Synthesis and Antitumor and Antiviral Properties of 5-Alkyl-2′-deoxyuridines, 3′,5′-Cyclic Monophosphates, and Neutral Cyclic Triesters

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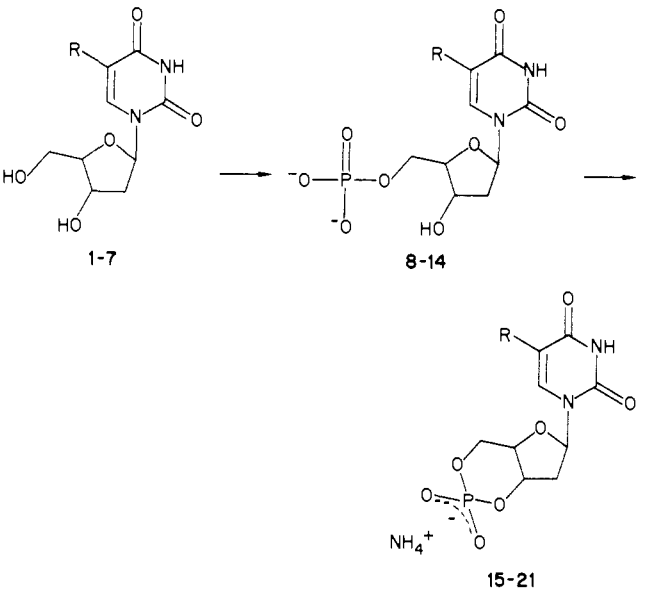
A series of 5-alkyl-2′-deoxyuridine 3′,5′-cyclic monophosphates (5-R-cdUMP's, R = Et, *i*-Pr, *n*-Pr, *n*-Bu, *n*-Pent, *n*-Hex, *n*-Oct) was prepared and tested in culture systems as antitumor and antiviral agents in comparison to the 5-alkyl-2′-deoxyuridines (5-R-dUrd's) themselves. Only the 5-Et- and 5-*n*-Bu-cdUMP showed appreciable cytostatic activities against murine L1210 and human lymphoblast Raji cells (ID_{50} range: 28–82 μg/mL). 5-Et-dUrd itself was much more active (ID_{50} = 1.6 and 2.9 μg/mL). The 5-*i*-Pr-, 5-*n*-Pr-, and 5-*n*-Bu-dUrd's were inactive, but activity increased again for groups with chain lengths of five carbons or greater. 5-Et-cdUMP and 5-Et-dUrd had greatly reduced activities against deoxythymidine kinase deficient (TK^-) L1210 and Raji cells. 5-Et-cdUMP evidently is not an efficient prodrug source of the corresponding 5′-monophosphate where the TK^- cells are concerned. Of the 5-R-cdUMP's, 5-Et-cdUMP displayed reasonably good antiviral potency against herpes simplex types 1 and 2 (MIC_{50}, mostly 7–70 μg/mL) and vaccinia virus (MIC, 70 μg/mL). The activity was nonetheless 10- to 100-fold less than that for 5-Et-dUrd. The other 5-R-dUrd's generally showed decreasing antiviral activity with increasing 5-R chain length. Methyl and/or benzyl neutral triesters of certain 5-R-cdUMP's were inactive as antivirals and largely inactive against tumor cells in culture. In contrast to the 5′-monophosphates, the 5-R-cdUMP's failed to inhibit thymidylate synthetase from L1210 cells.

5-Alkyl-2′-deoxyuridines (5-R-Urd's),[1] in particular the 5-ethyl[2] and 5-*n*-propyl[3] compounds, are known to possess selective antiviral activity in cell culture, i.e. against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), as well as useful in vivo potency.[4] The antitumor properties of these thymidine analogues are not particularly remarkable. Drawbacks of 5-Et-dUrd as a drug include its low lipophilicity, high aqueous solubility, and rapid degradation to 5-ethyluracil[5] and 5-(1-hydroxyethyl)uracil.[6] Prodrug forms of 5-Et-dUrd potentially avoid these problems. Indeed the 5′-*O*-acyl derivatives of 5-Et-dUrd have improved solubilities and lipophilicities while retaining high potency against HSV-1, HSV-2, and vaccinia virus in cell cultures.[7] As a result, 5-Et-dUrd is much more inhibitory to tumor growth in mice as the 5′-pivaloyl carboxylic ester[8] than as the nucleoside itself, presumably because of improved transport properties. Prodrugs also have the potential to overcome drug resistance stemming from mutation-induced changes in enzyme activity.

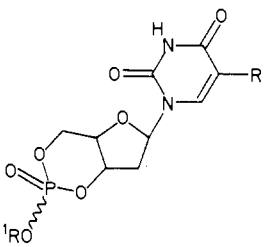
To these ends we have prepared a series of 3′,5′-cyclic monophosphates (**15–21**), based on the 5-alkyl-2′-deoxyuridines **1–7**, and tested them as antivirals and antitumor agents in cell systems. Since 5-alkyl-dUrd's must be phosphorylated at the 5′-position prior to incorporation into DNA, cells deficient in thymidine kinase (TK^- cells) are not likely to respond to 5-R-dUrd's. However, the introduction of the 5′-monophosphate in the 3′,5′-cyclic diester form, followed by hydrolytic cleavage of the C3′-OP bond, can potentially overcome drug resistance resulting from TK deficiency. Newly discovered phosphodiesterases have been shown to convert pyrimidine 3′,5′-cyclic monophosphates to the 5′-monophosphates and to be widely distributed in tissues.[9] Of special interest is the report of such activity in disrupted L1210 cells.[10] Evidence that 3′,5′-cyclic monophosphates may be transported to some degree through cell membranes has been presented.[11]

Neutral nucleoside cyclic 3′,5′-monophosphate triesters are known to penetrate cells very readily,[12] and **22–25**, therefore, hold the same prodrug potential as **15–21** once they are converted, perhaps by nonenzymatic means, to the cyclic diester.

