Discovery of a New Family of Inhibitors of Human Cytomegalovirus (HCMV) Based upon Lipophilic Alkyl Furano Pyrimidine Dideoxy Nucleosides: Action via a Novel Non-Nucleosidic Mechanism

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Following our discovery of the potent anti-varicella zoster virus action of lipophilic alkyl furano pyrimidine 2′-deoxynucleosides, we now report that 2′,3′-dideoxy sugar analogues are devoid of anti-VZV activity but are potent and selective inhibitors of human cytomegalovirus (HCMV). The present compounds are active in vitro at ca. 1 μM with cytotoxicity only above 200 μM. Importantly, we have discovered that the new agents do not act as nucleoside analogues, despite their nucleosidic structure, and time of addition studies revealed that the compounds may inhibit HCMV at an event in the replication cycle of the virus that precedes DNA synthesis. They represent new leads in the discovery of improved therapies for HCMV, particularly in view of their novel mechanism of action.

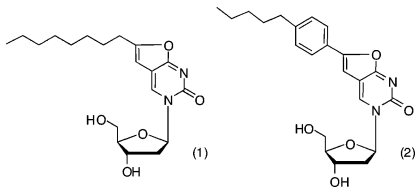
Introduction

We have recently reported on highly potent and selective inhibitors of varicella-zoster virus (VZV) with unusual bicyclic furanopyrimidine structures.[1−3] The structures were particularly novel with regard to their SARs on account of the requirement for a long alkyl chain on the base for antiviral action, although we later found that a *p*-alkylphenyl group could substitute, and indeed with a substantial boost in potency.[4]

These compounds have emerged as among the most potent inhibitors of VZV reported to date, with activity at concentrations as low as 1 nM.[4] They are also exquisitely selective, with selectivity index values exceeding 1 000 000, and indeed with complete selectivity for VZV over all other viruses tested, including the related members of the herpes virus family (HSV-1, -2, HCMV).[1−4] This degree of specificity is unusual among antiviral nucleosides and may be attributed to the presence of the obligatory lipid moiety on the base. Thus, in every structural variation reported to date the compounds of this family are entirely VZV-specific.[1−4] Lead structures are **1** and **2** (Chart 1) with *n*-octyl and *p*-pentylphenyl side chains, respectively.[1,4]

We have reported on extensive SAR studies in both the base and sugar moieties of these leads,[2,3] and in the latter region we have noted a substantial preference for 2′-deoxyribose over ribose and arabinose.[2] However, we were interested in other unusual sugars and particularly highly deoxygenated ones given their prevalence among current anti-retrovirals.[5] Thus, we sought the synthesis of a series of alkyl furano pyrimidines with a 2′,3′-dideoxy sugar.

Chart 1. Structures of Some Potent Anti-VZV Furano Pyrimidine Deoxy Nucleosides



Results and Discussion

Chemistry. The synthesis of previous, 2′-deoxyribose members of this family was conducted starting from 5-iodo-2′-deoxyuridine (IDU),[1] and we proposed to utilize an analogous route here, with 5-iodo-2′,3′-dideoxyuridine as a key synthon. Thus, following original procedures of Horwitz,[6] 2′-deoxyuridine (**3**) (Scheme 1) was 3′,5′-dimesylated and treated with aqueous sodium hydroxide to afford **4** in 71% yield. Upon treatment with NaH in DMF, this yielded the d4 dideoxy didehydro nucleoside **5** in good yield which gave dideoxyuridine (**6**) in almost quantitative yield upon catalytic hydrogenation. The next step was 5-iodination to give the key synthon **7**.

While this reaction has been reported in the literature,[7,8] we could not locate a detailed experimental protocol using our preferred method of ICl/MeOH. Lin[9] reported the use of iodic acid and iodine in AcOH, carbon tetrachloride, and water, with a yield of 31%, but we wondered whether such acidic conditions might lead to base cleavage in the acid-labile dideoxy sugar. Indeed, our initial ICl-mediated reaction, using 2.5 equiv of reagent at room temperature in methanol led to complete base cleavage as judged by TLC. Use of potassium carbonate as a heterogeneous buffer led to no reaction at all, cleavage, or iodination. Repeating the reaction in the absence of base but with reduced equivalents of reagent did give a good yield of product. However, when analyzed it was revealed to be the 5-iodo-6-methoxy

