39.8%: $[\alpha]^{23}D$ −53.0° (c 0.5, 0.2 N HOAc); TLC (silica gel) R_f^1 0.13, R_f^2 0.32, R_f^3 0.45; TLC (cellulose) R_f^1 0.69, R_f^2 0.90, R_f^3 0.91; TLE E(Glu), 0.26. Amino acid analysis gave Glu, 1.0; His, 0.98; Trp, 0.50; Ser, 0.87; Tyr, 0.99; Gly, 1.97; Leu, 1.0; δ-*N*-*i*-Pr-Orn[8], 1.0; Pro, 0.95; NH_3, 1.01. Anal. ($C_{57}H_{79}N_{15}O_{13}\cdot CH_3COOH\cdot 3H_2O$).

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References and Notes

(1) H. Matsuo, Y. Baba, R. M. G. Nair, A. Arimura, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **43**, 1334 (1971).
(2) R. Burgus, M. Butcher, M. Amoss, N. Ling, M. W. Monahan, J. Rivier, R. Fellows, R. Blackwell, W. Vale, and R. Guillemin, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 278 (1972).
(3) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963); *Adv. Enzymol.*, **32**, 221 (1969).
(4) W. Konig and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
(5) J. Meienhofer, *Horm. Proteins Pept., 1973*, **2**, 98 (1973).
(9) A. Arimura, J. A. Vilchez-Martinez, and A. V. Schally, *Proc. Soc. Exp. Biol. Med.*, **146**, 17 (1974).
(10) A. Arimura, J. A. Vilchez-Martinez, D. H. Coy, E. J. Coy, Y. Hirotsu, and A. V. Schally, *Endocrinology*, **95**, 1174 (1974).
(11) J. A. Vilchez-Martinez, D. H. Coy, A. Arimura, E. J. Coy, Y. Hirotsu, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **59**, 1226 (1974).
(12) T. Daane and A. F. Parlow, *Endocrinology*, **88**, 653 (1971).
(13) M. Fujino, S. Kobayashi, M. Obayashi, T. Fukuda, S. Shinagawa, I. Yamazaki, R. Nakayama, W. R. White, and R. H. Rippel, *Biochem. Biophys. Res. Commun.*, **49**, 698 (1972).
(14) N. Yanaihara, K. Tsuji, C. Yanaihara, T. Hashimoto, T. Kaneko, H. Oka, A. Arimura, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **51**, 165 (1973).
(15) W. Arnold, G. Flouret, R. Morgan, R. Rippel, and W. F. White, *J. Med. Chem.*, **17**, 314 (1974).
(16) M. Monahan, J. Rivier, W. Vale, N. Ling, G. Grant, M. Amoss, R. Guillemin, R. Burgus, E. Nicolaides, and M. Rebstock, "Chemistry and Biology of Peptides", J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1972, p 601.
(17) Y. Okada, K. Katumura, Y. Baba, A. Arimura, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **53**, 1180 (1973).
(18) D. H. Coy, E. J. Coy, and A. V. Schally, *J. Med. Chem.*, **16**, 1140 (1973).
(19) J. M. Stewart, G. Betz, K. L. Hill, M. J. Pfile, and G. R. Matsueda, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **33**, 231 (1974).
(20) N. Yanaihara, T. Hashimoto, C. Yanaihara, K. Tsuji, Y. Kenmochi, F. Ashizawa, T. Kaneko, H. Oka, S. Saito, A. Arimura, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **52**, 64 (1973).
(21) M. Monahan, W. Vale, J. Rivier, G. Grant, and R. Guillemin, Abstracts, 55th Meeting of the Endocrine Society, Chicago, Ill., 1973, p 145.
(22) D. H. Coy, E. J. Coy, Y. Hirotsu, J. A. Vilchez-Martinez, A. V. Schally, J. W. Van Nispen, and G. I. Tesser, *Biochemistry*, **13**, 3550 (1974).
(23) J. K. Chang, R. H. Williams, A. J. Humphries, W. G. Johansson, K. Folkers, and C. Y. Bowers, *Biochem. Biophys. Res. Commun.*, **47**, 727 (1972).
(24) N. Yanaihara, C. Yanaihara, T. Hashimoto, Y. Kenmochi, T. Keneko, S. Saito, A. V. Schally, and A. Arimura, *Biochem. Biophys. Res. Commun.*, **49**, 1280 (1972).
(25) J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis", W. H. Freeman, San Francisco, Calif., 1969.
(26) E. Kaiser, R. L. Colescott, C. D. Bossinger, and P. T. Cook, *Anal. Biochem.*, **34**, 595 (1970).
(27) T. Wieland, C. Birr, and H. Wissenbach, *Angew. Chem.*, **81**, 782 (1969).
(28) G. R. Marshall in "Pharmacology of Homonal Polypeptides and Proteins", N. L. Back and R. Pasletti, Ed., Plenum Press, New York, N.Y., 1968, p 48.
(29) D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *J. Biol. Chem.*, **237**, 1563 (1962).
(30) J. Greenstein, S. Birnbaum, and M. Otey, *J. Am. Chem. Soc.*, **75**, 1994 (1953).
(31) K. Dittmer, W. Herz, and S. J. Cristol, *J. Biol. Chem.*, **173**, 323 (1948).
(32) T. N. Pattabiraman and W. B. Lawson, *Biochem. J.*, **126**, 659 (1972).

