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Novel Pyrimidine and Purine Derivatives of L-Ascorbic Acid: Synthesis and Biological Evaluation

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The novel pyrimidine derivatives **1**−**6** of 2,3-dibenzyl-4,5-didehydro-5,6-dideoxy-L-ascorbic acid were synthesized by the condensation of pyrimidine bases with 5,6-diacetyl-2,3-dibenzyl-L-ascorbic acid (DDA). Both N-9 (**7**) and N-7 (**8**) regioisomers were obtained in the reaction of 6-chloropurine with 5-acetyl-6-bromo-2,3-dibenzyl-L-ascorbic acid (ABDA), while the reaction of 6-(*N*-pyrrolyl)purine with ABDA afforded exclusively the N-9 isomer **9**. Structures of all newly prepared compounds were deduced from the chemical shifts in ^{1}H and ^{13}C NMR spectra, as well as connectivities in 2D homo- and heteronuclear correlation spectra. An unambiguous proof of the structure and conformation of **7** was obtained by X-ray crystallographic analysis. Compounds **1**−**9** were found to exert cytostatic activities against malignant cell lines: pancreatic carcinoma (MiaPaCa2), breast carcinoma (MCF7), cervical carcinoma (HeLa), laryngeal carcinoma (Hep2), murine leukemia (L1210/0), murine mammary carcinoma (FM3A), and human T-lymphocytes (Molt4/C8 and CEM/0), as well as antiviral activities against varicella-zoster virus (TK$^+$VZV and TK$^-$VZV) and cytomegalovirus (CMV). The compound **6** containing a trifluoromethyl-substituted uracil ring exhibited marked antitumor activity. The N-7-substituted purine regioisomer **8** had greater inhibitory effects on the murine L1210/0 and human CEM/0 cell lines than the N-9 isomer **7**. Compound **9** with the 6-purine-substituted pyrrolo moiety had a more pronounced selective cytostatic activity against human (Molt4/C8 and CEM/0) cell lines than murine (L1210/0 and FM3A/0) and human (MiaPaCa2, MCF7, HeLa, and Hep2) tumor cell lines and normal fibroblasts (Hef522). The compound **6** exhibited the most potent antiviral activities against TK$^+$VZV, TK$^-$VZV, and CMV, albeit at concentrations that were only slightly lower than the cytotoxic concentrations.

Introduction

L-Ascorbic acid and its derivatives have been of multifold biological and pharmacological interest.[1−4] Thus, some derivatives of L-ascorbic acid, e.g., 6-bromo-, 6-amino-, and *N,N*-dimethyl-6-amino-6-deoxy-L-ascorbic acid, have been found to inhibit the growth of certain human malignant tumor cells lines: cervical carcinoma (HeLa), laryngeal carcinoma (Hep2), and pancreatic carcinoma (MiaPaCa2).[5] Furthermore, several nucleosides containing a 5-substituted pyrimidine moiety have been shown to inhibit growth of the murine mammary carcinoma (FM3A TK$^-$/HSV 1 TK$^+$) cells transformed with the herpes simplex virus type 1 (HSV-1) TK gene.[6−9] Searching for compounds related to these classes of biologically and pharmacologically active molecules, we have prepared new types of molecules containing pyrimidine (**1**−**6**) or purine (**7**−**9**) moieties connected via an acyclic chain to 2,3-dibenzyl-4,5-didehydro-5,6-dideoxy-L-ascorbic acid (Figure 1). The

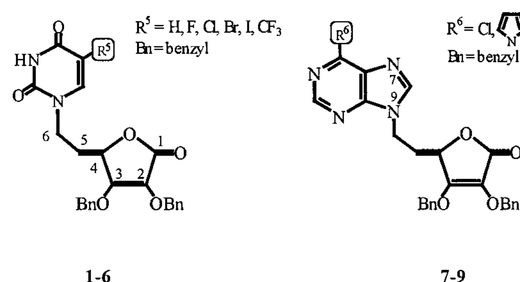

Figure 1. Pyrimidine (**1**−**6**) and purine (**7**−**9**) derivatives of 2,3-dibenzyl-4,5-didehydro-5,6-dideoxy-L-ascorbic acid.

synthesis, ^{1}H NMR, and X-ray crystal structure, as well as biological studies, are reported in this paper.

Chemistry

Compounds **1**−**6** were prepared by the general Vorbrüggen method[10,11] involving silylation of the uracil and its 5-substituted derivatives with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) in the presence of $(NH_4)_2SO_4$ and subsequent reaction of the intermediates thus obtained with 5,6-diacetyl-2,3-dibenzyl-L-ascorbic acid (DDA)[12,13] and trimethylsilyl triflate as Friedel−Crafts catalyst (Scheme 1).

