Synthesis of 1-[[2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl]-5-benzyluracil and Its Amino Analogue, New Potent Uridine Phosphorylase Inhibitors with High Water Solubility[1]

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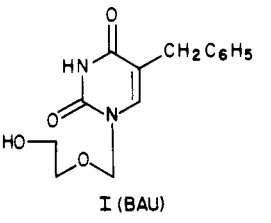
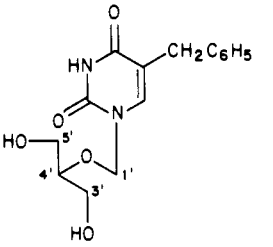
Acyclic nucleosides 1-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]-5-benzyluracil (DHPBU) (1) and 1-[[2-hydroxy-1-(aminomethyl)ethoxy]methyl]-5-benzyluracil (AHPBU) (2) have been synthesized by direct coupling of bis(trimethylsilyl)-5-benzyluracil with the corresponding chloromethyl ether, followed by removal of the blocking groups. Compounds 1 and 2 were found to be very potent inhibitors of uridine phosphorylase isolated from Sarcoma 180 cells, with a K_i value of 0.098 and 0.020 μM, respectively, and exhibited no apparent cytotoxicity against Sarcoma 180 host cells. Furthermore, 1 and 2 have shown excellent water solubility (270 and >300 mg/mL at 25 °C, respectively), which is a factor critical for the formulation that often limits the usefulness of a particular compound as a chemotherapeutic agent.

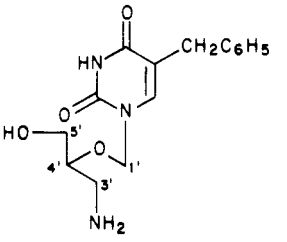
It has been known two mammalian pyrimidine nucleoside phosphorylases, namely, uridine phosphorylase and thymidine phosphorylase, can catalyze the reversible phosphorolysis of pyrimidine nucleosides. Many pyrimidine nucleosides are substrates for one, or both, of these phosphorylases. For instance, both 5-fluorouridine (FUR) and 2'-deoxy-5-fluorouridine (FUdR) are readily cleaved by pyrimidine nucleoside phosphorylases to 5-fluorouracil[3-5] and the corresponding sugar 1-phosphate and thereby inactivated by these enzymes. Earlier, Baker and Kelley[6,7] have synthesized a series of 5-substituted uracil derivatives as potential uridine and thymidine phosphorylases inhibitors. Among these compounds, they found that 5-benzyluracil exerted significant inhibitory effect on uridine phosphorylase from Walker 256 carcinoma (K_i = 5.3 μM).[6] Recently, Niedzwicki et al.[8] have reported the synthesis of 5-benzyl-1-[(2-hydroxyethoxy)methyl]uracil (BAU) (I) and other related derivatives. BAU was found to be a potent inhibitor of uridine phosphorylase (K_i = 0.098 μM)[8,9] and potentiated the anticancer activity of FUdR.[10] Now we report the synthesis of 1-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]-5-benzyluracil (1) (DHPBU) and 1-[[2-hydroxy-1-(aminomethyl)ethoxy]methyl]-5-benzyluracil (AHPBU) (2), two new potent uridine phosphorylase inhibitors with excellent water solubility. Compound 1 and 2 were found to be very potent inhibitors of uridine phosphorylase isolated from Sarcoma 180 neoplastic cells (K_i = 0.098 and 0.020 μM, respectively) and exhibited excellent water solubility (270 and >300 mg/mL at 25 °C, respectively), which is a factor critical for the formulation of chemotherapeutic agents.



I (BAU)



1 (DHPBU)



2 (AHPBU)

Chemistry. Bis(trimethylsilyl)-5-benzyluracil (3), which was prepared by refluxing 5-benzyluracil with hexamethyldisilazane, in the presence of a catalytic amount of ammonium sulfate, was alkylated with 1 molar ratio of the corresponding chloromethyl ether derivatives 4[11] and 5[12] in dry toluene at room temperature for 24 h and then under reflux for another 24 h to give acyclic nucleosides 6 and 7, respectively. Compound 6 was debenzylated by catalytic hydrogenation (10% Pd–C) under 45 psi of hydrogen pressure and room temperature to afford the desired free acyclic nucleoside 1. The phthaloyl protective group in 7 was removed by refluxing 7 with hydrazine in EtOH for 2 h[12] to yield 8. The removal of the benzyl protecting group in 8 could not be achieved by the same catalytic hydrogenation conditions that were used for the deblocking of 6. Instead, 8 was debenzylated by hydrogenation in EtOH with use of $PdCl_2$ as a catalyst at room temperature and under 50 psi of hydrogen pressure for 24 h. Upon removal of the catalyst and the solvent, the

