molecular pharmaceutics

articles

Synthesis and Biological Activity of *N*-2,3-Dihydroxypropyl-*N*-4-chlorobutyl Nucleoside Phosphoramidate Prodrugs

Weidong Wu[†] and Richard F. Borch*

Department of Medicinal Chemistry and Molecular Pharmacology and Cancer Center, Purdue University, West Lafayette, Indiana 47907

Received January 31, 2006

Abstract: *N*-2,3-Dihydroxypropyl-*N*-4-chlorobutyl phosphoramidate prodrugs of thymidine and 5-fluoro-2′-deoxyuridine (5-FdU) **2**−**4** were synthesized as analogues of the known prodrugs **1a**,**b** to assess the effects of the dihydroxypropyl moiety on prodrug activation, log *P*, and growth inhibitory activity in vitro. The thymidine analogues **2** and **3** were prepared as model compounds for kinetics and log *P* studies. ^{31}P NMR kinetics following hydrogenolysis of **2** showed that the thymidine *N*-dihydroxypropyl phosphoramidate released thymidine monophosphate with a half-life of 212 min under model physiologic conditions compared to ∼2 min for the corresponding *N*-methyl phosphoramidate reported previously. The measured log *P* for compound **3** was 1.1 log units lower than that of the analogous **1b**, confirming that the dihydroxypropyl group significantly decreased prodrug lipophilicity. The dihydroxypropyl prodrug **4** showed cell growth inhibition activity in the NCI 60 cell line panel similar to that of the *N*-methyl analogue **1a** previously reported.

Keywords: Prodrug; phosphoramidate; nucleoside

Introduction

There are a number of important molecules and drugs that have biological activity as the free phosphates; however, the charged nature of these compounds makes intracellular delivery inefficient. We[1−4] and others[5] have developed strategies to circumvent this problem that involve the design of neutral phosphoramidate prodrugs capable of passing through the cell membrane. Once inside the cell, our phosphoramidate prodrugs (**A** in Scheme 1, R_1 = Me, R_2 = 5-nitrofurfuryl) undergo intracellular activation (reduction) to generate an unstable phosphoramidate anion intermediate **B**, which in turn undergoes spontaneous cyclization and P−N bond cleavage by water to liberate the active free phosphate **D**. Application of this prodrug strategy to deliver nucleoside monophosphate intracellularly has resulted in the synthesis of *N*-methyl-*N*-4-chlorobutyl phosphoramidate drugs of 5-FdU (**1a**) and cytarabine (ara-C) with demonstrated biological activities.[3,4] However, introduction of the prodrug moieties increases significantly the lipophilicity of the molecule compared to the parent nucleoside. In the case of compounds more lipophilic than nucleosides, this increased

* To whom correspondence should be addressed. Mailing address: Purdue University, 201 South University St., West Lafayette, IN 47907-2064. Phone: 765-494-1403. Fax: 765-494-1414. E-mail: rickb@pharmacy.purdue.edu.

† Current address: LaserGen, Inc., 8052 El Rio St., Houston, TX 77054.

(1) Freel Meyers, C. L.; Hong, L.; Joswig, C.; Borch, R. F. Synthesis and biological activity of novel 5-fluoro-2′-deoxyuridine phosphoramidate prodrugs. *J. Med. Chem.* **2000**, *43*, 4313−8.

(2) Freel Meyers, C. L.; Borch, R. F. Activation mechanisms of nucleoside phosphoramidate prodrugs. *J. Med. Chem.* **2000**, *43*, 4319−27.

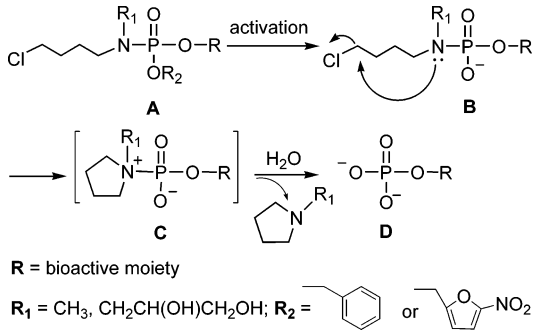
(3) Tobias, S. C.; Borch, R. F. Synthesis and biological studies of novel nucleoside phosphoramidate prodrugs. *J. Med. Chem.* **2001**, *44*, 4475−80.

(4) Tobias, S. C.; Borch, R. F. Synthesis and Biological Evaluation of a Cytarabine Phosphoramidate Prodrug. *Mol. Pharm.* **2004**, *1*, 112−6.

