fessor Earl Huyser of the University of Kansas for his assistance with the stopped-flow experiments.

Registry No. **1b**, 87414-25-3; **5**, 2365-48-2; **6**, 363-03-1; **7a**, 102830-51-3; **7b**, 102830-53-5; **8a**, 102830-52-4; **8b**, 102830-54-6; **9b**, 102851-29-6; **10**, 87414-26-4; **11**, 102830-55-7; **12**, 102851-30-9; **13**, 102830-57-9; **14**, 102830-56-8; 5-(2,5-dimethoxyphenyl)-2′-deoxyuridine, 76756-32-6; 5-(2,5-dimethoxyphenyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine, 87414-24-2; thymidylate synthase, 9031-61-2; 5-*p*-benzoquinonyl-2′-deoxyuridine-5′-phosphate, 87414-22-0.

Carbocyclic Analogues of 5-Halocytosine Nucleosides[1]

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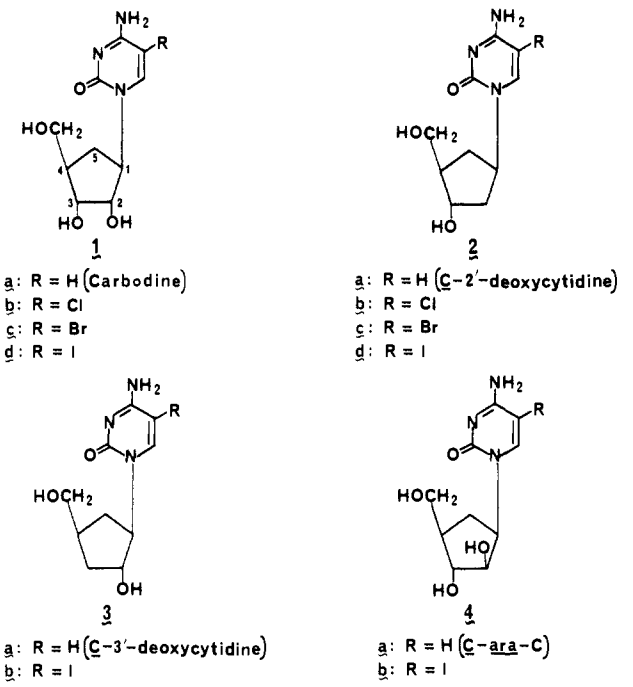
Carbocyclic analogues of 5-halocytosine nucleosides were prepared by direct halogenation of the carbocyclic analogues of cytidine, 2′-deoxycytidine, 3′-deoxycytidine, or ara-C. The 5-chloro and 5-bromo derivatives of the cytidine (carbodine) and of the 2′-deoxycytidine analogues and the 5-iodo derivatives of all four of the cytosine nucleoside analogues were prepared. All of the *C*-5-halocytosine nucleosides, as well as the parent *C*-cytosine nucleosides, were tested against a strain of herpes simplex virus type 1 (HSV-1) that induces thymidine kinase in host cells. Carbodine, 5-bromocarbodine, *C*-2′-deoxycytidine, *C*-5-bromo-2′-deoxycytidine, the four *C*-5-iodocytosine nucleosides, and *C*-ara-C inhibited replication of this strain of HSV-1 in cultured cells. Most of these compounds were tested also against the type 2 virus (HSV-2) in vitro and were active. The greatest activity observed was exerted by *C*-5-iodo-2′-deoxycytidine in inhibiting replication of HSV-1 in L929 cells. In tests against these DNA viruses, carbodine, a ribofuranoside analogue that had been shown previously to be highly active against human influenza A virus in vitro, was the most active compound against HSV-2 and one of the most active compounds against HSV-1 in Vero cells. 5-Bromocarbodine was active against influenza virus, but it was less active than carbodine.

The carbocyclic analogue (**1a**, carbodine) of cytidine was synthesized from the carbocyclic analogue of uridine.[3] Carbodine is active against lymphoid leukemia L1210 in mice,[3,4] is metabolized to the triphosphate in mammalian cells,[5,6] and selectively inhibits the replication of influenza virus (strains A_0/PR/8/34 and A_2/Aichi/2/68) in cultures of kidney cells.[6] However, carbodine was not effective against influenza A virus infections in mice when it was administered intraperitoneally or intranasally.[6] *C*-2′-Deoxycytidine (**2a**) and *C*-3′-deoxycytidine (**3a**), synthesized by the methods used for carbodine,[2,4] were not active (at 200 mg/kg per day, qd 1–9) against L1210 leukemia in mice[2] nor were these compounds active against influenza virus in cultured cells.[6] The carbocyclic analogue (**4a**, *C*-ara-C) of arabinofuranosylcytosine (ara-C) is quite active against L1210 leukemia in mice (T/C, 204% at 150 mg/kg per day, qd 1–9);[7] it also showed modest activity in tests against influenza virus in vitro.[6] Some 5-halo derivatives of these fundamental carbocyclic analogues of the cytosine nucleosides have been prepared and evaluated for antiviral activity. The results of these investigations are described in this report. Earlier, we had synthesized 5-methylcarbodine and reported its modest activity in vitro against influenza virus;[6] and, recently, Herdewijin et al.[8] described the synthesis and antiherpetic activity in vitro of *C*-5-(2-bromovinyl)-2′-deoxycytidine.

Chart I



Chemistry. 5-Chlorocarbodine (**1b**), *C*-5-chloro-2′-deoxycytidine (**2b**), 5-bromocarbodine (**1c**), and *C*-5-bromo-2′-deoxycytidine (**2c**) were prepared by direct halogenation of the parent analogues (**1a** and **2a**). Acetylation of the hydroxyl groups of **1a** or **2a** was allowed to

(1) In this report, as in earlier publications of this series, carbocyclic analogues of nucleosides are named by prefixing *C*- to the names of the corresponding true nucleosides; e.g., *C*-5-halocytosine nucleosides should be read as "carbocyclic analogues of 5-halocytosine nucleosides". However, the carbocyclic analogue (*C*-cytidine) of cytidine was also named *carbodine*;[2] therefore, the *C*-cytidines described in this report are named as derivatives of carbodine. In the Experimental Section, carbocyclic analogues of nucleosides are named, in accordance with the systematic nomenclature employed by *Chemical Abstracts*, as cyclopentyl derivatives of 4-amino-2(1*H*)-pyrimidinone.

