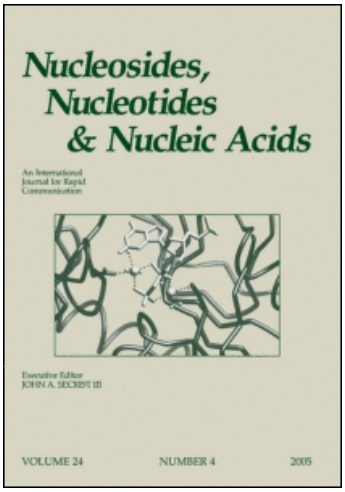

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Synthesis of (*R* - and (*S*)-1-[[2-Hydroxy-1-(aminomethyl)ethoxy]methyl]-5- benzyluracil, Potent Inhibitors of Uridine Phosphorylase

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SYNTHESIS OF (R) - AND (S)-1-[[2-HYDROXY-1-(AMINOMETHYL)ETHOXY]METHYL]-5-BENZYLURACIL, POTENT INHIBITORS OF URIDINE PHOSPHORYLASE

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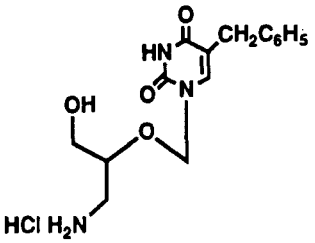
Abstract

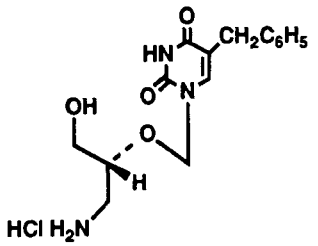
Optically pure (R)- and (S)-1-[[2-hydroxy-1-(aminomethyl) ethoxy]methyl]-5-benzyluracil [(R)-AHPBU and (S)-AHPBU, respectively], two potent uridine phosphorylase inhibitors, have been synthesized via multi-step syntheses starting from independent chiral compounds. The activity of (R)-AHPBU, (S)-AHPBU, and (R,S)-AHPBU which have been previously synthesized, on the inhibition of uridine phosphorylase from Sarcoma-180 cells has been studied and compared. The K_i values for (R,S)-, (R)- and (S)-AHPBU were determined to be 15±1.3, 17±3.7 and 16±2.0 nM, respectively. This indicates that (R) and (S) optical enantiomers have the same affinity for binding to uridine phosphorylase. These acyclic pyrimidine amino nucleoside analogues represent a new class of potent uridine phosphorylase inhibitors, which has a bulky hydrophobic substituent at the 5-position in the uracil base, yet has remarkably high water solubility.

Introduction

Recently[3], we have reported the synthesis of (R,S)-[[2-hydroxy-1-(aminomethyl) ethoxy] methyl]-5-benzyluracil (1), (R,S)-AHPBU, a new potent uridine phosphorylase inhibitor with excellent aqueous solubility (> 300 mg/mL), which is a factor critical for the formulation of chemotherapeutic agents. Since there is a chiral center at carbon-4 in the acyclic carbon chain of the (R,S)-AHPBU structure, this unique property offered us an opportunity to synthesize the (R)- and (S)-AHPBU optical enantiomers independently, and to study their binding affinity to uridine phosphorylase separately. Now, we would like to report the synthesis of (R)- and (S)-AHPBU, compounds 2 and 3, from independent starting chiral materials, and the findings of their biological properties. These inhibitors may have utilities to potentiate the antitumor effects of nucleosides such as 5-fluoro-2′-deoxyurudine[4,5].



