Synthesis and structural elucidation of 1-(3-*C*-ethynyl-4-thio-β-D-ribofuranosyl)cytosine (4′-thioECyd)

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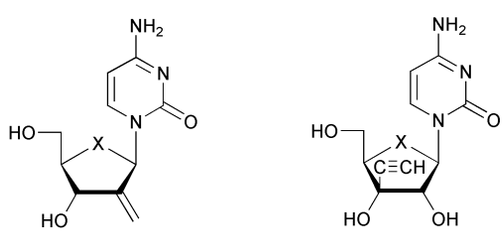
A practical synthesis of 1,4-anhydro-4-thio-D-ribitol (**5**) *via* 1,4-dibromo-1,4-dideoxy-2,3,5-tri-*O*-benzyl-L-lyxitol (**12**) is described. This method reduced our previous eleven step procedure starting from D-ribose by three steps. The Pummerer reaction of 1,4-anhydro-2-*O*-(2,4-dimethoxybenzoyl)-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-sulfinyl-D-ribitol (**6**) in the presence of N^4-benzoylcytosine afforded the 4′-thiocytidine derivative **7b** on a large scale. Starting with the resulting **7b**, 1-(3-*C*-ethynyl-4-thio-β-D-ribofuranosyl)cytosine (**4**; 4′-thioECyd), of which the 4′-oxo congener ECyd (**3**) is a new type of potent antineoplastic nucleoside developed in our group, was synthesized *via* elaborate protection and deprotection procedures, and successive reaction with cerium trimethylsilylacetylide. X-Ray crystal structures of 4′-thioECyd (**4**) and ECyd (**3**) are presented in this paper. Although striking differences in bond lengths and angles were observed in C1′–S4′ and C4′–S4′, and C4′–S4′–C1′, the overall structures of each compound, including the sugar puckering mode and the *syn*/*anti* conformation around the glycosyl bond, were similar.

Introduction

The 4′-thionucleosides, in which the furanose ring oxygen is replaced by a sulfur atom, have been of particular interest because of their biological properties, such as antiviral and antitumor activities.[1] One of the most straightforward methods in the design of new biologically interesting 4′-thionucleosides is to target bioisosteric compounds of the biologically active 4′-oxonucleosides. Since 4′-thionucleosides show different susceptibility to enzymes of nucleoside metabolism, this tactic sometimes gives 4′-thio congeners which are more biologically active than the parent 4′-oxonucleosides.[2] For example, 4′-thio-2′-deoxy-2′-methylidenecytidine (**2**; 4′-thioDMDC)[3] showed higher antineoplastic activity than 2′-deoxy-2′-methylidenecytidine (**1**; DMDC), which has been synthesized in our group (Fig. 1).[4]

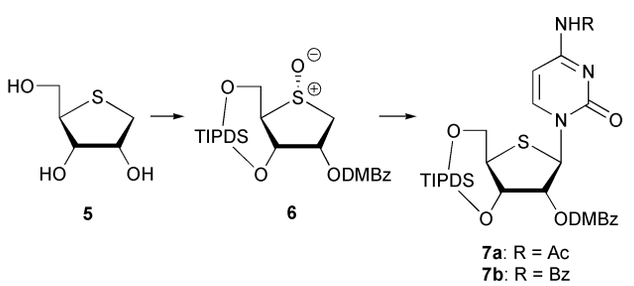


1: X = O (DMDC)
2: X = S (4′-thioDMDC)
3: X = O (ECyd)
4: X = S (4′-thioECyd)

Fig. 1

As part of our ongoing program for synthesizing new biologically active nucleosides, we have reported the synthesis and potent antitumor activities of 1-(3-*C*-ethynyl-β-D-ribofuranosyl)cytosine (**3**; ECyd).[5] ECyd first undergoes phosphorylation by uridine-cytidine kinase (UCK) to give ECyd 5′-monophosphate, which is successively converted to the active metabolite, ECyd 5′-triphosphate. The resulting 5′-triphosphate strongly inhibits cell growth and induces apoptosis as an RNA polymerase inhibitor.[6] Thus, ECyd is expected to be a new type of antitumor nucleoside, and is currently under investigation in Phase I clinical trials.

In view of the above background, we decided to synthesize 1-(3-*C*-ethynyl-4-thio-β-D-ribofuranosyl)cytosine (**4**; 4′-thioECyd) and to evaluate its antitumor activity. In contrast to the synthesis and biological evaluation of the 2′-deoxy-4′-thiocytidine derivatives, none of the 4′-thiocytidine derivatives have as yet been synthesized. This consideration also prompted us to commence this investigation. We have already reported the stereoselective synthesis of 4′-thioribonucleosides *via* the Pummerer reaction.[7] In this method, the reaction is carried out using protected sulfoxide **6**, prepared from D-ribose *via* 1,4-anhydro-4-thio-D-ribitol (**5**), and a silylated nucleobase to give a 4′-thioribonucleoside, such as the 4′-thiocytidine derivative **7a** (Scheme 1). Since the resulting **7** seemed a promising starting



TIPDS = 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl
DMBz = 2,4-dimethoxybenzoyl

Scheme 1

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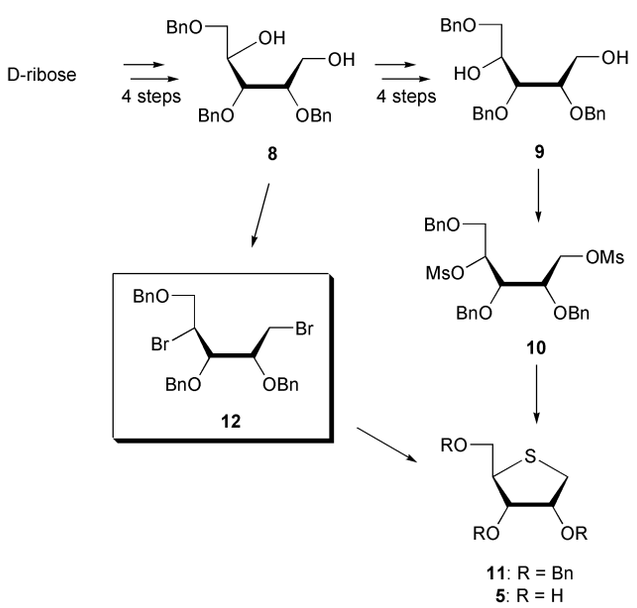
material to synthesize the desired **4**, a large scale synthesis of **7** was required. To achieve this, the development of an improved synthesis of **5** from D-ribose was essential. Thus, we first investigated the practical synthesis of **5**, and succeeded in reducing our previous eleven step synthesis by three steps. In addition, this method is suitable for large scale production. Consequently, compound **7b** was obtained in multi tens of grams using the Pummerer reaction. In this paper, we describe in detail the improved synthesis of **5** and conversion of **7b** to the desired **4**. We discuss the X-ray structures of ECyd (**3**) and 4′-thioECyd (**4**) and their cytotoxicity against tumor cells *in vitro*.