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0022-2623/86/1829-1720$01.50/0

proceed in a mixture of pyridine and acetic acid, and then a solution of the halogen in carbon tetrachloride was added. This method is similar to the method described by Fukuhara and Visser[9] for the preparation of 5-chloro- and 5-bromocytidine. Each total crude product was treated with ammonia in methanol to remove acetyl groups, and the resulting product was then purified by chromatography on a cation-exchange resin. 5-Iodocarbodine (**1d**), *C*-5-iodo-2′-deoxycytidine (**2d**), and *C*-5-iodo-3′-deoxycytidine (**3b**) were prepared by treating the parent cytosines (**1a**–**3a**) with iodine and iodic acid in a two-phase mixture containing carbon tetrachloride, acetic acid, and water. This method was developed by Chang and Welch[10] for the preparation of 5-iodocytidine and 5-iodo-2′-deoxycytidine. The 5-iodo derivatives were also purified by chromatography on a cation-exchange resin. *C*-5-Iodo-ara-C (**4b**) was obtained by treating *C*-ara-C (**4a**) with iodine in a solution of pyridine and acetic acid.

Data from the 300-MHz proton NMR spectra of the 5-halocytosines in Me_2SO-d_6 solutions are tabulated in Table III. The positions of the protons are designated by the numbering system for the cyclopentane ring (structure 1). The spectra include two broad singlets arising from amino group resonances. One of the chemical shifts appears at δ 7.60–7.67; the position of the other broad peak varies with the halogen at position 5 as follows: 5-chloro (**1b**, **2b**), δ 7.0–7.1; 5-bromo (**1c**, **2c**), δ 6.7–6.8; 5-iodo (**1d**, **2d**, **3b**, **4b**), δ 6.4–6.5. In the 100-MHz proton NMR spectra[2,7] of the parent *C*-cytosine nucleosides (**1a**, **2a**, **3a**, **4a**), the amino-group chemical shift appeared as a singlet at about δ 6.96. The 300-MHz spectra (Table III) of these compounds, which were determined subsequently for comparison with the spectra of the 5-halocytosine derivatives, also include only one amino-group chemical shift. Thus, the substituent at position 5 of the pyrimidine ring increases the barrier to rotation around the exocyclic C–N bond and, therefore, causes the two hydrogens of the amino group to be nonequivalent at 26 °C on the NMR time scale. Becker et al.[11] observed two peaks for the NH_2 group in the proton NMR spectra of 5-methyldeoxycytidine and 5-iododeoxycytosine.

In the 300-MHz spectra of all of these *C*-cytosine nucleosides (**1**–**4**), the chemical shifts of the two methylene protons at position 5 of the cyclopentane ring appear as separate multiplets. The signals from the downfield proton at position 5 and the proton at position 4 are overlapping multiplets in the spectra of the ribofuranoside analogues (**1a**–**d**) and the arabinofuranosyl analogues (**4a**,**b**). In the spectra of the *C*-2′-deoxycytidines (**2a**–**d**), the signals from the proton at position 4 and one of the protons at position 2 are overlapping multiplets. In contrast, all five of the protons at nonhydroxylated positions 3,4,5 of the *C*-3′-deoxycytidines (**3a**,**b**) produced separated multiplets in the 300-MHz spectra. (In the 100-MHz spectrum[2] of **3a**, the five protons produced a broad unresolved multiplet at δ 0.9–2.4.) In the spectra of **1a**–**d** and of **3a**,**b**, the signals from the CH_2 protons of the hydroxymethyl groups are unresolved multiplets centered, respectively, at approximately δ 3.4 and 3.3, whereas they are separate multiplets centered at δ 3.4 and 3.5 in the spectra of **2a**–**d** and of **4a**,**b**.

Biological Evaluation. All of the *C*-5-halocytosine nucleosides were tested for inhibition of cytopathogenic effects produced by herpes simplex virus type 1 (HSV-1). Most of the analogues that demonstrated significant activity against HSV-1 were then tested against the type 2 virus (HSV-2). The antiviral drug ara-A was employed as a positive control in evaluations of the analogues against either HSV-1 or HSV-2. The results of these antiviral tests are summarized in Table I. In addition, data obtained from tests of the parent *C*-cytosine nucleosides (**1a**, **2a**, **3a**, **4a**) against HSV are summarized in Table I. The results of tests of the latter compounds against influenza virus have been reported,[6] but data from evaluations of these compounds against HSV have not been recorded previously except for the modest activity of carbodine and *C*-ara-C vs. strain HF of HSV-1.[4]

Carbodine (**1a**), *C*-2′-deoxycytidine (**2a**), *C*-ara-C (**4a**), the 5-iodo derivatives (**1d**, **2d**, **4b**) of these three parent *C*-cytosine nucleosides, and *C*-5-bromo-2′-deoxycytidine (**2c**) showed significant, selective activity in tests against strain E-377 of HSV-1 replicating in Vero cells. Carbodine (VR, 2.8) and *C*-5-iodo-2′-deoxycytidine (**2d**; VR, 2.3) were the most active among these analogues. Both were comparable in activity to ara-A; the ratios of the virus ratings of **1a** and **2d** to the VR of ara-A (tested simultaneously with each analogue) were 0.93 and 1.15, respectively. The remaining active compounds (**1d**, **2a**, **2c**, **4a**, **4b**) were less inhibitory to HSV-1 in Vero cells (VR, 1.1–1.9; VR/VR of ara-A, 0.38–0.65); *C*-5-bromocarbodine exhibited borderline activity.

