Synthesis and antiviral activity of a series of new cyclohexenyl nucleosides

Ping Gu[1], Jordi Morral[1], Jing Wang[1], Jef Rozenski[1], Roger Busson[1], Arthur Van Aerschot[1], Erik De Clercq[2] and Piet Herdewijn[1]*

[1]Laboratory of Medicinal Chemistry and [2]Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

*Corresponding author: Tel: +32 1633 7387; Fax: +32 1633 7340; E-mail: piet.herdewijn@rega.kuleuven.ac.be

A series of new cyclohexenyl nucleosides is synthesized by coupling the heterocyclic bases with a protected cyclohexenyl precursor under Mitsunobu conditions. The compounds were evaluated for their antiviral and cytostatic activity. Pronounced activity against herpes simplex virus type 1 and type 2 was observed for the 2,6-diaminopurine analogue.

Keywords: synthesis, antiviral, cyclohexenyl nucleosides

Introduction

The development of new nucleoside analogues as antiviral agents has remained an attractive research field. Due to their hydrolytic stability, carbocyclic nucleosides have taken a particular place in the design process of new antiviral agents (Marquez, 1996). Most of these compounds are cyclopentane derivatives and less work has been done on conformationally more rigid carbocyclic analogues such as cyclohexane nucleosides. Some of these cyclohexane nucleosides were synthesized in the past but they were devoid of antiviral activity (Schaeffer *et al.*, 1964, 1968; Pérez-Pérez, 1995; Mikhailov *et al.*, 1996; Maurinsh *et al.*, 1997). However, the more flexible cyclohexene nucleosides look more promising. Indeed, introduction of a double bond into the cyclohexane ring could facilitate the phosphorylation of the nucleoside analogue and their eventual incorporation in DNA and, hence, lead to antiviral activity (Wang *et al.*, 2000). A cyclohexene ring is more flexible than a cyclohexane ring and is more prone to conformational changes that might be needed for substrate/inhibitor recognition during enzymatic reactions.

Therefore, cyclohexenyl guanine (Cycl-G) **1** and cyclohexenyl adenine (Cycl-A) **2** were synthesized and their biological activities were investigated (Wang *et al.*, 2000). In particular, D-cyclohexenyl-G has found to exhibit potent and selective activity against herpes viruses [herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicella-zoster virus (VZV), cytomegalovirus (CMV)], in analogy with that of the known antiviral drugs acyclovir and ganciclovir (Wang *et al.*, 2000). This activity could be explained by the intercellular phosphorylation of Cycl-G to its triphosphate in virus-infected cells, as deduced from the low activity of cyclohexenyl G against thymidine kinase-deficient (TK^-) viral strains (Figure 1).

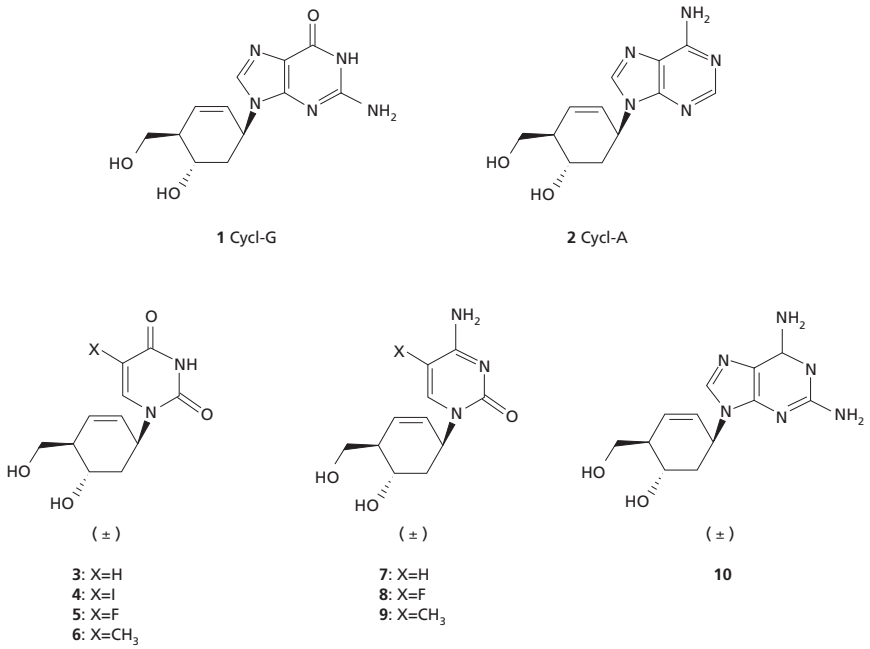
These results prompted us to synthesize other cyclohexenyl nucleosides **(3–10)** in order to study the effects of base modification on antiviral activity and toxicity. We recently reported a straightforward procedure for obtaining the racemic **1** using as the key step a Diels-Alder reaction (Wang *et al.*, 2001). The compounds envisaged here were obtained analogously as racemic mixtures, as separation of the (+) and (–) enantiomers proved tedious. In case of a positive biological evaluation, the synthesis of the separate enantiomers could be envisaged.

