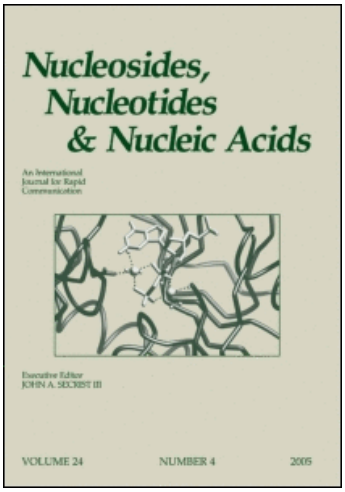

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ENZYMATIC SYNTHESIS OF 2',5'-DIDEOXY PURINE NUCLEOSIDES AND RELATED COMPOUNDS

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ABSTRACT. A wide range of 2',5'-dideoxy-nucleosides, including 6-substituted purine, pyrazolo[3,4-*d*]pyrimidine and 1-deazapurine derivatives, has been enzymatically prepared using purine nucleoside phosphorylase. Specificity towards cleavage by bacterial versus mammalian purine nucleoside phosphorylase was evaluated.

Nucleosides have proved to be of considerable biological interest as antiviral and antitumor agents, and several compounds in this class have found utility as therapeutic agents. A new combination therapy approach has recently been reported, which offers the potential for reducing the side effects of therapeutic agents such as nucleosides. This approach, which has been termed "suicide gene therapy," involves introduction of an exogenous gene into tumor cells, together with systemic administration of a masked or depot form of a chemotherapeutic agent. The gene introduced results in the production of an exogenous protein, which in turn converts the depot form of the therapeutic agent to the active entity. Normal cells are not affected by this process, since they do not contain the exogenous gene and therefore cannot metabolize the non-toxic precursor, whereas tumor cells are destroyed by the toxic compound generated from the depot.

Several approaches to suicide gene therapy have been described.[1-5] One variation involves a retroviral vector containing the *Herpes simplex* virus thymidine kinase gene in combination with the nucleoside analog Ganciclovir (9-[1,3-dihydroxy-2-propoxymethyl]guanine).[1,2] Phosphorylation of Ganciclovir by the expressed thymidine kinase gene present in the tumor cells produced

toxic metabolites which were incorporated into DNA and RNA, resulting in tumor cell death. Systemic toxicity was avoided since the thymidine kinase enzyme in normal human cells possesses a different specificity than that derived from the transfected bacterial gene, so that toxic metabolites were not produced systemically. A disadvantage of Ganciclovir, however, is that after phosphorylation by thymidine kinase, the phosphorylated derivatives cannot readily pass through cell membranes and affect neighboring tumor cells. A related approach used the *Herpes simplex* thymidine kinase gene in combination with 5-iodo-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (FIAU).[1] Treatment with a vector for the cytosine deaminase gene together with 5-fluorocytosine has also been used.[3]

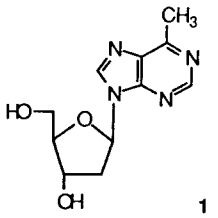
A promising approach to suicide gene therapy employs the gene for bacterial purine nucleoside phosphorylase (PNP) in conjunction with 9-(2-deoxy-β-D-ribofuranosyl)6-methylpurine (**1**, Figure 1).[4,5] Profound toxicity was observed in human melanoma cells after treatment with **1**, which was converted by *E. coli* PNP to the highly toxic 6-methylpurine. In contrast to the Ganciclovir approach, 6-methylpurine was capable of freely diffusing across cell membranes and was therefore able to exert an effect on neighboring tumor cells. This ability to destroy neighboring tumor cells increases the potential for suicide gene therapy, and has been termed the "bystander effect."[2] Bystander toxicity in this case was estimated to be at least 100 cells killed for each cell expressing PNP.

Another approach to suicide gene therapy described a method for specifically killing tumor cells expressing certain oncoproteins.[6] In this approach, the target oncoprotein binds to exogenously introduced gene products, resulting in transcriptional activation of a toxic gene. As an example, a construct with mutant p53 as a control element in combination with bacterial PNP and **1** was able to specifically kill cells overexpressing the mutant p53 gene in cell culture.

In view of the interest in suicide gene therapy with the PNP system, we have prepared a series of 2',5'-dideoxy nucleosides in an attempt to identify a compound with superior activity to that of **1**. The synthesis of these compounds, together with an initial evaluation of their specificity towards bacterial versus human PNP, is described in this report.

