A control rat was injected with saline and sacrificed 30 min later. Tissues were prepared as above with the addition of authentic [^{125}I]-**6** (10 μCi) to the supernatant prior to centrifugation. The supernatants were concentrated by evaporation of acetonitrile to a volume of approximately 100 μL (to 10% of the starting volume). The samples were applied to alumina TLC plates and eluted with $CHCl_3$. The R_f values of **6** and desmethyl **6** were 0.58 and 0.08, respectively. The extraction efficiency of radioactivity from the supernatant was 93% for heart tissue and 83% for blood for both control and experimental samples. The results with blood show 22% of the radioactivity co-migrates with desmethyl **6**. The remaining radioactivity (78%) is present as [^{125}I]-**6**. There was less metabolite in heart with 5% as ^{125}I-labeled desmethyl **6** and the remaining 95% as [^{125}I]-**6**. The controls showed 97% and 99% as [^{125}I]-**6** in blood and heart, respectively.

Acknowledgment. We are grateful to Linder Markham for preparing the manuscript and to Susan Fisher for help in performing the tissue distribution studies. We also thank the Phoenix Memorial Laboratory of the University of Michigan for use of their research facilities. We greatly appreciate the helpful synthetic suggestions of Dr. G. Leonard Watkins in the early phases of this study. This work was supported by the National Institutes of Health, NINCDS Grant 3 P01 NS-15665-0852.

Synthesis and Antiviral Activity of Certain 4- and 4,5-Disubstituted 7-[(2-Hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidines

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In vitro evaluation of a series of previously prepared tubercidin analogues revealed that certain 5-halogen-substituted analogues were active against human cytomegalovirus (HCMV) at concentrations lower than those that produced comparable cytotoxicity in uninfected cells. In contrast, tubercidin was cytotoxic at all antiviral concentrations. Even though the antiviral selectivity of the 5-substituted compounds was slight, this observation led us to prepare a series of acyclic analogues. Treatment of the sodium salt of 4-chloropyrrolo[2,3-*d*]pyrimidine (**2**) with (2-acetoxyethoxy)methyl bromide (**2a**) provided the acyclic nucleoside 4-chloro-7-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**3**). A nucleophilic displacement of the 4-chloro group with methoxide, methylamine, and dimethylamine yielded the corresponding 4-substituted compounds **4**, **5**, and **6**, respectively, in good yield. Electrophilic substitution (chlorination, bromination, and iodination) was effected at the C-5 position of compound **3** with *N*-chlorosuccinimide, *N*-bromosuccinimide, and iodine monochloride, respectively, in methylene chloride. Removal of the acetyl group from these intermediates (**7a–9a**) with methanolic ammonia at room temperature afforded the 5-chloro (**7b**), 5-bromo (**8b**), and 5-iodo (**9b**) derivatives of 4-chloro-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine. Treatment of compounds **7b–9b** with methanolic ammonia at an elevated temperature produced the corresponding 5-halotubercidin analogues **10**, **11**, and **12**, respectively. An alternate procedure for the preparation of these 4,5-disubstituted 7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidines involved an electrophilic substitution prior to the condensation of the heterocycle with **2a**. Treatment of **2** with *N*-chlorosuccinimide and *N*-bromosuccinimide gave compounds **13a** and **13b**, respectively. The condensation of **13a** and **13b** with **2a** and subsequent treatment with methylamine and ethylamine furnished the corresponding 5-halo-4-substituted-pyrrolo[2,3-*d*]pyrimidines **14a**, **14b**, **14c**, and **14d**, respectively. Evaluation of the target compounds (**4–6**, **7b–9b**, **10–12**, and **14a–14d**) for cytotoxicity and activity against HCMV and herpes simplex virus type 1 (HSV-1) revealed that all compounds except the 5-halogen-substituted compounds **10**, **11**, and **12** were inactive. Compounds **10**, **11**, and **12** were active against both viruses at noncytotoxic concentrations. The activity of compound **11** was particularly noteworthy, being at least 10-fold more potent than acyclovir.

The most important antiviral drug discovered during the past several years is the acyclic analogue of guanosine, 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir). This compound potently and selectively inhibits the in vitro and in vivo replication of herpes simplex viruses[1,2] and is clinically efficacious in the treatment of certain herpesvirus infections.[3-6] The biochemical basis for the antiviral activity of acyclovir involves its specific phosphorylation to the corresponding monophosphate by a herpesvirus-encoded pyrimidine deoxynucleoside kinase.[1,7] The monophosphate is phosphorylated further by cellular kinases[8,9] to acyclovir triphosphate, a potent and selective inhibitor of the virus-encoded DNA polymerase.[1,10-12] The combination of specificity and selectivity for virus-encoded enzymes leads to a paucity of cytotoxic effects by the drug in uninfected cells and assures the usefulness of acyclovir as an antiviral agent.

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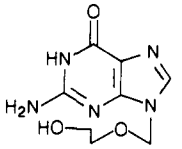


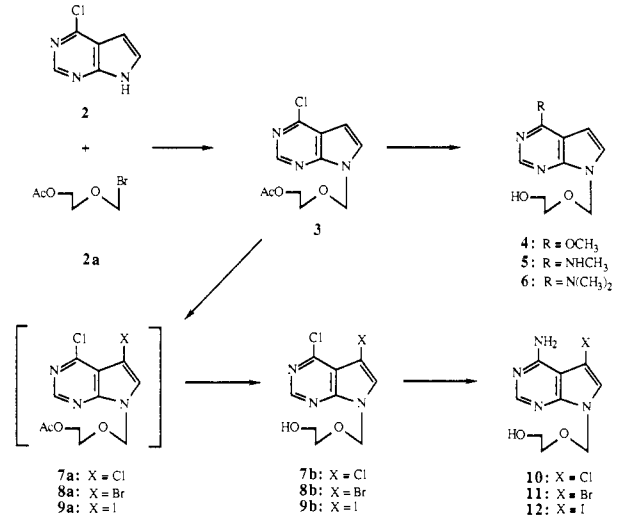
acyclovir

The success of acyclovir as an antiviral drug has prompted intensive efforts by several groups to prepare and evaluate many structurally related acyclic analogues.[13-16] In our laboratory, the synthesis of pyrrolo[2,3-*d*]pyrimidine nucleosides as potential antitumor agents has been under active investigation for a number of years.[17-19] More recently, we also have been investigating this class of nucleosides as potential antiviral drugs. We have found that arabinosyl- and deoxyribosylpyrrolo[2,3-*d*]pyrimidines are active against HCMV and HSV-1.[20] In contrast, a series of 2,4-disubstituted acyclic analogues was not active.[21] These observations led us to examine a series of ribosyl analogues and to initiate the synthesis of additional acyclovir analogues in which the guanine base was replaced by selected 4- and 4,5-disubstituted pyrrolo[2,3-*d*]pyrimidines. We report herein the evaluation of such compounds for antiviral activity and the unexpected observation that 5-halogen-substituted analogues are active against HCMV.

