reducing radioligand binding by 50% were determined.

Estrogen and Antiestrogen Assays. Estrogenic and antiestrogenic properties were determined by stimulation of the uterine growth or by inhibition of the uterine growth stimulated by estrone, respectively, using immature NMRI mice as described previously.[4,5] Female mice (body weight, 10–12 g; age, 20 days at test beginning, 10 mice per group) were injected sc daily for 3 days with solutions of the test compounds in olive oil (0.1 mL/mouse). The uteri were removed 24 h after the last injection, fixed with Bouin's solution, dried, and weighed.

Hormone-Dependent, Transplantable MXT Mammary Tumor of the BDF1 Mouse.[17,18] The MXT tumor used in these studies was the MXT line 3.2 provided by Dr. Bogden, Laboratory of Experimental Oncology, EG & G Bogden Laboratories, Worcester, MA, in a frozen state. The tumor was transplanted in pieces of about 2 mm^3 subcutaneously in female, 8-weeks-old BDF1-mice (body weight, 20 ± 1.6 g; Charles River Wiga, West Germany). After the tumor had reached a diameter of about 1 cm, it was transplanted to 20 mice to determine the hormone dependence. After transplantation the animals were randomly distributed in two groups of 10. The animals of one group were ovariectomized. The tumor grew well in control animals but only very slowly in the ovariectomized mice. Take rate of control animals was >95%. In an experiment to determine the tumor inhibiting activity of new compounds, transplantation is carried out as above (one tumor piece/animal). After transplantation, the animals are randomly distributed into groups of 10. Starting with the first day after transplantation, the test compounds were injected sc three times a week (Monday, Wednesday, Friday) as olive oil solutions (0.1 mL/mouse). The duration of treatment was 6 weeks. At the end of treatment, the animals were killed by cervical dislocation and weighed. The tumors were removed, washed in 0.9% sodium chloride solution, blotted dry, and weighed, and the average tumor weight was calculated. The uteri were also removed and prepared as described in ref 6 to serve as an indicator of the estrogenic side effects of the compounds.

Acknowledgment. We thank Dr. A. Bogden and Dr. G. Leclercq for providing us the MXT tumor and for helpful discussions in setting up the MXT-tumor model, M. Beer, K. Röhrl, and G. Tondl for skillful technical assistance, and the M. Scheel-Foundation, Deutsche Krebshilfe and the Verband der Chemischen Industrie, Fonds der Chemischen Industrie for financial support.

Registry No. 4, 1835-04-7; 5, 14046-55-0; 6, 6026-75-1; *meso*-7, 98876-44-9; (±)-7, 98876-45-0; *meso*-8, 98876-46-1; (±)-8, 98900-86-8; *meso*-9, 98876-47-2; (±)-9, 98876-48-3; 10, 98876-49-4; 10 (demethylated), 98876-50-7; 11, 98876-51-8; 11 (demethylated), 98876-52-9; 12, 98876-53-0; 12 (demethylated), 98876-54-1; 13, 98876-55-2; 14, 98876-56-3; 15, 98876-57-4; $(CH_3)_2CHCOCl$, 79-30-1; CH_3CH_2COCl, 79-03-8; *m*-BrC_6H_4OMe, 2398-37-0; veratrole, 91-16-7.

Supplementary Material Available: ^1H NMR data (Tables VIII and IX) of methoxy- and acetoxy-substituted 2-phenylindenes (2 pages). Ordering information is given on any current masthead page.

Synthesis and Antiviral Activity of the Carbocyclic Analogues of 5-Ethyl-2′-deoxyuridine and of 5-Ethynyl-2′-deoxyuridine

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The carbocyclic analogue of the antiviral agent 5-ethyl-2′-deoxyuridine (EDU) was synthesized by two routes. The pivotal step in the first route is the reaction of lithium dimethylcuprate with the carbocyclic analogue of 5-(bromomethyl)-2′-deoxyuridine dibenzoate (**6**). The second route is based on the synthesis of the carbocyclic analogue of 5-ethynyl-2′-deoxyuridine (**12**) by a coupling reaction catalyzed by bis(triphenylphosphine)palladium(II) chloride and copper(I) iodide, a method reported recently (Robins and Barr) for the synthesis of the true nucleoside 5-ethynyl-2′-deoxyuridine (**1b**). The carbocyclic analogue of EDU inhibits the replication of type 1 and type 2 herpes simplex viruses in Vero cells. The carbocyclic analogue of 5-ethynyl-2′-deoxyuridine has modest activity against herpes simplex virus, types 1 and 2.

5-Ethyl-2′-deoxyuridine (**1a**, EDU), synthesized originally from 5-ethyluracil,[1-3] was shown to have antiviral activity against herpes simplex and vaccinia viruses.[2-6] EDU inhibits the replication of both type 1 (HSV-1) and type 2 (HSV-2) herpes simplex viruses in cells in culture.[7-9] EDU, as well as related 5-substituted 2′-deoxyuridines, inhibits the replication of strains of HSV-1 that induce thymidine kinase, but it is ineffective against strains that lack the capacity to induce virus thymidine kinase in infected cells (TK^- strains).[7,8] Its affinities for thymidine kinases induced by HSV-1 and HSV-2 are about the same as the affinities of thymidine for these kinases, but the analogue binds much less firmly to cytoplasmic and mitochondrial thymidine kinases from human cells.[8] Furthermore, it has been reported that EDU is active in vivo against HSV-1 and HSV-2 encephalitis,[9,10] was somewhat more effective in vitro against clinical isolates of HSV-2 than against clinical isolates of HSV-1,[11] inhibits replication in cultured cells of several strains of Varicella-Zoster virus,[12] and is not immunosuppressive[13] or mutagenic.[3,14]

