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SYNTHESIS AND BIOLOGICAL ACTIVITY OF CARBOCYCLIC DERIVATIVES OF THE POTENT ANTIVIRAL AGENT 9-[2-(PHOSPHONOMETHOXY)ETHYL]GUANINE (PMEG)[1]

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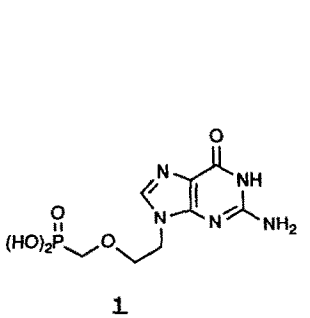
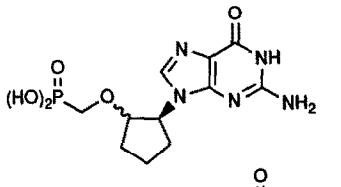
Abstract: Carbocyclic analogues of the potent nucleotide analogue 9-[2-(phosphonomethoxy)-ethyl]guanine (PMEG) were prepared and evaluated for antiviral activity against herpes simplex virus type 2 and human immunodeficiency virus.

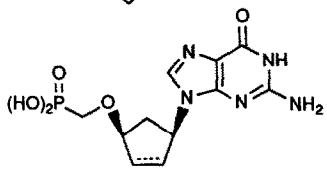
The nucleotide analogue 9-[2-(phosphonomethoxy)ethyl]guanine (**1**, PMEG) is an exceptionally potent antiviral agent which has demonstrated in vitro activity against both herpesviruses and retroviruses.[3,4] In vivo, PMEG has been shown to have activity against herpes simplex virus types 1 and 2 at doses as low as 0.1 mg/kg/day; however, toxicity is observed at doses higher than 5 mg/kg/day, giving PMEG a limited margin of selectivity.[4] As part of our efforts to improve upon the selectivity of PMEG,[5] we have prepared related guanine derivatives **2** - **5** in which the acyclic chain connecting the phosphonomethoxy group and guanine base is replaced with a carbocyclic ring. An important feature of this approach is that incorporation of the ring into the PMEG skeleton provides conformational constraints which are not present in the acyclic system. Furthermore, there is ample precedent in the nucleoside area that introduction of a carbocyclic ring offers a viable strategy for generating nucleoside mimics with a broad spectrum of antiviral activity.[6] The 1,2-substituted cyclopentane derivatives **2** and **3** were chosen as synthetic targets in order to explore the orientation



