Nucleosides and Nucleotides. 180. Synthesis and Antitumor Activity of Nucleosides That Have a Hydroxylamino Group Instead of a Hydroxyl Group at the 2′- or 3′-Position of the Sugar Moiety[1]

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The design and synthesis of potential antitumor antimetabolites 2′-deoxy-2′-(hydroxylamino)-uridine (**15**), -cytidine (**19**, 2′-DHAC), and -adenosine (**35**), their regioisomers, 3′-deoxy-3′-(hydroxylamino)uridine (**40**) and -cytidine (**45**, 3′-DHAC), and their 2′-deoxy analogues, 2′,3′-dideoxy-3′-(hydroxylamino)uridine (**49**) and -cytidine (**52**, 3′-dDHAC), are described. We measured the pK_a values of the hydroxylamino group in **15** and **40** using ^{13}C NMR spectroscopy as a function of pH to be 2.9 and 3.4, respectively. We also found that these nucleosides gradually decomposed in neutral solution but not in acidic solution. This decomposition may be related to the generation of aminoxy radicals at the sugar moiety. The in vitro cytotoxicity of these nucleosides was evaluated using L1210 and KB cells. 2′-DHAC (**19**) inhibited the growth of L1210 and KB cells, with IC_{50} values of 1.58 and 1.99 μM, respectively. 3′-DHAC (**45**) and 3′-dDHAC (**52**) were also cytotoxic against L1210 cells, with IC_{50} values of 4.03 and 1.84 μM, respectively, but not against KB cells. The cytotoxicity of 2′-DHAC (**19**) and 3′-DHAC (**45**) against L1210 cells in vitro was reversed by the addition of cytidine, while that of 3′-dDHAC (**52**) was reversed by 2′-deoxycytidine. 2′-DHAC (**19**) and 3′-dDHAC (**52**) mainly inhibited DNA synthesis in L1210 cells, while 3′-DHAC (**45**) inhibited RNA synthesis. We also evaluated the antitumor activities of 2′-DHAC (**19**) and 3′-DHAC (**45**) against murine Meth-A fibrosarcoma cells in vivo. 2′-DHAC (**19**) was more active than 3′-DHAC (**45**) when administered intravenously on days 1−10 consecutively at 10 mg/kg/day. 2′-DHAC (**19**) inhibited tumor growth at a rate of 66.9%.

Introduction

Uridine/cytidine kinase (UCK)-dependent chemotherapy is highly promising because the activity of UCK is much higher in various human tumor tissues than in nonneoplastic tissues.[2,3] We have been developing 1-(3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl)cytosine (ECyd) as a new type of antitumor agent, especially for solid tumors.[4] This nucleoside is a relatively good substrate of UCK,[4c,e] and the resulting 5′-monophosphate (ECMP) is further metabolized to its corresponding 5′-triphosphate (ECTP),[4f] which is a potent and rather pure inhibitor of RNA polymerase.[4d,f] Since RNA synthesis occurs in every cell cycle except the M phase, the inhibition of RNA synthesis is one of the most important targets of cancer chemotherapy, especially for slow-growing solid tumors. Therefore, it is important to design nucleoside antimetabolites which are good substrates of UCK for progress in cancer chemotherapy.

Hydroxylamine derivatives have interesting chemical properties. They can be readily reduced to amines and readily oxidized to nitrons. Additionally, the oxidation of hydroxylamine by ceric sulfate[5] and OH radicals[6] produces NH_2O· radicals. Furthermore, hydroxyurea, which contains a hydroxylamino moiety, potently inhibits DNA synthesis through the inhibition of ribonucleotide diphosphate reductase.[7] Therefore, if such a substituent can be introduced into the sugar moiety of a nucleoside, the result may be a unique nucleoside with a variety of biological activities.

Recently, we designed and synthesized 2′-deoxy-2′-(hydroxylamino)cytidine (2′-DHAC, **19**).[8] This nucleoside showed potent cytotoxicity against mouse leukemic L1210 and human epidermoid KB cells in vitro and antileukemic activity against the mouse P388 model in vivo. 2′-DHAC (**19**) was found to be phosphorylated by UCK and to predominantly inhibit DNA synthesis. We also detected 2′-NHO· radicals of 2′-DHAC (**19**) in neutral solution at room temperature by ESR, from which we anticipated that the 5′-diphosphate of 2′-DHAC (**19**) would inhibit ribonucleotide diphosphate reductase, which is a rate-determining enzyme of DNA synthesis. As described above, we now have two potent antitumor nucleoside antimetabolites that are both phosphorylated by UCK: one inhibits RNA synthesis, while the other inhibits DNA synthesis. In this paper, we report the improved synthesis of 2′-DHAC (**19**) and 2′-DHAU (**15**) in detail and the synthesis of their regioisomers, 3′-deoxy-3′-(hydroxylamino)cytidine (**45**, 3′-DHAC) and -uridine (**40**, 3′-DHAU), and their 2′-deoxy analogues, 2′,3′-dideoxy-3′-(hydroxylamino)uridine (**49**) and -cytidine (**52**, 3′-dDHAC). We also de-

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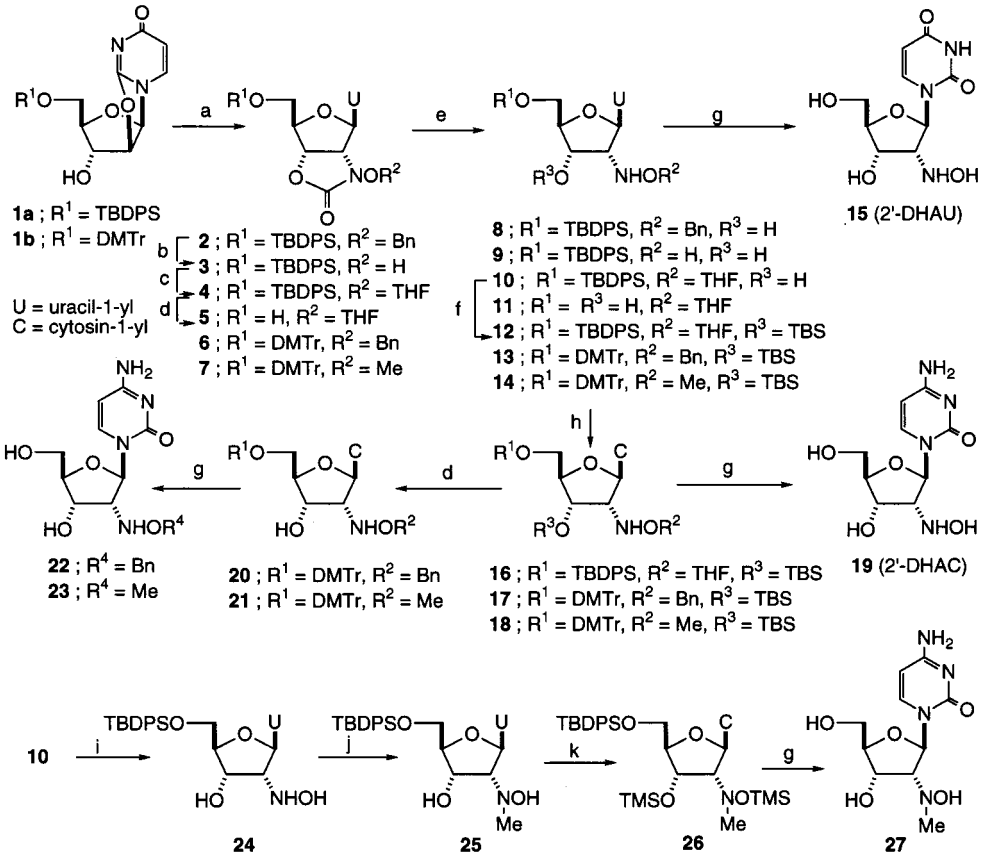
Scheme 1[a]



[a] (a) See ref 9; (b) H_2, Pd−C, EtOH, rt; (c) DHF, PPTS, THF, rt; (d) TBAF, THF, rt; (e) Cs_2CO_3, MeOH, rt; (f) TBSCl, imidazole, DMF, rt; (g) HCl, ROH; (h) (i) TPSCl, DMAP, Et_3N, MeCN, 0 °C, rt, (ii) 28% NH_4OH, rt; (i) $TsOH·H_2O$, MeOH, rt; (j) (i) aq HCHO, MeOH, rt, (ii) $NaBH_3CN$, rt; (k) (i) TMSCl, Et_3N, DMAP, MeCN, rt, (ii) TPSCl, (iii) 28% NH_4OH, rt.

scribe the chemical and antitumor properties of these nucleosides with some discussion of the underlying mechanisms.

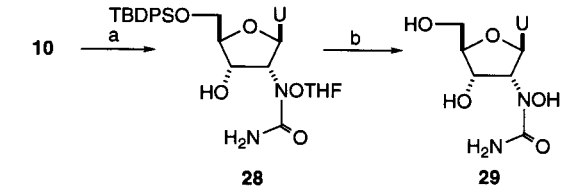
Chemistry

Recently, Sebesta et al. reported a new synthetic method for preparing 2′-(alkoxyamino)uridines via the intramolecular nucleophilic substitution of 2,2′-*O*-anhydrouridine derivatives.[9] This method is highly useful for synthesizing 2′-deoxy-2′-hydroxylamino pyrimidine nucleosides, such as 2′-DHAU (**15**) and 2′-DHAC (**19**) (Scheme 1). 5′-*O*-[*tert*-Butyldiphenylsilyl (TBDPS)]-2,2′-*O*-anhydrouridine (**1a**) was subsequently treated with *N*,*N*′-carbonyldiimidazole and then *O*-benzylhydroxylamine. The resulting 3′-*O*-(benzyloxyamino)carbonyl derivative, without purification, was converted into the corresponding 2′-(benzyloxyamino)-2′-*N*,3′-*O*-carbonyl derivative **2** in good yield.[9] When **2** was treated with Cs_2CO_3 in MeOH, the cyclic carbamate was effectively cleaved to give 2′-*O*-(benzyloxyamino)-2′-deoxyuridine derivative **8** in good yield. However, hydrogenation of the benzyl group of **8** using 10% Pd−C catalyst under a H_2 atmosphere did not give the desired 2′-hydroxylamino derivative **9**. On the other hand, hydrogenation of **2** under the same conditions gave *N*-hydroxyl derivative **3** in 79% yield. Since the cyclic carbamate moiety of **3** was not effectively cleaved by Cs_2CO_3 in MeOH, the *N*-hydroxyl group was again protected with a tetrahydrofuranyl group to give **4**, which would be deprotected in the final stage of the reaction sequence. The 5′-*O*-silyl group of **4** was deprotected with tetrabutylammonium fluoride (TBAF) to give **5**, and the cyclic carbamate moiety of **5** was cleaved with Cs_2CO_3 in MeOH to give **11**. Finally, the THF group was removed with 10% HCl in EtOH to give the desired 2′-deoxy-2′-(hydroxylamino)uridine (**15**, 2′-DHAU) as a hydrochloride. However, since this method for preparing **15** is rather lengthy, we devised a new route to prepare **4**. (2-Tetrahydrofuranyl)oxyamine ($THFONH_2$) was prepared via the reaction of *N*-hydroxyphthalimide with 2,3-dihydrofuran in the presence of pyridinium *p*-toluenesulfonate, followed by treatment with hydrazine.[10] $THFONH_2$ was used instead of $BnONH_2$ for the first reaction with **1a** to give **4** in 87% yield.

The desired cytosine counterpart **19** was synthesized from **10**, which was obtained from **4** as described above. Protection of the 3′-hydroxyl group of **10** with a *tert*-butyldimethylsilyl group gave **12**, which was converted into cytosine nucleoside **16** in the usual manner, followed by deprotection of **16** with 30% HCl in EtOH to give 2′-deoxy-2′-(hydroxylamino)cytidine (**19**, 2′-DHAC) as a dihydrochloride.

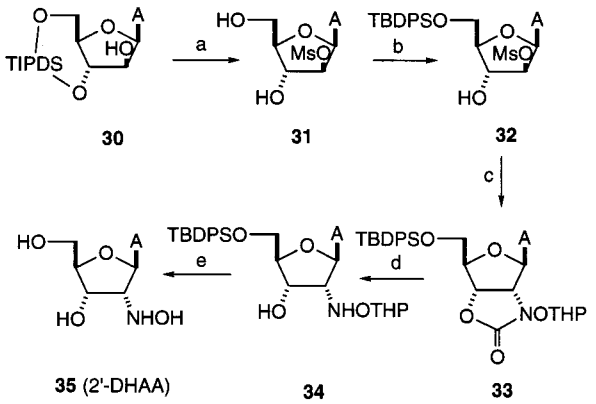
Recently, we reported that 2′-DHAC showed potent cytotoxicity against murine leukemia L1210 and human epidermoid KB cells in vitro and antileukemic activity against murine leukemia P388 models.[8] To further investigate the importance of the hydroxylamino group at the 2′-position, 2′-NHOMe, -NHOBn, and -NMeOH derivatives, **23**, **22**, and **27**, respectively, were also synthesized as shown in Scheme 1. 5′-*O*-DMTr-2,2′-*O*-

Scheme 2[a]



[a] (a) 28% NH_4OH, dioxane, rt; (b) HCl/EtOH, 0 °C.

Scheme 3[a]



[a] (a) (i) MsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C, (ii) TBAF, THF, rt; (b) TBDPSCl, imidazole, DMA, rt; (c) (i) Im_2CO, pyridine, rt, (ii) NH_2OTHF, rt, (iii) DBU, THF, rt; (d) Cs_2CO_3, MeOH, rt; (e) 4 M HCl, MeOH, rt.

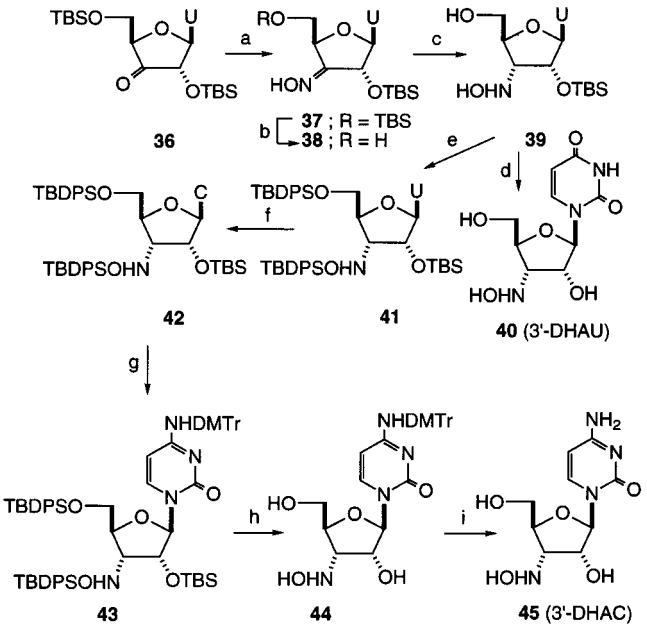
anhydrouridine (**1b**) was selected as a starting material. In a reaction sequence similar to the synthesis of 2′-DHAC (**19**), **1b** was converted into **22** and **23**. Compound **27** was prepared from **10**, the THF group of which was first deprotected to **24**, followed by reductive methylation in the presence of $NaBH_3CN$ and aqueous HCHO to give 2′-NMeOH derivative **25**. After trimethylsilylation of the hydroxy groups of **25**, the uracil moiety was converted into a cytosine by the usual method, followed by deprotection to furnish **27**.

Since hydroxyurea is known to inhibit DNA synthesis due to inhibition of ribonucleotide diphosphate reductase,[7] hydroxyurea derivative **29** was also prepared as shown in Scheme 2. Compound **10** was treated with NH_3/MeOH to give **28**, which was deprotected to furnish **29**.

