Synthesis and Cytotoxicity of 4-Amino-5-oxopyrido[2,3-*d*]pyrimidine Nucleosides

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A number of nucleoside analogues have been either used clinically as anticancer drugs or evaluated in clinical studies, while new nucleoside analogues continue to show promise. In this article, we report synthesis and cytotoxicity of a series of new pyrido[2,3-*d*]pyrimidine nucleosides. 2-Amino-3-cyano-4-methoxypyridine was converted, in two steps, to 4-amino-5-oxopyrido[2,3-*d*]pyrimidine. A variety of 1-*O*-acetylated pentose sugar derivatives were condensed with silylated 4-amino-5-oxopyrido[2,3-*d*]pyrimidine, followed by protection, to afford a series of 4-amino-5-oxopyrido[2,3-*d*]pyrimidine nucleosides. Further derivatizations provided an additional group of pyrido[2,3-*d*]pyrimidine nucleosides. These nucleosides were evaluated for in vitro cytotoxicity to human prostate cancer (HTB-81) and mouse melanoma (B16) cells as well as normal human fibroblasts (NHF). A number of compounds (**1a**,**b**, **2a**−**c**,**f**, **3f**+**4d**) showed significant cytotoxicity to cancer cells, with 4-amino-5-oxo-8-(β-D-ribofuranosyl)pyrido-[2,3-*d*]pyrimidine (**1b**) being the most potent proliferation inhibitor (EC_{50}: 0.06−0.08 μM) to all types of cells tested. However, a selective inhibition to the cancer cells was observed for 4-amino-5-oxo-8-(β-D-xylofuranosyl)pyrido[2,3-*d*]pyrimidine (**2b**), which is a potent inhibitor of HTB-81 (EC_{50}: 0.73 μM) and has a favorable in vitro selectivity index (28).

Introduction

Nucleoside analogues have been used clinically as cancer chemotherapy for years, and some new analogues are being evaluated in clinical studies.[1,2] However, it is quite common that nucleoside anticancer drugs are associated with various adverse effects.[1] Therefore, there is a need to search for new nucleoside analogues that can selectively inhibit cancer cell proliferation. In the past three decades, tremendous efforts have been directed to the search of nucleoside anticancer and antiviral drugs. Besides other nucleoside analogues, a large number of sangivamycin and toyocamycin analogues have been synthesized and evaluated for anticancer activities since these two compounds were isolated four decades ago.[3−8] Among many interesting sangivamycin and toyocamycin analogues are 4-amino-5-oxo-8-(β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine-6-carboxamide[9] and 4-amino-7-oxo-8-(β-D-ribofuranosyl)-pyrido[2,3-*d*]pyrimidine-5-carboxamide.[10] These two pyrido[2,3-*d*]pyrimidine nucleosides have a 4-aminopyrimidine ring which is identical to that in adenosine and a fused pyridone ring which replaces the fused imidazole ring in adenosine. Because of their structural similarity to adenosine, the novel nucleoside analogues in this class might be substrates or inhibitors in the metabolic and signal transduction pathways in which adenosine is involved.

To explore the possibility of pyrido[2,3-*d*]pyrimidine nucleosides as potential anticancer and antiviral drugs, 4-amino-5-oxopyrido[2,3-*d*]pyrimidine riboside (**1b**) was synthesized at ICN Pharmaceuticals, Inc. and turned out to be a very potent inhibitor of cancer cell proliferation.[11] However, compound **1b** showed a similar in vitro cytotoxicity profile to normal human cells, which indicated that the compound lacks selectivity between cancer and normal cells. The cytotoxicity of ribonucleoside analogues most likely results from nonselective inhibition of RNA polymerases by the triphosphates of ribonucleoside analogues, or from their nonselective incorporation into RNAs and subsequent inactivation of functional RNAs.[12,13] Since nucleoside antiviral drugs such as AZT and D4T achieve selectivity through sugar modifications, it may be worth while exploring the possibility to find selective anticancer drugs through sugar modifications. In fact, sugar modifications have proved to be useful for reducing toxicity of neplanocin A, an antibiotic nucleoside, by introducing substituents at the C-6′ position of the nucleoside[14] and for selective inhibition of tumor growth by 3′-*C*-ethynylcytidine.[13]

Recently, we synthesized and evaluated a series of pyridopyrimidine nucleosides in search of selective inhibitors of cancer cells. In this article we report the synthesis and cytotoxicity of 4-amino-5-oxopyrido[2,3-*d*]pyrimidine nucleosides having a variety of modified sugar moieties as shown in Chart 1.

Chemistry

Schemes 1−3 show the synthesis of modified sugar derivatives. The reaction of the keto sugar **7**[15] with methylmagnesium bromide, followed by protection of the 3-hydroxyl group with benzoyl, gave the 3-β-*C*-methyl derivative **8** by a similar procedure as published.[16] Treatment of **8** with TFA/water (9:1) gave **10**, which was subjected to acetylation in pyridine. Unexpectedly, the benzoyl group at the O-3 migrated to the

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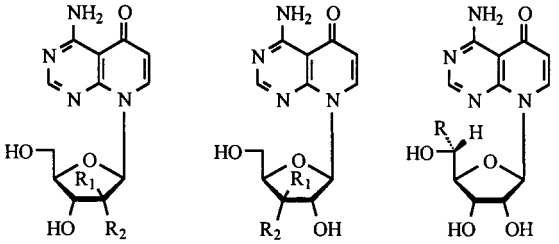
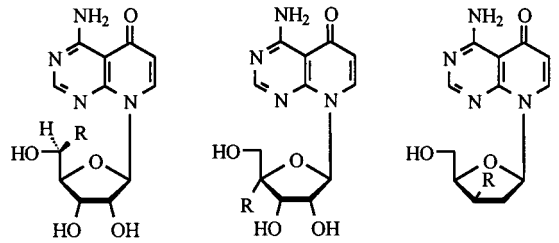
Chart 1



1a $R_1 = R_2 = H$
1b $R_1 = H$, $R_2 = OH$
1c $R_1 = OH$, $R_2 = H$
1d $R_1 = Me$, $R_2 = OH$
1e $R_1 =$ ethynyl, $R_2 = OH$
1f $R_1 + R_2 = =CH_2$

2a $R_1 = R_2 = H$
2b $R_1 = OH$, $R_2 = H$
2c $R_1 = Me$, $R_2 = OH$
2d $R_1 = OH$, $R_2 =$ ethynyl
2e $R_1 + R_2 = =CH_2$
2f $R_1 =$ ethynyl, $R_2 = OH$

3a R = methyl
3b R = ethyl
3c R = propyl
3d R = allyl
3e R = vinyl
3f R = ethynyl

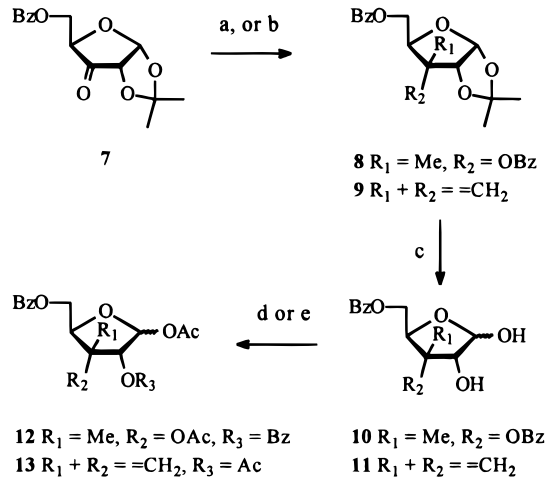


4a R = methyl
4b R = ethyl
4c R = vinyl
4d R = ethynyl

5a R = methyl
5b R = ethyl
5c R = vinyl
5d R = CH_2OH

6a R = H
6b R = OH

Scheme 1[a]



7

8 $R_1 = Me$, $R_2 = OBz$
9 $R_1 + R_2 = =CH_2$

12 $R_1 = Me$, $R_2 = OAc$, $R_3 = Bz$
13 $R_1 + R_2 = =CH_2$, $R_3 = Ac$

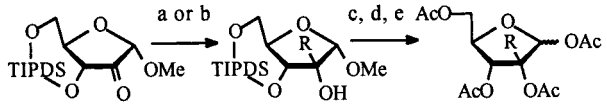
10 $R_1 = Me$, $R_2 = OBz$
11 $R_1 + R_2 = =CH_2$

[a] (a) 1. MeMgBr, ether, 0 °C, 7 h, 2. BzCl, pyridine, 75 °C, 3 days, 42% (2 steps); (b) $Ph_3P=CH_2$, ether, rt, 24 h, 47%; (c) TFA/H_2O, 0 °C, 2 h; (d) 1. Ac_2O, pyridine, rt, overnight, 2. Ac_2O, DMAP, Et_3N, CH_2Cl_2, rt, 3 h, 42% (3 steps); (e) Ac_2O, pyridine, rt, 64% (2 steps).

O-2 position to yield 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-*C*-methyl-D-ribofuranose as the major product, which was further acetylated in dichloromethane in the presence of DMAP and triethylamine to give **12**. The Wittig reaction[17] of **7** with methyltriphenylphosphorane gave the 3′-*C*-methylene derivative **9**, which was subjected to the similar treatment as **8** to give **13**.

By a similar procedure as used for preparation of **8**, the 2-*C*-methyl derivative **15**[18] was obtained from the reaction of the keto sugar **14**[17] with methylmagnesium bromide. Similarly, the reaction of **14** with ethynylmagnesium bromide gave the 2-*C*-ethynyl derivative **16**. Both Grignard reactions gave exclusively the 2-*β*-*C*-substituted products without formation of the α-isomers, which is consistent with the published results from similar Grignard reactions.[18] Compounds **15** and **16** were separately treated with TBAF, followed by two successive acetylations, to give **17** and **18**, respectively.

Scheme 2[a]



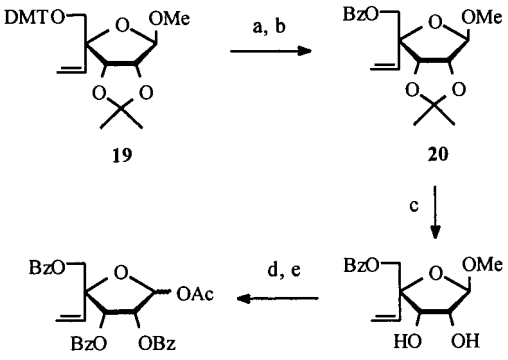
14

15 R = methyl
16 R = ethynyl

17 R = methyl
18 R = ethynyl

[a] (a) MeMgBr, THF, rt, 2 h, 53%; (b) HC≡CMgBr, THF, rt, 3 h, 51%; (c) TBAF, THF, rt, 2 h; (d) Ac_2O, DMAP, Et_3N, CH_2Cl_2, rt, 24 h; (e) Ac_2O, AcOH, H_2SO_4, rt, 5 h, 62% (3 steps) for **17**, 79% (3 steps) for **18**.

