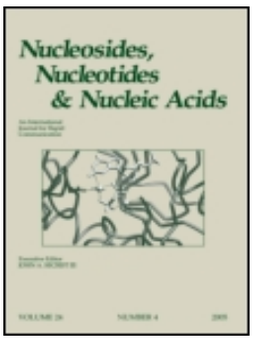

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SYNTHESIS OF 5-HALOGENO-6-AMINO-2'-DEOXYURIDINES AND THEIR ANALOGS AS POTENTIAL INHIBITORS OF THYMIDINE PHOSPHORYLASE

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ABSTRACT. 5-Halogeno-6-amino-2'-deoxyuridines were synthesized from 2'-deoxyuridine as potential thymidine phosphorylase (ThdPase) inhibitors. Among the compounds synthesized, 5-bromo-6-amino-2'-deoxyuridine (**6**) and 5-iodo-6-amino-2'-deoxyuridine (**9**) were found to inhibit ThdPase activity with IC50 values of 1.3 μM and 6.5 μM, respectively. In vitro cell culture studies showed that compound (**6**) can significantly enhance the cytotoxic effects of 5-fluoro-2'-deoxyuridine against a human colon cancer HCT-8 cell line.

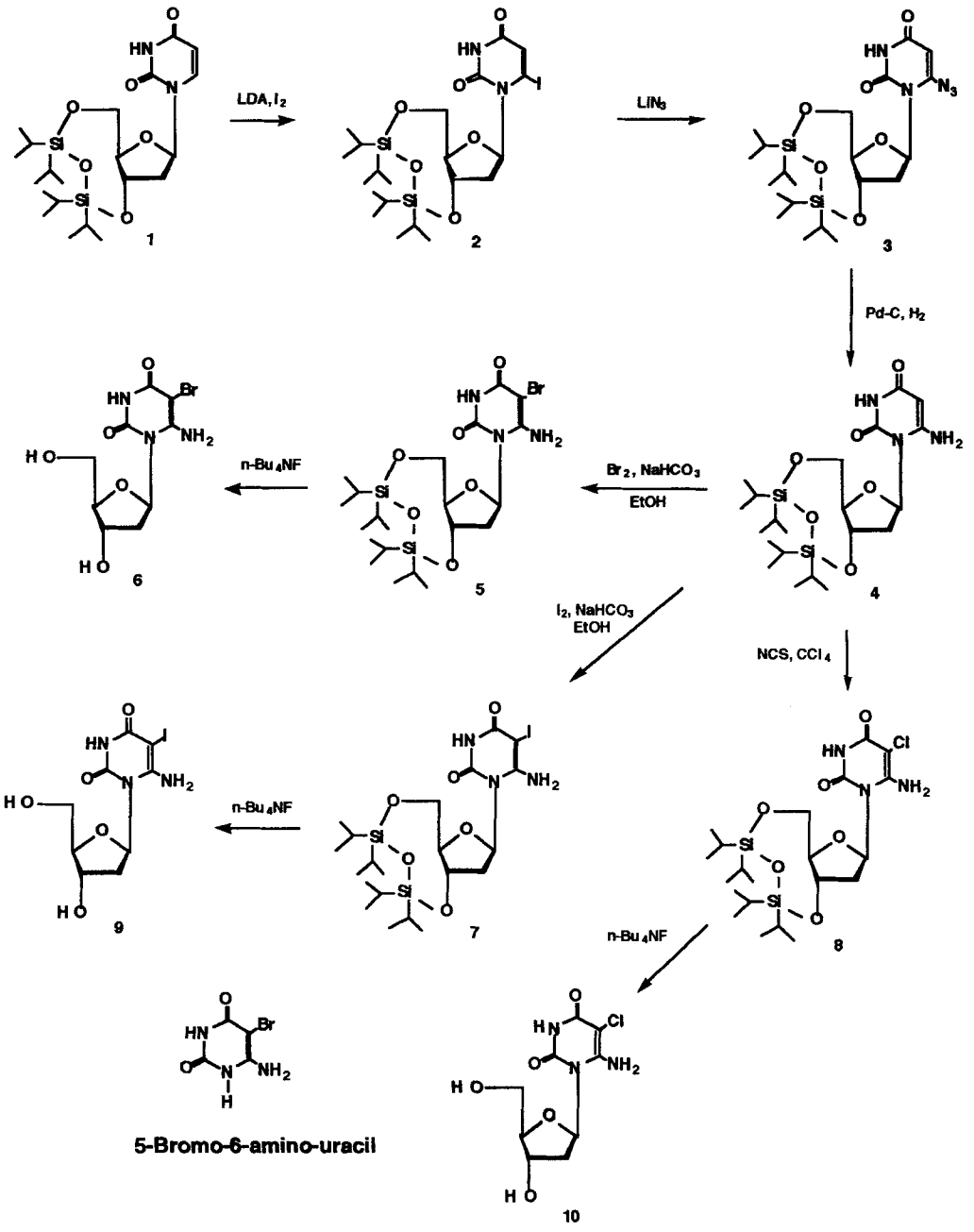
Thymidine phosphorylase (ThdPase) is one of two main pyrimidine nucleoside phosphorylases present within the cell, and it catalyzes the reversible phosphorolysis of thymidine, deoxyuridine and other pyrimidine 2'-deoxyribosides except for 4-aminosubstituted compounds such as deoxycytidine, to their respective base forms and 2-deoxyribose-1-phosphate (d-R-1-P) [1-3]. This enzyme also catalyzes the transfer of the deoxyribosyl moiety of one deoxynucleoside to a base to form a second deoxynucleoside[4-6]. Finally, it is now well-appreciated that ThdPase is responsible for the activation and deactivation of 5-substituted pyrimidine ribonucleoside analogs that have been associated with potential anticancer activity[7].