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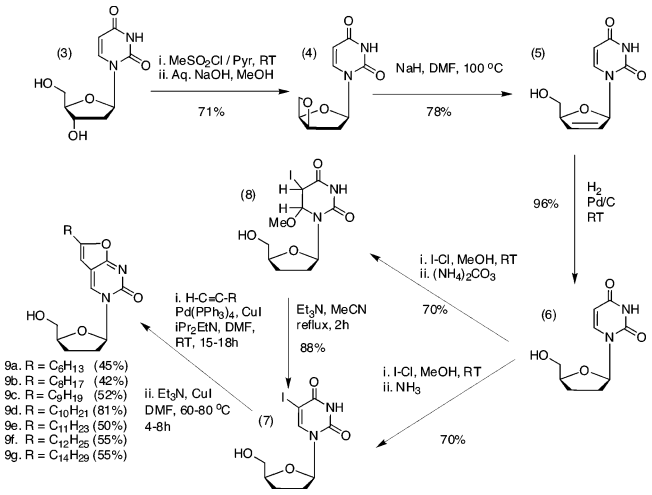
Scheme 1. Synthetic Route to 2′,3′-Dideoxy Alkylfurano Pyrimidine Antiviral Nucleosides



adduct (**8**). Similar products with other halogens have been previously noted by Walker.[10]

We found that **8** could be smoothly converted to the desired product **7** by treatment with triethylamine at reflux. This led us to modify the workup procedure of the iodination reaction, and we found that the use of ammonia rather than ammonium bicarbonate lead to the direct conversion of **6** into **7** in one step. Now, following procedures that we have extensively published,[1−4] we reacted **7** with a series of terminal alkynes (Scheme 1) and cyclized the resulting 5-alkynyl nucleosides in situ with copper to give the target furano pyrimidines **9a**−**g**. The yields of these compounds were ca. 40−80% which is somewhat similar to that noted for previous 2′-deoxy analogues.[1] Spectroscopic and analytical data fully confirmed the structure and purity of these compounds, and they were thus assayed for inhibition of a series of herpes viruses, by methods we have reported.[11]

Antiviral Activity. The target compounds **9a**−**g** were evaluated for their ability to inhibit the replication of VZV in vitro, using two strains of thymidine kinase-competent virus and also two strains of thymidine kinase-deficient VZV. Data (not shown) indicate an almost complete lack of antiviral effect at nontoxic concentrations, up to ca. 50 μM. This is in marked contrast to the parent deoxynucleoside analogues **1** which are active at ca. 0.01 μM. Thus, the dideoxy series **9a**−**g** are at least 10 000-fold less active than the previous compounds. This is taken to highlight the importance of a 3′-hydroxyl group for anti-VZV activity in this family. Whether this is necessary to enable 5′-phosphorylation, and thus activation of the nucleosides, and/or for the activity of the resulting nucleotides at their putative viral target remains unclear.[12,13] However, prior studies have shown the absolute requirement for VZV thymidine kinase for activity, strongly implying the necessity of 5′-phosphorylation for activity.[1,12]

Since different members of the herpes viral family have different phosphorylation profiles, it was of interest to test these compounds against other members of the family. Thus, **9a**−**g** were tested as inhibitors of two strains of HCMV, AD-169 and Davis. The data presented in Table 1 show a clear selective anti-HCMV effect for certain homologues of the series. Thus, the

Table 1. Anti-HCMV Activity and Cytotoxicity for Dideoxy Alkyl Furano Pyrmidines **9a**−**g** and Reference Compounds Ganciclovir, Cidofovir, and Foscarnet

		EC_{50}(μM)[a]			
compd	R	HCMV AD169	HCMV Davis	MCC[b] μM	CC_{50} [c] μM
9a	6	32	>50	200	143
9b	8	2.5	3.0	20	112
9c	9	1.2	1.3	58	>200
9d	10	2.6	3.2	>20	>200
9e	11	1.4	1.2	50	>200
9f	12	3.7	5.0	200	>200
9g	14	25	40	200	>200
ganciclovir	−	2.7	3.9	>150	>150
cidofovir	−	0.6	1.0	>150	158
foscarnet	−	53	62	>400	500

