Synthesis and Biological Activity of a New Class of Cytotoxic Agents: *N*-(3-Oxoprop-1-enyl)-Substituted Pyrimidines and Purines

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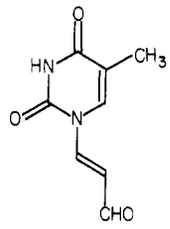
Received November 7, 1983

The 1-(3-oxoprop-1-enyl) derivatives of thymine and cytosine and the corresponding 9-substituted derivatives of adenine and guanine (products of degradation of DNA by bleomycin, Fe^{2+}, and O_2) have been synthesized and tested for biological activity. The thymine and adenine compounds are highly cytotoxic to a variety of tumor cell lines and inhibit macromolecular synthesis in cultured HeLa cells. Structure–activity studies, based primarily on the pyrimidine derivatives, reveal that the most potent inhibition occurs when the propenal group is located on the 3-nitrogen of a 2′-deoxyribonucleoside. The 3-(3-oxoprop-1-enyl) derivatives of thymidine, 2′-deoxyuridine, and 5-iodo-2′-deoxyuridine powerfully and selectively inhibit incorporation of thymidine into DNA at concentrations ($IC_{50} \approx 0.5\ \mu M$) comparable to those observed with idoxuridine. Active compounds in this series react readily with nucleophiles containing primary amino and sulfhydryl groups. The results of this study provide a basis for the development of a new class of cytotoxic agents.

In recent studies dealing with the action of bleomycin on DNA, we described the isolation of four analogous degradation products, **1**–**4**, and suggested an integrated



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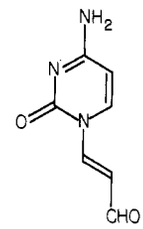

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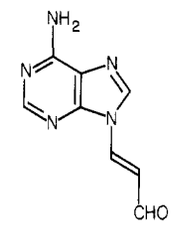

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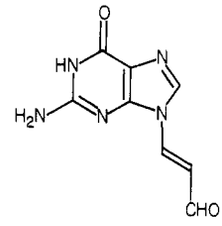
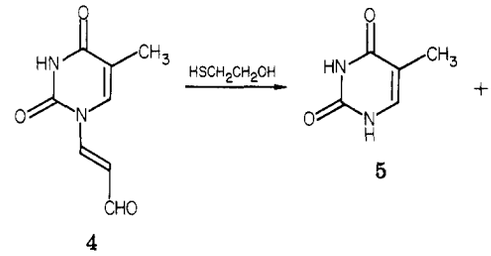


4

mechanism for their production.[1] Subsequently, we found that **1** and **3** inhibit macromolecular synthesis in HeLa cells and are cytotoxic to a variety of tumor cells in culture.[2] Incorporation of [^{3}H]thymidine into DNA is inhibited by both **1** and **3**; **1** also inhibits protein synthesis. Thus, it would appear that the cytotoxicity of bleomycin, at least in part, may be due to the formation of these compounds.

Rationale for Synthetic Work. There are several mechanisms by which **1** and **3** might exert their biological effects, for example, by inhibiting one or more enzymes that act on structurally related substrates. The reactivity of **1** with β-mercaptoethanol (eq 1) suggests that addition-elimination reactions with sulfhydryl or primary amino groups in an enzyme could readily take place. In this case, base propenals could be viewed as alkylating agents.



4 **5**

$$HOCH_2CH_2SCH{=}CHCHO \quad (1)$$

Alternatively, the aldehyde moiety may form a Schiff base or cyclic amino acetal with an amino group in the active site; such a reaction by contrast would not involve loss of the heterocyclic base. Possibly, both nucleophilic mechanisms may be operative. However, evidence presented later indicates that the loss of the heterocyclic base is very rapid in a cell culture system.

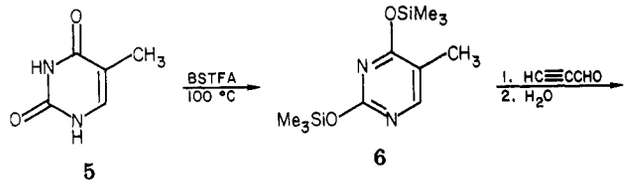
In the addition-elimination mechanism as described above, the heterocyclic base of the base propenal acts as a leaving group. The readiness with which the base leaves depends to a large degree on the ability of the ring to delocalize the negative charge on the nitrogen atom involved. In contrast, the rate of 1,4-addition of a nucleophile is controlled by the electron deficiency of the participating double bond, the principal influence in such cases being the electron-withdrawing aldehyde group. The electron deficiency of the aldehyde group would be the principal factor in the initial step of a 1,2-addition process, such as the formation of imines or amino acetals.

Based on the foregoing considerations and the fact that a number of unsaturated aldehydes appear to exert their biological effects through 1,4-addition reactions,[3] we elected to carry out a limited structure–activity relationship study (a) by varying the nature of the heterocyclic base (b) by replacing the aldehyde with other electron-withdrawing groups, and (c) by altering the nuclear position of the three-carbon side chain. In addition, we prepared and tested a series of structurally related nucleoside propenals.