Synthesis and Antiviral Activity of 5- and 5'-Substituted Thymidine Analogs

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The 5'-*O*-*p*-tolylsulfonyl derivatives of 5-chloro-, 5-bromo-, and 5-iodo-2'-deoxyuridine were synthesized and converted into the corresponding 5-halo-5'-azido-2',5'-dideoxyuridines (**5**–**7**). Reduction of 5-chloro-5'-azido-2',5'-dideoxyuridine (**5**) afforded 5-chloro-5'-amino-2',5'-dideoxyuridine (**10**, ACIU); however, similar efforts to prepare 5-bromo-5'-amino-2',5'-dideoxyuridine (**11**) and 5-iodo-5'-amino-2',5'-dideoxyuridine (**12**) by reduction of the corresponding 5'-azido precursor resulted in the formation of 5'-amino-2',5'-dideoxyuridine (**9**). 5-Bromo-5'-amino-2',5'-dideoxyuridine (**11**, ABrU) and 5-iodo-5'-amino-2',5'-dideoxyuridine (**12**, AIU) were prepared by halogenation of the 5-mercuriacetate of 5'-amino-2',5'-dideoxyuridine. The 5'-amino-2',5'-dideoxy analogs of 5-methyl-, 5-chloro-, 5-bromo-, and 5-iodo-2'-deoxyuridine possess antiviral activity against herpes simplex virus but exhibit no inhibitory activity against sarcoma 180 (murine) or Vero (monkey) cells in culture.

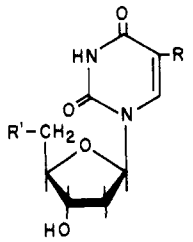
Baker et al.[1] first indicated that amino sugar nucleosides can possess biological activity. They reported that the activity of puromycin against a mammary adenocarcinoma and *Trypanosoma equipserdum* in mice is due to the in vivo enzymatic formation of N^6-dimethyl-3'-amino-adenosine. This nucleoside was found also to be an inhibitor of RNA synthesis as well as of cell division.[2] Other nucleosides that possess an amino substitutent in place of a sugar hydroxyl have been synthesized subsequently[3d,5] and some have been reported to exhibit antiviral or antineoplastic activity. Since thymidine analogs, such as 5-iodo-2'-deoxyuridine, 5-bromo-2'-deoxyuridine, 5-trifluoromethyl-2'-deoxyuridine, 5-ethyl-2'-deoxyuridine, 6-azathymidine, etc.,[3] have been shown to inhibit neoplastic cell and/or virus replication, the synthesis and biological activity of 5'-amino- (and 5'-azido-) thymidine

analogs appeared worthy of examination.

