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Sulfonyl-Containing Nucleoside Phosphotriesters and Phosphoramidates as Novel Anticancer Prodrugs of 5-Fluoro-2′-deoxyuridine 5′-Monophosphate (FdUMP)

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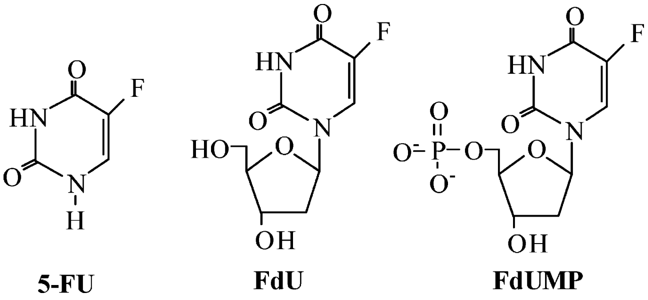
Abstract: A series of sulfonyl-containing 5-fluoro-2′-deoxyuridine (FdU) phosphotriester and phosphoramidate analogues were designed and synthesized as anticancer prodrugs of FdUMP. Stability studies have demonstrated that these compounds underwent pH dependent β-elimination to liberate the corresponding nucleotide species with half-lives in the range of 0.33−12.23 h under model physiological conditions in 0.1 M phosphate buffer at pH 7.4 and 37 °C. Acceleration of the elimination was observed in the presence of human plasma. Compounds with an FdUMP moiety (**4**−**9**) were considerably more potent than those without (**1**−**3**) as well as 5-fluorouracil (5-FU) against Chinese hamster lung fibroblasts (V-79 cells) in vitro. Addition of thymidine (10 μM) reversed the growth inhibition activities of only 5-FU and the compounds with an FdUMP moiety, but had no effect on those without. These results are consistent with thymidylate synthase as the target of the prodrugs.

Keywords: 5-FU; nucleotide prodrug; sulfonyl; β-elimination

Introduction

5-Fluoro-2′-deoxyuridine 5′-monophosphate (FdUMP) is the major metabolite responsible for the anticancer activity of 5-FU (Chart 1). FdUMP inhibits the enzyme thymidylate synthase (TS, EC 2.1.1.45), which catalyzes the reductive methylation of 2′-deoxyuridine 5′-monophosphate (dUMP) to thymidylate (dTMP).[1] Since TS catalyzes one of the rate-limiting steps in DNA synthesis, modulation of TS has often been targeted in the design of anticancer agents.[2] Direct administration of FdUMP should offer therapeutic advantages over 5-FU with respect to bypassing metabolic activation and possible toxic side effects caused by the incorporation of 5-FU into DNA or RNA.[3] FdUMP, however, does not readily penetrate cell membranes due to the presence of the phosphate group and is susceptible to degradation by nonspecific phosphohydrolase. Several nucleotide prodrug (pronucleotide) approaches have been attempted to circumvent the delivery problems of FdUMP.[4−9] The major challenge of developing FdUMP prodrugs has been in the

Chart 1. Structures of 5-FU, FdU, and FdUMP



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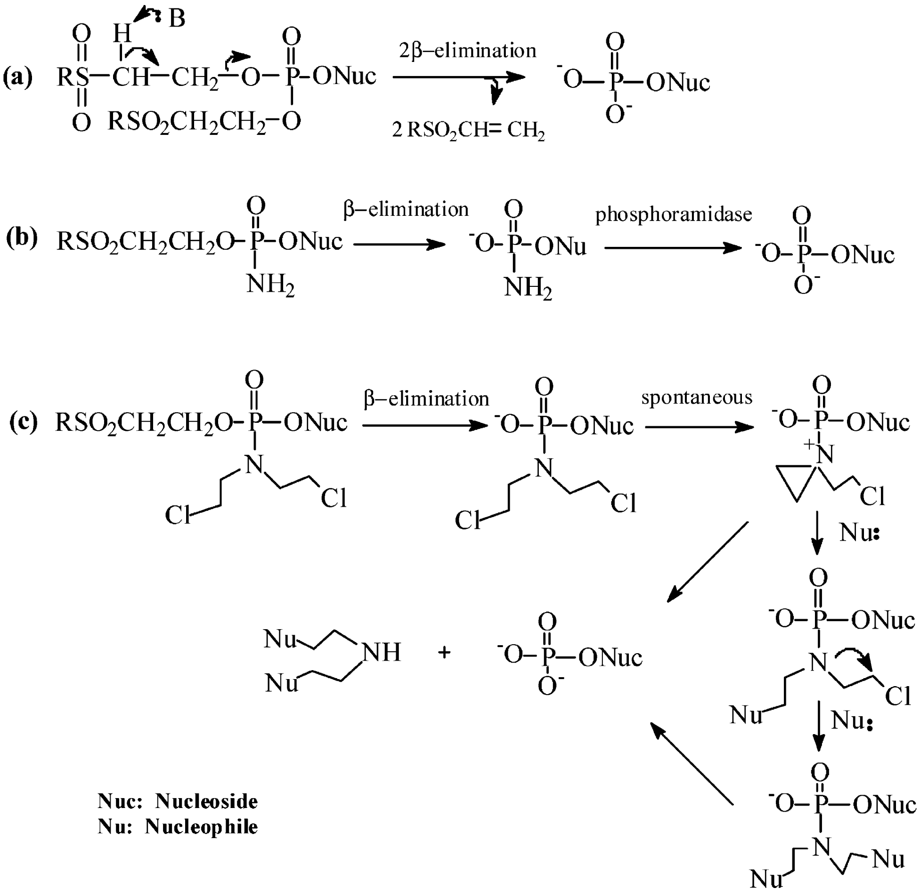
Scheme 1



selection of a suitable phosphate masking group. Sulfonylethyl groups have been successfully exploited as promoieties for phosphoramidate mustard derivatives.[10,11] It is hypothesized that the sulfonylethyl groups may also serve as useful phosphate masking groups in the development of FdUMP prodrugs.

A series of sulfonylethyl prodrugs of FdUMP was designed and synthesized in the forms of phosphotriester, phosphoramidate monoester, and bifunctional bis(2-chloroethyl) phosphoramidate monoesters. The proposed activation pathways of the prodrugs are shown in Scheme 1. All of the prodrugs are expected to undergo β-elimination reaction as the first activation step. For the phosphotriester prodrugs, FdUMP is expected to be released after two β-elimination reactions (Scheme 1a). However, for the phosphoramidate prodrugs, a further P−N bond cleavage is required to liberate FdUMP. Cleavage of the P−N bond has been proposed either through the enzymatic hydrolysis of a putative enzyme phosphoramidase[12,13] (Scheme 1b) or, in the case of bis(2-chloroethyl) phosphoramidate anion, by a spontaneous hy-

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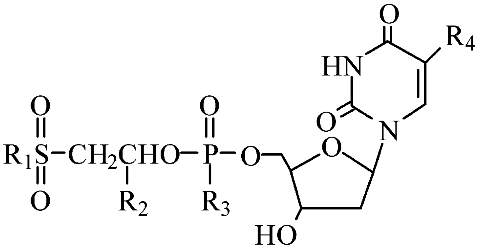
Chart 2. Structures of Sulfonyl Pronucleotides



1. R_1= p-tolyl, R_2= H, R_3=O-p-tolylsulfonylethyl, R_4=H;
2. R_1= p-tolyl, R_2= H, R_3=NH_2, R_4=H;
3. R_1= p-tolyl, R_2= H, R_3=$N(CH_2CH_2Cl)_2$, R_4=H;
4. R_1= p-tolyl, R_2= H, R_3=O-p-tolylsulfonylethyl, R_4=F;
5. R_1= p-tolyl, R_2= H, R_3=NH_2, R_4=F;
6. R_1= p-tolyl, R_2= H, R_3=$N(CH_2CH_2Cl)_2$, R_4=F;
7. R_1= p-nitrophenyl, R_2= H, R_3=O-p-nitrophenylsulfonyl, R_4=F;
8. R_1= p-nitrophenyl, R_2= H, R_3=$N(CH_2CH_2Cl)_2$, R_4=F;
9. R_1= p-tolyl, R_2= CH_3, R_3=O-p-tolylsulfonyl-1-methylethyl, R_4=F;

drolysis of the resulting aziridinium intermediates or its alkylating derivatives (Scheme 1c).[14]

In this study, compounds **4**, **5**, **6**, **8**, and **9,** which contain 5-FU moiety, were synthesized as target compounds; compounds **1**, **2**, and **3**, which contain 2′-deoxyuridine (dU), were synthesized as model compounds (Chart 2). The growth inhibitory activities of all these compounds were evaluated. A thymidine-reversed growth inhibition study was also performed.

Experimental Section

Chemistry. All experiments involving water-sensitive compounds were conducted under scrupulously dry conditions. Triethylamine was dried and stored over calcium hydride. Acetonitrile was dried by refluxing over calcium hydride and stored over activated 4 Å molecular sieves. Tetrahydrofuran was dried over sodium, and benzophenone was used as indicator. Dichloromethane was dried by refluxing with P_2O_5 overnight and stored over activated 4 Å molecular sieves. Evaporation of solvents was carried out on a rotary evaporator under reduced pressure. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and were uncorrected. ^1H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker 400 MHz spectrometer (^1H, 400 MHz; ^{13}C, 100 MHz). All ^{13}C and ^{31}P NMR spectra were recorded proton-decoupled. All NMR spectra were run at ambient temperature. Tetramethylsilane (TMS) was used as the internal reference. Deuterium exchange and COSY experiments were performed in order to confirm proton assignments. APT experiments were performed to confirmed carbon assignment. Chemical shifts are given in δ-values. Many proton NMR signals were split due to the presence of diastereomers. Abbreviations for the signal patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), or multiplet (m), broad (br), doubled doublet (dd), doubled triplet (dt), tertiary carbons (C tert), quarternary carbons (C quat). Elemental analyses were carried out by Atlantic Microlab Inc., Norcross, GA. Mass spectrometry was performed by Mass Consortium, San Diego, CA. Column chromatography refers to flash chromatography carried out using Merck silica gel 60 (40−60 μm) as stationary phase.

