Synthesis and Antitumor and Antiviral Properties of 5-Halo- and 5-(Trifluoromethyl)-2′-deoxyuridine 3′,5′-Cyclic Monophosphates and Neutral Triesters

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The title diesters (11–15; halo substituents F, Cl, Br, I) were prepared by DCC-induced cyclization of the precursor 5′-monophosphate or direct halogenation of the 2′-deoxyuridine 3′,5′-cyclic monophosphate. Antitumor activities of 11–15 in cell systems (L1210 and Raji/0) were compared to those of the corresponding nucleosides and 5′-monophosphates. Thus, the 5-F- and 5-CF_3-2′-deoxyuridines proved to be highly active derivatives [ID_{50} values (μg/mL) for L1210, 0.002 and 0.06, respectively], with the 5′-monophosphates showing comparable potencies. The corresponding 3′,5′-cyclic monophosphate diesters were 20–30 times less potent but nonetheless highly cytostatic. All derivatives including 11–15 had greatly increased ID_{50} values for the thymidine kinase deficient (TK^-) L1210 and Raji cells. The 3′,5′-cyclic diesters (11–15) evidently are not efficient prodrug sources of the nucleoside 5′-monophosphates in TK^- cells. They also proved to be 100- to 2000-fold less efficient inhibitors of L1210 thymidylate synthetase than were the 5′-monophosphates. The 5-substituted 2′-deoxyuridines and their 5′-monophosphates were potent inhibitors of herpes simplex virus (MIC_{50} mostly 0.07–10 μg/mL) and vaccinia virus (MIC_{50} 0.07–0.2 μg/mL), with antiviral activity decreasing in the order 5-I, 5-Br > 5-CF_3 > 5-Cl > 5-F. The 3′,5′-cyclic monophosphates (11–15) were for the most part 10- to 40-fold less active than the 5′-monophosphates in the virus assay systems (e.g., MIC_{50} for the 5-Br and 5-I derivatives ranged 1–20 μg/mL). By contrast 11–15 were considerably more potent inhibitors of vaccinia virus growth (MIC_{50} 0.4–2 μg/mL). As the neutral 3′,5′-cyclic methyl phosphate triesters (16–18), the 5-I and 5-Br compounds were less potent in antiviral and cytostatic agents than the 3′,5′-cyclic diesters, while the 5-iodo benzyl triester was in several cases as active as the 3′,5′-cyclic diester. The title compounds (11–15) appear to require extracellular hydrolysis to the nucleoside before functioning as antitumor or antiviral agents.

5-X-2′-Deoxyuridines (5-X-dUrd), where X is an electronegative substituent such as F, Cl, Br, CF_3, NO_2, CHO, or C≡CH, are nucleoside analogues that have received widespread recent study as potential antiviral[1] and antitumor[1c,2] agents. These analogues are highly cytostatic toward L1210 leukemia cells and show potent, but often unselective, antiviral activity.[3] Nonetheless, the 5-CF_3 (trifluridine)[4] and 5-I (idoxuridine) compounds are used clinically, usually in topical applications.[1] 5-F-dUrd is a clinically useful antitumor agent[2d,5] that, unfortunately, suffers from toxic side effects, e.g. on bone marrow cells, and also is readily degraded by thymidine phosphorylase to 5-fluorouracil.[6] Recent attempts at prodrug delivery of 5-F-dUrd as a neutral 5′-phosphate triester,[7,8] phosphoramidate,[8] or monocharged 5′-diester[9] have met with variable success. Prodrug 5-F-Ura derivatives of increased selectivity such as ftorafur[10] and 5′-deoxy-5-fluoro-2′-deoxyuridine also have been prepared.[11] The 5-X-dUrd's typically require thymidine kinase in the virus-infected or cancer cell for phosphorylation at the 5′-position,[1,2] after which they act as thymidylate synthetase inhibitors,[12] interfere with DNA polymerase, or are incorporated into DNA. Mutant virus strains or tumor cells may display resistance to 5-X-dUrd's by virtue of an altered or absent thymidine kinase activity.[13]

In the present research we have attempted to overcome these problems through the synthesis of 5-X-2′-deoxyuridine 3′,5′-cyclic monophosphates, 5-X-cdUMP's (11–15; X = F, Cl, Br, I, CF_3), as potential prodrug sources of 5-X-dUMP's or the corresponding 5-X-uracils. The antiviral and antitumor activities of 11–15 in cell systems were compared to those of the corresponding nucleosides and 5′-monophosphates (X = F, Br, I, CF_3). In addition, certain neutral 3′,5′-cyclic monophosphate triesters, 16–18, of the 5-iodo- and 5-bromo-2′-deoxyuridines were tested. Inhibition of L1210 thymidylate synthetase by the 5′-monophosphates and corresponding 3′,5′-cyclic mono-

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Table I. Synthetic Data for 5-Halo- and 5-(Trifluoromethyl)-2′-deoxyuridine 3′,5′-Cyclic Monophosphates 11–15

