(±)-2-Amino-3,4-dihydro-7-[2,3-dihydroxy-4-(hydroxymethyl)-1-cyclopentyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ones: New Carbocyclic Analogues of 7-Deazaguanosine with Antiviral Activity

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5-Allyl-2-amino-4,6-dihydroxypyrimidine (**3**) was chlorinated and ozonized to yield (2-amino-4,6-dichloropyrimidin-5-yl)acetaldehyde (**5**). Acetalization of **5** with ethanol afforded a new pyrimidine intermediate **6** which can lead to 2-amino-3,4-dihydro-7-alkyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ones and therefore to carbocyclic analogues of 7-deazaguanosine. The 7-substituent was a cyclopentyl analogue of the arabinofuranosyl moiety in **10a**, lyxofuranosyl moiety in **10b**, and ribofuranosyl moiety in **10c**. Compounds **10a** and **10b** exhibited selective inhibitory activities against the multiplication of HSV1 and HSV2 in cell culture. Repeated administration of compound **10a** at 10 mg/kg ip to mice infected with HSV2 increased the number of survivors and lengthened significantly the mean survival time.

Much effort continues to be expended in the development of nucleoside analogues with selective antiviral properties. Among the very potent antiherpetic drugs discovered over the past few years, the most potent are acyclic analogues of guanosine such as acyclovir which selectively inhibit the replication of herpes simplex virus (HSV).[1-6] The mechanism of action involves their selective initial phosphorylation by the HSV-encoded thymidine kinase and explains why they are much less toxic to the noninfected cells.[1] Thus, replacement of the cyclic carbohydrate moiety of guanosine by acyclic chains mimicking the carbohydrate moiety seems to bring about selective activation of the nucleoside analogue in the virus-infected cells. Another possibility of simulating the ribosyl part of nucleosides lies in the replacement of the furanosyl oxygen by a methylene group as in aristeromycin,[7,8] a natural carbocyclic analogue of adenosine. Carbocyclic analogues of guanosine or 8-azaguanosine have already been synthesized[9] that demonstrated significant antiviral activity, but no carbocyclic analogue of 7-deazaguanosine has been prepared, up to date, although this structural feature has been found to occur in several natural nucleosides like 7-cyano-7-deazaguanosine,[10] cadeguomycin,[11] kanagawamycin,[12] and dapiramycin.[13]

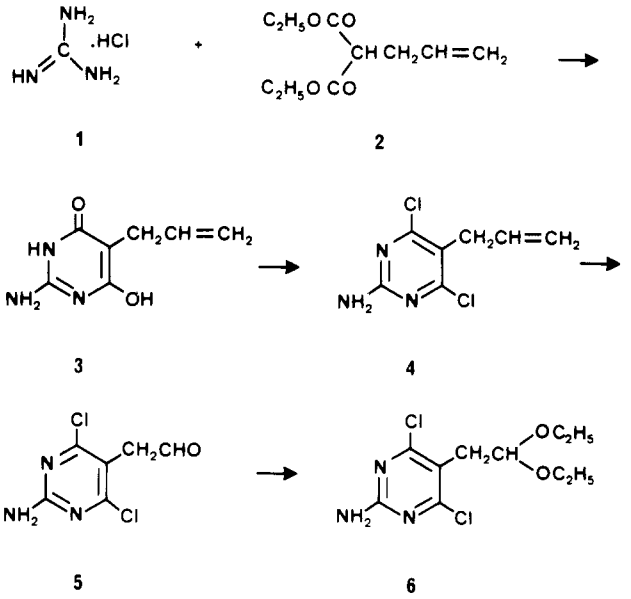
We synthesized recently a series of carbocyclic analogues of 7-deazaadenosine. These carbocyclic derivatives of the pyrrolo[2,3-*d*]pyrimidine ring were found to be inactive against HSV type 1[14] whereas some pyranosyl derivatives exhibited antiviral activity against vaccinia virus and Sindbis virus.[15]

On the basis of the above literature data[1-13] and as part of our ongoing program on the synthesis of pyrrolo[2,3-*d*]pyrimidine nucleoside analogues, it was deemed of interest to prepare some carbocyclic analogues of 7-deazaguanosine in order to evaluate their antiviral activity.