In addition to its critical role in maintaining the homeostasis of deoxynucleotides and thymidine metabolism, thymidine phosphorylase was recently found to have sequence homology with platelet-derived endothelial cell growth factor (PD-ECGF), a protein with documented angiogenic properties. Given this observation, various angiogenic model systems have confirmed that thymidine phosphorylase, itself, has angiogenic activity [8-12]. When mutant proteins with point mutations in the critical catalytic domain of the enzyme were expressed by site-directed mutagenesis, it was shown that these proteins lost their angiogenic properties identifying the catalytic domain of the enzyme as being critical for angiogenic activity.

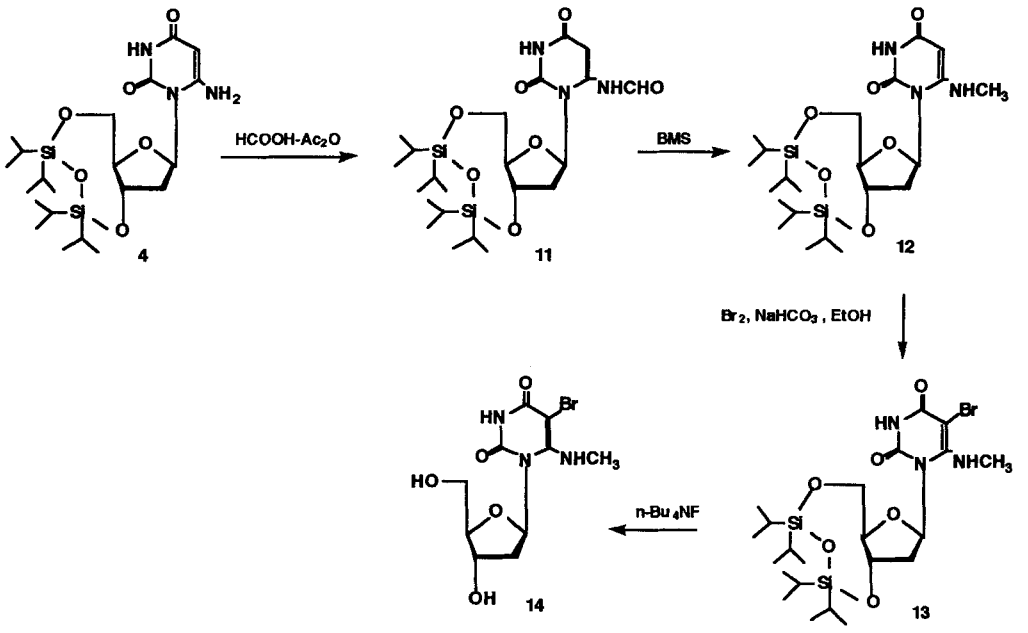
Recent investigations have shown that the levels of thymidine phosphorylase are markedly elevated in some human breast tumors when compared to normal breast tissue[12]. Bicknell et al [12] demonstrated that while overexpression of thymidine phosphorylase in human breast MCF-7 cancer cells had no stimulatory effect on cell growth *in vitro*, it significantly enhanced tumor growth *in vivo*, an effect presumably mediated via an angiogenic-mediated pathway. Taken collectively, these studies suggest that thymidine phosphorylase may represent an important target for cancer chemotherapy in terms of inhibiting critical pathways in pyrimidine metabolism as well as inhibiting the process of tumor angiogenesis. While there has been an interest in developing potent inhibitors of this enzyme, only a few such compounds have been identified to date [13-24, 25]. In the present paper, we report on the synthesis of a novel class of 5-halogeno-6-amino-2'-deoxyuridines and their analogs as potential new inhibitors of thymidine phosphorylase.

Chemical Synthesis: The synthesis of 5-halogeno-6-amino-2'-deoxyuridines is outlined in Scheme 1. 3',5'-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (**1**) was lithiated with LDA at -78° then treated with iodine to give 6-iodo-3',5'-O-(tetraisopropyl-disiloxane-3'-5'-diyl)-2'-deoxyridine (**2**) according to the method of Tanaka[26]. Reaction of the 6-iodo compound (**2**) with sodium azide in DMF afforded 6-azido-3',5'-(tetraisopropyl-disiloxane-3',5'-diyl)-2'-deoxyuridine (**3**). The latter was reduced by catalytic hydrogenation to 6-amino-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (**4**). Bromination of 6-amino compound (**4**) with bromine in ethanol in the presence of sodium bicarbonate gave 5-bromo-6-amino-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (**5**). The 5-bromo-6-amino derivative (**5**), after treatment with tetra-*n*-butylammonium fluoride, afforded 5-bromo-6-amino-2'-deoxyuridine (**6**). 6-Amino-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (**4**) reacted with a mixture of iodine and sodium bicarbonate in ethanol to afford 5-iodo-6-amino-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (**7**). 5-Chloro-6-amino-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (**8**) was prepared by chlorination of **4** with N-chlorosuccinimide in carbon tetrachloride. Reaction of **4** with chlorine in ethanol and sodium bicarbonate did not form **8**, due to the decomposition by oxidation. Deprotection of compounds **7** and **8** with tetra-*n*-butylammonium fluoride gave the corresponding 5-iodo-6-amino-2'-deoxyuridine (**9**) and 5-chloro-6-amino-2'-deoxyuridine (**10**).

