for 20 min, sodium hydroxide solution (0.9 g in 5 mL of water) was slowly added, and the mixture was stirred for an additional 10 min. The precipitate was filtered and washed consecutively with cold water and ethanol. Recrystallization from methanol afforded 2.0 g (74%) of pure product.

N,N'-[1,6-Hexanediylbis[(aminocarbonyl)amino]]bis-[acetamide] (16). To a cooled (0 °C) solution of acetylhydrazine (7.04 g, 95 mmol) in dry THF was added 1,6-diisocyanatohexane (4.0 g, 24 mmol) in dry THF. The product precipitated instantly. Filtration followed by washing with dry THF and ether afforded pure product (5.9 g, 78%).

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5-Quinone Derivatives of 2'-Deoxyuridine 5'-Phosphate: Inhibition and Inactivation of Thymidylate Synthase, Antitumor Cell, and Antiviral Studies

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Both photochemical aromatic substitution and palladium(0)-catalyzed biaryl coupling reactions have been employed in the synthesis of 5-substituted 2'-deoxyuridines. The former procedure was useful in the preparation of the 3,4-dimethyl-2,5-dimethoxyphenyl derivative **12a** and the 3,4,6-trimethyl-2,5-dimethoxyphenyl derivative **12b**. The latter reaction was efficient in the preparation of the 2-(3-methyl-1,4-dimethoxynaphthyl) derivative **14**. These compounds and their nucleotides (**20a-c**) were converted to the corresponding quinone nucleosides **19a-c** and nucleotides **6-8** by an oxidative demethylation reaction using ceric ammonium nitrate and silver(II) oxide, respectively. The kinetics and products of the reaction of the quinone nucleosides **19a,b** with methyl thioglycolate showed rapid addition to the quinone ring in the trisubstituted derivative **19a** and somewhat slower redox reactions with the tetrasubstituted quinones **19b** and **19c**. All six nucleotides had high affinity for the title enzyme from *Lactobacillus casei* with K_i values ranging from 0.59 to 3.6 μM; the most effective compounds were the dimethyl quinone **6** and the naphthoquinone **8**. Somewhat higher inhibitory constants were observed with the quinones against the L1210 enzyme. The dimethyl quinone nucleotide **6** showed time-dependent inactivation (k_{inact} = 0.015 s^{-1}) against the *L. casei* enzyme, a rate saturation effect, and substrate protection in accord with the kinetic expression for an active-site-directed alkylating agent. The apparent second-order rate of this reaction (2.5×10^4 M^{-1} s^{-1}) is one-twentieth the rate ($k_{cat.}$) of the normal enzymatic reaction leading to product. None of the compound exhibited sufficient activity in the antitumor cell or antiviral assays to warrant further study.

The inhibition of thymidylate synthase is recognized as a viable approach to the control of cancer and holds promise for the development of agents for the treatment of DNA viral infections.[1] This enzyme catalyzes a unique two-step reductive alkylation reaction to form a new carbon–carbon bond in the conversion of 2'-deoxyuridine 5'-phosphate to thymidine 5'-phosphate, a vital precursor for DNA synthesis. The acknowledged mechanism for the first step of the enzymatic reaction,[2] cysteine thiol addition to carbon 6 of the substrate followed by reaction of the carbanion generated at carbon 5 with the electrophilic cofactor, 5,10-methylenetetrahydrofolic acid, can be utilized in the development of mechanism-based inhibitors for this enzyme.

Studies in these laboratories have been directed to the development of pseudosubstrates for the enzyme that have three primary chemical features: (1) high affinity, (2) enhanced nucleophilic reactivity, and (3) the potential for the formation of a chemically reactive intermediate. 5-Nitro-2'-deoxyuridine 5'-phosphate was one such agent that fulfilled the first two requirements.[3] More recently attempts to incorporate the third feature, generation of a chemically reactive intermediate in the enzymatic reaction, entailed the synthesis of 5-*p*-benzoquinonyl-2'-deoxyuridine 5'-phosphate (**1**).[4] This substrate analogue had high affinity (K_i = 2.0 μM), rapidly inactivated the enzyme (k_{inact} = 0.065 s^{-1}), displayed substrate protection in accord with the kinetic equation (eq 1), and was not reversible on prolonged dialysis with 2-mercaptoethanol solution. One potential mechanism for the reaction (pathway a, Scheme I) initially proposed is the addition of the enzyme nucleophile (Cys-198 thiol anion) to carbon 6 of **1** to give the unstable intermediate **2**, which has a high probability for rearrangement to the stable covalent enzyme-inhibitor product **3**. If the reaction followed this pathway, the product, **3**, with the enzyme covalently bonded to an sp^2 carbon, should be stable.

Recognizing the fact that quinones also are thiol reagents leads to a second mechanism (pathway b, Scheme I) for enzyme inactivation wherein the enzymatic thiol anion adds directly to the quinone ring to generate **5**. The result would be the same and the usual features used to verify mechanism-based inactivation would be observed. How-

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‡ Biochemical Services Laboratory, University of Kansas.
§ University of Leuven.

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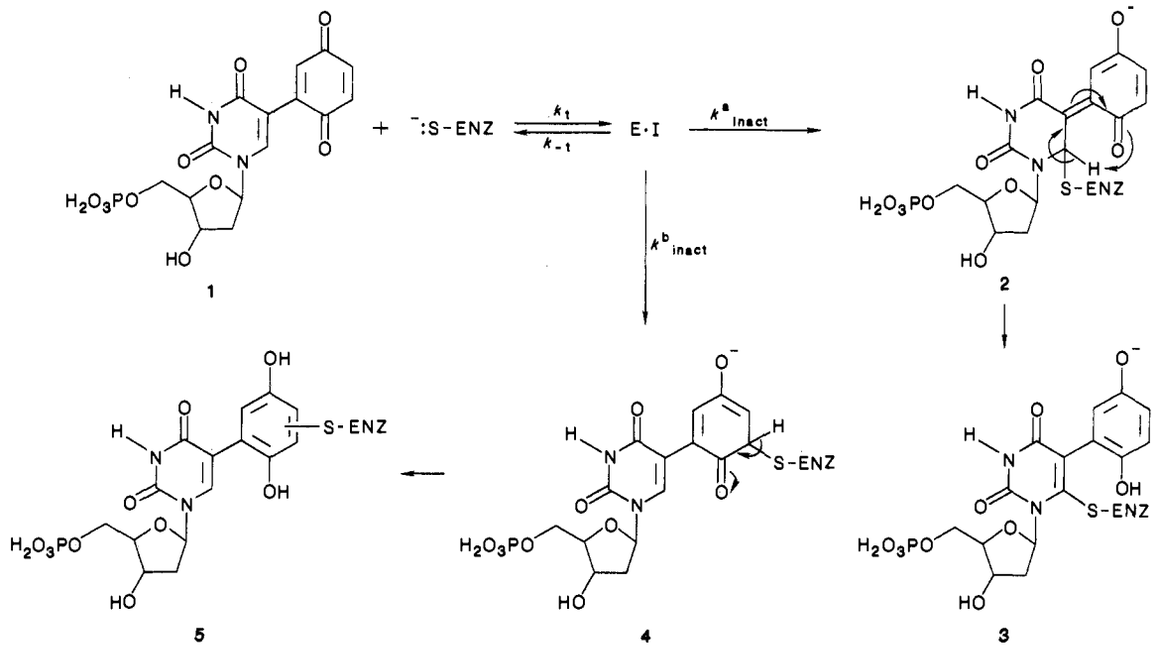
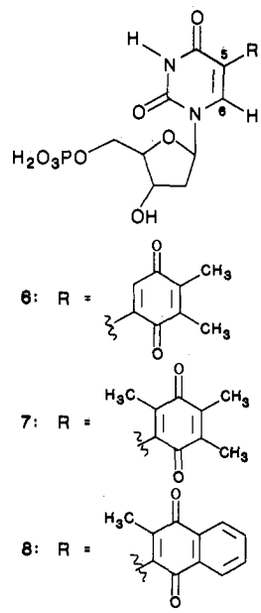
Scheme I. Mechanisms for the Inactivation of Thymidylate Synthase by 5-*p*-Benzoquinonyl-2′-deoxyuridine 5′-Phosphate (1)



ever, inactivation via pathway b would classify 1 as an affinity label and not a mechanism-based inhibitor. More recent studies on model reactions employing thiol addition to the nucleoside of 1 did indeed show addition to the three unsubstituted carbons of the quinone ring.[5] Since we failed to observe thiol addition to carbon-6 in these studies, it is highly probable that the enzyme inactivation by 1 also proceeds by thiol addition to the quinone ring.

One approach to direct the nucleophilic addition to carbon-6 of the pyrimidine ring is to prevent reaction at the quinone carbons by prior substitution at these positions. To achieve this goal, the synthesis and enzyme inhibition by the dimethyl quinone **6**, the trimethyl quinone **7**, and the methylnaphthoquinone **8**, was initiated.



Chemistry

The strategy for the synthesis of the target compounds was to couple the methyl-substituted 1,4-dimethoxyaryl derivative with the preformed nucleoside, phosphorylate the 5-aryl-substituted nucleoside, and follow with an oxidative demethylation reaction leading to the desired quinone derivatives **6**, **7**, or **8**. Two routes for the synthesis of 5-aryl-substituted pyrimidine nucleosides have been reported. The first to be explored,[6,7] an aromatic photosubstitution reaction, is convenient; however, the yields are seldom above 30%. The second, a palladium(0) biaryl coupling reaction,[8] although less direct than the photochemical method, affords higher yields. The synthesis of both **6** and **7** was completed by using the former method.

5-Iodo-2′-deoxyuridine **9** was converted to the trimethylsilylated derivative **10** (Scheme II). A solution of compound **10** and 2,3-dimethyl-1,4-dimethoxybenzene (**11a**) in acetonitrile was irradiated at 310 nm for 48 h at 40 °C to give, after removal of the trimethylsilyl groups, a 15% yield of the photocoupled product, 5-(3,4-dimethyl-2,5-dimethoxyphenyl)-2′-deoxyuridine (**12a**). The required aryl precursor, 2,3-dimethyl-1,4-dimethoxybenzene (**11a**) was prepared in five steps from 2,3-dimethylphenol by adaptation of literature procedures.[9] The second derivative in this series also was prepared by irradiation of **10** with 2,3,5-trimethyl-1,4-dimethoxybenzene (**11b**); however, optimum yields (25%) were obtained by photolysis at 254 nm. The starting aromatic derivative **11b** was prepared from 2,3,5-trimethyl-1,4-dihydroxybenzene.[10]

It had been observed that the ultraviolet absorption pattern for 5-aryl-substituted pyrimidine nucleosides was dependent on substitution ortho to the biaryl bond.[6,7] In the absence of ortho substituents on the phenyl ring, coplanarity of the two aromatic rings was evident in the substantial bathochromic shift from 262 to 275 nm or

