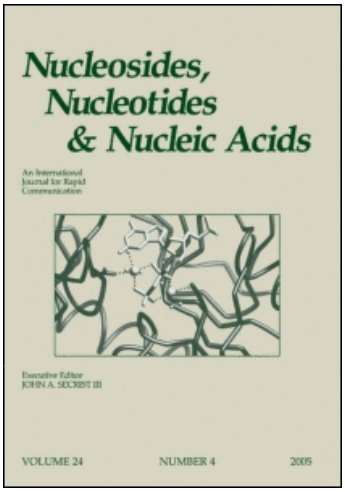

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 2′-DEOXY-4′-THIO-PYRAZOLO[3,4-*d*]PYRIMIDINE NUCLEOSIDES

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▫ *The coupling of 4-aminopyrazolo [3, 4-d]pyrimidine with the appropriate thio sugar gave a 3:1 ratio of α,β blocked 4-amino-1-(2-deoxy-4-thio-D-erythropentofuranosyl)-1H pyrazolo[3,4-d]pyrimidine nucleosides. The mixture was deblocked, both the anomers were separated, and the β-anomer was readily deaminated by adenosine deaminase. The nucleosides have been characterized, and their anomeric configurations have been determined by proton NMR. All three nucleosides were evaluated against a panel of human tumor cell lines for cytotoxicity in vitro. The details of a convenient and high yielding synthesis of these nucleosides are described.*

INTRODUCTION

For some years, we have been pursuing the synthesis of a variety of 4′-thionucleosides as anticancer and anti-infective agents. In that regard, we have prepared 4′-thionucleosides with a variety of different 4-thio sugars attached to both the normal purine and pyrimidine bases as well as analogs of those bases.[1–4] One of our goals in this program is to gain a further understanding about the active site of deoxycytidine kinase, so that we are better able to prepare potential drugs that are activated by this enzyme.

One of the altered bases that has drawn attention for many years is pyrazolo[3,4-*d*]pyrimidine. Our recent search of the research literature revealed that

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TABLE 1 Cytotoxicity Data: IC50 (μM)

Compound	CCRF-CEM (Leukemia)	CAKI-1 (Renal)	DLD-1 (Colon)	NCI-H23 (Lung)	SK-MeC-28 (Melanoma)	SNB-7 (CNS)
4	>100	>100	>100	>100	>100	>100
5	>100	>100	>100	>100	>100	>100
6	>100	>100	>100	>100	>100	>100

no nucleosides of pyrazolo[3,4-*d*]pyrimidine with 2′deoxy-4′-thiosugar have been reported, although there has been significant interest in these kinds of nucleosides with normal oxygen sugars.[5–8] Keeping these facts in mind, we prepared three new 2′-deoxy-4′-thio-pyrazolo[3,4-*d*]pyrimidine nucleosides. Chemical syntheses and biological data on these compounds are reported herein (Table 1).

CHEMISTRY

The synthesis of 1-O-acetyl-2-deoxy-4-thio-3,5-di-O-p-toluoyl-D-*erythro*-pentofuranose **2** has been reported earlier.[9] Tin (IV) chloride catalyzed coupling of thio sugar **2** with 4-aminopyrazolo[3,4-*d*]pyrimidine **1** in acetonitrile at room temperature for 24 h afforded nucleosides 4-amino-1-(2-deoxy-3,5-di-O-toluoyl-4-thio-D-*erythro*pentofuranosyl)-1-H-pyrazolo[3,4-*d*]pyrimidine **3** as an anomeric mixture (β:α ratio ~1:3) as a major product in 60% yield. Purification by silica gel chromatography gave the pure material as a white solid, but separation of anomers was not accomplished. Repeated fractional crystallization also failed to give pure anomers. Deprotection of compound **3** with sodium methoxide provided a mixture of **4** and **5** which has a better resolution on TLC as compared to the blocked parent **3**. Both these anomers were separated by silica gel column chromatography using chloroform-methanol-ammonium hydroxide 70:29:1 as eluent to afford 4-amino-1-(2-deoxy-4-thio-β-D-*erythro*pentofuranosyl)-1-H-pyrazolo[3,4-*d*]pyrimidine **4** and 4-amino-1-(2-deoxy-4-thio-α-D-*erythro*pentofuranosyl)-1-H-pyrazolo[3,4-*d*]pyrimidine **5**. Conversion of **4** to the corresponding 4-oxo-1-(2-deoxy-4-thio-β-D-*erythro*pentofuranosyl)-1-H-pyrazolo[3,4-*d*]pyrimidine **6** was accomplished by treating **4** with adenosine deaminase in water at room temperature for 46 hours. Compound **6** was purified on a XAD-4 resin column using 0.5 N ammonium hydroxide as eluent to obtain pure **6** in 75% yield. On the other hand, 4-amino-1-(2-deoxy-4-thio-α-D-*erythro*pentofuranosyl)-1-H-pyrazolo[3,4-*d*]pyrimidine **5** did not give the corresponding deaminated compound when treated with adenosine deaminase. All of these compounds were characterized by MS, NMR, and elemental analysis. The assignment of the anomeric configurations of all the compounds were made by NOE difference spectroscopy (Figure 1).