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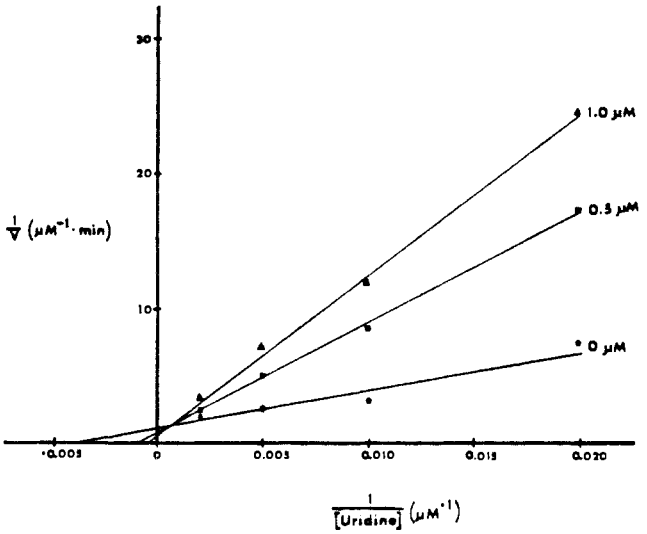


Figure 1. Lineweaver–Burk plots for uridine phosphorylase from Sarcoma 180 cells in the presence of 0, 0.5, and 1.0 μM DHPBU (1); 3 μg of cytosolic protein/assay.

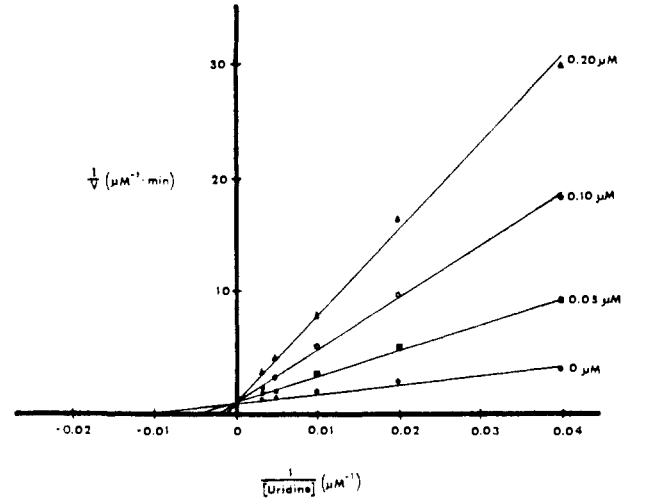


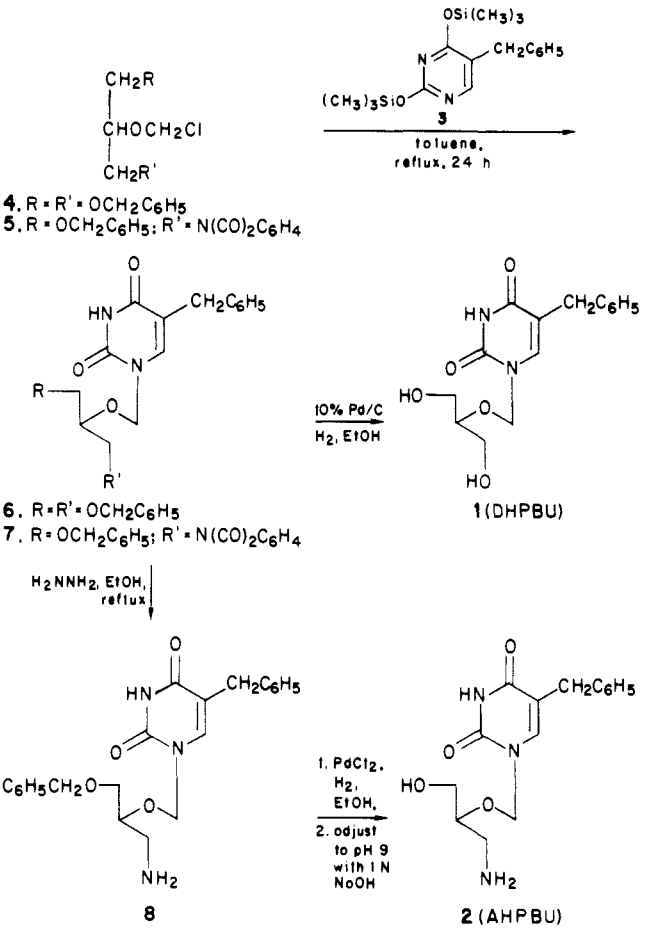
Figure 2. Lineweaver–Burk plots for uridine phosphorylase from Sarcoma 180 cells in the presence of 0, 0.05, 0.10, and 0.20 μM AHPBU (2); 26 μg of cytosolic protein/assay.

