# A Facile, Alternative Synthesis of 4′-Thioarabinonucleosides and Their Biological Activities

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4′-Thioarabinonucleosides, which are potential antiviral agents, were synthesized from D-glucose. 1,4-Anhydro-4-thioarabitol (**8**), which can be derived from diacetone glucose in nine steps, was subjected to Pummerer rearrangement after protection of the hydroxyl groups to give 1-*O*-acetyl-4-thioarabinose (**11**), which was condensed with nucleobases to give 4′-thioarabinonucleosides. The 5-substituted-4′-thioaraU (**6a**−**e**) derivatives showed anti-HSV-1 activity ($ED_{50}$ = 0.43−3.50 $\mu$g/mL). 4′-ThioaraG (**6h**) and 2,6-diaminopurine 4′-thioarabinonucleoside (4′-thioaraDAP, **6g**) showed antiviral activity against several herpes viruses and were particularly potent against human cytomegalovirus (0.010 and 0.022 $\mu$g/mL, respectively).

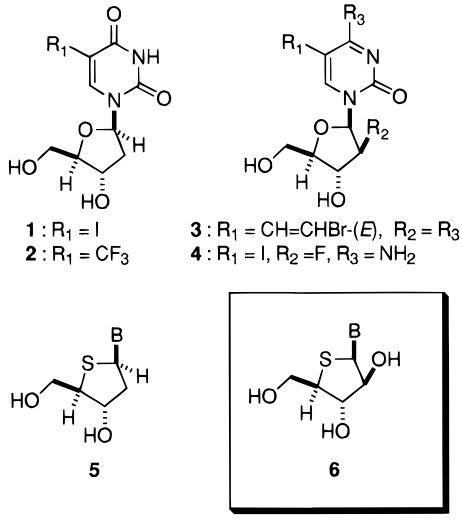
## Introduction

The development of new effective antiviral agents is essential for overcoming diseases that are caused by viruses, such as acquired immunedeficiency syndrome (AIDS). The first effective antiviral agents were 5-substituted-2′-deoxyuridines, such as 5-iodo-2′-deoxyuridine[1] (5-IdUrd, **1**; Chart 1) and 5-(trifluoromethyl)-2′-deoxyuridine[2] (5-$CF_3$dUrd, **2**), which are used for the treatment of herpes simplex virus (HSV) infections. Since then, many efforts have been made to synthesize various nucleoside antimetabolites. As a consequence, some 2′-modified derivatives, *e.g.*, 5-(bromovinyl)-1-(β-D-arabinofuranosyl)uracil[3] (BVaraU, **3**) and 5-iodo-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cytosine[4] (FIAC, **4**), have been shown to have potent anti-HSV activities. The former is an arabinosyl nucleoside, and the latter is a unique fluorine-containing nucleoside. Furthermore, both derivatives have 2′-"up" stereochemistry (*arabino* configuration), which is important for the recognition of virus-encoded thymidine kinase, but not host kinases.

In 1991, another novel class of antiviral agents was independently reported by Walker[5] and Secrist.[6] The 2′-deoxy-4′-thionucleosides **5**, 4′-thio congeners of the first generation of antivirals described above, were shown to have potent antiviral activities and cytotoxicities.[5,6] These findings triggered the further investigation of 4′-thionucleosides as potential therapeutic agents for treating virus infection and cancer. Although there were reports of the synthesis of some 4′-thionucleosides before 1991,[7−11] most of these studies focused only on their antitumor activities.[9,10] Welcome's group recently illustrated that 2′-deoxy-4′-thiopurine nucleosides possess potent anti-human cytomegalovirus activity[12] and 2′-deoxy-4′-thiopyrimidine nucleosides possess anti-HSV-1 activity.[13]

These results drew our attention to the design and synthesis of 4′-thionucleosides as potential antiviral agents: some 2′-modified 4′-thionucleosides, such as 4′-thioarabinonucleosides and 2′-deoxy-2′-fluoro-4′-thioarabinonucleosides, which represent the 4′-thio counterparts mentioned above, could be promising. Although the synthesis of the former group has been reported previously,[8,9,11] their biological effects, including their antiviral activities, remain unknown, except for those of 4′-thioaraC.[9] Recently, we explored a new strategy for the synthesis of 2′-substituted-4′-thionucleosides.[14] This method should be applicable to the synthesis of 4′-thioarabinonucleosides. Hence, we report here a facile, alternative synthesis of 4′-thioarabinonucleosides **6**, beginning from D-glucose. Their antiviral activities are also discussed.[15]

**Chart 1**




## Results and Discussion

**Chemistry.** We previously reported that 1,4-anhydro-4-thioarabitol (**8**) could be derived from diacetone glucose (**7**) in nine steps.[14] Compound **8**, a versatile intermediate for the synthesis of the target nucleosides, was benzylated to give the tribenzyl derivative **9** in good yield. Previously, we reported the trimethysilyl triflate (TMSOTf)-catalyzed Pummerer-type glycosylation reac-

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**Scheme 1**

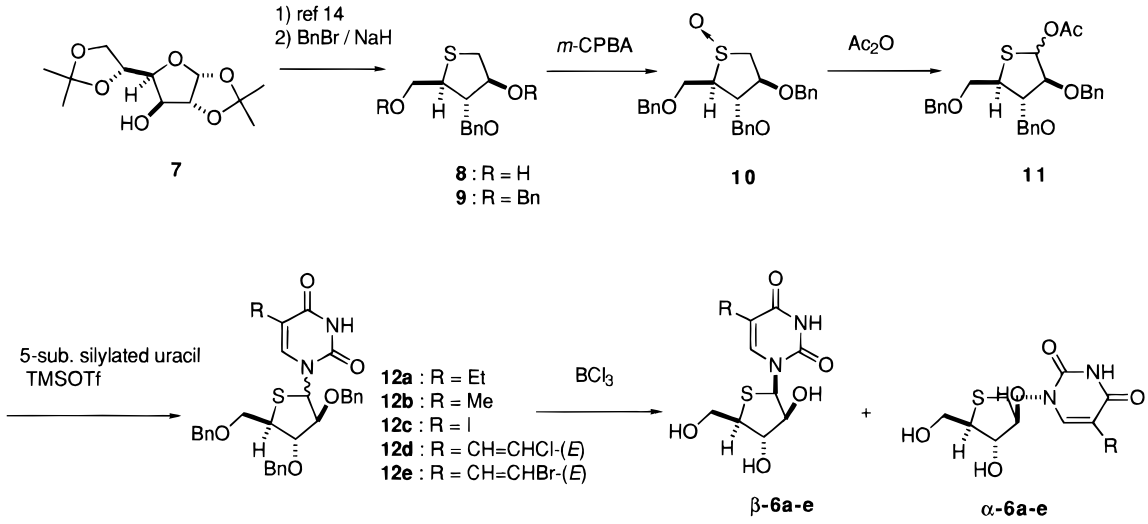



**Table 1.** Chemical Yield and α,β-Selectivity of Glycosylation Reaction

| compd no. | R | yield from **11** (%) (isolated yield of β-anomer, %) | α/β ratio[a] |
|---|---|---|---|
| **6a** | Et | 35 (11) | 2.30 |
| **6b** | Me | 50 (14) | 2.16 |
| **6c** | I | 45 (22) | 1.58 |
| **6d** | −CH=CHCl-(*E*) | 37 (11) | 2.36 |
| **6e** | −CH=CHBr-(*E*) | 52 (13) | 2.22 |

[a] α,β-Ratios were determined by HPLC analysis of free nucleosides.