6-Amino-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (**4**) was formylated with acetic-formic anhydride to give 6-formamido-3',5'-(tetraisopropyl-disiloxane-3',5'-diyl)-2'-deoxyuridine (**11**). The latter was reduced with borane-dimethyl sulfide complex to yield 6-methylamino-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (**12**). Bromination of **12** was accomplished by treating with bromine in ethanol containing sodium bicarbonate to give 5-bromo-6-methylamino-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (**13**). 5-Bromo-6-methylamino-2'-deoxyuridine (**14**) was obtained by desilylation of **13** with tetra-*n*-butylammonium fluoride (Scheme 2).



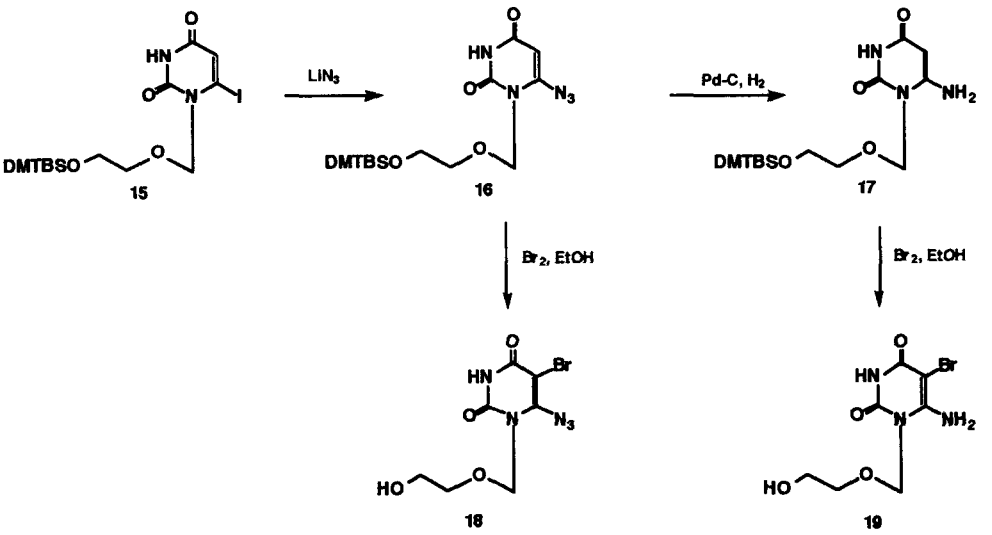
Scheme 1



Scheme 2

1-[2'-(*t*-Butyl-dimethylsilyloxy)ethoxy]methyl-6-iodouracil (**15**) [6] was converted into 1-[2'-(*t*-butyl-dimethylsilyloxy)ethoxy]methyl-6-azidouracil (**16**) by the treatment with lithium azide in dry DMF which in turn was hydrogenated to 1-[2'-(*t*-butyl-dimethylsilyloxy)ethoxy]methyl-6-aminouracil (**17**). Treatment of compounds 16 and 17 with bromine in ethanol gave the corresponding 1-[2'-(hydroxyethoxy)methyl]-5-bromo-6-aminouracil (**18**) and 1-[2'-(hydroxyethoxy)-methyl]-5-bromo-6-azidouracil (**19**) (Scheme 3).

Enzyme Assay: All 2'-deoxyuridine and 5- and 6-substituted uracil analogs described in the Experimental section were tested as potential inhibitors of thymidine phosphorylase using enzyme isolated from lysed human blood platelets. Earlier studies had shown that either intact and/or lysed human platelets as well as ThdPase isolated from human platelets represent relevant model systems for identifying inhibitor compounds of thymidine degradation[27,28]. Using either intact or lysed human platelets and isolated pure enzyme, Desgranges et al[28] demonstrated that 5-bromo-6-aminouracil was the most potent inhibitor of ThdPase when compared to other 5- and 6-subsituted uracil analogs. This compound remains the most potent ThdPase inhibitor identified to date, and for this reason, it was used in the present study as a standard for comparison. The concentration required to



Scheme 3

TABLE 1: Inhibition of ThdPase by 5-halogeno, 6-amino-2'-deoxyuridine analogs.

Compound	IC50 (μM)
5-Bromo-6-aminouracil	6.0 ± 0.20
5-Bromo-6-amino-2'-deoxyuridine (**6**)	1.3 ± 0.15
5-Iodo-6-amino-2'-deoxyuridine (**9**)	6.5 ± 0.21

inhibit 50% of phosphorolysis of 1 mM thymidine was defined as the IC50, and the IC50 values of the parent base compound and the two 5-halogen substituted nucleoside analogs are presented in Table 1. Our analysis revealed that none of the other compounds described in the Experimental section displayed ThdPase inhibitory activity (data not shown).

The nucleoside analog, 5-bromo-6-amino-2'-deoxyuridine (**6**), was 4.6-fold more potent an inhibitor of ThdPase than the free base, 5-bromo-6-aminouracil [25]. This difference in inhibitory activity was shown to be significant ($p < 0.0.5$). In contrast, 5-iodo-6-amino-2'-deoxyuridine analog (**9**) inhibited ThdPase to a similar degree as the free base. Of note however, the solubility of the two 2'-deoxyuridine nucleoside analogs (**6** and **9**) was

