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Synthesis and crystal structure of 2′-deoxy-2′-fluoro-4′-thioribonucleosides: substrates for the synthesis of novel modified RNAs

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Abstract

We report herein the synthesis of appropriately protected 2′-deoxy-2′-fluoro-4′-thiouridine (**5**), -thiocytidine (**7**), and -thioadenosine (**35**) derivatives, substrates for the synthesis of novel modified RNAs. The synthesis of **5** and **7** was achieved via the reaction of 2,2′-*O*-anhydro-4′-thiouridine (**3**) with HF/pyridine in a manner similar to that of its 4′-*O*-congener whereas the synthesis of **35** from 4′-thioadenosine derivatives was unsuccessful. Accordingly, **35** was synthesized via the glycosylation of the fluorinated 4-thiosugar **25** with 6-chloropurine. The X-ray crystal structural analysis revealed that 2′-deoxy-2′-fluoro-4′-thiocytidine (**8**) adopted predominately the same C3′-*endo* conformation as 2′-deoxy-2′-fluorocytidine.
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Keywords: Nucleoside; Fluorination; 2′-Deoxy-2′-fluoro-4′-thionucleoside; X-ray structure

1. Introduction

Thus far, a large number of nucleoside derivatives have been synthesized and incorporated into oligonucleotides (ONs) for the purpose of applying them to nucleic acid-based therapeutics including antisense,[1] short interfering RNA (siRNA) strategies,[2] and aptamers isolated by SELEX technology.[3] In our group, we have been intensely studying the synthesis of a series of 4′-thionucleosides[4] and their application with the aim of developing a functional ON.[5] Among the 4′-thionucleic acids investigated, 4′-thioRNA, which consists of 4′-thioribonucleosides, seems to be a most promising candidate for functional ONs because of its higher nuclease resistance, its hybridization property, and its utility in siRNA strategy and aptamers.[5,6] As a part of our continuing research project on 4′-thioRNA, we envisioned the development of new 4′-thionucleoside units to enhance the potential of 4′-thioRNA.

Chemical modification of the 2′-position of nucleoside units is considered to be a highly reliable method for increasing nuclease resistance and hybridization properties of the resulting RNA molecules.[7] For example, 2′-fluoroRNA (2′-FRNA), which consists of 2′-deoxy-2′-fluoronucleosides, has the aforementioned favorable properties due to the fluorine group on the 2′-position.[8] In addition, 2′-FRNA can be obtained through chemical as well as enzymatic synthesis from the corresponding nucleoside triphosphates via transcription by T7 RNA polymerase.[9] Accordingly, a selection of aptamers by SELEX composed of 2′-FRNA was examined, and one of them has been approved as Macugen®, which is the first example of a therapeutic aptamer.[10]

In view of this information, we planned to synthesize 2′-deoxy-2′-fluoro-4′-thionucleoside derivatives, a hybrid of 4′-thioribonucleoside and 2′-deoxy-2′-fluoronucleoside, as new 4′-thionucleoside units. The synthesis of the 2′-deoxy-2′-fluoro-4′-thiouridine and -thiocytidine derivatives **5** and **7** can be achieved from 2,2′-*O*-anhydro-4′-thiouridine (**3**) in a manner similar to the one used for its 4′-*O*-congener while the synthesis of the 2′-deoxy-2′-fluoro-4′-thioadenosine derivative **35** was carried out via condensation between an appropriate

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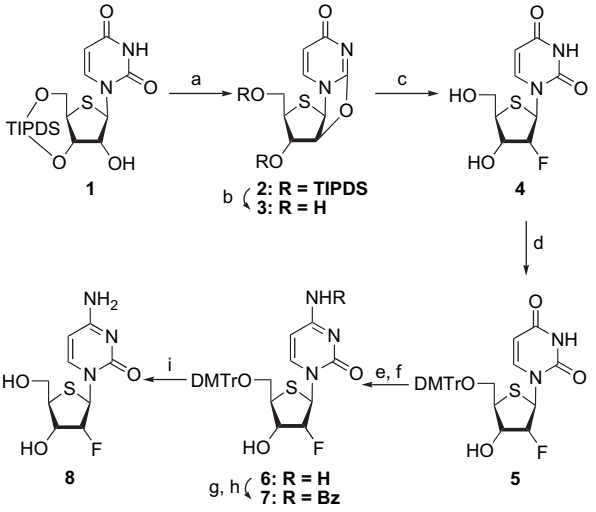
2-fluoro-4-thiosugar and 6-chloropurine. Herein, we wish to report in detail the synthesis of the target compounds. The crystal structure of 2′-deoxy-2′-fluoro-4′-thiocytidine (**8**) is also presented.

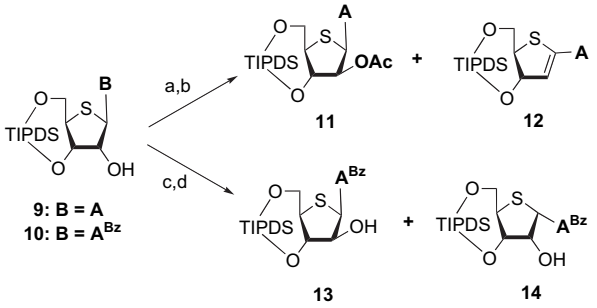
2. Results and discussion

2′-Deoxy-2′-fluorouridine was first prepared in 1961 by treatment of 2,2′-*O*-anhydrouridine with anhydrous HF.[11] This synthetic method was then improved by using HF/pyridine and became a general protocol to afford 2′-deoxy-2′-fluoropyrimidine nucleosides.[8a,12] For the synthesis of the corresponding purine derivatives, introduction of a fluorine atom at the 2′-position was achieved via an S_N2 type reaction from arabinofuranosyl purine derivatives.[8a,13] With these methods as examples, the synthesis of 2′-deoxy-2′-fluoro-4′-thionucleosides was attempted (Scheme 1). Thus, the 4′-thiouridine derivative **1**[4a,5a] was converted into the 2,2′-*O*-anhydro derivative **2** by treatment with trifluoromethanesulfonic anhydride (Tf_2O) in the presence of dimethylaminopyridine (DMAP). The 3′,5′-*O*-TIPDS group of **2** was removed by ammonium fluoride to give **3**.[14] When **3** was treated with HF/pyridine in dioxane in a steel container at 125 °C, the 2′-deoxy-2′-fluoro-4′-thiouridine (**4**) was obtained in 88% yield as is the case of its 4′-*O*-congener.[8a] Compound **4** was then protected with a dimethoxytrityl (DMTr) group to give **5**. After acetylation of the 3′-hydroxyl group of **5**, the resulting compound was converted into the cytosine derivative **6** by the usual method. To convert **6** into **7**, **6** was heated with benzoic anhydride in DMF in the absence of an organic amine, such as triethylamine, which is the usual method for selective protection of the amino group of the cytosine base.[15] However, in this case, partial deprotection of the 5′-*O*-DMTr group of **7** was observed. Accordingly, **6** was treated with triethylsilyl chloride (TESCl) to protect the 3′-hydroxyl group, followed by benzoyl chloride (BzCl) in pyridine. Then, the TES group at the 3′-position of the resulting product was removed by tetrabutylammonium fluoride (TBAF) to give **7** in 70% yield in three steps. Thus compounds **5** and **7**, precursors of the phosphoramidite unit for the ON synthesis, were prepared effectively. In addition, treatment of **6** with trifluoroacetic acid (TFA) in CH_2Cl_2 afforded the 2′-deoxy-2′-fluoro-4′-thiocytidine (**8**), which was used for structural analysis.



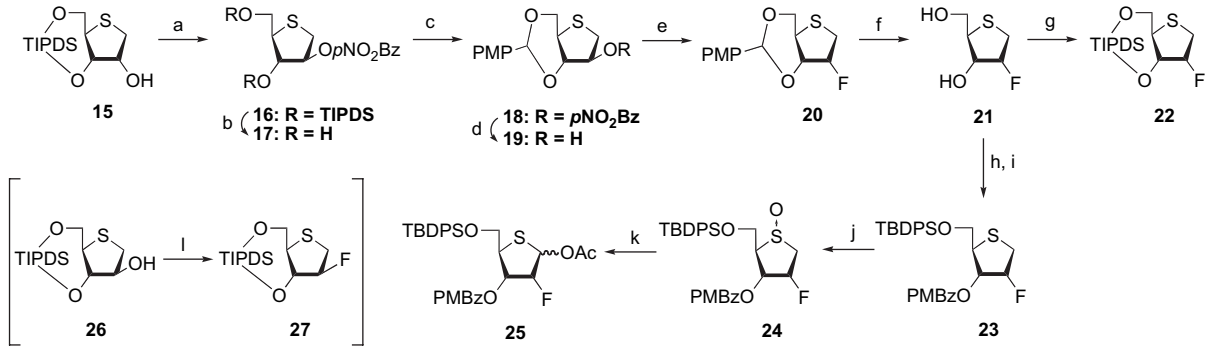
Scheme 1. (a) Tf_2O, DMAP, CH_2Cl_2; (b) NH_4F, MeOH, reflux; (c) HF·pyridine, dioxane, 125 °C; (d) DMTrCl, pyridine; (e) Ac_2O, Et_3N, DMAP, CH_3CN; (f) TPSCl, Et_3N, DMAP, CH_3CN, then NH_4OH; (g) TESCl, pyridine, then BzCl; (h) TBAF, THF; (g) 2% TFA in CH_2Cl_2.

In order to synthesize the adenosine derivative, preparation of 4′-thioarabinofuranosyl adenine derivatives such as **11** and **13** was first examined (Scheme 2). After treatment of the 3′,5′-*O*-TIPDS derivative **9**[5a] with Tf_2O in pyridine, the resulting 2′-*O*-triflate was treated with LiOAc in DMF containing hexamethylphosphoramide (HMPA) at room temperature. However, the desired product **11** was obtained in only 19% yield along with 49% of 1′,2′-unsaturated derivative **12**. Under the same conditions, its 4′-*O*-congener afforded the corresponding *arabino*-derivative in good yield as reported in the literature.[16] Modification of the nucleophile to NaOAc or CsOAc did not improve the chemical yield of **11**. As an alternative method, oxidation of **10**[4d] followed by reduction of the resulting 2′-keto derivative was examined. However, the desired *arabino*-derivative **13** was obtained in only 28% yield along with the α-derivative **14**. These results would arise from the higher acidity of the α-hydrogen of 4′-thionucleoside (i.e., the H-1′ protons) as compared with that of 4′-*O*-congener,[17] and would be a common characteristic of 4′-thionucleoside derivatives.[4c]



Scheme 2. (a) Tf_2O, DMAP, pyridine, CH_2Cl_2; (b) LiOAc, HMPA, DMF; (c) Ac_2O, DMSO; (d) $NaBH_4$, MeOH.

