Antihypertensive Screen. After an initial training period, 24 male, Okamoto-Aoki strain, spontaneously hypertensive rats (Taconic Farms, Germantown, NY) were distributed into six groups of four animals with approximately equal mean systolic blood pressures. The six groups were studied concurrently in a 2-day procedure. Test compounds were randomly assigned to each group. Five groups received test substances, and one control group received vehicle only. On 2 consecutive mornings, a group of four rats was orally dosed with a test substance that had been dissolved or suspended in water at concentrations such that 0.1 mL of solution was administered per 10 g of body weight. Immediately after dosing on day 2, all 24 rats were put in restrainers and then into a heated chamber (30.0 ± 1.0 °C) for 4 h. Systolic blood pressures (tail cuff) were recorded with photoelectric transducers at 1, 2, 3, and 4 h after drug administration. The coccygeal arteries of the rats were simultaneously occluded by inflated tail cuffs that were automatically inflated to 300 mmHg and then deflated. Tail pulses were simultaneously recorded, along with a pressure curve on a recorder. Four consecutive (at 3-s intervals) traces were recorded for each rat at each hour after dosing. The systolic pressure was considered to be the pressure at the appearance of the first pulse. The mean systolic pressure of each rat at each observation time in both drug-treated and control groups was calculated. Systolic pressures in the controls varied over the range 180 to 220 mmHg during the 4-h measurement period. The mean values of the respective drug-treated and control groups were then compared by using a 1-tail Student's t test. Statistical significance was considered to be $p \leq 0.05$.

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Registry No. **4**, 84060-08-2; **6** (R = Y = H), 84060-09-3; **6** (R = CH_3; Y = H), 84060-10-6; (±)-**8**, 84787-02-0; (±)-**8**·HCl, 84787-28-0; (±)-**9**, 84787-01-9; (±)-**9**·HCl, 84787-29-1; (±)-**10**, 84787-03-1; (±)-**10**·HCl, 84787-30-4; (±)-**11**·HCl, 84787-14-4; (±)-**12**·HCl, 84787-15-5; (±)-**13**·HCl, 84787-16-6; (±)-**14**·HCl, 84787-17-7; (±)-**15**·HCl, 84787-18-8; (±)-**16**·HCl, 84787-19-9; (±)-**17**·HCl, 84787-20-2; (±)-**18**·HCl, 84787-21-3; (±)-**19**·HCl, 84787-22-4; (±)-**20**·HCl, 84787-04-2; (±)-**21**, 84787-05-3; (±)-**22**·HCl, 84787-06-4; (±)-**23**·HCl, 84787-07-5; (±)-**24**·HCl, 84787-08-6; (±)-**25**·HCl, 84787-09-7; **26**, 84060-43-5; **26**·HCl, 84060-31-1; **27**·HCl, 84787-10-0; **28**·HCl, 84787-11-1; **29**·HCl, 84787-12-2; **30**·HCl, 84787-13-3; **31**·HCl, 84060-33-3; (±)-**32**, 84787-23-5; (±)-**33**·HCl, 84787-24-6; (±)-**34**·HCl, 84787-25-7; (±)-**35**·HCl, 84787-26-8; **36**·HCl, 84787-27-9; **37**·HCl, 84070-64-4; catechol, 120-80-9; *cis*-2,3-bis(chloromethyl)oxirane, 50703-46-3; *dl-threo*-2-oxiranyl-1,4-benzodioxan, 65347-66-2; *dl-erythro*-2-oxiranyl-1,4-benzodioxan, 65347-62-8; 2-hydroxy-5-fluoroacetophenone, 394-32-1; *trans*-1,4-dichloro-2-butene, 110-57-6; 2-[(4-chloro-*trans*-2-butenyl)oxy]-5-fluoroacetophenone, 84786-98-1; (±)-2-[(4-chloro-*trans*-2,3-epoxybutyl)oxy]-5-fluorophenyl acetate, 84786-99-2; *dl-erythro*-7-fluoro-2-oxiranyl-1,4-benzodioxan, 84787-00-8; *N*-(*tert*-butoxycarbonyl)aniline, 3422-01-3; *N*-(*tert*-butoxycarbonyl)-4-piperidinone, 79099-07-3; 2-phenoxyethyl bromide, 589-10-6.

Nucleic Acid Related Compounds. 40. Synthesis and Biological Activities of 5-Alkynyluracil Nucleosides[1]

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Coupling of terminal alkynes with 5-iodo-1-(2,3,5-tri-*O*-*p*-toluyl-β-D-arabinofuranosyl)uracil and 5-iodo-3′,5′-di-*O*-*p*-toluyl-2′-deoxyuridine proceeded readily in triethylamine with catalytic quantities of bis(triphenylphosphine)-palladium(II) chloride and copper(I) iodide. The resulting products were deprotected to give 5-alkynyl-1-β-D-arabinofuranosyluracil and 5-alkynyl-2′-deoxyuridine nucleosides. The 5-ethynyl, followed by 5-propynyl, products had the highest antiviral potency, with the 2′-deoxy derivatives being more effective than the arabinosyl compounds. Activity was weak at hexynyl and disappeared at heptynyl. Inclusion of an ω-hydroxy function diminished the antiviral effect. None of the 5-alkynyluracil nucleosides tested had sufficient selectivity to qualify as a candidate antiviral drug. Several of the compounds exerted an inhibitory action on thymidylate synthetase, with 5-ethynyl-2′-deoxyuridine being the most cytotoxic against L1210 cells.

