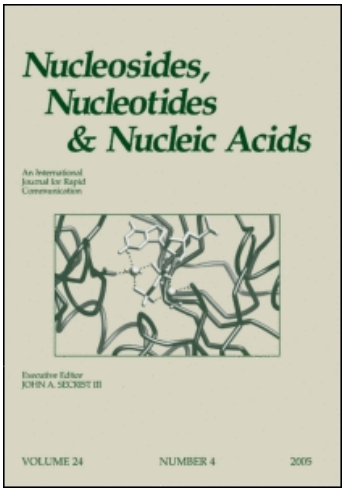

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SYNTHESIS OF CARBOCYCLIC OROTIDINE ANALOGS AS POTENTIAL OROTIDINE DECARBOXYLASE INHIBITORS

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SYNTHESIS OF CARBOCYCLIC OROTIDINE ANALOGS AS POTENTIAL OROTIDINE DECARBOXYLASE INHIBITORS

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ABSTRACT

An asymmetric synthesis of carbocyclic orotidine **15** and its monophosphate **16** were accomplished via the key intermediate cyclopentanone **4**, which was prepared from D-γ-ribonolactone in steps. None of synthesized the compounds inhibited orotidine 5′-monophosphate decarboxylase (EC 4.1.1.23) or orotate phosphoribosyltransferase (EC 2.4.2.10)

INTRODUCTION

Orotidine 5′-monophosphate decarboxylase (ODCase, EC 4.1.1.23) plays a key role in the de novo biosynthesis of pyrimidine nucleotides[1,2]. ODCase catalyzes the last step of the de novo pyrimidine nucleotide biosynthetic pathway whereby orotidine 5′-monophosphate (OMP) is converted to uridine 5′-monophosphate (UMP). From UMP, all pyrimidine nucleotides

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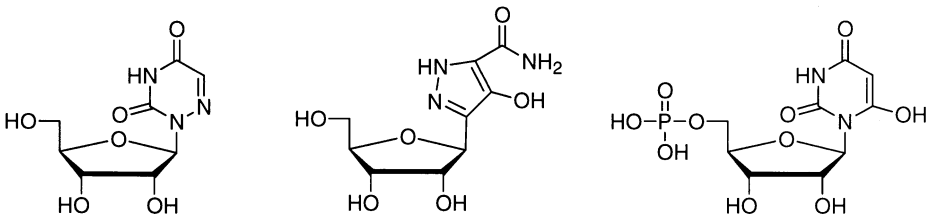


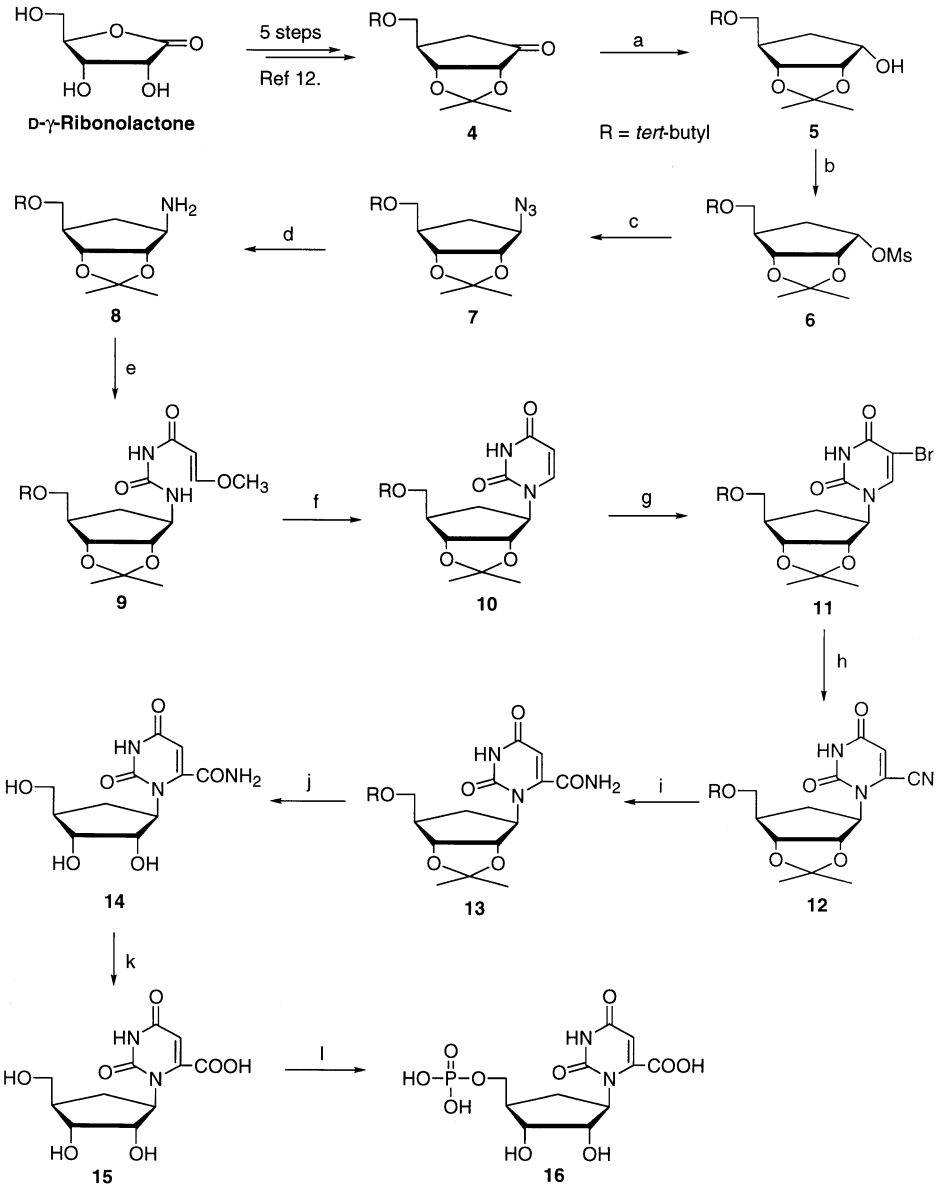
Figure 1. ODCase inhibitor.