Since **11** and **13** were not obtained in sufficiently high yields, condensation between an appropriate 2-fluoro-4-thiosugar derivative and a nucleobase was envisioned as the next tactic. To determine the plausibility of this method, introduction of a fluorine atom at the 2α-position of the 4-thiosugar was required. As a fluorination reagent, diethylaminosulfur trifluoride (DAST) is widely used, and the reaction generally proceeds via an S_N2 type pathway. However, in the reaction of the 4′-thionucleoside and the 4-thiosugar derivatives with DAST, several reported examples suggest that participation of the sulfur atom may give fluorinated compounds with retention of the stereochemistry, as depicted in the conversion of **26** into the fluorinated compound **27**.[18,19] With these previous results in mind, we anticipated the synthesis of the fluorinated 4-thiosugar, as shown in Scheme 3. The hydroxyl group at the 2-position of **15**[4a] was inverted by the Mitsunobu reaction to give **16** as the *p*-nitrobenzoyl derivative. Deprotection of the



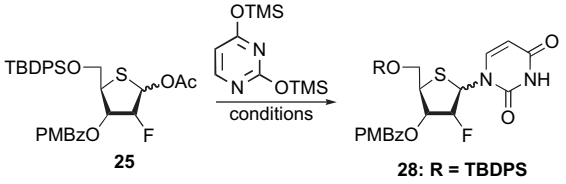
Scheme 3. (a) *p*-Nitrobenzoic acid, PPh_3, DIAD, THF; (b) TBAF, AcOH, THF; (c) *p*-methoxybenzaldehyde dimethylacetal, CSA, DMF; (d) $MeNH_2$, MeOH; (e) PBSF, DBU, dioxane, reflux; (f) 80% aq AcOH; (g) $TIPDSCl_2$, pyridine; (h) TBDPSCl, imidazole, DMF; (i) PMBzCl, pyridine; (j) O_3, CH_2Cl_2, −78 °C; (k) Ac_2O, reflux; (l) DAST, CH_2Cl_2, −15 °C.

3,5-*O*-TIPDS group of **16** by tetrabutylammonium fluoride (TBAF) afforded **17**. To protect the resulting 3- and 5-hydroxyl groups, **17** was treated with *p*-methoxybenzaldehyde dimethylacetal in the presence of camphorsulfonic acid (CSA) to give the 3,5-*O*-*p*-methoxybenzylidene acetal derivative **18**. To the best our knowledge, 3,5-*O*-benzylidene acetal type protecting groups in the furanosides are rare,[20] and thus the easy formation of **18** is worth noting. Structural differences arising from bond lengths and angles between furanosides and thiofuranosides such as **17** would account for this result. The *p*-nitrobenzoyl group of **18** was then removed to give **19**, the substrate for the fluorination. When **19** was heated with perfluoro-1-butanesulfonyl fluoride (PBSF)[21] in the presence of Hünigs base in THF, a fluorinated compound, later confirmed as **20**, was obtained in 29% yield along with the recovered compound **19** (54%). Therefore, the solvent was changed to dioxane to increase the reaction temperature. Accordingly, the fluorinated compound **20** was obtained in 88% yield when **19** was treated with PBSF in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dioxane under reflux. To confirm the stereochemistry of the fluorination, the *p*-methoxybenzylidene acetal group of the fluoro compound (i.e., compound **20**) was removed with aqueous acetic acid, and the resulting product (i.e., compound **21**) was converted into a 3,5-*O*-TIPDS derivative (i.e., compound **22**), whose ^1H NMR spectrum was not identical with that of **27**.[18] Hence, the fluorination on the 2α-position of **19** was achieved by treatment with PBSF without participation of the sulfur atom on the 4-position. Since the Mitsunobu reaction to afford **16** also proceeded with inversion of the stereochemistry, the participation of the sulfur atom reported so far might be a specific phenomenon in the reaction with DAST. Further investigations will be required for a more detailed discussion.

To install the nucleobase at the C-1 position of the resulting fluoro-thiosugar, conversion of **21** into an appropriate substrate for the glycosylation was considered. Watts et al. reported the synthesis of the 2′-fluoro-5-methyl-4′-thioarabinouridine derivative (α/β=0.7:1) via a Lewis acid catalyzed glycosylation with assistance of a benzoyl group at the 3-position of the fluoro-thiosugar.[18] With their report as a reference, we prepared the 1-*O*-acetyl derivative **25**, the substrate for the glycosylation, as shown below. After protection of the 5-OH of **21** with a *tert*-butyldiphenylsilyl (TBDPS) group, a *p*-methoxybenzoyl (PMBz) group instead of the expected benzoyl group was introduced at the 3-position to provide more efficient neighboring group participation and gave **23**. To convert **23** into the corresponding 1-*O*-acetate **25**, **23** was at first treated with ozone in CH_2Cl_2 at −78 °C to give the sulfoxide **24** quantitatively. Subsequent treatment of **24** with Ac_2O under reflux gave the desired **25** as a diastereomeric mixture (α/β=1:1).

Next, the glycosylation of **25** with a nucleobase was examined. To optimize the reaction conditions (Lewis acid and solvent), the glycosylation with uracil was first examined to ease the detection of α- and β-isomer (Table 1). When $SnCl_4$ was used as a Lewis acid, decomposition of **25** was observed and no coupling products were obtained, while the reaction in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a Lewis acid afforded the 4′-thiouridine derivatives. Thus, when **25** was treated with silylated uracil in CH_3CN in the presence of TMSOTf, **28** was obtained in 77% yield as a diastereomeric mixture (α/β=1:1). In order to improve the β-selectivity, the reaction solvent was changed to CH_2Cl_2 and CCl_4.[18] In our case, however, such attempts resulted in recovery of **25** or partial deprotection of the TBDPS group at the 5′-position to give **29** without improvement of the β-selectivity. To determine whether the participation came from the 3-*O*-protecting group in the glycosylation, we also prepared substrates possessing a

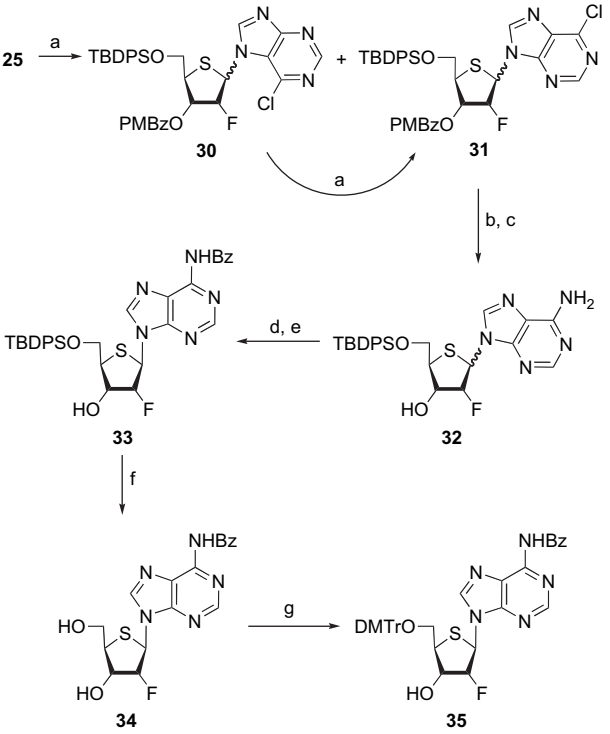
Table 1
Glycosylation of silylated uracil with **25**



Entry	Conditions	Result
1	$SnCl_4/CH_3CN$	Decomposition
2	$TMSOTf/CH_3CN$	**28** (77%, α/β=1:1)
3	$TMSOTf/CH_2Cl_2$	**28** (27%, α/β=0.8:1) **25** (43%)
4	$TMSOTf/CCl_4$	**28** (12%, α/β=0.6:1) **29** (27%, α/β=1.1:1)

2,4-dimethoxybenzoyl, a *p*-nitrobenzoyl, and a methoxymethyl group instead of the PMBz group of **25**, and subjected each of them to the same reaction. However, all substrates afforded 4′-thiouridine derivatives in a similar α/β ratio (from 1:1 to 0.8:1; data not shown), and thus it was concluded that the expected participation from the 3-*O*-acyl groups to give the β-selectivity did not occur. Thus far, several examples suggesting participation from the protecting groups at the 3-position have been reported to give the glycosylation products in a preferable β ratio.[18,22] Therefore, our results are probably due to the steric hindrance of the fluorine atom, arising from a larger van der Waals radius than that of a proton and longer C–F bond length than that of C–H,[23] on the α-face of the thiosugar. In addition, the 2-fluoro-4-thiosugar derivative is expected to adopt the C3-*endo* conformation but not the C2-*endo* conformation due to a *gauche* effect between the electronegative F2 and O4 atoms (the sugar puckering of 4′-thionucleoside derivative will be described later), and the 3-*O*-protecting group will locate in a pseudoequatorial orientation. In this conformation, the 3-*O*-protecting group will locate far from its C1 position to afford preferable β-selectivity (Fig. 1). Although this is, of course, not the structure of the thionium cation intermediate, this consideration would also be one of the explanations for our results.