Potent antiviral and antitumor activities have been demonstrated with 5-substituted uracil nucleosides. A series of 5-(2-substituted-vinyl)-2′-deoxyuridine compounds has been shown to contain highly selective inhibitors of herpes virus replication, especially against HSV-1 (herpes simplex virus type 1).[2] The *E* configuration of the vinyl side chain at C-5 has been shown to be important for the 5-(2-bromovinyl) compounds.[3,4] Substituents at C-2 of the vinyl group, including Cl, Br, I, CF_3, and CH_3, provide good activity.[2] The HSV-1 induced thymidine kinase of the infected cell promotes selective phosphorylation of these (*E*)-5-(2-substituted-vinyl)-dUrd compounds relative to the native enzyme of the host cells.[4] The subsequently produced 5′-triphosphate of BVDU, (*E*)-5-(2-bromovinyl)-2′-deoxyuridine, inhibits the HSV-1 DNA polymerase to a significantly greater extent than it does the native cellular DNA polymerases α and β.[5] This may amplify the antiherpes selectivity of BVDU.

In contrast with the 5-(2-substituted-vinyl)-dUrd series, 5-substituted-dUrd derivatives in which X = F, CF_3, NO_2, CHO, C≡CH, etc. show little selectivity against viral-in-

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(1) For the preceding paper in this series, see Robins, M. J.; Barr, P. J. *J. Org. Chem.*, in press. All alkynes and alkynyl products are the terminal or 1-isomers. For simplicity, the alkyn-1-yl designation is assumed by alkynyl.
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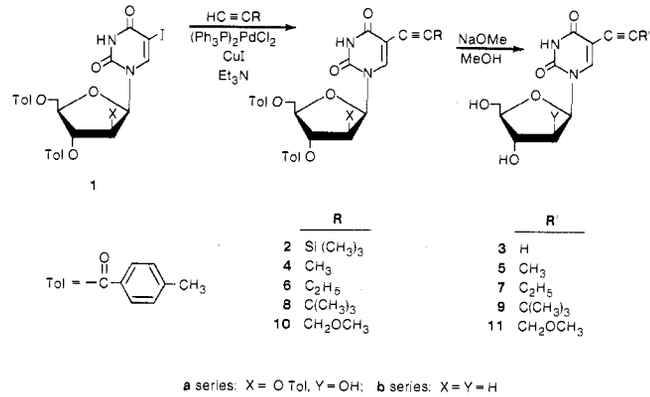
fected cells.[2] These compounds are highly toxic in the host cells and suppress the proliferation of murine leukemia (L1210) in culture at very low concentrations (0.001–0.2 μg/mL).[2] Thymidylate synthetase has been identified as a primary site of action for these 5-substituted-2′-deoxyuridine compounds in the inhibition of L1210 cell growth.[6] Phosphorylation of these nucleoside agents to their corresponding 5′-monophosphate esters is a prerequisite for inhibition of this enzyme.[7]

The only 5-alkynyluracil nucleoside that has been evaluated for antiviral and antitumor effects previously is 5-ethynyl-2′-deoxyuridine. This compound was found to inhibit the replication of HSV-1, HSV-2 (herpes simplex virus type 2), and VV (vaccinia virus) at concentrations of 0.1–1 μg/mL.[8,9] However, this corresponded closely to the concentration that inhibited the proliferation and metabolism (as monitored by incorporation of dUrd into DNA) of the primary rabbit kidney host cells. Potent inhibitory effects of 5-ethynyl-2′-deoxyuridine on the growth of L1210 cells in culture have been reported with ID_{50} values of 0.09 μg/mL[6] and 0.006 μg/mL.[10] Several of our 5-alkynyl-2′-deoxyuridine 5′-monophosphate derivatives have recently been shown to be inhibitors of isolated thymidylate synthetase.[11] Since the viral-induced thymidine kinase is capable of accepting 5-(2-substituted-vinyl)-2′-dUrd compounds as substrates,[4] it seemed reasonable that the linear 5-alkynyl-2′-dUrd analogues would also function as substrates.

We now report the synthesis of selected 5-alkynyluracil arabinosides and 2′-deoxyribosides and the investigation of the inhibitory effects of 19 of these compounds on virus replication, host cell metabolism, and tumor cell proliferation. Evaluation of structure–activity trends for the 5-alkynyl-dUrd and -*ara*-U derivatives has been pursued with respect to chain length, branching, and ω-hydroxy substitution of the alkynyl side chain, as well as the effect of the substituent at C-2′ of the sugar moiety (an "up" H or OH group).

