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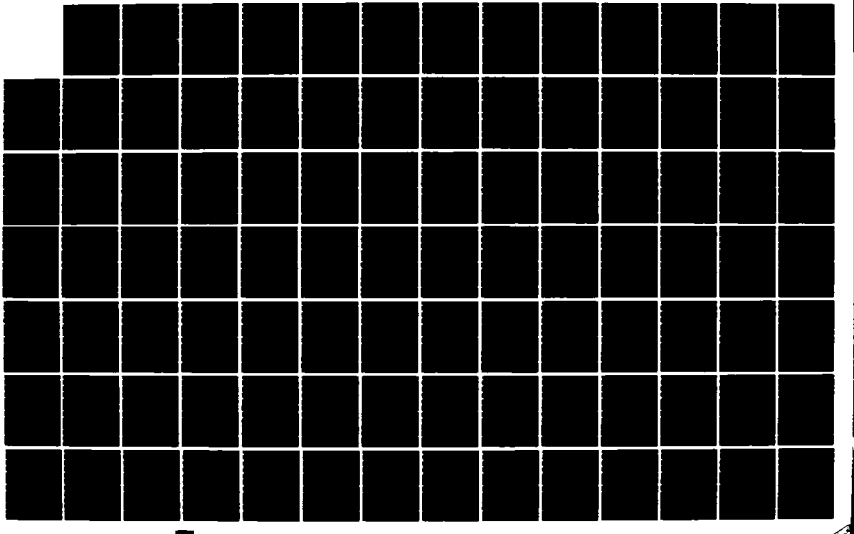

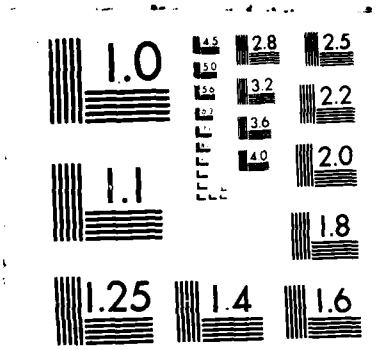

NEW INOSINE AND GUANOSINE ANALOGS AS INHIBITORS OF PARASITIC INFECTIONS

ANNUAL AND FINAL REPORT

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A number of pyrazolo[d]pyrimidine, pyrrolo[d]pyrimidine, s-triazolo[4,3-c]pyrimidine, s-triazolo[3,4-f]as-triazine, purine and imidazo[4,5-c]pyridine bases and nucleosides

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A number of heterocycles and nucleosides in the pyrazolo[3,4-d]pyrimidine, pyrazolo[3,4-f]-as-triazine, pyrrolo[2,3-d]pyrimidine, pyrrolo[3,2-d]pyrimidine, s-triazolo[3,4-f]-as-triazine, s-triazolo[4,3-c]pyridine, s-triazolo[1,5-a]-s-triazine, imidazo[4,5-c]pyridine and purine ring system have been prepared as potential antiparasitic agents. New synthetic procedures have been developed during the course of these synthetic studies. The compounds thus synthesized were tested at the Department of Medicinal Chemistry, Division of Experimental Therapeutics, Walter Reed ARmy Institute of Research, Washington, D. C., for their antiparasitic activities. The ED_{50} value for 1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine-4(5H)-thione is similar to that for allopurinol ribonucleoside against Leishmania amastigotes. 3-Bromo-allopurinol ribonucleoside (BK-15661) is more active than allopurinol ribonucleoside against L. tropica in vitro. 7-Deazainosine has a low ED_{50} dose (0.2μM), but only 80% of the organisms (L. tropica) were eliminated at 4 μM. 2-Methylinosine (BK-48428) has shown significant antitryanosomal activity (ED_{50} of 0.21 μM). 3-Deazaguanosine

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have been prepared and tested in vitro and in vivo against several parasites having substantial implications from a global epidemiological stand point. Considerable differences in antiparasitic activity with these compounds were noted. Formycin B had an ED_{50} of 0.04 μM against L. tropica in vitro and eliminated 90% of organism at $\leq$ 0.20 μM. Thioformycin B is significantly active against L. donovani in vitro (87% suppression), and cured 5 out of 5 mice infected with T. cruzi at 13.3 mg/kg. Oxoformycin also exhibited potent antiparasitic activity against T. rhodesiense in vivo.

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(BK-17405) is more active than allopurinol or allopurinol ribonucleoside against L. tropica in vitro (ED_{50} of 3.6 μM), and has shown significant activity against L. donovani in animals (76% suppression). Thioformycin B is also very potent against L. donovani in vivo (87% suppression). Selenoformycin B is more active than thioformycin B, but less active than formycin B against L. tropica promastigotes in vitro with an ED_{50} of 0.2 μM. Oxoformycin (BK-71338) exhibited potent activity against T. rhodesiense in vivo (5 out of 5 cures at 106, 212 and 424 mg/kg). A combination of sinefungin and oxoformycin could provide a rational approach to antiparasitic chemotherapy.

SUMMARY

The need for orally administrable, nontoxic antiparasitic agents has led to investigation of the antileishmanial activity of hypoxanthine and inosine analogs such as allopurinol (pyrazolo[3,4-d]pyrimidin-4(5H)-one), allopurinol riboside (1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one) and formycin B (3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidin-7(6H)-one). In vitro, these compounds are active against promastigotes and against amastigotes within macrophages, a clinically comparable model. In vivo, allopurinol is active against mucous leishmaniasis in Aotus monkeys, and formycin B is active against visceral disease in hamsters. Formycin B is more potent than allopurinol riboside and is an effective inhibitor of certain species of Leishmania which are resistant to allopurinol riboside. Allopurinol is currently in clinical trial against human visceral disease. In vitro biochemical studies have shown that the antiparasitic activity of these compounds is due to metabolism of the drugs into analogs of inosine and adenosine nucleotides by the organisms. Inhibition of guanosine nucleotide utilization may also be important for antiparasitic activity.

Both 4-APP (4-aminopyrazolo[3,4-d]pyrimidine) and 4-APP riboside (1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine) were found to be several-fold more active than allopurinol against promastigotes of the isolates of American Leishmania braziliensis and mexicana. The success of allopurinol, 4-APP, and their corresponding ribonucleosides, as well as the C-nucleoside formycin B as antiparasitic agents has generated considerable interest in the synthesis and antiparasitic evaluation of pyrazolo[d]pyrimidine derivatives and related compounds.

During the last two years, a number of pyrazolo[3,4-d]pyrimidine, pyrazolo[4,3-d]pyrimidine, s-triazolo[3,4-f]-as-triazine, s-triazolo[1,5-a]-s-triazine, imidazo[4,5-c]pyridine and purine derivatives related to allopurinol, allopurinol riboside and formycin B have been prepared and tested for their antiparasitic properties in vitro and in vivo. The ED_{50} for the elimination of Leishmania amastigotes from infected macrophages by 1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-thione is similar to that for allopurinol riboside. No more than 60% of organisms were eliminated by these nucleosides at the highest concentrations tested (72 to 190 μM). 3-Bromo-allopurinol riboside (BK-15661) was found to be more active (ED_{50} >15 μM) than allopurinol riboside against L. tropica in vitro. Formycin B had an ED_{50} of 0.04 μM against L. tropica and eliminated 90% of organisms at ≤0.20 μM, and obviously the most active agent tested in vitro. 7-Deazainosine had a low ED_{50} dose (0.2 μM), but only 80% of the organisms (L. tropica) were eliminated at 4 μM. The thio derivatives of formycin B (BJ-63911) and 7-deazainosine (BJ-84125) were much less active and much less toxic than their respective parent compounds in the human macrophage model. However, recently thio-formycin B has been shown to have significant activity against L. donovani in vivo (87% suppression). 3-Deazaguanosine (BK-17405) was more active than allopurinol or allopurinol riboside against L. tropica in vitro (ED_{50} of 3.6 μM), and has shown significant activity against L. donovani in animals (76% suppression) suggesting that guanosine derivatives may have potential as antiparasitic agents. Oxoformycin (BK-71338) exhibited potent activity against T. rhodesiense in vivo (5 out of 5 cures at 106 mg/kg).

In addition to the above compounds, certain purine analogs synthesized recently exhibited significant antitrypanosomal activity *in vitro*. 2-Methylinosine (BK-48428) had an ED_{50} of 0.21. The heterocycle 3-cyanoallopurinol (BK-49818) was found to be significantly active against trypanosomes (ED_{50} of 0.39) and malaria *in vitro*, whereas 4-amino-3-cyanopyrazolo[3,4-*d*]pyrimidine (BK-49827) had an ED_{50} of 2.03 against trypanosoma species. Another trisubstituted pyrazolo[3,4-*d*]pyrimidine, 3-bromo-4,6-diaminopyrazolo[3,4-*d*]-pyrimidine showed potent activity (ED_{50} 1.56) against trypanosomes. Selenoformycin B was more active than thioformycin B, but less active than formycin B against *L. tropica* promastigotes *in vitro* with an ED_{50} of 0.2 μM. Although the EC_{50} (concentration of drug that inhibits the growth rate of cells by 50%) value of allopurinol riboside is similar to that of formycin B (7.5 μM) for *T. cruzi* epimastigotes, the observed EC_{50} value of 1-methylformycin B is 0.6 μM. More of 1-methylformycin B is made available for further studies. The observation that the inosine and guanosine analogs like formycin B, thioformycin B, oxoformycin, oxoformycin B and 3-deazaguanosine exhibit potent antiparasitic activity *in vivo* gives high hope to the future of antiparasitic chemotherapy.

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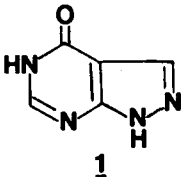
I. INTRODUCTION

Parasitic diseases are the most widespread of all the major human diseases and currently affect three billion people.[1] They cause malnutrition, debilitation, blindness, disfiguring lesions and death but, in spite of this, have been consistently neglected for the past several decades. Five of these parasitic diseases--malaria, schistosomiasis, filariasis, trypanosomiasis and leishmaniasis--are caused by protozoa or helminths and affect some 600 million people.[2] Chemotherapeutic research on these diseases has been severely underfunded. Effective drug treatment is nonexistent for all of these tropical parasitic infections. Although chloroquine (CQ) is generally considered to be one of the most fascinating, useful and versatile drugs developed during the modern era of synthetic organic chemistry,[3,4], CQ is now inadequate for controlling malaria because of the development of resistance. Mel B is too toxic for African sleeping sickness and diethyl carbamazine has poor efficacy in treating filariasis. No vaccines exist for any of these diseases.[1] In 1975 the World Health Organization (WHO) published a document, "Tropical Diseases Today--The Challenge and the Opportunity," in which the research areas covered by the new special program for research in tropical diseases are discussed.[5] A billion people are at risk of contracting one or more of these diseases.[6] Parasitic infections represent the greatest unsolved medical problem in our world today. It is interesting that although more than one billion dollars was spent on cancer research alone in the United States last year, only 40 million dollars was spent worldwide on tropical parasitic infections during the same period.[7]

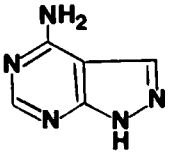
The biochemical rationale which influenced the selection of the pyrazolo-pyrimidine and related ring systems in the present work is based upon the general observation that the protozoan and helminth parasites have been shown

to be dependent on salvage pathways for purine nucleotide requirements.[8] The lack of a de nova metabolic pathway for purine biosynthesis in these parasites places an obligatory requirement for preformed purines and purine nucleosides as a necessity for the survival of the parasite. The parasite obtains its purines directly from the host in the form of adenine, hypoxanthine, adenosine or inosine and will accept certain pyrazolo[3,4-d]pyrimidines in place of purines.[9] Analogs of these naturally occurring heterocyclic bases and nucleosides might therefore show great potential as antiparasitic agents. Allopurinol [pyrazolo[3,4-d]pyrimidin-4(5H)-one, 1], initially synthesized by Robins[10] in 1956, was the first such analog shown by Frank and coworkers[11] to



1

Allopurinol



2

4-APP

inhibit the growth of the trypanosomid flagellate Crithidia fasciculata. Dewey and Kidder[12] showed that hypoxanthine could reverse the inhibition in this parasite and suggested that allopurinol might be an effective anti-trypanosomal agent. This has indeed proven to be the case. In vivo studies[13] have shown that mice infected with Trypanosoma cruzi showed no infection 275 days after successive treatments with allopurinol. Berens et al.[14] have confirmed that allopurinol is an effective agent in vitro against T. cruzi and showed that both the blood stream and the intracellular forms of T. cruzi metabolize allopurinol in the same manner as has been shown for the epimastigotes in vitro.[14-16] A similar observation has been made with trypanosoma species in vitro.[14,16,17] However, allopurinol ribonucleoside has been shown to be 10-fold more active against Leishmania braziliensis and 300-fold more active against Leishmania donovani than allopurinol in inhibiting the

growth of Leishmania promastigotes in vitro.[16,18,19] Both allopurinol and its ribonucleoside are equally effective in preventing the transformation of the intracellular form (amastigote) of L. donovani to the extracellular promastigote form.[18] Recently, Avila and coworkers [20] administered low, nontoxic doses of 4-aminopyrazolo[3,4-d]pyrimidine (2, 4-APP) to T. cruzi-infected mice and obtained a 100% survival at day 300 as compared to 100% mortality in the controls (at day 14). 4-APP has shown to be effective in the treatment of experimental Chagas disease in mice.[21,22] The beneficial results obtained with allopurinol on human cutaneous leishmaniasis suggest that this compound may be a candidate for a successful treatment of this disease.[22]

A study of the mechanism of inhibition in Trypanosomes,[15] as well as in Leishmania species,[18] reveals that allopurinol (1) is converted to allopurinol ribofuranoside 5'-phosphate (3) by a unique enzyme of the parasite, nucleoside phosphoribosyltransferase. Selective amination of 3 by the enzymes adenylosuccinate synthetase and succino-AMP lyase produced the 5'-phosphate of 4-APP ribonucleoside (4), which is eventually incorporated into RNA of the parasite as the 5'-triphosphate (5) resulting in cytotoxicity to these parasites (Figure 1). This conversion, which is analogous to the conversion of IMP to AMP, does not take place in mammalian cells.[23] These unusual metabolic transformations of 3 reveal significant biochemical differences between the host and the parasite, which should provide great potential for chemotherapeutic exploitation.[24]

Both 4-APP (2) and its ribonucleoside were found to be several-fold more active than allopurinol against promastigotes of the isolates of American Leishmania braziliensis and mexicana.[25,26] In light of these antiparasitic properties of allopurinol, 4-APP and their corresponding ribonucleosides, we

initiated a program to synthesize certain nucleosides of the pyrazolo[3,4-d]-pyrimidine ring.

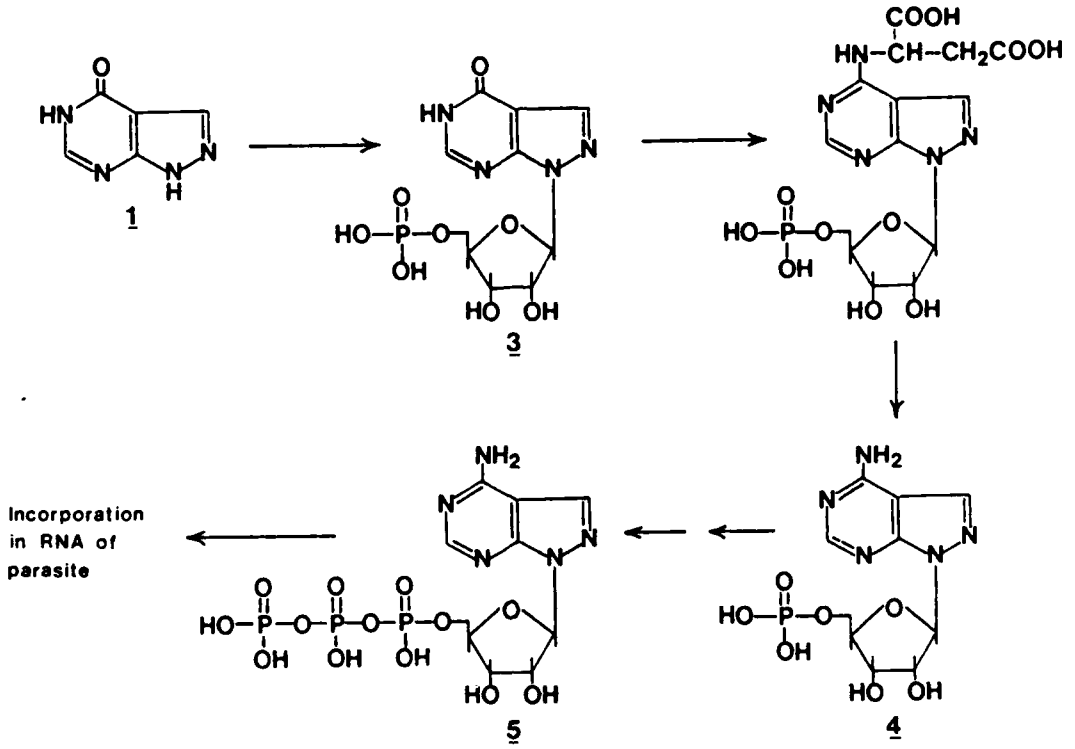
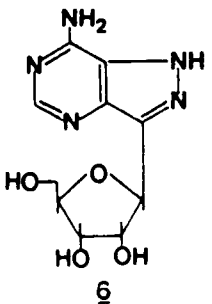
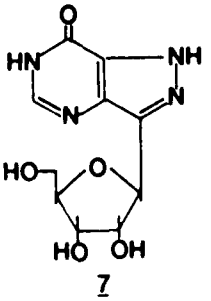


Figure 1

Like pyrazolo[3,4-d]pyrimidines, pyrazolo[4,3-d]pyrimidine nucleosides continue to be of considerable interest both from chemical and biological points of view. Because of the structural resemblance to purine nucleosides and the unusual biological properties[27] of the naturally-occurring C-nucleoside antibiotics formycin [28,29] (6) and formycin B [30] (7), there has



6
Formycin



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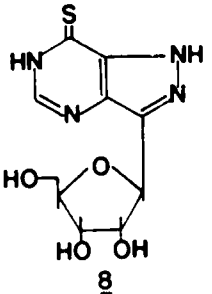
Formycin B



8
Thioformycin B

been an ongoing effort to synthesize nucleoside derivatives of the pyrazolo-[4,3-d]pyrimidine ring system. Formycin B is an analog of inosine with a sQepWn carbon-carbon glycosidic linkage and is a potent inhibitor of promastigote and amastigote forms of Leishmania.[31-34] Formycin B has recently been shown to be a potent inhibitor of the growth of T. cruzi (the causative agent of Chagas' disease) epimastigotes in culture.[35] Thioformycin B (3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine-7(6H)-thione, BJ-63911, 8) has also shown significant activity (ED_{50} 3.6 μM) against L. tropica in human monocyte-derived macrophages in vitro [26] and is much less toxic than formycin B. The EC_{50} value of allopurinol riboside is very similar to that of formycin B [35] for T. cruzi epimastigotes. However, formycin B is much more potent than allopurinol riboside and is an effective inhibitor of some species of Leishmania which are resistant to allopurinol riboside.[34]

The metabolic profile for formycin B is remarkably similar to that of allopurinol. The enzyme nucleoside phosphotransferase of the parasite catalyzes the 5'-phosphorylation to formycin B 5'-phosphate, which is subsequently acted upon by adenylosuccinate synthetase and lyase to form formycin 5'-phosphate, which is eventually incorporated as the triphosphate into RNA of the parasite, and presumed to be responsible for the antiparasitic activity.[34] Spector and Jones[36] have recently found that formycin B 5'-phosphate is a strong inhibitor of leishmanial guanosine 5'-monophosphate (GMP) reductase and is a weak inhibitor of human GMP reductase. GMP reductase is the enzyme which converts GMP to IMP.[37] This is the only known enzyme by which guanine nucleotides can be converted to IMP, the pivotal precursor of both adenine and guanine nucleotides. In view of this, Spector and Jones [36] speculate that the inhibition of leishmanial GMP reductase by formycin B

5'-phosphate may be a contributory factor in the antileishmanial activity of formycin B.

