General Method for the Preparation of MTX(G_n) Polylysine Conjugates. In a typical procedure 137 mg of poly(L-lysine) (M_r 52 000) was dissolved in 5 mL of 0.01 M potassium phosphate buffer containing 0.15 M NaCl at pH 7.2. A solution of 28 μmol/mL of MTX or the appropriate MTX(G_n) was prepared in the same manner as above, and 1 mL was added to the polylysine solution. Then 0.5 mL of a solution (200 mg/mL) of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) was added, and the reaction mixture was mixed and allowed to stand at 25 °C in the dark for 18 h. The reaction mixture was then applied on a column (1 × 25 cm) of Sephadex G 25, previously equilibrated with the buffer used for making the reagents. The column was eluted with 0.01 M potassium phosphate buffer, and the first yellow band eluted from the column was collected and dialyzed with a bag made of spectra/por (Fisher) membrane with a MW cutoff of 3500 for 4 days against 4 L of distilled water. The water was changed every 24 h. The dialyzed material was diluted with distilled water to a known volume, and methotrexate or MTX-polyglutamate content was estimated by UV spectroscopy with a standard curve. All conjugates exhibited the typical UV absorption of MTX or its corresponding polyglutamates, adducing independent evidence for the structural ingegrity of the 4-amino-4-deoxy-N^{10}-methylpteroylglutamate part of the molecule. The MTX(G_n) solutions thus prepared were lyophilized to a fine solid, and the weights were determined to estimate the number of MTX or its polyglutamates covalently bound to the polylysine molecule.

Aqueous solutions of all conjugates were analyzed by HPLC for the presence of free MTX or MTX-polyglutamates.[27] A mixture of polylysine and MTX or its polyglutamates on HPLC analyses monitored at 299 nm gave single-absorption peaks corresponding to MTX or its polyglutamates that were quantitatively eluted from the column.

Acknowledgment. This investigation was supported by Grants CA 32687, CA 27101 (M.G.N.), CA 25933 (J.G.), and CA 10914 (R.L.K.) from the National Cancer Institute, USPHS/Department of Health and Human Services.

Registry No. **1a**, 41600-13-9; **1b**, 41600-14-0; **1c**, 73610-81-8; **1d**, 80801-54-3; **1e**, 124126-66-5; **1f**, 63948-16-3; **1g**, 89-38-3; **1h**, 124153-32-8; **1i**, 29701-38-0; **1j**, 124126-67-6; **1k**, 105099-94-3; **3**, 59-05-2; **4**, 45120-30-7; **5**, 82911-69-1; **6**, 84793-07-7; **7**, 124126-68-7; 4-amino-4-deoxy-N^{10}-methylpteroic acid, 19741-14-1; 4-amino-4-deoxy-10-deazapteroic acid, 33047-42-6; L-lysine homopolymer, 25104-18-1; poly(L-lysine), SRU, 38000-06-5; D-lysine homopolymer, 26853-89-4; poly(D-lysine), SRU, 26913-90-6; N^{10}-TFA-pteroic acid, 37793-53-6; dihydrofolate reductase, 9002-03-3.

Synthesis and Antiviral and Cytotoxic Activity of Iodohydrin and Iodomethoxy Derivatives of 5-Vinyl-2′-deoxyuridines, 2′-Fluoro-2′-deoxyuridine, and Uridine

Rakesh Kumar,[†] Lihua Xu,[†] Edward E. Knaus,[†] Leonard I. Wiebe,*[,†] Dorothy R. Tovell,[‡] D. Lorne Tyrrell,[‡] and Theresa M. Allen[§]

Faculty of Pharmacy and Pharmaceutical Sciences, Department of Infectious Diseases and Medical Microbiology, and Department of Pharmacology, University of Alberta, Edmonton, Canada T6G 2N8. *Received April 7, 1989*

A series of new 5-(1-hydroxy-2-iodoethyl)-2′-deoxyuridine and uridine compounds (**11**, **16**) was synthesized by the regiospecific addition of HOI to the vinyl substituent of 5-vinyl-2′-deoxyuridine (**10a**), 5-vinyl-2′-fluoro-2′-deoxyuridine (**10b**), 5-vinyluridine (**10c**), and (*E*)-5-(2-iodovinyl)-2′-deoxyuridine (**4b**). Treatment of the iodohydrins **11a–c** with methanolic sulfuric acid afforded the corresponding 5-(1-methoxy-2-iodoethyl) derivatives (**12a–c**). In contrast, reaction of 5-(1-hydroxy-2-iodoethyl)-2′-deoxyuridine (**11a**) with sodium carbonate in methanol afforded a mixture of 5-(1-hydroxy-2-methoxyethyl)-2′-deoxyuridine (**13**) and 2,3-dihydro-3-hydroxy-5-(2′-deoxy-β-D-ribofuranosyl)-furano[2,3-*d*]pyrimidin-6(5*H*)-one (**14**). The most active compound, 5-(1-methoxy-2-iodoethyl)-2′-deoxyuridine (**12a**, ID_{50} = 0.1 μg/mL), which exhibited antiviral activity (HSV-1) 100-fold higher than that of the 5-(1-hydroxy-2-iodoethyl) analogue (**11a**), was less active than IVDU or acyclovir (ID_{50} = 0.01–0.1 μg/mL range). The C-5 substituent in the 2′-deoxyuridine series was a determinant of cytotoxic activity, as determined in the in vitro L1210 screen, where the relative activity order was $CH(OH)CHI_2$ (**16**) > $CH(OMe)CH_2I$ (**12a**) > $CH(OH)CH_2I$ (**11a**) ≃ $CH(OH)CH_2OMe$ (**13**). The 2′-substituent was also a determinant of cytotoxic activity in the 5-(1-hydroxy-2-iodoethyl) (**11a–c**) and 5-(1-methoxy-2-iodoethyl) series of compounds, where the relative activity profile was 2′-deoxyuridine > 2′-fluoro-2′-deoxyuridine > uridine (**11a** > **11b** ≥ **11c**; **12a** > **12b** > **12c**). The most active cytotoxic agent (**16**), possessing a 5-(1-hydroxy-2,2-diiodoethyl) substituent (ED_{50} = 0.77 μg/mL), exhibited an activity approaching that of melphalan (ED_{50} = 0.15 μg/mL). All compounds tested, except for **13** and **14**, exhibited high affinity (K_i = 0.035–0.22 mM range relative to deoxyuridine, K_i = 0.125) for the murine NBMPR-sensitive erythrocyte nucleoside transport system, suggesting that these iodohydrins are good permeants of cell membranes.

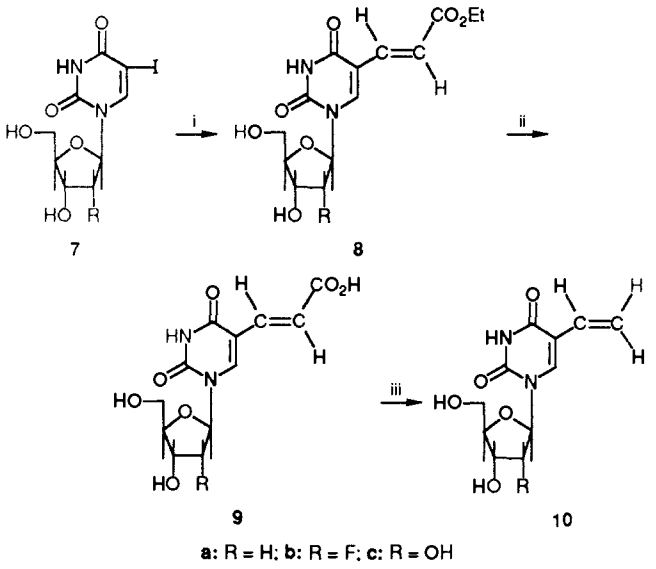
In an earlier study we reported the syntheses of the bromohydrin (**1a**) and chlorohydrin (**1b**) derivatives of 5-vinyl-2′-deoxyuridine (VDU, **2**).[1] 5-(1-Hydroxy-2-chloroethyl)-2′-deoxyuridine (**1b**) and 5-(1-methoxy-2-bromoethyl)-2′-deoxyuridine (**3a**) were moderately active (ID_{50} = 0.1–1.0 μg/mL, relative to acyclovir, ID_{50} = 0.01 μg/mL) against herpes simplex virus type 1 (HSV-1), but demonstrated only weak cytotoxicity against murine L1210 cells in vitro. This weak activity is a strong contrast to the potent in vitro anti-HSV-1 activity exhibited by BVDU (**4a**), IVDU (**4b**), and 5-(2-chloroethyl)-2′-deoxyuridine (CEDU, **5**).[2,3] BVDU and VDU are rapidly catabolized to the corresponding pyrimidine bases by the action of pyrimidine phosphorylase enzymes,[4,5] whereas CEDU is

[†] Faculty of Pharmacy and Pharmaceutical Sciences.
[‡] Department of Infectious Diseases and Medical Microbiology.
[§] Department of Pharmacology.

