Notes

Synthesis and Biological Effects of Acyclic Pyrimidine Nucleoside Analogues

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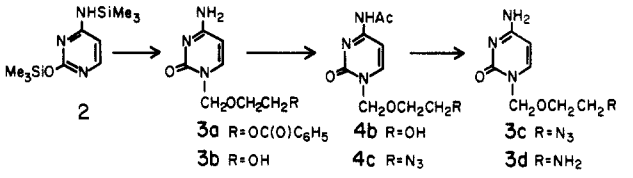
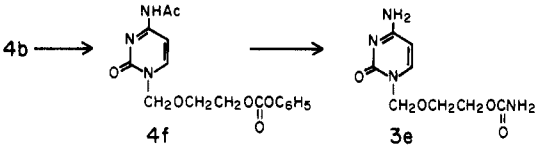
A series of nucleoside analogues has been prepared, wherein the cyclic carbohydrate moiety is replaced by aliphatic side chains attached to cytosine, thymine, uracil, and 5-fluorouracil. The 1-[(2-hydroxyethoxy)methyl] derivatives of these heterocycles were synthesized by reacting the silylated bases with 2-(chloromethoxy)ethyl benzoate, followed by removal of the protecting groups with methanolic ammonia. The hydroxy group of a number of these derivatives was subsequently replaced by an azido, amino, or carbamoyloxy moiety. The 1-(2-oxo-3-butyl) and 1-(2-oxo-3-nonyl) derivatives of cytosine were also prepared, their synthesis being accomplished by condensation of the silylated heterocycle with the appropriate α-halo ketone. At 10^{-4} M concentrations, the newly prepared compounds were inactive against leukemia L-1210 cells in culture. However, a number of the agents inhibited the in vitro growth of *Escherichia coli* K-12, the most potent among these, 1-[(2-hydroxyethoxy)methyl]-5-fluorouracil, being active at an IC_{50} of 1.2 μM. This compound was equally active in preventing the growth of a 5-fluorouracil resistant strain of *E. coli*. Some of the analogues were also found to selectively interfere with herpes simplex virus replication in vitro. None of the cytosine derivatives tested served as either substrates or inhibitors of human liver cytosine nucleoside deaminase.

Analogues of purine or pyrimidine nucleosides in which the cyclic carbohydrate moiety is replaced by acyclic chains mimicking the carbohydrate moiety have long been considered potentially capable of interfering with the activity of various enzymes for which the natural nucleosides or nucleotides serve as substrates. For example, Baker and Kelley[1,2] prepared a series of alkyl, aryl, and aralkyl derivatives of uracil as potential inhibitors of cytosine nucleoside deaminase, and Schaeffer and his co-workers[3-5] synthesized a comprehensive series of acyclic adenine derivatives as inhibitors of adenosine deaminase. Following this lead, we undertook the synthesis of similar derivatives containing the pyrimidine moiety, with the objective of evaluating their activity toward cytosine nucleoside deaminase and cyclic pyrimidine specific phosphodiesterase.[6] While this work was in progress, Kelley et al.[7] presented a preliminary report on the preparation of some 5-substituted 1-[(2-aminoethoxy)methyl]uracils as candidate antiviral compounds, and Schaeffer et al.[8] and Elion et al.[9] reported the selective inhibition of herpes simplex virus replication by 9-[(2-hydroxyethoxy)-methyl]guanine. Evaluation of the potential antiherpetic activity of the newly prepared compounds was therefore included in the biological screen. A variety of aliphatic nucleoside analogues have also been prepared by other investigators, and some of these agents have exhibited varied biological effects.[10-18]

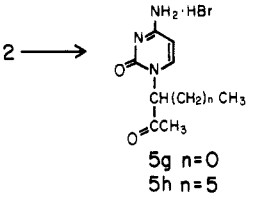
Scheme I



Scheme II



Scheme III



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In this paper we describe the synthetic procedures used for obtaining the 1-[(2-hydroxyethoxy)methyl] and related derivatives of various pyrimidine bases and report the effect they have on the in vitro growth of leukemia L-1210 and *E. coli* K12 cells, on HSV replication, and on the activity of human liver cytosine nucleoside deaminase.