(1) Giloni, L.; Takeshita, M.; Johnson, Francis; Iden, C.; Grollman, A. P. *J. Biol. Chem.* **1981**, *256*, 8608.
(2) Takeshita, M.; Johnson, Francis; Pillai, K. M. R.; Grollman, A. P. *Cancer Res.*, in press.
(3) Schauenstein, E.; Esterbauer, H.; Zollner, H. "Aldehydes in Biological Systems"; Pion Limited: London, 1977; pp 25–102.

0022-2623/84/1827-0954$01.50/0

Chemical Synthesis. Methods for the synthesis of the desired compounds or related substances have not been reported previously. However, we found that a number of the required pyrimidines can be synthesized by an adaptation of the methods developed by Butts[4] and by Robins and Hatfield[5] for the simple alkylation of this type of heterocycle. The reaction, as applied to thymine, is shown in eq 2 and has been reported briefly by us previ-



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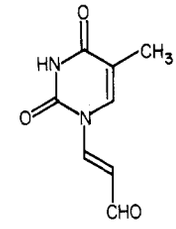
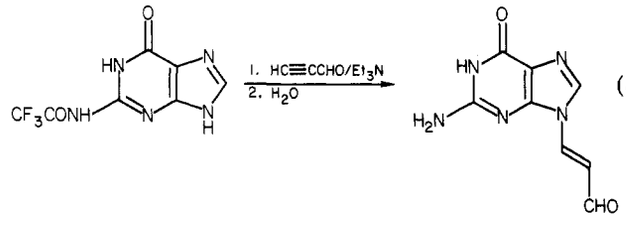


1 (2)

ously.[1] The general method gives good yields and was used for the synthesis of a series of the related pyrimidine derivatives (**7**, **8**, and **10–14**). A modified variation of this procedure was used for the synthesis of the cytosine propenal compound **2**. Compounds **12** and **13** were prepared by substituting cyanoacetylene or 1-butyn-3-one, respectively, for 2-propynal in the reaction. The reduction product (**15**) of **1** was prepared by using sodium borohydride, and the oxime **16** was obtained by standard methods.

Different procedures were required to prepare the purine derivatives **3** and **4**. The former was obtained by adding 2-propynal to a solution of adenine suspended in dimethylformamide at 20 °C in the presence of sodium methoxide. By analogy, the corresponding ester **17** was prepared with ethyl acetylenecarboxylate. The guanine derivative **4** proved more difficult to synthesize because of the insolubility of this base in all solvents tested. However, alkylation of the 2-(trifluoroacetyl) derivative[6] in DMF in the presence of triethylamine, followed by decomposition of the product by water at pH 5, did afford crude **4** (eq 3). The product was purified by HPLC.



4 (3)

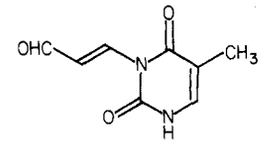
Compound **9** and the nucleoside derivatives **18–26** were prepared by treating a solution of the corresponding nucleoside in DMF at −70 °C with the appropriate acetylenic electrophile. After 1 h, the reaction mixture was allowed to come slowly to room temperature. Removal of the solvent by evaporation and purification of the resulting product by TLC afforded the required compound.

(4) Butts, W. C. *Anal. Biol. Chem.* **1972**, *46*, 187.
(5) Robins, L. J.; Hatfield, P. W. *Can. J. Chem.* **1982**, *60*, 547.
(6) Shapiro, R.; Cohen, D. I.; Shiuey, S. J.; Maurei, H. *Biochemistry* **1969**, *8*, 238.

Results and Discussion

The biological activity of the various base propenals and related compounds was examined in several different systems. All compounds were tested for their ability to inhibit macromolecular synthesis in HeLa cells, as measured by incorporation of radioactive thymidine, uridine, and leucine, respectively, into DNA, RNA, and protein. Compounds that showed significant inhibition of DNA or RNA synthesis ($IC_{50} < 30\ \mu M$) in this assay were tested for inhibition of cell growth (cytotoxicity). In selecting these parameters, we do not imply that the primary biological mode of action of these compounds has been firmly established. The results of this structure–activity study are presented in Table I.

Of the degradation products produced when bleomycin, Fe^{2+}, and O_2 act on DNA (compounds **1–4**), **1** and **3** proved most cytotoxic. Transposition of the propenal group from N-1 to N-3, as in compounds **8** and **9**, increases the inhibitory activity. However, these compounds contain N-1 substituents (CH_3 or $CH_2OCH_2CH_2OH$), and despite repeated efforts, we were unable to prepare the isomer of **2**, namely, **27**, necessary to establish the effect of transposition alone.



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Compounds substituted at C-5 in the thymine nucleus exhibited reduced capacity to inhibit DNA synthesis ($CH_3 > H > F > CF_3 > CH_2OH$). It is noteworthy that the uracil derivative **7** displays significant activity against RNA and protein synthesis, as do compounds **8** and **9**.

