A Novel Synthesis of 2′-Modified 2′-Deoxy-4′-thiocytidines from D-Glucose[1]

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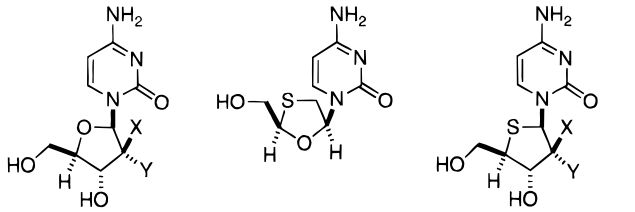
Novel 2′-deoxycytidine antimetabolites, specifically several 2′-modified 2′-deoxy-4′-thiocytidines, were synthesized as potential new antineoplastic agents. Methyl 3-*O*-benzylxylofuranoside was converted to a 1,4-anhydro-4-thioarabitol **24**. Protection of the primary alcohol of **24** gave a common intermediate (**15**) which was useful for the synthesis of various 2′-modified 2′-deoxy-4′-thionucleosides. Oxidation of the secondary hydroxyl group of **15**, followed by the Wittig reaction or treatment with (diethylamido)sulfur trifluoride (DAST) produced 2-deoxy-2-methylene (**26**) and 2-deoxy-2,2-difluoro (**34**) derivatives, respectively. Unique Pummerer-type glycosylation between the corresponding sulfoxides and trimethylsilylated N^4-acetylcytosine produced 2′-deoxy-2′-methylene- (**10**) and 2′-deoxy-2′,2′-difluoro-4′-thiocytidines (**11**). On the other hand, treatment of **15** with DAST introduced a fluorine atom with retention of the 2′-stereochemistry, yielding **40**. In contrast, the Mitsunobu reaction of 3-*O*-benzoyl derivative **53** which was obtained from **15** in five steps, using diphenylphosphoryl azide gave azide derivative **54** with inverted stereochemistry. These derivatives were converted to the corresponding 1-*O*-acetyl derivatives *via* the usual Pummerer rearrangement, which were in turn used to synthesize 4′-thiocytidines **12** and **58**. Among the 2′-modified 4′-thiocytidines obtained, 2′-methylene (**10**) and 2′-fluoro (**12**) derivatives were found to have potent antineoplastic properties *in vitro*.

The design of new effective agents for cancer chemotherapy is an important goal of synthetic chemists working in the field of medicinal chemistry. Nucleoside antimetabolites are considered to be promising candidates: 1-β-D-arabinosylcytosine (araC, **1**, Chart 1) is clinically used for the treatment of acute myeloblastic leukemia,[2] and a new 2′-deoxycytidine analogue, gemcitabine (2′-deoxy-2′,2′-difluorocytidine, **2**), has been introduced as a chemotherapeutic agent for solid tumors.[3] Some nucleoside analogues are also used to inhibit reverse transcriptase coded by human immunodeficiency virus (HIV),[4,5] a causative agent of acquired immunodeficiency syndrome (AIDS). In particular, 3′-thiacytidine (3TC, **7**)[5] has recently been approved as an anti-HIV agent by the United States Food and Drug Administration. On the basis of these considerations, our search for new antineoplastic and antiviral agents has focused on the synthesis of new nucleoside derivatives. Among the numerous nucleoside analogues which have been synthesized, 2′-deoxycytidine mimics are considered to be very promising. The success of araC has prompted many chemists to synthesize various 2′-substituted 2′-deoxycytidine derivatives. As a result, several effective derivatives have been found: 2′-deoxy-2′,2′-difluorocytidine (gemcitabine, **2**),[3] 1-(2-deoxy-2-*C*-methyl-β-D-*arabino*-pentopuranosyl)cytosine (SMDC, **3**),[6] 1-(2-*C*-cyano-2-deoxy-β-D-*arabino*-pentofuranosyl)cytosine (CNDAC, **4**),[7] 1-(2-deoxy-2-*C*-methylene-β-D-*erythro*-pentofuranosyl)cytosine (DMDC, **5**),[8] and 1-(2-azido-2-deoxy-β-D-arabinofuranosyl)cytosine (cytarazid, **6**).[9] These compounds have been shown to possess a broad spectrum of

Chart 1



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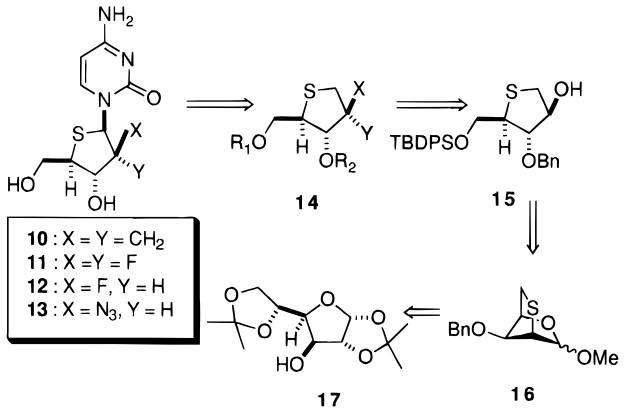
potent antitumor activities against various solid tumors, as well as leukemias.[3,6−9]

Another novel class of nucleoside antimetabolites is 4′-thionucleosides. The first example of 4′-thionucleosides was reported in 1964 by Reist *et al.*, who synthesized the 4′-thio counterpart of naturally occurring adenosine.[10] In 1968, 9-(4-thio-D-xylofuranosyl)adenine and 9-(4-thio-D-arabinofuranosyl)adenine were synthesized.[11] Since then, several examples of 4′-thionucleosides have been reported,[12] including 4′-thio-araC (**8**), which is as active as araC itself.[13] However, the difficulty of synthesizing 4′-thionucleosides has impaired further production of these analogues, and only a few examples are known.[14] Moreover, biologically interesting 2′-deoxy-4′-thionucleosides had not been synthesized before 1990, except for a report of a 5-fluorouracil derivative.[14] In 1991, Walker[15] and Secrist[16] independently reported the synthesis of pyrimidine 2′-deoxy-4′-thionucleosides. The method developed by Walker, which included a new and efficient synthesis of 2-deoxy-4-thioribose,[17] was particularly useful. At the same time, Uenishi and his co-workers achieved an elegant, alternative synthesis of 2′-deoxy-4′-thionucleosides using the Sharpless asymmetric epoxidation.[18] On the basis of these investigations, 2′-deoxy-4′-thionucleosides have been shown to have potent anti-herpes virus activities, and some analogues, especially 4′-thiothymidine and 2′-deoxy-4′-thiocytidine (**9**), have been shown to possess potent cytotoxicity.[15,16,18] Notably, the 4′-thionucleosides are resistant to hydrolytic cleavage of glycosyl linkage catalyzed by nucleoside phosphorylase.[19] This is a major advantage of 4′-thionucleosides, since several "4′-oxy" antivirals have fatal drawbacks with regard to their metabolic stability caused by nucleoside phosphorylase.[20] In addition, the potent antiviral activity and cytotoxicity of 4′-thionucleosides

Scheme 1



suggest that they are well-recognized as substrates by both viral and host cells kinases. Thus, the 4′-thionucleosides have received considerable attention as potential antiviral agents.[21]

The potent cytotoxicity of 2′-substituted cytidine analogues and the specific properties of 4′-thionucleosides prompted us to design and synthesize 2′-modified 2′-deoxy-4′-thiocytidines **10**−**13** as potential antineoplastic agents (Chart 1). As mentioned above, although several alternative synthetic methods regarding 4′-thionucleosides have been reported, these methods are limited to the 2′-deoxy derivatives. Imbach and his colleagues[22] reported the synthesis of 4′-thio*ribo*nucleosides using the intramolecular Mitsunobu reaction as reported by Walker.[15] However, the choice of a 4′-thio*ribo*nucleoside as a starting material might circumscribe the diversity of 2′-substituents on 4′-thionucleosides: it would not be applicable to the synthesis of 4′-thiogemcitabine, considering the original synthesis of the parental gemcitabine.[3a] Thus, the development of a new synthetic method would be a key to increasing the success of our project.

Strategy for the Synthesis of 2′-Modified 2′-Deoxy-4′-thiocytidines. To prepare various 2′-deoxy-2′-substituted 4′-thiocytidines, a sole intermediate to which various substituents could be introduced at the 2′-position would be advantageous. Thus, the intermediate **14** should be ideal, if a glycosidic bond could be formed at the α-methylene of the sulfide in this molecule (Scheme 1). The Pummerer rearrangement could be suitable for introducing a base moiety at the 1-position of **14**. Considering the synthesis of intermediate **14**, a recent work on the synthesis of 3TC conducted by Chu *et al.* was suggestive.[5d] They used an anhydrothiosugar (**18**) to construct the oxathiolane skeleton of 3TC[5d] (Scheme

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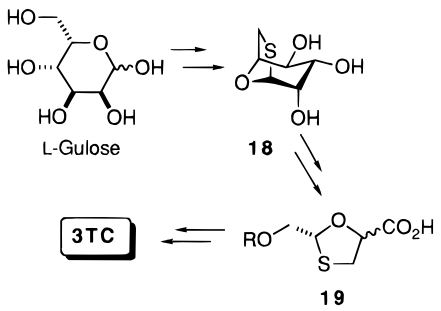
Scheme 2



2). Following Chu's tactics, three sets of chiral centers at the 2′-, 3′-, and 4′-positions of 4′-thionucleosides could be transferred from the chiralities of the 4-, 3-, and 5-carbons of inexpensive D-glucose *via* anhydrothiosugar **16**. This encouraged us to synthesize the title compounds by using a readily available D-glucose as a starting material.

Synthesis of the 4-Thiosugar Portion. Diisopropylideneglucose **17** was converted to 3-*O*-benzylxylose **21** using known chemistry. 3-*O*-Benzylxylose **21** was then subjected to acidic methanolysis to produce an anomeric mixture of methyl 3-*O*-benzylxyloside (**22**) in high yield (Scheme 3). The anomers were easily separated by a silica gel column. The separated α- and *β*-anomers of **22** were mesylated, which produced α- and *β*-**23**, and then treated with sodium sulfide in DMF to yield bicyclic α- and *β*-**16** in yields of 78 and 73%, respectively. We found that α-**16** was less stable than *β*-**16** and was converted to *β*-**16** if allowed to stand in $CDCl_3$ overnight. This means that the conversion of α- to *β*-**16** occurred under slightly acidic conditions. In addition, *β*-**16** was detected on TLC when α-**23** was reacted with sodium sulfide in DMF, but no α-**16** was formed in the reaction of *β*-**23** under the same conditions. These results suggest that steric repulsion between 1-OMe and H-3 could make α-**16** unstable and accelerate the conversion of the α- to the *β*-anomer, as shown in Scheme 4.

During the course of our investigation, Yuasa *et al.* independently reported the synthesis of *β*-**16**, in which they synthesized *β*-**16** in moderate yield by the intramolecular cyclization of methyl 3,5-di-*O*-benzyl-5-(benzylthio)-α(and *β*)-D-xylofuranoside with an iodine, triphenylphosphine, and imidazole system.[23] They reported that *β*-**16**, but no α-**16**, was formed in moderate yield from the reaction. They reasoned that the transition state of the α-isomer lacked an anomeric effect, which made the formation of α-**16** unfavored. However, our experimental results show that α-**16** is unstable and is easily converted to *β*-**16**. Thus, even in Yuasa's case, it is likely that α-**16**, which would be formed by the cyclization reaction of methyl 5-(benzylthio)-α-D-xylofuranoside, would readily be anomerized and converted to its *β*-isomer. This might result in the predominant formation of *β*-**16**, as described above. Hydrolysis of α,*β*-**16**, followed by borohydride reduction, gave 1,4-anhydro-4-thioarabitol **24** in 90% yield. The primary alcohol of **24** was selectively protected with a *tert*-butyldiphenylsilyl (TBDPS) group to give the common intermediate **15** in 87% yield.

Synthesis of 4′-ThioDMDC. As a first target among 2′-modified 2′-deoxy-4′-thiocytidine analogues, we chose a 4′-thio analogue of DMDC. DMDC is a 2′-deoxycytidine derivative developed by Matsuda *et al.*[8] which has potent antineoplastic activity both *in vitro* and *in vivo*. Thus, 4′-thioDMDC should also be a potential antineoplastic agent. The secondary hydroxyl group of **15** was oxidized with DMSO−Ac_2O,[24] to give **25**, which, without purification, was treated with methylenetriphenylphosphorane to give **26** in 74% yield from **15** (Scheme 5). Compound **26** was oxidized with *m*-CPBA to the corresponding sulfoxide, which was treated with Ac_2O at 110 °C to give exclusively one product (**27**), but not the desired **28** (Chart 2). This was confirmed by ^1H and ^{13}C NMR spectra: in the ^1H NMR spectrum, a new singlet signal, with 2H integration, at 4.65 ppm was observed instead of exo-methylene signals. In addition, ^{13}C NMR of **27** showed three peaks which corresponded to methylene carbons (C2, C5, and benzyl methylene) at 70.18, 64.84, and 61.02 ppm (confirmed by the DEPT spectrum, data not shown). This result showed that Pummerer rearrangement had occurred at the allylic position.[25] We concluded that **27** may be a substrate for the glycosylation reaction, since similar reactions have recently been reported.[26] Treatment of **27** with a trimethylsilylated N^4-acetylcytosine and trimethylsilyl trifluoromethanesulfonate (TMSOTf) at 0 °C gave the 4′-thioDMDC analogue **29** in 25% yield (3.8:1 mixture of the anomers) along with **30** (26% yield) which was attacked at the allylic position. The formation of **30** increased with a prolonged reaction time and an increase in the reaction temperature (data not shown). This tendency suggested the possibility that **30** may have an *O*-alkylated structure, which might arise from an allylic rearrangement of **29**. However, the UV spectra of **30**, which were consistent with those of **29** in both neutral and acidic methanol, suggest an *N*-glycosylated structure (see Experimental Section). In the HMBC spectrum of **30**, cross peaks corresponding to 2′-CH_2/C-2 and C-6 were observed (data not shown). Since this ruled out the *O*-alkylated structure, the structure of **30** was unambiguously determined, as depicted in Scheme 5.

