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Synthesis and antiviral properties of carbocyclic 3′-oxa-2′,3′-dideoxyguanosine and its 7-deazaguanosine analogue

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Summary

To evaluate analogues of the antiviral agent (*R*)-9-(3,4-dihydroxybutyl)guanine in which the side-chain C-3 hydroxyl oxygen is part of a five-membered ring, carbocyclic 3′-oxa-2′,3′-dideoxyguanosine (**4**) and carbocyclic 3′-oxa-2′,3′-dideoxy-7-deazaguanosine (**5**) have been synthesized in 17 and 14 steps, respectively, from 5-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylofuranose. Compounds **4** and **5** and their 6-chloro precursors were evaluated against a wide variety of DNA and RNA viruses. Only **4** showed any marginal activity and this was limited to HSV-1 and HSV-2. Even though **4** was less potent towards these latter two viruses than acyclovir, its mechanism and target of action is proposed to resemble that of acyclovir. The only toxicity observed for these compounds was observed in the cell growth assay with human embryonic lung cells.

Antiviral; Nucleoside; Purine; Pyrrolo[2,3-*d*]pyrimidine; Buciclovir; Analog

Introduction

The last 10 years have seen intensive interest in the search for antiviral agents

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(Diana et al., 1989; Mansuri and Martin, 1991). One impetus for this activity is due to the development of sugar modified nucleosides prominently represented by the ribofuranosyl truncated derivatives 9-[(2-hydroxyethoxy)methyl]guanine (**1**, acyclovir or ACV) (Elion et al., 1977), which is used clinically for treating herpes simplex infections (Mansuri and Martin, 1987; Mansuri and Martin, 1988), and 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (**2**, ganciclovir or DHPG) (Martin et al., 1983), which in some cases is more effective than **1** (Field et al., 1983) (for example, in the management of cytomegalovirus infections in immunocompromised patients) (Mar et al., 1983; Collaborative DHPG Treatment Group, 1986).

Stimulated by the results with **1** and **2**, other analogue studies led to the carba derivative (*R*)-9-(3,4-dihydroxybutyl)guanine (**3**, buciclovir or (*R*)-DHBG) (Larrson et al., 1983), which is active against herpes simplex types 1 and 2 in vitro and in vivo (Ericson et al., 1985; Datema et al., 1986). As part of our research on carbocyclic nucleosides as the basis for the design of antiviral agents (Koga et al., 1990; Patil et al., 1992; Chen et al., 1992), carbocyclic 3′-oxa-2′,3′-dideoxyguanosine (**4**) arose as a derivative of **3** lacking the C-3′ hydroxyl hydrogen atom yet still functionally capable of triphosphate formation at the C-5′ center, a likely requirement (Field et al., 1983; Smee et al., 1983; Larsson et al., 1986; Karkas et al., 1986; Datema et al., 1987; Reardon and Spector, 1989) for antiviral activity. To further explore the antiviral potential of carbocyclic 7-deazaguanine nucleosides (Legraverend et al., 1985), carbocyclic 3′-oxa-2′,3′-dideoxy-7-deazaguanosine (**5**) was also desired. The synthesis and antiviral data for **4** and **5** are reported here. (It should be noted that during the preparation of this manuscript **4**, which was prepared by a slightly different route than we have utilized, and its potent anti-

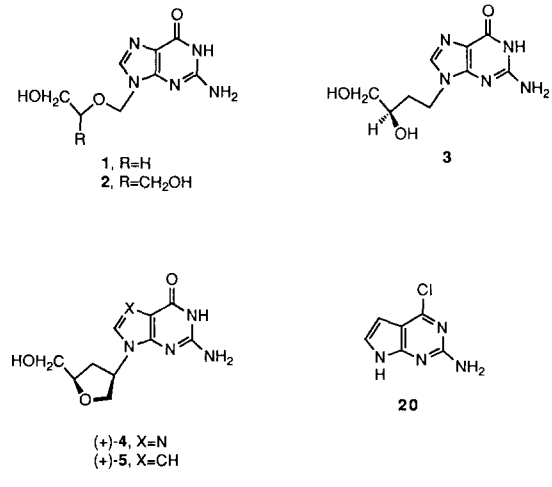

Fig. 1. Structures 1–5, 20.

HIV properties were reported (Huryn et al., 1992; Jones et al., 1992)).

