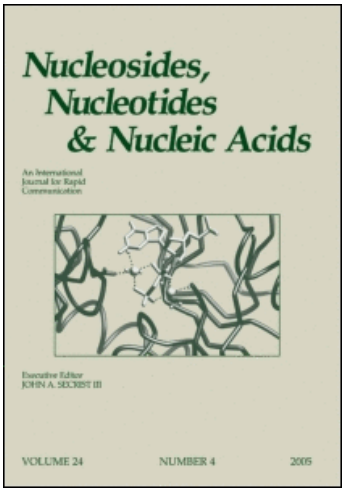

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Synthesis of Phosphonate Analogues of the Antiviral Cyclopropane Nucleoside A-5021

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SYNTHESIS OF PHOSPHONATE ANALOGUES OF THE ANTIVIRAL CYCLOPROPANE NUCLEOSIDE A-5021

Tomoyuki Onishi, Takaaki Sekiyama, and Takashi Tsuji ▫ *Pharmaceutical Research Laboratories, Ajinomoto Co. Inc., Kawasaki, Japan*

▫ *A series of phosphonate analogues of the antiviral cyclopropane nucleoside A-5021 were synthesized from (1S*, 7R*)-3,5-dioxa-4,4-diphenylbicyclo[5.1.0]octane-l-methanol by a 10-step process. In contrast to the potent antiherpetic activity of A-5021, they were all devoid of antiviral activity.*

Keywords Phosphate analogues, A-5021

INTRODUCTION

Phosphonate derivatives of antiviral nucleosides have been attractive targets as potential antiviral agents, since they do not require mono-phosphorylation, the first step in the activation of antiviral nucleosides. Among these, the *R*-enantiomer of ganciclovir phosphonate (SR-3773, 1) (Figure 1) has potent and selective in vitro activity against human cytomegalovirus (HCMV)[1,2] and in comparative studies is more effective than ganciclovir against murine cytomegalovirus (MCMV) infections in healthy and immunosuppressed mice.[3] Acyclovir phosphonate 2 also shows potent in vitro activity against HCMV and MCMV, whereas 2 is less potent than ganciclovir.[3]

We previously reported the synthesis and antiviral activity of (1*S*,2*R*)-9-[[1,2-*bis*(hydroxymethyl)-cyclopropan-l-yl]methyl]guanine (A-5021, 3) (Figure 2).[4–6] A-5021 has a unique structure with two asymmetric centers on the cyclopropane ring as an acyclosugar moiety. A-5021 shows extremely potent antiherpetic activity against herpes simplex virus (HSV) and varicella zoster virus, and has a greater therapeutic effect than acyclovir in animal models.[7] Since HCMV lacks viral thymidine kinase, which is responsible for the activation of A-5021,[8] we expected that the phosphonate analogues of A-5021 may have better anti-HCMV activity and

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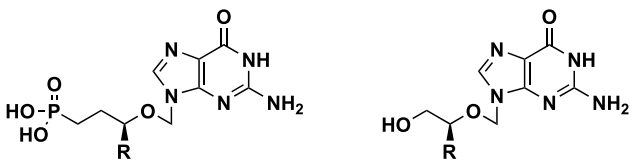


FIGURE 1

started to explore their synthesis. We report here the synthesis of phosphonate analogues of A-5021 and their antiviral activities.

RESULTS AND DISCUSSION

Since A-5021 is monophosphorylated at the 2′-site of cyclopropane by viral thymidine kinases,[8] compound 4 (Figure 2), in which the hydroxymethyl group of A-5021 at the 2′-site is substituted by a (dihydroxyphosphinyl)ethyl group, was synthesized as the primary target. (1*S**,7*R**)-3,5-Dioxa-4,4-diphenylbicyclo[5.1.0]octane-l-methanol 5, a useful intermediate for the synthesis of antiviral cyclopropane nucleosides, was used as a starting material (Scheme 1).[9] To obtain both (1*R**,2*R**)- and (1*R**,2*S**)-2-(dihydroxyphosphinyl)ethyl-1-hydroxymethylcyclopropane regioisomers, 1,1-dihydroxycyclopropane 9 was used as a key synthetic intermediate. In the presence of a catalytic amount of *p*-TsOH, transacetalization of [1,3]-dioxepane 5 proceeded to give [1,3]-dioxane 6 in 43% yield. Compounds 5 and 6 could be separated by silica gel column chromatography. A diethoxyphosphinyl group was successfully introduced by Swern oxidation of 6 followed by a Horner-Wittig reaction.[10–12] Both reactions proceeded with good yields (87% and 82%, respectively) to give olefin 8. Catalytic hydrogenation of 8 in the presence of acetic acid gave 1,1-dihydroxycyclopropane 9 in 64% yield.