1 (R,S)-AHPBU-HCl



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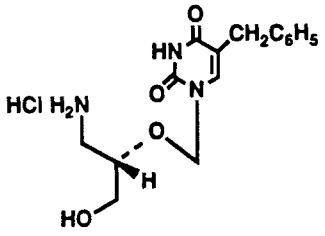
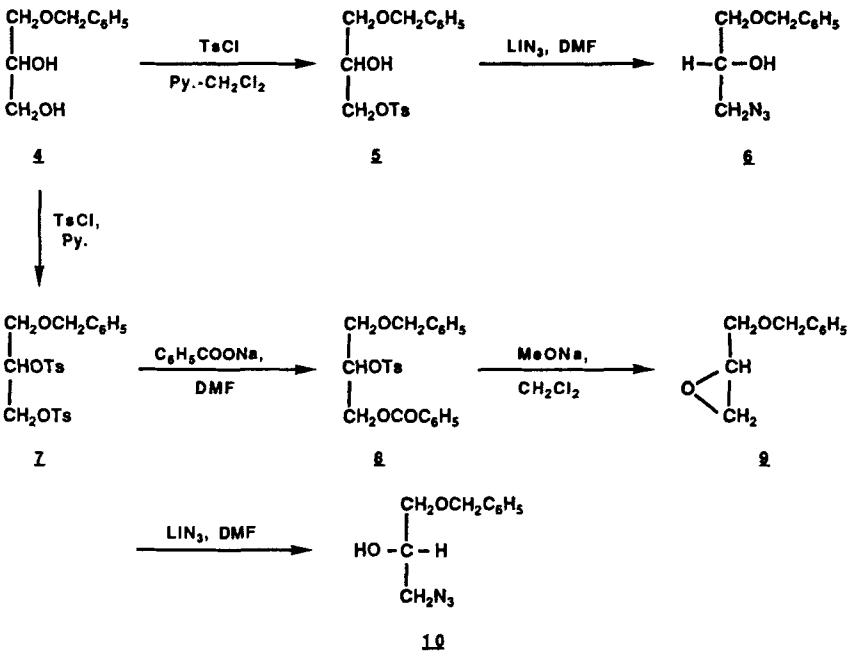


3 (S)-AHPBU-HCl

Chemistry

The optically-active compound 1-O-benzyl-D-glycerol (4), which was prepared from D-mannitol according to the procedures of Howe and Malkin[6], was used as the common starting material for the synthesis of the key chiral intermediates, (R)- and (S)-1-benzyloxy-3-azido-2-propanol, compounds 6 and 10, respectively. Treatment of the diol 4 with p-toluenesulfonyl chloride in a mixture of pyridine and CH_2Cl_2 (1:10) at 0-4°C gave the corresponding 3-p-toluenesulfonate 5[7], which was further reacted with lithum azide in DMF at 80-90°C to furnish the (R)-azido enantiomer 6. Compounds 7-9 were obtained by following the methodology of Anisuzzaman and Owen[8]. The diol 4 was converted to 1-O-benzyl-2,3-di-O-p-toluenesulfonyl-D-glycerol (7), and was then treated with sodium benzoate in DMF to produce the benzoate ester 8, selectively. Treatment of compound 8 with sodium methoxide, resulted in an internal S_N2 displacement reaction, yielding (R)-benzyl 2,3-epoxypropyl ether 9 with inversion of configuration at carbon-2. Ring opening of the epoxide 9 with lithium azide in DMF at room temperature afforded the other desired intermediate (S)-azido enantiomer 10 (Scheme I).

Treatment of the chiral alcohols 6 and 10 with paraformaldehyde and anhydrous hydrogen chloride gas in 1,2-dichloroethane at 0°C afforded the corresponding chloromethyl ethers 11 and 12. Each was then coupled with bis(trimethylsilyl)-5-benzyluracil (13)[3] in refluxing toluene under anhydrous condition to yield the protected azido acyclic nucleosides 14 and 15, respectively. Hydrogenation of compounds 14 and 15 in ethanol at 50 psi of hydrogen pressure, in the presence of 10% palladium on charcoal, gave the corresponding amino derivatives. The removal of the benzyl protecting group in compounds 14 and 15 could not be achieved by the same catalytic hydrogenation conditions. However, by converting the amino derivatives first to their corresponding hydrochloric acid salts,