Materials and methods

Chemistry

For all reactions, analytical grade solvents were used. All moisture-sensitive reactions were carried out in oven-dried glassware (100°C) under a nitrogen atmosphere. Anhydrous solvent 1,4-dioxane was refluxed on sodium/benzophenone and distilled. Melting points were determined in capillary tubes with a Büchi SMP-20 cap. apparatus and were uncorrected. ^{1}H NMR was determined with a Varian Unity 500 MHz spectrometer with tetramethylsilane (TMS) as internal standard for ^{1}H NMR spectra and a 200 MHz Varian Gemini apparatus was used for ^{13}C NMR determination with DMSO-d_6 (39.6 ppm) or $CDCl_3$ (76.9 ppm) as internal standard for the ^{13}C NMR spectra (s, singlet; d, doublet; dd, double doublet; t, triplet; br s, broad singlet; br d, broad doublet; m, multiplet). Exact mass measurements were performed on a quadrupole time-of-flight mass spectrometer (Q-Tof-2, Micromass, Manchester, UK) equipped with a standard electrospray-ionization (ESI) interface; samples were infused in *i*-PrOH/H_2O 1:1 at 3 μl/min. Precoated

Figure 1. Chemical structures of cyclohexenyl nucleosides



aluminum sheets (Fluka Silica gel/TLC-cards, 254 nm) were used for TLC; the spots were examined with UV light, or sprayed with sulfuric acid/anisaldehyde or 1% potassium permanganate solution. Column chromatography was performed on ICN silica gel 63-200 60 Å. Elemental analyses were done at the University of Konstanz, Germany, and were in agreement with calculated values within 0.4% error margin. The names of the compounds accorded to the rules of IUPAC and were checked with a nomenclature program (ACD-Labs, Version 4.08, Sept. 1999, Adv. Chem. Dev., Inc., Toronto, Canada).

*(±)-(4aR,7R,8aS)-2-phenyl-4a,7,8,8a-tetrahydro-4H-1,3-benzodioxin-7-ol **(11)***

The preparation of this compound has been described previously (Wang *et al.*, 2001).

*(±)-1-[(1S,4R,5S)-5-hydroxy-4-(hydroxymethyl)-2-cyclohexen-1-yl]-2,4(1H,3H)-pyrimidinedione **(3)***

A suspension of 1.73 g (8 mmol) of N^3-benzoyluracil **(12)**, 0.93 g (4 mmol) of (±)-(4aR,7R,8aS)-2-Phenyl-4a,7,8,8a-tetrahydro-4H-1,3-benzodioxin-7-ol **(11)**, 1.15 g (8 mmol) sodium benzoate and 2.62 g (10 mmol) triphenylphosphine in 100 ml of anhydrous dioxane was stirred under nitrogen. A solution of 1.60 ml (10 mmol) of diethylazodicarboxylate (DEAD) in 40 ml anhydrous dioxane was slowly added over a period of 3 h. The mixture was further stirred overnight at room temperature and filtered, and the filtrate was distilled *in vacuo* to remove the solvent. The residue was dissolved in 100 ml of methanol saturated with ammonia and stirred overnight at room temperature. Evaporation and coevaporation with methanol left colourless oil that was purified on silica gel EtOAc/n-Hexane 5–40% (R_f=0.4). Compound **21** was obtained as a white solid 0.51 g (1.6 mmol, yield 36%).

Compound **21** (300 mg, 0.91 mmol) was treated with 20 ml of 80% trifluoroacetic acid solution at room temperature for 2 days. After evaporation and coevaporation with toluene and methanol, the residue was dissolved in 10 ml water and extracted with ether. The water layer was concentrated and the resulting white solid was purified by column chromatography (CH_3OH/EtOAc 0–8%, R_f=0.3). Crystallization from CH_3OH/EtOAc afforded **3** as a white crystal (138 mg, 0.55 mmol, overall yield 23%).

mp. 171°C; ^{1}H NMR (DMSO-d_6) δ 1.78 (m, 2H, H-2′, 2′′, 2.08 (dt, 1H, J=2.5 Hz, 5.5 Hz, H-4′), 3.51 (m, 2H, -C<u>H</u>$_2$OH), 3.72 (m, 1H, H-3′), 4.70 (br s, 1H, -CH$_2$O<u>H</u>), 4.78 (br s, 1H, 3′-O<u>H</u>), 5.10 (m, 1H, H-1′), 5.54 (d, 1H, J=8.0 Hz, H-5), 5.61 (ddd, 1H, J=2.5 Hz, 3.5 Hz, 10.0 Hz, H-6′), 5.98 (ddd, 1H, J=2.0 Hz, 3.0 Hz, 10.2 Hz, H-5′), 7.44 (d, 1H, J=8.0 Hz, H-6), 11.28 (br s, 1H, -N<u>H</u>); ^{13}C NMR (DMSO-d_6) δ 34.6 (C-2′), 46.0 (C-4′), 50.1 (C-1′), 61.7 (-<u>C</u>H$_2$OH), 63.1 (C-3′), 101.1 (C-5), 125.3 (C-6′), 134.8 (C-5′), 142.6 (C-6), 151.1 (C-2), 163.6 (C-4) ppm. HRMS calcd. for $C_{11}H_{15}N_2O_4$ $(M+H)^+$: 239.1032, found 239.1019.

(±)-1-[(1S,4R,5S)-5-hydroxy-4-(hydroxymethyl)-2-cyclohexen-1-yl]-5-iodo-2,4(1H,3H)-pyrimidinedione ***(4)***

Starting with 0.46 g (2 mmol) of compound **11**, 1.36 g (4 mmol) of N^3-benzoyl-5-iodouracil **(13)**, 1.0 g (4 mmol) triphenylphosphine, 0.58 g (4 mmol) of sodium benzoate and 0.72 ml (4 mmol) of DEAD in 20 ml anhydrous dioxane, using the same procedure as described for **3**, after purification by column chromatography (CH_3OH/CH_2Cl_2 0–10%, R_f=0.5), 203 mg (0.56 mmol, overall yield 29%) of compound **4** was obtained.

