Synthesis and Antiviral and Cytostatic Evaluations of the New C-5 Substituted Pyrimidine and Furo[2,3-*d*]pyrimidine 4′,5′-Didehydro-L-ascorbic Acid Derivatives

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The novel C-5 alkynyl substituted pyrimidine (**1**−**11**) and furo[2,3-*d*]pyrimidine derivatives (**12**−**22**) of L-ascorbic acid were synthesized by coupling of 5-iodouracil-4′,5′-didehydro-5′,6′-dideoxy-L-ascorbic acid with terminal alkynes by using Sonogashira cross-coupling reaction conditions. The new compounds were evaluated for their cytostatic and antiviral activities. Among all evaluated compounds, the octynyl-substituted uracil derivative of L-ascorbic acid (**3**) exhibited the most pronounced cytostatic activities against all examined tumor cell lines (IC_{50} = 2−12 μM). Pyrimidine derivatives of L-ascorbic acid containing *p*-substituted phenylacetylene groups (**8**−**11**) displayed also a rather pronounced (IC_{50} = 3−37 μM) inhibitory effect toward all tumor cell lines. From the bicyclic series of compounds, 6-butylfuro[2,3-*d*]pyrimidine derivative (**12**) and 6-*p*-bromophenylfuro[2,3-*d*]pyrimidine derivative (**19**) showed the highest cytostatic activity (IC_{50} = 4.5−20 μM), particularly against malignant leukemia (L1210) and T-lymphocyte (Molt4/C8 and CEM) cells. Compounds **3** and **9** showed specific albeit moderate activity against cytomegalovirus (CMV, Davis strain, EC_{50} = 1.8 and 3.8 μM, respectively, for compounds **3** and **9**) at a ∼5-fold lower concentration than that required to show cytotoxicity.

Introduction

L-Ascorbic acid is one of the most important biomolecules. It acts as an antioxidant and radical scavenger widely distributed in aerobic organisms.[1] Thus, it protects cellular compounds against oxidative damage by free radicals and oxidants.[2] It was found that L-ascorbic acid derivatives induce apoptosis in tumor cells,[3] possess immunostimulant activity,[4,5] and/or protect against the lipid peroxidation of the biomembrane.[6,7] Drug conjugation with L-ascorbic acid can improve the delivery of drugs into the brain, via interaction with the sodium-dependent ascorbate transporter (SVCT2[a]).[8] Furthermore, many nucleoside analogues with interesting biological properties have arisen by substitution at the 5-position of the uracil base in the 2′-deoxyuridine series.[9] The 5-(2-substituted) vinyl-2′-deoxyuridines, in particular (*E*)-5-(2-bromovinyl)-2′-deoxyuridine, have emerged as potent and selective inhibitors of herpes virus replication, particularly against HSV-1 (herpes simplex virus type 1) and VZV (varicella-zoster virus).[10−12] Pyrimidine nucleosides containing C-5 alkynyl groups have been shown to possess significant antiviral and/or anticancer properties.[13] The 5-alkynyluracil nucleosides with a longer alkynyl chain at the C-5 position exhibited appreciable antiviral activity in contrast to the corresponding alkyl derivatives that showed decreasing antiviral activity with increasing C-5 side chain length.[13−16] Moreover, bicyclic nucleoside analogues represent highly specific and extremely potent antiviral agents.[17−19] SAR studies on furo[2,3-*d*]pyrimidine nucleoside analogues have shown that the length of the alkyl chain at the C-6 position of the furopyrimidine ring plays a crucial role in their anti-VZV potency. Compounds with shorter alkyl chains ($\leq C_6$) had little or no activity, while those with a C7 or C11 side chain had moderate activity, and those with C8 to C10 alkyl chains had the highest potency.[19−21] We have reported that some pyrimidine and purine derivatives of 4′,5′-didehydro-5′,6′-dideoxy-L-ascorbic acid exerted pronounced cytostatic activities against malignant tumor cell lines.[22−24] Recently we have also reported that some C-5 substituted pyrimidine derivatives of L-ascorbic acid exhibited selective albeit slight inhibitory activity against certain human tumor cell lines and moderate but not highly specific inhibitory potential against herpes simplex viruses type 1 and 2, vaccinia virus, and Punta Toro virus.[25] These results prompted us to synthesize a series of the novel C-5 pyrimidine derivatives of L-ascorbic acid containing an acetylene with longer side chain and *p*-alkylphenyl acetylene (**1**−**11**), and variously C-6 substituted furo[2,3-*d*]pyrimidine (**12**−**22**) moiety (Figure 1). The

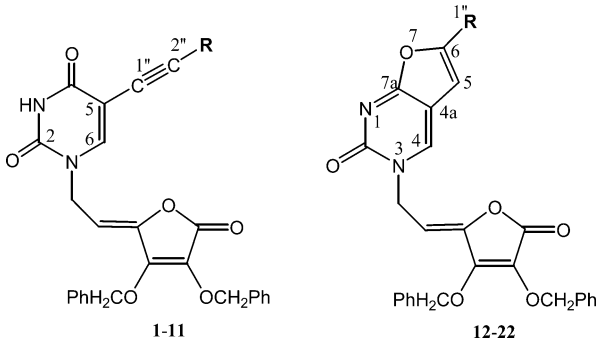

Figure 1. The C-5 alkynylated pyrimidine (**1**−**11**) and 6-alkylfuro-[2,3-*d*]pyrimidine (**12**−**22**) derivatives of L-ascorbic acid.

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[a] Abbreviations: CMV, cytomegalovirus; TK^+ VZV, varicella-zoster virus (thymidine kinase positive strain); TK^- VZV, varicella-zoster virus (thymidine kinase deficient strain); HSV-1, herpes simplex virus type 1 HSV-2, herpes simplex virus type 2; SVCT2, sodium-dependent transporter of L-ascorbic acid; IUAA, 5-iodouracil-2′,3′-di-*O*-benzyl-4′,5′-didehydro-5′,6′-dideoxy-L-ascorbic acid; ABAA, 5′,6′-di-*O*-acetyl-2′,3′-di-*O*-benzyl-L-ascorbic acid; *S*-DHPA, (*S*)-9-(1,3-dihydroxypropyl)adenine.

