Pergamon

Bioorganic & Medicinal Chemistry, Vol. 4, No. 4, pp 609–614, 1996

0968-0896/96 $15.00 + 0.00

S0968-0896(96)00048-X

Studies on the Synthesis and Biological Activities of 4′-(*R*)-Hydroxy-5′-(*S*)-hydroxymethyl-tetrahydrofuranyl Purines and Pyrimidines

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Abstract—A series of 4′-(*R*)-hydroxy-5′-(*S*)-hydroxymethyl-tetrahydrofuranyl purines and pyrimidines were synthesized by the reaction of 3,4-epoxy-5-(*S*,*trans*)-dimethoxymethyl-tetrahydrofuran and nucleobases under the catalysis of potassium *tert*-butoxide and 18-crown-6. Compounds **6a**, **6c** and **7b** have shown significant inhibition on the growth of HL-60 cells. The phosphotriester and phosphodiester of isonucleoside **8a–d** were synthesized and cytotoxic activities were reported. The conformation of isonucleosides in solution was studied by ^{1}H NMR. Copyright © 1996 Elsevier Science Ltd

Introduction

A number of analogues of nucleosides have been found to possess anticancer and antiviral activities.[1] Modification in the sugar part of nucleosides has led to the development of novel nucleoside analogues including acyclic[2] and carbocyclic nucleoside analogues,[3] nucleoside with a four-membered ring[4] and isonucleoside.[5] The therapeutic use of some nucleosides, for example ddA and ddI, is limited by the instability of their glycosidic bond at low pH and under enzymatic deactivations.[6] For more stable and effective anticancer and antiviral agents, there is considerable interest in the investigation of isonucleosides in which the nucleobase is linked to the position of ribose other than C_1. A series of isomeric of 2′,3′-dideoxynucleosides which contain a modified carbohydrate moiety have been synthesized and some of the compounds exhibited significant and selective anti-HIV activity.[7] New regioisomers of AZT, AZU, BVDU and IDU have also been investigated.[8] Kakefuda reported the synthesis and biological evaluations of ring-expanded oxetanocin analogues which have a nucleobase at the 2-position of tetrahydrofuran ring.[9] Efforts have primarily focused on modification of the sugar moiety of these molecules. We have reported the synthesis of fluoro-isonucleosides and their anti-cancer activities.[10] We report here on the synthesis of 3′-(*S*)-nucleobase-4′-(*R*)-hydroxy-5′-(*S*)-hydroxymethyl-tetrahydrofuran and the preliminary discussion of the relationship between the anticancer activity and conformation of these compounds.

Key words: isonucleoside, cytotoxicity, anti-HSV type I and HSV type II, conformation, stereochemistry.

Chemistry

3-(*R*)-Hydroxy-4-(*S*)-tosyl-5-(*S*)-dimethoxymethyl-tetrahydrofuran (**2**) prepared from 1′,2′-*O*-isopropylidene-α-D-xylose (**1**) in very good yield[7a] was treated with potassium carbonate in methanol at room temperature to yield 95% of 3,4-epoxy-5-(*S*,*trans*)-dimethoxymethyl-tetrahydrofuran (**3**). The substituted nucleobase was formed by the reaction of the epoxide **3** with corresponding nucleobase in the presence of potassium *tert*-butoxide and crown ether. The yield of substituted purine was higher than that of substituted pyrimidine, but guanine gave especially low yield due to its poor solubility. Two regioisomers **4** and **5** were obtained and separated with **4** as the main product. The structures of compounds **4** and **5** were identified by ^{1}H NMR COSY and NOESY spectra after deprotection. In the case of **6a**, for example, a strong NOE signal between H_8 of adenine and $H_{2'}$, $H_{4'}$, $H_{5'}$ of the tetrahydrofuran ring was observed. The dimethoxymethyl group in **4** was hydrolyzed in 3% TFA at 80 °C and reduced by $NaBH_4$ at room temperature to give corresponding **6** in good yield. The halogen substituted derivatives **7a** and **7c** were prepared from **6a** and **6c** by the general procedure. Compound **6c** was treated with $POCl_3$ and triazole followed by concd NH_4OH to give **7b**.

McGuigan et al. reported that introduction of bis-(trihaloethyl) substituents to the phosphate site of nucleoside analogues can increase both lipophilicity and hydrolytic lability and consequently increase their biological activity.[11] Thus compounds **8a–d** were synthesized from the phosphorylation of corresponding isonucleosides.

