4f, 130798-51-5; **4g**, 139896-76-7; **4h**, 132004-34-3; **4i**, 139896-77-8; **4j**, 139896-78-9; **4k**, 132004-30-9; **4l**, 139896-79-0; **4m**, 132004-35-4; **4n**, 132004-36-5; **4o**, 132004-32-1; **4p**, 132004-33-2; **4q**, 139896-80-3; **4r**, 139896-81-4; **4s**, 139896-82-5; **4t**, 139896-83-6; **4u**, 139896-84-7; **7b**, 130798-57-1; **7d**, 130798-58-2; **7f**, 130829-27-5; **7g**, 139896-86-9; **7m**, 139896-87-0; **7n**, 139896-88-1; **7r**, 139896-89-2; **7s**, 139896-90-5; **8**, 101861-63-6; **10**, 67976-94-7; **11**, 15733-83-2; **12**, 139896-91-6; **13**, 139896-92-7; **14**, 139896-93-8; **16b**, 53995-82-7; **17a**, 91348-45-7; **17b**, 137836-40-9; **15**, 139896-85-8; **18a**, 137836-30-7; **18b**, 137836-28-3; **19a**, 137836-31-8; **19b**, 137836-29-4; **20**, 1477-50-5; **21**, 123158-59-8; *m*-chloroaniline, 108-42-9; 4,6-dichloroaniline, 626-43-7; 3-nitroaniline, 99-09-2; 2-carboxymethoxy-4-hydroxyquinoline, 5965-59-3; methyl acrylate, 96-33-3; ethyl 2-mercaptoacetate, 623-51-8.

Synthesis and Antiviral Activity of 1-Cyclobutyl-5-(2-bromovinyl)uracil Nucleoside Analogues and Related Compounds

William A. Slusarchyk,* Gregory S. Bisacchi, A. Kirk Field, Deborah R. Hockstein, Glenn A. Jacobs, Bridget McGeever-Rubin, Joseph A. Tino, A. V. Tuomari, Gregory A. Yamanaka, Marian G. Young, and Robert Zahler

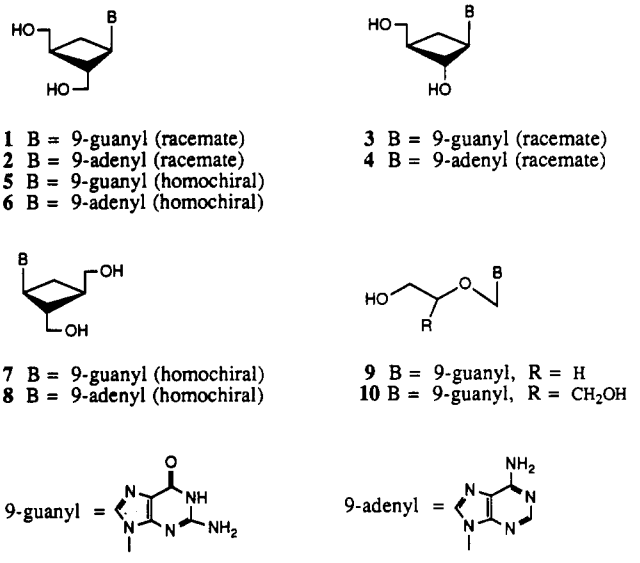
Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 08543-4000.
Received June 24, 1991

A series of racemic (1α(*E*),2β,3α)-1-[2,3-bis(hydroxymethyl)cyclobutyl]-5-(2-halovinyl)uracils was synthesized and evaluated in cell culture. The bromovinyl, iodovinyl, and chlorovinyl analogues, **13**, **15**, and **16**, respectively, are all potent inhibitors of varicella zoster virus (VZV), but are less inhibitory to the replication of human cytomegalovirus (HCMV) and herpes simplex viruses 1 and 2 (HSV-1, HSV-2). The excellent anti-VZV activities of **13**, **15**, and **16** coupled with their virtual inability to inhibit WI-38 cell growth indicate high in vitro therapeutic indices. VZV thymidine kinase readily converts these compounds to their respective monophosphates but not to their corresponding diphosphates. Compound **13a**, the (1′*R*) enantiomer of the bromovinyl analogue **13**, was also synthesized, and its potency is comparable to that of the racemate. A lower homologue **14**, (1α(*E*),2β,3α)-1-[2-hydroxy-3-(hydroxymethyl)cyclobutyl]-5-(2-bromovinyl)uracil, was found to be inactive against VZV, HCMV, HSV-1, and HSV-2.

Introduction

We recently reported the synthesis and antiherpes activity of the 9-guanyl and 9-adenyl cyclobutyl nucleoside analogues **1**–**8** (Chart I).[1] The racemates **1** and **2**, and the corresponding lower homologue racemates **3** and **4**, are all potent inhibitors of a broad spectrum of herpesviruses, including herpes simplex virus 1 and 2 (HSV-1, HSV-2), varicella zoster virus (VZV), and human cytomegalovirus (HCMV). The guanine analogue **1** exhibits in vitro antiherpes activity superior to that of acyclovir (**9**)[2] and comparable to that of ganciclovir (**10**).[2c] The lower homologue **3** displays activity equivalent to that of acyclovir against HSV-1, HSV-2, and VZV, and is comparable to ganciclovir against HCMV. The enantiomers of "natural" configuration (**5** and **6**) have activity equal to or greater than that

Chart I

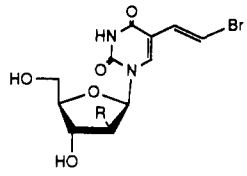


(1) (a) Slusarchyk, W. A.; Young, M. G.; Bisacchi, G. S.; Hockstein, D. R.; Zahler, R. Synthesis of SQ-33,054, a Novel Cyclobutane Nucleoside With Potent Antiviral Activity. *Tetrahedron Lett.* **1989**, *30*, 6453–6456. (b) Slusarchyk, W. A.; Bisacchi, G. S.; Hockstein, D. R.; Young, M. G.; Field, A. K.; McGeever-Rubin, B.; Tuomari, A. V.; Zahler, R. SQ-33,054: A Potent Member of a New Class of Nucleoside-Analog Antivirals. *29th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Houston, TX, 1989; Abstract 1330. (c) Field, A. K.; Tuomari, A. V.; McGeever-Rubin, B.; Terry, B. J.; Mazina, K. E.; Haffey, M. L.; Hagen, M. E.; Clark, J. M.; Braitman, A.; Slusarchyk, W. A.; Young, M. G.; Zahler, R. (±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)cyclobutyl]guanine [(±)-BHCG or SQ33054]: a Potent and Selective Inhibitor of Herpesviruses. *Antiviral Res.* **1990**, *13*, 41–52. (d) Jacobs, G. A.; Tino, J. A.; Zahler, R. Synthesis of SQ-32,829, A New Nucleoside Antiviral Agent. *Tetrahedron Lett.* **1989**, *30*, 6955–6958. (e) Jacobs, G. A.; Slusarchyk, W. A.; Spergel, S. H.; Tino, J. A.; Field, A. K.; Tuomari, A. V.; Zahler, R. *In Vitro* Activity of SQ-32,829, a New Nucleoside-Analog Antiviral Agent. *29th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Houston, TX, 1989; Abstract 1328. (f) Zahler, R.; Jacobs, G. A. Purinyl and Pyrimidinyl Cyclobutanes and Their Use As Antiviral Agents. U.S. Patent 4,918,075, 1990. (g) Bisacchi, G. S.; Braitman, A.; Cianci, C. W.; Clark, J. M.; Field, A. K.; Hagen, M. E.; Hockstein, D. R.; Malley, M. R.; Mitt, T.; Slusarchyk, W. A.; Sundeen, J. E.; Terry, B. H.; Tuomari, A. V.; Weaver, E. R.; Young, M. G.; Zahler, R. Synthesis and Antiviral Activity of Enantiomeric Forms of Cyclobutyl Nucleoside Analogues. *J. Med. Chem.* **1991**, *34*, 1415–1421.

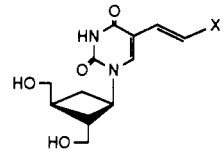
(2) (a) Thiers, B. H. Acyclovir in the Treatment of Herpesvirus Infections. *Dermatol. Clin.* **1990**, *8*, 583–587. (b) O'Brien, J. J.; Campoli-Richards, D. M. Acyclovir An Updated Review of its Antiviral Activity, Pharmacokinetic Properties and Therapeutic Efficacy. *Drugs* **1989**, *37*, 233–309. (c) Reines, E. D.; Gross, P. A. Antiviral Agents. *Med. Clin. North Am.* **1988**, *72*, 691–715.