C-5-Iodo-2′-deoxycytidine (**2d**) showed higher activity (VR, 6.9) and greater potency ($MIC_{50} < 0.4$ μg/mL) against HSV-1 replicating in L929 cells. The ratio of the VR of **2d** to the VR of ara-A in the same experiment indicated that **2d** was more active than ara-A by a factor of about 3.5. Likewise, *C*-2′-deoxycytidine (**2a**) was more active (VR, 2.6; VR ratio, 1.2) and potent (MIC_{50}, <26 μg/mL) against HSV-1 replicating in primary rabbit kidney cells (PRK) than against this virus in Vero cells. *C*-ara-C was also active in this host cell–virus system. The differences in the effectiveness of **2a** and **2d** in inhibiting virus-induced cytopathogenic effects in different host cells illustrate further the phenomenon of differences in antiviral activity observed in different host cells.[12,13]

In tests against HSV-2 replicating in Vero cells, the following analogues were active: carbodine (VR, 3.2; VR ratio, 1.9), *C*-2′-deoxycytidine (VR, 1.9; VR ratio, 1.1), *C*-5-bromo-2′-deoxycytidine (VR, 2.6; VR ratio, 1.44), *C*-ara-C (VR, 2.4; VR ratio, 1.4), *C*-5-iodo-ara-C (VR, 2.2; VR ratio, 1.2). The ratios of the virus ratings of the *C*-cytosine nucleosides to the VR of ara-A (tested simultaneously with each analogue) were greater than these VR ratios in tests of the same compounds against HSV-1 in the same host-cell line. *C*-5-Iodo-2′-deoxycytidine also exhibited significant activity against HSV-2 in L929 cells (VR, 2.1; VR ratio, 0.7) and in Vero cells (VR, 1.0; VR ratio, 0.6).

Strain E-377 of HSV-1 is a TK^+ strain (i.e., it induces thymidine kinase in host cells); strain HF is a TK^- strain. As stated above, carbodine and *C*-ara-C displayed modest or borderline activity against strain HF (ref. 4, cf. Table I). Four *C*-2′-deoxycytidines (**2a**, **2c**, **2d**, **4b**) that inhibit replication of strain E-377 in one or two cell lines and *C*-3′-deoxycytidine, which is not inhibitory to strain E-377, were tested against strain HF replicating in H.Ep.-2 cells. All four were ineffective. These results might suggest that *C*-5-bromo- and *C*-5-iodo-2′-deoxycytidine are deaminated

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Table I. Evaluation of Carbocyclic Analogues of Cytosine Nucleosides vs. Herpes Simplex Viruses[a]

	HSV-1					HSV-2[b]			
compound	host cells[c]	strain	VR	MIC_{50}, μg/mL	ratio[d] VR/VR of ara-A	host cells	VR	MIC_{50}, μg/mL	ratio VR/VR of ara-A
carbodine (1a)	H.Ep.-2	HF	0.8[e]	71					
	Vero	377	2.8	1.4	0.93	Vero	3.2	2.4	1.9
5-chlorocarbodine (**1b**)	Vero	377	0.4		0.11				
5-bromocarbodine (**1c**)	Vero	377	0.7	300	0.19				
5-iodocarbodine (**1d**)	Vero	377	1.5	98	0.5	Vero	0		
C-2′-deoxycytidine (**2a**)	PRK	377	2.6	<26	1.2				
	Vero	377	1.2	196	0.6	Vero	1.9	44	1.1
	H.Ep.-2	HF	0						
C-5-chloro-2′-deoxycytidine (**2b**)	Vero	377	0.3		0.1	Vero	0.7	270	0.3
C-5-bromo-2′-deoxycytidine (**2c**)	Vero	377	1.9	30	0.53	Vero	2.6	62	1.44
	H.Ep.-2	HF	0						
C-5-iodo-2′-deoxycytidine (**2d**)	L929	377	6.9	<0.4	3.45	L929	2.1	31	0.7
	Vero	377	2.3	75	1.15	Vero	1.0	340	0.6
	H.Ep.-2	HF	0						
C-3′-deoxycytidine (3a)	PRK	377	0						
	Vero	377	0						
	H.Ep.-2	HF	0						
C-5-iodo-3′-deoxycytidine (**3b**)	Vero	377	0.5	700	0.17				
C-ara-C (**4a**)	PRK	377	1.8	30	0.58				
	Vero	377	1.3	20	0.65	Vero	2.4	13	1.4
	H.Ep.-2	HF	0.6[e]	95					
C-5-iodo-ara-C (**4b**)	Vero	377	1.1	247	0.38	Vero	2.2	197	1.2
	H.Ep.-2	HF	0						

[a] The antiviral activity of each compound is expressed as a virus rating (VR), and the potency is given as a minimum inhibitory concentration (MIC_{50}). The VR is a weighted measurement of antiviral activity that takes into account both the degree of inhibition of virus-induced cytopathogenic effects and the degree of cytotoxicity produced by the test compound.[15] A virus rating (VR) equal to or greater than 1.0 indicates definite and significant antiviral activity, a VR of 0.5–0.9 indicates marginal to moderate antiviral activity, and a VR less than 0.5 usually indicates no significant antiviral activity. The MIC_{50} is the concentration of the tested compound required to inhibit virus-induced cytopathogenic effects by 50%. [b] Strain MS of HSV-2 was used in these experiments. [c] Vero cells are African green monkey kidney cells. PRK cells are primary rabbit kidney cell cultures. H.Ep.-2 cells are human epidermoid carcinoma cells. L929 cells are mouse connective tissue cells, clone L. Monolayer cultures of these cell lines were used. Cell culture media for these cell lines are described in the Experimental Section. [d] VR/VR of ara-A is the ratio of the VR of the *C*-cytosine nucleoside to the VR to ara-A (employed as a positive control) in the same experiment. [e] VR from ref 4.

