Synthesis and Biological Properties of Novel Phosphotriesters: A New Approach to the Introduction of Biologically Active Nucleotides into Cells

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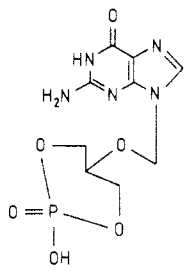
A series of aryl bis(3′-*O*-acetylthymidin-5′-yl) phosphate derivatives have been synthesized in order to find a suitable aryl derivative which would hydrolyze to the bis(nucleosid-5′-yl) phosphate under physiological conditions. The 4-(methylsulfonyl)phenyl derivative was selected and 4-(methylsulfonyl)phenyl bis[(*E*)-5-(2-bromovinyl)-2′-deoxyuridin-5′-yl] phosphate (**6d**) and bis[2-(guanin-9-ylmethoxy)ethoxy]-4-(methylsulfonyl)phenyl phosphate (**7b**) were prepared. The former compound (**6d**) was stable in human serum and only following hydrolysis to the 5′–5′-linked diester (half-life of 17 h at pH 7.7) was it enzymatically degraded very rapidly by phosphodiesterases. Compounds **6d** and **7b** were evaluated for antiherpesvirus effects, both in vitro and in vivo. Their antiviral spectrum and potency was remarkably similar to that of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) and 9-[(2-hydroxyethoxy)-methyl]guanine (ACV), suggesting that they only act as prodrugs of BVDU and ACV, respectively. However, compound **6d** did show unexpected toxicity, which could be explained by the liberation of BVDUMP following penetration of the triester into the cell.

Most of the currently licensed antiviral drugs and many of the drugs used in anticancer chemotherapy are nucleoside analogues.[1] For the majority of these analogues to show biological activity, the action of a kinase to form the 5′-monophosphate is required. The latter is often metabolized further before the active compound is formed.

Nucleotides themselves do not usually penetrate cells at a sufficient rate to show any significant chemotherapeutic effect. Therefore, to produce a successful chemotherapeutic agent of this type, one requires a (viral or cellular) kinase substrate and this puts a severe limitation on compound design. Normally, kinases have significant specificity such that any substantial deviation from the structure of the normal substrate immediately results in loss of activity and hence there is a great incentive to produce a phosphate prodrug so that a monophosphate can be liberated inside a cell.

Two recently discovered, interesting examples of what might be possible if phosphates or their analogues could be liberated inside cells have been shown by the cyclic 3′,5′-monophosphate of 9-[(1,3-dihydroxy-2-propoxy)-methyl]guanine[2,3] (**1**) and the phosphonate analogue (*S*)-9-[3-hydroxy-2-(phosphonylmethoxy)propyl]adenine[4,5] (**2**), which have a very different spectrum of antiviral activity when compared with that of the parent compounds DHPG and (*S*)-HPMPA, respectively.



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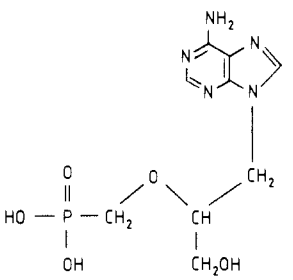
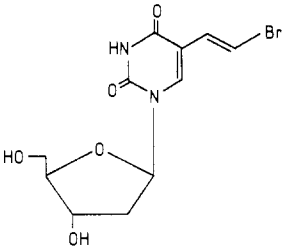


(2)

We agree with the assessment of Montgomery and coworkers[6] that, in many cases, ability to liberate a nucleotide of a known active compound inside a cell might be counterproductive. There are, however, also many examples where the ability to liberate a nucleotide of an otherwise inactive compound could be very useful, particularly in the search for chemotherapeutic agents active against viruses such as the human immunodeficiency virus (HIV), which rely on cellular kinases for initial activation to be eventually targeted at the reverse transcriptase. Indeed, it has been unequivocally shown that the potent anti-HIV agents 3′-azido-3′-deoxythymidine (AZT), 2′,3′-dideoxycytidine (ddCyd), and 2′,3′-didehydro-3′-deoxythymidine (D4T) solely depend on cellular kinases to be converted to their corresponding 5′-triphosphate metabolites.[7-9]

For many years we have been synthesizing potential phosphate prodrugs, with limited success.[10,11] One of the main problems is devising an assay system which can show unequivocally whether or not the principles involved are sound. Recently, the development of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) (**3**) and 9-[(2-hydroxyethoxy)methyl]guanine (ACV) (**4**) as herpes simplex virus



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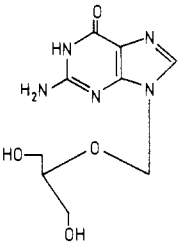

(4)

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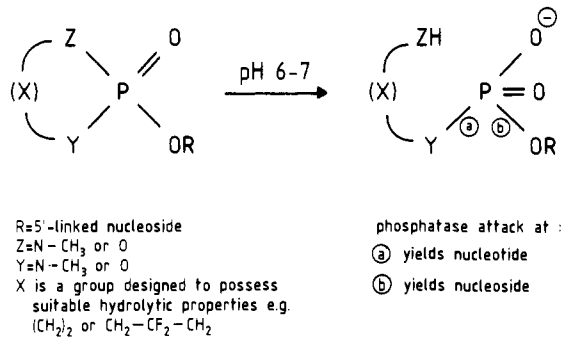
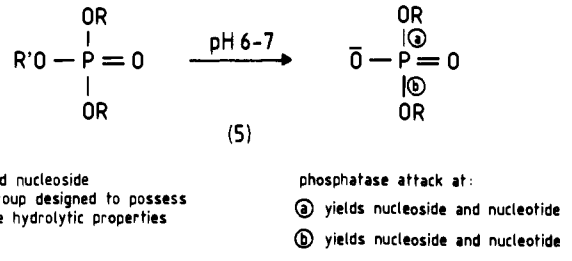
Scheme I



Scheme II



(HSV) inhibitors which depend upon the viral thymidine kinase (TK) for activation and the isolation of drug-resistant virus strains which owe this property to an inability to phosphorylate the drug has lead to the possibility of establishing meaningful in vitro and in vivo assays. Infection by these resistant (TK^-) virus strains is not affected by BVDU and/or ACV, and thus if the monophosphate of either drug could be liberated inside such an infected cell, the infection should be susceptible.

We realize that there are still problems even with this system. For example, a TK^- strain may also be thymidylate kinase negative, in which case, although BVDUMP may be liberated, its progress to BVDUTP would still be blocked. Even if capable of being further phosphorylated, presumably, if the monophosphate were to be liberated intracellularly, its further direction to biochemical transformation depends upon its concentration and the K_m for the relevant enzymes.