The reaction conditions having been fixed (although the β-selectivity was not satisfactory), we next attempted the glycosylation with 6-chloropurine (Scheme 4). Thus, the thiosugar **25** was treated with silylated 6-chloropurine in CH_3CN in the presence of TMSOTf at room temperature and the reaction mixture was heated under reflux. Upon consumption of **25**, the reaction was quenched and the desired N-9 isomer **31** was obtained in only 9% yield along with 78% yield of the N-7 isomer **30**. Both **30** and **31** were obtained as diastereomeric mixtures (α/β=1:1). A gradual isomerization of **30** to **31** on TLC analysis was observed on heating with **31**, being obtained in 37% yield along with 39% yield of **30** after 16 h of refluxing. However, further elongation of the reaction time resulted in a reduction of the chemical yields of both **30** and **31**. Accordingly, the separated compound **30** was subjected again to the glycosylation conditions in the presence of the silylated 6-chloropurine giving **31** in 40% yield. Using these individualized procedures, we were able to obtain **31** in 52% yield from **25**. Then, **31** was heated in NH_3/EtOH, followed by $MeNH_2$/MeOH at room temperature to remove the PMBz group, to give **32**. When the exocyclic amino group of **32** was benzoylated, the α- and β-isomers could be separated by flash chromatography to give the desired β-isomer **33** as the sole product. Deprotection of **33** by TBAF in THF afforded **34**. The resulting **34** was treated with DMTrCl in pyridine to give the desired **35** as the precursor of the phosphoramidite unit for the ON synthesis. Likewise, glycosylation with the silylated 2-amino-6-chloropurine was also examined to synthesize a 2′-deoxy-2′-fluoro-4′-thioguanosine derivative. Although the glycosylation with 2-amino-6-chloropurine proceeded well to give the corresponding N-7 isomer, no isomerization to the desired N-9 isomer was achieved under any conditions examined (data not shown). Therefore, efficient methods to prepare 2′-deoxy-2′-fluoro-4′-thioguanosine are still required, and these results will be reported along with the properties of the corresponding modified RNA in due course.



Scheme 4. (a) Silylated 6-chloropurine, TMSOTf, CH_3CN, rt, then reflux; (b) NH_3, EtOH, 80 °C; (c) $MeNH_2$, MeOH; (d) BzCl, pyridine; (e) 1 N NaOH, EtOH; (f) TBAF, THF; (g) DMTrCl, pyridine.

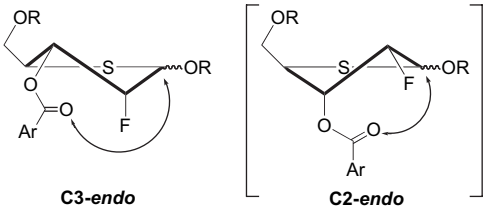


Figure 1. Possible conformation of 2-fluoro-4-thiosugar derivative.

The structure of 2′-deoxy-2′-fluoro-4′-thiocytidine (**8**) was confirmed by X-ray analysis. As can be seen in Figure 2, introduction of the fluorine atom on the α-face of the thiosugar was confirmed. To date, we and others have reported X-ray structures of a few 4′-thionucleoside derivatives, which were compared with those of 4′-*O*-congeners.[4b,24] In summary, the overall structures including the sugar puckering and the *syn*/*anti* conformation around the glycosyl bond of the 4′-thionucleoside derivatives were essentially similar to the corresponding 4′-*O*-congeners despite a marked conformational change in the carbohydrate ring. To elucidate the structure of **8**, important geometric parameters for **8** were compared with those of 2′-deoxy-2′-fluorocytidine (dfC),[25] and these data have been summarized in Table 2. Among the parameters, striking differences in the bond lengths and angles were observed in C1′–S4′ and C4′–S4′, and C4′–S4′–C1′, respectively. Thus, the bond lengths C1′–S4′ and C4′–S4′ were 1.8287 and 1.8295 Å, respectively, while the reported bond lengths of dfC were much shorter (i.e., 1.424 and 1.446 Å, respectively).

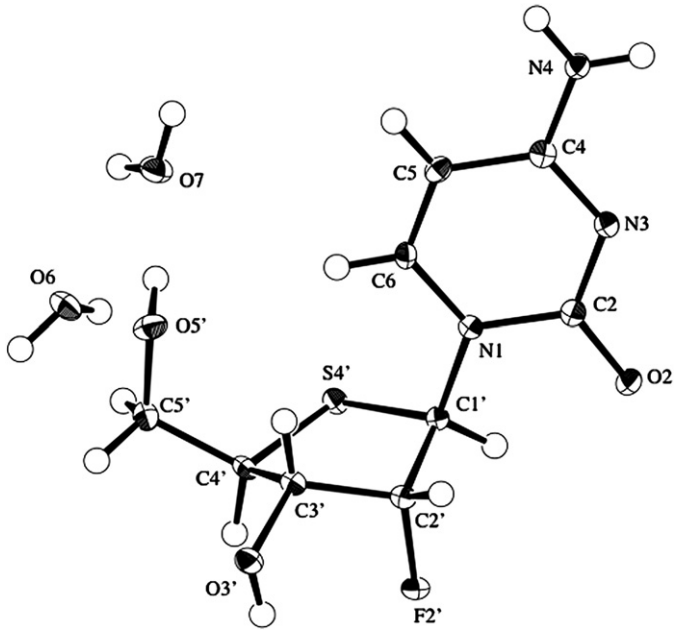


Figure 2. The crystal structure of 2′-deoxy-2′-fluoro-4′-thiocytidine (**8**).

The other bond lengths including the glycosidic bond (C1′–N1) were quite similar to those of dfC. In contrast to the longer bond length of **8**, the bond angle C4′–S4′–C1′ in the thiosugar is 95.2°, which is 15.1° less than that of dfC. The other bond angles in the two sugar moieties do not differ markedly. In spite of the partial structural differences between **8** and dfC, their overall structures can be concluded to be similar. Thus, the cytosine bases are both in the *anti* conformation with the glycosidic torsion χ (S4′–C1′–N1–C2)=−138.37° and χ (O4′–C1′–N1–C2)=−150.4°. In addition, the C5′–C4′ bond orientations are both *gauche*–*gauche*, i.e., γ (O5′–C5′–C4′–C3′)=51.32° for **8** and γ (O5′–C5′–C4′–C3′)=51.2° for dfC. Concerning the sugar puckering, every torsion angle of the sugar ring (ν_0–ν_4) shows some scattering; however, **8** and dfC are both suggested to be in a North-type puckered conformation (i.e., C3′-*endo* conformation). Thus, the thiosugar of **8** was estimated to have the pseudorotation phase angle P=−0.9° and the maximum puckering amplitude ν_m=45.7° while those of the furanose ring in dfC were reported as P=22.1° and ν_m=38.2°, respectively.[25,26] Torsion angles involving the fluorine atom, each of which is similar, are also listed in the table. The C3′-*endo* conformation of dfC is explained by the aforementioned *gauche* effect between the electronegative F2′ and O4′ atoms (F2′–C2′–C1′–O4′ fragment).[27] Since a sulfur atom is less electronegative than an oxygen atom, the *gauche* effect for the F2′–C2′–C1′–S4′ fragment in **8** is expected to be weaker compared with that of dfC.[28] However, at least in the crystals, this difference was not observed and both compounds adopted predominately the C3′-*endo* conformation. Contrary to the crystal structures, the structures in solution were somewhat different. Thus, the coupling constant of $J_{1',2'}$ of dfC in its ^{1}H NMR spectrum was reported as 1.7 Hz in DMSO-d_6, which supported the predominant C3′-*endo* conformation in solution (the $J_{3',4'}$ value of dfC was not given in the literature).[8b] In contrast, the J value of **8** in DMSO-d_6 was found to be $J_{1',2'}$=4.5 and $J_{3',4'}$=5.6 Hz, respectively (see Section 3). Although **8** is estimated to prefer the C3′-*endo* conformation from the equation to calculate the sugar puckering,[29] the differences in the $J_{1',2'}$ values between **8** and dfC should be taken into consideration in further structural investigations.

Table 2
Geometric parameters: bond lengths, angles, and torsion angles that represent important structural features of **8** and 2′-deoxy-2′-fluorocytidine (dfC)[a]

	8	dfC
Bond lengths (Å)		
C1′–N1	1.4699(19)	1.477(4)
C1′–X4′[b]	1.8287(15)	1.424(4)
C1′–C2′	1.533(2)	1.524(5)
C2′–C3′	1.520(2)	1.513(4)
C3′–C4′	1.539(2)	1.530(5)
C4′–X4′[b]	1.8295(16)	1.446(3)
Bond angles (°)		
N1–C1′–X4′[b]	113.85(10)	108.4(2)
X4′–C1′–C2′[b]	105.78(9)	106.3(2)
C1′–C2′–C3′	107.86(12)	103.3(2)
C2′–C3′–C4′	107.44(12)	101.5(2)
C3′–C4′–X4′[b]	105.99(10)	103.3(2)
C4′–X4′–C1′[b]	95.23(6)	110.3(2)
Torsion angles (°)		
X4′–C1′–N1–C2[b] (χ)	−138.37(11)	−150.4(2)
O5′–C5′–C4′–C3′ (γ)	51.32(17)	51.2(4)
C4′–X4′–C1′–C2′ (ν_0)	13.18(10)	−2.9(3)
X4′–C1′–C2′–C3′[b] (ν_1)	−35.35(14)	−21.4(3)
C1′–C2′–C3′–C4′[b] (ν_2)	45.72(16)	35.8(3)
C2′–C3′–C4′–X4′[b] (ν_3)	−34.17(14)	−37.8(3)
C3′–C4′–X4′–C1′[b] (ν_4)	11.83(11)	25.8(3)
F2′–C2′–C1′–X4′[b]	79.68(12)	91.9(3)
F2′–C2′–C1′–N1	−154.85(11)	−149.7(2)
F2′–C2′–C3′–C4′	−69.03(15)	−77.4(3)
F2′–C2′–C3′–O3′	55.29(16)	48.2(3)

[a] SDs (standard errors) are given in parentheses.
[b] X represents S in the case of **8** and O in the case of dfC.

In conclusion, we have synthesized the appropriately protected 2′-deoxy-2′-fluoro-4′-thiouridine **5**, -thiocytidine **7**, and -thioadenosine **35** derivatives as substrates for new modified RNAs. The synthesis of **5** and **7** was effectively carried out via 2,2′-*O*-anhydro-4′-thiouridine (**3**) by treatment with HF/pyridine in a manner similar to that of its 4′-*O*-congener. Unlike the pyrimidine derivatives, the synthesis of **35** from the 4′-thioadenosine derivatives was unsuccessful. Accordingly, its synthesis was achieved via the glycosylation of the fluorinated 4-thiosugar **25** with 6-chloropurine. The X-ray crystal structural analysis of **8** revealed that both 2′-deoxy-2′-fluorocytidine (dfC) and **8** adopted predominately the C3′-*endo* conformation.