(1) Kumar, R.; Wiebe, L. I.; Hall, T. W.; Knaus, E. E.; Tovell, D. R.; Tyrrell, D. L.; Allen, T. M.; Fathi-Afshar, R. *J. Med. Chem.* **1989**, *32*, 941.
(2) De Clercq, E.; Descamps, J.; De Somer, P.; Barr, P.; Jones, A. S.; Walker, R. T. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2947.
(3) Griengl, H.; Bodenteich, M.; Hayden, W.; Wanek, E.; Streicher, W.; Stutz, P.; Bachmayer, H.; Ghazzouli, I.; Rosenwirth, B. *J. Med. Chem.* **1985**, *28*, 1679.

0022-2623/90/1833-0717$02.50/0

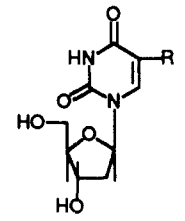
Scheme I[a]



a: R = H; **b**: R = F; **c**: R = OH

[a] Reagents: (i) CH_2=$CHCO_2Et$, $(Ph_3P)_2PdCl_2$, Et_3N (**7b,c**); (ii) 0.5 *N* KOH (**8b,c**); (iii) DMF, Et_3N, 100 °C (**9b,c**).

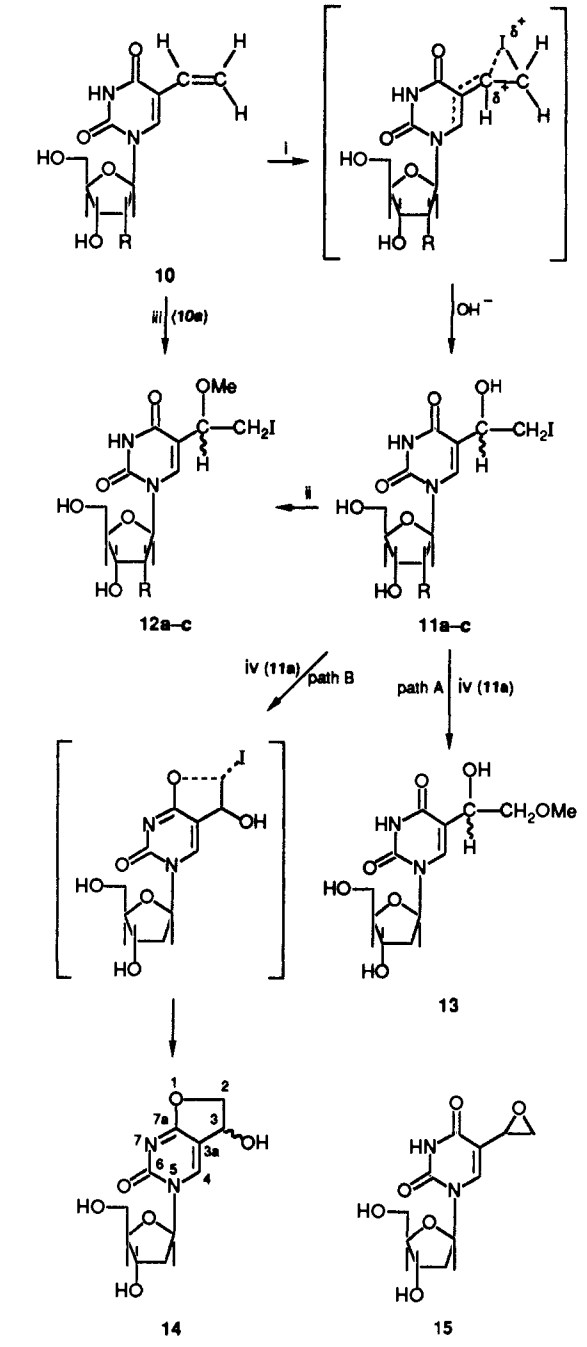
thought to undergo inactivation by hydroxylation of the C-5 substituent, in a manner analogous to that reported for 5-ethyl-2′-deoxyuridine (EDU, **6**).[6] Although the structural requirements at C-5 are reasonably well-defined for antiherpetic activity, in vivo catabolism necessitates further structural manipulation to obtain agents which are more effective clinically. It has been demonstrated that in vivo stabilization of pyrimidine nucleosides against phosphorolysis can be achieved by incorporation of a fluorine atom in the 2′-deoxyribo position of the furanosyl moiety,[7] but there is little qualitative or mechanistic knowledge of the biotransformation of 2-carbon substituents at C-5 of pyrimidine nucleosides.



1a: R = CH(OH)CH_2Br
b: R = CH(OH)CH_2Cl
2: R = CH=CH_2
3a: R = CH(OMe)CH_2Br
b: R = -CH(OMe)CH_2Cl
4a: R = (*E*)-CH=CHBr
b: R = (*E*)-CH=CHI
5: R = CH_2CH_2Cl
6: R = Et

Iodohydrin and iodomethoxy derivatives of IVDU (**4b**), VDU (**10a**), VFRU (**10b**), and VU (**10c**) have now been synthesized for evaluation as antiherpetic (HSV-1) and

Scheme II[a]



a: R = H; **b**: R = F; **c**: R = OH

[a] Reagents: (i) I_2, KIO_3, H_2O, 5 N H_2SO_4, 55 °C; (ii) 5 N H_2SO_4, MeOH; (iii) ICl, MeOH, 50 °C; (iv) Na_2CO_3, MeOH.

cytotoxic agents. This study represents an extension of previous investigations to acquire structure–activity relationships (SARs) by elaboration of the C-5 vinyl substituents of anti-HSV-1 pyrimidine nucleosides.[8-10] The synthesis of the 5-(1-hydroxy-2-iodethyl) derivative **11a** was also of interest since it is a potential metabolite of IVDU, which could account for a previously reported, but

(4) Walker, R. T.; Balzarini, J.; Coe, P. L.; De Clercq, E.; Harnden, M. R.; Jones, A. S.; Noble, S. A.; Rahim, S. G. *Nucleic Acids Res., Symp. Ser.* **1982**, No. 11, 215.
(5) Goodchild, J.; Wadsworth, H. J.; Sim, I. S. *Nucleosides Nucleotides* **1986**, *5*, 571.
(6) Szinai, E.; Bihari, M.; Ujszaszy, K.; Veres, Z.; Szaboles, A.; Holly, S.; Gacs-Baitz, E.; Otvos, L. *Bio-organic Heterocycles*; Van der Plas, H. C., Otvos, L., Simonyi, M., Eds.; Elsevier Press: Amsterdam, 1985; 335.
(7) Iwashina, T.; Tovell, D. R.; Xu, L.; Tyrrell, D. L.; Knaus, E. E.; Wiebe, L. I. *Drug Des. Delivery* **1988**, *3*, 309.
(8) Barr, P. J.; Jones, A. S.; Verhelst, G.; Walker, R. T. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1665.
(9) Coe, P. L.; Harnden, M. R.; Jones, A. S.; Noble, S. A.; Walker, R. T. *J. Med. Chem.* **1982**, *25*, 1329.
(10) Goodchild, J.; Roderick, A. P.; Raper, R. H.; Sim, I. S.; Upton, R. M.; Viney, J.; Wadsworth, H. J. *J. Med. Chem.* **1983**, *26*, 1252.

unidentified, polar metabolite of IVDU.[11]

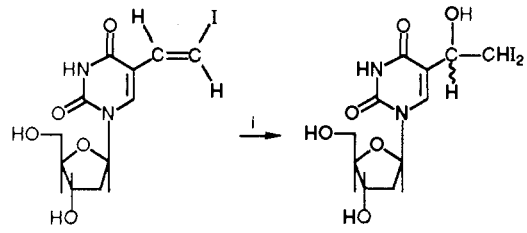
Chemistry

5-Vinyl-2′-deoxyuridine (**10a**) was readily prepared by the palladium acetate–triphenylphosphine-catalyzed reaction of 5-iodo-2′-deoxyuridine with vinyl acetate using the method of Rahim et al.[12] In contrast, similar reactions employing 5-iodo-2′-fluoro-2′-deoxyuridine (**7b**) and 5-iodouridine (**7c**) afforded low yields of **10b,c**, respectively, which were difficult to isolate from the complex reaction mixture obtained. Therefore, the 5-vinyl derivatives **10b,c** were synthesized by reaction of **7b,c** with ethyl acrylate and $(Ph_3P)_2PdCl_2$ by using the procedure illustrated in Scheme I.[13] Thus, hydrolysis of **8b,c** yielded the corresponding 5-(1-propenoic acids) **9b,c**, which were subsequently decarboxylated to afford the required 5-vinyl derivatives (**10b,c**).

