(4) H. W. Kosterlitz, J. A. H. Lord, and A. J. Watt, "Agonist and Antagonist Actions of Narcotic Analgesic Drugs", Macmillan, London, 1972.
(5) G. A. Deneau, J. E. Villarreal, and M. H. Seevers in "Problems of Drug Dependence", National Academy of Sciences, Washington, D.C., 1973.
(6) K. Verebey, J. Volavka, S. J. Mule, and R. B. Resnick, *Clin. Pharmacol. Ther.*, **20**, 315 (1975).
(7) M. Gordon, A. W. Pircio, F. S. Caruso, and I. J. Pachter, paper presented to the Committee on Problems of Drug Dependence, Mexico City, N.M., 1974.
(8) E. Kutter, A. Herz, H. J. Teschemacher, and R. Hess, *J. Med. Chem.*, **13**, 801 (1970).
(9) J. Knoll, *Neuropharmacology*, **14**, 921 (1975).
(10) J. R. Smythies, private communication.
(11) G. H. Loew and D. S. Berkowitz, *J. Med. Chem.*, **18**, 656 (1975).
(12) J. J. Kaufman, H. Popkie, and W. Koski, *J. Am. Chem. Soc.*, **98**, 1342 (1976).
(13) J. J. Kaufman and E. Kerman, *Int. J. Quantum Chem.*, **8**, 289 (1974).
(14) D. S. Berkowitz and G. H. Loew in "Opiates and Endogenous Opioids", Elsevier/North Holland, Amsterdam, 1976.
(15) A. P. Feinberg, I. Creese, and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 4215 (1976).
(16) R. A. Y. Jones, A. R. Katritsky, A. C. Richards, and R. J. Wyatt, *J. Chem. Soc. B*, 122 (1970).
(17) D. C. Palmer and M. J. Strauss, *Chem. Rev.*, **77**, 1 (1977).
(18) A. F. Casy in "Modern Pharmacology", Vol. 1, R. M. Featherstone, Ed., Marcel Dekker, New York, N.Y., 1973, p 1.
(19) S. Diner, J. P. Malrieu, F. Jordan, and M. Gilliert, *Theor. Chim. Acta*, **15**, 100 (1969).
(20) G. H. Loew and J. R. Jester, *J. Med. Chem.*, **18**, 1051 (1975).
(21) G. H. Loew, D. S. Berkowitz, and R. Newth, *J. Med. Chem.*, **19**, 863 (1976).
(22) I. L. Karle, *Acta Crystallogr., Sect. B*, **30**, 1682 (1974).
(23) R. J. Sime, M. Dobler, and R. L. Sime, private communication.
(24) R. L. Sime, R. Forehand, and R. J. Sime, *Acta Crystallogr., Sect. B*, **31**, 2236 (1975).
(25) J. Pople and D. Beveridge, "Approximate Molecular Orbital Theory", McGraw-Hill, New York, N.Y., 1970.
(26) L. Gylbert, *Acta Crystallogr., Sect. B*, **29**, 1630 (1973).
(27) I. L. Karle, R. D. Gilardi, A. V. Fratini, and J. Karle, *Acta Crystallogr., Sect. B*, **25**, 1469 (1969).
(28) R. J. Sime, M. Dobler, and R. L. Sime, *Acta Crystallogr., Sect. B*, **32**, 809 (1976).
(29) G. H. Loew, H. Weinstein, and D. Berkowitz in "Environmental Effects on Molecular Structure and Properties", B. Pullman, Ed., D. Reidel Publishing Co., Dordrecht, Holland, 1976.

Notes

A Novel Synthesis and Biological Activity of Several 5-Halo-5′-amino Analogues of Deoxyribopyrimidine Nucleosides

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A novel synthetic procedure has been developed for the large-scale synthesis of 5-chloro-, 5-bromo-, and 5-iodo-5′-amino-2′,5′-dideoxyuridine (**4c–e**) as well as of two new analogues, 5-iodo-5′-amino-2′,5′-dideoxycytidine and 5-fluoro-5′-amino-2′,5′-dideoxyuridine (**4a** and **4b**), in good yield. The starting materials, 5-halo-2′-deoxyuridine and 5-halo-2′-deoxycytidine, are readily available and the method is straightforward. This report describes the synthesis and the biologial activities of these compounds.

