Waters U6K valve (2.0 mL loop) onto a 150 × 4.6 mm Nucleosil 5 C_{18} column. The mobile phase (MeOH/H_2O/formic acid, 30:70:1) was delivered at a flow rate of 1.0 mL/min by a Waters Model 6000A pump, equipped with a Beckman Model 153 UV detector set at 280 nm with a range of 0.2 absorbance units. Chromatograms were recorded on a Houston Instruments Omniscribe Recorder, and data were collected with an Apple IIe computer equipped with an Interactive Microwave Chromatochart computer board and software for 16 min for each sample.

Identification of O-Methylated Products from Incubation of the *trans*-Dihydroxy-1,2,3,4,4a,5,6,10b-octahydrobenzo-[*f*]quinolines 11 and 12 with Catechol-*O*-methyltransferase. *trans*-Dihydroxy-1,2,3,4,4a,5,6,10b-octahydrobenzo[f]quinolines 11 and 12 were incubated separately at 37 °C with catechol-*O*-methyltransferase (COMT), *S*-adenosyl-L-methionine (SAM), and $MgCl_2$. Final concentrations were 13 mg of SAM (0.025 mmol), 0.07 mg of COMT (activity rated 1480 units/mg; 100 units produce 0.10 μmol of product/h), 1.5 mL of 0.05 M $MgCl_2$, 1.5 mL of 0.1 M phosphate buffer (pH 7.6), 4 mg (0.012 mmol) of 11 or 12, and doubly distilled H_2O, to make a total volume of 15 mL. As controls, incubations were run in which COMT, SAM, or substrate molecule (11, 12) was omitted. After 3 h the reactionwas stopped by addition of 250 μL of $HClO_4$. Alumina (1 g) was added; the suspensions were sonicated for 5 min, filtered through 0.2-μm Arco disc filters, and injected into the HPLC column in 10.0-μL aliquots as described for the standards.

Synthesis, Antitumor Activity, and Antiviral Activity of 3-Substituted 3-Deazacytidines and 3-Substituted 3-Deazauridines

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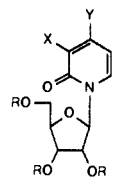
Novel 3-substituted analogues of 4-amino-1-β-D-ribofuranosyl-2(1*H*)-pyridinone (3-deazacytidine, **3**) and 4-hydroxy-1-β-D-ribofuranosyl-2(1*H*)-pyridinone (3-deazauridine, **4**) have been synthesized and tested for antitumor and antiviral activity. Thus the 3-chloro (**9a**), 3-bromo (**9b**), and 3-nitro (**9c**) analogues of **3** and the 3-chloro (**9d**), 3-bromo (**9e**), and 3-nitro (**9f**) analogues of 4 were prepared by standard glycosylating procedures. Novel requisite heterocycles 4-amino-3-chloro-2(1*H*)-pyridinone (**7a**) and 4-amino-3-bromo-2(1*H*)-pyridinone (**7b**) were prepared by halogenating 4-amino-2(1*H*)-pyridinone (**5**). Requisite heterocycles 4-amino-3-nitro-2(1*H*)-pyridinone (**7c**), 3-chloro-4-hydroxy-2(1*H*)-pyridinone (**7d**), 3-bromo-4-hydroxy-2(1*H*)-pyridinone (**7e**), and 4-hydroxy-3-nitro-2-(1*H*)-pyridinone (**7f**) were synthesized by known procedures from 4-hydroxy-2(1*H*)-pyridinone (**6**). Structure proof of target nucleosides was provided by independent synthesis, ^1H NMR, and UV. Compounds **9a–f** were devoid of activity against intraperitoneally implanted L1210 leukemia in mice. Compound **9f** displayed significant activity against rhinovirus type 34 grown in WISH cells. 4-Amino-3-fluoro-1-β-D-ribofuranosyl-2(1*H*)-pyridinone (1) displayed good activity against intraperitoneally implanted P388 leukemia in mice, but it was devoid of activity against M5076 sarcoma, amelanotic (LOX) melanoma xenograft, and subrenal capsule human mammary carcinoma MX-1 xenograft in mice. Compound 1 also displayed significant activity against rhinovirus type 34.

We recently reported that 4-amino-3-fluoro-1-β-D-ribofuranosyl-2(1*H*)-pyridinone (1) exhibits significant activity



1: X = F, Y = NH_2, R = H

2: X = F, Y = NH_2, R = $COCH_3$

3: X = H, Y = NH_2, R = H

4: X = H, Y = OH, R = H

against L1210 leukemia in mice (%T/C_{max} = 230).[1] This activity was retained by **2** (%T/C_{max} = 205), the triacetate of **1**. We have now further investigated the activity of **1** against P388 leukemia in mice, as well as its activity against three different nonleukemic tumors in mice and herein report those results.

The 3-unsubstituted analogue 4-amino-1-β-D-ribofuranosyl-2(1*H*)-pyridinone (**3**, 3-deazacytidine) is not reported to display any in vivo antitumor activity, although it is reported to display modest cytotoxicity against L1210 leukemia cells in vitro (ID_{50} = 5 × 10^{-5} M).[2] In order to explore further the structure–activity relationship of substitution on the 3-position of **3**, we have now prepared the 3-chloro (**9a**), 3-bromo (**9b**), and 3-nitro (**9c**) analogues, and have tested them for cytotoxicity against L1210 leukemia cells in vitro and for antitumor activity against L1210 leukemia in mice.

Compound **4**, 4-hydroxy-1-β-D-ribofuranosyl-2(1*H*)-pyridinone (3-deazauridine), in which the 4-amino group of **3** is replaced with hydroxy, is reported to display activity against L1210 leukemia in mice (%T/C_{max} = 165).[2] It was even tested clinically where it exhibited slight antileukemic activity.[3] We found it of interest to substitute the 3-position of **4** as we had done with some success with **3**. We have now prepared the 3-chloro (**9d**), 3-bromo (**9e**), and 3-nitro (**9f**) analogues of **4** and also tested them for antitumor activity. We attempted to synthesize, by a number of routes, the 3-fluoro analogue of **4** (**9** where X = F, Y = OH), without success.