(5) For an excellent review, see: Wagner, C. R.; Iyer, V. V.; McIntee, E. J. Pronucleotides: Toward the in Vivo Delivery of Antiviral and Anticancer Nucleotides. *Med. Res. Rev.* **2000**, *20*, 417−51.

10.1021/mp060006g CCC: $33.50
Published on Web 05/12/2006

Scheme 1 . Activation Mechanism of the Phosphoramidate Prodrug



lipophilicity can have an adverse effect on drug properties. Thus we were interested in exploring the effects of hydrophilic modification of the phosphoramidate structure. An advantage of this system is that the phosphoramidate substituents on the prodrug can be modified without affecting the structure of the phosphate delivered. Therefore we designed model nucleoside phosphoramidate compounds bearing a hydrophilic side chain, the *N*-2,3-dihydroxypropyl-*N*-4-chlorobutyl phosphoramidates of thymidine and 5-FdU **2**−**4** (Figure 1). Herein we report the synthesis of compounds

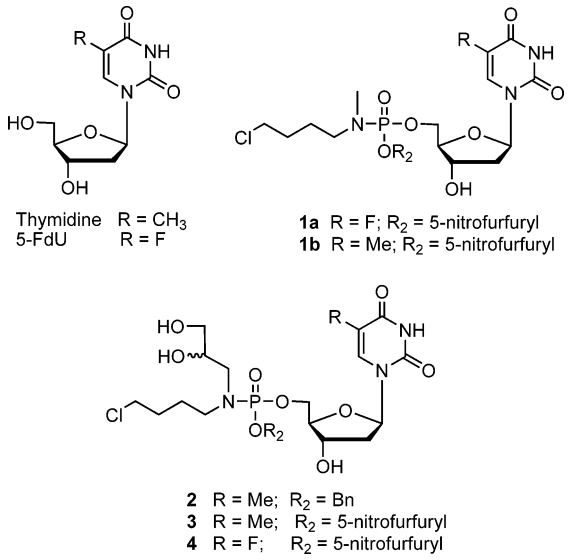


Figure 1. Structures of thymidine, 5-FdU, and their corresponding phosphoramidate prodrugs.

2−**4**, the activation kinetics and log *P* values of model compounds **1b**, **2**, and **3**, and the growth inhibitory activity of 5-FdU prodrug **4**.

Experimental Section

Materials and Methods. NMR spectra were recorded on a Bruker DPX 300 spectrometer equipped with a 5 mm multinuclear probe, and ^{1}H NMR (300 MHz) spectra are referenced to the residual solvent peak. ^{31}P NMR (121 MHz) spectra were acquired using broadband gated decoupling and are referenced using 1% triphenylphosphine oxide in benzene-d_6 as the coaxial reference (triphenylphosphine oxide/benzene-d_6 has a chemical shift of +25.17 ppm relative to 85% phosphoric acid). Mass spectra were obtained from the mass spectrometry laboratory at Purdue University. Analytical TLC on silica gel was performed on polyester plates coated with silica gel 60 F_{254} and were visualized by UV light or using one of the following stains: (i) 5% phosphomolybdic acid in ethanol or (ii) 1% 4-(*p*-nitrobenzyl)pyridine (NBP) in acetone followed by heating and treatment with 3% KOH in methanol. Flash silica gel chromatography was performed on silica gel 60 (230−400 mesh). All anhydrous reactions were carried out under argon using oven-dried flasks. Anhydrous solvents were either distilled from appropriate drying agents or obtained from commercial sources. Thymidine and 5-fluorodeoxyuridine were dried overnight under vacuum over P_2O_5 and coevaporated with anhydrous pyridine prior to use. All other chemical reagents were obtained from commercial sources.

^{31}P NMR Study. Kinetic experiments were carried out as described previously with slight modification.[2,3] Briefly, compound **2** (30 mg) was dissolved in anhydrous DMF (0.2 mL), Pd/Al_2O_3 (5 wt %, 10 mg) was added, and the mixture was stirred under 1 atm of H_2 at room temperature for 30 min. After the hydrogenolysis was complete as indicated by TLC, the reaction mixture was filtered though a 0.45 μm syringe filter to remove the catalyst. Then a portion of the filtrate (80 μL) was mixed with cacodylate buffer (500 μL, 0.4 M, pH 7.4) which was prewarmed to 37 °C. 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 30 min for 14 h. The integration of the peak areas was used to determine the relative concentration of the reactant and product. The relative concentration of the starting nucleoside phosphoramidate anion is represented as a percentage of total area at each time point, and the data was analyzed by linear regression of the log concentration values at each time point.

log *P* Determination. A 1−2 mg sample of thymidine prodrug **1b** or **3** was dissolved in octanol (400 μL), water (400 μL) was added, and the mixture was agitated vigorously for 2 min. The mixture was poured into a sealed disposable pipet and allowed to stand for 15 min. The tip of the pipet was removed, and samples of each layer were collected separately and analyzed by reverse phase HPLC. The concentration ratios were determined by measuring the peak areas from the octanol and water layers, respectively. The values reported represent the mean ± SD for three separate determinations.

