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Nucleosides and Nucleotides. 132. Synthesis and Biological Evaluations of Ring-Expanded Oxetanocin Analogues: Purine and Pyrimidine Analogues of 1,4-Anhydro-2-deoxy-D-arabitol and 1,4-Anhydro-2-deoxy-3-hydroxymethyl-D-arabitol[1]

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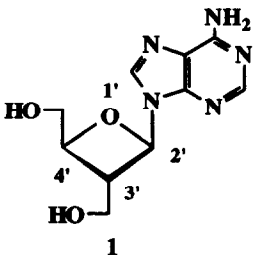
Abstract: (2*R*)-2-*C*-(Adenin-9-yl)-1,4-anhydro-2-deoxy-D-arabitol (**2**) and (2*R*, 3*R*)-2-*C*-(adenin-9-yl)-1,4-anhydro-2,3-dideoxy-3-*C*-hydroxymethyl-D-arabitol (**3**), and their 2,6-diaminopurine analogues **4** and **5** were synthesized from corresponding 1,4-anhydro-D-ribitol derivatives, which were readily obtained from D-glucose. The corresponding guanine isonucleosides **6** and **7** were obtained from **4** and **5** by enzymatic deamination with adenosine deaminase. Pyrimidine counterparts **8** and **9** were synthesized via construction of the pyrimidine moiety from amino derivatives of the 1,4-anhydro-D-arabitol derivatives. Antiviral activity of these ring-expanded derivatives of oxetanocins towards HSV-1, HSV-2, HCMV, and HBV *in vitro* was examined along with their cytotoxicity against L1210 and KB cells *in vitro*.

INTRODUCTION

Oxetanocin A (OXT-A, **1**), which was isolated from *Bacillus megaterium NK84-0218*, has a unique structure bearing an oxetanose instead of a furanose in the sugar moiety of the nucleoside.[2] Due to its unique structure and biological activity, including anti-HIV activity, various analogues of OXT-A both at the sugar and the base moieties have been synthesized to improve its chemotherapeutic index.[3] A guanine congener of OXT-A (OXT-G) and a carbocyclic OXT-G also had potent antiviral activities against HSV and HBV,[3] and their 5'-triphosphates[4] were found to be incorporated into DNA molecules and to terminate the elongation. The 2-fluoroadenine analogue was much more potent against HIV-1 than OXT-A, due in part to not being a substrate of adenosine deaminase.[3b] Therefore, these OXT analogues would be phosphorylated by virus and/or cellular kinases similarly to other antiviral nucleosides. Although the sugar moiety of OXT analogues



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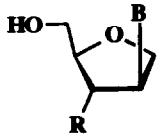
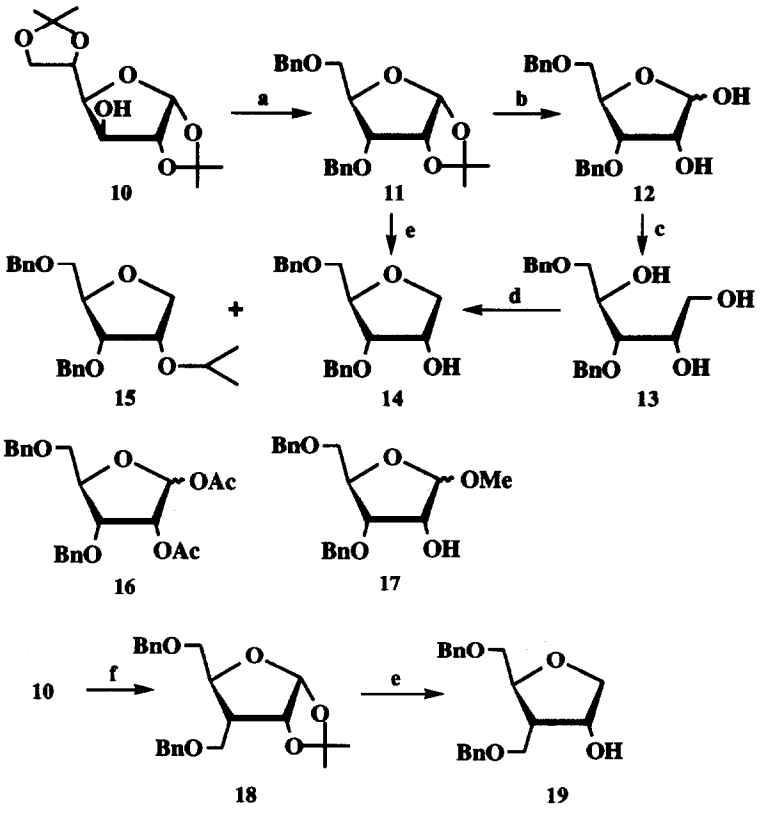


2 ; B = adenine, R = OH
3 ; B = adenine, R = CH_2OH
4 ; B = 2,6-diaminopurine, R = OH
5 ; B = 2,6-diaminopurine, R = CH_2OH
6 ; B = guanine, R = OH
7 ; B = guanine, R = CH_2OH
8 ; B = cytosine, R = OH
9 ; B = cytosine, R = CH_2OH

are constituted by the oxetanose and the cyclobutane ring with two primary hydroxyl groups at 3' and 4' positions, it is surprising that such nucleosides were recognized as substrates of kinases. Therefore, further modified-sugar nucleosides could be selectively recognized by the less substrate-specific viral kinases without affecting cellular enzymes. In our efforts to find out new antiviral sugar-modified nucleosides we design ring-expanded oxetanocin analogues, purine and pyrimidine derivatives of 1,4-anhydro-D-arabitol analogues **2-9**, which are isonucleosides without having glycosyl linkages. From these nucleosides, we would expect to avoid enzymatic deactivations such as glycosyl bond cleavage reactions by phosphorylases and also chemical stability due to having no glycosyl linkage in the molecules. In this report, we describe the synthesis of these nucleosides and their antiviral activity.[5]

Chemistry. To synthesize the target nucleosides, we required tetrahydrofuran derivatives **14** and **19** in large quantities. First, we examined the synthesis of 1,4-anhydro-3,5-di-*O*-benzyl-D-ribitol (**14**) from 1,2:5,6-di-*O*-isopropylidene-α-D-glucose (**10**). Conversion of **10** to 3,5-di-*O*-benzyl-1,2-isopropylidene-α-D-ribofuranose (**11**) was done using modifications of the literature methods.[6] After hydrolysis of the isopropylidene group by aqueous 80% AcOH, **12** was obtained, which was treated with $NaBH_4$ in MeOH to

Scheme 1[a]

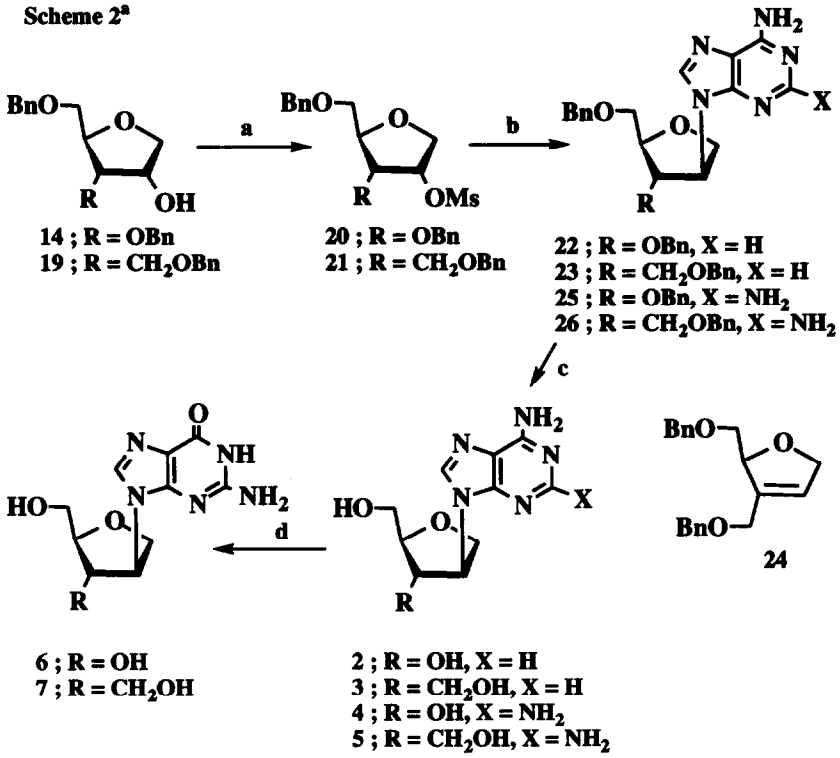


[a]Reagents and conditions: a) ref. 6; b) 80% AcOH, 100 °C; c) $NaBH_4$, MeOH; d) Ph_3P, DEAD, THF, 60 °C; e) Et_3SiH, TMSOTf, CH_2Cl_2, -18 °C to room temperature; f) ref. 8, 9.

afford the triol **13** in 74% yield from **11**. On treatment of **13** with diethyl azodicarboxylate and triphenylphosphine in THF, the desired **14** was obtained. However, even after column chromatographic purifications, a large amount of diethyl hydrazinodicarboxylate was contaminated with **14** and this was hardly separable. Since this sequence to prepare **14** was rather lengthy and had separation problems, we next examined the reduction of the anomeric position of **11** regioselectively.

When **11** was treated with Et_3SiH[7] in the presence of $BF_3{\cdot}Et_2O$ in CH_2Cl_2 at 0 °C to room temperature, the desired tetrahydrofuran-3-ol **14** was obtained in 53% yield. Using trimethylsilyl trifluoromethanesulfonate (TMSOTf), the yield was improved to 70%. However, formation of (2*R*, 3*R*, 4*R*)-3-benzyloxy-2-[(benzyloxy)methyl]-4-(isopropoxy)tetrahydrofuran (**15**) was observed. We next examined the reaction using di-*O*-acetate **15**, methyl riboside **17** (these were obtained from **12**), or **12**, which were treated under the same conditions, however, the desired **14** was obtained in 40-50% yield along with several unidentified compounds in each case (data not shown). Similarly, (3*S*)-1,4-anhydro-5-*O*-benzyl-3-*C*-benzyloxymethyl-3-deoxy-D-ribitol (**19**) was prepared from **18**, which was obtained from **10** according to the reference procedures,[8,9] in 85% yield using Et_3SiH and TMSOTf (Scheme 1).