Results and Discussion

Biological Evaluation of Literature Compounds. A series of tubercidin analogues previously prepared in our laboratory[22-24,31] were evaluated for activity against HCMV and for cytotoxicity in uninfected human foreskin fibroblasts (HFF cells) and in a human neoplastic cell line (KB cells). Table I presents data that show that tubercidin (**1**) and its unsubstituted analogue (**1a**, "7-deazanebularin") were very active in reducing HCMV plaque formation but also were very cytotoxic. Compounds substituted only at the 4-position by moieties other than amino either were active against HCMV with corresponding cytotoxicity (compounds **1c**, **1d**, **1f**, **1h**) or were relatively inactive. Substitution of the 5-position of tubercidin with halogen gave compounds (**1k**, **1n**, **1s**) that were as active as tubercidin against HCMV but with slightly reduced cytotoxicity. In a small series of 5-bromotubercidin analogues, replacement of the 4-amino group by SH or SCH_3 or methylation of the amino group reduced or eliminated activity. As a consequence of the activity and modest selectivity seen with 5-halotubercidins, our synthetic efforts with acyclic pyrrolopyrimidines[21] were broadened to include 5-halogen-substituted analogues.

Scheme I



Chemistry. The sodium salt glycosylation method[25] was used as the condensation procedure. This method has been used for the synthesis of various 2′-deoxynucleosides of pyrrolo[2,3-*d*]pyrimidines.[26] The sodium salt of 4-chloropyrrolo[2,3-*d*]pyrimidine (**2**), generated by the treatment of the heterocycle with sodium hydride in dimethylformamide, was condensed with (2-acetoxyethoxy)methyl bromide[27] (**2a**) in DMF (Scheme I). The blocked acyclic nucleoside analogue 4-chloro-7-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**3**) was obtained in a 40% yield, after column chromatography. The site of glycosylation was confirmed to be at N-7 on the basis of ^1H NMR and ultraviolet absorption spectroscopic studies.[28] The UV spectrum of **3** showed no bathochromic shift in λ_{max} relative to the λ_{max} observed for compound **2**. This essentially ruled out the possibility of either N-1 or N-3 substitution.[29] In the ^1H NMR spectrum of **3**, the C-5 and C-6 aromatic protons appeared as two separate doublets at δ 7.55 and 6.57 (J = 3.6 Hz). This provides strong support for the N-7 assignment[30,31] since if a hydrogen was residing at N-7, a different splitting pattern would have been observed for the C-5 and C-6 hydrogens.

A nucleophilic displacement of the chloro group from **3** with sodium methoxide in methanol furnished a good yield of 4-methoxy-7-[(2-hydroxyethoxy)methyl]pyrrolo-

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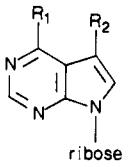
Table I. Antiviral Activity and Cytotoxicity of Tubercidin Analogues



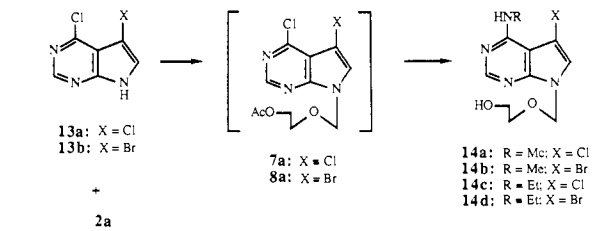
compound				50% inhibitory concentration, μM		
		substituent			cytotoxicity	
no.	source[a]	R_1	R_2	plaque reduction assay: HCMV	HFF[b]	KB[c]
1a	22b	H	H	0.4	2	1
1[d]	22b	H_2N	H	0.5	0.4	0.6[e]
1b	22b	H_3CNH	H	37[f]	>100[g]	>100
1c	22b	$(H_3C)_2N$	H	1.2[e]	2[e]	9
1d	22b	H_2NHN	H	4[e]	2[e]	13
1e	22b	$H_{10}C_5N$	H	>100	>100	>100
1f	22b	HO	H	3.5[e]	10[e]	17
1g	22b	H_3CO	H	10[e]	46[e]	>100
1h	22a	H_3CSe	H	4	1.5	4
1i	22b	HS	H	>100	>100	>100
1j	22b	C_7H_7S	H	>100	>100	11[g]
1k	31	H_2N	Cl	4.4[e]	10[e]	6.5[e]
1l	24	H	Br	23[e]	31[e]	22
1m	31	Cl	Br	29[e]	31[e]	2
1n	31	H_2N	Br	0.5[e]	4.5[e]	2.3[e]
1o	31	H_3CNH	Br	>100[e]	>100[e]	>100
1p	31	$(H_3C)_2N$	Br	16	10	3
1q	31	HS	Br	39	>100	>100
1r	31	H_3CS	Br	>100	>100	19
1s	31	H_2N	I	0.4[e]	2[e]	2.1[e]
1t	31	Cl	I	31[e,f]	13[e]	4

[a] Literature reference for preparation of compound. [b] Visual cytotoxicity scored on HFF cells at time of HCMV plaque enumeration. [c] Average percent inhibition of DNA, RNA, and protein synthesis determined in KB cells as described in the text. [d] Tubercidin. [e] Average I_{50} concentration derived from two or three experiments. [f] Significant reduction in size of plaques at concentrations showing plaques.

[2,3-*d*]pyrimidine (**4**). Treatment of **3** with methylamine and dimethylamine afforded 4-(methylamino)-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**5**) and 4-(dimethylamino)-7-[(2-hydroxyethoxy)methyl]pyrrolo-[2,3-*d*]pyrimidine (**6**), respectively. A comparison of the spectroscopic properties of **4**, **5**, and **6** with the corresponding 4-substituted 7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidines (**1g**, **1b**, **1c**)[28a] provided additional support for our structural assignment for **3**; vide supra.