We report in this paper the synthesis and antiherpetic activity of three carbocyclic nucleosides of 2-amino-3,4-dihydro-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (7-deazaguanine), which have been obtained via the new key intermediate 2-amino-4,6-dichloro-5-(2,2-diethoxyethyl)-pyrimidine.

Chemistry. We first sought to prepare the unknown pyrimidine intermediate (**6**), which could lead to carbocyclic analogues of 7-deazaguanosine. For this purpose, we used a reaction sequence similar to the one used by Montgomery and Hewson[16] for the synthesis of a carbocyclic analogue of 7-deazaadenosine (Scheme I).

Scheme I



5-allyl-2-amino-4,6-dihydroxypyrimidine (**3**), prepared in 64% yield by the reaction of guanidine hydrochloride

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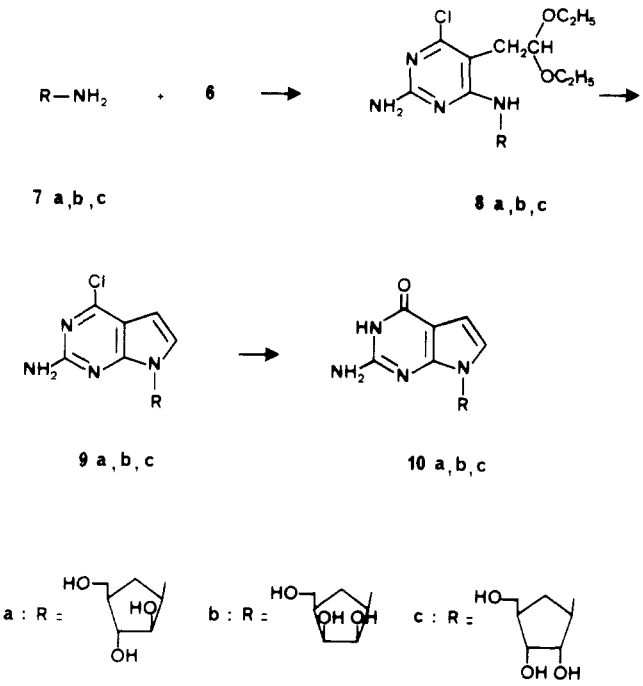
Scheme II



with diethyl allylmalonate, was converted to the 4,6-dichloro derivative (**4**) by treatment with $POCl_3$ and diethylaniline in the presence of PCl_5, in fair yield (50–60%). In the absence of PCl_5, the yield was lower (20–30%). The (2-amino-4,6-dichloropyrimidin-5yl)acetaldehyde (**5**) was obtained from **4** by ozonolysis of the allyl group in ethyl acetate–methanol at −78 °C and subsequent reduction of the ozonide by known procedure[16] (Scheme I).

The amines **7a–c** (2 equiv) (Scheme II), prepared according to Vince and Daluge,[17,18] were tentatively condensed with the aldehyde **5** (1 equiv) in 1-butanol and triethylamine in excess under argon. However, only the amine **7c** led to the expected pyrrolo[2,3-*d*]pyrimidine **9c**. In the two other cases, i.e. with **7a** and **7b**, no expectd **9a** or **9b**, respectively, could be isolated even by adding a large excess of amine. According to NMR spectra (Me_2SO-d_6) the Schiff base was formed in majority in these two cases, which was characterized by a doublet at 5.55 ppm and a multiplet at 3.21 ppm (irradiation of the multiplet at 3.21 ppm transformed the doublet into a singulet at 5.55 ppm). This is why the diethylacetal derivative **6** of **5** had to be prepared by using standard methods[16] and should be used in further investigations to avoid any imine formation. The acetals **8** cyclized to 2-amino-4-chloro-7-alkyl-7*H*-pyrrolo-[2,3-*d*]pyrimidine (**9**) on treatment with dilute aqueous HCl (0.2 N) at room temperature. The guanosine-like compounds **10** were then obtained by hydrolysis of the remaining chlorine atom of **9** in 1 N HCl at 100 °C.

Biological Results. Compounds **10a**, **10b**, and **10c** were examined for their inhibitory activities against the multiplication of DNA viruses (HSV1, HSV2, adenovirus) and RNA viruses (RV-1B, RV31, Coxsackie A21 virus, influenza (H_3N_2), parainfluenza, Sindbis virus). All three compounds, at the highest concentraton studied (100 μg/mL) were devoid of cytotoxicity in both confluent and proliferating MRC5 cells. No antiviral activity was detected against adenovirus, RV-1B, Coxsackie, influenza, parainfluenza, and Sindbis virus. At this concentration, a small inhibition (50%) of the cytopathic effect in RV31-infected cells was observed only with compound **10c**. In contrast, at 100 μg/mL compounds **10a** and **10b** were found to completely inhibit the cytopathic effect of HSV1 and HSV2, while compound **10c** was inactive.