Scheme 2[a]



14 ; R = OBn
19 ; R = CH_2OBn

20 ; R = OBn
21 ; R = CH_2OBn

22 ; R = OBn, X = H
23 ; R = CH_2OBn, X = H
25 ; R = OBn, X = NH_2
26 ; R = CH_2OBn, X = NH_2

6 ; R = OH
7 ; R = CH_2OH

2 ; R = OH, X = H
3 ; R = CH_2OH, X = H
4 ; R = OH, X = NH_2
5 ; R = CH_2OH, X = NH_2

[a]Reagents and conditions: a) MsCl, Et_3N, DMAP, CH_2Cl_2, room temperature; b) nucleobase, K_2CO_3, 18-crown-6, DMF, 120 °C; c) BCl_3, CH_2Cl_2, -78 °C to -18 °C; d) adenosine deaminase, pH 7.5, 37 °C.

Methanesulfonylation of **14** and **19** gave the corresponding mesylates **20** and **21** in 96% and 97% yields, respectively, which were then treated with nucleobases in the presence of K_2CO_3 and 18-crown-6 in

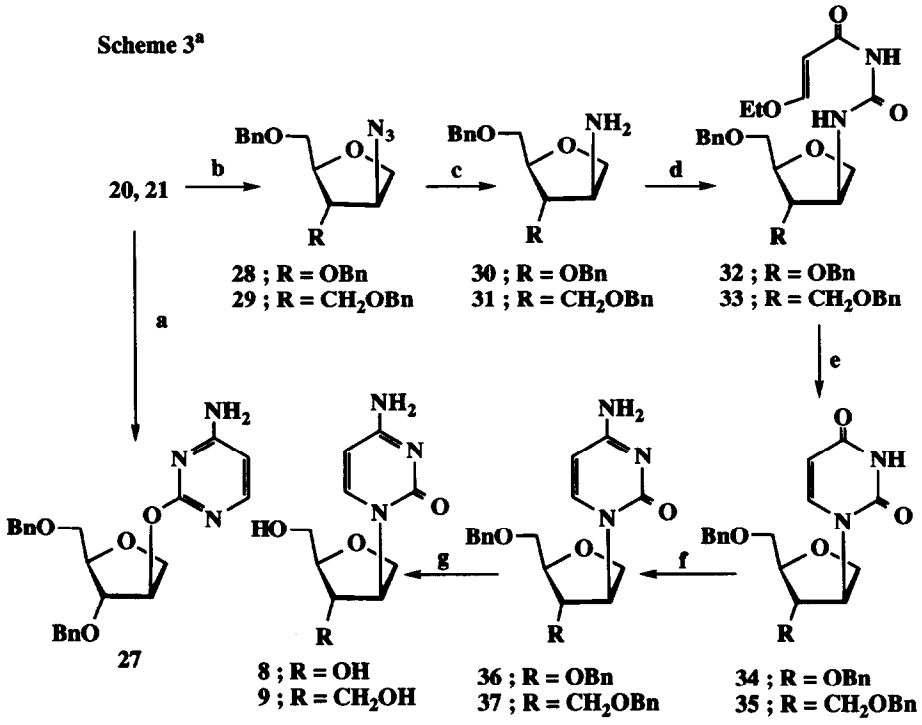
DMF at 120 °C (Scheme 2).[10] Reactions of **20** with adenine gave the desired nucleosides **22** in 50% yield. However, under the same conditions, **23** was obtained only in 37% yield, along with an elimination product, (2*S*)-2,3-bis[(benzyloxy)methyl]-2,5-dihydrofuran **24** in 49% yield. At lower temperatures, the reactions did not proceed well and at higher temperatures, the yields of the desired compounds were reduced. Structures of these nucleosides were identified by their UV, ^{1}H NMR, MS, and elemental analyses. Deblocking of the benzyl groups in these nucleosides was done using BCl_3 in CH_2Cl_2 at -78 °C to furnish free ring-enlarged oxetanocin derivatives **2** and **3** in good yields.

For the synthesis of guanine derivatives **6** and **7**, similar substitution reactions of **20** and **21** with 2-amino-6-chloropurine gave only a complex mixture. However, the reactions with 2,6-diaminopurine afforded **25** and **26** in 62% and 32% yields, respectively. The benzyl groups in both derivatives were removed similarly and the resulting **4** and **5** were treated with adenosine deaminase from calf intestine. Although the reactions catalyzed by the deaminase proceeded very slowly at 37 °C in phosphate buffer (pH 7.5), the desired guanine analogues **6** and **7** were obtained in 76% and 62%, respectively, after 10 days. Substrate specificity of the adenosine deaminase was well characterized and for substrates the presence of a primary alcohol at the 5' positions was essential.[11] The slow rate of the reactions therefore may be due to different distances between the base moiety and the hydroxyl group with naturally occurring adenosine and synthetic 2,6-diaminopurine riboside within the active site of the enzyme.

We next examined the synthesis of pyrimidine congeners (Scheme 3). Nucleophilic substitutions of **20** with cytosine under the same conditions described in the synthesis of purine analogues afforded a one cytosine analogue **27** in 55% yield. However, the UV spectrum of **27** in acidic MeOH differed from that characteristic of the corresponding N^1-alkylcytosine derivative and was akin to the *O*-alkyl derivative. Therefore, pyrimidine derivatives were constructed from amino sugars **30** and **31**, which were readily accessible from **20** and **21** by nucleophilic substitution with LiN_3, followed by hydrogenation using Lindlar catalyst under hydrogen. The uracil ring was constructed by the procedures of Shaw and Warrener.[12] The uracil derivatives **34** and **35** were converted into cytosine congeners **36** and **37** by a conventional method followed by debenzylation with BCl_3 in CH_2Cl_2 giving the desired nucleosides **8** and **9** in good overall yields.

Biological Activities. We evaluated all the ring-expanded oxetanocin analogues synthesized, together with ACV and DHPG as reference compounds, for antiviral activities against several pathogenic viruses (HSV-1, HSV-2, HCMV, and HBV), because oxetanocins and their analogues have been shown to be effective against these viruses. We also evaluated the compounds for cytotoxic effects on proliferation of mouse leukemia L1210 cells and human oral epidermoid carcinoma KB cells. The results are summarized in Table 1. Of these, compounds **6** and **7**, which have a guanine as the nucleobase, had obvious antiviral activities against HSV-1 (EC_{50} = 4.1, and 2.8 μg/mL, respectively) and HSV-2 (EC_{50} = 6.1, and 2.2 μg/mL, respectively), while not showing cytotoxic effects on either L1210 or KB cells at a concentration up to 100 μg/mL. However, their anti-HSV potency was approximately 10-fold lower than that of ACV, and these were inactive against other viruses tested. These selective antiviral activities of **6** and **7** against HSV-1 and -2 may indicate that these guanine derivatives could be phosphorylated specifically by herpes virus-encoded thymidine kinase in infected cells as ACV.

It was noteworthy that adenine derivative **3** had a significant antiviral activity against HBV (EC_{50} = 0.68 μg/mL), with somewhat lower activity against other viruses tested (EC_{50}: HSV-1, 9.0; HSV-2, 10.2;



[a]Reagents and conditions: a) cytosine, K_2CO_3, 18-crown-6, DMF, 120 °C; b) LiN_3, DMF, 100 °C; c) Lindlar cat. H_2, MeOH-AcOEt; d) EtOCH=CHCONCO, benzene-DMF, -18 °C to room temperature; e) 2.8% NH_4OH, acetone, 70 °C or 2 N H_2SO_4, dioxane, 100 °C; f) TPSCl, Et_3N, DMAP, CH_3CN, then conc. NH_4OH; g) BCl_3, CH_2Cl_2, -78 °C to -18 °C or BF_3·OEt, Ac_2O then NH_3/MeOH.

Table 1. **Antiviral Activity and Cytotoxicity of Ring-Expanded Oxetanocin Analogues.**[a]

	EC_{50} (μg/mL)				IC_{50} (μg/mL)	
compds	HSV-1	HSV-2	HCMV	HBV	L1210	KB
2	19.8	23.6	19.2	> 10	18	> 100
4	> 100	> 100	> 100	> 100	> 100	> 100
6	4.1	6.1	> 100	> 100	> 100	> 100
8	> 100	> 100	> 100	> 100	> 100	> 100
3	9.0	10.2	1.16	0.68	2.7	52
5	29.0	29.2	27.0	6.6	> 100	> 100
7	2.8	2.2	95	> 100	> 100	> 100
9	13.7	21.5	28.3	> 100	56	> 100
ACV	0.23	0.34	ND	ND	ND	ND
DHPG	ND	ND	1.34	ND	ND	ND

[a]Assay methods see Experimental Section. ND: not determined.

and HCMV, 1.2 μg/mL, respectively). Although **3** showed undesirable cytotoxicity against rapidly growing tumor cell lines (IC_{50} = 2.7 μg/mL on L1210 cells and IC_{50} = 52 μg/mL on KB cells, respectively), it may not affect normal cells in stationary phase. From the antiviral spectra, it was suggested that guanine derivatives **6** and **7** and adenine derivative **3** might have different antiviral mechanisms of action.

On the other hand, cytosine derivatives **8** and **9**, and diaminopurine derivatives **4** and **5** showed only insignificant effects in these evaluation systems.

These preliminary biological results suggested that the ring-expanded oxetanocin analogues, having a nucleobase at the 2-position of tetrahydrofuran ring, could be antimetabolites to natural nucleosides having a base at the 1-position, in which both hydroxyl and hydroxymethyl groups at the 3-position of the analogues could be functioned as the 3'-hydroxyl group of natural nucleosides. Based on the non-glycosidic structure of them, these would be stable chemically as well as biologically, which would be desired for their pharmacological use. Detailed biological evaluation of these compounds is now under investigation.

EXPERIMENTAL SECTION

General Methods. Melting points were measured on a Yanagimoto MP-3 micromelting point apparatus and are uncorrected. The ^1H NMR spectra were recorded on a JEOL JNM-GX 270 (270 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). All exchangeable protons were detected by addition of D_2O. The nOe measurements were done using JEOL JNM-GX 270 and the solutions (about 0.05 M) were degassed by bubbling N_2 through them followed by ultrasound sonication. UV absorption spectra were recorded with a Shimadzu UV-240 spectrophotometer. Mass spectra (MS) were measured on a JEOL JMX-DX303 spectrometer. TLC was done on Merck Kieselgel F254 precoated plates. The silica gel used for column chromatography was YMC gel 60A (70-230 mesh).