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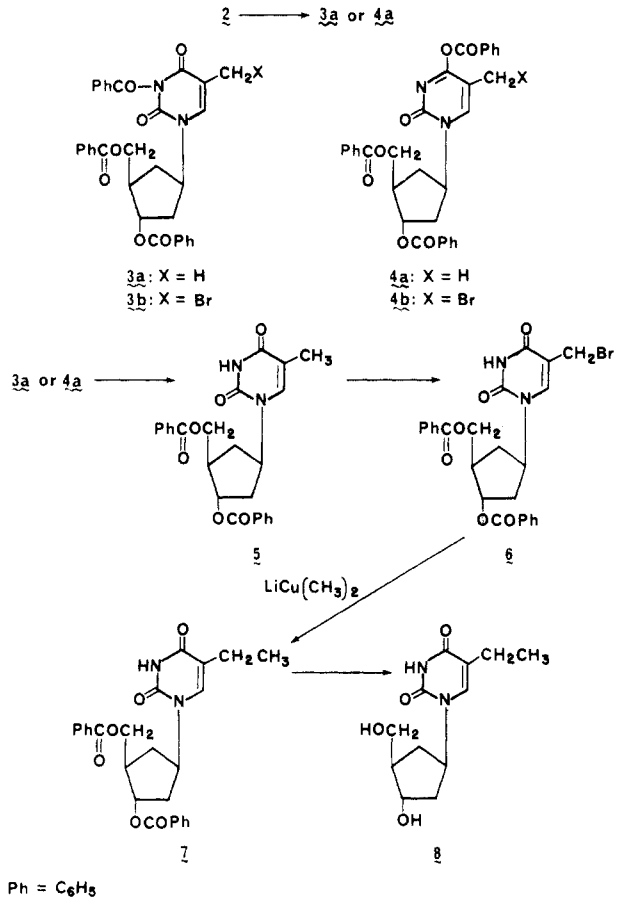
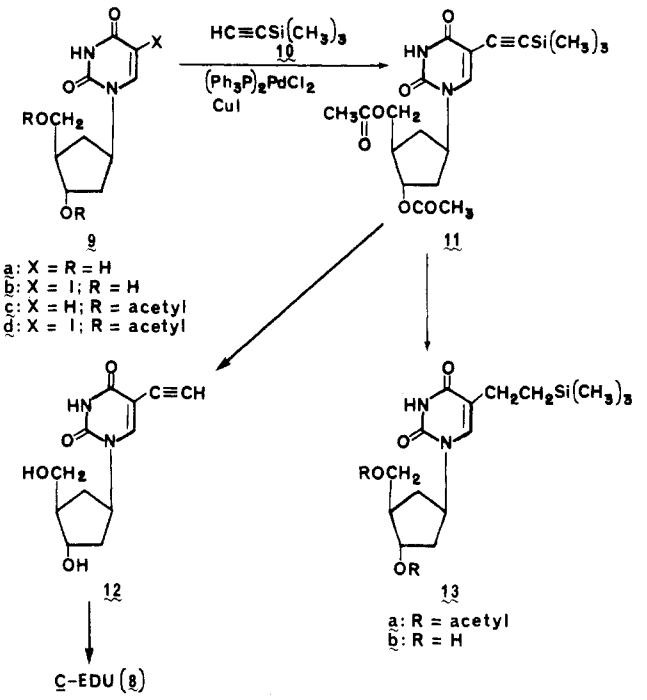
Scheme I

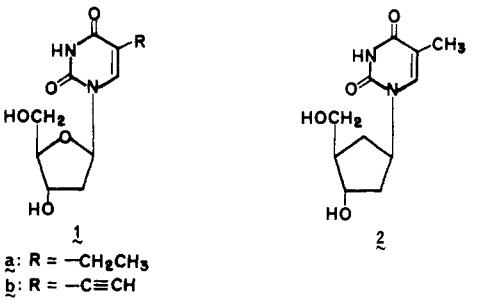


However, EDU and other 5-substituted 2′-deoxyuridines are degraded by thymidine phosphorylase to the corresponding 5-substituted uracils,[15] and 5-ethyluracil and 5-(1-hydroxyethyl)uracil (the major metabolite) have been identified as metabolites of EDU in vivo (rats).[16] We have pointed out earlier[17-19] that carbocyclic analogues of nucleosides, because they possess a cycloalkyl–heterocycle bond in place of the glycosidic bond of true nucleosides, are not subject to cleavage by nucleoside phosphorylases. Some carbocyclic analogues, therefore, may be more effective in various kinds of biological activities than are the corresponding true nucleosides. Both the favorable actions of EDU and the disadvantage posed by the phosphorolysis of this compound provide incentives for synthesizing the carbocyclic analogue (*C*-EDU[20]). In addition, the carbocyclic analogue of thymidine (*C*-thymidine, **2**), synthesized earlier,[21,22] has antineoplastic activity[22] (L1210 leukemia)

Scheme II



and antiviral activity[23,24] (HSV-1 and HSV-2). It was reasonable, therefore, to expect that *C*-EDU as an analogue of EDU and a homologue of *C*-thymidine would, at least, retain the antiviral activity of EDU and might, perhaps, be more effective. For these reasons, we synthesized the carbocyclic analogue of the interesting antiviral agent EDU.