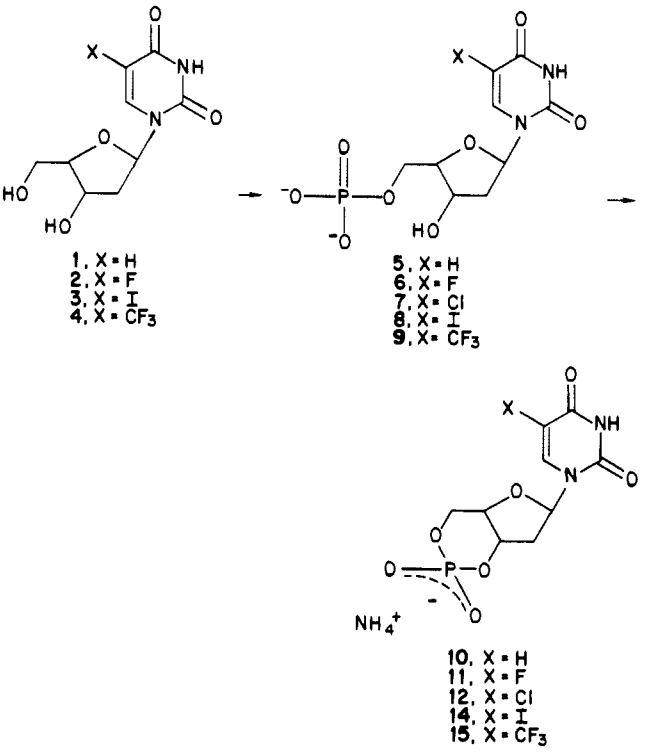
compd	5-X	formula	anal.[a]	% yields[b]	R_f[e] 1[g]	R_f[e] 2
11	F	$C_9H_{13}FN_3O_7P$	C, H, F, N, P	70	0.41 0.43[f]	0.51
12	Cl	$C_9H_{13}ClN_3O_7P$	C, H, Cl, N, P	43 88[c]	0.45 0.47[f]	0.51
13	Br	$C_9H_{13}BrN_3O_7P$	C, H, Br, N, P	83[c]	0.47 0.48[f]	0.51
14	I	$C_9H_{13}IN_3O_7P$	C, H, I, N, P	68	0.48 0.51[f]	0.51
15	CF_3	$C_{10}H_{13}F_3N_3O_7P$	C, H, F, N, P	50[d]	0.54 0.66[f]	0.58

[a] Found = calcd ± 0.4%. [b] Isolated yields based on ring closure of the corresponding 5′-monophosphates, unless otherwise indicated. [c] Direct halogenation. [d] 5′-Mononucleotide contaminated by the 3′-isomer (ca. 20%). [e] On silica gel TLC sheets, unless otherwise specified. [f] On cellulose TLC plates. [g] Solvent systems (see the Experimental Section).

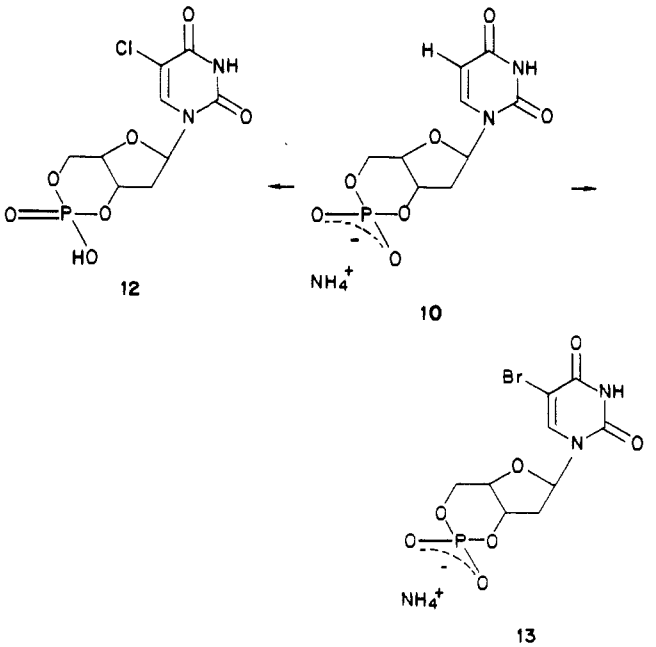
phosphates (11–15) was also investigated.

The drug design rationale for these studies is straightforward. Cyclic diesters 11–15 may be able to be transported through cell membranes[14] and then undergo hydrolysis to the 5′-monophosphate. Certain recently discovered cyclic nucleotide phosphodiesterases which have been recently found to hydrolyze pyrimidine (cytidine and uridine) cyclic 3′,5′-monophosphates[15] are potential catalysts of this reaction. Disrupted L1210 leukemia cells indeed display pyrimidine phosphodiesterase activity.[15] Selectivity would be based on different modifications or distributions of this enzyme in virus-infected or malignant cells vs. normal cells. Moreover, interaction with thymidine phosphorylase[16] might convert 11–15, or their 5′-monophosphate hydrolysis products, into the pyrimidine base that could then become involved in DNA synthesis via uridine phosphorylase or orotate phosphoribosyltransferase.[17] Either route would avoid the necessity of phosphorylation by thymidine kinase (TK) to activate the nucleoside in TK$^-$-deficient, drug-resistant tumor cells or virus-infected cells. The neutral phosphate triesters similar to 16–18 are readily transported into cells,[18] although they

Scheme I



Scheme II



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require noncatalystic hydrolytic conversion to a diester form before being potentially subject to PDE-catalyzed hydrolysis.

Results and Discussion

Chemistry. Nucleoside 3′,5′-cyclic monophosphates **11**, **12**, **14**, and **15** were prepared by cyclization of the *N*,*N*′-dicyclohexyl-4-morpholinecarboxamidine salt of the corresponding 5′-monophosphates **6**–**9** under the influence of dicyclohexylcarbodiimide, DCC (Scheme I).[19] Cyclic nucleotide **12** also was made by direct Cl_2 chlorination of 2′-deoxyuridine 3′,5′-cyclic monophosphate, **10**, prepared according to the literature (Scheme II).[19] *N*-Bromosuccinimide (NBS) bromination of **10** afforded **13**. The direct bromination of **5**[20] and of uridine 3′,5′-cyclic monophosphate[21] by NBS had been reported earlier. Yields of **11**–**15**, based on precursor 5′-monophosphates **6**–**9** and the 3′,5′-cyclic phosphate **10**, were 43–88% (Table I). Attempted direct iodination of **10** using I_2/HNO_3, a procedure successful for the preparation of **3**,[22] failed as a result of cleavage of the glycosidic bond under the reaction conditions.