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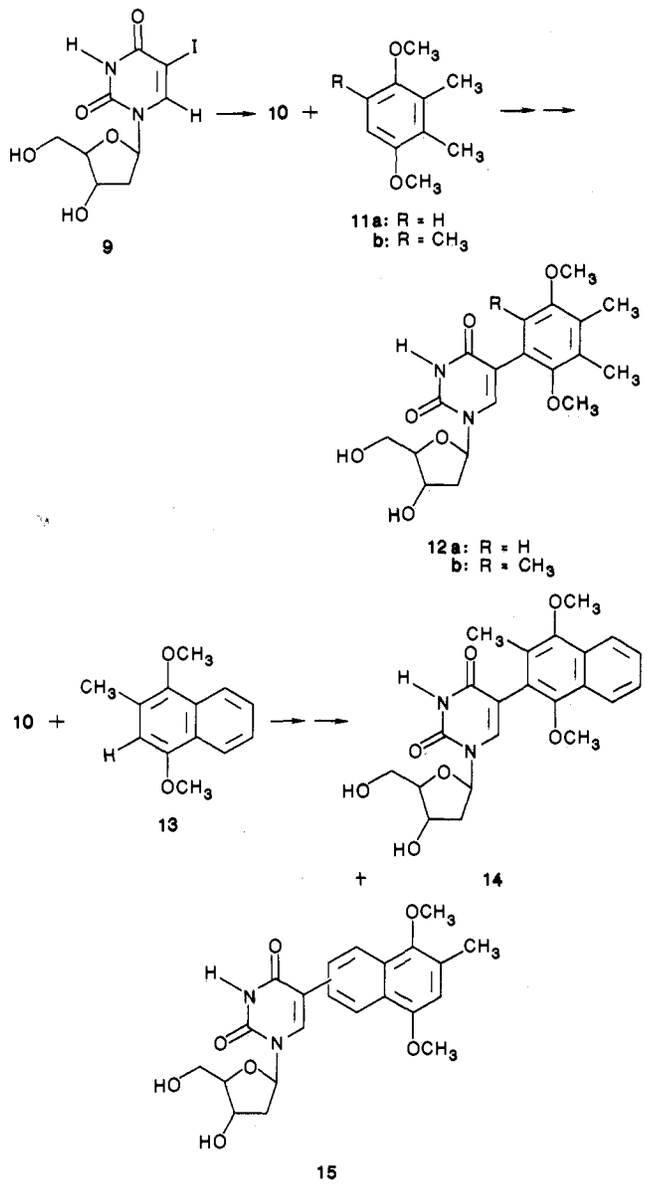
Scheme II. Aromatic Photosubstitution Reactions for the Synthesis of Biaryl Coupled Products **12a**, **12b**, and **14**



greater. When an ortho substituent was present, the ultraviolet maximum normally was in the range of 265–270 nm, which suggests that the two rings are out-of-plane. The ultraviolet maxima for compounds **12a** and **12b** were 269 and 271 nm. The ^1H and ^{13}C NMR of these compounds showed one isomer for **12a**; however, two rotomers of the trimethyl derivative **12b** were observed in equal amounts. The ^1H chemical shifts for the protons on the pyrimidine carbon-6 and one of the methoxy groups were observed as doublets. The ^{13}C NMR of **12b** also showed two signals for the carbons at 5 and 6 of the pyrimidine ring, carbons 1, 4, and 5 of the sugar, and one of the methoxy carbons of the phenyl ring.

Ito and co-workers[11] reported the photochemical coupling reaction between 5-bromo-1,3-dimethyluracil and 1,4-dimethoxynaphthalene gave the regiospecific 3-substituted naphthyl derivative. Application of the same method for the synthesis of the naphthyl derivative by irradiation of a solution of trimethylsilyl iodo nucleoside **10** and 2-methyl-1,4-dimethoxynaphthalene (**13**) at 254 nm for 40 h gave a mixture of regioisomers in 33% yield. The desired 2-naphthyl derivative **14** was separated in 18% overall yield from **15**, a mixture of isomers resulting from coupling at positions 5–8. The former product had the expected out-of-plane ultraviolet maxima at 271 nm whereas the isomeric mixture **15** had an ultraviolet maxima at 320 nm characteristic of extended conjugation in 5-aryl-substituted pyrimidine nucleosides. Key features of the NMR spectra were the high-field ^1H resonance in **15** assigned to the 2-naphthyl proton and absent in **14**. In addition, the presence of rotamers in **14** was confirmed by the two singlets observed for the ^{13}C NMR resonance peaks for carbon 5 of the pyrimidine ring, one of the naphthyl methoxy carbons, the methyl carbon, and carbon 5 of the sugar moiety.

Since the only method for separation of the isomeric mixture of **14** and **15** was preparative high-performance LC, other synthetic routes were explored. It had been observed that photolysis of a mixture of 2-chloro-1,4-dimethoxybenzene and the trimethylsilyl derivative of 2′-deoxyuridine gave 5-(2,5-dimethoxyphenyl)-2′-deoxyuridine.[7] A 4% yield of **14** was obtained after irradiation of a mixture of protected 2′-deoxyuridine and 2-bromo-3-methyl-1,4-dimethoxynaphthalene[12] at 310 nm for 24 h.

The most productive method for the preparation of **14** was application of the palladium(0) biaryl coupling reaction;[8] however, in the present case the initial palladium complex formed (**16**, Scheme III) was that from the 5-iodo nucleoside **10**. This complex was added to a solution of the lithium salt **18a** formed by the treatment of 2-bromo-3-methyl-1,4-dimethoxynaphthalene[12] (**17**) with *n*-butyllithium to give a 16% yield of **14**. The yield was increased to 50% by prior treatment of the lithium derivative **18a** with zinc chloride to give **18b**, which was then treated with the palladium–nucleoside complex **16**.

The oxidative demethylation of 1,4-dimethoxyphenyl derivatives has been accomplished by the use of electrochemical oxidation,[13] silver(II) oxide,[14,15] ceric ammonium nitrate,[16,17] and manganese dioxide.[18] We had used the silver(II) oxide method for the preparation of compound **1**; however, the yields were not good. The nucleosides **12a**, **12b**, and **14** were converted to the respective quinones **19a–c** in over 60% yield by treatment of an acetonitrile solution of the dimethoxy nucleosides with an aqueous solution of ceric ammonium nitrate; the reaction was normally completed in a few minutes (Scheme IV). In all cases oxidation of the carbon-5 hydroxyl group of the sugar to the aldehyde and acid constituted the other products of the reaction.

The nucleosides **12a**, **12b**, and **14** were converted to the corresponding nucleotides **20a–c** by standard procedures and resolved on DEAE-Sephadex. Oxidative demethylation of the nucleotides was unsuccessful with ceric ammonium nitrate because of precipitation. The addition of nitric acid to a suspension of silver(II) oxide and the nu-

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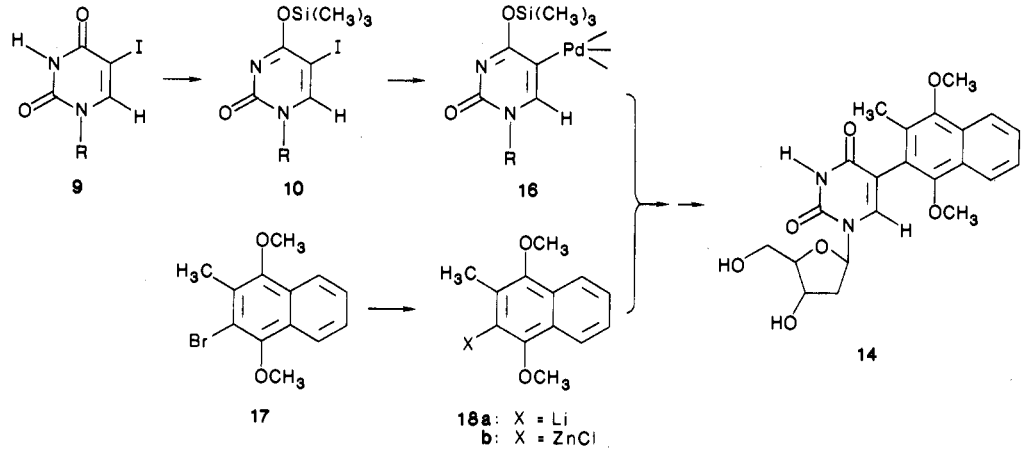
Scheme III. Palladium(0) Biaryl Coupling Reaction for the Synthesis of 14



Scheme IV. Oxidative Demethylation of Dimethoxyaryl Nucleosides and Nucleotides

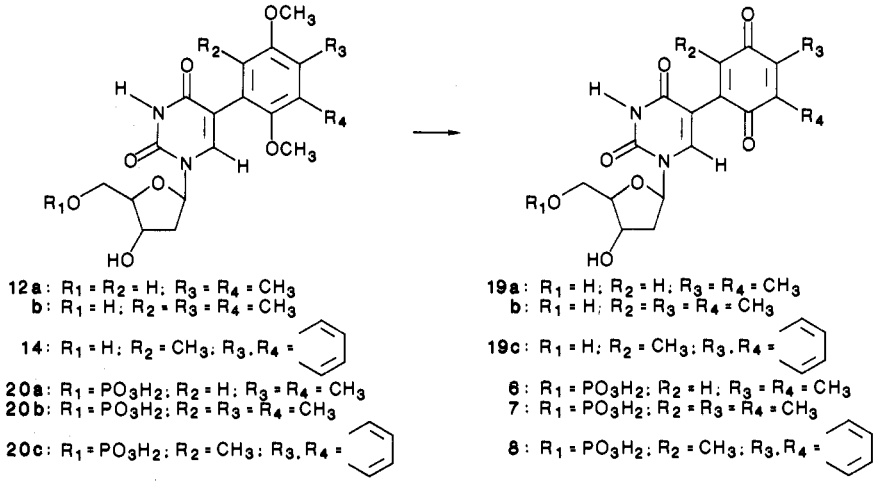


Table I. Kinetic Studies of the Reaction of Quinone-Substituted Nucleosides **19a–c** with Methyl 2-Thioglycolate (21) at 25 °C

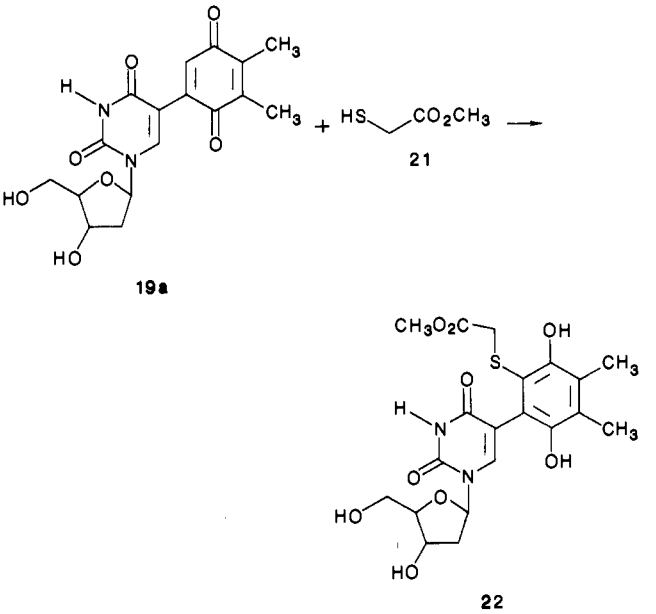
quinone (mM)	solvent	thiol concn, mM	k_{obsd}, min^{-1}	k_2, M^{-1} s^{-1}
19a (0.31)	acetonitrile	15	0.008	
		31	0.010	
		62	0.023	0.0055
19b (0.75)	0.1 M phosphate (pH 7.0)	7.5	0.017	
		22.5	0.050	
		37.5	0.12	
		75	0.27	0.064
19c (0.55)	0.1 M phosphate (pH 7.0)	38.5	0.0023	
		55	0.0087	
		82.5	0.014	
		110	0.027	0.0055

cleotide in aqueous dioxane afforded the desired quinones **6–8** in 45–65% yields (Scheme IV).

