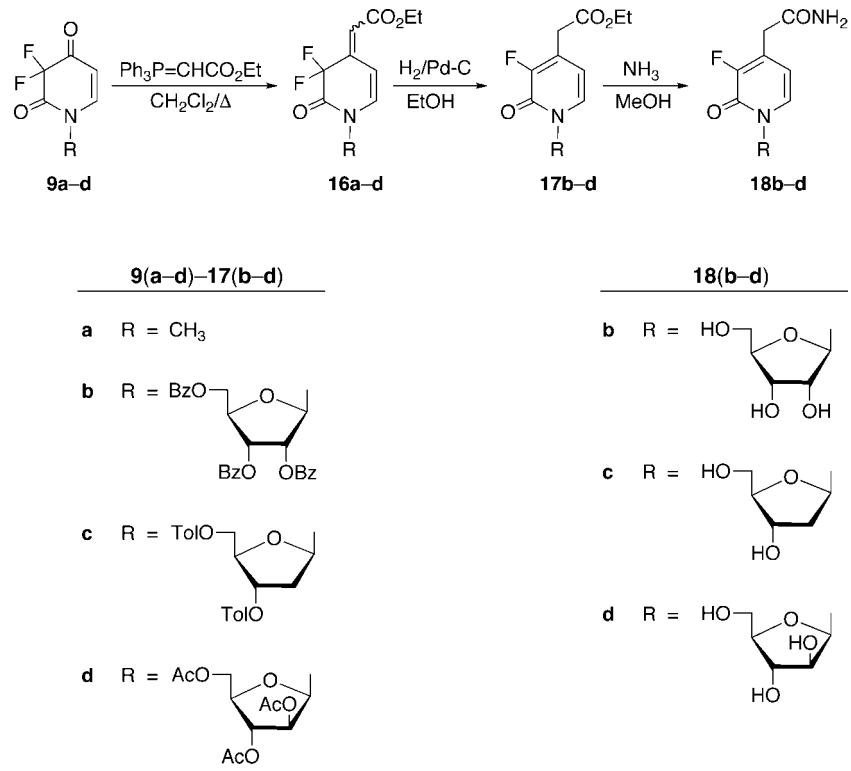
Several methods for the preparation of α-fluoro- and α,α-difluoro-substituted carbonyl compounds are available, but the safety profile and solubility properties of 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) (F-TEDA-BF_4) (Figure 2) are advantageous.[15] Treatment of a suspension of 4-hydroxy-1-methylpyridin-2(1*H*)-one (1-methyl-3-deazauracil) (**8a**) (Scheme 1) in acetonitrile with F-TEDA-BF_4 (>2 equiv) gave 3,3-difluoro-1-methylpyridine-2,4(1*H*,3*H*)-dione (3-deaza-3,3-difluoro-1-methyluracil) (**9a**) in 89% yield. Treatment of **8a** with one equivalent of the reagent resulted in conversion of ∼50% of **8a** to **9a** without observed buildup of a monofluorinated intermediate (TLC). This is consistent with enhancement of the acidity of the vicinal enol at C4 upon fluorination at C3, which would increase proton dissociation from O4 and produce the more reactive fluorinated enolate. Fluorination of 3-deazauridine (**1**) under these conditions was not successful, but analogous treatment of 2′,3′,5′-tri-*O*-benzoyl-3-deazauridine[3a] (**8b**) and 3-deaza-1-[2-deoxy-3,5-di-*O*-(4-methylbenzoyl)-β-D-*erythro*-pentofuranosyl]uracil[3a] (**8c**) gave the 3-deaza-3,3-difluorouridine **9b** (77%) and 2′-deoxy **9c** (76%) derivatives. Acetylation of 1-(β-D-arabinofuranosyl)-3-deazauracil[3a] gave the 4,2′,3′,5′-tetra-*O*-acetyl derivative, which underwent selective deacylation of the phenolic O4 in superheated methanol. Fluorination of 1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)-3-deazauracil (**8d**) and 1-(2,3,5-tri-*O*-benzoyl-β-D-arabinofuranosyl)-3-deazauracil (**8d′**, prepared by benzoylation and O4 debenzoylation) gave the 3-deaza-3,3-difluoro products **9d** (84%) and **9d′** (70%). Deacylation of the protected difluoro nucleoside derivatives was not successful.

Treatment of **9a** with methylmagnesium chloride in tetrahydrofuran at −78 °C gave the Grignard addition product **10a** (80%). Analogous treatment of **9b** gave diastereomers, and one isomer of **10b** (27%) was isolated by chromatography. The low temperature conditions allowed selective addition at C4, but competing attack at the benzoyl carbonyl groups occurred. Debenzoylation of **10b** gave the ribonucleoside adduct **13b** (80%). Greater byproduct formation was observed with the 2′-deoxy ester **9c**, and a slightly contaminated isomer of **10c** (20%) was obtained. Deprotection of **10c** gave the 2′-deoxy adduct **13c** (84%).

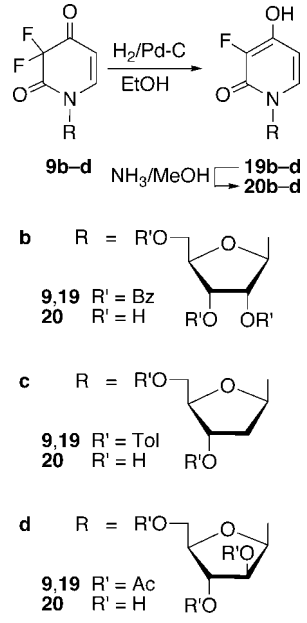
Addition of phenylmagnesium bromide to **9a** gave **11a** (88%). Analogous treatment of **9b** gave **11b** (43%), which was debenzoylated to give **14b** (98%). Compound **9c** gave **11c** (56%), which was deprotected to give **14c** (89%). Treatment of **9a** with ethynylmagnesium bromide gave **12a** (63%). Analogous treatment of **9b** gave diastereomers (2:1) of **12b** (53%), which were debenzoylated to give the diastereomeric ribonucleosides **15b** (77%). Addition to **9c** gave diastereomers (8:1) of **12c** (54%), which were deprotected to give the 2′-deoxynucleosides **15c** (91%). Complex mixtures (TLC) were formed upon treatment of the arabinosyl triacetate **9d** with Grignard reagents, and no significant improvement was observed with the tribenzoate **9d′**.

Treatment of **9a** with (ethoxycarbonylmethylene)triphenylphosphorane gave a single diastereomer of **16a** (97%) (Scheme 2). Analogous treatment of tribenzoate **9b** gave **16b** in quantitative yield. Attempted debenzoylation of **16b** by several procedures resulted in extensive decomposition. A solution of **16b** was stirred with hydrogen at atmospheric pressure over a 10% Pd−C catalyst. Hydrogenation of the exocyclic double bond and spontaneous elimination of hydrogen fluoride occurred to generate **17b** (85%) with a conjugated pyridin-2(1*H*)-one ring. Treatment of **17b** with methanolic

Scheme 2



Scheme 3



ammonia effected debenzoylation of the sugar moiety and ammonolysis of the appended carbethoxy group to give 4-(carboxamidomethyl)-3-deaza-3-fluorouridine (**18b**) (61%). The same sequence with **9c** gave Wittig product **16c** (92%), reduced 2-pyridone **17c** (68%), and 4-(carboxamidomethyl)-3-deaza-2′-deoxy-3-fluorouridine (**18c**) (90%). Wittig treatment of the arabinosyl triacetate **9d** gave **16d** (86%), which was hydrogenated to give **17d** (66%). Ammonolysis of **17d** gave 1-(β-D-arabinofuranosyl)-4-(carboxamidomethyl)-3-fluoropyridin-2(1*H*)-one (**18d**) (67%). In contrast with the attempted Grignard additions, Wittig treatment of **9d** gave the arabinosyl compound **16d** successfully. It is noteworthy that attempts to remove ester protecting groups from the difluoro intermediates (C4 being either C═O or C═C) resulted in extensive decomposition. Removal of one fluorine substituent from C3 with generation of a conjugated 2-pyridone ring gave compounds that underwent deacylation without complications.

The spontaneous elimination of hydrogen fluoride observed upon hydrogenation of the exocyclic C4 double bond with compounds **16b**−**d** (**16** → **17**) suggested a possible route to 3-deaza-3-fluorouridine (**20b**) (Scheme 3). Hydrogenolysis of C−F bonds is usually difficult. However, the large increase in stability gained by conjugation of the lone-pair electrons on nitrogen (through the C6−C5 and C4−C3 double bonds) with the C2 carbonyl group in a 2-pyridone ring would be expected to lower the energy barrier for catalyst-assisted C−F cleavage. Hydrogenolysis (H_2/10% Pd−C, atmospheric pressure) of tribenzoate **9b** resulted in isolation of 3-deaza-3-fluorouridine tribenzoate (**19b**) (63%). Deacylation of **19b** gave 3-deaza-3-fluorouridine (**20b**) (47%). The same sequence was applied to **9c** to give **19c** (75%) and then 3-deaza-2′-deoxy-3-fluorouridine (**20c**) (62%), and to **9d** to give **19d** (69%) and then 1-(β-D-arabinofuranosyl)-3-fluoro-4-hydroxypyridin-2(1*H*)-one (**20d**) (65%).

Biological Evaluation

Compounds **13b**, **13c**, **14b**, **14c**, **15b**, **15c**, **18b**, **18c**, **18d**, **20b**, **20c**, and **20d** were evaluated against herpes simplex virus-1 (HSV-1) (KOS), HSV-1 TK^- (KOS, ACV^r), HSV-2 (G), vaccinia virus, and vesicular stomatitis virus (VSV) in human embryonic lung (HEL) cell cultures; VSV, Coxsackie virus B4, and respiratory syncytial virus in HeLa cells; parainfluenza-3 virus, reovirus-1, Sindbis virus, and Punta Toro virus in Vero cells; cytomegalovirus (AD-169 and Davis strains) and varicella-zoster virus (OKA TK^+ and 07/1 TK^- strains) in HEL cells; MSV-induced transformation of C3H/3T3 cells; and HIV-1 and HIV-2 in human T-lymphocyte (CEM) cells. Viral replication was not inhibited at concentrations ≤100 μM. The cytostatic/cytotoxic effects on several tumor cell lines including murine

Downloaded by Jonathan Berry on September 11, 2009 | http://pubs.acs.org
Publication Date (Web): April 20, 2009 | doi: 10.1021/jm900203h

Downloaded by Jonathan Berry on September 11, 2009 | http://pubs.acs.org
Publication Date (Web): April 20, 2009 | doi: 10.1021/jm900203h

leukemia L1210 and human lymphocyte Molt4/C8 and CEM cells were also evaluated. None of the test compounds were inhibitory against tumor cell proliferation (≤500 μM) except 3-fluoro-3-deazauridine (**20b**), which was cytostatic against L1210 (CC_{50} = 6.7 ± 1.0 μM), Molt4/C8 (CC_{50} = 4.4 ± 0.4 μM), and CEM cells (CC_{50} = 9.6 ± 3.5 μM) (comparative data for 3-deazauridine (**1**) are: 1.3 ± 0.4 μM (L1210) and 11 ± 2.6 μM (CEM)).

Conclusions

Treatment of 4-hydroxypyridin-2(1*H*)-one (3-deazauracil) derivatives with F-TEDA-BF_4 resulted in formation of 3-deaza-3,3-difluorouracil compounds. Fluorination at C3 apparently produced an enol with sufficiently enhanced acidity to support increased proton dissociation, and rates of fluorination of that fluorinated enolate should be greater than for the starting enol. In harmony with this hypothesis, treatment with one equivalent of the reagent gave approximately equal amounts of difluorinated product and starting material. Attempted debenzoylation of 2′,3′,5′-tri-*O*-benzoyl-3-deaza-3,3-difluorouridine resulted in extensive decomposition.

Addition of Grignard reagents at C4 of 3-deaza-3,3-difluoro-1-methylpyridine-2,4(1*H*,3*H*)-dione and the protected ribo- and 2′-deoxynucleosides was successful, and deacylation gave the 4-hydroxy-4-(methyl, phenyl, and ethynyl) ribo- and 2′-deoxynucleoside adducts. However, treatment of the acetyl- or benzoyl-protected arabinonucleosides with Grignard reagents gave intractable mixtures.