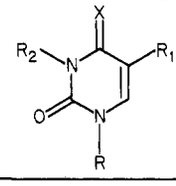
Among the structurally simpler pyrimidines (series **2–17**), changes in the aldehyde group reduce or abolish inhibitory activity against macromolecular synthesis. This is evident from the data for compounds **15** (reduction product), **16** (oxime), and **12**, **13**, and **17** (substitution of CN, $COCH_3$, and CO_2Et, respectively, for CHO).

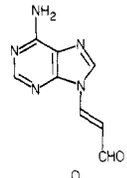
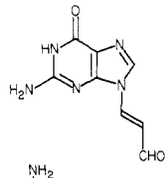
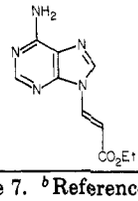
In the nucleoside series, in which the propenal group necessarily is located at N-3, several compounds, namely, those of the 2′-deoxyribosyl series (**18**, **21**, and **24**), are quite cytotoxic and show markedly increased inhibition of DNA synthesis in comparison with **2**. In this series, the corresponding methyl ketone **25** retains considerable inhibitory activity, whereas the related nitrile **26** is inactive. The alcohol **23** inhibits DNA synthesis, although to a lesser extent than **18**.

Compounds containing ribosyl or arabinosyl groups in place of deoxyribose show reduced ability to inhibit DNA synthesis. Compounds **19**, **23**, and **25** are the only pyrimidyl nucleosides tested that inhibit RNA synthesis to roughly the same extent as DNA synthesis; **19** is also cytotoxic.

To determine the general reactivity of base propenals toward nucleophiles, we measured the half-lives of **1**, **3**, and **18** in the presence of glutathione or aminoethanol in solutions buffered at pH 7.2 (Table II). The experiment indicates little difference in reaction rates among the several structurally different substrates tested, implying that the addition–elimination reaction suggested above must be implemented by site-directive effects in the substrate to obtain selective inhibition. The requirements of a 2′-deoxyribose sugar and a carbonyl group in the side chain to obtain optimal activity against DNA synthesis supports this argument.

Table I. Biological Activity of the Base Propenals and Related Compounds



no.	R	R_1	R_2	X	inhibn of biosynth:[a] IC_{50}, μM DNA	RNA	protein	cytotoxicity:[b] ID_{50}, μM
			A. Pyrimidine Derivatives					
2	CH=CHCHO	H	H	NH	>100	>100	>100	15
7	CH=CHCHO	H	H	O	30	30	30	4
1	CH=CHCHO	CH_3	H	O	23	100	25	1
8	CH_3	CH_3	CH=CHCHO	O	10	15	15	3
9	$CH_2OCH_2CH_2OH$	CH_3	CH=CHCHO	O	20	20	20	6
10	CH=CHCHO	F	H	O	60	90	40	3
11	CH=CHCHO	CF_3	H	O	60	>100	80	
12	CH=CHCN	CH_3	H	O	>100	>100	>100	
13	$CH=CHCOCH_3$	CH_3	H	O	>100	>100	>100	
14	CH=CHCHO	CH_2OH	H	O	>100	>100	>100	
15	$CH=CHCH_2OH$	CH_3	H	O	>100	>100	>100	
16	CH=CHCH=NOH	CH_3	H	O	>100	>100	>100	
			B. Pyrimidyl Nucleoside Derivatives					
18	2′-deoxyribosyl	CH_3	CH=CHCHO	O	0.5	20	15	1
19	ribosyl	H	CH=CHCHO	O	10	3	>100	9
20	ribosyl	CH_3	CH=CHCHO	O	>100	100	>100	
21	2′-deoxyribosyl	I	CH=CHCHO	O	0.3	80	15	20
22	arabinosyl	CH_3	CH=CHCHO	O	40	100	>100	40
23	2′-deoxyribosyl	CH_3	$CH=CHCH_2OH$	O	5	5	>100	60
24	2′-deoxyribosyl	H	CH=CHCHO	O	0.8	15	15	1
25	2′-deoxyribosyl	H	$CH=CHCOCH_3$	O	1.5	6	>100	6
26	2′-deoxyribosyl	H	CH=CHCN	O	100	100	>100	
			C. Purine Derivatives					
3					30	>100	>100	2
4					>100	50	>100	24
17					60	100	100	

[a] Reference 7. [b] Reference 2.

Table II. Reactivity of Selected Base-Propenals with Nucleophiles[a]

no.	nucleophile added: glutathione: $T_{1/2}$, min	aminoethanol: $T_{1/2}$, h
1	3.5	18
3	2.1	15
18	8.0	25

[a] Solutions were made 0.4 M in substrate and 0.2 M in nucleophile. Reactions were monitored by disappearance of substrate and appearance of free base, as analyzed by HPLC.

The facile addition–elimination reaction between base propenals and nucleophiles seems likely to be an important aspect of their biological activity. However, the almost equally reactive nitrile analogues **12** and **26** lack biological activity, emphasizing the importance of a carbonyl group in the side chain. This result could imply interaction with a second group in the active site, a point of view supported by the activity of the butenone **25**, a compound that might be expected to be less reactive chemically, in an addition–elimination reaction, than **26**.

