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IDENTIFICATION OF NOVEL NUCLEOTIDE PHOSPHONATE ANALOGS WITH POTENT ANTI-HCMV ACTIVITY

Paul Nguyen-Ba, Nathalie Turcotte, Leonard Yuen, Jean Bédard, Miguel Quimpère, and Laval Chan*

BioChem Therapeutic Inc., 275 Armand-Frappier Blvd., Laval, Québec, Canada, H7V 4A7

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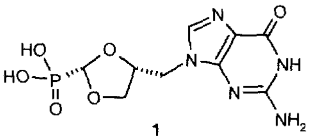
Abstract: We have recently described the discovery of new leads in the area of anti-HCMV research. Further structure - activity relationship studies have allowed us to identify potent and selective anti-HCMV nucleotide analogs. The synthesis as well as structure - activity relationship studies are described.

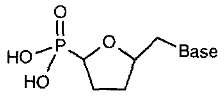
Introduction

Infection by the Human cytomegalovirus (HCMV) poses serious problems for immunocompromised individuals such as AIDS patients and organ transplant recipients.[1] In the previous paper,[2] we described the identification of a novel class of dioxolane phosphonate analogs of which the guanine analog **1** was found to be moderately active against HCMV and the activity appeared to reside in the enantiomer depicted. These findings provided us with the impetus to pursue our endeavors in this area of research. In this paper, we will describe the synthesis and structure - activity relationship (SAR) studies of the tetrahydrofuran phosphonate analogs **2**.[3]



1

IC_{50} 45 µg/mL
CC_{50} >100 µg/mL



2

Chemistry

Scheme 1 depicts our approach to the target molecules **2**. The commercially available (Aldrich) (*S*)-(+)-dihydro-5-(hydroxymethyl)-2(3*H*)-furanone **3** was converted to the corresponding bromide **4** as previously reported.[4] Reduction of the lactone followed by acetylation under standard conditions gave acetate **5** in 66% yield as a 1:1 mixture of isomers. The phosphonate group was then introduced by a Lewis acid catalyzed Arbuzov reaction, thus treatment of acetate **5** with $TiCl_4$ in methylene chloride at -30 °C, followed by triethylphosphite gave phosphonates **6** in 80% yield as a 1:1 mixture of *cis* and *trans* isomers.

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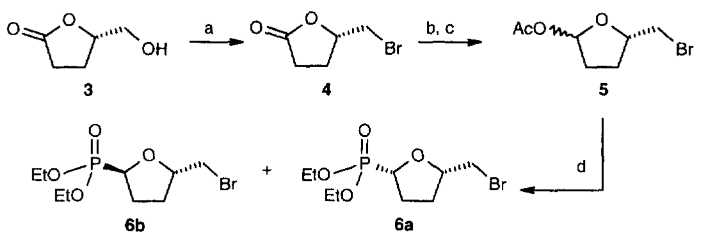
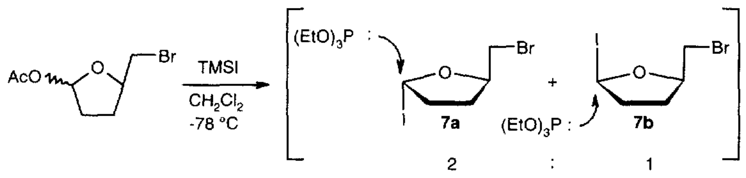


Scheme 1. (a) CBr_4, Ph_3P, MeCN, 84%. (b) DIBAL-H, toluene, -78 °C, 77%. (c) Ac_2O, pyridine, DMAP, CH_2Cl_2, 86%. (d) $TiCl_4$, $P(OEt)_3$, CH_2Cl_2, -30 °C, 86%.

Interestingly, the use of TMSI as a Lewis acid[5] gave a 2:1 *cis/trans* mixture of phosphonate **6** indicating that the intermediate glycosyl iodide **7** is formed as a 1:2 *cis/trans* ratio prior to S_N2 attack by phosphite (Scheme 2).



Scheme 2.

The mixture of bromophosphonates **6a** and **6b** was readily separable by flash chromatography (10 - 20% acetone in hexanes) and the relative stereochemistry was assigned by an NOE experiment (Figure 1) on the bromo-phosphonic acids **8a** and **8b** (obtained from TMSBr hydrolysis of **6a** and **6b**). Irradiation of H_b resulted in 2% enhancement of H_a thereby confirming the *cis* relationship between the phosphonic acid and the methylene group of **8a**.[6] No such effect was observed for the case of **8b**, instead an enhancement was observed for H_c indicating that in this case a *trans* relationship between the phosphonic acid and the methylene moiety is present. This effect could not be quantified due to a neighboring interfering signal.