are synthesized. This enzymatic reaction by ODCase is the most "efficient" enzymatic reaction described to date[3], with the enzyme-catalyzed V/K value exceeding the spontaneous reaction by a factor of 10^{23}. Thus, inhibitors of ODCase are potentially useful as chemotherapeutic agents that act by interfering with the de novo biosynthesis of pyrimidine nucleotides[4].

To date, only a few ODCase inhibitors have been reported[5,6]. 6-Azauridine (AzUrd, **1**) (Fig. 1) was shown to have activity against experimental tumors in vitro as well as in vivo[5]. Clinical trials demonstrated significant antileukemic activity, although the remissions observed were partial and temporary[7]. Investigation of the cytotoxic mechanism of AzUrd, after intracellular phosphorylation to its nucleoside 5′-monophosphate derivative, revealed that the primary biochemical effect of AzUrd was the inhibition of ODCase[6]. The resulting depletion of the pyrimidine nucleotide pools[8] appeared to account for the inhibition of cells growth by AzUrd, since the inhibition could be prevented by adding a natural pyrimidine nucleoside, uridine or cytidine, to the cell medium[9]. A naturally occurring C-nucleoside, pyrazofurin (**2**) is phosphorylated by adenosine kinase by mammalian cell to pyrazofurin 5′-monophosphate, a potent inhibitor of ODCase ($K_i = 5 \times 10^{-9}$ M). Pyrazofurin exhibited anticancer activity in some leukemia patients but toxicity limits its general usage as an antitumor agent[10]. Pyrazofurin was believed to produce its effects via the inhibition of ODCase[11]. On the basis of these observations, it was of interest to synthesize carbocyclic orotidine **15** and its phosphate analog **16** as potential inhibitors of ODCase.

RESULTS AND DISCUSSION

For the synthesis of the target compounds, we utilized the known intermediate, (-)-cyclopentanone **4** as a chiral starting material, prepared in 5 steps from D-γ-ribonolactone[12] as shown in Sch. 1. Stereo- and regio-selective reduction of the carbonyl group of the cyclopentanone **4** by sodium borohydride in MeOH for 1 h at 0°C in the presence of cerium (III) chloride gave exclusively the α- alcohol **5** in quantitative yield. The stereoselectivity of the



Scheme 1. Reagents: (a) $NaBH_4$, MeOH, 0°C, 1h; (b) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0°C, 2h; (c) NaN_3, DMF, 140°C, 18h; (d) 10% Pd/C, EtOH, rt, 20psi, 5h; (e) β-methoxyacryloyl-isocyanate, DMF, −20 to 20°C, 10h; (f) 30% NH_4OH, EtOH, 80–100°C, 12h; (g) Br_2, DMF, rt, 1h; (h) NaCN, 18-crown-6, rt, 1 day; (i) 0.5N NaOH, 0°C to rt 1h; (j) CF_3CO_2H/H_2O (2:1, v/v), 50°C, 3h; (k) 0.5N NaOH, 90°C, 5h; (l) $POCl_3$, pyridine, H_2O, CH_3CN, 0°C, 4h.