Next, 9 was treated with 1 equiv. of benzoyl chloride in the presence of triethylamine to give a mixture of monobenzoyl esters 10 and 11 in 47% yield (Scheme 2). Although 10 and 11 could not be separated by normal-phase silica gel

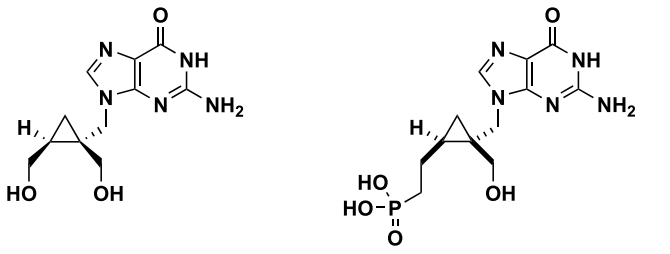
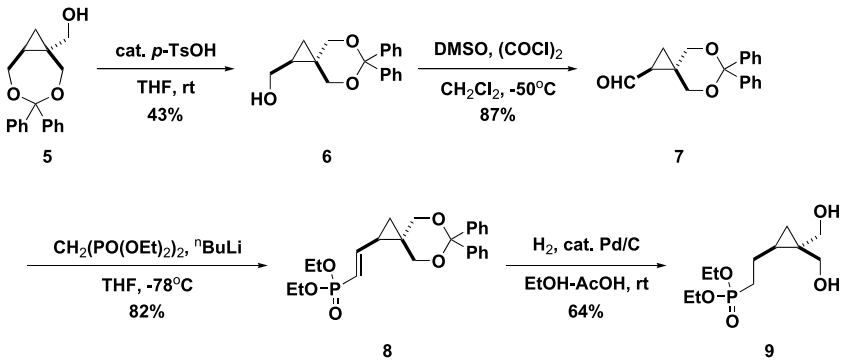


FIGURE 2

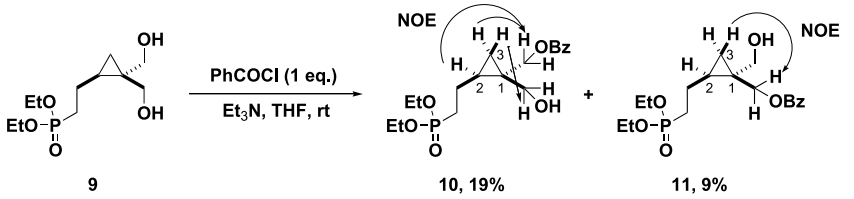


SCHEME 1

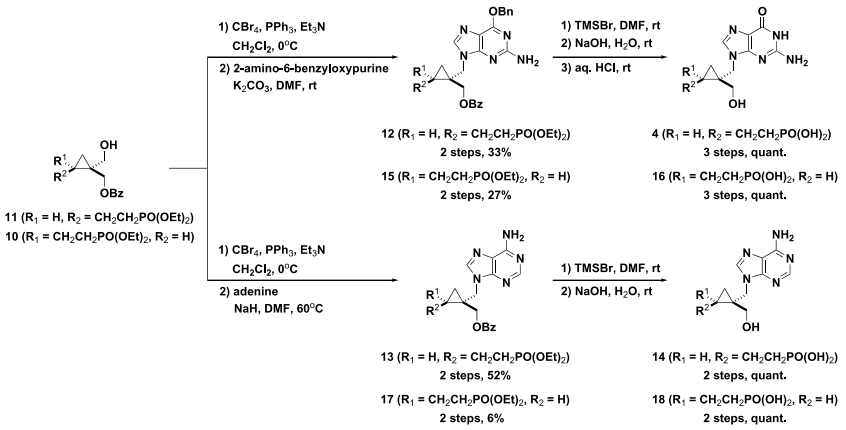
column chromatography, these isomers could be separated by reversed-phase C18 silica gel column chromatography eluting with 30% acetonitrile to give 10 and 11 in respective yields of 19% and 9%. The structure of each isomer could be clearly determined by NOESY.

