# Nucleosides and Nucleotides. 158. 1-(3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl)-cytosine, 1-(3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl)uracil, and Their Nucleobase Analogues as New Potential Multifunctional Antitumor Nucleosides with a Broad Spectrum of Activity[1]

Hideshi Hattori,[†] Motohiro Tanaka,[‡] Masakazu Fukushima,[§] Takuma Sasaki,[‡] and Akira Matsuda*[,†]

*Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan, Cancer Research Institute, Kanazawa University, Takara-machi, Kanazawa 920, Japan, and Cancer Research Laboratory-2, Taiho Pharmaceutical Company Ltd., Hanno 357, Japan*

*Received July 25, 1996*[⊗]

We previously designed 1-(3-*C*-ethynyl-*β*-D-*ribo*-pentofuranosyl)uracil (EUrd) as a potential multifunctional antitumor nucleoside antimetabolite. It showed a potent and broad spectrum of antitumor activity against various human tumor cells *in vitro* and *in vivo*. To determine the structure−activity relationship, various nucleobase analogues of EUrd, such as 5-fluorouracil, thymine, cytosine, 5-fluorocytosine, adenine, and guanine derivatives, were synthesized by condensation of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-3-*C*-ethynyl-α,*β*-D-*ribo*-pentofuranose (**6**) and the corresponding pertrimethylsilylated nucleobases in the presence of $SnCl_4$ or TMSOTf as a Lewis acid in $CH_3CN$ followed by debenzoylation. The *in vitro* tumor cell growth inhibitory activity of these 3′-*C*-ethynyl nucleosides against mouse leukemia L1210 and human nasopharyngeal KB cells showed that 1-(3-*C*-ethynyl-*β*-D-*ribo*-pentofuranosyl)cytosine (ECyd) and EUrd were the most potent inhibitors in the series, with $IC_{50}$ values for L1210 cells of 0.016 and 0.13 μM and for KB cells of 0.028 and 0.029 μM, respectively. 5-Fluorocytosine, 5-fluorouracil, and adenine nucleosides showed much lower activity, with $IC_{50}$ values of 0.4−2.5 μM, while thymine and guanine nucleosides did not exhibit any activity up to 300 μM. We next evaluated the tumor cell growth inhibitory activity of ECyd and EUrd against 36 human tumor cell lines *in vitro* and found that they were highly effective against these cell lines with $IC_{50}$ values in the nanomolar to micromolar range. These nucleosides have a similar inhibitory spectrum. The *in vivo* antitumor activities of ECyd and EUrd were compared to that of 5-fluorouracil against 11 human tumor xenografts including three stomach, three colon, two pancreas, one renal, one breast, and one bile duct cancers. ECyd and EUrd showed a potent tumor inhibition ratio (73−92% inhibition relative to the control) in 9 of 11 and 8 of 11 human tumors, respectively, when administered intravenously for 10 consecutive days at doses of 0.25 and 2.0 mg/kg, respectively, while 5-fluorouracil showed potent inhibitory activity against only one tumor. Such excellent antitumor activity suggests that ECyd and EUrd are worth evaluating further for use in the treatment of human cancers.

## Introduction

Inhibition of the nucleotide biosynthesis of tumor cells by antimetabolites is a classic but still important approach in cancer chemotherapy.[2] Although a nucleoside antimetabolite has been recognized as one of the most important agents in cancer chemotherapy, only 5-fluorouracil (5-FU) and its prodrugs are currently used clinically in Japan against solid tumors, such as breast carcinoma, colorectal carcinoma, gastric adenocarcinoma, and pancreatic adenocarcinoma. 2′-Deoxycytidine analogues, such as 1-(2-deoxy-2-methylene-*β*-D-*erythro*-pentofuranosyl)cytosine (DMDC),[3] 2′-deoxy-2′,2′-difluorocytidine (gemcitabine),[4] and 1-(2-*C*-cyano-2-deoxy-*β*-D-*arabino*-pentofuranosyl)cytosine (CNDAC),[5] have been developed as potent antitumor agents which, unlike 1-*β*-D-arabinofuranosylcytosine (araC), are effective not only against leukemias and lymphomas but also against a wide variety of solid tumors *in vitro* as well as *in vivo*. The antitumor activity of these nucleosides is believed to be due mainly to their inhibition of DNA synthesis in tumor cells, in which they are metabolized to their corresponding 5′-diphosphates and 5′-triphosphates and inhibit ribonucleoside diphosphate reductase (RDPR) and/or DNA polymerases. However, many solid tumor cells grow slowly, and a drug has few chances to encounter S-phase, in which DNA synthesis occurs. Therefore, it is not enough for nucleoside antimetabolites to only inhibit DNA synthesis in tumor cells. However, it has been reported that these 2′-deoxycytidine analogues also inhibit RNA synthesis to some extent.[3g,4,5e] Although their inhibition of RNA synthesis would not be a major mechanism of tumor cell death, it is conceivable that this inhibition may contribute to the antitumor efficacy against slow-growing solid tumors. Therefore, in our search for more potent inhibitors of tumor cell growth, we designed a nucleoside antimetabolite which inhibited both DNA and RNA syntheses.

To inhibit RNA synthesis, a nucleoside antimetabolite should be recognized as a substrate and/or an inhibitor of ribonucleoside- and ribonucleotide-metabolizing enzymes, such as nucleoside and nucleotide kinases, CTP synthetase, and RNA polymerases, and therefore should

* Author to whom all correspondence and reprint requests should be addressed.
[†] Hokkaido University.
[‡] Kanazawa University.
[§] Taiho Pharmaceutical Co. Ltd.
[⊗] Abstract published in *Advance ACS Abstracts,* November 1, 1996.

S0022-2623(96)00537-7 CCC: $12.00 

have two hydroxyl groups at the 2′- and 3′-positions with a *ribo*-configuration.

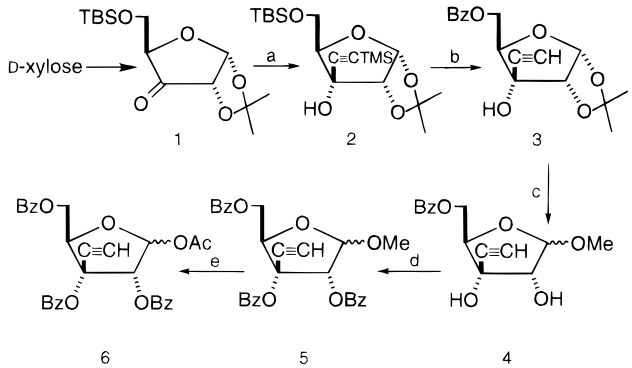
On the other hand, RDPR is one of the most important target enzymes for the inhibition of DNA synthesis.[6] RDPR catalyzes the conversion of all of the four main ribonucleoside 5′-diphosphates (rNDPs) to the corresponding 2′-deoxyribonucleoside 5′-diphosphates (dNDPs) in a *de novo* pathway. The resulting dNDPs are further phosphorylated to the corresponding 2′-deoxyribonucleoside 5′-triphosphates (dNTPs), which are essential for DNA synthesis, by nucleoside diphosphate kinase. *Escherichia coli* RDPR, which is a prototype of mammalian enzymes, is the best characterized. The enzyme consists of two subunits, R1 and R2, each of which is a homodimer. The R2 subunit contains a tyrosyl radical stabilized by a $\mu$-oxo-bridged binuclear iron(III) center.[7] This radical is transferred to certain amino acid residues in the active site of the R1 subunit in the catalytic site, which initiates the enzyme-catalyzed reduction of the 2′-hydroxyl group in rNDPs, by abstraction of the 3′-hydrogen atom in the ribose moiety of the rNDPs.[8] The amino acid residue corresponding to radical formation has been postulated to be Cys439 based on the results of site-directed mutagenesis of the R1 subunit.[9] X-ray crystallographic studies of the R1 subunit have also suggested that Cys439 is near the 3′-hydrogen of rNDP.[10] Therefore, as a functional group to react effectively and directly with the thiyl radical, we selected an acetylene group to be introduced at the 3′-position, since acetylene derivatives are well-known to react with thiyl radicals to form alkylthio vinyl sulfides.[11]

On the basis of these considerations, we previously designed 1-(3-*C*-ethynyl-$\beta$-D-*ribo*-pentofuranosyl)uracil (EUrd) as a potential multifunctional antitumor nucleoside and found that it showed potent tumor cell growth inhibitory activity against various human tumor cell lines *in vitro* and strong antitumor activity against murine leukemia P388 and a variety of human solid tumor xenografts *in vivo*.[12] In this paper, we describe the synthesis of various nucleobase analogues of EUrd, such as 5-fluorouracil, thymine, cytosine, 5-fluorocytosine, adenine, and guanine derivatives, to determine the structure−activity relationship of tumor cell growth inhibitory activity *in vitro*. We also evaluated the antitumor activity of EUrd and its cytosine analogue against various human tumor xenografts *in vivo*.