Chemistry. The compounds listed in Table I were synthesized by the procedures outlined in Schemes I–III. 1-[[2-(Benzoyloxy)ethoxy]methyl]cytosine (**3a**) was prepared by reacting 2-(chloromethoxy)ethyl benzoate (**1**) and the silyl derivative of cytosine (**2**) in dry toluene, **1** having been obtained by reaction of ethylene glycol monobenzoate[19] with paraformaldehyde and anhydrous hydrogen chloride in 1,2-dichloroethane at 0 °C.[20] Conversion of **3a** to 1-[(2-hydroxyethoxy)methyl]cytosine (**3b**)[21] was accomplished at 5 °C using methanolic ammonia. Synthesis of the analogous uracil, thymine, and 5-fluorouracil derivatives (**6a**, **7a**, **8a**, **6b**, **7b**, and **8b**) was achieved by similar procedures. The 4-amino group of **3b** was selectively acetylated using acetic anhydride in refluxing absolute ethanol[22] to provide **4b**, which was directly converted to the azido derivative **4c** by reaction with triphenylphosphine, lithium azide, and carbon tetrabromide in anhydrous dimethylformamide at room temperature.[23] Treatment of **4c** with ammonia in dry methanol yielded **3c**, which was catalytically reduced to the amino derivative **3d** using palladium on charcoal in methanol (Scheme I). The 2-azido- and 2-[(aminoethoxy)methyl] derivatives of thymine (**7c** and **7d**) were prepared similarly from **7b**. The carbamoyloxy derivative **3e** was synthesized by reacting **4b** with phenyl chloroformate in pyridine and methylene chloride, followed by treatment of the intermediate **4f** with concentrated ammonium hydroxide at room temperature (Scheme II). The cytosine derivatives **5g** and **5h** were prepared by treatment of **2** in dry toluene with 3-bromo-2-butanone or 3-bromo-2-nonanone,[24] respectively (Scheme III). 1-Isopropylcytosine (**9**) was prepared using the general procedure of Ward and Baker[25] (method A) in which **2** was reacted with 2-iodopropane in acetonitrile–hexamethyldisilazane. 4-Methoxy-1-methyl-2-pyrimidinone (**10**) and 1,N^4,N^4-trimethylcytosine (**11**) were prepared according to literature procedures,[26,27] with minor modification.[28]

Biological Evaluation. The effect the newly prepared compounds exerted on the in vitro growth of *E. coli* K12 cells is summarized in Table I. Among these compounds, the 5-fluorouracil derivatives **8a** and **8b** with IC_{50} values of 9.0 and 1.2 μM were the most potent inhibitors. These two agents retained much of their activity against a strain of *E. coli* K12 resistant to 10^{-3} M 5-fluorouracil, indicating that their inhibitory effect was not mediated via their conversion to free 5-fluorouracil. The fact that their ability to inhibit *E. coli* cell growth is diminished by the natural pyrimidines (Table II) in the same order of effectiveness as are 5-fluorouracil and its nucleoside derivatives[29] would suggest that the inhibition occurs along the metabolic path leading to DNA synthesis.

Significant growth inhibitory activity was also effected by the nonyl ketone (**5h**) and by the 1-[(2-hydroxyethoxy)methyl] (**3b**) derivatives of cytosine. The side chain of the latter compound is held to mimic the carbohydrate moiety of cytidine, its hydroxyl function corresponding to the 5′-hydroxy group of the intact nucleoside. It is of interest to note, therefore, that the replacement of this hydroxy group with an azido (**3c**) or amino (**3d**) function or its protection with a benzoyl group (**3a**) led to a pronounced decrease in cell growth inhibitory activity, as did the acetylation of the exocyclic amino group. The 1-[(2-hydroxyethoxy)methyl] derivative of uracil (**6b**) was less active an inhibitor than was the cytosine analogue, and with thymine as the heterocycle (**7b**), activity was abolished unless the hydroxy group was replaced by an amino or azido function. The metabolic site at which these agents exert their effect remains to be determined. It is noteworthy that simple alkyl derivatives, such as 4-methoxy-1-methyl-2-pyrimidinone (**10**), 1,N^4,N^4-trimethylcytosine (**11**), and 1,3-dimethyluracil (**12**), also demonstrated significant inhibitory activity in this test system (Table III).

The compounds listed in Tables I and III were also examined for their effect upon leukemia L-1210 cell growth in culture but were found to be inactive against this cell line, even at 10^{-4} M concentrations.

An initial evaluation of the ability of the newly prepared agents to inhibit the replication of herpes simplex virus type 1 (KOS) in CV-1 monkey kidney cells was carried out by use of a disk assay. The results showed that compounds **3a**, **3b**, and **4f** at the 5×10^{-4} M concentrations at which they were applied to the disks selectively interfered with viral replication. Quantitative assays are currently being carried out to define their potency more closely.

The unblocked cytosine derivatives listed in Table I were also evaluated for their ability to serve as substrates or inhibitors of a cytosine nucleoside deaminase partially purified from human liver, but they were found to be inactive with respect to both functions. The capacity of the compounds to inhibit cyclic CMP specific phosphodiesterase will be reported elsewhere.

