Carbocyclic Analogues of 5′-Amino-5′-deoxy- and 3′-Amino-3′-deoxythymidines[1]

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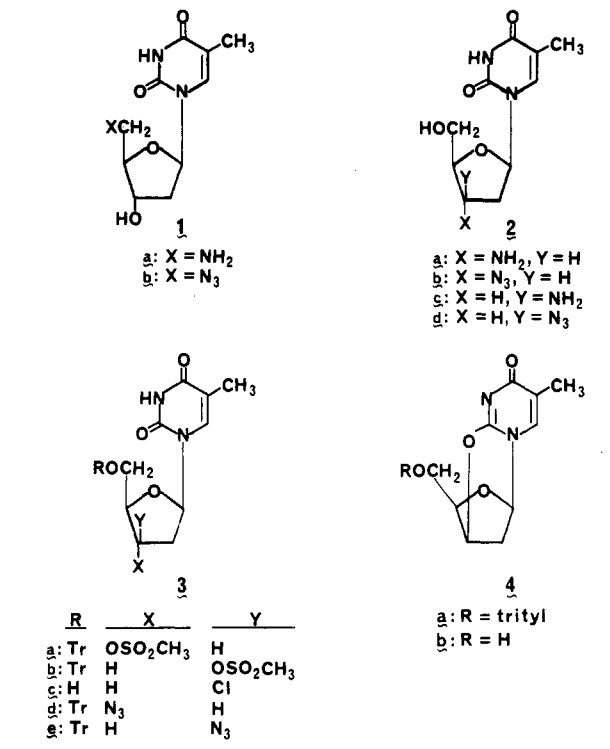
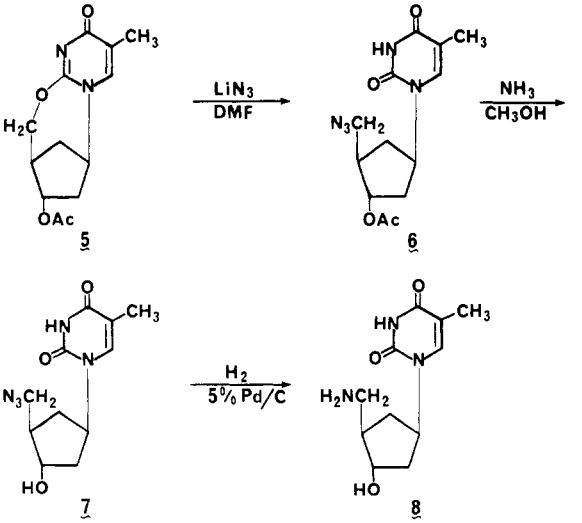
The carbocyclic analogue of 5′-amino-5′-deoxythymidine was synthesized from the carbocyclic analogue of 2,5′-*O*-anhydrothymidine acetate. The carbocyclic analogues of 3′-amino-3′-deoxythymidine and of 1-(3′-amino-2′,3′-dideoxylyxofuranosyl)thymine (an all-cis structure) were synthesized from the carbocyclic analogues of 5′-*O*-trityl-2,3′-*O*-anhydrothymidine and 5′-*O*-trityl-3′-*O*-(methylsulfonyl)thymidine, respectively. The carbocyclic analogue of 5′-amino-5′-deoxythymidine inhibited cytopathogenic effects (CPE) induced by a TK^+ strain of type 1 herpes simplex virus replicating in L929 (mouse connective tissue) cells, but it did not inhibit CPE in Vero cells. In contrast, the *all-cis*-3′-azido-3′-deoxythymidine analogue demonstrated modest inhibition of CPE in Vero cells, but not in L929 cells.

5′-Amino-5′-deoxythymidine (Chart I; **1a**, 5′-amino-5′-dThd) and the 5′-azido derivative (**1b**) were synthesized initially by Horwitz et al.[2] Subsequent studies have shown that 5′-amino-5′-dThd is a good inhibitor of thymidine kinase from tumor cells[3,4] and a weak inhibitor of thymidylate kinase;[5] that it is phosphorylated to the 5′-*N*-diphosphate in cells infected with type 1 herpes simplex virus (HSV-1), but not by a mixture of thymidine kinase and thymidylate kinase from uninfected mammalian cells;[6] and that its 5′-*N*-triphosphate is incorporated into DNA.[7] 5′-Amino-5′-dThd is a selective inhibitor of HSV-1 replication in cultured cells, but it is not an effective inhibitor of mouse neoplastic cells (L1210 leukemia and Sarcoma 180).[8,9] In contrast, 3′-amino-3′-deoxythymidine[10,11] (**2a**, 3′-amino-3′-dThd) has only slight antiviral activity,[9] but it has potent activity against L1210 leukemia,[9] sarcoma 180,[9] and P815 mouse leukemia[12] cells in culture (ED_{50} = 1 μM, 5 μM, 0.08 mcg/mL, respectively). In tests against L1210 leukemia in vivo, Lin, Fisher, and Prusoff[13] showed that 3′-amino-3′-dThd can increase markedly the survival times of treated mice, and Chen, Woods, and Prusoff[14] also presented evidence that inhibition of the DNA polymerase reaction is a major site of the inhibitory effects of 3′-amino-3′-dThd. In this report, we describe syntheses and initial biological evaluations of the carbocyclic analogues of 5′-amino-5′-dThd (**1a**), 3′-amino-3′-dThd (**2a**), the all-cis isomer (**2c**) of 3′-amino-3′-dThd, and the azido precursors (**1b**, **2b**, **2d**).