Precursor 5′-monophosphates **6**, **8**, and **9** resulted from Yoshikawa phosphorylation[23] of the requisite 2′-deoxyribonucleosides **2**–**4** in 35–56% yields. 5′-Monophosphate **9** was contaminated by lesser amounts of the 3′-monophosphate (see Experimental Section), a not uncommon occurrence,[24] which was of little importance in this work since both monophosphates yield **15** on DCC cyclization. Direct chlorination of **5** (Cl_2 in CCl_4) led to the precursor 5-chloro-2′-deoxyuridine 5′-monophosphate (**7**; 83% yield). This halogenation is closely parallel to that previously demonstrated for the preparation of 5-chlorouridine 5′-monophosphate from uridine 5′-monophosphate.[25]

The formation of both the 5′- and 3′-monophosphates from phosphorylation of **4** was evidenced by the ^{13}C NMR spectrum of the product monophosphate fractions isolated. The major component, **9**, showed $^3J_{PC}$ of 8.1 Hz for C4′ and $^2J_{PC}$ of 4.8 Hz for C5′ at reasonable δ values for the respective carbons.[26] The C3′ chemical shift of the minor component was 2.5 ppm downfield relative to that of its 5′-substituted counterpart, while C5′ was shifted 4.0 ppm upfield. Although the ^{31}P signals of the monophosphate mixture could not be separated at 121.5 MHz, the 5′-monophosphate/3′-monophosphate ratio was readily found to be 4/1 by integration of the well-separated ^1H NMR signals for H6 at δ 8.71 (3′-monophosphate) and δ 8.51 (5′-monophosphate). This mixture of isomers was used in the biological testing of the monophosphate of **4** (Tables III–V).

Cyclic diesters **11**–**15** were isolated by anion-exchange chromatography on DEAE Sephadex A-25 (HCO_3^-) under conditions that failed to separate the starting 5′-monophosphate from the 3′,5′-cyclic monophosphates. Hence,

Table II. Proton-Decoupled Carbon-13 NMR Data for 11–15 in D_2O

	nitrogen base							deoxyribose ring								
	chem shift[e]					coupling const[d]		δ[e]					coupling const[d]			
compd	C2	C4	C5	C6	CF_3			C1′	C2′	C3′	C4′	C5′	$^3J_{PC2'}$	$^2J_{PC3'}$	$^3J_{PC4'}$	$^2J_{PC5'}$
11[a]	150.49	160.00	140.20	126.86		25.9 ($^2J_{FC4}$)	232.6 ($^1J_{FC5}$)	86.64	35.52	76.77	75.79	67.90	8.2	4.4	4.7	7.2
12[a]	151.45	162.56	*c*	139.88				87.21	35.93	77.03	76.19	68.17	8.3	4.3	4.9	7.2
13[a]	151.21	162.33	97.55	142.02				86.71	35.47	76.61	75.70	67.79	8.5	4.3	4.8	7.2
14[a]	152.03	163.90	69.77	147.46				87.14	35.94	77.11	76.14	68.26	8.5	4.4	4.2	7.3
15[b,f]	153.30	164.38	107.36	146.23	124.82	33.8 ^2J(FC5)	6.5 ^3J(FC6)	89.93	37.45	78.59	77.91	69.80	8.4	4.4	4.7	7.2

[a] At 25.2 MHz. [b] At 75.5 MHz. [c] Not observed. [d] In Hz. [e] In ppm. [f] Relative to external $Me_3SiCH_2CH_2CO_2Na$.

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Table III. Cytostatic Activity of the 5-Halo- and 5-(Trifluoromethyl)-dUrd, -dUMP, and -cdUMP Analogues

compd	ID_{50},[a] μg/mL L1210/0[b]	L1210/BdUrd[c]	Raji/0[d]	Raji/TK⁻[e]
5-fluoro-dUrd (2)	0.002	1.80	0.004	1.55
5-chloro-dUrd	21.3	339	8.54	>1000
5-bromo-dUrd	33.8	>1000	17.5	>1000
5-iodo-dUrd (3)	16	>500	8.0	>500
5-(trifluoromethyl)-dUrd (4)	0.006	47	0.150	180
5-fluoro-dUMP (6)	0.003	3.0	0.013	2.41
5-bromo-dUMP	34.6	457	26	>1000
5-iodo-dUMP (8)	31	>500	6.8	>500
5-(trifluoromethyl)-dUMP (9)	0.017	38	0.169	212
5-fluoro-cdUMP (11)	0.060	7.4	0.164	19
5-chloro-cdUMP (12)	337	>1000	337	>1000
5-bromo-cdUMP (13)	457	>1000	224	>1000
5-iodo-cdUMP (14)	≥500	>500	54	>500
5-(trifluoromethyl)-cdUMP (15)	0.116	22	1.81	≥500

[a] Inhibitory dose-50 or dose required to inhibit tumor cell proliferation by 50%. [b] Murine leukemia L1210 cells, designated L1210/0. [c] L1210/BdUrd is a mutant murine leukemia L1210 cell line, selected from the parental L1210/0 cell line by its ability to grow in the presence of 260 μg/mL of 5-bromo-2′-deoxyuridine (BdUrd). This cell line is deficient in thymidine kinase activity.[12b] [d] Human lymphoblast Raji cells, designated Raji/0. [e] Thymidine kinase deficient Raji cell line, designated Raji/TK⁻.[12b]

Table IV. Inhibition of L1210 dTMP Synthetase by 5-Halo- and 5-(Trifluoromethyl)-dUMP and -cdUMP Analogues

compd	K_i/K_m[a]
5-fluoro-dUMP (6)	0.018
5-fluoro-cdUMP (11)	38.0
5-chloro-cdUMP (12)	>356
5-bromo-dUMP	2.16
5-bromo-cdUMP (13)	>354
5-iodo-dUMP (8)	3.54
5-iodo-cdUMP (14)	>355
5-(trifluoromethyl)-dUMP (9)	0.061
5-(trifluoromethyl)-cdUMP (15)	30.4

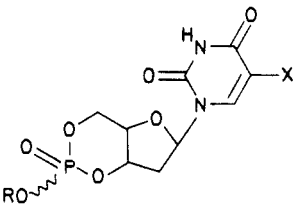
[a] The average K_m value was 1.28 μM. The inhibition was competitive with respect to dUMP for all dUMP and cdUMP analogues tested.

complete reaction of the 5′-monophosphate during DCC cyclization was a necessity.