Scheme 3[a]



19

20

22

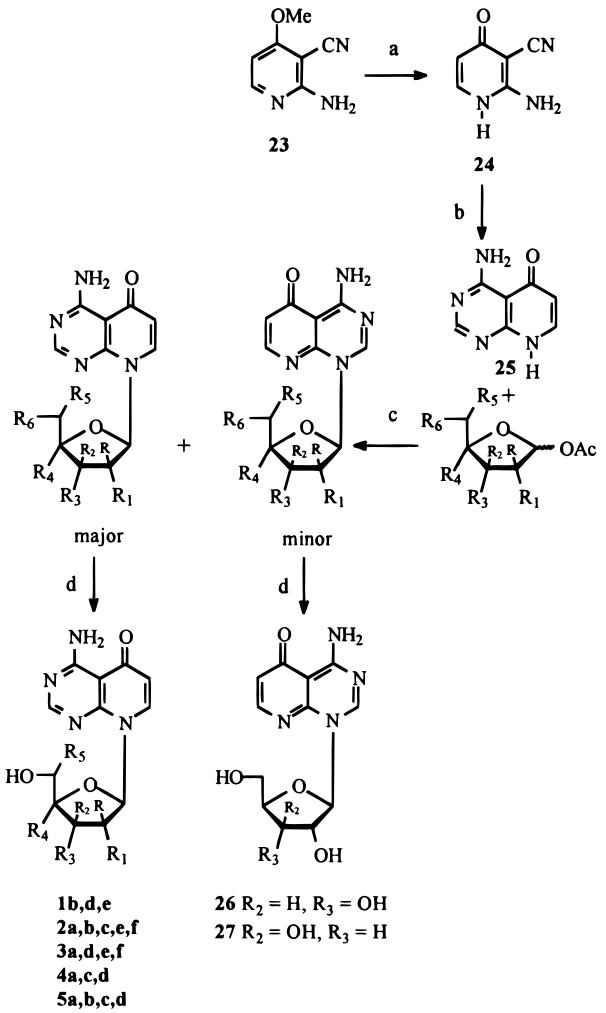
21

[a] (a) 80% AcOH, rt, 2.5 h; (b) BzCl, pyridine, rt, 18 h; (c) TFA/H_2O, 0 °C, 2 h; (d) same as step b; (e) Ac_2O, AcOH, H_2SO_4, rt, 6 h, 67% (5 steps).

The 4-*C*-vinyltetra-*O*-acyl derivative **22** was obtained in good yield from **19**[19] by a sequence of reactions: removal of DMT, benzoylation, removal of isopropylidene with TFA at low temperature, benzoylation again, and acetylation.

Most of the pyrido[2,3-*d*]pyrimidine nucleosides reported in this article were synthesized via the Vorbrüggen condensation of the 1-*O*-acetyl pentose sugars with silylated 4-amino-5-oxopyrido[2,3-*d*]pyrimidine (Scheme 4). The substituted pyridine **23**[20] was treated with trimethylsilyl iodide to remove the methyl group to give **24**, which was refluxed with formamidine acetate in ethoxyethanol to effect a cyclization. 4-Amino-5-oxopyrido[2,3-*d*]pyrimidine (**25**) was obtained in very good yield. It was noted that methyl iodide liberated during the formation of **24** needed to be removed from the reaction mixture by a slow argon flow to avoid the formation of undesired 2-amino-3-cyano-N^1-methyl-4-pyridone (not shown). According to a published procedure of Vorbrüggen reaction,[21] **25** was treated with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) and the resulting silylated form of **25** was condensed, in a one-pot reaction, with the 1-*O*-acetylated sugars in the presence of trimethylsilyl triflate. These condensations always gave a mixture of two products having the 1′-*β*-configuration which resulted from coupling at the N-1 position (minor) and the N-8 position (major) of **25**. The reaction course

Scheme 4[a]

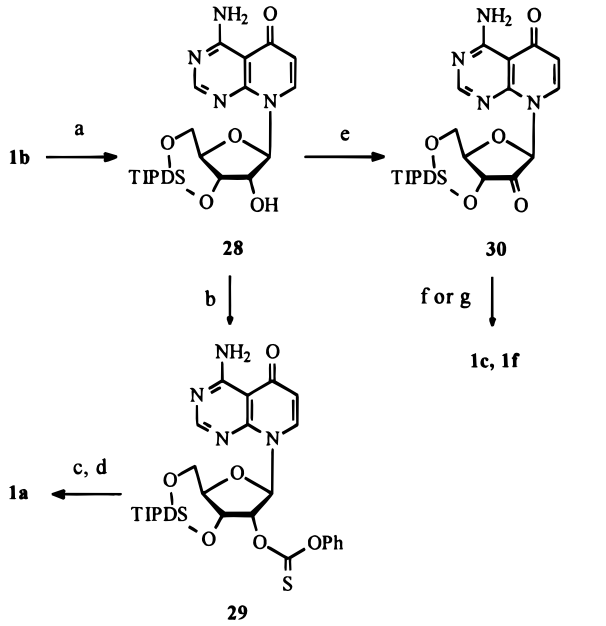


[a] (a) Me_3SiI, CH_3CN, reflux, 20 h, 80%; (b) $HC(=NH)NH_2 \cdot AcOH$, 2-ethoxyethanol, reflux, 30 h, 89%; (c) BSA, $ClCH_2CH_2Cl$, TMSOTf, reflux, 3 days; (d) NH_3/MeOH, rt, 20 h.

was clearly shown by TLC that the N-1 coupled products were formed first and then slowly converted to the N-8 coupled products during heating. However, an equilibrium was reached after 2−3 days, and an extended heating did not lead to an increase in the amount of the N-8 coupled products. The N-8 coupled products were usually obtained in 40−60% yields and the N-1 coupled products in 20−30% yields. The coupling products from **25** and a variety of sugar derivatives were treated with ammonia in methanol to give the 4-amino-5-oxopyrido[2,3-*d*]pyrimidine nucleosides **1b,d,e**, **2a**−**c,e,f**, **3a,d**−**f**, **4a,c,d**, and **5a**−**d**, respectively. The L-isomer (**1b**-L, not shown) of **1b** was prepared by the same procedure as **1b** from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-L-ribofuranose. Compounds **26** and **27** are shown here as representatives of the group of the N-1 coupled nucleosides.

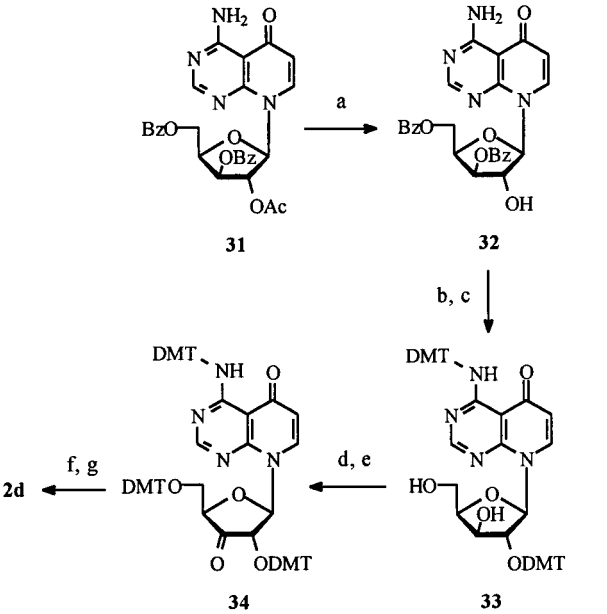
Further derivatizations yielded **1a**,**c**,**f**, and **2d** (Schemes 5 and 6). Compound **1b** was converted to the 3′,5′-tetraisopropyldisiloxy (TIPDS) derivative **28**, which was then converted to the 2′-phenoxythiocarbonyl derivative **29**. A Barton type deoxygenation[22,23]of **29**, followed by removal of the TIPDS group with TBAF, afforded **1a** in good yield. By a similar procedure as described for the preparation of arabinoadenosine from

Scheme 5[a]



[a] (a) TIPDS-Cl, pyridine, rt, 20 h, 92%; (b) PhOC(=S)Cl, DMAP, CH_3CN, rt, 2 h; (c) SiH, ACCN, 105 °C, 15 h; (d) TBAF, THF, rt, 2 h, 71% (3 steps); (e) DMSO, DCC, TFA, pyridine, rt, 18 h, 84%; (f) 1. $NaB(OAc)_3H$, THF, 0 °C, 20 h, 60%, 2. NH_4F, MeOH, 45 °C, 28 h, 25% (2 steps); (g) 1. $Ph_3P{=}CH_2$, ether, 0 °C, 4 h, 2. same as part 2 in step f, 42% (2 steps).

Scheme 6[a]



[a] (a) NH_2NH_2, pyridine, AcOH, rt, 20 h; (b) DMT-Cl, pyridine, 60 °C, 4 days; (c) NH_3/MeOH; (d) DMT-Cl, pyridine, 0 °C, 2 h; (e) DMSO, DCC, TFA, pyridine, rt, 18% (5 steps); (f) HC≡CMgBr, ether, 0 °C, 8 h; (g) 80% AcOH, 49% (2 steps).

adenosine,[24] **28** was oxidized to the 2′-keto derivative **30**, which was reacted with sodium (triacetoxy)borohydride, followed by removal of TIPDS, to give the arabinoside **1c**. The 2′-*C*-methylene derivative **1f** was prepared from **30** via a Wittig reaction and a subsequent deprotection of TIPDS by a similar procedure as used for other nucleosides.[25]

The 3′-*C*-ethynyl derivative **2d** was prepared starting from **31**, an N-8 coupled product from the Vorbrüggen condensation of **25** and 1,2-di-*O*-acetyl-3,5-di-*O*-benzoyl-

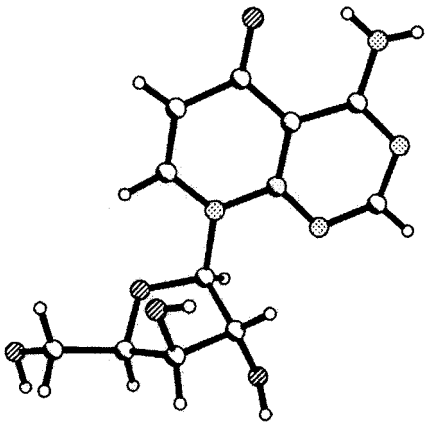


Figure 1. X-ray crystal structure of compound **2b**.

D-xylofuranose.[26] Selective deacetylation of **31** with hydrazine[27] gave **32** in good yield, which was subsequently protected with two 4,4′-dimethoxytrityl (DMT) groups at the O-2′ and N-4 positions. After debenzoylation with ammonia, the resulting **33** was protected with an additional DMT at the O-5′ position, followed by oxidation of the 3′-hydroxyl, to give the 3′-keto derivative **34**. The Grignard reaction of **34** with ethynylmagnesium bromide, followed by removal of DMT, gave the 3′-*C*-ethynyl nucleoside **2d**. The 2′-deoxy xyloside **6b** was prepared from **32** via a Barton type deoxygenation[22,23] and a subsequent debenzoylation. Compounds **3b, 4b,** and **3c** were prepared by catalytic hydrogenation of **3f, 4d,** and **3d**, respectively. The 2′,3′-dideoxy derivative **6a** was prepared via a selective protection of the 5′-hydroxyl group of **1a** with a *tert*-butyldiphenylsilyl group, a subsequent Barton type deoxygenation,[22,23]and the deprotection of the silyl group.

Stereochemistry

The sugar derivatives **10**, **17**, and **18** were prepared via highly selective reactions[16,18] using **7** and **14** as the starting material, respectively. The use of **7** and **14** could predict the stereochemistry of the products since the α-face of these ribose derivatives is much more hindered than the *β*-face, as shown in Schemes 1 and 2. The resulting substituents must be on the *β*-face of the ribose[16,18] since only one product was formed in each reaction. The pyrido[2,3-*d*]pyrimidine nucleosides have the aforementioned two isomers: the N-1 coupled and N-8 coupled nucleosides. In all the condensations, the two nucleosides were always formed as the final coupling products. All the major coupling products have much higher R_f on silica gel TLC regardless of solvents used. The two types of the fully deprotected nucleosides have quite distinct proton NMR spectra. The amino groups in all the major nucleosides have two doublet peaks, one at ca. 9.6 ppm and the other at ca. 8.2 ppm, whereas the amino groups in all the minor nucleosides also have two doublet peaks but with much higher chemical shifts, one at ca. 11.2 ppm and the other at ca. 9.2 ppm. According to the differences mentioned above, we could clearly divide the nucleosides into two groups but still could not assign which is which. Thanks to the X-ray crystal structure of **2b** (Figure 1), we were able unambiguously to assign the major nucleosides including **2b** as the N-8 coupled nucleosides.