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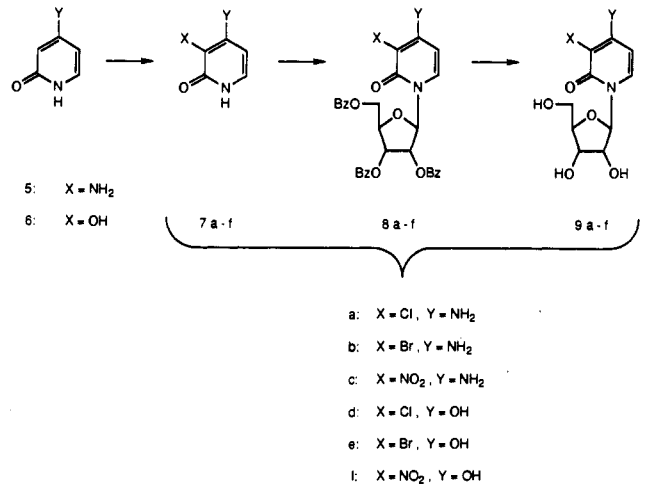
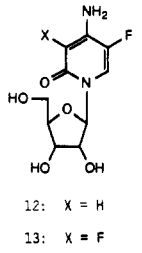
Scheme I



In addition to their reported antitumor activity, **3** and **4** are also reported to display significant activity against a broad spectrum of RNA viruses, including rhinovirus types 1A, 13, and 56; influenza A and B; parainfluenza 1 (Sendai); and vesicular stomatis virus.[4,5] The target compounds **9a–f** as well as the previously synthesized **1**, **2**, **12**, and **13**[1] were therefore also tested for antiviral activity and compared to that of **3** and **4**.

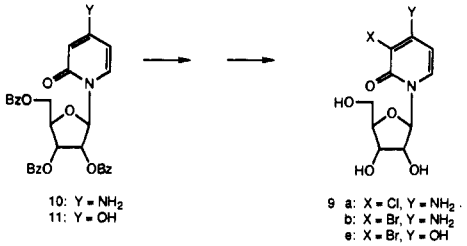


Chemistry

The six target compounds **9a–f** were synthesized according to Scheme I. Thus 4-amino-2(1*H*)-pyridinone[6] (**5**) was allowed to react with *N*-chlorosuccinimide in acetic acid to give 4-amino-3-chloro-2(1*H*)-pyridinone (**7a**). Compound **5** was treated with *N*-bromosuccinimide in acetic acid to provide 4-amino-3-bromo-2(1*H*)-pyridinone (**7b**). That halogenation occurred on the 3-position was obvious from the NMR data (see the Experimental Section). The synthesis of 4-amino-3-nitro-2(1*H*)-pyridinone (**7c**) from 4-hydroxy-3-nitro-2(1*H*)-pyridinone (**7f**) has been described.[7]

4-Hydroxy-2(1*H*)-pyridinone[8] (**6**) was allowed to react with *N*-chlorosuccinimide in acetic acid to give 3-chloro-4-hydroxy-2(1*H*)-pyridinone (**7d**). That halogenation occurred on the 3-position was again obvious from the NMR data. 3-Bromo-4-hydroxy-2(1*H*)-pyridinone[9] (**7e**) and 4-hydroxy-3-nitro-2(1*H*)-pyridinone[10] (**7f**) were synthesized by known procedures from **6**.

Scheme II



Treatment of **7a,b,d,e,f** with excess 1,1,1,3,3,3-hexamethyldisilazane and a catalytic amount of ammonium sulfate and of **7c** with bis(trimethylsilyl)trifluoroacetamide gave the unisolated trimethylsilyl derivatives. These were treated with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in 1,2-dichloroethane with trimethylsilyl trifluoromethanesulfonate[11] as catalyst (for **7a,b,d,e**) or with tin(IV) chloride[12] as catalyst (for **7c,f**) to give the expected β tribenzoyl derivatives **8a–f** as the major products. Subsequent deblocking with sodium methoxide gave the target compounds **9a–f**.

The structures of **9a** and **9b** were confirmed by independent syntheses as depicted in Scheme II. Thus reaction of the known[13] **10** with *N*-chlorosuccinimide and *N*-bromosuccinimide, respectively, followed by deblocking, yielded **9a** and **9b** identical with those obtained by Scheme I. This establishes that the ribofuranose of **9a** and **9b** is attached at N-1 and that its configuration is β. The structure of **9c** was not similarly proven by independent synthesis but was assigned in analogy to those of **9a** and **9b** since it was synthesized by the same general method (Scheme I) and since its NMR spectrum was similar to those of **9a** and **9b**. In particular the NMR spectrum of **9c** exhibited an NH_2 absorption as did those of **9a** and **9b** (thus excluding exocyclic ribosylation) and an absorption for the H-1′ proton which was similar to those of **9a** and **9b** in terms of chemical shift and coupling constant.

The structure of **9e** was also proven by independent synthesis as depicted in Scheme II. Compound **9e** has been synthesized from **11**[14] (Scheme II) and described.[15] The physical constants and in particular the NMR spectrum of **9e** prepared by us according to Scheme I were similar, but not identical, with those published for **9e** prepared by Scheme II. We therefore resynthesized **9e** by Scheme II. The NMR spectrum, as well as other physical constants, of **9e** synthesized by us according to Scheme II agrees very well with that of **9e** synthesized by Scheme I. This then establishes that the ribofuranose of **9e** is attached at N-1 and that its configuration is β. The structures of **9d** and **9f** were not similarly confirmed by independent synthesis but were assigned in analogy to that of **9e**, in part because the method of synthesis of **9d** and **9f** was the same as for **9e**. Also the NMR, UV–vis, and IR spectra of **9d** are very similar to those of **9e**, strongly indicating that the ribofuranose in **9d** is also attached at N-1 and that its configuration is also β. The NMR spectrum of **9f** is very similar to that of **9e**, specifically the absorption of the H-1′ proton in terms of chemical shift and coupling constant. This again indicates that the ribofuranose in **9f** is attached

