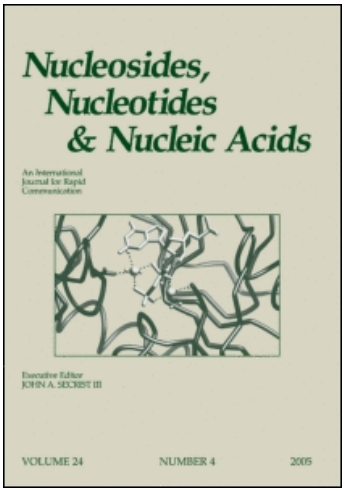

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Inhibition of Cytidine Deaminase by Derivatives of 1-(β-D-Ribofuranosyl)-Dihydropyrimidin-2-One (Zebularine)

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NUCLEOSIDES & NUCLEOTIDES, 11(10), 1781-1793 (1992)

INHIBITION OF CYTIDINE DEAMINASE BY DERIVATIVES OF 1-(β-D-RIBOFURANOSYL)-DIHYDROPYRIMIDIN-2-ONE (ZEBULARINE)

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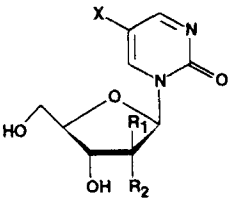
Abstract: The 2'-deoxy and *ara* derivatives of 1-β-(D-ribofuranosyl)-1,2-dihydropyrimidin-2-one (zebularine) were synthesized by improved routes and tested for their inhibitory properties against cytidine deaminase. It was shown that the K_i's of both compounds were comparable to that of the parent zebularine in inhibition studies with purified enzyme. In contrast to zebularine, 2'-deoxy and *ara* zebularine showed only nominal cytotoxicity against MOLT-4 and L1210 cells *in vitro*. A model compound for the inhibition of deoxycytidylate deaminase, 2'-deoxyzebularine 5'-monophosphate (**6**), was also prepared.

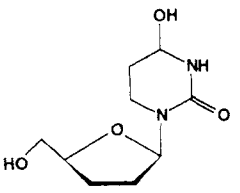
Analogues of deoxycytidine, such as cytosine arabinoside (*ara*-C) must be phosphorylated intracellularly in order to display antitumor or antiviral activity.[1] These drugs are also subject to metabolic degradation by cytidine deaminase (CDA) and deoxycytidylate deaminase (dCMPD) which function, respectively, at the nucleoside and monophosphate level. The balance among these anabolic and catabolic processes is critical for the maintenance of adequate levels of the active nucleotide forms. In addition, the products of these enzymatic deaminations are usually devoid of biological activity. The major sites of deamination in humans are the liver and spleen, and the high levels of CDA in these tissues are responsible for the rapid inactivation of these

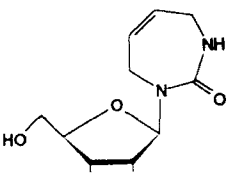
drugs.[2] As expected, inhibitors of CDA have been shown to prolong the plasma half-life of these drugs.[3]

Derivatives of 4-desaminocytidine (zebularines, **1**) have recently been evaluated with regard to their antitumor[4] and antiviral[5] potential. Although devoid of any prophylactic capacity against the pathogenic affects of human immuodeficiency virus type 1 (HIV-1), it was shown that the parent compound zebularine (**1a**) and its 5-fluoro analogue (**1b**) exhibited reasonable activity against L1210 leukemia *in vivo*.[4] Moreover, compounds **1a** and **1b** are known inhibitors of CDA,[6] and a distinct therapeutic advantage could be derived by the simultaneous administration of these drugs with cytidine analogues which are susceptible to enzymatic deamination.[4,7] Since cytidine analogues with 2'-deoxy- and *ara*-pentofuranosyl sugars are also good substrates for CDA,[8] we wanted to compare the CDA inhibitory activity of zebularine with that of the corresponding 2-deoxy- and *ara*-pentofuranosyl analogues. In addition, 2'-deoxyzebularine 5'-monophosphate was synthesized and its inhibitory activity against dCMPD was investigated.



