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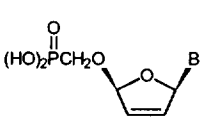
Synthesis and Antiviral Activity of Phosphonate Derivatives of Enantiomeric Dihydro-2*H*-Pyranyl Nucleosides

María-Jesús Pérez-Pérez[+], Jan Balzarini[#], Jef Rozenski[+], Erik De Clercq[#] and Piet Herdewijn[+*]

Laboratories of [+]Medicinal Chemistry and [#]Experimental Chemotherapy, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium.

Abstract : A synthetic approach to phosphonate derivatives of 2,5-*cis*-substituted dihydro-2*H*-pyranyl nucleosides has been developed and both series of enantiomers (**3** and **4**) have been prepared. The key step in the synthetic pathway was the introduction of the phosphonomethoxy moiety on pentopyranosyl glycals through a Ferrier-type rearrangement. The heterocyclic base was then incorporated under Mitsunobu conditions. The resulting nucleoside derivatives were more stable towards acidic degradation than their natural isomers. However, they were found to be inactive against the replication of human immunodeficiency virus (HIV), herpes simplex virus (HSV) and other herpes viruses [i.e. varicella-zoster virus (VZV), cytomegalovirus (CMV)] in cell culture, which could at least partially be ascribed to an inefficient phosphorylation by cellular enzymes (i.e. GMP kinase).

2',3'-Dideoxynucleosides (i.e. ddC, ddI) and their unsaturated analogues (i.e. d4T) are potent inhibitors of HIV replication.[1] To exert their antiviral activity, these compounds have to be transformed to their corresponding triphosphates by the action of cellular kinases. In this activation process, the generation of the monophosphate is, in most of the cases, the limiting step. A particularly successful strategy to circumvent this first phosphorylation step is the synthesis of phosphonate derivatives of nucleosides, where the phosphonomethoxy moiety is introduced as an isosteric and isoelectronic function of the monophosphate[2,3]. This approach has been followed with success in the series of acyclic nucleosides[2] and the series of unsaturated furanosyl nucleosides (**1**)[4], both showing potent anti-HIV activity. Despite the great number of modified 2',3'-dideoxynucleoside derivatives described, there are only a few reports on their ring-enlarged six-membered analogues,[5-10] and no phosphonate derivative of such structures has been reported. Therefore, we have considered of interest the synthesis and antiviral evaluation of the six-membered ring analogues of the nucleoside phosphonate derivative **1**, namely the α-L series (**2**)[11] and the isonucleosides (**3**). In the former, the extra methylene unit compared to **1** has been introduced between the oxygen atom and the phosphonomethoxy moiety, while in the latter, the extra CH_2 is inserted between the oxygen and the heterocyclic base. Both series can be considered as 2,5-substituted cyclic allylethers. In the present communication, we report on the synthetic strategy to obtain the isonucleosides **3** and their enantiomers **4**, exemplified by the synthesis of the guanine derivatives. The antiviral evaluation of these compounds and their stability in acidic conditions are also described.



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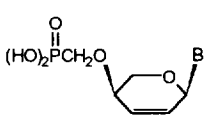

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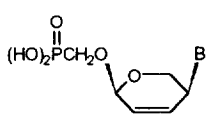

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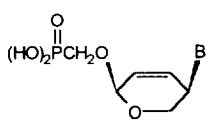
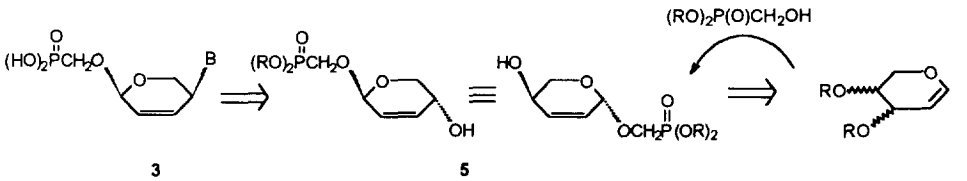


4

The key intermediate for the synthesis of the isonucleosides **3** (Scheme 1) is the *trans* alcohol **5**, where the heterocyclic base can be introduced under Mitsunobu conditions[12] to yield the desired *cis* nucleosides. For the synthesis of the intermediate **5**, which carries a phosphonomethoxy moiety in the anomeric position, we have made use of the allylic rearrangement initially described by Ferrier[13] on treatment of glycals with alcohols in the presence of acids.