RESULTS AND DISCUSSION

The compounds required for this study could in principle be prepared either by chemical synthesis or by enzymatic methods. Enzymatic processes



1

Figure 1

possess the advantage of normally producing the β-isomer exclusively, whereas chemical methods frequently produce a mixture of isomers which need to be separated by chromatography, crystallization, or some other method. Since relatively small amounts of each material were needed for biological testing, the enzymatic approach was selected in this work as a rapid and convenient method for generating a broad range of analogs without the need for extensive chemical synthesis and purification.

Several reports of nucleoside synthesis by enzymatic methods have been previously described. Bacterial PNP has been used in conjunction with thymidine or uridine phosphorylase to prepare purine nucleosides from the parent purine via a sugar-1-phosphate intermediate[7] and the method has been used to prepare a wide variety of nucleosides.[8,9,10] A related approach used 7-methylguanosine as the ribosyl donor,[11] which offered the advantage that the by-product 7-methylguanine was highly insoluble and could be readily removed from the mixture by filtration. Deoxyribosyltransferases from *Lactobacillus* have also been used to prepare nucleoside analogs.[12-14]

One of the potential disadvantages of **1** is that it possesses a 5'-hydroxyl group and is therefore capable of being phosphorylated by kinases which are known to be present in a wide variety of human tissues. The phosphorylated derivatives might be incorporated into DNA, thus potentially producing undesired toxic effects in normal cells, as well as tumor cells. A series of nucleoside derivatives of 6-methylpurine has been previously prepared and tested in cell culture, and it was postulated that cytotoxicity may correlate with the efficiency with which they are converted into nucleotides by adenosine kinase.[15] 5'-Deoxy compounds might be able to reduce this undesirable toxicity towards normal cells, since they cannot be directly phosphorylated to toxic metabolites. In addition, cleavage of a 5'-deoxy nucleoside by bacterial PNP

would generate a 5'-deoxy sugar-1-phosphate, which might interfere with the biochemical pathways involved with ribose or deoxyribose metabolism, as well as a potentially toxic purine base. Such a "double barreled" mechanism has the potential of increasing the effectiveness of the suicide gene therapy approach.

The deoxyribosyl transfer reaction catalyzed by PNP normally employs a pyrimidine nucleoside, such as thymidine or uridine, as the glycosyl donor and a purine analog as the glycosyl acceptor. We have used this methodology to synthesize a series of 2',5'-dideoxy-ribofuranosyl-purine nucleosides from 5'-deoxythymidine (Figure 2). To our knowledge the versatility of this system has not been studied in detail for 5'-deoxy derivatives, although two compounds of this type have been prepared enzymatically.[8,14] The glycosyl donor 5'-deoxythymidine (**2**) was synthesized from 5'-iodothymidine[16] in 50% yield by treatment with tri-n-butyl tin hydride in the presence of the radical initiator 1,1'-azobis(cyclohexanecarbonitrile).

Synthesis of Purine Nucleosides.

A series of purine nucleosides was prepared from the corresponding purine bases using the PNP/thymidine phosphorylase system. We initially concentrated our efforts on the synthesis of N_6-substituted derivatives, since prior researchers showed that *E. coli* PNP recognized 6-modified purine nucleosides as substrates, whereas modifications at positions 2, 7 and 8 of the purine ring were less well tolerated.[17] The purines were either commercially available or prepared from 6-chloropurine by reaction with the appropriate amine. In general, the progress of the glycosyl transfer could be monitored by HPLC and the presence of a later eluting peak indicated that nucleoside synthesis had taken place. The compounds were isolated by reversed phase HPLC, using a C_4 column with a water/acetonitrile gradient. The 6-methylpurine derivative **3** could readily be prepared by this method from **2** and 6-methylpurine, and the 2'-deoxy derivative **1** was prepared in the same way from thymidine, although 6-trichloromethylpurine was not a substrate. In terms of N_6-substituted derivatives, the dimethylamino and furfurylamino compounds **4** and **5** were prepared from N_6-dimethylaminopurine and N_6-furfurylaminopurine respectively. N_6-Allylaminopurine, N_6-bis-hydroxyethyladenine, N_6-trimethylamino-purine, N_6-piperidinopurine, and N_6-triazolopurine were not substates for PNP, even though the more bulky furfurylamino compound was tolerated as a substrate.

