Nucleosides and Nucleotides. 122. 2′-C-Cyano-2′-deoxy-1-β-D-arabinofuranosylcytosine and Its Derivatives. A New Class of Nucleoside with a Broad Antitumor Spectrum[1]

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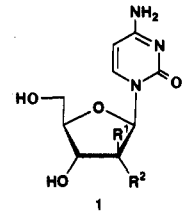
Design, synthesis, and tumor cell growth inhibitory effects of 2′-C-cyano-2′-deoxy-1-β-D-arabinofuranosyl derivatives of cytosine (**1i**, CNDAC), thymine (**6a**), uracil (**6c**), and adenine (**6d**) have been described. The synthesis of the target compounds was achieved from the corresponding 2′-keto nucleosides **2a–d**. Cyanohydrins of **2a–d** were converted to thionocarbonates, which were deoxygenated to give the desired 2′-β-cyano-2′-deoxy derivatives **5a–d**, followed by deprotection to furnish the target nucleosides. Of these nucleosides, CNDAC was the most potent inhibitor of cell growth with an IC_{50} value of 0.53 μM against L1210 cells. In vitro cytotoxicity of CNDAC against human tumor cell lines was also examined; compared with that of 1-β-D-arabinofuranosylcytosine (*ara*-C) and 5-fluorouracil (5-FU), CNDAC was more cytotoxic to several cell lines refractory to *ara*-C. The in vivo effect of CNDAC on M5076 mouse reticulum cell sarcoma was very strong; 99% tumor volume inhibition on day 20 was achieved when it was administered orally on days 1, 4, 7, 10, 13, and 16 at a dose of 400 mg/kg/day, while 5′-deoxy-5-fluorouridine (5′-DFUR) and 5-FU caused only 50% inhibition at a dose of 500 mg/kg/day and 28% inhibition at a dose of 50 mg/kg/day, respectively, on the same schedule. These results indicated that CNDAC may have potential as a new antineoplastic agent with a broad antitumor spectrum.

1-β-D-Arabinofuranosylcytosine (*ara*-C, **1a**) is recognized as one of the most effective drugs for the treatment of adult acute myeloblastic leukemia.[2,3] During studies of metabolism and pharmacology of **1a**, it has been found to have several drawbacks including its short half-life in plasma due to rapid deamination to chemotherapeutically inactive 1-β-D-arabinofuranosyluracil by the action of cytidine deaminase, development of resistance, and ineffectiveness against solid tumors.[4-7] Consequently, with the objective of overcoming these problems, efforts have been made to develop a number of prodrugs[8] or to substitute certain functional groups other than the hydroxyl group at the 2′-position. By the latter approach, a number of 2′-deoxy-2′-substituted-β-D-arabinofuranosylcytosines have been synthesized (Chart I).[9-20] For these nucleosides to hav antitumor activity, they must be phosphorylated at the 5′-position before they could inhibit certain target enzymes to cause tumor cell death, and therefore the relationship between their antitumor activity and substrate specificity for deoxycytidine kinase seems to be important when they act as antimetabolites.

We have synthesized (2′*S*)-2′-deoxy-2′-*C*-methylcytidine (SMDC, **1f**) and (2′*S*)-2′-deoxy-2′-*C*-ethylcytidine (SEDC, **1g**) from uridine.[16-18] Against L1210 cells in vitro, **1f** was as potent as **1a** but **1g** was not effective up to 100 μg/mL.[18] Additionally, 2′-deoxy-2′-(methylthio)-β-D-arabinofuranosylcytosine (**1h**) did not show any cytotoxicity toward L5178Y cells in vitro up to 100 μg/mL.[19] Therefore, it appears that increasing bulkiness of the 2′-substituent greatly reduced cytotoxicity, which might be related to a reduction in substrate specificity for deoxycytidine kinase.

Chart I



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	R^1	R^2
a	OH	H
b	F	H
c	F	F
d	N_3	H
e	NH_2	H
f	CH_3	H
g	CH_2CH_3	H
h	SCH_3	H
i	CN	H

Another approach to design a new type of antitumor nucleosides is to construct analogues that have a chemically reactive functionality at the 2′-β position of 2′-deoxycytidine, which should be chemically stable at the nucleoside level but would be expected to be reactive after incorporation into DNA.[20,21] Introduction of an electron-withdrawing group at the 2′-β position of 2′-deoxycytidine is expected to increase the acidity of the 2′-α proton. If such a nucleoside is enzymatically phosphorylated to the corresponding 5′-triphosphate and incorporated into DNA, the electron-withdrawing group will be in a β position relative to the 3′-phosphate diester and the anomeric carbon where the electron-withdrawing nucleobase is attached. In this case, β elimination would produce (i) a DNA strand break (path A) or formation of an abasic site (path B) as shown in Figure 1. Since strand breaks in DNA by radiation therapy have been hypothesized to cause tumor cell death, it is worth examining whether nucleosides with such chemical reactivities inhibit tumor cell growth. As an example of a nucleoside with an electron-withdrawing group at the 2′-β position of 2′-deoxycytidine, we chose the cyano group as an example. We have already reported the synthesis of 2′-C-cyano-2′-deoxy-1-β-D-arabinofuranosylcytosine (CNDAC, **1i**).[20] CNDAC is quite

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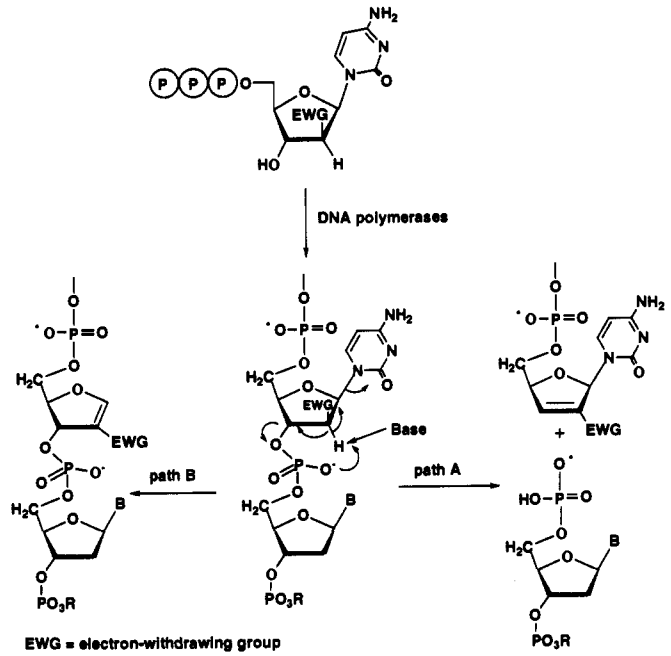


Figure 1. Possible reaction pathways of a DNA containing a deoxycytidine having an electron-withdrawing group at the 2′-β position.

cytotoxic to various human tumor cells including sarcomas, osteosarcomas, and fibrobrastomas in vitro as well as in vivo with a unique spectrum different from that of *ara*-C.[22] This report deals with the detailed synthesis of CNDAC and its derivatives and also the inhibitory effects of CNDAC on various human tumor cell lines in vitro as well as in vivo.