The compounds **7**−**9** were obtained by an alternative route which involved direct condensation of 6-chloro-

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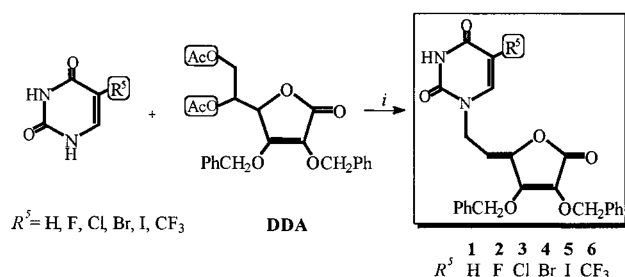
Table 1. Chemical Shifts (δ, ppm)[a] and H−H Coupling Constants (*J*, Hz)[b] in ^{1}H NMR Spectra for Compounds **1**−**9**

compd	H-6	H-2 H-8	NH	H-5′	H-6′	C_6H_5	OCH_2
1[c]	7.38−7.17 (1H)		9.17 (s, 1H)	5.44 (1H) *J* = 7.69 (t)	4.52 (2H) *J* = 7.69 (d)	7.38−7.17 (10H, m)	5.21 (s, 2H) 5.18 (s, 2H)
2	7.40−7.21 (1H)		9.79 (s, 1H)	5.43 (1H) *J* = 7.69 (t)	4.52 (2H) *J* = 7.69 (d)	7.40−7.21 (10H, m)	5.23 (s, 2H) 5.21 (s, 2H)
3	7.42−7.23 (1H)		9.23 (s, 1H)	5.43 (1H) 7.69 (t)	4.56 (2H) *J* = 7.69 (d)	7.42−7.23 (10H, m)	5.24 (s, 2H) 5.22 (s, 2H)
4	7.54 (s, 1H)		9.33 (s, 1H)	5.45 (1H) *J* = 7.69 (t)	4.57 (2H) *J* = 7.69 (d)	7.42−7.22 (10H, m)	5.25 (s, 2H) 5.23 (s, 2H)
5	7.63 (s, 1H)		9.50 (s, 1H)	5.44 (1H) *J* = 7.69 (t)	4.57 (2H) *J* = 7.70 (d)	7.41−7.23 (10H, m)	5.24 (s, 2H) 5.22 (s, 2H)
6	8.41 (s, 1H)		11.87 (s, 1H)	5.59 (1H) *J* = 6.39 (t)	4.63 (2H) *J* = 6.23 (d)	7.43−7.35 (10H, m)	5.32 (s, 2H) 5.16 (s, 2H)
7		8.75 (s, 1H) 8.15 (s, 1H)		5.53 (1H) *J* = 7.56 (t)	5.12 (2H) *J* = 7.69 (d)	7.38−7.19 (10H, m)	5.22 (s, 4H)
8		8.30 (s, 1H) 8.88 (s, 1H)		5.50 (1H) *J* = 7.57 (t)	5.31 (2H) *J* = 7.18 (d)	7.39−7.19 (10H, m)	5.22 (s, 4H)
9[d]		8.69 (s, 1H) 8.03 (s, 1H)		5.56 (1H) *J* = 7.57 (t)	5.10 (2H) *J* = 7.44 (d)	7.38−7.18 (10H, m)	5.21 (s, 4H)

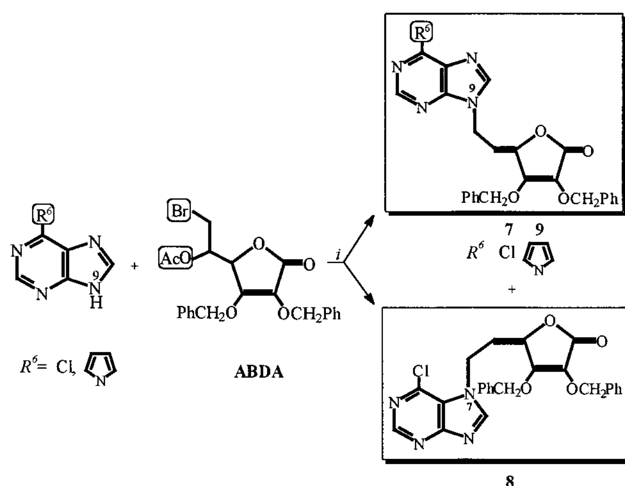
[a] $CDCl_3$, chemical shifts referred to TMS. Multiplicity of coupling and number of protons are given in parentheses: s, singlet; d, doublet; t, triplet; *m*, complex multiplet. [b] Digital resolution ± 0.28 Hz. [c] Chemical shift for H-5: 5.69 ppm (d, 1H), *J* = 7.69 Hz. [d] Chemical shifts for pyrrolo moiety H-2″,5″: 8.31 ppm (2H, t), *J* = 2.05 Hz and H-3″,4″: 6.42 ppm (2H, t), *J* = 2.05 Hz.

Scheme 1[a]



[a] Reagents and conditions: (i) HMDS, $(NH_4)_2SO_4$/argon atmosphere/reflux/3 h; then trimethylsilyl triflate/dry acetonitrile/55−70 °C/12 h.

Scheme 2[a]



[a] Reagent and conditions: (i) triethylamine/dry DMF/70 °C/11 h.

purine with 5-acetyl-6-bromo-2,3-dibenzyl-L-ascorbic acid (ABDA)[12,13] using a modified procedure to that for the preparation of purine and pyrimidine trihydroxyacyclo-nucleosides[14] (Scheme 2). This reaction afforded both N-9 and N-7 isomers in a ratio of 4:1. On the contrary, reaction of 6-(*N*-pyrrolyl)purine with ABDA gave only the N-9 regioisomer.