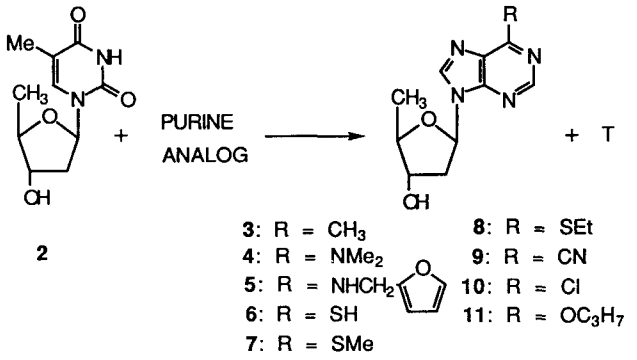


Figure 2

6-Thio derivatives were of particular interest due to the demonstrated antitumor activity of 6-thiopurine analogs[18] together with the fact that *E. coli* PNP has been shown to exhibit an increased affinity for nucleosides with sulfur substituents at the 6-position.[17] Purine analogs prepared in this series included the 6-thio (**6**), methylthio (**7**) and ethylthio (**8**) derivatives. 6-Cyanopurine, 6-chloropurine, 6-propoxypurine, 2,6-dichloropurine, 1-methyl-adenine, adenine-1-oxide and 2-amino-6-chloropurine all produced the corresponding nucleosides **9-15** respectively (Figures 2 and 3), whereas 2-aminopurine and 3-methyladenine were unreactive.

A nucleoside of the poorly soluble thioguanine could not be obtained, even though 2'-deoxythioguanosine has been previously prepared in good yield using a deoxyribosyl transferase from *Lactobacillus*.[12]

Other Heterocycles.

In addition to the purines described above, several other heterocylic systems were evaluated as potential substrates for PNP. Krenitsky et al. have shown that certain imidazo[4,5-*c*]pyridines (3-deazapurines) could be used as substrates, so that the enzyme is known to accept heterocycles other than purines.[8] Both 4-keto- and 4-thio-pyrazolo[3,4-*d*]pyrimidine were found to be substrates, giving the dideoxy compounds **16** and **17**, and the corresponding 2'-deoxy compounds were also prepared for comparison. The 2',5'-dideoxy derivative of 4-amino-pyrazolo[3,4-*d*]pyrimidine could not be prepared, even though the corresponding synthesis of the 2'-deoxy compound was successful.

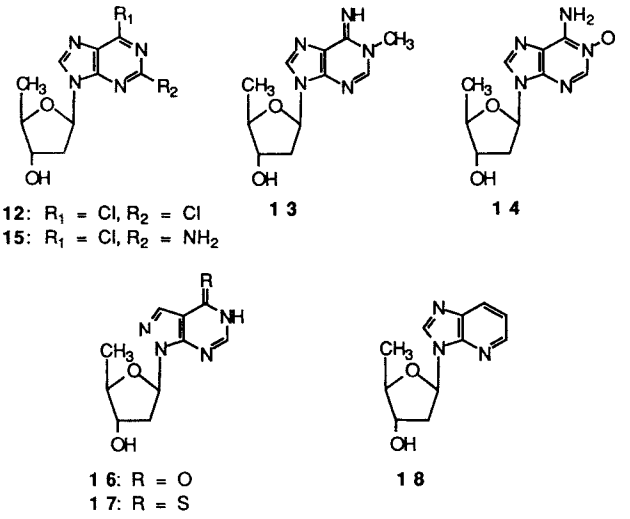


Figure 3

Several 4,6-disubstituted pyrazolo[3,4-*d*]pyrimidines, such as the 4-keto-6-thio-, 4-amino-6-keto-, and 6-keto-4-thio- derivatives, were also investigated, but none were tolerated as substrates.

Other heterocycles which were evaluated included imidazo[4,5-*b*]pyridine and indole-related ring systems. Imidazo[4,5-*b*]pyridine (1-deazapurine) gave the expected nucleoside **18**, and the site of substitution was assigned by comparison of the ultraviolet spectra of **18** versus data reported for the corresponding ribofuranosyl and 2'-deoxyribofuranosyl derivatives.[13,19] This comparison suggested that glycosylation occurred at the N_9- rather than the N_7-position. Indole derivatives which were examined included 4-nitro-, 4-methoxy-, 4-hydroxy-, 5-hydroxy- and 5-amino-indole, but none of these yielded the desired nucleoside. Similarly, 7-azaindole, indazole and 6-aminoindazole were found not to be substrates. In some instances the lack of reactivity may be due to the poor solubility of the heterocycle, whereas in most cases the material was quite soluble but was presumably not recognized as a substrate.

The intermediate 5'-iodo-thymidine was also evaluated as a glycosyl donor, but an incubation with 6-methylpurine in the presence of PNP and thymidine phosphorylase did not yield any product. The presence of the bulky iodo group is presumably unacceptable to the enzyme, whereas the more

compact deoxy group is much less likely to produce unfavorable steric interactions.

Cleavage by Human vs Bacterial PNP.

A preliminary study was carried out to compare the susceptibility of the nucleosides prepared to human versus bacterial PNP, since a difference in specificity is essential for the suicide gene therapy approach to be effective in vivo. In this study, samples were incubated with fixed amounts of both enzymes and the percentage of degradation after one hour was determined by HPLC. These results are shown in Table 1.

