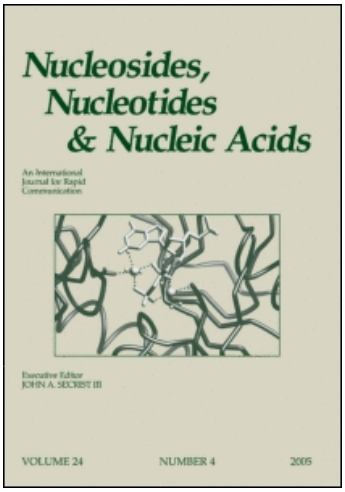

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ANTIHERPES SIMPLEX VIRUS ACTIVITY OF 9-[4-HYDROXY-3-(HYDROXYMETHYL)-1-BUTYL]GUANINE[1]

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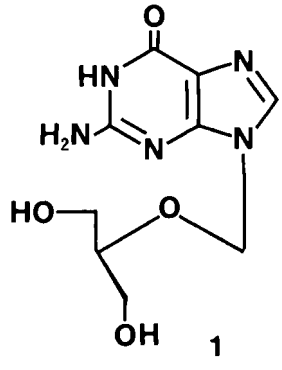
Abstract. The carba analogue 2 of DHPG (1) was found to be highly inhibitory to herpes simplex virus type 1 replication but less active against the type 2 virus.

INTRODUCTION

A number of nucleoside analogues have recently been reported to be selective antiviral agents.[2] We[3] and others[4] have described the synthesis of 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG, 1) an



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acyclic analogue of 2'-deoxyguanosine.[5] DHPG is an exceptionally potent and selective inhibitor of herpes virus replication. In part, DHPG is selective because it is phosphorylated to its monophosphate by

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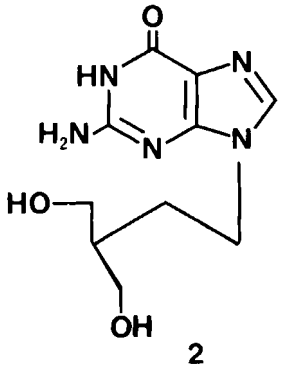
a virus-specified thymidine kinase present only in infected cells.[6,7] The resulting monophosphate is then converted by cellular enzymes to the corresponding triphosphate of DHPG.[7] The nucleoside triphosphate analogue prevents herpes virus replication by inhibition of the virus-specified DNA polymerase.[8,9] Additional selectivity is realized because the nucleoside triphosphate is a better inhibitor of the viral than the host DNA polymerase.[8] The triphosphate also acts as an alternative substrate for the polymerase and once incorporated into the DNA leads to inhibition of chain elongation.[9] DHPG exhibits a broad spectrum of action being active against not only herpes simplex virus types 1 and 2[3,4a,6,10,11] but also cytomegalovirus,[4a,6,10,12] varicella-zoster,[4a,13] and Epstein-Barr virus.[4a,10,14]

We have been synthesizing a number of analogues of DHPG in order to determine the effect of structural modifications on biological activity and now report the antiherpes activity of the carba analogue **2**. The actual synthesis of **2** was previously reported without experimental details or biological data by Pandit and coworkers in 1972.[15] The substitution of a methylene for the ether oxygen of nucleosides has many other precedents. For instance, the carbocyclic analogue of adenosine **3** (aristeromycin) was first synthesized in racemic form[16] and later isolated as a natural product with antimicrobial activity.[17] More recently the carbocyclic analogues of ara A (**4**)[18] and 3-deazaadenosine (**5**)[19] have been synthesized and shown to exhibit antiviral activity.



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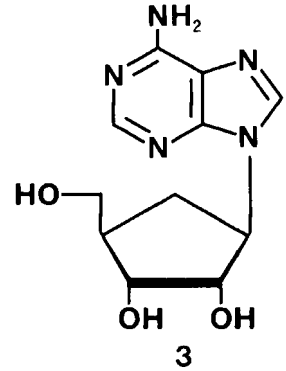

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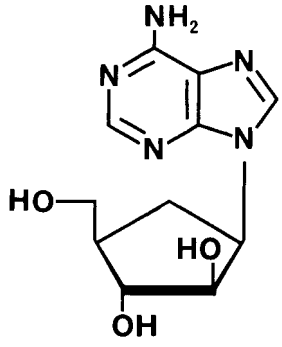