3,5-Di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose (11). Compound **10** (16.0 g, 61.5 mmol) was dissolved in 80% AcOH (200 mL) and the mixture was stirred for 9 h at room temperature. The reaction mixture was concentrated *in vacuo* and coevaporated with EtOH (x 3). A solution of $NaIO_4$ (19.7 g, 92.3 mmol) in H_2O (80 mL) was added to a solution of the residue in MeOH (80 mL) at 0 °C and the mixture was stirred for 0.5 h at room temperature. Ethylene glycol (5 mL) was added to the reaction mixture and the mixture was further stirred for 0.5 h. Insoluble materials were removed by filtration and the filtrate was concentrated *in vacuo*. A solution of $NaBH_4$ (3.49 g, 92.3 mmol) in H_2O (80 mL) was added to a solution of the residue in MeOH (80 mL) at 0 °C and the mixture was stirred for 0.5 h at room temperature. The reaction mixture was neutralized by addition of AcOH and then concentrated *in vacuo*. The residue was coevaporated with EtOH (x 3) and toluene (x 3). A solution of the residue in DMF (80 mL) was added dropwise to a suspension of NaH (60%, 6.15 g, 154 mmol) in DMF (120 mL) at 0 °C and the mixture was stirred for 0.5 h at room temperature under argon. BnBr (21.9 mL, 185 mmol) was added to the stirred mixture at 0 °C and the mixture was stirred for 13 h at room temperature under argon. 1 M NH_4Cl (200 mL) was added to the reaction mixture at 0 °C and the mixture was stirred for 0.5 h at room temperature. H_2O (200 mL) was added to the mixture and the whole was extracted with Et_2O (3x200 mL), the separated organic phase was washed with H_2O (3 x 150 mL), brine (300 mL), and dried (Na_2SO_4). The solvent was concentrated and the residue was purified on a silica gel column (7.6 x 15 cm) with 20% AcOEt in hexane to

give **11** (16.3 g, 72%, as a colorless oil): EI-MS *m/z* 370 (M^+), 355 (M^+-CH_3), 279 (M^+-Bn); ^{1}H-NMR ($CDCl_3$) 7.35–7.26 (10 H, m, Ph x 2), 5.76 (1 H, d, H-1, $J_{1,2}$ = 3.7 Hz), 4.73 and 4.57 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.57-4.55 (1 H, m, H-2), 4.54 and 4.49 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.18 (1 H, ddd, H-4, $J_{4,5a}$ = 2.2, $J_{4,5b}$ = 3.7, $J_{4,3}$ = 9.1 Hz), 3.86 (1 H, dd, H-3, $J_{3,4}$ = 9.1, $J_{3,2}$ = 4.4 Hz), 3.75 (1 H, dd, H-5a, $J_{a,4}$ = 2.2, $J_{a,b}$ = 9.4 Hz), 3.56 (1 H, dd, H-5b, $J_{b,4}$ = 3.7, $J_{b,a}$ = 9.4 Hz).

3,5-Di-*O*-benzyl-D-ribofuranose (12). A solution of **11** (1.38 g, 3.73 mmol) in aqueous 80% AcOH (20 mL) was heated at 100 °C for 9 h. The solvent was removed *in vacuo* and the residue was coevaporated with EtOH (x 3). The residue was purified on a silica gel column (3.3 x 10.5 cm) with hexane/AcOEt (2:1) to give **12** (1.08 g, 87.8% as a white solid): EI-MS *m/z* 370 (M^+), 330 (M^+), 239 (M^+-Bn); ^{1}H-NMR ($CDCl_3$) 7.39–7.26 (10 H, m, Phx2), 5.27 (0.5 H, dd, H-1 (α), $J_{1,2}$ = 4.4, $J_{1,OH}$ = 9.3 Hz), 5.23 (0.5 H, d, H-1 (β), $J_{1,OH}$ = 7.3 Hz), 4.63 and 4.45 (4 H, m, PhCH_2O x 2), 4.30-3.96 (3 H, m, H-2, 3, 4), 3.67-3.43 (2 H, m, H-5a, b), 3.63 (0.5 H, d, 1-OH (α), $J_{OH,1}$ = 9.3 Hz), 3.38 (0.5 H, d, 1-OH (β), $J_{OH,1}$ = 7.3 Hz), 2.88 (0.5 H, d, 2-OH (α), $J_{OH,2}$ = 8.1 Hz), 2.69 (0.5 H, d, 2-OH (β), $J_{OH,2}$ = 3.3 Hz).

(2*S*, 3*S*, 4*R*)-3,5-Dibenzyloxypentan-1,2,4-triol (13). $NaBH_4$ (118 mg, 3.12 mmol) was added to a solution of **12** (515 mg, 1.56 mmol) in MeOH (10 mL). After being stirred for 6 h at room temperature, the mixture was neutralized with AcOH and the solvent was removed *in vacuo*. The residue was purified on a silica gel column (3.3 x 6.5 cm) with 1-2% EtOH in $CHCl_3$ to give **13** (499 mg, 77% as a syrup): EI-MS *m/z* 241 (M^+-Bn); ^{1}H-NMR ($CDCl_3$) 7.35-7.23 (10 H, m, Ph x 2), 4.93 (1 H, d, 4-OH, $J_{OH,4}$ = 4.9 Hz), 4.71 (1 H, d, 2-OH, $J_{OH,2}$ = 5.0 Hz), 4.61 and 4.49 (each 2 H, s, PhCH_2O), 4.43 (1 H, t, 1-OH, $J_{OH,1}$ = 6.0 Hz), 3.93-3.91 (1 H, m, H-3), 3.67-3.36 (6 H, m, H-1a, b, 2, 4, 5a, b).

1,4-Anhydro-3,5-di-*O*-benzyl-D-ribitol (14). (**a**) A solution of TMSOTf (499 μL, 2.58 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a solution of **11** (477 mg, 1.29 mmol) in CH_2Cl_2 (20 mL) at 0 °C and the mixture was stirred for 0.5 h at room temperature under argon. Et_3SiH (1.03 mL, 6.45 mmol) was added to the stirred mixture at 0 °C and the mixture was stirred for 18 h at room temperature under argon. Saturated aqueous $NaHCO_3$ (5 mL) was added to the mixture and then H_2O (50 mL) was added, the whole was extracted with CH_2Cl_2 (2 x 40 mL), the separated organic phase was washed with saturated $NaHCO_3$ (50 mL), brine (50 mL), and dried (Na_2SO_4). The solvent was removed and the residue was purified on a silica gel column (3.3 x 6.5 cm) with hexane/AcOEx (1:1) to give **14** (301 mg, 75% as a yellowish syrup): EI-MS *m/z* 314 (M^+), 223 (M^+-Bn); ^{1}H-NMR ($CDCl_3$), 7.39–7.26 (10 H, m, Ph x 2), 4.59 (2 H, S, PhCH_2O), 4.57 and 4.50 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.22 (1 H, m, H-5), 3.98 (1 H, ddd, H-3, $J_{3,4}$ = 8.8, $J_{3,OH}$ = 4.4, $J_{3,2a}$ = 4.0 Hz), 4.05 (1 H, d, H-2b, $J_{b,a}$ = 9.5 Hz), 4.06-4.02 (1 H, m, H-5), 3.98 (1 H, dd, H-4, $J_{4,5}$ = 5.9, $J_{4,3}$ = 8.8 Hz), 3.79 (1 H, dd, H-2a, $J_{a,3}$ = 4.0, $J_{a,b}$ = 9.5 Hz), 3.60 (1 H, dd, H-6a, $J_{a,b}$ = 10.6, $J_{a,5}$ = 7.0 Hz), 3.51 (1 H, dd, H-6b, $J_{b,a}$ = 10.6, $J_{b,5}$ = 4.4 Hz), 2.67 (1 H, d, 3-OH, $J_{OH,3}$ = 4 .4 Hz). High-resolution MS *m/z* Calcd for $C_{19}H_{24}O_4$ (M^+): 314.1519. Found: 314.1490. (**b**) DEAD (241 μL, 1.53 mmol) was added to a solution of **13** (339 mg, 1.02 mmol) and Ph_3P (402 mg, 1.53 mmol) in THF (10 mL). The mixture was heated at 60 °C for 17 h under argon and then the solvent was removed *in vacuo*. The residue was chromatographed over silica gel (2.8 x 6.5 cm) with 20% AcOEt in hexane.

1,4-Anhydro-3,5-di-*O*-benzyl-2-*O*-isopropyl-D-ribitol (15). EI-MS *m/z* 356 (M^+), 313 (M^+-ipr), 265 (M^+-Bn); ^{1}H-NMR ($CDCl_3$) 7.33-7.27 (10 H, m, Phx2), 4.68 and 4.52 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.57 and 4.50 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.13-4.08 (1 H, m, H-3),

4.02 (1 H, d, H-2a, $J_{a,b}$ = 10.4 Hz), 4.05-3.93 (1 H, m, H-3), 3.88 (1 H, dd, H-4, $J_{4,5} = J_{4,3}$ = 5.5 Hz), 3.82 (1 H, dd, H-2b, $J_{b,3}$ = 6.6, $J_{b,a}$ = 10.4 Hz), 3.71-3.62 (1 H, m, OC*H*Me$_2$), 3.61 (1 H, dd, H-6a, $J_{a,5}$ = 3.3, $J_{a,b}$ = 10.4 Hz), 3.50 (1 H, dd, H-6b, $J_{b,5}$ = 4.4, $J_{b,a}$ = 10.4 Hz), 1.22-1.17 (6 H, m, OCH*Me*2).

