Nucleosides and Nucleotides. 123. Synthesis of 1-(2-Deoxy-2-isocyano-β-D-arabinofuranosyl)cytosine and Related Nucleosides as Potential Antitumor Agents[1]

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2′-Deoxy-2′-isocyano-1-β-D-arabinofuranosylcytosine (8, NCDAC) has been synthesized as a potential antitumor antimetabolite from a corresponding 2′-azido-2′-deoxy-1-β-D-arabinofuranosyluracil derivative **2a**. Uracil and thymine analogues **6a** and **6b** of 8 were also prepared. Attempts to synthesize 2′-deoxy-2′-isocyanocytidine (**14b**) failed due to the insertion of the 2′-α isocyano group into the 3′-OH group, affording the 2′,3′-oxazoline derivative **15b**. Stability of the isocyano derivative **6a** and 2′,3′-oxazoline derivative **15a** under basic and acidic conditions were examined. The isocyano group in **6a** was stable in basic conditions but unstable even in weakly acidic conditions to furnish the corresponding 2′-β formamide derivative **17**. Compound **15a** was easily hydrolyzed the corresponding 2′-α formamide derivative **16** on treatment with H_2O at room temperature. The cytotoxicity of 8, **6a**, and **6b** was examined in mouse and human tumor cells in vitro and compared with that of *ara*-C. Of these nucleosides, 8 was moderately cytotoxic to these cell lines. In vivo antitumor activity of 8 against Lewis lung carcinoma cells was also investigated and 8 showed only moderate tumor volume inhibition.

Introduction of a certain functional group into the 2′-β position of 2′-deoxycytidine is an interesting approach for designing a new type of antitumor nucleosides. A classical example of this strategy was 1-β-D-arabinofuranosylcytosine (*ara*-C), which is now used for the treatment of adult acute myeloblastic leukemia.[2] However, *ara*-C has a only narrow spectrum of antitumor activity and is less effective on solid tumors. One of our approaches to designing new antitumor nucleosides that have a broad spectrum of activity toward leukemia and solid tumors is to have a chemically reactive functionality at the 2′-β position of 2′-deoxycytidine. As an example, we have synthesized 2′-*C*-cyano-2′-deoxy-1-β-D-arabinofuranosylcytosine (CNDAC), which not only had cytotoxicity against a wide variety of human tumor cells in vitro but also had a prominent antitumor activity toward P388 mouse leukemia and M5076 mouse reticulum cell sarcoma in vivo.[1,3,4] CNDAC also showed excellent antitumor activity against HT-1080 human fibrosarcoma, which is refractory to *ara*-C, in chick embryos or athymic mice.[5] In this case, introduction of a cyano group at the 2′-β position made the 2′-α proton more acidic. Therefore, we expected that when CNDAC was incorporated into DNA, β-elimination reactions would happen to produce either a DNA strand break or formation of an abasic site. Although this hypothesis has not been fully confirmed, the approach of introducing a chemically reactive group into the 2′-β position should be further developed to find other types of functionality.

Isocyanides are unique in being the only stable functional group incorporating a bivalent carbon, which is expected to have chemical reactivity as a carbenoid and/or as a nucleophile.[6] When such a functional group is incorporated into the 2′-β position of 2′-deoxycytidine, it would be expected to have reactivities inherited in the functional group with nucleoside- and nucleotide-metabolizing enzymes to inhibit them. Although such a nucleoside has to be phosphorylated at the 5′-position to have antitumor activity, it is worth examining whether they inhibit tumor cell growth differently from other types of antimetabolites. This report deals with the synthesis of 2′-deoxy-2′-isocyano-1-β-D-arabinofuranosylcytosine (NCDAC) and an attempt to synthesize 2′-deoxy-2′-*C*-isocyanocytidine (NCDC), and also the inhibitory effects on tumor cell lines in vitro as well as in vivo.

Chemistry. A straightforward method for the synthesis of the target nucleoside is to synthesize a corresponding 2′-amino-2′-deoxy-1-β-D-arabinofuranosyl pyrimidine nucleoside. An original method for the synthesis of the amino-sugar pyrimidine nucleoside was developed by Bobek, starting from D-glucose in a multistep total synthesis.[7] However, this method is too far from our purposes for preparation on a large scale. We have developed a new method to introduce an azide group into the 2′-β position of uridine, which could be easily converted to an antineoplastic nucleoside, 2′-azido-2′-deoxy-1-β-D-arabinofuranosylcytosine, cytarazid, in a good overall yield.[8,9] Therefore, we used 1-[2-azido-2-deoxy-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl)-uracil (**2a**) as a starting material to synthesize the target 2′-deoxy-2′-isocyano-1-β-D-arabinofuranosylcytosine (8, NCDAC).

