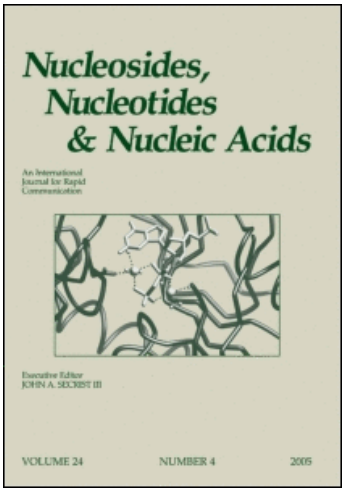

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Synthesis and Activities of the Phosphonate Isostere of the Monophosphate of (±)Cyclobut-G

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SYNTHESIS AND ACTIVITIES OF THE PHOSPHONATE ISOSTERE OF THE MONOPHOSPHATE OF (±)Cyclobut-G

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ABSTRACT. Synthesis of the novel phosphonate isostere of the monophosphate of (±)Cyclobut-G and its biological activities against HSV-1 and HSV-2 in Vero cells are reported. The cyclobutane ring was constructed by a [2+2] thermal cycloaddition. The relative stereochemistry of the substituents on the ring was determined by NOE experiments.

A novel class of antiviral compounds was developed which is based on the structure of oxetanocin,[1] a natural product isolated from *Bacillus megaterium* which contains an oxetane ring in an N-glycoside linkage with adenine. Analogues of oxetanocin have been synthesized by several independent laboratories and shown to have potent antiviral activities.[2-4] Cyclobut-G[5] (see Fig. 1) is a carbocyclic oxetanocin analogue which has a guanine base and shows promise as a therapeutic agent against HIV-1 and herpesviruses (including cytomegalovirus).[2,5,6] Racemic cyclobut-G has been reported to show a 5-fold[7] and 25-fold[4] difference in *in vitro* activity against tk^+ and tk^- strains of HSV-1 and HSV-2. We report here the synthesis and activity of the metabolically stable phosphonate isostere of (±)Cyclobut-G monophosphate (see Fig. 1), which is the first intermediate in the phosphorylation of (±)Cyclobut-G. Several notable examples of phosphonate isosteres which are potent and selective antiviral agents such as (S)-9-[3-hydroxy-2-(phosphonyl-methoxy)propyl]-adenine,[8] (S)-1-[3-hydroxy-2-phosphonylmethoxy)propyl]cytosine, and 1-[2-(phosphonylmethoxy)ethyl]cytosine[9] have been reported recently.

The synthetic strategy for the synthesis of the phosphonate isostere of (±)Cyclobut-G monophosphate is shown in Scheme 1. The [2+2] cycloaddition of