^{13}C NMR spectroscopy (Table II) fully identified products **11–15**. Chemical shift values for the phosphate and 2′-deoxyribose rings are completely consistent with those noted for other nucleoside 3′,5′-cyclic monophosphates.[27] The C3′ and C4′ chemical shifts given correspond to the recently published revised assignments of these carbons.[28] Particularly diagnostic of the presence of the phosphate ring are the phosphorus–carbon coupling constants displayed by C2′, C3′, C4′, and C5′. The C5 shieldings of **11–14** are consistent with the presence of the halogen substitution. An X-ray study of the structure of **11** was recently reported.[29]

^1H NMR spectra of **12**, **13**, and **15** confirmed the 5-substitution as evidenced by the presence of sharp singlet at δ 7.62–8.22.[30] The β-anomeric configuration gave rise to the expected[31] doublet of doublet pattern at δ 6.10–6.37. Mass spectrometry also supported the structure of **15**. Acid hydrolysis of **11** and **14**, to give the corresponding base (TLC), showed them to be more stable than the cyclic 3′,5′-monophosphates based on thymidine[19] and a series of 5-alkyl-2′-deoxyuridines.[32] The bases appeared within 1–1.5 h. The reactants were half-consumed in 11–13 h and completely consumed within ca. 22–25 h.

The preparation of the neutral monophosphate triesters, **16–18**, from reaction of the silver salts of the precursor 3′,5′-cyclic diester with CH_3I or $PhCH_2I$ was reported earlier.[33]



16: X = Br, R = CH_3
17: X = I, R = CH_3
18: X = I, R = CH_2Ph

Cytostatic Activity. Among the 5-substituted 2′-deoxyuridine (dUrd) derivatives tested for their inhibitory effects on L1210/0 and Raji/0 cell growth, 5-F-dUrd (**2**) and 5-CF_3-dUrd (**4**) were by far the most active. These compounds were about 2000–20 000 times more inhibitory to L1210 cell growth and 50–2000 times more inhibitory to Raji cell growth than 5-Cl-dUrd, 5-Br-dUrd, and 5-I-dUrd (**3**) (Table III). While the 5-halo- and 5-CF_3-dUMP's showed similar cytostatic effects against L1210 and Raji cell proliferation as compared to their dUrd counterparts, the corresponding cdUMP derivatives were generally less inhibitory. *5-CF_3-cdUMP and 5-F-cdUMP, nonetheless, were highly potent inhibitors of cell proliferation* (ID_{50} 0.06–1.8 μg/mL).

For all compounds, the ID_{50} values for the thymidine kinase deficient L1210/BdUrd and Raji/TK⁻ cells were significantly higher than the ID_{50} values for the wild-type parental cell lines, and for the most active compounds, namely 5-F- and 5-CF_3-dUrd, -dUMP, and -cdUMP, the difference in ID_{50} was about 100–8000-fold. Thus, the cell growth inhibitory effect of these compounds would seem highly dependent on the presence of TK activity in the host cell, as shown previously for the free nucleosides.[12b] It is likely that such nucleosides must first be phosphorylated to the 5′-monophosphate before they can exert their cytostatic action. Even the dUMP and the cdUMP derivatives showed a decreased inhibitory effect on the proliferation of TK-deficient cell lines. One may infer that the 5′-monophosphates are first hydrolyzed outside the cell to their corresponding nucleosides before they are intracellularly phosphorylated. Evidently, there is no efficient membrane transport and/or conversion of the cdUMP's to dUMP's in the TK-deficient cell lines. Likely this is true with the normal cell lines as well.

The neutral cyclic triesters **16–18** showed generally reduced cytostatic activities compared to **13** and **14**. ID_{50} values for **17** were all >200 μg/mL against the cell lines listed in Table III, while for **16** and **18** minimal ID_{50} values >100 μg/mL were established. The exception was the

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Table V. Antiviral Activity of the 5-Halo- and 5-(Trifluoromethyl)-dUrd, -dUMP, and cdUMP Analogues

	MIC_{50},[a] μg/mL							
compd	HSV-1 (KOS)[b]	HSV-1 (F)	HSV-1 (McIntyre)	HSV-2 (G)	HSV-2 (196)	HSV-2 (Lyons)	vaccinia virus	vesicular stomatitis virus
5-fluoro-dUrd (2)	6	8	4	8	150	10	0.1	>200
5-chloro-dUrd	2	0.7	2	1	2	2	0.2	>400
5-bromo-dUrd	0.2	0.2	0.2	0.2	0.2	0.07	0.07	>400
5-iodo-dUrd (3)	0.2	0.2	0.2	0.2	0.4	0.1	0.2	>400
5-(trifluoromethyl)-dUrd (4)	0.7	0.7	0.7	0.6	1	1	0.2	>40
5-fluoro-dUMP (6)	10	10	10	7	100	20	0.2	>200
5-bromo-dUMP	0.2	0.2	0.2	0.2	0.2	0.07	0.07	>400
5-iodo-dUMP (8)	0.2	0.4	0.2	0.4	0.7	0.2	0.2	>400
5-(trifluoromethyl)-dUMP (9)	0.7	0.7	0.4	0.7	0.7	0.6	0.2	>400
5-fluoro-cdUMP (11)	>400	>400	>400	≥300	≥400	≥200	1.5	>400
5-chloro-cdUMP (12)	20	100	100	40	150	20	2	>400
5-bromo-cdUMP (13)	2	2	2	2	20	2	1	>400
5-iodo-cdUMP (14)	2	2	7	10	20	7	2	>400
5-(trifluoromethyl)-cdUMP (15)	10	20	10	10	40	10	0.4	>400
BVDU[c]	0.02	0.02	0.02	1	100	1	2	>400

[a] Minimum inhibitory concentration required to reduce virus-induced cytopathogenicity in primary rabbit kidney cell cultures by 50%. Cytotoxicity, as revealed by a microscopically detectable alteration of normal cell morphology, was not observed at a concentration up to 400 μg/mL, except for **2**, **6**, **15** (cytotoxic at 200 μg/mL), **4**, and **9** (cytotoxic at 40 μg/mL). [b] HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2. [c] Reference compound: (*E*)-5-(2-bromovinyl)-2′-deoxyuridine.[38]

