two changes of 1 L each of buffer A, to remove the inhibitor, immediately before use as a source of the decarboxylase enzyme. Using this method, an enzyme preparation having a specific activity of about 15 nmol of CO_2 min^{-1} (mg of protein)$^{-1}$ was routinely obtained.

Assay of *S*-Adenosyl-L-methionine Decarboxylase Activity. *S*-Adenosyl-L-methionine decarboxylase activity was determined by a modification of the method of Pegg and Williams-Ashman.[16] The standard assay medium contained 0.1 M sodium phosphate buffer (pH 7.0), 2.5 mM dithiothreitol, 2.5 mM putrescine, 0.2 mM *S*-[1-^{14}C]adenosyl-L-methionine (2.0×10^5 cpm), and the enzyme preparation (50 μL) in a total volume of 1.0 mL. Reactions were carried out in 16 × 100 mm test tubes sealed with rubber stoppers carrying a polypropylene well containing 0.3 mL of ethanolamine. After incubation for 30 min at 37 °C, the reaction was terminated and $^{14}CO_2$ released by the injection of 0.5 mL of 5 N H_2SO_4 through the rubber stopper. The tubes were incubated for an additional 45 min at 25 °C, and the well and its contents were transferred into a glass scintillation vial containing 10 mL of scintillation cocktail. The samples were stored in the dark overnight and counted. All counts were corrected for the nonenzymatic production of $^{14}CO_2$, which was determined by replacing the enzyme fraction in the incubation medium with an equal volume of buffer A. This method gave quantitative recovery of the $^{14}CO_2$ released from sodium [^{14}C]-bicarbonate.

Enzyme Inhibition Studies. The incubation media contained 0.1 M sodium phosphate buffer, pH 7.0; 2.5 mM dithiothreitol; 2.5 mM putrescine; *S*-[1-^{14}C]adenosyl-L-methionine (2.0×10^5 cpm); *S*-adenosyl-L-methionine at concentrations of 2.0×10^{-5}, 4.0×10^{-5}, 8.0×10^{-5}, and 1.60×10^{-4} M; the inhibitor at concentrations of 6.25×10^{-5}, 1.25×10^{-4}, and 2.50×10^{-4} M; and 0.1 mL of the enzyme preparation in a total volume of 1.0 mL. The incubation conditions and the measurement of $^{14}CO_2$ released were as described under "Assay of *S*-Adenosyl-L-methionine Decarboxylase Activity".

Effects on Enzymatic Activity of Preincubation with Inhibitors or Inhibitors and Sodium Cyanoborohydride. The preincubation media contained enzyme fraction in buffer A, 0.25 mL; bovine serum albumin, 0.3 mg; inhibitor at concentrations of 0.0, 0.2, or 0.4 mM; and sodium cyanoborohydride, 1.0 mM, when appropriate, in a final volume of 0.3 mL adjusted to pH 7.0. The mixtures were incubated at 37 °C, and 25-μL aliquots were removed at 0, 40, and 80 min and assayed for decarboxylase activity as described under "Assay of *S*-Adenosyl-L-methionine Decarboxylase Activity", at a substrate concentration of 0.2 mM. Under these conditions, the presence of the inhibitors and/or sodium cyanoborohydride did not change the measured enzyme activity at 0 min.

Acknowledgment. The authors gratefully acknowledge the financial support of M.P. by the American Foundation for Pharmaceutical Education (1978–1980) and Melendy Summer Fellowship.

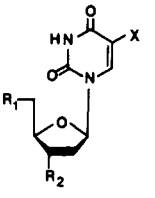
Synthesis and Biological Activities of 5-(Hydroxymethyl, azidomethyl, or aminomethyl)-2′-deoxyuridine and Related 5′-Substituted Analogues[1a]

George T. Shiau, Raymond F. Schinazi,[1b] Ming S. Chen, and William H. Prusoff*

Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510. *Received August 6, 1979*

The synthesis of 5-(azidomethyl)-2′-deoxyuridine (**10**) has been accomplished by two independent methods. The first involved tosylation of 5-(hydroxymethyl)-2′-deoxyuridine (**1**) to furnish a mixture of two mono- and a ditosyl nucleosides which were converted into the corresponding 5-(azidomethyl) (**10**), 5-(azidomethyl)-5′-azido (**14**), and 5-(hydroxymethyl)-5′-azido (**15**) derivatives of 2′-deoxyuridine. The second method was more selective and required the formation of the intermediate 5-(bromomethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine (**8**), followed by displacement of the bromo group by lithium azide and deacetylation. Catalytic hydrogenation of the azides **9**, **10**, **14**, and **15** gave the corresponding amines **16**, **2**, **6**, and **7**, respectively. Compounds **1**, **2**, **10**, and **16** inhibited the growth of murine Sarcoma 180 and L1210 in culture, and the activity of **2** was prevented by 2′-deoxypyrimidine nucleosides but not by purine nucleosides. The replication of herpes simplex virus type 1 (HSV-1) was strongly inhibited only by **1** and **10**. Studies on the binding of the various thymidine analogues to HSV-1 encoded pyrimidine deoxyribonucleoside kinase indicate that **1** and **10** have good affinity for the enzyme.

Modifications at the C-5 position of pyrimidine deoxyribonucleosides have produced a number of compounds with selective biological activity.[2,3] Some of these derivatives are phosphorylated in cell culture with subsequent incorporation into DNA leading to a stable modified DNA. The findings that 5-(hydroxymethyl)-2′-deoxyuridine (**1**) inhibited the replication of *Escherichia coli* 15T$^-$ by Green et al.,[4] had significant cytotoxic activity on a variety of tumor cell lines by Langen and co-workers[5–7] as well as on normal cells (bovine fetal kidney) by Meldrum et al.,[8] and had antiviral activity against vaccinia and herpes simplex



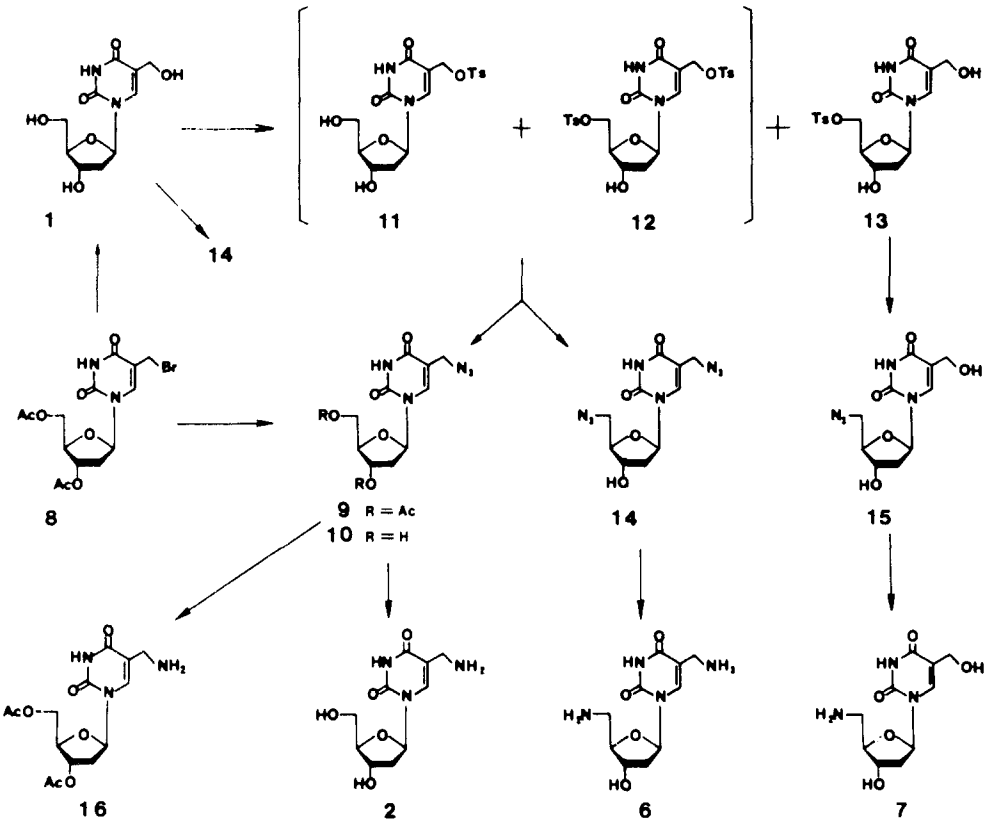
	R_1	R_2	X
1	OH	OH	CH_2OH
2	OH	OH	CH_2NH_2
3	NH_2	OH	I
4	NH_2	OH	CH_3
5	OH	NH_2	CH_3