Chemistry. Two synthesis routes to *C*-EDU were investigated. The first route, outlined in Scheme I, begins with *C*-thymidine (**2**). Treatment of **2** with a molar excess of benzoyl chloride in pyridine by a procedure employed previously[25] for the benzoylation of carbocyclic analogues of uracil nucleosides produced a tribenzoyl derivative[26] (**3a** or **4a**) of **2**. Hydrolysis of the tribenzoyl derivative in a weakly acidic medium gave *C*-thymidine dibenzoate (**5**), and bromination of **5** with *N*-bromosuccinimide, by a procedure similar to that of Bärwolf and Langen[28,29] for

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(26) Similarities between chemical shifts of analogous protons in the ^{1}H NMR spectra of *C*-thymidine dibenzoate (**5**) and of the tribenzoyl derivative (**3a** or **4a**) indicate that the structures of the pyrimidine moieties are the same and suggest, therefore, that **3a** is the structure of the tribenzoyl derivative. The evidence is consistent with the assignment, based on ^{13}C NMR data, of the *N*-acyl structure to several tetraacyl uridines.[27]

Table I. Evaluation of *C*-EDU and Compounds 11–13 for Inhibition of the Replication of Herpes Simplex Virus in Vero Cells[a]

compd	exp no.	HSV-1, strain 377 VR[b]	HSV-1, strain 377 MIC_{50}[c] mcg/mL	HSV-1, strain 377 MIC_{50}[c] μM/mL	HSV-2, strain MS VR[b]	HSV-2, strain MS MIC_{50}[c] mcg/mL	HSV-2, strain MS MIC_{50}[c] μM/mL
C-EDU (8)	1	2.4	45	0.17	1.1	230	0.89
	2	2.1	70	0.27	1.1	180	0.7
C-5-ethynyl-2'-deoxyuridine (12)	1	0.9	250	0.99	1.1	210	0.83
EDU (1a)[d]	2	2.3	20	0.08	2.2	37	0.14
ara-A[d,e]	1	2.1	18	0.07	1.7	22	0.08
	2	2.0	15	0.06	1.3	45	0.17
C-thymidine[f]	2	1.8	24	0.10	1.2	57	0.24
11	3	0			0		
13b	3	0.5	320	0.98	0.1		
ara-A[d,g]	3	2.4	5	0.02	1.2	24	0.09

[a] The antiviral activity of each compound is expressed as a virus rating (VR), and the potency is given as a minimum inhibitory concentration (MIC_{50}). The VR, determined by the general method of Ehrlich et al.,[37] is a weighted measurement of antiviral activity that takes into account both the degree of inhibition of virus-induced cytopathogenic effects and the degree of cytotoxicity produced by the test compound. [b] A virus rating (VR) equal to or greater than 1.0 indicates definite and significant antiviral activity, a VR of 0.5–0.9 indicates marginal to moderate antiviral activity, and a VR less than 0.5 usually indicates no significant antiviral activity. [c] The MIC_{50} is the concentration of the tested compound required to inhibit virus-induced cytopathogenic effects by 50%. [d] The true nucleoside EDU and the antiviral drug 9-β-D-arabinofuranosyladenine (*ara*-A) were positive controls in these experiments. [e] Positive control for 8, 12, and 1a. [f] In tests of *C*-thymidine vs. strain 377 of HSV-1 and strain MS of HSV-2 replicating in *rabbit kidney cells*, the results were as follows: VR = 5.4, MIC_{50} = 0.8 for HSV-1; VR = 3.2, MIC_{50} = 7 for HSV-2.[23] [g] Positive control for 11 and 13b.

the bromination of thymidine diacetate, afforded an impure specimen of the 5-bromomethyl derivative (**6**). The mass spectrum (FAB) and the proton NMR spectrum of this material confirmed that it was composed principally of **6**, but the NMR spectrum also showed the presence of some succinimide and of a small amount of **5**. Since it was difficult to remove these components from **6**, a reactive compound, the crude material was used for the next step. Treatment of **6** with lithium dimethylcuprate[30,31] furnished the desired *C*-EDU dibenzoate (**7**), and removal of the benzoyl groups with ammonia in methanol furnished *C*-EDU (**8**).

Tribenzoyl-*C*-thymidine (**3a** or **4a**) was prepared initially in order to obtain the fully benzoylated 5-bromomethyl derivative (**3b** or **4b**) for use as an intermediate. Treatment of the latter compound (prepared by a procedure similar to that employed for the preparation of **5**) with lithium dimethylcuprate produced a crude product that contained, according to mass spectral analysis, some of the desired tribenzoyl *C*-EDU, but the route via **5** appears to be superior.

The second route, outlined in Scheme II, begins with *C*-5-iodo-2'-deoxyuridine[23,24] (**9b**) and parallels the synthesis route to EDU described recently by Robins and Barr.[32] The crucial step in the second route is the reaction of ethynyltrimethylsilane (**10**) with *C*-5-iodo-2'-deoxyuridine diacetate[24] (**9d**) in the presence of the bis(triphenylphosphine)palladium(II) chloride–copper(I) iodide catalyst in triethylamine. The reaction of alkynes with aromatic halides catalyzed by (triphenylphosphine)palladium(II) catalysts had been reported by Heck[33] and by Cassar,[34] and the use of bis(triphenylphosphine)palladium(II) chloride and copper(I) iodide in diethylamine to catalyze the reaction of alkynes with iodoarenes, bromoalkenes, and bromopyridines has been reported by Sonogashira et al.[35] Austin et al.[36] employed ethynyltrimethylsilane (**10**) with palladium(II) acetate–triphenylphosphine in triethylamine, followed by removal of the trimethylsilyl group in basic media, to obtain ethynylbenzene derivatives.