Chemistry. Introduction of a certain substituent into the 2′-β position of pyrimidine nucleosides is rather difficult because the intramolecular nucleophilic attack of the 2-carbonyl group of the pyrimidine base on the 2′-position bearing a leaving group predominates over the intermolecular nucleophilic substitution. Reduction of the nucleophilicity of the 2-carbonyl group by N^3-benzoyl group gave rise to a 2′-β azido derivative of uridine in good yield under Mitsunobu reaction conditions using diphenyl phosphorazidate.[15,23] However, the reaction with diethylphosphoryl cyanide or hydrogen cyanide under similar conditions was unsuccessful. On the other hand, alkyl addition reactions to the 2′-keto nucleoside followed by deoxygenation of the methyl oxalyl ester of the resulting *tert* alcohol gave the desired (2′*S*)-2′-alkyl-2′-deoxy derivatives.[16-18,24] This method was also applied to the synthesis of 2′-deoxy-2′-ethynyl derivatives.[25] Since this deoxygenation method proceeded under neutral conditions, it is compatible with the target cyano nucleosides possessing an acidic 2′-α proton. Thus, we applied this method for the synthesis of 2′-*C*-cyano-2′-deoxy-1-β-D-arabinofuranosyl nucleosides through a cyanohydrin formation followed by deoxygenation of the hydroxyl group.[26,27]

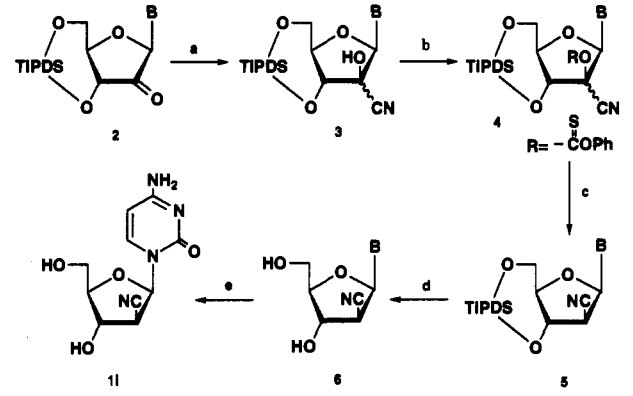
Initially, we started using 5-methyluridine derivatives. When 2′-keto nucleoside **2a**[21] was treated with NaCN in a mixture of aqueous $NaHCO_3$ solution and Et_2O, the desired cyanohydrin **3a** was obtained in 98% yield as an isomeric mixture. Next, **3a** was treated with phenyl chlorothionoformate in the presence of triethylamine and *p*-(dimethylamino)pyridine (DMAP) in CH_3CN at room temperature to give the corresponding thiocarbonate **4a**, on which without purifications, radical deoxygenation was done using Bu_3SnH in the presence of AIBN in toluene. Only one nucleosidic product (**5a**) was obtained from the reaction mixture as a foam. Removal of the silyl protecting group of **5a** was tried using tetrabutylammonium fluoride (TBAF) in the presence of acetic acid in THF to furnish the desired 2′-*C*-cyano-2′-deoxy-1-β-D-arabinofuranosylthymine (**6a**) in 78% yield as a crystal. Without addition of acetic acid in the reaction media, a greatly reduced yield of **6a** was observed because of cleavage of the glycosidic bond releasing thymine. The configuration at the 2′-position was confirmed by NOE experiments (see Experimental Section). Therefore, radical deoxygenation of **4a** proceeded stereospecifically to furnish the arabinonucleoside **5a** due to the steric hindrance of the β face.[16-18,25]

In the cytosine series, we started with N^4-acetyl-1-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-*erythro*-pentofuran-2-ulosyl]cytosine (**2b**). Compound **2b** was treated with NaCN as above to afford an epimeric mixture of 2′-cyanohydrins **3b**, after 35 h. However, we found that use of phosphate buffer (1 M, pH 7.5) instead of water remarkably shortened the reaction time of formation of the cyanohydrin. Compound **3b**, without purification, was further treated with phenyl chlorothionoformate in the presence of 1 equiv of DMAP in CH_3CN to afford thiocarbonate **4b**. This nucleoside was, without purification, heated with Bu_3SnH and AIBN in toluene at 100 °C, giving the crystalline deoxygenated nucleoside **5b** in 57% yield from **2b**. The configuration at the 2′-position was also confirmed by NOE experiments to be 2′-β. Deblocking of the silyl group was done with TBAF in the presence of AcOH in THF to provide **6b** in 84% yield. Removal of the N^4-acetyl group was attempted with methanolic ammonia at room temperature to give only cytosine (data not shown). Acid-catalyzed deprotection of **6b** was also attempted with MeOH in the presence of AcOH[28] under refluxing conditions for 5 days to furnish the desired 2′-*C*-cyano-2′-deoxy-1-β-D-arabinofuranosylcytosine (CNDAC, **1i**) in 39% yield after chromatographic purifications. On treatment of **6b** with 1% HCl in MeOH, the reaction proceeded much faster to furnish CNDAC as a hydrochloride in 89% yield. The configuration at the 2′ position of **6b** and its hydrochloride, **1i**·HCl was also confirmed by an NOE experiment to be the 2′-β. Therefore, it was not changed during the acid treatment.

The uracil and adenine congeners **6c** and **6d** were also synthesized by similar sequences described in Scheme I. In the synthesis of the adenine derivative **6d**, protection of the exocyclic amino group was not necessary in all the reaction sequences. Quite recently, Velazquez and Camarasa reported the synthesis of **6c,d** and **1i** in a similar manner in low yields.[29] However, they could not isolate **1i** due to its instability under basic conditions in the deprotection step. It should be noted that deprotection of the TIPDS groups in the sugar moiety of **5** with TBAF should be done with added acetic acid to make the reaction mixture acidic.

Next, a model study of the stability of **6** when incorporated into DNA was done (Scheme II). Compound **6b** was treated with dimethoxytrityl chloride to afford **7b** in good yield. Attempts to introduce a phosphate group into the 3′-hydroxyl group of **7b** failed when we used 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite under standard conditions for oligonucleotide synthesis. However, reaction of **7b** with *N,N*′-thiocarbonyldiimidazole in *N,N*-dimethylformamide (DMF) for 2 h at room temper-

Scheme I[a]



a series B = Thymin-1-yl

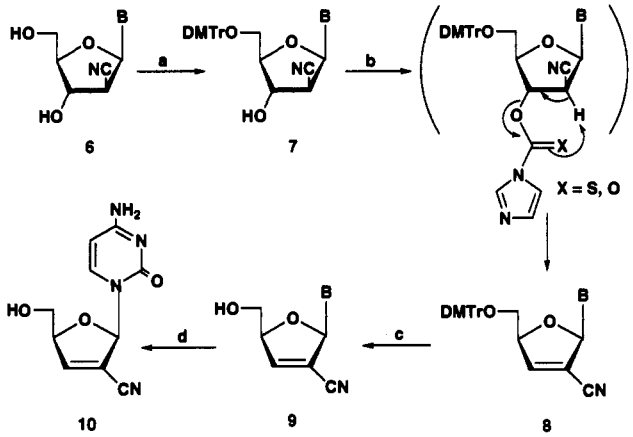
b series B = N^4-Acetylcytosin-1-yl

c series B = Uracil-1-yl

d series B = Adenin-1-yl

[a] (a) NaCN in 1 M phosphate buffer (pH 7.5), room temperature; (b) phenyl chlorothionoformate, Et_3N, DMAP in CH_3CN, 0 °C; (c) Bu_3SnH, AIBN in toluene, 100 °C; (d) TBAF, AcOH in THF, room temperature; (e) HCl/MeOH, 0 °C.

Scheme II[a]



a series B = Thymin-1-yl

b series B = N^4-Acetylcytosin-1-yl

[a] (a) DMTrCl in pyridine, room temperature; (b) *N,N'*-thiocarbonyldiimidazole or *N,N'*-carbonyldiimidazole in DMF, room temperature; (c) aqueous AcOH, room temperature; (d) 1% HCl/MeOH, room temperature.

ature furnished a β-elimination product, the 2'-*C*-cyano-2',3'-didehydro-2',3'-dideoxy nucleoside **8b** in 73% yield.[30] Such a *syn* elimination proceeded smoothly using a thionocarbonate but in the case of a carbonate or an ester, forced conditions are usually required. However, on treatment of **7b** with *N,N'*-carbonyldiimidazole for 3 h at room temperature in DMF, **8b** was obtained almost in quantitative yield. These reactions provided chemical evidence for the sensitivity of CNDAC to β elimination when the 3'-alcohol was activated. Such chemical reactivity would be expected from CNDAC if its nucleotide form were incorporated into DNA. Compound **8b** was detritylated to afford **9b** followed by deacetylation, giving **10**. Under a similar sequence, the thymine derivative **9a** was also prepared.