(1) (a) Part of this work was presented at the 176th National Meeting of the American Chemical Society, Miami Beach, Fl., Sept 10–18, 1978, CARB 27. (b) Present address: Department of Pediatrics, Division of Infectious Diseases and Immunology, Emory University School of Medicine, Atlanta, Ga. 30303.

(2) W. H. Prusoff and B. Goz, *Handb. Exp. Pharmakol.*, **38**, 272–345 (1975).
(3) E. de Clercq and P. F. Torrence, *J. Carbohydr., Nucleosides, Nucleotides*, **5**, 187 (1978).
(4) M. Green, H. Barner, and S. S. Cohen, *J. Biol. Chem.*, **228**, 621 (1957).
(5) P. Langen and D. Bärwolff, *Acta Biol. Med. Ger.*, **34**, K7 (1975).
(6) S. Waschke, J. Reefschlager, P. Langen, and D. Bärwolff, *Nature (London)*, **255**, 629 (1975).
(7) D. Bärwolff and P. Langen, *Nucleic Acids Res.*, **S1**, s29 (1975).
(8) J. B. Meldrum, V. S. Gupta, and J. R. Saunders, *Antimicrob. Agents Chemother.*, **6**, 393 (1974).

0022-2623/80/1823-0127$01.00/0

Scheme I



virus by DeClercq et al.[9] prompted us to prepare the corresponding 5-(aminomethyl) derivative **2**, since we have shown that replacement of a hydroxyl by an amino function in pyrimidine nucleosides may confer to the congener interesting biological properties. For example, whereas 5-iodo-5′-amino-2′,5′-dideoxyuridine (**3**) and the corresponding 5′-aminothymidine analogue **4** selectively inhibit the replication of herpes simplex virus type 1 (HSV-1), 3′-amino-3′-deoxythymidine (**5**) has potent cytostatic activity in culture.[10,11] A number of derivatives of **2** containing various substituents on the amino function have been prepared,[12-14] some of which are potent inhibitors of thymidylate synthetase, and recently[15] tritium-labeled **2** was synthesized as a precursor to labeled **1**. Hampton et al.[16] prepared **2** in good yield by conversion of 5-iodo-2′-deoxyuridine to 5-cyano-2′-deoxyuridine, which was subsequently hydrogenated with rhodium–alumina. However, the biological activity of the free aminomethyl nucleoside had not been reported. Hence, 5-(aminomethyl)-2′-deoxyuridine (**2**), 5-(aminomethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine (**16**), 5-(hydroxymethyl)-5′-amino-2′,5′-dideoxyuridine (**7**), and 5-(aminomethyl)-5′-amino-2′,5′-dideoxyuridine (**6**) were synthesized and evaluated for antiviral and antineoplastic activity. The present report also examines the effect of these nucleoside analogues and their precursors on the activity of HSV-1 encoded pyrimidine deoxyribonucleoside kinase and discusses a structure–activity relationship among these compounds.

Chemistry. Two approaches were taken in order to prepare one of the key intermediates, 5-(azidomethyl)-2′-deoxyuridine (**10**). This analogue, which is easily converted to the amino compound **2** by hydrogenation, was chosen as an intermediate, since it was also required for evaluation as a potential photosensitizing agent. The methods[12,17] for preparing this compound prior to the report by Hampton et al.,[16] which appeared during the preparation of this manuscript, are laborious; therefore, a new method was devised. Barwolff and Langen[18] have recently described a simple synthesis of 5-(bromomethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine (**8**) which involves selective photochemical monobromination of the C-5 methyl group of the readily available 3′,5′-di-*O*-acetylthymidine[19] with bromine in carbon tetrachloride. This unstable product has been shown to react with a variety of nucleophiles.[5] Therefore, we prepared compound **9** by displacement of the bromo function of compound **8** with lithium azide in DMF at 80 °C to give 5-(azidomethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine (**9**). This compound was isolated as an oil and was converted either to the deblocked nucleoside (**10**) with methanolic ammonia or to the 5-

(9) E. de Clercq, J. Descamps, P. F. Torrence, E. Krajewska, and D. Shugar, Current Chemotherapy I, Proceedings of the Tenth International Congress of Chemotherapy, Zurich, Sept 18, 1977, 1978, p 352.
(10) M. S. Chen, D. C. Ward, and W. H. Prusoff, *J. Biol. Chem.*, **251**, 4833 (1976).
(11) T. S. Lin and W. H. Prusoff, *J. Med. Chem.*, **21**, 109 (1978).
(12) A. Kampf, C. J. Pillar, W. J. Woodford, and M. P. Mertes, *J. Med. Chem.*, **19**, 909 (1976).
(13) M. S. Edelman, R. L. Barfknecht, R. Huet-Rose, S. Boguslawski, and M. P. Mertes, *J. Med. Chem.*, **20**, 669 (1977).
(14) D. Bärwolff and P. Langen, *Nucleic Acids Res.*, **S4**, s227 (1978).
(15) D. Bärwolff, D. Murawski, and P. Langen, FEBS Post-Congress Symposium, Antimetabolites in Biochemistry, Biology and Medicine, Prague, Czechoslovakia, July 10–12, 1978, p 23.
(16) A. Hampton, F. Kappler, and R. R. Chawla, *J. Med. Chem.*, **22**, 621 (1979).
(17) V. R. Brossmer and E. Rohm, *Hoppe-Seyler's Z. Physiol. Chem.*, **348**, 1431 (1967).
(18) D. Bärwolff and P. Langen, in "Nucleic Acid Chemistry", Part I, L. B. Townsend and R. S. Tipson, Eds., Wiley, New York, 1978, p 75. We thank the authors for a preprint of the paper.
(19) A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 816 (1955).

(aminomethyl)-3',5'-di-*O*-acetyl-2'-deoxyuridine (**16**) by catalytic hydrogenation of pure **9**. This amino analogue, **16**, is more lipid soluble than **2** and could act biologically as a prodrug. Compound **10** was separated from unreacted thymidine by column chromatography on silica, followed by fractional crystallization (Scheme I). The 5-(aminomethyl) analogue **2** was then readily obtained by hydrogenation of **10** in the presence of palladium on charcoal in an overall 24% yield. The absence of possible impurities such as **1** (which can arise from hydrolysis of **8** or deamination of **2**) and thymidine was ascertained using a high-pressure liquid chromatography method. Similarly, the possible presence of compound **1** or dThd in the preparation of compound **10** at the 0.01% level was eliminated by high-pressure LC analysis.