4 5 6

7 8 9

10

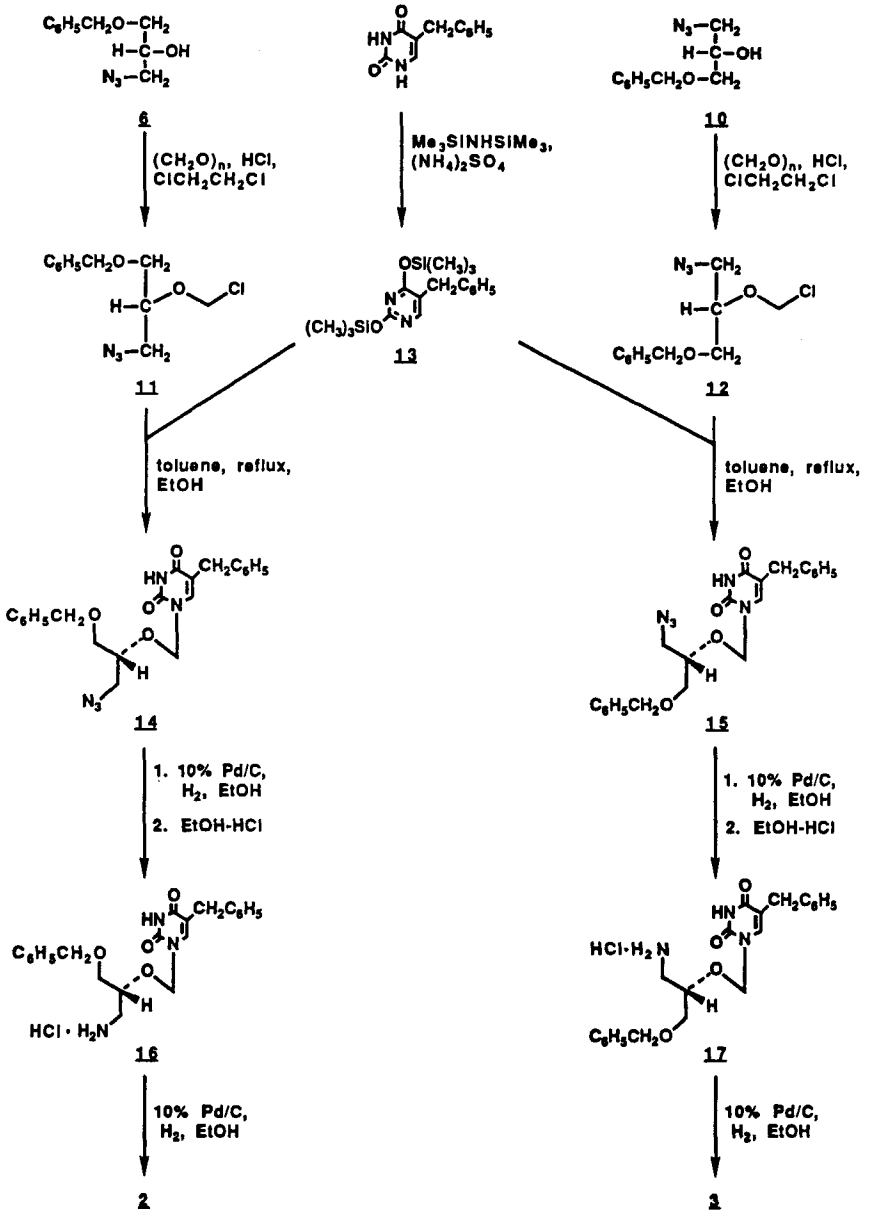
[a]Configuration is represented by Fischer projection convention.

Scheme I[a]

compounds 16 and 17, and then following the same reduction conditions as just mentioned, the respective final deblocked acyclic nucleosides 2 and 3 were obtained (Scheme II).

Biochemical Evaluation

As reported previously[3], (R,S)-AHPBU up to a concentration of 1 mM produced no growth inhibition of cultured S-180 cells. Furthermore, this analogue was found to be a potent inhibitor of uridine phosphorylase with a K_i of approximately 20 nM. However, because this compound was a mixture of (R) and (S) isomers, we wanted to determine if one enantiomer offers any enzymatic binding advantage over the other isomer. The effectiveness of (R,S)-, (R)- and (S)-AHPBU on the inhibition of uridine phosphorylase from S-180 cells is shown in Figures 1, 2 and 3. All three compounds produced competitive inhibition with respect to uridine. Linear regression analysis was used both to obtain the slopes of the Lineweaver-Burk plots and to determine the K_i values from the replots. The K_i values for (R,S)-, (R)- and (S)-AHPBU were calculated to be 15±1.3, 17±3.7 and 16±2.0 nM, respectively. This indicates that the (R) and (S) enantiomers have the same affinity for binding to uridine phosphorylase. Thus, this type of compound represents a new class of potent uridine phosphorylase inhibitors which are acyclic uridine amino nucleoside analogues with a bulky hydrophobic substituent at the C-5 position in the pyrimidine base but with marked high water solubility.