Our previous attempts at phosphate prodrug synthesis always required at some stage the action of a phosphatase on a phosphodiester (liberated by hydrolysis of a cyclic triester) to liberate the nucleoside monophosphate. Unfortunately, the preferred reaction appeared to be that to liberate nucleoside (Scheme I). The current work describes the synthesis of aryl bis(nucleosid-5'-yl) phosphates, which upon enzymatic attack should yield 1 equiv each of nucleoside and nucleotide (Scheme II).

Chemistry

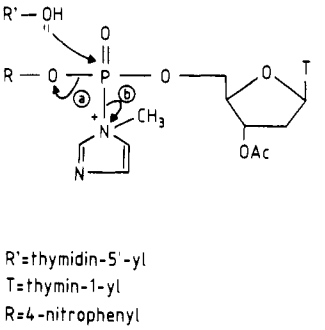
A series of compounds of type 5 (Scheme II, Table I) were synthesized to give a range of stability to hydrolysis under physiological conditions. As model compounds, R was thymidin-5-yl and a range of phenols were used for R'.

3'-*O*-Acetylthymidine was made in the usual way and the first triester synthesized was where R = 4-chlorophenyl (**5b**). The required phosphorylating agent, 4-chlorophenyl phosphorodichloridate is commercially available and the product (**5b**) could be expected to have reasonable stability. The method of synthesis devised by Pfleiderer and co-workers[12] was used, and a rather low yield of analytically pure product was obtained following a flash chromatographic separation of the reaction mixture. Subsequently, this low yield was shown to be related to the degree of purity of the phosphorylating agent, which should be redistilled immediately before use.

Table I. Properties of Phosphotriesters (**5a–f**) where R = 3'-*O*-Acetylthymidin-5'-yl

compd	R'	pK_A of ROH	^{31}P NMR	$t_{1/2}$, h
5a	4-(methylthio)phenyl	9.53	−5.757	
5b	4-chlorophenyl	9.38	−5.986	
5c	2-chlorophenyl	8.48	−6.271	
5d	4-(methylsulfonyl)phenyl	7.83	−6.579	17
5e	2,5-dichlorophenyl	7.50	−6.559	33
5f	4-nitrophenyl	7.16	−6.852	6

Scheme III



Attention was then turned to the alkylarylsulfide derivative (**5a**) and the corresponding sulfone (**5d**). The former was expected to be rather stable but could be oxidized to the sulfone as the last step in the synthesis to yield what would be expected to be a much more labile triester. This principle has been applied before.[13] 4-(Methylthio)phenyl phosphorodichloridate was prepared by a modification of the method described by Reese and co-workers,[14] but using 1-methylimidazole as catalyst. Triester **5a** was prepared as before but 1-methylimidazole was used as catalyst in preference to 1-benzylimidazole, as the former could be more rigorously purified. The product **5a** could be easily oxidized to sulfone **5d** with 3 equiv of 4-chloroperoxybenzoic acid.

The 2-chlorophenyl- and 2,5-dichlorophenyl triesters (**5c** and **5e**, respectively) were also made in an analogous way. The isolated yield of the former was quite high (64%), but due to the lability of the latter, only a 10% yield of analytically pure material could be isolated.

The final compound in this series, **5f**, was expected to be the most unstable. First attempts to prepare the triester resulted in the isolation of a compound which contained the imidazole moiety. It is probable that the properties of the intermediate formed in this reaction are such that the 4-nitrophenol (a) leaves in preference to the imidazole (b) (Scheme III). The resulting product must then undergo a P–N to P–C bond migration as the product clearly did not contain a positively charged nitrogen atom, and NMR evidence (^1H and ^{31}P) was consistent with the formation of a bond between P and C-5 of the imidazole ring.[15] However, if the reaction was performed with a reduced amount of catalyst and with the addition of triethylamine to quench the HCl liberated, although this

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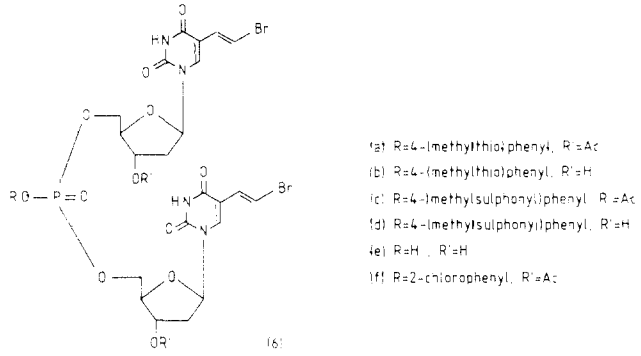
reaction is much slower, an analytically pure sample of 4-nitrophenyl triester **5f** could be isolated in poor (12%) yield.

The stability of these six triesters (**5a–f**) was investigated by a variety of means. The hydrolysis of **5f** could be followed colorimetrically as 4-nitrophenol is liberated. These results were then confirmed by HPLC analysis of the hydrolysate at different times and this latter method was then used to measure the rate of hydrolysis of the other esters. To confirm the identity of the product, the hydrolysis of **5e** was also followed by ^{31}P NMR, which confirmed that the product of hydrolysis formed at the rate determined by HPLC analysis was indeed the 5′–5′-linked phosphodiester. The results of these studies are given in Table I.

As can be seen, there is a reasonable correlation between the pK_a of the phenolic protecting group, the chemical shift of the ^{31}P NMR signal, and the half-life of the corresponding phosphotriester in buffered aqueous solution at pH 7.7. The exception is 2,5-dichloro derivative **5e**, where steric factors could be important. The results show quite clearly that under physiological conditions **5a–c** are far too stable and show no significant hydrolysis within 24 h. The decision was made to investigate further derivatives of the type **5d** for two reasons. Firstly, the stability of these compounds was of the right order and secondly they could be formed at the last stage of synthesis from the corresponding much more stable derivative **5a**.

The stability of **5a** enables the 3′-*O*-acetyl protecting group to be removed at this stage with potassium carbonate in aqueous methanol with no resulting hydrolysis of the 4-(methylthio)phenyl phosphotriester.

Thus, the corresponding 5′–5′-linked phosphotriester **6a** from (*E*)-5-(2-bromovinyl)-2′-deoxyuridine was synthesized.

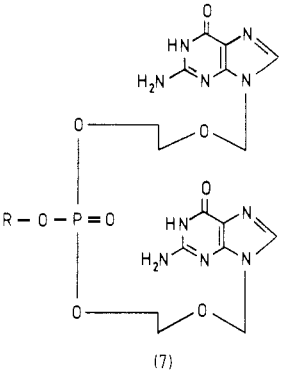


(6)
(a) R=4-(methylthio)phenyl, R'=Ac
(b) R=4-(methylthio)phenyl, R'=H
(c) R=4-(methylsulphonyl)phenyl, R'=Ac
(d) R=4-(methylsulphonyl)phenyl, R'=H
(e) R=H, R'=H
(f) R=2-chlorophenyl, R'=Ac

A preliminary investigation showed that the oxidation of methylthio to methylsulfonyl (**6a** → **6c**) could be achieved with 4-chloroperoxybenzoic acid, toward which the bromovinyl group was inert, and thus the acetyl groups of **6a** were removed to give **6b**, which was subsequently oxidized to **6d**.