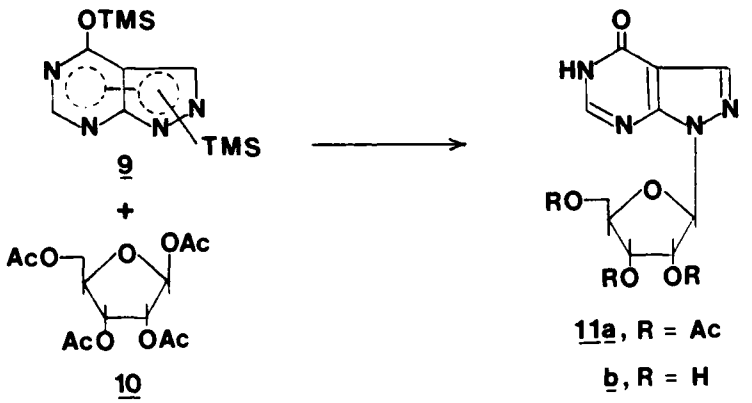
The success of allopurinol, 4-APP, allopurinol ribonucleoside and formycin B as antiparasitic agents has stimulated a great deal of effort toward the chemical synthesis of a number of pyrazolopyrimidine heterocycles, the corresponding nucleosides and certain related compounds. During the last seventeen months we continued out synthetic program designed to provide the selected allopurinol ribonucleoside and formycin B derivatives, as well as certain related compounds. Fifty-five such compounds were prepared and submitted to the Contracting Officer's Technical Representative, Department of Medicinal Chemistry, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C., for antiparasitic evaluation. The progress made in the synthetic aspect may be divided into ten major categories:

1. Synthesis of Pyrazolo[3,4-d]pyrimidine Nucleosides
2. Synthesis of Pyrazolo[4,3-d]pyrimidine Nucleosides
3. Synthesis of s-Triazolo[3,4-f]-as-triazines
4. Synthesis of s-Triazolo[4,3-c]pyrimidines
5. Synthesis of s-Triazolo[1,5-a]-s-triazine Nucleosides
6. Synthesis of Pyrrolo[3,2-c]pyridines
7. Synthesis of Imidazo[4,5-c]pyridines
8. Synthesis of Pyrrolo[d]pyrimidines
9. 9-(2-Deoxy-β-D-ribo-hexopyranosyl)- derivatives of adenine and hypoxanthine
10. Neplanocin D

II. CHEMISTRY AND DISCUSSION

1. Synthesis of Pyrazolo[3,4-d]pyrimidine Nucleosides

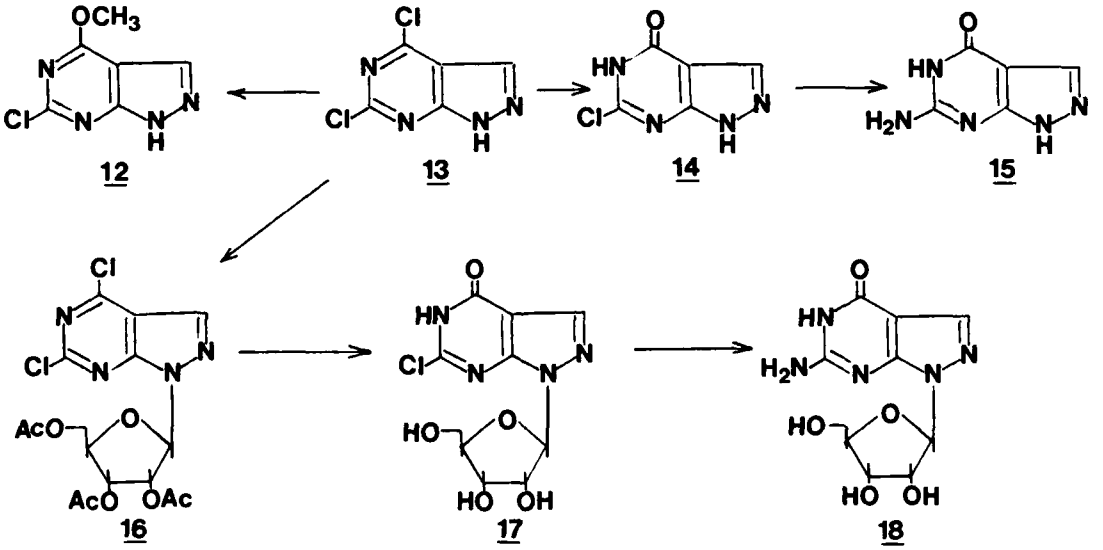
Allopurinol ribonucleoside [1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one] has been shown to be 10-fold more active against Leishmania braziliensis and 300-fold more active against Leishmania donovani than the aglycon allopurinol in inhibiting the growth of Leishmania promastigotes in vitro.[16,18] However, both allopurinol and its ribonucleoside are equally effective in preventing the transformation of the intracellular form (amastigote) of Leishmania donovani to the extracellular promastigate form.[18] In order to study the antiparasitic properties of allopurinol ribonucleoside in greater detail, more of this nucleoside was prepared as follows:



The silylation of allopurinol (1)[10] with hexamethyldisilazane in anhydrous pyridine under anhydrous conditions furnished the bis-trimethylsilyl derivative (9). Since 9 is susceptible toward hydrolysis (cleavage of the Si-N bond), it was always prepared immediately before utilization in the condensation reaction. The condensation of 9 with 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (10) in boiling p-dioxane in the presence of boron trifluoride diethyl ether gave a complex reaction mixture, from which the desired product 1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one

(11a) was isolated in a 32% yield. Removal of the protecting groups from the carbohydrate moiety of 11a was accomplished with sodium methoxide in methanol at room temperature to obtain allopurinol ribonucleoside (11b). That complete deacetylation had occurred was evident by the absence of any absorption peaks at δ 2.1 ppm in the ^{1}H NMR spectrum of 11b, and all the physicochemical properties of 11b are identical with those reported in the literature.[38]

In order to study the structure-activity relationship, we also prepared the guanine and guanosine analogs in the pyrazolo[3,4-d]pyrimidine ring system. We were desirous of finding a procedure which would direct the glycosyl attachment to N_1 and give an intermediate which could then be readily converted into various desired guanine derivatives. We also chose to study the general glycosylation procedure of Vorbrüggen and coworkers,[39] which



involves the coupling of a fully acylated sugar with the requisite trimethylsilylated heterocycle in the presence of trimethylsilyl trifluoromethanesulfonate ($Me_3SiOSO_2CF_3$) as a catalyst. The readily available 4,6-dichloropyrazolo[3,4-d]pyrimidine (13)[40] served as a starting material for these studies.

Selective replacement of the chlorine atom at position 4 of 13 was accomplished by the reaction of cold sodium methoxide with 13 to give 4-methoxy-6-chloropyrazolo[3,4-d]pyrimidine (12, BK-74446).[40] Similarly, when 13 was treated with the usual nucleophilic reagents under relatively mild reaction conditions, the corresponding 4-substituted-6-chloropyrazolo[3,4-d]-pyrimidine was obtained. Thus, 13 and dilute sodium hydroxide gave 6-chloro-pyrazolo[3,4-d]pyrimidin-4(5H)-one (14, 6-chloroallopurinol).[40] Reaction of 14 and alcoholic ammonia heated to 200°C gave a good yield of the guanine analog, 6-aminopyrazolo[3,4-d]pyrimidin-4(1H,5H)-one (15).[40]

Trimethylsilylation of 13 was accomplished by heating with hexamethyl-disilazane (HMDS) in the presence of ammonium sulfate to give the syrupy trimethylsilyl derivative. Without further purification, the trimethylsilyl derivative was treated with one equivalent of 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose in the presence of 1.5 molar equivalent of $Me_3SiOSO_2CF_3$[41] in dry acetonitrile at ambient temperature for 16 hours. Under these conditions the protected nucleoside 16 was obtained as the only major nucleoside product. Purification was achieved on a silica gel column by Prep LC techniques to give 4,6-dichloro-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine (16) as a pale yellow syrup in 74% yield. In one instance when the trimethyl silylation of 13 was incomplete, a small amount of the N_2-isomer 4,6-dichloro-2-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine was isolated and characterized. Treatment of 16 with boiling aqueous sodium hydroxide and methanol gave an excellent yield of the intermediate 6-chloro-1-β-D-ribo-furanosylpyrazolo[3,4-d]pyrimidin-4-one (17), which on subsequent amination with methanolic ammonia at 120°C for 20 hours provided the guanosine analog 6-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4-one (18)[38] in good

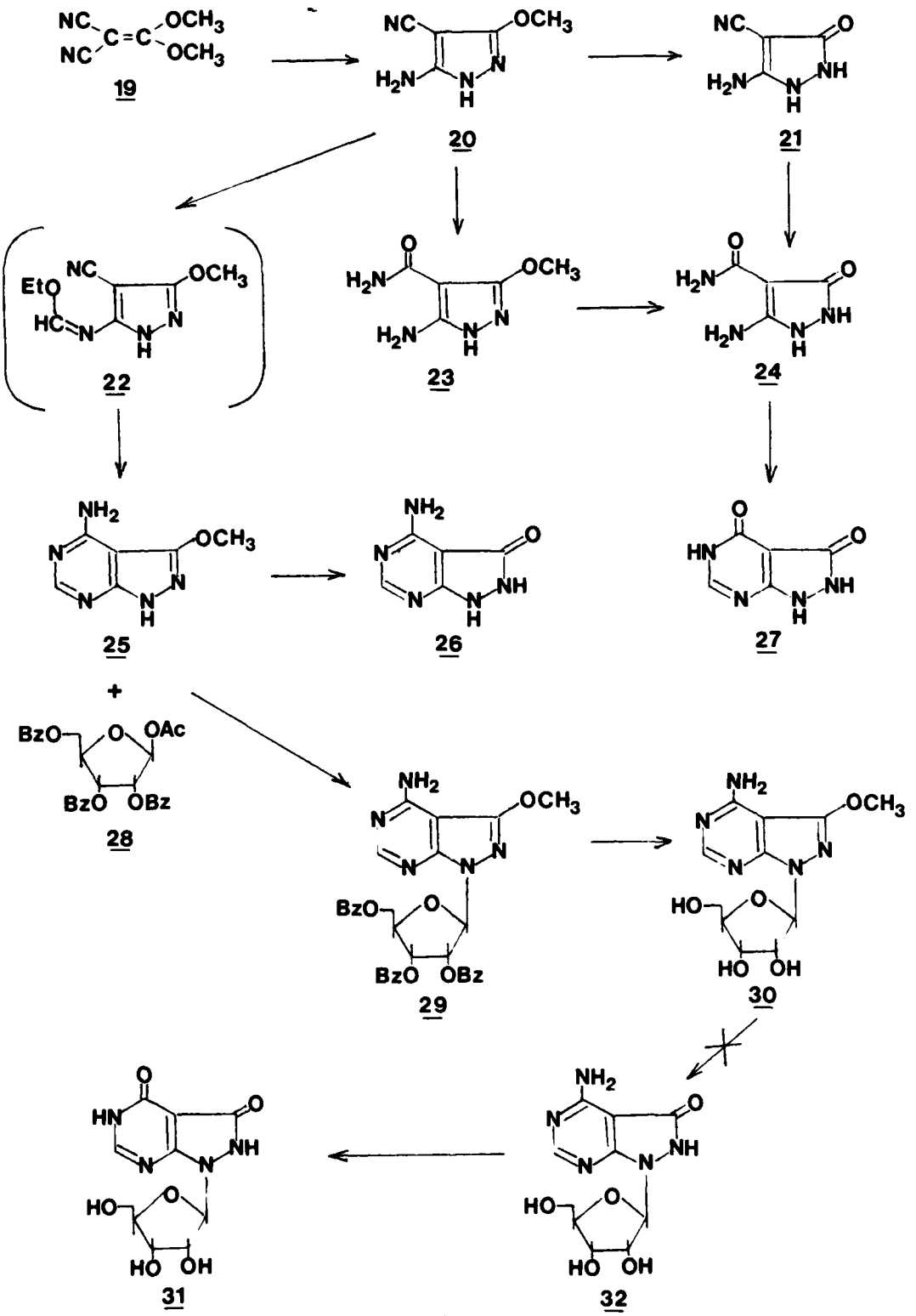
yield. The nucleoside 18 was found to be identical in all respects with that reported by Davoll and Kerridge.[42]

We further extended these synthetic studies to obtain certain selected 3-substituted pyrazolo[3,4-d]pyrimidines and their corresponding ribonucleosides. In view of the potent antiparasitic activity of allopurinol and 4-aminopyrazolo[3,4-d]pyrimidine (4-APP), the syntheses of the corresponding 3-oxo derivatives of these aglycons (26 and 27) and their ribonucleosides (32 and 31) is of particular interest. The synthesis of these target compounds was envisioned by following the Scheme I.

Treatment of dicyanoketene dimethyl acetal (19)[43] with hydrazine readily gave 5-amino-3-methoxypyrazole-4-carbonitrile (20). Demethylation of the ether linkage of 20 with chlorotrimethylsilane in the presence of sodium iodide furnished 5-amino-4-cyanopyrazol-3(1H,2H)-one (21) in a 48% yield. Hydrolysis of the carbonitrile function of 21 with ammonium hydroxide in the presence of hydrogen peroxide gave 5-amino-4-carbamoylpyrazol-3(1H,2H)-one (24). Alternatively, compound 24 was also prepared from 5-amino-3-methoxypyrazole-4-carboxamide (23), which in turn was obtained by the reaction of 20 with concentrated H_2SO_4 at room temperature. Treatment of 23 with chlorotrimethylsilane gave the desired 24. Ring annulation of 24 with boiling formamide gave a 36% yield of 3-oxoallopurinol (27, BK-98768).

A similar methodology was used to obtain 3-oxo-4-APP (26). When 5-amino-3-methoxypyrazole-4-carbonitrile (20) was heated under reflux with triethyl orthoformate, a good yield of the intermediate 3-methoxy-5-(ethoxymethyleneamino)pyrazole-4-carbonitrile (22), which when allowed to react with methanolic ammonia at 90°C for 3 hours gave 4-amino-3-methoxypyrazolo[3,4-d]pyrimidine (25, BL-05580), isolated in a 62% yield. Ether group cleavage of

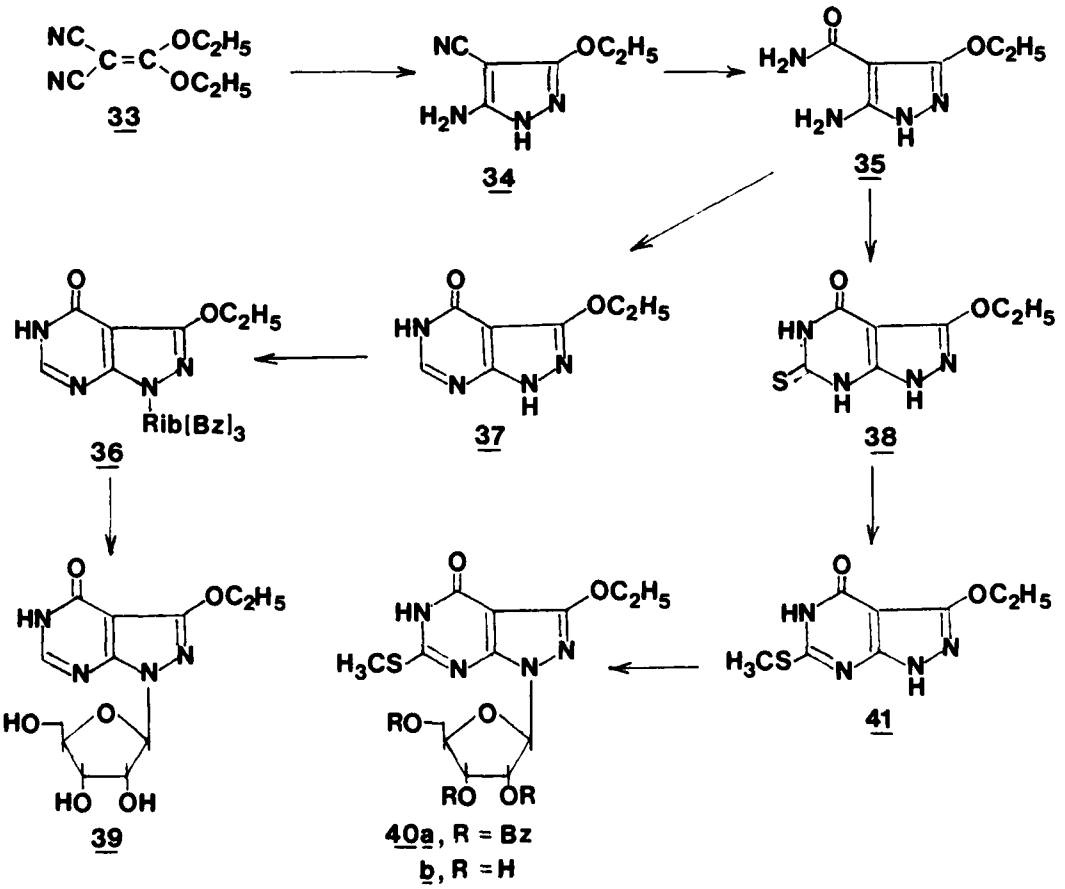
Scheme I



25 with chlorotrimethylsilane in the presence of sodium iodide afforded 4-aminopyrazolo[3,4-d]pyrimidin-3(1H,2H)-one (3-oxo-4-APP, 26).

By using the new, high-temperature glycosylation procedure developed recently in our laboratory,[44] unsilylated 25 was reacted with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (28) in the presence of boron trifluoride etherate in boiling nitromethane, which afforded after column chromatographic purification 4-amino-3-methoxy-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-pyrazolo[3,4-d]pyrimidine (29) as a syrup. Debenzoylation of 29 with sodium methoxide in methanol gave 4-amino-3-methoxy-1-β-D-ribofuranosylpyrazolo-[3,4-d]pyrimidine (30) in almost quantitative yield. However, the attempted cleavage of the ether linkage of 30 to obtain 32, and subsequently 31, were failed in our hands. Extensive glycosidic cleavage was observed.

