mmol) of mercuric acetate. First a solution formed, followed by crystallization of product. After 1 h, ether was added, and the precipitate was collected: yield 98 mg (84%); mp 230 °C dec.

This compound was converted to the corresponding 5-iodo derivative upon treatment at room temperature with iodine or iodine monochloride. ^{1}H NMR of 2-[(*N*-succinoylamino)-methyl]-5-mercuriothiophene (**61b**): 8.46 (1 H, t, NH), 7.13 (1 H, d, Ar-4), 6.68 (1 H, d, Ar-3), 4.37 (2 H, d, CH_2N), 2.43 (2 H, CH_2), 2.35 (2 H, CH_2).

5-Iodothiophene-2-acetic Acid (61a; R^4 = COOH). Compound **60a** (75 mg, 0.22 mmol) was suspended in 5 mL of dimethylformamide containing 5% DMSO. Iodine crystals (77 mg, 0.31 mmol) were added with stirring. A solution formed within 1 min. Hydrochloric acid (1 M) was added, and the mixture was extracted three times with ethyl acetate. The combined organic layers were dried (Na_2SO_4) and evaporated. The product was purified by column chromatography on silica gel. R_f of product (silica, ethyl acetate/petroleum ether) was 0.79.

The identical product was obtained as follows: *N*-Iodosuccinimide (236.2 mg, 1.05 mmol) was added to a stirred suspension of compound **59a** (326.2 mg, 0.95 mmol) in methanol (30 mL). After 16 h the suspension was filtered and the methanol removed in vacuo. The remaining oil was redissolved in ethyl acetate and washed with 0.5 N HCl, and the product was extracted into a 0.5 N NaOH solution. The basic fraction was washed with CH_2Cl_2, acidified to pH 1.0 with 1 N HCl, and extracted with EtOAc. The product was chromatographed on a silica gel column (eluent, 17/2/1 $CHCl_3$/MeOH/AcOH), and the solvents were removed from the product fractions in vacuo. Acetic acid was removed by azeotropic distillation with petroleum ether. The light yellow oil was redissolved in ethyl acetate, and ether was added, forming a precipitate which was removed by filtration. Evaporation of the solvent gave compound **61a** as a waxy yellow solid (110 mg, 43%).

Peptide Derivatives of Thiophene-2-acetic Acid. Compound **58** reacted with L-tyrosylglycine (274 mg, 1.15 mmol) in dimethylformamide to give *N*-(thiophene-2-acetyl)-L-tyrosylglycine (230 mg, 55% yield). Upon mercuration in dimethylformamide as for compound **60a**, *N*-(5-mercuriothiophene-2-acetyl)-L-tyrosylglycine (40% yield) was obtained.

2-[(*N*-Succinoylamino)methyl]thiophene [63a; R = $(CH_2)_2$COOH]. Thiophene-2-methanamine (Aldrich, 2.37 g, 21 mmol) was dissolved in 20 mL of tetrahydrofuran and treated with a solution of succinic anhydride (2.1 g, 21 mmol) in 20 mL of dimethylformamide. After $^1/_2$ h, ethyl acetate (50 mL) was added, and the mixture was extracted with citric acid (1 M) three times and with water. The organic layer was dried ($MgSO_4$). Solvent was removed and petroleum ether was added, causing white crystals of **62a** to precipitate: Yield 9.4%; mp 130 °C. The NMR and mass spectra were consistent with the assigned structure. UV spectrum λ_{max} 233 nm (log ϵ 4.019).

Biochemical Assays. Stock solutions of xanthines were prepared in the millimolar concentration range in dimethyl sulfoxide and stored frozen. Solutions were warmed to 50 °C prior to dilution in aqueous medium. Inhibition of binding of 1 nM [^{3}H]-N^6-(phenylisopropyl)adenosine to A_1-adenosine receptors in rat cerebral cortical membranes was assayed as described.[17] Inhibition of binding by a range of concentrations of xanthines was assessed in triplicate in at least three separate experiments. IC_{50} values, computer generated by using a nonlinear regression formula on the Graphpad program, were converted to K_i values by using a K_D value for [^{3}H]PIA of 1.0 nM and the Cheng–Prusoff equation.[19]

Inhibition of binding of [^{3}H]-5′-(*N*-ethylcarbamoyl)adenosine to A_2-adenosine receptors in rat striatal membranes was measured as described,[18] except that 5 mM theophylline was used to define nonspecific binding. N^6-Cyclopentyladenosine was present at 50 nM to inhibit binding of the ligand at A_1-adenosine receptors. Inhibition of binding by a range of concentrations of xanthines was assessed in triplicate in at least three separate experiments. IC_{50} values were converted to K_i values by the method of Bruns et al.,[18] using a conversion factor derived from the affinity of [^{3}H]-5′-(*N*-ethylcarbamoyl)adenosine at A_2 receptors and the Cheng–Prusoff equation.[19]

Acknowledgment. This project has been supported in part by National Institutes of Health SBIR Grant 1 R34 AM 37728-01 to Research Biochemicals, Inc.

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Synthesis of Acyclonucleoside Hydroxamic Acids as Inhibitors of Ribonucleotide Reductase

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N-Hydroxy-α-(2-hydroxyethoxy)-1(2*H*)-pyrimidineacetamides **1–3** were synthesized as potential antitumor agents whose mechanism of action would involve inhibition of ribonucleoside diphosphate reductase (RDPR, EC 1.17.4.1). Acyclonucleoside esters **6–8** were prepared by the stannic chloride catalyzed reaction of methyl chloro[2-(phenylmethoxy)ethoxy]acetate (**5**) with various silylated pyrimidines, generated in situ from the bases and bis(trimethylsilyl)acetamide. Catalytic didebenzylation of hydroxamate **11** gave **1**, while **2** and **3** were synthesized by the reaction of lactones **14** and **22**, respectively, with hydroxylamine. In vitro acyclonucleoside hydroxamic acids **1–3** were 3–10-fold less potent than hydroxyurea against calf thymus cytidine diphosphate reductase. 5-Fluorouracil derivative **2** is nearly equipotent with hydroxyurea in inhibiting the growth of HeLa cells, while **1** is a much weaker inhibitor and cytidine derivative **3** is devoid of activity at 200 μg/mL.

The enzyme ribonucleoside diphosphate reductase (RDPR) catalyzes the reduction of ribonucleotides to 2′-deoxyribonucleotides. This reaction is the rate-limiting step in the de novo biosynthesis of DNA and, as such, ribonucleotide reductase is a key enzyme target in the chemotherapy of cancer.[1] The enzyme from *E. coli* is prototypical of all known eukaryotic and viral-coded reductases: it consists of two nonidentical subunits, proteins B1 and B2, which are in turn each composed of two similar or identical polypeptide chains for an overall α,α',β_2 structure.[2] Protein B1 contains binding sites for both substrates and allosteric effectors and also contains the

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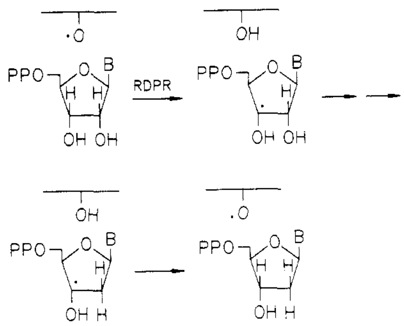
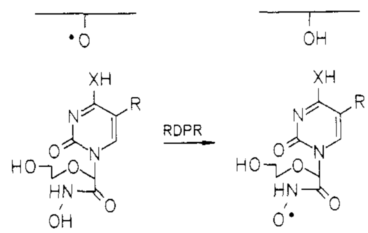
Scheme I



Scheme II



1, X=O, R=H
2, X=O, R=F
3, X=NH, R=H

redox-active thiols which reduce the 2′-hydroxyl group of the ribose moiety. Protein B2 contains a tyrosyl radical (tyrosine 122) stabilized by an adjacent iron center composed of two antiferromagnetically coupled Fe^{3+} ions linked by a μ-oxo bridge.[3] This tyrosyl radical has been postulated to participate in the catalytic process by first abstracting a 3′-hydrogen atom from a ribonucleotide substrate and later by donating a hydrogen atom back to the 3′-position after reduction of the 2′-hydroxyl has occurred (Scheme I).[4]