Adenine analogue **35** (2′-DHAA) was synthesized as shown in Scheme 3. For the synthesis of **35**, 3′,5′-*O*-TIPDS-arabinofuranosyladenine (**30**),[11] readily prepared from 3′,5′-*O*-TIPDS-adenosine in two steps, was selected as a starting material. If the 2′-hydroxyl group of the arabinofuranosyladenine can be activated, a similar intramolecular nucleophilic substitution can be realized from the 3′-hydroxyl group. The 2′-hydroxyl group of **30** was first mesylated and then treated with TBAF to give the desired 2′-OMs derivative **31**, the 5′-position of which was protected by a TBDPS group to give **32**. As in the cleavage of the 2,2′-*O*-anhydro linkage of **1**, **32** was treated first with *N*,*N*′-carbonyldiimidazole and then with $THPONH_2$, followed by DBU to give **33**, which was, without purification, further treated with Cs_2CO_3 in MeOH to give **34** with the desired *ribo*-configuration, in 40% yield from **32**. Deprotection of **34** gave 2′-DHAA (**35**) as a dihydrochloride.

It is important to better understand the structure−activity relationship of 2′-DHAC (**19**) to find more potent

Scheme 4[a]

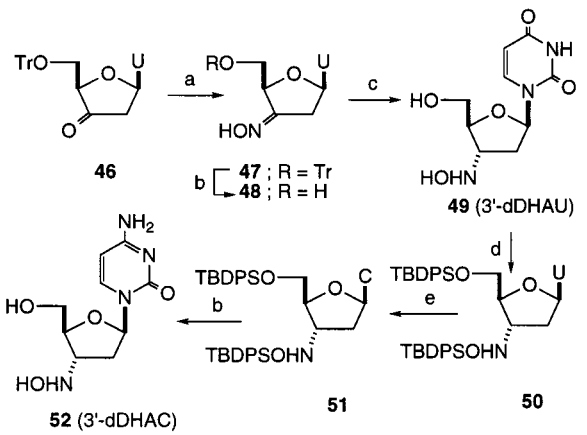


[a] (a) NH_2OH·HCl, pyridine, rt; (b) aq TFA, rt; (c) $NaBH_4$, AcOH, 0 °C; (d) TBAF, THF, rt; (e) TBDMSCl, imidazole, DMF, rt; (f) (i) TPSCl, DMAP, Et_3N, MeCN, 0 °C, rt, (ii) 28% NH_4OH, rt; (g) DMTrCl, Et_3N, CH_2Cl_2, rt; (h) TBAF, AcOH, THF, rt; (i) concd HCl, EtOH, 0 °C.

analogues. We synthesized regioisomers of 2′-DHAC (**19**) and 2′-DHAU (**15**), such as 3′-DHAU (**40**) and 3′-DHAC (**45**), and their 2′-deoxy analogues, such as 3′-dDHAU (**49**) and 3′-dDHAC (**52**). Stereoselective reduction of 3′-keto nucleosides to ribonucleosides was achieved with the assistance of the 5′-free hydroxyl group.[12] The stereoselective addition of some carbanions into the 3′-keto nucleosides from the β-face was also recently accomplished.[4e,13] Therefore, the desired 3′-hydroxylamino derivatives with a *ribo*-configuration can be obtained by hydride reduction of the corresponding 3′-oximes[14] when the 5′-hydroxyl group is not protected. As shown in Scheme 4, the desired oxime derivative **37** was readily obtained from 3′-ketouridine derivative **36** in quantitative yield. The 5′-protecting group of **37** was removed by treatment with aqueous 80% trifluoroacetic acid (TFA) to give **38**. The oxime group was reduced using $NaBH_4$ in AcOH to give the desired **39**, which was deprotected to yield 3′-DHAU (**40**). The stereochemistry of the sugar moiety was confirmed at **41**, which was obtained after further protection of the hydroxy groups with a TBDPS group. When the 3′β-proton of **41** was irradiated, NOE enhancements were observed at the 2′β-proton (9.3%) and at the 4′-proton (1.2%). The cytosine counterpart **45** was prepared from **39** by the usual method, after protection of the 5′-hydroxy group with a TBDPS group. However, isolation of **45** after deprotection of **42** was troublesome due to its highly polar character. Therefore, to ease the isolation, the 4-NH_2 group of **42** was dimethoxytritylated to yield **43**, the protecting groups of which at the sugar moiety were removed by treatment with TBAF to give **44**. Finally, **44** was treated with concentrated HCl in EtOH to give 3′-DHAC (**45**) as a dihydrochloride.

The synthesis of 3′-dDHAU (**49**) and 3′-dHAC (**52**) is shown in Scheme 5. Treatment of **46**[15] with NH_2OH·HCl in pyridine gave oxime derivative **47** in good yield.

Scheme 5[a]



[a] (a) $NH_2OH \cdot HCl$, pyridine, rt; (b) concd HCl, MeOH, 0 °C; (c) $NaBH_4$, AcOH, 0 °C; (d) TBDPSCl, imidazole, DMF, rt; (e) (i) TPSCl, DMAP, Et_3N, MeCN, 0 °C, rt, (ii) 28% NH_4OH, rt.

However, deprotection of the trityl group of **47** with HCl/MeOH gave **48** along with uracil in a ratio of ca. 1:1. Therefore, the glycosyl linkage of the oxime derivative was still unstable against acid treatment, while the 2′-deoxy-3′-keto nucleoside **46** was more labile against acid treatment.[15] Further treatment of the crude mixture of **48** with $NaBH_4$ in AcOH gave 3′-dDHAU (**49**) in 51% yield from **47**. The hydroxyl groups of **49** were silylated to give **50**, which was converted into cytosine derivative **51** in the usual manner, and deprotection of **51** was carried out under concentrated HCl in MeOH to give 3′-dDHAC (**52**) as a dihydrochloride. The stereochemistry of the sugar moiety of **52** was confirmed using the NOE technique. For example, when the 2′β-proton of **52** was irradiated, NOE enhancement (15.3%) was observed at the 3′β-proton, while 1.8% enhancement was observed at the 3′β-proton when the 2′α-proton was irradiated.

We recently reported that generation of 2′-NHO· radicals can be detected by their ESR spectrum at room temperature when 2′-DHAC is dissolved in phosphate buffer at pH 7.0.[8] However, none of these radicals are detected in the same buffer at pH 1.0 (data not shown). Simic and Hayon studied the generation of aminoxy radicals in hydroxylamine as a function of pH by hydroxyl radicals and concluded that protonation to hydroxylamine reduces the ability to produce aminoxy radicals due to the decreased reactivity of electrophilic hydroxy radicals.[6] Therefore, we became interested in the pK_a values of our nucleosides. We selected 2′-DHAU (**15**) and 3′-DHAU (**40**) as models and measured their pK_a values at the NHOH groups using ^{13}C NMR spectroscopy. The dependence of the chemical shifts of these nucleosides as a function of pH was measured, and the results are shown in Figure 1. For 2′-DHAU (**15**), the chemical shift for the 2′-carbon changes as a function of pH, and the pK_a value was calculated to be 2.9, while the pK_a value was calculated to be 3.4 from the change in the chemical shift of the 4′-carbon of 3′-DHAU as a function of pH. On the basis of these data, the hydroxylamino groups of both compounds would not be protonated in pH 7.0 buffer, and these results are consistent with the conclusion of Simic and Hayon.[6]

However, the pK_a value of *N*-methylhydroxylamine has been reported to be 5.96.[16] Therefore, the pK_a values of 2′-DHAU (**15**) and 3′-DHAU (**40**) are quite low. We anticipate that the low pK_a values of these hydroxylamino groups are due in part to approximation of the vicinal hydroxyl group such as in intramolecular hydrogen bonding. The pK_a value of 2′-amino-2′-deoxyuridine and its derivatives has been reported to be 6.2, and this low pK_a value is explained by the presence of the vicinal hydroxyl group as well as either the oxygen or the nitrogen of the molecule.[17]

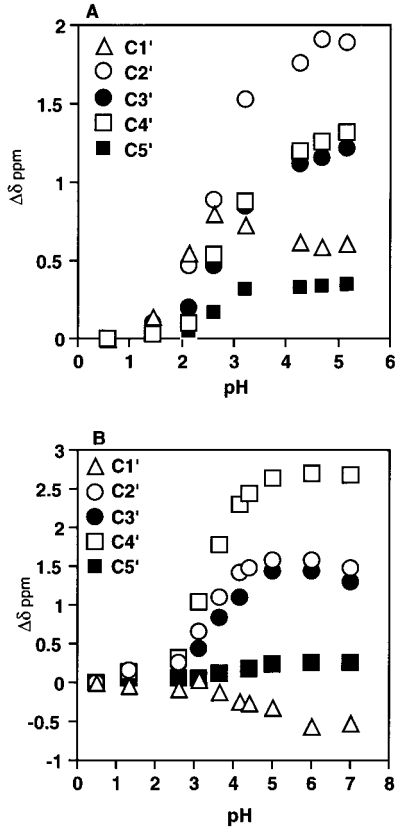


Figure 1. Measurement of the pK_a of the hydroxylamino group of 2′-DHAU (A) and 3′-DHAU (B) as a function of pH by ^{13}C NMR spectroscopy. See Experimental Section for the method.

While measuring the pK_a values of these nucleosides, we observed some changes as functions of time and pH. We previously anticipated that the generation of aminoxy radicals from 2′-DHAC 5′-diphosphate would be important for inhibiting ribonucleotide reductase, which might inhibit DNA synthesis in tumor cells. However, if the formation of the aminoxy radical from 2′-DHAC (**19**) itself is related to decomposition of the nucleoside, it would reduce the antitumor activity of 2′-DHAC (**19**). Therefore, we studied stabilities of 2′-DHAC (**19**) and 3′-DHAC (**45**) as functions of time and pH, and the results are shown in Figure 2. Incubation of 2′-DHAC (**19**; 1 mM) in phosphate buffer (100 mM, pH 7.0) was carried out at 37 °C, and the stability was checked by HPLC. The peak that was attributed to the starting material gradually disappeared, and several broad peaks were detected. One of the products was assigned to be cytosine, but other products could not be assigned because each peak contained several compounds based on their ^1H NMR spectra. The $t_{1/2}$ of 2′-DHAC (**19**) was calculated to be 2.6 h. When the reaction mixture was bubbled with argon, the degradation rate was greatly reduced, while bubbling with oxygen accelerated deg-

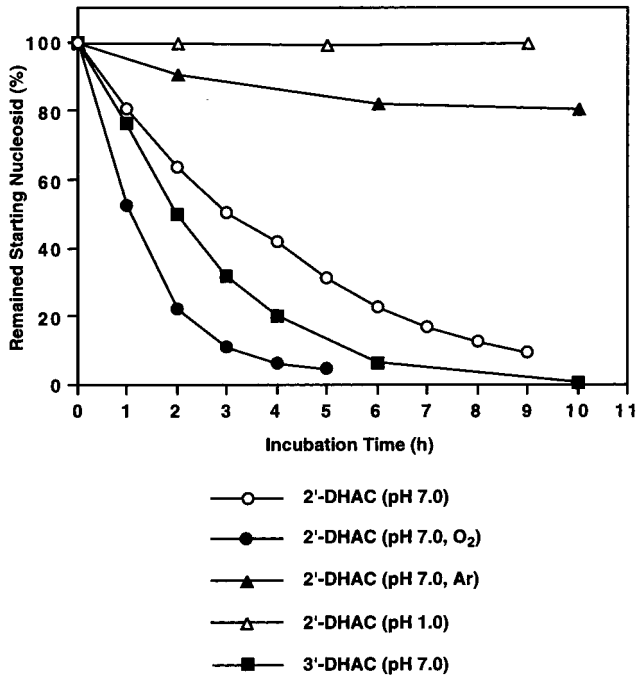


Figure 2. Degradation of 2′-DHAC (**19**) and 3′-DHAC (**45**). See Experimental Section for the method.

radation with a $t_{1/2}$ of 1.1 h. On the other hand, when 2′-DHAC was dissolved in HCl−KCl solution at pH 1.0, degradation was not detected either with or without oxygen bubbling. In addition, 3′-DHAC (**45**) was also found to decompose at a much faster rate than 2′-DHAC (**19**), with a $t_{1/2}$ of 1.9 h at pH 7.0 buffer without oxygen or argon bubbling. Therefore, the decomposition of these nucleosides is closely related to the generation of aminoxy radicals.

Biological Activity

The inhibitory activity of the hydroxylamino nucleosides against the in vitro growth of murine leukemia L1210 and human epidermoid KB cells was evaluated using the MTT assay (Table 1).[18] 2′-DHAC (**19**) inhibited the growth of L1210 and KB cells (IC_{50} values of 1.58 and 1.99 μM, respectively) more potently than 2′-DHAU (**15**) and 2′-DHAA (**35**) (IC_{50} values of 34.5 and 27.3 μM, and 27.5 and 49.6 μM, respectively). To examine the structure−cytotoxicity relationship, the in vitro cytotoxicities of **22** and **23**, which have *O*-substituted hydroxylamines, and **27**, which has an NMe hydroxylamine, were tested. Compounds **22** and **23** did not show any cytotoxicity up to 300 μM, while **27** showed weak cytotoxicity against L1210 cells but not against KB cells. These data suggest that the OH group in the hydroxylamino group of 2′-DHAC (**19**) is essential for its cytotoxicity. Since this OH group is also essential for generating the aminoxy radical, these data support a relationship between radical formation and cytotoxicity. For 3′-hydroxylamino derivatives, 3′-DHAC (**45**), 3′-DHAU (**40**), and 3′-dDHAC (**52**), but not 3′-dDHAU (**49**), were almost equally active against the growth of L1210 cells, with IC_{50} values of 4.03, 3.63, and 1.84 μM, respectively. Interestingly, these 3′-hydroxylamino nucleosides were not cytotoxic against KB cells, while 2′-hydroxylamino nucleosides were almost equally active against both types of cells. Hydroxyurea derivative **29** was also inactive against both types of cells. As discussed below, these nucleosides should be activated by certain nucleoside kinases to exhibit the cytotoxicity. As a result, **29** would not act as a hydroxyurea derivative to inactivate ribonucleotide reductase since it would not be phosphorylated.

Table 1. Inhibitory Effects of Various 2′- and 3′-Hydroxylamine-Substituted Uracil and Cytosine Nucleosides on the Growth of L1210 and KB Cells in Vitro[a]

	IC_{50} (μM)	
compounds	L1210	KB
2′-DHAC (**19**)	1.58	1.99
2′-DHAU (**15**)	34.5	27.3
2′-DHAA (**35**)	27.5	49.6
22	>300	>300
23	>300	>300
27	37.7	269
29	>300	>300
3′-DHAC (**45**)	4.03	254
3′-DHAU (**40**)	3.63	>300
3′-dDHAU (**49**)	>300	>300
3′-dDHAC (**52**)	1.84	>300

[a] The inhibition of tumor cell growth in vitro was assayed as described elsewhere.[18] 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: percent inhibition (%) = [1 − OD (540 nm) of sample well/OD (540 nm) of control well] × 100. IC_{50} (μg/mL) is the concentration that inhibits cell growth by 50%.

Next, we evaluated the in vitro cytotoxicities of 2′-DHAC (**19**), 3′-DHAC (**45**), and 3′-dDHAC (**52**) against 10 human tumor cell lines, including three leukemias, one colon, two pancreas, one fibro, one breast, one melanoma, and one hepatoma tumor cell lines. The results are summarized in Table 2. 2′-DHAC (**19**) was effective against nine cell lines with IC_{50} values in the micromolar range and was inactive against one pancreas adenocarcinoma cell line (PANC-1). However, 3′-DHAC (**45**) was active only against MCF-7 mammary adenocarcinoma cells, and 3′-dDHAC (**52**) was effective against only two leukemic cell lines, HL-60 and CCRF-CEM.

We also evaluated the in vivo antitumor activity of 2′-DHAC (**19**) against P388 mouse leukemia cells that had been implanted intraperitoneally into female CDF1 mice. When 2′-DHAC (**19**) was administered intraperitoneally on days 1−5 consecutively at a dose of 20 mg/kg/day, it showed antitumor activity with a T/C value of 167%, while all of the tumor-bearing mice (6 mice were used as a control) that were not administered the drug died on day 10.[8] Under the same schedule, when 50 mg/kg/day 3′-DHAC (**45**) was administered intraperitoneally, it showed antitumor activity with a T/C value of 144%. We also evaluated the antitumor potencies of 2′-DHAC (**19**) and 3′-DHAC (**45**) against murine Meth-A fibrosarcoma cells in vivo, and the results are summarized in Table 3. When the drug was administered intravenously on days 1−10 consecutively, these nucleosides showed dose-dependent antitumor activities to reduce the tumor size. 2′-DHAC (**19**) was more active than 3′-DHAC (**45**), and at 10 mg/kg/day it inhibited tumor growth at a rate of 66.9%.

