Novel C-6 Fluorinated Acyclic Side Chain Pyrimidine Derivatives: Synthesis, ^{1}H and ^{13}C NMR Conformational Studies, and Antiviral and Cytostatic Evaluations

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Received December 15, 2006

The synthetic route for introduction of a fluoroalkyl (**7**−**12**, **14**), fluoroalkenyl (**15** and **16**), fluorophenylalkyl (**17**, **19**, **20**, and **22**), and fluorophenylalkenyl (**18**, **21**) side chain at C-6 of the pyrimidine involved the lithiation of the pyrimidine derivatives **3** and **3a** and subsequent nucleophilic addition or substitution reactions of the organolithium intermediate thus obtained with various electrophiles. Conformational properties of the novel fluorinated pyrimidine derivatives were assessed by the use of 1D difference NOE enhancements and C−F coupling constants. Compounds **4**−**22** were evaluated for their antiviral and cytostatic activities. Of all compounds evaluated, the 5-bromopyrimidine derivatives **5** and **6** showed the highest inhibitory activities. Among the series of fluoroalkylated pyrimidines, which is generally more active than the series of fluorophenylalkylated pyrimidines, compounds **8** and **14** displayed moderate cytostatic activities against the tested tumor cell lines. Moreover, compound **8** containing a 2-fluoromethylpropyl side chain expressed some but not highly specific activity against varicella-zoster virus (VZV). From C-6 fluorophenylalkylated pyrimidine derivatives, **17a** and **21** showed a slight activity against cytomegalovirus (CMV), VZV, and Coxsackie B4 virus, respectively. Besides, compounds **17a** and **21** showed no cytotoxic effect.

Introduction

Pyrimidines are biologically important molecules and valuable heterocyclic nuclei for the design of pharmaceutical agents.[1,2] A great number of C-5 and C-6 substituted pyrimidine nucleosides have been prepared in view of their various biological activities.[3] Uracil derivatives substituted either at the C-5 or the C-6 position and their nucleosides have considerable importance in the field of antiviral chemotherapy.[4] Furthermore, 6-substituted pyrimidines, as for example 1-[(2-hydroxyethyl)-methyl]-6-(phenylthio)thymine (HEPT)[5−8] and 3,4-dihydro-2-alkoxy-6-benzyl-4-oxopyrimidines (DABOs),[9] show a potent and selective activity against human immunodeficiency virus type-1 (HIV-1). The excellent biological activities exhibited by these 6-substituted uracil derivatives provide a new emphasis to explore the chemistry and biological activities of these pyrimidine derivatives.[10] A large number of nucleosides such as thymidine analogues and acyclic guanosine derivatives show antiviral activities against herpes viruses.[11] The antiviral activity of these compounds is due to their selective and efficient activation through monophosphorylation by the viral enzyme in the intact cells.[12,13] Therefore, radiolabeling of these antiviral agents, such as ganciclovir and penciclovir, with the positron-emitting isotope ^{18}F allows noninvasive imaging of the viral thymidine kinase enzyme activity by means of positron-emission tomography (PET).[14−17] Besides, the PET technique has proven to be vital in early detection of cancer. This is critical to successful treatment and long-term cure of cancer, staging of primary tumors, and monitoring the efficacy of chemo- or radiotherapy response.[19−21]

Recently, we have reported that, among fluorinated propyl or propenyl acyclic pyrimidine derivatives, compounds containing the 2-hydroxy-3,3,3-trifluoro-1-propenyl side chain exhibited a pronounced effect against breast carcinoma (MCF-7), while the compound with a 2-fluoromethyl-2-acetoxypropyl chain exhibited a moderate effect against cervical carcinoma (HeLa).[22] We have also reported that ^{18}F-radiolabeled pyrimidine-based acyclic nucleosides in which the acyclic sugar moiety is attached in the 6-position rather that at N-1 of the pyrimidine ring can be suitable candidates for the development of nontoxic PET-tracer molecules that are specifically and efficiently phosphorylated by the herpes simplex virus type 1 thymidine kinase (HSV-1 TK).[14,15] The pronounced biological activities exhibited by this class of compounds led us to synthesize new types of C-6 fluoroalkylated (**7**−**12** and **14**−**16**) and fluorophenylalkylated (**17**−**22**) pyrimidine derivatives (Figure 1) as model compounds for development of tracer molecules in positron-emission tomography (PET).

Chemistry

Synthesis. 2,4-Dimethoxy-6-methylpyrimidine (**3**) as a key precursor was prepared according to the procedure described in the literature.[21,23] Radical reaction of **3** with *N*-bromosuccinimide in acetic acid gave mono-, di-, and tribrominated pyrimidine derivatives **4**−**6**. The new 6-acyclic chain (**7**−**12** and **14**−**16**, Scheme 1) and C-6 fluorophenylalkyl chain (**17**−**22**, Scheme 2) substituted pyrimidine derivatives were synthesized by lithiation, which has been studied extensively as an important carbon−carbon bond-forming reaction.[14,24] Lithiation of the 6-methyl group of the pyrimidine (**3**) and subsequent addition of the lithiated derivative to fluoroacetone and 1,1,1-trifluoroacetone afforded 2-fluoromethyl-2-hydroxypropyl (**7**)

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10.1021/jm0614329 CCC: $37.00
Published on Web 06/01/2007

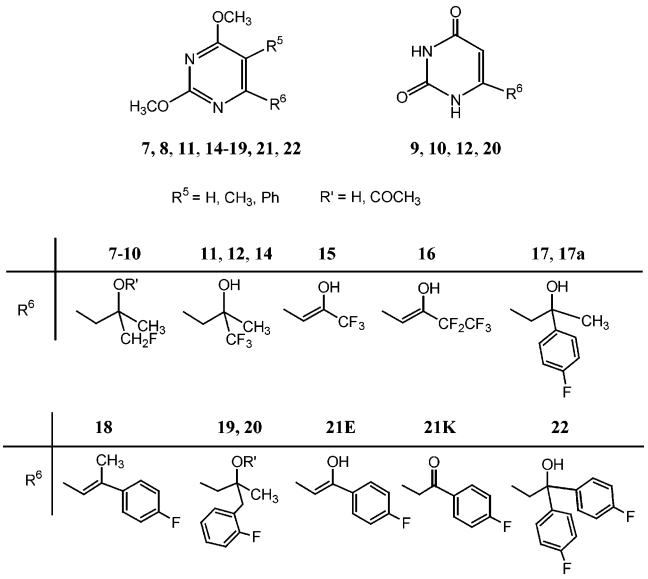


Figure 1. The new C-6 fluoroalkylated (**7**−**12** and **14**−**16**) and fluorophenylalkylated (**17**−**22**) pyrimidine derivatives.

and 2-trifluoromethyl-2-hydroxypropyl (**11**) pyrimidine derivatives. Nucleophilic substitution reaction of the organolithium intermediate with methyl trifluoroacetate and ethyl pentafluoropropionate gave 2,4-dimethoxypyrimidine derivatives containing a fluoro-substituted 2-hydroxy-1-propenyl side chain at C-6 (**15** and **16**, Scheme 1). Treatment of **7** and **11** with acetyl chloride effected a conversion to the 2,4-dimethoxypyrimidine with an acetylated side chain (**8**), pyrimidine derivatives containing free 2,4-diketo and hydroxyl functionalities (**9** and **12**), and a 2,4-dihydroxypyrimidine derivative with an acetylated hydroxyl group (**10**). The Stille reaction,[25−27] which is another efficient carbon−carbon bond-forming reaction, was applied for the introduction of the phenyl ring at C-5 of the pyrimidine moiety in **13** using tributylphenylstannane and dichlorobis(triphenylphosphine)palladium(II) as a catalyst. Subsequent nucleophilic addition of lithiated **13** to 1,1,1-trifluoroacetone afforded the pyrimidine derivative **14** with a trifluoroalkylated C-6 side chain (Scheme 1).

A fluorophenylalkyl side chain at C-6 of the pyrimidine moiety was introduced by lithiation and subsequent nucleophilic addition of pyrimidine derivative **3** and **3a** to 4-fluoroacetophenone, 2-fluorophenylacetone, and 4,4′-difluorbenzophenone to give **17**, **17a**, **19**, and **22**, respectively (Scheme 2). Reaction of lithiatied intermediate **3** with ethyl 4-fluorobenzoate afforded the C-6 substituted pyrimidine derivative containing unsaturated side chain, which exists as both the keto (**21K**) and enol (**21E**) tautomer (Scheme 2, Supporting Information). In the reaction with acetyl chloride, compound **17** yielded unsaturated 2,4-dimethoxypyrimidine **18** as a product of the elimination reaction, while compound **19** gave acetylated pyrimidine-2,4-dione derivative **20** (Scheme 2).