Table VI. Antiviral Activity and Cytotoxicity of Triesters of 2′-Deoxyuridine 3′,5′-Cyclic Monophosphates

	MIC_{50},[a] μg/mL							
compd	HSV-1[b] (KOS)	HSV-1 (F)	HSV-1 (McIntyre)	HSV-2 (G)	HSV-2 (196)	HSV-2 (Lyons)	vaccinia virus	vesicular stomatitis virus
16	>200	>200	>200	>200	>200	70	10	>200
17	150	150	70	300	300	150	20	>400
18	20	7	20	20	70	7	2	>200
BVDU[c]	0.02	0.02	0.07	4	70	7	4	>400
BVDUMP[c]	0.02	0.02	0.02	20	>400	100	7	>400

[a] Minimum inhibitory concentration required to reduce virus-induced cytopathogenicity in primary rabbit kidney cell cultures by 50%. Cytotoxicity measured as minimal concentration required to cause a microscopically detectable alteration of normal cell morphology (μg/mL): **16**, >200; **17**, >400; **18**, >200. [b] HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2. [c] Reference compounds: (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) and its 5′-monophosphate (BVDUMP).

comparatively easily hydrolyzable[18c,d] benzyl ester, **18**, against Raji/0 cells (ID_{50} 69 ± 8). This value is comparable to that for diester **14** (Table III).

According to our previous studies, the principal target for the cytostatic action of 5-substituted dUrd derivatives is thymidylate (dTMP) synthetase.[34] Therefore, we also evaluated the 5-substituted dUMP and cdUMP derivatives for their potential inhibitory effects on partially purified dTMP synthetase from L1210 cells (Table IV). There was a striking difference between the K_i/K_m values of the dUMP's and the cdUMP's for dTMP synthetase: the dUMP derivatives proved to be at least 100–2000-fold more inhibitory than their cdUMP counterparts. 5-F-dUMP (**6**) and 5-CF_3-dUMP (**9**) were the most potent inhibitors of the enzyme (K_i/K_m 0.018 and 0.061, respectively). These observations indicate that to achieve an inhibitory effect on dTMP synthetase the 5-substituted dUMPs may not have their 5′-monophosphate linked to the C-3′ hydroxyl group of the deoxyribose moiety. Thus, the cdUMP derivatives would first have to be hydrolyzed at C-3′ before they could act as potential inhibitors of dTMP synthetase.

Antiviral Activity. In agreement with previously published data,[35-37] 5-fluoro-, 5-chloro-, 5-bromo-, 5-iodo-, and 5-(trifluoromethyl)-dUrd are efficient inhibitors of herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), and vaccinia virus (Table V). Their antiviral potencies decrease in the order of 5-I-dUrd ~ 5-Br-dUrd > 5-CF_3-dUrd > 5-Cl-dUrd > 5-F-dUrd; and the two most active congeners, 5-I- and 5-Br-dUrd, show MIC_{50} values within the range of 0.1–1 μg/mL. The antiviral potencies of the 5-halo- and 5-CF_3-dUMP's are very similar to those of the corresponding dUrd analogues (Table V), which is consistent with previous observations[39] that 5-alkyl-dUMP's, i.e. 5-ethyl- and 5-propyl-dUMP, have potencies as antiviral agents equal to those of their dUrd counterparts.

For these compounds, the conversion of the dUMP to the cdUMP (**11–15**) normally resulted in a significant (approximately 10- to 40-fold) decrease in antiviral activity. Thus, 5-fluoro-cdUMP (**11**) was virtually inactive as an inhibitor of HSV replication, although it was still effective in inhibiting the replication of vaccinia virus (Table V).

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For 5-I- and 5-Br-cdUMP, the MIC_{50} ranged from 1 to 20 μg/mL. Vaccinia virus was more sensitive to the antiviral effects of the 5-halo- and 5-trifluoromethyl derivatives than were HSV-1 and HSV-2. This was particularly true for 5-F-dUrd, -dUMP, and -cdUMP. Also, 5-CF_3-cdUMP, by contrast to other series, was almost as potent an inhibitor of vaccinia virus replication as the corresponding dUrd and dUMP analogues. The potency of 5-F-cdUMP against vaccinia virus also is notable in view of its much reduced activity compared to the nucleoside and 5′-monophosphate where the other viruses are concerned. The generally reduced activity of the 5-X-cdUMP's suggests that they either do not readily enter the virus-infected cells or are not converted easily to 5-X-dUrd or 5-X-dUMP intracellularly. The 5-X-cdUMP's may indeed be prodrugs releasing the 5-X-dUrd's by extracellular hydrolysis. There is no evidence that they can function as sources of the 5-halouracils.

The results in Table VI for the neutral methyl and benzyl triesters of **13** and **14** show the activities for the methyl esters to be greatly reduced. Benzyl ester **18** is considerably more active than methyl ester **17** and in fact is as potent as **14** against HSV-1 (Lyons) and vaccinia virus. A parallel increase in cytostatic activity of the benzyl triester was noted above. Such benzyl esters are known to be more readily hydrolyzed than methyl esters to the cyclic 3′,5′-monophosphate,[18c,d] which probably accounts for the activity order **18** > **17**.

Related Studies. The 5-halocytidine[40] and 5-halouridine 3′,5′-cyclic monophosphates[41] (5-X-cCMP) and 5-X-cUMP) were recently studied (X = F, Cl, Br, I). As with the 5-X-cdUMPs, the 5-F compounds showed the most potent antitumor activities but were less active than the corresponding nucleoside or 5′-monophosphate. No special advantage of 5-F-cUMP against TK^- cells could be demonstrated. 5-F-cCMP displayed moderate virus ratings, but 5-F-cUMP was essentially inactive as an antiviral agent except against vaccinia virus.

