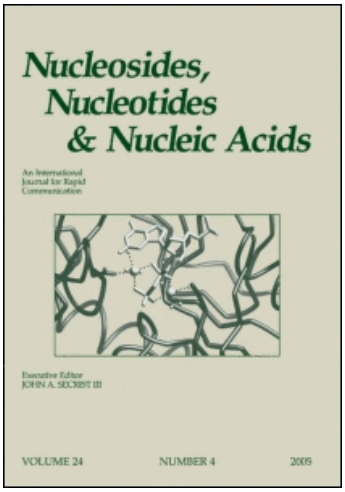

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SYNTHESIS OF CYCLOBUTANE ANALOGUES OF THE ANTIVIRAL CYCLOPROPANE NUCLEOSIDE A-5021

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

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ABSTRACT

Cyclobutane analogues of the antiviral cyclopropane nucleoside A-5021 were synthesized from 1-cyano-1,2-bis(methoxycarbonyl)cyclobutane via 1) isolation of both diastereomers by crystallization, 2) reduction to aminodiol, 3) coupling with 2-amino-4,6-dichloropyrimidine, and 4) guanine ring formation. Despite their structural resemblance to A-5021, the compounds were devoid of antiherpetic activity.

INTRODUCTION

We recently reported the synthesis and antiviral activity of (1′*S*,2′*R*)-9-[[1′,2′-bis(hydroxymethyl)-cycloprop-1′-yl]methyl]guanine (A-5021, 1) (Fig. 1)[1–4]. A-5021 shows extremely potent antiherpetic activity against herpes simplex virus and varicella zoster virus, and greater therapeutic effectiveness than acyclovir in animal models[5]. A-5021 has a unique structure with two asymmetric centers on the cyclopropane ring of the acyclosugar moiety.

*Corresponding author.

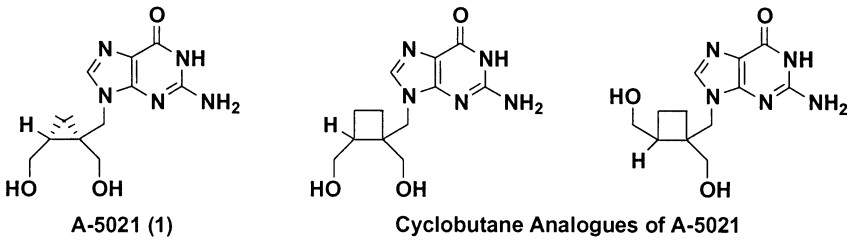
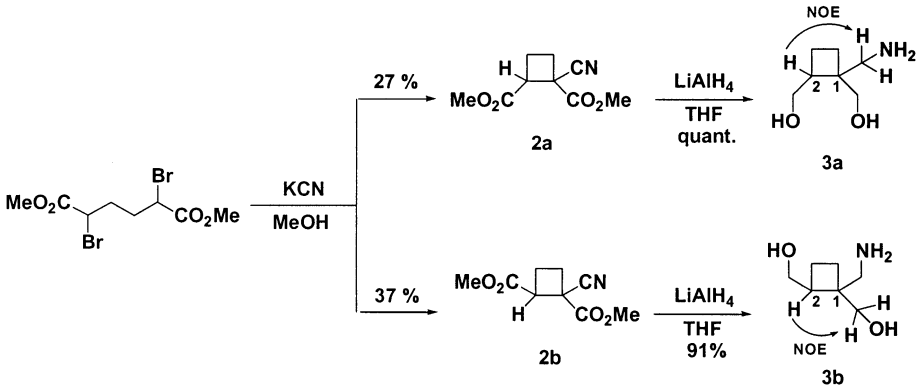


Figure 1.

