from acetone/MeOH to give a white solid that rapidly absorbed water from the air. The hydrated salt weighed 1.0 g, mp 221–224 °C dec. Anal. ($C_{13}H_{10}NNaO_2S \cdot 0.5H_2O$) C, H, N.

Method M. Anhydro-2-phenyl-3-hydroxyoxazolo[3,2-*a*]pyridinium Hydroxide (39). A rapidly stirred solution of mandelic acid (7.6 g, 0.05 mol) in DMF (50 mL) was treated portionwise with NaH (5.1 g of 50% dispersion in mineral oil, 0.105 mol). When the evolution of hydrogen was complete, 2-bromopyridine (11.9 g, 0.075 mol) was added and the mixture heated at 90 °C for 6 h. Addition of Et_2O to the cooled mixture gave a white solid that was filtered off, washed with Et_2O, and then dissolved in water. Acidification to pH 1 with HCl afforded 6.0 g (52%) of 2-phenyl-2-(2-pyridyloxy)acetic acid as a solid, which was recrystallized from EtOAc/hexane, mp 117–119.5 °C dec. Anal. ($C_{13}H_{11}NO_3$) C, H, N.

This acid (2.5 g, 0.011 mol) in pyridine (3 mL) was treated with 3 mL of Ac_2O for 15 min. Addition of Et_2O gave a red solid that was filtered off and recrystallized from EtOAc to give 1.3 g (56%) of **39**, mp 148–153 °C. Anal. ($C_{13}H_9NO_2$) C, H, N.

Determination of Activity against *T. foetus*. (a) In Vitro. A broth dilution assay using microtiter plates was used to determine the minimum lethal concentration (MLC) of the test compounds. Each compound was serially diluted (200–0.2 μg/mL) in duplicate in Kupferberg's medium[31] (pH 7.2) using a microdiluter. To each well containing 50 μL of diluted compound was added 50 μL of Kupferberg's broth containing 15 parasites/mm^3. Immediately following the addition of the parasites, two drops of sterile mineral oil were placed in each well of the microtiter plate to cover the surface of the growth medium. Plates were then incubated at 30 °C for 48 h after which they were examined microscopically for the presence of viable parasites.

(b) In Vivo Assay. A hamster vaginal infection model[24] was used to determine in vivo activity. Female Golden Syrian hamsters, 2–3 weeks old (~50 g), were infected intravaginally with *T. foetus* recovered from infected reservoir hamsters with Kupferberg's medium. The challenge contained a minimum of 150 parasites/mm^3.

Three days postinfection treatments were begun on groups of five animals per test compound. Each animal received a single intravaginal treatment of 2% (w/w) formulation and two subcutaneous treatments (100 mg/kg) each day for four days.[32] Flagyl, used as a positive control, consistently gave 100% negative vaginal washings using these doses with this treatment schedule. A group of 20 hamsters was treated with placebo. All placebo-treated animals remained infected throughout each experiment.

On day 1 posttreatment and day 4 posttreatment, the vagina of each animal was washed with Kupferberg's medium (0.5 mL) and the washings were examined microscopically for viable parasites.

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Registry No. 1, 32044-03-4; **2**, 97522-57-1; **3**, 97522-58-2; **4**, 97522-59-3; **5**, 43091-16-3; **6**, 97522-60-6; **7**, 97522-61-7; **8**, 97522-62-8; **9**, 97522-63-9; **10**, 97522-64-0; 11, 97522-65-1; **12**, 97522-66-2; **13**, 97522-67-3; **14**, 97522-68-4; **15**, 43091-17-4; **16**, 97522-69-5; **17**, 97522-70-8; **18**, 97522-71-9; **19**, 97522-72-0; **20**, 97522-73-1; **21**, 43091-18-5; **22**, 97522-74-2; **23**, 43091-14-1; **24**, 43091-13-0; **25**, 35143-57-8; **26**, 32002-92-9; **27**, 97522-75-3; **28**, 97522-76-4; **29**, 97522-77-5; **30**, 97522-78-6; **31**, 97522-79-7; **32**, 97522-80-0; **33**, 97522-81-1; **34**, 97522-82-2; **35**, 43091-21-0; **36**, 43091-22-1; **37**, 66085-20-9; **38**, 97522-83-3; **39**, 97522-84-4; **40**, 97522-85-5; **41**, 97522-86-6; **42**, 18100-80-6; $PhCH(Br)CO_2H$, 4870-65-9; $PhCH(SH)CO_2^-Na^+$, 62289-54-7; PhCH(Br)CN, 5798-79-8; $PhCH(Br)CO_2Et$, 2882-19-1; butyl potassium xanthate, 871-58-9; 2-[(4-chlorobenzyl)thio]pyridine, 74032-43-2; *m*-nitromandelic acid, 42164-79-4; 2,2′-dipyridyl disulfide, 2127-03-9; 2-mercaptopyridine, 2637-34-5; mandelic acid, 90-64-2; 2-bromopyridine, 109-04-6; 2-phenyl-2-(2-pyridyloxy)acetic acid, 97522-87-7; 2-chloro-3-nitropyridine, 5470-18-8.