(3*S*)-1,4-Anhydro-5-*O*-benzyl-3-*C*-benzyloxymethyl-3-deoxy-D-ribitol (19). Compound **18** (1.00 g, 2.60 mmol) was conversed as above to give **19** (0.721 g, 84.5% as a colorless syrup), after silica gel column chromatographic purification (3.7 x 7.0 cm) with hexane/AcOE (1:1-1:2): EI-MS *m/z* 328 (M^+), 237 (M^+-Bn); ^{1}H-NMR ($CDCl_3$) 7.39-7.27 (10 H, m, Phx2), 4.59 and 4.54 (each 1 H , d, PhC*H*$_2$O, *J* = 12.1 Hz), 4.56 and 4.47 (each 1H, d, PhC*H*$_2$O, *J* = 11.5 Hz), 4.50-4.47 (1 H, m, H-3), 4.09 (1 H, dd, H-5, $J_{5,6a}$ = 3.9, $J_{5,6b}$ = 5.5 Hz), 4.03 (1 H, dd, H-2a, $J_{a,3}$ = 4.4, $J_{a,b}$ = 9.9 Hz), 3.78 (1 H, dd, H-2b, $J_{b,3}$ = 2.2, $J_{b,a}$ = 9.9 Hz), 3.70 (2 H, d, H-7a,b, $J_{7,4}$ = 6.6 Hz), 3.60 (1 H, dd, H-6a, $J_{a,b}$ = 10.4, $J_{a,5}$ = 3.9 Hz), 3.51 (1 H, dd, H-6b, $J_{b,a}$ = 10.4, $J_{b,5}$ = 5.5 Hz), 2.62 (1 H, br s, 3-OH), 2.36 (1 H, ddd, H-4, $J_{4,5}$ = 5.5, $J_{4,7}$ = 6.6, $J_{4,3}$ = 6.1 Hz). High-resolution Ms *m/z* Calcd for $C_{20}H_{24}O_4$ (M^+): 328.1675. Found: 328.1656.

1,4-Anhydro-3,5-di-*O*-benzyl-2-*O*-methanesulfonyl-D-ribitol (20). MsCl (149 μL, 1.92 mmol) was added to a solution of **14** (301 mg, 0.96 mmol), DMAP (12 mg, 0.1 mmol), and Et_3N (334 μL, 2.40 mmol) in CH_2Cl_2 (15 mL) at 0 °C and the mixture was stirred for 2 h at room temperature under argon. H_2O (50 mL) was added to the reaction mixture and the whole was extracted with CH_2Cl_2 (2 x 40 mL), the separated organic phase was washed with saturated aqueous $NaHCO_3$ (40 mL), brine (40 mL), and dried (Na_2SO_4). The solvent was concentrated and the residue was purified by a silica gel column (2.8 x 11 cm) with hexane/AcOEt (2:1) to give **20** (364 mg, 97%, crystallized from Et_2O, as colorless needles): mp 70-71 °C; EI-MS *m/z* 392 (M^+), 301 (M^+-Bn); ^{1}H-NMR ($CDCl_3$) 7.38-7.26 (10 H, m, Phx2), 5.28 (1 H, ddd, H-3, $J_{3,4}$ = 6.6, $J_{3,2a}$ = 4.4, $J_{3,2b}$ = 2.2 Hz), 4.73 and 4.50 (each 1 H, d, PhC*H*$_2$O, *J* = 11.5 Hz), 4.57 and 4.48 (each 1 H, d, PhC*H*$_2$O, *J* = 12.1 Hz), 4.18 (1 H, dd, H-2a, $J_{a,3}$ = 4.4, $J_{a,b}$ = 11.0 Hz), 4.10 (1 H, dd, H-2b, $J_{b,3}$ = 2.2, $J_{b,a}$ = 11.0 Hz), 4.13-4.02 (2 H, m, H-4, 5), 3.69 (1 H, dd, H-6a, $J_{a,5}$ = 2.8, $J_{a,b}$ = 11.0 Hz), 3.52 (1 H, dd, H-6b, $J_{b,5}$ = 3.8, $J_{b,a}$ = 11.0 Hz), 3.05 (3 H, s, OSO_2CH_3). *Anal.* Calcd for $C_{20}H_{24}O_6S$: C, 61.21; H, 6.16; N, 8.17. Found: C, 61.03; H, 6.24; N, 8.31.

(3*S*)-1,4-Anhydro-5-*O*-benzyl-3-*C*-benzyloxymethyl-3-deoxy-2-*O*-methanesulfonyl-D-ribitol (21). Compound **19** (721 mg, 2.2 mmol) was converted as above to give **21** (875 mg, 98% as a colorless syrup): EI-MS *m/z* 406 (M^+), 315 (M^+-Bn); ^{1}H-NMR ($CDCl_3$) 7.37-7.28 (10 H, m, Ph x 2), 5.29-5.27 (1 H, m, H-3), 4.59 and 4.53 (each 1 H, d, PhC*H*$_2$O, *J* = 12.1 Hz), 4.52 and 4.47 (each 1 H, d, PhC*H*$_2$O, *J* = 12.1 Hz), 4.14 (1 H, dd, H-2a, $J_{a,3}$ = 1.7, $J_{a,b}$ = 11.0 Hz), 4.09 (1 H, dd, H-2b, $J_{b,3}$ = 2.8, $J_{b,a}$ = 11.0 Hz), 3.99 (1 H, ddd, H-5, $J_{5,6a}$ = 4.4, $J_{5,6b}$ = 3.9, $J_{5,4}$ = 9.3 Hz), 3.72 (1 H, d, H-7a, $J_{a,b}$ = 9.3 Hz), 3.67 (1 H, dd, H-7b, $J_{b,4}$ = 5.0, $J_{b,a}$ = 9.3 Hz), 3.54 (1 H, dd, H-6a, $J_{a,5}$ = 4.4, $J_{a,b}$ = 10.4 Hz), 3.52 (1 H, dd, H-6b, m, $J_{b,5}$ = 3.9, $J_{b,a}$ = 10.4 Hz), 2.95 (3 H, s, OSO_2CH_3), 2.75-2.64 (1 H, m, H-4). High-resolution MS *m/z* Calcd for $C_{21}H_{26}O_6S$ (M^+): 406.1451. Found: 406.1449.

(2*R*)-2-*C*-(Adenin-9-yl)-1,4-anhydro-3,5-di-*O*-benzyl-2-deoxy-D-arabitol (22). Adenine (108 mg, 0.80 mmol), K_2CO_3 (111 mg, 0.80 mmol), and 18-crown-6 (106 mg, 0.40 mmol) were added to a solution of **20** (157 mg, 0.40 mmol) in DMF (12 mL) and the mixture was stirred for 20 h at 120 °C under argon. After this cooled to room temperature, it was concentrated and the residue was partitioned between H_2O (40 mL) and $CHCl_3$ (3 x 20 mL). The separated organic phase was washed with brine (30 mL) and dried (Na_2SO_4). The solvent was removed and the residue was purified on a silica gel column (2.2 x 5.5

cm) with 2-4% EtOH in $CHCl_3$ to give **22** (86 mg, 49.5%, crystallized from EtOH as white needles): mp 134.5-135 °C; EI-MS *m/z* 431 (M^+), 340 (M^+-Bn); ^{1}H-NMR ($CDCl_3$) 8.40 and 8.07 (each 1 H, s, H-2 and H-8), 7.34-7.17 (10 H, m, Ph x 2), 5.62 (2 H, br s, 6-NH_2), 5.22-5.21 (1 H, m, H-3'), 4.83 and 4.57 (1 H, d, PhCH_2O, J = 11.7 Hz), 4.53 and 4.47 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.34-4.26 (2 H, m, H-2'a,b), 4.11-4.04 (2 H, m, H-4',5'), 3.69 (1 H, dd, H-6'a, $J_{a,5'}$ = 2.9, $J_{a,b}$ = 10.6 Hz), 3.57 (1 H, dd, H-6'b, $J_{b,5'}$ = 4.0, $J_{b,a}$ = 10.6 Hz). *Anal.* Calcd for $C_{24}H_{25}N_5O_3$: C, 66.81; H, 5.84; N, 16.23. Found: C, 67.00; H, 5.85; N, 16.19.

(2*R*, 3*R*)-2-*C*-(Adenin-9-yl)-1,4-anhydro-5-*O*-benzyl-3-*C*-benzyloxymethyl-2-deoxy-D-arabitol (23) and **1,4-anhydro-5-*O*-benzyl-3-*C*-benzyloxymethyl-2,3-didehydro-2,3-dideoxy-D-*ribo*-pentitol (24)**. Compound **23** was obtained from the reaction of **19** (198 mg, 0.49 mmol) and adenine (132 mg, 0.97 mmol) in the presence of K_2CO_3 (135 mg, 0.97 mmol) and 18-crown-6 (129 mg, 0.49 mmol) in DMF (15 mL) for 22 h at 120 °C. The mixture was purified on a silica gel column (2.8 x 5.5 cm) with $CHCl_3$ to 4% EtOH in $CHCl_3$. From the $CHCl_3$ fractions, **24** (74 mg, 49%) was obtained as a syrup and from 4% EtOH in $CHCl_3$ fractions, **23** (81 mg, 37%, crystallized form EtOH) was obtained. Physical data for **23**: mp 108-109.5 °C; EI-MS *m/z* 445 (M^+), 354 (M^+-Bn); ^{1}H-NMR ($CDCl_3$) 8.35 and 8.18 (each 1 H, s, H-2 and H-8), 7.38-7.25 (10 H, m, Ph x 2), 5.60 (2 H, br s, 6-NH_2), 5.20-5.16 (1 H, m, H-2'), 4.66 and 4.59 (each 1 H, d, PHCH_2O, J = 12.1 Hz), 4.54 and 4.49 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.15 (1 H, dd, H-2'a, $J_{a,3'}$ = 2.2, $J_{a,b}$ = 9.9 Hz), 4.13-4.04 (2 H, m, H-2'b, H-5'), 3.82 (1 H, dd, H-6'a, $J_{a,5'}$ = 2.9, $J_{a,b}$ = 10.6 Hz), 3.71-3.65 (2 H, m, H-6'b, 7'a), 3.61 (1 H, dd, H-7'b, $J_{b,4'}$ = 5.1, $J_{a,b}$ = 9.2 Hz), 2.79-2.70 (1 H, m, H-4'). *Anal.* Calcd for $C_{25}H_{27}N_5O_3$: C, 67.40; H, 6.11; N, 15.72. Found: C, 67.63; H, 6.08; N, 15.51. Physical data for **24**: EI-MS *m/z* 310 (M^+), 219 (M^+-Bn); ^{1}H-NMR ($CDCl_3$) 7.37-7.23 (10 H, m, Ph x 2), 5.90 (1 H, d, H-3, J = 1.7 Hz), 4.96-4.93 (1 H, m, H-5), 4.76-4.63 (2 H, m, H-7a, b), 4.59 and 4.53 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.52 and 4.46 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.09 (2 H, d, H-2a, b, $J_{a,b}$ = 12.1 Hz), 3.65 (1 H, dd, H-6a, $J_{a,5}$ = 3.3, $J_{a,b}$ = 10.4 Hz), 3.56 (1 H, dd, H-6b, $J_{b,5}$ = 4.9, $J_{b,a}$ = 10.4 Hz).