Several 5-halo analogues of 2′-deoxyuridine and 2′-deoxycytidine have excellent biological activity as either antineoplastic or antiviral agents. These include 5-fluoro-2′-deoxyuridine, 5-iodo-2′-deoxyuridine, 5-iodo-2′-deoxycytidine, etc.[1-3] In an attempt to modify their toxicity with retention of antiviral activity, the 5′-hydroxyl moiety of several nucleoside analogues has been replaced with an amino group.[4,5] Of these, 5-iodo-5′-amino-2′,5′-dideoxyuridine (AIU, AIdUrd) has retained the antiviral activity of the parent compound, 5-iodo-2′-deoxyuridine (IdUrd), albeit with a lesser potency,[6,7] but remarkably has none of the toxicities associated with the 5′-hydroxyl analogues.[6-8] Although the original method of preparation of AIdUrd[4] provided a sufficient amount of compound for our in vitro studies[6,9,10] and topical use in initial animal experiments,[7,10] there was a need for an improved method of synthesis to provide the large amounts required for systemic administration in our proposed animal test systems. Starting material (IdUrd), 300 g, yields 10–15% product of AIdUrd. A novel synthetic procedure was developed which is applicable for the large-scale synthesis of 5-chloro-, 5-bromo-, and 5-iodo-5′-amino-2′,5′-dideoxyuridine (**4c–e**) as well as of two new analogues, 5-iodo-5′-amino-2′,5′-dideoxycytidine and 5-fluoro-5′-amino-2′,5′-dideoxyuridine (**4a** and **4b**). The present report describes the synthesis and the biological activities of these compounds.

Chemistry. The synthesis of a variety of 5-halo-5′-substituted deoxyribopyrimidine nucleoside analogues is outlined in Scheme I. Tosylation of 5-halo-2′-deoxyribonucleosides **1a–e** with *p*-toluenesulfonyl chloride in dry pyridine at 3 °C gave the corresponding 5-halo-5′-*o*-*p*-tolylsulfonyl derivatives **2a–e**. Compounds **2a–e** reacted with lithium azide in *N,N*-dimethylformamide at 70–75 °C for 2 h to afford the 5-halo-5′-azido analogues **3a–e**.[4,5,11] Treatment of **3a–e** with triphenylphosphine[12] in pyridine at room temperature, followed by hydrolysis with concentrated ammonium hydroxide, yielded a series of novel 5-halo-5′-amino nucleoside analogues **4a–e**. The physical properties, yields of the last step conversion, and elemental analyses are listed in Table I. Compounds **4d** and **4e** have been synthesized previously by treatment of the 5-mercuriacetate of 5′-amino-2′,5′-dideoxyuridine with bromine and iodine, respectively.[4,13] Although this method

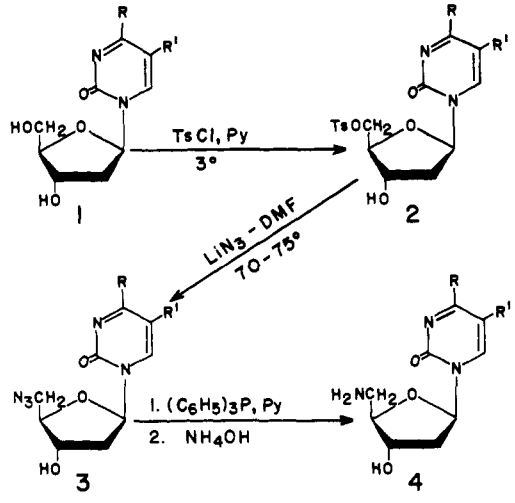
0022-2623/78/1821-0106$01.00/0

Table I. Physical Properties, Yield, and Elemental Analyses of Various 5-Halo-5'-substituted Deoxyribopyrimidine Nucleoside Analogues