3. Experimental section

3.1. General methods

Physical data were measured as follows: melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 270, 400, or 500 MHz and 67.5, 100, or 125 MHz instruments in $CDCl_3$ or DMSO-d_6 as the solvent with tetramethylsilane as

an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). All exchangeable protons were detected by the addition of D_2O. Mass spectra were measured on JEOL JMS-D300 spectrometer. TLC was done on Merck Kieselgel F_{254} precoated plates. Silica gel used for column chromatography was Merck silica gel 5715. All measurements for X-ray study were made on a Rigaku RAXIS RAPID imaging plate area detector with graphite monochromated Cu Kα radiation.

*3.2. 2,2′-O-Anhydro-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4′-thiouridine (**2**)*

To a solution of **1** (5.8 g, 11.5 mmol) in dry CH_2Cl_2 (120 mL) was added DMAP (5.6 g, 46.0 mmol) followed by Tf_2O (3.9 mL, 23.0 mmol) at 0 °C. After being stirred for 2 h at room temperature, the reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$. The mixture was concentrated in vacuo, and the residue was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$ followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column, eluted with MeOH in $CHCl_3$ (0–4%), to give **2** (5.2 g, 94%) as a white foam. An analytical sample was crystallized from EtOH: mp, 175–176 °C; FAB-LRMS *m/z* 485 (MH^+); FAB-HRMS calcd for $C_{21}H_{37}N_2O_5SSi_2$ (MH^+) 485.1961, found 485.1975; ^{1}H NMR ($CDCl_3$) δ: 7.19 (d, 1H, *J*=7.6 Hz), 6.09 (d, 1H, *J*=7.5 Hz), 5.67 (d, 1H, *J*=7.6 Hz), 5.23 (t, 1H, *J*=7.5 Hz), 4.47 (dd, 1H, *J*=7.5 and 9.9 Hz), 4.11 (dd, 1H, *J*=2.6 and 12.9 Hz), 3.86 (dd, 1H, *J*=2.1 and 12.9 Hz), 3.48 (ddd, 1H, *J*=2.1, 2.6, and 12.9 Hz), 1.13–0.93 (m, 28H); ^{13}C NMR ($CDCl_3$) δ: 171.7, 158.7, 134.8, 110.5, 87.1, 76.8, 61.5, 58.5, 57.3, 50.7, 18.5, 17.3, 17.2, 17.2, 17.1, 17.0, 16.9, 16.8, 13.8, 13.4, 12.5. Anal. Calcd for $C_{21}H_{36}N_2O_5SSi_2$: C, 52.03; H, 7.49; N, 5.78. Found: C, 51.74; H, 7.95; N, 5.31.

*3.3. 2,2′-O-Anhydro-4′-thiouridine (**3**)*[14]

To a solution of **2** (180 mg, 0.37 mmol) in MeOH (4 mL) was added NH_4F (140 mg, 3.7 mmol), and the reaction mixture was heated under reflux for 30 min. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was purified by a silica gel column, eluted with MeOH in $CHCl_3$ (20–30%), to give **3** (90 mg, quant.) as a white solid: FAB-LRMS *m/z* 243 (MH^+); FAB-HRMS calcd for $C_9H_{11}N_2O_4S$ (MH^+) 243.0439, found 243.0444; ^{1}H NMR (DMSO-d_6) δ: 7.80 (d, 1H, *J*=7.5 Hz), 6.18 (d, 1H, *J*=7.3 Hz), 5.88–5.84 (m, 2H), 5.35 (d, 1H, *J*=7.3 Hz), 5.20 (t, 1H, *J*=5.2 Hz), 4.67–4.66 (m, 1H), 3.41–3.14 (m, 3H).

*3.4. 1-(2-Deoxy-2-fluoro-4-thio-β-D-ribofuranosyl)uracil (**4**)*

To a solution of **3** (250 mg, 1.0 mmol) in MeOH (16 mL) was added HF/pyridine (70%, 260 μL, 10 mmol), and the reaction mixture was heated for 48 h at 150 °C in a steel container. The reaction mixture was neutralized with $NaHCO_3$. The solution was filtered through a Celite pad, which was washed with MeOH. The combined filtrate and washings were concentrated in vacuo. The residue was purified by a silica gel column, eluted with MeOH in $CHCl_3$ (5–6%), to give **4** (228 mg, 87%, crystallized from EtOH): mp 137–137.5 °C; FAB-LRMS *m/z* 263 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 11.4 (br s, 1H), 8.09 (d, 1H, *J*=8.0 Hz), 6.07 (dd, 1H, *J*=4.8 and 13.4 Hz), 5.77 (d, 1H, *J*=5.4 Hz), 5.69 (d, 1H, *J*=8.0 Hz), 5.19 (t, 1H, *J*=5.2 Hz), 5.20–5.05 (ddd, 1H, *J*=4.8, 8.3, and 50.4 Hz), 4.19–4.12 (m, 1H), 3.73–3.60 (m, 2H), 3.31–3.26 (m, 1H); ^{13}C NMR (DMSO-d_6) δ: 162.8, 150.8, 141.1, 102.3, 95.5 (d, *J*=228 Hz), 71.2 (d, *J*=16.7 Hz), 61.5, 60.5 (d, *J*=27.4 Hz), 52.0. Anal. Calcd for $C_9H_{11}FN_2O_4S$: C, 41.22; H, 4.23; N, 10.68. Found: C, 41.12; H, 4.05; N, 10.63.

*3.5. 1-[5-O-(4,4′-Dimethoxytrityl)-2-deoxy-2-fluoro-4-thio-β-D-ribofuranosyl]uracil (**5**)*

To a solution of **4** (622 mg, 2.3 mmol) in dry pyridine (23 mL) was added DMTrCl (1.2 g, 3.5 mmol), and the reaction mixture was stirred for 2 h at room temperature. The reaction was quenched by the addition of ice. The mixture was concentrated in vacuo. The residue was diluted with AcOEt, which was washed with H_2O and saturated aqueous $NaHCO_3$, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column, eluted with MeOH in $CHCl_3$ (0–2%), to give **5** (1.34 g, quant.) as an ivory foam: FAB-LRMS *m/z* 565 (MH^+); FAB-HRMS calcd for $C_{30}H_{29}FN_2O_6S$ (M^+) 564.1730, found 564.1728; ^{1}H NMR ($CDCl_3$) δ: 8.34 (br s, 1H), 7.97 (d, 1H, *J*=8.2 Hz), 7.43–7.24 (m, 9H), 6.87–6.84 (m, 4H), 6.20 (dd, 1H, *J*=3.3 and 13.0 Hz), 5.47 (d, 1H, *J*=8.2 Hz), 5.04–4.90 (dt, 1H, *J*=3.3 and 50.0 Hz), 4.29–4.20 (m, 1H), 3.79 (s, 6H), 3.61–3.49 (m, 3H), 2.25 (dd, 1H, *J*=1.5 and 6.7 Hz); ^{13}C NMR ($CDCl_3$) δ: 158.7, 150.2, 144.2, 140.7, 135.0, 134.9, 130.2, 130.1, 128.1, 128.0, 127.2, 113.3, 102.9, 96.6 (d, *J*=189 Hz), 87.4, 73.6 (d, *J*=19.1 Hz), 62.2, 55.2, 49.4.

*3.6. 1-[5-O-(4,4′-Dimethoxytrityl)-2-deoxy-2-fluoro-4-thio-β-D-ribofuranosyl]cytosine (**6**)*

To a solution of **5** (545 mg, 0.97 mmol) in dry acetonitrile (10 mL) were added Et_3N (400 μL, 2.9 mmol), Ac_2O (270 μL, 2.9 mmol), and DMAP (11 mg, 0.09 mmol), and the reaction mixture was stirred for 20 min at room temperature. The reaction was quenched by the addition of ice. The solvent was removed in vacuo and the residue was diluted with AcOEt. The organic layer was washed with H_2O and saturated aqueous $NaHCO_3$ followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in dry acetonitrile (10 mL), and Et_3N (400 μL, 2.9 mmol), TPSCl (880 mg, 2.9 mmol), and DMAP (350 mg, 2.9 mmol) were added to the solution. The mixture was stirred for 2 h at room temperature. After the starting material was consumed, concentrated NH_4OH (28%, 20 mL) was added

and the reaction mixture was kept for 22 h at room temperature. The whole mixture was concentrated in vacuo. The residue was diluted with H_2O and the aqueous layer was extracted with $CHCl_3$ (×3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column, eluted with MeOH in $CHCl_3$ (0–6%), to give **6** (450 mg, 82% in two steps) as a yellow foam: FAB-LRMS *m/z* 564 (MH^+); FAB-HRMS calcd for $C_{30}H_{31}FN_3O_5S$ (MH^+) 564.1969, found 564.1962; ^1H NMR (DMSO-d_6) δ: 7.85 (d, 1H, J=7.4 Hz), 7.40–7.22 (m, 9H), 6.90–6.89 (m, 5H), 6.07 (dd, 1H, J=2.8 and 14.8 Hz), 5.68 (br s, 2H), 4.95–4.84 (dt, 1H, J=2.8 and 49.8 Hz), 4.13–4.08 (m, 1H), 3.73 (s, 6H), 3.44–3.30 (m, 3H); ^{13}C NMR ($CDCl_3$) δ: 165.8, 158.6, 156.7, 144.6, 142.2, 135.6, 135.5, 130.3, 128.3, 128.0, 127.1, 113.3, 97.0 (d, J=190 Hz), 86.9, 72.6 (d, J=16.2 Hz), 63.5 (d, J=29.5 Hz), 55.3, 49.2.