5′-*O*-Dimethoxytrityl-2′-deoxyuridine (10b) was prepared according to the published procedures.[15,16] A solution of 2′-deoxynucleoside (1.0 mmol) and 4,4′-dimethoxytrityl chloride (DMTCl) (1.1 mmol) in 10 mL of anhydrous pyridine was stirred at room temperature for 5 h. To the reaction mixture was then added 1 mL of methanol, and the mixture was stirred for 30 min before being concentrated by rotary evaporator. The residue was partitioned between ethyl acetate (250 mL) and H_2O (250 mL). The organic layer was washed with water five times, dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography to give a white crystalline product: yield 80.4% (426 mg); mp 113 °C; R_f 0.40 (CH_2Cl_2/MeOH = 95:5); ^1H NMR ($CDCl_3$) δ 9.39 (1H, br s, NH), 7.80 (1H, d, J = 8.0 Hz, C6H), 7.40

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(2H, m, arom H), 7.29 (7H, m, arom H), 6.85 (4H, m, arom H), 6.34 (1H, t, $J = 6.1$ Hz, C1′H), 5.42 (1H, d, $J = 8.1$ Hz, C5H), 4.58 (1H, m, C3′H), 4.06 (1H, q, $J = 7.5$ Hz, C4′H), 3.80 (6H, s, $-OCH_3$), 3.46 (2H, m, $C5'H_2$), 2.48 (1H, m, C2′H), 2.36 (1H, m, C2′H).

5′-*O*-(Dimethoxytrityl)-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyuridine (10a) was prepared according to published procedures.[15,16] A mixture of **10b** (6.0 mmol), imidazole (14.0 mmol), and *tert*-butyldimethylsilyl chloride (7.5 mmol) was dissolved in 3 mL of anhydrous DMF, and the resulting reaction mixture was stirred overnight at room temperature. To the solution was added 100 mL of water, and the mixture was extracted with ethyl acetate (3 × 100 mL). The combined organic layer was dried (Na_2SO_4) and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography to give product as a white solid: yield 96% (3.71 g); mp 89−90.5 °C; R_f 0.66 (CH_2Cl_2/MeOH = 95:5); ^{1}H NMR ($CDCl_3$) δ 7.92 (1H, d, $J = 8.2$ Hz, C6H), 7.40 (2H, m, arom H), 7.30 (7H, m, arom H), 6.86 (4H, m, arom H), 6.30 (1H, t, $J = 6$ Hz, C1′H), 5.39 (1H, d, $J = 8.2$ Hz, C5′H), 4.54 (1H, q, $J = 6.2$ Hz, C3′H), 3.97 (1H, m, C4′H), 3.82 (6H, s, OCH_3), 3.52 (1H, m, C5′H), 3.36 (1H, m, C5′H), 2.40 (1H, m, C2′H), 2.23 (1H, m, C2′H), 0.87 (9H, s, $C(CH_3)_3$), 0.03 (6H, d, $J = 20$ Hz, $Si(CH_3)_2$).

3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxyuridine (10) was prepared according to the published procedures.[15,16] To **10a** (1.8 mmol) was added 10 mL of 80% (v/v) aqueous acetic acid at room temperature, and the mixture was stirred for 15 min. The mixture was evaporated to dryness in vacuo. To the residue was added 50 mL of water, and the mixture was neutralized with 0.1 N sodium hydroxide. The solution was extracted with ethyl acetate (3 × 100 mL). The extracts were collected and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the residue was purified by column chromatography to give white crystalline product: yield 79% (486 mg); mp 206.5−207 °C; R_{f1} 0.40 (CH_2Cl_2/MeOH = 95:5), R_{f2} 0.6 (EtOAC); ^{1}H NMR ($CDCl_3$) δ 9.29 (1H, br s, exch D_2O, NH), 7.69 (1H, d, $J = 8.1$ Hz, C6H), 6.20 (1H, t, $J = 6.6$ Hz, C1′H), 5.75 (1H, d, $J = 8.1$ Hz, C5H), 4.50 (1H, m, C3′H), 3.94 (2H, m, C4′H and C5′H), 3.77 (1H, d, $J = 9.9$ Hz, C5′H), 2.85 (1H, br s, exch D_2O, OH), 2.29 (2H, m, C2′H), 0.91 (9H, s, $C(CH_3)_3$), 0.10 (6H, s, $Si(CH_3)_2$).

5′-*O*-(Dimethoxytrityl)-5-fluoro-2′-deoxyuridine (11b) was prepared as described in **10b** using 5-fluoro-2′-deoxyuridine on a 1.0 mmol scale in 71% yield (389 mg): mp (dec) 70−73 °C; R_f 0.44 (CH_2Cl_2/MeOH = 95:5); ^{1}H NMR ($CDCl_3$) δ 8.78 (1H, br s, NH), 7.83 (1H, d, $J = 8.0$ Hz, C6H), 7.45 (2H, m, arom H), 7.32 (7H, m, arom H), 6.86 (4H, m, arom H), 6.30 (1H, t, $J = 6$ Hz, C1′H), 4.56 (1H, q, C3′H), 4.07 (1H, q, $J = 7.5$ Hz, C4′H), 3.81 (6H, s, OCH_3), 3.45 (2H, m, $C5'H_2$), 2.48 (1H, m, C2′H), 2.28 (1H, m, C2′H).

5′-*O*-(Dimethoxytrityl)-3′-*O*-(*tert*-butyldimethylsilyl)-5-fluoro-2′-deoxyuridine (11a) was prepared from **11b** on a 6.0 mmol scale as described in **10a**: yield 93% (3.70 g); mp (dec) 83−85 °C; R_f 0.91 (EtOAc/hexane = 1:1); ^{1}H NMR ($CDCl_3$) δ 7.91 (1H, d, $J = 6.1$ Hz, C6H), 7.42 (2H, m, arom H), 7.29 (7H, m, arom H), 6.86 (4H, m, arom H), 6.29 (1H, t, $J = 6.0$ Hz, C1′H), 4.50 (1H, m, C3′H), 4.01 (1H, m, C4′H), 3.82 (6H, s, OCH_3), 3.40 (2H, m, $C5'H_2$), 2.20 (1H, m, C2′H), 2.07 (1H, m, C2′H), 0.88 (9H, s, $C(CH_3)_3$), 0.05 (6H, d, $J = 18$ Hz, $Si(CH_3)_2$).

3′-*O*-(*tert*-Butyldimethylsilyl)-5-fluoro-2′-deoxyuridine (11) was prepared from **11a** on a 1.8 mmol scale as described in **10**: yield 66% (428 mg); mp137−142 °C; R_{f1} 0.42 (CH_2Cl_2/MeOH = 95:5), R_{f2} 0.65 (EtOAC); ^{1}H NMR ($CDCl_3$) δ 8.83 (1H, br s, exch D_2O, NH), 7.98 (1H, d, $J = 6.4$ Hz, C6H), 6.25 (1H, m, C1′H), 4.49 (1H, m, C3′H), 3.98 (2H, m, C4′H and C5′H), 3.83 (1H, m, C5′H), 2.32, 2,23 (2H, 2m, C2′H), 0.92 (9H, s, $C(CH_3)_3$), 0.10 (6H, s, $Si(CH_3)_2$).

2-(*p*-Tolylthio)ethanol (12) was prepared as described previously[11] in 83% yield (13.9 g, 0.1 mol scale); R_f 0.34 (EtOAc/hexane = 1:4); ^{1}H NMR (DMSO) δ 7.26 (2H, m, arom H), 7.12 (2H, d, $J = 8.4$ Hz, arom H), 3.56 (2H, t, $J = 7.2$ Hz, CH_2O), 2.99 (2H, t, $J = 6.8$ Hz, SCH_2), 2.26 (3H, s, arom CH_3).

2-(*p*-Nitrophenylthio)ethanol (13) was prepared as described previously[11] in 51% yield (10 g, 0.1 mol scale); mp 60−60.5 °C; R_f 0.30 (EtOAc/hexane = 1:1); ^{1}H NMR (DMSO) δ 8.12 (2H, m, arom H), 7.51 (2H, m, arom H), 3.66 (2H, m, CH_2O), 3.21 (2H, t, $J = 6.4$ Hz, SCH_2).

2-(*p*-Tolylthio)-1-methylethanol (14) was prepared by adding sodium hydride (0.91 g, 22.7 mmol, 60% dispersion in mineral oil) to a stirring solution of thiocresol (2.83 g, 22.7 mmol) in anhydrous THF (25 mL) at ice-bath temperature. The resulting cloudy suspension was transferred to a dropping funnel and added dropwise to a solution of propylene oxide (1.32 g, 22.7 mmol) in anhydrous THF (25 mL) at ice-bath temperature with stirring. The reaction mixture was stirred for 1 h at this temperature and warmed to room temperature for another 1 h. The solvent was removed, and the residue was dissolved in 100 mL of ethyl acetate. The organic layer was washed with 100 mL of 0.1% hydrochloric acid and water (2 × 100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtrated, and evaporated in vacuo. The resulting residue was purified by flash chromatography to give the product as a pale yellow oil: yield 61% (2.52 g); R_f 0.77 (EtOAc/hexane = 1:4); ^{1}H NMR (DMSO) δ 7.24 (2H, m, arom H), 7.12 (2H, d, $J = 8.4$ Hz, arom H), 3.74 (1H, m, C1H), 2.97 (1H, dd, C2H), 2.83 (1H, dd, C2H), 2.23 (3H, s, arom CH_3), 1.15 (3H, d, $J = 6.0$ Hz, CH_3); ^{13}C NMR (100 MHz, DMSO) δ 21.3 (positive, CH_3), 23.3 (positive, arom CH_3), 42.6 (C2, negative), 66.1 (C1, positive), 129.5 (tert arom), 130.5 (tert arom), 134.0 (quat arom), 135.9 (quat arom); MS, 182, 138 (M − 44), 123, 91, 77, 65, and 45.

Diisopropylammonium tetrazolide (22) was prepared according to the published procedures.[17] To a stirred solution of tetrazole (0.28 g, 4 mmol) in anhydrous acetonitrile (10 mL) was added diisopropylamine (0.81 g, 8 mmol). A white precipitate was formed immediately, and the product was collected by filtration. The solid was washed with dry acetonitrile and dried in a vacuum overnight to yield quantitatively white crystalline product.

Bis[2-(*p*-tolylthio)ethyl] *N*,*N*-diisopropylphosphoramidite (15) was prepared according to the published procedure with modifications.[18,19] Bis(diisopropylamino)chlorophosphine (1.2 mmol) was added to a stirred solution of **12** (1.0 mmol) in anhydrous THF (5 mL) containing triethylamine (1.5 mmol) at room temperature under N_2. TLC analysis, after 20 min, indicated complete conversion of the thioethanol into a product with a higher R_f value. Removal of the $Et_3N{\cdot}HCl$ salt and concentration in vacuo afforded the desired intermediate as a viscous liquid. The residue was then dissolved in anhydrous CH_2Cl_2 (5 mL), compound **12** (0.8 mmol) together with **22** (0.4 mmol) was added at room temperature, and the mixture was stirred for 1 h. The resulting mixture was washed with cold saturated sodium bicarbonate solution (2 × 5 mL) and cold brine (5 mL). The organic layer was dried with anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified by preparative thin-layer chromatography with an appropriate solvent containing 1% triethylamine to obtain the product as an oily residue: yield 68% (315 mg); R_f 0.9 (EtOAc/hexane = 1:4 containing 1% triethylamine); ^{1}H NMR ($CDCl_3$) δ 7.34−7.28 (4H, m, arom H), 7.11 (4H, d, J = 8.0 Hz, arom H), 3.81−3.73 (4H, m, CH_2O), 3.58 (2H, m, NCH), 3.13−3.09 (4H, m, SCH_2), 2.34 (6H, s, arom CH_3), 1.16 (12H, d, J = 6.8 Hz, $NC(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ 147.90.