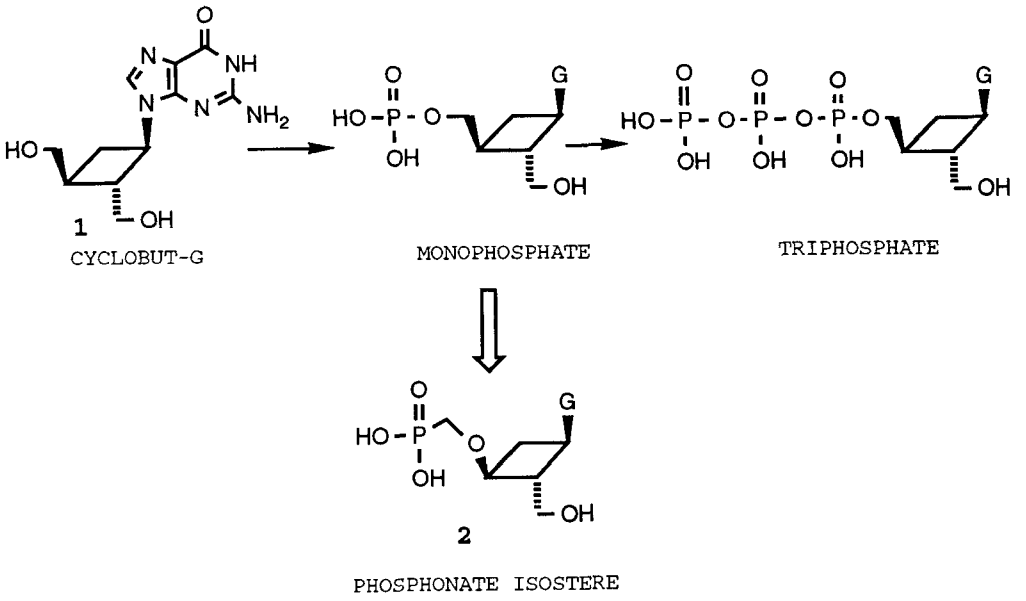
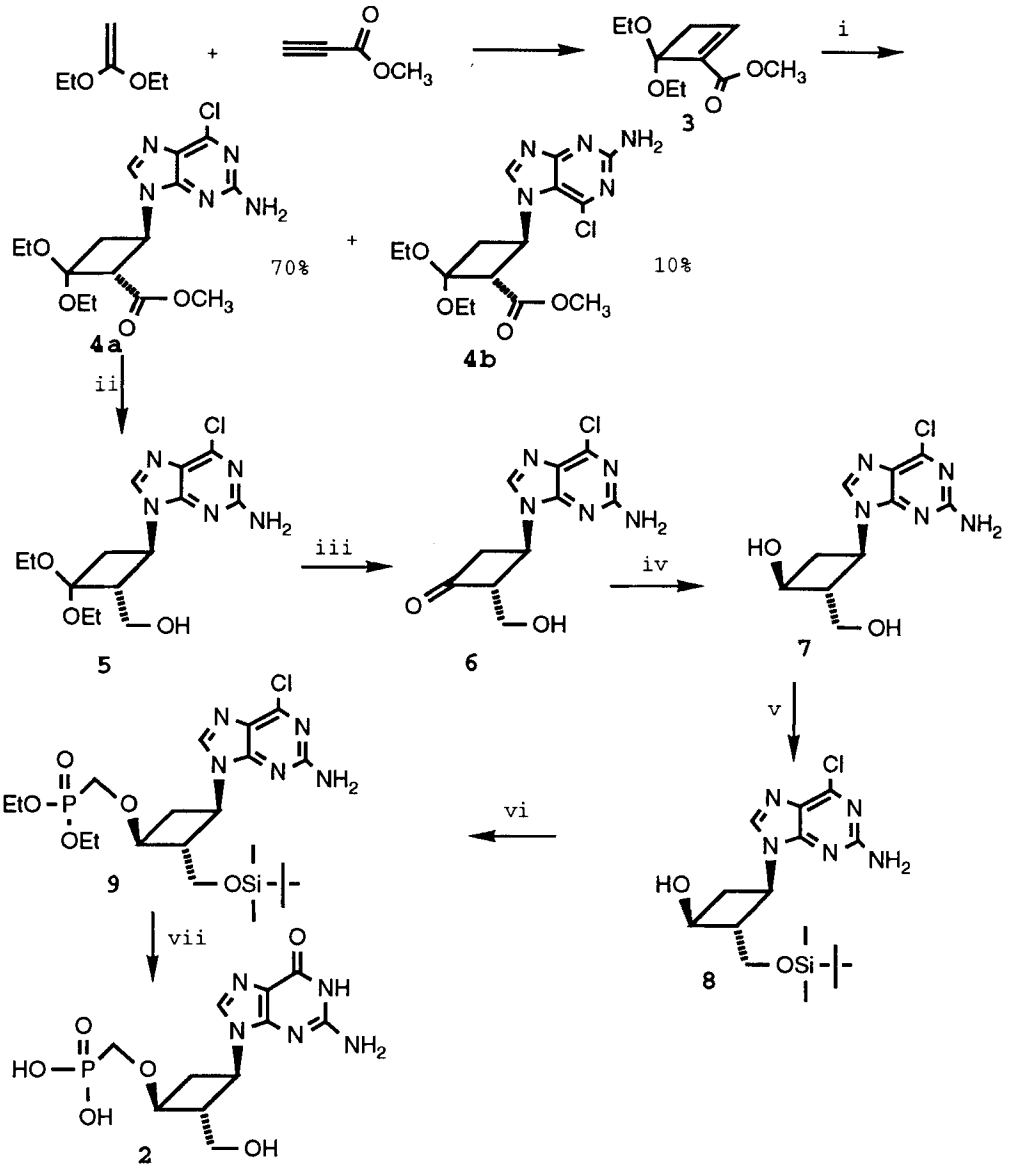