mp. 207°C; ^{1}H NMR (DMSO-d_6) δ 1.81 (m, 2H, H-2′, 2″), 2.10 (m, 1H, H-4′), 3.59 (m, 2H, -C<u>H</u>$_2$OH), 3.75 (m, 1H, H-3′), 4.72–4.83 (m, 2H, -CH$_2$O<u>H</u>, 3′-O<u>H</u>), 5.08 (m, 1H, H-1′), 5.65 (ddd, 1H, J=2.2 Hz, 3.9 Hz, 10.0 Hz, H-6′), 6.03 (ddd, 1H, J=1.7 Hz, 3.2 Hz, 10.0 Hz, H-5′), 7.86 (s, 1H, H-6), 11.61 (br s, 1H, -N<u>H</u>); ^{13}C NMR (DMSO-d_6) δ 34.5 (C-2′), 46.1 (C-4′), 50.7 (C-1′), 61.3 (-<u>C</u>H$_2$OH), 62.8 (C-3′), 68.2 (C-5), 124.9 (C-6′), 135.4 (C-5′), 146.7 (C-6), 150.6 (C-2), 160.7 (C-4) ppm. HRMS calcd. for $C_{11}H_{14}IN_2O_4$ $(M+H)^+$: 365.0000, found 365.0013.

(±)-5-fluoro-1-[(1S,4R,5S)-5-hydroxy-4-(hydroxymethyl)-2-cyclohexen-1-yl]-2,4(1H,3H)-pyrimidinedione ***(5)***

Following the same procedure as described for **3**, and starting with 0.93 g (4 mmol) of 11, 1.87 g (8 mmol) of N^3-benzoyl-5-fluorouracil **(14)**, 2.62 g (10 mmol) of triphenylphosphine, 1.15 g (8 mmol) of sodium benzoate and 1.60 ml (10 mmol) of DEAD in 40 ml anhydrous dioxane, 550 mg of compound **23** was obtained. After purification by column chromatography (CH_3OH/CH_2Cl_2 0–10%, R_f=0.3), the desired fluorouracil derivative **5** was obtained as a white crystal in 44% yield (112 mg, 0.44 mmol).

mp. 208°C; ^{1}H NMR (DMSO-d_6) δ 1.74–1.84 (m, 2H, H-2′, 2″), 2.08 (m, 1H, H-4′), 3.50–3.58 (ddd, 2H, J=5.1 Hz, 10.5 Hz, 22.5 Hz, -C<u>H</u>$_2$OH), 3.79 (m, 1H, H-3′), 4.71 (t, 1H, J=5.3 Hz, -CH$_2$O<u>H</u>), 4.76 (d, 1H, J=4.4 Hz, 3′-O<u>H</u>), 5.09 (m, 1H, H-1′), 5.62 (ddd, 1H, J=2.2 Hz, 3.4 Hz, 10.0 Hz, H-6′), 5.95 (ddd, 1H, J=1.9 Hz, 3.2 Hz, 10.0 Hz, H-5′), 7.73 (d, 1H, J=7.1 Hz, H-6), 11.81 (br s, 1H, -N<u>H</u>); ^{13}C NMR (DMSO-d_6) δ 34.1 (C-2′), 46.0 (C-4′), 50.5 (C-1′), 61.5 (-<u>C</u>H$_2$OH), 62.9 (C-3′), 125.1 (C-6′), 126.8 127.5 (C-6), 135.2 (C-5′), 137.4 142.0 (C-5), 149.7 (C-2), 157.6 (C-4) ppm. HRMS calcd. for $C_{11}H_{14}FN_2O_4$ $(M+H)^+$: 257.0938, found 257.0900.

(±)-1-[(1S,4R,5S)-5-hydroxy-4-(hydroxymethyl)-2-cyclohexen-1-yl]-5-methyl-2,4(1H,3H)-pyrimidinedione ***(6)***

To a solution of 0.83 g (3.6 mmol) of (±)-(4aR,7R,8aS)-2-Phenyl-4a,7,8,8a-tetrahydro-4H-1,3-benzodioxin-7-ol **(11)**, 1.64 g (7.2 mmol) of N^3-benzoylthymine **(15)**, 1.04 g (7.2 mmol) of sodium benzoate and 1.88 g (7.2 mmol) of triphenylphosphine in 100 ml of anhydrous dioxane, a solution of 1.41 ml (7.2 mmol) of DIAD in 30 ml was drop wise added under nitrogen environment. The mixture was kept stirring overnight at room temperature. The reaction was filtered and evaporated. The crude **20** was directly treated with 100 ml saturated ammonia/methanol solution for 6 h. After evaporation and coevaporation with methanol, a colourless oil of thymine analogue **24** was obtained and it was further treated with 40 ml 80% trifluoroacetic acid water solution for 2 days. Workup purification on silica gel (CH_3OH/CH_2Cl_2 0–10%, R_f=0.3) and recrystallization from CH_3OH/EtOAc yielded a white crystal **6** (144 mg, 0.57 mmol, overall yield 16%).

mp. 227°C; ^{1}H NMR (DMSO-d_6) δ 1.73 (d, 3H, J=1.0 Hz, -C<u>H</u>$_3$), 1.77 (br t, 2H, J=5.7 Hz, H-2′, 2″), 2.09 (m, 1H, H-4′), 3.54 (m, 2H, -C<u>H</u>$_2$OH), 3.79 (m, 1H, H-3′), 4.76 (t, 1H, J=5.2 Hz, -CH$_2$O<u>H</u>), 4.80 (d, 1H, J=4.2 Hz, 3′-O<u>H</u>), 5.10 (m, 1H, H-1′), 5.60 (ddd, 1H, J=2.2 Hz, 3.4 Hz, 10.0 Hz, H-6′), 5.96 (ddd, 1H, J=2.1 Hz, 3.0 Hz, 10.1 Hz, H-5′), 7.31 (d, 1H, J=1.2 Hz, H-6), 11.31 (br s, 1H, -N<u>H</u>); ^{13}C NMR (DMSO-d_6) δ 12.2 (-CH$_3$), 34.4 (C-2′), 46.1 (C-4′), 49.6 (C-1′), 61.7 (-<u>C</u>H$_2$OH), 63.1 (C-3′), 108.7 (C-5), 125.7 (C-6′), 134.6 (C-5′), 138.4 (C-6), 151.1 (C-2), 164.3 (C-4) ppm. HRMS calcd. for $C_{12}H_{17}N_2O_4$ $(M+H)^+$: 253.1188, found 253.1180.