We then studied the electrophilic substitution of compound **3** with several electrophilic reagents. Treatment of compound **3** with *N*-chlorosuccinimide, *N*-bromosuccinimide, and iodine monochloride furnished the 5-chloro (**7a**), 5-bromo (**8a**), and 5-iodo (**9a**) derivatives of 4-chloro-7-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine, respectively. Subsequent deacetylation of these intermediates with methanolic ammonia at room temperature yielded 4,5-dichloro-7-[(2-hydroxyethoxy)-methyl]pyrrolo[2,3-*d*]pyrimidine (**7b**), 5-bromo-4-chloro-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**8b**) and 4-chloro-5-iodo-7-[(2-hydroxyethoxy)methyl]pyrrolo-[2,3-*d*]pyrimidine (**9b**), respectively. Assignment of the halogen group to position 5 of these compounds was accomplished on the basis of the ^{1}H NMR spectra.[30,31] Previous work from this laboratory[32] has already established the position of the aromatic protons at C-2, C-5, and C-6 in the pyrrolo[2,3-*d*]pyrimidine ring system. The ^{1}H NMR spectrum of compound **3** in DMSO-d_6 exhibited absorption peaks at δ 8.6 (1 proton singlet, C-2-H), 7.88 (1 proton doublet, C-6-H, $J_{6,5}$ = 3.5 Hz), and 6.72 (1 proton doublet, C-5-H, $J_{5,6}$ = 3.5 Hz). The above assignment of C-6-H to the absorption peak centered at δ 7.88 and C-5-H

Scheme II



to the δ 6.72 absorption peak was made on the basis of reported[33] spectral data for pyrrole and the bicyclic pyrrole derivative, indole. In pyrrole and indole, the proton adjacent (C-2-H) to the ring nitrogen exhibits an absorption peak at a lower field than the absorption peak observed at the C-3-H proton. Assignment of the halogen group to position 5 in **3** was ascertained by the ^{1}H NMR spectra[32] of compounds **7b–9b**, which revealed absorption peaks at δ 8.69–8.74 (1 proton singlet, C-2-H) and δ 8.13–8.15 (1 proton singlet C-6-H). The disappearance of two separate doublets at δ 7.88 (C-6-H) and δ 6.72 (C-5-H) in **3** and the appearance of a singlet at δ 8.13–8.15 in **7b–9b** clearly confirmed electrophilic substitution in the pyrrole ring at C-5. The shift to lower field for both absorption peaks (C-2-H and C-6-H) in **7b–9b** was expected due to the deshielding effect of the halogen group at C-5.

On the basis of previous work from our laboratory,[30,31] it was assumed that the initial displacement of a halogen group would occur at the C-4 position. This was confirmed by the amination of compounds **7a–9a** with methanolic ammonia in a sealed reaction vessel at 135 °C, which af-

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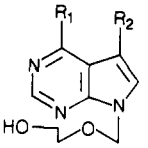
Table II. Antiviral Activity and Cytotoxicity of 4-Substituted and 4,5-Disubstituted 7-[(2-Hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidines



compound			50% inhibitory concentration, μM				
	substituent		plaque reduction assay		cytotoxicity		
no.	R_1	R_2	HCMV	HSV-1	HFF[a]	BSC[a]	KB[b]
4	OCH_3	H	>100[c]	>100	100	>100	80
5	$NHCH_3$	H	>100	>100	>100	>100	>100
6	$N(CH_3)_2$	H	>100	>100	>100	>100	31
7b	Cl	Cl	>100	>100	>100	>100	
8b	Cl	Br	>100	>100	>100	>100	
9b	Cl	I	>100	>100	>100	>100	
10	NH_2	Cl	16[d]	77	>100[d]	>100	350[d,e]
11	NH_2	Br	3.9[d]	16[d]	100[d]	>100[d]	100[d,e]
12	NH_2	I	24[d]	100	100[d]	>100	94[d,e]
14a	MeNH	Cl	>100	>100	>100	>100	>100
14b	MeNH	Br	>100[d]	>100	>100[d]	>100	
14c	EtNH	Cl	>100	>100	>100	>100	
14d	EtNH	Br	>100	>320	>100	>320	
acyclovir			63[d]	2.6[d]	>100	>100	>100
ganciclovir (DHPG)			8.8[f]	3.0[d]	>100	>100	1000

[a] Visual cytotoxicity scored on uninfected HFF or BSC-1 cells at time of HCMV or HSV-1 plaque enumeration. [b] Average percent inhibition of DNA, RNA, and protein synthesis determined in KB cells as described in the text. [c] I_{50} concentration not reached at highest concentration tested (100 μM). [d] Average of two to five experiments. [e] Effect on RNA and protein synthesis only. The I_{50} for effect on [^{3}H]dThd incorporation was 0.82, 0.13, and 1.2 μM for compounds **10**, **11**, and **12**, respectively. [f] Average of 23 experiments.

forded 4-amino-5-chloro-7-[(2-hydroxyethoxy)methyl]-pyrrolo[2,3-*d*]pyrimidine (**10**), 4-amino-5-bromo-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**11**), and 4-amino-5-iodo-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**12**), respectively.

An alternative approach for the synthesis of **7a** and **8a** was to treat compound **2** with *N*-chlorosuccinimide and *N*-bromosuccinimide. This furnished a very high yield of 4,5-dichloropyrrolo[2,3-*d*]pyrimidine (**13a**) and 5-bromo-4-chloropyrrolo[2,3-*d*]pyrimidine (**13b**), which were condensed with **2a** (Scheme II) to afford 4,5-dichloro- (**7a**) and 5-bromo-4-chloro-7-[(2-acetoxyethoxy)methyl]pyrrolo-[2,3-*d*]pyrimidine (**8a**), respectively. These compounds were then treated with methylamine and ethylamine at 130 °C in a sealed reaction vessel to provide 5-chloro-4-(methylamino)-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]-pyrimidine (**14a**), 5-bromo-4-(methylamino)-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**14b**), 5-chloro-4-(ethylamino)-7-[(2-hydroxyethoxy)methyl]-pyrrolo[2,3-*d*]pyrimidine (**14c**), and 5-bromo-4-(ethylamino)-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**14d**), respectively.