NCI Human Tumor Cell Line Screen. Details of the methodology are described at http://dtp.nci.nih.gov/branches/btb/ivclsp.html (accessed April 7, 2006). Briefly, cells are grown in supplemented RPMI 1640 medium for 24 h and then incubated with drug for 48 h at five concentrations from 10^{-8} to 10^{-4} M. The assay is terminated by addition of cold trichloroacetic acid, and the cells are fixed and stained with sulforhodamine B. Bound stain is solubilized, and the absorbance is read on an automated plate reader. Percentage growth inhibition is calculated from time zero, control

growth, and the five concentration level absorbances. The inhibitory concentrations reported represent the average of two independent experiments.

***N*-Allyl-*N*-4-chlorobutylamine Hydrochloride (6).** Anhydrous HCl(g) was bubbled through a solution of 4-allylamino-1-butanol (**5**)[6] (3.57 g, 27.6 mmol) in anhydrous CH_2Cl_2 (50 mL) at 0 °C until the mixture turned litmus paper red, and then thionyl chloride (4 mL, 54.8 mmol) was added dropwise. The reaction mixture was warmed to room temperature and stirred overnight. The mixture was concentrated in vacuo, and the residue was coevaporated with CH_2Cl_2 (3 × 50 mL) to remove excess thionyl chloride. The amine hydrochloride **6** (4.13 g, 81%) was obtained as a brown solid and was used without further purification. ^{1}H NMR ($CDCl_3$): δ 9.61 (bs, 1H), 6.00 (m, 1H), 5.44 (m, 2H), 3.53 (m, 4H), 2.89 (m, 2H), 1.90 (m, 4H). MS (ESI): 148/150 $[M + H]^+$ for free amine.

***N*-Allyl-*N*-4-chlorobutyl Phosphoramidic Dichloride (7).** Triethylamine (2.12 mL, 15.2 mmol) was added dropwise to a stirred solution of amine hydrochloride **6** (1.40 g, 7.6 mmol) and $POCl_3$ (0.7 mL, 7.6 mmol) in anhydrous CH_2Cl_2 (40 mL) at 0 °C under argon. The mixture was warmed to room temperature and stirred overnight. Saturated NH_4Cl solution was added, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layer was dried over Na_2SO_4 and concentrated; the residue was purified by silica gel chromatography (hexane/ethyl acetate 9:1) to give phosphoramidic dichloride **7** as an oil (1.68 g, 84%). ^{1}H NMR ($CDCl_3$): δ 5.77 (m, 1H), 5.29 (m, 2H), 3.81 (m, 2H), 3.54 (m, 2H), 3.26 (m, 2H), 1.74 (m, 4H). ^{31}P NMR ($CDCl_3$): δ −7.78.

5′-Thymidyl 1-Benzotriazolyloxy *N*-Allyl-*N*-4-chlorobutyl Phosphoramidate (9). *N*-Allyl-*N*-4-chlorobutyl phosphoramidic dichloride **7** (530 mg, 2.0 mmol) was dissolved in anhydrous THF (2 mL) and added dropwise to a stirred solution of 1-hydroxybenzotriazole (540 mg, 4.0 mmol) and pyridine (0.32 mL, 4.0 mmol) in anhydrous THF (5 mL) at room temperature under argon. The reaction mixture was stirred for 4 h; at this time ^{31}P NMR indicated that phosphoramidic bis(1-benzotriazolyl) ester **8** was formed. The mixture was centrifuged at 10 000 rpm for 10 min to separate pyridine hydrochloride, and the supernatant was added to a stirred solution of thymidine (242 mg, 1.0 mmol) in anhydrous pyridine (5 mL) at room temperature. *N*-Methylimidazole (0.16 mL, 2.0 mmol) was added to the above solution, and the reaction mixture was stirred for 4 h at room temperature under argon. Solvent was removed in vacuo, and the residue was dissolved in CH_2Cl_2 (20 mL), washed with saturated NH_4Cl solution (10 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/ethyl acetate/ethanol 10:10:1) to give **9** (225 mg, 40%, mixture of diastereomers at phosphorus as indicated by ^{1}H and ^{31}P NMR) as a white foam. ^{1}H NMR ($CDCl_3$) for diastereomers: δ 9.01 and 8.93 (2s, 1H, 1:1 mixture), 7.99−7.39 (m, 5 H), 6.28 (m, 1H), 5.70 (m, 1H), 5.25 (m, 2H), 4.41 (m, 2H), 4.12−3.70 (m, 3H), 3.52 (m, 4H), 3.22 (m, 2H), 2.40−2.00 (m, 2H), 1.90 and 1.77 (2s, 3H, 1:1 mixture), 1.70 (m, 4H). ^{31}P NMR ($CDCl_3$): δ −12.88 and −13.08 (1:1 mixture). MS (ESI): 569/571 $[M + H]^+$.

