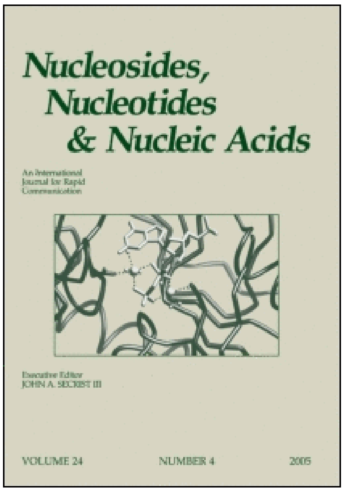

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SYNTHESIS AND ANTIVIRAL ACTIVITY OF THE 3-DEAZA ANALOGUE of 9-((1,3-DIHYDROXY-2-PROPOXY)METHYL)GUANINE[1]

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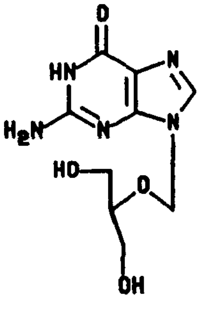
Abstract. The synthesis of 3-deaza-DHPG **2** and its positional isomer **10** via the acid catalysed fusion of imidazole **3** and acetoxymethylglycerol **4** is described. Analogue **2** has activity against herpes simplex viruses and cytomegalovirus.

INTRODUCTION

We[5] and others[6] have previously reported on the synthesis and antiviral activity of 9-((1,3-dihydroxy-2-propoxy)methyl)guanine (**1**, DHPG, ganciclovir). The selective antiviral activity of DHPG against herpes simplex virus types 1 and 2 (HSV 1 and 2) has been attributed to its preferential phosphorylation in virus infected cells by a virus specified thymidine kinase (TK).[7-9] The DHPG monophosphate thus formed is then converted by cellular kinases to the triphosphate, the active antiviral agent. Despite the absence of a virus specified kinase in cytomegalovirus (CMV) infected cells, DHPG has proven effective against this virus in vitro and in vivo[10] and is being used in the clinic for the treatment of cytomegalovirus infections in immunocompromised patients.[11] The mode of antiviral activity exhibited by DHPG against CMV, although less well understood than for herpes simplex viruses, has been examined by Freitas et al.[10] Recently, a report by Beauchamp et al.[12] described the evaluation of 1-((1,3-dihydroxy-2-propoxy)-methyl)cytosine which also possesses antiviral activity against CMV.

3-Deazaguanine is a synthetic purine analogue first described by Cook, et al. which has shown, along with a number of its glycosylated derivatives,[13-18] potent antiviral and antitumor activities. As a result of our continued interest in outlining the structure-activity requirements of compounds related to DHPG, we now describe the preparation of 6-amino-1-((1,3-dihydroxy-2-propoxy)methyl)imidazo[4,5-C]pyridin-4-one (**2**, 3-deaza-DHPG) and report on its antiviral activity.



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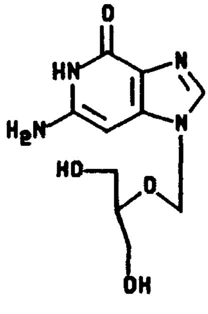