Bis[2-(*p*-nitrophenylthio)ethyl] *N*,*N*-diisopropylphosphoramidite (16) was prepared as described for **15** using **13** on a 1 mmol scale. The product was obtained as an oil: yield 60% (315 mg); R_f 0.73 (EtOAc/hexane = 1:1 containing 1% triethylamine); ^{1}H NMR ($CDCl_3$) δ 8.15−8.12 (4H, m, arom H), 7.40−7.31 (4H, m, arom H), 3.91−3.78 (4H, m, CH_2O), 3.60−3.52 (2H, m, NCH), 3.29−3.20 (4H, m, SCH_2), 1.19 (12H, d, J = 6.8 Hz, $NC(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ 149.16.

Bis[2-(*p*-tolylthio)-1-methylethyl] *N*,*N*-diisopropylphosphoramidite (17) was prepared as described for **15** using **14** on a 1 mmol scale. The product was obtained as an oil: yield 65% (320 mg); R_f 0.89 (EtOAc/hexane = 1:5 containing 1% triethylamine); ^{1}H NMR ($CDCl_3$) δ 7.28 (4H, m), 7.09 (4H,d, J = 7.6 Hz), 4.02−3.99 (2H, m), 3.59−3.56 (2H, m), 3.32−3.15 (2H, m), 2.90−2.83 (2H, m), 2.32 (6H, s), 1.14 (12H, d, J = 6.8 Hz); ^{31}P NMR δ 146.26, 145.63.

2′-Deoxy-3′-*tert*-butyldimethylsilyl-uridin-5′-yl-[2-(*p*-tolylthio)ethyl] *N*,*N*-diisopropylphosphoramidite (18) was prepared as described for **15** using 1 mmol of **12** and 0.8 mmol of **10**. The product was obtained as an oil: yield 51% (based on nucleoside 261 mg); R_f 0.27 (EtOAC/hexane = 1:2 containing 1% triethylamine); ^{1}H NMR ($CDCl_3$) δ 7.92 (1H, br m, NH, exch D_2O), 7.30 (1H, m, C6H), 7.28 (2H, m, arom H), 7.10 (2H, m, arom H), 6.10 (1H, m, C1′H), 5.69 (1H, d, J = 8.12 Hz, C5H), 4.50 (1H, m, C3′H), 4.16 (1H, m, C4′H), 3.91−3.70 (4H, m, C5′H_2, CH_2O), 3.58 (2H, m, NCH), 3.10 (2H, m, SCH_2), 2.30 (3H, s, arom CH_3), 2.36, 2.12, (2H, 2m, C2′H), 1.18 (12H, d, J = 7.0 Hz, $NC(CH_3)_2$), 0.90 (9H, s, $SiC(CH_3)_3$), 0.07 (6H, s, $Si(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ 149.30, 148.84.

5-Fluoro-2′-deoxy-3′-*tert*-butyldimethylsilyl-uridin-5′-yl-[2-(*p*-tolylthio)ethyl] *N*,*N*-diisopropylphosphoramidite (19) was prepared as described for **15** using 1 mmol of **12** and 0.8 mmol of **11**. The product was obtained as an oil: yield 50% (263 mg); R_f 0.30 (EtOAC/hexane = 1:2 containing 1% triethylamine); ^{1}H NMR ($CDCl_3$) δ 7.27 (3H, m, C6H, arom H), 7.09 (2H, m, arom H), 6.08 (1H, m, C1′H), 4.51 (1H, m, C3′H), 4.18 (1H, m, C4′H), 3.90−3.71 (4H, m, C5′H_2, CH_2O), 3.59 (2H, m, NCH), 3.12 (2H, m, SCH_2), 2.31 (3H, s, arom CH_3), 2.34, 2.12, (2H, 2m, C2′H_2), 1.17 (12H, d, J = 7.0 Hz, $NC(CH_3)_2$), 0.91 (9H, s, $SiC(CH_3)_3$), 0.09 (6H, d, J = 3.1 Hz, $Si(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ 147.90, 147.42.

3′-*tert*-Butyldimethylsilyl-2′-deoxyuridin-5′-yl bis[2-(*p*-tolylthio)ethyl] phosphate (1b) was prepared according to the published procedures with modifications.[17] 1*H*-Tetrazole (0.45 M in acetonitrile, 2.7 mL, 1.2 mmol) was added to a stirred solution of **10** (1 mmol) and **15** (1.2 mmol) in anhydrous acetonitrile (1 mL) at room temperature. After 1 h, the reaction mixture was cooled to −40 °C, and *tert*-butyl hydroperoxide (TBHP) (1.3 mmol) was added; the reaction mixture was then allowed to warm to room temperature over 1 h. The solvent was removed, and the residue was partitioned between CH_2Cl_2 and water. The organic layer was washed with saturated sodium bicarbonate (2 × 5 mL) and water and dried over anhydrous sodium sulfate. The solvent was evaporated to dryness under reduced pressure followed by preparative TLC purification to give the desired product: yield 58% (based on nucleoside, 419 mg); R_f 0.31 (CH_2Cl_2/MeOH = 95:5); ^{1}H NMR ($CDCl_3$) δ 7.55 (2H, d, J = 8.16 Hz, C6H) 7.31−7.28 (4H, m, arom H), 7.13−7.10 (4H,m, arom H), 6.27 (1H, t, J = 6.5 Hz, C1′H), 5.72 (1H, d, J = 8.2 Hz, C5H), 4.26 (1H, m, C3′H), 4.16 (4H, m, CH_2O), 4.08 (2H, m, C5′H_2), 3.11 (4H, m, SCH_2), 2.42 (1H, m, C2′H), 2.13 (1H, m, C2′H), 2.33 (6H, s, arom CH_3), 0.91 (9H, s, $C(CH_3)_3$), 0.08 (6H, s, $Si(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ 0.08.

5-Fluoro-3′-*tert*-butyldimethylsilyl-2′-deoxyuridin-5′-yl bis[2-(*p*-tolylthio)ethyl] phosphate (4b) was prepared as described for **1b** using 1.0 mmol of **15** and 0.8 mmol of **11**. The product was obtained as a viscous oil: yield 59% (350

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mg); R_f 0.35 (CH_2Cl_2/MeOH = 95:5); ^{1}H NMR ($CDCl_3$) δ 9.02 (1H, br s, NH), 7.72 (1H, d, J = 8.2 Hz, C6H), 7.30 (2H, m, arom H), 7.12 (2H, m, arom H), 6.27 (1H, m, C1′H), 4.43 (1H, m, C3′H), 4.26−4.02 (7H, 3m, $C5'H_2$, CH_2O, C4′H), 3.13 (2H, m, SCH_2), 2.33 (3H, s, arom CH_3), 2.34, 2.06 (2H, 2m, $C2'H_2$), 0.90 (9H, s, $SiC(CH_3)_3$), 0.1 (6H, s, $Si(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ 0.05.

5-Fluoro-3′-*tert*-butyldimethylsilyl-2′-deoxyuridin-5′-yl bis[2-(*p*-nitrophenylthio)ethyl] phosphate (7b) was prepared as described for **1b** using 1.0 mmol of **16** and 0.8 mmol of **11**. The product was obtained as a viscous oil: yield 60% (385 mg); R_f 0.33 (CH_2Cl_2/MeOH = 90:10); ^{1}H NMR ($CDCl_3$) δ 8.92 (1H, br s, NH), 8.16 (4H, m, arom H), 7.78 (1H, d, J = 8.18 Hz, C6H), 7.59 (4H, m, arom H), 6.18 (1H, m, C1′H), 4.41 (1H, m, C3′H), 4.37−4.31 (7H, 3m, CH_2O, $C5'H_2$, C4′H), 3.52 (4H, m, SCH_2), 2.43, 2.08 (2H, 2m, $C2'H_2$), 0.89 (9H, s, $SiC(CH_3)_3$), 0.1 (6H, s, $Si(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ 0.01.

5-Fluoro-3′-*tert*-butyldimethylsilyl-2′-deoxyuridin-5′-yl bis[2-(*p*-tolylthio)-1-methylethyl] phosphate (9b) was prepared as described for **1b** using 1.0 mmol of **17** and 0.8 mmol of **11**. The product was obtained as a viscous oil: yield 58% (356 mg); R_f 0.46 (CH_2Cl_2/MeOH = 95:5); ^{1}H NMR ($CDCl_3$) δ 8.62 (1H, br s, NH), 7.75 (1H, m, C6H), 7.30 (4H, m, arom H), 7.11 (4H, m, arom H), 6.29 (1H, m, C1′H), 4.66 (2H, m, $C5'H_2$), 4.50 (1H, m, C3′H), 4.22 (2H, m, CH_2O), 4.03 (1H, m, C4′H), 3.17, 2.96 (4H, 2m, SCH_2), 2.33 (3H, s, arom CH_3), 2.30, 2.08 (2H, 2m, $C2'H_2$), 0.9 (9H, s, $SiC(CH_3)_3$), 0.09 (6H, s, $Si(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ −1.33, −1.46, −1.48, −1.70.

***O,O*′-3′-*tert*-Butyldimethylsilyl-2′-deoxyuridin-5′-yl [2-(*p*-tolylthio)ethyl] H-phosphonate (20)** was prepared according to the published procedures.[18] To a solution of **18** (1 mmol) in acetonitrile (5 mL) were added 1*H*-tetrazole (35 mg, 0.5 mmol) and water (0.1 mL), and the mixture was stirred for 30 min at room temperature. The solvent was removed in vacuo and the residue was partitioned between CH_2Cl_2 (25 mL) and water (25 mL). The water layer was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuo and purified by preparative TLC to afford the product as an oil: yield 35% (194 mg); R_f 0.40 (CH_2Cl_2/MeOH = 95:5); ^{1}H NMR ($CDCl_3$) δ 9.89 (1H, br s, NH), 7.47 (1H, m, C6H), 7.24−7.21 (2H, m, arom H), 7.04 (2H, d, J = 7.9 Hz, arom H), 6.85 (1H, 2d, $^1J_{HP}$ = 717 Hz, PH), 6.19 (1H, m, C1′H), 5.66 (1H, d, J = 8.0 Hz, C5H), 4.38−3.94 (6H, 3m, $C5'H_2$, C3′H, CH_2O, C4′H), 3.07 (2H, m, SCH_2), 2.23 (3H, s, arom CH_3), 2.24, 2.06 (2H, 2m, $C2'H_2$), 0.81 (9H, s, $C(CH_3)_3$), 0.01 (6H, s, $Si(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ 10.15, 9.39.

