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Synthesis of 4′-substituted 2′-deoxy-4′-thiocytidines and its Evaluation for Antineoplastic and Antiviral Activities

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Keywords: nucleoside; thio-sugar; glycal; glycosidation; antineoplastic activity; antiviral activity

TOC GRAPHIC

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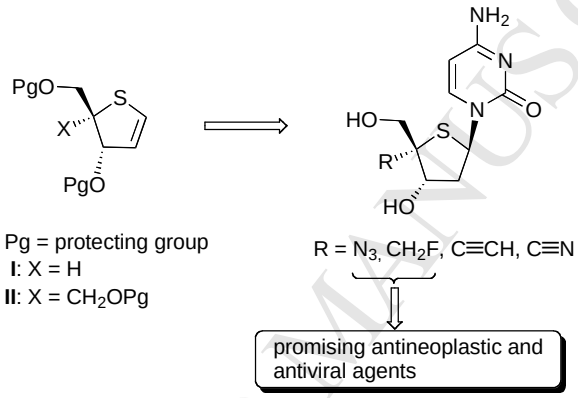


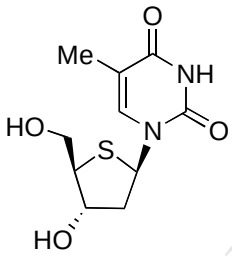
Abstract

4′-Azido- (**7**), 4′-*C*-fluoromethyl- (**8**) 4′-*C*-ethynyl- (**9**) and 4′-*C*-cyano- (**10**) 2′-deoxy-4′-thiocytidines have been synthesized. In this study, it was found that the isolated yield of 4′-thiouracil nucleoside **13** in a Lewis acid-promoted Vorbrüggen-type glycosidation utilizing **12** was better than that of the electrophilic glycosidation reaction between silylated uracil and **11**. This improved result prompted us to perform the glycosidation utilizing **36** and **43** for the synthesis of **37** and **44**. Introduction of the azido group was carried out by nucleophilic substitution in the 4′-benzoyloxy derivative **22a**. On the other hand, **9** and **10** were synthesized by way of the chemical manipulation of the hydroxymethyl group at the 4′-position of **46**.

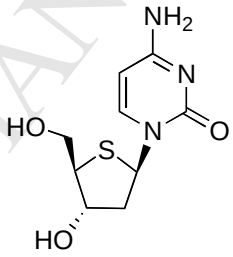
Evaluation of the antineoplastic activity of **2** and **7**-**10** against human B-cell (CCRF-SB) and T-cell leukemia (Molt-4) cell lines revealed that 4′-azido- (**7**) and 4′-*C*-fluoromethyl- (**8**) derivatives exhibited cytotoxic activity whereas no cytotoxicity was observed in the 4′-*C*-ethynyl- (**9**) and 4′-*C*-cyano- (**10**) derivatives as well as the parent compound **2**. Compound **7** was also found to possess promising antiviral activity against VZV and HSV-1 without any cytotoxity against HEL host cells. It is noteworthy that **7** exhibited potent inhibitory activities against the thymidine kinase-deficient (TK^-) mutant of VZV and HSV-1.

1. Introduction

Nucleoside analogues are recognized as an important class of biologically active compounds, especially as antiviral and antitumor agents.[1-21] Among their sugar-modified analogues, 4′-thionucleosides, in which the oxygen atom in the furanose ring is replaced with a sulfur atom, have attracted much attention since the discovery of the antiviral and antitumor activities of 4′-thiothymidine (**1**) and 2′-deoxy-4′-thiocytidine (**2**) (Figure 1).[22-24]



4'-thiothymidne (**1**)



2'-deoxy-4'-thiocytidine (**2**)

Figure 1. Structures of compounds **1** and **2**.

Recently, synthesis of 2′-substituted derivatives **3-6** of **2** and their evaluation for antineoplastic activity has been reported (Figure 2).[25] In this study, 4′-thioFAC **4** has emerged as a highly potent antineoplastic agent. Furthermore, some 2′-deoxy-4′-thiocytidine derivatives exhibited antiviral activities.[25b]

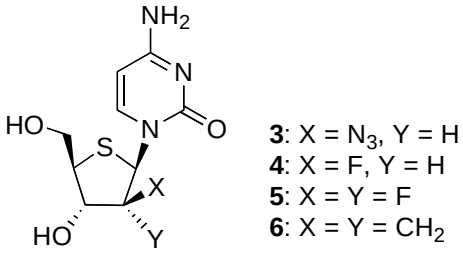


Figure 2. Structures of 2'-substituted 2'-deoxy-4'-thiocytidines **3**-**6**.

To expand the structure-activity relationships of **2**, we have been interested in the antineoplastic and antiviral activities of 4′-substituted congeners **7**-**10** because introduction of a substituent into the 4′-position of nucleoside has been found to lead to biologically-active agents.[26] Herein, we describe the results of the synthesis of **7**-**10** and their evaluation for antineoplastic and antiviral activities.

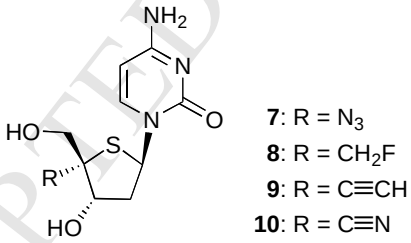


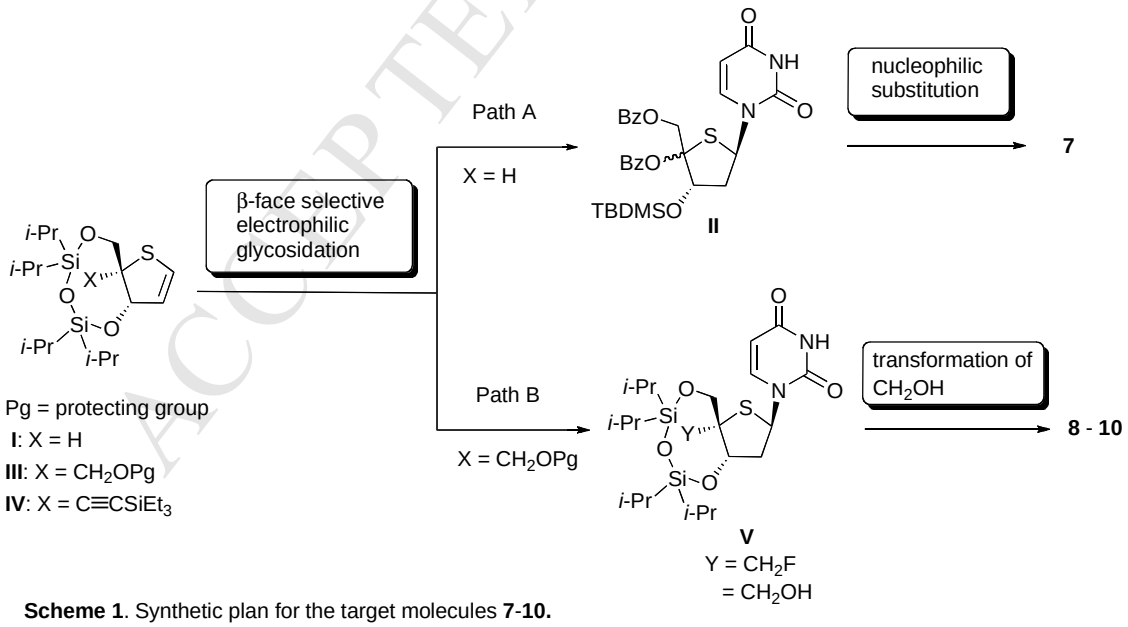
Figure 3. Structures of the target molecules **7**-**10**.

2. Results and Discussion

2.1. Chemistry

The synthetic plan is outlined in Scheme 1. 4′-Azido-2′-deoxy-4′-thiocytidine **7** would be obtained on the basis of the synthetic protocol of the respective thymidine

nucleoside (Path A).[27] Thus, 4-thiofuranoid glycal **I** is subjected to electrophilic glycosidation and subsequent $Pb(OBz)_4$-mediated dibenzoyloxylation to give 4′-benzoyloxy-2′-deoxy-4′-thiouridine derivative **II**.[28] Lewis-acid promoted nucleophilic substitution of **II** with $TMSN_3$ and conversion of the uracil moiety to cytosine base would provide the first target molecule **7**. In the synthesis of the 4′-branched 2′-deoxy-4′-thiocytidines **8**-**10**, glycal **III** might be a suitable glycosyl donor on the basis of the recently published procedure in which **9** has been already synthesized utilizing **IV** as a glycosyl donor (Path B).[29] The chemical transformation of the substituent at the 4′-position of **V** would furnish **8**-**10**.



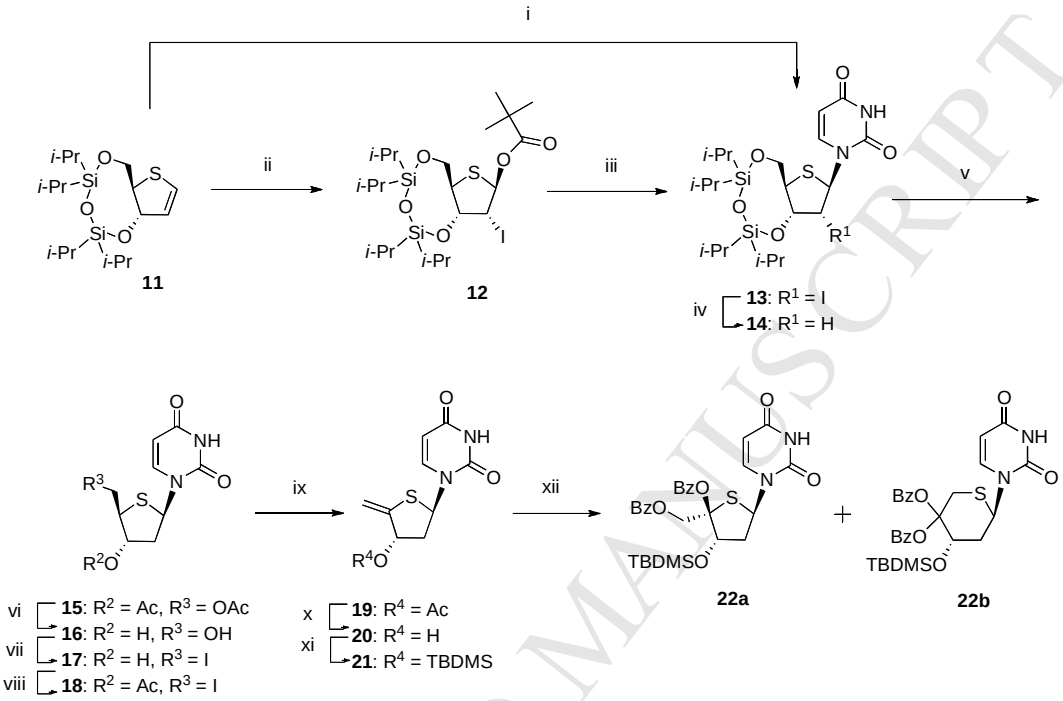
Scheme 1. Synthetic plan for the target molecules **7**-**10.**

Initially, preparation of 4′-benzoyloxy-2′-deoxy-4′-thiouridine derivative **II** was

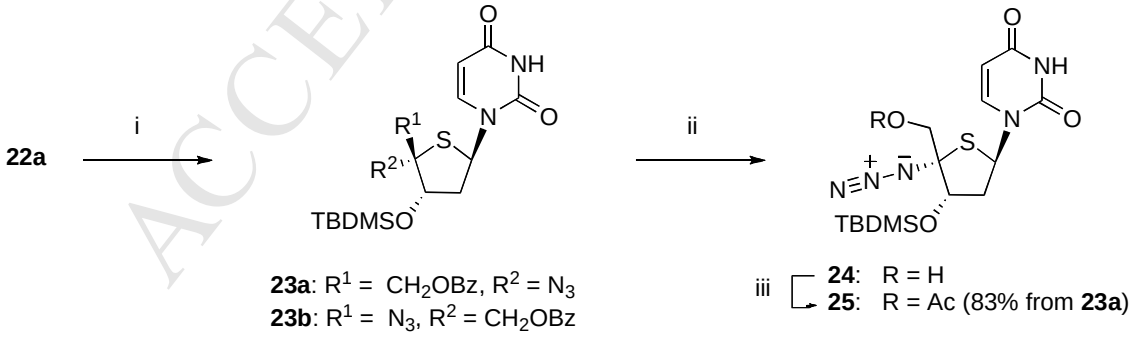
carried out (Scheme 2). *N*-Iodosuccimide (NIS)-mediated electrophilic glycosidation between 3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-4-thiofuranoid glycal **11** and silylated uracil gave the β-anomer of the protected 2′-deoxy-2′-iodo-4′-thiouridine **13** as a sole product, albeit the isolated yield of **13** was moderate (57% yield).[30,31] To improve the isolated yield of **13**, Lewis-acid promoted Vorbrüggen-type glycosidation was examined. Thus, the desired glycosyl donor **12** was prepared as single stereoisomer in 93% isolated yield by reacting **11** with pivalic acid in the presence of NIS in CH_3CN-CH_2Cl_2. When **12** was reacted with silylated uracil in the presence of TMSOTf in CH_3CN/CH_2Cl_2, **13** was isolated in 87% yield. The isolated yield in the two **ste**p sequence for the synthesis of **13** was superior to that of the electrophilic glycosidation; 81% (**11**→**12**→**13**) vs. 57% (**11**→**13**). Bu_3SnH-Mediated radical reduction of **13** using Et_3B as initiator in toluene at –60 °C provided **14** in 98% yield. Compound **14** was converted to di-*O*-acetate **15** in quantitative yield in two steps in one pot manner. 2′-Deoxy-4′-thiourudine **16** was obtained from **15** by treating with methanolic ammonia. 4′,5′-Unsaturated uracil nucleoside **19** was prepared in 47% yield in 4 steps from **15**; 1) reaction of **16** with I_2/Ph_3P/pyridine, 2) acetylation of **17**, 3) elimination of HI of **18** with DBN. Compound **19** was converted to **20** and subsequent silylation of **20** gave **21** in 61% yield in two steps from **19**. When **21** was treated with $\underline{Pb(OBz)_4}$ in PhMe at rt, a

mixture of two products was formed. The target molecule 4′-benzoyloxy-2′-deoxy-4′-thiouracil nucleoside **22a** was obtained in 63% yield with the α-L-configuration as evidenced by HMBC correlation (H-5′/5′-OCOPh) and NOE experiment (H-2′b (α-H)/CH_2-5′a, Si-Me/CH_2-5′a and Si-*tert*-Bu/CH_2-5′b). The other product was the ring-expanded compounds **22b** (28%), their thiopyranosyl structures being evident from the observed HMBC correlations between C-1′/H-5′a and C-1′/H-5′b. By comparing with the result obtained by the $Pb(OAc)_4$-mediated di-acetoxylation,[27] in which the respective 4′-acetoxynucleoside was obtained in 42% yield along with the pyranosyl counterpart (34% yield), the use of $Pb(OBz)_4$ gave improved isolated yield of the desired 4′-benzoyloxy-4′-thionucleoside. With 4′-benzoyloxy nucleoside **22a** in hand, nucleophilic substitution was examined. Thus, when **22a** was reacted with Me_3SiN_3 (5 equiv) in the presence of $SnCl_4$ in CH_2Cl_2 at –30 °C, the desired 4′-α-azido derivative **23a** was obtained in 61% isolated yield along with the 4′-β-azido **23b** (20% yield) (Scheme 3). The depicted stereochemistry of **23a** was assigned on the basis of NOE experiment of the respective 5′-*O*-acetyl derivative **25** (H-6/H-5′a and H-6/H-5′b) derived in two steps from **23a** via **24**. Compound **25** was converted to the respective cytosine nucleoside **27** (Scheme 4) through ammonolysis of the 4-*O*-(2,4,6-triisopropylbenzenefulfonyl) (TPS) ester **26**. Compound **27** was

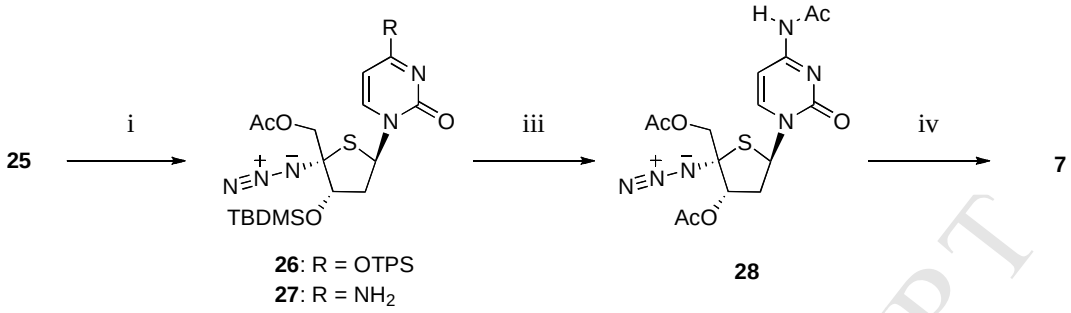
transformed into the tri-acetate **28**, which was subjected to treatment of NH_3 in MeOH to give the target molecule **7**.