To prepare the phosphonate analogue of A-5021 4, alcohol 11 was converted to a bromide by treatment with CBr_4-Ph_3P in the presence of triethylamine, and the resulting bromide was used without further isolation for the alkylation of 2-amino-6-benzyloxypurine at the 9-position to give fully protected phosphonate 12 in 33% yield from 11 (Scheme 3). Removal of the protecting groups by a three-step sequence gave the desired product 4. The adenine analogue 14 was also synthesized using adenine instead of 2-amino-6-benzyloxypurine. The (1*R**,2*S**)-2-(dihydroxyphosphinyl)ethyl-1-hydroxymethylcyclopropane regioisomers 16 and 18 were synthesized from 10 using the same method.

The antiherpetic activities of 4, 14, 16, and 18 were measured by a growth-inhibition assay against HCMV AD169 strain[5] and by a quantitative CPE reduction assay against HSV-1 Tomioka strain;[4] however, no inhibitory activity was observed at up to 100 μg/mL. They also showed no cytotoxic effect in Vero cells up to 100 μg/mL. Anti-HIV activity was also evaluated,[13] but they were devoid of such activity. Since phosphonates are not "perfect mimics" for monophosphates, the steps required for virus inhibition, either further phosphorylation or DNA polymerase inhibition, might be impaired in these compounds. Poor cellular uptake of the phosphonate analogues of A-5021 would be another possible reason for the apparent lack of activity.[14]



SCHEME 2



SCHEME 3

EXPERIMENTAL SECTION

The reagents used were the highest quality available commercially. Unless otherwise noted, organic extracts were dried over anhydrous Na_2SO_4 and temperature refers to the temperature of the bath. ^{1}H-NMR and NOESY spectra were recorded with a Varian XL-300 300-MHz and a JEOL JNM-GX-400 400-MHz spectrometer, using tetramethylsilane as an internal standard. Mass spectra were recorded on a JEOL JMS-DX300 spectrophotometer, and accurate masses were measured on a JEOL JMS-HX110 spectrometer. Silica gel column chromatography was conducted on silica gel 60 (70–230 mesh; Merck Art. no. 7734). Preparative reversed-phase column chromatography was conducted on a Merck LiChroprep RP-18 column (40–63 μm).

5,7-Dioxa-6,6-diphenyl-1-hydroxymethylspiro[2.5]octane (6). To a solution of (1*S**,7*R**)-3,5-dioxa-4,4-diphenylbicyclo[5.1.0]octane-1-methanol (10.00 g, 33.7 mmol) in dry THF (67.4 mL) was added *p*-toluenesulfonic acid monohydrate (321 mg, 1.69 mmol). After the solution was stirred for 12 h at room temperature, a saturated aqueous solution of sodium hydrogen carbonate was added, and the resulting mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate, and the solvent was then removed under reduced pressure. The residue was purified by silica gel chromatography using a step gradient of 20% and 25% ethyl acetate in hexane, to give 6 (4.32 g, 43%) as a white solid. ^{1}H-NMR ($CDCl_3$) δ 0.39 (t, J = 5.5 Hz, 1H), 0.66 (dd, J = 5.5, 8.7 Hz, 1H), 1.33–1.43 (m, 1H), 2.15–2.25 (m, 1H), 3.37 (d, J = 11.7 Hz, 1H), 3.41 (dd, J = 1.8, 11.4 Hz, 1H), 3.78 (dd, J = 2.1, 12.0 Hz, 1H), 3.88–3.99 (m, 1H), 4.17 (d, J = 11.4 Hz, 1H), 4.21 (d, J = 12.0 Hz, 1H), 7.18–7.59 (m, 10H); FAB MASS, 297 (MH^+).

5,7-Dioxa-6,6-diphenyl-1-formylspiro[2.5]octane (7). To a solution of oxalyl chloride (2.14 g, 16.9 mmol) in dichloromethane (39 mL) was

