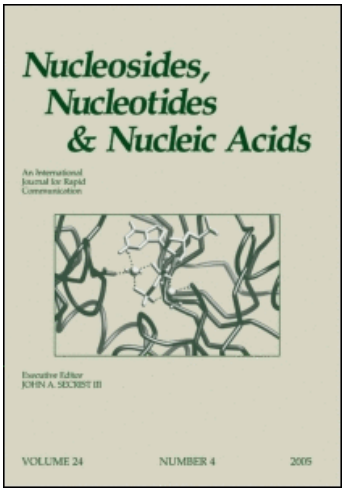

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SYNTHESIS AND ANTIVIRAL EVALUATION OF TWO NEW CARBOCYCLIC PYRROLO [2,3-d] PYRIMIDINE NUCLEOSIDES.

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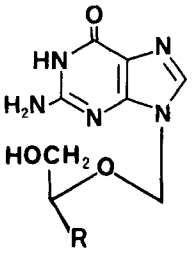
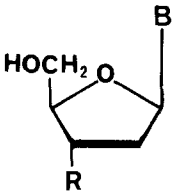
ABSTRACT : Two new carbocyclic pyrrolo[2,3-d]pyrimidine nucleoside analogs related to the antiherpetic agent RP54 247 (**8**) have been synthesized. These compounds exhibited no antiviral activity in vitro toward herpes simplex virus type 1, human cytomegalovirus, and human immunodeficiency virus 1.

With the exception of amantadine and rimantadine, which are influenza virus inhibitors, all other antivirals presently licensed are nucleoside analogs. Acyclovir (**1**) is now widely used for the treatment of herpes simplex virus (HSV-1, HSV-2) and varicella zoster virus (VZV) infections. More recently, ganciclovir (**2**) has been approved for the management of cytomegalovirus (CMV) infection in immunocompromised patients[1,2] (Fig. I).

Renewed interest in nucleoside analogs has appeared since it has been shown that 3'-azido-3'-deoxythymidine (**3**) (AZT, zidovudine) is an effective inhibitor of human immunodeficiency virus (HIV)[3], the causative agent of the acquired immunodeficiency syndrome (AIDS). At the present time, AZT is the only drug approved for the treatment of AIDS[4]. However, toxicity observed in patients treated with AZT[4,5] (mainly anemia and granulocytopenia) and the fact that, at least in cell cultures, HIV replication can emerge in the presence of the drug[5b], emphasize the need for new drugs in this area. The mode of action of AZT





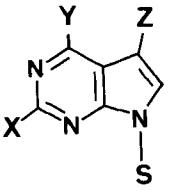
1 R = H (acyclovir)
2 R = CH_2OH (ganciclovir)

3 (AZT) R = N_3 ; B = thymine
4 a R = N_3 ; B = guanine
b R = H ; B = guanine

5 R = H ; B = cytosine
6 R = H ; B = adenine

FIG. 1 Structures of potent antiherpes (1), anticytomegalovirus (2), and anti-HIV (3, 4, 5, 6) compounds.

TABLE I. Structures of some pyrrolo[2,3-d]pyrimidine nucleosides with antiviral properties.



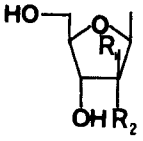
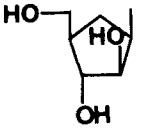
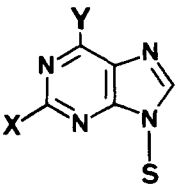
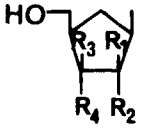
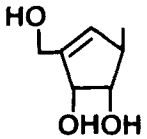
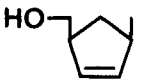
S	Compound	X	Y	Z	R_1	R_2	Ref.
	7a	H	NH_2	$CHOHCH_3$	H	OH	8
	b	H	NH_2	$CH(OCH_3)CH_3$	H	OH	8
	c	H	NH_2	CN	H	OH	9
	d	H	NH_2	CN	OH	H	9
	e	H	NH_2	$CONH_2$	H	OH	9
	f	H	NH_2	$CONH_2$	OH	H	9
	8 (RP54247)	NH_2	OH	H	-	-	10,11

TABLE II. Structures of some carbocyclic nucleosides with anti-HSV (**9a**,**b**,**d**, **10**) or anti-HIV properties (**11**) and of the inactive compound **9c**.