Treatment of the 3,3-difluoro-2,4-dione intermediates (including the arabinosyl triacetate) with (ethoxycarbonylmethylene)-triphenylphosphorane gave exocyclic alkenes at C4. However, attempted deacylations of the Wittig products gave complex mixtures. Palladium-catalyzed hydrogenation at atmospheric pressure resulted in saturation of the exocyclic double bond and spontaneous elimination of hydrogen fluoride to give 4-(carbethoxymethyl)-3-deaza-3-fluorouracil derivatives. Ester ammonolysis gave the deprotected 4-(carboxamidomethyl)-3-deaza-3-fluorouracil nucleosides. Palladium-catalyzed hydrogenolysis of one C−F bond of the 3,3-difluoro compounds was effected at atmospheric pressure, and deacylation gave 3-deaza-3-fluorouridine and its 2′-deoxy and arabino analogues.

In summary, methodology for the synthesis of 3,3-difluoropyridine-2,4-diones has been developed. Grignard additions and Wittig olefinations provided C4 adducts and alkenes, respectively. Hydrogenation of exocyclic alkenes was accompanied by spontaneous elimination of hydrogen fluoride to produce conjugated 2-pyridones. Hydrogenolysis of one C−F bond of the *gem*-difluoro intermediates gave conjugated 3-fluoro-4-hydroxypyridin-2(1*H*)-ones. The unprotected nucleoside derivatives were evaluated in viral-infected and cancer cell cultures. None of these compounds showed antiviral activity at concentrations ≤100 μM. They also exhibited no cytostatic/cytotoxic activity at ≤500 μM except for 3-fluoro-3-deazauridine (**20b**), which showed cytostatic activity against murine leukemia L1210 cells and human lymphocyte Molt4/C8 and CEM cells (CC_{50} values of 4.4 μM−9.6 μM).

Experimental Section

Flame- or oven-dried glassware was used, and solvents were dried immediately prior to use. Reaction progress was monitored by TLC (preparative TLC was performed on ANALTECH plates (20 cm × 20 cm, 1000 μ)). UV spectra were obtained with solutions in MeOH. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were obtained with solutions in $CDCl_3$ (internal reference δ 7.27 for ^{1}H and 77.23 for ^{13}C) unless otherwise noted (MeOH-d_4: internal reference ^{1}H (δ 4.87) and ^{13}C (49.15)). All ^{19}F NMR spectra were obtained at 282 MHz with CBr_3F (δ 0.0) as internal standard. F-TEDA-BF_4 (Selectfluor, >95% F^+) and "2,4-dihydroxypyridine" (3-deazauracil) were purchased from Aldrich. Tested compounds **13b**, **13c**, **14b**, **14c**, **15c**, **18b**, **18c**, **18d**·H_2O, **20b**, and **20c** had elemental analysis values for C, H, and N within ±0.4% of theory. Compound **15b** had (C 47.22 calcd, 46.76 found), H, N within ±0.4% of theory, and **20d**·1.5 H_2O had (C 41.67 calcd, 42.12 found), H, N within ±0.4% of theory (well within the 95% purity limit).

Procedure A: Fluorination. A suspension of the 4-hydroxypyridin-2(1*H*)-one derivative and Selectfluor (2−3 equiv) in CH_3CN was stirred at ambient temperature under an atmosphere of dry nitrogen. Volatiles were flash evaporated, the residue was suspended in CH_2Cl_2, and the suspension was filtered (microglass fiber filter). The product was isolated by flash chromatography.

Procedure B: Grignard Addition. A solution of the 3,3-difluoropyridine-2,4-(1*H*,3*H*)-dione derivative in THF was treated with a Grignard reagent (1.1−1.8 equiv) at −78 °C and stirred until no starting material remained (1−3 h). MeOH/H_2O (1:1) was slowly added, and the reaction mixture was allowed to warm to ambient temperature and partitioned ($NaHCO_3$/H_2O//CH_2Cl_2). The organic phase was dried (Na_2SO_4), volatiles were evaporated, and the residue was purified by chromatography.

Procedure C: Ammonolysis. A solution of the protected nucleoside in NH_3/MeOH (saturated at 0 °C) was stirred at ambient temperature until deprotection was complete. Volatiles were evaporated, and the residue was purified by flash chromatography.

Procedure D: Wittig Olefination. A solution of the 3,3-difluoropyridine-2,4(1*H*,3*H*)-dione derivative and (ethoxycarbonylmethylene)triphenylphosphorane in CH_2Cl_2 was heated at reflux until no starting material remained. Volatiles were evaporated, and the product was isolated by flash chromatography.

4-Hydroxy-1-methylpyridin-2(1*H*)-one (8a). A suspension of 3-deazauracil (100 mg, 0.9 mmol) and TMSCl (20 μL) in HMDS (2 mL) was heated at reflux under dry N_2 until a clear solution was obtained. Excess HMDS was removed under vacuum (130 °C), and dried CH_3CN (2 mL) was added. A solution of iodomethane (1.14 g, 8.03 mmol) in CH_3CN (3 mL) was added, and the reaction mixture was heated at 55−60 °C until methylation was complete (3−3.5 h) and then allowed to cool to ambient temperature. AcOH/MeOH (0.1 M) was added, and the mixture was stirred for 1 h. The solid was filtered, washed (CH_2Cl_2), and recrystallized to give **8a**[16] (50 mg) (∼45% recrystallization recovery).

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-4-hydroxypyridin-2(1*H*)-one (8d). A solution of 4-hydroxy-1-(β-D-arabinofuranosyl)pyridin-2(1*H*)-one[3a] (200 mg, 0.822 mmol) and DMAP (3 mg, 0.024 mmol) in Ac_2O (3.0 mL) was stirred overnight at ambient temperature. The reaction mixture was partitioned ($NaHCO_3$/H_2O//CH_2Cl_2), and the aqueous phase was extracted (CH_2Cl_2, 3×). Volatiles were evaporated from the combined organic layers, and the residue was chromatographed (EtOAc/hexanes, 7:3) to give 4-acetoxy-1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)pyridin-2(1*H*)-one (300 mg, 89%) as a white foam: UV max 299 nm, min 247 nm. ^{1}H NMR δ 7.53 (d, J = 8.0 Hz, 1H), 6.40 (d, J = 3.8 Hz, 1H), 6.22 (d, J = 2.6 Hz, 1H), 6.07 (dd, J = 2.6, 7.7 Hz, 1H), 5.50 (dd, J = 1.5, 4.1 Hz, 1H), 5.03 (dd, J = 1.5, 3.3 Hz, 1H), 4.35 (d, J = 5.5 Hz, 2H), 4.21−4.14 (m, 1H), 2.21, 2.07, 2.05, 1.85 (4 × s, 4 × 3H). ^{13}C NMR δ 170.5, 169.6, 168.2, 167.5, 162.5, 160.1, 134.2, 109.4, 102.1, 85.0, 80.8, 76.6, 73.9, 62.8, 21.1, 20.8, 20.7, 20.3. HRMS (FAB$^+$) m/z calcd for $C_{18}H_{22}NO_{10}$ $(M + H)^+$ 412.1245, found 412.1237.

A solution of this material (300 mg, 0.729 mmol) in MeOH (15 mL) was heated in a pressure tube (90 °C for 24 h and then 105 °C for an additional 24 h). Volatiles were evaporated, and the residue was chromatographed (EtOAc/hexanes, 9:1) to give **8d** (193 mg, 72%) as a white solid. ^{1}H NMR δ 7.46 (d, J = 7.8 Hz, 1H), 6.40 (d, J = 3.9 Hz, 1H), 6.09 (dd, J = 2.4, 7.8 Hz, 1H), 5.87 (d, J = 2.7 Hz, 1H), 5.48 (dd, J = 1.5, 3.9 Hz, 1H), 5.05−5.03 (m, 1H), 4.39 (dd, J = 4.8, 12.0 Hz, 1H), 4.33 (dd, J = 6.6, 11.8 Hz, 1H), 4.18 (dd, J = 5.7, 9.0 Hz, 1H), 2.09, 2.08, 1.85 (3 × s, 3 ×

H). ^{13}C NMR δ 170.6, 169.8, 169.1, 168.4, 164.2, 134.2, 102.5, 98.6, 84.9, 80.6, 76.6, 74.1, 62.9, 20.8, 20.7, 20.3. HRMS (FAB$^+$) *m/z* calcd for $C_{16}H_{19}NO_9Na$ (M + Na)$^+$ 392.0958, found 392.0968.

1-(2,3,5-Tri-*O*-benzoyl-β-D-arabinofuranosyl)-4-hydroxypyridin-2(1*H*)-one (8d′). Benzoyl chloride (5 mL, 43 mmol) was added to a solution of 4-hydroxy-1-(β-D-arabinofuranosyl)pyridin-2(1*H*)-one[3a] (1.4 g, 5.7 mmol) in pyridine (20 mL), the reaction mixture was stirred overnight at ambient temperature, and MeOH was added. Volatiles were evaporated, and the residue was chromatographed (EtOAc/hexanes, 3:7) to give 1-(2,3,5-tri-*O*-benzoyl-β-D-arabinofuranosyl)-4-benzoyloxypyridin-2(1*H*)-one (3.52 g, 94%) as a yellow oil. ^{1}H NMR δ 8.15−7.24 (m, 21H), 6.74 (d, J = 3.9 Hz, 1H), 6.44 (d, J = 2.4 Hz, 1H), 6.28 (dd, J = 2.4, 7.8 Hz, 1H), 6.04 (d, J = 3.9 Hz, 1H), 5.68 (d, J = 3.0 Hz, 1H), 4.94−4.85 (m, 2H), 4.63−4.58 (m, 1H). ^{13}C NMR δ 166.4, 165.6, 164.8, 163.4, 162.8, 160.9, 134.5, 134.3, 134.0, 133.8, 133.5, 130.5, 130.2, 129.9, 129.8, 129.5, 128.9, 128.83, 128.79, 128.73, 128.70, 128.61, 128.57, 109.9, 102.7, 85.9, 81.9, 77.8, 75.4, 63.5.

A suspension of this material (3.52 g, 5.34 mmol) in AcOH/MeOH (1:3, 50 mL) was heated in a pressure tube (7 days at 120 °C). Volatiles were evaporated, and the residue was chromatographed (EtOAc/hexanes, 4:6) to give recovered starting material (1.0 g) plus **8d′** (1.95 g, conversion yield 66%) as a light-yellow oil. ^{1}H NMR δ 8.16−7.24 (m, 16H), 6.68 (d, J = 3.9 Hz, 1H), 6.07 (dd, J = 2.4, 7.6 Hz, 1H), 5.95−5.92 (m, 2H), 5.64 (d, J = 3.4 Hz, 1H), 4.86 (d, J = 4.6 Hz, 2H), 4.56 (dd, J = 4.5, 8.2 Hz, 1H). ^{13}C NMR δ 169.2, 166.4, 165.6, 164.7, 164.3, 134.5, 134.0, 133.8, 133.5, 130.3, 129.9, 129.8, 129.6, 128.8, 128.7, 128.62, 128.55, 102.9, 99.3, 85.4, 81.5, 77.7, 75.5, 63.6. HRMS (FAB$^+$) *m/z* calcd for $C_{31}H_{25}NO_9Na$ (M + Na)$^+$ 578.1427, found 578.1437.