Scheme 2. Synthesis of 4'-benzoyloxy-2'-deoxy-4'-thiouridine derivative **22a**. Reagents and Coditions: (i) 1) uracil, BSA, CH_3CN, 2) NIS, CH_2Cl_2 (57%);(ii) NIS, benzoic acid, CH_3CN (93%) ;(iii) 1)uracil, BSA, CH_3CN, 2) TMSOTf, CH_2Cl_2 (87%), (iv) Bu_3SnH, Et_3B, toluene (98%); (v) 1) Bu_4NF then Ac_2O (100%), (vi) NH_3/MeOH, (vii) $!_2$, Ph_3P, pyridine, dioxane, (viii) Ac_2O, i-Pr_2NEt, DMAP, CH_3CN; (ix) 1) DBN, CH_3CN (47% in 4 stepsfrom **15**), (x) NH_3/MeOH, (xi) TBDMSCl, imidazole, DMF (61% in 2 steps from **19**); (xii) $Pb(OBz)_4$, toluene, **22a** (63%) and **22b** (28%)



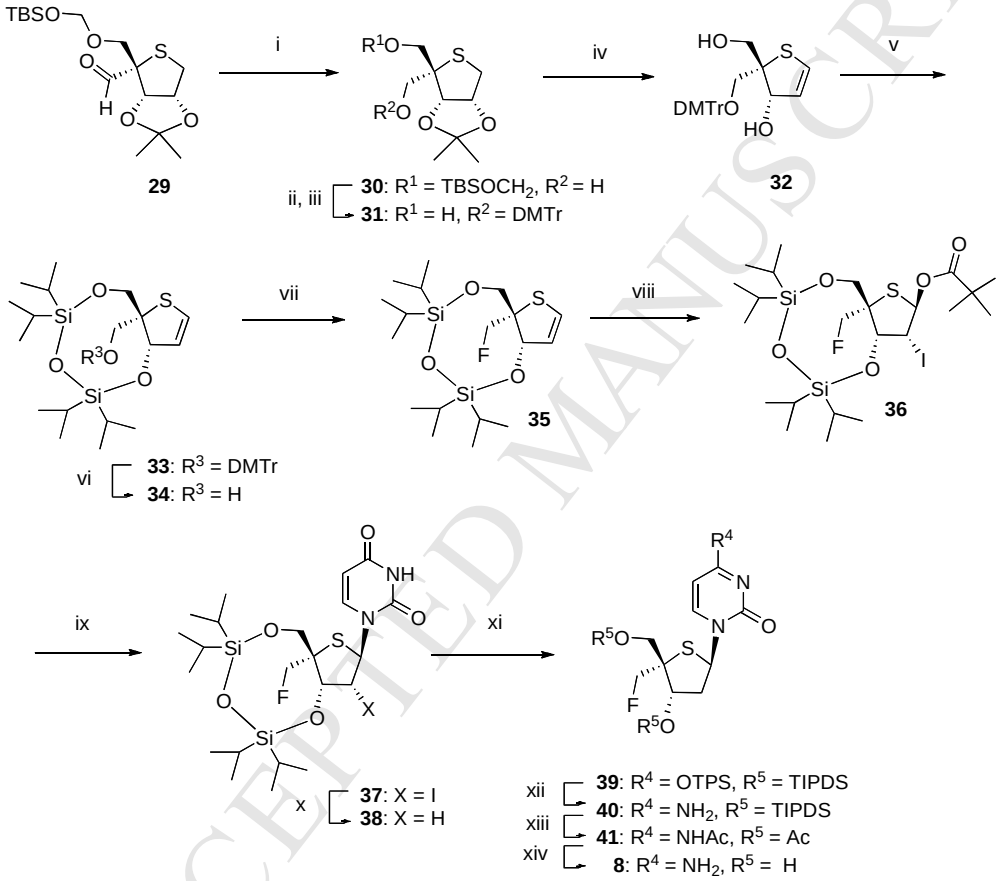
Scheme 3. Synthesis of 4'-azido-2'-deoxy-4'-thiouridine derivative **25**. Reagents and Coditions: (i) $TMSN_3$, $SnCl_4$, CH_2Cl_2, **23a** (61%) and **23b** (20%); (ii) NaOMe, MeOH; (iii) Ac_2O, *i*-Pr_2NEt, DMAP, CH_3CN, (83% from **23a**)



Scheme 4. Synthesis of 4'-azido-2'-deoxy-4'-thiocytidine **7**. Reagents and Coditions: (i) TPSCl, K_2CO_3, CH_3CN; (ii) NH_4OH, THF; (iii) 1) Bu_4NF, THF, 2) Ac_2O, *i*-Pr_2NEt, DMAP, CH_3CN (75% from **25**); (iv) NaOMe, MeOH (88%)

We then turned our attention to the synthesis of 4′-branched 2′-deoxy-4′-thiocytidines **8**-**10**. Initially, preparation of the glycosyl donor **36** for 4′-*C*-fluoromethyl congener **8** was carried out (Scheme 5). The aldehyde **29** obtained from D-ribose according to the published procedure[29] was converted into **31** through **30** in three steps. Compound **31** was subjected to β-elimination by reacting with *tert*-butyl lithium to give the 4-thiofuranoid glycal **32** in 89% isolated yield. Protection of the hydroxyl groups of **32** with 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDS) group (85% yield) followed by TsOH-mediated methanolysis (98% yield) of the dimethoxytrityl ether **33** gave the 4-*C*-α-hydroxymethyl glycal **34**. Reaction of **34** with DAST in the presence of Na_2CO_3 at −10 °C gave 4-*C*-fluoromethyl glycal **35** in 85% yield. The desired glycosyl donor **36** could be obtained in 97% yield as a single stereoisomer under the identical conditions as for the synthesis of **12**. Next, TMSOTf-mediated glycosidation between **36** and silylated uracil gave 2′-deoxy-4′-thiouracil nucleoside **37** in 72% isolated yield. Tin-radical mediated

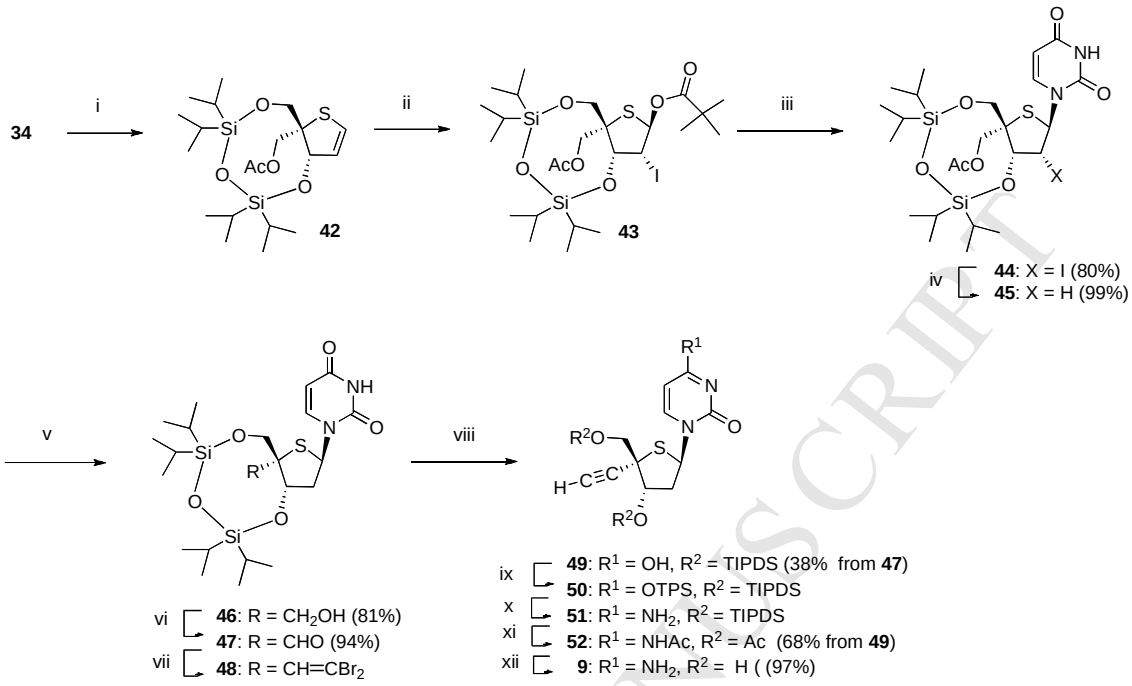
reduction of **37** gave **38** (99% yield). Cytosine nucleoside **40** could be obtained from **38** through **39** as for the synthesis of **27**. Transformation of **40** to the acetate **41** (59% yield in 3 steps from **38**) followed by removal of the protecting group of **41** (78% yield) gave the target 4′-*C*-fluoromethyl-2′-deoxy-4′-thiocytidine **8**.



Scheme 5. Synthesis of 4'-*C*-fluoromethyl-2'-deoxy-4'-thiocytidine **8**. Reagents and Coditions: (i) 1) $NaBH_4$, MeOH (98%), (ii) DMTrCl, Et_3N, CH_2Cl_2, (iii) Bu_4NF, THF (95% in 2 steps); (iv) 1) *tert*-BuLi,THF, 2) AcOH (89%); (v) 1) TIPDSCl, imidazole, DMF (85%), (vi) 1% TsOH/MeOH, $CHCl_3$ (98%); (vii) DAST, $NaHCO_3$, CH_2Cl_2 (85%); (viii) pivalic acid, NIS, CH_3CN-CH_2Cl_2 (97%); (ix) 1) uracil, BSA, CH_3CN, 2) TMSOTf (72%), (x) O_2, Bu_3SnH, Et_3B,toluene (99%); (xi) 1) TPSCl, K_2CO_3, CH_3CN, (xii) NH_4OH, THF, (xiii) Bu_4NF thef Ac_2O (59% in 3 steps), (xiv) NaOMe, MeOH (78%)

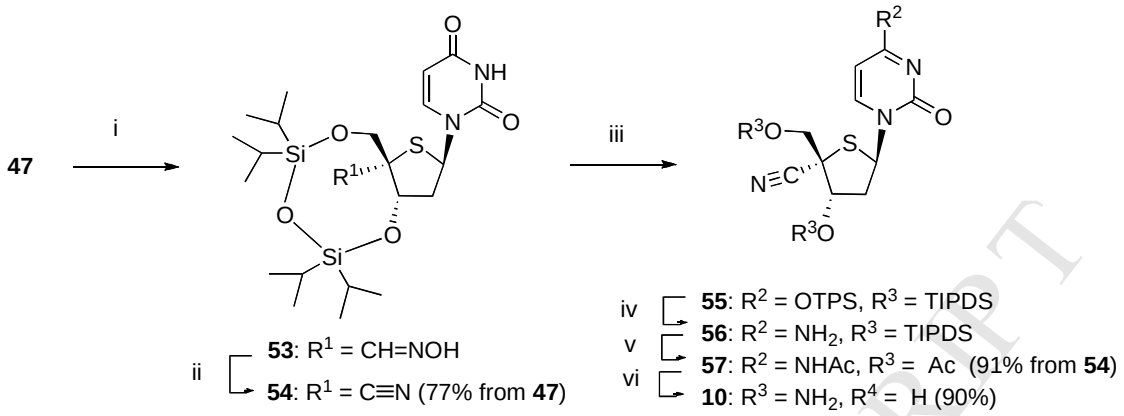
Finally, synthesis of 4′-*C*-ethynyl- (**9**) and 4′-*C*-cyano- (**10**) 2′-deoxy-4′-thiocytidine was carried out (Schemes 6 and 7). 4-*C*-Acetoxymethyl glycal **42**, which was obtained from **34** in 96% yield, was used as starting material. According to the synthetic

sequence for **8**, **42** was converted to glycosyl donor **43** (95% yield) and the subsequent glycosidation utilizing **43** provided **44** in 80% yield. Radical reduction of **44** gave **45** (99% yield) and subsequent treatment of **45** with methanolic ammonia furnished 4′-*C*-hydroxymethyl derivative **46** in 81% yield. Oxidation of the primary alcohol of **46** with Dess-Martin periodinane provided the aldehyde **47** in 94% yield. Reaction of **47** with Ph_3P=CHBr at –40 °C gave dibromoolefine **48** which was subsequently treated with butyl lithium to give 4′-*C*-ethynyl nucleoside **49** in 38% yield in two steps.[32] Compound **49** was converted to the cytosine derivative **52** as for the synthesis of **27**. Compound **52** was transformed into 4′-*C*-ethynyl-2′-deoxy-4′-thiocytidine **9** by treatment with methanolic ammonia. All spectral data of **9** were consisted with that of the previously synthesized compound.[29]



Scheme 6. Synthesis of 4'-*C*-ethynyl-2'-deoxy-4'-thiocytidine **9**. Reagents and Coditions: (i) 1) Ac_2O, *i*-Pr_2NEt, DMAP, CH_3CN (98%), (ii) DNIS, benzoic acid, CH_3CN (95%); (iii) 1) uracil, BSA, CH_3CN, 2) TMSOTf, CH_2Cl_2 (80%); (iv) Bu_3SnH, Et_3B, toluene (99%); (v) NH_3-MeOH (81%); (vi) Dess-Martin periodinane, CH_2Cl_2 (94%); (vii) $BrCH_2PPh_3^+Br^-$, KO*tert*-Bu, THF; (viii) BuLi, THF (38% from **47**); (ix) 1) TPSCl, K_2CO_3, CH_3CN, (x) NH_4OH, THF, (xi) Bu_4NF thef Ac_2O (68% from **49**), (xii) NH_3-MeOH (97%)

For the synthesis of **10**, the aldehyde **47** was converted to the oxime **53** by reacting H_2NOH in pyridine and subsequent treatment of **53** with $MsCl/Et_3N$ in CH_2Cl_2 gave the 4′-*C*-cyano nucleoside **54** in 77% yield in two steps (Figure 7). Compound **54** was converted to cytosine nucleoside **57** in 91% yield in 3 steps (**54**→**55**→**56**→**57**). Finally, the target 4′-*C*-cyano-2′-deoxy-4′-thiocytidine **10** could be obtained from **57** in 90% yield by treatment with methanolic ammonia.



Scheme 7. Synthesis of 4'-*C*-cyano-2'-deoxy-4'-thiocytidine **10**. Reagents and Coditions: (i) 1) H_2NOH, pyridine;(ii) MsCl, Et_3N, CH_2Cl_2 (77% from **47**); (iii) 1) TPSCl, K_2CO_3, CH_3CN, (iv) NH_4OH, THF, (v) Bu_4NF thef Ac_2O (91% from **54**), (vi) NH_3-MeOH (90%)

2.2. Biological evaluation

The synthesized 4′-substituted 2′-deoxy-4′-thiocytidines (**7**-**10**) were evaluated for their cytotoxic activities in two human cancer cell lines: B-cell leukemia (CCRF-SB) and T-cell leukemia (Molt-4) cell lines employing MTT assay. The resulting cytotoxic activity data of **7**-**10** as well as the positive control doxorubicin were presented in Table 1. Although **2** has been reported to show high cytotoxicity against human T-cell leukemia CCRF-CEM ($\underline{IC_{50}}$ 3.5 μM for a mixture of β and α-anomer of **2**), no cytotoxicity was observed against both CCRF-SB and Molt-4. Similarly, 4′-ethynyl- (**9**) and 4′-cyano- (**10**) 2′-deoxy-4′-thiocytidine did not show cytotoxic activity against both the above cell lines. On the other hand, 4′-azido-(**7**) exhibited cytotoxicity ($\underline{IC_{50}}$ 7.14 μM for CCRF-SB and 2.72 μM for Molt-4). Most potent cytotoxicity was seen in the

case of the 4′-fluoromethyl derivative (**8**); (IC_{50} 3.19 μM for CCRF-SB and 2.24 μM for Molt-4) although the potency of **7** and **8** was one tenth (for CCRF-SB) and one hundredth (for Molt-4) of that of reference doxorubicin.