## Synthesis

The synthesis of 3-*C*-ethynyl-$\beta$-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.[13] Addition of LiC≡CTMS to **1** gave the desired $\beta$-adduct **2** in 99% yield with high stereoselectivity.[14] Desilylation of **2** with tetrabutylammonium fluoride (TBAF) followed by benzoylation gave **3** in 93% yield. Treatment of **3** with aqueous 20% HCl in MeOH for 6 h at room temperature gave methyl 5-*O*-benzoyl-3-*C*-ethynyl-$\alpha$,$\beta$-D-*ribo*-pentofuranoside (**4**), which was subsequently benzoylated in a mixture of BzCl and DMAP in anhydrous pyridine at 100 °C to give **5** in 87% yield. When this benzoylation was performed at room temperature, benzoylation of the tertiary alcohol at the 3-position did not occur.[15] Furthermore, when **3** was treated with anhydrous HCl/MeOH at room temperature for 2 days, followed by benzoylation, **5** was obtained

**Scheme 1**[a]




[a] Conditions: (a) TMSC≡CH, BuLi, THF, −78 °C, 99%; (b) (i) $Bu_4NF$, THF, rt, (ii) BzCl, pyridine, rt, 93% in two steps; (c) aqueous 20% HCl, MeOH, rt; (d) BzCl, DMAP, pyridine, 100 °C, 87% in two steps; (e) concentrated $H_2SO_4$, AcOH, $Ac_2O$, rt, 93%.

in 72% yield along with methyl 5-*O*-benzoyl-3-*C*-ethynyl-2,3-*O*-isopropylidene-$\beta$-D-*ribo*-pentofuranose in 24% yield. Subsequent acetolysis of **5** 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*-ethynyl-$\alpha$,$\beta$-D-*ribo*-pentofuranose (**6**) in 93% yield as a syrup.

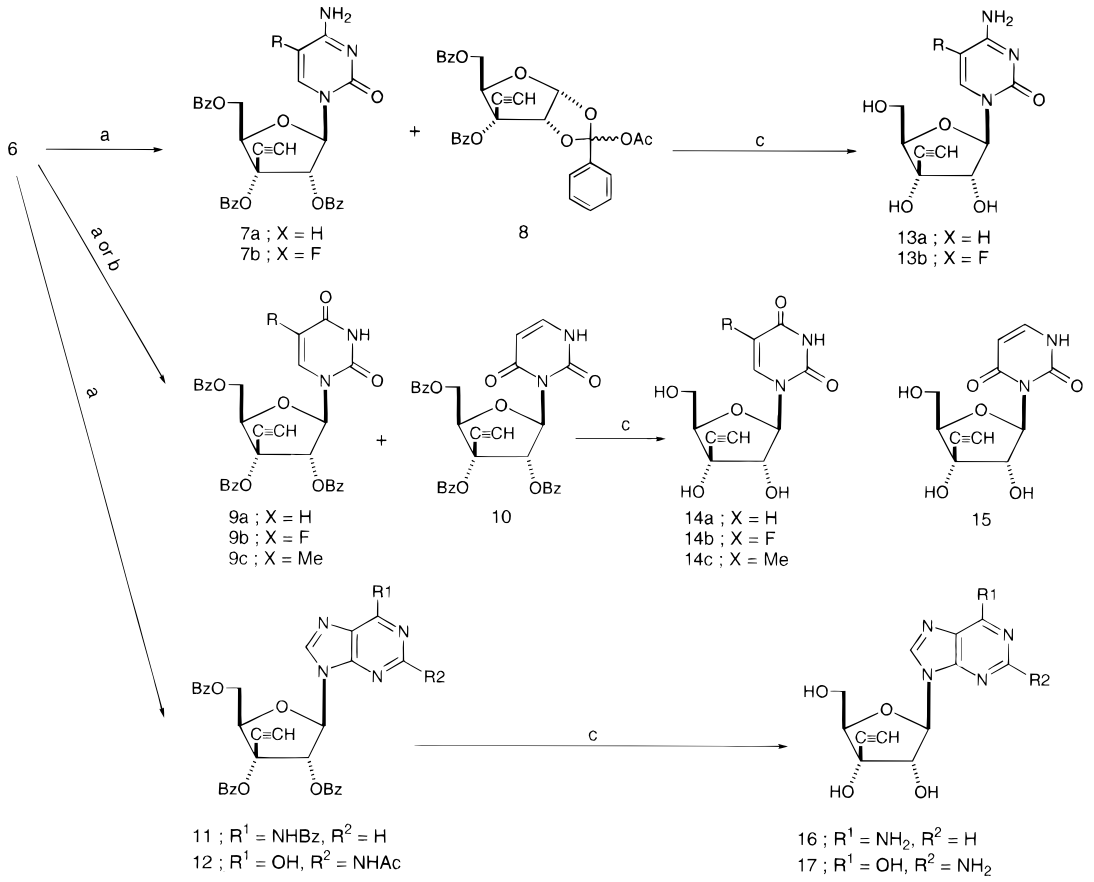
We next investigated the condensation of **6** with pertrimethylsilylated nucleobases under Vorbrüggen conditions.[16] When 1.2 equiv of bis(TMS)cytosine was reacted with **6** in the presence of 2 equiv of $SnCl_4$ in $CH_3CN$, the desired 1-(2,3,5-tri-*O*-benzoyl-3-*C*-ethynyl-$\beta$-D-*ribo*-pentofuranosyl)cytosine (**7a**) was obtained as a major product in 59% yield along with **8** in 26% yield. To improve the yield of **7a**, increased amounts of bis(TMS)cytosine and $SnCl_4$ to 4 and 5 equiv, respectively, were used to give **7a** in 81%. A further increase in the above reagents gave only a complex mixture. In a similar manner, 5-fluorocytosine, 5-fluorouracil, thymine, $N^6$-benzoyladenine, and $N^2$-acetylguanine nucleosides **7b**, **9b**,**c**, **11**, and **12**, respectively, were prepared in good yields by the condensation of **6** and the corresponding pertrimethylsilylated nucleobases. During the condensation with $N^2$-acetylguanine, the N9-riboside **12** was obtained in 99% yield without detection of its N7-isomer, which is usually observed during glycosylation reactions of guanine derivatives with appropriate sugars.

Condensation of bis(TMS)uracil (4 equiv) with **6** in $CH_3CN$ in the presence of $SnCl_4$ (4 equiv) gave a mixture of N1-$\beta$-nucleoside **9a** and N3-$\beta$-nucleoside **10** in yields of 58% and 20%, respectively. The structures of these nucleosides were identified by comparison of their UV spectra after deblocking (see the Experimental Section). However, when trimethylsilyl triflate (4 equiv; TMSOTf)[17] was used as a Lewis acid instead of $SnCl_4$, the yield of the desired **9a** was improved to 95%.

For deblocking of these nucleosides, **7a**,**b**, **9a**−**c**, and **10**−**12** were treated with $NH_3$/MeOH at room temperature to give the corresponding 3′-*C*-ethynyl nucleosides **13a**,**b**, **14a**−**c**, and **15**−**17** in good yields as crystals. The structures of these nucleosides were confirmed by $^1$H-NMR, mass spectrometry, and IR and UV spectrophotometry, along with elemental analyses.

Recently, Jung *et al.* reported the stereoselective addition of ethynylcerium reagents to 5′-hydroxy-3′-keto nucleosides from the $\beta$-face to give 3′-*C*-ethynyl-$\beta$-D-*ribo*-pentofuranosyl nucleosides, although the reaction of 5′-*O*-TBDMS-3′-keto derivatives with the same reagents

**Scheme 2**[a]




[a] Conditions: (a) pertrimethylsilylated nucleobase, $SnCl_4$, $CH_3CN$; (b) pertrimethylsilylated nucleobase, TMSOTf, $CH_3CN$; (c) $NH_3$/MeOH.

gave 3′-*C*-ethynyl-β-D-*xylo*-pentofuranosyl nucleosides.[18] However, for preparing the various nucleobase analogues of the 3′-*C*-ethynyl-β-D-*ribo*-pentofuranosyl nucleosides in this study, our glycosylation method is superior because once an appropriate sugar precursor for the condensation is synthesized, condensation with the appropriate nucleobases gives a variety of the desired nucleosides.