The expected products of hydrolysis (**6e** and the 5′-mononucleotide) of **6d** were required as standards so that the hydrolysis of **6d** could be followed. Diester **6e** was prepared by hydrolyzing the 2-chlorophenyl triester (**6f**) under alkaline conditions, at which time the 3′-*O*-acetyl groups were removed as well to give **6e**. The other expected product of synthesis, the 5′-mononucleotide, was synthesized directly from the nucleoside by using the procedure described by Yoshikawa[16] as modified by Sagi et al.[17]

The synthesis of the corresponding triester (**7b**) from acyclovir (ACV) should theoretically be easier, as there is no other hydroxyl group to consider. Although, the lack of solubility of ACV caused some problems.



(7)
(a) R=4-(methylthio)phenyl
(b) R=4-(methylsulphonyl)phenyl

Pyridine had to be used as a solvent and even then the required compound **7a** could only be isolated in low (19%) yield. This triester could not be conveniently oxidized to the corresponding sulfone as the only solvent in which it would dissolve was DMSO, which is not compatible with a 4-(chlorophenyl)peroxybenzoic acid oxidation.

Thus, 4-(methylsulfonyl)phenyl phosphorodichloridate was synthesized and used to produce the corresponding triester **7b** directly. Standard conditions were used except that the reaction was initially carried out with ultrasonic treatment to increase the contact between the reagents. Even so, due to solubility problems and the instability of the product, only low yields could be obtained. Compound **7b** had a half-life (17 h) similar to that of compound **5d**.

Results and Discussion

Enzymatic Hydrolyses. Diester compound **6e** was subjected to a variety of enzymatic hydrolyses. The results (data not shown) reveal that, as expected, the diester is a substrate for snake venom phosphodiesterase and the enzymes present in human serum to give the final product (*E*)-5-(2-bromovinyl)-2′-deoxyuridine. Compound **6e** was hydrolyzed to the extent of 80% in 4 h. Triester **6d** was stable to enzymatic attack and was only further hydrolyzed enzymatically following chemical hydrolysis, which at pH 7.7 has a half-life of 17 h. This implies that the triester has plenty of time in which to penetrate the cell before hydrolysis to the diester occurs, which is then subsequently a substrate for enzymatic hydrolysis.

Antiviral Effects. Compounds **6d** and **7b**, the 5′–5′-dimer of BVDU and corresponding dimer of ACV, respectively, were evaluated for their activity against a panel of several HSV-1, HSV-2, and HSV-1 TK$^-$ strains, vaccinia virus, and vesicular stomatitis virus. Compounds **6d** and **7b** were markedly active against the wild-type (TK$^+$) HSV-1 strains; **7b** was also markedly active against the TK$^+$ HSV-2 strains (Table II). However, neither **6d** nor **7b** showed appreciable activity against the TK$^-$ HSV-1 strains. When **6d** and **7b** had been preincubated in medium containing 10% fetal calf serum for 24 h at 37 °C prior to the addition to the cells, they became (slightly) more active (Table II). The parent compounds BVDU and ACV, that were included as reference compounds, proved clearly more inhibitory to HSV-1, HSV-2, and vaccinia

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Table II. Cytotoxicity and Antiviral Activity of **6d** in Primary Rabbit Kidney Cell Cultures

compound	minimum cytotoxic concn,[a] μg/mL	minimum inhibitory concentration,[b] μg/mL									
		herpes simplex virus-1			herpes simplex virus-2			vaccinia virus	vesicular stomatitis virus	TK⁻ herpes simplex virus-1	
		KOS	F	McIntyre	G	196	Lyons			B2006	VMW 1837
					As Such						
6d	>400	2	2	2	>200	>400	>400	>400	>400	>400	>400
7b	400	0.7	0.7	1	0.4	0.4	0.4	>200	>200	150	70
					Upon Preincubation at 37 °C for 24 h						
6d	>400	0.2	0.2	0.7	150	>400	200	70	>400	>400	300
7b	400	0.4	0.4	0.7	0.2	0.3	0.4	>200	>200	150	40
BVDU	>400	0.02	0.02	0.02	4	150	40	7	>400	200	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%.

virus than **6d** and **7b** (whether preincubated or not). The antiviral activity spectrum of **6d** was remarkably similar to that of BVDU and that of **7b** was similar to ACV (Table II), suggesting that under our experimental conditions the 5′–5′-dimers of BVDU and ACV must have been degraded to their parent compounds and, thus, actually act as prodrugs of BVDU and ACV, respectively. This would explain why in our cell culture system in vitro the 5′–5′-dimer of BVDU and ACV did not affect the replication of TK⁻ HSV-1. Alternatively, the 5′-monophosphate derivative may be liberated inside the cell but not further converted to the 5′-triphosphate derivative of BVDU and ACV. Regardless of whether hydrolysis actually occurs in the cells, our results provide clear evidence that both compounds **6d** and **7b** were at least subject to hydrolytic attack in an intact biological in vitro cell model.

To investigate whether **6d** and **7b** may affect TK⁻ HSV-1 infection in vivo, a new animal model, based on intracutaneous infection of nu/nu mice with the VMW 1837 variant of HSV-1 was introduced. This variant was isolated from an immunosuppressed patient with a chronic HSV-1 infection that had become resistant to acyclovir treatment.[18] The HSV-1 variant isolated from this patient was resistant to acyclovir and BVDU in vitro,[19] and plaque autoradiography (carried out by J. Christophers, Department of Virology, University Hospital, South Manchester, Great Britain) indicated that it was composed of 92% TK⁻ HSV-1 and 8% TK⁺ HSV-1. The VMW 1837 variant HSV-1 was found pathogenic for athymic–nude (nu/nu) mice following intracutaneous inoculation: it caused skin lesions and/or paralysis of the hind legs.

Compound **6d** delayed the appearance of skin lesions and paralysis when applied topically at 0.3% to nu/nu mice infected with HSV-1 (VMW 1837) (Table III). At a concentration of 3%, **6d** shortened the mean day of death (15 days) (as compared to the placebo group) (35.2 days), which might reflect the toxicity of the compound at this concentration. When evaluated under the same conditions, BVDU did not affect the mean survival time, nor the appearance of lesions whether it was applied at 0.3, 1, or 3%. However, such treatment proved effective in delaying the appearance of skin lesions and paralysis, as well as increasing the life span, of nu/nu mice infected with the wild type HSV-1 (KOS) (Table III).