Table 1. Antineoplastic activities of **2** and **7**-**10**

compound	IC_{50} (μM) [a]	
	CCRF-SB [b]	Molt-4 [c]
7	7.14	2.72
8	3.19	2.24
9	> 100	> 100
10	> 100	100
2	> 100	> 100
doxorubicin	0.28	0.060

a MTT assay
b human B-cell leukemia
c human T-cell leukemia

Next, antiviral activities of **7** - **10** were also evaluated against the following viruses: human cytomegalovirus strains AD-169 and Davis, varicella-zoster virus (VZV) strain OKA, VZV/TK^- strain 07-1, human cytomegalovirus AD-164 and Davis, herpes simplex virus type-1 (HSV-1) strain KOS, thymidine kinase-deficient (TK^-) HSV-1 KOS strain resistance to ACV, HSV-2 strain G. These assays were based on the inhibition of virus-induced cytopathicity or plaque formation (for HCMV and VZV) in human embryonic lung fibroblasts (HEL).

The parent compound **2** was found to be highly toxic to host HEL cell. 4′-ethynyl- (**9**)

and 4′-cyano- (**10**) 2′-deoxy-4′-thiocytidines have not possessed remarkable antiviral activities. On the other hand, promising antiviral results could be obtained in the case of 4′-azido- (**7**) and 4′-fluoromethyl- (**8**) 2′-deoxy-4′-thiocytidine. By comparing the activities of **7** with that of **8**, **7** was found to show more potent inhibitory activities against most of the above cell lines and less cytotoxicity to HEL host cell than that of **8**. It was noteworthy that antiviral activity of **7** against VZV (TK^+, OKA) (EC_{50} of 0.49 μM), which was ten times more potent than that (EC_{50} of 5.31 μM) of Acyclovir. Its antiviral activity against VZV (TK^-, 07-1) was also potent (0.76 μM of EC_{50}) while the inhibitory activity of Acyclovir was less potent (53.51 μM of EC_{50}). Furthermore, the growth inhibitory activity (EC_{50} of 0.56 μM) of **7** for HSV-1 (TK^- KOS ACV^r) was found not to diminish its activity, by comparing to that (EC_{50} of 0.75 μM) of HSV-1 (KOS) and the activity was three times more potent than that of Ganciclovir (EC_{50} of 1.8 μM).

Table 2. Antiviral activities of **7**-**10**

compound	Antiviral activities EC_{50} (μM) [a]				Antiviral activities EC_{50} (μM) [b]			Cytotoxicity CC_{50} (μM) [c]
	HCMV (AD-169 strain)	HCMV (Davis strain)	VZV (TK^+, OKA)	VZV (TK^-, 07-1)	HSV-1 (KOS)	HSV-1 (TK^- KOS ACV^r)	HSV-2 (G)	cell growth
7	7.64	6.34	0.43	0.76	0.75	0.55	0.70	>100
8	28.1	10.64	2.38	1.65	0.39	1.13	0.71	0.57
9	>100	>100	20.0	38.07	8.28	6.47	2.98	26.75
10	>20	>100	20.0	41.5	21.5	28.5	13.4	ND
2	<0.42	0.44	<0.42	0.42	ND	ND	ND	<0.16
Gancclovir	3.31	5.89			0.4	1.8	0.032	>350
Acyclovir			5.31	53.51	0.6	>250	0.9	>250

a: Effective concentration required to reduce virus plaque formation by 50%. Vurus input was 100 plaque forming units(PFU).
b: Required to reduce virus-induced cytopathogenicity by 50%.
c: Cytotoxic concentration required to reduce human enbrryomic lung cell (HEL) growth by 50%.
ND: not determined

3. Conclusions

In this study, 4′-azido- (**7**), 4′-*C*-fluoromethyl- (**8**) 4′-*C*-ethynyl- (**9**) and 4′-*C*-cyano- (**10**) 2′-deoxy-4′-thiocytidine have been synthesized. It was found that isolated yield of 4′-thiouracil nucleoside **13** in Lewis acid-promoted Vorbrüggen-type glycosidation utilizing **12** was better than that of electrophilic addition reaction between silylated uracil and **11**. This improved result prompted us to perform the glycosidation utilizing **36** and **43** for the synthesis 4′-thionucleosides **37** and **44**. Introduction of the azido group was carried out by nucleophilic substitution of 4′-benzoyloxy derivative **22a** according to the published protocol for the synthesis of 4′-azido-4′-thiothymidine. In the cases of **9** and **10**, chemical manipulation of the hydroxymethyl group of **46** was utilized because the susceptibility of the ethynyl group for electrophilic NIS and the

inertness of the reactivity of 4-*C*-cyano-4-thiofuranoid glycal exerted by electron-withdrawing character of the cyano group. Therefore, an alternative route to **9** was provided in this study.

Evaluation of the antineoplastic activity of **2** and **7**-**10** against human B-cell (CCRF-SB) and T-cell leukemia (Molt-4) cell lines revealed that 4′-azido- (**7**) and 4′-*C*-fluoromethyl- (**8**) derivatives exhibited cytotoxic activity whereas no cytotoxicity was observed in 4′-*C*-ethynyl- (**9**) and 4′-*C*-cyano- (**10**) as well as the parent compound 2′-deoxy-4′-thiocytidine (**2**). Compound **7** was also found to possess promising antiviral activity against VZV and HSV-1 without any cytotoxity against HEL host cell. It is noteworthy that **7** exhibited potent inhibitory activities against thymidine kinase-deficient (TK^-) mutant of VZV and HSV-1.

4. Methods

4.1. General Methods

Melting points are uncorrected. ^1H and ^{13}C NMR spectra were recorded either at 400 MHz or at 500 MHz. Chemical shifts are reported relative to Me_4Si. Mass spectra (MS) were taken in FAB or ESI modex. Column chromatography was carried out on silica gel. Thin-layer chromatography (TLC) was performed on silica gel. When

necessary, analytical samples were purified by high performance liquid chromatography (HPLC). THF was distilled from benzophenone ketyl.

4.2.

1-*O*-Pivaloyl-2-deoxy-2-iodo-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-ribofuranose (12)

To a solution of **11** (428.3 mg, 1.14 mmol) in CH_3CN (4.0 mL)/CH_2Cl_2 (3.0 mL) was added pivalic acid (582.1 mg, 5.70 mmol) and NIS (513.0 mg, 2.28 mmol) at 0 °C under Ar atmosphere and the mixture was stirred for 6 h. The reaction mixture was partitioned between $CHCl_3$/saturated $NaHCO_3$-0.2M $Na_2S_2O_3$ and silica gel column chromatography（hexane/ethyl acetate = 40/1）of the organic layer gave **12** (637.4 mg, 93%) as a syrup; ^{1}H NMR ($CDCl_3$) δ 1.04-1.09 (28H, m, Si-*i*-Pr), 1.18 (9H, s, $C(CH_3)_3$), 3.50-3.52 (1H, m, H-4), 3.61 (1H, dd, $J_{2,3}$ = 4.6 and $J_{3,4}$ = 9.2 Hz, H-3), 3.91 (1H, dd, $J_{4,5a}$ = 2.9 and $J_{5a,5b}$ = 12.9 Hz, H-5a), 4.06 (1H, dd, $J_{4,5b}$ = 2.9 and $J_{5a,5b}$ = 12.9 Hz, H-5b), 4.57 (1H, d, $J_{2,3}$ = 4.6 Hz, H-2), 6.03 (1H, s, H-1); NOE experiment: H-1/H-4, H-2/H-5b'; ^{13}C NMR ($CDCl_3$) δ: 12.8, 12.8, 13.2, 13.3, 17.1, 17.2, 17.3, 17.3, 17.4, 17.4, 17.5, 26.8, 38.6, 38.8, 52.7, 59.4, 83.6, 176.6. FAB-MS(*m*/*z*): 1027(M^+＋H); ESI-MS (*m*/*z*) 625 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{22}H_{43}O_5INaSSi_2$: 625.13066, found: 625.13083 (M^+＋Na).

4.3.

1-[2-Deoxy-2-iodo-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-ribofuranosyl]uracil (13)

To a suspension of uracil (64.4 mg, 0.57 mmol) in CH_3CN (2.5 mL) was added BSA (0.28 mL, 1.14 mmol) at rt under Ar atmosphere and the mixture was stirred for 1 h. To the clear solution was added a solution of **12** (229.0 mg, 0.38 mmol) in CH_3CN (4.0 mL)/CH_2Cl_2 (2.0 mL) and TMSOTf (0.27 mL, 1.52 mmol) at 0 °C under Ar atmosphere and the mixture was stirred 7 h. The reaction mixture was partitioned between $CHCl_3$/saturated $NaHCO_3$-0.2M $Na_2S_2O_3$ and silica gel column chromatography (hexane/ethyl acetate = 2/1) of the organic layer gave **13** (202.9 mg, 87%) as a foam; ^{1}H NMR ($CDCl_3$) δ 0.87-0.97 and 1.06-1.14 (28H, each as m, Si-*i*-Pr), 3.33 (1H, dd, $J_{2',3'}$ = 4.6 and $J_{3',4'}$ = 9.2 Hz, H-3′), 3.63-3.65 (1H, m, H-4′), 4.02 (1H, d, $J_{5'a,5'b}$ = 12.9 Hz, H-5′a), 4.13 (1H, dd, $J_{4',5'b}$ = 3.4 and $J_{5'a,5'b}$ = 12.9 Hz, H-5′b), 4.44 (1H, d, $J_{2',3'}$ = 4.6 Hz, H-2′), 5.70 (1H, dd, $J_{5,NH}$ = 2.3 and $J_{5,6}$ = 8.6 Hz, H-5), 6.03 (1H, s, H-1′), 8.44 (1H, dd, $J_{5,6}$ = 8.6 Hz, H-6), 8.50 (1H, br, NH); NOE experiment: H-6/H-3′, H-6/H-5′b, H-2′/H-5′a, H-2′/H-5′b; ^{13}C NMR ($CDCl_3$) δ: 12.5, 13.1, 13.3, 13.5, 16.9, 16.9, 17.1, 17.2, 17.4, 17.5, 38.2, 53.5, 58.0, 67.5, 71.0, 101.6, 141.2, 150.6, 163.5. ESI-MS (*m*/*z*) 635 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd

for $C_{21}H_{37}O_5N_2INaSSi_2$: 635.08986, found: 635.09042 (M^+ + Na).

4.4.

1-[2-Deoxy-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-ribofuranosyl]uracil (14)

To a solution of **13** (229.0 mg, 0.38 mmol) in toluene (8.0 mL) was added Bu_3SnH (0.25 mL, 0.92 mmol) and Et_3B (0.23 mL, 0.23 mmol) at –60 °C under Ar atmosphere and the mixture was stirred under O_2 atmosphere overnight. The reaction mixture was purified by silica gel column chromatography（hexane/ethyl acetate = 2/1）on silica gel to give **14** (219.9 mg, 98%) as a foam; ^{1}H NMR ($CDCl_3$) δ 0.92-1.14 (28H, each as m, Si-*i*-Pr), 2.26 (1H, dd, $J_{2'a,\,3'}$ = 5.2 and $J_{2'a,\,2'b}$ = 13.5 Hz, H-2′a), 2.46-2.58 (1H, ddd, $J_{1',\,2'b}$ = 6.9, $J_{2'b,\,3'}$ = 14.6 and $J_{2'a,\,2'b}$ = 13.5 Hz, H-2′b), 3.32-3.34 (1H, m, H-4′), 3.97 (1H, d, $J_{5'a,5'b}$ = 12.6 Hz, H-5′a), 4.13 (1H, dd, $J_{4',5'b}$ = 3.4 and $J_{5'a,5'b}$ = 12.6 Hz, H-5′b), 4.40 (1H, ddd, $J_{2'a,\,3'}$ = 5.2, $J_{2'b,\,3'}$ = 14.6 and $J_{3',\,4'}$ = 8.0 Hz, H-3′), 5.70 (1H, dd, $J_{5,\,NH}$ = 2.3 and $J_{5,\,6}$ = 8.0 Hz, H-5), 5.99 (1H, d, $J_{1',2'b}$ = 6.9 Hz, H-1′), 8.31 (1H, d, $J_{5,6}$ = 8.0 Hz, H-6), 8.57 (1H, br, NH); ^{13}C NMR ($CDCl_3$) δ: 12.4, 13.1, 13.2, 16.9, 16.96, 17.01, 17.3, 17.4, 17.5, 43.4, 54.2, 58.06, 58.11, 70.2, 101.6, 141.5, 150.8, 163.5. FAB-MS (*m*/*z*) 509 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{21}H_{38}O_5N_2NaSSi_2$: 509.19322, found: 509.19326 (M^+ + Na).

4.5. 1-[3,5-Di-*O*-acetyl-2-deoxy-4-thio-β-D-ribofuranosyl]uracil (15)

To a solution of **14** (287.1 mg, 0.59 mmol) in THF (5.0 mL) was added Bu_4NF (1M THF solution) (1.30 mL, 1.30 mmol) at 0 °C under Ar atmosphere and the mixture was stirred under Ar atmosphere 2 h. To the reaction mixture was added Ac_2O (0.22 mL, 2.36 mL) at 0 °C under Ar atmosphere and the reaction mixture was stirred overnight. The reaction mixture was partitioned between $CHCl_3$/saturated $NaHCO_3$ and silica gel column chromatography (3% MeOH in CH_2Cl_2) of the organic layer gave **15** (201.5 mg, 100%) as foam; ^{1}H NMR ($CDCl_3$) δ 2.13 and 2.14 (6H, each as s, Ac), 2.21 (1H, ddd, $J_{1',2'a}$ = 4.0, $J_{2'a,3'}$ = 9.2 and $J_{2'a,2'b}$ = 10.3 Hz, H-2′a), 2.59 (1H, ddd, $J_{1',2'b}$ = 2.9, $J_{2'b,3'}$ = 6.3 and $J_{2'a,2'b}$ = 10.3 Hz, H-2′b), 3.72 (1H, ddd, $J_{3',4'}$ = 2.3, $J_{4',5'a}$ = 6.3 and $J_{4',5'b}$ = 10.3 Hz, H-4′), 4.21 (1H, dd, $J_{4',5'a}$ = 7.5 and $J_{5'a,5'b}$ = 11.7 Hz, H-5′a), 4.34 (1H, dd, $J_{4',5'b}$ = 5.8 and $J_{5'a,5'b}$ = 11.7 Hz, H-5′b), 5.38-5.40 (1H, m, H-3′), 5.87 (1H, d, $J_{5,6}$ = 8.0 Hz, H-5), 6.54 (1H, d, $J_{1',2'a}$ = 4.0 and $J_{1',2'b}$ = 2.9 Hz, H-1′), 7.81 (1H, d, $J_{5,6}$ = 8.0 Hz, H-6), 9.49 (1H, br, NH); ^{13}C NMR ($CDCl_3$) δ: 20.8, 21.0, 39.7, 52.5, 61.4, 64.9, 76.4, 103.7, 140.0, 150.7, 162.8, 170.1, 170.5; ESI-MS (*m*/*z*) 351 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{13}H_{16}O_6N_2NaS$: 351.06213, found: 351.06268 (M^+ + Na).