## Biological Activity

*In vitro* tumor cell growth inhibitory activity of 3-*C*-ethynyl-β-D-*ribo*-pentofuranose nucleosides ECyd (**13a**), EFCyd (**13b**), EUrd (**14a**), EFUrd (**14b**), EMUrd (**14c**), EAdo (**16**), and EGuo (**17**) against mouse leukemia L1210 and human nasopharyngeal KB cell lines was investigated first. The results are summarized in Table 1. ECyd was the most effective inhibitor of tumor cell proliferation in this series of nucleosides, with $IC_{50}$ values of 16 and 28 nM against L1210 and KB cells, respectively. EUrd was 8 times less active against L1210 cells than ECyd but was equally active against KB cells. The introduction of a fluorine atom to the 5-position of ECyd and EUrd reduced the activity about 20−50-fold, and EMUrd had no activity against either cell line. These data may reflect the substrate specificity of the first activation enzymes of these nucleosides, such as uridine/cytidine kinase, which would recognize the bulky substituents at the 5-position. EAdo exhibited activity similar to EFCyd, while EGuo did not have such properties.

We next evaluated ECyd and EUrd against 36 human solid tumor cell lines, including eight stomach, four colon, ten lung, three breast, two pancreas, two bladder, two osteo, one leiomyo, one fibro, two melanoma, and one nasopharyngeal tumor cell lines *in vitro*. The results are summarized in Table 2. ECyd was effective against 22 cell lines with $IC_{50}$ values in the nanomolar to subnanomolar range and was ineffective against two cell lines. EUrd had potent inhibitory activity against 12 cell lines with $IC_{50}$ values in the subnanomolar range and was ineffective against six cell lines. We previously compared the *in vitro* tumor cell growth inhibitory activity of EUrd against several human tumor cell lines with those of 5-fluorouridine (FUrd), 2′-deoxy-5-fluorouridine (FdUrd), and 5-fluorouracil (5-FU) and found that EUrd was about 6−650 times more potent than

**Table 1.** Inhibitory Effects of Various 3′-*C*-Ethynyl-β-D-*ribo*-pentofuranosyl Nucleosides on the Growth of L1210 and KB Cells *in Vitro*[a]

| | $IC_{50}$ (μM) | |
|---|---|---|
| compounds | L1210 | KB |
| ECyd (**13a**) | 0.016 | 0.028 |
| EFCyd (**13b**) | 0.53 | 0.46 |
| EUrd (**14a**) | 0.13 | 0.029 |
| EFUrd (**14b**) | 2.5 | 1.4 |
| EMUrd (**14c**) | >300 | >300 |
| EAdo (**16**) | 0.69 | 0.83 |
| EGuo (**17**) | >300 | >300 |

[a] Tumor cell growth inhibitory activity assay *in vitro* was performed following the method of Carmichael *et al*.[21] Tumor cells ($2 \times 10^3$ cells/well) were incubated in the presence or absence of the compounds for 72 h. MTT reagent was added to each well, and the plate was incubated for an additional 4 h. The resulting MTT formazan was dissolved in DMSO, and the OD (540 nm) was measured. Percent inhibition was calculated as follows: % inhibition = [1 − OD (540 nm) of sample well/OD(540 nm) of control well] × 100. $IC_{50}$ (μM) represents the concentration at which cell growth was inhibited by 50%.

**Table 2.** Inhibitory Effects of ECyd and EUrd on the Growth of Human Tumor Cell Lines *in Vitro*[a]

| | | $IC_{50}$ ($\mu$M) | |
|---|---|---|---|
| cell lines | origin | ECyd | EUrd |
| MKN-28 | stomach adenocarcinoma | 0.21 | 0.065 |
| MKN-45 | | 0.0088 | 0.052 |
| KATO-III | | 0.031 | 0.031 |
| NUGC-4 | | 0.024 | 0.22 |
| ST-KM | | 0.047 | 0.086 |
| KKLS | | 0.021 | 0.23 |
| STSA-1 | | 0.15 | 1.9 |
| NAKAJIMA | | 0.17 | 0.78 |
| Colo320DM | colon adenocarcinoma | 0.02 | 0.06 |
| HCT-15 | | 0.0082 | 0.075 |
| SW-48 | | >1.9 | >1.9 |
| SW-480 | | 0.26 | 0.34 |
| PC-8 | lung adenocarcinoma | 0.090 | 0.33 |
| PC-9 | | 0.11 | 0.19 |
| PC-10 | | 0.16 | >1.9 |
| QG-56 | lung squamous-cell carcinoma | 0.045 | 0.15 |
| QG-95 | | 0.21 | 0.28 |
| Lu-65 | lung large-cell carcinoma | 0.032 | 0.089 |
| QG-90 | lung small-cell carcinoma | 0.13 | 1.0 |
| QG-96 | | 0.39 | 0.38 |
| NCI-H-82 | | 0.12 | 0.23 |
| NCI-H-417 | | 0.043 | 0.11 |
| MDA-MB-321 | breast adenocarcinoma | 0.024 | >1.9 |
| YMB-1-E | | 0.16 | >1.9 |
| MCF-7 | | 0.069 | 0.2 |
| PANC-1 | pancreas adenocarcinoma | >1.9 | >1.9 |
| Mia-PaCa-2 | | 0.015 | 0.054 |
| T24 | bladder carcinoma[b] | 0.028 | 0.21 |
| KK-47 | | 0.12 | 0.089 |
| HOS | osteosarcoma | 1.0 | 1.8 |
| MG-63 | | 0.15 | >1.9 |
| A431 | leiomyosarcoma | 0.033 | 0.29 |
| HT-1080 | fibrosarcoma | 0.073 | 0.16 |
| A375 | melanoma | 0.026 | 0.021 |
| SK-MEL-28 | | 0.047 | 0.086 |
| KB | pharyngeal carcinoma | 0.028 | 0.029 |

[a] Tumor cell growth inhibitory activity assay *in vitro* was done as described for Table 1. [b] Transitional cell.

5-FU and comparable to FUrd and FdUrd.[12] Therefore, the activity of ECyd was superior to that of EUrd and 5-FU derivatives *in vitro*. On the basis of the mean graph analysis[19] (data not shown), both ECyd and EUrd have a similar inhibitory activity spectrum and therefore may have a similar mechanism of action for antitumor activity.

We next examined the antitumor effects of ECyd and EUrd[20] against human tumors (three stomach, three colon, two pancreas, one renal, one breast, and one bile duct cancers) as xenografts in nude mice in comparison with 5-FU. The results are shown in Table 3. In this experiment, the tumor inhibition ratio (%) was used as a parameter of antitumor activity, and day 15 was chosen for the final evaluation of the effects of treatment on tumor growth. ECyd had a potent antitumor effect on 9 of 11 tumor xenografts and a moderate effect on Hucc-T1 bile duct and BxPC-3 pancreas tumor xenografts, while EUrd showed a similar potency to ECyd for 8 of 11 tumors but was only moderately effective against NUGC-3 stomach, Hucc-T1 bile duct, and BxPC pancreas tumors, when given in a daily intravenous dose of 0.25 and 2 mg/kg, respectively, for 10 consecutive days. We next compared the antitumor activity of 5-FU, which has been used clinically against solid tumors, especially stomach, colon, and breast cancers. When 5-FU was administered intravenously 14 consecutive days at a dose of 15 mg/kg, it had a potent antitumor effect only on H-81 stomach tumor but showed moderate antitumor effects on other human tumors. To show a potent antitumor effect against the human tumor xenografts used in this study, only one-eighth as much ECyd was required as EUrd. This reflects the *in vitro* potency of both nucleosides. The doses of ECyd, EUrd, and FU used in this study were each the respective maximum nontoxic dose. Since multiple-dose schedules were not tested, we may not have used these drugs under optimal conditions.

Although the optimal therapeutic schedule of ECyd and EUrd is not yet known, such excellent antitumor activity suggests that ECyd and EUrd are worth further evaluation for use in the treatment of human cancer. Further studies are in progress to determine whether the mechanism responsible for their antitumor properties is related to the inhibition of both DNA and RNA syntheses. If ECyd and EUrd act as multifunctional antitumor nucleosides, they would represent a new approach in anticancer chemotherapy.