Table II. Evaluation of Selected Carbocyclic Analogues of Cytosine Nucleosides against Influenza Virus[a]

		influenza				
compound	cell culture medium[b]	host cells[c]	strain[d]	VR	MIC_{50}, μg/mL	ratio VR/ribavirin VR[e]
carbodine (1a)	1	MDCK	A_0	3.5 ± 0.3[f]	2.6 ± 0.8[f]	0.73[g]
	2	RhMK	A_2	2.6[e]	2.1 ± 1.6[f]	0.81[g]
5-chlorocarbodine (**1b**)	3	MDCK	A_0	0.4		0.1
5-bromocarbodine (**1c**)	3	MDCK	A_0	1.5	150	0.36
5-iodocarbodine (**1d**)	4	MDCK	A_0	0.2		
C-2′-deoxycytidine (**2a**)	1	MDCK	A_0	0		
C-3′-deoxycytidine (**3a**)	1	MDCK	A_0	0.1[f]		
C-5-iodo-3′-deoxycytidine (**3b**)	4	MDCK	A_0	0		
	5	MDCK	A_0	0.5[f]		
C-ara-C (**4a**)	1	MDCK	A_0	0.8	312	0.2

[a] See footnote a of Table I. [b] Cell culture media 1–5 are defined in the Experimental Section. [c] MDCK cells are Madin–Darby canine kidney cells; RhMK cells are Rhesus monkey kidney cells. [d] A_0 = influenza virus strain A_0/PR/8/34; A_2 = influenza virus strain A_2/Aichi/2/68. [e] VR/VR of ribavirin is the ratio of the VR of the *C*-cytosine nucleoside to the VR of ribavirin (employed as a positive control) in the same experiment. [f] From ref 6. [g] Ratio of the average VR of carbodine to the average VR of ribavirin; average virus ratings from ref 6.

to *C*-5-bromo- and *C*-5-iodo-2′-deoxyuridine, respectively, because the latter compounds are active against strain E-377 in PRK cells and not against strain HF in H.Ep.-2 cells.[14] However, *C*-2′-deoxycytidine (**2a**) is active against strain E-377 in PRK cells, but its deamination product, *C*-2′-deoxyuridine, is not.[14] Therefore, **2a** must be active per se or the rate of deamination must be slow. In addition, the activity of *C*-5-bromo-2′-deoxycytidine and of *C*-5-iodo-2′-deoxycytidine against the TK^+ strain (E-377) and the lack of activity vs. the TK^- strain (HF) suggest that these compounds are activated by the HSV-specific pyrimidine deoxynucleoside kinase. Investigations of the phosphorylation of *C*-cytosine nucleosides in virus-infected cells may elucidate the latter question, and studies of enzymatic deamination of *C*-cytosine nucleosides may clarify the relationship between deamination and antiviral activity. In addition to these studies, several of the *C*-5-halocytosine nucleosides, particularly *C*-5-iodo-2′-deoxycytidine, appear to be sufficiently promising for further investigations of their antiviral activity.

The ribofuranoside analogues (**1b–d**) and the 3′-deoxyribofuranoside analogue (**3b**) were tested for inhibition of influenza virus, an RNA virus. The results, together with data[6] from tests of the parent *C*-cytidines, are summarized in Table II. 5-Bromocarbodine was active (VR, 1.5; VR/VR of ribavirin, 0.36) against strain A_0/PR/8/34, but it was less active than carbodine. The ratio (not previously

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Table III. Proton NMR Spectra of Carbocyclic Analogues of Cytosine Nucleosides at 26 °C[a]

compound	5-CH_2	4-CH	CH_2O	3-CH or 3-CH_2	2-CH or 2-CH_2	1-CH	NH_2	cyt CH 5-H	cyt CH 6-H	other
carbodine (**1a**)	1.23 m 1.98 m[b]	1.93 m[b]	3.40 m[c]	3.73 m	3.99 m	4.58 m[d]	6.98 s	5.68 d *J* = 7.27	7.58 d *J* = 7.27	3.35 s[c] (H_2O), 4.53 d, *J* = 4.3 (3-OH), 4.63 t,[d] *J* = 5.15 ($HOCH_2$), 4.70 d, *J* = 6.6 (2-OH)
5-chlorocarbodine (**1b**)	1.25 m 2.00 m[b]	1.93 m[b]	3.41 m[c]	3.72 m	4.05 m	4.60 m[d]	7.06 br s 7.67 br s		7.97 s	3.37 s[c] (H_2O), 4.4–5.0 m[d] (3 × OH)
5-bromocarbodine (**1c**)	1.27 m 2.00 m[b]	1.92 m[b]	3.40 m[c]	3.72 m	4.05 m	4.58 m[d]	6.83 br s 7.67 br s		8.02 s	3.37[c] (H_2O), 4.3–5.0 m[d] (3 × OH)
5-iodocarbodine (**1d**)	1.28 m 1.98 m[b]	1.93 m[b]	3.40 m[c]	3.71 m	4.04 m	4.57 m[d]	6.45 br s 7.64 br s		8.00 s	3.33 s[c] (H_2O), 4.53 m[d] (3-OH), 4.62 m[d] (5-OH), 4.76 m (2-OH)
C-2′-deoxycytidine (**2a**)	1.32 m 2.05 m	1.82 m[e]	3.39 m 3.48 m	3.98 m	1.82 m[e]	5.01 m	7.00 s	5.69 d	7.61 d	3.36 s (H_2O), 4.59 t ($HOCH_2$), 4.69 d (3-OH)
C-5-chloro-2′-deoxycytidine (**2b**)	1.38 m 2.07 m	1.90 m[f]	3.40 m 3.49 m	3.98 m	1.77 m 1.90 m[f]	4.97 m	7.04 br s 7.66 br s		7.97 s	3.33 s (H_2O), 4.58 m ($HOCH_2$), 4.67 m (3-OH)
C-5-bromo-2′-deoxycytidine (**2c**)	1.40 m 2.06 m	1.91 m[f]	3.40 m 3.49 m	3.97 m	1.77 m 1.91 m[f]	4.96 m	6.84 br s 7.67 br s		8.02 s	3.34 s (H_2O), 4.61 m ($HOCH_2$), 4.70 m (3-OH)
C-5-iodo-2′-deoxycytidine (**2d**)	1.42 m 2.07 m	1.91 m[f]	3.41 m 3.51 m	3.97 m	1.78 m 1.91 m[f]	4.95 m	6.46 br s 7.66 br s		8.02 s	3.36 s (H_2O), 4.59 m ($HOCH_2$), 4.68 m (3-OH)
C-3′-deoxycytidine (**3a**)	1.32 m 1.96 m	2.15 m	3.31 m[g]	1.56 m 1.75 m	4.14 m	4.44 m	6.97 s	5.68 d	7.57 d	3.35 s (H_2O),[g] 4.57 m ($HOCH_2$), 4.94 m (2-OH)
C-5-iodo-3′-deoxycytidine (**3b**)	1.41 m 1.94 m	2.13 m	3.32 m[g]	1.54 m 1.75 m	4.20 m	4.39 m	6.45 br s 7.63 br s		8.00 s	3.34 s (H_2O),[g] 4.55 t ($HOCH_2$), 4.95 s (2-OH)
C-ara-C (**4a**)	1.67 m 1.85 m[h]	1.85 m[h]	3.39 m 3.53 m	3.56 m	3.75 m	4.88 m	6.92 br s	5.62 d	7.54 d	3.36 s (H_2O), 4.61 m ($HOCH_2$), 4.87 m (3-OH), 5.01 d (2-OH)
C-5-iodo-ara-C (**4b**)	1.68 m 1.88	1.82 m	3.42 m 3.52 m	3.56 m	3.73 m	4.88 m	6.43 br s 7.60 br s		7.84 s	3.33 s (H_2O), 4.61 t ($HOCH_2$), 4.89 m (3-OH), 5.10 d (2-OH)