Results

Chemistry

Whereas previous synthetic efforts in this class of nucleosides (Huryn et al., 1992; Jones et al., 1992) focused on coupling 2-amino-6-chloropurine with an appropriately substituted tetrahydrofuran possessing a C-4 tosylate (Huryn et al., 1992) or mesylate (Jones et al., 1992), our approach employed a standard procedure for preparing carbocyclic guanosines (Patil and Schneller, 1991) via reduction of 5-(4-chlorophenylazo)pyrimidines (see **15** of Fig. 2) followed by ring closure of the resultant amine (**16** of Fig. 2). In this direction, it was first necessary to prepare (1*R*,4*R*)-4-(*t*-butyldimethylsilyloxy)methyl-3-oxa-1-cyclopentylamine (**12**). Thus, by modification of procedures that began the preparation of related carbocyclic nucleosides (Huryn et al., 1989; Frank et

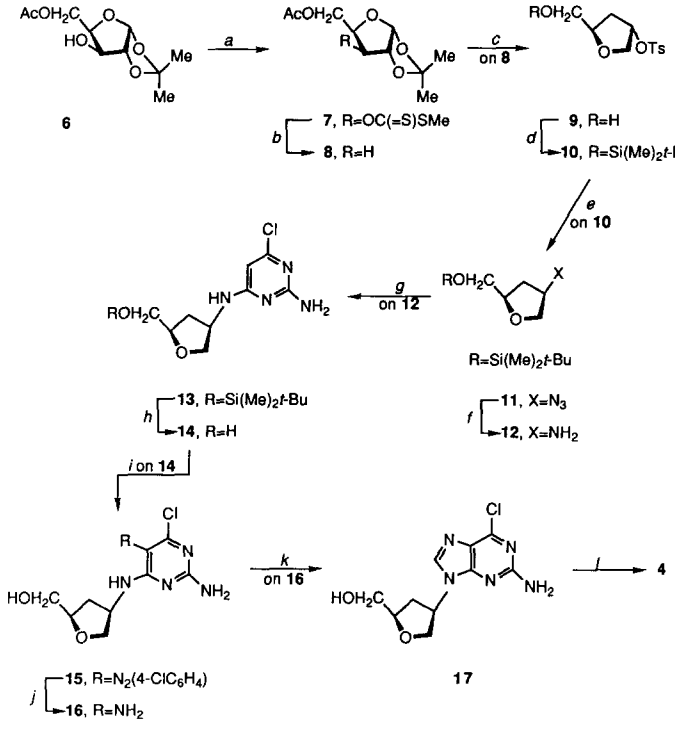

Fig. 2. Reaction conditions: *a*, (i) NaH in THF, 0°C; (ii) CS_2 then MeI; *b*, Bu_3SnH in toluene followed by α,α'-azobis(isobutyronitrile); *c*, (Huryn et al., 1989; Frank et al., 1990; Huryn et al., 1992); *d*, *t*-$BuMe_2SiCl$/imidazole in DMF; *e*, NaN_3 in DMF; *f*, H_2/10% Pd-C in MeOH; *g*, 2-amino-4,6-dichloropyrimidine and Et_3N in 1-BuOH; *h*, CsF and HF·pyridine in MeCN; *i*, 4-chlorophenyldiazonium chloride in H_2O and AcOH containing AcONa; *j*, Zn in AcOH; *k*, $(EtO)_3CH$/HCl in DMF; *l*, 0.5 M NaOH, reflux.

al., 1990; Huryn et al., 1992), the synthesis of **12** began with the conversion of 5-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**6**) (De Bernardo et al., 1985) into its 3-*O*-[(methylthio)thiocarbonyl] derivative **7** (Fig. 2). It should be noted that the 3-*O*-(imidazolylthiocarbonyl) derivative of **6** has been reported (De Bernardo et al., 1985), but we found (i) that the use of carbon disulfide and methyl iodide for preparing **7** to be a more convenient preparation and (ii) that the subsequent Barton reaction (Barton and McCombie, 1975; De Bernardo et al., 1985) on **7** was easily worked-up to give 5-*O*-acetyl-3-deoxy-1,2-*O*-isopropylidene-α-D-eythropentose (**8**) (De Bernardo et al., 1985).

Conversion of **8** into **9** followed a literature route (Huryn et al., 1989; Frank et al., 1990; Huryn et al., 1992) with comparable yields. Silylation of **9** to **10** was followed by azide displacement of the tosyl group of **10** to give (1*R*,4*R*)-4-(*t*-butyldimethylsilyloxy)methyl-3-oxa-1-cyclopentylazide (**11**). Reduction of **11** to **12** followed by reaction of **12** with 2-amino-4,6-dichloropyrimidine yielded **13** (Fig. 2). Fluoride promoted removal of the silyl protecting group from **13** gave **14**, which, in turn, was subjected to C-5 diazo coupling with 4-chlorophenyldiazonium chloride to result in **15**. Completion of the preparation of **4** followed a routine sequence (Patil and Schneller, 1991): reduction of **15** to amine **16**, ring closure with triethyl orthoformate to the precursor **17** and, finally, basic hydrolysis to (+)-2-amino-9-[(1*R*,4*R*)-4-hydroxymethyl-3-oxa-1-cyclopentyl]-9*H*-purin-6(1*H*)-one (carbocyclic 3′-oxa-2′,3′-dideoxyguanosine, **4**).

