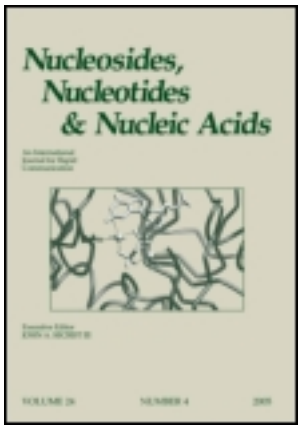

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Nucleosides and Nucleotides. 232. Synthesis of 2′-C-Methyl-4′-thiocytidine: Unexpected Anomerization of the 2′-Keto-4′-thionucleoside Precursor

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NUCLEOSIDES AND NUCLEOTIDES. 232. SYNTHESIS OF 2′-*C*-METHYL-4′-THIOCYTIDINE: Unexpected Anomerization of the 2′-Keto-4′-thionucleoside Precursor

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□ *The synthesis of 2′-C-methyl-4′-thiocytidine (***16***) is described. Since the 2′-keto-4′-thiocytidine derivative* **2β** *unexpectedly isomerized to* **2α** *and the methylation of* **2β** *proceeded predominantly from the less hindered α-face to give* **7**, *the desired product* **16** *was synthesized via the Pummerer reaction of the sulfoxide* **14** *and* N^4*-benzoylcytosine.*

Keywords Nucleoside; 4′-Thionucleoside; 2′-Keto-4′-thionucleoside; 2′-Branched-chain-4′-thionucleoside; 2′-*C*-methyl-4′-thiocytidine

INTRODUCTION

Branched-chain sugar nucleosides are some of the most attractive nucleoside derivatives for the development of antitumor and antiviral agents. Consequently, much attention has been directed toward modification of these derivatives, especially at the 2′-position, which has been extensively investigated with a number of biologically active 2′-branched-chain sugar nucleoside derivatives having been reported.[1,2] Our group also has been engaged in the synthesis of 2′-branched-chain sugar pyrimidine nucleosides, and have found that 2′-*C*-methylcytidine,[3,4] 2′-deoxy-2′(*S*)-methylcytidine,[5] 2′-deoxy-2′-methylidenecytidine (DMDC),[6,7] and

In honor and celebration of the life and career of John A. Montgomery.

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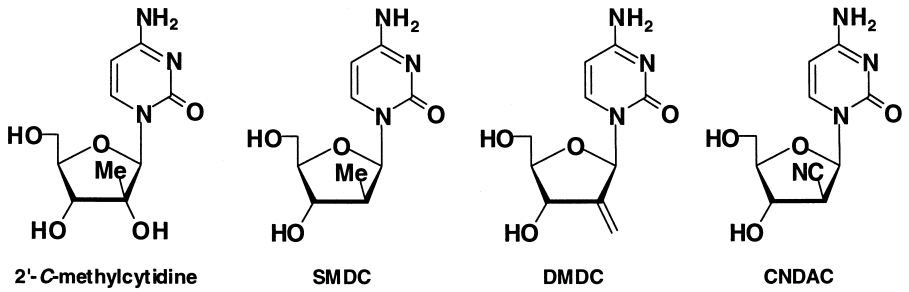


FIGURE 1 The structures of 2′-branched-chain sugar pyrimidine nucleosides.

2′-deoxy-2′(*S*)-cyanocytidine (CNDAC)[8] possess potent antitumor activity (Figure 1).