3.7. N^4-Benzoyl-1-[5-O-(4,4′-dimethoxytrityl)-2-fluoro-4-thio-β-D-ribofuranosyl]cytosine (7)

To a solution of **6** (175 mg, 0.31 mmol) in dry pyridine (3 mL) was added TESCl (68 μL, 0.40 mmol), and the reaction mixture was stirred for 1.5 h at room temperature. After the starting material was consumed, BzCl (43 μL, 0.37 mmol) was added and the reaction mixture was stirred for an additional 1.5 h at the same temperature. The reaction was quenched by the addition of ice and concentrated in vacuo. The residue was diluted with AcOEt, which was washed with H_2O and saturated aqueous $NaHCO_3$ followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was then dissolved in THF (3 mL) and treated with TBAF (1 M in THF, 0.62 mL, 0.62 mmol) at 0 °C. After being stirred for 10 min at the same temperature, the reaction mixture was concentrated in vacuo, and the mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with H_2O and saturated aqueous $NaHCO_3$ followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column, eluted with MeOH in $CHCl_3$ (0–1%), to give **7** (144 mg, 70% in three steps) as a white foam: FAB-LRMS *m/z* 668 (MH^+); FAB-HRMS calcd for $C_{37}H_{35}FN_3O_6S$ (MH^+) 668.2231, found 668.2229; ^1H NMR ($CDCl_3$) δ: 9.23 (br s, 1H), 8.64 (d, 1H, J=8.0 Hz), 7.90 (d, 2H, J=7.4 Hz), 7.56–7.23 (m, 13H), 6.87–6.85 (m, 4H), 6.10 (d, 1H, J=13.1 Hz), 5.18–5.08 (br d, 1H, J=49.8 Hz), 4.35–4.28 (m, 1H), 3.79 (s, 6H), 3.66–3.52 (m, 3H); ^{13}C NMR ($CDCl_3$) δ: 162.5, 158.5, 155.5, 146.3, 144.0, 135.4, 135.3, 133.0, 132.7, 130.1, 128.7, 128.2, 127.9, 127.7, 127.0, 122.8, 113.1, 96.5 (d, J=189 Hz), 87.0, 72.4 (d, J=17.9 Hz), 64.5 (d, J=29.8 Hz), 61.6, 55.1, 49.2.

3.8. 1-(2-Deoxy-2-fluoro-4-thio-β-D-ribofuranosyl)-cytosine (8)

Compound **6** (200 mg, 0.35 mmol) was dissolved in 2% TFA in CH_2Cl_2 (3 mL), and the reaction mixture was stirred for 1 h at room temperature. The solvent was removed in vacuo, and the residue was purified by a silica gel column, eluted with MeOH in $CHCl_3$ (0–30%), to give **8** (45 mg, 48% crystallized from H_2O): mp, 159–160.5; FAB-LRMS *m/z* 262 (MH^+); ^1H NMR (DMSO-d_6) δ: 8.06 (d, 1H, J=7.5 Hz), 7.27 and 7.24 (each br s, each 1H), 6.16 (dd, 1H, J=4.5 and 13.9 Hz), 5.78 (d, 1H, J=7.5 Hz), 5.71 (d, 1H, J=5.5 Hz), 5.25 (t, 1H, J=5.3 Hz), 5.07–4.92 (ddd, 1H, J=3.3, 4.5, and 40.5 Hz), 4.12 (dddd, 1H, J=3.3, 5.5, 5.6, and 9.1 Hz), 3.73 (ddd, 1H, J=4.5, 5.3, and 11.7 Hz), 3.63 (dt, 1H, J=5.3 and 11.7 Hz), 3.31 (ddd, 1H, J=4.5, 5.3, and 5.6 Hz); ^{13}C NMR (DMSO-d_6) δ: 166.2, 157.0, 143.1, 97.0 (d, J=190.8 Hz), 96.4, 72.1 (d, J=16.6 Hz), 62.4 (d, J=27.7 Hz), 62.3, 52.3. Anal. Calcd for $C_9H_{12}FN_3O_3S\cdot 2H_2O$: C, 36.36; H, 5.42; N, 14.13. Found: C, 36.20; H, 5.42; N, 14.07.

3.9. 9-[2-O-Acetyl-3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-arabinofuranosyl]adenine (11) and 9-[2-deoxy-3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-erythro-pent-1-enofuranosyl]adenine (12)

To a solution of **9** (500 mg, 0.95 mmol) in dry CH_2Cl_2 (10 mL) were added DMAP (460 mg, 3.8 mmol) and Tf_2O (240 μL, 1.4 mmol) at 0 °C. After being stirred for 40 min at room temperature, the reaction was quenched by the addition of ice. The solvent was removed in vacuo and the residue was diluted with AcOEt. The organic layer was washed with H_2O and saturated aqueous $NaHCO_3$ followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in DMF (10 mL) containing HMPA (4 mL), and LiOAc (470 mg, 5.7 mmol) was added to the solution. After being stirred for 18 h at room temperature, the mixture was concentrated in vacuo, and the residue was partitioned between AcOEt and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (2:1 to 1:2), to give **11** (100 mg, 19%) as a yellow foam and **12** (240 mg, 49%) as an orange foam.

Physical data for **11**: FAB-LRMS *m/z* 568 (MH^+); FAB-HRMS calcd for $C_{24}H_{42}N_5O_5SSi_2$ (MH^+) 568.2445, found 568.2454; ^1H NMR ($CDCl_3$) δ: 8.40 (s, 1H), 8.31 (s, 1H), 6.12 (d, 1H, J=6.3 Hz), 5.65 (br s, 2H), 5.48 (dd, 1H, J=6.3 and 10.3 Hz), 4.65 (t, 1H, J=10.3 Hz), 4.20 (dd, 1H, J=3.3 and 12.2 Hz), 4.06 (dd, 1H, J=2.4 and 12.2 Hz), 3.37 (ddd, 1H, J=2.4, 3.3, and 10.3 Hz), 1.11–0.98 (m, 28H); ^{13}C NMR ($CDCl_3$) δ: 169.9, 155.6, 153.0, 150.9, 140.6, 119.8, 76.6, 71.8, 59.8, 53.6, 46.4, 20.5, 17.6, 17.5, 17.4, 17.4, 17.1, 17.0, 16.9, 16.7, 13.5, 13.2, 12.4.

Physical data for **12**: FAB-LRMS *m/z* 508 (MH^+); FAB-HRMS calcd for $C_{22}H_{38}N_5O_3SSi_2$ (MH^+) 508.2233, found 508.2244; ^1H NMR ($CDCl_3$) δ: 8.44 (s, 1H), 7.92 (s, 1H), 6.39 (d, 1H, J=2.6 Hz), 5.65 (br s, 2H), 5.64 (dd, 1H, J=2.6 and 4.4 Hz) 4.17 (dd, 1H, J=3.7 and 11.0 Hz), 4.12–4.07 (m, 1H), 4.02–3.97 (m, 1H), 1.12–1.01 (m, 28H); ^{13}C NMR ($CDCl_3$) δ: 156.0, 153.8, 149.9, 138.8, 132.3, 120.0, 113.2, 80.9, 65.0, 57.7, 17.7, 17.5, 17.5, 17.4, 17.3, 17.2, 17.0, 16.9, 13.6, 13.1, 12.5.

3.10. N^6-Benzoyl-9-[3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-arabinofuranosyl]adenine (**13**) and N^6-benzoyl-9-[3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-α-D-ribofuranosyl]adenine (**14**)

A mixture of **10** (200 mg, 0.32 mmol) and Ac_2O (1.5 mL) in DMSO (3 mL) was stirred at room temperature for 2.5 h. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$. The mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$ followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in MeOH (6 mL), and $NaBH_4$ (42 mg, 1.1 mmol) was added to the solution. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo, and the residue was partitioned between AcOEt and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column, eluted with MeOH in $CHCl_3$ (0–2%), to give **13** (56 mg, 28%) as a yellow foam and **14** (110 mg, 53%) as a yellow foam.

Physical data for **13**: FAB-LRMS *m/z* 630 (MH^+); FAB-HRMS calcd for $C_{29}H_{44}N_5O_5SSi_2$ (MH^+) 630.2602, found 630.2609; ^{1}H NMR ($CDCl_3$) δ: 9.17 (br s, 1H), 8.69 (s, 1H), 8.56 (s, 1H), 8.01 (d, 2H, *J*=7.9 Hz), 7.60–7.50 (m, 3H), 6.09 (d, 1H, *J*=6.2 Hz), 4.50–4.47 (m, 1H), 4.38 (t, 1H, *J*=9.2 Hz), 4.20–4.17 (m, 1H), 4.00–3.97 (m, 1H), 3.69 (br s, 1H), 3.35–3.32 (m, 1H), 1.11–0.98 (m, 28H); ^{13}C NMR ($CDCl_3$) δ: 164.9, 152.6, 152.3, 149.5, 143.3, 133.7, 132.7, 128.8, 128.0, 123.0, 77.3, 74.5, 60.0, 56.4, 47.5, 17.6, 17.5, 17.4, 17.2, 17.1, 17.0, 16.9, 13.5, 13.2, 13.1, 12.6.

Physical data for **14**: FAB-LRMS *m/z* 630 (MH^+); FAB-HRMS calcd for $C_{29}H_{44}N_5O_5SSi_2$ (MH^+) 630.2602, found 630.2589; ^{1}H NMR ($CDCl_3$) δ: 8.99 (br s, 1H), 8.78 (s, 1H), 8.57 (s, 1H), 8.02 (d, 2H, *J*=7.0 Hz), 7.63–7.51 (m, 3H), 6.46 (d, 1H, *J*=4.5 Hz), 4.50–4.47 (m, 2H), 4.13 (dd, 1H, *J*=2.8 and 12.3 Hz), 4.04–3.92 (m, 2H), 2.90 (br s, 1H), 1.16–1.00 (m, 28H).