Thiol Addition Studies

The chemical reactivity and the products of the reactions of the quinone nucleosides **19a–c** with a thiol was examined with use of methyl thioglycolate (**21**) since this thiol has a pK_a value (8.0) that approximates that of the active-site thiol of thymidylate synthase.[19,20] The reaction

Scheme V. Product of the Reaction of the Dimethyl Quinone **19a** with Methyl 2-Thioglycolate (21)



of the dimethyl quinone **19a** with an excess of the thiol **21** was not measurable in buffer; however, the calculated second-order rate was determined in acetonitrile to be

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Table II. Inhibition of Thymidylate Synthase from *L. casei* and Murine Leukemia Cells (L1210) by Dimethoxyaryl and Quinone Derivatives of 2'-Deoxyuridine 5'-Phosphate[a]

	L. casei enzyme[b]		L1210 enzyme[c]	
compd	K_m, μM	K_i, μM	K_m, μM	K_i, μM
1[b]	7.6	2.0[d]		
20a	5.25	3.6		
20b	5.25	1.4		
20c	5.25	2.4		
6	6.98	0.59[d]	2.46	6.1
7	6.98	1.3	0.57	5.1
8	6.41	0.61	0.60	1.9

[a] All compounds showed inhibition that was competitive with substrate. [b] See ref 4 for methods and the data for compound 1. [c] See ref 21 for methods. [d] Determined by using eq 1 from the inactivation studies in the absence of 2-mercaptoethanol.

0.0055 M^{-1} s^{-1} (Table I). The corresponding reactions using **19b** and **19c** were sufficiently slow in pH 7 buffer to calculate rates. The value for the reaction of the trimethyl quinone **19b** with **21** in buffer (k_2 = 0.064 M^{-1} s^{-1}) was approximately 10 times faster than the reaction of **21** with the naphthoquinone **19c** (k_2 = 0.0055 M^{-1} s^{-1}).

The sole product observed in the reaction of the dimethyl quinone **19a** with a 40-fold excess of the thiol **21** in acetonitrile was the thiol addition product to carbon 6 of the quinone ring, the fully substituted hydroquinone, compound **22** (Scheme V). The structure was assigned principally from the mass spectral data, which confirmed the molecular ion and the two fragments resulting from cleavage of the pyrimidine–phenyl bond: the thiol-substituted hydroquinone (*m/e* 211) and deoxyuridine (*m/e* 228). Further verification of the structure of **22** was derived from the proton NMR that showed the pyrimidine carbon-6 proton (7.84 ppm) and the absence of the quinone proton observed in the starting material at 7.09 ppm. Treatment of a buffered aqueous solution of the trimethyl quinone **19b** with a 20-fold excess of the thiol again afforded a single product that was identified as the hydroquinone **23** from the NMR and mass spectral fragmentation pattern. The naphthoquinone **19c** on treatment with the thiol **21** also gave the hydroquinone product **24**. The two hydroquinones **23** and **24** were characterized as the corresponding tetraacetates **25** and **26**.

Enzyme Inhibition and Inactivation Studies

The nucleotides prepared in this study (**20a–c**, **6–8**) were examined for inhibition of thymidylate synthase purified from *L. casei* and from murine leukemia (L1210) cells. Crystalline thymidylate synthase from *L. casei* was assayed by previously reported procedures.[4] All six nucleotides showed inhibition that was competitive with substrate with inhibitory constants (K_i) ranging from 0.6 to 3.6 μM (K_m = 5.3 to 6.9) (Table II). The most potent compound in the series was the naphthoquinone **8**; the quinones in general were more active than the corresponding dimethoxy derivatives. The quinone nucleotides **6–8** were examined for inhibition of the murine leukemia enzyme by a reported procedure.[21] The K_m value for this enzyme ranged from 0.6 to 2.4 μM in these studies. As noted in the bacterial enzyme, the naphthoquinone **8** was the most active (Table II). The inhibition by all three quinones was competitive with the substrate.

The inactivation of *L. casei* thymidylate synthase was studied with use of phosphate rather than Tris as the buffer and in the absence of 2-mercaptoethanol since the latter would be expected to interact with the quinones.[4] The incubations were run at 30 °C in the absence of cofactor; aliquots were assayed at various times for the percentage of remaining active enzyme. With an excess of inhibitor, a plot of the logarithm of the percentage of active enzyme vs. time was linear. The initial time points were used to determine the rate constant (k_{inact}) for enzyme inactivation according to the kinetic equation (eq 1) de-

$$1/k_{inact} = \left[\frac{K_i[S]}{K_m k_{inact}} + \frac{K_i}{k_{inact}}\right]\frac{1}{[I]} + \frac{1}{k_{inact}} \quad (1)$$

veloped by Kitz and Wilson[22] and used in a similar study involving compound 1.[4] The calculated observed inactivation rates at a given concentration of inhibitor are expressed as k_{obsd} and the maximum rates using an exces-

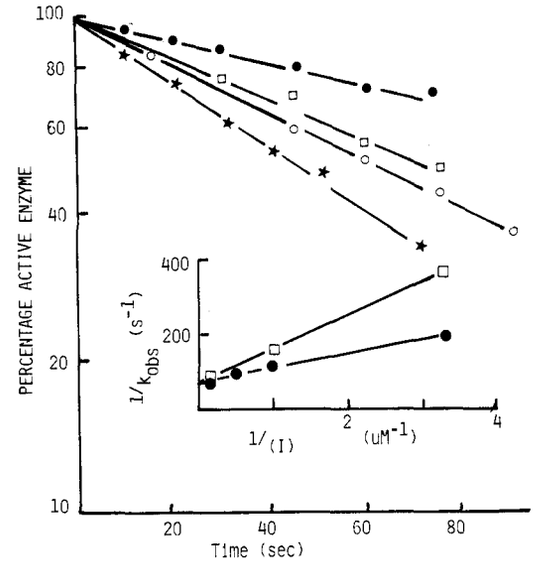

Figure 1. Semilog plot of the percentage of active thymidylate synthase (sp act. 3.2 μM mg^{-1} min^{-1}) vs. time (seconds) preincubated in the presence of varying concentrations of 5-(3,4-dimethyl-2,5-benzoquinonyl)-2'-deoxyuridine 5'-phosphate (**6**): 0.3 μM (●), 1.0 μM (□), 2.0 μM (○), 6.0 μM (★). Inset: double-reciprocal plot of the observed rate for the inactivation of *L. casei* thymidylate synthase vs. concentration of added inhibitor **6** in the absence (●) and presence (□) of 15 μM substrate 2'-deoxyuridine 5'-phosphate.

Table III. Kinetic Constants for the Inactivation of *L. casei* Thymidylate Synthase by 5-Quinone-Substituted 2'-Deoxyuridine Nucleosides **19a** and **19c** and 5'-Phosphates **6** and **7**[a]

compd	concn, μM	substrate concn, μM	k_{obsd}, s^{-1}	k_{inact},[b] s^{-1}
1[c]				0.065
19a	10	0	4×10^{-5}	
6[d]	0.3	0	0.0052	
	1.0	0	0.0092	
	2.0	0	0.011	
	6.0	0	0.015	0.015
	0.3	15	0.0028	
	1.0	15	0.0066	
	6.0	15	0.011	0.014
7	90	0	0.0084	

[a] The compounds were incubated with enzyme in 0.1 M phosphate buffer at pH 6.8 at 30 °C. Aliquots were removed at various times and assayed for remaining enzyme activity by using 50 μM substrate in a 60-s assay. [b] Calculated from the double-reciprocal plots of k_{obsd} vs. inhibitor concentration according to eq 1. [c] See ref 4. [d] The concentration of enzyme was 0.24 μM.

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Table IV. Inhibitory Effect of 5-Substituted Derivatives of 2′-Deoxyuridine on the Proliferation of Murine Leukemia (L1210), Human B-Cell Lymphoblast (Raji), and Human T-Cell Lymphoblast (Molt/4F) Cells[a]

compound	av ID_{50},[b] μg/mL		
	L1210	Raji	Molt/4F
3,4-dimethyl-2,5-dimethoxyphenyl **12a**	>100	>100	>100
3,4,6-trimethyl-2,5-dimethoxyphenyl **12b**	>100	>100	>100
2-(3-methyl-1,4-dimethoxynaphthyl) **14**	>100	55.6–10	>100
p-benzoquinonyl	35.7–7.8	>100	>100
3,4-dimethyl-*p*-benzoquinonyl **19a**	40.5–6.7	>100	66.8–25.9
3,4,6-trimethyl-*p*-benzoquinonyl **19b**	65.6–19.1	51.1–6.5	29.7–3.1
3-methyl-1,4-naphthoquinonyl **19c**	27.4–0.6	33.5–1.6	30.9–4.9
nitro	0.003		

[a] Methods are described in ref 23a. [b] Concentration required to inhibit tumor cell growth by 50%.

sively high concentration of inhibitor are expressed as k_{inact}; S and I are concentrations of substrate and inhibitor and K_m and K_i are the respective Michaelis and inhibitory constants.

The trimethyl quinone nucleotide **7** inactivated the enzyme only at very high concentrations. The control rate for enzyme loss (no inhibitor) was 0.003 s^{-1}; the observed rate in the presence of 90 μM **7** was 0.0083 s^{-1}. The dimethyl quinone **6** was examined at four concentrations (Figure 1); saturation was evident in comparing the 6 and 15 μM concentrations (Table III). The calculated k_{inact} from the double-reciprocal plot (Figure 1, insert) was 0.015 s^{-1} and the K_i was found to be 0.59 μM. This compares to a K_i value of 1.8 μM determined from the Lineweaver–Burk plot for the inhibitory constant. In the presence of 15 μM substrate, the observed rates of inactivation by **6** were decreased; the calculated k_{inact} from eq 1 in this study was essentially the same (0.014 s^{-1}) as predicted for inactivation proceeding through the enzyme–inhibitor complex. Recovery of activity was examined by dialysis studies. The activity was reduced to 12% of the control value by treatment with 6 μM **6** for 2 min. Dialysis of the inhibited and control enzyme in 50 mM 2-mercaptoethanol showed a 60% recovery of activity after 16 h of dialysis at 2 °C.

In an effort to detect nonspecific inactivation, 10 μM dimethyl quinone nucleoside **19a** was incubated with the enzyme. The observed rate of inactivation at this concentration was 0.0024 min^{-1}, 300 times slower than the k_{obsd} for the nucleotide at the same concentration.

Antitumor Cell and Antiviral Studies

These studies were performed by using previously published procedures.[23] The dimethoxy nucleosides **12a,b** and **14** showed no inhibitory effect on the proliferation of murine leukemia (L1210), human B-cell lymphoblasts (Raji), and human T-cell lymphoblasts (Molt/4F); the IC_{50} for this series exceeded 100 μg/mL (Table IV). The nucleoside of compound **1** and the quinones **19a–c** were also examined in these cultures. The results in Table IV show that the highest activity resides in the naphthoquinone **19c**; however, when compared to the standard (nitro), they can be considered as inactive. The antiviral results (Table V) followed the same trend. The most active compound, the nucleoside of compound **1**, had some activity against HSV-1 and HSV-2, but only at a much higher concentration than the standard compounds (bromo, iodo).

