69002-85-3; **15**, 37984-36-4; **16**, 127792-26-1; **17**, 24542-90-3; **17** (free acid), 15307-71-8; **18**, 122794-97-2; **19**, 127792-27-2; **20**, 100754-91-4; **20** (free acid), 100754-93-6; **21**, 127792-28-3; **22**, 127792-29-4; **23**, 70172-31-5; **24**, 118423-38-4; **25**, 127792-30-7; **26**, 127792-31-8; **27**, 127792-46-5; **27** (free acid), 127792-32-9; **28**, 64118-84-9; **29**, 127792-33-0; **30**, 127792-34-1; **31**, 127792-47-6; **31** (free acid), 127792-35-2; **32**, 90233-41-3; **32** (free acid), 90233-40-2; **33**, 70172-33-7; **34**, 106610-60-0; **35**, 69002-86-4; **36**, 69002-84-2; **38**, 15307-93-4; **39**, 15308-01-7; **40**, 15362-40-0; **41**, 15307-81-0; **42**, 127792-36-3; **43**, 127792-37-4; **44**, 127792-38-5; **45**, 127792-39-6; **46**, 127792-40-9; **47**, 73328-72-0; **48**, 127792-41-0; $ClCH_2COCl$, 79-04-9; oxalyl chloride, 79-37-8; *N,N*-dimethyl-[2-[(3,5-dichlorophenyl)amino]phenyl]acetamide, 127792-48-7; 2,6-dichloroaniline, 608-31-1; potassium 2-iodophenylacetate, 100754-92-5; potassium cyanide, 151-50-8; 2-bromo-5-fluorobenzyl bromide, 112399-50-5; (2-bromo-5-fluorophenyl)acetonitrile, 127792-49-8; potassium 2-bromo-5-fluorophenylacetate, 127792-50-1; 3,5-dichloroaniline, 626-43-7; *N,N*-dimethyl-2-iodophenylacetamide, 75117-26-9; cyclooxygenase, 39391-18-9.

Supplementary Material Available: Tables listing the correlations between the parameters used in the regression equations, the crystal data, final atomic positional parameters, bond distances, and angles of compounds **4**, **12**, **30**, **32**, and **33** (18 pages). Ordering information is given on any current masthead page.

Synthesis and Biological Evaluation of Some Cyclic Phosphoramidate Nucleoside Derivatives

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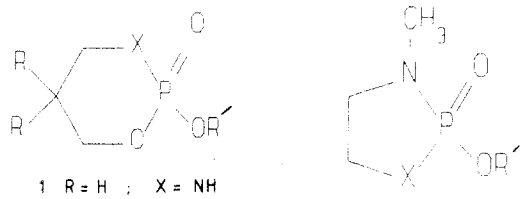
(*E*)-5-(2-Bromovinyl)-2′-deoxy-5′-*O*-(3-methyl-2-oxo-5-formyl-1,3,2-oxazaphosphacyclopentan-2-yl)uridine has been synthesized and, under physiological conditions and without the necessity for enzyme activity, has been shown to yield the 5′-nucleotide in vitro. Unfortunately this compound is not sufficiently stable in solution for it to be tested in vivo. The biological properties of this and some related derivatives of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine and acyclovir have been evaluated in in vitro and in vivo systems designed to show the effects of any intracellular liberation of the nucleotide. Although some of the derivatives are probably acting as prodrugs of the active nucleosides, there is no evidence for the liberation of meaningful concentrations of the 5′-nucleotide by any of the compounds.

Organic phosphomonoesters, such as nucleotides, do not readily enter living cells and in any case are often dephosphorylated before they can penetrate the membrane. Many drugs, particularly nucleoside analogues, owe their activity to the fact that in the living cell they are metabolized in the first instance to their phosphomonoesters, and it might be of therapeutic value if a class of neutral phosphomonoester prodrugs could be synthesized that may penetrate the cell wall and then subsequently be converted to a biologically active drug.

For the past decade, we have synthesized a number of cyclic phosphotriester and phosphoramidate derivatives of 5-substituted 2′-deoxyuridine analogues (**1–5**), in order to see if they would have such properties.[1,2] Similar compounds have been synthesized by other workers.[3,4]

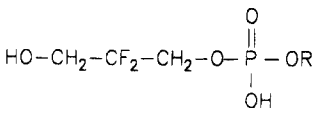


1 R = H ; X = NH
2 R = H ; X = O
3 R = F ; X = O

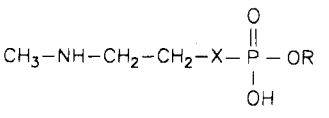
4 X = O
5 X = N - CH_3

R′ = 2′- deoxy - 5 - fluorouridin - 5′- yl

These compounds (**1–5**) were evaluated for their ability (a) to inhibit the growth of leukemia L1210 cells in cell culture and (b) to act as thymidylate synthase inhibitors either in cell culture or against the isolated enzyme. Compounds **1** and **2**, which have stable ring systems, were essentially inactive whereas compounds **3–5**, which are hydrolyzed under physiological conditions to acyclic phosphorus intermediates, showed considerable inhibitory activity against L1210 cells. This activity appeared to be due to the inhibition of thymidylate synthase in the cells, although compounds **1c–2a,2b** themselves did not inhibit the isolated enzyme. However, these compounds were not active against a variant of L1210 cells which was resistant to 5-bromo-2′-deoxyuridine (presumably because of lack of thymidine kinase activity) and against which one might have expected to see some activity if indeed the 5′-mononucleotide was being liberated inside the cell. Thus, the activity seen in the normal L1210 cell line was probably due to the production of 5-fluoro-2′-deoxyuridine outside the cell which was then subsequently phosphorylated once inside the cell. Although compounds **3** and **4** are hydrolyzed at physiological pH, the products have been shown to be of the type **6** and **7**, respectively.



(6)



X=O or NCH_3

(7)

† University of Birmingham.
‡ Rega Institute for Medical Research.

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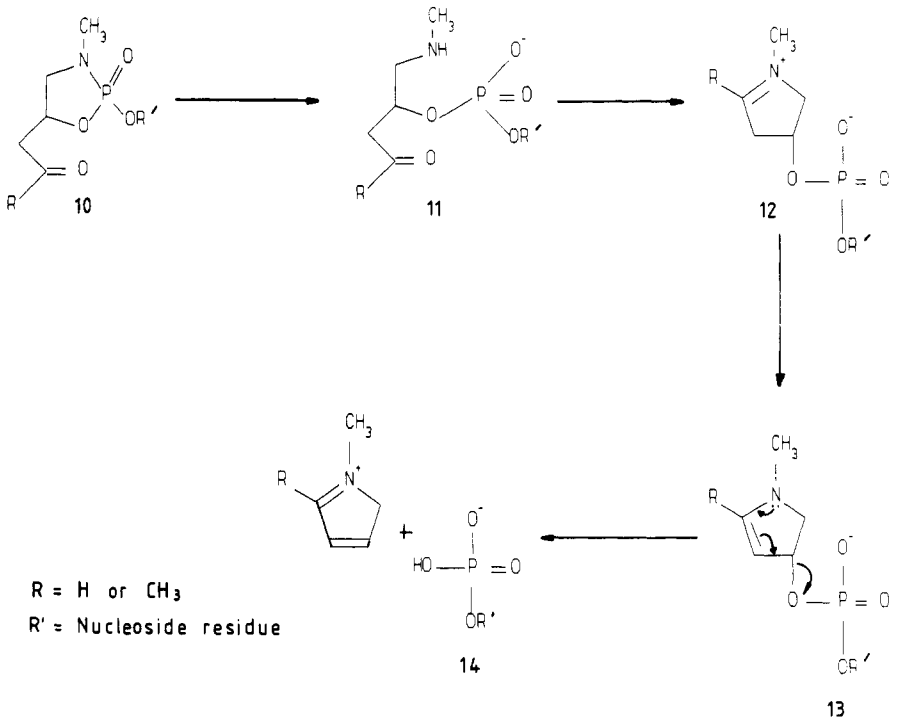
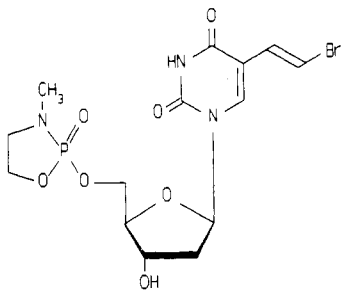
Scheme I