4.6. 1-[3-*O*-Acetyl-2,5-dideoxy-4-thio-β-D-*glycero*-4-eno-β-D-ribofuranosyl]uracil (19)

Compound **15** (198.5 mg, 0.60 mmol) was treated with methanolic ammonia (10 mL) at rt overnight. The reaction mixture was evaporated to dryness and dried *in vacuo* overnight. To a solution of **16** in dioxane (8.0 mL) was added pyridine (0.12 mL, 1.50 mmol), Ph_3P (314.7 mg, 1.20 mmol) and I_2 (304.6 mg, 1.20 mmol) at rt under Ar atmosphere and the reaction mixture was stirred for 4 h. The reaction mixture was quenched with MeOH and silica gel column chromatography（3% MeOH in CH_2Cl_2）of the reaction mixture gave **17** (209.1 mg, 98%) as a solid. To a solution of **17** (209.1 mg, 0.61 mmol) in CH_3CN (5.0 mL) was added *i*-Pr_2NEt (0.53 mL, 3.05 mmol), Ac_2O (0.17 mL, 1.83 mmol) and DMAP (37.9 mg, 0.31 mmol) at rt under Ar atmosphere and the reaction mixture was stirred for 5 h. The reaction mixture was partitioned between $CHCl_3$/saturated $NaHCO_3$ and silica gel column chromatography（hexane/ethyl acetate = 1/2）of the organic layer gave **18** (178.9 mg, 74%) as a foam. To a solution of **18** in CH_3CN (4.0 mL) was added DBN (0.23 mL, 1.83 mmol) at rt under Ar atmosphere and the reaction mixture was stirred overnight. The reaction mixture was neutralized with AcOH, partitioned between $CHCl_3$/saturated $NaHCO_3$ and silica gel column chromatography（hexane/ethyl acetate = 1/2）of the organic layer gave **19** (101.0 mg, 65% from) as a foam; ^{1}H NMR ($CDCl_3$) δ 2.13 (3H, s, Ac), 2.21 (1H, ddd, $J_{1',2'a}$ = 8.4, $J_{2'a,3'}$ = 5.2 and $J_{2'a,2'b}$ = 14.0 Hz, H-2′a), 2.59 (1H, ddd, $J_{1',2'b}$ = 6.3, $J_{2'b,3'}$ = 3.4 and $J_{2'a,}$

$_{2'b}$ = 14.0 Hz, H-2′a), 5.26 (1H, s, H-5′a), 5.51 (1H, s, H-5′b), 5.84 (1H, dd, $J_{2'a,3'}$ = 5.2 and $J_{2'b,3'}$ = 3.4 Hz, H-3′), 5.87 (1H, d, $J_{5,6}$ = 8.0 Hz, H-5), 6.73 (1H, dd, $J_{1',2'a}$ = 8.4 and $J_{1',2'b}$ = 6.3 Hz, H-1′), 7.59 (1H, d, $J_{5,6}$ = 8.0 Hz, H-6), 9.73 (1H, br, NH); ^{13}C NMR ($CDCl_3$) δ: 21.4, 41.4, 61.9, 77.1, 104.2, 110.3, 140.1, 144.6, 151.0, 163.5, 170.3. FAB-MS(*m*/*z*): 291 (M^+＋Na); ESI-HRMS (*m*/*z*): calcd for $C_{11}H_{12}O_4N_2NaS$: 291.04100, found: 291.04126 (M^+＋Na).

4.7.

1-[3-*O*-(*tert*-Butyldimethylsilyl)-2,5-dideoxy-4-thio-β-D-*glycero*-4-eno-β-D-ribofura nosyl]uracil (21)

Compound **19** (259.3 mg, 1.00 mmol) was treated with methanolic ammonia (25 mL) at rt for 4 h. The reaction mixture was evaporated to dryness and dried *in vacuo* overnight. To a solution of **20** in DMF (15.0 mL) was added imidazole (340.4 mg, 5.0 mmol) and TBDMSCl (452.1 mg, 3.0 mmol) at rt under Ar atmosphere and the reaction mixture was stirred overnight. The reaction mixture was partitioned between ethyl acetate/H_2O and silica gel column chromatography（hexane/ethyl acetate = 2/1）of the organic layer gave **21** (208.7 mg, 61%) as a foam.; ^{1}H NMR ($CDCl_3$) δ 0.11 and 0.12 (6H, each as s, Si-Me), 0.91 (9H, s, Si-*tert*-Bu), 2.06-2.08 (1H, m, H-2′a), 2.39-2.44 (1H, m, H-2′b), 4.74 (1H, t, $J_{2'a,3'}$ = $J_{2'b,3'}$ = 5.2 Hz, H-3′), 5.12 (1H, s, H-5′a), 5.32 (1H,

s, H-5′b), 5.82 (1H, d, $J_{5,6}$ = 8.0 Hz, H-5), 6.57 (1H, dd, $J_{1',2'a}$ = $J_{1',2'b}$ = 6.9 Hz, H-1′), 7.63 (1H, d, $J_{5,6}$ = 8.0 Hz, H-6), 9.42 (1H, br, NH); ^{13}C NMR ($CDCl_3$) δ : –4.73, –4.65, 18.1, 25.7, 29.7, 44.4, 61.4, 76.3, 103.2, 106.4, 140.3, 148.7, 150.3, 162.5. FAB-MS(*m*/*z*): 1027(M^++H); ESI-MS (*m*/*z*) 363 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{15}H_{24}O_3N_2NaSSi$: 363.11691, found: 363.11719 (M^++Na).

4.8. Dibenzoyoxylation of 21 with $Pb(OBz)_4$: Formation of 22a and 22b

To a solution of **21** (146.8 mg, 0.43 mmol) in toluene (7.0 mL) was added $Pb(OBz)_4$ (1.34 g, 1.94 mmol) at rt under Ar atmosphere and the mixture was stirred overnight. The reaction mixture was filtered through a celite pad. The filtrate was neutralized with Et_3N and partitioned between $CHCl_3$/saturated aq $NaHCO_3$. Column chromatography (hexane/ethyl acetate = 2/1-1/1) of the organic layer gave **22a** (158.3 mg, 63%, foam,) and **22b** (71.4 mg, 28%, foam).

4.8.1. Physical data of 22a: ^{1}H NMR ($CDCl_3$) δ 0.06 and 0.18 (6H, each as s), 0.94 (9H, s, Si-*tert*-Bu), 2.34 (1H, ddd, $J_{1',2'a}$ = 10.0, $J_{2'a,3'}$ = 3.4 and $J_{2'a,2'b}$ = 12.9 Hz, H-2′a), 2.57-2.63 (1H, m, H-2′b), 4.87 (1H, t, $J_{2'a,3'}$ = $J_{2'b,3'}$ = 3.4 Hz, H-3′), 4.90 (1H, d, $J_{5'a,5'b}$ = 12.0 Hz, H-5′a), 5.34 (1H, d, $J_{5'a,5'b}$ = 12.0 Hz, H-5′b), 5.66 (1H, d, $J_{5,6}$ = 8.0 Hz, H-5), 6.73 (1H, dd, $J_{1',2'a}$ = 10.0 and $J_{1',2'b}$ = 5.7 Hz, H-1′), 7.35-7.38, 7.47-7.52, 7.58-7.66, 7.89-7.94 and 8.02-8.06 (11H, each as m, Ph and H-6), 9.18 (1H, br, NH); NOE

experiment: : H-2′b (α-H)/CH_2-5′a, Si-Me/CH_2-5′a and Si-*tert*-Bu/CH_2-5′a; HMBC: CH_{2a}-5′/$CH_3\underline{C}O$-5′ and CH_{2b}-5′/$CH_3\underline{C}O$-5′; ^{13}C NMR ($CDCl_3$) δ: –5.1, –4.5, 17.9, 42.2, 61.6, 62.1, 101.1, 103.7, 128.4, 128.7, 129.5, 129.6, 133.2, 133.8, 140.1, 149.9, 150.6, 163.2, 164.5, 165.4; ESI-MS (*m*/*z*) 605 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{29}H_{34}O_7N_2NaSSi_2$: 605.17482, found: 605.17505 (M^+ + Na).

4.8.2. Physical data of 22b: ^1H NMR ($CDCl_3$) δ –0.08 and 0.14 (6H, each as s, Si-Me), 0.88 (9H, s, Si-*tert*-Bu), 2.30 (1H, ddd, $J_{1',2'a}$ = 2.9, $J_{2'a,3'}$ = 4.6 and $J_{2'a,\,2'b}$ = 13.5 Hz, H-2′a), 2.63 (1H, ddd, $J_{1',2'b}$ = 12.3, $J_{2'b,3'}$ = 1.7 and $J_{2'a,\,2'b}$ = 13.5 Hz, H-2′b), 3.93 (1H, d, $J_{5'a,5'b}$ = 13.7 Hz, H-5′a), 4.09 (1H, dd, $J_{3',5'b}$ = 1.7 and $J_{5'a,5'b}$ = 13.7 Hz, H-5′b), 5.38-5.39 (1H, m, H-3′), 5.74 (1H, d, $J_{5,6}$ = 8.0 Hz, H-5), 6.33 (1H, dd, $J_{1',2'a}$ = 2.9 and $J_{1',2'b}$ = 12.3 Hz, H-1′), 7.33 (1H, d, $J_{5,6}$ = 8.0 Hz, H-6), 7.41-7.45, 7.49-7.51, 7.56-7.57, 7.62-7.64, 8.00-8.01 and 8.10-8.11 (10H, each as m, Ph), 8.92 (1H, br, NH); ^{13}C NMR ($CDCl_3$) δ: –5.1, –4.4, 17.8, 25.7, 29.5, 39.1, 48.7, 68.5, 100.9, 103.4, 128.4, 128.7, 130.0, 133.6, 133.8, 140.3, 149.9, 163.2, 163.5, 164.1; HMBC: C-1′/H-5′a and C-1′/H-5′b. FAB-MS (*m*/*z*) 605 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{29}H_{34}O_7N_2NaSSi$: 605.17482, found: 605.17481 (M^+ + Na).

4.9. Reaction of 22a with azidotrimethylsilane: formation of 1-[4-Azido-5-*O*-benzoyl-3-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-4-thio-β-D-ribofuranosyl]uracil

(23a) and 1-[4-Azido-5-*O*-benzoyl-3-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-4-thio-α-L-ribofuranosyl]uracil (23b)

To a solution of **22a** (40.5 mg, 0.069 mmol) in dichloromethane (2.5 mL) was added azidotrimethylsilane (46.5 μL, 0.35 mmol) and $SnCl_4$ (1 M dichloromethane solution) (0.21 mL, 0.21 mmol) at −50 °C under Ar atmosphere and the mixture was stirred at −30°C for overnight. The reaction mixture was partitioned between $CHCl_3$ and saturated $NaHCO_3$ and preparative TLC (hexane/ethyl acetate = 2/1) of the organic layer gave **23a** (21.3 mg, 61%, syrup) and **23b** (10.0 mg, 20%, syrup).

4.9.1. Physical data for 23a: IR (neat) 2113 cm^{-1} (N_3); ^1H NMR ($CDCl_3$) δ 0.13 and 0.16 (6H, each as s, Si-Me), 0.94 (9H, s, Si-*tert*-Bu), 2.35 (1H, ddd, $J_{1',2'a}$ = 9.7, $J_{2'a,3'}$ = 2.9 and $J_{2'a,2'b}$ = 13.5 Hz, H-2′a), 2.51 (1H, ddd, $J_{1',2'b}$ = 6.3, $J_{2'b,3'}$ = 1.1 and $J_{2'a,2'b}$ = 13.5 Hz, H-2′b), 4.27 (1H, br, H-3′), 4.67 (1H, d, $J_{5'a,5'b}$ = 11.5 Hz, H-5′a), 4.86 (1H, d, $J_{5'a,5'b}$ = 11.5 Hz, H-5′b), 5.90 (1H, d, $J_{5,6}$ = 8.6 Hz, H-5), 6.78 (1H, dd, $J_{1',2'a}$ = 9.7 and $J_{1',2'b}$ = 6.3 Hz, H-1′), 7.46-7.49, 7.59-7.64 and 8.03-8.05 (5H, each as s, Ph) 7.63 (1H, d, $J_{5,6}$ = 8.6 Hz, H-6), 8.57 (1H, br, NH); ^{13}C NMR ($CDCl_3$) δ −4.9, −4.8, 18.1, 25.7, 42.6, 59.8, 67.1, 77.4, 84.0, 103.5, 128.6, 129.0, 129.8, 133.8, 140.1, 150.3, 162.7, 165.9; FAB-MS (*m*/*z*) 526 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{22}H_{29}O_5N_5NaSSi$:

526.15509, found: 526.15570 (M^+ + Na).

4.9.2. Physical data for 23b: IR (neat) 2120 cm^{-1} (N_3); ^{1}H NMR ($CDCl_3$) δ ^{1}H NMR ($CDCl_3$) δ 0.15 and 0.19 (6H, each as s, Si-Me), 0.95 (9H, s, Si-*tert*-Bu), 2.27 (1H, ddd, $J_{1',2'a}$ = 7.4, $J_{2'a,3'}$ = 4.0 and $J_{2'a,2'b}$ = 13.5 Hz, H-2′a), 2.59 (1H, ddd, $J_{1',2'b}$ = 6.3, $J_{2'b,3'}$ = 5.2 and $J_{2'a,2'b}$ = 13.5 Hz, H-2′b), 4.52 (1H, t, $J_{2'a,3'}$ = $J_{2'b,3'}$ = 4.6 Hz, H-3′), 4.54 (1H, d, $J_{5'a,5'b}$ = 11.5 Hz, H-5′a), 4.58 (1H, d, $J_{5'a,5'b}$ = 11.5 Hz, H-5′b), 5.68 (1H, d, $J_{5,6}$ = 8.0 Hz, H-5), 6.60 (1H, dd, $J_{1',2'a}$ = 7.4 and $J_{1',2'b}$ = 6.3 Hz, H-1′), 7.48-7.52, 7.62-7.65 and 8.05-8.07 (5H, each as s, Ph) 7.64 (1H, d, $J_{5,6}$ = 8.0 Hz, H-6), 8.54 (1H, br, NH); ^{13}C NMR ($CDCl_3$) δ –5.0, –4.5, 17.9, 25.6, 42.8, 61.9, 67.7, 78.6, 87.9, 104.2, 128.6, 129.1, 129.9, 133.6, 140.0, 150.3, 162.3, 165.7; FAB-MS (*m*/*z*) 526 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{22}H_{29}O_5N_5NaSSi$: 526.15509, found: 526.15588 (M^+ + Na).

4.10. 1-[5-*O*-Acetyl-4-azido-3-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-4-thio-β-D-ribofuranosyl]uracil (25)

To a solution of **23a** (60.1 mg, 0.12 mmol) in MeOH (3.5 mL) was added NaOMe (9.7 mg, 0.18 mmol) at 0 °C under Ar atmosphere and the mixture was stirred at rt overnight. Silica gel column chromatography (2% MeOH in CH_2Cl_2) of the reaction mixture gave **24** (42.1 mg, 88%, syrup). To a solution of **24** (42.1 mg, 0.11 mmol) in CH_3CN (3.0 mL) was added *i*-Pr_2NEt (63 μL, 0.36 mmol), Ac_2O (23 mL, 0.24 mmol)

and DMAP (7.3 mg, 0.06 mmol) at rt under Ar atmosphere and the mixture was stirred overnight. The reaction mixture was partitioned between $CHCl_3$ and saturated $NaHCO_3$ and silica gel column chromatography (hexane/ethyl acetate = 1/1) of the organic layer gave **25** (45.6 mg, 94%) as a syrup: IR (neat) 2113 cm^{-1} (N_3); ^1H NMR ($CDCl_3$) δ 0.15 and 0.18 (6H, each as s, Si-Me), 0.96 (9H, s, Si-*tert*-Bu), 2.15 (3H, s, Ac), 2.22 (1H, ddd, $J_{1',2'a}$ = 8.6, $J_{2'a,3'}$ = 4.0 and $J_{2'a,2'b}$ = 13.6 Hz, H-2′a), 2.54 (1H, ddd, $J_{1',2'b}$ = 6.2, $J_{2'b,3'}$ = 4.4 and $J_{2'a,2'b}$ = 13.6 Hz, H-2′b), 4.21 (1H, d, $J_{5'a,5'b}$ = 11.6 Hz, H-5′a), 4.38 (1H, d, $J_{5'a,5'b}$ = 11.6 Hz, H-5′b), 4.40 (1H, dd, $J_{2'a,3'}$ = 4.0 and $J_{2'b,3'}$ = 4.4 Hz, H-3′), 5.83 (1H, d, $J_{NH,5}$ = 2.0 and $J_{5,6}$ = 8.0 Hz, H-5), 6.61 (1H, dd, $J_{1',2'a}$ = 8.6 and $J_{1',2'b}$ = 6.2 Hz, H-1′), 7.65 (1H, d, $J_{5,6}$ = 8.0 Hz, H-6), 8.55 (1H, br, NH); NOE experiment: H-6/H-3′ and H-6/H-5′a; ^{13}C NMR ($CDCl_3$) δ –5.0, –4.8, 18.2, 20.7, 42.7, 60.0, 67.3, 77.6, 83.9, 103.6, 140.0, 150.2, 162.5, 170.2; FAB-MS (*m*/*z*) 464 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{17}H_{27}O_5N_5NaSSi$: 464.13944, found: 464.14079 (M^+＋Na).