In support of the addition–elimination hypothesis, it has been found by tracer studies that when HeLa cells are treated in culture with **18**, several effects can be observed: (1) the absorption of **18** by the cells is faster than that of thymidine; (2) the base propenal **18** rapidly vanishes from the system at rates comparable to our kinetic studies (Table II). Experiments are now underway to determine if **18** is converted to a phosphorylated species in an attempt to obtain further evidence for the site-directed effect. These and other experiments will be reported elsewhere.[2]

In summary, the following tentative conclusions concerning the structure–activity relationships may be drawn: The location of the *N*-propenal group does not appear to be a crucial determinant of biological activity, but compounds substituted with this group at N-3 are better inhibitors than those substituted at N-1. The presence of a 2′-deoxyribose moiety at N-1 in this series increases biological activity substantially and increases the selectivity of the compound, as measured by inhibition of the incorporation of thymidine into DNA. Biological activity, represented by inhibition of DNA synthesis and cytotoxicity, is optimized in compound **18** in which a *trans*-propenal group is located at N-3 and the sugar residue is that found in DNA, i.e., 2′-deoxyribose. Significant changes in the aldehyde function or substitution of cytosine or guanine for thymine or uracil markedly reduce or abolish biological activity.

Further work on these structure–activity relationships and the mechanism(s) of action of this novel class of cytotoxic agents will be the subject of future publications.

Experimental Section

NMR spectra were measured on a Varian CFT-20. IR spectra were taken on a Perkin-Elmer 257 instrument. Mass spectra were taken on a Hewlett-Packard HP5983 GC/MS spectrometer (low resolution) or a Kratos MS-30 (high resolution). All new compounds reported were checked for homogeneity by TLC analysis in two solvent systems. The following systems were used: A, EtOAc; B, EtOAc/*i*-PrOH/H_2O (76.15:9); C, 5% MeOH/EtOAc; D, 10% MeOH/EtOAc; E, EtOAc/*i*-PrOH/H_2O (70:20:10).

1-(3-Oxoprop-1-enyl)thymine (1). A mixture of thymine (504 mg, 4 mmol) and BSTFA (7 mL), protected from moisture, was heated at 120 °C for 2 h. At the end of this time, all thymine had dissolved, indicating complete silylation. The solution was cooled to room temperature, propargylaldehyde (432 mg, 8 mmol) was added in one lot, and the mixture was stirred for 1 h. Water was then added very slowly to the reaction mixture. The precipitate obtained was separated and washed with cyclohexane until the filtrate was colorless, and the residual solid was crystallized from methanol to afford **1** as a white powder (580 mg, 76.7%): mp 215–218 °C; homogeneous by TLC analysis in solvent systems A and B; NMR (Me_2SO-d_6) δ 1.84 (s, 3 H, CH_3), 6.47 (dd, J_1 = 7.8 Hz, J_2 = 14.8 Hz, 1 H, =C*H*CHO), 8.04 (s, 1 H, H-6), 8.17 (d, J = 14.8 Hz, 1 H, C*H*=CHCHO), 9.58 (d, J = 7.7 Hz, 1 H, CHO); IR (Nujol) 1735 (CHO) cm^{-1}; mass spectrum, m/z 180.0523 (M^+, calcd for $C_8H_8N_2O_3$, 180.0533). Compounds **7** and **9–15** were also synthesized by this method.

1-(3-Oxoprop-1-enyl)uracil (7): yield 82%; mp 210–212 °C; homogeneous by TLC analyses in solvent systems A and B; NMR (Me_2SO-d_6) δ 5.87 (d, J = 8.1 Hz, 1 H, H-5), 6.49 (dd, J_1 = 7.6 Hz, J_2 = 14.5 Hz, 1 H, =C*H*CHO), 8.07 (s, 1 H, H-6), 8.15 (d, J = 14.5 Hz, 1 H, C*H*=CHCHO), 9.63 (d, J = 7.6 Hz, 1 H, CHO); mass spectrum, m/z 166.0371 (M^+, calcd for $C_7H_6N_2O_3$, 166.0376).

5-Fluoro-1-(3-oxoprop-1-enyl)uracil (10): yield 68%; mp 240–242 °C; homogeneous by TLC analysis in solvent systems A and B; NMR (Me_2SO-d_6) δ 6.32 (dd, J_1 = 7.5 Hz, J_2 = 14.8 Hz, 1 H, =CHCHO), 8.02 (s, 1 H, H-6), 8.10 (d, J = 14.8 Hz, 1 H, C*H*=CHCHO), 9.56 (d, J = 7.5 Hz, 1 H, CHO); mass spectrum, m/z 184.0273 (M^+, calcd for $C_7H_5N_2O_3F$, 184.0282).

