Synthesis and Antitumor Activities of Novel Pyrimidine Derivatives of 2,3-*O*,*O*-Dibenzyl-6-deoxy-L-ascorbic Acid and 4,5-Didehydro-5,6-dideoxy-L-ascorbic Acid

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Received July 31, 2000

The new pyrimidine derivatives of 2,3-*O*,*O*-dibenzyl-6-deoxy-L-ascorbic acid (**8**−**10**) were synthesized by condensation of uracil and its 5-fluoro- and 5-trifluoromethyl-substituted derivatives with 4-(5,6-epoxypropyl)-2,3-*O*,*O*-dibenzyl-L-ascorbic acid (**7**), while pyrimidine derivatives of 4,5-didehydro-5,6-dideoxy-L-ascorbic acid (**14**−**17**) with free C-2′ and C-3′ hydroxy groups in the lactone ring were obtained by debenzylation of **11**−**13** with boron trichloride. *Z*-Configuration of the C4′═C5′ double bond and position of the benzyl group in the lactone ring of **14** were deduced from their ^{1}H and ^{13}C NMR spectra and connectivities in COSY, ROESY, and HMBC spectra. The exact stereostructure of **13** was confirmed by its X-ray crystal structure analysis. Of all the compounds in the series, compound **16** containing a 5-fluoro-substituted uracil ring showed the most significant antitumor activities against murine leukemia L1210/0 (IC_{50} = 1.4 μg/mL), murine mammary carcinoma FM3A/0 (IC_{50} = 0.78 μg/mL), and, to a lesser extent, human T-lymphocyte cells Molt4/C8 (IC_{50} = 31.8 μg/mL) and CEM/0 cell lines (IC_{50} = 20.9 μg/mL).

Introduction

L-Ascorbic acid and its derivatives have been found to possess antitumor and antiviral activities.[1−3] Thus, L-ascorbic acid inhibited apoptosis induced by oxidative stress in HL-60 myeloid leukemia cell.[4] Some derivatives of L-ascorbic acid, e.g. 6-bromo-, 6-amino-, and *N*,*N*-dimethyl-6-amino-6-deoxy-L-ascorbic acid, inhibited the growth of certain human malignant tumor cell lines: cervical carcinoma (HeLa), laryngeal carcinoma (Hep2), and pancreatic carcinoma (MiaPaCa2).[3,5] Furthermore, vitamin C and vitamin K-3 combination showed synergistic antitumor activity against human prostatic carcinoma cell lines (DU145).[6] Sodium 5,6-benzylidene-L-ascorbate and its related compounds induced DNA fragmentation in human myelogenous leukemic cell lines.[7] In addition, several nucleosides containing a 5-substituted pyrimidine moiety were shown to inhibit growth of murine mammary carcinoma (FM3A TK$^-$/HSV 1 TK$^+$) cells transformed with the herpes simplex virus type 1 (HSV-1) TK gene.[8−10] L-Ascorbic acid-2-phosphate was shown to afford long-lasting antiviral activity against several human cytomegalovirus (CMV) strains in human foreskin fibroblasts (HFF) and endothelial cells (EC).[1]

In our previous studies we demonstrated that pyrimidine and purine derivatives of 2,3-dibenzyl-4,5-didehydro-5,6-dideoxy-L-ascorbic acid exerted 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).[11] The compound containing a trifluoromethyl-substituted uracil ring displayed the most potent cytostatic activity particularly against human Molt4/C8 cells with an IC_{50} of 0.9 μM.[11] The synthesis of novel pyrimidine derivatives of 2,3-*O*,*O*-dibenzyl-6-deoxy-L-ascorbic acid (**8**−**10**) and 4,5-didehydro-5,6-dideoxy-L-ascorbic acid (**14**−**17**) (see Figure 1) and their evaluation for antitumor and antiviral activities are described herein.

Chemistry

The syntheses of 5,6-*O*,*O*-diacetyl-2,3-*O*,*O*-dibenzyl-L-ascorbic acid[12] (**4**) and 4-(5,6-epoxypropyl)-2,3-*O*,*O*-dibenzyl-L-ascorbic acid (**7**), key intermediates for coupling with pyrimidine bases, are outlined in Scheme 1. Treatment of L-ascorbic acid (**AA**) with acetyl chloride in acetone afforded the 5,6-ketal of L-ascorbic acid (**1**).[12] Benzylation of the C-2′ and C-3′ hydroxy groups of the lactone ring in **1** was accomplished using K_2CO_3 and benzyl chloride in DMF to provide **2**.[12,13] Deblocking of the 5,6-*O*,*O*-protected derivative of L-ascorbic acid **2** with acetic acid in methanol gave 2,3-*O*,*O*-dibenzyl-L-ascorbic acid (**3**).[12] Subsequent acetylation of **3** was carried out with acetic anhydride in pyridine and CH_2Cl_2 to give **4** using an analogous procedure for the preparation of 5-*O*-