Biological Evaluation of New Compounds. New compounds were evaluated more extensively than those presented in Table I. Plaque reduction assays were used to measure activity against both HCMV and HSV-1. Cytotoxicity of each compound was determined visually in normal human diploid cells (HFF cells) and in monkey kidney cells (BSC-1 cells). In some cases cytotoxicity was measured in a human neoplastic cell line (KB cells) with labeled precursor uptake. Table II shows that although nearly all compounds were inactive against HCMV and HSV-1, the 5-Cl, -Br, and -I analogues (**10**, **11**, **12**) were active against both viruses. The 5-Br compound (**11**) showed the most potent activity, inhibiting HCMV at an I_{50} concentration of 3.9 μM. Alteration of the 4-position by replacement of the amino group with chlorine (**7b**, **8b**, **9b**) or by conversion to methylamino or ethylamino (**14a–d**) completely abolished activity against both viruses. Little visual cytotoxicity was observed with any of the compounds in Table II. Compounds **10**, **11**, and **12**, however, did inhibit the incorporation of [^{3}H]dThd into acid precipitable material (Table II, footnote *e*). Whether or not this inhibition produces cytotoxicity that is not detectable otherwise is under investigation.

The activity of compounds **10**, **11**, and **12** against HCMV is quite surprising because acyclovir is only marginally active against this virus (see Table II and ref 34). In contrast to acyclovir, compound **11** is more active against HCMV but less active against HSV-1. It also appears to be more active against HCMV than is ganciclovir (Table II and ref 35). Compound **11** ultimately may prove to be more cytotoxic than acyclovir or ganciclovir but a detailed comparison is dependent upon additional studies.

Experimental Section

General Procedures. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were determined at 270 MHz with a BRUKER WP 270 SY. The chemical shift values are expressed in δ values (parts per million) relative to the standard chemical shift of the solvent DMSO-d_6. Ultraviolet spectra were recorded on a Hewlett-Packard 8450 A spectrophotometer. Infrared spectra were measured on a Perkin-Elmer 281 spectrophotometer. Elemental analysis were performed by M-H-W Laboratories, Phoenix, AZ. Thin-layer chromatography (TLC) was run on silica gel 60F-254 plates (Merck Reagents). E. Merck silica gel (230–400 mesh) was used for flash column chromatography. Detection of components on TLC was made by UV light absorption at 260 nm. Evaporations were carried out under reduced pressure (water aspirator) with the bath temperature below 30 °C, unless specified otherwise.

4-Chloro-7-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (3). Sodium hydride (0.09 g, 60% in mineral oil) was added to a solution of 4-chloropyrrolo[2,3-*d*]pyrimidine[30] (**2**, 0.3

(34) Crumpacker, C. S.; Schnipper, L. E.; Azia, J. A.; Levin, M. D. *Antimicrob. Agents Chemother.* **1979**, *15*, 642.
(35) Smee, D. F.; Martin, J. C.; Verheyden, J. P. H.; Matthews, T. R. *Antimicrob. Agents Chemother.* **1983**, *23*, 676.

g) in dry DMF (6 mL) in small portions at 0–5 °C under a nitrogen atmosphere. When all hydrogen evolution had ceased, (2-acetoxyethoxy)methyl bromide (**2a**) (0.45 g) was added dropwise with stirring at room temperature. The reaction mixture was then stirred at room temperature for an additional 3 h. After that period of time, water (50 mL) was added and the product was extracted with EtOAc. The EtOAc layer was separated, washed with cold water, and dried over anhydrous Na_2SO_4 and the solvent then evaporated under reduced pressure to afford a thick syrup. This syrup was applied to the top of a silica gel column (20 × 2 cm) and the column was eluted with 1% MeOH in $CHCl_3$. All fractions containing UV-absorbing material were combined, and the solvent from these fractions was evaporated to yield a colorless syrup. This syrup was crystallized from MeOH as colorless needles to yield 0.21 g of **3** (40.21%): mp 67–68 °C; ^{1}H NMR (DMSO-d_6) δ 8.5 (s, 1, C2-H), 7.88 (d, 1, J = 3.6 Hz, C6-H), 6.72 (d, 1, J = 3.6 Hz, C5-H), 5.7 (s, 2, N7-CH_2), 4.03 (m, 2, OCH_2), 3.52 (m, 2, CH_2), 1.92 (s, 3, OAc): UV λ_{max} nm ($\epsilon \times 10^4$) (pH 7) 223 (1.2), 276 (2.4), (pH 1) 225 (1.2), 274 (0.25), (pH 11) 227 (0.8), 276 (0.2). Anal. ($C_{11}H_{12}ClN_3O_3 \cdot 0.5MeOH$) N; C: calcd, 50.43; found, 50.91; H: calcd, 5.60; found, 5.08.

4-Methoxy-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (4). Compound **3** (0.74 g) was dissolved in dry MeOH (20 mL) and sodium methoxide (0.34 g) was then added to this solution. The reaction mixture was heated at reflux temperature for 1 h, and the pH was then adjusted to 7 with glacial acetic acid. The solvent was evaporated at 50 °C in vacuo to give a solid mass. The solid was dissolved in EtOAc (100 mL) and the EtOAc solution washed with water (3 × 30 mL) and then dried over anhydrous Na_2SO_4. The solvent was removed in vacuo to give a syrup. This syrup was purified by column chromatography on silica gel. The column (15 × 3 cm) was eluted with 35% acetone in hexane. All fractions containing UV-absorbing material were combined, and the solvent from these fractions evaporated at 30 °C to give an oil which crystallized from hexane as colorless crystals: 0.35 g (54.68%); mp 61–62 °C; ^{1}H NMR (DMSO-d_6) δ 8.46 (s, 1, C2-H), 7.55 (d, 1, J = 3.7 Hz, C6-H), 6.57 (d, 1, J = 3.7 Hz, C5-H), 5.6 (s, 2, N7-CH_2), 4.5 (m, 2, CH_2), 3.4 (m, 2, CH_2), 4.6 (s, 1, exchangeable with D_2O, OH), 4.05 (s, 3, OMe); UV λ_{max} nm ($\epsilon \times 10^4$) (pH 7) 221 (5.0), 262 (3.2), (pH 1) 228 (5.2), 271 (2.4), (pH 11) 225 (2.4), 264 (2.2). Anal. ($C_{10}H_{13}N_3O_3$) C, H, N.