added a solution of dimethylsulfoxide (2.65 g, 33.9 mmol) in dichloromethane (8.4 mL) at −50°C. After the solution was stirred for 2 min, a solution of 6 (4.56 g, 15.4 mmol) in dichloromethane (16 mL) was added. After this solution was stirred for 15 min, triethylamine (10.8 mL) was added. After this mixture was stirred for 5 min, it was allowed to warm to room temperature. To the solution was added water, and the resulting mixture was extracted with dichloromethane. The organic layer was washed with a saturated aqueous solution of sodium chloride and dried over anhydrous sodium sulfate, and then the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography using a gradient of hexane to 9% ethyl acetate in hexane, to give 7 (3.95 g, 87%) as a white solid. ^{1}H-NMR ($CDCl_3$) δ 1.11 (dd, J = 5.4, 8.1 Hz, 1H), 1.49 (t, J = 5.4 Hz, 1H), 2.09 (ddd, J = 3.2, 5.4, 8.1 Hz, 1H), 3.68 (dd, J = 1.6, 11.6 Hz, 1H), 3.99 (d, J = 11.6 Hz, 1H), 4.02 (dd, J = 1.6, 11.8 Hz, 1H), 4.14 (d, J = 11.8 Hz, 1H), 7.21–7.41 (m, 6H), 7.46–7.58 (m, 4H), 9.73 (d, J = 3.2 Hz); FAB MASS, 295 (MH^+).

1-(*E*)-(Diethoxyphosphinyl)ethenyl-5,7-dioxa-6,6-diphenylspiro[2.5]octane (8). To a solution of tetraethyl methylenediphosphonate (1.69 g, 5.88 mmol) in dry THF (7.8 ml) was added a solution of 1.6 M *n*-butyllithium in hexane (3.68 mL, 5.88 mmol) at −78°C. After this solution was stirred for 1 h, a solution of 7 (1.73 g, 5.88 mmol) in dry THF (7.8 mL) was added. After this mixture was stirred for an additional 1 h, a saturated aqueous solution of ammonium chloride was added. The resulting mixture was extracted with ethyl acetate, and the organic layer was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography using a step gradient of 17% to 67% ethyl acetate in hexane, to give 8 (2.08 g, 82%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ 0.90 (t, J = 5.6 Hz, 1H), 1.08 (dd, J = 5.6, 8.4 Hz, 1H), 1.29 (t, J = 7.1 Hz, 3H), 1.31 (t, J = 7.1 Hz, 3H), 1.60–1.72 (m, 1H), 3.75 (d, J = 11.6 Hz, 1H), 3.83 (d, J = 11.6 Hz, 1H), 3.98 (s, 2H), 3.99–4.10 (m, 4H), 5.75 (ddd, J = 0.6, 16.8, 19.5 Hz, 1H), 6.46 (ddd, J = 9.6, 16.8, 26.4 Hz, 1H), 7.22–7.38 (m, 6H), 7.45–7.55 (m, 4H); FAB MASS, 429 (MH^+).

1,1-Bis(hydroxymethyl)-2-[(diethoxyphosphinyl)ethyl]cyclopropane (9). To a solution of 8 (2.07 g, 4.83 mmol) in ethanol (48.3 mL) was added palladium carbon (10%) (0.1 g) and the mixture was stirred in a hydrogen atmosphere pressurizing to 20 PSI for 13 h. To this mixture was added acetic acid (2.4 mL) and the mixture was stirred for an additional 4.5 h. The mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography using a gradient of dichloromethane to 5% methanol in dichloromethane, to give 9 (819 mg, 64%) as a colorless oil. ^{1}H-NMR (CD_3OD) δ 0.28 (t, J = 5.0 Hz, 1H), 0.68 (dd, J = 5.0, 8.4 Hz, 1H), 0.90–1.00 (m, 1H), 1.37 (t, J = 7.1 Hz, 6H), 1.56–2.12 (m, 4H), 3.45 (d, J = 11.1 Hz, 1H), 3.55 (d, J = 11.1 Hz, 1H), 3.58 (d, J = 11.6 Hz, 1H), 3.83 (d, J = 11.6 Hz, 1H), 4.07–4.18 (m, 4H); ESI MASS, 289.5 ($M + Na^+$).

(1*R,2*R**)-1-Benzoyloxymethyl-2-(diethoxyphosphinyl)ethyl-1-hydroxymethyl-cyclopropane (10) and (1*R**,2*S**)-1-benzoyloxymethyl-2-(diethoxyphosphinyl)ethyl-1-hydroxymethyl-cyclopropane (11).** To a mixture of 9 (605 mg, 2.27 mmol) and triethylamine (460 mg, 4.55 mmol) in dichloromethane (11 mL) was added a solution of benzoyl chloride (320 mg, 2.27 mmol) in dichloromethane (11 mL) at room temperature. After this mixture was stirred for 1.5 h, a saturated aqueous solution of sodium hydrogen carbonate was added. The resulting mixture was extracted with dichloromethane, and the organic layer was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography to give a mixture of 10 and 11 (386 mg, 47%). Isomers 10 and 11 were isolated by chromatography on a column of reversed-phase C18 silica gel eluting with 30% acetonitrile in water, to give 10 (157 mg, 19%) and 11 (73.1 mg, 9%).