The palladium-catalyzed reaction of *C*-IdUrd diacetate (**9d**) with **10** furnished the 5-(trimethylsilylethynyl) derivative **11** in 75% yield (after purification), and removal of both the trimethylsilyl and the acetyl groups of **11** in sodium methoxide–methanol solution[32] afforded the desired *C*-5-ethynyl-2'-deoxyuridine (**12**). Catalytic hydrogenation of **12** furnished *C*-EDU. The 5-(trimethylsilyl)-ethyl derivative **13b** was obtained similarly by hydrogenation of **11** and deacetylation of the reduction product **13a** in ammonia–methanol. The specimens of *C*-EDU obtained by the two routes of Schemes I and II were shown to be identical by thin-layer chromatography, high-pressure liquid chromatography, melting point determinations, and comparisons of their infrared spectra.

Biological Evaluation. *C*-EDU, *C*-5-ethynyl-2'-deoxyuridine (**12**), and *C*-5-[(trimethylsilyl)ethyl]-2'-deoxyuridine (**13b**) were evaluated for antiviral activity in vitro against HSV-1 and HSV-2. EDU (**1a**) and 9-β-D-arabinofuranosyladenine (*ara*-A) were positive controls in these experiments. The results of these tests, which are summarized in Table I, show that both *C*-EDU (**8**) and *C*-5-ethynyl-2'-deoxyuridine (**12**) have significant activity against both HSV-1 and HSV-2. Strain 377 of HSV-1 and strain MS of HSV-2 induce virus-specific thymidine kinases; i.e., they are TK^+ strains. The results of Table I indicate, further, that *C*-EDU is as active (VR, 2.1–2.4) vs. HSV-1 as EDU and *ara*-A are, but it is less potent (MIC_{50}) than are these positive controls. *C*-EDU and the *C*-5-ethynyl derivative (**12**) have significant, and comparable, activity against HSV-2, but these analogues are less active and less potent than EDU and *ara*-A. As pointed out above, however, the carbocyclic analogues are not subject

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to the action of phosphorylases, which cleave EDU.[15,16]

Experimental Section[38]

General Methods. Decomposition and melting temperatures (mp) were determined in capillary tubes heated in a Mel-Temp apparatus. Ultraviolet spectra (UV) were recorded with a Cary Model 17 spectrophotometer, and absorption maxima are reported in nanometers; sh = shoulder. Solutions for ultraviolet spectral determinations were prepared by diluting a 5-mL aliquot of a water or ethanol solution to 50 mL with 0.1 N hydrochloric acid, phosphate buffer (pH 7), or 0.1 N sodium hydroxide. Absorption maxima of these solutions are reported as being determined at pH 1, 7, or 13, respectively. Infrared spectra (IR) were recorded with a Nicolet MX-10 Fourier transform spectrometer, and the spectra were determined with specimens in pressed potassium bromide disks; s = strong, vs = very strong, sh = shoulder, w = weak. Mass spectral data (MS) were taken from low-resolution, electron-impact spectra determined at 70 eV with a Varian/MAT 311 A spectrometer or from spectra determined by the fast-atom-bombardment (FAB) method. The peaks listed are those arising from the molecular ion (M), those attributable to the loss of certain fragments (M minus a fragment), and some other prominent peaks. Fragments containing the complete pyrimidine moiety are designated P plus an atom or group. Proton nuclear magnetic resonance spectra were determined at 300.64 MHz with a Nicolet 300 NB NMR spectrometer. The internal standard was tetramethylsilane; s = singlet, t = triplet, q = quartet, qn = quintet, m = multiplet. Thin-layer chromatography (TLC) was performed on plates of silica gel, the developing solvent is specified parenthetically, and developed plates were examined by ultraviolet light.

Tribenzoyl Derivative of *C*-Thymidine (3a or 4a).[26] A solution consisting of 5.0 mL (43 mmol) of benzoyl chloride, 2.0 g (8.3 mmol) of *C*-thymidine (**2**), and 100 mL of dry pyridine was heated at 57–58 °C for 70 h. The reaction solution was added slowly to a water–ice mixture (800 mL), and the amorphous precipitate that formed was extracted into portions of chloroform (total, 300 mL). The chloroform solution was washed successively with three 100-mL portions of 0.1 N hydrochloric acid, saturated sodium bicarbonate solution, and water. The organic layer was dried with magnesium sulfate, filtered, and concentrated in vacuo to a yellow gum (weight, 4.3 g). This material was purified by chromatography on a column of silica gel 60 (150 g) with 9:1 chloroform–methanol as the developing and eluting solvent. The fractions that contained the tribenzoyl derivative were selected by TLC and were combined and concentrated in vacuo to a white glass; yield, 4.02 g (88%). When cyclohexane was added to an ethyl acetate solution of the glassy product, an amorphous precipitate formed. The solvents were evaporated under reduced pressure, and the white glass was kept in vacuo at 56 °C for 3 h: MS (electron-impact; direct-probe temperature, 250 °C), *m/e* 552 (M), 524, 482, 447 (M – C_6H_5CO), 430 (M – C_6H_5COOH), 326, 325, 203, 126, 122 (C_6H_5COOH), 105 (C_6H_5CO); ^1H NMR ($CDCl_3$) δ 1.77 m and 2.52 m (CH_2, position 5 of the cyclopentyl group), 1.91 s (CH_3), 2.37 m (CH_2, position 2 of the cyclopentyl group), 2.75 m (CH, position 4 of the cyclopentyl group), 4.52 d (CH_2 of $CH_2OCOC_6H_5$), 5.22 qn (CH, position 1 of the cyclopentyl group), 5.46 m (CH, position 3 of the cyclopentyl group), 7.15 s (CH, position 6 of the pyrimidinyl group), 7.32–7.67 m (CH, positions 3, 4, and 5 of the phenyl groups), 7.86–8.06 m (CH, positions 2 and 6 of the phenyl groups). Anal. ($C_{32}H_{28}N_2O_7 \cdot 0.5EtOAc$) C, H, N.