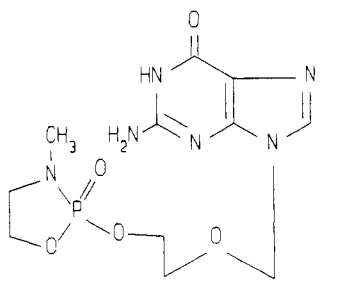


10 11 12 13 14

These derivatives then require enzymatic hydrolysis, where the choice is either bond a to liberate nucleotide or bond b to liberate nucleoside. The preferred reaction appears to be the latter. The work we describe here is in two parts: firstly the preparation of compounds analogous to **4** where the nucleoside is either (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) **8** or acyclovir **9** and secondly attempts to synthesize corresponding compounds of type **10** so that, by hydrolysis under physiological conditions without the intervention of enzymes, only the 5′-mononucleotide is produced.



8



9

As shown in Scheme I, compound **10** could undergo hydrolysis of the phosphoramidate bond, as previously described, to give the acyclic structure (**11**), which, because of the presence of the carbonyl containing side chain, would immediately cyclize to give **12**, which would then spontaneously rearrange (**13**) and eliminate the 5′-mononucleotide (**14**). Alternatively the parent compound could undergo a direct β-elimination, and this may form the basis for the instability of this compound.

Chemistry

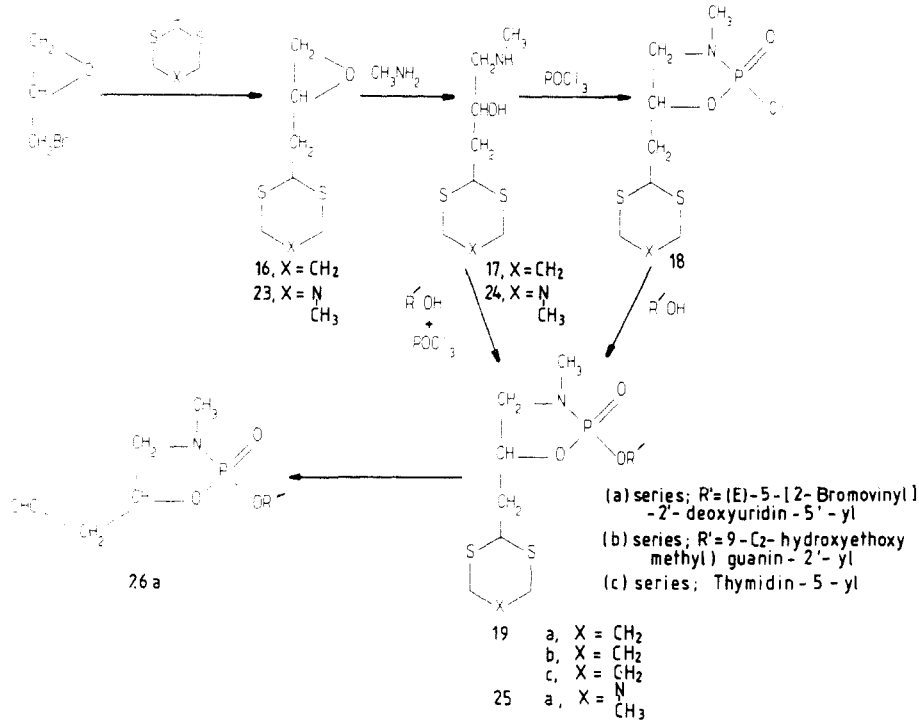
A series of compounds including **8**, **9**, and type **10** were synthesized to give a range of cyclic phosphate prodrugs that, being uncharged, should readily penetrate the cell wall and then under physiological conditions liberate the biologically active phosphomonoester once inside the cell.

Earlier evidence of Jones and co-workers[1,2] based on hydrolytic studies of 5′-*O*-3″-methyl-1″,3″,2″-oxazaphosphacyclopentan-2″-ylthymidine 2″-oxide (**4**, R′ = thymidin-5′-yl) and other similar five-membered cyclic phosphotriesters showed that these compounds were hydrolyzed in a few minutes at physiological pH. What we did not then appreciate, but has become clear in recent studies, is that their rate of hydrolysis is due to general-acid catalysis and thus was dependent upon the buffer used and its concentration. Thus compound **4** (R′ = thymidin-5′-yl) was completely hydrolyzed in 45 min in 0.5 M sodium acetate, pH 5.8, whereas in water at pH 5.8, 90% was unchanged after 24 h.

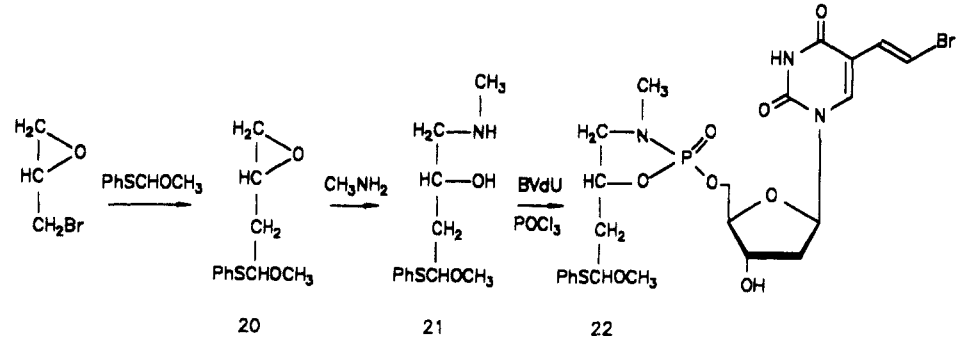
Thus it was decided to synthesize (*E*)-5-(2-bromovinyl)-2′-deoxy-5′-*O*-3″-methyl-1″,3″,2″-oxazaphosphacyclopentan-2″-yluridine 2″-oxide (**8**) and 2-*O*-(3-methyl-2-oxo-1,3,2-oxazaphosphacyclopentan-2-yl)-9-[(2-hydroxyethoxy)methyl]guanine (**9**) and test them for their ability to affect the replication of a range of herpesviruses in cell culture and in an animal model.

We realize that there is probably very little chemotherapeutic value in the synthesis of these precise analogues, as both acyclovir and BVDU depend for their selectivity and hence lack of toxicity on the fact that they are normally only substrates for the viral thymidine kinase. The purpose here was solely to use these analogues to see whether we could devise an assay using TK^- herpes strains that are normally resistant to acyclovir and/or BVDU. If their monophosphates could be liberated inside the cell, the viral infection either in vitro or in vivo might become susceptible to the drug, thus proving that this type of compound is indeed a phosphate prodrug.

Scheme II



Scheme III



The synthesis of the target acyclovir derivative appeared to require the protection of the guanine group and initial steps were taken to make the (9-fluorenylmethoxy)-carbonyl (FMOC)[5] group. However, the final product had such poor solubility properties that it was decided to try the synthesis without amino protection which, in the event, was accomplished satisfactorily.