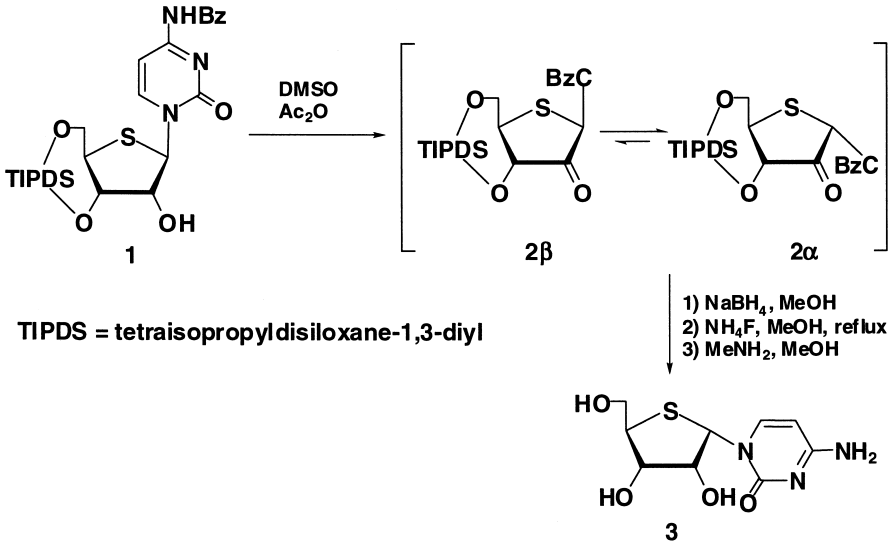
4′-Thionucleosides are nucleoside derivatives in which the furanose ring oxygen is replaced by a sulfur atom. This series of nucleoside analogs has long been recognized as a potential target of antimetabolites, and much effort has been expended in the design and synthesis of 4′-thionucleoside analogs.[9,10] Thus far, several groups have reported the synthesis of 2′-branched-chain sugar 4′-thionucleosides,[11–13] and 4′-thio-2′-deoxy-2′-methylidenecytidine (4′-thioDMDC) has been found to be a potent antineoplastic 4′-thionucleoside derivative.[14] Since the activity of 4′-thioDMDC was higher than that of DMDC, the synthesis and biological evaluation of the 4′-thio-congeners of 2′-branched-chain sugar pyrimidine nucleosides prepared in our laboratory would be an ideal approach for developing new biologically active nucleosides. In this article, we describe the synthesis and antitumor activity of 2′-*C*-methyl-4′-thiocytidine (**16**).

RESULTS AND DISCUSSION

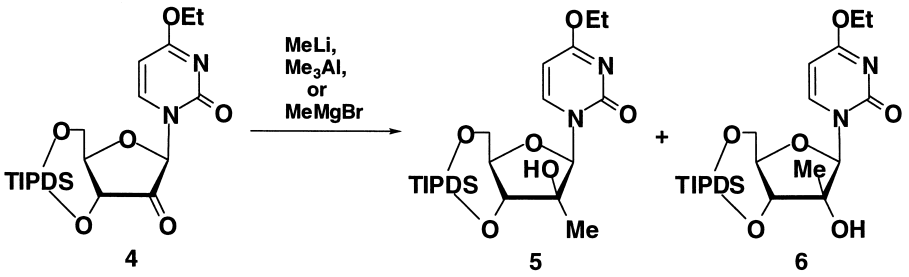
The 2′-branched-chain sugar pyrimidine nucleosides reported by us have been prepared from the corresponding 2′-ketonucleoside as the common precursor. Therefore, it was thought that a 2′-keto-4′-thionucleoside would be the ideal key compound for the preparation of the 4′-thio-congeners of 2′-branched-chain sugar pyrimidine nucleosides, including the target compound. To date, there have been no reports on a systematic study of the synthesis and reactivity of the 2′-keto-4′-thionucleoside derivative. Yoshimura et al. prepared the 2-keto-4-thiosugar derivative, a precursor of 4′-thioDMDC, by oxidation of the hydroxyl group at the 2-position with DMSO-Ac_2O without affecting the sulfur atom of the 4-thiosugar.[14] These conditions were examined as the first choice for the 2′-keto-4′-thionucleoside derivative synthesis. When **1**[15–17] was treated with DMSO-Ac_2O overnight according to the method reported by Yoshimura et al. two inseparable compounds were obtained after column chromatography