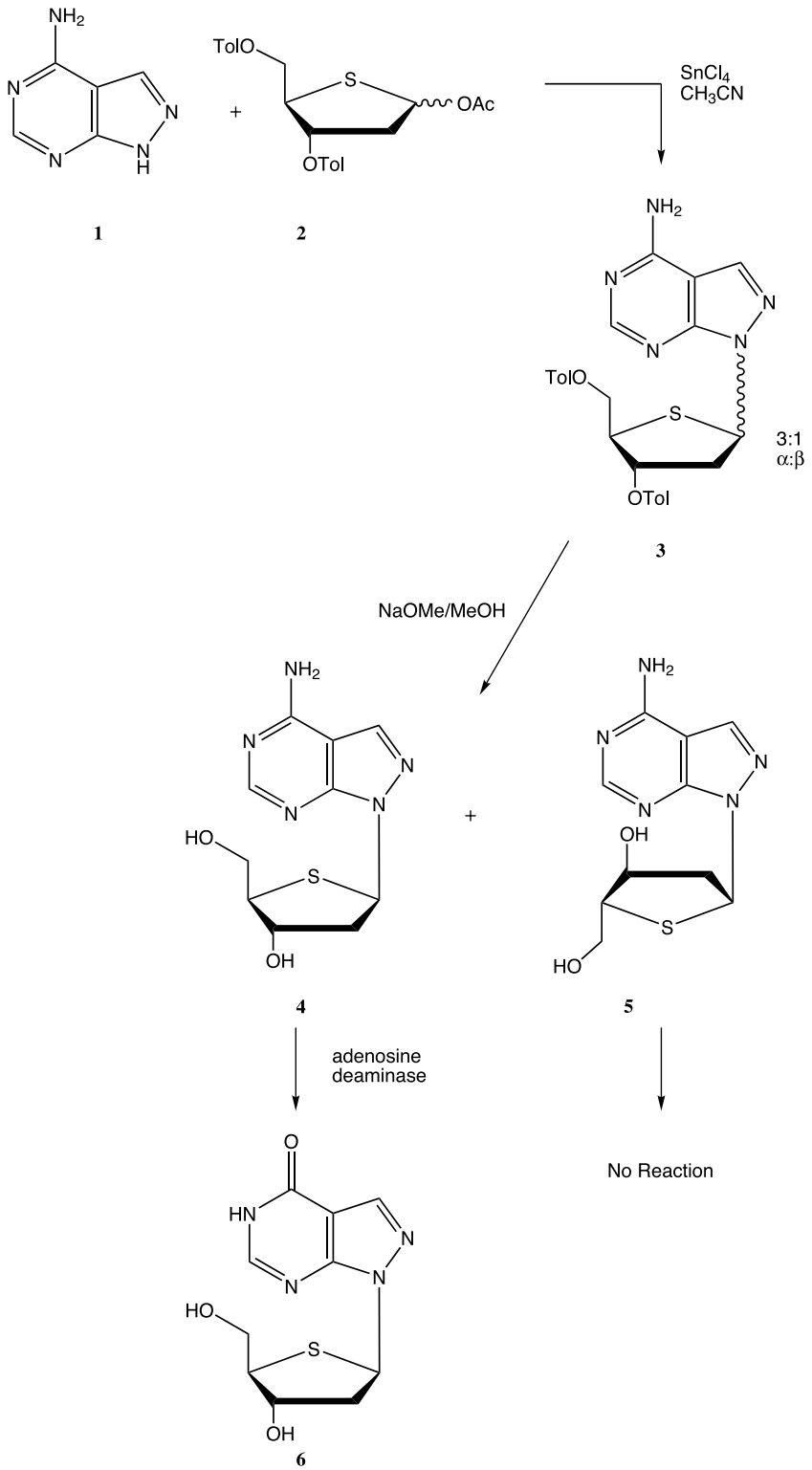

FIGURE 1

BIOLOGICAL RESULTS

4-Amino-1-(2-deoxy-4-thio-β-D-*erythro*pentofuranosyl)-1-H- pyrazolo[3,4-*d*]pyrimidine **4**, 4-amino-1-(2-deoxy-4-thio-α-D-*erythro*pentofuranosyl)-1-H- pyrazolo[3,4-*d*]pyrimidine ***5*** and 4-oxo-1-(2-deoxy-4-thio-β-D-*erythro*pentofuranosyl)-1-H- pyrazolo[3,4-*d*]pyrimidine **6**.

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