Several of the compounds are as efficient as, or are superior to, **1** in terms of specificity of cleavage by the bacterial enzyme, but not the human enzyme. These analogs include the 6-methylthio, 6-ethylthiopurine, and 6-propoxypurine derivatives **7**, **8** and **11** respectively. Other derivatives such as the dimethylamino derivative **4** and the 6-chloropurine compound **10** are much less specific since they undergo cleavage by both *E. coli* and human PNP. Previous studies have shown that mammalian and bacterial PNP have quite different structural requirements. Modifications to the N_6-position could be tolerated by the bacterial enzyme,[7,9] whereas modifications at these positions tended to reduce their effectiveness as substrates for the human enzyme.[20] Methylation at N_1 was also tolerated by the bacterial enzyme, as shown by the activity of **13**, although the N_1-oxide **14** was not a substrate for either enzyme. Bzowska et al. have postulated that the N_1-position is a binding site for the mammalian, but not the bacterial enzyme.[20]

EXPERIMENTAL

General Procedures. ^{1}H NMR spectra were recorded using a Varian Unity Plus 500 MHz spectrometer in DMSO-d_6 unless otherwise stated. HPLC purification was performed using a Waters 600E system controller equipped with a multisolvent delivery system and a Model 991 photodiode array detector. Analytical HPLC was carried out using Waters C_4 Nova-Pak cartridges (8 × 100 mm) using a gradient of 0-50% acetonitrile in water. Reagents were purchased from Aldrich Chemical Co., Milwaukee, WI or Lancaster Synthesis Inc., Windham, NH. Mammalian and bacterial PNP and thymidine phosphorylase were purchased from Sigma Chemical Co., St. Louis, MO. Electrospray mass

TABLE 1

Cleavage of 2',5'-Dideoxynucleosides by PNP

COMPOUND	PURINE ANALOG	% CLEAVAGE*	
		E. coli	Human
	Inosine	51	57
1	6-Methyl	57	0
3	6-Methyl	40	0
4	6-Dimethylamino	37	18
5	6-Furfurylamino	58	0
6	6-Thio	62	12
7	6-Methylthio	47	0
8	6-Ethylthio	55	0
9	6-Cyano	69	35
10	6-Chloro	69	10
11	6-Propoxy	36	0
12	2,6-Dichloro	49	0
13	6-Amino-1-methyl	42	0
14	6-Amino-1-oxide	0	0
15	2-Amino-6-chloro	46	0
16	4-Keto-pyrazolopyrimidine	0	0
17	4-Thio-pyrazolopyrimidine	0	0
18	1-Deazapurine	32	0

* Analogs (1 mM) were incubated with 1 unit of each enzyme for 1 hour at 37°.

spectrometry was performed on an APPI I Perkin Elmer SCIEX mass spectrometer by Mass Consortium Corporation, San Diego, CA.

5'-Deoxythymidine (2). 1,1'-Azobis(cyclohexanecarbonitrile) (1.56 g, 6.39 mmol) was added to a solution of 5'-iodothymidine[16] (4.5 g, 12.78 mmol) and tributyltin hydride (10.3 mL, 38.35 mmol) in dry THF (200 mL). The reaction was stirred overnight at room temperature, and analysis of the reaction mixture by silica TLC using ethyl acetate/methylene chloride (9:1) indicated quantitative conversion of starting material. The reaction was concentrated under reduced pressure to yield a white solid which was redissolved in ethyl acetate/methylene chloride (9:1) and applied to a flash column. The desired material was eluted using the same solvent to yield 1.45 g (50%) of pure **2** as a white solid. The product was analyzed on a C4 analytical HPLC column with a gradient of 0-50% acetonitrile in water. The desired product was eluted at 24.60 min.

UV λ_{max} (pH 7.2) 257 (ε 6.75 $\times$ 10^3). ^{1}H NMR δ 11.2 (bs, 1H, NH), 7.4 (s, 1H, H_6), 6.0 (t, 1H, J = 6.5 Hz), 5.2 (d, 1H, J = 4.5 Hz, 3'-OH), 3.9 (m, 1H, $H_{3'}$), 3.7 (m, 1H, $H_{4'}$), 2.0-2.2 (m, 2H, $H_{2'}$, $_{2''}$), 1.8 (s, 3H, 5-CH_3), 1.2 (d, 3H, J = 6.5 Hz, 5'-CH_3). Electrospray MS: *m/z* 227 (MH^+), 249 (MNa^+), 225 (M^-), calcd. for $C_{10}H_{14}O_4N_2$: (M) 226.09.