Antitumor Activity. A first screening for tumor cell growth inhibitory effects of the compounds synthesized was done using cultured murine leukemia L1210 and human oral epidermoid carcinoma KB cells for 72-h incubations, which are summarized in Table I. Among them, CNDAC was the most effective nucleoside in the series; IC_{50} values were 0.53 and 5.2 μM, respectively. Although these values are about ten times less potent against L1210 and about two times less potent against KB than those of *ara*-C, they are comparable to those of 2'-deoxy-2'-methylidenecytidine (DMDC),[21,31] which is now in phase I clinical trial in Japan, SMDC (**1f**),[18] and cytarazid (**1d**).[13,15] N^4-Acetyl-CNDAC (**6b**) was less potent than CNDAC itself, and other pyrimidine analogues, CNDAT (**6a**) and CNDAU (**6c**), were almost inactive up to 100 μg/mL in both cell lines. On the other hand, CNDAA (**6d**) showed cytotoxicity at lower concentrations against L1210 cells, although the concentration was about ten times higher than that of CNDAC. Elimination products **9a,b** were somewhat cytotoxic to both cell lines but were less effective than the corresponding CNDAC and **6b**. The thymine derivative **9a** was more potent than the cytosine counterpart **10**. Next we compared the cytotoxicity of CNDAC, CNDAA, **10**, and *ara*-C against 15 types of human solid tumor cell lines and the results are listed in Table II. CNDAC has a broader spectrum of activity than that of *ara*-C. Together with previous results[22] of cytotoxicity toward various human tumor cell lines, CNDAC showed potent tumor cell growth inhibitory activity toward a wide variety of human tumor cells with a spectrum quite different from that of *ara*-C. On the other hand, CNDAA was almost inactive toward all the cell lines used in this experiment. While 2-chlorodeoxyadenosine has recently been introduced for the treatment of hairy cell leukemia,[7] and is also activated by deoxycytidine kinase, it is devoid of activity against human solid tumor cells.[33] Therefore, there is a consistent lack of activity for these adenine analogues. Compound **10** does not have the 3'-hydroxyl group in the sugar moiety and is much less effective than CNDAC. These results indicated that CNDAC itself would be an active precursor for the cytotoxicity but not its degradation side product. However, differences in substrate specificities between nucleoside kinases such as deoxycytidine kinase and thymidine kinase to recognize the 2'-β-cyano group might explain these results.

We also evaluated in vivo antitumor activity of CNDAC against M5076 mouse reticulum cell sarcomas implanted subcutaneously into the axillary region of female $BD2F_1$ mice and compared the activity with that of 5-fluorouracil

Table I. In Vitro Cell Growth Inhibitory Activity of the Cyano Nucleosides against Murine Leukemia L1210 and Human Oral Epidermoid Carcinoma KB Cells[a] (IC_{50}, μM)[b]

compds	L1210	KB
CNDAC (1i·HCl)	0.53	5.2
CNDAT (**6a**)	6.7%[c]	−6.0%[c]
6b	25.3	230
CNDAU (**6c**)	10.2%[c]	8.4%[c]
CNDAA (**6d**)	5.8	163
9a	12.4	30.4
9b	68.8	97.8
10	261	46.8%[c]
ara-C	0.05	2.3

[a] Tumor cell growth inhibitory activity assay in vitro was done following the method of Carmichael et al.[32] Each tumor cell (2×10^3/well) was incubated in the presence or absence of compounds for 72 h. Then, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added. After incubation for 4 h more, 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. [b] IC_{50} (μM) was given as the concentration at 50% inhibition of cell growth. [c] Inhibition at 100 μg/mL.

Table II. Inhibitory Effects of CNDAC (1i), CNDAA (6d), 10, and *ara*-C on the Growth of Various Human Tumor Cell Lines in Vitro[a]

cell lines	origin	IC_{50},[b] μM CNDAC (1i-HCl)	CNDAA (6e)	10	*ara*-C (1a)
PC-8	lung adenocarcinoma	15.9	>360	>430	1.1
PC-9	lung adenocarcinoma	15.3	>360	>430	6.6
PC-13	lung large cell carcinoma	86.8	>360	363	>410
QG-95	lung squamous cell carcinoma	138	>360	427	>410
ST-KM	stomach adenocarcinoma	9.71	>360	350	>410
MKN-45	stomach adenocarcinoma	15.9	>360	376	57.6
KATO-III	stomach adenocarcinoma	55.6	>360	31.6	>410
KKLS	stomach adenocarcinoma	350	>360	>430	>410
SW-48	colon adenocarcinoma	>350	>360	273	>410
Colo-320	colon adenocarcinoma	1.40	>360	150	0.04
MCF-7	breast adenocarcinoma	1.75	>360	299	0.62
OST	osteosarcoma	22.2	>360	235	>410
MNNG/HOS	osteosarcoma	23.6	>360	205	10.7
HT-1080	fibrosarcoma	0.53	>360	248	0.53
A-375	melanoma	1.40	308	145	>410

[a] See Table I for the tumor cell growth inhibitory assay.

(5-FU) and 5′-deoxy-5-fluorouridine (5′-DFUR), which has recently been introduced in clinical trials as a prodrug of 5-FU against solid tumors. The M5076 cells were known to be sensitive to alkylating agents and nitrosoureas but apparently were unresponsive to antimetabolites such as methotrexate and 5-FU.[34] In this experiment, tumor volume inhibition and T/C values were used as the parameters of antitumor activity and day 20 was chosen as the day of evaluation as shown in Table III. When CNDAC was administered orally on days 1, 4, 7, 10, 13, and 16, at a dose of 400 mg/kg/day, it showed 99% inhibition of tumor volume on day 20 (T/C = 133%). Even at a dose of 200 mg/kg/day under the same schedule of drug administration, the T/C was calculated to be 136% with 93% tumor volume inhibition. In these cases, long-time survivors were observed as described in Table III. However, 5-FU and 5′-DFUR administered in the same way were much less effective than CNDAC, and long-time survivors were not observed. As was reported by us,[20] CNDAC had excellent activities on the treatment of murine leukemic P388 with a T/C (%) of >600 for intraperitoneal administration once each day on days 1–10 at a dose of 20 mg/kg/day and five out of six mice survived over 60 days, while under the same conditions *ara*-C had only a T/C value of 225% without observation of long-time survivors. We also reported that CNDAC was quite effective against solid tumors, refractory to *ara*-C, such as HT1080 human fibrosarcoma implanted in chick embryos or athymic mice.[22] Thus, CNDAC showed quite different antitumor activities in vivo from those of *ara*-C, although it is an analogue of *ara*-C. When the activity of CNDAC was compared with 5-FU and its prodrug, 5′-DFUR, against M5076 mouse reticulum cell sarcoma, it was also superior to 5-FU and 5′-DFUR, while its in vitro inhibitory activity toward human cell lines did not appear promising enough.

Table III. Antitumor Activity of CNDAC, 5′-DFUR, and 5-FU against M5076 Mouse Reticulum Cell Sarcoma by Oral Administration in Mice[a]

compds	dose, mg/kg/dose	weight change (g)[b]	inhibition (%) on day 20	T/C (%)[c]	60-day survivors
CNDAC	400	1.1	99	133	1/3
	300	1.0	97	120	0/6
	200	2.0	93	136	2/6
	100	1.7	79	118	0/6
	50	1.0	63	123	0/6
5′-DFUR	750	0.8	50	92	0/6
	500	2.5	19	104	0/6
	250	2.0	15	86	0/6
	125	1.8	18	98	0/6
	62.5	2.8	8	88	0/6
5-FU	50	1.4	28	79	0/6
	40	2.1	17	79	0/6
	30	2.4	9	86	0/6
	20	2.2	6	85	0/6
	10	2.4	1	94	0/6
control		2.9	a	a	0/9

[a] M5076 cells (10^6) were implanted subcutaneously into the axillary region of female BDF_1 mice and each drug was given orally on days 1, 4, 7, 10, 13, and 16. Average volume of tumors in control mice 20 days after tumor inoculation was 1480.3 mm^3 and median survival of untreated control mice was 42 days. [b] The changes in body weight of mice were obtained by subtracting the average body weight on day 1 from that on day 20. [c] Tumor growth inhibition and T/C % were calculated from the following equations: Tumor growth inhibition (%) = (1 − Vt/Vc) × 100; Vt = average value of tumor volume on the 20th day in the treated group and Vc = average value of tumor volume on the 20th day in the untreated control group. Tumor volume = 0.5 × length × width^2. T/C (%) = Dt/Dc × 100; Dt = median survival day of treated mice and Dc = median survival day of untreated control mice.