4.11. 1-[4-Azido-*N*^{4},3,5-tri-*O*-acetyl-2-deoxy-4-thio-β-D-ribofuranosyl]cytosine (28)

To a solution of **25** (45.6 mg, 0.10 mmol) in CH_3CN (4.0 mL) was added K_2CO_3 (55.3 mg, 0.40 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (121.1 mg, 0.40 mmol) under Ar atmosphere at 0 °C and the mixture was stirred at 60 °C overnight. The reaction mixture was filtered through a celite pad and washed with CH_2Cl_2. Neutral

silica gel column chromatography (hexane/ethyl acetate = 7/1) of the filtrate gave **26** (62.4 mg, 0.088 mmol, 88%). To a solution of **26** in THF (4.0 mL) was added ammonium hydroxide (4.0 mL) at −10 °C and the mixture was stirred overnight at rt. The reaction mixture was evaporated to dryness and dried *in vacuo*. To a solution of **27** was added tetrabutylammonium fluoride (1M THF solution) (0.22 mL, 0.22 mmol) at 0 °C under Ar atmosphere and the mixture was stirred at rt for 2h. To the reaction mixture was added Ac_2O (41.5 μL, 0.44 mmol) at 0 °C and the mixture was stirred at rt overnight. The reaction mixture was partitioned between chloroform and saturated $NaHCO_3$ and silica gel column chromatography (2% MeOH in CH_2Cl_2) of the organic layer gave **28** (30.7 mg, 85% from **26**) as a syrup: ^{1}H NMR ($CDCl_3$) δ 2.15, 2.17 and 2.28 (9H, each as s, Ac), 2.57 (1H, dt, $J_{1',2'a} = J_{2'a,3'} = 5.2$ and $J_{2'a,2'b} = 13.7$ Hz, H-2′a), 2.53 (1H, ddd, $J_{1',2'b} = 6.9$, $J_{2'b,3'} = 8.0$ and $J_{2'a,2'b} = 13.7$ Hz, H-2′b), 4.40 (1H, d, $J_{5'a,5'b}$ = 11.5 Hz, H-5′a), 4.42 (1H, d, $J_{5'a,F} = 1.2$ and $J_{5'a,5'b}$ = 11.5 Hz, H-5′b), 5.40 (1H, dd, $J_{2'a,3'} = 5.2$ and $J_{2'b,3'} = 8.0$ Hz, H-3′), 6.52 (1H, dd, $J_{1',2'a} = 5.2$ and $J_{1',2'b} = 6.9$ Hz, H-1′), 7.58 (1H, d, $J_{5,6} = 8.0$ Hz, H-5), 8.23 (1H, d, $J_{5,6} = 8.0$ Hz, H-6), 10.37 (1H, br, NH); ^{13}C NMR ($CDCl_3$) δ: 20.6, 20.7, 24.9, 38.2, 59.9, 67.4, 75.4, 81.0, 98.0, 144.6, 155,4, 162.8, 169.8, 167.0, 171.1. FAB-MS(*m*/*z*): 433 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{15}H_{18}O_6N_6NaS$: 433.09007, found: 433.09062 (M^+ + Na).

4.12. 1-[4-Azido-2-deoxy-4-thio-β-D-ribofuranosyl]cytosine (7)

To a solution of **28** (29.9 mg, 0.073 mmol) in MeOH (3.0 mL) was added NaOMe (11.9 mg, 0.22 mmol) at 0 °C and the mixture was stirred at rt overnight. The reaction mixture was chromatographed on silica gel (20% MeOH in CH_2Cl_2) to give **7** (18.4 mg, 88%) as a solid: mp 126-128 °C (dec); IR (neat) 2107 cm^{-1} (N_3); ^1H NMR (DMSO-d_6) δ 2.39 (1H, ddd, $J_{1',2'a}$ = 4.6, $J_{2'a,3'}$ = 5.2 and $J_{2'a,2'b}$ = 13.7 Hz, H-2′a), 2.55 (1H, ddd, $J_{1',2'b}$ = 7.4, $J_{2'b,3'}$ = 8.6 and $J_{2'a,2'b}$ = 13.7 Hz, H-2′b), 3.78 (1H, d, $J_{5'a,5'b}$ = 11.5 Hz, H-5′a), 3.83 (1H, d, $J_{5'a,5'b}$ = 11.5 Hz, H-5′b), 4.43 (1H, dd, $J_{2'a,3'}$ = 5.2 and $J_{2'b,3'}$ = 7.4 Hz, H-3′), 5.95 (1H, d, $J_{5,6}$ = 7.4 Hz, H-5), 6.38 (1H, dd, $J_{1',2'a}$ = 4.6 and $J_{1',2'b}$ = 7.4 Hz, H-1′), 8.10 (1H, d, $J_{5,6}$ = 7.4 Hz, H-6), 8.55; ^{13}C NMR ($CDCl_3$) δ 41.9, 60.1, 66.9, 76.2, 86.8, 97.0, 143.0, 158.1, 167.0; ESI-MS (*m*/*z*) 285 (M^+ + H); ESI-HRMS (*m*/*z*): calcd for $C_9H_{13}O_3N_6S$: 285.07644, found: 285.07653 (M^+ + H).

4.13. 1,4-Anhydro-4-*C*-(hydroxymethyl)-2,3-*O*-isopropylidene-4-thio-D-ribitol (30)

To a solution of **29** (3.50 g, 9.65 mmol) in MeOH (35 mL) was added $NaBH_4$ (730.1 mg, 19.30 mmol) at 0 °C under Ar atmosphere, and the mixture was stirred for 1 h at rt. The reaction mixture was neutralized with acetic acid and partitioned between chloroform/saturated $NaHCO_3$. Silica gel column chromatography

(hexane/ethyl acetate = 4/1) of the organic layer gave **30** (3.00 g, 98%) as a foam: ^{1}H NMR ($CDCl_3$) δ: 0.12 (6H, s, SiMe), 9.39 (9H, s, Si-*tert*-Bu), 1.33 and 1.55 (6H, each as s, isop-CH_3), 2.63 (1H, t, $J_{OH,CH2}$ = 6.9 Hz, OH), 2.91 (1H, dd, $J_{1a,\,2}$ = 2.3 and $J_{1a,\,1b}$ = 12.6 Hz, H-1a), 3.16 (1H, dd, $J_{1b,\,2}$ = 5.2 and $J_{1a,\,1b}$ = 12.6 Hz, H-1b), 3.68 (1H, d, $J_{5a,\,5b}$ = 10.3 Hz, H-5a), 3.81 (1H, d, $J_{5a,\,5b}$ = 10.3 Hz, H-5b), 3.83 (1H, dd, $J_{OH,\,CH2a}$ = 6.9 and $J_{CH2a,CH2b}$ = 11.5 Hz, C$\underline{H}_{2a}$OH), 3.95 (1H, dd, $J_{OH,\,CH2b}$ = 6.9 and $J_{CH2a,CH2b}$ = 11.5 Hz, C$\underline{H}_{2b}$OH), 4.70 (1H, d, $J_{2,3}$ = 5.8 Hz, H-3), 4.88 (1H, d, $J_{CH2a,CH2b}$ = 5.2 Hz, SiOC$\underline{H}_{2a}$O), 4.89 (1H, d, $J_{CH2a,CH2b}$ = 5.2 Hz, SiOC$\underline{H}_{2b}$O), 4.96(1H, ddd, $J_{1a,\,2}$ = 2.3, $J_{1b,\,2}$ = 5.2 and $J_{2,3}$ = 5.8 Hz, H-2); ^{13}C NMR ($CDCl_3$) δ: –5.0, 18.1, 24.5, 25.7, 26.4, 36.8, 63.4, 63.8, 71.7, 84.6, 86.2, 90.3, 111.5; ESI-MS (*m*/*z*) 387 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{16}H_{32}O_5NaSSi$: 387.16319, found: 387.16312 (M^+ + Na).

4.14.

1,4-Anhydro-4-*C*-(4′,4′′-dimethoxytrityloxymethyl)-2,3-*O*-isopropylidene-4-thio-D-ribitol (31)

To a solution of **30** in dichloromethane (35.0 mL) was added Et_3N (4.1 mL, 29.52 mmol) and DMTrCl (7.50 g, 22.14 mmol) at rt and the mixture was stirred at 70 °C for 5 h. The reaction mixture was partitioned between chloroform/saturated $NaHCO_3$

and silica gel column chromatography (hexane/ethyl acetate = 7/1-1/1) of the organic layer gave the respective dimethoxytritylated product. To a THF (30.0 mL) solution of the crude product was added Bu_4NF (2.89 g, 11.07 mmol) at 0 °C and the mixture was stirred at rt overnight. The reaction mixture was chromatographed on silica gel (hexane/ethyl acetate = 2/1) to give **31** (3.77 g, 95%) as a foam: ^{1}H NMR ($CDCl_3$) δ: 1.32 and 1.44 (6H, each as s, isop-CH_3), 2.39 (1H, dd, $J_{OH,CH2a}$ = 4.6 and $J_{OH,CH2b}$ = 8.6 Hz, OH), 2.86 (1H, dd, $J_{1a,\,2}$ = 1.2 and $J_{1a,\,1b}$ = 13.2 Hz, H-1a), 3.06 (1H, dd, $J_{1b,\,2}$ = 4.6 and $J_{1a,\,1b}$ = 13.2 Hz, H-1b), 3.35 and 3.58 (2H, each as d J_{gem} = 9.2 Hz, $\underline{CH_2}$ODMTr), 3.55 (1H, d, $J_{OH,5a}$ = 8.6 and $J_{5a,\,5b}$ = 11.8 Hz, CH_2-5a), 3.69 (1H, d, $J_{OH,5b}$ = 5.2 and $J_{5a,\,5b}$ = 11.8 Hz, CH_2-5b), 3.80 (6H, s, OMe), 4.76 (1H, d, $J_{2,3}$ = 5.8 Hz, H-3), 4.98 (1H, ddd, $J_{1a,\,2}$ = 1.2, $J_{1b,\,2}$ = 4.6 and $J_{2,3}$ = 5.8 Hz, H-2), 6.83-6.86, 7.20-7.23, 7.27-7.33, 7.37-7.39 and 7.48-7.50 (13H, each as m, Ph); ^{13}C NMR ($CDCl_3$) δ: 24.9, 26.4, 36.3, 55.3, 63.9, 64.7, 65.8, 84.4, 84.6, 86.7, 111.0, 113.2, 113.3, 126.9, 127.9, 128.2, 130.1, 130.2, 135.8, 135.9, 144.7, 158.6; ESI-MS (*m*/*z*) 545 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{30}H_{34}O_6NaS$: 545.19683, found: 545.19684 (M^+＋Na).

4.15.

1,4-Anhydro-2-deoxy-4-*C*-(dimethoxytrityloxymethyl)-3,5-*O*-(1,1,3,3-tetraisopr

opyldisiloxane-1,3-diyl)-D-*erythro*-pent-1-entiol (33)

To a solution of **31** (3.77 g, 7.21 mmol) in THF (65 mL) was added *tert*-BuLi (1.77 M in hexane solution) at −70 °C under Ar atmosphere and the mixture was stirred overnight. The reaction mixture was impregnated with neutral silica gel and chromatographed over silica gel (hexane/ethyl acetate = 50/1) to give **32** (2.97 g, 89% yield) as a syrup. To a solution of **32** (2.97 g, 6.39 mmol) in DMF (20.0 mL) was added imidazole (1.04 g, 15.34 mmol) and 1,3-dichrolo-1,1,3,3-tetraisopropyldisiloxane (2.45 mL, 7.67 mmol) at 0 °C under Ar atmosphere and the mixture was stirred overnight. The reaction mixture was partitioned between ethyl acetate/H_2O and silica gel column chromatography (hexane/ethyl acetate = 50/1) of the organic layer gave **33** (3.84 g, 85%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 0.75-1.12 (28H, m, Si-*i*-Pr), 3.46 and 3.57 (2H, each as d, $J_{5a, 5b}$ = 9.2 Hz, CH_2-5), 3.78 and 3.79 (6H, each as s, OMe), 4.11 and 4.31 (2H, each as d, J_{gem} = 11.5 Hz, $\underline{CH_2}$ODMTr), 5.34 (1H, dd, $J_{1,3}$ = 2.5 and $J_{2,3}$ = 1.9 Hz, H-2), 5.41 (1H, dd, $J_{1, 3}$ = 1.9 and $J_{2, 3}$ = 6.2 Hz, H-3), 6.11 (1H, dd, $J_{1, 2}$ = 6.2 and $J_{1, 3}$ = 2.5 Hz, H-1), 6.78-6.84, 7.16-7.18, 7.24-7.26, 7.36-7.42 and 7.31-7.33; ^{13}C NMR ($CDCl_3$) δ: 12.5, 12.6, 13.0, 13.1, 16.9, 17.10, 17.14, 17.2, 17.3, 17.4, 17.5, 17.6, 55.0, 60.2, 66.7, 67.0, 84.0, 85.8, 96.0, 112.8, 112.78, 123.83, 126.0, 126.3, 127.5, 128.6, 130.2,

130.3, 136.2, 136.7, 145.4, 158.1; ESI-MS (*m*/*z*) 729 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{39}H_{54}O_6NaSSi_2$: 729.30718, found: 729.30945 (M^+＋Na).

4.16.

1,4-Anhydro-2-deoxy-4-*C*-hydroxymethyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-D-*erythro*-pent-1-entiol (**34**)

To a chloroform (30.0 mL) solution of **33** (2.74 g, 5.43 mmol) was added 1% TsOH in MeOH (10 mL) at −10 °C and the mixture was stirred for 2 h. The reaction mixture was partitioned between chloroform/saturated $NaHCO_3$ and silica gel column chromatography (hexane/ethyl acetate = 20/1) of the organic layer gave **34** (1.54 g, 98%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 1.05-1.16 (28H, m, Si-*i*-Pr), 2.12 (1H, dd, *J* = 5.1 and 9.0 Hz, OH), 3.95 (1H, dd, $J_{OH,5a}$ = 9.0 and $J_{5a,5b}$ = 11.8 Hz, $\underline{CH_{2a}}OH$), 4.02 (1H, dd, $J_{OH,5b}$ = 5.1 and $J_{5a,5b}$ = 11.8 Hz, $\underline{CH_{2b}}OH$), 4.12 (2H, s, CH_2-5), 5.47 (1H, dd, $J_{1,3}$ = 2.3 and $J_{2,3}$ = 1.7 Hz, H-2), 5.44 (1H, dd, $J_{1,3}$ = 1.7 and $J_{2,3}$ = 6.4 Hz, H-3), 6.06 (1H, dd, $J_{1,2}$ = 6.4 and $J_{1,3}$ = 2.3 Hz, H-1); ^{13}C NMR ($CDCl_3$) δ:12.8, 13.0, 13.3, 13.4, 17.26, 17.28, 17.36, 17.44, 17.6, 17.65, 17.68, 62.5, 66.3, 67.9, 84.2, 96.3, 123.5, 127.5; ESI-MS (*m*/*z*) 427 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{18}H_{36}O_4NaSSi_2$: 427.17650, found: 427.17644 (M^+＋Na).