General Procedure for Enzymatic Synthesis. Incubations were carried out on a small scale and generally contained 1.36 μmol of the purine analog, 1.1 equivalents of 5'-deoxythymidine (or thymidine), 146 units of thymidine phosphorylase, and 100 units of purine nucleoside phosphorylase in 15 mL of 0.02 M phosphate buffer. Mixtures were shaken gently at 37°C for 3-5 days and the progress of the reaction was followed by HPLC using a reversed phase C_4 column with a gradient of 0-80% acetonitrile in water over 60 minutes. The suspensions were filtered and the filtrates were passed through a 0.45 μ filter and injected onto a C_4 Vydac HPLC column. The products were eluted using a gradient of 0-80% acetonitrile in water with a flow rate of 40 mL/min. The appropriate fractions were injected onto an analytical C_4 column for a purity check, and combined and concentrated under reduced pressure to remove the organic solvent. The aqueous solution containing the desired material was applied to a second C_4 Vydac column and eluted as above to remove minor amounts of contaminants. Fractions containing pure product were combined, concentrated to remove organic solvent, and freeze dried to give the desired nucleoside.

9-(2,5-Dideoxy-β-D-ribofuranosyl)-6-methylpurine (3). The desired product was eluted at 18.61 min. UV λ_{max} (pH 7.2) 251 (ε 7.26 $\times$ 10^3). ^{1}H NMR δ 8.8 (s, 1H, H_8), 8.7 (s, 1H, H_2), 6.3 (t, 1H, J = 6.5 Hz, $H_{1'}$), 5.3 (d, 1H, J = 4.5 Hz, 3'-OH), 4.2 (m, 1H, $H_{3'}$), 3.9 (m, 1H, $H_{4'}$), 2.8 (m, 1H, $H_{2'}$), 2.7 (s, 3H, 6-CH_3), 2.3 (m, 1H, $H_{2''}$), 1.2 (d, 3H, J = 6.5 Hz, 5'-CH_3).
Electrospray MS: *m/z* 235 (MH^+), 257 (MNa^+), 233 (M^-), calcd. for $C_{11}H_{14}N_4O_2$: (M) 234.26.

6-Dimethylamino-9-(2,5-dideoxy-β-D-ribofuranosyl)-purine (4). The desired product was eluted at 21.6 min. UV λ_{max} (pH 7.2) 258 (ε 7.4 $\times$ 10^3).^{1}H NMR δ 8.29 (s, 1H, H_8), 8.20 (s, 1H, H_2), 6.29 (t, 1H, J = 6.5 Hz, $H_{1'}$), 5.30 (d, 1H, 3'-OH), 4.18 (m, 1H, $H_{3'}$), 3.8 (m, 1H, $H_{4'}$), 3.4 (s, 6H, $N(CH_3)_2$), 2.7

(m, 1H, $H_{2'}$), 2.2 (m, 1H, $H_{2''}$), 1.24 (d, 3H, J = 6.5 Hz, 5'-CH_3). Electrospray MS: *m/z* 264 (MH^+), 302 (MK^+), 262 (M^-), calcd. for $C_{12}H_{17}N_5O_2$: (M) 263.13.

9-(2,5-Dideoxy-β-D-ribofuranosyl)-6-furfurylaminopurine (5). HPLC elution at 23.67 min. UV λ_{max} (pH 7.2) 264 (ε 5.3 × 10^3). ^1H NMR δ 8.3 (s, 1H, H_8), 8.24 (s, 1H, H_2), 8.2 (bs, 1H, NH), 7.5 (s, 1H, furan-H_5), 6.43 (t, 1H, J = 3.5 Hz, furan-H_4), 6.28 (t, 1H, J = 6.5 Hz, $H_{1'}$), 6.21 (d, 1H, J = 3 Hz, furan-H_3), 5.29 (d, 1H, J = 4.0 Hz, 3'-OH), 4.7 (m, 2H, CH_2), 4.2 (m, 1H, $H_{3'}$), 3.8 (m, 1H, $H_{4'}$), 2.8 (m, 1H, $H_{2'}$), 2.6 (m, 1H, $H_{2''}$), 1.25 (d, 3H, J = 6.0 Hz, 5'-CH_3). Electrospray MS: *m/z* 316 (MH^+), 354 (MK^+), 314 (M^-), calcd. for $C_{15}H_{17}N_5O_3$: (M) 315.13.