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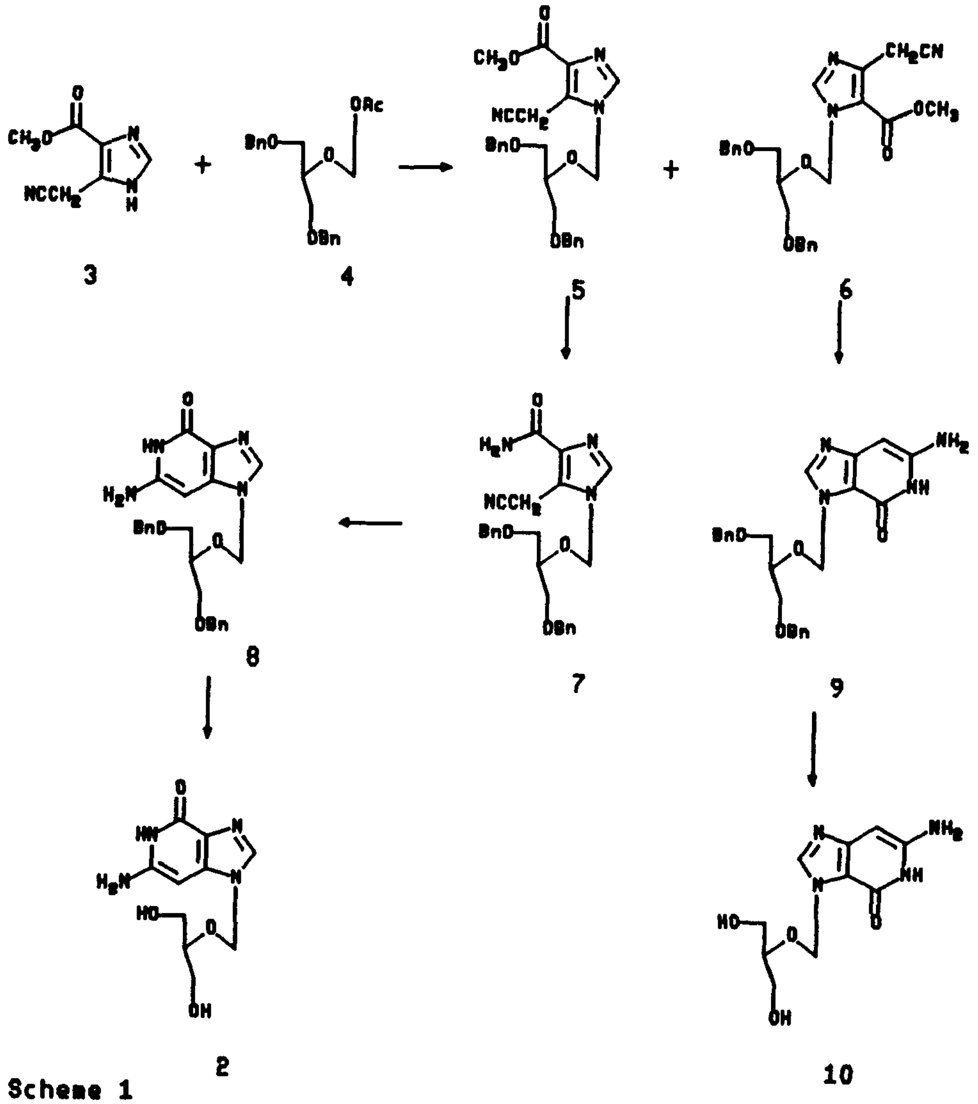
RESULTS AND DISCUSSION

Chemistry. Our approach to 3-deaza-DHPG followed closely the original strategy described by Cook and colleagues[13] for the synthesis of 3-deazaguanosine. The requisite methyl-5(4)-(cyanomethyl)-imidazole-4(5)-carboxylate (**3**) was prepared as described by Robins et al.[19] Imidazole **3** was coupled with 1,3-dibenzyl-2-acetoxymethyl-glycerol (**4**)[5] (Scheme 1) by heating a slurry of the two in a fusion type acid catalysed reaction to afford N-1 and N-3 substituted adducts **5** and **6** in 19% and 30% yield respectively (Scheme 1). The isomers were assigned on the basis of the reported characteristic ultra violet absorption spectra. The preponderance of the undesired isomer **6** is similar to the observation of others[14] under these reaction conditions involving imidazole **3**. Alternate coupling methods involving persilylated **3** and tin tetrachloride did not lead to a significant amount of desired product.