Discussion

The inhibition constants against *L. casei* thymidylate synthase for both the dimethoxyaryl- and the quinone-substituted nucleosides are within a narrow range and not unlike other aryl-substituted derivatives that have been studied.[24] The reasonably high affinity again confirms the observation that there does not appear to be any severe steric restrictions imposed by the enzyme for large substituents on carbon 5 of the pyrimidine ring.[25] The highest affinity noted is for the naphthoquinone **8** with a K_i value one-tenth that of the substrate K_m.

Since the K_i values for these compounds were measured in the standard assay containing 2-mercaptoethanol, the values for the quinone derivatives could arise not from the starting quinone but rather could be due to inhibition by a product of the reaction of the quinone with the thiol present in the assay. The chemical reactivity of these quinones was therefore established in model studies. It was found that the reaction of the dimethyl quinone nucleoside **19a** with excess methyl thioglycolate in pH 7 buffer was completed within a second. Addition of the thiol of the unsubstituted quinone position in the model nucleoside **19a** was observed to give the hydroquinone **22** (Scheme V). Given the time course of the assay and the concentration of 2-mercaptoethanol, the K_i value (1.8 μM) calculated for the quinone **6** in the presence of thiol is most probably the value for the inhibitory constant of the reaction product and not of the unchanged quinone. The K_i value for compound **6** calculated from the inactivation studies using eq 1 was found to be 0.59 μM. This is a valid number since the calculation was made from results obtained in the absence of 2-mercaptoethanol.

The same question can be raised with the quinones **7** and **8**. Both the trimethyl quinone (**19b**) and the naphthoquinone (**19c**) were reduced by methyl thioglycolate. Of these, the most reactive quinone nucleoside (**19b**) has a second-order rate of 0.064 M^{-1} s^{-1} for reaction with methyl thioglycolate at pH 7 (Table II). Assuming the reactivity of the two thiols to be similar, the 1000-fold excess of 2-mercaptoethanol (50 mM) in the assay compared to a 5 μM concentration of **7** would result in over 80% of the quinone remaining at the end of the 60-s assay. From these results it is reasonable to assume that the calculated inhibitory constants for **7** and the less reactive quinone **8** are valid for the starting compounds.

The reactivity of the nucleoside of quinone **1** with methyl thioglycolate was found to be high in pH 7 buffer; however, a second-order rate constant of 0.53 M^{-1} s^{-1} was found in acetonitrile.[5] This is 100 times faster than the reaction of the corresponding dimethyl quinone **19a**. Both electrophilicity and steric hinderance can account for the difference. For the former monosubstituted quinone, the meta and para isomers were formed. For the latter compound, only ortho substitution is possible. Rozeboom and co-workers,[26] on the basis of theoretical studies, suggested

(23) (a) De Clercq, E.; Balzarini, J.; Torrence, P. F.; Mertes, M. P.; Schmidt, C. L.; Shugar, D.; Barr, P. J.; Jones, A. S.; Verhelst, G.; Walker, R. T. *Mol. Pharmacol.* **1981**, *19*, 321–330. (b) De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R. T.; Jones, A. S.; Torrence, P. F.; Shugar, D. *J. Infect. Dis.* **1980**, *141*, 563–574.

(24) Chang, G.; Mertes, M. P., unpublished results.

(25) De Clercq, E.; Balzarini, J.; Descamps, J.; Bigge, C. F.; Chang, C. T.-C.; Kalaritis, P.; Mertes, M. P. *Biochem. Pharmacol.* **1981**, *30*, 495–502.

(26) Rozeboom, M. D.; Tegmo-Larsson, I. M.; Houk, K. N. *J. Org. Chem.* **1981**, *46*, 2338–2345.

Table V. Cytotoxicity and Antiviral Activity of 5-Substituted Derivatives of 2-Deoxyuridine in Primary Rabbit Kidney Cell Cultures against Herpes Simplex Virus (HSV), Vaccinia Virus, and Vesicular Stomatitis Virus[a]

compound	minimal inhib concn,[b] μg/mL	minimal inhibitory concentration,[c] μg/mL							
		HSV-1 (KOS)	HSV-1 (F)	HSV-1 (McIntyre)	HSV-2 (G)	HSV-2 (196)	HSV-2 (Lyons)	vaccinia virus	vesicular stomatitis virus
p-benzoquinonyl		20	20	150	70	70	70	70	>200
3,4-dimethyl-*p*-benzoquinonyl **19a**	200	150	>100	70	>100	70	70	>100	>100
3,4,6-trimethyl-*p*-benzoquinonyl **19b**	>200	>200	>200	>200	>200	>200	>200	>200	>100
3-methyl-1,4-naphthoquinonyl **19c**	40	>10	>10	>10	>10	>10	>10	>10	>10
bromo	>400	0.02	0.07	0.02	2	10	20	7	>400
iodo	>400	0.2	0.7	0.7	0.7	1	0.7	0.7	>400

[a] Methods are described in ref 23b. [b] Required to cause a microscopically detectable alteration of normal cell morphology. [c] Required to reduce virus-induced cytopathogenicity by 50%.

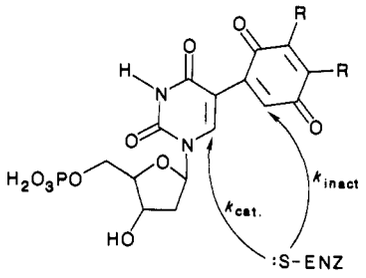
that donor and conjugating substituents on *p*-benzoquinone give regioselective nucleophilic addition in the order meta, para, ortho. Wilgus and co-workers noted formation of ortho adducts only when the quinone was substituted with an electron-accepting group.[27]

p-Quinones are recognized to be highly reactive reagents that can undergo both redox and electrophilic reactions.[28] While it is commonly accepted that the reaction with thiols is nucleophilic addition, we did find that the products of reaction of the fully substituted quinones **19b** and **19c** with thiol **21** gave reduction to the hydroquinone. A striking difference was noted in the rates of reduction of the trimethyl quinone **19b** and the naphthoquinone **19c**; the former is 10 times more readily reduced than the naphthyl derivative. A difference of +0.12 V reported[29] for the redox potentials for 2-methylnaphthoquinone (−5 mV vs. NHE) and trimethylbenzoquinone (+114 mV vs. NHE) suggests that this difference in rate is reasonable. We did not observe reaction of the thiol **21** with carbon 6 of the pyrimidine ring for any of the quinones studied.

The inactivation of *L. casei* thymidylate synthase by the dimethyl quinone **6** was time-dependent, demonstrated saturation kinetics, and was subject to substrate protection in accord with eq 1. These kinetic features are characteristic for both affinity labels (alkylating type inhibitors) and mechanism-based inhibitors. The analogous unsubstituted quinone **1** also fulfilled these requirements.[4] However, more recent model studies[5] suggest that **1** acts as an affinity label and alkylates an active-site nucleophile (pathway b, Scheme I). It was found that while the nucleoside of **1** also inactivated the enzyme, the reaction was considerably slower than the nucleotide **1**. For this reason the nucleoside (**19a**) of **6** was examined and found to inactivate the enzyme at a rate 300 times slower than the nucleotide **6**.

From the results of the model studies of the reactivity of **19a** with the thiol **21**, inactivation of thymidylate synthase by the dimethyl quinone **6** also could take place by the mechanism show in pathway b, Scheme I. In this case the nucleophile would add ortho to the pyrimidine ring. Considering the reaction rate differences in the model reactions (Table I) wherein thiol addition to the nucleoside of **1** is 100 times faster than addition to **19a**, a similar difference in reactivity should be noted with the enzyme. A difference was observed; however, it was not as striking; compound **1** had a k_{inact} value of 0.065 s^{-1} compared to a k_{inact} value of 0.015 s^{-1} for **6**.

Scheme VI. Proposed Mechanism for the Inactivation of Thymidylate Synthase by Quinone Nucleotides **1** (R = H) and **6** (R = CH_3)



A more valid comparison of the reactivity must take into account the affinity of the inhibitor for the enzyme. The K_i value for **1** was 2.0 μM and that for compound **6** was 0.59 μM; both values were obtained from data calculated according to eq 1. The apparent second-order rate for enzyme inactivation normally determined from the ratio of k_{inact}/K_i is 3.2×10^4 M^{-1} s^{-1} for compound **1** and 2.5×10^4 M^{-1} s^{-1} for compound **6**. The unexpected similarity in the overall rate of enzyme inactivation is not in accord with the predicted reactivity[26,27] nor does it agree with the 100 times difference observed in the model bimolecular reactions. If the chemical reactivity is the same then it strongly suggests that the chemistry of the enzyme–inhibitor reaction in both cases is similar. The only two reactive sites for nucleophilic addition in compound **6** are carbon 6 of the pyrimidine ring and the ortho position on the quinone ring. Both of these sites also are available for reaction in compound **1**. This leads to the proposal that **1** and **6** interact in the enzyme–inhibitor complex by nucleophilic addition to the same site on the inhibitor. Furthermore, we could not find the product of addition to carbon 6 of the pyrimidine ring of nucleosides of **1** and **6** with a thiol with the same pK_a as the enzyme active-site thiol. Therefore it is proposed that the nucleophile is the active-site thiol and it undergoes addition to the ortho carbon of the quinone ring in both **1** and **6**.

There is no substantial reason for considering that the inhibitors bind to the enzyme with different orientations in the two quinone rings. Given that the mode of binding is the same, the active-site thiol that normally adds to carbon 6 of the pyrimidine ring is now positioned such that addition to the ortho quinone carbon is favorable as illustrated in Scheme VI. Further support for this proposal is derived from the calculated $k_{cat.}$ for product formation in the enzyme-catalyzed reaction. With use of the specific activity of the *L. casei* enzyme (3.2 μM min^{-1} mg^{-1}) and the substrate affinity constant ($K_m \sim 5$ μM), the apparent second-order rate ($k_{cat.}$) is 7.6×10^5, which is only 20 times greater than the inactivation rates of **1** or **6**.

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(28) Finley, K. T. *The Chemistry of Quinoid Compounds*, Part 2; Patai, S., Ed.; Wiley: New York, 1974.
(29) Swallow, A. J. *Functions of Quinones in Energy Conserving Systems*; Trumpowere, B. L., Ed.; Academic: New York, 1982; p 59–72.

The trimethyl quinone 7 was found to have high affinity for thymidylate synthase; yet, the inactivation rate in the preincubation studies using saturating concentrations was only 3 times the rate of enzyme loss in the absence of inhibitor. The naphthoquinone 8, also having high affinity, rapidly inactivated the enzyme.[30]

Experimental Section

Materials and Methods. Melting points were measured on a Thomas-Hoover capillary melting point apparatus and were corrected. Proton NMR spectra were measured at 60 MHz with a Varian T-60, 80 MHz with a Varian FT-80, or 300 MHz with a Varian XL300 spectrometer. ^{13}C NMR spectra were obtained by using either a Varian FT-80 or a Varian XL-300 spectrometer. Chemical shifts are reported in ppm downfield from the internal standard (Me_4Si). UV spectra were measured either with a Cary Model 219 or a Cary 118 recording spectrophotometer. IR spectra were obtained with either a Beckman IR-33 or an IBM FT-IR. Microanalyses were obtained from a Hewlett-Packard 185B and mass spectra from a Varian CH5 or a Ribermag R-10-10 quadrupole mass spectrometer.

