Nucleosides and Nucleotides. 175. Structural Requirements of the Sugar Moiety for the Antitumor Activities of New Nucleoside Antimetabolites, 1-(3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl)cytosine and -uracil[1]

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We previously designed 1-(3-*C*-ethynyl-*β*-D-*ribo*-pentofuranosyl)uracil (EUrd) and its cytosine congener (ECyd) as potential multifunctional antitumor nucleoside antimetabolites. They showed potent and broad-spectrum antitumor activity against various human and mouse tumor cells in vitro and in vivo. To clarify the structure−activity relationship of the sugar moiety, various 3′-*C*-carbon-substituted analogues, such as 1-propynyl, 1-butynyl, ethenyl, ethyl, and cyclopropyl derivatives, of ECyd and EUrd were synthesized. We also prepared 3′-deoxy analogues and 3′-homologues of ECyd and EUrd with different configurations to determine the role of the 3′-hydroxyl group and the length between the 3′-carbon atom and the ethynyl group and a 2′-ethynyl derivative of ECyd to determine the spatial requirements of the ethynyl group. The in vitro tumor cell growth inhibitory activities of these nucleosides against mouse leukemic L1210 and human KB cells showed that ECyd and EUrd were the most potent inhibitors in the series, with IC_{50} values of 0.016 and 0.13 μM for L1210 cells and 0.028 and 0.029 μM for KB cells, respectively. Only 3′-*C*-1-propynyl and -ethenyl derivatives of ECyd showed greatly reduced cytotoxicity. We found that the cytotoxic activity of these nucleosides predominantly depended on their first phosphorylation by uridine/cytidine kinase.

Introduction

The development of nucleoside antimetabolites is important for progress in anticancer chemotherapy. We recently designed 1-(3-*C*-ethynyl-*β*-D-*ribo*-pentofuranosyl)uracil (EUrd) as a potential multifunctional antitumor antimetabolite which was expected to inhibit the synthesis of both DNA and RNA in tumor cells.[2] EUrd shows potent tumor cell growth inhibitory activity against a variety of human tumor cell lines in vitro and strong antitumor activity against not only murine leukemia P388 but also a variety of human solid tumor xenografts in vivo. To clarify the structure−activity relationship of the nucleobase moiety, we synthesized various analogues and found that 1-(3-*C*-ethynyl-*β*-D-*ribo*-pentofuranosyl)cytosine (ECyd) showed more potent activity against human tumor cell lines in vitro than EUrd, and lower doses were required to produce maximum antitumor effects against human tumor xenografts.[3,4] Since the in vitro cytotoxicity of ECyd is significantly reduced in the presence of cytidine or uridine, ECyd may have to be phosphorylated by uridine/cytidine kinase (UCK) to show its cytotoxicity.[4] The substrate function of ECyd for human cytidine deaminase is extremely low.[4] ECyd has been shown to strongly inhibit RNA synthesis by inhibiting RNA polymerases[5] and to slightly inhibit DNA synthesis.[4] At 12 h after treatment, ECyd induces apoptotic cell death in human gastric cancer MKN 45 cells with the wild-type *p53* gene.[4]

To clarify further structural requirements for the antitumor activity of ECyd and EUrd, we describe here the synthesis and in vitro cytotoxicity of various 3′-*C*-carbon-substituted analogues, such as 1-propynyl, 1-butynyl, ethenyl, ethyl, and cyclopropyl derivatives, of ECyd and EUrd. We also prepared 3′-deoxy analogues and 3′-homologues of ECyd and EUrd with different configurations to determine the role of the 3′-hydroxyl group and the required length between the 3′-carbon atom and the ethynyl group. To determine the spatial requirements of the ethynyl group, the 2′-ethynyl derivative of ECyd was also synthesized. Moreover, the phosphorylation of selected nucleosides by partially purified uridine/cytidine kinase from mouse Sarcoma-180 ascites cells and its relationship to cytotoxicity are also described.

Chemistry

The synthesis of 3-*C*-propynyl- and -butynyl-*β*-D-*ribo*-pentofuranose derivatives is illustrated in Scheme 1. The 3-ulose derivative **1** was easily obtained from D-xylose in four steps in good yield.[6] Addition of $LiC{\equiv}CCH_3$ (or $LiC{\equiv}CCH_2CH_3$) to **1** gave the desired *β*-adduct **2a** (or **2b**) with high stereoselectivity.[3,7] Desilylation of **2a**,**b** with tetrabutylammonium fluoride (TBAF) followed by benzoylation of the hydroxyl group at the 5-position yielded **3a**,**b**, which were treated with 20% HCl in aqueous MeOH at room temperature to give

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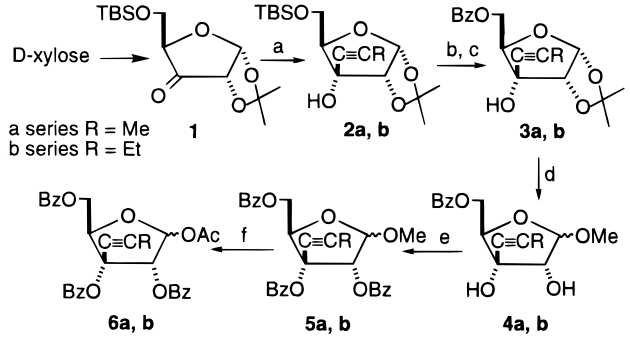
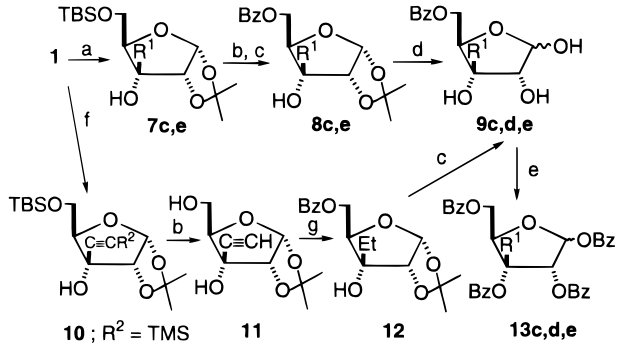
Scheme 1[a]



[a] (a) LiC≡CMe or LiC≡CEt, THF; (b) TBAF, THF; (c) BzCl, pyridine; (d) HCl/aq MeOH; (e) BzCl, DMAP, pyridine; (f) cH_2SO_4, AcOH, Ac_2O.

Scheme 2[a]

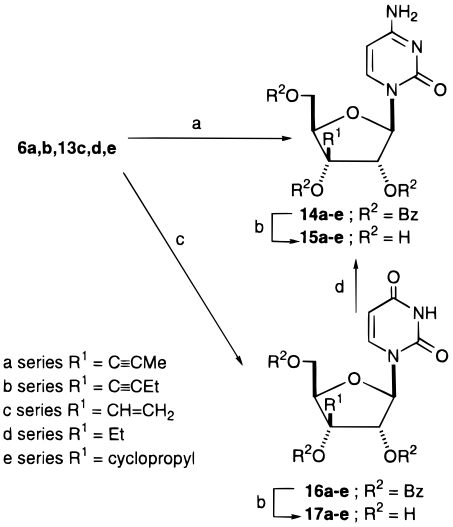


[a] (a) H_2C=CHMgBr or cyclopropyllithium, THF; (b) TBAF, THF; (c) BzCl, pyridine; (d) aq HCl, THF; (e) BzCl, DMAP, pyridine; (f) ref 3; (g) H_2, Pd−C, MeOH.

methyl 5-*O*-benzoyl-3-*C*-propynyl(and butynyl)-α,*β*-*ribo*-pentofuranosides (**4a**,**b**). Compounds **4a**,**b** were subsequently benzoylated with a mixture of BzCl and DMAP in anhydrous pyridine at 100 °C to give **5a**,**b**. Subsequent acetolysis of **5a**,**b** using concentrated H_2SO_4 in a mixture of AcOH and Ac_2O gave 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-3-*C*-propynyl(and butynyl)-α,*β*-*ribo*-pentofuranoses (**6a**,**b**), which were used for condensation with persilylated uracil and cytosine.

Although 3′-*C*-ethenyl and -ethyl derivatives can be prepared from ECyd and EUrd by partial or complete reduction of the ethynyl group by hydrogenolysis, the products and the starting material could not be adequately separated by any chromatography conditions we tested, even using HPLC, since these compounds had very similar retention times. Since ECyd and EUrd are highly potent tumor cell growth inhibitors, contamination by these nucleosides should be avoided when 3′-*C*-ethenyl and -ethyl derivatives are tested against tumor cells. Therefore, 3-*C*-ethenyl and -ethyl derivatives were synthesized using a condensation method similar to that described above. Reaction of **1** with vinylmagnesium bromide in THF at −78 °C gave the desired *β*-adduct **7c**, whose 5-*O*-TBS group was then deprotected by TBAF (Scheme 2). Subsequent benzoylation gave **8c**. The isopropylidene group of **8c** was removed by hydrolysis using 1.5% HCl in aqueous THF at reflux temperature to yield **9c**. The remaining hydroxyl groups of **9c** were benzoylated with BzCl in pyridine to give 1,2,3,5-tetra-*O*-benzoyl-3-*C*-ethenyl-α,*β*-D-*ribo*-pentofuranose (**13c**). Since the 3-tertiary alcohol was hardly benzoylated, 10 equiv of BzCl and 100 °C were required to complete the reaction. The 3-*C*-cyclopropyl derivative **13e** was prepared in a similar manner.

Scheme 3[a]



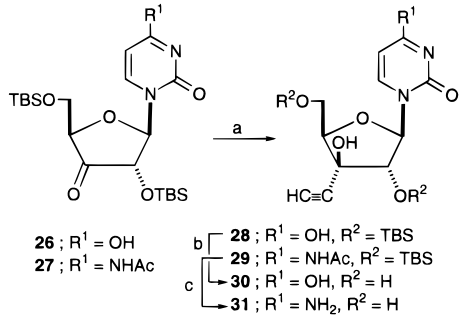
[a] (a) Persilylated cytosine, $SnCl_4$, MeCN; (b) NH_3/MeOH; (c) persilylated uracil, TMSOTf, MeCN; (d) TPSCl, DMAP, then NH_4OH.

Reaction of **1** with ethylmagnesium bromide, however, gave the desired 3-*C*-ethyl derivative together with considerable amounts of a reduced secondary alcohol (data not shown).[8] Therefore, the 3-*C*-ethyl derivative was prepared from **10**.[3] Compound **10** was first desilylated with TBAF to give **11**, which was hydrogenated and then benzoylated to give **12**. Hydrolysis of the 1,2-*O*-isopropylidene group, as described above, gave **9d**, which was subsequently benzoylated to furnish **13d**.

Condensation of **6a**,**b** with persilylated cytosine was carried out in the presence of $SnCl_4$ as a Lewis acid in MeCN at room temperature to give the corresponding cytosine nucleosides **14a**,**b** in good yields (Scheme 3). More time was needed for the condensation with bulkier terminal substituents at the ethynyl group. For the condensation of persilylated uracil with **6a**,**b**, trimethylsilyl triflate (TMSOTf) was used instead of $SnCl_4$ as a Lewis acid to avoid the formation of undesired *N*3-isomer[3] and gave predominantly the desired *N*1-nucleosides **16a**,**b** in good yields. For the synthesis of the 3′-*C*-ethenyl, -ethyl, and -cyclopropyl nucleosides, the corresponding sugar units **13c**−**e** were reacted with persilylated uracil in the presence of TMSOTf in MeCN to give **16c**−**e**, which were then transformed into the corresponding cytosine nucleosides **14c**−**e** by the usual procedure. Treatment of these protected nucleosides **14a**−**e** and **16a**−**e** with NH_3/MeOH gave the desired nucleosides **15a**−**e** and **17a**−**e**, respectively, in good yields.

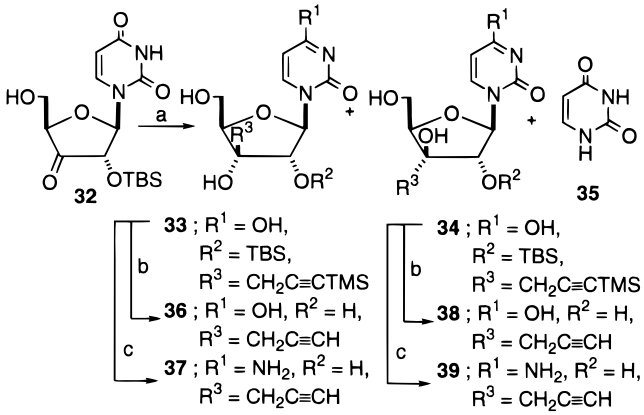
To further study the structure−activity relationship of ECyd and EUrd, we synthesized 1-(3-*C*-ethynyl-*β*-D-*xylo*-pentofuranosyl)cytosine (**31**) and -uracil (**30**) (Scheme 4), which are the 3′-epimers of ECyd and EUrd. Reaction of 2′,5′-di-*O*-TBS-3′-ketouridine derivative **26** with HC≡CMgBr in THF at −78 °C gave *xylo*-adduct **28** with high stereoselectivity.[9−11] In a similar manner, N^4-acetyl-3′-ketocytidine derivative **27** was converted into

Scheme 4[a]



[a] (a) HC≡CMgBr, THF; (b) NH_4F, MeOH; (c) HCl/MeOH.