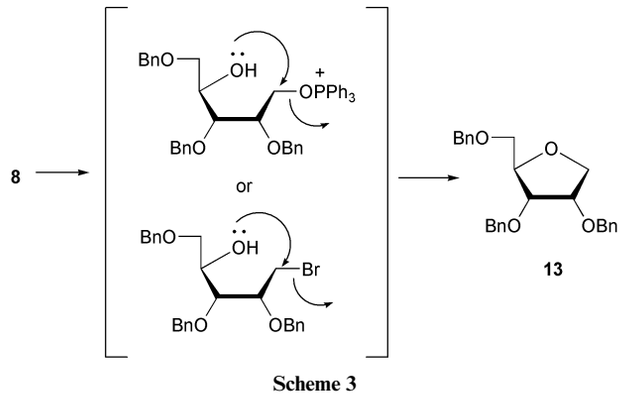
Results and discussion

As summarized in Scheme 2, 1,4-anhydro-4-thio-D-ribitol (**5**) was originally prepared on a multigram scale from D-ribose in eleven steps.[7b] However, inversion of the secondary hydroxy group of **8** by the Mitsunobu reaction, followed by cyclization to give **11** makes this a tedious method. Therefore, we attempted to simplify the synthetic route to **5** from **8**. Nagasawa, *et al*. reported that α,ω-dibromoalkanes cyclize effectively to give cyclic sulfides by treatment with sodium sulfide.[8] Thus, we thought that **11** could be prepared in fewer steps if we could substitute not only the primary but also the secondary hydroxy group by bromide with inversion of stereochemistry to give **12**.

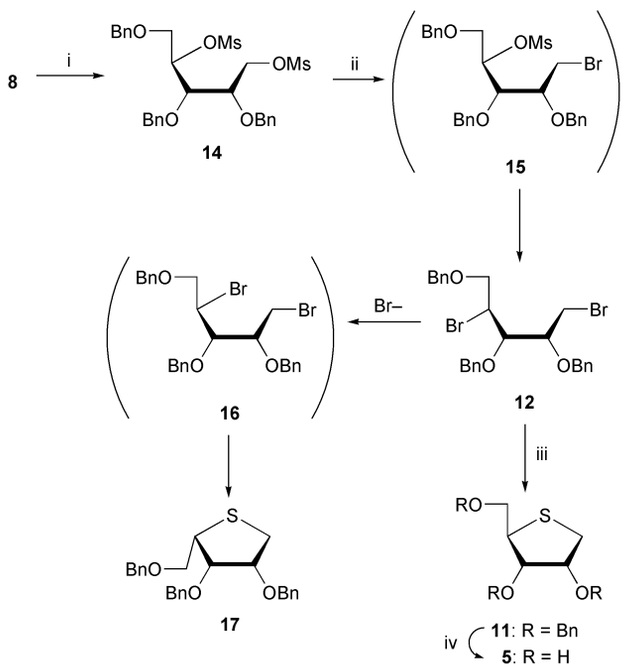


Scheme 2

Based on these considerations, a direct substitution of the hydroxy groups with bromide was first examined. When compound **8** was treated with NBS in the presence of triphenylphosphine,[9] however, 2,3,5-tri-*O*-benzyl-1,4-anhydro-D-ribitol (**13**)[10] was obtained as the major product (81% yield) along with 12% yield of the desired dibromo derivative **12**. It is thought that this result is due to the low reactivity of the secondary hydroxy group relative to the primary hydroxy group. Consequently, a nucleophilic attack of the oxygen of the secondary hydroxy group on the activated 1-position took place prior to the desired substitution (Scheme 3). Since no other conditions to give **12** preferentially in one-pot were found, a stepwise method was next examined. As shown in Scheme 4, compound **8** was converted to the dimesylate **14** in 95% yield by treatment with methanesulfonyl chloride in pyridine. Interestingly, in contrast, treatment of **8** with toluene-*p*-sulfonyl chloride or 4-nitrobenzenesulfonyl chloride under the same conditions afforded **13** as the sole product, but not the desired disulfonate. Nucleophilic bromination *via* an S_N2



Scheme 3



Scheme 4 *Reagents*: (i) MsCl, pyridine; (ii) LiBr, MEK, reflux; (iii) $Na_2S·9H_2O$, DMF, 100 °C; (iv) BCl_3, CH_2Cl_2, −78 °C.

mechanism was then examined. Lithium bromide and tetrabutylammonium bromide were tested as bromination reagents in various solvents such as acetonitrile, DMF, 1,3-dimethylimidazolidin-2-one and methyl ethyl ketone (MEK). Among the attempts, the best result was obtained when **14** was treated with ten equiv. of well-dried lithium bromide in MEK under reflux conditions to give **12** in 56% yield. The resulting **12** cyclized effectively to give **11**, as in the case of **10**. As a practical large scale synthesis, **11** was prepared from **8** in 65% yield in three steps without purification of the intermediates **14** and **12** (see Experimental Section). To conduct these successive modifications, it should be noted that TLC analysis of the bromination is important for the increased yield of **11**. The monobrominated derivative **15** is first observed in the early period of the reaction and is gradually converted to the less polar product **12**. In the synthesis of **11** from **8** on large scale, however, a small portion of the undesired **17** (less than 10%) was obtained along with **11** as an inseparable mixture, despite the fact that TLC confirmed the disappearance of the dimesylate **14** and the monobrominated derivative **15**. This could be attributed to further substitution of **12** by the bromonium ion to give **16**. Since **16** was not separable from **12** by TLC, it is recommended that one quenches the reaction immediately after the disappearance of **15**. Fortunately, the undesired **17** could be removed later during the conversion to give **6**, the substrate for the Pummerer reaction. The conditions for debenzylation of **11** were also improved. In our previous

method, the reaction was carried out at less than −90 °C, which was difficult to control on large scale. A reaction temperature of at least −78 °C is required. As a result, **5** was obtained in 68% yield when a solution of **11** in CH_2Cl_2 was added dropwise to a precooled solution of BCl_3 in CH_2Cl_2. Consequently, we developed the practical large scale synthesis of **5** from **8**, and succeeded in paring three steps from the original protocol (44% yield in four steps).