5′-(5-Fluoro-2′-deoxyuridyl) 1-Benzotriazolyloxy *N*-Allyl-*N*-4-chlorobutyl Phosphoramidate (10). Following the same procedure as described for the synthesis of **9,** phosphoramidic bis(1-benzotriazolyl) ester **8**, generated by the reaction of *N*-allyl-*N*-4-chlorobutyl phosphoramidic dichloride **7** (420 mg, 1.6 mmol) with 1-hydroxybenzotriazole (430 mg, 3.2 mmol) and pyridine (0.26 mL, 3.2 mmol) in anhydrous THF (6 mL) at room temperature for 4 h under argon, was added to a solution of 5-fluoro-2′-deoxyuridine (100 mg, 0.4 mmol) and *N*-methylimidazole (0.13 mL, 1.6 mmol) in anhydrous pyridine (3 mL), and the reaction mixture was stirred for 20 h at room temperature under argon. Solvent was removed in vacuo, and the residue was coevaporated with toluene (5 mL × 2) to remove trace pyridine. Then the residue was purified by silica gel chromatography (hexane/ethyl acetate/ethanol 20:20:1 to 10:10:1) to give **10** (86 mg, 38%, mixture of diastereomers at phosphorus as indicated by ^{1}H and ^{31}P NMR) as a white foam along with recovered 5-fluoro-2′-deoxyuridine (50 mg, 50%). ^{1}H NMR ($CDCl_3$) for diastereomers: δ 9.23 (b, 1H), 8.00−7.38 (m, 5 H), 6.10 (m, 1H), 5.74 (m, 1H), 5.23 (m, 2H), 4.44 (m, 2H), 4.14−3.74 (m, 3H), 3.51 (m, 4H), 3.18 (m, 2H), 2.40 (m, 1H), 2.18 (m, 1H), 1.69 (m, 4H). ^{31}P NMR ($CDCl_3$): δ −12.65 and −13.08 (1:1 mixture). MS (ESI): 573/575 $[M + H]^+$.

5′-Thymidyl Benzyl *N*-Allyl-*N*-4-chlorobutyl Phosphoramidate (11). A mixture of benzotriazolyloxy phosphoramidate **9** (350 mg, 0.62 mmol) and DMAP (305 mg, 2.5 mmol) in benzyl alcohol (5 mL, 48 mmol) was stirred overnight at room temperature under argon. The mixture was concentrated in vacuo, and the residue was purified by silica gel chromatography (methylene chloride/methanol, 20:1) to give **11** (272 mg, 81%, mixture of diastereomers at phosphorus as indicated by ^{1}H and ^{31}P NMR) as a white foam. ^{1}H NMR ($CDCl_3$) for diastereomers: δ 9.02 and 8.96 (2s, 1H, 1:1 mixture), 7.34 (m, 6H), 6.25 (m, 1H), 5.66 (m, 1H), 5.20 (m, 2H), 4.99 (m, 2H), 4.45 (m, 1H), 4.15 (m, 2H), 4.00 (m, 1H), 3.61−3.46 (m, 4H), 3.02 (m, 2H), 2.37 (m, 1H), 2.05 (m, 1H), 1.85 and 1.78 (2s, 3H, 1;1 mixture), 1.65 (m, 4H). ^{31}P NMR ($CDCl_3$): δ −14.21 (b). HRMS (ESI): calcd for $C_{24}H_{33}ClN_3NaO_7P$ 564.1642 $[M + Na]^+$, found 564.1647.