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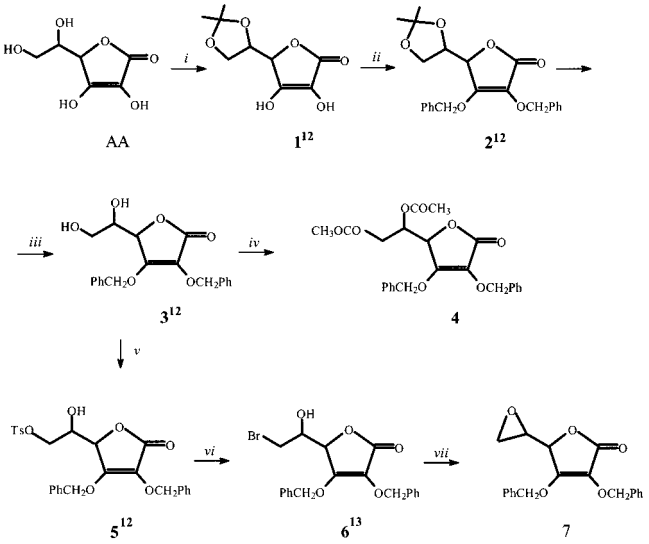
10.1021/jm0009540 CCC: $19.00
Published on Web 11/17/2000

Scheme 1[a]



[a] Reagents and conditions: (i) acetyl chloride/acetone/rt/3−4 h; (ii) benzyl chloride, K_2CO_3/dry DMF; (iii) 50% acetic acid/MeOH/100 °C/5 h; (iv) acetic anhydride/pyridine/CH_2Cl_2/−10−24 °C/2 h; (v) toluene-4-sulfonyl chloride/CH_2Cl_2/pyridine/0−24 °C; (vi) NaBr/acetone/130 °C/7 h; (vii) Na_2CO_3/acetonitrile/rt/1 h.

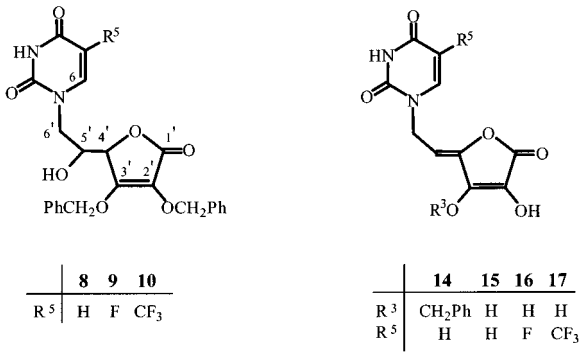


Figure 1. Pyrimidine derivatives of 2,3-*O*,*O*-dibenzyl-6-deoxy-L-ascorbic acid (**8**−**10**), 3-*O*-benzyl-4,5-didehydro-5,6-dideoxy-L-ascorbic acid (**14**), and 4,5-didehydro-5,6-dideoxy-L-ascorbic acid (**15**−**17**).

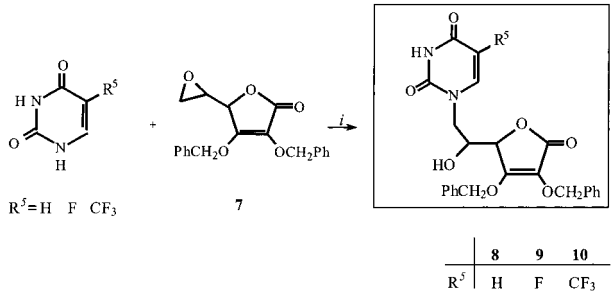
acetyl-6-bromo-6-deoxy-2,3-*O*,*O*-dibenzyl-L-ascorbic acid.[13] 2,3-*O*,*O*-Dibenzyl-6-*O*-tosyl-L-ascorbic acid (**5**) was obtained by tosylation of the 6-hydroxy group in **3** using toluene-4-sulfonyl chloride in pyridine.[12] Bromination of **5** with sodium bromide in acetone gave 6-bromo-2,3-*O*,*O*-dibenzyl-L-ascorbic acid (**6**).[13] 4-(5,6-Epoxypropyl)-2,3-*O*,*O*-dibenzyl-L-ascorbic acid (**7**) was obtained by reaction of **6** with sodium carbonate in acetonitrile.

Pyrimidine derivatives of 2,3-*O*,*O*-dibenzyl-6-deoxy-L-ascorbic acid (**8**−**10**) were prepared by silylation of the uracil and its 5-substituted derivatives with 1,1,1,3,3,3-hexamethyldisilazane and subsequent condensation of the intermediates thus obtained with **7** (Scheme 2).

Coupling of **4** with uracil and its 5-substituted derivatives gave pyrimidine derivatives of 2,3-*O*,*O*-dibenzyl-4,5-didehydro-5,6-dideoxy-L-ascorbic acid (**11**−**13**; Scheme 3).[11] Debenzylation of **11**−**13** was accomplished by treatment with boron trichloride in CH_2Cl_2, affording **14**−**17** (Scheme 3).[14,15]

^{1}H and ^{13}C NMR Studies. The assignment of ^{1}H and ^{13}C NMR spectra was performed on the basis of chemical shifts and the magnitude and multiplicity of H−H spin−spin coupling, as well as connectivities in the COSY,

Scheme 2[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.

ROESY, and HMBC spectra (see Table 1 and Experimental Section).

