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DESIGN AND SYNTHESIS OF A NOVEL CLASS OF NUCLEOTIDE ANALOGS WITH ANTI-HCMV ACTIVITY

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Abstract: A novel class of cyclic nucleotide analogs has shown anti-HCMV activity. The synthesis as well as structure - activity relationship studies are presented.

Introduction

HCMV infections pose a serious problem for individuals whose immune system has been compromised by disease, such as AIDS or by medication as in organ transplant recipients. Although the current therapies for treatment of Human Cytomegalovirus (HCMV) are effective, they all suffer from serious toxic side effects such as nephrotoxicity (foscarnet and HPMPC) and myelotoxicity (ganciclovir); there is therefore a need for better HCMV drugs.

Targeting viral polymerase with nucleosides analogs has proved to be one of the most successful approaches to inhibit viral replication. Since all the nucleoside analogs require conversion to the mono-, di- and finally the tri-phosphates prior to the incorporation in viral DNA (or RNA), strategies to circumvent the first phosphorylation have been developed. It has been shown by De Clercq and Holy that acyclic phosphonomethoxy nucleotide analogs like HPMPA, can lead to broad-spectrum antiviral agents,[1] which bypass the first crucial phosphorylation and can be converted by cellular kinases to their mono- and diphosphate ester derivatives.[2] HPMPC (Vistide™), a cytosine analogue, has recently been approved for treatment of HCMV infections.[3]

Based on HPMPC and on our experience in the chemistry of acetal and thioacetal nucleoside analogs,[4] we have designed a novel class of nucleotide analogs, by tethering the oxygen moiety of the terminal hydroxy group to the carbon α to the phosphonate. The outcome is a series of dioxolane nucleotide analogs **1** where the phosphonate group is directly attached to C-2 of the ring and the base is separated from the ring by a methylene group.

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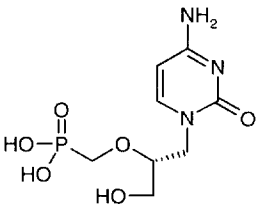
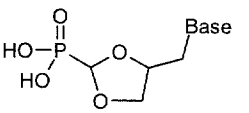
Since it is not known that antiviral activity will reside in a particular configuration, our strategy was first to synthesise racemic nucleotide analogs, and if there were antiviral activity, synthesis of the enantiomers of the active analogs would follow.



HPMPC



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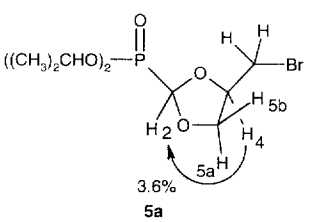
Synthesis

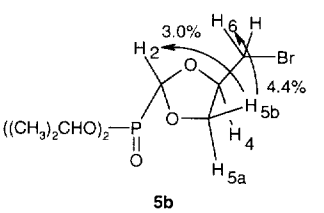
The dioxolane ring was constructed by reacting 3-bromo-1,2-propanediol **2** with trimethylorthoformate in the presence of a catalytic amount of pTSA to give a 1:1 mixture of *cis* and *trans* orthoester **3** in 96% yield (Scheme 1). The methoxyorthoester **3** was subsequently converted to the isopropoxyorthoester **4** under acid conditions, in a quantitative yield. The ratio of *cis* and *trans* isomers was about 1:1 as determined by ^{1}HNMR.

The next step was the introduction of a phosphonate moiety at C-2 of the dioxolane ring. This was achieved via an Arbuzov-type reaction by adding phosphorus trichloride, triisopropylphosphite and zinc chloride to the orthoester **4**, under neat conditions at 0 °C,[5] to give a mixture of the phosphonates **5a** and **5b** (2:5 ratio) in 72% yield. The mixtures of *cis* and *trans* isomers were separated by flash chromatography and used independently for the next step.

The relative stereochemistry was assigned by an NOE experiment (Figure 1) on the phosphonate **5a** and **5b**. Irradiation of H-4 of **5a** resulted in a 3.6% enhancement of H-2, thereby confirming the *cis* relationship between the phosphorus and the methylene group. No such effect was observed in the case of **5b**. Instead when H-5b was irradiated, an enhancement was observed with H-2 and H-6, which indicates that these 3 protons are on the same side of the molecule thereby requiring a *trans* relationship between the phosphorus and the methylene group.