0022-2623/92/1835-1799$03.00/0

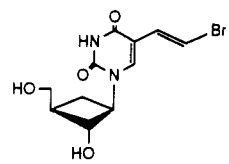
Chart II



11 R = H
12 R = OH



13 X = Br (racemate)
13a X = Br (1'R)
15 X = I (racemate)
16 X = Cl (racemate)



14 (racemate)

of the corresponding racemates (1 and 2), whereas the enantiomers of "unnatural" configuration (7 and 8) are devoid of activity.[1g] Several other laboratories have independently reported the syntheses and properties of compounds 1–7.[3]

We now report the synthesis and antiviral activities of the corresponding 5-(bromovinyl)uracil analogues, 13 and 14 (Chart II). The 5-(bromovinyl)uracil moiety is found in BVDU (11) and BVaraU (12), and both compounds are potent and selective inhibitors of HSV-1 and VZV in cell culture.[4] BVaraU is also highly effective in vivo against HSV-1[4f,5a,b] and simian varicella virus (SVV),[5c] and it is currently undergoing clinical evaluation for the treatment of VZV.[5d]. Similarly, BVDU is effective in the oral treatment of VZV in immunocompromised patients and the topical treatment of clinical HSV-1 keratitis;[6] however, the utility of BVDU in therapy may be limited. Pyrimidine nucleoside phosphorylases in infected cell culture or isolated from human blood platelets rapidly degrade BVDU by cleaving the glycoside linkage between the uracil group and the ribose moiety.[7] Enzymatic cleavage should not occur between the uracil and cyclobutane rings in compounds 13 and 14 because of the absence of a glycosidic linkage. Racemic compounds 13[8] and 14 were evaluated,

(3) (a) Norbeck, D. W.; Kern, E.; Hayashi, S.; Rosenbrook, W.; Sham, H.; Herrin, T.; Plattner, J. J.; Erickson, J.; Clement, J.; Swanson, R.; Shipkowitz, N.; Hardy, D.; Marsh, K.; Arnett, G.; Shannon, W.; Broder, S.; Mitsuya, H., Cyclobut-A and Cyclobut-G: Broad-Spectrum Antiviral Agents with Potential Utility for the Therapy of AIDs. *J. Med. Chem.* **1990**, *33*, 1281–1285. (b) Honjo, M.; Maruyama, T.; Sato, Y.; Horii, T. Synthesis of the Carbocyclic Analogue of Oxetanocin A. *Chem. Pharm. Bull.* **1989**, *37*, 1413–1415. (c) Katagiri, N.; Sato, H.; Kaneko, C. Highly Stereoselective Synthesis of Carbocyclic Analogues of Oxetanocin. *Chem. Pharm. Bull.* **1990**, *38*, 288–290. (d) Ichikawa, Y.; Narita, A.; Shiozawa, A.; Hayashi, Y.; Narasaka, K. Enantio- and Diastereo-selective Synthesis of Carbocyclic Oxetanocin Analogues. *J. Chem. Soc., Chem. Commun.* **1989**, 1919–1921. (e) Nishiyama, Y.; Yamamoto, N.; Yamada, Y.; Daikoku, T.; Ichikawa, Y.; Takahashi, K. Anti-Herpesvirus Activity of Carbocyclic Oxetanocin G *In Vitro*. *J. Antibiot.* **1989**, *42*, 1854–1859. (f) Norbeck, D. W.; Plattner, J. J.; Rosen, T. J.; Pariza, R. J.; Sowin, T. J.; Garmaise, D. L.; Hannick, S. M. New N-Cyclobutyl Analogues of Pyrimidine Nucleoside(s) With Antiviral and Antitumour Activities, and New Process Intermediates. Eur. Pat. Appl. EP 366059, 1990. (g) Hsiao, C.; Hannick, S. M. Efficient Synthesis of Protected (2S,3S)-2,3-Bis(hydroxymethyl)cyclobutanone, Key Intermediates for the Synthesis of Chiral Carbocyclic Analogues of Oxetanocin. *Tetrahedron Lett.* **1990**, *46*, 6609–6612. (h) Ichikawa, Y.; Yamazaki, M.; Matsuo, K.; Aoyama, K.; Matsumura, F.; Nishiyama, Y.; Matsubara, K.; Nagahata, T.; Hoshino, H.; Seki, J. New 1-Hydroxymethyl-4-pyrimidinyl or Purinyl Cyclobutanone Derivatives with Antiviral and Antitumour Activities, and New Epoxide Intermediates, Eur. Pat. Appl. 330992, 1989. (i) Kohlbrenner, W. E.; Carter, C. D.; Fesik, S. W.; Norbeck, D. W.; Erickson, J. Efficiency of Phosphorylation of the Cyclobut-G (A-69992) Enantiomers by HSV-1 Thymidine Kinase Does Not Correlate With Their Anti-Herpesvirus Activity, *Biochem. Pharmacol.* **1990**, *40*, R5-R10.

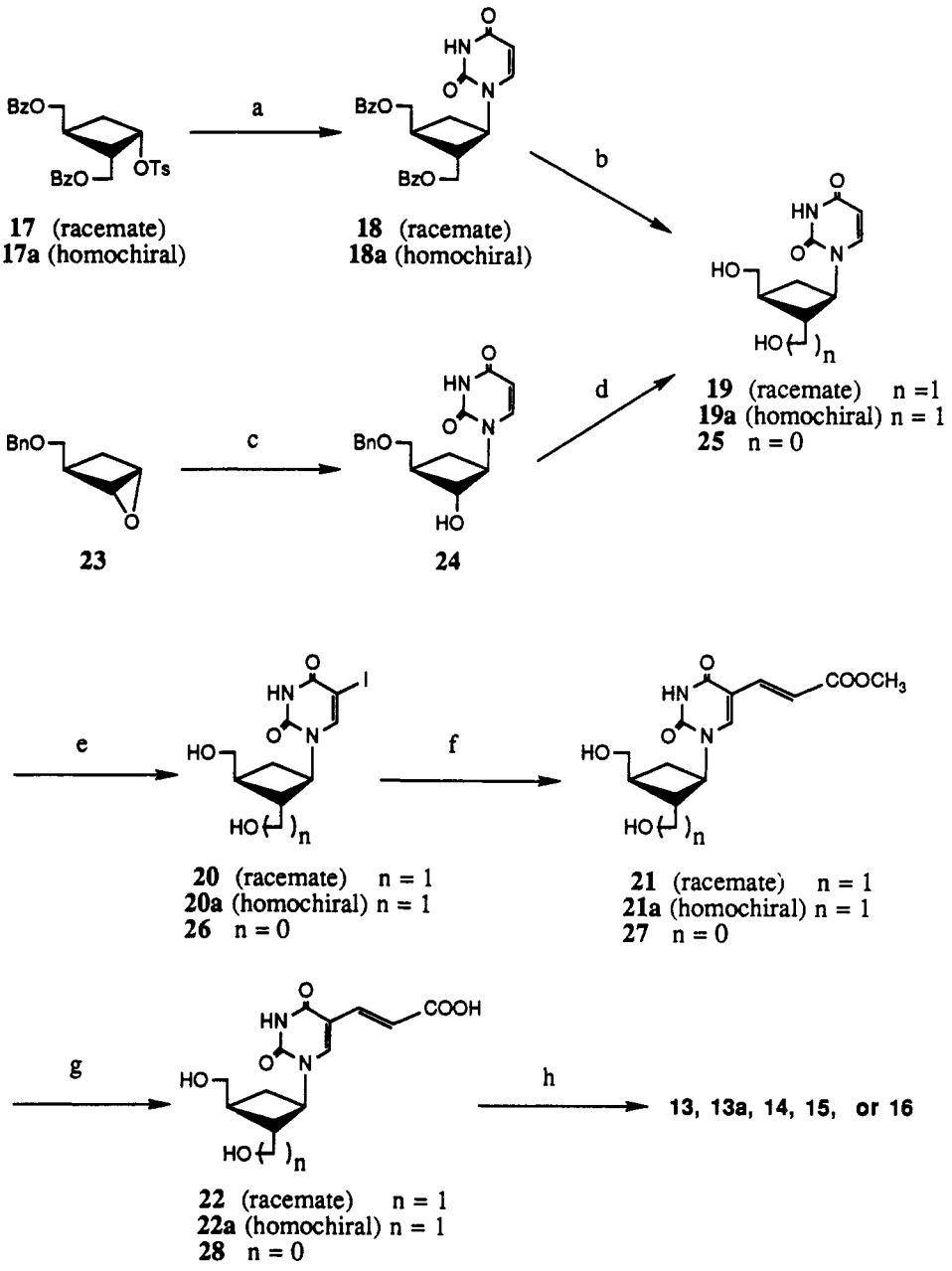
(4) (a) De Clercq, E.; Descamps, J.; De Somer, P.; Barr, P. J.; Jones, A. S.; Walker, R. T. (E)-5-(2-Bromovinyl)-2'-deoxyuridine: A Potent and Selective Anti-herpes Agent. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2947–2951. (b) De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R. T.; Jones, A. S.; Torrence, P. F.; Sugar, D. Comparative Efficacy of Antiherpes Drugs Against Different Strains of Herpes Simplex Virus. *J. Infect. Dis.* **1980**, *141*, 563–574. (c) Shigeta, S.; Yuokota, T.; Iwabucki, T.; Baba, M.; Konno, K.; Ogata, M.; De Clercq, E. Comparative Efficacy of Antiherpes Drugs Against Various Strains of Varicella-Zoster Virus. *J. Infect. Dis.* **1983**, *147*, 576–584. (d) Machida, H.; Sakata, S.; Kuninaka, A.; Yoshino, H. Antiherpesviral and Anticellular Effects of 1-β-D-Arabinofuranoyl-E-5-(2-Halogenovinyl)uracils *Antimicrob. Agents Chemother.* **1981**, *20*, 47–52. (e) Machida, H.; Kuninaka, A.; Yoshino, H. Inhibitory Effects of Antiherpesviral Thymidine Analogs Against Varicella-Zoster Virus. *Antimicrob. Agents Chemother.* **1982**, *21*, 358–361. (f) Machida, H.; Sakata, S. In Vitro and In Vivo Antiviral Activity of 1-β-D-Arabinofuranosyl-E-5-(2-bromovinyl)uracil (BV-araU) and Related Compounds. *Antiviral Res.* **1984**, *4*, 135–141. (g) Machida, H. Comparison of Susceptibilities of Varicella-Zoster Virus and Herpes Simplex Viruses to Nucleoside Analogs. *Antimicrob. Agents Chemother.* **1986**, *29*, 524–526.