When compound **7b** was evaluated in nu/nu mice infected with HSV-1 (VMW 1837) (Table III), it was found to increase the mean survival time as well as the time needed for the skin lesions and paralysis to appear, whether it was applied at 0.3, 1, or 3%. In this respect, compound **7b** and ACV proved about equally effective. ACV was also effective in delaying the appearance of skin lesions and paralysis and increasing the life span of nu/nu mice infected with the wild type HSV-1 (KOS) (Table III).

Table III. Topical Treatment of Intracutaneous HSV-1 (KOS) or TK⁻ HSV-1 (VMW 1837) Infection of Nu/nu Mice[a]

compd	concn (v/v in DMSO)	mean day of appearance of skin lesions and/or paralysis of the hind legs	mean time of survival,[b] days
		HSV-1 (KOS) Infection	
BVDU	3%	9.4 ± 1.5	13.4 ± 1.9
	1%	9.4 ± 4.3	16 ± 9.6
	0.3%	6.2 ± 0.8	9.4 ± 3.3
placebo	DMSO	4.8 ± 0.8	6.6 ± 0.9
ACV	3%	12 ± 1.4	23.2 ± 6.6
	1%	14.2 ± 6.5	22.2 ± 6.7
	0.3%	8.2 ± 2.6	11.6 ± 3.0
placebo	DMSO	4.6 ± 0.5	6.6 ± 0.9
		TK⁻ HSV-1 (VMW 1837) Infection	
6d	3%	9.7 ± 3.9	15 ± 3.9
	1%	10.4 ± 4.6	24.2 ± 14.5
	0.3%	16.7 ± 5.6	37.8 ± 14.7
BVDU	3%	10.8 ± 4.8, 9.4	31.4 ± 10.4, 34.2
	1%	9.7 ± 5.5, 10.6	38.6 ± 12.7, 30.6
	0.3%	8.8 ± 4.4	30.6 ± 16.2
placebo	DMSO	10 ± 4.7	35.2 ± 14.7
7b	3%	29.7 ± 4.5	40.2 ± 6.5
	1%	23.6 ± 13.5	40.6 ± 9.4
	0.3%	17.6 ± 13.4	33.4 ± 14.1
ACV	3%	29.0 ± 3.6	40.4 ± 5.3
	1%	16.7 ± 9.0	35.6 ± 11.7
	0.3%	17.0 ± 16.7	43.4 ± 7.9
placebo	DMSO	6.8 ± 0.8	20.4 ± 10.6

[a] Results obtained from different experiments (with five mice per group). Where more than one value is given per group, it represents data obtained for this group on a different occasion. [b] Mice infected with HSV-1 (KOS) or TK⁻ HSV-1 (VMW 1837) were followed for 28 or 49 days, respectively. Those mice that were still alive at the end of the experiment were taken into account as 28- or 49-day survivors, respectively.

Interpretation of the in vivo data is complicated by several factors. Firstly, on a weight basis, only 66% of compound **6d** and **7b** is potentially BVDU or ACV, which makes the protective effect of the compounds more impressive. Secondly, the VMW 1837 variant is a mixture of TK⁻ and TK⁺, whereas in the control animals, BVDU and ACV, which are presumably active against TK⁺ strain, are present from the time of initial infection; in the animals treated with triester **6d** and **7b**, until hydrolysed, no antiviral agent is present. The determined half-life of **6d** is 17 h, during which time the TK⁺ infection could be expected to have progressed significantly when compared with that in the control animals. Thus the increased ef-

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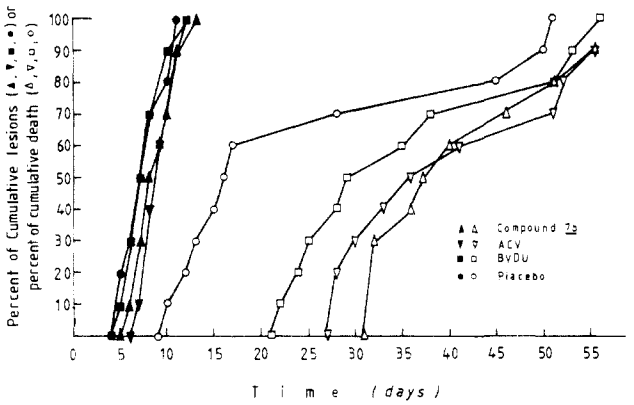

Figure 1. Topical treatment of intracutaneous TK⁻ HSV-1 (VMW 1837) infection of nu/nu mice. Effects of compounds **7b**, ACV, BVDU, and placebo (DMSO) on the development of herpetic skin lesions, paralysis of the hind legs, and mortality of nu/nu mice inoculated intracutaneously with "plaque purified" TK⁻ HSV-1 (BMW 1837). The compounds were applied at 1% (**7b**) or 0.67% (ACV, BVDU) in DMSO. There were 10 mice per group.

ficacy of **6d** may be due to the slow release of either BVDU or BVDUMP, or both. The former is effective against TK⁺ infection and the latter against TK⁻ and TK⁺.

Some evidence for the liberation of BVDUMP is provided by the toxicity seen when the dose of **6d** is increased. Experiments in which the corresponding triester of acyclovir was used (**7b**) indicated that this toxicity could not be due to the liberation of the phenol upon triester hydrolysis, as **7b** had no detectable toxicity. In the light of recent findings that BVDUMP causes severe toxicity when accumulating intracellularly,[20] it is likely that the toxicity shown by **6d** is due to the liberation of BVDUMP as both the phenol and BVDU, the only possible other products are not toxic at the concentrations used. It may thus be possible to liberate a nucleoside monophosphate inside cells. This opens the way to the synthesis of other prodrugs where it could be beneficial to overcome a potential blockage in a metabolic pathway because a necessary intermediate is not a kinase substrate.

A final experiment was carried out in nu/nu mice which had been infected with "plaque purified" TK⁻ HSV-1 (VMW 1837) (obtained from J. Christophers). In this experiment compound **7b** was applied at 1%, and ACV and BVDU were applied at 0.67%. As shown in Figure 1, neither **7b** nor ACV or BVDU proved effective in delaying the appearance of lesions (herpetic skin lesions and/or paralysis of the hind legs). Within 12 days after infection all mice showed lesions irrespective of whether they had been treated with **7b**, ACV, BVDU, or placebo. However, all three forms of treatment achieved a significant increase in the mean survival time of the infected mice. While 50% of the placebo-treated mice had died after 16 days, the 50% mortality points for the BVDU, ACV, and **7b** groups were reached only after 29, 36, and 37 days, respectively. Thus, in this last experiment (Figure 1), as in the previous one (Table III), no significant differences were noted in the behavior of **7b** and ACV, which suggests that both drugs act in a similar fashion and that, considering the structure of **7b** as compared to ACV, the former must act as a prodrug for the latter.