Although the route described above is simple, like most photochemical reactions it suffers the disadvantage of not being practical for large-scale preparations. Hence, a second approach was examined which involved the preparation of **1** by the base-catalyzed hydroxymethylation of 2'-deoxyuridine with paraformaldehyde at 60 °C according to the method of Baker et al.[20] The formation of the product was monitored by TLC. Although this reaction was slow in comparison to the preparation of **1** by hydrolysis and deacetylation of **8**, larger amounts of **1** are readily prepared by this latter procedure.

Reaction of **1** with *p*-toluenesulfonyl chloride in a mole ratio of 1:2 in anhydrous pyridine at 0 °C for 3 days gave the disulfonate **12** as the only product (Scheme I). However, when a 1:1 mole ratio was used at 0 °C for 1 day, a mixture of the two monosulfonates **11** and **13** and a disulfonate **12** was obtained, as well as some unreacted starting material. Partial purification of this mixture by column chromatography furnished **13** as a pure compound and a mixture of **11** and **12** containing traces of **13**. This mixture, which was difficult to resolve, was treated directly with lithium azide at 80 °C to give, after column chromatography on silica, pure azides **10** and **14**. In the same manner, **13** was converted to the azide **15**. Hydrogenation of the individual azides **10**, **14**, and **15** over palladium on charcoal furnished, after cation-exchange chromatography, the respective amines **2**, **6**, and **7**. All the compounds mentioned above were fully characterized by ^{1}H NMR, UV, IR spectra and by elemental analysis and were found to be consistent with the assigned structures.

Biological Results and Discussion

A. Cell Culture, Animal, and Viral Studies. The compounds synthesized were evaluated for cytotoxic and antiviral activities in cell culture. Both 5-CH_2N_3-dUrd (**10**) and 5-CH_2NH_2-dUrd (**2**) are potent inhibitors of the growth of murine L1210 cells, but the diamino derivative **6**, as well as compounds **7**, **14**, and **15**, was not active. A free 5'-hydroxyl function appears to be necessary for the cytotoxic effect to occur. Compound **2** exerted a 93% inhibition of the replication of L1210 cells in cell culture at 7 μM and has an ED_{50} in the region of 2 μM (Figure 1). Similarly, compound **10** exerted a 96% inhibition at 200 μM with an ED_{50} of about 90 μM (Figure 2).. Hence, this compound is significantly less inhibitory than compound **2** to the replication of L1210 cells in culture.

A similar study was made with Sarcoma 180 cells in cell culture. Figure 3 shows a plot of the percentage inhibition of the replication of S180 cells against the concentration (log scale) of compounds **1**, **2**, and **10**. The growth of Sarcoma 180 cells was almost completely inhibited by

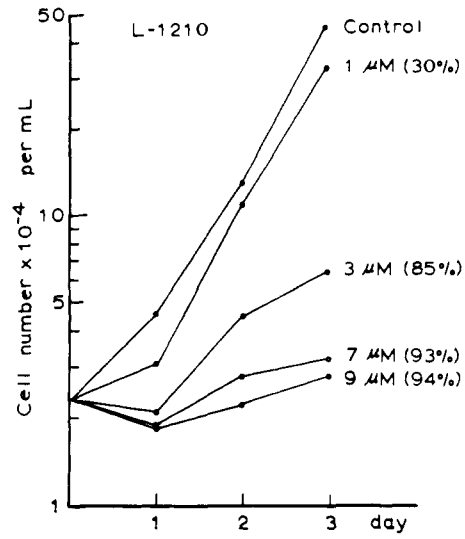


Figure 1. Effect of 5-(aminomethyl)-2'-deoxyuridine (**2**) on the replication of L1210 cells in cell culture.

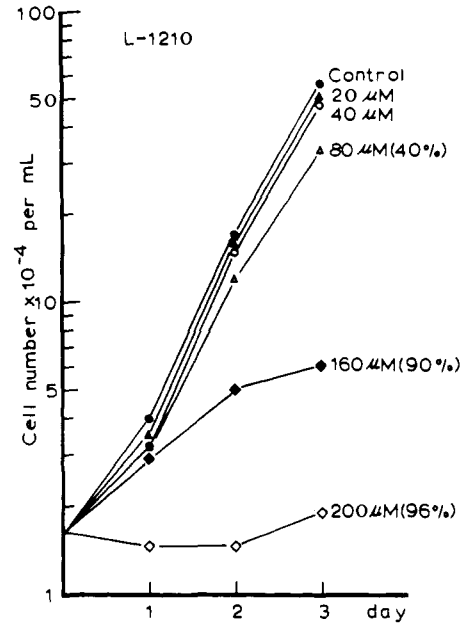


Figure 2. Effect of 5-(azidomethyl)-2'-deoxyuridine (**10**) on the replication of L1210 cells in cell culture.

compound **2** at only 1 μM, and the ED_{50} was estimated to be 0.16 μM. The corresponding azido analogue, compound **10**, was about 130-fold less inhibitory (ED_{50} = 21 μM). For comparison, the effect of 5-(hydroxymethyl)-2'-deoxyuridine (**1**) on the replication of Sarcoma 180 cells (ED_{50} = 8.5 μM) and of Ehrlich ascites carcinoma cells[5] (ED_{50} = 4 μM) has been included, and it can be seen that compound **2** is 25- to 50-fold more potent. Compounds **6**, **7**, **14**, and **15** were not inhibitory to the growth of S180 cells. The inhibition of growth by the aminomethyl derivative **2**, even at a concentration (1 μM) that is almost completely inhibitory, can be significantly prevented by the inclusion of dThd or dUrd (10 μM) in the media. However, neither purine 2'-deoxyribonucleosides (10 μM), such as dAdo or dGuo, nor ribonucleosides (10 μM), such as Urd, Ado, or Guo, prevented the inhibitory effect. Figure 4 shows that the inhibitory effect of **2** was almost completely prevented by dThd at a concentration five times that of the test compound.

Preliminary animal experiments on mice bearing L1210 leukemia cells were performed. Compound **2** was dissolved

(20) B. R. Baker, T. J. Schwan, and D. V. Santi, *J. Med. Chem.*, **9**, 66 (1966).