[a] 50% effective concentration or compound concentration required to inhibit HCMV-induced cytopathicity in human embryonic lung (HEL) fibroblast cell cultures by 50% as measured at day 7 postinfection. [b] Minimal cytotoxic concentration or compound concentration required to cause a microscopically visible alteration of HEL cell morphology as measured at day 7 postinfection. [c] 50% cytostatic concentration or compound concentration required to inhibit HEL cell proliferation by 50% as measured at day 4 after addition of the compounds to the cell culture. The detailed antiviral assay procedures are described in ref 16 (Snoeck et al., 2002).

shortest member of the series, the hexyl compound **9a**, shows only modest antiviral action, as indeed does the longest homologue, the tetradecyl compound **9g**. The intermediate members, with chain lengths C8−C12 (**9b**−**f**) all show antiviral activity at ca. 1−5 μM with cytotoxicity in excess of 200 μM. Thus, they show a similar anti-HCMV profile to the leading drug for CMV, ganciclovir (**10**). The compounds are less potent than cidofovir but more active than foscarnet (Table 1). Further assays (data not shown) reveal these compounds **9a**−**g** to be inactive vs HIV-1, HIV-2, HSV-1, and HSV-2. Thus, this new family of 2′,3′-dideoxy alkyl furano pyrimidines emerge as reasonably potent and selective antivirals with high specificity for HCMV. Given their novel structure, it appears likely that they may be complementary in their profiles to existing nucleosides such as ganciclovir, and extensive biological evaluation is currently underway to probe this notion. However, it has not escaped our attention that the present compounds offer very significantly higher lipophilicity over previous anti-HCMV nucleosides such as **10**. Thus, **9c** has a calculated logP value of 3.7 versus −2.5 for **10**, making **9c** more than 1 000 000 times more lipophilic.[14] This may be of advantage with regard to the onward clinical development of these agents particularly with regard to cellular access and tissue distribution. Such studies are currently underway in our laboratories.

Given the nucleoside type structures of **9a**−**g**, and indeed our prior experience with **1** and **2**, we presumed that these new anti-HCMV compounds were acting via a 'conventional' nucleoside mechanism involving 5′-phosphorylation and activity as a phosphorylated form. The interaction of the putative 5′-triphosphate with HCMV DNA polymerase would be the most obvious mechanism. However, to our surprise, several pieces of data indicated this not to be the case and, indeed, that this new family of agents acts via a novel non-nucleosidic mechanism. First, antiviral time of addition studies revealed that a delay of addition of compound **9c** for 24−36 h lead to a reduction in activity (Figure 1). Thus,

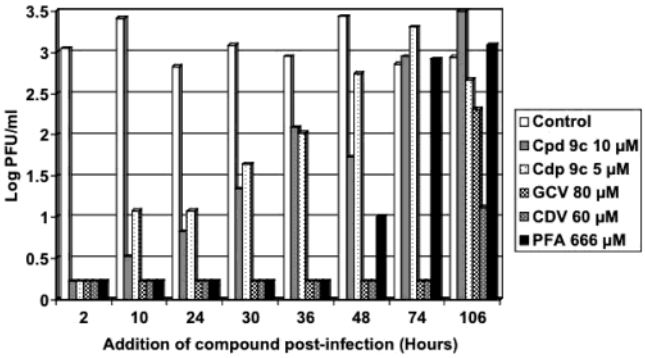


Figure 1. Time of addition study. A chart showing the effect of delayed compound addition on HCMV replication. GCV = ganciclovir; CDV = cidofovir, PFA = foscarnet.

the compound afforded a reduction in virus yield of 2.8 logs when added after 2 h of infection compared to 2.0 and 1.2 logs when added, respectively, after 24 and 48 h postinfection. By contrast, ganciclovir and cidofovir, two compounds known to affect viral DNA synthesis, **10** showed no reduction in activity when addition was delayed as late as 74 h after infection of cells (Figure 1, and data not shown). By the use of appropriate standards (see Experimental Section), we were able to deduce that **9a**−**g** must act at a stage of viral replication that is earlier than DNA synthesis. Viral entry is a likely mechanism of action, but this is not proven yet. Second, the SAR as presented in Table 1, with no significant variation in potency as the side chain is varied from C8 (**9b**) to C12 (**9f**) is in marked contrast to the analogous position with the anti-VZV compounds of type (1); here only a two-carbon variation (C6 to C8) leads to a >100-fold change (increase) in potency. Given the often rather precise structural requirements of nucleoside kinases and DNA polymerases, the 'flat' anti-HCMV SAR of **9b**−**f** is unusual and suggestive of a non-nucleoside mechanism.