The synthesis of **5** (Fig. 3) was accomplished by reaction of **12** with 2-(2-amino-4,6-dichloropyrimidin-5-yl)acetaldehyde dimethyl acetal (Siddiqi and Schneller, unpublished results) to give **18**, which was not fully characterized but readily cyclized to **19** upon treatment with hydrochloric acid at room temperature. Reaction of **19** with hydrochloric acid under reflux yielded (+)-2-amino-7-[(1*R*,4*R*)-4-hydroxymethyl-3-oxa-1-cyclopentyl]-7*H*-pyrrolo[2,3-

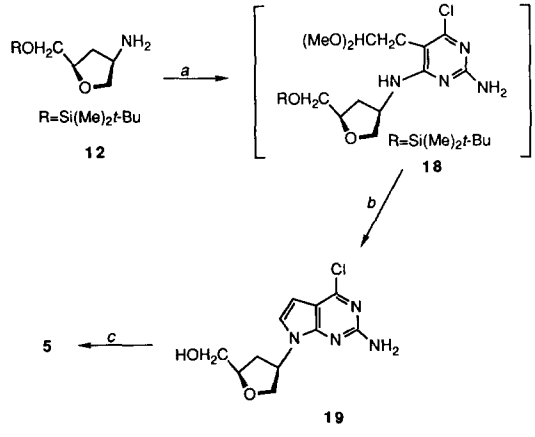

Fig. 3. Reaction conditions: *a*, 2-(2-amino-4,6-dichloropyrimidin-5-yl)acetaldehyde dimethyl acetal and Et_3N in 1-BuOH; *b*, 2 N HCl in dioxane, room temperature; *c*, 2 N HCl in MeOH, reflux.

TABLE 1

Inhibitory effects of compounds **4**, **5**, **17**, and **19** on the replication of DNA viruses and RNA viruses

Virus strain[a]	Cell[a]	MIC[b] (μg/ml)[c]							
		17	**4**	**19**	**5**	BVDU	Ribavirin	C-c^{3}Ado	Acyclovir[d]
HSV-1 (KOS)	E_6SM	300	2	> 400	150	0.04	> 400	> 400	0.02
TK$^-$ HSV-1 (B2006)	E_6SM	200	250	> 400	125	200	300	100	100
TK$^-$ HSV-1 (VMW 1837)	E_6SM	300	150	> 400	300	40	> 400	> 400	40
HSV-2 (G)	E_6SM	300	4	300	300	70	> 400	150	0.02
CMV (AD-169)	HEL	> 40	> 40	> 10	> 40	ND[e]	ND	ND	15
CMV (Davis)	HEL	> 40	> 40	> 10	> 40	ND	ND	ND	15
Vaccinia	E_6SM	300	> 400	20	300	0.7	70	2	> 200
Vesicular stomatitis	E_6SM	> 400	300	> 400	> 400	> 400	> 400	2	ND
Vesicular stomatitis	HeLa	> 400	> 200	> 200	> 400	> 400	70	2	ND
Coxsackie B4	HeLa	> 200	> 200	> 200	> 400	> 400	70	> 400	ND
Coxsackie B4	Vero	> 400	> 400	> 200	> 200	> 400	> 400	> 400	ND
Polio 1	HeLa	> 200	> 200	> 200	> 400	> 400	70	> 400	ND
Parainfluenza 3	Vero	100	200	> 200	150	> 400	20	0.4	ND
Reo 1	Vero	> 400	> 400	> 400	> 400	> 400	150	0.7	ND
Sindbis	Vero	> 400	> 400	> 200	> 400	> 400	40	20	ND
Semliki forest	Vero	> 400	> 400	> 200	> 200	> 400	70	150	ND
HIV-1 (III_B/LAI)	MT-4	222	242	> 500	> 500	ND	ND	ND	ND
HIV-2 (ROD)	MT-4	243	220	> 500	> 500	ND	ND	ND	ND
Cell morphology	E_6SM	> 400	> 400	> 400	> 400	> 400	> 400	> 400	400
Cell morphology	HeLa	> 400	> 400	> 400	> 400	> 400	> 400	> 400	ND
Cell morphology	Vero	> 400	> 400	> 400	> 400	> 400	> 400	> 400	ND
Cell growth	HEL	27	190	ND	152	ND	ND	ND	200
Cell growth	MT-4	> 500	> 500	> 500	> 500	ND	ND	ND	ND

[a]For abbreviations, see text (Experimental Section).

[b]Minimum inhibitory concentration required (i) to reduce virus-induced cytopathicity by 50%, (ii) to cause a microscopically detectable alteration of normal cell morphology, or (iii) to inhibit cell growth by 50%.

[c]All concentrations are expressed in μg/ml, except for data obtained with MT-4 cells, which are expressed in μM.

[d]Data taken from the literature (Boryski et al., 1991).

[e]ND, not determined.

d]pyrimidin-4(3*H*)-one (carbocyclic 3′-oxa-2′,3′-dideoxy-7-deazaguanosine, **5**). Attempts were unsuccessful for preparing **19** by reaction of 2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**20**) (Seela et al., 1983) with **9** (or **10**, which would have required desilylation) in the presence of base.