Chemistry. Prior syntheses of 5-ethynyl-2′-deoxyuridine had involved construction of the heterocyclic base, condensation with a 2-deoxy sugar derivative, and separation of the resulting anomeric mixture.[10,12] We have recently[1,13] reported a convenient and high-yield procedure for coupling the readily available protected 5-iodouracil nucleosides[14] with terminal alkynes. After deprotection, the resulting 5-alkynyl-2′-deoxyuridine products were obtained in good overall yields from 2′-deoxyuridine (and

Scheme I

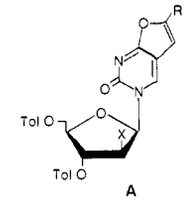


a series: X = O Tol, Y = OH; b series: X = Y = H

with absolute β-anomeric purity). Pichat has noted a more complex coupling method that gives analogous compounds with variable yields.[15]

Coupling of 5-iodo-1-(2,3,5-tri-*O*-*p*-toluyl-β-D-arabinofuranosyl)uracil[14] (**1a**) (see Scheme I) with trimethylsilylacetylene in triethylamine was catalyzed by bis(triphenylphosphine)palladium(II) chloride and copper(I) iodide.[16] The resulting product **2a** (83% yield) was deprotected with methanolic sodium methoxide. This treatment effected concomitant removal of the acetylenic trimethylsilyl group[13] to give the desired parent 5-ethynyl-1-β-D-arabinofuranosyluracil (**3a**) in 87% yield. This product was somewhat sensitive to heating and manipulation in warm solvents (probably resulting from attack of the arabino O-2′ at C-6 of the uracil ring).

In our initial work on this coupling procedure,[1] we did not obtain the desired product from treatment of 5-iodo-3′,5′-di-*O*-*p*-toluyl-2′-deoxyuridine[14] (**1b**) with commercial propyne under the usual conditions. More careful examination has now revealed that the commercial cylinder did not contain authentic propyne. Generation of propyne[17] from 1,2-dibromopropane and coupling of this synthetic alkyne with **1a** and **1b** proceeded without difficulty to give **4a** and **4b**, respectively. A minor quantity of cyclized byproduct (A), the 3-glycosyl-6-substituted-furano[2,3-



A

d]pyrimidin-2-one,[1] was observed as a fluorescent spot on thin-layer chromatography (TLC). The 2′-deoxy derivative (A, X = H; R = CH_3) was isolated in ~2% yield and characterized spectroscopically. The corresponding arabino byproduct (A, X = OTol; R = CH_3) was isolated in 14% yield. Deprotection of **4a** and **4b** gave 5-propynyl-1-β-D-arabinofuranosyluracil (**5a**) and 5-propynyl-2′-deoxyuridine (**5b**).

Butyne also underwent catalytic coupling with **1a** and **1b** in triethylamine at atmospheric pressure to give **6a** and **6b**. Deprotection of **6a** gave 5-butynyl-1-β-D-arabinofuranosyluracil (**7a**). The 2′-deoxy analogue (**7b**) was obtained previously.[13] Coupling of **1a** with 3,3-dimethyl-

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Table I. Antiviral and Antimetabolic Activities of 5-Substituted 2'-Deoxyuridines (dUrd) and Arabinosyluracils (*ara*-U) in Primary Rabbit Kidney (PRK) Cell Cultures

entry[a]	compound	ID_{50},[b] μg/mL HSV-1	HSV-2	VV	dThd incorp	dUrd incorp
	dUrd Derivatives					
1	5-propynyl-dUrd (**5b**)	0.3	0.7	0.4	3	0.7
2	5-butynyl-dUrd	4	32	7	13	2
3	5-(but-3-en-1-ynyl)-dUrd	3	23	3	38	2.5
4	5-pentynyl-dUrd	4	90	7	9	3
5	5-(3,3-dimethylbutynyl)-dUrd	4	19	7	6	2
6	5-hexynyl-dUrd	108	350	45	45	59
7	(*Z*)-5-hexenyl-dUrd	⩾400	>200	>400	5	34
8	5-hexyl-dUrd	400	350	300	7	52
9	5-heptynyl-dUrd	>400	>400	70	9	33
10	5-(phenylethynyl)-dUrd	>400	>400	>400	23	77
11	5-(3-hydroxypropynyl)-dUrd	36	37	10	68	8
12	5-(4-hydroxybutynyl)-dUrd	18	47	10	73	13
13	5-(5-hydroxypentynyl)-dUrd	⩾400	>400	125	>200	>156
14	5-[4-(*p*-toluenesulfonyloxy)butynyl]-dUrd	33	63	10	13	44
15	5-(3-methoxypropynyl)-dUrd (**11b**)	1	2	2	16	2
	ara-U Derivatives					
16	5-ethynyl-*ara*-U (**3a**)	4	6	250	50	80
17	5-propynyl-*ara*-U (**5a**)	20	50	200	92	>100
18	5-butynyl-*ara*-U (**7a**)	200	⩾400	>400	120	215
19	5-(3,3-dimethylbutynyl)-*ara*-U (**9a**)	>400	>400	>400	⩾400	>400
	Reference Compounds					
	EyDU 5-ethynyl-dUrd	0.5	1	0.1	3	0.05
	IDU 5-iodo-dUrd	0.15	0.3	0.2	1.5	0.12
	AraT 5-methyl-*ara*-U	0.3	0.25	10	200	⩾300
	BVDU (*E*)-5-(2-bromovinyl)-dUrd	0.007	2.5	5	90	25