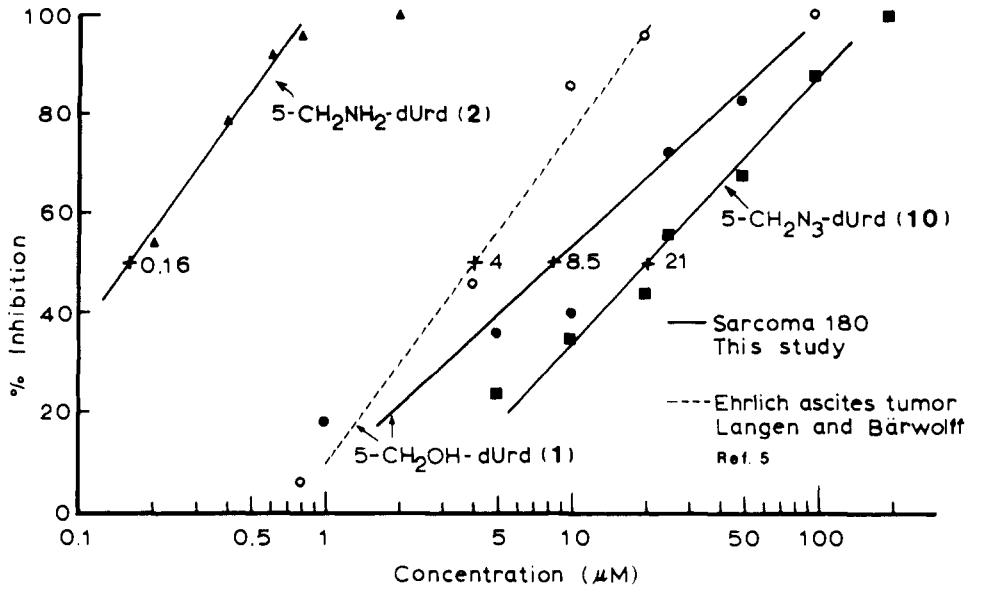


Figure 3. A comparison of the inhibitory effect of several nucleoside analogues on the replication of S180 cells in culture. Numbers without brackets (marked with + on the line) represent the ED_{50}.

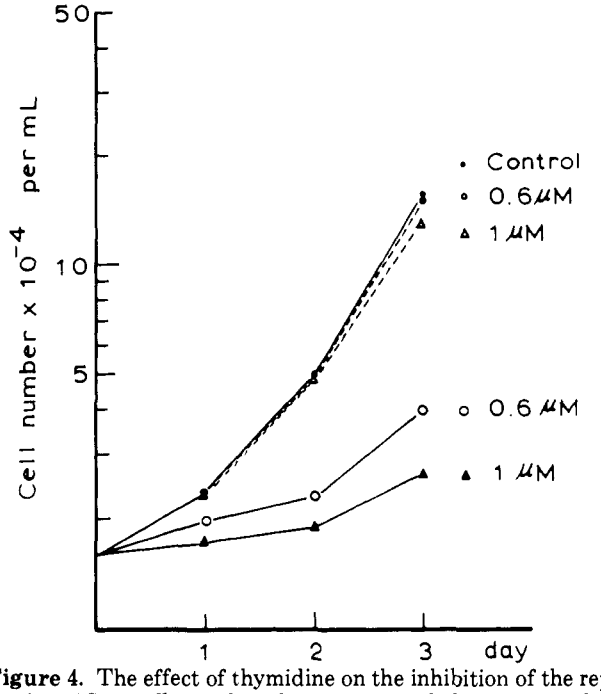


Figure 4. The effect of thymidine on the inhibition of the replication of S180 cells in culture by 5-(aminomethyl)-2′-deoxyuridine (**2**). Solid line: compound **2** alone; broken line: compound **2** plus 5 μM thymidine. The concentration of compound **2** is indicated in the figure.

in distilled sterile water and given ip either once daily for 6 days at 10, 100, and 400 mg kg^{-1} or twice daily for 2 days at 200 mg kg^{-1}, but neither toxicity nor significant prolongation of life was noted. The more lipid-soluble compound **16** was also tested as a prodrug at 200 and 400 mg kg^{-1} once a day for 6 days without an increase in life span. Since nucleosides are generally rapidly excreted, a regimen that maintains an appropriate blood level for an adequate amount of time will have to be used for proper evaluation.

The antiviral activity of several of these compounds was investigated, and the azido analogue **10** was found to be a potent inhibitor of HSV-1 (Table I). 5-CH_2OH-dUrd (**1**) was also a potent inhibitor of HSV-1; the virus titer was only 0.05% of the control at a concentration of 200 μM. However, DeClercq et al.[9] found **1** to be cytostatic (cell number reduced by about 30%) at a dose which was

Table I. Kinetic Inhibition Constants (K_i) of Various Nucleoside Analogues for Herpes Simplex Virus Type 1 Pyrimidine 2′-Deoxyribonucleoside Kinase and Their Antiviral Activity (HSV-1)

		antiviral activity	
compd	K_i, μM	concn, μM	% control
5-CH_2OH-dUrd (1)	3.5	200	0.05
5-CH_2NH_2-dUrd (2)	36	400	100
5-CH_2NH_2-5′-NH_2-dUrd (6)	>500	400	100
5-CH_2OH-5′-NH_2-dUrd (7)	530	400	100
5-CH_2N_3-dUrd (10)	0.8	200	0.5
5-CH_2OH-5′-N_3-dUrd (14)	62	400	100
5-CH_2N_3-5′-N_3-dUrd (15)	31	200	100
5-I-2′-dUrd	0.4	5	2
5-I-5′-NH_2-2′,5′-d_2Urd	5.0	200	4.5 ± 3.2

about half that required to produce a 50% inhibition of HSV replication. Since the azido analogue (**10**) is an effective inhibitor of the host Vero cells (ED_{50} = 110 μM), the antiviral activity (ED_{50} = 36 μM) is not considered to be specific. None of the other analogues had any significant antiviral activity.

B. Enzyme Studies. The kinetic constants (K_i) for the various amino and azido nucleosides with HSV-1 encoded pyrimidine deoxyribonucleoside kinase are shown in Table I. All these compounds competed competitively with dThd as the substrate. The azidomethyl derivative **10** had the strongest affinity for the enzyme, and the K_i was of the same order of magnitude as the K_m for the natural substrate (dThd, K_m = 0.6–1 μM),[21] indicating that the relatively long azidomethylene function was well tolerated at the 5 position. Replacement of one of the 5-methyl protons of dThd by a hydroxyl function (compound **1**) decreased the binding affinity fourfold, whereas replacing it by the basic amino group (compound **2**) increased the K_i approximately 40-fold. Similar studies by Hampton et al.[16] with a mammalian thymidine kinase also found that **2** was a weak competitive inhibitor with respect to thymidine having a K_i of 1.70 mM. The explanation for differences in K_i values for **2** found by these workers[16] and ourselves is unknown, but may be related to the source of the en-

(21) R. F. Schinazi, M. S. Chen, and W. H. Prusoff, *J. Med. Chem.*, 21, 1141 (1978).

zyme. The effect was even more severe when, in addition to $-CH_2OH$ or $-CH_2NH_2$ in position 5 of the pyrimidine moiety, the 5'-hydroxyl was also replaced by an amino group; a greater than 500-fold loss in binding was observed in compounds **6** and **7**. Modification of the 5' position from a hydroxymethylene group to an azidomethylene group also markedly decreased the affinity of the analogues relative to the parent compounds. These findings are consistent with earlier work.[22] The K_i of compounds **1** and **10** toward host Vero cell thymidine kinase is 47 and 750 μM, respectively, with both compounds showing linear competitive inhibition. The K_m for thymidine for Vero cell thymidine kinase is 0.5 μM.