***O,O*′-5-Fluoro-3′-*tert*-butyldimethylsilyl-2′-deoxyuridin-5′-yl [2-(*p*-tolylthio)ethyl] H-phosphonate (21)** was prepared as described for **20** using **19** on a 1 mmol scale. The product was obtained as an oil: yield 33% (189 mg); R_f 0.41 (CH_2Cl_2/MeOH = 95:5); ^{1}H NMR ($CDCl_3$) δ 7.67 (1H, m, C6H), 7.28 (2H, m, arom H), 7.08 (2H, d, J = 8.0 Hz, arom H), 6.94 (1H, 2d, $^1J_{HP}$ = 720 Hz, PH), 6.23 (1H, m, C1′H), 4.41, 4.25, 4.01 (6H, 3m (1:4:1), C3′H, $C5'H_2$, CH_2O, C4′H), 3.13 (2H, m, SCH_2), 2.29 (1H, m, C2′H), 2.27 (3H, s, arom CH_3), 2.02 (1H, m, C2′H), 0.86 (9H, s, $C(CH_3)_3$), 0.06 (6H, s, $Si(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ 10.11, 9.60.

***O,O*′-2′-Deoxy-3′-*tert*-butyldimethylsilyl-uridin-5′-yl [2-(*p*-tolylthio)ethyl] phosphoramidate (2b)** was synthesized according to the published procedures.[20,21] Compound **20** (0.5 mmol) was dissolved in CCl_4 (10 mL) and cooled at ice-bath temperature. The NH_3 gas was bubbled into the solution through a syringe, and the resulting reaction mixture was stirred for 2 h at the same temperature. Dichloromethane (10 mL) was added, and the organic layer was washed with 0.37% aqueous hydrochloric acid (20 mL) and brine (20 mL). The organic phase was dried by anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by preparative TLC gave the product: yield 22% (62 mg); R_f 0.34 (CH_2Cl_2/MeOH = 90:10); ^{1}H NMR (acetone-d_6) δ 10.27 (1H, br s, NH, exch D_2O), 7.81 (1H, m, C6H), 7.34 (2H, m, arom H), 7.16 (2H, m, arom H), 6.32 (1H, t, J = 5.7 Hz, C6H), 5.63 (1H, d, J = 7.2 Hz, C5H), 4.63 (1H, m, C5′H), 4.33 (2H, br s, NH_2, exch D_2O), 4.24 (2H, m), 4.18 (2H, m), 4.10 (1H, m), 3.23 (2H, t, J = 6.1 Hz, SCH_2), 2.31 (3H, s, arom CH_3), 2.25 (2H, m), 0.93 (9H, s, $C(CH_3)_3$), 0.15 (6H, s, $Si(CH_3)_2$); ^{31}P NMR (acetone-d_6) δ 12.27, 12.16.

***O,O*′-5-Fluoro-2′-deoxy-3′-*tert*-butyldimethylsilyl-uridin-5′-yl [2-(*p*-tolylthio)ethyl] phosphoramidate (5b)** was prepared as described for **2b** using **21** on a 0.5 mmol scale: yield 24% (70 mg); R_f 0.34 (CH_2Cl_2/MeOH = 90:10); ^{1}H NMR (acetone-d_6) δ 10.73 (1H, br s, NH, exch D_2O), 7.97 (1H, m, C6H), 7.33 (2H, m, arom H), 7.16 (2H, m, arom H), 6.30 (1H, t, J = 5.9 Hz, C1′H), 4.62 (1H, m, C3′H), 4.33 (2H, br s, NH_2, exch D_2O), 4.22, 4.20, 4.14 (5H, 4m, $C5'H_2$, C4′H, CH_2O, C3′H), 3.22 (2H, m, SCH_2), 2.30 (3H, s, arom CH_3), 2.27 (2H, m, C2′H), 0.92 (9H, s, $C(CH_3)_3$), 0.14 (6H, s, $Si(CH_3)_2$); ^{31}P NMR (acetone-d_6) δ 12.35, 12.19.

***O,O*′-2′-Deoxy-3′-*tert*-butyldimethylsilyl-uridin-5′-yl [2-(*p*-tolylthio)ethyl] *N,N*-bis(2-chloroethyl) phosphoramidate (3b)** was synthesized according to a published procedure with modifications.[22] A solution of 20% aqueous sodium hydroxide (0.2 mL) was added slowly at ice-bath temperature with stirring to a mixture of **20** (0.5 mmol), bis(2-chloroethyl)amine hydrochloride (116 mg, 0.65 mmol), tetrabromomethane (83 mg, 0.25 mmol), dichloromethane (2 mL), and benzyltriethylammonium chloride (40 mg, 0.18 mmol). After the addition had been completed, stirring was continued for 1 h at ice-bath temperature and then for another 1 h at room temperature. Dichloromethane (10 mL) was then added to the mixture; the organic layer was separated, washed with 5% aqueous hydrochloride (5 mL) and water (2 × 5 mL),

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and dried over anhydrous Na_2SO_4. Evaporation of solvent, followed by preparative TLC purification, afforded the product as a viscous oil: yield 7% (based on nucleoside, 24 mg); R_f 0.52 (CH_2Cl_2/MeOH = 9:1); ^1H NMR (acetone-d_6) δ 10.02 (1H, br s, NH, exch D_2O), 7.67 (1H, m, C6H), 7.32 (2H, m, arom H), 7.12 (2H, m, arom H), 6.23 (1H, m, C1′H), 5.64 (1H, m, C5H), 4.51 (1H,m, C5′H), 4.30 (3H, m, C5′H, CH_2O), 4.18 (2H, m, C4′H, C3′H), 3.64 (4H, m, CH_2Cl), 3.48 (4H, m, NCH_2), 3.20 (2H, m, SCH_2), 2.32 (3H, s, arom CH_3), 2.20 (2H, 2m, C2′H), 0.91 (9H, s, $C(CH_3)_3$), 0.13 (6H, s, $Si(CH_3)_2$); ^{31}P NMR (acetone-d_6) δ 10.47, 10.39.

***O,O*′-5-Fluoro-2′-deoxyuridin-5′-yl [2-(*p*-tolylthio)ethyl] *N,N*-bis(2-chloroethyl) phosphoramidate (6a)** was synthesized according to the published procedure with modifications.[7] Compound **12** (4 mmol) was dissolved in CH_2Cl_2 (10 mL) and cooled to −78 °C. Phosphorus trichloride (2 mL, 2.0 M in CH_2Cl_2) was added slowly followed by the dropwise addition of diisopropylethylamine (1 mL, 5.74 mmol). The reaction mixture was stirred at −78 °C for 15 min. Bis(2-chloroethyl)amine hydrochloride (4 mmol, 713.9 mg) was suspended in anhydrous CH_2Cl_2 and added to the reaction mixture. Diisopropylethylamine (2 mL, 11.5 mmol) was added dropwise, and the reaction mixture was warmed to −60 °C and stirred for 20 min. 5-Fluoro-2’-deoxyuridine (0.8 mmol) was coevaporated with anhydrous pyridine (4 × 30 mL), redissolved in pyridine (10 mL), and cooled to −45 °C. This mixture of nucleoside in pyridine was then titrated with the previous reaction mixture containing *N*,*N*-bis(2-chloroethyl)amine thioethyl chlorophosphine until nucleoside disappeared. The reaction mixture was oxidized by the dropwise addition of *tert*-butyl hydroperoxide (0.5 mL, 5.0−6.0 M in decane) at −45 °C and warmed to 0 °C over 30 min. Saturated aqueous NH_4Cl (50 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuo. Toluene (10 mL) was added and coevaporated several times (3 × 10 mL) to remove pyridine. The residue was then passed through a short plug of silica gel ($CHCl_3$/MeOH = 9:1) to remove any remaining amine hydrochloride salts and then purified by preparative TLC to afford desired product: yield 53% (based on the nucleoside, 303 mg); R_f 0.52 (CH_2Cl_2/MeOH = 9:1); ^1H NMR (acetone-d_6) δ 9.52 (1H, br s, NH, exch D_2O), 7.49 (1H, 2d, J = 8.0 Hz, C6H), 7.30 (2H, m, arom H), 7.14 (2H, m, arom H), 6.24 (1H, m, C1′H), 4.65 (1H, br s, OH), 4.52, 4.45 (2H, 2m, C5′H), 4.40−4.18 (4H, m, C4′H, C3′H, CH_2O), 3.66 (4H, m, CH_2Cl), 3.50 (4H, m, NCH_2), 3.16 (2H, m, SCH_2), 2.30 (3H, s, arom CH_3), 2.26, 2.18 (2H, 2m, C2′H); ^{31}P NMR (acetone-d_6) δ 10.47, 10.39.

***O,O*′-5-Fluoro-2′-deoxyuridin-5′-yl [2-(*p*-nitrophenylthio)ethyl] *N,N*-bis(2-chloroethyl) phosphoramidate (8a)** was prepared as described for **6a** using **13** on a 4 mmol scale: yield 47% (based on the nucleoside, 280 mg); R_f 0.33 (CH_2Cl_2/MeOH = 9:1); ^1H NMR (acetone-d_6) δ 8.12 (2H, m, arom H), 7.69 (1H, m, C6H), 7.56 (2H, m, arom H), 6.12 (1H, m, C1′H), 4.55 (1H, br s, OH), 4.45 (1H, m, C5′H), 4.34−4.28 (5H, m, C5′H, C4′H, C3′H, CH_2O), 3.51 (2H, m, SCH_2), 3.65 (4H, m, CH_2Cl), 3.40 (4H, m, NCH_2), 2.24 (2H, m, C2′H); ^{31}P NMR (acetone-d_6) δ 10.69, 10.55.

2′-Deoxyuridin-5′-yl bis[2-(*p*-tolylthio)ethyl] phosphate (1a) was prepared by adding tetra-*n*-butylammonium fluoride (0.15 mL, 0.15 mmol, 0.1 M in THF) at 0 °C to a stirred solution of **1b** (0.15 mmol) in THF (3 mL). The mixture was stirred for 1 h at 0 °C. The solvent was removed under reduced pressure, and the residue was purified by preparative TLC to give the product: yield 78% (71 mg); R_f 0.67 (CH_2Cl_2/MeOH = 9:1); ^1H NMR ($CDCl_3$) δ 9.48 (1H, br s, NH), 7.54 (1H, d, J = 8.2 Hz, C6H), 7.29 (4H, m, arom H), 7.11 (4H, m, arom H), 6.27 (1H, t, J = 6.5 Hz, C1′H), 5.72 (1H, J = 8.2 Hz, C5H), 4.47 (1H, m, C5′H), 4.26 (2H, m, C5′H, C4′H), 4.16 (4H, m, CH_2O), 4.07 (1H, m, C3′H), 3.11 (4H, m, SCH_2), 2.33 (3H, s, arom CH_3), 2.43, 2.18 (2H, 2m, C2′H); ^{31}P NMR ($CDCl_3$) δ −0.08.