^{1}H and ^{13}C NMR Studies. The structures of the novel compounds were determined on the basis of analysis of chemical shifts and H−H coupling constants in ^{1}H and ^{13}C NMR spectra (see Table 1 and Experimental Section). These data, as well as connectivities in heteronuclear ^{1}H and ^{13}C NMR spectra, showed that the lactone ring in the condensation reaction of nucleoside bases was preserved, whereas elimination of water in the 4,5-position occurred. This was also corroborated with IR spectra which showed a strong absorption band in the range 1764−1777 cm^{-1}. This band could be ascribed to the C=O stretching vibration of the lactone ring. Comparison of the NMR data from Table 1 indicated that strong electron-withdrawing substituents (F, Cl, Br, I, and CF_3) at position C-5 in the pyrimidine ring caused deshielding of the protons H-6 and N*H*.

The chemical shifts pattern of pyrimidine, purine, and lactone moieties in both ^{1}H and ^{13}C NMR spectra are consistent with those observed for related purine,[15,16] pyrimidine,[16] and ascorbic acid derivatives.[17] Structures of N-9 (**7**) and N-7 (**8**) regioisomers were also confirmed by ^{1}H and ^{13}C NMR. These observations are in accord with those found earlier.[18,19] The most pronounced differences in N-9 and N-7 isomers were found for the proton signals H-2 and H-8 of the purine skeleton. Thus, the proton H-8 in the N-7 isomer (**8**) was shifted downfield (8.88 ppm) relative to the corresponding one (8.15 ppm) in the N-9-substituted molecule (**7**). On the contrary, the signal H-2 was shifted upfield (8.30 ppm) in the N-7 derivative relative to the H-2 signal (8.75 ppm) in the N-9 derivative. Furthermore, the equivalent *N*-methylene protons were more deshielded (5.31 ppm) than the corresponding ones in the N-9 isomer (5.12 ppm). Vicinal coupling constant of protons in the side chain was greater in the N-9 than in the N-7 regioisomer.

X-ray Crystal Structure Analysis. Stereostructure of compound **7** was determined by its X-ray crystallographic analysis. Solid-state conformation with atom numbering of **7** is displayed in Figure 2. The skeleton of the molecule consists of a planar lactone ring connected with the purine moiety by the acyclic chain which contains a double bond between atoms C4 and C5. That bond is conjugated to the lactone ring. The plane defined by the atoms O2−O3−O4−C5 directly connected to the

Table 2. Inhibitory Effects of Compounds **1**−**9** on the Growth of Malignant Tumor Cell Lines and Normal Fibroblasts (Hef522)

compd	IC_{50}[a] (μM)								
	L1210/0	FM3A/0	Molt4/C8	CEM/0	MiaPaCa2	MCF7	HeLa	Hep2	Hef522
1	12.9	17.7	4.8	13.0	30	20	50	40	50
2	5.1	3.4	10.9	15.1	40	60	40	20	40
3	12.2	16.9	6.1	6.0	40	60	40	20	30
4	7.5	17.1	3.9	3.5	80	40	30	90	30
5	7.5	22.6	3.9	3.3	80	70	30	10	40
6	2.0	3.6	0.9	1.6	30	40	20	40	60
7	11.6	16.4	14.8	15.9	60	40	30	60	50
8	4.1	11.4	6.8	4.4	40	>100	30	60	20
9	110	≥200	3.9	5.8	>100	>100	70	>100	>100

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

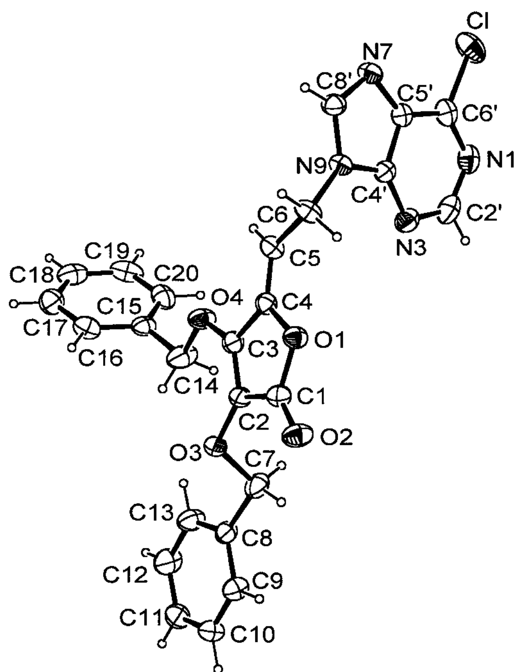


Figure 2. ORTEP[20,21] view with the labeling scheme for **7**. Displacement ellipsoids are drawn at the 20% probability level.

lactone ring is coplanar with the lactone ring. Such coplanarity is also observed for the plane defined by the adjacent atoms of the purine and benzene rings. Conformation of this molecule was determined by the dihedral angles between the planes of the lactone and other rings: C2−O3−C7−C8 [−171,3(2)°], C3−O4−C14−C15 [171,3(2)°], and C4−C5−C6−N9 [140,8(3)°].

