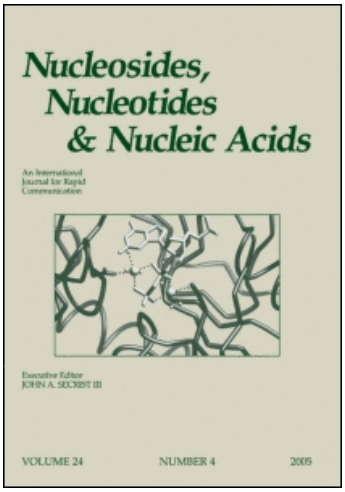

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SYNTHESIS AND ANTICANCER ACTIVITY OF 4-AMINO-5-OXO-8-(β-D-XYLOFURANOSYL) PYRIDO[2,3-*d*]PYRIMIDINE

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ABSTRACT

4-Amino-5-oxo-8-(β-D-xylofuranosyl)pyrido[2,3-*d*]pyrimidine (**4**) was recently synthesized and evaluated in our laboratories for anticancer activities. This compound showed potent *in vitro* inhibitory effects on the growth of HTB-81 prostate cancer cells and Daudi-lymphoma. *In vivo* studies showed that the compound could inhibit HTB-81 tumor growth in syngeneic mice by 93% at a daily dose of 8.5 mg/kg for 10 days.

Certain nucleosides as anticancer drugs have been used clinically for many years and new nucleoside analogues continue to show promise (1,2). However, current nucleoside anticancer drugs are commonly associated with various adverse effects (1). Apparently, there is a need to search for nucleoside analogues that can selectively inhibit cancer cell proliferation. In the past decades, a large number of sangivamycin (**1**) and toyocamycin (**2**) analogues have been synthesized (3–8) as potential anticancer and antiviral agents. Unfortunately, no compound in this group could be used clinically, primarily owing to their toxicity to normal cells. Considered as sangivamycin analogues, 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) were synthesized,

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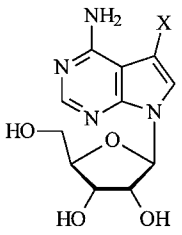
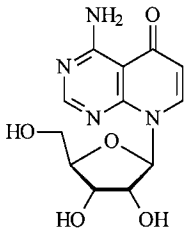
but their biological activity was not reported. To explore the possibility of pyrido[2,3-*d*]pyrimidine nucleosides as anticancer and antiviral drugs, 4-amino-5-oxo-8-(β-D-ribofuranosyl)-pyrido[2,3-*d*]pyrimidine (**3**) was synthesized at ICN Pharmaceuticals and identified as a very potent inhibitor of cancer cell proliferation (11). However, **3** also showed a similar *in vitro* cytotoxicity profile to normal human cells. The cytotoxicity of ribonucleoside analogues likely results from non-selective inhibition of RNA polymerases by their triphosphates, or from their non-selective incorporation into RNAs (12,13). We anticipated that a selective inhibition of cancer cell proliferation might be achieved if a ribonucleoside could selectively enter or be selectively metabolized in certain cancer cells. Recently, we synthesized and evaluated a series of pyrido[2,3-*d*]-pyrimidine nucleosides having modified sugars in search of selective inhibitors of cancer cells (14). 4-Amino-5-oxo-8-(β-D-xylofuranosyl)pyrido[2,3-*d*]pyrimidine (**4**) was identified as a potent inhibitor of some cancer cells with certain selectivity. In this communication, we report the synthesis and anticancer activities of **4**.



1 X = $CONH_2$
2 X = CN



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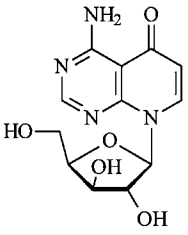

4

CHEMISTRY

Compound **5** (14) was treated with *N*,*O*-bis(trimethylsily)acetamide (BSA) in 1,2-dichloroethane at 60°C for 2 h and then the resulting silylated base was condensed with 1-*O*-acetylated xylofuranose derivative **6** (14). Compound **7** was formed readily and then slowly converted to **8** until an equilibrium was reached after 2-3 days. Compounds **7** and **8** were separated by flash chromatography and then subjected to a treatment with methanolic ammonia to give **9** and **4**, respectively. By this approach, the by-product **9** was formed in a significant amount. In order to avoid the formation of **7**, compound **5** was acetylated to give **11**, which was silylated and then condensed with **6** to give **12** directly after several hours. By the second approach, the N-1 coupled product was not formed and the purification of the final product was simplified. It can be noted that the yield of the final product **4** by the second approach was not greatly improved. It was observed that the acetyl on the N4 of the base was not stable enough under the condensation conditions.