Antiviral results

Compounds **4**, **5**, **17**, and **19** were evaluated against a wide variety of both DNA and RNA viruses (Table 1). Marginal activity (MIC: 2–4 μg/ml) was noted only with **4** against HSV-1 and HSV-2. The compound was virtually inactive against TK^- mutants of HSV-1. Similarly, only very weak activity (MIC: 200–250 μM) was noted with compounds **4** and **17** against HIV-1 and HIV-2 (Table 1). Interestingly, the IC_{50} values obtained here with **4** against HIV-1 and HIV-2 are somewhat higher than those reported previously for this compound (ED_{50} = 10–50 μM in ATH8 cells (Huryn et al., 1992) and IC_{50} = 4.8 μg/ml in MT-4 cells (Jones et al., 1992)).

Discussion

By comparing the antiviral activity spectrum of a new antiviral compound with the activity spectrum of known antiviral agents, it should be possible to reach a tentative conclusion as to the mechanism (and target) of action of the new compound. Thus, comparing the antiviral effects shown by **4** with the effects of the reference compounds BVDU [(*E*)-5-(2-bromovinyl)-2′-deoxyuridine], ribavirin, C-c^3Ado (carbocyclic 3-deazaadenosine) and acyclovir (Table 1), it is clear that the activity spectrum of **4** most closely resembles that of acyclovir: both compounds are about equally active against HSV-1 and HSV-2 (although **4** is much less potent than acyclovir) and inactive against TK^- HSV-1 and vaccinia virus. This observation suggests that **4** and acyclovir follow a similar course of action (i.e., specific phosphorylation by the virus-encoded thymidine kinase, eventually followed by the inhibition of viral DNA synthesis) but that **4** may be less efficiently converted to the requisite phosphates. Such an occurrence could, in turn, be responsible for the weaker antiviral activity of **4** compared to **3** and suggests that a free C-3′ hydroxyl group is a necessary requirement for the sequence of events resulting in the antiviral characteristics of **3**.

Materials and Methods

Melting points were recorded on a Mel-Temp capillary melting point apparatus and are uncorrected. Combustion analyses were performed by M-H-W Laboratories, Phoenix, AZ. ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL FX90Q spectrometer in DMSO-d_6 or $CDCl_3$ referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The spin multiplicities are indicated by the

symbols *s* (singlet), *d* (doublet), *t* (triplet), and *m* (multiplet). Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. Reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm E. Merck silica-gel 60-F_{254} precoated silica-gel plates with visualization by irradiation with a Mineralight UVGL-25 lamp or exposure to iodine vapor. Column chromatography was performed on Fluka flash chromatography silica-gel 60 (particle size 0.035–0.07 mm; 220–440 mesh ASTM) by eluting with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials.

*5-O-Acetyl-1,2-O-isopropylidene-3-O-[(methylthio)thiocarbonyl]-α-D-xylofuranose (**7**)*

At 0°C, a solution of 5-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**6**) (De Bernardo et al., 1985) (1.8 g, 7.7 mmol) in dry THF (20 ml) was added dropwise to a suspension of NaH (0.33 g, 11.0 mmol) in THF (20 ml) and this mixture stirred for 1 h at the same temperature. Then, CS_2 (2.26 g, 30.0 mmol, 1.8 ml) was added dropwise and the resultant mixture was stirred for another h at which time MeI (4.22 g, 30.0 mmol, 1.85 ml) was added. The solution was allowed to warm to room temperature and stirred overnight. After cooling the mixture to 0°C, a little ice was carefully added to the mixture to destroy unreacted NaH. Water (100 ml) was added to the mixture, which was then extracted with Et_2O (3 × 200 ml). The organic layer was separated, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by column chromatography (hexane-AcOEt, 3:1) to give **7**, which was recrystallized from Et_2O as white crystals (2.03 g, 81%): mp 84°C; ^{1}H NMR ($CDCl_3$) δ 1.33 (**s**, 3 H, isopropylidene Me), 1.54 (**s**, 3 H, isopropylidene Me), 2.07 (**s**, 3 H, acetyl Me), 2.59 (**s**, 3 H, SMe), 4.23–4.31 (**m**, 2 H, H-5), 4.51–4.69 (**m**, 2 H, H-2 and H-4), 5.96-5.99 (**m**, 2 H, H-1 and H-3); ^{13}C NMR ($CDCl_3$) δ 19.34, 20.75, 26.27, 26.71, 61.33, 77.04, 82.94, 84.08, 104.99, 112.36, 170.27, 214.96. Anal. Calcd. for $C_{12}H_{18}O_6S_2$: C, 44.70; H, 5.63. Found: C, 44.82; H, 5.73.

