An Alternative Synthesis of the Antineoplastic Nucleoside 4′-ThioFAC and Its Application to the Synthesis of 4′-ThioFAG and 4′-Thiocytarazid

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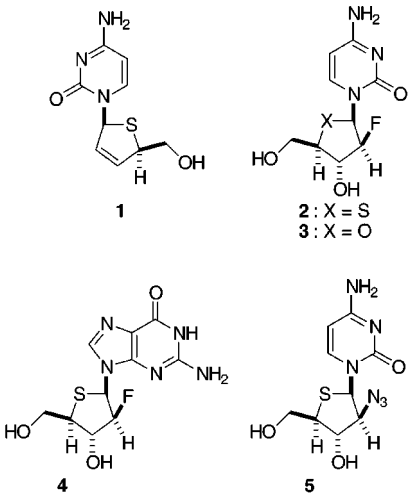
Previously, we synthesized 4′-thioFAC, a novel antineoplastic cytosine nucleoside, by developing an original method. However, several problems remained. To overcome these problems, we have developed an alternative method for the synthesis of 4′-thionucleosides. In the original synthesis, carbons from C1 to C5 of D-glucose were used. The new method also starts from D-glucose but uses carbons closer to the tail (C2−C6). A dibenzoyl derivative obtained by this approach was brominated at the anomeric position to give a 1-bromide derivative. Fusion of the 1-bromide and persilylated acetylcytosine, followed by deprotection, predominantly gave a β-anomer of 4′-thioFAC. The reaction of 2,6-diaminopurine with the 1-bromide in the presence of TMS triflate gave a glycosylated product in good yield. After deprotection, the resulting 1:1 anomeric mixture of free nucleosides was treated with adenosine deaminase to give a β-anomer of 4′-thioFAG, a guanine congener of 4′-thioFAC, selectively. Using a similar approach, we synthesized 4′-thiocytarazid, which was not possible using the original method.

Introduction

The discovery of the potent antiherpes virus activity of 2′-deoxy-4′-thionucleosides has stimulated the development of 4′-thionucleosides as potential antiviral agents.[1,2] The resistance of the glycosidic bond of 4′-thionucleosides to enzymatic hydrolysis catalyzed by nucleoside phosphorylase[3] is one of the critical points in nucleoside antiviral therapeutics. Several 4′-thionucleosides have been reported to be active against herpes viruses.[1,2,4,5] In addition, some have also shown antiretrovirus activity: e.g., L-2′,3′-dideoxy-didehydro-4′-thiocytidine (L-D4C, **1**, Chart 1) has potent antihuman immunodeficiency virus activity.[6] Another important feature of 4′-thionucleosides is their antineoplastic activity.[1,7] Recently, our attention has been focused on the synthesis and antineoplastic activity of 2′-substituted-4′-thiocytidine analogues.[8,9] Among them, 1-(2-deoxy-2-fluoro-β-D-4-thio-*arabino*-pentofuranosyl)cytosine (4′-thioFAC, **2**) is noteworthy.[9]

Chart 1



4′-ThioFAC has prominent and broad antitumor activities against various human solid tumor cell lines in vitro as well as in vivo.[9,10] Furthermore, 4′-thioFAC is an orally active antitumor agent; it has shown therapeutic effects on nude mice bearing colon and stomach cancers when given orally.[10]

Despite the unique biological activities of 4′-thionucleosides, the difficulty of synthesizing 4′-thionucleosides has impeded the investigation of structure−activity relationships (SAR). Thus, the production of new 4′-thionucleoside analogues has often depended upon the development of new synthetic methods.[8] In fact, for the synthesis of 2′-substituted-4′-thiocytidine, we exploited a novel synthetic method, which was successfully used to synthesize

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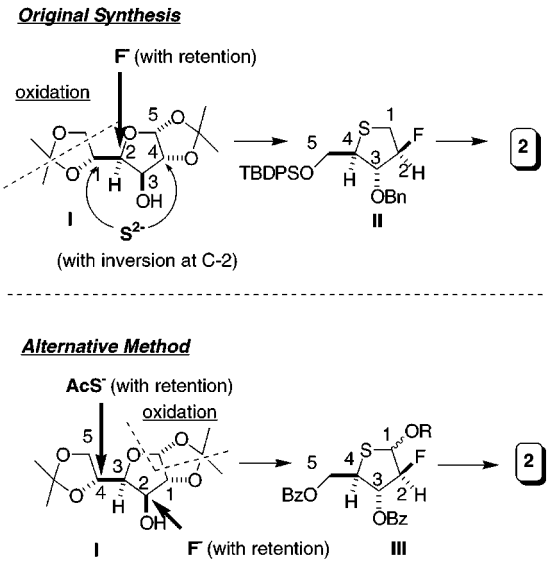
4′-thioFAC.[9] However, our original synthesis of 4′-thioFAC has not been optimized. Several problems make it unsuitable for large-scale preparation. First, an expensive silyl protecting group (*tert*-butyldiphenylsilyl group) and difficult-to-handle reagents, such as DAST, BBr_3, and mcpba, were used.[9] In addition, in the most serious problem in the synthesis of 4′-thioFAC, the undesired α-isomer was predominantly formed (α: β = 2.5:1) and had to be separated from the desired β-isomer by a complicated purification method.[9] To overcome these drawbacks, the development of an alternative synthetic method that can selectively produce β-4′-thioFAC is strongly desired.

With regard to these considerations, the improved synthesis of 1-(2-deoxy-2-fluoro-β-D-*arabino*-pentofuranosyl)cytosine (FAC, **3**), a 4′-oxy counterpart of 4′-thioFAC reported by Watanabe, was suggestive.[11] In addition, Wistler et al. achieved the synthesis of pyrimidine 4′-thioarabino nucleosides by a similar approach.[12] Thus, we sought to apply Watanabe's method to the synthesis of 4′-thioFAC. In this paper, we describe the alternative synthesis of a 2-fluoro-4-thiosugar derivative, and its stereoselective coupling with persilylated N^4-acetylcytosine, which led to 4′-thioFAC.[13] By applying this new method, we synthesized 1-(2-deoxy-2-fluoro-β-D-4-thio-*arabino*-pentofuranosyl)guanine (4′-thioFAG, **4**) and 1-(2-azido-2-deoxy-β-D-4-thio-*arabino*-pentofuranosyl)cytosine (4′-thiocytarazid, **5**). The former, previously synthesized by us, has potent antiherpes virus activities in vitro and had to be prepared on a multigram scale for the in vivo antiviral assays in our laboratory. The latter, which has been previously designed as a potential antitumor agent, could not be obtained by the original method. We also describe the synthesis of both compounds.

Results and Discussion

Alternative Synthesis of 4′-ThioFAC. Scheme 1 shows the plan for the alternative synthesis of **2** in comparison with the original synthesis. In the original synthesis, the glucose carbons from C1 to C5 were used for a 4-thiosugar intermediate **II**, the chiralities of C2, C3, and C4 of which were transferred from C4, C3, and C2 of glucose, respectively.[9] The glycosidic bond of **2** was formed via Pummerer rearrangement, followed by the Lewis acid-catalyzed glycosylation reaction, which resulted in the predominant formation of the undesired α-anomer.[9] Although the alternative method also begins with D-glucose, it uses carbons closer to the tail (C2−C6), as in the case of Watanabe's FAC synthesis.[11] Thus, the three sets of chiral centers from C2 to C4 in the intermediate **III** are transferred from C3 to C5 of D-glucose, respectively. The syntheses of 5- and 4-thiosugar derivatives, which involve the introduction of a sulfur unit at C5 of glucose and other sugars, have been reported previously.[14] However, there have been few reports concerning the synthesis of 4′-thionucleosides by

Scheme 1



expanding these chemistries. To improve the poor β-selectivity at the glycosylation step, we investigated the nucleophilic substitution of a 1-α-bromide of a 4-thiosugar derivative, in place of the Lewis-acid-catalyzed reaction, in the alternative synthesis.