Recently, a series of D- (BDCRB) and L- (Maribavir) benzimidazoles have been found active against herpes viruses including HCMV. Both types of compounds act in a different way. BDCRB blocks the processing and maturation of viral DNA whereas Maribavir inhibits the HCMV-specific pUL97 and interferes with DNA synthesis.[15] It is believed that the series of compounds described in our study clearly inhibit HCMV replication in a different manner and at least at a step in the replication cycle of HCMV that is earlier than BDCRB and Maribavir.

In conclusion, we report the discovery of a new family of potent and selective anti-HCMV agents based upon dideoxy alkyl furano pyrimidines. Their high inherent lipophilicity distinguishes them from the currently approved anti-HCMV therapies and may offer advantages in their onward development.

Experimental Section

The numbering of the bicyclic ring follows the recommended IUPAC nomenclature guidelines. The naming of the compounds follows IUPAC nomenclature and/or standard accepted nomenclature for nucleoside chemistry. ^{1}H and ^{13}C NMR spectra were recorded in a Bruker AVANCE DPX 300 spectrometer (300 MHz and 75 MHz, respectively) and autocalibrated to the deuterated solvent reference peak. All ^{13}C NMR spectra were proton-decoupled. Low resolution mass spectra were obtained with a Fisons VG Platform 8070 micromass spectrometer with electrospray (ES+) mode, using a mobile phase of acetonitrile /water (1:1). Accurate mass MS were obtained courtesy of the University of Birmingham, Department of Chemistry (Birmingham, UK) in FAB mode. All solvents used were anhydrous and used as supplied by Aldrich. All nucleosides and solid reagents were dried for several hours while being heated under high vacuum over phosphorus pentoxide. All glassware was oven dried at 130 °C for several hours or overnight and allowed to cool in a desiccator or under a stream of dry nitrogen. For thin-layer chromatography, precoated aluminum-backed (60 F-54,0.2 mm thickness; supplied by E. Merck AG, Darmstadt, Germany) were used and were developed by ascending method. After solvent evaporation, compounds were defected by quenching of the fluorescence, at 254 or 336 nm depending on the compound, on irradiation with a UV lamp. For column chromatography: Glass columns were slurry-packed in the appropriate eluent or preabsorbed onto silica gel. Fractions containing the product were identified by TLC and pooled, and the solvent was removed in vacuo Flash column chromatography was performed with a aid of a hand pump. Microanalytical studies were done by the courtesy of the University of London, Department of Chemistry (London, U.K).

1-[2,6-Dioxa-bicyclo [3.2.0]hept-3-yl]-1*H*-pyrimidine-2, 4-dione (4).[6] Methanesulfonyl chloride (12.56 g, 109.64 mmol, 2.5 equiv, 8.5 mL) was added to a stirred solution of 2′-deoxyuridine (10.0 g, 43.85 mmol) in anhydrous pyridine (75 mL) at 0 °C under N_2. The resulting solution was stirred for further period of 1.5 h at room temperature. The solvent was then removed under high vacuum, and the oil obtained was washed with ice−water (100 mL). The resulting oil was heated under gentle reflux with aqueous sodium hydroxide (10.5 g, 263 mmol, 6.0 equiv) in methanol (250 mL) for 3 h. The contents of the flask were cooled, and the solution was neutralized with 1 N HCI (pH ∼ 7.00). The solvent was removed in vacuo, the residue was extracted with boiling acetone (250 mL × 4), and the solvent from the combined extract was removed in vacuo. The crude product was purified by flash column chromatography eluting with 10% methanol in dichloromethane to afford an off white solid (6.52 g, 71%). ^{1}H NMR(d_6-DMSO): δ 11.37 (1H, S, 3-NH), 8.18 (1H, d, J = 8.2 Hz, H-6), 6.52 (1H, t, J = 5.3 Hz, H-1′), 5.74 (1H, d, J = 8.1 Hz, H-5), 5.49 (1H, m, H-3′), 4.93 (1H, m, H-5′), 4.69 (1H, m, H-5′), 4.04 (1H, m, H-4′), 2.49 (2H, m, H-2′).