[a] Proton NMR spectra were determined at 300.64 MHz (Nicolet NT 300NB NMR spectrometer). Me_2SO-D_6 was the solvent, and tetramethylsilane was the internal standard; m = multiplet, s = singlet, d = doublet, t = triplet, br s = broad singlet, and *J* is in Hertz. The numbering of positions on the cyclopentane ring is the same as the numbering in the systematic names in the Experimental Section. [b] The multiplets produced by the proton at position 4 and the downfield proton of the methylene group at position 5 overlap; the listed chemical shifts are the approximate centers of the two overlapping multiplets. [c] Addition of D_2O to the Me_2SO-D_6 solutions of the ribofuranoside analogues showed that the chemical shift of the OCH_2 protons of this type of carbocyclic analogue is an unresolved multiplet that overlaps the peak arising from water of solvation. [d] In the spectra of the ribofuranoside analogues, the chemical shift of the proton at position 1 is in the region of the chemical shifts of the hydroxyl groups. Addition of D_2O to the Me_2SO-D_6 solutions showed that the signal from 1-CH is a multiplet. [e] Approximate center of overlapping multiplets (δ 1.65–1.95) arising from 2-CH_2 and 4-CH. The individual chemical shifts are estimated to be δ 1.76, 1.82, and 1.88. [f] Approximate center of overlapping multiplets (δ 1.82–2.00) arising from 4-CH and the downfield proton of 2-CH_2. [g] The singlet resulting from water of solvation was superimposed on the CH_2O multiplet (an apparent doublet). [h] Center of unresolved multiplets arising from the proton at position 4 and the downfield proton of 5-CH_2.

recorded) of an average virus rating of carbodine to an average virus rating of ribavirin is 0.73.

Experimental Section

General Methods. Decomposition and melting temperatures (mp) were determined in capillary tubes heated in a Mel-Temp apparatus. Ultraviolet spectra (UV) were recorded with a Cary Model 17 spectrophotometer, and absorption maxima are reported in nanometers; sh = shoulder. Solutions for ultraviolet spectral determinations were prepared by diluting a 5-mL aliquot of a water or an ethanol solution to 50 mL with 0.1 N hydrochloric acid, phosphate buffer (pH 7), or 0.1 N sodium hydroxide. Absorption maxima of these solutions are reported as being determined at pH 1, 7, or 13, respectively. Infrared spectra (IR) were recorded with a Nicolet 10-MX Fourier transform spectrometer, and the spectra were determined with specimens in pressed potassium bromide disks; s = strong, vs = very strong, sh = shoulder, w = weak. Mass spectral data (MS) were taken from low-resolution, electron-impact spectra determined at 70 eV with a Varian/MAT 311 A spectrometer or from spectra determined by the fast atom bombardment (FAB) method. The peaks listed are those arising from the molecular ion (M), the pseudomolecular ion (M + 1), those attributable to the loss of certain fragments (M minus a fragment), and some other prominent peaks. Fragments containing the complete pyrimidine moiety are designated P plus an atom or group. Proton nuclear magnetic resonance spectra were determined at 300.64 MHz with a Nicolet NT 300NB NMR spectrometer. The internal standard was tetramethylsilane; s = singlet, t = triplet, q = quartet, qn = quintet, m = multiplet. Thin-layer chromatography (TLC) was performed on plates of silica gel, the developing solvent is specified parenthetically, and developed plates were examined by ultraviolet light.

(±)-4-Amino-1-[(1α,2β,3β,4α)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]-5-chloro-2(1*H*)-pyrimidinone (1b). A suspension of 100 mg (0.42 mmol) of carbodine (**1a**) in a mixture of 10 mL of glacial acetic acid and 5 mL of pyridine was stirred

at room temperature for 20 h in a stoppered flask. A solution of 33 mg (0.47 mmol) of chlorine in 0.5 mL of carbon tetrachloride was added to the homogeneous solution, and the reaction solution was stirred at room temperature for 24 h. The solution was concentrated in vacuo to dryness, several portions of methanol were evaporated from the residue, the gummy residue was dissolved in 10 mL of 5% NH_3 in methanol, and the solution was stirred in a stoppered flask for 4 days. The brown solution was concentrated to dryness. The syrupy, crude **1b** was dissolved in water and poured onto a column that contained 15 mL of a cation-exchange resin (Amberlite CG-120, H^+ form). The column was washed thoroughly with water and then with 0.5 N aqueous ammonia, and the basic eluate (40 mL) was concentrated to a glassy residue. Several portions of methanol were evaporated from the residue, which was then triturated with ethyl acetate: yield of tan solid, 60 mg (50%); mp, becomes a foam at about 112 °C (capillary inserted at 50°, 3°/min); TLC, 1 spot (7:3 2-propanol–1 M NH_4OAc); UV max 218 (ε 10 200) and 301 (9500) at pH 1, 218 (10 400) and 290 (6200) at pH 7, 290 (6800) at pH 13; MS (FAB), *m/e* 276 (M + 1). Anal. ($C_{10}H_{14}ClN_3O_4 \cdot {}^1/_2H_2O$) C, H, N.