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5-(Haloalkyl)-2′-deoxyuridines: A Novel Type of Potent Antiviral Nucleoside Analogue

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Syntheses of 5-(2-haloethyl)-2′-deoxyuridines, 5-(3-chloropropyl)-2′-deoxyuridines, and 5-(2-chloroethyl)-2′-deoxycytidine are described. The antiviral activities of these compounds were determined in cell culture against herpes simplex virus types 1 and 2. All compounds were shown to possess significant and selective antiviral activity. The most potent derivative, 5-(2-chloroethyl)-2′-deoxyuridine (CEDU), inhibited HSV-1 at concentrations below 0.1 μg/mL. It exerted measurable inhibitory effects on cell proliferation only at concentrations higher than 100 μg/mL. In vivo CEDU reduced the mortality rate of HSV-1-infected mice at concentrations lower than 5 mg/kg per day when given intraperitoneally and orally. Thus, it proved to be more effective in this in vivo model than the reference compounds (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) and 9-[(2-hydroxyethoxy)methyl]guanine (ACV).

One of the most potent and selective antiviral agents, (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) inhibits effectively herpes simplex type 1 virus (HSV-1) and varicella zoster virus (VZV) replication in vitro[1,2] and in vivo[3-7] and shows high promise for the treatment of diseases caused by these viruses.[8-11] Studies of structure–activity relationships of BVDU and related compounds seemed to indicate that optimal anti-HSV-1 activity in cell culture was associated with analogues in which the 5-substituent

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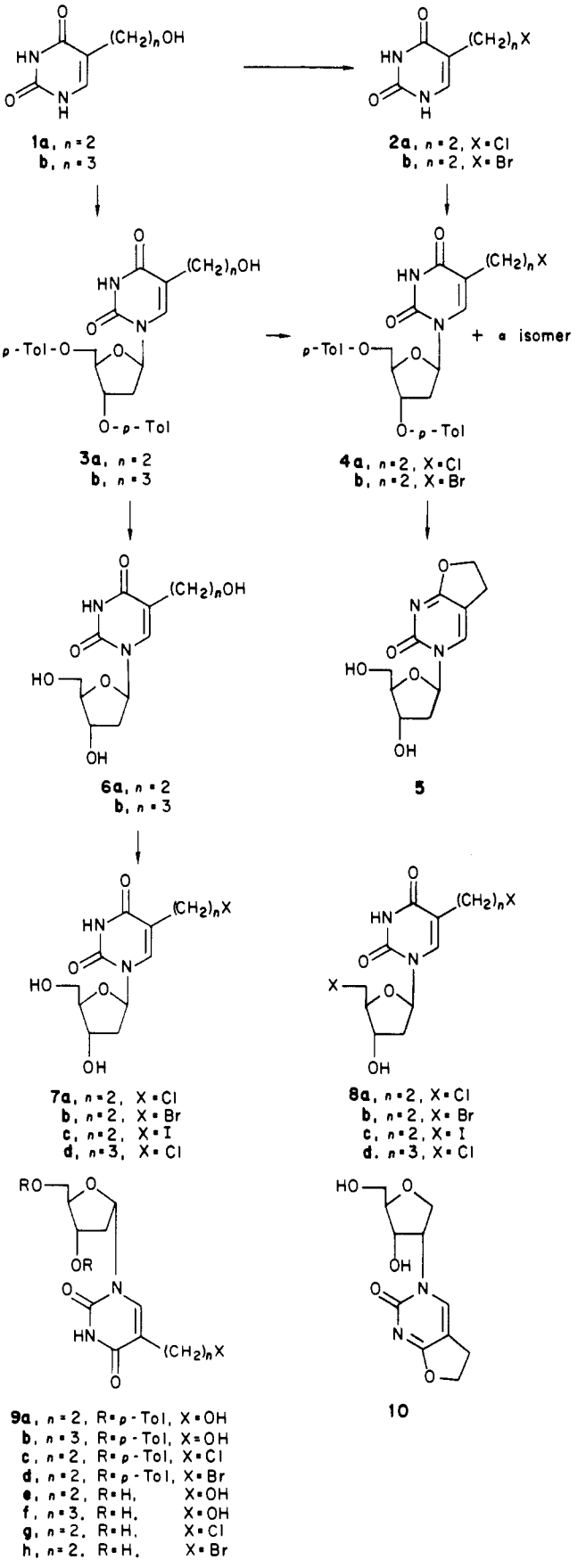
was unsaturated and conjugated with the pyrimidine ring.[12-14] The length of the side chain as well as its hydrophobicity and inductive effects also influenced antiviral activity.

In continuation of our work on structure–activity relationships of 5-substituted 2′-deoxyuridines[15] we synthesized a series of 5-haloalkyl derivatives and tested them for antiviral activity.

Chemistry

Starting from 5-(2-hydroxyethyl)uracil (**1a**)[16-18] and 5-(3-hydroxypropyl)uracil (**1b**),[19,20] respectively, the 5-(haloalkyl)-2′-deoxyuridines **7** have been obtained via halogenation, glycosylation, and deprotection with variation of the sequence of these reaction steps (Scheme I). The exchange of the hydroxy group with chlorine, bromine, or iodine has been performed with triphenylphosphine/CCl_4,[21] triphenylphosphine/*N*-bromosuccinimide,[22] or triphenylphosphine/*N*-bromosuccinimide/tetrabutylammonium iodide,[22] respectively. Glycosylation was performed by reaction of the silylated uracil derivatives **1** or **2** with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranose[23] in the presence of trimethylsilyl triflate.[24] With chloroform as solvent predominant formation of the β anomers was observed.[15] Thus, in some cases it was possible to obtain the pure β compounds by simple recrystallization. Recently, Walker[25] stressed the advantage of that solvent for glycosylation reactions in nucleoside chemistry. The corresponding α anomers **9** had to be isolated by column chromatography.