Inhibitors of ribonucleotide reductase generally fall into one of four categories:[5] (1) metal chelators, (2) mechanism-based (k_{cat}) inhibitors,[4] (3) inhibitors of the B1 subunit, including the natural negative allosteric effectors, and (4) inhibitors of the B2 subunit. This last class of compounds, which includes hydroxyurea, the ferrous complexes of α-(*N*)-heterocyclic thiosemicarbazones, guanazole, and 2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole (IMPY), all inhibit ribonucleotide reductase by destroying the catalytically essential tyrosyl free radical.[6]

Hydroxyurea is the only ribonucleotide reductase inhibitor currently in clinical use as an anticancer drug; however, its weak in vitro and in vivo effectiveness[5,7] have led to the synthesis of other urea[8] and hydroxamic acid derivatives[7] as potential inhibitors. In an attempt to design hydroxamic acids which more closely resemble the substrates, we have synthesized pyrimidine acyclonucleosides **1–3**, which contain a hydroxamic acid moiety ideally situated to destroy the tyrosyl radical of ribonucleotide reductase by donation of a hydrogen atom (Scheme II).

Chemistry

Condensation of 2-(benzyloxy)ethanol[9] with a mixture of the hydrate and methyl hemiacetal of methyl glyoxylate[10] in refluxing benzene with azeotropic removal of water and methanol gave the hemiacetal **4** (Scheme III), which was converted directly to **5** with methanesulfonyl chloride in CH_2Cl_2 at −18 to −10 °C. α-Chloro ether **5** was not purified; the crude material consisted of 67–75% **5** and 14–9% $PhCH_2OCH_2CH_2OMs$.[11] Lower reaction temperatures favored the formation of **5**. Condensation of **5** with silylated uracil, 5-fluorouracil, and N^4-acetylcytosine,[12] generated in situ from the bases and *N,O*-bis(trimethylsilyl)acetamide (BSA),[13] in the presence of 0.11 equiv of stannic chloride[14] gave pyrimidine acyclonucleosides **6**, **7**, and **8** in yields of 85, 66, and 71% based on the actual amount of α-chloro ether **5** present in the crude starting material. The IR, ^1H and ^{13}C NMR, and mass spectra are all consistent with the assigned structures. In particular, the ^1H NMR ($CDCl_3$) spectra of **6** and **8** exhibited singlets at δ 6.16 and 6.27 for the anomeric protons. The anomeric proton for **7** appeared as a doublet (J = 1.5 Hz) due to long-range spin–spin coupling with the 5-fluorine;[15] after D_2O exchange, the ^{19}F NMR spectrum showed a doublet of doublets (J = 5.7 and 1.5 Hz) centered at δ −164.35. The UV spectra are in accord with published data for N^1-substituted pyrimidine nucleosides.[16]

The most direct route for introduction of the hydroxamate moiety, i.e. reaction of the ester with NH_2OH,[17] was unsuccessful. In methanol at room temperature for 3 days, **15** failed to react with NH_2OH; in refluxing methanol, loss of the cytosine residue was observed. Consequently alternative routes to **1–3** were developed. Esters **6** and **7** were saponified to the corresponding acids **9** and **10**, which were then coupled to *O*-benzylhydroxylamine with EEDQ (Scheme IV). Didebenzylation of uracil derivative **11** was smoothly accomplished with PdO-catalyzed transfer hydrogenation[16b] to give **1**. However, the 5-fluoro derivative **2** could not be obtained in sufficient purity from **12** by this method. This problem, along with the numerous difficulties initially encountered in the synthesis of **3** (vide infra), required the development of a mild method of generating the hydroxamic acid as the final step in the synthesis. Lactones **13**, **14**, and **22** (vide infra) seemed ideally suited for our purposes if the lactones were sufficiently reactive toward NH_2OH; such a route would eliminate the need for any subsequent deprotection steps. Catalytic transfer hydrogenation of **9** and **10** followed by lactonization of the resulting crude hydroxy acids with *N,N′*-dicyclohexylcarbodiimide (DCC) in pyridine[18] gave lactones **13** and **14** in 46 and 68% yield, respectively

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(4) For a review of the mechanism of RDPR, see: Ashley, G. W.; Stubbe, J. *Pharmacol. Ther.* **1985**, *30*, 301.
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(12) Brown, D. M.; Todd, A.; Varadarajan, S. *J. Chem. Soc.* **1956**, 2384.
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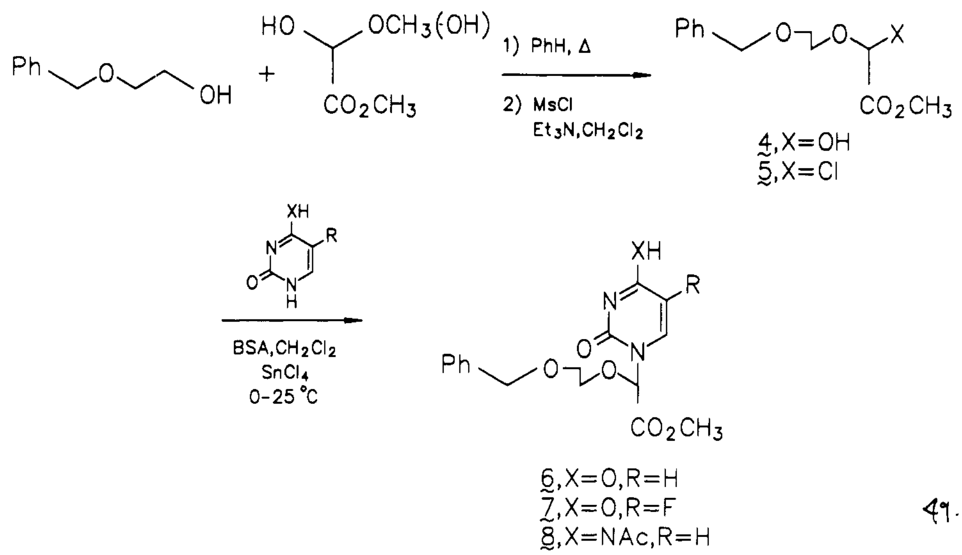
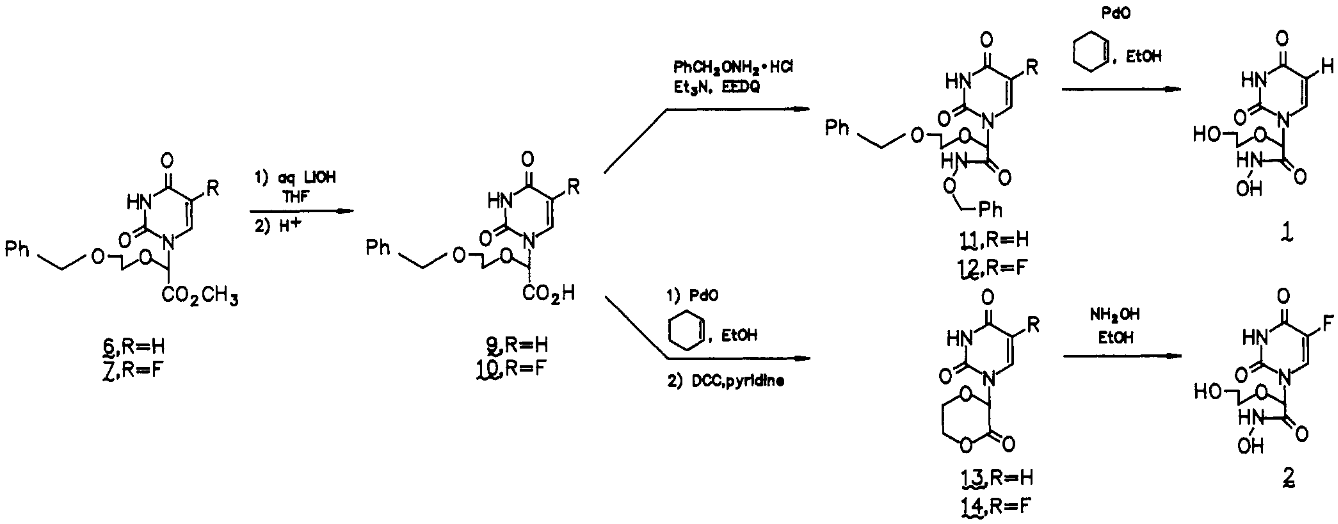
(18) Woodward, R. B.; Bader, F. E.; Bickel, H.; Frey, A. J.; Kierstead, R. W. *Tetrahedron* **1958**, *2*, 1.