Experimental Section

Melting points were determined with a Mel-Temp apparatus and are uncorrected. UV spectra were obtained on a Beckman Model 25 spectrophotometer. For measuring UV spectra at pH 7, the compounds were dissolved in 0.05 M KH_2PO_4 buffer. NMR spectra were recorded on either a Varian A-60A or XL-100 spectrophotometer, and δ values are reported downfield from a tetramethylsilane internal standard, except in the case of D_2O solutions where an external Me_4Si standard was used. IR spectra were obtained on a Perkin-Elmer 457 spectrophotometer. Dry column chromatography was carried out in nylon foil tubes using Silica Woelm DCC (ICN 404526). Bands were visualized with

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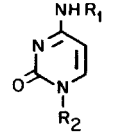
Table I. Some Physical, Chemical, and Biological Properties of Acyclic Pyrimidine Nucleoside Analogues



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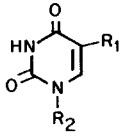

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compd	R_1	R_2	mp, °C	yield,[a] %	recrystn solvent	formula[b]	anal.	*E. coli* IC_{50},[c] μM	*E. coli*/FU IC_{50},[d] μM
3a	H	$CH_2OCH_2CH_2OC(O)C_6H_5$	187-188	45	H_2O[e]	$C_{14}H_{15}N_3O_4$	C, H, N	900	
3b	H	$CH_2OCH_2CH_2OH$	160-161[f]	85	EtOH	$C_7H_{11}N_3O_3$	C, H, N	87	
3c	H	$CH_2OCH_2CH_2N_3$	161-162	75	H_2O	$C_7H_{10}N_6O_2$	C, H, N	440	
3d	H	$CH_2OCH_2CH_2NH_2$	166-170 dec[g]	100[g]	*g*	$C_9H_{14}N_4O_3$[h]	C, H, N[h]	>1000	
3e	H	$CH_2OCH_2CH_2OC(O)NH_2$	176-177	95	EtOH	$C_8H_{12}N_4O_4$	C, H, N	910	
4b	$C(O)CH_3$	$CH_2OCH_2CH_2OH$	145-146	61	EtOH	$C_9H_{13}N_3O_4$	C, H, N	>1000	
4c	$C(O)CH_3$	$CH_2OCH_2CH_2N_3$	130-131	45	CH_2Cl_2-pet. ether	$C_9H_{12}N_6O_3$	C, H, N	>1000	
4f	$C(O)CH_3$	$CH_2OCH_2CH_2OC(O)OC_6H_5$	132-133	59	EtOH	$C_{16}H_{17}N_3O_6$	C, H, N	>1000	
5g	H (HBr salt)	$CH(CH_3)C(O)CH_3$	210-211 dec	47	EtOH	$C_8H_{11}N_3O_2HBr$	C, H, N, Br	560	
5h	H (HBr salt)	$CH(n\text{-}C_6H_{13})C(O)CH_3$	*i*	19	MeOH[i]	$C_{19}H_{25}N_7O_5$[i]	H, N; C[i,j]	30	
6a	H	$CH_2OCH_2CH_2OC(O)C_6H_5$	100-101	72	MeOH	$C_{14}H_{14}N_2O_5$	C, H, N	100	
6b	H	$CH_2OCH_2CH_2OH$	138-139	80	EtOH	$C_7H_{10}N_2O_4$	C, H, N	320	
7a	CH_3	$CH_2OCH_2CH_2OC(O)C_6H_5$	115-116	76	EtOH	$C_{15}H_{16}N_2O_5$	C, H, N	860	
7b	CH_3	$CH_2OCH_2CH_2OH$	140-141	94	EtOH	$C_8H_{12}N_2O_4$	C, H, N	>1000	
7c	CH_3	$CH_2OCH_2CH_2N_3$	143-144	64	H_2O	$C_8H_{11}N_5O_3$	C, H, N	250	
7d	CH_3	$CH_2OCH_2CH_2NH_2$	128-129	38	*k*	$C_{10}H_{15}N_3O_4$[l]	C, H, N[l]	310	
8a	F	$CH_2OCH_2CH_2OC(O)C_6H_5$	116-117	51	EtOH-H_2O	$C_{14}H_{13}N_2O_5F$	C, H, N, F	9.0	180
8b	F	$CH_2OCH_2CH_2OH$	154-155	72	*n*-PrOH	$C_7H_9N_2O_4F$	C, H, N, F	1.2	4.2

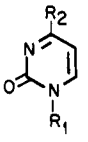
[a] Yield of product after purification by crystallization or chromatography. No effort was made to optimize yields. [b] Elemental analyses were within 0.40% of theoretical values unless indicated otherwise. [c] IC_{50} is the concentration required for 50% inhibition of *E. coli* K-12 growth. [d] A strain of *E. coli* K-12 resistant to 10^{-3} M 5-fluorouracil. [e] pH 5.5. [f] Literature[21] mp 158-159 °C. [g] Refers to product obtained as a white solid after evaporation from an EtOH-Et_2O mixture, which resisted crystallization. [h] Formula and analyses are for the side-chain *N*-acetyl derivative, mp 194-195 °C. [i] Refers to the 2,4-dinitrophenylhydrazone derivative, mp 196-197 °C. [j] C: calculated, 52.89; found, 52.44. [k] Crystallization occurred after concentrating an aqueous solution to a syrup. [l] Formula and analyses are for the side-chain *N*-acetyl derivative, mp 180-181 °C.