1a; R_1=H, R_2=OH, X=H
1b; R_1=H, R_2=OH, X=F
1c; R_1=H, R_2=H, X=H
1d; R_1=OH, R_2=H, X=H
1e; R_1=F, R_2=H, X=H

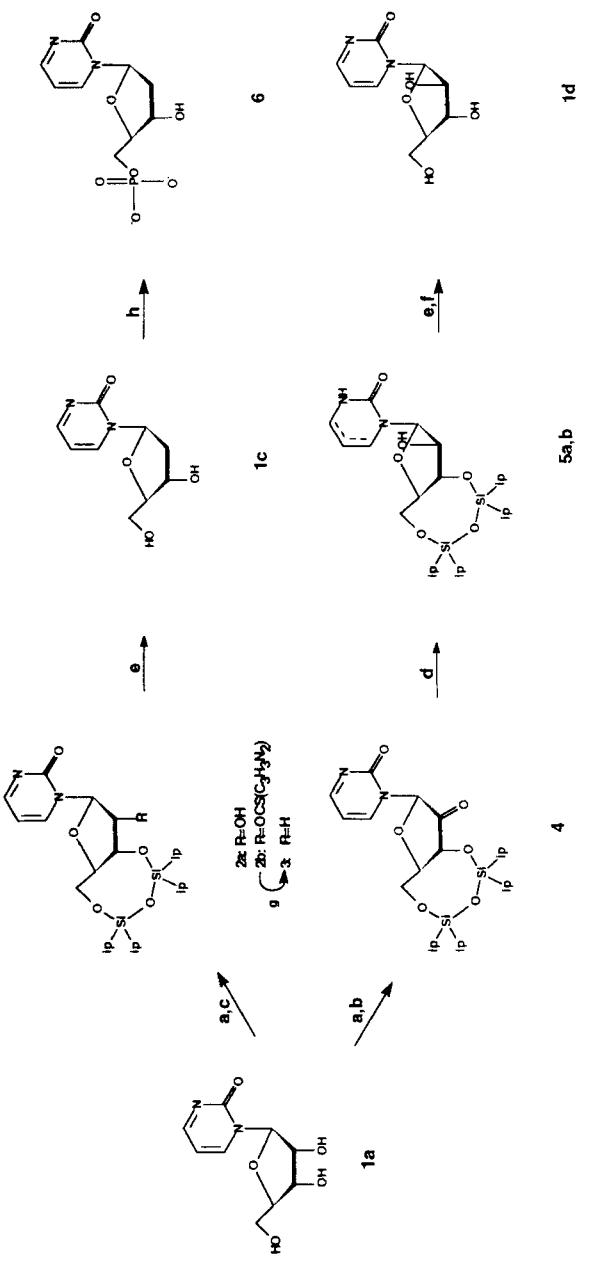




Chemistry. Compounds **1c** and **1d** were previously prepared by reductive desulfuration of the corresponding 4-thiouracil derivatives in only moderate yield.[9] Since we had access to large amounts of zebularine, we developed alternate routes to **1c** and **1d** from zebularine itself as outline in Scheme 1. This approach allows for the construction of several zebularine congeners. Treatment of **1a** in dry pyridine with Markiewicz's reagent[10] (1,3-dichloro-1,1,3,3,-tetraisopropyldisiloxane) gave the 3',5' cyclic TIPS derivative **2a** in nearly quantitative yield. This intermediate was processed to 2'-deoxyzebularine via deoxygenation of the 2'- thiocarbonylimidazole precursor **2b** with tris(trimethylsilyl)silane[11] in very good yield. Previous attempts at deoxygenation of **2b** with tri-*n*-butyltin hydride proved inferior with respect to yield and by-product formation. Deprotection of the TIPS group was realized with unexpected ease by cooling a THF solution of **3** to -10°C before addition of a stoichiometric amount of tetra-*n*-butylammonium fluoride. Care had to be exercised in this step due to the propensity of the pyrimidinone ring to undergo base- or nucleophile-catalyzed ring-opening and decomposition.[12]

