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# CONSTRAINED ANALOGUES OF 2'-NOR CYCLIC NUCLEOSIDE MONOPHOSPHATES

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**Abstract.** The synthesis of constrained analogues of 2'-nor-cyclic nucleosides monophosphates containing a thiomethylene tether was readily accomplished from the oxathiolane intermediate **4**. The uracil, cytosine, and 5-bromocytosine spirophosphate analogues **22**, **16**, and **25** were inhibitory to HCMV replication in Flow 2002 cells. © 1997 Elsevier Science Ltd.

The acyclonucleotide analogue, 9-[(2-hydroxy-1,3,2-dioxaphosphorinan-5-yl)oxymethyl]guanine P-oxide (**1**, 2'-nor-cGMP, DHPG-CP)[1] of the clinically approved agent ganciclovir (GCV, Cytovene) has potent broad spectrum antiviral activities against DNA viruses.[2] In plaque reduction assays, **1** is equivalent to GCV against human cytomegalovirus (HCMV) but substantially more potent against varicella-zoster virus.[1] In rodents, **1** is effective against infections caused by herpes simplex virus type 1 and 2 and CMV.[2,3] Compelling evidence has demonstrated that **1** is neither catabolized to GCV intracellularly nor cleaved to form GCV monophosphate which would then be converted by host enzymes to the active triphosphate form.[2-4] To date the mode of action of **1** has not been clarified although its activity, being independent of activation by viral kinases and metabolic conversion to a triphosphate derivative, is attributed to its resemblance structurally to the second messenger cGMP.[2-4] The limited structure-activity relationship in this series of nucleotides prompted us to investigate conformationally constrained analogues of **1** as inhibitors of viral replication. The target molecules of general formula **2** (Figure 1) possess a thiomethylene tether that would result in spirophosphate analogues of 4'-substituted 3'-thia dideoxynucleoside analogues (2,2,5-trisubstituted 1,3-oxathiolanes).[5] In addition to the guanine derivatives, pyrimidine analogues particularly cytosine derivatives were also regarded as useful target molecules in view of the recent reports describing the enhanced safety profile of cyclic HPMPC (**3**), currently in clinical trials for CMV retinitis, as compared to HPMPC (Vistide).[6] Herein, we describe the synthesis and antiviral (HSV-1, HSV-2, HIV, and HCMV) activities of spirophosphates **2**.

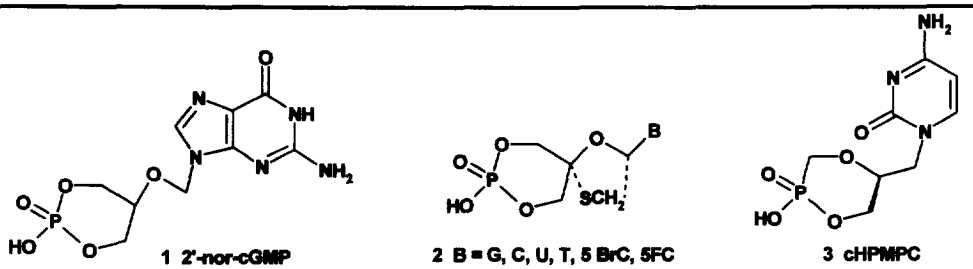
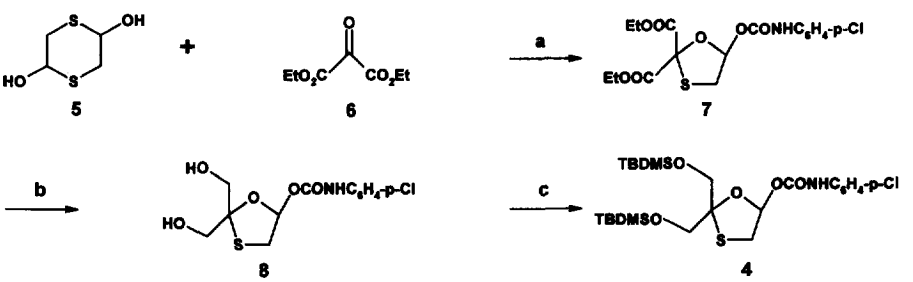