4-(Methylamino)-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (5). A mixture of **3** (0.5 g) and methylamine (40 mL, 40% w/v) was heated in a sealed reaction vessel at 100 °C for 2 h. The solvent was evaporated at 50 °C in vacuo to give a syrup. This syrup was subjected to column chromatography. Elution of the silica gel column (20 × 3 cm) with 5% MeOH in $CHCl_3$ yielded a colorless crystalline compound, after evaporation at 40 °C under reduced pressure of all the appropriate UV-absorbing fractions. This solid was recrystallized from EtOAc to afford **5** as colorless needles (0.11 g) (26.87%): mp 130–131 °C; ^{1}H NMR (DMSO-d_6) δ 8.17 (s, 1, C2-H), 7.45 (s, 1, exchangeable with D_2O, NH), 7.23 (d, 1, J = 3.5 Hz, C6-H), 6.57 (d, 1, J = 3.5 Hz, C5-H), 5.51 (s, 2, N7-CH_2), 4.5 (m, 1, exchangeable with D_2O, OH), 3.42 (m, 4, CH_2), 2.95 (d, 3, J = 4.71 Hz, CH_3); UV λ_{max} nm ($\epsilon \times 10^4$) (pH 7) 214 (2.5), 273 (1.7), (pH 1) 229 (2.0), 275 (1.6), (pH 11) 224 (0.7), 274 (1.6). Anal. ($C_{10}H_{14}N_4O_2$) C, H, N.

4-(Dimethylamino)-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (6). A mixture of **3** (9.25 g) and dimethylamino (40% w/v, 30 mL) was heated in a sealed reaction vessel at 125 °C for 3 h. The solvent was removed at 30 °C in vacuo to give a thick colorless syrup. This syrup was subjected to column chromatography; elution of the silica gel column (20 × 3 cm) with 5% MeOH in $CHCl_3$ gave a thick syrup after evaporation of all the appropriate UV-absorbing fractions. This syrup was crystallized from EtOAc as colorless prisms to yield 0.12 g of **6** (57.1%): mp 120–121 °C; ^{1}H NMR (DMSO-d_6) δ 8.18 (s, 1, C2-H), 7.4 (d, 1, J = 3.6 Hz, C6-H), 6.74 (d, 1, J = 3.6 Hz, C5-H), 5.5 (s, 2, N7-CH_2), 4.6 (br s, 1, exchangeable with D_2O, OH), 3.45 (m, 4, CH_2), 3.31 (s, 6, CH_3); UV λ_{max} nm ($\epsilon \times 10^4$) (pH 7) 214 (1.4), 282 (1.0), (pH 1) 231 (0.9), 280 (0.8), (pH 11) 226 (0.6) 283 (1.0). Anal. ($C_{11}H_{16}N_4O_2$) C, H, N.

4,5-Dichloro-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (7b). *N*-Chlorosuccinimide was added to a solution of **3** (0.35 g) in dry CH_2Cl_2 (15 mL). The reaction mixture was stirred at room temperature for 8 days. At that time TLC established a complete disappearance of starting material. Water (50 mL) was added to the mixture and the aqueous solution was extracted with $CHCl_3$ (3 × 30 mL). The chloroform extracts were combined and washed with water and then dried over anhydrous Na_2SO_4. The solvent was removed at 40 °C in vacuo and the resulting thick syrup was subjected to column chromatography. Elution of the silica gel column (15 × 2 cm) with benzene–chloroform (1:1) yielded a colorless oil (single spot on TLC), after evaporation of all the UV-absorbing fractions at reduced temperature and pressure. This oil was crystallized from EtOH to afford colorless needles of the acetylated intermediate, 4,5-dichloro-7-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**7a**). This was dissolved in dry MeOH (15 mL) and to this solution was added 25 mL of MeOH which had previously been saturated with ammonia at 0 °C. The reaction mixture was stirred in a pressure bottle at room temperature for 20 h. The solvent was evaporated at 30 °C in vacuo and the semisolid mass was subjected to column chromatography. Elution of the product from a silica gel column (15 × 2 cm) with 2% MeOH in $CHCl_3$ yielded a colorless compound, after evaporation of all the appropriate UV-absorbing fractions. This solid was recrystallized from MeOH to furnish 0.11 g of **7b** (40.7%): mp 142–143 °C; ^{1}H NMR (DMSO-d_6) δ 8.74 (s, 1, C2-H), 8.13 (s, 1, C6-H), 5.66 (s, 2, N7-CH_2), 4.65 (t, 1, J = 5.3 Hz, exchangeable with D_2O, OH), 3.42 (m, 4, CH_2); UV λ_{max} nm ($\epsilon \times 10^4$) (pH 7) 230 (3.3), 271 (0.6), 292 (0.6), (pH 1) 230 (2.8), 292 (0.6), (pH 11) 236 (2.8), 271 (0.4), 294 (0.4). Anal. ($C_9H_9Cl_2N_3O_2$) C, H, N.

4-Amino-5-chloro-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (10). Compound **7b** (0.07 g) was covered with methanolic ammonia (20 mL) and heated in a sealed reaction vessel at 135 °C for 10 h. The solvent was evaporated in vacuo to give a thick syrup. This syrup was subjected to column chromatography and elution of the silica gel column (20 × 3 cm) with 5% MeOH in $CHCl_3$ furnished colorless needles of **10**, after evaporation of appropriate UV-absorbing fractions (0.04 g, 66.6%): mp 149–150 °C; ^{1}H NMR (DMSO-d_6) δ 8.14 (s, 1, C2-H), 7.56 (s, 1, C6-H), 6.92 (br s, 2, exchangeable with D_2O, NH_2), 5.49 (s, 2, N7-CH_2), 4.64 (br s, 1, exchangeable with D_2O, OH), 3.43 (m, 4, CH_2); UV λ_{max} nm ($\epsilon \times 10^4$) (pH 7) 214 (2.5), 278 (1.3), (pH 1) 233 (2.8), 280 (1.3), (pH 11) 227 (1.4), 278 (1.3). Anal. ($C_9H_{11}N_4O_2Cl \cdot {}^1/_4H_2O$) C, H, N.