The ^{1}H NMR spectra of compounds **8**−**10** exhibit signals of the pyrimidine ring and benzyl groups in positions 2′ and 3′ of the lactone ring and signals for the protons H-4′, H-5′, H-6′, and 5′-OH of the aliphatic chain. The H−H coupling patterns in the side chain are singlet for H-4′, multiplet for H-5′, doublet of doublet for H-6′, and multiplet for 5′-OH.

In the ^{1}H NMR spectra of compounds **14**−**17**, besides signals of the pyrimidine skeleton which are in agreement with those reported for other pyrimidine derivatives,[11,14,15] signals for H-5′ and H-6′ in the side chain as well as for the hydroxy groups at the 2- and 3-positions of the lactone ring were observed. Side chain H−H coupling patterns in all compounds are doublet for H-6′ and triplet for H-5′. To determine the position of substitution of the benzyl group in the lactone ring in monobenzylated derivative **14**, its HMBC spectrum was recorded. This spectrum showed a cross-peak arising from three-bond coupling between carbon atom C-3′ of the lactone ring and methylene protons CH_2-Ph confirming substitution of the benzyl group at position 3. The structure of **14** was also verified by the ROESY spectrum which displayed interaction for methylene protons (CH_2-Ph) and phenyl protons of the benzyl group and methine proton H-5′. These observations also corroborate the *Z*-stereostructure around the C4′═C5′ double bond. In the hypothetical *E*-configuration, an interaction between protons H-6 of the pyrimidine ring and methylene protons of the benzyl group should have been seen. The lack of this cross-peak in the ROESY spectrum of **14** is in agreement with the proposed *Z*-geometry. Similarly, in the ROESY spectra of compounds **15**−**17** with C-2′ and C-3′ free hydroxy groups, interactions between protons of the C-3′ hydroxy group and methine proton H-5′ were found indicating *Z*-configuration of the C4′═C5′ double bond.

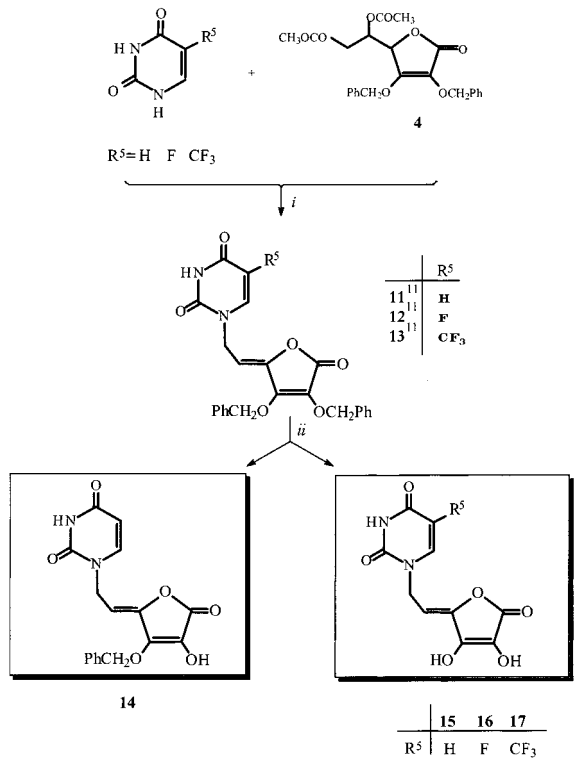
The ^{13}C NMR data are given in the Experimental Section. Generally, the ^{13}C NMR spectra for **14**−**17** showed four signals for the pyrimidine ring, four signals for the lactone moiety, and two signals for carbons in the side chain, and for **8**−**10** and **14** the spectra displayed additional peaks for the phenyl and methylene protons of the benzyl group. The C-3′ benzyl substitution in **14** caused differences in chemical shifts for carbons C-2′ (ca. −3 ppm) and C-3′ (ca. 2 ppm) of the lactone ring. The most pronounced differences in the ^{13}C NMR spectrum of **9** and **16** caused by substitution

Table 1. Chemical Shifts (δ, ppm)[a] and H−H Coupling Constants (J, Hz)[b] in ^{1}H NMR Spectra for Compounds **4**, **7**−**10**, and **14**−**17**