(5) (a) Topke, H.; Graf, M.; Wutzler, P.; Herrmann, G.; Reefschlager, Evaluation of (E)-5-(2-Bromovinyl)- and 5-Vinyl-1-β-D-arabinofuranosyluracil (BrVaraU, VaraU) in the Treatment of Experimental Herpes Simplex Virus Type 1 Keratitis in Rabbits: Comparison With (E)-5-(2-Bromovinyl)-2'-deoxyuridine (BrVUdR). *J. Antiviral Res.* **1988**, *19*, 273–280. (b) Machida, H.; Ikeda, T.; Ashida, N. Comparison of Antiviral Efficacies of 1-β-D-Arabinofuranosyl-E-5-(2-Bromovinyl)uracil (Brovavir) and Acyclovir Against Herpes Simplex Virus Type 1 Infections in Mice. *Antiviral Res.* **1990**, *14*, 99–108. (c) Soike, K.; Huang, J.; Tu, J-I.; Stouffer, B.; Swerdel, M.; Olsen, S.; Bonner, D. P.; Tuomari, A. V.; Field, A. K. Oral Bioavailability and Anti-Simian Varicella Efficacy of BVaraU in Monkeys. *30th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Atlanta, GA, 1990; Abstract 1102. (d) Sherman, J.; Devault, A.; Natarajan, C.; Harkins, J.; Hedden, B.; Stouffer, B.; Whigan, D.; Kassalow, L.; Grasela, D.; Sugarman, A.; Reilly, K. SQ32,756 (BV-araU): Characteristics and Pharmacokinetics in Healthy Young and Elderly Male Volunteers. *30th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Atlanta, GA, 1990; Abstract 234.

(6) (a) De Clercq, E.; Degreef, H.; Wildiers, J.; De Jonge, G.; Drochmans, A.; Descamps, J.; De Somer, P. Oral (E)-5-(2-Bromovinyl)-2'-deoxyuridine in Severe Herpes Zoster. *Br. Med. J.* **1980**, *281*, 1178–1179. (b) Maudgel, P. C.; De Clercq, E.; Missotten, L. Efficacy of Bromovinyldeoxyuridine in the Treatment of Herpes Simplex Virus and Varicella-Zoster Virus Eye Infections. *Antiviral Res.* **1984**, *4*, 281–291.

(7) (a) Ayisi, N. K.; Wall, R. A.; Wanklin, J.; Machida, H.; De Clercq, E.; Sacks, S. L. Comparative Metabolism of E-5-(2-Bromovinyl)-2'-deoxyuridine and 1-β-D-Arabinofuranosyl-E-5-(2-bromovinyl)uracil in Herpes Simplex Virus-Infected Cells. *Mol. Pharmacol.* **1987**, *31*, 422–429. (b) Desgranges, C.; Razaka, G.; Rabaud, M.; Bricaud, H.; Balzarini, J.; De Clercq, E. Phosphorolysis of (E)-5-(2-Bromovinyl)-2'-deoxyuridine (BVDU) and Other 5-Substituted-2'-deoxyuridines By Purified Human Thymidine Phosphorylase and Intact Blood Platelets. *Biochem. Pharmacol.* **1983**, *32*, 3583–3590.

Scheme I.[a]



[a] (a) Uracil, K_2CO_3, 18-crown-6, DMSO; or uracil tetrabutylammonium salt, DMF; (b) $NaOCH_3$, CH_3OH; (c) uracil, NaH, 18-crown-6, sulfolane; (d) $Pd(OH)_2/C$, cyclohexene, EtOH; (e) I_2, aqueous HNO_3, dioxane; (f) $Pd(OAc)_2$, Et_3N, Ph_3P, methyl acrylate, dioxane; (g) 2 N KOH; (h) NBS, $KHCO_3$, DMF for 13, 13a, and 14; NIS, $KHCO_3$, DMF for 15; NCS, aqueous $NaHCO_3$ for 16.

and 13 was found to have promising antiviral activity. We also describe the synthesis and antiherpes activities of the homochiral analogue 13a and the racemic iodovinyl and chlorovinyl compounds 15 and 16, respectively.

Chemistry

The racemic compound 13 was synthesized from the known racemic tosylate 17[1a] (Scheme I). Reaction of 17 with uracil in the presence of potassium carbonate and 18-crown-6 in dimethyl sulfoxide provided 18 in 27% yield. Subsequently, it was found that the yield of 18 could be increased to 37% by reacting the tetrabutylammonium salt of uracil with 17 in dimethylformamide. Deprotection of 18 with catalytic sodium methoxide in methanol provided the diol 19 (92%), which was reacted with iodine in aqueous nitric acid/dioxane[9] to give the iodouracil 20 (85%). Treatment of 20 with methyl acrylate, palladium(II) acetate, triphenylphosphine, and triethylamine in dioxane[10] afforded methyl ester 21 (75%), which was saponified to acid 22 (88%). Subsequent treatment of 22 with *N*-bromosuccinimide and potassium bicarbonate in dimethylformamide[11] provided racemate 13 (75%). The

(8) Zahler, R.; Young, M. G.; Slusarchyk, W. A.; Jacobs, G. A.; Bisacchi, G. S.; Haffey, M. L.; McGeever-Rubin, B.; Tuomari, A. V.; Yamanaka, G. A.; Field, A. K. SQ33,912: A Selective Inhibitor of Varicella-Zoster. *Antiviral Res.* **1990**, *Suppl. 1*, 54.

(9) Michelson, A. M. 5-Halogenouridine 5'-(Dihydrogen Phosphates). In *Synthetic Procedures in Nucleic Acid Chemistry*; Zorbach, W., Tipson, R., Eds.; Wiley Interscience: New York, 1968; Vol. 1, pp 491–492.

(10) Colla, L.; Busson, R.; DeClercq, E.; Vanderhaeghe, H. Synthesis of Aliphatic Nucleoside Analogues With Potential Antiviral Activity. *Eur. J. Med. Chem.* **1982**, *17*, 569–576.

Table I. Antiviral and Growth Inhibition Activities in Cell Cultures

	ID_{50} (μM)[a]					
antiviral[b]	**13a**	**13**	**15**	**16**	BVaraU (**12**)	acyclovir (**9**)
VZV strains						
Ellen	0.06–0.2	0.03–0.15	0.03–0.05	0.2–0.4	0.001–0.003	2–4
Ito	0.015	0.01–0.05	0.05–0.1	0.02–0.05	0.003–0.007	0.4–2
Oka		0.3–0.6			0.001–0.003	1–4
9021	0.06–0.15	0.2–0.6	0.13	0.45–1.1	0.001–0.003	1–4
pplla	0.06–0.2	0.06–0.2	0.13–0.26		0.001–0.003	2–4
40a2 (TK^-)		>300				110–220
Kanno-Kohmura (TK^-)	75–150	>300	>260	>220	>72	44–110
HSV-1 (Schooler)	1.5–3	<6	5–13	1.7–3.5	0.06–0.14	0.2–0.4
HSV-2 (186)	>300	>300	>260	>280	60–120	0.4–0.8
HCMV (AD169)	>300	>300	130–260	180–350	>290	20–40
WI-38 growth inhibn	≥750	≥750	>800	>400	>75	≥750
therapeutic index[c]	$\geq 1.3 \times 10^4$	$\geq 2.5 \times 10^4$	$\geq 2.7 \times 10^4$	$\geq 2.0 \times 10^3$	$\geq 7.5 \times 10^4$	$\geq 3.8 \times 10^2$

[a] All ID_{50} values show the range of repeat assays. [b] All plaque reduction assays were done on WI-38 cell monolayers. [c] ID_{50} for WI-38 cell growth inhibition/ID_{50} anti-VZV (strain Ellen).

(1′*R*) homochiral compound **13a** was prepared analogously from the homochiral tosylate **17a**.[1g] The racemic iodo analogue **15** was prepared in 73% from racemic acid **22** by reaction with *N*-iodosuccinimide and potassium bicarbonate in dimethylformamide,[11] and the racemic chloro compound **16** was obtained in 16% by treatment of **22** with *N*-chlorosuccinimide and sodium bicarbonate in water.[11]

The racemic bromovinyl compound **14** was prepared from the known racemic epoxide **23**.[1d] Coupling of uracil with **23** using catalytic sodium hydride in sulfolane in the presence of 18-crown-6 afforded compound **24**, which was debenzylated with palladium hydroxide and cyclohexene in ethanol to give diol **25**. Sequential iodination, reaction with methyl acrylate, saponification, and bromination as described above provided the desired product **14**.

Biological Results and Discussion

The antiviral potencies of compounds **13**, **13a**, **15**, **16**, BVaraU (**12**), and acyclovir (**9**) are shown in Table I, where ID_{50} is defined as the concentration of drug required to achieve 50% plaque reduction compared to virus controls. The (bromovinyl)-, (chlorovinyl)-, and (iodovinyl)uracil racemic analogues (**13**, **15**, and **16**, respectively) have excellent activity ($ID_{50} < 0.4$ μM) against VZV (Ellen). Expanded evaluation of **13** and its (1′*R*) enantiomer **13a** revealed that excellent activity is also observed against other VZV laboratory strains (Ito, Oka, pplIa) and a recent clinical isolate (9021). On average, **13**, **13a**, **15**, and **16** are 10-fold more potent than acyclovir (**9**) and 10- to 100-fold less potent than BVaraU (**12**). As in the case of BVaraU, the antiviral activities of **13**, **13a**, **15**, and **16** are reduced against VZV strains deficient in thymidine kinase activity (TK^-), indicating that antiviral activity is dependent upon phosphorylation by virally encoded kinases. Compounds **13**, **13a**, **15**, and **16** are also active against HSV-1 (ID_{50} = 1.5–13 μM), but are less active against HSV-2 ($ID_{50} > 260$ μM) and HCMV ($ID_{50} > 130$ μM). The lower homologue (bromovinyl)uracil compound, **14**, is devoid of activity ($ID_{50} > 300$ μM) against the HSV-1, HSV-2, HCMV, and VZV strains evaluated.