Scheme I



While there is no problem in removing the *p*-toluoyl protecting groups from the chloro derivatives **4a** and **9c** with sodium methanolate, the bromoethyl compounds **4b** and **9d** cyclized to furanopyrimidines **5** and **10**. Their

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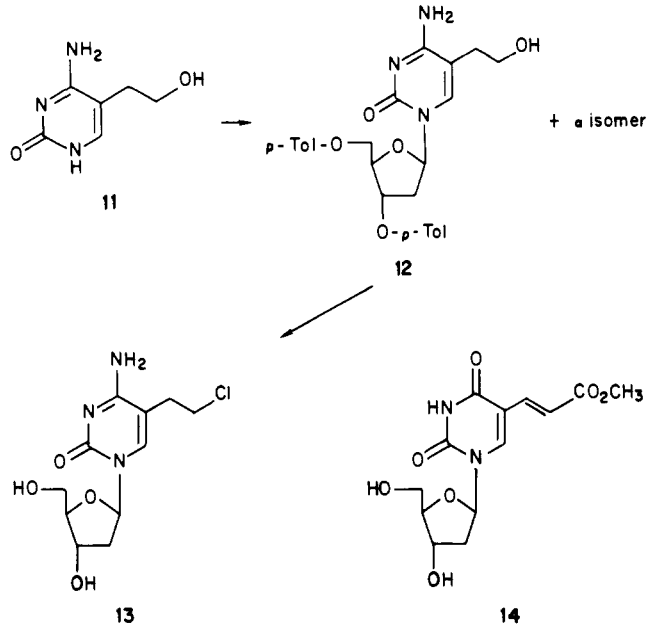
Scheme II



structures are proven by ^1H NMR spectroscopy. Instead of the triplet for e.g. CH_2Br in **4b** at 3.53 ppm (J = 7.5 Hz), a new triplet at 4.66 ppm (J = 7.5 Hz) for $COCH_2$ is observed. Hence, for the preparation of the bromo and iodo compounds **7** and **9** the halogen had to be introduced into the unprotected nucleoside analogues **6** and **9e**. As expected, a small amount of the dihalogenated products **8** is also formed as indicated by the disappearance of the ^1H NMR signals at 5.0 ppm for 5'-CH_2OH.

The cytidine analogue **13** has been synthesized according to Scheme II starting from the already known cytosine derivative **11**.[16] The reaction sequence was similar to the uridine series, but separation into pure anomers could only be achieved at the stage of the unprotected compound **13**. Configuration of all anomers was established by ^1H NMR spectroscopy.[26,27] The β anomers gave the characteristic pseudotriplet at 6.3 ppm (J = 7 Hz), whereas the α anomers showed a double doublet at 6.2 ppm (J = 3, 7 Hz). The hydroxypropyl compound **6b** was also prepared from the acrylic ester **14**[13] by catalytic hydrogenation followed by reduction of the ester group with $LiBH_4$.

Biological Results and Discussion

Antiviral activities and the toxic effects on host cells are summarized in Table I. All derivatives exert visible toxic effects only at high concentrations (≥100 μg/mL). The three 5-(2-haloethyl)-2'-deoxyuridines (**7a–c**) exhibit significant and selective inhibitory activity on HSV-1 replication; against HSV-2 they are much less active. The most potent compound of this series is 5-(2-chloroethyl)-2'-deoxyuridine (**7a**, CEDU); it inhibits HSV-1 at lower concentrations than the reference compounds araA and PFA; it is comparable to IDU and ACV and 10-fold less effective than BVDU. For the 5-bromo and 5-iodo derivatives 30–100-fold higher concentrations than for CEDU are required to give the same inhibitory effect. In the 5-halovinyl series of 2'-deoxyuridines, however, the 5-bromo derivative (BVDU) had proved to be 1.5- to 3-fold more potent than its 5-iodo and 5-chloro analogues.[28]

Table I. In Vitro Activity of Test Compounds Against HSV-1 and HSV-2[a]

compd	MTC, μg/mL	MIC, μg/mL HSV-1	MIC, μg/mL HSV-2
5	330	>100	>100
6a	330	100	100
6b	330	>100	>100
7a (CEDU)	330	0.03	3
7b	100	3	100
7c	330	1	30
7d	100	0.3	30
8a	330	100	>100
8b	330	30	100
8c	100	30	>100
8d	330	>100	>100
9g	100	3	100
9h	330	3	>100
10	330	>100	>100
14	100	0.3	30
BVDU	100	0.003	3
IDU	100	0.3	0.3
ACV	330	0.3	3
araA	100	10	10
PFA	330	10	30

[a] The lowest concentration of compound causing at least 25% inhibition of HSV-1- or HSV-2-induced cytopathic effect (MIC) and the lowest concentration causing visible toxic effects to uninfected cells (MTC) were determined in HEp-2 cells as described in the Experimental Section.