compd	H-5	H-6	NH	H-4′	H-5′	H-6′	2′-OH, 3′-OH
4[c]				4.81 (1H)	5.36−5.32 (1H)	4.33 (1H) J = 11.53; 6.92 (dd); 4.23 (1H) J = 11.66; 5.51 (dd)	
7[d]				4.56 (1H) J = 3.93 (d)	3.14−3.10 (1H) (m)	2.87−2.81 (2H) (m)	
8[e]	5.66 (1H) J = 7.7 (d)	7.24−7.14 (1H)	9.59 (s, 1H)	4.51 (s, 1H)	5.26−5.17 (1H) (m)	3.63 (1H) J = 13.59; 8.72 (dd); 4.03 (1H) J = 13.71; 3.97 (dd)	
9[f]		7.44−7.20 (1H)	10.15 (b, 1H)	4.57 (s, 1H)	5.17−5.08 (1H) (m)	3.98 (1H) J = 13.07 (d); 3.58 (1H) J = 13.72; 9.87 (dd)	
10[g]		7.83 (s, 1H)	10.21 (s, 1H)	4.47 (s, 1H)	4.93 (1H) (m)	3.82 (1H) J = 13.59 (d); 3.46 (1H) J = 11.92 (dd)	
14[h]	5.54 (1H) J = 7.84; 2.24 (dd)	7.60 (1H) J = 7.86 (d)	11.28 (1H) J = 1.51		5.31 (1H) J = 6.95 (t)	4.47 (2H) J = 6.96 (d)	10.35
15	5.57 (1H) J = 7.83; 1.78 (dd)	7.63 (1H) J = 7.86 (d)	11.29 (s, 1H)		5.38 (1H) J = 6.98 (t)	4.50 (2H) J = 6.99 (d)	11.20, 9.70
16		8.08 (1H) J = 6.69 (d)	11.82 (s, 1H)		5.39 (1H) J = 6.81 (t)	4.47 (2H) J = 6.82 (d)	11.29, 9.62
17		8.39 (s, 1H)	11.85 (s, 1H)		5.42 (1H) J = 6.68 (t)	4.59 (2H) J = 6.68 (d)	11.29, 9.60

[a] $CDCl_3$ except for **14**−**17** (DMSO-d_6); 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. See Figure 1 and Schemes 1−3 for structures. [c] Chemical shifts for OCOCH_3: 1.94 (3H, s), 2.04 (3H, s); C$_6H_5$: 7.22−7.38 (10H, m); OCH_2: 5.22−5.16 (4H, m). [d] Chemical shifts for C$_6H_5$: 7.46−7.19 (10H, m); OCH_2: 5.22−5.06 (4H, m). [e] Chemical shifts for 5′-OH: 4.28 (1H, m); C$_6H_5$: 7.36−7.34 (10H, m); OCH_2: 5.13−5.07 (4H, m). [f] Chemical shifts for 5′-OH: 4.20 (1H, m); C$_6H_5$: 7.44−7.20 (10H, m); OCH_2: 5.08 (s, 2H); 5.06 (s, 2H). [g] Chemical shifts for 5′-OH: 4.06 (1H, m); C$_6H_5$: 7.35−7.15 (10H, m); OCH_2: 5.09−5.06 (4H, m). [h] Chemical shifts for C$_6H_5$: 7.44−7.35 (5H, m); OCH_2: 5.44 (s, 2H).

Scheme 3[a]



[a] Reagents and conditions: (i) HMDS, $(NH_4)_2SO_4$/argon atmosphere/reflux/3 h, then trimethylsilyl triflate/dry acetronitrile/55−70 °C/12 h; (ii) BCl_3, CH_2Cl_2/−78 °C/2 h.

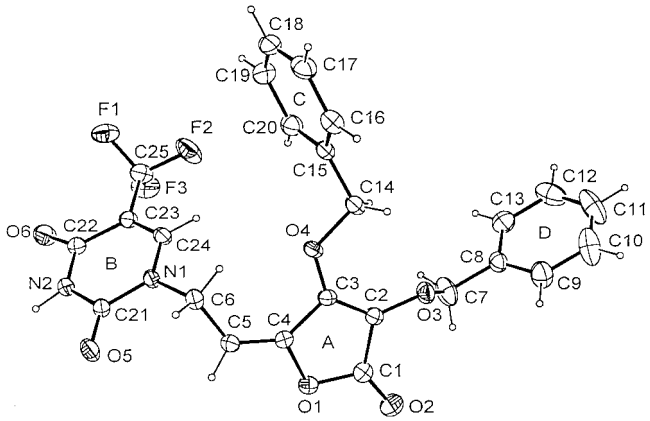


Figure 2. ORTEP[17] view with the labeling scheme for **13**.

of the fluoro atom at C-5 in the pyrimidine ring were observed for atoms C-4, C-5, and C-6. Thus, carbon C-5 was deshielded in **9** and **16** by ca. 39 ppm with respect to that of the unsubstituted uracil derivatives **14** and **15**, while carbons C-4 and C-6 in **9** and **16** were shielded by ca. 6 and 18 ppm, respectively. These differences in substituent-induced chemical shifts (SCS/ppm) for the same carbons in **10** and **17** that carry a trifluoromethyl group at the C-5 position were much smaller. Thus, SCS for C-4, C-5, and C-6 in **17** were ca. −4, 2, and 1 ppm, respectively. The magnitude of one-bond (ca. 245 Hz) and two-bond (ca. 38 Hz) C−F couplings at C-4, C-5, and C-6 in **9**, **16**, and **17** are in accord with the literature data for fluorinated derivatives.[16]

X-ray Crystal Structure Analysis. To determine the exact stereostructure of **13**,[11] its X-ray crystal structure analysis was undertaken. The perspective view with atom numbering and packing of the molecules in the unit cell of **13** are displayed in Figures 2[17] and 3. The skeleton of the molecule consists of a planar lactone ring connected to the pyrimidine moiety by an acyclic chain which contains a double bond between atoms C-4 and C-5. That bond is conjugated to the lactone ring and exists in the *E*-configuration. The planes defined by the atoms directly bonded to all four moieties − lactone (A), pyrimidine (B), and two phenyl (C and D) rings − are coplanar with those rings. Conformation of the molecule is defined by dihedral angles between the planes of the lactone ring (A) and other rings (B, C, and D): C4−C5−C6−N1 [114.2(3)°], C3−O4−C14−C15 [167.7(3)°], C2−O3−C7−C8 [174.8(3)°]. Details of the conformation are described as follows. The angles between planes of the