3,3-Difluoro-1-methylpyridine-2,4(1*H*,3*H*)-dione (9a). Treatment of **8a** (1.02 g, 8.16 mmol), Selectfluor (6.90 g, 18.5 mmol), and CH_3CN (25 mL) by procedure A (3 h; chromatography with EtOAc/hexanes, 8:2) gave **9a** (1.17 g, 89%) as a yellow oil that solidified on standing: UV max 318 nm (ε 20 500); min 243 nm (ε 500). ^{1}H NMR δ 7.29 (d, J = 8.8 Hz, 1H), 5.73 (dt, J = 3.2, 8.6 Hz, 1H), 3.37 (s, 3H). ^{13}C NMR δ 183.4 (t, J = 23.2 Hz), 164.0 (t, J = 28.7 Hz), 149.0, 105.3 (t, J = 2.0 Hz), 100.8 (t, J = 253.6 Hz), 35.7. ^{19}F NMR δ −112.0. HRMS (EI$^+$) *m/z* calcd for $C_6H_5F_2NO_2$ (M$^+$) 161.0288, found 161.0291.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-3,3-difluoropyridine-2,4(1*H*,3*H*)-dione (9b). Treatment of **8b** (2.05 g, 3.69 mmol), Selectfluor (4.10 g, 11.0 mmol), and CH_3CN (20 mL) by procedure A (2.5 h; chromatography with EtOAc/hexanes, 4:6) gave 9b (1.69 g, 77%) as a white foam: UV max 313, 230 nm; min 259, 211 nm. ^{1}H NMR δ 8.11−7.34 (m, 16H), 6.33 (d, J = 6.1 Hz, 1H), 5.88 (dd, J = 3.8, 6.0 Hz, 1H), 5.68 (t, J = 6.1 Hz, 1H), 5.58 (dt, J = 3.0, 8.8 Hz, 1H), 4.85 (dd, J = 2.4, 12.0 Hz, 1H), 4.75−4.72 (m, 1H), 4.68 (dd, J = 3.5, 12.1 Hz, 1H). ^{13}C NMR δ 182.4 (t, J = 23.2 Hz), 166.2, 165.6, 163.6 (t, J = 29.2 Hz), 142.3, 134.22, 134.16, 134.1, 130.2, 130.1, 129.8, 129.3, 129.1, 128.9, 128.85, 128.77, 128.4, 106.8, 101.0 (t, J = 255.1 Hz), 87.0, 81.3, 73.4, 71.5, 63.9. ^{19}F NMR δ −111.4 (d, J = 335.7 Hz), −113.7 (d, J = 332.7 Hz). HRMS (FAB$^+$) *m/z* calcd for $C_{31}H_{23}F_2NO_9Na$ (M + Na)$^+$ 614.1239, found 614.1223.

1-[2-Deoxy-3,5-di-*O*-(4-methylbenzoyl)-β-D-*erythro*-pentofuranosyl]-3,3-difluoropyridine-2,4(1*H*,3*H*)-dione (9c). Treatment of **8c** (1.20 g, 2.59 mmol), Selectfluor (2.90 g, 7.78 mmol), and CH_3CN (25 mL) by procedure A (22 h; chromatography with EtOAc/CH_2Cl_2, 1:9) gave **9c** (974 mg, 76%) as a white solid: UV max 314, 240, min 268, 215 nm. ^{1}H NMR (500 MHz) δ 7.94 (d, J = 8.3 Hz, 2H), 7.89 (d, J = 8.3 Hz, 2H), 7.65 (d, J = 8.8 Hz, 1H), 7.29−7.26 (m, 4H), 6.36 (dd, J = 5.4, 8.3 Hz, 1H), 5.62 (dd, J = 1.5, 6.3 Hz, 1H), 5.55−5.53 (m, 1H), 4.74 (dd, J = 2.9, 12.2 Hz, 1H), 4.68 (dd, J = 3.2, 12.2 Hz, 1H), 4.56 (d, J = 2.0 Hz, 1H), 2.74 (ddd, J = 1.5, 5.4, 14.2 Hz, 1H), 2.44, 2.43 (2 × s, 2 × 3H), 2.33−2.27 (m, 1H). ^{13}C NMR (125 MHz) δ 182.6 (t, J = 22.9 Hz), 166.2, 163.3 (t, J = 29.0 Hz), 145.0, 142.0, 130.0, 129.7, 129.6, 129.5, 126.5, 126.3, 106.2, 100.8 (t, J = 254.4 Hz), 85.0, 83.5, 74.8, 64.1, 37.9, 22.0. ^{19}F NMR δ −111.4 (d, J = 335.4 Hz), −114.1 (d, J = 335.4 Hz). HRMS (FAB$^+$) *m/z* calcd for $C_{26}H_{24}F_2NO_7$ (M + H)$^+$ 500.1522, found: 500.1531.

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-3,3-difluoropyridine-2,4(1*H*,3*H*)-dione (9d). Treatment of **8d** (250 mg, 0.677 mmol), Selectfluor (770 mg, 2.06 mmol), and CH_3CN (2.5 mL) by procedure A (16 h; chromatography with EtOAc/hexanes, 8:2) gave **9d** (230 mg, 84%) as a white foam: UV max 312 nm, min 244 nm. ^{1}H NMR δ 7.66 (d, J = 9.0 Hz, 1H), 6.20 (d, J = 4.2 Hz, 1H), 5.76 (dt, J = 3.2, 9.0 Hz, 1H), 5.43 (dd, J = 2.1, 4.3 Hz, 1H), 5.11 (dd, J = 2.3, 3.5 Hz, 1H), 4.45 (dd, J = 6.6, 12.0 Hz, 1H), 4.36 (dd, J = 3.9, 12.0 Hz, 1H), 4.24−4.20 (m, 1H), 2.15, 2.13, 2.06 (3 × s, 3 × 3H). ^{13}C NMR δ 182.4 (t, J = 22.9 Hz), 170.7, 169.8, 168.8, 163.1 (t, J = 29.2 Hz), 143.4, 105.0, 100.8 (t, J = 254.6 Hz), 84.1, 80.9, 76.0, 74.9, 62.7, 20.9, 20.8, 20.6. ^{19}F NMR δ −111.1 (d, J = 333.2 Hz), −114.6 (d, J = 335.4 Hz). HRMS (FAB$^+$) *m/z* calcd for $C_{16}H_{18}F_2NO_9$ (M + H)$^+$ 406.0951, found 406.0969.

1-(2,3,5-Tri-*O*-benzoyl-β-D-arabinofuranosyl)-3,3-difluoropyridine-2,4(1*H*,3*H*)-dione (9d′). Treatment of **8d′** (35 mg, 0.063 mmol), Selectfluor (70 mg, 0.19 mmol), and CH_3CN (2 mL) by procedure A (5 h; chromatography with EtOAc/hexanes, 8:2) gave **9d′** (26 mg, 70%) as a white solid: UV 313, 231 nm, min 259, 211 nm. ^{1}H NMR δ 8.11−7.36 (m, 16H), 6.46 (d, J = 4.2 Hz, 1H), 5.88 (dd, J = 1.7, 4.2 Hz, 1H), 5.67 (dd, J = 1.7, 3.7 Hz, 1H), 5.54 (dt, J = 3.2, 8.9 Hz, 1H), 4.95 (dd, J = 6.4, 12.2 Hz, 1H), 4.83 (dd, J = 3.7, 12.0 Hz, 1H), 4.61−4.56 (m, 1H). ^{13}C NMR δ 182.6 (t, J = 23.17 Hz), 166.6, 165.8, 165.2, 163.4 (t, J = 29.5 Hz), 143.6, 134.6, 134.5, 134.0, 130.4, 130.14, 130.11, 129.7, 129.3, 129.1, 129.0, 128.6, 128.1, 105.3, 101.0 (dd, J = 253.4, 256.3 Hz), 84.7, 81.7, 77.8, 76.1, 63.3. ^{19}F NMR δ −110.0 (d, J = 335.4 Hz), −115.5 (d, J = 335.4 Hz). HRMS (FAB$^+$) *m/z* calcd for $C_{31}H_{24}F_2NO_9$ (M + H)$^+$ 592.1419, found 592.1420.

3,3-Difluoro-3,4-dihydro-4-hydroxy-1,4-dimethylpyridin-2(1*H*)-one (10a). Treatment of **9a** (100 mg, 0.621 mmol), MeMgCl/THF (3 M; 350 μL, 1.1 mmol), and THF (1.0 mL) by procedure B (2 h; chromatography with CH_2Cl_2/MeOH, 20:1) gave **10a** (88 mg, 80%) as a red oil: UV 263 nm (ε 2900), min 219 nm (ε 630). ^{1}H NMR δ 6.04 (d, J = 8.1 Hz, 1H), 5.20 (ddd, J = 2.4, 3.6, 8.2 Hz, 1H), 3.15 (s, 3H), 2.40 (bs, 1H), 1.45 (d, J = 2.2 Hz, 3H). ^{13}C NMR δ 161.6 (dd, J = 28.7, 30.7 Hz), 130.3, 112.0 (d, J = 1.5 Hz), 111.9 (t, J = 252.8 Hz), 71.0 (dd, J = 22.4, 25.4 Hz), 34.3, 20.3 (t, J = 2.5 Hz). ^{19}F NMR δ −121.3 (d, J = 268.4 Hz), −130.2 (d, J = 268.7 Hz). HRMS (EI$^+$) *m/z* calcd for $C_7H_9F_2NO_2$ (M$^+$) 177.0601, found 177.0600.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-3,3-difluoro-3,4-dihydro-4-hydroxy-4-methylpyridin-2(1*H*)-one (10b). Treatment of **9b** (500 mg, 0.845 mmol), MeMgCl/THF (3 M; 290 μL, 0.87 mmol), and THF (7 mL) by procedure B (1 h; chromatography with EtOAc/hexanes, 2:8) gave **10b** (140 mg, 27%) as a white foam: UV 314, 231 nm, 291, 210 nm. ^{1}H NMR δ 8.11−7.26 (m, 15H), 6.48 (d, J = 8.0 Hz, 1H), 6.40 (d, J = 7.3 Hz, 1H), 5.83 (dd, J = 2.4, 6.1 Hz, 1H), 5.64 (t, J = 6.7 Hz, 1H), 5.30 (dd, J = 4.6, 8.1 Hz, 1H), 4.75−4.65 (m, 3H), 3.09 (s, 1H), 1.43 (d, J = 2.0 Hz, 3H). ^{13}C NMR δ 166.5, 166.2, 165.7, 161.5 (dd, J = 28.7, 32.7 Hz), 134.3, 134.1, 133.9, 130.2, 130.1, 130.0, 129.8, 129.3, 129.0, 128.91, 128.87, 128.8, 128.7, 128.1, 124.1, 114.7 (d, J = 3.0 Hz), 111.7 (dd, J = 251.8, 258.4 Hz), 84.8, 80.6, 72.6, 72.0, 69.6 (dd, J = 23.2, 26.2 Hz), 64.4, 19.1. ^{19}F NMR δ −116.2 (d, J = 267.0 Hz), −134.8 (d, J = 267.0 Hz). HRMS (FAB$^+$) *m/z* calcd for $C_{32}H_{27}F_2NO_9Na$ (M + Na)$^+$ 630.1552, found 630.1552.

3,3-Difluoro-3,4-dihydro-4-hydroxy-4-methyl-1-(β-D-ribofuranosyl)pyridin-2(1*H*)-one (13b). Deacylation of **10b** (156 mg, 0.257 mmol) by procedure C (20 mL, 1 d; chromatography with MeOH/CH_2Cl_2, 1:9) gave **13b** (61 mg, 80%) as an oil that was crystallized (MeOH/EtOAc) to give **13b**: mp 187−188 °C; UV max 258 nm (ε 3600), min 215 nm (ε 740). ^{1}H NMR (MeOH-d_4, 500 MHz) δ 6.67 (d. J = 8.3 Hz, 1H), 5.87 (d, J = 4.4 Hz, 1H), 5.33 (dd, J = 4.4, 8.3 Hz, 1H), 4.11−4.08 (m, 2H), 3.94 (dd, J = 3.4, 6.3 Hz, 1H), 3.79 (dd, J = 2.9, 12.2 Hz, 1H), 3.69 (dd, J = 3.4, 12.2 Hz, 1H), 1.40 (d, J = 2.0 Hz, 3H). ^{13}C NMR (MeOH-d_4, 125 MHz) δ 163.3 (dd, J = 29.0, 31.3 Hz), 125.8, 114.0 (d, J = 3. 0 Hz), 113.5

Downloaded by Jonathan Berry on September 11, 2009 | http://pubs.acs.org
Publication Date (Web): April 20, 2009 | doi: 10.1021/jm900203h

Downloaded by Jonathan Berry on September 11, 2009 | http://pubs.acs.org
Publication Date (Web): April 20, 2009 | doi: 10.1021/jm900203h

(dd, J = 249.9, 256.0 Hz), 88.7, 86.1, 74.4, 71.8, 70.4 (dd, J = 22.5, 25.6 Hz), 62.8, 20.0. ^{19}F NMR (MeOH-d_4) δ −117.6 (d, J = 264.9 Hz), −132.6 (d, J = 264.9 Hz). HRMS (EI$^+$) m/z calcd for $C_{11}H_{15}F_2NO_6$ (M^+) 295.0868, found 295.0853.