(±)-4-Amino-1-[(1S,4R,5S)-5-hydroxy-4-(hydroxymethyl)-2-cyclohexen-1-yl]-2(1H)-pyrimidinone ***(7)***

A premixed solution of 1,2,4-triazole (309 mg, 4.48 mmol) and phosphoroxychloride (120 µl, 1.28 mmol) in 30 ml dried pyridine was added to 210 mg (0.64 mmol) of (±)-1-[(4aR,7S,8aS)-2-phenyl-4a,7,8,8a,-tetrahydro-4H-1,3-benzodioxin-7-yl]-4-amino-2(1H)-pyrimidinone **(21)**. The mixture was stirred at room temperature. After 16 h, the mixture was cooled to 0°C in an ice bath and ammonia gas was bubbled for 12 min and the reaction was left for 10 min further at room temperature. Evaporation and coevaporation with toluene and methanol, a yellow syrup was obtained which was further treated with 80% trifluoroacetic acid solution (30 ml) for 2 days at room temperature. The reaction mixture was concentrated, and coevaporated with toluene and methanol. The residue was chromatographed on silica gel (CH_3OH/CH_2Cl_2 5–20%, R_f=0.5) to yield **7** (62.4 mg, 0.26 mmol, overall yield 41%) as a light yellow solid.

mp. 129°C; ^{1}H NMR (DMSO-d_6) δ 1.78 (m, 2H, H-2′, 2″), 2.08 (m, 1H, H-4′), 3.48–3.56 (ddd, 2H, J=5.4 Hz, 10.6 Hz, 21.4 Hz, -C<u>H</u>$_2$OH), 3.68 (m, 1H, H-3′), 4.67 (br s, 1H, -CH$_2$O<u>H</u>), 4.74 (br s, 1H, 3′-O<u>H</u>), 5.15 (m, 1H, H-1′), 5.58 (ddd, 1H, J=2.2 Hz, 3.7 Hz, 10.0 Hz, H-6′), 5.82 (d, 1H, J=7.5 Hz, H-5), 6.00 (ddd, 1H, J=2.0 Hz, 3.2 Hz, 10.0 Hz, H-5′), 7.58 (d, 1H, J=7.3 Hz, H-6), 7.64, 8.02 (br

d, 2H, -N<u>H</u>$_2$); ^{13}C NMR (DMSO-d$_6$) δ 34.8 (C-2′), 46.2 (C-4′), 51.0 (C-1′), 61.7 (-<u>C</u>H$_2$OH), 62.9 (C-3′), 93.5 (C-5), 125.2 (C-6′), 134.9 (C-5′), 144.7 (C-6), 152.8 (C-2), 163.3 (C-4) ppm. HRMS calcd. for $C_{11}H_{16}N_3O_3$ (M+H)$^+$: 238.1192, found 238.1179.

*(±)-4-Amino-5-fluoro-1-[(1S,4R,5S)-5-hydroxy-4-(hydroxymethyl)-2-cyclohexen-1-yl]-2(1H)-pyrimidinone **(8)***

Following the procedure used for preparation of **7**, and started from 172 mg (0.5 mmol) of (±)-1-[(4aR,7S,8aS)-2-phenyl-4a,7,8,8a-tetrahydro-4H-1,3-benzodioxin-7-yl]-5-fluoro-2,4 (1H,3H)-pyrimidinone (**23**), 1,2,4-triazole (242 mg, 3.5 mmol), phosphoroxychloride (93 μl, 1 mmol) in 30 ml dry pyridine, the 5-fluorocytosine analogue was obtained (59.9 mg, 0.2 mmol) in an overall yield of 47%.

mp. 264°C; ^{1}H NMR (DMSO-d$_6$) δ 1.71–1.82 (m, 2H, H-2′, 2′′), 2.05 (m, 1H, H-4′), 3.56 (m, 2H, -C<u>H</u>$_2$OH), 3.70 (m, 1H, H-3′), 4.68 (t, 1H, J=5.1 Hz, -CH$_2$O<u>H</u>), 4.70 (d, 1H, J=4.4 Hz, 3′-O<u>H</u>), 5.09 (m, 1H, H-1′), 5.60 (ddd, 1H, J=2.2 Hz, 3.9 Hz, 10.0 Hz, H-6′), 5.98 (m, 1H, H-5′), 7.37, 7.59 (br d, 2H, -N<u>H</u>$_2$), 7.60 (d, 1H, J=7.1 Hz, -N<u>H</u>); ^{13}C NMR (DMSO-d$_6$) δ 35.0 (C-2′), 46.3 (C-4′), 51.0 (C-1′), 61.4 (-<u>C</u>H$_2$OH), 62.5 (C-3′), 125.4 (C-6′), 127.5 (C-6), 127.9 (C-5′), 135.4 (C-5), 154.0 (C-2), 157.4 (C-4) ppm. HRMS calcd. for $C_{11}H_{15}FN_3O_3$ (M+H)$^+$: 256.1097, found 256.1080.