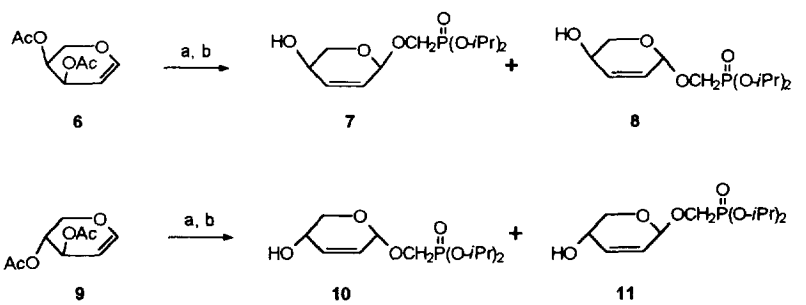
Scheme 1



3 **5**

Reaction of 3,4-di-*O*-acetyl-L-arabinal[14] (**6**) with diisopropyl hydroxymetylphosphonate[15] in the presence of trimethylsilyl triflate, followed by treatment with methanolic ammonia, afforded two isomeric *O*-glycosides (**7** and **8**) in a ratio of 1:3 and global yield of 65% (Scheme 2). On the other hand, a similar reaction sequence starting from 3,4-di-*O*-acetyl-D-xylal[16] (**9**) afforded the unsaturated sugars **10** and **11**, also in a 1:3 ratio and with a global yield of 72%.[17]

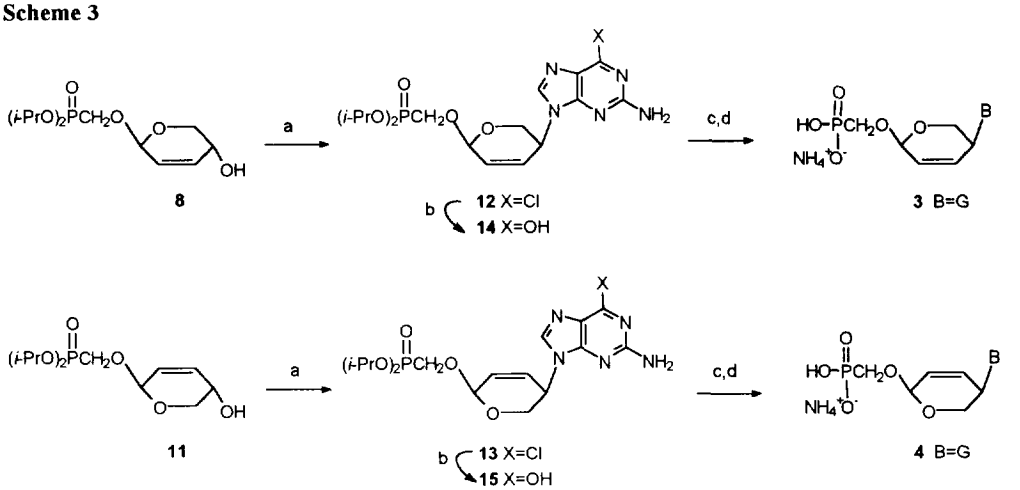
Scheme 2



(a) (*i*-PrO)$_2$P(O)CH$_2$OH, TMSTf, CH$_3$CN; (b) NH$_3$/MeOH.