According to our plan, 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**6**), which was easily accessible from 1,2:5,6-di-*O*-isopropylidene-α-D-glucose (**I**) in two steps,[15] should be fluorinated at the 3-position. Instead of the original method reported by Watanabe,[11] we used an alternative method, which was developed by a Bristol-Myers group,[16] with slight modification. Compound **6** was treated with sulfuryl chloride and imidazole to give imidazoyl sulfate **7**, which was fluorinated by treatment with potassium fluoride in refluxing 2-methoxyethanol to give 3-fluoro derivative **8** in 77% yield from **6**. Notably, the substitution reaction of **7** with a fluoro anion proceeded under conditions that were more gentle (125 °C in 2-methoxyethanol) than the previous examples, in which a higher reaction temperature (210 °C in acetamide[11] or 160 °C in 2,3-butanediol[16]) was required. In addition, the reaction of **7** did not require any additive, such as HF, which was used in the original method of the Bristol-Myers group.[16] As expected, the 3-fluoro derivative **8** consisted of a single stereoisomer, the inverted stereochemistry at C3 of which was confirmed by converting the final compound. The 3-fluoro derivative **8** was selectively deblocked at the 5,6-isopropylidene group under acidic conditions. Selective benzoylation at the primary hydroxyl group of the resulting diol gave 6-benzoate **9** in 82% yield from **8**. Mesylation at the C5 position of **9**, followed by treatment with sodium methoxide, resulted in debenzoylation and the subsequent formation of epoxide to give 5,6-epoxide **10** in 82% yield with inversion of the stereochemistry at C5. Treatment of the 5,6-epoxide **10** with thiourea in refluxing methanol gave a 5,6-thiirane derivative **11**, the C5 configuration of which had to be controlled by introducing a thio functional group, since it was to be a D-thiosugar. The 5,6-thiirane ring of **11** was cleaved by treatment with

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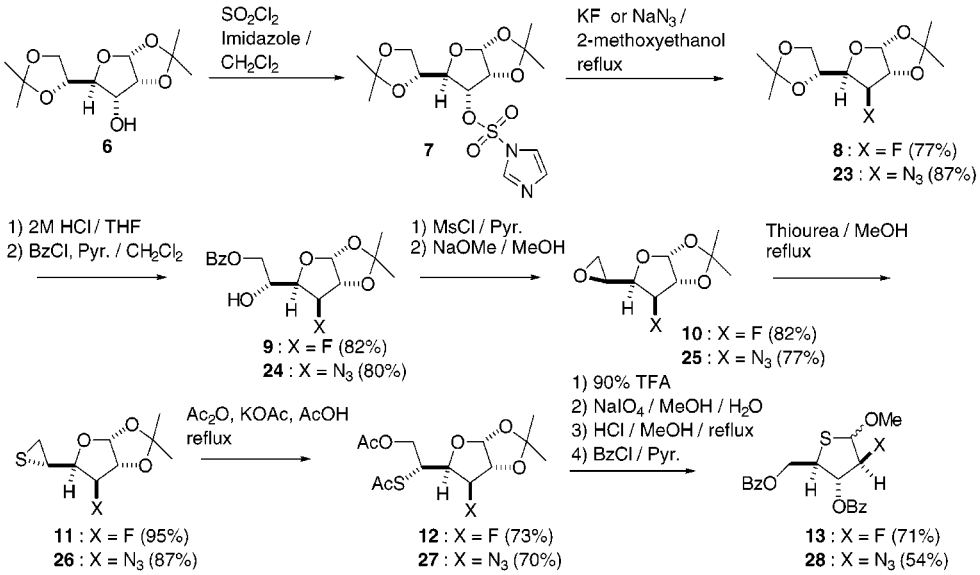
Scheme 2



potassium acetate in refluxing acetic anhydride and acetic acid (5:1) for 2 days,[14] to give a diacetate derivative **12** in 73% yield. Conversion of the diacetate derivative **12** to a desired 2-fluoro-4-thiosugar **13** was achieved by the following 4 steps: (1) selective acidic hydrolysis of the isopropylidene group by treatment with 90% trifluoroacetic acid at 0 °C, (2) oxidative scission of the resulting diol with sodium periodate, (3) deacetylation and subsequent cyclization to a thiofuranose by treatment with acidic methanol, and (4) benzoylation of the free hydroxyl groups by treatment with benzoyl chloride in pyridine. By these procedures, the 2-fluoro-4-thiosugar **13** was obtained in 58% yield from **12** (Scheme 2).

As mentioned above, the major drawback of the original synthesis of 4′-thioFAC was the formation of the undesired α-anomer over that of the biologically active β-anomer. Therefore, in this alternative synthesis we had to improve the β-stereoselectivity at the glycosylation step. Considering our previous results[4,8,9] and those of others,[1,2,17−19] the Lewis acid-catalyzed glycosylation reaction of 4-thiosugars tends to give undesired α-anomers as major products. Furthermore, the Lewis acid-catalyzed reaction of a 2-fluoroarabinose derivative predominantly forms an α-substituted product, due to the strong electrostatic effect of a fluoro substituent, which impairs the approach of nucleophiles from the β-side.[20] Therefore, the previous syntheses of FAC and related compounds were achieved by the nucleophilic substitution of the 1-α-bromo-2-fluoroarabinose derivative with nucleobases.[11,20,21] Bromination of the anomeric position of the 2-fluoroarabinose derivative, under the usual conditions, selectively gave the 1-α-bromide, which was applied to persilylated pyrimidines to give β-2-fluoroarabino pyrimidine nucleosides exclusively.[11,20,21] Thus, the condensation of silylated cytosine and 1-bromo-4-thiosugar, which would be derived from **13**, is an attractive choice to overcome the most serious problem.

Compound **13** was subjected to acetolysis of the 1-methoxy group to give 1-acetate **14** (90% yield), which was treated with HBr/acetic acid in dichloromethane to give the corresponding 1-bromide **15**. The instability of **15** made it difficult to confirm its structure and determine the ratio of α- and β-anomers based on the ^{1}H NMR spectrum. As in the case of the 4-oxy derivative mentioned above, we assumed that the 1-bromide **15** might largely be composed of an α-bromide, due to the stereoelectronic effect of the 2-fluoro substituent. Thus, soon after bromination of the 1-acetate **14**, the resulting 1-bromide **15** was subjected to the glycosylation of persilylated N^4-acetylcytosine without any purification or inspection.

In general, a 1-halogeno-sugar undergoes anomerization in a polar solvent. When persilylated pyrimidines are reacted with 1-α-halogeno sugar in such a solvent, the anomerization causes an increase in the unfavorable formation of α-nucleosides. Walker reported that β-2′-deoxynucleoside could selectively form when the protected 1-chloro-2-deoxyribose reacted in chloroform.[22] Howell et al. fully investigated the glycosylation of the 1-bromo-2-fluoroarabinose derivative by an S_N2 displacement,[20] which was first reported by Watanabe and Fox as mentioned above, to synthesize a series of antiviral 2′-fluoroarabino-nucleosides, and obtained results similar to those reported by Walker.[20] On the basis of these results, our first attempt at the glycosylation reaction by the condensation of **15** with persilylated N^4-acetylcytosine was performed in 1,2-dichloroethane, but poor results were obtained. The reaction in refluxing 1,2-dichloroethane gave only trace amounts of the glycosylated product. Similarly, other conditions with less-polar solvents such as dichloromethane and carbon tetrachloride gave only trace amounts or none of the glycosylated products (data not shown). However, the latter unreacted mixture in carbon tetrachloride was subjected to another

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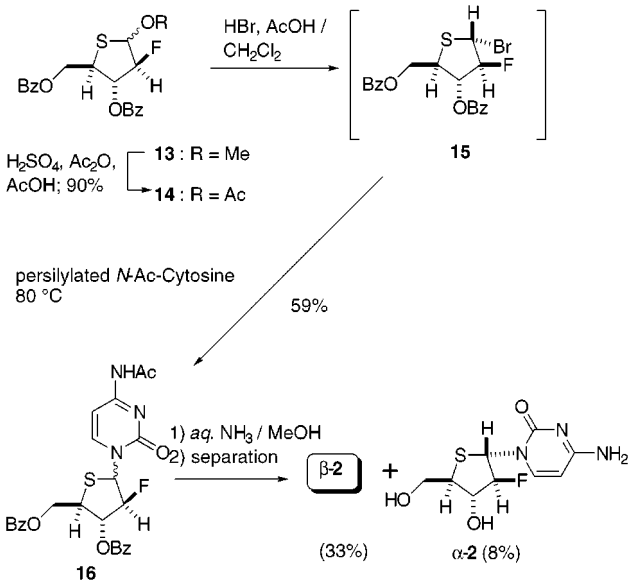
Scheme 3

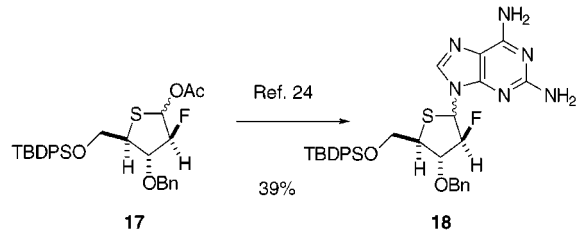


fusion reaction, after evaporation of the solvents, to give a 1:1 mixture of α- and *β*-anomers of **16** in 61% yield (data not shown). The loss of stereoselectivity in the glycosylation reaction may have been due to the pretreatment of **15** in refluxing carbon tetrachloride, which caused the anomerization of α-bromide (vide supra). After many efforts were made to optimize the reaction conditions, we found that the glycosylated product **16** was formed in a yield of 59% when the 1-bromide **15** was fused with the persilylated N^4-acetylcytosine at 80 °C for 5 h under reduced pressure. Compound **16** was deprotected by treatment with concentrated NH_4OH/MeOH to give the desired 4′-thioFAC (**2**) as an anomeric mixture. HPLC analysis of the crude products clearly showed that the desired *β*-anomer of 4′-thioFAC (**2**) was predominantly formed (α:*β* = 1:4), as we expected. The fusion reaction was critical for the progression of the glycosylation reaction and the predominant formation of *β*-anomer. The structures of *β*-**2** and α-**2** were confirmed by comparison of the instrumental analysis data, e.g., ^{1}H NMR, FAB mass spectra, and retention time of HPLC, after isolation (Scheme 3).