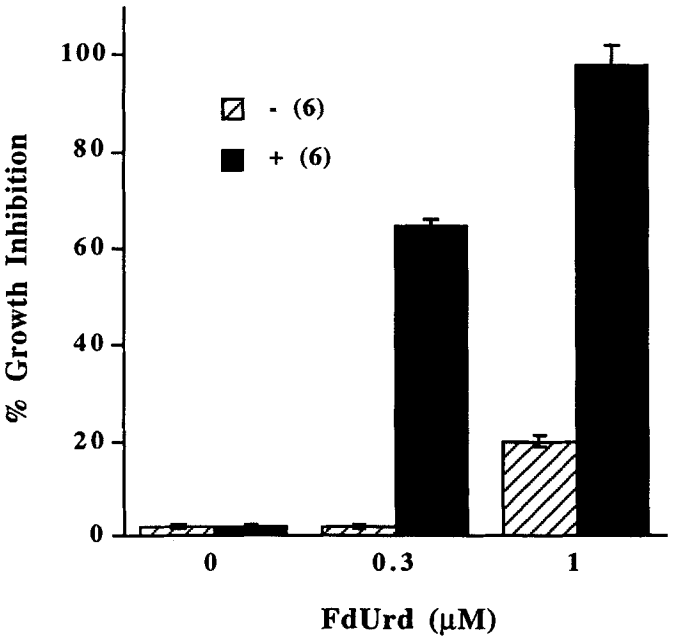


Figure 1. Effect of (6) on the cytotoxicity of FdUrd in HCT-8 cells.

significantly greater than that of the free base making each of these compounds far easier to work with and formulate.

Cell Culture Studies: Previous studies demonstrated that transfection of ThdPase into human colon cancer cells significantly enhanced the sensitivity of these cells to the fluoropyrimidine 5-FU, and that this action was mediated by enhancement of the metabolic activation of 5-FU[29]. Specifically, the increased expression of ThdPase resulted in a significant increase in the intracellular levels of the 5-FU metabolite 5-fluoro-2'-deoxyuridine-5'-monophosphate, FdUMP. Since ThdPase catalyzes the reversible phosphorolysis of 2'-deoxyuridine compounds, we investigated whether inhibitors of the enzyme might potentiate the cytotoxic activity of the fluoropyrimidine analog 5-fluoro-2'-deoxyuridine (FdUrd) by inhibiting its phosphorolytic cleavage. For these studies, we used 5-bromo-6-amino-2'-deoxyuridine as this compound was identified in the enzyme studies presented in Table 1 as the most potent inhibitor of ThdPase. The human colon cancer HCT-8 cell line was employed for these cytotoxicity studies as we had previously shown that this cell line does not express uridine phosphorylase activity, the other pyrimidine nucleoside phosphorylase present in cells[29]. As shown in Fig 1, FdUrd, alone, at concentrations of 0.3 μM and 1 μM had minimal growth inhibitory activity, resulting in 0 and 20% growth

inhibition, respectively. When 5-bromo-6-amino-2'-deoxyuridine (100 μM) was given 30 minutes prior to FdUrd, growth inhibition by 0.3 and 1 mM FdUrd was increased significantly to 65.5% ($p < 0.001$) and 100% ($p < 0.001$), respectively. Importantly, 5-bromo-6-amino-2'-deoxyuridine, at a concentration of 100 μM, alone, did not show growth inhibitory effects against HCT-8 cells.

EXPERIMENTAL

Melting points were determined on a Gallenkamp apparatus and were uncorrected. The uv absorption maxima and extinction coefficients were obtained using a Perkin-Elmer Model Lambda 3A recording spectrophotometer. The NMR spectra were run on a Brucker WM-400. All analyses were performed by the Baron Consulting Co., Orange, CT.

6-Iodo-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (2): 3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (**1**, 235 mg, 0.5 mmol) was dissolved in 5 ml of dry THF and cooled to -78°. To this solution, 1.25 ml of 1.2 M solution of LDA was gradually added and stirring was continued at -78° for 1.5 hr. It was then treated with a solution of 500 mg of iodine in 1.5 ml of dry THF. The reaction mixture was stirred for two hrs and then quenched with 0.2 ml of acetic acid. The solvent evaporated under reduced pressure. The residue was partitioned between methylene chloride and water and the methylene chloride layer was washed with water and aqueous sodium thiosulfate. The solvent was evaporated and the residue chromatographed on silica gel column. Elution with 10% ether in methylene chloride gave 202 mg (68 %) of 6-iodo derivative (**2**). ^{1}H-NMR (deuteriochloform): δ 1.01-1.16 (m, 28 H, *i*-Pr), 2.34-2.44 (m, 1 H, 2'-H), 2.84-2.92 (m, 1 H, 2'-H), 3.80-3.87 (m, 1 H, 4'-H), 4.01 (d, 2 H, 5'-H, J = 5.6 Hz), 4.99-5.05 (m, 1 H, 3'-H), 6.17 (dd, 1 H, J = 9.37, 3.04 Hz), 6.40 (d, 1 H, 5-H, J = 2.4 Hz), 8.39 (br, 1 H, NH).

Anal. Calcd for $C_{21}H_{37}IN_2O_6Si_2$: C, 42.28; H, 6.25; N, 4.70. Found: C, 41.93 H, 6.51 N, 4.42.

6-Azido-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine(3): A mixture of 298 mg (0.5 mmol) of iodo compound **2**, 163 mg of sodium azide and 3 ml of dry DMF was stirred at room temperature for 2 hrs. DMF was evaporated under reduced pressure at 35-40°C. The residue was extracted with methylene chloride and washed with water. After removal of solvent under reduced pressure, the residue was subjected to chromatography on silica gel and eluted with 5% ether in

methylene chloride to give the azido compound (**3**), 210 mg (82%), m.p. 110-113°, ^{1}H-NMR (deuteriochloroform): δ 1.00-1.16 (m, 28H, *i*- Pr), 2.30-2.39 (m, 1 H, 2'-H), 3.73-3.79 (m, 1 H, 4'-H), 3.98-4.01 (m, 2 H, 5'-H), 4.92-5.00 (m, 1 H, 3'-H), 5.45 (d, 1 H, 5-H, J = 2.2 Hz), 6.28 (dd, 1 H, 1'-H, J = 9.5, 3.4 Hz).