To estimate the metabolic pathways of 2′-DHAC (**19**), 3′-DHAC (**45**), and 3′-dDHAC (**52**), the inhibitory effects

Table 2. Inhibitory Effects of 2′-DHAC (**19**), 3′-DHAC (**45**), and 3′-dDHAC (**52**) on the Growth of Various Human Tumor Cells in Vitro[a]

cell line	origin	IC_{50} (μM)		
		2′-DHAC (**19**)	3′-DHAC (**45**)	3′-dDHAC (**52**)
K562	chronic myelogenous leukemia	2.77	14.9	27.1
HL-60	promyelocytic leukemia	5.76	213	4.90
CCRF-CEM	acute lymphoblastic leukemia	1.73	170	6.05
Colo320DM	colon adenocarcinoma	8.93	133	>300
MiaPaCa-2	pancreas adenocarcinoma	4.00	60.5	>300
PANC-1	pancreas adenocarcinoma	69.1	>300	>300
HT-1080	fibrosarcoma	2.53	107	>300
MCF-7	mammary adenocarcinoma	1.30	6.91	30.3
A375	melanoma	0.89	28.2	27.7
HuH7	hepatoma	1.47	274	>300

[a] See Table 1 for the assay method.

Table 3. Antitumor Effects of 2′-DHAC (**19**) and 3′-DHAC (**45**) on Murine Meth-A Fibrosarcoma in Vivo[a]

drug	dose (mg/kg × 10)	tumor size (mg)	tumor inhibition rate (%)
control		740.4 ± 222.6	
2′-DHAC (**19**)	2	309.3 ± 10.6	58.2[b]
	10	245.3 ± 95.4	66.9[b]
3′-DHAC (**45**)	2	500.4 ± 109.6	32.4
	10	404.7 ± 192.4	45.3

[a] Meth-A cells (5×10^5/mouse) were subcutaneously transplanted into BALB mice. Drugs were intravenously administered the day after transplantation for 10 days. [b] $p < 0.05$.

of these nucleosides on the growth of L1210 cells were examined in the presence or absence of common nucleosides, such as cytidine, 2′-deoxycytidine, thymidine, and adenosine. L1210 cells (10^4 cells/mL) were treated with graded concentrations of **19**, **45**, or **52** separately and in combination with each common nucleoside (final concentration; 100 μM) for 72 h (Figure 3). The inhibitory effects of 2′-DHAC (**19**) and 3′-DHAC (**45**) on the growth of L1210 cells were reduced by the addition of cytidine (Figure 3A,B, respectively). Therefore, the antitumor activity of these nucleosides may require phosphorylation by UCK. On the other hand, the cytotoxicity of 3′-dDHAC (**52**) was reduced by the addition of 2′-deoxycytidine (Figure 3C). This experiment suggested that 3′-dDHAC (**52**) may be phosphorylated by deoxycytidine kinase (dCK).

We next examined the effects of these nucleosides on DNA and RNA synthesis in L1210 cells by measuring the incorporation of [^{3}H]thymidine and [^{3}H]uridine as precursors, respectively, in the presence or absence of these nucleosides (Figure 4). As a result, 2′-DHAC (**19**; 20 μg/mL) predominantly inhibited DNA synthesis in L1210 cells while slightly inhibiting RNA synthesis (Figure 4A), while 3′-DHAC (**45**; 50 μg/mL) inhibited RNA synthesis time-dependently (Figure 4B). On the other hand, the inhibition of DNA synthesis was a major mechanism of the cytotoxicity of 3′-dDHAC (**52**; 50 μg/mL) (Figure 4C). However, the inhibition of DNA and/or RNA synthesis by 3′-DHAC (**45**) and 3′-dDHAC (**52**) seemed to require a time lag. Interestingly, changes in the position of the hydroxylamino group in the sugar moiety apparently alter the inhibitory effects on DNA and RNA synthesis.

Since the cytotoxicities of these nucleosides were reduced by the addition of certain common nucleosides, such as cytidine or 2′-deoxycytidine, while the hydroxyurea derivative **29** was not effective, these nucleosides may need to be phosphorylated by UCK or dCK to their 5′-monophosphates, which would be further phosphorylated by certain nucleotide kinases to their 5′-di- and 5′-triphosphates. As described above, the pK_a values of the hydroxylamino groups were quite low, and therefore these groups are not protonated at physiological pH. This characteristic might be important for them to be substrates for kinases. The relationship between aminoxy radical formation and antitumor activity is not fully understood. However, the generation of such radicals is at least partly related to their antitumor activities, since the structure−activity relationship of 2′-DHAC (**19**) shows that the NOH group is required for cytotoxicity and also for the generation of radicals. Considering pyrimidine nucleotide metabolism, we anticipated that 2′-DHAC (**19**), after phosphorylation to its 5′-diphosphate with the generation of aminoxy radicals, might inhibit ribonucleoside diphosphate reductase (RDR), which is responsible for the de novo biosynthesis of deoxyribonucleotides required for DNA synthesis, and 2′-DHACTP might be responsible for inhibiting RNA polymerase. 3′-DHAC (**45**) or 3′-dDHAC (**52**) might inhibit RNA polymerases or DNA polymerases at their 5′-triphosphates.

In conclusion, we designed and synthesized several nucleosides with a hydroxylamino group at the sugar moiety and expected some chemical reactivity of the hydroxylamino group. These nucleosides were cytotoxic against several tumor cell lines both in vitro and in vivo. Relatively stable aminoxy radical formation was observed in 2′-DHAC (**19**)[8] and 3′-DHAC (**45**), which might be responsible for their antitumor activities and may also lead to their degradation. Since the activity of uridine/cytidine kinase is much higher in various human tumor tissues than in nonneoplastic tissues,[2,3] 2′-DHAC (**19**) and 3′-DHAC (**45**) should be expected to exhibit antitumor activities in a tumor-specific manner, if prodrugs could be devised to prevent their decomposition in serum fluids.

Experimental Section

Melting points were measured on a Yanagimoto MP-3 micromelting point apparatus (Yanagimoto, Japan) and are uncorrected. Fast atom bombardment mass spectrometry (FAB-MS) was done on a JEOL JMS-HX110 instrument at an ionizing voltage of 70 eV. The ^{1}H NMR spectra were recorded on a JEOL JNM-GX 270 (270-MHz) or Bruker ARX 500 (500-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), m (multiplet), or br (broad). All exchangeable protons were detected by disappearance on the addition of D_2O. UV

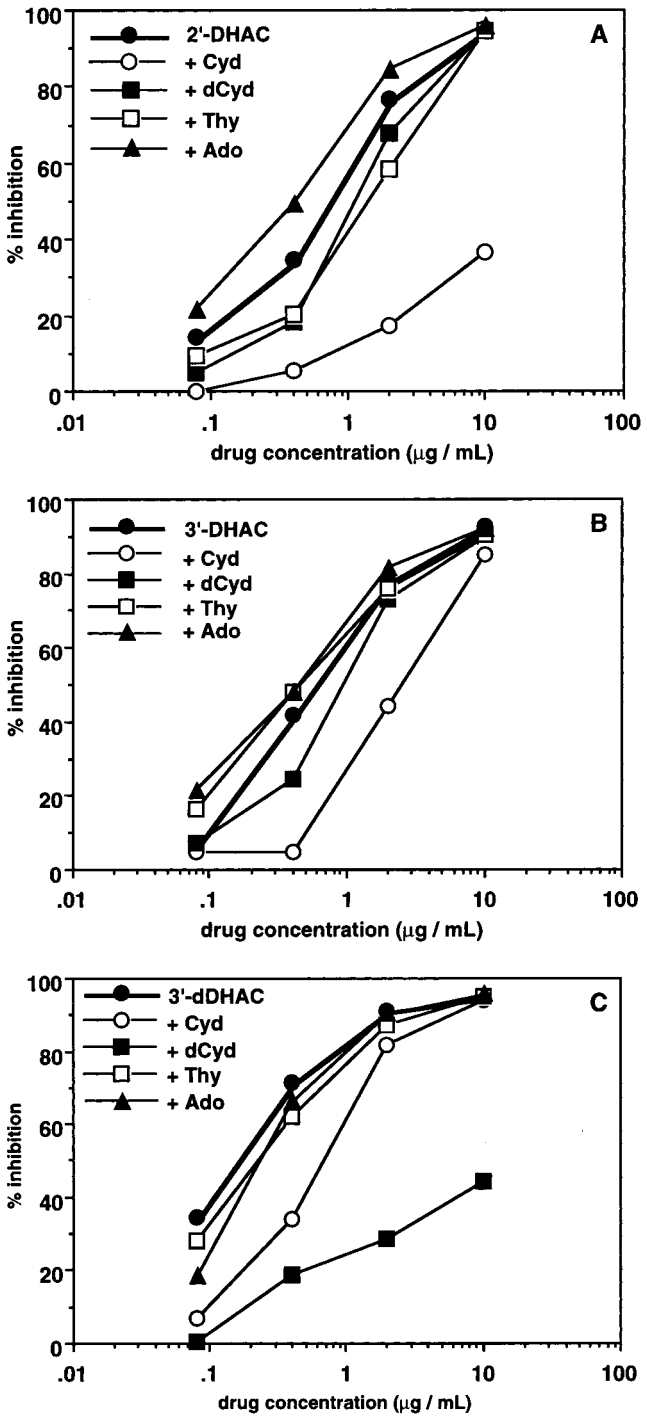


Figure 3. Competitive effects of common nucleosides on the cytotoxicities of 2′-DHAC (A), 3′-DHAC (B), and 3′-dDHAC (C) against the growth of L1210 cells.

absorption spectra were recorded with a Shimadzu UV-240 spectrophotometer. IR spectra were recorded with a JEOL A-102 spectrometer. TLC was done on Merck Kieselgel F254 precoated plates (Merck, Germany). The silica gel used for column chromatography was YMC gel 60A (70−230 mesh) (YMC Co., Ltd., Japan).

2′-(Benzyloxyamino)-5′-*O*-(*tert*-butyldiphenylsilyl)-2′-*N*,3′-*O*-carbonyl-2′-deoxyuridine (2). This compound was prepared according to the previous method.[9] From **1a** (2.30 g, 4.95 mmol), **2** (2.50 g, 82% as a white foam) was obtained: ^{1}H NMR ($CDCl_3$) 8.40 (br s, 1 H, 3-NH), 7.61−7.32 (m, 16 H, H-6, Ph), 6.77 (d, 1 H, H-1′, $J_{1',2'}$ = 8.1 Hz), 5.16−5.04 (m, 3 H, H-3′, benzylic), 4.24 (dd, 1 H, H-2′, $J_{2',1'}$ = 8.1, $J_{2',3'}$ = 2.1 Hz), 4.18 (m, 1 H, H-4′), 3.91 (dd, 1 H, H-5′a, $J_{5'a,4'}$ = 4.0, J_{gem} = 11.2 Hz), 3.81 (dd, 1 H, H-5′b, $J_{5'b,4'}$ = 4.6, J_{gem} = 11.3 Hz), 1.05 (s, 9 H, *t*-Bu).

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′-*N*,3′-*O*-carbonyl-2′-deoxy-2′-(hydroxylamino)uridine (3). A mixture of **2** (15.2 g, 24.8 mmol) and Pd−C (10%, 2.0 g) in EtOH (300 mL) was stirred at room temperature under H_2 atmosphere for 24 h. The mixture was filtrated through a Celite pad, and the filtrate was concentrated in vacuo. The residue was crystallized from EtOH and Et_2O to give **3** (10.3 g, 79% as a white crystalline solid): LRMS (FAB) *m*/*z* 522 [$(M - H)^+$, 100%]; ^{1}H NMR (DMSO-d_6) 12.50 (br s, 1 H, 2′-NO*H*), 10.30 (br s, 1 H, 3-N*H*), 7.68−7.37 (m, 11 H, H-6, Ph), 5.99 (m, 1 H, H-1′), 5.54 (d, 1 H, H-5, $J_{5,6}$ = 7.9 Hz), 5.17 (dd, 1 H, H-3′, $J_{3',2'}$ = 8.3, $J_{3',4'}$ = 5.5 Hz), 4.71 (d, 1 H, H-2′, $J_{2',3'}$ = 8.3 Hz), 4.20 (m, 1 H, H-4′), 3.91−3.83 (m, 2 H, H-5′), 1.00 (s, 9 H, *t*-Bu).

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′-*N*,3′-*O*-carbonyl-2′-deoxy-2′-[(2-tetrahydrofuranyl)oxyamino]uridine (4). Method A. A suspension of **3** (10.3 g, 19.7 mmol) in THF (200 mL) containing DHF (2.97 mL, 39.3 mmol) and PPTS (988 mg, 3.93 mmol) was stirred at room temperature for 11 h. The solvent was removed in vacuo, and the residue was dissolved in EtOAc (250 mL), washed with H_2O (250 mL × 2) and brine (200 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 50:1) to give **4** (11.7 g, quantitative as a white foam): LRMS (FAB) *m*/*z* 594 (MH^+, 7%); ^{1}H NMR ($CDCl_3$) 8.90, 8.79 (each br s, each 1 H, 3-NH), 7.65−7.31 (m, 11 H, H-6, Ph), 6.22, 6.03 (each d, each 1 H, H-1′, $J_{1',2'}$ = 3.4 Hz), 5.65−5.41 (m, 2 H, H-5, THF), 5.18, 5.16 (each dd, each 1 H, H-3′, $J_{3',2'}$ = 8.1, 8.5 Hz, $J_{3',4'}$ = 4.4, 5.8 Hz), 4.27 (m, 1 H, H-4′), 4.08−3.83 (m, 4 H, H-5′, THF), 2.17−1.75 (m, 4 H, THF), 1.10, 1.06 (each s, each 9 H, *t*-Bu). Anal. ($C_{30}H_{35}N_3O_8Si$) C, H, N.

Method B. A mixture of **1a** (232 mg, 0.5 mmol) and *N*,*N*-carbonyldiimidazole (122 mg, 0.75 mmol) in pyridine (5 mL) was stirred at room temperature for 24 h. A pyridine solution (1 mL) of NH_2OTHF (206 mg, 2 mmol) was added to the above mixture, and the mixture was stirred at the same temperature for a further 5 h. The solvent was removed in vacuo, and the residue taken up with AcOEt (20 mL) was washed with H_2O (10 mL × 3) and brine (10 mL), dried (Na_2SO_4), and concentrated in vacuo. DBU (15 μL, 0.1 mmol) was added to a solution of the above residue in THF (5 mL), and the mixture was stirred at room temperature for 24 h. The solvent was removed, and the residue was purified on a silica gel column to give **4** (257 mg, 87% from **1a**).

(2-Tetrahydrofuranyl)oxyamine. DHF (15.2 mL, 200 mmol) and PPTS (5.00 g, 19.9 mmol) were added to a stirred solution of *N*-hydroxyphthalimide (16.3 g, 100 mmol) in 1,4-dioxane (100 mL) at room temperature. After the mixture was stirred at room temperature for 1 h, the mixture was poured into H_2O (600 mL). The resulting precipitates were collected by filtration to give 18.8 g (81%) of white crystals: mp 128 °C; LRMS (FAB) *m*/*z* 234 (MH^+, 40%); HRMS (FAB) calcd for $C_{12}H_{12}NO_4$ 234.0766, found 234.0759; ^{1}H NMR ($CDCl_3$) 7.83−7.72 (m, 4 H, Ph), 5.79 (dd, 1 H, H-1), 4.34, 4.01 (each m, each 1 H, H-4), 2.32−1.93 (m, 4 H, H-2, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) 163.47, 133.99, 128.82, 123.11, 108.56, 69.00, 30.80, 22.56.