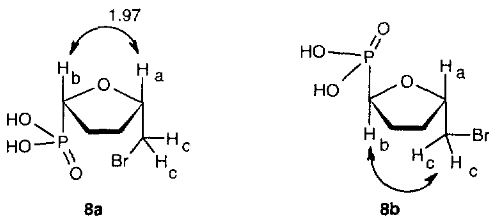
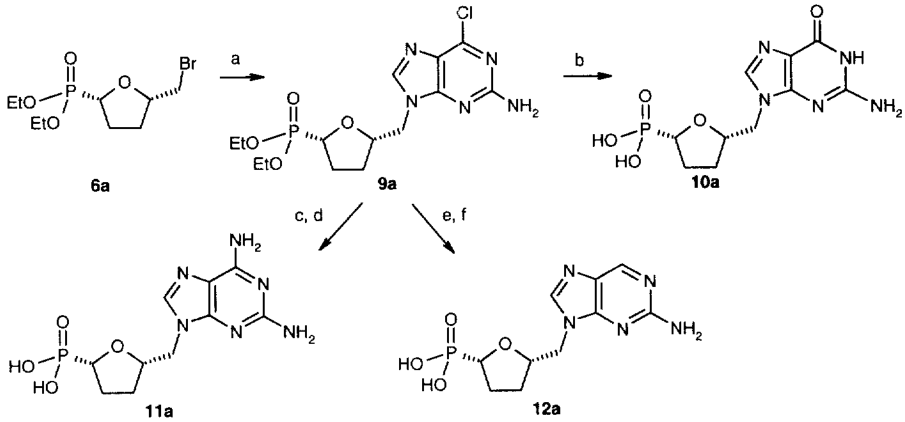


Figure 1. Nuclear Overhauser Effect (NOE) of **8a** and **8b** in $(CD_3)_2SO$ at room temperature, performed on a Bruker 400 MHz.

Condensation of bromide **6a** with 2-amino-6-chloropurine in the presence of Cs_2CO_3[7] in DMF at 95 °C gave 6-chloropurine **9a** in 45% yield. The phosphonate ester was deprotected by treatment with excess bromotrimethylsilane (TMSBr)[8] followed by concomitant hydrolysis of the resulting trimethylsilyl ester and the 6-chloropurine to guanine **10a** by refluxing in water. The phosphonic acid was sufficiently acidic to smoothly effect the last transformation. An overall yield of 8% starting with the commercially available (*S*)-(+)-dihydro-5-(hydroxymethyl)-2(3*H*)-furanone **3** was obtained. The intermediate 6-chloropurine **9a** was also converted to the diaminopurine analog **11a** by treatment with ammonia in ethanol at 100 °C followed by deprotection of the phosphonate. The 2-aminopurine phosphonate analog **12a** was obtained by phase transfer reduction of **9a** prior to phosphonate deprotection. The corresponding *trans* analogs were similarly obtained starting from **6b**. The optical antipodes of the compounds described were also prepared in order to compare their biological activity and to ascertain that in the tetrahydrofuran case, activity also resides in the same conformation as in dioxolane **1**.



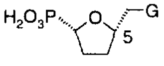
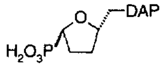
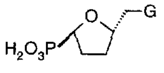
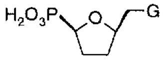
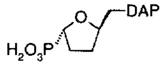
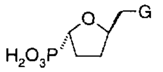
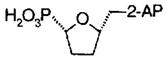
Scheme 3. (a) Cs_2CO_3, 2-amino-6-chloropurine, DMF, 95 °C, 45%. (b) TMSBr, CH_2Cl_2, then H_2O, 100 °C, 66%. (c) NH_3, EtOH, 100 °C. (d) TMSBr, CH_2Cl_2, then MeOH, 82%. (e) Cyclohexene, PdO, Et_3N, EtOH, reflux, 78%. (f) TMSBr, CH_2Cl_2, then MeOH, 93%.

Results and Discussions

In Vitro Evaluation. The anti-HCMV activity of the tetrahydrofuran phosphonates was measured using a plaque reduction assay in the WI-38 cell line and the cytotoxicity was determined by inhibition of cell proliferation in the same cell line.[9] These results were compared with that of ganciclovir (GCV) and HPMPC (Table 1). None of the 2,6-diaminopurines **11**, **14** or the 2-aminopurine derivatives **12** showed any inhibitory effect against HCMV; the antiviral effect was restricted to the *cis* and *trans* guanine analogs **10a** and **10b**. It is also apparent that the stereochemistry at C-5 is crucial for antiviral activity, only **10a** and **10b** showed activity whereas the corresponding optical antipodes **13a** and **13b** were inactive. The two active guanine analogs were further tested in the MRC-5 cell line and the results are shown in Table 1. The *cis* isomer **10a** had an IC_{50} of 1.6

μg/mL and a CC_{50} of >10 μg/mL and was found to be equipotent to GCV, whereas the *trans* isomer **10b** with an IC_{50} of 0.11 μg/mL and CC_{50} of 28 μg/mL was comparable to HPMPC in both antiviral activity and cytotoxicity.