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Table I. Activity of 1 against Intraperitoneally Implanted P388 Leukemia in Mice[a]

dose, mg/kg per inj	T/C[b]	net log kill[c]	wt chg[d]
600	185	3.27	-5.5
400	177, 189	2.75, 3.27	-4.9, -3.6
200	158, 176	1.47, 2.43	-3.4, -2.7
100	149, 156	0.83, 1.15	-2.5, -2.0
50	133	-0.26	-2.7

[a] CD_2F_1 mice in groups of six were inoculated intraperitoneally with 10^6 cells of P388 leukemia. Treatment (ip) was initiated 24 h after tumor inoculation and continued once-a-day for 5 days. [b] (Mean survival time of treated mice in days/mean survival time of control mice) × 100. %T/C ≥ 120 indicates moderate activity. %T/C ≥175 indicates good activity. [c] Difference between estimated tumor burdens before the first and just after the last treatment. This value is based on the observed doubling time for the tumor in that test and the median survival after the last treatment.[18] It includes an estimate of the regrowth of the tumor cell population between treatments. A net cell kill of zero indicates the tumor cells are equal to the original inoculum, 10^6, after therapy. A positive net cell kill indicates less than the original 10^6 inoculum of tumor cells is left after therapy and a negative net cell kill indicates that the tumor actually grew during the course of therapy. [d] Mean weight change (day 5 wt minus day 1 wt) of the treated mice minus the mean weight change of the control mice in grams.

at N-1 and that its configuration is β.

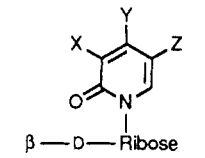
Biology

Compound 1 was tested at the NCI against intraperitoneally implanted P388 leukemia in mice by standard NCI protocol.[16,17] The results are illustrated in Table I. Compound 1 displayed significant activity at several dose levels, with a $\%T/C_{max}$ = 189. Based on this and its previously reported[1] activity against L1210 leukemia in mice, it was then tested at the NCI against three solid tumors in mice by standard NCI protocol:[16,17] M5076 sarcoma, amelanotic (LOX) melanoma xenograft, and subrenal capsule human mammary carcinoma MX-1 xenograft. No activity was observed in these solid tumors.

The newly synthesized compounds **9a–f** and the reference compounds **3** and **4** were tested for their ability to inhibit the growth of L1210 leukemia cells in culture by the method of Baguley and Nash.[19] The ID_{50} (μM), the micromolar concentration required to inhibit the growth of the cells by 50% are reported in Table II. Also included for comparison are the previously reported values for **1**, **2**, **12**, and **13**.[1] Whereas substitution by a fluorine on the 3-position of 3-deazacytidine (**3**) to give **1** resulted in a slight increase in the modest cytotoxicity, substitution by a chlorine on the same position to give **9a** or bromine to give **9b** resulted in loss of cytotoxicity. No conclusion can be drawn about the 3-nitro derivative **9c** since it was not tested at a high enough concentration to determine an ID_{50}. In the 3-deazauridine series, substitution by a chlorine and a nitro group on the 3-position of 3-deazauridine (**4**) to give **9d** and **9f**, respectively, resulted in slight decreases in the more potent cytotoxicity of **4**. In the same series, substitution by a bromine on the 3-position greatly reduced cytotoxicity.

Compounds **9a–f** were then tested at the NCI against intraperitoneally implanted L1210 leukemia in mice by

Table II. Inhibition of L1210 Leukemia Cell Growth in Vitro[a]



no.	X	Y	Z	ID_{50},[b] μM
3	H	NH_2	H	28.6
1	F	NH_2	H	11.8
2	F	NH_2	$H(Ac)_3$	13.4
9a	Cl	NH_2	H	>36.1[c]
9b	Br	NH_2	H	>31.1[c]
9c	NO_2	NH_2	H	>3.48[d]
13	F	NH_2	F	>35.9[c]
12	H	NH_2	F	>38.4[c]
4	H	OH	H	1.09
9d	Cl	OH	H	3.50
9e	Br	OH	H	19.8
9f	NO_2	OH	H	1.68

[a] See ref 19 for method. [b] The concentration required to inhibit the growth of cells by 50%. [c] Inactive at 10 μg/mL, the highest dose tested. [d] Inactive at 1 μg/mL, the highest dose tested.

standard NCI protocol.[16,17] All were inactive (%T/C <125).

3-Deazacytidine (**3**) and 3-deazauridine (**4**) had previously been shown to exhibit activity against several different strains of rhinovirus.[4,5] Therefore the antiviral activities of **9a–f**, **1**, **2**, **12**, and **13** were determined using rhinovirus type 34 and compared with the activity of **3** and **4** in the same system. The results are summarized in Table III. Antiviral activity was determined by measuring the inhibition of virus-induced cytopathic effect (CPE)[20] on WISH cells, and was expressed as the minimum inhibitory concentration (MIC) of the compound that inhibited CPE by 50%. The cytotoxicity of the compounds against uninfected WISH cells was then evaluated by microscopically observing the lowest concentration of compound which reduced cell viability by 25% or noticeably altered morphology in 25% of the cells. This was expressed as the minimum cytotoxic concentration (MCC). The antiviral index (AI) of the compounds, a measure of antiviral efficacy,[21] was then calculated by dividing the MCC by the MIC. Compound **1** displayed better efficacy in this test with an AI of 307.7 than 3-deazacytidine (**3**) itself, with an AI of 125.0. The AI of **2**, the prodrug of **1**, fell off considerably. Replacement of the fluorine in the 3-position of **1** with chlorine to give **9a**, bromine to give **9b**, and nitro to give **9c** resulted in less efficacious compounds than **3**, as did substitution by fluorine on the 5-position of **3** to give **12** and disubstitution by fluorine on the 3- and 5-positions to give **13**. In the 3-deazauridine series, 3-nitro-3-deazauridine (**9f**), with an AI of 48.2, was more efficacious than 3-deazauridine itself (**4**), with an AI of 7.0. The 3-chloro (**9d**) and 3-bromo (**9e**) derivatives in this series were not active.

