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SYNTHESIS AND ANTI-HCMV ACTIVITY OF 9-[[(ETHOXYHYDROXY-PHOSPHINYL)METHOXY]METHOXY]GUANINE, AN ISOSTERE OF PMEG MONOETHYL ESTER

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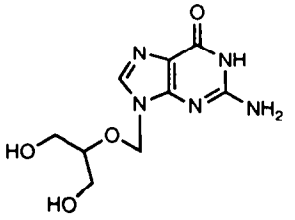
Abstract : The synthesis and anti-HCMV activity of novel 9-[[(ethoxyhydroxyphosphinyl)-methoxy]methoxy]guanine **5**, an isostere of PMEG monoethyl ester, are described. It has been demonstrated that phosphonate **5** showed comparable or greater anti-HCMV activity than ganciclovir against the AD-169 and Davis strains in tissue culture.

Human cytomegalovirus (HCMV) is one of the most important pathogens in immunologically immature or compromised hosts such as neonates, organ transplant recipients, cancer patients, and AIDS patients.[1] So far, only 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG, ganciclovir, **1**) and the trisodium salt of phosphonoformic acid (PFA, foscarnet, **2**) have been approved for the treatment of HCMV infections.[2] Ganciclovir has been found to be one of the most potent inhibitors of HCMV.[2] However, prolonged use of ganciclovir is often associated with serious side effects such as anemia and neutropenia.[3] Moreover, virus resistance to ganciclovir often develops during treatment.[4] It seems, therefore, still imperative to find novel chemotherapeutic agents active against HCMV, preferably, through a different mechanism of action.



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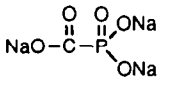

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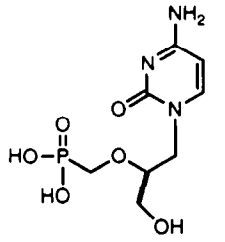

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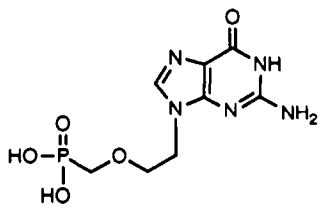

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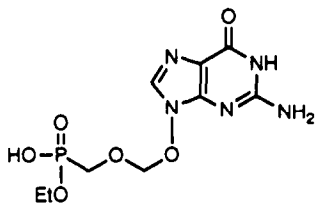
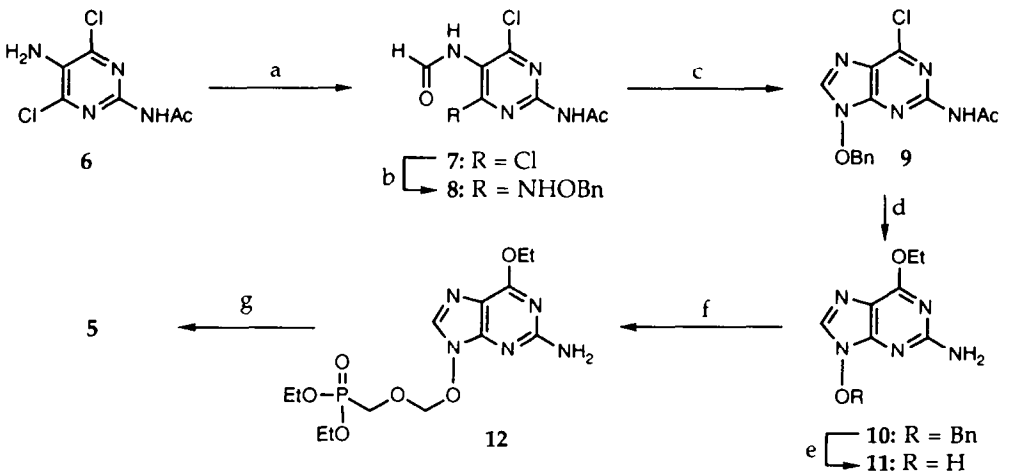