Scheme 5[a]

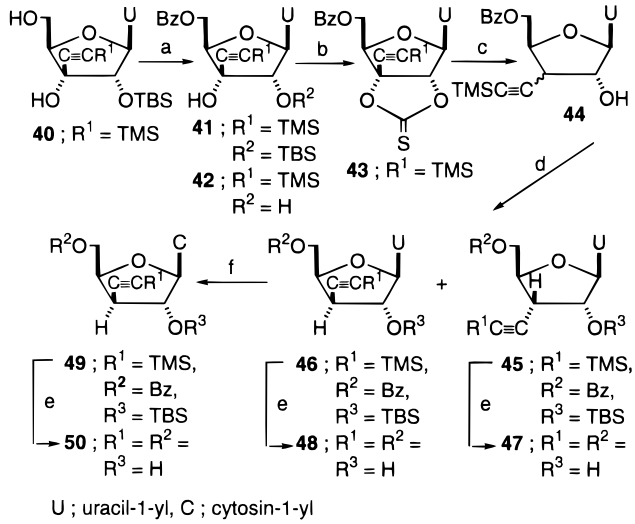


[a] (a) BuLi, $BrCH_2C{\equiv}CTMS$, THF; (b) NH_4F, MeOH; (c) (i) Bz_2O, DMAP, MeCN, (ii) TPSCl, DMAP, MeCN, (iii) NH_4OH, (iv) NaOMe, MeOH, (v) NH_4F, MeOH.

xylo-derivative **29**. These nucleosides were deprotected by the usual method to give the desired nucleosides **30** and **31**.

Previously, Jung et al. reported that treatment of 2′-*O*-TBS-3′-ketouridine derivative **32** with cerium (trimethylsilyl)acetylide in THF at −78 °C gave the *ribo*-pentofuranosyl derivative in good yield.[10] Therefore, we tried to synthesize 3′-*C*-2-propynyl derivatives, 3′-homologues of ECyd and EUrd, using this method (Scheme 5). However, attempts to prepare a cerium reagent by reacting 3-bromo-1-(trimethylsilyl)-1-propyne with BuLi followed by treatment with dehydrated $CeCl_3$ failed. On the other hand, reaction of **32** with a lithium salt of 1-(trimethylsilyl)-1-propyne (prepared by the reaction of 3-bromo-1-(trimethylsilyl)-1-propyne with BuLi) gave 1-[2-*O*-TBS-3-*C*-(3-(trimethylsilyl)-2-propynyl)-*β*-D-*ribo*-pentofuranosyl]uracil (**33**) in 12% yield along with its 3′-epimer **34** in 29% yield. From this reaction mixture, uracil (**35**) was obtained in 10% yield. In this reaction, 6 equiv of the reagent was required to consume all of the starting material. Compound **33** was deprotected by NH_4F in MeOH to give the desired uridine derivative **36**, which was converted into the cytosine derivative **37** using the usual method. *Xylo*-epimers **38** and **39** were also prepared by the same method. The stereochemistry at the 3′-position of each compound was confirmed using the NOE technique. For example, when the 1′-position of **36** was irradiated, NOE enhancement (5.8%) was observed at the 3′-down-OH but not at the methylene protons of the propynyl group, while 2.8% enhancement at the 3′-up-OH was observed when the 6-proton of the uracil base of **38** was irradiated.

Scheme 6[a]

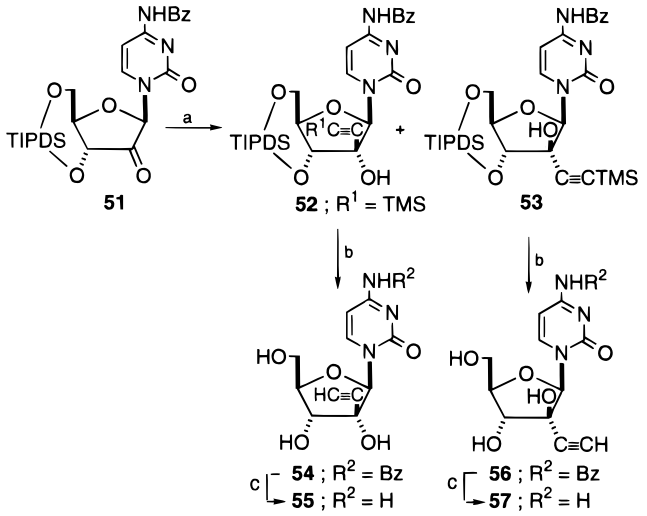


[a] (a) (i) Bz_2O, DMAP, MeCN, (ii) HCl/MeOH; (b) 1,1′-thiocarbonyldiimidazole, DMAP, MeCN, CH_2Cl_2; (c) Bu_3SnH, AIBN, toluene; (d) TBSCl, imidazole, DMF; (e) NaOMe/MeOH, then HCl/MeOH; (f) (i) TPSCl, DMAP, Et_3N, MeCN, (ii) NH_4OH.

To determine whether the 3′-OH in ECyd and EUrd is required for their cytotoxicity, we synthesized their 3′-deoxy analogues, **47**, **48**, and **50**, as illustrated in Scheme 6. The starting material **40** was synthesized as described previously.[10b] Selective benzoylation of the 5′-hydroxyl group of **40** gave **41**, and subsequent acid hydrolysis of the 2′-*O*-TBS group of **41** gave **42**. Treatment of **42** with 1,1′-thiocarbonyldiimidazole in MeCN gave 2′,3′-cyclic thiocarbonate **43**, which, without purification, was treated with Bu_3SnH in the presence of AIBN in toluene under reflux conditions to give an inseparable mixture of the corresponding 3′-deoxy derivatives **44** in a ratio of about 4:1 in a yield of 92%. This mixture was separable on a silica gel column after *tert*-butyldimethylsilylation of the 2′-hydroxyl group of **44** to give **45** and **46**, respectively. Since a propargyl radical at the 3′-position is more stable than the corresponding 2′-secondary radical, we could not detect any 2′-deoxy derivatives by TLC. The structures of these nucleosides were confirmed by NOE experiments. Compound **46** was converted into the corresponding cytosine nucleoside **49**. These nucleosides were deprotected to give **47**, **48**, and **50**.

We previously reported that the reaction of 1-[3,5-*O*-[1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDS)]-*β*-D-*erythro*-2-pentulofuranosyl]uracil and $LiC{\equiv}CR$ gave only α-adducts.[12] However, the reaction of N^4-benzoylcytosine derivative **51** with $LiC{\equiv}CTMS$ in THF at −78 °C gave the desired *β*-adduct **52** in 8.5% yield together with the usual α-adduct **53** in 68% yield (Scheme 7). The stronger coordination effects of a lithium cation to the 2-carbonyl oxygen of the cytosine base moiety than to that of uracil should give the *β*-adduct **52** in this case, since the *β*-addition of MeMgBr[8,13,14] to the 2′-keto group of 4-ethoxy-1-[3,5-*O*-(TIPDS)-*β*-D-*erythro*-2-pentulofuranosyl]-2(1*H*)-pyrimidinone was explained by chelation of the metal between 2- and 2′-carbonyls at the base and sugar moieties, which delivered the methyl carbanion from the sterically more hindered *β*-face. These protected nucleosides were deblocked in the usual

Scheme 7[a]



[a] (a) LiC≡CTMS, THF; (b) TBAF, THF; (c) NH_3/MeOH.

manner to give the corresponding free nucleosides **55** and **57**, respectively.

The structure of these nucleosides was confirmed by ^{1}H NMR, mass, IR, and UV spectrophotometries along with elemental analyses. The purities of **15d**, **17d**, **48**, and **50** were confirmed by HPLC without contamination by ECyd or EURd.

Biological Activity

The in vitro tumor cell growth inhibitory activities of the newly synthesized nucleosides against murine leukemia L1210 and human epidermoid KB cells were evaluated using MTT assay,[15] and these activities were compared to those of ECyd and EURd. The results are summarized in Table 1. Among the nucleosides, ECyd was the most potent inhibitor of tumor cell growth against L1210 cells, with an IC_{50} value of 0.016 μM. Against KB cells, ECyd and EURd were equally potent, with IC_{50} values of 0.028 and 0.029 μM, respectively. Compound **15a**, which has a methyl group instead of the terminal hydrogen atom of the ethynyl group in ECyd, showed 1500- and 520-fold lower activity against L1210 and KB cells, respectively. Compound **15b**, which has a longer ethyl group, had dramatically lower activity against both of the cells. Although **15c**, which has a relatively bulky ethenyl group instead of an ethynyl group, showed better activity than **15b**, its activity was negligible compared to that of ECyd. Both the 3′-*C*-ethyl and -cyclopropyl derivatives, **15d**,**e**, did not show any significant growth inhibitory activity. Additionally, the 3′-epimer, 3′-homologue and its epimer, and 3′-deoxy derivative of ECyd, **31**, **37**, **39**, and **50**, respectively, did not show any activity up to 350 μM against either of the cells. Moreover, the 2′-ethynyl derivative and its epimer, **55** and **57**, had no activity against either of the cells. This association between the 3′-substituent and cytotoxicity in the cytidine derivatives is similar to that in the uridine series (Table 1). Since ECyd and EURd were proposed to be phosphorylated to their 5′-monophosphates by UCK in a competition study with cytidine or uridine,[4] the bulkiness of the substituents would be important for recognition of these substrates by the kinase. Although **30**, **31**, **47**, **48**, and **50** have an ethynyl group at their 3′-position, they do not have a *cis*-diol at the 2′- and 3′-positions and therefore would not be substrates of UCK.

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

	IC_{50} (μM)	
compd	L1210	KB
ECyd	0.016	0.028
15a	24.5	14.6
15b	129	108
15c	60.8	74.3
15d	269	258
15e	>350	>350
31	>350	>350
37	>350	>350
39	>350	>350
50	>350	>350
55	>350	>350
57	>350	>350
EURd	0.13	0.029
17a	354	70.8
17b	338	226
17c	148	51.8
17d	>350	>350
17e	>350	>350
30	>350	>350
36	>350	>350
38	>350	>350
47	>350	>350
48	>350	>350

[a] Tumor cell growth inhibitory activity assay in vitro was done following the literature method.[15] Each tumor cell (2×10^3 cells/well) was incubated in the presence or absence of compounds for 72 h. MTT reagent was added to each well, and the plate was incubated 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. IC_{50} (μM) is given as the concentration at 50% inhibition of cell growth.

Table 2. Phosphorylation of ECyd, EURd, and Their Related Compounds by UCK from Mouse Sarcoma-180 Ascites Cells and Their Cytotoxicity against Sarcoma-180 Cells in Vitro

compd	phosphorylating activity[a] (pmol/min/assay)	relative activity (%)	cytotoxicity[b] IC_{50} (μM)
Cyd	812	100	
Urd	755	93	
ECyd	207	26	0.0034
EURd	141	17	0.013
15a	160	20	13.9
15b	0	0	>30
15c	0	0	>30
15d	0	0	>30
17c	9.3	1.1	>30
50	6.9	0.9	>30

[a] See the Experimental Section for the assay method. The values are means of duplicate experiments. [b] See Table 1 for the assay method.

To further examine the structure−cytotoxicity relationship of the sugar moiety of ECyd analogues, we compared the first phosphorylations of selected analogues by partially purified UCK from mouse Sarcoma-180 ascites cells. The results are summarized in Table 2 along with their in vitro tumor cell growth inhibitory activity (IC_{50}) against S-180 cells. For uridine, cytidine, ECyd, and EURd, radiolabeled nucleosides are available, and the substrate activity was measured in terms of the conversion of each nucleoside into the corresponding 5′-monophosphate. However, for other ECyd analogues, radiolabeled nucleosides are not available, and phos-

phorylation by UCK was quantitated in terms of the consumption of these nucleosides using HPLC. When cytidine and uridine were used as substrates, the phosphorylating activity of UCK was 812 and 755 pmol/min, respectively. ECyd and EUrd were phosphorylated by 26% and 17%, respectively, relative to the phosphorylation of cytidine. Therefore, the substrate specificity of UCK is reflected in ECyd and EUrd, and these analogues are relatively good substrates of UCK. Although **15a** was a substrate of the kinase (its relative activity was 20%), the cytotoxicity (IC_{50} = 13.9 μM) of **15a** against S-180 cells was 1000-fold less than that of EUrd. Again, **17c** and **50** were slightly phosphorylated by the kinase but were not cytotoxic. The phosphorylation of other analogues **15b**−**d** was not detected by this method, and these data correlate with their cytotoxicity. Therefore, these data suggest that the first phosphorylation of nucleosides by UCK is important for expression of their cytotoxicity. The substrate specificity of UCK from mouse S-180 ascites cells reflects the in vitro cytotoxicity of these compounds against L1210 and KB cells. The target enzyme of ECyd and EUrd that is responsible for their cytotoxicity against tumor cells has been proposed to be RNA polymerases.[5] Therefore, after phosphorylation by UCK, these 5′-monophosphates should be further converted into the corresponding 5′-triphosphates. In the case of **15a**, **17c**, and **50**, the efficiency of further phosphorylations by nucleotide kinases and/or their inhibitory activities against RNA polymerases remain to be elucidated.

In conclusion, the substrate specificity of UCK regarding ECyd and EUrd analogues is closely related to their cytotoxicities against the tumor cells used in this study. Only an ethynyl group at the 3′β-position in cytidine and uridine was sufficiently tolerated by UCK. Other bulkier substituents, such as 1-butynyl, ethyl, cyclopropyl, and even ethenyl groups, did not provide good substrates for UCK. The presence of a 2′,3′-*cis*-diol in ECyd and EUrd with the *ribo*-configuration was essential for its cytotoxicity. A further study is needed to elucidate the effect of these substituents on sugar puckering and its relationship to the substrate specificity of UCK.