NMR Assignment and Conformational Studies. The chemical identity of **4**−**22** has been confirmed by ^{1}H, ^{19}F, and ^{13}C NMR measurements. Respective chemical shifts are reported in Table 1 and the Supporting Information. The analysis of the ^{1}H NMR spectra of all compounds was relatively simple because they contain a small number of signals. H-1′ chemical shifts in **7, 8**, **10**, **11**, **12**, **14**, **17**, **17a**, **19**, **20**, **21K**, and **22** were observed from 2.59 to 4.40 ppm, which correspond to C1′−C2′ single-bond derivatives. In contrast, analogs with a C1′═C2′ double bond (**15**, **16**, **18**, and **21E**) exhibit H-1′ chemical shifts between 6.09 and 6.59 ppm. H-5 resonances exhibit distinct chemical shift ranges for uracil (δ 5.26−5.61) and its dimethoxy derivatives (δ 6.21−6.61, Table 1). ^{19}F NMR resonances were well resolved (Supporting Information). ^{1}H decoupled ^{13}C NMR spectra showed C−F coupling constants that enabled straightforward identification of fluorinated carbon atoms and their neighbors.

Conformational studies of **8** and **19** were carried out as a function of temperature. ^{1}H NMR spectra were collected at −90, −50, and 20 °C and showed no significant temperature changes.

Conformational properties of **11**, **12**, **18**, and **19** were assessed with the use of 1D difference NOE enhancements. The saturation of H-1′a (δ 2.82) and H-1′b (δ 3.00) protons in **11** resulted in different NOE enhancements at H-5 (Figure 2). Furthermore, the saturation of H-1′a also showed higher NOE enhancement at the C2′-Me group than saturation of H-1′b. These NOE enhancements suggest a predominant conformation in which H-1′a is spatially closer to the C2′-Me group and H-1′b is closer to the H-5 proton. Restricted rotation across the C6−C1′ bond is also confirmed by the fact that H-1′ protons are not isochronous. This conformer is probably stabilized by the N1···HO hydrogen bond (Figure 2).[22] The saturation of H-1′a (δ 2.64) and H-1′b (δ 2.83) in **12** resulted in NOE enhancements of 5.4% and 4.1% at the H-5 proton, respectively. H-1′a also showed stronger NOE of 1.4% at the C2′-Me group, while there was no NOE between H-1′b and C2′-Me. In **12**, H-1′a is therefore spatially closer to both H-5 and C2′-Me protons in comparison to H-1′b. NOE enhancement of 8.8% at H-1′ was observed upon saturation of H-5 in **18**. Furthermore, the saturation of the H-1′ proton showed 8.2% NOE enhancement at H-5 protons. These strong NOE enhancements clearly indicate the trans geometry along the C1′═C2′ double bond. **18** adopts a conformation where H-5 and H-1′ protons are spatially close.

The hydroxyl proton of **15** was found at δ 13.01 ppm, which offers a clear indication of a relatively strong hydrogen bond. As a result, C2′ is strongly deshielded (δ 171.40). Immediately after **15** was dissolved in DMSO-d_6, the predominant set of signals (>96%) was observed (Table 1 and Supporting Information). After ca. 1 day the second form (∼30%) with sharp NMR signals appeared, which has been assigned to dihydroxy form **15H** (Figure 3, Supporting Information).

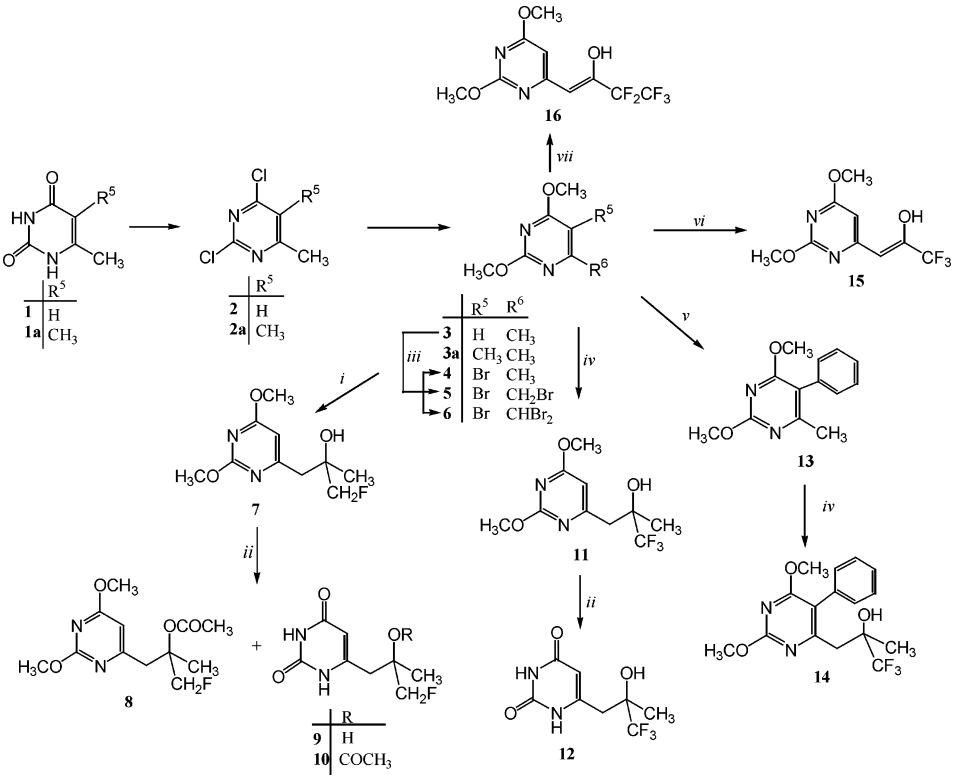
Biological Results

Antiviral Activities. The compounds **4**−**22** were evaluated for their inhibitory activities against cytomegalovirus (CMV[a] AD-169 and Davis strain) in human embryonic lung (HEL) cells; varicella-zoster virus (TK$^+$VZV, thymidine kinase positive and TK$^-$VZV, thymidine kinase deficient strains), parainfluenza virus-3, reovirus-1, Sindbis, Punta Toro virus in Vero cell cultures; and Coxsackie B4 virus in HeLa cell cultures (Tables 2 and 3). Their activities were compared with those of acyclovir, ganciclovir, cidofovir, brivudin, (*S*)-DHPA, and ribavirin (Tables 2 and 3).

5-Bromo-6-dibromomethyl-substituted pyrimidine derivative **6** showed moderate inhibitory potential against TK$^+$VZV (EC_{50} = 8.8 μM) and TK$^-$VZV (EC_{50} = 10.5 μM). Besides, compound **8** containing a 2-fluoromethylpropyl side chain showed a slight but not highly specific activity against TK$^+$-VZV (EC_{50} = 8.1 μM) and TK$^-$VZV (EC_{50} = 7.4 μM). Deprotection of the 2,4-diketo and hydroxyl functionalities of **8** caused a loss of activity for **7**, **9**, and **10**. The compound containing the C-6 fluorophenylalkyl chain in C-5 methyl-

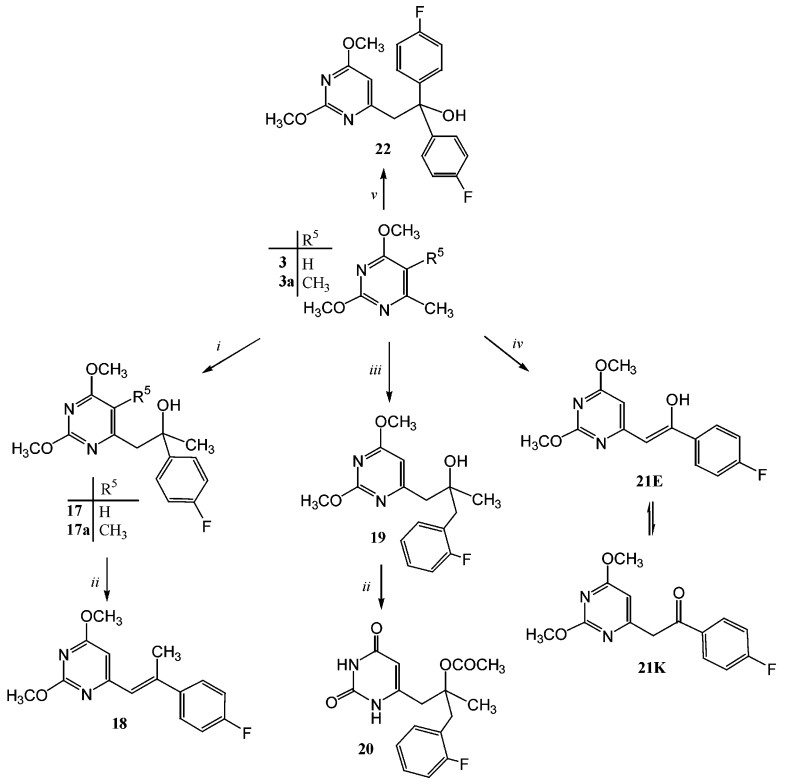
[a] Abbreviations: TK$^+$VZV, varicella-zoster virus (thymidine kinase positive); TK$^-$VZV, varicella-zoster virus (thymidine kinase deficient strains); CMV, cytomegalovirus.