Experimental Section

Chemical Methods and Materials. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. ^1H NMR spectra were recorded on a Brucker 270 HX spectrometer in Me_2SO-d_6 solution unless otherwise stated. Chemical shifts are reported in δ units, parts per million downfield from internal tetramethylsilane. Chemical shifts and coupling constants (J values in hertz) are first order. Double-resonance studies and deuterium exchange support the assignment of the protons, and integrations were consistent with peak assignment. Signals are quoted as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). IR spectra (KBr) were recorded on a Perkin-Elmer Model 21 IR spectrophotometer. UV absorption spectra were obtained on a Beckman Model 25 spectrophotometer. Most of the column chromatography was carried out on an adjustable Chromaflex (Kontes) column (47 × 2 in.; organic solvent resistant) used in the ascending mode. TLC was performed on plastic film coated with silica gel Merck F-254 (EM Laboratories, Inc., Elmsford, N.Y.). Unless otherwise noted, the solvent used was $CHCl_3$–EtOH (4:1, v/v). In the LC analysis, distilled, deionized H_2O and glass distilled CH_3CN were both degassed. The elemental analyses were carried out by Baron Consulting Co., Analytical Services, Orange, CT, and are within ±0.4% of the theoretical values. All other reagents were commercial products of the highest purity.

5-(Hydroxymethyl)-2'-deoxyuridine (1). Method 1. This compound was prepared according to the method reported by Baker et al.[20] with modification. Paraformaldehyde (25.0 g) was added to 2'-deoxyuridine (20.0 g, 87.6 mmol) dissolved in a solution of KOH (0.5 N, 200 mL). The reaction mixture was stirred and heated at 60 °C. After 1 day, the pH of the mixture dropped from 12.0 to 9.5, and 40% of the starting material was converted to a new substance as indicated by TLC and ^1H NMR. A sharp new peak appeared at δ 9.6 in an NMR spectrum of the reaction solution, suggesting that the neutralization of the base was occurring due to formic acid generated by the Cannizarro reaction of formaldehyde under these conditions. Two portions of a solution of KOH (0.5 N, 100 mL) were added on the 2nd and 3rd day to adjust the pH to 10.5. Analysis by TLC showed that a maximum 60% conversion to product had occurred after 6 days, with no increase in the desired product with longer reaction times or additional reagents. The reaction mixture was neutralized with Dowex 50W-X8 (H^+) resin. The resin was filtered and thoroughly washed with water, and the filtrate was evaporated to a syrup, which was purified on a silica gel column using $CHCl_3$–EtOH (4:1) as eluant. Fractions were collected, examined by TLC, and similar fractions combined. Three main fractions were pooled. The first fraction was concentrated to afford rectangular prisms of product **1**: yield 3.51 g (15.5%); mp 179–180 °C (lit.[23] 176–179 °C); TLC R_f 0.25; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 2 265 nm (10.3), at pH 12 265 (7.5); λ_{min} ($\epsilon \times 10^{-3}$) at pH 2 233 nm (3.0), at pH 12 244 (5.2); NMR δ 11.31 (br s, 1, NH-3), 7.72 (s, 1, H-6), 6.18 (dd, 1, $J_{1',2'}$ = 6.8 Hz, $J_{1',2''}$ = 6.7 Hz, H-1'), 5.25 (d, 1, J = 3.9 Hz, OH-3'), 4.96 (t, 1, J = 4.3 Hz, OH-5'), 4.90 (t, 1, J = 5.2 Hz, 5-CH_2OH), 4.21 (m, 1, H-3'), 4.11 (d, 2, J = 4.3 Hz, CH_2-5), 3.76 (m, 1, H-4'), 3.53 (t, 2, J = 3.87 Hz, CH_2-5'), 2.06 (m, 2, CH_2-2'). Anal. ($C_{10}H_{14}N_2O_6$) C, H, N. The second portion was a mixture of product and starting material in a 3 to 2 ratio as estimated by TLC, yield 2.30 g (11%). The third portion was unreacted starting material, yield 0.82 g (4%).

Method 2. The title compound was also prepared according to the method described by Bärwolff and Langen[18] (see also the experimental for the preparation of compound **10**).

5-(Hydroxymethyl)-5'-*O*-(*p*-tolylsulfonyl)-2'-deoxyuridine (13). To a solution of **1** (4.00 g, 15.5 mmol) in anhydrous pyridine (100 mL) cooled in an ice–water bath was added *p*-toluenesulfonyl chloride (3.54 g, 18.6 mmol), and the mixture was left stirring at 3 °C for 1 day. EtOH (10 mL) was added to terminate the reaction, and then the solvent was evaporated under vacuum at 30 °C to furnish a gum, which was chromatographed on a silica gel column eluting with $CHCl_3$–EtOH (4:1). Two main fractions were isolated and pooled. The first fraction (A) was concentrated from which colorless rectangular prisms formed, which were shown to be **13**: yield 0.75 g (12%); mp 165–167 °C; TLC R_f 0.52; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 2 265 nm (12.0), at pH 12 265 (8.8); λ_{min} ($\epsilon \times 10^{-3}$) at pH 2 254 nm (6.4), at pH 12 249 (6.4); NMR δ 11.38 (s, 1, NH-3), 7.81, 7.47 (d, 2 each, J = 8.4 Hz, Ts-5'), 7.43 (s, 1, H-6), 6.14 (dd, 1, $J_{1',2'}$ = 6.8 Hz, $J_{1',2''}$ = 6.6 Hz, H-1'), 5.47 (d, 1, J = 4.4 Hz, OH-3'), 4.96 (t, 1, J = 5.3 Hz, 5-CH_2OH), 4.24 (m, 1, H-3'), 4.20 (d, 2, $J_{5',4'}$ = 4.0 Hz, CH_2-5'), 4.15 (d, 2, J = 5.3 Hz, CH_2-5), 3.86 (m, 1, H-4'), 2.41 (s, 3, CH_3), 2.11 (m, 2, CH_2-2'). Anal. ($C_{17}H_{20}N_2O_8S$) H, N; C: calcd, 49.50; found, 51.10. The second fraction (B) contained a mixture of **11** and **12**, since on subsequent reaction with lithium azide (see below) compounds **10** and **14** could be isolated in crystalline form. Fraction B was slightly contaminated with **13**.

5-(Azidomethyl)-2'-deoxyuridine (10). Method 1. Fraction B (see preparation of **13**) was evaporated in vacuo to a syrup and then coevaporated with dry DMF (3 × 5 mL). The residue was redissolved in DMF (30 mL) and then LiN_3 (1.20 g) was added. The mixture was heated and stirred for 3 h at 85 °C. The solvent was then evaporated in vacuo at 40 °C, and the residue was dissolved in a minimum amount of $CHCl_3$–EtOH (4:1) and chromatographed on a silica gel column eluting with the same solvent. Three main fractions were collected and pooled. The first fraction on evaporation gave **14** as fine white needles: yield 1.35 g (28% based on **1**); mp 172–173 °C; TLC R_f 0.68 (for proof of structure, see the preparation of **14**). The second fraction on concentration (10 mL) furnished, overnight at room temperature, white rectangular prisms of **10**. The crystals were filtered, washed with EtOH, and dried: yield 0.35 g (8% based on **1**); mp 138–139 °C (lit.[12] 133–135 °C); TLC R_f 0.44; IR ν 2100 cm^{-1} (N_3); UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 2 265 nm (11.0), at pH 12 265 (7.6); λ_{min} ($\epsilon \times 10^{-3}$) at pH 2 236 nm (4.3), at pH 12 244 (5.5); NMR δ 11.55 (s, 1, NH-3), 8.03 (s, 1, H-6), 6.15 (dd, 1, $J_{1',2'}$ = 6.5 Hz, $J_{1',2''}$ = 6.2 Hz, H-1'), 5.25 (d, 1, J = 4.4 Hz, OH-3'), 5.04 (t, 1, J = 5.2 Hz, OH-5'), 4.24 (m, 1, H-3'), 4.06 (s, 2, CH_2-5), 3.78 (m, 1, H-4'), 3,57 (m, 2, CH_2-5'), 2.11 (m, 2, CH_2-2'). Anal. ($C_{10}H_{13}N_5O_5$) C, H, N. The third fraction contained a mixture of **10** and **15**, which was not resolved by rechromatography.