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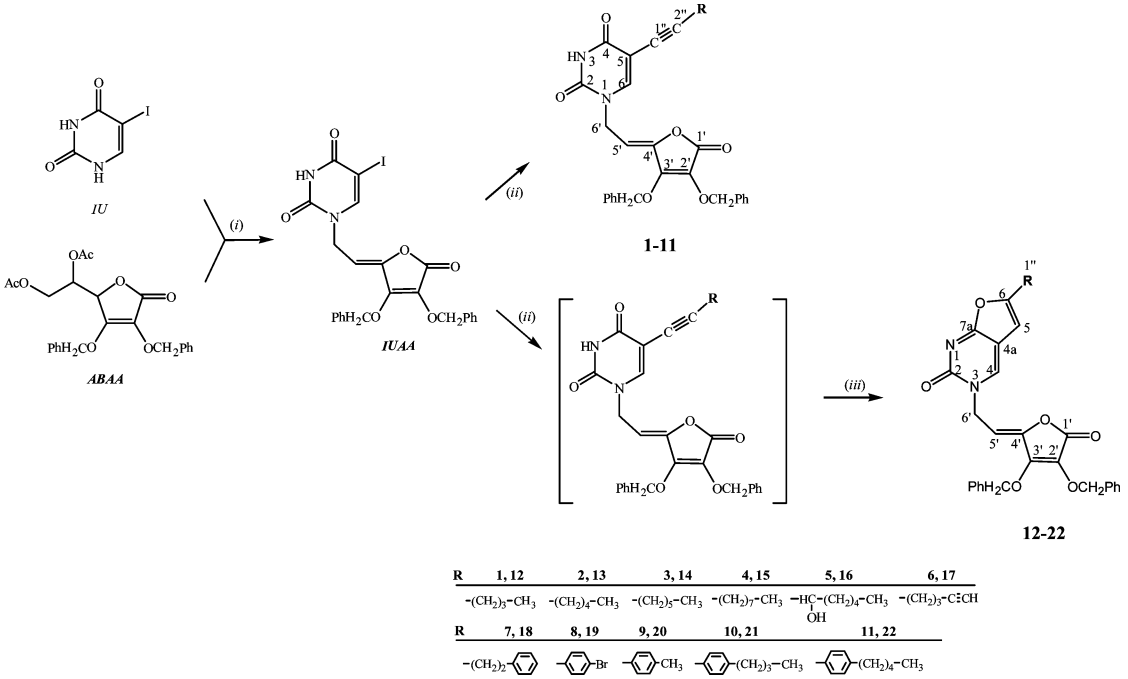
Scheme 1. Synthesis of the C-5 Substituted Pyrimidine (**1**−**11**) and Furo[2,3-*d*]pyrimidine (**12**−**22**) Derivatives of 4′,5′-Didehydro-5′,6′-dideoxy-L-ascorbic Acid[a]



R	1, 12	2, 13	3, 14	4, 15	5, 16	6, 17
	-(CH$_2$)$_3$-CH$_3$	-(CH$_2$)$_4$-CH$_3$	-(CH$_2$)$_5$-CH$_3$	-(CH$_2$)$_7$-CH$_3$	-HC(OH)-(CH$_2$)$_4$-CH$_3$	-(CH$_2$)$_3$-C≡CH

R	7, 18	8, 19	9, 20	10, 21	11, 22
	-(CH$_2$)$_2$-Ph	-Ph-Br	-Ph-CH$_3$	-Ph-(CH$_2$)$_3$-CH$_3$	-Ph-(CH$_2$)$_4$-CH$_3$

[a] Reagents and conditions: (*i*) HMDS, $(NH_4)_2SO_4$, reflux, then TMS-triflate, CH_3CN, 60 °C; (*ii*) R−C≡CH, *i*Pr_2EtN, $(PPh_3)_4Pd$, CuI, DMF, rt; (*iii*) Et_3N, CuI, 60 °C.

principal aim of this study was to evaluate the cytostatic and antiviral potencies of this class of novel compounds.

Chemistry

5-Iodouracil-2′,3′-di-*O*-benzyl-4′,5′-didehydro-5′,6′-dideoxy-L-ascorbic acid (IUAA),[22] the key precursor for cross-coupling with alkynes, was synthesized by condensation of 5′,6′-di-*O*-acetyl-2′,3′-di-*O*-benzyl-L-ascorbic acid (ABAA)[26,27] and 5-iodouracil. IUAA was obtained as a mixture of *Z* and *E* isomers in which the *Z* isomer predominated (in the range 80−90%). These stereoisomers were separated by column chromatography and pure *Z* isomer is applied in subsequent reactions (Scheme 1). Different substituents at the C-5 position of the pyrimidine ring were introduced by reaction of IUAA under optimized Sonogashira Pd(0)-catalyzed reactions.[28−33] It is important to note that this reaction must be run at room temperature to avoid the formation of byproducts produced by heating the reaction mixture. Reaction of IUAA with various terminal alkynes under Sonogashira conditions gave 5-substituted uracil derivatives **1**−**11** (Scheme 1). The targeted 6-alkylfuro[2,3-*d*]pyrimidine-2-one L-ascorbic acid derivatives (**12**−**22**) were synthesized by Sonogashira coupling of terminal alkynes with IUAA and copper(I)-promoted *in situ* cyclization.[30,34] These fused bicyclic pyrimidine analogues were prepared through an *O*-heteroannulation process using base and CuI as a catalyst (Supporting Information, Scheme S1). This 5-endo-dig (electrophile) cyclization of akynyl derivatives (**1**−**11**) involving the C-4 pyrimidine oxygen and acetylenic bond gave the targeted bicyclic nucleoside compounds (**12**−**22**).

The structures of the novel C-5 alkynyl substituted pyrimidine (**1**−**11**) and furo[2,3-*d*]pyrimidine derivatives (**12**−**22**) were determined on the basis of analysis of chemical shifts and H−H coupling constants in ^{1}H (Experimental Section) and ^{13}C NMR spectra (Supporting Information). The cyclization is accompanied by a new vinylic proton H-5 signal (^{1}H NMR, δ 6−7 ppm) and dramatic downfield shift of C-5 and C-6 for **12**−**22** in the ^{13}C NMR spectra in comparison with acetylenic carbons C-1″ and C-2″ for **1**−**11** (e.g., C-1″ at 89.41 ppm for **1** → C-5 at 94.41 ppm for **12**, C-2″ at 97.35 ppm for **1** → C-6 at 138.11 ppm for **12**).

Biological Results

Antiviral Activity. The compounds **1**−**22** were evaluated for their inhibitory activities against varicella-zoster virus (TK^+-VZV, thymidine kinase-positive, and TK^-VZV, thymidine kinase-deficient strains) and cytomegalovirus (CMV, AD-169 and Davis strains) in human embryonic lung (HEL) cells; vesicular stomatitis virus in HeLa cell culture; parainfluenza-3-virus in Vero cell culture; herpes simplex virus type 1 and 2 (HSV-1 and HSV-2) in HEL cell culture; reovirus-1, Sindbis, and Punta Toro virus in Vero cell cultures; and Coxsackie B4 virus in HeLa cell cultures (Tables 1 and 2, and Supporting Information). Their activities were compared with those of ganciclovir [9-[(1,3-dihydroxy-2-propoxy)methyl]guanine], cidofovir [(*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine], acyclovir [9-(3-hydroxyethoxymethyl)guanine], brivudin [(*E*)-5-(2-bromovinyl)-2′-deoxyuridine], (*S*)-DHPA [(*S*)-9-(1,3-dihydroxypropyl)adenine], and ribavirin [1-(β-D-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide] (Tables 1 and 2).