**Figure 1**

From the outset, it was envisaged that cyclic phosphates **2** could be readily prepared from the corresponding nucleosides, which in turn are available via a Vorbrüggen type coupling of a silylated base with a suitably functionalised oxathiolane derivative. The oxathiolane derivative **4**, which was chosen as the key intermediate of our synthesis, was prepared efficiently by the synthetic route depicted in Scheme 1. Heating of a suspension of 2,5-dihydroxy-1,4-dithiane (**5**) in diethyl ketomalonate (**6**) and pyridine under an argon atmosphere for 15 min produced 2,2-diethoxycarbonyl-5-hydroxyoxathiolane intermediate which was not isolated. After cooling to room temperature, a solution of this crude material in dichloromethane was treated with 4-chlorophenylisocyanate in the presence of a catalytic amount of diisopropylethylamine to provide the expected carbamate **7** as a white solid. The stability of the *p*-chlorophenylcarbamoyl group facilitated the reduction of the ester groups with sodium borohydride in a 1:1 mixture of methanol and dichloromethane to give the corresponding diol **8** in excellent yield. Subsequent treatment of this diol with *tert*-butyldimethylsilyl chloride and imidazole in dimethylformamide under the usual conditions afforded the key oxathiolane intermediate **4** in excellent yield.

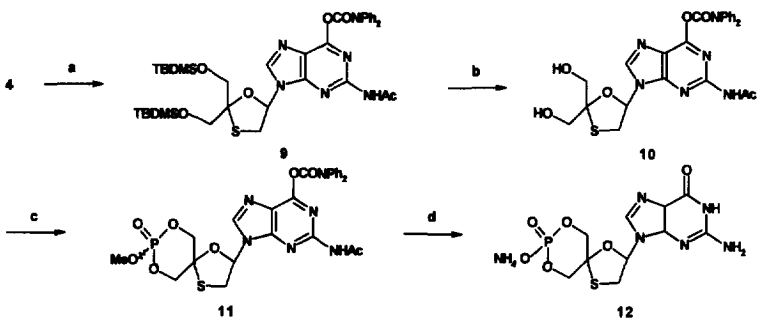



**Conditions and reagents:**
**(a) (i) py, 70 °C; (ii) DIEA, 4-chlorophenylisocyanate, $CH_2Cl_2$ 52%; (b) $NaBH_4$, MeOH-$CH_2Cl_2$ (1:1) 90%; (c) TBDMSCl Im., DMF 93%**

**Scheme 1**

Coupling of **4** with the silylated 2-acetamido-6-diphenylcarbamoyloxypurine[7] promoted by iodotrimethylsilane at room temperature provided regioselectively the desired N-9 nucleoside **9** in 49% after purification by column chromatography (Scheme 2). Unmasking of the hydroxyl functions was accomplished by treating **9** with tetra-*n*-butylammonium fluoride in tetrahydrofuran in the presence of acetic acid to afford **10** in good yield.