Table I. Antiherpetic Activity of Compounds **10a** and **10b** in Cell Culture[a]

		HSV2		
			log inhibn of viral yield	
compd	HSV1 MIC,[b] μg/mL	MIC, μg/mL	at 25 μg/mL	at 100 μg/mL
10a	25	25	2.5	3.7
10b	100	25	1.5	2.5

[a] See Experimental Section. [b] Minimal inhibitory concentration.

As shown in Table I, **10a** had a MIC of 25 μg/mL against both HSV1 and HSV2 while **10b** was less active against HSV1 (MIC = 100 μg/mL).

The inhibitory activity on the most sensitive virus (HSV2) was also confirmed by titrating the viral yield in MRC5, at 3 days post-fection. Compound **10b** inhibited HSV2 multiplication by 1.5 log at 25 μg/mL and by 2.5 log at 100 μg/mL. Compound **10a** was even more effective in inhibiting the virus by 2.5 log and 3.7 log, respectively, at 25 and at 100 μg/mL.

These results prompted us to perform animal studies with the latter compound. This compound (**10a**) was administered ip in multiple treatments (at 2 h before infection and at 2, 18, 24, and 48 h after infection) at 1–10 mg/kg to mice infected (ip) with HSV2. At the end of the experiment (14 days post-infection) the number of survivors (over total infected mice) was $^3/_{15}$ for the control mice receiving saline and respectively $^6/_{15}$ and $^8/_{15}$ for those treated with the compound at 1 and 10 mg/kg. The mean survival time was 8.6 days for the control mice and respectively 9.9 and 10.6 days for mice treated with compound **10a** at 1 and 10 mg/kg. However, it was only at the highest dose (10 mg/kg) that the prolongation of survival time was statistically significant ($p = 0.0047$).

The results presented in the present paper indicate that carbocyclic analogues of 7-deazaguanosine exhibit selective antiherpetic activity in cell culture and in an animal model of herpes virus infection. Further studies are needed to fully appreciate the therapeutic potential of these compounds as compared to other antiviral compounds.[1-3]

Experimental Section

Chemistry.[19] Melting points were determined with a Reichert hot-stage microscope and are uncorrected. Proton NMR spectra were obtained with a Varian XL 100 (100-MHz) spectrometer operating in the Fourier transform mode unless otherwise stated. ^1H NMR spectra (400 MHz) were obtained with a Brucker AM 400W spectrometer. Notations used in the NMR data are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiple. Chemical shifts (δ) in ppm are reported relative to tetramethylsilane. The elemental analyses were performed by the Service Central de Microanalyses, CNRS-ICSN (91190 Gif-sur-Yvette, France) and are within ±0.4% of the theoretical values. Ultraviolet (UV) spectra were determined in ethanolic solution

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(19) As noticed recently[9b] in accordance with Chemical Abstracts nomenclature, compounds **8** and **9** are named as derivatives of 1,2-cyclopentanediols. Compounds **10**, which have an oxo substituent on the heterocyclic ring, are named as cyclopentylpyrrolo[2,3-*d*]pyrimidines.

with a Cary 118 spectrophotometer; the number in parentheses is the extinction coefficient. The thin-layer chromatographic analyses (TLC) were performed with 0.2-mm-thin layers of silica gel coated on plastic plates (Kieselgel 60F254, Merck). The preparative separations were carried out on columns packed with 230–400-mesh silica gel (Kieselgel 60, Merck) under pressure (1–6 bars).