2′,3′-Didehydro-2′,3′-dideoxyuridine (5).[6] Sodium hydride (3.2 g, 60%, 80.00 mmol, 3.1 equiv) was added to a stirred solution of 1-[2,6-dioxa-bicyclo[3.2.0]hept-3-yl]-1*H*-pyrimidine-2,4-dione (**4**) (5.42 g, 25.80 mmol) in dry *N*,*N*-dimethylformamide, and the mixture was heated at 100 °C for 3 h under N_2. Excess of sodium hydride was destroyed on careful addition of dry ice and a small amount of water, and the solvent was removed under high vacuum. The residue obtained was dissolved in methanol and the solution was made neutral (pH ∼ 7.0) on addition of 1 N HCl. Solvent was removed in a vacuum, the final traces of *N*,*N*-dimethylformamide were removed under high vacuum, and the crude residue obtained was purified by flash column chromatography eluting with 10% methanol in dichloromethane to afford a white solid (4.22 g, 78%). ^{1}H NMR (d_6-DMSO): δ 11.32 (1H, s, 3-NH), 7.77 (1H, d, J = 8.0 Hz, H-6), 6.83 (1H, m, H-1′), 6.42 (1H, m, H-3′), 5.94 (1H, m, H-2′), 5.61 (1H, d, J = 8.0 Hz, H-5), 4.99 (1H, t, J = 4.7 Hz, 5′-OH), 4.79 (1H, m, H-4′), 3.60 (2H, m, H-5′).

2′,3′-Dideoxyuridine (6). Palladium on carbon (10%) (180 mg, 0.05 equiv) was added to a solution of 2′,3,-didehydro-2′,3′-dideoxyuridine (**5**) (3.6 g, 17.1 mmol) in methanol (150 mL), and the resulting suspension was hydrogenated at 1 atm for 3.5 h at room temperature. The catalyst was filtered, and the solvent was evaporated in vacuo to give an off white solid (3.5 g, 96%). ^{1}H NMR (d_6-DMSO): δ 7.95 (1H, d, J = 8.0 Hz, H-6), 5.96 (1H, dd, H-1′), 5.59 (1H, J = 8.0 Hz, H-5), 4.04 (1H, m, H-4′), 3.66 (1H, m, H-5′), 3.56 (1H, m, H-5′), 2.00−1.63 (4H, m, H-2′ and H-3′), 3-NH was not to be seen.

2′,3′-Dideoxy-5-iodo-6-methoxyuridine (8). Iodine monochloride (661 mg, 4.07 mmol, 1.35 equiv) was added to a

solution of 2′3′-dideoxyuridine (**6**) (640 mg, 3.02 mmol) in methanol (40 mL), and the resulting solution was stirred at room temperature for 10 min. The pH of the solution was made ~7.00 by adding a solution of ammonium bicarbonate (1 N). The solvent was removed under high vacuum, and the residue obtained was purified by flash column chromatography eluting with 13% methanol in dichloromethane to afford a pale brown oil (780 mg, 70%) which on trituration with dichloromethane gave an off white solid. ^{1}H NMR (d_6-DMSO): δ 10.65 (1H,S, 3-NH), 5.78 (1H, t, H-1′), 5.05 (1H, d, H-6), 4.80 (1H, bS, 5′-OH), 4.65 (1H, d, H-5), 3.85 (1H, S, H-4′), 3.54 (2H, m, H-5′), 3.30 (3H, S, OCH_3), 2.10 (2H, m, H-2′), 1.80 (2H, m, H-3′). MS [ES$^+$] *m/e* 393 (100%, MNa$^+$). Accurate mass $C_{10}H_{15}N_2O_5$ Na requires 392.9923, observed 392.9912.

2′,3′-Dideoxy-5-iodoridine (7) (Method 1). Iodine monochloride (3.75 g, 23.113 mmol, 1.4 equiv) was added to a solution of 2′,3′-dideoxyuridine (**6**) (3.5 g, 16.509 mmol) in methanol (120 mL), and the solution was stirred at room temperature for 2 h. The solution was made neutral on addition of 10% aqueous ammonia solution. The solvent was removed under high vacuum, the dark brown residue obtained was dissolved in ethyl acetate (300 mL), and the excess of iodine from this solution was removed by extracting it with a 0.5 M aqueous solution of sodium thiosulfate. The organic layer was washed with water (50 mL × 2) and dried over anhydrous $MgSO_4$ and filtered. The solvent from the organic layer was removed under high vacuum to obtain a yellowish oil which on addition of ethyl acetate afforded an off white solid (4.31 g, 70%). ^{1}H NMR (d_6-DMSO): δ 11.62 (1H, S, 3-NH), 8.59 (1H, S, H-6), 5.89 (1H, m, H-1′), 5.23 (1H, t, J = 4.6 Hz, 5′-OH), 4.06 (1H, m, H-4′), 3.77 (1H, m, H-5′), 3.55 (1H, m, H-5′), 2.25 (1H, m, H-2′), 2.04 (1H, m, H-2′), 1.83 (2H, m, H-3′).