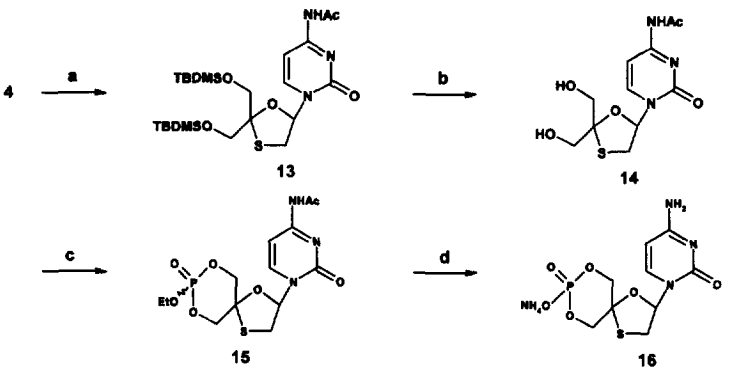
Subsequent reaction of the diol **10** with excess methyl dichlorophosphate at 0 °C in dichloromethane gave the corresponding cyclic phosphate derivative **11** as a mixture of two isomers after purification by column chromatography. Conversion of **11** to the guanine spirophosphate **12** was accomplished by removal of the protecting groups on the purine moiety by treatment with hydrazine in tetrahydrofuran, followed by reaction of the crude product thus obtained with sodium iodide at 90 °C in dimethylacetamide and purification by reversed phase HPLC.



**Conditions and reagents:**
**(a) TMSI, bis-silylated 2-acetamido-6-diphenylcarbamoyloxypurine, $ClCH_2CH_2Cl$ 49%; (b) TBAF-AcOH (1:1), THF, 72%; (c) (i) Methyl dichlorophosphate, py, $CH_2Cl_2$, 0 °C; (ii) column chromatography 10% MeOH-EtOAc, 49%; (d) (i) $N_2H_4$, THF; (ii) NaI, DMA, 90 °C; (iii) reverse-phase HPLC (eluent aq $NH_4OAc$) 27% from 11**

**Scheme 2**

A similar approach was employed for the preparation of the cytosine spirophosphate (Scheme 3). Iodotrimethylsilane promoted coupling of **4** with bis-silylated *N*-acetylcytosine gave the expected nucleoside **13** in excellent yield. Tetra-N-butylammonium fluoride induced cleavage of the silyl protecting groups afforded the corresponding diol **14** which upon reaction with ethyl dichlorophosphate produced the cyclic phosphate **15** again as a mixture of two isomers. The ethyl group was cleaved from the phosphate moiety using sodium iodide under the same conditions described above to furnish the cytosine spirophosphate **16** in good yield.



**Conditions and reagents:**
**(a) TMSI bis-silylated *N*-acetylcytosine, $CH_2Cl_2$ 67% (b) TBAF-AcOH (1:1), THF 94%; (c) Ethyl dichlorophosphate, $CH_2Cl_2$ 48%; (d) (i) NaI, DMA, 90 °C; (ii) $NH_3$, MeOH, (iii) reverse-phase HPLC, 60% from 15**

**Scheme 3**

Due to the good anti-HCMV activity of **16** (Table 1) a number of other structurally related pyrimidine analogues, namely the uracil, thymine, 5-bromocytosine, and 5-fluorocytosine derivatives were synthesized. The nucleotides were prepared from the corresponding nucleosides **17-20** (Fig. 2) by the same synthetic route described above for the cytosine analogue.