A variety of derivatives (i.e., the 5-alkynylpyrimidines **2**, **3**, **7**, **9**, and **10** and the furo[2,3-*d*]pyrimidine **14**) showed a slight anti-CMV (Davis strain) activity (EC_{50} = 1.8−8.4 μM) in HEL cell cultures (MCC, minimum cytotoxic concentration, 6−20-fold higher than the EC_{50} values). Such activity is within the same order of magnitude as that of ganciclovir (EC_{50} = 2.6 μM) (Table 1). However, it should also be mentioned that they show cytostatic activities at concentrations that are only 3−5-fold higher than the antiviral active concentrations. Except for compound **14**, which was able to inhibit the replication of both laboratory CMV strains with EC_{50} values of 20 μM (AD-169) and 6.0 μM (Davis), the compounds had no significant activity against the AD-169 strain of CMV.

Table 1. Activity of the Compounds **1**−**22** against Varicella-Zoster Virus (VZV) and Cytomegalovirus (CMV) in Human Embryonic Lung (HEL) Cells

	antiviral activity, EC_{50} (μM)[a]				cytotoxicity (μM)	
			CMV			
compd	TK^+VZV OKA strain	TK^-VZV 07/1 strain	AD-169 strain	Davis strain	cell morphology (MCC)[b]	cell growth (CC_{50})[c]
1	17.5	>20	>20	>20	≥100	≥68
2	>4	>4	>4	3.1	20	12.3
3	>1.6	>1.6	>1.6	1.8	20	10
4	>20	>20	>20	>20	100	≥84
5	51	49	≥100	28.4	≥100	≥33
6	>20	>20	>20	≥20	≥20	≥86
7	10.2	11.9	>20	8.4	60	≥28.5
8	7.3	9.3	>4	5.2	≥4	≥10.9
9	>4	>4	10	3.8	60	≥20.4
10	>4	>4	8.2	3.1	60	≥9.3
11	3.3	>4	≥4	2.4	≥4	≥8.6
12	>4	>4	>4	≥2.9	20	≥8.3
13	>20	>20	>20	≥14.5	100	41
14	>20	>20	20	6.0	100	41
15	>20	>20	>20	>20	100	>100
16	78	>100	>100	>100	≥100	>100
17	>100	80	>100	≥69	>100	>100
18	>20	>20	>20	12	100	>100
19	>20	>20	11	8.9	≥20	44
20	>20	>20	>20	≥14.5	100	46
21	>20	>20	>20	>20	≥20	50
22	>20	>20	>20	≥20	≥20	50
ganciclovir	–	–	6.5	2.6	≥1575	262
cidofovir	–	–	0.67	0.67	≥1270	133
acyclovir	1.0	32	–	–	>1778	545
brivudin	0.014	168	–	–	≥1201	270

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque-forming units (PFU). [b] Minimum cytotoxic concentration causing a microscopically detectable alteration of normal cell morphology. [c] Cytostatic concentration required to reduce cell growth by 50%.

Table 2. Activity of the Compounds **1**−**22** against Vesicular Stomatitis Virus in HeLa Cell Culture, Parainfluenza-3-virus in Vero Cell Culture, and Herpes Simplex Viruses Type 1 and 2 (HSV-1 and HSV-2) in HeL Cell Culture

	minimum inhibitory concentration[a] (μM)				
compd	vesicular stomatitis virus	Parainfluenza-3-virus	HSV-1 (KOS)	HSV-2 (G)	minimum cytotoxic concn[b] (μM)
1	>16	>3.2	>16	>16	80
2	>8	4.8	8	>8	40
3	>8	>8	>1.6	>1.6	40
4	>4	>4	>4	>4	20
5	40	>8	>8	>8	200
6	>40	>8	>8	>8	200
7	>8	4.8	>8	>8	40
8	>8	>1.6	>1.6	>1.6	40
9	>8	8	>1.6	>1.6	40
10	8	>8	>1.6	>1.6	40
11	>4	>4	>4	>4	20
12	>8	>1.6	>1.6	>1.6	40
13	40	>8	>8	>8	200
14	>8	>8	>8	>8	40
15	>40	>40	>40	>40	200
16	>40	>40	120	120	200
17	>40	>40	>40	>40	200
18	>40	40	>40	>40	200
19	>8	>8	>8	>8	40
20	>40	>8	>8	>8	200
21	>40	40	>40	>40	200
22	>40	>40	>40	>40	200
brivudin	>250	>250	0.08	10	>250
(*S*)-DHPA	>250	50	–	–	>250
ribavirin	>250	150	250	250	30
acyclovir	–	–	0.4	0.4	>250
ganciclovir	–	–	0.032	0.0064	>100

[a] Required to reduce virus-induced cytopathogenicity by 50%. [b] Required to cause a microscopically detectable alteration of normal cell morphology.

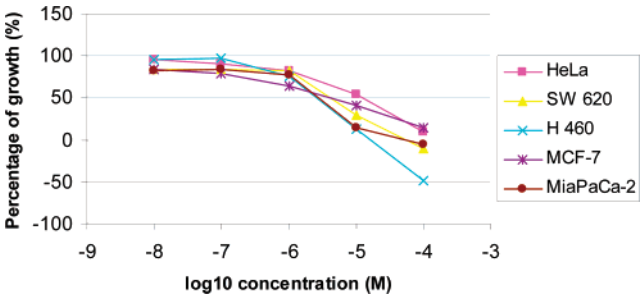


Figure 2. Dose−response profiles for compound **3** tested on various human tumor cell lines in vitro.

C-5 alkynyl pyrimidine derivatives of L-ascorbic acid containing phenylacetylene (**7**) and *p*-bromophenylacetylene (**8**) side chains showed EC_{50} values against varicella-zoster virus (OKA and 07/1 strains) in the range of 7−12 μM (Table 1). However, compound **8** produced an alteration of the cellular morphology at concentrations similar to those at which the compound inhibited viral plaque formation (MCC ≥ 4), and both compounds **7** and **8** showed cytostatic effects for HEL cells (CC_{50} ≥ 28.5 and 10.9, respectively, for compound **7** and **8**), resulting in a poor selectivity. On the contrary, the furo[2,3-*d*]pyrimidine derivatives **12**−**22** did not exhibit an inhibitory effect against varicella-zoster virus. Furthermore, 5-alkynyluracil (**2**, **7**, **9** and **10**) and furo[2,3-*d*]pyrimidine (**13**) derivatives displayed also some specific activity against parainfluenza-3-virus (EC_{50}: **2**, 4.8 μM; **7**, 4.8 μM; **9**, 8 μM), herpes simplex virus type 1 (EC_{50}: **2**, 8 μM) and vesicular stomatitis virus (EC_{50}: **10**, 8 μM; **13**, 40 μM) (Table 2). None of the compounds were active against vaccinia virus, respiratory syncytial virus, reovirus-1, Sindbis virus, Coxsackie virus B4, and Punta Toro virus (Supporting Information).