Thus, (*E*)-5-(2-bromovinyl)-2′-deoxyuridine and acyclovir were treated separately with phosphoryl trichloride and the resulting phosphorodichloridate cyclized by addition of *N*-methylethanolamine. Following the usual workup and silica gel chromatography, the compounds **8**, **9** were isolated in 86 and 45% yields, respectively.

Attention was then turned to the synthesis of compounds of type **10** (R = H) with the aldehyde side chain on the oxazaphos ring to give compounds that would hydrolyze under physiological conditions to give the mononucleotide without the necessity for enzymic hydrolysis of a phosphodiester. We have previously described attempts to use [(phenylthio)methyl]trimethylsilane[6] as a formyl synthon[7] but 1,4-silyl group migration from carbon to oxygen in the preparation of a key intermediate meant that this method was not suitable.

Thus, the common formyl synthon 1,3-dithiane was used. Initial attempts were made to synthesize the phosphorylating agent **18** by reacting compound **17** (Scheme II) with phosphoryl trichloride. The product (**18**), however, was a very poor phosphorylating agent and only low yields (16%) of the derivatives of structure **19** could be obtained. Thus, the phosphorodichloridate of the nucleosides was made in situ and reacted with compound **17**, as shown in Scheme II, to give compounds **19a–c** in good (<50%) yield.

All attempts to deprotect the dithianes[8-12] **19a–c** using a wide range of reagents suggested in the literature failed as the oxazaphos ring is by far the most labile part of the molecule. The use of other potential aldehyde synthons such as (methylthio)methyl *p*-toluoyl sulfone and methoxymethyl phenyl sulfide[13] was investigated but without success (Scheme III) as it was also impossible to regenerate the aldehyde function without breaking the oxazaphos ring.

Finally, some success was achieved with use of the dithiazine synthon (Scheme III, X = NCH_3) to produce

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Table I. Cytotoxicity and Antiviral Activity of 5′-Phosphoramidate Derivatives of (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine, Acyclovir, and Thymidine in Primary Rabbit Kidney Cell Cultures

		minimum inhibitory concentration,[b] μg/mL									
compd	min cytotoxic concn,[a] μg/mL	herpes simplex virus-1 (KOS)	herpes simplex virus-1 (F)	herpes simplex virus-1 (McIntyre)	herpes simplex virus-2 (G)	herpes simplex virus-2 (196)	herpes simplex virus-2 (Lyons)	vaccinia virus	vesicular stomatitis virus	herpes simplex virus-1 TK⁻ B2006	herpes simplex virus-1 TK⁻ VMW 1837
8	>400	0.02	0.07	0.04	10	>400	200	20	>400	>400	>400
22	>400	0.1	0.1	0.4	>400	>400	>400	≥400	>400	>400	>400
19a	≥400	200	150	>200	150	>200	>200	200	>200	150	300
19c	≥400	>400	–	–	300	–	–	>400	>400	–	–
19b	≥40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
9	≥400	0.2	–	–	0.2	–	–	400	>400	–	–
BVDU	≥400	0.02	0.01	0.01	2	100	20	7	>400	100	300
ACV	≥400	0.2	0.1	0.2	0.02	0.07	0.1	150	>400	20	10

[a] Required to cause a microscopically detectable alteration of normal cell morphology. [b] Required to reduce virus-induced cytopathogenicity by 50%. The data represent average values for two separate experiments.

Table II. Cytostatic Effects of 5′-Phosphoramidate Derivatives of (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine, Acyclovir, and Thymidine against Murine and Human Tumor Cell Lines

	50% inhibitory dose,[a] μg/mL					
compd	L1210	FM3A/0	FM3A TK⁻	FM3A TK⁻/HSV-2 TK⁺	Raji	Molt/4F
8	90	48	1.3	0.0008	>100	>100
22	>100	82	12.4	0.003	>100	>100
19a	>100	48	32	0.018	>100	>100
19c	>100	>100	–	–	>100	>100
19b	>100	>100	–	–	>100	>100
9	>100	>100	–	–	>100	>100
BVDU	22	9.4	0.31	0.0005	73	>100
BVDUMP	41	17	0.65	0.0009	>100	>100

[a] Required to inhibit tumor cell proliferation by 50%.

compound **25a**. This compound could be deprotected with mercuric chloride and mercuric oxide in aqueous acetonitrile to give the free aldehyde (**26a**), which could be completely characterized. Unfortunately, the aldehyde has so far proved to be too unstable to be of any use as a phosphate prodrug. The white solid obtained initially decomposes quickly to a brown gum and thus it appears that the only hope for a phosphate prodrug in this area might be compounds of the type **25a**, which might in vivo be substrates for sulfur oxidases such that the aldehyde is liberated in situ.

Using conditions (50 mM sodium acetate, pH 5.8) previously described[13] and monitoring the reaction by HPLC, we ascertained that, on the basis of the mobility, the only product formed from **25a** was (*E*)-5-(2-bromovinyl)-2′-deoxyuridine 5′-phosphate; therefore, in principle, the decomposition of these compounds will indeed yield the nucleotide without the necessity for any enzymatic intervention.

Results and Discussion

Cytostatic and Antiviral Activity. When evaluated against several strains of herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2, TK⁻ HSV-1, vaccinia virus (VV), and vesicular stomatitis virus (VSV), none of the 5′-phosphoramidate derivatives of BVDU, acyclovir, or thymidine, containing the cyclic dithiane side chain (compounds **19a**, **19b**, and **19c**), showed an appreciable antiviral activity (Table I). In contrast, the unsubstituted 5′-phosphoramidate derivatives of BVDU (**8**) and acyclovir (**9**) were potent inhibitors of HSV-1 and HSV-2 replication. In this respect, compounds **8** and **9** proved almost as potent as antiherpetic compounds as BVDU and acyclovir themselves (Table I). Interestingly, compounds **8** and **22** were devoid of any activity against TK⁻ HSV-1 replication. These observations point to the pivotal role of the HSV-1 encoded TK in the antiviral action of the BVDU derivatives. The data also suggest that compounds **8** and **22** do not directly release the 5′-monophosphate derivative of BVDU. Otherwise, these prodrugs should have shown activity against the TK⁻ HSV-1 variants. Compounds **19c** and **9** were also evaluated for their inhibitory effects on the replication of various RNA viruses (including Coxsackie virus B4, poliovirus-1, parainfluenza-3 virus, reovirus-1, Sindbis virus, and Semliki forest virus). They were found inactive against these viruses at a concentration of 100–200 μg/mL (data not shown). It is interesting that compounds **8** and **9** are active but **19a/b** are inactive. We can only presume that following cyclic phosphoramidate hydrolysis to give the acyclic phosphodiesters, compounds **8** and **9** are substrates for phosphodiesterases whereas **19a/b**, which still contain the dithiane ring structure, are not further broken down.

