Nucleosides and Nucleotides. 185. Synthesis and Biological Activities of 4′α-*C*-Branched-Chain Sugar Pyrimidine Nucleosides

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A series of 4′α-*C*-branched-chain pyrimidine nucleosides was synthesized from 2′-deoxycytidine or uridine. In the 2′-deoxycytidine series, the substituent at the 4′α-position affected cytotoxicity against L1210 mouse leukemic cells in vitro in the order Me (**23**) > CN (**22**) > C≡CH (**21**) > CH=CH_2 (**19**) > Et (**24**) > CH=CHCl (**20**). However, uridine and cytidine derivatives with ethynyl and cyano groups at the 4′α-position did not show any cytotoxicity. The antiviral activities of these nucleosides against HSV-1, HSV-2, and HIV-1 in vitro were also examined. Compounds **22** and **23** showed antiviral activities against HSV-1 and HSV-2 without showing significant toxicity to the host cells (MRC-5 cells). Although almost all of the nucleosides showed anti-HIV-1 activities, they were also cytotoxic to the host cells (MT-4).

Nucleoside antimetabolites play an important role in cancer and viral chemotherapy. Several branched-chain sugar nucleosides have been synthesized and evaluated as potential antitumor or antiviral agents. Some of these nucleosides, such as 1-(2-deoxy-2-methylene-β-D-*erythro*-pentofuranosyl)cytosine (DMDC),[1−3] 1-(2-cyano-2-deoxy-β-D-*arabino*-pentofuranosyl)cytosine (CNDAC),[4−7] 1-(2-deoxy-2-fluoromethylene-β-D-*erythro*-pentofuranosyl)cytosine (FMDC),[8] and 1-(3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl)cytosine (ECyd)[9−11] and its uracil congener (EUrd),[9−11] have shown potent antitumor activities both in vitro and in vivo. Recently, some 4′α-*C*-branched 2′-deoxynucleosides, such as 4′α-*C*-methyl-2′-deoxycytidine[12,13] and 4′α-*C*-fluoromethyl-2′-deoxycytidine,[14] have also been reported to have potent antileukemic activities. Moreover, we recently found that 4′α-*C*-ethyl-, -ethenyl-, and -ethynylthymidines showed antiviral activities against herpes simplex virus type-1 (HSV-1) and human immunodeficiency virus type-1 (HIV-1) in vitro.[15] Although the activities of these nucleosides are not superior to those of reference nucleosides such as acyclovir (ACV) and zidovudine (AZT), they are essentially not cytotoxic to host RPMI 18226 and MT-4 cells. On the basis of these findings, we became interested in the biological activities of 4′α-*C*-carbon-substituted pyrimidine nucleosides. Since there are currently few examples of 4′α-*C*-branched nucleosides, to clarify the structure−activity relationships, we synthesized a series of 4′α-*C*-branched-chain sugar nucleosides and evaluated their cytotoxicity against L1210 mouse leukemic and KB human pharyngeal carcinoma cells and their antiviral activity against HSV-1, HSV-2, and HIV-1 in vitro.

Chemistry

In a 2′-deoxycytidine series, the biological activities of 4′α-*C*-methyl (**23**),[12,13] -fluoromethyl,[14] and -cyano (**22**)[16] derivatives have been reported. The former two nucleosides have been synthesized starting from D-glucose. However, this method is rather lengthy, and overall yields are quite low. On the other hand, Moffatt's group has introduced a hydroxymethyl group at the 4′α-position of nucleosides using an appropriately protected nucleoside 5′-aldehyde under Cannizzaro reaction conditions.[17−19] Since this method is much shorter and effective, we adopted this method to synthesize our target nucleosides: 4′α-*C*-ethenyl, -chloroethenyl, -ethynyl, and -ethyl derivatives of 2′-deoxycytidine. We also prepared the known 4′α-*C*-methyl and -cyano derivatives using the same intermediate to evaluate their biological activities. Ribonucleoside counterparts, such as 4′α-*C*-ethynyl- and -cyanouridines and -cytidines, were also prepared from uridine.

We used N^4-benzoyl-3′-*O*-[*tert*-butyldimethylsilyl (TBS)]-2′-deoxycytidine (**3**) as a starting material, which was readily prepared from 2′-deoxycytidine in four steps (Scheme 1). The 5′-OH of **3** was oxidized by a slightly modified original method using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) hydrochloride instead of 1,3-dicyclohexylcarbodiimide (DCC).[18] The desired aldehyde **4** was obtained in a good yield and subsequently treated with aqueous HCHO and NaOH in dioxane for 14 h at room temperature. However, the desired 4′-*C*-hydroxymethyl derivative **5** was not obtained, perhaps due to deprotection of the benzoyl and TBS groups. Therefore, the crude **4** was treated under the same conditions for only 10 min; $NaBH_4$ was then added to reduce the resulting aldehyde at the 4′-position to give **5** in 44% yield. This aldol reaction followed by reduction

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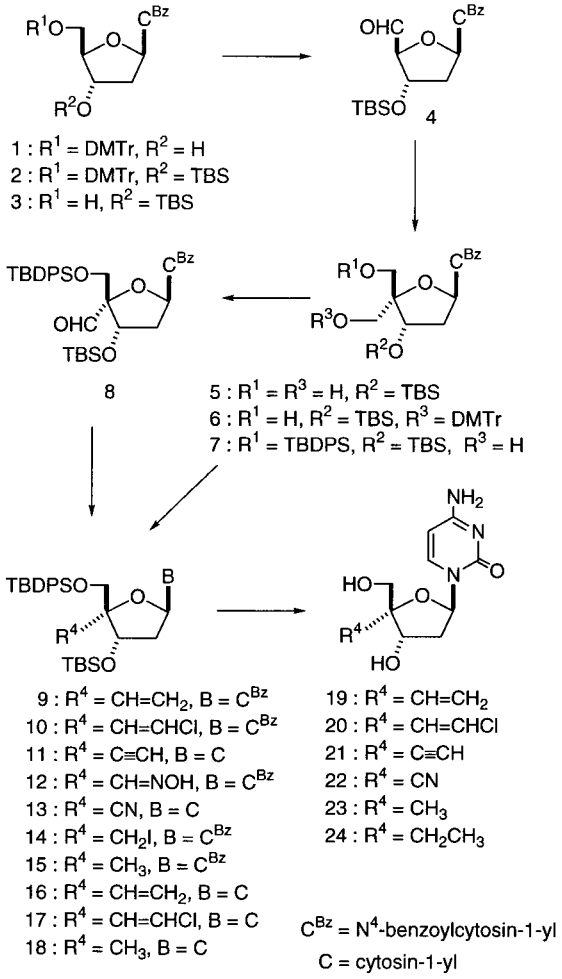
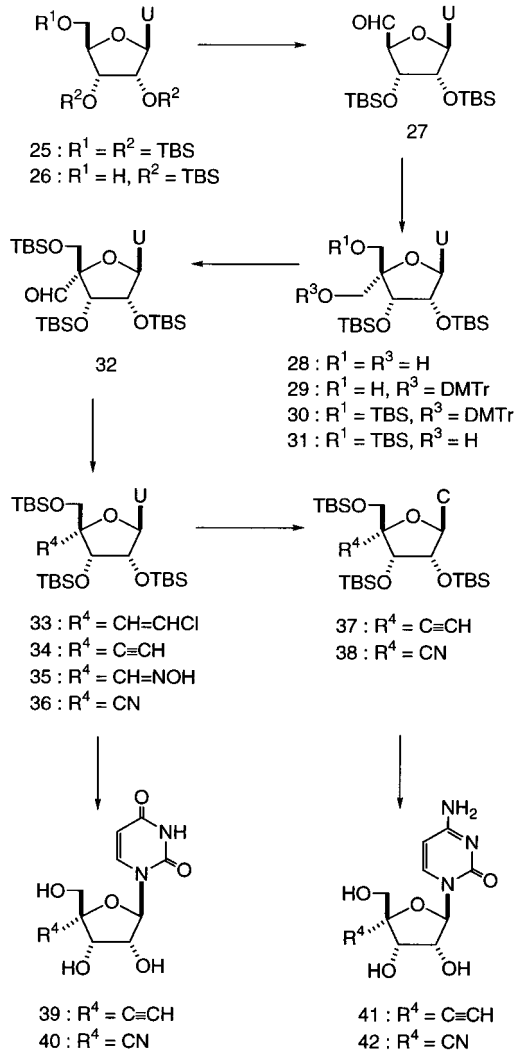
Scheme 1



Scheme 2



is better than the original Cannizzaro conditions when the starting materials have base-labile protecting groups. After manipulation of the protecting groups, the resulting **7** was oxidized to the corresponding 4′α-*C*-aldehyde under Swern conditions with a slight modification[20] to give **8** in 82% yield as crystals, which was used as a common intermediate to prepare our target nucleosides.