Experimental Section

General Methods. 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 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).

5-*O*-(*tert*-Butyldimethylsilyl)-1,2-*O*-isopropylidene-3-*C*-(1-propynyl)-α-D-*ribo*-pentofuranose (2a). A hexane solution of BuLi (1.63 M, 1.84 mL, 3 mmol) was added dropwise over 30 min to a solution of propyne (about 0.5 mL) in THF (5 mL) at −78 °C under Ar. A solution of **1** (302 mg, 1 mmol) in THF (2.5 mL) was added dropwise over 10 min to the above mixture at the same temperature with stirring. After 30 min, the reaction was quenched by addition of aqueous NH_4Cl (1 M, 5 mL). The solution was extracted by EtOAc (30 mL × 3), and the separated organic phase was washed with brine (3 mL × 3), dried (Na_2SO_4), and concentrated to dryness in vacuo. The residue was purified on a silica gel column (2 × 9 cm) with 5% EtOAc in hexane to give **2a** (320 mg, 93% as a syrup): LRMS (FAB) *m*/*z* 327 (M^+ − Me); IR (neat) 2255 cm^{-1} (C≡C). Anal. ($C_{17}H_{30}O_5Si$) C, H.

5-*O*-(*tert*-Butyldimethylsilyl)-1,2-*O*-isopropylidene-3-*C*-(1-butynyl)-α-D-*ribo*-pentofuranose (2b). As described for the synthesis of **2a**, **2b** was similarly prepared from **1**. From **1** (302 mg, 1 mmol) and 1-butyne (about 0.4 mL), **2b** (259 mg, 73% as a syrup) was obtained. **2b**: LRMS (FAB) *m*/*z* 341 (M^+ − Me); IR (neat) 2245 cm^{-1} (C≡C). Anal. ($C_{18}H_{32}O_5Si$) C, H.

5-*O*-Benzoyl-1,2-*O*-isopropylidene-3-*C*-(1-propynyl)-α-D-*ribo*-pentofuranose (3a). A THF solution of TBAF (1 M, 10 mL, 10 mmol) was added to a solution of **2a** (3.42 g, 10 mmol) in THF (30 mL). The mixture was stirred for 20 min at room temperature, concentrated to dryness, and coevaporated with pyridine (×3). BzCl (2.9 mL, 25 mmol) was added to a solution of the above residue in pyridine (50 mL) at 0 °C. The whole was stirred for 4 h at room temperature and concentrated to dryness. The residue was partitioned between EtOAc (100 mL) and H_2O (50 mL). The separated organic phase was further washed with aqueous saturated $NaHCO_3$ (50 mL × 3), dried (Na_2SO_4), and concentrated to dryness in vacuo. The residue was purified on a silica gel column with 5−15% EtOAc in hexane to give **3a** (2.47 g, 74% as a white powder, which was crystallized from hexanes−EtOAc): mp 120−122 °C; LRMS (FAB) *m*/*z* 333 (MH^+). Anal. ($C_{18}H_{20}O_6$) C, H.

5-*O*-Benzoyl-3-*C*-(1-butynyl)-1,2-*O*-isopropylidene-α-D-*ribo*-pentofuranose (3b). Compound **3b** was obtained as described for the synthesis of **3a**. From **2b** (3.56 g, 10 mmol), **3b** (2.98 g, 86% as a white powder, which was crystallized from hexanes−EtOAc) was obtained. **3b**: mp 110−113 °C; LRMS (FAB) *m*/*z* 347 (MH^+); IR (neat) 2245 cm^{-1} (C≡C). Anal. ($C_{19}H_{22}O_6$) C, H.

Methyl 2,3,5-Tri-*O*-benzoyl-3-*C*-(1-propynyl)-α,β-D-*ribo*-pentofuranose (5a). Compound **3a** (2.3 g, 6.9 mmol) was treated with a mixture of AcCl (13.4 mL, 179 mmol), H_2O (22.1 mL), and MeOH (74.9 mL) for 8 h at room temperature. The mixture was neutralized with Et_3N (30 mL), and the solvent was removed in vacuo. The residue dissolved in EtOAc (80 mL) was washed with H_2O (45 mL) and aqueous saturated $NaHCO_3$ (45 mL × 3). The separated organic phase was dried (Na_2SO_4), concentrated, and coevaporated with pyridine (×3). BzCl (8 mL, 69 mmol) was added to a mixture of the above residue and DMAP (91.27 g, 10.4 mmol) in pyridine (110 mL) at 0 °C. The mixture was heated for 24 h at 100 °C, and the cooled mixture was concentrated and coevaporated with toluene (×3) in vacuo. The residue dissolved in EtOAc (150 mL) was washed with H_2O (50 mL) and aqueous saturated $NaHCO_3$ (50 mL × 3). The separated organic phase was dried (Na_2SO_4) and concentrated to dryness in vacuo. The residue was purified on a silica gel column with 0−10% EtOAc in hexane to give **5a** (2.8 g, 80% as a yellowish syrup): LRMS (FAB) *m*/*z* 515 (MH^+), 483 (M^+ − OMe). Anal. ($C_{30}H_{26}O_8$) C, H.

Methyl 2,3,5-Tri-*O*-benzoyl-3-*C*-(1-butynyl)-α,β-D-*ribo*-pentofuranose (5b). Compound **5b** was prepared as described for the synthesis of **5a**. From **3b** (2.2 g, 6.4 mmol), **5b** (3.0 g, 88% as a yellowish syrup) was obtained. **5b**: LRMS (FAB) *m*/*z* 529 (MH^+), 497 (M^+ − OMe). Anal. ($C_{31}H_{28}O_8$) C, H.

1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-3-*C*-(1-propynyl)-α,β-D-*ribo*-pentofuranose (6a). Concentrated H_2SO_4 (1.04 mL) was added to a solution of **5a** (2.57 g, 5 mmol) in a mixture of AcOH (16.6 mL) and Ac_2O (2.1 mL) at 0 °C. The mixture was stirred for 30 min at room temperature and diluted with $CHCl_3$ (50 mL), which was successively washed with H_2O (5 mL), aqueous saturated $NaHCO_3$ (15 mL × 3), and H_2O (5 mL ×

2). The separated organic phase was dried (Na_2SO_4) and concentrated to dryness in vacuo. The residue was purified on a silica gel column with 10−20% EtOAc in hexane to give **6a** (2.64 g, 98% as a syrup): LRMS (FAB) *m*/*z* 543 (MH^+), 483 ($M^+ - OAc$). Anal. ($C_{31}H_{26}O_9$) C, H.

1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-3-*C*-(1-butynyl)-α,*β*-D-*ribo*-pentofuranose (6b). Compound **6b** was prepared as described for the synthesis of **6a**. From **5b** (2.69 g, 5.1 mmol), **6b** (2.37 g, 83% as a yellowish syrup) was obtained. **6b**: LRMS (FAB) *m*/*z* 557 (MH^+), 513 ($M^+ - Ac$), 493 ($M^+ - OAc$). Anal. ($C_{32}H_{28}O_9$) C, H.

5-*O*-(*tert*-Butyldimethylsilyl)-1,2-*O*-isopropylidene-3-*C*-ethenyl-α-D-*ribo*-pentofuranose (7c). A solution of **1** (3.02 g, 10 mmol) in THF (40 mL) was added dropwise over 30 min to a THF solution of vinylmagnesium bromide (1 M, 30 mL, 30 mmol), at −15 °C under Ar. The mixture was stirred for 2 h at the same temperature. The reaction was quenched by addition of aqueous NH_4Cl (1 M, 50 mL), which was extracted by EtOAc (35 mL × 3). The separated organic phase was washed with brine (30 mL × 3), dried (Na_2SO_4), and concentrated to dryness in vacuo. The residue was purified on a silica gel column with 5% EtOAc in hexane to give **7c** (2.11 g, 64% as a syrup): LRMS (FAB) *m*/*z* 315 ($M^+ - Me$), 273 ($M^+ - t$-Bu). Anal. ($C_{16}H_{30}O_5Si$) C, H.

5-*O*-(*tert*-Butyldimethylsilyl)-3-*C*-cyclopropyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentofuranose (7e). A hexane solution of BuLi (1.7 M, 14.7 mL, 25 mmol) was added dropwise over 30 min to a solution of cyclopropyl bromide (2.1 mL, 25 mmol) at −78 °C under Ar. The mixture was stirred for 30 min at the same temperature. A solution of **1** (4.5 g, 15 mmol) in THF (25 mL) was added dropwise over 10 min to the above solution. After being stirred for 3 h, the reaction was quenched by EtOAc (30 mL × 3), and the separated organic phase was washed with brine (15 mL × 3), dried (Na_2SO_4), and concentrated to dryness in vacuo. The residue was purified on a silica gel column with 5% EtOAc in hexane to give **7e** (3.3 g, 65% as a white powder, which was crystallized from hexane): mp 57−59 °C; LRMS (FAB) *m*/*z* 345 (MH^+), 329 ($M^+ - Me$). Anal. ($C_{17}H_{32}O_5Si$) C, H.

5-*O*-Benzoyl-3-*C*-ethenyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentofuranose (8c). After desilylation of **7c** (1.67 g, 5.1 mmol) by TBAF in THF, BzCl (1.72 mL, 15 mmol) was added to the residue in pyridine (35 mL) at 0 °C. The mixture was stirred for 4 h at room temperature. Workup was done as described for the synthesis of **3a** to give **8c** (1.46 g, 90% as a white powder, which was crystallized from hexane/EtOAc). **8c**: mp 110−111 °C; LRMS (FAB) *m*/*z* 321 (MH^+), 305 ($M^+ - Me$). Anal. ($C_{17}H_{20}O_6$) C, H.

5-*O*-Benzoyl-3-*C*-cyclopropyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentofuranose (8e). Compound **8e** was prepared as described for the synthesis of **8c**. From **7e** (3.0 g, 8.7 mmol), **8e** (2.49 g, 86% as a white powder, which was crystallized from hexane/EtOAc) was obtained. **8e**: mp 109−111 °C; LRMS (FAB) *m*/*z* 355 (MH^+), 319 ($M^+ - Me$). Anal. ($C_{18}H_{22}O_6$) C, H.

3-*C*-Ethynyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentofuranose (11). Compound **10**[3] (8.0 g, 20 mmol) in THF (60 mL) was desilylated by TBAF (1 M, 21 mL, 21 mmol) for 20 min at room temperature to give **11** (4.1 g, 96% as a white powder): LRMS (FAB) *m*/*z* 215 (MH^+).

5-*O*-Benzoyl-3-*C*-ethyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentofuranose (12). A mixture of **11** (214 mg, 1 mmol) and 10% Pd-C (50 mg) in MeOH (18 mL) was stirred under atmospheric pressure of H_2 for 1 h at room temperature. Insoluble materials were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified on a silica gel column with 5% MeOH in $CHCl_3$ to give 3-*C*-ethyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentofuranose (209 mg, 96% as a white powder, which was crystallized from EtOH): mp 97−100 °C; LRMS (FAB) *m*/*z* 219 (MH^+). Anal. ($C_{10}H_{18}O_5$) C, H. BzCl (1.74 mL, 15 mmol) was added to a solution of the above residue (2.18 g, 10 mmol) in pyridine (50 mL) at 0 °C. The mixture was stirred for 1.5 h at room temperature, and the solvent was removed and coevaporated with toluene (×3). The residue taken up with EtOAc (100 mL) was successively washed with H_2O (40 mL) and aqueous saturated $NaHCO_3$ (40 mL × 3). The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified on a silica gel column with 5−20% EtOAc in hexane to give **12** (2.94 g, 91% as a white powder, which was crystallized from hexanes−EtOAc): mp 83−86 °C (lit.[16] mp 84−85 °C); LRMS (FAB) *m*/*z* 323 (MH^+), 307 ($M^+ - Me$). Anal. ($C_{17}H_{22}O_6$) C, H.

1,2,3,5-Tetra-*O*-benzoyl-3-*C*-ethenyl-α,*β*-D-*ribo*-pentofuranose (13c). A solution of **8c** (1.1 g, 3.4 mmol) in a mixture of aqueous HCl (1 M, 25 mL) and THF (25 mL) was heated under reflux for 8 h. The mixture was cooled to room temperature and neutralized with Et_3N. The solvent was removed in vacuo, and the residue taken up with EtOAc (50 mL) was successively washed with H_2O (30 mL) and aqueous saturated $NaHCO_3$ (25 mL × 3). The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was coevaporated with pyridine (×3) and dissolved in pyridine (60 mL). BzCl (4 mL, 34 mmol) and DMAP (629 mg, 5.1 mmol) were added to the above solution at 0 °C. The mixture was heated at 100 °C for 24 h. The solvent was removed in vacuo and coevaporated with toluene (×3). The residue taken up with EtOAc (100 mL) was washed with H_2O (50 mL) and aqueous saturated $NaHCO_3$ (50 mL × 3). The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified on a silica gel column with 0−10% EtOAc in hexane to give **13c** (1.6 g, 63% as a syrup): LRMS (FAB) *m*/*z* 471 ($M^+ - OBz$).

1,2,3,5-Tetra-*O*-benzoyl-3-*C*-ethyl-α,*β*-D-*ribo*-pentofuranose (13d). Compound **13d** was prepared as described for the synthesis of **13c**. From **12** (1.75 g, 5.43 mmol), **13d** (2.3 g, 72% as a yellowish syrup) was obtained. **13d**: LRMS (FAB) *m*/*z* 595 (MH^+), 473 ($M^+ - OBz$).