reaction from **4** to **5** is probably due to the electronic effect as well as the steric hindrance of the oxygens of the isopropylidene group, which prevents a nucleophile attack (hydrogen) from the same side of the isopropylidene group. The α- alcohol **5** was converted to its methanesulfonate ester (mesylate **6**), which was subsequently displaced with NaN_3 in DMF for 18 h at 140°C to give the carbocyclic azide **7** in 82% from **5**. Reduction of the azido group of **7** by 10% Pd/C in EtOH at 20 psi for 5 h gave the amino derivative **8** in 87% yield. The uracil derivative **10** was then synthesized by the modified procedure of Shealy et al.[13] Reaction of **8** with β-methoxyacryloyl isocyanate in anhydrous DMF at −20 to 20°C gave the acryloylurea **9** in 87% yield, which was then cyclized with 30% NH_4OH at 100°C in a steel bomb to afford the uracil derivative **10** in 87% yield. Treatment of **10** with bromine in anhydrous DMF at room temperature for 1 h gave the 5-bromouracil analog **11** in 87% yield, which was converted to the 6-cyanourcail analog **12** by the known procedure[14]. Treatment of **12** with 0.5 N NaOH solution for 1 h at room temperature gave the 6-carboxamide uracil derivative **13** in 69% yield. Deprotection of compound **13** with trifluoroacetic acid/H_2O (2:1, v/v) for 3 h at 50°C provided the 6-carboxamide uracil analog **14** in 71% yield, which was further converted to the carbocyclic orotidine **15** by treatment with 0.5 N NaOH for 5 h at 90–100°C in 71% yield. Treatment of **15** with phosphoryl chloride in the presence of pyridine and water in acetonitrile gave the carbocyclic orotidine 5′-monophosphate analog **16** in 72% yield[15].

Carbocyclic 6-carboxamide uridine (**14**), carbocyclic orotidine (**15**) and carbocyclic orotidine 5′-monophosphate (**16**) were tested as potential ligands of ODCase as well as orotate phosphoribosyltransferase (OPRTase, EC 2.4.2.10), the enzyme which precedes ODCase in the de novo pyrimidine biosynthetic pathway, since these two enzymes work in tandem and have similar substrate specificities. Table 1 shows the binding of the carbocyclic orotidine analogs to ODCase and OPRTase from human and mouse livers as measured by percent inhibition of the two enzymes. 6-Azauridine (**1**) and 6-hydroxyuridine (barbituric acid) 5′-monophosphate (**3**) were included as positive controls since these two compounds are known as good inhibitors of ODCase[4].

The results in Table 1 show that the carbocyclic orotidine analogs do not bind to either ODCase or OPRTase from human and mouse livers whereas 6-azauridine (**1**) and 6-hydroxyuridine 5′-monophosphate (**3**) inhibited ODCase from both species and OPRTase from human liver. The results also indicate that the human hepatic enzymes are more sensitive to inhibition by 6-azauridine (**1**) and 6-hydroxyuridine 5′-monophosphate (**3**) than the enzymes from mouse liver.

Since the aglycon moieties are similar in both the carbocyclic orotidine analogs and the natural orotidine nucleosides and nucleotides, the lack of binding of the carbocyclic orotidine analogs suggest that the conformation and/or puckering of the carbocyclic moiety is significantly different from

Table 1. Percent Inhibition of ODCase and OPRTase by Carbocyclic Orotidine Analogs

	% Inhibition			
	ODCase		OPRTase	
Compound	Human Liver	Mouse Liver	Human Liver	Mouse Liver
Carbocyclic 6-carboxamide uridine (**14**)	0.0	0.0	0.0	0.0
Carbocyclic orotidine (**15**)	0.0	0.0	0.0	0.0
Carbocyclic orotidine 5′-monophosphate (**16**)	0.0	0.0	0.0	0.0
6-Azauridine (**1**)	60.6	35.0	25.0	0.0
6-Hydroxyuridine 5′-monophosphate (**3**)	94.5	92.6	26.8	0.0

orotidine which prevents the binding to ODCase. The validity of this proposition would require further experimentations.

EXPERIMENTAL SECTION

Melting points were determined on a Mel-temp II laboratory device and are uncorrected. NMR spectra were recorded on a Brucker AMX 400 Fourier transform spectrometer; chemical shifts are reported in parts per million (δ), and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and dd (doublet of doublets). UV spectra were obtained on a Beckman DU-7 or DU-650 spectrophotometer. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Dry 1,4-dioxane, benzene were obtained by distillation from CaH_2 prior to use. Dry THF was obtained by distillation from Na and benzophenone when the solution became purple.

