Synthesis and Biological Studies of Novel Nucleoside Phosphoramidate Prodrugs

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A novel approach to the intracellular delivery of nucleotides using phosphoramidate-based prodrugs is described. Specifically, we have developed phosphoramidate prodrugs of the anticancer nucleotide 5-fluoro-2′-deoxyuridine-5′monophosphate (FdUMP). These phosphoramidate prodrugs contain an ester group that undergoes intracellular activation liberating phosphoramidate anion, which undergoes spontaneous cyclization and P−N bond cleavage to yield the nucleoside monophosphate quantitatively. In vitro evaluation of 5-fluoro-2′-deoxyuridine phosphoramidate prodrugs **2a** and **3b** against L1210 mouse leukemia cells show potent inhibition of cell growth (IC_{50} 0.5−3 nM). Cell-based thymidylate synthase inhibition studies show that, in contrast to FUdR, the nitrofuran compound **2a** is of comparable potency in wild type vs thymidine kinase deficient LM cells. This result indicates that the activation of this novel prodrug occurs via the proposed mechanism of intracellular delivery. However, naphthoquinone **3b** has an IC_{50} value for thymidylate synthase inhibition that is comparable to FUdR in thymidine kinase deficient cells. Further studies revealed that **3b** rapidly decomposes to the nucleotide in cell culture medium, suggesting that the naphthoquinone analogue is not sufficiently stable to function as a nucleotide prodrug.

Introduction

Purine and pyrimidine nucleoside analogues have shown promise as anticancer agents.[1] Several modified nucleoside inhibitors of thymidylate synthase (TS; EC 2.1.1.45), a target enzyme for the control of cell growth, have been developed. However, these compounds require intracellular metabolism to the nucleotide in order to exert inhibitory activity.[2,3] Although the nucleoside may be membrane permeable, it relies on intracellular phosphorylation to generate the active compound. Various mechanisms of resistance to such analogues have developed in in vitro and in vivo experimental systems.[4] Among these are the increased activity of catabolic enzymes such as phosphatases and the decreased levels of anabolic enzymes such as kinases that lead to the decreased accumulation of the active metabolite.[5] These problems have encouraged the development of prodrugs that can release the nucleotide inside tumor cells in order to bypass the need for intracellular phosphorylation.[6−9]

FdUMP is an anticancer agent that inhibits TS and is a candidate for the prodrug approach. TS is an enzyme that catalyzes the formation of thymidine-5′-monophosphate (TMP) from 2′-deoxyuridine-5′-monophosphate (dUMP).[2] Aromatic amino acid phosphoramidate prodrugs and cyclosaligenyl prodrugs of FdUMP have been synthesized and studied in vitro.[8,9] The aromatic amino acid nucleotide prodrugs described by Wagner et al. are converted to the phosphoramidate; this intermediate undergoes slow conversion to the nucleotide via P−N bond cleavage by an unknown phosphoramidase to yield FdUMP. Although the prodrugs were found to be stable in media and human serum, they were ultimately converted to FUdR and not FdUMP intracellularly.[9] It was found that these prodrugs were ∼30 000-fold less cytotoxic in thymidine kinase deficient cells compared to the wild-type cells. The cyclosaligenyl prodrugs are proposed to hydrolyze by cleavage of the phenolic ester bond followed by spontaneous decomposition to yield FdUMP.[8] These prodrugs were found to degrade to FdUMP in medium and, although they showed inhibitory activity comparable to FUdR in wild-type cell lines, they were ∼200-fold less potent in thymidine kinase deficient cells. Hence the inability of the aromatic amino acid phosphoramidate prodrugs and cyclosaligenyl prodrugs to exhibit inhibition of TS activity in intact thymidine kinase deficient cells suggests that FdUMP is not delivered intracellularly. Our approach involves the development of nucleoside phosphoramidate prodrugs that undergo enzymatic reduction followed by P−N bond cleavage to release the active nucleotide.[10,11] These phosphoramidate prodrugs contain a delivery group and a masking group. The delivery group functions as a substrate for intracellular enzymatic reduction which leads to expulsion of phosphoramidate anion,[12] and the masking group consists of a haloalkylamine group that undergoes spontaneous cyclization and P−N bond cleavage following activation. Previously we reported the synthesis and biological activity of a series of haloethyl nucleoside phosphoramidate prodrugs bearing a nitrofuryl ester, which showed growth inhibition of L1210 cell proliferation in the nanomolar range.[11] However, further mechanistic studies on model thymidine phosphoramidate compounds revealed that, after cyclization