Scheme 1[a]



[a] Reagents and conditions: (i) LDA, THF, fluoroacetone; (ii) acetyl chloride, H_2O; (iii) NBS, acetic acid; (iv) LDA, THF, 1,1,1-trifluoroacetone; (v) Bu_3SnPh, $(PPh_3)_2PdCl_2$, THF; (vi) LDA, THF, methyl trifluoroacetate; (vii) LDA, THF, ethyl pentafluoropropionate.

Scheme 2[a]



[a] Reagents and conditions: (i) LDA, THF, 4-fluoroacetophenone; (ii) acetyl chloride, H_2O; (iii) LDA, THF, 2-fluorophenylacetone; (iv) LDA, THF, ethyl 4-fluorobenzoate; (v) LDA, THF, 4,4′-difluorobenzophenone.

substituted pyrimidine **17a** had marginal activity against the CMV AD-169 strain (EC_{50} = 45 μM), CMV Davis strain (EC_{50} = 20 μM), TK^+VZV (EC_{50} = 80 μM), and TK^-VZV (EC_{50} = 45 μM). On the other hand, its C-5 unsubstituted pyrimidine congener **17** was virtually devoid of antiviral activity. Moreover, C-6 fluorophenylalkenyl pyrimidine **21** displayed moderate activity against Coxsackie B4 virus (EC_{50} = 24 μM) and CMV Davis strain (EC_{50} = 38 μM).

Table 1. ^{1}H NMR Chemical Shifts (δ/ppm) and H−H/H−F Coupling Constants (*J*/Hz)

compd	−CH_3	H-1	−OCH_3	H-5	Ph	OH
4	−	2.51 (s)	3.97 (s) 3.99 (s)	−	−	−
5	−	4.55 (s)	3.89 (s) 3.99 (s)	−	−	−
6	−	7.27 (s)	3.97 (s) 4.00 (s)	−	−	−
7[a]	1.08 (d) $^4J_{HF}$ = 2.0	2.72 (s)	3.86 (s) 3.87 (s)	6.45 (s)	−	5.05 (s)
8[b]	1.50 (d) $^4J_{HF}$ = 2.3	3.15 (dd) $^4J_{HF}$ = 1.6, ^2J = 13.4 3.18 (dd) $^4J_{HF}$ = 1.4, ^2J = 13.4	3.96 (s) 3.97 (s)	6.35 (s)	−	−
9[c]	1.04 (d) $^4J_{HF}$ = 1.3	(covered with H_2O)	−	5.28 (s)	−	−
10[d]	1.43 (d) $^4J_{HF}$ = 2.2	2.88 (d) $^2J_{HF}$ = 13.8 2.74 (d) $^2J_{HF}$= 13.9	−	5.31 (s)	−	−
11	1.29 (s)	2.82 (d) ^2J = 13.6 3.00 (d) ^2J = 13.6	3.97 (s) 3.98 (s)	6.47 (s)	−	−
12	1.37 (s)	2.64 (d) ^2J = 14.0 2.83 (d) ^2J = 14.0	−	5.61 (s)	−	−
13	−	2.14 (s)	3.80 (s) 3.91 (s)	−	7.55−7.63 (m)	−
14	1.28 (s)	2.70 (d) ^2J = 15.5 2.93 (d) ^2J = 15.5	3.94 (s) 4.03 (s)	−	7.17−7.44 (m)	−
15	−	6.09 (s)	3.95 (s) 4.00 (s)	6.52 (s)	−	13.01 (s)
15H[e]	−	2.99 (s)	3.88 (s) 3.89 (s)	6.61 (s)	−	7.25 (s)
16	−	6.47 (s)	4.01 (s) 4.07 (s)	6.32 (s)	−	−
17	1.53 (s)	3.06 (d) ^2J = 14.0 3.12 (d) ^2J = 14.0	3.89 (s) 3.90 (s)	6.21 (s)	H3: 6.98 (m) H2: 7.44 (m)	−
17a[f]	1.57 (s)	3.29 (d) ^2J = 15.3 3.02 (d) ^2J = 15.3	3.91 (s) 3.94 (s)	−	H3: 6.89 (m) H2: 7.34 (m)	6.80 (s)
18	2.58 (d) $^7J_{HF}$ = 1.4	6.59 (m)	3.96 (s) 4.01 (s)	6.44 (s)	H3: 7.11 (m) H2: 7.57 (m)	−
19[g]	1.10 (s)	2.76 (d) ^2J = 13.7 2.82 (d) ^2J = 13.7	3.95 (s) 3.96 (s)	6.42 (s)	7.06−7.36 (m)	−
20[h]	1.02 (s)	2.95 (d) ^2J = 14.0 2.76 (d) ^2J = 13.9	−	5.26 (s)	7.14−7.35 (m)	−
21E	−	6.33 (s)	3.92 (s) 3.97 (s)	6.35 (s)	H3: 7.31 (m) H2: 7.88 (m)	14.31 (s)
21K	−	4.40 (s)	3.81 (s) 3.89 (s)	6.56 (s)	H3: 7.37 (m) H2: 8.10 (m)	−
22	−	3.60 (s)	3.85 (s) 3.88 (s)	6.34 (s)	H3: 6.98 (m) H2: 7.46 (m)	−

[a] Additional signals: 4.21 (CH_2F, d, $^2J_{HF}$ = 47.8 Hz), 4.22 (CH_2F, d, $^2J_{HF}$ = 47.8 Hz). [b] Additional signals: 1.98 ($COCH_3$, s), 4.63 (CH_2F, d, $^2J_{HF}$ = 47.3 Hz). [c] Additional signals: 4.09 (CH_2F, d, $^2J_{HF}$ =47.6 Hz), 4.10 (CH_2F, d, $^2J_{HF}$ = 47.7 Hz). [d] Additional signals: 2.00 ($COCH_3$, s), 4.56 (CH_2F, dd, $^2J_{HF}$ = 34.8 Hz, ^2J = 9.9 Hz), 4.72 (CH_2F, dd, $^2J_{HF}$ = 35.3 Hz, ^2J = 9.9 Hz), 10.75 and 11.04 (NH). [e] Initial ratio between **15**/**15H** was 25:1. It dropped to 7:3 after 1 day and to 1:1 after 4 days. [f] Additional signal: 1.97 (CH_3-5, s) [g] Additional signals: 2.85 (H-3′, dd, $^4J_{HF}$ = 1.3 Hz, ^2J = 13.7 Hz), 2.91 (H-3′, dd, $^4J_{HF}$ = 1.3 Hz, ^2J = 13.7 Hz). [h] Additional signals: 1.91 ($COCH_3$, s), 3.17 (H-3′, d, ^2J = 13.9 Hz), 3.24 (H-3′, d, ^2J = 14.1 Hz); 10.73 and 11.02 (NH).

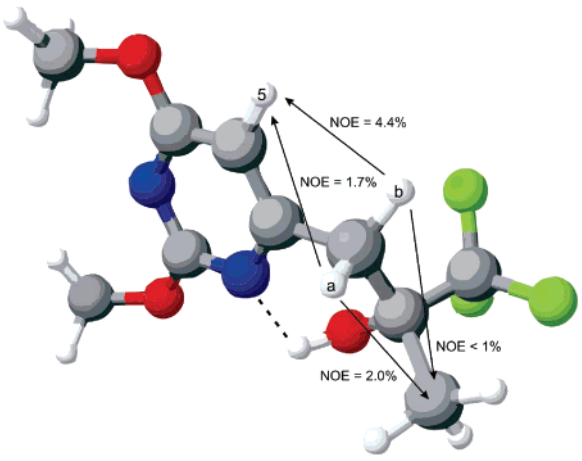


Figure 2. The predominant conformation of **11** in solution as established by 1D NOE experiments. The key NOE enhancements are shown. The proposed conformation is probably stabilized through the formation of the N1···HO hydrogen bond.