[a]Configuration is represented by Fischer projection convention.

Scheme II[a]

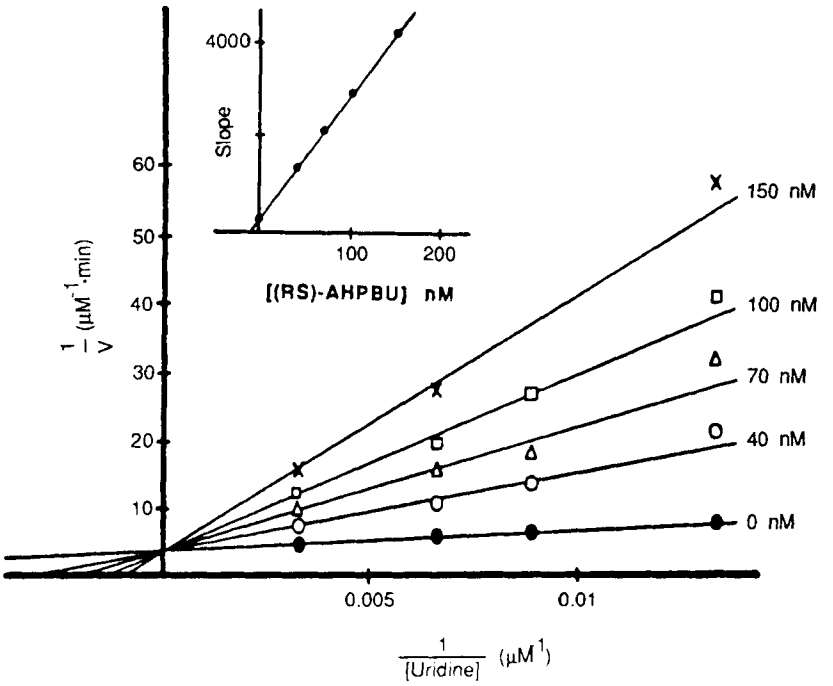


Figure 1. Lineweaver-Burk plot for inhibition of uridine phosphorylase from Sarcoma 180 cells in the presence of 0, 40, 70, 100, and 150 nM (R,S)-AHPBU.

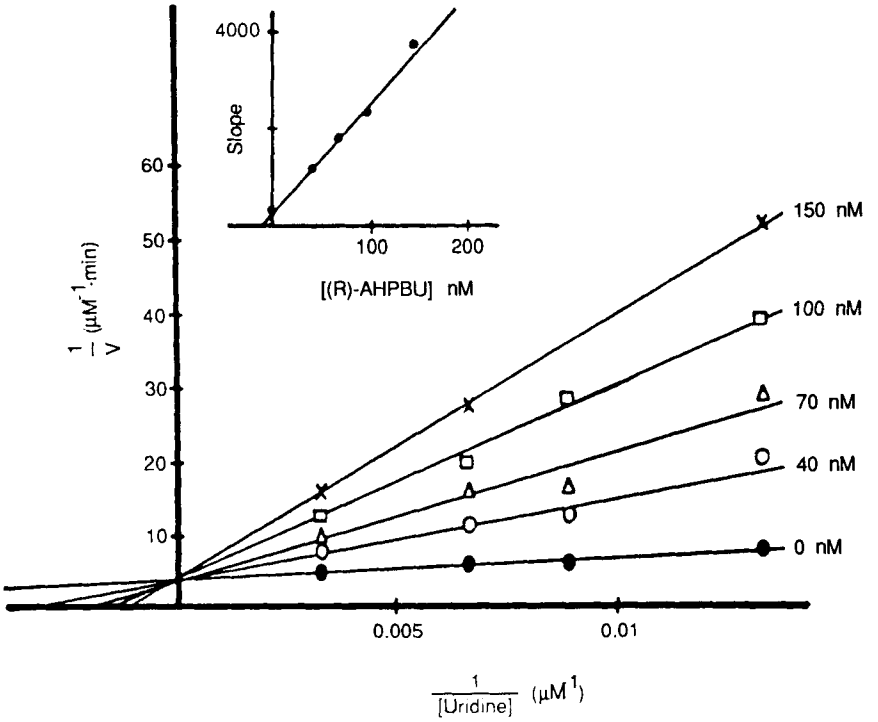


Figure 2. Lineweaver-Burk plot for inhibition of uridine phosphorylase from Sarcoma 180 cells in the presence of 0, 40, 70, 100 and 150 nM (R)-AHPBU.

