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Synthesis and Antiviral Activity of Acyclovir-5′-(Phenyl Methoxy Alaninyl) Phosphate as a Possible Membrane-Soluble Nucleotide Prodrug

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Abstract—We describe a synthesis of acyclovir-5′-(phenyl methoxy alaninyl) phosphate (**2**) from acyclovir (**1**). This compound was designed to act as a lipophilic, membrane-soluble prodrug of the free nucleotide. However, the biological activities of this derivative against a range of viruses indicated poor intracellular phosphate delivery, in marked contrast to the earlier successful delivery of several dideoxy anti-HIV nucleotides.

An important goal of medicinal chemistry is to continue to strive for improvements in current antiherpetics such as, acyclovir (**1**).[1] Although acyclovir, and recent analogues and prodrugs, make an important contribution to the therapy of herpes infections, they do have some limitations. Thus, **1** is somewhat less active against varicella Zoster Virus (VZV) and greatly less active against human cytomegalovirus (HCMV) than against herpes simplex (HSV), it has a low oral bioavailability, and herpetic mutations which are resistant to the compound have emerged in the clinic.[2] The replication of acyclovir resistant mutants is inhibited in the clinic using foscarnet, a pyrophosphate analogue which interacts with the pyrophosphate binding site of viral DNA polymerase and does not require activation by a herpes virus encoded enzyme.[3,4] Also, enhanced acyclovir bioavailability is provided by the L-valyl ester, valaciclovir, which is cleaved in the gut and the liver by valaciclovir hydrolase.[5] The bioavailability of acyclovir is also enhanced by 5′-hydroxyethoxymethyl-9-[2,6-diaminopurine] and 5′-hydroxyethoxymethyl-9-[2-amino-6-chloropurine] which are converted to acyclovir by adenosine deaminase.[6]

We have previously prepared masked phosphate prodrugs of the anti-HIV agents 3′-azido-3′-deoxythymidine[7] (zidovudine) and 3′-deoxy-2′,3′-didehydrothymidine[8] (d4T) as lipophilic membrane-soluble nucleotide prodrugs. The 5′-blocked phosphate derivatives are more lipophilic than the parent nucleosides and may 'bypass'[9] the nucleoside kinase-mediated 5′ phosphorylation step, which is normally required for biological activity. In particular, the 5′-(phenyl methoxy alaninyl) phosphate derivatives of d4T were found to be potent against HIV, non-toxic to uninfected cells, and to act in a nucleoside kinase-independent fashion.[10] We herein report the preparation of acyclovir-5′-(phenyl methoxy alaninyl) phosphate (**2**), intending this product to act as an intracellular nucleotide delivery motif, thus being nucleoside kinase-independent. An important feature of acyclovir is that HSV and VZV thymidine kinase enzymes convert the parent drug to the corresponding monophosphate, whereas the more specific host TK will not.[11] It is therefore possible that a successful intracellular delivery of the monophosphate may lead to a reduced antiviral selectivity in these cases; however, this would not be so for HCMV for example where potency should be significantly enhanced, and furthermore activity should also be increased against TK-altered, or TK-deleted acyclovir resistant HSV.

The methodology developed by us for the preparation of 5′-phosphoramidate derivatives from zidovudine[7] and d4T[8] failed for acyclovir, due to the insolubility of the nucleoside in the optimal solvent (tetrahydrofuran).

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The solubility of the nucleoside was enhanced sufficiently however by protecting the guanine base of acyclovir using *N*,*N*-dimethylformamide dimethyl acetal[12] (98%) and the N^2-DMF protected acyclovir was thus successfully phosphorylated (51%). The DMF protecting group was then removed (90%), by refluxing in propanol, to generate the intended product (**2**)[13] as a 6:5 mixture of phosphate diastereoisomers (Fig. 1) in 45% overall yield. An alternative synthesis using the method of Uchiyama[14] gave **2** from unprotected **1** in one-step, albeit in reduced yield (11%).

The biological activities of acyclovir-5′-(L-methoxy alaninyl phenyl) phosphate (**2**) against herpes simplex virus type 2 (HSV2), HCMV, and VZV were measured, by comparison to acyclovir (**1**) (Table 1).