Synthesis of 4′-ThioFAG. Recently, we have reported a novel antiviral 4′-thionucleoside, 4′-thioFAG (**4**), which has potent antiviral activities against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in vitro.[23,24] 4′-ThioFAG (**4**) also has potent activity against human cytomegalovirus (HCMV).[23,24] To further evaluate this compound, in vivo assays were needed. However, the original synthesis of **4** had several problems,[24] which needed to be overcome before **4** could be prepared on a multigram scale. As in the case of 4′-thioFAC, the biggest problem was the glycosylation step. The Lewis acid-catalyzed glycosylation reaction of 2,6-diaminopurine with 2-fluoro-4-thioarabinose derivative **17** gave the desired 4′-thionucleoside **18** in 39% yield as a mixture of α- and *β*-anomers (α:*β* = 1:1.5)[24] (Scheme 4).

The success of the improved synthesis of 4′-thioFAC encouraged us to apply this method to the synthesis of

Scheme 4



4′-thioFAG. To construct a glycosidic linkage of 4′-thioFAG, the S_N2 displacement of 1-bromide **15** using metal salts of purine bases seems to be promising, since the stereoselective formation of *β*-nucleoside is expected. Although we investigated the glycosylation reaction by using metal salts of 2,6-dichloropurine or 2-amino-6-chloropurine and 1-bromide **15** under various conditions, none of the reactions was successful.

Therefore, improvement of the synthesis of 4′-thioFAG mainly focused on increasing the yield of the glycosylation reaction of 2,6-diaminopurine, which was catalyzed by a Lewis acid. We used both 1-acetate **14** and 1-bromide **15** as glycosyl donors and investigated the glycosylation reaction of 2,6-diaminopurine under various Lewis acid conditions. Of the conditions we tested, the reaction of 1-bromide **15** in the presence of TMS triflate as a Lewis acid in acetonitrile at 60 °C gave the best results, in which the glycosylated product **19** was obtained in 75% yield (α:*β* = 1.2:1). The use of stannic chloride, instead of TMS triflate, caused a decrease in the yield of **19**. Likewise, the reaction of 1-acetate **14** also resulted in a decrease in **19** (data not shown). An anomeric mixture of the resulting **19** was deprotected by treatment with concentrated NH_4OH/MeOH to give a mixture of α- and *β*-anomers of 2,6-diamino derivative **20** in good yields.

To prepare **4**, it was necessary to hydrolyze at the 6-amino group and separate the resulting biologically active *β*-**4** from the inactive α-anomer. This could be achieved by using adenosine deaminase, which can discriminate a natural *β*-form from an α-form around a glycosidic bond. In fact, in a small-scale reaction of **20**, adenosine deaminase selectively recognized *β*-**20**.[24] Therefore, we could easily separate *β*-4′-thioFAG **4** from the unhydrolyzed α-2,6-diaminopurine derivative α-**20** by simple reversed-phase column chromatography. However, the low water-solubility of **20** made it difficult to reproduce the same reaction in a large-scale synthesis in which the volume of reaction buffer had to be decreased to half of that in the small-scale reaction because of the efficiency of column chromatography. Since this slowed the reaction rate, the reaction time had to be prolonged to complete the hydrolysis. As a result, undesired α-4′-thioFAG, which no longer could be separated from **4**, was also obtained. We overcame this problem by stopping the hydrolysis at an appropriate time. After unreacted *β*-**20** and undesired α-**20** were separated from *β*-**4**, hydrolysis of the 6-amino group by adenosine deaminase was repeated. Overall, 4′-thioFAG (**4**), the structure of which was identical to that synthesized by the original method,[24] was obtained from **20** in 34% yield (Scheme 5).

Synthesis of 4′-Thiocytarazid. As a final target of this project, we chose 4′-thiocytarazid. Cytarazid, (1-(2-azido-2-deoxy-*β*-D-*arabino*-pentofuranosyl)cytosine), a parent compound of 4′-thiocytarazid, is known to have potent antineoplastic activities against solid tumors as well as

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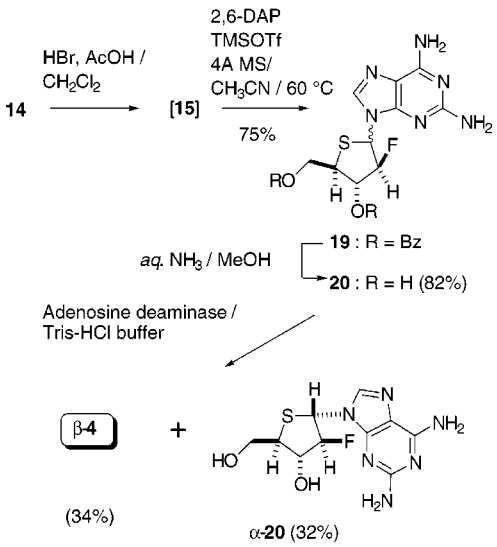
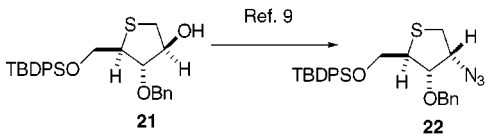
Scheme 5



Scheme 6



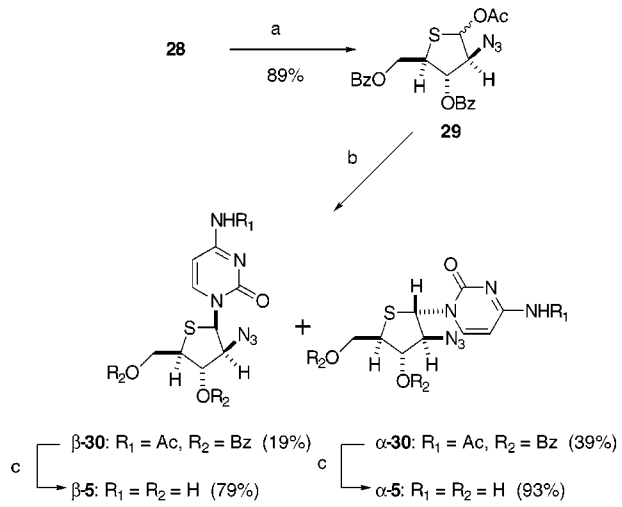
leukemia.[25,26] Thus, we selected 4′-thiocytarazid as a potential antitumor agent and attempted to synthesize it in our previous study. However, the introduction of an azido group at the 2-position of the key intermediate **21** by the Mitsunobu reaction resulted in the unexpected formation of a ribo-azido derivative **22**.[9] Consequently, we obtained 4′-thioazidocytidine, which was less active against tumor cell lines[9] (Scheme 6).

In contrast to the previous synthesis, in the above-mentioned alternative synthesis, the 2′-substituent is introduced in an earlier stage. In addition, the original synthesis of cytarazid by Bobek et al.[25] was achieved by adapting a reaction scheme similar to Watanabe's FAC synthesis, where a fluorine is simply substituted for an azido group. Therefore, we were hopeful that 4′-thiocytarazid could be synthesized using the alternative synthesis.

The introduction of an azido functional group at the 3-position of **6** has already been reported by Bobek et al.[25] However, we thought that we might be able to apply the reaction we used for the fluorination of **6** to C3 azidation, in which nucleophilic displacement of the 3-imidazoyl sulfate **7** was simply performed with sodium azide, instead of potassium fluoride. Indeed, treatment of **7** with sodium azide in refluxing 2-methoxyethanol gave a 3-azido derivative **23** in 87% yield from **6**. As depicted in Scheme 1, the 3-azido derivative **23** was further converted to 2-azido-4-thioarabinose **28**, and the reaction scheme was identical to that in the synthesis of **13**. All of the chemical yields in each step were comparable to those in the 2-fluoro series.

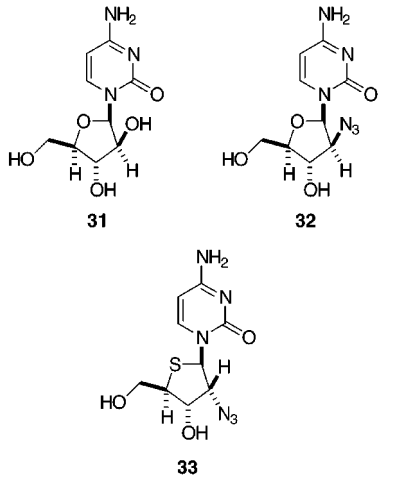
As with the alternative synthesis of 4′-thioFAC, a 1-acetoxy-2-azido derivative **29**, which was obtained by

Scheme 7[a]



[a] (a) H_2SO_4, Ac_2O, AcOH; (b) silylated N^4-acetylcytosine, TMSOTf, CH_3CN, reflux; (c) concntrated NH_4OH, MeOH.