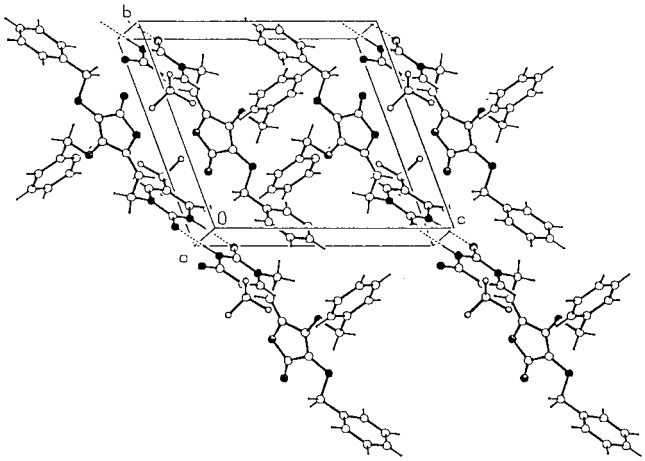


Figure 3. Packing diagram in the unit cell of **13**.

rings A/B and A/D were 72° and 74°, respectively, whereas planes B and D were nearly coplanar. A similar sterical relationship was found for planes B, C, and D. The angles between rings B/C and C/D were 101.44(10)° and 101.55(13)°; that is they are almost identical. An intramolecular hydrogen bond C24−H24···F2 [2.676(4) Å] in the pyrimidine moiety was observed. The molecules in the crystal structure are linked together by intermolecular hydrogen bonds of 2.862(4) Å between N-2 and O-5 atoms of symmetrically related molecules (symmetry code: $-x + 1, -y, -z + 2$), as shown in the packing diagram of Figure 3.[18]

The shortening of the σ-bond C23−C25 compared to the corresponding unfluorinated derivatives resulted from the perfluoroalkyl effect of the trifluoromethyl group.[19]

Biological Results and Discussion

Antitumor Activity. Inhibitory effects of **8**−**10** and **14**−**17** on the proliferation of murine leukemia (L1210/0), murine mammary carcinoma (FM3A/0), and human T-lymphocyte cells (Molt4/C8 and CEM/0) as well as their cytotoxicity for human embryonic lung (fibroblast) cells (HEL) and murine embryo fibroblasts (MEF) are displayed in Table 2. The inhibitory effects are compared with antitumor activities of 5-fluorouracil (5-FU) and 5-fluorodeoxyuridine (5-FdUrd). A growth experiment with MEF was performed in the presence of 5-FU, 5-FdUrd, and **16**.

In comparison with 2,3-*O*,*O*-dibenzyl-6-deoxy-L-ascorbic acid derivatives **8**−**10** the corresponding 5,6-dideoxy-L-ascorbic acid derivatives[11] **11**−**13** (Scheme 3) showed approximately 10-fold less pronounced cytostatic activity of all the examined tumor cells. 5-Fluorouracil derivatives of 4,5-didehydro-5,6-dideoxy-L-ascorbic acid **12**[11] and **16** (Scheme 3) expressed better inhibitory effects on the growth of all tumor cell lines than the corresponding 6-deoxy derivative of L-ascorbic acid (**9**). Of all the studied compounds the 5-fluorouracil derivatives of 4,5-didehydro-5,6-dideoxy-L-ascorbic acid (**16**) exhibited the most pronounced inhibitory activity against FM3A/0 cells (IC_{50} = 0.78 μg/mL). None of the compounds in Table 2 showed any selectivity for tumor cells, either human or murine.

Antiviral Activity. Compounds **8**−**10** and **14**−**17** were also evaluated against varicella-zoster virus (VZV), cytomegalovirus (CMV) in human embryonic lung (HEL) cells, and human immunodeficiency virus (HIV) in acutely infected MT-4 cells. None of the compounds showed appreciable antiviral activity (IC_{50} > 50 μg/mL) against any of these viruses (data not shown).

Conclusions

The present work describes the synthesis and antitumor and antiviral evaluation of the novel pyrimidine derivatives of 2,3-*O*,*O*-dibenzyl-6-deoxy-L-ascorbic acid (**8**−**10**) and 4,5-didehydro-5,6-dideoxy-L-ascorbic acid (**14**−**17**). Benzyl substitution at C-3′ of the lactone ring in **14** and the *Z*-configuration of the C4′═C5′ double bond in **14**−**17** were elucidated by both ^{1}H and ^{13}C NMR spectra. The stereostructure of **13** was unambiguously confirmed by its X-ray structural analysis.

Compound **16**, containing a 5-fluorouracil ring, was found to exhibit appreciable antitumor cell activity, particularly against the murine leukemia L1210/0 (IC_{50} = 1.4 μg/mL) and murine mammary carcinoma FM3A/0 (IC_{50} = 0.78 μg/mL) cell lines.