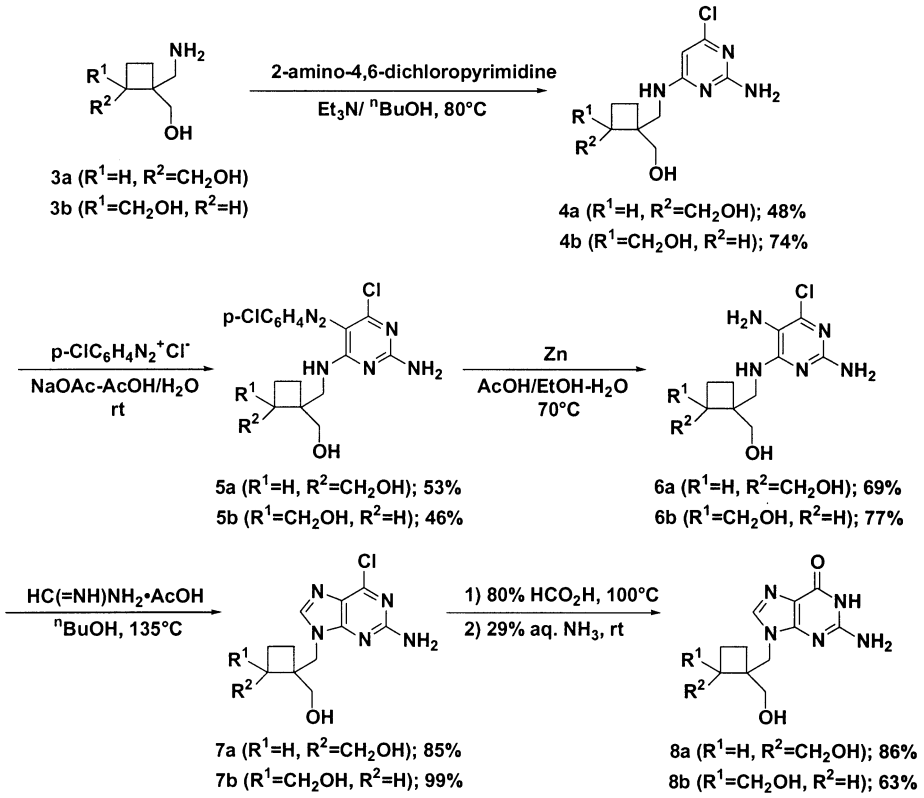
For a better understanding of the structure-activity relationship, preparation of its cyclobutane analogues would be useful. We report here the synthesis of its cyclobutane analogues and their antiviral activity.

RESULTS AND DISCUSSION

1-Cyano-1,2-bis(methoxycarbonyl)cyclobutane, a mixture of the Z-isomer **2a** and the E-isomer **2b**, which was prepared from dimethyl α,α′-dibromoadipate and KCN[6], was used as a starting material (Sch. 1). Since no information is available regarding the purification of its diastereomers, we attempted to isolate them. Although these isomers could not be separated by column chromatography, the Z-isomer **2a** was successfully crystallized from cold methanol in 27% yield. The *E*-isomer **2b** was also obtained after concentration of the mother liquor in 37% yield as a 9:1 mixture of **2b** and **2a**. The structure of each isomer was determined after conversion to an aminodiol. Reduction of **2a** and **2b** with $LiAlH_4$ in THF afforded aminodiol **3a** and **3b**, respectively. Their structures could be clearly determined by NOESY.



Scheme 1.



Scheme 2.

To prepare guanine derivatives from aminodiol **3a** and **3b**, cyclization from a 2,5,6-triamino-4-chloropyrimidine derivative was employed (Sch. 2)[7]. This method is especially useful when an appropriate amine is available as an intermediate. The *Z*-isomer **3a**, the structure that resembles that of A-5021, was reacted with 2-amino-4,6-dichloropyrimidine in the presence of Et_3N to afford the diamine **4a** in 48% yield. The nitrogen atom was introduced to the 5 position of the pyrimidine ring by diazo coupling with 4-chlorobenzenediazonium chloride to give azo compound **5a** in 53% yield. Reduction of **5a** with zinc in aqueous acetic acid gave the desired 2,5,6-triamino-4-chloropyrimidine derivative **6a** in 69% yield. The 2-amino-6-chloropurine derivative **7a** was obtained in 85% yield by acid-catalyzed reaction of **6a** with formamidine acetate. Finally, **7a** was treated with 80% HCO_2H[8] to give the guanine derivative **8a** in 86% yield. Thus, the cyclobutane analogue (**8a**) of the antiviral cyclopropane nucleoside A-5021 was synthesized. The other isomer **8b** was also synthesized from the *E*-isomer **3b** using the same method.