*(±)-4-Amino-1-[(1S,4R,5S)-5-hydroxy-4-(hydroxymethyl)-2-cyclohexen-1-yl]-5-methyl-2(1H)-pyrimidinone **(9)***

Starting with (±)-1-[(4aR,7S,8aS)-2-phenyl-4a,7,8,8a-tetrahydro-4H-1,3-benzodioxin-7-yl]-5-methyl-2,4(1H,3H)-pyrimidinone **(24)** (148 mg, 0.44 mmol), 1,2,4-triazole (240 mg, 3.48 mmol), phosphoroxychloride (81 μl, 0.86 mmol) and 15 ml dry pyridine, using the same procedure as described for **7**, and purified on silica gel (CH$_3$OH/CH$_2$Cl$_2$ 5–20%, R$_f$=0.4), a white solid was obtained, which was crystallized from CH$_2$Cl$_2$/CH$_3$OH (60 mg, 0.2 mmol, overall yield 55%).

mp. 277°C; ^{1}H NMR (DMSO-d$_6$) δ 1.76–1.80 (mt, 2H, H-2′, 2′′), 1.83 (s, 3H, -C<u>H</u>$_3$), 2.09 (m, 1H, H-4′), 3.54 (m, 2H, -C<u>H</u>$_2$OH), 3.70 (m, 1H, H-3′), 4.70–4.78 (br, 2H, -CH$_2$O<u>H</u>, 3′-O<u>H</u>), 5.08 (m, 1H, H-1′), 5.58 (ddd, 1H, J=2.1 Hz, 3.6 Hz, 10.0 Hz, H-6′), 5.96 (ddd, 1H, J=2.6 Hz, 3.0 Hz, 10.2 Hz, H-5′), 7.48 (d, 1H, J=1.2 Hz, H-6), 8.07–8.57 (br d, 2H, -N<u>H</u>$_2$); ^{13}C NMR (DMSO-d$_6$) δ 12.7 (-<u>C</u>H$_3$), 34.4 (C-2′), 46.2 (C-4′), 50.9 (C-1′), 61.7 (-<u>C</u>H$_2$OH), 62.9 (C-3′), 101.3 (C-5), 125.4 (C-6′), 135.0 (C-5′), 142.6 (C-6), 151.6 (C-2), 162.1 (C-4) ppm. HRMS calcd. for $C_{12}H_{18}N_3O_3$ (M+H)$^+$: 252.1348, found 252.1330.

*(±)-(1S,2R,5S)-5-(2,6-diamino-9H-purin-9-yl)-2-(hydroxymethyl)-3-cyclohexen-1-ol **(10)***

To a mixture of (±)-(4aR,7R,8aS)-2-phenyl-4a,7,8,8a-tetrahydro-4H-1,3-benzodioxin-7-ol **(11)** 500 mg (2.2 mmol), 2-amino-6-chloropurine (821 mg, 4.8 mmol) and triphenylphosphine (1.15 g, 4.4 mmol) in dry dioxane 30 ml under N$_2$, at room temperature, was slowly added a solution of DIAD (0.86 ml, 4.4 mmol) in dry dioxane 20 ml over a period of 3 h. The reaction mixture was stirred at room temperature for 2 days, filtered and concentrated. The residue was submitted to column chromatography (CH$_3$OH/CH$_2$Cl$_2$ 1%, R$_f$=0.3), a mixture of compound **25** and triphenylphosphine oxide was obtained. Due to the difficult separation, we decided to deprotect **25** before final purification.

A solution of impure compound **25** (about 1.4 g) in 100 ml methanol saturated with ammonia is heated in a Parr pressure reactor for 8 h at 100°C. After evaporation, the obtained residue was purified by flash chromatography (CH$_3$OH/CH$_2$Cl$_2$ 0–8%, R$_f$=0.3) to give 270 mg (0.74 mmol) of 26 as yellow syrup. The benzylidene moiety was removed with 80% trifluoroacetic acid solution. After evaporation and coevaporation with toluene and methanol, the residue was purified by flash chromatography (CH$_3$OH/CH$_2$Cl$_2$ 5–20%, R$_f$=0.5) to give diaminopurine analogue **10** (107 mg, 0.39 mmol, overall yield 18%), which was crystallized from CH$_2$Cl$_2$/CH$_3$OH to give a white crystal.

mp. 268°C; ^{1}H NMR (DMSO-d$_6$) δ 1.87 (m, 1H, H-2′), 1.97 (dt, 1H, J=4.0 Hz, 13.2 Hz, H-2′′), 2.12 (m, 1H, H-4′), 3.52–3.66 (m, 3H, -C<u>H</u>$_2$OH, H-3′), 4.70 (t, 1H, J=5.2 Hz, -CH$_2$O<u>H</u>), 4.76 (d, 1H, J=5.4 Hz, 3′-O<u>H</u>), 4.99 (m, 1H, H-1′), 5.77 (ddd, 1H, J=2.5 Hz, 3.9 Hz, 10.0 Hz, H-6′), 5.83 (br s, 2H, -N<u>H</u>$_2$), 5.98 (ddd, 1H, J=1.6 Hz, 2.9 Hz, 9.9 Hz, H-5′), 6.68 (br s, 2H, -N<u>H</u>$_2$) 7.60 (s, 1H, H-8); ^{13}C NMR (DMSO-d$_6$) δ 35.9 (C-2′), 46.6 (C-4′), 47.9 (C-1′), 61.6 (-<u>C</u>H$_2$OH), 62.8 (C-3′), 113.7 (C-5), 125.2 (C-6′), 133.7 (C-5′), 136.1 (C-8), 151.5 (C-4), 156.4 (C-6) 160.5 (C-2) ppm. HRMS calcd. for $C_{12}H_{17}N_6O_2$ (M+H)$^+$: 277.1413, found 277.1416.