The synthesis of new compounds within that class of molecules guided by the results of biological screening will be performed shortly.

Experimental Section

General Methods. New compounds were 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 (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. Solvent system used for column chromatography was CH_2Cl_2:MeOH, 30:1. Ad-

Table 2. Inhibitory Effects of Compounds **8**−**10** and **14**−**17** on the Growth of Malignant Tumor Cell Lines

	tumor cell growth IC_{50} [a] (μg/mL)					cytotoxicity (μg/mL)	
compd	L1210/0	FM3A/0	Molt4/C8	CEM/0	cell morphology (MCC)[b]	cell growth (CC_{50})[c]	MEF[d]
8	70.8	74.1	67.8	68.6	>50	>50	
9	65.6	52.2	81.2	74.0	50	33.4	
10	26.0	38.4	47.4	19.0	50	31.7	
14	>200	>200	>200	>200	>50	>50	
15	>200	>200	>200	>200	>50	>50	
16	1.4	0.78	31.8	20.9	>50	23	0.92
17	76.1	162	>200	>200	>50	>50	
5-FU	0.04	0.02	2.9	1.2			0.20
5-FdUrd	0.0003	0.0008	2.6	0.003			0.0001

[a] 50% Inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%. [b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of normal HEL cell morphology. [c] Cytotoxic concentration required to reduce normal HEL cell growth by 50%. HEL cells, human embryonic lung (fibroblast) cells. [d] MEF, murine embryo fibroblasts.

ditional purification of compounds **8**−**10** and **14**−**17** by recrystallization from ethanol afforded their analytical samples.

The electron impact mass spectra were recorded with an EXTREL FT MS 2001 instrument with ionizing energy 70 eV. 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 DMSO-d_6 or $CDCl_3$ 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. COSY, ROESY, and HMBC digital resolution in ^{1}H spectra was 0.28 Hz, while in ^{13}C spectra it was 0.65 Hz per point.

5,6-*O*,*O*-Isopropylidene-L-ascorbic acid (1),[12] 2,3-*O*,*O*-dibenzyl-5,6-*O*,*O*-isopropylidene-L-ascorbic acid (2),[12] 2,3-*O*,*O*-dibenzyl-L-ascorbic acid (3), 2,3-*O*,*O*-dibenzyl-6-*O*-tosyl-L-ascorbic acid (5),[12] and 6-bromo-2,3-*O*,*O*-dibenzyl-L-ascorbic acid (6)[13] were synthesized in accord with the original procedures given in the literature.

5,6-*O*,*O*-Diacetyl-2,3-*O*,*O*-dibenzyl-L-ascorbic Acid (4). To a cooled (−10 °C) solution of **3** (2 g, 5.6 mmol) in CH_2Cl_2 (80 mL) and pyridine (28 mL) was added dropwise acetic anhydride (28 mL, 296 mmol). Reaction mixture was stirred at room temperature for 2 h and then the solvent was evaporated. The crude product was submitted to column chromatography, yielding pure **4** (1.64 g, 67%, mp 83−86 °C): ^{13}C NMR ($CDCl_3$) δ 168.16 (C-1′), 154.77 (C-2′), 120.98 (C-3′), 73.34 (C-4′), 67.28 (C-5′), 61.66 (C-6′), 169.84, 169.06 (*C*OCH$_3$), 20.23, 20.07 (CO*C*H$_3$) 135.17, 128.65−127.71 (C_6H_5), 73.34, 73.29 (*C*H$_2$Ph).

4-(5,6-Epoxy)-2,3-*O*,*O*-dibenzyl-L-ascorbic Acid (7). To a solution of **6** (1.3 g, 3.1 mmol) in acetonitrile (25 mL) was added an aqueous solution of Na_2CO_3 (0.33 g, 3.1 mmol) and reaction mixture was stirred at room temperature for 1 h. Solvent was then removed under reduced pressure and the oily residue was extracted with chloroform. Organic layers were dried over $MgSO_4$. Recrystallization of oily product from methanol gave white crystals of **7** (0.896 g, 85.5%, mp 85−88 °C): ^{13}C NMR ($CDCl_3$) δ 168.5 (C-1′), 120.46 (C-2′), 156.05 (C-3′), 74.09 (C-4′), 50.33 (C-5′), 43.68 (C-6′), 135.42, 129.08−127.64 (C_6H_5), 73.68, 73.46, (*C*H$_2$Ph); MS *m*/*z* 339 (MH^+).

1-[2,4-Dioxo-(1*H*,3*H*)-pyrimidin-1-yl]-2-(2,3-*O*,*O*-dibenzyl-2-buten-4-olidylidene)ethan-2-ol (8). A suspension of anhydrous uracil (70% excess with respect to the compound **7**, 353 mg, 3.15 mmol) and $(NH_4)_2SO_4$ (30 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 4-(5,6-epoxy)-2,3-*O*,*O*-dibenzyl-L-ascorbic acid (**7**) (332 mg, 0.92 mmol) dissolved in anhydrous acetonitrile (5 mL) was added. 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 (10 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 of the oily residue afforded **8** (303 mg, 73%, mp 113−116 °C): UV (methanol) λ_{max} 206 (log ϵ 2.88), λ_{max} 250 (log ϵ 2.61); ^{13}C NMR ($CDCl_3$) δ 151.46 (C-2), 164.19 (C-4), 100.59 (C-5), 147.14 (C-6), 169.49 (C-1′), 157.79 (C-2′), 121.01 (C-3′), 75.86 (C-4′), 65.45 (C-5′), 51.21 (C-6′), 73.82, 72.97 (*C*H$_2$Ph), 136.15, 129.01−128.08 (C_6H_5); MS *m*/*z* 450 ($M^{+\bullet}$). Anal. ($C_{24}H_{22}N_2O_7$) C, H, N.