Conclusion

The 3-fluoro analogue (**1**) of 3-deazacytidine was shown to exhibit good activity against intraperitoneally implanted P388 leukemia in mice, comparable to its activity against intraperitoneally implanted L1210 in mice. However this antitumor activity was totally lost when **1** was tested in mice against M5076 sarcoma, amelanotic (LOX) melanoma xenograft, and the subrenal capsule human mammary

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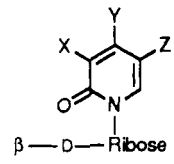
Table III. Inhibition of Rhinovirus Type 34 Induced Cytopathic Effect (CPE) and WISH Cell Toxicity



no.	X	Y	Z	inhibn of rhinovirus type 34 Induced CPE: MIC,[a] μg/mL	WISH cell toxicity: MCC,[b] μg/mL	antiviral index (AI)[c]
3	H	NH_2	H	3.2	400	125.0
1	F	NH_2	H	1.3	400	307.7
2	F	NH_2	H(Ac_3)	25.4	>800	31.5
9a	Cl	NH_2	H	53.7	400	7.5
9b	Br	NH_2	H	173.3	800	4.6
9c	NO_2	NH_2	H	>1000	>1000	NA[d]
13	F	NH_2	F	28.3	400	14.1
12	H	NH_2	F	50.7	800	15.8
4	H	OH	H	57.1	400	7.0
9d	Cl	OH	H	>1000	>1000	NA[d]
9e	Br	OH	H	>1000	>1000	NA[d]
9f	NO_2	OH	H	8.3	400	48.2

[a] The minimum inhibitory concentration (MIC) was the lowest calculated concentration of drug that inhibited rhinovirus type 34 induced CPE by 50%. [b] The lowest concentration of drug reducing cell viability by 25% or noticeably altering morphology of 25% of the cells was considered the minimum cytotoxic concentration (MCC). [c] The AI is defined as MCC/MIC. [d] Not applicable due to inactivity.

carcinoma MX-1 xenograft. The newly synthesized 3-substituted derivatives (**9a–f**) of 3-deazacytidine (**3**) and 3-deazauridine (**4**) were all devoid of activity against L1210 leukemia in mice. Two compounds, **1** and the 3-nitro analogue (**9f**) of 3-deazauridine (**4**), displayed good activity against rhinovirus type 34. The activity of these two compounds against other RNA viruses is currently being explored.

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. Mass spectra, where unnoted, were determined on a Finnigan 4000 mass spectrometer with a INCOS 2300 data system using direct introduction, electron impact at 70 eV and 150 °C. Mass spectra, where noted, were determined on VC 7070E/HF mass spectrometer with fast atom bombardment (FAB) using xenon target gas and the sample in a thioglycerol matrix. ^{1}H NMR spectra were determined at 100 MHz on an IBM WP100SY or at 200 MHz on a Varian XL-200 in Me_2SO-d_6 with tetramethylsilane as an internal standard. Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter with a 10-cm, 1-mL microcell. UV spectra were determined on a Cary C118 UV–vis spectrophotometer. All compounds had infrared spectra (potassium bromide) consistent with their structure as determined on a Nicolet 205X FT/IR. Elemental analyses were determined by Parke-Davis' microanalytical laboratory. TLC was performed with E. Merck silica gel 60 F-254 precoated glass plates (0.25 mm). Flash column chromatography was performed with E. Merck silica gel, 230–400 mesh. Concentrations were performed on a Yamato rotary evaporator at ≤45 °C (20 Torr).

Scheme I. 4-Amino-3-chloro-2(1*H*)-pyridinone (7a). To a solution of 0.25 g (0.0023 mol) of 4-amino-2(1*H*)-pyridinone (**5**) in 5 mL of AcOH was added 0.33 g (0.0025 mol) of *N*-chlorosuccinimide. The solution was stirred for 2 h and then concentrated, coevaporating with EtOH. The residue was dissolved in MeOH and treated with 5 g of flash silica gel. The MeOH was evaporated and the powder was applied to a column of 100 g of flash silica gel packed in CH_2Cl_2. Elution with CH_2Cl_2/MeOH (10:1) gave material which was triturated in ether to give 0.22 g (67%) of the product as a white solid: mp 264–267 °C; ^{1}H NMR (100 MHz, DMSO) δ = 5.80 (d, $J_{H\text{-}5\text{-}H\text{-}6}$ = 7 Hz, 1, H-5), 6.33 (s, 2, NH_2, exchangeable), 7.03 (d, $J_{H\text{-}6\text{-}H\text{-}5}$ = 7 Hz, 1, H-6), 10.87 (s, 1, NH, exchangeable); MS *m/e* = 144 (M^+). Anal. ($C_5H_5ClN_2O$) C, H, N.

4-Amino-3-bromo-2(1*H*)-pyridinone (7b). To a solution of 0.25 g (0.0023 mol) of **5** in 5 mL of AcOH was added 0.44 g (0.0025 mol) of *N*-bromosuccinimide. The solution was stirred for 2 h and then concentrated, coevaporating with EtOH. The residue was dissolved in MeOH and treated with 5 g of flash silica gel. The MeOH was evaporated and the powder was applied to a column of 100 g of flash silica gel packed in CH_2Cl_2. Elution with CH_2Cl_2/MeOH (10:1) gave 0.30 g (70%) of the product as an off-white solid: mp 255–258 °C; ^{1}H NMR (100 MHz, DMSO) δ = 5.80 (d, $J_{H\text{-}5\text{-}H\text{-}6}$ = 7 Hz, 1, H-5), 6.32 (s, 2, NH_2, exchangeable), 7.05 (d, $J_{H\text{-}6\text{-}H\text{-}5}$ = 7 Hz, 1, H-6), 10.85 (s, 1, NH, exchangeable); MS *m/e* = 188 (M^+), 190 (M^+ + 2). Anal. ($C_5H_5BrN_2O$) C, H, N.