(1*S*,2*S*,3*R*,4*R*)-4-(*tert*-Butoxymethyl)-2,3-(isopropylidenedioxy)-cyclopentan-1-ol (5). $NaBH_4$ (1.01 g, 0.026 mol) was added to a solution of compound **4** (5 g, 0.02 mol) and $CeCl_3 \cdot 7H_2O$ (7.69 g, 0.02 mol) in MeOH (80 mL) at 0°C. After stirring 1 h at 0°C, cold water was added and the mixture was extracted with EtOAc (2×300 mL). The organic phase was combined, washed with brine (2×200 mL) and then dried over anhydrous Na_2SO_4, and filtered. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (30% EtOAc in n-hexane) to give **5** (4.8 g, 95%) as a syrup: $[\alpha]^{26}_{D} = -16.95°$ (c 1.59, $CHCl_3$); ^{1}H NMR

(400 MHz, $CDCl_3$) δ 1.13 (s, 9H, *tert*-butyl), 1.34 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 1.83 (m, 2H, 5a,b-H), 2.19 (m, 1H, 4-H), 2.44 (d, $J = 9.0$ Hz, 1H, OH, D_2O exchangeable), 3.20 (dd, $J = 4.5$, 8.8Hz,1H, 6a-H), 3.31 (dd, $J = 4.5$, 8.8 Hz, 1H, 6b-H), 4.23 (m, 1H, 1-H), 4.44 (m, 2H, 2-H and 3-H); Anal. Calcd for $C_{13}H_{24}O_4$: C, 63.91; H, 9.90. Found: C, 64.09; H, 9.87.

(1*S*,2*S*,3*R*,4*R*)-4-(*tert*-Butoxymethyl)-2,3-(isopropylidenedioxy)-1-[(methylsulfonyl)oxy]-cyclopentane (6). Methane sulfonyl chloride (4.73 g, 41.27 mmol) was added dropwise at 0°C to a solution of compound **5** (6.50 g, 26.6 mmol) and triethylamine (7.29 g, 72.04 mmol) in 170 mL of CH_2Cl_2. After stirring for 45 min, the reaction mixture was quenched with cold water (270 mL). The aqueous layer was extracted with CH_2Cl_2 (3×200 mL), combined, washed with brine (2×200 mL) and then dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give the crude product **6** (quantitative yield) which was used next without further purification.

(1*R*,2*S*,3*R*,4*R*)-1-azido-4-(*tert*-butoxymethyl)-2,3-(isopropylidenedioxy)-cyclopentane (7). A solution of **6** in dry DMF (300 mL) in the presence of sodium azide (17.29 g, 0.268 mol) was heated at 140°C for 18 h with stirring. After cooling to room temperature, the reaction mixture was filtered, and the filtrate was concentrated to dryness. The residue was partitioned with EtOAc (150 mL) and water (50 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness. The resulting oil was purified by silica gel column chromatography (1–4% EtOAc in n-hexane) to give **7** (5.90 g, 82.6% from **5**) as a oil: $[\alpha]^{26}_D = -46.94°$ (c 1.11, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (s, 9H, *tert*-butyl), 1.30 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.71 (m, 1H, 5a-H), 2.29 (m, 2H, 4-H and 5b-H), 3.29 (dd, $J = 6.7$, 8.8 Hz, 1H, 6a-H), 3.37 (dd, $J = 7.0$, 8.8 Hz, 1H, 6b-H), 3.96 (m, 1H, 1-H), 4.40 (dd, $J = 2.3$, 6.1 Hz, 1H, 3-H), 4.48 (dd, $J = 2.0$, 6.1 Hz, 1H, 2-H); HR-FAB MS Obsd; m/z 270.1823. Calcd for $C_{13}H_{24}N_3O_3$; m/z 270.1818 $(M+H)^+$; Anal. Calcd for $C_{13}H_{23}N_3O_3 \cdot 0.13$-EtOAc: C, 57.95; H, 8.65; N, 14.99. Found: C, 58.25; H, 8.71; N, 14.76.

(1*R*,2*S*,3*R*,4*R*)-4-(*tert*-Butoxymethyl)-2,3-(isopropylidenedioxy)-1-cyclopentanamine (8). A suspension of compound **7** (4.0 g, 3.3 mmol) and 10% Pd/C (1.0 g) in absolute EtOH (140 mL) was shaken under 20 psi of H_2 for 5 h. The reaction mixture was filtered and the filtrate was evaporated to give crude **8** (3.6 g, quantitative yield) which was used for next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (s, 9H, *tert*-butyl), 1.28 (s, 3H, CH_3), 1.36 (m, 1H, 5a-H), 1.45 (s, 3H, CH_3), 1.89 (br s, 2H, NH_2), 2.24–2.36 (m, 2H, 4-H and 5b-H), 3.34–3.43 (m, 3H, 1-H and 6a,b-H), 4.21 (dd, $J = 2.6$, 6.2 Hz, 1H, 3-H), 4.48 (dd, $J = 2.8$, 6.2 Hz, 1H, 2-H; HR-FAB MS Obsd; m/z 244.1965. Calcd for $C_{13}H_{26}NO_3$; m/z 244.1913 $(M+H)^+$;

Anal. Calcd for $C_{13}H_{25}NO_3 \cdot 0.16H_2O$: C, 63.41; H, 10.37; N, 5.69. Found: C, 63.09; H, 10.16; N, 5.59.