The *ara*-zebularine analogue was prepared from the same intermediate by inverting the stereochemistry of the 2'-α-hydroxyl group. Swern oxidation[13] of **2a** at -78°C using a four-fold excess of oxidant gave the 2'-ulose **4** in excellent yield after chromatography. Unfortunately, 3-5% of unreacted starting material was eluted with the ketone due to the similarities in Rf of the two components on silica gel in a variety of solvents. Selective α-face addition of hydride ion occured with concomitant reduction of the pyrimidinone ring[14] (i.e. 1,4 and 1,6 reduction) upon addition of $NaBH_4$ at 0°C to give the *ara* hydroxy derivatives **5a** and **5b**. After chromatography, none of the *ribo* nucleoside was evident by NMR analysis. At this point, it was more expedient to deblock the TIPS group prior to re-oxidation of the base. To this end, **5** was treated with tetra-*n*-butylammonium fluoride in THF at 0°C to give a mixture of dihydropyrimidinone *ara*-nucleosides which were reoxidized to the target **1c** by the silyl-mediated oxidation method of Kelley.[15] The 2-deoxyzebularine nucleotide **6** was prepared in moderate yield according to the method of Holy[9] using $POCl_3$ in triethylphosphate. After initial separation by paper chromatography, the semi-crude monophophate (**6**, triethylammonium salt) was purified by partisil strong anion exchange (SAX) HPLC using a lithium formate buffer. The nucleotide proved to be homogeneous by ^1H NMR and HPLC.

Scheme 1



a: TIPS-Cl, Pyr, 25°C; b: $(COCl)_2$, DMSO, CH_2Cl_2, TEA, -78 - 0°C; c: CS(Imidazole)$_2$, DMF, 25°C; d: $NaBH_4$, MeOH;
e: TBAF, THF, -10°C; f: BSTFA, AIBN, CH_3CN, O2, reflux; g: $(TMS)_3SiH$, AIBN, Toluene, 85°C; h: $POCl_3$, TMP.

TABLE 1. K_i Values of Zebularine Congeners[a]

Compound	K_i (μM), CDA (Mouse Kidney)
1a	2.3
1b	0.3
1c	2.9
1d	2.6
1e	17.5
7	0.22
8	0.07

a; Deaminase activity measured as the decrease in absorbance at 282 nm (conversion of cytidine to uridine) in phosphate buffer (pH 7.0) at 37°C, with cytidine (1.0×10^{-4} M) as substrate.

Results and Discussion. The two synthetic zebularine congeners **1c** and **1d** were evaluated as inhibitors of purified mouse kidney cytidine deaminase. The relevant K_i's of these and other dihydropyrimidinone derivatives against CDA are compiled in Table 1. The values of the prototypical CDA inhibitor, tetrahydrouridine (**7**, THU),[16] and of the diazepinone riboside **8**,[17] are included for comparison. Compounds **1c** and **1d** were shown to inhibit the enzyme with a potency almost identical to that of the parent zebularine (**1a**). The strongest inhibitor of CDA in the zebularine series is still the 5-fluoro analogue **1b** with a potency approaching that of THU.[6] Like zebularine, all of these compounds probably function as transition state inhibitors of CDA after undegoing hydration at the active site of the enzyme.[18] The 2'-fluoro-2'-deoxy-arabinosylzebularine **1e** was 4-5 fold less potent than the 2'-deoxy or 2'-*ara* derivatives, but nevertheless showed reasonable inhibition (K_i = 17.5 μM) furthering the notion that modifications at the 2'-position of pyrimidine nucleosides are well tolerated by CDA.[8] This behavior is consistent with that of cytidines as substrates for

CDA where halogen substitution at 2' in the *ara* configuration did not abolish deamination, but substrate binding was attenuated significantly as the size of the substituent increased.[8] The presence of the 2'-β-fluorine may force an adjustment in the disposition (syn or anti) of the aglycone with respect to the furanose ring or alters the furanose ring puckering into a less favorable conformation required for efficient substrate binding. Replacement of a fluorine atom for hydrogen or hydroxyl groups on the furanose ring of nucleosides has been shown to strongly modulate their biological activity.[19]

The 2'-deoxy and *ara*-zebularine analogues **1c** and **1d** were also evaluated for cytotoxicity against L1210 leukemia and MOLT-4 cells (human peripheral blood lymphoblasts) *in vitro*. Both compounds showed essentially no cytotoxicity against these cell lines at concentrations of > 100 μM.

The 2'-deoxyzebularine nucleotide **6** showed potent inhibitory activity against partially purified dCMPD (IC_{50} = 0.15 μM). A comprehensive study of dCMPD inhibition by **6** and the implications of this step in the biochemical pharmacology of zebularine derivatives will be the subject of a subsequent publication.