(±)-1-[(1α,3β,4α)-3-Hydroxy-4-(hydroxymethyl)cyclopentyl]-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione Dibenzoate (5). A solution consisting of 1.15 g (2.08 mmol) of tribenzoyl-*C*-thymidine (**3a** or **4a**), 133 mL of ethanol, 60 mL of water, and 7.2 mL of 1 N hydrochloric acid was heated under gentle reflux for 24 h. The reaction solution was concentrated to remove ethanol, and the aqueous mixture (containing a colorless syrup) was extracted twice with 50-mL portions of chloroform. The total chloroform extract was washed with saturated aqueous sodium bicarbonate solution, dried with magnesium sulfate, filtered, and concentrated under reduced pressure to a colorless gum. The residue was mixed well with ethanol (15 mL); the mixture (containing white crystals) was stored in a freezer (–20 °C); and the crystalline product was collected by filtration, washed with cold ethanol, and dried in vacuo: yield, 725 mg (78%); mp 173–176 °C; mass spectrum (FAB), *m/e* 449 (M + 1), 327 (M – C_6H_5COO); ^1H NMR ($CDCl_3$) δ 1.73 m and 2.50 m (CH_2, position 5 of the cyclopentyl group), 1.89 s (CH_3), 2.35 m (CH_2, position 2 of the cyclopentyl group), 2.77 m (CH, position 4 of the cyclopentyl group), 4.54 d (CH_2OCOPh), 5.24 qn (CH, position 1 of the cyclopentyl group), 5.47 m (CH, position 3 of the cyclopentyl group), 7.06 s (CH, position 6 of the pyrimidinyl group), 7.42 m (CH, positions 3 and 5 of the phenyl groups), 7.56 m (CH, position 4 of the phenyl groups), 8.01 m (CH, positions 2 and 6 of the phenyl groups). Anal. ($C_{25}H_{24}N_2O_6$) C, H, N.

(±)-5-(Bromomethyl)-1-[(1α,3β,4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-2,4(1*H*,3*H*)-pyrimidinedione Dibenzoate (6). A mixture of *N*-bromosuccinimide (149 mg, 0.84 mmol), 300 mg (0.67 mmol) of *C*-thymidine dibenzoate (**5**), and dry carbon tetrachloride (34 mL) was boiled under reflux for 1 h and simultaneously was exposed to light (provided by a 150-W flood lamp). The colorless solution was decanted from a waxy precipitate and was concentrated in vacuo under anhydrous conditions to a gummy residue; yield, 99% (350 mg). The mass spectrum and the ^1H NMR spectrum of this material demonstrated that it was predominantly the desired 5-(bromomethyl)uracil derivative (**6**): MS (FAB), *m/e* 447 (M – Br), 405 (M – C_6H_5COO), 323 (M – P); ^1H NMR ($CDCl_3$) δ 1.82 m and 2.55 m (CH_2, position 5 of the cyclopentyl group), 2.41 s (CH_2, position 2 of the cyclopentyl group), 2.8 m (CH, position 4 of the cyclopentyl group), 4.23 d (CH_2Br), 4.57 d (CH_2OCOPh), 5.21 qn (CH, position 1 of the cyclopentyl group), 5.52 m (CH, position 3 of the cyclopentyl group), 7.49 s (CH, position 6 of the pyrimidinyl group), 7.45 m (CH, positions 3 and 5 of the phenyl groups), 7.58 m (position 4 of the phenyl groups), 8.04 m (positions 2 and 6 of the phenyl groups).

(±)-5-Ethyl-1-[(1α,3β,4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-2,4(1*H*,3*H*)-pyrimidinedione (*C*-EDU, 8) via Scheme I. A solution of 350 mg (0.664 mmol) of the 5-(bromomethyluracil derivative (**6**) in a mixture of dry ether (10 mL) and dry tetrahydrofuran (5 mL) was prepared under an argon atmosphere and was cooled to –15 °C. A solution of the lithium dimethylcuprate reagent was then prepared as follows. A suspension of cuprous iodide (1.43 g) in dry ether (25 mL) was prepared under an argon atmosphere and was cooled to –15 °C. To this stirred suspension was added dropwise (during approximately 10 min) a 1.5 M solution (10 mL) of methyllithium in ether. A yellow precipitate formed initially but the reaction mixture later became clear. A portion (5.6 mL) of the solution of lithium dimethylcuprate (calculated to contain 1.20 mmol of the reagent) was added dropwise to the stirred, cold (–15 °C) solution of the 5-(bromomethyl)uracil derivative, and the resulting mixture was kept at 3 °C overnight. An aqueous solution of ammonium chloride (5%, 15 mL) was added to the reaction mixture, and the resulting mixture was stirred at room temperature for 1 h and then extracted twice with chloroform (2 × 30 mL). The total chloroform extract was washed with water, dried over magnesium sulfate, filtered, and concentrated under reduced pressure to a syrup. *C*-EDU dibenzoate (**7**) was isolated by chromatography on silica gel 60 with 1:1 ethyl acetate–benzene as the developing and eluting solvent. The collection of fractions was monitored by TLC; fractions containing *C*-EDU dibenzoate were combined and concentrated in vacuo to a white solid foam: yield, 40% (120 mg); mass spectrum (FAB), *m/e* 463 (M + 1), 341 (M – C_6H_5COO).