Chart 2



acetolysis of **28**, was subjected to the glycosylation reaction. Although we tried the nucleophilic substitution of the corresponding 1-bromide prepared from **29** with persilylated acetylcytosine, the reaction gave none of the desired product. This seemed to be due to the instability of the generated 1-bromide. Thus, we prepared the corresponding 1-chloride, the nucleophilic displacement of which with persilylated cytosine derivative was also unsuccessful (data not shown). Alternatively, compound **29** was directly coupled with persilylated acetylcytosine in the presence of TMS triflate to give protected 4′-thiocytarazid **30**. By simple silica gel column chromatography, the anomers of **30** could be separated, giving β-**30** and α-**30** in 19% and 39% yields, respectively, from **29**. Both β- and α-**30** were deblocked by treatment with concentrated NH_4OH/MeOH to give β- and α-anomers of 4′-thiocytarazid **5** in 79% and 93% yields, respectively (Scheme 7).

The antineoplastic activities of 4′-thiocytarazid **5** were evaluated, and the results are summarized in Table 1, in comparison with those of araC **31**, cytarazid **32**, 4′-thioazidocytidine **33**, and 4′-thioFAC **2** (Chart 2 and Table 1). The β-anomer of 4′-thiocytarazid β-**5** showed only moderate inhibitory activity against human T-cell leukemia (CCRF-HSB-2), which was comparable to that of ribo-isomer **33**. Against solid tumor (KB cells), both

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(26) Matsuda, A.; Yasuoka, J.; Sasaki, T.; Ueda, T. *J. Med. Chem.* **1991**, *34*, 999−1002.

Table 1. Antitumor Activities of 2′-Substituted 4′-Thiocytidines

		IC_{50} (μg/mL)	
compd	no.	CCRF-HSB-2	KB
β-4′-thiocytarazid	β-**5**	7.8	42
α-4′-thiocytarazid	α-**5**	>100	>100
4′-thioazidocytidine	**33**	8.6	82
4′-thioFAC	**2**	0.051	0.015
cytarazid	**32**	0.15	1.0
araC	**31**	0.052	0.26

β-**5** and **33** were inactive. The α-anomer of **5** showed no activity against either cell line. Compared with its parent compound cytarazid **31**, 4′-thiocytarazid β-**5** was 50 times less active against CCRF-HSB-2 and 40 times less active against KB cells. These results suggest that the antineoplasic activities of 4′-thiocytidine analogues depend more closely on the nature of the 2′-substituents than those of their 4′-oxy counterparts. Deoxycytidine kinase, a key enzyme that converts deoxycytidine antimetabolites to their active form, may strictly recognize the stereo- and electrochemical properties of 2′-substituents of 4′-thiocytidines.

In conclusion, we have adapted an alternative synthetic route for 4′-thionucleosides, which is more practical than our original method. Using this method, we synthesized 4′-thioFAC, 4′-thioFAG, and 4′-thiocytarazid. Although the antineoplastic activity of 4′-thiocytarazid was rather disappointing, this compound could be synthesized by the alternative method but not by the original method.

Experimental Section

General Information. Melting points are uncorrected. ^{1}H NMR spectra were recorded at 400 MHz (^{1}H) and at 100 MHz (^{13}C) using $CDCl_3$ or DMSO-d_6 with tetramethylsilane as internal standard. Mass spectra were obtained by the fast atom bombardment (FAB) mode. Silica gel for chromatography was Merck Kieselgel 60. All anhydrous reactions were performed under an argon atmosphere.

3-Deoxy-3-fluoro-1,2:5,6-di-*O*-isopropylidene-α-D-*gluco*-pentofuranose (8). To a solution of 1,2:5,6-di-*O*-isopropylidene-α-D-*allo*-pentofuranose (**6**) (19.48 g, 74.8 mmol) in CH_2Cl_2 (200 mL) was added sulfuryl chloride (12.16 mL, 151 mmol). After 30 min of stirring at 0 °C, imidazole (50.92 g, 748 mmol) was added. The whole was stirred at room temperature for 2.5 h. The reaction was quenched with saturated $NaHCO_3$ and extracted with CH_2Cl_2 (×3). The combined organic layers were washed with brine and dried (Na_2SO_4). After being concentrated under reduced pressure, the residue was dissolved in 2-methoxyethanol (200 mL). To this mixture was added potassium fluoride (spray-dried, 43.44 g, 748 mmol). The mixture was kept under reflux for 7 h. After cooling to room temperature, the solvent was removed under reduced pressure. The residue was partitioned between AcOEt and water, and the organic layer was washed with brine and then dried (Na_2SO_4). After evaporation of the solvents, the residue was purified over a silica gel column (8.0 × 9.5 cm, 4% AcOEt in *n*-hexane) to give **8** (15.05 g, 77%) as a syrup: ^{1}H NMR ($CDCl_3$) δ ppm 5.95 (1H, d, J = 3.9 Hz), 5.01 (1H, dd, J = 2.2, 49.8 Hz), 4.70 (1H, dd, J = 3.9, 10.7 Hz), 4.29 (1H, ddd, J = 8.3, 5.9, 4.9 Hz), 4.12 (1H, dd, J = 5.9, 8.8 Hz), 4.11 (1H, ddd, J = 2.2, 8.3, 29.0 Hz), 4.03 (1H, dd, J = 4.9, 8.8 Hz), 1.50, 1.45, 1.37, 1.33 (each 3H, s); FAB-MS m/z 263 ($M + H^+$). Anal. Calcd for $C_{12}H_{19}FO_5 \cdot 0.25H_2O$: C, 54.03; H, 7.37. Found: C, 54.31; H, 6.97.

6-*O*-Benzoyl-3-deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-*gluco*-pentofuranose (9). A mixture of **8** (2.38 g, 9.07 mmol) in THF (10 mL) and 2 M HCl (10 mL) was stirred at room temperature for 1 h. After the mixture was neutralized by sodium bicarbonate, the insoluble salts were removed by filtration and the filtrate was concentrated under reduced pressure. The residue was extracted with $CHCl_3$ (×2), and the combined organic layers were washed with brine and then dried (Na_2SO_4). After the filtrate was concentrated under reduced pressure, the residue was passed through a silica gel column (3.7 × 14 cm, 50−67% AcOEt in *n*-hexane) to give a 5,6-diol derivative (1.81 g, 90%). To a solution of the 5,6-diol derivative (1.74 g, 7.83 mmol), pyridine (697 μL, 8.61 mmol), and DMAP (10 mg) in CH_2Cl_2 (30 mL) was added dropwise BzCl (992 μL, 8.61 mmol) in CH_2Cl_2 (20 mL) at 0 °C. The mixture was stirred at 0 °C for 5 h. After the reaction was quenched with MeOH, the mixture was extracted with $CHCl_3$ (×3). The combined organic layers were washed with 0.5 N HCl (×2), saturated $NaHCO_3$, and brine and dried (Na_2SO_4). After concentration, the residue was purified over a silica gel column (8.1 × 12 cm, 10−20% AcOEt in *n*-hexane) to give **9** (2.15 g, 91%) as a crystal: mp 130−132 °C (crystallized from *n*-hexane); ^{1}H NMR ($CDCl_3$) δ ppm 8.09−8.05 (2H, m), 7.60−7.42 (3H, m), 5.99 (1H, d, J = 3.9 Hz), 5.14 (1H, dd, J = 2.0, 49.8 Hz), 4.74−4.70 (2H, m), 4.46 (1H, dd, J = 5.9, 12.2 Hz), 4.27−4.18 (2H, m), 2.83 (1H, br, D_2O exchangeable), 1.47, 1.33 (each 3H, s); FAB-MS m/z 327 ($M + H^+$). Anal. Calcd for $C_{16}H_{19}FO_6$: C, 58.89; H, 5.87. Found: C, 58.78; H, 5.82.

5,6-Anhydro-3-deoxy-3-fluoro-1,2-*O*-isopropylidene-β-L-*ido*-pentofuranose (10). To a solution of **9** (8.80 g, 27.0 mmol) in pyridine (45 mL) was added MsCl (3.34 mL, 43.2 mmol), and the mixture was stirred at room temperature for 3.5 h. After quenching with ice−water, the solvent was removed under reduced pressure. The residue was partitioned between AcOEt and water. The organic layer was washed with 0.5 N HCl (×2), saturated $NaHCO_3$, and brine and then dried (Na_2SO_4). After the filtarte was concentrated under reduced pressure, the residue was dissolved in MeOH (50 mL) containing NaOMe (28%, 6.6 mL, 32.4 mmol). The mixture was stirred at room temperature for 40 min. After evaporation, the residue was partitioned between AcOEt and water, washed with brine, and then dried (Na_2SO_4). After the filtrate was concentrated under reduced pressure, the residue was purified over a silica gel column (200 mL, 10−20−40−60% AcOEt in *n*-hexane) to give **10** (4.84 g, 89%) as a syrup: ^{1}H NMR ($CDCl_3$) δ ppm 6.04 (1H, d, J = 3.9 Hz), 4.96 (1H, dd, J = 2.4, 50.3 Hz), 4.71 (1H, dd, J = 3.9, 11.2 Hz), 3.89 (1H, ddd, J = 2.4, 5.9, 30.3 Hz), 3.22 (1H, ddd, J = 2.9, 4.4, 5.9 Hz), 2.88 (1H, t, J = 4.4 Hz), 2.71 (1H, dd, J = 2.9, 4.9 Hz), 1.47, 1.33 (each 3H, s); FAB-MS m/z 205 ($M + H^+$). Anal. Calcd for $C_9H_{13}FO_4$: C, 52.94; H, 6.42. Found: C, 52.67; H, 6.17.