3-Chloro-4-hydroxy-2(1*H*)-pyridinone (7d). A suspension of 2.00 g (0.018 mol) of 4-hydroxy-2(1*H*)-pyridinone (**6**) and 2.88 g (0.022 mol) of *N*-chlorosuccinimide in 250 mL of AcOH was heated to reflux, becoming a solution. TLC showed that starting material remained. Another 0.58 g (0.0043 mol) of *N*-chlorosuccinimide was added and the suspension was heated under reflux for 10 min. The suspension was cooled and the solid was collected by filtration. Recrystallization from MeOH gave 1.33 g (50.8%) of the product as a white solid: mp >250 °C (lit.[22] mp ~310 °C dec); ^{1}H NMR (100 MHz, DMSO) δ = 6.02 (d, $J_{H\text{-}5\text{-}H\text{-}6}$ = 7.5 Hz, 1, H-5), 7.23 (d, $J_{H\text{-}6\text{-}H\text{-}5}$ = 7.5 Hz, 1, H-6), 11.25 (br s, 1, OH or NH), 11.52 (br s, 1, OH or NH); MS *m/e* = 145 (M^+). Anal. ($C_5H_4ClNO_2$) C, H, N.

4-Amino-3-chloro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyridinone (8a). Treatment of 2.13 g (0.0147 mol) of **7a** analogous to the treatment of **7d** to give **8d** gave 8.55 g (98.7%) of crude product. This was dissolved in CH_2Cl_2 and applied to a column of 500 g of flash silica gel packed in CH_2Cl_2/EtOAc (5:1). Elution with the same solvent gave 5.00 g (57.7%) of the product as a white foam: ^{1}H NMR (100 MHz, DMSO) δ = 6.2 (d, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 3 Hz, 1, H-1'), 6.70 (s, 2, NH_2, exchangeable); MS (FAB) *m/e* = 589 (M^+ + 1). Anal. ($C_{31}H_{25}ClN_2O_8$) C, H, N.

4-Amino-3-bromo-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyridinone (8b). Treatment of 1.16 g (0.00613 mol) of **7b** analogous to the treatment of **7d** to give **8d** gave 3.80 g (97.9%) of crude product. This was dissolved in CH_2Cl_2 and applied to a column of 250 g of flash silica gel packed in CH_2Cl_2/EtOAc (5:1). Elution with the same solvent gave 1.57 g (40.5%) of the product as a white foam: ^{1}H NMR (200 MHz, DMSO) δ = 6.16 (d, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 3.1 Hz, 1, H-1'), 6.61 (br s, 2, NH_2, exchangeable); MS (FAB) *m/e* = 633 (M^+), 635 (M + 2). Anal. ($C_{31}H_{25}BrN_2O_8$) C, H, N.

4-Amino-3-nitro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyridinone (8c). A suspension of 1.55 g (0.010

(22) Den Hertog, H. J.; Schogt, J. C. M.; De Bruyn, J.; Deklerk, A. *Recl. Trav. Chim. Pays-Bas* **1950**, *69*, 673.

mol) of 4-amino-3-nitro-2(1*H*)-pyridinone (**7c**) and 12.5 g (0.0486 mol) of bis(trimethylsilyl) trifluoroacetamide in 25 mL of CH_3CN was heated under reflux for 3 h. The resulting solution was concentrated. The residue was dissolved in 50 mL of 1,2-dichloroethane. The solution was treated with 5.04 g (0.010 mol) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, followed by 1.67 mL (0.0144 mol) of $SnCl_4$, and stirred at room temperature for 3 h. The solution was then poured into an ice/saturated $NaHCO_3$ solution. Approximately 200 mL of $CHCl_3$ was added to the suspension, and the layers were separated. The H_2O layer was extracted with $CHCl_3$. The organic layers were combined, dried ($MgSO_4$), and concentrated to give 5.4 g (90%) of the product as a yellow foam. A small amount of this material was recrystallized from EtOH/EtOAc to give the product as an amorphous, yellow solid: mp indefinite; NMR (100 MHz, DMSO) δ = 6.15 (d, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 3 Hz, 1, H-1'), 8.27 (br s, 2, NH_2). Anal. ($C_{31}H_{25}N_3O_{10}$) C, H, N.

3-Chloro-4-hydroxy-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyridinone (8d). A suspension of 0.20 g (0.0014 mol) of **7d** in 20 mL of 1,1,1,3,3,3-hexamethyldisilazane and a catalytic amount of ammonium sulfate was heated under reflux for 18 h. The resulting solution was concentrated. The residue was dissolved in 50 mL of 1,2-dichloroethane. The solution was cooled in a cold-water bath and treated with 0.69 g (0.00137 mol) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, followed by 0.32 mL (0.00164 mol) of trimethylsilyl trifluoromethanesulfonate. The solution was stirred at room temperature for 18 h and then poured into an ice/saturated $NaHCO_3$ solution. The mixture was filtered through Celite, and the layers were separated. The water layer was extracted with CH_2Cl_2. The organic layers were combined, dried ($MgSO_4$), and concentrated to give 0.81 g (100%) of a white foam. This was dissolved in CH_2Cl_2 and applied to a column of 100 g of flash silica gel packed in CH_2Cl_2. Elution with CH_2Cl_2/MeOH (50:1) gave 0.45 g (56%) of the product as a white foam: ^{1}H NMR (100 MHz, DMSO) δ = 6.17 (d, $J_{H\text{-}5\text{-}H\text{-}6}$ = 7.5 Hz, 1, H-5), 6.28 (d, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 3 Hz, 1, H-1'); MS (FAB) m/e = 590 (M^+ + 1). Anal. ($C_{31}H_{24}ClN_4O_9$) C, H, N.