tion using sulfoxides as glycosyl donors for the preparation of 4′-thioDMDC and 4′-thiogemcitabine.[14] However, application of this approach to sulfoxide **10**, obtained from **9** by *m*-CPBA oxidation, gave the α-4′-thionucleoside exclusively (data not shown). Therefore, we selected 1-acetate **11**, in place of **10**, for the glycosylation reaction. The tribenzyl derivative **9** was subjected to Pummerer rearrangement (*m*-CPBA oxidation followed by treatment with acetic anhydride) to give **11** as an anomeric mixture (1.7:1). Next, Vorbrüggen glycosylation[16] between **11** and persilylated 5-ethyluracil was investigated. The reaction of silylated 5-ethyluracil with **11** in the presence of TMSOTf in 1,2-dichloroethane proceeded efficiently (Scheme 1). However, the resulting α,β-anomers of protected nucleosides **12a** could not be separated using a silica gel column. An α,β-mixture of **12a** was deprotected using boron trichloride ($BCl_3$), and the resulting anomers were separated by HPLC to give α- and β-5-ethyl-4′-thioarabinouridine (**6a**) in yields of 24% and 11% from **11**, respectively. The other 5-substituted-pyrimidine arabinonucleosides (**6b**−**e**) were synthesized in a similar manner, and the results are summarized in Table 1. The structure of these 4′-thioarabinonucleosides (**6b**−**e**) was confirmed by instrumental analysis. The anomeric configuration of these nucleosides was easily assessed by comparing the chemical shifts of H-4′ protons; those of α-anomers were shifted downfield due to the deshielding effect of the C-2 keto group of the uracil moiety.[14,17] The α,β-ratios of the glycosylation reactions were approximately 1.5−2.4:1, and α-isomers were predominant.

We next tried to synthesize purine analogues using **11**. In contrast to pyrimidine derivatives, the reactions between **11** and purine derivatives were complicated. As a first target, we tried to synthesize 4′-thioarabinosyladenine (4′-thioaraA). The glycosylation reaction of pertrimethylsilylated adenine with **11**, under the conditions described above (method A), gave 4′-thionucleosides **13**, which were debenzylated to give α- and β-**14** in yields of 15% and 7.3%, respectively. The results of the instrumental analysis of α- and β-**14** were consistent with the desired structures, except for their UV spectra, which showed red shift absorption at 275 and 273 nm, respectively. This red shift in their UV spectra relative to that of adenosine suggests that glycosylation had occurred at the 7-position of the adenine base. None of

**Scheme 2**

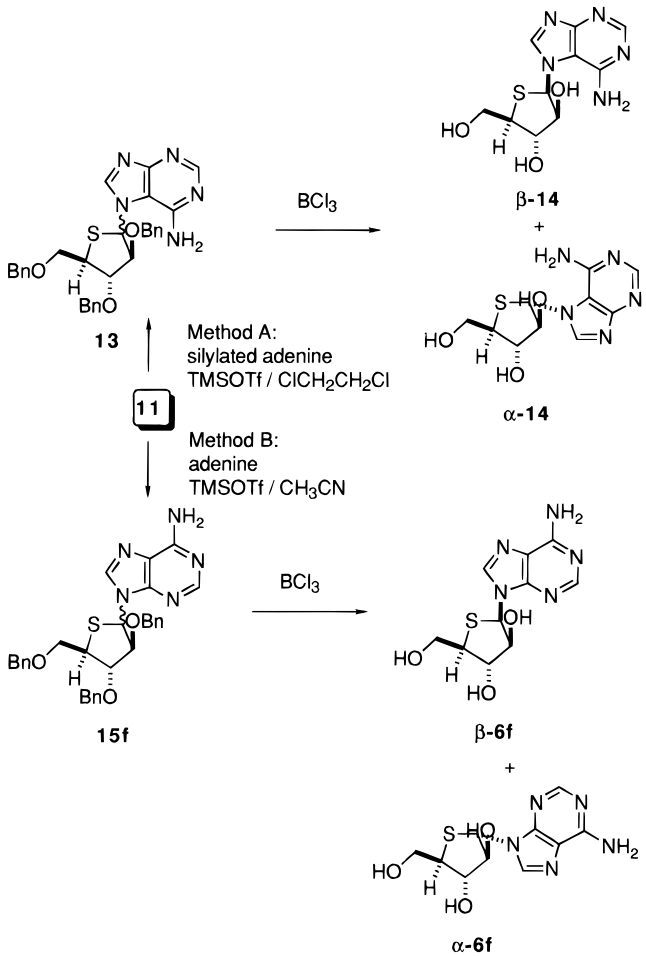
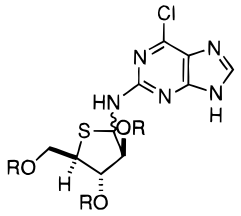


**Table 2.** Antiviral Activities of Pyrimidine 4′-Thioarabinonuleosides

| | | antiviral activities, $ED_{50}$ ($\mu$g/mL) | | | | anticell proliferative activity, $IC_{50}$ ($\mu$g/mL) |
|---|---|---|---|---|---|---|
| compd no. | R | HSV-1[a,e] | HSV-2[b,e] | VZV[c,e] | HCMV[d,e] | CCRF-HSB-2[f] |
| α-**6a** | Et | >100 | >100 | >50 | >50 | >100 |
| β-**6a** | Et | 0.43 | 6.5 | >50 | >50 | >100 |
| α-**6b** | Me | >100 | >100 | >50 | >50 | 42 |
| β-**6b** | Me | 0.77 | 4.6 | 6.6 | 44 | >100 |
| α-**6c** | I | >100 | >100 | >50 | >50 | >100 |
| β-**6c** | I | 3.50 | 13.6 | 27.1 | >50 | >100 |
| α-**6d** | −CH=CHCl-(*E*) | >100 | >100 | >50 | >50 | >100 |
| β-**6d** | −CH=CHCl-(*E*) | 0.97 | 71 | >50 | >50 | >100 |
| α-**6e** | −CH=CHBr-(*E*) | >100 | >100 | >50 | >50 | >100 |
| β-**6e** | −CH=CHBr-(*E*) | 0.82 | 58 | 0.20 | >50 | >100 |
| araC | | ND | ND | ND | ND | 0.0086 |
| BVAU | | 0.036 | 62 | 0.0013 | >50 | >100 |
| acyclovir | | 0.14 | 0.23 | 2.7 | 6.9 | >100 |

[a] VR-3 strain. [b] HSV-2 MS strain. [c] VZV Oka strain. [d] HCMV AD 169 strain. [e] Plaque reduction assay. [f] MTT assay.