Our interest in amino sugar nucleosides was prompted also by the recent observation that 5'-amino-5'-deoxythymidine[6] is a potent inhibitor of mammalian thymidine kinase,[7] an enzyme that shows enhanced activity in many tumor and virus-infected systems.[8] This compound is also a modest inhibitor of thymidylate kinase.[9] Baker and his colleagues stated that an inhibition of thymidine kinase might be a necessary adjunct to achieve "thymidine-less death" of cancer cells during blockade of dihydrofolate reductase or thymidylate synthetase.[10] 5-Substituted 2'-deoxyuridines are competitive inhibitors of thymidine kinase,[11] provided the 5-substituent is sterically similar to the normal methyl moiety.[12] In addition, 5-iodo-2'-deoxyuridine, which upon incorporation into DNA sensitizes mammalian cells, viruses, and bacteria to ionizing and ultraviolet radiation, has been shown (as a substrate) to sensitize *Escherichia coli* thymidine kinase to radiation inactivation.[4]

We have, therefore, synthesized several 5'-azido and 5'-amino analogs of 5-halo-2',5'-dideoxyuridines as potential inhibitors of viral or neoplastic cell replication as well as potential radiation sensitizers of thymidine- and TMP kinase.

Chemistry. The compounds prepared for this study are listed below.



1, R = Cl; R' = OTs
2, R = Br; R' = OTs
3, R = I; R' = OTs
4, R = H; R' = OTs
5, R = Cl; R' = N_3
6, R = Br; R' = N_3
7, R = I; R' = N_3
8, R = H; R' = N_3
9, R = H; R' = NH_2
10, R = Cl; R' = NH_2
11, R = Br; R' = NH_2
12, R = I; R' = NH_2
13, R = CH_3; R' = NH_2

Reist et al.[13] unambiguously proved that thymidine can be selectively tosylated at the 5'-hydroxyl with *p*-toluenesulfonyl chloride in pyridine at 0°. The 5'-*O*-*p*-tolylsulfonyl derivatives of 5-fluoro-2'-deoxyuridine and of 2'-deoxyuridine have also been prepared via direct tosylation of the unblocked nucleosides.[14] The 5-halo-5'-*O*-*p*-tolylsulfonyl-2'-deoxyuridines (compounds **1-3**) and 5'-*O*-*p*-tolylsulfonyl-2'-deoxyuridine (**4**) were synthesized in the same manner. The relatively small amounts of 3'-monotolylsulfonate and 3',5'-ditolylsulfonate that did form were readily distinguished by their greater mobilities on a thin-layer chromatogram (TLC). These versatile tolylsulfonyl derivatives afford the preparation of numerous 5'-substituted nucleosides by displacement of the tolylsulfonyl group.

Treatment of compounds **1-4** with sodium or lithium azide in DMF at 80–100° gave the corresponding 5'-azido derivatives **5-8**. Compounds **5-7** do not inhibit the replication of sarcoma 180 cells and are only slightly active against herpes simplex virus production (Table I). Antiviral and cytotoxic activity has been reported with 5'-azido-5'-deoxy derivatives of other nucleosides.[3d] Catalytic reduction of 5-chloro-5'-azido-2',5'-dideoxyuridine (**5**) with platinum oxide gave a low yield of the desired amino nucleoside (**10**). However, considerable dehalogenation to 5'-amino-2',5'-dideoxyuridine also occurred during the process.