1,2,3,5-Tetra-*O*-benzoyl-3-*C*-cyclopropyl-α,*β*-D-*ribo*-pentofuranose (13e). Compound **13e** was prepared as described for the synthesis of **13c**. From **8e** (2.0 g, 6 mmol), **13e** (2.4 g, 67% as a yellowish syrup) was obtained. **13e**: LRMS (FAB) *m*/*z* 501 ($M^+ - Bz$), 485 ($M^+ - OBz$).

General Method for the Synthesis of 3′-*C*-Substituted Ribonucleosides. Stannic chloride (2.5 mmol for cytosine) or TMSOTf (2 mmol for uracil) was added to a solution of persilylated pyrimidine [2 mmol, prepared from the pyrimidine (2 mmol) and $(NH_4)_2SO_4$ (7 mg) in HMDS (6 mL)] and the sugar (**6a**,**b**, or **13c**−**e**; 0.5 mmol) in MeCN (4 mL) at 0 °C. The whole was stirred for the indicated period at room temperature. The mixture was diluted with $CHCl_3$ (12 mL) and aqueous saturated $NaHCO_3$ (5 mL) with vigorous stirring for 30 min. The precipitate was removed by filtration through a Celite pad, which was washed well with $CHCl_3$. The combined filtrate and washings were washed with H_2O (5 mL × 2) and aqueous saturated $NaHCO_3$ (5 mL). The separated organic phase was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified on a silica gel column to give the desired protected nucleoside, which was then treated with NH_3/MeOH (saturated at 0 °C) for 2 days at room temperature. The solvent was removed in vacuo, and the residue was purified on a silica gel column with 5−20% MeOH in $CHCl_3$ to furnish the desired 3′-*C*-substituted ribonucleosides.

1-[3-*C*-(1-Propynyl)-*β*-D-*ribo*-pentofuranosyl]cytosine Hydrochloride (15a). The reaction of persilylated cytosine (2 mmol) with **6a** (0.5 mmol) in the presence of $SnCl_4$ (0.29 mL, 2.5 mmol) for 2.5 h at room temperature gave **14a** (214 mg, 72% as a foam): LRMS (FAB) *m*/*z* 594 (MH^+); ^1H NMR ($CDCl_3$) 8.15−7.31 (m, 15 H, Bz × 3), 7.82 (d, 1 H, H-6, $J_{6,5}$ = 7.5 Hz), 6.52 (d, 1 H, H-1′, $J_{1',2'}$ = 4.6 Hz), 6.03 (d, 1 H, H-2′, $J_{2',1'}$ = 4.6 Hz), 5.74 (d, 1 H, H-5, $J_{5,6}$ = 7.5 Hz), 4.95−4.86 (m, 3 H, H-4′, 5′), 1.86 (s, 3 H, 3′-C≡CCH$_3$). From **14a** (150 mg, 0.25 mmol), **15a** (63 mg, 89% as a pale-yellow solid, which was crystallized as an HCl salt from Et_2O/EtOH) was obtained. **15a**: mp 162−165 °C; LRMS (EI) *m*/*z* 281 (M^+); IR (Nujol) 2245 cm^{-1} (C≡C); UV λ_{max} (H_2O) 272 nm (ϵ 8600), λ_{max} (0.5 M HCl) 280 nm (ϵ 12 200), λ_{max} (0.5 M NaOH) 273 nm (ϵ 8900); ^1H NMR (DMSO-d_6 + D_2O) 8.26 (d, 1 H, H-6, $J_{6,5}$ = 7.9 Hz), 6.15

(d, 1 H, H-5, $J_{5,6}$ = 7.9 Hz), 5.78 (d, 1 H, H-1′, $J_{1',2'}$ = 5.7 Hz), 4.08 (d, 1 H, H-2′, $J_{2',1'}$ = 5.7 Hz), 3.95−3.94 (m, 1 H, H-4′), 3.75 (dd, 1 H, H-5′a, $J_{5'a,4'}$ = 4.7 Hz, J_{gem} = 12.0 Hz), 3.67 (dd, 1 H, H-5′b, $J_{5'b,4'}$ = 2.5 Hz, J_{gem} = 12.0 Hz), 1.81 (s, 3 H, 3′-C≡CCH_3). Anal. ($C_{12}H_{15}N_3O_5$·HCl·0.2H_2O) C, H, N.

1-[3-*C*-(1-Propynyl)-β-D-*ribo*-pentofuranosyl]uracil (17a). The reaction of persilylated uracil (2 mmol) with **6a** (0.5 mmol) in the presence of TMSOTf (0.39 mL, 2 mmol) for 8 h at room temperature gave **16a** (241 mg, 81% as a foam): LRMS (FAB) *m*/*z* 595 (MH^+); ^{1}H NMR ($CDCl_3$) 8.14−7.30 (m, 15 H, Bz × 3), 7.97 (br s, 1 H, NH), 7.78 (d, 1 H, H-6, $J_{6,5}$ = 8.0 Hz), 6.32 (d, 1 H, H-1′, $J_{1',2'}$ = 4.5 Hz), 5.96 (d, 1 H, H-2′, $J_{2',1'}$ = 4.5 Hz), 5.75 (dd, 1 H, H-5, $J_{5,6}$ = 8.0 Hz, $J_{5,NH}$ = 2.0 Hz), 4.93−4.84 (m, 3 H, H-4′,5′), 1.91 (s, 3 H, 3′-C≡CCH_3). From **16a** (236 mg, 0.4 mmol), **17a** (87 mg, 77% as a white powder, which was crystallized from aqueous MeOH) was obtained. **17a**: mp 211−213 °C; LRMS (EI) *m*/*z* 283 (MH^+); IR (Nujol) 2240 cm^{-1} (C≡C); UV λ_{max} (H_2O) 262 nm (ε 9700), λ_{max} (0.5 M HCl) 262 nm (ε 9600), λ_{max} (0.5 M NaOH) 263 nm (ε 7700); ^{1}H NMR (DMSO-d_6) 11.33 (br s, 1 H, NH), 7.97 (d, 1 H, H-6, $J_{6,5}$ = 8.2 Hz), 5.80 (d, 1 H, H-1′, $J_{1',2'}$ = 6.9 Hz), 5.75 (d, 1 H, 2′-OH, *J* = 6.5 Hz), 5.70 (s, 1 H, 3′-OH), 5.68 (dd, 1 H, H-5, $J_{5,6}$ = 8.2 Hz, $J_{5,NH}$ = 2.0 Hz), 5.04 (t, 1 H, 5′-OH, *J* = 4.6 Hz), 4.10 (dd, 1 H, H-2′, $J_{2',1'}$ = 6.9 Hz, $J_{2',OH}$ = 6.5 Hz), 3.87−3.85 (m, 1 H, H-4′), 3.73−3.62 (m, 2 H, H-5′), 1.83 (s, 3 H, 3′-C≡CCH_3). Anal. ($C_{12}H_{14}N_2O_6$·0.2H_2O) C, H, N.

1-[3-*C*-(1-Butynyl)-β-D-*ribo*-pentofuranosyl]cytosine Hydrochloride (15b). The reaction of persilylated cytosine (4 mmol) with **6b** (1 mmol) in the presence of $SnCl_4$ (0.59 mL, 5 mmol) for 19 h at room temperature gave **14b** (412 mg, 68% as a foam): LRMS (FAB) *m*/*z* 608 (MH^+); ^{1}H NMR ($CDCl_3$) 8.15−7.31 (m, 15 H, Bz × 3), 7.84 (d, 1 H, H-6, $J_{6,5}$ = 7.4 Hz), 6.54 (d, 1 H, H-1′, $J_{1',2'}$ = 4.6 Hz), 6.04 (d, 1 H, H-2′, $J_{2',1'}$ = 4.6 Hz), 5.72 (d, 1 H, H-5, $J_{5,6}$ = 7.4 Hz), 4.97−4.85 (m, 3 H, H-4′,5′), 2.25−2.20 (m, 2 H, 3′-C≡CC$H_2$$CH_3$), 1.08 (t, 3 H, 3′-C≡C$CH_2CH_3$, *J* = 7.5 Hz). From **14b** (336 mg, 0.55 mmol), **15b** (154 mg, 95% as a pale-yellow foam, which was crystallized as an HCl salt from *i*-PrOH) was obtained. **15b**: mp 181−184 °C; LRMS (FAB) *m*/*z* 295 (M^+); IR (Nujol) 2240 cm^{-1} (C≡C); UV λ_{max} (H_2O) 271 nm (ε 8600), λ_{max} (0.5 M HCl) 280 nm (ε 12 300), λ_{max} (0.5 M NaOH) 273 nm (ε 9900); ^{1}H NMR (DMSO-d_6 + D_2O) 8.27 (d, 1 H, H-6, $J_{6,5}$ = 7.8 Hz), 6.18 (d, 1 H, H-5, $J_{5,6}$ = 7.8 Hz), 5.77 (d, 1 H, H-1′, $J_{1',2'}$ = 5.5 Hz), 4.08 (d, 1 H, H-2′, $J_{2',1'}$ = 5.5 Hz), 3.96−3.95 (m, 1 H, H-4′), 3.77−3.67 (m, 2 H, H-5′), 2.22−2.17 (m, 2 H, 3′-C≡CC$H_2$$CH_3$), 1.05 (t, 3 H, 3′-C≡C$CH_2CH_3$, *J* = 7.5 Hz). Anal. ($C_{13}H_{17}N_3O_5$·HCl) C, H, N.

1-[3-*C*-(1-Butynyl)-β-D-*ribo*-pentofuranosyl]uracil (17b). The reaction of persilylated uracil (2 mmol) with **6b** (0.5 mmol) in the presence of TMSOTf [0.39 mL, 2 mmol; after 48 h, further amounts of TMSOTf (0.19 mL, 1.0 mmol) were added] for 53 h at room temperature gave **16b** (294 mg, 97% as a foam): LRMS (FAB) *m*/*z* 609 (MH^+); ^{1}H NMR ($CDCl_3$) 8.15−7.29 (m, 15 H, Bz × 3), 8.02 (br s, 1 H, NH), 7.82 (d, 1 H, H-6, $J_{6,5}$ = 8.2 Hz), 6.34 (d, 1 H, H-1′, $J_{1',2'}$ = 4.4 Hz), 5.97 (d, 1 H, H-2′, $J_{2',1'}$ = 4.4 Hz), 5.75 (dd, 1 H, H-5, $J_{5,6}$ = 8.2 Hz, $J_{5,NH}$ = 2.3 Hz), 4.93−4.84 (m, 3 H, H-4′,5′), 2.30−2.25 (m, 2 H, 3′-C≡CC$H_2$$CH_3$), 1.12 (s, 3 H, 3′-C≡C$CH_2CH_3$, *J* = 7.5 Hz). From **16b** (286 mg, 0.47 mmol), **17b** (129 mg, 93% as a white foam, which was crystallized from EtOH/Et_2O) was obtained. **17b**: mp 139−142 °C; LRMS (EI) *m*/*z* 297 (MH^+); IR (Nujol) 2245 cm^{-1} (C≡C); UV λ_{max} (H_2O) 263 nm (ε 9,500), λ_{max} (0.5 M HCl) 263 nm (ε 10 600), λ_{max} (0.5 M NaOH) 263 nm (ε 7600); ^{1}H NMR (DMSO-d_6) 11.31 (br s, 1 H, NH), 7.96 (d, 1 H, H-6, $J_{6,5}$ = 7.8 Hz), 5.79 (d, 1 H, H-1′, $J_{1',2'}$ = 6.7 Hz), 5.74 (d, 1 H, 2′-OH, *J* = 6.4 Hz), 5.67 (s, 1 H, 3′-OH), 5.66 (d, 1 H, H-5, $J_{5,6}$ = 7.8 Hz), 4.99 (t, 1 H, 5′-OH, *J* = 4.6 Hz), 4.08 (dd, 1 H, H-2′, $J_{2',1'}$ = 6.7 Hz, $J_{2',OH}$ = 6.4 Hz), 3.87−3.86 (m, 1 H, H-4′), 3.74−3.64 (m, 2 H, H-5′), 2.23−2.19 (m, 2 H, 3′-C≡CC$H_2$$CH_3$), 1.07 (t, 3 H, 3′-C≡C$CH_2CH_3$, *J* = 7.5 Hz). Anal. ($C_{13}H_{16}N_2O_6$·0.6$H_2O$) C, H, N.