The phosphorylations of **6a** and **6c** were performed by using phosphorylating agent N^1-benzotriazoyl-*n*-

butyl-2-chlorophenylphosphate (I) or N^1-benzotriazoyl-*n*-octyl-2-chlorophenylphosphate (II) to give compounds **8a–d** in 80% yields. Compound **8a** is a mixture of stereoisomers which showed two peaks in ^{31}P NMR at −6.58 and −6.64 ppm (1:1) corresponding to *S*p and *R*p. When phosphorylating agent II was used in this reaction, it was found that the reaction was stereoselective and **8b** or **8c** was formed as a single isomer (^{31}P NMR: **8b**: −5.81; **8c**: −6.82). Evidently the bulky octyl group and *o*-chlorophenyl in reagent II led to the stereoselective substitution in the phosphorylation reaction (Fig. 1).

Results and Discussion

The isonucleoside derivatives **6a–d**, **7a–c** and **8a–d** were evaluated in vitro for cytotoxicity in HL-60 cells and inhibitory effect on HSV-1 and HSV-2 (Table 1).

Compounds **6a**, **6c** and **7b** showed significant activities of cytotoxicity in HL-60 cells, the ED_{50} being 5.08, 1.60 and 8.06 μM, respectively. Compound **6c** also exhibited some inhibitory effect on HSV-1 and HSV-2. However, no increase in the cytotoxicity of compounds **8a–d** against HL-60 cells was observed when compared to the corresponding isonucleotides **6a** and **6c**. It might be speculated that compounds **6a**, **6c** and **7b** could be phosphorylated efficiently by nucleoside kinase in HL-60 cells and the lipophilicity of these compounds would not influence the cytotoxicity.

In order to investigate the relationship between the biological activities and structure of isonucleosides, the conformations of compounds **6a–d** and **7a** in solution have been studied by ^{1}H NMR spectroscopy. The chemical shifts and coupling constants of the relevant protons for compounds **6a–d** and **7a** were determined by means of simulated spectra of 1-D ^{1}H NMR. Table 2 shows the data of coupling constants. Their conformations were analysed using the method described by Altona.[12] The concept of pseudorotation with phase angle (P) and puckering amplitude (ψ m) have been used for the description of the geometry of the tetrahydrofuran ring. Table 3 shows the puckering parameters for compounds **6a–d** and **7a**. In the fast