Wittig reactions of **8** with $Ph_3P{=}CH_2$ and $Ph_3P{=}CHCl$ gave 4′α-*C*-ethenyl and -2-chloroethenyl derivatives **9** and **10** (*Z*:*E* = 20:1), respectively, in yields of 92%. Compound **10** was further treated with BuLi in THF at −78 °C to give debenzoylated 4′-*C*-ethynyl derivative **11** in 72% yield. Compound **8** was also converted into oxime **12**, which was then dehydrated in NaOAc−Ac_2O and debenzoylated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in MeOH to give 4′α-*C*-cyano derivative **13**. 4′α-*C*-Methyl derivative **15** was prepared from **7**. Using an iodine−imidazole−Ph_3P system, **7** was converted into the 4′-*C*-iodomethyl derivative **14**, which was hydrogenated with Pd/C in the presence of Et_3N to give **15** in 54% yield from **7**. The protecting groups of **9**−**18** were removed by usual methods to give the target compounds **19**−**23** in good yields. Hydrogenation with Pd/C of **19** gave ethyl derivative **24**.

We were unable to unequivocally establish the stereochemisty of the 4′-*C*-aldehyde group in **8** at this stage. However, NOE (0.9%) between the 4′α-*C*-methyl protons and H-1′ in the 4′-*C*-methyl derivative **23** confirmed the stereochemistry of the original formyl group in **8** to be 4′α.

Both 4′α-*C*-substituted uridine and cytidine derivatives were prepared from a common uracil intermediate, 1-(2,3,5-tri-*O*-TBS-4α-*C*-formyl-*β*-D-*ribo*-pentofuranosyl)uracil (**32**), since the uracil moiety can be easily converted into the corresponding cytosine moiety after transformation of the sugar moiety. The 5′-*O*-TBS group of 2′,3′,5′-tris-*O*-TBS-uridine **25** was selectively deprotected with TFA in aqueous THF to give **26** (Scheme 2). 4′-*C*-Hydroxymethyl-2′,3′-*O*-bis-TBS-uridine (**28**) was obtained from **26** using the reactions previously described with cytosine series. Oxidation of the hydroxyl group in **26**, followed by treatment of the resulting **27** under conditions similar to those described for the synthesis of **5**, gave the diol **28**. The 4′α-*C*-hydroxymethyl group in **28** was selectively protected with a DMTr group,[21] and the resulting free 5′-hydroxyl group was then protected with a TBS group, followed by deprotection of the DMTr group with AcOH to give **31**. Modified Swern oxidation of **31** gave the desired aldehyde **32**.

The formyl group in **32** was transformed into a cyano or ethynyl group by methods similar to those used for 2′-deoxycytidine derivatives. Compound **32** was converted into 4′α-*C*-[2(*Z*)-chloroethenyl] derivative **33** via

Table 1. Biological Activities of 4′α-*C*-Substituted Pyrimidine Nucleosides

	cytotoxicity IC_{50} (μM)[a]		antiviral activity					
			HSV-1[b]		HSV-2[b]		HIV-1[b]	
compd	L1210	KB	EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)	IC_{50} (μM)
19	21	>350	>350	>350	>350	>350	0.0086	0.18
20	200	>350	>350	>350	>350	>350	2.1	4.6
21	0.80	>350	200	>350	>350	>350	0.0022	0.16
22	0.33	>350	3.6	>350	21	>350	0.0012	0.17
23	0.16	>350	3.1	260	0.58	260	0.062	0.062
24	55	>350	>350	>350	>350	>350	0.013	0.77
39	>350	>350	ND[c]	ND	ND	ND	ND	ND
40	>350	>350	ND	ND	ND	ND	ND	ND
41	>350	>350	ND	ND	ND	ND	ND	ND
42	>350	>350	ND	ND	ND	ND	ND	ND
ACV	ND	ND	0.36	>350	1.1	>350	ND	ND
AZT	ND	ND	ND	ND	ND	ND	0.0041	3.2

[a] Tumor cells (2×10^3 cells/well) were incubated in the presence or absence of compounds for 72 h. MTT reagent was added to each well, and the plate was incubated for an additional 4 h. The resulting MTT-formazan was dissolved in DMSO, and the OD (540 nm) was measured. Percent inhibition was calculated as follows: % inhibition = [1 − OD (540 nm) of sample well/OD (540 nm) of control well] × 100. IC_{50} (μM) is the concentration that inhibits cell growth by 50%.[22] [b] To evaluate anti-HSV-1, anti-HSV-2, and anti-HIV-1 activities, HSV-1 Kos strain vs MRC-5 cells, HSV-2 G strain vs MRC-5 cells, and HIV-1 IIIb strain vs MT-4 cells were used, respectively. Briefly, cells were infected with viruses at a multiplicity of infection of 0.02. Immediately after the virus infection, a cell suspension (100 μL) was placed into each well containing various concentrations of the compounds (100 μL). After 4 days of incubation at 36 °C, the number of viable cells was determined by the MTT method. IC_{50} (μM) is the concentration that inhibits cell growth by 50%.[23,24] [c] ND, not determined.

the Wittig reaction. Compound **33** was then dehydrohalogenated with BuLi to give ethynyl derivative **34** and deprotected with HCl/dioxane in MeOH to give **39**. Compound **32** was also converted into the corresponding oxime **35**, which was further transformed into 4′α-*C*-cyano derivative **36**. Compound **36** was deprotected as described above to give **40**. Cytidine derivatives **37** and **38** were prepared from uridine derivatives **34** and **36**, respectively, using a 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl)−Et_3N−DMAP system, followed by NH_4OH treatment. Compounds **37** and **38** were desilylated with HF−Et_3N to give **41** and **42**, respectively.

Biological Activities

The cytotoxicities of a series of 4′α-*C*-branched nucleosides **19**−**21**, **24**, and **39**−**42**, including the known **22** and **23**, were investigated in vitro using mouse L1210 leukemia and human KB pharyngeal carcinoma cells, and the results are summarized in Table 1. As in previous studies,[12,13] 2′-deoxycytidine derivative **23**, which has a methyl group at the 4′α-position, showed significant antileukemic activities against L1210 cells, with IC_{50} values of 0.16 μM. The corresponding 4′α-*C*-cyano, -ethynyl, -ethenyl, and -ethyl derivatives **22**, **21**, **19**, and **24** also had antileukemic activity, with IC_{50} values of 0.33, 0.80, 21, and 55 μM, respectively. However, 4′α-*C*-[2(*Z*)-chloroethenyl] derivative **20** showed only insignificant activity. Therefore, in the 2′-deoxycytidine series, the substituents at the 4′α-position affect cytotoxicity in the order Me (**23**) > CN (**22**) > C≡CH (**21**) > $CH{=}CH_2$ (**19**) > Et (**24**) > CH=CHCl (**20**). Therefore, the activity seems to be related to the bulkiness of the 4′α-*C*-substituents. Since these 2′-deoxycytidine analogues can be phosphorylated by deoxycytidine kinase (dCK), the relative cytotoxicity might be dependent on the susceptibility to dCK. On the other hand, ribonucleoside derivatives **39**−**42** did not show any cytotoxicity. These ribonucleosides were thought to be phosphorylated by uridine/cytidine kinase, and the substrate specificity of this kinase might be more strict than that of dCK at the 4′α-position. However, none of the nucleosides described here were cytotoxic to KB cells. This difference between L1210 and KB cells might be related to the activity of certain activation enzymes, although any definite conclusions would require further studies.

The antiviral activities of the nucleosides against HSV-1, HSV-2, and HIV-1 were also examined in vitro (Table 1). Unlike the 4′α-*C*-substituted thymidines,[15] only 4′α-*C*- cyano and -methyl derivatives **22** and **23** were active against HSV-1 and HSV-2 without showing significant toxicity to the host cells (MRC-5 cells). On the other hand, almost all of the nucleosides described here showed significant anti-HIV-1 activities. However, all of these nucleosides showed significant cytotoxicity to the host cells (MT-4 cells). On the basis of these results together with our previous data,[15] the nucleobase moiety affects not only the activity but also the selectivity.

Experimental Section

Melting points were measured on a Yanagimoto MP-3 micromelting point apparatus (Yanagimoto, Japan) and are uncorrected. The ^1H NMR spectra were recorded on a JEOL AL-400 (400-MHz) or JEOL JNM-EX 270 (270-MHz) spectrometer 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 disappearance on the addition of D_2O. ^{13}C NMR spectra were recorded on a JEOL AL-400 (400-MHz) or JEOL JNM-EX 270 (270-MHz) spectrometer. Fast atom bombardment mass spectrometry (FAB-MS) was done on a JEOL JMS-HX110 instrument at an ionizing voltage of 70 eV. TLC was done on Merck silica gel 60 F_{254} precoated plates. The silica gel used for column chromatography was Merck silica gel 60 (70−230 mesh).

***N*⁴-Benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (3).** A suspension of dimethoxytrityl chloride (35.5 g, 105 mmol) and *N*⁴-benzoyl-2′-deoxycytidine (22.7 g, 68.5 mmol) in pyridine (140 mL) was stirred for 1 h at room temperature. MeOH (2 mL) was added to the mixture, and the mixture was concentrated in vacuo to give a residue which was partitioned

between EtOAc (400 mL) and H_2O (400 mL). The separated organic layer was dried (Na_2SO_4) and concentrated to give crude **1**. A mixture of the crude **1**, imidazole (11.2 g, 164 mmol), and TBSCl (12.4 g, 82.2 mmol) in DMF (140 mL) was stirred for 15 h at room temperature, quenched with EtOH (15 mL), and evaporated to dryness. EtOAc (500 mL) was added to the mixture, which was washed with H_2O (4 × 300 mL) and brine (200 mL). The organic layer was dried (Na_2SO_4) and evaporated. A mixture of the crude **2** in CH_2Cl_2 (500 mL) containing TFA (15.8 mL) was stirred for 2 h at room temperature, additional TFA (10.5 mL) was added, and the mixture was stirred for a further 1 h. The mixture was cooled to 0 °C in an ice bath and neutralized with 5 M NaOH. The separated organic layer was washed with saturated $NaHCO_3$ (2 × 200 mL), dried (Na_2SO_4), and concentrated. The residue was purified on a silica gel column with 0−5% MeOH in $CHCl_3$ to give the crude product **3**, which was crystallized from Et_2O−H_2O to give **3** (16.1 g, 51% as a white powder): mp 93−95 °C; FAB-MS *m*/*z* 446 (MH^+). Anal. ($C_{22}H_{31}N_3O_5Si\cdot H_2O$) C, H, N.