## 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) spectrometer or Bruker ARX 500 (500 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million ($\delta$), 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*-[2′-(trimethylsilyl)ethynyl]-α-D-*ribo*-pentofuranose (2).** A hexane solution of BuLi (1.62 M, 27.8 mL, 45 mmol) was added dropwise over 30 min to a solution of (trimethysilyl)-acetylene (6.3 mL, 45 mmol) in THF (60 mL) at −78 °C under Ar atmosphere. After being stirred for a further 30 min, a solution of 5-*O*-(*tert*-butyldimethylsilyl)-1,2-*O*-isopropylidene-α-D-*erythro*-pentos-3-ulose (**1**; 4.5 g, 15 mmol)[13] in THF (30 mL) was added dropwise over 10 min to the acetylide solution at the same temperature. The mixture was stirred for 3 h and then the reaction quenched by the addition of aqueous $NH_4Cl$ solution (1 M, 60 mL). The mixture was warmed to room temperature and extracted by EtOAc (35 mL × 3), which was further washed with brine (20 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 (7.5 × 8 cm), which was eluted with 5% EtOAc in hexane to give **2** (5.92 g, 99% as a white powder, which was crystallized from hexane): mp 84−86 °C; FAB-MS *m*/*z* 401 ($MH^+$), 383 ($M^+$ − OH); $^1$H-NMR ($CDCl_3$) 5.85 (d, 1 H, H-1, $J_{1,2}$ = 3.8 Hz), 4.56 (d, 1 H, H-2, $J_{2,1}$ = 3.8 Hz), 4.01−3.94 (m, 3 H, H-4, H-5), 3.05 (s, 1 H, 3-OH), 1.56, 1.37 (s, each 3 H, *i*-Pr), 0.91 (s, 9 H, *t*-Bu), 0.18 (s, 9 H, Me × 3), 0.06, 0.03 (s, each 3 H, Me). Anal. ($C_{19}H_{36}O_5Si_2$) C, H.

**5-*O*-Benzoyl-3-*C*-ethynyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentofuranose (3).** A THF solution of TBAF (1 M, 5.4 mL, 5.4 mmol) was added to a solution of **2** (1.44 g, 3.6 mmol) in THF (15 mL). The mixture was stirred for 10 min at room temperature, and the solvent was removed *in vacuo*. The residue was coevaporated with anhydrous pyridine (5 mL × 3) and dissolved in anhydrous pyridine (30 mL). BzCl (0.92 mL, 7.9 mmol) was added to the above solution at 0 °C, and the mixture was stirred for 2 h at room temperature. The solvent was removed *in vacuo*, and the residue was coevaporated with toluene (×3). The residue was taken up in EtOAc (50 mL) which was washed with $H_2O$ (25 mL) and aqueous saturated $NaHCO_3$ (25 mL × 3). The separated organic phase

**Table 3.** Antitumor Effects of ECyd, EUrd, and 5-FU on Human Tumor Xenografts in Nude Mice[a]

| | | tumor inhibition ratio (%) | | |
|---|---|---|---|---|
| | | ECyd[b] | EUrd[c] | 5-FU[d] |
| tumor | origin | 0.25 mg/kg × 10 | 2.0 mg/kg × 10 | 15 mg/kg × 14 |
| AZ-521 | stomach | 92 | 81 | 25 |
| H-81 | stomach | 84 | 92 | 83 |
| NUGC-3 | stomach | 80 | 54 | ND |
| CO-3 | colon | 83 | 90 | 49 |
| KM12C | colon | 88 | 81 | 59 |
| DLD-1 | colon | 78 | 73 | 50 |
| JRC-11 | renal | 73 | 75 | 69 |
| H-31 | breast | 76 | 82 | 68 |
| Hucc-T1 | bile duct | 55 | 64 | 1 |
| PAN-12 | pancreas | 75 | 81 | 40 |
| BxPC-3 | pancreas | 59 | 35 | 50 |

[a] Tumor mass (2 $mm^3$) was subcuanteously transplanted into nude mice. The tumor inhibition ratio was evaluated at day 15 and calculated as follows: inhibition ratio (%) = $(A - B)/A \times 100$, where $A$ is the average tumor weight in the control group and $B$ is that in the treated group. Each group consisted of eight nude mice. [b] ECyd was administered intravenously for 10 consecutive days when the tumor reached 60−100 $mm^3$. [c] EUrd was administered intravenously for 10 consecutive days when the tumor reached 60−100 $mm^3$. Some of the data were taken from ref 12. [d] 5-FU was administered intravenously for 14 consecutive days when the tumor reached 60−100 $mm^3$.

was dried ($Na_2SO_4$) and concentrated to dryness. The residue was purified on a silica gel column (4 × 17 cm), which was eluted with 0−10% EtOAc in hexane to give **3** (1.07 g, 93% as a yellowish oil): FAB-MS $m/z$ 319 ($MH^+$), 303 ($M^+ - Me$); $^1$H-NMR ($CDCl_3$) 8.12−7.42 (m, 5 H, Bz), 5.97 (d, 1 H, H-1, $J_{1,2}$ = 3.8 Hz), 4.76 (dd, 1 H, H-5a, $J_{5a,4}$ = 3.8 Hz, $J_{5a,b}$ = 12.0 Hz), 4.61 (dd, 1 H, H-5b, $J_{5b,4}$ = 7.4 Hz, $J_{5b,a}$ = 12.0 Hz), 4.60 (d, 1 H, H-2, $J_{2,1}$ = 3.8 Hz), 4.23 (dd, 1 H, H-4, $J_{4,5a}$ = 3.8 Hz, $J_{4,5b}$ = 7.4 Hz), 3.02 (s, 1 H, 3-OH), 2.63 (s, 1 H, 3-C≡CH), 1.62, 1.41 (s, each 3 H, *i*-Pr). Anal. ($C_{17}H_{18}O_6$) C, H.

**Methyl 2,3,5-Tri-*O*-benzoyl-3-*C*-ethynyl-α,*β*-D-*ribo*-pentofuranoside (5). (A)** A solution containing **3** (637 mg, 2.0 mmol) and camphorsulfonic acid (1.16 g, 5.0 mmol) in MeOH (27 mL) was heated under reflux for 3 days under Ar atmosphere. The solvent was removed, and the residue was taken up in EtOAc (30 mL), which was washed with $H_2O$ (15 mL) and aqueous saturated $NaHCO_3$ (15 mL × 3). The separated organic phase was dried ($Na_2SO_4$) and concentrated to dryness to give a crude **4**, which was coevaporated with anhydrous pyridine (×3) and dissolved into anhydrous pyridine (30 mL). BzCl (2.3 mL, 20 mmol) and DMAP (370 mg, 3.0 mmol) were added to the above mixture at 0 °C, and the whole was heated for 24 h at 100 °C. The solvent was removed *in vacuo* and coevaporated with toluene (×3). The residue was taken into EtOAc (20 mL), which was washed with $H_2O$ (10 mL) and aqueous saturated $NaHCO_3$ (10 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 (4 × 11 cm), which was eluted with 0−10% EtOAc in hexane to give **5** (825 mg, 83% as a yellowish oil): EI-MS $m/z$ 500 ($M^+$); $^1$H-NMR ($CDCl_3$) 8.12−7.30 (m, 15 H, Bz × 3), 6.03 (d, 0.67 H, *β*-H-1, $J_{1,2}$ = 1.2 Hz), 5.82 (d, 0.33 H, α-H-1, $J_{1,2}$ = 4.4 Hz), 5.47 (d, 0.33 H, α-H-2, $J_{2,1}$ = 4.4 Hz), 5.17 (d, 0.67 H, *β*-H-2, $J_{2,1}$ = 1.2 Hz), 5.10−4.78 (m, 3 H, α,*β*-H-4, H-5), 3.53 (s, 2 H, *β*-OMe), 3.45 (s, 1 H, α-OMe), 2.86 (s, 0.67 H, *β*-3-C≡CH), 2.78 (s, 0.33 H, α-3-C≡CH). The ratio of α: *β* anomer was 1:2. Anal. ($C_{29}H_{24}O_8$) C, H.

**(B)** Compound **3** (318 mg, 1 mmol) was treated with aqueous methanolic HCl [prepared from a mixture of AcCl (1.94 mL, 27.3 mmol), $H_2O$ (3.2 mL), and MeOH (8.86 mL)] for 6 h at room temperature. The mixture was neutralized with $Et_3N$ (4.5 mL), and the solvent was removed *in vacuo*. The residue was partitioned between EtOAc (15 mL) and aqueous saturated $NaHCO_3$ (7 mL × 3), and the separated organic phase was dried ($Na_2SO_4$) and concentrated to dryness. The residue was further treated with BzCl (1.16 mL, 10 mmol) and DMAP (184 mg, 1.5 mmol) in pyridine (15 mL) under heating at 100 °C for 24 h. The similar workup and purification gave **5** (435 mg, 87% as a yellowish oil; the ratio of α: *β* anomer was 1:2).