1-[2-Deoxy-3,5-di-*O*-(4-methylbenzoyl)-β-D-*erythro*-pentofuranosyl]-3,3-difluoro-3,4-dihydro-4-hydroxy-4-methylpyridin-2(1*H*)-one (10c). Treatment of **9c** (215 mg, 0.430 mmol), MeMgBr//THF/toluene (1:3) (1.4 M; 340 μL, 0.476 mmol), and THF (5 mL) by procedure B (3 h; radial chromatography with EtOAc/hexanes, 2:8) gave impure **10c**. Further purification (preparative-TLC, EtOAc/hexanes, 2:8) gave slightly contaminated **10c** (44 mg, 20%) as a light-yellow foam: UV max 315, 240 nm, min 291, 214 nm. ^{1}H NMR δ 7.94−7.90 (m, 4H), 7.28 (d, J = 2.4 Hz, 2H), 7.24 (d, J = 2.4 Hz, 2H), 6.38 (dd, J = 5.9, 8.5 Hz, 1H), 6.33 (d, J = 8.5 Hz, 1H), 5.58−5.50 (m, 1H), 5.16 (dt, J = 2.9, 5.6 Hz, 1H), 4.68−4.57 (m, 2H), 4.45 (t, J = 3.0 Hz, 1H), 2.76 (bs, 1H), 2.55−2.49 (m, 1H), 2.42 (s, 6H), 2.24−2.35 (m, 1H), 1.38 (s, 3H). ^{13}C NMR δ 166.3, 166.2, 161.1 (t, J = 30.5 Hz), 144.7, 144.5, 130.0, 129.8, 129.52, 129.46, 126.9, 126.5, 122.9, 113.7, 112.0 (t, J = 253.6 Hz), 83.7, 82.4, 74.9, 70.8 (dd, J = 22.0, 25.0 Hz), 64.3, 36.5, 21.88, 21.85. ^{19}F NMR δ −123.6 (d, J = 264.9 Hz), −128.2 (d, J = 264.9 Hz). HRMS (FAB$^+$) m/z for $C_{27}H_{27}F_2NO_7Na$ (M + Na)$^+$ 538.1653, found 538.1642.

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3,3-difluoro-3,4-dihydro-4-hydroxy-4-methylpyridin-2(1*H*)-one (13c). Deacylation of **10c** (110 mg, 0.213 mmol) by procedure C (15 mL, 2 d; chromatography with MeOH/CH_2Cl_2, 1:9) gave **13c** (50 mg, 84%) as a colorless oil that was crystallized (EtOH/$CHCl_3$/hexanes) to give **13c** (36 mg): mp 145−146 °C; UV max 258 nm (ε 3600), min 214 nm. ^{1}H NMR (MeOH-d_4, 500 MHz) δ 6.60 (d, J = 8.3 Hz, 1H), 6.26 (t, J = 6.8 Hz, 1H), 5.33 (dt, J = 2.9, 8.3 Hz, 1H), 4.36−4.34 (m, 1H), 3.87 (dd, J = 3.7, 7.1 Hz, 1H), 3.73 (dd, J = 3,7, 12.0 Hz, 1H), 3.68 (dd, J = 4.2, 12.0 Hz, 1H), 2.19−2.10 (m, 2H), 1.36 (d, J = 2.0 Hz, 3H). ^{13}C NMR (MeOH-d_4, 75 MHz) δ 163.1 (dd, J = 29.2, 30.7 Hz), 124.7, 114.8, 113.8 (dd, J = 253.1, 253.3 Hz), 88.6, 84.8, 72.6, 71.0 (dd, J = 21.4, 24.9 Hz), 63.3, 39.5, 20.6 (t, J = 2.8 Hz). ^{19}F NMR (MeOH-d_4) δ −122.2 (d, J = 264.9 Hz), −128.4 (d, J = 264.9 Hz). HRMS (EI$^+$) m/z calcd for $C_{11}H_{15}F_2NO_5$ 279.0918, found 279.0919.

3,3-Difluoro-3,4-dihydro-4-hydroxy-1-methyl-4-phenylpyridin-2(1*H*)-one (11a). Treatment of **9a** (32 mg, 0.20 mmol), PhMgBr/THF (1 M; 220 μL, 0.220 mmol), and THF (0.5 mL) by procedure B (3 h; chromatography with CH_2Cl_2/MeOH, 50:1) gave **11a** (42 mg, 88%) as a white powder: UV max 263 nm (ε 4700), min 235 nm (ε 2700). ^{1}H NMR δ 7.58−7.54 (m, 2H), 7.44−7.40 (m, 3H), 6.32 (d, J = 8.1 Hz, 1H), 5.48 (ddd, J = 1.8, 3.9, 8.2 Hz, 1H), 3.23 (s, 3H), 2.56−2.57 (m, 1H). ^{13}C NMR δ 161.1 (dd, J = 28.7, 31.2 Hz), 136.0, 132.6, 129.3, 128.6, 127.0 (d, J = 1.5 Hz), 111.6 (d, J = 1.8 Hz), 110.9 (dd, J = 252.3, 256.8 Hz), 74.9 (dd, J = 21.9, 25.4 Hz), 34.5. ^{19}F NMR δ −116.8 (d, J = 268.6 Hz), −128.3 (d, J = 268.6 Hz). HRMS (EI$^+$) m/z calcd for $C_{12}H_{11}F_2NO_2$ (M^+) 239.0758, found 239.0754.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-3,3-difluoro-3,4-dihydro-4-hydroxy-4-phenylpyridin-2(1*H*)-one (11b). Treatment of **9b** (330 mg, 0.558 mmol), PhMgBr/THF (1.0 M; 590 μL, 0.590 mmol), and THF (4 mL) by procedure B (1 h; chromatography with EtOAc/hexanes, 2:8) gave **11b** (162 mg, 43%) as a white foam: UV max 230 nm, min 212 nm. ^{1}H NMR (500 MHz) δ 8.14−7.19 (m, 20H), 6.53 (d, J = 8.8 Hz, 1H), 6.31 (d, J = 6.8 Hz, 1H), 5.88 (dd, J = 3.7, 6.1 Hz, 1H), 5.79 (t, J = 6.4 Hz, 1H), 5.38 (dt, J = 2.7, 8.3 Hz, 1H), 4.83 (dd, J = 2.7, 12.0 Hz, 1H), 4.68−4.63 (m, 2H), 2.92 (s, 1H). ^{13}C NMR (125 MHz) δ 166.4, 165.7, 165.6, 161.2 (t, J = 30.1 Hz), 135.1, 134.1, 134.0, 133.9, 130.2, 130.1, 129.9, 129.5, 129.3, 129.0, 128.9, 128.83, 128.81, 128.7, 128.5, 126.8, 125.8, 113.9 (t, J < 0.5 Hz), 111.1 (t, J = 255.6 Hz), 86.1, 80.7, 74.7 (dd, J = 21.3, 24.4 Hz), 72.4, 71.6, 64.2. ^{19}F NMR δ −121.0 (dd, J = 264.9), −124.7 (d, 264.9 Hz). HRMS (FAB$^+$) m/z calcd for $C_{37}H_{29}F_2NO_9Na$ (M + Na)$^+$ 692.1708, found 692.1708.

3,3-Difluoro-3,4-dihydro-4-hydroxy-4-phenyl-1-(β-D-ribofuranosyl)pyridin-2(1*H*)-one (14b). Deacylation of **11b** (240 mg, 0.358 mmol) by procedure C (20 mL, 1 d; chromatography with MeOH/CH_2Cl_2, 1:9) gave **14b** (125 mg, 98%) as an oil: UV max 257 nm (ε 3600), min 236 nm (ε 2400). ^{1}H NMR (MeOH-d_4, 500 MHz) δ 7.58 (d, J = 6.8 Hz, 2H), 7.40−7.34 (m, 3H), 6.89 (d, J = 8.3 Hz, 1H), 5.91 (d, J = 5.9 Hz, 1H), 5.56 (dt, J = 1.8, 8.5 Hz, 1H), 4.25 (t, J = 5.6 Hz, 1H), 4.15 (t, J = 4.9 Hz, 1H), 4.00 (dd, J = 3.7, 7.1 Hz, 1H), 3.79 (dd, J = 2.9, 12.2 Hz, 1H), 3.72 (dd, J = 3.9, 12.2 Hz). ^{13}C NMR (MeOH-d_4, 125 MHz) δ 163.3 (dd, J = 29.8, 30.5 Hz), 138.4, 129.8, 129.2, 128.3, 127.3, 114.1, 112.8 (dd, J = 249.9, 256.7 Hz), 88.8, 86.2, 74.8 (dd, J = 21.4, 24.4 Hz), 74.2, 71.9, 62.9. ^{19}F NMR (MeOH-d_4) δ −116.8 (d, J = 262.7 Hz), −126.8 (d, J = 264.9 Hz). HRMS (EI$^+$) m/z calcd for $C_{16}H_{17}F_2NO_6$ (M^+) 357.1024, found 357.1029.

1-[2-Deoxy-3,5-di-*O*-(4-methylbenzoyl)-β-D-ribofuranosyl]-3,3-difluoro-3,4-dihydro-4-hydroxy-4-phenylpyridin-2(1*H*)-one (11c). Treatment of **9c** (200 mg, 0.400 mmol), PhMgBr/THF (1.0 M; 600 μL, 0.600 mmol), and THF (5 mL) by procedure B (3 h; radial chromatography with EtOAc/CH_2Cl_2, 1:99) gave **11c** (130 mg, 56%) as a white solid: UV max 241 nm, min 216 nm. ^{1}H NMR δ 7.94−7.19 (m, 13H), 6.58 (d, J = 8.3 Hz, 1H), 6.39 (dd, J = 5.9, 8.5 Hz, 1H), 5.58 (d, J = 6.6 Hz, 1H), 5.39 (d, J = 8.3 Hz, 1H), 4.63 (dd, J = 3.4, 12.0 Hz, 1H), 4.59 (dd, J = 3.7, 12.5 Hz, 1H), 4.42 (d, J = 2.4 Hz, 1H), 3.51 (s, 1H), 2.58−2.51 (m, 1H), 2.42−2.31 (m, 1H), 2.40, 2.37 (2 × s, 2 × 3H). ^{13}C NMR δ 166.4, 166.2, 160.8 (dd, J = 29.2, 30.7 Hz), 144.7, 144.5, 135.8, 129.9, 129.7, 129.5, 129.4, 129.1, 128.4, 127.0, 126.7, 126.4, 125.2, 113.0, 111.1 (dd, J = 252.8, 257.3 Hz), 83.7, 82.4, 74.9, 74.2 (dd, J = 21.5, 24.9 Hz), 64.3, 36.4, 21.83, 21.78. ^{19}F NMR δ −118.2 (d, J = 264.9 Hz), −126.9 (d, J = 267.0 Hz). HRMS (FAB$^+$) m/z calcd for $C_{32}H_{29}F_2NO_7Na$ (M + Na)$^+$ 600.1810, found 600.1812.

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3,3-difluoro-3,4-dihydro-4-hydroxy-4-phenylpyridin-2(1*H*)-one (14c). Deacylation of **11c** (130 mg, 0.225 mmol) by procedure C (15 mL, 16 h; chromatography with MeOH/CH_2Cl_2, 1:9) gave **14c** (69 mg, 89%) as a white solid that was recrystallized (EtOH/$CHCl_3$) to give **14c**: mp 193−195 °C; UV max 257 nm (ε 3800), min 235 nm (ε 2600). ^{1}H NMR (MeOH-d_4, 300 MHz) δ 7.56−7.55 (m, 2H), 7.42−7.36 (m, 3H), 6.89 (d, J = 8.3 Hz, 1H), 6.30 (dd, J = 6.6, 7.6 Hz, 1H), 5.57 (ddd, J = 2.0, 3.9, 8.4 Hz, 1H), 4.42−4.38 (m, 1H), 3.91 (dd, J = 3.7, 7.1 Hz, 1H), 3.76 (dd, J = 3.6, 12.1 Hz, 1H), 3.70 (dd, J = 4.3, 11.8 Hz, 1H), 2.26 (ddd, J = 6.0, 7.7, 13.4 Hz, 1H), 2.16 (ddd, J = 3.3, 6.4, 13.4 Hz, 1H). ^{13}C NMR (MeOH-d_4, 75 MHz) δ 162.8 (dd, J = 28.7, 31.2 Hz), 138.5, 129.8, 129.2, 128.3, 126.9, 114.2, 112.8 (dd, J = 250.0, 257.1 Hz), 88.5, 84.8, 74.7 (dd, J = 21.2, 24.7 Hz), 72.6, 63.3, 39.5. ^{19}F NMR (MeOH-d_4) δ −116.8 (d, J = 264.9 Hz), −127.3 (d, J = 264.9 Hz). HRMS (FAB$^+$) m/z calcd for $C_{16}H_{17}F_2NO_5Na$ (M + Na)$^+$ 364.0972, found 364.0977.