S	Compound	X	Y	R_1	R_2	R_3	R_4	Ref
	**9a**	NH_2	OH	H	OH	OH	H	12
	b (cyclaradine)	H	NH_2	OH	H	H	OH	13
	c	NH_2	OH	H	OH	H	OH	14
	d	NH_2	NH_2	H	OH	H	OH	14
	**10** (neplanocin A)	H	NH_2	-	-			15
	**11** (carbovir)	NH_2	OH	-	-			16

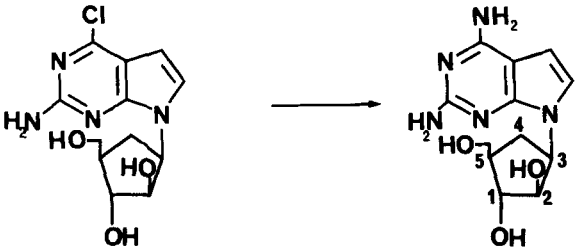
involves the preferential inhibition of HIV-coded reverse transcriptase by its 5'-triphosphate derivative[6a]. This mechanism may be common to other dideoxynucleosides such as **4**-**a**,**b**, **5** and **6**[6b,7].

Other types of nucleosides derived from pyrrolo[2,3-d]pyrimidine (**7a**,**b**,**c**,**e**, Table I) are known to inhibit different RNA viruses[8]. Ara-toyocamycin **7d** and ara-sangivamycin **7f** inhibit HSV-1 but even more powerfully human CMV in cell cultures[9]

Carbocyclic nucleoside analogs are also an important family of antiviral agents since anti-HSV activities have been noted with **9a**[12], **9b**[13] and **9d**[14] (Table II) whereas Neplanocin A (**10**) and 3-deazaneplanocin A inhibit vaccinia virus and other RNA viruses[15]. Moreover, some carbocyclic nucleosides such as carbovir **11** have been shown to exhibit anti-HIV activity[16] (Table II).

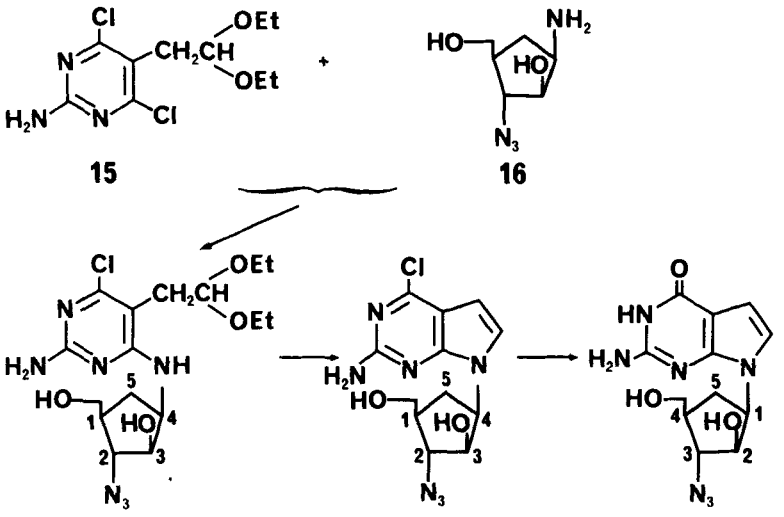
We have ourselves reported the preparation[10] and anti-HSV activities of RP 54 247 (8)[11], a carbocyclic pyrrolo[2,3-d]pyrimidine nucleoside, in cell cultures and in a HSV mouse model.