Compd	R	R'	Mp, °C	% yield	Recrystn solvent	Formula	Analyses[a]
2a	NH_2	I	147-150 dec	80	EtOH	$C_{16}H_{18}IN_3O_6S$	C, H, N
2b	OH	F	163-164 dec	86	EtOH-petr ether	$C_{16}H_{17}FN_2O_6S$	C, H, N
3a	NH_2	I	185-186 dec	65	Aq EtOH	$C_9H_{11}IN_6O_3$	C, H, N
3b	OH	F	134-135 dec	52	EtOH-Et_2O	$C_9H_{10}FN_5O_4$	C, H, N
4a	NH_2	I	190-191 dec	58	EtOH-Et_2O	$C_9H_{13}IN_4O_3$	C, H, N
4b	OH	F	199-200 dec	81	EtOH-Et_2O	$C_9H_{12}FN_3O_4 \cdot 0.5H_2O$	C, H, N
4c	OH	Cl	205-206 dec	92	EtOH	$C_9H_{12}ClN_3O_4$	C, H, N
4d	OH	Br	193-194 dec	80	EtOH	$C_9H_{12}BrN_3O_4$	H, N; C[b]
4e	OH	I	201-202 dec	53	EtOH	$C_9H_{12}IN_3O_4$	C, H, N

[a] All compounds listed above gave satisfactory C, H, and N analyses which were within ±0.4% of theoretical values. [b] C: calcd, 35.31; found, 34.84.

Scheme I



a R = NH_2, R' = I
b R = OH, R' = F
c R = OH, R' = Cl
d R = OH, R' = Br
e R = OH, R' = I

is a good procedure for the synthesis of small quantities of ^{125}I-labeled sample, it is impractical for bulk preparation. Catalytic hydrogenation of the 5'-azido derivatives **3b** and **3c** in the presence of 10% palladium on charcoal or platinum oxide gave a low yield of the desired amino nucleosides **4b** and **4c**,[4] because considerable dehalogenation to 5'-amino-2',5'-dideoxyuridine occurred during this process. Attempts to synthesize 5-iodo-5'-amino-2',5'-dideoxycytidine (**4a**), 5-bromo-5'-amino-2',5'-dideoxyuridine (**4d**), and 5-iodo-5'-amino-2',5'-dideoxyuridine (**4e**) by hydrogenation was similarly unsuccessful because reduction of the 5'-azido precursor was accompanied by loss of the halogen due to concomitant reduction of the carbon–halogen bond.

The present synthetic procedure affords a simple and straightforward synthesis to various novel 5-halo-5'-aminodeoxyribonucleoside analogues in excellent yield, and the procedure is readily scaled up for bulk preparation.

Biological Activity. The antiviral activity of various compounds listed in Table II was determined. Vero cells were grown to confluency in 25-cm^2 Falcon flasks using Dulbecco's medium supplemented with 10% fetal calf serum. The cells were then infected with Herpes simplex virus, type 1 (CL-101, obtained from Dr. Wilma Summers who originally received the virus from Dr. Saul Kit), at a multiplicity of infection (MOI) of 10. After a 1-h absorption period at 37 °C, the viral inoculum was removed and the flask washed once with phosphate-buffered saline. The test compounds indicated in Table II were dissolved in Dulbecco's medium supplemented with serum and then added to the flask. The infected cultures were incubated at 37 °C for 40 h and then frozen until virus titrations were performed. Virus was released by freezing and thawing the media–cell suspension one time. The cell lysates were diluted directly and the virus yield was assayed by plaque formation on Vero cells. The number of plaque-forming units (pfu) of virus in the drug-treated cultures relative to that found in the drug-free condition was determined and expressed as percent inhibition in Table II.

Table II. Effect of Various 5-Halo-5'-substituted Deoxyribopyrimidine Nucleoside Analogues on the Replication of Sarcoma 180 Cells, Vero Cells, and Herpes Simplex Virus Type 1 in Vitro

				% inhibn		
Compd	R	R'	Concn, μM	S-180	Vero cells	HSV-1
1a	NH_2	I	50		None	99.5
			800		None	
1b	OH	F	400	100	100	84.3
			0.1	100	100	
			0.01	100	None	
1e	OH	I	50	100	100	99.5
3a	NH_2	I	200	None	None	37.5
3b	OH	F	400	100	100	5
4a	NH_2	I	1600	None	None	
			400	None	None	92.8
4b	OH	F	400	100	100	85.6
			1	100	None	None
4c	OH	Cl	400	None	None	74.3
4d	OH	Br	200	None	None	86.2
4e	OH	I	200	None	None	96.1