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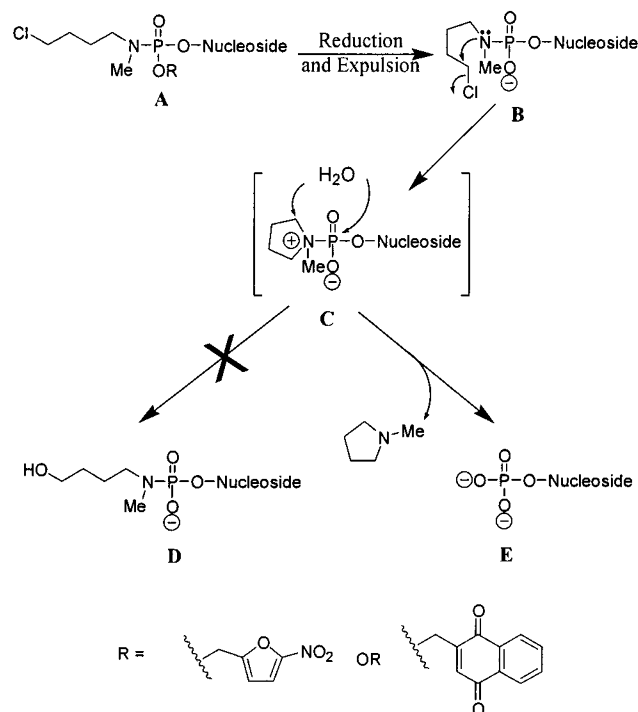
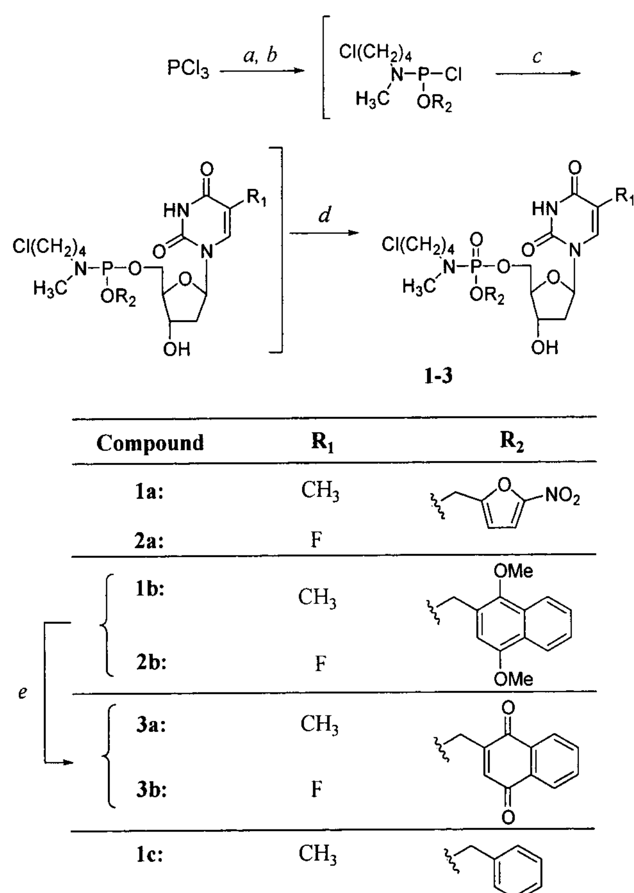
Scheme 1



Scheme 2[a]



Compound	R_1	R_2
1a:	CH_3	
2a:	F	
1b:	CH_3	
2b:	F	
3a:	CH_3	
3b:	F	
1c:	CH_3	

[a] Reagents and conditions: (a) 5-nitrofurfuryl alcohol, 1,4-dimethoxy-2-hydroxymethylnaphthalene or benzyl alcohol, DIEA, CH_2Cl_2, −70 °C, 20 min; (b) *N*-methyl-*N*-(4-chlorobutyl)amine hydrochloride, DIEA, CH_2Cl_2, −70 °C, 20 min; (c) thymidine or FUdR, pyridine, -45 °C; (d) *tert*-butyl hydroperoxide, −45 °C to 0 °C, 30 min; (e) $Ce(NH_4)_2(NO_3)_6$, CH_3CN/H_2O.

of the haloethyl phosphoramidate anion, nonselective nucleophilic attack of water at carbon and phosphorus of the aziridinium ion intermediate was observed.[13] Herein we describe the design, synthesis, and in vitro studies of a modified halobutyl phosphoramidate to provide novel prodrugs that undergo rapid and quantitative conversion to the active nucleotide.