Results of cytotoxicities showed that introduction of bromine at the C-5 and/or C-6 position of pyrimidine in parent

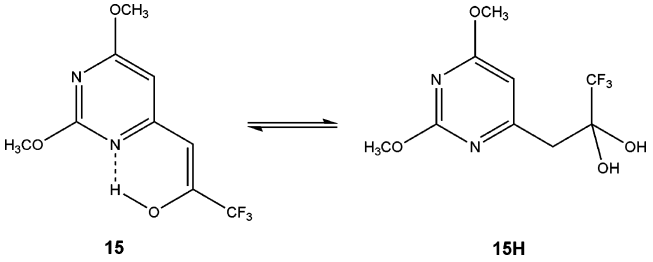


Figure 3. Hydration equilibrium of **15**. The predominant set of signals that corresponded to **15** (structure on the left) was observed immediately after it was dissolved in DMSO-d_6. After ca. 1 day at room temperature, the second form appeared, which has been assigned to dihydroxy form **15H**. After 4 days the ratio of **15**:**15H** was 1:1.

compounds **4**−**6** (CC_{50} = 1.7−38.7 μM) increased their cytotoxicity. Replacement of 5-bromine in **4** (CC_{50} = 18 μM) with 5-phenyl moiety in **13** and **14** resulted in somewhat reduced cytotoxicity (CC_{50} ∼ 35 μM). Comparison of cytotoxicity and antiviral potency of targeted fluoroalkylated and fluorophenylalkylated derivatives showed that fluorophenylalkylated derivatives **17a** and **21** possessed moderate antiviral activity and no cytotoxic effect (CC_{50} > 100 μM).

Table 2. Antiviral Activity of Compounds **4**−**22** against CMV and VZV in Human Embryonic Lung (HEL) Cells

	antiviral activity EC_{50} (μM)[a]				cytotoxicity (μM)	
	CMV					
compd	AD-169 strain	Davis strain	TK^+ VZV OKA strain	TK^- VZV 07/1 strain	cell morphology (MCC)[b]	cell growth (CC_{50})[c]
4	>100	>100	>100	88	≥100	18
5	>100	>100	>100	>100	>100	38.7
6	>20	>20	8.8	10.5	100	1.7
7	>100	>100	>100	>100	>100	>100
8	>14.7	>14.7	8.1	7.4	73.5	50
9	>100	>100	>100	>100	>100	>100
10	>100	>100	>100	>100	>100	>100
11	>100	>100	>100	>100	>100	>100
12	>100	>100	>100	>100	>100	>100
13	>20	>20	>20	>20	100	34
14	>0.8	>0.8	>20	>20	4	35
15	>80	>100	>100	>100	>100	>100
16	>100	>100	>100	>100	>100	≥60
17	>100	59	>100	>100	>100	>100
17a	45	20	80	45	>100	>100
18	>20	>20	>20	>20	100	88
19	>100	>100	>100	>100	≥100	>100
20	>100	>100	>100	>100	>100	>100
21	>100	38	>100	>20	>100	>100
22	>100	>100	>100	>100	>100	>100
acyclovir	−	−	1.0	32	>1778	649
ganciclovir	4.8	1.3	−	−	>1575	172
cidofovir	0.41	0.41	−	−	≥1270	51
brivudin	−	−	0.014	168	≥1201	408

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque-forming units (PFU). [b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of normal cell morphology at day 7 after addition of the compounds. [c] Cytostatic concentration required to inhibit cell proliferation by 50% at day 3 after addition of the compounds.

Table 3. Antiviral Activity of Compounds **4**−**22** against Parainfluenza-3 Virus, Reovirus-1, Sindbis Virus, and Punta Toro Virus in Vero Cell Cultures and Coxsackie B4 Virus in HeLa Cells

	antiviral activity EC_{50} (μM)[a]				
compd	Parainfluenza-3 virus	Reovirus-1	Sindbis virus	Punta Toro virus	Coxsackie B4 virus
4	>200	>200	>200	>200	>200
5	>8	>8	>8	>8	>40
6	40	>40	40	>40	>40
7	>200	>200	>200	>200	>200
8	>29	>29	>29	>29	>147
9	>196	>196	>196	>196 (588)	>200
10	>200	>200	>200	>200	>200
11	>200	>200	>200	>200	>200
12	>200	>200	>200	>200	>200
13	>40	>40	>40	40	>40
14	>40	>40	>40	>40	>40
15	>100	>100	>100	>100	>200
16	>100	>100	>100	>100	>100
17	120	200	>200	120	40
17a	>200	>200	>200	>200	>40
18	>40	>40	>40	40	>40
19	>130	>130	>130	>130	>200
20	>124	>124	>124	>124	>200
21	>200	>200	200	>200	24
22	>40	>40	>40	>40	>40
brivudin	>250	>250	>250	>250	>250
(*S*)-DHPA	>250	>250	>250	>250	>250
ribavirin	150	250	50	150	150

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 PFU.

In addition, none of the compounds described in this study were active against herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), vaccinia virus, vesicular stomatitis virus, or respiratory syncytial virus (data not shown).

Cytostatic Activities. The compounds **4**−**22** were evaluated for their activities against human malignant tumor cell lines: laryngeal carcinoma (Hep-2), cervical carcinoma (HeLa), pancreatic carcinoma (MiaPaCa-2), colon carcinoma (SW 620), breast carcinoma (MCF-7), murine leukemia (L1210), and human T-lymphocytes (Molt4/C8 and CEM) (Table 4). The 6-dibromomethyl-substituted pyrimidine derivative **6** showed pronounced cytotoxic activity, particularly against colon carcinoma (SW 620; IC_{50} = 0.4 μM). Its 6-bromomethyl pyrimidine derivative **5** displayed somewhat lower inhibitory activity than **6**, while 6-methyl-2,4-dimethoxy-5-bromopyrimidine (**4**) showed no activity whatsoever. In the series of fluoroalkyl pyrimidines, compounds **8** and **14** containing 2-fluoromethylpropyl and trifluoromethylpropyl chain, respectively, displayed reasonable cytostatic activities. On the contrary, compounds structurally related to **8**, with a deprotected hydroxyl group (**7**), 2,4-diketo groups (**10**), and both hydroxyl and 2,4-diketo groups (**9**) showed a lack of cytostatic activity. Interestingly, comparison of the cytostatic effect of the 6-(2-trifluoromethyl-2-hydroxypropyl)-2,4-dimethoxypyrimidine (**11**) with that of its phenyl-substituted analogue **14** indicated that introduction of the phenyl substitutent in the pyrimidine ring strongly enhanced the cytostatic activity of **14**. Besides, compound **16** with a multiple fluoro-substituted butenyl chain displayed some inhibitory activity, particularly against T-lymphocytes (CEM; IC_{50} = 9.7 μM).

Among the C-6 fluorophenylalkyl pyrimidine derivatives, the 2-fluorophenylpropenyl pyrimidine **18** showed cytostatic activity against Hep-2 (IC_{50} = 20 μM), HeLa (IC_{50} = 17 μM), and MCF-7 (IC_{50} = 16 μM) cells. Replacement of methyl with hydroxyl group in **21** abolished activity toward all tumor cells.

Finally, the activity of the most active pyrimidine derivatives was also evaluated on the normal human fibroblast cell line (WI 38) to determine potential selectivity with regard to nontumor cells. These results clearly demonstrated that the compounds **5**, **14**, **17a**, and **18** are less toxic to normal than tumor cells (Figure 4). Compound **5** has no influence on WI 38 fibroblasts up to 10^{-5} M concentration, while being strongly cytotoxic to all other tumor cell types. The influence of compounds **14** and **17a** is still more pronounced on tumor than on normal cells, although it is evident mostly at the highest tested concentration (10^{-4} M). Comparable growth-inhibitory differences are also seen with compound **18**, except on MiaPaCa-2 cell line.