Table 1. Cytotoxicity of 4-Amino-5-oxopyrido[2,3-*d*]pyrimidine Nucleosides

	EC_{50}[a] (μM)		
compd	HTB-81[b]	B16[c]	NHF[d]
1a	3.9	11.3	5.0
1b	0.06	0.06	0.08
1b-L	>100	>100	>100
1c	21.9	33.2	23.20
1d	46.7	85.5	>100
1e	29.3	81.0	65.1
1f	>100	>100	>100
2a	2.6	19.0	9.5
2b	0.73	5.9	20.7
2c	4.44	31.6	10.4
2d	>100	>100	>100
2e	31.1	60.5	41.1
2f	0.68	2.48	6.45
3c	20.4	51.6	41.1
3d	35.3	66.3	72.0
3a+**4a**[e]	10.0	18.3	16.5
3b+**4b**[f]	17.3	16.3	18.9
3f+**4d**[g]	6.1	17.2	15.3
5a	19.7	55.5	29.9
5b	36.7	96.0	65.1
5c	34.2	99.7	76.81
5d	>100	>100	>100
6a	43.3	47.1	60.5
6b	26.6	68.9	38.9
25	>100	>100	>100
26	7.3	NT[h]	7.3
27	>100	>100	>100
toyocamycin	0.012	0.005	0.004
daunomycin	0.058	0.16	0.22

[a] EC_{50} (μM) is the concentration of compound that caused a 50% reduction in absorbance at 490 nm relative to untreated cells using MTS assay. [b] HTB-81, human prostate cancer cells. [c] B16, mouse melanoma cells. [d] NHF, normal human fibroblasts. [e] Ratio of **3a** and **4a**, 2:3. [f] Ratio of **3b** and **4b**, 1:3 or 3:1. [g] Ratio of **3f** and **4d**, 3:2 or 2:3. [h] NT, not tested.

Four pairs of the 5′-substituted nucleosides, **3a**+**4a**, **3b**+**4b**, **3e**+**4c**, and **3f**+**4d**, were obtained as a mixture of the 5′(*R*)- and 5′(*S*)-isomers due to the use of the mixtures of the 5′(*R*)- and 5′(*S*)-substituted sugars as starting material. No efforts were made for separation and stereochemical assignments. Compound **2d** was assigned as the 3′-*C*-ethynylxylofuranosyl derivative since the isomeric 3′-*C*-ethynylribofuranosyl derivative **2f** was prepared from the known 1-*O*-acetyl-3-*C*-ethynylribofuranosyl derivative.[28]

In the crystal of **2b**, the unit cell contains a molecule of **2b** and a molecule of water. The bond length of C1′−N8 is 1.497 Å and the torsion angle of O4′−C1′−N8−C9 is −171.38°, which indicates that the pyrido[2,3-*d*]pyrimidine base in **2b** has the *anti* orientation. The pseudorotation phase angle *P*, calculated according to a widely used equation,[29] is 23.6°, which corresponds to a typical C3′-endo sugar pucker.

Cytotoxicity

Compounds **25**−**27** and **1b-L** and all the compounds in Chart 1 except **3e** and **4c** were evaluated for their cytotoxicity profile to human prostate cancer cells (HTB-81), mouse melanoma cancer cells (B16), and normal human fibroblasts (NHF). The results are shown in Table 1. Compounds **1a**,**b**, **2a**−**c**,**f**, and **3f**+**4d** showed significant cytotoxicity to the cancer cells, with **1b** being the most cytotoxic (EC_{50}: 0.06 μM). However, most of these compounds also showed cytotoxicity to normal human fibroblasts although they were slightly less cytotoxic. Compound **2b** seems more promising. Although it was not so cytotoxic to the cancer cells as **1b**,

the compound was still quite potent (EC_{50}: 0.73 μM). More importantly, **2b** was not very cytotoxic to the normal cells and has a favorable in vitro selectivity index (28). On the basis of the promising in vitro data, we have initiated in vivo studies and the results will be reported in due time. Evaluation of these pyridopyrimidine nucleosides against other types of cancer cells as well as a variety of viruses is under way.

Structure−Activity Relationship

As can be seen from Table 1, the modified sugar moieties have a striking effect on the cytotoxicity of these nucleosides to both cancer and normal cells. Considering the most cytotoxic compound **1b** as the parental compound, most of the 2′-*C*- and 4′-*C*-substituted pyridopyrimidine nucleosides have lost the cytotoxicity to both cancer and normal cells. However, certain modifications at the C-3′ position could be tolerated to a certain degree. Compounds **2a**−**c**,**f** which have a 3′-modified sugar moiety still retained potent cytotoxicity to the cancer cells. It was also noted that the mixtures of the 5′(*R,S*)-*C*-substituted nucleosides such as the mixture of **3f**+**4d** still had a significant cytotoxicity to the cancer cells. As expected, compound **1b**'s L-form (**1b-**L) did not show any cytotoxicity within the testing concentration range. Compound **26** which is a N-1 coupled nucleoside having an intact ribose showed a significant cytotoxicity to both HTB-81 and NHF cells, whereas compound **27**, another N-1 coupled nucleoside having xylose as the sugar moiety, and the heterocycle **25** had no cytotoxicity at the concentration range tested. The results clearly show that the intact ribofuranose is extremely important to the cytotoxicity, which implicated that the cytotoxicity was probably associated with the phosphorylation of the nucleosides by ribonucleoside kinases. This can also explain why **1b-**L did not show any cytotoxicity. Since some of the nucleosides having modifications at the C-3′ position showed certain promise as anticancer agents, further work will be focused on modifications at the C-3′ position and possibly at the C-5′ position of the pyrido[2,3-*d*]pyrimidine nucleosides.

Conclusion

We have reported the synthesis of a series of 4-amino-5-oxopyrido[2,3-*d*]pyrimidine nucleosides containing a variety of modified sugar moieties. These nucleosides were evaluated for cytotoxicity to human prostate cancer (HTB-81) and mouse melanoma (B16) cells as well as to normal human fibroblasts (NHF). The 2′-*C*- and 4′-*C*-substituted nucleosides showed only weak cytotoxicity to the cancer cells, while the 3′-*C*- and 5′-*C*-substituted nucleosides showed moderate to significant cytotoxicity. Compound **1b** containing the intact ribose was the most cytotoxic to both cancer and normal cells (EC_{50}: 0.06−0.08 μM), whereas compound **2b** was identified as a potent, selective inhibitor of the prostate cancer cells (HTB-81) with an EC_{50} of 0.73 μM and in vitro selectivity index of 28. Currently, compound **2b** is being evaluated in in vivo studies. Further evaluations of these pyrido[2,3-*d*]pyrimidine nucleosides against other types of cancer cells as well as further chemical modifications are under way.

Experimental Section

(A) Chemistry. ^{1}H NMR spectra were obtained on a Varian Mercury 300 spectrometer and tetramethylsilane was used as the internal standard. Mass spectral data were obtained on an electrospray mass spectrometer using negative ionization method from Mass Consortium, Corp., San Diego, CA. Elemental analysis was conducted by NuMega Resonance, Inc., San Diego. Melting points were measured on a capillary melting point measurement apparatus and are uncorrected. Anhydrous solvents were purchased from Aldrich or Fluka without further treatment unless noted. Thin-layer chromatography plates and silica gel for flash chromatography were supplied by ICN Biomedicals. Solvent ratios are based on volume in case that solvent mixture was used.

A usual workup procedure was used for most of the reactions in the Experimental Section: The mixture was diluted with ethyl acetate (or methylene chloride), washed sequentially with water (or brine), dilute sodium bicarbonate, and water, dried (Na_2SO_4), and concentrated to dryness at reduced pressure. Chromatography on silica gel columns was used to purify most of the compounds.

3,5-Di-*O*-benzoyl-1,2-*O*-isopropylidene-3-*C*-methyl-D-ribofuranose (8). A solution of **7**[15] (3.5 g, 12 mmol) in THF (15 mL) was added to a stirred solution of methylmagnesium bromide (3 M in ether, 20 mL) in a mixture of THF (45 mL) and diethyl ether (120 mL) at −5 °C. The reaction mixture was stirred at 0 °C for 7 h and then quenched with water. After the usual workup and a quick chromatography (5% methanol in dichloromethane), the resulting product was dissolved in pyridine (62 mL), and benzoyl chloride (2.75 mL, 24 mmol) was added at 0 °C. The reaction mixture was stirred at 75 °C for 3 days and quenched with methanol (5 mL). After the usual workup, the residue was subjected to chromatography (20% ethyl acetate in hexanes) to give 2.1 g of **8** (42%, 2 steps) as a foam: ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 3 H, Me), 1.51 (s, 3 H, Me), 1.60 (s, 3 H, Me), 4.51−4.68 (m, 3 H), 5.01 (d, J = 3.9 Hz, 1 H), 5.89 (d, J = 3.6 Hz, 1 H), 7.35−7.60 (m, 6 H, Bz), 7.97−8.12 (m, 4 H, Bz).

5-*O*-Benzoyl-3-deoxy-1,2-*O*-isopropylidene-3-*C*-methylene-D-ribofuranose (9) Sodium *tert*-pentoxide (1.9 g, 18 mmol) in benzene (19 mL) was added to a stirred suspension of methyltriphenylphosphonium bromide (7.0 g, 20 mmol) in ether (245 mL) under argon. After stirring at room temperature overnight, the mixture was cooled to 0 °C and a solution of **7**[15] (2.9 g, 9.8 mmol) in benzene (10 mL) was added. After stirring for 24 h at room temperature, the reaction mixture was diluted with ethyl acetate (500 mL). After the usual workup, the residue was subjected to chromatography (20% ethyl acetate in hexanes) to give 1.4 g of **9** (47%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 3 H, Me), 1.54 (s, 3 H, Me), 1.60 (s, 3 H, Me), 4.37−4.57 (m, 2 H), 4.96 (m, 1 H), 5.08 (m, 1 H), 5.30 (m, 1 H), 5.51 (m, 1 H), 5.92 (d, J = 3.9 Hz, 1 H), 7.41−7.60 (m, 3 H, Bz), 8.02−8.05 (m, 2 H, Bz).

1,3-Di-*O*-acetyl-2,5-di-*O*-benzoyl-3-*C*-methyl-D-ribofuranose (12). Compound **8** (2.0 g, 4.8 mmol) was dissolved in TFA/water (9:1, 15 mL) at −15 °C. The reaction mixture was stirred at −10 °C for 45 min and at 0 °C for 1 h 45 min and then concentrated to dryness under high vacuum at low temperature. The residue was dissolved in toluene and concentrated again to dryness. After a quick chromatography (20% ethyl acetate in dichloromethane), the resulting product **10** was dissolved in a mixture of pyridine (28 mL) and acetic anhydride (1.1 mL, 12 mmol). The reaction mixture was stirred at room temperature overnight and then poured into ice/water and extracted with ethyl acetate. After the usual workup, the residue was subjected to chromatography (0−5% ethyl acetate in dichloromethane) to give 1.2 g (56%) of 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-*C*-methyl-D-ribofuranose. This compound (0.93 g, 2.2 mmol) was dissolved in a mixture of dichloromethane (22 mL), triethylamine (0.75 mL), acetic anhydride (0.42 mL, 4.5 mmol) and DMAP (54 mg). The reaction mixture was stirred at room temperature for 3 h, then was diluted with dichloromethane. After the usual workup, the residue was subjected to chromatography (0−8% ethyl acetate in dichloromethane) to give 0.98 g of **12** (75%) as a foam: ^{1}H NMR ($CDCl_3$) δ 1.78 and 1.85 (2 s, 3 H, Ac), 1.98 and 2.02 (2 s, 3 H, Ac), 2.07 and 2.08 (2 s, 3 H, Me), 4.46−4.59 (m, 1 H), 4.70−4.87 (m, 2 H),

5.64 and 5.74 (2 s, 1 H), 6.17 and 6.25 (2 s, 1 H), 7.38−7.62 (m, 6 H), 7.95−8.13 (m, 4 H).