3-Bromo-4-hydroxy-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyridinone (8e). Treatment of 2.21 g (0.0116 mol) of 3-bromo-4-hydroxy-2(1*H*)-pyridinone (**7e**) analogous to the treatment of **7d** to give **8d** gave 1.73 g (23.5%) of slightly contaminated and 4.44 g (60.3%; total yield 83.8%) of pure product as a white foam: ^{1}H NMR (100 MHz, DMSO) δ = 6.16 (d, $J_{H\text{-}5\text{-}H\text{-}6}$ = 8 Hz, 1, H-5), 6.26 (d, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 2 Hz, 1, H-1'). Anal. ($C_{31}H_{24}BrNO_9$) C, H, N.

4-Hydroxy-3-nitro-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)-2(1*H*)-pyridinone (8f). A suspension of 5.00 g (0.032 mol) of 4-hydroxy-3-nitro-2(1*H*)-pyridinone (**7f**) in 100 mL of 1,1,1,3,3,3-hexamethyldisilazane and a catalytic amount of ammonium sulfate was heated under reflux for 18 h. The resulting solution was concentrated. The residue was dissolved in 100 mL of 1,2-dichloroethane and treated with 16.16 g (0.032 mol) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, followed by 5.39 mL (0.046 mol) of $SnCl_4$. The solution was stirred at room temperature for 18 h and poured into an ice/saturated $NaHCO_3$ solution. The mixture was filtered through Celite, and the layers were separated. The water layer was extracted with CH_2Cl_2. The organic layers were combined, dried ($MgSO_4$), and concentrated to give 23 g (>100%) of a foam. This was dissolved in CH_2Cl_2 and applied to a column of 1 kg of flash silica gel packed in CH_2Cl_2/MeOH (10:1). Elution with the same solvent gave 15.69 g (81.6%) of the product as a yellow foam: ^{1}H NMR (200 MHz, DMSO) δ = 5.76 (d, $J_{H\text{-}5\text{-}H\text{-}6}$ = 8.0 Hz, 1, H-5), 6.18 (d, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 2.8 Hz, 1, H-1'). Anal. ($C_{31}H_{24}N_2O_{11}$) H, N; C: calcd, 62.00; found, 60.12.

4-Amino-3-chloro-1-β-D-ribofuranosyl-2(1*H*)-pyridinone (9a). Treatment of 5.00 g (0.0084 mol) of **8a** analogous to the treatment of **8d** to give **9d** gave material which was recrystallized from EtOH to give 1.70 g (72.3%) of the product as a white solid: mp 197–199 °C; ^{1}H NMR (100 MHz, DMSO) δ = 5.92 (d, $J_{H\text{-}5\text{-}H\text{-}6}$ = 8 Hz, 1, H-5), 5.95 (d, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 3 Hz, 1, H-1'), 6.47 (s, 2, NH_2, exchangeable), 7.60 (d, $J_{H\text{-}6\text{-}H\text{-}5}$ = 8 Hz, 1, H-6); MS m/e = 276 (M^+). Anal. ($C_{10}H_{13}ClN_2O_5$) C, H, N.

4-Amino-3-bromo-1-β-D-ribofuranosyl-2(1*H*)-pyridinone (9b). Treatment of 1.57 g (0.00248 mol) of **8b** analogous to the treatment of **8d** to give **9d** gave material which was recrystallized from EtOH to give 0.54 g (68%) of the product as a white solid: mp 202–204 °C; ^{1}H NMR (100 MHz, DMSO) δ = 5.90 (d, $J_{H\text{-}5\text{-}H\text{-}6}$ = 7 Hz, 1, H-5), 5.97 (d, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 3 Hz, 1, H-1'), 6.43 (s, 2, NH_2, exchangeable), 7.62 (d, $J_{H\text{-}6\text{-}H\text{-}5}$ = 7 Hz, 1, H-6); MS m/e = 320 (M^+), 322 (M^+ + 2). Anal. ($C_{10}H_{13}BrN_2O_5$) C, H, N.

4-Amino-3-nitro-1-β-D-ribofuranosyl-2(1*H*)-pyridinone (9c). Treatment of 4.80 g (0.0080 mol) of **8c** analogous to the treatment of **8d** to give **9d** gave 2.2 g (96%) of material. This was recrystallized from 80 mL of H_2O to give 1.9 g (83%) of the product as light yellow crystals: mp 264–265 °C; ^{1}H NMR (100 MHz, DMSO) δ = 5.97 (d, $J_{H\text{-}5\text{-}H\text{-}6}$ = 7 Hz, 1, H-5), 6.0 (d, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 3 Hz, 1, H-1'), 7.83 (d, $J_{H\text{-}6\text{-}H\text{-}5}$ = 7 Hz, 1, H-6), 8.17 (s, 2, NH_2, exchangeable); MS m/e = 288 (M^+ + 1); $[\alpha]^{22}_D$ +18.6° (*c* 1.02, DMF). Anal. ($C_{10}H_{13}N_3O_7$) C, H, N.

3-Chloro-4-hydroxy-1-β-D-ribofuranosyl-2(1*H*)-pyridinone (9d). To 7.19 g (0.012 mol) of **8d** was added 132 mL (0.0132 mol) of 0.1 N $NaOCH_3$/MeOH and 50 mL of MeOH. The solution was stirred at room temperature for 18 h. To the solution was added 7.2 g of MeOH-washed IRC-50 (H^+) ion-exchange resin. The suspension was stirred for 1 h and the resin was filtered off and discarded. The filtrate was concentrated and coevaporated with EtOH and then $PhCH_3$ to give 4.83 g of an oil. This was dissolved in MeOH, treated with 35 g of flash silica gel, and the MeOH was evaporated. The resulting powder was applied to a column of 250 g of flash silica gel packed in CH_3CN/H_2O (10:1). Elution with the same solvent gave 2.84 g of a white foam. This was dissolved in a minimum amount of MeOH and precipitated with ether to give 2.51 g (69.1%) of the product as a hygroscopic, beige solid, containing 0.25 mol of ether and 0.35 mol of H_2O: mp >79 °C (indefinite); ^{1}H NMR (100 MHz, DMSO) δ = 6.0 (distorted d, 1, H-1'), 6.05 (d, $J_{H\text{-}5\text{-}H\text{-}6}$ = 8 Hz, 1, H-5), 7.77 (d, $J_{H\text{-}6\text{-}H\text{-}5}$ = 8 Hz, 1, H-6) (substantiates presence of 0.25 mol of Et_2O); UV_{max} (pH 1) 286 nm (ϵ = 5030); (pH 11) 261, 276 nm (ϵ = 5290, 5320); (H_2O) 278 nm (ϵ = 5270); $[\alpha]^{22}_D$ +36.4° (*c* 1.09, H_2O); MS m/e = 277 (M^+). Anal. ($C_{10}H_{12}ClNO_6 \cdot 0.25Et_2O \cdot 0.35H_2O$) C, H, N, H_2O.