Experimental Section

Materials. 5-Fluoro-2′-deoxyuridine (**2**) was a gift from Hoffman La Roche Inc., Nutley, NJ. 5-Iodo-2′-deoxyuridine (**3**) and 5-bromo-2′-deoxyuridine 5′-monophosphate, and 2′-deoxyuridine (**1**) were purchased from Sigma Chemical Co., St. Louis, MO. 5-Chloro-2′-deoxyuridine was from Calbiochem Behring Corp., Lucerne, Switzerland. 5-Trifluoromethyl-2′-deoxyuridine (**4**) was a gift from Burroughs Wellcome Co., Research Triangle Park, NC. Trimethyl and triethyl phosphate were vacuum distilled. Phosphoryl chloride, glacial acetic acid, and pyridine were freshly distilled from phosphorus pentoxide prior to use.

Chromatography. Precoated silica gel (Kieselgel 60 F_{254}, 0.2 mm × 20 cm × 20 cm; Merck, Darmstadt, FRG) and cellulose (Cellulose F_{254}, 0.1 mm × 20 cm × 20 cm; Merck, Darstadt FRG) TLC sheets were used to follow the reactions and check the purity of the products. Solvent systems used for TLC were (v/v) (1) isobutyric acid/25% ammonium hydroxide/water (66/1/33) and (2) 2-propanol/25% ammonium hydroxide/water (7/1/2). DEAE Sephadex A-25 for anion-exchange column chromatography was purchased from Pharmacia Fine Chemicals. DEAE Sephadex column chromatographic separations were monitored with the help of a Spectromom 195 spectrophotometer (MOM) equipped with a flow-through cell (Starna Ltd.) and a potentiometric recorder (Type OH 814/1; Radelkis).

Spectroscopy. Proton NMR spectra were recorded in D_2O with a Varian XL-100/15 FT NMR system operating at 100.1 MHz or with a Varian SC-300 FT NMR system operating at 300.3 MHz with dioxane (δ 3.70) for internal reference. Carbon-13 spectra were acquired in D_2O on a Varian XL-100/15 disk-augmented FT NMR system operating at 25.2 MHz and a Varian SC-300 FT NMR system operating at 75.5 MHz. Dioxane (67.71 ppm downfield from Me_4Si) or $Me_3SiCH_2CH_2CO_2Na$ was used as internal reference. Phosphorus-31 spectra were acquired in D_2O with a Varian SC-300 FT NMR system (121.5 MHz). Positive chemical shifts are (ppm) downfield from external 85% H_3PO_4. UV spectra were recorded at three different pH levels with Varian Cary 17D and Zeiss Specord UV–vis spectrophotometer systems. Infrared spectra were recorded in potassium bromide on a Nicolet 7199 FT IR spectrophotometer. Mass spectra were acquired on a Varian MAT 112S mass spectrometer with ionizing energy of 80 eV and an ion source temperature of 270 °C. Trimethylsilylation was carried out by the addition of 30 μL of BSTFA and 10 μL of pyridine to ca. 0.2 mg of compound in a glass capillary tube followed by a 1-h reaction at 100 °C.

General Procedure for the Synthesis of Nucleoside 5′-Monophosphate Diammonium Salts, 5, 6, 8, and 9. Compounds 1–4 (2 mmol) were dissolved in 5 mL of stirred trimethyl or triethyl phosphate. The solution was cooled to 0 °C. Phosphoryl chloride (4 mmol) was added in one portion with vigorous stirring. The solution was kept at 0 °C for several hours and then quenched by the addition of ice water containing ammonium bicarbonate (24 mmol). The solution thus obtained was applied to a DEAE Sephadex A-25 (HCO_3^-) column (2.5 × 60 cm). The column was washed with water until no more UV absorbance was observed. Then, compounds **5**, **6**, **8**, and **9** were eluted (20 mL/fraction in 10 min) with a linear gradient of water (1.5 L) and 0.75 M ammonium bicarbonate (1.5 L). Any inorganic salt contamination of the nucleotide was removed by extraction of the organic compound with dry pyridine (3 × 50 mL). The anion-exchange column chromatography was then usually repeated under the same conditions.

2′-Deoxyuridine 5′-Monophosphate Diammonium Salt (5). The reaction time was 16 h. Compound **5** appeared in fractions 76–93: yield 69%; R_f (solvent system 1) 0.30 (silica gel). Anal. ($C_9H_{19}N_4O_8P$) C, H, N, P.

5-Fluoro-2′-deoxyuridine 5′-Monophosphate Diammonium Salt (6). In this case the phosphoryl chloride was added in two 2-mmol portions 2 h apart.[24] Total reaction time was 10 h. Compound **6** appeared in fractions 83–101 (yield 56%). R_f: (solvent system 1) 0.31 (silica gel), 0.34 (cellulose); (solvent system 2) 0.07 (silica gel). Anal. ($C_9H_{18}FN_4O_8P$) C, H, F, N, P.

5-Iodo-2′-deoxyuridine 5′-Monophosphate Diammonium Salt (8). Gentle heating was required to dissolve **3** in triethyl phosphate. Alternatively, the volume of the triethyl phosphate was doubled. The reaction time was lengthened to 48 h. Compound **8** appeared in fractions 94–105 (yield 35%). R_f: (solvent system 1) 0.38 (silica gel). Anal. ($C_9H_{18}IN_4O_8P$) C, H, I, N, P. ^{13}C NMR (25.2 MHz): δ 40.25 (C2′), 65.86 (C5′, d, J_{PC} = 3.9 Hz), 69.97 (C5), 72.13 (C3′), 86.99 (C1′), 87.05 (C4′, d, J_{PC} = 5.1 Hz), 147.28 (C6), 152.37 (C2), 164.06 (C4).