FIGURE 1

ketene diethyl acetal with methyl propiolate[10] at 50°C provided the cyclobutene ester **3** in 55% yield. Michael-type addition of 2-amino-6-chloropurine to the α,ß-unsaturated ester **3** provided the *N*-9 alkylated product **4a** in 70%. A small amount (~10%) of *N*-7 alkylated product **4b** was also isolated. That alkylation occurs predominantly at *N*-9 is well precedented.[11] Although the product **4a** consisted of a 9:1 mixture of *trans/cis* isomers by NMR analysis, the mixture could be equilibrated to provide essentially pure *trans* product by treatment with DBU in acetonitrile overnight. The relative stereochemistry was confirmed by NOE studies, which showed no NOE between the C-1 and C-2 methines in the cyclobutane ring of **4a**. Reduction of the ester functionality in **4a** with LAH, followed by acid catalyzed hydrolysis of the diethyl ketal **5** provided the ketone **6** in 72% yield (2 steps). Stereoselective reduction of the ketone in **6** with sodium borohydride in methanol at 0°C provided a single alcohol **7** in 90% yield. The desired cis stereochemistry between the C-1 and C-3 methines of the cyclobutane was confirmed by a strong NOE effect between these protons. The primary

SYNTHETIC SCHEME 1.



Reagents: i, 2-amino-6-chloropurine/ DBU; ii, LAH; iii, 1N HCl; iv, $NaBH_4$; v, t-butyldimethylsilyl chloride/imidazole; vi, sodium hydride/ diethylphosphonomethyl triflate; vii, TMS-Br, then 1N HCl.

alcohol in **7** was selectively protected as the t-butyldimethylsilyl ether to give **8**. Condensation of the secondary alkoxide generated from **8** by exposure to sodium hydride, with diethyl phosphonomethyltriflate[12] provided the corresponding fully protected compound **9** in 80% yield. Dealkylation with trimethylsilyl bromide[13] and unmasking of the guanine base with 1N hydrochloric acid provided the phosphonate isostere of the monophosphate of (±)Cyclobut-G after purification by C-18 column chromatography in 83% yield.

The ability of the phosphonate **2** to inhibit HSV-1 (strain E-377) and HSV-2 (strain MS) induced cytopathogenic effects (CPE) in Vero cell monolayers was assessed using an MTT assay.[14] Against HSV-1, compound **2** reduced the viral CPE by 50% at 67 μg/ml, while the viability of uninfected control cells was unaffected at 320 μg/ml, the highest concentration tested. Although selective in nature, this antiviral activity is about two orders of magnitude less potent than that previously observed for cyclobut-G.[2,15] Moreover, the phosphonate **2** reduced HSV-2 induced CPE by only 25% at 167 μg/ml, while the IC_{50} of (±)cyclobut-G against this virus typically ranged from 1-5 μg/ml.[2,15] The effect of compound **2** on the yield of HCMV (strain AD169) in MRC5 cell monolayer cultures is summarized in Table I. Compared to the positive control ganciclovir and cyclobut-G, the anti-HCMV activity of the phosphonate was moderate.

In addition to these herpesviruses, compound **2** was also evaluated against HIV-1, a retrovirus and the etiologic agent of AIDS. However, unlike the parent compound cyclobut-G, the phosphonate did not exhibit activity against the cytopathic effect of the IIIB strain in ATH8 cells.[16] In summary, the phosphonate analogue **2** proved to be considerably less active *in vitro* than its parent, cyclobut-G, against all of the viruses examined. Further experiments are required to determine if the reduction in potency could be due to poor penetration of the membrane of the infected cell, inefficient phosphorylation to the "triphosphate," or weak inhibition of the putative nucleotide polymerase targets.

EXPERIMENTAL SECTION

^1H NMR spectra were obtained at 60 MHz or 300 MHz with a Varian EM-360 or GE QE-300 spectrometers. Proton chemical shifts are reported in δ units relative to tetramethylsilane. Mass spectra were obtained on a Finnigan MAT-312 instrument. TLC was performed on EM Merck F_{254} 0.25 mm silica gel plates. THF was distilled from sodium and benzophenone; dichloromethane was distilled from CaH_2;

Table I.

The Effect of the Phosphonate 2 on Yield of HCMV in MRC5 Cell Monolayer Cultures

Concentration of **2** (μg/ml)	HCMV Yield (log10 PFU*/ml)	Drug Cytotoxicity (MTT assay, % control)
320	<0.1	75
100	2.6	82
32	3.6	88
10	3.8	99
3.2	4.1	- -
0	4.9	- -
Ganciclovir (μg/ml)		
8.2	1.3	90

*PFU=plaque forming units in MRC5 cells.

dimethylformamide was distilled from barium oxide. All reactions were performed under argon unless specified otherwise.