Scheme I



1, 8, 15: R=Et
2, 9, 16: R=*i*-Pr
3, 10, 17: R=*n*-Pr
4, 11, 18: R=*n*-Bu
5, 12, 19: R=*n*-Pent
6, 13, 20: R=*n*-Hex
7, 14, 21: R=*n*-Oct



22, R=Et; R^1=CH_3
23, R=*i*-Pr; R^1=CH_3
24, R=*i*-Pr; R^1=$PhCH_2$
25, R=*n*-Bu; R^1=CH_3

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Table I. Synthetic Data for 5-Alkyl-2'-deoxyuridine 3',5'-Cyclic Phosphates **15–21**

compd	(5-R-)	formula	anal.[a]	% yields[b]	R_f[c] 1[d]	R_f[c] 2
15	CH_3CH_2-	$C_{11}H_{18}N_3O_7P$	C, H, N, P	48	0.49	0.74
16	$(CH_3)_2CH$-	$C_{12}H_{20}N_3O_7P$	C, H, N, P	62	0.52	0.79
17	$CH_3CH_2CH_2$-	$C_{12}H_{20}N_3O_7P$	C, H, N, P	41	0.53	0.79
18	$CH_3(CH_2)_2CH_2$-	$C_{13}H_{22}N_3O_7P$	C, H, N, P	69	0.55	0.84
19	$CH_3(CH_2)_3CH_2$-	$C_{14}H_{24}N_3O_7P$	C, H, N, P	74	0.56	0.84
20	$CH_3(CH_2)_4CH_2$-	$C_{15}H_{26}N_3O_7P$	C, H, N, P	45	0.57	0.85
21	$CH_3(CH_2)_6CH_2$-	$C_{17}H_{30}N_3O_7P$	C, H, N, P	91	0.60	0.87

[a] Found = calcd ± 0.4%. [b] Isolated material. [c] On silica gel TLC sheets. [d] Solvent system (see Experimental Section).

The diesters **15–21** might also function as inhibitors of thymidylate synthetase (TS),[13] as 5-R-2'-deoxyuridine 5'-monophosphates (5-R-dUMP's) show this ability.[14] TS inhibition is a further potential basis for development of drugs based on nucleoside derivatives.

Results and Discussion

Chemistry. 5-Alkyl-2'-deoxyuridine 3',5'-cyclic monophosphates **15–21** (Scheme I) were synthesized from the *N,N'*-dicyclohexyl-4-morpholinecarboxamidine salts of the corresponding 5'-monophosphates **8–14**. Dicyclohexylcarbodiimide (DCC) in pyridine was used as condensing agent for the ring closure as previously described for the preparation of other 2'-deoxyribonucleoside 3',5'-cyclic monophosphates.[15] Compounds **15–21** (yields 41–91%) were isolated by DEAE-Sephadex A-25 (HCO_3^-) anion exchange column chromatography (Table I). The preparations of **22–25** (mixtures of diastereomers) from reaction of MeI or $PhCH_2I$ with the silver salt of **15**, **16**, or **18** were reported previously.[16]

A modification of the original Yoshikawa reaction[17] for the preparation of 5'-monophosphates **8–13** (not for **14**) was published earlier by one of our laboratories.[18] To decrease the contamination of **8–14** by the 3'-isomers, a modified Yoshikawa phosphorylation procedure[19] was employed, although both isomers give only the desired 3',5'-cyclic nucleotides on ring closure. (The nonselectivity of the Yoshikawa reaction was later reported by several workers.[19–21]) A modified method for isolation of **8–14** used a simple procedure to desalt the reaction mixture prior to DEAE-Sephadex A-25 (HCO_3^-) anion exchange column chromatography. This column also separated the 5'-monophosphates, **8–14**, from the 3',5'-diphosphate byproducts of the Yoshikawa reaction. In some cases, an extraction of the organic compounds from the inorganic salts with dry pyridine after the chromatography, followed by a second anion exchange column chromatography, was necessary. Contamination by the 3'-monophosphate was not observed in the isolated 5'-mononucleotides **8–14** by the methods used (^1H NMR, ^{13}C NMR, TLC). The site of phosphorus substitution was readily assigned to the 5'-position using the geminal ($^2J_{POC5'}$ = 4–5 Hz) and vicinal ($^3J_{POCC4'}$ = 8–9 Hz) ^{31}P–^{13}C couplings.[22] The MS spectrum of **8** following silylation with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) also confirmed its structure as a monophosphate but did not allow the extent of possible 3'-phosphorylation to be determined.[23] The precursor 5-alkyl-2'-deoxyuridines, **1–7**, were synthesized in a modified Hilbert–Johnson reaction following precisely the procedure described earlier in the literature.[24]

The structures of cyclic nucleotides **15–21** were verified by ^1H and ^{13}C NMR spectroscopy. ^1H NMR spectroscopy confirmed the β nature of the glycosidic bond configuration at C1'. Thus, in each case the expected[25] doublet of