5′-Thymidyl 5-Nitrofurfuryl *N*-Allyl-*N*-4-chlorobutyl Phosphoramidate (12). A mixture of benzotriazolyloxy phosphoramidate **9** (570 mg, 1.0 mmol), 5-nitrofurfuryl alcohol (3.58 g, 25 mmol), and DMAP (488 mg, 4.0 mmol) in THF (1 mL) was stirred for 24 h at room temperature under argon. The mixture was concentrated in vacuo, and the residue was purified by silica gel chromatography (methylene chloride/methanol, 20:1) to give **12** (340 mg,

(6) Crich, D.; Ranganathan, K.; Huang, X. Generation and reaction of alkene radical cations under nonoxidizing conditions: synthesis of the pyrrolizidine nucleus. *Org. Lett.* **2001**, *3*, 1917−9.

59%, mixture of diastereomers at phosphorus as indicated by ^{1}H and ^{31}P NMR) as a brown foam. ^{1}H NMR ($CDCl_3$) for diastereomers: δ 9.15 (bs, 1H), 7.26 (d, 1H), 7.22 (m, 1H), 6.66 (d, 1H), 6.21 (m, 1H), 5.68 (m, 1H), 5.22 (m, 2H), 5.00 (m, 2H), 4.48 (m, 1H), 4.19 (m, 2H), 4.04 (m, 1H), 3.61 (m, 2H), 3.51 (m, 2H), 3.04 (m, 2H), 2.40 (m, 1H), 2.22 (m, 1H), 1.88 and 1.83 (2s, 3H, 1:1 mixture), 1.67 (m, 4H). ^{31}P NMR ($CDCl_3$): δ −14.01 (b). HRMS (ESI): calcd for $C_{22}H_{31}ClN_4O_{10}P$ 577.1466 $[M + H]^+$, found 577.1460.

5′-(5-Fluoro-2′-deoxyuridyl) 5-Nitrofurfuryl *N*-Allyl-*N*-4-chlorobutyl Phosphoramidate (13). A mixture of benzotriazolyloxy phosphoramidate **10** (280 mg, 0.49 mmol), 5-nitrofurfuryl alcohol (3.50 g, 24.4 mmol), and DMAP (240 mg, 2.0 mmol) in THF (0.5 mL) was stirred overnight at room temperature under argon. The mixture was concentrated in vacuo, and the residue was purified by silica gel chromatography (methylene chloride/methanol, 20:1) to give **13** (150 mg, 53%, mixture of diastereomers at phosphorus as indicated by ^{1}H and ^{31}P NMR) as a yellow foam. ^{1}H NMR ($CDCl_3$) for diastereomers: δ 9.51 and 9.47 (2s, 1H, 1:1 mixture), 7.74 and 7.68 (2d, 1H, 1:1 mixture), 7.26 (m, 1H), 6.67 (m, 1H), 6.18 (m, 1H), 5.76 (m, 1H), 5.21 (m, 2H), 5.04 (m, 2H), 4.50 (m, 1H), 4.22 (m, 2H), 4.04 (m, 1H), 3.62 (m, 2H), 3.51 (m, 2H), 3.04 (m, 2H), 2.19 (m, 2H), 1.69 (m, 4H). ^{31}P NMR ($CDCl_3$, 121 MHz: δ −14.11 and −14.32 (1:1 mixture). HRMS (ESI): calcd for $C_{21}H_{27}ClFN_4NaO_{10}P$ 603.1035 $[M + Na]^+$, found 603.1037.

5′-Thymidyl Benzyl *N*-2,3-Dihydroxypropyl-*N*-4-chlorobutyl Phosphoramidate (2). *N*-Allyl-*N*-4-chlorobutyl phosphoramidate **11** (250 mg, 0.46 mmol) and 4-methylmorpholine *N*-oxide (108 mg, 0.92 mmol) were dissolved in a mixture of THF/*t*-BuOH/H_2O (10 mL/4 mL/2 mL), to this solution was added an OsO_4/*t*-BuOH solution (2.5 wt %, 500 mg, 0.05 mmol), and the mixture was stirred at room temperature. After 1 day, additional portions of 4-methylmorpholine *N*-oxide (54 mg, 0.46 mmol) and OsO_4/*t*-BuOH solution (2.5 wt %, 250 mg, 0.025 mmol) were added and the mixture was stirred for an additional 2 days. To the mixture was added 5% $NaHSO_3$ (5 mL) solution to quench the reaction, and then the organic layer was separated and concentrated to an oily residue, which was purified by silica gel chromatography (methylene chloride/methanol 10:1) to give **2** (155 mg, 59%, mixture of diastereomers at both phosphorus and carbon as indicated by ^{1}H and ^{31}P NMR) as a white foam. ^{1}H NMR (DMSO-d_6) for diastereomers: δ 11.29 (bs, 1H, D_2O exchangeable), 7.47 (bs, 1H), 7.34 (m, 5H), 6.18 (m, 1H), 5.38 (m, 1H, D_2O exchangeable), 4.96 (m, 2H), 4.68 (m, 1H, D_2O exchangeable), 4.55 (m, 1H, D_2O exchangeable), 4.20 (m, 1H), 4.06 (m, 2H), 3.92 (m, 1H), 3.58 (m, 3H), 3.30 (m, 2H), 3.10−2.82 (m, 4H), 2.07 (m, 2H), 1.70 (bs, 3H), 1.60 (m, 4H). ^{31}P NMR (MeOH-d_4): δ −13.26 (m). HRMS (ESI): calcd for $C_{24}H_{35}ClN_3NaO_9P$ 598.1697 $[M + Na]^+$, found 598.1702.