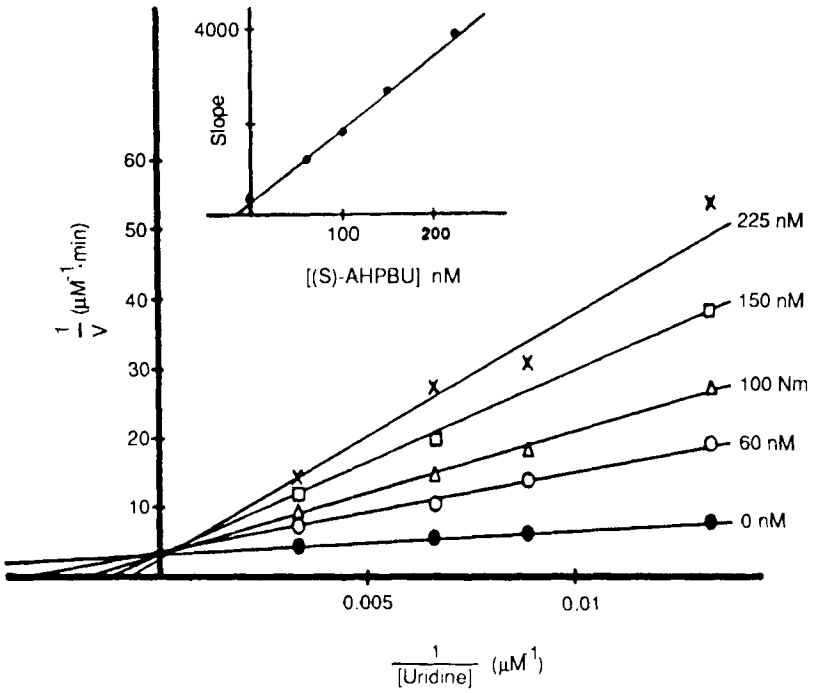


Figure 3. Lineweaver-Burk plot for inhibition of uridine phosphorylase from Sarcoma 180 cells in the presence of 0, 60, 100, 150, and 225 nM (S)-AHPBU.

Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 500 MHz on a Brucker WM-500 spectrometer, with Me_4Si as the internal reference. The UV spectra were recorded on a Beckman-25 spectrophotometer. IR spectra were taken on the Perkin-Elmer 21 spectrophotometer. The mass spectra (at 70 eV) were provided by Yale University Chemical Instrumentation Center. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. TLC was performed on EM precoated silical gel sheets containing a fluorescent indicator. Elemental analyses were carried out by the Baron Consulting Co., 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.

(S)-1-Benzyloxy-3-p-toluenesulfonyloxy-2-propanol (5)

Compound 5 was prepared by a modification of the methodology reported by Belleau and Puranen[7]. A solution of p-toluenesulfonyl chloride (3.9 g, 20 mmol) in 2.6 mL of pyridine and 30 mL of CH_2Cl_2 was added dropwise at 0°C, over a period of 2 h, to a stirred solution of 1-O-benzyl-D-glycerol (4, 3.5 g, 19 mmol) in 30 mL of CH_2Cl_2. The reaction mixture was stirred at 4°C for 20 h, and then washed with 20 mL of 0.1 N HCl, 20 mL of 5% sodium bicarbonate and 20 mL of water. The CH_2Cl_2 solution was dried (Na_2SO_4) and filtered. The filtrate was reduced to a small volume in vacuo and chromatographed on a

silica gel column (EtOAc-hexane, 2:3, R_f 0.35) to give 3.5 g (58%) of product: mp 51-52°C; $[\alpha]^{20}$ + 6.5 (c 5 in MeOH) [lit.[7], mp 48°C; $[\alpha]^{20}$ + 4.6 (c 5 in MeOH)]; NMR ($CDCl_3$) δ 2.39 (s, 3H, ϕ-CH_3), 2.68 (d, 1H, 2-OH, D_2O exchangeable), 3.46 (d, 2H, 1-CH_2), 3.85-4.13 (m, 1H, 2-CH), 4.02 (s, 2H, 3-CH_3), 4.44 (s, 2H, OCH_2Ar), 7.26 (s, 5H, C_6H_5), 7.29 (d, 2H, 3- and 5-H, tosyl), 7.72 (d, 2H, 2- and 6-H, tosyl).