Experimental Section

NMR spectra (^{1}H) were recorded on JEOL FX 90Q (90 MHz) and JEOL GX 270 (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 MS80 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 7734 (0.063–0.200 nm, 70–230 mesh) or 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 otherwise indicated.

Aryl Phosphorodichloridates. 4-Chlorophenyl, 2-chlorophenyl, 2,5-dichlorophenyl and 4-nitrophenyl phosphorodichloridate were obtained from commercial sources.

4-(Methylthio)phenyl Phosphorodichloridate. To a solution of freshly distilled phosphoryl chloride (45 mL, 0.5 mol) and 1-methylimidazole (0.15 mL) was added 4-(methylthio)phenol (14 g, 0.1 mol) and the solution was heated under reflux for 20 h. The excess of phosphoryl chloride was removed by distillation and the residue was distilled under reduced pressure to give the product (11 g, 42% yield): bp 135–142 °C (2 mmHg) [lit.[8] bp 120 °C (1 mmHg)]; ^{1}H NMR ($CDCl_3$) δ 2.39 (3 H, s, SCH_3), 7.22 (4 H, s, phenyl).

4-(Methylsulfonyl)phenol. To a solution of 4-(methylsulfonyl)phenol (7.0 g, 0.05 mol) in 30% aqueous methanol (100 mL) at 0 °C was added a solution of sodium periodate (10.7 g, 0.05 mmol) and the resulting suspension was stirred for 30 min. Water (500 mL) was then added and the precipitate was removed by filtration. The filtrate was cooled to 4 °C and a further portion of sodium periodate (10.7 g, 0.05 mmol) was added and the resulting suspension was stirred for 48 h when a further portion of sodium periodate (5.35 g, 0.025 mmol) was added. After stirring for a further 18 h, the precipitate was removed by filtration, the filtrate was extracted with ether, which was evaporated to dryness, and the residue was purified on a silica column using chloroform–methanol (9:1) as eluent to give the title compound (2.75 g, 32% yield). Anal. ($C_7H_8O_3S$) C, H.

4-(Methylsulfonyl)phenyl Phosphorodichloridate. 4-(Methylthio)phenol (3.0 g, 17 mmol) was heated under reflux with freshly distilled phosphoryl chloride (13.35 mL, 87 mmol) and 1-methylimidazole (0.05 mL) for 20 h. The excess of phosphoryl chloride was removed by distillation and the residue was distilled under reduced pressure to give the title compound [bp 185 °C (1 mmHg)] as a yellow oil, which solidified on cooling (500 mg, 10% yield). Anal. (free acid $C_7H_9O_6S$) C, H.

4-Chlorophenyl Bis(3′-*O*-acetylthymidin-5′-yl) Phosphate (5b). In one arm of an H-shaped reaction vessel, 3′-*O*-acetylthymidine (0.142 g, 0.5 mmol) was suspended in acetonitrile (1 mL). In the second arm, 1-benzylimidazole (0.27 g, 1.7 mmol) and 4-chlorophenyl phosphorodichloridate (0.053 mL, 0.3 mmol) were stirred in acetonitrile (0.75 mL) for 3 min, and the contents of the two arms were mixed and stirred for 20 h at room temperature. Phosphate buffer (pH 6.0, 0.2 M, 25 mL) was added, the mixture was extracted with chloroform, and the organic-soluble material was worked up in the usual way. The product (54 mg, 30% yield) was isolated by flash column chromatography using ethyl acetate as eluent: UV λ_{max} 264 nm (ϵ 17 360), λ_{min} 234 nm (ϵ 1400); ^{1}H NMR δ 1.74 (6 H, s, CH_3-5), 2.10 (6 H, s, $COCH_3$), 2.32 (4 H, m, H-2′), 4.16 (2 H, m, H-4′), 4.44 (4 H, m, H-5′), 5.20 (2 H, m, H-3′), 6.28 (2 H, t, H-1′), 7.1–7.4 (6 H, m, H-6 and phenyl), 9.70 (2 H, s, NH); ^{31}P NMR δ −5.986. Anal. ($C_{30}H_{34}N_4O_{14}ClP$) C, H, N.

4-(Methylthio)phenyl Bis(3′-*O*-acetylthymidin-5′-yl) Phosphate (5a). 4-(Methylthio)phenyl phosphorodichloridate (0.6 mL, 3.5 mmol) and 1-methylimidazole (1.5 L, 17 mmol) in acetonitrile (7.5 mL) were stirred for 5 min at room temperature and then added to a suspension of 3′-*O*-acetylthymidine (1.42 g, 5 mmol) in acetonitrile (10 mL), and the mixture was stirred for 3 days at room temperature. The product was isolated as described above except that ethyl acetate–ethanol (95:5) was used

(20) Balzarini, J.; De Clercq, E.; Verbruggen, A.; Ayusawa, D.; Shimizu, K.; Seno, T. *Mol. Pharmacol.* **1987**, *32*, 410.

as the column eluent. Fine purification was achieved with a Chromatotron eluting with chloroform–methanol (4:1) containing 2% triethylamine (460 mg, 25% yield): UV λ_{max} 257 nm (ϵ 26500), λ_{min} 231 nm (ϵ 8400); ^{1}H NMR δ 1.76 (6 H, s, CH_3-5), 2.09 (6 H, s, $COCH_3$), 2.30 (4 H, m, H-2′), 2.45 (3 H, s, SCH_3), 4.20 (2 H, m, H-4′), 4.40 (4 H, m, H-5′), 5.20 (2 H, m, H-3′), 6.19 (2 H, t, H-1′), 7.20 (4 H, m, phenyl), 7.49 (2 H, s, H-6), 11.36 (2 H, s, NH); ^{31}P NMR δ −5.757; FAB mass spectrum, m/z 753 $(M + H)^+$, 775 $(M + Na)^+$. Anal. ($C_{31}H_{37}N_4O_{14}PS\cdot H_2O$) C, H, N.