The target 5-(1-hydroxy-2-iodoethyl) derivatives of 2′-deoxyuridine (**11a**, 59%), 2′-fluoro-2′-deoxyuridine (**11b**, 72%), and uridine (**11c**, 65%) were prepared by reaction of the 5-vinyl analogues (**10a–c**) with iodine in the presence of the oxidizing agent iodic acid as illustrated in Scheme II. The ^{13}C NMR (*J* modulated spin echo) spectra provided conclusive evidence for the regiospecific addition of the HOI across the C-5 vinyl substituents of **10a–c**. For example, the iodine atom of **11a** is attached to a methylene carbon that exhibited a resonance at δ 14.58, whereas the hydroxyl substituent is attached to a methine carbon which resonates at δ 65.77. The ^{13}C NMR spectrum of **11b** exhibited dual resonances for the methylene carbon at δ 12.25 and 13.35, and for the chiral methine carbon it was at δ 67.45 and 68.25. Compound **11b** is a mixture of two diastereomers which differ in configuration (*R* and *S*) at the 1-position of the 5-(1-hydroxy-2-iodoethyl) substituent. This regiospecific addition is similar to that reported by Dalton et al.[14] for unsymmetrical olefins. Treatment of **11a–c** with methanolic sulfuric acid[15] afforded the corresponding 5-(1-methoxy-2-iodoethyl) derivatives (**12a–c**), which existed as a mixture of two diastereomers that could not be separated by TLC or silica gel column chromatography, in 81–94% yields. The 5-(1-methoxy-2-iodoethyl) analogue (**12a**) was also synthesized in 60% yield via a one-step reaction of 5-vinyl-2′-deoxyuridine (**10a**) with iodine monochloride in methanol.

Reaction of 5-(1-hydroxy-2-iodoethyl) derivative **11a** with sodium carbonate in methanol yielded two products. Displacement of the iodo substituent by methoxide anion afforded 5-(1-hydroxy-2-methoxyethyl) derivative **13** in 26% yield (path A), whereas, an intramolecular nucleophilic displacement of the iodo substituent yielded the bicyclic product 2,3-dihydro-3-hydroxy-5-(2′-deoxy-β-D-ribofuranosyl)furano[2,3-*d*]pyrimidin-6(5*H*)-one (**14**) in 17.5% yield (path B). This base-catalyzed cyclization reaction of **11a** to **14** is analogous to the reported conversion of 5-[2-[(methylsulfonyl)oxy]ethyl]uracil to 2,3-dihydrofurano[2,3-*d*]pyrimidin-6(5*H*)-one using *t*-BuOK in DMSO.[16] There was no evidence for the conversion of iodohydrin **11a** to oxiranyl derivative **15**. Further chemical transformation of **15**, if present, was discounted since the hydrolytic medium present in the isolation procedure would have afforded the corresponding 5-(1,2-dihydroxyethyl) analogue which was not detected.

Scheme III[a]



[a] Reagents: (i) I_2, KIO_3, HoAc, dioxane/water (3:7, v/v).

Reaction of (*E*)-5-(2-iodovinyl)-2′-deoxyuridine (**4b**) with iodine and potassium iodate afforded 5-(1-hydroxy-2,2-diiodoethyl)-2′-deoxyuridine (**16**) in 20% yield (see Scheme III). This substitution pattern was supported by the ^{13}C NMR spectrum which showed methine resonances at δ −9.44 (CHI_2) and 71.63 (*C*HOH).

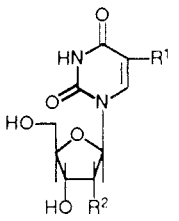
Results and Discussion

The antiviral activities of this new class of 5-[1-hydroxy(or methoxy)-2-iodoethyl]pyrimidine nucleosides (**11a–c**, **12a–c**) and related analogues (**10b**, **13**, **14**, **16**) were determined as the concentration to inhibit plaque formation by 50% (ID_{50}) in vero cells infected with herpes simplex virus type 1 (HSV-1, strain JLJ). 5-(1-Methoxy-2-iodoethyl) compound **12a** (ID_{50} = 0.1 μg/mL) exhibited an activity 100-fold greater than that of 5-(1-hydroxy-2-iodoethyl) analogue **11a** (ID_{50} > 10 μg/mL). The antiviral potency shown by **12a** was also superior to that reported for 5-(2-iodoethyl)-2′-deoxyuridine (MIC of 1 μg/mL).[3] Elaboration of the 5-(1-hydroxy-2-iodoethyl) substituent of **11a** to a 5-(1-hydroxy-2-methoxyethyl) (**13**) or 5-(1-hydroxy-2,2-diiodoethyl) substituent (**16**) does not enhance activity since all exhibited an ID_{50} > 10 μg/mL. These results indicate that the presence of the hydroxyl group in the C-5 substituent of these compounds is detrimental to antiviral activity. The most active 5-(1-methoxy-2-iodoethyl) compound (**12a**) exhibited an antiviral activity approaching that of IVDU and acyclovir. Compound **10b** was 10-fold less active than acyclovir (ID_{50} = 0.01 μg/mL), whereas **11b–c** and **12b–c** were inactive (ID_{50} > 100 μg/mL).

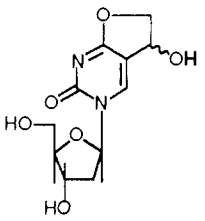
The cytotoxic activities for compounds **10b**, **11–14**, and **16** were determined by an in vitro L1210 assay. A comparison of the activities for the 2′-deoxyuridine analogues indicated that the 5-substituent was a determinant of activity where the relative activity order was **16** [CH(OH)CHI_2] > **12a** [CH(OMe)CH_2I] > **11a** [CH(OH)CH_2I] ≃ **13** [CH(OH)CH_2OMe]. In contrast, the C-5 substituent in the 2′-fluoro-2′-deoxyuridine series was not a determinant of activity, since the 5-vinyl (**10b**), 5-(1-hydroxy-2-iodoethyl) (**11b**), and 5-(1-methoxy-2-iodoethyl) (**12b**) analogues were relatively nontoxic. Because of the low toxicity of these compounds, ED_{50}'s were not determined, but percent survival studies indicated values in the 87–97% range at 10 μg/mL. The most active compound (**16**), possessing a 5-(1-hydroxy-2,2-diiodoethyl) substituent (ED_{50} = 0.77 μg/mL), exhibited cytotoxicity approaching that of the reference compound melphalan (ED_{50} = 0.15 μg/mL). Although **16** exhibited significant in vitro cyto-

(11) Samuel, J.; Gill, M. J.; Iwashina, T.; Tovell, D. R.; Tyrrell, D. L.; Knaus, E. E.; Wiebe, L. I. *Antimicrob. Agents Chemother.* **1986**, *29*, 320.
(12) Rahim, S. G.; Duggan, M. J. H.; Walker, R. T.; Jones, A. S.; Dyer, R. L.; Balzarini, J.; De Clercq, E. *Nucleic Acids Res.* **1982**, *10*, 5285.
(13) Jones, A. S.; Verhelst, G.; Walker, R. T. *Tetrahedron Lett.* **1979**, *45*, 4415.
(14) Dalton, D. R.; Dutta, V. P.; Jones, D. C. *J. Am. Chem. Soc.* **1968**, *90*, 5498.
(15) Hanzlik, R. P.; Edelman, M.; Michaely, J.; Scott, G. *J. Am. Chem. Soc.* **1976**, *98*, 1952.
(16) Fissekis, J. D.; Sweet, F. *J. Org. Chem.* **1973**, *28*, 264.