In summary, we have prepared compounds **1c** and **1d** by improved routes and found them to be potent inhibitors of CDA. The mono-nucleotide **6**, prepared from **1c** and purified to homogeneity, was shown to be a potent inhibitor of dCMPD. Consequently, the study of zebularine congeners as adjuvants in anticancer chemotherapy remains an interesting area of research.

Experimental

General. All chemical reagents were commercially available. Flash chromatography[20] (FC) was performed using silica gel 60, 220-440 mesh (Fluka) and analytical TLC was performed on Analtech Uniplates silica gel GF with the solvent mixtures indicated for FC. Proton and carbon NMR spectra were recorded at 250 and 62.9 MHz, respectively, on a Bruker AC-250 instrument. Chemical shifts are reported in ppm downfield from TMS and referenced to the solvent in which they were run. ^{13}C

NMR in D_2O were referenced against internal dioxane (66.5 ppm). Analyses were performed by Atlantic Microlabs, Inc., Norcross, GA, or by Galbraith Laboratories, Inc., Knoxville, TN.

CDA Inhibition Studies. Purification of mouse kidney cytidine deaminase and details of the deamination assay have been described previously.[6] In brief, the enzyme was extracted and partially purified from mouse kidney acetone powder (obtained from Sigma Chemical Co., St. Louis, Mo) by extraction at 55-60°C for 5 min with pH 8.0 phosphate buffer (0.05 M) and the extract was filtered through a Nalgene filter (0.45 μm grid membrane) to yield a clear yellow filtrate. The filtrate was fractionated with ammonium sulfate,[16] and the active fraction was dissolved in 2.0 ml of phosphate buffer (0.05 M, pH 7.0). The K_m for the deamination of cytidine using this preparation (5×10^{-5} M) was found to be in good agreement with the value reported previously by Tomchick, *et. al.*[21] CDA activity was measured by following the decrease in absorbance at 282 nm that characterizes the conversion of cytidine to uridine; all assays were performed at pH 7.0 (phosphate buffer) and 37°C with substrate (cytidine) at 1×10^{-4} M. Spectroscopic determinations were carried out with a Beckman model 34 kinetic spectrophotometric system with the recorder set for full scale deflection in the range 0.0 to 0.1 absorbance units. Candidate inhibitors were incubated with the enzyme for 2 min prior to the initiation of the enzymatic reaction by addition of cytidine.

1-(3,5-O-tetraisopropyldisiloxy-β-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one (**2a**).

Zebularine (2-oxo-pyrimidine riboside **1a**, 1.0 g, 4.35 mmol) was dissolved in dry pyridine (20 ml) under argon. The flask was cooled to 0°C and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPS-Cl, 2.05 g, 6.52 mmol) was added slowly by syringe. The cooling bath was removed and the mixture was stirred for 1.5 h. Water (25 ml) was added and the aqueous layer was extracted with ethyl acetate (3x). The combined organic extracts were washed with water and brine (2x), dried and concentrated. Flash chromatography (FC) on silica gel (CH_2Cl_2 - 1% MeOH/CH_2Cl_2, eluant) yielded 1.88 g (90%) of the desired 3',5'-cyclic derivative **2a**. ^{1}H NMR ($CDCl_3$) δ 8.56 (dd, J = 2.8 and 4.1 Hz, 1H, H4), 8.27 (dd, J = 2.8 and 6.7 Hz, 1H, H6), 6.29 (dd, J = 4.1 and 6.7 Hz, 1H, H5), 5.77 (s, 1H, H1'), 4.20 (br s, 2H, H2', H3'), 4.11 (center of AB quartet,

2H, H5', H5"), 3.57 (br s, 1H, H4'), 0.88-1.07 (m, 28H, isopropyl). ^{13}C NMR ($CDCl_3$) δ 166.01, 155.25, 143.46, 103.77, 91.87, 81.00, 74.83, 68.21, 59.86, 17.38, 17.33, 17.20, 16.89, 16.81, 16.73, 13.31, 12.84, 12.80, 12.38. Anal calcd for $C_{21}H_{38}N_2O_6Si_2$: C, 53.58; H, 8.44; N, 5.95; Si, 11.93. Found: C, 53.95; H, 8.44; N, 5.59; Si, 11.98.