(±)-4-Amino-1-[(1α,2β,3β,4α)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]-5-bromo-2(1*H*)-pyrimidinone (1c). A solution of carbodine (500 mg, 2.1 mmol) in 45 mL of glacial acetic acid and 25 mL of pyridine was protected from moisture and stirred at room temperature for 68 h. The solution was cooled to −10 °C, a solution of 370 mg of bromine in 1.25 mL of carbon tetrachloride was added in one portion, and the reaction solution was irradiated for 5 min at a distance of 10 cm by a 150-W flood lamp. The flood lamp was turned off immediately, and the orange reaction mixture was stirred at room temperature overnight. The solution was concentrated in vacuo, several portions of methanol were evaporated from the residue, the orange syrup that remained was dissolved in 50 mL of a 5% solution of ammonia in methanol, and the solution was stirred overnight in a stoppered flask. Volatile components were evaporated in vacuo, the gummy residue was dissolved in 50 mL of water, and the solution was poured onto a column that contained 50 mL of a cation-exchange resin (Amberlite CG-120, H^+ form). The column was washed thoroughly with water and then with 1 N aqueous ammonia. Fractions containing **1c** were identified by monitoring the basic effluent at 280 nm with a recording spectrophotometer. The fractions of the basic eluate containing **1c** were concentrated in vacuo to dryness, the white crystalline residue was triturated with ethanol, and the crystals were collected by filtration, washed with ethanol, and dried in vacuo at 78 °C: yield of **1c**, 450 mg (67%); mp 165–168 °C (capillary inserted at 100°, 3°/min); TLC, 1 spot (7:3 2-propanol–1 M NH_4OAc); UV max 217 (ε 11 300) and 303 (ε 11 200) at pH 1, 217 sh and 291 (7800) at pH 7, 292 (7800) at pH 13; IR (2000–1200-cm^{-1} region) 1660 s, 1645 sh, 1635 sh, 1600, 1570 sh, 1510, 1485, 1460 sh, 1450 sh, 1415, 1395, 1375, 1355 sh, 1340 sh, 1320, 1315, 1295, 1275 sh, 1255, 1220. Anal. ($C_{10}H_{14}BrN_3O_4 \cdot {}^1/_4H_2O$) C, H, N.

(±)-4-Amino-1-[(1α,2β,3β,4α)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]-5-iodo-2(1*H*)-pyrimidinone (1d). A two-phase mixture consisting of 241 mg (1 mmol) of carbodine, 144 mg (0.57 mmol) of iodine, 86 mg (0.49 mmol) of iodic acid, 0.66 mL of carbon tetrachloride, 0.33 mL of water, and 1 mL of glacial acetic acid was stirred vigorously and heated at 46–48 °C for 6 h. The mixture was stirred at room temperature overnight and then diluted with 5 mL of water and with 5 mL of CCl_4. The water layer was separated, extracted with CCl_4 until no more iodine was extracted into the organic layer, and diluted to 25 mL with water. The aqueous solution was applied to a column of 25 mL of a cation-exchange resin (Amberlite CG-120, H^+ form). The column was washed thoroughly with water and then with 0.5 N aqueous ammonia, and the basic eluate was concentrated in vacuo to a gummy residue that was triturated with ethanol. A tan, crystalline solid was filtered away, washed with ethanol, and dried in vacuo at 56 °C: yield, 188 mg (49%); mp 158–162 °C (capillary inserted at 100°, 3°/min); TLC, 1 spot (7:3 2-propanol–1 M NH_4OAc); UV max 223 (ε 13 400) and 313 (9300) at pH 1, 222 (15 300) and 297 (6500) at pH 7, 297 (6500) at pH 13; MS (electron-impact; direct-probe temperature, 150 °C), *m/e* 367 (M), 350 (M − OH), 349 (M − H_2O), 339 (M − CO), 336 (M − CH_2OH), 320, 318, 264 (P + C_2H_4), 238 (P + 2 H), 237 (P + H); IR (2000–1200-cm^{-1} region) 1650 s, 1635 s, 1625 s, 1590, 1565 sh, 1500, 1475, 1450 sh, 1420 w, 1390, 1335 sh, 1320 sh, 1315, 1295, 1250, 1235 w, 1215. Anal. ($C_{10}H_{14}IN_3O_4 \cdot H_2O$) C, H, N.

Additional **1d** (7% yield as a monohydrate) that was less pure (mp 150–154 °C) was obtained by washing the resin column with more 1 N aqueous ammonia, concentrating the eluate, and triturating the residue with ethanol.

(±)-4-Amino-1-[(1α,3β,4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-5-chloro-2(1*H*)-pyrimidinone (2b). Compound **2b** was prepared from 200 mg (0.89 mmol) of **2a** by the procedure described for the preparation of compound **1b** except that 3 portions of a chlorine solution were added [69 mg (0.97 mmol) of Cl_2 in 1.3 mL of carbon tetrachloride at the beginning, 16 mg (0.23 mmol) in 0.3 mL of CCl_4 after 24 h, and 11 mg (0.15 mmol) in 0.2 mL of CCl_4 after 48 h]. The solution was stirred at room temperature for 3 h after the last addition of chlorine and was concentrated in vacuo to dryness. The residue was dissolved in 20 mL of 5% NH_3 in methanol, and the solution was stirred in a stoppered flask for 60 h. The brown solution was concentrated to dryness, the syrupy residue was dissolved in water (50 mL), the solution was poured onto a column that contained 25 mL of a cation-exchange resin (Amberlite CG-120, H^+ form), and the column was washed with water until no more UV-absorbing materials were detected in the effluent. The column was then washed with 0.5 N aqueous ammonia, and product-containing fractions (determined by monitoring the column effluent at 280 nm with a recording spectrophotometer) were concentrated in vacuo to dryness. The residue was digested with 5 mL of boiling ethanol, and a yellow crystalline solid (90 mg) was collected by filtration. A second crop of amorphous solid (36 mg) was obtained by adding 10 mL of ether to the filtrate.