The cytotoxicity of the various test compounds on the uninfected host Vero cells was determined (Table II). Vero cells in Dulbecco's medium (2.5 mL) supplemented with 10% fetal calf serum were added to eight 25-cm^2 Falcon flasks at a concentration equivalent to 0.1 confluency for each compound under assay. After incubation at 37 °C in 5% CO_2–95% air for 1 day, the test compound, dissolved in 2.5 mL of the above growth medium, was added and two flasks were harvested immediately by decanting the medium, washing once with 5 mL of buffered saline, and then incubating at 37 °C for 15 min with a 5-mL solution of trypsin (0.125%) and EDTA (0.02%). The cells dislodged from the flask by this latter procedure were generally in clumps and were dispersed by repeated forceful pipetting of the suspension against the surface of the flask. To 1 mL of the well-dispersed cell suspension, 0.2 mL of trypan blue solution was added and the number of cells was counted using a haemocytometer. Each day for the next 3 days, two of the remaining flasks were harvested in the manner just described for determination of cell number.

The effect of these compounds on the replication of a neoplastic cell line in culture was also investigated. Mouse sarcoma 180 cells were maintained as suspension cultures in Fischer's medium supplemented with 10% horse serum at 37 °C in a humidified atmosphere of 5% CO_2–95% air. Under these conditions the generation time for sarcoma 180 cells is approximately 18 h. Each compound, at the given concentration (Table II), was added to sarcoma 180 cells ($\sim 2 \times 10^4$ cell/mL) which were in their exponential phase of growth. The increase in cell number of the drug-free culture (control) as well as that of the cultures supplemented with the tested compounds was determined after 18 and 42 h of growth. The percent inhibition in the drug-treated cultures relative to the control is shown in Table II.

Excellent antiviral activity in the complete absence of an inhibitory effect on the noninfected host Vero cells was found with compounds **1a** and **4a,c**–e (Table II). Replacement of the 5′-hydroxyl of 5-iodo-2′-deoxycytidine (**1a**) with either an azido (**3a**) or an amino group (**4a**) decreased the antiviral activity of the parent compound **1a**. The 5′-amino analogue has markedly greater antiviral activity than its azido precursor **3a**, thereby supporting the antiviral activity to be intrinsic to compound 4a rather than due to the presence of the starting material **1a** used for its synthesis. The lack of toxicity of compound **1a** is, as Greer and co-workers have shown,[14] related to the absence of adequate deoxycytidine deaminase activity. Those cells which have such enzyme activity convert compound **1a** into compound 1e, a compound with marked cytotoxicity.[6,14]

Replacement of the 5′-hydroxyl moiety of 5-chloro-, 5-bromo-, or 5-iodo-2′-deoxyuridine with an amino group produced compounds (**4c–e**) with antiviral activity of less potency, but also with complete absence of cytotoxicity not only for the host Vero cells but also for sarcoma 180 cells. As the van der Waals radii of the halogen in the 5 position of these 5′-aminopyrimidine deoxyribonucleoside analogues decreased from that of iodine (2.15) to bromine (1.95) to chlorine (1.80), there appears to be a small decrease in their antiviral activity. Although the difference in activity between compounds **4d** and 4e is reproducible, other preparations of these compounds made by a different procedure as described in ref 4 did *not* show any difference between these two compounds in their antiviral activity. A further decrease in the van der Waals radii of the halogen substituent in this position to that of fluorine (1.35) produced a compound (**4b**) with antiviral activity, but also one that had marked inhibitory activity of the replication of not only the host Vero cells but also that of the murine sarcoma 180 cells.

Compounds **1b** and **4b** have a fluorine atom in the 5 position of the pyrimidine moiety and inhibited the replication of the sarcoma 180 neoplastic cells to a markedly greater extent than that of the Vero cells. Since as little as 1% contamination of compound with the parent compound (**1b**) could account for this inhibition, the significance of this finding should be interpreted with caution. A 1% contamination of **4b** with compound **1b** would not be detected by the analytical procedures used. However, in support of the intrinsic antiviral activity of compound **4b** is the finding that its immediate precursor **3b** had no significant antiviral activity.