[a] Syntheses of the compounds of entry numbers 2–14 are described in ref 1. [b] ID_{50} = concentration required to reduce virus-induced cytopathogenicity or deoxy[*methyl*-^{3}H]thymidine (dThd) or deoxy[1′,2′-^{3}H]uridine (dUrd) incorporation by 50%. Average values for three HSV-1 strains (KOS, F, and McIntyre) and two HSV-2 strains (Lyons and G). Abbreviations used are: HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; VV, vaccinia virus.

butyne proceeded without difficulty to give 5-(3,3-dimethylbutynyl)-1-(2,3,5-tri-*O*-*p*-toluyl-β-D-arabinofuranosyl)uracil (**8a**), which was deprotected to give 5-(3,3-dimethylbutynyl)-1-β-D-arabinofuranosyluracil (**9a**). Minor quantities of byproduct A [X = OTol; R = C_2H_5 and $C(CH_3)_3$] were isolated from the respective coupling reactions. Only trace amounts (TLC) of these furano[2,3-*d*]pyrimidin-2-one byproducts were observed in the coupling reactions to give **2a** and **6b**. Analogous coupling of **1b** with 3-methoxypropyne gave 5-(3-methoxypropynyl)-1-(3,5-di-*O*-*p*-toluyl-β-D-*erythro*-pentofuranosyl)uracil (**10b**), which was deprotected to give 5-(3-methoxypropynyl)-2′-deoxyuridine (**11b**).

Antiviral Activity. Several 5-alkynyl-dUrd analogues exhibited a marked inhibition of HSV-1 replication in primary rabbit kidney (PRK) cells, the most potent being 5-propynyl- and 5-ethynyl-dUrd (see Table I). The 5-butynyl- and 5-pentynyl-dUrd derivatives were also potent inhibitors of HSV-1 replication, whereas 5-hexynyl- and 5-heptynyl-dUrd were not. It thus appears that in order to confer a substantial antiviral effect, the 5-alkynyl side chain should not exceed five carbons in line. As shown by 5-(3,3-dimethylbutynyl)-dUrd, a six-membered side chain may still be compatible with antiviral potency, if the six carbons are not bonded linearly. Substitution of a hydroxy (or methoxy) function at the terminal carbon of the alkynyl side chain weakened the antiviral potency, as exemplified with the three to five carbon chain compounds. Whereas 5-hexynyl-dUrd exhibited some activity, the (*Z*)-5-hexenyl analogue was totally devoid of antiviral activity. Only one 5-alkyl-dUrd analogue, 5-hexyl-dUrd, was included in the present series of compounds. Unlike other 5-alkyl-dUrd analogues with shorter C-5 side chains (e.g., 5-ethyl- and 5-propyl-dUrd), 5-hexyl-dUrd did not exert an appreciable antiviral effect. No antiviral activity was observed with 5-(phenylethynyl)-dUrd, which contrasts with the relatively significant antiherpes potency of 5-(phenylethyl)-dUrd.[18] This probably reflects a difference in substrate acceptance by the kinase, since 5-(phenylethynyl)-dUMP is a reasonable inhibitor of isolated thymidylate synthetase.[11]

As noted for the 5-alkynyl-dUrd series, the antiviral potency of the 5-alkynyl-*ara*-U analogues critically depended on the length of the C-5 side chain; thus, in order of decreasing potency: 5-ethynyl-*ara*-U > 5-propynyl-*ara*-U > 5-butynyl-*ara*-U > 5-(3,3-dimethylbutynyl)-*ara*-U (see Table I). A similar dependence on the C-5 side-chain length has been reported previously for 5-alkyl derivatives of *ara*-U: *ara*-T > 5-ethyl-*ara*-U > 5-propyl-*ara*-U > 5-butyl-*ara*-U.[19] All four 5-alkynyl-*ara*-U derivatives that were included in the present study proved less potent as antiviral agents than their 5-alkynyl-dUrd counterparts. In general, 5-substituted-*ara*-U analogues are less potent inhibitors of HSV replication than the corresponding 5-substituted-dUrd analogues, as exemplified by 5-ethyl-*ara*-U[20] vs. 5-ethyl-dUrd,[21] 5-nitro-*ara*-U[22] vs. 5-nitro-