5-Allyl-2-amino-4,6-dihydroxypyrimidine (3). Guanidine hydrochloride (Prolabo) (54 g, 0.56 mol) was added to cold absolute ethanol (400 mL) containing $NaOCH_2CH_3$ (114.92 g, 1.69 mol), and the mixture was stirred at 5 °C for 10 min before diethyl allylmalonate (Aldrich) (110 g, 0.56 mol) was added. The reaction mixture was then stirred at room temperature for 18 h. Acidification with concentrated HCl precipitated all the crude product, which was collected by filtration and washed with ethanol. Recrystallization from water afforded 60.4 g (64 %) of pure **3**: mp 272 °C; UV λ_{max} 266 nm (15 500); ^{1}H NMR (Me_2SO-d_6) δ 6.37 (s, 2 H, NH_2), 5.80 (m, 1 H, CH), 5.00 (m, 1 H, CH_2), 4.84 (m, 1 H, CH_2), 3.33 (br, N H, OH, DOH), 2.90 (d of t, 2 H, CH_2). Anal. ($C_7H_9N_3O_2 \cdot {}^1/_2H_2O$) C, H, N.

5-Allyl-2-amino-4,6-dichloropyrimidine (4). Dried 5-allyl-2-amino-4,6-dihydroxypyrimidine (**3**) (9.8 g, 55.9 mmol) was added in small portions to a solution of PCl_5 (12.3 g, 58.98 mmol) in $POCl_3$ (300 mL) at 60 °C. The temperature was raised to 120 °C, and diethylaniline (6 g) in $POCl_3$ (60 mL) was added dropwise. The reaction mixture was then refluxed for 4 h before it was evaporated to dryness. Hot water (200 mL) was added slowly to the residue (treatment of the residue with cold water would give lower yield of dichloropyrimidine **4**), and the resulting suspension was cooled and extracted with CH_2Cl_2 (250 mL × 3). The combined organic extracts were washed with cold water several times until the aqueous extract was above pH 5. The organic extract was dried ($MgSO_4$) and evaporated to dryness in vacuo. Recrystallization from methanol afforded pure **4** in 57% yield: mp 182 °C; UV λ_{max} 305, 237 nm (4500, 19 700); ^{1}H NMR (Me_2SO-d_6) δ 7.36 (s, 2 H, NH_2), 5.88 (m, 1 H, CH), 5.03 (m, 2 H, CH_2), 3.45 (m, 2 H, CH_2). Anal. ($C_7H_7Cl_2N_3$) C, H, Cl, N.

(2-Amino-4,6-dichloropyrimidin-5-yl)acetaldehyde (5). A solution of 5-allyl-2-amino-4,6-dichloropyrimidine (**4**) (3 g, 14.7 mmol) in ethyl acetate (150 mL) and methanol (30 mL) was ozonized at −78 °C for about 1.5 h on a Labo 76 (Trailigaz) apparatus that delivered about 5% ozone at a rate of 1 L/min. When all the 5-allyl-pyrimidine **4** had disappeared (according to TLC in petroleum ether–AcOEt 3:1 (v/v), the reaction mixture was flushed with oxygen for 30 in. NaI (9 g) and glacial acetic acid (9 mL) were added simultaneously to the cold reaction mixture, and the temperature was allowed to warm up to 20 °C with continuous stirring over a 90-min period. Sodium thiosulfate solution (67 g/100 mL of H_2O) was added to the reaction mixture until it became colorless. The resulting mixture was diluted with water (100 mL) and extracted with CH_2Cl_2 (100 mL × 4). The organic joined extracts were washed successively with H_2O (100 mL × 4), $NaHCO_3$ (100 mL), and saturated NaCl (100 mL) and dried ($MgSO_4$) before evaporation to dryness. Recrystallization from toluene afforded pure **5** in 70% yield: mp 180–182 °C; UV λ_{max} 304, 237 nm (3700, 15 200); ^{1}H NMR ($Me_2SO$$d_6$) δ 9.70 (s, 1 H, CH), 7.50 (s, 2 H, NH_2), 3.93 (s, 2 H, CH_2). Anal. ($C_6H_5Cl_2N_3O$) C, H, Cl, N.

2-Amino-4,6-dichloro-5-(2,2-diethoxyethyl)pyrimidine (6). A mixture of (2-amino-4,6-dichloropyrimidin-5-yl)acetaldehyde (**5**) (2 g, 9.7 mmol) and NH_4Cl (53.5 mg, 1 mmol) in absolute ethanol (40 mL) was refluxed for 3 h. The reaction solution was evaporated to dryness in vacuo and the residue was dissolved in hot hexane. After treatment with Norit and filtration, white crystals of **6** were obtained. Recrystallization from hexane afforded pure **6** in 77% yield: mp 146–149 °C; UV λ_{max} 304, 238 nm (4500, 18 300); ^{1}H NMR (Me_2SO-d_6) δ 7.36 (s, 2 H, NH_2), 4.68 (t, 1 H, CH), 3.54 (m, 4 H, OCH_2), 2.93 (d, 2 H, CH_2), 1.08 (t, 6 H, CH_3). Anal. ($C_{10}H_{15}Cl_2N_3O_2$) C, H, Cl, N.