Rayonet Models RPR-100 and RPR-208 photochemical reactors with the corresponding 254- and 310-nm lamps and quartz reaction tubes are products of Southern N.E. Ultraviolet Co. DEAE-sephadex A25 was obtained from Pharmacia Fine Chemicals. 2′-Deoxyuridine (B grade) was purchased from Calbiochem or Sigma. 5-Iodo-2′-deoxyuridine was purchased from ICN Nutritional Biochemicals. Tetrakis(triphenylphosphine)palladium(0) was a product of Aldrich Chemical Co. All solvents used for chromatography were redistilled. Silica (70–230 mesh) was a product of Merck. Tetrahydrofuran (THF) was redistilled from sodium/benzophenone ketyl. Pyridine was kept over potassium hydroxide for a few days and then distilled from barium oxide onto 3-Å molecular sieves. Acetonitrile was redistilled from calcium hydride and stored over 3-Å molecular sieves. Anhydrous reactions were performed under dry argon in glassware flame-dried and cooled under a stream of dry argon.

Tetrahydrofolic acid (H_4folate) was purchased from Sigma Chemical Co. in 65% purity in 50-mg ampules. A stock solution was prepared by mixing the crude H_4folate with a suspension containing 2-mercaptoethanol (0.5 mL of 10 M solution) and sodium bicarbonate (15 mg). The resulting mixture was deoxygenated by bubbling nitrogen through the suspension. After centrifugation the concentration of H_4folate was determined in the supernatant by spectrophotometric analysis based on the λ_{max} at 296 nm (ϵ 25 000).

The substrate [5-^{3}H]-2′-deoxyuridine 5′-phosphate at a specific activity of 21 Ci/mmol was purchased from Moravek Biochemicals, Industry, CA, and diluted with cold substrate purchased from Sigma Chemical Co., St. Louis, MO, to give a specific activity of 500 μCi/μmol. The studies with the murine leukemia (L1210) thymidylate synthase were performed with partially purified enzyme.[21]

Thymidylate synthase as a crude extract from methotrexate-resistant *L. casei* was purchased from the New England Enzyme Center as a dialyzed ammonium sulfate concentrate. The enzyme was purified and crystallized according to the method of Maley and co-workers[31] to give, after activation by dialysis for 24 h at 4 °C against 0.1 M potassium phosphate buffer at pH 6.8 containing 50 mM 2-mercaptoethanol, a preparation with a specific activity of 2.6–3.2 μM product mg^{-1} min^{-1} based on the radioisotope assay. The enzyme concentration was determined by absorbance at 278 nm by using an extinction coefficient of 1.05 × 10^6 and a dimer molecular weight of 70 000.

5-(2,5-Dimethoxy-3,4-dimethylphenyl)-2′-deoxyuridine (12a). A solution of 5-iodo-2′-deoxyuridine (**9**; 1.5 g, 4.24 mmol) and hexamethyldisilazane (1.5 g, 9.4 mmol) in 5 mL of anhydrous pyridine was stirred overnight under an argon atmosphere at room temperature. After removal of the pyridine in vacuo, the residue was dissolved in 500 mL of anhydrous acetonitrile. 1,4-Dimethoxy-2,3-dimethylbenzene[9] (**11a**; 2.0 g, 12 mmol) was added and the homogeneous solution was then placed in a quartz tube (50 × 5 cm) and deoxygenated by bubbling dry argon through a septum for 10 h. The quartz tube was then placed in the preparative photoreactor and the reaction mixture was irradiated at 310 nm for 48 h at 40 °C. The solvent was removed from the resulting dark brown solution, and the residue was stirred with 100 mL of 2% hydrochloric acid in methanol for 8 h at room temperature to assure removal of the silyl protecting groups.

After evaporation in vacuo, the residue was adsorbed on silica gel and eluted with 10% methanol in chloroform. The eluted fraction containing compound **12a** was then repurified by flash chromatography employing 10% isopropyl alcohol in methylene chloride to give compound **12a** in 15% yield (250 mg, 0.64 mmol); mp 106–108 °C. About 60% of the starting 5-iodo-2′-deoxyuridine (**9**) underwent photoreduction to give 2′-deoxyuridine. Ten percent of the starting 5-iodo-2′-deoxyuridine (**9**) was recovered: mass spectrum, *m/e* 392 (37, M$^+$), 303 (22), 276 (100, base peak, 5-(2,4-dimethoxy-3,4-dimethylphenyl)uracil), 261 (62), 190 (42), 149 (22), 117 (20, 2′-deoxyribose), 73 (72); ^1H NMR (CD_3OD) ppm 7.8 (s, 1 H, C_6H), 6.5 (s, 1 H, aromatic), 6.0–6.25 (t, 1 H, $C_{1'}H$), 4–4.2 (q, 1 H, $C_{4'}H$), 3.7 (d, 2 H, $C_{5'}H$), 3.55 (s, 3 H, OCH_3), 3.2 (s, 3 H, OCH_3), 2.15–2.1 (t, 2 H, $C_{2'}H$), 2.1–2.0 (s, 3 H, CH_3), 1.9 (s, 3 H, CH_3); ^{13}C NMR (CD_3OD) ppm 166 (C_4), 155 (C_2), 153 ($COCH_3$), 152 ($COCH_3$), 142 (C_6), 133, 130, 128, 125 (aromatic), 113 (C_5), 90.1 ($C_{1'}$), 87.8 ($C_{4'}$), 73.5 ($C_{3'}$), 64 ($C_{5'}$), 60 (OCH_3), 57.5 (OCH_3), 42.6 ($C_{2'}$), 16 (CH_3), 14 (CH_3); UV (H_2O) λ_{max} 269 nm (ϵ 9200), λ_{min} 255 nm (ϵ 8850); UV (0.1 N NaOH) λ_{max} 272 nm (ϵ 8000), λ_{min} 263 nm (ϵ 7900); UV (0.1 N HCl) λ_{max} 269 nm (ϵ 8900), λ_{min} 255 nm (ϵ 8600). Anal. ($C_{19}H_{24}N_2O_7 \cdot H_2O$, M_r 410.26). Calcd: C, 55.60; H, 6.39; N, 6.83. Found: C, 55.40; H, 6.28; N, 6.90.

5-(2,5-Dimethoxy-3,4,6-trimethylphenyl)-2′-deoxyuridine (12b). This compound was prepared by the same method described for **12a** with use of 5-iodo-2′-deoxyuridine (**9**; 500 mg, 1.4 mmol) and 1.9 g of 1,4-dimethoxy-2,3,5-trimethylbenzene[10] (**11b**; 10.6 mmol) dissolved in 200 mL of acetonitrile. The solution was irradiated with twelve 254-nm lamps in the preparative photochemical reactor at 40 °C until the reaction mixture turned to dark brown and there was no trimethylsilylated 5-iodo-2′-deoxyuridine (**10**) in the reaction mixture (36 h). Resolution on medium-pressure liquid chromatography with 7% ethanol/chloroform yielded 150 mg (25%) of compound **12b** as an amorphous white material (mp 108–112 °C). The starting 5-iodo-2′-deoxyuridine (**9**) underwent photoreduction to give a 50% conversion to 2′-deoxyuridine: mass spectrum, *m/e* 406 (3, M$^+$), 290 (100, base peak, 5-(2,5-dimethoxy-3,4,6-trimethylphenyl)-uracil), 275 (39), 260 (22), 259 (25), 244 (10), 230 (6), 204 (39), 117 (24), 73 (40); ^1H NMR (CD_3OD) ppm 7.3 (d, 1 H, C_6H), 6.35–5.9 (t, 1 H, $C_{1'}H$), 4.4–4.0 (q, 1 H, $C_{4'}H$), 3.7–3.55 (d, 2 H, $C_{5'}H$), 3.45 (s, 3 H, OCH_3), 3.25 (d, 3 H, OCH_3), 2.1–1.8 (m, 11 H, $C_{2'}H$, 3 CH_3); ^{13}C NMR (CD_3OD) ppm 165 (C_4), 154.5 (C_2), 154.2 ($COCH_3$), 152.2 ($COCH_3$), 141.62, 141.4 (C_6), 132.3, 130.6, 130.4, 129.2, 125.6 (aromatic) 112.5, 112.3 (C_5), 88.94, 88.9 ($C_{1'}$), 86.8, 86.6 ($C_{4'}$), 72.3, 72.2 ($C_{3'}$), 62.8, 62.6 ($C_{5'}$), 61.2, 61.1 (OCH_3), 60.4 (OCH_3), 41.6 ($C_{2'}$), 13.5 (CH_3), 13.01 (CH_3), 12.9 (CH_3); UV (H_2O) λ_{max} 271 nm (ϵ 10 000), λ_{min} 247 nm; UV (0.1 N HCl) λ_{max} 271 nm (ϵ 10 000), λ_{min} 247 nm; UV (0.1 N NaOH) λ_{max} 268 nm (ϵ 10 000), λ_{min} 254 nm. Anal. ($C_{20}H_{26}N_2O_7 \cdot H_2O$, M_r 424.5). Calcd: C, 56.60; H, 6.65; N, 6.60. Found: C, 56.39; H, 6.59; N, 6.41.

5-(1,4-Dimethoxy-3-methyl-2-naphthyl)-2′-deoxyuridine (14). Method A. Following the procedure described for **12a** using protected 5-iodo-2′-deoxyuridine (**10**; prepared from **9**, 300 mg, 0.85 mmol), 1,4-dimethoxy-2-methylnaphthalene[12] (**13**; 500 mg, 2.5 mmol) in 100 mL of acetonitrile was irradiated with twelve 254-nm lamps at 40 °C until the color turned dark brown and there was no more protected 5-iodo-2′-deoxyuridine (**10**) in the reaction mixture (40 h). This procedure yielded 160 mg of a mixture of 14 and the isomeric mixture **15**. Use of high-performance liquid chromatography and ^1H NMR produced data indicating that the product was present in more than one isomeric form, the desired compound 14 and other compounds indicated as products of substitution in the unsubstituted naphthalene ring (**15**). Compound 14, the major isomer, represented two-thirds of the product mixture; the t_R value of 7.4 min was obtained by HPLC with a solvent system of 65:35 methanol/water at a flow

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rate of 0.7 mL/min. The remainder of the mixture composed of isomers **15** (t_R = 9.1 and 9.56 min, using solvent system of 65:35 methanol/water at a flow rate of 0.7 mL/min) was separated by using preparative high-performance liquid chromatography employing 55% methanol in water as eluant. This yielded 98 mg (0.23 mmol, 18.3%) of compound **14** as an amorphous white material (mp 145–150 °C) and 45 mg (0.1 mmol, 8.4%) of the mixture **15**. No attempt was been made to separate the isomers of compound **15**. Forty percent of the starting 5-iodo-2′-deoxyuridine (**9**) underwent photoreduction to give 2′-deoxyuridine: mass spectrum (compound **14**), *m/e* (relative intensity) 427 (77, $M^+ + 1$), 312 (100, base peak, 5-(1,4-dimethoxy-3-methyl-2-naphthyl)uracil, 297 (90), 282 (34), 265 (34), 226 (41), 117 (14, 2′-deoxyribose), 98 (54), 81 (63); ^{1}H NMR (CD_3OD) 14 ppm 7.8–7.7 (m, 3 H, C_6H, 2 aromatic protons), 7.2–7.1 (m, 2 H, aromatic), 6.1–5.9 (t, 1 H, $C_{1'}H$) 4.2–4 (q, 1 H, $C_{4'}H$), 3.7 (s, 3 H, OCH_3), 3.5 (s, 3 H, OCH_3), 2.0 (s, 3 H, CH_3); ^{1}H NMR (CD_3OD) **15** ppm 7.8–7.7 (m, 2 H, C_6H, aromatic), 7.2–6.9 (m, 2 H, aromatic), 6.4 (s, 1 H, aromatic), 6.1–6.0 (t, 1 H, $C_{1'}H$), 4.1–4.0 (q, 1 H, $C_{4'}H$), 3.7–3.3 (m, 8 H, 2 OCH_3, $C_{5'}H$), 2.1–2.0 (t, 2 H, $C_{2'}H$); ^{13}C NMR (CD_3OD) 14 ppm 165 (C_4), 152 ($COCH_3$), 149 ($COCH_3$), 143.2 (C_6), 129.8, 128, 127.5, 126.4, 124.4, 123.3, 122.7 (aromatic) 111.6, 111.4 (C_5), 88.5 ($C_{2'}$), 86.6 ($C_{4'}$), 86.4 ($C_{3'}$), 71.8, 72.0 ($C_{5'}$), 62.3, 62.4 (OCH_3), 61.5 (OCH_3), 41.3, 41.2 ($C_{2'}$), 13.4, 13.3 (CH_3); UV (H_2O) λ_{max} 271 nm (ϵ 14 000), λ_{min} 256 nm; UV (0.1 N HCl) λ_{max} 271 nm (ϵ 13 500), λ_{min} 256 nm; UV (0.1 N NaOH) λ_{max} (sh) 265 nm (ϵ 13 300); UV **15** (CH_3OH) λ_{max} 320 nm, λ_{min} 289 nm. Anal. **14** ($C_{22}H_{24}N_2O_7 \cdot H_2O$, M_r 446.5). Calcd: C, 59.19; H, 5.87; N, 6.27. Found: C, 59.50; H, 5.98; N, 6.06.