(R)-1-Benzyloxy-3-azido-2-propanol (6)

A mixture of compound 5 (13 g, 38.6 mmol) and lithum azide (4.00 g, 81.7 mmol) in 40 mL of N, N-dimethylformamide was heated with stirring at 85-90°C for 3 h. The solvent was evaporated in vacuo to dryness. The remaining residue was dissolved in 30 mL of CH_2Cl_2 and washed with 10 mL of water (two times), dried (Na_2SO_4), and filtered. The filtrate was evaporated to dryness in vacuo to give the azide 6. Compound 6 was purified by silica gel column chromatography (EtOAc-hexane, 1:1, R_f 0.60) and isolated as an oil (7.22 g, 90% yield): NMR ($CDCl_3$) δ 2.70 (d, 1H, 2-OH, D_2O exchangeable), 3.31 (d, 2H, 3-CH_2), 3.49 (d, 2H, 1-CH_2), 3.77-4.01 (m, 1H, 2-CH), 4.53 (s, 2H, $ArCH_2O$), 7.33 (m, 5H, Ar). Anal. Calcd. for $C_{10}H_{13}N_3O_2$: C, 57.96; H, 6.32; N, 20.28. Found: C, 57.67; H, 6.47; N, 20.57.

Compounds 7, 8 and 9 were synthesized basically by the methodology of Anisuzzaman and Owen[8].

1-0-Benzyl-2, 3-di-0-p-toluenesulfonyl-D-glycerol (7):

mp 59-60°C (lit[8], mp 60-61°C); NMR ($CDCl_3$) δ 2.41 (s, 6H, two CH_3, tosyl), 3.56 (d, 2H, 1-CH_2), 4.13 (d, 2H, 3-CH_2), 4.39 (s, 2H, OCH_2Ar), 4.57-4.87 (m, 1H, 2-CH), 7.28-7.73 (m, 13H, Ar).

(S)-Benzyl-3-benzoyloxy-2-p-toluenesulphonyloxypropyl ether (8):

mp 104-105°C (lit[8], mp 106°C); NMR ($CDCl_3$) δ 2.30 (s, 3H, CH_3, tosyl), 3.70 (d, 2H, 1-CH_2), 4.49 (m, 4H, 3-CH_2 and OCH_2Ar), 4.77-5.03 (m, 1H, 2-CH), 7.06-7.90 (m, 14H, Ar).

(R)-Benzyl-2, 3-epoxypropyl ether (9)

An oil, purified by silica gel column chromatography (EtOAc-hexane, 1:1, R_f 0.75): NMR ($CDCl_3$) δ 2.45-2.73 (m, 2H, 3-CH_2), 2.98-3.16 (m, 1H, 2-CH), 3.32-3.77 (m, 2H, 1-CH_2), 4.53 (s, 2H, OCH_2Ar), 7.30 (s, 5H, Ar).

(S)-1-Benzyloxy-3-azido-2-propanol (10)

A mixture of (R)-benzyl 2, 3-epoxypropyl ether (9, 3.50 g, 21.3 mmol) and lithium azide (1.50 g, 30.6 mmol) in 15 mL of N, N-dimethylformamide was stirred at room temperature for 28 h. The solvent was removed under reduced pressure (~60°C). The resulting residue was dissolved in 25 mL of CH_2Cl_2 and washed with 20 mL of water (two times). The CH_2Cl_2 solution was dried (Na_2SO_4) and filtered. The filtrate was then reduced to a small volume in vacuo and chromatographed on a silica gel column (EtOAc-hexane, 1:1, R_f 0.6) to afford 4.0 g (90%) of 10 as an oil: NMR ($CDCl_3$) δ 2.42 (d, 1H, 2-OH, D_2O exchangeable), 3.07 (d, 2H, 3-CH_2), 3.28 (d, 2H, 1-CH_2), 3.45-3.76 (m, 1H, 2-CH), 4.23 (s, 2H, $ArCH_2O$), 7.03 (m, 5H,-C_6H_5). Anal. Calcd. for $C_{10}H_{13}N_3O_2$: C, 57.96; H, 6.32; N, 20.28. Found: C, 58.25; H, 6.60; N, 20.56.