(1) For a recent review of 5-substituted 2'-deoxyuridines as antivirals, see: De Clercq, E. In "Targets for the Design of Antiviral Agents"; De Clercq, E., Walker, R. T., Eds.; Plenum Press: New York, 1984; pp 203–230.
(2) (a) Teh, C.-Z.; Sacks, S. L. *Antimicrob. Agents Chemother.* **1983**, *23*, 637. (b) De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R. T.; Jones, A. S.; Torrence, P. F.; Shugar, D. *J. Infect. Dis.* **1980**, *141*, 563. (c) De Clercq, E.; Shugar, D. *Biochem. Pharmacol.* **1975**, *24*, 1073. (d) Gauri, K. K.; Malorny, G. *Naunyn Schmiedeberg's Arch. Pharmacol. Exp. Pathol.* **1967**, *257*, 21.
(3) (a) De Clercq, E.; Hempel, B.; Mauz, E., unpublished data, 1982. (b) Ruth, J. L.; Cheng, Y.-C. *J. Biol. Chem.* **1982**, *257*, 10261. (c) De Clercq, E.; Descamps, J.; Shugar, D. *Antimicrob. Agent Chemother.* **1978**, *13*, 545.
(4) Gauri, K. K. *Klin. Monatsbl. Augenheilkd.* **1968**, *153*, 837. Wassilew, S. W. *Z. Hautkrankh.* **1979**, *54*, 251. Elze, K. K. *Adv. Ophthalmol.* **1979**, *38*, 134.
(5) Kaul, R.; Kiefer, G.; Erhardt, S.; Hempel, B. *J. Pharm. Sci.* **1980**, *69*, 531.
(6) Kaul, R.; Keppeler, K.; Kiefer, G.; Hempel, B.; Fischer, P. *Chemosphere* **1982**, *11*, 539.
(7) Keppeler, K.; Kiefer, G.; De Clercq, E. *Arch. Pharm. (Weinheim, Ger.)* **1984**, *317*, 867.
(8) Gauri, K. K. In "Antiviral Chemotherapy: Design of Inhibitors of Viral Functions"; Gauri, K. K., Ed.; Academic Press: New York, 1981; pp 235–260.
(9) Helfman, D. M.; Kuo, J. F. *Biochem. Pharmacol.* **1982**, *31*, 43.
(10) Cheng, Y.-C.; Bloch, A. *J. Biol. Chem.* **1978**, *253*, 2522.
(11) Tsukamoto, T.; Suyama, K.; Germann, P.; Sonenberg, M. *Biochemistry* **1980**, *19*, 918. LePage, G. A.; Hersh, E. M. *Biochem. Biophys. Res. Commun.* **1972**, *46*, 1918. The phosphorothioate based on 3',5'-cyclic adenosine monophosphate (cAMPS) passes into renal tissues (Coulson, R.; Baraniak, J.; Stec, W. J.; Jastorff, B. *Life Sci.* **1983**, *32*, 1489.
(12) (a) Nargeot, J.; Nerbonne, J. M.; Engels, J.; Lester, H. A. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 2395. (b) Korth, M.; Engels, J. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1979**, *310*, 103. (c) Engels, J.; Schlaeger, E. J. *J. Med. Chem.* **1977**, *20*, 907. (d) Engels, J. *Bioorg. Chem.* **1979**, *8*, 9.
(13) Santi, D. V. In "Molecular Actions and Targets for Cancer Chemotherapeutic Agents"; Academic Press: New York, 1981; pp 285–300. Santi, D. V. *J. Med. Chem.* **1980**, *23*, 105.
(14) Balzarini, J.; De Clercq, E.; Mertes, M. P.; Shugar, D.; Torrence, P. F. *Biochem. Pharmacol.* **1982**, *31*, 3673.
(15) Drummond, G. I.; Gilgan, M. W.; Reiner, E. J.; Smith, M. *J. Am. Chem. Soc.* **1964**, *86*, 1626.
(16) Bēres, J.; Sāndor, P.; Kālmān, A.; Koritsānszky, T.; Ötvös, L. *Tetrahedron* **1984**, *40*, 2405.
(17) Yoshikawa, M.; Kato, T.; Takenishi, T. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 3505.
(18) Sāgi, J. T.; Szabolcs, A.; Szemzö, A.; Ötvös, L. *Nucleic Acids Res.* **1977**, *4*, 2767.
(19) Dawson, W. H.; Cargill, R. L.; Dunlap, R. B. *J. Carbohydr., Nucleosides Nucleotides* **1977**, *4*, 363.
(20) Bottka, S.; Tomasz, J. *Tetrahedron* **1979**, *35*, 2909.
(21) Manuscript in preparation for *J. Med. Chem.* from our laboratories.
(22) Stothers, J. B. In "Carbon-13 NMR Spectroscopy"; Academic Press: New York, 1972.
(23) Budzikiewicz, H.; Feistner, G. *Biomed. Mass Spectrom.* **1978**, *5*, 512.
(24) Szabolcs, A.; Sāgi, J.; Ötvös, L. *J. Carbohydr., Nucleosides, Nucleotides* **1975**, *2*, 197.

doublets structure (J_{HH} = ca. 8 and 3 Hz) in the region of 6.12–6.20 ppm for H1′ was observed. A sharp singlet signal for H6 in the region 7.18–7.31 ppm confirmed the alkyl substitution at the 5-position on the base.[26] δ values for ^{1}H signals arising from the sugar moiety of the molecules, generally seen as multiplet structures, were also in agreement with expectation. The most diagnostic evidence for the formation of the phosphate ring comes from the ^{13}C NMR spectra of **15–21** (Table II). Particular note should be taken of $\delta_{C4'}$ as well as $^3J_{PC2'}$, $^2J_{PC3'}$, $^3J_{PC4'}$, and $^2J_{PC5'}$, all of which are in the range seen for other cyclic 3′,5′-monophosphates.[26]

The structures of certain of **15–21** were further verified by characteristic UV absorptions (**15**), expected[12d,27] intense P=O (1240 cm^{-1}) and P—O (1085 cm^{-1}) bands (**15** and **20**), and mass spectrometry performed on a mixture of mono- and disilylated cyclic nucleotide (**15**).

A semiquantitative acid hydrolysis study of **15**, **16**, and **20**, and for comparison cTMP,[15] the 1-(2-deoxy-α-D-ribofuranosyl)-5-isopropyluracil 3′,5′-cyclic phosphate ammonium salt and the 5-isopropyl-2′-deoxyuridine 5′-monophosphate diammonium salt, **9**, was performed in 1 M hydrochloric acid at 37 °C. Only the changing relative amounts of the cyclic nucleotides and the corresponding 5-alkyluracils could be detected. For all of the cyclic nucleotides tested, the bases appeared within 2–6 min. The reactants were half-consumed in 0.5–1.5 h, as checked on silica gel TLC sheets by UV light (254 nm), and completely consumed within ca. 3–7 h. Under the same conditions, **9** remained unchanged even after 24 h. Although these observations are only qualitative, the tendencies observed are consistent with the hydrolysis results published earlier for pyrimidine 2′-deoxyribonucleoside 3′,5′-cyclic phosphates.[15] There was no significant effect of the 5-alkyl substituents or the anomeric configuration on the hydrolysis rate.