**1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-3-*C*-ethynyl-α,*β*-D-*ribo*-pentofuranose (6).** Concentrated $H_2SO_4$ (0.11 mL) was added to a solution of **5** (264 mg, 0.53 mmol) in a mixture of AcOH (1.75 mL) and $Ac_2O$ (0.22 mL) at 0 °C. The whole was stirred for 4 h at room temperature. The mixture was diluted with $CHCl_3$ (10 mL) and successively washed with $H_2O$ (0.4 mL), aqueous saturated $NaHCO_3$ (1.2 mL × 2), and $H_2O$ (0.4 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 (2.5 × 6 cm), which was eluted with $CHCl_3$ to give **6** (259 mg, 93% as an oil): EI-MS $m/z$ 528 ($M^+$), 485 ($M^+ - Ac$); $^1$H-NMR ($CDCl_3$) 8.17−7.32 (m, 15 H, Bz × 3), 6.77 (d, 0.33 H, α-H-1, $J_{1,2}$ = 4.6 Hz), 6.39 (d, 0.67 H, *β*-H-1, $J_{1,2}$ = 1.5 Hz), 6.19 (d, 0.67 H, *β*-H-2, $J_{2,1}$ = 1.5 Hz), 6.07 (d, 0.33 H, α-H-2, $J_{2,1}$ = 4.6 Hz), 5.07−4.79 (m, 3 H, α,*β*-H-4, H-5), 2.89 (s, 0.67 H, *β*-3-C≡CH), 2.81 (s, 0.33 H, α-3-C≡CH), 2.14 (s, 2 H, *β*-Ac), 2.00 (s, 1 H, α-Ac). The ratio of α: *β* anomer was 1:2. Anal. ($C_{30}H_{24}O_9$) C, H.

**1-(3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl)cytosine (13a).** Stannic chloride (0.29 mL, 2.5 mmol) was added to a solution of bis(TMS)cytosine [prepared from cytosine (222 mg, 2 mmol) and $(NH_4)_2SO_4$ (7 mg) in HMDS (2 mL)] and **6** (264 mg, 0.5 mmol) in $CH_3CN$ (4 mL) at 0 °C. The reaction mixture was stirred for 18 h at room temperature and then diluted with $CHCl_3$ (12 mL) and aqueous saturated $NaHCO_3$ (5 mL) with vigorous stirring for 30 min. The resulting precipitates were removed by filtration through a Celite pad, which was washed with $CHCl_3$. The filtrate was further 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 to dryness *in vacuo*. The residue was purified on a silica gel column (2 × 15 cm), which was eluted with 5% MeOH in $CHCl_3$ to give **7a** (235 mg, 81% as a foam): FAB-MS $m/z$ 580 ($MH^+$); $^1$H-NMR ($CDCl_3$) 8.15−7.31 (m, 15 H, Bz × 3), 7.76 (d, 1 H, H-6, $J_{6,5}$ = 7.5 Hz), 6.60 (d, 1 H, H-1′, $J_{1',2'}$ = 5.2 Hz), 6.06 (d, 1 H, H-2′, $J_{2',1'}$ = 5.2 Hz), 5.72 (d, 1 H, H-5, $J_{5,6}$ = 7.5 Hz), 4.96−4.89 (m, 3 H, H-4′, H-5′), 2.88 (s, 1 H, 3′-C≡CH).

Compound **7a** (200 mg, 0.35 mmol) was treated with methanolic ammonia (saturated at 0 °C, 9 mL) for 2 days at room temperature, and the solvent was removed *in vacuo*. The residue was purified on a silica gel column (2 × 15 cm), which was eluted with 5−20% MeOH in $CHCl_3$ to give **13a** (83 mg, 90% as a white powder, which was crystallized from aqueous MeOH): mp 233−235 °C; EI-MS $m/z$ 267 ($M^+$), 250 ($M^+ - OH$); IR (Nujol) 2115 $cm^{-1}$ (C≡C); UV $\lambda_{max}$ ($H_2O$) 270 nm ($\epsilon$ 9 100), $\lambda_{max}$ (0.5 N HCl) 280 nm ($\epsilon$ 13 400), $\lambda_{max}$ (0.5 N NaOH) 272 nm ($\epsilon$ 9 300); $^1$H-NMR (DMSO-$d_6$) 7.86 (d, 1 H, H-6, $J_{6,5}$ = 7.7 Hz), 7.25, 7.18 (br s, each 1 H, NH), 5.88 (d, 1 H, H-1′, $J_{1',2'}$ = 6.6 Hz), 5.84 (s, 1 H, 3′-OH), 5.79 (d, 1 H, 2′-OH, $J_{2'-OH,2'}$ = 6.6 Hz), 5.78 (d, 1 H, H-5, $J_{5,6}$ = 7.7 Hz), 5.06 (dd, 1 H, 5′-OH, $J_{5'-OH,5'a}$ = 4.4 Hz, $J_{5'-OH,5'b}$ = 5.5 Hz), 4.16 (t, 1 H, H-2′, $J$ = 6.6 Hz), 3.92−3.89 (m, 1 H, H-4′), 3.74−3.71 (m, 2 H, H-5′), 3.55 (s, 1 H, 3′-C≡CH). Anal. ($C_{11}H_{13}N_3O_5$) C, H, N.

**1-(3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl)uracil (14a). (A)** Trimethylsilyl triflate (0.39 mL, 2 mmol) was added to a solution of bis(TMS)uracil [prepared from uracil (225 mg, 2 mmol) and $(NH_4)_2SO_4$ (7 mg) in HMDS (2 mL)] and **6** (264 mg, 0.5 mmol) in $CH_3CN$ (4 mL) at 0 °C. The mixture was stirred for 5 h at room temperature and then diluted with $CHCl_3$ (12

mL), which was further stirred with aqueous saturated $NaHCO_3$ (5 mL) for 30 min. The whole was 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 to dryness *in vacuo*. The residue was purified by a silica gel column (2 × 9 cm), which was eluted with $CHCl_3$ to give **9a** (274 mg, 95% as a foam): FAB-MS *m*/*z* 581 ($MH^+$); $^1$H-NMR ($CDCl_3$) 8.13−7.30 (m, 15 H, Bz × 3), 8.05 (br s, 1 H, NH), 7.71 (d, 1 H, H-6, $J_{6,5}$ = 8.2 Hz), 6.38 (d, 1 H, H-1′, $J_{1',2'}$ = 5.0 Hz), 6.01 (d, 1 H, H-2′, $J_{2',1'}$ = 5.0 Hz), 5.74 (dd, 1 H, H-5, $J_{5,6}$ = 8.2 Hz, $J_{5,NH}$ = 2.0 Hz), 4.98−4.87 (m, 3 H, H-4′, H-5′), 2.92 (s, 1 H, 3′-C≡CH).

Compound **9a** (150 mg, 0.26 mmol) was treated with methanolic ammonia (saturated at 0 °C, 10 mL) for 2 days at room temperature. The solvent was removed *in vacuo*, and the residue was purified on a silica gel column (2 × 10 cm), which was eluted with 5−15% MeOH in $CHCl_3$ to give **14a** (59.4 mg, 85% as a white powder, which was crystallized from MeOH): mp 226−228 °C; EI-MS *m*/*z* 268 ($M^+$); IR (Nujol) 2110 $cm^{-1}$ (C≡C); UV $\lambda_{max}$ ($H_2O$) 261 nm ($\epsilon$ 9 900), $\lambda_{max}$ (0.5 N HCl) 261 nm ($\epsilon$ 10 200), $\lambda_{max}$ (0.5 N NaOH) 262 nm ($\epsilon$ 7 500); $^1$H-NMR (DMSO-$d_6$) 11.35 (br s, 1 H, NH), 7.99 (d, 1 H, H-6, $J_{6,5}$ = 8.2 Hz), 5.93 (s, 1 H, 3′-OH), 5.86 (d, 1 H, 2′-OH, $J_{2'-OH,2'}$ = 6.7 Hz), 5.83 (d, 1 H, H-1′, $J_{1',2'}$ = 7.3 Hz), 5.69 (d, 1 H, H-5, $J_{5,6}$ = 8.2 Hz), 5.13 (t, 1 H, 5′-OH, *J* = 4.5 Hz), 4.18 (dd, 1 H, H-2′, $J_{2',1'}$ = 7.3 Hz, $J_{2',2'-OH}$ = 6.7 Hz), 3.90−3.88 (m, 1 H, H-4′), 3.74−3.60 (m, 2 H, H-5′), 3.55 (s, 1 H, 3′-C≡CH). Anal. ($C_{11}H_{12}N_2O_6$) C, H, N.

**(B)** Stannic chloride (0.23 mL, 2.0 mmol) was added to a solution of bis(TMS)uracil [prepared from uracil (225 mg, 2 mmol) and $(NH_4)_2SO_4$ (7 mg) in HMDS (2 mL)] and **6** (264 mg, 0.5 mmol) in $CH_3CN$ (4 mL) at 0 °C. The mixture was stirred for 2 days at room temperature, and workup was done as described in the method A. The residue was purified on a silica gel column (2 × 9 cm), which was eluted with $CHCl_3$ to give **9a** (167 mg, 58% as a foam). Further elution of the column with 5% MeOH in $CHCl_3$ gave **10** (57 mg, 20% as a foam). Physical data of **10**: FAB-MS *m*/*z* 581 ($MH^+$); $^1$H-NMR ($CDCl_3$) 9.26 (br s, 1 H, NH), 8.14−7.29 (m, 16 H, Bz × 3, H-6), 6.92 (d, 1 H, H-1′, $J_{1',2'}$ = 6.8 Hz), 6.69 (d, 1 H, H-2′, $J_{2',1'}$ = 6.8 Hz), 5.82 (d, 1 H, H-5, $J_{5,6}$ = 7.6 Hz), 5.04−4.88 (m, 3 H, H-4′, H-5′), 2.82 (s, 1 H, 3′-C≡CH).