The antiviral and cytostatic activities observed for several of the pyrimidine derivatives of L-ascorbic acid are interesting, since these compounds may represent novel antiviral leads. Indeed, due to the lack of a free hydroxyl group in the ascorbic

Table 3. Inhibitory Effects of Compounds **1**−**22** on the Growth of Malignant Tumor Cell Lines.

	IC_{50} (μM)[a]							
compd	L1210	Molt4/C8	CEM	HeLa	MiaPaCa-2	SW 620	MCF-7	H-460
1	19 ± 6	15 ± 2	9.4 ± 5.0	16 ± 3	16 ± 2	23 ± 2	18 ± 1	NT[b]
2	10 ± 3	19 ± 14	8.2 ± 0.2	14 ± 0.5	13 ± 0.5	14 ± 3	15 ± 3	15 ± 1
3	6.8 ± 1.7	3.0 ± 1.1	2.0 ± 0.3	12 ± 2	3 ± 0.5	4 ± 0.3	4 ± 1.4	2.4 ± 0.4
4	220 ± 52	218 ± 36	81 ± 24	56 ± 35	>100	>100	>100	NT[b]
5	82 ± 42	167 ± 64	45 ± 1	35 ± 19	37 ± 6	23 ± 9	55 ± 11	>100
6	68 ± 38	177 ± 18	55 ± 13	38 ± 16	≥100	≥100	NT[b]	≥100
7	24 ± 9	36 ± 7	9.2 ± 0.4	16 ± 0.7	15 ± 0	16 ± 4	20 ± 3	18 ± 0.3
8	10 ± 1	15 ± 7	7.6 ± 0.6	8 ± 6	13 ± 0.3	7 ± 0.04	21 ± 6	11 ± 2.5
9	8.7 ± 1.4	37 ± 6	9.6 ± 1.1	14 ± 2	21 ± 3	18 ± 2	19 ± 7	23 ± 2.8
10	8.2 ± 2.3	9.3 ± 1.0	8.3 ± 0.2	4 ± 1	16 ± 0.3	7 ± 1.5	17 ± 11	12 ± 1
11	8.0 ± 0.0	6.9 ± 0.3	6.6 ± 0.4	3 ± 1	10 ± 9	6 ± 0.8	9 ± 4	NT[b]
12	4.5 ± 4.0	9.0 ± 0.3	7.7 ± 0.1	17 ± 2	15 ± 3	16 ± 3	16 ± 0.1	16 ± 2
13	38 ± 6	45 ± 5	25 ± 6	17 ± 4	17 ± 2	15 ± 4	19 ± 11	23 ± 1.6
14	41 ± 6	40 ± 8	10 ± 0	20 ± 1.5	22 ± 5	19 ± 2	52 ± 27	28 ± 11
15	117 ± 8	105 ± 7	96 ± 3	>100	>100	>100	NT[b]	>100
16	164 ± 20	142 ± 6	106 ± 7	60 ± 39	78 ± 20	>100	33 ± 21	64 ± 36
17	172 ± 19	190 ± 9	94 ± 40	>100	>100	>100	≥100	>100
18	80 ± 38	120 ± 39	49 ± 8	>100	>100	>100	>100	>100
19	9.5 ± 0.6	9.6 ± 0.8	8.3 ± 0.4	20 ± 4	14 ± 2	20 ± 9	13 ± 0.2	16 ± 0.4
20	44 ± 5	41 ± 2	35 ± 4	14 ± 0.1	16 ± 2	20 ± 0.6	20 ± 3	17 ± 1.6
21	38 ± 4	45 ± 2	38 ± 1	11 ± 0.1	13 ± 1.2	16 ± 0.5	16 ± 3	33 ± 4
22	40 ± 3	44 ± 3	36 ± 6	14 ± 2	11 ± 0.9	16 ± 0.5	12 ± 0.2	25 ± 7

[a] 50% inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%. [b] NT, not tested.

acid moiety of the molecules, it is excluded that the compounds can be phosphorylated. Therefore, their mechanism of antiviral action should be different from that of nucleoside analogues such as ganciclovir. The equal anti-VZV activity against TK^+ and TK^- strains is in agreement with these observations. It would now be imperative to further investigate the mechanism of antiviral action of these compounds. Also, additional efforts will be devoted to increase the antiviral selectivity by increasing the antiviral potency and/or by decreasing their cytostatic/cytotoxic activity.

Cytostatic Activity. Compounds **1**−**22** were evaluated for their cytostatic activities against the following malignant human tumor cell lines: murine leukemia (L1210), human T-lymphocytes (Molt4/C8, CEM), cervical carcinoma (HeLa), pancreatic carcinoma (MiaPaCa-2), colon carcinoma (SW 620), breast carcinoma (MCF-7), and non-small cell lung carcinoma (H-460) (Table 3). From C-5 alkynyl uracil series, compounds **3** (Figure 2) and **8**−**11** exhibited pronounced antiproliferative activities against all tumor cell lines (IC_{50} in the range 3−37 μM). Thus, correlation of the C-5 alkynyl side chains (**1**−**4**) clearly revealed that the cytostatic activity was strongly and nonlinearly dependent on the side chain length, showing that **3** with a C8 side chain had the strongest inhibitory activity. Among furo[2,3-*d*]pyrimidine derivatives, 6-butyl- (**12**) and 6-*p*-bromophenylfuro[2,3-*d*]pyrimidine (**19**) derivatives of L-ascorbic acid showed the highest cytostatic activities, particularly against malignant leukemia (L1210 IC_{50}: **12**, 4.5 μM; **19**, 9.5 μM) and T-lymphocytes (Molt4/C8 IC_{50}: **12**, 9 μM; **19**, 9.6 μM; CEM IC_{50}: **12**, 7.7 μM; **19**, 8.3 μM).

Conclusions

The novel C-5 alkynyl uracil derivatives of L-ascorbic acid (**1**−**11**) were prepared by Sonogashira coupling of 5-iodouracil derivatives of L-ascorbic acid with various terminal alkynes. Subsequent in situ cyclization of **1**−**11** catalyzed with base and copper(I) iodide gave fused bicyclic furo[2,3-*d*]pyrimidine derivatives (**12**−**22**). Comparison of cytostatic activities of 5-alkynyluracil (**1**−**11**) and furo[2,3-*d*]pyrimidine (**12**−**22**) series indicated that cyclization of the alkynyl side chain caused a significant reduction in the inhibitory effects, except for **12** and **19**, which showed cytostatic activities similar to those of their parent 5-alkynyluracil derivatives (**1** and **8**). Moreover, introduction of a *p*-substituted phenylacetylene group in **8**−**11** increased the cytostatic effects in comparison with their parent alkynyl chain analogues (**1**−**2** and **4**−**6**).