When **2a** was reduced by catalytic hydrogenation in the presence of AcOH, the desired amino derivative **3a** was obtained in 79% yield. Without addition of AcOH the yield of **3a** was reduced. Compound **3a** was then treated with acetic formic anhydride to afford the formamide derivative **4a** in 72% yield. However, since **3a** was rather unstable under neutral conditions due to addition of the amino group to the 6 position of the uracil moiety,[10] **2a**

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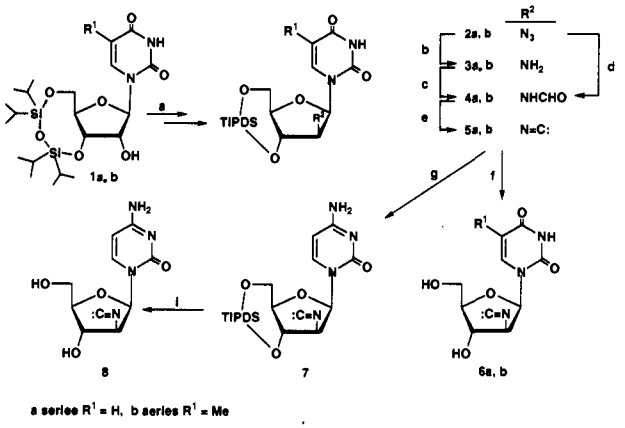
Scheme I[a]



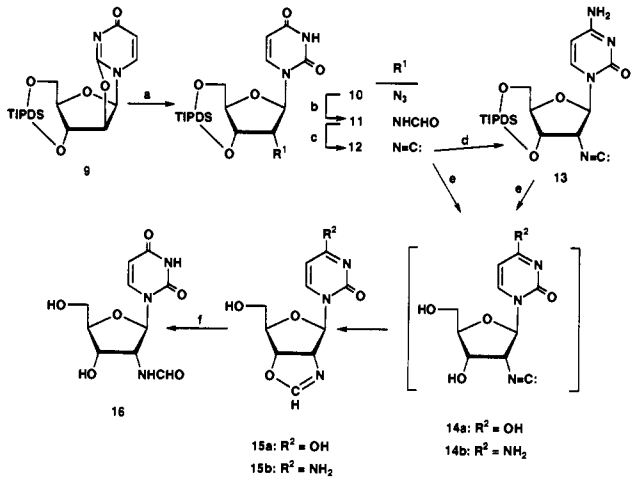
[a] (a) Reference 8; (b) H_2, Pd/C in EtOH and AcOH; (c) acetic formic anhydride in pyridine; (d) acetic formic anhydride, Ph_3P in toluene; (e) TsCl in pyridine; (f) TBAF in THF; (g) TPSCl, then NH_4OH.

was directly treated with acetic formic anhydride in the presence of triphenylphosphine,[11] giving **4a** in 59% yield. Upon heating of **4a** with TsCl in pyridine at 60 °C, the desired 1-[2-deoxy-2-isocyano-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]uracil (**5a**) was obtained in 69% yield. An IR spectrum of **5a** showed a characteristic sharp absorption of the isocyano group at 2140 cm^{-1} and in its ^1H NMR spectrum a doublet due to the H-1′ proton was observed at 6.24 ppm with a coupling constant of 6.1 Hz with the H-2′ proton. These spectral data together with elemental analyses are consistent with its structure. Using lower or higher temperatures, the yield was considerably reduced. Deprotection of the silyl group in **5a** was done using tetrabutylammonium fluoride (TBAF) in THF to furnish 2′-deoxy-2′-isocyano-1-β-D-arabinofuranosyluracil (**6a**, NCDAU) in 82% yield. The thymine derivative **6b** (NCDAT) was also synthesized in the same way. Amino derivative **3b** was stable in this case due to the electron-donating property of the 5-methyl group that prevented the addition reaction of the 2′-amino group to the 6-position.

Introduction of a (triisopropylphenyl)sulfonyl group into the O^4 position of **5a** was done using a phase-transfer method[12] and the resulting triisopropylbenzenesulfonate was treated with concentrated NH_4OH to afford cytosine derivative **7** in 85% yield, which was further treated with TBAF in THF furnishing 1-(2-deoxy-2-isocyano-β-D-arabinofuranosyl)cytosine (**8**, NCDAC) in 91% yield as a foam. Attempts to crystallize **8** as an HCl salt failed due to decomposition.

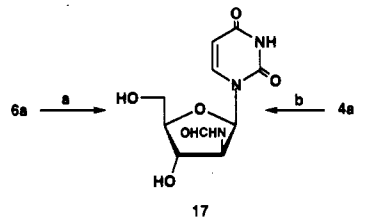
Synthesis of 1-(2-deoxy-2-isocyano-β-D-ribofuranosyl)-pyrimidines was also investigated. 2′-α Azide uridine derivative **10**, which was readily accessible from 2,2′-anhydrouridine derivative **9**,[13] was converted to the corresponding formamide derivative **11** on treatment with acetic formic anhydride and triphenylphosphine in toluene.[11] Compound **11** was further treated with TsCl in pyridine at 70 °C to afford the corresponding isocyano derivative **12** in 82% yield, which could also be converted to the cytosine derivative **13** as above. Deprotection of the silyl group in **12** was tried in the same way using TBAF. However, a product obtained from the reaction mixture did not show any peaks at around 2100–2200 cm^{-1} corresponding to the isocyano group expected to be in the structure of **14a** in its IR spectrum. In its ^1H NMR spectrum, a doublet due to H-1′ was observed at 5.74 ppm

Scheme II[a]



[a] (a) Reference 13; (b) acetic formic anhydride, Ph_3P in toluene; (c) TsCl in pyridine; (d) TPSCl, then NH_4OH; (e) TBAF in THF; (f) H_2O.