**Chart 2**



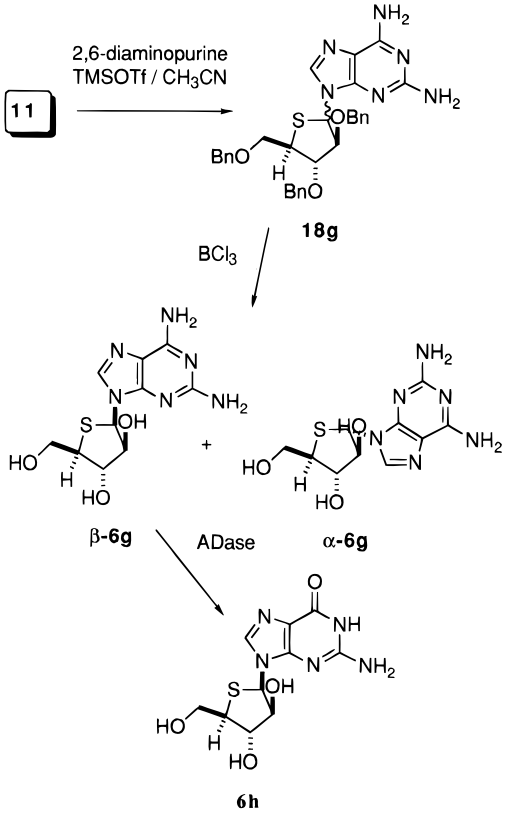

**16** : R = Bn
**17** : R = H

the 9-glycosylated products were found in the mixture after careful TLC analyses. We eventually found that the reaction of adenine, instead of pertrimethylsilylated adenine, and 1-*O*-acetyl-4-thioarabinose (**11**) in the presence of TMSOTf as a Lewis acid in acetonitrile[18] (method B) followed by deprotection of the product **15f** effectively gave α- and β-9-(4-thioarabinosyl)adenine (**6f**) in yields of 11% and 18% from **11**, respectively. Their UV as well as $^1$H NMR spectra and other instrumental analyses support the structure (Scheme 2). It is noteworthy that the formation of β-4′-thioaraA (β-**6f**), in sharp contrast to the pyrimidine derivatives, was predominant even though the total yield of the products decreased. The same conditions were used for the reaction with thymine. However, none of the desired 4′-thioaraT was obtained (data not shown).

To prepare the guanine derivative, we first attempted to use 2-amino-6-chloropurine as a nucleobase for the glycosylation reaction. This reaction gave an anomeric mixture of the products, which were deprotected by $BCl_3$ to give **17** in a low yield. The structure of **17** shown in Chart 2 was determined based on the following data: (1) In the $^1$H NMR spectrum, anomeric protons (5.26 and 5.51 ppm) were each coupled with a $D_2O$ exchangeable proton. (2) Both of the anomers, which were separated by HPLC, returned to an initial mixture when kept in an aqueous solution. (3) In the FAB mass spectra, both compounds showed molecular ion peaks at *m*/*z* 318. These results showed that glycosylation of 2-amino-6-chloropurine unexpectedly occurred at the 2-amino position.

Therefore, instead of using 2-amino-6-chloropurine, we selected 2,6-diaminopurine as a nucleobase for the glycosylation reaction. The reaction between 2,6-diaminopurine and **11** under the conditions described for the synthesis of 4′-thioaraA gave an anomeric mixture of 2,6-diamino-9-(4-thioarabinosyl)purine (**18g**) in a low

**Scheme 3**




yield, which was deprotected to give the α- and β-anomers of **6g** (5% and 9% yields from **11**, respectively). After separation by HPLC, the isolated β-**6g** was deaminated with adenosine deaminase to give 4′-thioarabinoguanosine **6h** in 97% yield from **6g** (Scheme 3).

**Biological Activities.** The antiviral effects of the synthesized pyrimidine and purine 4′-thioarabinonucleosides are summarized in Tables 2 and 3. None of the pyrimidine derivatives were cytotoxic against human T-cell leukemia cells (CCRF-HSB-2 cells) up to 100 $\mu$g/mL, except for α-4′-thioaraT (α-**6a**), which showed very weak cytotoxicity toward CCRF-HSB-2 ($IC_{50}$ = 42 $\mu$g/mL). All of the β-anomers of the 5-substituted 4′-thioaraU derivatives tested in this study were active against HSV-1 ($IC_{50}$'s ranging from 0.43 to 3.50 $\mu$g/mL) but showed weak activity against HSV-2 and no activity against HCMV. The 5-(halogenovinyl)uracil congeners **6d**,**e** exhibited very weak activity against HSV-2, as did BVaraU. These results show that β-stereochemistry

**Table 3.** Antiviral Activities of Purine 4′-Thioarabinonucleosides

| | | antiviral activities, $ED_{50}$ ($\mu$g/mL) | | | | anticell proliferative activity, $IC_{50}$ ($\mu$g/mL) |
|---|---|---|---|---|---|---|
| compd no. | B | HSV-1[a,e] | HSV-2[b,e] | VZV[c,e] | HCMV[d,e] | CCRF-HSB-2[f] |
| α-**6f** | A | >100 | >100 | >50 | >50 | >100 |
| β-**6f** | A | 18.4 | 13.5 | 1.85 | 1.36 | 14.8 |
| α-**6g** | DAP | >100 | >100 | >50 | >50 | >100 |
| β-**6g** | DAP | 0.52 | 0.40 | 0.11 | 0.022 | 0.20 |
| **6h** | G | 0.49 | 0.59 | 0.11 | 0.010 | 0.29 |
| araA | | 17.1 | 6.6 | 1.17 | 6.45 | 12.9 |
| araDAP | | 49 | >100 | 9.2 | 12.9 | 1.7 |
| araG | | 55 | 78 | 10.1 | 16.8 | 2.8 |
| DHPG | | 0.016 | 0.039 | 0.21 | 0.21 | 17.0 |

[a] VR-3 strain. [b] HSV-2 MS strain. [c] VZV Oka strain. [d] HCMV AD 169 strain. [e] Plaque reduction assay. [f] MTT assay.

around the glycosidic conformation is essential for phosphorylation by both virus-encoded and cellular kinases. On the other hand, 5(*E*)-(bromovinyl)-4′-thioaraU (β-**6e**) showed anti-VZV activity 10 times higher than acyclovir but 150 times lower than BVaraU. 5-(*E*)-(Chlorovinyl)-4′-thioaraU (β-**6d**), unlike β-**6e**, did not show any activity against VZV, whereas it did have significant activity against HSV-1. This is consistent with reports regarding 5-(chlorovinyl)-2′-deoxy-4′-thiouridine.[13] In 4′-oxy derivatives, however, both 5-(bromovinyl)- and 5-(chlorovinyl)araU were almost equally active against both VZV and HSV-1.[3] These differences, which could be related to the substrate specificity of the kinase, require further investigation.

β-Purine 4′-thioarabinonucleosides showed marked biological activities. All of the α-nucleosides were inactive, as in the case of the pyrimidine series described above. 4′-ThioaraA (β-**6f**), which behaved like araA, had moderate activity against VZV and HCMV and relatively weak activity against HSV-1, HSV-2, and the growth of CCRF-HSB-2 cells (Table 3). On the other hand, both the guanine (β-**6h**) and diaminopurine (β-**6g**) derivatives had potent anti-HCMV activities ($ED_{50}$ = 0.010 and 0.022 $\mu$g/mL, respectively). It is noteworthy that β-**6h** and β-**6g** were highly active antiviral agents, although both parental 4′-oxy congeners (araG and araDAP) were inactive or had only relatively weak activities. Thus, it appears as though the "4′-thio" substituents of β-**6h** and β-**6g** have greatly improved antiviral profiles. 4′-ThioaraG (β-**6h**) was 20 times and the diamino derivative (β-**6g**) 10 times more potent than ganciclovir, although both compounds were highly cytotoxic to CCRF-HSB-2 ($IC_{50}$ = 0.29 and 0.20 $\mu$g/mL, respectively). It is likely that 4′-thioaraGTP is an active metabolite in both cases because the diamino derivative β-**6g** was easily converted to 4′-thioaraG (β-**6h**) by adenosine deaminase. Recently, 2′-deoxy-4′-thio-2-amino-6-subsituted-purine nucleosides were reported to have potent anti-human hepatitis B virus and HCMV activities.[12] Among these, 2′-deoxy-4′-thioguanosine was also reported to be highly toxic to leukemic cells.[12] Although compounds β-**6g** and β-**6h** were cytotoxic, they had over a 10-fold greater effect on HCMV. Thus, it is possible that purine 4′-thioarabinonucleosides may lead to a new anti-HCMV agent. Future studies are needed to identify less cytotoxic and more selective active derivatives.