In summary, we have designed and synthesized a new type of antitumor nucleoside, 2′-*C*-cyano-2′-deoxy-1-β-D-arabinofuranosylcytosine (CNDAC). Although its in vitro activity against human tumor cell lines was not outstanding, it has significant antitumor activity in vivo against P388 mouse leukemia, M5076 mouse reticulum cell sarcoma, and HT1080 human fibrosarcoma, which is refractory to *ara*-C. From these results, its mode of action might be different from that of *ara*-C and to elucidate the differences between them would be important for further developments.

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. The NOE measurements were done using JEOL JNM-GX 270 and the solutions (about 0.05 M in DMSO-d_6) were degassed by bubbling N_2 through it followed by ultrasound sonication. UV absorption spectra were recorded with a Shimadzu UV-240 spectrophotometer. Mass spectra (MS) were measured in a JEOL JMX-DX303 spectrometer. IR spectra were 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-*C*-Cyano-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-pentofuranosyl]thymine (3a). Sodium cyanide (196 mg, 4 mmol) was added to a solution of **2a** (997 mg, 2 mmol) in a mixture of Et_2O–H_2O (2:1 v/v, 15 mL). The mixture was vigorously stirred for 36 h at room temperature. Ethyl acetate (50 mL) was added to the mixture and the whole was extracted with H_2O (3 × 10 mL). The separated organic phase was dried (Na_2SO_4) and

concentrated to dryness. The residue was purified by silica gel column chromatography (2.4 × 9.5 cm) with hexane:AcOEt (2:1) to give **3a** (1.03 g, 97.5% as a foam): MS *m/z* 482 (M^+ – isopr); IR ($CHCl_3$) ν C≡N 2250 cm^{-1}; NMR (100 MHz, $CDCl_3$) 9.20 and 8.52 (1 H, br s, 3-NH), 7.43 and 7.36 (1 H, d, H-6, $J_{6,Me}$ = 1.7 Hz), 6.22 and 6.00 (1 H, s, H-1'), 5.08 (1 H, br s, 2'-OH), 4.32-3.94 (4 H, m, H-3', 4', 5'a,b), 1.92 (3 H, d, 5-Me, $J_{Me,6}$ = 1.7 Hz), 1.12–1.07 (28 H, m, isopr).

1-[2-C-Cyano-2-deoxy-3,5-O-(tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]thymine (5a). Phenyl chlorothionocarbonate (39 μL, 0.28 mmol) was added to a solution of **3a** (100 mg, 0.19 mmol), DMAP (10 mg), and Et_3N (40 μL, 0.29 mmol) in CH_3CN (2 mL) under an argon atmosphere at 0 °C. The mixture was stirred for 3 h at 0 °C and then diluted with AcOEt (15 mL). The whole was washed with H_2O (3 × 5 mL) and the separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was coevaporated two times with toluene and was dissolved in toluene (2 mL). Bu_3SnH (80 μL, 0.3 mmol) was added to the above solution containing AIBN (5 mg) at 100 °C under an argon atmosphere. After being heated for 45 min, the solvent was removed in vacuo. The residue was purified over a silica gel column (1.6 × 10 cm) with 1% MeOH in $CHCl_3$ to give **5a** (71 mg, 73%, as a pale yellow foam): MS *m/z* 466 (M^+ – isopr): IR ($CHCl_3$) ν C≡N 2260 cm^{-1}; NMR (270 MHz, $CDCl_3$) 7.36 (1 H, d, H-6, $J_{6,Me}$ = 1.2 Hz), 6.28 (1 H, d, H-1', $J_{1',2'}$ = 7.3 Hz), 4.67 (1 H, dd, H-3', $J_{3',2'}$ = 9.3, $J_{3',4'}$ = 8.3 Hz), 4.17 (1 H, dd, H-5'a, $J_{5'a,4'}$ = 2.2, $J_{a,b}$ = 13.2 Hz), 4.04 (1 H, dd, H-5'b, $J_{5'b,4'}$ = 2.9, $J_{a,b}$ = 13.2 Hz), 3.78 (1 H, ddd, H-4', $J_{4',3'}$ = 8.3, $J_{4',5'a}$ = 2.2, $J_{4',5'b}$ = 2.9 Hz), 3.58 (1 H, dd, H-2', $J_{2',1'}$ = 7.3, $J_{2',3'}$ = 9.3 Hz), 1.94 (3 H, d, 5-Me, $J_{Me,6}$ = 1.2 Hz), 1.15–1.04 (28 H, m, isopr).

General Method for the Synthesis of 1-[2-C-Cyano-2-deoxy-3,5-O-(tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]pyrimidines and -adenine (5b,c,d). A solution of **2b**, **2c**, or **2d** (6 mmol) in a mixture of Et_2O (10 mL) and THF (5 mL) was added to a mixture of NaCN (1.0 g, 12 mmol) in phosphate buffer (1 M, pH 7.5, 10 mL). The mixture was vigorously stirred for 2 h at room temperature and then diluted with AcOEt (50 mL). The whole was transferred to a separate funnel. The separated organic phase was washed with H_2O (20 mL) and brine (20 mL), dried (Na_2SO_4), and concentrated to dryness in vacuo. The residue was coevaporated two times with toluene and dissolved in dry CH_3CN (40 mL). Triethylamine (1.25 mL, 9 mmol) was added to the above solution containing DMAP (200 mg) and phenyl chlorothionoformate (1.2 mL, 8.7 mmol) at 0 °C under an argon atmosphere. After the solution was stirred for 2 h at room temperature, the solvent was removed in vacuo. The residue was taken up in AcOEt (50 mL), which was washed with H_2O (2 × 20 mL). The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was coevaporated two times with toluene and dissolved in dry toluene (50 mL). Bu_3SnH (3.0 mL, 11.2 mmol) was added dropwise to the above solution containing AIBN (40 mg) at 100 °C. The mixture was further heated for 3 h at 100 °C and the solvent was removed in vacuo. The residue was purified on a silica gel column.

N^4-Acetyl-1-[2-C-cyano-2-deoxy-3,5-O-(tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]cytosine (5b). The reaction was done as above and the residue was purified on a silica gel column (3 × 25 cm) eluted with 10–30% AcOEt in hexane. The homogeneous product **5b** (1.69 g, 57%, crystallized from Et_2O–hexane) was obtained: mp 209–211 °C; MS *m/z* 493 (M^+ – isopr); IR ($CHCl_3$) ν C≡N 2250 cm^{-1}; NMR (270 MHz, $CDCl_3$) 9.92 (1 H, br s, 4-NH), 8.07 (1 H, d, H-6, $J_{6,5}$ = 7.7 Hz), 7.55 (1 H, d, H-5, $J_{5,6}$ = 7.7 Hz), 6.34 (1 H, d, H-1', $J_{1',2'}$ = 7.0 Hz), 4.63 (1 H, t, H-3', $J_{3',2'}$ = $J_{3',4'}$ = 8.1 Hz), 4.18 (1 H, dd, H-5'a, $J_{5'a,4'}$ = 2.4, $J_{a,b}$ = 13.4 Hz), 4.06 (1 H, dd, H-5'b, $J_{5'b,4'}$ = 2.7, $J_{a,b}$ = 13.4 Hz), 3.89 (1 H, ddd, H-4', $J_{4',3'}$ = 8.8, $J_{4',5'a}$ = 2.4, $J_{4',5'b}$ = 2.7 Hz), 3.72 (1 H, dd, H-2', $J_{2',1'}$ = 7.0, $J_{2',3'}$ = 8.8 Hz), 2.30 (3 H, s, Ac), 1.13–1.03 (28 H, m, isopr). Anal. ($C_{24}H_{40}N_4O_6Si_2$) C, H, N.