Scheme III[a]



[a] (a) Aqueous AcOH; (b) TBAF in THF.

with a $J_{1',2'} = 2.7$ Hz. Since a $J_{1',2'}$ value of the deprotection product **16** of **11** was 8.8 Hz, the value observed in this case was too small to be the expected product. Additionally, one proton signal as a singlet at 7.30 ppm, which did not disappear upon addition of D_2O, was observed and a proton due to the 3′-OH was not detected. From these data together with FAB-MS data, this nucleoside was assigned as [1-(uracil-1-yl)-2,3-dideoxy-β-D-ribofuranosido][2,3-*d*]-2-oxazoline (**15a**). Deprotection of cytosine derivative **13** with TBAF also gave rise to **15b**. Thus, although deprotection of the silyl group in **12** and **13** initially would give the corresponding free isocyano nucleosides **14a** and **14b**, since the isocyano group has a bivalent carbenic character, they inserted into the spatially proximate 3′-OH groups to furnish **15a** and **15b**. In the case of the arabino series, the generated isocyanide was not close enough to react to the 3′-OH due to the trans relationship. Such chemical reactivity, as expected, would affect their metabolism when reacting with enzymes.

During measurement of ^1H NMR, we have observed that **15a** was gradually decomposed when D_2O was added. When **15a** was treated in H_2O at room temperature for 2 days, 1-(2-deoxy-2-formamido-β-D-ribofuranosyl)uracil (**16**) was obtained in good yield, which was identical to the deprotection product of **11**. We also examined the stability of **6a** under basic and acid conditions. When **6a** was treated with H_2O or concentrated NH_4OH at room temperature, **6a** was completely recovered, while treatment with aqueous AcOH converted **6a** to 1-(2-deoxy-2-formamido-β-D-arabinofuranosyl)uracil (**17**), which was identical to the specimen of the deprotection product of **4a**. Therefore, the 2′-β isocyanide group was found to be stable under the neutral and basic conditions examined but was rather reactive under acidic conditions, proceeding to hydration via a protonation of the carbon atom of the isocyanide.

Table I. Tumor Cell Growth Inhibitory Activity of 2′-Deoxycytidine Analogues in Vitro[a]

compds	IC_{50} (μM)[b]			
	L1210[c]	KB[d]	LLC[e]	KATO III[f]
NCDAC	12.6	7.9	4.9	90
NCDAU	34	36.8	>100	>100
ara-C	0.12	0.64	5.6	0.3

[a] Each kind of cell (L1210: 4×10^{-3} well, KB: 2×10^{-3} well, LLC: 3×10^{-3} well, and KATO III: 3×10^{-3} well) was suspended in RPMI 1640 medium supplemented with 10% FCS (L1210: added with 50 mM 2-mercaptoethanol) and plated into 96-well culture plates. Each compound was dissolved in Dulbecco's modified PBS (Ca and Mg ion free), diluted with culture medium, and then added to the plates. After 72 h of incubation, IC_{50} values were calculated by the crystal violet staining method. [b] IC_{50} (μM) was given as the concentration at 50% inhibition of cell growth. [c] Mouse leukemia. [d] Human oral epidermoid carcinoma. [e] Lewis lung carcinoma. [f] Human stomach adenocarcinoma.

Table II. Antitumor Activity of NCDAC and *ara*-C against Lewis Lung Carcinoma Bearing Mice in Vivo[a]

compds	mg/kg/day	tumor weight (g): mean ± SD	tumor weight: inhibition ratio (%)	mean body weight change: on day 5	mean body weight change: on day 17
control		2.77 ± 0.62		0.4	3.3
NCDAC	100	1.08 ± 0.52	61.0[b]	0.4	2.0
	200	1.09 ± 0.20	60.6[b]	0.2	2.1
ara-C	100	0.47 ± 0.19	83.0[b]	−0.4	0.8
	200	0.34 ± 0.13	87.7[b]	−0.1	0.7

[a] 2-mm cube of LLC fragment was transplanted s.c. to male BDF1 mice (9 weeks old). Mice were allocated to control and test groups on day 0. NCDAC and *ara*-C were dissolved in saline at a concentration of 20 mg/mL and diluted. These were administered i.p. from day 1 to 5. Tumor weight inhibition ratio (%) was estimated by measurement of weight on day 17. [b] $p < 0.001$.

Antitumor Activity. We compared the in vitro cytotoxic spectrum of NCDAC and NCDAU with that of *ara*-C in L1210 mouse leukemia, Lewis lung carcinoma (LLC), KB human oral epidermoid carcinoma, and KATO III human stomach adenocarcinoma cell lines. As summarized in Table I, NCDAC had moderate cytotoxicity compared with that of *ara*-C. Against L1210, KB, and KATO III cells, *ara*-C was about 10 to 30 times more active than NCDAC, while against LLC, they had almost the same efficacy. The uracil counterpart, NCDAU, had much less activity and NCDAT did not show any cytotoxicity up to 100 μM toward L1210 cells. From these data, only the cytosine derivative, NCDAC, showed cytotoxicity to some extent and this would be related to the substrate specificity of nucleoside and/or nucleotide kinases. As shown in Table I, NCDAC had a similar activity against LLC to that of *ara*-C, so we also evaluated the in vivo antitumor activity of NCDAC against LLC implanted subcutaneously into male BDF1 mice, compared with that of *ara*-C. In this experiment, the tumor volume inhibition ratio was used as the parameter of antitumor activity and day 17 was chosen as the day of evaluation as shown in Table II. When *ara*-C was administered intraperitoneally on days 1 to 5 at a dose of 100 mg/kg/day, it showed an 83% inhibition of tumor volume. When we increased the dose to 200 mg/kg/day under the same schedule of drug administration, *ara*-C showed tumor volume inhibition of 88%. On the other hand, NCDAC showed less activity than that of *ara*-C at both doses.