Hydrazine hydrate (3.9 mL, 80 mmol) was added to a stirred solution of the above crystals (9.32 g, 40.0 mmol) in a mixture of $CHCl_3$ (50 mL) and MeOH (10 mL) at 0 °C. After the mixture was stirred at room temperature for 24 h, the mixture was concentrated in vacuo. The residue was dissolved with Et_2O (100 mL), washed with aqueous NaOH (6 M, 100 mL) and brine (100 mL), dried (Na_2SO_4), and concentrated in vacuo to give (2-tetrahydrofuranyl)oxyamine (2.00 g, 49% as a yellow oil): LRMS (EI) *m*/*z* 103 (M^+, 0.1%); HRMS (EI) calcd for $C_4H_9NO_2$ 103.0633, found 103.0637; ^{1}H NMR (DMSO-d_6) 5.83 (br s, 2 H, NH_2), 5.09 (dd, 1 H, H-1), 3.72 (m, 2 H, H-4), 1.89−1.66 (m, 4 H, H-2, H-3); ^{13}C NMR (MeOH-d_4) 106.79, 66.15, 30.11, 23.59. Anal. ($C_4H_9NO_2$·0.1AcOEt) C, H, N.

2′-*N*,3′-*O*-Carbonyl-2′-deoxy-2′-[(2-tetrahydrofuranyl)-oxyamino]uridine (5). A solution of **4** (2.37 g, 3.99 mmol) in THF (35 mL) was treated with TBAF (1 M in THF, 4.4 mL,

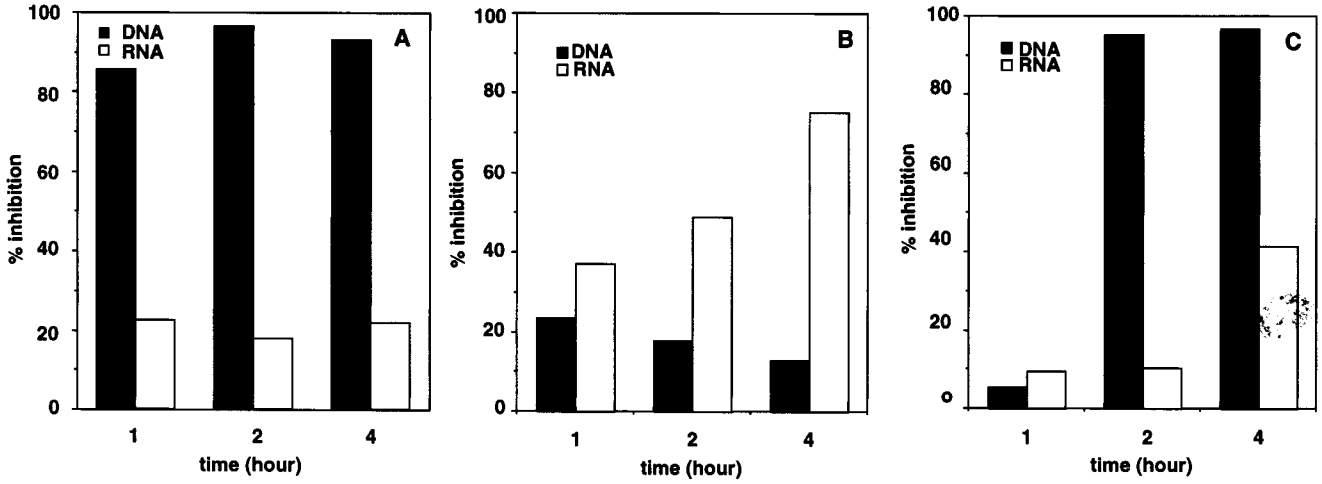


Figure 4. Effects of 2′-DHAC (A), 3′-DHAC (B), and 3′-dDHAC (C) on the incorporation of [^{3}H]thymidine and [^{3}H]uridine into DNA and RNA fractions of L1210 cells.

4.4 mmol) at room temperature for 24 h. The solvent was removed in vacuo. The residue taken up with EtOAc (250 mL) was washed with H_2O (50 mL × 2) and brine (50 mL), and the organic phase was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc:EtOH = 50:1) to give **5** (1.12 g, 79% as a white foam): LRMS (FAB) *m/z* 356 (MH^+, 16%); ^{1}H NMR (DMSO-d_6) 11.50 (br s, 1 H, 3-NH), 7.86, 7.68 (each d, 1 H, H-6, each $J_{5,6}$ = 8.0 Hz), 6.12, 6.08 (each d, 1 H, H-1′, $J_{1',2'}$ = 1.8, 4.0 Hz), 5.75, 5.65 (each d, 1 H, H-5, each $J_{5,6}$ = 8.0 Hz), 5.53, 5.51 (each m, 1 H, THF), 5.22, 5.11 (br t, 1 H, 5′-OH), 5.16−5.07 (m, 1 H, H-3′), 4.76−4.72 (m, 1 H, H-2′), 4.15−4.06 (m, 1 H, H-4′), 3.88−3.77 (m, 2 H, THF), 3.72−3.61 (m, 2 H, H-5′), 2.07−1.74 (m, 4 H, THF). Anal. ($C_{14}H_{17}N_3O_8 \cdot 0.2H_2O$) C, H, N.

2′-Deoxy-2′-[(2-tetrahydrofuranyl)oxyamino]uridine (11). A solution of **5** (300 mg, 0.844 mmol) and Cs_2CO_3 (824 mg, 2.53 mmol) in MeOH (10 mL) was stirred at room temperature for 23 h. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 15:2) to give **11** (236 mg, 85% as a white solid): mp 153−155 °C; LRMS (FAB) *m/z* 330 (MH^+, 76%); ^{1}H NMR (DMSO-d_6) 11.30 (br s, 1 H, 3-NH), 7.86, 7.85 (each d, 1 H, H-6, $J_{5,6}$ = 8.0, 8.2 Hz), 6.51, 6.42 (each d, 1 H, 2′-NH, $J_{2',NH}$ = 7.8, 8.8 Hz), 5.89, 5.88 (each d, 1 H, H-1′, $J_{1',2'}$ = 7.6, 7.7 Hz), 5.67−5.64 (m, 1 H, H-5), 5.54, 5.41 (each d, 1 H, 3′-OH, $J_{3',OH}$ = 4.5, 5.2 Hz), 5.20 (m, 1 H, THF), 5.09 (m, 1 H, 5′-OH), 4.18 (m, 1 H, H-3′), 3.86 (m, 1 H, H-4′), 3.72 (m, 1 H, H-2′), 3.68 (m, 2 H, THF), 3.55 (m, 2 H, H-5′), 1.87−1.56 (m, 4 H, THF). Anal. ($C_{13}H_{19}N_3O_7 \cdot {}^1/_3H_2O$) C, H, N.

2′-Deoxy-2′-(hydroxylamino)uridine Hydrochloride (15, 2′-DHAU). Compound **11** (408 mg, 1.24 mmol) was dissolved in EtOH (4.5 mL) containing concentrated HCl (500 μL) at 0 °C under argon atmosphere. After the mixture was stirred at the same temperature for 24 h, the solvent was removed in vacuo. The residue was coevaporated several times with EtOH, and the resulting crystals were collected by filtration to give **15** (353 mg, 96%): mp 160−162 °C; LRMS (FAB) *m/z* 352 [(MH + glycerin)$^+$, 9%]; ^{1}H NMR (MeOH-d_4) 7.97 (d, 1 H, H-6, $J_{5,6}$ = 8.1 Hz), 6.41 (d, 1 H, H-1′, $J_{1',2'}$ = 6.8 Hz), 5.77 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 4.64 (dd, 1 H, H-3′, $J_{2',3'}$ = 6.2, $J_{3',4'}$ = 3.0 Hz), 4.20 (dd, 1 H, H-2′, $J_{1',2'}$ = 6.8, $J_{2',3'}$ = 6.2 Hz), 4.15 (ddd, 1 H, H-4′, $J_{3',4'}$ = 3.0, $J_{4',5'a}$ = 2.6, $J_{4',5'b}$ = 2.9 Hz), 3.83 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 2.6, J_{gem} = 12.2 Hz), 3.75 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 2.9, J_{gem} = 12.2 Hz). Anal. ($C_9H_{13}N_3O_4 \cdot HCl \cdot 0.2H_2O$) C, H, N.

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′-deoxy-2′-[(2-tetrahydrofuranyl)oxyamino]uridine (10). A solution of **4** (1.20 g, 2.02 mmol) and Cs_2CO_3 (2.00 g, 6.14 mmol) in MeOH (15 mL) was stirred at room temperature for 24 h. The solvent was removed in vacuo, and the residue was dissolved in EtOAc (100 mL), washed with H_2O (100 mL × 3) and brine (100 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$:EtOH = 20:1) to give **10** (924 mg, 81% as a white crystalline solid): mp 147−148 °C; LRMS (FAB) *m/z* 568 (MH^+, 41%); ^{1}H NMR ($CDCl_3$) 8.39 (br s, 1 H, 3-NH), 7.89, 7.84 (each d, 1 H, H-6, each $J_{5,6}$ = 8.3 Hz), 7.68−7.40 (m, 10 H, Ph), 6.41, 6.37 (each d, 1 H, 2′-NH, $J_{2',NH}$ = 3.0, 3.8 Hz), 6.06, 5.95 (each d, 1 H, H-1′, $J_{1',2'}$ = 4.7, 7.3 Hz), 5.53−5.39 (m, 2 H, H-5, THF), 4.43 (m, 1 H, H-3′), 4.29 (m, 1 H, H-4′), 4.04 (m, 2 H, THF), 3.90 (m, 2 H, H-5′), 3.70−3.63 (m, 1 H, H-2′), 1.99−1.81 (m, 4 H, THF), 1.10, 1.06 (each s, 9 H, *t*-Bu). Anal. ($C_{29}H_{37}N_3O_7Si$) C, H, N.

3′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(*tert*-butyldiphenylsilyl)-2′-deoxy-2′-[(2-tetrahydrofuranyl)oxyamino]uridine (12). TBSCl (2.79 g, 18.5 mmol) and imidazole (1.51 g, 22.2 mmol) were added to a stirred solution of **10** (3.50 g, 6.17 mmol) in DMF (50 mL) at room temperature under argon atmosphere. After being stirred at room temperature for 24 h, the mixture was diluted with EtOAc (250 mL), washed with H_2O (250 mL × 3) and brine (250 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane:EtOAc = 3:2) to give **12** (4.20 g, quantitative as a white foam): LRMS *m/z* 682 (MH^+, 45%); ^{1}H NMR ($CDCl_3$) 8.19 (br s, 1 H, 3-NH), 7.80, 7.77 (each d, 1 H, H-6, $J_{5,6}$ = 8.0, 8.3 Hz), 7.68−7.37 (m, 10 H, Ph), 6.11, 6.09 (each d, 1 H, 2′-NH, $J_{2',NH}$ = 10.0, 10.1 Hz), 6.03, 5.99 (each d, 1 H, H-1′, $J_{1',2'}$ = 7.9, 8.3 Hz), 5.45 (d, 1 H, H-5, $J_{5,6}$ = 8.0 Hz), 5.32 (m, 1 H, THF), 4.43 (m, 1 H, H-3′), 3.92−3.68 (m, 5 H, H-2′, H-4′, H-5′), 1.91−1.68 (m, 4 H, THF), 1.10, 0.88 (each s, 9 H, *t*-Bu), 0.09, 0.07, 0.02, 0.00 (each s, 3 H, Me). Anal. ($C_{35}H_{51}N_3O_7Si_2$) C, H, N.

3′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(*tert*-butyldiphenylsilyl)-2′-deoxy-2′-[(2-tetrahydrofuranyl)oxyamino]cytidine (16). Triethylamine (205 μL, 1.47 mmol) was added dropwise to a stirred mixture of **12** (340 mg, 0.489 mmol), TPSCl (455 mg, 1.47 mmol), and DMAP (24 mg, 0.196 mmol) in MeCN (5 mL) at 0 °C under argon atmosphere. After being stirred at room temperature for 2 h, NH_4OH (28%, 5 mL) was added to the mixture. After 3 h, the resulting mixture was extracted with EtOAc (50 mL). The organic phase was washed with H_2O (20 mL × 3) and brine (20 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 10:1) to give **16** (302 mg, 91% as a white foam): LRMS (FAB) *m/z* 681 (MH^+, 31%); ^{1}H NMR ($CDCl_3$) 7.91−7.87 (m, 1 H, H-6), 7.70−7.36 (m, 12 H, 4-NH_2, Ph), 6.21−6.14 (m, 2 H, H-1′, 2′-NH), 5.41−5.34 (m, 2 H, H-5, THF-1″), 4.02−3.64 (m, 5 H, H-2′, H-4′, H-5′, THF), 1.94−1.69 (m, 4 H, THF), 1.11, 0.89 (each s, 9 H, *t*-Bu), 0.09, 0.08, 0.03, 0.01 (each s, 3 H, Me). Anal. ($C_{35}H_{53}N_4O_6Si_2$) C, H, N.

2′-Deoxy-2′-(hydroxylamino)cytidine Dihydrochloride (19, 2′-DHAC). Compound **16** (696 mg, 1.02 mmol) was

dissolved in a mixture of concentrated HCl (3 mL) and MeOH (7 mL) at room temperature under argon atmosphere. After the mixture was stirred at room temperature for 24 h, the resulting crystals were collected by filtration to give **19** (306 mg, 91%): mp 170−172 °C; LRMS (FAB) *m*/*z* 259 (MH^+, 42%); ^{1}H NMR (MeOH-d_4) 8.40 (d, 1 H, H-6, $J_{5,6}$ = 8.0 Hz), 6.45 (d, 1 H, H-1′, $J_{1',2'}$ = 6.0 Hz), 6.23 (d, 1 H, H-5, $J_{5,6}$ = 8.0 Hz), 4.68 (dd, 1 H, H-3′, $J_{2',3'}$ = 6.2, $J_{3',4'}$ = 3.4 Hz), 4.30 (dd, 1 H, H-2′, $J_{1',2'}$ = 6.0, $J_{2',3'}$ = 6.2 Hz), 4.23 (ddd, 1 H, H-4′, $J_{3',4'}$ = 3.4, $J_{4',5'a}$ = 2.6, $J_{4',5'b}$ = 2.8 Hz), 3.88 (dd, 1 H, H-5′a, $J_{4',5'b}$ = 2.6, J_{gem} = 12.3 Hz), 3.75 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 2.8, J_{gem} = 12.3 Hz). Anal. ($C_9H_{14}N_4O_6$·2HCl) C, H, N.

2,2′-*O*-Anhydro-1-[5′-*O*-(4,4′-dimethoxytrityl)-β-D-arabinofuranosyl]uracil (1b). A mixture of 2,2′-*O*-anhydrouridine (11.3 g, 50.0 mmol) and DMTrCl (19.5 g, 57.6 mmol) in pyridine (450 mL) was stirred at room temperature for 12 h under argon atmosphere. The solvent was removed in vacuo, and the residue was dissolved in AcOEt (500 mL), washed with H_2O (100 mL × 2) and brine (100 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 8:1) to give **1b** (25.1 g, 95% as a white foam): ^{1}H NMR ($CDCl_3$) 7.32−6.74 (m, 14 H, H-6, Ph), 6.14 (d, 1 H, H-1′, $J_{1',2'}$ = 5.8 Hz), 5.95 (d, 1 H, $J_{5,6}$ = 7.4 Hz), 5.27 (dd, 1 H, H-2′, $J_{1',2'}$ = 5.6, $J_{2',3'}$ = 5.6 Hz), 5.05 (d, 1 H, 3′-OH, $J_{3',OH}$ = 4.0 Hz), 4.47 (m, 1 H, H-4′), 4.38 (m, 1 H, H-3′), 3.75 (s, 6 H, OMe), 3.08 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 5.1, J_{gem} = 10.4 Hz), 2.96 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 7.0, J_{gem} = 10.3 Hz).