4-Ethynyl-3,3-difluoro-3,4-dihydro-4-hydroxy-1-methylpyridin-2(1*H*)-one (12a). Treatment of **9a** (38 mg, 0.236 mmol), HC_2MgBr/THF (0.5 M; 550 μL, 0.275 mmol), and THF (0.5 mL) by procedure B (2 h; chromatography with CH_2Cl_2/MeOH, 50:1) gave **12a** (28 mg, 63%) as a yellow oil: UV max 267 nm (ε 4600), min 220 nm (ε 1400). ^{1}H NMR (MeOH-d_4, 200 MHz) δ 6.31 (d, J = 8.1 Hz, 1H), 5.39 (ddd, J = 1.8, 4.0, 8.1 Hz, 1H), 3.12 (s, 3H), 3.11 (d, J = 1.1 Hz, 1H). ^{13}C NMR (MeOH-d_4, 75 MHz) δ 162.6 (dd, J = 28.2, 30.7 Hz), 132.1, 111.3 (t, J = 254.6 Hz), 101.5, 80.1, 76.3, 68.4 (dd, J = 23.2, 25.0 Hz), 34.4. ^{19}F NMR (MeOH-d_4) δ −118.3 (d, J = 265.5 Hz), −130.8 (d, J = 262.5 Hz). HRMS (EI$^+$) m/z calcd for $C_8H_7F_2NO_2$ 187.0445, found 187.0437.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-4-ethynyl-3,3-difluoro-3,4-dihydro-4-hydroxypyridin-2(1*H*)-one (12b). Treatment of **9b** (95 mg, 0.16 mmol), HC_2MgBr/THF (0.5 M: 350 μL, 0.18 mmol), and THF (1 mL) by procedure B (1 h, chromatography with EtOAc/hexanes, 2:8) gave **12b** (52 mg, 53%; diastereomers, ∼2:1) as a white foam: UV max 230 nm, min 211 nm. ^{1}H NMR (500 MHz) δ 8.12−7.31 (m, 16H), 6.55 (d, J = 7.8 Hz, 0.3H), 6.38 (d, J = 8.3 Hz, 1H), 6.32 (d, J = 6.3 Hz, 0.7H), 5.85−5.82 (m, 1H), 5.67 (t, J = 6.3 Hz, 0.7H), 5.62 (t, J = 6.6 Hz, 0.3H), 5.48 (dd, J = 4.4, 8.3 Hz, 0.3H), 5.33 (dt, J = 2.7, 8.3 Hz, 0.7H), 4.81−4.75 (m, 1H), 4.69−4.62 (m, 2H), 2.60 (s, 0.3H), 2.42 (d, J = 2.0 Hz, 0.7H). ^{13}C NMR (125 MHz) δ 166.4, 166.3, 165.7, 165.6, 165.4, 160.4 (t, J = 29.9 Hz), 134.3, 134.2, 134.1, 134.0, 133.9, 130.2, 130.12,

Downloaded by Jonathan Berry on September 11, 2009 | http://pubs.acs.org
Publication Date (Web): April 20, 2009 | doi: 10.1021/jm900203h

130.07, 129.94, 129.90, 129.1, 129.0, 128.9, 128.8, 128.6, 125.4, 125.1, 111.4, 111.1, 109.6 (t, J = 257.1 Hz), 109.4 (dd, J = 254.8, 260.9 Hz), 85.8, 85.0, 80.8, 80.7, 77.6, 76.2, 75.8, 72.8, 72.6, 71.9, 71.5, 67.7 (t, J = 24.4 Hz), 67.2 (t, J = 24.4 Hz), 64.4, 64.1. ^{19}F NMR δ −113.2 (d, J = 264.9 Hz), −120.4 (d, J = 260.6 Hz), −127.9 (d, J = 260.6 Hz), −131.8 (d, J = 264.9 Hz). HRMS (FAB$^+$) m/z calcd for $C_{33}H_{25}F_2NO_9Na$ $(M + Na)^+$ 640.1395, found 640.1404.

4-Ethynyl-3,3-difluoro-3,4-dihydro-4-hydroxy-1-(β-D-ribofuranosyl)pyridin-2(1*H*)-one (15b). Deacylation of **12b** (105 mg, 0.170 mmol) by procedure C (6 mL, 1 d; chromatography with MeOH/CH_2Cl_2, 1:9) gave **15b** ($C_{12}H_{13}F_2NO_6 \cdot H_2O$; 40 mg, 77%; diastereomers, ∼3:2) as a yellow oil: UV max 262 nm (ε 3700), min 221 nm (ε 1200). ^{1}H NMR (MeOH-d_4) δ 6.79 (d, J = 8.3 Hz, 0.4H), 6.74 (d, J = 8.3 Hz, 0.6H), 5.85 (d, J = 4.5 Hz, 0.4H), 5.84 (d, J = 4.3 Hz, 0.6H), 5.50−5.41 (m, 1H), 4.12−4.04 (m, 2H), 3.94 (dd, J = 3.5, 6.7 Hz, 1H), 3.81 (dd, J = 2.7, 12.2 Hz, 0.4H), 3.78 (dd, J = 2.9, 12.2 Hz, 0.6H), 3.70 (t, J = 3.8 Hz, 0.4H), 3.68 (t, J = 3.8 Hz, 0.6H), 3.14 (d, J = 1.0 Hz, 0.4H), 3.13 (d, J = 0.5 Hz, 0.6H). ^{13}C NMR (MeOH-d_4) δ 162.4 (dd, J = 28.7, 30.2 Hz), 162.2 (dd, J = 28.2, 31.2 Hz) (minor), 126.8 (minor), 126.4, 111.9, 111.6 (d, J = 2.0 Hz) (minor), 111.5 (dd, J = 253.3, 256.9 Hz), 111.3 (dd, J = 251.3, 258.8 Hz) (minor), 89.1, 89.0 (minor), 86.2, 86.1 (minor), 80.0 (d, J = 3.0 Hz), 79.9 (minor), 76.5, 74.64 (minor), 74.58, 71.8, 71.7 (minor), 68.2 (t, J = 24.2 Hz), 67.8 (minor), 62.8, 62.6 (minor). ^{19}F NMR (MeOH-d_4) δ −114.5 (d, J = 263.6 Hz) (minor), −118.4 (d, J = 261.8 Hz), −127.5 (dd, J = 3.7, 260.0 Hz), −129.9 (dd, J = 3.7, 263.6 Hz) (minor). HRMS (EI$^+$) m/z calcd for $C_{12}H_{13}F_2NO_6$ (M^+) 305.0711, found 305.0697.

1-[2-Deoxy-3,5-di-*O*-(4-methylbenzoyl)-β-D-*erythro*-pentofuranosyl]-4-ethynyl-3,3-difluoro-3,4-dihydro-4-hydroxypyridin-2(1*H*)-one (12c). Treatment of **9c** (265 mg, 0.531 mmol), HC_2MgBr/THF (0.5 M, 1.6 mL, 0.8 mmol), and THF (5 mL) by procedure B (3 h, radial chromatography with EtOAc/hexanes, 2:8) gave **12c** (150 mg, 54%; diastereomers, ∼8:1) as a yellow foam: UV max 241 nm, min 215 nm. ^{1}H NMR δ 7.93 (d, J = 7.8 Hz, 2H), 7.90 (d, J = 8.1 Hz, 2H), 7.25 (d, J = 8.3 Hz, 4H), 6.54 (d, J = 8.0 Hz, 0.2H), 6.45 (d, J = 8.3 Hz, 0.8H), 6.36 (dd, J = 5.9, 8.5 Hz, 1H), 5.56−5.54 (m, 1H), 5.34 (dt, J = 2.9, 8.2 Hz, 1H), 4.62 (d, J = 3.2 Hz, 2H), 4.47−4.45 (m, 1H), 3.88−3.86 (m, 1H), 2.63 (s, 1H), 2.55 (ddd, J = 1.6, 5.6, 14.3 Hz, 1H), 2.41 (s, 6H), 2.36−2.26 (m, 1H). ^{13}C NMR δ 166.4, 166.3, 160.0 (t, J = 29.5 Hz), 144.7, 144.5, 129.9, 129.7, 129.5, 129.4, 126.6, 126.4, 124.3, 110.6, 109.8, 109.7 (dd, J = 255.3, 257.8 Hz), 83.8, 82.5, 77.94, 77.90, 75.8, 74.8, 67.5 (t, J = 24.4 Hz), 64.3, 36.6, 21.83, 21.81. ^{19}F NMR δ −114.4 (d, J = 267.0 Hz), −120.0 (d, J = 260.6 Hz), −127.8 (d, J = 264.9 Hz), −131.3 (d, J = 267.0 Hz). HRMS (FAB$^+$) m/z calcd for $C_{28}H_{25}F_2NO_7Na$ $(M + Na)^+$ 548.1497, found 548.1492.

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-4-ethynyl-3,3-difluoro-3,4-dihydro-4-hydroxypyridin-2(1*H*)-one (15c). Deacylation of **12c** (130 mg, 0.247 mmol) by procedure C (20 mL, 2 d; chromatography with MeOH/CH_2Cl_2, 1:9) gave **15c** (65 mg, 91%; diastereomers, ∼4:1) as a white solid: UV max 262 nm (ε 3800), min 220 nm (ε 1000). ^{1}H NMR (MeOH-d_4) δ 6.64 (d, J = 8.1 Hz, 0.2H), 6.58 (d, J = 8.3 Hz, 0.8H), 6.11 (t, J = 7.0 Hz, 1H), 5.38−5.30 (m, 0.2H), 5.32 (dt, J = 2.9, 8.3 Hz, 0.8H), 4.21 (dd, J = 4.6, 7.6 Hz, 1H), 3.73 (dd, J = 3.7, 7.1 Hz, 1H), 3.62−3.51 (m, 2H), 2.99 (s, 1H), 1.99 (dd, J = 4.7, 6.9 Hz, 2H). ^{13}C NMR (MeOH-d_4) δ 162.0 (t, J = 29.5 Hz), 126.5 (minor), 125.8, 112.3, 111.8 (minor), 111.5 (dd, J = 253.3, 256.8 Hz), 88.61, 88.55 (minor), 84.7, 80.0 (minor), 79.95, 76.5, 72.5, 68.2 (t, J = 24.2 Hz), 63.2, 39.8, 39.7. ^{19}F NMR (MeOH-d_4) δ −114.5 (d, J = 262.7 Hz) (minor), −119.5 (d, J = 260.6 Hz), −127.2 (d, J = 260.6 Hz), −130.4 (dd, J = 4.3, 262.7 Hz) (minor). HRMS (EI$^+$) m/z calcd for $C_{12}H_{13}F_2NO_5$ (M^+) 289.0762, found 289.0758.