1-[3-*C*-(1-Ethenyl)-β-D-*ribo*-pentofuranosyl]uracil (17c). The reaction of persilylated uracil (2 mmol) with **13c** (0.5 mmol) in the presence of TMSOTf (0.39 mL, 2 mmol) for 39 h at room temperature gave **16c** (284 mg, 98% as a foam): LRMS (FAB) *m*/*z* 583 (MH^+); ^{1}H NMR ($CDCl_3$) 8.17−7.44 (m, 16 H, Bz × 3, H-6), 8.05 (br s, 1 H, NH), 6.52 (d, 1 H, H-1′, $J_{1',2'}$ = 7.7 Hz), 6.41 (dd, 1 H, 3′-C*H*c=CHaHb, $J_{c,a}$ = 17.4 Hz, $J_{c,b}$ = 11.1 Hz), 6.03 (d, 1 H, H-2′, $J_{2',1'}$ = 7.7 Hz), 5.54 (dd, 1 H, H-5, $J_{5,6}$ = 8.2 Hz, $J_{5,NH}$ = 2.2 Hz), 5.43−5.41 (m, 2 H, 3′-CHc=CHa,b), 5.25 (dd, 1 H, H-4′, $J_{4',5'a}$ = 3.2 Hz, $J_{4',5'b}$ = 3.7 Hz), 4.83 (dd, 1 H, H-5′a, $J_{5'a,4'}$ = 3.2 Hz, J_{gem} = 12.6 Hz), 4.71 (dd, 1 H, H-5′b, $J_{5'b,4'}$ = 3.7 Hz, J_{gem} = 12.6 Hz). From **16c** (279 mg, 0.48 mmol), **17c** (121 mg, 93% as a white foam, which was crystallized from aqueous MeOH) was obtained. **17c**: mp 219−222 °C; LRMS (EI) *m*/*z* 271 (MH^+); UV λ_{max} (H_2O) 261 nm (ε 10 000), λ_{max} (0.5 M HCl) 261 nm (ε 10 400), λ_{max} (0.5 M NaOH) 263 nm (ε 7700); ^{1}H NMR (DMSO-d_6) 11.32 (br s, 1 H, NH), 8.08 (d, 1 H, H-6, $J_{6,5}$ = 8.0 Hz), 6.05 (dd, 1 H, 3′-C*H*c=CHaHb, $J_{c,a}$ = 17.2 Hz, $J_{c,b}$ = 10.7 Hz), 5.95 (d, 1 H, H-1′, $J_{1',2'}$ = 8.0 Hz), 5.70 (dd, 1 H, H-5, $J_{5,6}$ = 7.8 Hz, $J_{5,NH}$ = 2.1 Hz), 5.48 (dd, 1 H, 3′-CHc=C*H*aHb, $J_{a,c}$ = 17.2 Hz, $J_{a,b}$ = 1.9 Hz), 5.46 (d, 1 H, 2′-OH, *J* = 7.0 Hz), 5.26 (dd, 1 H, 3′-CHc=CHa*H*b, $J_{b,c}$ = 10.7 Hz, $J_{b,a}$ = 1.9 Hz), 5.24 (t, 1 H, 5′-OH, *J* = 4.2 Hz), 4.90 (s, 1 H, 3′-OH), 4.11 (dd, 1 H, H-2′, $J_{2',1'}$ = 8.0 Hz, $J_{2',OH}$ = 7.0 Hz), 3.78−3.76 (m, 1 H, H-4′), 3.57−3.41 (m, 2 H, H-5′). Anal. ($C_{11}H_{14}N_2O_6$) C, H, N.

1-[3-*C*-(1-Cyclopropyl)-β-D-*ribo*-pentofuranosyl]uracil (17e). The reaction of persilylated uracil (2 mmol) with **13e** (0.5 mmol) in the presence of TMSOTf [0.39 mL, 2 mmol; after 48 h, further amounts of TMSOTf (0.39 mL, 2.0 mmol) were added] for 51 h at room temperature gave **16e** (269 mg, 90% as a foam): LRMS (FAB) *m*/*z* 597 (MH^+); ^{1}H NMR ($CDCl_3$) 8.18−7.45 (m, 15 H, Bz × 3), 7.93 (br s, 1 H, NH), 7.68 (d, 1 H, H-6, $J_{6,5}$ = 8.2 Hz), 6.48 (d, 1 H, H-1′, $J_{1',2'}$ = 7.7 Hz), 5.80 (d, 1 H, H-2′, $J_{2',1'}$ = 7.7 Hz), 5.60 (dd, 1 H, H-5, $J_{5,6}$ = 8.2 Hz, $J_{5,NH}$ = 1.7 Hz), 5.46 (dd, 1 H, H-4′, $J_{4',5'a}$ = 3.8 Hz, $J_{4',5'b}$ = 3.1 Hz), 4.98 (dd, 1 H, H-5′a, $J_{5'a,4'}$ = 3.8 Hz, J_{gem} = 12.7 Hz), 4.81 (dd, 1 H, H-5′b, $J_{5'b,4'}$ = 3.1 Hz, J_{gem} = 12.7 Hz), 2.09−0.56 (m, 5 H, 3′-cyclopropyl). From **16e** (265 mg, 0.44 mmol), **17e** (106 mg, 85% as a white foam, which was crystallized from EtOH/Et_2O) was obtained. **17e**: mp 179−181 °C; LRMS (EI) *m*/*z* 285 (MH^+); UV λ_{max} (H_2O) 261 nm (ε 8600), λ_{max} (0.5 M HCl) 261 nm (ε 8900), λ_{max} (0.5 M NaOH) 263 nm (ε 6900); ^{1}H NMR (DMSO-d_6) 11.27 (br s, 1 H, NH), 8.11 (d, 1 H, H-6, $J_{6,5}$ = 8.1 Hz), 5.88 (d, 1 H, H-1′, $J_{1',2'}$ = 7.8 Hz), 5.68 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 5.42 (d, 1 H, 2′-OH, *J* = 5.4 Hz), 5.16 (t, 1 H, 5′-OH), 4.16 (s, 1 H, 3′-OH), 4.03 (dd, 1 H, H-2′, $J_{2',1'}$ = 7.8 Hz, $J_{2',OH}$ = 5.4 Hz), 3.75−3.63 (m, 3 H, H-4′,5′), 1.04−0.20 (m, 5 H, 3′-cyclopropyl). Anal. ($C_{12}H_{16}N_2O_6$) C, H, N.

1-[3-*C*-(1-Ethyl)-β-D-*ribo*-pentofuranosyl]uracil (17d). The reaction of persilylated uracil (8 mmol) with **13d** (2 mmol) in the presence of TMSOTf [1.55 mL, 8 mmol; after 26 h, further amounts of TMSOTf (0.78 mL, 4 mmol) were added] for 52 h at room temperature gave **16d** (963 mg, 83% as a foam): LRMS (FAB) *m*/*z* 585 (MH^+); ^{1}H NMR ($CDCl_3$) 8.35 (br s, 1 H, NH), 8.19−7.46 (m, 16 H, Bz × 3, H-6), 6.48 (d, 1 H, H-1′, $J_{1',2'}$ = 7.8 Hz), 5.86 (d, 1 H, H-2′, $J_{2',1'}$ = 7.8 Hz), 5.48 (dd, 1 H, H-5, $J_{5,6}$ = 8.2 Hz, $J_{5,NH}$ = 1.9 Hz), 5.22 (dd, 1 H, H-4′, $J_{4',5'a}$ = 3.1 Hz, $J_{4',5'b}$ = 3.5 Hz), 4.87 (dd, 1 H, H-5′a, $J_{5'a,4'}$ = 3.1 Hz, J_{gem} = 12.7 Hz), 4.69 (dd, 1 H, H-5′b, $J_{5'b,4'}$ = 3.5 Hz, J_{gem} = 12.7 Hz), 2.90−2.04 (m, 2 H, 3′-C$H_2$$CH_3$), 0.95 (t, 3 H, 3′-$CH_2CH_3$, *J* = 7.4 Hz). From **16d** (369 mg, 0.63 mmol), **17d** (160 mg, 93% as a white foam, which was crystallized from EtOH/Et_2O) was obtained. **17d**: mp 220−222 °C (lit.[16] mp 217−218 °C); LRMS (EI) *m*/*z* 273 (MH^+); UV λ_{max} (H_2O) 262 nm (ε 9300), λ_{max} (0.5 M HCl) 261 nm (ε 9500), λ_{max} (0.5 M NaOH) 263 nm (ε 7300); ^{1}H NMR (DMSO-d_6) 11.26 (br s, 1 H, NH), 8.08 (d, 1 H, H-6, $J_{6,5}$ = 8.1 Hz), 5.89 (d, 1 H, H-1′, $J_{1',2'}$ = 7.7 Hz), 5.66 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 5.31 (d, 1 H, 2′-OH, *J* = 6.4 Hz), 5.15 (t, 1 H, 5′-OH), 4.49 (s, 1 H, 3′-OH), 3.91 (dd, 1 H, H-2′, $J_{2',1'}$ = 7.7 Hz, $J_{2',OH}$ = 6.4 Hz), 3.82−3.80 (m, 1 H, H-4′), 3.57−3.55 (m, 2 H, H-5′), 1.67−1.60 (m, 2 H, 3′-C$H_2$$CH_3$), 0.93 (t, 3 H, 3′-$CH_2CH_3$, *J* = 7.3 Hz). Anal. ($C_{11}H_{16}N_2O_6$·0.2$H_2O$) C, H, N.

General Procedure for Conversion of Uracil Nucleosides into Cytosine Nucleosides. Triethylamine (2 equiv) was added to a mixture of **16c**, **16d**, or **16e**, TPSCl (2 equiv),

and DMAP (2 equiv) in MeCN. The mixture was stirred for indicated period at room temperature. Concentrated NH_4OH (28%, 20 mL) was added to the mixture, and the whole was further stirred for 2.5 h at room temperature. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography with 0−5% MeOH in $CHCl_3$ to give the corresponding blocked cytosine derivatives **14c**−**e**, which were further treated with NH_3/MeOH (saturated at 0 °C, 10 mL) for 2 days at room temperature. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography with 0−20% MeOH in $CHCl_3$ to give the corresponding cytosine derivatives **15c**−**e**.

1-(3-*C*-Ethenyl-*β*-D-*ribo*-pentofuranosyl)cytosine (15c). From **16c** (400 mg, 0.96 mmol), **14c** (345 mg, 86% as a foam) was obtained. **14c**: LRMS (FAB) *m*/*z* 582 (MH^+); ^{1}H NMR ($CDCl_3$) 8.12−7.38 (m, 16 H, Bz × 3, H-6), 6.61 (d, 1 H, H-1′, $J_{1',2'}$ = 7.6 Hz), 6.35 (dd, 1 H, 3′-C*H*c=CHaHb, $J_{c,a}$ = 17.4 Hz, $J_{c,b}$ = 11.0 Hz), 6.03 (d, 1 H, H-2′, $J_{2',1'}$ =7.6 Hz), 5.61 (d, 1 H, H-5, $J_{5,6}$ = 6.9 Hz), 5.40−5.33 (m, 2 H, 3′-CHc=C*Ha*H*b*), 5.20−5.12 (m, 1 H, H-4′), 4.84−4.65 (m, 2 H, H-5′). From **14c** (323 mg, 0.55 mmol), **15c** (156 mg, 96% as a white powder, which was crystallized from Et_2O/EtOH) was obtained. **15c**: mp 194−197 °C; LRMS (El) *m*/*z* 270 (MH^+); UV λ_{max} (H_2O) 270 nm (ϵ 8100), λ_{max} (0.5 N HCl) 280 nm (ϵ 11 700), λ_{max} (0.5 N NaOH) 272 nm (ϵ 7400); ^{1}H NMR (DMSO-d_6) 7.93 (d, 1 H, H-6, $J_{6,5}$ = 7.5 Hz), 7.20, 7.17 (each br s, each 1 H, NH_2), 6.05 (dd, 1 H, 3′-C*H*c=CHaHb, $J_{c,a}$ = 17.2 Hz, $J_{c,b}$ = 10.6 Hz), 5.93 (d, 1 H, H-1′, $J_{1',2'}$ = 7.9 Hz), 5.75 (d, 1 H, H-5, $J_{5,6}$ = 7.5 Hz), 5.46 (dd, 1 H, 3′-CHc=C*Ha*Hb, $J_{a,c}$ = 17.2 Hz, $J_{a,b}$ = 2.0 Hz), 5.31 (d, 1 H, 2′-OH, *J* = 6.7 Hz), 5.23 (dd, 1 H, 3′-CHc=CHa*H*b, $J_{b,c}$ = 10.6 Hz, $J_{b,a}$ = 2.0 Hz), 5.21 (t, 1 H, 5′-OH, *J* = 4.5 Hz), 4.77 (s, 1 H, 3′-OH), 4.13 (dd, 1 H, H-2′, $J_{2',1'}$ = 7.9 Hz, $J_{2',OH}$ = 6.7 Hz), 3.76−3.75 (m, 1 H, H-4′), 3.55−3.39 (m, 2 H, H-5′). Anal. ($C_{11}H_{15}N_3O_5$) C, H, N.