2′-(*N*-Benzyloxyamino)-2′-*N*,3′-*O*-carbonyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)uridine (6). Compound **6** was prepared as described for the synthesis of **4** (method B). From **1b** (5.29 g, 10.0 mmol), **6** (4.89 g, 72% as a white foam) was obtained: ^{1}H NMR ($CDCl_3$) 9.03 (br s, 1 H, 3-NH), 7.44−6.78 (m, 19 H, H-6, Ph), 5.44 (d, 1 H, H-1′, $J_{1',2'}$ = 8.0 Hz), 5.08−5.02 (m, 4 H, H-5, H-3′, benzylic), 4.31 (dd, 1 H, H-2′, $J_{1',2'}$ = 8.1, $J_{2',3'}$ = 1.7 Hz), 4.18 (dd, 1 H, H-4′, $J_{3',4'}$ = 9.4, $J_{4',5'}$ = 4.7 Hz), 3.78 (s, 6 H, OMe), 3.42 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 5.2, J_{gem} = 10.5 Hz), 3.38 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 4.1, J_{gem} = 10.5 Hz).

2′-*N*,3′-*O*-Carbonyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2′-(*N*-methoxyamino)uridine (7). Compound **7** was prepared as described for the synthesis of **4** (method B). From **1b** (1.06 g, 2.01 mmol), **7** (924 mg, 77% as a white foam) was obtained: ^{1}H NMR ($CDCl_3$) 8.76 (br s, 1 H, 3-NH), 7.52 (d, 1 H, H-6, $J_{5,6}$ = 8.1 Hz), 7.37−6.82 (m, 13 H, Ph), 6.06 (d, 1 H, H-1′, $J_{1',2'}$ = 1.3 Hz), 5.50 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 5.32 (dd, 1 H, H-3′, $J_{2',3'}$ = 8.3, $J_{3',4'}$ = 5.7 Hz), 4.53 (dd, 1 H, H-2′, $J_{1',2'}$ = 1.3, $J_{2',3'}$ = 8.3 Hz), 4.30 (ddd, 1 H, H-4′, $J_{3',4'}$ = 5.7, $J_{4',5'a}$ = 3.4, $J_{4',5'b}$ = 4.4 Hz), 3.96 (s, 3 H, 2′-NOMe), 3.79 (s, 6 H, OMe), 3.55 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 3.4, J_{gem} = 10.7 Hz), 3.51 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 4.4, J_{gem} = 10.7 Hz).

2′-(*N*-Benzyloxyamino)-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)uridine (13). Compound **13** was prepared as described for the synthesis of **10**. From **6** (2.71 g, 4.00 mmol), **13** (2.66 g, 87% as a white foam) was obtained: LRMS (FAB) *m*/*z* 766 (MH^+, 3%); HRMS (FAB) calcd for $C_{43}H_{52}N_3O_8Si$ 766.3523, found 766.3535; ^{1}H NMR ($CDCl_3$) 8.30 (br s, 1 H, 3-NH), 7.60 (d, 1 H, H-6, $J_{5,6}$ = 8.2 Hz), 7.33−6.78 (m, 18 H, Ph), 6.05 (d, 1 H, 2′-NH, $J_{2',NH}$ = 9.4 Hz), 5.90 (d, 1 H, H-1′, $J_{1',2'}$ = 7.1 Hz), 5.31 (dd, 1 H, H-5, J = 2.0, 8.1 Hz), 4.64, 4.61 (each d, each 1 H, benzylic, each J = 11.7 Hz), 4.32 (dd, 1 H, H-3′, $J_{2',3'}$ = 5.3, $J_{3',4'}$ = 2.7 Hz), 3.97 (m, 1 H, H-4′), 3.75 (s, 6 H, OMe), 3.57 (m, 1 H, H-2′), 3.43 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 2.8, J_{gem} = 10.9 Hz), 3.27 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 2.6, J_{gem} = 10.9 Hz), 0.79 (s, 9 H, *t*-Bu), −0.04, −0.09 (each s, each 3 H, Me).

3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2′-(*N*-methoxyamino)uridine (14). Compound **14** was prepared as described for the synthesis of **10**. From **7** (850 mg, 1.41 mmol), **14** (828 mg, 85% as a white foam) was obtained: LRMS *m*/*z* 690 (MH^+, 1%); HRMS calcd for $C_{37}H_{48}N_3O_8$ 690.3210, found 690.3192; ^{1}H NMR ($CDCl_3$) 8.08 (br s, 1 H, 3-NH), 7.69 (d, 1 H, H-6, $J_{5,6}$ = 8.1 Hz), 7.29−6.73 (m, 13 H, Ph), 5.96 (d, 1 H, 2′-NH, $J_{2',NH}$ = 9.2 Hz), 5.88 (d, 1 H, H-1′, $J_{1',2'}$ = 6.8 Hz), 5.27 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 4.35 (dd, 1 H, H-3, $J_{2',3'}$ = 5.4, $J_{3',4'}$ = 3.0 Hz), 3.95 (ddd, 1 H, H-4′, $J_{3',4'}$ = 3.0, $J_{4',5'a}$ = 2.7, $J_{4',5'b}$ = 2.6 Hz), 3.70 (s, 6 H, OMe), 3.62 (ddd, 1 H, H-2′, $J_{1',2'}$ = 6.8, $J_{2',NH}$ = 9.2, $J_{2',3'}$ = 5.4 Hz), 3.43 (s, 3 H, 2′-NOMe), 3.38 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 2.7, J_{gem} = 10.1 Hz), 3.26 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 2.6, J_{gem} = 10.1 Hz), 0.82 (s, 9 H, *t*-Bu), 0.00, −0.02 (each s, each 3 H, Me).

2′-(*N*-Benzyloxyamino)-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)cytidine (17). This compound was prepared as described for the synthesis of **16**. From **13** (2.50 g, 3.26 mmol), **17** (2.19 g, 88% as a white foam) was obtained: LRMS (FAB) *m*/*z* 765 (MH^+, 1%); HRMS (FAB) calcd for $C_{43}H_{53}N_4O_7Si$ 765.3683, found 765.3686; ^{1}H NMR ($CDCl_3$) 7.86 (d, 1 H, H-6, $J_{5,6}$ = 7.8 Hz), 7.39−6.81 (m, 18 H, Ph), 6.19−6.15 (m, 2 H, H-1′, 2′-NH), 5.34 (d, 1 H, H-6, $J_{5,6}$ = 7.8 Hz), 4.76, 4.68 (each d, each 1 H, benzylic, each J = 11.7 Hz), 4.38 (dd, 1 H, H-3′, $J_{2',3'}$ = 4.9, $J_{3',4'}$ = 4.9 Hz), 4.07 (m, 1 H, H-2′), 3.79 (s, 6 H, OMe), 3.59−3.52 (m, 2 H, H-4′, H-5′a), 3.25 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 2.9, J_{gem} = 10.7 Hz), 0.80 (s, 9 H, *t*-Bu), 0.01, −0.09 (each s, each 3 H, Me).

3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2′-(*N*-methoxyamino)cytidine (18). This compound was prepared as described for the synthesis of **16**. From **14** (740 mg, 1.07 mmol), **18** (567 mg, 77% as a white foam) was obtained: LRMS (FAB) *m*/*z* 689 (MH^+, 0.2%); HRMS (FAB) calcd for $C_{37}H_{49}N_4O_7Si$ 689.3370, found 689.3367; ^{1}H NMR ($CDCl_3$) 7.91 (d, 1 H, H-6, $J_{5,6}$ = 7.0 Hz), 7.36−6.78 (m, 13 H, Ph), 6.10−6.08 (m, 2 H, H-1′, 2′-NH), 5.31 (d, 1 H, H-5, $J_{5,6}$ = 7.0 Hz), 4.37 (m, 1 H, H-3′), 4.03 (m, 1 H, H-2′), 3.75 (s, 6 H, OMe), 3.56 (m 1 H, H-4′), 3.51 (m, 1 H, H-5′), 3.49 (s, 3 H, 2′-NOMe), 3.24 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 2.8, J_{gem} = 12.3 Hz), 0.78 (s, 9 H, *t*-Bu), 0.00, −0.01 (each s, each 3 H, Me).

2′-(*N*-Benzyloxyamino)-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)cytidine (20). A mixture of **17** (2.00 g, 2.62 mmol) and TBAF (1 M in THF, 2.88 mL, 2.88 mmol) in THF (25 mL) was stirred at room temperature for 3 h. The solvent was removed in vacuo, and the residue was dissolved in AcOEt (150 mL), washed with H_2O (50 mL × 5) and brine (50 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 15:1) to give **20** (1.70 g, quantitative as a white foam): LRMS (FAB) *m*/*z* 651 (MH^+, 1%); HRMS (FAB) calcd for $C_{37}H_{39}N_4O_7$ 651.2818, found 651.2817; ^{1}H NMR ($CDCl_3$) 7.81 (d, 1 H, H-6, $J_{5,6}$ = 7.8 Hz), 7.38−6.81 (m, 18 H, Ph), 7.05 (br s, 1 H, 2′-NH), 5.93 (d, 1 H, H-1′, $J_{1',2'}$ = 5.9 Hz), 5.43 (d, 1 H, H-5, $J_{5,6}$ = 7.8 Hz), 4.75, 4.69 (each d, each 1 H, benzylic, each J = 11.7 Hz), 4.33 (m, 1 H, H-3′), 4.17 (m, 1 H, H-2′), 3.79 (s, 6 H, OMe), 3.67 (br s, 1 H, 3′-OH), 3.48 (m, 1 H, H-4′), 3.41 (dd, 1H, H-5′a, $J_{4',5'a}$ = 2.0, J_{gem} = 10.7 Hz), 3.28 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 4.9, J_{gem} = 10.7 Hz).

2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2′-(*N*-methoxyamino)cytidine (21). This compound was prepared as described for the synthesis of **20**. From **18** (527 mg, 0.764 mmol), **21** (419 mg, 95% as a white foam) was obtained: LRMS (FAB) *m*/*z* 575 (MH^+, 2%); HRMS (FAB) calcd for $C_{31}H_{35}N_4O_7$ 575.2505, found 575.2510; ^{1}H NMR ($CDCl_3$) 7.85 (d, 1 H, H-6, $J_{5,6}$ = 7.1 Hz), 7.39−6.84 (m, 13 H, Ph), 7.01 (s, 1 H, 2′-NH), 5.93 (d, 1 H, H-1′, $J_{1',2'}$ = 5.8 Hz), 5.44 (d, 1 H, H-5, $J_{5,6}$ = 7.1 Hz), 4.35 (m, 1 H, H-3′), 4.32 (m, 1 H, H-2′), 3.92 (br s, 1 H, 3′-OH), 3.80 (s, 6 H, OMe), 3.66 (m, 1 H, H-4′), 3.58 (s, 3 H, 2′-NOMe), 3.45 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 2.6, J_{gem} = 10.7 Hz), 3.35 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 4.2, J_{gem} = 10.7 Hz).

2′-(*N*-Benzyloxyamino)-2′-deoxycytidine Dihydrochloride (22). A mixture of **20** (1.60 g, 2.46 mmol) in concentrated HCl (2 mL) and EtOH (18 mL) was stirred at room temperature for 10 min. The solvent was removed in vacuo, and the residue was dissolved in H_2O (100 mL), washed with $CHCl_3$ (30 mL × 5), and concentrated in vacuo to give **22** (840 mg, 98% as a white crystalline solid): mp 210 °C dec; LRMS (FAB) *m*/*z* 349 (MH^+, 49%); HRMS (FAB) calcd for $C_{16}H_{21}N_4O_5$ 349.1510, found 349.1524; ^{1}H NMR (MeOH-d_4) 8.32 (d, 1 H, H-6, $J_{5,6}$ = 7.9 Hz), 7.40 (s, 5 H, Ph), 6.47 (d, 1 H, H-1′, $J_{1',2'}$ = 7.0 Hz), 6.13 (d, 1 H, H-6, $J_{5,6}$ = 7.9 Hz), 5.12, 5.07 (each d, each 1 H, benzylic, each J = 9.2 Hz), 4.64 (dd, 1 H, H-3′, $J_{2',3'}$

= 5.9, $J_{3',4'}$ = 2.5 Hz), 4.30 (dd, 1 H, H-2′, $J_{1',2'}$ = 6.7, $J_{2',3'}$ = 6.2 Hz), 4.20 (ddd, 1 H, H-4′, $J_{3',4'}$ = 2.5, $J_{4',5'a}$ = 2.5, $J_{4',5'b}$ = 2.5 Hz), 3.83 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 2.6, J_{gem} = 12.4 Hz), 3.77 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 2.7, J_{gem} = 12.4 Hz); ^{13}C NMR (100 MHz, MeOH-d_4) 160.58, 148.05, 145.70, 134.04, 130.44, 129.65, 95.71, 89.55, 86.57, 77.70, 70.88, 66.42, 62.29. Anal. ($C_{16}H_{20}N_4O_5$·2HCl) C, H, N.

2′-Deoxy-2′-(*N*-methoxyamino)cytidine Dihydrochloride (23). This compound was prepared as described for the synthesis of **22**. From **21** (200 mg, 0.348 mmol), **23** (102 mg, 85% as a white powder) was obtained: mp 170 °C dec; LRMS (FAB) *m*/*z* 273 (MH^+); HRMS (FAB) calcd for $C_{10}H_{17}N_4O_5$ 273.1198, found 273.1184; ^{1}H NMR (MeOH-d_4) 8.39 (d, 1 H, H-6, $J_{5,6}$ = 7.9 Hz), 6.44 (d, 1 H, H-1′, $J_{1',2'}$ = 6.4 Hz), 6.22 (d, 1 H, H-5, $J_{5,6}$ = 7.9 Hz), 4.65 (m, 1 H, H-3′), 4.32 (m, 1 H, H-2′), 4.22 (m, 1 H, H-4′), 3.94 (s, 3 H, 2′-NOMe), 3.89 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 2.7, J_{gem} = 12.1 Hz), 3.87 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 2.5, J_{gem} = 12.1 Hz). Anal. ($C_{10}H_{16}N_4O_5$·2HCl·$^4/_5$H$_2$O) C, H, N.

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′-deoxy-2′-(*N*-hydroxylamino)uridine (24). A mixture of **10** (114 mg, 0.201 mmol) and TsOH·H_2O (16 mg, 0.084 mmol) in MeOH (2 mL) was stirred at room temperature for 3 days, and the reaction mixture was neutralized by addition of saturated aqueous $NaHCO_3$. The solvent was removed in vacuo, and the residue was extracted with AcOEt (25 mL). The organic layer was washed with H_2O (25 mL) and brine (25 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 10:1) to give **24** (83.0 mg, 83% as a white foam): LRMS (FAB) *m*/*z* 498 (MH^+, 21%); HRMS (FAB) calcd for $C_{25}H_{32}N_3O_6Si$ 498.2058, found 498.2072; ^{1}H NMR ($CDCl_3$) 7.73 (d, 1 H, H-6, $J_{5,6}$ = 8.1 Hz), 7.67−7.36 (m, 10 H, Ph), 6.20 (d, 1 H, H-1′, $J_{1',2'}$ = 6.6 Hz), 5.42 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 4.55 (dd, 1 H, H-3′, $J_{2',3'}$ = 6.3, $J_{3',4'}$ = 3.6 Hz), 4.13 (m, 1 H, H-4′), 4.01 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 2.0, J_{gem} = 11.8 Hz), 3.82 (dd, 1 H, H-5′b, $J_{4',5'a}$ = 1.9, J_{gem} = 11.8 Hz), 3.67 (dd, 1 H, H-2′, $J_{1',2'}$ = 6.6, $J_{2',3'}$ = 6.3 Hz), 1.08 (s, 9 H, *t*-Bu). Anal. ($C_{25}H_{31}N_3O_6Si$·$^4/_5$H$_2$O) C, H, N.

