Notes

Synthesis and Cytotoxic Activity of 6-Vinyl- and 6-Ethynyluridine and 8-Vinyl- and 8-Ethynyladenosine

Stefano Manfredini,*,† Pier G. Baraldi,† Rita Bazzanini,†,⊥ Mirella Marangoni,†,§ Daniele Simoni,†,∥ Jan Balzarini,‡ and Erik De Clercq‡

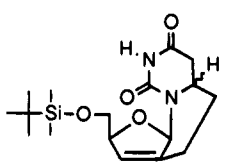
Departimento di Scienze Farmaceutiche, Università di Ferrara, Via Fossato di Mortara 17-19, I-44100 Ferrara, Italy and Rega Instituut, Katholieke Universiteit, Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

Received July 20, 1994®

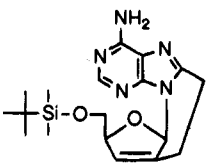
It is well-known that the introduction of vinyl and ethynyl moieties into nucleosides is of crucial importance for cytostatic, antiviral, or other biological activities. In this study 6- and 8-vinyl- and -ethynyluridine and -adenosine were prepared by a general procedure involving the palladium-catalyzed cross-coupling of trimethylsilylacetylene or vinyltributyltin. The introduction of a vinyl group at C-6 of uridine or an ethynyl group at C-8 of adenosine resulted in nucleoside derivatives showing cytostatic activity against several murine and/or human tumor cell lines. Interestingly, 8-vinyladenosine had pronounced selective inhibitory effects on human (Molt/4F and MT-4) versus murine (L1210 and FM3A) tumor cell lines.

Introduction

As reported by a number of authors, the introduction of alkyl, alkenyl and/or alkynyl groups into natural purine and pyrimidine nucleosides is of great interest in view of their potential biological activities.[1] In particular, modifications of the C-8 position in purine nucleosides and the C-6 position of pyrimidine nucleosides are of significant interest for their influence on the conformation of the glycosidic bond (i.e., selectivity on adenosine receptors[2]). Robins and Samano[3] have recently reported on the synthesis of 2′-deoxynucleoside-2′-spirocyclopropanes as mechanistic probes for ribonucleotide reductase (RR).[4] They have demonstrated that biomimetic radical reactions yield, through the spirocyclopropyl ring opening, 5,6-dihydro-6,2′-ethano-2′,3′-unsaturated cyclouridine (**1**) and 8,2′-ethano-2′,3′-unsaturated cycloadenosine (**2**). Moreover, Chattopadhyaya et al. have reported the intramolecular cyclization of olefins as a means for trapping carbon radicals at the 2′- and 3′-carbons of nucleosides,[5] and Tanaka et al. have recently demonstrated the usefulness of 6-(bromovinyl)uracil derivatives in radical-mediated cyclizations.[6] Taking these considerations into account, we envisaged compounds **5**, **9** and **13**, **14** as possible candidates for antitumor/antiviral agents.[7] The following considerations guided our approach: (a) potential antitumor/antiviral activities may be obtained by introduction of alkenyl and alkynyl groups at an opportune position of the nucleosidic base;[8] (b) a suitable function that could trap the incipient radical formed at position 2′ or 3′ of a ribonucleoside may serve as a probe for the proposed mechanism of action of ribonucleotide reductase; (c) alkynyl and vinyl moieties are known to be highly reactive with respect of the radical species; (d) the potential formation of cyclonucleosides, through addition to the unsaturated functionality (vinyl or ethynyl) of the incipient radical on the glycosylic portion, may account for the biological activity.[5]



1



2

Chemistry

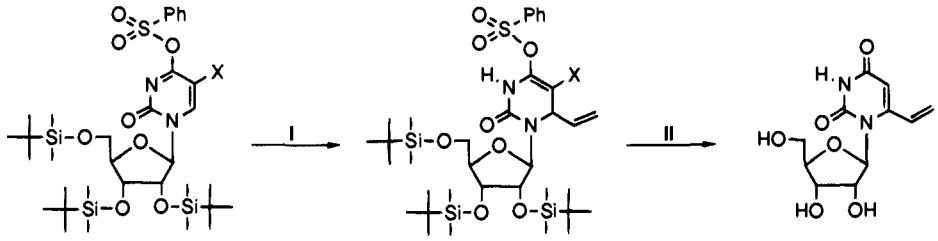
Initially, we were attracted by the possibility to obtain **5**[9] starting from compounds **4a** and/or **4b** (Scheme 1). A logical approach prompted us to study the oxidation of **4a** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) whereas treatment with bases of **4b** could reasonably afford deprotection of the (triisopropylphenyl)sulfonyl group (TPS) with concomitant elimination of hydrobromic acid. Compound **4a** has been already reported by Bichofberger,[10] and **4b** was simply prepared through reaction of the protected 5-bromouridine with triisopropylbenzenesulfonyl chloride (TPS-Cl). All attempts to achieve **5** as mentioned above were unsatisfactory: oxidation of **4a** afforded only traces of the target compound, the cleavage of the glycosylic bond being the principal reaction. However, better results, even not satisfactory, were obtained when **4b** was first deprotected with tetrabutylammonium fluoride (TBAF) in THF solution and *in situ* treated with tetrabutylammonium hydroxide: very limited amounts (16%) of the expected compound **5**, with concomitant disappearance of starting material, could be recovered.