5-*O*-Benzoyl-3-deoxy-1,2-di-*O*-acetyl-3-*C*-methylene-D-ribofuranose (13) was prepared from **9** (1.3 g, 4.5 mmol) by a similar procedure as described for **12**, except that the second acetylation in dichloromethane was not necessary: yield 0.97 g (64%, 2 steps); ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 3 H, Ac), 2.11 (s, 3 H, Ac), 4.39−4.54 (m, 2 H), 5.09 (m, 1 H), 5.48 and 5.55 (2 br s, 2 H), 5.73 (m, 1 H), 6.24 (s, 1 H), 7.42−7.60 (m, 3 H, Bz), 8.05−8.10 (m, 2 H, Bz).

2-*C*-Ethynyl-1-*O*-methyl-3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-α-D-ribofuranose (16). Ethynylmagnesium bromide (0.5 M in THF, 200 mL) was added to a stirred solution of **14**[17] (15 g, 37 mmol) in THF (30 mL) at −60 °C. The reaction mixture was stirred at room temperature for 3 h, poured into ice/water, diluted with ethyl acetate (600 mL) and acetic acid (10 mL). After the usual workup, the residue was subjected to chromatography (ethyl acetate/hexanes, 1:3) to give 8.1 g of **16** (51%) as a foam: ^{1}H NMR ($CDCl_3$) δ 1.00−1.10 (m, 28 H), 2.63 (s, 1 H), 3.51 (s, 3 H, OMe), 3.60 (s, 1 H, OH), 3.97 (m, 3 H), 4.07−4.12 (m, 1 H), 4.97 (s, 1 H).

1-*O*-Methyl-2-*C*-methyl-3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-α-D-ribofuranose (15) was prepared from the reaction (2 h) of **14**[17] (4.0 g, 9.9 mmol) and methylmagnesium bromide in THF by a similar procedure as described for **16**: yield 2.2 g (53%) as a colorless foam; ^{1}H NMR ($CDCl_3$) δ 0.87−1.05 (m, 28 H), 1.32 (s, 3 H, Me), 3.34 (s, 1 H, OH), 3.46 (s, 3 H, OMe), 3.61 (m, 1 H), 3.90 (m, 3 H), 4.58 (s, 1 H).

2-*C*-Ethynyl-1,2,3,5-tetra-*O*-acetyl-D-ribofuranose (18). Compound **16** (8.1 g, 18.8 mmol) was dissolved in THF (30 mL) and TBAF (1.0 M in THF, 28 mL) was added. The reaction mixture was stirred at room temperature for 2 h, then the solvent was evaporated. The residue was subjected to chromatography (5% methanol in ethyl acetate), and the resulting product was dissolved in a mixture of dichloromethane (35 mL) and triethylamine (15.4 mL, 111 mmol), followed by addition of DMAP (2.25 g, 18 mmol) and acetic anhydride (9.4 mL, 100 mmol). The reaction mixture was stirred at room temperature for 24 h and then subjected to the usual workup. After purification by chromatography (ethyl acetate/hexane, 45:55), the resulting product was dissolved in a mixture of acetic acid (53 mL), acetic anhydride (5.3 mL) and sulfuric acid (0.47 mL, 8.7 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 5 h, then poured into ice/water. After the usual workup, the residue was subjected to chromatography (ethyl acetate in hexanes, 45:55) to give 4.1 g of **18** (79%, 3 steps) as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 2.04, 2.08, 2.14, 2.16 and 2.16 (5 s, 12 H, Ac), 2.67 (s, 1 H), 4.08−4.44 (m, 4 H), 5.68 (m, 1 H), 6.46 (s, 1 H).

2-*C*-Methyl-1,2,3,5-tetra-*O*-acetyl-D-ribofuranose (17) was prepared from **15** (2.1 g, 5.0 mmol) by a similar procedure as described for **18**: total yield 1.0 g (62%) as a colorless syrup; ^{1}H NMR ($CDCl_3$) δ 1.65, 2.04, 2.09 and 2.11 (4 s, 15 H, Ac and Me), 4.21−4.41 (m, 3 H), 5.02 (d, J = 3.9 Hz, 1 H), 6.33 (s, 1 H).

1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-4-*C*-vinyl-D-ribofuranose (22). A solution of **19**[19] (0.89 g, 1.7 mmol) in 80% acetic acid (25 mL) was stirred at room temperature for 2.5 h, then the solvent was evaporated under reduced pressure. The residue was subjected to chromatography (1−2% methanol in dichloromethane), and the resulting foam was dissolved in pyridine (14 mL), followed by addition of benzoyl chloride (0.22 mL, 1.9 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 18 h, then cooled to 0 °C, quenched with methanol (5 mL), and concentrated under reduced pressure. After the usual workup and purification by chromatography (20% ethyl acetate in hexanes), the resulting oily foam (**20**) was treated with TFA/water following the same procedure as described for **10**. The crude was purified by chromatography (3% methanol in dichloromethane) to give **21**, which was dissolved in pyridine (8.0 mL) at 0 °C, followed by addition of benzoyl chloride (0.34 mL, 2.9 mmol). The reaction mixture was stirred at room temperature for 20 h, then cooled to 0 °C, quenched with methanol (1 mL), and concentrated under reduced pressure. After the usual workup, the residue was coevaporated with toluene and dissolved in a mixture of acetic acid (3.0 mL) and acetic anhydride (0.33 mL). Sulfuric acid (97%, 36 μL) in acetic acid (0.3 mL) was added at 5 °C. The reaction mixture was stirred at room temperature for 6 h and diluted with ethyl acetate and brine. After the usual workup, the residue was subjected to chromatography (30% ethyl acetate in hexanes) to give 0.6 g (67%, 5 steps) of **22** as a syrup (ratio of β/α anomers, 3:1): ^{1}H NMR ($CDCl_3$) δ 1.95 and 2.15 (2 s, 3 H, Ac), 4.41−4.67 (m, 2 H), 5.85 (m, 1 H), 5.37 and 5.49 (2 dd, J = 10.5 and 1.2 Hz, 1 H), 5.64 and 5.77 (2 dd, J = 17.4 and 1.5 Hz, 1 H), 5.80−6.12 (m, 3 H), 6.50 (s, 3/4 H), 6.73 (d, J = 4.5 Hz, 1/4 H), 7.26−8.15 (m, 15 H).

2-Amino-3-cyano-1*H*-pyrid-4-one (24). Trimethylsilyl iodide (7.5 mL, 55 mmol) was added to a stirred suspension of **23**[20] (7.5 g, 50 mmol) in acetonitrile (180 mL). The resulting mixture was heated at reflux under a flow of argon (to remove the resulting methyl iodide) for 20 h and cooled to room temperature. The resulting precipitate was filtered and washed with ethyl acetate and methanol to give 5.4 g (80%) of **24** as a brownish solid: ^{1}H NMR (DMSO-d_6) δ 5.68 (d, J = 7.5 Hz, 1 H), 6.94 (s, 2 H, NH_2), 7.28 (d, J = 7.8 Hz, 1 H), 10.6 (br s, 1 H, NH).

4-Amino-5-oxo-8*H*-pyrido[2,3-*d*]pyrimidine (25). A mixture of **24** (8.0 g, 59 mmol) in 2-ethoxyethanol (250 mL) and formamidine acetate (23 g, 220 mmol) was heated at reflux for 30 h, cooled to 80 °C, and filtered to give **25** (8.5 g, 89%) as a gray solid. Crystallization from a mixture of acetic acid and water gave a colorless solid: mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 6.09 (d, J = 7.5 Hz, 1 H), 7.23 (d, J = 7.8 Hz, 1 H), 8.06 and 8.41 (2 br d, 2 H, NH_2), 11.98 (br s, 1 H, NH). Anal. ($C_7H_6N_4O$) C, H, N.

General Procedure for the Vorbrüggen Reaction.[21] To a suspension of **25** (1.1 equiv in 1,2-dichloroethane (15 mL/mmol) was added *N*,*O*-bis(trimethylsilyl)acetamide (BSA; 4.0 equiv), and the reaction mixture was stirred at 75 °C for 2 h, then cooled to 55 °C. The 1-*O*-acetyl sugar derivative (1.0 equiv) in 1,2-dichloroethane (5 mL/mmol) and TMSOTf (1.5 equiv) were added successively, and the mixture was stirred at reflux for 3 days. The mixture was cooled to room temperature, concentrated under reduced pressure, diluted with ethyl acetate, washed successively with saturated sodium bicarbonate and brine, dried over anhydrous sodium sulfate, filtered, and concentrated to dryness. The residue was subjected to chromatography (15−25% acetone in dichloromethane) to give a major product (N-8 coupled product) and a minor compound (N-1 coupled product). A solution of the major product (1 equiv) in methanolic ammonia (25 mL/mmol) was stirred in a sealed flask at room temperature for 20 h. The solvent was evaporated, and the residue was subjected to chromatography (methanol in dichloromethane) to give the desired nucleoside as a colorless solid.

4-Amino-5-oxo-8-(β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (1b) and 4-Amino-5-oxo-1-(β-D-ribofuranosyl)-pyrido[2,3-*d*]pyrimidine (26). Compound **1b** was prepared from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose (1.7 g, 3.4 mmol) and **25** (0.60 g, 3.7 mmol) following the general procedure: total yield 0.47 g (48%, 2 steps) as a colorless solid; mp 222−223 °C (crystallized from water); ^{1}H NMR (DMSO-d_6) δ 3.65 (m, 2 H), 3.91 (m, 1 H), 3.99−4.09 (m, 2 H), 5.14 (d, J = 5.1 Hz, 1 H, OH), 5.19 (t, J = 4.8 Hz, 1 H, OH), 5.40 (d, J = 5.4 Hz, 1 H, OH), 6.20 (d, J = 8.1 Hz, 1 H), 6.58 (d, J = 4.8 Hz, 1 H), 8.19 and 9.57 (2 br d, J = 4.6 Hz, 2 H, NH_2), 8.29 (s, 1 H), 8.31 (d, J = 8.1 Hz, 1 H); MS 293 (M − H), 161 (base − H). Anal. ($C_{12}H_{14}N_4O_5$) C, H, N.

Compound **26** was obtained after debenzoylation of the minor N-1 product in 29% yield (2 steps) as a colorless solid: recrystallized from water, mp 245 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.60−3.82 (m, 2 H), 3.97 (m, 1 H), 4.08−4.18 (m, 2 H), 5.12 (d, J = 6.0 Hz, 1 H, OH), 5.36 (t, J = 4.8 Hz, 1 H, OH), 5.57 (d, J = 4.5 Hz, 1 H, OH), 6.23 (d, J = 6.3 Hz, 1 H), 6.37 (d, J = 2.7 Hz, 1 H), 7.90 (d, J = 6.6 Hz, 1 H), 8.98 (s, 1 H),

9.18 and 11.23 (2 br d, $J = 5.4$ Hz, 2 H, NH_2); MS 293 (M − H), 161 (base − H). Anal. ($C_{12}H_{14}N_4O_5$) C, H, N.