4-(Methylsulfonyl)phenyl Bis(3′-*O*-acetylthymidin-5′-yl) Phosphate (5d). To a solution of compound **5a** (250 mg, 0.34 mmol) in dry ethanol (30 mL), cooled to 0 °C, was added (from a syringe) a solution of 3-chloroperoxybenzoic acid (mcpba, 172 mg, 1.0 mmol) in dry ethanol (1 mL). The solution was left overnight at 5 °C, the ethanol was removed by evaporation, and the resulting residue was separated on a column of silica gel (type 9385) with chloroform–ethanol (15:1) as eluent. Further purification was achieved with a Chromatotron and the same solvent to give the product (146 mg, 56% yield): UV λ_{max} 263 nm (ϵ 18100), λ_{min} 237 nm (ϵ 6300); ^{1}H NMR δ 1.70 (6 H, s, CH_3-5), 2.08 (6 H, s, $COCH_3$), 2.32 (4 H, m, H-2′), 3.20 (3 H, s, SO_2CH_3), 4.20 (2 H, m, H-4′), 4.45 (4 H, m, H-5′), 5.20 (2 H, m, H-3′), 6.18 (2 H, t, H-1′), 7.40–7.49 (4 H, m, H-6 and phenyl), 7.88–7.97 (2 H, d, phenyl), 11.33 (2 H, s, NH); ^{31}P NMR δ −6.579. Anal. ($C_{31}H_{37}N_4O_{16}PS$) C, H, N.

2-Chlorophenyl Bis(3′-*O*-acetylthymidin-5′-yl) Phosphate (5c). This compound was prepared as described above for **5b**. The product (1.20 g, 64% yield) was isolated by flash column chromatography using ethyl acetate as eluent: UV λ_{max} 265 nm (ϵ 18200), λ_{min} 234.5 nm (ϵ 5500); ^{1}H NMR δ 1.70 (6 H, s, CH_3-5), 2.06 (6 H, s, $COCH_3$), 2.31 (4 H, m, H-2′), 4.22 (2 H, m, H-4′), 4.44 (4 H, m, H-5′), 5.19 (2 H, m, H-3′), 6.28 (2 H, t, H-1′), 7.2–7.6 (6 H, m, H-6 and phenyl), 11.35 (2 H, s, NH); ^{31}P NMR δ −6.271; FAB mass spectrum, m/z 763, 765 (M + H). Anal. ($C_{30}H_{34}ClN_4O_{14}P$) C, H, N.

2,5-Dichlorophenyl Bis(3′-*O*-acetylthymidin-5′-yl) Phosphate (5e). This compound was prepared as described above for **5b**. The product was finally purified with a Chromatotron with ethyl acetate as eluent (100 mg, 10% yield): UV λ_{max} 265 nm (ϵ 22700), λ_{min} 233 nm (ϵ 9000); ^{1}H NMR δ 1.68 (6 H, s, CH_3-5), 2.08 (6 H, s, $COCH_3$), 2.30 (4 H, m, H-2′), 4.21 (2 H, m, H-4′), 4.48 (4 H, m, H-5′), 5.20 (2 H, m, H-3′), 6.20 (2 H, t, H-1′), 7.3–7.6 (5 H, m, H-6 and phenyl), 11.32 (2 H, s, NH); ^{31}P NMR δ −6.559. Anal. ($C_{30}H_{33}Cl_2N_4O_{14}P$) C, H, N.

4-Nitrophenyl Bis(3′-*O*-acetylthymidin-5′-yl) Phosphate (5f). 4-Nitrophenyl phosphorodichloridate (0.385 g, 1.5 mmol), 1-methylimidazole (0.022 mL, 0.25 mmol), triethylamine (0.42 mL, 3 mmol), and acetonitrile (5 m) were stirred for 5 min at room temperature and then added to a suspension of 3′-*O*-acetylthymidine (0.72 g, 2.5 mmol) in acetonitrile (10 mL) and stirred. After 3 days at room temperature, a further portion of 4-nitrophenyl phosphorodichloridate (0.218 g, 0.5 mmol) with the appropriate amounts of 1-methylimidazole and triethylamine in acetonitrile was added and stirring was continued for another 3 days. The resulting mixture was then worked up in the usual way and the desired compound was finally purified with flash column chromatography and a Chromatotron with ethyl acetate as eluent (110 mg, 12% yield): UV λ_{max} 264 nm (ϵ 25000), λ_{min} 234 nm (ϵ 7000); ^{1}H NMR δ 1.80 (6 H, s, CH_3-5), 2.15 (6 H, s, $COCH_3$), 2.34 (4 H, m, H-2′), 4.25 (2 H, m, H-4′), 4.50 (4 H, m, H-5′), 5.25 (2 H, m, H-3′), 6.25 (2 H, t, H-1′), 7.50 (4 H, H-6 and phenyl), 8.25 (2 H, d, phenyl), 11.40 (2 H, s, NH); ^{31}P NMR δ −6.852; FAB mass spectrum, m/z 752 $(M + H)^+$, 774 $(M + Na)^+$.

4-(Methylthio)phenyl Bis(thymidin-5′-yl) Phosphate. Compound **5a** (60 mg, 0.08 mmol) was dissolved in a solution of methanol–water (9:1, 20 mL) containing potassium carbonate (11 mg) and stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was purified on a silica column using chloroform–ethanol (9:1) as eluent to give the product (35 mg, 66% yield): UV λ_{max} 257 nm (ϵ 22300), λ_{min} 231 nm (ϵ 8000); ^{1}H NMR δ 1.70 (6 H, s, CH_3), 2.10 (4 H, m, H-2′), 2.45 (3 H, s, SCH_3), 3.95 (2 H, m, H-4′), 4.29 (2 H, m, H-3′), 4.35 (4 H, m, H-5′), 5.45 (2 H, s, OH-3′), 6.19 (2 H, t, H-1′), 7.20 (4 H, m, phenyl), 7.44 (2 H, d, H-6), 11.30 (2 H, s, NH); ^{31}P NMR δ −6.192; FAB mass spectrum, m/z 669 $(M + H)^+$. Anal. ($C_{27}H_{33}N_4O_{12}PS\cdot 2H_2O$) C, H, N.

4-(Methylthio)phenyl Bis[(*E*)-5-(2-bromovinyl)-3′-*O*-acetyl-2′-deoxyuridin-5′-yl] Phosphate (6a). The title compound was prepared in a manner identical to that previously described for the corresponding thymidine analogue using (*E*)-5-(2-bromovinyl)-3′-*O*-acetyl-2′-deoxyuridine (1.13 g, 3 mmol) as starting material and allowing the reaction to proceed for 20 h at room temperature (780 mg, 55% yield): UV λ_{max} 292 nm (ϵ 29300), 250 nm (ϵ 46500); λ_{min} 271 nm (ϵ 22300); ^{1}H NMR δ 2.06 (6 H, s, $COCH_3$), 2.35 (4 H, m, H-2), 2.43 (3 H, s, SCH_3), 4.21 (2 H, m, H-4′), 4.40 (4 H, m, H-5′), 5.19 (2 H, m, H-3′), 6.15 (2 H, t, H-1′), 6.74 (2 H, d, vinylic, J = 14 Hz), 7.1 (4 H, m, phenyl), 7.25 (2 H, d, vinylic, J = 14 Hz), 11.60 (2 H, s, NH); ^{31}P NMR δ −4.885; FAB mass spectrum, m/z 933, 935, 937 $(M + H)^+$ (1:2:1). Anal. ($C_{33}H_{35}Br_2N_4O_{14}PS$) C, H, N.