Since *ara*-C showed good cytotoxicity to all the cell lines tested in this study, these cell lines should have deoxycytidine kinase activity, which is responsible for the first activation of *ara*-C to *ara*-CMP. The cytotoxicity of *ara*-C is known to correlate to inhibition of DNA polymerization after incorporation of *ara*-C into DNA molecules.[14] Although the substrate specificity of 2′-deoxycytidine analogues with a substituent at the 2′-β position for deoxycytidine kinase has not been thoroughly studied, we have found the smaller the better for the substituent to cause cytotoxicity,[4] which might reflect the substrate specificity of the deoxycytidine kinase. We have reported that (2′*S*)-2′-deoxy-2′-*C*-methylcytidine,[15,16] 2′-*C*-cyano-2′-deoxy-1-β-D-arabinofuranosylcytosine,[1,3] and 2′-azide-2′-deoxy-1-β-D-arabinofuranosylcytosine[7,8] were comparably effective in a variety of tumor cell lines. Since the size of the isocyano group would be similar to that of the substituents described above, the reason for its lesser potency would be due to the chemical instability of NCDAC and its metabolites during circulation: NCDAC was stable in basic conditions unlike CNDAC[1,3] but was unstable in acidic conditions, being converted into a formamide derivative. Therefore, as a choice of a chemically reactive functional group, the isocyano group at the 2′-β position of 2′-deoxycytidine was not fruitful and further searches for such functional groups are necessary.

Experimental Section

Melting points were measured on a Yanagimoto MP-3 micromelting point apparatus and are uncorrected. The ^1H NMR spectra were recorded on a JEOL JNM-FX 100 (100 MHz), JEOL JNM-GX 270 (270 MHz), or JEOL EX 400 (400 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 addition of D_2O. UV absorption spectra were recorded with a Shimadzu UV-240 spectrophotometer. Mass spectra (MS) were measured on a JEOL JMX-DX303 spectrometer. IR spectra was recorded with a Jasco IRA-I spectrometer. TLC was done on Merck Kieselgel F254 precoated plates. The silica gel used for column chromatography was YMC gel 60A (70-230 mesh).

1-[2-Amino-2-deoxy-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]uracil (3a). Compound **2a** (391 mg, 0.77 mmol) was hydrogenated under atmospheric pressure of hydrogen with 10% Pd/C (100 mg) in a mixture of AcOH (1 mL) and EtOH (9 mL) overnight at room temperature. The reaction mixture was filtered over Celite and the filtrate was concentrated to dryness. The residue was purified by silica gel column chromatography (2.4 × 9 cm) with hexane:AcOEt (3:1 to 2:1) and then $CHCl_3$ to give **3a** (295 mg, 79% as a foam). This compound showed positive in a ninhydrin test: EI-MS *m/z* 485 (M^+), 442 (M^+ − isopr), 337 (M^+ − uracil); NMR (100 MHz, $CDCl_3$) 8.39 (1 H, br s, 3-NH), 7.83 (1 H, d, H-6, $J_{6,5}$ = 8.1 Hz), 6.10 (1 H, d, H-1′, $J_{1',2'}$ = 6.8 Hz), 5.70 (1 H, d, H-5, $J_{5,6}$ = 8.1 Hz), 3.70–4.20 (5 H, m, H-2′,3′,4′,5′a,b), 1.05 (28 H, m, isopr).

1-[2-Deoxy-2-formamido-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]uracil (4a). (a) Acetic formic anhydride (0.25 mL) was added to a solution of **3a** (534 mg, 1.1 mmol) in pyridine (25 mL) under argon at 0 °C. The mixture was stirred for 15 min at 0 °C and MeOH (1 mL) was added. The solvent was removed by evaporation and the residue was partitioned between $CHCl_3$ and H_2O. The organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified over a silica gel column (2.8 × 10 cm) with 0–5% EtOH in $CHCl_3$ to give **4a** (410 mg, 72%, as a foam): FAB-MS *m/z* 514 ($M^+ + 1$), 470 (M^+ − isopr); NMR (DMSO-d_6) 10.22 (1 H, br s, 3-NH), 8.40 (1 H, br d, -N*H*CHO), 7.98 (1 H, s, -NHC*H*O), 7.51 (1 H, d, H-6, $J_{6,5}$ = 8.3 Hz), 5.95 (1 H, d, H-1′, $J_{1',2'}$ = 7.3 Hz), 5.54 (1 H, d, H-5, $J_{5,6}$ = 8.3 Hz), 3.50–4.80 (5 H. m, H-2′,3′,4′,5′a,b), 1.00 (28 H, m, isopr). (b) Acetic formic anhydride (0.03 mL) was added to a mixture of **2a** (110 mg, 0.22 mmol) and Ph_3P (74 mg, 0.28 mmol) in toluene (2 mL) under argon at 0 °C. The mixture was heated for 2 h at 70 °C and was concentrated in vacuo. The

residue was coevaporated with EtOH (3 × 5 mL) and was purified over a silica gel column (2.4 × 9 cm) with 0–4% EtOH in $CHCl_3$ to give **4a** (67 mg, 59%, as a foam).