(±)-(1α,2β,3β,5β)-3-[[2-Amino-4-chloro-5-(2,2-diethoxyethyl)pyrimidin-6-yl]amino]-5-(hydroxymethyl)-1,2-cyclopentanediol (8a). A mixture of (±)-(1α,2β,3β,5β)-3-amino-5-(hydroxymethyl)-1,2-cyclopentanediol (**7a**)[20] obtained from hydrolysis of its tetraacetate derivative (1 g, 3.17 mmol), 2-amino-4,6-dichloro-5-(2,2-diethoxyethyl)pyrimidine (**6**) (0.9 g, 3.2 mmol), and triethylamine (3 mL) in 1-butanol (40 mL) was heated at 100 °C under argon for 2 days. At this time, TLC ($CHCl_3$–EtOH 8:2 (v/v) showed only a minor spot of **6**. The reaction solution was evaporated to dryness under reduced pressure, and the residue was subjected to column chromatography prepared in $CHCl_3$. Elution with $CHCl_3$–EtOH 95:5 (v/v) afforded pure **8a** as a colorless oil in 42% yield: ^{1}H NMR (Me_2SO-d_6) δ 6.25 (s, 2 H, NH_2), 6.22 (d, 1 H, NH), 5.05 (d, 1 H, OH), 4.73 (d, 1 H, OH), 4.40 (m, 3 H, CH ethyl, OH, H-3′), 3.48 (m, 8 H, H-1′, H-2′, CH_2OH, 2 × CH_2 ethyl), 2.72 (m, 2 H, CH_2 ethoxy), 1.99 (m, 2 H, H-4′, H-5′), 1.21 (m, 7 H, H-4″, 2 × CH_3). Anal. ($C_{16}H_{27}ClN_4O_5$) C, H, Cl, N.

(±)-(1α,2α,3α,5α)-3-[[2-Amino-4-chloro-5-(2,2-diethoxyethyl)pyrimidin-6-yl]amino]-5-(hydroxymethyl)-1,2-cyclopentanediol (8b). A mixture of (±)-(1α,2α,3α,5α)-3-amino-5-(hydroxymethyl)-1,2-cyclopentanediol (**7b**) obtained as described[18] (555 mg, 3.7 mmol), 2-amino-4,6-dichloro-5-(2,2-diethoxyethyl)-pyrimidine (**6**) (1 g, 3.7 mmol), and triethylamine (3 mL) in 1-butanol (40 mL) was heated at 100 °C under argon for 2 days. The same treatment as for **8a** left **8b** as a colorless oil in 50% yield: ^{1}H NMR (Me_2SO-d_6) δ 6.30 (d, 1 H, NH), 6.20 (s, 2 H, NH_2), 4.80 (d, 1 H, OH, J = 6.1 Hz), 4.68 (d, 1 H, OH, J = 4.7 Hz), 4.53 (t, 1 H, CH ethyl, $J \sim$ 5.2 Hz), 4.39 (t, 1 H, CH_2OH), 4.33 (m, 1 H, H-3′), 4.04 (m, 1 H, H-1′), 3.86 (m, 1 H, H-2′), 3.54 (m, 6 H, CH_2OH, 2 × CH_2 ethyl), 2.69 (m, 4 H, 2 × CH_2 ethoxy), 2.06 (m, 2 H, H-4′, H-5′), 1.38 (m, 1 H, H-4″), 1.13 (m, 6 H, 2 × CH_3 ethyl). Anal. ($C_{16}H_{27}ClN_4O_5$) C, H, Cl, N.

