The effects compounds 11, 12, and 15 on the multiplication of mouse myeloma cells SP2 was assessed by growing these cells in suspension and determining their number at 0, 48, and 96 h. The test compounds were dissolved in the growth medium at a concentration of 100, 10, or 1 μg/mL or less.

Assays on Animals. For evaluation of the acute toxicity for mice, the compounds were suspended with 0.2 g of Tween 80 and 0.2 g of (carboxymethyl)cellulose/100 mL of distilled water and administered intraperitoneally to Swiss mice (Iffa Credo, 20–24 g) as a single dose of 100–2.000 mg/kg. The follow-up period was 14 days.

For evaluation of the effects of the compounds on herpetic encephalitis, young mice, weighing 10 g, were inoculated intracerebrally with 0.03 mL of an herpes simplex virus suspension containing ca. 4 $CCID_{50}$. The compounds were administered at 75 or 150 mg/kg per day, given as two daily intraperitoneal injections, for 4 days. This treatment was started immediately after virus inoculation. The animals were observed during 21 days.

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Synthesis and Antiviral Activity of (*E*)-5-(2-Bromovinyl)uracil and (*E*)-5-(2-Bromovinyl)uridine

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(*E*)-5-(2-Bromovinyl)uracil (BVU) and (*E*)-5-(2-bromovinyl)uridine (BVRU) were synthesized starting from 5-formyluracil via (*E*)-5-(2-carboxyvinyl)uracil or starting from 5-iodouridine via (*E*)-5-(2-carbomethoxyvinyl)uridine and (*E*)-5-(2-carboxyvinyl)uridine, respectively. Depending on the choice of the cell system, BVU and BVRU exhibited a marked activity against herpes simplex virus type 1 (HSV-1) in vitro. Although BVU and BVRU were less potent than the reference compound (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU), their antiviral activity spectrum was remarkably similar to that of BVDU. The latter findings suggest that BVU and BVRU are metabolically converted to BVDU or a phosphorylated product thereof. In vivo, BVU protected mice against a lethal disseminated HSV-1 infection.

BVDU [(*E*)-5-(2-bromovinyl)-2′-deoxyuridine]ranks among the most potent and selective inhibitors of herpes simplex virus type 1 (HSV-1) and varicella-zoster virus (VZV) replication.[1-3] It appears to be a promising drug for the systemic (oral) treatment of VZV infections in immunosuppressed patients,[4-6] as well as for topical treatment of HSV-1 keratitis.[7,8] However, BVDU is not a very efficient inhibitor of herpes simplex virus type 2 (HSV-2),[2] and in attempts to potentiate its activity against HSV-2, several derivatives of BVDU have been prepared with modification in either the pyrimidine or sugar moiety: i.e. IVDU [(*E*)-5-(2-iodovinyl)-2′-deoxyuridine],[1] (*E*)-5-(1-propenyl)-2′-deoxyuridine,[9,10] (*E*)-5-(3,3,3-trifluoro-1-propenyl)-2′-deoxyuridine,[11] (*E*)-5-(2-bromovinyl)-2′-deoxycytidine,[12] 1-β-D-arabinofuranosyl-(*E*)-5-(2-bromovinyl)uracil,[13,14] (*E*)-5-(2-bromovinyl)-3′-amino-2′,3′-dideoxyuridine,[15] and the carbocyclic analogues of BVDU and IVDU.[16]

All these BVDU analogues share a common activity spectrum, in that they are very active against HSV-1 and VZV, much less active against HSV-2, and inactive against dThd kinase-deficient (TK^-) strains of HSV-1. It has also become obvious that these analogues follow a mechanism of action that is similar, if not identical, to that of BVDU itself. The mode of action of BVDU is essentially based upon (i) a specific affinity for the virus-encoded dThd