Although several attempts were made to deprotect the benzyl group of **29**, none of the reaction conditions gave the debenzylated product, due to the instability of **29** under these conditions. Thus, it was necessary to remove the benzyl group prior to glycosylation. The reaction of **26** with boron trichloride (BCl_3)[27] proceeded smoothly to give the debenzylated product **31** at over 90% efficiency (Scheme 6). Pioneering works of Kita *et al.* led to the application of the Pummerer reaction to the synthesis of a C−C bond at the α-position of sulfoxides.[28] O'Neil and Hamilton also reported the syntheses of a tetrahydrothienylthymine and other derivatives using TMSOTf as a catalyst under similar reaction conditions.[29] On the basis of these reactions, we designed an alternative synthesis of 4′-thioDMDC which used sulfoxide **32** obtained from *m*-CPBA oxidation of **31**. Compound **32** was treated with silylated N^4-acetylcytosine (3 equiv) and

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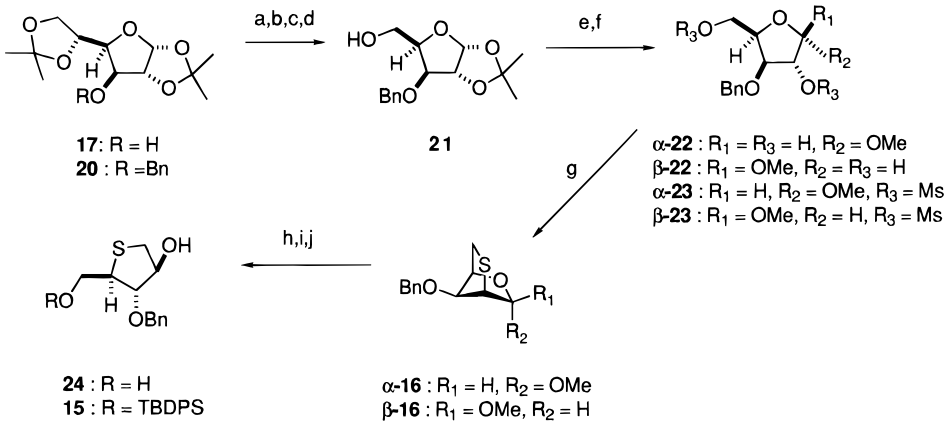
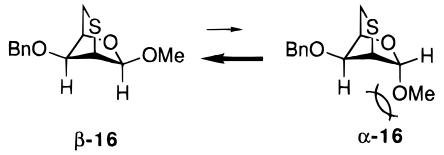
Scheme 3[a]

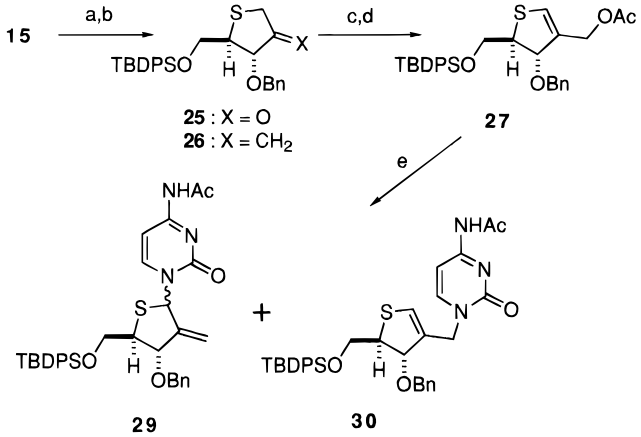


[a] (a) BnBr, NaH, DMF, THF; (b) 2 M HCl, THF; (c) $NaIO_4$, H_2O, MeOH; (d) $NaBH_4$, MeOH, 84% from **17**; (e) 5% HCl/MeOH, 91%; (f) MsCl, pyridine; (g) Na_2S, DMF, 100 °C, 78% (α-anomer) and 73% (*β*-anomer) from **22**; (h) 4 M HCl, THF; (i) $NaBH_4$, MeOH, 90% from **16**; (j) TBDPSCl, imidazole, DMF, 87%.

Scheme 4

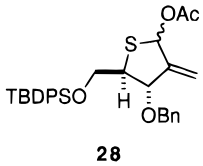


Scheme 5[a]



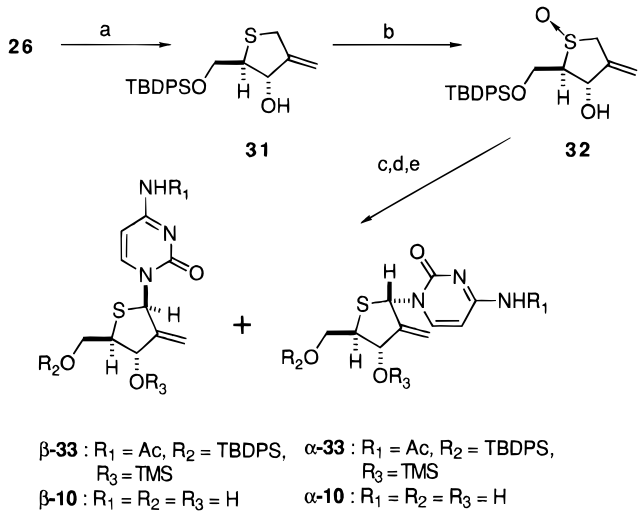
[a] (a) Ac_2O, DMSO; (b) $Ph_3P^+CH_3Br^-$, NaH, *tert*-amyl alcohol, THF, 74% from **15**; (c) *m*-CPBA, CH_2Cl_2, −78 °C; (d) Ac_2O, 110 °C, 68%; (e) silylated N^4-acetylcytosine, TMSOTf, $ClCH_2CH_2Cl$, **29** (25%) and **30** (24%).

Chart 2



TMSOTf (2 equiv) to produce the 4′-thioDMDC derivative α,*β*-**33** in 74% yield (α:*β* = 2.5:1).[30] Formation of allylic-substituted product was negligible. However, *β*-selective formation of **33** remains a problem. Finally, the α,*β*-mixture of **33** was deprotected by tetrabutylammonium fluoride (TBAF) followed by aqueous ammonia in methanol. Pure α- and *β*-anomers of **10** were obtained from the mixture by HPLC (α, 34%; *β*, 13%). α,*β*-Stereochemistry was assessed by NOE experiments of the separated isomers.[30] Comparison of the chemical shift of the H-4′ proton could be more convenient. In general, the resonance of each H-4′ proton in α-**10** was shifted downfield, compared with that of *β*-**10**, due to the deshielding effect of the C2-keto group of the pyrimidine ring.[31] A similar tendency was observed with the other 2′-modified derivatives of 2′-deoxy-4′-thiocytidine described below.

Scheme 6[a]



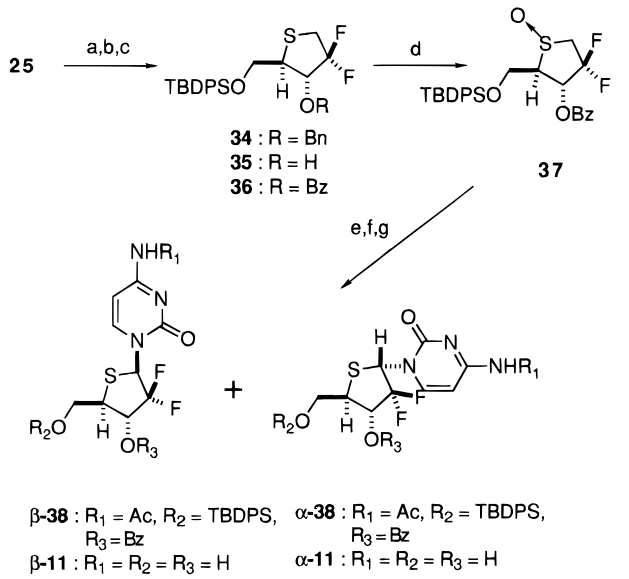
[a] (a) BCl_3, CH_2Cl_2, −78 °C, then MeOH, pyridine, 92%; (b) *m*-CPBA, CH_2Cl_2, −78 °C; (c) silylated N^4-acetylcytosine, TMSOTf, $ClCH_2CH_2Cl$, 0 °C, 74% from **31**; (d) TBAF, THF; (e) aqueous NH_3, MeOH, then HPLC separation, 34% (α-anomer) and 13% (*β*-anomer) from **33**.

Synthesis of 4′-Thiogemcitabine. To clarify the scope and limitations of our new strategy, this method was applied to the synthesis of other 2′-modified 4′-thiocytidines. We aimed at the synthesis of 4′-thiogemcitabine as a second example. Like DMDC, gemcitabine has prominent antineoplastic activities against various solid tumors, as well as leukemias,[3b] and has shown promising antitumor activities in clinical trials.[32] In fact, gemcitabine has been approved for the treatment of non-

(30) The stereochemistry at the anomeric carbon was determined by an NOE analysis of the free nucleoside **10** (minor isomer). It showed 7.1% NOE at the H-3′ proton when irradiated H-6.

(31) (a) Okabe, M.; Sun, R.-C.; Tam, S. Y.-K.; Todaro, L. J.; Coffen, D. L. *J. Org. Chem.* **1988**, *53*, 4780−4786. (b) Brånalt, J.; Kvarnström, I.; Classon, B.; Samuelsson, B. *J. Org. Chem.* **1996**, *61*, 3604−3610.

Scheme 7[a]



[a] (a) DAST, benzene, 0 °C, then room temperature 48%; (b) BCl_3, CH_2Cl_2, −78 °C, then MeOH, pyridine; (c) Bz_2O, Et_3N, DMAP, CH_3CN, 79% from **34**; (d) *m*-CPBA, CH_2Cl_2, −78 °C; (e) silylated N^4-acetylcytosine, TMSOTf, $ClCH_2CH_2Cl$, 0 °C, 57% from **36**; (f) TBAF, THF; (g) aqueous NH_3, MeOH, then HPLC separation, 36% (α-anomer) and 15% (*β*-anomer) from **38**.

small cell lung carcinoma (NSCLC) and pancreatic cancer in Europe.

DAST treatment[33] of ketone **25** produced the 2-deoxy-2,2-difluoro derivative **34** in 48% yield. To circumvent the problem described above, **34** was simultaneously deprotected and benzoylated to give **36** (Scheme 7), which was oxidized to produce **37**. Pummerer-type glycosylation of **37** was used as with 4′-thioDMDC, since the 1-*O*-acetyl derivative of **36** resisted Lewis acid-mediated glycosylation due to the difluoro substituent at the 2-position (data not shown).[34] Reaction of **37** with silylated N^4-acetylcytosine in the presence of TMSOTf resulted in a 57% yield of protected 4′-thiogemcitabine **38** as an anomeric mixture (α:*β* = 2.6:1).[35] Deprotection of **38**, followed by HPLC separation, gave the α- and *β*-derivatives of 4′-thiogemcitabine **11**.

Synthesis of 2′-Deoxy-2′-fluoro-4′-thioarabinosylcytosine (4′-ThioFAC). Monofluorosubstituted nucleosides, especially those with an "*arabino*" configuration, are also quite attractive. In 1970, 2′-deoxy-2′-fluoroarabinosylcytosine (FAC) was synthesized as an araC mimic[36] and was shown to have anti-leukemic activity.[36a] Adenine analogues of this class of nucleosides are also interesting: 2-chloro-9-(2-deoxy-2-fluoroarabinofuranosyl)adenine (Cl-F-araA) has been shown to have potent antitumor activities.[37] On the other hand, a series of 2′-fluoroarabinonucleosides are well-known as potent antiviral nucleosides, *e.g.*, 1-(2-deoxy-2-fluoro-*β*-D-arabinofuranosyl)-5-iodocytosine (FIAC) and 1-(2-deoxy-2-fluoro-*β*-D-arabinofuranosyl)thymine (FMAU).[38] The latter also has antitumor activity against murine leukemia.[39] Therefore, 4′-thio analogues of this class of nucleosides are promising as both antitumor and antiviral antimetabolites. In addition, to study the structure−activity relationship of the 2′-substituents of 2′-deoxy-4′-thiocytidines, a comparison of biological activities between di- and monosubstituted derivatives would be important. Hence, we sought to synthesize 4′-thioFAC as an antitumor candidate.

To prepare 4′-thioFAC, we envisaged the introduction of a fluorine atom at the 2-position of **15** with a retention of stereochemistry. Marquez and his co-workers reported that treatment of 1-(5-*O*-trityl-3-deoxy-4-thio-*threo*-pentofuranosyl)uracil with DAST gave the 2′-fluorinated 4′-thionucleoside with "*threo*" stereochemistry.[40] This result showed that the reaction proceeded *via* an episulfonium intermediate.[40,41] The ring sulfur atom is known to play a role in the case of a 4-thiosugar, which results in the formation of ring-contracted products.[24,41] Indeed, treatment of **15** with DAST produced a 2-deoxy-2-fluoro derivative **40** (69% yield) with an "*arabino*" configuration, which was confirmed after conversion to a free nucleoside (**12**) (*vide infra*). This result shows that the expected episulfonium intermediate **39** was generated *in situ* (Scheme 8). In the case of 4′-thioDMDC and 4′-thiogemcitabine, we have successfully applied a Pummerer-type glycosylation using the sulfoxides as glycosyl donors. However, similar reaction of the sulfoxide **41**, which was obtained from *m*-CPBA oxidation of **40**, resulted in the exclusive formation of the α-isomer (data not shown). To improve the *β*-selective formation of **43**, we planned to use 1-*O*-acetate **42** for the glycosylation reaction. The Pummerer rearrangement of **40** gave **42** in a 54% yield as an anomeric mixture. Compound **42** was treated with the silylated N^4-acetylcytosine in the presence of $SnCl_4$ to give an anomeric mixture of 2′-deoxy-2′-fluoro-4′-thiocytidines **43** in 93% yield (α:*β* = 2.9:1). Stepwise deprotection of **43** [1. BBr_3, 2. NH_4F in MeOH, 3. concentrated NH_4OH in MeOH], followed by reversed-phase column chromatography, gave pure α- and *β*-**12** (α, 43%; *β*, 17%). As in the case described above, the structure of *β*-**12** was unambiguously confirmed by an NOE experiment (Figure 1). NOE between H-2′ and H-4′ clearly indicates the 2′-"up" configuration of the molecule. This is also supported by a potent enhancement of the H-2′ signal with irradiation at the 1′ proton. Similarly, *β*-stereochemistry at the anomeric carbon was confirmed by an NOE of 8.0% at the H-3′ signal when H-6 was irradiated.