Anal. Calcd for $C_{21}H_{37}N_5O_6Si_2$: C, 49.29; H, 7.28; N, 13.69. Found: C, 49.39; H, 7.48; N, 13.65.

6-Amino-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (4): A suspension of 150 mg (0.29 mmol) of azido compound (**3**) and 30 mg of 10% Pd-C in 5 ml of ethanol was hydrogenated under atmospheric pressure. After the hydrogenation step was completed, it was filtered. Evaporation of ethanol under reduced pressure gave the amino compound (**4**), 125 mg (88%), m.p. 135-138°. ^{1}H-NMR (DMSO-d_6): δ 1.01-1.15 (m, 28 H, *i*-Pr), 2.18-2.27 (m, 1 H, 2'-H), 2.66-2.76 (m, 1 H, 2'-H), 3.62-3.68 (m, 1 H, 4'-H), 3.88-4.00 (m, 2 H, 5'-H), 4.65 (s, 1 H, 5-H), 4.81-4.90 (m, 1 H, 3'-H), 6.14 (dd, 1 H, 1'-H, J = 8.9, 3.9 Hz).

Anal. Calcd for $C_{21}H_{39}N_3O_6Si_2$: C, 51.93 ; H, 8.09 ; N, 8.65. Found: C, 51.54 ; H, 8.43 ; N, 8.34.

5-Bromo-6-amino-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (5): A suspension of 97 mg (0.2 mmol) of amino compound (**4**) and 150 mg of powdered sodium bicarbonate in 4 ml of ethanol was stirred at room temperature, to which a solution of 5% bromine in ethanol was dropwise added until the color of the reaction mixture remained light yellow. The solvent was removed at room temperature under reduced pressure. The residue was chromatographed on silica gel. Elution with 5% methanol in methylene chloride gave 95 mg (84%) of compound (**5**), ^{1}H-NMR (deuteriochloroform): δ 1.01-1.13 (m, 28 H, *i*-Pr), 2.42-2.48 (m, 2 H, 2'-H), 3.68-3.72 (m, 1 H, 4'-H), 4.03-4.07 (m, 2 H, 5'-H), 4.68-4.72 (m, 1 H, 3'-H), 5.97 (br, 2 H, NH2), 6.74 (dd, 1 H, 1'-H, J = 8.1, 5.8 Hz).

Anal. Calcd for $C_{21}H_{38}BrN_3O_6Si_2$: C, 44.67; H, 6.78; N,7.44. Found: C, 44.82; H, 7.06; N, 7.02.

5-Bromo-6-amino-2'-deoxyuridine (6): To a solution of 113 mg (0.2 mmol) of compound (**5**) in 3 ml of dry THF, there was added 0.4 ml of 1 M solution of tetra-*n*-butylammonium fluoride in THF. The mixture was stirred for 3 hrs. The crystalline product was filtered, washed with THF, and recrystallized form methanol to give 5-bromo-6-amino-2'-deoxyuridine, 51 mg (79%) , uv (water): λ_{max} 273 nm

(10827), ^{1}H-NMR (DMSO-d_6): δ 1.87-1.94 (m, 1 H, 2'-H), 2.40-2.55 (m, 1 H, 2'-H), 3.58-3.68 (m, 2 H, 5'-H), 3.76-3.81 (m, 1 H, 4'-H), 4.28-4.35 (m, 1 H, 3'-H), 6.66 (dd, 1 H, 1'-H, J = 9.5, 6.3 Hz).

Anal. Calcd for $C_9H_{12}BrN_3O_5$: C, 33.56; H, 3.75; N, 13.05. Found: C, 33.56; H, 3.88; N, 12.89.

5-Iodo-6-amino-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (7) : A suspension of 145.7 mg (0.3 mmol) of amino compound (**4**) and 250 mg of powdered sodium bicarbonate in 10 ml of ethanol was stirred at room temperature, to which a solution of 5% iodine in ethanol was added dropwise until the color of the reaction mixture remained light brown. The solvent was removed at room temperature under reduced pressure. The residue was chromatographed on silica gel. Elution with 5% methanol in methylene chloride gave 106 mg (57.8%) of compound (**7**), ^{1}H-NMR (deuteriochloroform): δ 1.02-1.15 (m, 28 H, *i*-Pr), 2.42-2.48 (m, 2 H, 2'-H), 3.69-3.75 (m, 1 H, 4'-H), 4.06 (d, 2 H, 5'-H, J = 2.4 Hz), 4.71 (dd, 1 H, 3'-H, J = 8.5, 17.0 Hz), 6.08 (br, 2 H, NH_2), 6.74 (dd, 1 H, 1'-H, J = 6.4, 7.7 Hz).

Anal. Calcd for $C_{21}H_{38}IN_3O_6Si_2$: C, 41.24; H, 6.26; N,6.87. Found: C, 41.58; H, 5.93; N, 6.67.