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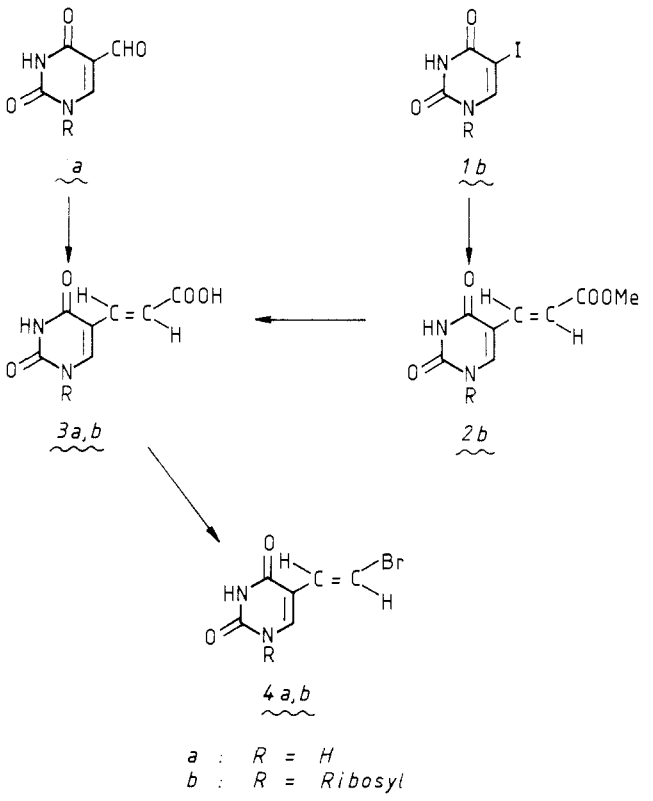
Scheme I



kinase[17] which ensures a preferential phosphorylation by the virus-infected cell,[18] and (ii) a specific interaction of BVDU 5′-triphosphate as either inhibitor[19,20] or substrate[20,21] of the viral DNA polymerase, which may eventually lead to the incorporation of BVDU into viral DNA.[22]

We now report on the antiviral activity of two new BVDU analogues, i.e. BVU [(*E*)-5-(2-bromovinyl)uracil] and BVRU [(*E*)-5-(2-bromovinyl)uridine]. Being a pyrimidine base and pyrimidine ribonucleoside respectively, BVU and BVRU cannot, as such, function via the dThd kinase–DNA polymerase pathway followed by BVDU. Yet, BVU and BVRU displayed an antiviral activity spectrum that was remarkably similar to that of BVDU, suggesting that somehow BVU and BVRU had to be processed via the dThd kinase–DNA polymerase pathway. Furthermore, BVU was found to exert a marked protective effect in mice against disseminated HSV-1 infection. These findings have both fundamental and practical implications, as to (i) the metabolic conversion of BVU and BVRU within the cell and (ii) their therapeutic potential in the treatment of herpes virus infections.

Chemistry. BVU (**4a**) was synthesized from 5-formyluracil (**1a**) via (*E*)-5-(2-carboxyvinyl)uracil (**3a**) (Scheme I) by published methods.[23-25] Compound **1a** was converted to **3a** upon treatment with malonic acid in the presence of piperidine; treatment of **3a** with *N*-bromosuccinimide in aqueous solution gave **4a**.

The synthesis of BVRU was achieved via an intermediate organopalladium derivative, essentially as described previously for BVDU.[26] BVRU (**4b**) was obtained from (*E*)-5-(2-carboxyvinyl)uridine (**3b**) by treatment with *N*-bromosuccinimide in *N*,*N*-dimethylformamide; **3b** itself was obtained from (*E*)-5-(2-carbomethoxyvinyl)uridine (**2b**) by alkaline hydrolysis. The latter was prepared by reaction of 5-iodouridine (**1b**) with methyl acrylate in dioxane in the presence of palladium(II) acetate, triphenylphosphine, and triethylamine. BVRU has also been synthesized by Ötvös and his colleagues.[27]

Biological Activity. BVDU is a potent inhibitor of the replication of HSV-1, irrespective of the choice of the cell system.[28] Depending on the nature of the cells, the minimum inhibitory concentration (MIC_{50}) of BVDU for HSV-1 may vary by 1 to 2 orders of magnitude (Table I).