(32) (a) Abbruzzese, J. L.; Grunewald, R.; Weeks, E. A.; Gravel, D.; Adams, T.; Nowak, B.; Mineishi, S.; Tarassoff, P.; Satterlee, W.; Raber, M. N.; Plunkett, W. *J. Clin. Oncol.* **1991**, *9*, 491−498. (b) Poplin, E. A.; Corbett, T.; Flaherty, L.; Tarassoff, P.; Redman, B. G.; Valdivieso, M.; Baker, L. *Invest. New Drugs* **1992**, *10*, 165−170. (c) Lund, B.; Kristjansen, P. E. G.; Hansen, H. H. *Cancer Treat. Rev.* **1993**, *19*, 45−55.

(33) An, S.-H.; Bobek, M. *Tetrahedron Lett.* **1986**, *27*, 3219−3222.

(34) Difluoro substituent at the 2′-position potentiates the stability of the glycosidic bond, due to its potent −*I* effect with destabilizing *β*-cation.

(35) The stereochemistry at the 1′-position was determined, after deprotection, by comparison of coupling constants of H-1′ with that of gemcitabine described in ref 3a.

(36) (a) Wright, J. A.; Wilson, D. P.; Fox, J. J. *J. Med. Chem.* **1970**, *13*, 269−272. (b) Reichman, U.; Watanabe, K. A.; Fox, J. J. *Carbohydr. Res.* **1975**, *42*, 233−240.

(37) (a) Secrist, J. A.; Shortnacy, A. T.; Montgomery, J. A. *J. Med. Chem.* **1988**, *31*, 405−410. (b) Montgomery, J. A.; Shortnacy-Fowler, A. T.; Clayton, S. D.; Riordan, J. M.; Secrist, J. A. *J. Med. Chem.* **1992**, *35*, 397−401. (c) Carson, D. A.; Wasson, D. B.; Esparza, L. M.; Carrera, C. J.; Kipps, T. J.; Cottam, H. B. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2970−2974.

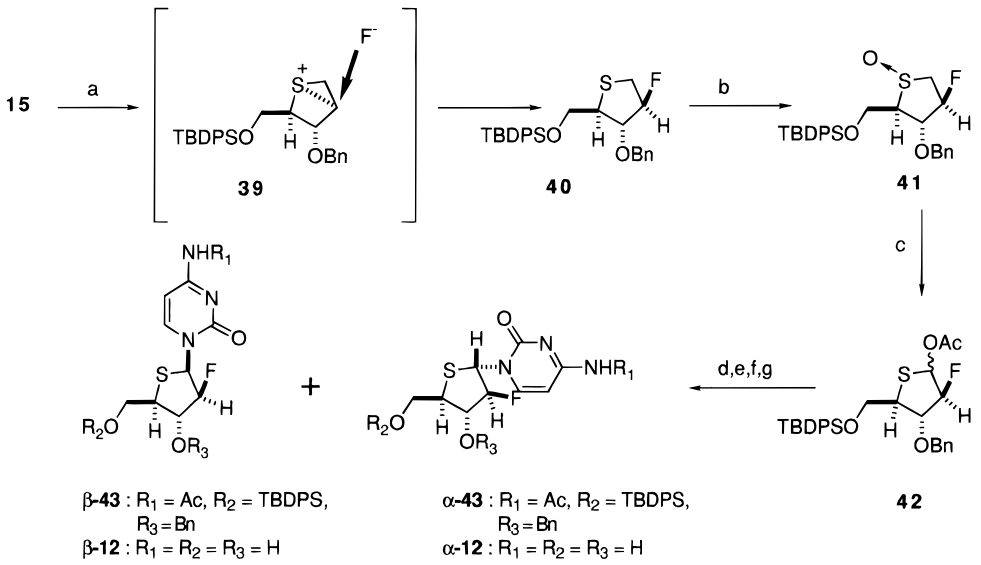
(38) (a) Watanabe, K. A.; Reichmen, U.; Hirota, K.; Lopez, C.; Fox, J. J. *J. Med. Chem.* **1979**, *22*, 21−24. (b) Watanabe, K. A.; Su, T.-L.; Klein, R. S.; Chu, C. K.; Matsuda, A.; Chun, M. W.; Lopez, C.; Fox, J. J. *J. Med. Chem.* **1983**, *26*, 152−156.

(39) Burchenal, J. H.; Chou, T.-C.; Lokys, L.; Smith, R. S.; Watanabe, K. A.; Su, T.-L.; Fox, J. J. *Cancer Res.* **1982**, *42*, 2598−2600.

(40) Jeong, L. S.; Nicklaus, M. C.; George, C.; Marquez, V. E. *Tetrahedron Lett.* **1994,** *35*, 7569−7572.

(41) Hughes, N. A.; Kuhajda, K.-M.; Miljkovic, D. A. *Carbohydr. Res.* **1994**, *257*, 299−304.

Scheme 8[a]



[a] (a) DAST, CH_2Cl_2, −78 °C, 77%; (b) *m*-CPBA, CH_2Cl_2, −78 °C; (c) Ac_2O, 100 °C, 77% from **40**; (d) silylated N^4-acetylcytosine, $SnCl_4$, CH_3CN, 93%; (e) BBr_3, then MeOH, saturated $NaHCO_3$; (f) NH_4F, MeOH, 60 °C; (g) aqueous NH_3, MeOH, then ODS column separation, 43% (α-anomer) and 17% (*β*-anomer) from **43**.

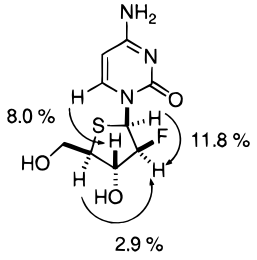
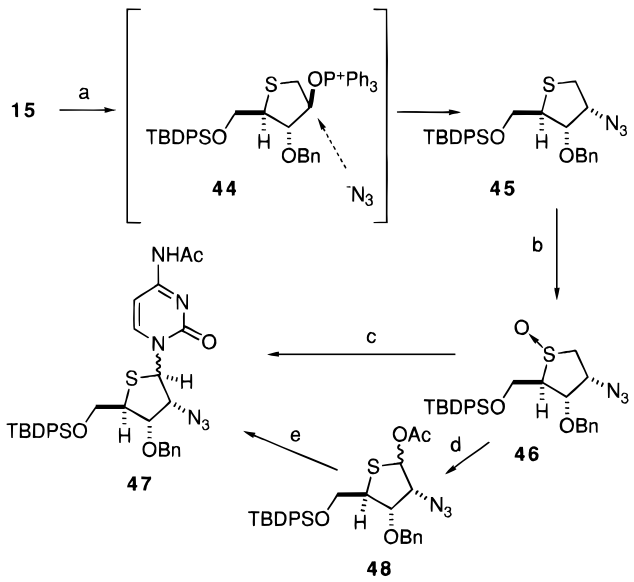


Figure 1. Summary of NOE experiment of *β*-**12**.

Synthesis of 2′-Azido-2′-deoxy-4′-thiocytidine. As a final target of our project, we intended to synthesize 4′-thiocytarazid. Akin to the 2′-deoxycytidine analogues described above, cytarazid, which has an azido group at the 2′-position with an *arabino* configuration, possesses potent antineoplastic activity.[9] Successful introduction of a fluorine substituent with a retention of stereochemistry encouraged us to apply the Mitsunobu reaction to prepare 4′-thiocytarazid. The Mitsunobu reaction of intermediate **15** using diphenylphosphoryl azide (DPPA)[9b] efficiently achieved the introduction of an azido group at the 2-position (80% yield, Scheme 9). The resulting product **45** consisted of only a single stereoisomer. However, we could not determine the stereochemistry at C-2 because **45** was not suitable for an NOE experiment, due to overlapping of the H-2 proton with H-4 and H-5 (see Experimental Section). The difficulty of assigning the stereochemistry forced us to convert **45** to 4′-thionucleoside **47**, the stereochemistry of which we attempted to determine. The Pummerer-type glycosylation of **45** *via* sulfoxide **46** gave an unsatisfactory result, with a low yield of **47**. On the other hand, the usual glycosylation reaction *via* 1-*O*-acetate **48**, as in the case of 4′-thioFAC, gave **47** in 59% yield. In contrast with the examples described above, the α,*β*-ratio of **47** was almost 1:1 in the glycosylation reaction and did not depend on the glycosyl donors (1-*O*-acetate *vs* sulfoxide). However, deprotection of the benzyl groups of **47** and **45** was not successful. Furthermore, **47** was an inseparable mixture of α,*β*-anomers, so that its ^1H NMR spectrum was rather complicated and not suitable for an NOE experiment.

Scheme 9[a]

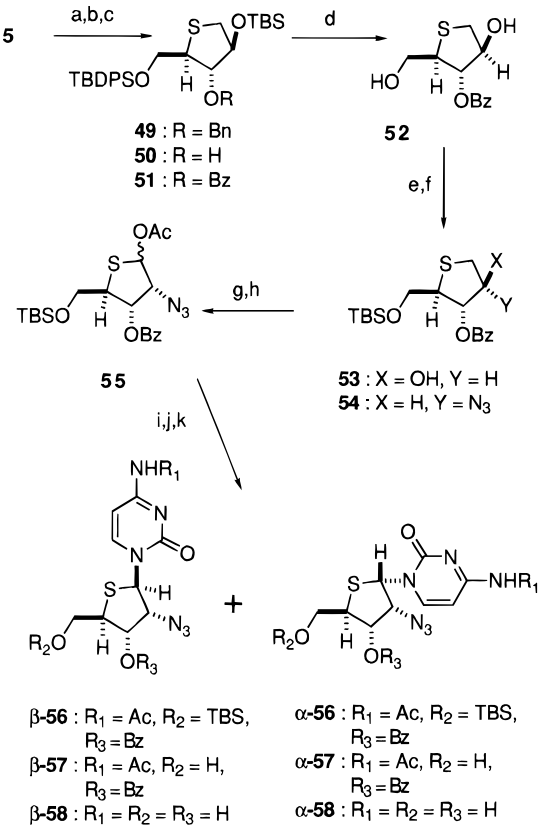


[a] (a) DPPA, DEAD, Ph_3P, THF, 80%; (b) *m*-CPBA, CH_2Cl_2, −78 °C; (c) silylated N^4-acetylcytosine, TMSOTf, $ClCH_2CH_2Cl$, 0 °C, 20% from **45**; (d) Ac_2O, 100 °C; 67% from **45**; (e) silylated N^4-acetylcytosine, TMSOTf, $ClCH_2CH_2Cl$, 0 °C, 59% from **48**.

Failing to obtain a free 4′-thionucleoside, we changed the substrate of the Mitsunobu reaction, in that the protecting group at the 3-position was changed from benzyl to an appropriate group. As shown in Scheme 10, 3-benzoylated derivative **53** was prepared from **15** in five steps. Bis-silyl-protected **49** was debenzylated and subsequently benzoylated to give **51**. The silyl protecting group of **51** was removed with ammonium fluoride in MeOH,[42] and selective protection of the primary hydroxyl group of **52** gave **53** (62% from **15**). The Mitsunobu reaction of **53** gave a single stereoisomer (**54**), as in the case of **45**, in 83% yield. Surprisingly, the results of an NOE experiment of **54** revealed that the products had a

(42) Zhang, W.; Robins, M. J. *Tetrahedron Lett.* **1992**, *33*, 1177−1180.

Scheme 10[a]



[a] (a) TBSOTf, pyridine, CH_2Cl_2, (b) BCl_3, CH_2Cl_2, −78 °C, then MeOH, pyridine; (c) Bz_2O, Et_3N, DMAP, CH_3CN, 93% from **15**; (d) NH_4F·HF, MeOH, 89%; (e) TBSCl, imidazole, DMF, 80%; (f) DPPA, DEAD, Ph_3P, THF, 83%; (g) *m*-CPBA, CH_2Cl_2, −78 °C; (h) Ac_2O, 100 °C, 53%; (i) silylated N^4-acetylcytosine, TMSOTf, $ClCH_2CH_2Cl$, 0 °C, 47%; (j) NH_4F·HF, MeOH, then silica gel column separation, 43% (α-anomer) and 45% (*β*-anomer); (k) aqueous NH_3, MeOH, 87% (α-anomer) and 97% (*β*-anomer).

ribo, rather than an *arabino*, configuration.[43] Compound **54** was further converted to 1-*O*-acetoxy derivative **55**, by Pummerer rearrangement, which was in turn subjected to Lewis acid-mediated glycosylation to give **56** (efficiency: 47%) as a 1:1 mixture of α,*β*-anomers.

Comparison of the ^{1}H NMR spectra of 3-benzoyl **54** and 3-benzyl **45** (see Experimental Section) confirms their *ribo* configurations at the 2-position. This strongly suggests that the Mitsunobu reaction occurred without participation of an episulfonium ion in both cases. This is also implied by the results of the α/*β* ratio of the glycosylation reaction and the resistance to the deprotection of benzyl groups. It is noteworthy that the mode of the Mitsunobu reaction is opposite that of a DAST reaction: in the DAST reaction of **15**, nucleophilic attack of the fluorine atom to the 2-position is rate-limiting due to its weak nucleophilicity. Thus, the contribution of the neighboring sulfur atom, as reported in the literature,[40] lowered the transition energy even though the intermediate episulfonium ion is highly strained. In sharp contrast, nucleophilic attack of the azide anion is faster than that of the fluoride anion and is even faster than the attack of the sulfur atom. Thus, the contribution by way of the strained episulfonium ion is not significant in the Mitsunobu reaction of **53**. As a consequence, the reaction proceeds with inversion.

(43) The stereochemistry of **54** was determined by an NOE analysis: a 1.5% NOE at H-5a was observed when H-2 was irradiated.

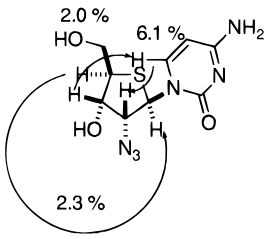


Figure 2. Summary of NOE experiment of *β*-**58**.