Results and Discussion

Chemistry. The proposed mechanism for the activation of these new compounds is shown in Scheme 1. It was anticipated that prodrug **A**, after undergoing enzymatic reduction and spontaneous expulsion to give **B**, would cyclize to generate intermediate **C**. Although by analogy to the haloethyl analogue this intermediate might undergo attack at carbon or phosphorus by water, it was hypothesized that nucleophilic attack at carbon of the pyrrolidinium ring would be disfavored compared to attack at phosphorus, and formation of the desired nucleotide **E** would predominate. With this hypothesis in mind, model nucleoside analogues **1a**−**c**, **3a**, and FdUMP prodrugs **2a**,**b** and **3b** were synthesized as shown in Scheme 2. The synthesis was accomplished in a one-pot method where a highly reactive phosphorus^{III} monochloro intermediate is generated and reacted with the nucleoside. Phosphorus trichloride is reacted with the corresponding alcohol in the presence of diisopropylethylamine followed by reaction with *N*-methyl-*N*-(4-chlorobutyl)amine hydrochloride to generate the monochloro intermediate. This intermediate is reacted with the nucleoside in situ and then oxidized with *tert*-butyl hydroperoxide to yield **1a**−**c** and **2a**,**b** in yields ranging from 34 to 71%. Intermediates **1b** and **2b** are then converted into the corresponding bioactive prodrugs **3a** and **3b** by oxidation with ceric ammonium nitrate.

^{31}P NMR Studies. The inhibitory activity of the FdUMP prodrugs relies on the rapid and quantitative release of the nucleotide after activation of the delivery group. To determine whether these compounds are releasing the active nucleotide, chemical activation of model compounds **1a**, **1c**, and **3a** was studied using ^{31}P NMR. Reduction of the nitrofuryl prodrug **1a** was accomplished using sodium dithionite; the ^{31}P NMR stack plot of the reaction under model physiologic conditions (0.4 M cacodylate buffer/CH_3CN, pH ∼ 7.4, 37 °C) is shown in Figure 1. The spectrum at 0 min represents **1a** in CH_3CN immediately prior to activation. Five minutes after reduction, three new peaks are present at −14.61, −21.03, and −23.45 ppm (reference 1% triphenyl phosphine oxide in benzene-d_6). The peak at −14.61 ppm corresponds to the phosphoramidate anion **B** (Scheme 1). This intermediate presumably cyclizes with a half-life of 2.0 min to form the pyrrolidinium zwitterion intermediate **C** (tentatively assigned at −21.03 ppm) that hydrolyzes spontaneously to thymidine monophosphate (−23.45 ppm; **E**) as the exclusive product. Attack by water at carbon of the pyrrolidinium intermediate was not observed.

Biological Activity: Growth Inhibtion. In vitro growth inhibitory activity in L1210 mouse leukemia cells was studied for compounds **2a** and **3b**. The growth inhibitory activity of these compounds was compared to previously synthesized compound **6**,[11] 5-fluorouracil (5-FU), and FUdR (Chart 1). All compounds exhibited

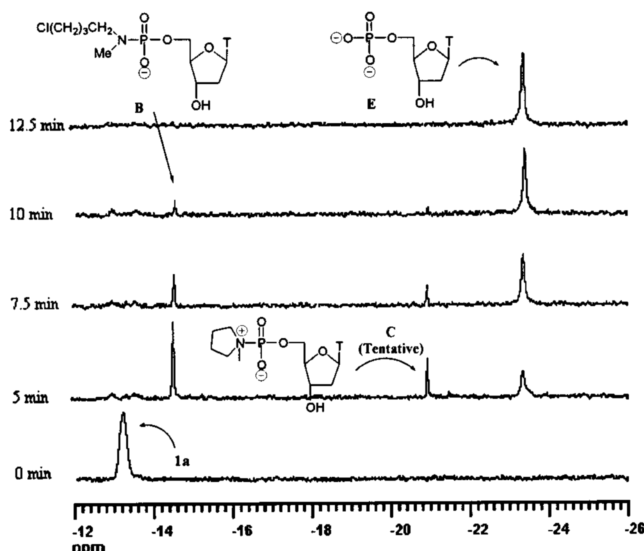


Figure 1. Reaction of phosphoramidate **1a** in cacodylate buffer (ca. 100 mM, pH 7.4, 37 °C). **B**: phoshoramidate anion intermediate. **C**: pyrrolidinium ion intermediate. **E**: thymidine 5′-monophosphate. Chemical shifts are reported relative to triphenylphosphine oxide reference. See Results and Discussion for details.