Table I. Antiviral (HSV-1) Activity, Cytotoxicity, and Transport Inhibition of 5-Substituted 2′-Deoxyuridines and Uridines



10–13, 16



14

no.	R^1	R^2	antiviral activity: ID_{50},[a] μg/mL	L1210 in vitro cytotoxicity: ED_{50},[b] μg/mL	L1210 in vitro cytotoxicity: % survival[c] (10 μg/mL)	P388 in vivo cytotoxicity: %T/C[d] (20 mg/kg)	transport inhibition constants: K_i,[e,f] mM ± SD
10b	$CH{=}CH_2$	F	0.1	ND[g]	87 ± 1	ND	ND
11a	$CH(OH)CH_2I$	H	>10	10.5	52 ± 1	98 ± 7	0.155 (0.15–0.16)
11b	$CH(OH)CH_2I$	F	>100	ND	93 ± 1	ND	ND
11c	$CH(OH)CH_2I$	OH	>100	ND	87 ± 5	ND	ND
12a	$CH(OMe)CH_2I$	H	0.1	6	42 ± 3	114 ± 36	0.035 (0.03–0.04)
12b	$CH(OMe)CH_2I$	F	>100	ND	97 ± 2	ND	ND
12c	$CH(OMe)CH_2I$	OH	>100	ND	108 ± 5	ND	ND
13	$CH(OH)CH_2OMe$	H	>10	12	37 ± 1	103 ± 13	0.61 (0.60–0.62)
14			>100	ND	103 ± 8	ND	8.0
16	$CH(OH)CHI_2$	H	>10	0.77	2 ± 1	112 ± 36	0.22
deoxyuridine							0.125 ± 0.021
IVDU	(*E*)-CH=CHI	H	<0.1				0.025 ± 0.001
acyclovir			0.01				
melphalan[h]				0.15			
5-fluorouracil						>135[i]	

[a] The concentration required to inhibit plaque formation in monolayers of Vero cells by 50%. [b] The concentration required for a 50% reduction in the number of surviving L1210 cells in vitro in suspension. [c] % survival ± SD (n = 3). [d] %T/C = treated/control. [e] Inhibition constant for the facilitated transport of [^{3}H]-thymidine by the murine erythrocyte NBMPR-sensitive nucleoside transporter. [f] The result is the mean of two determinations (range) except for 14 and 16 (n = 1) and 2′-deoxyuridine (n = 3). [g] ND = not determined. [h] 4-[*N*,*N*-Bis-(2-chloroethyl)amino]phenylalanine. [i] Value for a 20 mg/kg ip dose administered daily for either 5 or 9 days (ref 22).

toxic activity, it was inactive in the in vivo P388 leukemia screen, possibly indicative of rapid catabolism in vivo.

The design of nucleosides that have diagnostic and/or chemotherapeutic applications is complemented by a knowledge of the mechanisms through which they exert their effects in vivo. It is important to determine the ability of a drug to cross the cell membrane since entry into cells is a requisite for efficacy. Nucleosides are known to enter cells by a facilitated diffusion mechanism which is sensitive to structural changes in the nucleoside.[17,18] The murine erythrocyte study (see Table I) shows that iodohydrins **11a**, **16** and methoxy derivatives **12a**, **13** are strong competitive inhibitors of NBMPR-sensitive thymidine transport. This is indicative of rapid, nonconcentrative transport across the cell membrane and high bioavailability in those tissues in which facilitative transport is functional. It is interesting to observe that bicyclic compound **14**, with a K_i = 8.0 mM, does not appear to be transported by the NBMPR-sensitive transporter in erythrocytes. The existence or role of nucleoside transporters in central nervous system (CNS) membranes (viz, blood–brain barrier) has not been established, although it is generally accepted that stereospecific, saturable, carrier-mediated transport exists for some hydrophilic compounds.[19]

Penultimate-carbon hydroxylation has been reported to be a major metabolic reaction leading to the inactivation of 5-ethyl-2′-deoxyuridine (EDU, **6**) and 5-ethyluracil, via formation of the corresponding 5-(1-hydroxyethyl) derivatives.[6] As in the case of EDU, hydroxylation at the penultimate carbon of the C-5 substituent of CEDU (**5**) could be expected to occur, with consequent loss of antiviral activity. Although the penultimate-carbon hydroxylation product of CEDU, viz 5-(1-hydroxy-2-chloroethyl)-2′-deoxyuridine, retained some antiviral activity, there was a marked decrease in potency relative to the parent nucleoside.[1] A recent study of the enzyme-catalyzed conversion of the 5′-monophosphate ester of BVDU (**4a**) has demonstrated that nucleophilic substitution and reduction of the bromovinyl moiety is possible via a sequence of attack by thymidylate synthetase at C-6, conversion of the vinyl bromide to the allylic bromide, and subsequent nucleophilic attack at either carbon of the side chain.[20] Although this mode, which was based on attack by mercaptoethanol, resulted in debromination, it is reasonable to project a mechanism of nucleophilic attack by hydroxyl ions, leading to the formation of the corresponding bromohydrin. In the case of IVDU, a corresponding series of reactions would afford iodohydrin **11a**, which has now been synthesized. Although methoxy derivative **12a**, which is unlikely to be a metabolite, has moderate antiviral activity, iodohydrin **11a** was virtually devoid of activity. It is therefore tempting to speculate that an iodohydrin may represent a mechanism leading to inactivation of IVDU.

Experimental Section

Melting points were determined with a Büchi capillary apparatus and are uncorrected. Nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR) were determined for solutions in Me_2SO-d_6, CD_3OD, or pyridine-d_5 with Me_4Si as internal standard (^{1}H NMR) with a Bruker AM-300 spectrometer. ^{13}C NMR (*J* modulated spin echo) spectra were determined in all instances where methyl and methine carbon resonances appear as positive peaks and

(17) Gati, W. P.; Knaus, E. E.; Wiebe, L. I. *Mol. Pharmacol.* **1983**, *23*, 146.
(18) Gati, W. P.; Misra, H. K.; Knaus, E. E.; Wiebe, L. I. *Biochem. Pharmacol.* **1984**, *33*, 3325.
(19) Greig, N. H. *Cancer Treat. Rev.* **1987**, *14*, 1.
(20) Barr, P. J.; Oppenheimer, N. J.; Santi, D. V. *J. Biol. Chem.* **1983**, *258*, 13627.

methylene and quaternary carbon resonances appear as negative peaks. The quaternary carbon resonances for C-4 (**10c**), C-2 and C-4 (**11c**), C-4 (**12c**), and C-7a (14), which are always of low intensity, were not observed in the ^{13}C NMR spectra. Mass spectra were measured on an AEI MS-12 (CI, isobutane or ammonia), AEI MS-50 (HRMS) or a Hewlett-Packard 5995A (EI) spectrometer. Silica gel column chromatography was carried out with Merck 7734 (60–200 mesh) silica gel. 5-Vinyl-2′-deoxyuridine (**10a**),[12] (*E*)-5-(2-iodovinyl)-2′-deoxyuridine (**4b**),[13] and (*E*)-5-(2-carboxyvinyl)-2′-fluoro-2′-deoxyuridine (**9b**)[7] were prepared by using literature procedures. 5-Iodouridine (**7c**) was purchased from the Aldrich Chemical Co.

(*E*)-5-[2-(Ethoxycarbonyl)vinyl]uridine (8c). Dry triethylamine (1.25 mL), ethyl acrylate (0.65 mL, 6 mmol), and bis(triphenylphosphine)palladium(II) chloride (25 mg) were added consecutively to a solution of **7c** (1.5 g, 4.05 mmol) in dry acetonitrile (50 mL). The reaction was allowed to proceed at 80 °C with stirring for 24 h, cooled to 25 °C, and filtered, and the solvent was removed in vacuo. The residue obtained was purified by silica gel column chromatography, using CH_2Cl_2/MeOH (9:1, v/v) as eluent to yield **8c** (1.1 g, 79%) after recrystallization from $CHCl_3$/MeOH: mp 228 °C; ^1H NMR (Me_2SO-d_6) δ 1.24 (t, J = 7 Hz, 3 H, CH_2CH_3), 3.68 (m, 2 H, H-5′), 3.88 (m, 1 H, H-4′), 4.02 (m, 1 H, H-3′), 4.12 (complex m, 3 H, CH_2CH_3, H-2′), 5.10, 5.32, and 5.48 (d, $J_{CH,OH}$ = 6.4 Hz, 1 H each, 3′-OH, 4′-OH, 5′-OH, exchange with deuterium oxide), 5.78 (d, $J_{1',2'}$ = 5.5 Hz, 1 H, H-1′), 6.84 (d, J = 16 Hz, 1 H, CH=C*H*CO_2Et), 7.35 (d, J = 16 Hz, 1 H, C*H*=CHCO$_2$Et), 8.54 (s, 1 H, H-6), 11.7 (s, 1 H, NH, exchanges with deuterium oxide); ^{13}C NMR (Me_2SO-d_6) δ 14.19 (OCH_2CH_3), 59.74 (OCH_2CH_3), 60.21 (C-5′), 69.03 (C-2′), 73.88 (C-3′), 84.69 (C-4′), 88.65 (C-1′), 108.26 (C-5), 116.71 (CH=*C*HCO$_2$Et), 137.61 (*C*H=CHCO$_2$Et), 143.81 (C-6), 149.45 (C-2), 161.67 (C-4), 166.66 (CO_2); MS (EI) m/z 342 (M^+, 1.2). Anal. ($C_{14}H_{18}N_2O_8 \cdot {}^1/_4H_2O$) C, H, N.