Method B. By use of method A, a solution of 2′-deoxyuridine (250 mg, 1.1 mmol) and 2-bromo-1,4-dimethoxy-3-methylnaphthalene[12] (**17**; 500 mg) in 60 mL of anhydrous acetonitrile was irradiated for 24 h with thirteen 310-nm lamps to yield, after resolution, 19 mg (4%) of compound **14**. 2-Methyl-1,4-dimethoxynaphthalene (**13**) was identified as the photoreduced product of 2-bromo-1,4-dimethoxy-3-methylnaphthalene (**17**). Eighty percent of the starting 2′-deoxyuridine was recovered.

Method C. A solution of 5-iodo-2′-deoxyuridine (**9**; 500 mg, 1.4 mmol) and hexamethyldisilazane (1.5 g, 9.4 mmol) in 3 mL of anhydrous pyridine was stirred at room temperature overnight under an argon atmosphere. The solution was evaporated in vacuo at 30 °C. The residue and tetrakis(triphenylphosphine)palladium(0) (900 mg, 0.78 mmol) were mixed in dry tetrahydrofuran (20 mL), and the suspension was stirred vigorously at room temperature in the dark under an argon atmosphere. A clear yellow solution of palladium complex with protected 5-iodo-2′-deoxyuridine (**16**) was obtained after 20 min. The resulting solution was transferred via a syringe to another flask containing 5 mmol of a white suspension of 2-lithio-3-methyl-1,4-dimethoxynaphthalene (**18a**) in an ether and hexane mixture (prepared by the procedure described by Snyder and Rapaport[14c]) to give a clear yellow solution. The mixture was allowed to stir for 10 h at room temperature under a nitrogen atmosphere. The solvent was evaporated in vacuo, and the silyl protecting groups were removed by stirring the residue in 30 mL of 1% hydrochloric acid in methanol for 8 h. The solution was evaporated and the mixture resolved on silica gel with 10% ethanol in chloroform to yield 98 mg (0.22 mmol, 16%) of compound **14**. 2-Methyl-1,4-dimethoxynaphthalene (**13**) has been identified as the quenched product of the naphthalene lithium salt (**18a**) and 2′-deoxyuridine was obtained from the palladium complex.

Method D. A tetrahydrofuran solution containing 1.4 mmol of compound **16** (prepared by treating the protected 5-iodo-2′-deoxyuridine with tetrakis(triphenylphosphine)palladium(0) as described in method C) was transferred via a syringe to another flask containing 5 mmol of a clear yellow solution of the 2-zinc complex of 3-methyl-1,4-dimethoxynaphthalene (**18b**). The latter was prepared by treating 5 mmol of 2-lithio-3-methyl-1,4-dimethoxynaphthalene (**18a**) with 680 mg (5 mmol) of freshly fused zinc chloride in 20 mL of dry tetrahydrofuran. The mixture of compound **16** and compound **18b** was allowed to stir under a nitrogen atmosphere for 20 h at room temperature, resulting in a brown solution. The solvent was evaporated, and the silyl groups were removed by stirring the residue in 70 mL of 1% hydrochloric acid in methanol for 17 h. The solution was evaporated and the mixture resolved on silica gel with 10% ethanol in chloroform to yield 300 mg (0.7 mmol, 50%) of compound **14**.

5-(3,4-Dimethyl-2,5-benzoquinonyl)-2′-deoxyuridine (19a). A solution of ceric ammonium nitrate (200 mg, 0.36 mmol) in 0.5 mL of water was added dropwise to a solution of 5-(3,4-dimethyl-2,5-dimethoxyphenyl)-2′-deoxyuridine (**12a**; 50 mg, 0.127 mmol) in 1 mL of acetonitrile over 5 min. After the reaction mixture was stirred for 45 min at room temperature, the orange-yellow solution was extracted with 60 mL of ethyl acetate in 20-mL portions. The organic layer was dried over anhydrous sodium sulfate. The solvent was evaporated in vacuo and the residue containing compound **19a** was resolved by silica gel flash chromatography using 80% acetonitrile in methylene chloride to give compound **19a** in 65% yield (30 mg, 0.083 mmol) as an orange-yellow solid: mp 205–207 °C; mass spectrum, *m/e* 362 (22, M^+), 246 (18, 5-(3,4-dimethyl-2,5-benzoquinonyl)uracil), 117 (16, 2′-deoxyribose), 98 (100, base peak), 81 (86); UV (H_2O) λ_{max} 391 nm (ϵ 1200), λ_{mi} 360 nm (ϵ 1100), λ_{max} (sh) 310 nm (ϵ 6000); λ_{max} 255 nm (ϵ 15 500), λ_{min} 235 nm (ϵ 11 500); UV (0.1 N HCl) λ_{max} 391 nm (ϵ 1300), λ_{min} 362 nm (ϵ 1100), λ_{max} (sh) 310 nm (ϵ 6000), λ_{max} 255 nm (ϵ 15 000), λ_{min} 235 nm (ϵ 11 000); UV (0.1 N NaOH) λ_{max} 420 nm (ϵ 1600), λ_{min} 375 nm (ϵ 1300), λ_{max} (sh) 325 nm (ϵ = 3000), λ_{max} 258 nm (ϵ 15 500), λ_{min} 244 nm (ϵ 14 500); ^{1}H NMR (CD_3OD) ppm 8.22 (s, 1 H, C_6H), 7.1 (s, 1 H, C_6H, quinone ring), 6.2–6.3 (t, 1 H, $C_{1'}H$), 4.33–4.4 (q, 1 H, $C_{3'}H$). Anal. ($C_{17}H_{18}N_2O_7$, M_r 362.3). Calcd: C, 56.35; H, 5.01; N, 7.73. Found: C, 56.00; H, 5.00; N, 7,90.

Other products of this reaction were the result of oxidation of the 5′-hydroxyl group of the sugar moiety either to the aldehyde or to the carboxylic group.

5-(3,4,6-Trimethyl-2,5-benzoquinonyl)-2′-deoxyuridine (19b). By the method described for **19a**, a solution of ceric ammonium nitrate (548 mg, 1.0 mmol) in 1 mL of water was added dropwise to a solution of 5-(2,5-dimethoxy-3,4,6-trimethylpheny)-2′-deoxyuridine (**12b**; 100 mg, 0.25 mmol) in 1 mL of acetonitrile over 15 min. After 1 h at room temperature the starting dimethoxy compound **12b** was consumed. The product was purified by flash chromatography on silica gel using 7% isopropyl alcohol in ethyl acetate as the eluant to give compound **19b** in 80% yield (75 mg, 0.2 mmol): mp 125–127 °C; mass spectrum, *m/e* 377 (8.5, M^+), 283 (11), 261 (68, 5-(3,4,6-trimethyl-2,5-benzoquinonyl)uracil), 232 (3), 134 (7), 117 (55, 2′-deoxyribose), 98 (64), 81 (100, base peak); ^{1}H NMR (CD_3CN) ppm 9.2 (br, 1 H, NH), 7.8 (s, 1 H, C_6H), 6.25–6.1 (t, 1 H, $C_{1'}H$), 4.35–4.15 (q, 1 H, $C_{4'}H$), 3.75–3.9 (q, 1 H, $C_{3'}H$), 3.5–3.6 (d, 2 H, $C_{5'}H$), 2.2–2.4 (t, 2 H, $C_{2'}H$), 1.8–1.9 (2 s, 9 H, 3 CH_3); ^{13}C NMR (CD_3CN) ppm 188.2 (quinone carbonyl), 186.1 (quinone carbonyl), 163.3 (C_4), 151 (C_2), 144.7 (C_6), 142.3, 141.8, 141 (aromatic), 118.3 (C_5), 88.4 ($C_{1'}$), 86.3 ($C_{4'}$), 71.6 ($C_{3'}$), 62.2 ($C_{5'}$), 41.2 ($C_{2'}$), 14.3 (CH_3), 14.2 (CH_3), 12.5 (CH_3); UV (H_2O) λ_{max} (sh) 313 nm (ϵ 2500), λ_{max} 263 nm (ϵ 24 000), λ_{min} 236 nm (ϵ 8200); UV (0.1 N HCl) λ_{max} (sh) 313 (ϵ 2400, λ_{max} 263 (ϵ 24 000), λ_{min} 235 (ϵ 7000); UV (0.1 N NaOH) λ_{max} (sh) 325 nm (ϵ 1500), λ_{max} 264 nm (ϵ 21 500), λ_{min} 241 nm (ϵ 11 000). Anal. ($C_{18}H_{20}N_2O_7 \cdot 0.5H_2O$, M_r 385.4). Calcd: C, 56.10; H, 5.50; N, 7.26. Found: C, 55.99; H, 5.62; N, 7.26.

Other products obtained in the oxidation reaction were the oxidized product of the 5′-hydroxyl group of the sugar moiety, which was oxidized to the aldehyde group. This byproduct, which accounted for 20% of the starting material, was identified by the disappearance of the characteristic peak at 3.5–3.6 ppm for the $C_{5'}H_2$ in the ^{1}H NMR and mass spectroscopy. The characteristic peaks were *m/e* 404 (9, M + 1, 5′-aldehyde of **12b**), 375 (10, M + 1, 5′-aldehyde of **19b**), 115 (5′-aldehyde of 2′-deoxyribose).