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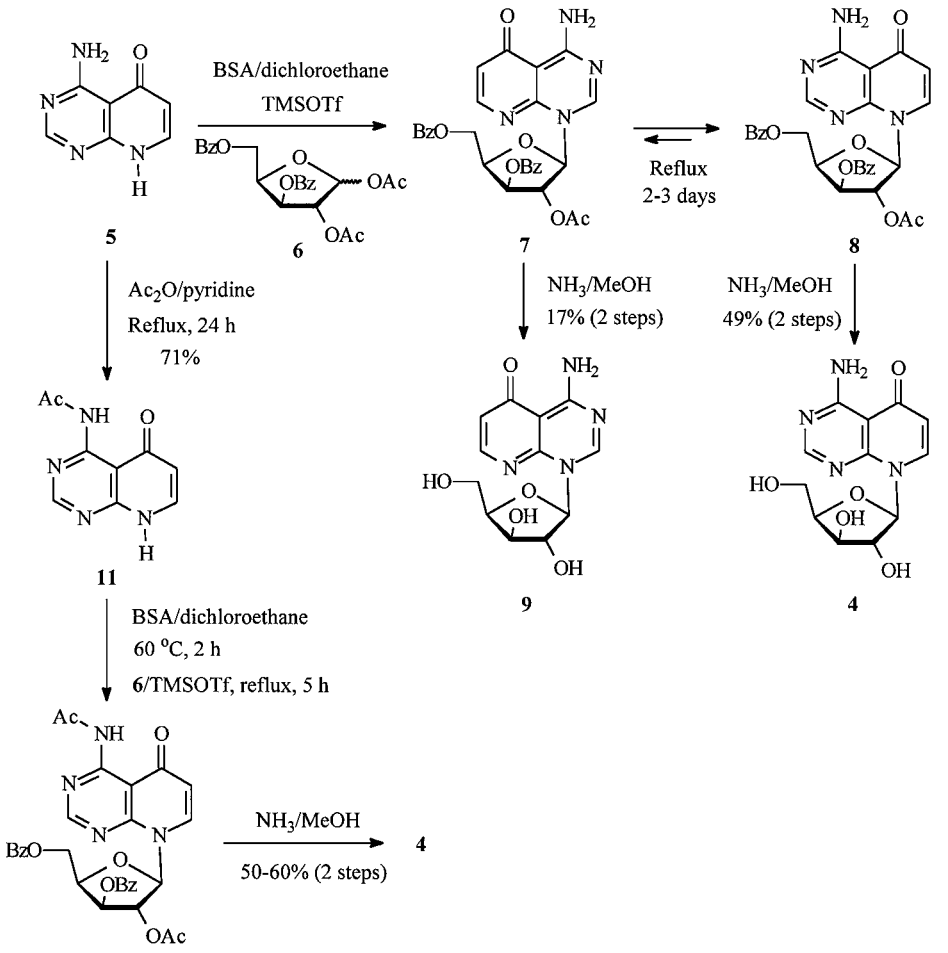
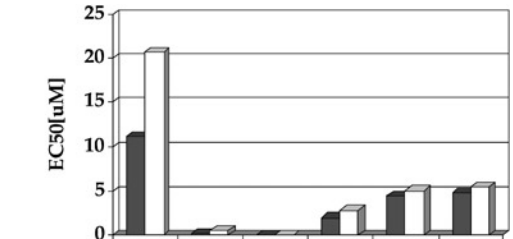
It is anticipated that the substitution of a benzoyl group for the acetyl would further increase the yield of **4**.



ANTICANCER ACTIVITIES

Our *in vitro* screening approach for anticancer activity was based on two assays: MTS (colorimetric) and DNA-Hoechst33342 binding assay (fluorescent method). Both assays were performed in parallel and under the same culture conditions (treated for 72 h). These methods were very reliable for following the growth of cells by mitochondrial activity (MTS Assay) or total amount of DNA in the cell culture. For the cancer cells tested, these two methods produced very similar results; however, in the case of normal cells, some discrepancies were noticed. The results shown in Figure 1 indicate that compound **4** has potent inhibitory effects on the growth of Daudi-lymphoma ($EC_{50} = 0.10\ \mu M$) and prostate



	NHF	HTB-81	Daudi	NB4	K562	B16
■ EC50-DNA	11.22	0.34	0.1	2.14	4.55	4.89
□ EC50-MTS	20.7	0.73	0.12	2.91	5.2	5.55

Figure 1. *In vitro* inhibitory effects of compound **4** on the growth of various cancer cells and normal human fibroblasts. EC_{50}-DNA is a concentration [μM] of the compound that caused a 50% reduction of total DNA amount after 72 h treatment relative to untreated cells using fluorescent method (Hoechst Dye). EC_{50}-MTS is a concentration [μM] of the compound that caused a 50% reduction in absorbance at 490 nm after 72 h treatment relative to untreated cells using MTS assay.

cancer HTB-81 cells ($EC_{50} = 0.73$ μM). The other types of cells tested were less sensitive to the compound, with normal human fibroblasts being least sensitive ($EC_{50} = 20.7$ μM).