Table II. Effect of Natural Pyrimidines on the Cytotoxicity of 1-[(2-Hydroxyethoxy)methyl]-5-fluorouracil (8b) to a 5-Fluorouracil-Resistant[a] Strain of *Escherichia coli*

metabolite present at 10^{-3} M concn	IC_{50},[b] μM
none	4.2
cytosine	7.4
uracil	8.4
cytidine	9.1
2′-deoxycytidine	9.5
uridine	240
2′-deoxyuridine	370
thymine	730
thymidine	1000

[a] Resistant to 10^{-3} M 5-fluorouracil. [b] IC_{50} is the concentration of 8b required for 50% inhibition of growth.

Table III. Effect of Various Simple Alkylpyrimidines on *E. coli* K-12 Growth



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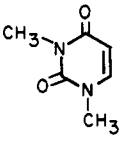

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compd	R_1	R_2	IC_{50},[a] μM
9	$CH(CH_3)_2$	NH_2	>1000
10	CH_3	OCH_3	61
11	CH_3	$N(CH_3)_2$	80
12[b]			15

[a] Concentration for 50% inhibition of growth. [b] Obtained from ICN Pharmaceuticals, Inc., K & K Labs Division, Life Sciences Group.

UV light and separated by slicing. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

1-[[2-(Benzoyloxy)ethoxy]methyl]cytosine (3a). Dry HCl was bubbled for 2.5 h through an ice-cooled mixture of ethylene glycol monobenzoate (5.00 g, 30.0 mmol) and paraformaldehyde (0.90 g, 30.0 mmol of CH_2O units) in dry 1,2-dichloroethane (5 mL). The mixture was dried ($MgSO_4$), filtered, and concentrated to give 6.18 g of a clear oil of 2-(chloromethoxy)ethyl benzoate (1; ~87% pure by NMR). Cytosine (2.98 g, 26.8 mmol) was trimethylsilylated by heating to 120–130 °C with 1,1,1,3,3,3-hexamethyldisilazane (HMDS; 10 mL) and a few milligrams of $(NH_4)_2SO_4$, with protection from moisture, until all solid had reacted. The resulting solution was coevaporated twice with 10-mL portions of dry toluene. Crude 1 (5.49 g) was added to the silylated cytosine 2 dissolved in hot (70–80 °C) dry toluene (30 mL). Following stirring at room temperature overnight, the mixture was heated to 70 °C for 3 h and, after cooling, 5 mL of EtOH was added. The mixture was concentrated, the residue was triturated with 95% EtOH, and the solid was filtered and dried to yield 4.44 g of crude **3a**. Crystallization from water (18 mL) and aqueous 1 N NaOH (7.5 mL) gave 3.47 g (12.0 mmol, 45%) of white crystals of **3a**: mp 184–185 °C. Recrystallization yielded an analytical sample: mp 187–188 °C; UV (MeOH) λ_{max} 270 nm (ε 8330); NMR (CF_3COOH) δ 3.71 and 4.17 (m, 4, CH_2CH_2), 5.02 (s, 2, OCH_2N), 5.88 (d, 1, C_5 H, J = 7.5 Hz), 7.02 and 7.63 (m, 8, NH_2, C_6H, ArH). Anal. ($C_{14}H_{15}N_3O_4$) C, H, N.

1-[(2-Hydroxyethoxy)methyl]cytosine (3b). To a saturated solution of dry NH_3 in MeOH (50 mL) at 0 °C was added **3a** (526 mg, 1.82 mmol). The mixture was kept at 4–5 °C for 5 days and then was concentrated, and the residue was partitioned between CH_2Cl_2 and H_2O. The H_2O layer was washed once with CH_2Cl_2 and then was concentrated to a clear syrup. The syrup was coevaporated with EtOH to give a white solid (356 mg), which was recrystallized from EtOH to yield 285 mg (1.54 mmol, 85%) of white crystals of **3b**: mp 159–160 °C. Recrystallization gave an analytically pure sample: mp 160–161 °C (lit.[21] mp 158–159 °C); UV (pH 7) λ_{max} 270 nm (ε 7540); NMR (D_2O) δ 3.67 (s, 4, CH_2CH_2), 5.18 (s, 2, OCH_2N), 5.99 (d, 1, C_5H, J = 7.5 Hz), 7.66 (d, 1, C_6 H, J = 7.5 Hz). Anal. ($C_7H_{11}N_3O_3$) C, H, N.

1-[(2-Azidoethoxy)methyl]cytosine (3c). Compound **4c** (491 mg, 1.95 mmol) was added to dry MeOH (35 mL) saturated with dry NH_3 at 0 °C. The tightly stoppered mixture was stirred overnight at 5 °C, filtered from a small amount of insoluble solid, and concentrated. The residue was dissolved in hot EtOH and reconcentrated to give off-white crystals (510 mg). These were recrystallized from H_2O (1 mL) to yield 307 mg (1.46 mmol, 75%) of white crystals of **3c**, mp 160–161 °C. Recrystallization from H_2O gave analytically pure **3c**: mp 161–162 °C; UV (pH 7) λ_{max} 269 nm (ε 7600); IR (KBr) 2105 cm^{-1} (N_3); NMR (D_2O) δ 3.94 and 4.25 (m, 4, $OCH_2CH_2N_3$), 5.69 (s, 2, NCH_2O), 6.48 (d, 1, C_5 H, J = 7.5 Hz), 8.14 (d, 1, C_6 H, J = 7.5 Hz). Anal. ($C_7H_{10}N_6O_2$) C, H, N.