Scheme 1

Reaction of the desired adduct 5, available in 19% yield, with ammonia lead to the isolation of amide 7 in 20% yield along with 5% of the fully cyclized product 8. Similar reaction of isomer 6 with ammonia lead directly to the cyclized product 9 in 57% yield. The difference in reactivity of isomers similar to 5 and 6 with ammonia has been observed previously.[14] Amide 7 was cleanly cyclized with aqueous sodium carbonate-ethanol to the protected 3-deaza analogue 8 in 89% yield. Final deprotection of 8 via transfer hydrogenation[20] afforded 3-deaza-DHPG 2 in 66% yield.

Table 1

Antiviral and Anticellular Activities of 2 and DHPG

Virus[a] or Cell[b]	ID_{50}, uM 2	DHPG
HSV 1 (F)	1.7	0.2
HSV 1 (FΔ305)[c]	14	6
HSV 1 (MP·PAA^r)[d]	55	0.8
HSV 1 (Shealey)	1.4	0.3
HSV 2 (G)	2.1	1.3
HSV 2 (Lovelace)	1.7	0.4
HSV 2 (MS)	2.5	1.8
HCMV (AD 169)	14	5
Vero Cells	>2000	480

a) Determined by plaque reduction assays in Vero cells (for herpes simplex viruses) or MRC-5 cells (for human cytomegalovirus) The virus strain is given in parenthesis.

b) Determined by cell proliferation assays.

c) Thymidine kinase mutant virus.

d) DNA polymerase mutant virus.

Likewise the positional isomer 9 was deprotected to give the "N-7" isomer of 3-deaza-DHPG **10**.

Antiviral Activity. In plaque reduction assays, **2** was less potent than DHPG against wild type strains of herpes simplex virus types 1 and 2 and human cytomegalovirus (HCMV) (Table 1). The potency difference between **2** and DHPG varied from less than two to seventy fold. Against the thymidine kinase mutant virus herpes 1 (FΔ305), DHPG was about 2.5 fold more potent than **2**, but both were less active against this virus than the wild type virus. The greatest difference between **2** and DHPG was observed against the DNA polymerase mutant HSV 1 (MP·PAA^r). In this case, DHPG was nearly 70-fold more potent than 2. The reduced antiviral potency of 2 relative to DHPG in the aforementioned cell line is in general agreement with the observed lower cytotoxicity exhibited by 2 as evidenced by the greater than four fold difference in the inhibition of Vero cell proliferation.

Each nucleoside was tested for its ability to be phosphorylated by purified HSV 1 (F) thymidine kinase to the 5'-monophosphate. In this assay which utilized gamma-^{32}P-ATP as the phosphate donor, 2 was phosphorylated about one third as rapidly as DHPG. This indicates that 2 is a less efficient substrate than DHPG for the enzyme; therefore, 2 may be less active than DHPG in vivo because of a slower conversion to phosphorylated derivatives.

The relatively good in vitro antiviral activity of 2 and its indication of lower cytotoxicity as compared to DHPG prompted further evaluation of 2 in vivo. In the first experiment, 2 and DHPG were administered s.c. twice a day for 5 days starting 24 hours after HSV-2 (G) i.p. inoculation. In this experiment 90% of saline treated mice died. DHPG groups receiving 20 and 10 mg/kg/day had only a 30% mortality rate which was statistically significant ($P < 0.005$), whereas all mice treated with 2 (10 and 20 mg/kg) died. Although it showed acitivity similar to DHPG against HSV 2 (G) in vitro, 2 is not effective in this HSV 2 model at doses comparable to those at which DHPG gives protection.

A second experiment (Table 2) was conducted to compare 2 and DHPG against a murine cytomegalovirus infection in mice. Each compound was highly protective in this study. DHPG appeared to be slightly more potent.

We conclude that the attachment of the (1,3-dihydroxy-2-propoxy)methyl grouping to the 3-deazaguanine base has resulted in a compound with in vivo potency comparable to that of DHPG against murine cytomegalovirus, but with substantially less activity than DHPG against HSV in vivo.