Chart I



Scheme I



Chemistry. 5′-Amino-5′-dThd (**1a**) has been synthesized by treating the unprotected 5′-(4-toluenesulfonate) with lithium azide in *N*,*N*-dimethylformamide[2,9] and then reducing the 5′-azido derivative (**1b**) with hydrogen[2,9] or

(1) The trivial name of a carbocyclic (cyclopentyl) analogue of a nucleoside is formed by prefixing *C*- to the trivial name of the corresponding nucleoside.

(2) Horwitz, J. P.; Thomson, A. J.; Urbanski; J. A.; Chua, J. *J. Org. Chem.* **1962**, *27*, 3045–3048.
(3) Neenan, J. P.; Rohde, W. *J. Med. Chem.* **1973**, *16*, 580–581.
(4) Cheng, Y.-C.; Prusoff, W. H. *Biochemistry* **1974**, *13*, 1179–1185.
(5) Cheng, Y.-C.; Prusoff, W. H. *Biochemistry* **1973**, *12*, 2612–2619.
(6) Chen, M. S.; Shiau, G. T.; Prusoff, W. H. *Antimicrob. Agents Chemother.* **1980**, *18*, 433–436.
(7) Letsinger, R. L.; Wilkes, J. S.; Dumas, L. B. *J. Am. Chem. Soc.* **1972**, *94*, 292–293.
(8) Lin, T.-S.; Neenan, J. P.; Cheng, Y.-C.; Prusoff, W. H. *J. Med. Chem.* **1976**, *19*, 495–498.
(9) Lin, T.-S.; Prusoff, W. H. *J. Med. Chem.* **1978**, *21*, 109–112.
(10) Miller, N.; Fox, J. J. *J. Org. Chem.* **1964**, *29*, 1772–1776.
(11) Horwitz, J. P.; Chua, J.; Noel, M. *J. Org. Chem.* **1964**, *29*, 2076–2078.
(12) Matsuda, A.; Watanabe, K. A.; Fox, J. J. *J. Org. Chem.* **1980**, *45*, 3274–3278.
(13) Lin, T.-S.; Fischer, P. H.; Prusoff, W. H. *Biochem. Pharmacol.* **1982**, *31*, 125–128.
(14) Chen, M. S.; Woods, K. L.; Prusoff, W. H. *Mol. Pharmacol.* **1984**, *25*, 441–445.

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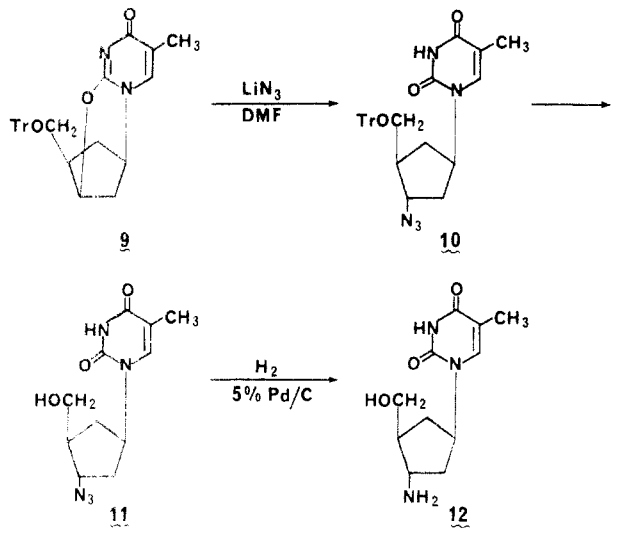
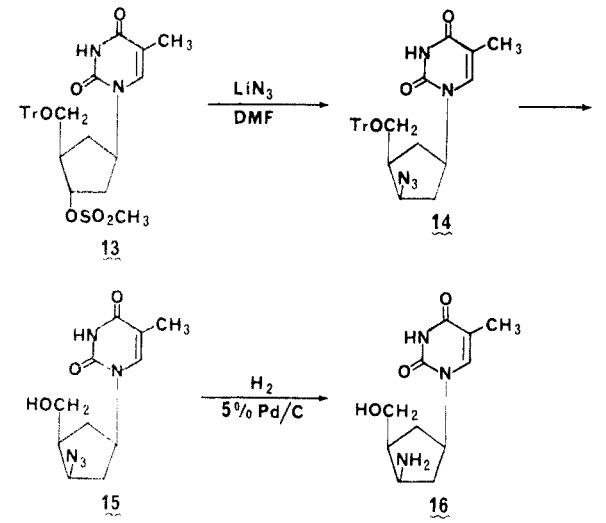
Scheme II



Scheme III

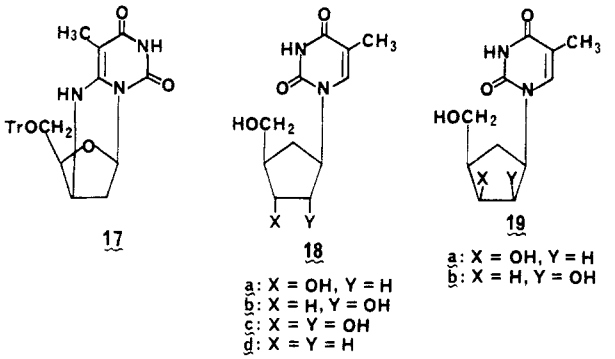


with triphenylphosphine.[15] Direct amination of radiolabeled thymidine 5′-(4-toluenesulfonate) furnished radioactive **1a**.[6] The synthesis route to *C*-5′-amino-5′-dThd (**8**) is outlined in Scheme I. The preparation of *C*-2,5′-*O*-anhydrothymidine acetate[16] (**5**) from *C*-thymidine[17,18] had been reported previously. Treatment of **5** with lithium azide in *N,N*-dimethylformamide afforded the azidomethyl derivative (**6**), and removal of the acetyl group and hydrogenolysis of the azido group by conventional procedures[2,8,9,11] furnished **8**. A precedent for the first step is the conversion of the isopropylidene derivative of 2,5′-*O*-anhydrouridine to the 5′-azido derivative.[2]