1-[2-C-Cyano-2-deoxy-3,5-O-(tetraisopropyldisiloxane-1,3-dilyl)-β-D-arabinofuranosyl]uracil (5c). The reaction was done as above and the residue was purified on a silica gel column (3 × 25 cm) eluted with 10–30% AcOEt in hexane. The homogeneous product **5c** (1.4 g, 48% as a foam) was obtained from the appropriate fractions. An analytical sample was crystallized from hexane–AcOEt: mp 156–157 °C.[29] Anal. ($C_{22}H_{37}N_3O_6Si_2$) C, H, N.

1-[2-C-Cyano-2-deoxy-3,5-O-(tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]adenine (5d). This reaction was done on a 1-mmol scale. The residue was purified on a silica gel column (3 × 10 cm) with 1–15% EtOH in $CHCl_3$ to give **5d** (336 mg, 67%, as a foam).[29]

General Method for the Deblocking of the Silyl Group. A THF solution of TBAF (1 M, 2 mL, 2 mmol) was added to a mixture of **5a**, **5b**, **5c**, or **5d** (1 mmol) in THF (5 mL) containing AcOH (0.06 mL, 1 mmol). The mixture was stirred for 10–15 min at room temperature and was concentrated to dryness in vacuo.

1-(2-C-Cyano-2-deoxy-β-D-arabinofuranosyl)thymine (6a). The residue was purified on a silica gel column (1.8 × 8 cm), which was eluted with 8% EtOH in $CHCl_3$ to afford **6a** (208 mg, 78%, crystallized from Et_2O–EtOH): mp 173.5–176 °C; MS *m/z* 267 (M^+); IR (Nujol) ν C≡N 2260 cm^{-1}; NMR (400 MHz, DMSO-d_6) 11.49 (1 H, br s, 3-NH), 7.85 (1 H, d, H-6, $J_{6,Me}$ = 1.1 Hz), 6.25 (1 H, d, 3'-OH, *J* = 6.0 Hz), 6.20 (1 H, d, H-1', $J_{1',2'}$ = 7.1 Hz), 5.30 (1 H, t, 5'-OH, *J* = 4.9 Hz), 4.47 (1 H, ddd, H-3', $J_{3',2'}$ = 8.8, $J_{3',OH}$ = 6.0, $J_{3',4}$ = 8.2 Hz), 3.89 (1 H, dd, H-2', $J_{2',1'}$ = 7.1, $J_{2',3'}$ = 8.8 Hz), 3.74 (1 H, ddd, H-5'a, $J_{5'a,4'}$ = 2.2, $J_{5'a,OH}$ = 4.9, $J_{a,b}$ = 11.1 Hz), 3.70 (1 H, ddd, H-4', $J_{4',3'}$ = 8.2, $J_{4',5'a}$ = 2.2, $J_{4',5'b}$ = 3.3 Hz), 3.62 (1 H, ddd, H-5'b, $J_{5'b,4'}$ = 3.3, $J_{5'b,OH}$ = 4.9, $J_{a,b}$ = 11.1 Hz), 1.78 (3 H, d, 5-Me, $J_{Me,6}$ = 1.1 Hz). When the H-2' was saturated, an NOE was observed at the H-1' about 15%. Also the H-6 was irradiated and an NOE was detected at the H-1' but not at the H-2'. Anal. ($C_{11}H_{13}N_3O_5$) C, H, N.

N^4-Acetyl-1-(2-C-cyano-2-deoxy-β-D-arabinofuranosyl)cytosine (6b). The residue was purified on a silica gel column (1.8 × 8 cm), which was eluted with 8% EtOH in $CHCl_3$ to afford **6b** (252 mg, 84%, crystallized from Et_2O–hexane): mp 210 °C dec; MS *m/z* 295 (M^+ + 1); IR (Nujol) ν C≡N 2260 cm^{-1}; NMR (400 MHz, DMSO-d_6) 10.97 (1 H, br, s, 4-NH), 8.36 (1 H, d, H-6, $J_{6,5}$ = 7.7 Hz), 7.26 (1 H, d, H-5, $J_{5,6}$ = 7.7 Hz), 6.27 (1 H, d, 3'-OH, *J* = 6.1 Hz), 6.22 (1 H, d, H-1', $J_{1',2'}$ = 7.1 Hz), 5.24 (1 H, br s, 5'-OH), 4.43 (1 H, ddd, H-3', $J_{3',2'}$ = $J_{3',4'}$ = 7.1, $J_{3',OH}$ = 6.1 Hz), 3.92 (1 H, t, H-2', $J_{2',3'}$ = $J_{2',1'}$ = 7.1 Hz), 3.84 (1 H, ddd, H-4', $J_{4',3'}$ = 7.1, $J_{4',5'a}$ = 2.8, $J_{4',5'b}$ = 3.3 Hz), 3.76 (1 H, br d, H-5'a, $J_{a,b}$ = 12.1 Hz), 3.63 (1 H, br d, H-5'b, $J_{a,b}$ = 12.1 Hz). Anal. ($C_{12}H_{14}N_4O_5 \cdot {}^1/_4H_2O$) C, H, N.

1-(2-C-Cyano-2-deoxy-β-D-arabinofuranosyl)uracil (6c). The residue was purified on a silica gel column (2 × 12 cm), eluted with 5–10% MeOH in $CHCl_3$ to afford **6c** (192 mg, 76%, crystallized from Et_2O–EtOH): mp 151 °C.[29] Anal. ($C_{10}H_{11}N_3O_5 \cdot {}^1/_2H_2O$) C, H, N.

1-(2-C-Cyano-2-deoxy-β-D-arabinofuranosyl)adenine (6d). The residue was purified on a silica gel column (3 × 12 cm), eluted with 0–20% EtOH in $CHCl_3$ to afford **6d** (229 mg, 83%, crystallized from EtOH): mp 193–195 °C.[29] Anal. ($C_{11}H_{12}N_6O_3$) C, H, N.

1-(2-C-Cyano-2-deoxy-β-D-arabinofuranosyl)cytosine (1i). A solution of **6b** (100 mg, 0.34 mmol) in MeOH (55 mL) containing AcOH (2.5 mL) was heated under reflux for 5 days and the solvent was removed in vacuo. The residue was purified on a silica gel column (1.8 × 7 cm) eluted with 12-15% MeOH in $CHCl_3$ to give **1i** (33 mg, 39%, crystallized from Et_2O–EtOH): mp 161–162 °C; FAB-MS *m/z* 253 (M^+ + 1); IR (KBr) ν C≡N 2260 cm^{-1}; NMR (400 MHz, DMSO-d_6) 7.83 (1 H, d, H-6, $J_{6,5}$ = 7.1 Hz), 7.27 (2 H, br, s, 4-NH_2), 6.17 (1 H, d, 3'-OH, *J* = 6.6 Hz), 6.15 (1 H, d, H-5, $J_{5,6}$ = 7.1 Hz), 5.79 (1 H, d, H-1', $J_{1',2'}$ = 7.6 Hz), 5.14 (1 H, t, 5'-OH, *J* = 4.9 Hz), 4.40 (1 H, ddd, H-3', $J_{3',2'}$ = 7.1, $J_{3',4'}$ = 7.7, $J_{3',OH}$ = 6.6 Hz), 3.77 (1 H, t, H-2', $J_{2',3'}$ = $J_{2',1'}$ = 7.1 Hz), 3.74 (1 H, ddd, H-4', $J_{4',3'}$ = 7.7, $J_{4',5'a}$ = 2.8, $J_{4',5'b}$ = 4.5 Hz), 3.73 (1 H, ddd, H-5'a, $J_{5'a,4'}$ = 2.8, $J_{5'a,OH}$ = 4.9, $J_{a,b}$ = 12.6 Hz), 3.60 (1 H, ddd, H-5'b, $J_{5'b,4'}$ = 2.8, $J_{5'b,OH}$ = 4.9, $J_{a,b}$ = 12.6 Hz). Anal. ($C_{10}H_{12}N_4O_4 \cdot {}^1/_6H_2O$) C, H, N.