Scheme II



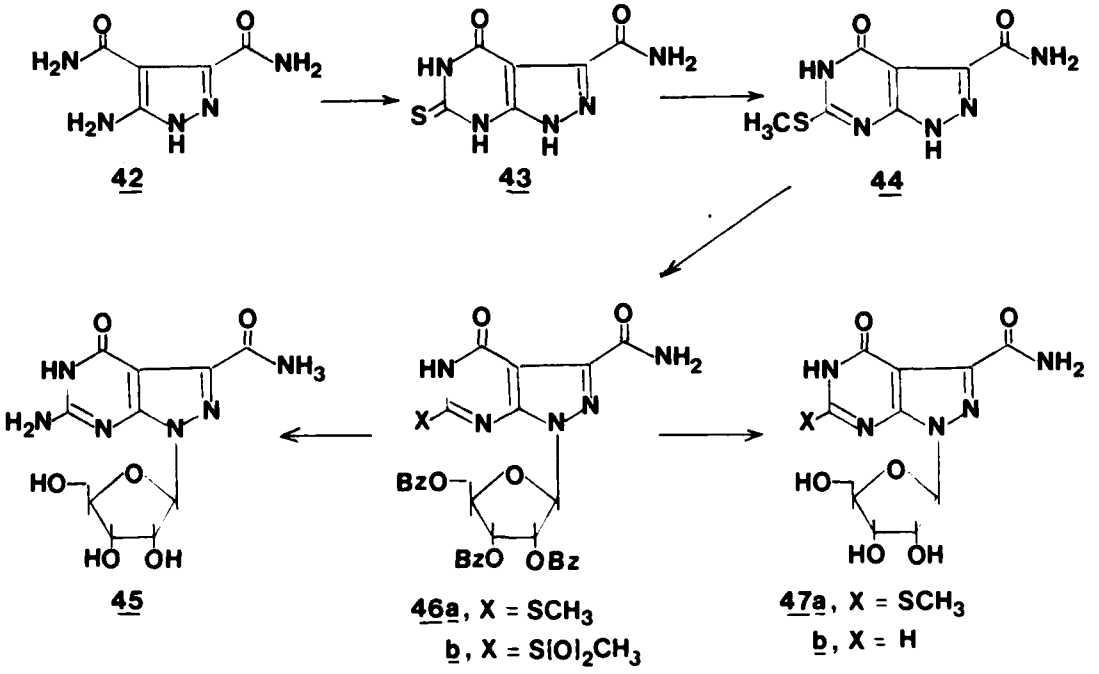
In an effort to obtain the desired 3-oxoallopurinol ribonucleoside, possibility of employing the suitably substituted allopurinol itself was subsequently considered (Scheme II). Treatment of dicyanoketene diethyl acetal (33)[43] with hydrazine gave 5-amino-3-ethoxypyrazole-4-carbonitrile (34), which on hydrolysis with concentrated H_2SO_4 at room temperature gave the corresponding carbamoyl derivative (35). Ring-closure of 35 with formamide at reflux temperature furnished 3-ethoxyallopurinol (37). Treatment of 35 with potassium ethyl xanthate in DMF at reflux temperature gave the ring-closed product 3-ethoxy-6-thiopyrazolo[3,4-d]pyrimidine-4(5H,7H)-dione (38), which on subsequent methylation with methyl iodide under alkaline conditions furnished one of the key intermediates 3-ethoxy-6-methylthiopyrazolo[3,4-d]pyrimidin-4(5H)-one (41).

The high-temperature glycosylation procedure[44] was also found to be very successful in the preparation of the ribonucleosides of 37 and 41. Thus, treatment of 37 with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (28) in the presence of boron trifluoride etherate in boiling nitromethane gave 3-ethoxy-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one (36), which on subsequent debenzoylation with sodium methoxide furnished 3-ethoxy-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one (39). Similar high-temperature glycosylation of 41 with 28 readily gave 3-ethoxy-6-methylthio-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrazolo-[3,4-d]pyrimidin-4(5H)-one (40a). Methoxide ion catalyzed debenzoylation of 40a afforded 3-ethoxy-6-methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one (40b) in a 77% yield. Further functional group manipulation studies with 39 and 40b to obtain the target compound 31 are in progress.

In view of the significant antiparasitic activity of 6-aminopyrazolo-[3,4-d]pyrimidin-4(5H)-one (6-aminoallopurinol, 15) against T. cruzi epimasti-

gotes *in vitro*,[22] the synthesis of 6-amino-1-β-D-ribofuranosyl-4(5H)-oxopyrazolo[3,4-d]pyrimidine-3-carboxamide (45) was undertaken. Our synthetic approach (Scheme III) starts with the readily preparable 6-thiopyrazolo-[3,4-d]pyrimidine-4(5H,7H)-dione-3-carboxamide (43). Thus, ring closure of 5-aminopyrazole-3,4-dicarboxamide (42)[45] with potassium ethyl xanthate gave an 82% yield of 43 (BK-74526). Methylation of 43 with methyl iodide furnished the versatile starting material 6-methylthio-4(5H)-oxopyrazolo[3,4-d]pyrimidine-3-carboxamide (44). Direct glycosylation of the nonsilylated 44 with the blocked benzoyl sugar 28 in the presence of the catalyst BF_3 etherate in a boiling polar aprotic solvent such as nitromethane gave a nucleoside product, identified as 6-methylthio-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-4(5H)-oxopyrazolo[3,4-d]pyrimidine-3-carboxamide (46a). No formation of other isomeric nucleosides was observed. Debenzoylation of 46a with sodium methoxide gave 6-methylthio-1-β-D-ribofuranosyl-4(5H)-oxopyrazolo[3,4-d]-

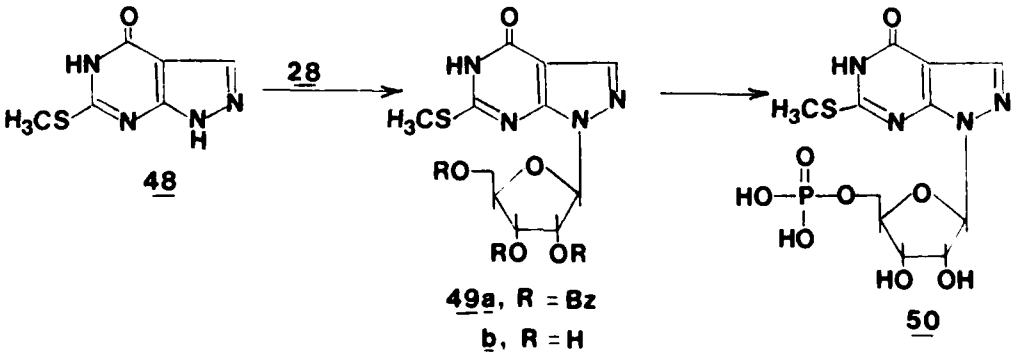
Scheme III



pyrimidine-3-carboxamide (47a). Dethiation of 46a with Raney nickel, followed by debenzoylation readily gave 4(5H)-oxo-1-β-D-ribofuranosylpyrazolo[3,4-d]-pyrimidine-3-carboxamide (47b), virtually identical to 3-carbamoylallopurinol ribofuranoside (BK-57409), prepared and recently reported from our laboratory.[44] This confirmed the structural assignment of 46a and the subsequent nucleosides derived therefrom.

Oxidation of 46a with m-chloroperoxybenzoic acid in dichloromethane gave the corresponding methylsulfonyl derivative 46b. In the ^1H NMR ($CDCl_3$) of 46b, all the protons except benzoyl and C_4'H and C_5'H_2 were shifted downfield as compared to those of 46a. The SO_2CH_3 protons had a considerable shift of 0.79 ppm, whereas the anomeric protons of 46b shifted by 0.24 ppm. This downfield shift in 46b would be expected due to the sulfonyl group. Treatment of 46b with liquid ammonia at 90°C for 48 hours gave a good yield of the target compound 45 (BK-74455).

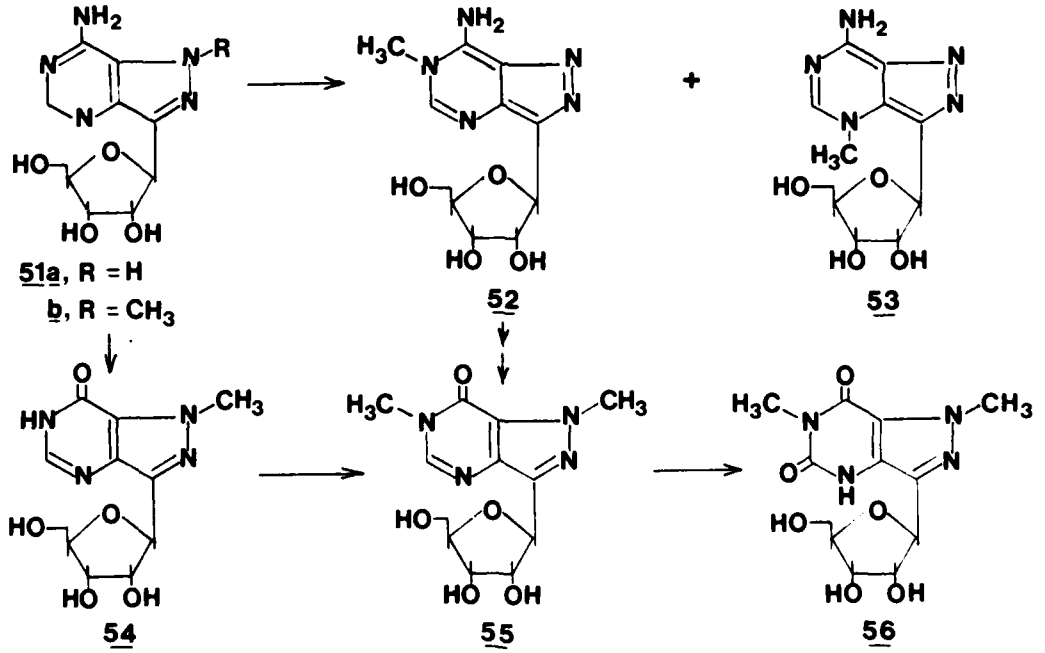
By using our new high-temperature glycosylation procedure, 6-methylthio-pyrazolo[3,4-d]pyrimidin-4(5H)-one (48, BK-49845)[10] was reacted with 28 in the presence of BF_3 etherate in boiling CH_3NO_2, which afforded 6-methylthio-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one (49a) as a syrup. Debenzoylation of 49a with sodium methoxide in methanol gave 6-methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one



(49b). Direct phosphorylation of 49b with $POCl_3$ in trimethylphosphate gave 6-methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one 5'-phosphate (50).

2. Synthesis of Pyrazolo[4,3-d]pyrimidine Nucleosides

Since formycin (6) and formycin B (7) are C-nucleosides, the glycosidic linkage is stable to chemical as well as enzymatic cleavage and thus not degraded by the host or the parasite.[34] Although the reported EC_{50} (concentration of drug that inhibits the growth rate of cells by 50%) value of allopurinol ribonucleoside[15] is similar to that of formycin B (7.5 μM)[35] for T. cruzi epimastigotes, the observed EC_{50} value of 1-methylformycin B (54, BK-62973) is 0.60 μM.[46] In view of these observations, we now prepared 6-methylformycin (52) and 1,6-dimethyloxoformycin B (56).

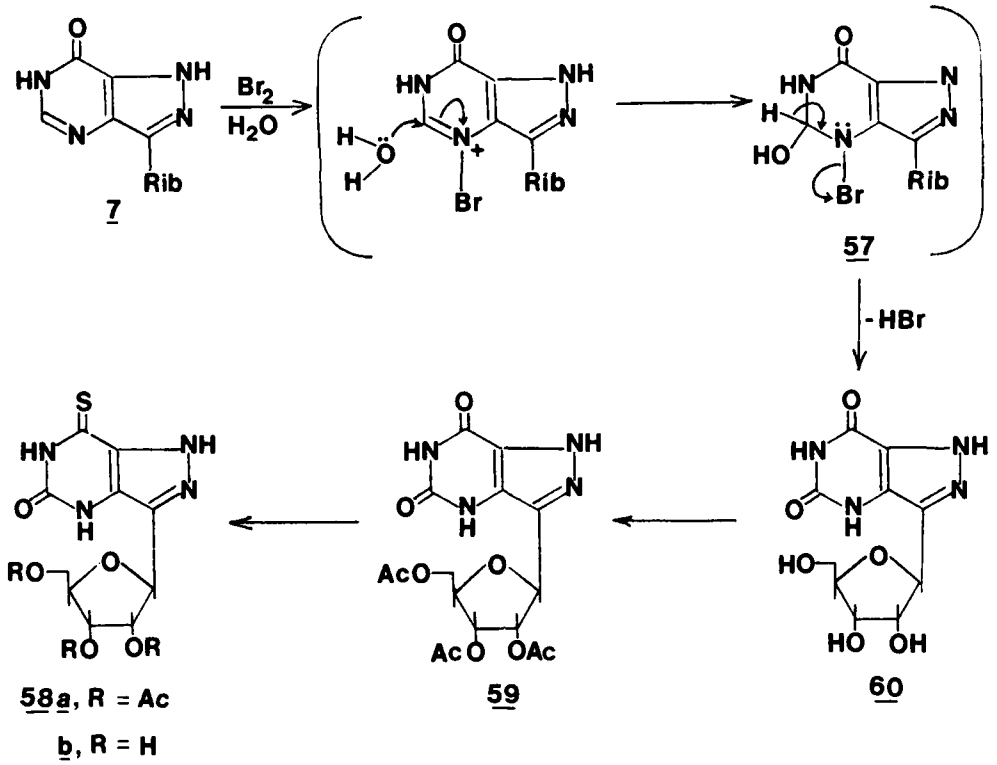


Methylation of formycin with methyl iodide in dimethylformamide, as reported by Lewis and Townsend,[47] gave a mixture of two major products, which were separated by fractional crystallization. The major product (50% isolated yield) of m.p. 230°C was isolated and identified as 7-amino-6-methyl-3-β-D-

ribofuranosylpyrazolo[4,3-d]pyrimidine (6-methylformycin, 52, BK-74419). The minor product (17% yield) of mp 268°C was found to be 4-methylformycin (53).[47] The ratio of the products 52 and 53 was found to be 2:1.

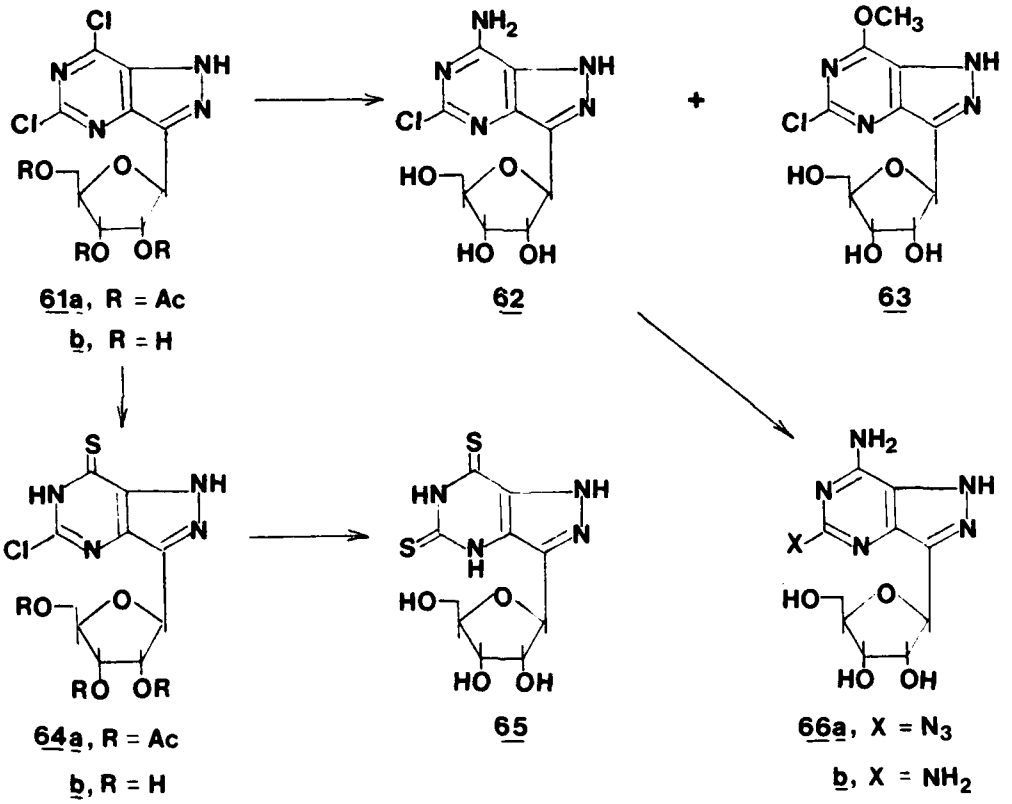
We have recently developed and reported[48] a simple and direct synthesis of oxoformycin B (3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine-5,7-(4H,6H)-dione) from formycin B. This procedure is considerably superior to previously reported[49] total synthesis of oxoformycin B. This simple, high-yield oxidation procedure used to prepare 1,6-dimethyloxoformycin B (56). Methylation of formycin with N,N-dimethylformamide dimethylacetal in DMF at 60°C overnight, followed by the treatment with methanolic ammonia, gave a 94% yield of 1-methylformycin (51b). Deamination of 51b with nitrosyl chloride to obtain 1-methylformycin B (54) has recently been described.[47] However, the isolated yield of 54 is less than 10%. We have now developed [50] a procedure for deamination of 51b with liquid nitrosyl chloride in DMF, which gave 54 in essentially quantitative yield, which was isolated as the monohydrochloride. The monohydrochloride salt has been isolated for the first time and adequately characterized. Neutralization of an aqueous solution of the hydrochloride salt with Dowex-50 (OH^-) resin gave free 1-methylformycin B (54, BK-62973). Further methylation of 54 with excess of N,N-dimethylformamide dimethyl acetal in DMF at 80-95°C for 2 days, followed by the treatment of the reaction product with ammonium hydroxide, gave a dimethylated compound identified as 1,6-dimethylformycin B (55). The fact that compound 55 is indeed 1,6-dimethylformycin B is confirmed by methylation of 6-methylformycin B [47] with N,N-dimethylformamide dimethyl acetal in DMF, followed by deacylation of the intermediate product. The isolated yield of 55 from these methods is over 85%. Treatment of 55 with bromine-water under our oxidation conditions [48] gave 1,6-dimethyloxoformycin B (56, BK-74393) in excellent yield. The disap-

pearance of the C_5 aromatic proton at δ 8.19 ppm in the ^1H NMR spectrum of 56 indicated that the oxidation had indeed taken place at position 5.