Table 2

Effect of Subcutaneous Treatments Twice Daily with 2 and DHPG on a Murine Cytomegalovirus Infection in Mice

Compound	Dose[a] (mg/kg/day)	Survivors/ Total	(%)	Mean Survival Time[b] (Days)	ED_{50} (mg/Kg/day)
Saline	-	0/16	(0)	6.1 ± 0.7[c]	
DHPG	2.5	2/16	(13)	6.6 ± 1.6	
DHPG	5	9/16	(56)[d]	7.4 ± 1.7	
DHPG	10	14/16	(88)[d]	6.0 ± 0.0	5.5
DHPG	20	15/16	(94)[d]	9.0 ± 0.0	
DHPG	40	16/16	(100)[d]	>14	
DHPG	80	16/16	(100)[d]	>14	
2	10	9/16	(56)[d]	6.6 ± 1.3	
2	20	12/16	(75)[d]	7.0 ± 1.1	<10
2	40	12/16	(75)[d]	7.5 ± 1.9	
2	80	16/16	(100)[d]	>14	

a) Half-daily doses were administered sub-cutaneously twice a day for 5 days starting 6 hours after virus inoculation.

b) Of mice that died. Survivors lived through 14 days.

c) Standard deviation.

d) Statistically significant ($p<0.002$), determined by the two-tailed Fisher exact test.

EXPERIMENTAL

Nuclear magnetic resonance spectra were recorded on a Varian EM-390 (^1H NMR, 90 MHz) and a Bruker WM-300 (^1H NMR, 300 MHz; ^{13}C NMR, 75.453 MHz) and chemical shifts are reported in parts per million downfield from internal tetramethylsilane. Ultraviolet spectra were recorded on a Hewlett Packard 8450A spectrometer. Spectroscopic data and elemental analyses were obtained by Syntex

Analytical Research. All chromatographic purifications were carried out on silica gel. Melting points were determined on a hot-stage microscope and are corrected.

Methyl 5-cyanomethyl-1-((1,3-dibenzyloxy-2-propoxy)methyl)-imidazole-4-carboxylate (5). A slurry of methyl 5(4)-cyanomethyl-imidazole-4(5)-carboxylate (3) (5 g, 30.2 mmol), 1,3-dibenzyloxy-2-acetoxymethylglycerol (4)[5] (12.5 g, 36.2 mmol) and bis (p-nitrophenyl) phosphate (180 mg) was heated for 4 h at 110 °C then allowed to cool to room temperature. The reaction mixture was partitioned between dilute Na_2CO_3 and dichloromethane then dried over $MgSO_4$ and evaporated to a brown oil. Chromatography eluting with 2% methanol/dichloromethane first afforded 2.58 g (19%) of 5 as an oil; ^{1} NMR δ 7.9 (s, 1H, H2), 7.32-7.23 (m, 10H, phenyl), 5.63 (s, 2H, H-1'), 4.43 (s, 2H, CH_2CN), 3.87 (m, 1H, H-4'), 3.79 (s, 3H, CH_3), 3.49 (m, 4H, H-3', H-5'). UV λ max (EtOH) 235 nm (ε 8890); IR (neat) 2200 (CN), 1710; Anal. Calcd for $C_{25}H_{27}N_3O_5$ (449.51): C, 66.80; H, 6.05; N, 9.35. Found: C, 67.02; H, 6.11; N, 9.10. Next eluted 4.14 g (30.5%) of the N^3-isomer 6 as an oil: ^{1}H NMR δ 8.15 (s, 1H, H-2), 7.36-7.24 (m, 10H, phenyl), 5.74 (s, 2H, H-1'), 4.42 (s, 4H, benzylic), 4.07 (s, 2H, CH_2CN), 3.93 (m, 1H, H-4'), 3.79 (s, 3H, CH_3), 3.44 (m, 4H, H-3' and H-5'); UV λ max (EtOH) 240 nm (ε 15900); IR (neat) 2260 (CN), 1720. Anal. Calcd for $C_{25}H_{27}N_3O_5$ (449.51): C, 66.80; H, 6.05; N, 9.35. Found: C, 66.94; H, 6.33; N, 9.42.