Table I. Antiviral Activity of BVU (**4a**) and BVRU (**4b**) in Different Cell Lines

cell line	MIC_{50},[a] μg/mL		
	4a	**4b**	BVDU
PRK	>400	7	0.007
BALB/3T3	>400	>400	0.1
MO	>400	>400	0.007
Vero	>400	>400	0.4
BS-C-1A	>400	>400	0.2
HSF (VGS)	7	1	0.04
E_1SM	7	1	0.02
E_6SM	7	4	0.02
M-21 (GM137)	1	4	0.02
T-21 (LR)	7	10	0.02
HEp-2	>200	150	0.2
HeLa	2	0.4	0.04

[a] Minimum inhibitory concentration required to reduce the cytopathogenicity of HSV-1 (KOS) by 50%. No microscopically detectable cytotoxicity was observed with any of the compounds at a concentration up to 400 μg/mL.

Table II. Antiviral Activity Spectrum of BVU (**4a**) and BVRU (**4b**) in E_6SM Cell Cultures

virus	MIC_{50},[a] μg/mL		
	4a	**4b**	BVDU
HSV-1 (KOS)	7	4	0.02
HSV-1 (F)	4	7	0.02
HSV-1 (McIntyre)	4	2	0.02
HSV-2 (G)	>400	>400	2
HSV-2 (196)	>400	>400	10
HSV-2 (Lyons)	>400	>400	4
TK^- HSV-1 (B2006)	>200	>200	40
vaccinia	>400	>400	7
vesicular stomatitis	>400	>400	>400

[a] Minimum inhibitory concentration required to reduce virus-induced cytopathogenicity by 50%.

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Table III. Reversal of Antiviral Activity of BVU (4a) and BVRU (4b) upon Addition of dThd

dThd concn, μg/mL	MIC_{50},[a] μg/mL		
	4a	4b	BVDU
300	>400	>400	>400
100	>400	>400	300
30	>400	>400	70
10	70	150	0.4
3	4	70	0.02
1	7	10	0.02
0	7	4	0.02

[a] Minimum inhibitory concentration required to reduce the cytopathogenicity of HSV-1 (KOS) in E_6SM cell cultures by 50%; dThd did not interfere with viral cytopathogenicity at the highest concentration tested.

In contrast with BVDU, BVU and BVRU proved totally inactive as HSV-1 inhibitors in a number of murine and simian cell lines. In various human cell lines, however, BVU and BVRU exhibited an MIC_{50} that was only 10- to 100-fold higher than that recorded for BVDU under the same conditions. This rather potent antiviral activity of BVU and BVRU was noted in both aneuploid (i.e. HeLa) and diploid (i.e. E_6SM) human cell cultures.

Neither BVU nor BVRU caused a microscopically detectable alteration of normal cell morphology at concentrations up to 400 μg/mL (Table I). When evaluated for their ability to induce sister chromatid exchange (SCE), an indicator of mutagenesis, in human lymphocytes, BVU and BVRU were positive at 200 μg/mL but negative at 50 μg/mL; similarly, BVDU was positive in inducing SCE at a concentration of 200 μg/mL but negative at 50 μg/mL (E. De Clercq and J.-J. Cassiman, unpublished data).

As further demonstrated in E_6SM cell cultures (Table II), BVU and BVRU were equally inhibitory to several HSV-1 strains but not to HSV-2, vaccinia virus, vesicular stomatitis virus, or TK^- HSV-1 (a dThd kinase deficient mutant of HSV-1). Thus, the antiviral activity spectrum of BVU and BVRU is remarkably similar to that of BVDU, which is inactive against vesicular stomatitis virus and only inhibitory to HSV-2, vaccinia virus or TK^- HSV-1 at a concentration 100- to 1000-fold higher than that required for inhibition of HSV-1 replication (in E_6SM cells, Table II; in PRK cells, ref 2).

The latter findings suggested that BVU and BVRU may operate through a similar mechanism as BVDU, and in particular their inactivity against TK^- HSV-1 indicated that their antiviral action, like that of BVDU, depended on a phosphorylation by the virus-encoded dThd kinase. That BVU and BVRU at some stage of their intracellular metabolism must be converted to BVDU, or a phosphorylated product thereof, became clear by experiments in which dThd was added to the cell culture medium. That the antiviral activity of BVDU is readily reversed upon the addition of dThd is a well-established phenomenon.[29] It now appeared that the antiviral effects of BVU and BVRU were equally well reversed by dThd: within the concentration range of 3–30 μg/mL, dThd abrogated their inhibitory effects on HSV-1 replication in E_6SM cells (Table III).