5′-Thymidyl 5-Nitrofurfuryl *N*-2,3-Dihydroxypropyl-*N*-4-chlorobutyl Phosphoramidate (3). Following the same procedure as described for the synthesis of **2**, *N*-allyl-*N*-4-chlorobutyl phosphoramidate **12** (270 mg, 0.47 mmol) was reacted with 4-methylmorpholine *N*-oxide (165 mg, 1.41 mmol) and OsO_4/*t*-BuOH solution (2.5 wt %, 360 mg, 0.036 mmol) in a mixture of THF/*t*-BuOH/H_2O (4 mL/1.6 mL/0.8 mL) at room temperature for 3 days. Workup and purification by silica gel chromatography (methylene chloride/methanol 10:1 to 5:1) gave **3** (123 mg, 43%, mixture of diastereomers at both phosphorus and carbon as indicated by ^{1}H and ^{31}P NMR) as a light-yellow foam. ^{1}H NMR (DMSO-d_6, 300 MHz) for diastereomers: δ 11.29 (bs, 1H, D_2O exchangeable), 7.66 (d, 1H), 7.46 (bs, 1H), 6.92 (d, 1H), 6.18 (m, 1H), 5.39 (m, 1H, D_2O exchangeable), 5.05 (m, 2H), 4.70 (m, 1H, D_2O exchangeable), 4.55 (m, 1H, D_2O exchangeable), 4.22 (m, 1H), 4.06 (m, 2H), 3.91 (m, 1H), 3.58 (m, 3H), 3.31 (m, 2H), 3.10−2.82 (m, 4H), 2.09 (m, 2H), 1.74 and 1.71 (2s, 3H), 1.60 (m, 4H). ^{31}P NMR (MeOH-d_4, 121 MHz): δ −13.38 (m). HRMS (ESI): calcd for $C_{22}H_{32}ClN_4NaO_{12}P$ 633.1341 $[M + Na]^+$, found 633.1338.

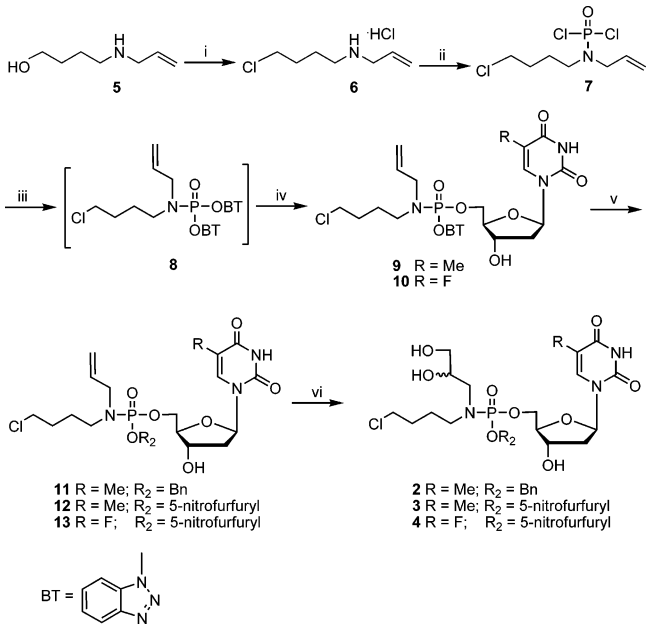
5′-(5-Fluoro-2′-deoxyuridyl) 5-Nitrofurfuryl *N*-2,3-Dihydroxypropyl-*N*-4-chlorobutyl Phosphoramidate (4). Following the same procedure as described for synthesis of **2**, *N*-allyl-*N*-4-chlorobutyl phosphoramidate **13** (130 mg, 0.22 mmol) was reacted with 4-methylmorpholine *N*-oxide (78 mg, 0.66 mmol) and OsO_4/*t*-BuOH solution (2.5 wt %, 330 mg, 0.03 mmol) in a mixture of THF/*t*-BuOH/H_2O (4 mL/1.6 mL/0.8 mL) at room temperature for 3 days. The same workup and purification by silica gel chromatography (methylene chloride/methanol 20:1 to 10:1) gave **4** (64 mg, 46%, mixture of diastereomers at both phosphorus and carbon as indicated by ^{1}H and ^{31}P NMR) as a light-yellow foam. ^{1}H NMR (MeOH-d_4) for diastereomers: δ 7.81 (m, 1H), 7.34 (d, 1H), 6.76 (d, 1H), 6.15 (m, 1H), 5.07 (m, 2H), 4.26 (m, 1H), 4.10 (m, 2H), 3.99 (m, 1H), 3.74 (m,1H), 3.50 (m, 2H), 3.43 (m, 2H), 3.09 (m, 4H), 2.18 (m, 2H), 1.66 (m, 4H). ^{31}P NMR (MeOH-d_4, 121 MHz): δ −13.58 (m). HRMS (ESI): calcd for $C_{21}H_{29}ClFN_4NaO_{12}P$ 637.1090 $[M + Na]^+$, found 637.1093.