1



2: *trans*-isomer
3: *cis*-isomer

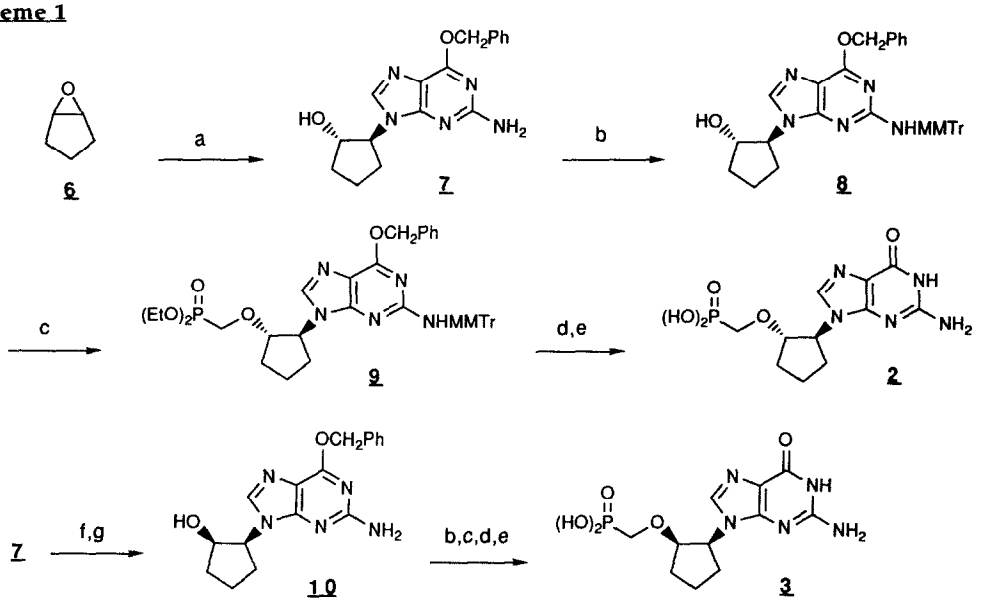


4: saturated
5: unsaturated

requirements of the phosphonomethoxy group relative to the base without altering the chain length between these key groups. In the 1,3-substituted cyclopentane derivative **4**, the guanine and phosphonate groups are oriented in the same relative configuration as the base and phosphate substituents in naturally-occuring guanosine nucleotides. The related unsaturated derivative **5** was also prepared.[7] This phosphonate derivative is an isosteric, isoelectronic analogue of the monophosphate of carbovir,[8] a carbocyclic nucleoside analogue which has received much attention as an anti-human immunodeficiency virus agent.

The synthesis of the 1,2-substituted cyclopentane derivatives **2** and **3** is outlined in Scheme 1. The ring-opening reaction of cyclopentane epoxide (**6**) with the lithium salt of 6-*O*-benzylguanine provided the N^9-alkylated *trans*-alcohol **7** in 43% yield, along with a 22% yield of the more polar N^7-isomer. Introduction of the phosphonomethoxy group on the secondary

Scheme 1



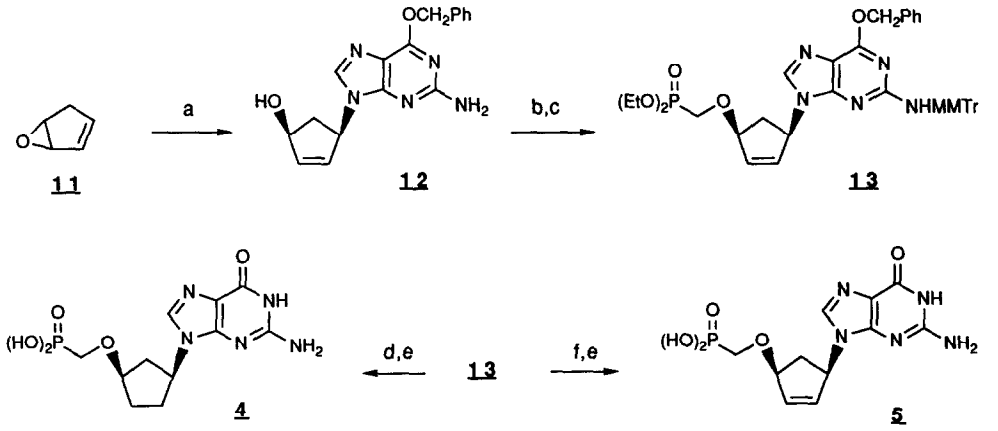
MMTr = monomethoxytrityl, (*p*-OMe-C_6H_4)Ph_2C-

(a) 6-*O*-benzylguanine/LiH, DMF, 60 °C (43% N^9-isomer, 22% N^7-isomer); (b) Et_3N, (*p*-MeO-C_6H_4)Ph_2CCl, 4-dimethylaminopyridine, CH_2Cl_2, rt (98%); (c) 2 equiv NaH, DMF, 80 °C, then 1.5 equiv $(EtO)_2P(O)CH_2OTs$, rt (72%); (d) 20% Pd(OH)2-C, 1:1 ethanol/cyclohexene, then 80% CH_3CO_2H, 60 °C (90%); (e) 10 equiv $(CH_3)_3SiBr$, DMF, rt (66%); (f) $PhCO_2H$, Ph_3P, diethylazodicarboxylate, THF (98%); (g) K_2CO_3, MeOH (98%).

hydroxyl required initial protection of the free amine in order to prevent the formation of significant amounts of bis-alkylated material. Thus, reaction of 7 with monomethoxytrityl chloride and triethylamine gave the protected guanine derivative 8 in quantitative yield. Generation of the sodium alkoxide of 8, followed by addition of diethyl (p-toluenesulfonyloxy)-methylphosphonate,[9] then provided phosphonate 9 in 72% yield. Sequential deprotection of the guanine base and the phosphonic acid moiety gave *trans*-cyclopentane derivative 2.[10] Access to the *cis*-isomer 3 was achieved by inversion of the hydroxyl group in 7 under Mitsunobu conditions. Basic hydrolysis of the intermediate benzoate afforded the *cis*-alcohol 10, which was converted to 3[10] as described for transformation of 7 to 2. Yields for each step were similar to those obtained in the *trans* series.