Virology

Antiviral activity determinations against herpesviruses were performed in either E$_6$SM or HEL cell cultures as previously described (De Clercq *et al.*, 1980). The origin of the viruses, HSV-1 (strains KOS, F and McIntyre), TK$^-$ HSV-1 (strain KOS ACVr), HSV-2 (strains G, 196 and Lyons), VZV (strains OKA and YS), TK$^-$ VZV (strains 07-1 and YS-R), vaccinia virus (VV), vesicular stomatitis virus (VSV) and CMV (strains AD169 and Davis) have been reported (De Clercq *et al.*, 1986). The assays for evaluating activity

against human herpesvirus 6 (HHV-6) have been described (De Clercq *et al.*, 2001). The cytotoxicity measurements were based on microscopically visible alteration of normal cell morphology (E_6SM) or inhibition of normal cell growth (HEL) as previously described (De Clercq *et al.*, 1981).

Results

(±)-(4aR,7R,8aS)-2-phenyl-4a,7,8,8a-tetrahydro-4H-1,3-benzodioxin-7-ol **(11)** is the key intermediate for the synthesis of cyclohexenyl nucleoside. A straightforward approach to synthesize compound **11** was recently reported (Wang *et al.*, 2001). This involved a Diels-Alder cycloaddition reaction to build up the six-membered ring skeleton, a Fraser-Reid reductive rearrangement and protection of the diol with a benzylidene group. This approach was followed for the synthesis of **11**, which was then used for base introduction.

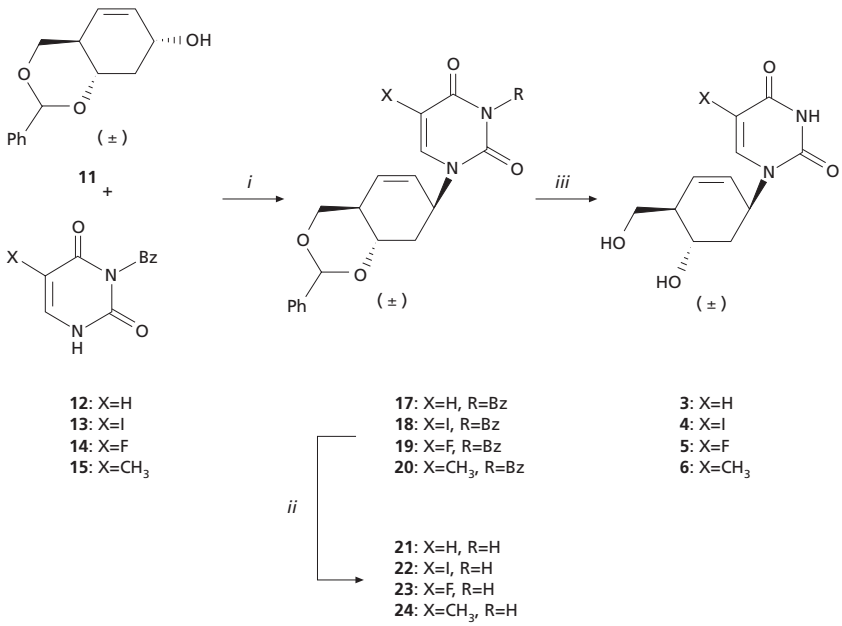
For the synthesis of the uracil analogue **3**, a Mitsunobu reaction was employed by using 1 eq. of compound **11**, 2 eq. of N^3-benzoyluracil **12**, 2 eq. of triphenylphosphine (Ph_3P), 2 eq. of sodium benzoate (PhCOONa), and 2 eq. of diethyl azodicarboxylate (DEAD) or diisopropyl azodicarboxylate (DIAD) in dioxane (Figure 2). The benzoyl group of **17** was removed by treatment with saturated ammonia/methanol and the benzylidene group was deprotected with 80% trifluoroacetic acid at room temperature. The uracil derivative **3** was obtained in 23% yield and, likewise, following the same procedure, starting from **13**, **14** and **15**, compounds **4**, **5** and **6** were obtained in 29, 44 and 16% overall yield, respectively.

Mitsunobu reaction using either N^3-benzoylated thymine or uracil, or N-benzoylcytosine afforded mainly the O^2-substituted nucleosides. Only by addition of sodium benzoate the desired pyrimidine analogues could be obtained in moderate yield. The Mitsunobu conditions used here were somewhat different (Varasi *et al.*, 1987; Hughes *et al.*, 1988) from the standard conditions (Jenny *et al.*, 1991), as we were unable to obtain the desired N^1-substituted pyrimidine nucleoside analogues using these standard conditions.

The cytosine, 5-methylcytosine and 5-fluorocytosine analogues **7**, **8** and **9** were obtained from their uracil counterparts (**21**, **23** and **24**, respectively) via the 4-triazolylpyrimidinone intermediates (Krug *et al.*, 1989) (Figure 3). Therefore, compounds **21**, **23** and **24** were treated with phosphorous oxychloride and 1, 2, 4-triazole, and subsequently treated with bubbling ammonia gas through the reaction mixture for 10–15 min. After chromatographic purification, the benzylidene group was removed with 80% trifluoroacetic acid solution to yield 7, 8 and 9 in overall yield of 15, 17 and 20%, respectively (starting from compound **11**).

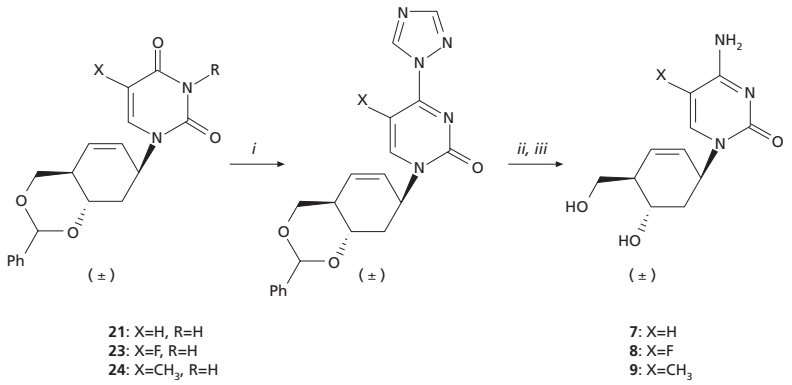
2-Amino-6-chloropurine was introduced under Mitsunobu conditions to afford **25**. This compound could be separated from its N-7 substituted isomer using column chromatography. Treatment of 25 with methanol saturated with ammonia in a Parr pressure reactor at 100°C (Verheggen *et al.*, 1995) gave, after removal of the benzylidene moiety, the desired 2,6-diaminopurine derivative **10** in combined yield of 18% (Figure 4).