Table I. Effect of Various Thymidine Analogs on the Growth of Herpes Simplex Virus Type 1 and of Sarcoma 180 Cells in Vitro

			Percent of control	
Compd[d]	R	R'	Herpes simplex virus[c]	Sarcoma 180
1[a]	Cl	OTs		65
2[a]	Br	OTs		65
5	Cl	N_3	81	100
6	Br	N_3	54	100
7	I	N_3	60	100
9	H	NH_2	101	100
10	Cl	NH_2	29	100
11[b]	Br	NH_2	1	
12[b]	I	NH_2	1	100
12	I	NH_2	0.02	100
13	CH_3	NH_2	3	100

[a] The concentration used was 100 μM. [b] Concentration used 200 μM. [c] Virus titer in absence of compound was 1.2×10^6 pfu and is equated to 100%. [d] Unless otherwise noted all compounds were tested at 400 μM.

Various attempts to synthesize 5-bromo-5'-amino-2',5'-dideoxyuridine (**11**) and 5-iodo-5'-amino-2',5'-dideoxyuridine (**12**) by hydrogenation failed because reduction of the 5'-azido precursor was accompanied by loss of the halogen during concomitant reduction of the carbon–halogen bond. However, 5-bromo-5'-amino-2',5'-dideoxyuridine (**11**, ABrU) and 5-iodo-5'-amino-2',5'-dideoxyuridine (**12**, AIU) were synthesized by halogenation of the 5-mercuriacetate[15] of 5'-amino-2',5'-dideoxyuridine (**9**). Compound **9** was synthesized from 2'-deoxyuridine via 5'-*O*-*p*-tolylsulfonyl- and 5'-azido-2',5'-dideoxyuridine intermediates. The physical properties and yields of these thymidine analogs are listed in Table II.

Biological Activity. The 5'-amino analogs of 5-methyl-, 5-chloro-, 5-bromo-, and 5-iodo-2',5'-dideoxyuridine (**10–13**) exert an antiviral effect against herpes simplex virus type 1 in cell culture. Furthermore, the antiviral activity of AIU (**12**) was accompanied by an unusual lack of toxicity to the host cell[16] as well as to experimental animals. Compounds **10**, **11**, and **13**, at concentrations that produced the antiviral activity listed in Table I, exerted no inhibitory effect on the replication of the uninfected host Vero cells. The potential toxicity of compounds **5**, **6**, **7**, and **9** was not evaluated since the amount of viral inhibitions found is not considered to be very significant.

Vero cells, a continuous line of African green monkey kidney cells,[17] 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 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. Medium, drug free or containing the drug concentrations indicated in Table I, was then added. 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 three times. The cell lysates were diluted directly and the virus yield was assayed by plaque formation on Vero cells. The percentage of plaque-forming units (pfu) of virus in the drug treated cultures relative to that found in the drug-free condition is presented in Table I.

Mouse sarcoma 180 cells were maintained as suspension cultures in Fischer's medium supplemented with 10%

Table II. Melting Points, Yields, and Elemental Analyses of Various Thymidine Analogs

Compd	R	R'	Mp, °C	% yield	Formula[a]	Recrystn solvent
1	Cl	OTs	140 dec	44	$C_{16}H_{17}ClN_2O_7S$	EtOH
2	Br	OTs	152 dec	52	$C_{16}H_{17}BrN_2O_7S$	EtOH
3	I	OTs	164-165 dec	46	$C_{16}H_{17}IN_2O_7S$	EtOH
4	H	OTs	163-164 dec	96	$C_{16}H_{18}N_2O_7S$	EtOH
5	Cl	N_3	172-173 dec	33	$C_9H_{10}ClN_5O_4$	2-PrOH
6	Br	N_3	176-177 dec	73	$C_9H_{10}BrN_5O_4$	2-PrOH
7	I	N_3	185 dec	59	$C_9H_{10}IN_5O_4$	2-PrOH
8	H	N_3	140-141	90	$C_9H_{11}N_5O_4$	EtOH-H_2O
9	H	NH_2	191-193 dec	72	$C_9H_{13}N_3O_4$	EtOH
10	Cl	NH_2	193 dec	14	$C_9H_{12}ClN_3O_4 \cdot 0.5H_2O$	EtOH-Et_2O-pet. ether
11	Br	NH_2	189-191 dec	69	$C_9H_{12}BrN_3O_4$	EtOH-Et_2O
12	I	NH_2	201-202 dec	77	$C_9H_{12}IN_3O_4$	EtOH-Et_2O

[a] All new compounds listed above gave satisfactory C, H, and N analyses except compound 10. Calcd: C, 39.94; N, 15.52. Found: C, 40.48; N, 14.90.