5-Cyanomethyl-1-((1,3-dibenzyloxy-2-propoxy)methyl)imidazole-4-carboxamide (7). A pressure vessel containing 5 (1.4 g, 3.11 mmol) was half filled (50 mL) with liquid NH_3 and then heated for 4h at 100 °C. The vessel was then opened and the contents allowed to evaporate at room temperature. The resulting residue was triturated with acetone which was then evaporated to afford an oil. This oil was redissolved in dichloromethane and washed with H_2O , dil HCl, and the organic phase was dried ($MgSO_4$) and evaporated to an oil. Chromatography (3% methanol/dichloromethane) separated 280 mg (20 %) of 7, 398 mg (29%) of unreacted 5, and 70 mg (5%) of 8. An analytical sample of 7 was obtained by crystallization from EtOH; mp 80-81 °C; ^{1}H NMR δ 7.91 (s, 1H, H-2), 7.49 (broad s, 1H, NH_2), 7.36-7.25 (m, 11H, phenyl, NH_2), 5.60 (s, 2H, H-1'), 4.44 (s, 4H, benzylic), 4.36 (s, 2H, CH_2CN), 3.86 (m, 1H, H-4'), 3.50 (m, 4H, H-3' and H-5'); UV λ max (methanol) 230 nm (ε 9670); IR (KBr) 2250 (CN), 1680. Anal. Calcd for $C_{24}H_{26}N_4O_4$ (434.50): C, 66.34; H, 6.03; N, 12.89. Found: C, 66.22; H, 6.04; N, 12.67.

6-Amino-1-((1,3-dibenzyloxy-2-propoxy)methyl)imidazo[4,5-C]-pyridin-4-one (8). A solution of 7 (313 mg, 0.72 mmol) ethanol (20 mL) and 10% Na_2CO_3 (2 mL) was heated at reflux for 1.5 h then evaporated. The solid residue was first washed with water and the residual solids were recrystallized from hot ethanol (charcoal) to give 280 mg (89%) of 8 as a white solid; mp 183-185 °C; ^{1}H NMR δ 7.74 (s, 1H, H-2), 7.35-7.22 (m, 10H, phenyl), 5.59 (broad s, 2H, NH_2), 5.47 (s, 3H, H-1' and H-7), 4.41 (s, 4H, benzylic), 3.85 (m, 1H, H-4'), 3.46 and 3.43 (ABX, J = 7 and 9Hz, 4H, H-3' and H-5'); UV λ max (methanol) 303 nm (ε 9400), 274 (12400). Anal. Calcd for $C_{24}H_{26}N_4O_4$ (434.50): C, 66.34; H, 6.03; N, 12.89. Found: C, 66.56; H, 5.77; N, 12.70.

6-Amino-1-((1,3-dihydroxy-2-propoxy)methyl)imidazo[4,5-C]-pyridin-4-one (2). A suspension of 8 (250 mg, 0.57 mmol), 1,4-cyclohexadiene (5 mL), ethanol (20 mL) and 20% $Pd(OH)_2/C$ (200 mg) was heated at reflux for 16 h and then diluted with hot water (70 mL), filtered hot through a Celite pad, and evaporated to a light blue solid. The solid was recrystallized from H_2O to afford 97 mg (66%) of 2 in two crops as a light blue solid; mp 219-220 °C; ^{1}H NMR δ 10.3 (broad s, 1H, NH), 7.74 (s, 1H, H-2), 5.61 (broad s, 2H, NH_2), 5.45 (s, 3H, H-7 and H-1'), after D_2O added 5.50 and 5.46 respectively, 4.65 (t, J = 5.1 Hz, 2H, OH's), 3.40 (m, 5H, H-4', H-5' and H-3'); ^{13}C NMR (22.62 MHz) δ 156.60 (C-4), 147.85 (C-6), 142.98 (C-3a), 139.11 (C-2), 123.34 (C-7a), 79.26 (C-4'), 73.21 (C-1'), 70.38 (C-7), 60.86 (C-3', C-5'); UV λ max (0.1 N HCl) 313 nm (sh) (ε 6800), 285 (12100); (0.1 N NaOH) 281 (11200). Anal. Calcd for $C_{10}H_{14}N_4O_4$: C, 47.24; H, 5.55; N, 22.04. Found: C, 47.49; H, 5.58; N, 22.00.