3-Bromo-4-hydroxy-1-β-D-ribofuranosyl-2(1*H*)-pyridinone (9e). Treatment of 4.32 g (0.0068 mol) of **8e** analogous to the treatment of **8d** to give **9d** gave after flash chromatography 2.76 g of a white foam. This was dissolved in EtOH and precipitated with ether to give 1.20 g (52.9%) of the product as a hygroscopic, beige solid, containing 0.25 mol of EtOH: mp >50 °C (indefinite) (lit.[15] mp 90–93 °C dec); ^{1}H NMR (200 MHz, DMSO) δ = 5.95 (d, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 3.0 Hz, 1, H-1'), 5.99 (d, $J_{H\text{-}5\text{-}H\text{-}6}$ = 7.8 Hz, 1, H-5), 7.74 (d, $J_{H\text{-}6\text{-}H\text{-}5}$ = 7.8 Hz, 1, H-6) (substantiates presence of 0.25 mol of EtOH) [lit.[15] ^{1}H NMR (60 MHz, DMSO) δ = 5.95 (d, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 3.0 Hz, 1, H-1'), 5.80 (d, $J_{H\text{-}5\text{-}H\text{-}6}$ = 8 Hz, 1, H-5), 7.50 (d, $J_{H\text{-}6\text{-}H\text{-}5}$ = 8 Hz, 1, H-6)]; UV_{max} (pH = 1) 287 nm (ϵ = 5890); (pH = 11) 262, 280 nm (ϵ = 5190, 5470); (H_2O) 280 nm (ϵ = 4960) [lit.[15] UV_{max} (pH = 1) 292 nm (ϵ = 6680); (pH = 11) 262, 282 nm (ϵ = 6200, 6340); (H_2O) 283 nm (ϵ = 7000)]; $[\alpha]^{22}_D$ +39.0° (*c* 1.03, H_2O) [lit.[15] $[\alpha]^{27}_D$ +54° (*c* 1.0, H_2O)]; MS m/e = 321 (M^+), 323 (M^+ + 2). Anal. ($C_{10}H_{12}BrNO_6 \cdot 0.25EtOH$) C, H, N.

4-Hydroxy-3-nitro-1-β-D-ribofuranosyl-2(1*H*)-pyridinone (9f). A suspension of 15.69 g (0.026 mol) of **8f** in 287 mL (0.0287 mol) of a 0.1 N $NaOCH_3$/MeOH solution was heated under reflux for 1 h. The suspension was diluted with 200 mL of MeOH and heated under reflux for an additional 1 h. The suspension was cooled and filtered to give a small amount of yellow solid. TLC indicated this material was mainly desired product but this crop was not further investigated. The MeOH filtrate from above was treated with IRC-50 (H^+) ion-exchange resin and stirred until neutral to pH paper. The resin was filtered off and discarded. The filtrate was concentrated and coevaporated with EtOH. The resulting solid was triturated in ether to give 7 g of a yellow solid. This was dissolved in MeOH and treated with 25 g of flash silica gel. The MeOH was evaporated and the resulting powder was applied to a column of 200 g of flash silica gel packed in CH_3CN/H_2O (10:1). Elution with the same solvent gave 6.2 g of a yellow solid. This was dissolved in hot MeOH and filtered. Ether was added to precipitate 4.17 g (50.1%) of the product, as its sodium salt containing 0.3 mol of MeOH, as a hydroscopic green solid: mp >100 °C (slowly dec.); ^{1}H NMR (200 MHz, DMSO) δ = 5.47 (d, $J_{H\text{-}5\text{-}H\text{-}6}$ = 8.2 Hz, 1, H-5), 5.91 (d, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 4.7 Hz, 1, H-1'), 7.33 (d, $J_{H\text{-}6\text{-}H\text{-}5}$ = 8.1 Hz, 1, H-6) (substantiates the presence of 0.3 MeOH); UV_{max} (pH = 1) 292, 350 nm (ϵ = 3110,

5260); (pH = 11) 336 nm (ϵ = 2980); (H_2O) 336 nm (ϵ = 3050); $[\alpha]^{22}_D$ +19.7° (c 1.18, H_2O). Anal. ($C_{10}H_{11}N_2O_8Na\cdot 0.3CH_3OH$) C, H, N, Na.