(R)-2-Chloromethyl-1-benzyloxy-3-azidopropyl ether (11)

Hydrogen chloride gas was bubbled into a stirred mixture of paraformaldehyde (2.5 g, 83 mmol) and 6 (5.2 g, 25 mmol) in 50 mL 1, 2-dichloroethane at 0°C until a clear solution was obtained (~1 h). The solution was dried with $CaCl_2$ to remove the accumulated water. Hydrogen chloride gas was then continually bubbled for an additional 2 h. The solution was dried ($CaCl_2$) again, and filtered. The filtrate was evaporated in vacuo to give a nearly quantitative yield of 11 as a clear oil: NMR ($CDCl_3$) δ 3.43 (d, 2H, 3-CH_2), 3.59 (d, 2H, 1-CH_2), 3.87-4.11 (m, 1H, 2-CH), 4.52 (s, 2H, ArCH_2O), 5.55 (S, 2H, OCH_2Cl), 7.33 (s, 5H, Ar). This compound was used immediately for the next preparation without further purification.

(S)-2-Chloromethyl 1-benzyloxy-3-azidopropyl ether (12)

The title compound was prepared from compound 10 as described for the synthesis of 11 to give nearly quantitative yield as an oil (4.91g), which had an identical NMR as compound 11. This compound was used immediately for the next preparation without further purification.

(R)-1-[[2-Benzyloxy-1-(azidomethyl)ethoxy]methyl]-5-benzyluracil (14)

Bis(trimethylsilyl)-5-benzyluracil (13)[3], which was prepared by refluxing 5-benzyluracil (4.5 g, 22 mmol) with hexamethyldisilazane (50 mL) and ammonium sulfate (0.8 g, 6.0 mmol) under anhydrous condition for 20 h, followed by the removal of excess reagent in vacuo, was alkylated with chloromethyl ether 11 (6.36 g, 24.9 mmol) in dry toluene (50 mL) under reflux for 20 h. The solvent was removed in vacuo, and the residue was treated with EtOH to give the acyclic nucleoside 14. Compound 14 was then purified by silica gel column chromatography (EtOAc-hexane, 1:1, R_f 0.50) to give 4.5g (48%) of product as a syrup: NMR ($CDCl_3$) δ 3.30 (d, 2H, 3′-CH_2N_3), 3.47 (d, 2H, 2′-CH_2), 3.60 (s, 2H, 5-CH_2Ar), 3.77-4.02 (m, 1H, 1′-CH), 4.46 (s, 2H, ArCH_2O), 5.15 (d, 2H, N$CH_2$0), 6.91 (s, 1H, 6-H), 7.10-7.30 (two s, 10H, Ar), 9.77 (s, 1H, 3-NH, D_2O exchangeable), Anal. Calcd. for $C_{22}H_{23}N_5O_4$: C, 62.70; H, 5.50; N, 16.62. Found: C, 62.41; H, 5.50; N, 16.89.

(S)-1-[[2-Benzyloxy-1-(azidomethyl)ethoxy]methyl]-5-benzyluracil (15)

A solution of bis(trimethylsilyl)-5-benzyluracil, which was prepared from 5-benzyluracil (3.70 g, 18.3 mmol) as described above, in dry toluene (50 mL) was reacted with chloromethyl ether 12 (4.91 g, 19.2 mmol), according to the methodology described previously for the synthesis of 14. The acyclic nucleoside 15 was obtained as a syrup (4.0 g, 43%): R_f 0.50 (EtOAc-hexane 1:1), NMR ($CDCl_3$) was identical with that of 14. Anal. Calcd. for $C_{22}H_{23}N_5O_4$: C, 62.70; H, 5.50; N, 16.62. Found: C, 62.39; H, 5.49; N, 16.90.