To determine the role of pyrimidine nucleoside phosphorylases in the antiviral activity of BVU and BVRU, their inhibitory effects on HSV-1 replication were measured in the presence of 6-aminothymine and benzylacyclouridine. 6-Aminothymine is an inhibitor of both dThd phosphorylase and Urd phosphorylase,[30,31] whereas benzylacyclouridine is a specific inhibitor of Urd phosphorylase.[32,33] Benzylacyclouridine did not affect the antiviral activity of BVU or BVRU, whereas 6-aminothymine blocked the antiviral activity of BVU but not that of BVRU (Table IV). Neither benzylacyclouridine nor 6-aminothymine influenced the antiviral activity of the parent compound BVDU.

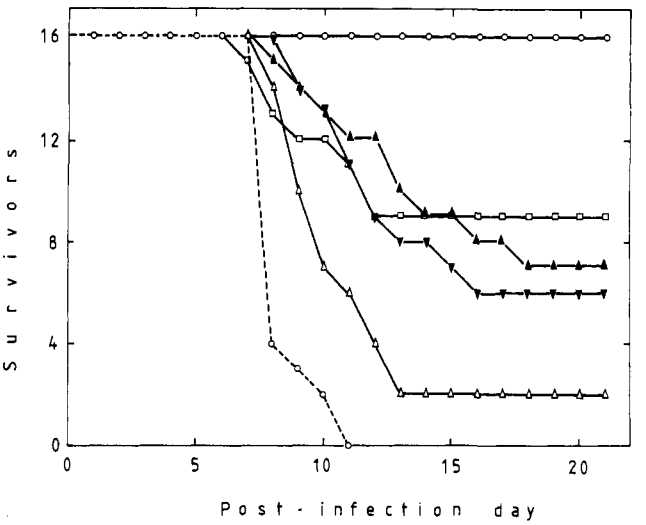

Figure 1. Effects of BVU and BVDU against disseminated HSV-1 infection in C_3H mice. The compounds were administered orally by gavage b.i.d. starting 8 h after intraperitoneal HSV-1 inoculation and continuing for 5.5 days. Symbols: O---O, control; O–O, BVU at 150 mg/kg; □–□, BVU at 50 mg/kg; △–△, BVU at 15 mg/kg; ▲–▲, BVDU at 15 mg/kg; ▼–▼, BVDU at 5 mg/kg.

The blocking effect of 6-aminothymine on BVU could theoretically be attributed to an inhibition of either Urd phosphorylase or dThd phosphorylase. To distinguish between these two possibilities, attempts were made to reverse the counter effect of 6-aminothymine by the addition of Urd or dUrd. In these experiments dUrd was chosen instead of dThd, since dUrd, unlike dThd (Table III), was by itself not detrimental to the antiviral activity of BVU. In fact, dUrd had a potentiating effect on the antiviral activity of BVU, since the MIC_{50} of the latter was decreased by 10-fold in the presence of 10 or 100 μg/mL of dUrd (Table V). More importantly, dUrd completely reversed the counteracting effect of 6-aminothymine on the antiviral activity of BVU. Its MIC_{50} for HSV-1 decreased from >400 to 7–10 μg/mL upon the addition of dUrd at 10 or 100 μg/mL (Table V).

In marked contrast with dUrd, Urd failed to reverse the counteracting effect of 6-aminothymine on the antiviral activity of BVU. The MIC_{50} of BVU (in the presence of 50 μg of 6-aminothymine/mL) for HSV-1 remained at >400 μg/mL even upon addition of 100 μg of Urd/mL (Table V). The antiviral activity of BVDU was not markedly altered by the addition of either Urd or dUrd, except for a 10- to 20-fold decrease in activity upon addition of 100 μg of dUrd/mL (Table V). This drop in antiviral activity is relatively small compared to the reversing effect of dThd on the antiviral activity of BVDU (Table III).