Antiherpetic activity of **8a** and **8b** was measured against HSV-1, and, quite surprisingly, no activity was found in spite of the structural similarity

between **8a** and the highly active A-5021 (1). The olefinic analogues of **1** were reported previously, and both *Z* and *E*-1,2-hydroxymethyl olefins showed moderate activity against HSV-1[1]. On the other hand, the *E*-isomer of **1** showed only weak activity[1]. Differences in torsion angles of the hydroxymethyl groups of these derivatives likely account for the differences in biological activity. It is also possible that the cyclobutane ring, which is the bulkiest in these series, may hinder the interaction with the target enzymes such as thymidine kinase or DNA polymerase. Detailed enzyme inhibitory studies will lead to better understanding of the inactivity of **8a** and **8b**.

EXPERIMENTAL SECTION

Reagents used were of the highest commercially available quality. 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 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). Quantitative CPE reduction assay against HSV-1 was performed using the neutral red dye uptake method as described previously[1].

Dimethyl 1-cyanocyclobutane-1α,2α-dicarboxylate (2a) and dimethyl 1-cyanocyclobutane-1α,2β-dicarboxylate (2b). A mixture of dimethyl 2,5-dibromoadipate (16.5 g, 50.0 mmol), KCN (9.2 g, 140 mmol) and MeOH (10 ml) was refluxed for 67 h. After addition of H_2O, **2a** and **2b** were extracted with EtOAc and the solvent was removed in vacuo. The residue was then dissolved in MeOH and the solution was cooled to ~20°C to afford **2a** as white crystals (2.7 g, 27%). After repeating this crystallization procedure five times, the mother liquor was concentrated to give a 9:1 mixture of **2b** and **2a** as a dark brown oil (3.7 g, 37%). **2a**; ^{1}H-NMR ($CDCl_3$) δ 2.28–2.40 (m, 1H), 2.51–2.76 (m, 3H), 3.67–3.77 (m, 1H), 3.70 (s, 3H), 3.80 (s, 3H); FAB MASS m/z 198 (MH^+). **2b**; ^{1}H-NMR ($CDCl_3$) δ 2.22–2.42 (m, 1H), 2.48–2.71 (m, 3H), 3.68–3.82 (m, 1H), 3.79 (s, 3H), 3.87 (s, 3H); FAB MASS m/z 198 (MH^+).

[1α,2α-Bis(hydroxymethyl)cyclobutane-1-yl]methylamine (3a). $LiAlH_4$ (103 mg, 2.72 mmol) was added to a solution of **2a** (179 mg, 0.907 mmol) in THF (2 ml) at 0°C. After stirring for 5 h at room temperature, a small amount of saturated Na_2SO_4 aqueous solution was added for quenching. After

filtration and washing with hot 2-propanol, the combined solution was concentrated to give **3a** as a colorless oil (132 mg, 100%). ^{1}H-NMR (CD_3OD) δ 1.55–1.79 (m, 3H), 1.88–2.04 (m, 1H), 2.25–2.42 (m, 1H), 2.74 (d, J = 13.8 Hz, 1H), 2.80 (d, J = 13.8 Hz, 1H), 3.54 (dd, J = 5.7, 11.1 Hz, 1H), 3.67 (d, J = 11.4 Hz, 1H), 3.68 (dd, J = 9.9, 11.1 Hz, 1H), 3.87 (d, J = 11.4 Hz, 1H); FAB MASS m/z 146 (MH^+).

[1α,2β-Bis(hydroxymethyl)cyclobutane-1-yl]methylamine (3b). $LiAlH_4$ (243 mg, 6.41 mmol) was added to a solution of **2b** (421 mg, 2.13 mmol) in THF (5 ml) at 0°C. After stirring for 1.5 h at room temperature, a small amount of saturated Na_2SO_4 aqueous solution was added for quenching. After filtration and washing with hot 2-propanol, the combined solution was concentrated to give **3b** as a pale yellow oil (282 mg, 91%). ^{1}H-NMR (CD_3OD) δ 1.54–1.84 (m, 3H), 1.92–2.03 (m, 1H), 2.27–2.38 (m, 1H), 2.85 (d, J = 13.2 Hz, 1H), 2.96 (d, J = 13.2 Hz, 1H), 3.52 (dd, J = 5.4, 11.1 Hz, 1H), 3.62 (d, J = 10.7 Hz, 1H), 3.64 (d, J = 10.7 Hz, 1H), 3.72 (dd, J = 9.9, 11.0 Hz, 1H); FAB MASS m/z 146 (MH^+).

Determination of the Structures of 3a and 3b by NOESY

In the case of the *Z*-isomer **3a**, NOE was observed between the proton at position 2 of the cyclobutane and the methylene proton of the aminomethyl group at position 1. On the other hand, in the case of the *E*-isomer **3b**, NOE was observed between the proton at position 2 of the cyclobutane and the methylene proton of the hydroxymethyl group at position 1.