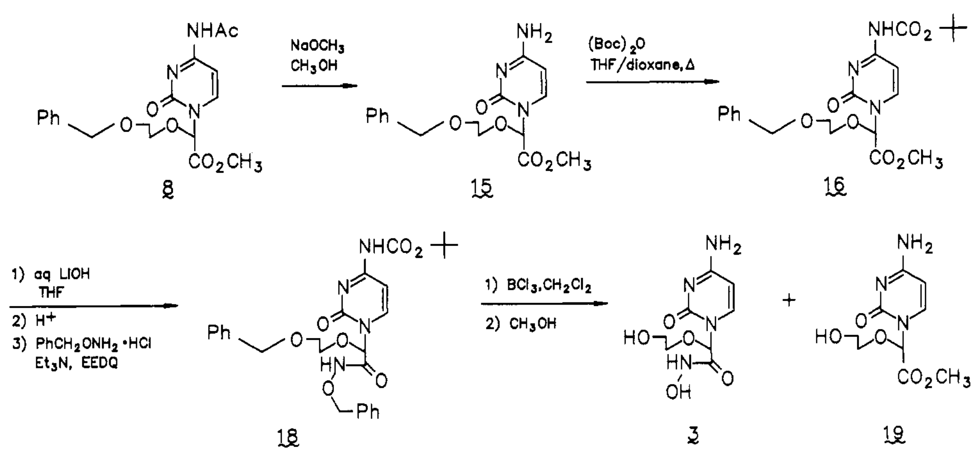
Scheme III



Scheme IV



Scheme V

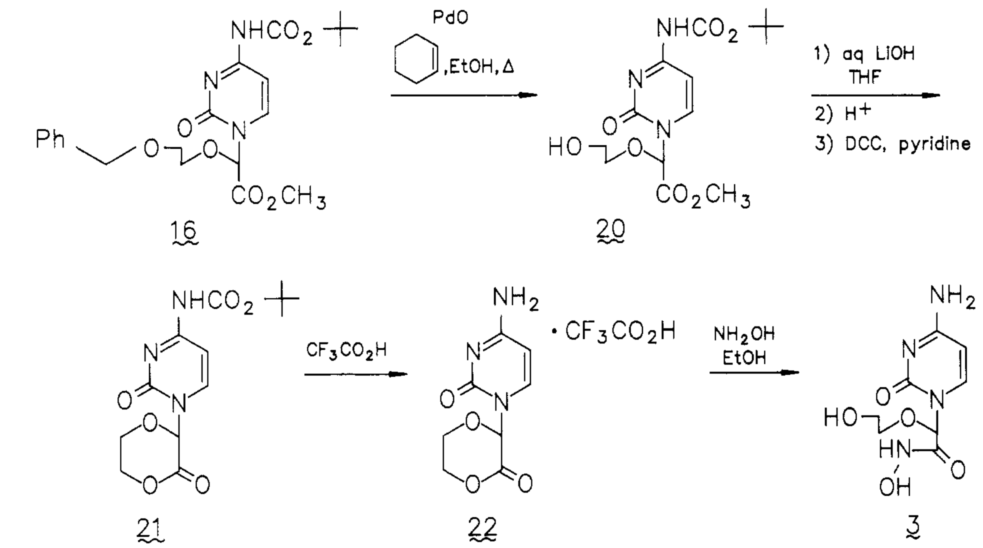


(unoptimized). Lactone **14** reacted with ethanolic NH_2OH within minutes at room temperature to give the desired hydroxamic acid **2**.

Prior to the development of the lactone route, we also attempted to prepare **3** from the dibenzyloxy derivative **18**. The base-labile acetyl group of **8** was removed by treatment with a catalytic amount of $NaOCH_3$ in methanol (Scheme V). The amino group was converted to the *t*-Boc derivative **16** using di-*tert*-butyl dicarbonate [$(Boc)_2O$][19] in refluxing THF/dioxane. Saponification of the ester **16** gave the corresponding acid **17**, which was coupled with *O*-benzylhydroxylamine as described above to give **18**. Treatment of a solution of **18** in CH_2Cl_2 at −50 to −30 °C with 1 M BCl_3/CH_2Cl_2 resulted in rapid cleavage of the two benzyl[20] and the *t*-Boc groups.[21] However, the ^1H

(19) Tarbell, D. S.; Yamamoto, Y.; Pope, B. M. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 730.

Scheme VI



NMR of the crude material obtained after treatment with CH_3OH showed the presence of two products in roughly equal amounts which were identified by GC/MS as hydroxamic acid **3**[22] and methyl ester **19**. Even after purification of **3** by reverse-phase column chromatography, attempted recrystallization from acetone/CH_3OH gave a 50/50 mixture of **3** and **19**. Since **3** prepared from lactone **22** (vide infra) was quite stable to alcohol, the BCl_3 cleavage of **18** evidently leads to a boron chelate of the hydroxamic acid which readily undergoes conversion to ester **19** upon exposure to CH_3OH. Transfer hydrogenation of **18** gave an inseparable mixture of products.

To avoid these problems, the aminolysis of lactone **22** was employed (Scheme VI). Benzyl ether **16** was hydrogenated to the alcohol **20**, which was converted to the lactone **21** in 61% yield (from **20**) as described above. Deprotection of **21** with trifluoroacetic acid (TFA) at 0 °C gave lactone **22**, isolated as the TFA salt, in quantitative yield. Treatment of **22** with 1.6 equiv of NH_2OH in ethanol rapidly gave the desired hydroxamic acid **3**. The acid-sensitive hydroxamic acid moiety must be introduced last, as reversal of these final two steps gives a mixture of lactone **22** and the TFA salt of **3**.

Biological Results

Acyclonucleoside hydroxamates **1**–**3** and hydroxyurea at concentrations ranging from 0 to 10 mM were tested for their effects on cytidine diphosphate (CDP) reductase activity in vitro with a partially purified preparation from calf thymus. All four compounds inhibited CDP reductase activity in a concentration-dependent manner. IC_{50}s as determined from a plot of CDP reductase activity (percent control) vs log [inhibitor] are 2.2 mM, 1.4 mM, 4.9 mM, and 0.5 mM for **1**, **2**, **3**, and hydroxyurea, respectively. The antiproliferative effects of these hydroxamates on HeLa cells in culture (Table I) paralleled their in vitro activities against the reductase. Test results for hydroxyurea and 5-fluorouracil (5-FU), a possible hydrolysis product of **2**, are included for comparison. 5-FU derivative **2** is nearly equipotent with hydroxyurea, while **1** was a much weaker inhibitor and cytidine derivative **3** was devoid of activity.

Table I. Effects of Acyclonucleoside Hydroxamates on the Growth of Human Cervical Carcinoma (HeLa) Cells in Culture

compd	concn, μg/mL	% inhibn	compd	concn, μg/mL	% inhibn
1	200	97	**3**	200	0
	100	47	hydroxyurea	50	90
	50	13		25	77
	25	19		10	63.5
2	50	80		5	34.3
	25	75		2.5	12.4
	10	66	5-fluorouracil	10	70
	5	40		1	55
	2.5	16.5		0.1	19

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Silica Gel 60 (230–400 mesh ASTM, EM Science) was used for all flash chromatographies. IR spectra were recorded on either a Perkin-Elmer Model 180 IR or a Model 1800 FT-IR spectrophotometer. ^{1}H NMR spectra (90-MHz) were recorded on a Varian EM 390 spectrometer. All other ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on a Varian VXR 300. ^{19}F chemical shifts are reported as ppm vs external $CFCl_3$. Mass spectra were recorded on either a Finnegan MAT 4600 or a MAT TSQ-46 mass spectrometer. UV spectra were recorded on a Carey Model 17 spectrophotometer.