***N*⁴-Benzoyl-1-[3-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-4α-hydroxymethyl-β-D-*ribo*-pentofuranosyl]cytosine (5).** A solution of **3** (13.9 g, 30.0 mmol) in EtOAc (100 mL) was dried (Na_2SO_4) and concentrated to dryness. A mixture of the above residue and EDC hydrochloride (17.3 g, 90.2 mmol) was dissolved in a mixture of benzene (100 mL) and DMSO (15 mL), and the mixture was stirred for 30 min at room temperature. The mixture was diluted with EtOAc (300 mL) and washed with H_2O (3 × 300 mL) and brine (300 mL). The separated organic layer was dried (Na_2SO_4) and evaporated to dryness. Hexane (300 mL) was added to a solution of the residue in EtOAc (120 mL). The resulting precipitate was collected by filtration and air-dried to give a crude **4**, which was dissolved in a mixture of aqueous 37% HCHO (5.5 mL) and 1,4-dioxane (70 mL) containing 2 M NaOH solution (24 mL). The mixture was stirred for 10 min at room temperature and neutralized with AcOH (4.8 mL). The mixture was evaporated to dryness, and the residue was partitioned between H_2O and $CHCl_3$. The organic layer was dried (Na_2SO_4) and evaporated to dryness. $NaBH_4$ (604 mg, 16.0 mmol) was added to a suspension of the residue in EtOH (240 mL) at 0 °C. After being stirred for 20 min at the same temperature, the mixture was quenched with AcOH (2.3 mL) and evaporated to dryness. The residue was partitioned between H_2O and $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$, dried (Na_2SO_4), and evaporated to dryness. The residue was purified on a silica gel column with 50−100% EtOAc in $CHCl_3$ to give **5** (6.20 g, 44% as a white foam): FAB-MS *m*/*z* 476 (MH^+). Anal. ($C_{23}H_{33}N_3O_6Si$) C, H, N.

***N*⁴-Benzoyl-1-[3-*O*-(*tert*-butyldimethylsilyl)-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-4α-hydroxymethyl-β-D-*ribo*-pentofuranosyl]cytosine (7).** A mixture of **5** (2.59 g, 5.45 mmol) and dimethoxytrityl chloride (2.30 g, 6.81 mmol) in pyridine (11 mL) was stirred for 50 min at room temperature and quenched with H_2O (80 mL). The mixture was diluted with EtOAc (100 mL) and washed with H_2O (100 mL) and brine (100 mL). The organic layer was dried (Na_2SO_4) and evaporated to dryness. The residue was purified on a silica gel column with 33% EtOAc in $CHCl_3$ to give crude **6** as a yellow foam. A solution of the crude **6** (2.77 g), imidazole (945 mg, 13.9 mmol), and TBDPSCl (1.2 mL, 4.6 mmol) in DMF (7.5 mL) was stirred for 1.5 h at room temperature and quenched with EtOH (0.4 mL). $CHCl_3$ (140 mL) was added to the mixture, which was washed with H_2O (3 × 100 mL). The organic layer was dried (Na_2SO_4) and evaporated to dryness. The residue in a mixture of THF (6 mL) and aqueous 80% AcOH (22 mL) was stirred for 12 h at room temperature. After addition of 28% NH_4OH (16 mL), the mixture was extracted with $CHCl_3$ (80 mL). The extract was washed with saturated $NaHCO_3$, dried (Na_2SO_4), and evaporated. The residue was purified on a silica gel column with 33−67% EtOAc in $CHCl_3$ to give a solid, which was crystallized from EtOAc−hexane to give **7** (1.86 g, 48% as fine colorless needles): mp 157−157.5 °C; FAB-MS *m*/*z* 714 (MH^+). Anal. ($C_{39}H_{51}N_3O_6Si_2$) C, H, N.

***N*⁴-Benzoyl-1-[3-*O*-(*tert*-butyldimethylsilyl)-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-4α-formyl-β-D-*ribo*-pentofuranosyl]cytosine (8).** Dimethyl sulfoxide (0.35 mL, 5.0 mmol) was added to a solution of trichloroacetic anhydride (0.66 mL, 3.6 mmol) in CH_2Cl_2 (6 mL) at −78 °C under an Ar atmosphere. The mixture was stirred for 20 min at the same temperature, and a solution of **7** (1.79 g, 2.50 mmol) in CH_2Cl_2 (6 mL) was added to the mixture, which was stirred for a further 30 min at the same temperature. After addition of Et_3N (1.67 mL, 12.0 mmol), the reaction mixture was allowed to warm to room temperature and stirred for 1 h. Water (30 mL) was added to the mixture, and the separated organic phase was washed with H_2O (30 mL), dried (Na_2SO_4), and evaporated. The residue was purified on a silica gel column with 33% EtOAc in $CHCl_3$ to give a solid, which was crystallized from EtOAc−hexane to give **8** (1.45 g, 82% as fine colorless needles): mp 174−174.5 °C; FAB-MS *m*/*z* 712 (MH^+). Anal. ($C_{39}H_{49}N_3O_6Si_2$) C, H, N.

***N*⁴-Benzoyl-1-[3-*O*-(*tert*-butyldimethylsilyl)-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-4α-ethenyl-β-D-*ribo*-pentofuranosyl]cytosine (9).** A hexane solution of BuLi (1.66 M, 2.89 mL, 4.80 mmol) was added to a suspension of methyltriphenylphosphonium bromide (1.72 g, 4.80 mmol) in THF (12 mL) at −78 °C under an Ar atmosphere. The mixture was stirred for 30 min at 0 °C, followed by addition of a solution of **8** (854 mg, 1.20 mmol) in THF (10 mL). The reaction mixture was stirred for 1 h at room temperature. A saturated NH_4Cl (20 mL) was added to the mixture, which was extracted with EtOAc (100 mL). The separated organic layer was washed with brine (100 mL), dried (Na_2SO_4), and concentrated in vacuo. EtOAc and hexane (each 20 mL) were added to the residue, and the resulting precipitate was filtered. The crude product was purified on a silica gel column with 6% MeOH in $CHCl_3$ to give a solid, which was crystallized from EtOAc−hexane to give **9** (782 mg, 92% as fine colorless needles): mp 181.5−182.5 °C; FAB-MS *m*/*z* 710 (MH^+). Anal. ($C_{40}H_{51}N_3O_5Si_2$) C, H, N.

***N*⁴-Benzoyl-1-[3-*O*-(*tert*-butyldimethylsilyl)-5-*O*-(*tert*-butyldiphenylsilyl)-4α-(2-chloroethenyl)-2-deoxy-β-D-*ribo*-pentofuranosyl]cytosine (10).** A hexane solution of BuLi (1.66 M, 1.21 mL, 2.0 mmol) was added to a suspension of chloromethyltriphenylphosphonium chloride (694 mg, 2.0 mmol) in THF (4 mL) at −78 °C under an Ar atmosphere. The mixture was stirred for 50 min at −78 °C, and a solution of **8** (356 mg, 0.50 mmol) in THF (3 mL) was added to the mixture. The mixture was warmed to 0 °C and stirred for 2.3 h. Saturated aqueous NH_4Cl (20 mL) was added to the mixture, which was extracted with EtOAc (25 mL). The separated organic layer was washed with brine (25 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified on a silica gel column with 50% EtOAc in hexane to give **10** (343 mg, 92% as a white foam): FAB-MS *m*/*z* 744 (MH^+). Anal. ($C_{40}H_{50}ClN_3O_5Si_2$) C, H, N.

1-[3-*O*-(*tert*-Butyldimethylsilyl)-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-4α-ethynyl-β-D-*ribo*-pentofuranosyl]cytosine (11). A hexane solution of BuLi (1.66 M, 2.89 mL, 4.8 mmol) was added to a solution of **10** (298 mg, 0.40 mmol) in THF (11 mL) at −78 °C under an Ar atmosphere. The mixture was stirred for 2 h at −78 °C, and a saturated aqueous NH_4Cl (20 mL) was added to the mixture, which was extracted with EtOAc (20 mL). The extract was washed with brine (20 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified on a silica gel column with 7−10% MeOH in $CHCl_3$ to give a crude product, which was purified on a silica gel column with EtOAc and 10% MeOH in $CHCl_3$ to give **11** (174 mg, 72% as a white foam): FAB-MS *m*/*z* 604 (MH^+). Anal. ($C_{33}H_{45}N_3O_4Si_2\cdot{}^1/_2H_2O$) C, H, N.