5-(3-Chloropropyl)-2'-deoxyuridine (**7d**) is a potent inhibitor of HSV-1 replication, too. As compared to CEDU, however, 10-fold higher minimal inhibitory concentrations (MIC) were determined against HSV-1. This demonstrates the importance of the length of the 5-haloalkyl side chain for antiviral activity, which has been shown before for the 5-vinyl series of 2'-deoxyuridines by Goodchild et al.[13]

Halogenation of the 5'-position significantly reduces antiviral activity (**8a–d**) as is expected for compounds that presumably must be phosphorylated at the 5'-position to exert their antiviral effect. It is not surprising either that the α anomers of 5-(bromoethyl)- and 5-(chloroethyl)-2'-deoxyuridine (**9g,h**) are less effective than the corresponding β anomers because the latter should be accepted preferentially by the enzymes involved in nucleoside phosphorylation and DNA synthesis. The 5-hydroxalkyl derivatives **6a,b** did not show antiviral activity.

5-(2-Chloroethyl)-2'-deoxycytidine (**13**) is the only representative of the cytidine series synthesized. It proved to be 10-fold less inhibitory to HSV-1 than the 2'-deoxyuridine analogue (**7a**, CEDU).

The most active representative of our 5-haloalkyl nucleoside analogues, CEDU, was chosen for further biological evaluation.

CEDU closely resembles BVDU in its pattern of virus specificity: the compound is highly active against HSV-1 and less active against HSV-2. Like BVDU,[2] it inhibits efficiently varicella zoster virus (VZV) replication but is inactive against cytomegalovirus (CMV) (details will be published elsewhere).

To estimate the toxic potential of CEDU in comparison to reference compounds growth of cells in tissue culture was monitored in the presence of various concentrations of test substances (Figure 1). CEDU and ACV at 330 μg/mL exert only a minimal inhibitory effect on the proliferation of HEp-2 cells; at 100 μg/mL no delay in cell division could be detected (data not shown). BVDU clearly is more toxic to the cells at 330 μg/mL, and this effect is also obvious at 100 μg/mL. IDU, which is known to be nonselective, completely blocks cell division at 330 μg/mL and still has some delaying effect at 30 μg/mL. Thus,

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Table II. In Vivo Activity of CEDU and Standard Compounds in Systemic HSV-1 Infection of Mice[a]

compd	treatment	percent survivors: doses, mg/kg per day							
		100	50	25	10	5	2.5	1	0
CEDU	ip	100	92	83	91	92	nt	75	
BVDU	ip	33	33	8	18	25	nt	33	
ACV	ip	91	100	100	83	75	nt	54	
control									13
CEDU	po	nt	100	100	nt	67	82	nt	
BVDU	po	nt	8	0	nt	0	0	nt	
ACV	po	nt	92	55	nt	9	0	nt	
control									0

[a] Twelve mice per group were inoculated intraperitoneally with HSV-1 (Brand) and treated with the indicated doses of test compounds either intraperitoneally or orally as described in the Experimental Section. nt = not tested.

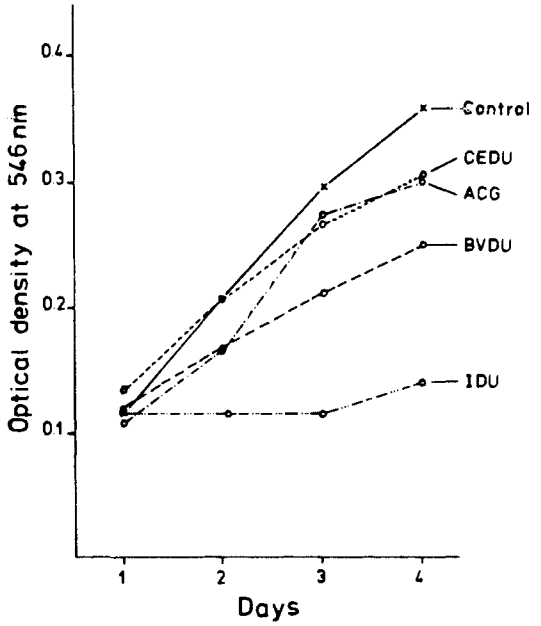

Figure 1. Growth inhibition of HEp-2 cells by CEDU and standard compounds. Cells in suspension were seeded into tissue culture plates at a low density to allow growth for 4 days before becoming contact inhibited. The test compounds were present in the culture fluid at a concentration of 330 μg/mL. Propagation of cells was monitored by staining of aliquots each day and determination of absorbance as described in the Experimental Section.

CEDU proved to be less cytotoxic than BVDU and IDU and is comparable to ACV in this regard.

In preliminary acute toxicity experiments in mice 1000 mg/kg of CEDU is tolerated without symptoms after peroral or intraperitoneal administration. In rats 450 mg/kg per day given perorally for 28 days did not induce any toxic symptoms.

The antiviral potential of CEDU could also be demonstrated in vivo. Intraperitoneal inoculation of mice with HSV-1 leads to systemic infection and finally to encephalitis and death of the animals. Table II shows that CEDU is highly effective in reducing the mortality rate of HSV-1-infected mice when given intraperitoneally or orally. Furthermore, it is protective at a dose as low as 1 mg/kg per day with intraperitoneal treatment and 2.5 mg/kg per day with oral treatment. At these doses BVDU and ACV are completely inactive orally and less active than CEDU intraperitoneally. In a further experiment, it could be demonstrated that CEDU is still highly protective when treatment is initiated as late as 24, 48, and 72 h post-infection, i.e. at a time when the virus has already reached the brain (Table III).