Table 1. Antineoplastic Activities of 2′-Modified 4′-Thionucleosides

comp (anomer)	2′-substituent	antineoplastic activities IC_{50} (*μ*g/mL) CCRF-HSB-2[a]	KB cells[b]
10 (α)	=CH_2	>10	ND[c]
10 (*β*)	=CH_2	0.0091	0.12
11 (α)	F_2	>10	ND
11 (*β*)	F_2	1.5	17
12 (α)	F (arabino)	>10	ND
12 (*β*)	F (arabino)	0.051	0.015
58 (α)	N_3 (ribo)	>10	ND
58 (*β*)	N_3 (ribo)	8.6	82
araC		0.052	0.26
DMDC		0.022	0.44

[a] MTT assay.[44] [b] Dye uptake method.[44] [c] Not determined.

The 5′-*O*-silyl group of the resulting 4′-thionucleoside **56** was deprotected with ammonium hydrogen fluoride in MeOH. The mixture of α- and *β*-isomers of **57** could easily be separated by silica gel column chromatography (α, 43%; *β*, 45%), and the separated anomers were converted to their free nucleosides **58** with aqueous ammonia (α, 87%; *β*, 97%; from α- and *β*-**57**, respectively). The NOE experiment of *β*-**53** supported its structure, *e.g.*, *β*-stereochemistry at the 1′-position and a *ribo* configuration (Figure 2).

Biological Aspects of 2′-Modified 4′-Thionucleosides. Antineoplastic activities of 2′-modified 2′-deoxy-4′-thiocytidines were evaluated[44] and are summarized in Table 1. All of the α-anomers of 2′-deoxy-4′-thiocytidine derivatives were inactive against human T-cell leukemia CCRF-HSB-2 cells. In contrast, the *β*-4′-thiocytidines showed significant cytotoxicity against the same cell line. *β*-4′-ThioDMDC (**10**) and *β*-4′-thioFAC (**12**) had especially potent antileukemic activities. These results suggest that the *β*-configuration of 4′-thiocytidines is essential for their cytotoxicity. Both 4′-thioDMDC (**10**) and 4′-thioFAC (**12**) were also effective against human solid tumor KB cells. The cytotoxicity of 4′-thioFAC (**12**) is particularly noteworthy, and even 4′-thioDMDC (**10**) has a greater activity against the same cell line than does the parental DMDC (**5**) (IC_{50} = 0.44 *μ*g/mL). In contrast, *β*-4′-thiogemcitabine (**11**) and *β*-2′-azido-4′-thiocytidine (**58**), the latter of which has an unexpected *ribo* configuration, were rather inactive. This result shows that the *arabino* configuration is also important for cytotoxicity, as in the usual 4′-oxy counterparts. It is interesting that 4′-thioDMDC (**10**) has potent antineoplastic activity, while 4′-thiogemcitabine (**11**) does not, even though both of the parent compounds DMDC (**5**) and gemcitabine (**2**) are highly active.[3,8] One plausible explanation for this difference is that the efficacy of phosphorylation of these analogues by deoxycytidine kinase, a key enzyme which

(44) Yoshimura, Y.; Kano, F.; Miyazaki, S.; Ashida, N.; Sakata, S.; Haraguchi, K.; Itoh, Y.; Tanaka, H.; Miyasaka, T. *Nucleosides Nucleotides* **1996**, *15*, 305−324.

converts 2′-deoxycytidine analogues such as araC,[2] DMDC,[8] CNDAC,[7] and gemcitabine[3] to their corresponding monophosphates, is different in the 4′-thiocytidine derivatives. The potent antitumor activities of 4′-thioDMDC (**10**) and 4′-thioFAC (**12**) were further confirmed by *in vivo* assay.[45,46]

In conclusion, we have designed and synthesized a novel class of 2′-modified 2′-deoxy-4′-thiocytidines which have methylene, azido, and both di- and monofluoro groups at the 2′-position. To prepare these derivatives, we developed a new synthetic route involving 2′-modified 2′-deoxy-4′-thionucleosides, starting from D-glucose. This should be a useful and general method for the synthesis of 2′-modified 2′-deoxy-4′-thionucleosides. Among the 2′-deoxy-4′-thiocytidines synthesized, 4′-thioDMDC and 4′-thioFAC were shown to have potent antineoplastic activities. Further evaluation of these compounds is underway, and the results will be reported elsewhere.

Experimental Section

General. 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 TMS as internal standard. Mass spectra were obtained by fast atom bombardment (FAB) mode. Silica gel for chromatography was Merck kieselgel 60.

3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-*xylo*-pentofuranose (21). A mixture of 1,2:5,6-di-*O*-isopropylideneglucose (**17**) (20.0 g, 76.9 mmol) and added 60% NaH (3.69 g, 92.2 mmol) in THF (150 mL) was stirred for 30 min. DMF (50 mL) and benzyl bromide (13.7 mL, 115 mmol) were added to this mixture, and the whole was stirred at room temperature for 2 h. The reaction was quenched by the addition of AcOH. The solvent was evaporated under reduced pressure. After water workup, the concentrated residue was purified by column chromatography over silica gel (7.9 × 14 cm; 2−10% AcOEt in hexane) to give **18** (26.1 g, 97%) as an oil. A mixture of 3-benzyl derivative **20** (26.1 g, 74.5 mmol) in THF (200 mL) and aqueous HCl (2 M, 200 mL) was stirred at room temperature overnight. After neutralization by $NaHCO_3$, precipitates were removed by filtration. The filtrate was concentrated under reduced pressure. The residue was extracted with $CHCl_3$, dried (Na_2SO_4), and concentrated. The residue was dissolved in MeOH (100 mL). An aqueous solution of $NaIO_4$ (17.5 g) in H_2O (100 mL) was added to this mixture. After being stirred for 30 min at room temperature, the reaction was quenched by glycerin (5 mL). Precipitates were removed by filtration, and the filtrate was concentrated. The residue was dissolved in MeOH (100 mL), and $NaBH_4$ (2.82 g) was added at 0 °C. The mixture was stirred at 0 °C for 30 min and neutralized by AcOH. The solvent was removed under reduced pressure. The residue was partitioned between AcOEt and H_2O. The organic phase was washed with brine and dried (Na_2SO_4). After the filtrate was concentrated under reduced pressure, the residue was purified by column chromatography over silica gel (3.7 × 17 cm; 33−50% of AcOEt in hexane) to give **21** (17.6 g, 84%): ^{1}H NMR ($CDCl_3$) δ 7.39−7.30 (5H, m), 5.99 (1H, d, J = 3.9 Hz), 4.72 (1H, d, J = 11.7 Hz), 4.64 (1H, d, J = 11.7 Hz), 4.28 (1H, dt, J = 3.4, 4.9 Hz), 4.02 (1H, d, J = 3.4 Hz), 3.94 (1H, ddd, J = 3.9, 4.9, 12.2 Hz), 3.85 (1H, ddd, J = 4.9, 8.8, 12.2 Hz), 2.11 (1H, dd, J = 3.9, 8.8 Hz), 1.49, 1.36 (each 3H, s). Anal. Calcd for $C_{15}H_{20}O_5 \cdot 0.5H_2O$: C, 62.27; H, 7.32. Found: C, 62.02; H, 7.20.

Methyl 3-*O*-Benzyl-α,β-D-*xylo*-pentofuranoside (22). Compound **21** (17.6 g, 62.7 mmol) was dissolved in 5% HCl/MeOH (150 mL), and the mixture was stirred at room temperature for 3 h. The reaction was quenched with $NaHCO_3$, and precipitates were removed by filtration. The filtrate was concentrated. The residue was purified by column chromatography over silica gel (8.3 × 12 cm; 0−1−2−4% MeOH in $CHCl_3$). The less polar fraction contained β-**22** (8.04 g), and the more polar fraction contained α-**22** (6.49 g, total 91%). **Data for α-22:** ^{1}H NMR ($CDCl_3$) δ 7.38−7.29 (5H, m), 4.80 (1H, d, J = 2.4 Hz), 4.72 (1H, d, J = 11.7 Hz), 4.59 (1H, d, J = 11.7 Hz), 4.35 (1H, ddd, J = 4.4, 4.9, 6.8 Hz), 4.31 (1H, ddd, J = 2.4, 3.9, 4.8 Hz), 4.10 (1H, dd, J = 3.9, 6.8 Hz), 3.80 (1H, ddd, J = 4.9, 6.6, 11.7 Hz), 3.76 (1H, ddd, J = 4.4, 6.6, 11.7 Hz), 3.43 (3H, s), 2.56 (1H, t, J = 6.6 Hz, D_2O exchangeable), 2.27 (1H, d, J = 4.8 Hz, D_2O exchangeable); EI-MS m/z 254 (M^+). Anal. Calcd for $C_{13}H_{18}O_5 \cdot 0.25H_2O$: C, 60.34; H, 7.21. Found: C, 60.08; H, 7.49. **Data for β-22:** mp 103−104 °C (crystallized from hexane−AcOEt); ^{1}H NMR ($CDCl_3$) δ 7.39−7.29 (5H, m), 4.96 (1H, d, J = 4.9 Hz), 4.85 (1H, d, J = 12.0 Hz), 4.61 (1H, d, J = 12.0 Hz), 4.29 (1H, dt, J = 4.9, 8.8 Hz), 4.26−4.23 (1H, m), 4.14 (1H, dd, J = 4.9, 6.8 Hz), 3.84 (1H, ddd, J = 3.9, 4.9, 12.7 Hz), 3.77 (1H, ddd, J = 4.4, 8.3, 12.7 Hz), 3.47 (3H, s), 2.66 (d, 1H, J = 8.8 Hz, D_2O exchangeable), 2.41 (1H, dd, J = 4.9, 8.3 Hz, D_2O exchangeable); EI-MS m/z 254 (M^+). Anal. Calcd for $C_{13}H_{18}O_5$: C, 61.41; H, 7.13. Found: C, 61.57; H, 7.43.

Methyl 2,5-Anhydro-3-*O*-benzyl-2,5-dideoxy-2-thio-α-D-*arabino*-pentofuranoside (α-16). A mixture of MsCl (5.60 mL, 72.4 mmol) and α-**22** (6.13 g, 24.1 mmol) in pyridine (60 mL) was stirred at room temperature for 1 h. H_2O was added to the mixture, and the solvent was removed under reduced pressure. The residue was partitioned between AcOEt and H_2O. The organic phase was washed with saturated $NaHCO_3$ and brine and then dried (Na_2SO_4). After concentration, the residue was dissolved in DMF (80 mL). To the mixture was added sodium sulfide (8.68 g, 36.2 mmol), and the mixture was kept at 100 °C for 1 h. After the solvent was removed under reduced pressure, the residue was partitioned between AcOEt and H_2O. The organic phase was washed with H_2O (×3) and brine and then dried (Na_2SO_4). After the solvent was removed under reduced pressure, the residue was purified by column chromatography over silica gel (4.7 × 16 cm; 10−20% AcOEt in hexane) to give α-**16** (4.75 g, 78%) as an oil: ^{1}H NMR ($CDCl_3$) δ 7.39−7.30 (5H, m), 5.13 (1H, d, J = 2.4 Hz), 4.66 (1H, d, J = 11.7 Hz), 4.53 (1H, d, J = 11.7 Hz), 4.36−4.35 (1H, br m), 4.29 (1H, t, J = 2.4 Hz), 3.51 (1H, t, J = 2.4 Hz), 3.47 (3H, s), 3.04 (1H, dd, J = 2.2, 10.5 Hz), 2.95 (1H, dd, J = 1.2, 10.5 Hz); EI-MS m/z 252 (M^+). Anal. Calcd for $C_{13}H_{16}O_3S \cdot 0.25H_2O$: C, 60.80; H, 6.48. Found: C, 60.68; H, 6.34.

Methyl 2,5-Anhydro-3-*O*-benzyl-2,5-dideoxy-2-thio-β-D-*arabino*-pentofuranoside (β-16). Compound β-**16** was synthesized as described for the preparation of α-**16**. After purification by silica gel column chromatography, β-**16** (73%) was obtained as an oil: ^{1}H NMR ($CDCl_3$) δ 7.36−7.29 (5H, m), 4.89 (1H, s), 4.62 (1H, d, J = 11.7 Hz), 4.52−4.48 (2H, m), 4.37−4.36 (1H, m), 3.34 (4H, s), 3.04 (1H, dd, J = 2.0, 10.3 Hz), 2.77 (1H, dd, J = 1.5, 10.3 Hz); EI-MS m/z 252 (M^+). Anal. Calcd for $C_{13}H_{16}O_3S \cdot 0.2H_2O$: C, 61.01; H, 6.46. Found: C, 61.05; H, 6.34.

1,4-Anhydro-3-*O*-benzyl-4-thio-D-arabitol (24). A solution of **16** (9.50 g, 1:1 mixture of α- and β-anomers, 37.7 mmol) in THF (200 mL) and aqueous HCl (4 M, 100 mL) was stirred at room temperature for 1 h. The reaction was quenched with $NaHCO_3$. Precipitates were removed by filtration, and THF was removed under reduced pressure. The whole was extracted with $CHCl_3$ (×2) and dried (Na_2SO_4). After concentration, the residue was dissolved in MeOH (150 mL), and $NaBH_4$ (1.43 g, 37.7 mmol) was added at 0 °C. After being stirred at 0°C for 45 min, the reaction was quenched by AcOH. After concentration, the residue was partitioned between $CHCl_3$ and H_2O. The water phase was extracted with $CHCl_3$ (×2), and the combined organic phase was washed with brine and dried (Na_2SO_4). After the solvent was removed under reduced pressure, the residue was purified by column chromatography over silica gel (6.3 × 13 cm; 20−33−50% AcOEt in hexane) to give **24** (8.18 g, 90%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 7.38−

(45) The biological results of 4′-thioDMDC have been appeared: Miura, S.; Tanaka, M.; Yoshimura, Y.; Satoh, H.; Sakata, S.; Machida, H.; Matsuda, A.; Sasaki, T. *Biol. Pharm. Bull.* **1996**, *19*, 1311−1315.

(46) 4′-ThioFAC administered i.p. daily for 8 consecutive days increased the life span of mice bearing P388 leukemia, showing T/C values of 163% and 205% at doses of 3 and 10 mg/kg/day, respectively. In the same assay, araC showed T/C values of 184% and 200% at doses of 3 and 10 mg/kg/day, respectively.