4-(Methylsulfonyl)phenyl Bis[(*E*)-5-(2-bromovinyl)-3′-*O*-acetyl-2′-deoxyuridin-5′-yl] Phosphate (6c). Compound **6a** (100 mg, 0.1 mmol) was oxidized with mcpba and the product was worked up as previously described to give the title compound (45 mg, 47% yield): UV λ_{max} 291 nm (ϵ 26000), 248 nm (ϵ 31000), λ_{min} 268 nm (ϵ 17500); ^{1}H NMR δ 2.07 (6 H, s, $COCH_3$), 2.40 (4 H, m, H-2′), 3.20 (3 H, s, SO_2CH_3), 4.22 (2 H, m, H-4′), 4.48 (4 H, m, H-5′), 5.21 (2 H, m, H-3′), 6.19 (2 H, t, H-1′), 6.79 (2 H, d, vinylic, J = 15 Hz), 7.24 (2 H, d, vinylic, J = 15 Hz), 7.45 (2 H, d, phenyl), 7.83 (2 H, d, H-6), 7.92 (2 H, d, phenyl), 11.65 (2 H, s, NH); ^{31}P NMR δ -6.999. Anal. ($C_{33}H_{35}Br_2N_4O_{16}PS$) C, H, N.

4-(Methylthio)phenyl Bis[(*E*)-5-(2-bromovinyl)-2′-deoxyuridin-5′-yl] Phosphate (6b). The 3′-*O*-acetyl groups were removed from compound **6a** as previously described to give the title compound (180 mg, 70% yield): UV λ_{max} 290 nm (ϵ 28000), λ_{max} 250 nm (ϵ 44400), λ_{min} 272 nm (ϵ 23000); ^{1}H NMR δ 2.20 (4 H, m, H-2′), 2.43 (3 H, s, SCH_3), 3.97 (2 H, m, H-4′), 4.27 (2 H, m, H-3′), 4.35 (4 H, m, H-5′), 5.49 (2 H, d, OH-3′), 6.20 (2 H, t, H-1′), 6.80 (2 H, d, vinylic, J = 14 Hz), 7.19 (4 H, m, phenyl), 7.25 (2 H, d, vinylic, J = 14 Hz), 7.78 (2 H, d, H-6), 11.61 (2 H, s, NH); ^{31}P NMR δ −6.192. Anal. ($C_{29}H_{31}Br_2N_4O_{12}PS$) C, H, N.

4-(Methylsulfonyl)phenyl Bis[(*E*)-5-(2-bromovinyl)-2′-deoxyuridin-5′-yl] Phosphate (6d). Compound **6b** was oxidized with mcpba as previously described to give the title compound (70% yield): UV λ_{max} 292 nm (ϵ 25500), λ_{max} 249 nm (ϵ 30700), λ_{min} 268.5 nm (ϵ 17000); ^{1}H NMR δ 2.07 (6 H, s, $COCH_3$), 2.40 (4 H, m, H-2′), 3.20 (3 H, s, SO_2CH_3), 4.22 (2 H, m, H-4′), 4.48 (4 H, m, H-5′), 5.21 (2 H, m, H-3′), 6.19 (2 H, t, H-1′), 6.79 (2 H, d, vinylic, J = 15 Hz), 7.24 (2 H, d, vinylic, J = 15 Hz), 7.45 (2 H, d, phenyl), 7.83 (2 H, d, H-6), 7.92 (2 H, d, phenyl), 11.65 (2 H, s, NH); ^{31}P NMR δ −6.192. Anal. ($C_{29}H_{31}Br_2N_4O_{12}PS$) C, H, N.

2-Chlorophenyl Bis[(*E*)-5-(2-bromovinyl)-3′-*O*-acetyl-2′-deoxyuridin-5′-yl] Phosphate (6f). The title compound was prepared in a manner identical to that described for compound **5c** starting from (*E*)-5-(2-bromovinyl)-3′-*O*-acetyl-2′-deoxyuridine (752 mg, 2 mmol) to give the product (610 mg, 66% yield): UV λ_{max} 292 nm (ϵ 25100), λ_{max} 249 nm (ϵ 31700), λ_{min} 270.5 nm (ϵ 17800); ^{1}H NMR δ 2.06 (6 H, s, $COCH_3$), 2.39 (4 H, m, H-2′), 4.25 (2 H, m, H-4′), 4.46 (4 H, m, H-5′), 5.21 (2 H, m, H-3′), 6.15 (2 H, t, H-1′), 6.76 (2 H, d, vinylic, J = 15 Hz), 7.21–7.30 (4 H, m, phenyl and vinylic, J = 15 Hz), 7.37 (1 H, d, phenyl), 7.55 (1 H, d, phenyl), 7.80 (2 H, d, H-6), 11.65 (2 H, s, NH); ^{31}P NMR δ −6.730. Anal. ($C_{32}H_{32}Br_2ClN_4O_{14}P$) C, H, N.

Bis[(*E*)-5-(2-bromovinyl)-2′-deoxyuridin-5′-yl] Phosphate (6e). Compound **6f** (550 mg, 0.6 mmol) was dissolved in dioxane (5 mL), sodium hydroxide solution (15 mL, 0.3 M) was added, and the mixture was stirred for 2 h at room temperature. The solution was adjusted to pH 2 by the addition of Dowex H^+ form ion-exchange resin; the filtrate and washings were combined, reduced to an oil, redissolved in water (30 mL), and extracted with ether (4 × 5 mL), and the aqueous layer was taken to dryness. The resulting residue was separated on a silica column using chloroform–methanol (3:2) as eluent and further purified on a DEAE-cellulose column with a gradient of triethylammonium bicarbonate. The product was finally converted to its sodium salt (192 mg, 43% yield): λ_{max} 295 nm (ϵ 21500), λ_{max} 248.5 nm (ϵ 25800), λ_{min} 272 nm (ϵ 13200); ^{1}H NMR δ 2.12 (4 H, m, H-2′), 3.88 (6 H, m, H-4′ and H-5′), 4.31 (2 H, m, H-3′), 5.42 (2 H, m, OH-3′), 6.21 (2 H, t, H-1′), 7.12 (2 H, d, vinylic, J = 14 Hz), 7.39 (2 H, d, vinylic, J = 14 Hz), 8.28 (2 H, s, H-6), 11.57 (2 H, s, NH);

^{31}P NMR δ -1.211; FAB mass spectrum, m/z 749, 751, 753 (M + H)$^+$ (1:2:1). Anal. ($C_{22}H_{24}Br_2N_4O_{12}PNa\cdot 2H_2O$) C, H.