residue was dissolved in water. The pH value of the aqueous solution was adjusted with 1 N NaOH to 9 and then spin evaporated in vacuo to dryness. The residue was crystallized from EtOH to give the amino analogue **2**. The synthetic sequences for the synthesis of compounds **1** (DHPBU) and **2** (AHPBU) are depicted in Scheme I.

Biological Evaluation. The initial screening of compounds **1** (DHPBU) and **2** (AHPBU) against uridine phosphorylase showed potent inhibitory activity with K_i values of 0.098 and 0.020 μM for **1** and **2**, respectively. The K_i values were calculated from the slopes of the Lineweaver–Burk plots for uridine phosphorylase as illustrated in Figures 1 and 2. The amino analogue **2** was found to be approximately 5 times more potent than either BAU (I) or DHPBU (**1**). Thus, it would be of interest to synthesize and study the biological and biochemical activity of its two optical enantiomers, (*R*)-**2** and (*S*)-**2**, separately. The synthesis of these two enantiomers is under way.

In addition, compounds **1** and **2** were also tested for cytotoxicity against Sarcoma 180 cells in vitro; these agents exhibited no apparent cytotoxicity up to 1000 μM, as shown in Figure 3. Low cytotoxicity to the host cells is an important requirement for pyrimidine phosphorylases inhibitors that are potentially useful potentiators of the

Scheme I



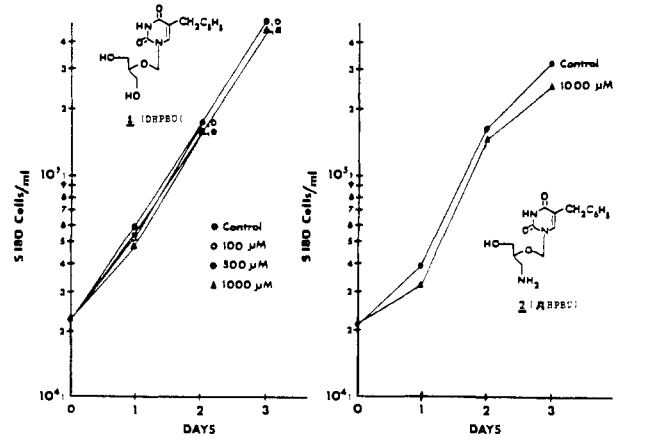


Figure 3. Cytotoxicity tests of (A) DHPBU (1) and (B) AHPBU (2) against Sarcoma 180 cells in vitro.

anticancer activity of certain pyrimidine nucleoside analogues. The more detailed results of the biological and biochemical studies of **1** and **2** and other related compounds will be published elsewhere.[13]

The water solubility of DHPBU (**1**) and AHPBU (**2**) and the known compound, BAU (I) were determined by UV spectroscopic methodology.[14] We have found that **1** and **2** were at least 150 and 166 times more soluble in water than BAU (I) (270 and >300 mg/mL, respectively, vs. 1.8 mg/mL, at 25 °C) and, therefore, it will easily be formulated. Furthermore, the amino analogue **2** can be readily converted to the corresponding hydrochloride or other

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salts, which should enhance the water solubility even further.