(±)-(1α,2β,3β,5β)-3-(2-Amino-4-chloro-7*H*-pyrrolo[2,3-*d*]-pyrimidin-7-yl)-5-(hydroxymethyl)-1,2-cyclopentanediol (9a). A solution of compound **8a** (500 mg, 1.28 mmol) in aqueous 0.2 N HCl (40 mL) was stirred at room temperature for 3 days. The reaction mixture was neutralized with concentrated NH_4OH and evaporated to dryness under reduced pressure. The residue was dissolved in AcOEt (200 mL), which was washed with water (3 × 50 mL). The AcOEt solution was dried ($MgSO_4$), and the insolubles were removed by filtration. After evaporation to dryness, **9a** was obtained by crystallization from ethanol: yield 69%; mp 228 °C; UV λ_{max} 237, 264, 314 nm (31 400, 3500, 5300); ^{1}H NMR (Me_2SO-d_6) δ 7.28 (d, 1 H, H-6, J = 3.9 Hz), 6.57 (s, 2 H, NH_2), 6.29 (d, 1 H, H-5, J = 3.9 Hz), 5.04 (m, 1 H, H-3′), 5.03 (d, 1 H, OH-2′, J = 5 Hz), 4.94 (d, 1 H, OH-1′, J = 4 Hz), 4.67 (t, 1 H, CH_2OH, J = 5 Hz), 3.84 (m, 1 H, H-2′), 3.73 (m, 1 H, H-1′), 3.55 (m, 2 H, CH_2OH), 2 (m, 3 H, H-4′, H-4″, H-5′). Anal. ($C_{12}H_{15}ClN_4O_3$) C, H, Cl, N.

(±)-(1α,2α,3α,5α)-3-(2-Amino-4-chloro-7*H*-pyrrolo[2,3-*d*]-pyrimidin-7-yl)-5-(hydroxymethyl)-1,2-cyclopentanediol (9b). A solution of **8b** (400 mg, 1.02 mmol) was treated with 0.2 N HCl as above to afford after crystallization from methanol 280 mg of **9b**: yield 91%; mp 203–204 °C; UV λ_{max} 237, 263, 316 nm (31 500, 3300, 5900); ^{1}H NMR (Me_2SO-d_6) δ 7.36 (d, 1 H, H-6, J = 3.8 Hz), 6.58 (s, 2 H, NH_2), 6.28 (d, 1 H, H-5, J = 3.8 Hz), 5.01 (q, 1 H, H-3′), 4.87 (d, 1 H, OH-1′, J = 4.2 Hz), 4.79 (d, 1 H, OH-2′, J = 6 Hz), 4.53 (t, 1 H, CH_2OH, J = 5.1 Hz), 4.09 (m, 1 H, H-1′), 4.04 (m, 1 H, H-2′), 3.67 (m, 1 H, CH_2OH), 3.53 (m, 1 H, CH_2OH), 2.16 (m, 1 H, H-4′), 2.11 (m, 1 H, H-5′), 1.86 (m, 1 H, H-4″). Anal. ($C_{12}H_{15}ClN_4O_3$) C, H, Cl, N.

(±)-(1α,2α,3β,5β)-3-(2-Amino-4-chloro-7*H*-pyrrolo[2,3-*d*]-pyrimidin-7-yl)-5-(hydroxymethyl)-1,2-cyclopentanediol (9c). A solution of (2-amino-4,6-dichloropyrimidin-5-yl)acetaldehyde (**5**) (280 mg, 1.36 mmol), (±)-(1α,2α,3β,5β)-3-amino-5-(hydroxymethyl)-1,2-cyclopentanediol (**7c**)[17] (400 mg, 2.72 mmol), and triethylamine (3 mL) in 1-butanol (20 mL) was heated at reflux for 8 h under Ar. The mixture was evaporated to dryness under reduced pressure, and the residue was subjected to column chromatography (prepared in $CHCl_3$). The column was first washed with $CHCl_3$, and $CHCl_3$–EtOH 95:5 (v/v) eluted **9c**, which crystallized in $CHCl_3$ upon concentration: yield 49%; mp 178 °C; UV λ_{max} 236, 263, 315 nm (30 000, 3100, 5300); ^{1}H NMR (Me_2SO-d_6) δ 7.31 (d, 1 H, H-6, J = 3.9 Hz), 6.56 (s, 2 H, NH_2), 6.35 (d, 1 H, H-5, J = 3.9 Hz), 4.82 (m, 1 H, H-3′), 4.75 (d, 1 H, OH-2′, J = 5 Hz), 4.66 (t br, 1 H, CH_2OH), 4.54 (d, 1 H, OH-1′, J = 4 Hz), 4.15 (m, 1 H, H-2′), 3.84 (m, 1 H, H-1′), 3.46 (m, 2 H, CH_2, CH_2OH), 2.11 (m, 2 H, H-5′, H-4′), 1.48 (m, 1 H, H-4″). Anal. ($C_{12}H_{15}ClN_4O_3$) C, H, Cl, N.