Bis[2-(guanin-9-ylmethoxy)ethyl] 4-(Methylthio)phenyl Phosphate (7a). 4-(Methylthio)phenyl phosphorodichloridate (0.6 mL, 3.5 mmol) and 1-methylimidazole (1.5 L, 17 mmol) in pyridine (10 mL) were stirred for 5 min at room temperature and then added to a suspension of 9-[(2-hydroxyethoxy)methyl]-guanine (1.12 g, 5 mmol) in pyridine (20 mL) and the mixture was stirred for 20 h at room temperature. The resulting clear solution containing a viscous oil was poured into ice-water (100 mL) and the oil was isolated as a brown solid after trituration. The product was purified by dissolving this solid in a small volume of dimethyl sulfoxide followed by the addition of methanol until crystallization occurred (300 mg, 19% yield): UV λ_{max} 254 nm (ϵ 38 600); ^1H NMR δ 2.45 (3 H, s, SCH_3), 3.70 (4 H, m, $POCH_2CH_2O$), 4.15 (4 H, m, $POCH_2CH_2O$), 5.35 (4 H, s, OCH_2N), 6.52 (4 H, s, 2-NH_2), 7.17 (4 H, m, phenyl), 7.82 (2 H, s, H-8), 10.67 (2 H, s, NH); ^{31}P NMR δ -6.326; FAB mass spectrum, m/z 635 (M + H)$^+$. Anal. ($C_{23}H_{27}N_{10}O_8PS\cdot 2H_2O$) C, H, N.

Bis[2-(guanin-9-ylmethoxy)ethyl] 4-(Methylsulfonyl)-phenyl Phosphate (7b). 4-(Methylsulfonyl)phenyl phosphorochloridate (87 mg, 0.3 mmol), 1-methylimidazole (0.15 L, 1.7 mmol), and pyridine (5 mL) were stirred for 5 min and then added to a suspension of 9-[(2-hydroxyethoxy)methyl]guanine (112 mg, 0.5 mmol) in pyridine (10 mL). The resulting suspension was subjected to sonication in a Ultrasound bath for 2 h and then stirred overnight at room temperature. The resulting gum and solution were added to ice-water (100 mL), and after trituration, filtration, and repeated washing with methanol, the product was obtained as a pale yellow solid (10 mg, 6% yield): UV λ_{max} 253 nm (ϵ 41 000); ^1H NMR δ 3.24 (3 H, s, SO_2CH_3), 3.69 (4 H, m, $POCH_2CH_2O$), 4.19 (4 H, m, $POCH_2CH_2O$), 5.35 (4 H, s, OCH_2N), 6.52 (4 H, s, 2-NH_2), 7.42 (2 H, d, phenyl), 7.82 (2 H, s, H-8), 7.95 (2 H, d, phenyl), 10.65 (2 H, s, NH); ^{31}P NMR δ -6.596; FAB mass spectrum, m/z 667 (M + H)$^+$. Anal. ($C_{23}H_{27}N_{10}O_{10}PS\cdot 2H_2O$) C, H.

In Vitro Antiviral Assays. The in vitro antiviral assays were based on an inhibition of virus-induced cytopathogenicity in primary rabbit kidney (PRK) cell cultures.[21] Briefly, confluent PRK cell cultures in 96-well microtiter trays were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the cell culture infective dose for 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 compounds. Viral cytopathogenicity was recorded as soon as it reached completion in the control virus-infected cell cultures.

The following virus strains were used in our studies: HSV-1 (KOS, F, and McIntyre), HSV-2 (G, 196, and Lyons), thymidine kinase-deficient (TK$^-$) HSV-1 (B2006 and VMW 1837), vaccinia virus, and vesicular stomatitis virus.

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 at 10^5 $CCID_{50}$/0.05 mL per mouse or wild-type HSV-1 (KOS) at $10^{4.7}$ PFU/0.05 mL per mouse, and treated topically with the indicated formulation four times a day for 5 days, starting immediately after virus infection. The appearance of skin lesions and paralysis of the hind legs and death of the mice were recorded daily.

Enzyme Studies. The examination of the hydrolysis of the test compounds by snake venom phosphodiesterase and human serum was performed as previously described.[22,23]

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Registry No. **3** 3′-*o*-acetyl derivative, 84218-88-2; **5a**, 125440-22-4; **5a** deacetyl derivative, 125440-37-1; **5b**, 125440-25-7; **5c**, 125440-28-0; **5d**, 125440-30-4; **5e**, 125440-32-6; **5f**, 125440-34-8; **6a**, 125440-23-5; **6b**, 125440-26-8; **6c**, 125440-29-1; **6d**, 125440-31-5; **6e**, 125440-33-7; **6e**·Na, 125515-24-4; **6f**, 125440-35-9; **7a**, 125440-24-6; **7b**, 125440-27-9; 4-(methylthio)phenyl phosphorodichloridate, 53676-21-4; 4-(methylsulfonyl)phenol, 14763-60-1; 4-(methylsulfonyl)phenyl phosphorodichloridate, 125440-36-0; *P*-(methylsulfonyl)phenol, phosphate, 28636-79-5; 3′-*O*-acetylthymidine, 21090-30-2; 9-[(2-hydroxyethoxy)methyl]guanine, 59277-89-3.

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Novel Benzamides as Selective and Potent Gastric Prokinetic Agents. 1. Synthesis and Structure-Activity Relationships of *N*-[(2-Morpholinyl)alkyl]benzamides

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With the purpose of obtaining more potent and selective gastric prokinetic agents than metoclopramide (**1**), a new series of *N*-[(2-morpholinyl)alkyl]benzamides (**17-52**) were synthesized and their gastric prokinetic activity was evaluated by determining effects on the gastric emptying of phenol red semisolid meal and of resin pellets solid meal in rats and mice. The morpholinyl moiety was newly designed after consideration of the side-chain structure of cisapride (**2**) and produced the desired activity when coupled with the 4-amino-5-chloro-2-methoxybenzoyl group of both metoclopramide and cisapride. Modification of the substituents of the benzoyl group markedly influenced the activity. In particular, 4-amino-*N*-[(4-benzyl-2-morpholinyl)methyl]-5-chloro-2-methoxybenzamide (**17**) and the 4-(dimethylamino) and 2-ethoxy analogues (**25** and **29**) of **17** showed potent and selective gastric prokinetic activity along with a weak dopamine D_2 receptor antagonistic activity.

Metoclopramide (**1**) is used clinically as a stimulant of upper gastrointestinal motility and as an antiemetic.[1] The gastric prokinetic action of metoclopramide is ascribed to stimulation of the gut motility by increase in acetylcholine

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