9-(2,5-dideoxy-β-D-ribofuranosyl)-6-thiopurine (6). HPLC elution at 34.52 min. UV λ_{max} (pH 7.2) 273, 240 (ε 6.5 ×10^3). ^1H NMR δ 8.8 (s, 1H, H_8), 8.7 (s, 1H, H_2), 6.4 (t, 1H, J = 6.5 Hz, $H_{1'}$), 5.35 (d, 1H, J = 4.5 Hz, 3'-OH), 4.2 (m, 1H, $H_{3'}$), 3.9 (m, 1H, $H_{4'}$), 2.8 (m, 1H, $H_{2'}$), 2.3 (m, 1H, $H_{2''}$), 1.2 (d, 3H, J = 6.9 Hz, 5'-CH_3). Electrospray MS: *m/z* 251 (M^-), calcd. for $C_{10}H_{12}O_2N_4S$: (M) 252.06.

9-(2,5-dideoxy-β-D-ribofuranosyl)-6-methylthiopurine (7). HPLC elution at 21.97 min. UV λ_{max} (pH 7.2) 280 (ε 7.1 × 10^3). ^1H NMR δ 8.7 (s, 1H, H_8), 8.6 (s, 1H, H_2), 6.36 (t, 1H, J = 6.5 Hz, $H_{1'}$), 5.33 (d, 1H, J = 4 Hz, 3'-OH), 4.23 (m, 1H, $H_{3'}$), 3.91 (m, 1H, $H_{4'}$), 2.86 (m, 1H, $H_{2'}$), 2.65 (s, 3H, S-CH_3), 2.3 (m, 1H, $H_{2''}$), 1.25 (d, 3H, J = 6.5 Hz, 5'-CH_3). Electrospray MS: *m/z* 267 (MH^+), 289 (MNa^+), 265 (M^-), calcd. for $C_{11}H_{14}N_4O_2S$: (M) 266.09

9-(2,5-Dideoxy-β-D-ribofuranosyl)-6-ethylthiopurine (8). HPLC elution at 24.28 min. UV λ_{max} (pH 7.2) 281 (ε 7.0 × 10^3). ^1H NMR δ 8.7 (s, 1H, H_8), 8.6 (s, 1H, H_2), 6.35 (t, 1H, J = 6.5 Hz, $H_{1'}$), 5.3 (d, 1H, J = 4.0 Hz, 3'-OH), 4.2 (m, 1H, $H_{3'}$), 3.9 (m, 1H, $H_{4'}$), 3.3 (q, 2H, J = 7.0 Hz, CH_2), 2.8 (m, 1H, $H_{2'}$), 2.3 (m, 1H, $H_{2''}$), 1.35 (t, 3H, J = 7.0 Hz, CH_3), 1.25 (d, 3H, J = 6.0 Hz, 5'-CH_3). Electrospray MS: *m/z* 281 (MH^+), 303 (MNa^+), 279 (M^-), calcd. for $C_{12}H_{16}O_2N_4S$: (M) 280.

6-Cyano-9-(2,5-dideoxy-β-D-ribofuranosyl)-purine (9). HPLC elution at 33.29 min. ^1H NMR δ 9.14 (s, 1H, H_8), 9.0 (s, 1H, H_2), 6.4 (t, 1H, J = 6.5 Hz, $H_{1'}$), 5.38 (d, 1H, J = 4.0 Hz, 3'-OH), 4.25 (m, 1H, $H_{3'}$), 3.9 (m, 1H, $H_{4'}$), 2.8 (m,

1H, $H_{2'}$), 2.3 (m, 1H, $H_{2''}$), 1.2 (d, 3H, J = 6.5 Hz, 5'-CH_3). Electrospray MS: *m/z* 268 (MNa^+), 244 (M^-), calcd. for $C_{11}H_{11}N_5O_2$: (M) 245.09.

6-Chloro-9-(2,5-dideoxy-β-D-ribofuranosyl)-purine (10). HPLC elution at 20.76 min. UV λ_{max} (pH 7.2) 259 (ε 6.8 × 10^3). ^1H NMR δ 8.85 (s, 1H, H_8), 8.80 (s, 1H, H_2), 6.40 (t, 1H, J = 6.7 Hz), 5.3 (d, 1H, J = 4.5 Hz, 3'-OH), 4.24 (m, 1H, $H_{3'}$), 3.93 (m, 1H, $H_{4'}$), 2.86 (m, 1H, $H_{2'}$), 2.34 (m, 1H, $H_{2''}$), 1.27 (d, 3 H, J = 7.0 Hz, 5'-CH_3). Electrospray MS: *m/z* 277 (MNa^+), 253 (M^-), calcd. for $C_{10}H_{11}ClN_4O_2$: (M) 254.1