4-Amino-5-oxo-8-(3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (28). TIPDS-Cl (1.3 mL, 4.1 mmol) was added to a stirred solution of **1b** (1.0 g, 3.4 mmol) in pyridine (10 mL) at 0 °C. The reaction mixture was stirred at room temperature for 20 h, then cooled to 0 °C, quenched with water (0.5 mL), and concentrated under reduced pressure. After the usual workup, the residue was subjected to chromatography (60% ethyl acetate in hexanes) to give 1.7 g of **28** (92%) as a white foam: ^{1}H NMR ($CDCl_3$): δ 0.95−1.10 (m, 28 H), 3.0 (m, 1 H, OH), 4.01−4.41 (m, 5 H), 5.95 and 9.85 (2 br m, 2 H, NH_2), 6.29 (d, $J = 8.1$ Hz, 1 H), 6.41 (s, 1 H), 8.13 (d, $J = 8.4$ Hz, 1 H), 8.42 (s, 1 H).

4-Amino-5-oxo-8-(2-deoxy-β-D-ribofuranosyl)pyrido-[2,3-*d*]pyrimidine (1a). A solution of **28** (0.65 g, 1.2 mmol), DMAP (0.30 g, 2.4 mmol), and phenyl chlorothionoformate (0.19 mL, 1.3 mmol) in acetonitrile (10 mL) was stirred at room temperature for 2 h and then concentrated. After the usual workup, the residue (**29**) was dissolved in toluene (10 mL), and tris(trimethylsilyl)silane (0.56 mL, 1.8 mmol) and 1,1′-azobis(cyclohexanecarbonitrile) (74 mg, 0.30 mmol) were added. The reaction mixture was heated at 80 °C for 2 h and at 105 °C for 15 h. The solvent was evaporated and the residue was dissolved in THF (5 mL). TBAF (1.0 M, 2.5 mL) was added and the reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated and the residue was subjected to chromatography (10% methanol in dichloromethane) to give 0.24 g of **1a** (71%, 3 steps) as a colorless solid: mp 187−188 °C (recrystallized from water); ^{1}H NMR (DMSO-d_6) δ 2.08 (m, 1 H), 2.26 (m, 1 H), 3.61 (m, 2 H), 3.86 (m, 1 H), 4.26 (m, 1 H), 5.10 (t, $J = 5.1$ Hz, 1 H, OH), 5.31 (d, $J = 4.2$ Hz, 1 H, OH), 6.19 (d, $J = 8.1$ Hz, 1 H), 6.85 (t, $J = 6.6$ Hz, 1 H), 8.18 and 9.57 (2 br d, $J = 4.6$ Hz, 2 H, NH_2), 8.27 (d, $J = 8.1$ Hz, 1 H), 8.29 (s, 1 H). Anal. ($C_{12}H_{14}N_4O_4$) C, H, N.

4-Amino-5-oxo-8-(β-D-arabinofuranosyl)pyrido[2,3-*d*]-pyrimidine (1c). To a mixture of **28** (1.7 g, 3.1 mmol) and DCC (1.3 g, 6.3 mmol) in DMSO (30 mL) at 0 °C were added a solution of pyridine (0.3 mL, 3.8 mmol) and TFA (0.14 mL, 1.9 mmol) in DMSO (5 mL). The reaction mixture was stirred at room temperature for 18 h and methanol (1 mL) was added. After stirring for 6 h at room temperature, the mixture was filtered and the filtrate subjected to the usual workup. Chromatography (4% methanol in dichloromethane) gave 1.4 g (84%) of **30** as a colorless solid.

Sodium borohydride (0.46 g, 12.3 mmol) was suspended in THF (50 mL), acetic acid (2.8 mL, 49.2 mmol) was added, and the mixture was stirred at 0 °C for 1 h. Compound **30** (1.3 g, 2.5 mmol) in THF (15 mL) was added and the reaction mixture was stirred at 0 °C for 20 h. The solvent was evaporated and the residue was subjected to chromatography (4% methanol in dichloromethane) to give 0.8 g of the arabinoside (60.4%) slightly contaminated by the riboside. Three recrystallizations from ethyl acetate gave 0.35 g of pure product, which was dissolved in a solution of ammonium fluoride (0.5 M in methanol, 3.8 mL) methanol (8 mL). The reaction mixture was stirred at 40−45 °C for 28 h and concentrated to dryness. The residue was subjected to chromatography (15% methanol in dichloromethane) to give 0.13 g of **1c** (25%, 2 steps) as a white foam. Crystallization from water gave a colorless solid: mp 218−219 °C; ^{1}H NMR (DMSO-d_6) δ 3.64 (t, $J = 5.1$ Hz, 2 H), 3.84 (m, 1 H), 3.93 (m, 1 H), 4.11 (m, 2 H), 5.12 (t, $J = 5.1$ Hz, 1 H, OH), 5.43 (d, $J = 5.4$ Hz, 1 H, OH), 5.51 (d, $J = 3.9$ Hz, 1 H, OH), 6.15 (d, $J = 8.1$ Hz, 1 H), 6.70 (d, $J = 4.2$ Hz, 1 H), 8.03 (d, $J = 8.4$ Hz, 1 H), 8.15 and 9.60 (2 br d, $J = 4.6$ Hz, 2 H, NH_2), 8.28 (s, 1 H). Anal. ($C_{12}H_{14}N_4O_5 \cdot 0.5H_2O$) C, H, N.

4-Amino-5-oxo-8-(2-deoxy-2-*C*-methylene-β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (1f). Sodium *tert*-pentoxide (0.20 g, 1.7 mmol) in benzene (1 mL) was added to a stirred suspension of methyltriphenylphosphonium bromide (0.66 g, 1.85 mmol) in ether (40 mL) under argon. After stirring at room temperature overnight, the mixture was cooled to 0 °C and a solution of **30** (0.45 g, 0.84 mmol) in ether (5 mL) was added, followed by addition of 5 mL of DMSO. After stirring at 0 °C for 4 h, the reaction mixture was diluted with ethyl acetate (50 mL). After the usual workup and chromatography (ethyl acetate/hexanes, 1:1), the resulting product was treated with ammonium fluoride in methanol as described for **1c**. Chromatography (9% methanol in dichloromethane) gave 100 mg of **1f** (42%, 2 steps) as a white foam. Recrystallization from water gave a colorless solid: mp 192−193 °C; ^{1}H NMR (DMSO-d_6) δ 3.56−76 (m, 3 H), 4.56 (m, 1 H), 5.04 (t, $J = 5.1$ Hz, 1 H, OH), 5.22 (br s, 1 H), 5.37 (br s, 1 H), 5.74 (d, $J = 6.3$ Hz, 1 H, OH), 6.18 (d, $J = 8.1$ Hz, 1 H), 7.31 (d, $J = 1.2$ Hz, 1 H), 7.91 (d, $J = 8.1$ Hz, 1 H), 8.24 and 9.53 (2 br d, $J = 4.5$ Hz, 2 H, NH_2), 8.32 (s, 1 H). Anal. ($C_{13}H_{14}N_4O_4$) C, H, N.

4-Amino-5-oxo-8-(2-*C*-methyl-β-D-ribofuranosyl)pyrido-[2,3-*d*]pyrimidine (1d) was prepared from **17** (0.48 g, 1.4 mmol) and **25** (0.48 g, 2.8 mmol) as described in the general procedure: yield 0.16 g (41%, 2 steps). Recrystallization from water gave a colorless solid: mp 193−194 °C; ^{1}H NMR (DMSO-d_6) δ 0.81 (s, 3 H, Me), 3.63−3.87 (m, 4 H), 5.12 (s, 1 H, OH), 5.24 (d, 1 H, $J = 6.6$ Hz, OH), 5.29 (t, $J = 4.2$ Hz, 1 H, OH), 6.17 (d, $J = 8.4$ Hz, 1 H), 6.65 (s, 1 H), 8.21 and 9.58 (2 br d, $J = 4.8$ Hz, 2 H, NH_2), 8.31 (m, 2 H). Anal. ($C_{13}H_{16}N_4O_5 \cdot 0.25H_2O$) C, H, N.

4-Amino-5-oxo-8-(2-*C*-ethynyl-β-D-ribofuranosyl)pyrido-[2,3-*d*]pyrimidine (1e) was prepared from **18** (1.0 g, 2.9 mmol) and **25** (0.95 g, 5.8 mmol) as described in the general procedure: yield 0.23 g (25%, 2 steps). Recrystallized from water to give a colorless solid: mp 130 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.15 (s, 1 H), 3.63 (m, 1 H), 3.83 (m, 2 H), 4.11 (m, 2 H), 5.32 (t, $J = 4.8$ Hz, 1 H, OH), 5.76 (d, 1 H, $J = 6.9$ Hz, OH), 6.17 (m, 2 H, incl. 1 OH), 6.75 (s, 1 H), 8.18 and 9.54 (2 br d, $J = 4.8$ Hz, 2 H, NH_2), 8.31 (s, 1 H), 8.37 (d, $J = 7.8$ Hz, 1 H). Anal. ($C_{14}H_{14}N_4O_5 \cdot 0.5H_2O$) C, H, N.

4-Amino-5-oxo-8-(3-deoxy-β-D-ribofuranosyl)pyrido-[2,3-*d*]pyrimidine (2a) was prepared from 1,2-di-*O*-acetyl-5-benzoyl-3-deoxy-D-xylose[30] (0.69 g, 2.1 mmol) and **25** (0.45 g, 2.8 mmol) as described in the general procedure: total yield 0.11 g (28%, 2 steps). Recrystallization from water gave **2a** as a colorless solid: mp 260−262 °C; ^{1}H NMR (DMSO-d_6) δ 1.77 (m, 1 H), 2.00 (m, 1 H), 3.60 (m, 1 H), 3.83 (m, 1 H), 4.25 (m, 1 H), 4.39 (m, 1 H), 5.20 (t, $J = 4.8$ Hz, 1 H, OH), 5.60 (d, $J = 3.9$ Hz, 1 H, OH), 6.16 (d, $J = 8.1$ Hz, 1 H), 6.37 (s, 1 H), 8.18 and 9.61 (2 br d, $J = 4.8$ Hz, 2 H, NH_2), 8.33 (s, 1 H), 8.42 (d, $J = 8.1$ Hz, 1 H); MS 277 (M − H), 161 (base − H). Anal. ($C_{12}H_{14}N_4O_4$) C, H, N.

4-Amino-5-oxo-8-(β-D-xylofuranosyl)pyrido[2,3-*d*]pyrimidine (2b) and 4-Amino-5-oxo-1-(β-D-xylofuranosyl)-pyrido[2,3-*d*]pyrimidine (27). Compound **2b** was prepared from 1,2-di-*O*-acetyl-3,5-di-*O*-benzoyl-D-xylofuranose[26] (0.69 g, 2.1 mmol) and **25** (0.66 g, 4.1 mmol) as described in the general procedure: total yield 0.12 g (49%, 2 steps). Recrystallization from water gave **2b** as a colorless solid: mp 190−192 °C; ^{1}H NMR (DMSO-d_6) δ 3.70−3.82 (m, 2 H), 3.95 (m, 1 H), 4.04 (d, $J = 4.2$ Hz, 1 H), 4.21 (m, 1 H), 4.81 (t, $J = 5.7$ Hz, 1 H, OH), 5.38 (d, $J = 3.0$ Hz, 1 H, OH), 5.81 (d, $J = 4.5$ Hz, 1 H, OH), 6.20 (d, $J = 8.4$ Hz, 1 H), 6.36 (s, 1 H), 8.13 (d, $J = 8.1$ Hz, 1 H), 8.18 and 9.60 (2 br d, $J = 4.5$ Hz, 2 H, NH_2), 8.32 (s, 1 H); MS 293 (M − H), 161 (base − H). Anal. ($C_{12}H_{14}N_4O_5 \cdot H_2O$) C, H, N.