3′-Amino-3′-dThd (**2a**) has been synthesized from 5′-*O*-trityl-3′-(*O*-methylsulfonyl)thymidine (**3a**)—evidently through the intermediacy of the 2,3′-*O*-anhydro derivative (**4a**),[10,12] from isolated anhydro derivatives (**4a,b**),[19] and from 1-(5′-*O*-trityl-3′-(*O*-methylsulfonyl)-2′-deoxylyxofuranosyl)thymine (**3b**)[9,11] or the analogous *cis*-3′-chloro derivative (**3c**).[20] These syntheses proceeded through the 3′-azido derivatives (**2b, 3d**)[9,11,19,20] or the analogous 3′-phthalimido derivative.[10] The synthesis of the carbocyclic analogue (**12**) of 3′-amino-3′-dThd is summarized in Scheme II. The synthesis began with *C*-thymidine and proceeded via *C*-2,3′-*O*-anhydrothymidine (**9**), which had been reported previously;[16] the synthesis route (**9–12**) paralleled a synthesis[19] of the corresponding true nucleoside (**2a**).

The synthesis of *C*-1-(3′-amino-2′,3′-dideoxylyxofuranosyl)thymine (**16**), the all-cis isomer of **12**, is outlined in Scheme III. The *O*-trityl *O*-(methylsulfonyl) derivative (**13**) was prepared from *C*-thymidine as previously described;[16] the remainder of the synthesis is analogous to the synthesis of **12**.

Matsuda, Watanabe, and Fox[12] isolated both 3′-azido-3′-deoxy-5′-*O*-tritylthymidine (**3d**) and 6,3′-imino derivative **17** after treating the *trans*-methanesulfonate (**3a**) with



sodium azide in boiling dimethylformamide for 24 h. Azide **3d** must have been formed via 2,3′-O-anhydro derivative **4a**, and the 6,3′-imino derivative (**17**) was apparently formed in the reaction mixture from the expected all-cis azide derivative (**3e**). Under less strenuous conditions, they obtained the all-cis azide (**3e**) in 47% yield and showed that it could be converted to **17**. During their investigations of azidothymidines, these investigators also showed that 5′-azido-5′-dThd (**1b**) is converted to the analogous 6,5′-imino derivative in boiling DMF.[12]

Similar reactions of the corresponding carbocyclic analogues may also be expected to occur under suitable reaction conditions, but the following data show that the carbocyclic analogues that were isolated are represented correctly in Schemes I–III. The infrared spectra of all of the compounds (**6, 7, 10, 11, 14, 15**) depicted as azides in Schemes I–III, include strong bands at 2115–2100 cm^{-1}, owing to the presence of the azido group. Mass spectral data (e.g., the molecular ion) and elemental analytical data are also in agreement with azido, rather than imino, derivatives. Furthermore, ultraviolet spectra of azido derivatives **11** and **15** and of amino derivatives **8, 12,** and **16** determined at approximately pH 1, 7, and 13 had absorption maxima at 272–273 nm (ϵ 10 000–10 900), 271–273 (ϵ 10 200–11 000), and 271–272 (ϵ 8000–8400), respectively (Table II). These data are in agreement with corresponding ultraviolet data for *C*-thymidine (**18a**), its all-cis isomer (**19a**), and other carbocyclic analogues (**18b–d, 19b**) of thymidine nucleosides (Table II). Since trityl 3′-azido-3′-dThd (**3d**) was obtained via **4a** by treating the *O*-(methylsulfonyl)thymidine (**3a**) with sodium azide,[12] the structure of the azide obtained by treating methylsulfonyl

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(17) Shealy, Y. F.; O'Dell, C. A. *J. Heterocycl. Chem.* **1976**, *13*, 1041–1047.
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(19) Glinski, R. P.; Khan, M. S.; Kalamas, R. L.; Sporn, M. B. *J. Org. Chem.* **1973**, *38*, 4299–4305.
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Table I. Evaluation of Carbocyclic Analogues of Azido- and Amino(dideoxypentofuranosyl)thymines for Antiviral Activity[a]

	HSV-1, strain 377				other viruses			
compd	expt no.[b]	host cells[c]	VR	ratio VR/VR of *ara*-A[d]	expt no.[b]	virus[e]	host cells[c]	VR
C-5′-azido-5′-dThd (**7**)	1	L929	0.8	0.27	1	HSV-2 MS	L929	0
					6	HSV-1 HF	H.Ep.-2	0
C-5′-amino-5′-dThd (**8**)	1	L929	3.0	1	1	HSV-2 MS	L929	0.5[f]
	2	L929	1.8	1.28	6	HSV-1 HF	H.Ep.-2	0.2
	3	Vero	0					
	4	Vero	0					
C-3′-azido-3′-dThd (**11**)	5	Vero	0					
C-3′-amino-3′-dThd (**12**)	2	L929	0.9	0.9				
	2	Vero	0					
	5	Vero	0					
C-3′-azido-2′,3′-dideoxylyxo-Thy (**15**)	1	L929	0.2		1	HSV-2 MS	L 929	0
	2	L929	0					
	2	Vero	0.6	0.43				
	3	Vero	1.0	0.50				
	4	Vero	0.8	0.44				
C-3′-amino-2′,3′-dideoxylyxo-Thy (**16**)	2	L929	0.4	0.4				
	2	Vero	0					
	4	Vero	0					