5,6-Anhydro-3-deoxy-3-fluoro-1,2-*O*-isopropylidene-5-thio-α-D-*gluco*-pentofuranose (11). A mixture of **10** (405 mg, 1.98 mmol) and thiourea (151 mg, 1.98 mmol) in MeOH (10 mL) was kept under reflux for 7 h. After cooling to room temperature, the solvent was removed under reduced pressure. The residue was partitioned between AcOEt and water. The organic layer was washed with brine and dried (Na_2SO_4). After the filtrate was concentrated under reduced pressure, the residue was purified over a silica gel column (2.1 × 10 cm, 5% AcOEt in *n*-hexane) to give **11** (386 mg, 88%) as a syrup: ^{1}H NMR ($CDCl_3$) δ ppm 6.01 (1H, d, J = 3.9 Hz), 4.93 (1H, dd, J = 2.0, 49.8 Hz), 4.72 (1H, dd, J = 3.9, 10.3 Hz), 3.60 (1H, ddd, J = 2.4, 8.8, 28.3 Hz), 3.10 (1H, dt, J = 5.4, 8.8 Hz), 2.65 (1H, dd, J = 1.5, 5.8 Hz), 2.41 (1H, d, J = 5.4 Hz), 1.46, 1.32 (each 3H, s); FAB-MS m/z 221 ($M + H^+$). Anal. Calcd for $C_9H_{13}FO_3S \cdot 0.13H_2O$: C, 48.56; H, 6.00. Found: C, 48.59; H, 5.76.

3-Deoxy-5,6-di-*O,S*-acetyl-3-fluoro-1,2-*O*-isopropylidene-5-thio-α-D-*gluco*-pentofuranose (12). A mixture of **11** (7.12 g, 22.1 mmol) and KOAc (3.46 g, 35.3 mmol) in acetic anhydride (75 mL) containing acetic acid (15 mL) was kept at 120 °C for 20 h. After cooling to room temperature, the solvent was removed under reduced pressure. The residue was partitioned between AcOEt and water. The organic layer was washed with saturated $NaHCO_3$ and brine and dried (Na_2SO_4). After the filtrate was concentrated under reduced pressure, the residue was purified over a silica gel column (220 mL, 15−20% AcOEt in *n*-hexane) to give **12** (5.20 g, 73%) as a crystal: mp 89−90 °C (crystallized from AcOEt−*n*-hexane); ^{1}H NMR

($CDCl_3$) δ ppm 5.98 (1H, d, J = 3.9 Hz), 4.96 (1H, dd, J = 2.0, 49.6 Hz), 4.68 (1H, dd, J = 3.9 Hz), 4.46−4.37 (3H, m), 4.11 (1H, dt, J = 3.9, 4.8 Hz), 2.36 (3H, s), 2.06 (3H, s), 1.49, 1.33 (each 3H, s); FAB-MS m/z 323 ($M + H^+$). Anal. Calcd for $C_{13}H_{19}O_6SF$: C, 48.44; H, 5.94. Found: C, 48.49; H, 6.00.

Methyl 2-deoxy-3,5-di-*O*-benzoyl-2-fluoro-4-thio-α- and *β*-D-*arabino*-pentofuranoside (13). A solution of **12** (480 mg, 1.49 mmol) in aqueous trifluoroacetic acid (90%, 4 mL) was stirred at 0 °C for 4.5 h. The solvent was removed under reduced pressure below 20 °C. The residue was partitioned between AcOEt and water and washed with saturated $NaHCO_3$. The water layer was extracted with AcOEt (×2) and the combined organic layers were washed with saturated $NaHCO_3$ and brine and then dried (Na_2SO_4). After the filtrate was concentrated under reduced pressure, the residue was dissolved in MeOH (6 mL). To this mixture was added dropwise $NaIO_4$ (319 mg, 1.49 mmol) in water (6 mL). After 25 min of stirring at room temperature, glycerin (0.2 mL) was added. The whole was stirred at room temperature for 25 min. After MeOH (30 mL) was added, insoluble salts were removed by suction. Most of MeOH was removed under reduced pressure, and the residual water solution was extracted with $CHCl_3$ (×3). The combined organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was dissolved in methanolic HCl (prepared from 0.35 mL of acetyl chloride and 6.65 mL of MeOH), and the mixture was kept under reflux for 2 h. After cooling to room temperature, the mixture was neutralized with $NaHCO_3$. The insoluble salts were removed by filtration. After the filtrate was concentrated under reduced pressure, the residue was coevaporated with pyridine (×3) and dissolved in pyridine (7 mL). To this mixture was added benzoyl chloride (3.61 mL, 31.3 mmol). The mixture was stirred at room temperature for 1 h. MeOH was added to the mixture, and the whole was stirred at room temperature for 30 min. After concentration under reduced pressure, the residue was partitioned between AcOEt and water. The organic layer was washed with 0.5 N HCl (×2), saturated $NaHCO_3$, and brine and dried (Na_2SO_4). After evaporation, the residue was purified over a silica gel column (2.4 × 12.5 cm, 2−4% AcOEt in *n*-hexane) to give α-**13** (less polar, 180 mg) and *β*-**13** (more polar, 231 mg, total 71%).

Data for α-13: an amorphous foam; ^1H NMR ($CDCl_3$) δ ppm 8.04−7.95 (4H, m), 7.60−7.33 (6H, m), 5.80−5.73 (1H, m), 5.28 (1H, ddd, J = 2.4, 3.9, 48.3 Hz), 5.22 (1H, dd, J = 2.0, 13.7 Hz), 4.59 (1H, dd, J = 6.8, 11.2 Hz), 4.49 (1H, ddd, J = 1.0, 6.4, 11.2 Hz), 4.05 (1H, q, J = 6.8 Hz), 3.42 (3H, s); FAB-MS m/z 391 ($M + H^+$). Anal. Calcd for $C_{20}H_{19}O_5SF$: C, 61.53; H, 4.91. Found: C, 61.29; H, 4.87.

Data for *β*-13: mp 75−80 °C (crystallized from AcOEt-*n*-hexane); ^1H NMR ($CDCl_3$) δ ppm 8.04−7.97 (4H, m), 7.60−7.31 (6H, m), 6.09 (1H, ddd J = 6.4, 8.8, 12.2 Hz), 5.29 (1H, ddd, J = 4.4, 8.8, 51.8 Hz), 4.96 (1H, d, J = 4.4 Hz), 4.64 (1H, dd, J = 6.4, 11.2 Hz), 4.50 (1H, dd, J = 6.4, 11.2 Hz), 3.69 (1H, q, J = 6.4 Hz), 3.44 (3H, s); FAB-MS m/z 391 ($M + H^+$). Anal. Calcd for $C_{20}H_{19}O_5SF$: C, 61.53; H, 4.91. Found: C, 61.94; H, 4.93.

1-*O*-Acetyl-2-deoxy-3,5-di-*O*-benzoyl-2-fluoro-4-thio-α,*β*-D-*arabino*-pentofuranose (14). A mixture of **13** (1.09 g, 2.80 mmol), acetic anhydride (10 mL), acetic acid (10 mL), and sulfuric acid (0.3 mL) was stirred at room temperature for 1 h. After being neutralized with NaOAc, the mixture was stirred at room temperature for 1 h and was partitioned between CH_2Cl_2 and water. The water layer was extracted with CH_2Cl_2 (×3). The combined organic layers were washed with saturated $NaHCO_3$ (×3) and brine and dried (Na_2SO_4). After concentration, the residue was coevaporated with MeOH (×2) and CH_2Cl_2. Crystallization of the residue from *n*-hexane gave **14** (933 mg, 80%): mp 85−93 °C; ^1H NMR ($CDCl_3$) δ ppm 8.06−7.94 (4H, m), 7.62−7.30 (6H, m), 6.24 (0.42H, dd, J = 2.0, 14.2 Hz), 6.18 (0.58H, d, J = 4.4 Hz), 6.08 (0.58H, ddd, J = 7.3, 9.3, 11.7 Hz), 5.85 (0.42H, dt, J = 3.9, 12.2 Hz), 5.39 (0.42H, ddd, J = 2.0, 3.9, 47.9 Hz), 5.31 (0.58H, ddd, J = 4.4, 9.3, 50.8 Hz), 4.69 (0.58H, dd, J = 6.4, 11.2 Hz), 4.55 (0.42H, dd, J = 7.8, 11.7 Hz), 4.49 (0.58H, dd, J = 6.4, 11.2 Hz), 4.47 (0.42H, dd, J = 1.5, 11.7 Hz), 4.11 (0.42H, ddd, J = 1.5, 4.4, 7.8 Hz), 3.74 (0.58H, q, J = 6.4 Hz), 2.12, 2.11 (total 3H, s); FAB-MS m/z 419 ($M + H^+$). Anal. Calcd for $C_{21}H_{19}O_6SF$: C, 60.28; H, 4.58. Found: C, 60.06; H, 4.56.