Biological Results and Discussion

Antitumor Activity. Compounds **1**−**9** were evaluated for their activity against malignant tumor cell lines: pancreatic carcinoma (MiaPaCa2), breast carcinoma (MCF7), cervical carcinoma (HeLa), laryngeal carcinoma (Hep2), murine leukemia (L1210/0), murine mammary carcinoma (FM3A), and human T-lymphocytes (Molt4/C8 and CEM/0).

Inhibitory effects of compounds **1**−**9** differ from one type of tumor cell to another. Better growth inhibition was achieved for the L1210/0, FM3A, Molt4/C8, and CEM/0 cell lines in comparison with the malignant MiaPaCa2, MCF7, HeLa, and Hep2 cells. The difference in IC_{50} was about 10 times (Table 2). Compound **6**, containing a trifluoromethyl group substituted at position C-5 of the uracil ring, showed the most significant cytostatic activity, particularly against human Molt4/C8 cells ($IC_{50} = 0.9$ μM, Table 2). The N-9-substituted purine regioisomer **7** showed slight inhibitory activity against malignant murine and human cells, whereas its N-7 isomer **8** displayed more pronounced inhibition on the majority of examined cell lines. The most pronounced differences in inhibitory effects of the N-9 and N-7 regioisomers **7** and **8** were observed for murine L1210/0 and human CEM/0 cell proliferation (for **7**: $IC_{50} = 11.6$ and 15.9 μM, as compared to **8**: $IC_{50} = 4.1$ and 4.4 μM, Table 2). Compound **9**, containing a pyrrolyl moiety at position C-6 of the purine ring, exhibited marked selectivity in cytostatic activity: This compound inhibited specifically the human Molt4/C8 ($IC_{50} = 3.9$ μM) and CEM/0 ($IC_{50} = 5.8$ μM) cells but not the other ones. It was also the most active antitumor agent from the purine series (**7**−**9**).

In comparison with ascorbic acid, all derivatives exhibited a better inhibitory effect on the growth of human tumor cell lines. As was shown earlier[2,5] ascorbic acid inhibited the growth of human tumor cell lines (about 25%) at a much higher concentration (2×10^{-3} M). At the concentrations used in our experiments (10^{-6}−10^{-4} M), ascorbic acid did not inhibit the growth of examined cell lines (results are not shown).

Antiviral Activity. The ability of novel compounds **1**−**9** to inhibit thymidine kinase-positive (TK^+) and thymidine kinase-deficient (TK^-) strains of varicella-zoster virus (VZV) was compared with the antiviral activity of acyclovir (ACV) and (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU). Inhibition of cytomegalovirus (CMV) by the test compounds was compared with 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG) and (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (HPMPC). The results presented in Table 3 show that compound **6** containing a trifluoromethyl group at C-5 of the uracil ring displayed more potent activity than the other compounds. Moreover, this compound was 6−10-fold more active than compound **1** with an unsubstituted uracil moiety. Also, compound **6** exhibited more potent activity against TK^+VZV/YS and TK^-VZV strains than ACV. Compound **6** also showed greater activity against cytomegalovirus than DHPG. Compounds **3**−**5** displayed almost identical activity against VZV and CMV. The N-7 isomer was more active against TK^-VZV (YS/R) than ACV and BVDU, while the N-9 derivative did not show appreciable antiviral activity. Compound **9**, which carries the 6-(*N*-pyrrolyl)purine

Table 3. Cytotoxicities and Antiviral Activities of Compounds **1**−**9** against Varicella-Zoster Virus (YS, OKA, 07/1, and YS/R Strains) and Cytomegalovirus (AD-169 and Davis Strains) in Human Embryonic Lung Cells

	antiviral activity IC_{50}[a] (μM)						cytotoxicity (μM)	
	TK^+VZV		TK^-VZV		CMV			
compd	YS	OKA	07/1	YS/R	AD-169	Davis	cell morph (MCC)[b]	cell growth (CC_{50})[c]
1	3.4	3.0	3.4	1.8	3.5	3.5	20	5
2	3.8	2.6	3.5	2.7	3.5	3.5	20	9
3	>2	1.3	>2	1.0	>2	>2	5	3
4	>2	1.3	>2	1.0	>2	>2	5	3
5	1.4	1.3	>2	1.0	>2	>2	5	3
6	0.5	0.3	0.5	0.3	>0.5	0.4	2	1
7	>5	>5	>5	>5	>5	>5	20	8
8	>5	5.0	>5	3.8	>5	>5	20	20
9	1.5	1.2	2.8	0.6	>0.5	>0.5	≥20	4
ACV	0.78	0.16	17	12	ND[d]	ND[d]	>50	>100
BVDU	0.005	0.001	>50	>50	ND[d]	ND[d]	>50	>100
DHPG	ND[d]	ND[d]	ND[d]	ND[d]	1	5	>50	>50
HPMPC	ND[d]	ND[d]	ND[d]	ND[d]	0.11	1	>50	ND[d]

[a] Inhibitory concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU). [b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology. [c] Cytotoxic concentration required to reduce cell growth by 50%. [d] ND, not determined.

moiety, was 20-fold more potent against TK^-VZV (YS/R) than ACV and BVDU.