Chart 1

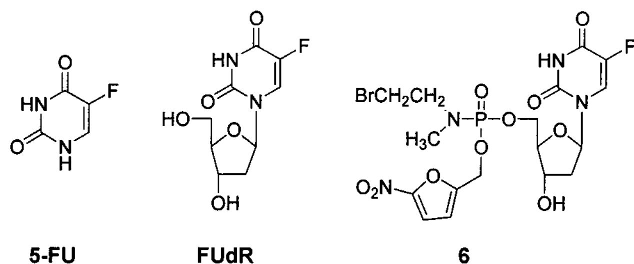


Table 1. Growth Inhibition in L1210 Mouse Leukemia Cells

	IC_{50}, nM			
	treatment time[a], h			
compound	2	8	24	48
2a	116	47	7.2	2.3
3b	44	16	3.1	0.63
6[b]	1027	347	56	16
5-FU	2500	1010	360	200
FUdR	45	23	4.1	0.64

[a] Cells were treated with drug for 2, 8, 24, and 48 h then spun down, resuspended in fresh drug-free medium, and returned to the incubator. Cell counts were determined 48 h after the start of drug treatment. [b] See ref 11.

potent inhibition of cell proliferation; the results are summarized in Table 1. Addition of thymidine (5 μM) resulted in reversal of growth inhibition when compared to cells that were not treated with thymidine, suggesting that the prodrugs are generating an inhibitor of thymidylate synthase. The halobutyl compounds **2a** and **3b** are more potent than the corresponding haloethyl analogue **6**. The increase in potency of compounds **2a** and **3b** coincides with the proposed mechanism where exclusive formation of the nucleotide is observed by ^{31}P NMR (Figure 1). As expected, compounds **2a** and **3b** are significantly more potent than 5-FU. Furthermore, it is worth noting that the naphthoquinone analogue **3b** exhibited 2−3-fold greater potency than the corresponding nitrofuryl analogue **2a**.

Table 2. Enzyme Inhibition in L1210, LM, and LM(TK−) Cells

	IC_{50}, nM[a]			
compound	L1210	LM	LM (TK−)	(TK−)/TK
2a	65	560	1060	1.9
3b	10	230	5900	25
FUdR	7.9[b]	260	5400	20

[a] Cells were treated with drug for 2 h, then pulsed for 1 h with labeled nucleoside. See experimental procedure for details. [b] Reported value = 7.6 nM.[16]

Enzyme Inhibition. Although our hypothesis of reduction and P−N bond cleavage to release the active nucleotide is supported by ^{31}P NMR mechanistic studies, the formation of 5-FU and FUdR following activation is also possible in this cell-based system. In the case where 5-FU is generated, it may be converted to FUdR by pyrimidine nucleoside phosphorylase and then rephosphorylated to FdUMP by thymidine kinase.[14] To determine whether the prodrug might be acting via formation of 5-FU and/or FUdR, a cell line that is thymidine kinase deficient was used. A tritium release assay developed by Kalman et al.[15] was used to measure intracellular inhibition of TS activity. In this assay, TS activity is measured by determining the tritiated water that is released from [5-^{3}H] 2′-deoxycytidine ([5-^{3}H]-dCyd).

To compare the effects of thymidine kinase on drug activity, wild type and thymidine kinase deficient LM cells were used. L1210 cells were also used in order to asseses the correlation between enzyme inhibition and growth inhibition. Table 2 shows the results obtained when compounds **2a**, **3b**, and FUdR were tested in this assay. FUdR is ~20-fold less potent in the thymidine kinase deficient cells as expected. Prodrug **2a** shows comparable enzyme inhibition in both wild type and thymidine kinase deficient LM cells, with the IC_{50} value in LM (TK−) cells about 2-fold higher when compared to the LM cells. Surprisingly, compound **3b** was comparable to FUdR in both the thymidine kinase deficient cells and the wild-type cells, suggesting that intracellular delivery of nucleotide was not occurring. This led us to study the stability of these prodrugs in cell culture media. Stability tests in serum-free media (Fisher's media, pH = 7.4, 37 °C) revealed that the naphthoquinone **3a** analogue decomposes to the nucleotide with a half-life of 3.5 min. In contrast, compound **1a** is stable in media with or without serum for >6 days. Presumably, naphthoquinone **3a** is rapidly converted to the nucleotide in media and is dephosphorylated prior to entry into the cell.

Conclusions

A novel prodrug approach for the intracellular delivery of nucleotides across the cell membrane has been developed. The synthesis of nucleoside phosphoramidates bearing nitrofuryl and naphthoquinone phosphoramidates that would function as prodrugs of FdUMP have been prepared. Modification of the previously reported[11] haloethyl moiety to a halobutyl moiety provides novel compounds that undergo rapid and quantitative conversion to the nucleotide. Compound **2a** shows excellent inhibition of cell proliferation and thymidylate synthase inhibition, with IC_{50} values in the nanomolar range. Most importantly, cell-based thymidy-

late synthase inhibition studies in thymidine kinase deficient cells indicate that the activation of compound **2a** occurs via the proposed mechanism involving direct intracellular conversion to FdUMP. However, the naphthoquinone analogue is rapidly degraded to the nucleotide in media and is further metabolized presumably to the nucleoside.