Conclusions

The novel type of nonconventional C-6 fluoroalkylated (**7**−**12**, **14**−**16**) and fluorophenylalkylated (**17**−**22**) pyrimidine nucleoside mimetics as model compounds for development of tracer molecules in positron-emission tomography (PET) were synthesized. Compounds **4**−**22** were evaluated for their antiviral and cytostatic activities. Among the all tested compounds, the 5-bromo-6-dibromomethyl-substituted pyrimidine derivative **6** exhibited the most prominent cytotoxic activity against the malignant tumor cells tested. In general, fluoroalkyl pyrimidine derivatives showed more pronounced cytostatic effects than the fluorophenyl pyrimidine series. Thus, fluoroalkylated compounds **8** and **14** showed appreciable inhibitory activity against all tested tumor cell lines. The lack of activities of analogues **7**, **9**, and **10** with deprotected functional groups might be explained by their lower lipophilicity and cell penetration. 5-Bromo-6-dibromomethyl-substituted pyrimidine **6** was 3-fold more potent against TK^-VZV than acyclovir and 16-fold more potent than brivudin. Besides, compound **8** was 16-fold more active against TK^-VZV than acyclovir and 84-fold more active than brivudin and 2-fold less active than acyclovir against TK^+-

Table 4. Inhibitory Effects of Compounds **4**−**22** on the Growth of Malignant Tumor Cell Lines

	IC_{50} (μM)[a]							
compd	Hep-2	HeLa	MiaPaCa-2	SW 620	MCF-7	L1210	Molt4/C8	CEM
4	>100	>100	>100	>100	>100	>200	>200	>200
5	7 ± 4	18 ± 6	2 ± 1	1 ± 1	8 ± 5	38 ± 36	97 ± 81	28 ± 28
6	2 ± 0.3	1 ± 0.1	2 ± 0.2	0.4 ± 0.09	6 ± 2	8.8 ± 0.2	8.5 ± 0.4	7.1 ± 1.4
7	>200	>200	>200	>200	>200	>200	>200	>200
8	17.7 ± 1.6	11.7 ± 0.8	39 ± 9	30 ± 19	18 ± 2	21.7 ± 4.0	47.8 ± 0	20.6 ± 15
9	>200	>200	>200	>200	>200	>200	>200	>200
10	>200	>200	>200	>200	>200	>200	>200	>200
11	>200	>200	>200	>200	>200	>200	>200	>200
12	>100	>100	>100	>100	>100	>500	>500	>500
13	>100	90 ± 4	>100	>100	>100	77 ± 23	70 ± 20	43 ± 11
14	19 ± 4	17 ± 2	20 ± 10	20 ± 1	17 ± 10	82 ± 53	118 ± 26	70 ± 2
15	>200	>200	>200	>200	>200	>200	80 ± 4	>200
16	55.4 ± 8	26.3 ± 10.8	≥100	45 ± 17	76 ± 20	80 ± 3	43 ± 7	9.7 ± 7
17	>100	>100	>100	>100	>100	>200	>200	>200
17a	43 ± 17	35 ± 10	65 ± 36	≥100	38 ± 30	195 ± 18	144 ± 11	136 ± 19
18	20 ± 13	17 ± 1	>100	56 ± 27	16 ± 0.1	>200	>200	74 ± 15
19	>200	>200	>200	>200	>200	>200	79 ± 9	156 ± 34
20	>200	>200	>200	>200	>200	>200	>200	>200
21	>100	>100	>100	≥100	≥100	>200	>200	192 ± 6
22	>100	>100	>100	>100	>100	≥500	>500	>500

[a] 50% inhibitory concentration, or compound concentration required to inhibit cell proliferation by 50%.

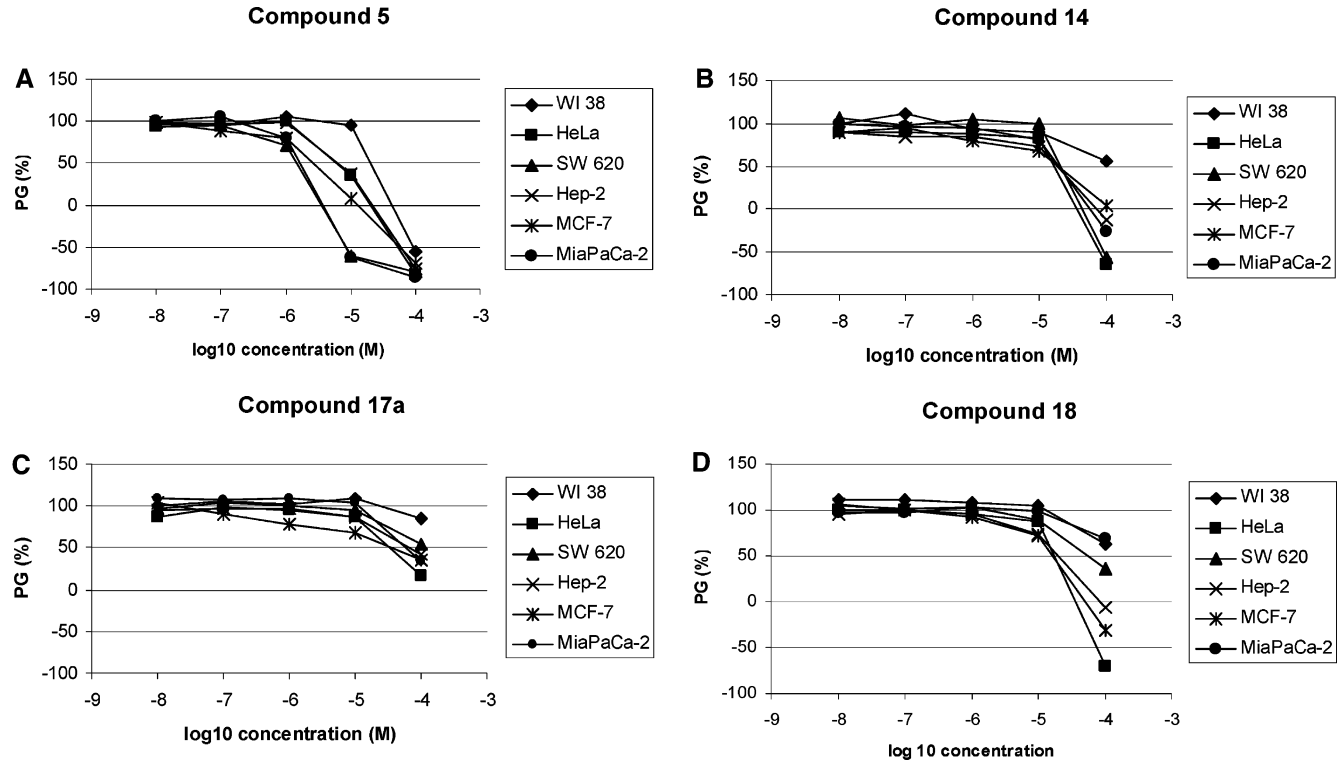


Figure 4. Dose−response profiles for compounds **5**, **14**, **17a**, and **18** tested on human tumor cell lines and normal fibroblasts (WI 38) in vitro.

VZV. Unlike C-5-unsubstituted alkylated pyrimidine derivatives **14** and **17**, their C-5 methyl-substituted congener **17a** showed some activity against CMV and VZV. C-6 fluorophenylalkenyl pyrimidine **21** was 6-fold more potent against Coxsackie B4 virus than ribavirin. Furthermore, fluorophenylalkylated pyrimidine derivatives **17a** and **21** showed no cytotoxic effect. In conclusion, from the fluoroalkylated pyrimidine series, compound **8** emerged as the most interesting leading compound with cytostatic and antiviral activities that could be used for further structural optimization.

Experimental Section

General Methods. Melting points (uncorrected) were determined with a Kofler micro hot-stage (Reichert, Wien). Precoated Merck silica gel 60F-254 plates were used for thin-layer chromatography (TLC) and the spots were detected under UV light (254 nm). Column chromatography (CLC) was performed using silica gel (0.063−0.2 mm, Kemika); the glass column was slurry-packed under gravity. Solvent systems used for the TLC and CLC were as follows: pethroleum ether:ethyl acetate = 5:1, pethroleum ether: ethyl acetate = 3:1, dichloromethane:methanol = 20:1, and dichloromethane:methanol = 5:1. The electron impact mass spectra were recorded with an EXTREL FT MS 2001 instrument with ionizing energy of 70 eV. Elemental analyses were performed in the Central Analytic Service, Rudjer Bošković Institute, Zagreb, Croatia. ^{1}H, ^{13}C, and ^{19}F NMR spectra were collected on a Varian Unity Inova 300 MHz NMR spectrometer equipped with 5 mm $^{1}H\{^{15}N-^{31}P\}$ indirect-detection probe with gradients. All data were recorded at 25 °C unless specified otherwise. NMR samples of all compounds were prepared in deuterated DMSO-d_6, except for **8**, **11**, **12**, **16**, **17**, **18**, **19**, and **22**, which were dissolved in CD_3OD.

Fluorine chemical shifts were externally referenced relative to CCl_3F (δ 0.0 ppm). Individual resonances were assigned on the basis of their chemical shifts; signal intensities; multiplicity of resonances; and H−H, C−H, and H−F coupling constants involved, as well as with the use of a series of 2D experiments (gHSQC and gHMBC).