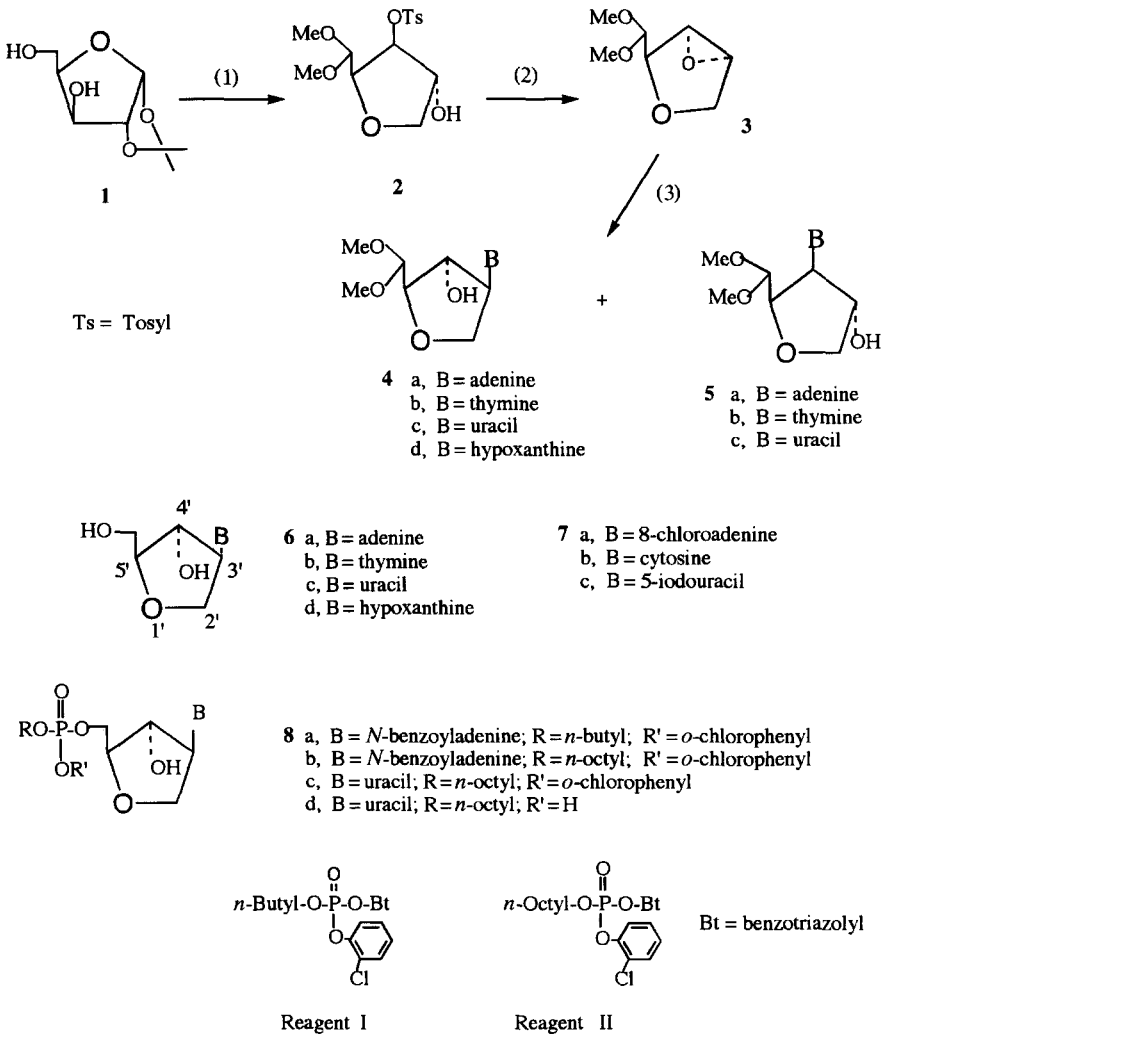


Figure 1. The synthesis and structures of isonucleosides. (1) Tosyl chloride, 20 °C, 4 days; 1% TFA, 75 °C, 8 h; 90% yield; (2) K_2CO_3/methanol, 95% yield; (3) nucleobase, *t*-BuoK, 18-crown-6, DMF, 20 °C, 30 min, 22–65% yield.

Table 1. The inhibitory effect of isonucleosides on HL-60 cells, HSV-1 and HSV-2

Compd	ED_{50} (μM)	IC_{50} (μM)	
	HL-60	HSV-1	HSV-2
6a	5.08	124	>200
6b	>100		
6c	1.6	68.5	137
6d	>100	>200	>200
7a	>100	>200	>200
7b	8.06	>200	>200
7c	>100	>200	>200
8a	190		
8b	4.5		
8c	170		
8d	9.3		

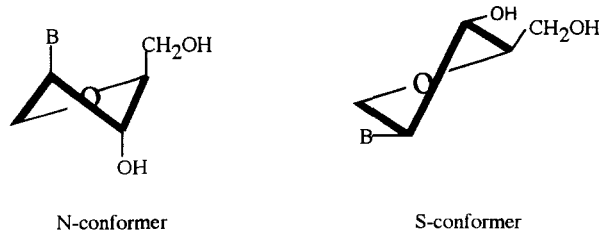
equilibrium between two broad classes of conformers (N-type referring to north in the pseudorotation wheel and S-type referring to south), the populations of the two conformers were described as X_N and X_S. Our result indicated that all the compounds adopted predominantly the S-type conformation (*C2′-endo/C3′-exo*). It seems that the cytotoxicity for HL-60 cells is reduced with an increase in the population of S-type conformer (Table 3). But such a relationship has not been observed in the cases of normal nucleosides or dideoxynucleosides (Fig. 2). Roey et al. have reported the correlation between preferred sugar ring conformation and activity of 3′-azido-deoxy and dideoxynucleoside analogues against HIV.[13] They described the analysis of the solid-state conformations of six active and two inactive anti-HIV nucleoside analogues and found that the active compounds have C3′-*exo* sugar

Table 2. Relevant vicinal couplings (Hz) in compounds **6a–d** and **7a** in DMSO-d_6

Coupling constant	**6a**	**6b**	**6c**	**6d**	**7a**
$^3J_{2',2''}$	−7.9	−7.2	−7.3	−7.0	−6.5
$^3J_{2',3'}$	5.2	4.8	6.2	4.7	5.3
$^3J_{2'',3'}$	5.3	5.2	4.4	4.2	5.8
$^3J_{3',4'}$	4.5	4.5	4.1	4.5	4.4
$^3J_{4',5'}$	4.2	5.6	5.6	5.4	4.0
$^3J_{5',6'}$	2.0	2.1	1.8	2.1	2.1
$^3J_{5',6''}$	2.4	2.8	2.2	2.3	2.8
$^3J_{6',6''}$	−9.5	−10.5	−9.6	−9.5	−8.8

Table 3. Puckering parameters for compounds **6a–d** and **7a**

Compd	$_m(S)=_m(N)$	P_N	P_S	X_N	ED_{50}
6a	37.08	1.0	176	0.40	5.08
6b	38.49	−2.0	172	0.39	>100
6c	38.08	−1.0	140	0.43	1.60
6d	41.76	2.0	164	0.35	>100
7a	25.74	2.0	139	0.37	>100



N-conformer S-conformer

Figure 2. The conformation of isonucleosides.

ring conformations and the inactive compounds have C3′-*endo* conformations. Jagannadh et al. investigated the sugar pucker of 2′,3′-dideoxynucleosides with anti-HIV activity in solution.[14] The results do not support what has been obtained from the X-ray diffraction studies. All these reports show that it is difficult to draw a relationship between the nature of the sugar pucker and biological activities. More data will be required for deducing more general and meaningful conclusions.