5-Iodo-6-amino-2'-deoxyuridine (9) : To a solution of 122 mg (0.2 mmol) of compound (**7**) in 1.2 ml of dry THF, there was added 0.2 ml of 1 M solution of tetra-*n*-butylammonium fluoride in THF. The whole was stirred for 3 hrs. The crystalline product was filtered, washed with THF, and recrystallized form methanol to give 5-iodo-6-amino-2'-deoxyuridine , 56 mg (75.9%) , uv (water): λ_{max} 276 nm (9201), ^{1}H-NMR (DMSO-d_6): δ 1.87-1.94 (m, 1 H, 2'-H), 2.40-2.50 (m, 1 H, 2'-H), 3.58-3.68 (m, 2 H, 5'-H), 3.76-3.85 (m, 1 H, 4'-H), 4.29-4.35 (m, 1 H, 3'-H), 6.65 (dd, 1 H, 1'-H, J = 9.5, 6.3 Hz).

Anal. Calcd for $C_9H_{12}IN_3O_5$: C, 29.29; H, 3.28; N, 11.38. Found: C, 29.69; H, 3.19; N, 11.10.

5-Chloro-6-amino-3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (8) : A suspension of 97 mg (0.2 mmol) of amino compound (**4**) and 404 mg of N-chloro-succinimide in 10 ml of carbon tetrachloride was refluxed until the reaction was complete (followed by TLC). It was washed with water and aqueous sodium bicarbonate. The solvent was removed at room temperature under reduced pressure, and the residue was subjected to chromatography on silica gel. Elution with 5% methanol in

methylene chloride gave 77 mg (74%) of compound (**8**), ^{1}H-NMR (deuteriochloroform): δ 1.04-1.17 (m, 28 H, *i*-Pr), 2.44-2.49 (m, 2 H, 2'-H), 3.66-3.73 (m, 1 H, 4'-H), 4.10 (d, 2 H, 5'-H, J = 2.4 Hz), 4.73 (dd, 1 H, 3'-H, J = 8.5, 17.0 Hz), 6.09 (br, 2 H, NH_2), 6.76 (dd, 1 H, 1'-H, J = 6.4, 7.7 Hz).

Anal. Calcd for $C_{21}H_{38}ClN_3O_6Si_2 \cdot H_2O$: C, 46.87; H, 7.49; N,7.81. Found: C, 47.18; H, 7.82; N, 7.84.

5-Chloro-6-amino-2'-deoxyuridine (10) : To a solution of 52 mg (0.1 mmol) of compound (**8**) in 0.6 ml of dry THF, there was added 0.1 ml of 1 M solution of tetra-*n*-butylammonium fluoride in THF. The mixture was stirred for 3 hrs. The crystalline product was filtered, washed with THF, and recrystallized from methanol to give 5-chloro-6-amino-2'-deoxyuridine , 21 mg (75.6%) , uv (water): λ_{max} 272 nm (9141), ^{1}H-NMR (DMSO-d_6): δ 1.88-1.95 (m, 1 H, 2'-H), 2.41-2.52 (m, 1 H, 2'-H), 3.58-3.68 (m, 2 H, 5'-H), 3.77--3.85 (m, 1 H, 4'-H), 4.29-4.35 (m, 1 H, 3'-H), 6.65 (dd, 1 H, 1'-H, J = 9.5, 6.3 Hz).

Anal. Calcd for $C_9H_{12}ClN_3O_5$: C, 38.93; H, 4.35; N, 15.13. Found: C, 38.24; H, 4.73; N, 15.04.

6-Formamido-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (11) : Formic acid (375 mg) was added to 675 mg of acetic anhydride at 0°C, heated gentle to 50-60°C for 2 hrs. and then cooled to 0°C. To this cold solution 485.7 mg (1 mmol.) of 6-Amino-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (**4**) was added and stirred at 0°C for 2 hrs. The volatile material was removed in vacuum. The residue was dissolved in methylene chloride, washed with water and aqueous sodium bicarbonate. Methylene chloride was evaporated under reduced pressure, and the residual oil was purified by chromatography on silica gel column. Elution of 2% ethanol in methylene chloride gave 475 mg (92%) of pure 6-formamido-3',5'-(tetraisopropyl-disiloxane1,3-diyl)-2'-deoxyuridine (**11**). ^{1}H-NMR (DMSO-d_6): δ 1.02-1.17 (m, 28 H, *i*-Pr), 2.37-2.46 (m, 1 H, 2'-H), 2.56-2.61 (m, 1H, 2'-H), 3.69-3.75 (m, 1 H, 4'-H), 4.03-412 (m, 2 H, 5'-H), 4.72 (dd, 1 H, 3'-H, J = 8.7, 17.3 Hz), 5.03 (s, 1H, 5-H), 6.71 (dd, 1 H, 1'-H, J = 4.5, 8.9 Hz), 7.93 (br, 1 H, 3-NH), 8.01 (s, 1 H, CHO).

Anal. Calcd for $C_{22}H_{39}N_3O_7Si_2$: C, 51.44; H, 7.65; N, 8.18. Found: C, 51.23; H, 7.39; N, 8.18.

6-Methylamino-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (12) : 6-Formamido-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (**11**, 257 mg, 0.5 mmol) was dissolved in 2.5 ml of dry THF and cooled to 0°C. To this solution was dropwise added 1 ml of borane-dimethyl sulfide complex (2M) in THF, and the reaction mixture was stirred overnight at room temperature. The solution was cooled to 0°C to which 2 ml of methanol was then added. The mixture was evaporated to dryness. The residue was partitioned between water and ether. The ethereal solution was washed with water and aqueous sodium bicarbonate. After removal of ether, the residue was chromatographed on silica gel column and eluted with 2% methanol in methylene chloride to give 178 mg (71.2%) of 6-methylamino-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (**12**). ^{1}H-NMR (DMSO-d_6): δ 1.02-1.14 (m, 28 H, *i*-Pr), 1.82 (s, 3 H, NMe), 2.36-2.56 (m, 2 H, 2'-H), 3.66-3.71 (m, 1 H, 4'-H), 4.05 (d, 2 H, 5'-H, J = 2.4 Hz), 4.72 (dd, 1 H, 3'-H, J = 7.8, 17.1 Hz), 5.12 (s, 1 H, 5-H), 6.76 (dd, 1 H, 1'-H, J = 5.4, 9.0 Hz), 7.93 (br, 1 H, 3-NH).