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(±)-2-Amino-3,4-dihydro-7-[(1α,2α,3β,4α)-2,3-dihydroxy-4-(hydroxymethyl)-1-cyclopentyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (10a). A solution of (±)-(1α,2β,3β,5β)-3-(2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-5-(hydroxymethyl)-1,2-cylclopentanediol (**9a**) (150 mg, 0.5 mmol) in 1 N HCl (20 mL) was heated at reflux for 6 h with stirring. The solvent was removed under reduced pressure and azeotroped with absolute ethanol. The residue was dissolved in water (1–2 mL), and the solution was neutralized (pH 7) with 6 N NaOH. After cooling, **10a** was collected by filtration and washed with cold water: yield 85%; mp 274–275 °C; UV λ_{max} 262 nm (15000), 280 (sh); ^{1}H NMR (400 MHz) (Me_2SO-d_6) δ 10.26 (s, 1 H, NH), 6.83 (d, 1 H, H-6, J = 3.7 Hz), 6.21 (s, 2 H, NH_2), 6.20 (d, 1 H, H-5, J = 3.7 Hz), 5.03 (d, 1 H, OH-2′, J = 4.9 Hz), 4.95 (d, 1 H, OH-3′, J = 3.7 Hz), 4.93 (m, 1 H, H-1′, $J_{1',2'}$ = 4.9 Hz, $J_{1',5'}$ = 7.3 Hz, $J_{1',5''}$ = 11 Hz), 4.71 (t, 1 H, CH_2OH), 3.77 (t, 1 H, H-2′, $J_{2',3'}$ = 1.5 Hz), 3.70 (dd, 1 H, H-3′, $J_{3',4'}$ = 3.3 Hz), 3.58 (m, 1 H, CH_2OH, ^2J = 10.5 Hz, ^3J = 6.5 Hz), 3.47 (m, 1 H, CH_2OH), 2.07 (m, 1 H, H-5′, $J_{5',5''}$ = 12 Hz, $J_{5',4'}$ = 7.9 Hz), 1.91 (m, 1 H, H-4′, $J_{4',5''}$ = 8.7 Hz), 1.85 (m, 1 H, H-5″). Anal. ($C_{12}H_{16}N_4O_4 \cdot {}^3/_2H_2O$) C, H, N.

(±)-2-Amino-3,4-dihydro-7-[(1α,2α,3α,4α)-2,3-dihydroxy-4-(hydroxymethyl)-1-cyclopentyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (10b). A solution of (±)-(1α,2α,3α,5α)-3-(2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-5-(hydroxymethyl)-1,2-cyclopentanediol (**9b**) (200 mg, 0.67 mmol) in 1 N HCl (25 mL) was treated as above (see preparation of **10a**) to yield **10b** in 75%: mp 212 °C; UV λ_{max} 262 nm (12000) 280 (sh); ^{1}H NMR (400 MHz) (Me_2SO-d_6) δ 10.27 (s, 1 H, NH), 6.92 (d, 1 H, H-6, J = 3.5 Hz), 6.20 (d, 1 H, H-5), 6.16 (s, 2 H, NH_2), 4.83 (q, 1 H, H-1′, $J_{1',2'}$ = 6.7 Hz, $J_{1',5'}$ = 7.9 Hz, $J_{1',5''}$ = 9.2 Hz), 4.82 (d, 1 H, OH-2′, J = 4.9 Hz), 4.71 (d, 1 H, OH-3′, J = 6.7 Hz), 4.55 (t, H, CH_2OH, J = 4.9 Hz), 4.08 (q, 1 H, H-3′, $J_{3',2'}$ = 4 Hz, $J_{3',4'}$ = 4.5 Hz), 3.95 (q, 1 H, H-2′, $J_{2',1'}$ = 6.7 Hz), 3.64 (m, 1 H, CH_2OH, ^2J = 10.5 Hz, ^3J = 5.5 Hz), 3.53 (m, 1 H, CH_2OH), 2 (unresolved m, 1 H, H-4′), 2 (unresolved m, 1 H, H-5′), 1.88 (m, 1 H, H-5″). Anal. ($C_{12}H_{16}N_4O_4 \cdot H_2O$) C, H, N.