2-Amino-4-chloro-6-[1′α,2′α-bis(hydroxymethyl)cyclobutane-1′-yl]methylaminopyrimidine (4a). A mixture of aminodiol **3a** (519 mg, 3.57 mmol), 2-amino-4,6-dichloropyrimidine (880 mg, 5.37 mmol) and Et_3N (720 mg, 7.12 mmol) in 1-butanol (40 ml) was heated for 63 h at 80°C. The mixture was concentrated in vacuo and the product was purified by silica gel chromatography eluting with a gradient of 1–10% MeOH in CH_2Cl_2 to give **4a** (472 mg, 48%) as a white solid (melting point?). ^{1}H-NMR (CD_3OD) δ 1.60–1.87 (m, 3H), 1.96–2.07 (m, 1H), 2.32–2.44 (m, 1H), 3.43 (d, J = 13.8 Hz, 1H), 3.48–3.66 (m, 2H), 3.56 (dd, J = 6.3, 11.0 Hz, 1H), 3.74 (dd, J = 9.3, 11.0 Hz, 1H), 3.80 (d, J = 11.4 Hz, 1H), 5.90 (s, 1H); FAB MASS m/z 273 (MH^+).

2-Amino-4-chloro-5-(4-chlorophenyl)azo-6-[1′α,2′α-bis(hydroxymethyl)-cyclobutane-1′-yl]methyl-aminopyrimidine (5a). A solution of $NaNO_2$ (100 mg, 1.45 mmol) in H_2O (0.59 ml) was added to a solution of 4-chloroaniline (182 mg, 1.42 mmol) in 2.5 mol/l HCl (2.4 ml) at 0°C. The resultant cold aqueous solution of 4-chlorobenzenediazonium chloride was added dropwise to a mixture of diamine **4a** (239 mg, 0.875 mmol), $NaOAc{\cdot}3H_2O$

(1.15 g), AcOH (4.2 ml) and H_2O (4.2 ml) at 0°C. After stirring for 12 h at room temperature, the mixture was cooled to 0°C. The precipitate was collected and washed with cold H_2O to give **5a** as a yellow solid (189 mg, 53%). ^{1}H-NMR (DMSO-d_6) δ 1.56–1.69 (m, 3H), 1.85–1.96 (m, 1H), 2.16–2.28 (m, 1H), 3.35–3.45 (m, 1H), 3.48–3.63 (m, 3H), 3.72–3.82 (m, 2H), 4.43 (t, J = 5.3 Hz, 1H), 4.93 (t, J = 4.8 Hz, 1H), 7.50–7.63 (m, 2H), 7.72–7.78 (m, 2H), 10.41–10.44 (m, 1H); FAB MASS m/z 411 (MH^+).

2,5-Diamino-4-chloro-6-[1′α,2′α-bis(hydroxymethyl)cyclobutane-1′-yl]-methyl-aminopyrimidine (6a). Zinc (136 mg, 2.08 mmol) was added to a solution of azo compound **5a** (185 mg, 0.451 mmol) and AcOH (0.54 ml) in 67% EtOH (20.7 ml) at 70°C, and the mixture was stirred for 15 h. After filtration of insoluble materials, the mixture was concentrated in vacuo and the product was purified by silica gel chromatography eluting with a gradient of 2–10% MeOH in CH_2Cl_2 to give **6a** as a white solid (melting point?) (89 mg, 69%). ^{1}H-NMR (CD_3OD) δ 1.62–1.76 (m, 2H), 1.76–1.91 (m, 1H), 1.96–2.08 (m, 1H), 2.36–2.49 (m, 1H), 3.56 (dd, J = 5.7, 11.3 Hz, 1H), 3.57 (d, J = 12.0 Hz, 1H), 3.58 (d, J = 13.8 Hz, 1H), 3.63 (d, J = 13.8 Hz, 1H), 3.74 (dd, J = 9.5, 11.3 Hz, 1H), 3.82 (d, J = 12.0 Hz, 1H); FAB MASS m/z 288 (MH^+).