1-[2,4-Dioxo-5-fluoro-(1*H*,3*H*)-pyrimidin-1-yl]-2-(2,3-*O*,*O*-dibenzyl-2-buten-4-olidylidene)ethan-2-ol (9). 5-Fluorouracil (60% excess, 205 mg, 1.58 mmol) was treated according to the procedure analogous to that for the preparation of compound **8** to give **9** (169 mg, 57%, mp 85−90 °C): UV (methanol) λ_{max} 208 (log ϵ 4.39), λ_{max} 240 (log ϵ 4.09); ^{13}C NMR ($CDCl_3$) δ 149.93 (C-2), 157.62 (C-4, J = 58.1 Hz), 139.86 (C-5, J = 236.7 Hz), 127.85 (C-6, J = 32.49 Hz), 169.78 (C-1′), 156.85 (C-2′), 121.25 (C-3′), 75.93 (C-4′), 66.60 (C-5′), 51.47 (C-6′), 135.42, 129.08−128.02 (C_6H_5), 73.96, 73.56 (*C*H$_2$Ph); MS *m*/*z* 468 ($M^{+\bullet}$). Anal. ($C_{24}H_{21}N_2O_7F$) C, H, N.

1-[2,4-Dioxo-5-(trifluoromethyl)-(1*H*,3*H*)-pyrimidin-1-yl]-2-(2,3-*O*,*O*-dibenzyl-2-buten-4-olidylidene)ethan-2-ol (10). 5-(Trifluoromethyl)uracil (60% excess, 283 mg, 1.57 mmol) was treated according to the procedure analogous to that for the preparation of compound **8** to give **10** (139 mg, 42%, mp 86−89 °C): UV (methanol) λ_{max} 208 (log ϵ 3.16), λ_{max} 248 (log ϵ 2.89); ^{13}C NMR ($CDCl_3$) δ 150.59 (C-2), 159.51 (C-4), 104.17 (C-5), 147.38 (C-6), 121.22 (CF$_3$, J = 270.39 Hz), 169.71 (C-1′), 156.66 (C-2′), 121.22 (C-3′), 75.76 (C-4′), 66.12 (C-5′), 51.80 (C-6′), 73.96, 73.78 (*C*H$_2$Ph), 135.26, 129.20−128.34 (C_6H_5); MS *m*/*z* 518 ($M^{+\bullet}$). Anal. ($C_{25}H_{21}N_2O_7F_3$) C, H, N.

1-[2,4-Dioxo-(1*H*,3*H*)-pyrimidin-1-yl]-2-(3-*O*-benzyl-2-hydroxy-2-buten-4-olidylidene)ethane (14) and 1-[2,4-Dioxo-(1*H*,3*H*)-pyrimidin-1-yl]-2-(2,3-dihydroxy-2-buten-4-olidylidene)ethane (15). To a solution of UDBnAA **(11)** (200 mg, 0.46 mmol) in dry CH_2Cl_2 at −78 °C under argon was added a 1 M solution of BCl_3 in CH_2Cl_2 (0.63 mL). The mixture was stirred at −78 °C for 2 h, then the temperature was raised to 10 °C and the reaction was continued for 2 h. A mixture of CH_2Cl_2/MeOH (1:1) was added and the solvent was then removed under reduced pressure. The crude product, purified by column chromatography, yielded **14** (41 mg, 25%, mp 189−190 °C) and **15** (49 mg, 39%, mp 202−204 °C). **14:** UV (methanol) λ_{max} 208 (log ϵ 4.43), λ_{max} 268 (log ϵ 4.32); ^{13}C NMR (DMSO-d_6) δ 150.79 (C-2), 163.71 (C-4), 101.24 (C-5), 145.34 (C-6), 164.66 (C-1′), 123.36 (C-2′), 141.10 (C-3′), 143.70 (C-4′), 100.95 (C-5′), 42.59 (C-6′), 136.12, 128.52−127.98 (C_6H_5), 72.17 (*C*H$_2$Ph); MS *m*/*z* 342 ($M^{+\bullet}$). Anal. ($C_{17}H_{14}N_2O_6$) C, H, N. **15:** UV (methanol) λ_{max} 208 (log ϵ 3.91), λ_{max} 264 (log ϵ 3.99); ^{13}C NMR (DMSO-d_6) δ 150.79 (C-2), 163.66 (C-4), 101.26 (C-5), 145.29 (C-6), 164.78 (C-1′), 121.49 (C-2′), 143.21 (C-3′), 144.57 (C-4′), 100.24 (C-5′), 42.60 (C-6′); MS *m*/*z* 252 ($M^{+\bullet}$). Anal. ($C_{10}H_8N_2O_6$) C, H, N.