(±)-2-Amino-3,4-dihydro-7-[(1α,2β,3β,4α)-2,3-dihydroxy-4-(hydroxymethyl)-1-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (10c). A solution of (±)-(1α,2α,3β,5β)-3-(2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-5-(hydroxymethyl)-1,2-cyclopentanediol (**9c**) (170 mg, 0.56 mmol) in 1 N HCl (20 mL) was treated as above (see preparation of **10a**) to yield 80% of **10c**: mp 196 °C; UV λ_{max} 262 nm (13000), 280 (sh); ^{1}H NMR (400 MHz) (Me_2SO-d_6) δ 10.29 (s, 1 H, NH), 6.85 (d, 1 H, H-6, J = 3.7 Hz), 6.27 (d, 1 H, H-5), 6.20 (s, 2 H, NH_2), 4.75 (d, 1 H, OH-2′, J = 6.1 Hz), 4.75 (t, 1 H, OH-5′, J = 5 Hz), 4.56 (d, 1 H, OH-3′, J = 3.6 Hz), 4.73 (q, 1 H, H-1′, $J_{1',2'}$ = 9.2 Hz, $J_{1',5'}$ = 10 Hz, $J_{1',5''}$ = 8 Hz), 4.07 (m, 1 H, H-2′, $J_{2',3'}$ = 5.5 Hz), 3.81 (m, 1 H, H-3′, $J_{3',4'}$ = 3.5 Hz), 3.47 (m, 2 H, CH_2OH), 2.11 (m, 1 H, H-5′, $J_{5',5''}$ = 13 Hz), 1.99 (m, 1 H, H-4′, $J_{4',5'}$ = 9 Hz, $J_{4',5''}$ = 8 Hz), 1.41 (m, 1 H, H-5″). Anal. ($C_{12}H_{16}N_4O_4 \cdot H_2O$) C, H, N.

Antiviral and Cytotoxicity Assays. Antiviral tests were carried out on the following viruses: herpes simplex type 1 and type 2 (HSV1, HSV2), adenovirus, rhinovirus 1B and 31 (RV1B, RV31), influenza virus A Victoria 3/75 (H_3N_2), parainfluenza, and Sindbis virus. With the exception of influenza virus multiplication, which was studied in MDCK cells (minimal Eagle's medium (MEM) with Earl's salts; trypsin 5 μg/mL, penicillin, and streptomycin sulfate at, respectively, 40 IU and 40 μg/mL), all other viruses were examined in MRC5 cells (basal medium Eagle (BME) with Earle's salts supplemented with 2% calf serum; antibiotics as above).

Confluent cell monolayers in 1.7 mL of medium were inoculated with 0.2 mL of virus (containing 10 × TC ID_{50}) and 0.1 mL of compound (concentrated 20×). Cultures were incubated at 37 °C until a generalized cytopathic effect (80–100% destruction) appeared (usually 3–5 days post-infection, depending on the type of virus studied). The minimal inhibitory concentration (MIC) was defined as the minimum concentration of the compound that was fully protecting cell monolayers from destruction. In the case of HSV2, production of infectious virus was titrated in MRC5 cells by determining the TC ID_{50} value (dose of virus capable of infecting 50% of the tissue cultures).[21] Cytotoxicity was determined in both confluent and proliferating uninfected MRC5 cell cultures. Confluent cultures were examined daily, for up to 6 days, under the microscope for signs of morphological abnormalities. For evaluation of the antiproliferative capacity, MRC5 cells were seeded in Limbro plates at the concentration of 7.5 × 10^4 cells/well (2 cm^2) in the presence of various concentrations of the compounds. After 3 days of culture, the number of cells in the newly formed monolayer was assessed by a colorimetric method.[22] OF1 mice (4-week-old females supplied by IFFA, Credo, France) were used for the in vivo evaluation of compound **10a**. The compound was administered intraperitoneally (ip) in 0.2 mL of saline at different times (−2 h, +2 h, +18 h, +24 h, +48 h) with respect to HSV2 infection (ip route). For each dosage, groups of 15 mice were used and control animals received multiple injections of saline. Results were interpreted for statistical significance by the generalized rank analysis test of Jonckheere.[23]

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