**3-(3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl)uracil (15).** Compound **10** (55 mg, 0.1 mmol) was treated with methanolic ammonia (saturated at 0 °C, 1.5 mL) for 2 days at room temperature. The solvent was removed *in vacuo*, and the residue was purified on a silica gel column (2 × 6 cm), which was eluted with 5−15% MeOH in $CHCl_3$ to give **15** (17 mg, 67% as a white powder, which was crystallized from aqueous MeOH): mp 255 °C dec; EI-MS *m*/*z* 250 ($M^+$ − OH), 237 ($M^+$ − $CH_2OH$); IR (Nujol) 2230 $cm^{-1}$ (C≡C); UV $\lambda_{max}$ ($H_2O$) 264 nm ($\epsilon$ 7 000), $\lambda_{max}$ (0.5 N HCl) 264 nm ($\epsilon$ 7 200), $\lambda_{max}$ (0.5 N NaOH) 293 nm ($\epsilon$ 9 900); $^1$H-NMR (DMSO-$d_6$) 11.20 (br s, 1 H, NH), 7.47 (d, 1 H, H-6, $J_{6,5}$ = 7.6 Hz), 6.09 (d, 1 H, H-1′, $J_{1',2'}$ = 8.3 Hz), 5.67 (s, 1 H, 3′-OH), 5.62 (d, 1 H, 2′-OH, $J_{2'-OH,2'}$ = 7.0 Hz), 5.60 (d, 1 H, H-5, $J_{5,6}$ = 7.6 Hz), 5.06 (dd, 1 H, H-2′, $J_{2',1'}$ = 8.3 Hz, $J_{2',2'-OH}$ = 7.0 Hz), 4.48−4.46 (m, 1 H, 5′-OH), 3.86−3.84 (m, 1 H, H-4′), 3.68−3.61 (m, 2 H, H-5′), 3.37 (s, 1 H, 3′-C≡CH). Anal. ($C_{11}H_{12}N_2O_6 \cdot {}^1/_2\ H_2O$) C, H, N.

**General Method for the Synthesis of 3′-*C*-Ethynyl Ribonucleosides**. Stannic chloride (3 mmol for $N^6$-benzoyladenine and $N^2$-acetylguanine, 2.5 mmol for 5-fluorocytosine, and 2 mmol for 5-fluorouracil and thymine) was added to a solution of pertrimethylsilylated nucleobases [2 mmol, prepared from the nucleobase (2 mmol) and $(NH_4)_2SO_4$ (7 mg, for the pyrimidine base) or pyridine (2 mL, for the purine base) in HMDS (6 mL)] and **6** (264 mg, 0.5 mmol) in $CH_3CN$ (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 blocked nucleosides, which were then treated with methanolic ammonia (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, which was eluted with 5−20% MeOH in $CHCl_3$ to give the desired 3′-*C*-ethynyl ribonucleosides.

**1-(3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl)-5-fluorocytosine (13b).** Reaction of pertrimethylsilylated 5-fluorocytosine (2 mmol) with **6** for 12 h at room temperature gave **7b** (224 mg, 75% as a foam): FAB-MS *m*/*z* 598 ($MH^+$); $^1$H-NMR ($CDCl_3$) 8.15−7.31 (m, 15 H, Bz × 3), 7.77 (d, 1 H, H-6, $J_{6,5-F}$ = 6.1 Hz), 6.54 (d, 1 H, H-1′, $J_{1',2'}$ = 5.1 Hz), 6.03 (d, 1 H, H-2′, $J_{2',1'}$ = 5.1 Hz), 4.97−4.96 (m, 2 H, H-5′), 4.91−4.89 (m, 1 H, H-4′), 2.90 (s, 1 H, 3′-C≡CH).

From **7b** (189 mg, 0.32 mmol), **13b** (81 mg, 89% as a white powder, which was crystallized from MeOH) was obtained. **13b**: mp 242 °C dec; EI-MS *m*/*z* 285 ($M^+$); IR (Nujol) 2115 $cm^{-1}$ (C≡C); UV $\lambda_{max}$ ($H_2O$) 280 nm ($\epsilon$ 7 800), $\lambda_{max}$ (0.5 N HCl) 290 nm ($\epsilon$ 11 400), $\lambda_{max}$ (0.5 N NaOH) 280 nm ($\epsilon$ 8 400); $^1$H-NMR (DMSO-$d_6$) 8.11 (d, 1 H, H-6, $J_{6,5-F}$ = 7.2 Hz), 7.82, 7.57 (br s, each 1 H, NH), 5.84 (dd, 1 H, H-1′, $J_{1',2'}$ = 6.9 Hz, $J_{1',5-F}$ = 1.9 Hz), 5.81 (s, 1 H, 3′-OH), 5.72 (d, 1 H, 2′-OH, $J_{2'-OH,2'}$ = 6.6 Hz), 5.14 (t, 1 H, 5′-OH, *J* = 4.5 Hz), 4.13 (dd, 1 H, H-2′, $J_{2',1'}$ = 6.9 Hz, $J_{2',2'-OH}$ = 6.6 Hz), 3.88−3.87 (m, 1 H, H-4′), 3.75−3.60 (m, 2 H, H-5′), 3.53 (s, 1 H, 3′-C≡CH). Anal. ($C_{11}H_{12}FN_3O_5$) C, H, F, N.

**1-(3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl)-5-fluorouracil (14b).** Reaction of pertrimethylsilylated 5-fluorouracil (2 mmol) with **6** for 6.5 h at room temperature gave **9b** (283 mg, 95% as a foam): FAB-MS *m*/*z* 599 ($MH^+$); $^1$H-NMR ($CDCl_3$) 8.16 (br s, 1 H, NH), 8.14−7.30 (m, 15 H, Bz × 3), 7.83 (d, 1 H, H-6, $J_{6,5-F}$ = 5.8 Hz), 6.35 (dd, 1 H, H-1′, $J_{1',2'}$ = 4.9 Hz, $J_{1',5-F}$ = 1.7 Hz), 5.79 (d, 1 H, H-2′, $J_{2',1'}$ = 4.9 Hz), 4.96−4.95 (m, 2 H, H-5′), 4.89−4.87 (m, 1 H, H-4′), 2.95 (s, 1 H, 3′-C≡CH).

From **9b** (276 mg, 0.46 mmol), **14b** (121 mg, 92% as a white powder, which was crystallized from MeOH) was obtained. **14b**: mp >210 °C dec; EI-MS *m*/*z* 286 ($M^+$), 251 ($M^+$ − OH); IR (Nujol) 2120 $cm^{-1}$ (C≡C); UV $\lambda_{max}$ ($H_2O$) 268 nm ($\epsilon$ 7 700), $\lambda_{max}$ (0.5 N HCl) 269 nm ($\epsilon$ 8 400), $\lambda_{max}$ (0.5 N NaOH) 269 nm ($\epsilon$ 6 900); $^1$H-NMR (DMSO-$d_6$) 11.87 (br s, 1 H, NH), 8.33 (d, 1 H, H-6, $J_{6,5-F}$ = 7.2 Hz), 5.93 (s, 1 H, 3′-OH), 5.83 (dd, 1 H, H-1′, $J_{1',2'}$ = 7.2 Hz, $J_{1',5-F}$ = 1.8 Hz), 5.82 (d, 1 H, 2′-OH, $J_{2'-OH,2'}$ = 6.4 Hz), 5.27 (t, 1 H, 5′-OH, *J* = 4.1 Hz), 4.18 (dd, 1 H, H-2′, $J_{2',1'}$ = 7.2 Hz, $J_{2',2'-OH}$ = 6.4 Hz), 3.92−3.91 (m, 1 H, H-4′), 3.77−3.64 (m, 2 H, H-5′), 3.56 (s, 1 H, 3′-C≡CH). Anal. ($C_{11}H_{11}FN_2O_6 \cdot {}^1/_3$ MeOH), C, H, F, N.

**1-(3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl)thymine (14c).** Reaction of pertrimethylsilylated thymine (2.0 mmol) with **6** for 27 h at room temperature gave **9c** (290 mg, 98% as a foam): FAB-MS *m*/*z* 595 ($MH^+$); $^1$H-NMR ($CDCl_3$) 8.17−7.34 (m, 17 H, Bz × 3, H-6, NH), 6.44 (d, 1 H, H-1′, $J_{1',2'}$ = 5.9 Hz), 6.06 (d, 1 H, H-2′, $J_{2',1'}$ = 5.9 Hz), 4.98−4.95 (m, 1 H, H-4′), 4.93−4.90 (m, 2 H, H-5′), 2.91 (s, 1 H, 3′-C≡CH), 1.72 (s, 3 H, 5-Me).