1-[2,4-Dioxo-5-fluoro-(1*H*,3*H*)-pyrimidin-1-yl]-2-(2,3-dihydroxy-2-buten-4-olidylidene)ethane (16). Compound **12** (290 mg, 0.64 mmol) was treated according to a procedure that was analogous to that for the preparation of compounds **14** and **15** to give **16** (13 mg, 6.8%, mp 242−244 °C): UV (methanol) λ_{max} 208 (log ϵ 4.09), λ_{max} 274 (log ϵ 4.13); ^{13}C NMR (DMSO-d_6) δ 149.41 (C-2), 157.40 (C-4, J = 25.77 Hz), 139.63 (C-5, J = 229.25 Hz), 129.78 (C-6, J = 33.60 Hz), 164.75 (C-1′), 121.48 (C-2′), 143.13 (C-3′), 144.46 (C-4′), 100.02 (C-5′), 42.92 (C-6′); MS *m*/*z* 270 ($M^{+\bullet}$). Anal. ($C_{10}H_7N_2O_6F$) C, H, N.

1-[2,4-Dioxo-(5-trifluoromethyl)-(1*H*,3*H*)-pyrimidin-1-yl]-2-(2,3-dihydroxy-2-buten-4-olidylidene)ethane (17). Compound **13** (250 mg, 0.5 mmol) was treated according to a procedure that was analogous to that for the preparation of compounds **14** and **15** to give **17** (16 mg, 9.1%, mp 292−294 °C): ^{13}C NMR (DMSO-d_6) δ 150.01 (C-2), 159.40 (C-4), 102.21 (C-5, J = 32.04 Hz), 147.28 (C-6), 122.71 (CF$_3$, J = 269.06 Hz), 164.75 (C-1′), 121.43 (C-2′), 143.24 (C-3′), 144.27 (C-4′), 100.11 (C-5′), 43.86 (C-6′); MS *m*/*z* 320.20 ($M^{+\bullet}$). Anal. ($C_{11}H_7N_2O_6F_3$) C, H, N.

X-ray Determination. The single crystal of **13** suitable for X-ray structure analysis was obtained by growth at room temperature of a very dilute solution of ethanol. The intensities were measured on a Philips PW1100 diffractometer upgraded by Stoe[20] using Mo Kα radiation (λ = 0.71073 Å) at 20 °C with the ω scan mode and corrected only for Lorentz polarization factor. During the data collection crystal decomposition of 21.3% was observed. The structure was solved by direct methods and refined by full-matric least-squares on F^2, using SHELXL93[21] program package. The hydrogen atoms were either located in a difference Fourier synthesis or generated and allowed to ride at a fixed distance from the attached atoms except for the hydrogen atom bonded to the pyrimidine nitrogen which was refined. A weighting scheme $w = 1/[\sigma^2 F_o + 0.0525P^2]$, where $P = (F_o^2 + 2F_c^2)/3$, was assumed for all observations. The final difference map contained no significant features.

Crystal data for **13**: $M_r = 500.42$, space group *P*-1; $a = 9.299(4)$, $b = 11.760(3)$, $c = 12.277(4)$ Å; $\alpha = 109.31(2)$, $\beta = 97.36(3)$, $\gamma = 107.49(3)°$; $V = 1169.2(7)$ Å^3, $Z = 2$, $F(000) = 516$, $d_x = 1.421$ g cm^{-3}; μ(Mo Kα) = 0.118 mm^{-1}, $S = 0.851$; $R/R_w = 0.0440/0.1007$ for 328 parameters and 1560 reflections with $I \geq 2\sigma(I)$, $R/R_w = 0.1357/0.2164$ for all 5609 independent reflections measured in the range $2.10° - 2\Theta - 27.96°$.

Materials for Biological Tests. Cell culturing: Antitumor activities against L1210/0 (murine leukemia), FM3A/0 (murine mammary carcinoma), Molt4/C8 (human T-lymphoblast), and CEM/0 (human T-lymphoblast) cell lines as well as inhibition of the proliferation of human embryonic cell (HEL) and murine embryo (MEF) fibroblasts were measured essentially as originally described for the mouse leukemia/L1210 cell lines.[22]

Antiviral activity assays: Antiviral activities against thymidine kinase-positive (TK^+) and -negative (TK^-) strains of varicella-zoster virus (VZV), cytomegalovirus (CMV),[23] and human immunodeficiency virus (HIV)[24] were determined as described previously.[23,24]

Acknowledgment. Support for this study by the Ministry of Science of Croatia (Project No. 175.003) is gratefully acknowledged. We thank Lizette van Berckelaer for excellent technical assistance in performing the antitumor cell activity assays, as well as Ann Absillis, Anita Van Lierde, Frieda De Meyer, Anita Camps, and Lies Vandenheurck for excellent technical assistance in performing the antiviral activity assays. We also thank Dr. Vanja Vela (Pliva Research Institute) for providing some starting materials and Dr. Oliver Zerbe (Department of ETH, Zürich) for recording and helpful discussions concerning one- and two-dimensional NMR spectra.

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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JM0009540