1-(3-*C*-Ethyl-*β*-D-*ribo*-pentofuranosyl)cytosine (15d). From **16d** (500 mg, 0.86 mmol), **14d** (390 mg, 78% as a foam) was obtained. **14d**: LRMS (FAB) *m*/*z* 584 (MH^+); ^{1}H NMR ($CDCl_3$) 8.17−7.43 (m, 16 H, Bz × 3, H-6), 6.66 (d, 1 H, H-1′, $J_{1',2'}$ = 6.7 Hz), 5.85 (d, 1 H, H-5, $J_{5,6}$ = 7.4 Hz), 5.50 (d, 1 H, H-2′, $J_{2',1'}$ = 6.7 Hz), 5.17 (m, 1 H, H-4′), 4.90 (dd, 1 H, H-5′a, $J_{5'a,4'}$ = 3.1 Hz, J_{gem} = 12.4 Hz), 4.71 (dd, 1 H, H-5′b, $J_{5'b,4'}$ = 3.4 Hz, J_{gem} = 12.4 Hz), 2.87−2.03 (m, 2 H, 3′-C*H*$_2$CH$_3$), 0.93 (t, 3 H, 3′-CH$_2$C*H*$_3$, *J* = 7.3 Hz). From **14d** (386 mg, 0.66 mmol), **15d** (159 mg, 89% as a white powder, which was crystallized from Et_2O/EtOH) was obtained. **15d**: mp 220−221 °C; LRMS (El) *m*/*z* 272 (MH^+); UV λ_{max} (H_2O) 271 nm (ϵ 8000), λ_{max} (0.5 N HCl) 280 nm (ϵ 12 300), λ_{max} (0.5 N NaOH) 272 nm (ϵ 8200); ^{1}H NMR (DMSO-d_6) 7.92 (d, 1 H, H-6, $J_{6,5}$ = 7.3 Hz), 7.22, 7.14 (each br s, each 1 H, NH_2), 5.85 (d, 1 H, H-1′, $J_{1',2'}$ = 7.6 Hz), 5.74 (d, 1 H, H-5, $J_{5,6}$ = 7.3 Hz), 5.19 (d, 1 H, 2′-OH, *J* = 6.4 Hz), 5.12 (t, 1 H, 5′-OH, *J* = 3.9 Hz), 4.39 (s, 1 H, 3′-OH), 3.94 (dd, 1 H, H-2′, $J_{2',1'}$ = 7.6 Hz, $J_{2',OH}$ = 6.4 Hz), 3.79 (m, 1 H, H-4′), 3.57−3.50 (m, 2 H, H-5′), 1.65−1.59 (m, 2 H, 3′-C*H*$_2$CH$_3$), 0.92 (t, 3 H, 3′-CH$_2$C*H*$_3$, *J* = 7.3 Hz). Anal. ($C_{11}H_{17}N_3O_5$·H_2O) C, H, N.

1-(3-*C*-Cyclopropyl-*β*-D-*ribo*-pentofuranosyl)cytosine (15e). From **16e** (440 mg, 0.74 mmol), **14e** (315 mg, 72% as a foam) was obtained. **14e**: LRMS (FAB) *m*/*z* 596 (MH^+); ^{1}H NMR ($CDCl_3$) 8.18−7.40 (m, 16 H, Bz × 3, H-6), 6.65 (d, 1 H, H-1′, $J_{1',2'}$ = 7.5 Hz), 5.77 (d, 1 H, H-2′, $J_{2',1'}$ = 7.5 Hz), 5.57 (d, 1 H, H-5, $J_{5,6}$ = 6.9 Hz), 5.41−5.39 (m, 1 H, H-4′), 4.97−4.86 (m, 2 H, H-5′), 2.08−0.52 (m, 5 H, 3′-cyclopropyl). From **14e** (312 mg, 0.52 mmol), **15e** (138 mg, 94% as a white powder, which was crystallized from Et_2O/EtOH) was obtained. **15e**: mp 205−208 °C; LRMS (EI) *m*/*z* 284 (MH^+); UV λ_{max} (H_2O) 270 nm (ϵ 8100), λ_{max} (0.5 M HCl) 279 nm (ϵ 11 400), λ_{max} (0.5 M NaOH) 263 nm (ϵ 7700); ^{1}H NMR (DMSO-d_6) 7.94 (d, 1 H, H-6, $J_{6,5}$ = 7.5 Hz), 7.17, 7.13 (each br s, each 1 H, NH_2), 5.85 (d, 1 H, H-1′, $J_{1',2'}$ = 7.6 Hz), 5.74 (d, 1 H, H-5, $J_{5,6}$ = 7.5 Hz), 5.31 (d, 1 H, 2′-OH, *J* = 6.3 Hz), 5.13−5.11 (m, 1 H, 5′-OH), 4.06 (s, 1 H, 3′-OH), 4.04 (dd, 1 H, H-2′, $J_{2',1'}$ = 7.6 Hz, $J_{2',OH}$ = 6.3 Hz), 3.71−3.62 (m, 3 H, H-4′,5′), 1.03−0.19 (m, 5 H, 3′-cyclopropyl). Anal. ($C_{12}H_{17}N_3O_5$·$^1/_3H_2O$) C, H, N.

1-(3-*C*-Ethynyl-*β*-D-*xylo*-pentofuranosyl)uracil (30). A THF solution of HC≡CMgBr (0.5 M, 12 mL, 6 mmol) was added to a solution of **26**[10b] (470 mg, 1 mmol) in THF (10 mL) at room temperature. The mixture was stirred for 60 min, and aqueous NH_4Cl (1 M, 10 mL) was added. The mixture was diluted with EtOAc (50 mL), washed with H_2O (10 mL × 3) and brine (25 mL), and dried (Na_2SO_4). The organic phase was concentrated to dryness, and the residue was purified on a silica gel column with 2% MeOH in $CHCl_3$ to give **28** (450 mg, 91% as a white solid): LRMS (EI) *m*/*z* 439 (M^+ − *t*-Bu); ^{1}H NMR ($CDCl_3$) 8.16 (br s, 1 H, NH), 7.93 (d, 1 H, H-6, $J_{5,6}$ = 8.1 Hz), 5.80 (s, 1 H, 3′-OH), 5.60 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 5.35 (br s, 1 H, H-1′), 4.37−4.30 (m, 2 H, H-5′), 4.09 (m, 2 H, H-2′,4′), 2.60 (s, 1 H, 3′-C≡CH), 0.90 (s, 18 H, *t*-Bu × 2), 0.22−0.14 (each s, each 3 H, Me × 4). Anal. ($C_{23}H_{40}N_2O_6Si_2$) C, H, N. A mixture containing **28** (300 mg, 0.6 mmol) and NH_4F (450 mg, 12 mmol) in MeOH (12 mL) was heated under reflux for 17 h. The mixture was allowed to cool to room temperature and concentrated to dryness, and the residue was purified on a silica gel column with 10−20% MeOH in $CHCl_3$ to give **30** (132 mg, 82% as a yellowish solid, which was crystallized from EtOH/hexane): mp 112 °C; LRMS (EI) *m*/*z* 268 (M^+); ^{1}H NMR (DMSO-d_6) 9.38 (br s, 1 H, NH), 7.73 (d, 1 H, H-6, $J_{6,5}$ = 8.0 Hz), 6.26 (d, 1 H, 2′-OH, *J* = 6.8 Hz), 6.13 (s, 1 H, 3′-OH), 5.67 (s, 1 H, H-1′), 5.62 (d, 1 H, H-5, $J_{5,6}$ = 8.0 Hz), 4.90 (t, 1 H, 5′-OH), 4.02 (dd, 1 H, H-4′, $J_{4',5'a}$ = 2.4 Hz, $J_{4',5'b}$ = 7.3 Hz), 3.89 (d, 1 H, H-2′, *J* = 6.0 Hz), 3.77 (ddd, 1 H, H-5′a, $J_{5'a,4'}$ = 2.4 Hz, $J_{5'a,OH}$ = 5.7 Hz, J_{gem} = 12.2 Hz), 3.70 (ddd, 1 H, H-5′b, $J_{5'b,4'}$ = 7.3 Hz, $J_{5'b,OH}$ = 5.7 Hz, J_{gem} = 12.2 Hz), 3.56 (s, 1 H, 3′-C≡CH). Anal. ($C_{11}H_{12}N_2O_6$·H_2O) C, H, N.

***N*^{4}-Acetyl-1-[2,5-di-*O*-(*tert*-butyldimethylsilyl)-3-*C*-ethynyl-*β*-D-*xylo*-pentofuranosyl]cytosine (29).** Oxidation of *N*^{4}-acetyl-1-[2,5-bis-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]cytosine (1.03 g, 2 mmol) was done according to the literature[10b] to give **27** (910 mg, 88% as a colorless foam). A THF solution of HC≡CMgBr (0.5 M, 12 mL, 6 mmol) was added dropwise to a solution of **27** (512 mg, 1 mmol) in THF (10 mL) at 0 °C. The mixture was stirred for 1 h at the same temperature. The reaction was quenched with an aqueous solution of NH_4Cl (1 M, 10 mL). The mixture was diluted with EtOAc (60 mL), washed with H_2O (20 mL × 2) and brine (20 mL), and dried (Na_2SO_4). The organic phase was concentrated to dryness, and the residue was purified on a silica gel column with 0−2% MeOH in $CHCl_3$ to give **29** (450 mg, 83% as a yellow foam): LRMS (EI) *m*/*z* 480 (M^+). Anal. ($C_{25}H_{43}N_3O_6Si_2$) C, H, N.

1-(3-*C*-Ethynyl-*β*-D-*xylo*-pentofuranosyl)cytosine (31). Acetyl chloride (0.5 mL) was added to MeOH (9.5 mL), and the mixture was stirred for 30 min at room temperature. To this mixture was added **29** (400 mg, 0.74 mmol), and the mixture was stirred for 2 days at room temperature. The mixture was neutralized with Et_3N and concentrated to dryness, and the residue was purified on a silica gel column with 17−25% MeOH in $CHCl_3$ to give a crude oil, which was further purified by charcoal chromatography with H_2O and then 10−100% MeOH in H_2O to give **31** (130 mg, 67% as a colorless crystalline solid): mp 126 °C; LRMS (EI) *m*/*z* 267 (M^+); ^{1}H NMR (DMSO-d_6) 7.69 (d, 1 H, H-6, $J_{6,5}$ = 7.5 Hz), 7.15, 7.05 (each br s, each 1 H, NH_2), 6.14 (d, 1 H, 2′-OH, *J* = 5.9 Hz), 6.03 (s, 1 H, 3′-OH), 5.58 (d, 1 H, H-5, $J_{5,6}$ = 7.5 Hz), 5.66 (s, 1 H, H-1′), 4.86 (t, 1 H, 5′-OH, *J* = 5.6 Hz), 4.00 (dd, 1 H, H-4′, $J_{4',5'a}$ = 2.2 Hz, $J_{4',5'b}$ = 7.1 Hz), 3.85 (d, 1 H, H-2′, $J_{2',OH}$ = 5.9 Hz), 3.78 (ddd, 1 H, H-5′a, $J_{5'a,4'}$ = 2.2 Hz, $J_{5'a,OH}$ = 5.9 Hz, J_{gem} = 12.0 Hz), 3.71 (ddd, 1 H, H-5′b, $J_{5'b,4'}$ = 7.1 Hz, $J_{5'b,OH}$ = 5.9 Hz, J_{gem} = 12.0 Hz), 3.50 (s, 1 H, 3′-C≡CH); ^{13}C NMR (MeOH-d_4) 167.78, 158.31, 143.46, 94.84, 94.53, 88.16, 83.68, 81.31, 77.52, 76.91, 61.38. Anal. ($C_{11}H_{13}N_3O_5$) C, H, N.

1-[2-*O*-(*tert*-Butyldimethylsilyl)-3-*C*-[3-(trimethylsilyl)-2-propynyl]-*β*-D-*ribo*-pentofuranosyl]uracil (33) and 1-[2-*O*-(*tert*-Butyldimethylsilyl)-3-*C*-[3-(trimethylsilyl)-2-propynyl]-*β*-D-*xylo*-pentofuranosyl]uracil (34). A hexane solution of BuLi (1.63 M, 27.9 mL, 45.5 mmol) was added dropwise over 20 min to a mixture of **32**[10b] (2.70 g, 7.57 mmol)

and 3-bromo-1-(trimethylsilyl)-1-propyne (6.4 mL, 45.2 mmol) in THF (25 mL) at −78 °C under Ar. The mixture was stirred at the same temperature for 25 min, and AcOH (3 mL) was added to the mixture, which was warmed to room temperature and concentrated in vacuo. The residue taken up with EtOAc (100 mL) was washed with H_2O (50 mL), aqueous saturated $NaHCO_3$ (50 mL) and brine (50 mL), and dried (Na_2SO_4). The solvent was removed in vacuo, and the residue was purified on a silica gel column with 40−50% EtOAc in hexane to give **33** (427 mg, 12% as a foam) and **34** (1.03 g, 29% as a foam). From the aqueous phases, uracil (85 mg) was obtained. **33**: LRMS (FAB) *m*/*z* 469 (MH^+); HRMS (FAB) calcd for $C_{21}H_{37}N_2O_6Si_2$ 469.2190, found 469.2168. Anal. ($C_{21}H_{36}N_2O_6Si_2$) C, H, N. **34**: LRMS (FAB) *m*/*z* 469 (MH^+); HRMS (FAB) calcd for $C_{21}H_{37}N_2O_6Si_2$ 469.2190, found 469.2215. Anal. ($C_{21}H_{36}N_2O_6S1_2 \cdot 0.4H_2O$) C, H, N.

1-[3-*C*-(2-Propynyl)-*β*-D-*ribo*-pentofuranosyl]uracil (36). A mixture of **33** (46 mg, 0.1 mmol) and NH_4F (74 mg, 2 mmol) in MeOH (2 mL) was heated under reflux for 29 h. The solvent was removed in vacuo, and the residue was purified on a silica gel column with 5% MeOH in $CHCl_3$ to give **36** (14 mg, 50% as a solid): mp 104 °C; LRMS (FAB) *m*/*z* 282 (M^+); ^{1}H NMR (DMSO-d_6) 11.07 (br s, 1 H, NH), 8.02 (d, 1 H, H-6, $J_{6,5}$ = 8.1 Hz), 5.89 (d, 1 H, H-1′, $J_{1',2'}$ = 7.9 Hz), 5.69 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 5.53 (br d, 1 H, 2′-OH), 5.31 (br s, 1 H, 5′-OH), 5.02 (s, 1 H, 3′-OH), 3.88 (br s, 2 H, H-2′,4′), 3.83−3.60 (br s, 2 H, H-5′), 2.82 (t, 1 H, 3′-CH_2C≡C*H*), the proton signals for 3′-CH_2C≡CH were overlapped with those for DMSO; ^{13}C NMR (MeOH-d_4) 166.41, 153.22, 143.57, 103.38, 88.91, 88.50, 80.99, 79.13, 78.43, 72.34, 62.33, 25.35. Anal. ($C_{12}H_{14}N_2O_6$) C, H, N.