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 a 400 μM concentration, was added to sarcoma 180 cells (~2 × 10^4 cells/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 thymidine analogs 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 I. In contrast to the antiviral activity exerted by these compounds, no significant inhibition of the replication of saroma 180 cells in culture was found.

Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are not corrected. The uv spectra were recorded on a Beckman Model-25 and/or a Beckman-DUR spectrophotometer. Elemental analyses were carried out by Baron Consulting Co., Analytical Services, Orange, Conn. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of theoretical values except for compound **10**. TLC and preparative TLC were performed on Eastman 6060 precoated silica gel sheets with fluorescent indicator and on Brinkman silica gel PF254 plates, respectively, using a $CHCl_3$–EtOH (9:1 v/v) solvent system. Compound **10** was developed in acetone as well as in 2-PrOH–concentrated NH_4OH–H_2O (7:1:2 v/v).

5-Iodo-5'-*O*-*p*-tolylsulfonyl-2'-deoxyuridine (3). To a stirred, chilled solution (ice bath) of 5-iodo-2'-deoxyuridine (3.54 g, 10 mmol) in 100 ml of pyridine was added portionwise *p*-toluenesulfonyl chloride (2.11 g, 11 mmol). After dissolution had occurred, the mixture was kept at 4° for 3 days. An additional 190 mg (1.0 mmol) of *p*-toluenesulfonyl chloride was then added and the mixture was allowed to stand overnight at room temperature. After addition of 10 ml of EtOH, the solvents were removed in vacuo. The residue was triturated with 100 ml of ice-cold water. The crude product was collected by filtration and dried under suction to give 4.36 g (86%) of a pink-white powder. The material was leached with hot toluene, cooled, and filtered, and the residue was dried to give 4.19 g of white powder. Recrystallization from ca. 800 ml of EtOH yielded 1.95 g of white crystals. A second crop from the concentrated supernatant fraction yielded 0.4 g of white crystals to produce a total yield of 46%. The compound decomposed at 164–165° with liberation of iodine vapor. Anal. ($C_{16}H_{17}IN_2O_7S$) C, H, N.

Compound 1 and 2 were prepared in a similar manner, except that the isolation procedure described for 5'-*O*-*p*-tolylsulfonylthymidine was used.[13] The melting point, yield, solvent used for recrystallization, and elemental analysis of these compounds are listed in Table II.

5'-*O*-*p*-Tolylsulfonyl-2'-deoxyuridine (4). To a solution of 2'-deoxyuridine (100 g, 0.35 mol) in 650 ml of dry pyridine (dry over KOH) was added *p*-toluenesulfonyl chloride (99.3 g, 0.52 mol) portionwise at 0° (ice bath). The reaction mixture was maintained at 0° for 1 h, with stirring, and then kept at 3° for another 23 h. The solvent (pyridine) was removed under reduced pressure (~0.1 mmHg) at 30° to give a thick syrup which was extracted with ether (5 × 300 ml). To the remaining syrupy residue was added 800 ml of ice–water. Upon vigorous rubbing with a spatula, the product solidified. The solid mass was pulverized and filtered, and the product was washed with water (5 × 500 ml), ether (10 × 250 ml), and petroleum ether (bp 30–60°, 3 × 200 ml). The dried product weighed 127.2 g (96%) and was used for the next preparation without further purification. An analytical sample was obtained by recrystallization of the crude product from EtOH: mp 163–164° dec (lit.[18] 156–157° dec). Anal. ($C_{16}H_{18}N_2O_7S$) C, H, N.