5

Recently, metabolically and chemically stable acyclic nucleoside phosphonate analogues, (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC, 3)[5] and 9-[2-(phosphonomethoxy)ethyl]guanine (PMEG, 4)[6] have been reported as potent and selective inhibitors against various DNA virus infections including HCMV. Since these phosphonate analogues are structural mimics of acyclic nucleoside monophosphates, they can bypass the initial enzymatic phosphorylation. Consequently, these compounds are highly effective against HCMV which does not encode a thymidine kinase and thymidine kinase-deficient strains of herpes simplex virus (HSV) and varicella zoster virus (VZV).[5] While HPMPC elicits a long-lasting antiviral effect against CMV *in vitro*[7] and *in vivo*[8], it has been observed that systemic administration of HPMPC was toxic to guinea pigs[9] and humans[10]. Although PMEG exhibits excellent antiviral activity against CMV, it also has high cellular toxicity. Thus, overall therapeutic index of PMEG did not exceed that of HPMPC.[9] Recently, Reist *et al.*[11] reported that the monoethyl esters of acyclic nucleoside phosphonates displayed comparable or even better activity against CMV than the corresponding diacid. The ethyl ester bond appears to be relatively stable and is cleaved only slowly *in vivo*, thus suggesting that the monoethyl ester of phosphonates could exert an antiviral effect without cleavage of the ester group.[12] The 3'-oxygen atom in the acyclic chain of PMEG has been demonstrated to play a crucial role for the enzymatic phosphorylation and thus for antiviral activity.[6] Harnden *et al.*[13] reported that an acyclic *N*-*O* linked nucleoside, 9-(3-hydroxypropoxy)guanine, had potent and selective anti-herpesvirus activity. Based on these findings, 9-[[(ethoxyhydroxyphosphinyl)methoxy]methoxy]guanine **5**, an isostere of PMEG monoethyl ester, has been designed to examine the role of an additional oxygen atom adjacent to the guanine base in anti-HCMV activity. The monoethyl ester **5** would be less polar than the corresponding diacid, therefore, could possibly penetrate the cell membrane more easily.

Scheme I[a]



[a](a) HCO_2H, Ac_2O, rt, 20 h; (b) $BnONH_2$·HCl, diisopropylethylamine, diglyme, 100 °C, 3 h; (c) (i) diethoxymethyl acetate, 120 °C, 2 h, (ii) NH_4OH, MeOH, rt, 1.5 h; (d) EtONa, EtOH, reflux, 1.5 h; (e) 10% Pd-C, H_2 (50 psi), EtOH, rt, 1.5 h; (f) diethyl chloromethoxymethanephosphonate, K_2CO_3, DMF, rt, 15 h; (g) 2*N* NaOH, 90 °C, 12 h

2-Acetamido-5-amino-4,6-dichloropyrimidine **6**, which was readily prepared from commercially available 2-amino-6-chloro-4-pyrimidinol in four steps according to a published procedure[14], was treated with formic acid in acetic anhydride to afford 2-acetamido-4,6-dichloro-5-formamidopyrimidine **7**[15] in quantitative yield. Replacement of one chloro atom of 4,6-dichloropyrimidine **7** was accomplished by treatment with *O*-benzylhydroxylamine hydrochloride in the presence of diisopropylethylamine in diglyme to give 2-acetamido-4-benzyloxyamino-6-chloro-5-formamidopyrimidine **8**[16] in 71% yield. Closure of the imidazole ring by heating at 120 °C with diethoxymethyl acetate followed by treatment with concentrated aqueous ammonia in methanol then gave 2-acetamido-9-benzyloxy-6-chloropurine **9**[17] in 80% yield. 6-Ethoxypurine **10**[18] was obtained from 6-chloropurine **9** by treatment with sodium ethoxide in refluxing ethanol in 88% yield. Reductive hydrogenation of 9-benzyloxypurine **10** in the presence of 10% palladium on activated carbon in an alcoholic medium afforded 2-amino-6-ethoxy-9-hydroxypurine **11**[19] in quantitative yield. Potassium salt of 9-hydroxypurine **11** was reacted with diethyl chloromethoxymethanephosphonate, *in situ* prepared from diethyl hydroxymethanephosphonate by chloromethylation with paraformaldehyde and hydrogen chloride in dichloromethane[20], in DMF to give 2-amino-9-[(diethylphosphono)methoxy]methoxy-6-ethoxypurine **12**. Because of difficulty of isolation of pure **12** from diethyl hydroxymethanephosphonate, the partially purified mixture was subsequently subjected to hydrolysis with 2*N* NaOH to afford the monoethyl phosphonate **5**[21] in 20% two step yield after purification by preparative HPLC on a C-18 reverse-phase bonded silica cartridge with water as the mobile phase.

Table I. Anti-HCMV Activity of Acyclic Nucleoside Phosphonates in Tissue Culture[a]

	EC_{50} (μg/mL)[b]		CC_{50} (μg/mL)[c]	TI[d]	
compound	AD-169	Davis	HEL 299	AD-169/HEL 299	Davis/HEL 299
5	1.8	0.6	>400	>200	>700
ganciclovir	1.9	0.8	>400	>200	>500

[a]Values are the mean of at least two independent experiments run in triplicates. [b]Concentration required to inhibit virus-induced CPE by 50% of the virus-infected control. [c]Concentration required to reduce the O.D. value by 50% of control. [d]Therapeutic index (ratio of CC_{50} to EC_{50}).