A solution of 115 mg (0.249 mmol) of *C*-EDU dibenzoate (**7**) in an ammonia–methanol solution (10 mL, 10% ammonia) was stirred at room temperature for 2.5–3 days. The reaction solution was concentrated under reduced pressure to a syrupy residue. *C*-EDU was isolated by applying a methanol solution of the residue to a preparative TLC plate of silica gel and developing the chromatogram with 5:1 chloroform–methanol. The principal band was scraped from the plate and extracted in a Soxhlet extractor with hot ethanol for 2 h. The ethanol extract was concentrated

(38) In accordance with *Chemical Abstracts* nomenclature, most of the *C*-2′-deoxyuridines described in this section are named as (±)-1-[(1α,3β,4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-2,4(1*H*,3*H*)-pyrimidinediones.

to dryness, a small amount of ethanol was added, the mixture was filtered, and the filtrate was concentrated under reduced pressure to a colorless syrup. The residue was triturated with ethyl acetate, and the resulting white crystalline solid was collected by filtration and dried in vacuo: yield, 41 mg (65%); mp 147–150 °C (capillary inserted at 50 °C, heating rate 3 °C/min); TLC, 1 spot (5:1 chloroform–methanol); MS (FAB), *m/e* 255 (M + 1), 141 (P + 2H); UV max 274 nm (ε 10 300) at pH 1, 273 (ε 10 500) at pH 7, and 271 (ε 8000) at pH 13; MS (electron-impact; direct-probe temperature, 200 °C), *m/e* 254 (M), 239 (M – CH_3), 224 (M – CH_2OH + H), 195 (M – CO – CH_2OH), 167 (P + C_2H_4), 141 (P + 2H), 140 (P + H); IR (1800–1300-cm^{-1} region) 1685 vs, 1670 vs, 1640, 1515 w, 1475, 1465, 1435 w, 1420, 1390, 1370, 1335, 1315; ^1H NMR (Me_2SO-d_6) δ 1.02 t (CH_3), 1.42 m and 2.05 m (CH_2, position 5 of the cyclopentyl group), 1.7–2.0 m (CH_2, position 2 of the cyclopentyl group), 1.91 m (CH, position 4 of the cyclopentyl group), 2.22 q (CH_2 of the ethyl group), 3.45 m (CH_2 of CH_2OH), 3.98 m (*CH*OH, position 3 of the cyclopentyl group), 4.60 t (OH of CH_2OH), 4.70 d (OH at position 3 of the cyclopentyl group), 4.96 m (CH, position 1 of the cyclopentyl group), 7.47 s (CH, position 6 of the pyrimidine ring), 11.16 s (NH of pyrimidine ring). Anal. ($C_{12}H_{18}N_2O_4$) C, H, N.

(±)-1-[(1α,3β,4α)-3-Hydroxy-4-(hydroxymethyl)cyclopentyl]-2,4(1*H*,3*H*)-pyrimidinedione Diacetate (9c). A solution (protected from atmospheric moisture) of 0.63 mL (6.0 mmol) of acetic anhydride, 10 mL of anhydrous pyridine, and 0.5 g (2.21 mmol) of *C*-2'-deoxyuridine[39] (**9a**) was stirred at room temperature for 21 h. The reaction solution was then concentrated under reduced pressure by means of a vacuum (oil) pump. The residue was mixed well with water (2 mL), the mixture was concentrated in vacuo, the solid residue was triturated with ethyl acetate (5 mL), and this mixture was placed in a refrigerator. The white crystalline solid was separated by filtration, washed with ethyl acetate, and dried in vacuo at 56 °C: yield, 476 mg (70%); mp 110–112 °C; TLC, 1 spot (9:1 chloroform–methanol); MS (electron-impact; direct-probe temperature, 20 °C), *m/e* 310 (M), 250 (M – CH_3COOH), 207, 189, 177, 147, 139 (P + C_2H_4), 134, 113 (P + 2H), 112 (P + H), 96. Anal. ($C_{14}H_{18}N_2O_6$) C, H, N.

(±)-1-[(1α,3β,4α)-3-Hydroxy-4-(hydroxymethyl)cyclopentyl]-5-iodo-2,4(1*H*,3*H*)-pyrimidinedione Diacetate (9d). *C*-IdUrd diacetate[24] (**9d**) was prepared previously by treating *C*-2'-deoxyuridine[39] with iodine and nitric acid and then treating *C*-IdUrd[23] with acetic anhydride in pyridine. A second method is the treatment of *C*-dUrd diacetate with iodine monochloride. This method was reported by Robins et al.[40] for the preparation of the true nucleoside IdUrd ditoluate.

A solution (protected from atmospheric moisture) of dichloromethane (17 mL), iodine monochloride (0.1 mL, 1.97 mmol), and *C*-dUrd diacetate (**9c**, 460 mg, 1.48 mmol) was boiled under reflux for 3 h. The reaction solution was cooled to room temperature and diluted with water (14 mL), and an aqueous solution of sodium hydrogen sulfite (2%) was added dropwise to the vigorously stirred solution until its purple color disappeared. Dichloromethane was evaporated under reduced pressure from the solution, and the resulting mixture, containing a white precipitate, was placed in a refrigerator overnight. The white solid precipitate was collected by filtration, washed well with cold water, and dried in vacuo at 56 °C: yield, 580 mg (89.6%); mp 176–178 °C. This material was recrystallized from methanol: weight of recrystallized product, 530 mg (91% recovery); mp 180–184 °C.