5-Bromo-5'-azido-2',5'-dideoxyuridine (6). A stirred mixture of compound **2** (3.68 g, 8.0 mmol) and NaN_3 (1.26 g, 19.4 mmol) in 70 ml of DMF was heated at 100° for 2 h. The mixture was cooled and filtered. The DMF was removed in vacuo at 50°. The yellow residue was dissolved in 200 ml of 50% aqueous MeOH, stirred for 0.5 h with 15 g of Dowex 50 (H^+) resin, and filtered. The solvents were removed in vacuo to a minimum remaining volume and chilled. The white precipitate was collected by filtration, dried, and recrystallized from 2-PrOH to yield 1.93 g (73%) of white powder, mp 176–177° with decomposition. Anal. ($C_9H_{10}BrN_5O_4$) C, H, N.

Compounds **5** and **7** were prepared by a similar procedure (Table II).

5'-Azido-2',5'-dideoxyuridine (8). A mixture of compound **4** (38.2 g, 0.1 mol) and LiN_3 (14.7 g, 0.3 mol) in 150 ml of DMF was heated to 75–80° for 2 h. The reaction mixture was cooled in an ice bath and filtered through a sintered glass funnel to remove any insoluble materials. The solvent was evaporated to dryness under diminished pressure at 60°. The residue was coevaporated several times with EtOH and then extracted with 2-PrOH–EtOH (1:1, 3 × 100 ml). The solution was clarified with Norite, filtered through a Celite pad, concentrated in vacuo to ~70 ml, and stored at −20° overnight. Compound **8** crystallized out as fine white crystals. The product was collected by filtration, washed with a small amount of ice-cooled EtOH–H_2O (1:1 v/v), ether, and petroleum ether (bp 30–60°), and then dried. The dried compound weighed 22.7 g (90%). An analytical sample was obtained by recrystallization of **8** from EtOH–H_2O (1:1, v/v): mp 140–141° (lit.[18] 139.5–140.5°). Anal. ($C_9H_{11}N_5O_4$) C, H, N.

5'-Amino-2',5'-dideoxyuridine (9). 5'-Azido-2',5'-dideoxyuridine (**8**) (17.6 g, 0.07 mol) was dissolved in 250 ml of EtOH–H_2O (1:1 v/v) and hydrogenated at room temperature, 35 psi of hydrogen pressure, in the presence of 3.0 g of 10% palladium on charcoal for 2 h. The catalyst was removed by filtration through a Celite pad. The filtrate was evaporated to dryness under reduced pressure, affording 15.0 g (95%) of crystalline residue. An analytical sample was obtained by recrystallization of the product from EtOH: mp 191–193° dec (lit.[18] mp 230° dec); uv $\lambda_{max}^{0.01\ N\ HCl}$ 261 nm (ε 10150); uv $\lambda_{min}^{0.01\ N\ HCl}$ 230 nm; uv $\lambda_{max}^{0.01\ N\ NaOH}$ 262 nm (ε 7615); u $\lambda_{min}^{0.01\ N\ NaOH}$ 241 nm. Anal. ($C_9H_{13}N_3O_4$) C, H, N.

5-Chloro-5'-amino-2',5'-dideoxyuridine (10). A solution of **5** (0.15 g, 0.52 mmol) in 50 ml of MeOH containing 40 mg of platinum oxide was shaken for 0.5 h under 20 psi of hydrogen pressure. Thin-layer chromatography (solvent, acetone) showed two major spots, both of which were ninhydrin-positive. The slower area had an R_f identical with authentic 5'-amino-2',5'-