β,γ-Imidoadenosine 5′-triphosphate (AMP-PNP); *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES); dithiothreitol (DTT); cytidine 5′-diphosphate, potassium tetraborate, hydroxyurea, and *Crotalus adamanteus* venom were purchased from Sigma. [U-^{14}C]Cytidine 5′-diphosphate, 527 mCi/mmol, was obtained from New England Nuclear and AG1X8 was purchased from Bio-Rad. DEAE-cellulose (DE52) was obtained from Whatman.

Methyl Chloro[2-(phenylmethoxy)ethoxy]acetate (5). A solution of 3.04 g (20.0 mmol) of 2-(benzyloxy)ethanol[9] and 2.84 g (25 mmol) of a 62/38 mixture of methyl glyoxylate methyl hemiacetal/hydrate[10] in 60 mL of benzene under nitrogen was slowly heated to reflux and 25 mL of distillate was collected over 1.5 h in a Dean–Stark trap. Concentration in vacuo of the pot residue gave 5.07 g of **4** as a pale, straw-colored liquid: ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.27 (s, 5 H), 4.99 (br d, 0.8 H, J = 8 Hz), 4.50 (s, 2 H), 4.28 (br d, 0.7 H), 3.91–3.51 (m, 4 H), 3.72 (s, <3 H).

To a stirred solution of crude **4** and 3.46 mL (24.8 mmol) of Et_3N in 35 mL of CH_2Cl_2 at −18 to −15 °C under nitrogen was added 1.91 mL (24.7 mmol) of MsCl dropwise via a syringe. After 1.25 h the solution was poured into ether/ice H_2O. The organic layer was separated, washed with brine, and dried ($MgSO_4$). Concentration in vacuo gave 5.09 g (5.17 g theoretical yield) of

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(21) (a) Hiskey, R. G.; Beacham, L. M., III; Matl, V. G.; Smith, J. N.; Williams, E. B., Jr.; Thomas, A. M.; Wolters, E. T. *J. Org. Chem.* **1971**, *36*, 488. (b) Schnabel, E.; Klostermeyer, H.; Berndt, H. *Justus Liebigs Ann. Chem.* **1971**, *749*, 90.

(22) The crude mixture, as well as the partially purified hydroxamic acid, gave positive $FeCl_3$ tests.

pale, straw-colored oil, which consisted of 75% 5 and 11% $PhCH_2OCH_2CH_2OMs$: ^1H NMR (90 MHz, $CDCl_3$) δ 7.26 (s, 5 H), 5.90 (s, 0.75 H), 4.48 (s, 2 H), 4.30 (m, 0.12 H, RCH_2OMs), 4.11–3.48 (m, >4 H), 3.76 (s, 3 H), 2.93 (s, 0.33 H, CH_3SO_3R).

Methyl 4-Acetamido-2-oxo-α-[2-(phenylmethoxy)ethoxy]-1(2*H*)-pyrimidineacetate (8). To a stirred mixture of 7.50 g (16 mmol, based on 2.2 mmol/g of 5 present) of crude 5 and 2.81 g (18.3 mmol) of N^4-acetylcytosine[12] in 40 mL of CH_2Cl_2 under argon was added 6.00 mL (24.3 mmol) of *N,O*-bis(trimethylsilyl)acetamide via a syringe. Solution occurred after about 20 min. After 2.25 h, the solution was cooled in an ice bath before 0.25 mL (2.1 mmol) of $SnCl_4$ was added. The ice bath was removed and the reaction mixture was allowed to stir at 25 °C for 17 h. The solution was poured into ice water and extracted twice with EtOAc/ether. The combined extracts were washed with water and brine and dried ($MgSO_4$). Concentration in vacuo gave 8.25 g of a tacky yellow solid. Recrystallization from cyclohexane/EtOAc gave 4.37 g (41% overall based on 2-(benzyloxy)ethanol, 71% based on actual amount of α-chloro ether 5 formed) of off-white crystals. Flash filtration through a short plug of silica gel eluted with EtOAc followed by a second recrystallization gave 8 as white crystals: mp 115.5–118 °C; IR (KBr) ν_{max} 3435, 1758, 1729, 1660, 1623, 1560, 1492, 1370, 1358, 1315, 1210, 1100 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.78 (s, 1 H), 7.88 (d, 1 H, J = 7.5 Hz), 7.44 (d, 1 H, J = 7.5 Hz), 7.38–7.26 (m, 5 H), 6.27 (s, 1 H), 4.52 (s, 2 H), 3.97–3.90 (m, 1 H), 3.84–3.58 (m, 3 H), 3.79 (s, 3 H), 2.28 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 170.60, 166.16, 163.15, 155.31, 144.78, 137.52, 128.35, 127.72, 127.57, 97.31, 83.30, 73.30, 70.83, 68.55, 53.30, 25.04; mass spectrum (chemical ionization, CH_4), m/z 416 (M^+ + 41), 404 (M^+ + 29), 376 (M^+ + 1); UV (CH_3OH) λ_{max} 249 (ε 15 700), 298 nm (ε 6740). Anal. ($C_{18}H_{21}N_3O_6$) C, H, N.

Methyl 3,4-Dihydro-2,4-dioxo-α-[2-(phenylmethoxy)ethoxy]-1(2*H*)-pyrimidineacetate (6). With use of the same procedure, 6 was obtained as a pale, straw-colored oil in 48% overall yield from 2-(benzyloxy)ethanol (85% based on α-chloro ether 5) after flash chromatography eluting with 50/50 EtOAc/CH_2Cl_2: ^1H NMR (90 MHz, $CDCl_3$) δ 10.1 (br s, 1 H), 7.39 (d, 1 H, J = 8 Hz), 7.29 (s, 5 H), 6.16 (s, 1 H), 5.68 (d, 1 H, J = 8 Hz), 4.48 (s, 2 H), 3.90–3.48 (m, 4 H), 3.74 (s, 3 H); mass spectrum (chemical ionization, CH_4), m/z 375 (M^+ + 41), 363 (M^+ + 29), 335 (M^+ + 1), 91; exact mass calcd for $C_{16}H_{19}N_2O_6$ 335.1243, found 335.1236.

Methyl 5-Fluoro-3,4-dihydro-2,4-dioxo-α-[2-(phenylmethoxy)ethoxy]-1(2*H*)-pyrimidineacetate (7). Similarly, after crystallization of the crude product mixture from anhydrous ether, 7 was obtained as white granules in 31% overall yield from 2-(benzyloxy)ethanol (66% based on α-chloro ether 5): mp 82.5–84.5 °C; IR (KBr) ν_{max} 3440, 3070, 1760, 1720, 1675, 1460, 1385, 1225, 1100 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.63 (br d, 1 H, J = 4.0 Hz), 7.53 (d, 1 H, J = 5.7 Hz), 7.38–7.25 (m, 5 H), 6.17 (d, 1 H, J = 1.5 Hz), 4.53 (s, 2 H), 3.96–3.87 (m, 1 H), 3.85–3.77 (m, 1 H), 3.82 (s, 3 H), 3.73–3.59 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 166.01, 156.73 (d, J = 26.9 Hz), 149.48, 140.73 (d, J = 239.4 Hz), 137.45, 128.43, 127.83, 127.64, 124.24 (d, J = 34.2 Hz), 81.94, 73.34, 70.54, 68.52, 53.42; ^{19}F NMR ($CDCl_3$) δ −164.26 to −164.32 (m, collapses to a dd, J = 5.7 and 1.5 Hz, after D_2O exchange); mass spectrum (chemical ionization, CH_4), m/z 393 (M^+ + 41), 381 (M^+ + 29), 353 (M^+ + 1), 221, 131, 91; UV (CH_3OH) λ_{max} 265 nm (ε 8540); UV (CH_3OH + concentrated HCl) λ_{max} 264 nm (ε 8390); UV (CH_3OH + aqueous KOH) λ_{max} 237 (ε 8350), 265 (sh) nm (ε 6650). Anal. ($C_{16}H_{17}FN_2O_6$) C, H, N.