[a] The antiviral activity of each compound is expressed as a virus rating (VR). The VR, determined by the general method of Ehrlich et al.,[21] 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. A 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. [b] Tests that were performed simultaneously can be identified by experiment number. [c] L929 cells are mouse connective tissue cells, clone L. Vero cells are African green monkey kidney cells. H.Ep.-2 cells are human epidermoid carcinoma cells, No. 2. [d] VR/VR of *ara*-A is the ratio of the VR of the thymidine analogue to the VR of *ara*-A (employed as a positive control) in the same experiment. [e] HSV-2 MS is strain MS (TK^+) of type 2 herpes simplex virus. HSV-1 HF is strain HF (TK^-) of type 1 herpes simplex virus. [f] VR/VR of *ara*-A is 0.36.

derivative **13** similarly with lithium azide in DMF might be **10** rather than the expected inversion product **14**. Under the conditions employed (100 °C, 20 h) for the reaction of **13** and lithium azide, a small amount (8% yield) of anhydro derivative **9** was isolated. However, the major product (55% yield) was the all-cis azido derivative (**14**); this assignment is confirmed by the fact that the proton NMR spectra (Table III) of the all-cis compounds **14–16** and of the deoxyribofuranoside analogues (**10–12**) clearly distinguish the former from the latter.

Biological Evaluation. The azido and amino derivatives of *C*-thymidine were evaluated for selective antiviral activity against DNA viruses by testing them for inhibition of the replication of type 1 herpes simplex virus (HSV-1) in host cells maintained in culture. A standard assay, which has been described previously,[21,22] for inhibition of virus-induced cytopathogenic effects was used to evaluate these compounds against HSV-1. In these experiments, 9-β-D-arabinofuranosyladenine (*ara*-A), an antiviral drug used in medical practice, was the positive control. The results of these tests, summarized in Table I, show that *C*-5′-amino-5′-dThd (**8**) displayed good activity in inhibiting the replication of strain 377 of HSV-1 in L929 cells and borderline activity against HSV-2. In two experiments with HSV-1 (377) in L929 cells, the ratios of the VR of **8** to the VR of *ara*-A were 1.0 and 1.28. However, **8** did not inhibit replication of strain 377 of HSV-1 in Vero cells or strain HF of HSV-1 in H.Ep.-2 cells. Strain 377 of HSV-1 and strain MS of HSV-2 induce virus-specific thymidine kinases, whereas strain HF of HSV-1 does not. Although **8** has good activity against HSV-1 (377) in L929 cells, it does not appear to be as active as some of the C-5-substituted 2′-deoxyuridines[23] or *C*-2′-deoxyribofuranosides[24] and C-ribofuranosides[25] of 2-amino-6-substituted-purines. Interestingly, *C*-1-(3′-azido-2′,3′-dideoxylyxofuranosyl)-thymine (**15**) showed modest activity vs. HSV-1 in Vero cells but did not inhibit replication of HSV-1 in L929 cells. None of the other amino and azido derivatives except for **12** showed selective activity in tests vs. strain 377 of HSV-1. Among these compounds, only azide **15** was tested against HSV-2; it was not active.

The differences in the effects of **8**, **12**, and **15** on the replication of HSV-1 (377) in different host cells are not without precedents. Variations in the effectiveness of several antiviral agents against a strain of HSV-1 or HSV-2 replicating in different cell lines have been reported. Cheng et al.[26] found that 5-ethyl-2′-deoxyuridine inhibits the replication of strain 333 of HSV-2 in HeLa cells, whereas De Clercq et al.[27] reported that this compound is not inhibitory to the same strain replicating in cultures of primary rabbit kidney cells or human skin fibroblasts. De Clercq[28] compared the activities of several well-known antiviral nucleosides against strain KOS of HSV-1 replicating in cultures of 12 different kinds of cells. The values of ED_{50} varied considerably among the different cells lines; one compound, 5-(bromoethenyl)-1-β-D-arabinofuranosyluracil, was highly active against HSV-1 KOS in several cell lines, but it was virtually inactive when the host cells were Vero or BS-C-1 cells. Also, Shealy et al. reported that *C*-thymidine was highly active against strain 377 of

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(22) Shannon, W. M.; Arnett, G.; Westbrook, L.; Shealy, Y. F.; O'Dell, C. A.; Brockman, R. W. *Antimicrob. Agents Chemother.* **1981**, *20*, 769–776.
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(24) Shealy, Y. F.; O'Dell, C. A.; Shannon, W. M.; Arnett, G. *J. Med. Chem.* **1984**, *27*, 1416–1421.
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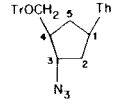
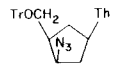
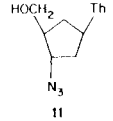
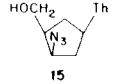
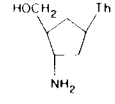
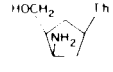
(27) De Clercq, E.; Krajewska, E.; Descamps, J.; Torrence, P. F. *Mol. Pharmacol.* **1977**, *13*, 980–984.
(28) De Clercq, E. *Antimicrob. Agents Chemother.* **1982**, *21*, 661–663.