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′-deoxy-2-(*N*-hydroxy-*N*-methylamino)uridine (25). A mixture of **24** (498 mg, 1.00 mmol) in MeOH (10 mL) containing aqueous HCHO (37%, 333 μL, 4.00 mmol) was stirred at room temperature for 3 h. $NaBH_3CN$ (943 mg, 15.0 mmol) was added to the above mixture at room temperature. After the mixture was stirred for 15 h, the solvent was removed in vacuo. The residue was dissolved in AcOEt (50 mL), washed with H_2O (20 mL × 3) and brine (20 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 20:1) to give **25** (308 mg, 60% as a white foam): LRMS (FAB) *m*/*z* 512 (MH^+, 100%); HRMS (FAB) calcd for $C_{26}H_{34}N_3O_6Si$ 512.2214, found 512.2220; ^{1}H NMR ($CDCl_3$) 7.86 (d, 1 H, H-6, $J_{5,6}$ = 8.1 Hz), 7.66−7.35 (m, 10 H, Ph), 6.62 (d, 1 H, H-1′, $J_{1',2'}$ = 7.9 Hz), 5.51 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 4.51 (m, 1 H, H-3′), 4.18 (m, 1 H, H-4′), 4.02 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 1.8, J_{gem} = 11.9 Hz), 3.83 (dd, 1 H, H-5′b, $J_{4',5'a}$ = 1.7, J_{gem} = 11.9 Hz), 3.31 (dd, 1 H, H-2′, $J_{1',2'}$ = 7.9, $J_{2',3'}$ = 5.5 Hz), 2.83 (s, 3 H, 2′-NMe), 1.12 (s, 9 H, *t*-Bu).

5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-*O*-(trimethylsilyl)-2′-deoxy-2′-[*N*-[(trimethylsilyl)oxy]-*N*-methylamino]cytidine (26). A mixture of **25** (250 mg, 0.489 mmol), TMSCl (311 mL, 2.45 mmol), Et_3N (681 mL, 4.89 mmol), and DMAP (24 mg, 0.196 mmol) in MeCN (5 mL) was stirred at room temperature under argon atmosphere for 2 h. TPSCl (443 mg, 2.30 mmol) was added to the mixture at 0 °C. After the mixture was stirred at room temperature for a further 3 h, NH_4OH (28%, 5 mL) was added to the mixture. After 2 h, the mixture was diluted with AcOEt (100 mL), washed with H_2O (30 mL × 3) and brine (30 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 20:1) to give **26** (125 mg, 39% as a white foam): LRMS (FAB) *m*/*z* 655 (MH^+, 23%); HRMS (FAB) calcd for $C_{32}H_{51}N_4O_5Si_3$ 655.3164, found 655.3140; ^{1}H NMR ($CDCl_3$) 7.77 (d, 1 H, H-6, $J_{5,6}$ = 7.4 Hz), 7.69−7.33 (m, 10 H, Ph), 6.61 (d, 1 H, H-1′, $J_{1',2'}$ = 6.4 Hz), 5.33 (d, 1 H, H-5, $J_{5,6}$ = 7.4 Hz), 4.48 (m, 1 H, H-3′), 3.94 (m, 1 H, H-4′), 3.90 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 2.9, J_{gem} = 11.1 Hz), 3.75 (dd, 1 H, H-5′b, $J_{4',5'a}$ = 1.9, J_{gem} = 11.1 Hz), 3.30 (m, 1 H, H-2′), 2.82 (s, 3 H, 2′-NMe), 1.11 (s, 9 H, *t*-Bu), 0.12, 0.08 (each s, each 9 H, Me).

2′-Deoxy-2′-(*N*-hydroxy-*N*-methylamino)cytidine Dihydrochloride (27). A mixture of **26** (115 mg, 0.176 mmol) in concentrated HCl (200 μL) and EtOH (1.8 mL) was stirred at room temperature for 5 h. The solvent was removed in vacuo. The residue was coevaporated several times with EtOH, dissolved in H_2O (25 mL), washed with $CHCl_3$ (25 mL × 3), and concentrated in vacuo. The residue was coevaporated several times with EtOH to give **27** (51 mg, 84% as a white crystalline solid): mp 205 °C dec; LRMS (FAB) *m*/*z* 273 (MH^+, 28%); HRMS (FAB) calcd for $C_{10}H_{17}N_4O_5$ 273.1198, found 273.1210; ^{1}H NMR (MeOH-d_4) 8.53 (d, 1 H, H-6, $J_{5,6}$ = 7.9 Hz), 6.61 (d, 1 H, H-1′, $J_{1',2'}$ = 5.6 Hz), 6.22 (d, 1 H, H-5, $J_{5,6}$ = 7.9 Hz), 4.69 (dd, 1 H, H-3′, $J_{2',3'}$ = 5.9, $J_{3',4'}$ = 3.6 Hz), 4.46 (dd, 1 H, H-2′, $J_{1',2'}$ = 5.6, $J_{2',3'}$ = 5.9 Hz), 4.22 (m, 1 H, H-4′), 3.87 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 2.4, J_{gem} = 12.3 Hz), 3.80 (dd, 1 H, H-5′b, $J_{4',5'a}$ = 2.1, J_{gem} = 12.3 Hz), 3.36 (s, 3 H, 2′-NMe); ^{13}C NMR (100 MHz, MeOH-d_4) 160.87, 148.12, 146.01, 95.76, 88.67, 86.02, 73.91, 61.91, 58.33, 46.91. Anal. ($C_{10}H_{16}N_4O_5$·2HCl·$^3/_5$H$_2$O·$^1/_3$EtOH) C, H, N.

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′-[*N*-carbamoyl-*N*-[(2-tetrahydrofuranyl)oxy]amino]-2′-deoxyuridine (28). A mixture of **10** (594 mg, 1.00 mmol) in 1,4-dioxane (5 mL) and NH_4OH (28%, 5 mL) was stirred at room temperature for 24 h. The solvent was removed in vacuo, and the residue was coevaporated several times with MeOH. The resulting white solid was collected by filtration, washed MeOH, and crystallized from EtOH to give **28** (380 mg, 62%): mp 173−174 °C; LRMS (FAB) *m*/*z* 611 (MH^+, 11%); HRMS (FAB) calcd for $C_{30}H_{39}N_4O_8Si$ 611.2537, found 611.2524; ^{1}H NMR (DMSO-d_6) 11.40 (br t, 1 H, 3-NH), 7.69−7.37 (m, 11 H, H-6, Ph), 6.55 (br s, 2 H, NH_2), 6.35, 6.26 (each d, 1 H, H-1′, *J* = 3.9, 4.9 Hz), 5.58−5.29 (m, 2 H, H-5, THF-1″), 4.69 (dd, 1 H, H-3′, $J_{2',3'}$ = 7.8, $J_{3',4'}$ = 4.4 Hz), 4.43 (m, 1 H, H-2′), 4.32, 4.26 (each t, 2 H, THF-4″, each *J* = 7.6 Hz), 3.98−3.74 (m, 3 H, H-4′, H-5′), 2.02−1.78 (m, 4 H, THF-2″, THF-3″), 0.99 (s, 9 H, *t*-Bu); ^{13}C NMR (100 MHz, $CDCl_3$) 163.79, 163.66, 162.84, 162.77, 150.30, 150.03, 040.04, 139.57, 135.22, 134.98, 132.61, 131.96, 131.86, 129.70, 129.61, 129.48, 127.58, 127.54, 127.42, 112.07, 109.82, 102.56, 102.13, 85.50, 85.20, 84.24, 68.91, 68.48, 68.37, 68.30, 66.80, 66.55, 62.68, 62.24, 31.25, 30.92, 30.63, 26.76, 26.68, 23.89, 23.82, 23.69, 19.25. Anal. ($C_{30}H_{38}N_4O_8Si$) C, H, N.

2′-(*N*-Carbamoyl-*N*-hydroxylamino)-2′-deoxyuridine (29). A mixture of **28** (100 mg, 0.164 mmol) in concentrated HCl (400 μL) and MeOH (1.6 mL) was stirred at 0 °C for 24 h. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 1:1) to give **29** (40 mg, 80% as a white solid): mp 163−164 °C; LRMS (FAB) *m*/*z* 303 (MH^+, 26%); HRMS (FAB) calcd for $C_{10}H_{15}N_4O_7$ 303.0939, found 303.0941; ^{1}H NMR (DMSO-d_6) 11.40 (br s, 1 H, 3-NH), 9.26 (br s, 1 H, 2′-NOH), 7.77 (d, 1 H, H-6, $J_{5,6}$ = 8.1 Hz), 6.37 (br s, 2 H, NH_2), 6.19 (d, 1 H, H-1′, $J_{1',2'}$ = 4.4 Hz), 5.62 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 4.98 (t, 1 H, 5′-OH, $J_{5',OH}$ = 5.1 Hz), 4.64 (dd, 1 H, H-3′, $J_{2',3'}$ = 7.6, $J_{3',4'}$ = 4.6 Hz), 4.18 (dd, 1 H, H-2′, $J_{1',2'}$ = 7.1, $J_{2',3'}$ = 7.1 Hz), 3.87 (m, 1 H, H-4′), 3.64 (ddd, 1 H, H-5′a, $J_{4',5'a}$ = 2.2, J_{gem} = 10.0, $J_{5',OH}$ = 4.9 Hz), 3.49 (m, 1 H, H-5′b).

2′-*O*-(Methylsulfonyl)-β-D-arabinofuranosyladenine (31). A mixture of **30** (550 mg, 1.19 mmol), Et_3N (248 μL, 1.78 mmol), DMAP (145 mg, 1.19 mmol), and MsCl (138 μL, 1.78 mmol) in CH_2Cl_2 (10 mL) was stirred at 0 °C for 2 h under argon atmosphere. The solvent was removed in vacuo, and the residue was dissolved in AcOEt (50 mL), washed with H_2O (20 mL) and brine (20 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 15:1) to give a white foam, which was treated with TBAF (1 M in THF, 2.20 mL, 2.20 mmol) in THF (10 mL) at 0 °C for 2 h. The reaction was quenched by addition of AcOH (126 μL), and the solvent was removed in vacuo. The residue was coevaporated several times

with MeOH and Et_2O, and the resulting crystals were collected by filtration to give **31** (280 mg, 0.811 mmol, 68%): mp 230 °C dec; LRMS (FAB) *m*/*z* 346 (MH^+, 9%); HRMS (FAB) calcd for $C_{11}H_{16}N_5O_6S$ 346.0820, found 346.0821; ^{1}H NMR (DMSO-d_6) 8.30, 8.20 (each s, each 1 H, H-2, H-8), 7.37 (br s, 2 H, NH_2), 6.52 (d, 1 H, $J_{1',2'}$ = 6.2 Hz), 6.07 (d, 1 H, 3′-OH, $J_{3',OH}$ = 5.6 Hz), 5.32 (dd, 1 H, H-2′, $J_{1',2'}$ = 6.2, $J_{2',3'}$ = 6.2 Hz), 5.18 (ddd, 1 H, H-3′, $J_{2',3'}$ = 6.2, $J_{3',OH}$ = 5.6, $J_{3',4'}$ = 6.4 Hz), 3.90 (m, 1 H, H-4′), 3.75 (m, 2 H, H-5′), 3.12 (s, 3 H, Me). Anal. ($C_{11}H_{15}N_5O_6S$·0.5MeOH) C, H, N.

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′-*O*-(methylsulfonyl)-β-D-arabinofuranosyladenine (32). A mixture of **31** (250 mg, 0.724 mmol), TBDPSCl (283 μL, 1.09 mmol), and imidazole (99.0 mg, 1.45 mmol) in DMF (7 mL) was stirred at room temperature for 12 h. The solvent was removed in vacuo, and the residue was dissolved in AcOEt (50 mL), washed with H_2O (50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was coevaporated several times with MeOH and Et_2O, and the resulting crystals were collected by filtration to give **32** (360 mg, 85%): mp 198−200 °C; LRMS (FAB) *m*/*z* 584 (MH^+, 100%); HRMS calcd for $C_{27}H_{34}N_5O_6SSi$ 584.1996, found 584.2002; ^{1}H NMR ($CDCl_3$) 8.23, 8.05 (each s, each 1 H, H-2, H-8), 7.70−7.35 (m, 10 H, Ph), 6.52 (d, 1 H, H-1′, $J_{1',2'}$ = 5.5 Hz), 5.67 (br s, 2 H, NH_2), 5.25 (dd, 1 H, H-2′, $J_{1',2'}$ = 5.5, $J_{2',3'}$ = 5.0 Hz), 4.90 (m, 1 H, H-3′), 4.08−3.99 (m, 3 H, H-4′, H-5′), 3.52 (br s, 1H, 3′-OH), 2.69 (s, 3 H, Me), 1.11 (s, 9 H, *t*-Bu). Anal. ($C_{27}H_{33}N_5O_6SSi$) C, H, N.

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′-deoxy-2′-[(2-tetrahydrofuranyl)oxyamino]adenosine (34). A mixture of **32** (1.40 g, 2.40 mmol) and *N*,*N*′-carbonyldiimidazole (779 mg, 4.80 mmol) in pyridine (20 mL) was stirred at room temperature for 30 min. NH_2OTHP (1.40 g, 12.0 mmol) was added to the mixture at room temperature. After the mixture was stirred for a further 4.5 h, the solvent was removed in vacuo. The residue was coevaporated several times with toluene and dissolved in THF (20 mL) containing DBU (1.40 mL, 9.60 mmol). The mixture was stirred at room temperature for 24 h, and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 40:1) to give **33** as white foam, which was treated with Cs_2CO_3 (3.00 g, 9.21 mmol) in MeOH (20 mL). After 24 h, the reaction mixture was neutralized by addition of aqueous HCl (1 M), and the mixture was extracted with AcOEt (150 mL). The organic phase was washed with H_2O (50 mL × 2) and brine (50 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 40:1) to give **34** (584 mg, 40% as a white foam): LRMS (FAB) *m*/*z* 605 (MH^+, 100%); HRMS (FAB) calcd for $C_{31}H_{41}N_6O_5Si$ 605.2905, found 605.2902; ^{1}H NMR ($CDCl_3$) 8.09, 8.28 (each s, 1 H, H-8), 8.11 (s, 1 H, H-2), 7.64−7.30 (m, 10 H, Ph), 7.23 (br s, 1 H, 2′-NH), 6.18, 6.06 (each d, 1 H, H-1′, $J_{1',2'}$ = 6.6, 7.3 Hz), 5.79 (br s, 2 H, 4-NH_2), 4.83, 4.81 (each m, 1 H, H-3′), 4.69 (br s, 1 H, 3′-OH), 4.54 (m, 1 H, H-2′), 4.38 (m, 1H, THP), 4.13 (ddd, 1 H, H-4′, *J* = 4.0, 4.0, 7.9 Hz), 3.99−3.91 (m, 2 H, THP), 3.83 (m, 1 H, H-5′a), 3.59 (m, 1 H, H-5′b), 1.97−1.27 (m, 6 H, THP), 1.02 (s, 9 H, *t*-Bu). Anal. ($C_{31}H_{40}N_6O_5Si$·0.5H_2O) C, H, N.

2′-Deoxy-2-(hydroxylamino)adenosine Dihydrochloride (35). A solution of **34** (400 mg, 0.661 mmol) in MeOH (2.5 mL) was treated with HCl (4 M in 1,4-dioxane, 2.5 mL) at room temperature for 24 h. The solvent was removed in vacuo, and the residue was dissolved in H_2O (15 mL), washed with $CHCl_3$ (15 mL × 3), and concentrated in vacuo. The residue was suspended with Et_2O, and the resulting crystals were collected by filtration to give **35** (233 mg, 99%): mp 125 °C dec; LRMS (FAB) *m*/*z* 283 (MH^+, 75%); HRMS (FAB) calcd for $C_{10}H_{15}N_6O_4$ 283.1154, found 283.1145; ^{1}H NMR (MeOH-d_4) 8.72, 8.47 (each s, each 1 H, H-2, H-8), 6.72 (d, 1 H, H-1′, $J_{1',2'}$ = 6.3 Hz), 4.84 (dd, 1 H, H-3′, $J_{2',3'}$ = 6.3, $J_{3',4'}$ = 3.3 Hz), 4.75 (dd, 1 H, H-2′, $J_{1',2'}$ = 6.3, $J_{2',3'}$ = 6.3 Hz), 4.26 (ddd, 1 H, H-4′, $J_{3',4'}$ = 3.2, $J_{4',5'a}$ = 3.0, $J_{4',5'b}$ = 3.2 Hz), 3.86 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 2.9, J_{gem} = 12.3 Hz), 3.80 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 3.2, J_{gem} = 12.2 Hz); ^{13}C NMR (MeOH-d_4) 151.83, 149.87, 145.70, 144.08, 120.37, 89.50, 85.57, 70.71, 67.25, 62.49. Anal. ($C_{10}H_{14}N_6O_4$·2HCl·H_2O) C, H, N.