3,4-Dihydro-2,4-dioxo-α-[2-(phenylmethoxy)ethoxy]-1-(2*H*)-pyrimidineacetic Acid Hemihydrate (9). To a stirred solution of 4.65 g (13.9 mmol) of 6 in 43 mL of THF was added 15 mL of 1 N LiOH and 8 mL of water. After 5 h, the solution was partially concentrated in vacuo, diluted with water, and washed with ether. The aqueous layer was acidified with cold, dilute HCl and extracted twice with ether/EtOAc. The combined extracts were washed with brine, dried ($MgSO_4$), and concentrated in vacuo to give 3.64 g (81%) of a colorless glass. Crystallization from ether/acetone gave 9 as white crystals: mp 74–77 °C; IR (KBr) ν_{max} 3430, 1680 (br), 1455, 1090 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.45 (s, 1 H), 7.55 (d, 1 H, J = 8.0 Hz), 7.41–7.24 (m, 5 H), 5.92 (s, 1 H), 5.64 (dd, 1 H, J = 8.0 and 2.1 Hz), 4.48 (s, 2 H), 3.84–3.54 (m, 4 H), 4.3–2.8 (br, H_2O); ^{13}C NMR (DMSO-d_6) δ 167.19, 163.06, 160.56, 141.29, 138.13, 128.11, 127.35, 127.31, 101.88, 82.24, 71.92, 69.05, 68.36; mass spectrum (chemical ionization, CH_4), m/z 361 (M^+ + 41), 349 (M^+ + 29), 321 (M^+ + 1), 91; FABMS (triethanolamine) m/z 319 (M^{-1} − 1); UV (CH_3OH) λ_{max} 258 nm (ε 9140); UV (CH_3OH + concentrated HCl) λ_{max} 258 nm (ε 9310); UV (CH_3OH + aqueous KOH) λ_{max} 257 nm (ε 6970). Anal. ($C_{15}H_{16}N_2O_6\cdot{}^1/_2H_2O$) C, H, N.

5-Fluoro-3,4-dihydro-2,4-dioxo-α-[2-(phenylmethoxy)ethoxy]-1(2*H*)-pyrimidineacetic Acid (10). Similarly, 10 was obtained in 95% yield. Crystallization from ether/acetone gave 80% of 10 as white crystals: mp 96–99.5 °C; IR (KBr) ν_{max} 3420, 3060, 1715, 1700, 1665, 1375, 1250, 1200, 1090 cm^{-1}; ^1H NMR (acetone-d_6) δ 10.80 (br s, 1 H), 7.72 (d, 1 H, J = 6.3 Hz), 7.37–7.22 (m, 5 H), 6.09 (d, 1 H, J = 1.3 Hz), 4.55 (s, 2 H), 3.98–3.85 (m, 2 H), 3.75–3.64 (m, 2 H); ^{13}C NMR (acetone-d_6) δ 167.43, 157.71 (d, J = 26.7 Hz), 150.39, 141.45 (d, J = 233.3 Hz), 139.26, 129.05, 128.34, 128.24, 125.49 (d, J = 34.6 Hz), 83.23, 73.48, 70.73, 69.57; ^{19}F NMR (acetone-d_6) δ −166.96 to −167.05 (m, collapses to a dd, J = 6.3 and 1.3 Hz, after D_2O exchange); mass spectrum (chemical ionization, CH_4), m/z (relative intensity) 379 (M^+ + 41, 6), 367 (M^+ + 29, 28), 339 (M^+ + 1, 100), 277 (19), 249 (16), 221 (59), 91 (30); UV (CH_3OH) λ_{max} 267 nm (ε 8150). Anal. ($C_{15}H_{15}FN_2O_6$) C, H, N.

3,4-Dihydro-2,4-dioxo-*N*-(phenylmethoxy)-α-[2-(phenylmethoxy)ethoxy]-1(2*H*)-pyrimidineacetamide (11). A solution of 1.62 g (5.06 mmol) of 9, 0.77 mL (5.5 mmol) of Et_3N, 0.88 g (5.5 mmol) of *O*-benzylhydroxylamine hydrochloride, and 1.61 g (6.51 mmol) of EEDQ in 20 mL of CH_2Cl_2 was allowed to stir at 25 °C under nitrogen for 6 days. The solution was diluted with ether/EtOAc, washed with dilute HCl, water, dilute $KHCO_3$, and brine, and dried ($MgSO_4$). Concentration in vacuo gave 2.18 g (100%) of a colorless semisolid. Crystallization from ether/acetone gave 1.13 g (53%) of 11 as white crystals: mp 95–97 °C (softens at 93 °C); IR (KBr) ν_{max} 3410, 3210, 1695 (br), 1460, 1390, 1275, 1250, 1090 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.74 (s, 1 H), 8.59 (br s, 1 H), 7.41–7.25 (m, 10 H), 6.97 (d, 1 H, J = 8.1 Hz), 6.10 (s, 1 H), 5.69 (dd, 1 H, J = 8.1 and 2.0 Hz), 4.88 (d, 1 H, J = 11.1 Hz), 4.75 (d, 1 H, J = 11.1 Hz), 4.47 (s, 2 H), 3.91–3.74 (m, 2 H), 3.63–3.58 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 162.69, 162.43, 150.86, 140.60, 137.06, 134.63, 129.39, 128.96, 128.66, 128.58, 128.23, 127.94, 103.32, 82.33, 78.33, 73.42, 70.32, 68.37; mass spectrum (chemical ionization, CH_4), m/z 466 (M^+ + 41), 454 (M^+ + 29), 426 (M^+ + 1), 320, 303, 107, 91; UV (CH_3OH) λ_{max} 257 nm (ε 10 200). Anal. ($C_{22}H_{23}N_3O_6$) C, H, N.

5-Fluoro-3,4-dihydro-2,4-dioxo-*N*-(phenylmethoxy)-α-[2-(phenylmethoxy)ethoxy]-1(2*H*)-pyrimidineacetamide (12). Similarly, 12 was obtained in 53% yield (based on 9% recovered 10) as a colorless semisolid after flash chromatography eluting first with 75/25 CH_2Cl_2/EtOAc and then with 60/40 EtOAc/CH_2Cl_2. Crystallization from ether/acetone gave white crystals: mp 101–101.5 °C; IR (KBr) ν_{max} 3250, 3190, 3070, 3040, 1728 and 1712 (br), 1680, 1495, 1470, 1455, 1387, 1250, 1110, 1095, 1085, 1070, 750, 740, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.40–7.26 (m, 10 H), 7.11 (d, 1 H, J = 5.5 Hz), 6.09 (d, 1 H, J = 1.4 Hz), 4.89 (d, 1 H, J = 11.2 Hz), 4.76 (d, 1 H, J = 11.2 Hz), 4.47 (s, 2 H), 3.89–3.74 (m, 2 H), 3.60 (t, 2 H, J = 4.4 Hz); ^{13}C NMR ($CDCl_3$) δ 162.37, 156.62 (d, J = 26.7 Hz), 149.72, 140.72 (d, J = 240 Hz), 136.97, 134.51, 129.38, 129.04, 128.64, 128.58, 128.22, 127.93, 124.68 (d, J = 33.6 Hz), 82.43, 78.32, 73.40, 70.43, 68.30; ^{19}F NMR ($CDCl_3$) δ −100.99 (d, J = 5.5 Hz); mass spectrum (chemical ionization CH_4), m/z 484 (M^+ + 41), 472 (M^+ + 29), 444 (M^+ + 1), 314, 107, 91. Anal. ($C_{22}H_{22}FN_3O_6$) C, H, N.