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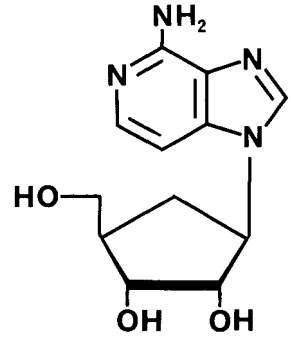

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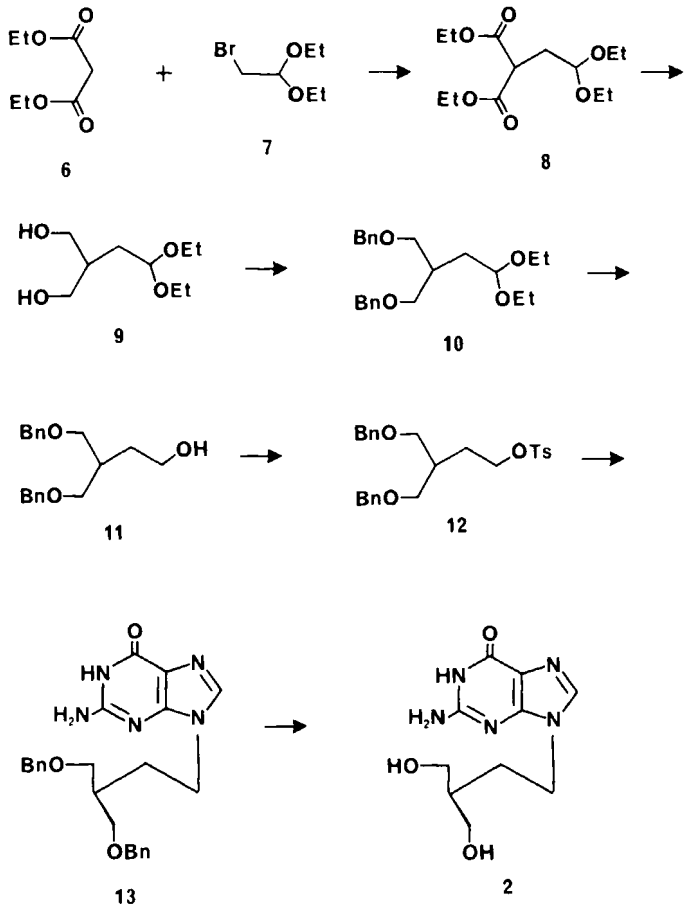
RESULTS AND DISCUSSION

Chemistry. Our synthesis of 2 (Scheme 1) is similar to that of Pandit's[15] and commences from diethyl malonate (**6**) which was alkylated with bromoacetaldehyde diethyl acetal (**7**) to give **8**.[20] Diester **8** was reduced with lithium aluminum hydride to furnish diol **9**.[21] Benzylation of **9** by successive treatment with sodium hydride and then benzyl bromide afforded **10** in 64% distilled yield. Hydrolysis of **10** (*p*-toluenesulfonic acid, tetrahydrofuran/water) followed by reduction (sodium borohydride) and distillation gave alcohol **11** in 81% yield. Reaction of **11** with *p*-toluenesulfonyl chloride furnished tosylate **12**. In a related project, we found that primary tosylates did not react well with the sodium salt of guanine; therefore, **12** was treated first with sodium iodide and then the sodium salt of guanine to give the protected analogue **13** in 8% yield from **11**. Cleavage of the benzyl ether functionalities of **13** by transfer hydrogenation[22] (20% palladium hydroxide on carbon, cyclohexene, ethanol, reflux) afforded 9-[4-hydroxy-3-(hydroxymethyl)-1-butyl]guanine (**2**) in 79% yield.

The structures of both **2** and **13** were confirmed by examination of their carbon NMR spectra. The absorptions of the purine carbons proved that the side chain was indeed at N^9.[23]



Scheme 1

Antiviral activity of 2. Although 2 was nearly as potent in vitro as DHPG (1) against herpes simplex virus type 1 (HSV-1), it was substantially less effective against herpes simplex virus type 2 (HSV-2) (Table 1). Also the carba analogue 2 was seven-fold less effective than DHPG in inhibiting human cytomegalovirus (HCMV) replication.