Experimental

Column chromatography was performed on silica gel (200–300 mesh, purchased from Qing-Dao Chemical Company, China). UV spectra were recorded with a Varian DMS-200 spectrophotometer. A ZAB-HS was used for mass spectra. ^1H, ^{13}C and ^{31}P NMR spectra were recorded with a VXR-300 and Bruker AM-500 with TMS as internal standard or 85% H_3PO_4 as external standard. The simulated spectra were prepared using program 'SPINS' on a VXR-300 at room temperature. Evaporations were carried out under reduced pressure with the bath temperature below 40 °C.

3,4-Epoxy-5-(*S,trans*)-dimethoxymethyl-tetrahydrofuran (3). A solution of 13.4 g of 3-(*R*)-hydroxy-4-(*S*)-tosyl-5-(*S*)-dimethoxymethyl-tetrahydrofuran (**2**)[15] dissolved in 200 mL methanol was treated with 6.7 g of potassium carbonate at room temperature for 1 h. After neutralization and evaporation, the residue was purified on silica gel column to give 6.14 g of compound **3** in 95% yield. ^1H NMR ($CDCl_3$, δ): 3.334 (3H, s, $-OCH_3$), 3.347 (3H, s, $-OCH_3$), 3.692 (1H, d, $H_{2''}$), 3.726 (1H, d, $H_{3'}$), 3.785 (1H, d, $H_{2'}$), 3.847 (1H, d, $H_{4'}$), 3.896 (1H, d, $H_{5'}$), 4.269 (1H, d, $H_{6'}$); ^{13}C NMR ($CDCl_3$, δ): 56.052 ($2\times OCH_3$), 56.540, 56.565 ($C_{3'}$, $C_{4'}$), 67.630 ($C_{2'}$), 77.806 ($C_{3'}$), 104.496 ($C_{6'}$).

3′-(*S*)-(Adenyl-9)-4′-(*R*)-hydroxy-5′-(*S*)-dimethoxymethyl-tetrahydrofuran (4a) and 3′-(*R*)-hydroxy-4′-(*S*)-(adenyl-9)-5′-(*S*)-dimethoxymethyl-tetrahydrofuran (5a). A mixture of adenine (4.1 g, 30 mmol), potassium *tert*-butoxide (3.36 g, 30 mmol) and compound **3** (3.2 g, 20 mmol) was dissolved into 100 mL of DMF containing 0.79 g of 18-crown ether-6. The mixture was heated at 80 °C for 68 h under nitrogen. After neutralization and evaporation, the residue was purified by silica gel chromatography eluting with chloroform and

methanol to give **4a** (3.84 g) in 65% yield and **5a** (0.22 g) in 3.7% yield.

Compound **4a**: mp 180–182 °C, UV (MeOH) λ_{max} = 260.8 nm, MS(FAB$^+$): 296 (M+1); elemental anal.: calcd C, 48.81; H, 5.76; N, 23.73, found C, 48.65; H, 5.80; N, 23.46. ^{1}H NMR (DMSO-d_6, δ): 3.30, 3.31 (6H, 2s, 2×—OCH_3), 3.78 (1H, m, $H_{5'}$), 4.17–4.21 (2H, m, $H_{2'}$, $H_{2''}$), 4.40 (1H, m, $H_{6'}$), 4.49 (1H, m, $H_{4'}$), 4.82 (1H, m, $H_{3'}$), 5.85 (1H, s, 4'-OH), 7.28 (2H, s, NH_2), 8.12 (1H, s, H_2), 8.16 (1H, s, H_8).

Compound **5a**: mp 204–206 °C, UV (MeOH) λ_{max} = 261.1 nm, MS(EI): 295 (M); elemental anal.: calcd C, 48.81; H, 5.76; N, 23.73, found C, 48.80; H, 5.80; N, 23.72. ^{1}H NMR (DMSO-d_6, λ): 3.17 (6H, s, 2×—OCH_3), 3.59–3.66 (2H, m, $H_{2'}$, $H_{2''}$), 4.13 (1H, m, $H_{3'}$), 4.50–4.52 (2H, m, $H_{5'}$, $H_{6'}$), 4.93 (1H, m, $H_{4'}$), 7.28 (1H, s, NH_2), 7.94 (1H, s, H_2), 8.16 (1H, s, H_8).