***N*⁴-Benzoyl-1-[3-*O*-(*tert*-butyldimethylsilyl)-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-4α-hydroxyiminomethyl-β-D-*ribo*-pentofuranosyl]cytosine (12).** A mixture of **8** (534 mg, 0.750 mmol) and $HONH_2\cdot HCl$ (104 mg, 1.50 mmol) in pyridine (6.0 mL) was stirred for 5 min at 50 °C and cooled to room temperature. The mixture was diluted with EtOAc (100 mL) and washed with H_2O (2 × 100 mL). The separated organic layer was dried (Na_2SO_4) and concentrated in vacuo,

and the crude product was crystallized from Et_2O to give **12** (495 mg, 91%, as a white powder): mp 207−207.5 °C; FAB-MS *m/z* 727 (MH^+). Anal. ($C_{39}H_{50}N_4O_6Si_2$) C, H, N.

1-[3-*O*-(*tert*-Butyldimethylsilyl)-5-*O*-(*tert*-butyldiphenylsilyl)-4α-cyano-2-deoxy-β-D-*ribo*-pentofuranosyl]cytosine (13). A suspension of **12** (451 mg, 0.62 mmol) and NaOAc (305 mg, 3.72 mmol) in Ac_2O (5.5 mL) was stirred for 2.5 h at 130 °C. The reaction mixture was cooled to room temperature, and saturated aqueous $NaHCO_3$ (100 mL) was added to the mixture, which was stirred for 30 min at room temperature. The mixture was extracted with EtOAc (100 mL), which was washed with saturated $NaHCO_3$ (2 × 100 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. A mixture of the above residue in MeOH (30 mL) containing DBU (0.19 mL, 1.24 mmol) was stirred for 45 min at room temperature, and silica gel was added to the mixture, which was evaporated to dryness. The residue was placed on top of a silica gel column, which was eluted with 7% MeOH in $CHCl_3$ to give a solid, which was crystallized from EtOH to give **13** (263 mg, 70% as a white powder): mp 258.5−260 °C; FAB-MS *m/z* 605 (MH^+). Anal. ($C_{32}H_{44}N_4O_4Si_2$) C, H, N.

***N*⁴-Benzoyl-1-[3-*O*-(*tert*-butyldimethylsilyl)-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-4α-iodomethyl-β-D-*ribo*-pentofuranosyl]cytosine (14).** A mixture of **7** (714 mg, 1 mmol), Ph_3P (1.05 g, 4 mmol), I_2 (508 mg, 2 mmol), and imidazole (272 mg, 4 mmol) in benzene (10 mL) was stirred for 30 h at 80 °C under an Ar atmosphere. The mixture was cooled to room temperature and diluted with EtOAc (50 mL), which was washed with saturated aqueous sodium thiosulfate (2 × 50 mL) and H_2O (50 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified on a silica gel column with 50−60% EtOAc in hexane to give a crude product, which was crystallized from EtOAc−hexane to give **14** (580 mg, 70% as fine colorless needles): mp 180−181.5 °C; FAB-MS *m/z* 824 (MH^+). Anal. ($C_{39}H_{50}IN_3O_5Si_2$) C, H, N.

***N*⁴-Benzoyl-1-[3-*O*-(*tert*-butyldimethylsilyl)-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-4α-methyl-β-D-*ribo*-pentofuranosyl]cytosine (15).** A mixture of **14** (453 mg, 0.55 mmol), 10% Pd/C (150 mg), and Et_3N (0.12 mL) in EtOH (9 mL) and EtOAc (9 mL) was stirred for 2 h at room temperature under a hydrogen atmosphere. The reaction mixture was filtered through a Celite pad, and the filtrate was evaporated. Water and $CHCl_3$ were added to the residue, and the separated organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified on a silica gel column with 11−17% EtOAc in $CHCl_3$ to give a solid, which was crystallized from EtOAc−hexane to give **15** (296 mg, 77% as colorless needles): mp 160−160.5 °C; FAB-MS *m/z* 698 (MH^+). Anal. ($C_{39}H_{51}N_3O_5Si_2$) C, H, N.

General Method for Deprotection of the *N*⁴-Benzoyl Group. A mixture of the *N*⁴-benzoyl derivative and DBU (1.5 equiv) in MeOH was stirred for 30 min at room temperature. Silica gel was added to the mixture, which was evaporated to dryness. The residue was placed on top of a silica gel column, which was eluted with MeOH in $CHCl_3$ to give the product.

1-[3-*O*-(*tert*-Butyldimethylsilyl)-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-4α-ethenyl-β-D-*ribo*-pentofuranosyl]cytosine (16). From **9** (639 mg, 0.90 mmol), **16** (486 mg, 89%) was obtained as fine colorless needles: mp 108−109.5 °C ($CHCl_3$−hexane); FAB-MS *m/z* 606 (MH^+). Anal. ($C_{33}H_{47}N_3O_4Si_2 \cdot {}^1/_2H_2O$) C, H, N.

1-[3-*O*-(*tert*-Butyldimethylsilyl)-5-*O*-(*tert*-butyldiphenylsilyl)-4α-(2-chloroethenyl)-2-deoxy-β-D-*ribo*-pentofuranosyl]cytosine (17). From **10** (313 mg, 0.42 mmol), **17** (229 mg, 85%) was obtained as colorless needles: mp 173−174 °C (EtOAc−hexane); FAB-MS *m/z* 640 (MH^+). Anal. ($C_{33}H_{46}ClN_3O_4Si_2$) C, H, N.

1-[3-*O*-(*tert*-Butyldimethylsilyl)-5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-4α-methyl-β-D-*ribo*-pentofuranosyl]cytosine (18). From **15** (244 mg, 0.350 mmol), **18** (203 mg, 98%) was obtained as a white foam: FAB-MS *m/z* 594 (MH^+). Anal. ($C_{32}H_{47}N_3O_4Si_2 \cdot {}^1/_3H_2O$) C, H, N.

General Method for Deprotection of the Silyl Groups. A mixture of the silyl-protected nucleoside and NH_4F (20 equiv) in MeOH was heated under reflux. The reaction mixture was cooled to room temperature, and silica gel was added to the mixture, which was evaporated to dryness. The residue was placed on top of a silica gel column, which was eluted with MeOH in $CHCl_3$.

1-(2-Deoxy-4α-ethenyl-β-D-*ribo*-pentofuranosyl)cytosine (19). From **16** (394 mg, 0.64 mmol), **19** (150 mg, 91%) was obtained as a white foam. An analytical sample was obtained as an HCl salt: mp 184−186 °C dec; ^{1}H NMR (DMSO-d_6) 7.82 (d, 1 H, H-6, $J_{6,5}$ = 7.5 Hz), 7.11, 7.04 (each br s, each 1 H, 4-NH_2), 6.04 (dd, 1 H, H-1′, $J_{1'-2'a}$ = 6.8, $J_{1'-2'b}$ = 4.4 Hz), 5.90 (dd, 1 H, 4′-CH_a=CH_bH$_c$, $J_{Ha,Hb}$ = 17.3, $J_{Ha,Hc}$ = 10.8 Hz), 5.69 (d, 1 H, H-5, $J_{5,6}$ = 7.5 Hz), 5.32 (dd, 1 H, 4′-CH_a=CH_bH$_c$, $J_{Hb,Ha}$ = 17.3, $J_{Hb,Hc}$ =2.1 Hz), 5.16−5.20 (m, 3 H, 4′-CH_a=CH_bH$_c$, 3′, 5′-OH), 4.37 (td, 1 H, H-3′, $J_{3',2'}$ = 7.0, $J_{3',OH}$ = 5.3 Hz), 3.52 (dd, 1 H, H-5′a, $J_{5'a,5'b}$ = 11.7, $J_{5'a,OH}$ = 5.6 Hz), 3.36 (d, 1 H, H-5′b, $J_{5'b,5'a}$ = 11.7, $J_{5'b,OH}$ = 5.6 Hz), 2.07 (ddd, 1 H, H-2′a, $J_{2'a,1'}$ = 6.8, $J_{2'a,2'b}$ = 12.9, $J_{2'a,3'}$ = 7.0 Hz), 1.99 (ddd, 1 H, H-2′b, $J_{2'b,1'}$ = 4.4, $J_{2'b,2'a}$ = 12.9, $J_{2'b,3'}$ = 7.0 Hz); ^{13}C NMR (DMSO-d_6) 165.43, 154.92, 141.08, 136.55, 114.70, 93.48, 89.01, 83.11, 69.15, 63.44, 39.63; FAB-MS *m/z* 254 (MH^+). Anal. ($C_{11}H_{16}ClN_3O_4$) C, H, N.