In summary, we have developed a new and facile synthesis of 4′-thioarabinonucleosides. Among these compounds, (*E*)-5-(bromovinyl)-4′-thioarabinosyluracil showed potent anti-HSV-1 and anti-VZV activity *in vitro*. In addition, 2,6-diaminopurine and guanine derivatives of 4′-thioarabinonucleosides exhibited potent anti-HCMV activity and cytotoxicity. Although 4′-thioaraG was cytotoxic, it had a 30-fold greater effect on HCMV. Thus, it could lead to a new class of anti-HCMV agents.

## Experimental Section

**General Methods.** Physical data were measured as follows: Melting points were determined on a Yanagimoto MP-500D micromelting point apparatus and are uncorrected. $^1$H NMR spectra were recorded on a JEOL JNM-GSX-400 instrument in $CDCl_3$ or DMSO-$d_6$ as the solvent with tetramethylsilane as internal standard. UV spectra were recorded with a Shimadzu UV-160A spectrophotometer. Low- and high-resolution mass spectra were taken on a JEOL JMS-AX500 spectrometer. THF was freshly distilled under argon from sodium/benzophenone before use, whereas dichloromethane was distilled from calcium hydride. All the reactions described below were performed under argon atmosphere and monitored by thin layer chromatography (TLC). TLC and preparative TLC were carried out on Merck precoated plates (Kieselgel 60F254). Silica gel for chromatography was Merck Kieselgel 60. BVaraU, acyclovir, ganciclovir, araA, araDAP, and araG were synthesized in the laboratory of Yamasa Corp. Adenosine deaminase (from bovine spleen, EC 3.5.4.4) was purchased from Sigma Co., Ltd., and used without further purification.

**1,4-Anhydro-2,3,5-tri-*O*-benzyl-4-thio-D-arabitol (9).** Sodium hydride (60%, 4.16 g, 104 mmol) was added to a solution of 1,4-anhydro-3-*O*-benzyl-4-thio-D-arabitol (**8**)[14] (5.0 g, 20.8 mmol) in DMF (100 mL) at 0 °C, and the mixture was stirred for 1 h. To this mixture were added DMF (50 mL) and benzyl chloride (16.8 mL, 146 mmol), and the whole was stirred at room temperature overnight. The reaction mixture was poured into ice water (150 mL) and extracted with AcOEt (×2). The organic phase was washed with brine and dried ($Na_2SO_4$). The filtrate was concentrated under reduced pressure, and the residue was purified on column chromatography over silica gel (14% AcOEt in hexane) to give **9** (5.54 g, 63%, syrup): $^1$H NMR ($CDCl_3$) $\delta$ 7.35−7.25 (15H, m), 4.90 (1H, m), 4.72−4.45 (6H, m), 4.11 (1H, m), 3.69 (1H, dd, $J$ = 7.3, 8.8 Hz), 3.56 (1H, ddd, $J$ = 3.4, 6.4, 7.3 Hz), 3.50 (1H, dd, $J$ = 6.4, 8.8 Hz), 3.08 (dd, 1H, $J$ = 4.9, 11.2 Hz), 2.90 (1H, dd, $J$ = 4.4, 11.2 Hz); EI MS $m/z$ 420 ($M^+$). Anal. ($C_{26}H_{28}O_3S$) C, H.

**1-*O*-Acetyl-2,3,5-tri-*O*-benzyl-D-4-thioarabinofuranose (11).** To a solution of **9** (2.88 g, 6.85 mmol) in $CH_2Cl_2$ (40 mL) was added dropwise a solution of *m*-CPBA (80%, 1.48 g, 6.85 mmol) in $CH_2Cl_2$ (40 mL) at −78 °C. The mixture was stirred at the same temperature for 30 min. The reaction was quenched by saturated $NaHCO_3$, and the whole was extracted with $CHCl_3$ (×2). The organic phase was washed with 10% sodium thiosulfate solution, saturated $NaHCO_3$ (×2), and brine and dried ($Na_2SO_4$). The solvent was removed under reduced pressure, and the residue was dissolved in acetic anhydride (34 mL). The mixture was kept at 100 °C for 3 h and then evaporated to dryness. The residue was purified on column chromatography over silica gel (9% AcOEt in hexane) to give **11** (1.79 g, 55%, syrup): $^1$H NMR ($CDCl_3$) $\delta$ 7.35−7.24 (15H, m), 6.07 (0.63H, d, $J$ = 3.9 Hz), 5.98 (0.37H, d, $J$ = 2.9 Hz),

4.83−4.48 (6H, m), 4.26 (0.37H, dd, $J$ = 2.9, 4.9 Hz), 4.18 (0.63H, dd, $J$ = 3.9, 8.8 Hz), 4.12 (0.63H, dd, $J$ = 6.8, 8.8 Hz), 4.03 (dd, 0.37H, $J$ = 4.9, 6.4 Hz), 3.76 (0.37H, m), 3.73−3.44 (2H, m), 3.40 (0.63H, m), 2.04 (3H, s); FAB MS $m/z$ 435 ($M^+ - COCH_3$). Anal. ($C_{28}H_{30}O_4S\cdot0.75H_2O$) C, H.

**5-Ethyl-1-(4-thio-D-arabinofuranosyl)uracil (6a).** A solution of 5-ethyluracil (841 mg, 6.0 mmol) in 1,1,1,3,3,3-hexamethyldisilazane (HMDS; 8.4 mL) and ammonium sulfate (8 mg) was kept under reflux overnight and concentrated to dryness. To this residue were added a solution of **9** (925 mg, 1.93 mmol) in 1,2-dichloroethane (37 mL) and TMSOTf (0.62 mL, 3.2 mmol). After the mixture stirred for 1.5 h at room temperature, saturated $NaHCO_3$ was added to the reaction mixture. The whole was extracted with $CHCl_3$, and the organic phase was washed with water (×2) and brine and dried ($Na_2SO_4$). The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography over silica gel (2% MeOH in $CHCl_3$) to give **12a** (841 mg, 78%).