After **5** was converted into **6**,[7b] the Pummerer reaction was performed on **6** in the presence of N^4-benzoylcytosine on a large scale to give a sufficient amount of **7b** for further use. An effective method for introducing an ethynyl group on the C-3′ position of nucleosides stereoselectively has been reported Jung *et al.* involving the reaction of 2′-*O*-TBDMS-3′-ketonucleosides with cerium (trimethylsilyl)acetylide,[11] the utility of which has also been confirmed by us.[12] This method should be applicable to 4′-thio congeners, and thus, conversion of **7b** into the 2′,5′-di-*O*-TBDMS derivative **23** was examined. Since attempts for selective deprotection of the 2,4-dimethoxybenzoyl (DMBz) group on the 2′-hydroxy group were unsuccessful, both acyl protective groups were removed at once. When **7b** was treated with methylamine in MeOH solution, **18** was obtained in 69% yield. When methanolic ammonia was used instead for this deacylation, a longer reaction time was required and resulted in a decreased yield of **18**. Selective benzoylation of the *N*-4 amino group was achieved by treatment of **18** with benzoic anhydride in DMF to give **19**. The silyl protective groups on the 3′ and 5′-hydroxy groups were easily removed to give N^4-benzoyl-4′-thiocytidine (**20**). Hakimelahi *et al.* reported selective silylation of ribonucleosides to give 2′,5′-di-*O*-silylated derivatives promoted by silver nitrate.[13] When **20** was treated with TBDMSCl in the presence of silver nitrate, however, the 5′-monoprotected compound was obtained, but none of the disilylated derivative (data not shown). All attempts at selective silylation of **20** did not work well. The desired **23** was effectively synthesized by elaborate protection and deprotection procedures. Thus, silylation of the 2′-hydroxy group of **19** was carried out by treatment with TBDMSOTf in the presence of 2,6-lutidine to give **21**. This reaction did not proceed under the usual conditions such as TBDMSCl and imidazole in DMF. The selective desilylation of 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDS) group was achieved to give **22**, when **21** was treated with tetrabutylammonium fluoride in the presence of an equimolar amount of acetic acid. Migration of the remaining TBDMS group to the adjacent 3′-hydroxy group was not observed under the conditions. As a practical synthesis, **22** was prepared from **19** without purification of **21** in 73% yield in 2 steps. Silylation of the 5′-hydroxy group by TBDMSOTf gave the desired 2′,5′-di-*O*-silylated derivative **23** (Scheme 5).

Compound **23** was then oxidized with DMSO–Ac_2O to give the 3′-keto derivative **24**. To direct the preferential nucleophilic addition to the ketone from the β face, assistance of a free hydroxy group at the 5′-position was needed.[11] Treatment of **24** with 90% aqueous TFA gave the hydroxy ketone **25**. Immediately after the usual water work-up, **25** was successively treated with cerium (trimethylsilyl)acetylide to give **26** in 64% yield in 2 steps stereoselectively, as in the case of the 4′-oxo derivatives. Deprotection of **26** by ammonium fluoride, followed by methylamine in MeOH, gave the desired product **4** quantitatively (Scheme 6).

The structure of **4** was confirmed by X-ray analysis (Fig. 2). As can be seen in Fig. 2a, the ethynyl group is introduced on the β-face of the thio sugar. To date, several X-ray structures of 4′-thionucleosides have been presented,[14] and it was revealed that a marked conformational change in the carbohydrate ring compared with the corresponding 4′-oxonucleoside was observed despite the resemblance of their overall structures, namely the sugar puckering mode and the *syn*/*anti* conformation around the glycosyl bond. To elucidate the structural differences, an X-ray structure of **3** is also presented in Fig. 2b. In Table 1, important conformational characteristics for the structures **3** and **4** are summarized. Among these data, striking differences in the bond lengths and angles were observed in

Table 1 Geometric parameters: bond lengths, angles, and torsion angles that represent important structural features of **4** and **3**[a]

	4′-thioECyd (4)	ECyd (3)
	Bond lengths/Å	
C1′–C2′	1.527 (3)	1.523 (3)
C2′–C3′	1.532 (3)	1.551 (3)
C3′–C4′	1.546 (3)	1.547 (4)
C1′–S4′[b]	1.818 (2)	1.408 (3)
C4′–S4′[b]	1.840 (3)	1.449 (3)
C1′–N1	1.473 (3)	1.458 (3)
	Bond angles (deg)	
C1′–C2′–C3′	108.6 (2)	101.7 (2)
C2′–C3′–C4′	107.2 (2)	101.4 (2)
C3′–C4′–S4′[b]	106.3 (2)	106.1 (2)
C4′–S14–C1′[b]	94.9 (1)	110.7 (2)
S4′–C1′–C2′[b]	107.4 (2)	106.3 (2)
S4′–C1′–N1[b]	112.2 (2)	109.1 (2)
	Torsion angles (deg)	
C4′–S4′–C1′–C2′[b] (ν_0)	−10.1 (2)	−19.5 (3)
S4′–C1′–C2′–C3′[b] (ν_1)	31.7 (2)	34.5 (2)
C1′–C2′–C3′–C4′ (ν_2)	−42.8 (3)	−35.2 (2)
C2′–C3′–C4′–S4′[b] (ν_3)	33.7 (2)	25.0 (2)
C3′–C4′–S4′–C1′[b] (ν_4)	−13.7 (2)	−4.0 (3)
S4′–C1′–N1–C2[b] (χ)	−134.6 (2)	−139.2 (2)
O5′–C5′–C4′–C3′ (γ)	58.1 (3)	58.6 (3)
	Pseudorotation parameters (deg)	
Phase angle (P)	182.2	167.5
Puckering amplitude (ν_m)	42.8	36.1

[a] Sds (standard errors) are given in parentheses. [b] S represents O4′ in the case of compound **3**.