1-[2-O-(1-imidazolylthiocarbonyl)-3,5-O-tetraisopropyldisiloxy-β-D-ribofuranosyl]-1,2-dihydropyrimidone-2-one (**2b**).

Compound **2a** (1 g, 2.14 mmol) was dissolved in dry DMF and 1,1'-thiocarbonyldiimidazole (1.25 g, 7.0 mmol) was added all at once. The mixture was stirred under argon at room temperature for 24 h. Water was added and the aqueous layer was extracted with ethyl acetate. The combined extracts were washed with water and brine (3x), dried and concentrated. FC on silica (50% EtOAc/pet ether- 100% EtOAc, eluant) yielded 1.071 g (87%) of the thiocarbonylimidazole derivative **2b**. ^1H NMR ($CDCl_3$) δ 8.64 (dd, J = 2.8 and 4.9 Hz, 1H, H4), 8.34 (t, J = 1.0 Hz, 1H, Im-H), 8.25 (dd, J = 2.8 and 6.8 Hz, 1H, H6), 7.65 (t, J = 1.5 Hz, 1H, Im-H), 7.05 (t, J = 0.8 Hz, 1H, Im-H), 6.37 (dd, J = 4.9 and 6.8 Hz, 1H, H5), 6.27 (d, J = 4.7 Hz, 1H, H2'), 5.97 (s, 1H, H1'), 4.53 (dd, J = 4.7 and 9.4, 1H, H3'), 4.31 (d, J = 13.6 Hz, 1H, H5'), 4.15 (br dt, $J_{3,4}$ = 9.4, 1H, H4'), 4.14 (dd, J = 2.6 and 13.6, 1H, H5"), 0.80-1.10 (m, 28H, isopropyl). ^{13}C NMR ($CDCl_3$) δ 182.18, 166.68, 154.82, 143.05, 136.80, 131.00, 118.19, 104.12, 89.76, 83.05, 82.54, 68.11, 59.48, 17.39, 17.22, 16.91, 16.88, 16.73, 13.32, 12.87, 12.48.

1-(2-deoxy-3,5-O-tetraisopropyldisiloxy-β-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one (**3**).

Compound **2a** (0.500 g, 0.865 mmol) was suspended in dry toluene under argon and AIBN (0.060 g, 0.400 mmol) was added. The mixture was heated to 80°C and tris(trimethylsilyl)silane (0.535 ml, 2.16 mmol) was added slowly by syringe. The mixture was stirred at this temperature for 45 min and the solvent evaporated. Purification of the crude residue by FC on silica gel (20% EtOAc/pet ether-50% EtOAc/pet ether, eluant) afforded 265 mg (67%) of the deoxygenated product **3** along with ca 5% of recovered starting material. ^1H NMR ($CDCl_3$) δ 8.58 (dd, J = 2.8 and 4.1 Hz, 1H, H4), 8.37 (dd, J = 2.8 and 6.7 Hz, 1H, H6), 6.32 (dd, J = 4.1 and 6.7 Hz,

1H, H5), 6.03 (d, J = 6.7 Hz, 1H, H1'), 4.33 (m, 1H, H3'), 4.19 (d, J = 13.5 Hz, 1H, H5'), 4.01 (dd, J = 2.7 and 13.5 Hz, 1H, H5"), 2.62 (ddd, J = 7.0, 11.1 and 13.4 Hz, 1H H2'), 2.40 (dd, J = 6.8 and 13.4 Hz, 1H, H2"). ^{13}C NMR ($CDCl_3$) δ 165.68, 155.35, 143.32, 103.57, 85.98, 85.33, 65.97, 59.57, 39.30, 17.46, 17.40, 17.27, 16.92, 16.85, 16.76, 13.37, 12.91, 12.37.

1-(2-deoxy-β-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one (**1c**).