dideoxyuridine.[18] The mixture was filtered through Celite, and the MeOH was removed in vacuo. The residue was dissolved in H_2O and applied to a column containing Dowex 50 (H^+) resin. The column was washed with 200 ml of H_2O, and the 5'-amino nucleosides were eluted with 200 ml of 1 N NH_4OH. After evaporation to dryness the material was subjected to preparative TLC (solvent, acetone) and two ninhydrin-positive areas appeared, the slower being compound **9** and the faster the desired product which was then eluted with EtOH. The product crystallized from a mixture of EtOH–Et_2O–petroleum ether (ca. 1:1:1 v/v) to give 14 mg of a tan powder. Addition of Et_2O to the mother liquors gave 5 mg of a white powder for a total yield of 14%. The product gradually turned red at 182°. It formed a red melt at 191° and decomposed. Anal. ($C_9H_{12}ClN_3O_4 \cdot 0.5H_2O$) C, H, N; C: calcd, 39.94; found, 40.48; N: calcd, 15.52; found, 14.90.

5-Bromo-5'-amino-2',5'-dideoxyuridine (11). To a solution of compound **9** (0.113 g, 0.5 mmol) in 100 ml of 0.05 M sodium acetate buffer, pH 6.0, was added mercuric acetate (0.8 g, 2.5 mmol). The solution was heated at 60 °C for 5 h and then cooled on ice. Chelex 100 resin (Bio-Rad), 20 g, was added and the slurry stirred for 15 min at 0 °C. The resin was removed by filtration and to the filtrate was added *N*-bromosuccinimide (0.177 g, 1.0 mmol). The solution was stirred at room temperature for approximately 30 min. The extent of bromination was followed spectrophotometrically at 300 nm[15] and the reaction terminated by the addition of aniline (in ethanol) when the absorbance increase plateaued. The reaction mixture was applied directly to a column (2 × 15 cm) of Dowex 50 (H^+) resin, the column washed with 200 ml of water, and the product eluted by the addition of 1 N NH_4OH. Solvent was removed at 40 °C under reduced pressure and the product crystallized from a mixture of EtOH–Et_2O to give 104 mg (69%). Anal. ($C_9H_{12}BrN_3O_4$) C, H, N.

5-Iodo-5'-amino-2',5'-dideoxyuridine (12). To a solution of compound **9** (4.5 g, 20 mmol) in 500 ml of 0.5 M sodium acetate buffer, pH 6.0, was added mercuric acetate (16 g, 50 mmol). The solution was heated at 50 °C overnight (~18 h), cooled to room temperature, and diluted to 1 l. with water. A solution of elemental iodine in ethanol (400 ml of 0.2 M I_2) was then added and the mixture stirred at room temperature for 90 min. Excess I_2 and HgI_2 were removed by extracting the solution four times with 700 ml of $CHCl_3$. The aqueous phase was applied directly to a column (2 × 25 cm) of Dowex 50 (H^+) resin and washed with 500 ml of water, and the product was eluted with 1 N NH_4OH (~100–200 ml). Solvent was removed by rotary evaporation at 30 °C until the volume was reduced to 10–15 ml. The product which crystallized during the solvent evaporation was collected by filtration to give 4.8 g of product. Addition of an EtOH–Et_2O mixture (1:2) to the mother liquor afforded an additional 0.6 g for a total yield of 5.4 g (77%). An analytical sample was obtained by recrystallization of the product from EtOH–Et_2O. Anal. ($C_9H_{12}IN_3O_4$) C, H, N.

5'-Amino-5'-deoxythymidine (13). The title compound was prepared according to the procedure reported by J. P. Horwitz and co-workers.[6]