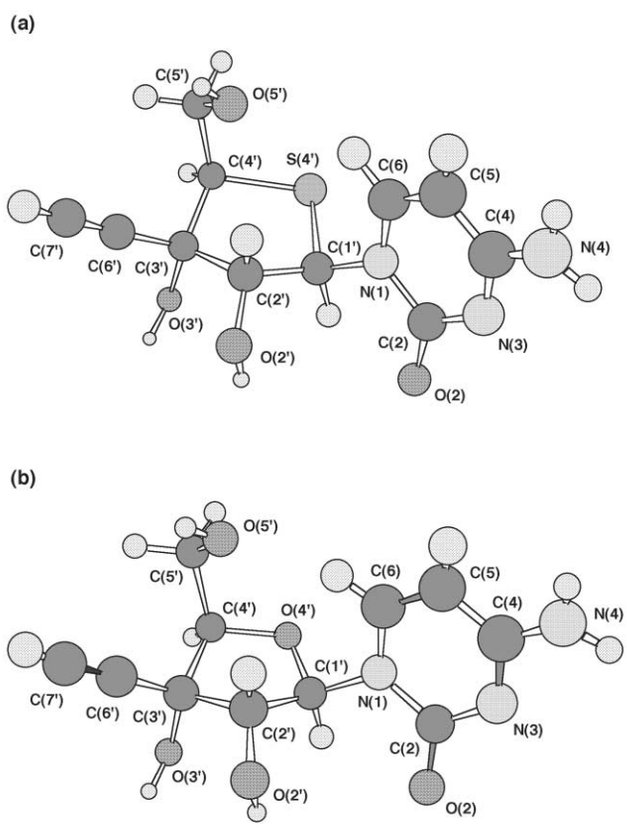
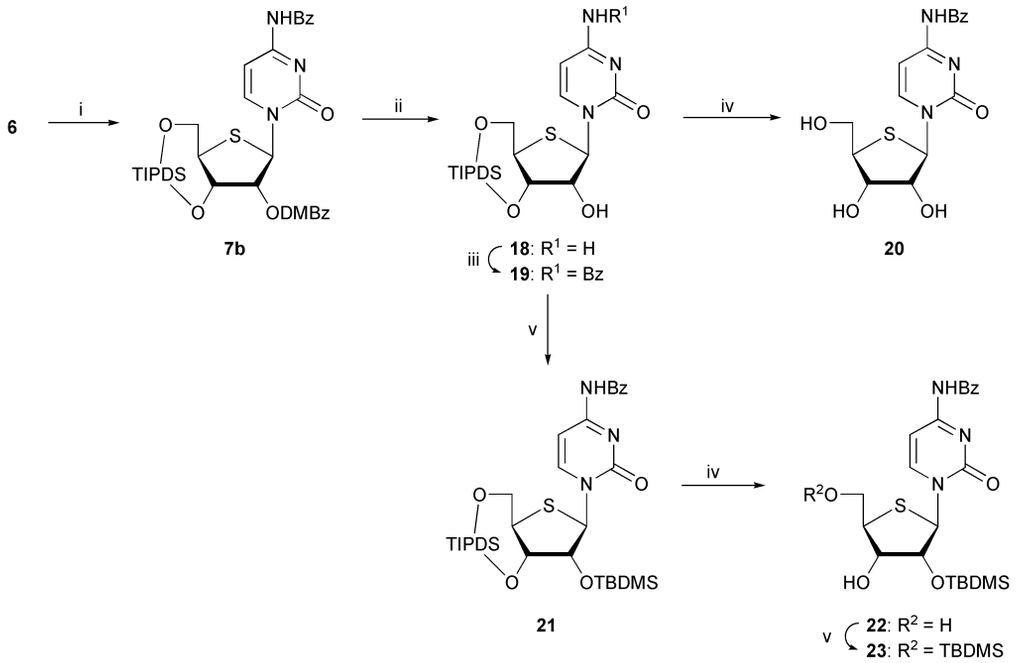
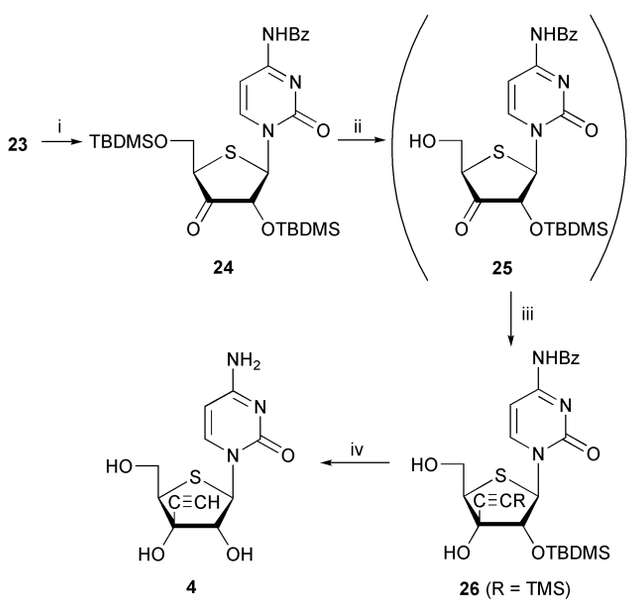


Fig. 2 The crystal structures of (a) 4′-thioECyd (**4**) and (b) ECyd (**3**).



Scheme 5 *Reagents*: (i) N^4-benzoylcytosine, TMSOTf, Et_3N, CH_2Cl_2–toluene; (ii) $MeNH_2$ in MeOH (40%); (iii) Bz_2O, DMF, 50 °C; (iv) TBAF, AcOH, THF; (v) TBDMSOTf, 2,6-lutidine, CH_2Cl_2.



Scheme 6 *Reagents*: (i) Ac_2O, DMSO; (ii) aq. TFA; (iii) lithium acetylide, $CeCl_3$, THF, −78 °C; (iv) NH_4F, MeOH, reflux, then $MeNH_2$ in MeOH (40%).

C1′–S4′ and C4′–S4′, and C4′–S4′–C1′. Thus, the bond lengths C1′–S4′ and C4′– S4′ of **4** were 1.818 (2) and 1.840 (3) Å, respectively, while the corresponding bond lengths of **3** were much shorter (*i.e.*, 1.408 (3) and 1.449 (3) Å, respectively). The other bond lengths including the glycosidic bond (C1′–N1) were quite similar to those of **3**. In contrast to the longer bond length of **4**, the bond angle C4′–S4′–C1′ in the thio sugar is 94.9°, which is 15.8° less than that of **3**. The other bond angles in the two sugar moieties do not differ markedly. In spite of the partial structural differences between **3** and **4**, their overall structures are quite similar. Thus, the cytosine bases are both in the *anti* conformation with the glycosidic torsion χ(S4′–C1′–N1–C2) = −134.6° and χ(O4′–C1′–N1–C2) = −139.2°. The 4′-thiosugar of **4** is found in a South-type puckered conformation with pseudorotation phase angle P = 182.2° and maximum puckering amplitude ν_m = 42.8°. The furanose ring of **3** also exhibits a South-type conformation with the values of P = 167.5° and ν_m = 36.1°, respectively. This type of conformation was also maintained in both structures in solution. The conformation of each sugar rings was analyzed on the basis of the coupling constant $J_{1',2'}$ in DMSO-d_6.[15] The J value of **4** was found to be 8.6 Hz, while that of **3** was 6.6 Hz. These data show that both compounds prefer a South-type puckered conformation in solid state and in solution.