Thus, CEDU proves to be one of the most potent inhibitors of HSV-1-induced disease in mice. In this in vivo model it clearly surpasses BVDU and ACV in activity. Obviously, the 10-fold lower MIC values obtained for BVDU as compared to CEDU were not predictive for the in vivo activity: CEDU reduces the mortality of infected mice at considerably lower concentrations than BVDU, especially when given orally. This finding may be explained by a more favorable pharmacokinetic behavior of CEDU as compared to BVDU or ACV. Similarly, recent studies have shown that DHPG is a more potent antiviral agent than ACV in vivo, though both are comparable in vitro.[29]

Table III. Effect of Delayed Treatment with CEDU on Mortality of Systemically Infected Mice[a]

compd	treatment	percent survivors: start of treatment (h pi)			
		3	24	48	72
CEDU	ip	100	100	80	60
control		10			
CEDU	po	80	90	100	70
control		0			

[a] Ten mice per group were inoculated intraperitoneally with HSV-1 (Brand) and treated with 25 mg/kg per day of CEDU either intraperitoneally or orally starting at the times indicated after virus infection.

To summarize, 5-(2-chloroethyl)-2′-deoxyuridine (CEDU) and some of its analogues were shown to possess significant and selective antiviral activity. Previous studies of structural requirements for antiherpes activity of 5-substituted deoxyuridines had yielded the result[13,14] that optimum inhibition of HSV-1 in cell culture occurred when the 5-substituent was unsaturated and conjugated with the pyrimidine ring. The in vitro comparison of BVDU with its saturated analogue CEDU (Table I) shows the former to be more active by a factor of 10, which supports this conclusion. However, though CEDU is less potent in vitro than BVDU, it is clearly superior to the reference compounds BVDU and ACV in vivo, especially when applied orally. Further evaluation of CEDU in other animal models has confirmed the high antiviral potential of the compound and will be published elsewhere (De Clercq, E.; Rosenwirth, B., submitted for publication in *Antimicrob. Agents Chemother.*). Obviously, pharmacokinetic and other effects in animals may influence considerably the antiviral activities of test compounds in vivo and may even override the potencies determined in cell cultures.

Experimental Section

Chemistry. Melting points were determined on a Büchi melting point apparatus and are uncorrected. Column chromatography was performed on silica gel 60, 230–400 mesh, ASTM, Merck, Darmstadt, with the following eluants: A, $CHCl_3$/acetone

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(9:1, v/v); B, $CHCl_3$/methanol (9:1, v/v). All compounds were characterized by ^1H NMR on a Bruker WH-90 spectrometer with Me_4Si as standard and Me_2SO-d_6 as solvent. The spectral data were fully consistent with the structures given.

5-(2-Hydroxyethyl)-1*H*,3*H*-pyrimidine-2,4-dione (1a) was prepared according to the literature[16-18] with slight modifications via (ureidomethylene)-γ-butyrolactone: yield after two steps 71%; mp 273–274 °C (lit.[16] mp 265–275 °C).

5-(3-Hydroxypropyl)-1*H*,3*H*-pyrimidine-2,4-dione (1b) was prepared in analogy to literature[19,20] methods via 5-(3-hydroxypropyl)-2-thiouracil: yield 27% over two steps; mp 252–254 °C (lit.[20] mp 251–253 °C).

4-Amino-5-(2-hydroxyethyl)-1*H*-pyrimidin-2-one (11) was obtained from 5-(2-acetoxyethyl)uracil[25] via 5-(2-acetoxyethyl)-4-thiouracil:[16] yield 50% over two steps; mp >200% dec. Literature[16] data: yield 60%; mp >200 °C dec. 11 could be used in the following step without further purification.

Glycosylation of 1, 2a, and 11. A suspension of 10 mmol of the respective pyrimidine in 15 mL of hexamethyldisilazane and 2 mL of trimethylchlorosilane was refluxed for 3 h. The resultant solution was evaporated in vacuo to an oil that was dissolved in 20 mL of xylene and reevaporated. To this syrup in 50 mL of dry $CHCl_3$ were added 3.88 g (10 mmol) 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranose[23] and 0.1 mL of trimethylsilyl triflate. After stirring at room temperature 1 h, the reaction mixture was extracted once with saturated $KHCO_3$ solution. The aqueous phase was washed with 50 mL of $CHCl_3$, and the combined organic layers were evaporated to dryness to leave a mixture of the anomers as oils.

1-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-5-(2-hydroxyethyl)-1*H*,3*H*-pyrimidine-2,4-dione (3a) and 1-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl)-5-(2-hydroxyethyl)-1*H*,3*H*-pyrimidine-2,4-dione (9a). The oil obtained as above starting from 1a was treated with 20 mL of boiling toluene. After cooling, a crystalline mixture of the anomers (4.27 g (84%)) with melting point 165–167 °C and an anomer ratio of $\beta/\alpha = 5/1$ was obtained. Usually this mixture was used in the next steps without further purification; analytical samples were obtained by column chromatography, solvent A. **3a**: mp 176–178 °C (ethanol). Anal. ($C_{27}H_{28}N_2O_8$) C, H, N. **9a**: mp 97–99 °C (ethanol). Anal. ($C_{27}H_{28}N_2O_8$) C, H, N.