4-Acetyl-1-(2-deoxy-3,5-di-*O*-benzoyl-2-fluoro-4-thio-α,*β*-D-*arabino*-pentofuranosyl)cytosine (16). To a solution of **14** (3.00 g, 7.20 mmol) in CH_2Cl_2 (20 mL) was added 30% HBr/acetic acid solution (6.0 mL) at room temperature. The mixture was stirred at room temperature for 20 min. The reaction was quenched by the addition of ice water. The whole was extracted with CH_2Cl_2 twice, and the combined organic layers were washed with saturated $NaHCO_3$ and ice water and then dried (Na_2SO_4). The solvent was removed under reduced pressure and the residual **15** was dissolved in CH_2Cl_2. To a solution of silylated N^4-acetylcytosine (prepared from N^4-acetylcytosine (1.65 g, 10.8 mmol) by refluxing with bis-(trimethylsilyl)acetamide (5.3 mL, 21.5 mmol) in 1,2-dichloroethane (20 mL) for 3 h) was added the CH_2Cl_2 solution of **15**. After evaporation, the neat mixture was kept at 80 °C for 5 h under reduced pressure (less than 4 mmHg). After cooling to room temperature, $CHCl_3$ was added, and the insoluble materials were removed by filtration. The filtrate was concentrated, and the residue was purified over a silica gel column (200 mL, 0−5% MeOH in $CHCl_3$) to give **16** (2.17 g, 59%) as an amorphous foam: ^1H NMR ($CDCl_3$) δ ppm 8.58 (0.2 H, br, D_2O exchangeable), 8.39 (0.8H, br, D_2O exchangeable), 8.38 (0.8H, d, J = 7.3 Hz), 8.37 (0.2H, d, J = 6.8 Hz), 8.12−8.04 (4H, m), 7.83−7.38 (7H, m), 6.96 (0.8H, dd, J = 3.9, 23.4 Hz), 6.38 (0.2H, dd, J = 2.0, 14.2 Hz), 5.92 (0.8H, dt, J = 2.0, 9.3 Hz), 5.86 (0.2H, dt, J = 2.9, 12.2 Hz), 5.52 (0.2H, dt, J = 2.0, 46.4 Hz), 5.39 (0.8H, ddd, J = 2.4, 3.4, 49.3 Hz), 4.75−4.56 (2H, m), 4.35−4.32 (0.2H, m), 4.04 (0.8H, t, J = 7.8 Hz), 2.26, 2.25 (total 3H, s); FAB-MS m/z 511 ($M + H^+$). Anal. Calcd for $C_{25}H_{22}N_3O_6SF \cdot 1.25H_2O$: C, 56.23; H, 4.62; N, 7.87. Found: C, 55.92; H, 4.27; N, 8.16.

1-(2-Deoxy-2-fluoro-4-thio-α- and *β*-D-*arabino*-pentofuranosyl)cytosine (α- and *β*-4′-ThioFAC, α- and *β*-2). A suspension of **16** (2.17 g, 4.24 mmol) in MeOH (30 mL) and concentrated NH_4OH (30 mL) was stirred at room temperature for 2 days. The solvents were removed under reduced pressure, and the residue was coevaporated with EtOH (×3). The residue was purified over a silica gel column (200 mL, 10−33% MeOH in $CHCl_3$). An anomeric mixture of **2** was separated by an ODS column (Wako sil 40C18, Wako Pure Chemical Industries, Ltd., Japan; 400 mL, H_2O only). The unseparable fractions were collected. The ODS column chromatography was repeated to give *β*-**2** (613 mg, 55%) and α-**2** (152 mg, 14%). All of the instrumental analyses data for *β*-**2** and α-**2** were identical to those reported previously.

9-(2-Deoxy-3,5-di-*O*-benzoyl-2-fluoro-4-thio-α,*β*-D-*arabino*-pentofuranosyl)-2,6-diaminopurine (19). Compound **15** was prepared from **14** (6.81 g, 16.3 mmol) as described in the synthesis of **16**. A mixture of **15** (as 16.3 mmol), 2,6-diaminopurine (7.34 g, 48.9 mmol), TMS triflate (18.9 mL, 97.6 mmol), and 4A molecular sieves (18.7 g) in CH_3CN (150 mL) was kept at 60 °C overnight. After cooling to room temperature, the reaction was quenched with saturated $NaHCO_3$. The whole was extracted with $CHCl_3$ (×3), and the organic layer was washed with brine and then dried (Na_2SO_4). After concentration under reduced pressure, the residue was purified over a silica gel column (400 mL, 0−1−3% MeOH in $CHCl_3$) to give **19** (6.20 g, 75%) as an amorphous foam: ^1H NMR ($CDCl_3$) δ ppm 8.09−7.39 (11H, m), 6.52 (0.5H, dd, J = 3.9, 21.0 Hz), 6.19 (0.5H, dd, J = 2.9, 14.2 Hz), 6.14 (0.5H, dt, J = 2.9, 9.3 Hz), 5.91 (0.5H, dd, J = 3.9, 6.3 Hz), 5.87 (0.5H, dt, J = 3.9, 63.5 Hz), 5.37 (2H, br, D_2O exchangeable), 5.31 (0.5H, dt, J = 3.9, 49.3 Hz), 4.77 (2H, br, D_2O exchangeable), 4.84−4.55 (2H, m), 4.41−4.36 (0.5H, m), 4.05−4.01 (0.5H, m); FAB-MS m/z 509 ($M + H^+$). Anal. Calcd for $C_{24}H_{21}N_6O_4SF \cdot 1.25H_2O$: C, 54.28; H, 4.46; N, 15.83. Found: C, 54.62; H, 4.22; N, 15.46.

9-(2-Deoxy-2-fluoro-4-thio-α,*β*-D-*arabino*-pentofuranosyl)-2,6-diaminopurine (20). A suspension of **19** (6.20 g, 12.2 mmol) in MeOH (160 mL) and concentrated NH_4OH (160 mL) was stirred at room temperature overnight. After MeOH was evaporated under reduced pressure, the mixture was washed

with $CHCl_3$ (×3). Insoluble materials were removed by filtration. The solvents were removed under reduced pressure. Crystallization of the residue from H_2O gave **20** (3.02 g, 82%) as an anomeric mixture: ^{1}H NMR (DMSO-d_3 + D_2O) δ ppm 8.18 (0.5H, s), 8.10 (0.5H, s), 6.06 (0.5H, t, J = 6.8 Hz), 6.00 (0.5H, dd, J = 6.3, 16.6 Hz), 5.52 (0.5H, dt, J = 6.8, 52.2 Hz), 5.09 (0.5H, ddd, J = 5.9, 7.3, 50.8 Hz), 4.42 (0.5H, dt, J = 7.3, 12.2 Hz), 4.19 (0.5H, dt, J = 7.3, 13.7 Hz), 3.86−3.73 (2H, m), 3.52 (0.5H, dd, J = 7.3, 11.2 Hz), 3.29 (0.5H, q, J = 5.8 Hz); FAB-MS m/z 301 (M + H$^+$). Anal. Calcd for $C_{10}H_{13}N_6O_3FS\cdot 0.5H_2O$: C, 38.83; H, 4.56; N, 27.17. Found: C, 38.64; H, 4.64; N, 27.21.

9-(2-Deoxy-2-fluoro-4-thio-β-D-*arabino*-pentofuranosyl)-guanine (4). To a suspension of **20** (3.02 g, 10.1 mmol) in Tris−HCl buffer (pH 7.0, 420 mL) was added adenosine deaminase (from bovine spleen, EC 3.5.4.4, purchased from Sigma Co., Ltd., 6.0 mL, 1400 units). The mixture was stirred at room temperature for 4 h. Another Tris−HCl buffer (30 mL) was added, and the mixture was stirred for 2 h. The reaction mixture was applied to a top of ODS column (Chromatorex, Fuji Silysia Chemical Ltd., 500 mL). From the eluate containing 1% CH_3CN, compound **4** (710 mg) was obtained after evaporation of the solvents. Similary, from the eluate containing 3% CH_3CN, unreacted **20** (1.46 g) was obtained. Hydrolysis of the recovered **20** by adenosine deaminase was repeated to give **4** (330 mg, total 34%) and α-**20** (0.98 g, 32%). All of the instrumental analyses data for **4** and α-**20** were identical to those reported previously.