2′,3′-Dideoxy-5-iodoridine (7) (Method 2). 2′,3′-Dideoxy-5-iodo-6-methoxyuridine (**8**) (100 mg, 0.27 mmol) was dissolved in acetonitrile (5 mL), and to this solution was added triethylamine (70 mg, 0.70 mmol, 2.6 equiv, 100 μL). The resulting solution was heated under reflux for 2 h. The solvent was removed in vacuo, and the oil obtained afforded an amorphous solid on trituration with diethyl ether (80 mg, 80%). ^{1}H NMR (d_6-DMSO): δ 11.62 (1H, S, 3N−H), 8.58 (1H, S, H-6), 5.89 (1H, m, H-1′), 5.23 (1H, t, J = 4.9 Hz, 5′-OH), 4.06 (1H, m, H-4′), 3.78 (1H, m, H-5′), 3.55 (1H, m, H-5′), 2.33−1.84 (4H, m, H-3′ and H-2′).

3-[2′,3-Dideoxy-ribo-β-D-furanosyl]-6-hexyl-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (9a). Diisopropylethylamine (267 mg, 2.07 mmol, 2 equiv, 0.36 mL), 1-octyne (342 mg, 3.106 mmol, 3 equiv, 0.45 mL), tetrakis(triphenylphosphine)palladium(0) (120 mg, 0.104 mmol, 0.1 equiv), and copper(II) iodide (39 mg, 0.206 mmol, 0.2 equiv) were added to a solution of 2′3′-dideoxy-5-iodoridine (**7**) (350 mg, 1.035 mmol) in dry *N*,*N*-dimethylformamide (25 mL) at room temperature under N_2 atmosphere, and the mixture was stirred at room temperature for 16 h under N_2. To the resulting solution were added copper(I) iodide (39 mg, 0.206 mmol, 0.2 equiv) and triethylamine (10 mL), and the solution was heated to 70−80 °C for 6 h. Solvent was removed under high vacuum. Dichloromethane and methanol (1:1 50 mL mixture) were added to the above residue, to this solution was added an excess of Amberlite IRA-400 (HCO_3^- form), and the resulting mixture was stirred at room temperature for 1 h. The resin was filtered and washed with methanol, and the combined filtrate was evaporated to dryness to afford a dark brown residue. This was purified by flash column chromatography, eluting with 6% methanol in ethyl acetate to afford a pale yellow oil (150 mg, 45%) which gave a white solid on trituration with ethyl acetate. ^{1}H NMR (d_6-DMSO): δ 8.82 (1H, S, H-4), 6.40 (1H, S, H-5), 5.98 (1H, m, H-1′), 5.19 (1H, t, J = 5.3 Hz, 5′-OH), 4.15 (1H, m, H-4′), 3.82 (1H, m, H-5′) 3.63(1H, m, H-5′), 2.62 (2H, t, J = 7.3 Hz, α-CH_2), 2.48 (1H, m, H-2′), 1.99 (1H, m, H-2′), 1.78 (2H, m, H-3′), 1.60−1.22 (8H, m, 4 × CH_2), 0.83 (3H, t, J = 6.9 Hz, CH_3). MS [ES$^+$] *m/e* 344 (10%), 343 (100%, MNa$^+$), 243 (15%, [base Na]$^+$). $C_{17}H_{24}N_2O_4$ Na requires 343.1634, observed 343.1633. Anal. ($C_{17}H_{24}N_2O_4$) C, H, N.