We have recently proposed[48] a plausible mechanism of this simple selective oxidation to obtain oxoformycin B from formycin B. This oxidation may be visualized simply as occurring by the direct attack of Br^+ at N_4, followed by the addition of water to C_5. It has been shown by X-ray crystallography that the N_4-C_5 double bond in formycin hydrobromide (bond length 1.284 A)[51,52] is shorter than that in formycin (1.313 A)[53] or formycin B (1.310 A), and is > 0.03 A shorter than in the normal purine nucleoside [corresponding N_3-C_2 double bond]. It is presumed that this increased double bond character leads to the formation of 57, followed by spontaneous elimination of the elements of hydrogen bromide to give oxoformycin B (60). Acetylation of 60 with acetic anhydride in the presence of 4-N,N-dimethylaminopyridine (DMAP) at room temperature for 12 hours gave a triacetylated product 3-(2,3,5-tri-O-acetyl-β-

D-ribofuranosyl)pyrazolo[4,3-d]pyrimidine-5,7(4H,6H)-dione (59, BK-95196) in a 91% yield. Treatment of 59 with purified P_2S_5 in anhydrous dioxane containing DMAP, at reflux temperature for 30 min. gave the thiated product 7-thio-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidine-5(4H,6H)-dione (58a), which on deacetylation with sodium methoxide in methanol furnished 7-thio-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine-5(4H,6H)-dione (58b, BK-98811) in a 89% yield.



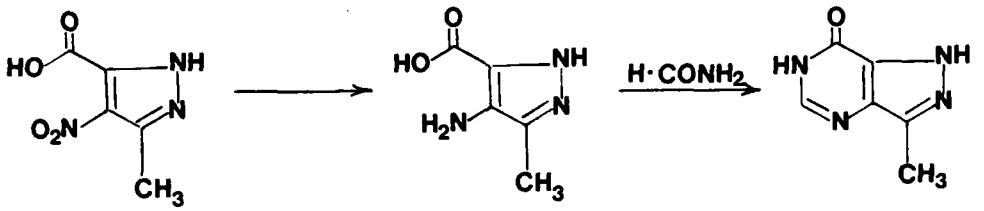
Triacetyloxoformycin B (59) was used for functional group manipulation studies to obtain hitherto inaccessible 5,7-disubstituted pyrazolo[4,3-d]-pyrimidine ribonucleosides. Chlorination of the sodium salt of 59 (generated *in situ* by the treatment of sodium hydride in anhydrous tetrahydrofuran) with phenylphosphonic dichloride gave the key intermediate 5,7-dichloro-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidine (61a) in 81% yield.

However, chlorination of 59 with boiling $POCl_3$ generally gave lower yield (~40%) of 61a, and the isolation of the pure product was cumbersome. Deacetylation of 61a under controlled conditions (10% NaOH in dioxane at room temperature for 3 hours) gave 5,7-dichloro-3-β-D-ribofuranosylpyrazolo[4,3-d]-pyrimidine (61b), which was isolated in a 46% yield, after extensive purification on a silica gel column. However, treatment of 61a with methanolic ammonia (saturated at 0°C) at room temperature gave a mixture of two nucleoside products. These nucleosides were separated on an open bed silica gel column using $CHCl_3$:MeOH (10:1, v/v) as the solvent. The fast moving (first eluted) nucleoside product was identified as 5-chloro-7-methoxy-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine (63, 63%) and the slower moving product was found to be 7-amino-5-chloro-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine (62, 25%, 5-chloroformycin). As expected, reaction of 61a with liquid ammonia gave exclusively 62, which was isolated in over 89% yield. Similarly, treatment of 61a with sodium methoxide in methanol at room temperature furnished 63 as the sole product in 87% yield. Compound 63 was converted into 62 by the treatment of liquid ammonia for 24 hours.

Thiation of 61a with thiourea in absolute ethanol at room temperature gave the monothio derivative 5-chloro-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidine-7(6H)-thione (64a). The isolated yield in this case was 68%. Deacetylation of 64a with freshly prepared sodium methylate in methanol furnished the deprotected nucleoside 5-chloro-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine-7(6H)-thione (64b). Further treatment of 64b with sodium hydrogen sulfide (prepared by saturating 1 N $NaOCH_3$ in CH_3OH with H_2S) gave the interesting dithio compound 3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine-5,7(4H,6H)-dithione (65). Compound 65 was fully characterized by spectroscopic and elemental analysis. When 5-chloroformycin

(62) was allowed to react with lithium azide in DMF at 120°C for 15 hours, 5-azido-7-amino-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine (66a) was formed which was isolated in 85% yield, and fully characterized. Further studies with 66a to obtain 5-aminoformycin (66b) by catalytic hydrogenation, are in progress.

As a part of this program we have also prepared 3-methylpyrazolo[4,3-d]-pyrimidin-7(6H)-one (69). The starting material 3-methyl-4-nitropyrazole-5-



67 68 69

carboxylic acid (67) was prepared by the method of Musante [54] and reduced in aqueous solution with sodium hydrosulfite to give 3-methyl-4-aminopyrazole-5-carboxylic acid (68) in about 60% yield.[55] Ring closure of 68 by boiling with formamide furnished the target compound 69.[55]

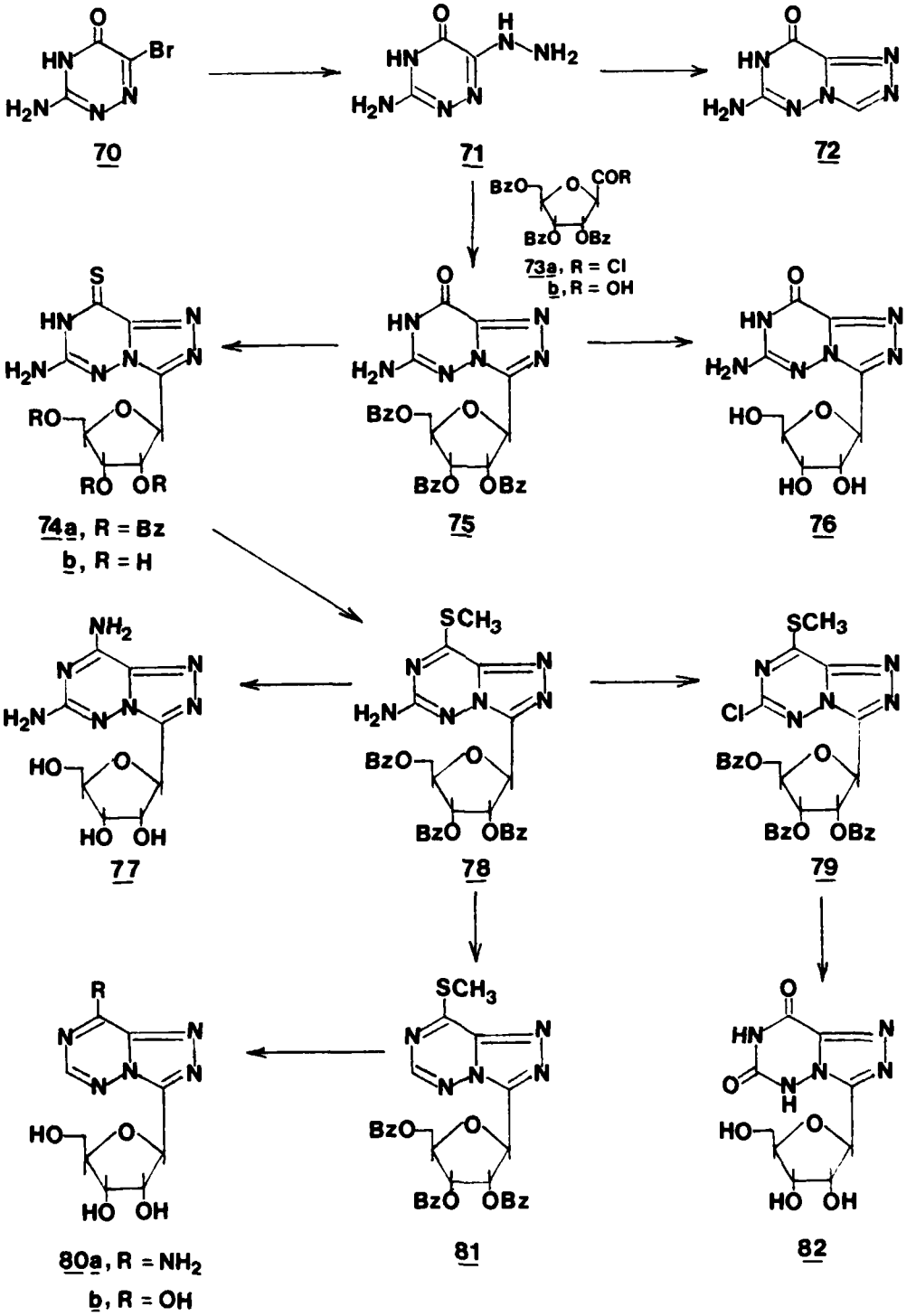
3. Synthesis of s-Triazolo[3,4-f]-as-triazines

s-Triazolo[3,4-f]-as-triazine ribonucleosides are of particular interest since these nucleosides may be looked upon as aza-analogues of formycin B. The parent s-triazolo[3,4-f]-as-triazine ring system is not studied adequately and the nucleoside derivatives of this ring system are not described in the literature until the present work. Since direct ribosylation of the parent ring system would be fraught with difficulties in structural assignments, we preferred the annulation of the triazine ring on to a carbohydrate derivative, and for this purpose, 3-amino-6-bromo-1,2,4-triazine-5(4H)-one (70, BK-48526) was found to be a viable starting material.

The bromine atom in 70 was reasonably labile, undergoing displacement reaction by hydrazine affording 3-amino-6-hydrazino-1,2,4-triazin-5(4H)-one (71, BK-57463).[56] When 71 was treated with hot formic acid, the guanine analog 6-amino-s-triazolo[3,4-f]-as-triazin-8(7H)-one (72, BK-57472)[56] was formed. The ir spectrum of 72 possessed an intense absorption at 1700 cm^{-1}, which is consistent with the carbonyl absorption reported for similar fused triazines,[57] leading to the conclusion that ring closure had occurred. Supporting evidence was obtained in the ^1H NMR spectrum which revealed a highly deshielded proton resonating at δ 9.50 ppm. Treatment of 71 with 1-chlorocarbonyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (73a)[58] in anhydrous THF in the presence of KOH, followed by heating in ethylene glycol at 200°C for 2 hours furnished a 40% yield of the ring-closed product 6-amino-3-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-s-triazolo[3,4-f]-as-triazin-8(7H)-one (75, BK-57481). Subsequently it was observed that the dehydrative coupling of 71 with 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl-1-carboxylic acid (73b)[58] in the presence of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) in ethanol is much more reproducible and gives better yield of 75. Debenzoylation of 75 with sodium methoxide in methanol gave the guanosine analog 6-amino-3-β-D-ribofuranosyl-s-triazolo[3,4-f]-as-triazin-8(7H)-one (76, BK-57507). Thiation of 75 with P_2S_5 in boiling dioxane, in the presence of DMAP, gave the intermediate compound 6-amino-3-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-s-triazolo-[3,4-f]-as-triazine-8(7H)-thione (74a). Debenzoylation of 74a with NaOMe/MeOH furnished the thioguanosine analog, 6-amino-3-β-D-ribofuranosyl-s-triazolo-[3,4-f]-as-triazine-8(7H)-thione (74b) in a good yield.

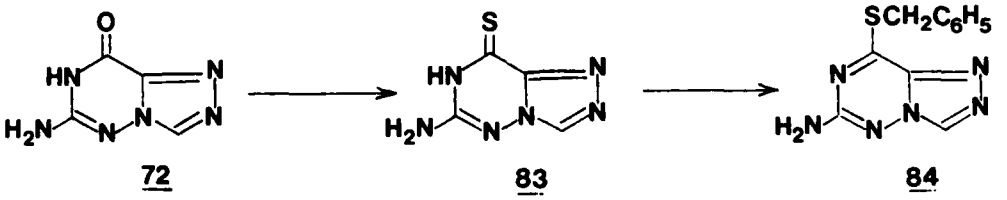
Compound 74a was found to be a key intermediate for the preparation of adenosine, inosine and xanthosine analogs. Methylation of the sodium salt of 74a (produced by the treatment of NaH) with methyl iodide in DMF:CH_2Cl_2 (1:1)

at room temperature gave the corresponding methylthio derivative (78). Compound 78 was isolated in more than 98% yield as analytically pure foam.



When compound 78 was allowed to react with CH_3OH/NH_3 at room temperature for 2 days, 6,8-diamino-3-β-D-ribofuranosyl-s-triazolo[3,4-f]-as-triazine (77) was formed. Treatment of 78 with tert-butyl nitrite (TBN) in the presence of $SbCl_3$ converted the amino group to an halide to afford 6-chloro-8-methylthio-3-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-s-triazolo[3,4-f]-as-triazine (79), which was isolated in a 77% yield and fully characterized. A similar treatment of 78 with anhydrous TBN and dry THF gave the deaminated product identified as 8-methylthio-3-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-s-triazolo-[3,4-f]-as-triazine (81). The ^1H NMR spectrum of 81 revealed the aromatic proton (C_5H) at δ 8.19 ppm, confirming the structural assignment, which was further corroborated by elemental and spectral analysis. Treatment of 81 with CH_3OH/NH_3 at 70-80°C in a steel reaction vessel for 15 hours gave the adenosine analog 80a. Nitrous acid deamination studies with 80a to obtain the inosine analog 80b are in progress. Similarly, efforts to obtain the xanthosine analog 82 from 79 by the treatment with aqueous alkali are underway.

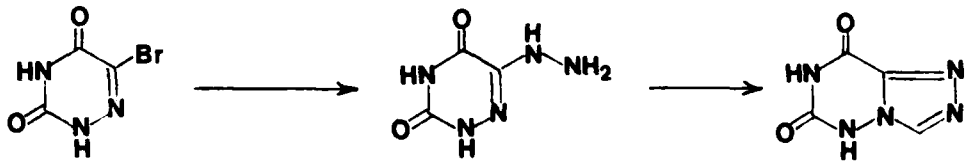
Several appropriately substituted s-triazolo[3,4-f]-as-triazines are also prepared during this study. Reaction of 72 with purified P_2S_5 in anhydrous



72 83 84

pyridine at reflux temperature for 6 hours afforded the thioguanine analog 6-amino-s-triazolo[3,4-f]-as-triazine-8(7H)-thione (83) in rather low yield (~40%). Benzylation of 83 with benzylbromide in DMF containing Na_2CO_3 at 40°C for 3 hours gave 6-amino-8-benzylthio-s-triazolo[3,4-f]-as-triazine (84) in good yield.

The xanthine analog s-triazolo[3,4-f]-as-triazine-6,8(5H,7H)-dione (87) was prepared by following the methodology used for the synthesis of 72. Thus, bromination of 6-azauracil with bromine in water, according to the procedure

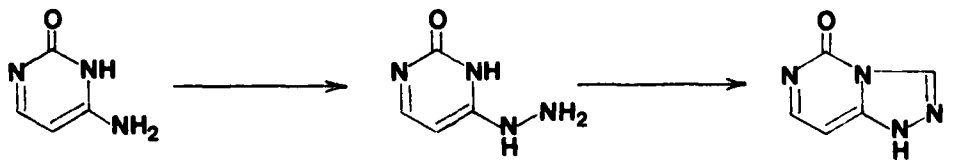


85 86 87

of Chang and Ulbricht,[59] gave 5-bromo-6-azauracil (85, BK-74437). Treatment of 85 with 97% hydrazine and water in a steel bomb at 140-145°C for 4 hours furnished 5-hydrazino-6-azauracil (86, BK-74400),[60] which when heated under reflux in 97% formic acid for 2 days ring closed to yield the xanthine analog s-triazolo[3,4-f]-as-triazine-6,8(5H,7H)-dione (87).

4. Synthesis of s-Triazolo[4,3-c]pyrimidines

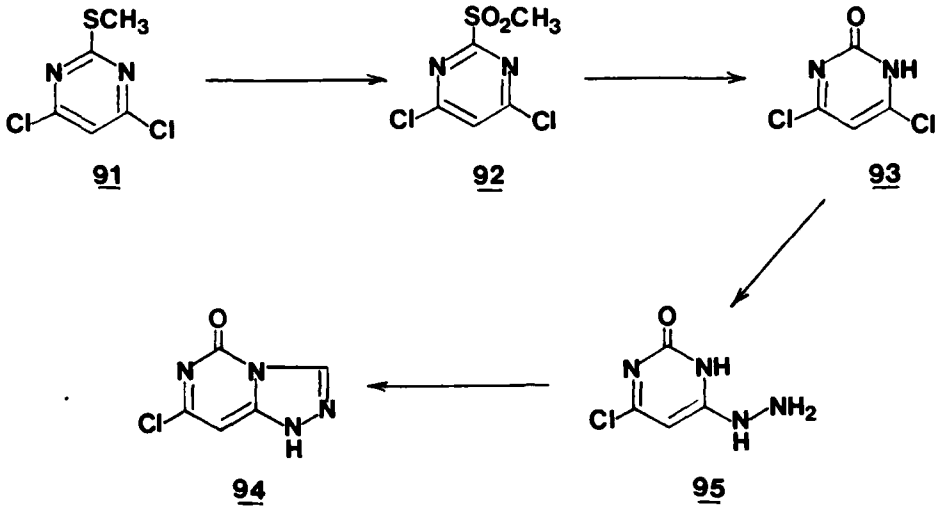
The synthesis of s-triazolo[4,3-c]pyrimidine nucleosides is of particular interest since it represents a class of purine nucleosides with both N_3 and N_7 missing. The synthesis of these nucleosides is envisioned starting from the parent heterocycle itself, and recently we initiated the synthetic work in this direction. A few selected aglycons have been prepared. The allopurinol



88 89 90

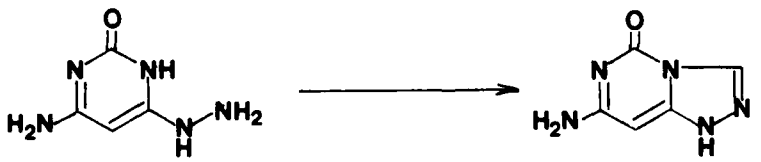
analog, s-triazolo[4,3-c]pyrimidin-5(1H)-one (90)[61] was prepared by a two-step sequence. Treatment of cytosine with 85% hydrazine hydrate at 80°C for 1 hour gave a 50% yield of 6-hydrazinopyrimidin-2-(1H)-one (89),[62] which when heated

under reflux in 97% formic acid for 3 hours ring-closed to give 90 in over 90% yield.