(2*R*)-1,4-Anhydro-3,5-di-*O*-benzyl-2-*C*-(2,6-diaminopurin-9-yl)-2-deoxy-D-arabitol (25). The reaction of **20** (547 mg, 1.4 mmol) in DMF (30 mL) with 2,6-diaminopurine (419 mg, 2.8 mmol), K_2CO_3 (385 mg, 2.8 mmol), and 18-crown-6 (547 mg, 1.4 mmol) for 45 h at 120 °C under argon gave **25** (383 mg, 62% as a yellowish syrup): EI-MS *m/z* 446 (M^+), 355 (M^+-Bn); ^{1}H-NMR ($CDCl_3$) 7.76 (1 H, s, H-8), 7.35-7.21 (10 H, m, Ph x 2), 5.41 (2 H, br s, 6-NH_2), 5.02-5.01 (1 H, m, H-3'), 4.80 and 4.56 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.70 (2 H, br s, 2-NH_2), 4.55 and 4.48 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.25 (2 H, d, H-2'a,b, $J_{2',3'}$ = 3.8 Hz), 4.06-4.02 (2 H, m, H-4', 5'), 3.65 (1 H, dd, H-6'a, $J_{a,5'}$ = 2.2, $J_{a,b}$ = 9.9 Hz), 3.57 (1 H, dd, H-6'b, $J_{b,5'}$ = 3.9, $J_{b,a}$ = 9.9 Hz). High-resolution MS *m/z* Calcd for $C_{24}H_{26}N_6O_3$ (M^+): 446.2069. Found: 446.2094.

(2*R*, 3*R*)-1,4-Anhydro-5-*O*-benzyl-3-*C*-benzyloxymethyl-2-*C*-(2,6-diaminopurin-9-yl)-2,3-dideoxy-D-arabitol (26). Compound **21** (416 mg, 1 mmol) was converted as described for the synthesis of **22** to give **26** (151 mg, 32% as a yellowish syrup): EI-MS *m/z* 460 (M^+), 369 (M^+-Bn); ^{1}H-NMR ($CDCl_3$) 7.84 (1 H, s, H-8), 7.39-7.26 (10 H, m, Ph x 2), 5.37 (2 H, br s, 6-NH_2), 5.00-4.95 (1 H, m, H-3'), 4.65 and 4.59 (each 1 H, d, PHCH_2O, J = 12.1 Hz), 4.62 (2 H, br s, 2-NH_2), 4.51 (2 H, s, PhCH_2O), 4.12 (1 H, dd, H-2'a, $J_{a,3'}$ = 3.9, $J_{a,b}$ = 9.9 Hz), 4.09-4.03 (2 H, m, H-2'b,5'), 3.79 (1 H, dd, H-6'a, $J_{a,5'}$ = 3.3, $J_{a,b}$ = 10.4 Hz), 3.68 (1 H, dd, H-6'b, $J_{b,5'}$ = 5.0, $J_{b,a}$ = 10.4 Hz), 3.64-3.57 (2 H, m,

H-7'a,b), 2.74-2.65 (1 H, m, H-4'). High-resolution MS *m/z* Calcd for $C_{25}H_{28}N_6O_3$ (M^+): 460.2223. Found: 460.2196.

(2*R*)-2-*C*-(Adenin-9-yl)-1,4-anhydro-2-deoxy-D-arabitol (2). A solution of BCl_3 (1 M hexane solution, 630 μL, 0.63 mmol) was added to a solution of **22** (68 mg, 0.16 mmol) in CH_2Cl_2 (5 mL) at -78 °C under argon. The mixture was stirred for 2.5 h at -78 °C and then further stirred for 4 h at -18 °C. MeOH (3 mL) was added to the mixture which was stirred for 12 h at room temperature. The mixture was concentrated *in vacuo* and coevaporated several times with MeOH. The residue was purified on a silica gel column (1.8 x 8 cm) with 15-20% MeOH in CH_2Cl_2 to give **2** (31 mg, 78%, crystallized from MeOH): mp 223-225 °C; FAB-MS *m/z* 251 (M^+); ^{1}H-NMR (DMSO-d_6) 8.19 and 8.15 (each 1 H, s, H-2, 8), 7.23 (2 H, br s, 6-NH_2), 5.75 (1 H, d, 4'-OH, $J_{OH,4'}$ = 5.5 Hz), 4.90 (1 H, t, 6'-OH, $J_{OH,6'}$ = 5.5 Hz), 4.89-4.83 (1 H, m, H-3'), 4.39 (1 H, dd, H-4', $J_{4',5'} = J_{4',3'}$ = 4.3, $J_{4',OH}$ = 5.5 Hz), 4.16 (1 H, dd, H-2'a, $J_{a,3'}$ = 6.6, $J_{a,b}$ = 9.9 Hz), 4.09 (1 H, dd, H-2'b, $J_{b,3'}$ = 5.1, $J_{b,a}$ = 9.9 Hz), 3.72-3.50 (3 H, m, H-5',6'a,b). *Anal.* Calcd for $C_{10}H_{13}N_5O_3$: C, 47.81; H, 5.22; N, 27.87. Found: C, 47.63; H, 5.19; N, 27.63.

(2*R*, 3*R*)-2-*C*-(Adenin-9-yl)-1,4-anhydro-2,3-dideoxy-3-*C*-hydroxymethyl-D-arabitol (3). Compound **23** (49 mg, 0.11 mmol) was debenzylated as described for the synthesis of **2** to give **3** (21 mg, 72%, crystallized from MeOH): mp 212-214 °C; FAB-MS *m/z* 265 (M^+); ^{1}H-NMR (DMSO-d_6) 8.27 and 8.13 (each 1 H, s, H-2, 8), 7.21 (2 H, br s, 6-NH_2), 5.04-5.01 (1 H, m, H-3'), 4.98 and 4.92 (each 1 H, t, 6'-OH, 7'-OH, $J_{OH,6'} = J_{OH,7'}$ = 5.5 Hz), 4.00 (1 H, dd, H-2'a, $J_{a,3'}$ = 4.0, $J_{a,b}$ = 9.9 Hz), 3.96 (1 H, dd, H-2'b, $J_{b,3'}$ = 5.9, $J_{b,a}$ = 9.9 Hz), 3.79-3.72 (1 H, m, H-5'), 3.71-3.52 (4 H, m, H-6'a,b, 7'a,b), 2.58-2.51 (1 H, m, H-4'). *Anal.* Calcd for $C_{11}H_{15}N_5O_3 \cdot 0.35\ H_2O$: C, 45.96; H, 5.89; N, 29.23. Found: C, 45.90; H, 5.86; N, 29.24.

(2*R*)-1,4-Anhydro-2-deoxy-2-*C*-(2,6-diaminopurin-9-yl)-D-arabitol (4). Compound **25** (115 mg, 0.26 mmol) was debenzylated as described for the synthesis of **2** to give **4** (53 mg, 77%, crystallized from MeOH): mp 181-182.5 °C; FAB-MS *m/z* 266 (M^+); ^{1}H-NMR (DMSO-d_6) 7.77 (1 H, s, H-8), 6.68 (2 H, br s, 6-NH_2), 5.78 (2 H, br s, 2-NH_2), 5.76 (1 H, d, 4'-OH, $J_{OH,4'}$ = 5.5 Hz), 4.90 (1 H, t, 6'-OH, $J_{OH,6'}$ = 5.5 Hz), 4.67 (1 H, ddd, H-3', $J_{3',4'}$ = 4.0, $J_{3',2'a}$ = 6.6, $J_{3',2'b}$ = 5.0 Hz), 4.32 (1 H, dd, H-4', $J_{4',5'} = J_{4',OH}$ = 5.5, $J_{4',3'}$ = 4.0 Hz), 4.12 (1 H, dd, H-2'a, $J_{a,3'}$ = 6.6, $J_{a,b}$ = 9.3 Hz), 3.96 (1 H, dd, H-2'b, $J_{b,3'}$ = 5.0, $J_{b,a}$ = 9.3 Hz), 3.66-3.53 (3 H, m, H-5',6'a,b). *Anal.* Calcd for $C_{10}H_{14}N_6O_3$: C, 45.11; H, 5.30; N, 31.56. Found: C, 44.93; H, 5.30; N, 31.54.

(2*R*,3*R*)-1,4-Anhydro-2-*C*-(2,6-diaminopurin-9-yl)-2,3-dideoxy-3-*C*-hydroxymethyl-D-arabitol (5). Compound **26** (156 mg, 0.34 mmol) was debenzylated as above and the residue was purified on a silica gel column (2.8 x 7 cm) with 20% MeOH in $CHCl_3$ to give **5** (67 mg, 70%, crystallized from MeOH). An analytical sample was obtained by a reverse phase HPLC (YMC D-ODS-5, 120 A) with 20% MeOH in H_2O: mp 231-232 °C; FAB-MS *m/z* 280 (M^+); ^{1}H-NMR (DMSO-d_6) 7.87 (1 H, s, H-8), 6.66 (2 H, br s, 6-NH_2), 5.75 (2 H, br s, 2-NH_2), 4.97 and 4.90 (each 1 H, t, 6'-OH, 7'-OH, $J_{OH,6'} = J_{OH,7'}$ = 5.0 Hz), 4.83-4.77 (1 H, m, H-3'), 3.90 (1 H, dd, H-2'a, $J_{a,3'}$ = 5.9, $J_{a,b}$ = 9.3 Hz), 3.86 (1 H, dd, H-2'b, $J_{b,3'}$ = 4.4, $J_{a,b}$ = 9.3 Hz), 3.78-3.52 (5 H, m, H-5', 6'a,b, 7'a,b), 2.53-2.49 (1 H, m, H-4'). *Anal.* Calcd for $C_{11}H_{16}N_6O_3 \cdot 0.4\ H_2O$: C, 45.96; H, 5.89; N, 29.23. Found: C, 45.90; H, 5.86; N, 29.24.