Scheme II. 9a. To a solution of 1.00 g (0.0018 mol) of 4-amino-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyridinone (**10**) in 50 mL of CH_2Cl_2, cooled to 0 °C by an ice bath, was added 0.27 g (0.0020 mol) of *N*-chlorosuccinimide. The solution was stirred cold for 2 h. At this time TLC [SiO_2, CH_2Cl_2/EtOAc (5:1)] indicated that the starting material had been converted to material with an R_f identical with that of **8a** prepared via Scheme I. The solution was washed three times with H_2O, dried ($MgSO_4$), and concentrated to give 1.16 g of a white foam. To this material was added 9 mL (0.0009 mol) of a 0.1 N $NaOCH_3$/MeOH solution and the solution was stirred for 18 h. To the solution was added 1 g of MeOH-washed IRC-50 (H^+) ion-exchange resin and the suspension was stirred for 1 h and the resin was filtered off and discarded. The filtrate was concentrated to give a material which was triturated with ether and then recrystallized from EtOH to give 0.26 g (52% over two steps) of the product as an off-white solid: mp 193–195 °C; ^1H NMR (200 MHz, DMSO) δ = 5.89 (d, $J_{H\text{-}5\text{-}H\text{-}6}$ = 7.8 Hz, 1, H-5), 5.94 (d, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 3.3 Hz, 1, H-1′), 6.44 (s, 2, NH_2, exchangeable), 7.58 (d, $J_{H\text{-}6\text{-}H\text{-}5}$ = 7.65 Hz, 1, H-6); MS *m/e* = 276 (M^+); the TLC [SiO_2, CH_2Cl_2/MeOH (4:1)] and IR (KBr) spectra of this product were the same as those of **9a** prepared via Scheme I. Anal. ($C_{10}H_{13}ClN_2O_5$) C, H, N.

9b. To a solution of 1.00 g (0.0018 mol) of **10**, cooled to 0 °C by an ice bath, was added 0.35 g (0.0020 mol) of *N*-bromosuccinimide. The solution was stirred cold for 2 h. At this time TLC [SiO_2, CH_2Cl_2/EtOAc (5:1)] indicated that the starting material had been converted to material with an R_f identical with that of **8b** prepared via Scheme I. The solution was washed three times with H_2O, dried ($MgSO_4$), and concentrated to give 1.26 g of a white foam. To this material was added 9 mL (0.0009 mol) of a 0.1 N $NaOCH_3$/MeOH solution, and the solution was stirred for 18 h. To the solution was added 1 g of MeOH-washed IRC-50 (H^+) ion-exchange resin and the suspension was stirred for 1 h and the resin was filtered off and discarded. The filtrate was concentrated to give a material which was triturated in ether then recrystallized from EtOH to give 0.35 g (60% over two steps) of the product as an off-white solid: mp 199–201 °C; ^1H NMR (200 MHz, DMSO) δ = 5.89 (d, $J_{H\text{-}5\text{-}H\text{-}6}$ = 7.7 Hz, 1, H-5), 5.93 (d, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 3.4 Hz, 1, H-1′), 6.40 (s, 2, NH_2, exchangeable), 7.61 (d, $J_{H\text{-}6\text{-}H\text{-}5}$ = 7.7 Hz, 1, H-6); the TLC [SiO_2, CH_2Cl_2/MeOH (4:1)] and IR (KBr) spectra of this product were the same as those of **9b** prepared by Scheme I. Anal. ($C_{10}H_{13}BrN_2O_5$) C, H, N.

9e. To a solution of 2.00 g (0.0036 mol) of 4-hydroxy-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyridinone (**11**) in 100 mL of $CHCl_3$, cooled by an ice bath, was added 0.70 g (0.0039 mol) of *N*-bromosuccinimide. The solution was stirred cold for 2 h. At this time TLC [SiO_2, CH_2Cl_2/MeOH (10:1)] indicated the starting material had been converted to material with an R_f identical with that of **8e** prepared by Scheme I. The solution was washed three times with H_2O, dried ($MgSO_4$), and concentrated to give 2.28 g of a yellow foam. To this material was added 39 mL (0.0039 mol) of a 0.1 N $NaOCH_3$/MeOH solution and the solution was stirred for 18 h. To the solution was added 2 g of MeOH-washed IRC-50 (H^+) ion-exchange resin and the suspension was stirred for 1 h and the resin was filtered off and discarded. The filtrate was concentrated to give a solid which was triturated in ether. This was dissolved in a minimum amount of MeOH and precipitated by the addition of ether to give 1.04 g of an off-white solid. This was dissolved in a minimum amount of MeOH and treated with 7 g of flash silica gel. The MeOH was evaporated and the resulting powder was placed on a column of 115 g of flash silica gel packed in CH_3CN/H_2O (20:1). Elution with the same solvent gave 0.96 g (81% over two steps) of the product as an off-white, hygroscopic solid containing 0.5 mol of H_2O: mp >80 °C (indefinite); ^1H NMR (100 MHz, DMSO) δ = 5.96 (d, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 3 Hz, 1, H-1′), 6.01 (d, $J_{H\text{-}5\text{-}H\text{-}6}$ = 8 Hz, 1, H-5), 7.77 (d, $J_{H\text{-}6\text{-}H\text{-}5}$ = 8 Hz, 1, H-6); $[\alpha]^{22}_D$ +41.9° (c 0.86, H_2O); MS *m/e* = 321 (M^+), (M^+ + 2); the TLC [SiO_2, CH_2Cl_2/MeOH (4:1)] and IR (KBr) spectra of this product were the same as those of **9e** prepared via Scheme I. Anal. ($C_{10}H_{12}BrNO_6\cdot 0.5H_2O$) C, H, N; H_2O: calcd, 2.72; found, 1.28.

Antiviral Evaluation. Antiviral activity was evaluated by observing inhibition of virus-induced cytopathic effect (CPE) of rhinovirus type 34 (RV) grown in WISH cells. For antiviral assays, cells were grown for 18–24 h to obtain monolayers. Serial 2-fold dilutions of drugs were placed on cells, 320 cell culture 50% infectious doses ($CCID_{50}$) of virus were added, and plates were sealed and incubated at 33 °C for 72 h. Experimental controls included cell control (cells + medium), virus controls (cells + medium + virus), and toxicity controls (cells + medium + drugs). At the end of the incubation period, the cells were observed microscopically for appearance of cell death due to virus replication or drug toxicity. Death was scored on a 0–4 scale with 4 representing 100% death. The minimum inhibitory concentration (MIC) was the lowest calculated concentration of drug that inhibited CPE by 50%. The lowest concentration of drug reducing cell viability by 25% or noticeably altering morphology of 25% of the cells was considered the minimum cytotoxic concentration (MCC). The two values can be used to calculate an antiviral index (AI), which is derived by the following formula: AI = MCC/MIC.

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