The antiviral activity of phosphonate **5** along with ganciclovir has been evaluated against two strains of HCMV, AD-169 and Davis, by CPE (cytopathic effect) inhibition assay.[22] Cytotoxicity of these compounds against HEL 299 (human embryonic lung fibroblast) cells have been also tested by MTT dye reduction method.[23] The phosphonate **5** showed therapeutic indices of >200 and >700 with EC_{50} values of 1.8 and 0.6 μg/mL against AD-169 and Davis, respectively, greater than, or comparable to ganciclovir in antiviral activity. On the basis of this excellent anti-HCMV activity *in vitro*, extensive *in vivo* studies of **5** against CMV are currently under way.

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15. **7** : mp 226 °C; ^{1}H NMR (DMSO-d_6) δ 2.17 (s, 3 H, $COCH_3$), 8.32 (s, 1 H, CHO), 10.23 (s,1 H, N*H*Ac), 11.16 (s, 1 H, N*H*CHO); ^{13}C NMR (DMSO-d_6) δ 24.88, 121.66, 154.57, 160.06, 160.85, 169.39.
16. **8** : mp 192.7-192.9 °C; ^{1}H NMR (DMSO-d_6) δ 2.12 (s, 3 H, $COCH_3$), 5.01 (s, 2 H, CH_2Ph), 7.52-7.70 (m, 5 H, Ar), 8.10 (s, 1 H, CHO), 9.26 (s, 1 H, N*H*OBn), 11.71 (s, 1 H, N*H*Ac), 12.19 (s, 1 H, N*H*CHO); ^{13}C NMR (DMSO-d_6) δ 23.85, 75.20, 108.46, 127.68, 128.32, 129.20, 137.74, 143.09, 147.36, 155.26, 159.53, 173.91.
17. **9** : mp 200.8-200.9 °C; ^{1}H NMR (DMSO-d_6) δ 2.24 (s, 3 H, $COCH_3$), 5.45 (s, 2 H, CH_2Ph), 7.41-7.51 (m, 5 H, Ar), 8.52 (s, 1 H, H-8), 10.93 (s, 1 H, N*H*Ac); ^{13}C NMR (DMSO-d_6) δ 24.57, 81.14, 123.06, 128.57, 129.43, 129.87, 133.18, 142.26, 148.26, 149.80, 152.34, 168.67.
18. **10** : mp 141-141.6 °C; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, *J* = 7.2 Hz, 3 H, OCH_2CH_3), 4.54 (q, *J* = 7.2 Hz, 2 H, OCH_2CH_3), 5.13 (s, 2 H, NH_2), 5.29 (s, 2 H, CH_2Ph), 7.16 (s, 1 H, H-8), 7.28-7.39 (m, 5 H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.42, 62.82, 80.85, 111.91, 128.80, 129.61, 129.80, 133.40, 135.55, 149.31, 159.67, 161.44.
19. **11** : mp 194-195 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.37 (t, *J* = 6.9 Hz, 3 H, OCH_2CH_3), 4.46 (q, *J* = 6.9 Hz, 2 H, OCH_2CH_3), 6.44 (s, 2 H, NH_2), 7.96 (s, 1 H, H-8), 11.66 (br s, 1 H, OH); ^{13}C NMR (DMSO-d_6) δ 14.84, 62.28, 110.86, 137.11, 150.69, 160.24, 160.84.
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21. **5** : mp 192.5-193.5 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.09 (t, *J* = 6.9 Hz, 3 H, OCH_2CH_3), 3.71-3.81 (m, 4 H, OCH_2CH_3 and OCH_2P), 5.21 (s, 2 H, NH_2), 6.89 (s, 2 H, OCH_2O), 8.03 (s, 1 H, H-8), 11.31 (br s, 1 H, NH); ^{13}C NMR (DMSO-d_6) δ 16.93 (d, *J* = 5.8 Hz, $POCH_2CH_3$), 59.10 (d, *J* = 5.5 Hz, $POCH_2CH_3$), 65.27 (d, *J* = 151.4 Hz, OCHP), 101.01 (d, *J* = 8.3 Hz, OCH_2O), 112.60, 134.57, 134.60, 146.93, 154.34, 156.90.
22. HEL 299 cells (ATCC CCL 137) in stationary phase were infected with virus at an M.O.I. (multiplicity of infection) of 2-4 $CCID_{50}$ (50% cell culture inhibitory dose) per well of 96-well plates. After a 2 h adsorption period at 37 °C in 5% CO_2 incubator, the liquid was aspirated off and 100μL of Dulbecco's modified Eagle medium (DMEM) (GIBCO)/2% fetal bovine serum (FBS) (GIBCO) containing a compound was applied to each well in triplicate for each concentration. After 7 days of incubation at 37 °C in 5% CO_2 incubator, Giemsa staining was performed. The antiviral activity was measured by microscopic observation of CPE and expressed as EC_{50}, a concentration of the compound required to inhibit virus-induced CPE by 50%.
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