In order to produce higher amounts of compound **5**, we planned a different strategy (Scheme 2): based on

† Università di Ferrara.
⊥ Current address: Laboratoire de Chimie Bioorganique, Université de Montpellier II, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France.
§ Current address: Laboratory of Medicinal Chemistry, Rega Instituut, Katholieke Universiteit, Leuven.
∥ Current address: Istituto Farmacochimico, Università di Palermo, Italy.
‡ Katholieke Universiteit.
® Abstract published in *Advance ACS Abstracts*, November 15, 1994.

0022-2623/95/1838-0199$09.00/0

Scheme 1[a]



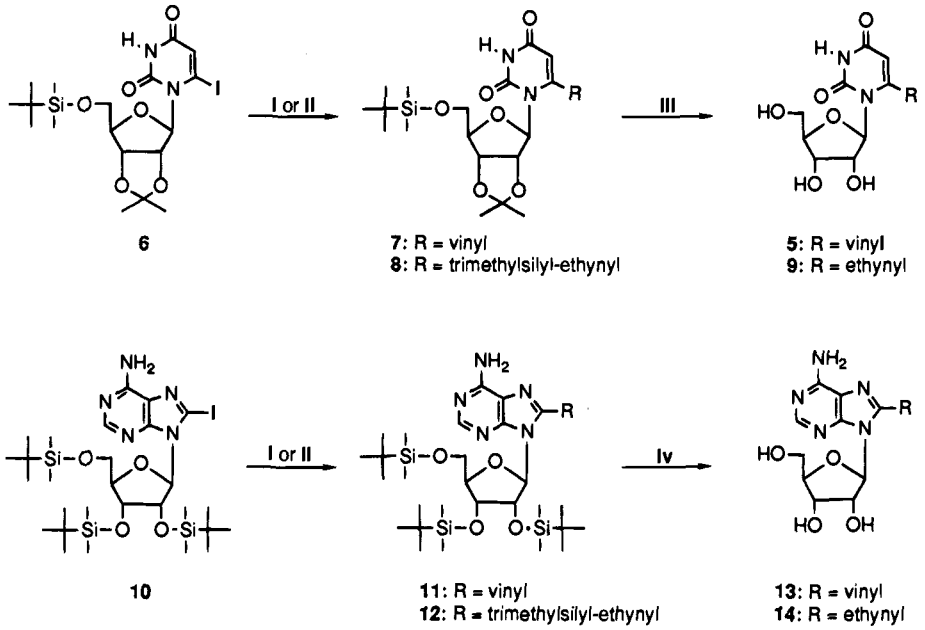
3a: X = H
3b: X = Br

4a: X = H
4b: X = Br

5

[a] (i) Vinylmagnesium bromide, THF, 0 °C; (ii) (1) TBAF, THF, (2) Bu_4NOH, H_2O.

Scheme 2[a]



6

7: R = vinyl
8: R = trimethylsilyl-ethynyl

5: R = vinyl
9: R = ethynyl

10

11: R = vinyl
12: R = trimethylsilyl-ethynyl

13: R = vinyl
14: R = ethynyl

[a] (i) Tributylvinylstannane, $(Ph_3P)_4Pd$, DMF; (ii) (trimethylsilyl)acetylene, $(Ph_3P)_2PdCl_2$, Et_3N; (iii) CF_3CO_2H/H_2O 50%; (iv) NH_4F, MeOH, 70 °C.