1-[(2-Aminoethoxy)methyl]cytosine (3d). Compound **3c** (250 mg, 1.19 mmol) was dissolved in hot MeOH (50 mL) and stirred with 10% Pd on charcoal (25 mg) under H_2 at atmospheric pressure and room temperature for 4 h. The mixture was filtered through Celite Filter Aid and concentrated to give 227 mg (1.23 mmol, 100% crude yield) of a white solid of **3d**, which was resistant to crystallization: mp 166–170 °C dec; UV (pH 7) λ_{max} 268 nm; NMR (D_2O) δ 3.28 (m, 2, CH_2NH_2), 4.08 (t, 2, OCH_2CH_2, J = 5.5 Hz), 5.66 (s, 2, NCH_2O), 6.48 (d, 1, C_5H, J = 7.5 Hz), 8.13 (d, 1, C_6H, J = 7.5 Hz). N-Acetylation of the side-chain amino group was carried out by treating **3d** (100 mg, 0.544 mmol) in ice-cold absolute MeOH (2 mL) with acetic anhydride (0.057 mL, 0.598 mmol). After 30 min at 0 °C, the solution was stirred overnight at room temperature and concentrated, and the residue was purified by dry column (20 mm) chromatography, using 25 g of silica gel and EtOH as the eluent. The band which migrated from the origin was sliced from the nylon foil column and eluted with MeOH. The MeOH elutate was evaporated to give a white solid (66 mg), which was recrystallized from EtOH to give 43 mg of the *N*-acetyl derivative of **3d**: mp 194–195 °C; UV (pH 7) λ_{max} 269 nm (ε 7440). Anal. ($C_9H_{14}N_4O_3$) C, H, N.

1-[[2-(Carbamoyloxy)ethoxy]methyl]cytosine (3e). Compound **4f** (145 mg, 0.418 mmol) was stirred with concentrated NH_4OH (25 mL) overnight at room temperature and then was concentrated to a syrup. Upon standing, the syrup crystallized, and the crystals were triturated with CH_2Cl_2 and Et_2O to yield 90 mg (0.395 mmol, 95%) of white crystals of **3e**. Recrystallization from EtOH, twice in succession, afforded an analytically pure sample of **3e**: mp 176–177 °C; UV (pH 7) λ_{max} 269 nm (ε 7100); IR (KBr) 1700 cm^{-1} (carbamate C=O); NMR (D_2O) δ 4.30 and 4.66 (m, 4, CH_2CH_2), 5.70 (s, 2, NCH_2O), 6.50 (d, 1, C_5 H, J = 7.5 Hz), 8.14 (d, 1, C_6 H, J = 7.5 Hz). Anal. ($C_8H_{12}N_4O_4$) C, H, N.

1-[(2-Hydroxyethoxy)methyl]-N^4-acetylcytosine (4b). To a hot solution of **3b** (3.9 g, 21.1 mmol) in absolute EtOH (400 mL) was added acetic anhydride (Ac_2O, 4.0 mL). The mixture was stirred and refluxed with protection from moisture. Five additional aliquots of Ac_2O were added at hourly intervals, and the solution was refluxed an additional hour after the last addition. A small quantity of insoluble solid was filtered off and the filtrate was concentrated to a syrup. The syrup was dissolved with H_2O, after evaporation of the solvent the process was repeated with EtOH, and then the residue was crystallized from EtOH–Et_2O to give 2.90 g (12.8 mmol, 61%) of white crystals of **4b**: mp 144–145 °C. Recrystallization from EtOH gave analytically pure **4b**: mp 145–146 °C; UV (pH 7) λ_{max} 297 nm (ε 7180), 247 (15500); NMR (D_2O) δ 2.68 (s, 3, $NHCOCH_3$), 4.17 (s, 4, CH_2CH_2), 5.82 (s, 2, NCH_2O), 7.81 (d, 1, C_5H, J = 7.5 Hz), 8.58 (d, 1, C_6H, J = 7.5 Hz). Anal. ($C_9H_{13}N_3O_4$) C, H, N.