Comparison of the cytotoxicity of **1**−**9** (Table 3) indicates that all compounds showed relatively low selectivity in their antiviral potencies.

Conclusions

New types of compounds consisting of pyrimidine (**1**−**6**) or purine (**7**−**9**) moieties connected by an acyclic chain to 2,3-dibenzyl-4,5-didehydro-5,6-dideoxy-L-ascorbic acid were prepared and evaluated for their antitumor and antiviral activities.

The stereostructure of compound **7** was unambiguously demonstrated by its X-ray structural analysis. The compounds **1**−**9** exhibited greater inhibitory activities against L1210/0, FM3A, Molt4/C8, and CEM/0 cells than the MiaPaCa2, MCF7, HeLa, and Hep2 ones. Compound **6**, containing a trifluoromethyl-substituted uracil ring, was found to be the most active against human Molt4/C8 cells. The most pronounced differences in inhibitory effects of the N-9- and N-7-substituted purine regioisomers **7** and **8** were observed for murine L1210/0 and human CEM/0 cell proliferation. Compound **9** with the purine substituted at position 6 with a pyrrolo moiety showed the most selective inhibitory activity against human Molt4/C8 and CEM/0 cell proliferation as compared to normal fibroblast Hef522 cell proliferation. The most potent (yet relatively aspecific) antiviral effects against TK^+VZV, TK^-VZV, and CMV were found for compound **6**.

Experimental Section

General Methods. All new compounds were fully characterized by ^{1}H and ^{13}C NMR, electron impact mass spectra, and elemental analysis (C, H, N). Melting points of compounds were determined with a Kofler micro hot-stage apparatus (Reichert, Wien) and are uncorrected. 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 was performed using silica gel (0.05−0.2 mm, Merck); glass column was slurry-packed under gravity. Additional purification of compounds **1**−**9** by recrystallization from ethanol afforded analytical samples of **1**−**9**. Acetonitrile was first refluxed several hours over P_2O_5, distilled from P_2O_5, and finally refluxed over CaH_2 and distilled.

The electron impact mass spectra were recorded with an EXTREL FT MS 2001 instrument with ionizing energy of 70 eV. The IR spectra were recorded on a Nicolet-Magna IR 760 spectrometer and UV spectra on a Hewlett-Packard 8452 spectrometer. Elemental analyses were performed by the Central Analytical Service, Ruđer Bošković Institute, Zagreb. Results were within ±0.4% of the theoretical values and are indicated by symbols of the elements. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer, operating at 75.46 MHz for the ^{13}C resonance. The samples were dissolved in $CDCl_3$ and measured at 21 °C in 5-mm NMR tubes. The ^{1}H and ^{13}C chemical shift values are in ppm, referred to TMS. Digital resolution in ^{1}H spectra was 0.28 Hz, while in ^{13}C spectra it was 0.65 Hz/point.

1-(Uracil-1-yl)-2-(2,3-*O,O*-dibenzyl-2-buten-4-olidylidene)ethane (1). A suspension of anhydrous uracil (60% excess compared with DDA, 176 mg, 1.57 mmol) and $(NH_4)_2SO_4$ (15 mg) in HMDS (10 mL) was heated under reflux for 3 h in argon atmosphere. Evaporation of HMDS under reduced pressure gave an oily product to which was added DDA (433 mg, 0.98 mmol) dissolved in anhydrous acetonitrile (10 mL). After the reaction mixture was cooled (−20 °C), trimethylsilyl trifluoromethanesulfonate (0.5 mL, 2.76 mmol) was added dropwise and the mixture was heated at 55−70 °C for 12 h. The reaction was terminated by diluting with CH_2Cl_2 (40 mL) and adding ice-cold $NaHCO_3$ solution (40 mL). The mixture was extracted several times with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and evaporated. Silica gel column chromatography (CH_2Cl_2:MeOH − 40:1) of the oily residue afforded **1** (179 mg, 42%, mp 95−97 °C): IR (KBr, ν/cm^{-1}) 3033 (=C(5′)-H), 2821−2970 (C−H), 1774 (C=O), 1680 (C=C), 1654−1680 (C=O); UV (methanol) λ_{max} 208 (log ϵ 4.37), λ_{max} 270 (log ϵ 4.30); ^{13}C NMR ($CDCl_3$) δ 150.73 (C-2), 163.67 (C-4), 102.69 (C-5), 144.08 (C-6), 163.77 (C-1′), 147.67 (C-2′), 123.60 (C-3′), 144.98 (C-4′), 100.98 (C-5′), 42.49 (C-6′), 73.93, 73.32 (C-7′,7″), 135.51, 135.07 (C-8′,8″), 128.84−127.74 (C-9′,10′,11′,9″,10″,11″); MS *m/z* 432 ($M^{+\bullet}$). Anal. ($C_{24}H_{20}N_2O_6$) C, H, N.