Compound **12a** (199 mg, 0.356 mmol) was dissolved in $CH_2Cl_2$ (3 mL). To this mixture was added a $CH_2Cl_2$ solution of $BCl_3$ (1 M, 2.14 mL, 2.14 mmol) at −78 °C. After 30 min of stirring at −78 °C, the mixture was allowed to warm to −20 °C. After 2 h of stirring at −20 °C, the reaction was quenched by saturated $NaHCO_3$ and Celite filtration and then extracted with $CHCl_3$. The separated water phase was concentrated under reduced pressure. The residue was purified on column chromatography over silica gel (10−20% MeOH in $CHCl_3$), and the anomers were separated by HPLC (YMC-PACK SIL-06 20 × 250mm, YMC Co., Ltd., Japan; 7% MeOH in $CHCl_3$, flow rate 7 mL/min; retention time α, 23 min, β, 22 min) to give α-**6a** (34 mg, 24%) and β-**6a** (12 mg, 11%) as amorphous solids. β-Anomer (β-**6a**): UV $\lambda_{max}$ ($H_2O$) 272 nm (ε 9600); $^1$H NMR (DMSO-$d_6$) δ 11.25 (1H, s, $D_2O$ exchangeable), 7.91 (1H, s), 6.08 (1H, d, $J$ = 5.9 Hz), 5.73 (1H, d, $J$ = 5.4 Hz, $D_2O$ exchangeable), 5.44 (1H, d, $J$ = 4.9 Hz, $D_2O$ exchangeable), 5.24 (1H, t, $J$ = 4.4 Hz, $D_2O$ exchangeable), 3.99 (1H, q, $J$ = 5.4 Hz), 3.94 (1H, q, $J$ = 5.8 Hz), 3.74 (1H, dd, $J$ = 5.4, 11.2 Hz), 3.67 (1H, dd, $J$ = 4.4, 11.2 Hz), 3.15 (1H, dt, $J$ = 4.4, 5.4 Hz), 2.21 (2H, q, $J$ = 7.3 Hz), 1.04 (3H, t, $J$ = 7.3 Hz); EI MS $m/z$ 288 ($M^+$). Anal. ($C_{11}H_{16}N_2O_5S\cdot0.5H_2O$) C, H, N. α-Anomer (α-**6a**): UV $\lambda_{max}$ ($H_2O$) 271 nm (ε 10 200); $^1$H NMR (DMSO-$d_6$) δ 11.25 (1H, s, $D_2O$ exchangeable), 7.78 (1H, s), 5.77 (1H, d, $J$ = 7.3 Hz), 5.69 (1H, d, $J$ = 5.4 Hz, $D_2O$ exchangeable), 5.52 (1H, d, $J$ = 4.9 Hz, $D_2O$ exchangeable), 4.91 (1H, t, $J$ = 5.1 Hz, $D_2O$ exchangeable), 4.01 (1H, q, $J$ = 7.3 Hz), 3.85 (1H, dt, $J$ = 3.9, 10.7 Hz), 3.66−3.61(1H, m), 3.56 (1H, ddd, $J$ = 3.4, 3.9, 7.8 Hz), 3.44 (1H, dt, $J$ = 7.8, 10.7 Hz), 2.27 (2H, q, $J$ = 7.5 Hz), 1.05 (3H, t, $J$ = 7.5 Hz); EI MS $m/z$ 288 ($M^+$). Anal. ($C_{11}H_{16}N_2O_5S\cdot0.5H_2O$) C, H, N.

**5-Methyl-1-(4-thio-D-arabinofuranosyl)uracil (6b).** From **11** (925 mg, 1.93 mmol), α- and β-**6b** were obtained as described in the synthesis of **6a**. Anomers were separated by HPLC (YMC-PACK SIL-06 20 × 250mm, YMC Co., Ltd., Japan; 15% MeOH in $CHCl_3$, flow rate 8 mL/min; retention time α, 20 min, β, 18 min) to give α-**6b** (137 mg, 36%) and β-**6b** (53 mg, 14%). β-Anomer (β-**6b**): mp 108−111 °C (crystallized from $H_2O$); UV $\lambda_{max}$ ($H_2O$) 272 nm (ε 10 500); $^1$H NMR (DMSO-$d_6$) δ 11.27 (1H, s, $D_2O$ exchangeable), 7.95 (1H, s), 6.09 (1H, d, $J$ = 5.4 Hz), 5.73 (1H, d, $J$ = 5.4 Hz, $D_2O$ exchangeable), 5.44 (1H, d, $J$ = 4.4 Hz, $D_2O$ exchangeable), 4.02 (1H, dt, $J$ = 5.4, 6.4 Hz), 3.95 (1H, dt, $J$ = 5.9, 6.4 Hz), 3.78 (1H, dd, $J$ = 4.9, 11.2 Hz), 3.70 (1H, dd, $J$ = 5.9, 11.2 Hz), 3.17−3.14 (1H, m), 1.78 (3H, s); EI MS $m/z$ 274 ($M^+$). Anal. ($C_{10}H_{14}N_2O_5S\cdot0.75H_2O$) C, H, N. α-Anomer (α-**6b**): an amorphous solid; UV $\lambda_{max}$ ($H_2O$) 271 nm (ε 10 500); $^1$H NMR (DMSO-$d_6$) δ 11.29 (1H, s, $D_2O$ exchangeable), 7.85 (1H, s), 5.76 (1H, d, $J$ = 7.8 Hz), 5.69 (1H, d, $J$ = 5.9 Hz, $D_2O$ exchangeable), 5.54 (1H, d, $J$ = 4.9 Hz, $D_2O$ exchangeable), 5.25 (1H, t, $J$ = 5.1 Hz, $D_2O$ exchangeable), 4.91 (1H, t, $J$ = 5.1 Hz, $D_2O$ exchangeable), 4.01 (1H, dt, $J$ = 7.8, 8.3 Hz), 3.87−3.83 (1H, m), 3.65 (1H, dt, $J$ = 3.4, 8.3 Hz), 3.56 (1H, dt, $J$ = 3.4, 8.3 Hz), 3.39−3.36 (1H, m), 1.84 (3H, s); EI MS $m/z$ 274 ($M^+$). Anal. ($C_{10}H_{14}N_2O_5S\cdot0.5H_2O$) C, H, N.

**5-Iodo-1-(4-thio-D-arabinofuranosyl)uracil (6c).** From **11** (503 mg, 1.05 mmol), α- and β-**4c** were obtained as described in the synthesis of **6a**. Anomers were separated by HPLC (YMC-PACK D-ODS-5 20 × 250mm, YMC Co., Ltd., Japan; 7% $CH_3CN$ in $H_2O$, flow rate 8 mL/min; retention time α, 42 min, β, 46 min) to give α-**6c** (92 mg, 23%) and β-**6c** (87 mg, 22%). β-Anomer (β-**6c**): mp 189−190 °C (crystallized from $H_2O$); UV $\lambda_{max}$ ($H_2O$) 292 nm (ε 8300), 217 (ε 11 400); $^1$H NMR (DMSO-$d_6$) δ 11.67 (1H, s, $D_2O$ exchangeable), 8.55 (1H, s), 6.34 (1H, d, $J$ = 5.9 Hz), 5.79 (1H, d, $J$ = 5.4 Hz, $D_2O$ exchangeable), 4.97 (1H, d, $J$ = 4.4 Hz, $D_2O$ exchangeable), 4.32 (1H, t, $J$ = 4.9 Hz, $D_2O$ exchangeable), 3.99 (1H, dt, $J$ = 5.8, 5.9 Hz), 3.95−3.93 (1H, m), 3.71 (1H, dd, $J$ = 5.4, 11.2 Hz), 3.66 (1H, dd, $J$ = 5.4, 11.2 Hz), 3.18−3.16 (1H, m); EI MS $m/z$ 386 ($M^+$). Anal. ($C_9H_{11}IN_2O_5S$) C, H, N. α-Anomer (α-**6c**): mp 196−198 °C (crystallized from $H_2O$); UV $\lambda_{max}$ ($H_2O$) 291 nm (ε 8300), 217 (ε 11 900); $^1$H NMR (DMSO-$d_6$) δ 11.69 (1H, s, $D_2O$ exchangeable), 8.42 (1H, s), 5.74 (1H, d, $J$ = 6.8 Hz), 5.73 (1H, d, $J$ = 6.8 Hz, $D_2O$ exchangeable), 5.50 (1H, d, $J$ = 4.9 Hz, $D_2O$ exchangeable), 4.93 (1H, t, $J$ = 4.9 Hz, $D_2O$ exchangeable), 4.05 (1H, dt, $J$ = 6.4, 6.8 Hz), 3.84 (1H, ddd, $J$ = 4.2, 4.9, 11.2 Hz), 3.72 (1H, dt, $J$ = 6.4, 7.3 Hz), 3.47 (1H, ddd, $J$ = 4.2, 7.3, 7.8 Hz), 3.43 (1H, ddd, $J$ = 4.9, 7.8, 11.2 Hz); EI MS $m/z$ 386 ($M^+$). Anal. ($C_9H_{11}IN_2O_5S\cdot H_2O$) C, H, N.