5-Iodo-2′-deoxycytidine (**1a**) was first sythesized by Chang and Welch[15] and studies of its antiviral activity in vitro and against experimental herpetic keratitis have been reviewed.[2] This compound, in agreement with the studies of Schildkraut et al.,[14] is equally as potent an antiviral agent as 5-iodo-2′-deoxyuridine (1e), but without the toxicity of the latter in many systems for the reason indicated above. Both compound **1a** and 1e are more potent as antiviral agents than the 5′-amino analogue of 5-iodo-2′-deoxyuridine (4e), but the latter has the advantage of complete absence of toxicity both in a variety of cell culture systems as well as in animals.[10]

Experimental Section

Melting points were taken on a Thomas-Hoover Unimelt apparatus and are not corrected. A Perkin-Elmer 15 instrument was used to determine the IR spectra. The UV spectra were taken on a Beckman-25 spectrophotometer. The NMR spectra were recorded at 270 MHz on a Bruker 270HX spectrometer. The elemental analyses were carried out by Baron Consulting Co., Orange, Conn.

5-Iodo-5′-*o*-*p*-tolylsulfonyl-2′-deoxycytidine (2a). To a suspension of 5-iodo-2′-deoxycytidine (1a) (17.65 g, 50.00 mmol) in 250 mL of dry pyridine at 0 °C (ice bath) was added *p*-toluenesulfonyl chloride (11.50 g, 60.00 mmol). The reaction mixture was stirred at 0 °C for 1 h and then stored at 3 °C in the dark with stirring for an additional 24 h. At the end of the reaction a clear solution was obtained, to which 15 mL of methanol was added. After standing for 30 min, the solvent was removed under diminished pressure at room temperature to afford a syrup which was coevaporated several times with methanol. The residue was triturated with ice-cooled water to give a crystalline mass which was broken down to fine particules with a spatula. The solid material was collected by filtration, washed thoroughly with water, a small amount of ice-cooled ethanol, and ether, and dried in vacuo. The compound was recrystallized from ethanol to yield 20.24 g (80%) of product.

5-Iodo-5′-azido-2′,5′-dideoxycytidine (3a). A mixture of compound **2a** (2.80 g, 5.52 mmol) and lithium azide (0.41 g, 8.28 mmol) in 30 mL of DMF was heated to 70–75 °C (oil bath) for 2 h. The solvent was evaporated to dryness under reduced pressure. The residue was coevaporated several times with ethanol and triturated with ether. The white solid was collected by filtration, washed with ice-cooled water, a small amount of ethanol, and ether, and then dried under reduced pressure. The product was recrystallized from aqueous ethanol to afford 1.35 g (65%): IR (KBr) ν_{max} 4.78 μ (azido).

5-Iodo-5′-amino-2′,5′-dideoxycytidine (4a). A mixture of the 5′-azido derivative **3a** (7.55 g, 19.97 mmol) and triphenylphosphine (8.38 g, 31.95 mmol) in 250 mL of pyridine was stirred magnetically at room temperature and a clear solution was obtained after 1 h. The reaction mixture was stirred for another 30 min and after addition of 25 mL of concentrated NH_4OH solution stirred at room temperature for an additional 3 h. The solvent was evaporated below 30 °C under reduced pressure to yield a gummy syrup which was coevaporated several times with ethanol and triturated with ether to form a crystalline mass which was pulverized and extracted with benzene (5 × 150 mL) and ether (5 × 200 mL). The insoluble solid powder was collected by filtration, washed with more benzene and ether, then dried, and extracted with water (3 × 250 mL). The insoluble material was removed by filtration and the aqueous solution evaporated to dryness in vacuo. The residue was dissolved in boiling ethanol (150 mL) and filtered through a sintered glass funnel. Ether (1500 mL) was added to the filtrate with stirring, and fine crystals formed. The solution was maintained at 0 °C for several hours, during which time more crystals formed. The fine pale yellow crystals were collected by filtration, washed thoroughly with ether, and dried under reduced pressure to afford 3.42 g of product. The filtrate and the ether washings were combined and kept at −20 °C overnight during which time more crystals precipitated out to yield an additional 0.58 g of product. The total yield was 4.10 g (58%): UV λ_{max} (0.01 N HCl) 299 nm (ϵ 7680); UV λ_{min} (0.01 N HCl) 260 nm; UV λ_{max} (0.01 N NaOH) 291 nm (ϵ 6270); UV λ_{min} (0.01 N NaOH) 263 nm; NMR (Me_2SO-d_6) δ 2.05 (m, 2, H-2′), 2.74 (d, 2, H-5′), 3.69 (m, 1, H-4′), 4.15 (m, 1, H-3′), 5.17 (br s, 3, C-3′ OH, C-5′ NH_2), 6.06 (t, 1, $J_{1',2'}$ = 6.62 Hz, H-1′), 6.63 (br s, 1, C-4 C-NH_a), 7.85 (br s, 1, C-4 NH_b), 8.28 (s, 1, H-6).