By comparison of the antiviral activities of the two series of compounds, we can infer that the C-5 alkynylpyrimidine derivatives of L-ascorbic acid showed a better antiviral potential. Compounds **2**, **3**, **7**, **9**, and **10** showed some anti-CMV activity (Davis strain) with EC_{50} values similar to those of the reference anti-CMV compound ganciclovir; however, their selectivities were relatively limited. Compound **14** was the only derivative able to inhibit the replication of both CMV laboratory strains. The mechanism of action of these new derivatives, although still unclear, is different from that of ganciclovir, making these compounds novel antiviral leads to be further explored.

Experimental Section

General Methods. The bicyclic ring has been numbered in accordance with the recommended IUPAC nomenclature guidelines. Melting points (uncorrected) were determined with a Kofler micro hot-stage (Reichert, Wien). High-field one- and two-dimensional ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 spectrometer, operating at 75.46 MHz for the ^{13}C resonance. The samples were dissolved in DMSO-d_6 and measured in 5 mm NMR tubes. The ^1H and ^{13}C NMR chemical shift values (δ) are expressed in ppm referred to TMS and coupling constants (J) in hertz. 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. Precoated E. Merck silica gel 60F-254 plates were used for thin layer chromatography (TLC) and for preparative TLC, and the spots were detected under UV light (254 nm). Column chromatography (CLC) was performed using Fluka silica gel (0.063−0.2 mm); glass columns were slurry-packed under gravity. Compound purity was analyzed by HPLC with DAD detector. The novel compounds **1**−**22** are soluble in dichloromethane, acetonitrile, tetrahydrofuran, acetone, and dimethyl sulfoxide.

General Procedure for the Preparation of 5-Substituted Uracil Derivatives of L-Ascorbic Acid (1−11). To a stirred solution of 5-iodouracil-2′,3′-di-*O*-benzyl-L-ascorbic acid (200 mg, 0.36 mmol) in anhydrous dimethylformamide (10 mL) were added diisopropylethylamine (0.13 mL, 0.72 mmol), the acetylene deriva-

tive (0.54 mmol), tetrakis(triphenylphosphine)palladium(0) (41.5 mg, 0.036 mmol), and copper(I) iodide (13.4 mg, 0.072 mmol). The mixture was stirred for 20 h at room temperature under nitrogen and then evaporated to dryness and purified by column chromatography (initial eluent CH_2Cl_2, followed by CH_2Cl_2:MeOH = 60:1).

1-[5-(Hex-1″-yn-1″-yl)uracil-1-yl]-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (1). The procedure was carried out using hexyne (0.06 mL, 0.54 mmol). Additional purification by preparative thin layer chromatography gave brown oil **1** (76 mg, 35.1%). ^{1}H NMR: 11.52 (1H, s, NH), 7.94 (1H, s, H-6), 7.22−7.41 (10H, m, Ph), 5.53 (1H, t, J = 6.46 Hz, H-5′), 5.27 (2H, s, OCH_2), 5.19 (2H, s, OCH_2), 4.51 (2H, d, J = 7.01 Hz, H-6′), 2.41 (2H, m, H-3″), 1.48 (2H, m, H-4″), 1.27 (2H, m, H-5″), 0.83 (3H, m, CH_3) ppm. MS: *m*/*z* calcd 512.19, found 512.6 ($M^{+\cdot}$). Anal. ($C_{30}H_{28}N_2O_6$) C, H, N.

1-[5-(Hept-1″-yn-1″-yl)uracil-1-yl]-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (2). The procedure was carried out using heptyne (0.07 mL, 0.54 mmol), which gave oil **2** (92 mg, 48.7%). ^{1}H NMR: δ 11.52 (1H, s, NH), 7.92 (1H, s, H-6), 7.22−7.41 (10H, m, Ph), 5.40 (1H, t, J = 6.61 Hz, H-5′), 5.30 (2H, s, OCH_2), 5.14 (2H, s, OCH_2), 4.51 (2H, d, J = 6.71 Hz, H-6′), 2.35 (2H, t, J = 6.82 Hz, H-3″), 1.46 (2H, m, H-4″), 1.26−1.35 (4H, m, H-5″, H-6″), 0.87 (3H, t, J = 6.87 Hz, CH_3) ppm. MS: *m*/*z* calcd 526.21, found 526.6 ($M^{+\cdot}$). Anal. ($C_{31}H_{30}N_2O_6$) C, H, N.

1-[5-(Oct-1″-yn-1″-yl)uracil-1-yl]-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (3). The procedure was carried out using octyne (0.08 mL, 0.54 mmol), and additional purification by preparative thin layer chromatography afforded oil **3** (71 mg, 36.5%). ^{1}H NMR: δ 11.38 (1H, s, NH), 8.14 (1H, s, H-6), 7.31−7.45 (10H, m, Ph), 5.29 (1H, t, J = 6.40 Hz, H-5′), 4.99 (2H, s, OCH_2), 4.87 (2H, s, OCH_2), 3.98 (2H, d, J = 7.08 Hz, H-6′), 2.83 (2H, m, H-3″), 1.21−1.48 (8H, m, H-4″−7″), 0.81 (3H, m, CH_3) ppm. MS: *m*/*z* calcd 540.23, found 540.6 ($M^{+\cdot}$). Anal. ($C_{32}H_{32}N_2O_6$) C, H, N.

1-[5-(Dec-1″-yn-1″-yl)uracil-1-yl]-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (4). The procedure was carried out using decyne (0.4 mL, 0.54 mmol), which gave oil **4** (39.2 mg, 19.1%). ^{1}H NMR: δ 11.42 (1H, s, NH), 8.43 (1H, s, H-6), 7.24−7.41 (10H, m, Ph), 5.51 (1H, t, J = 6.48 Hz, H-5′), 5.27 (2H, s, OCH_2), 5.17 (2H, s, OCH_2), 4.81 (2H, d, J = 7.16 Hz, H-6′), 2.67 (2H, m, H-3″), 1.66 (2H, m, H-4″), 1.22−1.27 (10H, m, H-5″−9″), 0.87 (3H, m, CH_3) ppm. MS: *m*/*z* calcd 568.26, found 568.7 ($M^{+\cdot}$). Anal. ($C_{34}H_{36}N_2O_6$) C, H, N.