(±)-1-[(1α,3β,4α)-3-Hydroxy-4-(hydroxymethyl)cyclopentyl]-5-[(trimethylsilyl)ethynyl]-2,4(1*H*,3*H*)-pyrimidinedione Diacetate (11). A suspension of 750 mg (1.72 mmol) of *C*-IdUrd diacetate (**9d**) in triethylamine (75 mL) was vigorously deoxygenated by bubbling argon through the mixture. To this mixture, under an atmosphere of argon, was added (trimethylsilyl)acetylene (**10**, 0.75 mL), bis(triphenylphosphine)palladium(II) chloride (25 mg), and cuprous iodide (25 mg), and the resulting mixture was stirred under argon at 50 °C for 3 h. The reaction mixture was concentrated to dryness in vacuo; the dark, solid residue was dissolved in chloroform (100 mL); and the chloroform solution was washed twice with 50-mL portions of a 10% aqueous solution of the disodium salt of ethylenediaminetetraacetic acid and twice with 50-mL portions of water. The resulting chloroform solution was dried with magnesium sulfate, filtered, and concentrated in vacuo to a solid residue. The desired product was isolated by chromatography of the crude product on a column of silica gel 60 (50 g) with 9:1 chloroform–methanol as developing and eluting solvent. Fractions that were shown by TLC to contain the desired product were combined and concentrated in vacuo to a pale buff-colored solid; weight, 640 mg (91.8% yield). A hot solution of this material in ethyl acetate was diluted with cyclohexane (12 mL), and the resulting solution was allowed to cool to room temperature and then placed in a refrigerator overnight. A white crystalline precipitate was collected by filtration, washed with cold cyclohexane, and dried in vacuo: yield, 525 mg (75%); mp 181–184 °C; UV max 298 nm (ε 14 600) and 237 (ε 12 200) at pH 1, 297 nm (ε 14 800) and 236 (ε 12 400) at pH 7, 288 nm (ε 9900) and 232 (ε 12 200) at pH 13; MS (electron-impact; direct-probe temperature, 20 °C, *m/e* 406 (M), 391 (M – CH_3), 346 (M – CH_3COOH), 331, 309, 289, 273, 271, 235 (P + C_2H_4), 208 (P + H), 199 (M – P), 193; IR (2200–1200-cm^{-1} region) 2155 (C≡C), 1740 vs, 1725 s, 1680 vs, 1610, 1470, 1460 w, 1450 w, 1435, 1405, 1380, 1365, 1350 sh, 1340 sh, 1315, 1305, 1290, 1265 vs, 1255 sh, 1240, 1230; medium-strong IR bands at 1040, 890, and 845 cm^{-1}. Anal. ($C_{19}H_{26}N_2O_6Si$) C, H, N.

(±)-5-Ethynyl-1-[(1α,3β,4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-2,4(1*H*,3*H*)-pyrimidinedione (12). A solution of 375 mg (0.92 mmol) of the trimethylsilyl)ethynyl derivative **11** in 15 mL of a 0.2 M solution of sodium methoxide in methanol was stirred under an argon atmosphere overnight at room temperature. The solution was diluted with water, and the proton form of a cation-exchange resin was added to the stirred mixture until the solution was approximately neutral (about pH 6). The resin was removed by filtration, the filtrate (combined with copious methanol washings of the residue) was treated with activated charcoal, and the resulting filtrate (plus methanol washings) was concentrated in vacuo. The amorphous residue was triturated with 9:1 chloroform–methanol, the solvents were evaporated in vacuo, the solid residue was dissolved in a mixture of ethanol (3 mL) and ether (10 mL), the mixture (containing a small amount of a curdy precipitate) was refrigerated and then filtered, and the filtrate was concentrated in vacuo to a gummy residue. The residue was triturated with ethyl acetate (4 mL) to induce crystallization, and the crystalline solid was collected by filtration, washed with ethyl acetate, and dried in vacuo at 56 °C: yield, 140 mg (61%); mp 190–193 °C; MS (electron-impact; direct-probe temperature, 20 °C), *m/e* 250 (M), 206, 169, 163 (P + C_2H_4), 155, 137 (P + 2H), 136 (P + H); UV max 292 nm (ε 11 900) and 230 (ε 10 100) at pH 1, 292 nm (ε 12 000) and 230 (ε 10 200) at pH 7, 288 nm (ε 9600) and 231 (ε 11 300) at pH 13. Anal. ($C_{12}H_{14}N_2O_4 \cdot 0.1H_2O$) C, H, N.

(±)-5-Ethyl-1-[(1α,3β,4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-2,4(1*H*,3*H*)-pyrimidinedione (*C*-EDU, 8) via Scheme II. A mixture of the 5-ethynylpyrimidine **12** (50 mg, 0.20 mmol), commercial 5% palladium-on-calcium carbonate containing lead (60 mg), quinoline (0.2 mL), and acetone (10 mL) was treated with hydrogen at approximately atmospheric pressure. After 20 min of stirring in the hydrogen atmosphere, the mixture was filtered to remove the catalyst, and the filtrate (including ethanol washings of the catalyst) was concentrated to dryness in vacuo. The crude product was purified by chromatography on a column of silica gel 60 (15 g) with 9:1 chloroform–methanol as the developing and eluting solvent. Effluent fractions that were shown by means of a recording UV monitor to contain the desired product were combined and concentrated to dryness. The residue was triturated with ethyl acetate (2 mL), and the white crystalline product (*C*-EDU) was collected by filtration, washed with ethyl acetate, and dried in vacuo at 56 °C: yield, 63% (32 mg); mp 148–151 °C (capillary inserted at 45 °C, heating rate 3 °C/min); TLC, 1 spot (5:1 chloroform-methanol). The melting point of a mixture of this material and the specimen of *C*-EDU obtained via the 5-(bromomethyl) derivative **6** (Scheme I) was 147–150 °C (capillary inserted at 50 °C, 3 °C/min). When this material and the specimen of *C*-EDU obtained via **6** and **7** were applied side-by-side on a TLC plate of silica gel, they moved side-by-side

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(40) Robins, M. J.; Barr, P. J.; Giziewicz, J. *Can. J. Chem.* **1982**, *60*, 554–557.