5-Fluoro-2′-deoxyuridin-5′-yl bis[2-(*p*-tolylthio)ethyl] phosphate (4a) was prepared as described for **1a** using **4b** on a 0.15 mmol scale: yield 75% (70 mg); R_f 0.70 (CH_2Cl_2/MeOH = 9:1); ^1H NMR (acetone-d_6) δ 10.50 (1H, br s, NH), 7.87 (1H, d, J = 6.2 Hz, C6H), 7.34 (4H, m, arom H), 7.16 (4H, m, arom H), 6.31 (1H, t, J = 6.1 Hz, C1′H), 4.49 (1H, m, C5′H), 4.30 (2H, m, C5′H, C4′H), 4.18 (4H, m, CH_2O), 4.15 (1H, m, C3′H), 3.22 (4H, m, SCH_2), 2.30 (6H, s, arom CH_3), 2.32, 2.07 (2H, 2m, C2′H); ^{31}P NMR ($CDCl_3$) δ −0.03.

5-Fluoro-2′-deoxyuridin-5′-yl bis[2-(*p*-nitrophenylthio)ethyl] phosphate (7a) was prepared as described for **1a** using **7b** on a 0.15 mmol scale: yield 70% (72 mg); R_f 0.65 (CH_2Cl_2/MeOH = 9:1); ^1H NMR (acetone-d_6) δ 10.15 (1H, br s, NH), 8.21 (4H, m, arom H), 7.82 (1H, d, J = 6.2 Hz, C6H), 7.63 (4H, m, arom H), 6.2 (1H, t, J = 6.1 Hz, C1′H), 5.59 (1H, m, C5′H), 4.36 (2H, m, C5′H, C4′H), 4.33 (4H, m, CH_2O), 4.11 (1H, m, C3′H), 3.55 (4H, m, SCH_2),, 2.42, 2.08 (2H, 2m, C2′H); ^{31}P NMR ($CDCl_3$) δ −0.01.

5-Fluoro-2′-deoxyuridin-5′-yl bis[2-(*p*-tolylthio)-1-methylethyl] phosphate (9a) was prepared as described for **1a** using **9b** on a 0.15 mmol scale: yield 81% (79 mg); R_f 0.74 (CH_2Cl_2/MeOH = 9:1); ^1H NMR (acetone-d_6) δ 10.48 (1H, br s, NH), 7.87 (1H, m, C6H), 7.34 (4H, m, arom H), 7.14 (4H, m, arom H), 6.31 (1H, m, C1′H), 4.64 (2H, m, CHO), 4.50 (1H, m, C5′H), 4.22 (2H, m, C5′H, C4′H), 4.11 (1H, m, C3′H), 3.27, 3.08 (4H, 2m, SCH_2), 2.30 (6H, s, arom CH_3), 2.26 (2H, m, C2′H), 1.42 (6H, m, CH_3); ^{31}P NMR (acetone-d_6) δ −1.27, −1.31, −1.43, −1.55.

***O,O*′-2′-Deoxyuridin-5′-yl [2-(*p*-tolylthio)ethyl] phosphoramidate (2a)** was prepared as described for **1a** using **2b** on a 0.15 mmol scale: yield 83% (57 mg); R_f 0.34 (CH_2Cl_2/MeOH = 9:1); ^1H NMR (acetone-d_6) δ 10.17 (1H, br s, NH, exch D_2O), 7.81 (1H, d, J = 8.1 Hz, C6H), 7.33 (2H, m, arom H), 7.15 (2H, m, arom H), 6.32 (1H, m, C1′H), 5.64 (1H, d, J = 8.0 Hz, C5H), 4.52−4.45 (3H, br m, C5′H, C4′H), 4.21−4.10 (5H, 3m, CH_2O, C3′H, PNH_2), 3.23 (2H, t, SCH_2), 3.01 (1H, b, OH), 2.30 (3H, s, arom CH_3), 2.21 (2H, 2m, C2′H); ^{31}P NMR (acetone-d_6) δ 12.53, 12.36.

_O,O′_-2′-Deoxyuridin-5′-yl [2-(_p_-tolylthio)ethyl] _N,N_-bis(2-chloroethyl) phosphoramidate (3a) was prepared as described for **1a** using **3b** on a 0.15 mmol scale: yield 68% (59 mg); R_f 0.48 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (acetone-d_6) δ 10.17 (1H, br s, NH, exch D_2O), 7.92 (1H, 2d, J = 8.1 Hz, C6H), 7.34 (2H, m, arom H), 7.16 (2H, m, arom H), 6.33 (1H, m, C1′H), 5.62 (1H, m, C5H), 4.81 (1H, br s, OH), 4.50 (1H, m, C5′H), 4.33−4.12 (5H, m, C5′H, C4′H, C3′H, CH_2O), 3.65 (4H, m, CH_2Cl), 3.50 (4H, m, NCH_2), 3.24 (2H, m, SCH_2), 2.31 (3H, s, arom CH_3), 2.24 (2H, 2m, C2′H); ^{31}P NMR δ 10.61, 10.40.

_O,O′_-5-Fluoro-2′-deoxyuridin-5′-yl [2-(_p_-tolylthio)ethyl] phosphoramidate (5a) was prepared as described for **1a** using 5**b** on a 0.15 mmol scale: yield 53% (37 mg); R_f 0.34 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (acetone-d_6) δ 7.94 (1H, 2d, J = 8.0 Hz, C6H), 7.32 (2H, m, arom H), 7.15 (2H, m, arom H), 6.30 (1H, t, J = 6.5 Hz, C1′H), 4.52 (1H, m, C5′H), 4.40 (2H, m, C5′H, C4′H), 4.23 (2H, m, CH_2O), 4.11 (3H, m, C3′H, PNH_2), 3.22 (2H, m, SCH_2), 2.29 (3H, s, arom CH_3), 2.23 (2H, 2m, C2′H); ^{31}P NMR δ 12.55, 12.37.

2′-Deoxyuridin-5′-yl′ bis[2-(_p_-tolylsulfonyl)ethyl] phosphate (1) was prepared according to the procedure described previously.[23] An excess of aqueous H_2O_2 (30%, 2 mL) and 0.3 M ammonium molybdate (1.5 mL) were added to a stirring solution of **1a** (0.5 mmol) in acetone (10 mL). The reaction mixture was stirred at room temperature for 2 h and then evaporated in vacuo. The residue was partitioned between CH_2Cl_2 (20 mL) and water (20 mL), and the water layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined extract was washed with saturated brine (2 × 20 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated in vacuo, and the residue was purified by preparative TLC to afford the product: yield 77% (258 mg); R_f 0.65 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (acetone-d_6) δ 10.02 (1H, br s, NH), 7.85 (4H, m, arom H), 7.66 (1H, d, J = 8.0 Hz, C6H), 7.49 (4H, d, J = 8.0 Hz, arom H), 6.32 (1H, m, C1′H), 5.64 (1H, d, J = 8.0 Hz, C5H), 4.50 (1H, br, OH), 4.44 (1H, m, C3′H), 4.36, 4.25 (4H, m, CH_2O), 4.13 (2H, m, C5′H_2), 4.04 (1H, m, C4′H), 3.64 (4H, m, SO_2CH_2), 2.47 (6H, s, arom CH_3), 2.31, 2.22 (2H, 2m, C2′H_2); ^{31}P NMR (acetone-d_6) δ −0.90. Anal. ($C_{27}H_{33}N_2O_{12}S_2P$) C, H, N.

5-Fluoro-2′-deoxyuridin-5′-yl bis[2-(_p_-tolylsulfonyl)ethyl] phosphate (4) was prepared as described for **1** using **4a** on a 0.5 mmol scale: yield 84% (290 mg); R_f 0.50 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (acetone-d_6) δ 10.48 (1H, br s, NH), 7.83 (5H, m, arom H, C6H), 7.49 (4H, m, arom H), 6.34 (1H, m, C1′H), 4.47 (1H, m, C3′H), 4.36 (4H, m, CH_2O), 4.17 (2H, m, C5′H_2), 4.06 (1H, m, C4′H), 3.62 (4H, m, SO_2CH_2), 2.47 (6H, s, arom CH_3), 2.32, 2.22 (2H, 2m, C2′H); ^{31}P NMR (acetone-d_6) δ −0.79. Anal. ($C_{27}H_{32}FN_2O_{12}S_2P\cdot 2H_2O$) C, H, N, S.

5-Fluoro-2′-deoxyuridin-5′-yl bis[2-(_p_-tolylsulfonyl)-1-methylethyl] phosphate (9) was prepared as described for **1** using **9a** on a 0.5 mmol scale: yield 92% (330 mg); R_f 0.55 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (acetone-d_6) δ 7.87 (5H, m, arom H, C6H), 7.49 (4H, m, arom H), 6.32 (1H, m, C1′H), 4.91 (2H, m, CHO), 4.50 (1H, m, C3′H), 4.26 (2H, 2m, C5′H_2), 4.10 (1H, m, C4′H), 3.66, 3.50 (4H, 2m, SO_2CH_2), 2.47 (6H, s, arom CH_3), 2.07 (2H, m, C2′H_2), 1.47 (6H, m, CH_3); ^{31}P NMR (acetone-d_6) δ −2.16, −2.40, −2.95 (1:2:1). Anal. ($C_{29}H_{36}N_2O_{12}S_2PF\cdot{}^1/_2H_2O$) C, H, N, S, F.

_O,O′_-2′-Deoxyuridin-5′-yl [2-(_p_-tolylsulfonyl)ethyl] phosphoramidate (2) was prepared as described for **1** using **2a** on a 0.5 mmol scale: yield 78% (190 mg); R_f 0.35 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (acetone-d_6) δ 10.13 (1H, br s, NH, exch D_2O), 7.85 (2H, m, arom H), 7.78 (1H, m, C6H), 7.48 (2H, d, J = 8.0 Hz, arom H), 6.31 (1H, m, C1′H), 5.64 (1H, m, C5H), 4.80 (1H, s, OH), 4.51 (1H, m, C3′H), 4.33 (4H, m, CH_2O, PNH_2), 4.09−4.04 (3H, m b, C5′H_2, C4′H), 3.66 (2H, m, SO_2CH_2), 2.46 (3H, s, arom CH_3), 2.32, 2.21 (2H, 2m, C2′H_2); ^{31}P NMR (acetone-d_6) δ 12.15, 12.04. Anal. ($C_{18}H_{24}N_3O_9SP$) C, H, N, S.