4.17.

1,4-Anhydro-2-deoxy-4-*C*-fluoromethyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-D-*erythro*-pent-1-entiol (35)

To a solution of **34** (523.3 mg, 1.29 mmol) in CH_2Cl_2 (12.0 mL) was added Na_2CO_3 (410.2 mg, 3.87 mmol) and DAST (0.51 mL, 3.87 mmol) at 0 °C under Ar atmosphere and the mixture was stirred overnight. The reaction mixture was partitioned between chloroform/saturated $NaHCO_3$ and silica gel column chromatography (hexane/ethyl acetate = 40/1) of the organic layer gave **35** (447.3 mg, 85%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 1.00-1.17 (28H, m, Si-*i*-Pr), 4.11 and 4.13 (2H, each as d, $J_{5a,5b}$ = 11.5 Hz, CH_2-5), 4.69 (1H, dd, $J_{H,F}$ = 46.4 and $J_{5a,5b}$ = 9.2 Hz, $\underline{CH_{2a}}F$), 4.67 (1H, dd, $J_{H,F}$ = 46.4 and $J_{5a,5b}$ = 9.2 Hz, $\underline{CH_{2b}}F$), 5.47 (1H, dd, $J_{1,2}$ = 2.9 and $J_{2,3}$ = 1.7 Hz, H-2), 5.54 (1H, dd, $J_{1,3}$ = 1.7 and $J_{2,3}$ = 6.0 Hz, H-3), 6.08 (1H, dd, $J_{1,2}$ = 6.0 and $J_{1,3}$ = 2.9 Hz, H-1); ^{13}C NMR ($CDCl_3$) δ: 12.6, 12.8, 13.0, 13.2, 17.0, 17.1, 17.27, 17.30, 17.4, 17.46, 17.49, 64.9, 65.0, 65.96, 66.00, 76.8, 77.1, 77.3, 80.9, 82.3, 84.68, 84.72; ESI-MS (*m*/*z*) 429 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{18}H_{35}O_3FNaSSi_2$: 429.17217, found: 429.17219 (M^+＋Na).

4.18.

1-*O*-Pivaloyl-2-deoxy-4-*C*-fluoromethyl-2-iodo-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-ribofuranose (36)

To a solution of **35** (226.9 mg, 0.56 mmol) in CH_3CN (5.0 mL)/CH_2Cl_2 (5.0 mL) was added pivalic acid (630 mg, 2.80 mmol) and NIS (286 mg, 2.80 mmol) at 0 °C under Ar atmosphere and the mixture was stirred for 6 h. The reaction mixture was partitioned between $CHCl_3$/saturated $NaHCO_3$-0.2M $Na_2S_2O_3$ and silica gel column chromatography（hexane/ethyl acetate = 50/1）of the organic layer gave **36** (346.1 mg, 97%) as a syrup; ^{1}H NMR ($CDCl_3$) δ 1.04-1.12 (28H, m, Si-*i*-Pr), 1.20 (9H, s, *tert*-Bu), 3.77 (1H, dd, J = 1.2 and $J_{5a,5b}$ = 11.5 Hz, CH_{2a}-5), 4.11 (1H, d, $J_{5a,5b}$ = 11.5 Hz, CH_{2b}-5), 4.26 (1H, dd, $J_{2,F}$ = 2.9 and $J_{2,3}$ = 5.5 Hz, H-2), 4.77 (1H, dd, $J_{3,F}$ = 1.2 and $J_{2,3}$ = 5.5 Hz, H-3), 5.00 (1H, dd, $J_{F,H2a}$ = 47.5 and $J_{CH2a,CH2b}$ = 9.2 Hz, CH_{2a}F), 5.15 (1H, dd, $J_{F,H2a}$ = 47.5 and $J_{CH2a,CH2b}$ = 9.2 Hz, CH_{2b}F), 6.11 (1H, s, H-1); NOE experiment: H-1/CH_{2a}-4, H-1/ CH_{2b}--4, H-2/ CH_{2a}-5, H-2/ CH_{2b}-5; ^{13}C NMR ($CDCl_3$) δ: 12.8, 12.9, 13.0, 13.2, 16.9, 17.1, 17.2, 17.3, 17.4, 17.5, 26.8, 38.2, 38.6, 51.8, 60.4, 60.5, 70.18, 70.22, 79.2, 79.3, 82.7, 83.9, 96.1, 129.5, 176.0, 195.5. ESI-MS (*m*/*z*) 657 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{23}H_{44}O_5FINaSSi_2$: 657.13689, found: 657.13707 (M^+＋Na).

4.19.

1-[2-Deoxy-4-fluoromethyl-2-iodo-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-ribofuranosyl]uracil (37)

To a suspension of uracil (19.1 mg, 0.17 mmol) in CH_3CN (2.0 mL) was added BSA

(82 μL, 0.33 mmol) at rt under Ar atmosphere and the mixture was stirred for 1 h. To the solution was added a solution of **36** (71.7 mg, 0.11 mmol) in CH_3CN (2.0 mL)/CH_2Cl_2 (1.0 mL) and TMSOTf (99.4 μL, 1.53 mmol) at −10 °C under Ar atmosphere. After being stirred for 1 h, the mixture was stirred at 0 °C for 1h and then at rt overnight. The reaction mixture was partitioned between $CHCl_3$/saturated $NaHCO_3$ and silica gel column chromatography（hexane/ethyl acetate = 2/1-1/1）of the organic layer gave **37** (50.9 mg, 72%,) as a syrup: ^{1}H NMR ($CDCl_3$) δ 0.96-1.18 (28H, m, Si-*i*-Pr), 4.04 (1H, dd, $J_{5'a,F}$ = 1.7 and $J_{5'a,5'b}$ = 12.6 Hz, CH_{2a}-5′), 4.09 (1H, d, $J_{5'a,5'b}$ = 12.6 Hz, CH_{2b}-5′), 4.13 (1H, dd, $J_{3',F}$ = 3.5 and $J_{2',3'}$ = 6.9 Hz, H-3′), 4.65 (1H, d, $J_{2',3'}$ = 6.9 Hz, H-2′), 4.95 (1H, dd, $J_{F,H2a}$ = 47.6 and $J_{CH2a,CH2b}$ = 9.8 Hz, CH_{2a}F), 5.35 (1H, dd, $J_{F,H2b}$ = 47.6 and $J_{CH2a,CH2b}$ = 9.8 Hz, CH_{2b}F), 5.78 (1H, d, $J_{5,6}$ = 8.6 Hz, H-5), 6.17 (1H, s, H-1′), 8.14 (1H, d, $J_{5,6}$ = 8.6 Hz, H-6), 9.90 (1H, br, NH); NOE experiment: H-6/ CH_{2b}-5′, H-6/H-2′, H-1′/CH_{2b}-4′, H-2′/ CH_{2b}-5′, H-2′/CH_{2a}-5′; ^{13}C NMR ($CDCl_3$) δ: 12.8, 13.0, 13.2, 13.5, 17.0, 17.2, 17.3, 17.4, 17.47, 17.49, 63.6, 63.7, 64.3, 64.4, 68.2, 74.09, 74.14, 85.0, 86.3, 102.5, 140.7, 150.3, 163.3. FAB-MS(*m*/*z*): 667 (M^+＋Na); ESI-HRMS (*m*/*z*): calcd for $C_{22}H_{38}O_5N_2FINaSSi_2$: 667.09609, found: 667.09656 (M^+＋Na).

4.20.

1-[2-Deoxy-4-*C*-fluoromethyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-ribofuranosyl]uracil (38)

To a solution of **37** (91.5 mg, 0.14 mmol) in toluene (4.0 mL) was added Bu_3SnH (75 μL, 0.42 mmol) and Et_3B (70 μL, 0.07 mmol) at −70 °C under Ar atmosphere and the mixture was stirred under O_2 atmosphere overnight. The reaction mixture was chromatographed on silica gel（hexane/ethyl acetate = 2/1）to give **38** (72.0 mg, 99%) as a syrup; ^{1}H NMR ($CDCl_3$) δ 0.92-1.16 (28H, m, Si-*i*-Pr), 2.25 (1H, dd, $J_{2'a,3'}$ = 6.3 and $J_{2'a,2'b}$ = 14.4 Hz, H-2′a), 2.69-2.76 (1H, m, H-2′b), 3.95 (1H, d, $J_{5'a,5'b}$ = 12.0 Hz, CH_{2a}-5′), 4.00 (1H, d, $J_{5'a,5'b}$ = 12.0 Hz, CH_{2b}-5′), 4.56 (1H, dd, $J_{F,H2a}$ = 47.0 and $J_{CH2a,CH2b}$ = 9.8 Hz, $CH_{2a}F$), 4.60-4.65 (1H, m, H-3′), 4.68 (1H, dd, $J_{F,H2b}$ = 47.0 and $J_{CH2a,CH2b}$ = 9.8 Hz, $CH_{2b}F$), 5.76 (1H, d, $J_{5,6}$ = 8.0 Hz, H-5), 6.05 (1H, s, H-1′), 8.23 (1H, d, $J_{5,6}$ = 8.0 Hz, H-6), 9.81 (1H, br, NH); ^{13}C NMR ($CDCl_3$) δ: 12.5, 13.1, 13.2, 13.3, 17.05, 17.08, 17.2, 17.3, 17.4, 17.46, 17.51, 42.1, 57.3, 61.8, 61.9, 62.0, 72.4, 83.8, 85.2, 96.2, 102.1, 141.1, 150.8, 163.6. FAB-MS (*m*/*z*) 541 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{22}H_{39}O_5N_2FNaSSi_2$: 541.19945, found: 541.19965 (M^+＋Na).

4.21.

1-[N^4,3,5-Tri-*O*-acetyl-2-deoxy-4-*C*-fluoromethyl-4-thio-β-D-ribofuranosyl]cytosine (41)

To a solution of **38** (45.8 mg, 0.088 mmol) in CH_3CN (5.0 mL) was added K_2CO_3 (96.7 mg, 0.70 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (212.0 mg, 0.70 mmol) under Ar atmosphere at 0 °C and the mixture was stirred at 70 °C overnight. The reaction mixture was filtered through a celite pad and washed with CH_2Cl_2. The filtrate was chromatographed on neutral silica gel (hexane/ethyl acetate = 7/1) to give **39**. To the solution of **39** in THF (1.0 mL) was added ammonium hydroxide (3.0 mL) at −10 °C and the mixture was stirred overnight at rt. The reaction mixture was evaporated to dryness and silica gel column chromatography (5% MeOH in CH_2Cl_2) of the residue gave **40**. To a solution of **40** (27.5 mg, 0.053 mmol) in THF (2.0 mL) was added Bu_4NF (1M THF solution) (0.13 mL, 0.13 mmol) at 0 °C under Ar atmosphere and the mixture was stirred for 2h. To the reaction mixture was added Ac_2O (25 μL, 0.27 mmol) at 0 °C and the mixture was stirred at rt overnight. The reaction mixture was partitioned between chloroform and saturated $NaHCO_3$ and silica gel column chromatography (2% MeOH in CH_2Cl_2) of the organic layer gave **41** (18.5 mg, 59% in 3 steps) as a syrup: ^{1}H NMR ($CDCl_3$) δ 2.14, 2.15 and 2.29 (9H, each as s, Ac), 2.46 (1H, ddd, $J_{1',2'a}$ = 7.2, $J_{2'a,3'}$ = 4.6 and $J_{2'a,2'b}$ = 14.3 Hz, CH_{2a}-2′), 2.53 (1H, ddd, $J_{1',2'b}$ = 5.8, $J_{2'b,3'}$ = 4.6 and $J_{2'a,2'b}$ = 14.3 Hz, CH_{2b}-2′), 4.36 (1H, dd, $J_{5'a,F}$ = 1.2 and $J_{5'a,5'b}$ = 11.5 Hz, CH_{2a}-5′), 4.50 (1H, dd, $J_{5'b,F}$ = 1.2, and $J_{5'a,5'b}$ = 11.5 Hz, CH_{2b}-5′), 4.55 (1H, dd, $J_{H4'a,F}$ = 46.5 and

$J_{CH2a,CH2b}$ = 9.8 Hz, $CH_{2a}F$), 4.73 (1H, dd, $J_{H4'b,F}$ = 46.5 and $J_{CH2a,CH2b}$ = 9.8 Hz, $CH_{2b}F$), 5.47 (1H, t, $J_{2'a,3'} = J_{2'b,3'}$ = 4.6 Hz, H-3′), 6.52 (1H, dd, $J_{1',2'a}$ = 7.2 and $J_{1',2'b}$ = 5.8 Hz, H-1′), 7.55 (1H, d, $J_{5,6}$ = 8.0 Hz, H-5), 8.34 (1H, d, $J_{5,6}$ = 8.0 Hz, H-6), 9.64 (1H, br, NH); ^{13}C NMR ($CDCl_3$) δ: 13.2, 17.15, 17.17, 20.8, 24.9, 41.0, 61.3, 61.8, 61.9, 64.75, 64.78, 74.8, 81.3, 82.8, 97.9, 145.1, 155.6, 162.6, 169.7, 170.3, 170.9. ESI-MS (*m*/*z*): 424 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{16}H_{20}O_6N_3FNaS$: 424.09491, found: 424.09556 (M^+ + Na).

4.22. 1-[2-deoxy-4-*C*-fluoromethyl-4-thio-β-D-ribofuranosyl]cytosine (8)

To a solution of **41** (18.7mg, 0.047 mmol) in MeOH (2.5 mL) was added NaOMe (7.6 mg, 0.14 mmol) at 0 °C under Ar atmosphere and the mixture was stirred at rt overnight. Silica gel column chromatography (20% MeOH in CH_2Cl_2) of the reaction mixture gave **8** (10.1 mg, 78%) as a solid: mp 129-131 °C; ^{1}H NMR (CD_3OD) δ 2.36 (1H, ddd, $J_{1',2'a}$ = 8.0, $J_{2'a,3'}$ = 4.6 and $J_{2'a,2'b}$ = 12.9 Hz, CH_{2a}-2′), 2.46-2.51 (1H, m, CH_{2b}-2′), 3.79 (1H, dd, $J_{5'a,F}$ = 1.2 and $J_{5'a,5'b}$ = 11.8 Hz, CH_{2a}-5′), 3.82 (1H, dd, $J_{5'b,F}$ = 1.2, and $J_{5'a,5'b}$ = 11.8 Hz, CH_{2b}-5′), 4.43-4.44 (1H, m, H-3′), 4.60 (1H, dd, $J_{H4'a,F}$ = 47.5 and $J_{CH2a,CH2b}$ = 9.2 Hz, $CH_{2a}F$), 4.81 (1H, dd, $J_{H4'b,F}$ = 47.5 and $J_{CH2a,CH2b}$ = 9.2 Hz, $CH_{2b}F$), 5.97 (1H, d, $J_{5,6}$ = 7.5 Hz, H-5), 6.50 (1H, t, $J_{1',2'a} = J_{1',2'b}$ = 8.0 Hz, H-1′), 8.20 (1H, d, $J_{5,6}$ = 8.0 Hz, H-6); ^{13}C NMR (CD_3OD) δ: 44.2, 62.1, 65.0, 67.2, 67.4, 75.0, 83.9,

85.3, 97.2, 143.6, 158.4, 167.1; ESI-MS (*m*/*z*) 298 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{10}H_{14}O_3N_3FNaS$: 298.06321, found: 298.06324 (M^+ + Na).

4.23.