5-(3-Methyl-1,4-napthoquinon-2-yl)-2′-deoxyuridine (19c). By the method described for **19a**, a solution of ceric ammonium nitrate (500 mg, 0.912 mmol) in 1 mL of water was added dropwise with stirring to a solution of 5-(1,4-dimethoxy-3-methyl-2-naphthyl)-2′-deoxyuridine (**14**; 150 mg, 0.35 mmol) in 1 mL of acetonitrile over 30 min at room temperature. After the reaction mixture was stirred for 2 h, the reaction reached completion. Purification gave 82 mg of compound **19c** (59%): mp 121–123 °C; mass spectrum **19c**, *m/e* 399 (32, $M^+ + 1$), 283 (50, 5-(3-methyl-1,4-naphthoquinon-2-yl)uracil + 1), 117 (55, 2′-deoxyribose), 98 (93), 81 (100), base peak); ^{1}H NMR (CD_3CN) ppm 9.25 (br, 1 H, NH), 7.81 (m, 5 H, C_6H and aromatic), 6.1–7.3 (t, 1 H, $C_{1'}H$), 4.2–4.4 (q, 1 H, $C_{4'}H$), 3.75–3.95 (q, 1 H, $C_{3'}H$), 3.6–3.7 (d, 2 H, $C_{5'}H$), 2.2–2.35 (doublet of doublets, 2 H, $C_{2'}H$), 2.1 (s, 3 H,

CH_3); ^{13}C NMR (CD_3CN) ppm 151 (C_2), 149 (aromatic CCH_3), 140.5 (C_6), 135.2, 134.8, 134, 127.3, 126.8 (aromatic), 119 (C_5), 88.4 ($C_{1'}$), 86.3 ($C_{4'}$), 71.8 ($C_{3'}$), 62.5 ($C_{5'}$), 15 (CH_3); UV (H_2O) λ_{max} 333 nm (ϵ 3800), λ_{min} 303 nm (ϵ 3100), λ_{max} 265 nm (ϵ 22 500), λ_{min} 260 nm (ϵ 22 000), λ_{max} 255 nm (ϵ 24 500), λ_{min} 233 nm (ϵ 10 800); UV (0.1 N HCl) λ_{max} 333 nm (ϵ 3800), λ_{min} 303 nm (ϵ 3100), λ_{max} 265 nm (ϵ 22 000), λ_{min} 260 nm (ϵ 20 800) λ_{max} 255 nm (ϵ 23 000), λ_{min} 232 nm (ϵ 10 400); UV (0.1 N NaOH) λ_{max} 340 nm (ϵ 3500), λ_{min} 305 nm (ϵ 2300) λ_{max} 265 nm (ϵ 20 000), λ_{min} 261 nm (ϵ 17 000), λ_{max} 254 nm (ϵ 24 000), λ_{min} 250 nm (ϵ 22 000). Anal. ($C_{20}H_{18}N_2O_7 \cdot H_2O$, M_r 416.6). Calcd: C, 57.69; H, 4.84; N, 6.73. Found: C, 57.40; H, 4.80; N, 6.80.

Other oxidation products obtained from this reaction were the result of oxidation of the 5′-hydroxy group of the sugar moiety either to an aldehyde group or to the carboxylic acid. These were characterized by mass spectrometry and ^1H NMR.

5-(3,4-Dimethyl-2,5-dimethoxyphenyl)-2′-deoxyuridine 5′-Phosphate Bis(triethylammonium) Salt (20a). The nucleoside 5-(3,4-dimethyl-2,5-dimethoxyphenyl)-2′-deoxyuridine (**12a**; 100 mg, 0.255 mmol) was mixed at 0 °C with 0.535 mL of a freshly prepared solution containing (v/v) phosphorus oxychloride (2.01), acetonitrile (5.87), water (0.22), and pyridine (1.9). After the mixture was stirred at 0 °C for 4 h, 1 mL of water was added dropwise to the homogeneous solution of the reaction mixture; the resulting mixture was neutralized to pH 7.5 by the addition of a 2.0 M solution of triethylammonium bicarbonate buffer (pH 7.5). After the mixture was diluted to 80 mL with water, it was resolved on a DEAE-Sephadex column (2 × 30 cm) with a gradient composed of 500 mL each of 0.01 and 0.6 M triethylammonium bicarbonate (pH 7.5). The product **20a** was obtained in 36% yield and the 3′,5′-diphosphate in 30% yield: UV (H_2O) λ_{max} 269 nm, λ_{min} 255 nm.

5-(2,5-Dimethoxy-3,4,6-trimethylphenyl)-2′-deoxyuridine 5′-Phosphate Bis(triethylammonium) Salt (20b). By the same method described for **20a**, 5-(2,5-dimethoxy-3,4,6-trimethylphenyl)-2′-deoxyuridine (**12b**; 90 mg, 0.22 mmol) was mixed at 0 °C with 0.45 mL of the phosphorylating reagent and the mixture stirred at 0 °C for 4 h. The product was resolved in 45% yield; about 5% of the starting nucleoside underwent phosphorylation at the 3′-hydroxyl group and 40% at the 3′,5′-position to give the diphosphate product. The elution pattern was 5′-phosphate, 3′-phosphate, and 3′,5′-diphosphate in that order; UV (H_2O) **20b** λ_{max} 271 nm, λ_{min} 247 nm.

5-(1,4-Dimethoxy-3-methyl-2-naphthyl)-2′-deoxyuridine 5′-Phosphate Bis(triethylammonium) Salt (20c). By the same method described for **20a** the nucleoside 14 (103 mg, 0.24 mmol) was mixed at 0 °C with 0.5 mL of a freshly prepared phosphorylation reagent. The product **20c** was obtained in 50% yield and the 3′,5′-diphosphate in 40% yield: UV (H_2O) **20c** λ_{max} 271, λ_{min} 256 nm.

5-(3,4-Dimethyl-2,5-benzoquinonyl)-2′-deoxyuridine 5′-Phosphate (6). The ammonium salt of 5-(3,4-dimethyl-2,5-dimethoxyphenyl)-2′-deoxyuridine 5′-phosphate (**20a**; 12 mg, 0.0237 mmol) was dissolved in 0.3 mL of dioxane, and 15 mg of freshly prepared silver(II) oxide (0.12 mmol) in 0.3 mL of water was added. The reaction was started by the addition of 0.02 mL of 6 N HNO_3 to the vigorously stirred solution. Over a 15-min period a deep yellow color developed and the precipitate dissolved slowly. The solution was neutralized to pH 6 by addition of 0.05 mL of 2 M triethylammonium bicarbonate, and the entire mixture was diluted with sufficient water (2 mL) to give a final concentration of salts of less than 0.1 M. This solution was applied at room temperature to a DEAE-Sephadex column (2 × 30 cm) and resolved by use of a linear gradient of 500 mL of 0.01 M and 500 mL of 0.6 M triethylammonium bicarbonate. The column fractions containing compound **6** were passed through a Dowex 50 (H^+) column (5 × 10 cm) and the resulting acidic solution of compound **6** was freeze-dried to give the product as a yellow solid in 50% yield. Twenty percent was recovered as unreacted starting material; UV (H_2O) λ_{max} 391 nm, λ_{min} 360 nm, λ_{max} (sh) 310 nm, λ_{max} 255 nm, λ_{min} 235 nm.

5-(3,4,6-Trimethyl-2,5-benzoquinonyl)-2′-deoxyuridine 5′-Phosphate (7). By the procedure described for **6**, the ammonium salt of 5-(3,4,6-trimethyl-2,5-dimethoxyphenyl)-2′-deoxyuridine 5′-phosphate (**20b**; 25 mg, 0.048 mmol) was dissolved in 0.2 mL of *p*-dioxane, and 25 mg of freshly prepared silver(II) oxide (0.2 mmol) in 0.4 mL of water was added. The reaction was started by the addition of 0.04 mL of 6 N HNO_3 to the vigorously stirred solution. Over a 15-min period a deep yellow color developed and the precipitate dissolved slowly. Compound **7** was obtained in 76% yield. Ten percent of the starting material was recovered; UV (H_2O) λ_{max} (sh) 313 nm, λ_{min} 310 nm, λ_{max} 263 nm, λ_{min} 235 nm.

5-(3-Methyl-1,4-naphthoquinon-2-yl)-5-deoxyuridine 5′-Phosphate (8). By the procedure described for **6**, the ammonium salt of 5-(1,4-dimethoxy-3-methyl-2-naphthyl)-2′-deoxyuridine 5′-phosphate (**20c**; 25 mg, 0.036 mmol) was dissolved in 0.4 mL of dioxane and 25 mg of freshly prepared silver(II) oxide (0.2 mmol) in 0.4 mL of water was added. The reaction was started by the addition of 0.04 mL of 6 N HNO_3 to the vigorously stirred solution. Over a 10-min period a yellow color developed and the precipitate dissolved slowly. The reaction mixture was filtered through glass wool and Florisil. The reaction mixture was resolved to give the product in 45% yield. Ten percent of the starting material was recovered; UV (H_2O) λ_{max} 333 nm, λ_{min} 303 nm, λ_{max} 265 nm, λ_{min} 260 nm, λ_{max} 255 nm, 250 nm (sh), λ_{min} 235 nm.

5-[3,4-Dimethyl-6-[(carbomethoxymethyl)thio]-2,5-dihydroxyphenyl]-2′-deoxyuridine (22). To a solution of 5-(3,4-dimethoxy-2,5-benzoquinonyl)-2′-deoxyuridine (**19a**; 10 mg, 0.028 mmol) in 3 mL of acetonitrile was added a solution of methyl thioglycolate (**21**; 1.12 mmol) in 0.1 mL acetonitrile. The resulting mixture was allowed to stir at room temperature under an argon atmosphere. The reaction was monitored by disappearance of the bright yellow color and formation of a colorless solution (30 min). The solvent was evaporated in vacuo and the residue was redissolved in deuteriated acetonitrile and reevaporated. The resulting residue showed a single spot on TLC. The single spot was compound **22**, which showed an R_f value of 0.4 with 80% acetonitrile in methylene chloride: UV (H_2O) λ_{max} (sh) 305 nm, λ_{max} 265 nm, λ_{min} 252 nm; ^1H NMR (CD_3CN) ppm 7.84 (s, 1 H, C_6H); mass spectrum, *m/e* 468 (1, M^+), 436 (1), 352 (10, 5-[3,4-dimethyl-6-[(carbomethoxymethyl)thio]-2,5-dihydroxyphenyl]-uracil) 117 (14, 2′-deoxyribose).

5-(3,4,6-Trimethyl-2,5-dihydroxyphenyl)-2′-deoxyuridine (23). A solution of methyl thioglycolate (**21**; 56.4 mg, 0.53 mmol) in 0.1 mL acetonitrile was added to a solution of 5-(3,4,6-trimethyl-2,5-benzoquinonyl)-2′-deoxyuridine (**19b**; 10 mg, 0.027 mmol) in 3 mL of 0.1 N phosphate buffer, pH 7.0. The resulting reaction mixture was allowed to stir at room temperature under an argon atmosphere for 12 h. The solvent and excess methyl thioglycolate were evaporated in vacuo, and the residue was redissolved in deuteriated acetonitrile and evaporated. The resulting compound **23** showed a UV spectrum (0.1 N phosphate buffer, pH 7) of λ_{max} 266 nm, λ_{min} 246 nm; ^1H NMR (CD_3CN) ppm 7.85–7.83 (2 s, 1 H, C_6H), 6.4–6.32 (2 t, 1 H, $C_{1'}H$); mass spectrum, *m/e* 379 (1, $M^+ + 1$), 263 (33, 5-(3,4,6-trimethyl-2,5-dihydroxyphenyl)uracil), 117 (44, 2′-deoxyribose).