4-[(Ethoxycarbonyl)methylene]-3,3-difluoro-3,4-dihydro-1-methylpyridin-2(1*H*)-one (16a). Treatment of **9a** (26 mg, 0.16 mmol), Ph_3PCHCO_2Et (69 mg, 95%, 0.19 mmol), and CH_2Cl_2 (4 mL) by procedure D (4 h, chromatography with EtOAc/hexanes, 1:1) gave **16a** (36 mg, 97%) as a dark-yellow oil: UV max 219, 349 nm, min 279 nm. ^{1}H NMR δ 6.90 (d, J = 7.32 Hz, 1H), 6.34 (s, 1H), 6.31 (d, J = 7.1 Hz, 1H), 4.24 (q, J = 7.1 Hz, 2H), 3.24 (s, 3H), 1.32 (t, J = 7.1 Hz, 3H). ^{13}C NMR δ 165.2, 161.4 (t, J = 30.2 Hz), 141.8 (t, J = 19.1 Hz), 133.5, 119.6 (t, J = 7.5 Hz), 107.4 (t, J = 245.8 Hz), 102.4 (t, J = 3.5 Hz), 61.1, 35.1, 14.3. ^{19}F NMR δ −98.3. HRMS (EI$^+$) m/z calcd for $C_{10}H_{11}F_2NO_3$ (M^+) 231.0707, found 231.0701.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-4-[(ethoxycarbonyl)-methylene]-3,3-difluoro-3,4-dihydropyridin-2(1*H*)-one (16b). Treatment of **9b** (203 mg, 0.343 mmol), Ph_3PCHCO_2Et (250 mg, 95%, 0.682 mmol), and CH_2Cl_2 by procedure D (4 h, chromatography with EtOAc/hexanes, 4:6) gave **16b** (227 mg, 100%) as a yellow-green foam: UV max 229, 336 nm; min 210, 262 nm. ^{1}H NMR δ 8.14−7.26 (m, 15H), 6.87 (d, J = 8.8 Hz, 1H), 6.62 (d, J = 8.8 Hz, 1H), 6.36−6.32 (m, 2H), 5.87 (dd, J = 3.9, 6.1 Hz, 1H), 5.68 (t, J = 6.1 Hz, 1H), 4.80 (dd, J = 3.9, 13.5 Hz, 1H), 4.75−4.65 (m, 2H), 4.22 (q, J = 7.2 Hz, 2H), 1.30 (t, J = 7.2 Hz, 3H). ^{13}C NMR δ 166.3, 165.5, 165.4, 164.8, 161.0 (t, J = 30.7 Hz), 140.3 (t, J = 18.9 Hz), 134.0, 133.8, 130.1, 130.0, 129.8, 129.4, 129.0, 128.8, 128.7, 128.5, 126.9, 120.2 (t, J = 7.3 Hz), 107.4 (t, J = 247.0 Hz), 103.9, 86.5, 80.7, 73.0, 71.3, 64.0, 61.2, 14.3. ^{19}F NMR δ −97.9 (d, J = 294.8 Hz), −102.3 (d, J = 294.8 Hz). HRMS (FAB$^+$) m/z calcd for $C_{35}H_{30}F_2NO_{10}$ $(M + H)^+$ 662.1838, found 662.1845.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-4-[(ethoxycarbonyl)-methyl]-3-fluoropyridin-2(1*H*)-one (17b). Treatment of **16b** (1.17 g, 1.77 mmol) and 10% Pd−C (100 mg) by procedure E [THF/EtOH (1:1), 16 mL, 4 h; chromatography with EtOAc/hexanes, 3:7] gave **17b** (0.97 g, 85%) as a light-yellow foam: UV max 230, 283 nm; min 212, 258 nm. ^{1}H NMR δ 8.12−7.32 (m, 16H), 6.61 (d, J = 4.6 Hz, 1H), 6.05 (dd, J = 5.9, 7.3 Hz, 1H), 5.96 (t, J = 5.6 Hz, 1H), 5.82 (dd, J = 4.8, 5.7 Hz, 1H), 4.87 (dd, J = 2.7, 12.0 Hz, 1H), 4.80−4.76 (m, 1H), 4.70 (dd, J = 4.2, 12.0 Hz, 1H), 4.17 (q, J = 7.1 Hz, 2H), 3.54 (s, 2H), 1.26 (t, J = 7.1 Hz, 3H). ^{13}C NMR δ 168.8 (d, J = 2.0 Hz), 166.2, 165.4, 165.3, 155.6 (d, J = 26.7 Hz), 150.1 (d, J = 249.3 Hz), 133.8, 133.7, 130.1, 130.0, 129.8, 129.5, 128.81, 128.76, 128.72, 128.6, 128.1 (d, J = 12.6 Hz), 127.2 (d, J = 6.0 Hz), 107.0 (d, J = 1.5 Hz), 88.9, 80.7, 75.0, 71.1, 63.8, 61.7, 33.6 (d, J = 2.0 Hz), 14.2. ^{19}F NMR δ −135.2. HRMS (EI$^+$) m/z calcd for $C_{35}H_{30}FNO_{10}$ (M^+) 643.1854, found 643.1848.

4-[(Aminocarbonyl)methyl]-3-fluoro-1-(β-D-ribofuranosyl)pyridin-2(1*H*)-one (18b). Treatment of **17b** (150 mg, 0.233 mmol) by procedure C (10 mL, 1 d; chromatography with MeOH/CH_2Cl_2, 1:9 → 2:8) gave **18b** (43 mg, 61%) as an oil. A portion of this material (17 mg) was crystallized (EtOH) to give white crystals of **18b** (11 mg): mp 180−182 °C; UV max 300 nm (ε 5100), min 250 nm. ^{1}H NMR (D_2O, 500 MHz) δ 7.74 (d, J = 7.3 Hz, 1H), 6.47 (t, J = 6.8 Hz, 1H), 6.14 (d, J = 2.9 Hz, 1H), 4.30 (t, J = 3.7 Hz, 1H), 4.20−4.19 (m, 2H), 3.98 (dd, J = 2.2, 12.9 Hz, 1H), 3.84 (dd, J = 3.9, 12.7 Hz, 1H), 3.67 (s, 2H). ^{13}C NMR (D_2O, 125 MHz) δ 174.2, 156.6 (d, J = 25.9 Hz), 149.7 (d, J = 243.4 Hz), 131.3 (d, J = 13.0 Hz), 127.9 (d, J = 5.3 Hz), 109.1, 90.6, 84.0, 74.9, 69.1, 60.6, 34.4. ^{19}F NMR (D_2O) δ −138.7 (d, J = 4.2 Hz). HRMS (EI$^+$) m/z calcd for $C_{12}H_{15}FN_2O_6$ (M^+) 302.0914, found 302.0914.

1-[2-Deoxy-3,5-di-*O*-(4-methylbenzoyl)-β-D-*erythro*-pentofuranosyl]-4-[(ethoxycarbonyl)methylene]-3,3-difluoro-3,4-dihydropyridin-2(1*H*)-one (16c). Treatment of **9c** (200 mg, 0.400 mmol), Ph_3PCHCO_2Et (210 mg, 95%, 0.572 mmol), and CH_2Cl_2 (5 mL) by procedure D (3.5 h; chromatography with EtOAc/hexanes, 2:8) gave **16c** (210 mg, 92%) as a yellow-green foam: UV max 239, 338 nm, min 214, 289 nm. ^{1}H NMR δ 7.93 (d, J = 8.1 Hz, 2H), 7.92 (d, J = 8.1 Hz, 2H), 7.26 (d, J = 7.8 Hz, 2H), 7.25 (d, J = 8.1 Hz, 2H), 6.83 (d, J = 8.6 Hz, 1H), 6.71 (d, J = 8.8 Hz, 1H), 6.40 (dd, J = 5.7, 8.4 Hz, 1H), 6.31 (s, 1H), 5.59 (d, J = 6.6 Hz, 1H), 4.67 (d, J = 3.2 Hz, 1H), 4.52−4.98 (m, 1H), 4.22 (q, J = 7.2 Hz, 2H), 2.64−2.58 (m, 1H), 2.42 (s, 6H), 2.36−2.29 (m, 1H), 1.30 (t, J = 7.1 Hz, 3H). ^{13}C NMR δ 166.1, 166.0, 164.8, 160.5 (t, J = 30.7 Hz), 144.6, 144.4, 140.6 (t, J = 19.1 Hz), 129.9, 129.6, 129.5, 129.4, 126.5 (t, J = 6.7 Hz), 119.6 (t, J = 7.4 Hz), 107.4 (t, J = 246.6 Hz), 103.4 (t, J = 3.0 Hz), 84.2, 82.7, 74.8, 64.2, 60.9, 37.0, 21.8, 21.7, 14.2. ^{19}F NMR δ −97.3 (d, J = 294.8 Hz), −102.3

(d, J = 294.8 Hz). HRMS (FAB$^+$) m/z calcd for $C_{30}H_{29}F_2NO_8Na$ (M + Na)$^+$ 592.1759, found: 592.1764.

1-[2-Deoxy-3,5-di-*O*-(4-methylbenzoyl)-β-D-*erythro*-pentofuranosyl)-4-[(ethoxycarbonyl)methyl]-3-fluoropyridin-2(1*H*)-one (17c). Treatment of **16c** (210 mg, 0.369 mmol) and 10% Pd−C (35 mg) by procedure E [THF/EtOH (1:1), 10 mL; 5 h, chromatography with EtOAc/hexanes, 3:7] gave **17c** (138 mg, 68%) as a foam: UV max 240, 300 nm; min 216, 268 nm. ^{1}H NMR (500 MHz) δ 7.93 (d, J = 8.3 Hz, 2H), 7.84 (d, J = 8.3 Hz, 2H), 7.46 (d, J = 7.3 Hz, 1H), 7.24 (d, J = 8.3 Hz, 2H), 7.20 (d, J = 8.3 Hz, 2H), 6.59 (dd, J = 5.6, 8.0 Hz, 1H), 6.02 (t, J = 6.6 Hz, 1H), 5.59 (dd, J = 2.2, 4.6 Hz, 1H), 4.69 (dd, J = 3.4, 12.2 Hz, 1H), 4.66 (dd, J = 3.9, 12.2 Hz, 1H), 4.60−4.58 (m, 1H), 4.14 (q, J = 7.2 Hz, 2H), 3.51 (s, 2H), 2.96 (ddd, J = 1.8, 5.8, 14.3 Hz, 1H), 2.40 (s, 3H), 2.38 (s, 3H), 2.30−2.24 (m, 1H); 1.23 (t, J = 7.1 Hz, 3H). ^{13}C NMR δ 169.0 (d, J = 2.0 Hz), 166.3, 166.2, 155.4 (d, J = 26.7 Hz), 149.8 (d, J = 247.8 Hz), 144.7, 144.5, 130.1, 129.8, 129.6, 129.5, 128.0 (d, J = 12.1 Hz), 126.8, 126.6, 126.2 (d, J = 5.6 Hz), 106.8, 86.6, 83.6, 75.1, 64.4, 61.7, 39.3, 33.6 (d, J = 2.5 Hz), 21.92, 21.88, 14.3. ^{19}F NMR δ −136.7 (d, J = 4.3 Hz). HRMS (FAB$^+$) m/z calcd for $C_{30}H_{30}FNO_8Na$ (M + Na)$^+$ 574.1853, found: 574.1855.

4-[(Aminocarbonyl)methyl]-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3-fluoropyridin-2(1*H*)-one (18c). Treatment of **17c** (130 mg, 0.236 mmol) by procedure C (20 mL, 2 d; chromatography with MeOH/CH_2Cl_2, 1:9) gave **18c** (61 mg, 90%) as a white solid that was recrystallized (EtOH) to give **18c**: mp 163−165 °C; UV max 300 nm (ε 4700), min 247 nm (ε 900). ^{1}H NMR (MeOH-d_4) δ 7.85 (dd, J = 1.5, 7.3 Hz, 1H), 6.43 (t, J = 6.3 Hz, 1H), 6.35−6.30 (m, 1H), 4.59 (bs, 2H), 4.38−4.34 (m, 1H), 4.01−3.96 (m, 1H), 3.80 (dd, J = 3.3, 12.1 Hz, 1H), 3.72 (dd, J = 4.1, 12.1 Hz, 1H), 3.54−3.52 (m, 2H), 2.48 (ddd, J = 3.9, 6.2, 13.6 Hz, 1H) 2.16−2.08 (m, 1H). ^{13}C NMR (MeOH-d_4) δ 173.6, 157.1 (d, J = 26.2 Hz), 150.7 (d, J = 243.7 Hz), 132.0 (d, J = 12.1 Hz), 128.9 (d, J = 6.0 Hz), 108.9 (d, J = 2.0 Hz), 89.4, 87.7 (d, J = 1.5 Hz), 72.0, 62.7, 42.6, 35.4 (d, J = 2.0 Hz). ^{19}F NMR (MeOH-d_4) δ −138.6 (d, J = 4.3 Hz). HRMS (FAB$^+$) m/z calcd for $C_{12}H_{15}FN_2O_5Na$ (M + Na)$^+$ 309.0863, found 309.0858.

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-4-[(ethoxycarbonyl)-methylene]-3,3-difluoro-3,4-dihydropyridin-2(1*H*)-one (16d). Treatment of **9d** (150 mg, 0.370 mmol), Ph_3PCHCO_2Et (271 mg, 95%, 0.739 mmol), and CH_2Cl_2 (5 mL) by procedure D (3 h; chromatography with EtOAc/hexanes, 3:7) gave **16d** (152 mg, 86%) as a yellow-green foam: UV max 219, 338 nm, min 264 nm. ^{1}H NMR δ 6.92 (d, J = 8.8 Hz, 1H), 6.69 (d, J = 8.8 Hz, 1H), 6.33 (s, 1H), 6.18 (d, J = 4.4 Hz, 1H), 5.42 (dd, J = 2.6, 4.5 Hz, 1H), 5.14 (dd, J = 2.6, 4.0 Hz, 1H), 4.41 (dd, J = 4.3, 11.8 Hz, 1H), 4.35 (dd, J = 5.6, 12.0 Hz, 1H), 4.23 (q, J = 7.2 Hz, 2H), 4.19−4.12 (m, 1H), 2.131, 2.126, 2.02 (3 × s, 3 × 3H), 1.31 (t, J = 7.1 Hz, 3H). ^{13}C NMR δ 170.6, 169.8, 169.0, 165.0, 160.5 (t, J = 31.0 Hz), 140.5 (t, J = 18.9 Hz), 128.1, 119.8 (t, J = 7.6 Hz), 107.3 (t, J = 246.8 Hz), 102.1 (t, J = 3.5 Hz), 83.8, 80.1, 76.0, 75.0, 62.8, 61.1, 20.9, 20.8, 20.5, 14.3. ^{19}F NMR δ −97.4 (d, J = 294.8 Hz), −103.8 (d, J = 294.8 Hz). HRMS (EI$^+$) m/z calcd for $C_{20}H_{23}F_2NO_{10}$ (M$^+$) 475.1290, found 475.1288.