As described for **26**, compound **27** was obtained in 17% yield. Recrystallization from water gave **27** as a colorless solid: mp 200 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.74−3.87 (m, 2 H), 3.95 (m, 1 H), 4.13 (d, $J = 3.9$ Hz, 1 H), 4.32 (m, 1 H), 4.88 (t, $J = 5.7$ Hz, 1 H, OH), 5.37 (d, $J = 3.0$ Hz, 1 H, OH), 5.90 (d, $J = 3.9$ Hz, 1 H, OH), 6.19 (s, 1 H), 6.23 (d, $J = 6.6$ Hz, 1 H), 7.92 (d, $J = 6.3$ Hz, 1 H), 8.52 (s, 1 H), 9.13 and 11.17 (2 br d, $J = 5.2$ Hz, 2 H, NH_2); MS 293 (M − H), 161 (base − H). Anal. ($C_{12}H_{14}N_4O_5$) C, H, N.

4-Amino-5-oxo-8-(3-*C*-methyl-β-D-ribofuranosyl)pyrido-[2,3-*d*]pyrimidine (2c) was prepared from **12** (0.27 g, 0.59 mmol) and **25** (0.12 g, 0.71 mmol) as described in the general procedure: total yield 0.10 g (55%, 2 steps). Recrystallization from methanol gave **2c** as a colorless solid: mp 225−226 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 3 H, Me), 3.54−3.68 (m, 2 H),

3.85 (m, 1 H), 3.99 (m, 1 H), 4.81 (s, 1 H, OH), 5.23 (t, J = 4.2 Hz, 1 H, OH), 5.34 (d, J = 6.6 Hz, 1 H, OH), 6.25 (d, J = 8.1 Hz, 1 H), 6.79 (d, J = 7.8 Hz, 1 H), 8.20 and 9.60 (2 br d, J = 4.8 Hz, 2 H, NH_2), 8.29 (s, 1 H), 8.44 (d, J = 8.1 Hz, 1 H); MS 307 (M − H), 161 (base − H). Anal. ($C_{13}H_{16}N_4O_5$) C, H, N.

4-Amino-5-oxo-8-(3-deoxy-3-*C*-methylene-*β*-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (2e) was prepared from **13** (0.54 g, 1.6 mmol) and **25** (0.34 g, 2.1 mmol) as described in the general procedure: total yield 0.12 g (28%, 2 steps). Recrystallization from water gave **2e** as a colorless solid: mp 137−138 °C; ^{1}H NMR (DMSO-d_6) δ 3.68−3.74 (m, 2 H), 4.62 (m, 2 H), 5.14 (t, J = 5.1 Hz, 1 H, OH), 5.20 (d, J = 9.9 Hz, 2 H), 5.87 (d, J = 4.8 Hz, 1 H, OH), 6.26 (d, J = 7.8 Hz, 1 H), 6.58 (d, J = 6.6 Hz, 1 H), 8.23 and 9.58 (2 br d, J = 4.8 Hz, 2 H, NH_2), 8.27 (d, J = 8.1 Hz, 1 H), 8.30 (s, 1 H). Anal. ($C_{13}H_{14}N_4O_4\cdot0.5H_2O$) C, H, N.

4-Amino-5-oxo-8-(3-*C*-ethynyl-*β*-D-xylofuranosyl)pyrido-[2,3-*d*]pyrimidine (2d). Hydrazine hydrate (0.99 mL, 20 mmol) was added to a solution of **31**, the major coupling product from the condensation of **25** and 1,2-di-*O*-acetyl-3,5-di-*O*-benzoyl-D-xylofuranose[26] (3.7 g, 6.8 mmol), in a mixture of pyridine (48 mL) and acetic acid (12 mL). The reaction mixture was stirred at room temperature for 20 h, then acetone (20 mL) was added. The solvent was evaporated and the crude **32** was dissolved in pyridine (55 mL). DMT-Cl (5.6 g, 16 mmol) was added and the reaction mixture was stirred at 60 °C for 4 days, then cooled to 0 °C, quenched with methanol (5 mL). After the usual workup, the residue was subjected to chromatography (0−6% methanol in dichloromethane) to give 3.2 g of the fully protected nucleoside, which was added to methanolic ammonia (73 mL). The reaction mixture was sealed and stirred at room temperature for 20 h. The solvent was evaporated and the residue (**33**) was dissolved in pyridine (28 mL). DMT-Cl (1.0 g, 3.1 mmol) was added at 0 °C and the mixture was stirred at the same temperature for 2 h. Methanol (5 mL) was added and the solvent was evaporated. After the usual workup, the residue was dissolved with DCC (1.2 g, 5.6 mmol) in a mixture of toluene (4 mL) and DMSO (17 mL). Dichloroacetic acid (0.12 mL, 1.4 mmol) was added at 0 °C and the reaction mixture was stirred at room temperature for 20 h. After the usual workup, the residue was subjected to chromatography (0−5% ethyl acetate in dichloromethane) to give 1.5 g (18%, 5 steps) of the keto nucleoside **34**.

Compound **34** (0.85 g, 0.7 mmol) in THF (18 mL) was added to a solution of ethynylmagnesium bromide (0.5 M in THF, 5 mL) in ether (18 mL), and the reaction mixture was stirred at 0 °C for 8 h, quenched with water, diluted with ethyl acetate and 2 mL of acetic acid. After the usual workup and chromatography (0−5% ethyl acetate in dichloromethane), the residue was dissolved in 80% acetic acid (8.2 mL). The reaction mixture was stirred at room temperature for 5 h and then at 50 °C for 1 h. The solvent was evaporated and the residue was subjected to chromatography (15% methanol in dichloromethane) to give 0.11 g of **2d** (49%, 2 steps) as a white foam. Recrystallization from water gave a colorless solid: mp 240−242 °C; ^{1}H NMR (DMSO-d_6) δ 3.56 (s, 1 H), 3.81 (m, 2 H), 3.98 (d, J = 6.0 Hz, 1 H), 4.14 (m, 1 H), 5.96 (t, J = 5.7 Hz, 1 H, OH), 6.09 (s, 1 H, OH), 6.20 (d, J = 8.4 Hz, 1 H), 6.33 (d, J = 6.3 Hz, 1 H, OH), 6.36 (s, 1 H), 8.12 (d, J = 8.4 Hz, 1 H), 8.20 and 9.59 (2 br d, J = 5.1 Hz, 2 H, NH_2), 8.32 (s, 1 H). Anal. ($C_{14}H_{14}N_4O_5\cdot0.25H_2O$) C, H, N.

4-Amino-5-oxo-8-(3-*C*-ethynyl-*β*-D-ribofuranosyl)pyrido-[2,3-*d*]pyrimidine (2f) was prepared from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-3-*C*-ethynyl-D-ribofuranose[28] and **25** according to the general procedure: total yield 38% (2 steps) as a colorless solid; ^{1}H NMR (DMSO-d_6) δ 3.56 (s, 1 H), 3.68−3.82 (m, 2 H), 3.97 (m, 1 H), 4.29 (t, J = 6.6 Hz, 1 H), 5.21 (t, J = 4.4 Hz, 1 H, OH), 5.86 (d, J = 6.6 Hz, 1 H, OH), 5.99 (s, 1 H, OH), 6.25 (d, J = 7.8 Hz, 1 H), 6.72 (d, J = 6.9 Hz, 1 H), 8.21 and 9.57 (2 d, J = 5.1 Hz, 2 H, NH_2), 8.30 (s, 1 H), 8.37 (d, J = 8.1 Hz, 1 H). Anal. ($C_{14}H_{14}N_4O_5\cdot0.25H_2O$) C, H, N.

4-Amino-5-oxo-8-(5(*R*)-*C*-methyl-*β*-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (3a) and 4-amino-5-oxo-8-(5(*S*)-*C*-methyl-*β*-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (4a) were prepared as a mixture from 1-*O*-acetyl-5(*R*,*S*)-*C*-methyl-2,3,5-tri-*O*-benzoyl-D-ribofuranoses[19] (*R*/*S* ratio, 2:3 or 3:2; 0.65 g, 1.3 mmol) and **25** (0.24 g, 1.5 mmol) as described in the general procedure: total yield 150 mg (25%, 2 steps) as a colorless solid (5′(*R*/*S*)-isomers, 2:3); ^{1}H NMR (DMSO-d_6) δ 1.11 and 1.17 (2 d, J = 6.6 and 6.3 Hz, 3 H, Me), 3.70−3.78 (m, 1 H), 3.86 (m, 1 H), 3.98−4.15 (m, 2 H), 5.08−5.20 (m, 2 H, OH), 5.33 and 5.37 (2 d, J = 5.1 and 5.4 Hz, 1 H, OH), 6.21−6.24 (m, 1 H), 6.60 and 6.67 (2 d, J = 4.5 and 5.4 Hz, 1 H), 8.21 and 9.58 (2 br m, 2 H, NH_2), 8.27−8.47 (m, 2 H). Anal. ($C_{13}H_{16}N_4O_5\cdot0.25H_2O$) C, H, N.

4-Amino-5-oxo-8-(5(*R*)-*C*-ethyl-*β*-D-ribofuranosyl)pyrido-[2,3-*d*]pyrimidine (3b) and 4-Amino-5-oxo-8-(5(*S*)-*C*-ethyl-*β*-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (4b). A suspension of a mixture of **3f** and **4d** (*R*/*S* ratio, 1:3 or 3:1; 0.14 g, 0.45 mmol) and 10% Pd/C (200 mg) in methanol (50 mL) was shaken in a hydrogenation apparatus (3 psi hydrogen) at room temperature for 2 h, and the catalyst was filtered and washed with methanol. The solvents were evaporated, and the residue was subjected to chromatography (10% methanol in dichloromethane) to give a mixture of **3b**+**4b** (*R*/*S* ratio, 1:3 or 3:1; 0.12 g, 79%) as a colorless solid (two isomers, 1:3): ^{1}H NMR (DMSO-d_6) δ 0.88−0.94 (m, 3 H, CH_3CH$_2$), 1.46−1.56 (m, 2 H, CH$_3$CH_2), 3.54 (m, 1 H), 3.80 and 3.86 (2 m, 1 H), 4.00−4.12 (m, 2 H), 5.09 (m, 1 H, OH), 5.15 and 5.24 (2 d, J = 6.0 and 5.4 Hz, 1 H, OH), 5.32 and 5.38 (2 d, J = 5.1 and 5.4 Hz, 1 H, OH), 6.22 (d, J = 7.8 Hz, 1 H), 6.59 and 6.65 (2 d, J = 4.5 and 5.7 Hz, 1 H), 8.20 and 9.59 (2 br d, J = 4.5 Hz, 2 H, NH_2), 8.29−8.47 (m, 2 H). Anal. ($C_{14}H_{18}N_4O_5$) C, H, N.