1-[(2-Azidoethoxy)methyl]-N^4-acetylcytosine (4c). Ph_3P (2.18 g, 8.31 mmol), LiN_3 (939 mg, 19.2 mmol), and **4b** (1.45 g, 6.39 mmol) were dissolved in anhydrous DMF (45 mL), the solution was cooled on ice, and CBr_4 (3.18 g, 9.58 mmol) was added with stirring. The tightly stoppered mixture was then kept at room temperature for 24 h and MeOH (7.5 mL) was added to quench the reaction. The solvent was evaporated in vacuo, and the residue was purified by dry column chromatography using 200 g of silica gel and developing the 30-mm diameter column with EtOAc. The band with the second highest mobility was

eluted with EtOAc–EtOH. The eluate was concentrated to give 718 mg (2.85 mmol, 45%) of off-white crystals of **4c**: mp 116–124 °C. Recrystallization from CH_2Cl_2–petroleum ether gave white crystals (493 mg): mp 130–131 °C; UV (pH 7 with 4% EtOH) λ_{max} 297 nm (ϵ 6320), 248 (14000); IR (KBr) 2080 cm^{-1} (N_3); NMR (Me_2SO-d_6) δ 2.12 (s, 3, CH_3CO), 3.44 and 3.78 (m, 4, CH_2CH_2), 5.27 (s, 2, NCH_2O), 7.20 (d, 1, C_5 H, J = 7.5 Hz), 8.13 (d, 1, C_6 H, J = 7.5 Hz). Anal. ($C_9H_{12}N_6O_3$) C, H, N.

1-[[2-[(Phenoxycarbonyl)oxy]ethoxy]methyl]-N^4-acetylcytosine (4f). A solution of phenyl chloroformate (0.073 mL, 0.55 mmol) in dry CH_2Cl_2 (2 mL) was added dropwise to **4b** (125 mg, 0.55 mmol) in dry pyridine (6 mL) kept cool in an ice bath. After the addition was completed, the mixture was stirred at 0 °C for 30 min and then at room temperature for 4 h. The resulting solution was concentrated in vacuo and the residual syrup was dissolved in CH_2Cl_2. The solution was extracted three times with H_2O and then was dried over $MgSO_4$ and concentrated to 142 mg of a thick frothy syrup. This product was crystallized from EtOH to give 113 mg (0.326 mmol, 59%) of white crystals of **4f**: mp 131–132 °C. Recrystallization from EtOH yielded 72 mg of analytically pure **4f**: mp 132–133 °C; UV (pH 7 with 4% EtOH) λ_{max} 297 nm (ϵ 6430), 248 (14000); NMR ($CDCl_3$) δ 2.26 (s, 3, $COCH_3$), 3.98 and 4.41 (m, 4, OCH_2CH_2O), 5.40 (s, 2, NCH_2O), 7.34 (m, 6, C_5 H and ArH), 7.78 (d, 1, C_6 H, J = 7.5 Hz), 9.56 (br s, 1, NH). Anal. ($C_{16}H_{17}N_3O_6$) C, H, N.

1-(2-Oxo-3-butyl)cytosine Hydrobromide (5g). Cytosine (5.00 g, 45.0 mmol) was trimethylsilylated using 12.5 mL of HMDS as described in the procedure for **3a**. The residual solid was dissolved in dry toluene (30 mL) while heating to 55 °C, and 3-bromo-2-butanone (4.7 mL, 45 mmol) was added. The mixture was stirred at 55–60 °C for 2.5 h, additional 3-bromo-2-butanone (3.0 mL) was added, and heating at 55 °C was continued for 1 h and 40 min. EtOH (25 mL) was added, causing the separation of an amorphous solid that gradually crystallized at room temperature to give 8.68 g (33.1 mmol, 74%) of **5g** as a light tan solid. The product was decolorized with activated charcoal (Norit A) in H_2O and recrystallized from EtOH to yield 5.50 g (21.0 mmol, 47%) of white crystals of **5g**: mp 210–211 °C dec; UV (pH 7) λ_{max} 272 nm (ϵ 9100); NMR (D_2O) δ 1.66 (d, 3, CH_3CH, J = 7.5 Hz), 2.32 (s, 3, CH_3CO), 5.19 (q, 1, CH_3CH, J = 7.5 Hz), 6.24 (d, 1, C_5 H, J = 7.5 Hz), 7.83 (d, 1, C_6 H, J = 7.5 Hz). Anal. ($C_8H_{11}N_3O_2$·HBr) C, H, N, Br.

1-(2-Oxo-3-nonyl)cytosine Hydrobromide (5h). Cytosine (500 mg, 4.50 mmol) was refluxed for 1 h in HMDS (2.0 mL) containing a trace of $(NH_4)_2SO_4$. The mixture was coevaporated with dry toluene (5 mL), and the residue was dissolved in dry toluene (5 mL) and concentrated. With protection from moisture, 3-bromo-2-nonanone[24] (0.62 mL, 3.40 mmol) was added dropwise to a solution of the silylated cytosine in toluene (5 mL), and the mixture was stirred at 55–60 °C for 5 h and at room temperature overnight. After the mixture was treated with EtOH (1 mL), the precipitate was removed by filtration and washed with a small amount of EtOH to give a light tan solid (651 mg). Separation of the product from unreacted cytosine was achieved by column chromatography, using 60 g of silica gel which had been preequilibrated with 10% by weight of the developing solvent, and developing the 20-mm diameter column with $CHCl_3$–EtOH (1:1). The highest mobility band was eluted with MeOH and then with EtOH–$CHCl_3$ (1:1), and the combined eluate was concentrated to give 212 mg (0.639 mmol, 19%) of a light brown, stiff syrup of **5h** which was resistant to crystallization: UV (1 N HCl) λ_{max} 281 nm; UV (0.01 N NaOH) λ_{max} 273 nm; NMR ($CDCl_3$) δ 0.87 (m, 3, CH_2CH_3), 1.28 [m, 8, $(CH_2)_4CH_3$], 1.96 (br m, 2, $NCHCH_2$), 2.23 (s, 3, $COCH_3$), 5.28 (m, 1, $NCHCH_2$), 5.88 (d, 1, C_5 H, J = 7.5 Hz), 7.22 (d, 1, C_6 H, J = 7.5 Hz). The 2,4-dinitrophenylhydrazone derivative of **5h** was prepared,[30] mp 196–197 °C. Anal. ($C_{19}H_{25}N_7O_5$) H, N; C: calcd, 52.89; found, 52.44.