1-[4α-[2(*Z*)-Chloroethenyl]-2-deoxy-β-D-*ribo*-pentofuranosyl]cytosine (20). From **17** (192 mg, 0.3 mmol), **20** (47 mg, 54%) was obtained as colorless needles: mp 205−206 °C (EtOAc−hexane); ^{1}H NMR (DMSO-d_6) 7.93 (d, 1 H, H-6, $J_{6,5}$ = 7.4 Hz), 7.11, 7.05 (each br s, each 1 H, 4-NH_2), 6.41 (d, 1H, 4′-CH_a=CH_bCl, $J_{Hb,Ha}$ = 8.0 Hz), 6.11 (t, 1 H, H-1′, $J_{1'-2'}$ = 5.9 Hz), 5.96 (d, 1 H, 4′-CH_a=CH_bCl, $J_{Ha,Hb}$ = 8.0 Hz), 5.70 (d, 1 H, H-5 $J_{5,6}$ = 7.4 Hz), 5.36 (d, 1 H, 3′-OH, $J_{OH,3'}$ = 4.7 Hz), 5.29 (d, 1 H, 5′-OH, $J_{OH,5'}$ = 5.6 Hz), 4.39 (td, 1 H, H-3′, $J_{3',2'}$ = 6.2, $J_{3',OH}$ = 4.7 Hz), 3.64 (dd, 1 H, H-5′a, $J_{5'a,5'b}$ = 12.0, $J_{5'a,OH}$ = 5.6 Hz), 3.58 (dd, 1 H, H-5′b, $J_{5'b,5'a}$ = 12.0, $J_{5'b,OH}$ = 5.6 Hz), 2.09 (ddd, 1 H, H-2′a, $J_{2'a,1'}$ = 5.9, $J_{2'a,2'b}$ = 13.2, $J_{2'a,3'}$ = 6.2 Hz), 2.05 (ddd, 1 H, H-2′b, $J_{2'b,1'}$ = 5.9, $J_{2'b,2'a}$ = 13.2, $J_{2'b,3'}$ = 6.2 Hz); ^{13}C NMR (DMSO-d_6) 165.37, 154.89, 140.94, 129.08, 119.76, 93.63, 88.91, 83.64, 69.86, 62.15; FAB-MS *m/z* 288, 290 (MH^+). Anal. ($C_{11}H_{14}ClN_3O_4$) C, H, N.

1-(2-Deoxy-4α-ethynyl-β-D-*ribo*-pentofuranosyl)cytosine (21). From **11** (167 mg, 0.276 mmol), **21** (49 mg, 67%) was obtained as a white powder: mp 219.5−221 °C (EtOH); ^{1}H NMR (DMSO-d_6) 7.76 (d, 1 H, H-6, $J_{6,5}$ = 7.8 Hz), 7.17, 7.10 (each br s, each 1 H, 4-NH_2), 6.12 (dd, 1 H, H-1′, $J_{1'-2'a}$ = 7.2, $J_{1'-2'b}$ = 4.7 Hz), 5.70 (d, 1 H, H-5, $J_{5,6}$ = 7.8 Hz), 5.45 (d, 1 H, 3′-OH, $J_{OH,3'}$ = 5.4 Hz), 5.38 (t, 1 H, 5′-OH, $J_{OH,5'}$ = 5.9 Hz), 4.29 (td, 1 H, H-3′, $J_{3',2'}$ = 7.3, $J_{3',OH}$ = 5.4 Hz), 3.63 (dd, 1 H, H-5′a, $J_{5'a,5'b}$ = 12.1, $J_{5'a,OH}$ = 5.9 Hz), 3.56 (dd, 1 H, H-5′b, $J_{5'b,5'a}$ = 12.1, $J_{5'b,OH}$ = 5.9 Hz), 3.47 (s, 1 H, 4′-ethynyl), 2.24 (ddd, 1 H, H-2′a, $J_{2'a,1'}$ = 7.2, $J_{2'a,2'b}$ = 13.2, $J_{2'a,3'}$ = 7.3 Hz), 2.05 (ddd, 1 H, H-2′b, $J_{2'b,1'}$ = 4.7, $J_{2'b,2'a}$ = 13.2, $J_{2'b,3'}$ = 7.3 Hz); ^{13}C NMR (DMSO-d_6) 165.50, 154.90, 141.03, 93.98, 84.35, 83.27, 81.33, 78.58, 69.29, 63.72, 39.03; FAB-MS *m/z* 252 (MH^+). Anal. ($C_{11}H_{13}N_3O_4 \cdot {}^2/_3H_2O$) C, H, N.

1-(4α-Cyano-2-deoxy-β-D-*ribo*-pentofuranosyl)cytosine (22). From **13** (212 mg, 0.350 mmol), **22** (56.1 mg, 62% as a white powder) was obtained: mp 230−231 °C (MeOH) dec; ^{1}H NMR (DMSO-d_6) 7.60 (d, 1 H, H-6, $J_{6,5}$ = 7.3 Hz), 7.25, 7.22 (each br s, each 1 H, 4-NH_2), 6.32 (t, 1 H, H-1′, $J_{1'-2'}$ = 6.8 Hz), 6.19 (d, 1 H, 3′-OH, $J_{OH,3'}$ = 5.1 Hz), 5.74 (d, 1 H, 5′-OH, $J_{OH,5'}$ = 6.1 Hz), 5.72 (d, 1 H, H-5, $J_{5,6}$ = 7.3 Hz), 4.41 (ddd, 1 H, H-3′, $J_{3',2'a}$ = 6.5, $J_{3',2'b}$ = 5.4, $J_{3',OH}$ = 5.1 Hz), 3.72 (dd, 1 H, H-5′a, $J_{5'a,5'b}$ = 11.6, $J_{5'a,OH}$ = 6.1 Hz), 3.66 (dd, 1 H, H-5′b, $J_{5'b,5'a}$ = 11.6, $J_{5'b,OH}$ = 6.1 Hz), 2.25 (ddd, 1 H, H-2′a, $J_{2'a,1'}$ = 6.8, $J_{2'a,2'b}$ = 13.7, $J_{2'a,3'}$ = 6.5 Hz), 2.05 (ddd, 1 H, H-2′b, $J_{2'b,1'}$ = 6.8, $J_{2'b,2'a}$ = 13.7, $J_{2'b,3'}$ = 5.4 Hz); ^{13}C NMR (DMSO-d_6) 165.58, 155.02, 141.53, 117.98, 95.00, 86.17, 85.56, 71.02, 63.34, 37.86; FAB-MS *m/z* 253 (MH^+). Anal. ($C_{10}H_{12}N_4O_4 \cdot {}^1/_4H_2O$) C, H, N.

1-(2-Deoxy-4α-methyl-β-D-*ribo*-pentofuranosyl)cytosine (23). From **18** (160 mg, 0.270 mmol), **23** (38 mg, 58%) was obtained as a white powder: mp 200−201 °C (EtOH); ^{1}H NMR (DMSO-d_6) 7.81 (d, 1 H, H-6, $J_{6,5}$ = 7.4 Hz), 7.02, 6.95 (each br s, each 1 H, 4-NH_2), 6.00 (t, 1 H, H-1′, $J_{1'-2'}$ = 6.3 Hz), 5.62 (d, 1 H, H-5, $J_{5,6}$ = 7.4 Hz), 5.02 (d, 1 H, 3′-OH, $J_{OH,3'}$

= 5.0 Hz), 4.97 (d, 1 H, 5′-OH, $J_{OH,5'}$ = 5.5 Hz), 4.10 (ddd, 1 H, H-3′, $J_{3',2'a}$ = 4.9, $J_{3',2'b}$ = 6.3, $J_{3',OH}$ = 5.0 Hz), 3.33 (dd, 1 H, H-5′a, $J_{5'a,5'b}$ = 11.7, $J_{5'a,OH}$ = 5.5 Hz), 3.31 (dd, 1 H, H-5′b, $J_{5'b,5'a}$ = 11.7, $J_{5'b,OH}$ = 5.5 Hz), 2.12 (ddd, 1 H, H-2′a, $J_{2'a,1'}$ = 6.3, $J_{2'a,2'b}$ = 13.2, $J_{2'a,3'}$ = 4.9 Hz), 2.05 (ddd, 1 H, H-2′b, $J_{2'b,1'}$ = 6.3, $J_{2'b,2'a}$ = 13.2, $J_{2'b,3'}$ = 6.3 Hz), 0.99 (s, 3 H, 4′-CH_3); ^{13}C NMR (DMSO-d_6) 165.34, 154.92, 141.08, 93.53, 87.24, 83.63, 70.41, 65.90, 40.51,17.79; FAB-MS *m/z* 242 (MH^+). Anal. ($C_{10}H_{15}N_3O_4 \cdot {}^1/_3H_2O$) C, H, N.