(*E*)-5-(2-Carboxyvinyl)uridine (9c). A solution of **8c** (0.62 g, 1.8 mmol) in 0.5 N KOH (30 mL) was stirred at 25 °C for 2 h prior to neutralization with Dowex 50X 8-200 ion-exchange resin (H^+). The mixture was filtered, the solvent was removed in vacuo, and the residue was recrystalized from methanol to yield **9c** as white crystals (0.56 g, 99%): mp 266 °C; ^1H NMR (Me_2SO-d_6) δ 3.68 (m, 2 H, H-5′), 3.88 (m, 1 H, H-4′), 4.06 (complex m, 2 H, H-2′, H-3′), 5.10, 5.33, and 5.50 (m, 1 H each, 2′-OH, 3′-OH, 5′-OH, exchange with deuterium oxide), 5.80 (d, $J_{1',2'}$ = 4.5 Hz, 1 H, H-1′), 6.8 (d, J = 15.6 Hz, 1 H, CH=C*H*CO_2H), 7.30 (d, J = 15.6 Hz, 1 H, C*H*=CHCO$_2$H), 8.50 (s, 1 H, H-6); ^{13}C NMR (Me_2SO-d_6) δ 60.26 (C-5′), 69.06 (C-2′), 74.03 (C-3′), 84.69 (C-4′), 88.37 (C-1′), 109.77 (C-5), 125.96 (CH=*C*HCO$_2$H), 130.82 (*C*H=CHCO$_2$H), 140.07 (C-6), 149.75 (C-2), 161.94 (C-4), 170.23 (CO_2H). Compound **9c** was used immediately in subsequent reactions.

5-Vinyl-2′-fluoro-2′-deoxyuridine (10b). A solution of **9b** (0.24 g, 0.76 mmol) in triethylamine (0.7 mL) and dimethylformamide (6 mL) was heated at 100 °C for 6 h. Removal of the solvent in vacuo gave a residue which was dissolved in methanol (2 mL) prior to adsorption onto silica gel (2 g). The solvent was removed in vacuo and this solid was placed on the top of a silica gel column. Elution with chloroform/methanol (19:1, v/v) yielded **10b** as a white solid (40 mg, 19%): mp 205 °C dec.; ^1H NMR (CD_3OD) δ 3.78 (d, J_{gem} = 12.1 Hz of d, $J_{4',5'}$ = 2.5 Hz, 1 H, H-5′), 3.94–4.05 (m, 2 H, H-4′, H-5′), 4.34 (d, $J_{3',F}$ = 20.6 Hz of d, $J_{3',4'}$ = 4.2 Hz of d, $J_{2',3'}$ = 4.2 Hz of d, $J_{3',OH}$ = 4.2 Hz, 1 H, H-3′), 5.05 (d, $J_{2',F}$ = 53.6 Hz of d, $J_{2',3'}$ = 4.2 Hz of d, $J_{1',2'}$ = 1.8 Hz, 1 H, H-2′), 5.15 (d, J_{cis} = 11.2 Hz of d, J_{gem} = 2.2 Hz, 1 H, CH=C*H*H′), 5.92 (d, J_{trans} = 17.8 Hz of d, J_{gem} = 2.2 Hz, 1 H, CH=CH*H*′), 6.02 (d, $J_{1',F}$ = 16.4 Hz of d, $J_{1',2'}$ = 1.8 Hz, 1 H, H-1′), 6.43 (d, J_{trans} = 17.8 Hz of d, J_{cis} = 11.2 Hz, 1 H, C*H*=CH$_2$), 8.32 (s, 1 H, H-6); ^{13}C NMR (CD_3OD) δ 60.71 (C-5′), 69.08 and 69.31 (d, C-3′), 84.79 (C-4′), 89.54 and 90.00 (d, C-1′), 93.98 and 96.44 (d, C-2′), 113.36 (C-5), 115.37 (CH=*C*H$_2$), 129.09 (*C*H=CH$_2$), 138.82 (C-6), 151.43 (C-2), 164.49 (C-4); mass calcd for $C_{11}H_{13}N_2O_5F$ (HRMS) 272.0805, found 272.0806. Anal. ($C_{11}H_{13}N_2O_5F \cdot CH_3OH$) C, H, N.

5-Vinyluridine (10c). Triethylamine (0.7 mL) was added to a solution of **9c** (0.20 g, 0.64 mmol) in dimethylformamide (5 mL) and water (0.1 mL), and the mixture was heated at 100 °C for 20 h. Removal of the solvent in vacuo gave an oil which was purified by silica gel column chromatography using chloroform/methanol (93:7, v/v) as eluent to yield **10c** (81 mg, 42%): mp 250–255 °C dec (lit.[21] mp 255–260 °C dec); ^1H NMR (CD_3OD) δ 3.75 and 3.88 (two m, 1 H each, H-5′), 4.02 (m, 1 H, H-4′), 4.18 (m, 2 H, H-2′, H-3′), 5.15 (d, J_{cis} = 11 Hz, of d, J_{gem} = 2.2 Hz, 1 H, CH=C*H*H′), 5.92 (d, $J_{1',2'}$ = 3.1 Hz, 1 H, H-1′), 5.95 (d, J_{trans} = 18 Hz of d, J_{gem} = 2.2 Hz, 1 H, CH=CH*H*′), 6.43 (d, J_{trans} = 18 Hz of d, J_{cis} = 11 Hz, 1 H, C*H*=CHH′), 8.28 (s, 1 H, H-6); ^{13}C NMR (CD_3OD) δ 61.99 (C-5′), 71.07 (C-2′), 75.98 (C-3′), 86.31 (C-4′), 91.02 (C-1′), 113.39 (C-5), 115.28 (CH=*C*H$_2$), 129.11 (*C*H=CH$_2$), 139.09 (C-6), 151.82 (C-2).

5-(1-Hydroxy-2-iodoethyl)-2′-deoxyuridine (11a). A solution of **10a** (1.19 g, 4.7 mmol), iodine (0.76 g, 3 mmol), potassium iodate (0.235 g, 1.1 mmol) in water (50 mL), acetonitrile (10 mL), and sulfuric acid (400 μL of 5 N) was maintained at 55 °C for 2 h with stirring. Removal of the solvent in vacuo gave a residue which was purified by silica gel column chromatography using chloroform/methanol (94:6, v/v) as eluent to yield **11a** (1.1 g, 59%) as a white solid after recrystallization from methanol: mp 123 °C dec; ^1H NMR (pyridine-d_5) δ 2.69 (complex m, 2 H, H-2′), 3.72 (d, J_{gem} = 11 Hz of d, J_{vic} = 7.26 Hz, 1 H, CHI), 4.12 (d, J_{gem} = 11 Hz of d, J_{vic} = 2.4 Hz, 1 H, CH′I), 4.24 (complex m, 2 H, H-5′), 4.59 (m, 1 H, H-4′), 4.92–5.23 (complex m, 4 H, 3′-OH, 5′-OH, CHO*H*CH$_2$I, H-3′, hydroxyls exchange with deuterium oxide), 5.34 (d, J_{vic} = 7.26 Hz of d, J_{vic} = 2.4 Hz, 1 H, C*H*OHCH$_2$I), 7.12 (d, J = 6 Hz, of d, J = 6 Hz, 1 H, H-1′), 8.86 (s, 1 H, H-6); ^{13}C NMR (Me_2SO-d_6) δ 14.58 (*C*H$_2$I), 39.41 (C-2′), 61.51 (C-5′), 65.77 (CHOHCH$_2$I), 70.47 (C-3′), 84.17 (C-1′), 87.30 (C-4′), 115.13 (C-5), 137.11 (C-6), 149.99 (C-2), 162.03 (C-4); MS (CIMS, NH_3) m/z 288 (M^+ – HI + NH_4, 5.5) 116 (deoxyribosyl, 24). Microanalyses were obtained for **12a** since **11a** was hygroscopic.