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Table II. Inhibitory Effects of 5-Substituted 2'-Deoxyuridines (dUrd) and Arabinosyluracils (*ara*-U) on the Growth and Metabolism of Murine Leukemia L1210 Cells

entry[a]	compound	ID_{50},[b] μg/mL		
		cell growth	dThd incorp	dUrd incorp
	dUrd Derivatives			
1	5-propynyl-dUrd (5b)	164	45	11
2	5-butynyl-dUrd	≥1000	61	10
3	5-(but-3-en-1-ynyl)-dUrd	>1000	107	3.5
4	5-pentynyl-dUrd	>1000	229	7
5	5-(3,3-dimethylbutynyl)-dUrd	287	48	9
6	5-hexynyl-dUrd	±1000	47	52
7	(*Z*)-5-hexenyl-dUrd	361	39	40
8	5-hexyl-dUrd	301	25	51
9	5-heptynyl-dUrd	219	45	48
10	5-(phenylethynyl)-dUrd	494	120	132
11	5-(3-hydroxypropynyl)-dUrd	>1000	439	19
12	5-(4-hydroxybutynyl)-dUrd	>1000	444	20
13	5-(5-hydroxypentynyl)-dUrd	280	>1000	136
14	5-[(4-*p*-toluenesulfonyloxy)butynyl]-dUrd	>200	112	140
15	5-(3-methoxypropynyl)-dUrd (11b)	254	74	4
	ara-U Derivatives			
16	5-ethynyl-*ara*-U (3a)	45	>1000	1000
17	5-propynyl-*ara*-U (5a)	36	>1000	>1000
18	5-butynyl-*ara*-U (7a)	>1000	>1000	>1000
19	5-(3,3-dimethylbutynyl)-*ara*-U (9a)	330	>1000	>1000
	Reference Compounds			
	EyDU 5-ethynyl-dUrd	0.09[c]	28[c]	0.16[c,d]
	IDU 5-iodo-dUrd	61[c]	4.3[c]	0.82[c,d]
	AraT 5-methyl-*ara*-U	6.3		
	FDU 5-fluoro-dUrd	0.001[c]	80[c]	0.003[c,d]

[a] Syntheses of the compounds of entry numbers 2–14 are described in ref 1. [b] ID_{50} = concentration required to reduce cell growth or deoxy[*methyl*-^{3}H]thymidine (dThd) or deoxy[1',2'-^{3}H]uridine (dUrd) incorporation by 50%. [c] As reported previously.[6] [d] [2-^{14}C]dUrd was used instead of [1',2'-^{3}H]dUrd.

dUrd,[23] 5-(propynyloxy)-*ara*-U[22] vs. 5-(propynyloxy)-dUrd,[24] and (*E*)-5-(2-bromovinyl)-*ara*-U vs. (*E*)-5-(2-bromovinyl)-dUrd[25,26] (although the latter two compounds are equally effective inhibitors of HSV-1 in some cell systems, e.g., human fibroblast cells).[26,27]

For most compounds, the pattern of susceptibility of HSV-2 and VV was similar to that noted for HSV-1 (Table I), although HSV-2 tended to be less susceptible to inhibition than HSV-1. Striking differences were observed in the susceptibility of HSV-1 and HSV-2 to the inhibitory effects of 5-butynyl-dUrd, 5-(but-3-en-1-ynyl)-dUrd, 5-pentynyl-dUrd, and 5-(3,3-dimethylbutynyl)-dUrd, although these differences did not attain the same amplitude as that recorded for BVDU (see also ref 9). Unlike their dUrd counterparts, 5-ethynyl-*ara*-U and 5-propynyl-*ara*-U were much less inhibitory to VV than HSV-1. Other examples of 5-substituted-*ara*-U analogues that have significant antiherpes potency but minimal antivaccinia potency are 5-ethyl-*ara*-U[20] and (*E*)-5-(2-bromovinyl)-*ara*-U.[28] Thus, it may be expected that transition from the 5-substituted-dUrd to the 5-substituted-*ara*-U series causes a relatively greater loss of antivaccinia than of antiherpes potency, with a resulting increase in the antiherpes specificity.

Antimetabolic Activity. As parameters of cytotoxicity, we determined the inhibitory effects of the compounds on incorporation of [*methyl*-^{3}H]dThd and [1',2'-^{3}H]dUrd into DNA of uninfected PRK cells. Those compounds that inhibit dUrd incorporation to a markedly greater extent than dThd incorporation may be assumed to act significantly at the thymidylate synthetase level.[6] Indeed, the conversion of dUMP to dTMP is the only metabolic step that distinguishes the pathways that lead to the incorporation of dUrd and dThd into DNA. Based on these premises, several potent inhibitors of thymidylate synthetase have been recognized, for example, 5-fluoro-dUrd, 5-(trifluoromethyl)-dUrd, 5-nitro-dUrd,[23] 5-ethynyl-dUrd,[8] 5-formyl-dUrd,[29] and the 5-oxime and 5-dithiolane derivatives of 5-formyl-dUrd.[30] In keeping with this pattern, several 5-alkynyl-dUrd analogues [e.g., 5-butynyl-dUrd, 5-(3-hydroxypropynyl)-dUrd, 5-(3-methoxypropynyl)-dUrd, 5-(4-hydroxybutynyl)-dUrd, and, in particular, 5-(but-3-en-1-ynyl)-dUrd] also could be considered as rather effective inhibitors of dTMP synthetase, albeit not as potent as 5-ethynyl-dUrd (Table I). In fact, the potency of the 5-alkynyl-dUrd analogues as "in vivo" inhibitors of dTMP synthetase, as reflected by the ID_{50} for dUrd incorporation into DNA, decreased with increasing length of the C-5 side chain: 5-ethynyl-dUrd > 5-propynyl-dUrd > 5-butynyl-dUrd > 5-pentynyl-dUrd > 5-hexynyl-dUrd. However, this may reflect the efficiency of phosphorylation of these thymidine analogues.[11]