the study of Tanaka and co-workers[11] on the position 6 of pyrimidine bases, we envisaged the key intermediate **6**[11] as a suitable candidate. This compound is amenable of conversion to both the desired derivatives **7** and **8** through a coupling reaction with a suitable source of alkynyl or alkenyl functions. The ethynyl derivative **8** was prepared in 68% yield, by a methodology developed previously[12] and involving the coupling of **6** and (trimethylsilyl)acetylene with $(Ph_3P)_2PdCl_2$. For the introduction of the vinyl moiety, the palladium-catalyzed cross-coupling reaction with organotin reagents is known to be a valuable approach,[13] and applications of this procedure for the alkylation of purine and pyrimidine nucleosides have been recently reported.[8,14,15] Either tetraalkyltin or alkyltributyltin reagents were employed on a suitable halide precursor to give the corresponding alkyl nucleoside. We used vinyltributyltin in DMF in the presence of catalytic amount of $(Ph_3P)_4Pd$ to obtain in almost quantitative yield the expected **7**.[16] Deprotection of **7** and **8** at both 5′-*O*-*tert*-butyldimethylsilyl (TBDMS) and 2′,3′-*O*-isopropylidene groups was performed with 50% aqueous trifluoroacetic acid to give **5** and **9**[17] (with concurrent removal of the trimethylsilyl group) in good yield (Scheme 2). Moreover compound **5** has also been prepared from 6- ethynyluridine (**9**) by direct hydrogenation on Pd−$BaSO_4$ catalyst. However, overreduction, formation of side products, and difficulties in the monitoring of the reduction led to very limited yields (20%).

The above-described approach was extended to protected 8-iodoadenosine (**10**)[15] to give in 85 and 63% yields **11** and **12**, respectively (Scheme 2). Difficulties in the deprotection step have been encountered; however, the use of NH_4F in methanol[18] instead of the standard TBAF procedure gave the final compounds **13** and **14** in 50 and 77% yield, respectively. These difficulties in the deprotection of silylated-vinyl adenosine have also been encountered by Van Aerschot et al.[8] and overcome through a tranisent silylation step. The NH_4F/methanol deprotection avoided the conjugate addition reaction that occurs by the use of TBAF.

Finally, extension of our procedure to the preparation of the cytidine analogs of compounds **5** and **9** failed. Surprisingly, we did not succeed in converting 6-iodo-4-(1,2,4-triazol-1-yl)-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-isopropylideneuridine,[19,20] into the expected coupling products.

Results and Discussion

6- and 8-vinyl- and -ethynyluridine and -adenosine have been prepared, starting from the corresponding iodo-substituted intermediates. Our methodology further extend the chemistry developed for the function-

Table 1. Inhibitory Effects of Nucleoside Derivatives **5**, **9**, **13**, and **14** on the Proliferation of Murine Leukemia L1210, Murine Mammary Carcinoma FM3A, and Human T-Lymphoblast Molt/4F and MT-4 Cells

	IC_{50} (μM)[a]			
compd	L1210	FM3A	Molt/4F	MT4
5	21 ± 0.7	24	14 ± 0.12	11
9	>372	>372	>372	286 ± 78
13	190 ± 38	145	6.5 ± 0.1	15 ± 5
14	54 ± 24	27	25 ± 0.1	22 ± 7

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

alization of position 6 on uridine and position 8 on adenosine and allows a general procedure for the preparation of either 6- and 8-vinyl- or -ethynyluridine and -adenosine based on a palladium-catalyzed cross-coupling strategy. Whereas 6-ethynyluridine (**9**) was devoid of any marked cytostatic activity against murine (L1210 and FM3A) and human (Molt/4F and MT-4) cells [50% inhibitory concentration (IC_{50}) ≥ 286 μM (Table 1)], its 6-vinyl-substituted counterpart (**5**) inhibited tumor cell proliferation with an IC_{50} of 12–24 μM against the four tumor cell lines investigated. Whereas 8-ethynyladenosine (**14**) inhibited the proliferation of all four cell lines (IC_{50} 22–54 μM), 8-vinyladenosine (**13**) was poorly cytostatic against the murine tumor cells (L1210 and FM3A), but had a more pronounced inhibitory effect on human Molt/4F and MT-4 cell proliferation (IC_{50}: 6.5–15 μM). These data may be suggestive of differences in the metabolism of 8-vinyladenosine in human *versus* murine tumor cell lines.

Thus, the introduction of a vinyl moiety at the C-6 of uridine, or a vinyl or ethynyl group at the C-8 of adenosine, which induce the opposite conformation of the glycosidic bond if compared to the natural nucleosides, made these nucleosides markedly more cytostatic than the natural ones. The pronounced cytostatic activity of 8-vinyladenosine (**13**) together with its previously reported antiviral activity (i.e., herpes simplex virus type 1, vaccinia virus, and respiratory syncytial virus)[8] make this compound particularly interesting. Moreover, in constrast with the trend observed in the 5-vinyl- and 5-ethynyl-substituted 2'-deoxyuridine series,[21] 6 vinyluridine is a potent cytostatic compound, whereas the 6-ethynyl derivative is inactive.