91 92 93 95 94

A viable precursor required for the glycosylation studies to obtain the target s-triazolo[4,3-c]pyrimidine nucleosides is 7-chloro-s-triazolo[4,3-c]-pyrimidin-5(1H)-one (94, BL-05599). The synthesis of 94 was accomplished from the readily available [63] 4,6-dichloro-2-methylthiopyrimidine (91). Oxidation of 91 with m-chloroperoxybenzoic acid in anhydrous dichloromethane afforded the corresponding methylsulfonyl derivative 92, which when treated with 1 N sodium hydroxide at reflux temperature gave 4,6-dichloropyrimidin-2-one (93). Reaction of 93 with anhydrous hydrazine gave 4-chloro-6-hydrazinopyrimidin-2(1H)-one (95), which on subsequent ring-closure by boiling in formic acid



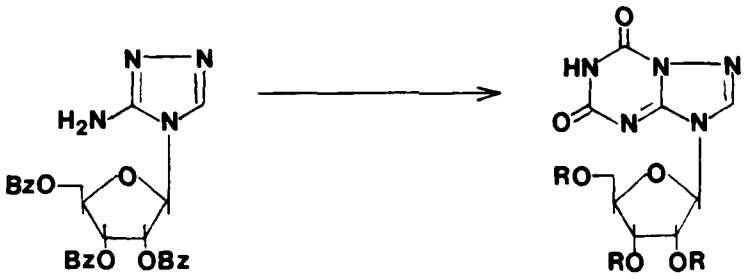
96 97

furnished 94. Similarly, ring-annulation of 4-amino-6-hydrazinopyrimidin-2(1H)-one (96) with either formic acid or triethyl orthoformate gave the

guanine analog 7-amino-s-triazolo[4,3-c]pyrimidin-5(1H)-one (97). The glycosylation studies with 90, 94 and 97 are in progress.

5. Synthesis of s-Triazolo[1,5-a]-s-triazine Nucleosides

In view of the significant antiparasitic activity of oxoformycin B, the synthesis of the xanthosine analog in the s-triazolo[1,5-a]-s-triazine (5-azapurine) ring system was undertaken. The synthesis of this analog was visualized via the ring-closure of 3-amino-4-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,2,4-triazole (98). Compound 98 has previously been reported from



98

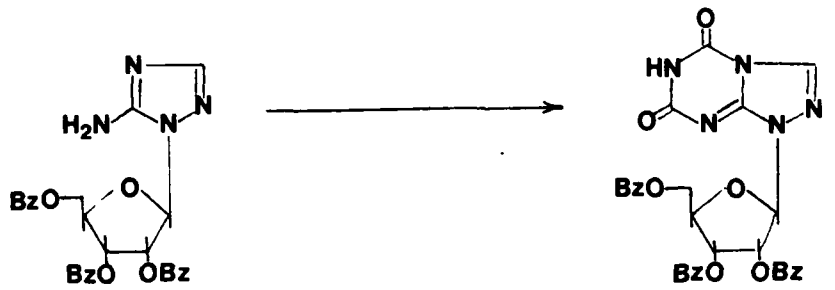
99a, R = Bz

b, R = H

from our laboratory[64] and has been fully characterized. Initially, we prepared the target xanthosine analog 99b by the treatment of 98 with chlorocarbonylisocyanate in anhydrous tetrahydrofuran at room temperature, which gave a 98% yield of 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-s-triazolo-[1,5-a]-s-triazine-5,7(6H)-dione (99a) as the sole product. Debenzoylation of 99a with sodium methoxide in methanol afforded the crystalline xanthosine analog 1-β-D-ribofuranosyl-s-triazolo[1,5-a]-s-triazine-5,7(6H)-dione (99b, BK-57490). Over the period of time, the overall yield of 99b was substantially increased and additional 2.0 g of the material was submitted to WRAIR.

A similar treatment of 5-amino-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,2,4-triazole (100)[64] with chlorocarbonylisocyanate in dry THF gave the

blocked nucleoside 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-s-triazolo-[4,3-a]-s-triazine-5,7(6H)-dione (101). But our attempts to deblock 101 under a variety of conditions failed, and only the ring opened product was isolated.

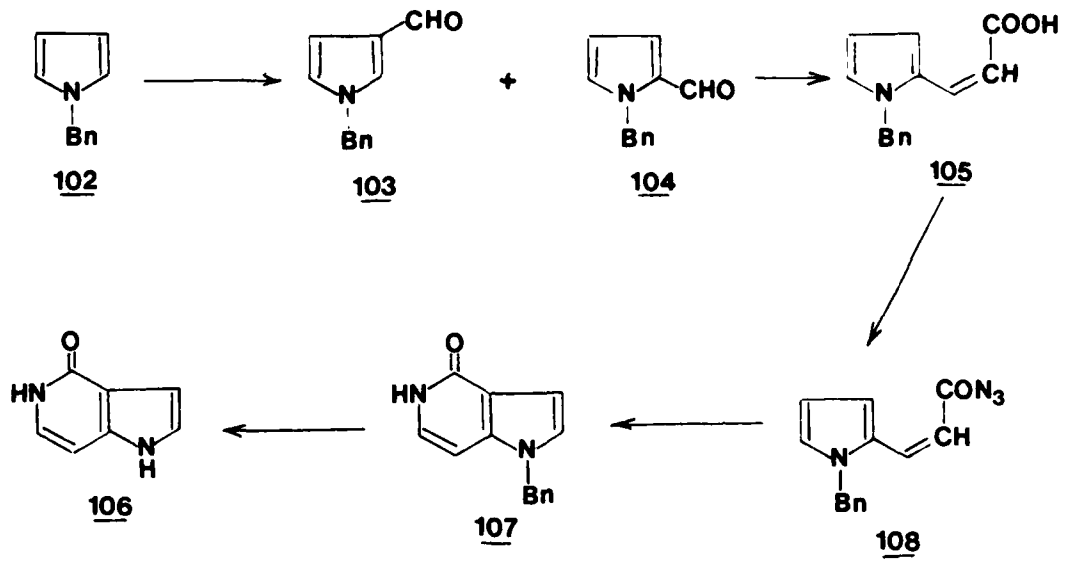


100 101

6. Synthesis of Pyrrolo[3,2-c]pyridines

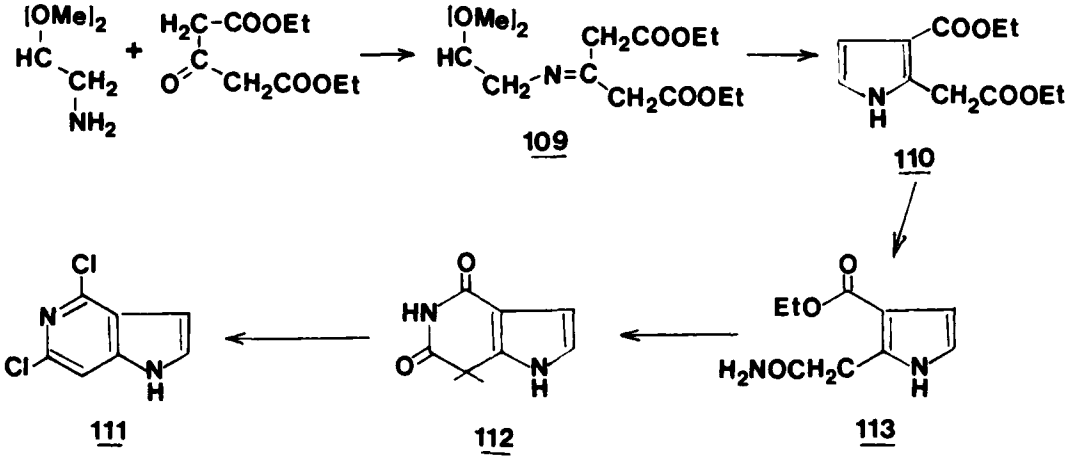
In recent years several unnatural nucleosides have been prepared,[65] which resemble at first glance the natural purine nucleosides, but actually differ in some minor aspect. However, these so-called "counterfeits" have often exhibited considerable biological activity, e.g. significant antiparasitic activity of 9-deazainosine against Leishmania donovani, Trypanosoma cruzi and Trypanosoma gambiense and its very little toxicity for mouse L cells.[66] In some cases the observed inactivity was correlated with lack of appropriate binding; thus, the function of the various nitrogen atoms of purine nucleosides as binding sites for certain important nucleic acid enzymes has become the subject of considerable interest.[67] In view of these observations we have now prepared 2,7-dideazaallopurinol and the corresponding xanthine analog.

Pyrrolo[3,2-c]pyridin-4(5H)-one (2,7-dideazaallopurinol, 106, BK-98786) was prepared as reported.[68] N-Benzylpyrrole (102)[69] was converted to N_1-benzylpyrrolo[3,2-c]pyridin-4(5H)-one (107) by a four-step reaction sequence. Formylation of 102 with $POCl_3$/DMF under Vilsmeier reaction conditions gave a mixture of the corresponding 2- and 3-formyl derivatives (104 and 103, respectively). Condensation of 104 with malonic acid gave the acrylic



acid derivative 105, which was further transformed into N_1-benzylpyrrole-2-acrylazide (108).[68] Ring annulation of 108 in diphenylether afforded 107. Debenzylation of 107 with sodium in liquid ammonia gave the target compound 2,7-dideazaallopurinol, 106 (BK-98786).

The xanthine analog, pyrrolo[3,2-c]pyridine-4,6(5H,7H)-dione (112) was prepared by following the procedure of Schneller et al.[70] Condensation of

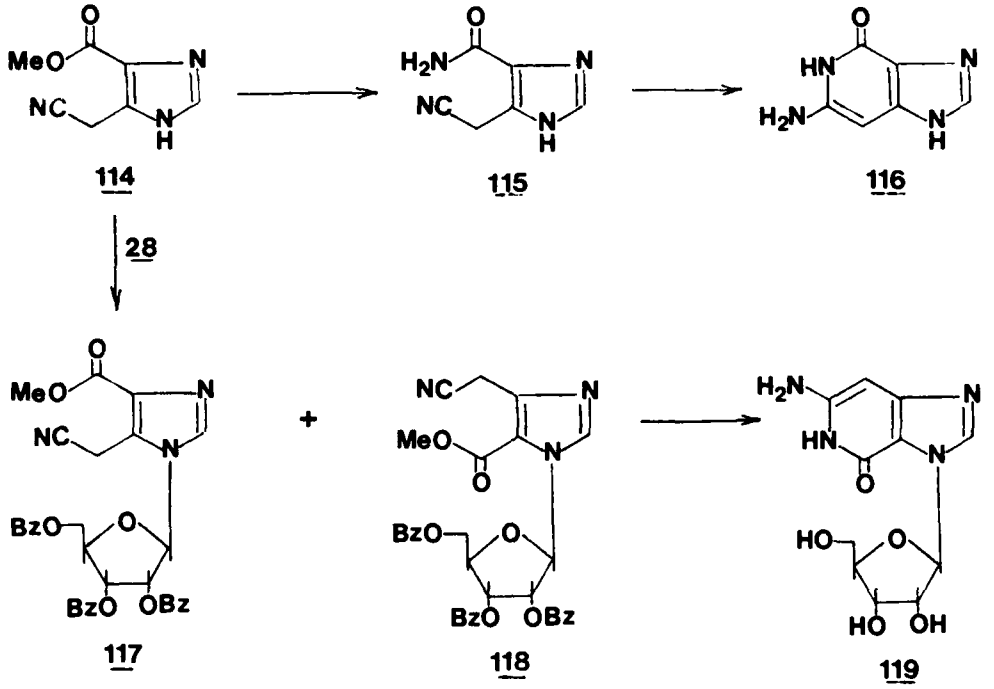


aminoacetaldehyde dimethylacetal and diethyl 1,3-acetonedicarboxylate give the Schiff base 109 in a 78% yield. Ring-closure of 109 by the treatment of HBr in dichloromethane gave ethyl 3-ethoxycarbonylpyrrole-2-acetate (110).[71] The diester 110 was converted into 3-ethoxycarbonylpyrrole-2-acetamide (113) with ammonium hydroxide which, in turn, upon treatment with sodium hydroxide solution cyclized to 112.[70] Chlorination of 112 with phenylphosphonic dichloride gave 4,6-dichloropyrrolo[3,2-c]pyridine (111, BK-74508).[72] Glycosylation studies with 111 are in progress.

7. Synthesis of Imidazo[4,5-c]pyridines

6-Aminoimidazo[4,5-c]pyridin-4(5H)-one (3-deazaguanine, 116) has been shown to inhibit the growth of Escherichia coli B, in vitro.[73] The 7-ribosyl derivative of 3-deazaguanine (6-amino-3-β-D-ribofuranosylimidazo[4,5-c]-pyridin-4(5H)-one, 119) showed antibacterial activity against several Gram-negative strains in vivo without any appreciable toxicity to the host.[74] The antibacterial action of 119 has been ascribed to its cleavage to 3-deaza-guanine in E. coli B infected cells.[75] Recently, 3-deazaguanosine was shown to be a potent antileishmanial agent,[26] which is at least 20 times more active than 3-deazaguanine or allopurinol ribonucleoside against Leishmania tropica in vitro. In order to study the antiparasitic properties in greater detail more of 3-deazaguanine (116) and 7-ribosyl-3-deazaguanine (119) have been prepared as reported from our laboratory.[76]

The key intermediate in the synthesis of both 116 and 119 is methyl 5(4)-cyanomethylimidazole-4(5)-carboxylate (114), which was obtained in 80% yield from methyl 5(4)-carbamoylmethylimidazole-4(5)-carboxylate[77] and refluxing $POCl_3$. Treatment of 114 with liquid ammonia at 100°C for 2 days gave 5(4)-cyanomethylimidazole-4(5)-carboxamide (115) in a 77% yield. Compound 115 was smoothly cyclized to 3-deazaguanine (116) with aqueous sodium



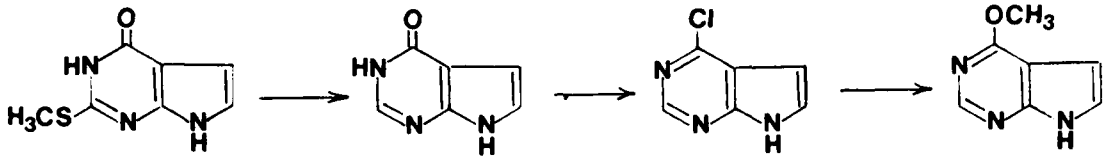
carbonate.[76] Glycosylation of the trimethylsilyl derivative of 114 with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose in the presence of 0.72 molar equivalent of $SnCl_4$, gave a mixture of N_1- and N_3-glycosylimidazole derivatives (117 and 118, respectively), which were separated on an open bed silica gel column to obtain pure 118. When 118 was treated with liquid ammonia at 110°C for 2 hours, the cyclized product 119 was formed,[76] and was isolated in more than 80% yield.

8. Synthesis of Pyrrolo[d]pyrimidines

The pyrrolo[d]pyrimidine ring system, consisting of pyrrole fused with a pyrimidine ring, exists in two forms. The pyrrolo[2,3-d]pyrimidine ring system is found in nature, is sometimes referred to as 7-deazapurine, and differs from purine only in that the N_7 of purine has been replaced by -CH=. The other ring system, pyrrolo[3,2-d]pyrimidine is referred to as 9-deaza-purine. Because of their structural resemblance to purines, the derivatives

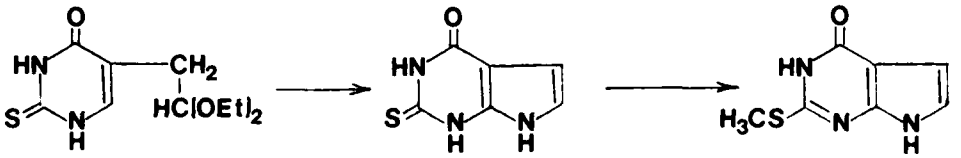
of pyrrolo[d]pyrimidine exhibit unusual biological properties. We have now prepared the allopurinol analog in pyrrolo[2,3-d]pyrimidine ring system and xanthine analog in pyrrolo[3,2-d]pyrimidine ring system for evaluation of their antiparasitic properties.

The starting material needed for the preparation of pyrrolo[2,3-d]-pyrimidin-4(3H)-one (2-deazaallopurinol, 121) was 2-methylthiopyrrolo[2,3-d]-pyrimidin-4(3H)-one (120) and was prepared as reported by Davoll.[78]



120 121 122 123

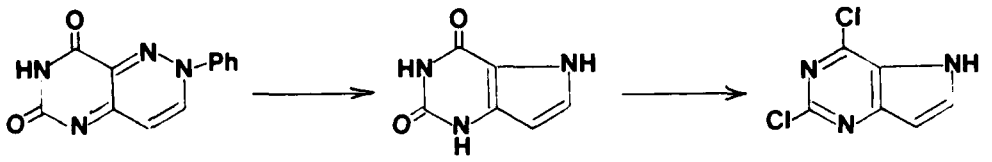
Desulfurization of 120 with Raney nickel in an ammoniacal solution gave 121 (BK-74517), which on chlorination with $POCl_3$ at reflux temperature furnished 4-chloropyrrolo[2,3-d]pyrimidine (122, BK-98795).[79] When 122 was allowed to react with freshly prepared sodium methoxide in methanol at reflux temperature, 4-methoxypyrrolo[2,3-d]pyrimidine (123, BK-98802)[79] was formed. Ring-closure of 2-thio-5-(2,2-diethoxyethyl)pyrimidine-4(1H,3H)-dione (124) in the presence of an acid gave 2-thiopyrrolo[2,3-d]pyrimidine-4(1H,3H)-dione



124 125 126

(125, BL-01500),[78] which on further methylation with methyliodide afforded 2-methylthiopyrrolo[2,3-d]pyrimidin-4(3H)-one (126, BL-01519).[78]

The xanthine analog pyrrolo[3,2-d]pyrimidine-2,4(1H,3H)-dione (128) was prepared as reported by Klein and coworkers.[80] The viable precursor 6,8-dioxo-7H-2-phenylpyrimido[5,4-c]pyridazine (127) was prepared from



127 128 129

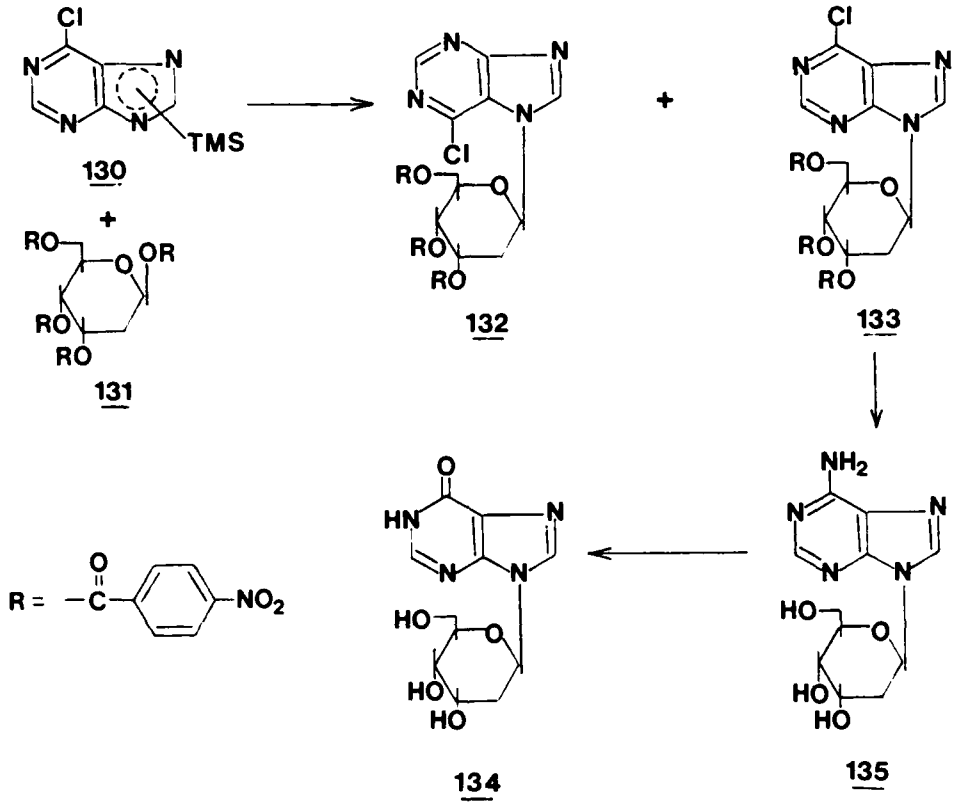
6-methyl-5-phenylazouracil.[81] Treatment of 6-methyl-5-phenylazouracil with tert-butoxybis(dimethylamino)methane in DMF afforded 127, which was converted by hydrogenolysis to 128. Chlorination of 128 with phenylphosphonic dichloride gave the known 2,4-dichloropyrrolo[3,2-d]pyrimidine (129, BK-98777).[82] Glycosylation studies with 129 are in progress.