3'-(*S*)-(Adenyl-9)-4'-(*R*)-hydroxy-5'-(*S*)-hydroxymethyl-tetrahydrofuran (6a). A solution of 1.74 g of compound **4a** in 200 mL water containing 0.3% TFA was heated at 80 °C for 3 h then cooled to 0 °C. After neutralization, the mixture was treated with 0.27 g of $NaBH_4$ at room temperature for 40 min, then cooled to 0 °C and neutralized again. The residue was evaporated and purified on silica gel column to give compound **6a** 1.37 g in 90% yield.

Compound **6a**: mp 226–228 °C, $[\alpha]_D$ +43.4, UV (MeOH) λ_{max} = 260 nm, MS(EI): 252 (M), elemental anal.: calcd C, 47.81; H, 5.18; N, 27.89, found: C, 47.57; H, 5.21; N, 27.50. ^{1}H NMR (DMSO-d_6, δ): 3.52 (1H, m, $H_{6''}$), 3.59 (1H, m, $H_{6'}$), 4.08 (1H, m, $H_{2'}$), 4.15 (1H, m, $H_{2''}$), 4.38 (1H, m, $H_{4'}$), 4.68 (1H, m, $H_{5'}$), 4.85 (1H, m, $H_{3'}$), 5.76 (1H, m, OH), 7.24 (2H, s, NH_2), 8.14 (1H, s, H_2), 8.18 (1H, s, H_8). ^{13}C NMR (DMSO-d_6, δ): 61.049 ($C_{6'}$), 62.267 ($C_{3'}$), 69.823 ($C_{2'}$), 75.978 ($C_{4'}$), 118.877 ($C_{5'}$), 139.656 (C_8), 149.649 (C_4), 152.696 (C_2), 156.108 (C_6).

3'-(*R*)-Hydroxy-4'-(*S*)-(thymin-1-yl)-5'-(*S*)-dimethoxy-methyl-tetrahydrofuran (5b) and 3'-(*S*)-(thymin-1-yl)-4'-(*R*)-hydroxy-5'-(*S*)-hydroxymethyl-tetrahydrofuran (6b). The procedure of reaction is the same as the synthesis for **4a** and **5a**, but the reaction was carried out at 120 °C for 78 h to give **4b** in 34% yield and **5b** in 5.2% yield. Compound **4b** was treated with a 0.3% TFA water solution and $NaBH_4$ as described for **6a** to give **6b** in 70% yield.

Compound **5b**: UV (MeOH) λ_{max} = 272.7 nm, MS(FAB$^-$): 285 (M−2H). ^{1}H NMR (DMSO-d_6, δ): 3.241 (3H, s, OCH_3), 3.270 (3H, s, OCH_3), 3.586 (3H, s, CH_3), 3.612 (2H, m, $H_{2',2''}$), 3.753 (1H, m, $H_{3'}$), 3.861 (2H, m, $H_{5'}$, $H_{6'}$), 4.290 (1H, m $H_{4'}$), 7.197 (1H, s, H_6), 11.301 (1H, s, NH).

Compound **6b**: mp 148–150 °C, $[\alpha]_D$ +33.2, UV (MePH) λ_{max} = 272 nm, MS(EI): 242 (M); elemental anal.: calcd C, 49.79; H, 5.39; N, 11.62, found C, 49.25; H, 5.78; N, 11.27. ^{1}H NMR (DMSO-d_6, δ): 3.53 (2H, m, $H_{6'}$, $H_{6''}$), 3.64 (1H, m, $H_{5'}$), 3.82 (1H, m, $H_{2'}$), 3.98 (1H, m, $H_{2''}$), 4.11 (1H, m, $H_{4'}$), 4.79 (1H, m, $H_{3'}$), 5.65 (1H, s, OH), 7.52 (1H, s, H_6), 11.23 (1H, s, NH). ^{13}C NMR (DMSO-d_6, δ): 12.34 (CH_3), 60.62 ($C_{6'}$), 62.90 ($C_{3'}$), 69.46 ($C_{2'}$), 75.55 ($C_{4'}$), 85.79 ($C_{5'}$), 109.74 (C_5), 138.44 (C_6).

3'-(*R*)-Hydroxy-4'-(*S*)-(uracil-1-yl)-5'-(*S*)-dimethoxy-methyl-tetrahydrofuran (5c) and 3'-(*S*)-(uracil-1-yl)-4'-(*R*)-hydroxy-5'-(*S*)-hydroxymethyl-tetrahydrofuran (6c). The above procedures for **4a** and **4b** were adopted for the syntheses of **4c** and **5c** giving 22.1% and 4.7% yields, respectively. Compound **4c** was treated with 0.3% TFA and $NaBH_4$ to give **6c** in 90% yield.