1-[3-*C*-(2-Propynyl)-*β*-D-*xylo*-pentofuranosyl]uracil (38). Compound **34** was deprotected as described for the synthesis of **36**. From **34** (100 mg, 0.2 mmol), **38** (58 mg, 96% as a solid) was obtained. **38**: mp 218 °C; LRMS (FAB) *m*/*z* 282 (M^+); ^{1}H NMR (DMSO-d_6) 11.24 (br s, 1 H, NH), 7.78 (d, 1 H, H-6, $J_{6,5}$ = 8.1 Hz), 6.03 (d, 1 H, 2′-OH), 5.58 (m, 2 H, H-5,1′), 5.27 (s, 1 H, 3′-OH), 4.80 (t, 1 H, 5′-OH), 3.91 (dd, 1 H, H-2′, $J_{2',1'}$ = 0.9 Hz, $J_{2',OH}$ = 5.6 Hz), 3.87 (dd, 1 H, H-4′, $J_{4',5'a}$ = 3.6 Hz, $J_{4',5'b}$ = 6.3 Hz), 3.83 (ddd, 1 H, H-5′a, $J_{5'a,4'}$ = 3.6 Hz, $J_{5'a,OH}$ = 5.6 Hz, J_{gem} = 12.0 Hz), 3.69 (ddd, 1 H, H-5′b, $J_{5'b,4'}$ = 6.3 Hz, $J_{5'b,OH}$ = 5.6 Hz, J_{gem} = 12.0 Hz), 2.78 (t, 1 H, 3′-CH_2C≡C*H*), 2.48 (d, 2 H, 3′-CH_2C≡CH); ^{13}C NMR (MeOH-d_4) 167.03, 152.80, 143.63, 101.55, 93.71, 87.23, 83.29, 81.01, 72.58, 62.09, 24.55. Anal. ($C_{12}H_{14}N_2O_6 \cdot H_2O$) C, H, N.

1-[3-*C*-(2-Propynyl)-*β*-D-*ribo*-pentofuranosyl]cytosine (37). A mixture of **33** (187 mg, 0.4 mmol), Bz_2O (272 mg, 1.2 mmol), and DMAP (147 mg, 1.2 mmol) in MeCN (4 mL) was stirred for 1 h at room temperature. The mixture was diluted with EtOAc (20 mL), which was washed successively with aqueous HCl (0.1 M, 20 mL), aqueous saturated $NaHCO_3$ (20 mL), and H_2O (20 mL × 2), and dried (Na_2SO_4). The solvent was removed in vacuo. A mixture of the residue, Et_3N (112 μL, 0.8 mmol), 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl; 242 mg, 0.8 mmol), and DMAP (98 mg, 0.8 mmol) in MeCN (4 mL) was stirred for 12 h at room temperature, and then NH_4OH (28%, 3 mL) was added to the mixture, which was further stirred for 30 min at room temperature. The solvent was removed in vacuo, and the residue taken up with EtOAc (20 mL) was washed with H_2O (20 mL × 6). The organic phase was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was purified on a silica gel column with 0−3% MeOH in $CHCl_3$ to give protected **37**, which was treated with MeOH (5 mL) containing NaOMe (5 M, 24 μL) at room temperature for 27 h. The mixture was neutralized with aqueous HCl (1 M), and the solvent was removed in vacuo. A mixture of the residue and NH_4F (300 mg, 8 mmol) in MeOH (5 mL) was heated under reflux for 1.5 h. The solvent was removed, and the residue was extracted with $CHCl_3$. The aqueous phase was absorbed to an active charcoal column which was washed well with H_2O and then with 50% aqueous MeOH to give **37** (57 mg, 51% as a solid): mp 210−213 °C; LRMS (FAB) *m*/*z* 250 ($M^+ - CH_2OH$); ^{1}H NMR (DMSO-d_6 + D_2O) 7.87 (d, 1 H, H-6, $J_{6,5}$ = 7.4 Hz), 5.85 (d, 1 H, H-1′, $J_{1',2'}$ = 7.9 Hz), 5.74 (d, 1 H, H-5, $J_{5,6}$ = 7.4 Hz), 3.90 (d, 1 H, H-2′, $J_{2',1'}$ = 7.9 Hz), 3.85 (br s, 1 H, H-4′), 3.72 (dd, 1 H, H-5′a, J_{gem} = 12.1 Hz), 3.61 (dd, 1 H, H-5′b, J_{gem} = 12.1 Hz), 2.77 (br s, 1 H, 3′-CH_2C≡C*H*), 2.53 (dd, 1 H, 3′-CH_2C≡CH, J_{gem} = 17.0 Hz); ^{13}C NMR (MeOH-d_4) 167.15, 158.70, 144.31, 96.50, 90.90, 88.26, 80.75, 78.94, 78.51, 71.97, 62.10, 25.10. Anal. ($C_{12}H_{15}N_3O_5 \cdot 0.8H_2O$) C, H, N.

1-[3-*C*-(2-Propynyl)-*β*-D-*xylo*-pentofuranosyl]cytosine (39). Compound **39** was prepared as described for the synthesis of **37**. From **34** (100 mg, 0.2 mmol), **39** (58 mg, quantitative as a solid) was obtained. **39**: mp 137 °C; LRMS (FAB) *m*/*z* 281 (M^+); HRMS (EI) calcd for $C_{12}H_{13}N_3O_5$ 281.1010, found 281.1017; ^{1}H NMR (DMSO-d_6 + D_2O) 7.21 (d, 1 H, H-6, $J_{6,5}$ = 7.6 Hz), 5.82 (d, 1 H, H-5, $J_{5,6}$ = 7.6 Hz), 5.53 (s, 1 H, H-1′), 3.90−3.88 (m, 2 H, H-2′,4′), 3.83 (dd, 1 H, H-5′a, J_{gem} = 11.9 Hz), 3.69 (dd, 1 H, H-5′b, J_{gem} = 11.9 Hz), 2.75 (br s, 1 H, 3′-CH_2C≡C*H*), the proton signals for 3′-CH_2C≡CH were overlapped with those for DMSO; ^{13}C NMR (MeOH-d_4) 166.70, 157.07, 144.49, 94.97, 94.61, 87.26, 83.20, 81.08, 80.88, 72.39, 61.87, 24.38. Anal. ($C_{12}H_{15}N_3O_5 \cdot 0.5$MeOH) C, H, N.

1-[5-*O*-Benzoyl-3-*C*-[(trimethylsilyl)ethynyl]-*β*-D-*ribo*-pentofuranosyl]uracil (42). Bz_2O (220 mg, 0.97 mmol) was added to a solution of **40**[10b] (370 mg, 0.81 mmol) and DMAP (119 mg, 0.97 mmol) in a mixture of MeCN and CH_2Cl_2 (1:1, 8 mL) at 0 °C. After being stirred for 45 min at the same temperature, EtOH (1 mL) was added to the mixture. The solvent was removed in vacuo, and the residue taken up with EtOAc (10 mL) was washed with aqueous HCl (0.1 M), aqueous saturated $NaHCO_3$, H_2O, and brine, dried (Na_2SO_4), and evaporated to dryness to give a crude **41**. The residue containing **41** was further treated with HCl/MeOH (5%, 12 mL) at room temperature for 15 min, which was neutralized with aqueous saturated $NaHCO_3$. The solvent was removed in vacuo, and the residue was purified on a silica gel column with 3−5% MeOH in $CHCl_3$ to give **42** (201 mg, 56% as a solid): LRMS (FAB) *m*/*z* 445 (MH^+). Anal. ($C_{21}H_{24}N_2O_7Si$) C, H, N.

1-[5-*O*-Benzoyl-3-deoxy-3-*C*-[(trimethylsilyl)ethynyl]-*β*-D-*ribo*- and -*xylo*-pentofuranosyl]uracil (44). A mixture of **42** (440 mg, 1 mmol) and *N*,*N*′-thiocarbonyldiimidazole (310 mg, 1.6 mmol) in a mixture of MeCN and CH_2Cl_2 (1:1, 20 mL) was stirred for 2.5 h at room temperature. Water (1 mL) was added to the mixture, and the whole was diluted with CH_2Cl_2 (100 mL), which was washed with H_2O (50 mL) and brine (50 mL). The organic phase was dried (Na_2SO_4), concentrated to dryness, and coevaporated several times with toluene. The residue was dissolved in toluene (70 mL) containing AIBN (16 mg) and Bu_3SnH (810 μL, 3 mmol), which was heated under reflux for 90 min. The solvent was removed in vacuo, and the residue taken up with EtOAc (50 mL) was washed with aqueous KF (1 M, 50 mL) and brine (20 mL × 2). The organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified on a silica gel column with 0−2% MeOH in $CHCl_3$ to give **44** (390 mg, 92% as a foam): LRMS (FAB) *m*/*z* 429 (MH^+); HRMS (FAB) calcd for $C_{21}H_{25}N_2O_6Si$ 429.1482, found 429.1495. Anal. ($C_{21}H_{24}N_2O_6Si \cdot 0.2H_2O$) C, H, N.

1-[5-*O*-Benzoyl-2-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-3-*C*-[(trimethylsilyl)ethynyl]-*β*-D-*xylo*-pentofuranosyl]-uracil (46) and 1-[5-*O*-Benzoyl-2-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-3-*C*-[(trimethylsilyl)ethynyl]-*β*-D-*ribo*-pentofuranosyl]uracil (45). A mixture of **44** (280 mg, 0.65 mmol), *tert*-butyldimethylsilyl chloride (245 mg, 1.63 mmol), and imidazole (133 mg, 1.95 mmol) in DMF (6.5 mL) was stirred for 20 h at 80 °C under Ar. The mixture was partitioned between EtOAc (20 mL) and H_2O (10 mL × 3). The organic phase was further washed with aqueous HCl (1 M, 20 mL), aqueous saturated $NaHCO_3$ (10 mL), and H_2O (10 mL) and dried (Na_2SO_4). The solvent was removed in vacuo, and the residue was purified on a silica gel column with 20% EtOAc in hexane to give **46** (255 mg, 72% as a foam) and **45** (71 mg, 20% as a foam). **46**: LRMS (FAB) *m*/*z* 543 (MH^+); HRMS calcd for $C_{27}H_{39}N_2O_6Si_2$ 543.2346, found 543.2352. Anal. ($C_{27}H_{38}N_2O_6Si_2 \cdot 0.2H_2O$) C, H, N. **45**: LRMS (FAB) *m*/*z* 543 (MH^+); HRMS calcd for $C_{27}H_{39}N_2O_6Si_2$ 543.2346, found 543.2349. Anal. ($C_{27}H_{38}N_2O_6Si_2$) C, H, N.

1-[5-*O*-Benzoyl-2-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-3-*C*-[(trimethylsilyl)ethynyl]-*β*-D-*xylo*-pentofuranosyl]cytosine (49). A mixture of **46** (120 mg, 0.22 mmol), TPSCl (133 mg, 0.44 mmol), Et_3N (60 μL, 0.44 mmol), and DMAP (54 mg, 0.44 mmol) in MeCN (2 mL) was stirred for 19 h at room temperature, and then NH_4OH (28%, 2 mL) was added to the mixture, which was further stirred for 45 min at the same temperature. The solvent was removed in vacuo, and the residue was purified on a silica gel column with 2% MeOH in $CHCl_3$ to give **49** (115 mg, 96% as a foam): LRMS (FAB) *m*/*z* 542 (MH^+); HRMS (FAB) calcd for $C_{27}H_{40}N_3O_5Si_2$ 542.2506, found 542.2505. Anal. ($C_{27}H_{39}N_3O_5Si_2$) C, H, N.

1-(3-Deoxy-3-*C*-ethynyl-*β*-D-*xylo*-pentofuranosyl)uracil (48). Compound **46** (100 mg, 0.18 mmol) was stirred in MeOH (2 mL) containing MaOMe (5 M, 40 μL) at room temperature. After 3.5 h, HCl/MeOH (2%, 2 mL) was added to the mixture, which was stirred further for 16 h at the same temperature. The solvent was removed and coevaporated several times with EtOH. The residue was purified on a silica gel column with 10% MeOH in $CHCl_3$ to give **48** (48 mg, quantitative as a foam): LRMS (EI) *m*/*z* 252 (M^+); HRMS (FAB) calcd for $C_{11}H_{12}N_2O_5$ 252.0745, found 252.0723; ^{1}H NMR (D_2O) 7.92 (d, 1 H, H-6, $J_{6,5}$ = 8.1 Hz), 5.88 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 5.83 (d, 1 H, H-1′, $J_{1',2'}$ = 3.0 Hz), 4.58 (dd, 1 H, H-2′, $J_{2',1'}$ = 3.0 Hz, $J_{2',3'}$ = 3.5 Hz), 4.53 (ddd, 1 H, H-4′, $J_{4',3'}$ = 6.3 Hz, $J_{4',5'a}$ = 6.3 Hz, $J_{4',5'b}$ = 4.3 Hz), 4.04 (dd, 1 H, H-5′a, $J_{5'a,4'}$ = 6.3 Hz, J_{gem} = 12.3 Hz), 3.97 (dd, 1 H, H-5′b, $J_{5'b,4'}$ = 4.3 Hz, J_{gem} = 12.3 Hz), 3.30 (ddd, 1 H, H-3′, $J_{3',2'}$ = 3.5 Hz, $J_{3',4'}$ = 6.3 Hz, $J_{3',C\equiv CH}$ = 2.6 Hz), 2.70 (d, 1 H, 3′-C≡CH, $J_{C\equiv CH,3'}$ = 2.6 Hz); ^{13}C NMR (MeOH-d_4) 166.23, 152.38, 142.46, 102.06, 92.77, 82.35, 81.29, 80.24, 76.01, 63.14, 40.75. Anal. ($C_{11}H_{12}N_2O_5$·0.3MeOH) C, H, N.