(R)-1-[[2-Hydroxy-1-(aminomethyl)ethoxy]methyl]-5-benzyluracil hydrochloride (2)

A mixture of compound 14 (2.0 g, 4.75 mmol) and 0.7 g of 10% Pd-C in 100 mL of EtOH was shaken with hydrogen at 50 psi and room temperature for 6 h. The catalyst was removed by filtration and the filtrate was evaporated to afford the amino derivative as a syrup: NMR (Me_2SO-d_6) δ 2.72 (d, 2H, NCH_2C), 3.36-3.61 (m, 5H, 1′-H, 2′-CH_2 and 5′-CH_2Ar), 4.32 (s, 2H, ArCH_2O), 5.12 (s, 2H, NCH_2O), 5.28 (s, 2H, 3′-NH_2, D_2O exchangeable), 7.10-7.20 (m, 1OH, Ar), 7.51 (s, 1H, 6-H). The amino derivative was converted to its hydrochloric acid salt (16) and debenzylated by shaking with 1.0 g of Pd-C in 100 mL of EtOH at 50 psi of hydrogen pressure for 33h. Upon removal of the catalyst and solvent,

the residue was crystallized from EtOH-EtOAc to give 0.88 g (55%) of white crystals: mp 285-287°C dec; $[\alpha]^{23}$ = + 11.5 (c 3, EtOH); UV (0.1 N HCl) λ_{max} 266 nm (ε 8795); UV (0.1 N NaOH) λ_{max} 266 nm (ε 6209); NMR (Me_2SO-d_6) δ 2.83-3.01 (m, 2H, 2′-CH_2), 3.47 (m, 2H, NCH_2C); 3.54 (s, 2H, 5-CH_2Ar), 3.82 (m, 1H, 1′-H), 5.04 (s, 2′-OH, D_2O exchangeable), 5.18 (m, 2H, NCH_2O), 7.19-7.26 (m, 5H, Ar), 7.74 (s, 1H, 6-H), 8.01-8.04 (m, 3H, NH^+_3, D_2O exchangeable), 11.37 (s, 1H, 3-NH, D_2O exchangeable); MS m/e 306 (MH^+). Anal. Calcd. for $C_{15}H_{19}N_3O_4HCl$: C, 52.71; H, 5.90; N, 12.29. Found: C, 52.30; H, 6.21; N, 11.90.

(S)-1-[[2-Hydroxyl-1-(aminomethyl)ethoxy]methyl]-5-benzyluracil hydrochloride (3)

Compound 3 was synthesized from compound 15 (2.5g, 5.9 mmol) via the blocked amino hydrochloric acid salt 17 by the same methodology as described for the synthesis of compound 2: yield, 1.4g (70%); mp 267-269°C dec (from EtOH-EtOAc); $[\alpha]^{23}$ = -11.15 (c 3, EtOH); UV (0.1 HCl) λ_{max} 266 nm (ε 9363); UV (0.1 N NaOH) λ_{max} 266 nm (ε 6478); NMR was identical with that of 2; MS m/e 306 (MH^+). Anal. Calcd. for $C_{15}H_{19}N_3O_4HCl$: C, 52.71; H, 5.90; N, 12.29. Found: C, 53.01; H, 5.62; N, 12.40.

Biochemical Procedures

Exponentially growing S-180 cells were harvested by centrifugation essentially as described[9]. The cell pellet was resuspended in buffer containing 25 mM HEPES, pH 7.5, 4 mM dithiothreitol and 3 mM $MgCl_2$. The cell suspension was frozen and thawed three times before the addition of KCl to a final concentration of 0.15 M. Cells were disrupted by sonication followed by the addition of glycerol to 10% (v/v) before centrifugation at 15,000 X g for 5 min. The supernatant was used as the source of uridine phosphorylase.

Uridine phosphorylase was assayed using [2-^{14}C]uridine essentially as described[10]. The reaction was linear with time and enzyme concentration. Care was taken to ensure reaction linearity at all uridine concentrations used in the kinetic analysis. Each condition described represents the average of duplicate determinations. Linear regression analysis was used to calculate K_i values. The correlation coefficient of the replot for (R,S)-AHPBU, (R)-AHPBU and (S)-AHPBU (Figures 1, 2 and 3, respectively) was 0.9995, 0.9988 and 0.9983.

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