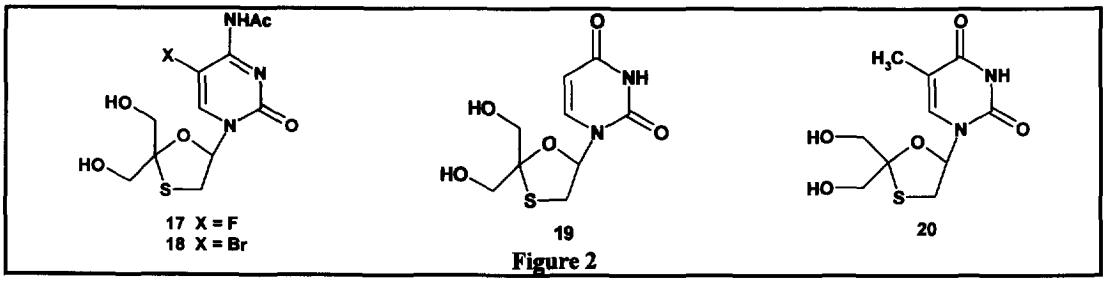
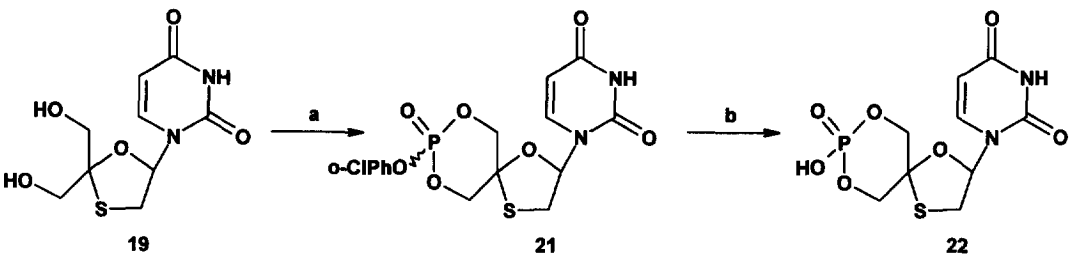



**Figure 2**

However in these cases, 2-chlorophenyldichlorophosphate was employed for the preparation of the cyclic monophosphate moiety instead of ethyldichlorophosphate due to the relative ease of unmasking this group by simple treatment with aqueous sodium hydroxide. Scheme 4 illustrates a representative preparation for the uracil spirophosphate **22** via the *o*-chlorophenyl intermediate **21**. Following this protocol, the spirophosphate derivatives **23**, **24** and **25** (Fig. 3) were obtained from **20**, **17**, and **18**, respectively, in 60%, 45%, and 15% overall yield.




**Conditions and reagents:**
**(a) (i) *o*-chlorophenyl dichlorophosphate, py, $CH_2Cl_2$; (ii) chromatography (7% MeOH-EtOAc) 66%; (b) aq NaOH**

**Scheme 4**

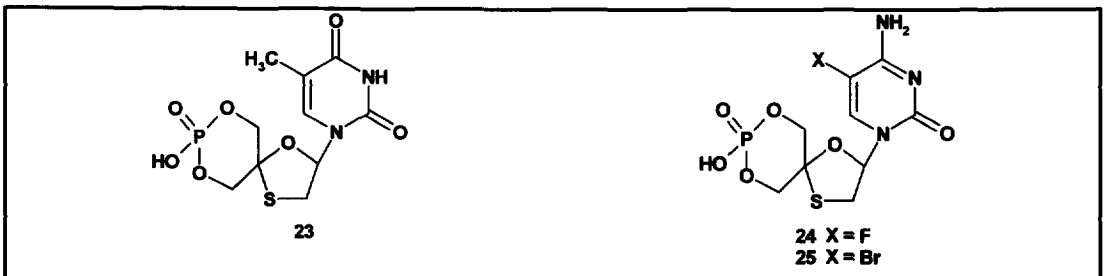



**Figure 3**

The nucleotides **12**, **16**, **22**, **23**, **24**, and **25** were tested in plaque reduction assays against HCMV (WF1 strain) in Flow 2002 cells,[8] HSV-1 (KOS strain) and HSV-2 (186 strain) in Vero cells and HIV-1 (RF strain) in MT-4 cells. None of the nucleotides inhibited HIV-1, HSV-1 or HSV-2 at concentrations up to 100 μg/mL. Unfortunately, the guanine derivative **12** did not inhibit HCMV plaque formation at concentrations up to 100 μg/mL. However, the uracil spirophosphate **22**, cytosine and 5-bromocytosine analogues **16** and **25** showed good inhibitory activity against HCMV being 25- to 110-fold weaker than the control GCV (Table 1). In comparison, the corresponding nucleosides **19**, **14**, and **18** were not inhibitory to HCMV at concentrations up to 100 μg/mL.