In the mouse encephalitis model[6] in which mice were challenged with HSV-2 (strain G), 2 was not effective at a dose of up to 20 mg/kg/day (Table 2). Additionally 2 did not prolong the mean survival time. The lack of activity of 2 as compared to DHPG in this in vivo model is consistent with the in vitro test data showing 2 to be less effective than 1 against HSV-2.

TABLE 1. Antiviral activities of carba-DHPG (2) and DHPG (1) in cell culture.

Virus[a]	ID_{50} (μM)[b] 2	1
HSV-1 (F)	0.5	.2
HSV-2 (G)	4.0	0.5
HCMV (AD 169)	45	7.0

[a]The strain is given in parentheses.
[b]Determined by plaque assays in Vero (HSV) or MRC-5 (HCMV) cells.

TABLE 2. Effects of oral treatment with carba-DHPG (2) and DHPG (1) on HSV-2 induced mortality in mice.

Drug	mg/kg[a]	Survivors/ Total	Survivor Increase[b]	Mean Survival Time (days)	Mean Survival Time Increase[c]
Saline		1/20 (5)[d]		9.4 ± 1.9[e]	
DHPG (1)	20	17/20 (85)	< .001	12.7 ± 1.5	< .001
	10	16/20 (80)	< .001	12.3 ± 1.0	< .001
	5	14/20 (70)	< .001	12.0 ± 1.3	< .001
Carba-DHPG (2)	20	1/20 (5)	NS	9.7 ± 1.8	NS
	10	4/20 (20)	NS	9.3 ± 1.5	NS
	5	1/20 (5)	NS	9.3 ± 1.6	NS

[a]Mice were infected intraperitoneally and an oral dose was administered once daily (at 24 h intervals) for 4 days starting 24 h after inoculation.
[b]Probability (Fisher exact test).
[c]Probability (Mann-Whitney test).
[d]Percent survivors.
[e]Standard deviation.

TABLE 3. Effects of subcutaneous treatment with carba-DHPG (2) and DHPG (1) in a mouse HSV-1 intracerebral infection.

Drug	mg/kg[a]	Survivors/ Total	Survivor Increase[b]	Mean Survival Time (days)	Mean Survival Time Increase[c]
Saline		0/20 (0)[d]		6.2 ± 1.4[e]	
DHPG (1)	30	12/21 (57)	< .001	9.6 ± 3.0	.001
	10	10/21 (48)	< .001	11.3 ± 4.8	< .01
	3.0	10/21 (48)	< .001	8.7 ± 2.6	< .01
	1.0	7/21 (33)	< .05	8.2 ± 3.4	NS
	0.3	2/22 (9)	NS	6.9 ± 2.1	NS
Carba-DHPG (2)	100	2/17 (12)	NS	8.2 ± 1.8	< .001
	30	6/21 (29)	< .05	7.4 ± 2.2	NS
	10	7/21 (33)	< .05	7.9 ± 2.8	< .01

[a]Mice were infected intracerebrally and a subcutaneous dose of 2 or 1 was administered twice daily (at 12 h intervals) for 4 days starting 24 h post-infection.
[b]Probability (Fisher exact test).
[c]Probability (Mann-Whitney test).
[d]Percent survivors.
[e]Standard deviation.

Because carba-DHPG (2) and DHPG (1) are essentially equivalent against HSV-1 in vitro, we decided to also investigate their potency against a mouse intracerebral HSV-1 infection.[24] In fact, 2 was effective, (Table 3) and at a dose of 10 mg/kg/day there were 7 out of 21 survivors in the carba-DHPG group compared to 10 out of 21 in the DHPG group. Possibly because of toxicity, 2 was not effective at 100 mg/kg/day.

Although carba-DHPG (2) is nearly as active as DHPG against HSV-1, the lower activity of 2 against HSV-2 and cytomegalovirus limits its potential utility.

EXPERIMENTAL

Nuclear magnetic resonance spectra were recorded on a Varian EM-390 (^{1}H NMR, 90 MHz) and a Bruker WM-300 (^{1}H 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 spectometer. 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.