1-(2-C-Cyano-2-deoxy-β-D-arabinofuranosyl)cytosine Hydrochloride (1i·HCl). A solution of **6b** (400 mg, 1.4 mmol) in 1% HCl/MeOH (30 mL) was kept for 50 min at room temperature. The solvent was removed in vacuo and coevaporated several times with EtOH, and the resulting solid was crystallized from EtOH–Et_2O to furnish **1i·HCl** as a hydrochloride (360 mg, 89%): mp 175–176 °C; FAB-MS *m/z* 253 (M^+ + 1-HCl): IR (KBr) ν C≡N 2270 cm^{-1}: NMR (400 MHz, DMSO-d_6) 9.80 (1 H, br s, 4-NH),

8.75 (1 H, s, 4-NH), 8.30 (1 H, d, H-6, $J_{6,5}$ = 7.7 Hz), 6.21 (1 H, d, H-1', $J_{1',2'}$ = 7.2 Hz), 6.12 (1 H, d, H-5, $J_{5,6}$ = 7.7 Hz), 4.43 (1 H, dd, H-3', $J_{3',2'}$ = 7.1, $J_{3',4'}$ = 7.7 Hz), 3.97 (1 H, t, H-2', $J_{2',3'}$ = $J_{2',1'}$ = 7.1 Hz), 3.83 (1 H, ddd, H-4', $J_{4',3'}$ = 7.7, $J_{4',5'a}$ = 2.8, $J_{4',5'b}$ = 3.3 Hz), 3.76 (1 H, dd, H-5'a, $J_{5'a,4'}$ = 2.8, $J_{a,b}$ = 12.6 Hz), 3.62 (1 H, dd, H-5'b, $J_{5'b,4'}$ = 3.8, $J_{a,b}$ = 12.6 Hz). Anal. ($C_{10}H_{12}N_4O_4 \cdot HCl \cdot {}^1/_2EtOH$) C, H, N.

1-[2-*C*-Cyano-2-deoxy-5-*O*-(dimethoxytrityl)-β-D-arabinofuranosyl]thymine (7a). Dimethoxytrityl chloride (508 mg, 1.5 mmol) was added to a solution of **6a** (267 mg, 1 mmol) in dry pyridine (7 mL). The mixture was stirred for 1.5 h at room temperature and the solvent was removed in vacuo. The residue was purified on a silica gel column (1.8 × 8.5 cm), which was eluted with 1-2% EtOH in $CHCl_3$ to afford **7a** (565 mg, quantitatively, as a foam): FAB-MS *m/z* 570 ($M^+ + 1$); IR (KBr) ν C≡N 2260 cm^{-1}; NMR (400 MHz, DMSO-d_6) 8.40 (1 H, br s, NH), 7.50 (1 H, d, H-6, $J_{6,Me}$ = 1.2 Hz), 7.47–7.26 (9 H, m, Ph), 6.90–6.80 (4 H, m, Ph), 6.27 (1 H, d, H-1', $J_{1',2'}$ = 6.8 Hz), 4.74 (1 H, t, H-2', $J_{2',3'}$ = $J_{2',1'}$ = 6.8 Hz), 3.93 (1 H, ddd, H-4', $J_{4',3'}$ = 6.6, $J_{4',5'a}$ = 3.2, $J_{4',5'b}$ = 3.7 Hz), 3.79 (6 H, s, MeO), 3.62 (1 H, dd, H-3', $J_{3',2'}$ = 6.8, $J_{3',4'}$ = 6.6 Hz), 3.61 (1 H, m, H-5'a), 3.30 (1 H, d, 3'-OH), 3.30 (1 H, m, H-5'b), 1.67 (3 H, d, 5-Me, $J_{Me,6}$ = 1.2 Hz).

2'-*C*-Cyano-2',3'-didehydro-2',3'-dideoxy-5'-*O*-(dimethoxytrityl)-5-methyluridine (8a). *N,N'*-Thiocarbonyldiimidazole (94 mg, 0.53 mmol) was added to a solution of **7a** (200 mg, 0.35 mmol) in DMF (3 mL). The mixture was stirred for 2 h at room temperature and then diluted with AcOEt (20 mL), which was washed with H_2O (3 × 10 mL). The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified on a silica gel column (2 × 6.5 cm), which was eluted with hexane–AcOEt (1:1–1:2) to afford **8a** (162 mg, 84% as a foam): FAB-MS *m/z* 551 (M^+); IR (KBr) ν C≡N 2230 cm^{-1}; NMR (400 MHz, $CDCl_3$) 8.40 (1 H, br s, NH), 7.47–7.26 (9 H, m, Ph), 6.90–6.80 (4 H, m, Ph), 7.10 (1 H, d, H-1', $J_{1',3'}$ = 1.8 Hz), 7.06 (1 H, dd, H-3', $J_{3',1'}$ = 1.8, $J_{3',4'}$ = 4.0 Hz), 5.10 (1 H, d, H-4', $J_{4',3'}$ = 4.0 , $J_{4',5'a}$ = 2.6, $J_{4',5'b}$ = 3.3 Hz), 4.12 (3 H, s, MeO), 3.61 (1 H, dd, H-5'a, $J_{5'a,4'}$ = 2.6, $J_{a,b}$ = 11.0 Hz), 3.45 (1 H, dd, H-5'b, $J_{5'b,4'}$ = 3.3, $J_{a,b}$ = 11.0 Hz), 1.90 (3 H, d, 5-Me, $J_{Me,6}$ = 1.1 Hz).

2'-*C*-Cyano-2',3'-didehydro-2',3'-dideoxy-5-methyluridine (9a). A solution of **8a** (112 mg, 0.2 mmol) in AcOH (3 mL) was stirred for 1 h at room temperature and the solvent was removed in vacuo. The residue was coevaporated several times with EtOH and further purified on a silica gel column (1.6 × 8.5 cm), which eluted with 8% EtOH in $CHCl_3$ to give **9a** (22 mg, 44%, crystallized from Et_2O–hexane): mp 191–192 °C; FAB-MS *m/z* 250 (M^+); IR (Nujol) ν C≡N 2220 cm^{-1}; NMR (400 MHz, DMSO-d_6) 11.53 (1 H, br s, NH), 7.81 (1 H, d, H-6, $J_{6,Me}$ = 1.1 Hz), 7.63 (1 H, d, H-1', $J_{1',3'}$ = 1.7 Hz), 7.02 (1 H, dd, H-3', $J_{3',1'}$ = 1.7, $J_{3',4'}$ = 3.9 Hz), 5.33 (1 H, t, 5'-OH, J = 4.9 Hz), 5.05 (1 H, dd, H-4', $J_{4',3'}$ = 3.9, $J_{4',5'a}$ = $J_{4',5'b}$ = 2.8 Hz), 3.74 (1 H, ddd, H-5'a, $J_{5'a,4'}$ = 2.8, $J_{5'a,OH}$ = 4.9, $J_{a,b}$ = 12.6 Hz), 3.67 (1 H, ddd, H-5'b, $J_{5'b,4'}$ = 2.8, $J_{5'b,OH}$ = 4.9, $J_{a,b}$ = 12.6 Hz), 1.75 (3 H, d, 5-Me, $J_{Me,6}$ = 1.1 Hz). Anal. ($C_{11}H_{11}N_3O_4$) C, H, N.

***N*⁴-Acetyl-1-[2-*C*-cyano-2-deoxy-5-*O*-(dimethoxytrityl)-β-D-arabinofuranosyl]cytosine (7b).** Reaction of **6b** (194 mg, 0.66 mmol) with dimethoxytrityl chloride in pyridine gave **7b** (326 mg, 83% as a foam): FAB-MS *m/z* 597 ($M^+ + 1$); IR (KBr) ν C≡N 2260 cm^{-1}; NMR (400 MHz, $CDCl_3$) 8.80 (1 H, br s, 4-NH), 8.19 (1 H, d, H-6, $J_{6,5}$ = 7.6 Hz), 7.41–7.14, 6.89–6.76 (14 H, m, Ph, H-5), 6.30 (1 H, d, H-1', $J_{1',2'}$ = 6.1 Hz), 4.79 (2 H, m, H-2',3'-OH), 4.08 (2 H, m, H-2',4'), 3.79 (6 H, s, MeO), 3.56 (2 H, m, H-5'a,b), 2.09 (3 H, s, Ac).