1-(5-Fluorouracil-1-yl)-2-(2,3-*O,O*-dibenzyl-2-buten-4-olidylidene)ethane (2). 5-Fluorouracil (60% excess, 237 mg, 1.82 mmol) was treated according to the procedure analogous to that for the preparation of compound **1**, to give **2** (255 mg, 50%, mp 165−167 °C): IR (KBr, ν/cm^{-1}) 3035 (=C(5′)-H), 2828−2970 (C−H), 1777 (C=O), 1693 (C=C), 1659−1693 (C=O); UV (methanol) λ_{max} 208 (log ϵ 4.23), λ_{max} 276 (log ϵ 4.09); ^{13}C NMR ($CDCl_3$) δ 149.48 (C-2), 157.19 (C-4, J = 25.5 Hz), 140.62 (C-5, J = 240.2 Hz), 127.91 (C-6), 163.68 (C-1′), 147.56 (C-2′), 123.69 (C-3′), 145.35 (C-4′), 105.52 (C-5′), 42.61 (C-6′), 73.95, 73.36 (C-7′,7″), 135.46, 135.01 (C-8′,8″), 129.09−127.77 (C-9′,10′,11′,9″,10″,11″); MS *m/z* 450 ($M^{+\bullet}$). Anal. ($C_{24}H_{19}N_2O_6F$) C, H, N.

1-(5-Chlorouracil-1-yl)-2-(2,3-*O,O*-dibenzyl-2-buten-4-olidylidene)ethane (3). 5-Chlorouracil (60% excess, 266 mg, 1.82 mmol) was treated according to the procedure analogous to that for the preparation of compound **1**, to give **3** (312 mg, 59%, mp 168−170 °C): IR (KBr, ν/cm^{-1}) 3033 (=C(5′)-H), 2820−2960 (C−H), 1768 (C=O), 1710 (C=C), 1627−1695 (C=O), 835 (C−Cl); UV (methanol) λ_{max} 208 (log ϵ 4.11), λ_{max} 284 (log ϵ 3.99); ^{13}C NMR (CDCl$_3$) δ 149.73 (C-2), 159.14 (C-4), 109.29 (C-5), 140.78 (C-6), 163.69 (C-1′), 147.56 (C-2′), 123.74 (C-3′), 145.39 (C-4′), 100.18 (C-5′), 42.84 (C-6′), 73.98, 73.41 (C-7′,7″), 135.46, 135.00 (C-8′,8″), 129.45−127.82 (C-9′,-10′,11′,9″,10″,11″); MS *m/z* 466 (M$^{+\bullet}$). Anal. ($C_{24}H_{19}N_2O_6Cl$) C, H, N.

1-(5-Bromouracil-1-yl)-2-(2,3-*O,O*-dibenzyl-2-buten-4-olidylidene)ethane (4). 5-Bromouracil (60% excess, 611 mg, 3.2 mmol) was treated by the procedure analogous to that for the preparation of compound **1**, to give **4** (518 mg, 50%, mp 181−183 °C): IR (KBr, ν/cm^{-1}) 3033 (=C(5′)-H), 2853−2924 (C−H), 1774 (C=O), 1700 (C=C), 1649−1686 (C=O); UV (methanol) λ_{max} 208 (log ϵ 4.50), λ_{max} 284 (log ϵ 4.31); ^{13}C NMR (CDCl$_3$) δ 150.05 (C-2), 159.36 (C-4), 96.92 (C-5), 143.35 (C-6), 163.69 (C-1′), 147.58 (C-2′), 123.71 (C-3′), 145.27 (C-4′), 100.31 (C-5′), 42.84 (C-6′), 73.96, 73.36 (C-7′,7″), 135.46, 135.00 (C-8′,8″), 128.86−127.79 (C-9′,10′,11′,9″,10″,11″); MS *m/z* 511 (M$^{+\bullet}$). Anal. ($C_{24}H_{19}N_2O_6Br$) C, H, N.

1-(5-Iodouracil-1-yl)-2-(2,3-*O,O*-dibenzyl-2-buten-4-olidylidene)ethane (5). 5-Iodouracil (60% excess, 762 mg, 3.3 mmol) was treated according to the procedure analogous to that for the preparation of compound **1** to give **5** (473 mg, 42%, mp 171−173 °C): IR (KBr, ν/cm^{-1}) 3028 (=C(5′)-H), 2834 (C−H), 1776 (C=O), 1697 (C=C), 1647 (C=O); UV (methanol) λ_{max} 208 (log ϵ 4.39), λ_{max} 290 (log ϵ 4.23); ^{13}C NMR (CDCl$_3$) δ 150.33 (C-2), 160.36 (C-4), 68.27 (C-5), 148.35 (C-6), 163.69 (C-1′), 147.58 (C-2′), 123.68 (C-3′), 145.15 (C-4′), 100.46 (C-5′), 42.78 (C-6′), 73.95, 73.35 (C-7′,7″), 135.44, 135.00 (C-8′,8″), 128.85−127.77 (C-9′,10′,11′,9″,10″,11″); MS *m/z* 558 (M$^{+\bullet}$). Anal. ($C_{24}H_{19}N_2O_6I$) C, H, N.