In this paper, we describe the synthesis and antiviral evaluation of two derivatives of 8, namely 12 and 13. Compound 12 was synthesized with the purpose of increasing the anti-HSV activity of 8[10,11] as in the case of the anti-HSV compound 9d with reference to its monoamino congener 9c which was inactive. Compound 12 was also synthesized in order to study its anti-CMV activity already observed with other pyrrolo[2,3-d]pyrimidine nucleosides 7[9] (Table I). Compound 13 which has an azido group in the 3'-position was synthesized as potential anti-HIV agent, owing to the good anti-HIV activity of 3 (AZT), 4a, and carbovir (11). Compound 8 was also evaluated for anti-CMV and anti-HIV properties.



14 12

The 2,4-diaminopyrrolo [2,3-d] pyrimidine derivative 12 was isolated after reaction of the chloro derivative 14[10] with ammonia at 90°C for 3 days in a



15 16 17 18 13

stainless-steel bomb. Condensation of azido-cyclopentylamine 16 and pyrimidine 15[10] has been performed in 1-butanol at 100°C for 3 days under inert atmosphere.

Amine 16 was obtained by mild acidic hydrolysis of 4-α-acetamido-3α-acetoxy-2β-azido-1α-cyclopentanemethyl acetate[17] followed by column chromatography on Dowex 50 (H^+) in methanol. Treatment of 17 with dilute aqueous HCl at room temperature led to the aldehyde which cyclised directly to the pyrrolo[2,3-d]pyrimidine 18. Hydrolysis of 18 with 1N HCl under reflux conditions gave 7-deazaguanine derivative 13.

The inhibition of the cytopathic effect (CPE) of HSV-1 and CMV (Davis strain) in stationary MRC_5 cells was studied as reported previously[11].

Compounds 12 and 13 were devoid of anti-HSV and anti-CMV activity at concentration $\leqslant$ 100 μg/ml. However, partial inhibition (70 %) of CMV multiplication was observed with the parental compound 8 at 100 μg/ml. Higher concentrations ($\leqslant$ 300 μg/ml) were used in the anti-HIV assay (see experimental section) where 20 % and 50 % inhibition of cell growth was noticed at 300 μg/ml with 12 and 13, respectively. An inhibition of 50 % of CEM cell growth was noticed in a 7-days assay for compound 8 at 30 μg/ml. These two compounds (12 and 13) as well as RP 54247 (8) were totally devoid of anti-HIV activity.

In conclusion, replacement of 7-deazaguanine in 8 by a 7-deaza-2,6-diamino purine (12) led to a loss of anti-HSV activity. Anti-CMV activity previously observed with other 7-deaza adenosines (7d, 7f) was detected with compound 8 only at 100 μg/ml but compounds 12 and 13 were found inactive.

The introduction of a polymerase chain terminator (azido-group) at the 3' position in 13, was insufficient to make this compound active against HIV, despite its structural features related to the guanosine analogs 4a and carbovir 11.

EXPERIMENTAL SECTION

Chemistry. The melting points were taken on a Kofler hot stage apparatus and are uncorrected. Nuclear magnetic resonance (^1H NMR) spectra were obtained with a Varian XL100 at 100 MHz and with a Bruker AM 400 W spectrometer at 400 MHz. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane. The numbering used for NMR data of compounds 12, 17, 18 and 13 is shown on the schemes. Elemental analyses were performed by the "Service de Microanalyses", CNRS-ICSN, 91190 Gif sur Yvette, France. The preparative chromatographies were carried out in glass columns packed with 230-400 mesh silica gel (Kiesel gel 60, Merk) under low pressure (1-10 bars).

(±)-(1α,2β,3β,5β)-3-[2,4-Diamino-7H-pyrrolo[2,3-d]pyrimidin -7yl] -5-hydroxymethyl-1,2-cyclopentanediol (12).