(ca. 7:1 ratio). Although the ratio of the two compounds varied depending on the oxidation reaction time (from 1:4 for 2.5 h to 7:1 for 24 h), neither compound could be prepared as the sole product. To determine the structures of the oxidation product, the mixture, consisting of a 7:1 ratio, was successively treated with sodium borohydride, ammonium fluoride, and methylamine. Consequently, the analytical data of the resulting major product was identical with those of 4′-α-thiocytidine (**3**) (Scheme 1).[18] Unexpected anomerization of the 2′-keto-4′-thiocytidine derivative, i.e., from **2*β*** to **2*α***, occurred not only during oxidation but also during chromatographic purification. Such anomerization of 2′-ketonucleosides had not been observed in the corresponding 4′-oxo-congener.[7] In addition, since the corresponding 3′-keto-4′-thiocytidine derivative synthesized in our previous study was also inactive for epimerization at the 4′-position,[15] we reasoned that this equilibrium should be characteristic of the 2′-keto-4′-thiocytidine derivative. The unexpected anomerization of the 2′-keto-4′-thiocytidine derivative can be explained by the higher acidity of the α-hydrogen (i.e., the H-1′ proton of **2*β***) of the thioether and the higher stability of the resulting carbanion versus those of the corresponding ether.[19,20] Since other oxidation conditions including SO_3-pyridine complex and Dess-Martin periodinane, had resulted in a diminished ratio and chemical yield of **2*β***, we decided to use DMSO-Ac_2O, which proved to be the best choice for obtaining **2*β*** in a satisfactory ratio.

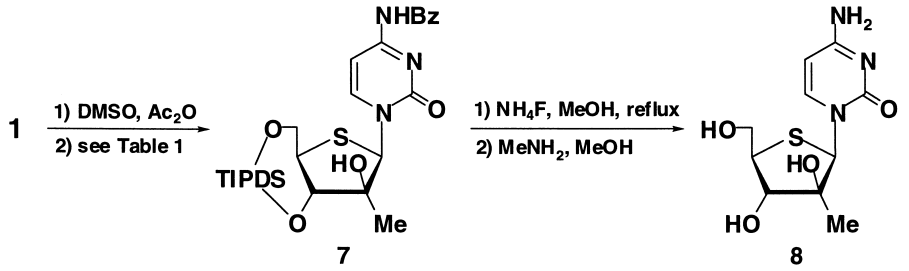
In our study of the 2′-*C*-methylcytidine synthesis, we found that the stereoselectivity of the carbonyl methylation of the 2′-ketonucleoside derivative