Results and Discussion

We envisioned that bis-hydroxylation of *N*-allyl-*N*-4-chlorobutyl phosphoramidates **11**−**13** would provide facile access to the desired *N*-2,3-dihydroxypropyl-*N*-4-chlorobutyl phosphoramidates of thymidine and 5-FdU (Scheme 2). Compounds **11**−**13** could be synthesized using a strategy similar to the one previously employed for the *N*-methyl-*N*-chlorobutyl phosphoramidates.[7] Briefly, selective phosphorylation on the 5′-hydroxyl group of thymidine and 5-FdU using highly reactive phosphoramidic bis(1-benzotriazolyloxy) ester **8**, generated in situ from phosphoramidic dichloride **7** and 1-hydroxybenzotriazole (HOBT), gave the benzotriazolyloxy esters **9** and **10**, respectively. The OBT moiety was then displaced by either benzyl alcohol or 5-nitrofurfuryl alcohol in the presence of DMAP to furnish the *N*-allyl-*N*-4-chlorobutyl phosphoramidates **11**−**13**. OsO_4/NMO bis-

(7) Wu, W.; Freel Meyers, C. L.; Borch, R. F. A novel method for the preparation of nucleoside triphosphates from activated nucleoside phosphoramidates. *Org. Lett.* **2004**, *6*, 2257−60.

Scheme 2 [a]



[a] Reagents and conditions: (i) HCl(g), $SOCl_2$; (ii) $POCl_3$, Et_3N; (iii) HOBT, pyridine; (iv) thymidine or 5-FdU, *N*-methylimidazole; (v) BnOH or 5-nitrofurfuryl alcohol, DMAP; (vi) OsO_4, NMO.

hydroxylation[8] of **11**−**13** afforded the final *N*-2,3-dihydroxypropyl-*N*-4-chlorobutyl phosphoramidates **2**−**4** as mixtures of diastereomers at both phosphorus and carbon of the hydroxypropyl group.

The ability of these modified phosphoramidates to release nucleoside monophosphate after reductive activation was studied in a ^{31}P NMR kinetics experiment using thymidine analogue **2**.[2,3] Removal of the benzyl group was accomplished using catalytic hydrogenolysis in DMF,[7] the resulting phosphoramidate anion (**B** in Scheme 1, $R_2 = CH_2$-$CH(OH)CH_2OH$) was dissolved in buffer, and the reaction was monitored by ^{31}P NMR (0.4 M cacodylate buffer/DMF, pH 7.4, 37 °C). The result is shown in Figure 2. The first spectrum represents benzyl ester **2** in DMF prior to catalytic hydrogenolysis (multiple peaks indicate **2** as a mixture of diastereomers). Subsequent spectra show the conversion of **B** to thymidine 5′-monophosphate **D** in cacodylate buffer. The half-life of the conversion is 221 min, corresponding to a rate of disappearance $k = 0.0031$ min^{-1}. The relatively slow conversion of the *N*-2,3-dihydroxypropyl phosphoramidate to nucleotide compared to the *N*-methyl analogue ($t_{1/2} \leq 5$ min)[3] was expected since the inductive effect of the hydroxyl groups will reduce the nucleophilicity of the nitrogen and thus the rate of cyclization (**B** to **C** in Scheme 1).