A solution of 750 mg (2.51 mmol) of (±)-(1α,2β,3β,5β)-3-[2-amino-4-chloro-(7H)-pyrrolo[2,3-d]pyrimidin-7-yl]-5-hydroxymethyl-1,2-cyclopentanediol (14)[10] in 200 mL of liquid ammonia was heated at 85°C for 3 days in a stainless-steel bomb with stirring. The ammonia was evaporated with a current of nitrogen and the product purified by column chromatography under pressure (10 bars). Elution of 12 was obtained with CH_2Cl_2 : EtOH (8 : 2, v/v). Yield 40 % ; m.p. 136-138°C (EtOH).

^1H NMR (100 MHz) (Me_2SO-d_6) :δ 1.91 (m, 3H, CH_2-4, H-5) ; 3.53 (m, 2H, CH_2OH) ; 3.77 (m, 2H, H-1, H-2) ; 4.63 (t, 1H, $CH_2\underline{OH}$) ; 4.88 (d, 1H, OH) ; 4.93 (d, 1H, OH) ; 5.08 (m, 1H, H-3) ; 5.49 (s, 2H, NH_2) ; 6.30 (d, 1H, H-6, J_{6-5}= 3.5 Hz) ; 6.43 (s, 2H, NH_2) ; 6.89 (d, 1H, H-6, J_{6-5}= 3.5 Hz). Anal. Calcd. for $C_{12}H_{17}N_5O_3 \cdot C_2H_6O$: C, 51.68 ; H, 7.08 ; N, 21.53. Found : C, 51.39 ; H, 7.12 ; N, 21.23.

(±)-(1α,2β,3α,4α)-4-[[2-Amino-4-chloro-5-(2,2-diethoxyethyl)-pyrimidin-6-yl]-amino]-2-azido-3-hydroxycyclopentanemethanol (17).

A mixture of pyrimidine 15 (2,2 g, 7.8 mmol) azido cyclopentylamine 16 (1.1 g, 6.4 mmol) and triethylamine (3 g) in 1-butanol (50 mL) was stirred for two days at 100°C under an argon atmosphere. After evaporation under reduced pressure, the residue was purified by gel column chromatography. Elution with hexane : EtOAc (7 : 3, v/v) separated the unreacted pyrimidine 15, and 17 was then obtained as an oil which crystallized after solvent evaporation. Recrystallization from EtOAc yielded 47 % of pure product ; m.p. 119°C. ^1H NMR (400 MHz) (Me_2SO-d_6) : δ 1.13 (tt, 6H, 2 x CH_3) ; 1.35 (m, 1H, H-5, $^2J_{5-5}$= 12.8 Hz, $^3J_{5-1}$= 9.2 Hz) ; 1.88 (m, 1H, H-1) ; 2.19 (m, 1H, H-5) ; 2.70 (m, 2H, CH_2 pyrim.) ; 3.47 (m, 4H, 2 x CH_2) ; 3.64 (m, 2H, CH_2OH) ; 3.69 (m, 1H, H-2, J_{2-3}= 3.7 Hz, J_{2-1}= 6.1 Hz) ; 3.90 (m, 1H, H-3, J_{3-OH}= 4.7 Hz) ; 4.26 (m, 1H, H-4, J_{4-NH} = 7.2 Hz, J_{4-3}= 6 Hz) ; 4.50 (t, 1H, CH-ethyle) ; 4.88 (t, 1H, $CH_2\underline{OH}$, J = 5.1 Hz) ; 5.64 (d, 1H, OH, J = 4.7 Hz) ; 6.27 (d, 1H, NH, J = 7.2 Hz) ; 6.36 (s, 2H, NH_2) ; Anal. Calcd. for $C_{16}H_{26}N_7O_4Cl$: C, 46.20 ; H, 6.25 ; N, 23.58. Found : C, 46.19 ; H, 6.22 ; N, 23.34.

(±)-(1α,2β,3α,4α)-4-[2-Amino-4-chloro-7H-pyrrolo[2,3-d]pyrimidin-7-yl]-2-azido-3-hydroxycyclopentanemethanol (18).