_O,O′_-2′-Deoxyuridin-5′-yl [2-(_p_-tolylsulfonyl)ethyl] _N,N_-bis(2-chloroethyl) phosphoramidate (3) was prepared as described for **1** using **3a** on a 0.5 mmol scale: yield 89% (273 mg); R_f 0.44 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR ($CDCl_3$) δ 9.02 (1H, br s, NH, exch D_2O), 7.82 (2H, m, arom H), 7.59, 7.49 (1H, 2d, J = 8.0 Hz, C6H), 7.41 (2H, d, J = 8.1 Hz, arom H), 6.25 (1H, m, C1′H), 5.77 (1H, m, C5H), 4.39−4.61 (3H, m, C3′H, CH_2O), 4.35, 4.22 (2H, 2m, C5′H_2), 4.10 (1H, m, C4′H), 3.75 (1H, b m, OH, exch D_2O), 3.63 (4H, m, CH_2Cl), 3.50 (2H, m, SO_2CH_2), 3.44 (4H, m, NCH_2), 2.48 (3H, s, arom CH_3), 2.46, 2.26 (2H, 2m, C2′H_2); ^{31}P NMR ($CDCl_3$) δ 10.87, 10.79. Anal. ($C_{22}H_{30}N_3Cl_2O_9SP\cdot{}^1/_2H_2O$) C, H, N, S.

_O,O′_-5-Fluoro-2′-deoxyuridin-5′-yl [2-(_p_-tolylsulfonyl)ethyl] phosphoramidate (5) was prepared as described for **1** using **5a** on a 0.5 mmol scale: yield 56% (142 mg); R_f 0.36 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (acetone-d_6/D_2O) δ 10.61 (1H, br s, NH, exch D_2O), 7.92 (1H, 2d, J = 6.9 Hz, C6H), 7.87 (2H, m, arom H), 7.48 (2H, d, J = 8.0 Hz, arom H), 6.30 (1H, t, J = 6.6 Hz, C1′H), 4.82 (1H, br s, OH), 4.46 (1H, m, C3′H), 4.35 (3H, m, CH_2O), 4.32 (2H, m, PNH_2, exch D_2O), 4.12 (2H, m, C5′H_2), 4.06 (1H, m, C4′H), 3.64 (2H, m, SO_2CH_2), 2.46 (3H, s, arom CH_3), 2.38, 2.26 (2H, 2m, C2′H_2); ^{31}P NMR (acetone-d_6) δ 12.28, 12.09. Anal. ($C_{18}H_{23}FN_3O_9SP\cdot H_2O$) C, H, N, S, F.

_O,O′_-5-Fluoro-2′-deoxyuridin-5′-yl [2-(_p_-tolylsulfonyl)ethyl] _N,N_-bis(2-chloroethyl) phosphoramidate (6) was prepared as described for **1** using **6a** on a 0.5 mmol scale: yield 86% (272 mg); R_f 0.48 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR ($CDCl_3$) δ 9.84, 9.79 (1H, 2 br s, NH, exch D_2O), 7.8 (2H, m, arom H), 7.68 (1H, d, J = 8.0 Hz, C6H), 7.40 (2H, d, J = 8.0 Hz, arom H), 6.25 (1H, m, C1′H), 4.50, 4.44, 4.37, 4.29 (5H, 4m, C3′H, CH_2O, C5′H_2, C4′H), 4.10 (1H, m, C4′H), 3.66−3.60 (4H, m, CH_2Cl), 3.48 (2H, m, SO_2CH_2), 3.40 (4H, m, NCH_2), 2.47 (3H, s, arom CH_3), 2.43,

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(23) Hardy, P. M.; Rydon, H. N.; Thompson, R. C. The Use of β-Methylthioethyl Esters for the Protection of carboxy Groups in Peptide Synthesis: Removal through the β-Methylsulfonylethyl Ester. *Tetrahedron Lett.* **1968**, *21*, 2525−6.

2.22 (2H, m, C2′H); ^{31}P NMR (acetone-d_6) δ 10.86, 10.76. Anal. ($C_{22}H_{29}N_3Cl_2O_9SPF{\cdot}{}^1/_2H_2O$) C, H, N, S.

***O,O*′-5-Fluoro-2′-deoxyuridin-5′-yl [2-(*p*-nitrophenylsulfonyl)ethyl] *N,N*-bis(2-chloroethyl) phosphoramidate (8)** was prepared as described for **1** using **8a** on a 0.5 mmol scale: yield 71% (235 mg); R_f 0.33 (CH_2Cl_2/MeOH = 9:1); ^{1}H NMR (acetone-d_6) δ 10.51 (1H, br s, NH, exch D_2O), 8.54 (2H, m, arom H), 8.31 (2H, m, arom H), 7.93, 7.82 (1H, 2d, J = 8.0 Hz, C6H), 6.28 (1H, m, C1′H), 4.70 (1H, br s, OH), 4.48 (3H, m, CH_2O, C3′H), 4.20 (2H, m, C5′H_2), 4.08 (1H, m, C4′H), 3.89 (2H, m, SO_2CH_2), 3.67 (4H, m, CH_2Cl), 3.36 (4H, m, NCH_2), 2.30 (2H, m, C2′H_2); ^{31}P NMR (acetone-d_6) δ 10.83, 10.60. Anal. ($C_{21}H_{26}N_4Cl_2O_{11}SPF$) C, H, N, S.

Stability Studies. Stability Test of Compounds 1 and 4 in 0.1 M Phosphate Buffer at 37 °C at pH values of 5.8, 6.4, and 7.4. Each incubation mixture consisted of 0.5 mL of preincubated 0.1 M phosphate buffer and 1.5 mM (in DMSO) of compound **1** or **4**. Incubations were carried out for varying time intervals in a shaking water bath (Dubnoff metabolic shaking incubator, Precision Scientific) at 37 °C. Incubations were initiated by the addition of the drugs and terminated by the addition of 0.1 mL of 2.0 M acetate buffer (pH 5) and 0.4 mL of ethanol and immediate cooling in crushed ice. An aliquot of the resulting mixtures (15 uL) was spotted on silica gel TLC plates, developed, and quantitatively analyzed by using the Uniscan video densitometer. The results were expressed as the percentage of starting material remaining after each incubation period in comparison to the zero time incubation control sample. These results were plotted against the respective incubation times, and the half-lives were calculated from the rate constant of the decay equation.

Stability Test of Compounds 5, 6, 8, and 9 in 0.1 M Phosphate Buffer, pH 7.4 at 37 °C. Each incubation mixture consisted of 0.5 mL of preincubated 0.1 M pH 7.4 phosphate buffer and a 1.5−3 mM (in DMSO) concentration of the compound tested. The protocol used for the study was essentially the same as described in the procedure previously for compounds **1** and **4**.

Stability Test of Compounds 4−6, 8, and 9 in the Presence of Human Plasma. Lyophilized human plasma (Sigma Chemical Co., St. Louis, MO) was used for this study, and it was reconstituted immediately prior to the experiment. The protocol used for the study was essentially the same as in phosphate buffer except the following:

Each incubation mixture contained 0.4 mL of reconstituted solution of human plasma in 0.1 M phosphate buffer pH 7.4, instead of the buffer alone. After the termination of the incubation, the vials were cooled in crushed ice and centrifuged at high speed (3000g) for 20 min to precipitate the proteins. Subsequently, an aliquot of the resulting supernatant was spotted on TLC plates, developed, and analyzed as described above in the procedure for phosphate buffer.

In Vitro Growth Inhibition Assay. Chinese hamster lung fibroblast (V-79) cells were obtained from ATCC (Rockville, MD) and maintained in MEM (Gibco, Grand Island, NY) supplemented with 10% heat inactivated fetal bovine serum, Pen G sodium, streptomycin sulfate, amphotericin, essential amino acids, and vitamins at 37 °C in a humidified atmosphere with 5% CO_2 in air. Growth inhibition was determined using the sulforhodamine B (SRB) assay. Approximately 1×10^4 cells/200 μL of media were plated in 96 well plates and allowed to attach for 24 h. Drugs were dissolved in DMSO, and serial dilutions were carried out in media, such that the final concentration of DMSO was 1% or less. At the end of the incubation, cultures were treated with 50 μL of 50% cold trichloroacetic acid for 1 h at 4 °C followed by washing with water. The fixed cells were stained with 100 μL of 0.4% sulforhodamine B solution in 1% acetic acid for 30 min, washed with 1% acetic acid, and then dissolved in Tris buffer (10 mM). The absorbance at 570 nm was then determined on a plate reader. IC_{50} values were determined from plots of the percent of the control vs drug concentrations.

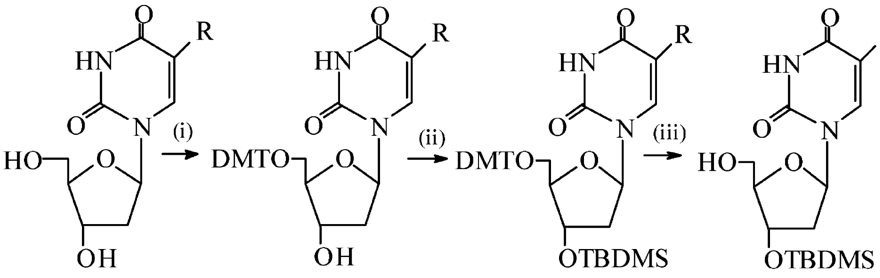
Thymidine-Reversed Growth Inhibition Assay. The protocol used for this study was essentially the same as in the growth inhibition assay for 48 h except that the medium contained 10 μM thymidine.

Results

Chemistry. The 3′-protected nucleosides **10** and **11** were prepared by treating 2′-deoxynucleosides with dimethoxytrityl chloride and *tert*-butyldimethylsilyl chloride followed by detritylation (Scheme 2). The 2-thioethanol starting materials were prepared by reaction of thiocresol or *p*-nitrothiophenol with 2-chloroethanol to produce **12** and **13**, respectively. Reaction of thiocresol with propylene oxide yielded **14** (Scheme 3).