(2*R*)-1,4-Anhydro-2-deoxy-2-*C*-(guanin-9-yl)-D-arabitol (6). Adenosine deaminase (Boehringer Manheim Yamanouchi 102105, 1,000 μL, 1,000 U) was added to a solution of **4** (50 mg, 0.19 mmol) in potassium phosphate buffer (0.5 M, pH 7.5, 20 mL) and the mixture was stirred for six days at 37

°C. Further amount of adenosine deaminase (250 µL, 250 U) was added to the mixture, which was stirred for further four days. The mixture was diluted with H_2O (80 mL) and was put on a charcoal column, washed with H_2O and then eluted with aqueous 50-95% EtOH to give **6** (38 mg, 76%, crystallized from H_2O) as white needles: mp >300 °C; FAB-MS *m/z* 268 (M^++1); ^{1}H-NMR (DMSO-d_6) 10.60 (1 H, br s, 1-NH), 7.77 (1 H, s, H-8), 6.46 (2 H, br s, 2-NH_2), 5.71 (1 H, d, 4-OH, $J_{OH,4'}$ = 5.5 Hz), 4.91 (1 H, t, 6'-OH, $J_{OH,6'}$ = 5.5 Hz), 4.65 (1 H, ddd, H-3', $J_{3',4'}$ = 4.4, $J_{3',a}$ = 6.6, $J_{3',b}$ = 5.0 Hz), 4.27 (1 H, dd, H-4', $J_{4',5'} = J_{4',OH}$ = 5.5, $J_{4',3'}$ = 4.4 Hz), 4.09 (1 H, dd, H-2'a, $J_{a,3'}$ = 6.6, $J_{a,b}$ = 9.3 Hz), 3.93 (1 H, dd, H-2'b, $J_{b,3'}$ = 5.0, $J_{a,b}$ = 9.3 Hz), 3.66-3.59 (2 H, m, H-5', 6'a), 3.53 (1 H, dd, H-6'b, $J_{b,5'} = J_{b,6'OH}$ = 5.5, $J_{b,a}$ = 12.6 Hz). *Anal.* Calcd for $C_{10}H_{13}N_5O_4 \cdot 7/6\ H_2O$: C, 41.67; H, 5.36; N, 24.29. Found: C, 41.99; H, 5.46; N, 23.89.

(2*R*, 3*R*)-1,4-Anhydro-2,3-dideoxy-2-*C*-(guanin-9-yl)-3-*C*-hydroxymethyl-D-arabitol (7). Compound **5** (50 mg, 0.18 mmol) was converted as above and the reaction mixture was purified on a charcoal column with aqueous 50-95% EtOH to give **7** (31 mg, 62%, crystallized from aqueous MeOH). An analytical sample was obtained by a reverse phase HPLC (YMC D-ODS-5, 120 A) with 15% MeOH in H_2O: mp 280-282 °C; FAB-MS *m/z* 282 (M^++1); ^{1}H-NMR (DMSO-d_6) 10.54 (1 H, br s, 1-NH), 7.85 (1 H, s, H-8), 6.41 (2 H, br s, 2-NH_2), 5.96 and 4.87 (each 1 H, d, 6'-OH, 7'-OH, $J_{OH,6'} = J_{OH,7'}$ = 5.5 Hz), 4.80-4.75 (1 H, m, H-3'), 3.89 (1 H, dd, H-2'a, $J_{a,3'}$ = 6.0, $J_{a,b}$ = 9.3 Hz), 3.83 (1 H, dd, H-2'b, $J_{b,3'}$ = 4.0, $J_{a,b}$ = 9.3 Hz), 3.76-3.48 (5 H, m, H-5', 6'a,b, 7'a,b), 2.51-2.49 (1 H, m, H-4'). *Anal.* Calcd for $C_{11}H_{15}N_5O_4 \cdot 0.5\ H_2O$: C, 45.52; H, 5.56; N, 24.13. Found: C, 45.67; H, 5.55; N, 24.14.

1,4-Anhydro-3,5-di-*O*-benzyl-2-*O*-(cytosin-2-yl)-D-arabitol (27). Cytosine (32 mg, 0.29 mmol), K_2CO_3 (39 mg, 0.29 mmol), and 18-crown-6 (37.5 mg, 0.14 mmol) was added to a solution of **14** (56 mg, 0.14 mmol) in DMF (10 mL) and the mixture was stirred for 23 h at 120 °C under argon. After this cooled to room temperature, the solvent was removed *in vacuo* and the residue was partitioned between H_2O (20 mL) and $CHCl_3$ (3 x 10 mL). The separated organic phase was washed with brine (2 x 20 mL) and dried (Na_2SO_4). The solvent was removed *in vacuo* and the residue was purified on a silica gel column (1.6 x 6 cm) with 1% EtOH in $CHCl_3$ to give **27** (32 mg, 55% as a yellowish syrup): EI-MS *m/z* 407 (M^+), 316 (M^+-Bn); UV λmax 273 nm (MeOH), 232, 260 nm (acidic); ^{1}H-NMR ($CDCl_3$) 8.00 (1 H, d, H-6, $J_{6,5}$ = 5.5 Hz), 7.31-7.26 (10 H, m, Ph x 2), 6.10 (1 H, d, H-5, $J_{5,6}$ = 5.5 Hz), 5.39-5.37 (1 H, m, H-3'), 4.87 (2 H, br s, 4-NH_2), 4.73 and 4.63 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.60 and 4.53 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.20 (1 H, dd, H-2'a, $J_{a,3'}$ = 4.4, $J_{a,b}$ = 9.3 Hz), 4.09 (1 H, d, H-2'b, $J_{b,a}$ = 9.3 Hz), 4.11-4.07 (2 H, m, H-4',5'), 3.70-3.63 (2 H, m, H-6'a,b).

(2*R*)-1,4-Anhydro-2-azido-3,5-di-*O*-benzyl-2-deoxy-D-arabitol (28). LiN_3 (279 mg, 5.71 mmol) was added to a solution of **14** (448 mg, 1.14 mmol) in DMF (15 mL) and the mixture was stirred for 6 h at 100 °C under argon. After this cooled to room temperature, it was diluted with H_2O (70 mL) and the whole was extracted with Et_2O (2 x 40 mL). The separated organic phase was washed with H_2O (3 x 30 mL), brine (50 mL), and dried (Na_2SO_4). The solvent was concentrated and the residue was purified on a silica gel column (2.8 x 14 cm) with 10-20% AcOEt in hexane to give **28** (345 mg, 90% as a colorless oil): EI-MS *m/z* 248 (M^+-Bn); IR (neat) ν 2090 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 7.38-7.28 (10 H, m, Phx2), 4.64-4.58 (4 H, m, PhCH_2O), 4.02 (1 H, dd, H-2a, $J_{a,3}$ = 5.5, $J_{a,b}$ = 9.9 Hz), 3.99 (1 H, dd, H-2b, $J_{b,3}$ = 3.9, $J_{a,b}$ = 9.9 Hz), 4.03-3.91 (3 H, m, H-3, 4, 5), 3.64-3.51 (2 H, m, H-6a,b).

(2*R*, 3*R*)-1,4-Anhydro-2-azido-5-*O*-benzyl-3-*C*-benzyloxymethyl-2,3-dideoxy-D-arabitol (29). Compound **19** (874 mg, 2.15 mmol) was converted as above and the residue was purified on a silica gel column (3.6 x 9 cm) with 10-20% AcOEt in hexane to give **29** (708 mg, 93% as a colorless oil): EI-MS *m/z* 262 (M^+-Bn); IR (neat) ν 2090 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 7.38-7.26 (10 H, m, Ph x 2), 4.58-4.47 (4 H, m, PhCH_2O), 3.96 (1 H, dd, H-2a, $J_{a,3}$ = 4.4, $J_{a,b}$ = 9.3 Hz), 3.89 (1 H, dd, H-2b, $J_{b,3}$ = 3.9, $J_{a,b}$ = 9.3 Hz), 3.99-3.83 (2 H, m, H-3,5), 3.60 (2 H, d, H-7a,b, J = 4.9 Hz), 3.56 (1 H, dd, H-6a, $J_{a,5}$ = 5.5, $J_{a,b}$ = 9.3 Hz), 3.47 (1 H, dd, H-6b, $J_{b,5}$ = 6.0, $J_{a,b}$ = 9.3 Hz), 2.38-2.29 (1 H, m, H-4).

(2*R*)-2-Amino-1,4-anhydro-3,5-di-*O*-benzyl-2-deoxy-D-arabitol (30). A suspension of **28** (293 mg, 0.86 mmol) and Lindlar catalyst (86 mg) in MeOH/AcOEt (1:1, 20 mL) was vigorously stirred for 6 h at room temperature under atmospheric pressure of H_2. Insoluble materials were removed by filtration and the filtrate was concentrated *in vacuo*. The residue was purified on a silica gel column (2.8 x 7 cm) with 2-4% EtOH in $CHCl_3$ to give **30** (257 mg, 95% as a yellowish syrup) which is positive to the ninhydrin test: EI-MS *m/z* 313 (M^+), 222 (M^+-Bn); ^{1}H-NMR ($CDCl_3$) 7.34-7.22 (10 H, m, Ph x 2), 4.61 and 4.60 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.53 (2 H, d, PhCH_2O, J = 12.1 Hz), 4.00 (1 H, dd, H-2a, $J_{a,3}$ = 3.9, $J_{a,b}$ = 9.3 Hz), 3.99 (1 H, dd, H-2b, $J_{b,3}$ = 4.4, $J_{a,b}$ = 9.3 Hz), 3.74-3.71 (2 H, m, H-4, 5), 3.67 (1 H, dd, H-6a, $J_{a,5}$ = 3.3, $J_{a,b}$ = 10.4 Hz), 3.59 (1 H, dd, H-6b, $J_{b,5}$ = 4.4, $J_{b,a}$ = 10.4 Hz), 3.48-3.45 (1 H, m, H-3).