The data indicates that (**2**) is roughly equi-active with acyclovir against HCMV and VZV. Thus, either acyclovir or acyclovir-5′-monophosphate may have been generated from **2** in the HCMV and VZV assays; assays of **2** against TK-deficient strains of virus may confirm which of these possible metabolites contributes most to the observed activity. However, it is notable that the phosphoramidate (**2**) is inactive against HSV-2, in contrast to the moderate activity noted for acyclovir (**1**). There are several explanations for this inactivity of the pro-drug. Firstly, cellular uptake of **2** may be poor in the HSV-2 infected cells, as these (Vero) differ from those of the HCMV and VZV assays (MRC-5). Alternatively, some of the required enzymes for activation of the pro-drug (e.g. carboxyl esterase) may be poorly active in the Vero cell line, or may have altered substrate specificity such that **2** is poorly processed.

The generally poor antiviral data for **2** contrast sharply with the high potency and selectivity when this phosphoramidate pro-drug motif was applied by us to the anti-HIV agents d4T,[10] ddA[16] and d4A.[17] This was considered to correspond to differences in the required activating enzymes from cell to cell and/or compound to compound. In order to further probe the unusual antiviral profile noted in Table 1, and in particular the surprisingly low anti-HSV-2 activity of **2**, we carried out several further biochemical studies. Firstly, carrying out the HSV-2 assay in MRC-5 cells (as used for the VZV and CMV assays). Here we noted some activity (EC_{50} 61 μM), but considerably less than that noted for acyclovir (**1**). Furthermore, pre-incubation of **2** with

Table 1.[15]

	EC_{50} (μM) HCMV	EC_{50} (μM) HSV2	EC_{50} (μM) VZV	CC_{50} (μM)
	MRC5	Vero	MRC5	Vero
1	50	3	5–20	>500
2	12	≥100	16	>500

MRC-5 cells for 4 days prior to viral inoculation lead to no enhancement in antiviral activity; further indicating that cellular delivery was not a problem with these compounds (as indeed their high lipophilicities would have predicted).

To pursue further the origins of the relatively poor antiviral efficacy of **2** we attempted to probe the possible enzyme-mediated hydrolysis of this compound. Thus, we incubated compound **2** and the previously reported d4T analogue[10] which has notable anti-HIV potency, with pig liver esterase. We have recently noted that the activation of such pro-drugs to their free amino acyl phosphoramidates to be an apparently necessary condition for antiviral action, and this simple assay may therefore have a valuable predictive role.[18] After 21 h incubation,[19] both **2** and the analogous d4T derivative were noted to be almost entirely processed to more polar species. Indeed, isolation of crude products from the incubation of **2** revealed only one major compound, characterised as the amino acyl phosphoramidate (**3**) (Fig. 2). We suggest that this arises through carboxyl esterase cleavage of **2** to the intermediate **4**, which spontaneously hydrolyses under the conditions of the assay to generate **3**.

The novel compound **3** was characterised by P-31 NMR, where the achirality of the phosphate centre is apparent from the loss of 'doublet' structure notable for chiral phosphoramidates such as **2**. Mass spectral data also confirmed the molecular mass of **3**.

Thus, in conclusion, we note that the phenyl methoxy alaninyl phosphoramidate of acyclovir (**2**) is readily prepared using phosphorochloridate chemistry, on a *N*-protected nucleoside. The compound is efficiently processed by carboxyl esterase to give the novel amino acyl phosphoramidate (**3**), which might be regarded as evidence for likely intracellular nucleotide release, and antiviral action. However, in a range of herpes virus