The 5-iodouracil intermediates **20** and **26** were also tested for antiviral activity. The importance of a 5-iodouracil group in imparting antiherpesvirus activity to nucleoside analogues is well-known, and 5-iodo-2′-deoxyuridine (idoxuridine)[4g,6b] is a classical example. In plaque reduction assays, the racemic 5-iodouracil intermediate **20** exhibited modest activity against HSV-1 (ID_{50} = 6–14 μM) and VZV (Ellen) (ID_{50} = 14–28 μM) and was not active at 280 μM against HSV-2 and HCMV. The lower homologue compound **26** was inactive ($ID_{50} > 300$ μM) against HSV-1, HSV-2, VZV, and HCMV.

Compounds were also evaluated for inhibition of cell growth. None of the compounds evaluated killed cells, and inhibition of cell growth to half that observed for untreated control cultures (ID_{50}) was greater than the highest concentrations tested (750–800 μM for compounds **13a**, **13**, and **15**; 400 μM for compound **16**). The therapeutic index (ID_{50} cell growth/ID_{50} VZV (Ellen)) for compounds **13**, **13a**, and **15** is at least 13 000, and for compound **16**, at least 2000, indicating excellent selectivity for anti-VZV activity.

The activity of BVaraU against VZV depends upon conversion of the nucleoside analogue to its corresponding mono- and diphosphate by VZV thymidine kinase, and subsequent conversion to the triphosphate by cellular kinases.[12] We evaluated compounds **13**, **13a**, **15**, **16**, acyclovir, and BVaraU, in comparison to thymidine, as substrates for phosphorylation by purified VZV thymidine kinase (Table II). As expected, both thymidine and BVaraU are readily phosphorylated to their mono- and diphosphates, whereas acyclovir is solely converted to its monophosphate at a relatively slow rate. The racemic compounds **13**, **15**, and **16** are excellent substrates for VZV thymidine kinase, with conversion to the respective monophosphates, but not to the diphosphates.

The (1′*R*) enantiomer **13a** is phosphorylated at only 5% the rate of racemate **13**. This relatively slower phosphorylation rate of the "natural" (1′*R*) enantiomer by VZV thymidine kinase is similar to the case of the (1′*R*) enantiomer **5** ((*R*)-BHCG) and racemate **1** ((±)-BHCG) upon phosphorylation by HSV-1 TK. (*R*)-BHCG is poorly phosphorylated by purified HSV-1 thymidine kinase when compared to (±)-BHCG or the inactive, "unnatural" enantiomer (*S*)-BHCG (**7**).[3i,13] However, in HSV-1 infected

(11) (a) Jones, A. S.; Verhelst, G.; Walker, R. T. The Synthesis of the Potent Anti-Herpes Virus Agent E-5(2-Bromovinyl)-2′-deoxyuridine and Related Compounds. *Tetrahedron Lett.* **1979**, *45*, 4415–4418. (b) Ashwell, M.; Jones, A. S.; Kumar, A.; Sayers, J. R.; Walker, R. T.; Sakuma, T.; DeClercq, E. The Synthesis and Antiviral Properties of (E)-5-(2-Bromovinyl)-2′-deoxyuridine-Related Compounds. *Tetrahedron* **1987**, *43*, 4601–4608.

(12) Yokota, T.; Konno, K.; Mori, S.; Shigeta, S.; Kumagai, M.; Watanabe, Y.; Machida, H. Mechanism of Selective Inhibition of Varicella Zoster Virus Replication by 1-β-D-Arabinofuranosyl-E-5-(2-bromovinyl)uracil. *Mol. Pharmacol.* **1989**, *36*, 312–316.

(13) Terry, B. J.; Cianci, C. W.; Hagen, M. Inhibition of Herpes Simplex Virus Type 1 DNA Polymerase by [1R(1α,2β,3α)]-9-[2,3-Bis(hydroxymethyl)cyclobutyl]guanine. *Mol. Pharmacol.* **1991**, *40*, 591–596.

cells, the limited phosphorylation rate of (*R*)-BHCG is compensated by rapid conversion to the corresponding di- and triphosphates.[14] (*R*)-BHCG triphosphate, but not (*S*)-BHCG triphosphate, is a potent inhibitor of HSV-1 DNA polymerase.[1g]. In future studies, it will be important to evaluate the metabolism of **13a** in VZV infected cells and the capacity of **13a** triphosphate to inhibit VZV DNA polymerase.

Experimental Section

Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were obtained with a JEOL GX-270 or GSX-270 270-MHz spectrometer with tetramethylsilane as internal reference, unless otherwise specified. Chemical shifts are expressed in δ units (parts per million). Ultraviolet spectra were recorded on a Shimadzu UV-265 spectrometer. Mass spectra (CI or FAB) were obtained on a Finnigan TSQ or VG-ZAB-2F mass spectrometer. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. TLCs were run on Merck silica gel 60 F254 plates, and purities of samples, when measured by densitometry at 254 nm, were determined on Shimadzu CS-930 and CS-9000 TLC scanners. Melting points (uncorrected) were determined on a Thomas-Hoover capillary apparatus. Diaion CHP 20P is a reverse-phase resin for chromatography and was obtained from Mitsubishi Chemical Industries Limited.

(1α,2β,3α)-1-[2,3-Bis[(benzoyloxy)methyl]cyclobutyl]-2,4(1*H*,3*H*)-pyrimidinedione (18). Procedure A. To a solution of uracil (1.26 g, 11.23 mmol, dried at 50 °C/1 mm for 16 h) and 18-crown-6 (1.98 g, 7.49 mmol) in dry DMSO (9 mL) at 50 °C was added 2.07 g (14.98 mmol) of K_2CO_3 and 3.7 g (7.49 mmol) of (1α,2β,3β)-3-[[(4-methylphenyl)sulfonyl]oxy]-1,2-cyclobutanedimethanol, dibenzoate ester (**17**).[1a] The mixture was heated to 100 °C, additional DMSO (3 mL) was added, and the mixture was stirred at 100 °C for 24 h. The solvents were removed in vacuo to give a residue, which was purified by chromatography on a column of Merck silica gel (700 mL) using a gradient of toluene to 3% isopropyl alcohol in toluene to give 850 mg of **18** as a colorless amorphous solid. Impure fractions, when dissolved in toluene and left to stand, gave an additional 35 mg of **18** for a total of 885 mg of **18** (27% yield): ^{1}H NMR ($CDCl_3$) δ 2.16 (m, 1 H), 2.54 (m, 2 H), and 2.98 (m, 1 H) [H-2′, H-3′, H-4′], 4.47 (m, 4 H, CH_2O), 4.73 (m, 1 H, H-1′), 5.69 (d, J = 8.2 Hz, 1 H, vinylic H), 7.47 (m, 7 H, aromatics and vinylic H), 8.01 (m, 4 H, aromatics), 9.62 (br s, 1 H, NH); MS (CI) 435 $(M + H)^+$, 433 $(M - H)^-$. The sample was homogeneous (UV detection) by TLC [R_f 0.40, toluene–isopropyl alcohol (9:1)].

Procedure B. To 8.145 g (72.6 mmol) of uracil in 300 mL of DMF was added a solution of 40% tetra-*n*-butylammonium hydroxide in water (42.3 mL, 68 mmol). The mixture was stirred at room temperature for 30 min, and then the clear solution was concentrated at 45 °C/1 mm. The residue was concentrated five times from DMF (150-mL portions) and then dried overnight at room temperature in vacuo to give crude dried uracil tetra-*n*-butylammonium salt as an amorphous solid. To this solid under argon was added 90 mL of dry DMF followed by 9.0 g (18 mmol) of **17**. The mixture was stirred at 80 °C for 38 h, cooled to room temperature, and stirred overnight. Acetic acid (7.2 mL) was added, and the solvents were removed in vacuo at 45 °C (1 mm). The resulting residue was taken up in EtOAc (200 mL) and water (200 mL). Slightly damp Bio-Rad AG-MP-50 (K^+ form) resin (ca. 182 g) was added, the pH was adjusted from 3.2 to 7.0 using 1 N KOH, and the mixture was stirred for 45 min. The mixture was filtered, and the filter cake was washed with water and EtOAc. The layers in the filtrate were separated, and the EtOAc layer was dried (Na_2SO_4) and evaporated to a residue (6.5 g). Chromatography of the residue on a column of Merck silica gel (2 L, packed in toluene) by elution with toluene and then 2 and 4% isopropyl alcohol in toluene afforded, after drying overnight at room temperature in vacuo, 2.90 g (37% yield) of **18** as a colorless amorphous solid: homogeneous (UV detection) by TLC [toluene–isopropyl alcohol (9:1)]; ^{1}H NMR ($CHCl_3$) identical with that for **18** obtained by procedure A.

(1α,2β,3α)-1-[2,3-Bis(hydroxymethyl)cyclobutyl]-2,4-(1*H*,3*H*)-pyrimidinedione (19). To a suspension of **18** (885 mg, 2.04 mmol) in dry CH_3OH (25 mL) was added a solution of 25% $NaOCH_3$ in CH_3OH (264 μL, 1.22 mmol). The mixture was stirred at 40 °C for 3 h under nitrogen. The solvents were removed in vacuo, and the residue was dissolved in water (5 mL). The pH was lowered to 7 with 1 N HCl, and the resulting mixture was purified on a CHP 20P resin column (200 mL) using a step gradient of water, 2% and 4% CH_3CN in water, to give, after concentration in vacuo, 423 mg (92% yield) of **19** as a white solid: ^{1}H NMR (DMSO-d_6) δ 1.81 (m, 1 H), 1.92 (m, 1 H), 2.21 (m, 1 H), and 2.47 (m, 1 H overlapping DMSO) [H-2′, H-3′, H-4′], 3.44 (m, 4 H, OCH_2), 4.45 (m, 1 H, H-1′), 4.49 (m, 2 H, OH, D_2O exchangeable), 5.58 (d, J = 8.2 Hz, 1 H, H-5), 7.77 (d, J = 8.2 Hz, 1 H, H-6), 11.13 (br s, 1 H, NH); ^{13}C NMR (DMSO-d_6) δ 28.24, 32.73, 45.98, 49.58, 61.70 and 62.97 (CH_2O), 100.81 (C-5), 142.33 (C-6), 150.86 (C-2), 163.27 (C-4): MS (FAB) 227 $(M + H)^+$. The sample was homogeneous (UV detection) by TLC [R_f 0.56, $CHCl_3$–CH_3OH–NH_4OH (6:3:1)].