4-Amino-5-oxo-8-(5(*R*)-*C*-propyl-*β*-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (3c) was prepared from **3d** (85 mg, 0.25 mmol) by a similar procedure as described for **3b**: yield 55 mg (65%). Recrystallization from water/methanol gave **3c** as a colorless solid: mp 218−220 °C; ^{1}H NMR (DMSO-d_6) δ 0.88 (t, J = 6.6 Hz, 3 H, CH_3CH$_2$), 1.30−1.50 (m, 4 H, CH$_3$CH_2CH_2), 3.67 (m, 1 H), 3.78 (m, 1 H), 4.10 (m, 2 H), 5.10 (d, 1 H, J = 4.5 Hz, OH), 5.14 (d, J = 5.7 Hz, 1 H, OH), 5.32 (d, J = 5.4 Hz, 1 H, OH), 6.22 (d, J = 8.4 Hz, 1 H), 6.66 (d, J = 5.4 Hz, 1 H), 8.21 and 9.59 (2 br d, J = 4.5 Hz, 2 H, NH_2), 8.30 (m, 2 H). Anal. ($C_{15}H_{20}N_4O_5$) C, H, N.

4-Amino-5-oxo-8-(5(*R*)-*C*-allyl-*β*-D-ribofuranosyl)pyrido-[2,3-*d*]pyrimidine (3d) was prepared from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-5(*R*)-*C*-allyl-D-ribofuranose[19] (0.65 g, 1.2 mmol) and **25** (0.23 g, 1.4 mmol) as described in the general procedure: total yield 0.15 g (39%, 2 steps). Recrystallization from water gave **3d** as a colorless solid: mp 208−209 °C; ^{1}H NMR (DMSO-d_6) δ 2.14−2.30 (m, 2 H), 3.71−3.82 (m, 2 H), 4.08−4.15 (m, 2 H), 5.03−5.14 (m, 3 H, incl. 1 OH), 5.31 (d, J = 5.4 Hz, 1 H, OH), 5.36 (d, J = 5.7 Hz, 1 H, OH), 5.80−5.93 (m, 1 H), 6.22 (d, J = 8.1 Hz, 1 H), 6.65 (d, J = 5.7 Hz, 1 H), 8.22 and 9.58 (2 br d, J = 4.8 Hz, 2 H, NH_2), 8.29 (d, J = 9.0 Hz, 1 H), 8.30 (s, 1 H). Anal. ($C_{15}H_{18}N_4O_5$) C, H, N.

4-Amino-5-oxo-8-(5(*R*,*S*)-*C*-vinyl-*β*-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (3e) and 4-amino-5-oxo-8-(5(*R*,*S*)-*C*-vinyl-*β*-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (4c) were prepared as a mixture from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-5(*R*,*S*)-*C*-vinyl-D-ribofuranoses[19] (*R*/*S* ratio, 1:1; 0.41 g, 0.77 mmol) and **25** (0.15 g, 0.93 mmol) as described in the general procedure: total yield 0.095 g (39%, 2 steps) as a colorless solid (5′(*R*/*S*)-isomers, 1:1); ^{1}H NMR (DMSO-d_6) δ 3.85−3.92 (m, 1 H), 4.00−4.19 (m, 2 H), 4.25 (m, 1 H), 5.10−5.19 (m, 2 H, including 1 OH), 5.27−5.41 (m, 2 H, include. 1 OH), 5.59 (m, 1 H, OH), 6.23 (m, 1 H), 6.58 and 6.72 (2 d, J = 5.1 and 7.2 Hz, 1 H), 8.21 and 9.58 (2 br m, 2 H, NH_2), 8.29−8.44 (m, 2 H). Anal. ($C_{14}H_{16}N_4O_5\cdot0.2H_2O$) C, H, N.

4-Amino-5-oxo-8-(5(*R*,*S*)-*C*-ethynyl-*β*-D-ribofuranosyl)-pyrido[2,3-*d*]pyrimidine (3f) and 4-amino-5-oxo-8-(5(*R*,*S*)-*C*-ethynyl-*β*-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (4d) were prepared as a mixture from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-5(*R*,*S*)-*C*-ethynyl-D-ribofuranoses[19] (*R*/*S* ratio, 1:1; 1.5 g, 2.8 mmol) and **25** (0.50 g, 3.1 mmol) as described in the general procedure: total yield 0.43 g (51%, 2 steps) as a colorless solid (5′(*R*/*S*)-isomers, 1:1); ^{1}H NMR (DMSO-d_6) δ 3.42 and 3.50 (2 d, J = 1.8 Hz, 1 H), 3.92 (m, 1 H), 4.02−4.20 (m, 2 H), 4.44−

4.50 (m, 1 H), 5.28 and 5.33 (2 d, J = 4.2 and 4.8 Hz, 1 H, OH), 5.40 and 5.46 (2 d, J = 6.0 Hz, 1 H, OH), 6.11 and 6.16 (2 d, J = 5.4 and 5.7 Hz, 1 H, OH), 6.24 (m, 1 H), 6.68 and 6.77 (2 d, J = 6.0 and 6.9 Hz, 1 H), 8.20−8.31 (m, 2 H), 8.23 and 9.57 (2 br d, J = 3.3 Hz, 2 H, NH_2). Anal. ($C_{14}H_{14}N_4O_5$) C, H, N.

4-Amino-5-oxo-8-(4-*C*-methyl-*β*-D-ribofuranosyl)pyrido-[2,3-*d*]pyrimidine (5a) was prepared from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-4-*C*-methyl-D-ribofuranose[19] (0.60 g, 1.2 mmol) and **25** (0.23 g, 1.4 mmol) as described in the general procedure: total yield 0.13 g (39%, 2 steps). Recrystallization from water gave **5a** as a colorless solid: mp 124−127 °C; ^{1}H NMR (DMSO-d_6) δ 1.13 (s, 3 H, Me), 3.4 (m, 2 H), 3.99 (t, J = 5.1 Hz, 1 H), 4.29 (m, 1 H), 5.11 (d, J = 4.8 Hz, 1 H, OH), 5.27 (d, J = 6.6 Hz, 1 H, OH), 5.31 (t, J = 5.1 Hz, 1 H, OH), 6.24 (d, J = 8.1 Hz, 1 H), 6.71 (d, J = 6.6 Hz, 1 H), 8.19 and 9.59 (2 br d, J = 4.6 Hz, 2 H, NH_2), 8.30 (s, 1 H), 8.32 (d, J = 8.1 Hz, 1 H). Anal. ($C_{13}H_{16}N_4O_5$) C, H, N.

4-Amino-5-oxo-8-(4-*C*-ethyl-*β*-D-ribofuranosyl)pyrido-[2,3-*d*]pyrimidine (5b) was prepared from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-4-*C*-ethyl-D-ribofuranose[19] (0.70 g, 1.3 mmol) and **25** (0.26 g, 1.6 mmol) as described in the general procedure: total yield 0.15 g (39%, 2 steps). Recrystallization from methanol gave **5b** as a colorless solid: mp 230−232 °C; ^{1}H NMR (DMSO-d_6): δ 0.85 (t, J = 7.5 Hz, 3 H, CH_3CH$_2$), 1.55−1.70 (m, 2 H, CH$_3$CH_2), 3.50 (d, J = 4.8 Hz, 2 H), 4.02 (t, J = 4.9 Hz, 1 H), 4.34 (m, 1 H), 5.07 (d, J = 4.5 Hz, 1 H, OH), 5.22 (m, 2 H, OH), 6.25 (d, J = 8.1 Hz, 1 H), 6.72 (d, J = 7.5 Hz, 1 H), 8.19 and 9.59 (2 br d, J = 4.6 Hz, 2 H, NH_2), 8.30 (m, 2 H). Anal. ($C_{14}H_{18}N_4O_5$) C, H, N.

4-Amino-5-oxo-8-(4-*C*-vinyl-*β*-D-ribofuranosyl)pyrido-[2,3-*d*]pyrimidine (5c) was prepared from **22** (0.60 g, 1.1 mmol) and **25** (0.24 g, 1.5 mmol) as described in the general procedure: total yield 0.17 g (52%, 2 steps). Recrystallization from methanol gave **5c** as a colorless solid: mp 211−213 °C; ^{1}H NMR (DMSO-d_6) δ 3.32−3.38 (m, 1 H), 3.51−3.57 (m, 1 H), 4.18−4.28 (m, 2 H), 5.10−5.32 (m, 4 H, incl. 2 OH), 5.43 (t, J = 5.1 Hz, 1 H, OH), 5.97 (dd, J = 10.8 and 17.4 Hz, 1 H), 6.26 (d, J = 8.1 Hz, 1 H), 6.73 (d, J = 6.0 Hz, 1 H), 8.22 and 9.59 (2 br d, J = 4.6 Hz, 2 H, NH_2), 8.30 (s, 1 H), 8.36 (d, J = 8.4 Hz, 1 H). Anal. ($C_{14}H_{16}N_4O_5$) C, H, N.

4-Amino-5-oxo-8-(4-*C*-hydroxymethyl-*β*-D-ribofurano-syl)pyrido[2,3-*d*]pyrimidine (5d) was prepared from 1-*O*-acetyl-4-*C*-benzoyloxymethyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose[19] (1.8 g, 2.8 mmol) and **25** (0.47 g, 2.9 mmol) as described in the general procedure: total yield 0.36 g (35%, 2 steps). Recrystallization from water gave **5b** as a colorless solid: mp 156−158 °C; ^{1}H NMR (DMSO-d_6) δ 3.6 (m, 4 H), 4.13 (m, 1 H), 4.27 (m, 1 H), 4.67 (br m, 1 H, OH), 5.16 (d, J = 3.9 Hz, 1 H, OH), 5.23 (t, J = 4.6 Hz, 1 H, OH), 5.32 (br m, 1 H, OH), 6.25 (d, J = 8.1 Hz, 1 H), 6.77 (d, J = 6.9 Hz, 1 H), 8.20 and 9.58 (2 br d, J = 4.8 Hz, 2 H, NH_2), 8.30 (s, 1 H), 8.31 (d, J = 7.8 Hz, 1 H). Anal. ($C_{13}H_{16}N_4O_6$) C, H, N.

4-Amino-5-oxo-8-(2,3-dideoxy-*β*-D-ribofuranosyl)pyrido-[2,3-*d*]pyrimidine (6a). DMAP (0.2 g, 1.6 mmol) and TBDPS-Cl (0.43 mL, 1.6 mmol) were added to a solution of **1a** (0.41 g, 1.5 mmol) in pyridine (15 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2 h, then water (0.1 mL) was added, and the solvent was evaporated. After the usual workup, the residue was converted to **6a** by a similar procedure as described for **1a**: yield 65 mg (18%, 4 steps). Recrystallization from ethyl acetate/methanol gave **6a** as a colorless solid: mp 174−176 °C; ^{1}H NMR (DMSO-d_6) δ 1.80−2.10 (m, 3 H), 2.35−2.45 (m, 1 H), 3.58 (m, 1 H), 3.76 (m, 1 H), 4.10 (m, 1 H), 5.13 (t, J = 5.1 Hz, 1 H, OH), 6.15 (d, J = 8.1 Hz, 1 H), 6.60 (m, 1 H), 8.15 and 9.60 (2 br d, J = 4.8 Hz, 2 H, NH_2), 8.28 (s, 1 H), 8.39 (d, J = 8.1 Hz, 1 H). Anal. ($C_{12}H_{14}N_4O_3$) C, H, N.