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-4-[(ethoxycarbonyl)-methyl]-3-fluoropyridin-2(1*H*)-one (17d). Treatment of **16d** (152 mg, 0.320 mmol) and 10% Pd−C (30 mg) by procedure E [THF/EtOH (1:1), 10 mL; 5 h, chromatography with EtOAc/hexanes, 3:7] gave **17d** (97 mg, 66%) as a white foam: UV max 298 nm, min 247 nm. ^{1}H NMR δ 7.36 (dd, J = 1.6, 7.4 Hz, 1H), 6.52 (d, J = 3.9 Hz, 1H), 6.14 (dd, J = 5.9, 7.3 Hz, 1H), 5.58 (dd, J = 1.7, 3.9 Hz, 1H), 5.12 (dd, J = 1.6, 3.3 Hz, 1H), 4.42 (d, J = 5.4 Hz, 2H), 4.27−4.22 (m, 1H), 4.18 (q, J = 7.2 Hz, 2H), 3.58 (d, J = 1.7 Hz, 2H), 2.15, 2.12, 1.92 (3 × s, 3 × 3H), 1.26 (t, J = 7.2 Hz, 3H). ^{13}C NMR δ 170.8, 169.9, 169.0 (d, J = 2.0 Hz), 168.4, 155.2 (d, J = 27.2 Hz), 149.6 (d, J = 248.3 Hz), 128.2 (d, J = 12.1 Hz), 128.0 (d, J = 6.0 Hz), 105.9 (d, J = 1.5 Hz), 85.3, 81.2, 76.7, 74.3, 63.0, 61.8, 33.7 (d, J = 2.5 Hz), 21.02, 20.96, 20.6, 14.4. ^{19}F NMR δ −136.4 (d, J = 4.2 Hz). HRMS (FAB$^+$) m/z calcd for $C_{20}H_{24}FNO_{10}Na$ (M + Na)$^+$ 480.1282, found 480.1283.

4-[(Aminocarbonyl)methyl]-1-(β-D-arabinofuranosyl)-3-fluoropyridin-2(1*H*)-one (18d). Treatment of **17d** (120 mg, 0.262 mmol) by procedure C (10 mL, 1 d; chromatography with MeOH/CH_2Cl_2, 1:9) gave **18d** (53 mg, 67%) as an oil: UV max 303 nm (ε 5300), min 249 nm (ε 400). ^{1}H NMR (MeOH-d_4) δ 7.75 (dd, J = 1.5, 7.3 Hz, 1H), 6.39 (d, J = 3.9 Hz, 1H), 6.33 (dd, J = 6.2, 7.2 Hz, 1H), 4.32 (dd, J = 2.4, 3.9 Hz, 1H), 4.13 (t, J = 2.8 Hz, 1H), 4.06−4.02 (m, 1H), 3.86−3.84 (m, 2H), 3.57 (t, J = 1.6 Hz, 2H). ^{13}C NMR (MeOH-d_4) δ 173.6, 157.1 (d, J = 26.7 Hz), 150.6 (d, J = 243.2 Hz), 131.9 (d, J = 12.6 Hz), 130.7 (d, J = 5.5 Hz), 107.7 (d, J = 2.0 Hz), 89.0, 87.3, 78.0, 76.7, 62.8, 35.5. ^{19}F NMR (MeOH-d_4) δ −139.4 (d, J = 4.3 Hz). HRMS (FAB$^+$) m/z calcd for $C_{12}H_{15}FN_2O_6Na$ (M + Na)$^+$ 325.0812, found: 325.0826.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-3-fluoro-4-hydroxypyridin-2(1*H*)-one (19b). Treatment of **9b** (790 mg, 1.34 mmol) and 10% Pd−C (70 mg) by procedure E [THF/EtOH (1:1), 10 mL, 5 h; chromatography with EtOAc/hexanes, 6:4] gave **19b** (480 mg, 63%) as a light-yellow foam: UV max 228, 276 nm, min 215, 258 nm. ^{1}H NMR δ 9.51 (bs, 1H), 8.08−7.25 (m, 16H), 6.60 (d, J = 4.9 Hz, 1H), 5.99 (t, J = 7.3 Hz, 1H), 5.89 (t, J = 5.9 Hz, 1H), 5.75 (t, J = 5.4 Hz, 1H), 4.84 (dd, J = 2.9, 12.2 Hz, 1H), 4.76−4.73 (m, 1H), 4.69 (dd, J = 4.4, 12.2 Hz, 1H). ^{13}C NMR δ 166.4, 165.6, 165.4, 157.3 (d, J = 22.7 Hz), 152.3 (d, J = 10.6 Hz), 138.5 (d, J = 232.2 Hz), 133.9, 133.8, 133.7, 130.1, 130.0, 129.9, 129.5, 128.9, 128.8, 128.69, 128.66, 127.9 (d, J = 5.0 Hz), 102.6, 88.3, 80.6, 75.1, 71.1, 63.9. ^{19}F NMR δ −165.5 (d, J = 4.3 Hz). HRMS (FAB$^+$) m/z calcd for $C_{31}H_{24}FNO_9Na$ (M + Na)$^+$ 596.1333, found 596.1327.

3-Fluoro-4-hydroxy-1-(β-D-ribofuranosyl)pyridin-2(1*H*)-one (20b). Deacylation of **19b** (255 mg, 0.445 mmol) by procedure C (20 mL, 1 d; chromatography with MeOH/CH_2Cl_2, 1:9) gave **20b** (55 mg, 47%) as an oil, which was crystallized (MeOH/EtOAc) to give **20b**: mp 182−184 °C; UV max 281 nm (ε 4000), min 245 nm (ε 1800). ^{1}H NMR (MeOH-d_4, 500 MHz) δ 7.68 (dd, J = 1.5, 7.8 Hz, 1H), 6.09−6.06 (m, 1H), 4.17−4.13 (m, 2H), 4.04−4.02 (m, 1H), 3.89 (dd, J = 2.7, 12.4 Hz, 1H), 3.76 (dd, J = 3.4, 12.2 Hz, 1H), 3.65−3.64 (m, 1H); ^{13}C NMR (MeOH-d_4, 75 MHz) δ 159.0 (d, J = 21.6 Hz), 154.1 (d, J = 10.1 Hz), 139.3 (d, J = 229.1 Hz), 130.5 (d, J = 5.0 Hz), 102.5, 91.7, 86.0, 76.8, 70.8, 62.0. ^{19}F NMR (MeOH-d_4) δ −169.6 (d, J = 6.5 Hz). HRMS (FAB$^+$) m/z calcd for $C_{10}H_{12}FNO_6$ (M + H)$^+$ 262.0728, found 262.0726.

1-[2-Deoxy-3,5-di-*O*-(4-methylbenzoyl)-β-D-*erythro*-pentofuranosyl)-3-fluoro-4-hydroxypyridin-2(1*H*)-one (19c). Treatment of **9c** (100 mg, 0.200 mmol) and 10% Pd−C (20 mg) by procedure E [THF/EtOH (1:1), 5 mL, 24 h; chromatography with EtOAc/hexanes, 8:2] gave **19c** (75 mg, 75%) as a foam: UV max 240, 274 nm, min 220, 268 nm. ^{1}H NMR δ 7.95 (d, J = 8.3 Hz, 2H), 7.85 (d, J = 8.1 Hz, 2H), 7.44 (d, J = 7.6 Hz, 1H), 7.25 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 8.1 Hz, 2H), 6.58 (t, J = 6.7 Hz, 1H), 6.09 (t, J = 7.1 Hz, 1H), 5.58 (d, J = 5.6 Hz, 1H), 4.76−4.64 (m, 2H), 4.60−4.54 (m, 1H), 2.94 (dd, J = 5.0, 14.3 Hz, 1H), 2.41, 3.37 (2 × s, 2 × 3H), 2.32−2.22 (m, 1H). ^{13}C NMR δ 166.4, 166.2, 157.1 (d, J = 22.2 Hz), 152.6 (d, J = 10.1 Hz), 144.6 (d, J = 7.0 Hz), 138.5 (d, J = 232.2 Hz), 130.0, 129.7, 129.5, 129.4, 126.9 (d, J = 5.0 Hz), 126.6, 126.4, 102.5, 86.3, 83.4, 75.0, 64.3, 39.4, 21.9, 21.8. ^{19}F NMR δ −165.6 (d, J = 6.4 Hz). HRMS (FAB$^+$) m/z calcd for $C_{26}H_{24}FNO_7Na$ (M + Na)$^+$ 504.1434, found 504.1421.

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3-fluoro-4-hydroxypyridin-2(1*H*)-one (20c). Deacylation of **19c** (120 mg, 0.249 mmol) by procedure C (15 mL, 2 d; chromatography with MeOH/CH_2Cl_2, 1:9) gave **20c** ($C_{10}H_{12}FNO_5$; 38 mg, 62%) as a yellow oil: UV max 281 nm (ε 5300), min 246 nm (ε 1900). ^{1}H NMR (MeOH-d_4) δ 7.68 (dd, J = 1.8, 7.9 Hz, 1H), 6.36 (t, J = 6.5 Hz, 1H), 6.02 (t, J = 7.4 Hz, 1H), 4.29 (dt, J = 3.3, 6.4 Hz, 1H), 3.88 (dd, J = 3.5, 7.2 Hz, 1H), 3.72 (dd, J = 3.4, 12.2 Hz, 1H), 3.64 (dd, J = 4.0, 12.1 Hz, 1H), 2.35 (ddd, J = 3.7, 6.2, 13.6 Hz, 1H), 2.08−1.99 (m, 1H). ^{13}C NMR (MeOH-d_4) δ 158.6 (d, J = 22.2 Hz), 153.8 (d, J = 10.1 Hz), 139.2 (d, J = 229.1 Hz), 130.0 (d, J = 5.0 Hz), 102.4, 89.2, 87.1, 72.1, 62.8, 42.6. ^{19}F NMR (MeOH-d_4) δ −167.2 (d, J = 6.4 Hz).

Downloaded by Jonathan Berry on September 11, 2009 | http://pubs.acs.org
Publication Date (Web): April 20, 2009 | doi: 10.1021/jm900203h

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-3-fluoro-4-hydroxypyridin-2(1*H*)-one (19d). Treatment of **9d**(100 mg, 0.247 mmol) and 10% Pd−C (10 mg) by procedure E [THF/EtOH (1:1), 5 mL, 5 h; chromatography with EtOAc/hexanes, 8:2] gave **19d** (66 mg, 69%) as a light-yellow foam: UV max 282 nm, min 248 nm. ^{1}H NMR δ 7.34 (dd, J = 0.5, 6.6 Hz, 1H), 6.47 (d, J = 3.9 Hz, 1H), 6.23 (t, J = 7.3 Hz, 1H), 5.53 (dd, J = 1.5, 3.9 Hz, 1H), 5.08 (d, J = 1.7 Hz, 1H), 4.40 (d, J = 5.4 Hz, 2H), 4.23−4.20 (m, 1H), 2.13, 2.11, 1.90 (3 × s, 3 × 3H). ^{13}C NMR δ 170.9, 170.0, 168.7, 156.9 (d, J = 22.7 Hz), 152.9 (d, J = 9.6 Hz), 138.0 (d, J = 232.2 Hz), 128.8 (d, J = 5.0 Hz), 101.7, 85.1, 80.9, 76.6, 74.2, 63.0, 20.9, 20.8, 20.4. ^{19}F NMR δ −166.0 (d, J = 6.4 Hz). HRMS (FAB$^+$) m/z calcd for $C_{16}H_{18}FNO_9Na$ $(M + Na)^+$ 410.0863, found: 410.0871.