5-Bromo-4-chloro-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (8b). A mixture of compound **3** (0.2 g) and *N*-bromosuccinimide (0.10 g) was dissolved in dry CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 20 h. The solvent was evaporated at 40 °C under reduced pressure to give a semisolid mass which was recrystallized from MeOH as a light brown solid (**8a**, 0.11 g, 50%). The solid (0.3 g) was dissolved in dry MeOH (10 mL) and to this solution was added MeOH saturated with ammonia (20 mL). The reaction mixture was stirred in a pressure bottle at room temperature for 20 h. The solvent was removed at 70 °C under reduced pressure in vacuo and the semisolid mass was purified by column chromatography. Elution of the silica gel column (15 × 2 cm) with 2% MeOH in $CHCl_3$ yielded a colorless syrup after evaporation of the appropriate UV-absorbing fractions. Trituration of this syrup with ether gave a colorless compound, which was recrystallized from $CHCl_3$ to give 0.11 g of **8b** (38.02%): mp 135–136 °C; ^{1}H NMR (DMSO-d_6) δ 8.72 (s, 1, C2-H), 8.15 (s, 1, C6-H), 5.66 (s, 2, N7-CH_2), 4.63 (t, 1, J = 5.45 Hz, 3.2 Hz, exchangeable with D_2O, OH), 3.46 (m, 4, CH_2); UV λ_{max} nm ($\epsilon \times 10^4$) (pH 7) 230 (2.5), 270 (0.3), 298 (0.35), (pH 1) 231 (2.7), 270 (0.3), (pH 11) 232 (2.6), 370 (0.3), 301 (0.35). Anal. ($C_9H_9ClBrN_3O_2$) C, H, N.

4-Amino-5-bromo-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (11). Compound **8b** (0.7 g) was dissolved in dry MeOH (10 mL) and MeOH saturated with ammonia (40 mL) was then added to this solution. The reaction mixture was heated at 125 °C in a sealed reaction vessel for 10 h. The solvent was removed at 40 °C under reduced pressure and the resulting solid was then subjected to column chromatography. Elution of the silica gel column (20 × 2 cm) with 5% MeOH in $CHCl_3$ yielded a solid, after evaporation of the appropriate UV-absorbing fractions, which was recrystallized from $CHCl_3$ to afford **11**: 0.18 g (31.25%), mp 163–164 °C; ^{1}H NMR (DMSO-d_6) δ 8.13 (s, 1, C2-H), 7.55 (s, 1, C6-H), 6.81 (br s, 2, exchangeable with D_2O, NH_2), 5.5 (s, 2 N7-CH_2), 4.62 (m, 1, exchangeable with D_2O, OH),

3.42 (m, 4, CH_2); UV λ_{max} nm ($\epsilon \times 10^4$) (pH 7) 211 (1.9), 278 (0.9), (pH 1) 233 (2.0), 280 (0.94), (pH 11) 227 (0.9), 278 (0.95). Anal. ($C_9H_{11}N_4O_2Br$) C, H, N.

4-Chloro-5-iodo-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-d]pyrimidine (9b). To a solution of compound **3** (0.82 g) in dry CH_2Cl_2 (25 mL) was added dropwise iodine monochloride (0.38 mL) with stirring under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 20 h. The solvent was evaporated at 40 °C under high pressure and the dark purple syrup was placed on the top of a silica gel column. Elution of the column (15 × 2 cm) with $CHCl_3$ yielded a dark colored syrup, after evaporation of the appropriate UV-absorbing fractions, which on trituration with dry ether gave a crystalline product (0.3 g) as colorless needles. These needles were covered with MeOH saturated with ammonia (30 mL) at 0 °C and the reaction mixture was stirred at room temperature in a pressure bottle for 20 h. The solvent was evaporated under vacuum and the solid was recrystallized from MeOH to furnish colorless needles of **9b**: 0.21 g (80.75%), mp 154–155 °C; ^{1}H NMR (DMSO-d_6) δ 8.69 (s, 1, C2-H), 8.14 (s, 1, C6-H), 5.66 (s, 2, N7-CH_2), 4.57 (t, 1, J = 3.0 Hz, 3.3 Hz, exchangeable with D_2O, OH), 3.46–3.33 (m, 4, CH_2); UV λ_{max} nm ($\epsilon \times 10^4$) (pH 7) 225 (1.8), 296 (0.9), (pH 1) 233 (2.1), 280 (0.95), (pH 11) 228 (0.8), 280 (0.85). Anal. ($C_9H_9ClIN_3O_2$) C, H, N.

4-Amino-5-iodo-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-d]pyrimidine (12). Compound **9b** (0.3 g) was covered with MeOH saturated with ammonia (25 mL) and the reaction mixture was heated at 130 °C in a sealed vessel for 10 h. The solvent was concentrated in vacuo to give a semisolid mass which was recrystallized from MeOH to afford **12**: 0.20 g (71.4%), mp 169–170 °C; ^{1}H NMR (DMSO-d_6) δ 8.7 (s, 1, C2-H), 8.3 (s, 1, C6-H), 5.7 (s, 2, N7-CH_2), 4.6 (t, 1, J = 4 Hz, exchangeable with D_2O); UV λ_{max} nm ($\epsilon \times 10^4$) (pH 7) 210 (2.0), 280 (0.9), (pH 1) 230 (1.8), 279 (0.9), (pH 11) 228 (0.9), 278 (0.9). Anal. ($C_9H_{11}N_4O_2I$) C, H, N.

4,5-Dichloropyrrolo[2,3-d]pyrimidine (13a). 4-Chloropyrrolo[2,3-d]pyrimidine (**2**, 2.00 g, 0.013 mol) was suspended in 100 mL of dry CH_2Cl_2. *N*-Chlorosuccinimide (1.84 g, 0.14 mol) was then added and the stirred suspension was heated at reflux temperature for 90 min. The solvent was removed in vacuo to yield a solid. This solid was triturated with water (3 × 300 mL), collected by vacuum filtration, and dried in vacuo at 40 °C for 12 h to afford 2.10 g (89%) of **13a**: mp 222–224 °C dec; R_f 0.46 (10% MeOH–$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.22 (s, 1, C-6), 8.93 (s, 1, C-2), 13.2 (br s, 1, N-7, exchangeable with D_2O). Anal. ($C_6H_3N_3Cl_2$) C, H, N.