4-Benzyloxy-3-(benzyloxymethyl)-1-butanal diethyl acetal (10). To a stirred suspension of hexane prewashed NaH (13.4 g, 50%, 280 mmol) in DMF (400 mL) under N_2 was added **9**[21] (23.2 g, 121 mmol) as a solution in DMF (100 mL) over 15 min. After H_2 evolution ceased, benzyl bromide (64.9 mL, 264 mmol) was added over 0.5 h as a solution in DMF (100 mL). After 18 h, the solution was evaporated to dryness and the residue partition between ether and water. The organic phase was dried over $MgSO_4$ and evaporated to dryness. The residue was distilled (bp 186-190°C/1 torr) to give 28.8 g (64%) of **10** as a clear oil; ^{1}H NMR (300 MHz, $CDCl_3$) 7.30 (m, 10H, phenyl), 4.60 (t, J = 6 Hz, 1H, OCHO), 4.48 (s, 4H, benzylic), 3.40-3.68 (m, 8H, CH_2O), 2.11 (heptet, J = 6 Hz, 1H, CH), 1.72 (t, J = 6 Hz, 2H, CH_2), 1.17 (t, J = 6 Hz, 6H, CH_3). Anal. Calcd for $C_{23}H_{32}O_4$ (372.50): C, 74.16; H, 8.66. Found: C, 74.29; H, 8.67.

4-Benzyloxy-3-benzyloxymethyl-1-butanol (11). A solution of **10** (28.2 g, 75.7 mmol) and -toluenesulfonic acid (0.35 g, 1.8 mmol) in THF (70 mL) and water (6 mL) was heated at reflux for 8 h then evaporated to dryness. The residue was dissolved in ethyl acetate washed with saturated $NaHCO_3$, dried over $MgSO_4$ and evaporated to dryness. A solution of the residue and $NaBH_4$ (2.36 g, 62.4 mmol) in methanol (70 mL) was stirred at room temperature for 0.5 h, the reaction was quenched with acetone and the solvent was evaporated. The resulting oil was dissolved in ethyl acetate, washed with 10% HCl,

saturated $NaHCO_3$ and water, dried over $MgSO_4$ and evaporated to dryness. The residue was distilled (bp 198-204°C/1 torr) to give 18.4 g (81%) of **11** as a clear oil; ^1H NMR (90 MHz, $CDCl_3$) δ 7.30 (s, 10H, phenyl), 4.45 (s, 4H, benzylic), 3.30-3.80 (m, 6H, CH_2O), 2.86 (s, broad, 1H, OH), 2.80 (septet, J = 6 Hz, 1H, CH), 1.64 (q, J = 6 Hz, 2H, CH_2). Anal. Calcd for $C_{19}H_{24}O_3$ (300.40): C, 75.97; H, 8.05. Found: C, 75.76; H, 8.13.

4-Benzyloxy-3-benzyloxymethyl-1-butyl tosylate (12). A solution of **11** (10.93 g, 36.3 mmol) and *p*-toluenesulfonyl chloride (9.46 g, 49.6 mmol) in pyridine (160 mL) was kept at 4°C for 18 h, then water (4 mL) was added. After 1 h, the solution was poured into ice water and extracted with ethyl acetate. The extract was washed with 5% HCl, saturated $NaHCO_3$ and brine, dried over $MgSO_4$ and evaporated to give **12** as a clear oil which was used directly in the next reaction. An analytical sample was prepared by chromatography (1:6 ethyl acetate/hexane); ^1H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, J = 8 Hz, 2H, tosylate), 7.22-7.37 (m, 12H, phenyl, tosylate), 4.42 (s, 4H, benzylic), 4.11 (t, J = 6 Hz, 2H, CH_2OTs), 3.43, 3.38 (ABX, J = 6 and 10 Hz, 4H, CH_2O), 2.42 (s, 3H, CH_3), 2.03 (heptet, J = 6 Hz, 1H, CH), 1.77 (q, J = 6 Hz, 2H, CH_2). Anal. Calcd for $C_{26}H_{30}O_5S$ (454.59): C, 68.70; H, 6.65; S, 7.05. Found: C, 68.60; H, 6.66; S, 7.07.