*2,5-Diamino-4-[(1R,4R)4-hydroxymethyl-3-oxa-1-cyclopentyl]amino-6-chloropyrimidine (**16**)*

To gently refluxing dry toluene (500 ml) under N_2 tributyltin hydride (46.6 g, 0.16 mol, 97%) was added in one portion. To this mixture was added α,α′-azobis(isobutyronitrile) (200 mg) and the resulting mixture brought to reflux. Next, a solution of **7** (49.0 g, 0.152 mol) in dry toluene (200 ml) was added with stirring under N_2. Refluxing was then continued for 1 h. After cooling the mixture, it was evaporated to dryness under reduced pressure and the residue subjected to column chromatography (hexane-AcOEt, 2:1) to give pure 5-*O*-acetyl-3-deoxy-1,2-*O*-isopropylidene-α-D-erythropentose (**8**) (De Bernardo et al., 1985) (31 g, 94%) as an oil; ^{1}H NMR ($CDCl_3$) δ 1.32 (**s**, 3 H, isopropylidene Me), 1.52 (**s**, 3 H, isopropylidene Me), 1.50–1.80 (**m**, 1 H, H-3), 1.95–2.20 (**m**, 1 H, H-3), 2.07 (**s**, 3 H, acetyl Me), 3.90–4.22 (**m**, 2 H, H-5), 4.30–4.55 (**m**, 1 H, H-

4), 4.75 (*t*, 1 H, H-2), 5.81 (*d*, 1 H, H-1); ^{13}C NMR ($CDCl_3$) δ 20.91, 26.33, 26.92, 35.43, 65.12, 75.79, 80.45, 105.86, 111.33, 170.70.

Compound **8** was converted into (1*S*, 4*R*)-4-hydroxymethyl-3-oxa-1-cyclopentyl tosylate (**9**) following literature conditions (Huryn et al., 1989; Frank et al., 1990; Huryn et al., 1992). Then, a solution of **9** (990 mg, 3.64 mmol), imidazole (619 mg, 9.10 mmol) and *t*-butyldimethylsilyl chloride (660 mg, 4.37 mmol) in dry DMF (20 ml) was stirred for 5 h at room temperature. After this period, ice-H_2O (100 ml) and Et_2O (100 ml) were added to the reaction mixture. The organic layer was separated and the aqueous phase was extracted with Et_2O (2 × 100 ml). The ether phases were combined, washed with H_2O (2 × 100 ml), dried (Na_2SO_4), and evaporated under reduced pressure. The residue contained (1*S*,4*R*)-4-(*t*-butyldimethylsilyloxy)methyl-3-oxa-1-cyclopentyl tosylate (**10**) (1.39 g, 99%), which was an oil of sufficient purity to be used in the next step for preparing **11**: ^{1}H NMR ($CDCl_3$) δ 0.05 (**s**, 6 H, 2 × MeSi), 0.83 (**s**, 9 H, $SiCMe_3$), 1.99 (**m**, 2 H), 2.35 (**s**, 3 H, aryl Me), 3.50–4.25 (**m**, 5 H), 4.98 (**m**, 1 H), 7.32 (**d**, 2 H, Ar), 7.70 (**d**, 2 H, Ar); ^{13}C NMR ($CDCl_3$) δ −5.5, 21.6, 26.2, 29.7, 34.8, 65.3, 73.2, 78.9, 82.5, 127.7, 130.4, 134.3, 145.0.

A mixture of **10** (26 g, 67.4 mmol) and NaN_3 (22 g, 337 mmol) in DMF (500 ml) was stirred at 120°C for 12 h. After cooling this mixture and removal of the DMF under reduced pressure, H_2O (100 ml) and Et_2O (100 ml) were added to the residue and the Et_2O phase separated. The H_2O phase was extracted with additional Et_2O (2 × 100 ml). The Et_2O fractions were combined, washed with H_2O (3 × 100 ml), dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue remaining was (1*R*,4*R*)-4-(*t*-butyldimethylsilyloxy)methyl-3-oxa-1-cyclopentylazide (**11**) (16.5 g, 95.3%) as an oil that was used as obtained in the next step: ^{1}H NMR ($CDCl_3$) δ 0.07 (**s**, 6 H, Me_2Si), 0.87 (**s**, 9 H, $SiCMe_3$), 1.6–2.3 (**m**, 3 H, H-1 and H-5), 3.4–4.1 (**m**, 5 H, H-2, H-4 and $C\underline{H}_2OSi$); ^{13}C NMR ($CDCl_3$) δ −5.5, 26.7, 30.5, 34.7, 61.6, 65.8, 73.3, 80.2.