(1α,2β,3α)-1-[2,3-Bis(hydroxymethyl)cyclobutyl]-5-iodo-2,4(1*H*,3*H*)-pyrimidinedione (20). To a suspension of **19** (423 mg, 1.87 mmol) in dioxane (38 mL, purified by passage through basic alumina) were added iodine (950 mg, 3.74 mmol) and 0.8 M HNO_3 (2.5 mL, 2 mmol), The mixture was stirred under argon at 95 °C for 1.5 h and then cooled to room temperature. A solution of saturated aqueous sodium thiosulfate was added until the dark red color faded, and the mixture was concentrated in vacuo to a residue. Passage of this material through a column of CHP 20P resin (150 mL) using a gradient of water to 50% CH_3CN in water afforded, after concentration in vacuo, 557 mg (85% yield) of **20** as a white solid: mp 170–171 °C; ^{1}H NMR (DMSO-d_6) δ 1.90 (m, 2 H), 2.21 (m, 1 H), and 2.50 (m, 1 H, + DMSO overlapping) [H-2′, H-3′, H-4′], 3.45 (m, 4 H, CH_2O), 4.40 (m, 1 H, H-1′), 4.62 (m, 2 H, OH, D_2O exchangeable), 8.18 (s, 1 H, H-6), 11.50 (br s, 1 H, NH); ^{13}C NMR (DMSO-d_6) δ 28.59, 32.91, 46.18, 50.96, 62.02 and 63.00 (CH_2O), 68.38 (C-5), 147.11 (C-6), 150.97 (C-2), 161.08 (C-4); UV (H_2O, pH 1.5) λ_{max} 294.1 nm (ϵ 8090), (H_2O, pH 6.0) λ_{max} 293.9 nm (ϵ 8110), (H_2O, pH 11.5) λ_{max} 282.7 nm (ϵ 6000); MS (FAB) 353 $(M + H)^+$, 351 $(M - H)^-$; 98.9% pure by TLC [UV densitometry, R_f 0.40, $CHCl_3$–CH_3OH–NH_4OH (6:3:1)]. Anal. ($C_{10}H_{13}IN_2O_4 \cdot 0.25H_2O$) C, H, N.

(1α(*E*),2β,3α)-3-[1-[2,3-Bis(hydroxymethyl)cyclobutyl]-1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl]-2-propenoic Acid, Methyl Ester (21). Dry dioxane (12.2 mL, purified by passage through basic alumina) was deoxygenated in vacuo. To the deoxygenated dioxane under argon were added 48.6 mg (0.21 mmol) of $Pd(OAc)_2$, 110 mg (0.42 mmol) of triphenylphosphine, and 0.77 mL (5.53 mmol) of triethylamine (freshly distilled from CaH_2). The mixture was placed in a bath at 70 °C and stirred for 20 min to give a deep red solution. Compound **20** (1.43 g, 4.05 mmol) was added, followed by methyl acrylate (0.73 mL, 8.1 mmol), and the mixture was stirred at 70 °C for 4 h. The reaction was filtered, and the filtrate was diluted with CH_2Cl_2, absorbed on Baker silica gel, and applied to a column (650 mL) of Merck silica gel packed in CH_2Cl_2. Elution with CH_2Cl_2 and then 5 and 10% CH_3OH in CH_2Cl_2 afforded 1.05 g of a colorless solid consisting by ^{1}H NMR integration of 945 mg (75% yield) of **21** and 0.15 equiv (60 mg) of triethylammonium iodide: ^{1}H NMR (CD_3OD) δ 2.07 (m, 2 H), 2.42 (m, 1 H), and 2.66 (m, 1 H) [H-2′, H-3′, H-4′], 3.65 (m, 4 H, CH_2O), 3.74 (s, 3 H, CH_3O), 4.58 (m, 1 H, H-1′), 6.92 (d, J = 15.8 Hz, 1 H, vinylic H), 7.42 (d, J = 15.8 Hz, 1 H, vinylic H), 8.17 (s, 1 H, H-6); ^{1}H NMR (DMSO-d_6) δ 1.87 (m, 1 H), 1.94 (m, 1 H), 2.25 (m, 1 H), and 2.55 (m, 1 H, + DMSO overlapping) [H-2′, H-3′, H-4′], 3.46 (m, 4 H, CH_2O), 3.68 (s, 1 H, CH_3O), 4.50 (m, 1 H, H-1′), 4.56 (m, 2 H, OH), 6.89 (d, J = 15.8 Hz, 1 H, vinylic H), 7.47 (d, J = 15.8 Hz, 1 H, vinylic H), 8.38 (s, 1 H, H-6), 11.52 (br s, 1 H, NH). In the DMSO-d_6 spectrum, a triplet at 1.17 ppm and multiplet at 3.08 ppm were attributed to triethylammonium iodide. The sample was homogeneous (UV detection) by TLC [R_f 0.62, $CHCl_3$–CH_3OH–NH_4OH (6:3:1)].

(1α(*E*),2β,3α)-3-[1-[2,3-Bis(hydroxymethyl)cyclobutyl]-1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl]-2-propenoic Acid

(14) Yamanaka, G. A.; Tuomari, A. V.; Hagen, M.; McGeever-Rubin, B.; Terry, B. J.; Haffey, M.; Bisacchi, G. S.; Field, A. K. Selective Activity and Cellular Pharmacology of (1R,1α,2β,3α)-9-[2,3-Bis(hydroxymethyl)cyclobutyl]guanine in Herpesvirus-Infected Cells. *Mol. Pharmacol.* **1991**, *40*, 446–453.

(22). A solution of 240 mg of the above preparation of 21 (0.70 mmol) in 2.45 mL of 2 N KOH was stirred at room temperature for 1 h and then filtered. The filtrate was cooled to 0–5 °C, and the pH was adjusted to 2.0 using 6 N HCl. The solids were collected by filtration, washed with a minimum amount of cold water, air-dried, and then dried overnight in vacuo (0.1 mm) over P_2O_5 at room temperature to give 182 mg (88% yield) of 22 as a white solid: ^{1}H NMR (DMSO-d_6) δ 1.87 (m, 1 H), 1.94 (m, 1 H), 2.25 (m, 1 H), and 2.55 (m, 1 H) [H-2′, H-3′, H-4′], 3.46 (m, 4 H, CH_2O), 4.51 (m, 1 H, H-1′), 4.60 (br s, 2 H, OH, D_2O exchangeable), 6.80 (d, J = 15.8 Hz, 1 H, vinylic H), 7.39 (d, J = 15.8 Hz, 1 H, vinylic H), 8.34 (s, 1 H, H-6), 11.5 (br s, 1 H, NH), 12.1 (br s, 1 H, COOH); homogeneous (UV detection) by TLC [R_f 0.13, $CHCl_3$–CH_3OH–NH_4OH (6:3:1)].

(1α(*E*),2β,3α)-1-[2,3-Bis(hydroxymethyl)cyclobutyl]-5-(2-bromoethenyl)-2,4(1*H*,3*H*)-pyrimidinedione (13). To a stirred solution of 22 (180 mg, 0.60 mmol) in dry DMF (3.25 mL) under argon at room temperature was added 183 mg (1.83 mmol) of $KHCO_3$ followed by a solution of *N*-bromosuccinimide (109 mg, 0.61 mmol) in DMF (1.25 mL). After 6 h, additional *N*-bromosuccinimide (11 mg) in DMF (0.2 mL) was added, and stirring was continued for 1 h. The reaction was filtered, and the filtrate was concentrated in vacuo (50 °C/1 mm) to a residue. Concentration of this residue in vacuo from water and then chromatography on a column of CHP 20P resin (30 mL) using a gradient of water to 50% CH_3CN in water gave, after lyophilization from water, 149 mg (75% yield) of 13 as a colorless solid: mp 157–159 °C; ^{1}H NMR (DMSO-d_6) δ 1.82 (m, 1 H), 1.92 (m, 1 H), 2.26 (m, 1 H) and 2.50 (m, 1 H + DMSO) [H-2′, H-3′, H-4′], 3.45 (m, 4 H, CH_2O), 4.48 (m, 1 H, H-1′), 4.55 (m, 2 H, OH, D_2O exchangeable), 6.92 (d, J = 13.5 Hz, 1 H, vinylic H), 7.26 (d, J = 13.5 Hz, 1 H, vinylic H), 8.02 (s, 1 H, H-6), 11.41 (br s, 1, NH); ^{13}C NMR (DMSO-d_6) δ 28.70, 32.78, 46.40, 49.76, 61.55 and 63.48 (CH_2O), 105.96 (C-5), 109.04 (C-α), 129.97 (C-β), 141.51 (C-6), 149.63 (C-4), 161.64 (C-2); UV (H_2O, pH 7.2, phosphate buffer) λ_{max} 253.4 nm (ε 15 100), 298.0 nm (ε 12 500); MS (FAB) 331 and 333 (M + H)$^+$, 329 and 331 (M − H)$^-$; 99.0% pure by TLC [UV densitometry, R_f 0.68, $CHCl_3$–CH_3OH–NH_4OH (6:3:1)]; ($C_{12}H_{15}BrN_2O_4$·0.5H_2O) C, H, N.

[1*R*-(1α,2β,3α)]-1-[2,3-Bis[(benzoyloxy)methyl]cyclobutyl]-2,4(1*H*,3*H*)-pyrimidinedione (18a) was prepared from [1*S*-(1α,2β,3β)]-3-[[(4-methylphenyl)sulfonyl]oxy]-1,2-cyclobutanedimethanol, dibenzoate ester (**17a**)[1g] (2.25 g, 4.55 mmol) in the same manner as described for 18 (procedure B) to afford 18a as a colorless glassy solid (36% yield): ^{1}H NMR;[15] homogeneous (UV detection) by TLC [toluene–isopropyl alcohol (9:1)].