5-(1-Hydroxy-2-iodoethyl)-2′-fluoro-2′-deoxyuridine (11b). A solution of **10b** (54 mg, 0.2 mmol), iodine (25 mg, 0.2 mmol), potassium iodate (12 mg, 0.056 mmol), water (3 mL), and sulfuric acid (15 μL of 5 N) was stirred at 55 °C for 2 h, and then the solvent was removed in vacuo. The residue was purified by silica gel column chromatography using methanol/chloroform (4:96, v/v) as eluent to afford **11b** (60 mg, 72): mp 105 °C dec; ^1H NMR (CD_3OD) (mixture of two diastereomers in a ratio of 1:1) δ 3.42 (m, 1 H, CHI), 3.60 (m, 1 H, CH′I), 3.76 (m, 1 H, H-5′), 3.95 (m, 1 H, H-5′), 4.04 (m, 1 H, H-4′), 4.32 (m, 1 H, H-3′), 4.56 (m, 1 H, C*H*OHCH$_2$I), 5.05 (m, 1 H, H-2′), 6.05 (m, 1 H, H-1′), 8.04 (s, 1 H, H-6); ^{13}C NMR (CD_3OD) δ 12.25 and 13.35 (*C*H$_2$I), 61.46 and 61.51 (C-5′), 67.45 and 68.25 (*C*HOHCH$_2$I), 69.75 and 69.95 (d, C-3′), 84.96 (C-4′), 89.64, 89.82, 90.09, and 90.27 (2 d, C-1′, C-1′ couples with F in each diastereomer), 93.68 and 96.14, and 93.80 and 96.29 (2 d, C-2′), 116.57 (C-5), 139.54 and 139.69 (C-6), 151.73 (C-2), 164.36 (C-4). Anal. ($C_{11}H_{14}N_2O_6FI \cdot H_2O$) C, H, N.

5-(1-Hydroxy-2-iodoethyl)uridine (11c). A solution of **10c** (70 mg, 0.26 mmol), iodine (31 mg, 0.24 mmol), potassium iodate (15 mg, 0.07 mmol), water (2 mL), acetonitrile (2 mL), and sulfuric acid (15 μL of 5 N) was heated at 55 °C with stirring for 30 min and then the solvent was removed in vacuo. The residue was purified by silica gel column chromatography using methanol/chloroform (7:93, v/v) as eluent to yield **11c** as a white solid (70 mg, 65%) after recrystallization from methanol: mp 105 °C dec; ^1H NMR (CD_3OD) (mixture of two diastereomers in a ratio of 1:1) δ 3.46 and 3.65 (2 m, 1 H each, C*H*$_2$I), 3.76 and 3.88 (2 m, 1 H each, H-5′), 4.06 (m, 1 H, H-4′), 4.20 (m, 2 H, H-2′, H-3′), 4.55 (m, 1 H, C*H*OHCH$_2$I), 5.98 (d, $J_{1',2'}$ = 4.6 Hz, 1 H, H-1′), 8.04 (2 s, closely spaced, 1 H total, H-6); ^{13}C NMR (CD_3OD) δ 13.50 (*C*H$_2$I), 62.59 (C-5′), 67.42 (*C*HOHCH$_2$I), 71.49 (C-2′), 75.92 (C-3′), 86.38 (C-4′), 91.05 (C-1′), 116.60 (C-5), 139.72 (C-6). Anal. ($C_{11}H_{15}N_2O_7I$) C, H, N.

5-(1-Methoxy-2-iodoethyl)-2′-deoxyuridine (12a), Method A. A solution of sulfuric acid in methanol (0.4 mL, 5 N) was added to a solution of **11a** (0.143 g, 0.36 mmol) in methanol (15 mL). The reaction was allowed to proceed for 24 h at 25 °C with stirring prior to neutralization with methanolic sodium hydroxide. Removal of the solvent in vacuo, dissolution of the residue in methanol (5 mL), adsorption onto silica gel (2 g), removal of the

(21) Sharma, R. A.; Bobek, M. *J. Org. Chem.* **1975**, *40*, 2377.
(22) "Instruction 271F"; Developmental Therapeutic Program; Division of Cancer Treatment, National Cancer Institute: Bethesda, Maryland, November 1983.

solvent in vacuo and application of this material to the top of a silica gel column followed by elution with chloroform/methanol (97:3, v/v) yielded **12a** (0.14 g, 94%) as a viscous oil. Trituration with cold ether gave **12a** as a white solid: mp 115 °C dec; ^{1}H NMR (Me_2SO-d_6) (mixture of two diastereomers in a ratio of 1:1) δ 2.18 (m, 2 H, H-2′), 3.25 and 3.27 (2 s, 3 H total, OMe), 3.38 (m, 1 H, C*H*I), 3.55 (complex m, 1 H, C*H*′I), 3.6 (m, 2 H, H-5′), 3.85 (m, 1 H, H-4′) 4.17 (m, 1 H, C*H*OCH$_3$), 4.3 (m, 1 H, H-3′), 5.07 and 5.30 (2 br s, 1 H each, 3′-OH, 5′-OH, exchange with deuterium oxide), 6.2 (2 d, overlapping, J = 6 Hz of d, J = 6 Hz, 1 H total, H-1′), 7.86 and 7.90 (2 s, 1 H total, H-6), 11.52 (s, 1 H, NH, exchanges with deuterium oxide); ^{13}C NMR (CD_3OD) δ 8.92 and 10.12 (*C*H$_2$I), 41.60 and 41.49 (C-2′), 57.71 and 57.56 (OMe), 62.86 and 62.98 (C-5′), 72.21 and 72.33 (C-3′), 76.73 and 77.66 (*C*HOMeCH$_2$I), 86.68 and 86.95 (C-1′), 88.98 and 89.07 (C-4′), 113.42 and 113.54 (C-5), 140.05 (C-6), 151.91 (C-2), 164.52 (C-4); MS (EI) m/z 412 (M^+). Anal. ($C_{12}H_{17}N_2O_6I$) C, H, N.

Method B. A solution of iodine monochloride (97.2 mg, 0.6 mmol) in methanol (1 mL) was added to the solution of **10a** (76.2 mg, 0.3 mmol) in methanol (3 mL) and the reaction mixture was heated at 50 °C for 1 h. Removal of the solvent in vacuo gave a residue which was purified by silica gel column chromatography using chloroform/methanol (97:3, v/v) as eluent to yield **12a** (74.2 mg, 60%) as a viscous oil that was a mixture of two diastereomers in a ratio of 1:1 as indicated by ^{1}H NMR (Me_2SO-d_6). The ^{1}H NMR and ^{13}C NMR spectral data were identical with that obtained for **12a** prepared by method A. Trituration with cold ether gave **12a** as a white solid, mp 113–115 °C dec.

5-(1-Methoxy-2-iodoethyl)-2′-fluoro-2′-deoxyuridine (12b). Methylation of **11b** (46 mg, 0.11 mmol) using method A, as described for the synthesis of **12a**, with a reaction time of 36 h and purification of the product by silica gel column chromatography using chloroform/methanol (98:2, v/v) as eluent afforded **12b** as an oil (43 mg, 91%). Trituration with cold ether gave **12b** as a white solid: mp 135–140 °C dec; ^{1}H NMR (CD_3OD) (mixture of two diastereomers in a ratio of 1:1) δ 3.32 and 3.27 (2 s, 3 H total, OMe), 3.40 (m, 1 H, CHI), 3.58 (m, 1 H, CH′I), 3.78 and 3.98 (2 m, 1 H each, H-5′), 4.05 (m, 1 H, H-4′), 4.20 (m, 1 H, C*H*OMe), 4.33 (m, 1 H, H-3′), 5.04 (m, 1 H, H-2′), 6.04 (m, 1 H, H-1′), 8.10 and 8.16 (2 s, 1 H total, H-6); ^{13}C NMR (CD_3OD) δ 8.68 and 9.61 (*C*H$_2$I), 57.59 and 57.80 (O*C*H$_3$), 61.10 and 61.18 (C-5′), 69.51, 69.60, 69.72, and 69.81 (2 d, C-3′ of two diastereomers), 77.81 and 76.97 (*C*HOMe), 84.98 (C-4′), 89.53, 89.85, 90.00, and 90.30 (2 d, C-1′, C-1′ couples with F in each diastereomer), 93.92 and 96.40 (d, C-2′), 113.73 (C-5), 140.11 and 140.17 (C-6), 151.73 (C-2), 164.49 (C-4); MS m/z 430 (M^+, 3). Anal. ($C_{12}H_{16}N_2O_6FI$·H_2O) C, H, N.