A mixture of **11** (16.5 g, 64.2 mmol) and 10% Pd-C (50 mg) in MeOH (50 ml) was subjected to hydrogenation (30 psi) for 12 h. The mixture was filtered to remove the catalyst and the filtrate evaporated to dryness to provide crude (1*R*,4*R*)-4-(*t*-butyldimethylsilyloxy)methyl-3-oxa-1-cyclopentylamine (**12**) (13.3 g, 89.6%) as an oil: ^{1}H NMR ($CDCl_3$) δ 0.1 (**s**, 6 H, Me_2Si), 0.90 (**s**, 9 H, $SiCMe_3$), 1.35–2.45 (**m**, 2 H, H-5), 3.4–4.1 (**m**, 6 H, H-1, H-2, H-4, and $C\underline{H}_2OSi$); ^{13}C NMR ($CDCl_3$) δ −5.5, 26.0, 29.5, 36.5, 52.5, 65.7, 75.6, 79.3.

A mixture of **12** (3.4 g, 14.7 mmol), 2-amino-4,6-dichloropyrimidine (2.4 g, 17.7 mmol) and Et_3N (5 ml) in 1-BuOH (50 ml) was heated at 120°C for 12 h. After evaporation of the mixture to dryness under reduced pressure, H_2O (100 ml) was added to the residue and the new mixture was extracted with $CHCl_3$ (3 × 100 ml). The $CHCl_3$ solution was dried (Na_2SO_4) and then evaporated to dryness under reduced pressure. The residue was then purified by column chromatography (hexane-AcOEt, 2:1) to give 2-amino-4-[(1*R*,4*R*)-4-(*t*-butyldimethylsiloxy)methyl-3-oxa-1-cyclopentyl]amino-6-chloropyrimidine (**13**) (3.6 g, 68.1%) as an oil: ^{1}H NMR ($CDCl_3$) δ 0.12 (**s**, 6 H, Me_2Si), 0.93 (*s*, 9 H,

$SiCMe_3$), 1.6–2.5 (**m**, 2 H, H-5′), 3.5–4.6 (**m**, 6 H, H-1′, H-2′, H-4′, and $C\underline{H}_2OSi$) 5.71 (**s**, 1 H, H-5), 5.83 (**s**, 2 H, NH_2), 5.95 (**d**, 1 H, NH); ^{13}C NMR ($CDCl_3$) δ −5.24, 18.53, 26.97, 34.09, 51.55, 65.26, 73.66, 78.79, 93.27, 158.94, 162.75, 163.41.

A solution of **13** (9.5 g, 26.5 mmol), CsF (4.4 g, 28.9 mmol), and hydrogen fluoride-pyridine complex (1.5 ml) in MeCN (100 ml) and MeOH (10 ml) was stirred at room temperature overnight. After removal of the solvent under reduced pressure, H_2O (50 ml) was added to the residue and the new mixture was extracted with AcOEt. The organic layer was dried and then evaporated to dryness under reduced pressure to give 2-amino-4-[(1*R*,4*R*)-4-hydroxymethyl-3-oxa-1-cyclopentyl]amino-6-chloropyrimidine (**14**) (6.17 g, 95.2%) as a solid that was used as obtained in the next step: ^1H NMR (DMSO-d_6) δ 1.4–1.7 and 2.1–2.45 (**m**, 2 H, H-5′), 3.2–4.0 (**m**, 5 H, H-2′, H-4′, and $C\underline{H}_2OH$), 4.2–4.5 (**m**, 1 H, H-1′), 4.81 (*t*, 1 H, OH), 5.75 (**s**, 1 H, H-5), 6.38 (**s**, 2 H, $\underline{N}H_2$), 7.24 (*d*, 1 H, NH); ^{13}C NMR (DMSO-d_6) δ 34.5, 51.0, 63.8, 72.1, 79.1, 92.3, 157.9, 163.0, 164.0.

A cold (0–5°C, ice-MeOH bath) solution of 4-chlorophenyldiazonium chloride was prepared by adding a solution of sodium nitrite (2.1 g, 32 mmol) in H_2O (17 ml) to a solution of 4-chloroaniline (3.6 g, 28 mmol) dissolved in 12 N HCl (17 ml) and H_2O (47 ml). The cold solution of 4-chlorophenyldiazonium chloride was added, dropwise, to a mixture of **14** (6.0 g, 24 mmol), AcONa trihydrate (50 g), AcOH (129 ml) and H_2O (129 ml) at room temperature. The resulting mixture, which turned yellow, was stirred at room temperature overnight. The mixture was then cooled in an ice-bath and the yellow precipitate that resulted was isolated by filtration, washed with cold H_2O, and dried to give 2-amino-5-(4-chlorophenylazo)-4-[(1*R*,4*R*)-4-hydroxymethyl-3-oxa-1-cyclopentyl]amino-6-chloropyrimidine (**15**) (6.0 g, 63.6%) as a yellow solid that was used as obtained in the next step: ^1H NMR (DMSO-d_6) δ 1.6–2.6 (**m**, 2 H, H-5′), 3.5–4.2 (**m**, 5 H, H-2′, H-4′, and $C\underline{H}_2OH$), 4.72 (**m**, 1 H, H-1′), 7.56 (**s**, 2 H, NH_2), 7.62 (**d**, 2 H, Ar), 7.72 (**d**, 2 H, Ar), 10.35 (**d**, 1 H, NH); ^{13}C NMR (DMSO-d_6) δ 34.0, 50.9, 63.2, 72.9, 79.0, 122.7, 130.0, 133.1, 150.6, 154.0, 161.0, 164.9.