1-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-5-(3-hydroxypropyl)-1*H*,3*H*-pyrimidine-2,4-dione (3b) and 1-(2-Deoxy-3,5-di-*O*-p-toluoyl-α-D-*erythro*-pentofuranosyl)-5-(3-hydroxypropyl)-1*H*,3*H*-pyrimidine-2,4-dione (9b). These two anomers were obtained as a chromatographically inseparable mixture through glycosylation of 1b that were used in the following step without further purification.

5-(2-Chloroethyl)-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-1*H*,3*H*-pyrimidine-2,4-dione (4a) and 5-(2-Chloroethyl)-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl)-1*H*,3*H*-pyrimidine-2,4-dione (9c). The anomeric mixture obtained from **2a** was recrystallized from ethanol to yield 3.43 g (65%) of **4a**, mp 167 °C. Anal. ($C_{27}H_{27}ClN_2O_7$) C, H, N, Cl. Evaporation of the mother liquor and column chromatography with solvent A gave 0.79 g (15%) of **9c**, mp 142–144 °C. Anal. ($C_{27}H_{27}ClN_2O_7$) C, H, N, Cl.

4-Amino-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl)-5-(2-hydroxyethyl)-1*H*-pyrimidine-2-one (12). The residue obtained by glycosylation of 11 was dissolved in 100 mL of ethanol/water (80/20, v/v) and the resultant mixture refluxed for 20 min. After removal of the solvents in vacuo, the anomeric mixture of 12 was purified by column chromatography with solvent B to yield 4.0 g (79%, syrup). Anal. ($C_{27}H_{29}N_3O_7$) C, H, N.

Halogenation of 1a and 3a/9a. Triphenylphosphine (5.2 g, 20 mmol) was added to a solution of 10 mmol of the corresponding pyrimidine derivative in 50 mL of DMF. After the mixture was stirred for 10 min, 15 mmol of the halogenating agent (carbon tetrachloride/pyridine or *N*-bromosuccinimide (NBS)) were added, and the solution was kept at room temperature until no more starting material could be detected by TLC (usually 1.5 h). Then, 5 mL of 1-butanol was added, and the solvents were removed in vacuo. Starting from **3a**, 2.9 g (55%) of **4a** identical with the material described above was obtained after recrystallization from 50 mL of ethanol.

5-(2-Chloroethyl)-1*H*,3*H*-pyrimidine-2,4-dione (2a). Treatment of the reaction mixture obtained through chlorination of 1a with 70 mL of solvent B gave 1.65 g (90%) of **2a**, which was sufficiently pure to be used in the following step; mp 260–262 °C (ethanol). Anal. ($C_6H_7ClN_2O_2$) C, H, N.

5-(2-Bromoethyl)-1*H*,3*H*-pyrimidine-2,4-dione (2b).[18] The compound was isolated as described for **2a** to yield 1.47 g (67%), mp 253 °C (ethanol) (lit.[18] mp 262–263 °C).

5-(2-Bromoethyl)-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-1*H*,3*H*-pyrimidine-2,4-dione (4b) and 5-(2-Bromoethyl)-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl)-1*H*,3*H*-pyrimidine-2,4-dione (9d). Recrystallization of the bromination reaction mixture obtained from **3a/9a** gave 4.06 g (71%) of **4b**, mp 158–161 °C. Anal. ($C_{27}H_{27}BrN_2O_7$) C, H, N, Br. Evaporation of the mother liquor and column chromatography with solvent A gave 0.86 g (15%) of **9d**, mp 134–137 °C. Anal. ($C_{27}H_{27}BrN_2O_7$) C, H, N, Br.

Chlorination and Deblocking of 12. A solution of 13.19 g (26 mmol) of 12 and 9.4 g (36 mmol) of triphenylphosphine in 100 mL of DMF was treated with 6 mL of pyridine and 3 mL of CCl_4. The mixture was stirred at room temperature until no more starting material could be detected by TLC. After addition of 30 mL of 1-butanol the solvents were removed in vacuo; the residue was taken up in 100 mL ethanol and treated with 50 mL of 1 N NaOEt/EtOH. After stirring at room temperature for 10 min, the solution was neutralized with 1 N acetic acid and evaporated to dryness. The residue was dissolved in water. After washing with $CHCl_3$ and ether and evaporation to dryness, the residue was stirred with 10 mL of ethyl acetate/methanol (3/1, v/v). The precipitate was filtered off and recrystallized from water to yield 1.88 g (25%) of the pure β isomer 4-amino-5-(2-chloroethyl)-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1*H*-pyrimidin-2-one (13), mp 174 °C. Anal. ($C_{11}H_{16}ClN_3O_4$) C, H, N, Cl.

Deblocking of Nucleosides 3a/9a, 3b/9b, 4a, and 9c. A suspension of 10 mmol of the corresponding blocked 2'-deoxynucleoside (mixture of **3a/9a**, **3b/9b**, or pure anomers **4a** or **9c**) in 100 mL of dry ethanol was treated with 20 mL of 1 N NaOEt/EtOH and stirred at room temperature until the reaction was complete. After neutralization with 1 N acetic acid, the solvents were removed in vacuo, and the residue was taken up in water. The aqueous solution was extracted once with ether and the aqueous phase evaporated in vacuo to dryness.

The resulting β anomers **6a**, **6b**, and **7a** as well as the α anomer **9g** were obtained pure by recrystallization from ethanol; the α anomers **9e** and **9f** were isolated by evaporation of the corresponding mother liquors and column chromatography with solvent B.