1-[5-(3″-Hydroxyoct-1″-yn-1″-yl)uracil-1-yl]-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (5). The procedure was carried out using 3-hydroxy-1-octyne (0.08 mL, 0.54 mmol), which gave oil **5** (76 mg, 38%). ^{1}H NMR: δ 11.61 (1H, s, NH), 7.63 (1H, s, H-6), 7.32−7.42 (10H, m, Ph), 5.76 (1H, s, OH), 5.51 (1H, m, H-5′), 5.31 (2H, s, OCH_2), 5.14 (2H, s, OCH_2), 4.49 (2H, d, J = 6.76 Hz, H-6′), 1.97 (1H, m, CH), 1.24−1.32 (8H, m, CH_2), 0.86 (3H, m, CH_3) ppm. MS: *m*/*z* calcd 556.22, found 556.6 ($M^{+\cdot}$). Anal. ($C_{32}H_{32}N_2O_7$) C, H, N.

1-[5-(Hepta-1″,6″-diyn-1″-yl)uracil-1-yl]-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (6). The procedure was carried out using 1,6-heptadiyne (0.07 mL, 0.54 mmol), which gave oil **6** (87 mg, 46.3%). ^{1}H NMR: δ 11.54 (1H, s, NH), 8.09 (1H, s, H-6), 7.28−7.40 (10H, m, Ph), 5.36 (1H, t, J = 6.42 Hz, H-5′), 5.22 (2H, s, OCH_2), 5.11 (2H, s, OCH_2), 3.87 (2H, d, J = 6.92 Hz, H-6′), 2.31 (4H, m, H-3″, H-5″), 1.78 (1H, m, H-7″), 1.66 (2H, m, H-4″) ppm. MS: *m*/*z* calcd 522.18, found 522.6 ($M^{+\bullet}$). Anal. ($C_{31}H_{26}N_2O_6$) calcd 71.46 (C), 6.18 (H), 5.05 (N), found 71.27 (C), 7.00 (H), 5.06 (N).

1-[5-(4″-Phenylbut-1″-yn-1″-yl)uracil-1-yl]-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (7). The procedure was carried out using 4-phenylbutyne (0.05 mL, 0.54 mmol), which gave oil **7** (134 mg, 66.5%). ^{1}H NMR: δ 11.63 (1H, s, NH), 8.16 (1H, s, H-6), 7.18−7.42 (15H, m, Ph), 5.51 (1H, t, J = 6.18 Hz, H-5′), 5.31 (2H, s, OCH_2), 5.14 (2H, s, OCH_2), 4.51 (2H, d, J = 6.53 Hz, H-6′), 2.78 (2H, m, H-3″), 2.64 (2H, m, H-4″) ppm. MS: *m*/*z* calcd 560.19, found 560.6 ($M^{+\cdot}$). Anal. ($C_{34}H_{28}N_2O_6$) C, H, N.

1-[5-(*p*-Bromophenylethyn-1″-yl)uracil-1-yl]-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (8). The procedure was carried out using *p*-bromophenylacetylene (65 mg, 0.54 mmol), which gave oil **8** (141 mg, 66.7%). ^{1}H NMR: δ 11.65 (1H, s, NH), 8.17 (1H, s, H-6), 7.34−7.62 (14H, m, Ph), 5.51 (1H, t, J = 6.41 Hz, H-5′), 5.31 (2H, s, OCH_2), 5.14 (2H, s, OCH_2), 4.52 (2H, m, H-6′) ppm. MS: *m*/*z* calcd 610.07, found 611.4 ($M^{+\cdot}$). Anal. ($C_{32}H_{23}BrN_2O_6$) C, H, N.

1-[5-(*p*-Methylphenylethyn-1″-yl)uracil-1-yl]-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (9). The procedure was carried out using *p*-methylphenylacetylene (0.05 mL, 0.54 mmol), which gave oil **9** (137 mg, 72.9%). ^{1}H NMR: δ 11.67 (1H, s, NH), 8.14 (1H, s, H-6), 7.28−7.42 (14H, m, Ph), 5.55 (1H, t, J = 6.43 Hz, H-5′), 5.31 (2H, s, OCH_2), 5.14 (2H, s, OCH_2), 4.56 (2H, d, J = 6.42 Hz, H-6′), 2.32 (3H, s, CH_3) ppm. MS: *m*/*z* calcd 546.18, found 546.6 ($M^{+\cdot}$). Anal. ($C_{33}H_{26}N_2O_6$) C, H, N.

1-[5-(*p*-Butylphenylethyn-1″-yl)uracil-1-yl]-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (10). The procedure was carried out using *p*-butylphenylacetylene (0.09 mL, 0.54 mmol), which gave oil **10** (151 mg, 71.3%). ^{1}H NMR: δ 11.54 (1H, s, NH), 8.08 (1H, s, H-6), 7.24−7.39 (14H, m, Ph), 5.11 (1H, m, H-5′), 4.52 (2H, s, OCH_2), 4.26 (2H, s, OCH_2), 3.94 (2H, m, H-6′), 2.59 (2H, m, CH_2), 1.56 (2H, m, CH_2), 1.25 (2H, m, CH_2), 0.93 (3H, m, CH_3) ppm. MS: *m*/*z* calcd 588.23, found 588.7 ($M^{+\cdot}$). Anal. ($C_{36}H_{32}N_2O_6$) C, H, N.

1-[5-(*p*-Pentylphenylethyn-1″-yl)uracil-1-yl]-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (11). The procedure was carried out using *p*-pentylphenylacetylene (0.21 mL, 1.08 mmol), which gave powder **11** (76 mg, 35.1%, mp 59−61 °C). ^{1}H NMR: δ 11.67 (1H, s, NH), 8.11 (1H, s, H-6), 7.14−7.42 (14H, m, Ph), 5.53 (1H, t, J = 6.41 Hz, H-5′), 5.28 (2H, s, OCH_2), 5.14 (2H, s, OCH_2), 4.53 (2H, m, H-6′), 2.55 (2H, m, CH_2), 1.53−1.59 (6H, m, CH_2), 1.27 (3H, m, CH_3) ppm. MS: *m*/ *z* calcd 602.24, found 602.7 ($M^{+\cdot}$). Anal. ($C_{37}H_{34}N_2O_6$) C, H, N.

General Procedure for the Preparation of 6-Substituted Furo-[2,3-*d*]pyrimidin-2-ones of L-Ascorbic Acid (12−22). To a stirred solution of 5-iodouracil-2′,3′-di-*O*-benzyl-L-ascorbic acid (200 mg, 0.36 mmol) in anhydrous dimethylformamide (10 mL) were added diisopropylethylamine (0.13 mL, 0.72 mmol), acetylene derivative (0.54 mmol), tetrakis(triphenylphosphine)palladium(0) (41.5 mg, 0.036 mmol), and copper(I) iodide (13.7 mg, 0.072 mmol). The mixture was stirred for 20 h at room temperature, after which time TLC (CH_2Cl_2:MeOH = 40:1) showed complete conversion of the starting material. Copper(I) iodide (13.7 mg, 0.072 mmol) and triethylamine (20 mL) were then added to the mixture, which was subsequently stirred at 60 °C for 6 h. The reaction mixture was then concentrated *in vacuo* to dryness and product was purified by column chromatography (initial eluent: CH_2Cl_2, followed by: CH_2Cl_2:MeOH = 60:1). The appropriate fractions were combined, and the solvent was evaporated to give the pure product.