Antitumor Activity. The 5-alkyl-cdUMP's (**15–21**) were not appreciably cytostatic against the murine leukemia L1210/0 and human lymphoblast Raji/0 cell lines, except for **15** and **18** whose ID_{50} values ranged between 28 and 82 μg/mL (Table III). Even so, **15** was about 20–30 times less potent as an inhibitor of L1210/0 and Raji/0 cell proliferation than the corresponding dUrd analogue (**1**). By contrast, the dUrd precursor of **18** (compound **4**) was totally devoid of any significant cytostatic activity. 5-Et-dUrd was active against both L1210/0 and Raji/0 cells (ID_{50} = 1.6 and 2.9 μg/mL, respectively), while compounds **2–4** were not inhibitory to tumor cell proliferation ($ID_{50} \geq 500$ μg/mL). Compounds **5–7** showed some activity which increased with increasing length of their 5-substituent.

Both **1** and **15** showed greatly decreased cytostatic activity against the dThd kinase-deficient (TK$^-$) tumor cell lines. Based on this observation, one may infer that the conversion of **1** to its 5′-monophosphate by dThd kinase is essential for its cytostatic cell activity.[28,29] Diester **15** evidently does not function in TK$^-$ cells as an efficient prodrug source of the 5′-monophosphate. Quite possibly in normal cells, **15** is first hydrolyzed to the free nucleoside

Table II. Proton-Decoupled Carbon-13 Data[a] for **15-21**

no.	δ_{C2}	δ_{C4}	δ_{C5}	δ_{C6}	δ_{Ca}[e]	δ_{Cb}	δ_{Cc}	δ_{Cd}	δ_{Ce}	δ_{Cf}	δ_{Cg}	δ_{Ch}	$\delta_{C1'}$	$\delta_{C2'}$	$\delta_{C3'}$	$\delta_{C4'}$	$\delta_{C5'}$	$^3J_{PC2'}$, Hz	$^2J_{PC3'}$, Hz	$^3J_{PC4'}$, Hz	$^2J_{PC5'}$, Hz
15	150.13	163.20	116.00	135.83	19.68	13.25							84.00	34.74	75.48	75.35	65.74	8.1	4.3	4.3	6.7
16	149.82	162.83	119.99	134.66	25.90	21.14							84.56	34.85	75.73	75.46	65.73	8.4	4.5	4.5	6.9
17[b]	151.79	166.19	116.41	138.58	28.88	22.12	13.81						86.24	35.27	76.99	75.72	67.76	8.4	3.8	4.6	6.8
18	150.19	163.36	114.28	136.08	30.61	26.16	21.91	13.71					84.01	35.02	75.66	75.51	65.87	7.9	3.9	4.1	6.9
19	151.51	166.01	116.38	138.09	30.92	26.37	27.81	22.15	13.74				86.03	34.87	76.72	75.22	67.53	7.4	4.8	4.4	6.8
20[c]	150.08	163.25	114.66	136.52	31.01	26.25	28.32	28.32	22.00	13.83			83.90	34.62	75.68	75.22	65.66	8.5	4.3	4.3	6.7
21[c]	150.21	163.39	115.03	136.08	31.42	26.62	28.61	28.96	28.96	28.83	22.21	13.94	83.95	35.05	75.77	75.41	65.66	*d*	*d*	*d*	*d*

[a] Downfield chemical shifts from Me_4Si. D_2O solvent unless otherwise specified. [b] D_2O/Me_2SO-d_6. [c] Me_2SO-d_6/$CDCl_3$. [d] Not observed. [e] 5-Alkyl groups.

(25) Blackburn, B. J.; Lapper, R. D.; Smith, I. C. P. *J. Am. Chem. Soc.* **1973**, *95*, 2873.
(26) Lapper, R. D.; Smith, I. C. P. *J. Am. Chem. Soc.* **1973**, *95*, 2880.
(27) Taguchi, Y.; Mushika, Y. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 1528.
(28) Balzarini, J.; De Clercq, E.; Kiefer, G.; Keppeler, K.; Buchele, A. *Invest. New Drugs* **1984**, *2*, 35.
(29) Balzarini, J.; De Clercq, E.; Torrence, P. F.; Mertes, M. P.; Park, J. S.; Schmidt, C. L.; Shugar, D.; Barr, P. J.; Jones, A. S.; Verhelst, G.; Walker, R. T. *Biochem. Pharmacol.* **1982**, *31*, 1089.

Table III. Cytostatic Activity of Compounds 1–7 and 15–21

cell type	ID_{50},[a] μg/mL													
	1	2	3	4	5	6[b]	7	15	16	17	18	19	20	21
L1210/0[c]	1.6	≥500	>500	>500	476	172	66	55	≥500	>500	28.4	>500	>500	164
L1210/BdUrd[d]	>500	>500	>500	>500	477	195	72	>500	>500	>500	33.5	>500	>500	193
Raji/0[e]	2.9	>500	>500	>500	334	151	52	60	>500	>500	82	>500	>500	165
Raji/TK-[f]	>500	>500	>500	>500	328	173	140	>500	>500	>500	165	>500	>500	184

[a] Inhibitory dose-50 or dose required to inhibit tumor cell proliferation by 50%. [b] Compound received from M. J. Robins (Department of Chemistry, The University of Alberta, Edmonton, Alberta, Canada). [c] Murine leukemia L1210 cells, designated L1210/0. [d] 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 5-bromo-2'-deoxyuridine (BdUrd). This cell line is deficient in thymidine kinase activity.[29] [e] Human lymphoblast Raji cells, designated Raji/0. [f] Thymidine kinase deficient Raji cell line, designated Raji/TK-.[29]