(2*R*, 3*R*)-2-Amino-1,4-anhydro-5-*O*-benzyl-3-*C*-benzyloxymethyl-2-deoxy-D-arabitol (31). Compound **29** (708 mg, 2 mmol) was converted as above and the residue was purified on a silica gel column (3.3 x 10 cm) with 4-8% EtOH in $CHCl_3$ to give **31** (627 mg, 96% as a yellowish syrup) which is positive to the ninhydrin test: EI-MS *m/z* 328 (M^++1), 236 (M^+-Bn); ^{1}H-NMR ($CDCl_3$) 7.37-7.22 (10 H, m, Phx2), 4.61 and 4.56 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.52-4.45 (2 H, m, PhCH_2O), 3.92 (1 H, dd, H-2a, $J_{a,3}$ = 6.0, $J_{a,b}$ = 8.8 Hz), 3.90-3.86 (1 H, m, H-5), 3.66 (1 H, dd, H-6a or H-7a, $J_{a,5}$ = 2.8, $J_{a,b}$ = 10.4 Hz), 3.59 (1 H, dd, H-2b, $J_{b,3}$ = 5.5, $J_{a,b}$ = 8.8 Hz), 3.55-3.51 (3 H, m, H-6b, 7b), 3.38-3.21 (1 H, m, H-3), 2.11-2.02 (1 H, m, H-4).

(2*R*)-1,4-Anhydro-3,5-di-*O*-benzyl-2-deoxy-2-[*N*-(3-ethoxyacryloyl)ureido]-D-arabitol (32). A solution of 3-ethoxy-2-propenoyl isocyanate [prepared from 3-ethoxy-2-propenoyl chloride (221 mg, 1.64 mmol) and AgNCO (492 mg, 3.28 mmol) by heating in benzene] was added dropwise to a solution of **30** (257 mg, 0.82 mmol) at -18 °C and the mixture was stirred for 1 h at -18 °C, and then for further 1 h at room temperature under argon. The mixture was poured into saturated aqueous $NaHCO_3$ (30 mL) at 0 °C and the whole was extracted with AcOEt (3 x 30 mL). The separated organic phase was washed with H_2O (2 x 50 mL), brine (50 mL), and dried (Na_2SO_4). The solvent was concentrated and the residue was purified on a silica gel column (2.8 x 8 cm) with hexane/AcOEt (2:1) to give **32** (357 mg, 96% as a colorless syrup): EI-MS *m/z* 454 (M^+); ^{1}H-NMR ($CDCl_3$) 8.99 (1 H, br d, 1-NH, J = 7.7 Hz), 8.48 (1 H, br s, 3-NH), 7.59 (1 H, d, H-6, $J_{6,5}$ = 12.1 Hz), 7.33-7.26 (10 H, m, Ph x 2), 5.23 (1 H, d, H-5, $J_{5,6}$ = 12.1 Hz), 4.73 and 4.67 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.55 (2 H, d, PhCH_2O, J = 12.1 Hz), 4.49-4.43 (1 H, m, H-3'), 4.01 (2 H, q, OCH_2CH$_3$, J = 7.1 Hz), 4.05-3.87 (4 H, m, H-2'a,b, 4', 5'), 3.61-3.51 (2 H, m, H-6'a,b), 1.30 (3 H, t, OCH$_2$CH_3, J = 7.1 Hz).

(2*R*, 3*R*)-1,4-Anhydro-5-*O*-benzyl-3-*C*-benzyloxymethyl-2,3-dideoxy-2-[*N*-(3-ethoxyacryloyl)ureido]-D-arabitol (33). Compound **31** (627 mg, 1.91 mmol) was converted as above to give **33** (891 mg, 99% as a colorless syrup): EI-MS *m/z* 468 (M^+), 377 (M^+-Bn); ^{1}H-NMR ($CDCl_3$) 8.96 (1 H, br d, 1-NH, J = 7.1 Hz), 8.56 (1 H, br s, 3-NH), 7.60 (1 H, d, H-6, $J_{6,5}$ = 12.1 Hz), 7.35-7.23 (10

H, m, Ph x 2), 5.23 (1 H, d, H-5, $J_{5,6}$ = 12.1 Hz), 4.68 and 4.59 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.50-4.46 (1 H, d, PhCH_2O, J = 11.5 Hz), 4.34-4.27 (1 H, m, H-3'), 3.99 (1 H, dd, H-2'a, $J_{a,3'}$ = 6.1, $J_{a,b}$ = 9.3 Hz), 3.93 (2 H, q, OCH_2CH$_3$, J = 7.1 Hz), 3.96-3.92 (1 H, m, H-5'), 3.82 (1 H, dd, H-2'b, $J_{b,3'}$ = 4.4, $J_{a,b}$ = 9.3 Hz), 3.63 (1 H, dd, H-6'a or H-7'a, $J_{a,5'}$ = 3.3, $J_{a,b}$ = 10.4 Hz), 3.62-3.53 (2 H, m, H-6'b, 7'b), 3.52 (1 H, dd, H-6'b or H-7'b, $J_{b,5'}$ = 2.7, $J_{b,a}$ = 10.4 Hz), 2.32-2.28 (1 H, m, H-4'), 1.33 (3 H, t, OCH$_2$CH_3, J = 7.1 Hz).

(2*R*)-1,4-Anhydro-3,5-di-*O*-benzyl-2-deoxy-2-*C*-(uracil-1-yl)-D-arabitol (34). NH_4OH (2.8%, 18 mL) was added to a solution of **32** (356 mg, 0.78 mmol) in acetone (6 mL) in glass sealed tube and the mixture was stirred for 10 h at 75 °C. After this cooled to room temperature, it was concentrated to dryness and the residue was purified on a silica gel column (2.8 x 7.5 cm) with hexane/AcOEt (1:2) to give **34** (172 mg, 54% as a yellowish syrup): EI-MS *m/z* 408 (M^+), 317 (M^+-Bn); ^{1}H-NMR ($CDCl_3$) 8.35 (1 H, br s, 3-NH), 7.55 (1 H, d, H-6, $J_{6,5}$ = 7.7 Hz), 7.37-7.17 (10 H, m, Ph x 2), 5.30 (1 H, dd, H-5, $J_{5,6}$ = 7.7, $J_{5,NH}$ = 2.2 Hz), 5.14-5.12 (1 H, m, H-3'), 4.81 and 4.53 (each 1 H, d PhCH_2O, J = 12.1 Hz), 4.50-4.40 (each 1 H, d, PhCH_2O, J = 11.5 Hz), 4.17 (1 H, dd, H-2'a, $J_{a,3'}$ = 6.0, $J_{a,b}$ = 11.0 Hz), 4.11-4.04 (1 H, m, H-4'), 4.02 (1 H, d, H-2'b, $J_{b,a}$ = 11.0 Hz), 3.95 (1 H, ddd, H-5', $J_{5',6'a}$ = 2.2, $J_{5',6'b}$ = 2.8, $J_{5',4'}$ = 5.0 Hz), 3.80 (1 H, dd, H-6'a, $J_{a,5'}$ = 2.2, $J_{a,b}$ = 10.4 Hz), 3.60 (1 H, dd, H-6'b, $J_{b,5'}$ = 2.8, $J_{b,a}$ = 10.4 Hz). High-resolution MS *m/z* Calcd for $C_{23}H_{24}N_2O_5$ (M^+): 408.1686. Found: 408.1710.

(2*R*, 3*R*)-1,4-Anhydro-5-*O*-benzyl-3-*C*-benzyloxymethyl-2-deoxy-2-*C*-(uracil-1-yl)-D-arabitol (35). 2 N H_2SO_4 (7 mL) was added to a solution of **33** (847 mg, 1.81 mmol) in dioxane (20 mL) and the mixture was heated for 2 h at 100 °C with stirring. After this cooled to room temperature, it was neutralized by addition of 2 N NaOH at 0 °C. The mixture was diluted by H_2O (30 mL) and the whole was extracted with AcOEt (3 x 30 mL). The separated organic phase was washed with H_2O (50 mL), brine (50 mL), and dried (Na_2SO_4). The solvent was concentrated to dryness and the residue was purified on a silica gel column (3.7 x 8 cm) with hexane/AcOEt (1:2) to give **35** (687 mg, 90% as a colorless syrup): EI-MS *m/z* 422 (M^+), 331 (M^+-Bn); ^{1}H-NMR ($CDCl_3$) 8.28 (1 H, br s, 3-NH), 7.75 (1 H, d, H-6, $J_{6,5}$ = 8.2 Hz), 7.37-7.26 (10 H, m, Ph x 2), 5.35 (1 H, dd, H-5, $J_{5,6}$ = 8.2, $J_{5,NH}$ = 2.2 Hz), 5.15-5.11 (1 H, m, H-3'), 4.59 and 4.52 (each 1 H, d PhCH_2O, J = 11.5 Hz), 4.54-4.50 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 3.97-3.91 (3 H, m, H-5', H-6'a,b or H-7'a,b), 3.89 (1 H, dd, H-2'a, $J_{a,3'}$ = 2.2, $J_{a,b}$ = 10.4 Hz), 3.66 (1 H, dd, H-2'b, $J_{b,3'}$ = 3.3, $J_{b,a}$ = 10.4 Hz), 3.63-3.61 (2 H, m, H-6'a,b or H-7'a,b), 2.59-2.51 (1 H, m, H-4').

(2*R*)-1,4-Anhydro-3,5-di-*O*-benzyl-2-*C*-(cytosin-1-yl)-2-deoxy-D-arabitol (36). Et_3N (100 µL, 0.73 mmol) was added to a solution of **34** (149 mg, 0.36 mmol) in CH_3CN (10 mL) containing TPSCl (221 mg, 0.73 mmol) and DMAP (89 mg, 0.73 mmol) at 0 °C. The mixture was stirred for 3.5 h at room temperature under argon and then concentrated NH_4OH (28%, 6 mL) was added to the mixture, which was further stirred for 1.5 h at room temperature. The mixture was concentrated to dryness and the residue was purified on a silica gel column (2.3 x 8 cm) with 8% EtOH in $CHCl_3$ to give **36** (144 mg, 97% as a yellowish syrup): EI-MS *m/z* 407 (M^+), 316 (M^+-Bn); UV λmax (MeOH) 279 nm; ^{1}H-NMR ($CDCl_3$+D_2O) 7.57 (1 H, d, H-6, $J_{6,5}$ = 7.1 Hz), 7.32-7.14 (10 H, m, Ph x 2), 5.36 (1 H, d, H-5, $J_{5,6}$ = 7.1 Hz), 5.22-5.20 (1 H, m, H-3'), 4.83 and 4.52 (each 1 H, d PhCH_2O, J = 12.1 Hz), 4.46 and 4.37 (each 1 H, d, PhCH_2O, J = 11.5 Hz), 4.10 (1 H, dd, H-2'a, $J_{a,3'}$ = 5.5, $J_{a,b}$ = 11.0 Hz), 3.97-3.90 (3 H, m, H-2'b, 4', 5'), 3.72 (1 H, d, H-6'a, $J_{a,5'}$ = 2.2, $J_{a,b}$ = 11.0 Hz), 3.52 (1 H, dd, H-6'b, $J_{b,5'}$ = 3.3, $J_{a,b}$ = 11.0 Hz). High-resolution MS *m/z* Calcd for $C_{23}H_{25}N_3O_4$ (M^+): 408.1847. Found: 408.1849.