3,4-Dihydro-*N*-hydroxy-α-(2-hydroxyethoxy)-2,4-dioxo-1-(2*H*)-pyrimidineacetamide (1). A mixture of 0.39 g of $PdO\cdot xH_2O$ and 1.12 g (2.63 mmol) of 11 in 25 mL of 3/1 EtOH/cyclohexene was heated at reflux for 1.25 h. The warm solution was diluted with EtOH and filtered through filter aid. The filtrate was concentrated in vacuo and the residue was purified by flash chromatography eluting with 6% H_2O/CH_3CN to give a colorless oil. Two crystallizations from EtOAc/CH_3OH gave 0.21 g (33%) of 1 as pale, pink crystals: mp 162 °C dec; IR (KBr) ν_{max} 3390, 3275, 3175, 1735 and 1675 (br), 1475, 1385, 1255, 1140, 1080 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.42 (br s, 1 H), 11.11 (br, 1 H), 9.25 (br s, 1 H), 7.48 (d, 1 H, J = 8.0 Hz), 5.90 (s, 1 H), 5.67 (d, 1 H, J = 8.0 Hz), 4.79 (br s, 1 H), 3.57–3.47 (m, 4 H); ^{13}C NMR (DMSO-d_6) δ 163.03, 161.88, 150.76, 141.22, 101.86, 81.34, 71.33, 59.51; FABMS (50/50 glycerol/thioglycerol; HCl) m/z 246 (M^+

+ 1). Anal. ($C_8H_{11}N_3O_6$) C, H, N.

1-(3-Oxo-1,4-dioxan-2-yl)-2,4(1*H*,3*H*)-pyrimidinedione (13). A mixture of 1.47 g (4.59 mmol) of 9 and 0.04 g of PdO·xH$_2$O in 24 mL of 3/1 EtOH/cyclohexene was heated at reflux for 3.5 h. The cooled mixture was filtered through filter aid; the solids were rinsed with EtOH. The combined filtrate and washings were concentrated in vacuo. The crude hydroxy acid was dissolved in 8 mL of pyridine under nitrogen, and 1.17 g (5.67 mmol) of DCC was added with stirring. TLC after 18 h indicated that the reaction was incomplete. An additional 0.59 g (2.9 mmol) of DCC was added. After 3 h the mixture was filtered through filter aid; the filter cake was rinsed with hot acetone. The filtrate and washings were concentrated in vacuo, and the residue was recrystallized from acetone to remove some *N,N'*-dicyclohexylurea. The residue (1.31 g) was treated with 150 mL of hot EtOAc and filtered. The filtrate was concentrated in vacuo to give 0.50 g of white solid. Flash chromatography eluting with EtOAc gave 0.45 g (46%) of 13 as a white solid. Recrystallization from EtOAc gave fine, white crystals: mp 237–240 °C; IR (KBr) ν_{max} 3430, 3040, 1685 (v br), 1615, 1425, 1400, 1358, 1290, 1260, 1219, 1204, 1113, 1102, 947, 825 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.63 (br s, 1 H), 7.82 (d, 1 H, J = 7.9 Hz), 5.98 (s, 1 H), 5.69 (d, 1 H, J = 7.9 Hz), 4.64–4.45 (m, 2 H), 4.17–4.01 (m, 2 H); ^{13}C NMR (DMSO-d_6) δ 163.68, 163.26, 150.11, 144.71, 101.98, 81.26, 68.44, 61.68; mass spectrum (chemical ionization, CH$_4$), m/z 253 (M$^+$ + 41), 241 (M$^+$ + 29), 213 (M$^+$ + 1), 113, 73. Anal. ($C_8H_8N_2O_5$) C, H, N.

5-Fluoro-1-(3-oxo-1,4-dioxan-2-yl)-2,4(1*H*,3*H*)-pyrimidinedione (14). The above procedure was also used for the synthesis of 14. However, some esterification of the acid occurred during the debenzylation of 10. The acid/ester mixture was saponified in aqueous THF with 1 N LiOH and neutralized with 1 equiv of 1 N HCl prior to removal of the solvents. The mixture of hydroxy acid and LiCl was treated with DCC in pyridine as above. Workup followed by flash chromatography eluting with EtOAc gave a 68% yield of 14. Recrystallization from EtOAc gave 14 as white crystals: mp 216–217 °C; IR (KBr) ν_{max} 3440, 3270, 1720 (br), 1675, 1475, 1380, 1300, 1250, 1210, 950 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 12.19 (br s, 1 H), 8.34 (d, 1 H, J = 6.5 Hz), 5.94 (s, 1 H), 4.64–4.47 (m, 2 H), 4.21–4.03 (m, 2 H); ^{13}C NMR (DMSO-d_6) δ 163.39, 157.05 (d, J = 26.3 Hz), 148.83, 139.69 (d, J = 231.9 Hz), 128.71 (d, J = 34.9 Hz), 81.00, 68.36, 61.66; ^{19}F NMR (DMSO-d_6) δ −167.07 (d, J = 6.5 Hz); mass spectrum (chemical ionization, CH$_4$), m/z 271 (M$^+$ + 41), 259 (M$^+$ + 29), 231 (M$^+$ + 1). Anal. ($C_8H_7FN_2O_5$) C, H, N.

5-Fluoro-3,4-dihydro-*N*-hydroxy-α-(2-hydroxyethoxy)-2,4-dioxo-1(2*H*)-pyrimidineacetamide (2). To a stirred suspension of 0.60 g (2.6 mmol) of 14 in 12 mL of EtOH was added 2.6 mL of 1 N NH$_2$OH/EtOH (prepared by neutralization of HONH$_2$·HCl with NaOH). After 30 min the mixture was filtered to remove a small amount of unreacted lactone 14 (which was also present in the filtrate). The filtrate was concentrated in vacuo and the residue was recrystallized three times from EtOAc/CH$_3$OH to give 2 as a white powder: mp 154–155 °C dec; IR (KBr) ν_{max} 3390, 3210, 1700 (v br), 1470, 1385, 1245, 1125, 1075 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.84 (br, 1 H), 11.13 (br, 1 H), 9.26 (br s, 1 H), 7.82 (d, 1 H, J = 6.8 Hz), 5.87 (d, 1 H, J = 1.4 Hz), 4.80 (br s, 1 H), 3.59–3.49 (m, 4 H), 0.20 mol of EtOAc also present; ^{13}C NMR (DMSO-d_6) δ 161.70, 157.08 (d, J = 26.1 Hz), 149.44, 139.89 (d, J = 231.3 Hz), 125.51 (d, J = 34.1 Hz), 81.62, 71.63, 59.57, EtOAc also present; ^{19}F NMR (DMSO-d_6) δ −167.4 (d, J = 6.8 Hz); mass spectrum (chemical ionization, CH$_4$), m/z (relative intensity) 304 (M$^+$ + 41, 5), 292 (M$^+$ + 29, 3), 264 (M$^+$ + 1, 65), 231 (M$^+$ + 1 − NH$_2$OH, 100), 131 (37); exact mass calcd for $C_8H_{11}FN_3O_6$ 264.0632, found 264.0639.

Methyl 4-Amino-2-oxo-α-[2-(phenylmethoxy)ethoxy]-1-(2*H*)-pyrimidineacetate (15). A catalytic amount of CH$_3$ONa was added to a stirred suspension of 6.37 g (17.0 mmol) of 8 in 65 mL of CH$_3$OH. After 2.5 h (the reaction is usually complete in 30–40 min) the clear solution was concentrated in vacuo and the light yellow solid converted to *t*-Boc derivative 16 without further purification.