[1*R*-(1α,2β,3α)]-1-[2,3-Bis(hydroxymethyl)cyclobutyl]-2,4(1*H*,3*H*)-pyrimidinedione (19a) was prepared from 18a (701 mg, 1.62 mmol) in the same manner as described for 19 to afford 19a as a white solid after lyophilization (93% yield): ^{1}H NMR;[15] homogeneous (UV detection) by TLC [$CHCl_3$–CH_3OH–NH_4OH (6:3:1)].

[1*R*-(1α,2β,3α)]-1-[2,3-Bis(hydroxymethyl)cyclobutyl]-5-iodo-2,4(1*H*,3*H*)-pyrimidinedione (20a) was prepared from 19a (338 mg, 1.50 mmol) in the same manner as described for 20 to afford 20a as a white solid (79% yield): ^{1}H NMR;[15] homogeneous (UV detection) by TLC [$CHCl_3$–CH_3OH–NH_4OH (6:3:1)].

[1*R*-(1α(*E*),2β,3α)]-3-[1-[2,3-Bis(hydroxymethyl)cyclobutyl]-1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl]-2-propenoic Acid, Methyl Ester (21a). Dry dioxane (3.5 mL, purified by passage through basic alumina) was deoxygenated in vacuo. To the deoxygenated dioxane under argon were added 13 mg (0.058 mmol) of $Pd(OAc)_2$, 30 mg (0.116 mmol) of triphenylphosphine, and 0.21 mL (1.47 mmol) of triethylamine (freshly distilled from CaH_2). The mixture was placed in a bath at 70 °C and stirred for 15 min to give a deep red solution. Compound 20a (379 mg, 1.08 mmol, previously dried over P_2O_5 at 55 °C/1 mm for 2 h) was added, followed by methyl acrylate (194 μL, 2.16 mmol), and the mixture was stirred at 70 °C for 4 h. The reaction mixture was cooled to room temperature, diluted with CH_2Cl_2, filtered through Celite, and concentrated in vacuo. Treatment of the residue with CH_3OH gave, after collection by filtration, 195 mg of 21a as a colorless solid. Evaporation of the filtrate gave a residue, which was treated with CH_3OH to give 29 mg of additional 21a for a total of 224 mg of 21a (69% yield): homogeneous by ^{1}H NMR (CD_3OD);[15] homogeneous by ^{1}H NMR (DMSO-d_6) δ 1.86 (m, 1 H), 1.94 (m, 1 H), 2.25 (m, 1 H), and 2.57 (m, 1 H) [H-2′, H-3′, H-4′], 3.46 (m, 4 H, CH_2O), 3.68 (s, 3 H, CH_3O), 4.55 (m, 1 H, H-1′), 4.60 (m, 2 H, OH), 6.89 (d, J = 15.8 Hz, 1 H, vinylic H), 7.47 (d, J = 15.8 Hz, 1 H, vinylic H), 8.40 (s, 1 H, H-6), 11.53 (br s, 1 H, NH); homogeneous (UV detection) by TLC [$CHCl_3$–CH_3OH–NH_4OH (6:3:1)].

[1*R*-(1α(*E*),2β,3α)]-3-[1-[2,3-Bis(hydroxymethyl)cyclobutyl]-1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl]-2-propenoic acid (22a) was prepared from 21a (230 mg, 0.74 mmol) in the same manner as described for 22 to afford 22a as an amorphous white solid (94% yield): ^{1}H NMR;[15] homogeneous (UV detection) by TLC [$CHCl_3$–CH_3OH–NH_4OH (6:3:1)].

[1*R*-(1α(*E*),2β,3α)]-1-[2,3-Bis(hydroxymethyl)cyclobutyl]-5-(2-bromoethenyl)-2,4(1*H*,3*H*)-pyrimidinedione (13a) was prepared from 22a (196 mg, 0.66 mmol) in the same manner as described for 13 to afford 163 mg of 13a (74% yield) as a lyophilized colorless solid: mp 141–142 °C; $[\alpha]^{22}_D$ = −45° (c = 0.31, methanol); ^{1}H NMR;[15] UV (H_2O, pH 7.2, phosphate buffer) λ_{max} 253.4 nm (ε 13 900), λ_{max} 298.8 (ε 11 600); MS (FAB) 331 and 333 (M + H)$^+$; 99.0% pure by TLC [UV densitometry, R_f 0.68, $CHCl_3$–CH_3OH–NH_4OH (6:3:1)]; 99.4% pure by HPLC, AQ-303 C-18 column [acetonitrile–0.1 M triethylammonium acetate, pH 7 (18:88)]. Anal. ($C_{12}H_{15}BrN_2O_4$·0.75H_2O) C, H, N. HPLC analysis on a Chiracel OD column [ethanol–methanol–hexane (6:6:88)] indicated the presence of less than 0.06% of the (1*S*) enantiomer in 13.

(1α(*E*),2β,3α)-1-[2,3-Bis(hydroxymethyl)cyclobutyl]-5-(2-iodoethenyl)-2,4(1*H*,3*H*)-pyrimidinedione (15). To a stirred solution of 22 (118.5 mg, 0.4 mmol) in 4 mL of dry DMF under argon at room temperature was added 79 mg (8 mmol) of potassium acetate. The mixture was stirred for 30 min, *N*-iodosuccinimide (92.3 mg, 0.41 mmol) was added, and the mixture was heated at 50 °C, protecting the reaction from light. After 5 h, additional *N*-iodosuccinimide (37 mg, 0.164 mmol) was added, and heating was continued for 4 h longer. After stirring overnight at room temperature, additional *N*-iodosuccinimide (46 mg, 0.205 mmol) was added, and the mixture was heated at 60 °C for 3 h. The mixture was filtered and the solvent was removed in vacuo to give a residue, which was applied to a column of CHP 20P resin (25 mL) packed in water. Elution with a gradient of water to 50% CH_3CN in water, followed by lyophilization, gave 110 mg (73%) of 15 as a colorless solid: mp 165–167 °C; ^{1}H NMR (DMSO-d_6) δ 1.80 (m, 1 H), 1.94 (m, 1 H), 2.23 (m, 1 H), and 2.50 (m, 1 H + DMSO) [H-2′, H-3′, H-4′], 3.45 (m, 4 H, CH_2O), 4.45 (m, 1 H, H-1′), 4.55 (m, 2 H, OH, D_2O exchangeable), 7.21 (s, 2 H, vinylic H), 8.01 (s, 1 H, H-6), 11.42 (br s, 1 H, NH); ^{13}C NMR (DMSO-d_6) δ 28.67, 32.79, 46.38, 49.75, 61.59 and 63.46 (CH_2O), 77.57 (C-α), 110.86 (C-5), 136.98 (C-β), 141.33 (C-6), 149.62 (C-4), 161.68 (C-2); UV (H_2O, pH 7.2, phosphate buffer) λ_{max} 255 nm (ε 15 960), 303 nm (ε 13 900); MS (FAB) 379 (M + H)$^+$, 377 (M − H)$^-$; 99.4% pure by TLC [UV densitometry, $CHCl_3$–CH_3OH–NH_4OH (6:3:1)]. Anal. ($C_{12}H_{15}IN_2O_4$·0.25H_2O) C, H, N, I.

(1α(*E*),2β,3α)-1-[2,3-Bis(hydroxymethyl)cyclobutyl]-5-(2-chloroethenyl)-2,4(1*H*,3*H*)pyrimidinedione (16). To a stirred suspension of 22 (247 mg, 0.84 mmol) in 8.4 mL of water at room temperature was added 141 mg (1.68 mmol) of $NaHCO_3$. The mixture was stirred for 30 min, *N*-chlorosuccinimide (124 mg, 0.924 mmol) was added, and the mixture was heated at 90 °C for 6 h. Additional amounts of *N*-chlorosuccinimide (269 mg, 2 mmol) were added intermittently in four portions over the next 18 h while heating was continued at 90 °C. The aqueous mixture was cooled and applied to a column of CHP 20P resin (50 mL) packed in water. Elution with a gradient of water to 50% CH_3CN in water, followed by lyophilization, gave 38 mg (16%) of 16 as a pale yellow solid: mp 156–160 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.83 (m, 1 H), 1.96 (m, 1 H), 2.23 (m, 1 H) and 2.50 (m, 1 H + DMSO) [H-2′, H-3′, H-4′], 3.46 (m, 4 H, CH_2O), 4.45 (m, 1 H, H-1′), 4.55 (m, 2 H, OH, D_2O exchangeable), 6.66 (d, J = 13.5 Hz, 1 H, vinylic H), 7.20 (d, J = 12.9 Hz, 1 H, vinylic H), 7.99 (s, 1 H, H-6), 11.40 (br s, 1 H, NH); ^{13}C NMR (DMSO-d_6) δ 28.67, 32.79, 46.41, 49.78, 61.59 and 63.49 (CH_2O), 108.04 (C-5), 117.45 (C-α), 126.47 (C-β), 141.33 (C-6), 149.68 (C-4), 161.71 (C-2); UV (H_2O, pH 7.2, phosphate buffer) 251 nm (ε 12 700), 296 nm (ε 10 020); MS (CI) 287 and 289 (M + H)$^+$, 304 and 306 (M + NH_4)$^+$; 98.7% pure by TLC [UV densitometry, $CHCl_3$–CH_3OH–NH_4OH (6:3:1)].