(R_f ca. 0.5) when the chromatogram was developed with 5:1 chloroform–methanol. Similarly, reverse-phase HPLC (μBondapak C18 column; solvent 0.01 M aqueous ammonium dihydrogen phosphate–methanol (90:10), isocratic) of the two specimens monitored at 254 nm showed that their retention times (15.46 min and 15.45 min) were the same. The IR spectrum of the specimen obtained via **12** was identical with the IR spectrum of the specimen of *C*-EDU obtained via **6**. Thus, the melting temperatures, TLC, HPLC, and IR spectra show that the two specimens obtained by the routes outlined in Schemes I and II are identical.

Evidently, the commercial Pd–$CaCO_3$–lead catalyst was sufficiently active to reduce the ethynyl group to the ethyl group. The reduction of crude **12** (100 mg) in ethanol (10 mL) was also effected on 5% Pd–C at approximately atmospheric pressure. After 1 h in the hydrogen atmosphere, the mixture was filtered to remove the catalyst, and the filtrate (including ethanol washings of the catalyst) was concentrated to a residue that crystallized when it was triturated with ether. The white crystals were collected by filtration, washed with ether, and dried in vacuo at 56 °C: yield, 90% (92 mg). The IR and ^{1}H NMR spectra of this material showed that it was crude *C*-EDU.

(±)-1-[(1α,3β,4α)-3-Hydroxy-4-(hydroxymethyl)cyclopentyl]-5-[2-(trimethylsilyl)ethyl]-2,4(1*H*,3*H*)-pyrimidinedione (13b). A mixture of 173 mg (0.43 mmol) of the 5-(trimethylsilylethynyl)pyrimidine (**11**), ethanol (20 mL), and 5% palladium-on-charcoal (100 mg) was stirred for 45 min in an atmosphere of hydrogen at approximately atmospheric pressure. The mixture was filtered to remove the catalyst, and the filtrate (including ethanol washings of the catalyst) was concentrated in vacuo to a colorless syrup: yield, 173 mg (99%). The mass spectrum (FAB) of this material showed that it was **13a**: *m/e* 411 (M + 1), 395 (M – CH_3), 351 (395 – CH_3CO – H), 213 (P + 2H), 197.

A solution of **13a** (170 mg) in ammonia–methanol (17 mL, 10% ammonia) was stirred at room temperature overnight. The reaction solution was concentrated under reduced pressure to a syrupy residue. The desired product was isolated by chromatography on silica gel with 5:1 chloroform–methanol as the developing and eluting solvent. The collection of fractions was monitored by TLC; fractions containing the desired product were combined and concentrated under reduced pressure. Further concentrated in vacuo with a vacuum pump left a crystalline residue that was triturated with 1:1 ethyl acetate–cyclohexane (3 mL). The crystalline product was collected by filtration, washed with the same solvent, and dried in vacuo at 78 °C: yield, 68 mg (50%); mp 154–156 °C (capillary inserted at 100 °C, 3 °C/min); TLC, 1 spot (5:1 chloroform–methanol); MS (FAB), *m/e* 327 (M + 1), 311 (M – CH_3), 213 (P + 2H), 197. Anal. ($C_{15}H_{26}N_2O_4Si$) C, H, N.

Antiviral Evaluations in Vitro. The compounds listed in Table I were tested for inhibition of the cytopathogenic effects produced by strain 377 (TK^+) of HSV-1 or strain MS of HSV-2 replicating in Vero cells. The data summarized in Table I were acquired by methods and procedures described previously for the evaluation of compounds for antiviral activity in vitro.[41] The general assay method was described by Ehrlich et al.,[37] but some modifications were incorporated.

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(*o*- and *p*-Nitrobenzyloxycarbonyl)-5-fluorouracil Derivatives as Potential Conjugated Bioreductive Alkylating Agents[1]

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A series of (*o*- and *p*-nitrobenzyloxycarbonyl)-5-fluorouracil derivatives were synthesized by reacting *o*- or *p*-nitrobenzyl chloroformate with 5-fluorouracil in the presence of triethylamine in DMF or Me_2SO. The reductive activation of these agents was hypothesized to generate a reactive methide and 5-fluorouracil, two components that are capable of synergistic interaction through complementary inhibition. Measurement of the surviving fractions of EMT6 tumor cells treated with these agents in culture under conditions of hypoxia and aerobiosis resulted in equal cell kill regardless of the state of oxygenation. One of the synthesized agents, 3-(*p*-nitrobenzyloxycarbonyl)-5-fluorouracil (**4**), appeared to be superior to 5-fluorouracil in prolonging the survival time of mice bearing intraperitoneal implants of the P388 leukemia and Sarcoma 180.

The malignant cell subpopulations of solid tumors are markedly heterogeneous with respect to a number of properties, including the degree of oxygenation and the rate of proliferation. Oxygen deficiency occurs in these neoplasms as a result of an insufficient blood supply, a phenomenon that leads to hypoxic tumor cells with significant resistance to both X-irradiation and many of the drugs used in therapy. We have hypothesized that the environment of hypoxic neoplastic stem cells is more conducive to reductive reactions than that of well-oxygenated normal cells. Such a metabolic differential is theoretically exploitable through the use of prodrugs requiring reductive activation to form reactive electrophiles; we have called this class of drugs bioreductive alkylating agents. Our laboratory has demonstrated that the quinone antibiotic mitomycin C, which may be considered a prototype bioreductive alkylating agent, is (a) preferentially

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