1-[2-Deoxy-2-isocyano-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]uracil (5a). A mixture of **4a** (203 mg, 0.40 mmol) and TsCl (113 mg, 0.59 mmol) in pyridine (4 mL) was heated for 3 h at 60 °C. EtOH (1 mL) was added to the mixture, which was concentrated to dryness. The residue was partitioned between AcOEt and H_2O and the organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified on a silica gel column (2.4 × 11 cm) with hexane:AcOEt (4:1 to 2:1) to give **5a** (134 mg, 69%). Further elution of this column gave the starting material **4a** (35 mg). Physical data of **5a**: mp 168–170 °C (crystallized from Et_2O–hexane); EI-MS *m/z* 496 ($M^+ + 1$), 452 (M^+ – isopr); IR (Nujol) ν N=C 2140 cm^{-1}; NMR (270 MHz, $CDCl_3$) 8.54 (1 H, br s, 3-NH), 7.64 (1 H, d, H-6, $J_{6,5}$ = 8.3 Hz), 6.24 (1 H, d, H-1′, $J_{1',2'}$ = 6.1 Hz), 5.78 (1 H, d, H-5, $J_{5,6}$ = 8.3 Hz), 3.70–4.60 (5 H, m, H-2′,3′,4′,5′a,b), 1.05 (28 H, m, isopr). Anal. ($C_{22}H_{37}N_3O_6Si_2$) C, H, N.

1-(2-Deoxy-2-isocyano-β-D-arabinofuranosyl)uracil (6a). TBAF (1 M THF solution, 0.95 mL, 0.95 mmol) was added to a solution of **5a** (189 mg, 0.38 mmol) in THF (4 mL). The mixture was stirred for 30 min at room temperature and concentrated to dryness in vacuo. The residue was purified on a silica gel column (2 × 11 cm), eluted with 2–14% EtOH in $CHCl_3$ to give **6a** (79 mg, 82% as a foam): IR (Nujol) ν N=C: 2150 cm^{-1}; FAB-MS *m/z* 254 ($M^+ + 1$); NMR (DMSO-d_6) 11.49 (1 H, br s, 3-NH), 7.94 (1 H, d, H-6, $J_{6,5}$ = 8.2 Hz), 6.27 (1 H, d, 3′-OH, *J* = 5.5 Hz), 6.16 (1 H, d, H-1′, $J_{1',2'}$ = 6.0 Hz), 5.72 (1 H, d, H-5, $J_{5,6}$ = 8.2 Hz), 5.26 (1 H, t, 5′-OH, *J* = 5.0 Hz), 4.58 (1 H, dd, H-2′, $J_{2',1'}$ = 6.6, $J_{2',3'}$ = 7.1 Hz), 4.27–4.37 (1 H, m, H-3′, $J_{3',2'}$ = 7.1, $J_{3',4'} = J_{3',OH}$ = 5.5 Hz), 3.58–3.74 (3 H, m, H-4′,5′a,b). Anal. ($C_{10}H_{11}N_3O_5$) C, H, N.

1-[2-Deoxy-2-isocyano-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]cytosine (7). A mixture of **5a** (247 mg, 0.5 mmol), triisopropylbenzenesulfonyl chloride (272 mg, 0.9 mmol), and tetrabutylammonium bromide (20 mg) in a mixture of CH_2Cl_2 (15 mL) and 0.2 M aqueous Na_2CO_3 (20 mL) was vigorously stirred for 4 h at room temperature. The mixture was diluted with $CHCl_3$ (20 mL) and the organic phase separated was further washed with H_2O. The organic phase was dried (Na_2SO_4) and concentrated to dryness. Concentrated NH_4OH (28%, 2 mL) was added to a solution of the above residue in dioxane (5 mL) and the mixture was stirred for 2 h at room temperature. The solvent was removed in vacuo and the residue was purified on a silica gel column (2.7 × 8.5 cm) eluted with 0–10% EtOH in $CHCl_3$ to give **7** (211 mg, 85% as a foam): IR (Nujol) ν N=C 2140 cm^{-1}; MS *m/z* 494 (M^+), 451 (M^+ – isopr); NMR (270 MHz, $CDCl_3$) 7.61 (1 H, d, H-6, $J_{6,5}$ = 7.4 Hz), 6.37 (2 H, br s, 4-NH_2), 6.29 (1 H, d, H-1′, $J_{1',2'}$ = 5.6 Hz), 5.85 (1 H, d, H-5, $J_{5,6}$ = 7.4 Hz), 3.65–4.55 (5 H, m, H-2′,3′,4′,5′a,b), 1.05 (28 H, m, isopr).

1-(2-Deoxy-2-isocyano-β-D-arabinofuranosyl)cytosine (8). Compound **7** (241 mg, 0.49 mmol) was deblocked as described in the synthesis of **6a** to give **8** (112 mg, 91%, as a foam): IR (Nujol) ν N=C 2140 cm^{-1}; MS *m/z* 252 (M^+); NMR (DMSO-d_6) 7.82 (1 H, d, H-6, $J_{6,5}$ = 7.3 Hz), 6.40 (2 H, br s, 4-NH_2), 6.25 (1 H, br s, 3′-OH), 6.11 (1 H, d, H-1′, $J_{1',2'}$ = 6.6 Hz), 5.84 (1 H, d, H-5, $J_{5,6}$ = 7.3 Hz), 5.20 (1 H, br s, 5′-OH), 4.50 (1 H, br s, H-2′), 4.29 (1 H, m, H-3′), 3.59– 3.78 (3 H, m, H-4′,5′a,b). Anal. ($C_{10}H_{12}N_4O_4 \cdot 1/6H_2O$) C, H, N.