1-(6-Butylfuro[2,3-*d*]pyrimidin-2-on-3-yl)-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (12). The procedure was carried out using hexyne (0.12 mL, 1.08 mmol). Additional purification by preparative thin layer chromatography afforded oil **12** (91 mg, 49.3%). ^{1}H NMR: δ 8.56 (1H, s, H-4), 7.32−7.42 (10H, m, Ph), 6.56 (1H, s, H-5), 5.56 (1H, t, J = 6.67 Hz, H-5′), 5.30 (2H, s, OCH_2), 5.14 (2H, s, OCH_2), 4.79 (2H, d, J = 6.35 Hz, H-6′), 2.40 (2H, t, J = 6.80 Hz, H-1″), 1.30−1.54 (4H, m, CH_2), 0.89 (3H, m, CH_3) ppm. MS: *m*/*z* calcd 512.19, found 512.6 ($M^{+\cdot}$). Anal. ($C_{30}H_{28}N_2O_6$) C, H, N.

1-(6-Pentylfuro[2,3-*d*]pyrimidin-2-on-3-yl)-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (13). The procedure was carried out using heptyne (0.05 mL, 0.54 mmol), and additional purification by preparative thin layer chromatography gave oil **13** (79 mg, 41.6%). ^{1}H NMR: δ 8.59 (1H, s, H-4), 7.33−7.41 (10H, m, Ph), 6.44 (1H, s, H-5), 5.30 (1H, t, J = 6.62 Hz, H-5′), 5.22 (2H, m, OCH_2), 4.96 (2H, m, OCH_2), 4.06 (2H, m, H-6′), 2.53 (2H, t, J = 7.33 Hz, H-1″), 1.59 (2H, m, CH_2), 1.31 (4H, m, CH_2), 0.85 (3H, m, CH_3) ppm. MS: *m*/*z* calcd 526.21, found 526.6 ($M^{+\cdot}$). Anal. ($C_{31}H_{30}N_2O_6$) C, H, N.

1-(6-Hexylfuro[2,3-*d*]pyrimidin-2-on-3-yl)-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (14). The procedure was carried out using octyne (0.08 mL, 0.54 mmol), which gave oil **14** (106 mg, 54.5%). ^{1}H NMR: δ 8.45 (1H, s, H-4), 7.33−7.41 (10H, m, Ph), 6.43 (1H, s, H-5), 5.55 (1H, t, J = 6.59 Hz, H-5′), 5.30 (2H, s, OCH_2), 5.14 (2H, s, OCH_2), 4.75 (2H, d, J = 6.81 Hz, H-6′), 2.26 (2H, t, J = 7.22 Hz, H-1″), 1.62 (2H, m, CH_2), 1.19−1.28 (6H, m, CH_2), 0.84 (3H, m, CH_3) ppm. MS: *m*/*z* calcd 540.23, found 540.6 ($M^{+\cdot}$). Anal. ($C_{32}H_{32}N_2O_6$) C, H, N.

1-(6-Octylfuro[2,3-*d*]pyrimidin-2-on-3-yl)-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (15). The procedure was carried out using decyne (0.07 mL, 54 mmol), and additional purification by preparative thin layer chromatography gave oil **15** (64 mg, 32.1%). ^{1}H NMR: δ 8.59 (1H, s, H-4), 7.21−7.39 (10H, m, Ph), 6.43 (1H, s, H-5), 5.37 (1H, t, J = 6.61 Hz, H-5′), 5.18 (2H, s, OCH_2), 4.99 (2H, s, OCH_2), 4.22 (2H, m, H-6′), 2.63 (2H, t, J = 7.01 Hz, H-1″), 1.83 (2H, s, CH_2), 1.60 (2H, m, CH_2), 1.24−1.28 (8H, m, CH_2), 0.85 (3H, m, CH_3) ppm. MS: *m*/*z* calcd 568.26, found 568.7 ($M^{+\cdot}$). Anal. ($C_{34}H_{36}N_2O_6$) C, H, N.

1-(6-(1″-Hydroxyhex-1″-yl)furo[2,3-*d*]pyrimidin-2-on-3-yl)-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (16). The procedure was carried out using 3-hydroxy-1-octyne (0.08 mL, 0.54 mmol), which gave oil **16** (81 mg, 41.9%). ^{1}H NMR: δ 7.99 (1H, s, H-4), 7.32−7.41 (10H, m, Ph), 6.51 (1H, s, H-5), 5.62 (1H, s, OH), 5.33 (1H, t, J = 6.55 Hz, H-5′), 5.22 (2H, s, OCH_2), 5.14 (2H, s, OCH_2), 4.35 (2H, d, J = 6.70 Hz, H-6′), 1.97 (1H, m, CH), 1.24−1.32 (8H, m, CH_2), 0.86 (3H, m, CH_3) ppm. MS: *m*/*z* calcd 556.22, found 556.6 ($M^{+\cdot}$). Anal. ($C_{32}H_{32}N_2O_7$) C, H, N.

1-(6-(Pent-4″-yn-1″-yl)furo[2,3-*d*]pyrimidin-2-on-3-yl)-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (17). The procedure was carried out using 1,6-heptadyine (0.07 mL, 0.54 mmol), which gave oil **17** (94 mg, 50%). ^{1}H NMR: δ 8.24 (1H, s, H-4), 7.30−7.43 (10H, m, Ph), 6.48 (1H, s, H-5), 5.35 (1H, t, J = 6.42 Hz, H-5′), 5.27 (2H, s, OCH_2), 5.16 (2H, s, OCH_2), 3.89 (2H, d, J = 6.42 Hz, H-6′), 2.33 (2H, t, J = 6.76 Hz, H-1″), 1.76 (1H, m, H-5″), 1.36−1.51 (4H, m, H-2″, H-3″) ppm. MS: *m*/*z* calcd 522.18, found 522.6 ($M^{+\cdot}$). Anal. ($C_{31}H_{26}N_2O_6$) C, H, N.

1-(6-Phenylethylfuro[2,3-*d*]pyrimidin-2-on-3-yl)-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (18). The procedure was carried out using 4-phenylbutyne (0.05 mL). Additional purification by preparative thin layer chromatography afforded oil **18** (43 mg, 21.3%). ^{1}H NMR: δ 8.54 (1H, s, H-4), 7.16−7.33 (15H, m, Ph), 6.43 (1H, s, H-5), 5.42 (1H, t, J = 6.46 Hz, H-5′), 5.23 (2H, s, OCH_2), 5.18 (2H, s, OCH_2), 4.37 (2H, d, J = 6.82 Hz, H-6′), 2.62 (2H, m, H-1″), 2.33 (2H, m, H-2″) ppm. MS: *m*/*z* calcd 560.19, found 560.6 ($M^{+\cdot}$). Anal. ($C_{34}H_{28}N_2O_6$) C, H, N.