The data presented above collectively shows that DHPBU (1) and AHPBU (2) are very potent inhibitors of uridine phosphorylase. This activity, coupled with their high water solubility and low cytotoxicity, makes these agents potential candidates for combination chemotherapy.

We have recently learned that Chu et al.[15] have independently synthesized compound 1 by using a somewhat different methodology.

Experimental Section

Melting points were taken on a Thomas-Hoover Unimelt apparatus and are not corrected. The thin-layer chromatography was performed on EM silica gel 60 F_{254} sheets (0.2 mm). The UV spectra were recorded on a Beckman 25 spectrophotometer, and the NMR spectra were taken on a Varian T-60 or a WM 500 spectrometer at 60 or 500 MHz with Me_4Si as the internal reference. The elemental analyses were carried out by Baron Consulting Co., Analytical Services, Orange, CT. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values.

1-[[2-(Benzyloxy)-1-[(benzyloxy)methyl]ethoxy]methyl]-5-benzyluracil (6). Bis(trimethylsilyl)-5-benzyluracil (3), which was prepared by refluxing 5-benzyluracil (2.0 g, 9.9 mmol) with hexamethyldisilazane (100 mL) and a catalytic amount of ammonium sulfate for 12 h, followed by removal of the excess reagent in vacuo, was alkylated with 1 molar ratio of the corresponding chloromethyl ether derivative (4)[11] (3.3 g, 9.9 mmol) in dry toluene at room temperature for 24 h and then under reflux for another 24 h. The solvent was removed under vacuum, and the residue was treated with EtOH to give the blocked acyclic nucleoside 6. Compound 6 was purified by silica gel column chromatography ($CHCl_3$-EtOH, 20:1) and isolated as a syrup (2.4 g, 47% yield, R_f 0.75): NMR ($CDCl_3$) δ 3.24–3.55 (m, 7 H, 3'-, 4'-, and 5'-H, 5-CH_2), 4.42 (s, 4 H, Ar CH_2O), 5.14 (s, 2 H, NCH_2O), 7.18 (s, 1 H, H-6), 7.22 (m, 15 H, Ar).

1-[[2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl]-5-benzyluracil (1). Compound 6 (2.2 g, 4.05 mmol) was debenzylated by shaking with 0.2 g of 10% Pd–C in MeOH at 45 psi of hydrogen pressure and room temperature for 18 h. The catalyst was removed by filtration, and the solvent was evaporated to dryness in vacuo. The oil residue was then crystallized from $CHCl_3$ to yield 1.24 g (67% yield): mp 78–81 °C; UV (0.1 N HCl) λ_{max} 266 nm (ε 9340); UV (0.1 N NaOH) λ_{max} 266 nm (ε 6500); UV (H_2O) λ_{max} 266 nm (ε 9310); NMR (Me_2SO-d_6) δ 3.25–3.53 (m, 7 H, 3'-, 4'-, and 5'-H, 5-CH_2), 4.52 (t, 2 H, 3'- and 5'-OH, D_2O exchangeable), 5.12 (s, 2 H, NCH_2O), 7.14 (s, 5 H, Ar), 7.46 (s, 1 H, 6-H), 10.1 (br s, 1 H, 1-NH, D_2O exchangeable). Anal. ($C_{15}H_{18}N_2O_5$) C, H, N.

1-[[2-(Benzyloxy)-1-[(phthaloylimino)methyl]ethoxy]methyl]-5-benzyluracil (7). Compound 7 was synthesized by using the same method as described previously in the preparation of 6. Coupling bis(trimethylsilyl)-5-benzyluracil (9.9 mmol) with equal molar ratio of the respective chloromethyl ether 5[12] (3.27 g, 9.9 mmol) yielded 3.14 g (58%) of the desired product: mp 135–136 °C; NMR (Me_2SO-d_6) δ 3.20–3.55 (m, 7 H, 3'-, 4'-, and 5'-H, 5-CH_2), 4.45 (s, 2 H, Ar CH_2O), 5.32 (q, 2 H, NCH_2O), 7.10 (m, 5 H, 5-$CH_2C_6H_5$), 7.26 (m, 5 H, $OCH_2C_6H_5$), 7.75 [m, 4 H, $N(CO)_2C_6H_4$]. Anal. ($C_{30}H_{27}N_3O_6$), C, H, N.