6-Methyl-2,4-dimethoxy-5-bromopyrimidine (4), 6-Bromomethyl-2,4-dimethoxy-5-bromopyrimidine (5), and 6-Dibromomethyl-2,4-dimethoxy-5-bromopyrimidine (6). A mixture of compound **3** (5.0 g, 21.45 mmol) and acetic acid (115.5 mL) was refluxed for 20 min and *N*-bromosuccinimide was then added (11.75 g, 66.01 mmol). The reaction mixture was refluxed for an additional 3 h and then stirred overnight at room temperature. Acetic acid was evaporated at reduced pressure and the residue was dissolved in CH_2Cl_2 and then washed with $NaHCO_3$ and $Na_2S_2O_3$ solution. The organic layer was dried over $MgSO_4$ and the solvent was evaporated. After column chromatography with CH_2Cl_2 as eluent, compounds **4** (450 mg, 29.9%), **5** (100 mg, 4.8%), and **6** (470 mg, 18.3%) were isolated. **4**: mp = 69−70 °C; MS *m*/*z* 232, 234 [M^+, M + 2]. Anal. ($C_7H_9N_2O_2Br$) C, H, N. **5**: mp = 77−82 °C; MS *m*/*z* 299, 301, 303 [M − 2, M^+, M + 2]. Anal. ($C_7H_8N_2O_2$ Br_2) C, H, N. **6**: mp = 79−83 °C; MS *m*/*z* 386, 388, 390, 392 [M − 2, M^+, M + 2, M + 4]. Anal. ($C_7H_7N_2O_2Br_3$) C, H, N.

6-(2-Fluoromethyl-2-hydroxypropyl)-2,4-dimethoxypyrimidine (7). The solution of 2,4-dimethoxy-6-methylpyrimidine (**3**) (502 mg, 3.26 mmol) in THF (10 mL) was cooled at −70 °C and lithium diisopropylamide (2.4 mL, 2 M in THF/heptane/ethylbenzene) was added dropwise to the reaction mixture. The temperature was then raised to −55 °C and the reaction mixture was stirred for 30 min. Fluoroacetone (0.22 mL, 3.91 mmol) was added and the mixture was additionally stirred for 3 h and then neutralized with glacial acetic acid. The temperature was raised to room temperature and the reaction mixture was stirred further for 15 min. The solvent was evaporated and the residual yellow oily product was extracted with CH_2Cl_2 and water. The organic layer was dried over Na_2SO_4 and purified by silica gel column chromatography using pethroleum ether:ethyl acetate = 5:1 as eluent. After column chromatography, compound **7** (357 mg, 47.6%) was isolated as yellow oil. **7**: MS *m*/*z* 231 $[MH]^+$. Anal. ($C_{10}H_{15}N_2O_3F$) C, H, N.

6-(2-Fluoromethyl-2-acetoxypropyl)-2,4-dimethoxypyrimidine (8), 6-(2-Fluoromethyl-2-hydroxypropyl)-2,4-dihydroxypyrimidine (9), and 6-(2-Fluoromethyl-2-acetoxypropyl)-2,4-dihydroxypyrimidine (10). The compound **7** (197 mg, 0.85 mmol) was dissolved in acetyl chloride (5 mL). The reaction mixture was refluxed for 5 h. Water (1.5 mL) was then added and the reaction mixture was stirred overnight at room temperature. The solvent was evaporated at the reduced pressure and the remaining yellow oil was chromatographed on silica gel column using dichloromethane:methanol = 20:1 as eluent. Compounds **8** (93 mg, 40.3%), **9** (35 mg, 20.4%), and **10** (50 mg, 24.1%) were isolated. **8**: MS *m*/*z* 273 $[MH]^+$. Anal. ($C_{12}H_{17}N_2O_4F$) C, H, N. **9**: mp = 198−201 °C; MS *m*/*z* 201 [M − H]. Anal. ($C_8H_{11}N_2O_3F$) C, H, N. **10**: mp = 187−190 °C; MS *m*/*z* 245 $[MH]^+$. Anal. ($C_{10}H_{13}N_2O_4F$) C, H, N.

6-(2-Trifluoromethyl-2-hydroxypropyl)-2,4-dimethoxypyrimidine (11). The synthesis of **11** was performed analogously to that of **7**, using compound **3** (790 mg, 5.12 mmol) dissolved in THF (11 mL), LDA (3.8 mL, 2 M in THF/heptane/ethylbenzene), and trifluoroacetone (0.58 mL, 6.14 mmol) as reagents. After column chromatography with pethroleum ether:ethyl acetate = 5:1 as eluent, compound **11** (432 mg, 31.7%) was isolated as a yellow oil. **11**: MS *m*/*z* 267 $[MH]^+$. Anal. ($C_{10}H_{13}N_2O_3F_3$) C, H, N.

6-(2-Trifluoromethyl-2-hydroxypropyl)-2,4-dihydroxypyrimidine (12). A procedure analogous to the deprotection of **7** was used for the synthesis of **12**. Reagents used were **11** (307 mg, 1.15 mmol), acetyl chloride (5.5 mL), and water (2.5 mL). After column chromatography using dichloromethane:methanol = 20:1 as eluent, **12** (15.3 mg, 5.6%) was obtained. **12**: mp = 206−209 °C; MS *m*/*z* 237 [M − H]. Anal. ($C_8H_9N_2O_3F_3$) C, H, N.

6-Methyl-2,4-dimethoxy-5-phenylpyrimidine (13). To the solution of compound **4** (1.03 g, 4.41 mmol) in dry THF (35 mL) were added Bu_3SnPh (11.06 mL, 33.90 mmol) and $PdCl_2(PPh_3)_2$ (0.33 g, 0.44 mmol). The reaction mixture was refluxed overnight and then the solvent was evaporated. The residue was chromatographed on silica gel column using CH_2Cl_2 as eluent and compound **13** was isolated as a solid (290 mg, 28.2%). **13**: mp = 70−75 °C; MS 231 *m*/*z* $[MH]^+$. Anal. ($C_{13}H_{14}N_2O_2$) C, H, N.

6-(2-Trifluoromethyl-2-hydroxypropyl)-2,4-dimethoxy-5-phenylpyrimidine (14). The synthesis of **14** was the same as described for **7**. Reagents used were **13** (0.19 g, 0.82 mmol), THF (8 mL), LDA (0.63 mL, 2 M in THF/heptane/ethylbenzene), and trifluoroacetone (0.09 mL, 0.99 mmol). After column chromatography with dichloromethane:methanol = 5:1 as eluent, oily compound **14** was isolated (40 mg, 12.5%). **14**: MS 343 *m*/*z* $[MH]^+$. Anal. ($C_{16}H_{17}N_2O_3F_3$) C, H, N.

6-(2-Hydroxy-3,3,3-trifluoro-1-propenyl)-2,4-dimethoxypyrimidine (15). The synthesis of **15** was performed by a procedure analogous to that of **7** using compound **3** (1.090 g, 7.08 mmol) dissolved in THF (15 mL), LDA (5.24 mL, 2 M in THF/heptane/ethylbenzene), and methyl trifluoroacetate (0.85 mL, 8.49 mmol). After column chromatography with pethroleum ether:ethyl acetate = 5:1 as eluent, compound **15** was isolated as a colorless oil (751 mg, 42.4%). **15** and **15H**: mp = 70−72 °C; MS *m*/*z* 251 $[MH]^+$. Anal. ($C_9H_9N_2O_3F_3$) C, H, N.

6-(2-Hydroxy-4,4,4-trifluoro-3,3-difluoro-1-butenyl)-2,4-dimethoxypyrimidine (16). In the same manner as described for **7**, the synthesis of **16** was performed with **3** (737 mg, 4.78 mmol), LDA (3.54 mL, 2 M in THF/heptane/ethylbenzene), THF (12 mL), and ethyl pentafluoropropionate (0.85 mL, 5.74 mmol). After column chromatography using pethroleum ether:ethyl acetate = 3:1 as eluent, compound **16** (960 mg, 67.0%) was isolated as a yellow solid oil. **16**: mp = 57−60 °C; MS *m*/*z* 301 $[MH]^+$. Anal. ($C_{10}H_9N_2O_3F_5$) C, H, N.

6-[2-(4′-Fluorophenyl)-2-hydroxypropyl]-2,4-dimethoxypyrimidine (17). The procedure was the same as for compound **7**. Reagents used were compound **3** (606 mg, 3.9 mmol), LDA (2.91 mL, 2 M in THF/heptane/ethylbenzene), THF (10 mL), and 4-fluoroacetophenone (0.57 mL, 4.7 mmol). After column chromatography with pethroleum ether:ethyl acetate = 5:1 as eluent, compound **17** (659 mg, 57.8%) was isolated as a yellow oil. **17**: MS *m*/*z* 291 [M − H]. Anal. ($C_{15}H_{17}N_2O_3F$) C, H, N.