1-[[2-(Benzoyloxy)ethoxy]methyl]pyrimidines (6a, 7a, and 8a). These compounds were prepared according to the procedure used for the synthesis of **3a** with the following exceptions: 2-(chloromethoxy)ethyl benzoate (1) was added to the silylated pyrimidine dissolved in toluene at room temperature. **6a** was prepared by stirring the mixture at room temperature for 3 days and then heating to 50–60 °C for 1.25 h. For the preparation of **7a**, the reaction mixture was stirred at room temperature for 24 h without heating. **8a** was synthesized by stirring the mixture overnight at room temperature and then heating at 75–80 °C for 10 h. In order to remove any trace of 5-fluorouracil (5-FU) that may have remained in the preparation prior to its biological assay, **8a** was chromatographed on a silica gel 60 F-254 (2-mm thick layer) TLC plate (E. Merck 5766) using 1:1 $CHCl_3$–Me_2CO as the solvent. In this solvent, 5-FU has a lower R_f than **8a**. The single band obtained was scraped off the plate and was sequentially eluted with CH_2Cl_2, Me_2CO, and EtOH. The residue obtained after evaporation of the combined eluate was crystallized from EtOH–H_2O. This purified sample was also used in the synthesis of **8b**.

1-[(2-Hydroxyethoxy)methyl]pyrimidines (6b, 7b, and 8b). These compounds were prepared using the same procedure used for **3b**.

1-[(2-Azidoethoxy)methyl]thymine (7c). This compound was prepared by the same procedure used for **4c**, with the exception that, after the reaction mixture was concentrated, EtOH was added and the mixture was again concentrated. The residue was triturated with MeOH–$CHCl_3$ (1:1), and the crystals were filtered to yield **7c**.

1-[(2-Aminoethoxy)methyl]thymine (7d). This compound was prepared according to the procedure used for the preparation of **3d**, with the following modifications in the workup: following filtration of the reaction mixture, the filtrate was concentrated and dissolved in H_2O, and a small amount of residue removed by filtration. The aqueous filtrate was placed on a Bio-Rad AG 50W-X8 (50–100 mesh, H^+ form) ion-exchange column, the column was washed with H_2O until UV absorbance at 267 nm was negligible, and the product was eluted with 0.5 N NH_4OH to give a viscous syrup which recrystallized on standing. The crystals were triturated with CH_2Cl_2–EtOH (2:1) and filtered to give **7d**.

1-Isopropylcytosine (9). Compound **9** was prepared essentially by the method of Ward and Baker.[25] Cytosine (1.00 g, 9.00 mmol) was mixed with HMDS (4.0 mL) and a few milligrams of $(NH_4)_2SO_4$, and the mixture was heated at reflux until all of the cytosine had reacted (1 h). After the mixture cooled, 2-iodopropane (0.93 mL, 1.58 g, 9.30 mmol) in dry acetonitrile (10 mL) was added to the silylated cytosine, and the mixture was heated with protection from moisture at 50 °C for 19 h and was then heated an additional 24 h at 70 °C. The solution was cooled and EtOH (10–15 mL) was added with stirring. After 1 h the yellowish white solid was filtered off to give 708 mg of crystals of cytosine containing only a small amount of **9**, as determined by TLC and UV. On standing for 2 weeks, 581 mg (2.07 mmol, 23%) of white crystals of **9** in the form of the HI salt was recovered from the mother liquor: NMR (Me_2SO-d_6) δ 1.28 (d, 6, CH_3, J = 7 Hz), 4.74 (m, 3, NCH and NH_2), 5.98 (d, 1, C_5 H, J = 7.5 Hz), 7.97 (d, 1, C_6 H, J = 7.5 Hz). This crude product (100 mg), dissolved in H_2O, was placed on a small Bio-Rad AG1-X10 200–400 mesh (OH^- form, 8 mL) ion-exchange column, and **9** was eluted with water. Concentration of the eluate gave a white solid (76 mg), which was recrystallized from EtOH to give 26 mg of white crystals of **9**: mp 203 °C (lit.[31] mp 201–203 °C); UV (0.01 N HCl) λ_{max} 285 nm (ϵ 13100); UV (0.01 N NaOH) λ_{max} 275 nm (ϵ 8810).