N-{[(1*R*,2*S*,3*R*,4*R*)-4-(*tert*-Butoxymethyl)-2,3-(isopropylidenedioxy) cyclopentyl]aminocarbonyl}-3-methoxy-2-propenamide (9). Silver cyanate (7.60 g, dried in vacuo over phosphorus pentoxide in the dark at 100°C for 3 h) was added to a solution of β-methoxy acryloyl chloride (2.64 g, 22.1 mmol) in anhydrous benzene (30 mL). The resulting mixture was heated under reflux for 30 min and allowed to cool to room temperature. After the solid phase had settled, 22.5 mL of the supernatant solution which contained β-methoxy acryloyl isocyanate was added during 15 min to a solution of amine **8** (3.0 g, 12.33 mmol) in dried DMF (50 mL) at -15 to -20°C under nitrogen. The reaction mixture was stirred for 2 h at -15°C and then for 10 h at room temperature under nitrogen. The mixture was evaporated under reduced pressure and coevaporated with toluene (2×20 mL) to afford **9** (3.96 g, 87%) as a solid: $[\alpha]_D^{27} = -26.62°$ (c 0.57, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.17 (s, 9H, *tert*-butyl), 1.28 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.58 (m, 1H, 5′a-H), 2.28 (m, 1H, 4′-H), 2.36–2.43 (m, 1H, 5′b-H), 3.33–3.42 (m, 2H, 6′a,b-H), 3.73 (s, 1H, OCH_3), 4.20 (m, 1H, 3′-H), 4.45 (m, 2H, 1′-H and 2′-H), 5.35 (d, $J = 12.3$ Hz, 1H, 5-H), 7.67 (d, $J = 12.3$ Hz, 1H, 6-H), 8.72 (br s, 1H, NH), 9.35 (br s, 1H, NH); Anal. Calcd for $C_{18}H_{30}N_2O_6$: C, 58.36; H, 8.16; N, 7.56. Found: C, 58.28; H, 8.13; N, 7.60.

(1′*R*,2′*S*,3′*R*,4′*R*)-1-[4-(*tert*-Butoxymethyl)-2,3-(isopropylidenedioxy) cyclopentan-1-yl] uracil (10). Compound **9** (4.2 g, 11.34 mmol) was dissolved in ethanol (25 mL) and aqueous ammonium (30%, 11 mL). The reaction mixture was heated at 100°C in a steel bomb for 12 h. After cooling, the solution was evaporated to dryness. The residue was purified by silica gel column chromatography (50% EtOAc in n-hexane) to give **10** (3.21 g, 86.7%) as a white foam: $[\alpha]_D^{27} = -41.53°$ (c 0.88, $CHCl_3$); UV (MeOH) λ_{max} 266.0 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19 (s, 9H, *tert*-butyl), 1.30 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 1.97 (m, 1H, 5′a-H), 2.32–2.41 (m, 2H, 4′-H and 5′b-H), 3.43–3.50 (m, 2H, 6′a,b-H), 4.48 (dd, $J = 4.1$, 6.5 Hz, 1H, 3′-H), 4.65–4.75 (m, 2H, 1′-H and 2′-H), 5.72 (d, $J = 8.0$ Hz, 1H, 5-H), 7.35 (d, $J = 8.0$ Hz, 1H, 6-H), 8.63 (br s, 1H, NH); HR-FAB MS Obsd; m/z 339.1954. Calcd for $C_{17}H_{27}N_2O_5$; m/z 339.1920 $(M+H)^+$; Anal. Calcd for $C_{17}H_{26}N_2O_5$: C, 60.34; H, 7.74; N, 8.28. Found: C, 60.06; H, 7.70; N, 8.14.

(1′*R*,2′*S*,3′*R*,4′*R*)-1-[4-(*tert*-Butoxymethyl)-2,3-(isopropylidenedioxy) cyclopentan-1-yl]-5-bromouracil (11). Bromine (2.82 g, 17.73 mmol) in CCl_4 (20 mL) was added to a solution of compound **10** (3 g, 8.86 mmol) in anhydrous DMF (50 mL) at room temperature. The reaction mixture was stirred for 1 h at room temperature. The solvent was evaporated and the residue was purified by silica gel column chromatography (50% EtOAc in n-hexane) to

give **11** (3.3 g, 86.7%) as a white foam: $[\alpha]_D^{27} = -53.14°$ (c 1.09, $CHCl_3$); UV (MeOH) λ_{max} 283 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (s, 9H, *tert*-butyl), 1.31 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 1.93 (m, 1H, 5′a-H), 2.41–2.46 (m, 2H, 4′-H and 5′b-H), 3.47–3.50 (m, 2H, 6′a,b-H), 4.46 (m, 1H, 3′-H), 4.65 (t, $J = 6.09$ Hz, 1H, 2′-H), 4.81 (m, 1H, 1′-H), 7.71 (s, 1H, 6-H), 8.45 (br s, 1H, NH); HR-FAB MS Obsd; m/z 417.1008. Calcd for $C_{17}H_{26}BrN_2O_5$; m/z 417.1025 $(M+H)^+$; Anal. Calcd for $C_{17}H_{25}BrN_2O_5 \cdot 0.7$MeOH: C, 48.35; H, 6.37; N, 6.36. Found: C, 48.13; H, 6.13; N, 6.36.