Table II. Ultraviolet Absorption Data

compd	0.1 N HCl[a]		pH 7[b]		0.1 N NaOH[a]	
	λ_{max}, nm	ϵ	λ_{max}, nm	ϵ	λ_{max}, nm	ϵ
8	273	10000	273	10400	272	8100
11	273	10900	273	11000	271	8400
12	272	10000	273	10300	271	8000
15	273	10300	273	10400	271	8000
16	272	10300	271	10200	272	8100
18a–d[c]	273	10100–10400	273[d]	10100–10400	271–272	7900–8100
19a,b[e]	272–273	10000–10600	273	10200–10700	271	7900–8000

[a] See the Experimental Section. [b] Phosphate buffer. [c] Reference 17 for **18a**; ref 18 for **18b–d**. [d] UV of **18b** not determined at pH 7. [e] Reference 16 for **19b**; UV for **19a**[16] not reported previously.

Table III. Proton NMR Data for Carbocyclic Analogues of 3′-Azido- and 3′-Amino-2′,3′-(dideoxypentofuranosyl)thymines

compd	positions of protons: δ (multiplicity, no. of protons)									
	1	2	3	4	5	CH_2O	CH_3	pyrim C6	NH	other
 **10**	4.85 (m, 1 H)	1.96 (m, 1 H) 2.16 (m, 3 H)[a]	4.08 (m, 1 H)	2.16 (m, 3 H)[a]	1.56 (m, 1 H) 2.16 (m, 3 H)[a]	3.12 (d, 2 H)	1.76 (s, 3 H)	7.54 (s, 1 H)	11.22 (s)	7.33 (m, 15 H, tr)
 **14**	4.96 (m, 1 H)	1.71 (m, 1 H) 2.50 (m, 2 H)[b]	4.39 (m, 1 H)	2.50 (m, 2 H)[b]	1.36 (m, 1 H) 1.96 (m, 1 H)	3.02 (t, 1 H) 3.18 (m, 1 H)	1.73 (s, 3 H)	7.35 (m, 16 H)[c]	11.2 (s)	7.35 (m, 16 H, tr)[c]
 **11**	4.84 (m, 2 H)[e]	1.91 (m, 1 H) 2.10 (m, 3 H)[d]	4.06 (m, 1 H)	2.10 (m, 3 H)[d]	1.51 (m, 1 H) 2.10 (m, 3 H)[d]	3.48 (m, 2 H)	1.78 (s, 3 H)	7.57 (s, 1 H)	11.22 (s)	4.84 (m, 2 H, OH)[e]
 **15**	4.93 (m, 1 H)	1.75 (m, 1 H) 2.44 (m, 1 H)	4.23 (m, 1 H)	2.20 (m, 1 H)	1.46 (m, 1 H) 1.97 (m, 1 H)	3.55 (m, 2 H)	1.80 (s, 3 H)	7.47 (s, 1 H)	11.21 (s)	4.70 (t, 1 H, OH)
 **12**	4.96 (m, 1 H)	1.67 (m, 2 H)[f] 1.90 (m, 1 H)	3.18 (m, 1 H)	1.67 (m, 2 H)[f]	1.42 (m, 1 H) 2.01 (m, 1 H)	3.48 (m, 2 H)	1.78 (s, 3 H)	7.52 (s, 1 H)		
 **16**	4.88 (m, 1 H)	1.39 (m, 1 H) 2.24 (m, 1 H)	3.39 (m, 1 H)	1.90 (m, 2 H)[g]	1.54 (m, 1 H) 1.90 (m, 2 H)[g]	3.52 (m, 2 H)	1.78 (s, 3 H)	7.95 (s, 1 H)		

[a] Center of overlapping multiplets from protons at positions 2, 4, and 5. [b] Center of overlapping multiplets from protons at positions 2 and 4. [c] Center of overlapping multiplets from trityl and pyrimidine C6 protons. [d] Center of overlapping multiplets from protons at positions 2, 4, and 5. [e] Center of overlapping multiplets from protons at position 1 and OH of CH_2OH. [f] Center of overlapping multiplets from protons at positions 2 and 4. [g] Center of overlapping multiplets from protons at positions 4 and 5.

HSV-1 replicating in rabbit kidney cells,[23] but it was considerably less active against this strain in Vero cells.[29]

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; solutions for ultraviolet spectral determinations were prepared by diluting a 5-mL aliquot of a water or 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 MX-IE spectrometer from samples in potassium bromide disks (vs = very strong, s = strong, sh = shoulder). Mass spectral data (MS) were taken from low-resolution, electron-impact spectra determined at 70 eV unless indicated otherwise. The peaks listed are those arising from the molecular ion (M), those attributable to the loss of certain fragments (M minus a fragment), and some other prominent peaks. Fragments containing the complete thymine moiety may be designated Thy plus an atom or group. Proton NMR spectra (Table III) were determined at 300.64 MHz with a Nicolet 300 NB NMR spectrometer. The solvent was Me_2SO-d_6, and the internal standard was tetramethylsilane (s = singlet, m = multiplet, t = triplet). Thin-layer chromatography (TLC) was performed on plates of silica gel, and developed plates were examined by UV light (254 nm).