Experimental Section

Materials and Methods. NMR spectra were recorded using a 250 MHz Bruker spectrometer equipped with a 5 mm multinuclear probe. ^{1}H chemical shifts are reported in parts per million using tetramethylsilane as an internal reference. ^{31}P NMR spectra were obtained using broadband ^{1}H decoupling, and chemical shifts are reported in parts per million using 1% triphenyl phosphine oxide in benzene-d_6 as a coaxial insert (25.17 ppm relative to 85% phosphoric acid). ^{31}P NMR kinetics carried out at 37 °C were conducted using a Bruker variable temperature unit. Silica gel grade 60 was used to carry out all chromatographic purifications. HPLC analysis was done using a Beckman System Gold equipped with a 168 detector set to 250 nm, a 126 solvent module, and an econosphere C18 column (5 μM, 4 × 250 mm) from Alltech Associates. Mass spectral analysis was obtained from the mass spectrometry laboratory at Purdue University, West Lafayette, IN. All anhydrous reactions were carried out under argon, using flamed dried flasks, and all organic solvents were distilled prior to use. Radioactivity was measured using a Packard Tri-CARB liquid scintillation analyzer model 1900 TR. EcoLite liquid scintillation cocktail was obtained from ICN.

^{31}P NMR Studies. Kinetic experiments were carried out as described previously.[11] Briefly, the compound (15 mg) was dissolved in acetonitrile (80 μL); to this was added a solution of the activating agent (sodium dithionite; 3 equiv) in cacodylate buffer (500 μL, 0.4 M, pH = 7.4). The reaction mixture was transferred to a 5 mm NMR tube, and data acquisition was started with the probe maintained at 37 °C. Spectra were acquired every 2.5 min for 30 min and when necessary every 10 min for an additional hour. Time points were assigned to each data acquisition from the start of the reaction. The integration of the peak areas was used to determine the relative concentration of the intermediates and products.

In Vitro Growth Inhibition Assay. Stock solutions of drugs were prepared in absolute ethanol, and serial dilutions of drug were prepared. L1210 cells were suspended in Fisher's medium supplemented with 10% horse serum, 1% glutamine, and 1% antibiotic-antimycotic solution to give 10 mL volumes of cell suspension at a final density of 3−6 × 10^4 cells/mL. Appropriate volumes of the drug solution were transferred to the cell suspensions, and incubation was continued for 2, 8, 24, or 48 h. The cells were spun down, resuspended in fresh drug-free medium and returned to the incubator. Final cell counts were determined 48 h after the start of drug treatment. The data were analyzed by sigmoidal curve fit of cell count vs log(drug concentration) and the results expressed as the IC_{50} (the drug concentration that inhibits cell growth to 50% of control value).

In Vitro Enzyme Inhibition Assay. Thymidylate synthase inhibition was determined using the published assay.[15] Briefly, serial dilutions of drug were prepared in absolute ethanol such that 5 μL of drug solution added to 445 μL of cell suspension gave the desired final concentration. L1210 cells in exponential growth were suspended in Fischer's medium, and LM and LM (TK−) cells were suspended in Minimum Essential Medium to give a final density of 1.1 × 10^7 cells/mL. Aliquots of the cell suspension (445 μL) were placed in eppendorf tubes, drug solution (5 μL) was added, and the cells were incubated at 37 °C for 2 h. Measurement of thymidylate synthase activity was initiated by the addition of 50 μL of serum free media containing 1 μCi of [5-^{3}H]-2′-deoxycytidine (0.5 μM final concentration). The reaction mixture was incubated for 1 h in a shaking water bath at 37 °C, and then terminated by transferring 100 μL of the reaction mixture to eppendorf tubes containing 100 μL of 20% activated charcoal suspended in 4% aqueous perchloric acid (w/v). The tubes were vigorously vortexed and centrifuged using a minicentrifuge. The supernatant (100 μL) was transferred to 2 mL of scintillation cocktail and counted for 1 min. The data were analyzed by sigmoidal curve fit of counts per minute vs log(drug concentration) and the results expressed as the IC_{50} values. Background counts (<500 CPM) were obtained by quenching a solution of cells and [5-^{3}H]dCyd immediately after pulsing with the isotope. Negative controls were obtained by treating cells with EtOH only and incubating after addition of the isotope and then quenching.