Experimental Part

Chemistry. Melting points were obtained in open capillary tubes and are uncorrected. Reaction courses were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F254 Merck plates with detection under 254-nm UV lamp and/or by spraying the plates with 10% H_2SO_4/MeOH and heating. Nuclear magnetic resonance (^1H NMR, ^{13}C NMR) spectra were determined in DMSO-d_6 or $CDCl_3$ solution with a Bruker AC-200 spectrometer, and peak positions are given in parts per million downfield from tetramethylsilane as internal standard. Ultraviolet spectra were recorded on a JASCO 510 spectrometer. Column chromatography was performed with Merck 60–200-mesh silica gel. Room temperature varied between 22 and 25 °C. All drying operations were performed over anhydrous magnesium sulfate. Microanalysis were in agreement with calculated values within ±0.4%.

Starting Materials. Starting compounds **3a**,[10] **6**,[11] and **10**[15] were prepared as reported.

4-*O*-[(Triisopropylphenyl)sulfonyl]-5-bromo-2',3',5'-tris-*O*-(*tert*-butyldimethylsilyl)uridine (3b). Compound **3b** was prepared following and adapting the procedure described on the parent uridine derivative.[10] Chromatography (EtOAc/Hexane, 1:9) gave a foam (yield 87%): ^1H NMR ($CDCl_3$) δ 8.08 (s, 1H, H6), 7.21 (s, 2H, Ar), 5.96 (d, J = 2.2 Hz, 1H, H1'), 4.29 (m, 5H, H2', H3', H4', and 2 × CH-isop), 3.70 (m, 2H, H5'), 2.90 (m, 1H, CH-isop), 1.31 (d, J = 7 Hz, 12H, Me-isop), 1.29 (d, J = 7 Hz, 6H, Me-isop), 0.88–0.94 (3 s, 27H, tBu), 0.10–0.02 (m, 18H, SiMe).

4-*O*-[(Triisopropylphenyl)sulfonyl]-5-bromo-6-vinyl-5,6-dihydro-2',3',5'-tris-*O*-(*tert*-butyldimethylsilyl)uridine (4b). Compound **3b** (0.50 g, 0.53 mmol) was dissolved in anhydrous THF (5 mL) under positive argon pressure, and vinylmagnesium bromide 1.0 M in THF (2.5 mL, 2.5 mmol) was added at 0 °C. After 1 h at 0 °C, TLC indicated complete reaction and the mixture was then diluted with Et_2O (20 mL), treated wtih saturated NH_4Cl (20 mL), and washed with H_2O (50 mL). The organic layer was dried and evaporated to give a crude residue (1.1 g), which was purified by chromatography (EtOAc/hexane, 9:1, in the presence of traces of Et_3N) to give **4b** as an oil (0.49 g, yield 96%). The resulting compound was stable only if stored in solution (CH_2Cl_2) below −17 °C: ^1H NMR ($CDCl_3$) δ 7.23 (s, 2H, Ar), 6.39 (s, 1H, NH), 5.99–5.83 (m, 1H, vinyl), 5.90 (d, J = 6.5 Hz, 1H, H1'), 5.37 (d, J. = 17 Hz, 1H, vinyl), 5.22 (d, J = 10 Hz, vinyl), 4.64 (d, J = 4 Hz, 1H, H6) 4.19–3.93 (m, 5H, H2', H3', H4', and 2 × CH-isop), 3.70 (m, 2H, H5'), 2.90 (M, 1h, CH-isop), 1.31 (d, J = 7 Hz, 12H, 2 × Me-isop), 1.29 (d, J = 7 Hz, 6H, Me-isop), 0.88–0.94 (3s, 27H, tBu), 0.10–0.02 (m, 18H, SiMe).

Preparation of 6- and 8-Vinyl-Protected Uridine (7) and Adenosine (11). General Procedure. In a typical experiment in a 100 mL two-necked flask, halide (**6** and **10**) (0.95 mmol) was dissolved in dry DMF (20 mL), and $(Ph_3P)_4$-Pd (0.054 g, 0.0475 mmol) and tributylvinylstannane (1 mL, 4.76 mmol) were added. The mixture was stirred at 90 °C for 1.5 h under positive argon pressure (TLC, EtOAc/hexane, 3:7). When the reaction was complete, the solvent was evaporated, and the brown residue was dissolved in EtOAc (50 mL), washed with saturated NH_4Cl (1 × 20 mL), dried, and evaporated. The resulting brown oil was purified by chromatography (EtOAc/hexane, 3:7, in presence of traces of Et_3N).