5-Bromo-4-chloropyrrolo[2,3-d]pyrimidine (13b). *N*-Bromosuccinimide (5.93 g, 0.033 mol) was added to a suspension of compound **2** (4.35 g, 0.028 mol) in 200 mL of dry CH_2Cl_2. The reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated in vacuo to yield a brown solid which was triturated in H_2O, collected by filtration, recrystallized from MeOH, and dried in vacuo (40 °C) for 12 h to yield 5.57 g (84%) of **13b**: mp 215–217 °C dec (lit.[30] mp 229 °C, 82%).

5-Chloro-4-(methylamino)-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-d]pyrimidine (14a). Sodium hydride (0.28 g, 60% in mineral oil) was added to a stirred solution of **13a** (1.03 g, 0.0055 mol) in dry DMF (10 mL) under a nitrogen atmosphere. When all the hydrogen evolution had ceased, compound **2a** (1.3 g, 0.0066 mol) was added dropwise to the solution. The reaction mixture was stirred for an additional 30 min. Water (50 mL) was added and the product was extracted with EtOAc (3 × 25 mL). The extracts were combined, washed with water (3 × 50 mL), and dried over $MgSO_4$. The solvent was evaporated in vacuo to give a brown oil which was dissolved in a minimum amount of $CHCl_3$ and applied to the top of a column packed with wet silica gel in $CHCl_3$. Elution of the column with $CHCl_3$ yielded, after evaporation of all UV-absorbing fractions, 4,5-dichloro-7-[(2-acetoxyethoxy)-methyl]pyrrolo[2,3-d]pyrimidine (**7a**, 0.85 g, 51%) (identical in all respects with the previously prepared compound **7a**; vide supra). Compound **7a** (1.06 g) was then placed in a steel reaction vessel, methylamine (40% in water, 20 mL) was added, and the sealed reaction vessel was heated at 135 °C for 90 min. The solvent was removed in vacuo to yield a light brown oil which was crystallized from benzene–hexane (1:1, v:v) to afford colorless needles of **14a**: 0.16 g (18%), mp 95–96 °C; R_f 0.16 (7% MeOH in $CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 2.96 (d, 3, N-CH_3), 3.41 (s, 4, CH_2), 4.62 (m, 1, exchangeable with D_2O, OH), 5.48 (s, 2, N7-CH_2), 6.87 (q, 1, exchangeable with D_2O, NH), 7.47 (s, 1, C6-H), 8.20 (s, 1, C2-H); UV λ_{max} nm ($\epsilon \times 10^4$) (pH 7), 212 (1.01), 285 (0.48), (pH 1) 205 (0.64), 235 (0.73), 280 (0.46), (pH 11) 225 (0.27), 281 (0.47). Anal. ($C_{10}H_{13}N_4O_2Cl$) C, H, N.

5-Bromo-4-(methylamino)-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-d]pyrimidine (14b). Sodium hydride (2.2 g, 60% in mineral oil) was added to a stirred reaction mixture of compound **13b** (7.67 g, 0.033 mol) in dry DMF (66 mL) under a nitrogen atmosphere. When hydrogen evolution had ceased, (2-acetoxyethoxy)methyl bromide (**2a**, 7.91 g, 0.40 mol) was added dropwise to the stirred reaction mixture over a 45-min period at room temperature. Water (100 mL) was added and the pH of the solution was adjusted to 7 with glacial acetic acid. The solution was extracted with EtOAc (3 × 50 mL). The EtOAc extracts were combined, washed with water (3 × 100 mL), and then dried over anhydrous $MgSO_4$. The solvent was evaporated in vacuo to give a brown oil which was applied to the top of a column (2-in. diameter, 100 g of silica) packed with wet silica gel in $CHCl_3$. Elution of the column with chloroform and subsequent evaporation of all UV-absorbing fractions yielded a colorless oil which was crystallized from EtOH to afford 5-bromo-4-chloro-7-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-d]pyrimidine (**8a**, 5.52 g, 48%) (identical in all respects with the previously prepared compound **8a**; vide supra). This compound (**8a**, 1.39 g) was then covered with methylamine (40% in H_2O, 20 mL) and heated in a sealed steel reaction vessel at 135 °C for 90 min. The solvent was then evaporated in vacuo to yield a yellow oil which was crystallized from benzene–hexane (1:1 v:v) to afford **14b**: 0.83 g (69%), mp 117–118 °C; ^{1}H NMR (DMSO-d_6) δ 3.01 (d, 3, N-CH_3), 3.42 (s, 4, CH_2), 4.67 (m, 1, exchangeable with D_2O, OH), 5.42 (s, 2, N7-CH_2), 6.67 (q, 1, exchangeable with D_2O, NH), 7.53 (s, 1, C6-H), 8.22 (s, 1, C2-H); UV λ_{max} nm ($\epsilon \times 10^4$) (pH 7) 206 (0.48), 237 (0.54), 282 (0.35), (pH 1) 212 (0.71), 286 (0.35), (pH 11) 229 (0.2), 282 (0.35). Anal. ($C_{10}H_{13}N_4O_2Br$) C, H, N.

5-Chloro-4-(ethylamino)-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-d]pyrimidine (14c). A mixture of compound **7a** (1.10 g, 3.62 mmol) and ethylamine (70% in H_2O, 20 mL) was heated in a sealed steel reaction vessel at 130 °C for 2 h. The solvent was then evaporated in vacuo to yield a light yellow oil. This oil was crystallized from a mixture of benzene–hexane (1:1, v:v) to afford colorless needles of **14c**: 0.14 g (15%), mp 98–99 °C; R_f 0.16 (7% MeOH in $CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 1.17 (t, 3, NHCH$_2$CH_3), 3.48 (s, 4, CH_2), 3.51 (quin, 2, NCH_2CH$_3$), 4.63 (m, 1, exchangeable with D_2O, OH), 5.48 (s, 2, N7-CH_2), 6.76 (t, 1, exchangeable with D_2O, NH), 7.48 (s, 1, C6-H), 8.19 (s, 1, C2-H); UV λ_{max} nm ($\epsilon \times 10^4$) (pH 7) 213 (0.76), 286 (0.38), (pH 1) 206 (0.53), 236 (0.611), 280 (0.37), (pH 11) 229 (0.17), 282 (0.36). Anal. ($C_{11}H_{15}N_4O_2Cl$) C, H, N.