10 (colorless oil); ^{1}H-NMR ($CDCl_3$) δ 0.31 (t, J = 5.6 Hz, 1H), 0.84 (dd, J = 5.6, 8.7 Hz, 1H), 0.98–1.09 (m, 1H), 1.32 (t, J = 6.9 Hz, 6H), 1.65–1.99 (m, 4H), 2.86 (t, J = 6.2 Hz, 1H), 3.50 (dd, J = 6.2, 12.2 Hz, 1H), 3.90 (dd, J = 6.2, 12.2 Hz, 1H), 4.03–4.16 (m, 5H), 4.54 (dd, J = 1.1, 11.6 Hz, 1H), 7.41–7.48 (m, 2H), 7.54–7.60 (m, 1H), 8.02–8.08 (m, 2H); ESI MASS, 371.3 (MH^+).

11 (colorless solid); ^{1}H-NMR ($CDCl_3$) δ 0.45 (t, J = 5.3 Hz, 1H), 0.76 (dd, J = 5.3, 8.4 Hz, 1H), 1.03–1.14 (m, 1H), 1.29 (t, J = 7.1 Hz, 3H), 1.30 (t, J = 7.1 Hz, 3H), 1.47–2.01 (m, 4H), 3.30 (d, J = 11.7 Hz, 1H), 3.65 (d, J = 11.7 Hz, 1H), 3.98–4.15 (m, 4H), 4.41 (d, J = 11.7 Hz, 1H), 4.54 (d, J = 11.7 Hz, 1H), 7.39–7.44 (m, 2H), 7.51–7.58 (m, 1H), 8.02–8.08 (m, 2H); ESI MASS, 371.3 (MH^+).

Determination of the Structures of 10 and 11 by NOESY

In 10, NOE was observed between the proton at position 2 of the cyclopropane and the methylene proton of the benzoyloxymethyl group at position 1, between the proton at position 3(α) of cyclopropane and the methylene proton of the benzoyloxymethyl group at position 1, and between the proton at position 3(β) of cyclopropane and the methylene proton of the hydroxymethyl group at position 1. On the other hand, in 11, NOE was observed between the proton at position 3(β) of cyclopropane and the methylene proton of the benzoyloxymethyl group at position 1.

2-Amino-6-benzyloxy-9-[(1*R,2*R**)-1-benzoyloxymethyl-2-[(diethoxyphosphinyl)-ethyl]cyclopropan-1-yl]methylpurine (12).** To a mixture of 11 (36.6 mg, 0.099 mmol), triethylamine (5 mg, 0.049 mmol) and triphenylphosphine (47 mg, 0.178 mmol) in dichloromethane (1 mL) was added carbon tetrabromide (59 mg, 0.178 mmol) at 0°C. After this mixture was stirred for 1 h at 0°C, phosphate buffer (pH = 7) was added, and the resulting mixture was extracted with dichloromethane. The organic layer was dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure.

The residue was dissolved in dry DMF (1 mL), and to the solution were added 2-amino-6-benzyloxypurine (23.8 mg, 0.099 mmol) and potassium carbonate (13.6 mg, 0.099 mmol). After this mixture was stirred for 12 h at room temperature, insoluble substances were removed by filtration. The filtrate was concentrated under reduced pressure, and the residue was purified by preparative thin-layer chromatography, eluting with 10% methanol in dichloromethane, to give 12 (19.5 mg, 33%) as a colorless solid. ^{1}H-NMR (CD_3OD) δ 0.65 (t, J = 5.6 Hz, 1H), 1.14 (dd, J = 5.6, 8.7 Hz, 1H), 1.21 (t, J = 7.4 Hz, 3H), 1.24 (t, J = 7.4 Hz, 1H), 1.54–1.73 (m, 2H), 1.79–2.12 (m, 3H), 3.88–4.02 (m, 5H), 4.12 (d, J = 12.3 Hz, 1H), 4.46 (d, J = 13.5 Hz, 1H), 4.74 (d, J = 13.5 Hz, 1H), 5.38 (d, J = 12.3 Hz, 1H), 5.48 (d, J = 12.3 Hz, 1H), 7.30–7.45 (m, 5H), 7.47–7.60 (m, 5H), 8.00 (s, 1H); ESI MASS, 594.5 (MH^+).