From 5-(Bromomethyl)-3',5'-di-*O*-acetyl-2'-deoxyuridine (8). Method 2. Compound **8** was prepared according to the method of Bärwolff and Langen[18] from dried 3',5'-di-*O*-acetylthymidine:[19] NMR ($CDCl_3$) δ 7.25 (6-H). The product was obtained as a hygroscopic amorphous solid, which was stable when stored in a dessicator (P_2O_5) under vacuum: 71% yield; UV ($CHCl_3$) λ_{max} 274 nm; NMR δ ($CDCl_3$) 7.73 (6-H). A sample of this product was converted[18] to **1** in order to confirm the structure.

Crude **8** (3.8 g, 9.4 mmol) and dry LiN_3 (0.87 g, 17.8 mmol) were dissolved in dry DMF (50 mL) and heated for 2 h at 80 °C. The dark solution was then evaporated under reduced pressure and the residue extracted twice with EtOAc–H_2O (2:1, 50 mL). The organic phases were pooled and evaporated, and the syrup obtained was loaded onto a silica gel column. The column was eluted with EtOAc. Three main fractions were obtained. The product of the fast-moving fraction was not identified; TLC (EtOAc) R_f 0.68. The second fraction consisted of the 5-(azidomethyl) compound **9** (R_f 0.52) contaminated with 3',5'-di-*O*-acetylthymidine (R_f 0.32). The third fraction consisted mainly of the latter compound. The fractions containing the desired product were pooled and rechromatographed on a silica gel column eluting with EtOAc

(22) R. F. Schinazi, M. S. Chen, and W. H. Prusoff, *J. Med. Chem.*, **22**, 1273 (1979).
(23) R. E. Cline, R. M. Fink, and K. Fink, *J. Am. Chem. Soc.*, **81**, 2521 (1959).

(ascending column). Pure 5-(azidomethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine (**9**) was obtained as an oil: yield 2.2 g (63%); IR ν 2100 cm^{-1} (N_3); NMR ($CDCl_3$) δ 10.10 (s, 1, NH-3), 7.55 (s, 1, H-6), 6.28 (dd, 1, $J_{1',2'}$ = 5.7 Hz, $J_{1',2''}$ = 8.4 Hz, H-1′), 5.19 (d, 1, J = 6.2 Hz, H-3′), 4.38 (dd, 1, J_{gem} = 12.8 Hz, $J_{5',4'}$ = 4.8 Hz, H-5′), 4.29 (dd, 1, J_{gem} = 12.8 Hz, $J_{5'',4'}$ = 3.1 Hz, H-5″), 4.25 (m, 1, overlap with H-5″, H-4′), 4.19, 4.09 (2 d, 1 each, J_{gem} = 14.5 Hz, CH_2-5), 2.48 (dd, 1, J_{gem} = 14.1 Hz, $J_{2',1'}$ = 5.7 Hz, H-2′), 2.17 (m, 1, H-2″), 2.10, 2.08 (2 s, 3 each, OAc-3′,5′).

Compound **9** was dissolved in the minimum amount of MeOH and then a concentrated NH_4OH solution was added. The progress of the reaction was monitored by TLC (the deacetylated product has no mobility using EtOAc as solvent). After 2 h, the solvent was evaporated and the residue loaded onto a silica gel column, which was eluted with $CHCl_3$–EtOH (4:1). The main fractions obtained contained the desired product (R_f 0.44). These were pooled and evaporated under reduced pressure. The residue was redissolved in EtOH and left overnight at room temperature. White prisms formed and were recrystallized in EtOH to yield pure **10** which had identical physical properties with a sample obtained by method 1: 64% yield.

5-(Azidomethyl)-5′-azido-2′,5′-dideoxyuridine (14). A solution of **1** (2.00 g, 7.75 mmol) in anhydrous pyridine (50 mL) was cooled in an ice–water bath and then *p*-toluenesulfonyl chloride (3.54 g, 18.6 mmol) was added. The mixture was left stirring in the ice–water bath for 3 h and then kept in a refrigerator for 1 day. The solvent was evaporated under reduced pressure, and the residue was dissolved in water (50 mL) and then extracted with chloroform (3 × 50 mL). The aqueous layer was concentrated (5 mL) in vacuo, evaporated with DMF (3 × 5 mL) to a gum, redissolved in dry DMF (50 mL), and then LiN_3 (2.00 g, 40.9 mmol) was added. The suspension was heated in an oil bath at 90 °C for 2 h. After cooling to room temperature, the reaction mixture was filtered and evaporated at 40 °C in vacuo to a syrup. The syrup was shaken vigorously in a mixture of $CHCl_3$ (100 mL) and H_2O (30 mL). The resulting emulsion was left overnight at room temperature, and a white precipitate (1.5 g) which had formed was then filtered and dried. Crystallization from EtOH furnished fine needles: yield 0.92 g (38%); mp 172–173 °C; TLC R_f 0.68; IR ν 2100 cm^{-1} (N_3); UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 2 265 nm (10.4), at pH 12 265 (7.4); λ_{min} ($\epsilon \times 10^{-3}$) at pH 2 236 nm (3.4), at pH 12 246 (5.4); NMR δ 11.60 (br s, 1, NH-3), 7.85 (s, 1, H-6), 6.19 (dd, 1, $J_{1',2'}$ = 7.1 Hz, $J_{1',2''}$ = 6.6 Hz, H-1′), 5.44 (br s, 1, OH-3′), 4.22 (m, 1, H-3′), 4.10 (s, 2, CH_2-5), 3.86 (m, 1, H-4′), 3.57 (d, 2, $J_{4',5'}$ = 4.8 Hz, CH_2-5′), 2.26, 2.15 (2 m, 1 each, CH_2-2′). Anal. ($C_{10}H_{12}N_8O_4$) C, H, N.

5-(Hydroxymethyl)-5′-azido-2′,5′-dideoxyuridine (15). A mixture of **13** (0.60 g, 1.45 mmol) and LiN_3 (0.24 g, 5.0 mmol) in DMF (10 mL) was heated at 85 °C for 2 h. The solvent was evaporated under vacuum, and the residue was then chromatographed on a silica gel column eluting with $CHCl_3$–EtOH (4:1) to give fractions which on evaporation yielded an amorphous solid: yield 0.26 g (63%); mp 185 °C (dec); TLC, R_f 0.37; IR ν 2100 cm^{-1} (N_3); UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 2 264 nm (9.3), at pH 12 264 (7.0); λ_{min} ($\epsilon \times 10^{-3}$) at pH 2 236 nm (3.3), at pH 12 244 (5.5); NMR δ 11.37 (s, 1, NH-3), 7.57 (s, 1, H-6), 6.20 (dd, 1, $J_{1',2'}$ = 6.6 Hz, $J_{1',2''}$ = 7.1 Hz, H-1′), 5.44 (d, 1, J = 4.4 Hz, OH-3′), 4.99 (t, 1, J = 5.3 Hz, 5-CH_2OH), 4.21 (m, 1, H-3′), 4.15 (d, 2, J = 5.3 Hz, CH_2-5), 3.85 (m, 1, H-4′), 3.60 (dd, 1, J_{gem} = 13.7 Hz, $J_{5',4'}$ = 4.0 Hz, H-5′), 3.56 (dd, 1, J_{gem} = 13.7 Hz, $J_{5'',4'}$ = 6.2 Hz, H-5″), 2.19 (m, 2, CH_2-2). Anal. ($C_{10}H_{13}N_5O_5$) C, H, N.