The two crops of product were combined and dissolved in 12 mL of boiling ethanol, the solution was filtered, and the filtrate was diluted with 15 mL of ether. An amorphous precipitate was removed by filtration, washed with 2:1 ether–ethanol, and dried in vacuo at 56 °C: weight, 87 mg. This material was suspended in 4 mL of ethanol, the suspension was heated to boiling and cooled, and a white solid was filtered away, washed with cold ethanol, and dried in vacuo at 56 °C: yield, 63 mg (26%); mp 195–197 °C dec (capillary inserted at 100°, 3°/min); TLC, 1 spot (7:3 2-propanol–1 M NH_4OAc); UV max 218 (ε 11 200) and 302 (11 900) at pH 1, 218 (11 900) and 290 (7900) at pH 7, and 290 (8000) at pH 13; MS (FAB), *m/e* 260 (M + 1); IR (1800–1200-cm^{-1} region) 1685 s, 1645 s, 1615 s, 1500 s, 1460, 1440, 1400, 1375, 1355, 1340 sh, 1330, 1295 s, 1275, 1255, 1230, 1205. Anal. ($C_{10}H_{14}ClN_3O_3 \cdot {}^1/_2H_2O$) C, H, N.

(±)-4-Amino-1-[(1α,3β,4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-5-bromo-2(1*H*)-pyrimidinone (2c). Compound **2c** was prepared [beginning with 100 mg (2.1 mmol) of **2a**, 7 mL of glacial acetic acid, and 5 mL of pyridine] by the procedure described for the preparation of **1c** except that the solution was stirred at room temperature for 20 h. The solution was exposed to a flood lamp as described for the preparation of **1c**, and a solution of 78 mg of bromine in 0.25 mL of carbon tetrachloride was added in one portion. The solution was stirred at room temperature overnight.

Deacetylation with ammonia–methanol and column chromatography of **2c** on a cation-exchange resin were performed as described for **1c**. The basic eluate (approximately 30 mL) was concentrated to dryness. The residue was triturated with ethyl acetate, and a tan solid was collected by filtration and dried at 56 °C and then for 2 h at 78 °C: yield, 90 mg (66%); mp, 174–178 °C dec (capillary inserted at 115°, 3°/min); TLC, 1 spot (7:3 2-propanol–1 M NH_4OAc); UV max 217 (ε 11 200) and 303 (11 000) at pH 1, 217 sh and 290 (7600) at pH 7, 290 (7400) at pH 13; IR (2000–1200-cm^{-1} region) 1660 s, 1635 s, 1600, 1490 s, 1455, 1445 sh, 1415, 1375, 1360 sh, 1345, 1330 sh, 1310, 1295, 1270, 1230. Anal. ($C_{10}H_{14}BrN_3O_3 \cdot {}^1/_4H_2O$) C, H, N.

(±)-4-Amino-1-[(1α,3β,4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-5-iodo-2(1*H*)-pyrimidinone (2d). Compound **2d** was prepared from **2a** by the procedure described for the preparation of **1d**. The two-phase reaction mixture consisted of 850 mg (3.77 mmol) of **2a**, 544 mg (2.14 mmol) of iodine, 327 mg (1.86 mmol) of iodic acid, 8.5 mL of carbon tetrachloride, 2.9 mL of water, and 8.5 mL of glacial acetic acid. Product-containing fractions of the basic effluent (1 N aqueous ammonia) from the column of cation-exchange resin were filtered and concentrated

in vacuo to a residue (960 mg) that crystallized to a white solid when it was slurried with 10 mL of ethanol. The mixture was chilled, and the crystalline product was collected by filtration, washed with ethanol, and dried in vacuo at 78 °C: yield, 910 mg (67%); mp 196–198 °C dec (capillary inserted at 125°, 3°/min); TLC, 1 spot (7:3 2-propanol–1 M NH_4OAc); MS (electron impact; direct-probe temperature, 280 °C), m/e 351 (M), 334 (M – OH), 320 (M – CH_2OH), 304, 277, 264 (P + C_2H_4), 238 (P + 2 H), 237 (P + H); UV max 223 (ε 13 900) and 312 (9800) at pH 1, 221 (15 500) and 296 (6500) at pH 7, 297 (6700) at pH 13; IR (1800–1200-cm^{-1} region) 1660 s, 1620 vs, 1600 s, 1485 s, 1440 sh, 1385, 1350, 1330, 1305 sh, 1290, 1250 w, 1210 w. Anal. ($C_{10}H_{14}IN_3O_3 \cdot {}^1/_2H_2O$) C, H, N.

(±)-4-Amino-1-[(1α,2β,4α)-2-hydroxy-4-(hydroxymethyl)-cyclopentyl]-5-iodo-2(1*H*)-pyrimidinone (3b). Compound **3b** was prepared from 100 mg (0.44 mmol) of **3a** by the procedure described for the preparation of **1d**. The gummy residue obtained from the basic eluate of the resin column crystallized when it was triturated with ethanol. The off-white solid was filtered away, washed with cold ethanol, and dried in vacuo at 56 °C: yield, 92 mg (60%); mp 190–200 °C, incomplete melt (capillary inserted at 100°, 3°/min). This material was combined in boiling water (5 mL) with the white solid (55 mg) obtained by concentrating the filtrate to dryness, and the hot mixture was filtered. White crystals that formed in the cold filtrate were collected by filtration, washed with cold water, and dried in vacuo at 56 °C: yield, 68 mg (43%); mp 208–211 °C dec (capillary inserted at 50°, heating rate 3°/min); TLC, 1 spot (4:1 2-propanol–1 M NH_4OAc); UV max 223 (ε 13 600) and 313 (9700) at pH 1, 222 (15 400) and 298 (6500) at pH 7, 297 (6600) at pH 13; MS (electron-impact; direct-probe temperature, 150 °C), *m/e* 351 (M), 333 (M – H_2O), 323 (M – CO), 322, 308, 302, 292 (M – CO – CH_2OH), 264 (P + C_2H_4), 238 (P + 2 H), 196; IR (2000–1200-cm^{-1} region) 1650 s, 1645 sh, 1630 vs, 1600, 1565 sh, 1490 s, 1455, 1445, 1400, 1335 sh, 1325, 1310, 1285, 1270 sh, 1245, 1230. Anal. ($C_{10}H_{14}IN_3O_3 \cdot {}^1/_4H_2O$) C, H, N.