SCHEME 1



SCHEME 2



SCHEME 3

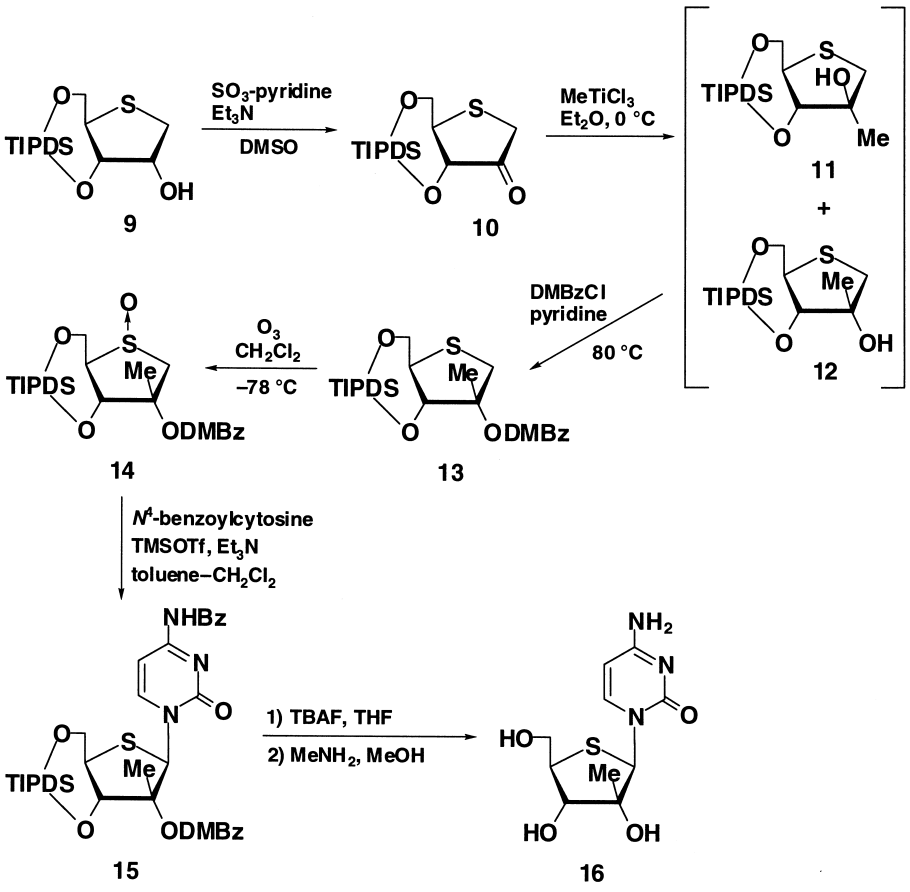
varied depending on the alkylating reagent (Scheme 2).[3] Thus, the reaction of **4** with MeLi and $AlMe_3$ afforded **5** in at least 80% yield as the sole product arising from nucleophilic attack at the less hindered α-face. While the reaction with MeMgBr gave **5** and **6** in a 1:0.8 ratio (52% and 43%, respectively) presumably due to a chelation-controlled process in which the Grignard reagent would chelate between the 2-carbonyl oxygen of the pyrimidine ring and the 2′-carbonyl oxygen of the sugar moiety. Accordingly, we next examined the carbonyl methylation of the 2′-keto-4′-thiocytidine using MeLi, $AlMe_3$, and MeMgBr (Scheme 3). Compound **1** was treated with DMSO-Ac_2O until the starting material had been consumed. The resulting **2β** containing **2α** (ca. **2α**:**2β** = 1:4) was worked up in water and then subjected to methylation under the conditions in Table 1. Although the reaction with MeMgBr gave the best chemical yield, all reactions afforded **7** as the sole product arising from nucleophilic attack on the less hindered α-face.

TABLE 1 Reaction of Compound **2β** with Organometallic Reagents

Entry	Reagent	Conditions	Yield of 7
1	MeLi	Et_2O, −78°C	26
2	Me_3Al	CH_2Cl_2, −78° to rt	54
3	MeMgBr	Et_2O, −78°C	55

Unlike the reaction with **4**, the expected chelation-controlled process causing nucleophilic attack on the β-face was not observed even in the reaction of **2*β*** with MeMgBr, probably due to the structural difference between **4** and **2*β***. Treatment of **7** with ammonium fluoride in MeOH, followed by methylamine, gave the free nucleoside **8** in 94% yield. The structure of **8** was confirmed by NOE experiment. Thus, the expected NOEs were observed at H-1′ (4.6%) and H-4′ (1.2%) upon irradiation of the methyl protons at the 2′-position.

Since we were unsuccessful in synthesizing 2′-*C*-methyl-4′-thiocytidine from **2*β***, we decided to adopt an alternative route, in which the methyl group would be introduced prior to the Pummerer reaction, as shown in Scheme 4. Thus, **9** was converted to the ketone **10**. The SO_3-pyridine complex proved to be superior to DMSO-Ac_2O for use in this oxidation due to the formation of the corresponding methylthiomethyl ether derivative



SCHEME 4

under latter conditions. When the resulting **10** was treated with $MeTiCl_3$, a mixture of **11** and **12** was obtained in 72% yield (**11**:**12** = 1:0.33). Although other reagents such as MeLi, $AlMe_3$, and MeMgBr were examined, none of these increased the ratio of the desired **12**. Since **11** and **12** were inseparable by silica gel column chromatography, the mixture was subsequently treated with dimethoxybenzoyl chloride (DMBzCl) in pyridine at 80°C to isolate **13** in 19% yield. After conversion of **13** into the sulfoxide **14**, the Pummerer reaction of **14** was carried out in the presence of N^4-benzoylcytosine, and the 2′-*C*-methyl-4′-thiocytidine derivative **15** was obtained in 23% yield. Although this reaction proceeded stereoselectively due to the effect of the DMBz group, the chemical yield was insufficient because of steric hindrance of the methyl substituent at the 2-position. Since no other coupling product was detected, the thiocarbocation intermediate generated from **14** under the Pummerer conditions would be decomposed prior to coupling with N^4-benzoylcytosine. Deprotection of **15** by tetrabutylammonium fluoride (TBAF) in THF, followed by treatment with methylamine gave the desired 2′-*C*-methyl-4′-thiocytidine (**16**) in 97% yield. Although the antileukemic activity of **8** and **16** was tested toward L1210 cells in vitro, neither compound inhibited cell growth at a concentration of 100 μg/mL, while the IC_{50} value of 2′-*C*-methylcytidine was 12 μg/mL.[3,4]