From **9c** (290 mg, 0.49 mmol), **14c** (115 mg, 83% as a white powder, which was crystallized from MeOH) was obtained. **14c**: mp 113−118 °C; EI-MS *m*/*z* 282 ($M^+$); IR (Nujol) 2115 $cm^{-1}$ (C≡C); UV: $\lambda_{max}$ ($H_2O$) 266 nm ($\epsilon$ 8 800), $\lambda_{max}$ (0.5 N HCl) 265 nm ($\epsilon$ 8 600), $\lambda_{max}$ (0.5 N NaOH) 267 nm ($\epsilon$ 7 000); $^1$H-NMR (DMSO-$d_6$) 11.30 (br s, 1 H, NH), 7.85 (d, 1 H, H-6, $J_{6,5-Me}$ = 1.0 Hz), 5.90 (s, 1 H, 3′-OH), 5.83 (d, 1 H, H-1′, $J_{1',2'}$ = 7.5 Hz), 5.81 (d, 1 H, 2′-OH, $J_{2'-OH,2'}$ = 6.7 Hz), 5.13 (t, 1 H, 5′-OH, *J* = 4.4 Hz), 4.19 (dd, 1 H, H-2′, $J_{2',1'}$ = 7.5 Hz, $J_{2',2'-OH}$ = 6.7 Hz), 3.88−3.86 (m, 1 H, H-4′), 3.74−3.65 (m, 2 H, H-5′), 3.56 (s, 1 H, 3′-C≡CH), 1.73 (d, 3 H, 5-Me, $J_{5-Me,6}$ = 1.0 Hz). Anal. ($C_{12}H_{14}N_2O_6 \cdot$MeOH) C, H, N.

**9-(3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl)adenine (16).** Reaction of pertrimethylsilylated $N^6$-benzoyladenine (2.0 mmol) with **6** for 7 h at room temperature gave **11** (261 mg, 74% as a foam): FAB-MS *m*/*z* 708 ($MH^+$); $^1$H-NMR ($CDCl_3$) 8.99 (br s, 1 H, NHBz), 8.75 (s, 1 H, H-8), 8.49 (s, 1 H, H-2), 8.16−7.31 (m, 15 H, Bz × 3), 6.58 (d, 1 H, H-1′, $J_{1',2'}$ = 4.8 Hz), 6.56 (d, 1 H, H-2′, $J_{2',1'}$ = 4.8 Hz), 5.07−5.03 (m, 2 H, H-5′), 4.98−4.94 (m, 1 H, H-4′), 2.95 (s, 1 H, 3′-C≡CH).

From **11** (234 mg, 0.33 mmol), **16** (80 mg, 83% as a white powder, which was crystallized from $H_2O$) was obtained. **16**: mp 149−152 °C; EI-MS *m*/*z* 291 ($M^+$), 274 ($M^+$ − OH); IR

(Nujol) 2110 $cm^{-1}$ (C≡C); UV $\lambda_{max}$ ($H_2O$) 256 nm ($\epsilon$ 12 700), $\lambda_{max}$ (0.5 N HCl) 257 nm ($\epsilon$ 12 500), $\lambda_{max}$ (0.5 N NaOH) 260 nm ($\epsilon$ 13 000); $^1$H-NMR (DMSO-$d_6$) 8.35 (s, 1 H, H-8), 8.14 (s, 1 H, H-2), 7.36 (br s, 2 H, $NH_2$), 5.99 (s, 1 H, 3′-OH), 5.88 (d, 1 H, 2′-OH, $J_{2'-OH,2'}$ = 7.2 Hz), 5.85 (d, 1 H, H-1′, $J_{1',2'}$ = 7.8 Hz), 5.60 (dd, 1 H, 5′-OH, $J_{5'-OH,5'a}$ = 7.4 Hz, $J_{5'-OH,5'b}$ = 4.0 Hz), 4.81 (dd, 1 H, H-2′, $J_{2',1'}$ = 7.8 Hz, $J_{2',2'-OH}$ = 7.2 Hz), 4.01−3.80 (m, 1 H, H-4′), 3.79−3.69 (m, 2 H, H-5′), 3.54 (s, 1 H, 3′-C≡CH). Anal. ($C_{12}H_{13}N_5O_4 \cdot {}^2/_3\ H_2O$) C, H, N.

**9-(3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl)guanine (17).** Reaction of pertrimethylsilylated $N^2$-acetylguanine (2.0 mmol) with **6** for 2 h at room temperature gave **12** (327 mg, 99% as a foam): FAB-MS *m*/*z* 662 ($MH^+$); $^1$H-NMR ($CDCl_3$) 12.26 (br s, 1 H, 1-NH), 10.36 (br s, 1 H, 2-NHAc), 8.38 (s, 1 H, H-8), 8.15−7.28 (m, 15 H, Bz × 3), 6.85 (d, 1 H, H-1′, $J_{1',2'}$ = 3.9 Hz), 6.32 (d, 1 H, H-2′, $J_{2',1'}$ = 3.9 Hz), 5.09−5.05 (m, 1 H, H-4′), 5.04−4.86 (m, 2 H, H-5′), 2.97 (s, 1 H, 3′-C≡CH), 2.36 (s, 3 H, 2-NHAc).

Compound **12** (320 mg, 0.49 mmol) was treated with NaOMe (0.25 M, 0.9 mL) in MeOH (30 mL) for 2 h at room temperature. The mixture was neutralized with Amberlite IRC-50S ($H^+$), and the resin was filtered off. The filtrate was concentrated, and the residue was dissolved in $H_2O$ (150 mL), which was extracted with $CHCl_3$ (100 mL × 2). The separated aqueous phase was concentrated to dryness *in vacuo*, and the residue was crystallized from $H_2O$ to give **17** (95 mg, 63%): mp 225 °C dec; EI-MS *m*/*z* 307 ($M^+$); UV $\lambda_{max}$ ($H_2O$) 253 nm ($\epsilon$ 13 000), $\lambda_{max}$ (0.5 N HCl) 252 nm ($\epsilon$ 12 100), $\lambda_{max}$ (0.5 N NaOH) 263 nm ($\epsilon$ 11 000); $^1$H-NMR (DMSO-$d_6$) 8.35 (s, 1 H, H-8), 8.14 (s, 1 H, H-2), 7.36 (br s, 2 H, $NH_2$), 5.99 (s, 1 H, 3′-OH), 5.88 (d, 1 H, 2′-OH, $J_{2'-OH,2'}$ = 7.2 Hz), 5.85 (d, 1 H, H-1′, $J_{1',2'}$ = 7.8 Hz), 5.60 (dd, 1 H, 5′-OH, $J_{5'-OH,5'a}$ = 7.4 Hz, $J_{5'-OH,5'b}$ = 4.0 Hz), 4.81 (dd, 1 H, H-2′, $J_{2',1'}$ = 7.8 Hz, $J_{2',2'-OH}$ = 7.2 Hz), 4.01−3.80 (m, 1 H, H-4′), 3.79−3.69 (m, 2 H, H-5′), 3.54 (s, 1 H, 3′-C≡CH). Anal. ($C_{12}H_{13}N_5O_5$) C, H, N.

**Acknowledgment.** This investigation was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Education, Science, Sports, and Culture of Japan and the 2nd Term Comprehensive Ten-Year Strategy for Cancer Control from the Ministry of Health and Welfare of Japan.

## References

(1) For part 157, see: Shuto, S.; Obara, T.; Yaginuma, S.; Matsuda, A. New Neplanocin Analogues. IX. A practical preparation of (6′*R*)-6′-*C*-methylneplanocin A (RMNPA), a potent antiviral agent, by the diastereoselective enzymatic deamination as a key-step, and the determination of Its 6′-configuration. *Chem. Pharm. Bull.*, in press.

(2) For a recent review, see: *Cancer Chemotherapeutic Agents*; Foye, W. D., Ed.; American Chemical Society: Washington, DC, 1995; Chapter 3, pp 47−109.