**(*E*)-5-(2-Chlorovinyl)-1-(4-thio-D-arabinofuranosyl)uracil (6d).** From **11** (462 mg, 0.967 mmol), α- and β-**6d** were obtained as described in the synthesis of **6a**. Anomers were separated by HPLC (YMC-PACK ODS-AQ 20 × 250 mm, YMC Co., Ltd., Japan; 15% $CH_3CN$ in $H_2O$, flow rate 8 mL/min; retention time α, 61 min, β, 56 min) to give α-**6d** (80 mg, 26%) and β-**6d** (34 mg, 11%). β-Anomer (β-**6d**): mp 241−243 °C (crystallized from $H_2O$); UV $\lambda_{max}$ ($H_2O$) 297 nm (ε 7300), 248 (ε 9700); $^1$H NMR (DMSO-$d_6$) δ 11.56 (1H, s, $D_2O$ exchangeable), 8.34 (1H, s), 7.18 (1H, d, $J$ = 13.4 Hz), 6.62 (1H, d, $J$ = 13.4 Hz), 6.07 (1H, d, $J$ = 5.9 Hz), 5.74 (1H, d, $J$ = 5.9 Hz, $D_2O$ exchangeable), 5.44 (1H, d, $J$ = 4.9 Hz, $D_2O$ exchangeable), 5.33 (1H, t, $J$ = 5.1 Hz, $D_2O$ exchangeable), 4.01 (1H, dt, $J$ = 5.9, 6.4 Hz), 3.95 (1H, dt, $J$ = 5.9, 6.4 Hz), 3.77−3.73 (2H, m), 3.17−3.14 (1H, m); EI MS $m/z$ 320 ($M^+$). Anal. ($C_{11}H_{13}ClN_2O_5S\cdot0.5H_2O$) C, H, N. α-Anomer (α-**6d**): mp 234−238 °C (crystallized from $H_2O$); UV $\lambda_{max}$ ($H_2O$) 295 nm (ε 12 200), 249 nm (ε 15 600); $^1$H NMR (DMSO-$d_6$) δ 11.60 (1H, s, $D_2O$ exchangeable), 8.25 (1H, s), 7.26 (1H, d, $J$ = 13.4 Hz), 6.73 (1H, d, $J$ = 13.4 Hz), 5.76 (1H, d, $J$ = 7.3 Hz), 5.74 (1H, d, $J$ = 5.9 Hz, $D_2O$ exchangeable), 5.59 (1H, d, $J$ = 4.4 Hz, $D_2O$ exchangeable), 4.94 (1H, t, $J$ = 5.4 Hz, $D_2O$ exchangeable), 4.04 (1H, dt, $J$ = 7.3, 7.8 Hz), 3.87 (1H, dt, $J$ = 3.9, 11.2 Hz), 3.68 (1H, dt, $J$ = 7.8, 8.3 Hz), 3.61 (dt, 1H, $J$ = 3.9, 8.3 Hz), 3.42−3.39 (1H, m); EI MS $m/z$ 320 ($M^+$). Anal. ($C_{11}H_{13}ClN_2O_5S$) C, H, N.

**(*E*)-5-(2-Bromovinyl)-1-(4-thio-D-arabinofuranosyl)uracil (6e).** From **11** (619 mg, 1.29 mmol), α- and β-**6e** were obtained as described in the synthesis of **6a**. The residue was purified on column chromatography over silica gel (15% MeOH in $CHCl_3$), and anomers were separated by HPLC (Wakosil-II 5C18 HG 20 × 250 mm, Wako Pure Chemicals Industries, Ltd., Japan; 20% $CH_3CN$ in $H_2O$, flow rate 8 mL/min; retention time α, 34 min, β, 32 min) to give α-**6e** (187 mg, 39%) and β-**6e** (63 mg, 13%). β-Anomer (β-**6e**): mp 216 °C dec (crystallized from 50% aqueous EtOH); UV $\lambda_{max}$ ($H_2O$) 297 nm (ε 10 400), 251 (ε 14 100); $^1$H NMR (DMSO-$d_6$) δ 11.58 (1H, s, $D_2O$ exchangeable), 8.36 (1H, s), 7.25 (1H, d, $J$ = 13.5 Hz), 6.89 (1H, d, $J$ = 13.5 Hz), 6.06 (1H, d, $J$ = 5.9 Hz), 5.75 (1H, d, $J$ = 5.9 Hz, $D_2O$ exchangeable), 5.45 (1H, d, $J$ = 4.9 Hz, $D_2O$ exchangeable), 5.34 (1H, t, $J$ = 5.1 Hz, $D_2O$ exchangeable), 4.02 (1H, dt, $J$ = 5.9, 6.8 Hz), 3.94 (1H, dt, $J$ = 3.4, 6.8 Hz), 3.77 (1H, dt, $J$ = 4.9, 11.2 Hz), 3.73 (1H, dt, $J$ = 5.4, 11.2 Hz), 3.15 (1H, dt, $J$ = 3.4, 4.9 Hz); EI MS $m/z$ 364, 366 ($M^+$). Anal. ($C_{11}H_{13}BrN_2O_5S$) C, H, N. α-Anomer (α-**6a**): mp 201−203 °C (crystallized from 50% aqueous EtOH); UV $\lambda_{max}$ ($H_2O$) 296 nm (ε 12 300), 251 (ε 15 300); $^1$H NMR (DMSO-$d_6$) δ 11.61 (1H, s, $D_2O$ exchangeable), 8.28 (1H, s), 7.33 (1H, d, $J$ = 13.7 Hz), 6.99 (1H, d, $J$ = 13.7 Hz), 5.77 (1H, d, $J$ = 7.3 Hz), 5.74 (1H, d, $J$ = 3.4 Hz, $D_2O$ exchangeable), 5.60 (1H, d, $J$ = 4.4 Hz, $D_2O$ exchangeable), 4.95 (1H, t, $J$ = 5.1 Hz, $D_2O$ exchangeable), 4.01 (1H, dt, $J$ = 7.8, 7.3 Hz), 3.86 (1H, dd, $J$ = 3.9, 11.2 Hz), 3.68 (1H, dt, $J$ = 7.8, 8.3 Hz), 3.61 (1H, dt, $J$ = 3.9, 8.3 Hz), 3.43 (1H, dd, $J$ = 8.3, 11.2 Hz); EI MS $m/z$ 364, 366 ($M^+$). Anal. ($C_{11}H_{13}BrN_2O_5S\cdot0.5H_2O$) C, H, N.