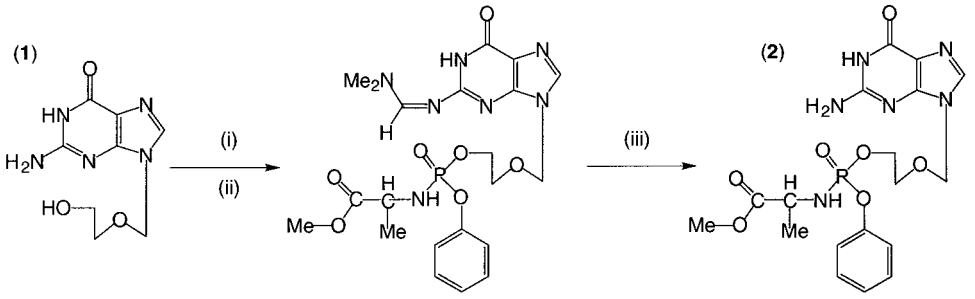
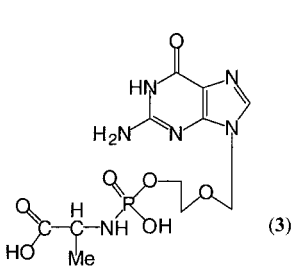
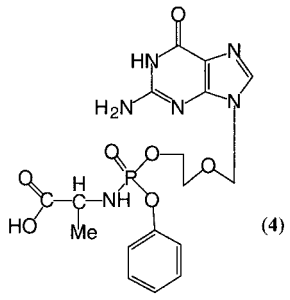


Figure 1. Reagents and conditions: (i) $Me_2NCH(OMe)_2$/DMF; (ii) (PhO)(L-MeOC(O)CHMeNH)P(O)Cl, NMI/THF; (iii) Δ/nPrOH.



(3)



(4)

Figure 2.

assays, compound **2** revealed only moderate antiviral action, and was certainly not significantly superior to acyclovir itself. This was considered to be due to the compound in this study **2** being a poor substrate for the enzymes that initiate activation of such phosphoramidate pro-drugs, or that such enzymes may be absent, or present in only low activity, in the cell lines in this study. However, the (slight) enhancement in the anti-HCMV activity of **2** over **1** indicates that there may be some interest in attempts to further increase the potency of the phosphoramidate by tuning of the pro-drug structure.