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-(2-hydroxyethyl)-1*H*,3*H*-pyrimidine-2,4-dione (6a): yield 1.93 g (71%); mp 160–161 °C. Anal. ($C_{11}H_{16}N_2O_6$) C, H, N.

1-(2-Deoxy-α-D-*erythro*-pentofuranosyl)-5-(2-hydroxyethyl)-1*H*,3*H*-pyrimidine-2,4-dione (9j): yield 0.41 g (15), syrup. Anal. ($C_{11}H_{16}N_2O_6$) C, H, N.

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-(3-hydroxypropyl)-1*H*,3*H*-pyrimidine-2,4-dione (6b): yield 1.09 g (38%); mp 139–142 °C. Anal. ($C_{12}H_{18}N_2O_6$) C, H, N.

1-(2-Deoxy-α-D-*erythro*-pentofuranosyl)-5-(3-hydroxypropyl)-1*H*,3*H*-pyrimidine-2,4-dione (9f): yield 0.38 g (13%), syrup. Anal. ($C_{12}H_{18}N_2O_6$) C, H, N.

5-(2-Chloroethyl)-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1*H*,3*H*-pyrimidine-2,4-dione (7a): yield 2.62 g (90%); mp 166–167 °C. Anal. ($C_{11}H_{15}ClN_2O_5$) C, H, N, Cl.

5-(2-Chloroethyl)-1-(2-deoxy-α-D-*erythro*-pentofuranosyl)-1*H*,3*H*-pyrimidine-2,4-dione (9g): yield 2.41 g (83%); mp 140–142 °C. Anal. ($C_{11}H_{15}ClN_2O_5$) C, H, N, Cl.

Cyclization and Deblocking of 4b and 9d. A solution of 1.5 g of **4b** or **9d**, respectively (2.6 mmol), in 100 mL of methanol, saturated with ammonia at 0 °C, was stirred at room temperature for 4 days. After removal of the solvent in vacuo, the residue was crystallized from acetone.

5-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-2,3-dihydro-5*H*-furano[2,3-*d*]pyrimidin-6-one (5): yield 0.40 g (56%); mp 206–208 °C. Anal. ($C_{11}H_{14}N_2O_5$) C, H, N.

5-(2-Deoxy-α-D-*erythro*-pentofuranosyl)-2,3-dihydro-5*H*-furano[2,3-*d*]pyrimidin-6-one (10): yield 0.44 g (66%); mp 184–186 °C. Anal. ($C_{11}H_{14}N_2O_5$) C, H, N.

Halogenation of the Deblocked Nucleosides 6a, 6b, and 9e. This halogenation was performed as described above; for iodination, a mixture of 15 mmol of NBS and 40 mmol of tetrabutylammonium iodide was used. The mono- and dihalogenated products **7**, **8**, **9g**, and **9h** were purified by column chromatography with solvent B. **7a** was obtained in 52% yield by this route.

1-(5-Chloro-2,5-dideoxy-β-D-*erythro*-pentofuranosyl)-5-(2-chloroethyl)-1*H*,3*H*-pyrimidine-2,4-dione (8a): yield 0.26 g (9%); mp 210–212 °C (ethanol). Anal. ($C_{11}H_{14}Cl_2N_2O_4$) C, H, N, Cl.

5-(2-Bromoethyl)-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1*H*,3*H*-pyrmidine-2,4-dione (7b): yield 1.47 g (44%); mp 161–163 °C (ethanol/chloroform). Anal. ($C_{11}H_{15}BrN_2O_5$) C, H, N, Br; C: calcd, 33.19; found, 33.89.

1-(5-Bromo-2,5-dideoxy-β-D-*erythro*-pentofuranosyl)-5-(2-bromoethyl)-1*H*,3*H*-pyrimidine-2,4-dione (8b): yield 0.64 g (16%); mp 175–177 °C (ethanol). Anal. ($C_{11}H_{14}Br_2N_2O_4$) C, H, N, Br.

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-(2-iodoethyl)-1*H*3*H*-pyrimidine-2,4-dione (7c): yield 0.34 g (9%); mp 160–161 °C (ethanol). Anal. ($C_{11}H_{15}IN_2O_5$) C, H, N, I; C: calcd, 26.85; found, 28.69.

1-(5-Iodo-2,5-dideoxy-β-D-*erythro*-pentofuranosyl)-5-(2-iodoethyl)-1*H*,3*H*-pyrimidine-2,4-dione (8c): yield 0.49 g (10%); mp 165–168 °C (ethanol). Anal. ($C_{11}H_{14}I_2N_2O_4$) C, H, N, I.

5-(3-Chloropropyl)-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1*H*,3*H*-pyrimidine-2,4-dione (7d): yield 1.43 g (47%); mp 107–110 °C (ethyl acetate). Anal. ($C_{12}H_{17}ClN_2O_5$) C, H, N, Cl.

1-(5-Chloro-2,5-dideoxy-β-D-*erythro*-pentofuranosyl)-5-(3-chloropropyl)-1*H*,3*H*-pyrimidine-2,4-dione (8d): yield 0.26 g (8%); mp 165–167 °C (ethanol). Anal. ($C_{12}H_{16}Cl_2N_2O_4$) C, H, N, Cl.