2-Amino-6-chloro-9-[1′α,2′α-bis(hydroxymethyl)cyclobutane-1′-yl]-methylpurine (7a). A solution of triamine **6a** (85.5 mg, 0.297 mmol) and formamidine acetate (34.0 mg, 0.327 mmol) in 1-butanol (3 ml) was heated to 135°C for 20 h. The mixture was concentrated in vacuo and the resultant residue was treated with 1 M HCl (3 ml) for 20 min. After neutralization using K_2CO_3, the product was extracted with EtOAc and the solvent was removed in vacuo to give **7a** as a white solid (75 mg, 85%). ^{1}H-NMR (CD_3OD) δ 1.54–1.77 (m, 2H), 1.94–2.10 (m, 2H), 2.48–2.62 (m, 1H), 3.41 (d, J = 12.0 Hz, 1H), 3.54 (dd, J = 6.2, 11.5 Hz, 1H), 3.68 (dd, J = 9.0, 11.5 Hz, 1H), 3.81 (d, J = 12.0 Hz, 1H), 4.31 (d, J = 14.4 Hz, 1H), 4.36 (d, J = 14.4 Hz, 1H), 8.09 (s, 1H); FAB MASS m/z 298 (MH^+).

9-[1′α,2′α-bis(hydroxymethyl)cyclobutane-1′-yl]methylguanine (8a). 2-Amino-6-chloropurine derivative **7a** (45.8 mg, 0.154 mmol) was heated in 80% formic acid (2 ml) at 100°C for 2 h. The mixture was concentrated in vacuo and the resultant residue was treated with 29% aq. ammonia (2 ml) for 1 h at room temperature. After evaporation, the product was purified by reversed-phase chromatography eluting with a gradient of 0–20% MeOH in H_2O to give **8a** as a white solid (melting point?) (37.0 mg, 86%). mp 299–301°C; ^{1}H-NMR (DMSO-d_6) δ 1.41–1.65 (m, 2H), 1.73–1.89 (m, 2H), 2.24–2.35 (m, 1H), 3.21–3.38 (m, 2H), 3.42–3.58 (m, 2H), 4.00 (d, J = 14.3 Hz, 1H), 4.05 (d, J = 14.3 Hz, 1H), 4.35 (t, J = 5.3 Hz, 1H), 4.94 (t, J = 5.4 Hz, 1H), 6.49 (brs, 2H), 7.62 (s, 1H); HRMS calcd for $C_{12}H_{18}O_3N_5$ (MH^+) 280.1410, found 280.1404.

2-Amino-4-chloro-6-[1′α,2′β-bis(hydroxymethyl)cyclobutane-1′-yl]methylaminopyrimidine (4b). A mixture of aminodiol **3b** (270 mg, 1.86 mmol), 2-amino-4,6-dichloropyrimidine (460 mg, 2.80 mmol) and Et_3N (380 mg, 3.76 mmol) in 1-butanol (20 ml) was heated for 65 h at 90°C. The mixture was concentrated in vacuo and the product was purified by silica gel chromatography eluting with a gradient of 2–10% MeOH in CH_2Cl_2 to give **4b** (394 mg, 74%) as a white solid. ^{1}H-NMR (CD_3OD) δ 1.62–1.84 (m, 3H), 1.96–2.08 (m, 1H), 2.28–2.42 (m, 1H), 3.49 (d, J = 11.1 Hz, 1H), 3.53 (d, J = 11.1 Hz, 1H), 3.56–3.66 (m, 2H), 3.62 (dd, J = 6.3, 10.9 Hz, 1H), 3.73 (dd, J = 8.9, 10.9 Hz, 1H), 5.91 (s, 1H); FAB MASS m/z 273 (MH^+).

2-Amino-4-chloro-5-(4-chlorophenyl)azo-6-[1′α,2′β-bis(hydroxymethyl)cyclobutane-1′-yl]methyl-aminopyrimidine (5b). A solution of $NaNO_2$ (158 mg, 2.29 mmol) in H_2O (0.94 ml) was added to a solution of 4-chloroaniline (288 mg, 2.26 mmol) in 2.5 M HCl (3.84 ml) at 0°C. The resultant cold aqueous solution of 4-chlorobenzenediazonium chloride was added dropwise to a mixture of diamine **4b** (377 mg, 1.38 mmol), $NaOAc \cdot 3H_2O$ (1.82 g), AcOH (6.7 ml) and H_2O (6.7 ml) at 0°C. After stirring for 12 h at room temperature, the mixture was cooled to 0°C. The precipitate was collected and washed by cold H_2O to give **5b** as a yellow solid (melting point?) (262 mg, 46%). ^{1}H-NMR (DMSO-d_6) δ 1.56–1.68 (m, 2H), 1.69–1.78 (m, 1H), 1.84–1.95 (m, 1H), 2.15–2.28 (m, 1H), 3.36–3.84 (m, 6H), 7.52–7.60 (m, 2H), 7.72–7.78 (m, 2H), 10.34–10.38 (m, 1H); FAB MASS m/z 411 (MH^+).