In conclusion, we have examined the synthesis of 2′-*C*-methyl-4′-thiocytidine (**16**) via the 2′-keto-4′-thiocytidine derivative **2*β*** and found that **2*β*** unexpectedly isomerized to its α-anomer **2*α*** under the oxidation conditions, and that the methylation of **2*β*** proceeded predominantly from the less hindered α-face to give **7** under all conditions. Accordingly, **16** was synthesized via the Pummerer reaction between the 2-*C*-methyl-4-thiosugar **14** and N^4-benzoylcytosine. The desired **16** showed no significant cytotoxicity toward L1210 cells. In our previous paper, we reported the synthesis of 1-(3-*C*-ethynyl-4-thio-β-D-ribofuranosyl)cytosine, which had no significant cytotoxicity.[15] From the results obtained in this and previous papers, it may be concluded that 4′-thioribocytidine derivatives are less susceptible to phosphorylation by cellular uridine-cytidine kinase. Since some 2′-deoxy-4′-thiocytidine derivatives are suggested to phosphorylate by cellular deoxycytidine kinase and show potent cytotoxicity,[21,22] further investigations of the susceptibility of 4′-thioribocytidine derivatives to uridine-cytidine kinase is needed.

EXPERIMENTAL SECTION

General Methods

Physical data were measured as follows: Melting points are uncorrected. ^1H and ^{13}C NMR spectra were recorded at 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), q (quartet), m (multiplet), or br (broad). All exchangeable protons were detected by addition of D_2O. TLC was done on Merck Kieselgel F254 precoated plates. Silica gel used for column chromatography was Merck silica gel 5715.

4′-α-Thiocytidine (3).[18] A mixture of **1**[15] (244 mg, 0.40 mmol) and Ac_2O (2.0 mL) in DMSO (4.0 mL) was stirred at room temperature overnight. The reaction was quenched by addition of saturated aqueous $NaHCO_3$, and the whole reaction mixture 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 and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (2:1–1:2), to give **2** (**2α**:**2β** = 7:1) as a mixture of diastereomers (227 mg, 93%). A solution of the resulting **2** in MeOH (7 mL) was treated with $NaBH_4$ (55 mg, 1.4 mmol) for 10 min at room temperature. The solvent was removed, and the residue was purified by a silica gel column, eluted with hexane/AcOEt (2:1–1:3), to give protected 4′-α-thiocytidine derivative. The resulting compound was then dissolved in MeOH (3 mL) and ammonium fluoride (122 mg, 3.3 mmol) was added to the solution. The whole reaction mixture was heated under reflux for 12 h. The solvent was removed in vacuo, and the residue was dissolved in methylamine in MeOH (40%, 5 mL). The reaction mixture was kept for 2 h at room temperature, and the solvent was removed in vacuo. The residue was purified by a silica gel column, eluted with 33% MeOH in $CHCl_3$, to give **3** (32 mg, 31% from **1**).