5-(1-Methoxy-2-iodoethyl)uridine (12c). A solution of sulfuric acid in methanol (3 drops of 5 N) was added to a solution of **11c** (41 mg, 0.1 mmol) in methanol (10 mL), the reaction was completed, and the product was purified, by using the procedure described for **12b**, to yield **12c** as a viscous oil (35 mg, 81%): ^{1}H NMR (CD_3OD) (mixture of two diastereomers in a ratio of 1:1) δ 3.32 and 3.34 (2 s, 3 H total, OMe), 3.40 (m, 1 H, CHI), 3.60 (m, 1 H, CH′I), 3.78 and 3.88 (2 m, 1 H each, H-5′), 4.06 (m, 1 H, H-4′), 4.20 (m, 3 H, H-2′, H-3′, C*H*OMe), 5.96 (m, 1 H, H-1′), 8.08 and 8.14 (2 s, 1 H total, H-6); ^{13}C NMR (CD_3OD) δ 8.71 and 9.81 (*C*H$_2$I), 57.53 and 57.74 (O*C*H$_3$), 62.26 (C-5′), 71.37 and 71.43 (C-2′), 76.07 (C-3′), 76.85 and 77.78 (*C*HOMe), 86.31 and 86.40 (C-4′), 91.10 (C-1′), 113.46 (C-5), 140.29 and 140.41 (C-6), 152.15 (C-2). Anal. ($C_{12}H_{17}N_2O_7I$·$^3/_2H_2O$) C, H, N.

5-(1-Hydroxy-2-methoxyethyl)-2′-deoxyuridine (13) and 2,3-Dihydro-3-hydroxy-5-(2′-deoxy-β-D-ribofuranosyl)-furano[2,3-*d*]pyrimidin-6-(5*H*)-one (14). Sodium carbonate (0.65 g, 6.1 mmol) was added to a solution of **11a** (0.50 g, 1.25 mmol) in methanol (200 mL), and the reaction was allowed to proceed for 6 h at 25 °C with stirring. Removal of the solvent in vacuo and purification of the residue obtained by silica gel column chromatography using chloroform/methanol (93:7, v/v) as eluent yielded **13** (100 mg, 26%) as a viscous oil. Trituration with cold ether gave **13** as a white solid (hygroscopic): mp 192 °C dec; ^{1}H NMR (Me_2SO-d_6), (mixture of two diastereomers in a ratio of 1:1) δ 2.14 (m, 2 H, H-2′), 3.27 and 3.28 (2 s, 3 H total, OMe), 3.42 (complex m, 1 H, C*H*OCH$_3$), 3.53 (complex m, 1 H, C*H*′OCH$_3$), 3.62 (m, 2 H, H-5′), 3.84 (m, 1 H, H-4′), 4.16 (m, 1 H, C*H*OHCH$_2$OCH$_3$), 4.28 (m, 1 H, H-3′), 4.78 (m, 1 H, CHO*H*, exchanges with deuterium oxide), 5.05 and 5.32 (2 br s, 1 H each, 3′-OH, 5′-OH, exchange with deuterium oxide), 6.20 (2 d, overlapping, J = 6 Hz of d, J = 6 Hz, 1 H total, H-1′), 7.76 and 7.79 (2 s, 1 H total, H-6), 11.14 (br s, 1 H, NH, exchanges with deuterium oxide); ^{13}C NMR (CD_3OD) δ 41.23 and 41.48 (C-2′), 57.29 (O*C*H$_3$), 62.83 and 62.92 (C-5′), 64.63 (*C*H$_2$OCH$_3$), 72.21 and 72.30 (C-3′), 78.86 and 79.04 (*C*HOH), 86.85 (C-1′), 88.85 (C-4′), 112.21 (C-5), 140.11 and 140.23 (C-6), 152.0 (C-2), 165.04 (C-4). Anal. For the 3′,5′-dibenzoate of **13** ($C_{26}H_{26}N_2O_9$·$^1/_2H_2O$) C, H, N.

Further elution, using chloroform/methanol (93:7, v/v) as eluent, yielded **14** (60 mg, 17.5%) as a white solid after recrystallization from methanol: mp 220 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.99 and 2.3 (2 m, 1 H each, H-2′), 3.63 (m, 2 H, H-5′), 3.90 (m, 1 H, H-4′), 4.23 (m, 1 H, H-3′), 4.36 (d, J_{gem} = 10.18 Hz of d, J_{vic} = 2.71 Hz, 1 H, furanyl CHCH*H*′), 4.71 (J_{gem} = 10.18 Hz of d, J_{vic} = 5.75 Hz, 1 H, furanyl CHC*H*H′), 5.14 (m, 2 H, furanyl C*H*OH, OH, hydroxyl exchanges with deuterium oxide), 5.29 (d, $J_{CH,OH}$ = 4.5 Hz, 1 H, OH, exchanges with deuterium oxide), 5.82 (d, $J_{CH,OH}$ = 6 Hz, 1 H, OH, exchanges with deuterium oxide), 6.14 (d, J = 6 Hz of d, J = 6 Hz, 1 H total, H-1′), 8.42 (s, 1 H, H-6); ^{13}C NMR (CD_3OD/D_2O) δ 41.96 (C-2′), 62.29 (C-5′), 67.62 (C-3), 71.49 (C-3′), 82.15 (C-2), 88.89 and 88.83 (C-4′ and C-1′), 109.53 (C-3a), 142.71 (C-4), 158.44 (C-6); MS (CIMS, isobutane) m/z 271 (M^+ + H). Anal. ($C_{11}H_{14}O_6N_2$·$^1/_4H_2O$) C, H, N.

5-(1-Hydroxy-2,2-diiodoethyl)-2′-deoxyuridine (16). A mixture of **4b** (0.151 g, 0.4 mmol), iodine (53.34 mg, 0.21 mmol), potassium iodate (23.54 mg, 0.11 mmol), glacial acetic acid (30 μL), and dioxane/water (3:7, v/v) (7 mL) was allowed to stir at 25 °C for 24 h. Removal of the solvent in vacuo and purification of the product by elution from a silica gel column using chloroform/methanol (94:6, v/v) as eluent yielded **16** as a white solid (41 mg, 20%): mp 73 °C (sublimation); ^{1}H NMR (Me_2SO-d_6) (mixture of two diastereomers in a ratio of 1:1) δ 2.12 (m, 2 H, H-2′), 3.58 (m, 2 H, H-5′), 3.84 (m, 1 H, H-4′), 4.28 (m, 2 H, H-3′, C*H*OHCHI$_2$), 5.0 and 5.31 (2 m, 1 H each, 3′-OH, 5′-OH, exchange with deuterium oxide), 5.46 (m, 1 H, C*H*I$_2$), 6.2 (m, 1 H, H-1′), 6.37 (m, 1 H, CHO*H*, exchanges with deuterium oxide), 7.88 and 7.84 (2 s, 1 H total, H-6), 11.54 (s, 1 H, NH, exchanges with deuterium oxide); ^{13}C NMR δ −9.44 (*C*HI$_2$), 39.51 (C-2′), 60.67 and 61.51 (C-5′), 70.44 and 70.64 (C-3′), 71.63 (*C*HOHCHI$_2$), 84.24 (C-1′), 87.44 and 87.90 (C-4′), 114.64 (C-5), 138.45 (C-6), 149.78 (C-2), 161.85 (C-4). Anal. For the 5′-benzoate of **16** ($C_{18}H_{18}N_2O_7I_2$·$^3/_4H_2O$) C, H, N.

In Vitro Antiviral Assay (HSV-1). Monolayers of mycoplasma-free vero cells in 60-mm plates were infected with HSV-1 (strain JLJ isolated from a patient with herpes simplex encephalitis). After a 1-h adsorption period at 37 °C, the cells were overlaid with 1% agarose in Eagle minimum essential medium (E-MEM) containing 2% fetal bovine serum and known concentrations (0.1, 1.0, and 10 μg/mL) of the agents being tested and 0.01, 0.1, and 1 μg/mL for acyclovir. Cells were stained with neutral red after incubation for 4 days at 37 °C in 5% CO_2 incubator, and the number of plaques were counted. The experiment was performed once. The concentration of test compound required to inhibit plaque formation by 50% (ID_{50}) was determined.

In Vitro L1210 Cytotoxic Assay. Mouse L1210 leukemia cells were cultivated as a suspension in Fischer's medium supplemented with 10% heat-inactivated horse serum and incubated at 37 °C in a humidified 5% CO_2 atmosphere to prepare a cell stock solution. The number of cells/mL of medium was determined with a Model ZF Coulter counter 48 h after incubation. The test compound was dissolved in ethanol/water (1:1, v/v; **11a**), water (**12a**, **13**), or ethanol (**10b**, **11b,c**, **12b,c**, **16**), and 10 μL of this solution was added to test wells containing 2 mL of suspended L1210 cells (10^5 cells/mL) such that 2 mL of the cell suspension had a test compound concentration of 50, 10, and 1 μg/mL of medium, respectively. Control wells were identical, except that the test compound was absent. Compounds for which ED_{50} values were obtained (**11a**, **12a**, **13**, **16**) had the following test compound concentrations (μg/mL of medium); 50, 25, 10, 5.0, 2.5, and 1. The concentrations of melphalan were 10, 1, 0.5, 0.25, 0.1, and 0.05 μg/mL of medium. All tests and controls were grown in triplicate. The percent cell survival was calculated by using the following formula: % survival = $(T_{48} - T_0)/(C_{48} - C_0) \times 100$, where T_{48} is the number of living cells/mL for each test drug concentration

at 48 h, T_0 is the cell number for test wells at time zero (normally 10^5), C_{48} is the number for the control at 48 h, and C_0 is the number for the control at time zero (normally, $T_0 = C_0 = 10^5$ cells/mL). Compounds exhibiting an $ED_{50} > 5$ μg/mL are considered to be inactive in this screen.