A solution of **17** (760 mg, 1.82 mmol) in aqueous HCl (0.2 N) containing 50 % EtOH was stirred for 3 days at room temperature. After addition of an excess of concentrated NH_4OH, the mixture was evaporated to dryness. The residue was redissolved in EtOAc (200 mL) and washed with water (20 mL x 3). The organic phase was dried ($MgSO_4$) and the solvent evaporated to yield an oil which crystallized on drying (550 mg). An analytical sample was obtained by recrystallization in EtOAc (92 %) ; m.p. 179°C. ^1H NMR (100 MHz) (Me_2SO-d_6) : δ 2.07 (m, 2H, H-5) ; 3.57 (m, 2H, H-1, H-2) ; 3.87 (m, 1H, H-3) ; 4.03 (m, 2H, CH_2OH) ; 4.94 (m, 2H, H-4) ; 5.48 (d, 1H, OH, J = 5 Hz) ; 6.30 (d, 1H, H-6 ; J = 3.7 Hz) ; 6.58 (s, 2H, NH_2) ; 7.26 (d, 1H, H-5 ; J = 3.7 Hz). Anal. Calcd. for $C_{12}H_{14}N_7O_2Cl$: C, 44.51 ; H, 4.32 ; N, 30.29. Found : C, 44.93 ; H, 4.33 ; N, 30.33.

(±)-2-Amino-7-[(1α,2α,3β,4α)-3-azido-2-hydroxy-4-(hydroxymethyl)-cyclopentyl]-3H,7H-pyrrolo[2,3-d]pyrimidin-4-one (13).

A solution of **18** (1.6 g, 4.94 mmol) in 2N HCl (100 mL) was heated at 100°C for 6 h. After cooling and neutralization by NH_4OH, water was evaporated until precipitation of **13**. A first crop of **13** was filtered. The aqueous phase was then extracted with EtOAc and the residue obtained after evaportion was subjected to silica gel column chromatography. Elution with CH_2Cl_2 : EtOH (9 : 1, v/v) yielded **13** which crystallized from EtOH-water (27 %) ; m.p. 212-213°C. ^1H NMR (400 MHz) (Me_2SO-d_6) : δ 1.94 (m, 1H, H-5) ; 2.00 (m, 1H, H-4) ; 2.17 (m, 1H, H-5) ; 3.58 (m, 2H, CH_2OH) ; 3.82 (t, 1H, H-3, J_{3-2}= 5.1 Hz) ; 3.97 (q, 1H, H-2, J_{2-OH}= 4.6 Hz, J_{2-1}= 5.1 Hz) ; 4.83 (m, 1H, H-1) ; 4.93 (m, 1H, CH_2OH) ; 5.45 (d, 1H, OH) ; 6.18 (s, 2H, NH_2) ; 6.22 (d, 1H, H-5, J_{5-6}= 3.5 Hz) ; 6.83 (d, 1H, H-6) ; 10.27 (s, 1H, NH). Anal. Calcd. for $C_{12}H_{15}N_7O_3$: C, 47.20 ; H ,4.95 ; N, 32.11. Found : C, 47.15 ; H, 4.84 ; N, 31.89.

Biology. To determine anti-HIV-1 activity, 25 µL of each compound dissolved in phosphate buffer saline (PBS) or PBS alone were distributed in triplicate in each well of a 96-well tissue culture place ; 125 µL of CEM cell suspension ($5x10^4$ cells/mL) were then added and the plates were incubated for 1 hour at 37°C (5 % CO_2). Cells were infected with 100 µL/well of a HIV-1 suspension (100-200 TC

ID_{50}) and cultured for 7 days ; mock infected cultures were carried out in parallel to determine the cytotoxicity. Cell viability was determined colorimetrically according to previously described essays[18]. The extent of the cytopathic effect was 60 % in infected, untreated cultures.

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