1,4-Anhydro-2-deoxy-4-*C*-acetoxymethyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-D-*erythro*-pent-1-entiol (**42**)

To a solution of **34** (293.6 mg, 0.73 mmol) in CH_3CN (7.0 mL) was added *i*-Pr_2NEt (0.38 mL, 2.19 mmol), Ac_2O (0.14 mL, 1.46 mmol) and DMAP (45.2 mg, 0.37 mmol) at 0 °C under Ar atmosphere and the mixture was stirred at rt overnight. The reaction mixture was partitioned between chloroform/saturated $NaHCO_3$ and silica gel column chromatography (hexane/ethyl acetate = 70/1) of the organic layer gave **42** (313.2 mg, 96%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 0.98-1.15 (28H, m, Si-*i*-Pr), 2.08 (3H, s, Ac), 4.08 (1H, d, $J_{5a,\,5b}$ = 12.0 Hz, CH_{2a}-5) 4.12 (1H, d, $J_{5a,\,5b}$ = 12.0 Hz, CH_{2b}-5), 4.50 (1H, d, J_{gem} = 11.5 Hz, $\underline{CH_{2a}}OAc$), 4.56 (1H, d, J_{gem} = 11.5 Hz, $\underline{CH_{2b}}OAc$), 5.41 (1H, dd, $J_{1,3}$ = 2.9 and $J_{2,3}$ = 2.3 Hz, H-3), 5.51 (1H, dd, $J_{1,\,2}$ = 6.2 and $J_{2,\,3}$ = 2.6 Hz, H-2), 6.04 (1H, dd, $J_{1,\,2}$ = 6.2 and $J_{1,\,3}$ = 2.9 Hz, H-1); ^{13}C NMR ($CDCl_3$) δ: 12.6, 12.86, 12.94, 13.2, 17.0, 17.2, 17.3, 17.36, 17.44, 17.47, 17.49, 62.5, 64.8, 66.5, 84.6, 123.2, 126.8, 170.9; ESI-MS (*m*/*z*) 469 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{20}H_{38}O_5NaSSi_2$: 469.18707, found: 469.18704 (M^+ +

Na).

4.24.

1-*O*-Pivaloyl-2-deoxy-4-*C*-acetoxymethyl-2-iodo-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-ribofuranose (43)

To a solution of **42** (313.2 mg, 0.70 mmol) in CH_3CN (5.0 mL)/CH_2Cl_2 (5.0 mL) was added pivalic acid (787.5 mg, 3.50 mmol) and NIS (357.5 mg, 3.50 mmol) at 0 °C under Ar atmosphere and the mixture was stirred for 8 h. The reaction mixture was partitioned between $CHCl_3$/saturated $NaHCO_3$-0.2M $Na_2S_2O_3$ and silica gel column chromatography（hexane/ethyl acetate = 40/1）of the organic layer gave **43** (448.3 mg, 95%) as a syrup; ^{1}H NMR ($CDCl_3$) δ 0.96-1.17 (28H, m, Si-*i*-Pr), 1.21 (9H, s, *tert*-Bu), 2.07 (3H, s, Ac), 3.76 (1H, d, $J_{5a,5b}$ = 11.5 Hz, CH_{2a}-5), 4.06 (1H, d, $J_{5a,5b}$ = 11.5 Hz, CH_{2b}-5), 4.23 (1H, d, $J_{2,3}$ = 5.2 Hz, H-2), 4.62 (1H, d, $J_{CH2a,CH2b}$ = 10.9 Hz, $\underline{CH_{2a}}$OAc), 4.78 (1H, d, $J_{2,3}$ = 5.7 Hz, H-3), 4.85 (1H, dd, $J_{CH2a,CH2b}$ = 10.9 Hz, $\underline{CH_{2b}}$OAc), 6.07 (1H, s, H-1); NOE experiment: H-1/C$\underline{H_{2a}}$OAc, H-1/C$\underline{H_{2b}}$OAc, H-2/H-5$_a$, H-2/H-5$_b$; ^{13}C NMR ($CDCl_3$) δ: 12.8, 13.1, 13.2, 16.9, 17.15, 17.21, 17.3, 17.4, 17.5, 20.8, 26.8, 37.9, 38.6, 59.8, 64.4, 71.1, 79.1, 83.8, 170.4, 176.1. FAB-MS (*m*/*z*) 697 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{25}H_{47}O_7INaSSi_2$: 697.15179, found: 697.15183 (M^++ Na).

4.25.

1-[4-*C*-Acetoxymethyl-2-deoxy-2-iodo-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-ribofuranosyl]uracil (44)

To a suspension of uracil (77.3 mg, 0.69 mmol) in CH_3CN (2.0 mL) was added BSA (0.34 mL, 1.38 mmol) at rt under Ar atmosphere and the mixture was stirred for 1 h. To the mixture was added a solution of **43** (154.4 mg, 0.23 mmol) in CH_3CN (3.0 mL)/CH_2Cl_2 (1.0 mL) and TMSOTf (0.42 mL, 2.30 mmol) at 0 °C under Ar atmosphere and the mixture was stirred at rt overnight. The reaction mixture was partitioned between $CHCl_3$/saturated $NaHCO_3$ and silica gel column chromatography（hexane/ethyl acetate = 2/1）of the organic layer gave **44** (126.7 mg, 80%) as a foam: ^{1}H NMR ($CDCl_3$) δ 0.89-1.17 (28H, each as m, Si-*i*-Pr), 2.14 (3H, s, Ac), 3.69 (1H, d, $J_{5'a,5'b}$ = 12.0 Hz, CH_{2a}-5′a), 4.02 (1H, d, $J_{5'a,5'b}$ = 12.0 Hz, CH_{2b}-5′b), 4.11 (1H, dd, $J_{1',2'}$ = 1.7 $J_{2',3'}$ = 7.1 Hz, H-2′), 4.45 (1H, d, $J_{CH2a,CH2b}$ = 11.5 Hz, $\underline{CH_{2a}}$OAc), 4.68 (1H, d, $J_{2',3'}$ = 7.1 Hz, H-3′), 5.13 (1H, dd, $J_{CH2a,CH2b}$ = 11.5 Hz, $\underline{CH_{2b}}$OAc), 5.77 (1H, d, $J_{NH,5}$ = 1.2 and $J_{5,6}$ = 8.0 Hz, H-5), 6.20 (1H, dd, $J_{1',2'}$ = 1.7 Hz, H-1′), 8.04 (1H, d, $J_{5,6}$ = 8.0 Hz, H-6), 9.53 (1H, br, NH); NOE experiment: H-6/CH_{2b}-5′, H-6/H-2′, H-1′/CH_{2b}-4′, H-2′/CH_{2b}-5′; ^{13}C NMR ($CDCl_3$) δ:13.0, 13.1, 13.3, 13.5, 17.2, 17.30, 17.32, 17.38, 17.42, 17.5, 17.6, 21.1, 35.1, 63.4, 65.4, 66.5, 68.4, 74.2, 96.3, 102.8, 140.8, 150.3,

163.0, 170.7; ESI-MS (*m*/*z*) 707 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{24}H_{41}O_7N_2INaSSi_2$: 707.11099, found: 707.11155 (M^+＋Na).

4.26.

1-[4-Acetoxymethyl-2-deoxy-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-ribofuranosyl]uracil (45)

To a solution of **44** (113.5 mg, 0.17 mmol) in toluene (5.0 mL) was added Bu_3SnH (92 μL, 0.34 mmol) and Et_3B (85 μL, 0.085 mmol) at –70 °C under Ar atmosphere and the mixture was stirred under O_2 atmosphere overnight. The reaction mixture was silica gel column chromatography（hexane/ethyl acetate = 1/1）on silica gel to give **45** (94.4 mg, 99%) as a syrup ^{1}H NMR ($CDCl_3$) δ 0.92-1.18 (28H, each as m, Si-*i*-Pr), 2.16 (3H, s, Ac), 2.26 (1H, dd, $J_{2'a,\,3'}$ = 6.3 and $J_{2'a,\,2'b}$ = 13.8 Hz, CH_{2a}-2′), 2.78 (1H, dt, $J_{1',\,2'b}$ = 7.5, $J_{2'b,\,3'}$ = $J_{2'a,\,2'b}$ = 13.8 Hz, CH_{2b}-2′), 3.89 (1H, d, $J_{5'a,5'b}$ = 12.6 Hz, H-5′a), 4.03 (1H, d, $J_{5'a,5'b}$ = 12.6 Hz, H-5′b), 4.25 (1H, d, $J_{CH2a,CH2b}$ = 12.5 Hz, $\underline{CH_{2a}}$OAc), 4.41 (1H, d, $J_{CH2a,CH2b}$ = 12.5 Hz, $\underline{CH_{2b}}$OAc), 4.63 (1H, dd, $J_{2'a,\,3'}$ = 6.3 and $J_{2'b,\,3'}$ = 13.8 Hz, H-3′), 5.76 (1H, d, $J_{NH,5}$ = 1.7 and $J_{5,6}$ = 8.0 Hz, H-5), 6.00 (1H, d, $J_{1',2'b}$ = 7.5 Hz, H-1′), 8.27 (1H, d, $J_{5,6}$ = 8.0 Hz, H-6), 9.51 (1H, br, NH); ^{13}C NMR ($CDCl_3$) δ: 12.6, 13.2, 13.46, 13.49, 17.25, 17.32, 17.48, 17.54, 17.6, 17.7, 42.0, 57.5, 61.7, 62.9, 65.9, 72.4, 102.1, 141.3, 150.9, 163.5, 170.8. ESI-MS (*m*/*z*) 581 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for

$C_{24}H_{42}O_7N_2NaSSi_2$: 581.21435, found: 581.21484 (M^+ + Na).

4.27.

1-[2-Deoxy-4-hydroxymethyl-3,5-*O*-(1,1,3,3-tetraisoproryldisiloxane-1,3-diyl)-4-thio-β-D-ribofuranosyl]uracil (46)

Compound **45** (249.8 mg, 0.45 mmol) was treated with methanolic ammonia (30 mL) at 3 °C for 2 days. The reaction mixture was evaporated and silica gel column chromatography（hexane/ethyl acetate = 2/1）of the residue gave **46** (188.0 mg, 81%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 0.97-1.18 (28H, each as m, Si-*i*-Pr), 2.22 (1H, dd, $J_{2'a,\,3'}$ = 6.3 and $J_{2'a,\,2'b}$ = 12.6 Hz, CH_{2a}-2′), 2.62 (1H, dd, $J_{OH,CH2a}$ = 7.5 and $J_{OH,CH2b}$ = 4.6 Hz, $CH_2O\underline{H}$), 2.81 (1H, dt, $J_{1',\,2'b}$ = 7.5, $J_{2'b,\,3'}$ = $J_{2'a,\,2'b}$ = 12.6 Hz, CH_{2b}-2′), 3.74 (1H, dd, $J_{OH,CH2a}$ = 7.5 and $J_{CH2a,CH2b}$ = 12.0 Hz, $C\underline{H_{2a}}OH$), 3.86 (1H, d, $J_{OH,CH2b}$ = 4.6 and $J_{CH2a,CH2b}$ = 12.0 Hz, $C\underline{H_{2b}}OH$), 3.98 (1H, d, $J_{5'a,5'b}$ = 12.6 Hz, CH_{2a}-5′), 4.11 (1H, d, $J_{5'a,5'b}$ = 12.6 Hz, CH_{2b}-5′), 4.69 (1H, dd, $J_{2'a,\,3'}$ = 6.3 and $J_{2'b,\,3'}$ = 12.6 Hz, H-3′), 5.75 (1H, d, $J_{5,6}$ = 8.0 Hz, H-5), 6.03 (1H, d, $J_{1',2'b}$ = 7.5 Hz, H-1′), 8.26 (1H, d, $J_{5,6}$ = 8.0 Hz, H-6), 9.66 (1H, br, NH); ^{13}C NMR ($CDCl_3$) δ: 12.3, 12.9, 13.10, 13.13, 16.90, 16.93, 16,96, 17.03, 17.19, 17.23, 17.3, 41.0, 56.7, 61.4, 63.2, 64.7, 72.1, 102.0, 140.9, 150.6, 163.1, ; ESI-MS (*m*/*z*) 539 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{22}H_{40}O_6N_2NaSSi_2$: 539.20378, found: 539.20441 (M^+ + Na).

4.28.

1-[2-Deoxy-4-*C*-formyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-ribofuranosyl]uracil (47)

To a solution of **46** (147.6 mg, 0.29 mmol) in CH_2Cl_2 (7.0 mL) was added Dess-Martin periodinane (123.2 mg, 0.44 mmol) at 0 °C under Ar atmosphere and the mixture was stirred for 5 h. The reaction mixture was partitioned between dichloromethane/saturated $NaHCO_3$ and silica gel column chromatography （hexane/ethyl acetate = 2/1）of the organic layer gave **47** (140.1 mg, 94%) as a syrup: δ 0.98-1.02 (28H, each as m, Si-*i*-Pr), 2.41 (1H, dd, $J_{2'a,\,3'}$ = 6.3 and $J_{2'a,\,2'b}$ = 13.8 Hz, CH_{2a}-2′), 2.93 (1H, dt, $J_{1',\,2'b}$ = 7.5, $J_{2'b,\,3'}$ = $J_{2'a,\,2'b}$ = 13.8 Hz, CH_{2b}-2′), 4.06 (1H, d, $J_{5'a,5'b}$ = 13.8 Hz, CH_{2a}-5′), 4.38 (1H, d, $J_{5'a,5'b}$ = 13.8 Hz, CH_{2b}-5′), 4.85 (1H, dd, $J_{2'a,\,3'}$ = 6.3 and $J_{2'b,\,3'}$ = 13.8 Hz, H-3′), 5.79 (1H, d, $J_{5,6}$ = 8.0 Hz, H-5), 6.22 (1H, d, $J_{1',2'b}$ = 7.5 Hz, H-1′), 8.34 (1H, d, $J_{5,6}$ = 8.0 Hz, H-6), 9.71 (1H, s, CHO), 9.82 (1H, br, NH); ^{13}C NMR ($CDCl_3$) δ: 12.3, 13.0, 13.3, 13.5, 16.7, 16.8, 16.9, 16.9, 17.25, 17.32, 17.4, 17.5, 41.9, 58.0, 58.1, 65.7, 71.9, 96.1, 102.2, 140.8, 150.5, 162.8. ESI-MS (*m*/*z*) 515 (M^+ + H); ESI-HRMS (*m*/*z*): calcd for $C_{22}H_{39}O_6N_2SSi_2$: 515.20619, found: 515.20726 (M^+＋H).

4.29.

1-[2-Deoxy-4-C-ethynyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-ribofuranosyl]uracil (49)

To a suspension of bromomethyltriphenylphosphonium bromide (148.3 mg, 0.34 mmol) in THF (2 mL) was added potassium *tert*-butoxide (1 M THF solution) (0.34 mL, 0.34 mmol) at –40 °C under Ar atmosphere and the mixture was stirred for 2 h. To the ylide solution was added a solution of **47** (29.5 mg, 0.057 mmol) in THF (2 mL) at –40 °C and the reaction mixture was stirred at -40 °C for 2 h. The reaction mixture was partitioned between chloroform/saturated NH_4Cl and silica gel column chromatography （hexane/ethyl acetate = 2/1） of the organic layer gave a mixture of **48** (17.6 mg, 46% yield). To a solution of **48** (17.6 mg, 0.026 mmol) in THF (2 mL) was added butyl lithium (1.55 M hexane solution) (0.14 mL, 0.21 mmol) at –10 °C under Ar atmosphere and the mixture was stirred for 16 h. The reaction mixture was partitioned between chloroform/saturated NH_4Cl and and silica gel column chromatography（hexane/ethyl acetate = 2/1） of the organic layer gave **49** (10.9 mg, 82%) as a syrup: IR (neat) : 2111 cm $^{-1}$(C≡CH); δ 0.98-1.16 (28H, each as m, Si-*i*-Pr), 2.21 (1H, dd, $J_{2'a,3'}$ = 5.2 and $J_{2'a,2'b}$ = 13.5 Hz, CH_{2a}-2′), 2.63 (1H, s, C≡CH), 2.93 (1H, ddd, $J_{1',2'b}$ = 7.5, $J_{2'b,3'}$ = $J_{2'a,2'b}$ = 13.5 Hz, CH_{2b}-2′), 4.02 (1H, d, $J_{5'a,5'b}$ = 12.6 Hz, CH_{2a}-5′), 4.05 (1H, d, $J_{5'a,5'b}$ = 12.6 Hz, CH_{2b}-5′), 4.45 (1H, dd, $J_{2'a,3'}$ = 5.2 and $J_{2'b,3'}$ = 13.5 Hz, H-3′), 5.71 (1H, dd, $J_{NH,5}$ = 2.3

and $J_{5,6}$ = 8.6 Hz, H-5), 6.22 (1H, d, $J_{1',2'b}$ = 7.5 Hz, H-1′), 8.22 (1H, d, $J_{5,6}$ = 8.6 Hz, H-6), 8.75 (1H, br, NH); ^{13}C NMR ($CDCl_3$) δ: 12.3, 13.1, 13.3, 13.4, 16.8, 16.90, 16.97, 16.99, 17.2, 17.3, 17.4, 17.5, 40.7, 57.0, 57.2, 61.7, 70.8, 76.3, 81.4, 101.9, 141.1, 150.5, 162.9. FAB-MS(*m*/*z*): 533 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{23}H_{38}O_5N_2NaSSi_2$: 533.19322, found: 533.19434(M^+ + H).