7: yield 95%; foam; ^1H NMR ($CDCl_3$) δ 9.1 (sbr, 1H, NH), 6.68 (dd, J = 10 Hz, J = 17 Hz, 1H, vinyl), 5.88 (d, J = 17 Hz, 1H, vinyl), 5.78 (s, 1H, H5), 5.75 (d, J = 2.2 Hz, 1H, H1'), 5.67 (d, J = 10 Hz, 1H, vinyl), 5.19 (dd, J = 2.2 Hz, J = 6.4 Hz, 1H, H2'), 4.84 (dd, J = 6.4 Hz, J = 4.4 Hz, 1H, H3'), 4.15 (m, 1H, H4'), 3.80 (m, 2H, H5'), 1.55 (s, 3H, Me-isop), 1.34 (s, 3H, Me-isop), 0.9 (s, 9H, tBu), 0.05 (s, 6H, SiMe).

11: yield 85%; mp 184–186 °C (EtOH/H_2O); ^1H NMR ($CDCl_3$) δ 8.28 (s, 1H, H2), 6.98 (dd, J = 12, Hz, J = 18 Hz, 1H, vinyl), 6.47 (dd, J = 2 Hz, J = 18 Hz, 1H, vinyl), 5.99 (d, J = 6 Hz, 1H, H1'), 5.95 (s, 2H, NH_2), 5.70 (dd, J = 2 Hz, J = 12 Hz, 1H, vinyl), 5.29 (dd, J = 6.2 Hz, J = 4.6 Hz, 1H, H2'), 4.50 (dd, J = 2.4 Hz, J = 4.6 Hz, 1H, H3'), 4.07 (m, 2H, H4', H5'), 3.78 (dd, J = 6.4 Hz, J = 13.6 Hz, 1H, H5'), 0.97–0.75 (3s, 27H, tBu), 0.16–0.02 (m, 18H, SiMe).

Preparation of 6- and 8-(Trimethylsilyl)ethynyl-Protected Uridine (8) and Adenosine (12). General Procedure. In a typical experiment in a 100 mL two-necked flask, freshly distilled dry Et_3N (40 mL) was vigorously purged with nitrogen for 30 min, and halide (**6** and **10**) (0.92 mmol) was added followed by (trimethylsilyl)acetylene (0.426 mL, 3.01 mmol), $(Ph_3P)_2PdCl_2$ (0.015 g, 0.0213 mmol), and CuI (0.01 g, 0.0526 mmol). This suspension was stirred at room temperature for 1 h and then at 80 °C for 2 h under positive argon pressure (TLC, EtOAc/hexane, 3:7). When the reaction was complete, the solvent was evaporated, and the brown residue was dissolved in CH_2Cl_2 (100 mL), washed with 2% disodium EDTA/H_2O (2 × 50 mL) and H_2O (2 × 50 mL), dried, and evaporated. The resulting brown oil was purified by column chromatography (EtOAc/hexane, 3:7, in presence of traces of Et_3N).

8: yield 68%; yellow syrup; ^1H NMR ($CDCl_3$) δ 9.77 (br, 1H, NH), 6.33 (d, J = 1.5 Hz, 1H, H1'), 5.94 (d, J = 2.2 Hz, 1H, H5), 5.19 (dd, J = 1.6 Hz, J = 6.6 Hz, 1H, H2'), 4.80 (dd, J = 4 Hz, J = 6.6 Hz, 1H, H3'), 4.17 (m, 1H, H4'), 3.82 (m, 2H, H5'), 1.53 (s, 3H, Me-isop), 1.35 (s, 3H, Me-isop), 0.88 (s, 9H, tBu), 0.29 (s, 9H, $SiMe_3$), 0.04 (s, 6H, $SiMe_2$).

12: yield 63%; yellow solid mp 85–87 °C (EtOH/H_2O); ^1H NMR ($CDCl_3$) δ 8.29 (s, 1H, H2), 6.15 (d, J = 6.8 Hz, 1H, H1'), 6.01 (s, 2H, NH_2), 5.38 (dd, J = 4.4 Hz, J = 7 Hz, 1H, H2'), 4.45 (d, J = 4.2 Hz, 1H, H3'), 4.18–4.07 (m, 2H, H4', H5'), 3.77 (dd, J = 4.4 Hz, J = 9 Hz, 1H, H5'), 0.97–0.78 (3s, 27H, tBu), 0.29–0.07 (m, 27H, SiMe).