1-(2-Deoxy-4α-ethyl-β-D-*ribo*-pentofuranosyl)cytosine (24). A mixture of **19** (53 mg, 0.21 mmol) and 10% Pd/C (30 mg) in MeOH (3 mL) was stirred for 30 min at room temperature under a hydrogen atmosphere. The mixture was filtered through a Celite pad, and the filtrate was evaporated. The residue was purified on a silica gel column with 20−25% MeOH in $CHCl_3$ to give **24** (50 mg, 93% as a white foam). An analytical sample was obtained as an HCl salt: mp 171−173 °C dec; ^{1}H NMR (DMSO-d_6) 7.84 (d, 1 H, H-6, $J_{6,5}$ = 7.4 Hz), 7.10, 7.03 (each br s, each 1 H, 4-NH_2), 6.07 (t, 1 H, H-1′, $J_{1'-2'}$ = 6.4 Hz), 5.68 (d, 1 H, H-5, $J_{5,6}$ = 7.4 Hz), 5.04 (d, 1 H, 3′-OH, $J_{OH,3'}$ = 4.9 Hz), 4.95 (d, 1 H, 5′-OH, $J_{OH,5'}$ = 5.0 Hz), 4.21 (ddd, 1 H, H-3′, $J_{3',2'a}$ = 3.9, $J_{3',2'b}$ = 6.4, $J_{3',OH}$ = 4.9 Hz), 3.37−3,45 (m, 2 H, H-5′), 2.14 (ddd, 1 H, H-2′a, $J_{2'a,1'}$ = 6.4, $J_{2'a,2'b}$ = 13.8, $J_{2'a,3'}$ = 3.9 Hz), 2.04 (ddd, 1 H, H-2′b, $J_{2'b,1'}$ = 6.4, $J_{2'b,2'a}$ = 13.8, $J_{2'b,3'}$ = 6.4 Hz), 1.59 (dq,1 H, *CHa*CH_3, $J_{6'a,6'b}$ = 13.0, $J_{6'a,7'}$ = 7.5 Hz), 1.49 (dq,1 H, *CHb*CH_3, $J_{6'b,6'a}$ = 13.0, $J_{6'b,7'}$ = 7.5 Hz), 0.84 (t, 3 H, CH_2*CH*$_3$, $J_{7',6'}$ = 7.5 Hz); ^{13}C NMR (DMSO-d_6) 159.18, 146.98, 144.36, 93.32, 90.46, 84.96, 70.25, 62.73, 40.61, 23.61, 8.29; FAB-MS *m/z* 256 (MH^+). Anal. ($C_{11}H_{18}ClN_3O_4$) C, H, N.

2′,3′-Bis-*O*-(*tert*-butyldimethylsilyl)uridine (26). A solution of uridine (48.8 g, 200 mmol), imidazole (136 g, 660 mmol), and TBSCl (100 g, 660 mmol) in DMF (150 mL) was stirred for 7 h at room temperature. The reaction was quenched with EtOH (30 mL) and then diluted with EtOAc (1 L). The mixture was washed with H_2O (8 × 800 mL), dried (Na_2SO_4), and concentrated in vacuo to give **25**. The crude **25** was suspended in a mixture of aqueous 80% AcOH (960 mL) and THF (80 mL). TFA (40 mL) was added to the mixture, and the whole was stirred for 1 h at room temperature and for 1.5 h at 0 °C. The resulting precipitate was collected by filtration and washed with aqueous 80% AcOH. NH_4OH (28%, 80 mL) was added to the filtrate, and the resulting precipitate was collected by filtration and washed with aqueous 80% AcOH. The combined precipitates were dissolved in $CHCl_3$ (1 L), which was washed with saturated $NaHCO_3$ (4 × 800 mL). The organic layer was dried (Na_2SO_4) and evaporated to give a crude product, which was crystallized from Et_2O to give **26** (55.8 g, 59% as a white powder): mp 226−227 °C; FAB-MS *m/z* 473 (MH^+). Anal. ($C_{21}H_{40}N_2O_6Si_2$) C, H, N.

1-[2,3-Bis-*O*-(*tert*-butyldimethylsilyl)-4α-hydroxymethyl-β-D-*ribo*-pentofuranosyl]uracil (28). EDC hydrochloride (40.3 g, 210 mmol) was added to a solution of **26** (33.1 g, 70 mmol) in a mixture of pyridine (7.4 mL, 91 mmol), TFA (3.5 mL, 45 mmol), DMSO (37 mL, 11 mmol), and benzene (250 mL). The mixture was stirred for 25 min at room temperature, diluted with EtOAc (250 mL), and washed with brine (4 × 200 mL) and H_2O (200 mL). The separated organic layer was dried (Na_2SO_4) and evaporated. The residue was purified on a silica gel column with 25−33% EtOAc in $CHCl_3$ to give **27** (24.6 g as a foam). An aqueous solution of HCHO (37%, 11 mL, 150 mmol) and 2 M NaOH (50 mL) was added to a solution of **27** in 1,4-dioxane (150 mL). The mixture was stirred for 50 min at room temperature, AcOH (10 mL) was added to the mixture, and the whole was evaporated to dryness. The residue was suspended in H_2O (500 mL), which was successively extracted with $CHCl_3$ (2 × 500 mL), and the combined organic layer was dried (Na_2SO_4) and evaporated. The residue was dissolved in EtOH (430 mL) and cooled to 0 °C. After addition of $NaBH_4$ (81.6 g, 45.0 mmol), the reaction mixture was stirred for 30 min at the same temperature and quenched with AcOH (5.1 mL). The mixture was evaporated, and the residue was suspended in $CHCl_3$ (1 L), which was washed with H_2O (800 mL) and saturated aqueous $NaHCO_3$ (800 mL). The organic layer was dried (Na_2SO_4) and evaporated to give a crude product, which was purified on a silica gel column with 33−67% EtOAc in $CHCl_3$ to give **28** (13.6 g, 39% as a white powder): mp 188−189.5 °C; FAB-MS *m/z* 503 (MH^+). Anal. ($C_{22}H_{42}N_2O_7Si_2 \cdot {}^1/_2H_2O$) C, H, N.

1-[2,3-Bis-*O*-(*tert*-butyldimethylsilyl)-4α-[(4,4′-dimethoxytrityl)oxymethyl]-β-D-*ribo*-pentofuranosyl]uracil (29). A mixture of **28** (2.26 g, 4.50 mmol) and dimethoxytrityl chloride (1.98 g, 5.85 mmol) in pyridine (10 mL) was stirred for 1.5 h at room temperature. The mixture was diluted with EtOAc (70 mL) and washed with H_2O (4 × 100 mL). The organic layer was dried (Na_2SO_4) and evaporated. The residue was purified on a silica gel column with 40−60% EtOAc in $CHCl_3$ to give a crude product, which was crystallized from EtOAc−hexane to give **29** (2.31 g, 64% as a white powder): mp 213-213.5 °C; FAB-MS *m/z* 804 (M^+). Anal. ($C_{43}H_{60}N_2O_9Si_2$) C, H, N.

1-[2,3,5-Tris-*O*-(*tert*-butyldimethylsilyl)-4α-hydroxymethyl-β-D-*ribo*-pentofuranosyl]uracil (31). A mixture of **29** (2.00 g, 2.48 mmol), TBSCl (561 mg, 3.72 mmol), and imidazole (760 mg, 11.2 mmol) in DMF (7 mL) was stirred for 13.5 h at room temperature. The reaction was quenched with EtOH (1 mL), and the mixture was diluted with EtOAc and washed with H_2O (5 × 100 mL). The organic layer was dried (Na_2SO_4) and evaporated to give **30**. The crude **30** was dissolved in a mixture of THF (10 mL) and aqueous 80% AcOH (35 mL). The mixture was stirred for 5 h at room temperature and then cooled to 0 °C. After addition of 28% NH_4OH (25 mL) to the mixture, the whole was extracted with EtOAc (80 mL), which was successively washed with H_2O (80 mL) and saturated aqueous $NaHCO_3$ (80 mL). The separated organic layer was dried (Na_2SO_4) and evaporated. The residue was purified on a silica gel column with 20% EtOAc in $CHCl_3$ to give **31** (1.32 g, 86% as a white foam): FAB-MS *m/z* 617 (MH^+). Anal. ($C_{28}H_{56}N_2O_7Si_3$) C, H, N.

1-[2,3,5-Tris-*O*-(*tert*-butyldimethylsilyl)-4α-formyl-β-D-*ribo*-pentofuranosyl]uracil (32). Compound **31** (1.23 g, 2.00 mmol) was stirred for 30 min in a mixture of DMSO (0.28 mL, 4.0 mmol) and trichloroacetic anhydride (0.53 mL, 2.90 mmol) in CH_2Cl_2 (5 mL) at −78 °C, followed by treatment with Et_3N (1.34 mL, 9.60 mmol). After workup as described for the synthesis of **8**, **32** (1.04 g, 85%) was obtained as a white foam: FAB-MS *m/z* 615 (MH^+). Anal. ($C_{28}H_{54}N_2O_7Si_3$) C, H, N.

1-[2,3,5-Tris-*O*-(*tert*-butyldimethylsilyl)-4α-[2(*Z*)-chloroethenyl]-β-D-*ribo*-pentofuranosyl]uracil (33). Compound **32** (71.4 mg, 0.116 mmol) was treated with Ph_3P=CHCl [prepared from BuLi (1.58 M, 0.294 mL, 0.464 mmol) and chloromethyltriphenylphosphonium chloride (161 mg, 0.464 mmol) in THF (1 mL) at −78 °C] for 45 min at 0 °C. After workup as described for the synthesis of **10**, **33** (60.2 mg, 80%) was obtained as a white foam: FAB-MS *m/z* 647 (MH^+). Anal. ($C_{29}H_{55}ClN_2O_6Si_3$) C, H, N.

1-[2,3,5-Tris-*O*-(*tert*-butyldimethylsilyl)-4α-ethynyl-β-D-*ribo*-pentofuranosyl]uracil (34). Compound **33** (477 mg, 0.737 mmol) in THF (20 mL) was treated with BuLi (1.58 M, 4.93 mL, 7.80 mmol) for 1 h at −78 °C. After workup as described for **11**, **34** (371 mg, 82%) was obtained as a white foam: FAB-MS *m/z* 611 (MH^+). Anal. ($C_{29}H_{54}N_2O_6Si_3$) C, H, N.