A mixture of **15** (6.0 g, 15.6 mmol), Zn dust (8.0 g, 200 mesh) and AcOH (4.5 ml) in EtOH (150 ml) and H_2O (150 ml) was refluxed under N_2 until the yellow color of **15** disappeared. The excess Zn was removed by filtration and the filtrate removed under reduced pressure. The brown residue was purified by column chromatography (CH_2Cl_2-MeOH, 20:1) to give **16** (2.5 g, 61.7%) as a white solid following recrystallization from MeOH: mp 150°C; ^1H NMR (DMSO-d_6) δ 1.71 (ddd, J = 12.6 Hz, 7.5 Hz, and 6.5 Hz, 1 H, H-5′), 2.3 (dt, J = 12.6 Hz and 7.5 Hz, 1 H, H-5′), 3.48 (*t*, J = 5.4 Hz, 2 H, $C\underline{H}_2OH$), 3.6 (**m**, 1 H, H-4′), 3.8 (**d**, J = 6.0 Hz, 1 H, H-2′), 3.85 (**s**, 2 H, NH_2), 3.9 (**d**, J = 6.0 Hz, 1 H, H-2′), 4.49 (dt, J = 12.6 Hz and 6.5 Hz, 1 H, H-1′), 4.8 (*t*, J = 5.4 Hz, 1 H, OH), 5.58 (**s**, 2 H, NH_2), 6.55 (**d**, J = 6.5 Hz, 1 H, NH); ^{13}C NMR (DMSO-d_6) δ 34.08, 51.36, 63.38, 72.27, 78.59, 113.33, 141.23, 154.61, 155.59. Anal. Calcd. for $C_9H_{14}ClN_5O_2$: C, 41.62; H, 5.43; N, 26.79. Found: C, 41.43; H, 5.54; N, 26.86.

2-Amino-6-chloro-9-[(1R,4R)4-hydroxymethyl-3-oxa-1-cyclopentyl]purine (**17**)

Concentrated HCl (2 ml) was added to a solution of **16** (2 g, 7.7 mmol) and triethyl orthoformate (70 ml) in DMF (10 ml) at 0°C. The solution was stirred overnight at room temperature. After evaporation of the volatiles under reduced pressure, 5% HCl (50 ml) was added to the residue and the new mixture stirred for 1 h. Following this period, neutralization of the mixture with 12 M NaOH and evaporation of the solvent under reduced pressure was carried out. The resulting residue was purified using column chromatography (CH_2Cl_2-MeOH, 20:1) to give **17** (1.5 g, 72%) as a white solid following recrystallization from MeOH: mp 211°C; ^{1}H NMR (DMSO-d_6) δ 2.05 (ddd, 1 H, H-5′), 2.55 (dt, 1 H, H-5′), 3.88 (**m**, 2 H, C$\underline{H_2}$OH), 3.95 (**m**, 3 H, H-2′ and H-4′), 4.9 (*t*, 1 H, OH), 5.00 (**m**, 1 H, H-1′), 6.89 (**s**, 2 H, NH_2), 8.25 (**s**, 1 H, H-8); ^{13}C NMR (DMSO-d_6) δ 33.70, 54.02, 62.25, 71.79, 79.59, 123.25, 141.13, 149.37, 153.86, 159.66. Anal. Calcd. for $C_{10}H_{12}ClN_5O_2$: C, 44.53; H, 4.49; N, 25.97. Found: C, 44.70; H, 4.50; N, 26.14.

2-Amino-9-[(1R,4R)4-hydroxymethyl-3-oxa-1-cyclopentyl]-9H-purin-6(1H)-one (**4**)

A solution of **17** (400 mg, 1.49 mmol) in 0.5 M NaOH (30 ml) was refluxed for 5 h. After neutralization, the volatiles were removed under reduced pressure and the residue was purified using column chromatography (CH_2Cl_2-MeOH, 9:1) to give **4** (250 mg, 67%) as a white solid after recrystallization from MeOH: mp 271.5–272°C; $[\alpha]_D^{25}$ + 27.87° (*c* 0.567, DMSO) [lit. (Huryn et al., 1992) reports $[\alpha]_D$ −19.09° (*c* 0.11, H_2O) and (Jones et al., 1992) $[\alpha]_D^{22}$ + 16.1° (*c* 0.59, MeOH); ^{1}H NMR (DMSO-d_6) δ 2.0–2.5 (**m**, 2 H, H-5′), 3.52 (**m**, 2 H, C$\underline{H_2}$OH), 3.95 (**m**, 3 H, H-2′ and H-4′), 4.91 (*t*, 1 H, OH), 4.94 (**m**, 1 H, H-1′), 6.46 (**s**, 2 H, NH_2), 7.83 (**s**, 1 H, H-8), 10.59 (**s**, 1 H, NH); ^{13}C NMR (DMSO-d_6) δ 33.97, 53.42, 62.41, 71.89, 79.47, 116.31, 135.11, 150.88, 153.37, 156.78. Anal. Calcd. for $C_{10}H_{13}N_5O_3 \cdot H_2O$: C, 44.60; H, 5.61; N, 26.01. Found: C, 44.89; H, 5.32; N, 25.79.