**7-(4-Thio-D-arabinofuranosyl)adenine (14).** From **11** (462 mg, 0.967 mmol), α- and β-**14** were obtained as described in the synthesis of **6a**. The residue was purified on column chromatography over silica gel (15% MeOH in $CHCl_3$), and anomers were separated by HPLC (Wakosil-II 5C18 HG 20 × 250 mm, Wako Pure Chemicals Industries, Ltd., Japan; 20% $CH_3CN$ in $H_2O$, flow rate 8 mL/min; retention time α, 34 min, β, 32 min) to give α-**14** (42 mg, 15%) and β-**14** (15 mg, 7.3%). β-Anomer (β-**14**): mp 163−167 °C (crystallized from $H_2O$); UV $\lambda_{max}$ ($H_2O$) 273 nm (ε 9300), 249 (sh, ε 6300); $^1$H NMR (DMSO-$d_6$) δ 8.89 (1H, s), 8.15 (1H, s), 6.80 (2H, br, $D_2O$ exchangeable), 6.08 (1H, d, $J$ = 5.9 Hz), 5.78 (1H, d, $J$ = 5.9 Hz, $D_2O$ exchangeable), 5.46 (1H, d, $J$ = 5.9 Hz, $D_2O$ exchangeable), 5.33 (1H, t, $J$ = 4.6 Hz, $D_2O$ exchangeable), 4.14−4.10 (1H, m), 3.87−3.83 (1H, m), 3.81−3.79 (2H, m), 3.16 (1H, ddd, $J$ = 4.4, 7.8, 8.3 Hz); FAB MS $m/z$ 284 (M + H$^+$). Anal. ($C_{10}H_{13}N_5O_3S\cdot0.75H_2O$) C, H, N. α-Anomer (α-**14**): mp 247−249 °C (crystallized from $H_2O$); UV $\lambda_{max}$ ($H_2O$) 275 nm (ε 9400), 251 (sh, ε 6200); $^1$H NMR (DMSO-$d_6$) δ 8.62 (1H, s), 8.22 (1H, s), 6.96 (2H, br, $D_2O$ exchangeable), 6.01 (1H, br, $D_2O$ exchangeable), 5.93 (1H, d, $J$ = 7.3 Hz), 5.63 (1H, d, $J$ = 4.4 Hz, $D_2O$ exchangeable), 5.03 (1H, t, $J$ = 5.1 Hz, $D_2O$ exchangeable), 4.16 (1H, dt, $J$ = 7.3, 8.3 Hz), 3.91 (1H, ddd, $J$ = 3.4, 5.1, 10.7 Hz), 3.80 (1H, t, $J$ = 8.3 Hz), 3.63 (1H, ddd, $J$ = 3.4, 7.8, 8.3 Hz), 3.53 (1H, ddd, $J$ = 5.1, 7.8, 10.7 Hz); FAB MS $m/z$ 284 (M + H$^+$). Anal. ($C_{10}H_{13}N_5O_3S\cdot0.55H_2O$) C, H, N.

**9-(4-Thio-D-arabinofuranosyl)adenine (6f).** A mixture of **11** (489 mg, 1.02 mmol), adenine (261 mg, 1.93 mmol), TMSOTf (0.75 mL, 3.88 mmol), and 4A molecular sieves (897 mg) was stirred at room temperature for 1 h. The reaction was quenched by saturated $NaHCO_3$. The whole was extracted with $CH_2Cl_2$, and the organic phase was washed with saturated $NaHCO_3$ and dried ($Na_2SO_4$). After the solvent was removed under reduced pressure, the residue was purified on column chromatography over silica gel (5% MeOH in $CHCl_3$), giving 378 mg of **15f** (0.697 mmol) which was dissolved in $CH_2Cl_2$ (5 mL).

To this mixture was added a $CH_2Cl_2$ solution of $BCl_3$ (1 M, 4.2 mL, 4.2 mmol) at −78 °C. After 1 h of stirring at −78 °C, the mixture was allowed to warm to −20 °C. After 2 h of stirring at −20 °C, the reaction was quenched by saturated $NaHCO_3$, and the mixture was extracted with $CHCl_3$. The separated water phase was concentrated under reduced pressure. The residue was purified on column chromatography over silica gel (17% MeOH in $CHCl_3$), and the anomers were separated by HPLC (Wakosil-II 5C18 HG 20 × 250 mm, Wako Pure Chemicals Industries, Ltd., Japan; 10% $CH_3CN$ in $H_2O$, flow rate 8 mL/min; retention time α, 13 min, β, 15 min) to give α-**6f** (32 mg, 11%) and β-**6f** (52 mg, 18%). β-Anomer (β-**6f**): mp 138−140 °C (crystallized from $H_2O$); UV $\lambda_{max}$ ($H_2O$) 261 nm (ε 13 300); $^1$H NMR (DMSO-$d_6$) δ 8.36 (1H, s), 8.13 (1H, s), 7.22 (2H, br, $D_2O$ exchangeable), 6.05 (1H, d, $J$ = 5.4 Hz), 5.72 (1H, br, $D_2O$ exchangeable), 5.51 (1H, d, $J$ = 2.9 Hz, $D_2O$ exchangeable), 5.19 (1H, br, $D_2O$ exchangeable), 4.18−4.11 (2H, m), 3.87 (1H, dd, $J$ = 3.9, 11.2 Hz), 3.78 (1H, dd, $J$ = 6.6, 11.2 Hz), 3.25 (1H, ddd, $J$ = 3.9, 5.9, 6.6 Hz); EI MS $m/z$ 283 (M$^+$). Anal. ($C_{10}H_{13}N_5O_3S\cdot2H_2O$) C, H, N. α-Anomer (α-**6f**): mp 250 °C (crystallized from $H_2O$); UV $\lambda_{max}$ (crystallized from $H_2O$) 261 nm (ε 13 600);$^1$H NMR (DMSO-$d_6$) δ 8.41 (1H, s), 8.15 (1H, s), 7.24 (2H, br, $D_2O$ exchangeable), 5.79 (1H, d, $J$ = 4.9 Hz, $D_2O$ exchangeable), 5.73 (1H, d, $J$ = 7.3 Hz), 5.61 (1H, d, $J$ = 4.4 Hz, $D_2O$ exchangeable), 4.93 (1H, t, $J$ = 4.6 Hz, $D_2O$ exchangeable), 4.56 (1H, dt, $J$ = 4.4, 7.3 Hz), 3.89 (1H, dt, $J$ = 3.9, 10.7 Hz), 3.75 (1H, dt, $J$ = 4.4, 7.8 Hz), 3.66 (1H, ddd, $J$ = 3.9, 7.8, 8.1 Hz), 3.50 (1H, dt, $J$ = 8.1, 10.7 Hz); EI MS $m/z$ 283 (M$^+$). Anal. ($C_{10}H_{13}N_5O_3S$) C, H, N.

**6-Chloro-2-[(4-thio-D-arabinofuranos-1-yl)amino]purine (17).** From **11** (461 mg, 0.967 mmol), α- and β-**17** were obtained as described in the synthesis of **6f**. The residue was purified on column chromatography over silica gel (15% MeOH in $CHCl_3$), and anomers were separated by HPLC (Wakosil-II 5C18 HG 20 × 250 mm, Wako Pure Chemicals Industries, Ltd., Japan; 20% $CH_3CN$ in $H_2O$, flow rate 8 mL/min; retention time α, 34 min, β, 32 min) to give α-**17** (18.7 mg, 6.2%, amorphous solid) and β-**17** (35.9 mg, 12%, amorphous solid). β-Anomer (β-**17**): $^1$H NMR (DMSO-$d_6$) δ 8.12 (1H, s), 6.96 (1H, br, $D_2O$ exchangeable), 5.51 (1H, brt, $J$ = 3.9 Hz), 5.47 (1H, d, $J$ = 4.9 Hz), 5.27 (1H, d, $J$ = 4.9 Hz, $D_2O$ exchangeable), 4.87 (1H, t, $J$ = 4.9 Hz, $D_2O$ exchangeable), 4.03 (1H, dt, $J$ = 5.4, 6.4 Hz), 3.97 (1H, dt, $J$ = 3.9, 6.4 Hz), 3.79 (1H, dt, $J$ = 4.9, 10.7 Hz), 3.46 (1H, ddd, $J$ = 4.9, 7.8, 10.7 Hz), 3.13 (1H, ddd, $J$ = 4.9, 5.4, 7.8 Hz); FAB MS $m/z$ 318 (M$^+$). Anal. ($C_{10}H_{12}N_5O_3SCl\cdot0.2EtOH\cdot0.8H_2O$) C, H, N. α-Anomer (α-**17**): $^1$H NMR (DMSO-$d_6$) δ 13.07 (1H, br), 8.17 (1H, s), 7.78 (1H, br, $D_2O$ exchangeable), 5.39−5.37 (2H, m, $D_2O$ exchangeable), 5.26 (1H, brdt, $J$ = 6.8 Hz), 4.75 (1H, t, $J$ = 5.1 Hz, $D_2O$ exchangeable), 3.99 (1H, dt, $J$ = 6.8, 7.3 Hz), 3.81 (1H, ddd, $J$ = 4.2, 5.1, 10.5 Hz), 3.64 (1H, dt, $J$ = 7.3, 7.8 Hz), 3.37 (1H, ddd, $J$ = 5.1, 8.1, 10.5 Hz), 3.30 (1H, ddd, $J$ = 4.2, 7.8, 8.1 Hz); FAB MS $m/z$ 318 (M$^+$). Anal. ($C_{10}H_{12}N_5O_3SCl\cdot0.8H_2O$) C, H, N.