7.27 (5H, m), 4.64 (2H, s), 4.38 (1H, dt, J = 2.9, 4.4 Hz), 3.96 (1H, t, J = 2.9 Hz), 3.78 (1H, dd, J = 2.9, 11.7 Hz), 3.66 (1H, dd, J = 3.9, 11.7 Hz), 3.65 (1H, br, D_2O exchangeable), 3.60 (1H, dt, J = 2.9, 3.9 Hz), 3.21 (1H, dd, J = 4.4, 11.2 Hz), 2.90 (1H, dd, J = 2.9, 11.2 Hz), 2.61 (1H, br, D_2O exchangeable); FAB-MS m/z 241 (M^+ + H). Anal. Calcd for $C_{12}H_{16}O_3S\cdot 0.2H_2O$: C, 59.09; H, 6.78. Found: C, 59.03; H, 6.88.

1,4-Anhydro-3-*O*-benzyl-5-*O*-(*tert*-butyldiphenylsilyl)-4-thio-D-arabitol (15). TBDPSCl (6.22 mL, 23.9 mmol) was added to a mixture of **24** (5.47 g, 22.8 mmol) and imidazole (1.63 g, 23.9 mmol) in DMF (90 mL) at 0 °C. The mixture was stirred at room temperature overnight. H_2O was added to the mixture, and the whole was stirred for 10 min. The solvent was removed under reduced pressure. The residue was partitioned between AcOEt and H_2O. The organic phase was washed with H_2O (×2) and brine and then dried (Na_2SO_4). After the solvent was removed under reduced pressure, the residue was purified by colum chromatography over silica gel (6.3 × 11 cm). The eluate with 2−4−10% AcOEt in hexane was collected, and the solvent was removed under reduced pressure to leave crystalline **15** (9.55 g, 87%): mp 46−47 °C; ^{1}H NMR ($CDCl_3$) δ 7.72−7.64 (4H, m), 7.46−7.26 (11H, m), 4.59 (1H, d, J = 12.0 Hz), 4.54 (1H, d, J = 12.0 Hz), 4.40 (1H, brt, J = 2.4 Hz), 4.00 (1H, t, J = 2.0 Hz), 3.74 (1H, dd, J = 4.4, 10.7 Hz), 3.69 (1H, dd, J = 5.4, 10.7 Hz), 3.56 (1H, dt, J = 2.0, 4.9 Hz), 3.24−3.20 (2H, m, 1H was D_2O exchangeable), 2.90 (1H, dd, J = 2.4, 11.2 Hz), 1.07 (9H, s); FAB-MS m/z 479 (M^+ + H). Anal. Calcd for $C_{28}H_{34}O_3SSi$: C, 70.25; H, 7.16. Found: C, 69.91; H, 7.34.

1,4-Anhydro-3-*O*-benzyl-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2-*C*-methylene-4-thio-D-*erythro*-pentitol (26). A mixture of **15** (21.4 g 44.9 mmol) and Ac_2O (120 mL) in DMSO (240 mL) was stirred at room temperature overnight. After dilution with H_2O, the whole was extracted by ether. The organic phase was washed with H_2O (×3), saturated $NaHCO_3$ (×2), and brine and then dried (Na_2SO_4). The solvent was removed under reduced pressure, and the residue was passed through a short silica gel column (eluate: 17% AcOEt in hexane), giving **25**. A mixture of methyltriphenylphosphonium bromide (49.6 g, 139 mmol), amyl alcohol (16.6 mL), and 60% NaH (6.10 g, 153 mmol) in THF (300 mL) was stirred at room temperature for 2 h. To this ylide solution was added a THF solution (120 mL) of **25** (20 g, 42.0 mmol) slowly at 0 °C, and then the solution was stirred at room temperature overnight. The reaction was quenched with 1 M NH_4Cl, and the whole was extracted with AcOEt (×2). The organic phase was washed with H_2O and brine and then dried (Na_2SO_4). The solvent was removed under reduced pressure, and the residue was purified by column chromatography over silica gel (9% AcOEt in hexane) to give **26** (14.7 g, 74%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 7.60−7.55 (4H, m), 7.43−7.27 (11H, m), 5.13, 4.94 (each 1H, s), 4.64 (1H, d, J = 12.7 Hz), 4.44 (1H, d, J = 12.7Hz), 4.35 (1H, s), 3.62−3.31 (4H, m), 3.21 (1H, d, J = 12.7 Hz), 0.99 (9H, s); FAB-MS m/z 475 (M^+ + H). Anal. Calcd for $C_{29}H_{34}O_2SSi$: C, 73.37; H, 7.22. Found: C, 73.12; H, 7.32.

(2*R*,3*S*)-3-(Acetoxymethyl)-4-(benzyloxy)-5-(((*tert*-butyldiphenylsilyl)oxy)methyl)-2,3-dihydrothiophene (27). To a solution of **26** (115 mg, 0.242 mmol) in CH_2Cl_2 (4 mL) at −78 °C was dropwise added 80% *m*-CPBA (52 mg, 0.242 mmol) in CH_2Cl_2 (3 mL). The mixture was stirred at the same temperature for 20 min. The reaction was quenched with saturated $NaHCO_3$, and the whole was extracted with $CHCl_3$ (×2). The organic phase was washed with a 10% sodium thiosulfate solution, saturated $NaHCO_3$ (×2), and brine and then dried (Na_2SO_4). The solvent was removed under reduced pressure, and the residue was dissolved in Ac_2O (4 mL). The mixture was kept at 110 °C for 1.5 h and concentrared. The residual Ac_2O was removed by codistillation with toluene (×3). The residue was partitioned between AcOEt and H_2O. The organic phase was washed with saturated $NaHCO_3$ (×2) and brine and then dried (Na_2SO_4). After the solvent was removed under reduced pressure, the residue was purified by column chromatography over silica gel (1.7 × 11.5 cm; 2−4% AcOEt in hexane) to give **27** (88 mg, 68%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 7.67−7.64 (4H, m), 7.46−7.25 (11H, m), 6.41 (1H, s), 4.87 (1H, d, J = 1.2 Hz), 4.66 (1H, d, J = 11.7 Hz), 4.65 (2H, s), 4.48 (1H, d, J = 11.7 Hz), 3.85 (1H, ddd, J = 1.2, 6.1, 9.0 Hz), 3.76 (1H, dd, J = 6.1, 10.3 Hz), 3.55 (1H, dd, J = 9.0, 10.3 Hz), 1.97 (3H, s), 1.07 (9H, s); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 170.62, 138.10, 135.55 (2C), 135.52 (2C), 133.03, 130.78, 129.84 (2C), 128.90, 128.43, 128.39, 128.34 (2C), 127.75 (4C), 127.69, 127.64, 85.97, 70.18, 64.84, 61.02, 54.63, 26.80 (3C), 20.83, 19.23; FAB-MS m/z 471 (M^+ − OAc − 2H). Anal. Calcd for $C_{31}H_{36}O_4SSi$: C, 69.89; H, 6.81. Found: C, 69.77; H, 6.98.

4-Acetyl-1-(3-*O*-benzyl-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2-*C*-methylene-4-thio-α,β-D-*erythro*-pentofuranosyl)cytosine (29) and (2*R*,3*S*)-3-((4-Acetylcytosin-1-yl)-methyl)-4-(benzyloxy)-5-(((*tert*-butyldiphenylsilyl)-oxy)methyl)-2,3-dihydrothiophene (30). Compound **26** (1.14 g, 2.40 mmol) was converted to **27** as described above. The obtained **27** was subjected to the following glycosylation reaction without purification: A mixture of **27**, silylated N^4-acetylcytosine (4.80 mmol), and TMSOTf (0.464 mL, 2.40 mmol) in $ClCH_2CH_2Cl$ (20 mL) was stirred at 0 °C for 30 min. The reaction was quenched by the addition of saturated $NaHCO_3$. The precipitated material was removed by suction. The filtrate was extracted with $CHCl_3$ (×3), which was washed with brine, then dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography over silica gel (0−1% MeOH in $CHCl_3$) to give less polar **29** (an amorphous foam of exclusive α-isomer; 369 mg, 25% from **26**) and more polar **30** (an amorphous foam; 356 mg, 24% from **26**). **Data for 29 (α-anomer):** UV (MeOH) λ_{max} 303, 249, 221 nm; UV (MeOH, H^+) λ_{max} 315, 222 nm;^{1}H NMR ($CDCl_3$) δ 9.06 (1H, br, D_2O exchangeable), 8.50 (1H, d, J = 7.8 Hz), 7.71−7.21 (16H, m), 6.74 (1H, s), 5.69 (1H, s), 5.19 (1H, s), 4.43 (1H, d, J = 11.7 Hz), 4.40 (1H, s), 4.29 (1H, d, J = 11.7 Hz), 3.77−3.71 (1H, m), 3.66 (1H, dd, J = 5.1, 10.3 Hz), 3.40 (1H, t, J = 10.3 Hz), 2.22 (3H, s), 1.01 (9H, s); FAB-MS m/z 626 (M^+ + H). Anal. Calcd for $C_{35}H_{39}N_3O_4SSi$: C, 67.17; H, 6.28; N, 6.71. Found: C, 67.03; H, 6.57; N, 6.33. **Data for 30:** UV (MeOH) λ_{max} 302, 248, 221 nm; UV (MeOH, H^+) λ_{max} 317, 221 nm; ^{1}H NMR ($CDCl_3$) δ 9.06 (1H, br, D_2O exchangeable), 7.65−7.16 (17H, m), 6.44 (1H, s), 4.74 (1H, s), 4.61 (1H, d, J = 11.7 Hz), 4.57 (1H, d, J = 14.2 Hz), 4.45 (1H, d, J = 14.2 Hz), 4.38 (1H, d, J = 11.7 Hz), 3.84−3.81 (1H, m), 3.73 (1H, dd, J = 6.4, 10.3 Hz), 3.49 (1H, t, J = 10.3 Hz), 2.20 (3H, s), 1.06 (9H, s); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 170.88, 162.66, 155.60, 148.33, 137.72, 135.52 (3C), 135.49 (3C), 132.93, 132.86, 131.99, 131.93, 131.89, 129.94, 129.91, 128.48, 128.02, 127.83 (3C), 127.36, 96.63, 86.43, 70.25, 64.80, 54.62, 48.08, 26.83 (3C), 24.78, 19.21; FAB-MS m/z 626 (M^+ + H). Anal. Calcd for $C_{35}H_{39}N_3O_4SSi\cdot H_2O$: C, 66.22; H, 6.35; N, 6.62. Found: C, 66.42; H, 6.46; N, 6.67.

1,4-Anhydro-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2-*C*-methylene-4-thio-D-*erythro*-pentitol (31). A solution of BCl_3 (7.76 mL of a 1 M CH_2Cl_2 solution, 7.76 mmol) was added slowly to a solution of **26** (1.84 g, 3.88 mmol) in CH_2Cl_2 (30 mL) at −78 °C. After being stirred at the same temperature for 1 h, pyridine (10 mL) and MeOH (20 mL) were added to the mixture. The mixture was stirred at −78 °C for 1 h and allowed to warm to room temperature. The solvent was removed under reduced pressure. After the residue was partitioned between $CHCl_3$ and H_2O, the organic phase was washed with aqueous HCl (0.5 M, ×2), saturated $NaHCO_3$, and brine and then dried (Na_2SO_4). The solvent was removed under reduced pressure. The residue was purified by column chromatography over silica gel (3.7 × 11 cm; 5−10% AcOEt in hexane) to give **31** (1.34 g, 92%): ^{1}H NMR ($CDCl_3$) δ 7.67−7.64 (4H, m), 7.46−7.37 (6H, m), 5.19 (1H, d, J = 1.0 Hz), 5.05 (1H, d, J = 1.5 Hz), 4.57 (1H, br dd, J = 4.9, 5.4 Hz), 3.79 (1H, dd, J = 5.4, 10.3 Hz), 3.64 (1H, dd, J = 9.3, 10.3 Hz), 3.52 (1H, dd, J = 1.0, 13.7 Hz), 3.45 (1H, dd, J = 1.5, 13.7 Hz), 3.31 (1H, dt, J = 5.4, 9.3 Hz), 2.61 (1H, d, J = 4.9 Hz, D_2O exchangeable), 1.07 (9H, s); FAB-MS m/z 385 (M^+ + H). Anal. Calcd for $C_{22}H_{28}O_2SSi\cdot 0.75H_2O$: C, 66.37; H, 7.47. Found: C, 66.56; H, 7.24.

4-Acetyl-1-(5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2-*C*-methylene-3-*O*-(trimethylsilyl)-4-thio-α(and β)-D-*erythro*-pentofuranosyl)cytosine (α,β-33). A solution of *m*-CPBA (80%, 509 mg, 2.36 mmol) in CH_2Cl_2 (15 mL) was added

dropwise to a solution of **31** (906 mg, 2.36 mmol) in CH_2Cl_2 (30 mL) at −78 °C. The mixture was stirred at the same temperature for 30 min. The reaction was quenched with 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 then dried (Na_2SO_4). The filtrate was concentrated to leave the crude sulfoxide **32**, which was dissolved in $ClCH_2CH_2Cl$ (35 mL). A mixture of silylated N^4-acetylcytosine (7.08 mmol) and TMSOTf (0.925 mL, 4.48 mmol) in $ClCH_2CH_2Cl$ (10 mL) was added to the solution of **32** at 0 °C. The mixture was stirred at the same temperature for 30 min. The reaction was quenched by the addition of saturated $NaHCO_3$. The precipitated material was removed by suction. The filtrate was extracted with $CHCl_3$ (×3). The organic phase was washed with brine, then dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography over silica gel (0−1% MeOH in $CHCl_3$) to give **33** (1.06 g, 74%) as an amorphous foam: UV (MeOH) λ_{max} 304, 249 nm; ^{1}H NMR ($CDCl_3$) δ 8.72 (1H, br, D_2O exchangeable), 8.37 (0.74H, d, J = 7.3 Hz), 7.89 (0.26H, d, J = 7.8 Hz), 7.68−7.63 (4H, m), 7.46−7.34 (7H, m), 6.90 (0.26H, s), 6.75 (0.74H, s), 5.29, 5.22 (each 0.74H, s), 5.15, 4.99 (each 0.26H, s), 4.71 (0.26H, d, J = 4.9 Hz), 4.65 (0.74H, d, J = 3.4 Hz), 3.78 (0.26H, dd, J = 4.9, 10.7 Hz), 3.68 (0.74H, dd, J = 4.9, 9.8 Hz), 3.64 (0.26H, dd, J = 6.8, 10.7 Hz), 3.55 (0.74H, dd, J = 8.3, 9.8 Hz), 3.50 (0.74H, ddd, J = 3.4, 4.9, 8.3 Hz), 3.33 (0.26H, dt, J = 4.9, 6.8 Hz), 2.24, 2.23 (total 3H, s), 1.10, 1.08 (total 9H, s), 0.12, 0.10 (total 9H, s); FAB-MS m/z 608 (M^+ + H). Anal. Calcd for $C_{31}H_{41}N_3O_4SSi_2$: C, 61.25; H, 6.80; N, 6.91. Found: C, 60.90; H, 7.08; N, 6.60.