1-(6-(*p*-Bromophenyl)furo[2,3-*d*]pyrimidin-2-on-3-yl)-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (19). The procedure was carried out using *p*-bromophenylacetylene (97.8 mg), and additional purification by preparative thin layer chromatography gave oil **19** (76 mg, 34.5%). ^{1}H NMR: δ 8.40 (1H, s, H-4), 7.27−7.43 (14H, m, Ph), 6.45 (1H, s, H-5), 5.24 (1H, t, J = 6.40 Hz, H-5′), 5.19 (2H, s, OCH_2), 4.99 (2H, s, OCH_2), 3.79 (2H, d, J = 6.90 Hz, H-6′) ppm. MS: *m*/*z* calcd 610.07, found 611.4 ($M^{+\cdot}$). Anal. ($C_{32}H_{23}BrN_2O_6$) C, H, N.

1-(6-(*p*-Methylphenyl)furo[2,3-*d*]pyrimidin-2-on-3-yl)-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (20). The procedure was carried out using *p*-methylphenylacetylene (0.05 mL), and additional purification by preparative thin layer chromatography gave oil **20** (56 mg, 28.4%). ^{1}H NMR: δ 8.56 (1H, s, H-4), 7.27−7.39 (14H, m, Ph), 6.37 (1H, s, H-5), 5.38 (1H, t, J = 6.43 Hz, H-5′), 5.23 (2H, s, OCH_2), 5.14 (2H, s, OCH_2), 4.47 (2H, d, J = 6.42 Hz, H-6′), 2.36 (3H, s, CH_3) ppm. MS: *m*/*z* calcd 546.18, found 546.6 ($M^{+\cdot}$). Anal. ($C_{33}H_{26}N_2O_6$) C, H, N.

1-(6-(*p*-Butylphenyl)furo[2,3-*d*]pyrimidin-2-on-3-yl)-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (21). The procedure was carried out using *p*-butylphenylacetylene (0.06 mL, 0.54 mmol), and additional purification by preparative thin layer chromatography gave oil **21** (71 mg, 33.5%). ^{1}H NMR: δ 7.75 (1H, s, H-4), 7.33−7.40 (14H, m, Ph), 6.40 (1H, s, H-5), 5.55 (1H, t, J = 6.39 Hz, H-5′), 5.21 (2H, s, OCH_2), 4.97 (2H, s, OCH_2), 4.07 (2H, d, J = 6.63 Hz, H-6′), 2.57 (2H, m, CH_2), 1.54 (2H, m, CH_2), 1.32 (2H, m, CH_2), 0.89 (3H, m, CH_3) ppm. MS: *m*/*z* calcd 588.23, found 588.7 ($M^{+\cdot}$). Anal. ($C_{36}H_{32}N_2O_6$) C, H, N.

1-(6-(*p*-Pentylphenyl)furo[2,3-*d*]pyrimidin-2-on-3-yl)-2(*Z*)-(2′,3′-di-*O*-benzyl-2′-buten-4′-olidylidene)ethane (22). The procedure was carried out using *p*-pentylphenylacetylene (0.07 mL, 0.54 mmol). Additional purification by preparative thin layer chromatography gave oil **22** (59 mg, 27.2%). ^{1}H NMR: δ 7.78 (1H, s, H-4), 7.23−7.33 (14H, m, Ph), 6.54 (1H, s, H-5), 5.48 (1H, d, J = 6.42 Hz, H-5′), 5.15 (2H, m, OCH_2), 4.88 (2H, m, OCH_2), 3.99 (2H, d, J = 6.36 Hz, H-6′), 2.48 (2H, m, CH_2), 1.89 (2H, m, CH_2), 1.11−1.21 (4H, m, CH_2), 0.78 (3H, m, CH_3) ppm. MS: *m*/*z* calcd 602.24, found 602.7 ($M^{+\cdot}$). Anal. ($C_{37}H_{34}N_2O_6$) C, H, N.

Antiviral Activity Assays. Antiviral activity against VZV, CMV, vesicular stomatitis virus, parainfluenza-3-virus, HSV-1, HSV-2, vaccinia virus, Coxsackie B4 virus, respiratory syncytial virus, reovirus-1, Sindbis virus, and Punta Toro virus was determined essentially as described previously.[35,36] Confluent human embryonic lung (HEL) fibroblasts were grown in 96-well microtiter plates and infected with the human cytomegalovirus (CMV) 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 minimum essential medium (MEM) containing different concentrations of the compounds. After incubation for 7 days at 37 °C, virus-induced cytopathogenicity was monitored microscopically before and after ethanol fixation and staining with Giemsa (for CMV and VZV). Antiviral activity was expressed as the EC_{50} or concentration required to reduce virus-induced cytopathogenicity by 50%. EC_{50} values were calculated from graphic plots of the percentage of cytopathogenicity as a function of concentration of the compounds. The cytostatic concentration was calculated as the CC_{50}, 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), the compound concentration that causes a microscopically detectable alteration of cell morphology of the confluent cell cultures that were exposed to the compounds.

Cytostatic Activity Assays. Cytostatic activity against L1210 (murine leukemia), Molt4/C8, and CEM (human T-lymphocytes) cells were measured essentially as originally described for the mouse leukemia (L1210) cell line.[37] 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 HeLa (cervical carcinoma), MiaPaCa-2 (pancreatic carcinoma), SW 620 (colon carcinoma), MCF-7 (breast carcinoma), and H-460 (non-small-cell lung 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 HeLa, MiaPaCa-2, SW 620, MCF-7, and H-460 were seeded into a series of standard 96-well microtiter plates on day 0. Test agents were then added in 5- and 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. After 72 h of incubation the cell growth rate was evaluated by the MTT assay, as described previously.[38,39] Each test point was performed in quadruplicate in three individual experiments. The results are expressed as IC_{50}, the concentration necessary for 50% 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 percentage of growth values above and below the reference value (i.e., 50%).

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Supporting Information Available: Mechanism for the synthesis of 6-alkylfuro[2,3-*d*]pyrimidine-2-one L-ascorbic acid derivatives (**12**−**22**) by Sonogashira coupling of terminal alkynes with IUAA and subsequent CuI-promoted in situ cyclization; ^{13}C NMR data for compounds **1**−**22**; activities of compounds **1**−**22** against vaccinia virus, respiratory syncytial virus, reovirus-1, Sindbis virus, Coxsackie virus B4, and Punta Toro virus; and cytotoxicity and elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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