Table IV. Antiviral Activity of Compounds 1–7 and 15–21

virus	MIC_{50},[a] μg/mL														
	1	2	3	4	5	6[b]	7	15	16	17	18	19	20	21	BVDU[c]
HSV-1 (KOS)[d]	0.7	7	2	20	20	≥400	>100	70	>200	150	>400	>400	>400	>200	0.02
HSV-1 (F)	2	2	7	70	10	≥400	>100	70	>200	300	>400	>400	>400	>200	0.02
HSV-1 (McIntyre)	0.2	1	2	20	40	300	>100	70	>200	150	>400	>400	>400	>200	0.02
HSV-2 (G)	0.4	40	10	150	70	400	100	10	>200	400	>400	>400	>400	>200	1
HSV-2 (196)	2	400	40	>400	>400	...	>100	150	>200	>400	>400	>400	>400	>200	100
HSV-2 (Lyons)	0.2	100	7	20	70	300	100	7	>200	400	300	>400	300	>200	1
vaccinia virus	0.7	300	200	300	300	300	7	70	>200	>400	300	>400	>400	20	2
vesicular stomatitis virus	>400	>400	>200	>400	>400	...	>100	>400	>200	>400	>400	>400	>400	>200	>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 7 (cytotoxic at 100 μg/mL), 16 (cytotoxic at 200 μg/mL), and 21 (cytotoxic at 200 μg/mL). [b] Compound received from M. J. Robins (Department of Chemistry, The University of Alberta, Edmonton, Alberta, Canada). [c] Reference compound: (*E*)-5-(2-bromovinyl)-2'-deoxyuridine.[36] [d] HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2.

before or during uptake by the cell; and though this may occur as well with the TK- systems, the necessary reconversion to the 5'-monophosphate is hampered. The inactivity of **15** in the TK- cell cultures also could be the result of poor cell-membrane transport or because it is not efficiently hydrolyzed within the cell to the 5'-monophosphate. The response of **15** toward the phosphodiesterases[9] which hydrolyze 3',5'-cyclic pyrimidine monophosphates has not been tested.

Since the activity of **18** is little changed by the absence of dThd kinase activity, its mechanism of action may be different. Similarly, the activity of **6**, **7**, and **21** is little dependent on dThd kinase.

Neutral triesters **22–25** also were tested with the tumor cell lines of Table III. ID_{50} values were in the range 190–410 μg/mL with the exception of $POCH_2Ph$ ester **24**, which showed an ID_{50} of 40.3 ± 7.5 μg/mL with the L1210/0 system. This activity was 10 times that of **23**, the $POCH_3$ analogue, which presumably is less readily hydrolyzed to the cyclic diester. No evidence for enhanced efficacy of **24** in TK-deficient cells was noted.

Thymidylate Synthetase Inhibition. Since 5-substituted dUMP derivatives may be considered as potential inhibitors of thymidylate (dTMP) synthetase,[14] we also evaluated the 5-alkyl-cdUMP's (**15–21**) for their inhibitory effects on partially purified dTMP synthetase from L1210 cells. None of the 5-alkyl-cdUMP's showed inhibition of dTMP synthetase at 450 μM (the highest concentration tested). By contrast, 5-Et-dUMP (K_i/K_m = 5.93[14]) and 5-*n*-Pr-dUMP (K_i/K_m = 6.00[14]) are inhibitory to L1210 thymidylate synthetase. This means that the 5'-monophosphate cannot be involved in a six-membered ring if the nucleotide is to bind strongly to thymidylate synthetase. It may also be significant that other studies[30] have shown that 5-F-cdUMP (K_i/K_m = 38.0) and 5-CF_3-cdUMP (K_i/K_m = 30.4) have demonstrable activities as L1210 dTMP synthetase inhibitors, which are nonetheless significantly lower than those of the corresponding 5'-monophosphates.

Antiviral Activity. Of the 5-alkyl-cdUMP's **15–21**, only **15** exhibited a distinct antiviral effect on the replication of herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), or vaccinia virus (Table IV). However, **15** was 25- to 100-fold less potent as an antiviral agent than the corresponding dUrd analogue (**1**). If **15** is indeed a prodrug source of the required 5'-monophosphate, it again apparently does not function very efficiently as such.

5-Ethyl-dUrd (**1**) was clearly the most potent antiviral agent among the 5-alkyl-dUrd's (**1–7**). As a rule, the antiviral potencies of the 5-alkyl-dUrd's decreased with the increasing length of the 5-substituent. Also, the alkyl-dUrd's **2–5** were more inhibitory to HSV-1 than to HSV-2 or vaccinia virus. Curiously, compound **7** was only effective against vaccinia virus. Some of the 5-alkyl-dUrd's, namely **1** (ref 2b), **2** (ref 3), **3** (ref 2b), and **4** (ref 31), have also been the subject of previous antiviral activity studies, and the MIC_{50} values reported here are in good agreement with those reported earlier. The 5-alkyl-dUMP's **8–14** were not examined for antiviral activity; it is assumed that their antiviral potencies are similar, if not identical, to those of the corresponding dUrd analogues, as has been directly demonstrated for 5-ethyl-dUMP,[32] 5-propyl-dUMP,[32] 5-fluoro-dUMP,[30] 5-bromo-dUMP,[30] 5-iodo-dUMP[30], and 5-trifluoromethyl-dUMP.[30]

Neutral phosphate triesters **22–25** were generally inactive against the viral strains of Table IV at the maximum concentrations tested: **22** (300 μg/mL), **23** (400 μg/mL), **24** (150 μg/mL), and **25** (200 μg/mL). Clearly, the triester prodrug approach leads to greatly reduced antiviral potency relative to the corresponding nucleoside or even the

(30) Bères, J.; Sàgi, G.; Bentrude, W. G.; Balzarini, J.; De Clercq, E.; Ötvös, L. *J. Med. Chem.*, in press.

(31) Sim, I. S.; Goodchild, J.; Meredith, D. M.; Porter, R. A.; Raper, R. H.; Viney, J.; Wadsworth, H. J. *Antimicrob. Agents Chemother.* **1983**, *23*, 416.