1-(3-Oxoprop-1-enyl)-5-(trifluoromethyl)uracil (11): yield 46%; mp 220–222 °C; homogeneous by TLC analysis in solvent systems A and B; NMR (Me_2SO-d_6) δ 6.25 (dd, J_1 = 7.8 Hz, J_2 = 14.7 Hz, 1 H, =C*H*CHO), 7.95 (s, 1 H, H-6), 8.05 (d, J_1 = 14.7 Hz, 1 H, CH=CHCHO) 9.68 (d, J = 7.5 Hz, 1 H, CHO); mass spectrum, m/z 234.0235 (M^+, calcd for $C_8H_5N_2O_3F_3$, 234.0250).

5-(Hydroxymethyl)-1-(3-oxoprop-1-enyl)uracil (14): yield 59%; mp 212–214 °C; homogeneous by TLC analysis in solvent systems A and B; NMR (Me_2SO-d_6) δ 4.05 (s, 2 H, CH_2OH), 6.26 (dd, J_1 = 7.6 Hz, J_2 = 14.6 Hz, 1 H, =C*H*CHO), 7.72 (s, 1 H, H-6), 7.98 (d, J = 14.6 Hz, 1 H, C*H*=CHCHO), 9.40 (d, J = 7.6 Hz, 1 H, CHO); mass spectrum, m/z 196.0478 (M^+, calcd for $C_8H_8N_2O_4$, 196.0481).

1-(2-Cyanoethenyl)thymine (12). Following the same method, starting from thymine and cyanoacetylene, **12** was obtained in 62% yield as colorless needles: mp 198–200 °C; homogeneous by TLC analysis in solvent systems B and C; NMR (Me_2SO-d_6) δ 1.86 (s, 3 H, CH_3), 5.64 (d, J = 9.8 Hz, 1 H, =CHCN), 7.52 (d, J = 9.8 Hz, 1 H, C*H*=CHCN), 7.95 (s, 1 H, H-6); IR (Nujol) 2200 (CN) cm^{-1}; mass spectrum, m/z 177.0527 (M^+, calcd for $C_8H_7N_3O_2$, 177.0534).

1-(3-Oxobut-1-enyl)thymine (13). Compound **13** was obtained as white needles in 72% yield from thymine and 1-butyn-3-one: mp 268–270 °C; homogeneous by TLC analysis in solvent systems B and C; NMR (Me_2SO-d_6) δ 1.82 (s, 3 H, CH_3), 2.24 (s, 3 H, $COCH_3$), 7.18 (d, J = 14.8 Hz, 1 H, =C*H*$COCH_3$), 7.94 (s, 1 H, H-6), 8.08 (d, J = 14.8 Hz, 1 H, C*H*=CHCOCH_3); mass spectrum, m/z 194.0679 M^+ calcd for $C_9H_{10}N_2O_3$, 194.0690).

1-[(2-Hydroxyethoxy)methyl]-3-(3-oxoprop-1-enyl)thymine (9). 1-[(2-hydroxyethoxy)methyl]thymine[5] (200 mg, 1 mmol) in anhydrous DMF (5 mL) containing triethylamine (101 mg, 1 mmol) was cooled to −70 °C. Propargylaldehyde (108 mg, 2 mmol) was added, and the reaction mixture was stirred for 1 h. The temperature was then allowed to rise slowly to 0 °C, and the solvent was removed in vacuo. The resulting solid was redissolved in MeOH, and the product was isolated by preparative TLC with solvent system C, to afford **9** as colorless plates (130 mg, 51%); this was homogeneous by TLC analysis in solvent systems B: mp 218–220 °C; NMR (Me_2SO-d_6) δ 1.85 (s, 3 H, CH_3), 3.30 (m, 4 H, OCH_2CH_2OH), 5.10 (s, 2 H, NCH_2O), 6.98 (dd, J_1 = 7.5 Hz, J_2 = 14.8 Hz, 1 H, =C*H*CHO), 7.73 (s, 1 H, H-6), 8.10 (d, J = 14.8 Hz, 1 H, C*H*=CHCHO), 9.52 (d, J = 7.6 Hz, 1 H, CHO); mass spectrum, m/z 194.1926 (M^+ calcd for $C_{11}H_{14}N_2O_3$, 194.0690).

1-(3-Hydroxyprop-1-enyl)thymine (15). A solution of **1** (18 mg, 0.1 mmol) in MeOH (5 mL) was cooled to 0 °C. Sodium borohydride (7.6 mg, 2 mmol) was added, and the solution was stirred for 1 h. The solvent was removed in vacuo, and the product was isolated by preparative TLC using solvent system C to afford **15** (5% MeOH in EtOAc) as pure white plates (15 mg, 75%): mp 152–154 °C; homogeneous by TLC analysis in solvent system B; NMR (Me_2SO-d_6) δ 1.82 (s, 3 H, CH_3), 4.05 (dd, J_1 = 1.5 Hz, J_2 = 5.5 Hz, 2 H, CH_2OH), 5.95 (m, 1 H, =C*H*CH_2OH), 7.01 (d, J = 14.4 Hz, 1 H, C*H*=C*H*CH_2OH), 7.82 (s, 1 H, H-6); mass spectrum, m/z 182.0678 (M^+, calcd for $C_8H_{10}N_2O_3$, 182.0692).