The synthetic procedures used for sulfonyl phosphotriester and phosphoramidate compounds were based on P(III) chemistry. The phosphoramidite intermediates **15**−**19** were prepared via a two-step sequence involving phosphorylating of thioethanol followed by an in situ displacement of diisopropylamine with an appropriate thioethanol (**12**, **13**, or **14)** or 3′-protected nucleoside (**10** or **11)** (Scheme 4). Subsequent conversion of phosphoramidite intermediates (**15**, **16**, and **17)** to phosphotriesters was accomplished by treatment with the appropriate nucleoside and 1*H*-tetrazole followed by in situ oxidation with *tert*-butyl hydroperoxide to obtain **1b**, **4b**, **7b**, and **9b**. Reaction of phosphoramidites **18** and **19** with 1*H*-tetrazole in acetonitrile/H_2O produced H-phosphonate intermediates **20** and **21,** respectively. The H-phosphonate intermediates were then converted to phosphoramidate intermediates **2b**, **3b**, and **5b** by oxidative coupling with ammonium or bis(2-chloroethyl)amine hydrochloride. However, the coupling reaction of **20** with bis-(2-chloroethyl)amine hydrochloride resulted a very low yield of **3b** (<10%). To improve the yield, an alternative one-pot strategy was applied (Scheme 5). Phosphorus trichloride was reacted with the corresponding thioethanol in the presence of diisopropylethylamine followed by reaction with bis(2-chloroethyl)amine hydrochloride to generate a highly reactive

Scheme 2[a]



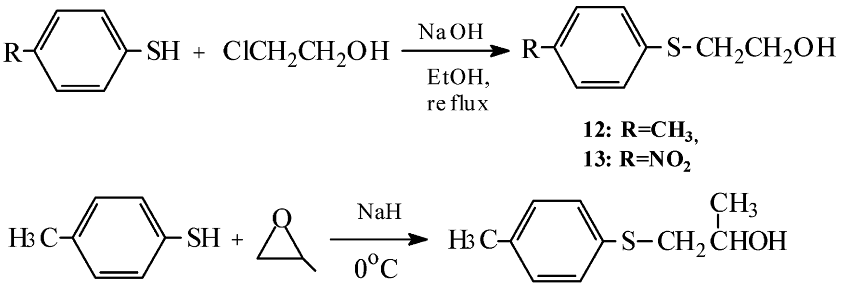
10 b: R = H
11 b: R = F

10 a: R = H
11 a: R = F

10: R = H
11: R=F

[a] Reagents and conditions: (i) 4,4′-dimethoxytrityl chloride (DMTCl), pyridine, rt; (ii) *tert*-butyldimethylsilyl chloride (TBDMSiCl), imidazole, DMF, overnight; (iii) 80% acetic acid, 20 min, rt.

Scheme 3



12: R=CH_3,
13: R=NO_2

14

monochloro intermediate, which was then reacted with the FdU in situ and oxidized by *tert*-butyl hydroperoxide to yield **6a** and **8a**. Intermediates **1a**−**5a**, **7a**, and **9a** were obtained by treatment of **1b**−**5b**, **7b**, and **9b** with tetra-*n*-butylammonium fluoride, respectively. The target sulfonyl compounds were prepared by oxidation of intermediates **1a**−**9a** with H_2O_2 in the presence of catalyst ammonium molybdate. However, compound **7** was not able to be obtained because it decomposed during the process of oxidation.

Phosphoramidate analogues were obtained as a mixture of diastereomers without further purification because they produced the identical nucleotides or phosphoramidate enantiomers after β-elimination activation. Certain compounds turned out to be very hygroscopic.

Stability Studies. The rates of β-elimination of the compounds tested were determined by measuring the rates of disappearance of the parent compounds with the use of quantitative TLC technique. Both compounds **1** and **4** underwent pH dependent β-elimination as shown in Table 1. The similar stability profiles of compounds **1** and **4** indicated that the influence of 5′-substituted pyrimidine on the rate of β-elimination was insignificant. Having established these observations, several target compounds were synthesized and examined for their stability in model physiological conditions and in the presence of human plasma. Results are presented in Table 2.

Our previous studies[11] have shown that the catalytic effect of human serum albumin (HSA) on the β-elimination rates of both Aldo and the sulfonylethyl analogues of PM are essentially equivalent. Consistent with these findings, the sulfonylethyl pronucleotides displayed a similar accelerated β-elimination profile. All of the tested compounds underwent faster decomposition in plasma than in phosphate buffer alone, and the enhancement of β-elimination was 1.4−3.5-fold (Table 2).

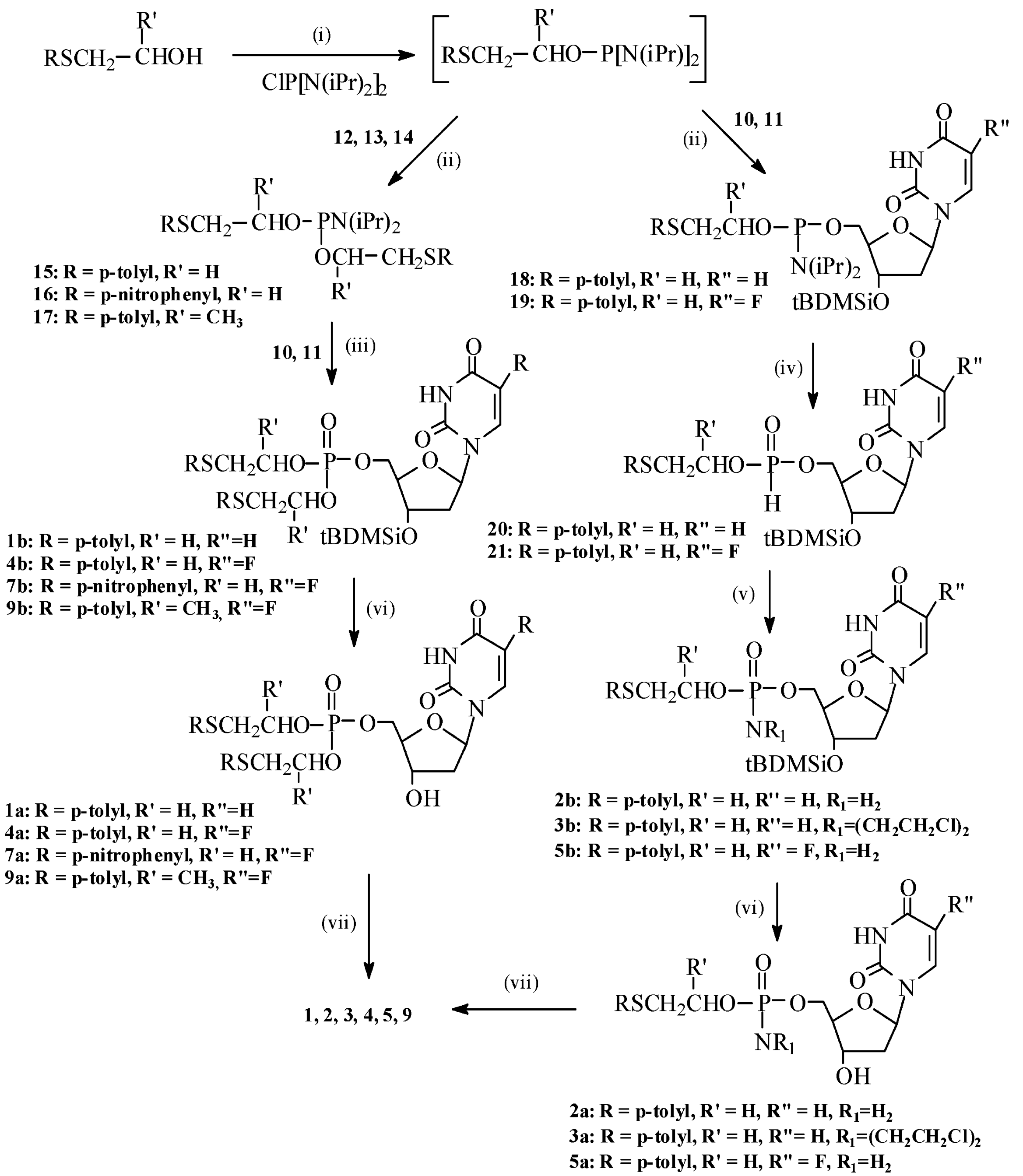
In Vitro Growth Inhibition Assay. Sulfonyl pronucleotides were evaluated for in vitro cytotoxicity against Chinese hamster lung fibroblasts (V-79 cells) using sulforhodamine B (SRB) assay under varying drug exposure times. Results are presented in Table 3. The model compounds (**1**, **2**, and **3**) and 5-FU were tested as negative and positive controls, respectively.

Thymidine-Reversed Growth Inhibition Assay. Addition of 10 μM of thymidine reversed the growth inhibition activities of only 5-FU and target compounds **5** and **6**, but had no effect on the model compounds (Figures1 and 2).

Discussion

Several sulfonylethyl-containing phosphotriester and phosphoramidate analogues of FdUMP have been synthesized and examined as novel anticancer nucleotide prodrugs. Consistent with our previous findings in sulfonyl-containing prodrugs of phosphoramide mustard,[11] the rate of β-elimination of sulfonyl pronucleotides was influenced by the substitution on the sulfonylethyl group and the types of leaving group. Compounds **5** and **6** exhibited greater stability than **4**, apparently due to the change of the nature of the leaving groups (e.g., phosphoramidic acid vs phosphoric acid) or the number of sulfonylethyl groups accessible for β-elimination. Moreover, compound **5** containing a P−NH_2 phosphorami-

Scheme 4[a]

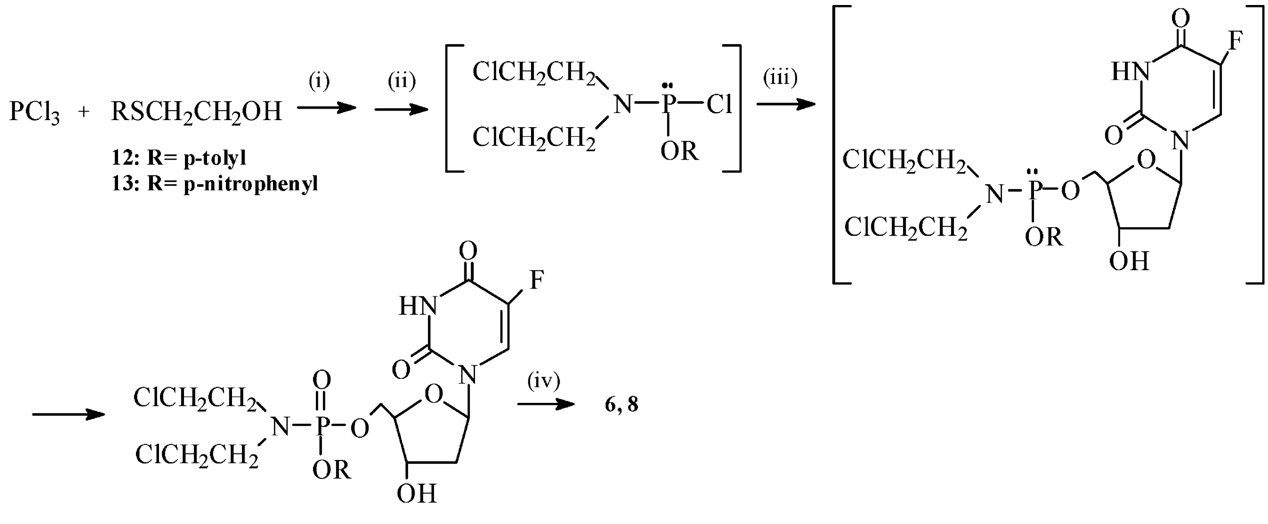


[a] Reagents and conditions: (i) triethylamine/THF, rt, 20 min; (ii) *N*,*N*′-diisopropylammonium tetrazolide/CH_2Cl_2, rt; (iii) 1*H*-tetrazole/acetonitrile, rt, 30 min followed by *tert*-butyl hydroperoxide at −40 °C over 30 min, then warm to rt for 1 h; (iv) 1*H*-tetrazole/acetonitrile, H_2O, rt, 30 min; (v) NH_3/CCl_4, 0 °C, 2 h; or 20% NaOH, bis(2-chloroethyl)amine hydrochloride, CH_2Cl_2/CCl_4, benzyltriethylammonium chloride, 0 °C; (vi) tetra-*n*-butylammonium fluoride/THF, 0 °C, 1b; (vii) H_2O_2/ammonium molybdate acetone, rt, 2 h.