1-(2-Deoxy-2-*C*-methylene-4-thio-α(and *β*)-D-*erythro*-pentofuranosyl)cytosine (4′-ThioDMDC, α,*β*-10). A mixture of **33** (238 mg, 0.391 mmol) and TBAF (0.78 mL of 1 M THF solution, 0.78 mmol) was stirred at room temperature for 15 min. After concentration, the residue was passed through a silica gel column. The eluate with 9% MeOH in $CHCl_3$ was collected and concentrated. The residue was dissolved in MeOH (5 mL) and aqueous ammonia (5 mL). The mixture was stirred at room temperature overnight. After the solvent was removed under reduced pressure, the residue was purified by HPLC (Develosil ODS-5, 10 × 250 mm, Nomura Chemical Co.; 1% CH_3CN in H_2O, flow rate 4 mL/min; retention time α, 27 min, *β*, 29 min) to give α-**10** (34 mg) and *β*-**10** (13 mg, total 47% yield). **Data for α-10:** mp 220−222 °C (dec, crystallized from H_2O); UV (H_2O) λ_{max} 276 nm (8300); UV (0.1 N HCl) λ_{max} 283 nm (11900), 219 nm (7600); ^{1}H NMR (DMSO-d_6) δ 7.61 (1H, d, J = 7.2 Hz), 7.24 (2H, br, D_2O exchangeable), 6.64 (1H, s), 5.85 (1H, br), 5.79 (1H, d, J = 5.9 Hz, D_2O exchangeable), 5.32 (1H, s), 4.98 (1H, t, J = 5.4 Hz, D_2O exchangeable), 4.80 (1H, s), 4.28 (1H, br t, J = 7.8 Hz), 3.81 (1H, ddd, J = 3.9, 5.4, 10.7 Hz), 3.45 (1H, ddd, J = 5.4, 7.8, 10.7 Hz), 3.37 (1H, dt, J = 3.9, 7.8 Hz); ^{13}C NMR (100.4 MHz, DMSO-d_6) δ 165.0, 155.4, 150.4, 143.1, 111.2, 95.3, 74.1, 62.9, 58.8, 54.1; FAB-MS m/z 256 (M^+ + H). Anal. Calcd for $C_{10}H_{13}N_3O_3S\cdot0.5H_2O$: C, 45.44; H, 5.34; N, 15.90. Found: C, 45.73; H, 5.36; N, 16.12. **Data for *β*-10:** mp 221−222 °C (crystallized from H_2O); UV (H_2O) λ_{max} 276 nm (8700); UV (0.1 N HCl) λ_{max} 282 nm (11800), 219 nm (7600); ^{1}H NMR (DMSO-d_6) δ 7.57 (1H, d, J = 7.3 Hz), 7.24, 7.20 (total 2H, br s, D_2O exchangeable), 6.66 (1H, s), 5.79 (1H, br d, J = 7.3 Hz), 5.62 (1H, d, J = 4.9 Hz, D_2O exchangeable), 5.32 (1H, s), 5.07 (1H, t, J = 5.4 Hz, D_2O exchangeable), 4.92 (1H, s), 4.49 (1H, br t, J = 4.9 Hz), 3.60 (1H, ddd, J = 5.4, 6.4, 11.2 Hz), 3.49 (1H, ddd, J = 5.4, 6.4, 11.2 Hz), 3.12 (1H, dt, J = 4.9, 6.4 Hz); ^{13}C NMR (100.4 MHz, DMSO-d_6) δ 165.3, 155.7, 149.7, 142.5, 112.7, 95.5, 74.9, 63.2, 60.4, 54.4; FAB-MS m/z 256 (M^+ + H). Anal. Calcd for $C_{10}H_{13}N_3O_3S$: C, 47.05; H, 5.13; N, 16.46. Found: C, 46.87; H, 5.06; N, 16.45.

1,4-Anhydro-3-*O*-benzyl-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2,2-difluoro-4-thio-D-*erythro*-pentitol (34). A benzene solution (11 mL) of **25** (1.75 g, 3.68 mmol) was added gradually to a solution of DAST (2 mL, 14.7 mmol) in anhydrous benzene (11 mL) at 0 °C. After being stirred at room temperature for 5 h, the mixture was poured into ice−water and extracted with ether (×2). The organic phase was washed with H_2O and brine, then dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography over silica gel (4 × 23 cm; 3−5% AcOEt in hexane) to give **34** (876 mg, 48%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 7.66−7.60 (4H, m), 7.47−7.27 (11H, m), 4.80 (1H, d, J = 11.7 Hz), 4.62 (1H, d, J = 11.7 Hz), 4.12−4.04 (1H, m), 3.77 (1H, ddd, J = 1.5, 6.8, 10.7 Hz) 3.66 (1H, ddd, J = 1.0, 6.4, 10.7 Hz), 3.48−3.55 (1H, m), 3.27 (1H, dt, J = 12.6 Hz, $J_{H,F}$ = 12.6, 16.1 Hz), 3.11 (1H, dd, J = 12.6 Hz, $J_{H,F}$ = 25.0 Hz), 1.04 (9H, s); FAB-MS m/z 499 (M^+ + H). Anal. Calcd for $C_{28}H_{32}F_2O_2SSi$: C, 67.44; H, 6.47. Found: C, 67.24; H, 6.53.

1,4-Anhydro-3-*O*-benzoyl-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2,2-difluoro-4-thio-D-*erythro*-pentitol (36). Compound **34** (1.31 g, 2.63 mmol) was debenzylated as described in the synthesis of **31**. A mixture of crude debenzylated product (0.922 g), Et_3N (0.54 mL, 3.84 mmol), Bz_2O (0.762 g, 3.36 mmol), and DMAP (54 mg) in CH_3CN (11 mL) was stirred at room temperature for 3 h. After concentration of the mixture to dryness, the residue was partitioned between ether and H_2O. The organic phase was washed with aqueous HCl (0.5 M) and saturated $NaHCO_3$ and then dried ($MgSO_4$). After the solvent was removed under reduced pressure, the residue was purified by column chromatography over silica gel (100 g; 3.5% AcOEt in hexane) to give **36** (1.06 g, 79%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 8.08−8.04 (2H, m), 7.68−7.58 (5H, m), 7.50−7.30 (8H, m), 5.79−5.72 (1H, m), 3.89 (1H, ddd, J = 1.0, 6.5, 10.7 Hz), 3.75 (1H, ddd, J = 1.5, 6.8, 10.7 Hz), 3.66−3.60 (1H, m), 3.38−3.20 (2H, m), 1.04 (9H, s); FAB-MS m/z 513. (M^+ + H). Anal. Calcd for $C_{28}H_{30}F_2O_3SSi$: C, 65.50; H, 5.90. Found: C, 65.77; H, 5.98.

4-Acetyl-1-(5-*O*-(*tert*-butyldiphenylsilyl)-3-*O*-benzoyl-2-deoxy-2,2-difluoro-4-thio-α(and *β*)-D-*erythro*-pentofuranosyl)cytosine (α,*β*-38). Compound **36** (1.05 g, 2.04 mmol) was subjected to the glycosylation reaction as described for the preparation of **33**. Purification by column chromatography over silica gel (130 g; 3% MeOH in $CHCl_3$) gave **38** (0.778 g, 57%) as an amorphous foam: ^{1}H NMR ($CDCl_3$) δ 8.86 (0.72H, br, D_2O exchangeable), 8.73 (0.28H, br, D_2O exchangeable), 8.29−8.23 (1H, m), 8.09−8.05 (0.56H, m), 8.00−7.96 (1.44H, m), 7.70−7.59 (5H, m), 7.52−7.30 (9H, m), 6.91−6.81 (1H, m), 5.90−5.81 (1H, m), 4.00-3.78 (2.72H, m), 3.71−3.67 (0.28H, m), 2.26 (2.16H, s), 2.25 (0.84H, s), 1.11 (2.52H, s), 1.06 (6.48H, s); FAB-MS m/z 664 (M^+ + H). Anal. Calcd for $C_{34}H_{35}F_2N_3O_5SSi\cdot0.25H_2O$: C, 61.10; H, 5.35; N, 6.29. Found: C, 61.08; H, 5.35; N, 6.22.

1-(2-Deoxy-2,2-difluoro-4-thio-α(and *β*)-D-*erythro*-pentofuranosyl)cytosine (4′-Thiogemcitabine, α,*β*-11). Compound **38** (0.773 g, 1.17 mmol) was deprotected, as described for the preparation of **10**, to give α-**11** (119 mg) and *β*-**11** (48 mg, total 51%). **Data for α-11:** mp 200−203 °C (crystallized from H_2O); UV (H_2O) λ_{max} 273 nm (8000); UV (0.1 N HCl) λ_{max} 280 nm (14 700), 217 nm (9100); ^{1}H NMR (DMSO-d_6) δ 7.85 (1H, dd, J = 2.5, 8.0 Hz), 7.36 (2H, br d, D_2O exchangeable), 6.58 (1H, dd, J = 9.5, 13.5 Hz), 6.38 (1H, br, D_2O exchangeable), 5.83 (1H, d, J = 8.0 Hz), 5.18 (1H, br, D_2O exchangeable), 4.27−4.16 (1H, m), 3.84−3.76 (1H, m), 3.59−3.48 (2H, m); FAB-MS m/z 280 (M^+ + H). Anal. Calcd for $C_9H_{11}F_2N_3O_3S$: C, 38.71; H, 3.97; N, 15.05. Found: C, 38.74; H, 4.04; N, 14.82. **Data for *β*-11:** UV (H_2O) λ_{max} 273 nm (9900); UV (0.1 N HCl) λ_{max} 280 nm (11 000), 219 nm (5700); ^{1}H NMR (DMSO-d_6) δ 8.05 (1H, d, J = 7.5 Hz), 7.35 (2H, br d, D_2O exchangeable), 6.36 (1H, br, D_2O exchangeable), 6.36 (1H, dd, J = 3.5, 12.0 Hz), 5.82 (1H, d, J = 7.5 Hz), 5.40 (1H, br, D_2O exchangeable), 4.16−4.04 (1H, m), 3.83−3.65 (2H, m), 3.16 (1H, m); FAB-MS m/z 280 (M^+ + H). Anal. Calcd for $C_9H_{11}F_2N_3O_3S\cdot0.5H_2O$: C, 37.50; H, 4.20; N, 14.58. Found: C, 37.87; H, 4.34; N, 14.14.

1,4-Anhydro-3-*O*-benzyl-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2-fluoro-4-thio-D-arabitol (40). To a solution of DAST (9.9 mL, 74.6 mmol) in anhydrous CH_2Cl_2 (80 mL) was gradually added **15** (23.8g, 49.7 mmol) in CH_2Cl_2 (80 mL) at −78 °C over 30 min. After being stirred at −78 °C for 3 h, saturated $NaHCO_3$ (200 mL) was added to the mixture. The whole was stirred at room temperature for 30 min. The separated H_2O phase was extracted with $CHCl_3$ (×2). The combined organic phase was washed with brine, then dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography over silica gel (7.9 × 14 cm; 5% AcOEt

in hexane) to give **40** (18.35g, 77%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 7.71−7.63 (4H, m), 7.47−7.28 (11H, m), 5.18 (1H, dq, J = 3.5, 50.5 Hz), 4.64 (1H, d, J = 12.0 Hz), 4.60 (1H, d, J = 12.0 Hz), 4.35 (1H, dt, J = 2.9, 11.2 Hz), 3.76 (1H, t, J = 9.5 Hz), 3.66 (1H, ddd, J = 2.0, 6.1, 10.5 Hz), 3.57−3.53 (1H, m), 3.19 (1H, ddd, J = 4.4, 12.2, 30.3 Hz), 3.06 (1H, ddd, J = 3.4, 12.2, 18.1 Hz), 1.05 (9H, s); FAB-MS m/z 423 ($M^+ - t$-Bu). Anal. Calcd for $C_{28}H_{33}FO_2SSi$: C, 69.96; H, 6.92. Found: C, 70.03; H, 6.98.

1-*O*-Acetyl-3-*O*-benzyl-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2-fluoro-4-thio-D-*arabino*-pentofuranose (42). A solution of *m*-CPBA (80%, 3.0 g, 13.9 mmol) in CH_2Cl_2 (170 mL) was added dropwise to a solution of **40** (6.66 g, 13.9 mmol) in CH_2Cl_2 (110 mL) at −78 °C. The mixture was stirred at the same temperature for 1 h. The reaction was quenched by the addition of saturated $NaHCO_3$. The whole was extracted with $CHCl_3$ (×2). The organic phase was washed with a 10% sodium thiosulfate solution, saturated $NaHCO_3$ (×2) and brine and then dried (Na_2SO_4). After the filtrate was concentrated, the residue was dissolved in Ac_2O (140 mL). The mixture was kept at 100 °C for 2 h. After concentration, the residue was partitioned between AcOEt and H_2O. The organic phase was washed with saturated $NaHCO_3$ and brine and then dried (Na_2SO_4). After the solvent was removed under reduced pressure, the residue was purified by column chromatography over silica gel (4.8 × 26 cm; 3−5% AcOEt in hexane) to give **42** (5.79 g, 77%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 7.68−7.62 (4H, m), 7.46−7.25 (11H, m), 6.06 (1H, d, J = 4.4 Hz), 5.11 (1H, ddd, J = 4.4, 8.3, 51.0 Hz), 4.78 (1H, d, J = 11.7 Hz), 4.60 (1H, d, J = 11.7 Hz), 4.38 (1H, ddd, J = 7.3, 8.3, 11.7 Hz), 3.81 (1H, dd, J = 4.4, 10.5 Hz), 3.74 (1H, dd, J = 5.9, 10.5 Hz), 3.34 (1H, ddd, J = 4.4, 5.9, 7.3 Hz), 2.05 (3H, s), 1.07 (9H, s); FAB-MS m/z 479 (M^+ − OAc). Anal. Calcd for $C_{30}H_{35}FO_4SSi\cdot H_2O$: C, 64.72; H, 6.70. Found: C, 64.97; H, 6.35.