1-[2-Amino-2-deoxy-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]thymine (3b). Compound **2b** (1.0 g, 1.9 mmol) was hydrogenated under atmospheric pressure of hydrogen with 10% Pd/C (30 mg) in a mixture of AcOH (3 mL) and EtOH (15 mL) overnight at room temperature. The reaction mixture was filtered over Celite and the filtrate was concentrated to dryness. The residue was purified by silica gel column chromatography (2.8 × 11 cm) with 0–4% EtOH in $CHCl_3$ to give **3b** (842 mg, 89%, crystallized from Et_2O–hexane). This compound was positive in a ninhydrin test: mp 125–126 °C; EI-MS *m/z* 499 (M^+), 456 (M^+ – isopr), 337 (M^+ – thymine); NMR (DMSO-d_6) 11.30 (1 H, br s, 3-NH), 7.30 (1 H, s, H-6), 5.92 (1 H, d, H-1′, $J_{1',2'}$ = 6.4 Hz), 3.71–4.00 (5 H, m, H-2′,3′,4′,5′a,b), 1.76 (3 H, br s, 5-Me), 1.05 (28 H, m, isopr). Anal. ($C_{22}H_{41}N_3O_6Si_2$) C, H, N.

1-[2-Deoxy-2-formamido-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]thymine (4b). Acetic formic anhydride (0.05 mL, 1.08 mmol) was added to a solution of **3b** (283 mg, 0.57 mmol) in pyridine (6 mL) under argon at 0 °C. The mixture was stirred for 15 min at 0 °C and MeOH was added (1 mL). The solvent was removed by evaporation and the residue was partitioned between $CHCl_3$ and H_2O. The organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified over a silica gel column (2.8 × 10 cm) with 0–5% EtOH in $CHCl_3$ to give **4b** (295 mg, 99%, as a foam): FAB-MS *m/z* 528 ($M^+ + 1$); NMR (DMSO-d_6) 11.27 (1 H, br s, 3-NH), 8.38 (1 H, d, -N*H*CHO), 7.98 (1 H, br s, –NHC*H*O), 7.23 (1 H, d, H-6, $J_{6,Me}$ = 1.0 Hz), 5.98 (1 H, d, H-1′, $J_{1',2'}$ = 7.6 Hz), 3.76–4.90 (5 H, m, H-2′,3′,4′,5′a,b), 1.74 (3 H, br s, 5-Me), 1.05 (28 H, m, isopr).

1-[2-Deoxy-2-isocyano-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]thymine (5b). A mixture of **4b** (113 mg, 0.21 mmol) and TsCl (52 mg, 0.32 mmol) in pyridine (2 mL) was heated for 1.5 h at 75 °C. EtOH (1 mL) was added to the mixture, which was concentrated to dryness. The residue was partitioned between $CHCl_3$ and H_2O and the organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified on a silica gel column (2 × 7 cm) with 0–1% EtOH in $CHCl_3$ to give **5b** (84 mg, 79%): mp 175.5–176.5 °C (crystallized from Et_2O–hexane); El-MS *m/z* 510 ($M^+ + 1$), 466 (M^+ – isopr); IR (Nujol) ν N=C 2140 cm^{-1}; NMR (DMSO-d_6) 11.49 (1 H, br s, 3-NH), 7.94 (1 H, d, H-6, $J_{6,5}$ = 8.2 Hz), 6.27 (1 H, d, 3′-OH, *J* = 5.5 Hz), 6.16 (1 H, d, H-1′, $J_{1',2'}$ = 6.0 Hz), 5.72 (1 H, d, H-5, $J_{5,6}$ = 8.2 Hz) , 5.26 (1 H, t, 5′-OH, *J* = 5.0 Hz), 4.58 (1 H, dd, H-2′), 4.27-4.37 (1 H, m, H-3′), 3.58–3.74 (3 H, m, H-4′,5′a,b). Anal. ($C_{23}H_{39}N_3O_6Si_2$) C, H, N.

1-(2-Deoxy-2-isocyano-β-D-arabinofuranosyl)thymine (6b). Compound **5b** (265 mg, 0.52 mmol) was desilylated as described in the synthesis of **6a** to give **6b** (135 mg, 97% crystallized from EtOH–hexane): mp 171.5-172.5 °C; IR (Nujol) ν N=C 2160 cm^{-1}; FAB-MS *m/z* 268 ($M^+ + 1$); NMR (DMSO-d_6) 11.49 (1 H, br s, 3-NH), 7.84 (1 H, s, H-6), 6.24 (1 H, d, 3′-OH, *J* = 5.5 Hz), 6.16 (1 H, d, H-1′, $J_{1',2'}$ = 6.6 Hz), 5.30 (1 H, t, 5′-OH, *J* = 4.9 Hz), 4.58 (1 H, dd, H-2′, $J_{2',1'}$ = 6.6 , $J_{2',3'}$ = 7.1 Hz), 4.33 (1 H, m, H-3′, $J_{3',2'}$ = 7.1, $J_{3',4'} = J_{3',OH}$ = 5.5 Hz), 3.59–3.76 (3 H, m, H-4′,5′a,b), 1.79 (3 H, s, 5-Me). Anal. ($C_{11}H_{13}N_3O_5$) C, H, N.