In spite of the overall structural resemblance of **4** to **3**, however, **4** did not show any significant cytotoxicity against L1210 and KB cells *in vitro* at the concentration of 100 μg mL^{-1}, while **3** was a strong inhibitor of tumor cell proliferation with IC_{50} values of 16 and 28 nM, respectively, against the same cell lines.[5] One explanation for this result would be the difference of susceptibility to nucleoside and/or nucleotide kinase(s). As described in the introduction, **3** is phosphorylated by UCK to give its 5′-monophosphate, which is successively converted to the active metabolite, ECyd 5′-triphosphate.[6] In these metabolic activations, the first phosphorylation is thought to be the most important step. Thus, we tested the first phosphorylation of **4** by partially purified UCK from mouse Sarcoma-180 cells, and compared the relative susceptibility to the enzyme with **3** and the natural substrate, cytidine. Consequently, **3** was phosphorylated 26% relative to cytidine,[12] while phosphorylation of **4** was not detected under the same conditions (data not shown). To our knowledge, only one other 4′-thioribonucleoside, that is, 4′-thioadenosine has been tested for susceptibility to bovine liver adenosine kinase, and it was revealed that this nucleoside was a poor substrate for the kinase.[16] As was the case for 4′-thioadenosine, the 4′-thiocytidine derivative, **4** was also a poor substrate for UCK. To date, potent biological activities of several 2′-deoxy-4′-thionucleoside derivatives have been reported.[2,3] These results strongly suggest that some 2′-deoxy-4′-thionucleosides would be good substrates for kinases such as thymidine kinase and deoxycytidine kinase. In contrast to the deoxynucleoside kinases, ribonucleoside kinases may discriminate structural changes in the sugar conformations, and do not metabolize 4′-thioribonucleosides to the corresponding 5′-phosphates.

In conclusion, we have developed an improved synthesis of the 1,4-anhydro-4-thio-D-ribitol (**5**), in which we succeeded in reducing our previous eleven step synthesis by three steps. This method facilitates a large scale synthesis of the 4′-thiocytidine derivative **7b**, which is thought to be a good substrate for

further derivatized 4′-thiocytidine analogs. Starting with the resulting **7b**, 4′-thioECyd (**4**) was synthesized *via* elaborate protection and deprotection procedures. In addition, X-ray crystal structural analyses of **4** and **3** have been done. The present results indicate that the overall structures of **4** and **3** are similar. Contrary to our expectations, 4′-thioECyd (**4**) showed no significant cytotoxicity against L1210 and KB cells *in vitro*. Further investigation is needed to elucidate the effect of the sulfur atom at the 4′-position of the nucleosides and their susceptibility to nucleoside and/or nucleotide kinases. The improved method presented in this work should be the driving force for further studies.

Experimental section

General methods

Physical data were measured as follows. Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 270 or 400 MHz and 100 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), m (multiplet), or br (broad). All exchangeable protons were detected by addition of D_2O. Mass spectra were measured on JEOL JMS-D300 spectrometer. X-Ray measurements were made on AFC-7R with graphite-monochromated Mo-Kα radiation or AFC-5R with graphite-monochromated CuKa radiation, Rigaku. TLC was done on Merck Kieselgel F254 precoated plates. Silica gel used for column chromatography was Merck silica gel 5715.

1,4-Anhydro-2,3,5-tri-*O*-benzyl-4-thio-D-ribitol (11)[7b]

To a solution of **8** (248.9 g, 0.59 mol) in dry pyridine (1 L) was added methanesulfonyl chloride (114 mL, 1.47 mol) at 0 °C. After the mixture was stirred for 1 h at the same temperature, the reaction was quenched by addition of ice. 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*, and the residue was coevaporated with toluene to give **14**. The crude **14** was dissolved in MEK (1.2 L), and well-dried lithium bromide (512 g, 5.89 mol) was added to the solution. The mixture was heated under reflux for 7 h. After being cooled to room temperature, the mixture was diluted with AcOEt and washed with H_2O, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo* to give crude **12**. The resulting **12** was dissolved in dry DMF (1.2 L), and sodium sulfide nanohydrate (142 g, 0.59 mol) was added to the solution. The mixture was heated at 100 °C for 1 h. After being cooled to room temperature, the mixture was diluted with AcOEt and washed with H_2O, 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 (50 : 1–10 : 1), to give **11** including less than 10% of **17** (162 g, 65% in three steps).

Physical data for 1,4-bis(*O*-methanesulfonyl)-2,3,5-tri-*O*-benzyl-D-ribitol (14)

Found: C, 58.13; H, 5.94. $C_{28}H_{34}O_9S_2$ requires C, 58.11; H, 5.92%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.35–7.24 (m, 15 H, Ph), 5.13 (ddd, 1 H, H-4, J = 7.5, 3.0, 3.2 Hz), 4.76–4.44 (m, 7 H, H-1a, CH_2Ph × 3), 4.32 (dd, 1 H, H-1b, J = 11.3, 4.0 Hz), 3.91 (dd, 1 H, H-3, J = 3.0, 7.5 Hz), 3.77–3.71 (m, 2 H, H-2, H-5a), 3.59 (dd, 1 H, H-5b, J = 3.2, 11.1 Hz), 3.00 and 2.93 (each s, each 3 H, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.10, 136.70, 136.58, 128.39, 128.32, 128.23, 128.13, 128.00, 127.77, 127.59, 81.42, 77.25, 75.89, 73.89, 72.30, 68.57, 67.49, 38.62, 37.66; FAB-LRMS *m*/*z* 579 (MH^+, 12.4%).

Physical data for 1,4-dibromo-1,4-dideoxy-2,3,5-tri-*O*-benzyl-L-lyxitol (12)

Found: C, 56.88; H, 5.15. $C_{26}H_{28}Br_2O_3$ requires C, 56.95; H, 5.15%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.27 (m, 15 H, Ph), 4.80–4.43 (m, 7 H), 3.99–3.67 (m, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.57, 137.23, 137.09, 128.30, 128.09, 128.01, 127.90, 127.86, 127.71, 127.60, 127.55, 77.89, 76.62, 74.94, 73.06, 72.09, 70.70, 53.39, 33.40 FAB-LRMS *m*/*z* 547, 549, and 551 (MH^+, MH^+ + 2, and MH^+ + 4, 2.6, 3.8 and 1.6%).

Physical data for 1,4-anhydro-2,3,5-tri-*O*-benzyl-4-thio-L-lyxitol (17)

^{1}H NMR (270 MHz, $CDCl_3$) δ 7.34–7.25 (m, 15 H, Ph), 4.87 and 4.69 (each d, each 1 H, CH_2Ph, J = 11.7 Hz), 4.55 and 4.48 (each s, each 2 H, CH_2Ph × 2), 4.19 (m, 1 H, H-3), 4.05 (ddd, 1 H, H-2, J = 2.6, 6.2, 9.1 Hz), 3.91 (m, 1 H, H-4), 3.08 (dd, 1 H, H-1a, J = 9.4, 9.7 Hz), 2.91 (dd, 1 H, H-1b, J = 6.2, 9.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.47, 137.97, 137.90, 128.29, 128.22, 128.11, 127.63, 127.56, 127.49, 127.38, 127.25, 83.40, 78.71, 73.49, 73.23, 72.05, 70.13, 45.64, 30.31; FAB-LRMS *m*/*z* 421 (MH^+, 12.5%); FAB-HRMS 421.1850 (MH^+, $C_{26}H_{29}O_3S$ requires *m*/*z* 421.1837).