4-Acetyl-1-(3-*O*-benzyl-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2-fluoro-4-thio-α(and *β*)-D-*arabino*-pentofuranosyl)cytosine (α,*β*-43). A mixture of **42** (108 mg, 0.20 mmol), silylated N^4-acetylcytosine (0.60 mmol), and $SnCl_4$ (0.40 mL of 1 M CH_2Cl_2 solution, 0.40 mmol) in anhydrous CH_3CN (5 mL) was stirred at room temperature for 1.5 h. The reaction was quenched by the addition of saturated $NaHCO_3$. The precipitated material was removed by suction. The filtrate was extracted with $CHCl_3$ (×3). The organic phase was washed with brine, then dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography over silica gel (1.5 × 6.5 cm; 0−1% MeOH in $CHCl_3$) to give α,*β*-**43** (117 mg, 93%) as a foam: UV (MeOH) λ_{max} 301, 250 nm; ^{1}H NMR ($CDCl_3$) δ 9.49 (1H, br, D_2O exchangeable), 8.31 (0.74H, d, J = 7.3 Hz), 8.22 (0.26H, dd, J = 1.7, 7.3 Hz), 7.67−7.61 (4H, m), 7.49−7.11 (12H, m), 6.70 (0.26H, d, J = 4.4, 19.0 Hz), 6.32 (0.74H, d, J = 14.2 Hz), 5.16 (1H, d, J = 46.9 Hz), 4.64 (0.26H, d, J = 11.7 Hz), 4.59 (0.26H, d, J = 11.7 Hz), 4.52 (0.74H, d, J = 11.7 Hz), 4.44 (0.74H, d, J = 11.7 Hz), 4.36 (1H, br d, J = 10.3Hz), 3.95−3.62 (3H, m), 2.27, 2.26 (total 3H, s), 1.08, 1.06 (total 9H, s); FAB-MS m/z 632 (M^+ + H). Anal. Calcd for $C_{34}H_{38}FN_3O_4SSi\cdot 0.5H_2O$: C, 63.72; H, 6.13; N, 6.56. Found: C, 63.75; H, 6.05; N, 6.77.

1-(2-Deoxy-2-fluoro-4-thio-α(and *β*)-D-*arabino*-pentofuranosyl)cytosine (4′-ThioFAC, α,*β*-12). BBr_3 (2.48 mL, 26.2 mmol) was added slowly to a solution of **43** (5.52 g, 8.74 mmol) in CH_2Cl_2 (80 mL) at −78 °C. After being stirred for 1.5 h at −78 °C, the reaction was quenched by the addition of MeOH (12 mL) and saturated $NaHCO_3$ (50 mL) and allowed to warm to room temperature. The whole was extracted with $CHCl_3$ (×3), and the organic phase was washed with saturated $NaHCO_3$ and brine, then dried (Na_2SO_4), and concentrated. The residue was dissolved in MeOH (80 mL) containing ammonium fluoride (3.24 g, 87.4 mmol). The mixture was kept at 60 °C for 2 h. After the solvent was removed under reduced pressure, the residual product was roughly purified by a silica gel column (4.9 × 14 cm; 2−20−30% MeOH in $CHCl_3$). The obtained N^4-acetyl derivative was suspended in MeOH (25 mL) and concentrated NH_4OH (25 mL). The mixture was stirred at room temperature overnight. The solvent was removed and coevaporated with EtOH (×3) under reduced pressure. The residue was purified by column chromatography over silica gel (4.4 × 11 cm; 10−20−30% MeOH in $CHCl_3$). Then, α and *β*-isomers were separated by reverse-phased ODS column chromatography (Wakosil 40C18, 6.4 × 25 cm; 0−2% CH_3CN in H_2O). The unseparable fractions were collected, concentrated, and rechromatographed (Wakosil 40C18, 3.7 × 18.5 cm; H_2O) to give α-**12** (991 mg) and *β*-**12** (386 mg, total 60% yield). **Data for α-12:** mp 204−205 °C (crystallized from EtOH); UV (H_2O) λ_{max} 274 nm (8600); UV (0.1 N HCl) λ_{max} 282 nm (12 300), 219 nm (6700); ^{1}H NMR (DMSO-d_6) δ 7.97 (1H, d, J = 7.3 Hz), 7.26, 7.24 (2H, br d, D_2O exchangeable), 6.15 (1H, dd, J = 5.9, 17.5 Hz), 5.91 (1H, d, J = 5.4 Hz, D_2O exchangeable), 5.79 (1H, d, J = 7.3 Hz), 5.06 (1H, dt, J = 6.4, 52.3 Hz), 5.03 (1H, dd, J = 4.9, 5.4 Hz, D_2O exchangeable), 4.09 (1H, ddt, J = 5.4, 6.8, 13.7 Hz), 3.77 (1H, dt, J = 4.9, 10.7 Hz), 3.59 (1H, dt, J = 4.9, 7.3 Hz), 3.41 (1H, ddd, J = 6.3, 7.8, 10.7 Hz); FAB-MS m/z 262 (M^+ + H). Anal. Calcd for $C_9H_{12}FN_3O_3S$: C, 41.37; H, 4.63; N, 16.08. Found: C, 41.53; H, 4.76; N, 15.83. **Data for *β*-12:** mp > 220 °C (dec, crystallized from H_2O); UV (H_2O) λ_{max} 274 nm (9000); UV (0.1 N HCl) λ_{max} 282 nm (15 600), 219 nm (9400);^{1}H NMR (DMSO-d_6) δ 7.98 (1H, dd, J = 1.0, 7.3 Hz), 7.26, 7.19 (2H, br d, D_2O exchangeable), 6.47 (1H, dd, J = 5.4, 13.2 Hz), 5.85 (1H, d, J = 4.9 Hz, D_2O exchangeable), 5.77 (1H, d, J = 7.3 Hz), 5.22 (1H, t, J = 5.4 Hz, D_2O exchangeable), 4.91 (1H, dt, J = 5.4, 50.8 Hz), 4.25 (1H, ddt, J = 4.9, 5.4, 11.2 Hz), 3.72 (1H, dt, J = 5.4, 11.2 Hz), 3.60 (1H, dt, J = 5.9, 11.2 Hz), 3.22 (1H, dt, J = 5.4, 5.9 Hz); FAB-MS m/z 262 (M^+ + H). Anal. Calcd for $C_9H_{12}FN_3O_3S\cdot 0.2H_2O$: C, 40.84; H, 4.66; N, 15.88. Found: C, 40.73; H, 4.62; N, 15.77.

1,4-Anhydro-2-azido-3-*O*-benzyl-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-4-thio-D-ribitol (45). A mixture of DEAD (0.47 mL, 3.0 mmol) and DPPA (0.65 mL, 3.0 mmol) in THF (3 mL) was added dropwise to a solution of **15** (480 mg, 1.0 mmol) and triphenylphosphine (786 mg, 3.0 mmol) in THF (14 mL) at 0 °C. After the solution was stirred at room temperature for 2 h, EtOH was added. After 30 min of stirring, the solvent was removed under reduced pressure. The residue was purified by column chromatography over silica gel (3.4 × 15 cm; 3.5% AcOEt in hexane) to give **45** (402 mg, 80%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.65−7.62 (4H, m), 7.46−7.29 (11H, m), 4.66 (1H, d, J = 12.2 Hz), 4.62 (1H, d, J = 12.2 Hz), 4.14 (1H, t, J = 3.4 Hz), 3.73−3.54 (4H, m), 3.05 (1H, dd, J = 8.3, 10.7 Hz), 2.93 (1H, dd, J = 5.9, 10.7 Hz), 1.06 (9H, s); FAB-MS m/z 504 (M^+ + H); IR (neat) 2104 cm^{-1} (N_3). Anal. Calcd for $C_{28}H_{33}N_3O_2SSi$: C, 66.76; H, 6.60; N, 8.34. Found: C, 67.00; H, 6.57; N, 8.21.

4-Acetyl-1-(2-azido-3-*O*-benzyl-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-4-thio-α(and *β*)-D-*ribo*-pentofuranosyl)cytosine (α,*β*-47). (a) From Sulfoxide. Sulfoxide **46** (768 mg, 1.50 mmol) was prepared from **45** as described in the synthesis of **42** (*m*-CPBA treatment). The crude products were passed through a silica gel column (2.8 × 18 cm; 1% MeOH in $CHCl_3$) to give **46** (773 mg, quant.). A mixture of **46** (104 mg, 0.2 mmol), silylated N^4-acetylcytosine (0.60 mmol), and TMSOTf (0.15 mL, 0.8 mmol) in $ClCH_2CH_2Cl$ (4 mL) was stirred at 0 °C for 2 h and then at room temperature overnight. The reaction was quenched by the addition of saturated $NaHCO_3$. The precipitated material was removed by suction. The filtrate was extracted with $CHCl_3$ (×3), and the organic phase was washed with brine, then dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography over silica gel (1.1 × 22 cm; 0−1% MeOH in $CHCl_3$) to give α,*β*-**47** (27 mg, 20%) as a foam.

(b) From 1-*O*-Acetate. A mixture of **48** (120 mg, 0.21 mmol), silylated N^4-acetylcytosine (0.63 mmol), and TMSOTf (0.15 mL, 0.8 mmol) in $ClCH_2CH_2Cl$ (4 mL) was stirred at 0 °C for 1 h and then at room temperature overnight. The reaction was quenched by the addition of saturated $NaHCO_3$. The precipitated material was removed by suction. The filtrate was extracted by $CHCl_3$ (×3), and the organic phase was washed with brine, then dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography over silica gel (1.1 × 22 cm; 0−1% MeOH in $CHCl_3$) to give α,*β*-**47** (81 mg, 59%) as a foam: ^{1}H NMR ($CDCl_3$) δ 9.51 (0.48H, br s, D_2O exchangeable), 9.25 (0.52H, br s, D_2O exchangeable), 8.45

(0.48H, d, J = 7.8 Hz), 8.41 (0.52H, d, J = 7.3 Hz), 7.68−7.20 (16H, m), 6.44 (0.52H, d, J = 5.4 Hz), 6.08 (0.48H, d, J = 3.9 Hz), 4.61 (0.52H, d, J = 11.7 Hz), 4.57 (0.48H, d, J = 11.7 Hz), 4.52 (0.52H, d, J = 11.7 Hz), 4.50 (0.48H, d, J = 11.7 Hz), 4.40 (0.52H, dd, J = 3.9, 5.4 Hz), 4.20 (0.52H, dd, J = 3.9, 6.8 Hz), 4.11 (0.52H, dt, J = 3.4, 6.8 Hz), 4.03 (0.48H, t, J = 3.9 Hz), 3.96−3.88 (1.52H, m), 3.82−3.75 (0.96H, m), 3.70 (0.48H, dt, J = 3.9, 7.8 Hz), 2.27 (1.44H, s), 2.26 (1.56H, s), 1.12 (4.32H, s), 1.07 (4.68H, s); FAB-MS m/z 655 (M^+ + H). Anal. Calcd for $C_{34}H_{38}N_6O_4SSi$·0.17AcOEt: C, 62.19; H, 5.92; N, 12.55. Found: C, 62.49; H, 5.99; N, 12.36.

1-*O*-Acetyl-2-azido-3-*O*-benzyl-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-4-thio-D-*ribo*-pentofuranose (48). A solution of **46** (490 mg, 0.94 mmol) in Ac_2O (10 mL) was kept at 100 °C for 1.5 h. After concentration, the residue was purified by column chromatography over silica gel (2.2 × 23 cm; 2−5% AcOEt in hexane) to give **48** (356 mg, 67%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.67−7.29 (15H, m), 6.19 (0.67H, d, J = 4.9 Hz), 5.83 (0.33H, d, J = 2.9 Hz), 4.71 (0.67H, d, J = 12.2 Hz), 4.66−4.59 (1.33H, m), 4.36 (0.33H, q, J = 3.9 Hz), 4.31 (0.67H, dd, J = 2.0, 4.9 Hz), 4.08 (0.33H, dd, J = 2.9, 3.9 Hz), 3.81 (0.67H, q, J = 4.9 Hz), 3.78−3.74 (0.67H, m), 3.65−3.60 (1H, m), 3.53 (0.66H, t, J = 4.9 Hz), 3.48 (0.67H, dd, J = 7.3, 11.2 Hz), 2.15 (2.01H, s), 2.04 (0.99H, s), 1.06 (9H, s); FAB-MS m/z 504 (M^+ − ^{t}Bu). Anal. Calcd for $C_{30}H_{35}N_3O_4SSi$: C, 64.14; H, 6.28; N, 7.48. Found: C, 63.87; H, 6.17; N, 7.39.

1,4-Anhydro-3-*O*-benzoyl-2-*O*-(*tert*-butyldimethylsilyl)-5-*O*-(*tert*-butyldiphenylsilyl)-4-thio-D-arabitol (51). A mixture of **15** (3.4 g, 7.1 mmol), pyridine (1.72mL, 21.3 mmol), and TBSOTf (2.5 mL, 10.7 mmol) in CH_2Cl_2 (70 mL) was stirred at room temperature for 2 h. After the addition of saturated $NaHCO_3$, the whole was extracted with $CHCl_3$. The organic phase was washed with brine, then dried (Na_2SO_4), and concentrated. The residual pyridine was removed by codistillation with toluene (×3) to give crude **49**, which was dissolved in CH_2Cl_2 (70 mL). A solution of BCl_3 (32 mL of 1.0 M CH_2Cl_2 solution, 32 mmol) was gradually added to the mixture at −78 °C. After being stirred for 6.5 h at the same temperature, the reaction was quenched by the addition of MeOH−pyridine (48 mL, 2:1). The mixture was allowed to warm to room temperature and was stirred for 1 h. After the most of organic solvents were removed under reduced pressure, the whole was extracted with AcOEt (×2). The organic phase was washed with H_2O and brine and then dried (Na_2SO_4). The solvent was removed under reduced pressure to leave crude **50**. A mixture of crude **50**, Bz_2O (2.40 g, 10.7 mmol), Et_3N (1.70 mL, 12.1 mmol), and DMAP (83 mg, 0.70 mmol) in CH_3CN (70 mL) was stirred at room temperature for 5 h. After the addition of saturated $NaHCO_3$, the whole was extracted with AcOEt (×2). The organic phase was washed with brine, then dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography over silica gel (3.6 × 24 cm; 5% AcOEt in hexane) to give **51** (4.01 g, 93%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 8.04−8.01 (2H, m), 7.67−7.30 (13H, m), 5.53 (1H, t, J = 3.4 Hz), 4.54−4.50 (1H, m), 4.01 (1H, dd, J = 7.3, 9.8 Hz), 3.79 (1H, dd, J = 7.3, 9.8 Hz), 3.52 (1H, dt, J = 3.4, 7.3 Hz), 3.10 (1H, dd, J = 4.9, 11.2 Hz), 2.87 (1H, dd, J = 4.4, 11.2 Hz), 1.04 (9H, s), 0.82 (9H, s), 0.07 (3H, s), 0.05 (3H, s); FAB-MS m/z 549 (M^+ − ^{t}Bu). Anal. Calcd for $C_{34}H_{46}O_4SSi_2$: C, 67.28; H, 7.64. Found: C, 67.38; H, 7.84.