Biological Assay Procedures. The procedures used for evaluating the effect of the agents on the growth of *E. coli* and leukemia L-1210 cells have been described previously.[32,33] The antiviral assay used is a modification of a filter paper disk method described by Herrmann.[34] One million monkey kidney cells (CV-1) were introduced into 35-mm diameter plastic tissue culture wells (Costar) in a modified Eagle medium containing 5% calf serum (EM5C). On the following day, the monolayers were infected with 200 PFU of HSV-1, strain KOS. After virus adsorption, the cells were overlaid with EM5C medium containing 1% 4K CPS methylcellulose. The compounds were applied to 0.5-in. analytical paper disks (Schleicher and Schuell), and after evaporation of the solvent, they were placed on the medium. EM5C medium containing 2% 15 CPS methylcellulose and 100

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μg/mL Neutral red was added 2 days after infection, and the results were read the following day. Cytosine nucleoside deaminase was partially purified from human liver, obtained at autopsy, and assayed ("after autopsy") according to the procedures of Wentworth and Wolfenden.[35] The analogues and the substrate 2′-deoxycytidine were added to the assay mixtures at 10^{-4} M concentrations.

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Carbocyclic Analogues of 5-Fluorouracil Nucleosides

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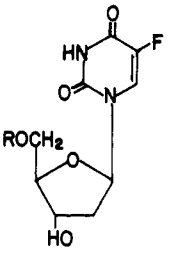
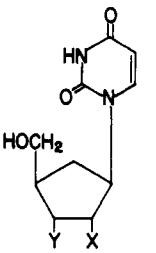
The carbocyclic analogues of 5-fluoro-2′-deoxyuridine (5-FdUrd, 1), 5-fluorouridine, and 5-fluoro-3′-deoxyuridine were prepared by fluorination of the uracil nucleoside analogues with elemental fluorine. The 5-FdUrd analogue (C-5-F-2′-dUrd, **6**) was enzymatically phosphorylated to the analogue of 5-FdUrd 5′-phosphate and inhibited the incorporation of 2′-deoxyuridine into DNA of murine colon 26 tumor cells and L-1210 cells in culture. Biochemical studies also indicated that C-5-F-2′-dUrd (**6**) was a less potent inhibitor of DNA synthesis in tumor cells than was 5-FdUrd (1). C-5-F-2′-dUrd was cytotoxic (ED_{50} = 2.5 mcg/mL) to L-1210 cells in culture; the other two analogues were less cytotoxic. C-5-F-2′-dUrd was inactive—or, at best, borderline active—in tests against P-388 leukemia in vivo.

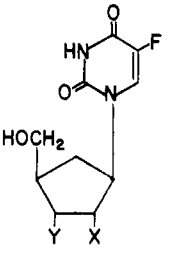
5-Fluorouracil (5-FUra)[1] is an important clinical anticancer drug, particularly in the treatment of gastrointestinal and mammary tumors.[2-6] 5-FUra proceeds through the same anabolic and catabolic steps as does uracil.[2,7] It may be transformed enzymatically to 5-fluorouridine[8,9] (5-FUrd), to 5-fluoro-2′-deoxyuridine[9] (5-FdUrd, 1), or (by reaction with 5-phosphoribosyl-1-pyrophosphate) to 5-fluorouridine 5′-phosphate[10,11] (5-FUMP)[1]. The nucleosides, 5-FUrd and 5-FdUrd (1), may, in turn, be converted by kinases to the nucleotides, 5-FUMP[7-9] and 5-fluoro-2′-deoxyuridine 5′-phosphate[9] (5-FdUMP, 2), and 5-FUMP may be phosphorylated further to the di- and triphosphate.[7] 5-FdUMP (2) is a potent inhibitor of thymidylate synthetase,[12] and 5-FUra, after metabolism to 5-FUTP, is incorporated into RNA;[13] however, 5-FUra and its nucleosides and nucleotides act at several biochemical loci and produce a multiplicity of biological effects.[2] Furthermore, the nucleotides may be converted to nucleosides by the action of phosphatases, and the nucleosides are subject to the action of phosphorylases, which cleave them to 5-FUra.[7,14] The result of these biochemical interconversions among 5-FUra, 5-FUrd, 5-FdUrd, and the nucleotides is that administration of any one of these derivatives may affect the sites of action of all of them. Thus, the anticipated effectiveness of 5-FdUrd (1), an immediate precursor of the thymidylate synthetase in-



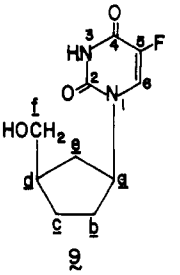
1: R = H
2: R = $PO(OH)_2$



3: X = H, Y = OH
4: X = OH, Y = H
5: X = Y = OH



6: X = H, Y = OH
7: X = OH, Y = H
8: X = Y = OH



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Letter a-f specify positions for NMR assignments.

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