Anal. Calcd for $C_{22}H_{41}N_3O_6Si_2$: C, 52.88; H, 8.27; N, 8.41. Found: C, 53.03; H, 8.11; N, 8.34.

5-Bromo-6-methylamino-3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-de-oxyuridine (13): This compound was prepared by bromination of 6-methylamino-3',5'-O-(tetra-isopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (**12**) in 75% yield by the method used for the preparation of 5-bromo-6-amino-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (**5**), m.p. 129-130°C. ^{1}H-NMR (DMSO-d_6): δ 1.02-1.18 (m, 28 H, *i*-Pr), 2.13 (s, 3 H, NMe), 2.25-2.51 (m, 2 H, 2'-H), 3.68-3.80 (m, 1 H, 4'-H), 4.05-4.15 (m, 2 H, 5'-H), 4.48-4.53 (m, 1 H, 3'-H), 4.95 (br, 1 H, NHMe), 6.52 (dd, 1 H, 1'-H, J = 5.4, 9.0 Hz), 7.65 (br, 1 H, 3-NH).

Anal. Calcd for $C_{22}H_{40}BrN_3O_6Si_2$: C, 45.67; H, 6.96; N, 7.26. Found: C, 45.77; H, 6.66; N, 6.96.

5-Bromo-6-methylamino-2'-deoxyuridine (14) : This compound was obtained by the deprotection of 5-bromo-6-methylamino-3',5'-(tetraisopropyl-disiloxane-1,3-diyl)-2'-deoxyuridine (**13**) with tetra-*n*-butylammonium fluoride by the method used for the preparation of 5-bromo-6-methylamino-2'-deoxyuridine (**6**). Compound **14** was obtained in 83% yield, uv (water): λ_{max} 273 nm (8950), ^{1}H-NMR (DMSO-d_6): δ 1.70-1.85 (m, 1 H, 2'-H), 2.14 (s, 3 H, NMe), 2.53-2.62 (m, 1 H, 2'-H), 3.55-3.65 (m, 2 H, 5'-H), 3.69-3.74 (m, 1 H, 4'-H), 4.30-4.37(m, 1 H, 3'-H), 6.67 (dd, 1 H, 1'-H, J = 9.5, 6.5 Hz).

Anal. Calcd for $C_{10}H_{14}BrN_3O_5$: C, 35.73; H, 4.20; N, 12.50. Found: C, 35.91; H, 4.42; N, 12.71.

1-[2'-(*t*-Butyl-dimethylsilyloxy)ethoxy]methyl-6-azidouracil (16): 1-[2'-(*t*-Butyl-dimethylsilyloxy)ethoxy]methyl-6-iodouracil (**15**, 2.13 g, 5 mmol) was dissolved in 10 ml of dry DMF and cooled to 0°C. To this solution, 0.77 g (15.7 mmol) of lithium azide was added and stirred at room temperature for half hour. DMF was removed under reduced pressure below 30°C, 15 ml of water was added and then extracted with ether. The ethereal solution was washed with water. After evaporation of ether, the product was purified by chromatography on silica gel column and eluted with 5% ether in methylene chloride to yield 1.38 g (79.1%) of pure 1-[2'-(*t*-butyl-dimethylsilyloxy)ethoxy]methyl-6-azidouracil (**16**). ^{1}H-NMR (DMSO-d_6): δ 0.02 (s, 3 H, CH_3Si), 0.04 (s, 3 H, CH_3Si), 0.87 (s, 9 H, *t*-Bu), 3.54-3.59 (m, 2 H, CH_2O), 3.61-3.69 (m, 2 H, CH_2O), 5.28 (s, 2 H, NCH_2O), 5.45 (s, 1 H, 5-H).

Anal. Calcd for $C_{13}H_{23}N_5O_4Si$: C, 45.73; H, 6.79; N, 20.51. Found: C, 45.55; H, 6.47; N, 20.19.

1-[2'-(*t*-Butyl-dimethylsilyloxy)ethoxy]methyl-6-aminouracil (17) : A sus-pension of 341 mg (1 mmole) of 1-[2'-(*t*-butyl-dimethylsilyloxy)ethoxy]methyl-6-azidouracil (**16**) and 20 mg of 10% Pd-C in 20 ml of ethanol was hydrogenated under atmospheric pressure. After the hydrogenation step was completed, it was filtered. Evaporation of the filtrate under reduced pressure gave 308 mg (97.7%) of 1-[2'-(*t*-butyl-dimethylsilyloxy)ethoxy]methyl-6-aminouracil (**17**). ^{1}H-NMR (DMSO-d_6): δ 0.02 (s, 3 H, CH_3Si), 0.04 (s, 3 H, CH_3Si), 0.87 (, S, 9 H, *t*-Bu), 3.51-3.57 (m, 2 H, CH_2O), 3.66-3.71(m, 2 H, CH_2O), 4.58 (s, 1 H, 5-H), 5.27 (s, 2 H, NCH_2O), 6.70 (br.s, 2 H, NH_2).