In Vivo P388 Screen. Compounds were tested for their ability to prolong the survival of $B_6D_2F_1$ mice (six per group) receiving an implant of P388 mouse leukemia cells (10^6 cells in 0.2 mL saline) ip on Day 0. The test sample was constituted in saline/ethanol (6:1, v/v) and injected ip (20 mg/kg) on Days 1–5 (0.2 mL). The mice were observed until death, and the mean survival times were compared to those of the controls receiving the same number of cells at Day 0 and sterile saline (0.2 mL) for the five following days. Significant in vivo antineoplastic activity is ascribed to compounds exhibiting a %T/C > 125.

Transport Studies. The transport of 2′-deoxyuridine (DU), (*E*)-5-(2-iodovinyl)-2′-deoxyuridine (**4b**), **11a**, **12a**, **13**, **14**, and **16** were determined with fresh murine erythrocytes exposed to various concentrations of extracellular test nucleoside and [6-^{3}H]thymidine. The concentration of [6-^{3}H]thymidine in the extracellular fluid at various times after this exposure was determined by counting aliquots by liquid-scintillation counting on a Beckman LS9000 or Searle Mark III counter. These procedures and the interpretation of results have been described in detail elsewhere.[17,18] The results are summarized in Table I.

Acknowledgment. We are grateful to the Medical Research Council of Canada (Grant MT-5965) (L.I.W. and E.E.K.) and the National Cancer Institute of Canada (T.M.A.) for financial support of this work, and to the Alberta Heritage Foundation for Medical Research for a postdoctoral fellowship (R.K.) and graduate studentship (L.X.).

Registry No. **4b**, 69304-48-9; **7c**, 1024-99-3; **8c**, 123881-85-6; **9b**, 121563-64-2; **9c**, 99394-52-2; **10a**, 55520-67-7; **10b**, 123881-86-7; **10c**, 55520-64-4; (*R*)-**11a**, 123881-87-8; (*S*)-**11a**, 123881-88-9; (*R*)-**11b**, 123881-89-0; (*S*)-**11b**, 123881-90-3; (*R*)-**11c**, 123881-91-4; (*S*)-**11c**, 123881-92-5; (*R*)-**12a**, 123881-93-6; (*S*)-**12a**, 123881-94-7; (*R*)-**12b**, 123881-95-8; (*S*)-**12b**, 123881-96-9; (*R*)-**12c**, 123881-97-0; (*S*)-**12c**, 123881-98-1; (*R*)-**13**, 123881-99-2; (*R*)-**13** 3′,5′-dibenzoate, 123882-03-1; (*S*)-**13**, 123882-00-8; (*S*)-**13** 3′,5′-dibenzoate, 123882-04-2; (*R*)-**14**, 123882-01-9; (*S*)-**14**, 123882-02-0; (*R*)-**16**, 123882-05-3; (*S*)-**16**, 123882-06-4; ethyl acrylate, 140-88-5.

Synthesis and Inhibitory Potency of Peptides Corresponding to the Subunit 2 C-Terminal Region of Herpes Virus Ribonucleotide Reductases[1]

Pierrette Gaudreau,*,† Hélène Paradis,‡ Yves Langelier,‡ and Paul Brazeau†

Neuroendocrinology Laboratory, Rm M-5218, Notre-Dame Hospital Research Center, and Institut du Cancer de Montréal, Notre-Dame Hospital, 1560 Sherbrooke Street East, Montréal, Canada H2L 4M1. *Received May 5, 1989*

H-Tyr^{329}-Ala^{330}-Gly^{331}-Ala^{332}-Val^{333}-Val^{334}-Asn^{335}-Asp^{336}-Leu^{337}-OH, the C-terminal end of herpes simplex virus ribonucleotide reductase subunit 2 (HSV R2), specifically inhibits viral enzyme activity by interacting with subunit 1 (HSV R1). In a previous structure–activity study, we identified four sites on the nonapeptide where the inhibitory potency could be modulated: a minimum active core 333–337, a spacer segment 330–332, and the N- and C-termini. To further explore the structural features of HSV R2-(329–337) that are required to obtain a potent inhibition, a series of analogues comprising modifications in these four regions were synthesized by solid-phase methodology. Changes in the segment 333–337 of the molecule decreased the inhibitory potency by more than 2-fold, except for the Ile^{334} substitution, which resulted in a 1.5-fold increase in potency. Replacement of Tyr^{329} by other aromatic or aliphatic amino acids diminished the nonapeptide activity from 1.4-fold to 5.9-fold. The spacer segment contributed to enhance potency. Modification with amino acids that could induce conformational changes, such as Pro or D-Ala, generated compounds with a similar or lower activity, respectively. Amidation or amino acyl addition at the carboxylic end was detrimental while acylation of the N-terminus was generally beneficial for the inhibitory potency. Disubstitution in position 332 and 334 by Thr and Ile, which are present in the C-terminal portion of varicella-zoster virus ribonucleotide reductase subunit 2, resulted in a peptide that is 4.0 times more potent than HSV R2-(329-337), while each monosubstitution alone generated peptides with 150% of the activity of HSV R2-(329-337) nonapeptide. These results indicate a synergistic effect of the disubstitution which confers to this analogue physicochemical properties enhancing its ability to interact with its R1 binding site.

Numerous herpesviruses, such as herpes simplex types I and II (HSV-1 and HSV-2), Epstein–Barr virus (EBV), varicella-zoster virus (VZV), pseudorabies virus (PRV), and equine herpesvirus type I (EHV-1), induce ribonucleotide reductase (RR) activities.[2-7] This enzyme, formed by the association of two nonidentical subunits (R1 and R2), catalyzes the reduction of ribonucleoside diphosphates to their deoxy forms and plays an important role in viral replication.[8-10] Cohen et al.[11,12] and Dutia et al.[13] demonstrated that a synthetic nonapeptide, H-Tyr^{329}-Ala^{330}-Gly^{331}-Ala^{332}-Val^{333}-Val^{334}-Asn^{335}-Asp^{336}-Leu^{337}-OH (HSV R2-(329–337)), corresponding to the RR enzyme's subunit 2 carboxyl terminus, specifically and reversibly inhibits HSV-1, HSV-2, and PRV RR activities. Studies on the mechanism of action of HSV R2-(329–337) have shown that it impedes binding of the RR subunits by interacting with R1,[14,15] thus impairing enzymatic activity. Previously, we determined the minimum active segment of this pep-

* Author to whom correspondence should be sent.
† Notre-Dame Hospital Research Center.
‡ Institut du Cancer de Montréal.

(1) Symbols and abbreviations are in accord with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (Eur. J. Biochem. 1984, 158, 9). All optically active amino acids are of the L configuration, unless otherwise specified. Additional abbreviations used are: Ac, acetyl; β-Ala, 3-aminopropionic acid; desamino-Tyr, 3-(4-hydroxyphenyl)-propionic acid; OMe, methoxy.
(2) Cohen, G. H. *J. Virol.* **1972**, *9*, 408.
(3) Cohen, G. H.; Factor, M. N.; Ponce De Leon, M. J. *J. Virol.* **1974**, *41*, 893.
(4) Lankinen, H.; Graslund, A.; Thelander, L. *J. Virol.* **1982**, *41*, 893.
(5) Cohen, J. C.; Henry, B. E.; Randall, C. C.; O'Callaghan, D. J. *Proc. Soc. Exp. Biol. Med.* **1977**, *155*, 395.
(6) Henry, B. E.; Glaser, R.; Hewetson, J.; O'Callaghan, D. J. *J. Virol.* **1978**, *89*, 262.
(7) Davidson, A. J.; Scott, J. E. *J. Gen. Virol.* **1986**, *67*, 1759.
(8) Preston, V. G.; Palfreyman, J. W.; Dutia, B. M. *J. Gen. Virol.* **1984**, *66*, 1581.

0022-2623/90/1833-0723$02.50/0