***N*⁴-Acetyl-2'-*C*-cyano-2',3'-didehydro-2',3'-dideoxy-5'-*O*-(dimethoxytrityl)cytidine (8b).** (a) Compound **7b** (326 mg, 0.55 mmol) was treated with *N,N'*-thiocarbonyldiimidazole (150 mg) in DMF (5 mL) for 2 h at room temperature. The mixture was diluted with AcOEt and was washed with water several times. The separated organic phase was dried (Na_2SO_4), concentrated, and purified on a silica gel column to afford **8b** (233 mg, 73% as a foam). An analytical sample was crystallized from Et_2O: mp 148–150 °C; FAB-MS *m/z* 579 ($M^+ + 1$); IR (KBr) ν C≡N 2220 cm^{-1}; NMR (400 MHz, DMSO-d_6) 9.22 (1 H, br s, 4-NH), 8.16 (1 H, d, H-6, $J_{6,5}$ = 7.3 Hz), 7.35–7.22 (9 H, m, Ph, H-5), 6.95 (1 H, dd, H-3', $J_{3',4'}$ = 4.0, $J_{3',1'}$ = 1.8 Hz), 6.90–6.84 (5 H, m, Ph), 6.68 (1 H, d, H-1', $J_{1',3'}$ = 1.8 Hz), 5.08 (1 H, ddd, H-4', $J_{4',3'}$ = 4.0, $J_{4',5'a}$ = 2.9, $J_{4',5'b}$ = 2.6 Hz), 3.82 (6 H, s, MeO), 3.71 (1 H, dd, H-5'a, $J_{5'a,4'}$ = 2.9, $J_{a,b}$ = 11.7 Hz), 3.59 (1 H, dd, H-5'b, $J_{5'b,4'}$ = 2.6, $J_{a,b}$ = 11.7 Hz), 2.24 (3 H, s, Ac). (b) Compound **7b** (372 mg, 0.63 mmol) was treated with *N,N'*-carbonyldiimidazole (220 mg, 1.2 mmol) in DMF (3 mL) for 3 h at room temperature. The solvent was removed in vacuo and the residue was purified on a silica gel column (2.4 × 13 cm), which was eluted with 0–5% MeOH in $CHCl_3$ to afford **8b** (330 mg, 90%).

***N*⁴-Acetyl-2'-*C*-cyano-2',3'-didehydro-2',3'-dideoxycytidine (9b).** A solution of **8b** (70 mg, 0.12 mmol) in aqueous AcOH (90%, 10 mL) was stirred for 2 h at room temperature and the solvent was removed in vacuo. The residue was coevaporated several times with EtOH and further purified on a silica gel column (1.8 × 13 cm), which was eluted with 0–5% MeOH in $CHCl_3$ to give **9b** (23 mg, 69%, crystallized from Et_2O–EtOH): mp 220 °C dec; MS *m/z* 276 (M^+); IR (Nujol) ν C≡N 2220 cm^{-1}; NMR (400 MHz, DMSO-d_6) 11.02 (1 H, br s, NH), 8.33 (1 H, d, H-6, $J_{6,5}$ = 7.1 Hz), 7.65 (1 H, d, H-1', $J_{1',3'}$ = 1.7 Hz), 7.24 (1 H, d, H-5, $J_{5,6}$ = 7 .1 Hz), 7.12 (1 H, dd, H-3', $J_{3',1'}$ = 1.7, $J_{3',4'}$ = 3.3 Hz), 5.30 (1 H, t, 5'-OH, J = 4.9 Hz), 5.12 (1 H, ddd, H-4', $J_{4',3'}$ = 3.3, $J_{4',5'a}$ = $J_{4',5'b}$ = 2.8 Hz), 3.74 (1 H, dd, H-5'a, $J_{5'a,4'}$ = 3.3, $J_{a,b}$ = 12.6 Hz), 3.67 (1 H, dd, H-5'b, $J_{5'b,4'}$ = 3.3, $J_{a,b}$ = 12.6 Hz), 2.12 (3 H, s, Ac). Anal. ($C_{11}H_{11}N_3O_4$) C, H, N.

2'-*C*-Cyano-2',3'-didehydro-2',3'-dideoxycytidine (10). Compound **9b** (30 mg, 0.11 mmol) was stirred in 1% HCl/MeOH (5 mL) for 1 h at room temperature. The reaction mixture was neutralized with Dowex-1 (OH^- form) and filtered to remove the resin. The filtrate was concentrated to dryness and the residue was crystallized from Et_2O/EtOH to give **10** (24 mg, 94%): mp 213–215 °C; MS *m/z* 234 (M^+); IR (KBr) ν C≡N 2220 cm^{-1}; NMR (270 MHz, DMSO-d_6) 9.94 (1 H, br s, NH), 8.84 (1 H, br s, NH), 8.21 (1 H, d, H-6, $J_{6,5}$ = 7.7 Hz), 7.70 (1 H, d, H-1', $J_{1',3'}$ = 1.6 Hz), 7.04 (1 H, dd, H-3', $J_{3',1'}$ = 1.6, $J_{3',4'}$ = 1.6 Hz), 6.23 (1 H, d, H-5, $J_{5,6}$ = 7.7 Hz), 5.12 (1 H, m, H-4', $J_{4',3'}$ = 1.6, $J_{4',5'a}$ = 3.3, $J_{4',5'b}$ = 2.7 Hz), 3.74 (1 H, dd, H-5'a, $J_{5'a,4'}$ = 3.3, $J_{a,b}$ = 9.3 Hz), 3.67 (1 H, dd, H-5'b, $J_{5'b,4'}$ = 2.7, $J_{a,b}$ = 9.3 Hz). Anal. ($C_{10}H_{10}N_4O_3 \cdot {}^3/_8MeOH$) C, H, N.

Antitumor Activity against M5076. The test animals used in this experiment were female BD2F1 mice (7–8 weeks old, 20–25 g), purchased from Charles River Japan Inc., Kanagawa, Japan. The mice were divided into experimental groups, each group containing six mice for the treated groups, three mice for the treated group at 400 mg/kg of CNDAC, and nine mice for the control group and all mice within each group were treated identically. Each mouse was implanted subcutaneously with 1 × 10^6 viable cells (the number of cells was calculated by Trypan blue dye exclusion under a microscope) of M5076 mouse reticulum cell sarcoma. CNDAC and 5-FU were dissolved in saline and 5'-DFUR was dissolved in a small amount of dimethylacetamide (DMA) and then mixed with 0.5% carboxymethyl cellulose solution. The final concentration of DMA was 5%. The drug solution was administered orally on the first, fourth, seventh, tenth, thirteenth, and sixteenth days after inoculation of sarcoma cells. The mice in the control group were not treated. The % inhibition of tumor growth and the period for which mice survived were observed. The antitumor activity was assessed by tumor growth inhibition and T/C values calculated as described in Table III.

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References

(1) Part 121: Ono, A.; Dan, A.; Matsuda, A. Synthesis of oligonucleotides carrying linker groups at 1'-position of the sugar residues. *Bioconjugate Chem.* In press.
(2) Keating, M. J.; McCredie, K. B.; Bodey, G. P.; Smith, T. L.; Gehan, E.; Freidreich, E. J. Improved prospects for long-term survival in adults with acute myelogenous leukemia. *JAMA* **1982**, *248*, 2481–2486.
(3) Livingston, R. B.; Carter, S. K. Cytosine arabinoside (NSC-63878)–Clinical brochure. *Cancer Chemother. Rep., Part 3* **1968**, *1* (No. 1), 179–205.