5-(Trifluoromethyl)-2′-deoxyuridine 5′-Monophosphate Diammonium Salt (9). The reaction time was 9 h. Product **9** was accompanied by a lesser amount of the 3′-phosphate (ca. 20%) as shown by ^1H and ^{13}C NMR. Both isomers appeared in fractions 85–107 (yield 36%). R_f: (solvent system 1) of 5′-isomer, 0.47 (silica gel), 3′-isomer, 0.50 (silica gel), 0.59 (cellulose, both isomers); (solvent system 2) 0.21 (silica gel, both isomers). Anal. ($C_{10}H_{18}F_3N_4O_8P$) C, H, F, N, P. ^{13}C NMR (75.5 MHz): (5′-isomer) δ 42.4 (C2′), 67.6 (C5′, d, J_{PC} = 4.8 Hz), 74.1 (C3′), 89.5 (C1′), 89.8 (C4′, d, J_{PC} = 8.1 Hz), 107.8 (C5, d, J_{FC} = 33.0 Hz), 125.3 (CF_3, d, J_{FC} = 269.5 Hz), 146.1 (C6, d, J_{FC} = 6.7 Hz), 154.1 (C2), 164.9 (C4); (3′-isomer) δ 41.8 (C2′), 63.6 (C5′), 76.6 (C3′, d, J_{PC} = 3.7 Hz), 89.4 (C1′), 89.6 (C4′), 146.7 (C6, d, J_{FC} = 6.7 Hz) (C2, C4, C5, and CF_3, not observed or overlapped by the signals of the 5′-isomer).

General Procedure for the Synthesis of Nucleoside 3′,5′-Cyclic Monophosphate Ammonium Salts 10–12, 14, and 15. Compounds **5–9** (1 mmol) were dissolved in 10 mL of water, and to this solution was added *N,N′*-dicyclohexyl-4-morpholinecarboxamidine (1 mmol) in 10 mL of pyridine. The solution was evaporated to dryness, and the solid residue was dried overnight under vacuum (0.13 Pa) over P_2O_5. The *N,N′*-dicyclohexyl-4-morpholinecarboxamidine salt of **5–9** was dissolved

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in 100 mL of pyridine. This solution was added dropwise to vigorously stirred DCC (5 mmol) in 100 mL of pyridine at reflux temperature. The stirred solution was kept at reflux temperature for an additional 1.5 h. The reaction mixture was evaporated to dryness, and 50 mL of ether and 50 mL of water were added to the solid residue. *N,N'*-Dicyclohexylurea was filtered off, and the aqueous phase was concentrated to a smaller volume. This solution was applied to a DEAE Sephadex A-25 (HCO_3^-) column (2.5 × 60 cm). The column was washed with water until no more UV absorbance at 260 nm was observed. Compounds **10–12**, **14**, and **15** were eluted (20 mL/fraction in 10 min) using a linear gradient of water (1.5 L) and 0.75 M ammonium bicarbonate (1.5 L).

2′-Deoxyuridine 3′,5′-Cyclic Monophosphate Ammonium Salt (10). Compound **10** appeared in fractions 81–90 (yield 69%). R_f: (solvent system 1) 0.41 (silica gel); (solvent system 2) 0.67 (silica gel). Anal. ($C_9H_{14}N_3O_7P$) C, H, N, P. ^{13}C NMR (25.2 MHz): δ 35.4 (C2′, d, J_{PC} = 8.1 Hz), 67.9 (C5′, d, J_{PC} = 7.2 Hz), 75.7 (C4′, d, J_{PC} = 4.7 Hz), 77.0 (C3′, d, J_{PC} = 4.4 Hz), 86.6 (C1′), 103.3 (C5), 143.1 (C6), 152.0 (C2), 166.8 (C4). ^1H NMR (100.1 MHz): δ 7.90 (H6, d, 1 H), 6.58 (H1′, dd, 1 H), 6.19 (H5, d, 1 H), 4.91 (H3′, q, 1 H), 4.40–4.75 (H5′, H5″, m, 2 H), 4.10–4.35 (H4′, m, 1 H), 2.68–2.95 (H2′, H2″, m, 2H).

5-Fluoro-2′-deoxyuridine 3′,5′-Cyclic Monophosphate Ammonium Salt (11). Compound **11** appeared in fractions 93–102 (yield 70%).

5-Chloro-2′-deoxyuridine 3′,5′-Cyclic Monophosphate Ammonium Salt (12). Method A. 2′-Deoxyuridine 5′-monophosphate diammonium salt (**5**; 0.900 g, 2.63 mmol) was dissolved in 22 mL of glacial acetic acid. To this solution was added chlorine (4.2 mmol) in carbon tetrachloride (6.4 mL) with stirring at ambient temperature. Stirring was continued for an additional 15 min. The solution then was boiled at 100 °C for 5 min to remove the chlorine and then evaporated to dryness. The residue was coevaporated three times with small volumes of methanol to remove the last traces of acetic acid. The solid residue was suspended in a small volume of ethanol, precipitated with absolute ether, filtered, washed with ether, and dried under vacuum over solid potassium hydroxide. Isolated was 0.747 g (2.18 mmol, 83%) of 5-chloro-2′-deoxyuridine 5′-monophosphate (**7**) as the free acid. R_f: (solvent system 1) 0.34 (silica gel). Anal. ($C_9H_{12}ClN_2O_8P$) C, H, Cl, N, P.

The cyclic diester was prepared by the above method. Compound **12** (ammonium salt) appeared in fractions 93–104 (yield 43%). ^1H NMR (100.1 MHz): δ 7.62 (H6, s, 1 H), 6.10 (H1′, dd, 1 H), 4.43 (H3′, q, 1 H), 3.9–4.3 (H5′, H5″, m, 2 H), 3.56–3.80 (H4′, m, 1 H), 2.25–2.43 (H2′, H2″, m, 2 H).

5-Iodo-2′-deoxyuridine 3′,5′-Cyclic Monophosphate Ammonium Salt (14). Compound **14** appeared in fractions 97–105 (yield 68%). UV (λ_{max}, nm, λ_{min}, nm): 285, 247 (pH 2); 285, 247 (pH 6); 278, 253 (pH 10). IR (cm^{-1}): $\gamma_{P=O}$ 1231, γ_{POC} 1080.