Compound **5c**: mp 110–112 °C, UV (MeOH) λ_{max} = 267.4 nm, MS(FAB$^-$): 271 (M−H); elemental anal.: calcd C, 48.53; H, 5.88; N, 10.29, found C, 48.75; H, 6.02; N, 10.32. ^{1}H NMR (DMSO-d_6, δ): 3.368 (1H, m, $H_{5'}$), 3.918–3.962 (2H, m, $H_{2'}$, $H_{2''}$), 4.155 (1H, m, $H_{4'}$), 4.398 (1H, m, $H_{6'}$), 4.658 (1H, m, $H_{3'}$), 5.605 (1H, s, H_5), 7.548 (1H, m, H_6).

Compound **6c**: mp 197–199 °C, $[\alpha]_D$ −4.12, UV (MeOH) λ_{max} = 268 nm, MS(FAB$^+$): 229 (M+H); elemental anal.: calcd C, 47.37; H, 5.26; N, 12.28, found C, 47.68; H, 5.42; N, 12.49. ^{1}H NMR (DMSO-d_6, δ): 3.50 (1H, m, $H_{6'}$), 3.55 (1H, m, $H_{5'}$), 3.61 (1H, m, $H_{6''}$), 3.84 (1H, m, $H_{2'}$), 3.99 (1H, m, $H_{2''}$), 4.09 (1H, m, $H_{4'}$), 4.77 (1H, m, $H_{3'}$), 5.58 (1H, m, H_5), 7.63 (1H, m, H_6).

3'-(*S*)-(Hypoxanthin-9-yl)-4'-(*R*)-hydroxy-5'-(*S*)-dimethoxymethyl-tetrahydrofuran (4 d) and 3'-(*S*)-(hypoxanthin-9-yl)-4'-(*R*)-hydroxy-5'-(*S*)-hydroxy-methyl-tetrafuran (6d). Compound **4a** (1 g) was dissolved into 30 mL of acetic acid and reacted with 1.15 g of $NaNO_2$ at room temperature for 48 h. After removal of acetic acid, the residue was purified on silica gel column eluting with chloroform and methanol to give compound **4d** in 60% yield. Compound **4d** was treated with 0.3% TFA and $NaBH_4$ as described for **6a** to give **6d** in 80% yield.

Compound **4d**: mp 190–192 °C, $[\alpha]_D$ +55.7, UV (MeOH) λ_{max} = 250.2 nm, MS(FAB$^+$): 297 (M+H); elemental anal.: calcd C, 48.65; H, 5.41; N, 18.92, found C, 48.61; H, 5.32; N, 18.74. ^{1}H NMR (DMSO-d_6, δ): 3.35 (6H, 2s, 2×OCH_3), 3.75 (1H, m, $H_{5'}$), 4.12 (1H, m, $H_{2'}$), 4.18 (1H, m, $H_{2''}$), 4.35 (1H, m, $H_{6'}$), 4.40 (1H, m, $H_{4'}$), 4.80 (1H, m, $H_{3'}$), 5.75 (1H, s, OH), 8.08 (2H, s, H_2, H_8), 12.2 (1H, s, NH). ^{13}C NMR (DMSO-d_6, δ): 54.59, 55.57 (2×OCH_3), 62.94 ($C_{3'}$), 69.76 ($C_{2'}$), 76.77 ($C_{4'}$), 85.18 ($C_{5'}$), 104.07 ($C_{6'}$), 124.36 (C_5), 139.12 (C_8), 145.81 (C_4), 148.74 (C_2).

Compound **6d**: mp 270–272 °C, UV (MeOH) λ_{max} = 250.5 nm, MS (FAB$^+$): 253 (M+H); elemental anal.: calcd C, 47.62; H, 4.76; N, 22.22, found C, 47.56;

H, 4.40; N, 22.02. ^{1}H NMR (DMSO-d_6, δ): 3.53 (1H, m, $H_{6''}$), 3.61 (1H, m, $H_{6'}$), 3.67 (1H, m, $H_{5'}$), 4.05 (1H, m, $H_{2'}$), 4.15 (1H, m, $H_{2''}$), 4.33 (1H, m, $H_{4'}$), 4.84 (1H, m, $H_{3'}$), 5.75 (1H, s, OH), 8.05 (1H, s, H2), 8.14 (1H, s, H_8), 12.13 (1H, s, NH).

3′-(*S*)-(8-Chloroadenyl-9)-4′-(*R*)-hydroxy-5′-(*S*)-hydroxymethyl-tetrahydrofuran (7a). Compound **6a** (0.65 g) was dissolved into 30 mL of DMF and 6 mL of acetic acid, the mixture was reacted with 1.27 g of *N*-chlorosuccimide and 10 mg of *m*-chlorobenzohydroperoxide at room temperature for 24 h. After neutralization and evaporation, the residue was purified by silica gel chromatography eluting with chloroform and methanol to obtain compound **7a** in 10% yield.