3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*gluco*-pentofuranose (23). To a solution of 1,2:5,6-di-*O*-isopropylidene-α-D-*allo*-pentofuranose (**6**) (10.4 g, 40 mmol) in CH_2Cl_2 (100 mL) was added sulfuryl chloride (6.48 mL, 80 mmol). After 30 min of stirring at 0 °C, imidazole (27.2 g, 400 mmol) was added. The whole was stirred at room temperature overnight. The reaction was quenched with saturated $NaHCO_3$ and extracted with CH_2Cl_2 (×3). The combined organic layers were washed with brine and dried (Na_2SO_4). After concentration under reduced pressure, the residue was dissolved in 2-methoxyethanol (100 mL). To this mixture was added sodium azide (7.80 g, 120 mmol). The mixture was kept under reflux for 7 h. After cooling to room temperature, the solvent was removed under reduced pressure. The residue was partitioned between AcOEt and water, and the organic layer was washed with brine and then dried (Na_2SO_4). After evaporation, the residue was purified over a silica gel column (7.3 × 10.5 cm, 4% AcOEt in *n*-hexane) to give **23** (9.90 g, 87%) as a syrup: ^{1}H NMR ($CDCl_3$) δ ppm 5.86 (1H, d, J = 3.4 Hz), 4.62 (1H, d, J = 3.9 Hz), 4.24 (1H, ddt, J = 1.0, 4.9, 5.9 Hz), 4.14 (1H, dd, J = 5.9, 8.8 Hz), 4.11−4.08 (2H, m), 3.98 (1H, dd, J = 4.9, 8.8 Hz), 1.51, 1.44, 1.37, 1.33 (each 3H, s); FAB-MS m/z 286 (M + H$^+$); IR (neat) 2112 cm^{-1} (N_3). Anal. Calcd for $C_{12}H_{19}N_3O_5\cdot 0.2H_2O$: C, 49.89; H, 6.77; N, 14.54. Found: C, 50.05; H, 6.55; N, 14.42.

3-Azido-6-*O*-benzoyl-3-deoxy-1,2-*O*-isopropylidene-α-D-*gluco*-pentofuranose (24). Compound **24** was synthesized from **23** (9.80 g, 34.3 mmol) as described in the synthesis of **9**. The residue was purified over a silica gel column (8.1 × 12 cm, 10−20% AcOEt in *n*-hexane) to give **24** (9.55 g, 80%) as a syrup: ^{1}H NMR ($CDCl_3$) δ ppm 8.07−8.05 (2H, m), 7.60−7.42 (3H, m), 5.90 (1H, d, J = 3.4 Hz), 4.72 (1H, dd, J = 2.0, 11.7 Hz), 4.65 (1H, d, J = 3.9 Hz), 4.45 (1H, dd, J = 5.4, 11.7 Hz), 4.26−4.17 (3H, m), 2.90 (1H, d, J = 4.4 Hz, D_2O exchangeable), 1.49, 1.33 (each 3H, s); FAB-MS m/z 350 (M + H$^+$); IR (KBr) 2112 cm^{-1} (N_3). Anal. Calcd for $C_{16}H_{19}N_3O_6\cdot 0.5H_2O$: C, 53.63; H, 5.63; N, 11.73. Found: C, 53.96; H, 5.48; N, 11.60.

5,6-Anhydro-3-azido-3-deoxy-1,2-*O*-isopropylidene-β-L-*ido*-pentofuranose (25). Compound **25** was synthesized from **24** (8.09 g, 23.2 mmol) as described in the synthesis of **10**. The residue was purified over a silica gel column (4.8 × 15 cm, 10−20−40−60% AcOEt in *n*-hexane) to give **25** (3.48 g) and a 5-mesyl derivative (1.27 g). Treatment of the 5-mesyl derivative (1.27 g, 3.80 mmol) with NaH (60%, 152 mg, 3.80 mmol) in THF (15 mL) for 30 min at 0 °C gave **25** (584 mg, total 77%) as a syrup: ^{1}H NMR ($CDCl_3$) δ ppm 5.96 (1H, d, J = 3.4 Hz), 4.67 (1H, d, J = 3.4 Hz), 3.98 (1H, d, J = 3.4 Hz), 3.94 (1H, dd, J = 3.4, 5.4 Hz), 3.21 (1H, ddd, J = 2.4, 4.4, 5.4 Hz), 2.89 (1H, t, J = 4.9 Hz), 2.70 (1H, dd, J = 2.4, 4.9 Hz), 1.49, 1.33 (each 3H, s); FAB-MS m/z 228 (M + H$^+$); IR (neat) 2114 cm^{-1} (N_3). Anal. Calcd for $C_9H_{13}N_3O_4$: C, 47.57; H, 5.77; N, 18.49. Found: C, 47.24; H, 5.53; N, 18.50.

5,6-Anhydro-3-azido-3-deoxy-1,2-*O*-isopropylidene-5-thio-α-D-*gluco*-pentofuranose (26). Compound **26** was synthesized from **25** (3.79 g, 16.7 mmol) as described in the synthesis of **11**. The residue was purified over a silica gel column (4.7 × 11.5 cm, 5% AcOEt in *n*-hexane) to give **26** (3.52 g, 87%) as a syrup: ^{1}H NMR ($CDCl_3$) δ ppm 5.93 (1H, d, J = 3.4 Hz), 4.66 (1H, d, J = 3.9 Hz), 4.02 (1H, d, J = 3.4 Hz), 3.62 (1H, dd, J = 3.4, 8.8 Hz), 3.10 (1H, ddd, J = 4.9, 5.9, 8.8 Hz), 2.66 (1H, dd, J = 1.5, 6.3 Hz), 2.41 (1H, dd, J = 1.5, 4.9 Hz), 1.47, 1.32 (each 3H, s); FAB-MS m/z 244 (M + H$^+$); IR (neat) 2110 cm^{-1} (N_3). Anal. Calcd for $C_9H_{13}N_3O_3S\cdot 0.25H_2O$: C, 43.63; H, 5.49; N, 16.96. Found: C, 43.33; H, 5.26; N, 16.89.

3-Azido-3-deoxy-5,6-di-*O*,*S*-acetyl-1,2-*O*-isopropylidene-5-thio-α-D-*gluco*-pentofuranose (27). Compound **27** was synthesized from **26** (3.329 g, 13.6 mmol) as described in the synthesis of **12**. The residue was purified over a silica gel column (4.3 × 12 cm, 5−10−15% AcOEt in *n*-hexane) to give **27** (3.31 g, 70%); mp 94−95 °C (crystallized from AcOEt−*n*-hexane); ^{1}H NMR ($CDCl_3$) δ ppm 5.89 (1H, d, J = 3.4 Hz), 4.66 (1H, d, J = 3.9 Hz), 4.43 (1H, dd, J = 3.4, 11.7 Hz), 4.39 (1H, dd, J = 3.4, 10.7 Hz), 4.33 (1H, dd, J = 4.9, 11.7 Hz), 4.06 (1H, ddd, J = 3.4, 4.9, 10.7 Hz), 4.00 (1H, d, J = 2.9 Hz), 2.38, 2.05 (each 3H, s), 1.50, 1.33 (each 3H, s); FAB-MS m/z 346 (M + H$^+$); IR (KBr) 2116 cm^{-1} (N_3). Anal. Calcd for $C_{13}H_{19}N_3O_6S$: C, 45.21; H, 5.54; N, 12.17. Found: C, 45.29; H, 5.54; N, 12.07.

Methyl 2-Azido-2-deoxy-4-thio-α,β-D-*arabino*-pentofuranoside (28). Compound **28** was synthesized from **27** (3.09 g, 8.95 mmol) as described in the synthesis of **13**. The residue was purified over a silica gel column (4.3 × 16 cm, 5−10% AcOEt in *n*-hexane) to give α-**28** (757 mg) and β-**28** (1.25 g, total 54%).

Data for α-28: mp 43−47 °C (crystallized from AcOEt−*n*-hexane); ^{1}H NMR ($CDCl_3$) δ ppm 8.08−7.93 (4H, m), 7.61−7.28 (6H, m), 5.52 (1H, t, J = 7.3 Hz), 5.06 (1H, d, J = 4.9 Hz), 4.60 (1H, dd, J = 6.4, 11.7 Hz), 4.44 (1H, dd, J = 6.4, 11.7 Hz), 4.34 (1H, dd, J = 4.9, 7.3 Hz), 4.06 (1H, dt, J = 6.4, 7.8 Hz), 3.42 (3H, s); FAB-MS m/z 414 (M + H$^+$); IR (KBr) 2112 cm^{-1} (N_3). Anal. Calcd for $C_{20}H_{19}N_3O_5S\cdot 0.25H_2O$: C, 57.47; H, 4.70; N, 10.05. Found: C, 57.40; H, 4.71; N, 9.83.