1-(β-D-Arabinofuranosyl)-3-fluoro-4-hydroxypyridin-2(1*H*)-one (20d). Deacylation of **19d** (115 mg, 0.297 mmol) by procedure C (15 mL, 16 h; chromatography with MeOH/CH_2Cl_2, 1:9) gave **20d** ($C_{10}H_{12}FNO_6 \cdot 1.5H_2O$; 50 mg, 65%) as an oil: UV max 282 nm (ε 5900), min 248 nm (ε 2400). ^{1}H NMR (MeOH-d_4) δ 7.64 (dd, J = 1.7, 7.8 Hz, 1H), 6.36 (d, J = 3.9 Hz, 1H), 6.12 (t, J = 7.6 Hz, 1H), 4.25 (dd, J = 2.7, 3.9 Hz, 1H), 4.10 (t, J = 2.8 Hz, 1H), 4.01−3.98 (m, 1H), 3.83−3.81 (m, 2H). ^{13}C NMR (MeOH-d_4) δ 158.6 (d, J = 22.2 Hz), 154.0 (d, J = 9.6 Hz), 139.2 (d, J = 228.1 Hz), 131.8 (d, J = 5.0 Hz), 101.2, 88.3, 86.9, 78.1, 76.8, 62.8. ^{19}F NMR (MeOH-d_4) δ −168.1 (d, J = 6.4 Hz). HRMS (FAB$^+$) m/z calcd for $C_{10}H_{12}FNO_6Na$ $(M + Na)^+$ 284.0546, found: 284.0547.

Antiviral Assays. The antiviral assays, other than the anti-HIV and anti-MSV assays, were based on inhibition of virus-induced cytopathicity in HEL [herpes simplex virus type 1 (HSV-1) (KOS), HSV-2 (G), vaccinia virus, and vesicular stomatitis virus], Vero (parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4, and Punta Toro virus), or HeLa (vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus) cell cultures. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures). After a 1 h virus adsorption period, residual virus was removed and the cell cultures were incubated in the presence of varying concentrations (200, 40, 8, ... μM) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. The methodology for the anti-HIV and anti-MSV assays was as follows: human CEM ($\sim 3 \times 10^5$ cells/cm^3) cells were infected with 100 $CCID_{50}$ of HIV(III_B) or HIV-2(ROD)/mL and seeded in 200 μL wells of a microtiter plate containing appropriate dilutions of the test compounds. After 4 days of incubation at 37 °C, HIV-induced CEM giant cell formation was examined microscopically. Murine C3H/3T3 embryo fibroblasts were seeded in 48-well plates and grown until confluency. Then Moloney murine sarcoma virus (MSV) was added at 75 focus-forming units to the cell cultures. After adding different concentrations of the test compounds to the MSV-infected cell cultures, the MSV-induced transformation of the cells was examined microscopically at day 6 postinfection.

Cytostatic Assays. Murine leukemia L1210 and human lymphocyte Molt4/C8 and CEM cells were seeded in 96-well microtiter plates at 50000 (L1210) or 75000 (Molt, CEM) cells per 200 μL well in the presence of different concentrations of the test compounds. After 2 (L1210) or 3 (Molt/CEM) days, the viable cell number was counted using a Coulter counter apparatus. The 50% cytostatic concentration (CC_{50}) was defined as the compound concentration required to inhibit tumor cell proliferation by 50%.

Acknowledgment. We thank Brigham Young University for graduate fellowships (H.Y. and K.M.), pharmaceutical companies for unrestricted funds (M.J.R.), and the Geconcerteerde Onderzoeksacties (GOA no. 05/19) for financial support. The technical assistance of Lizette van Berckelaer, Frieda De Meyer, Leentje Persoons, Lies Van den Heurck, and Steven Carmans is gratefully acknowledged.

References

(1) Nucleic Acid Related Compounds. 147. Paper 146. Robins, M. J.; Novak, I.; Wnuk, S. F.; Hansske, F.; Madej, D. Deoxygenative [1,2]-Hydride Shift Rearrangements in Nucleoside and Sugar Chemistry: Analogy with the [1,2]-Electron Shift in the Deoxygenation of Ribonucleosides by Ribonucleotide Reductases. *J. Org. Chem.* **2007**, *72*, 8216–8221.

(2) Robins, M. J.; Currie, B. L. The Synthesis of 3-Deazauridine [4-Hydroxy-1-(β-D-*ribo*-pentofuranosyl)-2-pyridone]. *Chem. Commun.* **1968**, 1547–1548.

(3) (a) Currie, B. L.; Robins, R. K.; Robins, M. J. The Synthesis of 3-Deazapyrimidine Nucleosides Related to Uridine and Cytidine and Their Derivatives. *J. Heterocycl. Chem.* **1970**, *7*, 323–329. (b) Currie, B. L.; Robins, M. J.; Robins, R. K. The Synthesis of 3-Deazaorotidine and Related Nucleoside Derivatives of 4-Hydroxy-2-pyridone. *J. Heterocycl. Chem.* **1971**, *8*, 221–224. (c) Robins, M. J.; Currie, B. L.; Robins, R. K.; Broom, A. D. A Novel Nucleophilic Displacement of Benzoate by Sulfur During Thiation of 3-Deazauridine Tribenzoate. *Can. J. Chem.* **1971**, *49*, 3067–3068.

(4) Bloch, A.; Dutschman, G.; Currie, B. L.; Robins, R. K.; Robins, M. J. Preparation and Biological Activity of Various 3-Deazapyrimidines and Related Nucleosides. *J. Med. Chem.* **1973**, *16*, 294–297.

(5) (a) Khare, G. P.; Sidwell, R. W.; Huffman, J. H.; Tolman, R. L.; Robins, R. K. Inhibition of RNA Virus Replication in Vitro by 3-Deazacytidine and 3-Deazauridine. *Proc. Soc. Exp. Biol. Med.* **1972**, *140*, 880–884. (b) Shannon, W. M.; Brockman, R. W.; Westbrook, L.; Shaddix, S.; Schabel, F. M. Inhibition of Gross-Leukemia Virus-Induced Plaque Formation in XC Cells by 3-Deazauridine. *J. Nat. Cancer Inst.* **1974**, *52*, 199–205.

(6) Yap, B. S.; McCredie, K. B.; Keating, M. J.; Bodey, G. P.; Freireich, E. J. Phase I-II Study of 3-Deazauridine in Adults with Acute Leukemia. *Cancer Treat. Rep.* **1981**, *65*, 521–524.

(7) (a) Moriconi, W. J.; Slavik, M.; Taylor, S. 3-Deazauridine (NSC 126849): An interesting Modulator of Biochemical Response. *Invest. New Drugs* **1986**, *4*, 67–84. (b) Momparler, R. L.; Momparler, L. F. Chemotherapy of L1210 and L1210/ARA-C Leukemia with 5-Aza-2′-deoxycytidine and 3-Deazauridine. *Cancer Chemother. Pharmacol.* **1989**, *25*, 51–54. (c) Momparler, R. L.; Bouffard, D. Y.; Momparler, L. F.; Marquet, J.; Zittoun, J.; Marie, J. P.; Zittoun, R. Enhancement of Antineoplastic Activity of Cytosine-Arabinoside Against Human HL-60 Myeloid Leukemic-Cells by 3-Deazauridine. *Int. J. Cancer* **1991**, *49*, 573–576. (d) Grem, J. L.; Plowman, J.; Rubenstein, L.; Hawkins, M. J.; Harrison, S. D. Modulation of Cytosine-Arabinoside Toxicity by 3-Deazauridine in a Murine Leukemia Model. *Leukemia Res.* **1991**, *15*, 229–236. (e) Gao, W.-Y.; Johns, D. G.; Mitsuya, H. Potentiation of the Anti-HIV Activity of Zalcitabine and Lamivudine by a CTP Synthase Inhibitor, 3-Deazauridine. *Nucleosides, Nucleotides Nucleic Acids* **2000**, *19*, 371–377.

(8) McPartland, R. P.; Wang, M. C.; Bloch, A.; Weinfeld, H. Cytidine 5′-Triphosphate Synthetase as a Target for Inhibition by the Antitumor Agent 3-Deazauridine. *Cancer Res.* **1974**, *34*, 3107–3111.

(9) Robins, M. J.; Kaneko, C.; Kaneko, M. Nucleic Acid Related Compounds. 35. Synthesis of 5-Bromo- and 5-Deuterio-3-deazauridine. *Can. J. Chem.* **1981**, *59*, 3356–3359.

(10) McNamara, D. J.; Cook, P. D.; Allen, L. B.; Kehoe, M. J.; Holland, C. S.; Teepe, A. G. Synthesis, Antitumor Activity, and Antiviral Activity of 3-Substituted 3-Deazacytidines and 3-Substituted 3-Deazauridines. *J. Med. Chem.* **1990**, *33*, 2006–2011.

(11) Welch, J. T.; Eswarakrishnan, S. *Fluorine in Bioorganic Chemistry*; Wiley: New York, 1991.

(12) (a) Akahoshi, F.; Ashinori, A.; Sakashita, H.; Yoshimura, T.; Eda, M.; Imada, T.; Nakajima, M.; Mitsutomi, N.; Kuwahara, S.; Ohtsuka, T.; Fukaya, C.; Miyazaki, M.; Nakamura, N. Synthesis, Structure−Activity Relationships, and Pharmacokinetic Profiles of Nonpeptidic Difluoromethylene Ketones as Novel Inhibitors of Human Chymase. *J. Med. Chem.* **2001**, *44*, 1297–1304. (b) Moore, C. L.; Leatherwood, D. D.; Diehl, T. S.; Selkoe, D. J.; Wolfe, M. S. Difluoro Ketone Peptidomimetics Suggest a Large S1 Pocket for Alzheimer's γ-Secretase: Implications for Inhibitor Design. *J. Med. Chem.* **2000**, *43*, 3434–3442. (c) Chorki, F.; Grellepois, F.; Crousse, B.; Ourevitch, M. Fluoro Artemisinins: Difluoromethylene Ketones. *J. Org. Chem.* **2001**, *66*, 7858–7863. (d) Repine, J. T.; Himmelsbach, R. J.; Hodges, J. C.; Kaktenbronn, J. S.; Sircar, I.; Skeean, R. W.; Brennan, S. T.; Hurley, T. R.; Lunney, E.; Humbelt, C. C.; Weishaar, R. E.; Rapundalo, S.; Ryan, M. J.; Taylor, D. G.; Olson, S. C.; Michniewicz, B. M.; Kornberg, B. E.; Belmont, D. T.; Taylor, M. D. Renin inhibitors containing esters at the P2-position. Oral activity in a derivative of methyl aminomalonate. *J. Med. Chem.* **1991**, *34*, 1935–1943.

Downloaded by Jonathan Berry on September 11, 2009 | http://pubs.acs.org
Publication Date (Web): April 20, 2009 | doi: 10.1021/jm900203h

(13) (a) Lewis, D. A.; Villafranca, J. J. Investigation of the Mechanism of CTP Synthetase Using Rapid Quench and Isotope Partitioning Methods. *Biochemistry* **1989**, *28*, 8454–8459. (b) von der Saal, W.; Villafranca, J. J. Cytidine-5′-triphosphate Synthetase Catalyzes the Phosphorylation of Uridine 5′-Triphosphate by Adenosine 5′-Triphosphate. *J. Am. Chem. Soc.* **1985**, *107*, 703–704.

(14) Snider, M. J.; Reinhardt, L.; Wolfenden, R.; Cleland, W. W. 15N Kinetic Isotope Effects on Uncatalyzed and Enzymatic Deamination of Cytidine. *Biochemistry* **2002**, *41*, 415–421.

(15) (a) Banks, R. E. Selectfluor Reagent F-TEDA-BF_4 in Action: Tamed Fluorine at Your Service. *J. Fluorine Chem.* **1998**, *87*, 1–17. (b) Lal, G. S.; Pez, G. P.; Syvert, R. G. Electrophilic NF Fluorinating Agents. *Chem. Rev.* **1996**, *96*, 1737–1755.

(16) den Hertog, H. J.; Buurman, D. J. Tautomerism of Hydroxypyridines (I) The Structure of 2,4-Dihydroxypyridine. *Rec. Trav. Chim. Pays-Bas* **1956**, *75*, 257–264.

JM900203H

Downloaded by Jonathan Berry on September 11, 2009 | http://pubs.acs.org
Publication Date (Web): April 20, 2009 | doi: 10.1021/jm900203h