1-[2,3,5-Tris-*O*-(*tert*-butyldimethylsilyl)-4α-hydroxyiminomethyl-β-D-*ribo*-pentofuranosyl]uracil (35). A mixture of **32** (923 mg, 1.50 mmol) and $HONH_2 \cdot HCl$ (208 mg, 3.00 mmol) in pyridine (12 mL) was stirred for 40 min at room temperature. After workup as described for the synthesis of **12**, **35** (938 mg, 99%) was obtained as a white foam: FAB-MS *m/z* 630 (MH^+). Anal. ($C_{28}H_{55}N_3O_7Si_3$) C, H, N.

1-[2,3,5-Tris-*O*-(*tert*-butyldimethylsilyl)-4α-cyano-β-D-*ribo*-pentofuranosyl]uracil (36). A mixture of **35** (882 mg, 1.40 mmol) and NaOAc (689 mg, 8.40 mmol) in Ac_2O (12.5 mL) was stirred for 2.5 h at 130 °C. After workup described for the synthesis of **13**, **36** (724 mg, 85%) was obtained as colorless needles: mp 170.5−171 °C (EtOAc−hexane); FAB-MS *m/z* 612 (MH^+). Anal. ($C_{28}H_{53}N_3O_6Si_3$) C, H, N.

General Method for Conversion of the Uracil Moiety to the Cytosine Moiety. TPSCl (2 equiv) was added to a

mixture of **34** (0.58 mmol) or **36** (0.66 mmol), DMAP (2 equiv), and Et_3N (2 equiv) in CH_3CN (4 mL). The mixture was stirred for 1.5 h at room temperature, and a mixture of 28% NH_4OH (2 mL) and CH_3CN (2 mL) was added to the mixture. After being stirred for 30 min, the mixture was diluted with $CHCl_3$ (100 mL) and washed with H_2O (100 mL), 0.1 M HCl (100 mL), and saturated aqueous $NaHCO_3$ (100 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified on a silica gel column.

1-[2,3,5-Tris-*O*-(*tert*-butyldimethylsilyl)-4α-ethynyl-β-D-*ribo*-pentofuranosyl]cytosine (37). Compound **37** (140 mg, 69%) was obtained as a white foam: FAB-MS *m*/*z* 610 (MH^+). Anal. ($C_{29}H_{55}N_3O_5Si_3 \cdot {}^1/_4H_2O$) C, H, N.

1-[2,3,5-Tris-*O*-(*tert*-butyldimethylsilyl)-4α-cyano-β-D-*ribo*-pentofuranosyl]cytosine (38). Compound **38** (282 mg, 80%) was obtained as a white foam: FAB-MS *m*/*z* 611 (MH^+). Anal. ($C_{28}H_{54}N_4O_5Si_3 \cdot {}^1/_2H_2O$) C, H, N.

1-(4α-Ethynyl-β-D-*ribo*-pentofuranosyl)uracil (39). A solution of **34** (122 mg, 0.2 mmol) in a mixture of MeOH (10 mL) and concentrated HCl (1.5 mL) was stirred for 24 h at room temperature. The mixture was evaporated, and the residue was purified on a silica gel column with 17% MeOH in $CHCl_3$ to give **39** (34.9 mg, 65% as a white foam): ^{1}H NMR (DMSO-d_6) 11.35 (br s, 1 H, 3-NH), 7.77 (d, 1 H, H-6, $J_{6,5}$ = 8.1 Hz), 5.88 (d, 1 H, H-1′, $J_{1'-2'}$ = 6.4 Hz), 5.67 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 5.53 (t, 1 H, 5′-OH, $J_{OH,5'}$ = 5.7 Hz), 5.37 (d, 1 H, 2′-OH, $J_{OH,2'}$ = 6.1 Hz), 5.29 (d, 1 H, 3′-OH, $J_{OH,3'}$ = 5.9 Hz), 4.12 (ddd, 1 H, H-2′, $J_{2',1'}$ = 6.4, $J_{2',3'}$ = 5.6, $J_{2',OH}$ = 6.1 Hz), 4.04 (dd, 1 H, H-3′, $J_{3',2'}$ = 5.6, $J_{3',OH}$ = 5.9 Hz), 3.56 (dd, 1 H, H-5′a, $J_{5'a,5'b}$ = 11.7, $J_{5'a,OH}$ = 5.7 Hz), 3.54 (dd, 1 H, H-5′b, $J_{5'b,5'a}$ = 11.7, $J_{5'b,OH}$ = 5.7 Hz), 3.49 (s, 1 H, 4′-ethynyl); ^{13}C NMR (DMSO-d_6) 162.93, 150.73, 140.74, 102.21, 87.26, 83.74, 81.13, 78.94, 72.56, 70.86, 65.47; FAB-MS *m*/*z* 269 (MH^+); FAB-HRMS calcd for $C_{11}H_{13}N_2O_6$ 269.0774, found 269.0786. Anal. ($C_{11}H_{12}N_2O_6 \cdot {}^1/_2H_2O$) C, H, N.

1-(4α-Cyano-β-D-*ribo*-pentofuranosyl)uracil (40). Triethylamine (1.12 mL, 8.0 mmol) and 1,4-dioxane (4 mL) were added to a solution of **36** (245 mg, 0.4 mmol) in CH_3CN (10 mL) containing 48% HF (0.26 mL, 8.0 mmol). The mixture was stirred for 22 h at 50 °C and evaporated. Water (20 mL) was added to the residue, and the mixture was washed with $CHCl_3$ (3 × 20 mL). The H_2O layer was loaded on Diaion PK 212 (H^+ form) column and eluted with H_2O. The eluate was evaporated to dryness, and the residue was purified on a silica gel column with 20% MeOH in $CHCl_3$ to give a foam, which was crystallized from EtOH−$CHCl_3$ to give **40** (32 mg, 36% as a white powder): mp 146−147 °C; ^{1}H NMR (DMSO-d_6) 11.36 (br s, 1 H, 3-NH), 7.72 (d, 1 H, H-6, $J_{6,5}$ = 7.9 Hz), 6.12 (d, 1 H, OH, $J_{OH,}$ = 5.3 Hz), 5.91 (d, 1 H, H-1′, $J_{1'-2'}$ = 5.9 Hz), 5.84 (t, 1 H, 5′-OH, $J_{OH,5'}$ = 5.9 Hz), 5.67 (d, 1 H, H-5, $J_{5,6}$ = 7.9 Hz), 5.66 (d, 1 H, OH, J_{OH} = 5.3 Hz), 4.16−4.27 (m, 2 H, H-2′, H-3′), 3.74 (dd, 1 H, H-5′a, $J_{5'a,5'b}$ = 11.9, $J_{5'a,OH}$ = 5.9 Hz), 3.67 (dd, 1 H, H-5′b, $J_{5'b,5'a}$ = 11.9, $J_{5'b,OH}$ = 5.9 Hz); ^{13}C NMR (DMSO-d_6) 162.78, 150.51, 140.94, 117.59, 102.38, 88.68, 84.13, 71.28, 70.63, 63.52; FAB-MS *m*/*z* 270 (MH^+). Anal. ($C_{10}H_{11}N_3O_6 \cdot {}^1/_5H_2O$) C, H, N.

1-(4α-Ethynyl-β-D-*ribo*-pentofuranosyl)cytosine Hydrochloride (41). A solution of **37** (110 mg, 0.2 mmol) in a mixture of MeOH (10 mL) and HCl in dioxane (4 M, 2 mL) was stirred for 24 h at room temperature. The resulting precipitates were collected by filtration and washed with a small amount of EtOH to give **41** (55.5 mg, 77% as a white powder, 1:1 mixture with dioxane): mp 201−203 °C dec; ^{1}H NMR (DMSO-d_6) 9.81 (br s, 1 H, NH), 8.70 (br s, 1 H, NH), 8.19 (d, 1 H, H-6, $J_{6,5}$ = 7.8 Hz), 6.17 (d, 1 H, H-5, $J_{5,6}$ = 7.8 Hz), 5.84 (d, 1 H, H-1′, $J_{1'-2'}$ = 5.1 Hz), 4.15 (dd, 1 H, H-2′, $J_{2',1'}$ = 5.1, $J_{2',3'}$ = 5.4 Hz), 4.09 (d, 1 H, H-3′, $J_{3',2'}$ = 5.4 Hz), 3.61 (d, 1 H, H-5′a, $J_{5'a,5'b}$ = 12.2 Hz), 3.58 (d, 1 H, H-5′b, $J_{5'b,5'a}$ = 12.2 Hz), 3.55 (s, 8 H, dioxane), 3.54 (s, 1 H, 4′-ethynyl); ^{13}C NMR (DMSO-d_6) 159.10, 147.34, 144.50, 94.40, 88.76, 84.44, 80.94, 79.39, 73.56, 70.46, 66.41 (dioxane), 65.02. Anal. ($C_{11}H_{14}ClN_3O_5 \cdot C_4H_8O_2 \cdot {}^1/_6H_2O$) C, H, N.