The phosphoramidate derivatives of BVDU (compounds **8**, **19a**, and **22**), thymidine (compound **19c**), and acyclovir (compounds **19b** and **9**) were also evaluated for their inhibitory activity against tumor cell proliferation in vitro (Table II). None of the compounds were cytostatic against human Raji and Molt/4F cells. The 5′-phosphoramidate derivatives of acyclovir and thymidine also failed to inhibit the proliferation of murine L1210 and FM3A/0 cells [50% inhibitory dose (ID_{50}): >100 μg/mL]. Also, the BVDU derivatives **8**, **22**, and **19a** were virtually inactive against both murine tumor cell lines. However, TK-deficient FM3A cells showed a marked increase in their sensitivity to the cytostatic activity of compounds **8** and **22** (40- and 7-fold, respectively). This phenomenon has also been observed for BVDU[14] and BVDUMP (Table II). Furthermore, FM3A TK⁻/HSV-2 TK⁺ cells that contain the gene of HSV-2 TK and also express HSV-2 TK activity proved exquisitely sensitive to the inhibitory effects of

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Table III. Intraperitoneal Treatment of Intracutaneous TK⁻ HSV-1 (VMW 1837) Infection of Nu/Nu Mice

compd	concn, mg/kg per day	mean day of appearance of skin lesions and/or paralysis of the hind legs	mean time of survival,[a] days
19b	100 (in CMC)	8.4	18
ACV	100 (in H_2O)	17.2	30.4
BVDU	100 (in H_2O)	10.2	33.8
placebo	(in CMC)	8.8	25
placebo	(in H_2O)	8.6	28.2

[a] Mice infected with TK⁻ HSV-1 (VMW 1837) were followed for 39 days. Those mice that were still alive at the end of the experiment were taken into account as 39-day survivors. Abbreviation: CMC, (carboxymethyl)cellulose.

compounds **8**, **22**, and **19a**. In this respect, compound **8** proved as potent an inhibitor of FM3A TK⁻/HSV-2 TK⁺ cell proliferation as BVDU[15] and BVDUMP. Compound **22** was only 5-fold and compound **19a** 20-fold less inhibitory to FM3A TK⁻/HSV-2 TK⁺ cell growth than BVDU. In a previous paper, we have reported that the cytostatic action of BVDU against HSV-2 TK or HSV-1 TK gene-transformed FM3A cells is due to an inhibition of thymidylate synthase by BVDUMP.[15] We demonstrated that, unlike FM3A/0 cells, HSV-2 TK gene-transformed cells extensively phosphorylated BVDU to its 5′-monophosphate, and that intracellular accumulation of BVDUMP is the molecular basis for the cytostatic activity of BVDU against FM3A TK⁻/HSV-2 TK⁺ cells. In context of these earlier observations, the fact that **8** and **22** did not cause an appreciable inhibition of FM3A/0 cell proliferation may be interpreted to mean that no BVDUMP is released intracellularly from the prodrugs **8** and **22**.

To investigate whether **8** and **19b** may affect TK⁻ HSV-1 infection in vivo, nu/nu mice were infected intracutaneously with the TK⁻ HSV-1 variant VMW 1837 and treated for 5 subsequent days with compound **8** (given topically as a 10% ointment), or compound **19b** (given intraperitoneally at 100 mg/kg per day in (carboxymethyl)cellulose). The control compounds (i.e. BVDU, acyclovir) were included in this study at the same concentrations and under similar experimental conditions as the test compounds. Compound **19b** did not delay the appearance of skin lesions or paralysis of the legs when administered intraperitoneally at 100 mg/kg per day (Table III). It even shortened the mean day of death (as compared to the placebo group), which might reflect toxicity of the compound at this concentration. When evaluated under the same conditions, acyclovir prolonged the mean day of appearance of lesions (as compared to the placebo group) but had no beneficial effect on survival of the mice. BVDU did not markedly affect the course of the disease when used at a dose of 100 mg/kg per day.

When compound **8** was evaluated in nu/nu mice infected with TK⁻ HSV-1 (VMW-1837), it was found to increase the mean survival time when applied topically at 10% (Table IV) as did BVDU and acyclovir when evaluated under identical conditions. Neither **19b** nor **8** proved superior to BVDU or acyclovir in their inhibitory effect against TK⁻ HSV-1 (VMW 1837) infection in vivo. These observations indicate that neither **19b** nor **8** directly release the 5′-monophosphate derivatives of acyclovir and BVDU in vivo, a conclusion that is in agreement with our in vitro data.

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Table IV. Topical Treatment of Intracutaneous TK⁻ HSV-1 (VMW 1837) Infection of Nu/Nu Mice

compd	concn (v/v in DMSO)	mean day of appearance of skin lesions and/or paralysis of the hind legs	mean time of survival,[a] days
8	10%	10.2	45.2
BVDU	10%	8.2	39.6
ACV	10%	40.8	46.2
placebo	DMSO	8.2	27.8

[a] Mice infected with TK⁻ HSV-1 (VMW 1837) were followed for 52 days. Those mice that were taken alive at the end of the experiment were taken into account as 52-day survivors, respectively.

Experimental Section

NMR spectra (^1H) were recorded on JEOL FX 90Q (90 MHz) and JEOL GX270 (270 MHz) instruments with Me_2SO-d_6 as solvent unless otherwise stated. ^{31}P spectra were recorded on either a JEOL FX 90Q (90 MHz) or a Bruker WH400 (operating at 162 MHz) using 85% H_3PO_4 as a reference. UV spectra were measured on a Perkin-Elmer 552 spectrophotometer and mass spectra were measured on a Kratos M580 mass spectrometer using fast atom bombardment. Glycerol, glycerol/methanol, or 3-nitrobenzyl alcohol were used as matrices, sometimes with NaCl added.

Column chromatography was carried out on silica gel, Kieselgel 60 type 9385 (0.040–0.063 nm, 230–400 mesh). Short-column chromatography was carried out with silica gel 60 GF_{250} type 7730.

Some separations were achieved with a chromatotron (Harrison Research Ltd.) using plates prepared from silica gel 60 PF_{254} type 7749.

All experiments were carried out under scrupulously dry conditions unless stated otherwise.

(*E*)-5-(2-Bromovinyl)-2′-deoxy-5′-*O*-3″-methyl-1″,3″,2″-oxazaphosphacyclopentan-2″-yluridine (8). Phosphoryl trichloride (0.5 mL, 5.5 mmol) was dissolved in 1,2-dimethoxyethane (10 mL) and *N*-methylmorpholine (0.33 g, 3.3 mmol). The solution was cooled to 0 °C and vigorously stirred while a solution of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (0.71 g, 2.1 mmol) and *N*-methylmorpholine (11 mg, 0.11 mmol) in 1,2-dimethoxyethane (10 mL) was added. The mixture was stirred at 20 °C for 4 h and then cooled to 0 °C and a solution of *N*-methylethanolamine (3.94 g, 52.5 mmol) and *N*-methylmorpholine (5.3 g, 52.5 mmol) added dropwise to the vigorously stirred mixture. After being stirred for 1 h at 20 °C, the resulting suspension was filtered and the precipitate washed with chloroform (2 × 50 mL). The combined filtrate and washings were evaporated under reduced pressure to give a pale orange oil. This was purified on a silica column which was eluted with ethanol–ethyl acetate (1:3). Pooling of the appropriate fractions and removal of the solvent under reduced pressure gave the product as a white powder (690 mg, 80%): UV λ_{max} 293.0 nm (ϵ 10 300), λ_{min} 276.0 nm (ϵ 8300), λ_{max} 250.0 nm (ϵ 13 750) (in distilled water); ^1H NMR [$(CD_3)_2SO$] δ 2.16 (2 H, m, H-2′), 2.58 (3 H, dd, NCH_3, J_{H-P} 10 Hz), 3.1–3.5 (2 H, m, H-4″), 3.9–4.4 (6 H, m, H-3′,4′,5′ and H_2-5″), 5.45 (1 H, d, OH-3′), 6.21 (1 H, t, H-1′), 6.95 (1 H, dd, vinylic, J = 14 Hz), 7.3 (1 H, dd, vinylic, J = 14 Hz), 7.85 (1 H, d, H-6), 11.6 (1 H, s, NH); FAB mass spectrum, m/z 476 (M + Na), 454 (M + H)$^+$. Anal. ($C_{14}H_{19}N_3O_7PBr$) C, H, N.