4.30. 1-[*N*^{4},3,5-Tri-*O*-acetyl-2-deoxy-4-*C*-ethynyl-4-thio-β-D-ribofuranosyl]cytosine (52)

To a solution of **49** (10.5 mg, 0.021 mmol) in CH_3CN (2.0 mL) was added K_2CO_3 (18.0 mg, 0.13 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (39.4 mg, 0.13 mmol) under Ar atmosphere at rt and the mixture was stirred at 80 °C for 6 h. The reaction mixture was filtered through a celite pad, washed with CH_2Cl_2 and the filtrate was evaporated to dryness. Silica gel column chromatography over neutral silica gel (hexane/ethyl acetate = 10/1) of the residue gave **50** (14.6 mg, 90%). To a solution of **50** in THF (0.5 mL) was added ammonium hydroxide (2 mL) at 0 °C and the mixture was stirred overnight at rt. The reaction mixture was evaporated to dryness and silica gel column chromatography (4% MeOH in CH_2Cl_2) of the residue gave crude **51**. To a THF (1.5 mL) solution of crude **51** was added Bu_4NF (1M THF solution) (53 μL, 0.053 mmol) at 0 °C under Ar atmosphere and the mixture was stirred for 2h. To the reaction

mixture was added Ac_2O (10.4 μL, 0.11 mmol) at 0 °C and the mixture was stirred at rt overnight. The reaction mixture was partitioned between chloroform and saturated $NaHCO_3$ and silica gel column chromatography (2% MeOH in CH_2Cl_2) of the organic layer gave **52** (6.3 mg, 76%) as a syrup: IR (neat): 2116 cm $^{-1}$(C≡CH); ^{1}H NMR ($CDCl_3$) δ 2.15, 2.16 and 2.27 (9H, each as s, Ac), 2.46 (1H, dt, $J_{1',2'a} = J_{2'a,3'} = 5.2$ and $J_{2'a,2'b} = 14.3$ Hz, CH_{2a}-2′), 2.53 (1H, dt, $J_{1',2'b} = J_{2'b,3'} = 6.9$ and $J_{2'a,2'b} = 14.3$ Hz, CH_{2b}-2′), 2.63 (1H, s, C≡CH), 4.37 (1H, d, $J_{5'a,5'b} = 11.5$ Hz, CH_{2a}-5′), 4.41 (1H, d, $J_{5'a,5'b} = 11.5$ Hz, CH_{2b}-5′), 5.42 (1H, dd, $J_{2'a,3'} = 5.2$ and $J_{2'b,3'} = 6.9$ Hz, H-3′), 6.51 (1H, dd, $J_{1',2'a} = 5.2$ and $J_{1',2'b} = 6.9$ Hz, H-1′), 7.50 (1H, d, $J_{5,6} = 8.0$ Hz, H-5), 8.34 (1H, d, $J_{5,6} = 8.0$ Hz, H-6), 9.08 (1H, br, NH); ^{13}C NMR ($CDCl_3$) δ: 20.8, 20.9, 25.0, 39.6, 55.7, 63.4, 66.6, 74.4, 76.3, 79.4, 97.3, 144.9, 155.4, 162.3, 169.9, 170.0. ESI-MS(*m*/*z*): 416 ($M^+ + Na$); ESI-HRMS (*m*/*z*): calcd for $C_{17}H_{19}O_6N_3NaS$: 416.08868, found: 416.08899 ($M^+ + Na$).

4.31. 1-[2-deoxy-4-*C*-ethynyl-4-thio-β-D-ribofuranosyl]cytosine (9)

Compound **52** (13.4 mg, 0.034 mg) was treated with methanolic ammonia (3.0 mL) at 0 °C overnight. The reaction mixture was evaporated to dryness and silica gel column chromatography (20% MeOH in CH_2Cl_2) of the residue gave **9** (8.8 mg, 97%) as a solid: mp 203-206 °C; IR (neat): 2110cm $^{-1}$(C≡CH); ^{1}H NMR (CD_3OD) δ 2.25 (1H, dt,

$J_{1',2'a}$ = $J_{2'a,3'}$ = 4.6 and $J_{2'a,2'b}$ = 13.2 Hz, CH_{2a}-2′), 2.54 (1H, ddd, $J_{1',2'b}$ = 6.7, $J_{2'b,3'}$ = 8.6 and $J_{2'a,2'b}$ = 13.2 Hz, CH_{2b}-2′), 2.86 (1H, s, C≡CH), 3.68 (1H, d, $J_{5'a,5'b}$ = 12.1 Hz, CH_{2a}-5′), 3.75 (1H, d, 1H, d, $J_{5'a,5'b}$ = 12.1 Hz, CH_{2a}-5′), 4.25 (1H, $J_{2'a,3'}$ = 4.6 and $J_{2'b,3'}$ = 8.6 Hz,, H-3′), 5.84 (1H, d, $J_{5,6}$ = 7.5 Hz, H-5), 6.20 (1H, dd, $J_{1',2'a}$ = 4.6 and $J_{1',2'b}$ = 6.9 Hz, H-1′), 8.23 (1H, d, $J_{5,6}$ = 7.5 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ: 42.9, 61.03, 61.10, 66.6, 74.7, 77.3, 83.1, 97.4, 143.9, 158.6, 167.4; ESI-MS (*m*/*z*) 290 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{11}H_{13}O_3N_3NaS$: 290.05698, found: 290.05711 (M^+＋Na).

4.32.

1-[4-*C*-Cyano-2-deoxy-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-ribofuranosyl]uracil (54)

To a solution of **47** (87.7 mg, 0.17 mmol) in pyridine (4.0 mL) was added $HONH_2$·HCl (35.4 mg, 0.51 mmol) at rt under Ar atmosphere and the mixture was stirred overnight. The reaction mixture was quenched with EtOH and evaporated to dryness. The residue was partitioned between ethyl acetate/H_2O and silica gel column chromatography（hexane/ethyl acetate = 1/1）of the organic layer gave **53** (86.7 mg, 96% yield). To a solution of **53** (86.7 mg, 0.16 mmol) in CH_2Cl_2 (4.0 mL) was added Et_3N (0.12 mL, 0.85 mmol) and MsCl (66.0 mL, 0.85 mmol) at 0 °C under Ar atmosphere and the mixture was stirred at rt overnight. The reaction mixture was partitioned

between chloroform/saturated $NaHCO_3$ and silica gel column chromatography （hexane/ethyl acetate = 2/1）of the organic layer gave **54** (65.9 mg, 80%) as a syrup; IR ($CHCl_3$): 2237^{-1} (C≡N); ^{1}H NMR ($CDCl_3$) δ 0.95-1.15 (28H, each as m, Si-*i*-Pr), 2.37 (1H, dd, $J_{2a,3'}$ = 5.8 and $J_{H2a,H2b}$ = 14.1 Hz, CH_{2a}-2′), 2.89 (1H, ddd, $J_{1',2'b}$ = 7.5, $J_{2'b,3'}$ = 5.2 and $J_{2'a,2'b}$ = 14.1 Hz, CH_{2b}-2′), 4.14 (1H, d, $J_{5'a,5'b}$ = 12.1 Hz, CH_{2a}-5′), 4.18 (1H, d, $J_{5'a,5'b}$ = 12.1 Hz, CH_{2b}-5′), 4.56 (1H, dd, $J_{2'a,3'}$ = 5.8 and $J_{2'b,3'}$ = 5.2 Hz, H-3′), 5.74 (1H, dd, $J_{5,NH}$ = 2.3 and $J_{5,6}$ = 8.6 Hz, H-5), 6.17 (1H, d, $J_{1',2'b}$ = 7.5 Hz, H-1′), 8.01 (1H, d, $J_{5,6}$ = 8.6 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ: 12.4, 13.0, 13.3, 16.8, 16.9, 16.9, 17.1, 17.2, 17.3, 17.5, 31.6, 41.0, 56.5, 57.3, 60.1, 71.5, 102.6, 117.6, 140.4, 150.6, 163.3. ESI-MS(*m*/*z*): 534 (M^+＋Na); ESI-HRMS (*m*/*z*): calcd for $C_{22}H_{37}O_5N_3NaSSI_2$: 534.18847, found: 534.18942(M^+＋Na).

4.33. 1-[*N*^{4},3,5-Tri-*O*-acetyl-4-C-cyano-2-deoxy-4-thio-β-D-ribofuranosyl]cytosine (57)

To a solution of **54** (50.9 mg, 0.099 mmol) in CH_3CN (5.0 mL) was added K_2CO_3 (81.5 mg, 0.59 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (178.7 mg, 0.59 mmol) under Ar atmosphere at rt and the mixture was stirred at 80 °C for 6 h. The reaction mixture was chromatographed over neutral silica gel (hexane/ethyl acetate = 5/1) to give **55** (72.1 mg, 94% yield). To a solution of **55** in THF (1.0 mL) was added

ammonium hydroxide (3.0 mL) at −10 °C and the mixture was stirred overnight at rt. The reaction mixture was partitioned between chloroform and H_2O and silica gel column chromatography (4-8% MeOH in CH_2Cl_2) of the organic layer gave crude **56**. To a solution of **56** in THF (4 mL) was added Bu_4NF (1 M THF solution) (0.25 mL, 0.25 mmol) at 0 °C under Ar atmosphere and the mixture was stirred for 2 h. To the reaction mixture wad added Ac_2O (47 μL, 0.50 mmol) and the mixture was stirred 2 h. The reaction mixture was partitioned between chloroform/saturated $NaHCO_3$ and silica gel column chromatography（2% MeOH in CH_2Cl_2）of the organic layer gave **57** (41.7 mg, 97% from **55**) as a syrup.: IR (neat) 2242 cm^{-1} (C≡N); ^1H NMR ($CDCl_3$) δ 2.18, 2.23 and 2.29 (9H, eacha as s, Ac), 2.72 (1H, dt, $J_{1',2'a} = J_{2'a,3'} = 5.2$ and $J_{2'a,2'b} = 14.3$ Hz, CH_{2a}-2′), 2.85 (1H, dt, $J_{1',2'b} = J_{2'b,3'} = 7.5$ and $J_{2'a,2'b} = 14.3$ Hz, CH_{2b}-2′), 4.43 (1H, d, $J_{5'a,5'b} = 11.5$ Hz, CH_{2a}-5′), 4.55 (1H, d, $J_{5'a,5'b} = 11.5$ Hz, CH_{2b}-5′), 5.51 (1H, dd, $J_{2'a,3'} = 5.2$ and $J_{2'b,3'} = 7.5$ Hz, H-3′), 6.45 (1H, dd, $J_{1',2'a} = 5.2$, $J_{1',2'b} = 7.5$Hz, H-1′), 7.60 (1H, d, $J_{5,6} = 7.5$ Hz, H-5), 8.12 (1H, d, $J_{5,6} = 7.5$ Hz, H-6), 9.64 (1H, br, NH); ^{13}C NMR ($CDCl_3$) δ: 27.5, 31.7, 38.0, 51.0, 52.1, 54.0, 61.7, 65.4, 74.6, 98.2, 116.5, 144.5, 155.5, 162.8, 169.7, 169.9, 208.4. ESI-MS(*m*/*z*): 417 (M^+＋Na); ESI-HRMS (*m*/*z*): calcd for $C_{16}H_{18}O_6N_4NaS$: 268.0756, found: 417.08444 (M^+＋Na).

4.34. 1-[2-deoxy-4-*C*-cyano-4-thio-β-D-ribofuranosyl]cytosine (10)

Compound **57** (22.5 mg, 0.057 mmol) was treated with methanolic ammonia (5.0 mL) at 0 °C overnight. The reaction mixture was evaporated to dryness and purified by silica gel column chromatography (20% MeOH in CH_2Cl_2) to give pure **10** (13.8 mg, 90%) as a solid: mp 252-253 °C (dec); IR (neat) 2235 cm^{-1} (C≡N); ^{1}H NMR (CD_3OD) δ 2.44-2.48 (1H, m, CH_{2a}-2′), 2.55-2.61 (1H, m, CH_{2b}-2′), 3.88 (1H, d, $J_{5'a,5'b}$ = 11.5 Hz, CH_{2a}-5′), 3.94 (1H, d, $J_{5'a,5'b}$ = 11.5 Hz, CH_{2b}-5′), 4.51 (1H, dd, $J_{2'a,3'}$ = 4.6 and $J_{2'b,3'}$ = 7.5 Hz,, H-3′), 5.93 (1H, d, $J_{5,6}$ = 7.5 Hz, H-5), 6.38 (1H, t, $J_{1',2'a} = J_{1',2'b}$ = 5.8 Hz, H-1′), 8.03 (1H, d, $J_{5,6}$ = 7.5 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ: 42.0, 60.8, 61.8, 65.9, 74.9, 97.2, 119.9, 143.1, 158.3, 167.3; ESI-MS (*m*/*z*) 291 (M^+ + Na); ESI-HRMS (*m*/*z*): calcd for $C_{10}H_{12}O_3N_4NaS$: 291.05223, found: 291.05226 (M^+ + Na).

4.35. Cytotoxicity test[33]

A cell suspension of human B-cell acute lymphoblastic leukemia cells, CCRF-SB or human T-cell acute lymphoblastic leukemia cells, Molt-4, containing 1.0 x 10^5 cells/mL, was prepared in RPMI 1640 medium supplemented with 10% fetal bovine serum; 100 μL of the cell suspension was seeded in a 96-well plate, and 100 μL of medium or phosphate-buffered saline (PBS) containing test compound in serial 2-fold dilution was added. Cells were incubated in a 5% CO_2 incubator at 37 °C. After 3 days, 10 μL of Cell

Count Reagent SF (Nacalai Tesque, Kyoto, Japan) was added to each well. After 4 h incubation at 37 °C, absorbance at 570 nm (test wavelength) and 690 nm (reference wavelength) were measured using a microplate reader (iMark, Bio-Rad). The percentage of cell growth inhibition was calculated by the following formula:

Inhibition (%) = [1 - (Tx – C_0/Cx – C_0)] x 100

Where T_x is absorbance at the end of incubation with test drug, T_x is absorbance at the end of incubation without test drug, and C_0 is absorbance at beginning of incubation. IC_{50} of the test compound was determined graphically from a dose-inhibition curve.

4.36. Antiviral assays[34]

HEL cells and the following virus strain were used: herpes simplex virus type-1 (HSV-1) strain KOS, thymidine kinase-deficient (TK^-) HSV-1 KOS strain resistant to ACV, HSV type-2 strain G, varicella-zoster virus (VZV) strain Oka, VZV/TK- strain 07-1, human cytomegarovirus (HCMV) strain Davis, vaccinia virus Lederle strain. These assays were based on the inhibition of virus-induced cytopathicity or plaque formation (for VZV) in human embryonic lung fibroblasts, African green monkey cells, human epithelial cells, Crandell feline kidney cells or median-darby canine kidney cells. Briefly, confluent cell cultures in microtitre 96-well plates were inoculated with 100

50% cell culture infectious doses (CCID 50) of virus (1 CCID 50 being the virus dose to infect 50% of the cell cultures) or with 20 plaque-forming units (PFU; for VZV). After 1-2 h adsorption period, residual virus was removed and the cell cultures were incubated in the presence of varying concentrations of the test compounds. Viral cytopathicity of plaque formation (VZV) was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the 50% effective concentration, that is, effective concentration required to reduce virus-induced cytopathogenecity or viral plaque formation (VZV) by 50%.

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