(1′*R*,2′*S*,3′*R*,4′*R*)-1-[4-(*tert*-Butoxymethyl)-2,3-(isopropylidenedioxy) cyclopentan-1-yl]-6-cyanouracil (12). NaCN (281 mg, 5.75 mmol) and 18-crown-6 (126 mg, 0.479 mmol) was added to a solution of compound **11** (2 g, 4.79 mmol) in anhydrous DMF (50 mL) at room temperature. The reaction mixture was stirred for 1 day and then diluted with cold water (50 mL) and carefully neutralize with aqueous d-acetic acid. The mixture was extracted with EtOAc (3 × 200 mL), washed with brine, dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was purified by silica gel column chromatography (40% EtOAc in n-hexane) to give **12** (1.42 g, 81.6%) as a white solid: mp: 69–71°C; $[\alpha]_D^{27} = -6.27°$ (c 0.58, $CHCl_3$); UV (MeOH) λ 292 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (s, 9H, *tert*-butyl), 1.31 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 1.93 (m, 1H, 5′a-H), 2.41–2.48 (m, 2H, 4′-H and 5′b-H), 3.34–3.57 (m, 2H, 6′a,b-H), 4.50 (m, 1H, 3′-H), 4.71 (m, 1H, 2′-H), 5.07 (m, 1H, 1′-H), 6.26 (s, 1H, 5-H), 8.28 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 25.47, 27.47, 27.69, 32.28, 44.59, 62.53, 66.99, 72.91, 81.11, 81.40, 111.09, 112.16, 113.55, 129.55, 149.05, 160.85; HR-FAB MS Obsd; m/z 364.1841. Calcd for $C_{18}H_{26}N_3O_5$; m/z 364.1872 $(M+H)^+$; Anal. Calcd for $C_{18}H_{25}N_3O_5 \cdot 0.19H_2O$: C, 58.94; H, 6.97; N, 11.45. Found: C, 59.01; H, 6.91; N, 11.25.

(1′*R*,2′*S*,3′*R*,4′*R*)-1-[4-(*tert*-Butoxymethyl)-2,3-(isopropylidenedioxy) cyclopentan-1-yl]-6-carboxamide uracil (13). Compound **12** (1.98 g, 59.28 mmol) was dissolved in 0.5 N NaOH (35 mL). The reaction mixture was stirred at 0°C and then warmed to room temperature. After stirring 1 hr, Dowex 50W × 8-200 ion exchange resin was added to neutralize the solution. After filtration resin, the mixture was extracted with EtOAc (200 mL × 2), dried over $MgSO_4$ and filtered, and then concentrated to dryness. The residue was purified by silica gel column chromatography (5% MeOH in $CHCl_3$) to give **13** (1.57 g, 69%) as a white solid: mp: 161–163°C; $[\alpha]_D^{25} = 28.64°$ (c 0.32, MeOH); UV (MeOH) λ_{max} 270.5 nm; ^{1}H NMR (400 MHz, DMSO-d_6 and D_2O) δ 1.09 (s, 9H, *tert*-butyl), 1.17 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.97–2.17 (m, 3H, 4′-H and 5′a,b-H), 3.21–3.28 (m, 1H, 6′a-H), 3.39–3.43 (m, 1H, 6′b-H), 4.12 (m, 1H, 3′-H), 4.36 (t, $J = 6.17$ Hz, 1H, 2′-H), 4.91 (m, 1H, 1′-H), 5.60 (s, 1H, 5-H); Anal. Calcd for $C_{18}H_{27}N_3O_6 \cdot 0.4H_2O$: C, 55.63; H, 7.21; N, 10.81. Found: C, 55.61; H, 7.21; N, 10.74.

(1*R*,2*S*,3*R*,4*R*)-2′,3′-Dihydroxy)-4′-hydroxymethyl-6-carboxamide uracil (14). Compound **13** (900 mg, 2.35 mmol) was dissolved in 60 mL of CF_3COOH/H_2O (v/v, 2:1). The solution was stirred for 3 h at 50°C. After cooling to room temperature, the solvent was evaporated under reduced pressure and coevaporated with EtOH (30 mL × 2). The residue was purified by silica gel column chromatography (20% MeOH in $CHCl_3$) to give **14** (475 mg, 71%) as a white solid which was washed with cold methanol: mp: 226–228°C; $[\alpha]_D^{25} = 6.36°$ (c 0.19, H_2O); UV (H_2O) λ_{max} 271.5 nm (ε 8 148) (pH 7), 271.0 nm (ε 7 805) (pH 2), 271.0 nm (ε 5 959) (pH 11); ^{1}H NMR (400 MHz, DMSO-d_6+D_2O) δ 1.80–2.01 (m, 3H, 4′-H and 5′a,b-H), 3.35–3.38 (m, 1H, 6′a-H), 3.52–3.56 (m, 1H, 6′b-H), 3.75–3.78 (m, 1H, 3′-H), 3.91–3.97 (m, 1H, 2′-H), 4.37 (t, $J = 6.26$ Hz, 1H, 1′-H), 5.64 (s, 1H, 5-H); HR-FAB MS Obsd; m/z 286.1060. Calcd for $C_{11}H_{16}N_3O_6$; m/z 286.1039 $(M+H)^+$; Anal. Calcd for $C_{11}H_{15}N_3O_6 \cdot 1.2H_2O$: C, 43.05; H, 5.72; N, 13.69. Found: C, 43.21; H, 5.59; N, 13.47.