6-[2-(4′-Fluorophenyl)-2-hydroxypropyl]-2,4-dimethoxy-5-methylpyrimidine (17a). The procedure was the same as for compound **7**. Reagents used were compound **3a** (610 mg, 3.70 mmol), LDA (2.71 mL, 2 M in THF/heptane/ethylbenzene), THF (10 mL), and 4-fluoroacetophenone (0.54 mL, 4.44 mmol). After column chromatography with pethroleum ether:ethyl acetate = 5:1 as eluent, compound **17a** (240 mg, 19.4%) was isolated as a yellow oil. **17a**: MS *m*/*z* 305 [M − H]. Anal. ($C_{16}H_{19}N_2O_3F$) C, H, N.

6-[2-(4′-Fluorophenyl)-1-propenyl]-2,4-dimethoxypyrimidine (18). Compound **17** (320 mg, 1.09 mmol) was dissolved in acetyl chloride (5 mL). The reaction mixture was refluxed for 5 h. Water (1.5 mL) was added and the reaction mixture was stirred overnight at room temperature. The solvent was evaporated at reduced pressure and the remaining yellow oil was chromatographed on silica gel column using dichloromethane:methanol = 20:1 as eluent and **18** (63 mg, 21.1%) was isolated as a white solid. **18**: mp = 55−60 °C; MS *m*/*z* 275 $[MH]^+$. Anal. ($C_{15}H_{15}N_2O_2F$) C, H, N.

6-[2-(2′-Fluorobenzyl)]-2-hydroxypropyl-2,4-dimethoxypyrimidine (19). A procedure analogous to that of **7** was used for the synthesis of **19**. Reagents used were **3** (968 mg, 6.28 mmol), LDA (4.65 mL, 2 M in THF/heptane/ethylbenzene), 2-fluorophenylacetone (1.05 mL, 7.54 mmol), and THF (15 mL). After column chromatography using pethroleum ether:ethyl acetate = 3:1 as eluent, compound **19** (743 mg, 37.3%) was isolated as a colorless oil. **19**: MS *m*/*z* 305 [M − H]. Anal. ($C_{16}H_{19}N_2O_3F$) C, H, N.

6-[2-(2′-Fluorobenzyl)-2-acetoxypropyl]-2,4-dihydroxypyrimidine (20). A procedure analogous to deprotection of **7** was used for the synthesis of **20**. Reagents used were **19** (353 mg, 1.15 mmol), acetyl chloride (5.5 mL), and water (2.5 mL). After column chromatography using dichloromethane:methanol = 20:1 as eluent, **20** (237 mg, 64.3%) was obtained. **20**: mp = 149−152 °C; MS *m*/*z* 321 $[MH]^+$. Anal. ($C_{16}H_{17}N_2O_4F$) C, H, N.

6-[2-(4′-Fluorophenyl)-2-hydroxy-1-etenyl]-2,4-dimethoxypyrimidine (21). In the same manner as described for **7**, the synthesis of **21** was performed with **3** (613 mg, 4.0 mmol), LDA (2.9 mL, 2 M in THF/heptane/ethylbenzene), THF (10 mL), and ethyl 4-fluorobenzoate (0.7 mL, 4.8 mmol). After column chromatography using pethroleum ether:ethyl acetate = 5:1 as eluent, **21** (576 mg, 52.1%) was isolated. **21E** and **21K**: mp = 98−102 °C; MS *m*/*z* 277 $[MH]^+$. Anal. ($C_{14}H_{13}N_2O_3F$) C, H, N.

6-[2,2-(Di-4-fluorophenyl)-2-hydroxyethyl]-2,4-dimethoxypyrimidine (22). The synthesis of **22** was performed by a procedure analogous to that of **7** using compound **3** (607 mg, 3.9 mmol) dissolved in THF (10 mL), LDA (2.91 mL, 2 M in THF/heptane/ethylbenzene), and 4,4′-difluorobenzophenone (1.026 g, 4.7 mol). After column chromatography with pethroleum ether:ethyl acetate = 5:1 as eluent, compound **22** (564 mg, 38.8%) was isolated. **22**: mp = 150−154 °C; MS *m*/*z* 371 [M − H]. Anal. ($C_{20}H_{18}N_2O_3F_2$) C, H, N.

Antiviral Activity Assays. Antiviral activity against HCMV, VZV, parainfluenza-3 virus, reovirus-1, Sindbis virus, Punta Toro virus, Coxsackie B4 virus, HSV-1, HSV-2, vaccinia virus, vesicular stomatitis virus, and respiratory syncytial virus was determined essentially as described previously.[28,29] Confluent human embryonic lung (HEL) fibroblasts were grown in 96-well microtiter plates and infected with the human cytomegalovirus (HCMV) strains Davis and AD-169 at 100 PFU per well. After a 2-h incubation period, residual virus was removed, and the infected cells were further incubated with the medium containing different concentrations of the compounds tested (in duplicate). After incubation for 7 days at 37 °C, virus-induced cytopathogenicity was monitored microscopically and after ethanol fixation and staining with Giemsa (for HCMV and VZV). Antiviral activity was expressed as the EC_{50} or concentration required to reduce virus-induced cytopathogenicy by 50%. EC_{50} values were calculated from graphic plots of the percentage of cytopathogenicity as a function of the concentration of the compounds. Cytostatic measurements based on the inhibition of HEL cell growth were performed as follows: HEL cells were seeded at a rate of 5×10^3 cells/well into 96-well microtiter plates and allowed to proliferate for 24 h. Then, medium containing different concentrations of the test compounds was added. After 3 days of incubation at 37 °C, the cell number was determined with a Coulter counter. The cytostatic concentration was calculated as the CC_{50}, or the compound concentration required to reduce cell growth by 50% relative to the number of cells in the untreated controls. CC_{50} values were estimated from graphic plots of the number of cells (percentage of control) as a function of the concentration of the test compounds. Cytotoxicity was expressed as minimum cytotoxic concentration (MCC) or the compound concentration that causes a microscopically detectable alteration of cell morphology of the confluent cell cultures that were exposed to the compounds.

Cytostatic Assays. Cytostatic activity against L1210 (murine leukemia), Molt4/C8, and CEM (human T-lymphocytes) cell lines was measured essentially as originally described for the mouse leukemia (L1210) cell line.[30] The Hep-2 (laryngeal carcinoma), HeLa (cervical carcinoma), MiaPaCa-2 (pancreatic carcinoma), SW 620 (colon carcinoma), MCF-7 (breast carcinoma) cells were cultured as monolayers and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C.

The Hep-2, HeLa, MiaPaCa-2, SW-620, and MCF-7 were seeded into a series of standard 96-well microtiter plates on day 0. Test agents were then added in five 10-fold dilutions (10^{-8}−10^{-4} M) and incubated for a further 72 h. Working dilutions were freshly prepared on the day of testing. The solvent was also tested for eventual inhibitory activity by adjusting its concentration to be the same as working concentrations. After 72 h of incubation, the cell growth rate was evaluated by MTT assay, as described previously.[22,31]

Each test point was performed in quadruplicate in three individual experiments. The results are expressed as IC_{50}, a concentration necessary for 50% of inhibition. Each result is a mean value from three separate experiments. The IC_{50} values for each compound were calculated from dose−response curves using linear regression analysis by fitting the test concentrations that gave PG (percentage of growth) values above and below the reference value (i.e., 50%).

Acknowledgment. Support for this study was provided by the Ministry of Science of the Republic of Croatia (Projects #125-0982464-2925, #125-0982464-2922, #098-0982464-2514 and #098-0982464-2393). We thank Lizette van Berckelaer for excellent technical assistance in performing (part of) the cytostatic cell activity assays, as well as Ann Absillis, Anita Van Lierde, Frieda De Meyer, Leentje Persoons, Anita Camps, and Lies Vandenheurck for excellent technical assistance in performing the antiviral activity assays.