1-[2-Deoxy-2-formamido-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]uracil (11). Acetic formic anhydride (0.09 mL) was added to a solution of **10** (430 mg, 0.84 mmol) and Ph_3P (490 mg, 1.1 mmol) in toluene at 0 °C. The mixture was heated at 60 °C for 2 h and was diluted with $CHCl_3$ (15 mL). The mixture was washed with H_2O and the separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified on a silica gel column (2.7 × 11 cm), which was eluted with 0–2% EtOH in $CHCl_3$ to afford **11** (317 mg, 74%, as a foam): El-MS *m/z* 513 (M^+), 470 (M^+ – isopr); NMR ($CDCl_3$) 8.29 (1 H, br s, 3-NH), 7.28 (1 H, d, H-6, $J_{6,5}$ = 8.1 Hz), 6.45 (1 H, br d, –N*H*CHO), 5.75 (1 H, dd, H-5, $J_{5,6}$ = 8.1 Hz), 5.64 (1 H, d, H-1′, $J_{1',2'}$ = 5.1 Hz), 3.90–4.80 (5 H, m, H-2′,3′,4′,5′a,b), 1.06 (28 H, m, ipr).

1-[2-Deoxy-2-isocyano-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]uracil (12). A solution of **11** (224 mg, 0.44 mmol) and TsCl (405 mg, 2.1 mmol) in pyridine (8 mL) was heated at 70 °C for 1 h. MeOH (2 mL) was added to the mixture and the solvent was removed in vacuo. The residue was partitioned between AcOEt and H_2O. The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified on a silica gel column (2.4 × 10 cm), which was eluted with hexane:AcOEt (4:1 to 2:1) to afford **12** (177 mg, 82%, as a foam): IR ($CHCl_3$) ν N=C 2140 cm^{-1}; El-MS *m/z* 495 (M^+), 469 (M^+ - NC); NMR ($CDCl_3$) 8.63 (1 H, br s, 3-NH), 7.76 (1 H, d, H-6, $J_{6,5}$ = 8.5 Hz), 5.90 (1 H, s, H-1′), 5.72 (1 H, d, H-5, $J_{5,6}$ = 8.5 Hz), 3.96–4.44 (5 H, m, H-2′,3′,4′,5′a,b), 1.06 (28 H, m, isopr).

1-[2-Deoxy-2-isocyano-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]cytosine (13). A mixture of **12** (168 mg, 0.34 mmol), triisopropylbenzenesulfonyl chloride (206 mg, 0.68 mmol), and tetrabutylammonium bromide (20 mg) in a mixture of CH_2Cl_2 (15 mL) and 0.2 M aqueous Na_2CO_3 (20 mL) was vigorously stirred for 2.5 h at room temperature. The mixture was diluted with $CHCl_3$ (20 mL). The separated organic phase was washed with H_2O, dried (Na_2SO_4), and concentrated to dryness. Concentrated NH_4OH (28%, 2 mL) was added to a solution of the above residue in dioxane (5 mL) and the mixture

was stirred for 2 h at room temperature. The solvent was removed in vacuo and the residue was purified on a silica gel column (2.4 × 7.5 cm) eluted with 0–10% EtOH in $CHCl_3$ to give **13** (157 mg, 94% as a foam): IR (Nujol) ν N=C 2140 cm^{-1}; El-MS m/z 494 (M^+), 451 (M^+ – isopr); NMR ($CDCl_3$) 7.83 (1 H, d, H-6, $J_{6,5}$ = 7.6 Hz), 7.34 (2 H, br s, 4-NH_2), 5.92 (1 H, s, H-1′), 5.77 (1 H, d, H-5, $J_{5,6}$ = 7.6 Hz), 3.95–4.38 (5 H, m, H-2′,3′,4′,5′a,b), 1.05 (28 H, m, isopr).

[1-(Uracil-1-yl)-2,3-dideoxy-β-D-ribofuranosido][2,3-*d*]-2-oxazoline (15a). A THF solution of TBAF (1 M, 0.9 mL, 0.9 mmol) was added to a solution of **12** (177 mg, 0.36 mmol) in THF (3 mL) at 0 °C. The mixture was stirred for 30 min at 0 °C. The solvent was removed in vacuo, and the residue was purified on a silica gel column (2 × 6 cm) and then eluted with 4–12% EtOH in $CHCl_3$ to give **15a** (81 mg, 89% as a foam): FAB-MS m/z 254 (M^+ + 1); NMR (DMSO-d_6) 11.40 (1 H, br s, NH), 7.77 (1 H, d, H-6, $J_{6,5}$ = 7.7 Hz), 7.30 (1 H, br s, oxazoline-H), 5.74 (1 H, d, H-1′, $J_{1',2'}$ = 2.7 Hz), 5.66 (1 H, d, H-5, $J_{5,6}$ = 7.7 Hz), 5.11 (1 H, t, 5′-OH, J = 5.5 Hz), 4.99 (1 H, dd, H-3′, $J_{3',2'}$ = 7.1, $J_{3',4'}$ = 4.4 Hz), 4.80 (1 H, br dd, H-2′, $J_{2',3'}$ = 7.1, $J_{2',1'}$ = 2.7 Hz), 3.90 (1 H, dd, H-4′, $J_{3',4'}$ = 4.4 Hz), 3.64 (2 H, dd, H-5′a,b). Anal. ($C_{10}H_{11}N_3O_5$) C, H, N.