5-(Aminomethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine (16). A solution of pure **9** (2.0 g, 5.4 mmol) in aqueous ethanol (15%, 30 mL) was hydrogenated under 30 psi of hydrogen pressure at room temperature for 3 h in the presence of 10% palladium on charcoal (0.2 g). The catalyst was removed by filtration on a Celite pad and the filtrate was evaporated to a gum. The gum was redissolved in hot ethanol and crystallized at 3 °C overnight to afford 1.6 g (87%) of product: mp 142–144 °C; ninhydrin positive (dark yellow); UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 2 264 nm (10.4), at pH 12 265 (7.7); λ_{min} ($\epsilon \times 10^{-3}$) at pH 2 232 nm (2.5), at pH 12 245 (5.6); NMR ($CDCl_3$) δ 7.50 (s, 1, H-6), 6.34 (dd, 1, $J_{1',2'}$ = 5.7 Hz, $J_{1',2''}$ = 8.4 Hz, H-1′), 5.22 (m, 1, H-3′), 4.36 (m, 2, CH_2-5′), 4.21 (m, 1, H-4′), 3.60 (s, 2, CH_2-5), 3.06 (br s, 3, NH-3, NH_2-5), 2.49 (m, 1, H-2′), 2.23 (m, 1, H-2″), 2.14, 2.12 (2 s, 3 each, OAc-3′,5′). Anal. ($C_{14}H_{19}N_3O_7$) C, H, N.

5-(Aminomethyl)-5′-amino-2′,5′-dideoxyuridine (6). A solution of **14** (0.90 g 2.9 mmol) in aqueous ethanol (15%, 30 mL) was hydrogenated at 30 psi at room temperature for 3 h in the presence of palladium on charcoal. The catalyst was filtered through Celite and the filtrate evaporated to dryness. The residue was dissolved in a minimum amount of water and the pH adjusted to 3 with HCl (0.5 N). The solution was loaded onto a column of AG 50W-X8 (H^+) ion-exchange resin, washed thoroughly with water, and then eluted with an NH_4OH solution (0.5 N). The eluate was evaporated under reduced pressure, and the residue was triturated in EtOH to afford an amorphous white powder: yield 0.48 g (65%); mp 115 °C dec; ninhydrin positive (dark yellow); UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 2 265 nm (10.2), at pH 12 265 (7.2), λ_{min} ($\epsilon \times 10^{-3}$) at pH 2 234 nm (3.2), at pH 12 244 (5.2); NMR δ 7.65 (s, 1, H-6), 6.17 (dd, 1, $J_{1',2'}$ = 6.2 Hz, $J_{1',2''}$ = 7.1 Hz, H-1′), 4.20 (m, 1, H-3′), 4.05 (br s, 6, NH-3, NH_2-5, OH-3′, NH_2-5′), 3.67 (m, 1, H-4′), 3.34 (s, 2, CH_2-5), 2.73 (d, 2, $J_{5',4'}$ = 5.3 Hz, CH_2-5′), 2.10 (m, 2, CH_2-2′). Anal. ($C_{10}H_{16}N_4O_4$) C, H, N.

5-(Aminomethyl)-2′-deoxyuridine (2). Treatment of **10** in a manner similar to that described for the preparation of **6** afforded **2** in a 68% yield. The product gave a characteristic dark yellow color with ninhydrin. This product can also be prepared in pure form from **8** without isolating any of the intermediate reaction products in an overall 23% yield (based on **8**): mp 173–176 °C dec (lit.[16] 184–186 °C, dec); UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 2 265 nm (10.6), at pH 12 265 (7.4); λ_{min} ($\epsilon \times 10^{-3}$) at pH 2 234 nm (3.0), at pH 12 246 (5.4); NMR δ 7.71 (s, 1, H-6), 6.19 (dd, 1, $J_{1',2'}$, = 6.2 Hz, $J_{1',2''}$ = 7.1 Hz, H-1′), 4.98 (br s, 5, NH-3, NH_2-5, OH-3′, OH-5′), 4.24 (m, 1, H-3′), 3.77 (m, 1, H-4′), 3.56 (m, 2, CH_2-5′), 3.32 (s, 2, CH_2-5), 2.09 (m, 2, CH_2-2′). Anal. ($C_{10}H_{15}N_3O_5$) C, H, N.

5-(Hydroxymethyl)-5′-amino-2′,5′-dideoxyuridine (7). Treatment of **15** in a manner similar to that described for the preparation of **6** afforded **7** in a 61% yield: mp 164 °C dec; ninhydrin positive (dark yellow); UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 2 264 nm (9.8), at pH 12 264 (7.5); λ_{min} ($\epsilon \times 10^{-3}$) at pH 2 234 nm (3.0), at pH 12 244 (5.7); NMR δ 7.62 (s, 1, H-6), 6.16 (t, 1, J = 7.1 Hz, H-1′), 5.0 (br s, 3, NH-3, OH-3′, OH-5), 4.18 (m, 1, H-3′), 4.14 (s, 2, CH_2-5), 3.66 (m, 1, H-4′), 3.33 (br s, 2, NH_2-5′), 2.71 (d, 2, J = 4.4 Hz, CH_2-5′), 2.08 (m, 2, CH_2-2′). Anal. ($C_{10}H_{15}N_3O_5$) C, H, N.

High-Pressure Liquid Chromatography. The main contaminants in the preparation of **10** by either method 1 or 2 are 5-(hydroxymethyl)-2′-deoxyuridine (**1**) and thymidine (dThd), respectively. The former is known to have potent biological properties, whereas the latter can prevent the activity of **10**. It was, therefore, essential to ascertain that **10** was devoid of impurities such as **1** or dThd. Using a Du Pont [illegible] fitted with a Partisil-10 ODS-2 (Whatman) column (25 cm × 4.6 mm i.d.), the following conditions resolved the three compounds in question: ambient temperature; mobile phase A = H_2O and B = CH_3CN, linear gradient 1–30% at 1% B/min; inlet pressure 700 psi; flow rate ~1.2 mL/min; detection at 254 nm; reproducibility ± 2%; limit of detection measured by relative ϵ_{254} for dThd was 0.004%. The retention times (t_r, min) were as follows: **1**, 2.5; **10**, 3.3; dThd, 3.9. The resolution can be improved by keeping the flow isocratic at 5% B/min until compound **1** (t_r = 4.5 min) followed by dThd (t_r = 9.8 min) eluted. The gradient was then started at 10.2 min at 1% B/min to a final composition of 30% B. Compound **10** eluted 5.1 min after the initiation of the gradient.

Biological Evaluation. The compounds reported in this paper were screened for cytotoxicity against murine L1210 and S180 cells, as well as for antiviral activity against HSV-1 (yield reduction assay) in Vero cells using the methodology described previously.[11,24] Compound **2** was screened for antineoplastic activity in CDF female mice bearing L1210 leukemia. The mice were inoculated with 1 × 10^5 L1210 cells and the drug was given ip 24 h later. The mice were weighed daily (see Discussion). No increase in life span was noted.