3-(3-Oxoprop-1-enyl)thymidine (18). Thymidine (488 mg, 2 mmol) was dissolved in anhydrous DMF (4 mL) containing triethylamine (202 mg, 2 mmol), and the mixture was stirred for 1 h at room temperature. The solution was cooled to −78 °C, propargylaldehyde (216 mg, 4 mmol) was added in one portion, and the reaction mixture was stirred at this temperature for 2 h and then allowed to come to room temperature slowly. Excess reagents and DMF were removed in vacuo, and the residue, in MeOH, was purified by preparative TLC using solvent system C. The desired product (**18**) was extracted from the silica gel by methanol and was obtained as almost colorless plates (220 mg, 37%): mp 121–122 °C; homogeneous by TLC analysis in solvent system B; IR (Nujol) 3425, 1733, 1690, 1660, 1295, 1260, 1095, 1040 cm^{-1}; NMR (Me_2SO-d_6) δ 1.85 (s, 3 H, CH_3), 6.19 (t, J = 6.1 Hz, 1 H, OCHN), 7.06 (dd, J_1 = 7.8 Hz, J_2 = 14.8 Hz, 1 H, =C*H*CHO), 7.95 (s, 1 H, H-6), 8.16 (d, J = 14.8 Hz, 1 H, C*H*=CHCHO), 9.60 (d, J = 7.8 Hz, 1 H, CHO); mass spectrum, m/z 296.

The following compounds (**19–22** and **24–26**) were synthesized by the method described for **18**.

3-(3-Oxoprop-1-enyl)uridine (19): yield 34%; pale yellow oil; homogeneous by TLC analysis in solvent systems D and E; NMR (Me_2SO-d_6) δ 5.79 (d, J = 3.5 Hz, 1 H, OCHN), 5.88 (d, J = 8.4 Hz, 1 H, H-5), 7.05 (dd, J = 7.7 Hz, J_2 = 14.8 Hz, 1 H, =C*H*CHO), 8.14 (d, J = 8.4 Hz, 1 H, H-6), 8.16 (d, J = 14.8 Hz, 1 H, C*H*=CHCHO), 9.60 (d, J = 7.7 Hz, 1 H, CHO); mass spectrum, m/z 298.

3-(3-Oxoprop-1-enyl)-2′α-hydroxythymidine (20): yield 32%; colorless oils; homogeneous by TLC analysis in solvent systems D and E; NMR (Me_2SO-d_6) δ 1.82 (s, 3 H, CH_3), 5.72 (d, J = 3.5 Hz, 1 H, OCHN), 7.02 (dd, J_1 = 7.8 Hz, J_2 = 14.7 Hz, 1 H, =CHCHO), 8.09 (d, J = 8.2 Hz, 1 H, H-6), 8.14 (d, J = 14.7 Hz, 1 H, C*H*=CHCHO), 9.60 (d, J = 7.7 Hz, 1 H, CHO); mass spectrum, m/z 312.

5-Iodo-3-(3-oxoprop-1-enyl)-2′-deoxyuridine (21): yield 25%; colorless powder; mp 98–100 °C; homogeneous by TLC analysis in solvent systems D and E; NMR (Me_2SO-d_6) δ 6.09 (t, J = 6.1

Hz, 1 H, OCHN), 7.04 (dd, J_1 = 7.8 Hz, J_2 = 14.8 Hz, 1 H, =CHCHO), 8.10 (d, J = 14.8 Hz, 1 H, CH=CHCHO), 8.59 (s, 1 H, H-6), 9.60 (d, J = 7.7 Hz, 1 H, CHO); mass spectrum, m/z 408.

3-(3-Oxoprop-1-enyl)-2′β-hydroxythymidine (22): yield 28%; homogeneous by TLC analysis; solvent systems D and E; NMR (Me_2SO-d_6) δ 1.80 (s, 3 H, CH_3), 5.70 (d, J = 3.5 Hz, 1 H, OCHN), 7.06 (dd, J_1 = 7.8 Hz, J_2 = 14.8 Hz, 1 H, =CHCHO), 7.90 (s, 1 H, H-6), 8.14 (d, J = 14.8 Hz, 1 H, CH=CHCHO), 9.60 (d, J = 7.8 Hz, 1 H, CHO); mass spectrum, m/z 314.

3-(3-Oxoprop-1-enyl)-2′-deoxyuridine (24): yield 34%; pale yellow oil; homogeneous by TLC analysis in solvent systems D and E; NMR (Me_2SO-d_6) δ 5.88 (d, J = 8.2 Hz, 1 H, H-5), 6.15 (t, J = 6.1 Hz, 1 H, OCHN), 7.04 (dd, J_1 = 7.8 Hz, J_2 = 14.8 Hz, 1 H, =CHCHO), 8.05 (d, J = 8.2 Hz, 1 H, H-6), 8.15 (d, J = 14.8 Hz, 1 H, CH=CHCHO), 9.60 (d, J = 7.8 Hz, 1 H, CHO); mass spectrum, m/z 282.