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References and Notes

(1) B. R. Baker, J. P. Joseph, and J. H. Williams, *J. Am. Chem. Soc.*, **77**, 1 (1955).
(2) (a) J. F. Gierthy and G. P. Studyzinski, *Cancer Res.*, **33**, 2673 (1973); (b) A. E. Farnham and D. T. Dubin, *J. Mol. Biol.*, **14**, 55 (1965); (c) G. P. Studyzinski and K. A. O. Ellem, *J. Cell Biol.*, **29**, 411 (1966); (d) N. Dickie, C. S. Alexander, and H. T. Nagasawa, *Biochim. Biophys. Acta*, **95**, 156 (1965).
(3) (a) A. Bloch in "Drug Design", Vol. 4, A. Ariens, Ed., Academic Press, New York, N.Y., 1973, p 285; (b) B. Goz and W. H. Prusoff, *Annu. Rev. Pharmacol.*, **10**, 143 (1970); (c) P. Roy-Burman, "Analogues of Nucleic Acid Components", Springer-Verlag, New York, N.Y., 1970; (d) T. Y. Shen, *Angew. Chem., Int. Ed. Engl.*, **9**, 678 (1970).
(4) (a) P. Voytek, P. K. Chang, and W. H. Prusoff, *J. Biol. Chem.*, **247**, 367 (1972); (b) R. Cysyk and W. H. Prusoff, *ibid.*, **247**, 2522 (1972).
(5) J. P. H. Verheyden, D. Wagner, and J. G. Moffatt, *J. Org. Chem.*, **36**, 250 (1971).
(6) J. P. Horwitz, A. J. Tomson, J. A. Urbanski, and J. Chua, *J. Org. Chem.*, **27**, 3045 (1962).
(7) (a) J. P. Neenan and W. Rhode, *J. Med. Chem.*, **16**, 580 (1973); (b) Y.-C. Cheng and W. H. Prusoff, *Biochemistry*, **12**, 2612 (1973).
(8) For review articles on thymidine phosphorylation see (a) J. S. Roth in "Protein Metabolism and Biological Function", C. P. Bianchi and R. Hilf, Ed., Rutgers University Press, New Brunswick, N.J., 1970, p 141; (b) J. E. Cleaver, "Thymidine Metabolism and Cell Kinetics", Wiley, New York, N.Y., 1967; (c) E. P. Anderson in "The Enzymes", Vol. IX, Part B, P. D. Boyer, Ed., Academic Press, New York, N.Y., 1973, p 49.
(9) Y.-C. Cheng and W. H. Prusoff, *Biochemistry*, **13**, 1179 (1974).
(10) B. R. Baker, T. J. Schwan, and D. V. Santi, *J. Med. Chem.*, **9**, 66 (1966).
(11) R. Okazaki and A. Kornberg, *J. Biol. Chem.*, **239**, 269 (1964).
(12) (a) W. Szybalski in "The Molecular Basis of Neoplasia", University of Texas Press, Austin, Texas, 1962, pp 147–171; (b) W. H. Prusoff, Y. S. Bakhle, and L. Sekely, *Ann. N.Y. Acad. Sci.*, **130**, 135 (1965); (c) C. Heidelberger, *Prog. Nucleic Acid Res. Mol. Biol.*, **4**, 1 (1965).
(13) E. J. Reist, A. Benitez, and L. Goodman, *J. Org. Chem.*, **29**, 554 (1964).
(14) (a) Y. Nishizawa, J. E. Casida, S. W. Anderson, and C. Heidelberger, *Biochem. Pharmacol.*, **14**, 1605 (1965); (b) G. Kowollik, G. Gaertner, G. Etzold, and P. Langen, *Carbohydr. Res.*, **12**, 301 (1970).
(15) R. M. K. Dale, D. C. Ward, D. C. Livingston, and E. Martin, *Nucleic Acid Res.*, **2**, 915 (1975).
(16) Y. C. Cheng, B. Goz, J. P. Neenan, D. C. Ward, and W. H. Prusoff, *J. Virol.*, **15**, 1284 (1975).
(17) J. E. Shannon and M. L. Macy, Ed., "Registry of Animal Cell Lines", 2nd ed, American Type Culture Collection, Rockville, Md., 1972, pp 81–82.
(18) M. W. Logue and N. J. Leonard, *J. Am. Chem. Soc.*, **94**, 2842 (1972).
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