(1*R*,2*S*,3*R*,4*R*)-2′,3′-Dihydroxy)-4′-hydroxymethyl-6-carboxylic uracil (carbocyclic orotidine, 15). Compound **14** (300 mg, 1.05 mmol) was dissolved in 20 mL of 0.5 N NaOH solution. The solution was stirred for 5 h at 90–100°C. After cooling to room temperature, the solution was added Dowex 50 (H^+) resin to adjust pH 2–3. After filtration of the resin, the filtrate was applied to DEAE-Sephadex column chromatography with the linear concentration gradient elution of triethylammonium bicarbonate (0–0.1 M). The fraction containing carbocyclic orotidine **15** was pooled and concentrated. The residue was dissolved in water and acidified through short Dowex 50 (H^+) resin column. The eluent was concentrated to give **15** as a white solid: $[\alpha]_D^{23} = 18.27°$ (c 0.22, H_2O); UV (H_2O) λ_{max} 271.0 nm (ε 8 345) (pH 7), 270.5 nm (ε 8 113) (pH 2), 270.0 nm (ε 7 588) (pH 11); ^{1}H NMR (400 MHz, DMSO-d_6+D_2O) δ 1.70–1.96 (m, 3H, 4′-H and 5′a,b-H), 3.26–3.31 (m, 1H, 6′a-H), 3.46–3.50 (m, 1H, 6′b-H), 3.82–3.89 (m, 1H, 3′-H), 4.11–4.17 (m, 1H, 2′-H), 4.32 (t, $J = 6.26$ Hz, 1H, 1′-H), 5.24 (s, 1H, 5-H); HR-FAB MS Obsd; m/z 287.2397. Calcd for $C_{11}H_{15}N_2O_7$; m/z 287.2381 $(M+H)^+$; Anal. Calcd for $C_{11}H_{14}N_2O_7$: C, 46.16; H, 4.93; N, 9.79. Found: C, 46.31; H, 4.88; N, 9.85.

(1*R*,2*S*,3*R*,4*R*)-2′,3′-Dihydroxy)-4′-phosphonoxymethyl-6-carboxylic uracil (16). Compound **15** (150 mg, 0.52 mmol) was added to a solution of phosphoryl trichloride (241 mg, 1.57 mmol) and water (14 mg, 0.78 mmol) in acetonitrile (2 mL) at 0°C. The reaction mixture was stirred for 4 h at 0°C then poured into ice water and stirred further for 1 h. After concentration to 2 mL, the concentrate was applied to a column Dowex (H^+) ion exchange resin. The column was washed with water to remove starting material and then the product was eluted with a linear gradient of water and 0.5 M formic acid. The fraction containing the compound **16** was pooled and concentrated

to give **16** as a white solid: $[\alpha]_D^{25} = 9.34°$ (c 0.21, H_2O); UV (H_2O) λ_{max} 270.5 nm (ε 8 342) (pH 7), 270.5 nm (ε 8 759) (pH 2), 270.5 nm (ε 7 750) (pH 11); ^1H NMR (400 MHz, DMSO-d_6+D_2O) δ 1.81–2.04 (m, 3H, 4′-H and 5′a,b-H), 3.25–3.32 (m, 1H, 6′a-H), 3.50–3.55 (m, 1H, 6′b-H), 3.80–3.85 (m, 1H, 3′-H), 3.88–3.93 (m, 1H, 2′-H), 4.32 (t, $J = 6.26$ Hz, 1H, 1′-H), 5.58 (s, 1H, 5-H); HR-FAB MS Obsd; m/z 367.2180. Calcd for $C_{11}H_{16}N_2O_{10}P$; m/z 367.2165 $(M+H)^+$; Anal. Calcd for $C_{11}H_{15}N_2O_{10}P$: C, 36.08; H, 4.13; N, 7.65. Found: C, 36.12; H, 4.05; N, 7.54.