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References and Notes

1. Easterbrook, P.; Wood, M. J. *J. Antimicrob. Chemother.* **1994**, *34*, 307.
2. Ehrlich, K. S.; Mills, J.; Chatis, P. *N. Engl. J. Med.* **1989**, *320*, 293.
3. Mao, J. C. H.; Robishaw, E. E.; Overby, L. R. *J. Virol.* **1975**, *15*, 1281.
4. Safrin, S.; Berger, T. G.; Gilson, I.; Wolfe, P. R.; Wofsy, C. B.; Mills, J.; Byron, K. K. *Ann. Intern. Med.* **1991**, *115*, 19.
5. Weller, S. I.; Blum, M. R.; Doucette, M.; Burnette, T.; Cederberg, D. M.; De Miranda, P. *Clin. Pharmacol. Therapeut.* **1993**, *54*, 595.
6. Robins, M. J.; Hatfield, P. W. *Can. J. Chem.* **1982**, *60*, 547.
7. McGuigan, C.; Devine, K. G.; Nicholls, S. R.; O'Connor, T. J.; Kinchington, D. *Antiviral Res.* **1991**, *15*, 255.
8. McGuigan, C.; Cahard, D.; Sheeka, H. M.; De Clercq, E.; Balzarini, J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1183.
9. McGuigan, C.; Cahard, D.; Sheeka, H. M.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **1996**, *39*, 1748.
10. Balzarini, J.; Karlsson, A.; Aquaro, S.; Perno, C.-F.; Cahard, D.; Naesens, L.; De Clercq, E.; McGuigan, C. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 7295.
11. Elion, G. B.; Furman, P. A.; Fyfe, J. A.; De Miranda, P.; Beauchamp, L.; Schaeffer, H. J. *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 5716.
12. Zemlicka, J.; Holy, A. *J. Chem. Soc., Chem. Commun.* **1967**, *32*, 3159.
13. Selected data for (**2**): *denotes major diastereoisomer: δp (DMSO-d_6) 5.0*, 4.8 (6:5); δ_H (DMSO-d_6) 10.62 (1 H, s, NH), 7.82*, 7.81 (1H, s, H-8), 7.37–7.12 (5H, M, Ph), 6.51 (2H, s, NH_2), 5.98–5.90 (1H, m, AlaNH), 5.37*, 5.35 (2H, s, H-1′), 4.11–4.02 (2H, m, H-5′), 3.82–3.78 (1H, m, AlaCH), 3.70–3.61 (2H, m, H-4′), 3.58, 3.56* (3H, s, OMe), 1.21–1.16 (3H, m, AlaMe); δ_c (DMSO-d_6) 177.3–177.1 (m, AlaCO), 160.3 (C6), 157.4 (C2), 154.9 (C4), 154.2*, 154.1 (d, J_{PC} = 3.5 Hz, 3.5 Hz, ipsoPh), 141.2 (C8), 133.1 (*meta*Ph), 128.0 (*para*Ph), 123.7, 123.6* (*ortho*Ph), 120.5 (C5), 75.4 (C1′), 71.1–71.0 (m, C4′), 68.5–68.4 (m, C5′), 55.4, 55.3* (AlaCH), 53.3*, 53.1 (AlaCH), 23.2–23.0 (m, AlaMe); LA FAB MS (thioglycerol matrix) *m/e* 489 (MNa^+ 40%), 467 (MH^+, 32%), 316 (MH^+ −base, 100%), 260 (MeOAlaPhOP(O)OH_2^+ 87%), 200 (260–HCO_2Me, 45%), 152 (base H^+, 79%), 77 (Ph^+, 40%); HR EI MS *m/e* MNa^+ obtained 489.1268, $C_{18}H_{23}N_6O_7NaP$ requires 489.1264; HPLC retention time (min) 20.79, 21.78 (overlapping signals) ACS quaternary system, using an Ultratech ODS2 5 μM 250×4.6 mm column, eluent A = water, B = acetonitrile, linear gradient conditions 82% A 0 min, 82% A 10 min, 20% A 30 min, 20% A 45 min, flow rate 1.00 mL/min); lR (cm^{-1}) 3390, 3054, 2985, 2833, 2361, 2307, 1740, 1686, 1654, 1605, 1540, 1490, 1457, 1420, 1377, 1345, 1265, 1213, 1153, 1062, 936, 897.
14. Uchiyama, M.; Aso, Y.; Noyori, R.; Hayakawa, Y. *J. Org. Chem.* **1993**, *58*, 373.
15. EC_{50} = the drug concentration (μM) required to reduce viral antigen production by 50%. CC_{50} = the concentration (μM) which reduces the viability of uninfected cells by 50%. See, for example: Rahim, S. G.; Trivedi, N. T.; Bogunovic-Batchelor, M. V.; Hardy, G. W.; Mills, G.; Selway, J. W. T.; Snowden, W.; Littler, E.; Coe, P. L.; Basnak, I.; Whale, R. F.; Walker, R. T. *J. Med. Chem.* **1996**, *39*, 789.
16. Balzarini, J.; Kruining, J.; Wedgwood, O.; Pannecouque, C.; Aquaro, S.; Perno, C. F.; Naesens, L.; Witvrouw, M.; Heijtink, R.; De Clercq, E.; McGuigan, C. *FEBS Letts.* **1997**, *410*, 324.
17. McGuigan, C.; Wedgwood, O. M.; De Clercq, E.; Balzarini, J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2359.
18. McGuigan, C.; Tsang, H.-W.; Sutton, P. W.; De Clercq, E.; Balzarini, J. *Antiviral Chem. Chemother.* **1998**, *9*, 109.
19. Esterase assay conditions: compound (**2**) (5.0 mg, 10.7 μM) was solubilised in acetone (0.1 mL) and 0.05 M pH 7.6 TRIZMA buffer (1 mL, made up in D_2O), and then exposed to pig liver esterase (40 mg, E.C. 3.1.1.1, Sigma UK, activity 19 units/mg solid). The mixture was transferred to a 5 mm NMR tube, incubated at 37 °C for 21 h, and then evaluated by P-31 NMR. The mixture was diluted with water (10 mL) and extracted with chloroform (2×10 mL). The combined organic layers were further extracted with water (10 mL) and then reduced to dryness. The combined aqueous layers were freeze dried. Aq phase: δp (D_2O) 8.52; MS (ES^-): *m/z* 258 (100%), 375 ($M-H^+$, 50%). Org phase: δp: no signals.