Compound **3** (0.265 g, 0.586 mmol) was dissolved in dry DMF under argon and cooled to -10°C (ice-salt bath). Tetra-*n*-butylammonium fluoride (1.0 ml of a 1.0 M solution in THF) was added over a period of 5 min by syringe. The cold mixture was stirred for 30 min whereupon the THF was evaporated at 20°C. The residue was applied to a column of C18 silica and eluted with water. However, the fractions containing the product were still contaminated with a by-product of the reagent. These were further purified by normal phase FC on silica (5-10% CH_3OH/CH_2Cl_2, eluant) which removed the majority of the contaminants (75% recovery). Final purification was effected by HPLC (C18, 4% CH_3CN/H_2O, eluant) to give pure 2'-deoxyzebularine **1c** in 51% yield. ^1H NMR (D_2O) δ 8.48 (br dd, J = 2.6 and 4.5 Hz, 1H, H4), 8.37 (dd, J = 2.6 and 6.7 Hz, 1H, H6), 6.62 (dd, J = 4.6 and 6.7 Hz, 1H, H5), 6.07 (t, J = 6.1 Hz, 1H, H1'), 4.26 (br q, 1H, H3'), 4.02 (br q, 1H, H4'), 3.73 (dd, J = 3.6 and 12.6 Hz, 1H, H5'), 3.63 (dd, J = 5.4 and 12.6 Hz, 1H, H5"). 2.50 (m, 1H, H2'), 2.20 (m, 1H, H2"). ^{13}C NMR (D_2O, 1 drop dioxane-d_8) δ 167.31, 157.46, 146.09, 107.3, 88.99, 88.48, 70.87, 61.78, 40.95. Anal calcd for $C_9H_{12}N_2O_4 \cdot 0.33\ H_2O$: C, 49.54; H, 5.81; N, 12.84. Found: C, 49.27; H, 5.80; N, 12.72.

1-(2-oxo-3,5-O-tetraisopropylsiloxy-β-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one (**4**).

To a cooled (-78°C) solution of oxalyl chloride (0.8 ml of a 2.0 M solution, 1.58 mmol) in CH_2Cl_2 (6 ml) was added DMSO (246 mg, 3.16 mmol in 2 ml of CH_2Cl_2) slowly by syringe over a period of 5 min. This was stirred for 10 min at -78°C and a solution of compound **2a** (370 mg, 0.79 mmol) and CH_2Cl_2 (4 ml) was added via canula (argon pressure) to the cold reaction mixture. The solution was maintained at this temperature for 1 h whereupon triethylamine (1.1 ml) was added and the solution

was warmed to 20°C. Water was added and the organic layer was separated. The aqueous part was extracted twice more with CH_2Cl_2 and the combined organic layers were washed with brine, dried and concentrated. FC (20%-60% EtOAc/pet ether) gave 315 mg (85%) of the ketone **3** slightly contaminated with starting alcohol. ^{1}H NMR ($CDCl_3$) δ 8.59 (dd, J = 2.7 and 4.0 Hz, 1H, H4), 7.68 (dd, J = 2.7 and 6.7 Hz, 1H, H6), 6.32 (dd, J= 4.0 and 6.7 Hz, 1H, H5), 5.15 (d, J = 8.0 Hz, 1H, H3'), 5.08 (s, 1H, H1'), 3.92-4.27 (m, 3H, H4', H5', H5"), 0.81-1.22 (m, 28H, Si-tetraisopropyl). ^{13}C NMR ($CDCl_3$) δ 204.4, 168.4, 154.5, 148.2, 104.6, 87.6, 81.3, 72.4, 63.6, 16.7-17.5 and 12.3-13.3 (m, tetraisopropyl). Anal. calcd. for $C_{21}H_{36}O_6N_2Si_2$: C, 53.81; H, 7.74; N, 5.97. Found: C, 53.49; H, 7.72; N, 5.59.

1-(β-D-arabinofuranosyl)-1,2-dihydropyrimidin-2-one **(1d)**.