(32) De Clercq, E.; Kulikowski, T.; Shugar, D. *Biochem. Pharmacol.* **1980**, *29*, 2883.

3′,5′-cyclic monophosphate (compare **15** vs. **22**). Unfortunately, the benzyl triester of the 5-Et-cdUMP is not available as it would be expected to be more readily hydrolyzed to the diester. The superior activity of the benzyl compared to methyl triester of 5-iodo-2′-deoxyuridine has been noted.[30]

Summary. The efficacy of the potential prodrug 3′,5′-cyclic monophosphates as antitumor and antiviral agents is reduced below that of the corresponding nucleosides. This finding is general as well for the 5-halocytidines,[33] 5-CF_3- and 5-halo-2′-deoxyuridines,[30] and 5′-halouridines.[34] Nonetheless, depending on the analogue in question, a reasonably high level of activity can be maintained. No particular action circumventing TK^- resistance is found in tumor cells. Whether other advantages may pertain to the 3′,5′-cyclic monophosphates, such as superior transport properties, awaits testing in animal systems. Inhibition of thymidylate synthetase activity by the nucleoside 3′,5′-cyclic phosphates also is much reduced compared to the 5′-monophosphates although in some systems it is not completely lost.[30]

Experimental Section

Chromatography. Precoated TLC plates (Keiselgel 60 F_{254}, 0.2 mm × 20 cm × 20 cm, Merck,. Darmstadt, F.R.G.) were used to follow the reactions and check the purity of the products. Solvent systems (v/v) for silica gel TLC were (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 was purchased from Pharmacia Fine Chemicals, Sweden. DEAE-Sephadex column chromatographic separations were performed with the help of a Spectromom 195 spectrophotometer (MOM, Hungary) equipped with a flow-through cell (Starna Ltd., England) and a potentiometric recorder (Type OH 814/1, Radelkis, Hungary).

Spectroscopy. Proton spectra were recorded with a Varian XL-100/15 FT NMR system at 100.1 MHz with use of dioxane (δ 3.70) for internal reference. Carbon-13 spectra were acquired on a Varian XL-100/15 disk-augmented FT NMR system operating at 25.2 MHz. Dioxane (67.71 ppm downfield from Me_4Si) served as internal reference except in Me_2SO-d_6/$CDCl_3$ where Me_4Si was used. UV spectra were recorded with a Zeiss Specord UV-VIS spectrophotometer at three different pH levels (pH 2, 10^{-2} M hydrochloric acid; pH 6, distilled water; pH 10, 10^{-4} M sodium hydroxide). IR spectra were recorded in potassium bromide on a Nicolet 7199 FT IR spectrophotometer. Mass spectra were acquired on an AEI MS-902 double-focusing instrument with ionizing energy of 70 eV and ion source temperatures of 140–170 °C. Samples were introduced by direct probe techniques.

Materials. Triethyl phosphate was vacuum distilled with the exclusion of atmospheric moisture. Phosphoryl chloride and pyridine were freshly distilled from phosphorus pentoxide prior to use. In some cases the 1-(2-deoxy-α-D-ribofuranosyl)-5-alkyluracil 3′,5′-cyclic monophosphate has also been prepared (see, e.g., acidic hydrolysis study).

General Procedure for the Synthesis of 5-Alkyl-2′-deoxyuridine 5′-Monophosphate Diammonium Salts 8–14. To a stirred solution of 5-alkyl-2′-deoxyuridine, **1–7** (2 mmol), in triethyl phosphate (5 mL) was added phosphoryl chloride at 0 °C in two 2-mmol portions separated by 2 h. After 7–16 h at this temperature, the reaction mixture was quenched with ice–water (20 mL). The pH of this solution was adjusted to 3 with 2 M sodium hydroxide. Then the solution was evaporated (<40 °C, 2 kPa) and the triethyl phosphate coevaporated several times with small portions (5 mL) of anhydrous ethanol. After the precipitated inorganic salts were filtered off, water (10 mL) was added to the filtrate, and the pH was adjusted to 7. This solution was applied to a DEAE-Sephadex A-25 (HCO_3^-) column (2.5 × 60 cm). The column was washed with water (ca. 0.5–1 L) until no more UV absorbance (260 nm) was observed in the eluate. Products **8–14** were then eluted (20 mL/10 min per fraction) with a linear gradient of water (1.5 L) and 0.75 M ammonium hydrogen carbonate (1.5 L). In an another workup, the pH of the hydrolyzed reaction mixture was adjusted to 7, after which it was applied to the above column. Anhydrous pyridine (3 × 50 mL) was used to extract the resulting 5′-nucleotides, homogeneous by UV (260 nm) and TLC, from the inorganic contamination, followed by a second anion exchange column chromatography.

5-Ethyl-2′-deoxyuridine 5′-Monophosphate Diammonium Salt (8). Compound **8** appeared in fractions 69–89 (yield 0.52 g, 70%): R_f (system 1) 0.41. EI-MS, *m/e* (relative intensity) 552, M^+ + 3 Me_3Si (24); 537, M^+ + 3 Me_3Si − 15 (6); 624, M^+ + 4 Me_3Si (3); 609, M^+ + 4 Me_3Si − 15 (2).

5-Isopropyl-2′-deoxyuridine 5′-Monophosphate Diammonium Salt (9). Compound **9** appeared in fractions 58–80 (yield 0.45 g, 59%): R_f (system 1) 0.43.

5-*n*-Propyl-2′-Deoxyuridine 5′-Monophosphate Diammonium Salt (10). Compound **10** appeared in fractions 77–96 (yield 0.47 g, 61%): R_f (system 1) 0.44.