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Table IV. Counteracting Effect of Inhibitors of Pyrimidine Nucleoside Phosphorylases on Antiviral Activity of BVU (**4a**) and BVRU (**4b**)

	MIC_{50},[a] μg/mL								
	4a			**4b**			BVDU		
inhibitor concn, μg/mL	E_6SM	HeLa	PRK	E_6SM	HeLa	PRK	E_6SM	HeLa	PRK
6-aminothymine									
100	>400	>200	>400	4	1	10	0.02	0.01	0.01
20	>400	>200	>400	3	0.4	10	0.02	0.01	0.01
4	>400	>200	>400	3	0.4	10	0.02	0.01	0.01
0	7	2	>400	4	0.2	10	0.02	0.02	0.01
benzylacyclouridine									
50	4	...[b]	...	10	...	...	0.04	...	...
10	4	...	...	4	...	...	0.02	...	...
0	4	...	...	4	...	...	0.02	...	...

[a] Minimum inhibitory concentration required to reduce the cytopathogenicity of HSV-1 (KOS) by 50%; 6-aminothymine and benzylacyclouridine did not interfere with viral cytopathogenicity at the highest concentration tested. [b] Not determined.

Table V. Reversal of the Counteracting Effect of 6-Aminothymine on Antiviral Activity of BVU (**4a**) by Urd and dUrd

Urd or dUrd concn, μg/mL	plus or minus 6-aminothymine (50 μg/mL)	MIC_{50},[a] μg/mL	
		4a	BVDU
Urd			
100	-	4	0.02
10	-	7	0.04
1	-	2	0.04
0	-	4	0.02
100	+	>400	0.02
10	+	>400	0.04
1	+	>400	0.02
0	+	>400	0.02
dUrd			
100	-	0.7	0.2
10	-	0.7	0.02
1	-	10	0.01
0	-	7	0.02
100	+	7	0.2
10	+	10	0.02
1	+	>400	0.01
0	+	>400	0.01

[a] Minimum inhibitory concentration required to reduce the cytopathogenicity of HSV-1 (KOS) in E_6SM cell cultures by 50%; neither Urd (or dUrd) nor 6-aminothymine interfered with viral cytopathogenicity at the concentrations tested.

When evaluated for its in vivo efficacy in a mouse model of disseminated HSV-1 infection,[34] BVU effected a significant increase in the survival rate, when administered at a dose of 150 or 50 mg/kg twice daily for 5.5 days (Figure 1). At a dosage of 50 mg/kg, BVU was about as active as BVDU at 15 mg/kg. In terms of the mean survival time (t), all BVU and BVDU dosage regimens conferred significant protection: t was raised from 205 h (control) to 440 h ($p < 0.001$) with BVDU at 5 mg/kg, 502 h ($p < 0.001$) with BVDU at 15 mg/kg, 277 h ($p < 0.01$) with BVU at 15 mg/kg, 512 h ($p < 0.001$) with BVU at 50 mg/kg, and >1000 h ($p < 0.001$) with BVU at 150 mg/kg. Statistical significance was assessed by logrank comparison of the survival times between the treated and untreated group (16 mice per group).

Discussion

The characteristic activity spectrum of BVU and BVRU, their inactivity against TK^- HSV-1 (Table II), and reversal of antiviral activity upon addition of dThd (Table III)

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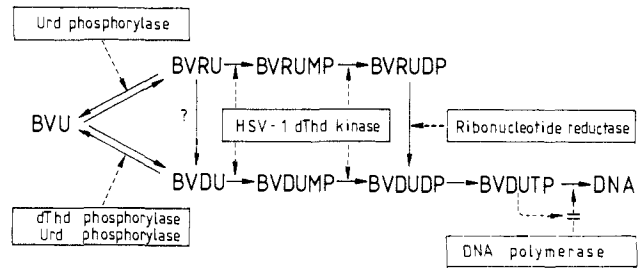
Scheme II



suggest that BVU and BVRU, at some stage of their intracellular metabolism, must be converted to BVDU (or BVDU 5′-mono-, di-, or triphosphate) (Scheme II). Support for a direct conversion of BVU to BVDU stems from (i) the counteracting effect of 6-aminothymine, a well-known inhibitor of pyrimidine nucleoside phosphorylases, on the antiviral activity of BVU (Table IV) and (ii) the reversal of this counteraction by dUrd (Table V). The formation of BVDU from BVU requires the transfer of a deoxyribosyl moiety, i.e. from dThd, and as such pentosyl transfer reaction readily occurs in vivo,[31] it may account for the protection conferred by BVU against disseminated HSV-1 infection in the mouse model (Figure 1).