(1α,2β,3α)-1-[2-Hydroxy-3-(phenylmethoxymethyl)cyclobutyl]-2,4(1*H*,3*H*)-pyrimidinedione (24). To a mixture of (3.80 g, 20 mmol) of **23**,[1d] 9.63 g (86 mmol) of uracil, and 4.49 g (17 mmol) of 18-crown-6 in 96 mL of sulfolane (dried over 3A molecular sieves) under argon at 55 °C was added 0.48 g of 60% NaH in mineral oil (12 mmol NaH). Additional sulfolane (30 mL) was added, and the mixture was stirred at 115 °C for 110 h and cooled to room temperature. Acetic acid (0.75 mL) was added, and the reaction was diluted with CH_2Cl_2 and filtered. The filtrate was concentrated in vacuo to remove CH_2Cl_2, and sulfolane was removed using a Kugelrohr apparatus (80–85 °C/0.05 mm). The residue was absorbed on Baker silica gel (25 g) using CH_2Cl_2 and applied to a column of Merck silica gel packed in CH_2Cl_2. Elution with CH_2Cl_2 (2 L) and then 3% CH_3OH in CH_2Cl_2 (6 L) gave 3.67 g of a colorless, amorphous solid consisting by ^1H NMR integration of 3.19 g (53% yield) of **24** and 0.48 g of 18-crown-6: ^1H NMR (DMSO-d_6) δ 1.35 (m, 1 H), 1.95 (m, 1 H), and 2.05 (m, 1 H) [H-3′, H-4′], 3.53 (m, 2 H, CH_2O), 3.89 (m, 1 H, H-2′), 4.32 (m, 1 H, H-1′), 4.42 (s, 2 H, benzyl CH_2), 5.50 (d, J = 7 Hz, 1 H, OH), 5.51 (d, J = 8 Hz, 1 H, H-5), 7.18–7.35 (m, 5 H, aromatics), 7.62 (d, J = 8 Hz, 1 H, H-6), 11.14 (br s, 1 H, NH). A singlet at 3.52 ppm was attributed to 18-crown-6. The sample was homogeneous (UV detection) by TLC [R_f 0.25, CH_2Cl_2-CH_3OH (19:1)].

(1α,2β,3α)-1-[2-Hydroxy-3-(hydroxymethyl)cyclobutyl]-2,4(1*H*,3*H*)-pyrimidinedione (25). A mixture of 3.67 g of the above preparation of **24** (10.6 mmol), 180 mL of 95% EtOH, 45 mL of cyclohexene, and 1.45 g of 20% $Pd(OH)_2/C$ was refluxed with stirring under argon for 4 h. The mixture was cooled to room temperature and filtered through a pad of Celite and Whatman No. 50 filter paper. Evaporation of the filtrate in vacuo followed by addition of ethanol and concentration in vacuo gave 2.76 g of a white solid consisting by ^1H NMR integration of 2.35 g (quantitative yield) of **25** and 0.41 g of 18-crown-6. The 2.76-g sample was homogeneous (UV detection) by TLC [R_f 0.44, $CHCl_3$–CH_3OH–NH_4OH (6:3:1)]. A sample of **25** was obtained free of 18-crown-6 after chromatography on a column of CHP 20P resin using a gradient of water to 50% CH_3CN in water and then lyophilization. It had the following: ^1H NMR (400 MHz, DMSO-d_6) δ 1.35 (dd, J = 10, 20 Hz, 1 H, H-4′), 1.91 (m, 1 H, H-3′), 2.05 (dd, J = 9, 20 Hz, 1 H, H-4′), 3.50 (m, 2 H, CH_2O), 3.97 (m, 1 H, H-2′), 4.39 (m, 1 H, H-1′), 4.52 (t, J = 5 Hz, 1 H, OH), 5.44 (d, J = 6 Hz, 1 H, OH), 5.61 (d, J = 8 Hz, 1 H, H-5), 7.65 (d, J = 8 Hz, 1 H, H-6), 11.19 (br s, 1 H, NH); UV (H_2O, pH 7.2, phosphate buffer) λ_{max} 269 nm (ε 10660), (0.1 N NaOH) λ_{max} 268 nm (ε 8590); MS (CI) 213 (M + H)$^+$; 99.8% pure by TLC [UV densitometry, R_f 0.43, $CHCl_3$–CH_3OH–NH_4OH (6:3:1)].

(1α,2β,3α)-1-[2-Hydroxy-3-(hydroxymethyl)cyclobutyl]-5-iodo-2,4(1*H*,3*H*)-pyrimidinedione (26). To 2.04 g of the above preparation of **25** containing 18-crown-6 (8.16 mmol) were added, under argon, 80 mL of dry dioxane (purified by passage through basic alumina), 4.14 g (16.3 mmol) of iodine, and 10.2 mL of 0.8 N HNO_3. The mixture was heated at 100 °C with stirring for 3 h, cooled to room temperature, and concentrated in vacuo to a dark residue. Excess iodine was removed from the mixture by repeated concentrations of the mixture from 95% EtOH, $CHCl_3$, and finally mixtures of 95% EtOH–$CHCl_3$, which afforded a yellow solid. Crystallization of this solid from hot water afforded in two crops, 2.253 g of **26** as a colorless solid. Concentration of the mother liquor gave a residue, which was dissolved in water with heating, and applied to a column of CHP 20P resin (120 mL) packed in water. Elution with water, and then 10%, 20%, and 30% CH_3CN in water gave, after recrystallization from water, an additional 0.27 g of **26** for a total yield of 2.52 g (91% yield) of **26** as a colorless solid: mp 99–101 °C; ^1H NMR (DMSO-d_6) δ 1.45 (m, 1 H), 1.90 (m, 1 H), and 2.05 (m, 1 H) [H-3′, H-4′], 3.51 (m, 2 H, CH_2O), 4.02 (m, 1 H, H-2′), 4.34 (m, 1 H, H-1′), 4.50 (t, J = 5 Hz, 1 H, OH, D_2O exchangeable), 5.46 (d, J = 6 Hz, 1 H,OH, D_2O exchangeable), 8.07 (s, 1 H, H-6), 11.59 (br s, 1 H, NH); UV (0.1 N HCl) λ_{max} 294 nm (ε 8260), (H_2O, pH 7.2 phosphate buffer) λ_{max} 294 (ε 8190), (0.01 N NaOH) λ_{max} 286 nm (ε 5750); MS (FAB) 339 (M + H)$^+$; 98.9% pure by TLC [UV densitometry, R_f 0.32, $CHCl_3$–CH_3OH–NH_4OH (6:3:1)]. Anal. ($C_9H_{11}IN_2O_4$·H_2O) C, H, N.

(1α(*E*),2β,3α)-3-[1-[1-Hydroxy-3-(hydroxymethyl)cyclobutyl]-1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl]-2-propenoic Acid Methyl Ester (27). A mixture of $Pd(OAc)_2$ (65 mg, 0.29 mmol), triphenylphosphine (149 mg, 0.57 mmol), and triethylamine (1.05 mL, 7.5 mmol, distilled from CaH_2) in dry dioxane (15 mL, purified by passage through basic alumina and degassed in vacuo) under argon was heated with stirring at 70 °C for 20 min to give a deep red solution. Compound **26** (1.86 g, 5.50 mmol, previously dried over P_2O_5 at 55 °C/1 mm) was added followed by methyl acrylate (1.0 mL, 11 mmol), and the reaction mixture was stirred at 70 °C for 2.5 h. After cooling to room temperature, the catalyst and triethylammonium salts were removed by filtration. Evaporation of the filtrate in vacuo gave a residue, which was absorbed on 8 g of Baker silica gel using CH_2Cl_2–CH_3OH (9:1) and applied to a column of Merck silica gel (235 g) packed in CH_2Cl_2. Elution with CH_2Cl_2 and then 5%, 10%, and 25% CH_3OH in CH_2Cl_2 afforded 1.23 g of a colorless amorphous solid consisting by ^1H NMR integration of 1.07 g (66% yield) of **27** and 0.16 g of triethylammonium iodide: ^1H NMR (DMSO-d_6) δ 1.47 (m, 1 H), 1.93 (m, 1 H), and 2.06 (m, 1 H) [H-3′, H-4′], 3.54 (m, 2 H, CH_2O), 3.69 (s, 3 H, CH_3O), 4.03 (m, 1 H, H-2′), 4.45 (m, 1 H, H-1′), 4.49 (t, J = 5.3 Hz, 1 H, OH), 5.46 (d, J = 6.5 Hz, 1 H, OH), 6.91 (d, J = 15.8 Hz, 1 H, vinylic H), 7.46 (d, J = 15.8 Hz, 1 H, vinylic H), 8.30 (s, 1 H, H-6), 11.60 (br s, 1 H, NH); MS (FAB) 295 (M − H)$^-$, 297 (M + H)$^+$. The sample was homogeneous (UV detection) by TLC [R_f 0.33, CH_2Cl_2–CH_3OH (9:1)].

(1α(*E*),2β,3α)-3-[1-[2-Hydroxy-3-(hydroxymethyl)cyclobutyl]-1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl]-2-propenoic Acid (28). To 1.22 g of the above preparation of **27** (3.58 mmol) was added 12.5 mL of 2 N KOH solution (25 mmol). The mixture was stirred under argon at room temperature for 2.5 h and then cooled to 0–5 °C. The pH was adjusted to 2.0 using 6 N HCl, and the precipitate was collected by filtration, washed with water, air-dried, and then dried over P_2O_5 at 55 °C (1 mm) for 2 h to give 771 mg (73% yield) of **28** as a white amorphous solid: ^1H NMR (DMSO-d_6) δ 1.47 (m, 1 H), 1.93 (m, 1 H), and 2.10 (m, 1 H) [H-3′, H-4′], 3.54 (m, 2 H, CH_2O), 4.04 (t, J = 7.6 Hz, 1 H, H-2′), 4.45 (m + br s, 2 H, H-1′ and OH), 5.47 (br s, 1 H, OH), 6.82 (d, J = 15.8 Hz, 1 H, vinylic H), 7.38 (d, J = 15.8 Hz, 1 H, vinylic H), 8.23 (s, 1 H, H-6), 11.55 (br s, 1 H, NH), 12.10 (br s, 1 H, COOH); homogeneous (UV detection) by TLC [R_f 0.11, $CHCl_3$–CH_3OH–NH_4OH (6:3:1)].