5a

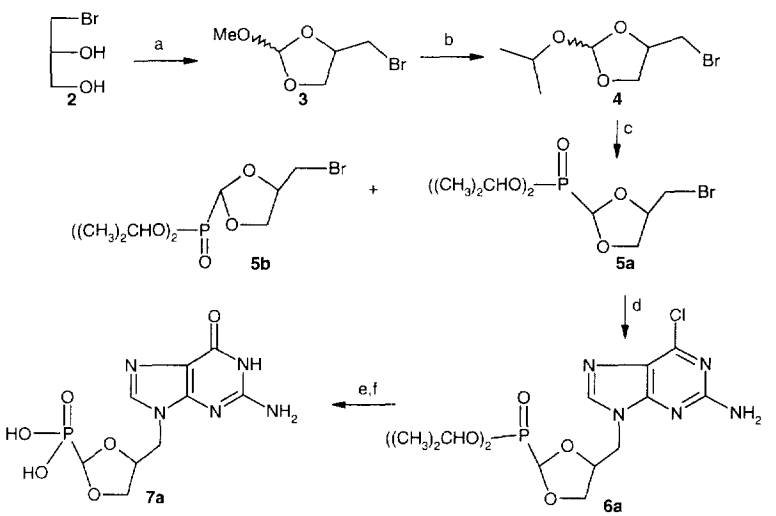


5b

Figure 1 Nuclear overhauser Effect (NOE) of **5a** and **5b** in $CDCl_3$ at room temperature, performed on a Varian 300 MHz.

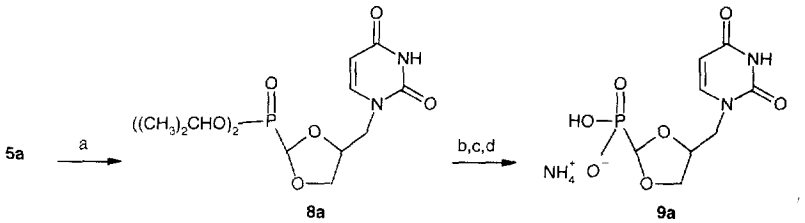
The bromide in **5a** was displaced with 2-amino-6-chloropurine in the presence of Cs_2CO_3 in DMF at 60 °C to provide compound **6a** in 30% yield. There was no evidence of other N-alkylated regioisomers. The phosphonate ester was deprotected by treatment with excess of iodotrimethylsilane followed by hydrolysis of the trimethylsilyl ester. The resulting phosphonic acid was sufficiently acidic to catalyse the hydrolysis of the 2-amino-6-chloropurine to guanine **7a**.

Similarly, **5b** was coupled with 2-amino-6-chloropurine to give the *trans* isomer **6b** in 59% yield. This increase in yield can be explained by the less hindered *trans* configuration of the bromide **5b**, allowing an easier approach of the purine to displace the bromide. Conversion of **6b** to **7b** was done in the same condition as previously described. Adenine **10a** and **10b** and cytosine **11a** and **11b** analogs were also prepared under the same conditions.



Scheme 1. (a) $CH(OMe)_3$/PhH/pTSA, 90 °C (96%); (b) i-propanol/PhH/pTSA, 90 °C (quant); (c) PCl_3/$P(OCH(CH_3)_2)_3$/$ZnCl_2$/0 °C - rt (72%); (d) 2-amino-6-chloropurine/DMF/Cs_2CO_3/60 °C (30%); (e) TMSI/CH_3CN:DMF; (f) H_2O, rt (53%).

As shown in Scheme 2, uracil analogue **8a** was prepared by displacement of bromide **5a** with uracil and NaH at 70 °C to give the desired nucleotide in 15% yield. After appropriate deprotection of the phosphonate esters with excess bromotrimethylsilane, followed by a purification on Sephadex DEAE A-25 column, the uracil analogue **9a** was obtained as the ammonium salt. The *trans* isomer **9b** as well as the thymine **12a** and **12b** analogs were prepared in the same manner.



Scheme 2. (a) Uracil/NaH/DMF/70 °C (15%); (b) TMSBr/CH_3CN/DMF; (c) H_2O, rt; (d) Sephadex DEAE A-25 gradient of 0 to 0.2 M of NH_4HCO_3 (58%).

Results

The nucleotide analogs were evaluated for HCMV activity at concentration up to 100 μg/mL and the results are summarized in Table 1. All of the compounds were found to be inactive except for the *cis* guanine analogue **7a,** which displayed anti-HCMV activity at 20 μg/mL in Flow2002 cell line. No toxicity was observed for these compounds.