5-(3,4,6-Trimethyl-2,5-diacetoxyphenyl)-2′-deoxyuridine 3′,5′-Diacetate (25). A solution of 200 mg of sodium borohydride (5.3 mmol) was added dropwise to a solution of 5-(3,4,6-trimethyl-2,5-benzoquinonyl)-2′-deoxyuridine (**19b**; 20 mg, 0.05 mmol) in 1 mL of ethanol. The resulting mixture was allowed to stir for 10 min under an argon atmosphere at room temperature to give the corresponding hydroquinone compound **23** in quantitative yield. Compound **23** showed an ultraviolet maximum at 268 nm and a minimum at 251 nm. The excess sodium borohydride was quenched with glacial acetic acid (0.3 mL). The excess acetic acid was removed by evaporation to dryness in vacuo. The residue was redissolved in 2 mL of a 1.0 M solution of tetra-*n*-butylammonium fluoride (2 mmol) in tetrahydrofuran containing 3 mL of acetic anhydride (31.8 mmol). The resulting reaction mixture was allowed to stir at room temperature under an argon atmosphere for 24 h. After 24 h no starting material could be detected on TLC. The solvent and the unreacted acetic anhydride were removed under vacuum, and the residue was resolved on a silica gel column and eluted with ethyl acetate (using flash chromatography) to give compound **25** in 80% yield (27 mg): ^1H NMR ($CDCl_3$) ppm 7.26 (2 s, 1 H, C_6H), 6.45 (2 t, 1 H, $C_{1'}H$), 5.2 (1 H, q, $C_{4'}H$), 4.26 (t, 2 H, $C_{5'}H$), 4.1 (q, 1 H, $C_{3'}H$), 2.33 (d, 2 H, $C_{2'}H$), 2.2 (s, 3 H, $COCH_3$), 2.15 (s, 3 H, $COCH_3$), 2.1–2.08 (2 s, 9 H, 3 CH_3), 2.05 (s, 3 H, $COCH_3$), 1.95 (s, 3 H, $COCH_3$); ^{13}C NMR ($CDCl_3$) ppm 170.78 ($COCH_3$), 151, 151.2 (C_6), 146 (C_2

pyrimidine ring), 142, 141, 140, 139, 136, 132, 129.3, 128.8 (aromatic), 121, 121.1 (C_5), 85.1, 84.7 ($C_{1'}$), 82.87, 82.65 ($C_{4'}$), 75.25, 74.37 ($C_{3'}$), 64.7, 63.8 ($C_{5'}$), 38.2, 37,92 ($C_{2'}$), 21.3, 20.9, 20.8, 20.65 (CH_3CO), 14.5, 14.3 (CH_3 at C_6 of the phenyl ring), 13.8 (CH_3), 13.5 (CH_3); mass spectrum, *m/e* 547 (2, M^+), 504 (3), 460 (2), 347 (11, 5-(3,4,6-trimethyl-2,5-diacetoxyphenyl)uracil), 201 (6, 2'-deoxyribose 3',5'-diacetate).

5-(3-Methyl-1,4-dihydroxy-2-naphthyl)-2'-deoxyuridine (24). A solution of methyl thioglycolate (**21**; 53 mg, 0.5 mmol) in 0.1 mL acetonitrile was added to a solution of 5-(3-methyl-1,4-naphthoquinon-2-yl)-2'-deoxyuridine (**19c**; 10 mg, 0.025 mmol) in 2 mL of 0.1 M phosphate buffer (pH 7.0). The resulting solution was allowed to stir at room temperature for 15 h under argon atmosphere. The solvent and the excess compound **21** were evaporated in vacuo, and the residue was redissolved in 0.5 mL deuteriated acetone and the solvent reevaporated in vacuo; UV spectrum (H_2O) λ_{max} 270 nm (sh), λ_{min} 260 nm; ^1H NMR (CD_3CN) ppm 8.3–7.9 (m, 5H, aromatic and C_6H), 6.5–6.3 (2t, 1 H, $C_{1'}H$); mass spectrum m/e 400 (1, M^+), 284 (6.5, 5-(3-methyl-2,5-dihydroxynaphthyl)uracil), 117 (16.5, 2'deoxyribose).

5-(3-Methyl-1,4-diacetoxy-2-naphthyl)-2'-deoxyuridine 3',5'-Diacetate (26). A solution of sodium borohydride (100 mg, 0.3, mmol) in 2 mL of absolute ethanol was added dropwise to a solution of 5-(3-methyl-2,5-naphthoquinonyl)-2'-deoxyuridine (**19c**; 50 mg, 0.125 mmol) in 1 mL of ethanol. The resulting solution was allowed to stir for 20 min under an argon atmosphere. The reduction reaction was monitored by disappearance of the bright yellow color to a very faint color. The excess sodium borohydride was quenched with 0.5 mL of glacial acetic acid. The solvent and the excess glacial acetic acid were evaporated under vacuum to dryness to give the corresponding hydroquinone nucleoside compound **24**. Compound **24** showed an ultraviolet maximum at 270 nm and a minimum at 261 nm. The residue was redissolved in 3 mL of acetic anhydride (31.8 mmol) and 2 mL of 1.0 M tetra-*n*-butylammonium fluoride (2.0 mmol). The resulting mixture was allowed to stir at room temperature. The reaction reached completion after 10 h. The solvent was evaporated in vacuo and the residue was resolved on a silica gel column (flash chromatography) with ethyl acetate to give compound **26** in 70% yield (50 mg): ^1H NMR ($CDCl_3$) ppm 7.34–7.26 (m, 5 H, aromatic + C_6H) 6.3–6.0 (m, 2 H, $C_{1'}H$), 5.3–5.0 (2 q, 1 H, $C_{4'}H$), 4.4–4.0 (m, 2 H, $C_{5'}H$), 2.47–2.27 (m, 2 H, $C_{2'}H$), 2.06 (s, 6 H, 2 $COCH_3$), 2.04 (s, 3 H, CH_3CO), 2.01 (s, 3 H, CH_3CO), 1.99 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) ppm 170.4 (CH_3CO), 169.5 ($COCH_3$), 168.4 (CH_3CO), 161.8 (C_4), 150.2 (C_2), 139.1, 140.4 (C_6), 126.9, 127.96 (aromatic C_1 and C_4 of the naphthalene moiety), 121.42, 121.5, 121.55, 122.25 (aromatic), 110.4, 110.6 (C_5), 84.53, 84.9 ($C_{1'}$), 82.5, 82.66 ($C_{4'}$), 64.43 ($C_{5'}$), 38.9, 39.1 ($C_{2'}$), 20.96, 21.08 (CH_3CO for two rotamers), 20.75 ($COCH_3$), 20.68 (CH_3CO), 20.02 (CH_3CO), 13.7, 14.2 (CH_3 for two rotamers); mass spectrum (CI, CH_4), 569 (2, $M^+ + 1$), 526 (4.7), 368 (8.5, 5-(3-methyl-1,4-diacetoxynaphthyl)uracil), 201 (4.7, 2'-deoxyribose 3',5'-diacetate).

Kinetic Studies. The quinone nucleosides in either acetonitrile or buffer containing thiol **21** were analyzed at 22 °C for time-dependent changes in the ultraviolet spectra by repetitive scan to determine the percentage of the unreacted starting quinone nucleoside.

Assay of *L. casei* Thymidylate Synthase. The enzyme was assayed by using the radioisotope assay described.[4] The assay mixture (0.1 mL) containing 34.5 mM 2-mercaptoethanol, 0.22 mM tetrahydrofolic acid, 1.15 mM sodium bicarbonate, 6.75 mM formaldehyde, 3 mM magnesium chloride, 6 mM potassium phosphate buffer at pH 6.8, 5 μL of the diluted enzyme solution, substrate, and, when indicated, inhibitor. The concentration of sodium bicarbonate solution and 2-mercaptoethanol depends on the concentration of the cofactor tetrahydrofolic acid. The control reaction contains all the reagents in the assay mixture except the cofactor tetrahydrofolic acid, H_4folate. The substrate [5-^3H]-2'-deoxyuridine 5'-phosphate at a specific activity of 500 μCi/umol was used in the following concentrations: 1.2, 2.0, 3.2, 6, and 16 μM. The assay was started by addition of the enzyme to the complete assay mixture and then incubated at 30 °C for 30 or 60 s. The incubation was stopped by the addition of 50 μL of 2% trichloroacetic acid. The resulting solution was vigorously mixed, 250 μL of a 20% aqueous charcoal suspension was added, the mixture was vortexed and allowed to stand at room temperature for 15 min, the suspension was filtered through a cotton-plugged Pasteur pipet, and 0.1 mL of the filtrate was counted in Beckman Ready Solve HP scintillation fluid. Counting efficiency was 40%; control samples lacking the cofactor were found to have less than 5% of the respective sample counts.

Assay of L1210 Thymidylate Synthase. The enzyme was prepared and partially purified according to the procedure reported by Balzarini et al.[21] The assay mixture contained 0.26 mM tetrahydrofolic acid, 5.0 mM formaldehyde, 15 mM 2-mercaptoethanol, 0.1 M sodium fluoride, 1.2–16 μM [5-^3H]-dUMP of 0.25 μCi, and an appropriate amount of the inhibitor in a total volume of 30 μL in 0.05 M potassium phosphate buffer at pH 7.5. The reaction was started by addition of 10 μL of the enzyme and the resulting mixture was incubated at 37 °C for 30 min. The reaction was stopped by addition of 160 μL of a charcoal suspension (100 mg/mL in 2% trichloroacetic acid). After centrifugation for 10 min at 1000*g*, 0.1 mL of the supernatant was assayed for radioactivity in a toluene-base scintillant.

Preincubation Studies. For the preincubation studies 2-mercaptoethanol was removed by passing the concentrated solution of the activated enzyme through a 1 × 20 cm column of Sephadex G-25 with 0.05 M phosphate buffer at a pH of 6.8. Fractions of 0.025 mL were collected and assayed for thymidylate synthase activity. The fractions showing the highest activity were combined and used. The enzyme preparation was stable for several days at 2 °C.

The enzyme in concentrations ranging from 0.1 to 0.4 μM was preincubated at 30 °C in a solution containing 10 mM potassium phosphate buffer at a pH of 6.8, 3 mM magnesium chloride, 0.12 mM EDTA, and varying concentrations of the nucleotide tested for inactivation of the enzyme. Substrate protection was evaluated by including the indicated concentration of substrate at a specific activity of 500 μCi/umol. After incubation for the indicated time period, the assay for the remaining active enzyme was started by the addition of 50 μL of a solution containing buffer and other components of the assay to a 50-μL aliquot of the preincubation mixture to give the same concentrations of reagents as used in the enzyme assay. A high substrate concentration (50 μM) was used in these assays to afford reasonably high velocity and to competitively reduce the enzyme inactivation by the inhibitor during the assay. The assay was run for 60 s and treated as described in the enzyme assay section. The inactivation of the enzyme was determined by comparing the velocity at the indicated incubation time with the velocity at time zero. The controls used for the preincubation studies contained all the components in the assay mixture including the inhibitor except the cofactor H_4folate. The stability of the enzyme during preincubation was determined in solution without the inhibitors.

Dialysis Studies. The enzyme was preincubated with varying concentrations of the inhibitor for 2 min. After assay for active enzyme the control and inhibited enzyme preparations were dialyzed against 3 L of 50 mM potassium phosphate buffer, pH 6.8, at 4 °C. Controls used in the dialysis study contained all of the components in the assay mixture except the inhibitor. Dialysis was performed with and without 50 mM 2-mercaptoethanol.

Antitumor Cell and Antiviral Studies. These were performed by previously described methods.[23]

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