1-(3-Deoxy-3-*C*-ethynyl-*β*-D-*ribo*-pentofuranosyl)uracil (47). Compound **47** was prepared as described for the synthesis of **48**. From **45** (50 mg, 0.09 mmol), **47** (23 mg, quantitative as a foam) was obtained. **47**: LRMS (EI) *m*/*z* 252 (M^+); HRMS (EI) calcd for $C_{11}H_{12}N_2O_5$ 252.0745, found 252.0770; ^{1}H NMR (D_2O) 7.94 (d, 1 H, H-6, $J_{6,5}$ = 8.1 Hz), 5.87 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 5.85 (s, 1 H, H-1′), 4.53 (d, 1 H, H-2′, $J_{2',3'}$ = 4.6 Hz), 4.35 (m, 1 H, H-4′, $J_{4',3'}$ = 10.3 Hz), 4.08 (br d, 1 H, H-5′a, J_{gem} = 12.2 Hz), 3.87 (br d, 1 H, H-5′b, J_{gem} = 12.2 Hz), 3.18 (dd, 1 H, H-3′, $J_{3',2'}$ = 4.6 Hz, $J_{3',4'}$ = 10.8 Hz), 2.24 (s, 1 H, 3′-C≡CH); ^{13}C NMR (MeOH-d_4) 166.37, 152.01, 142.37, 101.79, 93.74, 86.01, 78.70, 77.79, 74.50, 60.77, 36.13. Anal. ($C_{11}H_{12}N_2O_5$·0.57MeOH) C, H, N.

1-(3-Deoxy-3-*C*-ethynyl-*β*-D-*xylo*-pentofuranosyl)cytosine (50). Compound **50** was prepared as described for the synthesis of **47**. From **49** (100 mg, 0.18 mmol), **50** (59 mg, quantitative as a foam) was obtained. **50**: LRMS (EI) *m*/*z* 251 (M^+); HRMS (EI) calcd for $C_{11}H_{13}N_3O_4$ 251.0905, found 251.0913; ^{1}H NMR (DMSO-d_6 + D_2O) 7.69 (d, 1 H, H-6, $J_{6,5}$ = 7.5 Hz), 5.74 (d, 1 H, H-5, $J_{5,6}$ = 7.5 Hz), 5.65 (br s, 1 H, H-1′), 4.21 (dd, 1 H, H-4′, $J_{4',5'a}$ = 5.7 Hz, $J_{4',5'b}$ = 4.6 Hz), 4.18 (br s, 1 H, H-2′), 3.70 (dd, 1 H, H-5′a, $J_{5'a,4'}$ = 5.7 Hz, J_{gem} = 11.5 Hz), 3.67 (dd, 1 H, H-5′b, $J_{5'b,4'}$ = 4.6 Hz, J_{gem} = 11.5 Hz), 3.11 (s, 1 H, 3′-C≡CH), 3.02 (br s, 1 H, H-3′); ^{13}C NMR (MeOH-d_4) 166.59, 156.92, 143.39, 95.24, 94.43, 83.03, 81.88, 80.18, 76.21, 63.16, 40.75.

***N*^{4}-Benzoyl-1-[3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-*C*-[(trimethylsilyl)ethynyl]-*β*-D-*ribo*-pentofuranosyl]cytosine (52) and *N*^{4}-Benzoyl-1-[3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-*C*-[(trimethylsilyl)ethynyl]-*β*-D-*arabino*-pentofuranosyl]cytosine (53).** A hexane solution of BuLi (1.56 M, 10 mL, 16.1 mmol) was added dropwise over 15 min to a solution of (trimethylsilyl)acetylene (2.27 mL, 16.1 mmol) in THF (10 mL) at −78 °C under Ar. After being stirred for 30 min, a solution of *N*^{4}-benzoyl-1-[3,5-*O*-(TIPDS)-*β*-D-*erythro*-pentofuran-2-ulosyl]cytosine (**51**; 3.15 g, 5.4 mmol) in THF (10 mL) was added dropwise over 10 min to the above solution. The whole was stirred for 2.5 h at the same temperature and was quenched by addition of aqueous NH_4Cl (1 M, 25 mL). The mixture was extracted with EtOAc (50 mL × 3). The separated organic phase was washed with brine (100 mL), dried (Na_2SO_4), and concentrated to dryness in vacuo. The residue was purified on a silica gel column with 25% EtOAc in hexane to give **53** (2.49 g, 68% as a white foam). The column was successively eluted with 35% EtOAc in hexane and then 50% EtOAc in hexane. From the former elution, **51** (490 mg, as a foam) was recovered, and from the latter elution, **52** (310 mg, 8.5% as a white foam) was obtained. **53**: LRMS (EI) *m*/*z* 685 (M^+). Anal. ($C_{33}H_{51}N_3O_7Si_3$) C, H, N. **52**: LRMS (EI) *m*/*z* 685 (M^+). Anal. ($C_{33}H_{51}N_3O_7Si_3$) C, H, N.

1-(2-*C*-Ethynyl-*β*-D-*arabino*-pentofuranosyl)cytosine Hydrochloride (57). A THF solution of TBAF (1 M, 4.1 mL, 4.1 mmol) was added to a mixture of **53** (810 mg, 1.2 mmol) in THF (30 mL) containing AcOH (0.24 mL, 4.1 mmol). The mixture was stirred for 40 min at room temperature and concentrated to dryness in vacuo. The residue was purified on a silica gel column with 4% MeOH in $CHCl_3$ to give *N*^{4}-benzoyl-1-(2-*C*-ethynyl-*β*-D-*arabino*-pentofuranosyl)cytosine (**56**; 379 mg, 86% as a white powder): LRMS (EI) *m*/*z* 371 (M^+). Anal. ($C_{18}H_{17}N_3O_6$·0.8EtOH) C, H, N. Compound **56** (250 mg, 0.67 mmol) was treated with NH_3/MeOH (saturated at 0 °C, 10 mL) for 17 h at room temperature. The solvent was removed in vacuo, and the residue was purified on a silica gel column with 30% MeOH in $CHCl_3$ to give a white powder, which was crystallized from HCl/EtOH to give **57** (156 mg, 77%): mp 239−241 °C; ^{1}H NMR (DMSO-d_6) 9.78, 8.73 (each br s, each 1 H, NH_2), 7.99 (d, 1 H, H-6, $J_{6,5}$ = 7.8 Hz), 6.51 (br s, 1 H, 2′-OH), 6.14 (d, 1 H, H-5, $J_{5,6}$ = 7.8 Hz), 6.12 (s, 1 H, H-1′), 5.90 (br s, 1 H, 3′-OH), 3.87 (d, 1 H, H-3′, $J_{3',4'}$ = 4.4 Hz), 3.83 (dd, 1 H, H-4′, $J_{4',3'}$ = $J_{4',5'a}$ = 4.4 Hz, $J_{4',5'b}$ = 4.9 Hz), 3.66 (dd, 1 H, H-5′a, $J_{5'a,4'}$ = 4.4 Hz, J_{gem} = 11.7 Hz), 3.60 (dd, 1 H, H-5′b, $J_{5'b,4'}$ = 4.9 Hz, J_{gem} = 11.7 Hz), 3.59 (s, 1 H, 2′-C≡CH). Anal. ($C_{11}H_{13}N_3O_5$·HCl) C, H, N.

1-(2-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl)cytosine Hydrochloride (55). Compound **52** (360 mg, 0.53 mmol) was desilylated as described above to give *N*^{4}-benzoyl-1-(2-*C*-ethynyl-*β*-D-*ribo*-pentofuranosyl)cytosine (**54**), which was debenzoylated to give **55** (95 mg, 60% as an HCl salt): mp 212−213 °C; ^{1}H NMR (DMSO-d_6) 9.77 (br s, 1 H, NH), 8.77 (br s, 1 H, NH), 8.63 (d, 1 H, H-6, $J_{6,5}$ = 7.8 Hz), 6.37 (br s, 1 H, 2′-OH), 6.16 (d, 1 H, H-5, $J_{5,6}$ = 7.8 Hz), 5.87 (s, 1 H, H-1′), 4.00 (d, 1 H, H-3′, $J_{3',4'}$ = 8.8 Hz), 3.84 (dt, 1 H, H-4′, $J_{4',5'a}$ = $J_{4',5'b}$ = 2.4 Hz, $J_{4',3'}$ = 9.3 Hz), 3.80 (dd, 1 H, H-5′a, $J_{5'a,4'}$ = 2.4 Hz, J_{gem} = 12.7 Hz), 3.61 (dd, 1 H, H-5′b, $J_{5'b,4'}$ = 2.4 Hz, J_{gem} = 12.7 Hz), 3.53 (s, 1 H, 2′-C≡CH). Anal. ($C_{11}H_{13}N_3O_5$·HCl) C, H, N.

Purification of Uridine/Cytidine Kinase (UCK). Uridine/cytidine kinase (UCK, EC 2.7.1.48) was highly purified from ascitic Sarcoma-180 cells growing in mice. The tumor cells (63.5 g wet weight) were homogenized with 4 volumes of potassium phosphate buffer (10 mM, pH 7.5) containing DTT (5 mM), KCl (25 mM), and $MgCl_2$ (5 mM) and centrifuged at 105000*g* for 60 min. The resulting supernatant was treated with ammonium sulfate (30−50% saturation) and centrifuged at 105000*g* for 30 min. The precipitates were dissolved in potassium phosphate buffer (10 mM, pH 7.5) containing DTT (5 mM) and 5% glycerol, dialyzed against the same buffer, and applied to a Sephacryl S-200 column (3 × 105 cm) equilibrated with the same buffer. The active eluates containing UCK were subjected to a Bio-Gel HT hydroxyapatite column (4 × 16 cm) equilibrated with the same buffer. UCK was obtained by the linear gradient elution of 10−100 mM phosphate buffer. The eluates were dialyzed against the starting buffer and then purified by chromatography on a DEAE-Sepharose CI-6B column (3 × 15 cm). The purified enzyme preparation was found not to contain any other enzymes such as CMP kinase, CDP kinase, Cyd deaminase, or pyrimidine nucleoside phosphorylase. UCK activity was stable for more than 1 year when stored at −80 °C.

Assay of UCK. Phosphorylation of the test compounds by UCK was measured according to the method described by Ikenaka et al.[17]

(1) Radiochemical Assay. The substrate activity of radiolabeled Cyd, Urd, ECyd, and EUrd by partially purified UCK was assayed by a radiochemical method which quantitated the formation of the corresponding radioactively labeled 5′-monophosphates. The reaction mixture, in a total volume of 125 μL, consisted of 50 mM Tris-HCl buffer (pH 8.0), 10 mM ATP, 10 mM NaF, 5 mM $MgCl_2$, 1 mg/mL BSA, 0.6 mM tritiated nucleoside (75 nmol/tube, 20 μCi/tube), and 50 mL of the enzyme solution. The mixture was incubated at 37 °C for 15 min (for Cyd and Urd) or 60 min (for ECyd and EUrd), heated in a boiling water bath for 3 min, and centrifuged at 3000 rpm for 10 min; 10 μL of the supernatant was then spotted onto a 2.5 × 10-cm PEI-cellulose TLC plate (Merck TLC plates, PEI-cellulose F precoated) and developed with water. Phosphorylated compound at the origin was scraped into a vial and extracted with 0.5 mL of 1 M HCl; 10 μL of scintillator was then added, and the radioactivity was measured.

(2) HPLC Assay. Phosphorylation of Cyd, Urd, ECyd, EUrd, **15a**−**d**, **17c**, and **50** was measured by HPLC which quantitated the consumption of these nucleosides by partially purified UCK. The reaction mixture was similar to that described above except that tritium-labeled nucleoside was by nonlabeled nucleoside. After centrifugation of the boiled reaction mixture, 50 μL of the supernatant was diluted with 200 μL of water; 10 μL of the sample was then loaded onto a Chemcosorb 300-5C18 column (4.6 × 250 cm; Chemco Co., Ltd.) under the following chromatographic conditions: monitoring wavelength, 270 nm; flow rate, 1 mL/min; mobile phase, 1.5% MeOH for Cyd (7.1 min) and Urd (7.5 min), 2.5% MeOH for ECyd (7.1 min), EUrd (9.8 min), **15a** (8.5 min), **15c** (9.5 min), **15d** (8.3 min), and **17c** (10.4 min), or 7.5% MeOH for **15b** (9.3 min) and **50** (7.3 min) containing 0.01% trifluoroacetic acid. The numbers in parentheses indicate the retention time.

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Supporting Information Available: NMR data for the nontarget compounds (8 pages). Ordering information is given on any current masthead page.

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