***N*^{4}-Benzoyl-1-[2-*C*-methyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-*arabino*-pentofuranosyl]cytosine (7).** A mixture of **1** (302 mg, 0.50 mmol) and Ac_2O (2.5 mL) in DMSO (5.0 mL) was stirred for 2.5 h at room temperature. The reaction was quenched by addition of saturated aqueous $NaHCO_3$, and the whole reaction mixture 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 and concentrated in vacuo to give crude **2β**. The residue was dissolved in Et_2O (5 mL), and MeMgBr (3.0 M in Et_2O, 0.83 mL, 2.5 mmol) was added dropwise to the solution at −78°C. The mixture was stirred for 4 h at the same temperature, and the reaction was quenched by addition of saturated aqueous NH_4Cl. After warming 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 and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (3:1–1:1), to give **7**

(174 mg, 56% as a white foam): FAB-LRMS *m/z* 620 (MH^+, 25%); ^1H NMR ($CDCl_3$) δ: 8.86 (d, 1H, J = 7.6 Hz), 8.72 (br s, 1H), 7.90–7.88 (m, 2H), 7.64–7.49 (m, 4H), 5.87 (s, 1H), 4.19 (dd, 1H, J = 2.9 and 12.6 Hz), 4.01 (d, 1H, J = 10.0 Hz), 3.98 (d, 1H, J = 12.6 Hz), 3.72 (dd, 1H, J = 2.9 and 10.0 Hz), 2.83 (br s, 1H), 1.57 (s, 3H), 1.20–0.92 (m, 28H); ^{13}C NMR ($CDCl_3$) δ: 166.18. 161.78, 157.64, 147.09, 133.01, 132.86, 128.87, 128.43, 127.38, 127.17, 96.29, 80.21, 73.10, 66.48, 58.90, 47.57, 21.15, 17.70, 17.65, 17.57, 17.50, 17.40, 17.36, 16.99, 16.90, 13.73, 13.42, 13.27, 12.60. Anal. calcd for $C_{29}H_{45}N_3O_6SSi_2$: C, 56.19; H, 7.32; N, 6.78. Found: C, 56.19; H, 7.32; N, 6.56.

1-(2-*C*-Methyl-4-thio-β-D-*arabino*-pentofuranosyl)cytosine (8). A mixture of **7** (66 mg, 0.11 mmol) in MeOH (2 mL) containing ammonium fluoride (79 mg, 2.1 mmol) was heated under reflux for 1 h. The solvent was removed in vacuo, and the residue was dissolved in methylamine in MeOH (40%, 2 mL). The reaction mixture was kept for 15 min at room temperature, and the solvent was removed in vacuo. The residue was purified by a silica gel column, eluted with 33% MeOH in $CHCl_3$, to give **8** (28 mg, 94% as a white solid): FAB-LRMS *m/z* 274 (MH^+, 17%); ^1H NMR (DMSO-d_6) δ: 8.00 (d, 1H, J = 7.5 Hz), 7.12 and 7.03 (br s, each 1H), 6.14 (s, 1H), 5.67 (d, 1H, J = 7.5 Hz), 5.42 (d, 1H, J = 4.7 Hz), 5.18 (s, 1H), 5.09 (t, 1H, J = 5.3 Hz), 3.81–3.72 (m, 2H), 3.64–3.59 (m, 1H), 3.10 (m, 1H), 1.11 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 165.09, 155.90, 144.52, 93.04, 81.08, 78.83, 64.09, 63.32, 54.78, 21.03. Anal. calcd for $C_{10}H_{15}N_3O_4S$: C, 43.95; H, 5.53; N, 15.37. Found: C, 43.72; H, 5.43; N, 15.47.

5,5,7,7-Tetraisopropyl-tetrahydro-4,6,8-trioxa-1-thia-5,7-disilacyclopentacycloocten-3-one (10). To a solution of **9**[17] (1.98 g, 5.1 mmol) in DMSO (25 mL) was added SO_3-pyridine (4.0 g, 25.2 mmol) and triethylamine (7.0 mL, 51.0 mmol), and the whole reaction mixture was stirred for 2.5 h at room temperature. The reaction was quenched by addition of saturated aqueous $NaHCO_3$, and the mixture 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 and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (50:1), to give **10** (1.6 g, 81% as a yellow oil): FAB-LRMS *m/z* 391 (MH^+, 27%); ^1H NMR ($CDCl_3$) δ: 4.50 (d, 1H, J = 10.8 Hz), 4.18 (dd, 1H, J = 3.2 and 12.9 Hz), 3.94 (dd, 1H, J = 3.2 and 12.9 Hz), 3.40–3.29 (m, 3H), 1.12–1.03 (m, 28H); ^{13}C NMR ($CDCl_3$) δ: 207.00, 76.21, 59.53, 48.09, 34.18, 17.51, 17.44, 17.40, 17.20, 17.11, 17.07, 17.01, 16.72, 16.65, 13.76, 13.39, 12.91. Anal. calcd for $C_{17}H_{34}O_4SSi_2$: C, 52.26; H, 8.77. Found: C, 52.16; H, 8.61.