5′-Thymidyl 5-nitrofurfuryl *N*-methyl-*N*-(4-chlorobutyl) phosphoramidate (1a): Phosphorus trichloride (6.19 mL, 2.0 M in CH_2Cl_2) was cooled to −70 °C under argon. 5-Nitrofurfuryl alcohol (1.77 g; 12.38 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL) and added slowly followed by the dropwise addition of diisopropylethylamine (3.24 mL, 18.58 mmol). The reaction mixture was allowed to stir at −70 °C for 15 min. *N*-Methyl-*N*-(4-chlorobutyl)amine hydrochloride (1.96 g, 12.38 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL) and added dropwise to the reaction mixture. Diisopropylethylamine (6.47 mL, 37.15 mmol) was added dropwise, and the reaction mixture was stirred at −70 °C for 25 min. Thymidine (1.0 g, 4.13 mmol) was coevaporated with anhydrous pyridine (5 × 30 mL), dissolved in anhydrous pyridine (30 mL), and cooled to −45 °C. The CH_2Cl_2 reaction mixture was added dropwise to the solution of thymidine in pyridine until thymidine disappeared by TLC (10% MeOH/$CHCl_3$). After 35 min, the reaction mixture was oxidized with *tert*-butyl hydroperoxide (2.4 mL, 4.6 M in decane) at −40 °C and warmed to 0 °C over 30 min. Saturated NH_4Cl (15 mL) was added, the layers were separated, and the aqueous layer was extracted with $CHCl_3$ (5 × 20 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The crude reaction mixture was flushed through a plug of silica gel (10% MeOH/$CHCl_3$). Further purification by chromatography on silica gel (5% MeOH:$CHCl_3$) afforded **1a** (1.34 g; 59%) as a light yellow foam. R_f = 0.20 (5% MeOH/$CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.54 (m, 1H); 7.36 (s, 1H); 7.29 (d, 1H, J = 3.66 Hz); 6.69 and 6.67 (d, 1H, J = 3.66 Hz); 6.27 (q, 1H); 5.03 (m, 2H); 4.53 (m, 1H); 4.22 (m, 2H); 4.05 (m, 1H); 3.57 (t, 2H); 3.09 (m, 2H); 2.67 (d, 3H, J = 10.17 Hz); 2.61 (m, 1H); 2.25 (m, 1H), 1.89 (d, 3H); 1.73 (m, 4H). ^{31}P NMR ($CDCl_3$): δ −13.64, −13.75 (mixture of diastereomers). MS (ESI): m/z 551 $(M + H)^+$.

5′-Thymidyl-2-(1,4-dimethoxynaphthyl)methyl *N*-methyl-*N*-(4-chlorobutyl) phosphoramidate (1b): Phosphoramidate **1b** was prepared from 1,4-dimethoxy-2-hydroxymethylnaphthalene (180 mg, 0.825 mmol), phosphorus trichloride (0.41 mL, 2 M in CH_2Cl_2), *N*-methyl-*N*-(4-chlorobutyl)amine hydrochloride (130 mg, 0.825 mmol), thymidine (100 mg, 0.413 mmol), and *tert*-butyl hydroperoxide (0.24 mL, 4.6 M in decane) as described for compound **1a**. The reaction mixture was filtered through a plug of Celite and concentrated under reduced pressure. Column chromatography of the crude product (1:1 CH_2Cl_2/acetone) afforded **1b** (156 mg; 61%) as a light orange foam. R_f = 0.33 (1:1 CH_2Cl_2/acetone). ^{1}H NMR ($CDCl_3$): δ 8.58 (s, 1H); 8.23 (d, 1H, J = 7.50 Hz); 8.03 (m, 1H); 7.54 (m, 2H); 6.82 (s, 1H); 6.25 (m, 1H); 5.25 (m, 2H); 4.49 (m, 1H); 4.18 (m, 2H); 3.98 (s, 3H); 3.92 (s, 3H); 3.52 (t, 2H); 3.08 (m, 2H); 2.65 (d, 3H, J = 9.70 Hz); 2.37 (m, 1H); 2.17 (s, 3H) 1.71 (m, 4H). ^{31}P NMR ($CDCl_3$): δ −12.93. MS (ESI): m/z 626 $(M + H)^+$.