(±)-1-[(1α,3β,4α)-4-(Azidomethyl)-3-hydroxycyclopentyl]-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione (7). A solution of 200 mg (0.76 mmol) of **5**[16] and 121 mg (2.46 mmol) of lithium azide in 10 mL of dry dimethylformamide was heated at 100 °C in an atmosphere of dry nitrogen for 7 h. Volatile components were evaporated from the reaction mixture under reduced pressure, and the gummy residue was dissolved in methanol. The solution was applied to a preparative silica gel TLC plate, the plate was developed in 9:1 chloroform–methanol, and the product band was removed and extracted in a Soxhlet extractor with ethanol. The filtered extract was concentrated in vacuo to an orange, glassy residue: weight of **6**, 148 mg (64%); MS (direct-probe temperature 230 °C) *m/e* 307 (M), 279 (307–CO), 265 (307–N_3), 236 (M – CO – Ac), 220 (M – CO – OAc), 193, 192, 177, 153 (Thy + C_2H_4), 148, 147, 127 (Thy + 2 H), 126 (Thy + H); IR (strong and medium–strong bands, 2200–600-cm^{-1} region) 2100, 1735 sh, 1690 vs, 1470, 1450, 1370, 1270 sh, 1245 cm^{-1}.

A solution of 145 mg (0.47 mmol) of acetate **6** in 5 mL of ammonia–methanol (15% ammonia) was stirred at room temperature for 25 h and then concentrated to dryness under reduced pressure. The crude product was chromatographed in 95:5 chloroform–methanol (application and elution solvent) on a column of silica gel. Concentration of product-containing fractions (determined by TLC) afforded 104 mg (83%) of a colorless syrup. Attempts to crystallize this material from various solvents failed, and it was evaporated with several portions of ethanol: weight of **7**, 95 mg; TLC, 1 spot (60 mcg, 9:1 chloroform–methanol); IR (strong and medium–strong bands, 2200–600-cm^{-1} region) 2100 s, 1690 vs, 1475, 1275 cm^{-1}. Anal ($C_{11}H_{15}N_5O_3 \cdot {}^1/_8C_2H_5OH$) C, H, N.

(±)-1-[(1α,3β,4α)-4-(Aminomethyl)-3-hydroxycyclopentyl]-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione (8). A solution of 82 mg (0.31 mmol) of azide **7** in 10 mL of methanol that contained 25 mg of 5% palladium-on-charcoal was hydrogenated at atmospheric pressure and room temperature for 20 h. Hydrogen uptake could not be measured because reduction of the azide group resulted in evolution of nitrogen in the closed system. The catalyst was separated by filtration, and the combined filtrate and methanol washes were concentrated in vacuo to a syrupy residue that crystallized upon chilling. The residue was triturated with ethyl acetate, collected by filtration, washed with ethyl acetate, and dried in vacuo: yield of **8**, 42 mg (57% yield); mp, darkens above 170 °C, 186–189 °C dec (inserted at 100 °C, 3 °C/min); TLC, 1 spot (40 or 80 mcg applied, ethanol–concentrated ammonium hydroxide (4:1) as developing solvent); UV λ_{max} 211 (ε 8700) and 273 (ε 10 000) at pH 1, 209 (ε 9000) and 273 (ε 10 400) at pH 7, 272 (ε 8100) at pH 13; MS (direct-probe temperature 140 °C) *m/e* 239 (M), 222 (M – OH), 221 (M – H_2O), 210 (M – CH_2NH_2 + H), 193 (M – OH – CH_2NH_2 + H), 153 (Thy + C_2H_4), 127 (Thy + 2 H), 126 (Thy + H); IR (strong and medium–strong bands, 1800–600-cm^{-1} region) 1695 sh, 1665 s, 1615, 1500, 1475, 1465 sh, 1355, 1295, 1075, 1055, 910, 790 cm^{-1}. Anal ($C_{11}H_{17}N_3O_3 \cdot {}^1/_2CH_3OH$) C, H, N.

(±)-1-[(1α,3β,4α)-3-Azido-4-[(triphenylmethoxy)methyl]cyclopentyl]-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione (10). A solution of 1.40 g (3.01 mmol) of anhydrothymidine **9**[16] and 480 mg (9.80 mmol) of lithium azide in 72 mL of dry dimethylformamide was heated at 100 °C under an atmosphere of nitrogen for 72 h. An additional 480 mg (9.80 mmol) of lithium azide was added, and heating was continued for 7 days. Thin-layer chromatography indicated that reaction was complete, and the reaction mixture was cooled and filtered. The combined filtrate and dimethylformamide washes were evaporated in vacuo to dryness, and the residue was extracted with 50 mL of 98:2 chloroform–methanol. The mixture was filtered to remove inorganics, and the combined filtrate and washes were concentrated to dryness in vacuo. The residue was triturated with ether (25 mL), and the white solid that separated was filtered away, washed thoroughly with ether, and dried in vacuo at 56 °C: yield, 1.47 g (96%); mp 200–205 °C dec (inserted at 180 °C, 3 °C/min). Additional **10** (55 mg (4%)) was obtained by concentrating the first-crop filtrate to dryness and triturating the residue with methanol. The crops were combined and recrystallized from 55 mL of boiling methanol: weight, 975 mg (64% recovery); mp 205–207 °C dec (inserted at 180 °C, 3 °C/min): TLC, 1 spot (20 or 40 mcg, 95:5 chloroform–methanol as developing solvent); MS (field desorption; 13 mA; solvent Me_2SO) *m/e* 507 (M); IR (strong and medium–strong bands, 2200–600-cm^{-1} region) 2105 s, 1685 vs, 1650 s, 1450, 1255, 710 cm^{-1}. Anal ($C_{30}H_{29}N_5O_3$) C, H, N.