9-[(1*R,2*R**)-2-(Dihydroxyphosphinyl)ethyl-1-hydroxymethylcyclopropan-1-yl]-methylguanine (4).** To a solution of 12 (19.5 mg, 0.033 mmol) in dry DMF (0.4 mL) was added trimethylsilyl bromide (75.9 μL, 0.575 mmol) at room temperature. After this mixture was stirred for 43 h, the solvent was removed, coevaporating three times with methanol. The residue was dissolved in 1 M NaOH (0.7 mL). After this mixture was stirred for 2 h, 2 M HCl (0.7 mL) was added. After this mixture was stirred for an additional 20 min, 1 M NaOH (0.7 mL) was added. The mixture was purified by chromatography on a column of reversed-phase C18 silica gel eluting with water to give 4 (11.3 mg, 100%) as a white solid. ^{1}H-NMR (d^6-DMSO) δ 0.16 (t, J = 5.1 Hz, 1H), 0.85 (dd, J = 5.1, 8.4 Hz, 1H), 1.03–1.17 (m, 1H), 1.47–1.64 (m, 4H), 3.17 (d, J = 12.0 Hz, 1H), 3.36 (d, J = 12.0 Hz, 1H), 3.72 (d, J = 14.1 Hz, 1H), 4.08 (d, J = 14.1 Hz, 1H), 6.61 (bs, 2H), 7.86 (s, 1H), 10.74 (bs, 1H); ^{13}C-NMR (d^6-DMSO) δ 14.7, 22.2 [J(^{13}C,^{31}P) = 3.7 Hz], 23.3 [J(^{13}C,^{31}P) = 19.2 Hz], 27.0, 27.8 [J(^{13}C,^{31}P) = 134.7 Hz], 47.8, 60.1, 137.7, 151.1, 153.7, 156.3; ^{31}P-NMR (d^6-DMSO) δ 26.1; UV λ_{max} (0.1 M HCl) 281 sh nm (ε 7400), 255 (ε 11000); HRMS calcd for $C_{12}H_{19}N_5O_5P$ (MH^+) 344.1124, found 344.1129.

9-[(1*R,2*R**)-1-Benzoyloxymethyl-2-[(diethoxyphosphinyl)-ethyl]cyclopropan-l-yl]methyladenine (13).** To a mixture of 11 (36.6 mg, 0.099 mmol), triethylamine (5 mg, 0.049 mmol) and triphenylphosphine (47 mg, 0.178 mmol) in dichloromethane (1 mL) was added carbon tetrabromide (59 mg, 0.178 mmol). After this mixture was stirred for 1 h at 0°C, phosphate buffer (pH = 7) was added, and the resulting mixture was extracted with dichloromethane. The organic layer was dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The residue was dissolved in dry DMF (0.5 mL), and the solution was added to a suspension of adenine (13.3 mg, 0.099 mmol) and sodium hydride (60% oil dispersion, 3.9 mg, 0.099 mmol) in dry DMF (0.5 mL). After this mixture was stirred for 12 h at 60°C, insoluble substances were removed by filtration. The filtrate was concentrated under reduced pressure,

and the residue was purified by preparative thin-layer chromatography, eluting with 10% methanol in dichloromethane, to give 13 (24.8 mg, 52%) as a white solid. ^{1}H-NMR (CD_3OD) δ 0.69 (t, J = 5.4 Hz, 1H), 1.17–1.28 (m, 7H), 1.58–1.73 (m, 2H), 1.78–2.10 (m, 3H), 3.91–4.04 (m, 4H), 4.14 (d, J = 12.6 Hz, 1H), 4.16 (d, J = 14.6 Hz, 1H), 4.55 (d, J = 14.6 Hz, 1H), 4.72 (d, J = 12.6 Hz, 1H), 7.37–7.45 (m, 2H), 7.51–7.61 (m, 3H), 8.11 (s, 1H), 8.32 (s, 1H); ESI MASS, 488.3 (MH^+).