9. 9-(2-Deoxy-β-D-ribo-hexopyranosyl)- derivatives of Adenine and Hypoxanthine

During the course of these synthetic studies we also prepared certain sugar modified adenosine and inosine derivatives. The purine nucleosides considered in this study contain a ring-expanded carbohydrate moiety, in which a methylene group was inserted between the $C_{1'}$ and the $C_{2'}$ positions of D-ribose, viz, 2-deoxy-D-ribo-hexopyranose. Molecular structures of adenosine and 9-(2-deoxy-β-D-ribo-hexopyranosyl)adenine indicate slightly altered glycon. This type of nucleoside analogues could mimic either as ribonucleosides or as 2'-deoxyribonucleosides. It is of interest to notice that a large number of naturally-occurring nucleoside antibiotics contain a hexopyranosyl moiety, e.g. gougerotin, miharamycin, herbicidins, etc.

Two published accounts describe the chemical synthesis of 2-deoxy-β-D-ribo-hexopyranosyl nucleosides.[83,84] In the later report[84] Zorbach and Saeki attempted the synthesis of 1-(2-deoxy-D-ribo-hexopyranosyl)thymine and 9-(2-deoxy-D-ribo-hexopyranosyl)adenine. However, the final nucleosides were not adequately characterized, the reported yields were discouragingly low and, as a matter of fact, no assignment of anomeric configuration was made. In

view of this inadequacy we now undertook the synthesis and complete characterization of 2-deoxy-β-D-ribo-hexopyranosyl nucleosides of adenine and hypoxanthine.

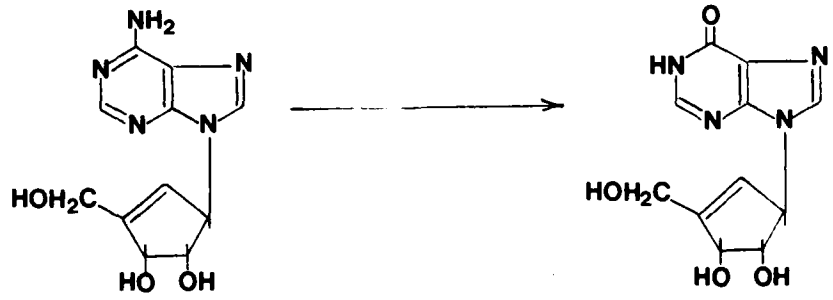


Glycosylation of the trimethylsilyl derivative of 6-chloropurine (130) with 2-deoxy-1,3,4,6-tetra-O-p-nitrobenzoyl-D-ribo-hexose (131)[85] in anhydrous CH_3CN in the presence of the catalyst TMS-triflate at room temperature gave a mixture of two nucleoside products, which were separated on a silica gel column. The pure nucleosides were identified as 6-chloro-9-(2-deoxy-3,4,6-tri-O-p-nitrobenzoyl-β-D-ribo-hexopyranosyl)purine (133) and the corresponding N_7-glycosyl isomer. Treatment of 133 with liquid ammonia at 100°C for 12 hours in a steel vessel, gave the desired adenosine analog 6-amino-9-(2-deoxy-β-D-ribo-hexopyranosyl)purine (135, BL-01484), which was fully characterized by spectral and elemental analysis. Deamination of 135

with aqueous nitrous acid at 25°C for 2 days gave the inosine analog 9-(2-deoxy-β-D-ribo-hexopyranosyl)hypoxanthine (134, BL-01493), in a 68% yield.

10. Neplanocin D

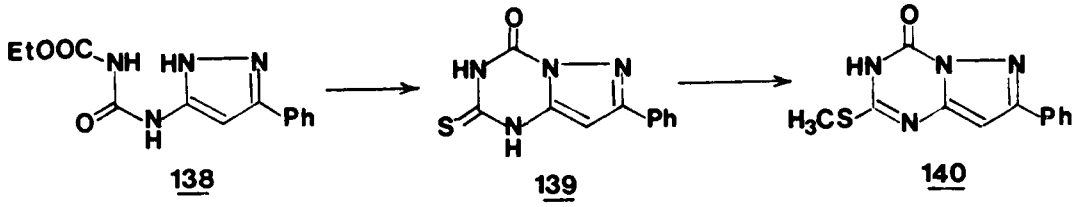
Neplanocins are a group of novel carbocyclic analogs of purine nucleosides which were isolated from the culture filtrate of Ampullarilla regularis A11079. These antibiotics exhibit potent antitumor properties. To the best of our knowledge no antiparasitic data for the inosine analog Neplanocin D has been reported. The culture filtrates of a fermentation beer produced by neplanocin A-producer CL-1018 was obtained from Warner-Lambert Pharmaceutical Research Division, Ann Arbor, Michigan. Isolation of the antibiotic neplanocin A was performed by the successive column chromatography on ion-exchange resin and charcoal, and by partition.[86] Neplanocin A (136) was



136 137

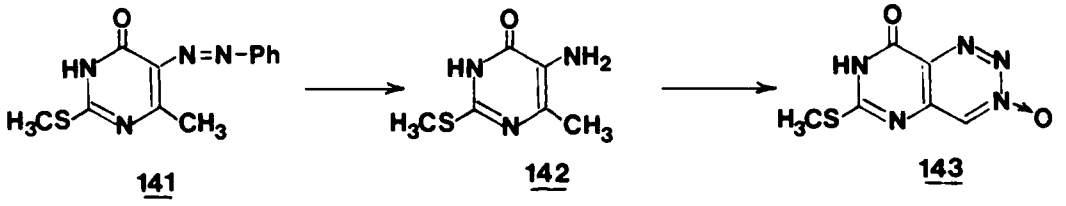
isolated as the major product, and fully characterized. Deamination of 136 with aqueous nitrous acid at 48°C for 4 hours gave the inosine analog neplanocin D (137, BL-05606). Repeated ion-exchange column chromatography and lyophilization gave analytically pure product.

Several heterocyclic precursors, needed for further glycosylation studies, have been prepared. Methylation of 7-phenyl-2-thiopyrazolo[1,5-a]-s-



138 139 140

triazin-4(1H,3H)-one (139),[87] which in turn was obtained by the ring closure of N-carbethoxy-N'-(5-phenylpyrazol-3-yl)thiourea (138), with methyl iodide in ethanol containing sodium hydroxide gave 2-methylthio-7-phenylpyrazolo[1,5-a]-s-triazin-4(3H)-one (140).[87]



141 142 143

Reduction of 6-methyl-2-methylthio-5-phenylazopyrimidin-4-one (141)[81] with sodium dithionate in 1 N sodium hydroxide solution at 65-70°C gave 5-amino-6-methyl-2-methylthiopyrimidin-4(3H)-one (142) in rather low yield (~45%). Diazolization of a solution of 142 in 1 N sodium hydroxide with aqueous nitrous acid gave the intermediate 2-methylthiopyrimido[4,5-d]-1,2,3-triazin-4(3H)-one N,-oxide (143). As in the case of 128, ring contraction of 143 is expected to yield a pyrazolo[4,3-d]pyrimidine derivative, which would be employed for C-glycosylation studies, and these studies are in progress.

III. EXPERIMENTAL

General Procedures. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR) spectra were determined at 89.6 MHz with a JEOL FX 90Q spectrometer. The chemical-shift values are expressed in δ values (parts per

million) relative to tetramethylsilane as an internal standard. The presence of solvent as indicated by elemental analysis was verified by ^1H NMR. Infrared spectra (IR) were obtained on a Beckman Acculab 2 spectrophotometer and ultraviolet spectra (UV; sh = shoulder) were recorded on a Cary Model 15 spectrophotometer. Elemental analyses were performed by Robertson Labs, Florham Park, NJ. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 plates (EM Reagents). E. Merek silica gel (230-400 mesh) was used for flash column chromatography. Preparative liquid chromatography (LC) was run utilizing the Waters Prep 500 LC system. All solvents used were reagent grade. Detection of components on TLC was by UV light and with 10% H_2SO_4 in MeOH spray followed by heating. Evaporations were carried out under reduced pressure with the bath temperature below 30°C.

1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one (Allopurinol ribonucleoside triacetate, 11a). A mixture of dry pyrazolo-[3,4-d]pyrimidin-4(5H)-one[10] (Allopurinol, 1, 27.20 g, 0.2 mole, dried at 110°C over P_2O_5 under vacuum, overnight), anhydrous pyridine (dried over KOH, 65.0 ml), anhydrous hexamethyldisilazane (HMDS, dried over molecular sieve, 50 ml) and ammonium sulfate (250 mg) was heated under reflux (oil bath temperature, 130-135°C) with the exclusion of moisture. Within 15 min. a clear solution was obtained. Heating was continued for further 3 hours, after which excess of pyridine and HMDS was removed by evaporation in vacuo. Co-evaporation with anhydrous benzene (3 x 100 ml) gave crystalline solid of 1,0,-bistrimethylsilyl allopurinol.

The bistrimethylsilyl allopurinol was dissolved in p-dioxane (dried over molecular sieve, 750 ml) to which was added 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (10, 63.6 g, 0.20 mole). The mixture was heated at gentle reflux under anhydrous conditions, as a solution of freshly distilled boron tri-

fluoride diethyl etherate (33.0 ml) in anhydrous p-dioxane (200 ml) was added dropwide. After the addition was complete, heating was continued for an additional 30 min. The reaction mixture was cooled to room temperature and carefully added to saturated aqueous sodium bicarbonate solution (1 lit). After stirring for 30 min. the mixture was filtered and the filtrate was extracted with chloroform (4 x 250 ml), dried over anhydrous sodium sulfate before the solvent was evaporated in vacuo to yield about 85 g of pale yellow foam containing two major nucleoside products. The mixture was separated on a silica gel (1000 g) column using chloroform:acetone (85:15, v/v) as the solvent. The mixture can also be separated on Waters preparative HPLC system. The N_1-glycosyl isomer eluted first. The fractions containing the homogeneous product was evaporated to dryness to yield 25.4 g (32.2%) of colorless foam. ^1H NMR (Me_2SO-d_6) δ 6.65 (d, 1, J = 3.5 Hz, $C_{1'}\underline{H}$), 8.11 (s, 1, $C_3\underline{H}$), 8.21 (s, 1, $C_6\underline{H}$), and other sugar protons, R_f = 0.30 ($CHCl_3$:acetone, 8:2 and 0.20 ether).

		C	H	N
Analysis :	Calcd for $C_{16}H_{18}N_4O_8$:	48.73	4.56	14.21
	Found:	48.56	4.42	14.37

1-β-D-Ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one (Allopurinol ribonucleoside, 11b). A solution of 11a (3.94 g, 10 mmol) in absolute methanol (100 ml) was adjusted to pH 10 with sodium methoxide and stirred at room temperature for 16 hours. The mixture was neutralized with Dowex-50 (H^+) resin and the resin was removed by filtration. Evaporation of the filtrate gave a solid which was crystallized from water to yield 2.40 g (89.5%); mp 205°C.

Chromatography: Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr): Major bands - ν 695, 770, 800, 835, 880, 995, 1010, 1050, 1075, 1130, 1255, 1395, 1425, 1440, 1505, 1530, 1580, 1700, 2900-3500 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 250 nm (ε 8,200);
λ_{max} (pH 7) 250 nm (ε 8,600);
λ_{max} (pH 11) 255 nm, sh (ε 8,600), 270 (11,500).

		C	H	N
Analysis :	Calcd for $C_{10}H_{12}N_4O_5$:	44.78	4.51	20.89
	Found:	44.75	4.58	20.67

6-Chloro-4-methoxypyrazolo[3,4-d]pyrimidine (12). To a solution of sodium methoxide prepared by dissolving 5.0 g of sodium in 150 ml of absolute methanol were added, in small portions, 4,6-dichloropyrazolo[3,4-d]pyrimidine[40] (6.0 g, 31.7 mmol). The solution was warmed until all the starting material had dissolved. The insoluble sodium chloride was filtered and the filtrate immediately evaporated to dryness. To the residue was added 100 ml of water, and the clear, yellow solution was then acidified with acetic acid. After cooling overnight, the solid that separated was collected by filtration to give 4.8 g of crude product, mp 175-180°C. Recrystallization from benzene raised the mp to 178-180°C [Lit.[40] mp 181-182°C].

Chromatography : Absorbent - silica gel
Solvent - Benzene:EtOAc, 4:1, v/v

Infrared (KBr) : Major bands - ν 700, 735, 775, 850, 920, 940, 960, 1140, 1160, 1245, 1295, 1330, 1360, 1380, 1470, 1570, 1595 and 3200 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 252 nm (ε 7,800);
λ_{max} (pH 7) 252 nm (ε 7,600);
λ_{max} (pH 11) 257 nm (ε 6,300), 285 sh (2,400).

			C	H	N	Cl
Analysis	:	Calcd for $C_6H_5ClN_4O$:	39.04	2.73	30.36	19.21
		Found:	38.82	2.56	30.07	19.07

6-Aminopyrazolo[3,4-d]pyrimidin-4(1H,5H)-one (6-Aminoallopurinol, 15).

Step I. 6-Chloropyrazolo[3,4-d]pyrimidin-4(1H,5H)-one (14). To 400 ml of a boiling and vigorously stirred 2 N KOH solution was added, a little at a time, 40 g (211 mmole) of 4,6-dichloropyrazolo[3,4-d]pyrimidine.[40] The addition took 15 min after which time decolorizing carbon was added, and the solution was gently boiled and stirred for 15 min. more. The solution was then filtered and the hot filtrate acidified with acetic acid and allowed to cool to room temperature. The light green solution was then filtered from approximately 1 g of solid impurities and the filtrate further cooled for 2 days in the refrigerator. The solid that separated was collected by filtration and dried to yield 24.1 g of crude material. This material was crystallized from water to give colorless crystals, mp > 300°C [Lit.[40] mp > 300°C].

			C	H	N
Analysis	:	Calcd for $C_5H_3ClN_4O$:	35.20	1.80	32.90
		Found:	35.40	2.10	33.00

Step II. 6-Aminopyrazolo[3,4-d]pyrimidin-4(1H,5H)-one (15). Compound 14 (10 g, 58.6 mmol) was added to ethanolic ammonia (100 ml, saturated at 0°C). The solution was placed in a high pressure bomb and heated at 200°C (inside temperature) for 12 hours. The solution was cooled and filtered. The white product was further purified by suspending the material in 300 ml of boiling water followed by the addition of just enough conc. HCl to effect solution. To the clear solution was added NH_4OH until a pH of 9 was reached. The product was immediately filtered from the hot solution and washed with water.

The yield of the purified product was 7.6 g, mp > 300°C [Lit.[40] mp > 300°C].

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 660, 765, 830, 935, 1140, 1235, 1335, 1450, 1500, 1580, 1670, 2910, 3100, 3320 and 3400 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 251 nm (ε 4,500);

λ_{max} (pH 7) 247 nm (ε 6,100);

λ_{max} (pH 11) 244 nm (ε 7,600), 265 (6,900).

Analysis :		C	H	N
	Calcd for $C_5H_5N_5O$:	39.74	3.33	46.34
	Found:	39.58	3.42	46.63

6-Amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one (6-Amino-allopurinol ribonucleoside, 18).

Step I. 4,6-Dichloro-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-d]-pyrimidine (16). A mixture of dry 4,6-dichloropyrazolo[3,4-d]pyrimidine[40] (8.0 g, 42.6 mmol), HMDS (50 ml) and $(NH_4)_2SO_4$ (25 mg) was heated under reflux for 5 hours, with the exclusion of moisture. Excess HMDS was removed by distillation to provide the trimethylsilyl derivative of 13 as a semi-solid. To the solution of the TMS derivative in dry CH_3CN (125 ml) was added 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (13.5 g, 42.6 mmol), followed by TMS-triflate[41] (14.2 g, 64 mmol). The clear reaction mixture was stirred at ambient temperature for 16 hours. The solvent was evaporated to dryness, the residual syrup dissolved in EtOAc (300 ml) and poured into a saturated aqueous $NaHCO_3$ solution (200 ml) with stirring. The organic layer was separated and washed with 5% $NaHCO_3$ solution (2 x 75 ml), followed by water (3 x 75 ml). The dried (Na_2SO_4) organic phase was evaporated and the residual syrup (15.7 g) was

purified on a silica gel column by Prep LC techniques using EtOAc:hexane (3:7, v/v) as the solvent. The title compound 16 was isolated as colorless gum, 14.0 g (74%).

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:acetone, 8:2, v/v

Infrared (neat): ν 780 (C-Cl), 1750 (OAc) cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 215 nm (ϵ 25,500), 269 (5,400);

λ_{max} (pH 7) 215 nm (ϵ 25,900), 269 (5,800);

λ_{max} (pH 11) 215 sh, nm (ϵ 29,000), 269 (6,300).

		C	H	N
Analysis :	Calcd for $C_{16}H_{16}Cl_2N_4O_7 \cdot \frac{1}{2}H_2O$:	42.12	3.76	12.28
	Found:	42.46	3.76	11.90

Step II. 6-Chloro-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one (17). A solution of 16 (4.10 g, 9.17 mmol) in 1 N NaOH (50 ml) was heated on a steam bath for 2 hours with occasional shaking. The resulting clear solution was cooled (5-10°C) and adjusted to pH 4 with Amberlite IRC-50 (H^+). The resin was removed by filtration and washed with hot water (3 x 25 ml). The combined filtrate and washings were evaporated to provide 17 as colorless solid, 2.60 g (96%). An analytical sample (0.20 g) was prepared by passing through a silica gel column (2 x 25 cms), eluted with CH_2Cl_2:MeOH (5:1, v/v), mp 130°C [Lit.[38] mp 130°C].