A second crop (240 mg, 16% recovery) was obtained by concentrating the filtrate to about one-fourth of its original volume: mp 203–205 °C dec (inserted at 180 °C, 3 °C/min). Compound **10** can also be purified by chromatography on a column of silica gel with chloroform–methanol (98:2) as eluting solvent.

(±)-1-[(1α,3β,4α)-3-Azido-4-(hydroxymethyl)cyclopentyl]-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione (11). A solution of 1.21 g (2.38 mmol) of azide **10** in 50 mL of 80% acetic acid was heated at reflux for ${}^1/_4$ h. The solution was concentrated in vacuo to remove volatiles, and the residue was extracted with 25 mL of water. The insoluble portion (triphenylmethanol) was filtered away, and the combined filtrate and water washes were evaporated in vacuo, yielding 570 mg of a viscous residue. The crude product was chromatographed in 95:5 chloroform–methanol on a column of silica gel. Fractions of the eluate containing **11** (determined by TLC) were combined and concentrated in vacuo to dryness. Trituration of the residue with ether afforded white crystals: yield, 472 mg (75%); mp 154–157 °C (inserted at 100 °C, 3 °C/min); TLC, 1 spot (40 or 80 mcg, 9:1 chloroform–methanol as developing solvent); UV λ_{max} 211 nm (ε 9700) and 273 (ε 10 900) at pH 1, 210 (ε 10 000) and 273 (ε 11 000) at pH 7, 271 (ε 8400) at pH 13; MS (direct-probe temperature, 150 °C) *m/e* 265 (M), 237 (M – N_2), 219 (M – N_2 – H_2O), 193, 178, 153 (Thy + C_2H_4), 127 (Thy + 2 H), 126 (Thy + H); IR (strong and medium–strong bands, 2200–600-cm^{-1} region) 2115 s, 1685 s, 1670 s, 1645, 1300, 1275, 1055, 585 cm^{-1}. Anal ($C_{11}H_{15}N_5O_3 \cdot {}^1/_4CH_3OH$) C, H, N.

(±)-1-[(1α,3β,4α)-3-Amino-4-(hydroxymethyl)cyclopentyl]-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione (12). Compound **12** was prepared, by the procedure described for the preparation of **8**, by catalytic hydrogenation of **11** (230 mg, 0.87 mmol) on 5% palladium–charcoal (60 mg) in methanol (20 mL). The residue that remained after concentration in vacuo crystallized when triturated with ethyl acetate. White, crystalline **12** was collected by filtration, washed with ethyl acetate, and dried in vacuo: yield 178 mg (86%); mp 188–192 °C dec (inserted at 100 °C, 3 °C/min); TLC, 1 spot (40 and 80 mcg applied, 7:3 2-propanol–1 M ammonium acetate as developing solvent); UV λ_{max} 272 (ε 10 000) at pH 1, 273 (ε 10 300) at pH 7, 271 (ε 8000) at pH 13; MS (direct-probe temperature, 20 °C) *m/e* 239 (M), 196, 127 (Thy + 2 H), 126 (Thy + H); IR (strong and medium–strong bands, 1800–600-cm^{-1} region) 1685 vs, 1470, 1455, 1445, 1385, 1365,

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1280, 1265, 905, 760 sh, 755, 480, 410 cm^{-1}. Anal ($C_{11}H_{17}N_3O_3 \cdot {}^1/_4H_2O$) C, H, N.

(±)-1-[(1α,3α,4α)-3-Azido-4-[(triphenylmethoxy)methyl]cyclopentyl]-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione (14). A solution of 2.42 g (4.32 mmol) of mesylate **13**[16] and 0.528 g (10.8 mmol) of lithium azide in 120 mL of dry dimethylformamide was heated at 100 °C for 20 h. The reaction mixture was concentrated in vacuo to dryness and thoroughly dried at high vacuum. The residue was dissolved in 5 mL of 95:5 chloroform–methanol and filtered to remove inorganics, and the filtrate was applied to a column containing 90 g of silica gel. The column was eluted with 95:5 chloroform–methanol, and product-containing fractions (determined by TLC) were combined and concentrated in vacuo to dryness. The residue crystallized when triturated with methanol, and **14** was collected by filtration, washed with cold methanol, and dried in vacuo: yield, 982 mg (45%); mp 210–212 °C (inserted at 110 °C, 3 °C/min); TLC, 1 spot (40 mcg applied, 95:5 chloroform–methanol); MS (direct-probe temperature, 250 °C) *m/e* 507 (M), 479 (M – N_2), 448, 447, 429, 401, 326, 264 (M – CPh_3), 260, 248 (M – $OCPh_3$), 423 (CPh_3), 183, 165, 127 (Thy + 2 H), 126 (Thy + H); IR (strong and medium-strong bands, 2200–800-cm^{-1} region) 2105, 1695, 1680, 1645, 1470, 1445, 1270, 1065 cm^{-1}. Anal. ($C_{30}H_{29}N_5O_3$) C, H, N. The filtrate deposited additional crystalline **14** upon standing: weight, 228 mg (10%); mp 206–210 °C (inserted at 100 °C, 3 °C/min).