9-[(1*R,2*R**)-2-(Dihydroxyphosphinyl)ethyl-1-hydroxymethylcyclopropan-1-yl]-methyladenine (14).** To a solution of 13 (24.8 mg, 0.051 mmol) in dry DMF (0.5 mL) was added trimethylsilyl bromide (117.5 μL, 0.890 mmol) at room temperature. After this mixture was stirred for 41 h, the solvent was removed, coevaporating three times with methanol. The residue was dissolved in 1 M NaOH (1.0 mL). After this mixture was stirred for 2 h, 2 M HCl (0.5 mL) was added. The mixture was purified by chromatography on a column of reversed-phase C18 silica gel using a gradient of water to 5% methanol in water, to give 14 (14.8 mg, 89%) as a white solid. ^{1}H-NMR (d^6-DMSO) δ 0.16 (t, J = 4.9 Hz, 1H), 0.88 (dd, J = 4.9, 8.6 Hz, 1H), 1.08–1.18 (m, 1H), 1.46–1.60 (m, 4H), 3.17 (d, J = 12.2 Hz, 1H), 3.34 (d, J = 12.2 Hz, 1H), 3.91 (d, J = 14.3 Hz, 1H), 4.26 (d, J = 14.3 Hz, 1H), 7.21 (bs, 2H), 8.11 (s, 1H), 8.13 (s, 1H); ^{13}C-NMR (d^6-DMSO) δ 14.8, 22.2 [J(^{13}C,^{31}P) = 3.7 Hz], 23.5 [J(^{13}C,^{31}P) = 20.2 Hz], 27.1, 27.9 [J(^{13}C,^{31}P) = 135.6 Hz], 47.9, 60.1, 118.4, 141.1, 149.8, 152.3, 155.9; ^{31}P-NMR (d^6-DMSO) δ 26.0; UV λ_{max} (0.1 M HCl) 260 sh nm (ε 14,400); HRMS calcd for $C_{12}H_{19}N_5O_4P$ (MH^+) 328.1175, found 328.1177.

2-Amino-6-benzyloxy-9-[(1*R,2*S**)-1-benzoyloxymethyl-2-[(diethoxyphosphinyl)-ethyl]cyclopropan-1-yl]methylpurine (15).** To a mixture of 10 (40.3 mg, 0.109 mmol), triethylamine (5.5 mg, 0.055 mmol) and triphenylphosphine (51.5 mg, 0.196 mmol) in dichloromethane (1.1 mL), was added carbon tetrabromide (65 mg, 0.196 mmol) at 0°C. After this mixture was stirred for 1.5 h at 0°C, phosphate buffer (pH = 7) was added, and the resulting mixture was extracted with dichloromethane. The organic layer was dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The residue was dissolved in dry DMF (1.1 mL), and to the solution were added 2-amino-6-benzyloxypurine (26.2 mg, 0.109 mmol) and potassium carbonate (15.0 mg, 0.109 mmol). After this solution was stirred for 15 h at room temperature, insoluble substances were removed by filtration. The filtrate was concentrated under reduced pressure, and the residue was purified by preparative thin-layer chromatography, eluting with 10% methanol in dichloromethane, to give 15 (17.2 mg, 27%) as a colorless solid. ^{1}H-NMR (CD_3OD) δ 0.94 (t, J = 5.5 Hz, 1H), 1.00 (dd, J = 5.5, 8.6 Hz, 1H), 1.25–1.41 (m, 1H), 1.37 (t, J = 7.2 Hz, 6H), 1.84–2.12 (m, 4H), 4.07–4.25 (m, 7H), 4.63 (d, J = 15.0 Hz, 1H), 5.42 (d, J = 12.3 Hz, 1H), 5.47 (d, J = 12.3 Hz, 1H), 7.32–7.44 (m, 5H), 7.49–7.57 (m, 3H), 7.68–7.74 (m, 2H), 7.98 (s, 1H); ESI MASS, 594.5 (MH^+).