**Table 1. Anti-HCMV of Spirophosphates in Flow Cells**

| Nucleotide | $PD_{50}$ μg/mL | $CD_{50}$ μg/mL |
|---|---|---|
| 12 | >100 | >100 |
| 16 | 11 | >100 |
| 22 | 2.5 | >100 |
| 23 | >100 | >100 |
| 24 | >100 | >100 |
| 25 | 2.5 | >100 |
| GCV | 0.1 | >100 |

In summary, we have described an efficient synthesis of 2,2-dihydroxy-1,3-oxathiolane nucleosides and their spirophosphate analogues as constrained analogues of 2'-nor-cyclic nucleoside monophosphates and demonstrated good activity against HCMV in Flow 2002 cells for the uracil, cytosine and 5-bromocytosine analogues.[9,10]

**Acknowledgments**
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**References and notes**

1. 2'-Nor-cGMP refers to the removal of the 2'CHOH moiety of 3',5'-guanosine monophosphate DHPG-CP cyclic monophosphate of 9-(1,3-dihydroxy-2-propoxymethyl)guanine. This compound is related to 1',2'-seco nucleosides. For a recent reference on 1',2'-seco nucleosides see, Racha, S.; Vargeese, C.; Vemishetti, P.; El-Subbagh, H. I.; Abushanab, E.; Panzica, R. P. *J. Med. Chem.* **1996**, *39*, 1130.
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8. Anti-HCMV testing. Subconfluent monolayers of Flow 2002 cells in 24-well tissue culture dishes were inoculated with 100 μL (containing 30 pfu) of CMV (WF1 strain) diluted in Glascow's MEM. After adsorption at 37 °C (2 h) the monolayers were overlaid with medium containing test compounds and 0.75% w/v carboxymethyl-cellulose. After incubation at 37 °C in 5% $CO_2$/air for 6-7 days the monolayers were fixed. Virus induced plaques were counted and the concentration of compound required to inhibit plaque formation by 50% compared to the untreated control cultures was calculated and expressed as the $PD_{50}$ value in micrograms per mL. $CD_{50}$ was assessed on virus-free cell layers. Visual assessment of the integrity of the treated monolayers at each compound dose was made.
9. Selected spetroscopic data of **12**: UV ($\lambda_{max}$): 254 nm ($H_2O$); $^1$H NMR (300 MHz, DMSO-$d_6$) δ 3.55 (d of d, 1H, $J$ = 5.0, 11.4 Hz), 3.77 ( d of d, 1H, $J$ = 6.6, 11.5 Hz), 3.82 - 4.09 (m, 3H), 4.09 - 4.22 (m, 1H), 6.28 (t, 1H, $J$ = 5.8 Hz), 6.60 (br. s, 2H), 6.80 - 7.60 (unresolved m, 4H), 7.98 (s, 1H), 10.50 - 10.90 (unresolved m, 1H); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) δ 34.44, 71.67 (d, $J$ = 6.5 Hz), 72.41 (d, $J$ = 5.7 Hz), 85.44, 88.66 (d, $J$ = 5.0 Hz), 116.93, 135.46, 151.35, 154.23, 157.03. **16**: UV ($\lambda_{max}$): 268.2 nm ($H_2O$); $^1$H NMR (300 MHz, DMSO-$d_6$) δ 3.15 (d of d, 1H, $J$ = 5.2, 