9-(2,5-dideoxy-β-D-ribofuranosyl)-6-propoxypurine (11). HPLC elution at 23.96 min. UV λ_{max} (pH 7.2) 246 (ε 6.6 × 10^3).^1H NMR δ 8.52 (s, 1H, H_8), 8.50 (s, 1H, H_2), 6.4 (t, 1H, J = 6.5 Hz, $H_{1'}$), 5.3 (d, 1H, 3'-OH), 4.50 (t, 2H, J = 6.5 Hz, CH_2), 4.2 (m, 1H, $H_{3'}$), 3.9 (m, 1H, $H_{4'}$), 2.80 (m, 1H, $H_{2'}$), 2.30 (m, 1H, $H_{2''}$), 1.80 (m, 2H, CH_2), 1.2 (d, 3H, J = 6.4 Hz, 4-CH_3), 0.98 (t, 3H, J = 7.5 Hz, CH_3). Electrospray MS: *m/z* 279 (MH^+), 301 (MNa^+), 277 (M^-), calcd. for $C_{13}H_{18}N_4O_3$: (M) 278.31.

2,6-Dichloro-9-(2,5-dideoxy-β-D-ribofuranosyl)-purine (12). HPLC elution at 40.42 min. ^1H NMR δ 8.8 (s, 1H, H_8), 6.3 (t, 1H, J = 6.5 Hz, $H_{1'}$), 5.37 (d, 1H, J = 3.5 Hz, 3'-OH), 4.2 (m, 1H, $H_{3'}$), 3.9 (m, 1H, $H_{4'}$), 2.7 (m, 1H, $H_{2'}$), 2.3 (m, 1H, $H_{2''}$), 1.27 (d, 3H, J = 6.0 Hz, 5'-CH_3). Electrospray MS: *m/z* 287 (M^-), calcd. for $C_{10}H_{10}Cl_2N_4O_2$: (M) 288.01.

9-(2,5-Dideoxy-β-D-ribofuranosyl)-1-methyladenine (13). HPLC elution at 19.02 min. UV λ_{max} (pH 7.2) 252 (ε 6.5 × 10^3). ^1H NMR δ 8.3 (s, 1H, H_8), 8.2 (s, 1H, H_2), 6.27 (t, 1H, J = 6.5 Hz, $H_{1'}$), 5.29 (d, 1H, J = 3.5 Hz, 3'-OH), 4.20 (m, 1H, $H_{3'}$), 3.8 (m, 1H, $H_{4'}$), 2.9 (bs, 3H, N-CH_3), 2.8 (m, 1H, $H_{2'}$), 2.5 (m, 1H, $H_{2''}$), 1.25 (d, 3H, J = 6.5 Hz, 5'-CH_3). Electrospray MS: *m/z* 250 (MH^+), 248 (M^-), calcd. for $C_{11}H_{15}N_5O_2$: (M) 249.12.

9-(2,5-dideoxy-β-D-ribofuranosyl)-adenine-N_1-Oxide (14). HPLC elution at 21.65 min. UV λ_{max} (pH 7.2) 259, 249 (ε 5.5 × 10^3). ^1H NMR δ 8.6 (s, 1H, H_8), 8.4 (s, 1H, H_2), 6.25 (t, 1H, J = 6.5 Hz, $H_{1'}$), 5.36 (bs, 1H, 3'-OH), 4.1 (m, 1H, $H_{3'}$), 3.8 (m, 1H, $H_{4'}$), 2.7 (m, 1H, $H_{2'}$), 2.2 (m, 1H, $H_{2''}$), 1.2 (d, 3H, J = 6.5, 5'-CH_3). Electrospray MS: *m/z* 252 (MH^+), 250 (M^-), calcd. for $C_{10}H_{13}N_5O_3$: (M) 251.

2-Amino-6-chloro-9-(2,5-dideoxy-β-D-ribofuranosyl)-purine (15). HPLC elution at 29.45 min. UV λ_{max} (pH 7.2) 254 (ε 7.26 $\times 10^3$). ^{1}H NMR δ 8.29 (s, 1H, H_8), 6.9 (s, 2H, 2-NH_2), 6.16 (t, 1H, J =7.0 Hz, $H_{1'}$), 5.28 (d, 1H, J = 4.5 Hz, 3'-OH), 4.14 (m, 1H, $H_{3'}$), 3.8 (m, 1H, $H_{4'}$), 2.73 (m, 1H, $H_{2'}$), 2.23 (m, 1H, $H_{2''}$), 1.24 (d, 3H, J = 6.5 Hz, 5'-CH_3). Electrospray MS: *m/z* 270 (MH^+), 292 (MNa^+), 268 (M^-), calcd. for $C_{10}H_{12}ClN_5O_2$: (M) 269.06.