5-(2-Bromoethyl)-1-(2-deoxy-α-D-*erythro*-pentofuranosyl)-1*H*,3*H*-pyrimidine-2,4-dione (9h): yield 1.14 g (34%); mp 131–133 °C (ethanol). Anal. ($C_{11}H_{15}BrN_2O_5$) C, H, N, Br.

Reduction of 14 to 6b. A solution of 2.8 g (9 mmol) of 14[23] in methanol was hydrogenated with palladium (5% on charcoal) at 3 bar for 15 h. After removal of the catalyst the solution was evaporated to dryness, and the residue was dissolved in 20 mL of hexamethyldisilazane, treated with 0.2 mL of trimethylchlorosilane, and refluxed for 2 h. Excess hexamethyldisilazane was removed in vacuo and the residue taken up in 30 mL of ether and treated with 175 mg (8 mmol) of $LiBH_4$. After the mixture was refluxed for 4 h, water was cautiously added and the mixture saturated with CO_2(g) and evaporated to dryness in vacuo. The residue was heated with 50 mL of methanol and filtered and the filtrate again evaporated to dryness. After column chromatography (CH_2Cl_2/MeOH, 8/2 (v/v) as eluant) 1.15 g (40%) of **6b** was obtained, identical with the material described above.

Biology

Materials. Cells. HEp-2 cells were obtained from the American Type Culture Collection, Rockville, MD.

Viruses. Herpes simples virus type 1 (HSV-1) strain Brand was originally isolated by H. Zur Hausen in Erlangen and HSV-2 strain K979 by B. Vestergaard in Copenhagen; they were kindly provided by M. Scriba, Vienna. Cytomegalovirus (CMV) strain Towne was obtained from B. Fleckenstein, Erlangen. Varicella zoster virus (VZV) strain Ellen was purchased from the American Type Culture Collection, Rockville, MD.

Mice. Hairless (hr/hr) mice were obtained from Bomholtgard, Ry, Denmark.

Compounds. The reference compound (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) was synthesized according to Jones et al.[30] and 9-[(2-hydroxyethoxy)-methyl]guanosine (Acyclovir, ACV) according to Schaeffer et al.[31] 9-β-D-Arabinofuranosyladenine (araA) were obtained from Sigma Chemical Corp., St. Louis, MO. 5-Iododeoxyuridine (IDU) was purchased from Mack, Illertissen, FRG.

Methods. In Vitro Antiviral Assays. Cytopathic effect (CPE) inhibition assays with HSV-1 and -2 were performed as follows: Serial 3-fold dilutions of test compound in Eagle's minimum essential medium (E-MEM) were prepared in flat-bottomed microtiter plates. Equal parts of virus in E-MEM and cell suspension in E-MEM plus 15% fetal calf serum were added. Virus-infected cultures without compound were included as control as were uninfected cells treated with compound. The cell concentration was adjusted to give a monolayer after 1–2 days of incubation, the virus dilution to induce a 90–100% complete CPE in the infected controls after 3 days. At this time, the plates were fixed and stained, and the extent of virus-induced CPE in infected controls and in drug-treated wells was estimated.

Cellular Toxicity Assay. HEp-2 cells suspended in culture medium containing test compound at various concentrations were seeded into 24-well tissue culture dishes (1×10^5 cells/well); four wells were used per variable. After 1, 2, 3, and 4 days aliquots of the cells were stained with 0.01% neutral red in phosphate-buffered saline (PBS) for 2 h at 37 °C, washed with PBS, and dried. The stained cells were dissolved in 1.5 mL/well ethanol/0.1 M NaH_2PO_4 (1/1), and the optical density of the solution was measured at 546 nm. The absorbance has been found to be proportional to the cell number.

In Vivo Antiviral Assay. Hairless mice (hr/hr) weighing 18–20 g were inoculated intraperitoneally with HSV-1 (Brand) at 2×10^5 PFU/0.2 mL per mouse and treated twice a day either intraperitoneally or orally (by gavage) with the doses of test compound indicated. Treatment was for 10 days starting 1 h after virus infection (Table II) unless indicated otherwise (Table III).

Statistical significance of the differences in the final mortality rates was assessed by the χ^2 test.

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Registry No. **1a**, 23956-12-9; **1b**, 53761-38-9; **2a**, 90301-68-1; **2b**, 53438-96-3; **3a**, 97974-93-1; **3b**, 97974-94-2; **4a**, 90301-69-2; **4b**, 97974-96-4; **5**, 97975-00-3; **6a**, 90301-60-3; **6b**, 97974-98-6; **7a**, 90301-59-0; **7b**, 90301-64-7; **7c**, 90301-67-0; **7d**, 97975-05-8; **8a**, 97975-02-5; **8b**, 97975-03-6; **8c**, 97975-04-7; **8d**, 97975-06-9; **9a**, 90301-62-5; **9b**, 97974-95-3; **9c**, 90301-63-6; **9d**, 97974-97-5; **9e**, 90301-66-9; **9f**, 97974-99-7; **9g**, 90301-61-4; **9h**, 90301-65-8; **10**, 97975-01-4; **11**, 89711-99-9; **β-12**, 90301-76-1; **13**, 90301-75-0; **14**, 96244-97-2; 5-(2-acetoxyethyl)uracil, 90301-77-2; 5-(2-acetoxyethyl)-4-thiouracil, 92223-84-2; 1-chloro-2-deoxy-3,5-di-*O*-*P*-toluoyl-D-*erythro*-pentofuranose, 3601-89-6.

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