1,4-Anhydro-2-*O*-(2,4-dimethoxybenzoyl)-2-*C*-methyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-D-ribitol (13). An Et_2O solution (20 mL) containing $TiCl_4$ (1.8 mL, 16.4 mmol) was cooled to −78°C, and MeLi (1.14 M in Et_2O, 14.4 mL, 16.4 mmol) was added to the solution at the same temperature. After being stirred for 10 min, the solution was warmed to 0°C, and a THF solution (20 mL) of **10** (1.6 g, 4.1 mmol) was added to the resulting $MeTiCl_3$ solution at the same temperature. The reaction mixture was stirred for 8.5 h at room temperature, and the reaction was quenched by addition of saturated aqueous NH_4Cl. The mixture was partitioned between AcOEt and H_2O, and the separated organic layer was washed with H_2O, followed by brine. The organic layer was dried and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (50:1–20:1), to give **11** and **12** as a mixture of diastereomers (1.19 g, 72%; **11**:**12** = 1:0.33). To a mixture of **11** and **12** (1.19 g, 2.83 mmol) in pyridine (14 mL) was added DMBzCl (1.7 g, 8.5 mmol), and the mixture was stirred for 16 h at 80°C. The reaction was quenched by addition of ice, and 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 and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (50:1–10:1), to give **13** (0.31 g, 19% as a colorless oil): FAB-LRMS *m/z* 593 (MNa^+, 8%); ^{1}H NMR ($CDCl_3$) δ: 7.94 (d, 1H, J = 8.9 Hz), 6.47–6.43 (m, 2H), 4.12 (dd, 1H, J = 2.6 and 12.5 Hz), 3.96–3.83 (m, 9H), 3.66 (ddd, 1H, J = 2.6, 3.3, and 9.2 Hz), 2.91 (d, 1H, J = 12.5 Hz), 1.77 (s, 3H), 1.12–0.85 (m, 28H); ^{13}C NMR ($CDCl_3$) δ: 163.99, 163.90, 161.60, 134.00, 112.87, 104.20, 98.72, 86.90, 80.65, 60.24, 55.82, 55.43, 49.82, 33.55, 21.56, 17.69, 17.45, 17.42, 17.38, 17.32, 14.16, 13.51, 13.41, 12.81. Anal. calcd for $C_{27}H_{46}O_7SSi_2$: C, 56.81; H, 8.12. Found: C, 56.83; H, 8.20.

1,4-Anhydro-2-*O*-(2,4-dimethoxybenzoyl)-2-*C*-methyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-sulfinyl-D-ribitol (14). Ozone was bubbled through a solution of **13** (306 mg, 0.54 mmol) in CH_2Cl_2 (5.0 mL) at −78°C. After 10 min, argon gas was bubbled through the solution 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 (1:1), to give **14** (251 mg, 80% as a colorless oil): FAB-LRMS *m/z* 587 (MH^+, 2%); FAB-HRMS calcd for $C_{27}H_{47}O_8SSi_2$ (MH^+) 587.2530, found 587.2562; ^{1}H NMR ($CDCl_3$) δ: 7.93 (d, 1H, J = 9.2 Hz), 6.47–6.43 (m, 2H), 4.58 (d, 1H, J = 12.9 Hz), 4.26 (dd, 1H, J = 3.0 and 12.9 Hz), 3.93 (d, 1H, J = 15.8 Hz), 3.88–3.77 (m, 7H), 3.55 (dd, 1H, J = 3.0 and 11.5 Hz), 3.14 (d, 1H, J = 15.8 Hz), 1.79 (s, 3H), 1.13–1.03 (m, 28H); ^{13}C NMR ($CDCl_3$) δ: 164.12, 163.90, 161.65, 134.03, 112.47,

104.27, 98.71, 84.29, 74.09, 73.38, 56.50, 56.01, 55.82, 55.45, 22.12, 17.35, 17.26, 17.20, 17.16, 14.21, 13.26, 13.18, 12.62.