1,4-Anhydro-4-thio-D-ribitol (5)[7b]

A solution of 1 M BCl_3 in CH_2Cl_2 (1.49 L, 1.49 mol) was cooled to −78 °C, and a solution of **11** (104.5 g, 0.24 mol) in CH_2Cl_2 (1 L) was added to the precooled solution over 3.5 h. After the solution was stirred at the same temperature for 2 h, the reaction was quenched by addition of a mixture of MeOH–CH_2Cl_2 (2 : 1, 900 mL) at −78 °C. The solvent was removed *in vacuo*, and the residue was coevaporated with MeOH. The residue was purified by a silica gel column, eluted with 4–16% MeOH in $CHCl_3$, to give **5** (25.5 g, 68% as a yellow oil).

N^4-Benzoyl-1-[2-*O*-(2,4-dimethoxybenzoyl)-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-ribofuranosyl]-cytosine (7b)

To a suspension of N^4-benzoylcytosine (14.4 g, 67.0 mmol) in dry toluene (350 mL) was added triethylamine (9.3 mL, 67.0 mmol) and TMSOTf (51.8 mL, 268.1 mmol), and the mixture was stirred at room temperature until giving a two-phase clear solution. Dry CH_2Cl_2 (200 mL) was added to the above solution, which gave an one-phase clear solution, and the whole was added to a solution of **6** (25.6 g, 44.7 mmol) in dry CH_2Cl_2 (200 mL) dropwise over 30 min *via* a cannula. Additional triethylamine (28.0 mL, 201.1 mmol) in dry toluene (100 mL) was added dropwise to the reaction mixture at 0 °C to initiate the Pummerer reaction. After being stirred for 15 min at room temperature, the reaction was quenched by addition of ice, 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 (3 : 1–1 : 3), to give **7b** (22.9 g, 67% as a white solid): Found: C, 57.76; H, 6.68; N 5.36. $C_{37}H_{51}N_3O_9SSi_2$ requires C, 57.71; H, 6.68; N, 5.46%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (br s, 1 H, NH), 8.61 (d, 1 H, H-6, J = 7.9 Hz), 7.92–7.85 (m, 3 H, *o*-DMBz, *o*-Bz), 7.65–7.50 (m, 4 H, H-5, *m*-Bz, *p*-Bz), 6.52–6.48 (m, 2 H, *m*-DMBz), 6.16 (s, 1 H, H-1′), 5.72 (d, 1 H, H-2′, J = 4.0 Hz), 4.49 (dd, 1 H, H-3′, J = 4.0, 9.2 Hz), 4.18 (dd, 1 H, H-5′a, J = 3.0, 12.9 Hz), 4.09 (d, 1 H, H-5′b, J = 12.9 Hz), 3.86 (s, 6 H, MeO × 2), 3.78 (dd, 1 H, H-4′, J = 9.2, 3.0 Hz), 1.14–0.87 (m, 28 H, TIPDS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.34, 164.10, 163.09, 161.84, 161.24, 154.75, 145.99, 133.78, 133.09, 132.85, 128.91, 127.44, 112.01, 104.45, 98.91, 96.56, 77.45, 71.42, 64.18,

58.12, 56.01, 55.53, 50.90, 17.62, 17.56, 17.52, 17.18, 17.02, 17.00, 13.51, 13.31, 13.27, 12.73; FAB-LRMS *m*/*z* 770 (MH^+, 8.3%).

1-[3,5-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-ribofuranosyl]cytosine (18)

Compound **7b** (1.4 g, 1.82 mmol) was dissolved in methylamine in MeOH solution (40%, 90 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 4% MeOH in $CHCl_3$, to give **18** (0.63 g, 69% as a white foam): Found: C, 50.19; H, 7.73; N, 8.16. $C_{21}H_{39}N_3O_5SSi_2$ requires C, 50.27; H, 7.83; N, 8.37%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (d, 1 H, H-6, J = 7.3 Hz), 7.17 (d, 2 H, NH_2), 5.86 (d, 1 H, 2′-OH, J = 4.1 Hz), 5.68 (d, 1 H, H-5, J = 7.3 Hz), 5.58 (s, 1 H, H-1′), 4.00–3.89 (m, 4 H, H-2′, H-3′, H-5′), 3.52 (m, 1 H, H-4′), 1.10–0.88 (m, 28 H, TIPDS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.52, 156.37, 142.28, 94.44, 78.63, 71.75, 65.85, 58.51, 49.75, 17.64, 17.58, 17.53, 17.51, 17.22, 17.20, 17.17, 17.06, 13.46, 13.29, 13.24, 12.48; FAB-LRMS *m*/*z* 502 (MH^+, 68.8%).

N^4-Benzoyl-1-[3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-ribofuranosyl]cytosine (19)

To a solution of **18** (6.9 g, 13.8 mmol) in DMF (140 mL) was added Bz_2O (4.7 g, 20.6 mmol), and the whole was stirred at 50 °C for 9 h. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ at 0 °C, 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 (3 : 1–2 : 1), to give **19** (7.8 g, 93% as a white foam): Found: C, 55.37; H, 7.15; N, 6.71. $C_{28}H_{43}N_3O_6SSi_2$ requires C, 55.50; H, 7.15; N, 6.94%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.75–8.72 (m, 2 H, NH, H-6, J = 7.3 Hz), 7.92–7.89 (m, 2 H, *o*-Bz), 7.65–7.49 (m, 4 H, H-5, *m*-Bz, *p*-Bz), 5.97 (s, 1 H, H-1′), 4.28 (dd, 1 H, H-3′, J = 3.3, 9.2 Hz), 4.23 (d, 1 H, H-2′, J = 3.3 Hz), 4.15 (dd, 1 H, H-5′a, J = 3.0, 12.9 Hz), 4.06 (d, 1 H, H-5′b, J = 12.9 Hz), 3.72 (dd, 1 H, H-4′, J = 9.2, 3.0 Hz), 2.90 (br s, 1 H, 2′-OH), 1.14–0.87 (m, 28 H, TIPDS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.93, 161.95, 155.25, 146.33, 133.13, 132.76, 129.76, 128.94, 128.14, 127.41, 96.28, 78.82, 72.03, 66.13, 58.42, 50.12, 17.61, 17.57, 17.50, 17.22, 17.17, 17.15, 17.05, 13.52, 13.31, 13.28, 12.57; FAB-LRMS *m*/*z* 606 (MH^+, 19.8%).