(4) Ellison, R. R.; Holland, J. F.; Weil, M.; Jacquillant, C.; Bernard, J.; Sawitsky, A.; Rosner, F.; Gussoff, B.; Silver, R. T.; Karanas, A.; Cuttner, J.; Spurr, C. L.; Hayes, D. M.; Blom, J.; Leone, L. A.; Haurani, F.; Kyle, R.; Huchison, J. L.; Forcier, R. J.; Moon, J. H. Arabinosyl cytosine: a useful agent in the treatment of acute leukemia in adults. *Blood* **1968**, *32*, 507–523.
(5) Prince, H. N.; Grumberg, E.; Buck, M.; Cleeland, R. A. comparative study of antitumor and antiviral activity of 1-β-D-arabinofuranosyl-5-fluorocytosine (FCA) and 1-β-D-arabinofuranosylcytosine (CA). *Proc. Soc. Exp. Biol. Med.* **1969**, *130*, 1080–1086.
(6) Ho, W. D. H. Distribution of kinase and deaminase of 1-β-D-arabinofuranosylcytosine in tissues of man and mouse. *Cancer Res.* **1973**, *33*, 2816–2820.
(7) Plunkett, W.; Gandhi, V. Cellular pharmacodynamics of anticancer drugs. *Semin. Oncol.* **1993**, *20*, 50–63.
(8) Hadfield, A. F.; Sartorelli, A. C. The pharmacology of prodrugs of 5-fluorouracil and 1-β-D-arabinofuranosylcytosine. *Adv. Pharmacol. Chemother.* **1984**, *20*, 21–67.
(9) Watanabe, K. A.; Su, T.-L.; Klein, R. S.; Chu, C. K.; Matsuda, A.; Chun, M. W.; Lopez, C.; Fox, J. J. Synthesis of antiviral nucleosides: 5-Substituted 1-(2-deoxy-2-halogeno-β-D-arabinofuranosyl)-cytosines and -uracils. Some structure-activity relationships. *J. Med. Chem.* **1983**, *26*, 152–156.
(10) Watanabe, K. A.; Reichman, U.; Hirota, K.; Lopez, C.; Fox, J. J. Synthesis and antiherpes virus activity of some 2'-fluoro-2'-deoxyarabinofuranosylpyrimidine nucleosides. *J. Med. Chem.* **1979**, *22*, 21–24.
(11) Su, T.-L.; Watanabe, K. A.; Shinazi, R. F.; Fox, J. J. Synthesis and antiviral effects of several 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-alkyluracils. Some structure-activity relationships *J. Med. Chem.* **1986**, *29*, 151–154, and references cited therein.
(12) Hertel, L. W.; Boder, G. B.; Stan Kroin, J.; Rinzel, S. M.; Poore, G. A.; Todd, G. C.; Grindey, G. B. Evaluation of the antitumor activity of gemcitabine (2',2'-difluoro-2'-deoxycytidine). *Cancer Res.* **1990**, *50*, 4417–4422.
(13) Bobek, M.; Cheng, Y. C.; Block, A. Novel arabinofuranosyl derivatives of cytosine resistant to enzymatic deamination and possessing potent antitumor activity. *J. Med. Chem.* **1978**, *21*, 660–661.
(14) Bobek, M.; Cheng, Y. C.; Mihich, E.; Block, A. Synthesis, biological effects, and biochemical properties of some 2'-azido- and 2'-amino-2'-deoxyarabinofuranosyl pyrimidines and purines. *Recent Results Cancer Res.* **1980**, *74*, 78–83.
(15) Matsuda, A.; Yasuoka, J.; Sasaki, T.; Ueda, T. Improved synthesis of 1-(2-azido-2-deoxy-β-D-arabinofuranosyl)cytosine (Cytarazid) and -thymine. Inhibitory spectrum of cytarazid on the growth of various human tumor cells in vitro. *J. Med. Chem.* **1991**, *34*, 999–1002.
(16) Matsuda, A.; Takenuki, K.; Itoh, H.; Sasaki, T.; Ueda, T. Radical deoxygenation of *tert*-alcohols in 2'-branched-chain sugar pyrimidine nucleosides: Synthesis and antileukemic activity of 2'-deoxy-2'(*S*)-methylcytidine. *Chem. Pharm. Bull.* **1987**, *35*, 3967–3970.
(17) Ueda, T.; Matsuda, A.; Yoshimura, Y.; Takenuki, K. Synthesis and biological activity of branched chain-sugar nucleosides. *Nucleosides Nucleotides* **1989**, *8*, 743–752.
(18) Matsuda, A.; Takenuki, K.; Sasaki, T.; Ueda, T. Radical deoxygenation of *tert*-alcohols in 1-(2-*C*-alkylpentofuranosyl)-pyrimidines: Synthesis of 2'*S*)-2'-deoxy-2'-*C*-methylcytidine, an antileukemic nucleoside. *J. Med. Chem.* **1991**, *34*, 234–239.
(19) Shibuya, S.; Ueda, T. Synthesis of 1-(2-deoxy-2-methylthio-β-D-arabinofuranosyl)-uracil and -cytosine. *J. Carbohydr. Nucleosides Nucleotides* **1980**, *7*, 49–56.
(20) 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.
(21) 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.
(22) 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.
(23) Matsuda, A.; Yasuoka, J.; Ueda, T. A new method for synthesizing the antineoplastic nucleosides 1-(2-azido-2-deoxy-β-D-arabinofuranosyl)cytosine (cytarazid) and 1-(2-amino-2-deoxy-β-D-arabinofuranosyl)cytosine (cytaramin) from uridine. *Chem. Pharm. Bull.* **1989**, *37*, 1659–1661.
(24) Takenuki, K.; Itoh, H.; Matsuda, A.; Ueda, T. On the stereoselectivity of alkyl addition reaction of pyrimidine 2'-ketonucleosides. *Chem. Pharm. Bull.* **1990**, *38*, 2947–2952.
(25) Yoshimura, Y.; Lino, T.; Matsuda, A. Stereoselective radical deoxygenation of *tert*-propargyl alcohols in sugar moiety of pyrimidine nucleosides: Synthesis of 2'-*C*-alkynyl-2'-deoxy-1-β-D-arabinofuranosylpyrimidines. *Tetrahedron Lett.* **1991**, *32*, 6003–6006.
(26) Calvo-Mateo, A.; Camarasa, M.-J.; Diaz-Ortiz, A.; De las Heras, F. G. 3'-*C*-Cyano-3'-deoxythymidine. *Tetrahedron Lett.* **1988**, *29*, 941–944.
(27) Camarasa, M.-J.; Diaz-Ortiz, A.; Calvo-Mateo, A.; De las Heras, F. G.; Balzarini, J.; De Clercq, E. Synthesis and antiviral activity of 3'-*C*-cyano-3'-deoxynucleosides. *J. Med. Chem.* **1989**, *32*, 1732–1738.
(28) Matsuda, A.; Inoue, H.; Ueda, T. Synthesis of 6-cyanocytidine and its derivatives. *Chem. Pharm. Bull.* **1978**, *26*, 2340–2345.
(29) Velazquez, S.; Camarasa, M.-J. Synthesis of 2'-*C*-cyano-2'-deoxy- and 2'-*C*-cyano-2',3'-dideoxy-β-D-arabinofuranosyl nucleosides. *Tetrahedron* **1992**, *48*, 1683–1694.
(30) Matsuda, A.; Satoh, M.; Nakashima, H.; Yamamoto, N.; Ueda, T. Synthesis and anti-HIV activity of 3'-cyano-2',3'-dideoxythymidine and 3'-cyano-2',3'-didehydro-2',3'-dideoxythymidine. *Heterocycles* **1988**, *27*, 2545–2548.
(31) 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 derivative. *Cancer Res.* **1991**, *51*, 2319–2323.
(32) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. Evaluation of a tetrazolium-based semiautomated colorimetric assay: Assessment of chemosensitivity testing. *Cancer Res.* **1987**, *47*, 936–942.
(33) Matsuda, A.; Sasaki, T. Unpublished results.
(34) Langdon, S. P.; Gescher, A.; Hickman, J. A.; Stevens, M. F. G. The chemosensitivity of a new experimental model-the M5076 reticulum cell sarcoma. *Eur. J. Cancer Clin. Oncol.* **1984**, *20*, 699–705.