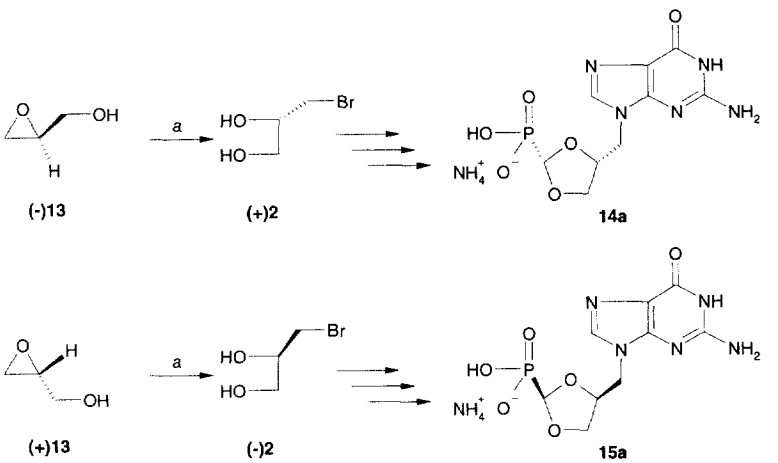
Since **7a** is racemic it was interesting to determine which of the enantiomers carries the antiviral activity.

Table 1. Anti-HCMV activity of 2-phosphonate-1,3-dioxolane nucleotide analogs in F2002 cell line.

Nucleotide	Base	IC_{50} (μg/mL)	CD_{50} (μg/mL)
7a	Guanine	20	>100
7b	Guanine	>100	>100
10a	Adenine	>100	>100
10b	Adenine	>100	>100
11a	Cytosine	>100	>100
11b	Cytosine	>100	>100
9a	Uracil	>100	>100
9b	Uracil	>100	>100
12a	Thymine	>100	>100
12b	Thymine	>100	>100
DHPG	-	0.1	>100

To prepare the chiral nucleotide analogs using the same route as in Scheme 1, a chiral synthesis of 3-bromo-1,2-propanediol was necessary since the compound is not commercially available. This was achieved by following literature conditions to open epoxides with halides.[6] (*S*)(-)-Glycidol **(-)13** was selectively opened by Li_2NiBr_4 at 0 °C to give the (2*S*)(+)-3-bromo-1,2-propanediol **(+)2**[7] in 65% yield (Scheme 3). Compound **(+)2** was then used to provide the *cis* guanine analogue **14a** and the *trans* guanine analogue **14b** under the same conditions described in the racemic synthesis. Similarly, (*R*)(+)-Glycidol **(+)13** gave (2*R*)(-)-3-bromo-1,2-propanediol **(-)2**[7] which was used to prepare the *cis* guanine analogue **15a** and the *trans* guanine analogue **15b.**

The chiral nucleotide analogs were evaluated for antiviral activity and the results summarised in Table 2. Only the 2*S*,4*R* configuration of the *cis* guanine analogue **14a** was found to be active at 45 μg/mL for HCMV in Flow2002 cell line. Compared to the racemic mixture, this is a decrease of potency by a factor of 2 however ganciclovir (DHPG) also showed a similar decrease in potency in the same assay. No toxicity was observed for these compounds.



Scheme 3. (a) LiBr, $NiBr_2$, THF/0 °C (65%).

Table 2. Anti-HCMV activity of chiral 2-(Dihydroxyphosphinoyl)-4-(guanin-9'-ylmethyl)-1,3-dioxolane in F2002 cell line.

Nucleotide	Geometry	Stereochemistry	α_D (H_2O)	IC_{50} (μg/mL)	CD_{50} (μg/mL)
14a	*cis*	2*S*,4*R*	+35.7° (*c* 0.26)	45	>100
14b	*trans*	2*R*,4*R*	+6.4° (*c* 0.25)	>100	>100
15a	*cis*	2*R*,4*S*	-34.4° (*c* 0.25)	>100	>100
15b	*trans*	2*S*,4*S*	-7.4° (*c* 0.27)	>30	>30
DHPG	-	-	-	0.2	>100

In summary, a novel class of anti-HCMV nucleoside analogs with moderate activity has been discovered.

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7. (2*S*)(+)-3-bromo-1,2-propanediol (+)**2** has an α_D of 4.9° (*c* 1.05, MeOH) and (2*R*)(-)-3-bromo-1,2-propanediol (-)**2** has an α_D of -5.1° (*c* 1.07, MeOH).