Data for β-28: mp 133−136 °C (crystallized from AcOEt−*n*-hexane); ^{1}H NMR ($CDCl_3$) δ ppm 8.03−7.92 (4H, m), 7.59−7.27 (6H, m), 6.04 (1H, dd, J = 6.8, 10.3 Hz), 4.95 (1H, d, J = 3.9 Hz), 4.60 (1H, dd, J = 6.4, 11.2 Hz), 4.51 (1H, dd, J = 6.4, 11.7 Hz), 4.08 (1H, dd, J = 3.9, 9.8 Hz), 3.68 (1H, q, J = 6.4 Hz), 3.42 (3H, s); FAB-MS m/z 414 (M + H$^+$); IR (KBr) 2104 cm^{-1} (N_3). Anal. Calcd for $C_{20}H_{19}N_3O_5S$: C, 58.10; H, 4.63; N, 10.16. Found: C, 58.20; H, 4.59; N, 10.09.

1-*O*-Acetyl-2-azido-2-deoxy-3,5-di-*O*-benzoyl-4-thio-α,β-D-*arabino*-pentofuranose (29). Compound **29** was synthesized from **28** (1.72 g, 4.16 mmol) as described in the synthesis of **14**. The residue was purified over a silica gel column (3.7 × 12 cm, 10−20% AcOEt in *n*-hexane) to give **29** (1.63 g, 89%) as a crystalline solid: mp 136−137 °C (crystallized from AcOEt−*n*-hexane); ^{1}H NMR ($CDCl_3$) δ ppm 8.05−7.90 (4H, m), 7.61−7.27 (6H, m), 6.14 (0.7H, d, J = 4.4 Hz), 6.01−5.97 (1H, m), 5.60 (0.3H, t, J = 6.4 Hz), 4.67 (0.7H, dd, J = 6.4, 11.2 Hz), 4.62 (0.3H, dd, J = 6.8, 11.7 Hz), 4.52−4.48 (1H, m), 4.43 (0.3H, dd, J = 6.8, 11.7 Hz), 4.20 (0.7H, dd, J = 4.4, 10.3 Hz), 4.10 (0.3H, q, J = 6.8 Hz), 3.33 (0.7H, q, J = 6.4 Hz), 2.13, 2.12 (total 3H, s); FAB-MS m/z 442 (M + H$^+$); IR (KBr) 2108 cm^{-1} (N_3). Anal. Calcd for $C_{21}H_{19}N_3O_6S$: C, 57.14; H, 4.34; N, 9.52. Found: C, 57.38; H, 4.40; N, 9.25.

4-Acetyl-1-(2-azido-2-deoxy-3,5-di-*O*-benzoyl-4-thio-α and β-D-*arabino*-pentofuranosyl)cytosine (30). To a solution of N^4-acetylcytosine (447 mg, 2.92 mmol) in CH_3CN (10 mL) was added BSA (1.59 mL, 6.42 mmol), and the mixture was kept under reflux for 3.5 h. After cooling to room temperature, **29** (645 mg, 1.46 mmol) and TMSOTf (282 μL, 1.46 mmol) were added. The mixture was kept under reflux

for 3 h. After cooling to room temperature, the solvents were removed under reduced pressure. The residue was dissolved in $CHCl_3$. To this mixture was added saturated $NaHCO_3$, and the insoluble materials were removed by suction. The separated water layer was extracted with $CHCl_3$ (×3), which was dried over Na_2SO_4. After concentration, the residue was purified over a silica gel column (2.4 × 17.5 cm, 50−66% AcOEt in *n*-hexane) to give α-**30** (less polar, 305 mg) and *β*-**30** (more polar, 148 mg, total 58%).

Data for α-30: an amorphous foam; ^{1}H NMR ($CDCl_3$) *δ* ppm 8.94 (1H, br, D_2O exchangeable), 8.37 (1H, d, *J* = 7.8 Hz), 8.07−8.04 (2H, m), 7.83−7.81 (2H, m), 7.59−7.38 (7H, m), 6.16 (1H, d, *J* = 3.4 Hz), 5.64 (1H, t, *J* = 3.4 Hz), 4.73−4.68 (2H, m), 4.58 (1H, dd, *J* = 7.3, 11.2 Hz), 4.28 (1H, dt, *J* = 3.4, 7.3 Hz), 2.27 (3H, s); FAB-MS *m*/*z* 535 (M + H$^+$); IR (KBr) 2116 cm^{-1} (N_3). Anal. Calcd for $C_{25}H_{22}N_6O_6S$·0.33AcOEt: C, 56.10; H, 4.41; N, 14.91. Found: C, 55.86; H, 4.10; N, 14.66.

Data for *β*-30: mp 206−208 °C (crystallized from AcOEt−*n*-hexane); ^{1}H NMR ($CDCl_3$) *δ* ppm 8.43 (1H, d, *J* = 7.8 Hz), 8.27 (1H, br, D_2O exchangeable), 8.09−8.03 (4H, m), 7.65−7.46 (6H, m), 7.40 (1H, d, *J* = 7.8 Hz), 6.80 (1H, d, *J* = 5.9 Hz), 5.77 (1H, t, *J* = 4.9 Hz), 4.79 (1H, dd, *J* = 6.8, 11.7 Hz), 4.73 (1H, t, *J* = 5.4 Hz), 4.65 (1H, dd, *J* = 7.3, 11.2 Hz), 3.96 (1H, dt, *J* = 4.9, 6.4 Hz), 2.24 (3H, s); FAB-MS *m*/*z* 535 (M + H$^+$); IR (KBr) 2120 cm^{-1} (N_3). Anal. Calcd for $C_{25}H_{22}N_6O_6S$·0.5H_2O: C, 55.24; H, 4.26; N, 15.46. Found: C, 55.45; H, 3.96; N, 15.14.

1-(2-Azido-2-deoxy-4-thio-*β*-D-*arabino*-pentofuranosyl)-cytosine (4′-Thiocytarazid, *β*-5). A suspension of *β*-**30** (173 mg, 0.324 mmol) in MeOH (8 mL) and concentrated NH_4OH (8 mL) was stirred at room temperature for 2 days. After the solvents were removed under reduced pressure, the residue was dissolved in water (50 mL) and purified over an ODS column (2.2 × 15 cm, H_2O only) to give *β*-**5** (73 mg, 79%): mp 182−185 °C (crystallized from CH_3CN-H_2O); ^{1}H NMR (DMSO-d_3) *δ* ppm 8.03 (1H, d, *J* = 7.3 Hz), 7.17, 7.09 (total 2H, br, D_2O exchangeable), 6.28 (1H, d, *J* = 6.8 Hz), 5.85 (1H, d, *J* = 5.9 Hz, D_2O exchangeable), 5.74 (1H, d, *J* = 7.3 Hz), 5.18 (1H, d, *J* = 4.9, 5.4 Hz, D_2O exchangeable), 4.23 (1H, dd, *J* = 6.8, 9.8 Hz), 3.92 (1H, ddd, *J* = 6.3, 8.3, 9.8 Hz), 3.79 (1H, ddd, *J* = 3.4, 4.4, 11.7 Hz), 3.68 (1H, dt, *J* = 5.9, 11.7 Hz), 3.18 (1H, ddd, *J* = 3.4, 5.9, 8.3 Hz); NOE, irradiate H-6 (8.03 ppm), observe H-3′ (3.92 ppm; 10.8%); irradiate H-2′ (4.23 ppm), observe H-4′ (3.18 ppm, 5.3%); irradiate H-3′, observe H-6 (13.6%); irradiate H-4′, observe H-2′ (6.6%); FAB-MS *m*/*z* 285 (M + H$^+$); IR (KBr) 2130 cm^{-1} (N_3). Anal. Calcd for $C_9H_{12}N_6O_3S$·0.25H_2O: C, 37.43; H, 4.36; N, 29.10. Found: C, 37.20; H, 4.02; N, 29.13.

1-(2-Azido-2-deoxy-4-thio-α-D-*arabino*-pentofuranosyl)-cytosine (α-5). Compound α-**30** (193 mg, 0.361 mmol) was treated as described above to give α-**5** (96 mg, 93%) as an amorphous foam: ^{1}H NMR (DMSO-d_3) *δ* ppm 7.97 (1H, d, *J* = 7.3 Hz), 7.27 (2H, br d, D_2O exchangeable), 6.07 (1H, d, *J* = 5.9 Hz, D_2O exchangeable), 5.92 (1H, d, *J* = 8.8 Hz), 5.81 (1H, d, *J* = 7.8 Hz), 4.96 (1H, d, *J* = 4.9, 5.9 Hz, D_2O exchangeable), 4.26 (1H, t, *J* = 9.3 Hz), 3.87−3.77 (2H, m), 3.59 (1H, dt, *J* = 3.4, 8.3 Hz), 3.41 (1H, ddd, *J* = 6.4, 7.8, 10.3 Hz); FAB-MS *m*/*z* 285 (M + H$^+$); IR (KBr) 2114 cm^{-1} (N_3). Anal. Calcd for $C_9H_{12}N_6O_3S$·0.3$(CH_3)_2CO$: C, 38.19; H, 4.47; N, 27.00. Found: C, 37.89; H, 4.57; N, 26.90.

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