3.11. 1,4-Anhydro-2-O-(p-nitrobenzoyl)-3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-D-arabitol (**16**)

To a mixture of **15** (1.0 g, 2.5 mmol), *p*-nitrobenzoic acid (840 mg, 5.0 mmol), and PPh_3 (1.3 g, 5.0 mmol) in dry THF (20 mL) was added a solution of DIAD (990 μL, 5.0 mmol) in dry THF (5 mL) dropwise via cannula. After the reaction mixture was stirred for 20 min at room temperature, the reaction was quenched by the addition of ice. The solvent was removed in vacuo. The residue was partitioned between AcOEt and H_2O. The separated organic layer was washed with H_2O and saturated aqueous $NaHCO_3$ followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (40:1 to 30:1), to give **16** (1.3 g, quant.) as a yellow oil: FAB-LRMS *m/z* 541 (M^+); FAB-HRMS calcd for $C_{24}H_{39}NO_7SSi_2$ (M^+) 541.1986, found 541.1978; ^{1}H NMR ($CDCl_3$) δ: 8.30 (d, 2H, *J*=8.8 Hz), 8.20 (d, 2H, *J*=8.8 Hz), 5.48 (dd, 1H, *J*=8.2 and 15.8 Hz), 4.52 (t, 1H, *J*=8.2 Hz), 4.07 (dd, 1H, *J*=3.4 and 12.5 Hz), 3.85 (dd, 1H, *J*=4.9 and 12.5 Hz), 3.35–3.31 (m, 1H), 3.29–3.26 (m, 1H), 2.79 (dd, 1H, *J*=8.2 and 10.9 Hz), 1.43–0.93 (m, 28H); ^{13}C NMR ($CDCl_3$) δ: 163.8, 150.4, 134.8, 130.6, 123.4, 79.9, 74.3, 61.7, 48.4, 28.5, 17.5, 17.4, 17.3, 17.1, 13.9, 13.4, 12.9, 12.6.

3.12. 1,4-Anhydro-2-O-(p-nitrobenzoyl)-4-thio-D-arabitol (**17**)

To a solution of **16** (2.6 g, 4.8 mmol) in THF (50 mL) containing AcOH (550 μL, 9.6 mmol) was added TBAF (1 M in THF, 9.6 mL, 9.6 mmol) at 0 °C. After being stirred for 1 h at the same temperature, the reaction mixture was concentrated in vacuo. The residue was purified by a silica gel column, eluted with MeOH in $CHCl_3$ (0–1%), to give **17** (1.4 g, quant.) as a white solid. An analytical sample was crystallized from EtOH/hexane: mp 139 °C; FAB-LRMS *m/z* 299 (M^+); FAB-HRMS calcd for $C_{12}H_{13}NO_6SSi_2$ (M^+) 299.046, found 299.0462; ^{1}H NMR (DMSO-d_6) δ: 8.35 (d, 2H, *J*=8.7 Hz), 8.16 (d, 2H, *J*=8.7 Hz), 5.64 (d, 1H, *J*=4.9 Hz), 5.30 (dd, 1H, *J*=5.0 and 9.6 Hz), 5.00 (t, 1H, *J*=5.4 Hz), 4.21 (dd, 1H, *J*=4.5 and 9.6 Hz), 3.72–3.66 (m, 1H), 3.44–3.38 (m, 1H), 3.25–3.18 (m, 2H), 2.90 (dd, 1H, *J*=5.0 and 11.6 Hz); ^{13}C NMR (DMSO-d_6) δ: 163.3, 150.0, 134.6, 130.4, 123.7, 81.0, 76.0, 63.6, 53.4, 31.2. Anal. Calcd for $C_{12}H_{13}NO_6S$: C, 48.16; H, 4.38; N, 4.68. Found: C, 48.22; H, 4.29; N, 4.72.

3.13. 1,4-Anhydro-3,5-O-(p-methoxybenzylidene)-4-thio-D-arabitol (**19**)

To a solution of **17** (7.2 g, 24 mmol) in dry DMF (240 mL) were added CSA (2.8 g, 12 mmol) and *p*-methoxybenzaldehyde dimethylacetal (16 mL, 96 mmol). The reaction mixture was stirred under reduced pressure (20 mmHg) at 35 °C for 7.5 h. The reaction mixture was cooled to room temperature and the reaction was quenched by the addition of saturated aqueous $NaHCO_3$. The whole mixture was concentrated in vacuo and the residue was diluted with AcOEt. The organic layer was washed with H_2O and saturated aqueous $NaHCO_3$ followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo to give **18**. The resulting **18** was dissolved in a MeOH solution of $MeNH_2$ (40%, 200 mL) and the mixture was kept for 3 h at room temperature. The solvent was removed in vacuo and the residue was coevaporated with MeOH. The residue was purified by a silica gel column, eluted with hexane/AcOEt (7:1–3:2), to give **19** (5.5 g, 85%) as a white solid. An analytical sample was crystallized from EtOH/hexane: mp 142 °C; FAB-LRMS *m/z* 269 (MH^+); FAB-HRMS calcd for $C_{13}H_{16}O_4S$ (MH^+) 269.0848, found 269.0843; ^{1}H NMR ($CDCl_3$) δ: 7.43 (d, 2H, *J*=8.8 Hz), 6.91 (d, 2H, *J*=8.8 Hz), 5.53 (s, 1H), 4.40 (dd, 1H, *J*=4.3 and 10.4 Hz), 4.33 (ddd, 1H, *J*=3.3, 8.2, and 10.4 Hz), 3.89–3.83 (m, 1H), 3.80 (s, 3H), 3.75–3.70 (m, 1H), 3.30–3.23 (m, 1H), 3.17 (dd, 1H, *J*=8.2 and 10.4 Hz), 2.74 (dd, 1H,

J=8.2 and 10.0 Hz), 2.45 (d, 1H, J=3.3 Hz); ^{13}C NMR ($CDCl_3$) δ: 160.0, 129.4, 127.4, 113.6, 102.1, 87.8, 73.4, 72.0, 55.3, 39.1, 30.2. Anal. Calcd for $C_{13}H_{16}O_4S$: C, 58.19; H, 6.01. Found: C, 57.83; H, 5.94.

3.14. 1,4-Anhydro-2-deoxy-2-fluoro-3,5-O-(p-methoxybenzylidene)-4-thio-D-ribitol (**20**)

To a solution of **19** (5.0 g, 19 mmol) in dry dioxane (370 mL) were added DBU (28 mL, 186 mmol) and perfluoro-1-butanesulfonyl fluoride (PBSF, 35 mL, 186 mmol) at 0 °C. The reaction mixture was heated under reflux for 15 min. The reaction mixture was cooled to room temperature and the reaction was quenched by the addition of saturated aqueous $NaHCO_3$. The solvent was removed in vacuo. The residue was diluted with AcOEt. The organic layer was washed with H_2O and saturated aqueous $NaHCO_3$ followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (5:1 to 1:1), to give **20** (4.4 g, 88%) as a white solid. An analytical sample was crystallized from EtOH/hexane: mp 131–132 °C; FAB-LRMS m/z 271 (MH^+); ^{1}H NMR ($CDCl_3$) δ: 7.45 (d, 2H, J=8.6 Hz), 6.89 (d, 2H, J=8.6 Hz), 5.53 (s, 1H), 5.40–5.26 (br d, 1H, J=53 Hz), 4.58 (dd, 1H, J=3.7 and 10.2 Hz), 3.91–3.86 (m, 1H), 3.80 (s, 3H), 3.71–3.59 (m, 2H), 3.22–3.03 (m, 2H); ^{13}C NMR ($CDCl_3$) δ: 160.0, 129.2, 127.5, 113.6, 102.3, 90.3 (d, J=185 Hz), 85.8 (d, J=17.4 Hz), 72.0, 55.3, 38.8, 32.2 (d, J=23.2 Hz). Anal. Calcd for $C_{13}H_{15}FO_3S$: C, 57.76; H, 5.59. Found: C, 57.79; H, 5.49.

3.15. 1,4-Anhydro-2-deoxy-2-fluoro-4-thio-D-ribitol (**21**)

A mixture of **20** (3.0 g, 11.1 mmol) and 80% aqueous AcOH (100 mL) was heated at 60 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was coevaporated with MeOH and purified by a silica gel column, eluted with hexane/AcOEt (2:1 to 1:1), to give **21** (1.4 g, 86%) as an ivory solid. An analytical sample was crystallized from EtOH: mp 76 °C; FAB-LRMS m/z 271 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 5.41 (d, 1H, J=6.3 Hz), 5.09–4.96 (dddd, 1H, J=2.8, 4.0, 6.3, and 53.2 Hz), 4.86 (t, 1H, J=4.5 Hz), 3.85–3.78 (m, 1H), 3.77–3.73 (m, 1H), 3.39–3.21 (m, 1H), 3.24 (m, 1H), 3.01 (ddd, 1H, J=4.0, 12.0, and 33.8 Hz), 2.83 (ddd, 1H, J=2.8, 12.0, and 19.4 Hz); ^{13}C NMR (DMSO-d_6) δ: 94.8 (d, J=180 Hz), 77.3, 62.1, 50.0 (d, J=1.6 Hz), 31.2 (d, J=21.6 Hz). Anal. Calcd for $C_5H_9FO_2S$: C, 39.46; H, 5.96. Found: C, 39.31; H, 5.63.

3.16. 1,4-Anhydro-2-deoxy-2-fluoro-3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-D-ribitol (**22**)

To a solution of **21** (85 mg, 0.56 mmol) in dry pyridine (5 mL) was added a solution of $TIPDSCl_2$ (268 μL, 0.84 mmol) in dry pyridine (1 mL) dropwise via cannula at 0 °C. After being stirred for 3 h at room temperature, the reaction was quenched by the addition of saturated aqueous $NaHCO_3$. The solvent was removed in vacuo, and the residue was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (30:1), to give **22** (202 mg, 91%) as a colorless oil: FAB-LRMS m/z 395 (MH^+); FAB-HRMS calcd for $C_{17}H_{36}FO_3SSi_2$ (MH^+) 395.1908, found 395.1908; ^{1}H NMR ($CDCl_3$) δ: 5.15–5.02 (br d, 1H, J=53.4 Hz), 4.17 (ddd, 1H, J=2.6, 9.9, and 28.4 Hz), 4.07 (dd, 1H, J=2.8 and 12.5 Hz), 3.93 (dd, 1H, J=2.0 and 12.5 Hz), 3.56–3.53 (m, 1H), 3.15–2.93 (m, 2H), 1.09–0.98 (m, 28H); ^{13}C NMR ($CDCl_3$) δ: 94.6 (d, J=186.0 Hz), 75.6 (d, J=18.0 Hz), 58.8, 48.5, 31.2 (d, J=22.8 Hz), 17.8, 17.7, 17.7, 17.6, 17.6, 17.5, 17.4, 13.9, 13.6, 13.0, 12.9.