5-*n*-Butyl-2′-deoxyuridine 5′-Monophosphate Diammonium Salt (11). Compound **11** appeared in fractions 85–103 (yield 0.40 g, 50%): R_f (system 1) 0.46; ^{13}C NMR data (D_2O) δ 167.2 (C4), 152.7 (C2), 138.5 (C6), 117.1 (C5), 86.7 (d, J_{PC} = 8.7 Hz, C4′), 86.4 (C1′), 72.3 (C3′), 66.0 (d, J_{PC} = 4.8 Hz, C5′), 39.8 (C2′).

5-*n*-Pentyl-2′-deoxyuridine 5′-Monophosphate Diammonium Salt (12). Compound **12** appeared in fractions 93–119 (yield 0.45 g, 55%): R_f (system 1) 0.50.

5-*n*-Hexyl-2′-deoxyuridine 5′-Monophosphate Diammonium Salt (13). Compound **13** appeared in fractions 70–101 (yield 0.38 g, 45%): R_f (system 1) 0.53.

5-*n*-Octyl-2′-deoxyuridine 5′-Monophosphate Diammonium Salt (14). Compound **14** appeared in fractions 101–119 (yield 0.48 g, 53%): R_f (system 1) 0.61.

General Procedure for the Synthesis of 5-Alkyl-2′-deoxyuridine 3′,5′-Cyclic Monophosphate Ammonium Salts 15–21. The 5-alkyl-2′-deoxyuridine 5′-monophosphate diammonium salt, **8–14** (1 mmol), was dissolved in distilled water (10 mL). To this solution was added *N,N′*-dicyclohexyl-4-morpholinecarboxamidine (1 mmol) dissolved in pyridine (10 mL), and the solution thus obtained was evaporated. The foamy residue was dried in vacuum over P_2O_5. The residual 5-alkyl-2′-deoxyuridine 5′-phosphate *N,N′*-dicyclohexyl-4-morpholinecarboxamidine salt was dissolved in dry pyridine (100 mL). This solution was added dropwise to a refluxing solution of dicyclohexylcarbodiimide (DCC, 5 mmol) in dry pyridine (100 mL) over a period of 2 h. The reflux temperature was maintained for an additional 1.5 h after which the solution was evaporated to dryness, and 50 mL each of ether and water were added. The insoluble dicyclohexylurea was filtered off, and the aqueous phase was concentrated to a smaller volume (ca. 20 mL) and applied to a DEAE-Sephadex A-25 (HCO_3^-) column (2.5 × 60 cm). The column was washed with water (ca. 0.5–1.0 L) until no more UV absorbance was observed after which the product, **15–21**, was eluted (20 mL/10 min per fraction) with use of a linear gradient of water (1.5 L) and 0.75 M ammonium bicarbonate (1.5 L).

5-Ethyl-2′-deoxyuridine 3′,5′-Cyclic Monophosphate Ammonium Salt (15). Compound **15** appeared in fractions 47–53 (yield 48%): UV λ_{max} 265, λ_{min} 235 (pH 2); λ_{max} 265, λ_{min} 234 (pH 6); λ_{max} 265, λ_{min} 245 nm (pH 10); IR (KBr) 1242 (P=O), 1088 (POC) cm^{-1}; EI-MS, *m/e* (relative intensity) 390, M^+ + Me_3Si (18); 375, M^+ + Me_3Si − 15 (18): 462, M^+ + 2 Me_3Si (3); 447, M^+ + 2 Me_3Si − 15 (23); ^1H NMR (Me_2SO-d_6/$CDCl_3$) δ 7.20 (H6, s, 1 H), 6.20 (H1′, dd, 1H), 4.41–4.80 (H3′, m, 1 H), 3.92–4.33 (H5′, H5″, m, 2 H), 3.47–3.83 (H4′, m, 1 H), 2.28 (CH_2CH_3, q, 2 H), 1.08 (CH_2CH_3, t, 3 H) H2′; H2″ peaks are overlapped with the CH_2CH_3.

5-Isopropyl-2′-deoxyuridine 3′,5′-Cyclic Monophosphate Ammonium Salt (16). Compound **16** appeared in fractions 41–48 (yield 62%).

5-*n*-Propyl-2′-deoxyuridine 3′,5′-Cyclic Monophosphate Ammonium Salt (17). Compound **17** appeared in fractions 54–61 (yield 41%): ^1H NMR (Me_2SO-d_6/D_2O) δ 7.22 (H6, s, 1 H), 6.12 (H1′, dd, 1 H), 4.37–4.72 (H3′, m, 1 H), 3.90–4.38 (H5′, H5″, m,

(33) Bēres, J.; Bentrude, W. G.; Kruppa, G.; McKernan, P. A.; Robins, R. K. *J. Med. Chem.* **1985**, *28*, 418.
(34) Bēres, J.; Bentrude, W. G.; Kālmān, A.; Pārkānyi, L.; Balzarini, J.; De Clercq, E. *J. Med. Chem.*, preceding paper in this issue.

2 H), 3.31-3.80 (H4′, m, 1 H), 2.04-2.38 (H2′, H2″, m, 2 H), 1.16-1.60 ($(CH_2)_2CH_3$, m, 4 H), 0.78 ($(CH_2)_2CH_3$, t, 3 H).

5-*n*-Butyl-2′-deoxyuridine 3′,5′-Cyclic Monophosphate Ammonium Salt (18). Compound **18** appeared in fractions 63-69 (yield 69%).

5-*n*-Pentyl-2′-deoxyuridine 3′,5′-Cyclic Monophosphate Ammonium Salt (19). Compound **19** appeared in fractions 65-76 (yield 74%): ^{1}H NMR (Me_2SO-d_6) δ 7.31 (H6, s, 1 H), 6.20 (H1′, dd, 1 H), 4.40-4.78 (H3′, m, 1 H), 3.95-4.30 (H5′, H5″, m, 2 H), 3.42-3.76 (H4′, m, 1 H), 2.18-2.41 (H2′, H2″, m, 2 H), 1.32 ($(CH_2)_4CH_3$, br s, 10 H), 0.87 ($(CH_2)_4CH_3$, t, 3 H).