Supporting Information Available: ^{19}F and ^{13}C NMR chemical shift values and elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Öğretir, C.; Yaman, M. AM1, PM3 and MNDO study of the tautomeric equilibria of 2-, 4- or 5-hydroxypyrimidine derivatives and their azo- and thio-analogs. *J. Mol. Struct. (Theochem)* **1999**, *458*, 217−226.
(2) Williams, M.; Kowaluk, E. A.; Arneric, S. P. Emerging molecular approaches to pain therapy. *J. Med. Chem.* **1999**, *42*, 1481−1500.
(3) Bradshaw, T. K.; Hutchinson, D. N. 5-Substituted pyrimidine nucleosides and nucleotides. *Chem. Soc. Rev.* **1977**, *6*, 43−62.
(4) Das, P.; Spears, C. P.; Shahinian, A. H.; Dasgupta, S. K.; Kundu, N. G. Palladium-catalysed synthesis of some biologically active 5, 6-disubstituted uracils. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2477−2480.
(5) Baba, M.; De Clercq, E.; Tanaka, H.; Ubasawa, M.; Takashima, H.; Sekiya, K.; Nitta, I.; Umezu, K.; Walter, R. T.; Mori, S. Highly potent and selective inhibition of human immunodeficiency virus type 1 by a novel series of 6-substituted acyclouridine derivatives. *Mol. Pharmacol.* **1991**, *39*, 805−810.
(6) Tanaka, H.; Takashima, H.; Ubasawa, M.; Sekiya, K.; Nitta, I.; Baba, M.; Shigeta, S.; Walker, R. T.; De Clercq, E.; Miyasaka, T. Synthesis and antiviral activity of deoxy analogs of 1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio)thymine (HEPT) as potent and selective anti-HIV-1 agents. *J. Med. Chem.* **1992**, *35*, 4713−4719.
(7) Balzarini, J.; Karlsson, A.; De Clercq, E. Human immunodeficiency virus type 1 drug-resistance patterns with different 1-[(2-hydroxy-ethoxy)methyl]-6-(phenylthio)thymine derivatives. *Mol. Pharmacol.* **1993**, *44*, 694−701.
(8) Baba, M.; De Clercq, E.; Tanaka, H.; Ubasawa, M.; Yuasa, S.; Walker, R. T.; Miyasaka, T. HEPT derivatives-6-benzyl-1-ethoxym-ethyl-5-isopropyluracil (MKC-442). *Nucleosides Nucleotides* **1995**, *14*, 575−583.
(9) Mai, A.; Artico, M.; Sbardella, G.; Quartarone, S.; Massa, S.; Loi, A. G.; De Montis, A.; Scintu, F.; Putzolu, M.; La Colla, P. Dihydro-(alkylthio)(naphthylmethyl)oxopyrimidines: Novel non-nucleoside reverse transcriptase inhibitors of the *S*-DABO series. *J. Med. Chem.* **1997**, *40*, 1447−1454.
(10) Botta, M.; Occhionero, F.; Nicoletti, R.; Mastromarino, P.; Conti, C.; Magrini, M.; Saladino, R. Synthesis and biological evaluation of 2-methoxy- and 2-methylthio-6-[(2′-alkylamino)ethyl]-4(3*H*)-pyrimidinones with anti-rubella virus activity. *Bioorg. Med. Chem.* **1999**, *7*, 1925−1931.
(11) Kulikowski, T. Structure−activity-relationships and conformational features of antiherpetic pyrimidine and purine nucleoside analogs—A review. *Pharm. World Sci.* **1994**, *16*, 127−138.
(12) Cheng, Y. C.; Grill, S. P.; Dutschman, G. E.; Nakayama, K.; Bastow, K. F. Metabolism of 9-(1,3-dihydroxy-2-propoxymethyl)guanine, a new anti-herpes virus compound, in herpes simplex virus-infected cells. *J. Biol. Chem.* **1983**, *258*, 12460−12464.

(13) Pospisil, P.; Pilger, B. D.; Schelling, P.; Wurth, C.; Scapozza, L.; Folkers, G.; Pongračić, M.; Mintas, M.; Raić-Malić, S. Synthesis, kinetics and molecular docking of novel 9-hydroxypropyl purine nucleoside analogues as ligands of herpesviral thymidine kinases. *Helv. Chim. Acta* **2002**, *85*, 3237−3250.
(14) Raić-Malić, S.; Johayem, A.; Ametamey, S.; Batinac, S.; De Clercq, E.; Folkers, G.; Scapozza, L. Synthesis, ^{18}F-Radiolabelling and biological evaluations of C-6 alkylated pyrimidine nucleoside analogues. *Nucleosides, Nucleotides Nucleic Acids* **2004**, *23*, 1707−1721.
(15) Johayem, A.; Raić-Malić, S.; Lazzati, K.; Schubiger, P. A.; Scapozza, L.; Ametamey, S. M. Synthesis and characterization of a C(6) nucleoside analogue for the in vivo imaging of the gene expression of herpes simplex virus type-1 thymidine kinase (HSV1 TK). *Chem. Biodiversity* **2006**, *3*, 274−283.
(16) Alauddin, M. M.; Conti, P. S. Synthesis and preliminary evaluation of 9-(4-[18F]-fluoro-3-hydroxymethylbutyl)guanine ([18F]FHBG): A new potential imaging agent for viral infection and gene therapy using PET. *Nucl. Med. Biol.* **1998**, *25*, 175−180.
(17) Alauddin, M. M.; Shahinian, A.; Gordon, E. M.; Bading, J. R.; Conti, P. S. Preclinical evaluation of the penciclovir analog 9-(4-[(18)F]-fluoro-3-hydroxymethylbutyl)guanine for in vivo measurement of suicide gene expression with PET. *J. Nucl. Med.* **2001**, *42*, 1682−1690.
(18) Eary, J. F.; Mankoff, D. A.; Spence, A. M.; Berger, M. S.; Olshen, A.; Link, J.; O'Sullivan, F.; Krohn, K. A. 2-[C-11]thymidine imaging of malignant brain tumors. *Cancer Res.* **1999**, *59*, 615−621.
(19) Mankoff, D. A.; Shields, A. F.; Link, J. M.; Graham, M. M.; Muzi, M.; Peterson, L. M.; Eary, J. F.; Krohn, K. A. Kinetic analysis of 2-[11C]thymidine PET imaging studies: Validation studies. *J. Nucl. Med.* **1999**, *40*, 614−624.
(20) van Eijkeren, M.; De Schrijver, A.; Goethals, P.; Poupeye, E.; Schelstraete, L.; Lemahieu, I.; De Potter, R. Measurement of short-term 11C-thymidine activity in human head and neck tumours using positron emission tomography (PET). *Acta Oncol.* **1993**, *31*, 539−543.
(21) Shields, A. F.; Lim, K.; Grierson, J.; Link, J.; Krohn, K. A. Utilization of labeled thymidine in DNA synthesis: Studies for PET. *J. Nucl. Med.* **1990**, *31*, 337−342.
(22) Prekupec, S.; Makuc, D.; Plavec, J.; Kraljević, S.; Kralj, M.; Pavelić, K.; Andrei, G.; Snoeck, R.; Balzarini, J.; De Clercq, E.; Raić-Malić, S.; Mintas, M. Antiviral and cytostatic evaluation of the novel 6-acyclic chain substituted thymine derivatives. *Antiviral Chem., Chemother.* **2005**, *16*, 327−338.
(23) Schlenker, J. Über 4,5-Dimethylpyrimidin. *Berichte* **1901**, *34*, 2812−2829.
(24) Middleton, W. J. New fluorinating reagents. Dialkylaminosulfur fluorides. *J. Org. Chem.* **1975**, *40*, 574−578.
(25) Agrofoglio, L. A.; Gillaizeau, I.; Saito, Y. Palladium-assisted routes to nucleosides. *Chem. Rev.* **2003**, *103*, 1875−1916.
(26) Stille, J. K. The palladium-catalyzed cross-coupling reactions of organotin reagents with organic electrophiles [new synthetic methods (58)]. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 508−524.
(27) Stille, J. K. Palladium catalyzed coupling of organotin reagents with organic electrophiles. *Pure Appl. Chem.* **1985**, *57*, 1771−1780.
(28) De Clercq, E.; Holý, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. A novel selective broad-spectrum anti-DNA virus agent. *Nature* **1986**, *323*, 464−467.
(29) Balzarini, J.; Naesens, L.; Slachmuylders, J.; Niphuis, H.; Rosenberg, I.; Holý, A.; Schellekens, H.; De Clercq, E. 9-(2-Phosphonylmethoxyethyl)adenine (PMEA) efficiently inhibits retrovirus replication in vitro and simian immunodeficiency virus infection in rhesus monkeys. *AIDS* **1991**, *5*, 21−28.
(30) De Clercq, E.; Balzarini, J.; Torrence, P. F.; Mertes, M. P.; Schmidt, C. L.; Shugar, D.; Barr, P. J.; Jones, A. S.; Verhelst, G.; Walker. R. T. Thymidylate synthetase as target enzyme for the inhibitory activity of 5-substituted 2′-deoxyuridines on mouse leukemia L1210 cell growth. *Mol. Pharmacol.* **1981**, *19*, 321−330.
(31) Opačić, N.; Barbarić, M.; Zorc, B.; Cetina, M.; Nagl, A.; Frković, D.; Kralj, M.; Pavelić, K.; Balzarini, J.; Andrei, G.; De Clercq, E.; Raić-Malić, S.; Mintas, M. The novel L- and D-amino acid derivatives of hydroxyurea and hydantoins: Synthesis, X-ray crystal structure study, and cytostatic and antiviral activity evaluations. *J. Med. Chem.* **2005**, *48*, 475−482.

JM0614329