6-Amino-3-((1,3-dibenzyloxy-2-propoxy)methyl)imidazo-[4,5-C]-pyridin-4-one (9). A pressure vessel containing 6 (2.2 g, 4.89 mmol), acetamide (400 mg) and saturated methanolic ammonia (30 ml) was kept for 24 h at 105 °C and then left for 3 days at room temperature. The vessel contents were then evaporated and the residue partitioned between dichloromethane and dil $NaHCO_3$, sat. NaCl. The organic phase dried ($MgSO_4$) and evaporated to an oil. Chromatography (5% methanol/dichloromethane) produced 1.1 g (52%) of 9 as a greenish oil; ^1H NMR ($CDCl_3$) δ 10.7 (broad s, 1H, NH), 7.82 (s, 1H, H-2), 7.33-7.24 (m, 10H, phenyl), 5.87 (s, 2H, H-1'), 5.64 (s, 1H, H-7), 4.44 (s, 4H, benzylic), 4.37 (broad s, 2H, NH_2), 4.13 (m, 1H, H-4'), 3.51 (m, 4H, H-3' and H-5'), UV λ max (methanol) 326 nm (ε 6790), 262 (7570). Anal. Calcd for $C_{24}H_{26}N_4O_4$ (434.50): C, 66.34; H, 6.03; N, 12.89. Found: C, 66.25; H, 6.21; N, 12.64.

6-Amino-3-((1,3-dihydroxy-2-propoxy)methyl)imidazo[4,5-C]-pyridin-4-one (10). A suspension of 9 (800 mg, 1.84 mmol), ethanol (80 ml), 1,4-cyclohexadiene (10 ml) and 20% Pd(OH_2/C) (700 mg) was heated at reflux for 2 h and then filtered hot through Celite. The filtrate was evaporated to a red solid which crystallized from H_2O to afford 100 mg (21%) of 10 as a tan solid; mp 222-224 °C, ^1H NMR δ 10.58 (broad s, 1H, NH), 8.07 (s, 1H, H-2), 5.71 (s, 2H, H-1'), 5.53 (s, 1H, H-7), 5.36 (broad s, 2H, NH_2), 4.66 (t, J = 5.6 Hz, 2H, OH), 3.61 (m, 1H, H-4'), 3.40 and 3.31 (after D_2O added, ABX, J = 6 and 11 Hz, 4H, H-3' and H-5'), ^{13}C NMR (22.62 MHz) 153.89, 152.82 (C-4, C-7a), 146.65, 145.22 (C-6, C-2), 113.06 (C-3a), 79.58 (C-1'), 78.05 (C-7), 73.76 (C-4'), 60.86 (C-3' and C-5'), UV λ max (0.1 N HCl) 320 nm (ε 5880), 279 (11400); (0.1 N NaOH); 311 (5660), 260 (5650). Anal. Calcd for $C_{10}H_{14}N_4O_4$ (254.25) C, 47.24; H, 5.55; N, 22.04. Found: C, 47.11; H, 5.37; N, 21.92.

Plaque and cytotoxicity assays. Experiments were conducted in Vero and MRC-5 cells as described previously.[7,10,21] The fifty percent inhibitory concentrations are defined as those causing a 50% reduction of virus plaque numbers compared to untreated controls.

Thymidine kinase assay. Thymidine kinase from herpes 1 infected cells was affinity purified by a published procedure.[21] The phosphorylation of DHPG and **2** was determined using a gamma-^{32}P-ATP transfer assay.[22]

Animal experiments. These studies were performed according to published methods.[7,10] Statistical significance of increases in numbers of survivors was made by the two-tailed Fisher exact test.

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