1-(2,5-dideoxy-β-D-ribofuranosyl)-4-keto-pyrazolo[3,4-d]-pyrimidine (16). HPLC elution at 16.75 min. UV λ_{max} (pH 7.2) 246 (ε 6.9 $\times 10^3$). ^{1}H NMR δ 8.1 (s, 1H, H_7), 8.0 (s, 1H, H_6), 6.5 (t, J = 6.0 Hz, $H_{1'}$), 5.3 (d, 1H, 3'-OH), 4.3 (m, 1H, $H_{3'}$), 3.9 (m, 1H, $H_{4'}$), 2.8 (m, 1H, $H_{2'}$), 2.3 (m, 1H, $H_{2''}$), 1.2 (d, J = 6.5 Hz, 5'-CH_3). Electrospray MS: *m/z* 237 (MH^+), 259 (MNa^+), 235 (M^-), calcd. for $C_{10}H_{12}N_4O_3$: (M) 236.09.

1-(2,5-Dideoxy-β-D-ribofuranosyl)-4-thio-pyrazolo[3,4-d]-pyrimidine (17). HPLC elution at 17.57 min. UV λ_{max} (pH 7.2) 304, 272 (ε 6.5 $\times 10^3$). ^{1}H NMR δ 8.2 (s, 1H, H_7), 8.14 (s, 1H, H_6), 6.4 (t, J = 6.3 Hz, $H_{1'}$), 5.3 (d, 1H, 3'-OH), 4.2 (m, 1H, $H_{3'}$), 3.9 (m, 1H, $H_{4'}$), 2.8 (m, 1H, $H_{2'}$), 2.3 (m, 1H, $H_{2''}$), 1.2 (d, J = 6.5 Hz, 5'-CH_3). Electrospray MS: *m/z* 275 (MNa^+), 291 (MK^+), 251 (M^-), calcd. for $C_{10}H_{12}N_4O_2S$: (M) 252.06.

9-(2,5-Dideoxy-β-D-ribofuranosyl)-1-deazapurine (18). HPLC elution at 27.69 min. UV λ_{max} (pH 7.2) 273, 242 (ε 6.8 $\times 10^3$). ^{1}H NMR δ 8.6 (s, 1H, H_2), 8.36 (d, 1H, J = 5.0 Hz, H_5), 8.1 (d, 1H, J = 9.5 Hz, H_7), 7.3 (m, 1H, H_6), 6.4 (t, 1H, J = 6.5 Hz, $H_{1'}$), 5.3 (bs, 1H, 3'-OH), 4.2 (m, 1H, $H_{3'}$), 3.9 (m, 1H, $H_{4'}$), 2.9 (m, 1H, $H_{2'}$), 2.29 (m, 1H, $H_{2''}$), 1.25 (d, 3H, J = 6.5 Hz, 5'-CH_3). Electrospray MS: *m/z* 220 (MH^+), 242 (MNa^+), 218 (M^-), calcd. for $C_{11}H_{13}N_3O_2$: (M) 219.10.

Specificity of glycosidic bond cleavage. The nucleoside analogs (1 mM) were incubated with 1 unit of *E. coli* PNP or 1 unit of human erythrocyte PNP in 250 μL of 0.02 M potassium phosphate, pH 7.2, at 37°C for one hour. The reactions were terminated by heating for 10 minutes at 97°C in a heating block to destroy the enzyme. Aliquots of 25 μL were removed and injected onto a C_4 reversed phase HPLC column which was eluted with a gradient of 0-50% acetonitrile in water, and the extent of degradation was determined by measurement of the area remaining under the peak corresponding to starting material. Cleavage of inosine, a substrate of both *E. coli* and mammalian PNP was also carried out under the same conditions as a positive control.

CONCLUSIONS

The PNP/thymidine phosphorylase enzyme system has been shown to provide a convenient method for the synthesis of small quantities of a wide range of 2',5'-dideoxy nucleosides, although it is less practical for the preparation of larger amounts because of the relatively low yields obtained. A variety of substitutions to the 6-position of the purine can be tolerated, although more bulky substituents were not accepted as substrates. Although most of the other heterocycles examined were found not to be substrates, selected pyrazolopyrimidine and 1-deazapurine analogs were converted into the corresponding dideoxy nucleosides. Yields were generally low (typically in the region of 10%), presumably due to the limited solubility of 5'-deoxythymidine and the lower substrate specificity of the 2, 5'-deoxyribose derivative. In terms of specificity of degradation by bacterial but not human PNP, the 6-furfurylaminopurine derivative **5**, and the 6-ethylthio compound **8** showed specificities similar to that for the 6-methylpurine nucleoside **1**. Introduction of the 5'-deoxy substituent somewhat diminished the extent of cleavage by bacterial PNP, as evidenced by a comparison of the degradation of **3** versus **1**.

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