2-[[(9′-Fluorenylmethoxy)carbonyl]amino]-9-[(2-hydroxyethoxy)methyl]guanine (15). 9-[(2-Hydroxyethoxy)methyl]guanine (0.5 g, 2.2 mmol) was suspended in pyridine (50 mL) and trimethylsilyl chloride (1.02 g, 9.4 mmol) was added. After the mixture was stirred for 15 min, a clear solution was formed and 9-fluorenylmethyl chloroformate (0.74 g, 2.8 mmol) was added. After stirring overnight at room temperature, the solution was cooled to −5 °C and distilled water (10 mL) was added. After stirring overnight, the solvent was removed by evaporation and the residue was suspended in distilled water (50 mL) and extracted with ethyl acetate–ether. The organic layer

contained a white precipitate and the solvent was evaporated to give a white solid. This was applied, preabsorbed onto silica gel (100 g, type 7734) column. The column was eluted with chloroform–methanol (9:1). Pooling of the appropriate fractions and evaporation of solvent gave a white solid. This was crystallized from methanol to give the product (440 mg, 45%): UV λ_{max} 260.0 nm (ϵ 31 500), λ_{min} 241.5 nm (ϵ 19 700) (ethanol + 0.5% DMSO); ^{1}H NMR (CD_3OD) δ 3.62 (4 H, m, OCH_2CH_2O), 5.55 (2 H, m, NCH_2O), 7.36 (4 H, m, phenyl), 7.75 (4 H, m, phenyl), 7.99 (1 H, s, H-8); FAB mass spectrum, m/z 448 (M + H). Anal. ($C_{23}H_{21}N_5O_5$·MeOH) C, H, N.

2-*O*-(3-Methyl-1,3,2-oxazaphosphacyclopentan-2-yl)-9-[(2-hydroxyethoxy)methyl]guanine (9). 9-[(2-Hydroxyethoxy)methyl]guanine (1 g, 4.44 mmol) was dissolved in dry triethyl phosphate (5 mL) and the mixture cooled to 0 °C under dry N_2. To this solution was added freshly distilled phosphoryl trichloride (0.68 g, 8.88 mmol) and mixture allowed to warm up to room temperature overnight. Then the mixture was cooled to 0 °C and a solution of *N*-methylethanolamine (0.6 g, 7.98 mmol) and *N*-methylmorpholine (0.81 g, 8.0 mmol) added dropwise with vigorous stirring. A white gum was immediately formed and was separated by decanting off the solution, which was stirred overnight. The solution yielded a white precipitate, which was recrystallized from methanol and dimethyl sulfoxide and was characterized as the required product (1.1 g, 70%): UV λ_{max} 252.5 (ϵ 14 085) in ethanol + 0.5% DMSO; ^{1}H NMR [$(CD_3)_2SO$] δ 3.4 (3 H, s, NCH_3), 3.5–4.2 (8 H, m, OCH_2CH_2O, 2 CH_2-3″,4″), 5.35 (2 H, s, NCH_2), 6.6 (2 H, 5, NH_2), 7.9 (1 H, d, H-8), 10.8 (1 H, bs, NH); FAB mass spectrum, m/z 345 (M + H)$^+$, sulfolane matrix. Anal. ($C_{11}H_{17}N_6O_5P$) C, H, N.

The white gum was crystallized from methanol and was characterized as being 9-[(2-chloroethoxy)methyl]guanine (0.27 g, 23%): UV λ_{max} 252.5 nm (ϵ 13 040) in ethanol + 0.5% DMSO; ^{1}H NMR [$(CD_3)_2SO$] δ 3.7 (4 H, s, CH_2CH_2O), 5.4 (2 H, s, NCH_2O), 6.5 (2 H, s, NH_2), 7.85 (1 H, s, NCH), 10.7 (1 H, s, NH); FAB mass spectrum, m/z 260 (M + H)$^+$. Anal. ($C_8H_{10}N_5O_3Cl$) C, H, N.

2-(2′,3′-Epoxypropyl)-1,3-dithiane (16). To a rapidly stirred solution of dry 1,3-dithiane (6.09, 50 mmol) in dry THF (100 mL) at −40 °C was added *n*-butyllithium in *n*-hexane (32.7 mL, 52 mmol). After 1.5 h the dithiane was metalated quantitatively as evidenced by TLC. To this solution of the anion was added epibromohydrin (6 g, 45 mmol) in dry THF (4 mL) with vigorous stirring. After 15 min the reaction mixture was quenched by the addition of phosphate buffer, pH 7.0, and the mixture extracted with dichloromethane (3 × 100 mL). The organic layer was dried (Na_2SO_4) and the solvent removed under reduced pressure to give a yellow oil. Final purification was achieved on a silica gel column eluting with hexane–ethyl acetate (8:2) to give 4.8 g (78%) of a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.5–1.8 (4 H, m, 2 CH_2S), 3.2–2.5 (7 H, m, CH_2-4,6, CH_2-3′, CH-2′), 4.3 (1 H, t, CH_2-2); bp 189–190 °C (0.2 mm of Hg); mass spectrum, m/e 176 (M$^+$), 145, 133, 119. Anal. ($C_7H_{12}OS_2$) C, H.

1-(*N*-Methylamino)-3-dithian-2-ylpropan-2-ol (17). Methylamine gas was bubbled through a methanolic solution of 2-(2′,3′-epoxypropyl)-1,3-dithiane (4 g, 23 mmol) in a thick-walled glass tube at −20 °C. The glass tube was sealed and left in a steel container at room temperature overnight. A crystalline solid is formed, which is washed with hexane and dry acetone to give the product, 3.9 g (82%): ^{1}H NMR ($CDCl_3$) δ 2.2–1.7 (4 H, m, 2 CH_2S), 2.5 (3 H, s, NCH_3), 2.6 (2 H, m, CH_2-4), 2.8 (4 H, m, CH_2-6,3′), 2.9 (2 H, s, NH, OH), 4.1–3.8 (1 H, m, CHOH), 4.4–4.2 (1 H, m, SCHS); mass spectrum, m/e 207 (M$^+$), 162, 132. Anal. ($C_8H_{17}NOS_2$) C, H, N.

2-[(2-Chloro-2-oxo-3-methyl-1,3,2-oxazaphosphacyclopentan-5-yl)methyl]-1,3-dithiane (18). Phosphoryl trichloride (1.37 g, 8.92 mmol) was dissolved in dry chloroform (6 mL) and the mixture cooled to −15 °C, while a solution of 1-(*N*-methylamino)-3-dithian-2-ylpropan-2-ol (1.85 g, 8.92 mmol) in dry chloroform (10 mL) and dry triethylamine (1.25 mL) was added dropwise with vigorous stirring. The mixture was left at 0 °C overnight. The solvent was then removed under reduced pressure at 35 °C and the residue extracted with dry acetone (4 × 50 mL). The acetone extracts were evaporated, and the residue was treated with dry chloroform (20 mL) and dry carbon tetrachloride (60 mL). After trituration of the solid that initially formed, crystals appeared, which were collected and dried to give 1.85 g (72%): ^{1}H NMR ($CDCl_3$) δ 2.4–1.8 (4 H, m, 2 CH_2, 1.2), 2.7 (3 H, d, NCH_3), 3.8–3.1 (6 H, m, 3 CH_2, 4′,5′,6′), 4.3–4.1 (1 H, m, SCHS), 5.1–4.8 (1 H, m, CHO); ^{31}P NMR δ +21.0; FAB mass spectrum, m/z 289 (M + H)$^+$. Anal. ($C_8H_{15}O_2NPS_2Cl$·$(C_2H_5)_3NHCl$) C, H, N.