Table 1 gives another set of data which shows the *in vitro* inhibitory effects of compound **4** on the growth of various cancer cells based on an assay using alamarBlue reaction. The EC_{50} values vary between 3.6-32 μM, indicating that this compound is not equally potent towards all types of cancer cells. Moreover, even among cell lines derived from the same types of tumors, compound **4** showed different potency. Thus, human prostate cancer cells HTB-81 and PC-3 have different sensitivities to the compound, with $EC_{50} = 0.78$ μM for HTB-81 and 16 μM for PC-3. Another type of prostate cancer cell, LyLu, is even less sensitive to the compound, with $EC_{50} = 55$ μM (not shown in Fig. 1 and Table 1). Similarly, two

Table 1. Inhibitory Effects of Compound **4** on the Growth of Various Cancer Cells

Cancer Cell Line	EC_{50} [μM]
MCF7/Breast	3.6
T47D/Breast	10.0
HT29/Colon	32.0
ACHN/Kidney	7.9
K562/Leu	10.0
HC4/Liver	9.3
HepG2/Liver	9.5
PC6/Lung	12.0
SKMEL5/Melanoma	16.0
PC3/Prostate	12.0

EC_{50} is the concentration of the compound that causes a 50% reduction in cell proliferation relative to untreated cells (alamarBlue reaction).

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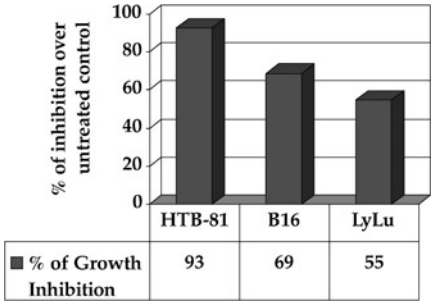

Figure 2. *In vivo* inhibition of tumor growth by compound **4**. % inhibition over untreated control: a percentage by which tumor growth is inhibited by compound **4** compared to the untreated. % = (U-T)/U, U is the average tumor weight of the untreated animals and T is the average tumor weight of the treated animals. Animals were treated I.p. daily for 10 days with a dose of 8.5 mg/kg of compound **4**. Human prostate cancer HTB-81 grew in nude mice; mouse melanoma B16 grew in syngeneic mice; human prostate cancer LyLu grew in syngeneic rats.

breast cancer cell lines (CF-7 and T47D) also showed different sensitivities to the compound. These results suggest that compound **4** may not be equally transported to all types of cancer cells or may be metabolized at different rates in different cancer cells.

Based on the *in vitro* inhibitory data, an *in vivo* study was initiated to evaluate the inhibitory effects of compound **4** on tumor growths in animals. As can be seen from Figure 2, compound **4** showed a strong inhibition on the growth of HTB-81 tumor in nude mice while less inhibition was observed for B16 tumor in syngeneic mice and Lylu tumor in syngeneic rats. These results are proportional to the *in vitro* inhibitory data (Fig. 1).

Figure 3 shows a dose-responsive inhibition of B16 tumor in syngeneic mice by compound **4**. Although the compound has an EC_{50} value of around 5 μM in

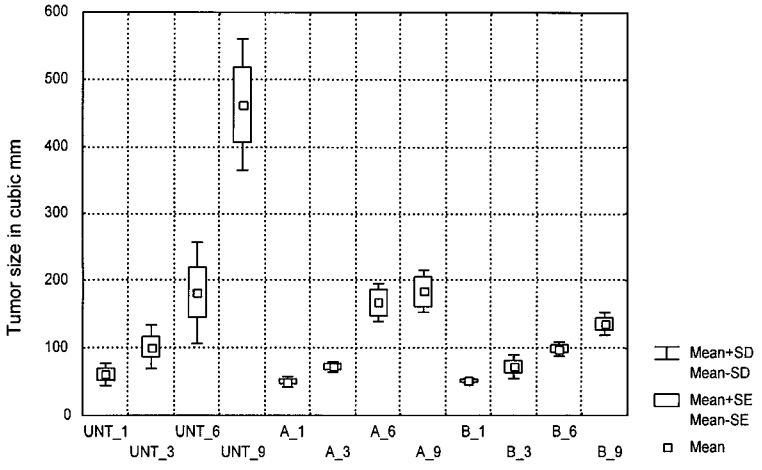

Figure 3. Compound **4** inhibits the growth of melanoma B16 in syngeneic mice in a dose-dependent manner. A = 1.7 mg/kg and B = 8.7 mg/kg.

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both total DNA and MTS assays, the *in vivo* study demonstrated that the compound could effectively inhibit the tumor growth (also see Fig. 2). The tumor inhibition by compound **4** at a dose of 8.7 mg/kg is significantly more effective than that at a dose of 1.7 mg/kg.

In summary, synthesis of 4-amino-5-oxo-8-(β-D-xylofuranosyl)pyrido[2,3-*d*]-pyrimidine (**4**) was explored. The compound showed potent *in vitro* inhibitory effects on the growth of human prostate cancer HTB-81 and Daudi-lymphoma while a moderate, broad-spectrum inhibition on various cancer cells was observed. An effective inhibition (by 93%) of HTB-81 tumor growth in nude mice was achieved at a dose of 8.5 mg/kg of compound **4**. Further studies are underway to evaluate the *in vivo* efficacy and toxicity of the compound.

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