N^4-Benzoyl-1-(4-thio-β-D-*ribo*-pentofuranosyl)cytosine (20)

To a solution of **19** (306 mg, 0.50 mmol) in THF (10 mL) was added AcOH (57 μL, 1.0 mmol) and TBAF (1 M in THF, 1.0 mL, 1.0 mmol) at 0 °C. After being stirred for 10 min at the same temperature, the solvent was removed *in vacuo*. The residue was suspended in EtOH, and collected by filtration. The solid was washed by EtOH, and dried to give **20** (164 mg, 89% as a white solid): Found: C, 52.82; H, 4.96; N, 11.28. $C_{16}H_{17}N_3O_5S$ requires C, 52.88; H, 4.72; N, 11.56%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 11.24 (br s, 1 H, NH), 8.57 (d, 1 H, H-6, J = 7.9 Hz), 8.00 (d, 2 H, *o*-Ar, J = 7.3 Hz), 7.64–7.47 (m, 3 H, *m*-Ar, *p*-Ar), 7.38 (d, 1 H, H-5, J = 7.9 Hz), 5.96 (d, 1 H, H-1′, J = 5.9 Hz), 5.53 (d, 1 H, 2′-OH, J = 5.9 Hz), 5.24 (d, 1 H, 3′-OH, J = 4.6 Hz), 5.18 (t, 1 H, 5′-OH, J = 5.3 Hz), 4.20 (m, 1 H, H-2′), 4.03 (m, 1 H, H-3′), 3.72 (ddd, 1 H, H-5′a, J = 5.9, 11.2, 5.3 Hz), 3.60 (ddd, 1 H, H-5′b, J = 4.6, 11.2, 5.3 Hz), 3.28 (m, 1 H, H-4′); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.19, 162.59, 155.19, 146.74, 133.01, 132.72, 128.41, 96.80, 77.20, 72.98, 64.43, 62.91, 53.08; FAB-LRMS *m*/*z* 364 (MH^+, 16.7%).

N^4-Benzoyl-1-[2-*O*-(*tert*-butyldimethylsilyl)-4-thio-β-D-ribofuranosyl]cytosine (22)

To a solution of **19** (1.8 g, 3.0 mmol) in dry CH_2Cl_2 (15 mL) was added 2,6-lutidine (3.5 mL, 30.0 mmol) and TBDMSOTf (3.4 mL, 15.0 mmol) at 0 °C. The mixture was stirred for 15 h at room temperature. The reaction was quenched by addition of 1 M aqueous HCl, and the mixture was stirred for 10 min. 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* to give crude **21**. Compound **21** was dissolved in THF (30 mL), and AcOH (0.34 mL, 6.0 mmol) and TBAF (1 M in THF, 6.0 mL, 6.0 mmol) were added to the mixture at 0 °C. After being stirred for 2 h at the same temperature, the reaction mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with H_2O, 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 : 1–1 : 1), to give **22** (1.04 g, 73% as a white solid): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (br s, 1 H, NH), 8.66 (d, 1 H, H-6, J = 7.5 Hz), 7.87 (d, 2 H, *o*-Bz, J = 7.5 Hz), 7.61–7.46 (m, 4 H, H-5, *m*-Bz, *p*-Bz), 5.87 (d, 1 H, H-1′, J = 3.6 Hz), 4.52 (dd, 1 H, H-2′, J = 3.6, 3.4 Hz), 4.17 (m, 1 H, H-3′), 4.08 (dd, 1 H, H-5′a, J = 3.0, 11.7 Hz), 3.93 (dd, 1 H, H-5′b, J = 3.2, 11.7 Hz), 3.75 (br s, 1 H, 5′-OH), 3.58 (ddd, 1 H, H-4′, J = 6.2, 3.0, 3.2 Hz), 2.82 (d, 1 H, 3′-OH, J = 5.8 Hz), 0.90 (s, 9 H, *t*-Bu), 0.18, 0.11 (each s, each 3 H, Me × 2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.50, 161.96, 155.05, 147.50, 133.10, 132.75, 128.88, 127.47, 96.90, 79.13, 74.78, 69.25, 62.13, 52.63, 25.84, 18.10, −4.45, −4.93; FAB-LRMS *m*/*z* 478 (MH^+, 25.5%); FAB-HRMS 478.1830 (MH^+, $C_{22}H_{32}N_3O_5SSi$ requires *m*/*z* 478.1832).

N^4-Benzoyl-1-[2,5-bis-*O*-(*tert*-butyldimethylsilyl)-4-thio-β-D-ribofuranosyl]cytosine (23)

To a solution of **22** (48.2 mg, 0.101 mmol) in dry CH_2Cl_2 (1 mL) was added 2,6-lutidine (70.5 μL, 0.60 mmol) and TBDMSOTf (69.5 μL, 0.30 mmol) at 0 °C. The mixture was stirred for 30 min at the same temperature. The reaction was quenched by addition of 1 M aqueous HCl, and the mixture was stirred for 10 min. 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 : 1–3 : 1), to give **23** (52.9 mg, 89% as a white foam): Found: C, 56.71; H, 7.58; N, 7.10. $C_{28}H_{45}N_3O_5SSi_2$ requires C, 56.82; H, 7.66; N, 7.10%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (d, 1 H, H-6, J = 7.0 Hz), 8.67 (br s, 1 H, NH), 7.90 (d, 2 H, *o*-Bz, J = 7.3 Hz), 7.70–7.49 (m, 4 H, H-5, *m*-Bz, *p*-Bz), 6.05 (d, 1 H, H-1′, J = 2.6 Hz), 4.29 (dd, 1 H, H-2′, J = 2.6, 3.2 Hz), 4.11 (m, 1 H, H-3′), 4.06 (dd, 1 H, H-5′a, J = 2.9, 11.1 Hz), 3.92 (dd, 1 H, H-5′b, J = 2.6, 11.1 Hz), 3.51 (ddd, 1 H, H-4′, J = 6.4, 2.9, 2.6 Hz), 2.29 (d, 1 H, 3′-OH, J = 8.2 Hz), 0.98, 0.94 (each s, each 9 H, *t*-Bu × 2), 0.27, 0.18, 0.17, 0.15 (each s, each 3 H, Me × 4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.41, 161.69, 155.01, 146.69, 132.99, 128.82, 128.60, 127.43, 96.54, 80.19, 73.53, 66.91, 62.04, 52.43, 26.12, 25.84, 18.78, 18.10, −4.37, −5.01, −5.12, −5.15; FAB-LRMS *m*/*z* 592 (MH^+, 7.3%).

N^4-Benzoyl-1-[2,5-bis-*O*-(*tert*-butyldimethylsilyl)-4-thio-β-D-ribofuran-3-ulosyl]cytosine (24)

A mixture of **23** (48.4 mg, 0.08 mmol) and Ac_2O (0.4 mL) in DMSO (0.8 mL) was stirred at room temperature for 2.5 h. The reaction was quenched by addition of saturated aqueous $NaHCO_3$, and the whole was stirred for 10 min. 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 (4 : 1–3 : 1), to give **24** (41.4 mg, 86% as a white foam): Found: C, 56.71; H, 7.29; N, 7.04. $C_{28}H_{43}N_3O_5SSi_2$ requires C, 57.01; H, 7.35; N, 7.12%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (br s, 1 H, NH), 8.32 (d, 1 H, H-6, J = 7.3 Hz), 7.90 (m, 2 H, *o*-Bz), 7.64–7.51 (m, 4 H, H-5, *m*-Bz, *p*-Bz), 6.22 (d, 1 H, H-1′, J = 7.3 Hz), 4.36 (d, 1 H, H-2′, J = 7.3 Hz), 4.18 (dd, 1 H, H-5′a, J = 4.7, 11.4 Hz), 3.89–3.86 (m, 2 H, H-4′, H-5′b), 0.95, 0.85 (each s, each 9 H, *t*-Bu × 2), 0.15, 0.12, 0.08, −0.01 (each s, each 3 H, Me × 4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.98, 166.12, 161.53, 155.02, 145.15, 133.20, 132.69, 128.92, 127.45, 97.67, 80.40, 63.75, 59.57, 52.20, 26.06, 25.53, 18.64, 18.14, −4.46, −5.11, −5.15, −5.18; FAB-LRMS *m*/*z* 590 (MH^+, 6.7%).