3-(2-Cyanoethenyl)thymidine (26). Cyanoacetylene was used in place of propargylaldehyde, and the desired product (**26**) was obtained as a faintly yellow oil (35% yield): homogeneous by TLC analysis in solvent systems B and C; NMR (Me_2SO-d_6) δ 1.85 (s, 3 H, CH_3), 6.17 (t, J = 6.1 Hz, 1 H, OCHN), 6.18 (d, J = 9.0 Hz, 1 H, =CHCN), 7.06 (d, J = 9.0 Hz, 1 H, CH=CHCN), 7.89 (s, 1 H, H-6); mass spectrum, m/z 293.

3-(3-Oxobut-1-enyl)thymidine (25). 1-Butyn-3-one was used in place of propargylaldehyde, and the desired product (**25**) was obtained as an oil (12% yield): homogeneous by TLC analysis in solvent systems C and B; NMR (Me_2SO-d_6) δ 1.85 (s, 3 H, CH_3), 2.27 (s, 3 H, $COCH_3$), 6.17 (t, J = 6.1 Hz, 1 H, OCHN), 7.19 (d, J = 15 Hz, 1 H, =CHCOCH$_3$), 7.91 (s, 1 H, H-6), 8.02 (d, J = 15 Hz, 1 H, CH=CHCOCH$_3$); mass spectrum, m/z 310.

3-(3-Hydroxyprop-1-enyl)thymidine (23). A solution of **18** (29.6 mg, 0.1 mmol) in MeOH (3 mL) was cooled to 0 °C. Sodium borohydride (7.2 mg, 2 mmol) was added, and the reaction mixture was stirred for 1 h and then allowed to come to room temperature slowly. The solvent was removed in vacuo, and the product was isolated by preparative TLC using solvent system D to afford **23** as a pure white solid in 90% yield: mp 158–160 °C; homogeneous by TLC analysis in solvent system E; NMR (Me_2SO-d_6) δ 1.82 (s, 1 H, CH_3), 4.15 (m, 2 H, CH_2OH), 6.19 (m, 1 H, =CHCH$_2$OH), 6.47 (t, 1 H, OCHN), 6.71 (d, J = 14.6 Hz, 1 H, CH=CHCH$_2$OH), 7.79 (s, 1 H, H-6); mass spectrum, m/z 298.

1-(3-Oxoprop-1-enyl)cytosine (2). Cytosine (333 mg, 3 mmol) was silylated by heating at 110 °C with BSTFA (6 mL) for 2 h. The pasty silyl derivative was suspended in anhydrous acetonitrile (5 mL), and propargyl aldehyde (324 mg, 6 mmol) was added. The reaction mixture was stirred for 4 h, and the solid that had separated was filtered, washed with ethyl acetate, and recrystallized from water to afford **2** as colorless needles (180 mg, 36.4%): mp 255–257 °C dec; homogeneous by TLC analysis in solvent systems D and E; NMR (Me_2SO-d_6) δ 5.92 (d, J = 7.7 Hz, 1 H, H-6), 6.38 (dd, J_1 = 7.6 Hz, J_2 = 14.7 Hz, 1 H, =CHCHO), 7.87 (s, 2 H, NH_2), 8.04 (d, J = 7.7 Hz, 1 H, H-5), 8.26 (d, J = 14.7 Hz, 1 H, CH=CHCHO), 9.55 (d, J = 7.6 Hz, 1 H, CHO); mass spectrum, m/z 165.0530 (M^+ calcd for $C_7H_7N_3O_2$, 165.0534).

9-(3-Oxoprop-1-enyl)adenine (3). A solution of sodium ethoxide (15 mg of sodium in 1 mL of ethanol) was added to adenine (135 mg, 1 mmol) dissolved in DMF (2 mL). The mixture was stirred for 1 h and then cooled to −40 °C. Propargylaldehyde (54 mg, 1 mmol) was added in one portion, and stirring was continued for 1 h at this temperature. The pale brown reaction mixture was then allowed to warm to room temperature and neutralized with aqueous ammonium chloride. The precipated solid was collected and recrystallized from water to afford **3** as pale yellow needles (100 mg, 52.9%): mp 258–260 °C; homogeneous by TLC analysis in solvent systems D and E; NMR (Me_2SO-d_6) δ 7.17 (dd, J_1 = 7.8 Hz, J_2 = 14.4 Hz, 1 H, =CHCHO), 7.55 (s, 2 H, NH_2), 8.28 (s, 1 H, H-3), 8.54 (d, J = 14.4 Hz, 1 H, CH=CHCHO), 8.64 (s, 1 H, H-8), 9.68 (d, J = 7.9 Hz, CHO); mass spectrum, m/z 189.0649 (M^+ calcd for $C_8H_7N_5O$, 189.0645).