3-[2′,3′-Dideoxy-ribo-β-D-furanosyl]-6-octyl-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (9b). This was prepared entirely as outlined for **9a** above. The product was purified by flash column chromatography, eluting with 5% methanol in ethyl acetate to afford a pale yellow oil (140 mg, 42%) which gave a white solid on trituration with ethyl acetate. ^{1}H NMR (d_6-DMSO): δ 8.85 (1H, S, H-4), 6.43 (1H, S, H-5), 6.01 (1H, m, H-1′), 5.22 (1H, t, J = 5.1 Hz, 5′-OH), 4.18 (1H, m, H-4′), 3.84 (1H, m, H-5′) 3.66 (1H, m, H-5′), 2.64 (2H, t, J = 7.2 Hz, α-CH_2), 2.48 (1H, m, H-2′), 1.97 (1H, m, H-2′), 1.78 (2H, m, H-3′), 1.63−1.25 (12H, m, 6 × CH_2), 0.85 (3H, t, J = 6.9 Hz, CH_3). MS [ES$^+$] *m/e* 372 (15%), 371 (100%, MNa$^+$), 271 (20%, [base Na]$^+$). $C_{19}H_{28}N_2O_4$ Na requires 371.1947, observed 371.1957. Anal. ($C_{19}H_{28}N_2O_4$) C, H, N.

3-[2′,3′-Dideoxy-ribo-β-D-furanosyl]-6-nonyl-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (9c). This was prepared entirely as outlined for **9a** above. The product was was purified by flash column chromatography, eluting with 7% methanol in ethyl acetate to afford a pale yellow oil (138 mg, 52%) which gave a white solid on trituration with diethyl ether. ^{1}H NMR ($CDCl_3$): δ 8.76 (1H, S, H-4), 6.25 (1H, m, H-1′), 6.18 (1H, S, H-5) 4.34 (1H, m, H-4′), 4.18 (1H, m, H-5′), 3.94 (1H, m, H-5′), 2.68 (3H, m, α-CH_2+H-2′), 2.22 (1H, m, H-2′), 1.99 (2H, m, H-3′), 1.74−1.31 (14H, m, 7 × CH_2), 0.93 (3H, t, J = 6.9 Hz, CH_3). MS [ES$^+$] *m/e* 386 (15%), 385 (100%, MNa$^+$), 285 (40%, [base Na]$^+$). $C_{20}H_{30}N_2O_4$ Na requires 385.2103, observed 385.2104. Anal. ($C_{20}H_{30}N_2O_4$·1.5H_2O) C, H, N.

3-[2′,3′-Dideoxy-ribo-β-D-furanosyl]-6-decyl-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (9d). This was prepared entirely as outlined for **9a** above. The product was purified by flash column chromatography, eluting with 6% methanol in ethyl acetate to afford a pale yellow oil (360 mg, 81%) which gave a white solid on trituration with ethyl acetate. ^{1}H NMR (d_6-DMSO): δ 8.85 (1H, S, H-4), 6.43 (1H, S, H-5), 6.01 (1H, m, H-1′) 5.21 (1H, t, J = 5.2 Hz, 5′-OH), 4.18 (1H, m, H-4′), 3.84 (1H, m, H-5′), 3.65 (1H, m, H-5′), 2.64 (2H, t, J = 7.3 Hz, α-CH_2), 2.48 (1H, m, H-2′), 1.97 (1H, m, H-2′), 1.78 (2H, m, H-3′), 1.63−1.18 (16H, m, 8 × CH_2), 0.85 (3H, t, J = 6.9 Hz, CH_3). MS [ES$^+$] *m/e* 399 (100%, MNa$^+$), 299 (50%, [base Na]$^+$). $C_{21}H_{32}N_2O_4$ Na requires 399.2260, observed 399.2254. Anal. ($C_{21}H_{32}N_2O_4$) C, H, N.

3-[2′,3′-Dideoxy-ribo-β-D-furanosyl]-6-undecyl-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (9e). This was prepared entirely as outlined for **9a** above. The product was purified by flash column chromatography, eluting with 6% methanol in ethyl acetate to afford an off white solid (145 mg, 50%). ^{1}H NMR ($CDCl_3$): δ 8.74 (1H, S, H-4), 6.25 (1H, m, H-1′), 6.17 (1H, S, H-5), 4.34 (1H, m, H-4′), 4.21 (1H, m, H-5′), 3.94 (1H, m, H-5′), 2.68 (2H, t, J = 7.5 Hz, α-CH_2), 2.53 (1H, m, H-2′), 2.28 (1H, m, H-2′), 1.99 (2H, m, H-3′), 1.74−1.13 (18H, m, 9 × CH_2), 0.93 (3H, t, J = 6.9 Hz, CH_3). MS [ES$^+$] *m/e* 429 (5%, MK$^+$), 414 (15%), 413 (80%, MNa$^+$), 313 (100%, [base Na]$^+$), 291 (20%, [base + 1]$^+$). Anal. ($C_{22}H_{34}N_2O_4$·3H_2O) C, H, N.