5′-*O*-[3-Methyl-2-oxo-5-[(1,3-dithian-2-yl)methyl]-1,3,2-oxazaphosphacyclopentan-2-yl]thymidine (19c). Dry pyridine (20 mL) was added to a mixture of thymidine (1.05 g, 4.3 mmol) and the cyclic phosphorochloridate (1.3 g, 4.3 mmol). The mixture was allowed to stir for 3 days at room temperature under dry N_2. The solvent was then removed under reduced pressure, the last traces being removed by coevaporation with methanol to give an orange oil which was separated on a silica gel column using ethyl acetate–ethanol (3:1) as eluant. Pooling of appropriate fractions gave a white solid (0.35 g, 16%): UV λ_{max} 267 nm (ϵ 11 055), λ_{min} 240 nm (ϵ 2350); ^{1}H NMR [$(CD_3)_2SO$] δ 1.7 (2 H, m, CH_2-5″), 1.8 (3 H, s, CH_3), 1.95–2.2 (5 H, m, CH-2′, CH-2″, CH-4″), 2.55 (3 H, d, NCH_3, J = 14 Hz), 2.8 (6 H, m, CH_2-5′, CH_2CH_2S), 3.4 (2 H, m, CH_2S), 3.9 (1 H, m, H-4′), 4.25 (1 H, m, SCHS), 4.65 (1 H, m, H-3′), 5.4 (1 H, D, OH-3′), 6.2 (1 H, t, H-1′), 7.5 (1 H, m, H-6), 11.3 (1 H, bs, NH); ^{31}P NMR δ +26.0; FAB mass spectrum, m/z 494 (M + H)$^+$, 516 (M + Na)$^+$. Anal. ($C_{18}H_{28}N_3O_5PS_2$) C, H, N.

(*E*)-5-(2-Bromovinyl)-2′-deoxy-5′-*O*-[3-methyl-2-oxo-5-[(1,3-dithian-2-yl)methyl]-1,3,2-oxazaphosphacyclopentan-2-yl]uridine (19a). To a suspension of triethyl phosphate (5 mL) and (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (0.7 g, 2.1 mmol) at 0 °C was added freshly distilled phosphoryl trichloride (0.5 mL, 5.5 mmol) with vigorous stirring. After the mixture was allowed to warm up to room temperature over 4 h, it was cooled to 0 °C, and *N*-methylmorpholine (0.38 g, 4 mmol) and 1-(*N*-methylamino)-3-dithian-2-ylpropan-2-ol (0.4 g, 3.8 mmol) in 1,2-dimethoxyethane (3 mL) were added dropwise with vigorous stirring under a dry stream of N_2. The mixture was allowed to warm up to room temperature and left overnight. Then the solvent was removed under reduced pressure to give a pale yellow oil which was purified on a silica gel column using ethyl acetate–ethanol (9:1) as eluant. Pooling of appropriate fractions gave a white solid (0.58 g, 47%): UV λ_{max} 295 nm (ϵ 12 250), λ_{min} 270 nm (ϵ 8750), λ_{max} 250 nm (ϵ 14 000) (in distilled water); ^{1}H NMR [$(CD_3)_2SO$] δ 1.8 (2 H, m, CH-5″), 2.0–2.2 (5 H, m, H-2′, CH_2-2″, CH-4″), 2.6 (3 H, dd, NCH_3), 3.4–3.9 (6 H, m, CH_2-5′, CH_2CH_2S), 3.95–4.4 (5 H, m, H-4′, H-3′, CH_2-5, SCHS), 5.5 (1 H, d, OH-3′), 6.25 (1 H, t, H-1′), 6.9 (1 H, d, vinylic, J = 15 Hz), 7.3 (1 H, d, vinylic, J = 15 Hz), 7.8 (1 H, s, H-6), 11.6 (1 H, s, NH); ^{31}P NMR δ +20; FAB mass spectrum, m/z 585 (M + H)$^+$. Anal. ($C_{19}H_{27}N_3O_7S_2PBr$) C, H, N.

2-*O*-[[3-Methyl-2-oxo-5-[(1,3-dithian-2-yl)methyl]-1,3,5-oxazaphosphacyclopentan-2-yl]oxy]acyclovir (19b). To a suspension of 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir) (1 g, 4.44 mmol) in triethyl phosphate (5 mL) at 0 °C was added freshly distilled phosphoryl trichloride (1.0 mL, 11 mmol) with vigorous stirring. After 4 h at room temperature the mixture was cooled to 0 °C and *N*-methylmorpholine (0.76 g, 8 mmol) and 1-(*N*-methylamino)-3-dithian-2-ylpropan-2-ol (0.8 g, 7.6 mmol) in 1,2-dimethoxyethane (6 mL) was added dropwise with vigorous stirring under dry N_2. The resulting suspension was subjected to sonication in an ultrasound bath for 2 h and then stirred overnight at room temperature. The resulting gum and solution was added to ice–water (100 mL), and after trituration, the solid material was filtered and washed with cold methanol, and the product obtained was a yellow solid (1.3 g, 62%): ^{1}H NMR [$(CD_3)_2SO$] δ 1.5 (2 H, m, CH_2-5″), 1.9–2.1 (3 H, m, CH_2-2″, CH-4″), 3.5 (3 H, d, NCH_3), 3.6–4.1 (9 H, m, OCH_2CH_2O, CH_2CHCH_2), 5.35 (2 H, s, NCH_2), 6.7 (2 H, bs, NH_2), 7.9 (1 H, d, H-8), 10.8 (1 H, bs, NH); FAB mass spectrum, m/z 477 (M + H)$^+$.

1-Methoxy-1-(phenylthio)-3,4-epoxybutane (20). This compound was prepared as described for **16**. The product was isolated in 87% yield by flash chromatography using ethyl acetate–hexane (2:8): ^{1}H NMR ($CDCl_3$) δ 1.75–2.1 (2 H, m, CH_3-3), 2.5 (1 H, m, CH-1), 2.75 (1 H, m, CH-1), 3.1 (1 H, m, CH-2), 3.55–3.48 (3 H, d, OCH_3), 4.8 (1 H, m, SCHO), 7.2–7.5 (5 H, m, Ph); mass spectrum, m/e 210, 166. Anal. ($C_{11}H_{14}O_2S$) C, H.

3-Hydroxy-4-(methylamino)-1-methoxy-1-(phenylthio)-butane (21). This compound was prepared as described for **17**. The product was crystallized from methanol in 82% yield: ^{1}H NMR ($CDCl_3$) δ 1.75–1.9 (3 H, m, CH_2-3 and CH-1), 2.2–2.8 (6

H, m, CH_3, NH, OH, and CH-1), 3.55 (3 H, s, OCH_3), 3.8–4.0 (1 H, m, CH-2), 4.9 (1 H, m, SCHO), 7.2–7.5 (5 H, m, Ph); FAB mass spectrum, m/z 242 $(M + H)^+$. Anal. ($C_{12}H_{19}NO_8S$) C, H, N.

(*E*)-5-(2-Bromovinyl)-2′-deoxy-5′-*O*-[5-[2-methoxy-2-(phenylthio)eth-1-yl]-3-methyl-2-oxo-1,3,2-oxazaphosphacyclopentan-2-yl]uridine (22). To a suspension of triethyl phosphate (5 mL) and (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (0.7 g, 2.1 mmol) at 0 °C was added freshly distilled phosphoryl trichloride (0.5 mL, 5.5 mmol) with vigorous stirring. The mixture was stirred overnight at room temperature and then cooled to 0 °C again, and *N*-methylimidazole (0.33 g, 4 mmol) and 3-hydroxy-4-(methylamino)-1-methoxy-1-(phenylthio)butane (0.92 g, 3.8 mmol) in 1,2-dimethoxyethane (3 mL) were added dropwise with vigorous stirring. The mixture was allowed to warm up to room temperature and left overnight. Then the solvent was removed under reduced pressure to give a pale yellow oil. Purification was achieved on a silica gel column using chloroform–ethanol (95:5) as eluant. Pooling of appropriate fractions gave a white solid (0.7 g, 54%): UV λ_{max} 293 nm (ϵ 12 985), λ_{min} 268 nm (ϵ 9478), λ_{max} 248 nm (ϵ 18 832); ^1H NMR [$(CD_3)_2SO$] δ 1.9 (2 H, m, CH_2-5″), 2.2 (2 H, m, CH_2-2′), 3.0 (3 H, m, CH-4″, CH_2-5′), 3.2 (3 H, d, OCH_3), 3.5 (3 H, s, NCH_3), 3.8–4.2 (4 H, m, H-3′,4′, CH_2-3″), 4.8 (1 H, m, 5CHO), 5.5 (1 H, d, OH-3′), 6.2 (1 H, t, H-1′), 6.9 (1 H, d, vinylic), 7.2–7.5 (6 H, m, PhS, vinylic), 7.8 (1 H, s, H-6), 11.6 (1 H, s, NH); FAB mass spectrum, m/z 619 $(M + H)^+$, 642 (M + Na). Anal. ($C_{23}H_{29}N_3O_8PSBr$) C, H, N.