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Table III. Chemical Data for Compounds Prepared in This Study

compd	yield, %	mp, °C	UV max,[a] nm (ε)	MS molecular ion calcd	MS molecular ion found	anal.	characteristic ^{1}H NMR peaks, δ
2a	83	199-200	283, 240 (12 600, 58 000)	694.2346	694.2360	C, H, N	0.18 (s, 9, $SiMe_3$), 6.49 (d, 1, H-1')
4a	72	212-214	283, 240 (10 600, 53 100)	636.2107	636.2096	C, H, N	1.96 (s, 3, $C{\equiv}CCH_3$), 6.48 (d, 1, H-1')
4b	90	239-241	283, 237 (11 700, 39 000)	502.1740	502.1738	C, H, N	1.90 (s, 3, $C{\equiv}CCH_3$), 6.24 (t, 1, H-1')
6a	66	163-166	283, 240 (10 900, 55 000)	650.2264	650.2256	C, H, N	1.08 (t, 3, $C{\equiv}CCH_2CH_3$), 6.49 (d, 1, H-1')
6b	85	224-225	284, 239 (11 400, 41 000)	516.1896	516.1873		1.04 (t, 3, $C{\equiv}CCH_2CH_3$), 6.25 (t, 1, H-1')
8a	72	206-209	284, 240 (11 200, 55 800)	678.2577	678.2571	C, H, N	1.18 (s, 9, CMe_3), 6.47 (d, 1, H-1')
10b	87	215-217	283, 238 (12 100, 35 700)			C, H, N	3.26 (s, 3, OCH_3), 6.27 (t, 1, H-1')
A (X = OTol; R = CH_3)[b]	14	177-181		636.2107	636.2119		2.30 (s, 3, $ArCH_3$), 6.58 (d, 1, H-1')
A (X = OTol; R = C_2H_5)	12	195-199		650.2264	650.2260		1.16 (t, 3, $ArCH_2CH_3$), 6.58 (d, 1, H-1')
A (X = OTol; R = CMe_3)	~1	247-252		678.2577	678.2557		1.24 (s, 9, $ArCMe_3$), 6.59 (d, 1, H-1')
A (X = H; R = CH_3)[c]	~2	185-188		502.1740	502.1720		2.31 (s, 3, $ArCH_3$), 6.32 (t, 1, H-1')
3a	87	205-207 dec	288, 229 (11 600, 10 500)	268.0695	268.0692	C, H, N[d]	4.06 (s, 1, C≡CH), 5.97 (d, 1, H-1')
5a	76	240-245 dec	290, 230 (10 900, 10 600)	282.0852	282.0853	C, H, N	1.98 (s, 3, $C{\equiv}CCH_3$), 5.96 (d, 1, H-1')
5b	94	187-190	291, 231 (10 200, 10 400)	266.0902	266.0903	C, H, N[e]	1.98 (s, 3, $C{\equiv}CCH_3$) 6.12 (t, 1, H-1')
7a	61	189-193	292, 232 (10 800, 11 700)	296.1008	296.1011	C, H, N	1.12 (t, 3, $C{\equiv}CCH_2CH_3$), 5.96 (d, 1, H-1')
9a	82	135-137	292, 231 (11 200, 11 600)	324.1321	324.1317	C, H, N[d]	1.23 (s, 9, CMe_3), 5.96 (d, 1, H-1')
11b	68	175-177	288, 230 (11 900, 10 600)	296.1008	296.1019	C, H, N	3.30 (s, 3, OCH_3), 6.13 (t, 1, H-1')

[a] UV spectra of all *p*-toluyl-protected compounds were determined in MeOH, and all deprotected nucleosides, in H_2O at pH 7.0. [b] 3-(2,3,5-Tri-*O*-*p*-toluyl-β-D-arabinofuranosyl)-6-methylfurano[2,3-*d*]pyrimidin-2-one. [c] 3-(3,5-Di-*O*-*p*-toluyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-6-methylfurano[2,3-*d*]pyrimidin-2-one. [d] With $0.5H_2O$. [e] With $0.25H_2O$.