1-(4α-Cyano-β-D-*ribo*-pentofuranosyl)cytosine (42). Compound **38** (222 mg, 0.363 mmol) was deprotected as for **40**. After workup, the resulting powder was dissolved in H_2O and purified on an ODS column (YMC-Pack R&D D-ODS-5-A 250-mm i.d. S-5 μm, 120 Å) with 0−2% CH_3CN in H_2O to give a solid, which was crystallized from H_2O to give **42** (43.9 mg, 41% as colorless needles): mp 224−225 °C dec; ^{1}H NMR (DMSO-d_6) 7.64 (d, 1 H, H-6, $J_{6,5}$ = 7.3 Hz), 7.26, 7.24 (each br s, each 1 H, 4-NH_2), 5.98 (d, 1 H, 3′-OH, $J_{OH-3'}$ = 5.3 Hz), 5.91 (d, 1 H, H-1′, $J_{1'-2'}$ = 4.0 Hz), 5.77 (t, 1 H, 5′-OH, $J_{OH-5'}$ = 5.9 Hz), 5.72 (d, 1 H, H-5, $J_{5,6}$ = 7.3 Hz), 5.54 (d, 1 H, 2′-OH, $J_{OH-2'}$ = 5.3 Hz), 4.14−4.21 (m, 2 H, H-2′, H-3′), 3.73 (dd, 1 H, H-5′a, $J_{5'a,5'b}$ = 11.9, $J_{5'a,OH}$ = 5.9 Hz), 3.65 (d, 1 H, H-5′b, $J_{5'b,5'a}$ = 11.9, $J_{5'b,OH}$ = 5.9 Hz); ^{13}C NMR (DMSO-d_6) 165.49, 154.97, 142.05, 117.90, 94.68, 90.53, 83.79, 71.76, 70.73, 63.70; FAB-MS *m*/*z* 269 (MH^+). Anal. ($C_{10}H_{12}N_4O_5 \cdot {}^1/_6H_2O$) C, H, N.

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Supporting Information Available: ^{1}H and ^{13}C NMR data for the nontarget compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Takenuki, K.; Matsuda, A.; Ueda, T.; Sasaki, T.; Fujii, A.; Yamagami, K. Design, synthesis, and antineoplastic activity of 2′-deoxy-2′-methylidenecytidine. *J. Med. Chem*. **1988**, *31*, 1063−1064.

(2) Matsuda, A.; Takenuki, K.; Tanaka, M.; Sasaki, T.; Ueda, T. Synthesis of new broad-spectrum antineoplastic nucleosides, 2′-deoxy-2′-methylidenecytidine (DMDC) and its derivatives. *J. Med. Chem*. **1991**, *34*, 812−819.

(3) Yamagami, K.; Fujii, A.; Arita, M.; Okumoto, T.; Sakata, S.; Matsuda, A.; Ueda, T.; Sasaki, T. Antitumor activity of 2′-deoxy-2′-methylidenecytidine, a new 2′-deoxycytidine derivatives. *Cancer Res*. **1991**, *51*, 2319−2323.

(4) Matsuda, A.; Nakajima, Y.; Azuma, A.; Tanaka, M.; Sasaki, T. 2′-*C*-cyano-2′-deoxy-1-β-D-arabinofuranosylcytosine (CNDAC): Design of a potential mechanism-based DNA-strand-breaking antineoplastic nucleoside. *J. Med. Chem*. **1991**, *34*, 2917−2919.

(5) Tanaka, M.; Matsuda, A.; Terao, T.; Sasaki, T. Antitumor activity of a novel nucleoside, 2′-*C*-cyano-2′-deoxy-1-β-D-arabinofuranosylcytosine (CNDAC) against murine and human tumors. *Cancer Lett*. **1992**, *64*, 67−74.

(6) Azuma, A.; Nakajima, Y.; Nishizono, N.; Minakawa, N.; Suzuki, M.; Hanaoka, K.; Kobayashi, T.; Tanaka, M.; Sasaki, T.; Matsuda, A. 2′-*C*-cyano-2′-deoxy-1-β-D-arabinofuranosylcytosine and its derivatives. A new class of nucleoside with a broad antitumor spectrum. *J. Med. Chem*. **1993**, *36*, 4183−4189.

(7) Azuma, A.; Hanaoka, K.; Kurihara, A.; Kobayashi, T.; Miyauchi, S.; Kamo, N.; Tanaka, M.; Sasaki, T.; Matsuda, A. Chemical stability of a new antitumor nucleoside, 2′-*C*-cyano-2′-deoxy-1-β-D-arabinofuranosylcytosine in alkaline medium: Formation of 2′-*C*-cyano-2′-deoxy-1-β-D-ribofuranosylcytosine and its antitumor activity. *J. Med. Chem*. **1995**, *38*, 3391−3397.

(8) McCarthy, J. R.; Matthews, D. P.; Stemerick, D. M.; Huber, E. W.; Bey, P.; Lippert, B. J.; Snyder, R. D.; Sunkara, P. S. Stereospecific method to *E* and *Z* terminal fluoro olefines and its application to the synthesis of 2′-deoxy-2′-fluoromethylene nucleosides as potential inhibitors of ribonucleoside diphosphate reductase. *J. Am. Chem. Soc*. **1991**, *113*, 7439−7440.

(9) Hattori, H.; Tanaka, M.; Fukushima, M.; Sasaki, T.; Matsuda, A. 1-(3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl)cytosine, 1-(3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl)uracil, and their nucleobase analogues as new potential multifunctional antitumor nucleosides with a broad spectrum of activity. *J. Med. Chem*. **1996**, *39*, 5005−5011.

(10) Hattori, H.; Nozawa, E.; Iino, T.; Yoshimura, Y.; Shuto, S.; Shimamoto, Y.; Nomura, M.; Fukushima, M.; Tanaka, M.; Sasaki, T.; Matsuda, A. Structural requirements of the sugar moiety for the antitumor activities of new nucleoside antimetabolites, 1-(3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl)cytosine and -uracil. *J. Med. Chem*. **1998**, *41*, 2892−2902.

(11) Takatori, S.; Kanda, H.; Takenaka, K.; Wataya, Y.; Matsuda, A.; Fukushima, M.; Shimamoto, Y.; Tanaka, M.; Sasaki, T. Antitumor mechanisms and metabolism of novel antitumor nucleoside analogues, 1-(3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl)-cytosine and 1-(3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl)uracil. *Cancer Chemother. Pharmacol.*, in press.

(12) Waga, T.; Nishizaki, T.; Miyakawa, I.; Ohrui, H.; Meguro, H. Synthesis of 4′-*C*-methylnucleosides. *Biosci. Biotechnol. Biochem.* **1993**, *57*, 1433−1438.
(13) Waga, T.; Ohrui, H.; Meguro, H. Synthesis and biological evaluation of 4′-*C*-methylnucleosides. *Nucleosides Nucleotides* **1996**, *15*, 287−304.
(14) Kitano, K.; Miura, S.; Ohrui, H.; Meguro, H. Synthesis of 4′-*C*-fluoromethylnucleosides as potential antineoplastic agents. *Tetrahedron* **1997**, *53*, 13315−13322.
(15) Sugimoto, I.; Shuto, S.; Mori, S.; Shigeta, S.; Matsuda, A. Synthesis of 4′-α-branched thymidines as a new type of antiviral agent. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 385−388.
(16) O-Yang, C.; Wu, H. Y.; Fraser-Smith, E. B.; Walker, K. A. M. Synthesis of 4′-cyanothymidine and analogues as potent inhibitors of HIV. *Tetrahedron Lett.* **1992**, *33*, 37−40.
(17) Youssefyeh, R. D.; Tegg, D.; Verheyden, J. P. H.; Jones, G. H.; Moffatt, J. G. Synthetic routes to 4′-hydroxymethylnucleosides. *Tetrahedron Lett.* **1977**, 435−438.
(18) Youssefyeh, R. D.; Verheyden, J. P. H.; Moffatt, J. G. 4′-Substituted nucleosides. 4. Synthesis of some 4′-hydroxymethyl nucleosides. *J. Org. Chem.* **1979**, *44*, 1301−1306.
(19) Jones, G. H.; Taniguchi, M.; Tegg, D.; Moffatt, J. G. 4′-Substituted nucleosides. 5. Hydroxymethylation of nucleoside 5′-aldehyde. *J. Org. Chem.* **1979**, *44*, 1309−1317.
(20) Marx, A.; Erdmann, P.; Senn, M.; Körner, S.; Jungo, T.; Petretta, M.; Imwinkelried, P.; Dussy, A.; Kulicke, K. J.; Macko, L.; Zehnder, M.; Giese, B. Synthesis of 4′-*C*-acylated thymidines. *Helv. Chim. Acta* **1996**, *79*, 1980−1994.
(21) O-Yang, C.; Kurtz, W.; Eugui, E. M.; McRoberts, M. J.; Verheyden, J. P. H.; Kurtz, L. J.; Walker, K. A. M. 4′-Substituted nucleosides as inhibitors of HIV: An unusual oxetane derivative. *Tetrahedron Lett.* **1992**, *33*, 41−44.
(22) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. Evaluation of a tetrazolium-based semiaautomated colorimetric assay. Assessment of chemosensitivity testing. *Cancer Res.* **1987**, *47*, 936−942.
(23) Takeuchi, H.; Baba, M.; Shigeta, S. An application of tetrazolium (MTT) colorimetric assay for the screening of anti-herpes simplex virus compounds. *J. Virol. Methods* **1991**, *33*, 61−71.
(24) Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdwijn, P.; Desmyter, J.; De Clercq, E. Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. *J. Virol. Methods* **1988**, *20*, 309−321.

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