3-[2′,3′-Dideoxy-ribo-β-D-furanosyl]-6-dodecyl-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (9f). This was prepared entirely as outlined for **9a** above. The product was purified by flash column chromatography eluting with 6% methanol in ethyl acetate to afford a pale yellow oil (230 mg, 55%) which gave a white solid on trituration with diethyl ether. ^{1}H NMR (d_6-DMSO): δ 8.82 (1H, S, H-4), 6.41 (1H, S, H-5), 6.03 (1H, m, H-1′), 5.09 (1H, bS, 5′-OH), 4.17 (1H, m, H-4′), 3.80 (1H, m, H-5′), 3.67 (1H, m, H-5′), 2.64 (2H, t, J = 7.3 Hz, α-CH_2), 2.45 (1H, m, H-2′), 1.97 (1H, m, H-2′), 1.78 (2H, m, H-3′), 1.63−1.25 (20H, m, 10 × CH_2), 0.87 (3H, t, J = 6.9 Hz, CH_3). MS [ES$^+$] *m/e* 443 (5%, MK$^+$), 428 (20%), 427 (100%, MNa$^+$), 327 (30%, [base Na]$^+$). $C_{23}H_{36}N_2O_4$ Na requires 427.2573, observed 427.2575. Anal. ($C_{23}H_{36}N_2O_4$) C, H, N.

3-[2′,3′-Dideoxy-ribo-β-D-furanosyl]-6-tetradecyl-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (9g). This was prepared entirely as outlined for **9a** above. The product was purified by flash column chromatography, eluting with 5% methanol in ethyl acetate to afford a white solid (250 mg, 56%). ^{1}H NMR ($CDCl_3$): δ 8.74 (1H, S, H-4), 6.25 (1H, m, H-1′), 6.17 (1H, S, H-5), 4.34 (1H, m, H-4′), 4.21 (1H, m, H-5′) 3.94 (1H, m, H-5′),

2.68 (2H, t, $J = 7.7$ Hz, α-CH_2), 2.56 (1H, m, H-2′), 2.56 (1H, m, H-2′), 2.23 (1H, m, H-2′), 1.99 (2H, m, H-3′), 1.76−1.30 (24H, m, 12 × CH_2), 0.93 (3H, t, $J = 6.9$ Hz, CH_3). MS [ES^+] *m/e* 471 (5%, MK^+), 456 (20%), 455 (100%, MNa^+), 355 (40%, [base Na]$^+$). $C_{25}H_{40}N_2O_4$ Na requires 455.2886, observed 455.2881. Anal. ($C_{25}H_{40}N_2O_4$) C, H, N.

Viruses. The laboratory HCMV strains Davis and AD-169 were used in the CPE reduction assays.

Antiviral Assays. Confluent HEL cells grown in 96-well microtiter plates were inoculated with HCMV at an input of 100PFU (plaque forming units) per well. After a 1−2 h incubation period, residual virus was removed, and the infected cells were further incubated with MEM (supplemented with 2% inactivated FCS, 1% L-glutamine, and 0.3% sodium bicarbonate) containing varying concentrations of the compounds. Antiviral activity was expressed as EC_{50} (50% effective concentration) or compound concentration required to reduce virus-induced cytopathicity after 7 days by 50% compared to the untreated control.

Cytotoxicity Assays. Confluent monolayers of HEL cells as well as growing HEL cells in 96-well microtiter plates were treated with different concentrations of the experimental drugs. Growing cells were incubated for 3 days. At that time, the cells were trypsinized, and the cell number was determined using a Coulter counter. The 50% cytotoxic concentration (CC_{50}) was defined as the compound concentration required to reduce the cell number by 50%. During the antiviral assay, the minimum cytotoxic concentration (MCC) was evaluated at day 7 as the lowest compound concentration giving microscopically visible cytotoxicity on the confluent cells.

Time of Addition Study. Compound **9c** at concentrations of 5 and 10 μM were added at different times after infection of HEL cells with HCMV (Davis strain). Following 4 days of incubation, culture supernatants were harvested and virus yield was determined by titration in HEL cells. Ganciclovir [GCV], cidofovir [CDV], and foscarnet [PFA] at concentrations of respectively, 80 μM, 60 μM, and 666 μM were added as standard controls.

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