Compound **4** (550 mg, 1.17 mmol) was dissolved in THF (10 ml) and the flask was cooled to 0°C. Sodium borohydride (65 mg, 1.76 mmol) was added and the mixture was stirred for 40 min at this temperature. Methanol (5 ml) was added and the ice bath was removed. After 1 h at room temperature the solvents were removed and the residue was evaporated from methanol (3x). The crude reaction mixture was taken up in water and extracted with ethyl acetate (3x). The combined extracts were washed with water, potassium phosphate buffer (pH 7) and brine, dried and concentrated. FC (30-50% EtOAc/pet ether, eluant) yielded 145 mg (72%) of a mixture of **5a** and **5b**. This was dissolved in THF (8 ml), cooled to -10°C and treated with tetra-*n*-butylammonium fluoride (1.5 ml of a 1.0 M solution in THF). After 20 min at this temperature, the THF was removed *in vacuo* and the residue was partially purified by silica gel chromatography (5% MeOH/CH_2Cl_2, eluant) to remove less polar by-products and reagent. The semi-pure, pyrimidinone-reduced nucleoside was dissolved in 10 ml of acetonitrile and BSTFA (2.5 ml) was added. A small amount (spatula tip) of AIBN was added and the mixture was heated to reflux. After 24 h at this temperature, the volatiles were removed and the residue was evaporated from methanol 3x. The syrup was redissolved in methanol and 0.5 ml of 0.1 N HCl was added. After stirring at room temperature for 5 min the mixture was neutralized with solid $NaHCO_3$, filtered and evaporated. The crude residue was quickly filtered through a small C18 column (water, eluant) and was then judged by analytical HPLC to be ~ 70% pure.

Final purification by reverse phase HPLC (10% CH_3CN/H_2O) gave the 2'-*ara* zebularine **1d** in 61% yield. ^1H NMR (D_2O) δ 8.50 (br dd, J = 4.4 and 2.6 Hz, 1H, H4), 8.28 (dd, J = 6.7 and 2.6 Hz, 1H, H6). 6.63 (dd, J = 6.7 and 4.4 Hz, 1H, H5), 6.10 (d, J = 4.6 Hz, 1H, H1'), 4.39 (br t, 1H, H2'), 3.99 (m, 2H, H3', H4'), 3.74 (AB of ABX, J_{gem} = 12.5 Hz, 2H, H5', H5''). 13C NMR (D_2O, drop of Dioxane-d_8) 167.5, 157.4, 147.3, 106.9, 88.6, 85.3, 76.3, 75.9, 61.6. Anal calcd for $C_9H_{12}N_2O_5$: C, 47.36; H, 5.30; N, 12.27. Found: C, 47.21; H, 5.36; N, 12.22.

1-(2-deoxy-β-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one 5'-monophosphate (**6**).

Compound **1c** (25 mg, 0.12 mmol) was suspended in 2.1 ml of triethylphosphate and stirred at 0°C. Freshly distilled $POCl_3$ (0.04 ml, 0.29 mmol) was added and the flask was stoppered and stirred for 2 h. The reaction was quenched at 0°C with Et_3NHCO_3 (1.0 M solution) and the pH was raised to between 8.0 and 8.5 with triethylamine. This solution was stirred an additional 4 h at 25°C and subsequently concentrated *in vacuo*. The residue was dissolved in 1 ml of H_2O and applied to Whatman 3MM paper and eluted for 10 h with 7:3 ethanol/0.01 M ammonium formate. The UV active band of intermediate mobility (0.5 Rf) was separated and eluted with dilute ammonia (~ 0.001 M). Analytical HPLC analysis on Partisil SAX showed a major peak with appropriate retention time (rt) for a monophosphate (0.01 M $NH_4^+HCOO^-$, 12.3 min) along with a small amount of starting nucleoside (rt = between 2-3 minutes). The peak at 12.3 min was collected from a semi-preparative run on SAX using 0.01 M lithium formate buffer. The fraction containing the monophosphate was lyophilized and the dry powder was triturated with absolute ethanol. This mixture was centrifuged for 10 min and HPLC analysis of both the supernatant and the pellet revealed almost no nucleotide in the supernatent. The pellet was dried and analyzed by NMR. The proton spectrum in D_2O showed a clean compound, identical in most respects to 2'-deoxyzebularine except for a slight downfield shift (0.2 ppm) of the 5 and 5' protons whose coupling patterns were complicated by an additional three-bond ^1H-^{31}P interaction. This material was used as such for enzyme inhibition studies with dCMPD.

Acknowledgements. The authors wish to thank Dr. David A. Cooney, Kathryn Dobbins and Emily Stowe, Biochemical Pharmacology section, LMC, NCI, for cytotoxicity studies and Dr. Harry Ford, Analytical Chemistry section, LMC, NCI, for the enzyme inhibition studies on compound **6**.

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Received 7/10/92
Accepted 9/3/92