(3) (a) Takenuki, K.; Matsuda, A.; Ueda, T.; Sasaki, T.; Fujii, A.; Yamagami, K. Design, synthesis, and antineoplastic activity of 2′-deoxy-2′-methylidenecytidine. *J. Med. Chem.* **1988**, *31*, 1063−1064. (b) Matsuda, A.; Takenuki, K.; Sasaki, T.; Ueda, T. Synthesis of a new broad spectrum of antineoplastic nucleoside, 2′-deoxy-2′-methylidenecytidine (DMDC) and its derivatives. *J. Med. Chem.* **1991**, *34*, 812−819. (c) Yamagami, K.; Fujii, A.; Arita, M.; Okumoto, T.; Sakata, S.; Matsuda, A.; Ueda, T.; Sasaki, T. Antitumor activity of 2′-deoxy-2′-methylidenecytidine. A new 2′-deoxycytidine derivative. *Cancer Res.* **1991**, *51*, 2319−2323. (d) Lin, T.-S.; Luo, M. Z.; Liu, M. C.; Clarkatzenburg, R. H.; Cheng, Y. C.; Prusoff, W. H.; Mancini, W. R.; Birnbaum, G. I.; Gabe, E.; Giziewicz, J. Synthesis and anticancer and antiviral activities of various 2′- and 3′-methylidene-substituted nucleoside analogues and crystal structure of 2′-deoxy-2′-methylidenecytidine hydrochloride. *J. Med. Chem.* **1991**, *34*, 2607−2615. (e) Baker, C. H.; Banzon, J.; Bollinger, J. M.; Stubbe, J.; Samano, V.; Robins, M. J. 2′-Deoxy-2′-methylenecytidine and 2′-deoxy-2′,2′-difluorocytidine 5′-diphosphates, potent mechanism-based inhibitors of ribonucleotide reductase. *J. Med. Chem.* **1991**, *34*, 1879−1884. (f) Cory, A. H.; Samano, V.; Robins, M. J.; Cory, J. G. 2′-Deoxy-2′-methylene derivatives of adenosine, guanosine, tubercidin, cytidine and uridine as inhibitors of L1210 cell growth in culture. *Biochem. Pharmacol.* **1994**, *47*, 365−371. (g) Ono, T.; Fujii, A.; Yamagami, K.; Hosoya, M.; Okumoto, T.; Sakata, S.; Matsuda, A.; Sasaki, T. Cell kill kinetics of an antineoplastic nucleoside, 1-(2-deoxy-2-methylene-*β*-D-*erythro*-pentofuranosyl)cytosine. *Biochem. Pharmacol.* **1996**, *52*, 1279−1285.

(4) Plunkett, W.; Huang, P.; Xu, Y.-Z.; Heinemann, V.; Grunewald, R.; Gandhi, V. Gemcitabine: Metabolism, mechanisms of action, and self-potentiation. *Semin. Oncol.* **1995**, *22* (Suppl. 11), 3−10 and references cited therein.

(5) (a) Matsuda, A.; Nakajima, Y.; Azuma, A.; Tanaka, M.; Sasaki, T. 2′-*C*-Cyano-2′-deoxy-1-*β*-D-arabinofuranosylcytosine (CNDAC): Design of a potential mechanism-based DNA-strand breaking antineoplastic nucleoside. *J. Med. Chem.* **1991**, *34*, 2917−2919. (b) Azuma, A.; Nakajima, Y.; Nishizono, N.; Minakawa, N.; Suzuki, M.; Hanaoka, K.; Kobayashi, T.; Tanaka, M.; Sasaki, T.; Matsuda, A. 2′-*C*-Cyano-2′-deoxy-1-*β*-D-arabinofuranosylcytosine and its derivatives: A new class of nucleoside with a broad antitumor spectrum. *J. Med. Chem.* **1993**, *36*, 4183−4189. (c) Tanaka, M.; Matsuda, A.; Terao, T.; Sasaki, T. Antitumor activity of a novel nucleoside, 2′-*C*-cyano-2′-deoxy-1-*β*-D-arabinofuranosylcytosine (CNDAC) against murine and human tumors. *Cancer Lett.* **1992**, *64*, 67−74. (d) Azuma, A.; Hanaoka, K.; Kurihara, A.; Kobayashi, T.; Miyauchi, S.; Kamo, N.; Tanaka, M.; Sasaki, T.; Matsuda, A. Chemical stability of a new antitumor nucleoside, 2′-C-cyano-2′-deoxy-1-*β*-D-*arabino*-pentofuranosylcytosine (CNDAC) in alkaline medium: Formation of 2′-C-cyano-2′-deoxy-1-*β*-D-*ribo*-pentofuranosylcytosine (CNDC) and its antitumor activity. *J. Med. Chem.* **1995**, *38*, 3391−3397. (e) Matsuda, A.; Azuma, A. 2′-*C*-Cyano-2′-deoxy-1-*β*-D-arabinofuranosylcytosine (CNDAC): A mechanism-based DNA-strand-breaking antitumor nucleoside. *Nucleosides Nucleotides* **1995**, *14*, 461−471.

(6) (a) Thelander, L.; Reichard, P. Reduction of ribonucleotides. *Ann. Rev. Biol.* **1979**, *48*, 133−158. (b) *Inhibitors of Ribonucleoside Diphosphate Reductase Activity*; Cory, J. G., Cory, A. H., Eds.; Pergamon Press: New York, 1989.

(7) Ling, J.; Sahlin, M.; Sjoberg, B.; Loehr, T. M.; Sanders-Loehr, J. Dioxygen is the source of the *μ*-oxo bridge in iron ribonucleotide reductase. *J. Biol. Chem.* **1994**, *269*, 5595−5601.

(8) Stubbe, J.; Ackles, D. On the mechanism of ribonucleoside diphosphate reductase from Escherichia coli. *J. Biol. Chem.* **1980**, *255*, 8027−8030.

(9) Mao, S. S.; Holler, T. P.; Bollinger, J. M., Jr.; Yu, G. X.; Johnston, M. I.; Stubbe, J. A model for the role of multiple cysteine involved in ribonucleotide reduction: Amazing and still confuzing. *Biochemistry* **1992**, *31*, 9733−9743.

(10) Uhlin, U.; Eklund, H. Structure of ribonucleotide reductase protein R1. *Nature* **1994**, *370*, 533−539.

(11) (a) Broka, C. A.; Reichert, D. E. Thiophenol promoted cyclization of enynes. *Tetrahedron Lett.* **1987**, *28*, 1503−1506. (b) Oswald, A. A.; Griesbaum, K.; Hundson, B. E. Organic sulfur compounds. XIII. Free-radical addition of thiols to phenylacetylene. *J. Am. Chem. Soc.* **1964**, *86*, 2877−2884.

(12) Matsuda, A.; Hattori, H.; Tanaka, M.; Sasaki, T. 1-(3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl)uracil as a broad spectrum antitumor nucleoside. *BioMed. Chem. Lett.* **1996**, *6*, 1887−1892.

(13) Yoshimura, Y.; Sano, T.; Matsuda, A.; Ueda, T. Synthesis of 6,3′-methanocytidine, 6,3′-methanouridine, and their 2′-deoxyribonucleosides. *Chem. Pharm. Bull.* **1988**, *36*, 162−167.

(14) Bevierre, M.-O.; De Mesmaeker, A.; Wolf, R. M.; Freier, S. M. Synthesis of 2′-*O*-methyl-6,3′-ethanouridine and its introduction into antisense oligonucleotides. *BioMed. Chem. Lett.* **1994**, *4*, 237−240.

(15) Nutt, R. F.; Dickinson, M. J.; Holly, F. W.; Walton, E. Branched-chain sugar nucleosides. III. 3′-C-Methyladenosine. *J. Org. Chem.* **1970**, *33*, 1789−1795.

(16) Niedballa, U.; Vorbruggen, H. A general synthesis of N-glycosides. I. Synthesis of pyrimidine nucleosides. *J. Org. Chem.* **1974**, *39*, 3654−3660.

(17) (a) Vorbruggen, H.; Krolikiewicz, K.; Bennua, B. Nucleoside synthesis with trimethylsilyl triflate and perchlorate as catalysts. *Chem. Ber.* **1981**, *114*, 1234−1255. (b) Vorbruggen, H.; Hofle, G. On the nucleoside synthesis. *Chem. Ber.* **1981**, *114*, 1256−1268.

(18) Jung, P. M. J.; Burger, A.; Biellmann, J.-F. Rapid and efficient stereocontrolled synthesis of *C*-3′-ethynyl ribo and xylonucleosides by organocerium additions to 3′-ketonucleosides. *Tetrahedron Lett.* **1995**, *36*, 1031−1034 and references cited therein.

(19) Paull, K. D.; Hamel, E.; Malspeis, L. Prediction of Biochemical Mechanism of Action from the In Vitro Antitumor Screen of the National Cancer Institute. In *Cancer Chemotherapeutic Agents*; Foye, W. O., Ed.; American Chemical Society: Washington, DC, 1995; pp 9−45.

(20) Some of tha data were taken from ref 12.

(21) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. Evaluation of a tetrazolium-based semiautomated colorimetric assay. Assessment of chemosensitivity testing. *Cancer Res.* **1987**, *47*, 936−942.

JM960537G