Enzyme Assay. HSV-1 encoded pyrimidine deoxyribonucleoside kinase was purified and used to determine the inhibition constants as described previously.[21] All kinetic constants

(24) T. S. Lin, J. P. Neenan, Y.-C. Cheng, and W. H. Prusoff, *J. Med. Chem.*, **19**, 495 (1976).

(K_i) were calculated from the replot of slope vs. inhibitor concentration from double-reciprocal plots. The thymidine concentrations used in these studies ranged from 0.3 to 2.3 μM and that of the inhibitor from three- to eightfold that of their K_i.

Acknowledgment. We are grateful to Mrs. J. J. Lee and Mrs. E. Lentz for their technical assistance. This investigation was supported by U.S. Public Health Service Research Grant CA-05262 from the National Cancer Institute. G.T.S. was supported in part by the USPHS Cancer Center Support Grant 1-PO1-CA-16359. We also acknowledge the support of Southern New England High Field NMR Facility made possible by a grant from the Biotechnology Resource Program of the National Institutes of Health (1-PO7-PR798).

Trapping of Metabolically Generated Electrophilic Species with Cyanide Ion: Metabolism of 1-Benzylpyrrolidine

Bert Ho and Neal Castagnoli, Jr.*

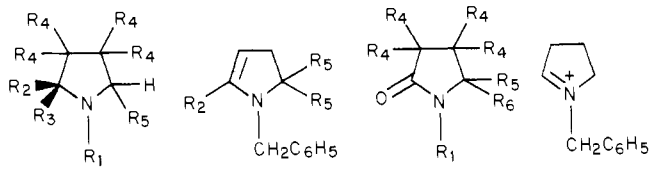
Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143.
Received June 29, 1979

Incubations of 1-benzylpyrrolidine (4) and specifically deuterium-labeled analogues of 4 with rabbit liver microsomal preparations in the presence of cyanide ion have led to the characterization of 1-benzyl-2-cyanopyrrolidine (**13**), *cis*- and *trans*-1-benzyl-2,5-dicyanopyrrolidine (**14a** and **14b**, respectively), and 1-benzyl-5-cyano-2-pyrrolidinone (**15**). The cyano adducts of the amine are thought to result from nucleophilic attack by cyanide ion on metabolically generated iminium species. The cyanolactam may be produced by mixed function oxidation of the dicyano compounds. Incubations of tritium-labeled 1-benzylpyrrolidine with rabbit liver microsomal preparations led to the reduced nicotinamide adenine dinucleotide phosphate dependent incorporation of the label into the macromolecular fraction isolated from the postincubates. Although the level of incorporation was low compared to the amount of cyano adducts formed, it is comparable to that reported for other metabolically activated cytotoxic agents. Attempts to identify the possible arene oxide rearrangement product 1-(4-hydroxybenzyl)pyrrolidine (**24**) as a metabolite of 4 were unsuccessful. The results have prompted us to postulate that metabolically generated iminium ions are capable of alkylating nucleophilic functionalities present on microsomal macromolecules.

The toxicity of a variety of lipophilic xenobiotics is thought to involve oxidative metabolism of the parent compound to reactive electrophiles which alkylate nucleophilic functionalities present on macromolecules.[1] Previous studies by us[2] and others[3] have shown that the tobacco alkaloid nicotine (1) is oxidatively metabolized by hepatic microsomal preparations to reactive intermediates, which in the presence of cyanide ion are converted to the isolable cyano adducts **2** and **3**. In an attempt to further characterize the metabolic pathway leading to the formation of such electrophilic intermediates, we have examined with the aid of specifically deuterium-labeled compounds and GC–EIMS the rabbit liver microsomal metabolism of the model *tert*-amine 1-benzylpyrrolidine (4) in the presence and absence of cyanide ion. Additionally, we have attempted to evaluate the possible toxicological significance of electrophilic metabolic intermediates derived from *tert*-amines by examining the metabolically dependent formation of covalent adducts between tritium-labeled 1-benzylpyrrolidine and microsomal macromolecules.

The GC analysis of the base fraction isolated from 1-benzylpyrrolidine postincubates showed the presence of two major and one minor metabolite (Figure 1a). In the absence of NADPH or when boiled microsomes were used, only the starting substrate 4 was observed in the GC tracing. The structure assignments (see below) and GC–EIMS characterizations of these compounds and unmetabolized 1-benzylpyrrolidine are summarized in Table I.

The GC–EIMS spectra of all four compounds are dominated by the tropylium ion ($C_6H_5CH_2^+$) at m/e 91. The appearance of this fragment ion in these spectra, as well as in the mass spectra of the cyano adducts discussed below, indicates that the benzylic carbon atom of 1-benzylpyrrolidine is not altered in these metabolically generated products. The possibility that substituent rearrangements might accompany the fragmentations was



1-4, 8, 9, 13, 14, and 16 — 5 — 6, 7, and 15 — 10

	R_1	R_2	R_3	R_4	R_5	R_6
1	CH_3	H	3-C_5H_4N	H	H	
2	CH_2CN	H	3-C_5H_4N	H	H	
3	CH_3	H	3-C_5H_4N	H	CN	
4	$CH_2C_6H_5$	H	H	H	H	
4-α,α-d_2	$CD_2C_6H_5$	H	H	H	H	
4-2,2-d_2	$CH_2C_6H_5$	^2H	^2H	H	H	
4-d_4	$CH_2C_6H_5$	H	H	^2H	H	
4-α,α-t_2	$CT_2C_6H_5$	H	H	H	H	
5		H			H	
5-d_1		^2H			H	
5-d_2		H			^2H	
6	$CH_2C_6H_5$			H	H	H
6-α,α-d_2	$CD_2C_6H_5$			H	H	H
6-5,5-d_2	$CH_2C_6H_5$			H	^2H	^2H
6-d_4	$CH_2C_6H_5$			^2H	H	H
7	$CH_2C_6H_5$			H	OH	H
7-d_1	$CH_2C_6H_5$			H	OH	^2H
8	$C(=O)C_6H_5$	H	H	H	H	
9	$CH_2C_6H_5$	OH	H	H	H	
13	$CH_2C_6H_5$	CN	H	H	H	
13-d_1	$CH_2C_6H_5$	CN	^2H	H	H	
13-d_2	$CH_2C_6H_5$	^2H	^2H	H	CN	
14a, 14b	$CH_2C_6H_5$	CN	H	H	CN	
14a-d_1, 14b-d_1	$CH_2C_6H_5$	CN	^2H	H	CN	
15	$CH_2C_6H_5$			H	CN	H
15-d_1	$CH_2C_6H_5$			H	CN	^2H
16	$CHCNC_6H_5$	H	H	H	H	

(1) Nelson, S. D.; Boyd, M. R.; Mitchell, J. R. *ACS Symp. Ser.* **1977** *no. 44*, 155–185. Miller, J. A. *Cancer Res.* **1970**, *30*, 559.
(2) Nguyen, T. L.; Gruenke, L. D.; Castagnoli, N., Jr. *J Med. Chem.* **1976**, *19*, 1168; Nguyen, T. L.; Gruenke, L. D.; Castagnoli, N., Jr. *Ibid.* **1979**, *22*, 259.
(3) Murphy, P. *J. Biol. Chem.* **1973**, *248*, 2796.

0022-2623/80/1823-0133$01.00/0