9-[(1*R,2*S**)-2-(Dihydroxyphosphinyl)ethyl-1-hydroxymethylcyclopropan-1-yl]-methylguanine (16).** To a solution of 15 (17.2 mg, 0.029 mmol) in dry DMF (0.3 mL) was added trimethylsilyl bromide (66.9 μL, 0.507 mmol) at room temperature. After the solution was stirred for 47 h, the solvent was removed, coevaporating three times with methanol. The residue was dissolved in 1 M NaOH (0.7 mL). After this mixture was stirred for 3 h, 2 M HCl (0.7 mL) was added. After this mixture was stirred for an additional 30 min, 1 M NaOH (0.7 mL) was added. The mixture was purified by chromatography on a column of reversed-phase C18 silica gel using a gradient of water to 5% methanol in water, to give 16 (10.0 mg, 100%) as a white solid. ^{1}H-NMR (d^6-DMSO) δ 0.48 (dd, J = 4.8, 8.5 Hz, 1H), 0.54 (t, J = 4.8 Hz, 1H), 0.93–0.99 (m, 1H), 1.52–1.72 (m, 4H), 2.76 (d, J = 11.2 Hz, 1H), 3.17 (d, J = 11.2 Hz, 1H), 3.72 (d, J = 14.2 Hz, 1H), 4.33 (d, J = 14.2 Hz, 1H), 6.48 (bs, 2H), 7.65 (s, 1H), 10.54 (bs, 1H); ^{31}P-NMR (d^6-DMSO) δ 26.3; UV λ_{max} (0.1 M HCl) 281 sh nm (ε 6900), 255 (ε 10200); HRMS calcd for $C_{12}H_{19}N_5O_5P$ (MH^+) 344.1124, found 344.1103.

9-[(1*R,2*S**)-1-Benzoyloxymethyl-2-[(diethoxyphosphinyl)-ethyl]cyclopropan-l-yl]-methyladenine (17).** To a mixture of 10 (40.3 mg, 0.109 mmol), triethylamine (5.5 mg, 0.055 mmol) and triphenylphosphine (51.5 mg, 0.196 mmol) in dichloromethane (1.1 mL) was added carbon tetrabromide (65 mg, 0.196 mmol) at 0°C. After the mixture was stirred for 1.5 h, phosphate buffer (pH = 7) was added, and the resulting mixture was extracted with dichloromethane. The organic layer was dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The residue was dissolved in dry DMF (0.5 mL), and the solution was added to a suspension of adenine (14.7 mg, 0.109 mmol) and sodium hydride (60% oil dispersion, 4.35 mg, 0.319 mmol) in dry DMF (0.6 mL). After this mixture was stirred for 15 h at 60°C, insoluble substances were removed by filtration. The filtrate was concentrated under reduced pressure, and the residue was purified by preparative thin-layer chromatography, eluting with 10% methanol in dichloromethane, to give 17 (3.1 mg, 6%) as a white solid. ^{1}H-NMR (CD_3OD) δ 0.98–1.07 (m, 2H), 1.32–1.41 (m, 1H), 1.38 (t, J = 7.2 Hz, 6H), 1.92–2.13 (m, 4H), 4.10–4.23 (m, 6H), 4.28 (d, J = 14.6 Hz, 1H), 4.81 (d, J = 14.6 Hz, 1H), 7.39–7.47 (m, 2H), 7.49–7.63 (m, 1H), 7.63–7.73 (m, 2H), 8.13 (s, 1H), 8.29 (s, 1H); ESI MASS, 488.4 (MH^+).

9-[(1*R,2*S**)-2-(Dihydroxyphosphinyl)ethyl-l-hydroxymethylcyclopropan-1-yl]-methyladenine (18).** To a solution of 17 (3.1 mg, 0.0064 mmol) in dry DMF (63.6 μL) was added trimethylsilyl bromide (14.7 μL, 0.111 mmol) at room temperature. After this mixture was stirred for 65 h, the solvent was removed, coevaporating three times with methanol. The residue was dissolved in 1 M NaOH (1.0 mL). After this mixture was stirred for 2 h, 2 M HCl (0.5 mL) was added. The mixture was purified by chromatography on a column of reversed-phase C18 silica gel using a gradient of water to 5% methanol in water, to

give 18 (2.1 mg, 100%) as a white solid. ^{1}H-NMR (D_2O) δ 0.58–0.78 (m, 2H), 1.04–1.11 (m, 1H), 1.65–1.85 (m, 4H), 3.07 (d, J = 12.0 Hz, 1H), 3.36 (d, J = 12.0 Hz, 1H), 3.99 (d, J = 14.8 Hz, 1H), 4.45 (d, J = 14.8 Hz, 1H), 7.98 (s, 1H), 8.32 (s, 1H); ^{31}P-NMR (d^6-DMSO) δ 26.5; HRMS calcd for $C_{12}H_{19}N_5O_4P$ (MH^+) 328.1175, found 328.1190.

Antiviral Activity

A quantitative CPE reduction assay against HSV-I was performed using the neutral red dye uptake method as described previously.[4] A growth-inhibition assay of MRC-5 cells against HCMV was performed as described previously.[5] An anti-HIV assay was also performed as described previously.[12]

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