5-Fluoro-5′-*o*-*p*-tolylsulfonyl-2′-deoxyuridine (2b). To a solution of 5-fluoro-2′-deoxyuridine (5.0 g, 20.3 mmol) in 25 mL

of pyridine at 0 °C (ice bath) *p*-toluenesulfonyl chloride (4.7 g, 24.4 mmol) was added. The reaction mixture was stirred at 0 °C for 2 h and then let stand in the refrigerator (3 °C) for another 22 h. Pyridine was removed under diminished pressure at room temperature. The syrup was extracted with ether (5 × 50 mL) and water (5 × 100 mL), and the residue was then dissolved in 100 mL of chloroform. To the chloroform solution 100 mL of petroleum ether (35–60 °C) was added slowly with stirring. The product precipitated out as a white solid which was collected by filtration and washed thoroughly with ether and petroleum ether. The product was recrystallized from ethanol–petroleum ether to give 7.01 g (86%).

5-Fluoro-5'-azido-2',5'-dideoxyuridine (3b). A mixture of **2b** (8.0 g, 20.0 mmol) and lithium azide (1.57 g, 32.0 mmol) in DMF (30 mL) was heated at 70–75 °C with stirring for 2 h. The mixture was cooled to room temperature and filtered. The solvent was removed in vacuo at 50–60 °C. The residue was dissolved in 200 mL of 50% aqueous ethanol, stirred for 30 min with 15 g of AG50W-X8 (H^+) resin to remove Li^+, and then filtered. The solvent was evaporated to dryness in vacuo. The residue was dissolved in a minimum volume of ethanol–ether and kept at −20 °C for a few days. The crystals were collected by filtration and dried to yield 2.82 g (52%) of product: IR (KBr) ν_{max} 4.76 μ (azido).

5-Iodo-5'-amino-2',5'-dideoxyuridine (4e). Compound **3e**[4] (5.10 g, 13.5 mmol) and triphenylphosphine (5.79 g, 22.1 mmol) were dissolved in 35 mL of pyridine and kept at room temperature with stirring for 1 h. Concentrated ammonium hydroxide (5 mL) was then added and the solution was allowed to stand for an additional 2 h with stirring. The solvent was evaporated to dryness under reduced pressure at room temperature to give a syrup which was washed thoroughly with ether (3 × 100 mL). The residue was then extracted with 1 N ammonium hydroxide solution (2 × 150 mL), and the excess triphenylphosphine and triphenylphosphine oxide were removed by filtration. The filtrate was extracted with benzene (3 × 100 mL) and with ether (3 × 150 mL) to remove residual triphenylphosphine and then evaporated to dryness in vacuo at 50 °C to yield 4.43 g (93%) of solid which was extracted twice with 150-mL portions of boiling ethanol. The insoluble material was collected by filtration, washed with ethanol and ether, and dried to afford 2.51 g (53%) of an analytically pure product: UV λ_{max} (0.01 N HCl) 285 nm (ϵ 7420); UV λ_{min} (0.01 N HCl) 247 nm; UV λ_{max} (0.01 N NaOH) 279 nm (ϵ 5530); UV λ_{min} (0.01 N NaOH) 252 nm.

Compounds **4b–d** were synthesized from the corresponding 5'-azido derivatives, **3b**, **3c**,[4] and **3d**,[4] in the same manner as described in the preparation of **4e** except that water was used as extracting solvent instead of 1 N ammonium hydroxide solution.

Acknowledgment. The authors wish to express their appreciation to Ms. Jung Ja Lee and Mrs. C. Chai for their excellent technical assistance. This research was supported by U.S. Public Health Service Research Grant CA-05262 from the National Cancer Institute and by the National Institutes of Health Research Grant No. 1-PO7-PR00798 from the Division of Research Resources.