1,2-Epoxy-3-(5,6-dihydro-5-methyl-4*H*-1,3,5-dithiazin-2-yl)propane (23). To a rapidly stirred solution of 5,6-dihydro-5-methyldithiazine (1.8 g, 13 mmol) in dry THF (26 mL) at −30 °C was added *n*-butyllithium in *n*-hexane (0.85 g, 14 mmol). After 10 min a thick white precipitate dissolved. After 15 min the reaction mixture was quenched by the addition of phosphate buffer, pH 7.0, and mixture extracted with dichloromethane (3 × 100 mL); the organic layer was dried (K_2CO_3) and solvent removed under reduced pressure to give a yellow oil. Final purification was achieved on a silica gel column eluting with hexane–ethyl acetate (7.5:2.5) to give (89%) of a colorless oil: ^1H NMR ($CDCl_3$) δ 1.9–2.1 (2 H, t, CH_2-3), 2.6 (3 H, s, CH_3), 2.7–2.9 (2 H, m, CH_2-1), 3.0–3.4 (1 H, m, CH-1′), 4.0–4.8 (5 H, m, 2 CH_2-3′,5′, CH-2); mass spectrum, m/e 191. Anal. ($C_7H_{13}NOS_2$) C, H, N.

1-(*N*-Methylamino)-2-hydroxy-3-(5,6-dihydro-5-methyl-4*H*-1,3,5-dithiazin-2-yl)propane (24). This compound was prepared by using the same procedure as for compound 17: ^1H NMR ($CDCl_3$) δ 1.7–1.9 (2 H, t, CH_2-3), 2.3–2.7 (8 H, m, 2 CH_3, OH, NH), 2.8 (2 H, m, CH_2-1), 4.05 (1 H, m, CH-2), 4.25 (1 H, m, 5-CH-5), 4.5–4.9 (4 H, m, 2 CH_2-5); FAB mass spectrum, m/z 223 $(M + H^+)$. Anal. ($C_8H_{18}NOS_2$) C, H, N.

(*E*)-5-(2-Bromovinyl)-2′-deoxy-5′-*O*-[3-methyl-2-oxo-5-(5,6-dihydro-5-methyl-4*H*-1,3,5-dithiazin-2-yl)-1,3,2-oxazaphosphacyclopentan-2-yl]uridine (25a). To a suspension of triethyl phosphate (10 mL) and (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (1.4 g, 4.2 mmol) at 0 °C was added freshly distilled phosphoryl trichloride (1.0 mL, 11.0 mmol) with vigorous stirring. After the mixture was allowed to warm up to room temperature over 3 h, it was cooled to 0 °C, and *N*-methylimidazole (0.66 g, 8 mmol) and 1-(*N*-methylamino)-2-hydroxy-3-(5,6-dihydro-5-methyl-4*H*-1,3,5-dithiazin-2-yl)propane (1.69 g, 7.6 mmol) in 1,2-dimethoxyethane (6 mL) were added dropwise with vigorous stirring under dry N_2. The mixture was stirred overnight at room temperature. The solvent was then removed under reduced pressure to give a yellow oil, which was purified on a silica gel (7734) column using ethyl acetate–ethanol (9:1) as eluant. Pooling of appropriate fractions gave a white solid (1.5 g, 59%): UV λ_{max} 293 nm (ϵ 10 500), λ_{min} 273 nm (ϵ 8490), λ_{max} 250 nm (ϵ 13 560) (in distilled water); ^1H NMR [$(CD_3)_2SO$] δ 2.0–2.3 (4 H, m, CH_2-2′ and CH_2-5″), 2.6 (6 H, m, 2 CH_3), 3.2 (2 H, m, CH_2-5′), 3.4 (1 H, m, CH-3′), 3.9–4.5 (2 H, m, CHO, SCHS), 4.5 (1 H, m, CH-4′), 4.5–4.9 (4 H, m, SCH_2N), 5.5 (1 H, s, OH), 6.3 (1 H, t, H-1′), 7.3 (1 H, m, vinylic), 7.7 (1 H, m, vinylic), 8.7 (1 H, s, H-6), 11.7 (1 H, s, H); FAB mass spectrum, pm 599 $(M + H)^+$, 621 $(M + Na)^+$. Anal. ($C_{19}H_{28}N_4O_7PS_2Br$) C, H, N.

Deprotection of (*E*)-5-(2-Bromovinyl)-2′-deoxy-5′-*O*-[3-methyl-2-oxo-5-(5,6-dihydro-5-methyl-4*H*-1,3,5-dithiazin-2-yl)-1,3,2-oxazaphosphacyclopentan-2-yl]uridine (26a). Compound **25a** (200 mg, 0.33 mmol) was dissolved in 85% aqueous acetonitrile (3 mL) and stirred at room temperature. Then 2.2 equiv of mercuric oxide (156 mg, 0.74 mmol) and mercuric chloride (200 mg, 0.72 mmol) was added. Reaction was monitored by TLC (in ethyl acetate–ethanol, 6:2); after 25 min, no starting material remained. The mixture was then quenched by adding acetonitrile (10 mL) and then 1 equiv of 0.1 M sodium sulfide (13 mL) was added. The black precipitate was filtered off and the clear supernatant was concentrated to a colorless oil under reduced pressure. Further purification was achieved on a silica gel column using ethyl acetate–ethanol (6:2) as eluant to give a white solid (41 mg, 25%): UV λ_{max} 293 nm (ϵ 11 000), λ_{min} 270 nm (ϵ 7950), λ_{max} 250 nm (ϵ 13 000); ^1H NMR [$(CD_3)_2SO$] δ 2.2 (2 H, m, H-2′), 3.3 (3 H, d, NCH_3), 3.8–4.4 (6 H, m, H-4′, CH_2CHCH_2), 5.5 (1 H, d, OH-3′), 6.25 (1 H, t, H-1′), 6.9 (1 H, d, vinylic, J = 15 Hz), 7.3 (1 H, d, vinylic, J = 15 Hz), 7.8 (1 H, s, H-6), 11.6 (1 H, s, NH). Anal. ($C_{16}H_{21}N_3O_8PBr(C_2H_5O)_3PCO$)) C, H, N.

Biological Section. Materials and Methods. Cells. FM3A cells (subclone F28-7), originally established from a spontaneous mammary carcinoma in a C3H/He mouse[14] and designated FM3A/0, were cultured as published earlier.[15] FM3A/TK$^-$ cells, which lack host cell thymidine kinase (TK) activity, and FM3A TK$^-$/HSV-2 TK$^+$ cells, which lack host cell TK activity but express HSV-2 TK activity, were derived from the FM3A/0 cell line as previously described.[15-18] The cells transformed by the HSV-2 TK gene were cultured in the same culture medium as the FM3A/0 and FM3A/TK$^-$ cells.