The crude reaction mixture from another experiment was purified by flash chromatography eluting with 90/10 EtOAc/CH$_3$OH. Two recrystallizations from EtOAc/cyclohexane/CH$_3$OH gave white crystals: mp 142.5–144 °C; IR (KBr) ν_{max} 3310, 1755, 1660, 1635, 1490, 1095, 785 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.54 (d, 1 H, J = 7.4 Hz), 7.39–7.25 (m, 7 H), 5.96 (s, 1 H), 5.76 (d, 1 H, J = 7.4 Hz), 4.48 (s, 2 H), 3.81–3.73 (m, 1 H), 3.71–3.55 (m, 3 H), 3.68 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 166.80, 165.72, 154.85, 141.68, 138.06, 128.02, 127.26, 127.21, 94.52, 82.79, 71.91, 68.86, 68.39, 52.51; mass spectrum (chemical ionization, CH$_4$), m/z 374 (M$^+$ + 41), 362 (M$^+$ + 29), 334 (M$^+$ + 1), 202, 91; UV (CH$_3$OH) λ_{max} 243 (ϵ 8100), 270 nm (ϵ 8030); UV (CH$_3$OH + concentrated HCl) λ_{max} 281 nm (ϵ 12500); UV (CH$_3$OH + aqueous KOH) λ_{max} 242 (ϵ 9020), and 268 nm (ϵ 7890). Anal. ($C_{16}H_{19}N_3O_5$) C, H, N.

Methyl 4-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-oxo-α-[2-(phenylmethoxy)ethoxy]-1(2*H*)-pyrimidineacetate (16). A solution of crude 15 from above and 4.80 mL (20.9 mmol) of di-*tert*-butyl dicarbonate in 110 mL of 50/50 THF/1,4-dioxane was allowed to stir at reflux for 4.5 h. The solvents were removed in vacuo, and the residue was purified by flash chromatography eluting with 70/30 EtOAc/CH$_2$Cl$_2$ to give 6.68 g (91%) of 16 as a colorless glass: IR (CHCl$_3$) ν_{max} 3410, 3015, 1760, 1672, 1495, 1225, 1150 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.48 (br s, 1 H), 8.01 (d, 1 H, J = 7.5 Hz), 7.37–7.24 (m, 5 H), 7.06 (d, 1 H, J = 7.5 Hz), 6.02 (s, 1 H), 4.47 (s, 2 H), 3.91–3.56 (m, 4 H), 3.71 (s, 3 H), 1.46 (s, 9 H); ^{13}C NMR (DMSO-d_6) δ 166.40, 163.64, 154.46, 151.95, 145.45, 138.16, 128.18, 127.43, 127.39, 94.97, 83.91, 81.18, 72.02, 69.86, 68.45, 52.81, 27.73; mass spectrum (chemical ionization, CH$_4$), m/z 474 (M$^+$ + 41), 462 (M$^+$ + 29), 434 (M$^+$ + 1), 378 (M$^+$ + 1-C$_4$H$_8$), 360, 334; exact mass calcd for $C_{21}H_{28}N_3O_7$ 434.1927, found 434.1925. Anal. ($C_{21}H_{27}N_3O_7$) H, N; C: calcd, 58.19; found, 57.10.

Synthesis and BCl$_3$ Cleavage of 4-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-oxo-*N*-(phenylmethoxy)-α-[2-(phenylmethoxy)ethoxy]-1(2*H*)-pyrimidineacetamide (18). To a stirred solution of 4.50 g (10.4 mmol) of 16 in 45 mL of THF was added 13 mL of 1.0 N LiOH and 13 mL of water. After 4.5 h, the solution was partially concentrated in vacuo and then diluted with water containing a small amount of NaCl and washed with ether. The aqueous layer was acidified with ice-cold, dilute HCl and extracted twice with ether/EtOAc. The combined extracts were washed with water and brine and dried (MgSO$_4$). Concentration in vacuo gave 3.88 g (89%) of 4-[[(1,1-dimethylethoxy)carbonyl]amino]-2-oxo-α-[2-(phenylmethoxy)ethoxy]-1-(2*H*)-pyrimidineacetic acid (17) as a white foam: ^{1}H NMR (90 MHz, CDCl$_3$) δ 9.80 (br s, 2 H), 7.92 (d, 1 H, $J \approx$ 7.5 Hz), 7.36–7.12 (m, 6 H), 6.23 (s, 1 H), 4.41 (s, 2 H), 4.12–3.46 (m, 4 H), 1.47 (s, 9 H).

A solution of 2.10 g (5.01 mmol) of 17, 0.83 mL (5.6 mmol) of Et$_3$N, 0.94 g (5.6 mmol) of PhCH$_2$ONH$_2$·HCl, and 1.60 g (6.47 mmol) of EEDQ in 20 mL of CH$_2$Cl$_2$ was allowed to stir under nitrogen for 8 days. The solution was then diluted with ether/EtOAc, washed with dilute HCl, water, dilute KHCO$_3$, and brine, and dried (MgSO$_4$). Concentration in vacuo gave 2.61 g of a pale, straw-colored foam, which was chromatographed with 82/18 EtOAc/CH$_2$Cl$_2$ as eluant to give 1.88 g (72%) of 18 as a white foam: ^{1}H NMR (90 MHz, CDCl$_3$) δ 10.6 (br, <1 H), 8.68 (br, <1 H), 7.72 (d, 1 H, $J \approx$ 7.5 Hz), 7.47–7.07 (m, 11 H), 6.36 (s, 1 H), 4.82 (br s, 2 H), 4.36 (s, 2 H), 3.83–3.38 (m, 4 H), 1.49 (s, 9 H).

To a stirred solution of 1.33 g (2.54 mmol) of 18 in 5 mL of CH$_2$Cl$_2$ at −50 °C under nitrogen was added 6.1 mL of 1 M BCl$_3$/CH$_2$Cl$_2$ dropwise, but rapidly, via a syringe. TLC after 30 min at −50 to −30 °C indicated residual 18 was present, so an additional 1.5 mL of 1 M BCl$_3$/CH$_2$Cl$_2$ was added. After 1 h, the solvents were removed in vacuo. The residue was suspended in 20–25 mL of CH$_2$Cl$_2$ and enough CH$_3$OH was added to dissolve the beige solid. The solvents were again removed in vacuo, and the residual solid was dissolved in water and washed twice with EtOAc/ether. The colorless aqueous layer was concentrated in vacuo to give a colorless glass, which gave a positive FeCl$_3$ test: ^{1}H NMR (90 MHz, DMSO-d_6) δ 9.5 (br, 0.5 H) 8.72 (br, 0.5 H), 7.91 (br d, 1 H), 7.39 (br, 3 H), 6.20 and 6.17 (2d of ca. equal intensity, 1 H total, $J_1 \approx J_2 \approx$ 7 Hz), 6.03 and 5.90 (2 s, 1 H total), 3.7–3.4 (m), 3.70 (s); GC/MS (Vydac C-18, 25 cm × 4.6 mm), eluting with water for 3, retention time 5.7 min, and then 90/10 H$_2$O/CH$_3$OH for 19, retention time 16.5 min; m/z (relative intensity) for 3, 245 (M$^+$ + 1, 16), 223 (48), 212 (M$^+$ + 1 − NH$_2$OH, 45), 151 (100), 134 (80); for 19, 244 (M$^+$ + 1, 100), 212 (M$^+$ + 1 − CH$_3$OH, 90).

Methyl 4-[[(1,1-Dimethylethoxy)carbonyl]amino]-α-(2-hydroxyethoxy)-2-oxo-1(2*H*)-pyrimidineacetate (20). A stirred mixture of 6.00 g (13.8 mmol) of **16** and 0.61 g of $PdO{\cdot}xH_2O$ in 68 mL of 3/1 EtOH/cyclohexene was allowed to stir at reflux for 1.5 h. Filtration through filter aid and concentration in vacuo gave 4.65 g (98%) of pale, straw-colored foam. ^1H NMR analysis indicated ~6% residual **16**. This was combined with 0.46 g of crude **20** (prepared from 0.61 g of **16**) and purified by flash chromatography eluting with EtOAc to obtain 0.34 (5%) of recovered **16** and 0.05 g (1%) of lactone **21**. Elution with 7.5% CH_3OH/EtOAc gave 4.03 g (77%) of **20** as a clear glass: IR ($CHCl_3$ film) ν_{max} 3230, 2980, 1745, 1662, 1628, 1500, 1370, 1230, 1150 cm^{-1}; (the NMR spectra of **20** were complicated by the presence of syn/anti tautomers of the carbamate) ^1H NMR ($CDCl_3$) δ 7.89 and 7.80 (2 d, 1 H total, J = 7.5 Hz for the d at δ 7.80), 7.30 (d, 1 H, J = 7.5 Hz), 7.27 (s, 1 H), 6.35 (s, <1 H), 3.88–3.70 (m, 8 H), 1.52 and 1.49 (2 s, 9 H total); mass spectrum (chemical ionization, CH_4), m/z 372 (M^+ + 29), 344 (M^+ + 1), 312 ($M^+ + 1 - CH_3OH$), 288, 244, 212, 112. Anal. ($C_{14}H_{21}N_3O_7$) C, H, N.