2-Amino-4-chloro-7-[(1R,4R)4-hydroxymethyl-3-oxa-1-cyclopentyl]-7H-pyrrolo[2,3-d]pyrimidine (**19**)

A mixture of **12** (4.0 g, 17.3 mmol), the dimethyl acetal of 2-(2-amino-4,6-dichloropyrimidin-5-yl)acetaldehyde (Siddiqi and Schneller, unpublished results) (6.5 g, 27.7 mmol), Et_3N (5 ml) in 1-BuOH (50 ml) was heated at 120°C for 24 h. After evaporation of the mixture under reduced pressure (to **18**), dioxane (100 ml) and 2 N HCl (20 ml) were added to the residue and the mixture stirred at room temperature for 12 h. Following neutralization of the reaction mixture with NH_4OH (28%), the new mixture was evaporated to dryness under reduced pressure. The resultant residue was purified by column chromatography (CH_2Cl_2-MeOH, 20:1) to give **19** (1.0 g, 21%) as a white solid: mp 58–59°C; ^{1}H NMR ($CDCl_3$) δ 2.03–2.54 (**m**, 2 H, H-5′), 3.86–4.50 (**m**, 5 H, H-2′, H-4′, and C$\underline{H_2}$OH), 5.28 (**m**, 1 H, H-1′), 5.72 (**s**, 2 H, NH_2), 6.32 (**d**,

J=3.76 Hz, 1 H, H-5), 7.10 (**d**, J=3.76 Hz, 1 H, H-6); ^{13}C NMR ($CDCl_3$) δ 34.18, 54.07, 63.38, 72.65, 79.96, 100.33, 110.35, 123.03, 152.34, 153.04, 158.62. Anal. Calcd. for $C_{11}H_{13}ClN_4O_2$: C, 49.17; H, 4.88; N, 20.85. Found: C, 49.07; H, 4.99; N, 20.71.

2-Amino-7-(1R,4R)-4-hydroxymethyl-3-oxa-1-cyclopentyl]-7H-pyrrolo[2,3-d]pyrimidin-4(3H)-one (**5**)

A solution of **19** (700 mg, 2.59 mmol) in MeOH (20 ml) and 2 M HCl (20 ml) was refluxed for 24 h. After cooling the reaction mixture, it was neutralized with 12 M NaOH solution and then evaporated to dryness under reduced pressure. The residue was purified using column chromatography (CH_2Cl_2-MeOH, 10:1) to give **5** (400 mg, 61.5%) that was recrystallized from MeOH as a white solid: mp 228–229°C; $[\alpha]_D^{25}$ + 10.77° (c 0.390, DMSO); ^{1}H NMR (DMSO-d_6) δ 1.70–2.02 and 2.29–2.61 (**m**, 2 H, H-5′), 3.45–3.65 (**m**, 2 H, C$\underline{H}_2$OH), 3.75–4.25 (**m**, 3 H, H-2′ and H-4′), 4.89 (t, 1 H, OH), 5.11 (**m**, 1 H, H-1′), 6.20 (**s**, 2 H, NH_2), 6.29 (**d**, J=3.76 Hz, 1 H, H-5), 6.85 (**d**, J=3.76 Hz, 1 H, H-6), 10.33 (**s**, 1 H, NH); ^{13}C NMR (DMSO-d_6) δ 34.40, 53.09, 62.79, 71.99, 79.53, 99.63, 101.84, 116.85, 150.12, 152.23, 158.73. Anal. Calcd. for $C_{11}H_{14}N_4O_3$: C, 52.79; H, 5.64; N, 22.34. Found: C, 52.71; H, 5.64; N, 22.18.

Antiviral activity assays

The antiviral assays were carried out as described in the literature (De Clercq et al., 1980; De Clercq et al., 1986; Balzarini et al., 1991). The sources of the viruses and cells have also been described in these previous publications. Abbreviations used for the viruses and cells are as follows: HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; TK$^-$, thymidine kinase deficient; CMV, cytomegalovirus; HIV-1, human immunodeficiency virus type 1; HIV-2, human immunodeficiency virus type 2; E_6SM, embryonic skin-muscle; HEL, human embryonic lung.

Cytotoxicity assays

Cytotoxicity measurements were based on a microscopically visible alteration of normal cell morphology (E_6SM, HeLa, Vero) or inhibition of cell growth (HEL, MT-4) as described previously (De Clercq et al., 1980; De Clercq et al., 1986; Balzarini et al., 1991).

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