Figure 2. Synthesis of compounds **3**, **4**, **5** and **6**



i) Ph_3P, PhCOONa, DEAD or DIAD, Dioxane, r.t. 2 days; ii) NH_3/CH_3OH, 12 h; iii) TFA/H_2O (80%).

Figure 3. Synthesis of compounds **7**, **8** and **9**



i) $POCl_3$, 1,2,4-1H-triazole, pyridine; ii) NH_3; iii) TFA/H_2O (80 %).

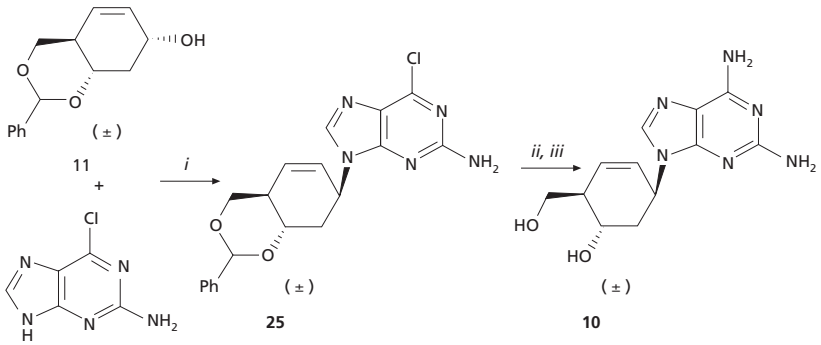
Discussion

The newly synthesized compounds **4**, **7**, **8**, **9** and **10** were investigated for their inhibitory effect on the cytopathogenicity of HSV-1, TK^- HSV-1, HSV-2, VV and VSV in human embryonic lung (HEL) cell cultures. Compounds **3**, **5** and **6** were tested against HSV-1, HSV-2 and VSV in human embryonic skin muscle (E_6SM) fibroblast cell cultures (Table 1). The antiviral activity was compared with that of known and approved antiviral drugs, one with a pyrimidine base moiety (brivudin) and two with a purine base moiety (acyclovir, ganciclovir). The sources of the viruses and the methodology used to monitor antiviral activity have been described previously (De Clercq *et al.*, 1980, 1986).

While compounds **3–5** did not display any meaningful activity, the analogues **6–10** demonstrated significant activity against HSV-1 and HSV-2. Of these compounds, **10** effected a 50% reduction of the cytopathogenicity induced by HSV-1 at a concentration of 1.4 μM. However, none of them approached the activity level of the reference compounds, brivudin, acyclovir or ganciclovir. The activity of the deoxycytidine analogues **7**, **8** and **9** against HSV-1 and HSV-2 was very similar. The diaminopurine derivative **10** is more potent against HSV-1 than against HSV-2. As the compounds were less active against the TK^- acyclovir-resistant (ACV^r) strain, intracellular phosphorylation by the virus-induced thymidine kinase must play an important role in their metabolic activation. Compounds were not active against VV at 210 μM. None of the compounds proved cytotoxic at a concentration up to 1000 μM, as monitored by microscopically detectable alteration of normal cell morphology. Compounds **3** and **6** were also evaluated for their activity against VZV and CMV. However, they exhibited no inhibitory effect on the cytopathogenicity of VZV (whether TK^+ or TK^-) or CMV in HEL cells (data not shown). Compounds **3**, **4** and **8–10** were not active against human herpesvirus type 6A or 6B in human T-lymphoblast HSB-2 and Molt-3 cells (De Clercq *et al.*, 2001) at concentrations up to 20 μM (data not shown).

From these results it is clear that only the diaminoguanine derivative, which can be considered as a precursor of cyclohexenyl-G, is of interest because of its moderate anti-HSV activity. Further research is needed to unravel its

Figure 4. Synthesis of compound **10**



i) Ph_3P, PhCOONa, DIAD, Dioxane, r.t. 2 days; ii) NH_3/CH_3OH, Parr bomb, 100°C, 14 h; iii) TFA/H_2O (80%).

Table 1. Antiviral activity against HSV-1, HSV-2, VV and VSV and cytotoxicity of compound **3–10**

	MIC (μM)										
Compound	3	4	5	6	7	8	9	10	D-Cycl G *	Acyclovir	Ganciclovir
Cell virus	E_6SM	HEL	E_6SM	E_6SM	HEL	HEL	HEL	HEL	E_6SM	HEL	HEL
HSV-1 (KOS)	1007	>220	936	190	40	63	191	1.4	0.01	0.4	0.02
HSV-1 (F)	ND§	220	ND	ND	13	38	64	2.3	0.01	0.6	<0.01
HSV-1 (McIntyre)	ND	659	ND	ND	202	38	191	6.9	0.01	0.6	0.01
HSV-1 (TK^- KOS ACV^r)	1007	1098	ND	ND	67	940	955	174	1.37	213	3.1
HSV-2 (G)	1007	659	>312	190	40	13	64	35	0.18	0.4	<0.01
HSV-2 (196)	ND	659	ND	ND	67	38	191	35	0.25	0.4	0.03
HSV-2 (Lyons)	ND	659	ND	ND	67	188	191	35	0.25	0.4	0.01
VV	>1679	>220	>312	>1585	1011	>1567	>1592	869	ND	>1775	>392
VSV	>1679	>220	>312	>1585	>1686	>1567	>1592	>290	ND	>1775	>392
MCC	>1679	≥1098	≥1561	>1585	>1686	>1567	>1592	≥1448	>1442	>1775	>392

MIC, minimum inhibitory concentration, or concentration required to reduce virus-induced cytopathogenicity by 50%; MCC, minimal cytotoxic concentration, or concentration required to cause a microscopically detectable alteration of normal cell morphology.
* Literature data taken from reference (Wang *et al.*, 2000). ND, not determined.

mode of action and to evaluate the individual (+) and (–) enantiomers of compound **10**.