N^4-Benzoyl-1-[2-*O*-(*tert*-butyldimethylsilyl)-3-*C*-(trimethylsilyl)ethynyl-4-thio-β-D-ribofuranosyl]cytosine (26)

A solution of **24** (628 mg, 1.06 mmol) in an aqueous TFA solution (90%, 5 mL) was stirred for 20 min at 0 °C. The reaction mixture was added to saturated aqueous $NaHCO_3$ at 0 °C, and the whole was stirred for 10 min. 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 coevaporated with toluene to give crude **25** as a white solid. The unstable hydroxy ketone **25** was used directly without further purification.

To a solution of (trimethylsilyl)acetylene (0.9 mL, 6.39 mmol) in dry THF (5 mL) was added *n*-BuLi (15% w/v in hexane, 4.0 mL, 6.39 mmol) at −20 °C. The mixture was stirred for 1 h at the same temperature. The resulting lithium acetylide solution was added to the anhydrous $CeCl_3$ suspension (1.57 g, 6.39 mmol) in THF (5 mL) *via* a cannula at −78 °C. The mixture was stirred for 1 h at the same temperature, and a solution of crude **25** in dry THF (5 mL) was added *via* a cannula at −78 °C. After being stirred for 30 min at −78 °C, the reaction was quenched by addition of AcOH (0.9 mL), and the temperature was raised to room temperature. The mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with H_2O, 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 : 1–2 : 1), to give **26** (390 mg, 64% as a white foam): ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.91 (br s, 1 H, NH), 8.22 (d, 1 H, H-6, J = 6.9 Hz), 7.91 (d, 2 H, *o*-Bz, J = 7.6 Hz), 7.61–7.48 (m, 4 H, H-5, *m*-Bz, *p*-Bz), 5.92 (d, 1 H, H-1′, J = 5.9 Hz), 4.89 (d, 1 H, H-2′, J = 5.9 Hz), 4.21 (m, 1 H, H-5′a, J = 10.9 Hz), 3.91 (dd, 1 H, H-5′b, J = 3.6, 10.9 Hz), 3.57 (dd, 1 H, H-4′, J = 7.6, 3.6 Hz), 3.31 (m, 2 H, 3′-OH, 5′-OH), 0.86 (s, 9 H, *t*-Bu), 0.18 (s, 9 H, TMS), 0.14 and −0.08 (each s, each 3 H, Me × 2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.58, 161.84, 154.96, 147.89, 132.96, 132.64, 128.68, 128.57, 127.53, 102.82, 97.75, 93.50, 82.25, 77.37, 67.21, 63.78, 54.84, 25.76, 17.89, −0.23, −4.07, −4.63; FAB-LRMS *m*/*z* 574 (MH^+, 54.2%); FAB-HRMS 574.2228 (MH^+, $C_{27}H_{40}N_3O_5SSi_2$ requires *m*/*z* 574.2227).

1-(3-*C*-Ethynyl-4-thio-β-D-ribofuranosyl)cytosine (4)

A solution of **26** (182 mg, 0.32 mmol) in MeOH (3 mL) containing ammonium fluoride (117 mg, 3.17 mmol) was heated under reflux for 1 h. The solvent was removed *in vacuo*, and the residue was dissolved in methylamine in MeOH (40%, 6 mL). The reaction mixture was kept for 1 h at room temperature, and the solvent was removed *in vacuo*. The residue was coevaporated with MeOH. The residue was purified by a silica gel column, eluted with 33% MeOH in $CHCl_3$, to give **4** (89.0 mg, 99% as a white solid, crystallized from MeOH): mp 237 °C dec; Found: C, 46.53; H, 4.71; N, 14.79. $C_{11}H_{13}N_3O_4S$ requires C, 46.64; H, 4.63; N, 14.83%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.97 (d, 1 H, H-6, J = 7.3 Hz), 7.15 (br d, 2 H, NH_2), 5.97 (d, 1 H, H-1′, J = 8.6 Hz), 5.93 (s, 1 H, 3′-OH), 5.76 (d, 1 H, H-5, J = 7.3 Hz), 5.58 (d, 1 H, 2′-OH, J = 7.9 Hz), 5.10 (dd, 1 H, 5′-OH, J = 4.6, 5.3 Hz), 4.22 (dd, 1 H, H-2′, J = 8.6, 7.9 Hz), 3.79–3.76 (m, 2 H, H-5′a, H-5′b), 3.54 (s, 1 H, 3′-C≡CH), 3.20 (dd, 1 H, H-4′, J = 4.0, 5.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.00, 155.76, 144.37, 94.84, 83.50, 80.39, 77.59, 74.87, 63.43, 61.49, 55.59; FAB-LRMS *m*/*z* 284 (MH^+, 22.9%).

X-Ray crystallography †

Crystal data for **4**: $C_{11}H_{13}N_3O_4S$, M = 283.30, orthorhombic, a = 10.887 (3), b = 10.892 (3), c = 10.385 (3) Å, V = 1231.4 (5) Å^3, T = 296 K, space group $P2_12_12_1$ (no. 19), Z = 4, μ(MoKα) = 2.78 cm^{-1}, 1655 reflections measured, 1637 unique (R_{int} = 0.000) which were used in all calculations. The final R was 0.037 ($I > 2.0\sigma(I)$).

Crystal data for **3**: $C_{11}H_{13}N_3O_5$, M = 267.24, orthorhombic, a = 10.657 (4), b = 10.894 (3), c = 10.362 (3) Å, V = 1202.9 (5) Å^3, T = 296 K, space group $P2_12_12_1$ (no. 19), Z = 4, μ(Cu-Kα) = 10.12 cm^{-1}, 2273 reflections measured, 2249 unique (R_{int} = 0.026) which were used in all calculations. The final R was 0.048 ($I > 4.0\sigma(I)$).

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† CCDC reference number(s) 186372–186373. See http://www.rsc.org/suppdata/p1/b2/b204993g/ for crystallographic files in .cif or other electronic format.

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