(1α(*E*),2β,3α)-5-(2-Bromoethenyl)-1-[2-hydroxy-3-(hydroxymethyl)cyclobutyl]-2,4(1*H*,3*H*)-pyrimidinedione (14). To compound **28** (444 mg, 1.5 mmol) and $KHCO_3$ (450 mg, 4.5 mmol) in 8 mL of dry DMF under argon was added dropwise a solution of *N*-bromosuccinimide (267 mg, 1.5 mmol) in 3 mL of DMF. The reaction was stirred at room temperature for 8 h. Filtration of the reaction mixture and evaporation of the filtrate at 55 °C (1 mm) gave a residue. Concentration of the residue from water (three times) gave crystals, which were collected by filtration, washed with water, and dried over P_2O_5 at 55 °C (1 mm) for 2 h to give 363 mg (76% yield) of compound **14** as a white solid: mp 146–147 °C; ^1H NMR (DMSO-d_6) δ 1.42 (m, 1 H), 1.92 (m, 1 H), and 2.08 (m, 1 H) [H-3′, H-4′], 3.53 (m, 2 H, CH_2O), 3.99 (m, 1 H, H-2′), 4.43 (m, 1 H, H-1′). 4.51 (m, 1 H, OH, D_2O exchangeable), 5.44 (d, J = 5.9 Hz, 1 H, D_2O exchangeable), 6.93 (d, J = 13.5 Hz, 1 H, vinylic H), 7.29 (d, J = 13.5 Hz, 1 H, vinylic H), 7.93 (s, 1 H, H-6), 11.50 (br s, 1 H, NH); ^{13}C NMR (DMSO-d_6) δ 22.65 (C-4′), 40.59 (C-3′), 56.12 (C-2′), 61.76 (C-1′), 71.29 (CH_2O), 106.34 (C-5), 109.45 (C-α), 129.84 (C-β), 141.12 (C-6), 149.79 (C-4), 161.63 (C-2); UV (H_2O, pH 7.2 phosphate buffer) λ_{max} 253.3 nm (ε 13800), 298.2 nm (ε 11600); MS (FAB) 317 and 319 (M + H)$^+$, 315 and 317 (M − H)$^-$; 98.6% pure by TLC [UV densitometry, R_f 0.61, $CHCl_3$–CH_3OH–NH_4OH (6:3:1)]. Anal. ($C_{11}H_{13}BrN_2O_4$·0.25H_2O) C, H, N.

Antiviral Assays in Cell Culture. Viruses, cells, and assays have been described in detail previously.[1c] In brief, herpes simplex virus type 1 (HSV-1) strain Schooler and HSV-2 strain 186 were prepared as extracts from infected Vero cell cultures. Human cytomegalovirus (HCMV) strain AD169 and varicella-zoster virus (VZV) strains Ellen, Ito, Kanno-Kohmura, Oka, ppIIa, 40a2, and 9021 were prepared as suspensions of infected WI-38 cells. VZV strain Ito is a BUdR resistant, acyclovir sensitive, TK altered clinical isolate, and VZV strain Kanno-Kohmura is a thymidine kinase deficient (TK$^-$) mutant of Kanno, provided by Dr. S. Shigeta, Fukushima Medical Center, Fukushima, Japan. VZV strain ppIIa is a clinical isolate, and strain 40a2 is a TK$^-$ mutant

Table II. Phosphorylation of Compounds by Purified VZV Thymidine Kinase

substrate	kinase activity,[a] pmol/min per mL monophosphate	diphosphate
thymidine	585	174
13a	63	ND[b]
13	1279	ND
15	736	ND
16	333	ND
BVaraU (12)	140	95
acyclovir (9)	13	ND

[a] Initial reaction velocities for mono- and diphosphate production were determined by HPLC using 100 μM substrate and purified VZV (strain Ellen) thymidine kinase. Details of the reaction are described in the Experimental Section. [b] ND = not detectable.

derived from ppIIa. Both were provided by Dr. J. Ostrove, NIH. VZV strain 9021 is a recent clinical isolate provided by Dr. L. Frenkel, Robert Wood Johnson Viral Diagnostic Laboratory. VZV strains Ellen (VR-58) and Oka (VR-795) were obtained from ATCC. WI-38 (CCL75) and Vero (CCL81) cells were obtained from ATCC and were grown in Eagles minimim essential medium with Earle's salts (EMEM) supplemented with 2 mM L-glutamine, 100 units/mL penicillin, 11 μg/mL streptomycin, and 10% FBS (Gibco Laboratories, Grand Island, NY).

Viruses were assayed on WI-38 cell monolayers. Viruses were absorbed to cell monolayers in 6-well culture plates (Costar, Cambridge, MA) for 1–2 h prior to addition of maintenance medium (EMEM plus supplements, 1% (carboxymethyl)cellulose, 2.5% FBS ± drug) containing duplicate dilutions of the test compound. Inhibition of plaque development for all viruses was evaluated after 4–6 days incubation at 37 °C. ID_{50} values were determined from the drug concentration which conferred 50% plaque reduction compared to virus controls. All titrations were done in duplicate and expressed as a range in repeat assays.

Cell Growth Inhibition. WI-38 were planted at 1×10^5 cells/mL in 12-well Costar cell culture plates. Twenty four hours later, the cell cultures were refed with growth medium containing serial dilutions of the antiviral compound. At 24-h intervals, quadruplicate cell cultures at each concentration were resuspended by trypsinization and counted for viable and dead cells using the criterion of trypan blue exclusion. Control cultures were similarly evaluated, and over the 96-h evaluation increased 3- to 5-fold. The ID_{50} for each compound was calculated as the concentration which inhibited growth by 50% relative to control cell cultures.

Varicella-Zoster Thymidine Kinase Assay. VZV thymidine kinase was purified from VZV-infected WI-38 cells by sequential DEAE-cellulose and thymidine-affinity chromatography.[16] The final product was >90% pure as judged by SDS PAGE and approximately 98% free of cellular kinase activities. The enzyme was assayed in a reaction mixture containing 200 mM Tris-Cl (pH 7.5), 6 mM $MgCl_2$, 3 mM ATP, 1 mM DTT, 100 μg/mL BSA, 100 μM nucleoside substrate, and 50 ng/mL purified VZV thymidine kinase. Reactions were incubated at 25 °C for intervals of up to 30 h and then quenched by heating at 80 °C for 2 min. Reaction products were analyzed and quantitated by HPLC. The values shown in Table II represent initial reaction velocities, which were linear for both the nucleoside and nucleoside-monophosphate kinase activities.

Acknowledgment. We thank Dr. Joan Newburger and her staff for the chiral HPLC analysis and Mr. Terry McCormick and his staff for the elemental analyses.

(15) Spectral data were identical to those of the corresponding racemate.

(16) Fyfe, J. A. Differential Phosphorylation of (E)-5-(2-Bromovinyl)-2′-deoxyuridine Monophosphate by Thymidylate Kinases from Herpes Simplex Viruses Types 1 and 2 and Varicella Zoster Virus. *Mol. Pharmacol.* **1982**, *21*, 432–437.

Molecular Modeling and Crystallographic Studies of 4-Amino-*N*-phenylbenzamide Anticonvulsants

Norma E. C. Duke† and Penelope W. Codding*

Departments of Chemistry and of Pharmacology and Therapeutics, University of Calgary, Calgary, Alberta T2N 1N4, Canada.
Received August 22, 1991

The molecular structures of five different MES-active *N*-phenylbenzamides were determined by X-ray diffraction methods, and the conformations of a series of active and inactive benzamides were analyzed by molecular mechanics calculations. The most active compounds adopt a similar, consistent conformation in both the experimentally determined crystallographic structures and in the calculated molecular mechanics structures. This conformation places one *o*-methyl group proximal to the NH group of the central amide plane and orients the methyl-substituted phenyl ring at an angle of 90° to 120° to the central amide plane. Intermolecular interactions in the crystal structures indicate that hydrogen bonding to the central amide group is the important interaction. The observed consistent conformation facilitates formation of hydrogen bonds to the carbonyl oxygen atom. The conformations of inactive compounds obstruct this interaction. These findings help to outline a model of some of the structural features which this series of benzamides must possess in order to demonstrate MES anticonvulsant activity.

Approximately 1% of the population suffers from epilepsy, but less than 50% of these people achieve seizure control with drug therapy, and only 70–80% experience partial seizure control.[1] Since current anticonvulsant drugs are often inadequate in the control of epileptic seizures, the search continues for different and more active compounds. With the exception of the benzodiazepines and NMDA receptor antagonists, receptor sites have not been identified for anticonvulsants; therefore, drug identification is usually conducted via in vivo screening tests. Two tests commonly used are the maximal electroshock (MES) test and the subcutaneous metrazole (scMET) test.[2] A new series of potent 4-aminobenzamide anticonvulsants

* Author to whom correspondence should be addressed.
† Current address: Department of Medical Microbiology and Infectious Diseases, University of Alberta, Edmonton, Alberta, Canada.

(1) Jones, G. L.; Woodbury, D. M. Principles of Drug Action: Structure Activity Relationships and Mechanisms. In *Antiepileptic Drugs*, 2nd ed.; Woodbury, D. M., Perry, J. K., Pippenger, C. E., Eds.; New York: Raven Press, 1982; pp 83–109.

(2) Porter, R. J.; Pitlick, W. H. Antiepileptic Drugs. In *Basic and Clinical Pharmacology*, 3rd ed.; Katzung, B. G., Ed.; Appleton and Lange: East Norwalk, CT, 1987; pp 262–278.

0022-2623/92/1835-1806$03.00/0