In both cases, the 1,4-*trans* isomers are obtained as the major compounds, which give us direct access to both series of enantiomeric nucleosides (**3** and **4**). Thus, treatment of **8** (Scheme 3) with 2-amino-6-chloropurine (2eq.) under Mitsunobu conditions [Ph$_3$P (2 eq.) and DEAD (2eq.)] afforded the *N*-9 nucleoside **12** in 42% yield. Similarly, and starting from the alcohol **11**, the nucleoside derivative **13** was obtained (35% yield).[18] Transformation of **12** and **13** to their guanine analogues (**14** and **15**, respectively) was carried out by reaction with 35% aqueous trimetylamine, followed by treatment with DBU[19] (**14**: 78%; **15**: 74% yield). Finally, the diisopropyl ester functions were removed by reaction with TMSBr,[20] followed by hydrolysis with aqueous ammonia. The phosphonate derivatives were isolated as their ammonium salts, after chromatography on XAD and DEAE-Sephadex-A25 ($HCO3^-$ form) with a gradient of H_2O-0.1M NH_4HCO_3 (**3**: 53% and **4**: 59% yield).

Scheme 3



(a) 2-Amino-6-chloropurine, Ph_3P, DEAD, dioxane, rt; (b) 35% Me_3N, and then DBU; (c) Me_3SiBr, 2,6-lutidine, DMF; (d) NH_4OH

The unsaturated derivatives of 2',3'-dideoxynucleosides are highly sensitive towards acidic degradation, in some cases limiting their therapeutic use.[21] Therefore, we were concerned about the stability in acid conditions of this new family of unsaturated nucleoside derivatives. Based on the fact that a phosphonomethoxy moiety is a poorer leaving group than a heterocyclic base, the isonucleosides (**3** and **4**) should be more stable towards acid degradation than their natural isomers **2**. Actually, the adenine derivative of **2** was hydrolyzed at pH=3 with release of the free base, having a half-life of 5 h at this pH. In contrast, the guanine derivatives of the isonucleosides **3** and **4** were much more stable at pH=3 and after 20 h ~96% of the compounds remained unaffected. These results confirm our hypothesis and are in agreement with the behaviour described for the furanosyl analogues **1**.[4]

Compounds **3** (B=G) and **4** (B=G) were found to be inactive in cell culture against all herpes- and retroviruses tested (HSV-1, HSV-2, VZV, CMV, HIV-1, HIV-2). This lack of antiviral activity may be due to the poor phosphorylation of the test compounds and/or the lack of affinity of the corresponding triphosphate analogues for the herpetic DNA polymerases or HIV reverse transcriptase. To investigate the first hypothesis, compounds **3** (B=G) and **4** (B=G) were tested as substrates for GMP kinase purified from porcine brain[22]. Under the experimental conditions where 0.2 μmol guanylate (GMP) and 9-(2-phosphonylmethoxyethyl)guanine (PMEG) were fully converted to their corresponding diphosphate analogues within ~20 sec and 20 min, respectively, by 0.56 unit of the enzyme, no phosphorylated products from **3** (B=G) and **4** (B=G) could be detected. Thus, the lack of phosphorylation of the guanine derivatives by GMP kinase may, at least, be one factor explaining their antiviral inactivity. However, it cannot be excluded that other purine/pyrimidine analogues of this series of compounds may be phosphorylated by other enzymes involved in nucleotide metabolism.

Acknowledgements

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17. In both cases the anomeric configuration of the *O*-glycosides was inequivocally establised from the coupling constants observed in the ^{1}H-NMR spectra of their hydrogenated derivatives.
18. The *N*-9 substitution was established from their UV and NMR parameters and comparison with published data (i.e. Toyota, A.; Katagiri, N.; Kaneko, C. *Heterocycles* **1993**, *36*, 1625-1630). In both cases, a small proportion of the *N*-7 isomers (10-20%) was detected.
19. DBU was added to favour the transformation of trimethyl ammonium intermediate to the guanine derivative.
20. The deprotection with TMSBr was carried out in the presence of an excess of 2,6-lutidine to avoid possible anomerization or acetal cleavage.
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