3.17. 1,4-Anhydro-5-O-(tert-butyldiphenylsilyl)-2-deoxy-2-fluoro-3-O-(p-methoxybenzoyl)-4-thio-D-ribitol (**23**)

To a solution of **21** (550 mg, 3.6 mmol) in DMF (24 mL) were added imidazole (880 mg, 13 mmol) and TBDPSCl (1.1 mL, 4.3 mmol), and the reaction mixture was stirred for 2 h at room temperature. The reaction was quenched by the addition of MeOH. The solution was concentrated in vacuo, and the residue was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in dry pyridine (24 mL), and p-methoxybenzoyl chloride (1.5 mL, 11 mmol) was added to the solution at 0 °C. The reaction mixture was stirred for 1.5 h at room temperature. The reaction was quenched by the addition of ice. The solution was concentrated in vacuo, and the residue was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (10:1), to give **23** (1.9 g, quant. in two steps) as a colorless oil: FAB-LRMS m/z 525 (MH^+); FAB-HRMS calcd for $C_{29}H_{34}FO_4SSi$ (MH^+) 525.1937, found 525.1937; ^{1}H NMR ($CDCl_3$) δ: 7.98 (d, 2H, J=7.4 Hz), 7.69–7.63 (m, 4H), 7.43–7.30 (m, 6H), 6.92 (d, 2H, J=8.6 Hz), 5.45–5.34 (m, 2H), 3.89–3.83 (m, 3H), 3.87 (s, 3H), 3.27–3.13 (m, 2H), 1.04 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 165.3, 163.7, 135.6, 132.9, 131.9, 129.7, 127.7, 121.6, 113.6, 92.1 (d, J=187 Hz), 76.3 (d, J=16.7 Hz), 64.4, 55.4, 48.2, 31.6 (d, J=21.4 Hz), 26.6, 19.1, 14.1.

3.18. 1,4-Anhydro-5-O-(tert-butyldiphenylsilyl)-2-deoxy-2-fluoro-3-O-(p-methoxybenzoyl)-4-sulfinyl-D-ribitol (**24**)

Ozone gas was bubbled through a solution of **23** (4.7 g, 9.1 mmol) in CH_2Cl_2 (60 mL) at −78 °C. After 2 h, argon gas was bubbled through the reaction mixture to remove excess ozone. The reaction mixture was allowed to warm to

room temperature and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (2:1 to 1:1), to give **24** (4.9 g, quant.) as a diastereomeric mixture (isomer a/isomer b=0.8:1): FAB-LRMS *m/z* 541 (MH^+); FAB-HRMS calcd for $C_{29}H_{34}FO_5SSi$ (MH^+) 541.1881, found 541.1876; ^1H NMR ($CDCl_3$) δ: 8.00 (d, 2H of isomer a, J=9.1 Hz), 7.83 (d, 2H of isomer b, J=9.1 Hz), 7.71–7.21 (m, 10H of isomers a and b), 6.94 (d, 2H of isomer a, J=9.1 Hz), 6.89 (d, 2H of isomer b, J=9.1 Hz), 5.72–5.61 (m, 1H of isomers a and b), 5.57–5.49 (m, 1H of isomers a and b), 4.43 (dd, 1H of isomer a, J=2.3 and 11.3 Hz), 4.35 (t, 1H of isomer b, J=10.3 Hz), 4.02–3.97 (m, 1H of isomers a and b), 3.89 and 3.87 (each s, each 3H of isomers a and b), 3.86–3.78 (m, 1H of isomer b), 3.62–3.60 (m, 1H of isomer a), 3.50–3.45 (m, 1H of isomer b), 3.35–3.26 (m, 2H of isomer a), 3.14–3.04 (m, 1H of isomer b), 1.07 and 1.02 (each s, each 9H of isomers a and b); ^{13}C NMR ($CDCl_3$) δ: 165.3, 165.2, 164.0, 163.9, 135.6, 135.5, 135.4, 133.0, 132.5, 132.1, 132.0, 132.0, 132.0, 130.1, 130.0, 129.9, 129.9, 128.0, 127.9, 127.8, 121.0, 120.9, 113.9, 113.7, 92.2 (d, J=188.8 Hz), 90.5 (d, J=185.8 Hz), 77.3, 74.3 (d, J=17.1 Hz), 73.1, 72.4 (d, J=17.1 Hz), 63.5, 58.6, 58.0 (d, J=22.8 Hz), 57.6, 55.6, 55.5, 54.4 (d, J=20.0 Hz), 29.7, 26.8, 26.7, 19.2.

*3.19. 1-O-Acetyl-5-O-(tert-butyldiphenylsilyl)-2-deoxy-2-fluoro-3-O-(p-methoxybenzoyl)-4-thio-α/β-D-ribofuranose (**25**)*

A mixture of **24** (360 mg, 0.67 mmol) and Ac_2O (2.5 mL) was heated under reflux for 40 min. The reaction mixture was cooled to room temperature and the reaction was quenched by the addition of ice. The mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$ followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (2:1 to 1:1), to give **25** (370 mg, 94%) as a diastereomeric mixture (isomer a/isomer b=0.4:1): FAB-LRMS *m/z* 583 (MH^+); FAB-HRMS calcd for $C_{31}H_{36}FO_6SSi$ (MH^+) 583.1986, found 583.1983; ^1H NMR ($CDCl_3$) δ: 8.06 (d, 2H of isomer b, J=8.6 Hz), 7.96 (d, 2H of isomer a, J=9.0 Hz), 7.73–7.26 (m, 10H of isomers a and b), 6.94–6.92 (m, 2H of isomers a and b), 6.35 (t, 1H of isomer b, J=4.5 Hz), 6.04 (dd, 1H of isomer a, J=1.8 and 9.9 Hz), 5.74–5.71 (m, 1H of isomer b), 5.66–5.57 (m, 1H of isomer a), 5.40–5.25 (m, 1H of isomers a and b), 3.90–3.70 (m, 6H of isomers a and b), 2.19 (s, 3H of isomer b), 2.10 (s, 3H of isomer a), 1.06 (s, 9H of isomer b), 1.03 (s, 9H of isomer a); ^{13}C NMR ($CDCl_3$) δ: 170.2, 169.4, 165.4, 165.2, 163.9, 163.8, 135.8, 135.7, 135.6, 135.6, 132.9, 132.7, 132.5, 132.3, 132.1, 130.1, 130.0, 129.9, 129.8, 127.9, 127.8, 127.7, 121.8, 121.4, 113.8, 113.7, 93.9 (d, J=188.3 Hz), 90.2 (d, J=206.3 Hz), 76.9, 76.8, 74.1 (d, J=16.9 Hz), 74.0 (d, J=16.7 Hz), 64.6, 63.2, 58.2, 55.5, 50.5, 50.5, 48.6, 48.6, 26.8, 26.7, 21.1, 21.0, 19.3.

*3.20. 6-Chloro-7-[5-O-(tert-butyldiphenylsilyl)-2-deoxy-2-fluoro-3-O-(4-methoxybenzoyl)-4-thio-α/β-D-ribofuranosyl]-9H-purine (**30**) and 6-chloro-9-[5-O-(tert-butyldiphenylsilyl)-2-deoxy-2-fluoro-3-O-(4-methoxybenzoyl)-4-thio-α/β-D-ribofuranosyl]-9H-purine (**31**)*

To a suspension of 6-chloropurine (247 mg, 1.6 mmol) in dry acetonitrile (10 mL) was added HMDS (510 μL, 2.4 mmol), and the mixture was heated under reflux until the reaction mixture turned to be a clear solution to give silylated 6-chloropurine. After the solution was cooled to room temperature, a solution of **25** (238 mg, 0.4 mmol) in dry acetonitrile (4 mL) and TMSOTf (217 μL, 1.2 mmol) were successively added to the solution of silylated 6-chloropurine at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was heated under reflux for 16 h. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$, and the reaction mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$ followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (4:1 to 1:1), to give a mixture of α/β-anomer (1:1) of **31** (98 mg, 37%) as a yellow foam and a mixture of α/β-anomer of **30** (104 mg, 39%) as an orange foam.

Physical data for **31**: FAB-LRMS *m/z* 677 (MH^+); FAB-HRMS calcd for $C_{34}H_{35}FN_4O_4SSi$ (MH^+) 677.1820, found 677.1816; ^1H NMR ($CDCl_3$) δ: 8.74 (s, 0.5H), 8.70 (s, 0.5H), 8.69 (d, 0.5H, J=2.2 Hz), 8.56 (s, 0.5H), 7.98 (d, 1H, J=8.6 Hz), 7.88 (d, 1H, J=9.1 Hz), 7.73–7.28 (m, 10H), 6.93–6.89 (m, 2H), 6.68 (dd, 0.5H, J=4.0 and 18.3 Hz), 6.37 (dd, 0.5H, J=4.0 and 13.1 Hz), 5.84–5.47 (m, 2H), 4.40–4.38 (m, 0.5H), 4.09–3.97 (m, 2.5H), 3.86 and 3.85 (each s, 3H), 1.11 and 1.08 (each s, 9H); ^{13}C NMR ($CDCl_3$) δ: 165.1, 165.0, 164.1, 164.1, 152.2, 152.1, 151.9, 151.6, 151.5, 151.2, 145.7, 145.7, 144.0, 135.6, 135.6, 132.6, 132.5, 132.5, 132.3, 132.1, 132.1, 131.7, 130.1, 130.1, 130.0, 130.0, 128.0, 127.9, 127.9, 127.9, 121.0, 120.7, 113.9, 113.9, 93.9 (d, J=197.9 Hz), 91.2 (d, J=196.7 Hz), 74.3 (d, J=15.6 Hz), 73.1 (d, J=15.6 Hz), 63.4, 63.5, 60.9, 60.6, 60.4, 57.1, 56.9, 55.6, 51.2, 49.9, 26.9, 26.8, 19.3, 19.3.