1-[5-(Trifluoromethyl)uracil-1-yl]-2-(2,3-*O,O*-dibenzyl-2-buten-4-olidylidene)ethane (6). 5-(Trifluoromethyl)uracil (60% excess, 500 mg, 2.78 mmol) was treated according to the procedure analogous to that for the preparation of compound **1**, to give **6** (430 mg, 52%, mp 181−183 °C): IR (KBr, ν/cm^{-1}) 3032 (=C(5′)-H), 2851−2960 (C−H), 1764 (C=O), 1670 (C=C), 1655−1670 (C=O); UV (methanol) λ_{max} 206 (log ϵ 4.44), λ_{max} 270 (log ϵ 4.39); ^{13}C NMR (CDCl$_3$) δ 150.35 (C-2), 159.81 (C-4), 102.41 (C-5, J = 31.33 Hz), 147.76 (C-6, J = 5.8 Hz), 122.14 (CF$_3$, J = 270.39 Hz), 164.05 (C-1′), 148.46 (C-2′), 123.07 (C-3′), 142.23 (C-4′), 104.05 (C-5′), 44.12 (C-6′), 74.19, 73.17 (C-7′,7″), 136.02, 135.65 (C-8′,8″), 129.08−128.30 (C-9′,10′,-11′,9″,10″,11″); MS *m/z* 500 (M$^{+\bullet}$). Anal. ($C_{25}H_{19}N_2O_6F_3$) C, H, N.

1-(6-Chloropurin-9-yl)-2-(2,3-*O,O*-dibenzyl-2-buten-4-olidylidene)ethane (7) and 1-(6-Chloropurin-7-yl)-2-(2,3-*O,O*-dibenzyl-2-buten-4-olidylidene)ethane (8). 6-Chloropurine (167 mg, 1.08 mmol) was dissolved in dry DMF (10 mL) and triethylamine (0.2 mL, 1.43 mmol). ABDA (500 mg, 1.08 mmol) was then added, and the reaction mixture was stirred at 70 °C for 5 h, the course of the reaction being monitored by TLC. Triethylamine hydrobromide separated gradually. After a further quantity of triethylamine (0.2 mL, 1.43 mmol) was added, heating of the mixture was continued at 70 °C for 6 h. The precipitated triethylamine hydrobromide was filtered off and the solvent evaporated. The crude oily product was purified by column chromatography (CH$_2$Cl$_2$:MeOH − 40:1) providing colorless crystals of **7** (124 mg, 61%, mp 121−122 °C) and the oily compound **8** (34 mg, 17%).

7: IR (KBr, ν/cm^{-1}) 3110 (C-8-H), 3030−3060 (=C(5′)-H), 2923 (C−H), 1775 (C=O), 1693 (C=C); UV (methanol) λ_{max} 206 (log ϵ 4.52), λ_{max} 264 (log ϵ 4.23); ^{13}C NMR (CDCl$_3$) δ 152.10 (C-2), 147.45 (C-4), 121.10 (C-5), 151.51 (C-6), 144.89 (C-8), 163.55 (C-1′), 151.11 (C-2′), 123.74 (C-3′), 145.12 (C-4′), 99.91 (C-5′), 38.23 (C-6′), 73.92, 73.36 (C-7′,7″), 135.41, 134.97 (C-8′,8″), 129.06−127.75 (C-9′,10′,11′,9″,10″,11″); MS *m/z* 474 (M$^{+\bullet}$). Anal. ($C_{25}H_{19}N_4O_4Cl$) C, H, N.

8: IR (KBr, ν/cm^{-1}) 3065 (C-8-H), 3033 (=C(5′)-H), 2957 (C−H), 1775 (C=O), 1697 (C=C); UV (methanol) λ_{max} 208 (log ϵ 4.50), λ_{max} 264 (log ϵ 4.20); ^{13}C NMR (CDCl$_3$) δ 152.62 (C-2), 142.95 (C-4), 122.19 (C-5), 149.16 (C-6), 148.93 (C-8), 163.37 (C-1′), 148.28 (C-2′), 123.80 (C-3′), 145.01 (C-4′), 100.38 (C-5′), 41.45 (C-6′), 73.93, 73.46 (C-7′,7″), 135.35, 134.92 (C-8′,8″), 129.34−127.80 (C-9′,10′,11′,9″,10″,11″); MS *m/z* 474 (M$^{+\bullet}$). Anal. ($C_{25}H_{19}N_4O_4Cl$) C, H, N.