General Procedures for Deprotection. Preparation of 6-Vinyl- (5) and 6-Ethynyluridine (9). The protected compound (1 mmol) was dissolved at 0 °C in aqueous trifluoroacetic acid (TFA) (20 mL), and the mixture was stirred overnight at room temperature. After evaporation the crude residue was purified by column chromatography (CH_2Cl_2/MeOH, 9:1).

5:[9] yield 88%; mp 203–205 °C (MeOH/Et_2O); UV (H_2O) λ_{max} 276 (ϵ 4260), λ_{min} 238 (ϵ 460), λ_{max} 212 (ϵ 2420); ^1H NMR (DMSO-d_6) δ 11.40 (sbr, 1H, NH), 6.79 (dd, J = 11 Hz, J = 17 Hz, 1H, vinyl), 5.91 (d, J = 17 Hz, 1H, vinyl), 5.75 (s, 1H, H5), 5.69 (d, J = 2.2 Hz, 1H, H1'), 5.60 (d, J = 11 Hz, 1H, vinyl), 5.23 (d, J = 5.6 Hz, 1H, OH2'), 4.97 (d, J = 6.2 Hz, 1H, OH3'), 4.80 (t, J = 5.6 Hz, 1H, OH5'), 4.40 (m, 1H, H2'), 4.01 (m, 1H, H3'), 3.69–3.43 (m, 3H, H4' and H5'). Anal. ($C_{11}H_{14}N_2O_6$) C, H, N.

9: yield 73%; mp 216 °C (MeOH/Et_2O), (lit.[17] mp 217–219 °C). Anal. ($C_{11}H_{12}N_2O_6$) C, H, N.

Preparation of 8-Vinyl- (13) and 8-Ethynyladenosine (14). The protected compound (1 mmol) was dissolved at room temperature in MeOH (20 mL), and NH_4F[18] (5 mmol) was added. The mixture was refluxed at 60–70 °C until complete conversion to deprotected compound (TLC, CH_2Cl_2/MeOH, 9:1). After evaporation, the crude residue was purified by column chromatography.

13: yield 50%; mp 241 °C (MeOH/Et_2O) (lit.[8] mp 245 °C dec); UV (H_2O) λ_{max} 228 (ϵ 25 700), λ_{min} 253 (ϵ 5900), λ_{max} 288 (ϵ 13 400); ^1H NMR (DMSO-d_6) δ 8.11 (s, 1H, H2), 7.44 (s, 2H, NH_2), 7.10 (dd, J = 11 Hz, J = 17 Hz, 1H, vinyl), 6.35 (dd, J = 2 Hz, J = 17 Hz, 1H, vinyl), 5.96 (d, J = 7 Hz, 1H, H1'), 5.77–5.67 (m, 2H, OH5' and vinyl), 5.38 (d, J = 7 Hz, 1H, OH2'), 5.22 (d, J = 4.6 Hz, 1H, OH3'), 4.81 (m, 1H, H2'), 4.12 (m, 1H, H3'), 3.99 (m, 1H, H4'), 3.56 (m, 2H, H5'). Anal. ($C_{12}H_{15}N_5O_4$) C, H, N.

14: yield 77%; mp 232–234 °C (MeOH/Et_2O); UV (H_2O) λ_{max} 228 (ϵ 20 000), λ_{min} 247 (ϵ 3900), λ_{max} 292 (ϵ 17 000); ^1H NMR ($CDCl_3$) δ 8.18 (s, 1H, H2), 7.70 (s, 2H, NH_2), 5.96 (d, J = 7 Hz, 1H, H1'), 5.57 (m, 1H, OH5'), 5.48 (d, J = 7 Hz, 1H, OH2'), 5.25 (d, J = 4.4 Hz, 1H, OH3'). Anal. ($C_{12}H_{13}N_5O_4$) C, H, N.

Biology. (a) Cells. Murine leukaemia L1210, murine mammary carcinoma FM3A, human T-lymphoblast Molt/4F, and human T-lymphocyte MT-4 cells were cultivated in Eagle's minimal essential medium (Gibco BRL, Paisley, Scotland) (L1210, FM3A) or RPMI-1640 medium (Gibco BRL) (Molt/4F, MT-4), supplemented with 10% fetal calf serum (Gibco BRL), 2 mM L-glutamine (Gibco BRL), and 0.075% $NaHCO_3$. Cells were subcultivated twice a week.