5′-Thymidyl 2-(1,4-naphthoquinonyl)methyl *N*-methyl-*N*-(4-chlorobutyl) phosphoramidate (3a): Ceric ammonium nitrate (550 mg, 1.0 mmol) in water (7 mL) was added dropwise over 15 min to a solution of **1b** (250 mg, 0.4 mmol) in CH_3CN (7 mL). The reaction mixture was stirred at room temperature for 15 min and extracted with $CHCl_3$ (3 × 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (5% MeOH/$CHCl_3$) to give **3a** (210 mg, 90%) as a yellow foam. R_f = 0.50 (10% MeOH/

$CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.08 (m, 2H); 7.79 (m, 2H); 7.05 (d, 1H, $J = 5.68$ Hz); 6.21 (t, 1H); 5.03 (m, 2H); 4.59 (m, 1H); 4.26 (m, 2H); 4.05 (m, 1H); 3.58 (t, 2H); 2.73 (d, 3H, $J = 10.07$ Hz); 2.27 (m, 2H); 1.91 (d, diastereomers, 3H); 1.59 (m, 4H). ^{31}P NMR ($CDCl_3$): δ −12.67, −12.99 (mixture of diastereomers). MS (ESI): *m/z* 596 (M + H)$^+$.

5′-Thymidyl benzyl *N*-methyl-*N*-(4-chlorobutyl) phosphoramidate (1c): Phosphoramidate **1c** was prepared from phosphorus trichloride (2.06 mL, 2 M in CH_2Cl_2), benzyl alcohol (0.43 mL, 4.13 mmol), *N*-methyl-*N*-(4-chlorobutyl)amine hydrochloride (648 mg, 4.13 mmol), thymidine (500 mg, 2.06 mmol), and *tert*-butyl hydroperoxide (1.17 mL, 4.6 M in decane) as described above for compound **1a**. Column chromatography of the crude product (5% MeOH/$CHCl_3$) afforded **1c** (676 mg; 64%) as a white foam. $R_f = 0.23$ (10% MeOH/$CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.41 (m, 1H); 7.40 (s, 5H); 6.29 (m, 1H); 5.06 (m, 3H); 4.39 (m, 1H); 4.16 (m, 2H); 4.02 (m, 1H); 3.54 (t, 2H); 3.04 (m, 2H); 2.61 (d, 3H, $J = 10.07$ Hz); 2.39 (m, 1H); 2.12 (m, 1H); 1.84 (d, diastereomers, 3H); 1.60 (m, 4H). ^{31}P NMR ($CDCl_3$): δ −13.27, −13.33 (mixture of diastereomers). MS (ESI): *m/z* 538 (M + Na)$^+$.

5-Fluoro-2′-deoxyuridyl 5-nitrofurfuryl *N*-methyl-*N*-(4-chlorobutyl) phosphoramidate (2a): Phosphoramidate **2a** was prepared from 5-nitrofurfuryl alcohol (233 mg, 1.63 mmol), phosphorus trichloride (0.81 mL, 2 M in CH_2Cl_2), *N*-methyl-*N*-(4-chlorobutyl)amine hydrochloride (257 mg, 1.63 mmol), 5-fluoro-2′-deoxyuridine (200 mg; 0.812 mmol), and *tert*-butyl hydroperoxide (0.44 mL, 4.6 M in decane) as described above for compound **1b**. Column chromatography of the crude product (1:1 CH_2Cl_2/acetone) afforded **2a** (155 mg; 34%) as a yellow foam. R_f = 0.34 (1:1 CH_2Cl_2/acetone). ^{1}H NMR ($CDCl_3$): δ 9.68 (m, 1H); 7.80 and 7.74 (d, 1H, $J = 6.22$ and 6.41 Hz); 7.30 (d, 1H, $J = 3.48$ Hz); 6.71 (d, 1H, $J = 3.30$); 6.22 (m, 1H); 5.03 (m, 2H); 4.52 (m, 1H); 4.24 (m, 2H); 4.07 (m, 1H); 3.57 (m, 2H); 3.07 (m, 2H); 2.68 (d, 3H, $J = 10.25$ Hz); 2.49 (m, 1H); 2.18 (m, 1H), 1.74 (m, 4H). ^{31}P NMR ($CDCl_3$): δ −15.59, −15.78 (mixture of diastereomers). Anal. ($C_{19}H_{25}ClFN_4O_{10}P$) C, H, N.