N^4-Benzoyl-1-[2-*O*-(2,4-dimethoxybenzoyl)-2-*C*-methyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-*ribo*-pentofuranosyl]cytosine (15). To a suspension of N^4-benzoylcytosine (130 mg, 0.6 mmol) in toluene (4 mL) was added triethylamine (84 μL, 0.6 mmol) and TMSOTf (0.47 mL, 2.4 mmol), and the mixture was stirred at room temperature until giving two-phase clear solution. After being added CH_2Cl_2 (2 mL) to the above solution, the whole solution was added to a solution of **14** (237 mg, 0.4 mmol) in CH_2Cl_2 (2 mL) dropwise over 15 min via a cannula. An additional triethylamine (0.25 mL, 1.8 mmol) in toluene (2 mL) was added dropwise to the reaction mixture. After being stirred for 5 min at room temperature, the reaction was quenched by addition of ice, and 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 and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (5:1–1:1), to give **15** (72 mg, 23% as a white foam): FAB-LRMS *m/z* 806 (MNa^+, 21%); FAB-HRMS calcd for $C_{38}H_{53}N_3O_9SSi_2Na$ (MNa^+) 806.2938, found 806.2942; ^1H NMR ($CDCl_3$) δ: 11.40 (br s, 1H), 7.99 (d, 1H, J = 7.3 Hz), 7.77 (d, 2H, J = 8.7 Hz), 7.62 (d, 1H, J = 7.3 Hz), 7.59–7.47 (m, 3H), 7.42 (d, 1H, J = 7.3 Hz), 6.83 (s, 1H), 6.63–6.53 (m, 2H), 4.18–4.11 (m, 3H), 3.82 (s, 6H), 3.75 (m, 1H), 1.52 (s, 3H), 1.14–0.87(m, 28H); ^{13}C NMR ($CDCl_3$) δ: 167.35, 164.23, 162.85, 162.63, 161.19, 154.74, 146.00, 133.45, 133.40, 132.96, 132.82, 128.47, 111.90, 105.32, 99.03, 96.57, 89.47, 76.81, 62.53, 59.38, 55.98, 55.78, 49.37, 17.62, 17.51, 17.40, 17.31, 17.17, 13.33, 13.02, 12.78, 12.73.

2′-*C*-Methyl-4′-thiocytidine (16). To a solution of **15** (24 mg, 0.03 mmol) in THF (0.5 mL) was added AcOH (3.5 μL, 0.06 mmol) and TBAF (1 M in THF, 60 μL, 0.06 mmol) at 0°C. After being stirred for 15 min 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 and concentrated in vacuo, and the residue was dissolved in methylamine in MeOH (40%, 1 mL). The reaction mixture was kept for 2 h at room temperature, and the solvent was removed in vacuo. The residue was purified by a silica gel column, eluted with 30% MeOH in $CHCl_3$, to give **16** (8 mg, 94% as a white solid): FAB-LRMS *m/z* 274 (MH^+, 21%); FAB-HRMS calcd for $C_{10}H_{16}N_3O_4S$ (MH^+) 274.0862, found 274.0864; ^1H NMR (DMSO-d_6) δ: 8.06 (d, 1H, J = 7.6 Hz), 7.19 and 7.11 (br s, each 1H), 5.96 (s, 1H), 5.73 (d, 1H, J = 7.6 Hz), 5.15–5.05 (m, 3H), 3.84 (ddd, 1H, J = 2.9, 4.2, and 11.2 Hz), 3.71 (ddd, 1H,

J = 4.2, 5.9, and 11.2 Hz), 3.48 (m, 1H), 3.30 (m, 1H), 0.91 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 164.99, 155.76, 142.68, 94.15, 81.58, 76.08, 66.37, 61.28, 52.42, 20.72.

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