Anal. Calcd for $C_{13}H_{25}N_3O_4Si$: C, 49.50; H, 7.98; N, 13.32. Found: C, 49.32; H, 7.63; N, 13.69.

1-(2'-Hydroxyethoxy)methyl-5-bromo-6-azidouracil (18) : A solution of 341 mg (1 mmol) of 1-[2'-(*t*-butyl-dimethylsilyloxy)ethoxy]-methyl-6-azidouracil (**16**) in 4 ml of ethanol was stirred at room temperature, to which a solution of 2% bromine in ethanol was added dropwise until the color of the reaction mixture remained light yellow. The solvent was removed at room temperature under reduced pressure . The residue was chromatographed on silica gel. Elution with 2% methanol in methylene chloride gave 210 mg (68.6%) of 1-(2'-hydroxyethoxy)methyl-5-bromo-6-azidouracil

(**18**). ^{1}H-NMR (DMSO-d_6): δ 3.48-3.53 (m, 4 H, CH_2O), 5.26 (s, 2 H, NCH_2O), 4.95 (br.s, 1 H, OH),

Anal. Calcd for $C_7H_8BrN_5O_4$: C, 27.47; H, 2.63; N, 22.88. Found: C, 27.75; H, 2.47; N, 23.01.

1-(2'-Hydroxyethoxy)methyl-5-bromo-6-aminouracil (19): A solution of 157.5 mg (0.5 mmol) of 1-[2'-(*t*-butyl-dimethylsilyloxy)ethoxy]-methyl-6-aminouracil (**17**) in 10 ml of ethanol was stirred at room temperature, to which a solution of 5% bromine in ethanol was added dropwise until the color of the reaction mixture remained light yellow. The solvent was removed at room temperature under reduced pressure. The residue was chromatographed on silica gel. Elution with 5% methanol in methylene chloride gave 94 mg (67.1%) of 1-(2'-hydroxy-ethoxy)methyl-5-bromo-6-aminouracil (**19**). uv (water): λ_{max} 273 nm (9655), ^{1}H-NMR (DMSO-d_6): δ 3.49-3.52 (m, 4 H, CH_2O), 5.26 (s, 2 H, NCH_2O), 4.75 (br.s, 1 H, OH), 6.65 (br.s, 2 H, NH_2).

Anal. Calcd for $C_7H_{10}BrN_3O_4$: C, 30.02; H, 3.60; N, 15.00. Found: C, 30.31; H, 3.45; N, 15.01.

Biological Studies:

1. Preparation of Thymidine Phosphorylase from Human Blood Platelets: Human blood (27 ml) was placed into three separate plastic tissue culture tubes (Corning 25200) with each tube containing 1 ml of sodium citrate solution. These samples were then centrifuged (1,200 rpm) for 10 min. at 25°C. The platelet-rich plasma (PRP) fraction was then pooled from all the samples to give a total volume of approximately 10 ml. To this sample was added 1.5 ml acid-citrate dextrose (ACD). After careful mixing, the mixture was centrifuged (2875 rpm) for 10 min. at room temperature. The supernatant was discarded, and the pellet containing the platelet fraction was resuspended in 1 ml of 1 mM EDTA, 0.15 M $MgCl_2$, 0.01M Tris-HCl (pH 7.4). The resuspended pellet was then subjected to sonication using three 2- to 3-second bursts (Bronson sonifier). The extracts were centrifuged at 12,000 rpm for 30 min at 4°C. The supernatants were then used as the source of thymidine phosphorylase. Previous studies have shown that human blood platelets do not contain uridine phosphorylase activity[26], and control experiments were performed to confirm this.

2. Enzyme Assay: The phosphorolysis of thymidine by lysed platelets was determined by an HPLC method. In a total volume of 1 ml, the reaction mixture contained thymidine (1 mM), 1 mM EDTA, 0.15 M NaCl, 1 mM sodium phosphate, 0.01 Tris-HCl (pH 7.4). The reaction was started by the addition of the enzyme source derived from the platelet homogenate, and all reactions were performed at 37°C for 30 min. The reaction was terminated with addition of 6% perchloric acid, and samples were then analyzed by HPLC.

HPLC analysis using a Water's C_{18} reverse-phase column was performed, and control experiments revealed that the retention time for thymidine, the natural substrate for ThdPase, was determined to be 9.3 min. and that for thymine, the product of this enzymatic reactions, was 6.2 min. Using this HPLC assay, it was demonstrated that 5-bromo-6-aminouracil, 5-bromo-6-amino-2'-deoxyuridine (**6**) and 5-iodo-6-amino-2'-deoxyuridine (**9**) were potent inhibitors of ThdPase with IC50 values of 6.0 μM, 1.37 μM and 6.49 μM, respectively.

3. *In Vitro* Cytotoxicity Studies: Plastic tissue culture tubes (15 ml, Corning 25200) were seeded with 4.9 ml suspensions of $2x10^4$ cells/ml of HCT-8 human colon cancer cells and incubated at 37°C. After a 24 hr incubation, cells were pre-treated with compound **6** (100 μM) for 30 min prior to FdUrd (0.3 or 1 μM). For control cells, sterile 0.9 % NaCl solution was added to the culture medium. The cells were then incubated with both drugs continuously for 72 hrs. At the end of this incubation period, cells were counted by the trypan blue method[30,31]. In each experiment, the non drug-treated controls were allowed to grow for at least three doublings. All experiments were performed in duplicate, and the data represent the mean ± S.E. from at least 3-5 different experiments. The IC_{50} value represents the concentration of drug that resulted in 50% inhibition of cell growth as determined form the plot of percentage of control growth (cell number) versus the logarithm of drug concentration.

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