References and Notes

(1) A. Bloch, Ed., "Chemistry, Biology and Chemical Use of Nucleoside Analogs", *Ann. N.Y. Acad. Sci.*, **255** (1975).
(2) W. H. Prusoff and D. C. Ward, *Biochem. Pharmacol.*, **25**, 1233 (1976).
(3) E. C. Herrmann, Jr., Ed., "Third Conference on Antiviral Substances", *Ann. N.Y. Acad Sci.*, **284** (1977).
(4) T. S. Lin, J. P. Neenan, Y. C. Cheng, W. H. Prusoff, and D. C. Ward, *J. Med. Chem.*, **19**, 495 (1976).
(5) T. S. Lin, C. Chai, and W. H. Prusoff, *J. Med. Chem.*, **19**, 915 (1976).
(6) Y. C. Cheng, B. Goz, J. P. Neenan, D. C. Ward, and W. H. Prusoff, *J. Virol.*, **15**, 1284 (1975).
(7) D. M. Albert, M. Lakov, P. N. Bhatt, T. W. Reid, R. E. Ward, R. C. Cykiert, T. S. Lin, D. C. Ward, and W. H. Prusoff, *J. Invest. Opthalmol.*, **15**, 470 (1976).
(8) D. M. Albert, D. H. Percy, C. A. Puliafito, E. Fritsch, T. S. Lin, D. C. Ward, and W. H. Prusoff, *Proc. Soc. Exp. Biol. Med.*, in press.
(9) M. S. Chen, D. C. Ward, and W. H. Prusoff, *J. Biol. Chem.*, **251**, 4833 (1976).
(10) W. H. Prusoff, D. C. Ward, T. S. Lin, M. S. Chen, G. T. Shaiu, C. Chai, E. Lentz, R. Capizzi, J. Idriss, N. H. Ruddle, F. L. Black, H. L. Kumari, D. Albert, P. N. Bhatt, G. D. Hsiung, S. Strickland, and Y. C. Cheng, *Ann. N.Y. Acad. Sci.*, **284**, 335 (1977).
(11) T. S. Lin and W. H. Prusoff, *J. Carbohydr., Nucleosides, Nucleotides*, **2**, 185 (1975).
(12) W. S. Mungall, G. L. Greene, G. A. Heavner, and R. Letsinger, *J. Org. Chem.*, **40**, 1695 (1975).
(13) R. M. K. Dale, D. C. Ward, D. C. Livingston, and E. Martin, *Nucleic Acid Res.*, **2**, 915 (1975).
(14) I. Schildkraut, G. M. Cooper, and S. Greer, *Mol. Pharmacol.*, **11**, 153 (1975).
(15) P. K. Chang and A. D. Welch, *Biochem. Pharmacol.*, **8**, 327 (1961).

Synthesis and Biological Activity of Several Amino Analogues of Thymidine

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3',5'-Diamino-3',5'-dideoxythymidine (**7**) was synthesized via a nine-step synthesis from thymidine in good overall yield. 3'-Amino-3'-deoxythymidine (**8**) and 5'-amino-5'-deoxythymidine (**12**) were prepared with a minor modification of the procedure reported by Horwitz and co-workers. Although the 5'-amino analogue **12** had potent antiviral activity relative to the 3'-amino analogue **8**, the latter is a potent inhibitor of the replication of both murine sarcoma 180 cells (ED_{50} = 5 μM) and of murine L1210 cells (ED_{50} = 1 μM) in vitro. Most unexpectedly, however, was the finding of complete lack of either antiviral or antineoplastic activity by the 3',5'-diamino analogue **7** which appears to have acquired the undesirable qualities of both the 3'-amino and 5'-amino analogues of thymidine.

Although the 3'- and the 5'-amino analogues of thymidine had been synthesized previously, their biological potential has not been explored.[1-3] The 5'-amino analogue of thymidine is a good competitive inhibitor of the phosphorylation of thymidine by thymidine kinase[4,5] and a modest inhibitor of thymidylate kinase.[6] Interest in amino analogues of nucleosides in general was stimulated by the findings that the 5'-amino analogues of thymidine and 5-iodo-2'-deoxyuridine have significant antiviral activity.[7-10]

The 5'-amino analogue of 5-iodo-2'-deoxyuridine (AIdUrd, AIU) has demonstrated potent antiviral activity in the complete absence of toxicity to cells in culture or to day-old or adult mice.[7-11] Therapy of experimental Herpes simplex keratitis in rabbits indicated[12] that AIdUrd when in aqueous solution (8 mg/mL) is almost as effective as

0022-2623/78/1821-0109$01.00/0