1-[2,5-*O*-Bis(*tert*-butyldimethylsilyl)-3-deoxy-3-(hydroxyimino)-β-D-*erythro*-pentofuranosyl]uracil (37). A solution of **36** (4.70 g, 9.98 mmol) and NH_2OH·HCl (1.74 g, 25.0 mmol) in pyridine (100 mL) was stirred at room temperature for 20 min. The solvent was removed in vacuo, and the residue was dissolved in AcOEt (250 mL), washed with H_2O (50 mL × 3) and brine (50 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane:AcOEt = 3:1) to give **37** (4.87 g, 9.98 mmol, quantitative as a white foam): LRMS (FAB) *m*/*z* 486 (MH^+, 20%); HRMS (FAB) calcd for $C_{21}H_{40}N_3O_6Si_2$ 486.2453, found 486.2429; ^{1}H NMR ($CDCl_3$) 8.67−8.31, 7.86 (each br s, each 2 H, 3-NH, 3′-NOH), 7.94 (d, 1 H, H-6, $J_{5,6}$ = 8.2 Hz), 7.78 (d, 1 H, H-6, $J_{5,6}$ = 8.1 Hz), 6.12 (d, 1 H, H-1′, $J_{1',2'}$ = 7.2 Hz), 5.97 (d, 1 H, H-1′, $J_{1',2'}$ = 2.9 Hz), 5.77, 5.71 (each dd, 1 H, H-5, *J* = 2.2, 8.1 Hz), 5.03 (m, 1 H, H-4′), 4.90 (d, 1 H, H-2′, $J_{1',2'}$ = 2.9 Hz), 4.81 (m, 1 H, H-4′), 4.62 (dd, 1 H, H-2′, *J* = 1.3, 7.2 Hz), 4.15 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 1.2, J_{gem} = 11.1 Hz), 4.10 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 2.3, J_{gem} = 11.8 Hz), 3.96 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 3.2, J_{gem} = 11.8 Hz), 3.86 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 1.8, J_{gem} = 11.1 Hz), 0.92, 0.90, 0.88, 0.86 (each s, each 9 H, *t*-Bu), 0.12, 0.11, 0.10, 0.08, 0.07, 0.06, 0.05, 0.04 (each s, each 3 H, Me); ^{13}C NMR ($CDCl_3$) 163.45, 163.14, 157.47, 155.45, 150.29, 150.12, 140.44, 139.71, 103.21, 102.46, 91.09, 86.51, 79.55, 74.68, 71.81, 63.21, 62.27, 25.90, 25.86, 25.63, 25.47, 18.47, 18.34, 18.16, 18.08, −4.72, −5.02, −5.20, −5.30, −5.35, −5.42, −5.45. Anal. ($C_{21}H_{39}N_3O_6Si_2$) C, H, N.

1-[2-*O*-(*tert*-Butyldimethylsilyl)-3-deoxy-3-(hydroxyimino)-β-D-*erythro*-pentofuranosyl]uracil (38). A solution of **37** (3.50 g, 7.20 mmol) in aqueous TFA (80%, 25 mL) was stirred at 0 °C for 30 min, and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (hexane:AcOEt = 1:1) to give **38** (2.64 g, 99% as a white foam): LRMS (FAB) *m*/*z* 372 (MH^+, 31%); HRMS (FAB) calcd for $C_{15}H_{26}N_3O_6Si$ 372.1589, found 372.1592; ^{1}H NMR ($CDCl_3$) 8.91 (br s, 1 H, 3′-NOH), 8.41 (br s, 1 H, 3-NH), 7.55 (d, 1 H, H-6, $J_{5,6}$ = 8.1 Hz), 5.83 (d, 1 H, H-5, $J_{5,6}$= 8.1 Hz), 5.62 (d, 1 H, H-1′, $J_{1',2'}$ = 6.6 Hz), 5.06 (dd, 1 H, H-2′, $J_{1',2'}$ = 6.6 Hz), 5.04 (m, 1 H, H-4′), 4.18 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 1.5, J_{gem} = 12.0 Hz), 3.99 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 2.0, J_{gem} = 12.0 Hz), 0.86 (s, 9 H, *t*-Bu), 0.35, 0.13 (each s, each 3 H, Me). Anal. ($C_{15}H_{25}N_3O_6Si$·0.5H_2O) C, H; N: calcd, 11.04; found, 10.33.

2′-*O*-(*tert*-Butyldimethylsilyl)-3′-deoxy-3-(hydroxylamino)uridine (39). A solution of **38** (400 mg, 1.09 mmol) in AcOH (3 mL) was added dropwise to a solution of AcOH (8 mL) containing $NaBH_4$ (82 mg, 2.18 mmol) at 0 °C. After the mixture was stirred at the same temperature for 2 h, the solvent was removed in vacuo. The residue was diluted with AcOEt (50 mL), washed with H_2O (20 mL × 3) and brine (20 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography (AcOEt) to give **39** (378 mg, 92% as a white foam): LRMS (FAB) *m*/*z* 374 (MH^+, 8%); HRMS (FAB) calcd for $C_{15}H_{28}N_3O_6Si$ 374.1746, found 374.1718; ^{1}H NMR ($CDCl_3$) 10.20 (br s, 1 H, 3-NH), 8.00 (d, 1 H, H-6, $J_{5,6}$ = 8.1 Hz), 6.82 (br s, 1 H, 3′-NHO*H*), 5.74 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 5.69 (d, 1 H, H-1′, $J_{1',2'}$ = 2.8 Hz), 5.64 (br s, 1 H, 3′-N*H*OH), 4.65 (dd, 1 H, H-2′, $J_{1',2'}$ = 2.8, $J_{2',3'}$ = 4.7 Hz), 4.10 (ddd, 1 H, H-4′, $J_{3',4'}$ = 6.9, $J_{4',5'a}$ = 1.3, $J_{4',5'b}$ = 1.3 Hz), 4.02 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 1.3, J_{gem} = 12.1 Hz), 3.85 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 1.3, J_{gem} = 12.1 Hz), 3.77 (br s, 1 H, 5′-OH), 3.66 (dd, 1 H, H-3′, $J_{2',3'}$ = 4.7, $J_{3',4'}$ = 6.9 Hz), 0.93 (s, 9 H, *t*-Bu), 0.16 (s, 6 H, Me); ^{13}C NMR (67.8 MHz, $CDCl_3$) 164.69, 150.78, 142.41, 101.60, 92.78, 81.92, 73.82, 61.74, 61.24, 25.72, 17.95, −4.81, −4.87. Anal. ($C_{15}H_{27}N_3O_6Si$·$^1/_3H_2O$) C, H, N.

3′-Deoxy-3-(hydroxylamino)uridine (40, 3′-DHAU). A mixture of **39** (371 mg, 1.00 mmol) and TBAF (1 M in THF, 1.20 mL, 1.20 mmol) in THF (10 mL) was stirred at room temperature for 1 h. The solvent was removed in vacuo, the residue was coevaporated several times with MeOH, and the resulting precipitates were collected by filtration to give **40** (185 mg, 71% as a white solid): mp 205−206 °C; LRMS (FAB)

m/z 352 ((MH + glycerin)$^+$, 7%); ^{1}H NMR (DMSO-d_6) 11.30 (br s, 1 H, 3-NH), 7.96 (d, 1 H, H-6, $J_{5,6}$ = 8.2 Hz), 7.46 (br s, 1 H, 3′-NHO*H*), 5.83 (d, 1 H, H-1′, $J_{1',2'}$ = 5.2 Hz), 5.71 (br s, 1 H, 3′-N*H*OH), 5.64 (d, 1 H, H-5, $J_{5,6}$ = 8.2 Hz), 5.13 (br s, 1 H, 5′-OH), 4.17 (m, 1 H, H-2′), 3.98 (ddd, 1 H, H-4′, $J_{3',4'}$ = 4.8, $J_{4',5'a}$ = 2.0, $J_{4',5'b}$ = 2.8 Hz), 3.67 (d, 1 H, H-5′a, J_{gem} = 11.8 Hz), 3.53 (d, 1 H, H-5′b, J_{gem} = 11.8 Hz), 3.36 (m, 1 H, H-3′); ^{13}C NMR (67.8 MHz, DMSO-d_6) 163.24, 150.80, 140.67, 101.67, 88.57, 81.93, 72.78, 62.77, 61.87. Anal. ($C_9H_{13}N_3O_6$·0.2MeOH) C, H, N.

2′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(*tert*-butyldiphenylsilyl)-3′-[*N*-(*tert*-butyldiphenylsilyl)oxyamino]-3′-deoxyuridine (41). A mixture of **39** (1.00 g, 2.68 mmol), TBDPSCl (2.10 mL, 8.25 mmol), and imidazole (618 mg, 9.08 mmol) in DMF (30 mL) was stirred at room temperature for 24 h. The solvent was removed in vacuo, and the residue was dissolved in AcOEt (150 mL), washed with H_2O (50 mL × 3) and brine (50 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane:AcOEt = 5:1) to give **41** (2.06 g, 90% as a white foam): LRMS (FAB) *m/z* 850 (M^+, 12%); HRMS (FAB) calcd for $C_{47}H_{63}N_3O_6Si_3$ 850.4099, found 850.4119; ^{1}H NMR ($CDCl_3$) 8.28 (br s, 1 H, 3-NH), 7.64−7.25 (m, 21 H, H-6, Ph), 5.85 (d, 1 H, H-1′, $J_{1',2'}$ = 2.4 Hz), 5.79 (d, 1 H, 3′-NH, $J_{3',NH}$ = 9.2 Hz), 5.16 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 4.31 (dd, 1 H, H-2′, $J_{1',2'}$ = 2.4, $J_{2',3'}$ = 4.8 Hz), 3.86 (m, 2 H, H-5′), 3.51 (dd, 1 H, H-4′, $J_{3',4'}$ = 8.9, $J_{4',5'}$ = 4.1 Hz), 3.31 (ddd, 1 H, H-3′, $J_{2',3'}$ = 4.8, $J_{3',NH}$ = 9.2, $J_{3',4'}$ = 8.9 Hz), 1.08, 1.04, 0.88 (each s, each 9 H, *t*-Bu), 0.16, 0.08 (each s, each 3 H, Me); NOE irradiate H-3′β, observe H-2′β (9.3%) and H-4′ (1.2%), no other NOE enhancement was observed. Anal. ($C_{47}H_{63}N_3O_6Si_3$) C, H, N.

2′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(*tert*-butyldiphenylsilyl)-3′-[*N*-(*tert*-butyldiphenylsilyl)oxyamino]-3′-deoxycytidine (42). Compound **41** (2.10 g, 2.47 mmol) was converted into **42** (2.02 g, 96% as a white foam) as described for the synthesis of **16**: LRMS (FAB) *m/z* 849 (MH^+, 8%); HRMS (FAB) calcd for $C_{47}H_{65}N_4O_5Si_3$ 849.4259, found 849.4225; ^{1}H NMR ($CDCl_3$) 7.71−7.16 (m, 23 H, H-6, 4-NH_2, Ph), 5.80 (s, 1 H, H-1′), 5.75 (d, 1 H, 3′-NH, $J_{3',NH}$ = 11.3 Hz), 5.03 (d, 1 H, H-5, $J_{5,6}$ = 7.4 Hz), 4.38 (d, 1 H, H-2′, $J_{2',3'}$ = 4.1 Hz), 3.91−3.80 (m, 2 H, H-5′), 3.52 (dd, 1 H, H-4′, $J_{3',4'}$ = 11.6, $J_{4',5'}$ = 4.2 Hz), 3.24 (ddd, 1 H, H-3′, $J_{2',3'}$ = 4.1, $J_{3',NH}$ = 11.3, $J_{3',4'}$ = 11.6 Hz), 1.06, 1.03, 0.88 (each s, each 9 H, *t*-Bu), 0.29, 0.16 (each s, each 3 H, Me). Anal. ($C_{47}H_{64}N_4O_5Si_3$·2H_2O) C, N; H: calcd, 7.74; found, 7.29.

***N*^{4}-(4,4′-Dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*tert*-butyldiphenylsilyl)-3′-[(*tert*-butyldiphenylsilyl)oxyamino]-3′-deoxycytidine (43).** A mixture of **42** (849 mg, 1.00 mmol), Et_3N (237 μL, 1.70 mmol), and DMTrCl (508 mg, 1.50 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature for 6 h. The solvent was removed in vacuo, and the residue was dissolved in AcOEt (50 mL), washed with H_2O (10 mL × 3) and brine (10 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane:AcOEt = 3:2) to give **43** (1.12 g, 97% as a white foam): LRMS (FAB) *m/z* 1151 (MH^+, 5%); HRMS (FAB) calcd for $C_{68}H_{83}N_4O_7Si_3$ 1151.5569, found 1151.5580; ^{1}H NMR ($CDCl_3$) 7.58−6.79 (m, 34 H, H-6, Ph), 6.67 (br s, 1 H, 4-NH), 5.77 (s, 1 H, H-1′), 5.73 (d, 1 H, 3′-NH, $J_{3',NH}$ = 9.0 Hz), 4.75 (d, 1 H, H-5, $J_{5,6}$ = 7.6 Hz), 4.33 (d, 1 H, H-2′, $J_{2',3'}$ = 3.9 Hz), 3.76 (s, 6 H, OMe), 3.76−3.64 (m, 2 H, H-5′), 3.27 (m, 1 H, H-4′), 3.00 (ddd, 1 H, H-3′, $J_{2',3'}$ = 3.9, $J_{3',NH}$ = 9.0, $J_{3',4'}$ = 11.4 Hz), 1.04, 0.97, 0.89 (each s, each 9 H, *t*-Bu), 0.28, 0.17 (each s, each 3 H, Me).

***N*^{4}-(4,4′-Dimethoxytrityl)-3′-deoxy-3′-(hydroxylamino)-cytidine (44).** A solution of **43** (505 mg, 0.438 mmol) in THF (4 mL) containing AcOH (94 μL, 1.58 mmol) was treated with TBAF (1 M in THF, 1.58 mL, 1.58 mmol) at room temperature for 3 h. The solvent was removed in vacuo, and the residue was dissolved in AcOEt (50 mL), washed with H_2O (10 mL × 5) and brine (10 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 8:1) to give **44** (217 mg, 88% as a white powder): LRMS (FAB) *m/z* 561 (MH^+, 3%); HRMS (FAB) calcd for $C_{30}H_{33}N_4O_7$ 561.2349, found 561.2339; ^{1}H NMR ($CDCl_3$) 7.70 (d, 1 H, H-6, $J_{5,6}$ = 7.4 Hz), 7.31−6.81 (m, 13 H, Ph), 6.96 (br s, 1 H, 4-NH), 5.64 (s, 1 H, H-1′), 5.13 (d, 1 H, H-5, $J_{5,6}$ = 7.4 Hz), 4.44 (m, 1 H, H-2′), 4.11 (m, 1 H, H-4′), 3.87 (d, 1 H, H-5′a, J_{gem} = 11.4 Hz), 3.84 (d, 1 H, H-5′b, J_{gem} = 11.4 Hz), 3.76 (s, 6 H, OMe), 3.59 (m, 1 H, H-3′).