(b) Inhibition of Tumor Cell Proliferation. All assays were performed in flat-bottomed 96-well microplates (Falcon) as previously described.[22,23] Briefly, the cells were suspended in growth medium and added to the microplate wells at a density of 5×10^4 L1210 or FM3A cells/well (200 μL) or 6.25×10^4 Molt/4 or MT-4 cells/well in the presence of varying concentrations of the test compounds. The cells were then allowed to proliferate for 48 (L1210 and FM3A), 72 (Molt/4), or 120 h (MT-4) at 37 °C in a humidified, CO_2-controlled atmosphere. At the end of the incubation period, the L1210, FM3A, and Molt/4F cells were counted in a Coulter counter (Coulter Electronics Ltd., Harpenden, Herts, U.K.). MT-4 cell cultures were stained by trypan blue to count the number of viable cells under the microscope. The IC_{50} was defined as the concentration of compound that reduced the number of viable cells by 50%. All values shown in Table 1 for L1210, Molt/4F, and MT-4 cells are means of at least two or three independent experiments; the data shown for FM3A cells are derived from one single experiment.

Acknowledgment. This work was supported by the Consiglio Nazionale delle Ricerche of Italy and also by the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (project no. 3.0026.91). We thank Prof. Morris J. Robins for fruitful discussions in devising the project, Dr. Norbert Bischofberger for helpful informations on the stability of compound **4a**, and Dr. Hiromichi Tanaka for his caring interest and for providing a sample of compound **6** for comparative purposes. We also thank Lizette van Berckelaer for excellent technical help.

References

(1) Hobbs, J. B. Purine and pyrimidine targets. In *Comprehensive Medicinal Chemistry*; Sammes, P. G., vol. Ed.; Pergamon Press: Oxford, UK, 1990; Vol. 2, pp 299–331.
(2) Jacobson, K. A. Adenosine (P1) and ATP (P2) receptors. Membrane and receptors. In *Comprehensive Medicinal Chemistry*; Emmett, J. C., vol. Ed.; Pergamon Press: Oxford, UK, 1990; Vol. 3, pp 601–642.
(3) Samano, V.; Robins, M. J. Synthesis and radical-induced ring-opening reactions of 2'-deoxyadenosine-2'-spirocyclopropane and its uridine analogue. Mechanistic probes for ribonucleoside reductases. *J. Am. Chem. Soc.* **1992**, *114*, 4007–4008.
(4) Reichard, P.; Ehrenberg, A. Ribonucleotide reductase a radical enzyme. *Science* **1983**, *221*, 514–519.
(5) Wu, J. C.; Xi, Z.; Gioeli, C.; Chattopadhyaya, J. Intramolecular cyclization—trapping of carbon radicals by olefins as mean to functionalize 2'- and 3'-carbons in β-D-nucleosides. *Tetrahedron* **1991**, *47*, 2237–2254.
(6) Kittaka, A.; Tanaka, H.; Odanaka, Y.; Ohnuki, K.; Yamaguchi, K.; Miyasaka, T. Vinyl radical-based cyclization of 6-substituted 1-(2-deoxy-D-erythro-pent-1-enofuranosyl)uracils: synthesis of anomeric spiro nucleosides. *J. Org. Chem.* **1994**, *13*, 3636–3641.
(7) Compounds **9** (ref 17) and **13** (ref 8) were reported by an alternative synthesis. During the preparation of this manuscript, compound **5** was reported (ref 9), but only biological activity against L1210 cell lines was mentioned.
(8) Van Aerschot, A.; Mamos, P.; Weyns, N. J.; Ikeda, S.; De Clercq, E.; Herdewijn, P. A. Antiviral activity of C-alkylated purine nucleosides obtained by cross-coupling with tetraalkyltin reagents. *J. Med. Chem.* **1993**, *36*, 2938–2942 and references cited therein.
(9) Megati, S.; Sodum, R.; Otter, G. M.; Klein, R. S.; Otter, B. A. Synthesis and in vitro cytotoxicity of 6-vinyluridine and related compounds. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 469–472.
(10) Bischofberger, N. Addition of Grignard reagents to 4-O-TPS pyrimidine nucleosides: synthesis of 6-substituted 5,6-dihydropyrimidine nucleoside derivatives. *Tetrahedron Lett.* **1989**, *30*, 1621–1623.
(11) Tanaka, H.; Hayakawa, H.; Miyasaka, T. Lithiation chemistry of uridine derivatives: access to a new anti HIV-1 lead. In *Nucleosides and Nucleotides as Antitumor and Antiviral Agents*; Chu, C. K., Backer, D. C., Eds.; Plenum Press: New York, 1993; pp 23–53 and previous papers in this series.
(12) Robins, M. J.; Manfredini, S.; Wood, S. G.; Wanklin, R. J.; Rennie, B. A.; Sacks, S. L. Nucleic acid related compounds. 65. New syntheses of 1-β-D-arabinofuranosyl-5(*E*)-(2-iodovinyl)uracil (IVaraU) from vinylsilane precursors. Radioiodine uptake as a marker for thymidine kinase positive herpes viral infections. *J. Med. Chem.* **1991**, *34*, 2275–2280.
(13) Stille, J. K. The palladium-catalyzed cross-coupling reactions of organotin reagents with organic electrophiles. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 508–524.
(14) Moriarty, R. M.; Epa, W. R.; Awasthi, A. K. Palladium catalyzed C-8 allylation and vinylation of adenosine, 2'-deoxyadenosine and 2',3'-dideoxyadenosine nucleosides. *Tetrahedron Lett.* **1990**, *31*, 5877–5880.
(15) Mamos, P.; Van Aerschot, A. A.; Weyns, N. J.; Herdewijn, P. A. Straightforward C-8 alkylation of adenosine analogues with tetraalkyltin reagents. *Tetrahedron Lett.* **1992**, *33*, 2413–2416.
(16) The biological studies were in progress when the synthesis of 6-ethenyl-2',3'-isopropilidene-5'-*O*-(methoxymethyl)uridine, involving a reversal cross-coupling strategy was reported. Palmisano, G.; Santagostino, M. Base-modified pyrimidine nucleosides. Efficient entry to 6-derivatized uridines by Sn-Pd transmetallation-coupling process. *Tetrahedron* **1993**, *49*, 2533–2542.
(17) Tanaka, H.; Haraguchi, K.; Koizumi, Y.; Fukui, M.; Miyasaka, T. Synthesis of 6-alkynylated uridines. *Can. J. Chem.* **1986**, *64*, 1560–1563.
(18) Zhang, W.; Robins, M. J. Removal of silyl protecting groups from hydroxyl functions with ammonium fluoride in methanol. *Tetrahedron Lett.* **1992**, *33*, 1177–1180.
(19) 6-Iodo-4-(1,2,4-triazol-1-yl)-5'-*O*-(*tert*-butyldimethylsilyl)-2',3'-isopropylideneuridine was obtained as a brown foam (89% yield), starting from the corresponding iodo-derivative **6**, by the procedure of Divakar and Reese (ref 20). ^1H NMR ($CDCl_3$): δ 9.20