(±)-4-Amino-1-[(1α,2α,3β,4α)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]-5-iodo-2(1*H*)-pyrimidinone (4b). A solution of 214 mg (0.89 mmol) of *C*-ara-C, 20 mL of acetic acid, and 10 mL of pyridine was stirred at room temperature overnight. Iodine (214 mg, 0.84 mmol) was added, the red solution was stirred for 2 days at room temperature, a second portion of iodine (214 mg) was added, and stirring was continued for 3 days. The reaction solution was concentrated to dryness in vacuo, and several portions of ethanol were evaporated from the dark residue, which was then kept in a vacuum (0.1 mm Hg) for several hours. The black, gummy residue was dissolved in 25 mL of a 9:1 water–ethanol mixture, the solution was applied to a column of a cation-exchange resin (20 mL of Amberlite CG-120, H^+ form), the column was washed with the water–ethanol mixture until elution of iodine and UV-absorbing materials was complete, and the column was then washed with 1 N aqueous ammonia. Product-containing fractions (determined by monitoring the effluent at 280 nm) were combined and concentrated to a white waxy solid (115 mg) that was recrystallized from boiling water (3.5 mL). White crystals were collected by filtration, washed with cold water, and dried in vacuo at 56 °C: yield, 50 mg (15%); mp, darkens above 180 °C, 192–195 °C dec (capillary inserted at 100°, 3°/min); TLC, 1 spot (7:3 2-propanol–1 M NH_4OAc); UV max 223 (ε 13 800) and 312 (10 200) at pH 1, 222 (16 100) and 296 (7000) at pH 7, 223 (15 600) and 297 (7100) at pH 13; MS (FAB), *m/e* 368 (M + 1); IR (1800–1400-cm^{-1} region) 1660, 1630 sh, 1620 s, 1585, 1570, 1505, 1475 s, 1450, 1415, 1395 w, 1385, 1360 w, 1330, 1320, 1290, 1260, 1225, 1210. Anal. ($C_{10}H_{14}IN_3O_4 \cdot {}^1/_4H_2O$) C, H, N.

Antiviral Evaluations in Vitro. The compounds listed in Tables I and II were tested for selective inhibition of virus-induced cytopathogenic effects (CPE) produced by HSV-1 (strain E-377/TK^+ or strain HF/TK^-), HSV-2 (strain MS), or human influenza virus (type A_0/PR-8/34) in infected cell cultures. HSV-1 was propagated and assayed in continuous-passage human epidermoid carcinoma of the larynx (H.Ep.-2) cells, in continuous-passage African green monkey kidney (Vero) cells, in continuous-passage mouse (L-929) fibroblasts, or in primary rabbit kidney (PRK) cell cultures. HSV-2 was propagated and assayed either in Vero or in L-929 cells. Human influenza virus was propagated and assayed either in continuous-passage Madin–Darby canine kidney (MDCK) cells or in primary rhesus monkey kidney (RhMK) cell cultures.

The host cells were grown as monolayer cultures in 96-well plastic microtitration plates (Microtest II plates; Falcon Plastics, Oxnard, CA). The following cell-culture media were used in tests of compounds for inhibition of replication of HSV-1 or HSV-2: (a) Eagle's minimum essential medium (MEM) supplemented either with 5% calf serum for seeding and growth or with 2% fetal bovine serum for cell maintenance during assay (HEp-2, Vero, and PRK cells); (b) Eagle's MEM supplemented with 5% calf serum (L-929 cells). The following media (1–5 of Table II) were used in the evaluations of compounds for inhibition of influenza virus replication: (1) Eagle's basal medium (BME) reinforced with twice the usual concentration of each of the vitamins and amino acids[16] and supplemented with 2% fetal bovine serum; (2) reinforced BME + 5% fetal bovine serum; (3) MEM + 2% fetal bovine serum; (4) MEM + 4 times the usual concentrations of vitamins, 0.1% glucose, 0.1% bovine serum albumin, and 15 μg/mL of trypsin; (5) the same as (4) except for 10 μg/mL of trypsin.

For assay, the cell culture medium was removed from replicate, preformed cell monolayers, and to each of the triplicate cultures was added 0.1 mL of virus suspension and 0.1 mL of maintenance medium containing the appropriate concentration of test compound or, for virus controls, 0.1 mL of virus suspension and 0.1 mL of maintenance medium alone. Virus was diluted in medium to yield a final virus concentration of 32 cell culture infectious dose 50% ($CCID_{50}$) units per well. Seven different concentrations of each test compound were evaluated against the challenge virus, and these were selected to range, in serial half-log_{10} dilutions, from cytotoxic to noncytotoxic levels. Cytotoxicity controls and normal uninfected cell controls were included in each test. Either 9-β-D-arabinofuranosyladenine (ara-A) or 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin) was evaluated concomitantly for antiviral activity in the same manner as the test compounds, and these antiviral nucleosides served as positive control drugs for the inhibition of herpes simplex virus and human influenza virus replication, respectively. The microtitration plates were sealed and incubated at 37 °C for 4 or 5 days, depending on the virus strain, after which the cell monolayers were examined microscopically and the virus-induced CPE were scored on a scale from 0 (no CPE) to 4 (100% CPE). A virus rating (VR) was then calculated from the CPE scores by a modification of the method of Ehrlich et al.,[15] which has been described previously.[6,17] The ratio of the VR of the test compound to the VR of the positive control drug was calculated, and the minimum inhibitory concentration 50% (MIC_{50}), in μg/mL, was determined for each active compound.

Acknowledgment. This investigation was supported by Grants R01-CA25701 and P01-CA34200 from the National Institutes of Health, Public Health Service. We are indebted to Marion C. Kirk and Christine G. Richards for some of the spectroscopic determinations and for elemental analyses and to Beverly Washburn and Carol Eldridge for technical assistance with the antiviral evaluations.

Registry No. **1a**, 62805-43-0; **1b**, 102871-20-5; **1c**, 102871-21-6; **1d**, 102871-22-7; **2a**, 69975-21-9; **2b**, 102871-23-8; **2c**, 102871-24-9; **2d**, 102871-25-0; **3a**, 74560-43-3; **3b**, 102871-26-1; **4a**, 74958-34-2; **4b**, 103000-80-2.

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