**2,6-Diamino-9-(4-thio-D-arabinofuranosyl)purine (6g).** From **11** (489 mg, 1.06 mmol), α- and β-**6g** were obtained as described in the synthesis of **6f**. Anomers were separated by HPLC (Wakosil-II 5C18 HG 20 × 250 mm, Wako Pure Chemicals Industries, Ltd., Japan; 10% $CH_3CN$ in $H_2O$, flow rate 8 mL/min; retention time α, 13 min, β, 14 min) to give α-**6g** (17 mg, 5%) and β-**6g** (28 mg, 9%). β-Anomer (β-**6g**): mp 292−295 °C (crystallized from $H_2O$); UV $\lambda_{max}$ ($H_2O$) 282 nm (ε 10 900), 258 (ε 9300); $^1$H NMR (DMSO-$d_6$) δ 7.93 (1H, s), 6.67 (2H, br, $D_2O$ exchangeable), 5.93 (1H, d, $J$ = 5.4 Hz), 5.77 (2H, br, $D_2O$ exchangeable), 5.74 (1H, d, $J$ = 4.9 Hz, $D_2O$ exchangeable), 5.51 (1H, d, $J$ = 4.9 Hz, $D_2O$ exchangeable), 5.19 (1H, br, $D_2O$ exchangeable), 4.12 (1H, dt, $J$ = 5.9, 6.8 Hz), 4.04 (1H, dt, $J$ = 5.4, 6.8 Hz), 3.83 (1H, dd, $J$ = 4.9, 10.7 Hz), 3.68 (1H, dd, $J$ = 6.8, 10.7 Hz), 3.22 (1H, ddd, $J$ = 4.9, 5.9, 6.8 Hz); EI MS $m/z$ 298 (M$^+$). Anal. ($C_{10}H_{14}N_6O_3S\cdot0.75H_2O$) C, H, N. α-Anomer (α-**6g**): mp 241−247 °C (crystallized from $H_2O$); UV $\lambda_{max}$ ($H_2O$) 281 nm (ε 10 900), 259 (ε 9600); $^1$H NMR (DMSO-$d_6$) δ 8.00 (1H, s), 6.67 (2H, br, $D_2O$ exchangeable), 5.78 (2H, br, $D_2O$ exchangeable), 5.75 (1H, br, $D_2O$ exchangeable), 5.58 (1H, br, $D_2O$ exchangeable), 5.56 (1H, d, $J$ = 7.3 Hz), 4.90 (1H, br, $D_2O$ exchangeable), 4.45 (1H, t, $J$ = 7.3 Hz), 3.86 (1H, dt, $J$ = 1.0, 11.0 Hz), 3.70 (1H, t, $J$ = 7.8 Hz), 3.63 (1H, m), 3.45 (1H, dt, $J$ = 8.1, 11.0 Hz); EI MS $m/z$ 298 (M$^+$). Anal. ($C_{10}H_{14}N_6O_3S\cdot H_2O$) C, H, N.

**9-(4-Thio-β-D-arabinofuranosyl)guanine (β-6h).** A solution of **6g** (20 mg, 0.067 mmol) and adenosine deaminase (43 μL, 10 units) in Tris-HCl buffer (5 mL, pH 7.0) was kept at 55 °C for 7.5 h. After dilution, the solution was applied to a column of adsorption resin (10 mL, Sepabeads SP 206, Mitsubishi Chemical Corp., Japan). After it was washed with 200 mL of water, the eluate of 10% aqueous EtOH was collected and concentrated under reduced pressure to leave crystalline β-**6h** (19.5 mg, 97%): mp 260−264 °C (crystallized from $H_2O$); UV $\lambda_{max}$ ($H_2O$) 273 nm (ε 10 500), 256 (ε 14 000);$^1$H NMR (DMSO-$d_6$) δ 10.56 (1H, br), 7.92 (1H, s), 6.44 (2H, br, $D_2O$ exchangeable), 5.86 (1H, d, $J$ = 5.4 Hz), 5.71 (1H, d, $J$ = 5.4 Hz, $D_2O$ exchangeable), 5.49 (1H, d, $J$ = 4.9 Hz, $D_2O$ exchangeable), 5.14 (1H, t, $J$ = 5.4 Hz, $D_2O$ exchangeable), 4.07 (1H, dd, $J$ = 5.4, 11.0 Hz), 4.03 (1H, dd, $J$ = 6.6, 11.0 Hz), 3.83 (1H, dt, $J$ = 5.4, 5.9 Hz), 3.67 (1H, dt, $J$ = 5.4, 5.9 Hz), 3.21 (1H, dt, $J$ = 5.4, 6.6 Hz); FAB MS $m/z$ 300 (M + H$^+$). Anal. ($C_{10}H_{13}N_5O_4S\cdot H_2O$) C, H, N.

**Antiviral Assays.** HEL cells and the following virus strains were used: HSV-1 VR-3 strain, HSV-2 MS strain, VZV Oka strain, and HCMV AD 169 strain. The origins of viruses and cells have been described previously.[3,19] Antiviral activities against these herpes viruses were determined by the plaque reduction assay as described earlier.[3,20]

**Cell Growth Inhibition Test.** A cell suspension of human T-cell acute lymphoblastoid leukemia cells, CCRF-HSB-2, containing 1.1 × 10$^5$ cells/mL was prepared in RPMI 1640 medium supplemented with 10% fetal bovine serum; 90 μL of the cell suspension was seeded in a 96-multiwell plate, and 10 μL of medium or phosphate-buffered saline (PBS) containing test compound in serial 0.5 $\log_{10}$ dilution was added. Cells were incubated in a 5% $CO_2$ incubator at 37 °C. After 3 days, 10 μL of MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Chemical Co., St. Louis, MO), prepared in a concentration of 5 mg/mL in PBS, was added to each well. After another 4 h incubation at 37 °C, 100 μL of extraction buffer (0.02 N HCl solution of 50% DMF containing

20% SDS) was added to each well to solubilize MTT-formazan. Absorbance at 570 nm (test wavelength) and 690 nm (reference wavelength) were measured using a microplate reader (MPR-A4i, Tosoh Co.). The percentage of cell growth inhibition was calculated by the following formula:

$$\text{inhibition (\%)} = [1 - (T_x - C_0/C_x - C_0)] \times 100$$

where $T_x$ is absorbance at the end of incubation with test drug, $C_x$ is absorbance at the end of incubation without drug, and $C_0$ is absorbance at beginning of incubation. $IC_{50}$ of the test compound was determined graphically from a dose−inhibition curve.

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