From a comparison of the ID_{50} values for HSV (or VV) replication and dUrd (or dThd) incorporation (Table I), it is immediately clear that none of the new dUrd analogues tested could be regarded as a selective antiviral agent. For most compounds the ID_{50} required to inhibit dUrd incorporation corresponded closely to the dose that was required to inhibit virus replication, suggesting that the inhibition of virus replication was merely the consequence of an inhibitory effect on host cell metabolism (primarily dTMP synthesis). Notable exceptions to this trend were 5-ethynyl-*ara*-U and 5-propynyl-*ara*-U. These compounds inhibited HSV-1 replication at a dose that was at least 5 to 20 times lower than the dose required to inhibit dUrd incorporation. Thus, substitution of arabinose for 2-deoxyribose in the 5-ethynyl and 5-propynyl compounds led to a dramatic decrease of antimetabolic activity (inhibition of dUrd incorporation) that was not accompanied by a commensurate decrease in antiherpes potency. As a result, 5-ethynyl-*ara*-U and 5-propynyl-*ara*-U are more specific, albeit less potent, in their antiherpes activity than 5-ethynyl-dUrd and 5-propynyl-dUrd. From data reported previously,[8,31] it appears that 5-vinyl-*ara*-U also has greater antiherpes specificity than its 5-vinyl-dUrd counterpart.

Antitumor Activity. None of the newly synthesized 5-alkynyluracil nucleosides proved to be a very effective inhibitor of tumor cell proliferation. Their ID_{50} values for inhibition of the growth of murine leukemia (L1210) cells were several orders of magnitude higher than the ID_{50} previously obtained for 5-ethynyl-dUrd and 5-fluoro-dUrd (see Table II). Those 5-alkynyl-dUrd analogues that appeared to be effective inhibitors of dTMP synthetase in PRK cells [i.e., 5-butynyl-dUrd, 5-(3-hydroxypropynyl)-dUrd, 5-(3-methoxypropynyl)-dUrd, 5-(4-hydroxybutynyl)-dUrd, and 5-(but-3-en-1-ynyl)-dUrd] also were found to inhibit dUrd incorporation into DNA of L1210 cells at a much lower dose than that required to inhibit dThd incorporation (Table II). Hence, these compounds also appear to be inhibitors of dTMP synthetase in L1210 cells. However, their inhibitory effects on dTMP synthetase were not sufficient to effect a substantial suppression of tumor cell growth.

Experimental Section

Chemistry. General procedures and instrumentation were as described in ref 1. ^{1}H NMR spectra were determined at 100 MHz in Me_2SO-d_6 with Me_4Si as internal standard. Mass spectra were measured at 70 eV by direct sample introduction (150–250 °C) with an AEI MS-50 spectrometer with coupled computer processing by the Mass Spectrometry Laboratory of the Department of Chemistry at The University of Alberta.

Progress of the coupling reactions was monitored by TLC with E. Merck 60 F_{254} precoated silica gel sheets. For enhancement of resolution, the sheets were predeveloped with Et_3N/MeOH (1:9) and dried at room temperature before samples were applied. TLC development was performed with Et_3N/acetone/cyclohexane (2:49:49) as solvent. Coupling products migrated faster than starting **1a** or **1b** in all cases. E. Merck silica gel 60 PF_{254} was used for preparative TLC. E. Merck silica gel 60 (230–400 mesh)

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was used for short column chromatography.

Et_3N was heated at reflux over CaH_2 for several hours and then distilled. Copper(I) iodide was purchased from Fisher Scientific Co. Bis(triphenylphosphine)palladium(II) chloride was prepared in 94% yield according to Burmeister and Basolo.[32] Trimethylsilylacetylene was purchased from Petrarch Systems, Inc., butyne and 3,3-dimethylbutyne from Farchan Division, Chemsampco, Inc., and 3-methoxypropyne from Aldrich Chemical Co. Propyne was prepared from 1,2-dibrompropane according to Hurd et al.[17]

Coupling of Terminal Alkynes with Protected 5-Iodouracil Nucleosides. A suspension of 5 mmol of **1a** or **1b**,[14] 75 mg of $(Ph_3P)_2PdCl_2$, and 75 mg of CuI in 200 mL of anhydrous Et_3N was stirred vigorously and purged with oxygen-free N_2. Excess alkyne (see below) was added, and the reaction mixture was stirred under N_2 in an oil bath at 50 °C for 2–6 h (progress of the reactions was monitored by TLC). After the starting **1a** or **1b** was completely reacted, the mixture was cooled and evaporated and then dried overnight in vacuo. The colored residue was dissolved in ~250 mL of CH_2Cl_2, washed with 2 × 100 mL of 2% Na_2EDTA/H_2O and 100 mL of H_2O, dried (Na_2SO_4), and filtered, and the filtrate was evaporated to dryness.

The resulting residues of two 2′-deoxy compounds (**4b** and **6b**) were dissolved in minimal volumes of hot CH_2Cl_2, 98% EtOH (200 mL) was added, and the mixtures were chilled overnight at 0 C before filtration. The products were recrystallized from 98% EtOH to give pure **4b** and **6b** with properties listed in Table III.

The dark-colored residues obtained with the arabinosyl analogues were dissolved in a small volume of CH_2Cl_2 and purified by passage through a short column of silica gel with $CHCl_3$ as the elution solvent. Appropriate fractions were evaporated, and the residues were crystallized and recrystallized from 98% EtOH to give the pure products listed in Table III.