11.5 Hz), 4.05 - 4.45 (m, 4H), 5.87 (d, 1H, $J$ = 7.5 Hz), 6.39 (d of d, 1H, $J$ = 5.2, 6.8 Hz), 6.90 - 7.50 (unresolved m, 2.5H), 7.80 (d, 1H, $J$ = 7.6 Hz), 7.65 - 8.10 (unresolved m, 2H); $^{13}$C NMR (75.5 MHz, (DMSO-$d_6$) δ 35.09, 71.57 (d, $J$ = 6.1 Hz), 72.51 (d, $J$ = 6.5 Hz), 87.09, 87.16, 95.19, 141.87, 153.31, 164.60. **22**: UV ($\lambda_{max}$) 204.7, 259.6 nm ($H_2O$); $^1$H NMR (300 MHz, DMSO-$d_6$) δ 3.24 ( d of d, 1H, $J$ = 6.9, 11.5 Hz), 3.44 (d of d, 1H, $J$ = 5.3, 11.5 Hz), 3.94 - 4.13 (m, 3H), 4.14 - 4.25 (m, 1H), 5.70 (d, 1H, $J$ = 8.0 Hz), 6.32 (d of d, 1H, $J$ = 5.3, 6.7 Hz), 6.60 - 7.80 (unresolved m, 3H), 7.79 (d, 1H, $J$ = 8.1 Hz); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) δ 34.33, 71.20 (d, $J$ = 5.8 Hz), 72.07 (d, $J$ = 5.5 Hz), 86.04, 88.00 (d, $J$ = 5.8 Hz), 102.80, 140.76, 150.55, 163.26. **23**: UV ($\lambda_{max}$) 210.4, 263.8 nm ($H_2O$); $^1$H NMR (300 MHz, DMSO-$d_6$) δ 1.81 (s, 3H), 3.22 (d of d, 1H, $J$ = 7.9, 11.3 Hz), the other d of d from the oxathiolane ring was obscured by the solvent signal, 3.92 - 4.28 (m, 4H), 6.32 (d of d, 1H, $J$ = 5.4, 7.7 Hz), 6.70 - 7.55 (unresolved m, 3H), 7.65 (s, 1H); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) δ 12.49, 33.85, 71.15 (d, $J$ = 5.7 Hz), 72.22 (d, $J$ = 5.8 Hz), 85.54, 87.13 (d, $J$ = 4.8 Hz), 110.61, 136.11, 150.58, 163.94. **24**: UV ($\lambda_{max}$) 239.1, 278.9 nm ($H_2O$); $^1$H NMR (300 MHz, DMSO-$d_6$) δ 3.14 (d of d, 1H, $J$ = 7.7, 11.3 Hz), the other d of d from the oxathiolane ring was obscured by the solvent signal, 3.90 - 4.35 (m, 4H), 6.30 (m, 1H), 6.95 - 7.50 (unresolved m, 3H), 7.71 (br. s, 1H), 6.95 - 7.50 (unresolved m, 3H), 7.71 (br. s, 1H), 7.94 (br. s, 1H), 7.98 (d, 1H, $J$ = 6.8 Hz); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) δ 34.74, 71.24 (d, $J$ = 5.7 Hz), 72.23 (d, $J$ = 5.7 Hz), 86.92, 87.23 (d, $J$ = 5.1 Hz), 125.60 (d, $J$ = 32 Hz), 136.65 (d, $J$ = 243 Hz), 153.23, 157.97 (d, $J$ = 13.7 Hz). **25**: UV ($\lambda_{max}$) 285.8 nm ($H_2O$); $^1$H NMR (300 MHz, DMSO-$d_6$) δ 3.21, (d of d, 1H, $J$ = 7.5, 11.1 Hz), the other d of d from the oxathiolane ring was obscured by the solvent signal, 3.90 - 4.30 (m, 4H), 6.29 ( d of d, 1H, $J$ = 5.0, 7.7 Hz), 6.90 - 7.60 (unresolved m, 4H), 8.04 (br. s, 1H), 8.08 (s, 1H); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) δ 34.67, 71.21 (d, $J$ = 5.7 Hz), 72.21 (d, $J$ = 5.6 Hz), 86.94, 87.42 (d, $J$ = 5.7 Hz), 72.21 (d, $J$ = 5.6 Hz), 86.94, 87.42 (d, $J$ = 5.1 Hz), 87.55, 141.95, 153.52, 162.30.
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