ENZYME STUDIES

Preparation of Cell Extracts

Mouse livers were obtained from female CD-1 mice (20–25 g) obtained from Charles River Laboratories. Human liver specimens were obtained from donors through the Alabama Regional Organ Bank. Livers were homogenized in 3 volumes of 50 mM Tris-Cl (pH 8.0) buffer containing, 5 mM $MgCl_2$ and 1 mM DTT at 4°C using a Brinkmann polytron homogenizer (Brinkmann Instruments, Westbury, NY). The homogenate was then centrifuged at 105,000 × g for 1 h at 4°C. The supernatant fluid (cytosol) was collected and used as the source of enzyme.

Enzyme Assay

ODCase activity was measured simultaneously with orotate phosphoribosyltransferase (OPRTase) activity, from human and mouse livers, by following the formation of orotidine 5′-monophosphate (OMP), orotidine, UMP, and uridine and uracil formation from [6-^{14}C]orotate. The standard assay mixture contained 50 mM Tris-Cl buffer (pH 8.0), 5 mM $MgCl_2$, 1 mM DTT, 2.5 mM 5-phosphoribosyl 1-pyrophosphate (PRPP), 5 μM [6-^{14}C]orotate (56.2 Ci/mol) (Moraveck Biochemical Inc), 0.9 mM of the compound to be tested, and 25 μL of enzyme extract in a final volume of 50 μL. Assays were run at 37°C under conditions where activity was linear with time and enzyme concentration. Reactions were started by the addition of extract and terminated after 30 min by boiling in a water bath for 2 min followed by freezing. Precipitated proteins were removed by centrifugation. The substrate was separated from products in the supernatant by thin layer chromatography (TLC). After removal of the precipitated proteins, a 10 μL of the supernatant fluid was spotted on prewashed PEI TLC plates (Brinkmann). The plates were developed first in distilled water to a front of 10 cm. They were then dried and redeveloped in 0.2 M lithium chloride. R_f values were OMP, 0.16; UMP, 0.51; orotate, 0.62; orotidine, 0.77 and uridine, 0.95. OPRTase activity was measured as the sum of OMP, orotidine, UMP, and uridine and uracil

combined while ODCase activity was measured as the sum of UMP, and uridine and uracil combined. The radioactivity in the spots was determined on a percentage basis using a Berthold LB-2821 Automatic TLC-Linear Analyzer. Protein concentrations were determined spectrophotometrically by the method of Bradford[16] using bovine γ-globulin (Bio-Rad Lab) as a standard. Enzyme specific activities (pmol/mim/mg protein) were estimated by computer programs written by Dr. F. N. M. Naguib.

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REFERENCES

1. Miller, B.G.; Hassell, A.M.; Wolfenden, R.; Milburn, M.V.; Short, S.A. Proc. Natl. Acad. Sci. U.S.A. **2000**, *97*, 2011–2016.
2. Wu, N.; Mo, Y.; Gao, J.; Pai, E.F. Proc. Natl. Acad. Sci. U.S.A. **2000**, *97*, 2017–2022.
3. Radzicka, A.; Wolfenden, R. Science **1995**, *267*, 90–93.
4. Niedzwicki, J.G.; Iltzsch, M.H.; EL Kouni, M.H.; Cha, S. Biochem. Pharmac. **1984**, *33*, 2383–2395.
5. Wotring, L.L.; Townsend, L.B. Cancer Res. **1989**, *49*, 289–294.
6. Handschumacher, R.E. J. Biol. Chem. **1960**, *235*, 2917–2919.
7. Fallon, H.J.; Frei, E.; Freireich, E. J. Am. J. Med. **1962**, *33*, 526–537.
8. Janeway, C.M.; Cha, S. Cancer Res. **1977**, *37*, 4382–4388.
9. Skoda, J.; Sorm, F. Collect. Czech, Chem. Commun. **1956**, *21*, 1328–1331.
10. Cadman, E.C.; Dix, D.E. Cancer Res. **1978**, *38*, 682–688.
11. Calabresi, P.; Parks, R.E. In *The Pharmacological Basis of Therapeutics*; Goodman, A.G., Goodman, L.S., Gilman, A., Eds.; Macmillan: New York, 1980; 6th Edn., 1256.
12. (a) Chun, B.K.; Chu, C.K. Tetrahedron Lett. **1999**, *40*, 3309–3312. (b) Ali, S.M.; Ramesh, K.; Borchardt, R.T. Tetrahedron Lett. **1990**, *31*, 1509–1512.
13. Shealy, Y.F.; O'Dell, C.A.; Thorpe, M.C. J. Heterocycl. Chem. **1981**, *18*, 383–389.
14. Matsuda, H.; Inoue, H.; Ueda, T. Chem. Pharm. Bull. **1978**, *26*, 2340–2345.
15. Sowa, T.; Ouchi, S. Bull. Chem. Soc. Jpn. **1975**, *48*, 2084–2090.
16. Bradford, M.M. Anal. Biochem. **1976**, *72*, 248–254.

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