1,1-Dimethylethyl [1,2-Dihydro-2-oxo-1-(3-oxo-1,4-dioxan-2-yl)-4-pyrimidinyl]carbamate (21). To a stirred solution of 4.03 g (11.7 mmol) of **20** in 60 mL of THF was added 26 mL of 1 N LiOH and 25 mL of water. After 1.25 h, the solution was concentrated in vacuo. The residue was dissolved in a solution prepared by addition of 3.40 mL (42 mmol) of pyridine to 27 mL of 1.0 N HCl. The solution was again concentrated in vacuo. Evaporation from a pyridine solution gave a white foam, which was combined with crude hydroxy acid from a similar experiment (from 3.11 mmol of **20**). The foam was dissolved with stirring in 32 mL of pyridine under nitrogen, and 4.36 g (21.1 mmol) of DCC was added. After 21 h, the pyridine was removed and the residue was partitioned between water and EtOAc; the mixture was filtered to remove 4.27 g of *N,N'*-dicyclohexylurea. The EtOAc layer was separated and concentrated in vacuo. Flash chromatography eluting with EtOAc gave 2.82 g (61%) of **21**. Recrystallization from EtOAc/cyclohexane gave fine, white crystals: mp 180.5 °C dec; IR (KBr) ν_{max} 3415, 2990, 1750, 1680, 1635, 1550, 1500, 1295, 1225, 1150 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.59 (s, 1 H), 8.18 (d, 1 H, J = 7.5 Hz), 7.06 (d, 1 H, J = 7.5 Hz), 5.95 (s, 1 H), 4.68–4.59 (m, 1 H), 4.51–4.43 (m, 1 H), 4.21–4.13 (m, 1 H), 4.10–4.00 (m, 1 H), 1.46 (s, 9 H); ^{13}C NMR (DMSO-d_6) δ 164.38, 163.35, 153.88, 151.87, 149.29, 94.90, 82.66, 81.32, 68.21, 61.65, 27.71; FABMS (glycerol) m/z (relative intensity) 312 (M^+ + 1, 44), 270 (13), 258 (17), 257 (39), 256 ($M^+ + 1 - C_4H_8$, 100), 212 (43), 112 (15). Anal. ($C_{13}H_{17}N_3O_6$) C, H, N.

4-Amino-1-(3-oxo-1,4-dioxan-2-yl)-2(1*H*)-pyrimidinone Trifluoroacetic Acid Salt (22). To 0.92 g (3.0 mmol) of **21** placed in a flask under nitrogen at 0 °C was added 15 mL of trifluoroacetic acid (TFA). The solution was allowed to stir at 0 °C for 1 h. The TFA was removed in vacuo and the residue was dissolved in acetone. Concentration in vacuo gave 0.96 g (100%) of a white solid. Recrystallization from acetone gave white crystals containing 5.7 mol % acetone, which could not be removed: mp 223 °C dec; IR (KBr) ν_{max} 3290, 1750, 1710, 1535, 1380, 1310, 1205, 1185, 1135, 1110, 955, 790, 730 cm^{-1}; ^1H NMR (DMSO-d_6, D_2O) δ 8.00 (d, 1 H, J = 7.7 Hz), 6.10 (d, 1 H, J = 7.7 Hz), 5.98 (s, 1 H), 4.68–4.49 (m, 2 H), 4.21–4.07 (m, 2 H), 2.11 (s, CH_3COCH_3); ^{13}C NMR (DMSO-d_6 after D_2O exchange) δ 164.16, 162.02, 149.79, 148.21, 95.41, 82.82, 69.23, 62.44; ^{19}F NMR (DMSO-d_6) δ −73.10; FABMS (glycerol), m/z (relative intensity) 304 (M^+ + 1 + glycerol, 26), 212 (M^+ + 1, 100), 112 (70), 101 (12); exact mass calcd for $C_8H_{10}N_3O_4$ 212.0671, found 212.0665. Anal. ($C_8H_9N_3O_4{\cdot}CF_3CO_2H$) H; C: calcd, 36.93; found, 37.37; N: calcd, 12.92; found, 12.50.

4-Amino-*N*-hydroxy-α-(2-hydroxyethoxy)-2-oxo-1(2*H*)-pyrimidineacetamide (3). To a stirred suspension of 0.43 g (2.0 mmol) of **22** in 6 mL of EtOH was added 2.1 mL of 1.0 M NH_2OH/EtOH. An additional 1.1 mL of 1.0 M NH_2OH/EtOH was added in three portions over 50 min until TLC analysis indicated the reaction was complete. The solution was concentrated in vacuo and the residue was chromatographed eluting with 85/15 CH_3CN/H_2O to give 0.20 g (40%) of a colorless glass. Two crystallizations from CH_3OH/EtOAc (filtering through filter aid) gave a fine, white, crystalline powder: mp 158.5 °C dec; IR (KBr) ν_{max} 3340, 3190, 1687, 1650, 1600, 1495, 1380, 1120, 1075, 810, 790 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.03 (br s, 1 H), 9.16 (br s, 1 H), 7.46 (d, 1 H, J = 7.5 Hz), 7.29 (br s, 1 H), 7.20 (br s, 1 H), 5.96 (s, 1 H), 5.75 (d, 1 H, J = 7.5 Hz), 4.76 (br s, 1 H), 3.55–3.38 (m, 4 H); ^{13}C NMR (DMSO-d_6) δ 166.43, 163.83, 156.91, 142.97, 95.89, 82.92, 71.68, 60.34; mass spectrum (chemical ionization, CH_4), m/z (relative intensity) 212 (78), 140 (9), 112 (100), 87 (23), 73 (22); FABMS (glycerol) m/z 245 (M^+ + 1); exact mass calcd for $C_8H_{10}N_3O_4$ 212.0671, found 212.0668; exact mass calcd for $C_4H_6N_3O$ 112.0511, found 112.0504. Anal. ($C_8H_{12}N_4O_5$) C, H; N: calcd, 22.94; found, 22.31.

Biological Studies. 1. CDP Reductase Assay. Ribonucleotide reductase from calf thymus was partially purified by following the procedure of Engström[23] through the DE52 step. CDP reductase was assayed by the following procedure. In brief, each assay (150 μL) contained the following: 0.5 unit of CDP reductase (unit = nanomole of CDP reduced/30 min at saturating concentrations of CDP); 4 mM $MgCl_2$; 2 mM AMP-PNP; 10 mM HEPES, pH 7.6; 6 mM DTT; 0.14 μCi [^{14}C]CDP; and a single concentration (0–10 mM) of inhibitor. CDP reductase activity was determined by incubating the total incubate at 37 °C for 30 min and then cleaving nucleotides and deoxynucleotides to their respective nucleosides and deoxynucleosides with rattlesnake venom and assaying for deoxycytidine by the Dowex-1-borate method of Steeper and Steuart.[24]

2. Inhibition of HeLa Cells in Culture. Exponentially growing HeLa cells were plated at a density of 0.5×10^5 cells/35-mm dish. The plates were incubated overnight for 18 h at 37 °C in a 5% CO_2 incubator. After 18-h incubation, the medium was replaced with fresh medium containing different concentrations of the compounds and was further incubated for 72 h with a medium change at 48 h. At the end of the incubation, cells were collected by trypsinization and counted with a culture counter. The cell number had increased to 9.5×10^5 in the controls. The data presented is an average of two experiments.

(23) Engström, Y.; Ericksson, S.; Thelander, L.; Åkerman, M. *Biochemistry* **1979**, *18*, 2941.
(24) Steeper, J. R.; Steuart, C. D. *Anal. Biochem.* **1970**, *34*, 123.