[1-(Cytosin-1-yl)-2,3-dideoxy-β-D-ribofuranosido][2,3-*d*]-2-oxazoline (15b). Compound **13** (150 mg, 0.30 mmol) was similarly treated with TBAF to give **15b** (65 mg, 87% as a foam): FAB-MS m/z 253 (M^+ + 1); NMR (DMSO-d_6) 7.67 (1 H, d, H-6, $J_{6,5}$ = 7.4 Hz), 7.24 (3 H, br d, 4-NH_2 and oxazoline-H), 5.70 (1 H, d, H-5, $J_{5,6}$ = 7.4 Hz), 5.64 (1 H, d, H-1′, $J_{1',2'}$ = 2.7 Hz), 4.95–5.01 (2 H, m, 5′-OH and H-3′), 4.72 (1 H, m, H-2′), 3.84 (1 H, dd, H-4′, $J_{3',4'}$ = 5.0 Hz), 3.56–3.67 (2 H, m, H-5′a, b).

1-(2-Deoxy-2-formamido-β-D-ribofuranosyl)uracil (16). (a) A solution of **15a** (30 mg, 0.11 mmol) in H_2O (2 mL) was stirred for 2 days and the solvent was removed in vacuo. The residue was purified on a silica gel column (1.8 × 5 cm) and eluted with 10% MeOH in $CHCl_3$ to give **16** (30 mg, 94%, as a foam): FAB-MS m/z 272 (M^+ + 1); NMR (400 MHz, DMSO-d_6) 11.25 (1 H, br s, NH), 8.15 (1 H, d, N*H*CHO, J = 8.2 Hz), 8.00 (1 H, s, NHC*H*O), 7.90 (1 H, d, H-6, $J_{6,5}$ = 8.2 Hz), 5.89 (1 H, d, H-1′, $J_{1',2'}$ = 8.8 Hz), 5.83 (1 H, d, 3′-OH, J = 4.4 Hz), 5.69 (1 H, d, H-5, $J_{5,6}$ = 8.2 Hz), 5.20 (1 H, t, 5′-OH, J = 4.9 Hz), 4.50 (1 H, m, H-2′), 4.08 (1 H, br s, H-3′), 3.94 (1 H, br s, H-4′), 3.59 (2 H, br d, H-5′a,b). (b) Compound **11** (100 mg) was deprotected with TBAF as described in the synthesis of **6a** to give **16** (27 mg, 52% as a foam) after silica gel column chromatographic purification.

1-(2-Deoxy-2-formamido-β-D-arabinofuranosyl)uracil (17). (a) A solution of **6a** (30 mg, 1.4 mmol) in H_2O (2 mL) containing AcOH (5 μL) was stirred for 5 days at room temperature. The solvent was removed in vacuo and the residue was purified on a silica gel column (1.7 × 5 cm) with 4–8% EtOH in $CHCl_3$ to give **17** (28 mg, 88%, as a foam): FAB-MS m/z 273 (M^+ + 1); NMR (400 MHz, DMSO-d_6) 11.22 (1 H, br s, NH), 8.19 (1 H, br d, N*H*CHO, J = 8.8 Hz), 7.97 (1 H, s, NHC*H*O), 7.75 (1 H, d, H-6, $J_{6,5}$ = 8.2 Hz), 6.04 (1 H, d, H-1′, $J_{1',2'}$ = 6.0 Hz), 5.57 (1 H, d, H-5, $J_{5,6}$ = 8.2 Hz), 4.48 (1 H, dd, H-2′, after addition of D_2O, it became a triplet), 4.35 (1 H, t, 5′-OH, J = 4.9 Hz), 4.03 (1 H, dd, H-3′, after addition of D_2O, it became a triplet), 3.58–3.80 (3 H, m, H-4′, 5′a,b). (b) Compound **4a** (60 mg, 0.12 mmol) was deprotected with TBAF as described in the synthesis of **6a** to give **17** (25 mg, 79% as a foam) after silica gel column chromatographic purification.

Cytotoxicity Test. Mouse L1210 leukemia, mouse Lewis lung carcinoma (LLC), KATO III human gastric cancer, and human oral epidermoid carcinoma KB cell lines were cultured in a RPMI 1640 medium supplemented with 10% FCS. In cytotoxicity test, cells (2–3 × 10^3 cells) harvested by treatment of 0.25% trypsin–0.02% EDTA solution, compounds dissolved in saline were added to the wells, and the cells were exposed to them for 3 days in a CO_2 incubator. Then, cells were fixed with 0.1% glutaraldehyde for 10 min. After being washed in running water, cells were stained with 0.01% crystal violet for 20 min and then washed again in running water. The dye was extracted with 50% EtOH–25 mM NaH_2PO_4 solution and absorbance at OD_{254} nm was measured by a plate reader (Corona, Tokyo Japan). The IC_{50} values were defined as the concentration needed for 50% reduction of optical density in each test.

Antitumor Test. A LLC fragment (2 × 2 × 2 mm) was implanted subcutaneously into the backs of 9-week-old male BDF1 mice (Japan Clea Inc.) on day 0. A group of seven mice was used for each dose level. The compounds tested were dissolved in saline and given intraperitoneously once a day for 5 days at a volume of 0.1 mL/10 g of mouse body weight. The percentage of inhibition of the tumor growth of LLC was calculated by comparing the average tumor weight of drug-treated and control animals (vehicle only) on day 17.

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