5-(Trifluoromethyl)-2′-deoxyuridine 3′,5′-Cyclic Monophosphate Ammonium Salt (15). Compound **15** appeared in fractions 91–103 (yield 50%). UV (λ_{max}, nm, λ_{min}, nm): 259, 228 (pH 2); 259, 226 (pH 7); 259, 239 (pH 11). EI–MS, *m/e* (relative intensity, %): 415, $M^+ + Me_4Si - 15$ (0.5); 488, $M^+ + 2\ Me_4Si - 15$ (1.5). ^1H NMR (300.3 MHz): δ 8.22 (H6, s, 1 H), 6.35 (H1′, dd, 1 H), 4.79 (H3′, q, 1 H), 4.40–4.65 (H5′, H5″, m, 2 H), 4.04–4.12 (H4′, m, 1 H), 2.67–2.81 (H2′, H2″, m, 2 H).

5-Chloro-2′-deoxyuridine 3′,5′-Cyclic Monophosphate (12). Method B. 2′-Deoxyuridine 3′,5′-cyclic monophosphate ammonium salt (**10**; 0.154 g, 0.50 mmol) was dissolved in 3 mL of glacial acetic acid. To this solution was added chlorine (0.8 mmol) in carbon tetrachloride (1.2 mL). The solution was stirred for 15 min at ambient temperature and then evaporated to dryness. The residue was coevaporated several times with small volumes of methanol (3 × 5 mL) to remove the last traces of acetic acid. The residue was then suspended in a small volume of ethanol (1 mL) and precipitated with ether. The white powder was filtered off, washed with ether, and dried under vacuum over solid potassium hydroxide. Isolated was 0.143 g (0.44 mmol, 88%) of 5-chloro-2′-deoxyuridine 3′,5′-cyclic phosphate (**12**) as the free acid.

5-Bromo-2′-deoxyuridine 3′,5′-Cyclic Monophosphate Ammonium Salt (13). 2′-Deoxyuridine 3′,5′-cyclic phosphate ammonium salt (**10**; 0.307 g, 1 mmol) was dissolved in 3 mL of glacial acetic acid. *N*-Bromosuccinimide (0.231 g, 1.73 mmol) was added with stirring. This solution was kept for an additional 15 min at room temperature with stirring and then evaporated to dryness. The residue was coevaporated several times with small volumes of methanol (3 × 5 mL) to remove the last traces of acetic acid. This residue was dissolved in 10 mL of water, applied to a DEAE Sephadex A-25 (HCO_3^-) column (3 × 30 cm) and eluted (20 mL/fraction in 10 min) with a linear gradient of water (2 L) and 1 M ammonium bicarbonate (2 L). Compound **13** as the ammonium salt appeared in fractions 69–78 (0.320 g, 0.83 mmol, 83%). ^1H NMR (100.1 MHz): δ 8.10 (H6, s, 1 H), 6.37 (H1′, dd, 1 H), 4.78 (H3′, q, 1 H), 4.2–4.7 (H5′, H5″, m, 2 H), 3.90–4.18 (H4′, m, 1 H), 2.59–2.78 (H2′, H2″, m, 2 H).

Acid Hydrolysis of Compounds 11 and 14. Compounds **11** and **14** (0.1 mM) were rapidly dissolved in cold (0 °C) 1 M hydrochloric acid (5 mL). These solutions were then incubated at 37 °C. Aliquots (0.1 mL) were removed at intervals. The reactions were quenched by adding 1.5 M ammonium bicarbonate solution (0.1 mL). The contents of these aliquots were then examined chromatographically on silica gel TLC sheets in solvent system 1. The hydrolysis products were detected by UV light at 254 nm by comparisons with authentic samples of the respective pyrimidine bases formed.

Antitumor assays were performed according to previously established procedures.[34] L1210/0, L1210/BdUrd, Raji/0, and Raji/TK$^-$ cell lines were characterized as described.[12b] Thymidylate synthetase assays were carried out with a partially purified L1210 cell extract as indicated in ref 12a.

Antiviral assays were performed as reported previously.[37] The origin and preparation of the virus stocks have also been documented in ref 37.

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Registry No. 1 (X = H), 951-78-0; 1 (X = Cl), 50-90-8; 1 (X = Br), 59-14-3; **2**, 50-91-9; **3**, 54-42-2; **4**, 70-00-8; **5** (X = H), 99966-07-1; **5** (X = H, free acid), 964-26-1; **5** (X = H, *N,N'*-dicyclohexyl-4-morpholinecarboxamidine salt), 99966-17-3; **5** (X = Br, free acid), 6666-38-2; **6**, 99966-08-2; **6** (free acid), 134-46-3; **6** (*N,N'*-dicyclohexyl-4-morpholinecarboxamidine salt), 99966-18-4; **7**, 99966-09-3; **7** (free acid), 64334-79-8; **7** (*N,N'*-dicyclohexyl-4-morpholinecarboxamidine salt), 99966-19-5; **8**, 49620-45-3; **8** (free acid), 1763-02-6; **8** (*N,N'*-dicyclohexyl-4-morpholinecarboxamidine salt), 99966-20-8; **9**, 99966-10-6; **9** (3′-phosphate), 99966-11-7; **9** (3′-phosphate, free acid), 99966-14-0; **9** (free acid), 345-02-8; **9** (3′-phosphate, *N,N'*-dicyclohexyl-4-morpholinecarboxamidine salt), 99966-21-9; **9** (*N,N'*-dicyclohexyl-4-morpholinecarboxamidine salt), 100019-66-7; **10**, 55727-02-1; **10** (free acid), 24853-46-1; **11**, 100019-64-5; **11** (free acid), 36519-08-1; **11** (pyrimidine base), 51-21-8; **12**, 99966-12-8; **12** (free acid), 99966-15-1; **13**, 99966-22-0; **13** (free acid), 99966-23-1; **14**, 99966-13-9; **14** (free acid), 94844-21-0; **14** (pyrimidine base), 696-07-1; **15**, 100019-65-6; **15** (free acid), 99966-16-2; **16**, 100100-60-5; **17**, 100100-61-6; **18**, 100100-62-7; thymidylate synthetase, 9031-61-2.