1-[[2-Hydroxy-1-(aminomethyl)ethoxy]methyl]-5-benzyluracil (2). The phthaloyl protective group in 7 was removed by refluxing 7 (3.30 g, 6.27 mmol) with hydrazine (2 mL) in 150 mL of EtOH for 2 h[12] to afford 8 (syrup, 2.0 g, 80% yield): NMR (Me_2SO-d_6) δ 2.70 (d, 2 H, NCH_2C), 3.35–3.59 (m, 5 H, 4'- and 5'-H, 5-CH_2), 4.32 (s, 2 H, Ar CH_2O), 5.10 (s, 2 H, NCH_2O), 5.28 (s, 2 H, 3'-NH_2, D_2O exchangeable), 7.10–7.20 (m, 10 H, Ar), 7.51 (s, 1 H, 6-H). The removal of the benzyl protecting group in 8 could not be achieved by the same catalytic hydrogenation conditions that were used for the deblocking of 6. Instead, 8 (2.10 g, 5.31 mmol) was debenzylated by hydrogenation in 100 mL of EtOH with use of $PdCl_2$ (1 g) as a catalyst at room temperature and under 50 psi of hydrogen pressure for 24 h. Upon removal of the catalyst and the solvent, the residue was dissolved in water. The pH value of the aqueous solution was adjusted with 1 N NaOH to 9 and then spin evaporated in vacuo to dryness. The residue was crystallized from EtOH to give 2 (0.7 g, 44% yield): mp 80–82 °C; UV (0.1 N HCl) λ_{max} 266 nm (ε 9100); UV (0.1 N NaOH) λ_{max} 266 nm (ε 6200); NMR (Me_2SO-d_6) δ 2.72 (d, 2 H, NCH_2C), 3.38–3.59 (m, 5 H, 4'- and 5'-H, 5-CH_2), 5.10 (s, 2 H, NCH_2O), 6.60 (br s, 2 H, 3'-NH_2, D_2O exchangeable), 7.10 (s, 5 H, Ar), 7.51 (s, 1 H, 6-H). Anal. ($C_{15}H_{19}N_3O_4$) C, H, N.

Biological Test Procedures. Mouse S-180 cells were maintained as suspension cultures in Fischer's medium supplemented with 10% horse serum at 37 °C in a humidified atmosphere of 5% CO_2–95% air. Under these conditions the generation time for S-180 cells is approximately 18 h. Each compound at the given concentration was added to S-180 cells (2×10^4 cells/mL), which was in their exponential phase of growth. The increase in cell number of the drug-free culture (control), as well as that of the cultures supplemented with the tested compounds, was determined after 24, 48, and 72 h of growth.

Biochemical Procedures. Compounds 1 and 2 were tested for their inhibitory activity against uridine phosphorylase isolated from Sarcoma 180 tumor cells by the methodology of Niedzwicki et al.[9,16] Determination of K_m for uridine at pH 8.0 for the Sarcoma 180 uridine phosphorylase preparation yielded a value of 108 μM. This value was calculated by the statistical method of Wilkinson,[17] and it represents the average of three determinations. The previously reported value for the K_m at pH 8.0 is 100 μM.

Uridine phosphorylase was assayed at pH 8.0 in buffer A (20 mM potassium phosphate, 1 mM EDTA, and 1 mM β-mercaptoethanol) containing between 1 and 10 μg of S-180 cytosolic protein and the desired concentration of inhibitors. The mixture was preincubated at 37 °C for 5 min and the reaction was initiated by the addition of an appropriate concentration of 2-[^{14}C]uridine in a total volume of 200 μL. At 0, 20, 40, 60, and 90 min after initiation of the reaction, 30-μL aliquots were removed and added to 10 μL of ice-cold 40% perchloric acid, and the mixture was agitated for 2 s to stop the reaction.

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Registry No. 1, 96328-44-8; 2, 96328-45-9; 3, 96328-46-0; 4, 74564-16-2; 5, 90503-17-6; 6, 96328-47-1; 7, 96328-48-2; 8, 96328-49-3; 5-benzyluracil, 18493-83-9; uridine phosphorylase, 9030-22-2.

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