5-Propynyl- and 5-(3-methoxypropynyl)-3′,5′-di-*O*-*p*-toluyl-2′-deoxyuridine (**4b** and **10b**) were quite insoluble in Et_3N. An alternative isolation of **4b** (and of **10b**) involved their filtration from the cooled reaction mixtures and washing with cold Et_3N. They then were dissolved in CH_2Cl_2 and processed as in the general procedure given above.

The mother liquors from crystallization of **4a,b** and **6a** were evaporated, and the residues were chromatographed by preparative TLC. Resolution was enhanced when the plates were predeveloped with Et_3N/MeOH (1:9) and air-dried at room temperature before the samples were applied. Development with Et_3N/acetone/cyclohexane (3:15:85) then gave good separation of the 5-alkynyl products from the fluorescent furano[2,3-*d*]pyrimidin-2-one byproducts. The zones were eluted with acetone, and the products were crystallized from 98% EtOH to give an additional crop of **4a,b** and **6a** plus the purified byproducts. Fractional crystallization separated **8a** and its cyclized byproduct.

Propyne was generated by the dropwise addition of 20.2 g (0.1 mol) of 1,2-dibromopropane to a refluxing mixture of 18 g (0.32 mol) of KOH in 40 mL of *n*-BuOH over a period of ~2 h. The gaseous propyne was introduced through a discharge tube into the stirred mixture of **1a** or **1b** and catalysts in Et_3N at room temperature. The general coupling procedure was then followed. Butyne (~15 equiv) was introduced slowly from a commercial cylinder (on a large balance), and the reactions were executed analogously. Approximately 3 equiv of the liquid trimethylsilylacetylene, 3,3-dimethylbutyne, and 3-methoxypropyne was used.

Deprotection of the para-toluylated intermediates was effected by stirring a 2-mmol sample of the compound in 40 mL of 0.2 M NaOMe/MeOH at room temperature for ~2 h (TLC, MeOH/$CHCl_3$, 2:8). Dowex 50W (H^+) resin was added, and stirring was continued until the solution was neutral (moistened pH paper). The mixture was filtered, the resin was washed with MeOH, and the combined filtrate was evaporated. The residue was treated with Et_2O and H_2O. The separated aqueous layer was washed with Et_2O and then evaporated. The residue was crystallized from EtOH (**5b** and **11b**), EtOH/H_2O (**3a** and **5a**), or EtOH with diffusion of Et_2O[33] (**7a**) or lyophilized (**9a**) to give the products listed in Table III.

Antiviral, Antimetabolic, and Antitumor Assays. The methodology for measuring the inhibition of virus-induced cytopathogenicity in PRK cell cultures and for measuring the inhibition of incorporation of [*methyl*-^{3}H]dThd and [1′,2′-^{3}H]dUrd into DNA of these cells has been described previously.[18,23,29] The procedures for monitoring the inhibition of L1210 cell growth and incorporation of [*methyl*-^{3}H]dThd and [1′,2′-^{3}H]dUrd into L1210 cell DNA also have been described.[6]

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Registry No. **1a**, 81777-55-1; **1b**, 31356-86-2; **2a**, 84558-89-4; **3a**, 84558-90-7; **4a**, 84558-91-8; **4b**, 84558-92-9; **5a**, 84558-93-0; **5b**, 84558-94-1; **6a**, 84558-95-2; **6b**, 77875-86-6; **7a**, 84558-96-3; **8a**, 84558-97-4; **9a**, 84558-98-5; **10b**, 84558-99-6; **11b**, 84559-00-2; A (X = OTol; R = CH_3), 84559-01-3; A (X = OTol; R = C_2H_5), 84559-02-4; A (X = OTol; R = CMe_3), 84559-03-5; A (X = H; R = CH_3), 84559-04-6; 5-butynyl-dUrd, 77875-96-8; 5-(but-3-en-1-ynyl)-dUrd, 84582-78-5; 5-pentynyl-dUrd, 77887-18-4; 5-(3,3-dimethylbutynyl)-dUrd, 77887-19-5; 5-hexynyl-dUrd, 77875-97-9; (*Z*)-5-hexenyl-dUrd, 84621-32-9; 5-hexyl-dUrd, 57741-93-2; 5-heptynyl-dUrd, 77875-98-0; 5-(phenylethynyl)-dUrd, 77887-20-8; 5-(3-hydroxypropynyl)-dUrd, 77875-99-1; 5-(4-hydroxybutynyl)-dUrd, 77876-00-7; 5-(5-hydroxypentynyl)-dUrd, 77876-01-8; 5-[4-(*p*-toluenesulfonyloxy)butynyl]-dUrd, 84559-05-7; trimethylsilylacetylene, 1066-54-2; bis(triphenylphosphine)palladium(II) chloride, 13965-03-2; copper(I) iodide, 7681-65-4; propyne, 74-99-7; butyne, 107-00-6; 3,3-dimethylbutyne, 917-92-0; 3-methoxypropyne, 627-41-8.

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