Concentration of later fractions from the silica gel column described in this procedure and crystallization of the crude residue in methanol afforded an 8% yield of **9**: mp 250–252 °C (inserted at 110 °C, 3 °C/min); TLC, 1 spot identical with authentic **9**[16] (40 and 80 mcg applied, 95:5 chloroform–methanol); MS (direct-probe temperature 260 °C) *m/e* 464 (M), 387 (M – Ph), 243 (CPh_3), 221 (M – CPh_3).

(±)-1-[(1α,3α,4α)-3-Azido-4-(hydroxymethyl)cyclopentyl]-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione (15). Azide **14** (450 mg, 0.89 mmol) was treated with 80% acetic acid (20 mL) and was purified according to the procedure described for the preparation of **11**. The glassy residue obtained by concentrating column fractions that contained product (determined by TLC) crystallized when triturated with ethyl acetate: yield of **15**, 178 mg (76%); mp 150–152 °C (inserted at 100 °C, 3 °C/min); UV λ_{max} 273 nm (ε 10300) at pH 1, 273 (ε 10400) at pH 7, 271 (ε 8000) at pH 13; MS (direct-probe temperature, 20 °C) *m/e* 266 (M + 1), 265 (M), 193, 180, 127 (Thy + 2 H), 126 (Thy + H); IR (strong and medium-strong bands, 2200–700-cm^{-1} region) 2110 s, 1705 s, 1680 sh, 1665 vs, 1340, 1280, 1265, 1025 cm^{-1}. Anal. ($C_{11}H_{15}N_5O_3$) C, H, N.

(±)-1-[(1α,3α,4α)-3-Amino-4-(hydroxymethyl)cyclopentyl]-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione (16). Compound **16** was prepared from **15** (125 mg, 0.47 mmol) and purified by the procedure described for the preparation of **8**. The residual colorless glass crystallized upon trituration with ethyl acetate and was filtered away, washed with ethyl acetate, and dried in vacuo at 56 °C for 2 h: yield of white crystals, 90 mg (80%); mp, sinters 162 °C, melts 165–168 °C with mild dec (inserted at 100 °C, 3 °C/min); TLC, 1 spot (40 or 80 mcg applied, 7:3 2-propanol–1 M ammonium acetate as developing solvent); UV λ_{max} 272 nm (ε 10300) at pH 1, 271 (ε 10200) at pH 7, 272 (ε 8100) at pH 13; MS (FABMS) *m/e* 240 (M + 1); IR (strong and medium-strong bands, 1800–400-cm^{-1} region) 1680, 1665 sh, 1640 sh, 1605 sh, 1370, 1290, 1075, 760, 585, 425 cm^{-1}. Anal ($C_{11}H_{17}N_3O_3$) C, H, N.

Antiviral Evaluations in Vitro. The compounds listed in Table I were tested for inhibition of the cytopathogenic effects produced by strain 377 (TK$^+$) or strain HF (TK$^-$) of HSV-1 or strain MS of HSV-2. The data summarized in Table I were acquired by methods and procedures described previously for the evaluation of compounds for antiviral activity in vitro.[22] The general assay method was described by Ehrlich et al.,[21] but some modifications were incorporated.

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Registry No. **5**, 87491-47-2; **6**, 100020-47-1; **7**, 100020-48-2; **8**, 100020-49-3; **9**, 87470-97-1; **10**, 100020-50-6; **11**, 100020-51-7; **12**, 100020-52-8; **13**, 87470-96-0; **14**, 100101-39-1; **15**, 100101-40-4; **16**, 100101-41-5.

Synthesis, Structure, and Antitumor and Antiviral Activities of a Series of 5-Halouridine Cyclic 3′,5′-Monophosphates

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A series of potential prodrug 5-halouridine 3′,5′-cyclic monophosphates (5-X-cUMPs, X = F, Cl, Br, I, **1–4**) has been prepared and tested for antitumor activity against murine leukemia L1210/0 and human lymphoblast Raji/0 cells and their deoxythymidine kinase deficient (TK$^-$) counterparts, as well as for antiviral activity in primary rabbit kidney cells infected with herpes simplex virus type 1 or 2, vaccinia virus, or vesicular stomatitis virus. The 5-halopyrimidine bases, nucleosides (5-X-U), and 5′-monophosphates (5-X-UMP) were tested for comparison. 5-F-cUMP (**1**) showed reasonably potent inhibition of tumor cell proliferation (ID_{50} = 0.33–1.6 μg/mL), while the remaining diesters displayed ID_{50}'s ranging from 210 to >1000 μg/mL. 5-F-cUMP was 70- to 300-fold less active than 5-F-dU in the same systems. With TK$^-$ L1210 cells, 5-F-cUMP was as potent as with the normal (L1210/0) line but was about fourfold less active with TK$^-$ Raji cells compared to Raji/0 cells. The 5-X-cUMPs showed little potency as antivirals. A single-crystal X-ray analysis of the ammonium salt of 5-I-cUMP confirmed its structure and showed the conformation of the phosphate ring to be the expected chair. The ribose pucker is near $^3_4$T, and the torsion angle about the β-glycosidic N(1)–C(1′) bond is in the syn range (–84.8°).

5-Fluorouracil (5-F-Ura) and 5-fluoro-2′-deoxyuridine (5-F-dU) are clinically useful antitumor agents.[1,2] 5-F-dU also shows highly selective anti-herpes activity (HSV-1) in cell cultures.[3] 5-Fluorouridine (5-FU) is an antitumor

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