It is unlikely that BVU is directly converted to BVRUMP by a pyrimidine phosphoribosyl transferase, since such phosphoribosyl transfer reaction does not offer an explanation for the clear-cut inhibitory effect of 6-aminothymine on the antiviral activity of BVU. It is also unlikely that BVU would be converted to BVRU by Urd phosphorylase, since (i) benzylacyclouridine, a well-known inhibitor of Urd phosphorylase, did not affect the antiviral activity of BVU, and (ii) the counter effect of 6-aminothymine on the antiviral activity of BVU was not reversed by Urd. The latter finding indicates that 6-aminothymine acts by preventing the conversion of BVU to BVDU and not by inhibiting the BVU → BVRU reaction.

To rationalize the antiviral activity of BVRU, several possibilities could be envisaged. It is unlikely that BVRU may be converted to BVDU via BVU: such sequence of events would imply two successive phosphorylase reactions, at least one of which should be sensitive to 6-aminothymine (namely the BVU → BVDU reaction); and 6-aminothymine was found not to counteract the antiviral activity of BVRU. A direct conversion of BVRU to BVDU, through substitution of 2′-dexoyribosyl for the ribosyl moiety, can a priori not be excluded, but direct evidence for such unusual pentosyl exchange is lacking. Finally, BVRU may serve as substrate of the HSV-1-encoded dThd kinase and, upon phosphorylation to its 5′-diphosphate form, be transformed to the corresponding BVDUDP by

ribonucleotide reductase. Whether BVRU is actually recognized as substrate by HSV-1 dThd kinase is an interesting possibility which is now being explored.

Experimental Section

Biological Evaluation. The procedures used for measuring inhibition of viral cytopathogenicity in cell culture have been described previously.[2] The origin of the virus strains is also mentioned in ref 2; the TK⁻ HSV-1 strain B2006 was originally isolated by Dubbs and Kit.[35] The different cell lines were designated as follows: PRK, primary rabbit kidney cells; BALB/3T3, murine fibroblasts derived from BALB/c mouse embryos; MO, murine fibroblasts derived from C_3H mouse embryos; Vero, a simian fibroblast cell line derived from African green monkey kidney; BS-C-1A, a simian epithelial cell line derived from African green monkey kidney; HSF, human skin fibroblasts (VGS strain); E_1SM and E_6SM, human embryonic skin–muscle fibroblasts; M-21, human fibroblasts (GM137 strain) monosomic for chromosome 21; T-21, human fibroblasts (LR strain) trisomic for chromosome 21; HEp-2, a human epithelial cell line derived from a larynx carcinoma; HeLa, a human epithelial cell line derived from a cervix carcinoma. The mouse model used to evaluate in vivo activity against disseminated HSV-1 infection has been described by Sim et al.[34]

(*E*)-5-(2-Carbomethoxyvinyl)uridine (2b). Palladium(II) acetate (0.6 g, 2.6 mmol), triphenylphosphine (1.44 g, 5.4 mmol), and redistilled triethylamine (10 mL) were combined in dry dioxane (80 mL) and stirred at 70 °C until a deep red coloration developed. Then 5-iodouridine (20 g, 54 mmol) and methyl acrylate (9.3 g, 108 mmol) were added, and the mixture was stirred at reflux for 60 min and filtered. The filtrate was stored at room temperature overnight and filtered under suction. The precipitate was washed with dichloromethane (4 × 100 mL) to give a light brown solid which was recrystallized from ethanol to give the title compound as a white fluffy solid (14 g, 86%, mp 204–207 °C): UV λ_{max} 302 nm (ϵ 17 000), λ_{min} 277 nm (ϵ 10 600), λ_{max} 269 nm (sh) (ϵ 10 800) (ethanol); ^{1}H NMR (Me_2SO-d_6) δ 11.5 (1 H, s, NH), 8.45 (1 H, s, H-6), 7.3 (1 H, d, vinylic H, J = 16 Hz), 6.9 (1 H, d, vinylic H, J = 16 Hz), 5.75 (1 H, d, H-1′), 5.4 (1 H, d, 2′-OH), 5.25 (1 H, t, 5′-OH), 5.0 (1 H, d, 3′-OH), 4.05 (2 H, m, H-2′ + H-3′), 3.8 (1 H, m, H-4′), 3.7-3.5 (5 H, m, H-5′ + CO_2CH_3). Anal. Calcd for $C_{13}H_{16}N_2O_8$: C, 47.56; H, 4.91; N, 8.53. Found: C, 47.72; H, 4.89; N, 8.50.