1-[6-(*N*-Pyrrolyl)purin-9-yl]-2-(2,3-*O,O*-dibenzyl-2-buten-4-olidylidene)ethane (9). 6-(*N*-Pyrrolyl)purine (153.72 mg, 0.83 mmol) was dissolved in dry DMF (10 mL). To this mixture were added triethylamine (0.2 mL, 1.43 mmol) and ABDA (383 mg, 0.83 mmol), and the reaction solution was stirred at 70 °C for 5 h. A further quantity of triethylamine (0.2 mL, 1.43 mmol) was added, and heating was continued at 70 °C for 6 h. The solution was filtered and evaporated in vacuo. The residual oil was purified by column chromatography (CH$_2$Cl$_2$:MeOH − 40:1) to give **9** (97 mg, 23%, mp 105−107 °C): IR (KBr, ν/cm^{-1}) 3028 (=C(5′)-H), 2929 (C−H), 1768 (C=O), 1690 (C=C); UV (methanol) λ_{max} 206 (log ϵ 4.62), λ_{max} 290 (log ϵ 4.64), λ_{max} 298 (log ϵ 4.60); ^{13}C NMR (CDCl$_3$) δ 152.30 (C-2), 147.62 (C-4), 121.90 (C-5), 152.93 (C-6), 142.93 (C-8), 163.75 (C-1′), 147.62 (C-2′), 123.62 (C-3′), 144.73 (C-4′), 100.75 (C-5′), 37.89 (C-6′), 73.93, 73.32 (C-7′,7″), 135.46 135.03 (C-8′,8″), 129.12−127.75 (C-9′,10′,11′,9″,10″,11″); MS *m/z* 505 (M$^{+\bullet}$). Anal. ($C_{29}H_{23}N_5O_4$) C, H, N.

X-ray Determination. Single crystals of **7** suitable for X-ray structure analysis were prepared by growth under slow evaporation at room temperature of a very dilute solution of ethanol. The intensities were measured on a Philips PW1100 diffractometer upgraded by Stoe[22] using Mo *K*α radiation (λ = 0.71073 Å) at 20 °C with the $\Theta - 2\Theta$ scan mode and corrected only for Lorentz polarization factor. During the data collection crystal decomposition of 6.2% was observed. The structure was solved by direct methods and refined on the basis of observed reflections [$I \geq 2\sigma(I)$], using SHELX-85[23] and SHELX-93[24] program packages. Hydrogen atoms were located either in a difference Fourier synthesis or generated and allowed to ride at a fixed distance from the attached carbon atoms with a fixed isotropic temperature factor for two groupings according to the stereochemical rules for sp^2- and sp^3-hybridized carbon atoms. A unit weight was assumed for all observations. Final difference map contained no significant features.

Crystal data for **7**: M_r = 474.89, space group $P2_1/c$, a = 9.653(1), b = 24.442(4), c = 6.692(2) Å, β = 98.79(2)°, V = 2259.9(6) Å^3, Z = 4, F(000) = 984, d_x = 1.396 g cm^{-3}, μ(Mo $K\alpha$) = 0.210 mm^{-1}, S = 0.956, R/R_w = 0.0590/0.1378 for 309 parameters and 2522 reflections, and R/R_w = 0.1378/0.2023 for all 6572 independent reflections measured in the range 2.13 − 2Θ − 30.00°.

Materials for Biological Tests. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Life Techology (Paisley, U.K.). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sigma (St. Louis, MO). All other chemicals were of analytical grade (Kemika, Zagreb, Croatia).

Cell Culturing. The human cell lines pancreatic carcinoma (MiaPaCa2), cervical carcinoma (HeLa), laryngeal carcinoma (Hep2), and breast carcinoma (MCF7) and the normal fibroblasts (Hef522) were cultured in DMEM supplemented with 10% FBS, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere with 5% CO$_2$ at 37 °C. All cells were grown as a monolayer culture. The cells were plated in 96-microwell plates at a concentration of 1.5 × 10^4/mL (MiaPaCa2, HeLa, Hep2, and MCF7) and 2 × 10^4/mL (Hef522). Twenty-four hours later, the test compounds were added and the cells were treated for an additional 72 h. Control cells were grown under the same conditions, but without addition of test compounds. The number of MiaPaCa2, HeLa, Hep2, MCF7, and Hef522 cells was determined by the MTT test, and percentage of growth was calculated. Each number was the mean from three parallel samples in three individual experiments. The variations between the experiments were

less than 10%. The results are expressed as IC_{50}. The compounds were dissolved in DMSO at a concentration of 10^{-1} M and diluted with DMEM to concentrations between 10^{-6} and 10^{-4} M. The concentration of DMSO was less than 0.1%, and at that concentration it did not affect cell growth.

Antitumor activity against L1210/0 (murine leukemia), FM3A/0 (murine mammary carcinoma), Molt4/C8 (human T-lymphoblast), and CEM/0 (human T-lymphoblast) cell lines was measured essentially as originally described for the mouse leukemia/L1210 cell lines.[25]

Antiviral Activity Assays. Antiviral activity against thymidine kinase-positive (TK^+) and -negative (TK^-) strains of varicella-zoster virus (VZV) and against cytomegalovirus (CMV) was determined as described previously.[26]

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Supporting Information Available: Tables of atomic coordinates and equivalent isotropic displacement parameters, bond lengths and angles, anisotropic displacement parameters, hydrogen coordinates, and isotropic displacement parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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