5-Bromo-4-(ethylamino)-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-d]pyrimidine (14d). A mixture of compound **8a** (1.48 g, 4.25 mmol) and ethylamine (70% in water, 15 mL) was heated in a sealed steel reaction vessel at 135 °C for 90 min. The solvent was then evaporated in vacuo to give a brown oil which was crystallized from benzene–hexane (1:1, v:v) as colorless needles of **14d** (0.48 g, 34%): mp 80–81 °C; R_f 0.14 (7% MeOH in $CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 1.18 (t, 3, NHCH$_2$CH_3), 3.42 (s, 4, CH_2), 3.54 (quin, 2, NCH_2CH$_3$), 4.63 (m, 1, exchangeable with D_2O, OH), 5.99 (s, 2, N7-CH_2), 5.69 (t, 1, exchangeable with D_2O, NH), 7.53 (s, 1, C6-H), 8.20 (s, 1, C2-H); UV λ_{max} nm ($\epsilon \times 10^4$) (pH 7) 213 (0.67), 286 (0.34), (pH 1) 206 (0.47), 238 (0.52), 282 (0.34), (pH 11) 230 (0.2), 282 (0.34). Anal. ($C_{11}H_{15}BrN_4O_2$) C, H, N.

Biological Evaluations. (a) Cells and Viruses. KB cells, an established human cell line derived from an epidermoid oral carcinoma, were routinely grown in minimal essential medium (MEM) with Hanks salts [MEM(H)] supplemented with 5% fetal bovine serum. African green monkey kidney (BSC-1) cells and diploid human foreskin fibroblasts (HFF cells) were grown in MEM with Earle salts [MEM(E)] supplemented with 10% fetal bovine serum. Cells were passaged according to conventional procedures as detailed previously.[36] A plaque-purified isolate, P_0, of the Towne strain of HCMV was used in all experiments and was a gift of Dr. Mark Stinski, University of Iowa. The S-148

(36) Shipman, C., Jr.; Smith, S. H.; Carlson, R. H.; Drach, J. C. *Antimicrob. Agents Chemother.* **1976**, *9*, 120.

strain of HSV-1 was provided by Dr. T. W. Schafer of Schering Corp. Stock preparations of HCMV and HSV-1 were prepared and titered as described elsewhere.[20]

(b) Assays for Antiviral Activity. HCMV plaque reduction experiments were performed with monolayer cultures of HFF cells by a procedure similar to that referenced above for titration of HCMV, with the exceptions that the virus inoculum (0.2 mL) contained approximately 50 PFU of HCMV and the compounds to be assayed were dissolved in the overlay medium. HSV-1 plaque reduction experiments were performed with monolayer cultures of BSC-1 cells. The assay was performed exactly as referenced above for HSV-1 titration assays except that the 0.2 mL of virus suspension contained approximately 100 PFU of HSV-1 and the compounds to be tested were dissolved in the overlay medium.

(c) Cell Cytotoxicity Assays. Two basic tests for cellular cytotoxicity were routinely employed for compounds examined in antiviral assays. Cytotoxicity produced in HFF and BSC-1 cells was estimated by visual scoring of cells not affected by virus infection in the plaque reduction assays described above. Drug-induced cytopathology was estimated at 35- and 60-fold magnification and scored on a zero to four plus basis on the day of staining for plaque enumeration. Cytotoxicity in KB cells was determined by measuring the effects of compounds on the incorporation of radioactive precursors into DNA, RNA, and protein as detailed elsewhere.[20]

(d) Data Analysis. Dose-response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentrations. Fifty-percent inhibitory (I_{50}) concentrations were calculated from the regression lines. The three I_{50} concentrations for inhibition of DNA, RNA, and protein synthesis were averaged to give the values reported in the tables for KB cell cytotoxicity. Samples containing positive controls (acyclovir, ganciclovir, and vidarabine) were used in all assays. Results from sets of assays were rejected if inhibition by the positive control deviated from its mean response by more than 1.5 standard deviations.

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N-Acylphenylalanines and Related Compounds. A New Class of Oral Hypoglycemic Agents

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N-Benzoyl-DL-phenylalanine (1) was found to possess hypoglycemic activity. A series of the analogues of compound 1 were prepared and evaluated for their blood glucose lowering activity. Both the steric effects of the phenylalanine moiety and the effects of variations in the acyl moiety were investigated. This study elucidated some of the structure-activity relationships and led to the development of *N*-(4-ethylbenzoyl)-D-phenylalanine (34), which was 50 times more potent than the initial compound 1.

Oral therapy of non-insulin-dependent diabetes mellitus (NIDDM) largely relies on the sulfonylureas and the biguanides.[1] Although the sulfonylureas are valuable therapy for NIDDM, they do have disadvantages, e.g., hypoglycemia, and primary or secondary failure of efficacy.[2] The use of biguanides has declined because of their fetal lactic acidosis side effect.[3] To seek another type of antidiabetic drug, we screened numerous compounds in 18-h-fasted normal mice for hypoglycemic effects. In the course of this screening, we found that *N*-benzoyl-DL-phenylalanine[4] (1) exhibited a slight blood glucose lowering activity at a oral dose of 500 mg/kg.[5,6] To determine the structural requirements for possessing hypoglycemic activity and to obtain more potent compounds, the component parts of compound 1 (the acyl moiety and the phenylalanine moiety) were systematically varied.

First, the steric effects in the phenylalanine moiety were investigated. We compared *N*-benzoyl-D-phenylalanine (2) with *N*-benzoyl-L-phenylalanine (3) and found the difference in the pharmacological potency of the enantiomers. The conformationally restricted analogues, such as *N*-benzoyl-D-3-carboxy-1,2,3,4-tetrahydroisoquinoline (6), *N*-benzoyl-L-3-carboxy-1,2,3,4-tetrahydroisoquionline (7), (*Z*)-α-benzamidocinnamic acid (11), and (*E*)-α-benzamidocinnamic acid (12), were synthesized to examine the conformational effect on activity. Secondly, the acyl moiety of compound 1 was varied in order to elucidate the effects of the acyl moiety on biological activity.

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