3′-Deoxy-3′-(hydroxylamino)cytidine Dihydrochloride (45, 3′-DHAC). A mixture of **44** (56.0 mg, 0.099 mmol) in concentrated HCl (100 μL) and EtOH (900 μL) was stirred at 0 °C for 24 h. The solvent was removed in vacuo. The residue was dissolved in H_2O (25 mL), washed with $CHCl_3$ (10 mL × 3), and concentrated in vacuo. The residue was suspended with *i*-PrOH and Et_2O, and the resulting crystals were collected by filtration to give **45** (32.0 mg, 98%): mp 241 °C dec; LRMS (FAB) *m/z* 259 (MH^+, 33%); ^{1}H NMR (MeOH-d_4) 8.25 (d, 1 H, H-6, $J_{5,6}$ = 7.8 Hz), 6.21 (d, 1 H, H-5, $J_{5,6}$ = 7.8 Hz), 5.93 (d, 1 H, H-1′, $J_{1',2'}$ = 4.6 Hz), 4.49 (dd, 1 H, H-2′, $J_{1',2'}$ = 4.6, $J_{2',3'}$ = 6.0 Hz), 4.45 (m, 1 H, H-4′), 3.93 (dd, 1 H, H-3′, $J_{2',3'}$ = 6.0, $J_{3',4'}$ = 5.8 Hz), 3.76 (dd, 1 H, H-5′a, $J_{4',5'b}$ = 2.5, J_{gem} = 12.2 Hz), 3.65 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 2.7, J_{gem} = 12.2 Hz); ^{13}C NMR (MeOH-d_4) 160.95, 148.46, 146.05, 95.01, 92.98, 81.87, 74.10, 62.37, 61.76. Anal. ($C_9H_{14}N_4O_5$·2HCl·0.5EtOH) C, H, N.

1-[2,3-Dideoxy-3-(hydroxyimino)-5-*O*-trityl-β-D-*erythro*-pentofuranosyl]uracil (47). A mixture of 5′-*O*-trityl-2′-deoxyuridine (2.82 g, 5.99 mmol) and Dess−Martin reagent (5.09 g, 12.0 mmol) in CH_2Cl_2 (50 mL) was stirred at room temperature for 24 h. The mixture was diluted with AcOEt (200 mL), washed with aqueous $NaHCO_3$ (50 mL), aqueous $Na_2S_2O_3$ (50 mL), and brine (50 mL), dried (Na_2SO_4) and concentrated in vacuo. A mixture of crude **46** and NH_2OH·HCl (2.00 g, 28.8 mmol) in pyridine (50 mL) was stirred at room temperature for 24 h. The solvent was removed in vacuo, and the residue was dissolved in AcOEt (200 mL), washed with H_2O (50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane:AcOEt = 1:2) to give **47** (2.10 g, 72% as a white foam): LRMS (FAB) *m/z* 484 (MH^+, 10%); HRMS (FAB) calcd for $C_{28}H_{26}N_3O_5$ 484.1872, found 484.1875; ^{1}H NMR ($CDCl_3$) 9.73, 9.70 (each br s, 1 H, 3′-NOH), 9.27, 9.20 (each br s, 1 H, 3-NH), 7.94, 7.83 (each d, 1 H, H-6, $J_{5,6}$ = 8.3 Hz), 7.38−7.20 (m, 15 H, Ph), 6.35, 6.34 (each dd, 1 H, H-1′, $J_{1',2'}$ = 6.9, 8.3 Hz), 5.28, 5.25 (each d, 1 H, H-5, $J_{5,6}$ = 8.3 Hz), 4.94, 4.71 (each m, 1 H, H-4′), 4.02−2.75 (m, 4 H, H-2′, H-5′).

2′,3′-Dideoxy-3′-(hydroxylamino)uridine (49, 3′-dDHAU). A mixture of **47** (2.00 g, 4.14 mmol) in concentrated HCl (1 mL) and MeOH (9 mL) was stirred at room temperature for 12 h, and the reaction mixture was neutralized by addition of saturated aqueous $NaHCO_3$. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 10:1) to give white solids (646 mg) as a mixture of **48** and uracil (about 1:1): LRMS (FAB) *m/z* 242 (MH^+, 41%); HRMS (FAB) calcd for $C_9H_{12}N_3O_5$ 242.0777, found 242.0792; ^{1}H NMR (DMSO-d_6) 11.30, 11.00 (each br s, 1 H, 3′-NOH), 10.90, 10.80 (each br s, 1 H, 3′-NH), 8.01, 7.82 (each m, 1 H, H-6), 6.19 (m, 1H, H-1′), 5.69−5.64 (m, 1 H, H-5), 5.02, 4.08 (m, 1 H, 5′-OH), 4.71, 4.47 (each m, 1 H, H-4′), 3.70−3.60 (m, 2 H, H-5′), 3.16−2.49 (m, 2 H, H-2′).

A solution of the above solids (580 mg) in AcOH (10 mL) was added dropwise to a solution of AcOH (10 mL) containing $NaBH_4$ (182 mg, 4.81 mmol) at 0 °C. After being stirred for 1 h, the solvent was removed in vacuo. The residue was coevaporated several times with toluene and purified by silica gel column chromatography ($CHCl_3$:MeOH = 3:1) to give **49** (456 mg, 1.87 mmol, 51% from **47** as a white foam): LRMS (FAB) *m/z* 244 (MH^+, 31%); HRMS (FAB) calcd for $C_9H_{13}N_3O_5$ 244.0933, found 244.0929; ^{1}H NMR (DMSO-d_6) 11.80 (br s, 1 H, 3-NH), 8.55 (d, 1 H, H-6, $J_{5,6}$ = 8.1 Hz), 6.72 (dd, 1 H, H-1′, $J_{1',2'a}$ = 6.8 Hz, $J_{1',2'b}$ = 6.8 Hz), 6.25 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 4.51 (m, 1 H, H-4′), 4.26−4.13 (m, 3 H, H-3′, H-5′), 2.81 (m, 1 H, H-2′a), 2.57 (ddd, 1 H, H-2′b, $J_{1',2'b}$ = 6.6, J_{gem} = 14.2, $J_{2'b,3'}$ = 6.6 Hz); ^{13}C NMR (MeOH-d_4) 162.91, 150.19, 140.44, 101.65, 84.50, 83.07, 62.30, 35.42, 21.73. Anal. ($C_9H_{13}N_3O_5$·1.5MeOH) C, H; N: calcd, 14.43; found, 13.87.

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-dideoxy-3′-[(*tert*-butyldiphenylsilyl)oxyamino]uridine (50). A mixture of **49** (319 mg, 1.31 mmol), TBDPSCl (1.02 mL, 3.94 mmol), and imidazole (446 mg, 6.55 mmol) in DMF (10 mL) was stirred at room temperature for 24 h. The mixture was diluted with AcOEt (200 mL), washed with H_2O (50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane:AcOEt = 2:1) to give **50** (809 mg, 85% as a white foam): LRMS (FAB) *m*/*z* 720 (MH^+, 49%); HRMS (FAB) calcd for $C_{41}H_{50}N_3O_5Si_2$ 720.3289, found 720.3306; ^{1}H NMR ($CDCl_3$) 8.95 (br s, 1 H, 3-NH), 7.74 (d, 1 H, H-6, $J_{5,6}$ = 8.1 Hz), 7.68−7.32 (m, 20 H, Ph), 6.20 (dd, 1 H, H-1′, $J_{1',2'a}$ = 6.3, $J_{1',2'b}$ = 7.6 Hz), 5.35 (dd, 1 H, H-5, J = 2.0, 8.1 Hz), 5.22 (d, 1 H, 3′-NH, $J_{3',NH}$ = 5.6 Hz), 3.95−3.82 (m, 3 H, H-3′, H-5′), 3.59 (m, 1 H, H-4′), 2.48 (ddd, 1 H, H-2′a, $J_{1',2'a}$ = 6.3, J_{gem} = 14.4, $J_{2'a,3'}$ = 4.2 Hz), 1.99 (ddd, 1 H, H-2′b, $J_{1',2'b}$ = 7.6, J_{gem} = 14.4, $J_{2'b,3'}$ = 7.6 Hz), 1.09, 1.05 (each s, each 9 H, *t*-Bu). Anal. ($C_{41}H_{49}N_3O_5Si_2\cdot0.9H_2O$) C, H, N.

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-dideoxy-3-[(*tert*-butyldiphenylsilyl)oxyamino]cytidine (51). Compound **50** (735 mg, 1.02 mmol) was converted into **51** (635 mg, 87% as a white foam) as described for the synthesis of **16**: LRMS (FAB) *m*/*z* 719 (MH^+, 1%); HRMS (FAB) calcd for $C_{41}H_{51}N_4O_4Si_2$ 719.3449, found 719.3442; ^{1}H NMR ($CDCl_3$) 7.86 (d, 1 H, H-6, $J_{5,6}$ = 7.3 Hz), 7.65−7.28 (m, 20 H, Ph), 6.18 (dd, 1 H, H-1′, $J_{1',2'a}$ = 6.3, $J_{1',2'b}$ = 6.3 Hz), 5.33 (d, 1 H, H-5, $J_{5,6}$ = 7.3 Hz), 5.19 (d, 1 H, 3′-NH, $J_{3',NH}$ = 6.8 Hz), 3.97 (ddd, 1 H, H-4′, $J_{3',4'}$ = 4.9, $J_{4',5'a}$ = 2.9, $J_{4',5'b}$ = 2.9 Hz), 3.86 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 2.9, J_{gem} = 11.2 Hz), 3.77 (m, 1 H, H-3′), 3.56 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 2.9, J_{gem} = 11.2 Hz), 2.62 (ddd, 1 H, H-2′a, $J_{1',2'a}$ = 6.3, J_{gem} = 14.2, $J_{2'a,3'}$ = 6.3 Hz), 1.97 (ddd, 1 H, H-2′b, $J_{1',2'b}$ = 6.3, J_{gem} = 14.2, $J_{2'b,3'}$ = 7.8 Hz), 1.07, 1.03 (each s, each 9 H, *t*-Bu); ^{13}C NMR ($CDCl_3$) 165.28, 155.52, 141.06, 135.63, 135.61, 135.40, 135.24, 132.96, 132.38, 129.79, 129.69, 127.71, 127.67, 127.48, 127.46, 93.61, 85.90, 82.57, 64.23, 61.75, 36.75, 27.45, 27.07, 19.43, 19.29. Anal. ($C_{41}H_{50}N_4O_4Si_2\cdot1.6H_2O$) C, H, N.

2′,3′-Dideoxy-3′-(hydroxylamino)cytidine Dihydrochloride (52, 3′-dDHAC). A mixture of **51** (72 mg, 0.100 mmol) in concentrated HCl (300 μL) and MeOH (700 μL) was stirred at room temperature for 4 h. The solvent was removed in vacuo. The residue was coevaporated several times with EtOH and suspended with EtOH and hexane, and the resulting crystals were collected by filtration to give **52** (32.0 mg, quantitative): mp 175 °C dec; LRMS (FAB) *m*/*z* 243 (MH^+, 28%); HRMS calcd for $C_9H_{15}N_4O_4$ 243.1093, found 243.1095; ^{1}H NMR (MeOH-d_4) 8.36 (d, 1 H, H-6, $J_{5,6}$ = 7.9 Hz), 6.27 (dd, 1 H, H-1′, $J_{1',2'a}$ = 6.6, $J_{1',2'b}$ = 6.6 Hz), 6.17 (d, 1 H, H-5, $J_{5,6}$ = 7.9 Hz), 4.54 (ddd, 1 H, H-4′, $J_{3',4'}$ = 2.6, $J_{4',5'a}$ = 2.6, $J_{4',5'b}$ = 3.3 Hz), 4.31 (ddd, 1 H, H-3′, $J_{2'a,3'}$ = 2.6, $J_{2'b,3'}$ = 8.6, $J_{3',4'}$ = 2.6 Hz), 3.93 (dd, 1 H, H-5′a, $J_{4',5'a}$ = 2.6, J_{gem} = 11.9 Hz), 3.82 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 3.3, J_{gem} = 11.9 Hz), 2.85 (ddd, 1 H, H-2′a, $J_{1',2'a}$ = 6.6, J_{gem} = 15.2, $J_{2'a,3'}$ = 2.6 Hz), 2.55 (ddd, 1 H, H-2′b, $J_{1',2'b}$ = 6.6, J_{gem} = 15.2, $J_{2'b,3'}$ = 8.6 Hz); NOE: irradiate H-2′β, observe H-1′ (1.9%), H-6 (5.8%), and H-3′β (15.3%), irradiate H-2′α, observe H-1′ (17.9%) and H-3′β (1.8%), no other NOE enhancement was observed; ^{13}C NMR (MeOH-d_4) 160.84, 148.00, 145.94, 94.61, 88.46, 82.73, 62.97, 62.56, 35.12. Anal. ($C_9H_{14}N_4O_4\cdot2HCl\cdot0.4H_2O$) C, H, N.

Determination of the pK_a's of 2′-DHAU (15) and 3′-DHAU (40) by ^{13}C NMR Spectroscopy. The nucleosides (50 mM) in 200 mM HCl−KCl, phosphate, borate, or acetate buffer (H_2O) adjusted to the pH given in Figure 1 and containing 10% D_2O were measured at 27 °C. Each data point is shown as $\Delta\delta$ (ppm) = δ (ppm) at the pH given in Figure 1 − δ (ppm) at pH 0.57 for **15** or pH 0.50 for **40**.

Degradation of 2′-DHAC (19) or 3′-DHAC (45). A solution of 2′-DHAC (3 mg, 0.009 mmol) or 3′-DHAC (3 mg, 0.009 mmol) in phosphate buffer (pH 7.0, 0.1 M, 10 mL) or KCl−HCl buffer (pH 1.0, 0.1 M, 10 mL) was incubated at 37 °C. At appropriate periods, samples of the reaction mixture (10 μL) were analyzed by C-18 HPLC (J′-sphere ODS-M80, 4.6 × 250 mm) with 1% aqueous MeOH as an eluent at a flow rate of 1 mL/min. Each peak was detected at 260 nm and showed the appropriate retention time. Each peak area ratio was calculated with cytidine as a reference.

Effects of Common Nucleosides on the in Vitro Cytotoxicities of 2′-DHAC (19), 3′-DHAC (45), and 3′-dDHAC (52). L1210 cells (10^4 cells/mL) (180 μL) were seeded in a 96-well microplate, and 10 μL of graded concentrations of 2′-DHAC (**19**), 3′-DHAC (**45**), or 3′-dDHAC (**52**) (final concentration: 10, 2, 0.4, 0.08 μg/mL) and 10 μL of each common nucleoside (final concentration: 50 μM) were simultaneously added in triplicate to each well. The plate was incubated for 3 days at 37 °C in a humidified atmosphere of 5% CO_2. The cytotoxicities of 2′-DHAC, 3′-DHAC, and 3′-dDHAC were evaluated by the MTT assay.

Effects of 2′-DHAC (19), 3′-DHAC (45), and 3′-dDHAC (52) on DNA and RNA Syntheses. After L1210 cells (2.5 × 10^5 cells/mL) were incubated for 24 h, 2′-DHAC (**19**), 3′-DHAC (**45**), or 3′-dDHAC (**52**) was added to the culture at 20 μg/mL and incubated for 1, 2, and 4 h. Before cell harvest, cells were pulse-labeled for 30 min with 1 μCi/mL of [^{3}H]thymidine (83.2 Ci/mmol) or [^{3}H]uridine (23.1 Ci/mmol) purchased from Amersham Life Sciences, Buckinghamshire, England. The radioactivity of the acid-insoluble fractions was measured by a Beckman LS 6500 liquid scintillation counter (Beckman Instruments, Fullerton, CA) using Scintisol EX-H (Dojin Chemicals, Kumamoto, Japan).

Antitumor Effects of 2′-DHAC (19) and 3′-DHAC (45) on Murine Fibrosarcoma Meth-A. Five-week-old female BALB/cCrj mice weighing about 20 g were purchased from Charles River Japan Inc., Atsugi, Japan. They were maintained on a standard diet and water throughout the experimental period under specific pathogen-free conditions. Six mice were used for each group in these experiments. Murine fibrosarcoma Meth-A was maintained weekly by serial transplantation by injection into the abdominal cavity of BALB/c mice. One-week-old Meth-A cells (5 × 10^5 cells) were subcutaneously transplanted to the right inguinal region of BALB/c mice. 2′-DHAC (**19**) and 3′-DHAC (**45**) were intravenously administered for 10 consecutive days at doses of 2 and 10 mg/kg from the day after transplantation. Three weeks after transplantation, mice were sacrificed and solid tumors were dissected out and weighed. The tumor inhibition ratio (%) was calculated as follows: tumor inhibition ratio (%) = (1 − T/C) × 100, where C is the mean tumor weight in the control group and T is that in the treated group. Effectiveness was statistically evaluated by Student's *t*-test. The criterion for statistical significance was $p < 0.05$.

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