5-*n*-Hexyl-2′-deoxyuridine 3′,5′-Cyclic Monophosphate Ammonium Salt (20). Compound **20** appeared in fractions 68-80 (yield 45%): IR (KBr) 1235 (P=O), 1081 (POC) cm^{-1}.

5-*n*-Octyl-2′-deoxyuridine 3′,5′-Cyclic Monophosphate Ammonium Salt (21). Compound **21** appeared in fractions 81-116 (yield 91%): ^{1}H NMR (Me_2SO-d_6/$CDCl_3$) δ 7.30 (H6, s, 1 H), 6.20 (H1′, dd, 1 H), 4.38-4.75 (H3′, m, 1 H), 3.92-4.35 (H5′, H5″, m, 2 H), 3.45-3.90 (H4′, m, 1 H), 2.10-2.40 (H2′, H2″, m, 2 H), 1.25 ($(CH_2)_7CH_3$, br s, 14 H), 0.80 ($(CH_2)_7CH_3$, br s, 3 H).

Acid Hydrolysis of Compounds 15, 16, and 20. Compounds **15**, **16**, and **20** and for comparison cTMP, 1-(2-deoxy-α-D-ribofuranosyl)-5-isopropyluracil 3′,5′-cyclic phosphate ammonium salt, and 5-isopropyl-2′-deoxyuridine 5′-phosphate diammonium salt, **9** (0.1 mM), were quickly dissolved individually in cold (0 °C) 1 M hydrochloric acid (5 mL). These solutions were then incubated at 37 °C in a thermostat. Aliquots (0.1 mL) were removed from the solutions at certain time intervals. The reactions were quenched by addition of a 1.5 M ammonium hydrogen carbonate 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 with use of authentic samples for identification.

Biology. Antitumor assays were performed according to previously established procedures.[35] L1210/0, L1210/BdUrd, Raji/0, and Raji/TK$^-$ cell lines were characterized as described.[29] Thymidylate synthetase assays were carried out with a partially purified L1210 cell extract as indicated in ref 14.

Antiviral assays were performed as reported previously.[2b] The origin and preparation of the virus stocks have also been documented in ref 2b.

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(35) De Clercq, E.; Balzarini, J.; Torrence, P. F.; Mertes, M. P.; Schmidt, C. L.; Shugar, D.; Barr, P. J.; Jones, A. S.; Verhelst, G.; Walker, R. T. *Mol. Pharmacol.* **1981**, *19*, 321.
(36) De Clercq, E.; Descamps, J.; De Somer, P.; Barr, P. J.; Jones, A. S.; Walker, R. T. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2947.

Oxidation of 5-Hydroxytryptamine and 5,7-Dihydroxytryptamine. A New Oxidation Pathway and Formation of a Novel Neurotoxin

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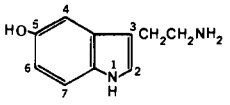
The electrochemical oxidation of 5-hydroxytryptamine (5-HT) in acidic solution proceeds through a minor route leading first to 5,7-dihydroxytryptamine (5,7-DHT) then to 4,5,7-trihydroxytryptamine and finally to 5-hydroxytryptamine-4,7-dione. The latter compound is a major electrochemical oxidation product of 5,7-DHT at pH 2 and 7 and a major autoxidation product at pH ≥6. Preliminary biological results indicate that 5-hydroxytryptamine-4,7-dione is a more potent central nervous system toxin than 5,7-DHT. These results show for the first time a chemical pathway from 5-HT to 5,7-DHT and suggest possible minor metabolic oxidative pathways for the neurotransmitter 5-HT to at least two powerful neurotoxins.

Over the past 30 years a number of reports have appeared concerned with the oxidation of the chemical neurotransmitter 5-hydroxytryptamine (5-HT) in biological systems.[1-9] However, these have been highly speculative



5-HT

reports and, in fact, neither the mechanisms nor even the products of these oxidation reactions are known. It has been suggested[1,2,4,9] that 5-HT might undergo chemical oxidation to a dihydroxytryptamine species. The possiblity of forming dihydroxy derivatives or similar species by biochemical oxidation of 5-HT is intriguing because of the known neurotoxic properties of compounds such as 5,6-dihydroxytryptamine (5,6-DHT) and 5,7-dihydroxytryptamine (5,7-DHT).[10-16] However, there is no evidence in the literature for any chemical pathway from 5-HT to such

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(1) Udenfriend, S. T. E.; Weissbach, H.; Peterson, R. E. *J. Biol. Chem.* **1956**, *219*, 335.
(2) Dalgliesh, C. E. In Proceedings of the Symposium on 5-Hydroxytryptamine; Pergamon Press: New York, 1957; p 58.
(3) Blum, J. J.; Ling, N. S. *Biochem. J.* **1959**, *73*, 530.
(4) McIsaac, W. M.; Page, I. H. *J. Biol. Chem.* **1959**, *234*, 858.
(5) Schneckloth, R. E.; McIsaac, W. M.; Page, I. H. *J. Am. Med. Assoc.* **1959**, *170*, 1143.
(6) Martin, G. M.; Benditt, E. P.; Eriksen, N. *Arch. Biochem. Biophys.* **1960**, *90*, 208.
(7) Barrass, B. C.; Roger, D. B.; Pinder, M.; Skeels, M. *Biochem. Pharmacol.* **1973**, *22*, 2891.
(8) Uemura, T.; Shimazu, J. *Biochem. Biophys. Res. Commun.* **1980**, *93*, 1074.
(9) Eriksen, N.; Martin, G. M.; Benditt, E. P. *J. Biol. Chem.* **1960**, *235*, 1662.
(10) Baumgarten, H. G.; Björklund, A.; Lachenmeyer, L.; Nobin, A.; Stenevi, U. *Acta Physiol. Scand. Suppl.* **1971**, *373*, 1.
(11) Baumgarten, H. G.; Evetts, K. D.; Holman, R. B.; Iversen, L. L.; Vogt, M.; Wilson, G. *J. Neurochem.* **1972**, *19*, 1587.

0022-2623/86/1829-0499$01.50/0