Physical data for **30**: FAB-LRMS *m/z* 677 (MH^+); FAB-HRMS calcd for $C_{34}H_{35}FN_4O_4SSi$ (MH^+) 677.1821, found 677.1827; ^1H NMR ($CDCl_3$) δ: 9.08 (s, 0.5H), 9.02 (d, 0.5H, J=1.7 Hz), 8.94 (s, 0.5H), 8.91 (s, 0.5H), 7.95 (d, 1H, J=9.1 Hz), 7.83 (d, 1H, J=9.7 Hz), 7.71–7.35 (m, 10H), 6.96–6.90 (m, 2.5H), 6.71 (dd, 0.5H, J=2.8 and 10.8 Hz), 5.68–5.45 (m, 2H), 4.28–4.24 (m, 0.5H), 4.08–3.91 (m, 2.5H), 3.89 and 3.86 (each s, 3H), 1.11 and 1.10 (each s, 9H); ^{13}C NMR ($CDCl_3$) δ: 165.1, 165.0, 164.2, 162.8, 162.5, 153.0, 152.6, 149.5, 147.7, 142.8, 142.6, 135.7, 135.6, 135.5, 132.5, 132.4, 132.4, 132.1, 130.2, 130.2, 130.1, 130.1, 128.1, 128.0, 122.9, 122.5, 120.7, 120.5, 114.0, 113.9, 95.0 (d, J=196.7 Hz), 91.4 (d, J=201.5 Hz), 74.6 (d, J=16.7 Hz), 72.2 (d, J=16.7 Hz), 63.8, 62.8 (d, J=29.9 Hz), 62.4, 60.3 (d, J=17.9 Hz), 55.6, 51.6, 49.6, 26.9, 26.8, 19.3, 19.2.

3.21. 9-[5-O-(tert-Butyldiphenylsilyl)-2-deoxy-2-fluoro-4-thio-α/β-D-ribofuranosyl]adenine (32)

Compound **31** (170 mg, 0.24 mmol) was dissolved in ethanolic ammonia (saturated at 0 °C, 5 mL) and the mixture was heated at 80 °C for 3.5 h in a steel container. The solvent was removed in vacuo. The residue was dissolved in a MeOH solution of $MeNH_2$ (40%, 5 mL) and the mixture was kept at room temperature for 1 h. The solvent was removed and the residue was coevaporated with MeOH. The residue was purified by a silica gel column, eluted with MeOH in $CHCl_3$ (0–3%), to give a mixture of α/β-anomer (1:1) of **32** (110 mg, 88%) as a white foam: FAB-LRMS *m/z* 524 (MH^+); FAB-HRMS calcd for $C_{26}H_{31}FN_5O_2SSi$ (MH^+) 524.1952, found 524.1963; ^{1}H NMR (DMSO-d_6) δ: 8.35 (s, 1H), 8.15 (s, 0.5H), 8.09 (s, 0.5H), 7.66–7.41 (m, 10H), 7.10 and 6.89 (each br s, 2H), 6.47 (dd, 0.5H, *J*=4.0 and 22.3 Hz), 6.17 (dd, 0.5H, *J*=4.0 and 13.7 Hz), 5.92–5.88 (m, 1H), 5.57–5.46 (dt, 0.5H, *J*=4.0 and 49.8 Hz), 5.19–5.07 (dt, 1H, *J*=4.0 and 49.8 Hz), 4.57–4.53 (m, 0.5H), 4.36–4.34 (m, 0.5H), 4.14–3.95 (m, 2H), 3.80–3.76 (m, 0.5H), 3.59–3.57 (m, 0.5H), 1.00 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 155.7, 155.6, 153.1, 152.9, 149.8, 149.7, 141.4, 139.4, 135.7, 135.6, 135.5, 132.7, 132.6, 132.6, 130.1, 130.1, 130.0, 127.9, 127.9, 127.9, 127.9, 119.9, 118.8, 96.8 (d, *J*=191.6 Hz), 93.8 (d, *J*=193.6 Hz), 75.2 (d, *J*=16.2 Hz), 73.0 (d, *J*=16.2 Hz), 64.8, 64.2, 57.9 (d, *J*=29.5 Hz), 56.4 (d, *J*=17.1 Hz), 52.8, 51.4, 26.9, 26.8, 19.3, 19.3.

3.22. N^6-Benzoyl-9-[5-O-(tert-butyldiphenylsilyl)-2-deoxy-2-fluoro-4-thio-β-D-ribofuranosyl]adenine (33)

To a solution of **32** (1.5 g, 2.8 mmol) in dry pyridine (30 mL) was added BzCl (1.7 mL, 14 mmol) at 0 °C and the mixture was stirred for 1 h at room temperature. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$. The reaction mixture was concentrated in vacuo, and the residue was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$ followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in MeOH (30 mL). To this solution, 1 N aqueous NaOH (10 mL) was added and the mixture was stirred for 30 min at room temperature. The reaction mixture was neutralized with 1 N aqueous HCl. The mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$ followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a flush silica gel column, eluted with hexane/AcOEt (1:1 to 0:1), to give **33** (740 mg, 42%) as a white foam: FAB-LRMS *m/z* 628 (MH^+); FAB-HRMS calcd for $C_{33}H_{35}FN_5O_3SSi$ (MH^+) 628.2214, found 628.2220; ^{1}H NMR ($CDCl_3$) δ: 9.14 (br s, 1H), 8.73 (s, 1H), 8.30 (s, 1H), 7.98 (d, 2H, *J*=7.7 Hz), 7.70–7.38 (m, 13H), 6.25 (dd, 1H, *J*=3.4 and 13.1 Hz), 5.42 (dt, 1H, *J*=3.4 and 49.8 Hz), 4.60–4.57 (m, 1H), 4.07–3.95 (m, 2H), 3.72–3.68 (m, 1H), 2.79 (br s, 1H), 1.08 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 164.6, 152.8, 151.6, 149.6, 141.8, 135.5, 133.4, 132.8, 132.5, 132.3, 130.1, 128.8, 127.9, 123.4, 96.3 (d, *J*=192 Hz), 73.7, 64.4, 60.0 (d, *J*=28.7 Hz), 51.0, 26.8, 19.2.

3.23. N^6-Benzoyl-9-(2-deoxy-2-fluoro-4-thio-β-D-ribofuranosyl)adenine (34)

To a solution of **33** (270 mg, 0.42 mmol) in THF (4 mL) was added TBAF (1 M in THF, 0.84 mL, 0.84 mmol) at 0 °C. After being stirred for 30 min at the same temperature, the reaction mixture was concentrated in vacuo. The residue was purified by a silica gel column, eluted with MeOH in $CHCl_3$ (0–2.5%), to give **34** (160 mg, 98%) as a white glass: FAB-LRMS *m/z* 390 (MH^+); FAB-HRMS calcd for $C_{17}H_{17}FN_5O_3S$ (MH^+) 390.1036, found 390.1032; ^{1}H NMR (DMSO-d_6) δ: 11.2 (br s, 1H), 8.82 (s, 1H), 8.77 (s, 1H), 8.03 (d, 2H, *J*=7.4 Hz), 7.64 (t, 1H, *J*=7.4 Hz), 7.54 (t, 2H, *J*=7.4 Hz), 6.29 (dd, 1H, *J*=4.5 and 13.1 Hz), 5.90 (d, 1H, *J*=5.7 Hz), 5.56–5.31 (dt, 1H, *J*=4.5 and 50.4 Hz), 5.32 (t, 1H, *J*=5.7 Hz), 4.51–4.47 (m, 1H), 3.89–3.84 (m, 1H, *J*=5.7 and 11.4 Hz), 3.74–3.69 (m, 1H, *J*=5.7 and 11.4 Hz), 3.45–3.43 (m, 1H); ^{13}C NMR (DMSO-d_6) δ: 165.6, 152.1, 151.8, 150.5, 143.2, 133.2, 132.5, 128.5, 125.8, 96.0 (d, *J*=191 Hz), 71.5 (d, *J*=16.2 Hz), 62.0, 59.0 (d, *J*=27.7 Hz), 52.3.

3.24. N^6-Benzoyl-9-[2-deoxy-2-fluoro-5-O-(4,4′-dimethoxytrityl)-4-thio-β-D-furanosyl]adenine (35)

Compound **35** (250 mg, 95%) was obtained as a yellow foam from **34** (150 mg, 0.38 mmol) as described above for the synthesis of **5**, after purification by silica gel column chromatography, eluted with MeOH in $CHCl_3$ (0–1.5%): FAB-LRMS *m/z* 692 (MH^+); FAB-HRMS calcd for $C_{38}H_{35}FN_5O_5S$ (MH^+) 692.2343, found 692.2343; ^{1}H NMR ($CDCl_3$) δ: 9.16 (br s, 1H), 8.71 (s, 1H), 8.26 (s, 1H), 7.98 (d, 2H, *J*=7.4 Hz), 7.60–7.21 (m, 12H), 6.84 (d, 4H, *J*=9.1 Hz), 6.23 (dd, 1H, *J*=3.4 and 13.7 Hz), 5.47–5.35 (dt, 1H, *J*=3.4 and 53.2 Hz), 4.51–4.47 (m, 1H), 3.75–3.71 (m, 1H), 3.63–3.53 (m, 2H), 3.39 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ: 164.6, 158.6, 152.8, 151.6, 149.6, 144.2, 141.9, 135.4, 133.4, 132.8, 130.0, 128.8, 128.1, 128.0, 127.9, 127.1, 123.5. 113.3, 96.3 (d, *J*=191 Hz), 87.0, 74.3 (d, *J*=17.9 Hz), 64.2, 60.1 (d, *J*=29.8 Hz), 55.2, 49.3.

3.25. X-ray crystallography

Crystal data for **8**: $C_9H_{12}FN_3O_3S\cdot 2H_2O$, *M*=297.30, orthorhombic, *a*=6.5004 (1), *b*=6.9843 (1), *c*=27.1636 (6) Å, *V*=1233.28 (4) Å^3, *T*=100 K, space group $P2_12_12_1$ (no. 19), *Z*=4, μ(Cu Kα)=27.02 cm^{-1}, 14,437 reflections measured, 2266 unique (R_{int}=0.034), which were used in the last least-squares refinement. The final *R* was 0.025 for 2234 reflections with $I>2\sigma(I)$.

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