(*E*)-5-(2-Carboxyvinyl)uridine (3b). A solution of (*E*)-5-(2-carbomethoxyvinyl)uridine (14 g, 43 mmol) in aqueous sodium hydroxide (1 N, 525 mL) was stirred overnight at room temperature. The solution was then cooled to 0 °C and brought to pH 2 by the addition of 4 N HCl. The mixture was left to stand in ice for 30 min and the white precipitate was filtered. The filtrate was evaporated to dryness under reduced pressure and water (100 mL) was added. The resulting precipitate was filtered and washed with water. The precipitates were combined and dried under vacuum to give the title compounds as a white solid (9 g, 67%): UV λ_{max} 300 nm (ϵ 14 000), λ_{min} 274 nm (ϵ 8800), λ_{max} 260 nm (ϵ 10 200) (ethanol). ^{1}H NMR (Me_2SO-d_6) δ 11.4 (1 H, s, NH), 8.45 (1 H, s, H-6), 7.3 (1 H, d, vinylic H, J = 16 Hz), 6.7 (1 H, d, vinylic H, J = 16 Hz), 5.75 (1 H, d, H-1′), 5.6–4.7 (3 H, br d, 2′,3′,5′-OH), 4.2–3.6 (5 H, m, H-2′, H-3′, H-4′ + H-5′). Anal. Calcd for $C_{12}H_{14}N_2O_8$: C, 45.86; H, 4.49; N, 8.91. Found: C, 45.82; H, 4.61; N, 8.72.

(*E*)-5-(2-Bromovinyl)uridine (4b). To a solution of (*E*)-5-(2-carboxyvinyl)uridine (4.32 g, 13.73 mmol) in dry DMF (20 mL) was added potassium carbonate (4.1 g, 40.1 mmol) and the solution was stirred at room temperature for 15 min. Then a solution of *N*-bromosuccinimide (2.45 g, 13.9 mmol) in DMF (20 mL) was added dropwise over 30 min at room temperature, and the solution was filtered under suction. The precipitate was washed with DMF (2 × 10 mL), and the filtrate and washings were combined. This solution was evaporated under high vacuum at 40 °C with the receiving flask cooled in liquid air. After 4 h a solid was obtained to which was added water. After vigorous shaking, the resulting precipitate was filtered and washed with water to give the title compound as an off-white solid (2 g, 41.6%). The filtrate was cooled at 0 °C overnight to yield a second crop of products as brown crystals (1.36 g, 28.3%). Overall yield: 3.36 g, 70%. Further purification was achieved by column chromatography with $CHCl_3$/MeOH (4/1) as the eluent (2.8 g, 58%): mp 161 °C; UV λ_{max} 294 nm (ϵ 10 200), λ_{min} 270 nm (ϵ 4200), λ_{max} 251 nm (ϵ 13 000)(ethanol); ^{1}H NMR (Me_2SO-d_6) δ 8.1 (1 H, s, H-6), 7.2 (1 H, d, vinylic H, J = 13 Hz), 6.77 (1 H, d, vinylic H, J = 13 Hz), 5.75 (1 H, d, H-1′), 5.5–4.8 (3 H, brd, 2′,3′,5′-OH), 4.2–4.0 (2 H, m, H-2′ + H-3′), 3.95–3.75 (1 H, m, H-4′), 3.7–3.55 (2 H, m, H-5′). Anal. Calcd for $C_{11}H_{13}BrN_2O_6$: C, 37.84; H, 3.75; N, 8.02. Found: C, 37.86; H, 3.61; N, 8.0.

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(35) Dubbs, D. R.; Kit, S. *Virology* **1964**, *22*, 493.