1,4-Anhydro-3-*O*-benzoyl-4-thio-D-arabitol (52). A mixture of **51** (4.78 g, 7.89 mmol) and NH_4F·HF (9.0 g, 158 mmol) in MeOH (100 mL) was stirred at room temperature for 43 h. After concentration, the residue was partitioned between AcOEt and H_2O. The organic phase was washed with H_2O and brine, then dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography over silica gel (3.4 × 10 cm; 5−50% AcOEt in hexane) to give **52** (1.78 g, 89%) as a crystal: mp 110−111 °C (crystallized from hexane−AcOEt); ^{1}H NMR (DMSO-d_6) δ 7.98−7.96 (2H, m), 7.70−7.66 (1H, m), 7.54 (2H, t, J = 7.8 Hz), 5.56 (1H, d, J = 4.4 Hz, D_2O exchangeable), 5.30 (1H, t, J = 3.9 Hz), 5.00 (1H, t, J = 5.4 Hz, D_2O exchangeable), 4.41−4.36 (1H, m), 3.75−3.70 (1H, m), 3.52−3.46 (1H, m), 3.39 (1H, dt, J = 3.9, 6.8 Hz), 3.07 (1H, dd, J = 5.4, 11.2 Hz), 2.80 (1H, dd, J = 4.4, 11.2 Hz); FAB-MS m/z 255 (M^+ + H). Anal. Calcd for $C_{12}H_{14}O_4S$: C, 58.66; H, 7.66. Found: C, 58.67; H, 7.58.

1,4-Anhydro-3-*O*-benzoyl-5-*O*-(*tert*-butyldimethylsilyl)-4-thio-D-arabitol (53). A mixture of **52** (1.77 g, 7.0 mmol), TBSCl (1.06 g, 7.0 mmol), and imidazole (524 mg, 7.7 mmol) in DMF (50 mL) was stirred at room temperature overnight. H_2O was added to the mixture, and the whole was stirred for an additional 10 min. The solvent was removed under reduced pressure. The residue was partitioned between AcOEt and H_2O, and the organic phase was washed with H_2O (×2) and brine and then dried (Na_2SO_4). After the solvent was removed under reduced pressure, the residue was purified by column chromatography over silica gel (3.6 × 20 cm; 5−16% AcOEt in hexane) to give **53** (2.1 g, 80%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 8.02−8.00 (2H, m), 7.60−7.57 (1H, m), 7.45 (2H, t, J = 7.8 Hz), 5.36 (1H, br s), 4.50−4.42 (2H, m, including D_2O exchangeable 1H), 4.12 (1H, dd, J = 3.4, 10.7 Hz), 3.72 (1H, dd, J = 3.4, 10.7 Hz), 3.67−3.65 (1H, m), 3.32 (1H, dd, J = 3.9, 11.7 Hz), 3.04 (1H, dd, J = 2.0, 11.7 Hz), 0.94 (9H, s), 0.15 (3H, s), 0.14 (3H, s); FAB-MS m/z 369 (M^+ + H). Anal. Calcd for $C_{18}H_{28}O_4SSi$: C, 58.66; H, 7.66. Found: C, 58.67; H, 7.58.

1,4-Anhydro-2-azido-3-*O*-benzoyl-5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-4-thio-D-ribitol (54). Compound **53** (680 mg, 1.85 mmol) was subjected to the Mitsunobu reaction as described for the synthesis of **45**. After silica gel column chromatography (2.7 × 18 cm; 3.5% AcOEt in hexane), 2-azido derivative **54** (605 mg, 83%) was obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 8.10−8.07 (2H, m), 7.62−7.58 (1H, m), 7.49−7.45 (2H, m), 5.56 (1H, t, J = 3.9 Hz), 4.32−4.28 (1H, m), 3.82 (1H, dd, J = 5.4, 10.3 Hz), 3.73 (1H, dd, J = 5.4, 10.3 Hz), 3.69−3.65 (1H, m), 3.17 (1H, dd, J = 5.9, 10.7 Hz), 3.09 (1H, dd, J = 7.3, 10.7 Hz), 0.89 (9H, s), 0.06 (6H, s); FAB-MS m/z 394 (M^+ + H). IR (neat) 2108 cm^{-1} (N_3). Anal. Calcd for $C_{18}H_{27}N_3O_3SSi$: C, 54.93; H, 6.91; N, 10.68. Found: C, 55.15; H, 6.86; N, 10.58.

1-*O*-Acetyl-2-azido-3-*O*-benzoyl-5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-4-thio-D-*ribo*-pentofuranose (55). Compound **54** (1.35 g, 3.44 mmol) was subjected to the Pummerer rearrangement as described for the synthesis of **46** and **48**. After silica gel column chromatography (3.6 × 20 cm; 2−5% AcOEt in hexane), **55** (816 mg, 53%) was obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 8.15−8.07 (2H, m), 7.63−7.59 (1H, m), 7.50−7.45 (2H, m), 6.37 (0.55H, d, J = 4.4 Hz), 5.92 (0.45H, d, J = 2.9 Hz), 5.74 (0.55H, dd, J = 1.5, 4.4 Hz), 5.70−5.68 (0.45H, m), 4.54 (0.45H, dd, J = 2.9, 3.9 Hz), 4.05 (0.55H, t, J = 4.4 Hz), 3.96 (0.55H, dd, J = 4.4, 10.7 Hz), 3.85−3.74 (1.9H, m), 3.65 (0.55H, dd, J = 4.4, 10.7 Hz), 2.24 (1.65H, s), 2.12 (1.35H, s), 0.91 (4.95H, s), 0.87 (4.05H, s), 0.09 (3.3H, s), 0.04 (2.7H, s); FAB-MS m/z 492 (M^+ − OAc). Anal. Calcd for $C_{20}H_{29}N_3O_5SSi$: C, 53.19; H, 6.47; N, 9.30. Found: C, 53.43; H, 6.50; N, 9.32.

4-Acetyl-1-(2-azido-3-*O*-benzoyl-5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-4-thio-α(and *β*)-D-*ribo*-pentofuranosyl)-cytosine (α,*β*-56). Compound **55** (596 mg, 1.32 mmol) was subjected to the glycosylation reaction as described for the synthesis of **47b**). After silica gel column chromatography (2.2 × 24 cm; 2% MeOH in $CHCl_3$), **56** (342 mg, 47%) was obtained as a foam: ^{1}H NMR ($CDCl_3$) δ 9.18 (1H, br s, D_2O exchangeable), 8.73 (0.5H, d, J = 7.8 Hz), 8.44 (0.5H, d, J = 7.8 Hz), 8.07−8.01 (2H, m), 7.63−7.60 (1H, m), 7.50−7.43 (3H, m), 6.63 (0.5H, d, J = 5.4 Hz), 6.35 (0.5H, d, J = 3.9 Hz), 5.60 (0.5H, dd, J = 4.4, 6.8 Hz), 5.53 (0.5H, dd, J = 3.9, 6.4 Hz), 4.81 (0.5H, dd, J = 4.4, 5.4 Hz), 4.42 (0.5H, t, J = 3.9 Hz), 4.12−3.84 (3H, m), 2.28 (3H, s), 0.94 (4.5H, s), 0.89 (4.5H, s), 0.13 (3H, s), 0.07 (3H, s); FAB-MS m/z 544 (M^+ + H). Anal. Calcd for $C_{24}H_{32}N_6O_5SSi$·0.2AcOEt: C, 52.97; H, 6.02; N, 14.94. Found: C, 53.03; H, 6.00; N, 14.88.

4-Acetyl-1-(2-azido-3-*O*-benzoyl-2-deoxy-4-thio-α(and *β*)-D-*ribo*-pentofuranosyl)cytosine (α,*β*-57). A mixture of **56** (319 mg, 0.59 mmol) and NH_4F·HF (336 mg, 5.90 mmol) in MeOH (10 mL) was stirred at room temperature overnight. After the solvent was removed under reduced pressure, the residue was purified by column chromatography over silica gel (1.6 × 27 cm; 2% MeOH in $CHCl_3$) to give less polar *β*-**57** (crystal; 115 mg, 45%) and more polar α-**57** (crystal; 110 mg,

43%). **Data for α-57:** mp 208−210 °C (dec, crystallized from MeOH); ^{1}H NMR (DMSO-d_6) δ 10.90 (1H, br s, D_2O exchangeable), 8.59 (1H, d, J = 7.3 Hz), 7.93 (2H, d, J = 7.3 Hz), 7.71−7.67 (1H, m), 7.50 (2H, t, J = 7.3 Hz), 7.27 (1H, d, J = 7.3 Hz), 6.55 (1H, d, J = 5.9 Hz), 6.70−6.68 (1H, m), 5.31 (1H, t, J = 5.4 Hz, D_2O exchangeable), 5.00 (1H, dd, J = 4.9, 5.9 Hz), 4.03−3.99 (1H, m), 3.73−3.69 (1H, m), 3.63−3.58 (1H, m), 2.12 (3H, s); FAB-MS m/z 431 (M^+ + H). Anal. Calcd for $C_{18}H_{18}N_6O_5S$: C, 50.23; H, 4.21; N, 19.52. Found: C, 50.35; H, 4.30; N, 19.29. **Data for β-57:** mp 204−206 °C (dec, crystallized from $CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 11.03 (1H, br s, D_2O exchangeable), 8.60 (1H, d, J = 7.8 Hz), 8.03−8.01 (2H, m), 7.75−7.71 (1H, m), 7.62−7.58 (2H, m), 7.33 (1H, d, J = 7.8 Hz), 6.32 (1H, d, J = 7.3 Hz), 5.73 (1H, t, J = 3.9 Hz), 5.50 (1H, br s, D_2O exchangeable), 4.85 (1H, dd, J = 3.9, 7.3 Hz), 3.80−3.68 (3H, m), 2.12 (3H, s); FAB-MS m/z 431 (M^+ + H). Anal. Calcd for $C_{18}H_{18}N_6O_5S\cdot 0.25H_2O$: C, 49.71; H, 4.29; N, 19.32. Found: C, 49.98; H, 4.19; N, 19.15.

1-(2-Azido-2-deoxy-4-thio-α(and β)-D-*ribo*-pentofuranosyl)cytosine (α,β-58). A suspension of β-**57** (89 mg, 0.21 mmol) in MeOH (3 mL) and concentrated NH_4OH (3 mL) was stirred at room temperature for 2 h. After the solvent was removed under reduced pressure, the remaining H_2O was removed by codistillation with EtOH (×3). The residue was purified by column chromatography over silica gel (1.0×14 cm; 17% MeOH in $CHCl_3$) to give β-**58** (58 mg, 97%) as an amorphous solid. In a similar way, α-**57** (90 mg, 0.21 mmol) was converted to α-**58** (an amorphous foam, 52 mg, 87%). **Data for α-58:** UV (H_2O) λ_{max} 275 nm (8700); UV (0.1 N HCl) λ_{max} 282 nm (12 500), 219 nm (6300); ^{1}H NMR (DMSO-d_6) δ 8.02 (1H, d, J = 7.8 Hz), 7.03 (2H, br s, D_2O exchangeable), 6.35 (1H, d, J = 5.4 Hz), 5.83 (1H, d, J = 3.9 Hz, D_2O exchangeable), 5.76 (1H, d, J = 7.8 Hz), 4.88 (1H, t, J = 4.4 Hz, D_2O exchangeable), 4.31−4.26 (2H, m), 3.75−3.70 (1H, m), 3.60−3.55 (1H, m), 3.48−3.42 (1H, m); FAB-MS m/z 285 (M^+ + H). IR (KBr) 2120 cm^{-1} (N_3). Anal. Calcd for $C_9H_{12}N_6O_3S\cdot 1.3MeOH$: C, 37.95; H, 5.32; N, 25.78. Found: C, 37.90; H, 5.14; N, 25.54. **Data for β-58:** UV (H_2O) λ_{max} 275 nm (8400); UV (0.1 N HCl) λ_{max} 282 nm (12 100), 219 nm (6400); ^{1}H NMR (DMSO-d_6) δ 7.97 (1H, d, J = 7.8 Hz), 7.28, 7.26 (total 2H, br d, D_2O exchangeable), 6.25 (1H, d, J = 8.3 Hz), 5.98 (1H, d, J = 4.9 Hz, D_2O exchangeable), 5.80 (1H, d, J = 7.8 Hz), 5.23 (1H, t, J = 5.4 Hz, D_2O exchangeable), 4.30 (1H, dd, J = 3.4, 7.3 Hz), 3.98 (1H, dd, J = 3.4, 8.3 Hz), 3.67−3.54 (2H, m), 3.28−3.24 (1H, m); FAB-MS m/z 285 (M^+ + H); IR (KBr) 2116 cm^{-1} (N_3). Anal. Calcd for $C_9H_{12}N_6O_3S\cdot 1.1MeOH$: C, 37.96; H, 5.17; N, 26.30. Found: C, 38.09; H, 4.87; N, 25.97.

***In Vitro* Assay for Antitumor Effects.** Cytotoxicities against CCRF-HSB-2 and KB cells were evaluated as reported previously.[44] Typically, cells (5000 cells/well) were exposed to drugs in a 96-well plate for 72 h and the growth inhibition rates were determined by MTT assay[47] or the dye uptake method.[48]

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