(2*R*, 3*R*)-1,4-Anhydro-5-*O*-benzyl-3-*C*-benzyloxymethyl-2-*C*-(cytosin-1-yl)-2-deoxy-D-arabitol (37). Compound **35** (485 mg, 1.15 mmol) was converted into the cytosine derivative as described for the synthesis of **36** to give **37** (436 mg, 90% as a yellowish syrup): EI-MS *m/z* 407 (M^+), 316 (M^+-Bn); ^{1}H-NMR ($CDCl_3$ + D_2O) 7.80 (1 H, d, H-6, $J_{6,5}$ = 7.1 Hz), 7.36-7.26 (10 H, m, Ph x 2), 5.38 (1 H, d, H-5, $J_{5,6}$ = 7.1 Hz), 5.27-5.22 (1 H, m, H-3'), 4.59 and 4.52 (each 1 H, d, PhCH_2O, J = 11.5 Hz), 4.52-4.48 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.00-3.97 (1 H, m, H-5'), 3.94 (2 H, d, H-6'a,b or H-7'a,b, J = 4.4 Hz), 3.87 (1 H, dd, H-2'a, $J_{a,3'}$ = 2.2, $J_{a,b}$ = 11.4 Hz), 3.71 (1 H, dd, H-6'a or H-7'a, $J_{a,5'}$ = 5.0, $J_{a,b}$ = 9.3 Hz), 3.68-3.62 (1 H, m, H-6'b or H-7'b), 3.64 (1 H, dd, H-2'b, $J_{b,3'}$ = 3.9, $J_{b,a}$ = 10.4 Hz), 2.54-2.47 (1 H, m, H-4'). High-resolution MS *m/z* Calcd for $C_{24}H_{27}N_3O_4$ (M^+): 421.2003. Found: 421.1998.

(2*R*)-1,4-Anhydro-2-*C*-(cytosin-1-yl)-2-deoxy-D-arabitol hydrochloride (8). Compound **36** (139 mg, 0.34 mmol) was debenzylated as described for the synthesis of **2**. The product was purified on a silica gel column (2.3 x 7 cm) with 25% MeOH in $CHCl_3$. The fractions containing **8** were concentrated and the residue was dissolved in a mixture of 1 N HCl (0.5 mL) and EtOH (3 mL). The mixture was concentrated to dryness and the residue was coevaporated three times with EtOH to give **8** as a hydrochloride (74 mg, 83%, crystallized from aqueous EtOH): mp 215-220 °C; FAB-MS *m/z* 228 (M^+); ^{1}H-NMR (DMSO-d_6) 9.60 and 8.52 (each 1 H, br s, 4-NH_2), 7.98 (1 H, d, H-6, $J_{6,5}$ = 7.7 Hz), 6.10 (1 H, dd, H-5, $J_{5,6}$ = 7.7 Hz), 5.80-5.60 (2 H, br s, 4'-, 6'-OH), 4.76 (1 H, ddd, H-3', $J_{3',4'}$ = 3.3, $J_{3',2'a}$ = 6.0, $J_{3',2'b}$ = 3.3 Hz), 4.15-4.11 (1 H, m, H-4', $J_{4',5'}$ = 6.0 Hz), 4.03 (1 H, dd, H-2'a, $J_{a,3'}$ = 6.0, $J_{a,b}$ = 10.4 Hz), 3.95 (1 H, dd, H-2'b, $J_{b,3'}$ = 3.3, $J_{b,a}$ = 10.4 Hz), 3.57 (1 H, dd, H-6'a, $J_{a,5'}$ = 2.2, $J_{a,b}$ = 12.6 Hz), 3.66-3.49 (2 H, m, H-5', 6'b). *Anal.* Calcd for $C_9H_{13}N_3O_4$·HCl·0.4 H_2O: C, 39.91; H, 5.51; N, 15.51. Found: C, 40.16; H, 5.53; N, 15.30.

(2*R*, 3*R*)-1,4-Anhydro-2-*C*-(cytosin-1-yl)-2-deoxy-2-*C*-hydroxymethyl-D-arabitol hydrochloride (9). BF_3·Et_2O (640 μL, 5.2 mmol) was added to a solution of **37** (273 mg, 0.65 mmol) in Ac_2O (8 mL) at 0 °C and the mixture was stirred for 2.5 h at room temperature under argon. Saturated aqueous $NaHCO_3$ (30 mL) was added slowly to the reaction mixture and the whole was extracted with $CHCl_3$ (2 x 30 mL). The separated organic phase was washed with brine (50 mL) and dried (Na_2SO_4). The solvent was removed *in vacuo* and the residue was coevaporated three times with EtOH, which was treated with NH_3/MeOH (saturated at 0 °C, 10 mL) for 13 h at room temperature. The solvent was removed *in vacuo* and the residue was purified on a silica gel column (2.8 x 8 cm) with 25% MeOH in $CHCl_3$. The fractions containing **9** was dissolved in a mixture of 1 N HCl (1 mL) and EtOH (5 mL). The mixture was concentrated *in vacuo* and the residue was coevaporated 3 times with EtOH to give **9** as a hydrochloride (152 mg, 84%, crystallized from aqueous EtOH): mp 197-203 °C; FAB-MS *m/z* 277 (M^+); ^{1}H-NMR (DMSO-d_6) 9.55 and 8.48 (each 1 H, br s, 4-NH_2), 8.10 (1 H, d, H-6, $J_{6,5}$ = 7.7 Hz), 6.09 (1 H, dd, H-5, $J_{5,6}$ = 7.7 Hz), 4.94-4.92 (1 H, m, H-3'), 3.95 (1 H, dd, H-2'a, $J_{a,3'}$ = 1.8, $J_{a,b}$ = 10.4 Hz), 3.95 (1 H, dd, H-2'b, $J_{b,3'}$ = 6.0, $J_{b,a}$ = 10.4 Hz), 3.71-3.67 (2 H, m, H-6'a or H-7'a,b), 3.59 (1 H, dd, H-6'a or H-7'a, $J_{a,5'}$ = 6.0, $J_{a,b}$ = 11.0 Hz), 3.55-3.53 (1 H, m, H-5'), 3.49 (1 H, dd, H-6'b or H-7'b, $J_{b,5'}$ = 5.0, $J_{b,a}$ = 11.0 Hz), 2.30-2.26 (1 H,m, H-4'). *Anal.* Calcd for $C_{10}H_{15}N_3O_4$·HCl: C, 43.25; H, 5.81; N, 15.13. Found: C, 43.31; H, 5.96; N, 14.96.

Antivirus Assays in General. African green monkey kidney cells (Vero) were used for anti-HSV-1 and anti-HSV-2 assays. Human fibroblastic diploid cells (human embryo lung fibroblast, HEL), were

used for the anti-HCMV assay. HB611 cells were used for the anti-HBV assay. Cells except HB611 cells were grown in Eagle's minimum essential medium containing 5% calf serum (Vero), or 10% fetal calf serum (HEL). HB611 cells were developed from human hepatoblastoma cells Huh6-c15[13] by integrating tandem arranged HBV genomes (3 copies).[14] HB611 cells were grown in Dulbecco's modified Eagle medium containing 10% fetal calf serum. HSV-1 strain KOS, HSV-2 strain 186, and HCMV strain AD169 were used.

Fresh confluent monolayers of Vero (for HSV) or HEL (for HCMV) cells in 12-well tissue culture plates were infected with 100 to 150 PFU of each virus strain. After a 1-h adsorption period at 37 °C, the cultures were mixed with the relevant culture medium and various concentrations of test compounds. The cultures infected with HSV or HCMV were fixed and stained at 2 and 7 days after infection, respectively. Plaques were counted with a dissecting microscope at x 10 magnification. Acyclovir (ACV) and Ganciclovir (DHPG) were purchased from Sigma Chemical Co.

Assay for Anti-Hepatitis B Virus Activity. Details of the assay method were described previously.[15,16] Briefly, confluent cultures of HB611 cells in 12-well tissue culture plates were treated with various concentrations of compounds for 15 consecutive days. Culture medium was exchanged every three days for the fresh one and test compounds dissolved in DMSO (final concentration of DMSO is 0.5%) were added at the same time. Intracellular HBV DNA forms were analyzed at the end of the treatment period. The cells were harvested and total DNA was prepared by lysing in 10 mM Tris-HCl (0.5 mL, pH 7.4), 5 mM EDTA, 1% SDS, and proteinase K (0.1 mg/mL). The lysate was incubated at 55 °C for 3 h followed by RNase A treatment. After phenol-chloroform extraction and EtOH precipitation, the DNA was dissolved in 10 mM Tris-HCl (pH 7.4) and 1 mM EDTA. The extracted total cellular DNA was digested with *Hind*III, which did not cleave within the HBV genomes. A sample of the DNA (2.5 μg) was electrophoresed in a 1.5% agarose gel with TAE buffer (40 mM Tris-acetate and 1 mM EDTA) followed by blotting onto a nylon filter as described by Southern.[15] The filter was hybridized with ^{32}P-labeled HBV DNA probe, washed twice with 0.1 x standard saline citrate (SSC) containing 0.1% SDS at 65 °C for 30 min, and autoradiographed. To evaluate the inhibitory activity of the compounds, we measured the radioactivity of band areas S, D1, D2, and I by an image analyzer (Fujix Bioimage Analyzer BAS2000) and calculated the inhibition percentage as follows[17]: Inhibition (%) = [1 - (S_{drug} + $D1_{drug}$ + $D2_{drug}$)/I_{drug}/(S_{cont} + $D1_{cont}$ + $D2_{cont}$)/I_{cont}] x 100. In this formula, S represents a single-stranded full sized linear minus strand, D1 represents a partially double-stranded DNA consisting of the S and an incompletely synthesized plus strand, and D2 represents the D1 molecule in circular form, as is found in virions. I represents the chromosomally integrated HBV genome. Division of (S + D1 + D2) by I can eliminate technical variations.

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