Murine leukemia L1210, human B-lymphoblast Raji, and T-lymphoblast Molt/4F cells were cultured as described previously.[19]

Inhibition of Cell Proliferation. The methods for evaluating the cytostatic effects of the compounds on the tumor cells have been described previously.[19] Briefly, (5–7.5) × 10^4 cells were suspended in growth medium and added to microplate wells in the presence of varying concentrations of the test compounds. The cells were then allowed to proliferate for 48 or 72 h at 37 °C in a humidified CO_2-controlled atmosphere. At the end of the incubation period, the cells were counted with a Coulter counter.

Antiviral Test Procedures. The antiviral test procedures were based on an inhibition of virus-induced cytopathogenicity in primary rabbit kidney cell cultures following previously established procedures.[20] Briefly, confluent cell cultures in microtiter trays were inoculated with 100 $CCID_{50}$ of virus [herpes simplex virus type 1 (HSV-1) (strain KOS, F or McIntyre), HSV-2 (strain G, 196 or Lyons, TK$^-$ HSV-1 (strain B2006 or VMW 1837), vaccinia virus, or vesicular stomatitis virus], 1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After 1 h of virus adsorption, residual virus was removed and the cell cultures were incubated in the presence of varying concentrations of the test compounds. Viral cytopathogenicity was recorded as soon as it reached completion in the control virus-infected cell cultures.

In Vivo Antiviral Assays. Twenty-five- to 30-day-old athymic nude (nu/nu) mice, weighing 15–20 g, were inoculated intracutaneously in the lumbosacral area with TK$^-$ HSV-1 (VMW 1837) at 10^5 $CCID_{50}$ 50 μL per mouse and treated topically or intraperitoneally at the indicated doses four times or two times a day, respectively, for 5 days, starting immediately after virus infection. The appearance of skin lesions, paralysis of the hind legs, and death of the mice were recorded daily.

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Registry No. 8, 127915-66-6; 8 dephosphorylated derivative, 69304-47-8; 9, 127915-67-7; 9 dephosphorylated derivative, 59277-89-3; 15, 127943-59-3; 16, 127915-68-8; 17, 127915-69-9; 18, 127915-70-2; 19a, 127915-71-3; 19b, 127915-77-9; 19c, 127915-78-0; 20, 119672-65-0; 21, 127915-72-4; 22, 127943-60-6; 23, 127915-73-5; 24, 127915-74-6; 25a, 127915-75-7; 26a, 127915-76-8; 9-[(2-chloroethoxy)methyl]guanine, 127302-85-6; thymidine, 50-89-5.

Synthesis and Antitumor Activity of Novel 4-Demethoxyanthracyclines

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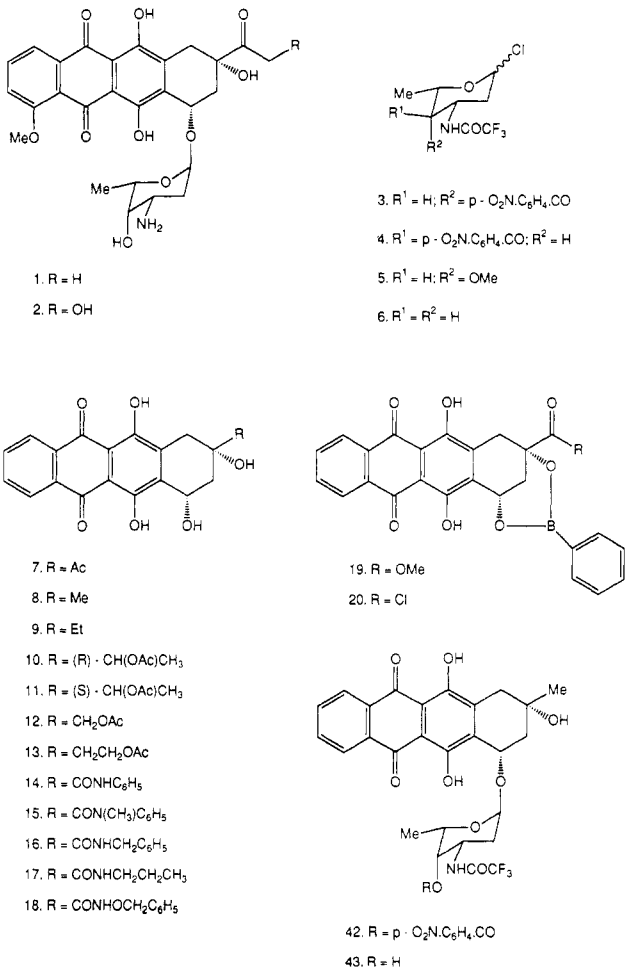
A versatile and efficient synthetic route to 4-demethoxyanthracyclinones has been utilized in the preparation of a number of aglycons having 9-alkyl, 9-(hydroxylalkyl), or 9-carbamoyl substituents. Silver trifluoromethanesulfonate catalyzed coupling of these aglycons with various daunosamine derivatives has yielded a series of novel anthracyclines which have been evaluated as antitumor agents. 9-Alkylanthracyclines **22**, **23**, **33**, and **34** have higher efficacy vs L-1210 leukemia than the parent 4-demethoxydaunorubicin (**21**), or the natural anthracyclines daunorubicin (**1**) and doxorubicin (**2**). 9-(Hydroxyalkyl) derivatives have in most cases high efficacy but are slightly less potent than **21**. 9-Methyl analogue **22** has higher efficacy vs P388 leukemia than other anthracyclines tested, while 9-(hydroxymethyl) derivative **37** retains similar efficacy to anthracyclines **1**, **2**, and **21** but is considerably more potent. The N-substituted 9-carbamoylanthracyclines are devoid of antitumor activity.

The anthracyclines daunorubicin (daunomycin, **1**; Chart I) and, particularly, doxorubicin (adriamycin, **2**) are widely used for the treatment of human tumors.[1-3] However, the clinical use of these agents is hampered by a number of undesirable side effects, particularly a dose-related and irreversible cardiotoxicity. Consequently a major goal for anthracycline research is the identification of new analogues with reduced toxicity, as well as a broader spectrum of antitumor activity, and this objective has stimulated considerable research activity in various industrial and academic laboratories.[4] Some structure–activity relationships have been established for the anthracyclines, but these are largely derived from the investigation of analogues obtained through chemical modification of fermentation-derived products.[5] This has limited the range of new structures since the natural products have labile functions at positions 7 and 9 which are essential for good biological activity. We have therefore undertaken to extend the understanding of structure–activity relationships by utilizing a new method of total synthesis which facilitates the preparation of the substantial quantities of novel anthracylinones necessary for subsequent glycosidation and biological evaluation.[6,7] We have selected as targets for synthesis a number of novel 4-demethoxyanthracyclines because 4-demethoxydaunorubicin (**21**) and 4-demethoxydoxorubicin are claimed to be more potent and less toxic than the parent compounds[8] and are effective in human therapy.[9,10] The choice of position 9 substituents is supported by various observations. Some natural products such as aclacinomycin A,B[11] and cinerubin A[12] which lack a C-13 oxo function show interesting antitumor activities. Besides being a potent antitumor agent, aclacinomycin A is claimed to be considerably less cardiotoxic than doxorubicin, while cinerubin A shows low but reproducible activity against a doxorubicin-resistant strain of P-388 leukemia.[13] In addition, both daunorubicin (**1**) and doxorubicin (**2**) have been converted to 13-deoxy derivatives with retention of considerable antitumor activity.[14] As one of the principal metabolic reactions of the anthracyclines **1** and **2** in man[15] and in animal tissues[16-19] involves reduction of the C-13 oxo function to give 13-dihydroanthracyclines, analogues which lack this function

Chart I



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