date linkage showed a longer half-life (∼2×) than the corresponding bis(2-chloroethyl) phosphoramidate analogue **6**. The electron donating *p*-tolylsulfonyl substituent in compound **6** reduces the acidity of the α-proton, thereby decreasing the β-elimination rate as compared to electron-withdrawing *p*-nitrophenylsulfonyl in analogue **8** (5.73 h vs 0.33 h). The β-methyl substitution in compound **9** has dramatically prolonged its half-life over **4** (7.05 h vs 0.95 h), and this may be explained by the electron-inductive effects as well as the steric hindrance of β-methyl in **9**.

The in vitro growth inhibition potency of target compounds (**4**−**6**, **8**, and **9**) increased with longer treatment times and showed much greater potency than the model compounds (**1**, **2**, and **3**) as well as 5-FU after 48 h. These results demonstrated that the presence of FdUMP is essential for the potent cytotoxic activity. Moreover, addition of thymidine completely reversed the growth inhibition activities of the target compounds and 5-FU but had no effects on the model compounds. The data are consistent with thymidylate synthase as the target of these prodrugs.

Scheme 5[a]



12: R= p-tolyl
13: R= p-nitrophenyl

6a: R = 2-(p-tolylthio)ethyl
8a: R = 2-(p-nitrophenylthio)ethyl

[a] Reagents and conditions: (i) diisopropylethylamine, CH_2Cl_2 (1:3), −78 °C, 15 min; (ii) bis(2-chloroethyl)amine hydrochloride, diisopropylethylamine, CH_2Cl_2, −78 to −60 °C, 20 min; (iii) FdU, pyridine, −45 °C; followed by *tert*-butyl hydroperoxide, −45 to 0 °C, 30 min; (iv) H_2O_2/ammonium molybdate, rt, 1 h.

Table 1. pH Dependent Stability Profiles of Sulfonyl Pronucleotides **1** and **4** in 0.1 M Phosphate Buffer at 37 °C

	half-life (h)[a]		
compd	pH 7.4	pH 6.4	pH 5.8
1	0.95 ± 0.02	5.20 ± 0.55	23.28 ± 1.54
4	0.96 ± 0.03	5.28 ± 0.87	22.21 ± 2.17

[a] Mean ± SD from three experiments.

Table 2. Half-Lives of Sulfonyl Pronucleotides in 0.1 M Phosphate Buffer, pH 7.4 at 37 °C and in the Presence of Human Plasma

	half-life[a] (h)	
compd	buffer	human plasma[b]
4	0.96 ± 0.03	0.27 ± 0.02
5	12.23 ± 0.15	3.39 ± 0.68
6	5.73 ± 0.69	1.87 ± 0.42
8	0.33 ± 0.03	0.19 ± 0.02
9	7.05 ± 0.27	2.20 ± 0.56

[a] Mean ± SD from three experiments. [b] Human plasma reconstituted in 0.1 M phosphate buffer, pH 7.4.

Table 3. In Vitro Growth Inhibition of Sulfonyl Pronucleotides against Chinese Hamster Lung Fibroblast (V-79) Cells

	IC_{50} (nM)[a]			
compd	2 h	6 h	24 h	48 h
1	9140 ± 2480	5320 ± 240	3380 ± 460	2390 ± 456
2	57200 ± 1870	15200 ± 4230	9280 ± 3050	5000 ± 693
3	50800 ± 12100	9830 ± 4850	6970 ± 4440	3330 ± 951
4	9860 ± 6380	6130 ± 1200	765 ± 109	4.00 ± 1.44
5	32100 ± 1980	10400 ± 2230	4050 ± 880	389 ± 88
6	23700 ± 18700	15100 ± 4790	1610 ± 210	67 ± 16
8	2190 ± 67	3370 ± 654	1920 ± 476	209 ± 66
9	247000 ± 39500	88100 ± 9760	365 ± 176	1.50 ± 1.27
5-FU	208000 ± 64800	37200 ± 5660	3370 ± 1220	3040 ± 1200

[a] IC_{50}: The drug concentration required to reduce cell survival to 50% of controls using V-79 cells in the SRB assay; mean ± SD from three experiments. [b] Treatment time.

Although studies had indicated that the phosphate binding region of thymidylate synthase with FdUMP is unlikely to accommodate the alkylating moiety in the phosphoramidates of these compounds,[24] alkylation of some other cellular targets may contribute to the growth inhibition. The bifunctional analogue **6**, which was expected to form the alkylating aziridinium ion, showed much less cytotoxicity (~17×) than **4**. Presumably compound **4** was better able to liberate FdUMP than **6**, which required further P−N bond cleavage. These data suggested that the rate of liberation of FdUMP is critical for the activity of these compounds. Moreover, the alkylating model compound **3** was more potent as compared to the nonalkylating model compound **2**. This suggested that, in addition to the release of FdUMP, the bifunctional pronucleotides **6** and **8** may have a mechanism of action resulted from the alkylating moiety. Similarly, compound **6** showed greater cytotoxic activity than the corresponding nonalkylating compound **5**. However, the change of lipophilicity and susceptibility to P−N bond cleavage may also account for their different activities. As compare the *p*-nitrophenyl substituted compound **8** to *p*-tolyl analogue **6**, **6** was found to be more cytotoxic than **8** after

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(24) Matthews, D. A.; Appelt, K.; Oatley, S. J.; Xuong, N. H. Crystal Structure of Escherichia Coli Thymidylate Synthase Containing Bound 5-Fluoro-2′-Deoxyuridylate and 10-Propargyl-5,8-Dideazafolate. *J. Mol. Biol.* **1990**, *214*, 923−36.

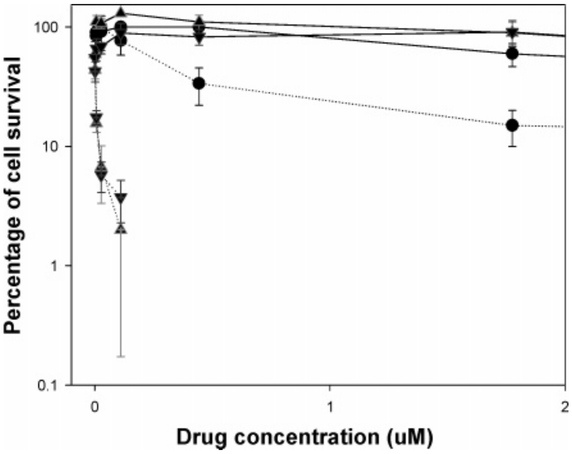

Figure 1. Thymidine-reversed growth inhibitory activity of 5-FU and compounds **5** and **6**. V-79 cells were maintained in the media containing 10 μM thymidine and treated with the compounds for 48 h: (●) 5-FU, (▲) (**5**), (▼) (**6**), (—) with thymidine, (- - -) without thymidine. Mean ± SD from three experiments.

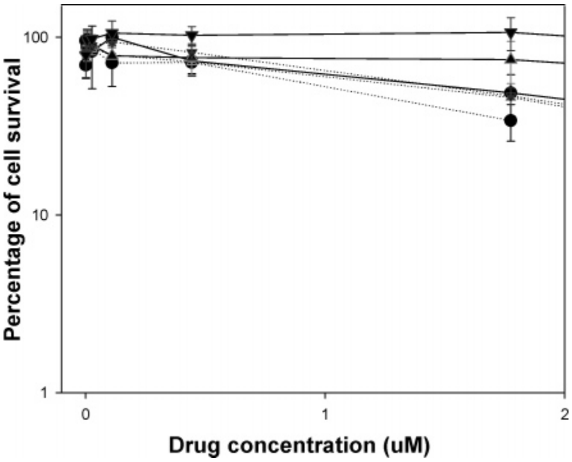

Figure 2. Absence of thymidine-reversed growth inhibitory activities of compounds **1**, **2**, and **3**. V-79 cells were maintained in the media containing 10 μM thymidine and treated with the compounds for 48 h: (●) (**1**), (▲) (**2**), (▼) (**3**), (—) with thymidine, (- - -) without thymidine. Mean ± SD from three experiments.

48 h treatment despite that the *p*-nitrophenyl substituted PM prodrug in our previous studies[11] had shown better activities both in vitro and in vivo than the corresponding *p*-tolyl substituted analogues.

Compound **9** was designed and synthesized to reduce the reactivity of the side product vinyl sulfone from *β*-elimination reaction. Although the systemic effects of the vinyl sulfone have not been evaluated, it was speculated that the electrophilic vinyl sulfone may contribute to the cytotoxicities of the model compounds as well as the acute cytotoxicity of the target compounds. Our results showed that compound **9** exhibited much less acute cytotoxicity (2 and 6 h) than **4**, presumably due to the less reactive methyl-substituted vinyl sulfone generated from **9** than the vinyl sulfone from **4**. However, compound **9** was more potent than **4** after 24 and 48 h treatments. Since compound **9** appeared to be more stable than **4**, it is speculated that compound **4** would be degraded more rapidly than compound **9** extracellularly. Furthermore, compound **9** should be more lipophilic than **4** due to the *β*-methyl substituent and therefore the cellular uptake might be increased as well.

Although extracellular generation of FdU or 5-FU from the prodrugs may occur, we have found that FdU did not exert any significant cytotoxicity in V-79 cells (data not shown) and 5-FU was less potent than all of the prodrugs, thereby suggesting that the contribution of cytotoxicity from extracellular 5-FU or FdU may be negligible. Further studies are warranted to clarify whether the higher potency of prodrugs than 5-FU is to what extent, if any, due to the intracellular generation of FdUMP or just simply more efficient delivery of 5-FU/FdU.

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Supporting Information Available: Table of elemental analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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