In order to determine the effect of dihydroxypropyl group substitution on the hydrophilicity of the prodrug, the log *P* values of **1b** and **3** were measured. The log *P* values for the thymidine analogues **1b** and **3** are $+1.08 \pm 0.03$ and -0.03 ± 0.01, respectively, indicating that replacement of the methyl group with the dihydroxypropyl group reduces the octanol:water partition coefficient by approximately 13-fold.

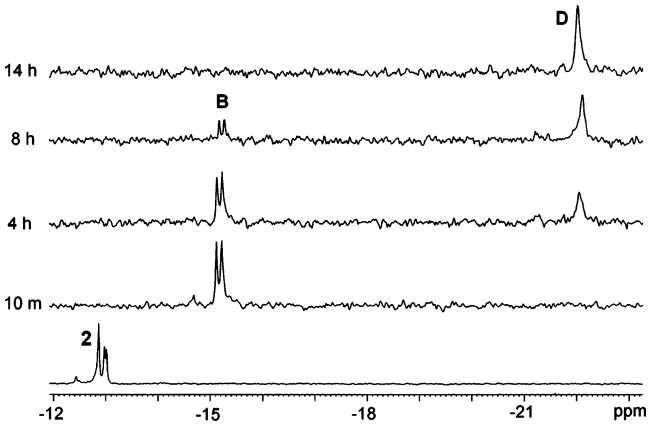


Figure 2. Reaction of phosphoramidate **2** in cacodylate buffer (0.4 M, pH 7.4, 37 °C) following chemical activation. **B**: Phosphoramidate anion intermediate (two peaks arise from dihydroxypropyl diastereomers). **D**: Thymidine 5′-monophosphate. Chemical shifts are reported relative to triphenylphosphine oxide in benzene-d_6. See Results and Discussion for details.

Table 1. GI_{50} Values for Compounds **1a** and **4** against Selected Cell Lines from the NCI 60 Cell Line Panel[a]

	GI_{50}, μM	
cell line	**1a**	**4**
MCF7	0.03	<0.01
SF-539	<0.01	0.07
SR	0.07	0.04
LOX IMVI	0.07	0.03
A549	0.03	<0.01
NCI-H460	<0.01	0.12
ACHN	0.04	0.03

[a] See the Experimental Section for details of the assay.

Prodrug **4** was tested in the NCI 60 cell line in vitro antitumor screening panel. A comparison of the results for **1a** and **4** in several of the most sensitive cell lines is shown in Table 1; the complete data set is available in the Supporting Information. Surprisingly, the dihydroxypropyl compound showed tumor cell growth inhibitory activity (median $GI_{50} = 0.41$ μM) that was comparable to that of the *N*-methyl analogue **1** (median $GI_{50} = 1.2$ μM), suggesting that a rapid rate of cyclization is not required to achieve growth inhibitory activity in vitro.

Conclusions

N-2,3-Dihydroxypropyl-*N*-4-chlorobutyl phosphoramidate prodrugs of thymidine and 5-FdU were synthesized. The new phosphoramidate prodrug represents a modification of the phosphoramidate structure previously reported by us with a more hydrophilic side chain in order to increase water solubility of the prodrug. Our results indicate that *N*-2,3-dihydroxypropyl phosphoramidates are as effective as the

(8) VanRheenen, V.; Kelly, R. C.; Cha, D. Y. An improved catalytic OsO_4 oxidation of olefins to -1,2-glycols using tertiary amine oxides as the oxidant. *Tetrahedron Lett.* **1976**, *17*, 1973−6.

N-methyl analogues in terms of activation and conversion to nucleoside monophosphates, although the conversion of *N*-2,3-dihydroxypropyl phosphoramidates to free phosphate is about 2 orders of magnitude slower than that of the *N*-methyl analogues. The goal of increasing hydrophilicity with the introduction of the dihydroxypropyl group has also been achieved. Finally, the ability of the newly synthesized nucleoside phosphoramidate prodrug to undergo intracellular activation and release nucleoside monophosphate is supported by the excellent growth inhibitory activity of the 5-FdU prodrug. Investigations are currently underway to study the effect of other modifications of the phosphoramidate structure on prodrug activation and activity.

Acknowledgment. Support from Purdue Cancer Center Support Grant P30 CA23168 for services provided by the NMR and Mass Spectrometry Shared Resources is appreciated. We thank Irene Boutselis for carrying out the log *P* determinations. Financial support from the National Cancer Institute (Grant R01 CA34619) is gratefully acknowledged.

Supporting Information Available: Table of GI_{50} values for the NCI 60 cell line panel. This material is available free of charge via the Internet at http://pubs.acs.org.

MP060006G