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : ν 1690 (C=O), 3260-3480 (NH, OH) cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 247 nm (ϵ 8,300);

λ_{max} (pH 7) 250 nm (ϵ 8,200);

λ_{max} (pH 11) 260 nm (ϵ 9,700).

		C	H	N	Cl
Analysis :	Calcd for $C_{10}H_{11}ClN_4O_5$:	39.68	3.66	18.51	11.71
	Found:	39.59	4.36	18.75	11.66

Step III. 6-Amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one (18). Compound 17 (1.0 g, 3.3 mmol) and MeOH/NH_3 (saturated at 0°C, 50 ml) were placed in a steel bomb (125 ml). The bomb was heated at 120°C for 20 hours. MeOH/NH_3 was evaporated, the residue was dissolved in MeOH, adsorbed on silica gel (~5 g) and placed on top of a silica gel column (2 x 25 cms, packed in EtOAc). The column was eluted with EtOAc:H_2O:n-PrOH (4:2:1, upper phase) and the appropriate homogeneous fractions were pooled and evaporated to dryness. Crystallization of the residue from water gave white needles, 0.70 g (75%), mp 262-263°C [Lit.[38] mp 262-263°C].

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 6:1, v/v

Infrared (KBr) : Major bands - ν 680, 730, 780, 850, 970, 1010, 1040, 1120, 1140, 1200, 1300, 1370, 1460, 1550, 1610, 1640, 1690, 2900-3500 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 252 nm (ε 13,600);

λ_{max} (pH 7) 252 nm (ε 14,400);

λ_{max} (pH 11) 263 nm (ε 11,000).

^1H NMR (Me_2SO-d_6): δ 5.88 (d, 1, J = 4.06 Hz, $C_1'H$), 6.72 (s, 2, NH_2), 7.84 (s, 1, C_3H), 10.77 (s, 1, N_5H), and other sugar protons.

		C	H	N
Analysis :	Calcd for $C_{10}H_{13}N_5O_5$:	42.40	4.62	24.73
	Found:	42.35	4.70	24.43

5-Amino-4-cyanopyrazol-3(1H,2H)-one (21). To a suspension of dicyanoketene dimethyl acetal[43] (19, 2.76 g, 20 mmol) in water (20 ml) was added hydrazine hydrate (1 ml) with stirring. An exothermic reaction was observed. The reaction mixture was heated on a steam bath for 5 min. and allowed to cool to 0-5°C. The product that separated was collected by filtration and dried to yield 2.62 g (95%) of 5-amino-3-methoxypyrazol-4-carbonitrile (20). To a solution of 20 (1.38 g, 10 mmol) in dry CH_3CN (100 ml) were added sodium iodide (1.50 g) and chlorotrimethylsilane (1.4 ml) and the resulting mixture was heated under reflux for 15 hours. After cooling the precipitated product was collected, washed with cold sodium bisulfite (5%) solution (2 x 25 ml), followed by cold water (2 x 25 ml). Crystallization of the residue from aqueous DMF gave off-white needles, 0.62 g (50%), mp > 350°C.

Chromatography : Absorbent - silica gel

Solvent - CH_3CN:1 M NH_4Cl, 7:3, v/v

Infrared (KBr) : Major bands - ν 710, 785, 1225, 1310, 1460, 1540, 1570, 1620, 2210, 3160, 3280 and 3400 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 228 nm (ε 10,200);

λ_{max} (pH 7) 220 nm, sh (ε 8,100);

λ_{max} (pH 11) 222 nm, sh (ε 8,800).

Analysis		C	H	N
	Calcd for $C_4H_4N_4O$:	38.71	3.25	45.15
	Found:	38.45	3.47	45.26

5-Amino-3-methoxypyrazole-4-carboxamide (23). Concentrated sulfuric acid (15 ml) was cooled to 10°C and finely powdered 20 (1.38 g, 10 mmol) was added with stirring so that the temperature did not rise above 25°C. The addition of 20 took about 5 min. The solution was stirred at room temperature for 3 hours and then poured with stirring into a mixture of 50 ml of water and 50 g

ice. The aqueous solution was neutralized with 50% aqueous sodium hydroxide solution, keeping the temperature below 20°C. The precipitated Na_2SO_4 was removed and the filtrate again adjusted to pH 7. The mixture was set aside overnight in the refrigerator. The product that separated was collected by filtration, dried and recrystallized from water to yield 1.40 g (89.7%), mp 242-244°C.

		C	H	N
Analysis :	Calcd for $C_5H_6N_4O_2$:	38.46	5.13	35.90
	Found:	38.54	5.33	36.12

5-Amino-4-carbamoylpyrazol-3(1H,2H)-one (24). Method 1. Compound 23 (7.75 g, 49.7 mmol) was combined with dry CH_3CN (250 ml) and sodium iodide (8.2 g, 55 mmol) at room temperature. Under nitrogen atmosphere, chlorotrimethylsilane (6.95 ml, 55 mmol) was added dropwise and the reaction mixture was heated under reflux for 24 hours. After cooling (0-5°C), the precipitated product was collected, washed with cold sodum bisulfite solution (5%, 2 x 25 ml), followed by cold water (2 x 25 ml). The solid material was crystallized from aqueous DMF to yield 5.90 g (79%), mp > 350°C.

Chromatography : Absorbent - silica gel

Solvent - CH_3CN:0.1 M NH_4Cl, 7:3, v/v

Infrared (KBr) : Major bands - ν 680, 720, 770, 800, 820, 1090, 1170, 1300, 1390, 1550, 1580, 1630, 1655 and 2750-3400 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 238 nm (ε 7,300);

λ_{max} (pH 7) 240 nm (ε 6,400);

λ_{max} (pH 11) 243 nm (ε 7,200).

		C	H	N
Analysis :	Calcd for $C_4H_6N_4O_2$:	33.81	4.26	39.42
	Found:	33.55	4.10	39.23

Method 2. 5-Amino-4-cyanopyrazole-3(1H,2H)-one (21, 1.24 g, 10 mmol) was dissolved in 15% ammonium hydroxide solution (25 ml). To the solution was added 30% H_2O_2 (5 ml) and the mixture was stirred at room temperature for 4 hours, before it was evaporated to dryness. The residue was washed with cold water and crystallized from aqueous DMF to yield 0.90 g (63.4%), mp > 350°C. This compound was identical in all respects to 24 prepared by Method 1.

Pyrazolo[3,4-d]pyrimidine-3,4(2H,5H)-dione (27, 3-Hydroxyallopurinol). A solution of 24 (1.42 g, 10 mmol) in formamide (10 ml) was heated at 180-190°C for 45 min. The cooled solution was diluted with water (20 ml) and evaporated to dryness. The residue was co-evaporated with water several times, and finely dissolved in water (10 ml) and adjusted to pH 8.5. The precipitated product was collected by filtration and crystallized from DMF:H_2O (20:2, v/v) as light-yellow needles, 0.55 g (36%), mp > 360°C.

Chromatography : Absorbent - silica gel

Solvent - CH_3CN:0.1 M NH_4Cl, 7:3, v/v

Infrared (KBr) : Major bands - ν 690, 760, 790, 875, 1050, 1220, 1330, 1460, 1520, 1600, 1680, 1715, 2820, 3050 and 3400 cm^{-1}.

^1H NMR (Me_2SO-d_6): δ 7.85 (s, 1, C_6H) and 11.64 (br s, 1, NH).

		C	H	N
Analysis :	Calcd for $C_5H_4N_4C_2 \cdot \frac{1}{2}H_2O$:	37.27	3.13	34.77
	Found:	37.08	3.21	34.64

4-Amino-3-methoxypyrazolo[3,4-d]pyrimidine (25). A solution of 5-amino-3-methoxypyrazol-4-carbonitrile (20, 1.38 g, 10 mmol) in triethyl orthoformate (freshly distilled, 25 ml) was heated under reflux for an hour. The reaction mixture was evaporated to dryness and the residual syrup was co-evaporated with toluene (3 x 25 ml) to yield the intermediate 3-methoxy-5-(ethoxymethyleneamino)pyrazole-4-carbonitrile (22), which was dissolved in MeOH/NH_3 (saturated at 0°C, 50 ml) and heated in a steel bomb at 90°C for 3 hours. After allowing to stand overnight at room temperature the MeOH/NH_3 was evaporated and the residue was crystallized from aqueous methanol as white needles, yield 1.0 g (62%), mp 258-259°C.

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 690, 735, 800, 910, 1080, 1120, 1170, 1260, 1300, 1410, 1440, 1480, 1530, 1590, 1640, 2910, 3180, 3300-3470 cm^{-1}.

^1H NMR (Me_2SO-d_6): δ 3.90 (s, 3, OCH_3), 7.0 (br s, 2, NH_2), 8.07 (s, 1, C_6H) and 12.37 (br s, 1, NH).

		C	H	N
Analysis :	Calcd for $C_6H_7N_5O$:	43.64	4.27	42.40
	Found:	43.48	4.35	42.48

4-Aminopyrazolo[3,4-d]pyrimidin-3(1H,2H)-one (26). To a solution of 25 (3.7 g, 22.4 mmol) in dry CH_3CN (150 ml) containing NaI (6.0 g, 40 mmol) was added chlorotrimethylsilane (3.65 g, 33.6 mmol) under nitrogen atmosphere, and the mixture was heated under reflux for 18 hours, whereupon more of NaI (3.4 g) and chlorotrimethylsilane (2.85 ml) were added. After refluxing for further 24 hours, additional NaI (6.8 g) and chlorotrimethylsilane (5.7 ml) were added and heating was continued for further 10 hours, at which time the

reaction was complete. After cooling to room temperature, the precipitated product was collected, washed with cold aqueous solution of sodium bisulfite (2 x 25 ml), followed by water (3 x 25 ml) and the crude product was crystallized from aqueous DMF to yield 1.97 g (58%), mp > 300°C.

Chromatography : Absorbent - silica gel

Solvent - CH_3CN:0.1 M NH_4Cl, 7:3, v/v

Infrared (KBr) : Major bands - ν 690, 780, 900, 1110, 1200, 1290, 1360, 1410, 1460, 1510, 1530, 1610, 1670 and 3100 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 231 nm (ε 14,000);

λ_{max} (pH 7) 235 nm (ε 12,100);

λ_{max} (pH 11) 233 nm (ε 9,000).

		C	H	N
Analysis :	Calcd for $C_5H_5N_5O \cdot \frac{3}{4}H_2O$:	36.48	3.98	42.54
	Found:	36.71	4.07	42.25

4-Amino-3-methoxy-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (30). Compound 25 (3.30 g, 20 mmol) and 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose (28, 15.12 g, 30 mmol) were combined with dry CH_3NO_2 (250 ml) and brought to reflux. To this suspension was added freshly distilled BF_3 etherate (8.3 g, 30 mmol) through the condenser by syringe. The resulting clear solution was refluxed for 30 min. and then evaporated to dryness. The residue was dissolved in EtOAc (500 ml) and washed with saturated aqueous $NaHCO_3$ solution (2 x 100 ml), followed by water (2 x 100 ml). After drying over anhydrous Na_2SO_4, the solvent was evaporated to an amber colored foam, which was purified on a silica gel column using $CHCl_3$:MeOH (40:1, v/v) as the solvent. The appropriate, homogeneous fractions were pooled and evaporated to yield 7.0 g (57.5%) of the blocked nucleoside 4-amino-3-methoxy-1-(2,3,5-tri-

O-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine (29) as a syrup, which was used directly for deblocking reaction. This was accomplished by combining the above syrup (5.2 g, 8.6 mmol) with sodium methoxide in methanol to a pH of 9. The solution was stirred at room temperature for 15 hours and then neutralized with Dowex-50 (H^+) resin. The resin was removed by filtration and the filtrate evaporated to dryness. The residual solid was crystallized from methanol to yield 2.20 g (94%) of the title compound, mp 185-187°C.

Chromatography : Absorbent - silica gel

Solvent - CH_2Cl_2:MeOH, 9:1, v/v

Infrared (KBr) : Major bands - ν 690, 780, 880, 930, 970, 1040, 1090, 1200, 1270, 1415, 1450, 1475, 1540, 1575, 1600, 1640, 2920, 3200 and 3350 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 230 nm (ε 19,900);

λ_{max} (pH 7 and 11) 248 nm, sh (ε 4,400), 278 (3,600).

Analysis :		C	H	N
Calcd for $C_{11}H_{15}N_5O_5 \cdot \frac{1}{4}H_2O$:		43.78	5.18	23.21
Found:		43.70	5.31	23.13

3-Ethoxypyrazolo[3,4-d]pyrimidin-4(5H)-one (37). 5-Amino-3-ethoxypyrazole-4-carboxamide (2.3 g, 8.1 mmol, prepared as described for 23 using dicyanoketene diethyl acetal, 33 [43]) and formamide (23 ml) were boiled in an open beaker at 180-190°C for 30 min. The reaction mixture was allowed to cool to 80°C and glacial acetic acid (3 ml) were added before the solution was again boiled for 10 min. Upon cooling, solid product separated, which was collected by filtration, washed with cold water (2 x 20 ml) and recrystallized from aqueous DMF to yield white crystals, 1.85 g (76%), mp 274-275°C.

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 720, 765, 790, 900, 1020, 1070, 1110, 1170, 1310, 1355, 1385, 1470, 1530, 1570, 1595, 1675, 1710, 2880-3560 cm^{-1}.

Ultraviolet : λ_{max} (pH 11) 268 nm (ε 3,800).

		C	H	N
Analysis :	Calcd for $C_7H_8N_4O_2 \cdot \frac{1}{4}H_2O$:	45.53	4.64	30.34
	Found:	45.74	4.57	30.47

3-Ethoxy-6-methylthiopyrazolo[3,4-d]pyrimidin-4(5H)-one (41). 5-Amino-3-ethoxypyrazole-4-carboxamide (3.4 g, 20 mmol) was combined with potassium ethyl xanthate (10.9 g, 68 mmol) in DMF (200 ml) and the mixture was heated under reflux for 3 hours. After cooling (0-5°C), the solid that separated was collected by filtration, dissolved in 2 N NaOH, warmed on a steam bath and acidified (pH 4) with glacial acetic acid. A floculent white precipitate that formed was collected and purified by acid-base treatment to yield 2.7 g (63.7%) of 3-ethoxy-6-thiopyrazolo[3,4-d]pyrimidine-4(5H,7H)-dione (38).

Compound 38 (2.5 g, 11.8 mmol) was dissolved in 1 N NaOH (50 ml) and methyl iodide (0.73 ml, 11.8 mmol) was added with stirring. The reaction mixture was stirred at room temperature for 6 hours and then acidified with glacial acetic acid. The product that separated was collected by filtration and crystallized from water-DMF to yield 2.1 g (79%) of the title compound, mp 248-250°C.

Chromatography : Absorbent - silica gel

Solvent - CH_2Cl_2:MeOH, 23:1, v/v

Infrared (KBr) : Major bands - ν 730, 840, 905, 965, 1020, 1050, 1135, 1250, 1300, 1330, 1415, 1475, 1510, 1620, and 2860-3200 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 233 nm (ε 13,900), 267 (9,000);
λ_{max} (pH 7) 233 nm (ε 16,300), 260 (10,000);
λ_{max} (pH 11) 236 nm (ε 19,000), 259 (11,300).

Analysis :		C	H	N	S
	Calcd for $C_8H_{10}N_4O_2S \cdot {}^6/_{10}H_2O$:	40.53	4.76	23.63	13.52
	Found:	40.61	4.63	23.78	13.55

3-Ethoxy-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-d]-pyrimidin-4(5H)-one (36). Compound 37 (2.75 g, 15.3 mmol) and 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose (28, 12.2 g, 23 mmol) were combined with dry CH_3NO_2 (200 ml) and brought to reflux. To this suspension was added freshly distilled BF_3 etherate (2.9 ml) through the condenser by syringe. The resulting clear solution was refluxed for 3 hours and then evaporated to dryness. The residue was dissolved in EtOAc (250 ml) and washed with saturated aqueous $NaHCO_3$ solution (2 x 50 ml), followed by water (2 x 75 ml). After drying the organic phase over anhydrous Na_2SO_4, the solvent was evaporated to dryness and the residual syrup was purified on a silica gel column using CH_2Cl_2:acetone (10:1, v/v) as the solvent. The appropriate homogeneous fractions were pooled, evaporated to dryness and the residual syrup was triturated with 10 ml of EtOAc. The solid that separated was collected and crystallized from methanol as colorless needles to yield 1.75 g (18%) of the title compound, mp 180-181°C.

Chromatography : Absorbent - silica gel
Solvent - CH_2Cl_2:acetone, 10:1, v/v

Infrared (KBr) : Major bands - ν 700, 790, 870, 890, 985, 1020, 1090, 1175, 1265, 1310, 1355, 1390, 1450, 1520, 1545, 1590, 1720, 2900-3260 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 230 nm (ε 22,200), 273 (7,500);
λ_{max} (pH 7) 230 nm (ε 20,300), 273 (6,200);
λ_{max} (pH 11) 230 nm (ε 24,700), 273 (6,200).

		C	H	N
Analysis :	Calcd for $C_{33}H_{28}N_4O_9$:	63.46	4.52	8.97
	Found:	63.32	4.57	8.90

3-Ethoxy-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one (39). A solution of compound 36 (3.2 g, 5.1 mmol) in absolute methanol (100 ml) was adjusted to pH 9 with sodium methoxide. The mixture stirred at room temperature for 15 hours and then neutralized with Dowex-50 (H^+) resin. The resin was removed by filtration and the filtrate evaporated to dryness. The residue was crystallized from methanol to yield 1.30 g (81%), mp 160-162°C.

Chromatography : Absorbent - silica gel
Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 670, 700, 795, 900, 990, 1050, 1080, 1090, 1185, 1235, 1320, 1390, 1430, 1480, 1545, 1600, 1680, 1730, 2960 and 3400 cm^{-1}.

Ultraviolet : λ_{max} (pH 1 and 7) 260 nm (ε 3,700);
λ_{max} (pH 11) 274 nm (ε 4,800).

		C	H	N
Analysis :	Calcd for $C_{12}H_{16}N_4O_6 \cdot \frac{1}{4}H_2O$:	45.50	5.25	17.69
	Found:	45.40	5.05	17.45

3-Ethoxy-6-methylthio-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrazolo-[3,4-d]pyrimidin-4(5H)-one (40a). Compounds 41 (0.50 g, 2.2 mmol) and 28 (1.67 g, 3.3 mmol) were reacted in the presence of BF_3 etherate (0.47 g, 3.3 mmol) in dry nitromethane (50 ml) in the same manner as described for the

preparation of 36. The product was purified on a silica gel column using CH_2Cl_2:acetone as the solvent, and crystallized from ether-ethyl acetate mixture to yield 0.9 g (61%) of the title compound, mp 213-214°C.