Compound **7a**: mp 214–216 °C, $[\alpha]_D$ +44.1, UV (MeOH) λ_{max} = 263.7 nm, MS(EI): 285 (M); elemental anal.: calcd C, 42.03; H, 4.20; N, 24.52, found C, 41.93; H, 3.98; N, 24.21. ^{1}H NMR (DMSO-d_6, δ): 3.56 (1H, m, $H_{6''}$), 3.66 (1H, m, $H_{6'}$), 3.71 (1H, m, $H_{5'}$), 4.13 (1H, m, $H_{2'}$), 4.29 (1H, m, $H_{2''}$), 4.75 (1H, m, $H_{4'}$), 4.97 (1H, m, $H_{3'}$), 5.56 (1H, s, OH), 7.36 (1H, s, NH_2), 8.14 (1H, s, H_2).

3′-(*S*)-(Cytosin-1-yl)-4′-(*R*)-hydroxy-5′-(*S*)-hydroxymethyl-tetrahydrofuran (7b). Compound **6c** (0.17 g) was reacted with 0.21 mL of acetic anhydride in 5 mL of pyridine at room temperature for 5 h. The mixture was added into ice-water and stirred for 30 min. The solution was extracted with chloroform. After evaporation, the dry residue was reacted with 0.21 mL of $POCl_3$, 1.41 mL of triethylamine and 0.72 g of triazole in 2 mL of acetonitrile at room temperature for 1.5 h, then the mixture was evaporated under vacuum. The residue was dissolved into chloroform and washed with saturated sodium bicarbonate and purified by silica gel chromatography to give a white solid. This solid was dissolved into 1 mL of concentrated NH_4OH and 1 mL of dioxane at room temperature for 12 h; after dryness, the residue was recrystallized from methanol to give compound **7b** in 45% yield.

Compound **7b**: mp 235–237 °C, $[\alpha]_D$ −17.0, UV (MeOH) λ_{max} = 281.2 nm, MS (FAB$^+$): 229 (M+2H); elemental anal.: calcd C, 47.58; H, 5.73; N, 18.50, found C, 47.11; H, 5.59; N, 18.61. ^{1}H NMR (DMSO-d_6, δ): 3.47–3.61 (3H, m, $H_{5'}$, $H_{6'}$, $H_{6''}$), 3.76 (1H, m, $H_{2'}$), 3.98 (1H, m, $H_{2''}$), 4.06 (1H, m, $H_{4'}$), 4.82 (1H, m, $H_{3'}$), 5.68 (1H, s, H_5), 7.06 (2H, s, NH_2), 7.60 (1H, s, C_6).

3′-(*S*)-(5-Iodouracil-1-yl)-4′-(*R*)-hydroxy-5′-(*S*)-hydroxymethyl-tetrahydrofuran (7c). Compound **6c** (0.1 g) was reacted with 0.12 g of iodine and 3.4 mL of 0.8 N nitric acid in 8.4 mL of dioxane at 130 °C for 5 h. The reaction was stopped by adding 85 mg of sodium sulfite at room temperature. After dryness, the residue was purified by silica gel chromatography eluting with chloroform and methanol to give compound **7c** in 80% yield.

Compound **7c**: mp 130–132 °C, UV (MeOH) λ_{max} = 282 nm, MS(FAB$^+$): 355 (M+H), elemental anal.: calcd C, 30.51; H, 3.11; N, 7.91, found C, 30.46; H, 3.11; N, 7.91. ^{1}H NMR (DMSO-d_6, δ): 3.40–3.65 (3H, m, $H_{5'}$, $H_{6'}$, $H_{6''}$), 3.93 (1H, m, $H_{2'}$), 3.98 (1H, m, $H_{2''}$), 4.16 (1H, m, $H_{4'}$), 4.76 (1H, m, $H_{3'}$), 8.13 (1H, s, H_6), 11.71 (1H, s, NH).

Phosphorylation of compounds 6a and 6c

General method. *N*-Hydroxybenzotriazole (HOBT) 1.35 g (10 mmol) was dissolved into 0.8 mL of pyridine and 20 mL of dioxane. A solution of 1.22 g (5 mmol) of *O*-chlorophenylphosphoryl dichloride in 4 mL of dioxane was added and the mixture was stirred at room temperature for 3 h under nitrogen. After filtration, the filtrate was kept for the preparation of phosphorylating reagent I and II. Then 1 mmol of prepared solution and 1 mmol of *n*-butanol or 1 mmol of *n*-octanol was mixed at room temperature and stirred for 20 min to give reagent I or II.