9-(3-Oxoprop-1-enyl)guanine (4). 2-(Trifluoroacetyl)guanine (247 mg, 1 mmol) was dissolved in anhydrous DMF (5 mL) containing triethylamine (101 mg, 1 mmol). The mixture was stirred for 1 h and then cooled to −70 °C, and propargylaldehyde (108 mg, 2 mmol) was added in one portion. Stirring was continued for 1 h, the mixture was allowed to warm to room temperature, and the solvents were removed in vacuo. The residual material was boiled with water (250 mL) and filtered. After treatment with charcoal, the filtrate was made slightly acidic (pH 5.5) with acetic acid, and, on standing, the aqueous solution furnished **4** as a colorless powder (45 mg, 14.4%). This product was further purified by HPLC: mp > 300 °C; NMR (Me_2SO-d_6) δ 6.16 (s, 2 H, NH_2), 6.88 (dd, J_1 = 7.8 Hz, J_2 = 14.7 Hz, 1 H, =CHCHO), 8.03 (d, J = 14.7 Hz, 1 H, CH=CHCHO), 8.38 (s, 3 H, H-8); mass spectrum, m/z 205.0568 (M^+ calcd for $C_8H_7N_5O_2$, 205.0594).

9-(2-Ethoxycarbonylethenyl)adenine (17). Adenine (405 mg, 3 mmol) was suspended in anhydrous DMF (8 mL). Sodium methoxide (from 30 mg of sodium and 3 mL of ethanol) was added, the reaction mixture was stirred for 1 h, and ethyl propiolate (254 mg, 3 mmol) was then added in one portion. The reaction mixture became brown, and the adenine went into solution. Stirring was continued for 2 h, the reaction mixture was neutralized with aqueous ammonium chloride, and the precipitated solid was filtered, washed with ethyl acetate and crystallized from MeOH to afford **17** as faintly yellow needles (171 mg, 30%): mp 228–230 °C; homogeneous by TLC analysis in solvent systems A and B; NMR (Me_2SO-d_6) δ 1.20 (t, 3 H, CH_3), 4.16 (q, 2 H, CH_2), 5.92 (d, J = 10.2 Hz, 1 H, =CHCOOC$_2$H$_5$), 7.45 (d, J = 10.2 Hz, 1 H, CH=CHCOOC$_2$H$_5$), 7.46 (br s, 2 H, NH_2), 8.21 (s, 1 H, H-3), 8.86 (s, 1 H, H-8), mass spectrum, m/z 233.0820 (M^+ calcd for $C_{10}H_{11}N_5O_2$, 233.0806).

Measurement of DNA, RNA, and Protein Synthesis in HeLa Cells. HeLa S_3 cells, grown in suspension culture in MEM supplemented with 5% fetal calf serum and 2 mM L-glutamine, were incubated with 0.5 μCi of [*methyl*-^{3}H]thymidine (mm Ci/mmol), [5,6-^{3}H]uridine (40 Ci/mmol), or L-[4,5-^{3}H]leucine (56 Ci/mmol). The rates of DNA, RNA, and protein synthesis were determined by measuring the respective uptake of these precursors into cold TCA-insoluble material.[7]

Inhibition of Cell Growth. HeLa cells (100 mL), grown in complete media to a density of 1.7×10^5/mL, were incubated for 16 h with varying concentrations of inhibitors. Cytotoxicity values represent the number of cells detected after staining with Trypan blue compared to that observed in the absence of inhibitor.[8]

Acknowledgment. This work was supported in part by Grant CH-240 from the American Cancer Society. We thank Dr. Charles Iden for his assistance in mass spectral analysis.

Registry No. **1**, 85394-19-0; **2**, 90029-72-4; **3**, 90029-73-5; **4**, 90029-74-6; **7**, 86798-57-4; **7** (R = H), 66-22-8; **8**, 90029-75-7; **9**, 90029-76-8; **10**, 90029-77-9; **10** (R = H), 51-21-8; **11**, 90029-78-0; **11** (R = H), 54-20-6; **12**, 90029-79-1; **13**, 90029-80-4; **14**, 90029-81-5; **14** (R = H), 4433-40-3; **15**, 90029-82-6; **16**, 90029-83-7; **17**, 90029-84-8; **18**, 90029-85-9; **19**, 90029-86-0; **19** (R^2 = H), 58-96-8; **20**, 90029-87-1; **20** (R^2 = H), 1463-10-1; **21**, 90029-88-2; **21** (R^2 = H), 54-42-2; **22**, 90029-89-3; **22** (R^2 = H), 605-23-2; **23**, 90029-90-6; **24**, 90029-91-7; **24** (R^2 = H), 951-78-0; **25**, 90029-92-8; **26**, 90029-93-9; thymine, 65-71-4; propargyl aldehyde, 624-67-9; cyanoacetylene, 1070-71-9; 1-butyn-3-one, 1423-60-5; thymidine, 50-89-5; cytosine, 71-30-7; adenine, 73-24-5; 2-(trifluoroacetyl)-guanine, 21323-85-3; ethyl acetylenecarboxylate, 623-47-2; 1-[(2-hydroxyethoxy)methyl]thymine, 68724-11-8.

(7) Huang, M. T.; Grollman, A. P. *Mol. Pharmacol.* **1972**, *8*, 538.
(8) Patterson, Jr., M. K. *Methods Enzymol.* **1979**, *58*, 141.