(s, 1H, triazole), 8.13 (s, 1H, triazole), 7.67 (s, 1H, H5), 6.30 (s, 1H, H1′), 5.20 (d, J = 6.4 Hz, 1H, H2′), 4.90 (dd, J = 6.4 Hz, J = 4.4 Hz, 1H, H3′), 4.25 (m, 1H, H4′), 3.90 (m, 2H, H5′), 1.55 (s, 3H, Me-isop), 1.34 (s, 3H, Me-isop), 0.9 (s, 9H, tBu), 0.05 (s, 6H, SiMe).

(20) Divakar, K. J.; Reese, C. B. 4-(1,2,4-triazol-1-yl)- and 4-(3-nitro-1,2,4-triazol-1-yl)-1-(β-D-2,3,5-tri-O-acetylarabinofuranosyl)pyrimidin-2(1H)-ones. Valuable intermediates in the synthesis of derivatives of 1-(β-D-arabinofuranosyl)cytosine (Ara-C). *J. Chem. Soc., Perkin Trans. 1* **1982**, 1171–1176.

(21) De Clercq, E.; Balzarini, J.; Torrence, P. F.; Mertes, M. P.; Schmidt, C. L.; Shugar, C. L.; Barr, P. J.; Jones, A. S.; Verhelst, G.; Walker, R. T. Thymidylate synthetase as target enzyme for the inhibitory activity of 5-substituted 2′-deoxyuridines on mouse leukemia L1210 cell growth. *Mol. Pharmacol.* **1981**, *19*, 321–330.

(22) Balzarini, J.; Karlsson, A.; Wang, L.; Bohman, C.; Horska, K.; Votruba, I.; Fridland, A.; Van Aerschot, A. A.; Herdewijn, P.; De Clercq, E. Eicar (5-ethynyl-1-β-D-ribofuranosyl-imidazole-4-carboxamide) a novel potent inhibitor of inosinate dehydrogenase activity and guanylate biosynthesis. *J. Biol. Chem.* **1993**, *268*, 24591–24598.

(23) Balzarini, J.; Bohman, C.; De Clercq, E. Differential mechanism of cytostatic effect of (E)-5-(2-bromovinyl)-2′-deoxyuridine, 9-(1,3-dihydroxy-2-propoxymethyl)guanine, and other antiherpetic drugs on tumor cells tranfected by the thymidine kinase gene of herpes simplex virus type 1 or type 2. *J. Biol. Chem.* **1993**, *268*, 6332–6377.

JM940465Y