Acknowledgements

We thank A Marchand and I Lagoja for their helpful suggestions and L Baudemprez for NMR analysis. We also thank A Van Lierde and F De Meyer for their excellent technical assistance and C Biernaux for her dedicated editorial help.

References

De Clercq E, Balzarini J, Torrence PF, Mertes MP, Schmidt CL, Shugar D, Barr PJ, Jones AS, Verhelst G & Walker RT (1981) Thymidylate synthetase as a target enzyme for the inhibitory activity of 5-substituted 2′-deoxyuridines on mouse leukemia L-1210 cell growth. *Molecular Pharmacology* **19:**321–330.

De Clercq E, Descamps J, Verhelst G, Walker RT, Jones AS, Torrence PF & Shugar D (1980) Comparative efficacy of antiherpes drugs against different strains of herpes simplex virus. *Journal of Infectious Diseases* **141:**562–574.

De Clercq E, Hol A, Rosenberg I, Sakuma I, Balzarini J & Maudgal P (1986) A novel selective broad-spectrum anti-DNA virus agent. *Nature* **323:**464–467.

De Clercq E, Naesens L, De Bolle L, Schols D, Zhang Y & Neyts J (2001) Antiviral agents active against human herpesviruses HHV-6, HHV-7 and HHV-8. *Reviews in Medical Virology* **11:**381–395.

Hughes DL, Reamer RA, Bergan JJ & Grabowski EJJ (1988) A mechanistic study of the Mitsunobu esterification reaction. *Journal of the American Chemical Society* **110:**6487–6491.

Jenny TF, Previsani N & Benner SA (1991) Carbocyclic analogs of nucleosides via modified Mitsunobu reactions. *Tetrahedron Letters* **32:**7029–7032.

Krug A, Schmidt S, Lekschars J, Lemke K & Cech D (1989) Transformation von uridin-zu cytidinderivaten durch selektive aminierung. (Transformation of uridine derivatives into cytidine via selective amination.) *Journal für Praktische Chemie* **331:**835–842.

Marquez VE (1996) Carbocyclic nucleosides. In *Advances in Antiviral Drug Design*, vol. 2; pp. 89-146. Edited by E De Clercq. London: JAI Press.

Maurinsh Y, Schraml J, De Winter H, Blaton N, Peeters O, Lescrinier E, Rozenski J, Van Aerschot A, De Clercq E, Busson R & Herdewijn P (1997) Synthesis and conformational study of 3-hydroxy-4-(hydroxymethyl)-L-cyclohexanyl purines and pyrimidines. *Journal of Organic Chemistry* **62:**2861–2871.

Mikhailov SN, Blaton N, Rozenski J, Balzarini J, De Clercq E & Herdewijn P (1996) Use of cyclohexene epoxides in the preparation of carbocyclic nucleosides. *Nucleosides, Nucleotides & Nucleic Acids* **15:**867–878.

Pérez-Pérez M–J, Rozenski J, Busson R & Herdewijn P (1995) Application of the Mitsunobu-type condensation reaction to the synthesis of phosphonate derivatives of cyclohexenyl and cyclohexanyl nucleosides. *Journal of Organic Chemistry* **60:**1531–1537.

Schaeffer HJ, Kaistha KK & Chakraborti SK (1964) Enzyme inhibitors II. Synthesis of trans-3-(6-substituted-9-purinyl)cyclohexanols as adenosine deaminase inhibitors. *Journal of Pharmaceutical Science* **53:**1371–1374.

Schaeffer HJ & Vince R (1968) Enzyme inhibitors XIX. The synthesis of some 1-hydroxy-2-hydroxymethyl-4-(6-substituted-9-purinyl)cyclohexanes as nucleoside analogues. *Journal of Medicinal Chemistry* **11:**15–20.

Varasi M, Walker KAM & Maddox ML (1987) A revised mechanism for the Mitsunobu reaction. *Journal of Organic Chemistry* **52:**4235–4238.

Verheggen I, Van Aerschot A, Van Meervelt L, Rozenski J, Wiebe L, Snoeck R, Andrei G, Balzarini J, Claes P, De Clercq E & Herdewijn P (1995) Synthesis biological evaluation, and structure analysis of a series of new 1,5-anhydrohexitol nucleosides. *Journal of Medicinal Chemistry* **38:**826–835.

Wang J, Froeyen M, Hendrix C, Andrei G, Snoeck R, De Clercq E & Herdewijn P (2000) The cyclohexene ring system as a furanose mimic: synthesis and antiviral activity of both enantiomers of cyclohexenylguanine. *Journal of Medicinal Chemistry* **43:**736–745.

Wang J, Morral J, Hendrix C & Herdewijn P (2001) A straightforward stereoselective synthesis of D- and L-5-hydroxy-4-hydroxymethyl-2-cyclohexenylguanine. *Journal of Organic Chemistry* **66:**8478–8482.

Received 9 December 2002; accepted 11 February 2003