α,ß-Unsaturated ester **3**. A solution of methylpropiolate (4.86 g, 58 mmole) and ketene diethylacetal (6.72 g, 58 mmole) in dichloromethane (60 ml) was heated at 50°C under nitrogen for 25 h. Removal of the solvent by rotary evaporation followed by short-path distillation at 38°C (~0.003 torr) provided a colorless oil (55%, 6.38 g). ^{1}H NMR ($CDCl_3$): δ 1.20 (t, 6H, *J*=7.2 Hz), 2.60 (d, 2H, *J*=1.2 Hz), 3.65 (q, 4H, *J*=7.2 Hz), 3.70 (s, 3H), 7.02 (t, 1H, *J*=1.2 Hz). Mass spectrum: m/e 200.

Synthesis of compound **4a**. To a solution of the unsaturated ester **3** (2.3 g, 11.5 mmole), and 1 equivalent of 2-amino-6-chloropurine in 50 ml of DMF was added 0.05 ml of DBU. After 2 h at room temp. (RT) the solvent was removed by rotary evaporation and the residue was dissolved in ethyl acetate (100 ml) and washed with satd. sodium bicarbonate. Drying over Na_2SO_4, concentration, and purification by

silica gel column chromatography (2% MeOH/98% CH_2Cl_2) provided **4a** as the major product (colorless oil) (2.98 g, 70%). TLC: Rf 0.27 (1:1 EtOAc-CH_2Cl_2). ^1H NMR ($CDCl_3$): δ1.20 (t, 3H, *J*=9 Hz), 1.28 (t, 3H, *J*=9 Hz), 2.90 (d, 2H, *J*=8.5 Hz), 3.50 (m, 2H), 3.65 (m, 1H), 3.75 (s, 3H), 3.77 (m, 1H), 4.02 (d, 1H, *J*=8.5 Hz), 5.08 (dt, 1H, *J*=8.5 Hz), 5.10 (brs, 2H), 7.86 (s, 1H). Anal. ($C_{15}H_{20}ClN_5O_4$). Calcd: C, 48.71; H, 5.45; N, 18.93. Found: C, 48.60; H, 5.36; N, 18.95.

Synthesis of alcohol **5**. To a solution of methyl ester **4a** (1.9 g, 5.1 mmole) in 70 ml of THF at 0°C was added 1.5 equivalent of lithium aluminum hydride. The reaction mixture was stirred for 1.5 h. After sequential addition of 0.4 ml of H_2O, 0.4 ml of 15% NaOH, and 1.2 ml of H_2O the reaction mixture was stirred vigorously for 20 min at RT. After filtration and concentration of the filtrate on rotary evaporator, the residue was purified by silica gel column chromatography to provide 98% of white solid (1.67 g, 5.0 mmole). TLC: Rf 0.2 (5:95 MeOH-CH_2Cl_2). ^1H NMR ($CDCl_3$): 1.25 (m, 6H), 2.66 (m, 1H), 3.00 (m, 2H), 3.55 (m, 4H), 3.86 (m, 1H), 4.00 (m, 1H), 4.67 (dt, 1H, *J*=9 Hz), 5.10 (br s, 2H), 7.90 (s, 1H). Anal. ($C_{14}H_{20}ClN_5O_3$). Calcd: C, 49.20; H, 5.90; N, 20.49. Found: C, 49.03; H, 5.95; N, 20.56.

Synthesis of hydroxy-ketone **6**. To a solution of compound **5** (2.0 g, 5.85 mmole) in 200 ml of acetone was added 30 ml of 1N HCl in 2 portions. The solution was stirred at RT overnight, concentrated on rotary evaporator, neutralized with 1N NaOH and extracted with EtOAc. The dried (Na_2SO_4) and filtered EtOAc solution was concentrated and the residue was purified by silica gel column chromatography to provide 76% yield of **6** as a white solid (1.2 g, 4.49 mmole). TLC: Rf 0.29 (1:9 MeOH-CH_2Cl_2). ^1H NMR (DMSO-d_6): δ 3.65-3.80 (m, 2H), 4.20 (m, 2H), 5.00 (t, 1H, *J*=4.5 Hz), 5.16 (m, 1H), 6.92 (br s, 2H), 8.49 (s, 1H). Anal. ($C_{10}H_{10}ClN_5O_2$). Calcd: C, 44.87; H, 3.77; N, 26.16. Found: C, 44.68; H, 3.70; N, 26.25.