9-[(4-Benzyloxy-3-benzyloxymethyl)-1-butyl]guanine (13). A solution of **12** (12.0 g, 26.3 mmol) and NaI (7.91 g, 52.8 mmol) in DMF (100 mL) was stirred at room temperature for 1.5 h. In a separate flask, guanine (10.2 g, 67.2 mmol) plus NaH (2.71 g, 50%, 56.4 mmol; prewashed with hexane) in DMF (200 mL) was stirred at room temperature for 1 h. The two solutions were combined, heated at 90°C for 1.5 h and then evaporated to dryness. The residue was chromatographed (1:10 methanol/dichloromethane) and selected fractions recrystallized from ethanol to give 0.94 g (8%) of **13**: mp 221-222°C; UV λmax (methanol) sh 275 nm (ε 9540), 253 (13700); ^1H NMR (300 MHz, Me_2SO-d_6) δ 10.54 (s, broad, 1H, NH), 7.64 (s, 1H, H-8), 7.23-7.36 (m, 10H, phenyl), 6.40 (s, broad, 2H, NH_2), 4.42 (s, 4H, benzylic), 4.01 (t, J = 6 Hz, 2H, CH_2N), 3.35-3.50 (m, 4H, CH_2O), 1.83 (m,

3H, CH, CH_2); ^{13}C NMR (75.453 MHz, Me_2SO-d_6) δ 156.72 (C-6), 153.34 (C-2), 151.05 (C-4), 138.41 (phenyl), 137.21 (C-8), 128.11, 127.30, 127.24 (phenyl), 116.57 (C-5), 72.05 (benzylic), 70.01 (CH_2O), 40.87 (CH_2N), 36.39 (CH), 29.01 (CH_2). Anal. Calcd for $C_{24}H_{27}N_5O_3$ (433.52): C, 66.49; H, 6.28; N, 16.16. Found: C, 66.37; H, 6.29; N, 16.15.

9-[(4-Hydroxy-3-hydroxymethyl)-1-butyl]guanine (2). A mixture of **13** (217 mg, 0.50 mmol), 20% $Pd(OH)_2/C$ (220 mg), cyclohexene (5 mL) and ethanol (15 mL) was heated at reflux for 18 h. The mixture was then diluted with 2:1 methanol/water (200 mL) and filtered hot through celite. The filtrate was evaporated to dryness and the residue recrystallized from water to give 101 mg (79%) of **2**: mp 273-275°C; λmax sh 273 nm (ε 10,050), 254 (14,100); ^1H NMR (Me_2SO-d_6, 300 MHz) δ 10.59 (s, broad, 1H, NH), 7.68 (s, 1H, H-8), 6.45 (s, 2H, NH_2), 4.44 (t, J = 5 Hz, 2H, OH), 4.01 (t, J = 6 Hz, 2H, NCH_2), 3.40 (m, 4H, CH_2O), 1.72 (q, J = 6 Hz, 2H, CH_2), 1.46 (heptet, J = 6 Hz, 1H, CH); ^{13}C NMR (75.453 MHz, Me_2SO-d_6) δ 156.68 (C-6), 153.13 (C-2), 151.00 (C-4), 137.48 (C-8), 116.41 (C-5), 79.01 (CH_2O), 40.97 (CH_2N), 40.74 (CH), 28.66 (CH_2). Anal. Calcd for $C_{10}H_{15}N_5O_3$ (253.26): C, 47.43; H, 5.97; N, 27.65. Found: C, 47.34; H, 6.00; N, 27.60.

Plaque Assays. Experiments were conducted with Vero cells infected with HSV-1 (F-strain) and HSV-2 (G-strain) or MRC-5 cells with HCMV (AD 169) and then treated with the nucleoside analogue as described previously.[6] Fifty percent inhibitory doses (ID_{50}) are defined as doses causing a 50% reduction in plaque numbers compared to untreated controls.

Animal Studies. Swiss-Webster female mice (Simonsen Laboratories, Gilroy, Calif.), weighing approximately 20 g each, were infected intraperitoneally with 5×10^4 plaque forming units of HSV-2 (strain G). This challenge was approximately equivalent to ten 50% lethal doses. Alternatively, the mice were infected intracerebrally with 250 plaque forming units of HSV-1 (strain Shealey). DHPG and **2** were administered subcutaneously once a day

for 4 days starting 24 h post-infection. Deaths were recorded for 21 days after infection.

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