The key step in the preparation of the 1,3-cyclopentane derivatives 4 and 5 involved the palladium-catalyzed ring opening[11] of cyclopentadiene epoxide (11) in the presence of 6-O-benzylguanine (Scheme 2). This reaction proceeded with high regio- and stereoselectivity to provide the cyclopentene derivative 12 in which the guanine base was introduced in a 1,4-relationship *cis* to the hydroxyl group. In addition, only the N^9-alkylated guanine isomer was obtained. Introduction of the phosphonomethoxy group was carried out as previously described to provide intermediate 13. Hydrogenolysis of 13 in the presence of acetic acid gave a

Scheme 2



(a) 6-O-benzylguanine, $Pd(PPh_3)_4$, PPh_3, DMF. 80 °C (77%, N^9-isomer only); (b) Et_3N, DMAP, (p-MeO-C_6H_4)Ph_2CCl, CH_2Cl_2, rt (92%); (c) 2 equiv NaH, DMF, 80 °C, then 1.5 equiv $(EtO)_2P(O)CH_2OTs$, rt (57%); (d) 20% $Pd(OH)_2$-C, 1:1 ethanol/cyclohexene, CH_3CO_2H, reflux (80%); (e) 10 equiv $(CH_3)_3SiBr$, DMF, rt (80-90%); (f) 80% CH_3CO_2H, 60 °C (90%).

saturated cyclopentane intermediate, which was then treated with bromotrimethylsilane to provide phosphonic acid **4**.[10] Conversion of **13** to the unsaturated cyclopentene derivative **5**[10] was achieved by treatment with acetic acid to effect removal of the guanine protecting groups, followed by subsequent deprotection of the phosphonic ester.

Compounds **2** - **5** were evaluated for antiviral activity against herpes simplex virus type 2 (HSV-2) and human immunodeficiency virus (HIV) (Table 1). Whereas the 50% inhibitory concentration (IC_{50}) for PMEG is 0.7 ug/mL against HSV-2, all of the carbocyclic derivatives of PMEG were inactive at concentrations up to 100 ug/mL. This result was somewhat surprising since both 1'- and 2'-methyl substituted derivatives of PMEG have been shown to retain significant anti-HSV 2 activity relative to PMEG (IC_{50} values of 3 and 11-25 ug/mL, respectively), indicating that branching on the chain connecting the phosphonomethoxy group and the guanine base is tolerated in the acyclic nucleotide series.[5] Apparently, introduction of both substituents onto the PMEG skeleton results in a cumulative decrease in activity against HSV 2. Against HIV, the *cis*-1,2-substituted cyclopentane isomer **3** showed moderate activity (IC_{50} = 20 ug/mL), while the *trans*-derivative **2** was inactive. This result indicates that the relative configuration of the guanine and phosphonate groups is important, at least in terms of activity versus HIV, and that the key groups prefer to be *cis* to one another as in natural nucleoside derivatives. In the 1,3-cyclopentyl series, the unsaturated derivative **5** displayed modest potency against HIV, although the corresponding saturated derivative **4** was inactive. It should be noted that compounds **2** - **5** are racemic, and it is likely that only one enantiomer will be responsible for the antiviral effect. In fact, we have prepared both enantiomers of the cyclopentene derivative **5**, and found that the isomer having the (1R,4S)-configuration has an IC_{50} of 26 uM against HIV, while its enantiomer is devoid of activity.[12] The configuration of the active enantiomer corresponds to the configuration of naturally-occuring nucleosides and to the active isomer of carbovir.[8]

Table 1

	IC_{50} (ug/mL)	
Compound	HSV-2 (vero cells)	HIV (CEM cells)
2	>100	>100
3	>100	20
4	>100	>100
5	>100	66
1 (PMEG)	0.7	0.2

An explanation for the diminished activity of these compounds is complicated by the fact that most nucleoside and nucleotide analogues must be sequentially metabolized to the corresponding triphosphate analogue in order to inhibit the target viral polymerase. Although the monophosphate analogues described here bypass the need for the first phosphorylation step, they must still be converted by cellular kinases to the triphosphate analogue.[13] Thus, the lack of activity may be due to inefficient phosphorylation or to an inability of the triphosphate to inhibit the viral enzyme. Taken together, our results indicate that modification of the PMEG skeleton by introduction of the carbocyclic ring results in a significant reduction in activity relative to the parent compound such that no increase in selectivity is realized.

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