N^6-Benzoyl derivative of **6a**† or **6c** (0.66 mmol) was dissolved into 2 mL of pyridine, then reacted with 0.21 mL of *N*-methyl imidazole and reagent I or II (0.66 mmol) at 20 °C for 1 h. The mixture was diluted with 5 mL of 1 M tetraethylammonium bromide and 5 mL of methylene chloride. The methylene chloride solution was washed with water and after dryness, the residue was purified by silica gel chromatography to give the correpsonding compounds **8a–c** in 80% yield. Compound **8c** was treated by general method to obtain deprotected compound **8d** in 86% yield.

Compound **8a**: MS (FAB$^-$): 600 (M−H); ^{31}P NMR ($CDCl_3$, δ): −6.641; −6.567. ^{1}H NMR ($CDCl_3$, δ): 0.897 (6H, m, 2×CH_3), 1.364 (4H, m, 2×CH_2), 1.676 (4H, m, 2×CH_2), 4.049–4.148 (10H, m, 2×$H_{5'}$, 2×$H_{6'}$, 2×$H_{6''}$, 2×OCH_2), 4.522–4.523 (4H, m, 2×$H_{2'}$, 2×$H_{2''}$), 4.699 (2H, m, $H_{4'}$), 5.038 (2H, m, $H_{3'}$), 7.033–8.088 P8H, m, aromatic ring), 8.681 (2H, d, H_2), 8.688 (2H, d, H_8).

Compound **8b**: MS (FAB$^+$): 658 (M+H); ^{31}P NMR ($CDCl_3$, δ): −5.811. ^{1}H NMR ($CDCl_3$, δ): 0.856 (3H, m, CH_3), 1.223 (10H, m, 5×CH_2), 1.664 (2H, m, CH_2), 4.157–4.226 (5H, m, $H_{5'}$, $H_{6'}$, $H_{6''}$, CH_2), 4.434–4.502 (2H, m, $H_{2'}$, $H_{2''}$), 4.722 (1H, m, $H_{4'}$), 5.050 (1H, m, $H_{3'}$), 7.073–7.593 (11H, m, NH_2, aromatic ring), 8.663 (2H, m, H_2, H_8).

Compound **8c**: MS (FAB$^+$): 529 (M−H); ^{31}P NMR ($CDCl_3$, δ): 0.868 (3H, m, CH_3), 1.239 (10H, m, CH_2), 1.630 (2H, m, CH_2), 3.961–4.240 (5H, m, $H_{5'}$, $H_{6'}$, $H_{6''}$, CH_2), 4.332–4.409 (2H, m, $H_{2'}$, $H_{2''}$), 4.474 (1H, m, $H_{4'}$), 4.942 (1H, m, $H_{3'}$), 5.671 (1H, m, H_5), 7.10–7.50 (5H, m, H_6, aromatic ring).

†Compound **6a** was treated by benzoic anhydride and pyridine as general procedure. The product was identified by MS and ^{1}H NMR.

Compound **8d**: MS (FAB^-): 491 (M−H); ^1H NMR (D_2O, δ): 0.615 (3H, m, CH_3), 1.022 (10H, m, CH_2), 1.371 (4H, m, CH_2), 3.566–3.617 (3H, m, $H_{5'}$, $H_{6'}$, $H_{6''}$), 3.881–3.944 (3H, m, $H_{4'}$, $H_{2'}$, $H_{2''}$), 4.003–4.026 (1H, m, $H_{3'}$), 5.660 (1H, s, H_5), 7.521 (1H, s, H_6).

Cytotoxicity of compounds 6, 7 and 8 in HL-60 cells

The cytotoxicity of compounds **6**, **7** and **8** against HL-60 cells was evaluated by colorimetric MTT assay.[16] The HL-60 cells were seeded in 96-well plates at a cell density of 5×10^3 cells/well and were incubated with the isonucleosides at different concentrations for 12 h. Twenty μL of MTT solution (5 mg/mL) were added to each well of microplate which was then incubated for 4 h at 37 °C in 5% CO_2 in air. The medium was then aspirated and the reduced product was solubilized in 100 μL of DMSO per well. The absorbance at 550 nm ws read with an ELISA reader (Bio-Rad).

Inhibition of HSV type 1 and HSV type 2 in vero cells

Inhibitory activity of compounds against the HSV-1 and HSV-2 multiplication in acutely infected cells was based on the inhibition of virus-induced cytopathogenicity in vero cells. The infected vero cells were incubated with the isonucleosides at different concentrations at 37 °C in 5% CO_2 in air for 3 days then evaluated by colorimetric MTT assay.[16]

Acknowledgment

We thank the National Natural Science Foundation of China for financial support.

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(*Received in Japan* 4 *September* 1995; *accepted* 16 *January* 1996)