Table 1
Antiviral activity (IC_{50}) and Cytotoxicity (CC_{50}) in WI-38 cell line

Nucleotide		$[\alpha]_D$ (in H_2O)	IC_{50} (μg/mL)	CC_{50} (μg/mL)	Nucleotide		$[\alpha]_D$ (in H_2O)	IC_{50} (μg/mL)	CC_{50} (μg/mL)
10a		+52° (*c* 0.10)	0.5-1 1.6*	10-50 >10*	**11b**		-17° (*c* 0.26)	50	>100
10b		-9° (*c* 0.26)	0.1-1 0.11*	10-100 28*	**14a**		-39° (*c* 0.48)	>50	>100
13a		-56° (*c* 0.40)	>10	100	**14b**		+16° (*c* 0.25)	>50	>100
13b		+9° (*c* 0.25)	>10	>100	**12a**		+65° (*c* 0.25)	100	>100
11a		+42° (*c* 0.48)	>50	>100	HPMPC		-	0.1-1 0.04*	10-50 19*
					Ganciclovir		-	2.1*	>100*

*Measured in MRC-5 cell line.

In Vivo Experiments. The in vivo efficacy of the *cis* tetrahydrofuran phosphonate **10a** was determined in mice infected with Murine Cytomegalovirus (MCMV). Since no convenient animal model for HCMV exists, the murine model serves as a useful surrogate for the determination of in vivo activity.[10] Tolerance studies as a function of body weight were also performed prior to compound administration, **10a** was well tolerated at doses of 12.5 and 25 mg/kg 3 times per day when administered ip (Figure 2), but at 50 mg/kg the rate of weight increase was less than placebo indicating presence of sub-acute toxicity. Complete protection from death was achieved at a dose of 2.5 mg/kg/dose 3 times a day for 4 days whereas to achieve the same degree of protection with GCV, a dose of 10 mg/kg was required (Figure 3). No cytotoxicity related deaths were observed at doses up to 20 mg/kg; however, at 40 mg/kg, **10a** was found to be toxic to the animals.

Figure 2. Tolerance of 10a in mice.

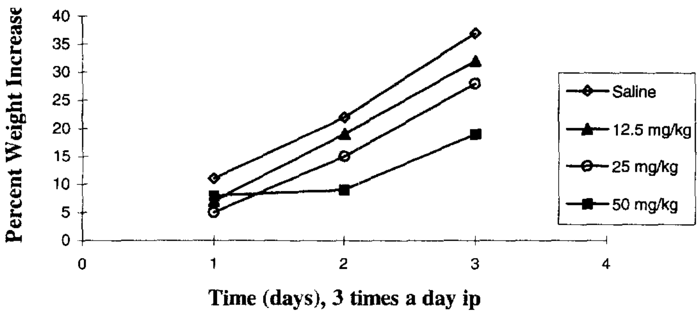
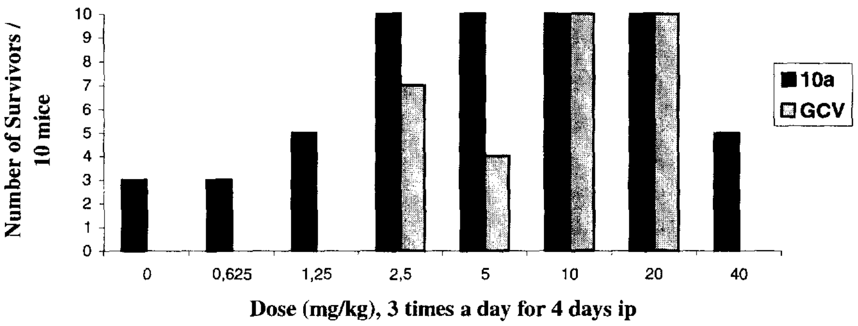


Figure 3. In Vivo MCMV Efficacy of 10a and GCV.



Summary

In summary, we have described the synthesis and antiviral activity of potent and selective anti-HCMV guanine phosphonate analogs **10a** and **10b**. The in vivo efficacy of **10a** in a murine model was demonstrated and shown to be superior to GCV. Furthermore, it is also interesting to speculate whether **10a** and **10b** belong to the family of antiviral L-nucleosides[11] since they approximate L-guanosine 5'-monophosphate in structure.

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