4-Amino-5-oxo-8-(2-deoxy-*β*-D-xylofuranosyl)pyrido-[2,3-*d*]pyrimidine (6b). Compound **32** (0.29 g, 0.58 mmol) and DMAP (0.21 g, 1.7 mmol) were dissolved in acetonitrile (12 mL) and phenyl chlorothionoformate (0.12 mL, 0.87 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 2 h and the solvent was removed. After the usual workup, the residue was dissolved in toluene (4.7 mL), followed by addition of tris(trimethylsilyl)silane (0.45 mL, 1.5 mmol) and 1,1′-azobis(cyclohexanecarbonitrile) (37 mg, 0.15 mmol). The reaction mixture was heated at reflux for 2 h, then cooled to room temperature. The solvent was evaporated and the residue was subjected to chromatography (0−2% methanol in dichloromethane). The product was dissolved in methanolic ammonia (11 mL) and the reaction mixture was sealed and stirred at room temperature for 24 h. The solvent was evaporated and the residue was subjected to chromatography (10% methanol in dichloromethane) to give 110 mg of **6b** (74%, 3 steps) as a colorless solid: mp 214−216 °C (recrystallized from methanol); ^{1}H NMR (DMSO-d_6) δ 1.96 (d, J = 14.4 Hz, 1 H), 2.62−2.72 (m, 1 H), 3.76 (m, 2 H), 3.94 (m, 1 H), 4.27 (m, 1 H), 4.78 (t, J = 5.4 Hz, 1 H, OH), 5.21 (d, J = 3.3 Hz, 1 H, OH), 6.23 (d, J = 8.1 Hz, 1 H), 6.70 (m, 1 H), 8.17 and 9.61 (2 br d, J = 4.9 Hz, 2 H, NH_2), 8.25 (d, J = 8.1 Hz, 1 H), 8.30 (s, 1 H). Anal. ($C_{12}H_{14}N_4O_4$) C, H, N.

(B) MTS Cytotoxicity Assay. All compounds tested in vitro were dissolved in DMSO (100 mM solution) and subsequently diluted in the culture medium before treatment of the cultured cells. Tested cells were plated in 96-well plates at a density of 4 × 10^3 cells/well/200 μL of the proper culture medium and treated with the compounds at concentrations of 0.78−100 μM. In parallel, the cells were treated with 1% of DMSO as control.

MTS Assay (Promega, G5430) was performed 72 h later according to instructions provided by Promega. This assay is based on the cellular conversion of the tetrazolium salt, MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt], into a formazan that is soluble in cell culture medium and is measured at 490 nm directly in 96-well assay plates without additional processing. Absorbance is directly proportional to the number of living cells in culture. Three types of cells were used in these studies: (1) normal human dermal fibroblasts (NDHF) from Clonetics, Inc., #CC-2509, cultivated in FGM medium provided by Clonetics, #CC-3132; (2) human prostate cancer cells DU-145 (HTB-81) provided by ATCC and cultivated in MEM (ICN, #1210254) supplemented with 10% fetal bovine serum (Hyclone, #SH30070.03) and 2 mM of L-glutamine (ICN, #1680149); (3) mouse melanoma B16 from ATCC cultivated in the same type of medium as HTB-81 cells.

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References

(1) Chapter 3. Antimetabolites. *Cancer Chemotherapeutic Agent;* Foye, W. O., Ed.; ACS Professional Reference Book; American Chemical Society: Washington, DC, 1995.
(2) *Nucleoside Analogues in Cancer Therapy*; Cheson, B. D., Keating, M. J., Plunkett, W., Eds.; Marcel Dekker: New York, 1997.
(3) Revankar, G. R.; Robins, R. K. The Synthesis and Chemistry of Heterocyclic Analogues of Purine Nuclesides and Nucleotides. In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum: New York, 1991; Vol. 2, pp 161−398.
(4) Bobek, M.; Bloch, A. Relationship between the Structure of Sangivamycin-Derived Nucleosides and Their effect on Leukemic Cell Growth and on Protein Kinase A and C Activity. *Nucleosides Nucleotides* **1994**, *13*, 429−435.
(5) Krawczyk, S. H.; Nassiri, M. R.; Kucera, L. S.; Kern, E. R.; Ptak, R. G.; Wotring, L. L.; Drach, J. C.; Townsend, L. B. Synthesis and Antiproliferative and Antiviral Activity of 2′-Deoxy-2′-fluoroarabinofuranosyl Analogues of the Nucleoside Antibiotics Toyocamycin and Sangivamycin. *J. Med. Chem*. **1995**, *38*, 4106−4114.
(6) Krawczyk, S. H.; Renau, T. E.; Nassiri, M. R.; Westerman, A. C.; Wotring, L. L.; Drach, J. C.; Townsend, L. B. Synthesis and Evaluation of Certain Thiosangivamycin Analogues as Potential Inhibitors of Cell Proliferation and Human Cytomegalovirus. *J. Med. Chem*. **1995**, *38*, 4115−4119.
(7) Finch, Rick A.; Revankar, Ganapathi R.; Chan, Pui K. Structural and Functional Relationships of Toyocamycin on NPM-translocation. *Anti-Cancer Drug Des*. **1997**, *12*, 205−215.

(8) Loomis, C. R.; Bell, R. M. Sangivamycin, A Nucleoside Analogue, Is a Potent Inhibitor of Protein Kinases C. *J. Biol. Chem*. **1988**, *263*, 1682−1692.
(9) Rizkalla, B. H.; Broom, A. D. Pyrido[2,3-*d*]pyrimidines. III. Synthesis of Some 8-(*β*-D-Ribofuranosyl)pyrido[2,3-*d*]pyrimidines Structurally Related to the Antibiotic Sangivamycin. *J. Org. Chem*. **1972**, *37*, 3980−3985.
(10) Anderson, G. L.; Broom, A. D. Pyridopyrimidines. 7. Ribonucleosides Structurally Related to the Antitumor Antibiotic Sangivamycin. *J. Org. Chem*. **1977**, *42*, 997−1000.
(11) An unpublished result at ICN Pharmaceuticals, Inc.
(12) Cohen, M. B.; Glazer, R. I. Cytotoxicity and Inhibition of Ribosomal RNA Processing in Human Colon Carcinoma cells. *Mol. Pharmacol*. **1985**, *27*, 308−313.
(13) Takatori, S.; Kanda, H.; Takenaka, K.; Wataya, Y.; Matsuda, A.; Fukushima, M.; Shimamoto, Y.; Tanaka, M.; Sasaki, T. Antitumor Mechanisms and Metabolism of the Novel Antitumor Nucleoside Analogues, 1-(3-C-ethynyl-*β*-D-ribo-pentofuranosyl)-cytosine and 1-(3-C-ethynyl-*β*-D-ribo-pentofuranosyl)uracil. *Cancer Chemother. Pharmacol.* **1999**, *44*, 97−104.
(14) Shuto, S.: Obara, T.; Kosugi, Y.; Saito, Y.; Toriya, M.; Yaginuma, S.; Shigeta, S.; Matsuda, A. New Neplanocin Analogues. III. 6′*R*-Configuration is Essential for the Activity of 6′-*C*-Methyl-3-deazaneplanocin A's. *Bioorg. Med. Chem. Lett*. **1994**, *4*, 605−608.
(15) Gosselin, G.; Puech, F.; Genu-Dellac, C.; Imbach, J.-L. 1,2-Di-*O*-acetyl-5-*O*-benzoyl-3-deoxy-3-fluoro-D-xylofuranose. A Versatile Precursor for the Synthesis of 3-Deoxy-3-fluoro-*β*-D-xylofuranosyl Nucleosides as Potential Antiviral Agents. *Carbohydr. Res.* **1993**, *249*, 1−17.
(16) Rosenthal, A.; Mikhailov, S. Branched-Chain Nucleosides. Synthesis of 3′-*C*-Ethyl (and 3′-*C*-Butyl)Uridine. *Carbohydr. Res*. **1980**, *79*, 235−242.
(17) Wang, G.; Girardet, J.-L.; Gunic, E. Conformationally Locked Nucleosides. Synthesis and Stereochemical Assignments of 2′-*C*,4′-*C*-Bridged Bicyclonucleosides. *Tetrahedron* **1999**, *55*, 7707−7724.
(18) Harry-O′kuku, R. E.; Smith, J. M.; Wolfe M. S. A Short, Flexible Route toward 2′-C-Branched Ribonucleosides. *J. Org. Chem*. **1997**, *62*, 1754−1759.
(19) Gunic, E.; Girardet, J.-L.; Pietrzkowski, Z.; Esler, C.; Wang, G. Synthesis and Cytotoxicity of 4′-*C*- and 5′-*C*-Substituted Toyocamycins. *Bioorg. Med. Chem*., in press.
(20) Mittelbach, M.; Kastner, G.; Junek, H. Zur Synthese von 4-Hydroxynicotinsaure aus Butadiendicarbonitrilen. *Arch. Pharm*. **1985**, *318*, 481−486.
(21) Devivar, R. V.; Kawashima, E.; Revankar, G. R.; Breitenbach, J. M.; Kreske, E. D.; Drach, J. C.; Townsend, L. B. Benzimidazole Ribonucleosides: Design, Synthesis, and Antiviral Activity of Certain 2-(Alkylthio)- and 2-(Benzylthio)-5,6-dichloro-1-(*β*-D-ribofuranosyl)benzimidazoles. *J. Med. Chem*. **1994**, *37*, 2942−2949.
(22) Robins, M. J.; Wilson, J. S. 2′-Deoxyadenosine and 2′-Deoxy-2′-Deuterioadenosine. Regiospecific and Stereoselective 2′-deoxygenation of a ribonucleosides. In *Nucleic Acid Chemistry*; Townsend, L. B., Tipson, R. S, Eds.; John Wiley and Sons: New York, 1991; Part 4, pp 194−200.
(23) Chatgilialoglu, C.; Driller, D.; Lesage, M. Tris(trimethylsilyl)-silane. A New Reducing Agent. *J. Org. Chem*. **1988**, *53*, 3641−3642.
(24) Robins, M. J.; Sarker, S.; Samano, V.; Wnuk, S. F. Nucleic Acid Related Compounds. 94. Remarkably High Stereoselective Reduction of 2′- and 3′-Ketonucleoside Derivatives to Give Arabino, Ribo, and Xylofuranosyl Nucleosides with Hydrogen Isotopes at C2′ and C3′. *Tetrahedron* **1997**, *53*, 447−456.
(25) Samano, V.; Robins, M. J. Stereoselective Addition of a Wittig Reagent to Give a Single Nucleoside Oxaphosphetane Diastereomer. Synthesis of 2′(and 3′)-methyleneuridine (and cytidine) Derivatives from Uridine Ketonucleosides. *Synthesis* **1991**, 283−288.
(26) Gosselin, G.; Bergogne, M.-C.; Imbach, J.-L. 1,2-Di-*O*-acetyl-3,5-di-*O*-benzoyl-D-xylofuranose. A Versatile Precursor for the Synthesis of Various *β*-D-Pentofuranosyl Nucleosides and Related analogues. In *Nucleic Acid Chemistry*; Townsend, L. B., Tipson, R. T., Eds.; Wiley: New York, 1991; Vol. 4, pp 41−45.
(27) Gosselin, G.; Imbach, J.-L. Nucléosides Synthèse XXII: Obtention, Comme Synthons Oligoxylonucléotidiques, de D-xylofuranosyl Adenines Spécifiquement Substitués. *J. Heterocycl. Chem*. **1982**, *19*, 597.
(28) Hattori, H.; Tanaka, M.; Fukushima, M.; Sasaki, T.; Matsuda, A. 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 Broad Spectrum of Activity. *J. Med. Chem*. **1996**, *39*, 5005−5011.
(29) Saenger W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; pp 9−50.
(30) Gosselin, G.; Mathe, C.; Bergogne, M.-C.; Aubertin, A.-M.; Kirn, A.; Sommadossi, J.-P.; Schinazi, R.; Imbach, J.-L. 2′- and/or 3′-Deoxy-*β*-L-Pentofuranosyl Nucleoside Derivatives: Stereospecific Synthesis and Antiviral Activities. *Nucleosides Nucleotides* **1995**, *14*, 611−617.

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