5-Fluoro-2′-deoxyuridyl 2-(1,4-dimethoxynaphthyl)methyl *N*-methyl-*N*-(4-chlorobutyl) phosphoramidate (2b): Phosphoramidate **2b** was prepared from phosphorus trichloride (0.41 mL, 2 M in CH_2Cl_2), 1,4-dimethoxy-2-hydroxymethylnaphthalene (177 mg, 0.823 mmol), *N*-methyl-*N*-(4-chlorobutyl)amine hydrochloride (128 mg, 0.812 mmol), 5-fluoro-2′-deoxyuridine (100 mg, 0.406 mmol), and *tert*-butyl hydroperoxide (0.23 mL, 4.6 M in decane) as described for **1b**. Column chromatography of the crude product (1:1 CH_2Cl_2/acetone) afforded **2b** (181 mg; 71%) as a light orange foam. R_f = 0.53 (1:1 CH_2Cl_2/acetone). ^{1}H NMR ($CDCl_3$): δ 8.22 (dd, 1H); 8.04 (dd, 1H); 7.74 and 7.66 (d, 1H, $J = 6.23$ & 6.39); 7.55 (m, 2H); 6.83 (s, 1H); 6.16 (m, 1H); 5.26 (m, 2H); 4.51 (m, 1H); 4.23 (m, 2H); 4.05 (m, 1H); 3.99 (s, 3H); 3.93 (s, 3H); 3.50 (m, 2H); 3.08 (m, 2H); 2.65 (d, 3H, $J = 9.70$ Hz); 2.42 (m, 2H); 1.66 (m, 4H). ^{31}P NMR ($CDCl_3$): δ −12.82, −12.90 (mixture of diastereomers). MS (FAB) $C_{27}H_{34}ClFN_3O_4P$ calculated 630.1784 (M + H)$^+$, found 630.1760.

5-Fluoro-2′-deoxyuridyl 2-(1,4-naphthoquinonyl)methyl *N*-methyl-*N*-(4-chlorobutyl) phosphoramidate (3b) was prepared from ceric ammonium nitrate (225 mg, 0.41 mmol) and **2b** (100 mg, 0.16 mmol) as described for compound **3a**. The crude product was purified by silica gel chromatography (10% MeOH/$CHCl_3$) to give **3b** (88 mg, 93%) as a yellow foam. $R_f = 0.50$ (10% MeOH/$CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.10 (m, 2H); 7.77 (m, 2H); 7.72 (m, 1H); 7.03 (s, 1H); 6.18 (m, 1H); 5.02 (m, 2H); 4.58 (m, 1H); 4.29 (m, 2H); 4.05 (m, 1H); 3.57 (m, 2H); 2.73 (d, 3H, $J = 10.07$ Hz); 2.47 (m, 1H); 2.31 (m, 1H); 1.75 (m, 4H). ^{31}P NMR ($CDCl_3$): δ −12.67, −12.99 (mixture of diastereomers). Anal. ($C_{25}H_{28}ClFN_3O_9P{\cdot}1.5\ H_2O$) C, H, N.

***N*-Methyl-*N*-4-hydroxybutylamine** was prepared according to the procedure of Kuznetsov et al.[16] Methylamine (53 mL, 1.20 mol) gas was condensed in a 12 in. pressure tube using a dry ice−acetone bath. 4-Chloro-1-butanol (24 mL, 0.24 mol) was slowly added to the methylamine. Methanol (23 mL) was cooled to −78 °C and added to the solution. The pressure tube was closed and warmed to room temperature, then heated in an oil bath at 80 °C. The reaction mixture was allowed to stir at this temperature for 16 h. The tube was cooled, the pressure was released, and methanol was distilled off to give a viscous orange oil. This mixture was washed with diethyl ether (3 × 20 mL), then slowly added to a solution of KOH in water (30 mL, 1.2 g/mL). The water layer was extracted with toluene, the toluene was removed under reduced pressure, and the product was purified by distillation to give a clear oil (10.14 g, 50%), bp 60 °C (2 mm). ^{1}H NMR ($CDCl_3$): δ 3.58 (t, 2H); 2.64 (t, 2H); 2.44 (s, 3H); 1.65 (m, 4H). MS (CI): *m/z* 104 (M + H)$^+$.

***N*-Methyl-*N*-(4-chlorobutyl) amine hydrochloride:** HCl gas was bubbled into a stirred solution of *N*-methyl-4-hydroxybutylamine (2.00 g, 19.38 mmol) in CH_2Cl_2 (10 mL) until the solution turned moist pH paper red (pH = 2). The reaction mixture was cooled to 0 °C, thionyl chloride (1.41 mL, 19.38 mmol) was added dropwise, and the reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure to give the product as a white solid (2.90 g, 95%), mp 119−121 °C. ^{1}H NMR ($CDCl_3$): δ 8.79 (s, 1H); 3.59 (t, 2H, $J = 5.95$); 2.97 (m, 2H); 2.70 (s, 3H); 2.06 (m, 2H); 1.95 (m, 2H); 1.63 (s, 1H). MS (CI): *m/z* 122 (M + H)$^+$.

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