2,5-Diamino-4-chloro-6-[1′α,2′β-bis(hydroxymethyl)cyclobutane-1′-yl]-methyl-aminopyrimidine (6b). Zinc (190 mg, 2.91 mmol) was added to a solution of azo compound **5b** (258 mg, 0.627 mmol) and AcOH (0.5 ml) in 67% EtOH (18.9 ml) at 70°C, and the mixture was stirred for 1.5 h. After filtration of insoluble materials, the mixture was concentrated in vacuo and the product was purified by silica gel chromatography eluting with a gradient of 2–10% MeOH in CH_2Cl_2 to give **6b** as a white solid (melting point?) (138 mg, 77%). ^{1}H-NMR (CD_3OD) δ 1.64–1.88 (m, 3H), 1.97–2.10 (m, 1H), 2.25–2.38 (m, 1H), 3.52 (d, J = 11.6 Hz, 1H), 3.54 (d, J = 11.6 Hz, 1H), 3.64 (d, J = 14.0 Hz, 1H), 3.65 (dd, J = 5.7, 11.0 Hz, 1H), 3.78 (dd, J = 9.2, 11.0 Hz, 1H), 3.78 (d, J = 14.0 Hz, 1H); FAB MASS m/z 288 (MH^+).

2-Amino-6-chloro-9-[1′α,2′β-bis(hydroxymethyl)cyclobutane-1′-yl]-methylpurine (7b). A solution of triamine **6b** (124 mg, 0.429 mmol) and formamidine acetate (50.0 mg, 0.472 mmol) in 1-butanol (4.5 ml) was heated to 135°C for 10 h. The mixture was concentrated in vacuo and the resultant residue was treated with 1 mol/1 HCl (4 ml) for 20 min. After neutralization using K_2CO_3, the product was extracted with AcOEt and the solvent was removed in vacuo to give **7b** as a white solid (melting point?) (127 mg, 99%). ^{1}H-NMR (CD_3OD) δ 1.49–1.63 (m, 1H), 1.84–1.96 (m, 1H), 1.96–2.14 (m, 2H),

2.46–2.59 (m, 1H), 3.32 (d, J = 11.7 Hz, 1H), 3.49 (d, J = 11.7 Hz, 1H), 3.73 (dd, J = 6.2, 11.1 Hz, 1H), 3.83 (dd, J = 8.6, 11.1 Hz, 1H), 4.44 (d, J = 14.4 Hz, 1H), 4.51 (d, J = 14.4 Hz, 1H), 8.10 (s, 1H); FAB MASS m/z 298 (MH^+).

9-[1′α,2′β-bis(hydroxymethyl)cyclobutane-1′-yl]methylguanine (8b). 2-Amino-6-chloropurine derivative **7b** (78.7 mg, 0.154 mmol) was heated in 80% formic acid (2 ml) at 100°C for 2 h. The mixture was concentrated in vacuo and the resultant residue was treated with 29% aq. ammonia (2 ml) for 1 h at room temperature. After evaporation, the product was purified by reversed-phase chromatography eluting with a gradient of 0–20% MeOH in H_2O to give **8b** as a white solid (46.7 mg, 63%). mp 302–304°C; 1H-NMR (DMSO-d_6) δ 1.33–1.45 (m, 1H), 1.67–1.78 (m, 1H), 1.78–2.00 (m, 2H), 2.32–2.42 (m, 1H), 3.08–3.29 (m, 2H), 3.46–3.66 (m, 2H), 4.10 (d, J = 14.3 Hz, 1H), 4.24 (d, J = 14.3 Hz, 1H), 4.50 (t, J = 4.8 Hz, 1H), 4.82 (t, J = 5.4 Hz, 1H), 6.46 (brs, 2H), 7.61 (s, 1H), 10.54 (brs, 1H); HRMS calcd for $C_{12}H_{18}O_3N_5$ (MH^+) 280.1410, found 280.1418.

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