Synthesis of diol **7**. To a solution of compound **6** (430 mg, 1.61 mmole) in 45 ml of methanol cooled to 0°C was added 100 mg of sodium borohydride in 3 portions. After 15 min, the reaction was quenched by addition of 1 ml of acetone, concentrated on rotary evaporator and the residue was purified by silica gel column chromatography to provide 85% yield of diol **7** as a white solid (370 mg, 1.38 mmole). TLC: Rf 0.10 (1:9 MeOH-CH_2Cl_2). ^1H NMR (DMSO-d_6):

δ 2.19 (m, 1H), 2.70 (m, 1H), 2.83 (m, 1H), 3.55 (m, 2H), 3.82 (m, 1H), 4.23 (m, 1H), 4.67 (t, 1H, *J*=4.5 Hz), 5.28 (d, 1H, *J*=6.5 Hz), 6.88 (br s, 2H), 8.27 (s, 1H). Anal. ($C_{10}H_{12}ClN_5O_2$). Calcd: C, 44.54; H, 4.48; N, 25.97. Found: C, 44.38; H, 4.54; N, 25.78.

Synthesis of mono-silyl ether **8**. To a solution of diol **7** (390 mg, 1.45 mmole) in 10 ml of DMF cooled to 0°C was added 2 equivalents of imidazole (200 mg) and 1.1 equivalents (240 mg) of t-butyldimethylsilyl chloride. After 1 h, the reaction was stopped and the DMF removed under vacuum. The crude product was purified by silica gel column chromatography to provide 50% yield of compound **8** as a white solid (275 mg, 0.72 mmole). TLC: Rf 0.2 (5:95 MeOH-CH_2Cl_2). Some starting diol was recovered (140 mg, 36%) and can be recycled. ^{1}H NMR (DMSO-d_6): δ -0.20 (s, 6H), 0.73 (s, 9H), 2.20 (m, 1H), 2.70 (m, 1H), 2.92 (m, 1H), 3.70-3.80 (m, 3H), 4.20 (m, 1H), 5.33 (d, 1H, *J*=6.2 Hz), 6.80 (br s, 2H), 8.28 (s, 1H). Anal. ($C_{16}H_{26}ClN_5O_2Si$). Calcd: C, 51.39; H, 7.01; N, 18.73. Found: C, 51.18; H, 6.97; N, 18.65.

Synthesis of diethyl phosphonate **9**. To a solution of compound **8** (155 mg, 0.42 mmole) in 10 ml of THF at 0°C was added 18.5 mg of sodium hydride (1.1 equivalents). After 10 min, diethyl phosphonomethyltriflate (0.108 ml, 1.2 equivalents) was added. After 1 h at 0°C, the reaction was quenched with methanol, concentrated on rotary evaporator. The residue was dissolved in EtOAc (150 ml) and washed with satd. brine, dried with Na_2SO_4 and concentrated. The crude product was purified by silica gel column chromatography to provided a 71% yield of compound **9** as a white solid (170 mg, 0.32 mmole). TLC: Rf 0.30 (5:95 MeOH-CH_2Cl_2). ^{1}H NMR (DMSO-d_6): δ -0.20 (s, 6H), 0.73 (s, 9H), 1.25 (t, 6H, *J*=7.5 Hz), 2.40 (m, 1H), 2.75 (m, 1H), 3.05 (m, 1H), 3.72 (m, 2H), 3.80 (m, 3H), 4.05 (q, 4H, *J*=7.5 Hz), 4.23 (m, 1H), 6.82 (br s, 2H), 8.32 (s, 1H). Anal. ($C_{21}H_{37}ClN_5O_5PSi$). Calcd: C, 47.23; H, 6.98; N, 13.11. Found: C, 47.31; H, 6.88; N, 12.97.

Synthesis of phosphonate **2**. To a solution of diethyl phosphonate **9** (169 mg, 0.32 mmole) in 2 ml of CH_3CN was added trimethylsilyl bromide (0.42 ml, 10 equivalents). The reaction mixture was kept at RT for 16 h and concentrated on the rotary evaporator. After drying for several h on high vacuum, the glassy solid was dissolved in 7 ml of 1N HCl and heated at 90°C for 2.5 h. The solution was concentrated

on the rotary evaporator and the residue was treated with 0.5 ml of conc. NH_4OH and concentrated to give a white solid which was purified by C-18 column chromatography (H_2O as the eluent) to provide a 87% yield of **2** as the mono-ammonium salt (101 mg, 0.28 mmole). ^1H NMR (D_2O): δ 2.45 (m, 1H), 3.00 (m, 2H), 3.67 (d, 2H, *J*=9 Hz), 3.82 (d, 2H, *J*=6 Hz), 3.96 (dt, 1H, *J*=6 Hz), 4.30 (dt, 1H, *J*=6 Hz), 8.00 (s, 1H). Anal. ($C_{11}H_{19}N_5O_6P \cdot H_2O$). Calcd: C, 34.74; H, 5.56; N, 22.09. Found: C, 34.63; H, 5.42; N, 22.20.

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