Chromatography : Absorbent - silica gel

Solvent - CH_2Cl_2:acetone, 20:1, v/v

Infrared (KBr) : Major bands - ν 700, 765, 890, 940, 1045, 1100, 1230, 1350, 1400, 1445, 1510, 1555, 1600, 1650, 2940, 3150 and 3320 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 238 nm (ε 32,500), 280 (16,400);

λ_{max} (pH 7) 241 nm (ε 26,800), 280 (15,100);

λ_{max} (pH 11) 232 nm (ε 25,100), 263 sh (9,400).

		C	H	N	S
Analysis :	Calcd for $C_{14}H_{18}N_4O_9S$:	60.89	4.51	8.35	4.78
	Found:	60.83	4.45	8.41	5.07

3-Ethoxy-6-methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one (40b). A solution of compound 40a (0.90 g, 1.34 mmol) in absolute methanol (25 ml) was adjusted to pH 9 with sodium methoxide. The mixture was stirred at room temperature for 15 hours and then neutralized with Dowex-50 (H^+) resin. The resin was removed by filtration and the filtrate evaporated to dryness. The residue was crystallized from water to yield 0.37 g (77%) of the title compound, mp 214-215°C.

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 785, 870, 980, 1040, 1070, 1100, 1190, 1310, 1355, 1380, 1430, 1540, 1560, 1680, 2940 and 3360 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 234 nm (ε 14,800), 275 (8,400);

λ_{max} (pH 7) 234 nm (ε 15,700), 274 (8,900);

λ_{max} (pH 11) 238 nm (ε 15,200), 261 (9,300).

		C	H	N	S
Analysis :	Calcd for $C_{13}H_{18}N_4O_6S$:	43.57	5.06	15.63	8.95
	Found:	43.40	5.00	15.61	8.82

6-Thiopyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dione-3-carboxamide (*43*). A mixture of 5-aminopyrazole-3,4-dicarboxamide[45] (*42*, 2.0 g, 12 mmol) and potassium ethyl xanthate (3.8 g, 24 mmol) in DMF (180 ml) was heated under reflux for 2 hours. The reaction mixture was cooled in an ice-bath and the precipitated product was collected by filtration. The solid was dissolved in 1 N NaOH, treated with charcoal, filtered and the filtrate acidified with glacial acetic acid. The light-yellow solid that separated was collected, washed with cold water (3 x 25 ml) and dried (at 100°C under vacuum) to yield 2.1 g (82%) of the title compound, mp > 300°C.

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:*n*-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 680, 820, 1050, 1130, 1160, 1250, 1385, 1420, 1540, 1600, 1660, 2920, 3120 and 3320 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 272 nm (ε 31,600);

λ_{max} (pH 7) 240 nm (ε 21,000), 284 (30,000);

λ_{max} (pH 11) 264 nm (ε 29,000).

		C	H	N	S
Analysis :	Calcd for $C_6H_5N_5O_2S \cdot \frac{1}{4}H_2O$:	33.91	2.57	32.47	14.86
	Found:	33.76	2.77	32.56	14.82

6-Methylthio-4(5H)-oxopyrazolo[3,4-d]pyrimidine-3-carboxamide (44). To a solution of 43 (2.1 g, 10 mmol) in 1 N NaOH (50 ml) was added methyl iodide (1.7 g, 12 mmol) and the mixture was stirred vigorously for 2 hours at room temperature. The solid that separated on acidification (AcOH, to pH 5) was collected by filtration, washed with cold water (2 x 50 ml) and dried to yield 2.1 g (93%), mp > 300°C.

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 800, 960, 1040, 1125, 1170, 1250, 1360, 1440, 1460, 1545, 1655, 1680 and 2850-3420 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 238 nm (ε 19,800), 266 (14,500);

λ_{max} (pH 7) 239 nm (ε 19,300), 265 (13,200);

λ_{max} (pH 11) 249 nm (ε 22,500), 283 sh (8,700).

		C	H	N	S
Analysis :	Calcd for $C_7H_7N_5O_2S$:	37.33	3.13	31.09	14.23
	Found:	37.15	3.22	30.95	14.15

6-Methylthio-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-4(5H)-oxopyrazolo-[3,4-d]pyrimidine-3-carboxamide (46a). In the same manner as for 36, the title compound was prepared using 44 (10.0 g, 44 mmol), 28 (33.0 g, 67 mmol) and BF_3 etherate (3 ml) in CH_3NO_2 (200 ml). The product was purified on a Prep LC system using 25% acetone in toluene as the eluting solvent. The unreacted sugar eluted first, followed by 46a, to yield 21.1 g (66%), mp 131-132°C (sinters), > 160°C (dec.).

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:acetone, 8:2, v/v

Infrared (KBr) : Major bands - ν 700, 775, 895, 970, 990, 1020, 1060, 1080, 1110, 1170, 1260, 1310, 1440, 1470, 1540, 1590, 1610, 1680, 1720, 3160 and 3400 cm^{-1}.

Ultraviolet : λ_{max} (MeOH) 230 nm (ε 62,000), 273 (16,100).

Analysis :	C	H	N	S
Calcd for $C_{33}H_{27}N_5O_9S$:	59.19	4.06	10.46	4.79
Found:	58.98	4.07	10.36	4.77

6-Methylthio-1-β-D-ribofuranosyl-4(5H)-oxopyrazolo[3,4-d]pyrimidine-3-carboxamide (47a). A solution of compound 46a (6.7 g, 10 mmol) in absolute methanol (200 ml) was adjusted to pH 9 with sodium methoxide. The mixture was stirred at room temperature for 15 hours and then neutralized with Dowex-50 (H^+) resin. The resin was removed by filtration and the filtrate evaporated to dryness. The residue was crystallized from water to yield 2.78 g (78%) of the title compound, mp 290-293°C (dec.).

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase.

Infrared (KBr) : Major bands - ν 740, 770, 1005, 1040, 1090, 1120, 1175, 1280, 1360, 1485, 1550, 1650, 2930, 3180, 3300 and 3380 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 240 nm (ε 20,500), 272 (17,000);

λ_{max} (pH 7) 243 nm (ε 21,300), 272 (14,300);

λ_{max} (pH 11) 248 nm (ε 24,800), 273 sh (11,200).

Analysis :	C	H	N	S
Calcd for $C_{12}H_{15}N_5O_6S \cdot \frac{1}{4}H_2O$:	39.83	4.32	19.35	8.86
Found:	39.65	4.28	19.05	8.79

6-Amino-1-β-D-ribofuranosyl-4(5H)-oxopyrazolo[3,4-d]pyrimidine-3-carboxamide (45).

Step I. 6-Methylsulfonyl-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-4(5H)-oxopyrazolo[3,4-d]pyrimidine-3-carboxamide (46b). To a solution of 46a (2.0 g, 3 mmol) in CH_2Cl_2 (50 ml) was added m-chloroperoxybenzoic acid (2.1 g, 12 mol). After stirring at room temperature for 18 hours, the solution was evaporated to dryness. The residue was triturated with ether (3 x 50 ml) and the ether insoluble solid was crystallized from a mixture of EtOH/hexane to yield 1.95 g (93%) of the title compound, mp 115-116°C (sinters, >150°C (dec.).

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:acetone, 8:2, v/v

Infrared (KBr) : ν 1120 and 1320 (SO_2CH_3), 1730 (C=O) cm^{-1}.

Ultraviolet : λ_{max} (MeOH) 229 nm (ε 63,100), 282 (14,700).

^1H NMR ($CDCl_3$) : δ 3.42 (s, 3, SO_2CH_3), 6.93 (d, 1, J = 2.5 Hz, C_1'H), 7.41 and 8.01 (m, 17, $3COC_6H_5$, $CONH_2$), and other sugar protons.

		C	H	N	S
Analysis :	Calcd for $C_{33}H_{27}N_5O_{11}S \cdot H_2O$:	55.08	4.06	9.73	4.45
	Found:	55.25	3.89	9.72	4.24

Step II. 6-Amino-1-β-D-ribofuranosyl-4(5H)-oxopyrazolo[3,4-d]pyrimidine-3-carboxamide (45). A mixture of 46b (6.5 g, 9 mmol) and liquid NH_3 (75 ml) was heated in a steel bomb at 90°C for 48 hours. The bomb was cooled in a dry ice-acetone bath and the NH_3 was allowed to evaporate. The residual solid was suspended in MeOH (50 ml), boiled for a few minutes and then filtered. The solid was again suspended in 0.1 N AcOH (50 ml), boiled for a few minutes and filtered to give 2.6 g (86%) of off-white solid, mp > 300°C.

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 690, 740, 770, 890, 1050, 1080, 1220, 1280, 1360, 1440, 1480, 1530, 1555, 1580, 1670, 2930 and 3200-3400 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 230 nm (ε 21,900), 254 sh (10,800), 282 (5,200);

λ_{max} (pH 7) 230 nm (ε 22,500), 254 sh (10,500), 284 (5,400);

λ_{max} (pH 11) 230 nm (ε 26,600), 254 (9,500), 286 (6,800).

		C	H	N
Analysis :	Calcd for $C_{11}H_{14}N_6O_6 \cdot \frac{1}{4}H_2O$:	39.95	4.38	25.41
	Found:	40.06	4.59	25.35

6-Methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one 5'-phosphate (50). To an ice-cooled (0-5°C) solution of freshly distilled trimethylphosphate (10 ml) and $POCl_3$ (0.6 ml) was added dry and powdered 6-methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one (0.57 g, 1.45 mmol). The mixture was stirred at ice-bath temperature for 2 hours, after which the solution was poured into crushed ice (50 g). The aqueous solution was neutralized with 2 N NaOH solution, extracted with chloroform (3 x 50 ml) and then placed on top of a charcoal column (2.5 x 25 cms). The column was washed with water (2 lit) till free of salts (Cl^- ions free). The nucleotide was then eluted with H_2O:EtOH:NH_4OH (10:10:1, v/v). The appropriate fraction was collected, evaporated to dryness and the aqueous solution of the residue was neutralized with Dowex-50 (H^+) resin. The resin was

removed by filtration and the filtrate was lyophilized to yield the title compound, 0.41 g (58%), mp 175-176°C (dec.).

Chromatography : Absorbent - silica gel

Solvent - CH_3CN:0.1 M NH_4Cl, 7:3, v/v

Infrared (KBr) : Major bands - ν 685, 770, 840, 900, 950, 1030, 1100, 1200, 1390, 1440, 1485, 1555, 1680, 2920 and 3400 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 266 nm (ε 13,800);

λ_{max} (pH 7) 269 nm (ε 14,200);

λ_{max} (pH 11) 236 nm (ε 18,500), 274 (13,600).

		C	H	N	S
Analysis :	Calcd for $C_{11}H_{15}N_4O_8SP$:	33.51	3.83	14.21	8.13
	Found:	33.98	4.10	14.56	8.24

7-Amino-6-methyl-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine (6-Methylformycin, 52). To a solution of formycin (51a, 2 g) in dry DMF (60 ml) was added methyl iodide (2 ml), and the mixture was stirred at room temperature for 26 hours. The reaction mixture was evaporated in vacuo on a steam bath. The residual syrup was co-evaporated with ethanol (3 x 50 ml) before it was tritrated with hot ether (3 x 50 ml). The aqueous solution of the residue was adjusted to pH 9.5 by the addition of 1 N NH_4OH. A white solid separated after stirring at room temperature of 30 min, and this was followed by refrigeration of 2 hours. The solid was collected by filtration and crystallized from water to yield 1.2 g (50%) of the title compound, mp 230°C [Lit.[47] mp 231-232°C].

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 670, 860, 885, 960, 1000, 1050, 1060, 1110, 1190, 1250, 1330, 1390, 1555, 1600, 1665, 3160 and 3300 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 227 nm (ε 12,500), 290 (7,300);
λ_{max} (pH 7) 233 nm (ε 14,300), 285 (6,600);
λ_{max} (pH 11) 235 nm (ε 14,800), 283 (10,300).

Analysis :		C	H	N
	Calcd for $C_{11}H_{15}N_5O_4$:	46.97	5.38	24.90
	Found:	46.70	5.39	24.60

1,6-Dimethyl-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine-5,7(4H)-dione (1,6-Dimethyloxoformycin B, 56).

Step I. 1,6-Dimethyl-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidin-7-one (55).

A mixture of 1-methylformycin B [50] (54, 1.0 g, 3.6 mmol), DMF (10 ml) and N,N-dimethylformamide dimethyl acetal (10 ml) was heated at 80-95°C for 48 hours. The reaction mixture was evaporated to dryness and the residue was co-evaporated with water (2 x 25 ml). The residual semi-solid was dissolved in concentrated NH_4OH (25 ml) and stirred at room temperature for 48 hours. Evaporation of the solution gave an oil which, on trituration with absolute ethanol, gave a solid. Crystallization of the solid from aqueous ethanol gave the title compound, 0.90 g (85%), mp 165-166°C [Lit.[48] mp 165-166°C].

Chromatography : Absorbent - silica gel
Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : ν 1690 (C=O), 3400 (OH, NH) cm^{-1}.

Ultraviolet : λ_{max} (pH 1 and 7) 273 nm (ε 7,400);
λ_{max} (pH 11) 273 nm (ε 7,700).

^{1}H NMR (Me_2SO-d_6): δ 3.50 (s, 3, N_6-CH_3), 4.19 (s, 3, N_1-CH_3), 4.92 (d, 1, J = 6.0 Hz, $C_{1'}$-H), 8.19 (s, 1, C_5-H), and other sugar protons.

		C	H	N
Analysis :	Calcd for $C_{12}H_{16}N_4O_5$:	48.65	5.44	18.91
	Found:	48.58	5.62	18.67

Step II. 1,6-Dimethyl-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine-5,7(4H)-dione (56). To a solution of 55 (0.50 g, 1.7 mmol) in water (2 ml) was added an aqueous solution of bromine (1 ml of bromine in 100 ml of water) over a period of 30 min. with stirring at room temperature. After stirring for 3 days at ~22°C, the reaction mixture was purged with nitrogen and concentrated to ~10 ml. The solid that separated on cooling (ice-bath) was collected by filtration, washed with cold methanol (2 x 10 ml) and dried. The product was crystallized from aqueous ethanol to yield 0.47 g (88%) of the title compound, mp 210°C [Lit.[48] mp 206-207°C].

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 770, 810, 920, 950, 1015, 1115, 1205, 1280, 1330, 1360, 1430, 1500, 1660, 1700, 2950 and 3340 cm^{-1}.

Ultraviolet : λ_{max} (pH 1 and 7) 240 nm (ε 5,900), 290 (6,100); λ_{max} (pH 11) 245 nm, sh (ε 5,900), 315 (6,900).

^{1}H NMR (Me_2SO-d_6): δ 3.21 (s, 3, N_6-CH_3), 4.08 (s, 3, N_1-CH_3), 4.82 (d, 1, J = 5.0 Hz, $C_{1'}$-H), 11.10 (s, 1, ring NH), and other sugar protons.

			C	H	N
Analysis	:	Calcd for $C_{12}H_{16}N_4O_6$:	46.15	5.16	17.94
		Found:	45.85	5.25	17.65

3-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidine-5,7(4H,6H)-dione (59). A mixture of oxoformycin B [48] (60, 2.66 g, 9.36 mmol), acetic anhydride (50 ml) and DMAP (200 mg) was heated on a steam bath till all the solid dissolved to form a clear solution. The reaction mixture was then stirred at room temperature for 12 hours. The solvent was evaporated and the residue was co-evaporated from ethanol (2 x 50 ml). The residue was purified on a silica gel column (2 x 30 cm) using $CHCl_3$:MeOH (10:1, v/v) as the solvent. The pure product was crystallized from water to yield 3.5 g (91%) of the title compound, mp 110°C.

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:acetone, 8:2, v/v

Infrared (KBr) : Major bands - ν 745, 895, 925, 950, 1030, 1050, 1070, 1215, 1240, 1370, 1425, 1680, 1720, 1735 and 2940-3310 cm^{-1}.

Ultraviolet : λ_{max} (pH 1 and 7) 284 nm (ε 5,100);

λ_{max} (pH 11) 293 nm (ε 3,700).

			C	H	N
Analysis	:	Calcd for $C_{16}H_{18}N_4O_9 \cdot \frac{1}{2}H_2O$:	45.83	4.57	13.36
		Found:	45.98	4.43	13.21

7-Thio-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine-5(4H,6H)-dione (58b). Step I. 7-Thio-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]-pyrimidine-5(4H,6H)-dione (58a). To a refluxing solution of 59 (2.0 g, 4.87

mmol) in dry dioxane (75 ml) was added purified P_2S_5 (2.0 g) and DMAP (0.2 g). After 30 min. an additional P_2S_5 (2.0 g) was added and refluxed for a total of 5 hr. The cooled reaction mixture was poured into water (300 ml) and stirred for an hour. The aqueous solution was extracted with chloroform (3 x 150 ml), the combined organic phase was washed with water (2 x 200 ml) and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified on a silica gel column using $CHCl_3$:MeOH (95:5, v/v) as the solvent. The pure product was crystallized from aqueous ethanol to yield the title compound, 1.60 g (77%), mp 196-197°C.

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 95:5, v/v

Infrared (KBr) : ν 1230 (C=S), 1690 (C=O), 1720, 1740 and 3210 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 256 nm (ε 4,200), 340 (15,100);

λ_{max} (pH 7) 256 nm (ε 3,400), 340 (17,800);

λ_{max} (pH 11) 227 nm (ε 12,800), 263 (5,500), 305 (1,900), 365 (4,700).

^{1}H NMR (Me_2SO-d_6): δ 1.98, 2.04, 2.10 (3s, 9, 3COC<u>H</u>$_3$), 5.22 (d, 1, J = 7.1 Hz, C_1'<u>H</u>), 11.64, 12.54, 13.6 (3s, 3, 3 ring N<u>H</u>), and other sugar protons.

		C	H	N	S
Analysis :	Calcd for $C_{16}H_{18}N_4O_8S$:	45.06	4.25	13.14	7.52
	Found:	45.28	4.43	12.88	7.70

Step II. 7-Thio-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine-5(4H,6H)-dione (58b). A solution of 58a (0.9 g, 2.1 mmol) in absolute methanol (50 ml) was adjusted to pH 10 by adding freshly prepared 1 N sodium methoxide in methanol. The mixture was stirred for 2 hours at room temperature and then neutralized with Dowex-50 (H^+) resin. The resin was removed by filtration and

washed several times with methanol. The combined filtrates were evaporated and the residue was crystallized from aqueous methanol to yield the title compound, 0.56 g (89%), mp 252°C.

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 690, 785, 825, 885, 990, 1030, 1070, 1120, 1150, 1230, 1320, 1345, 1370, 1430, 1460, 1505, 1595, 1675, 1700, 2900-3400 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 262 nm (ε 7,500), 310 sh (8,000), 342 sh (19,200);

λ_{max} (pH 7) 262 nm (ε 5,800), 310 sh (7,300), 343 (15,500);

λ_{max} (pH 11) 262 nm (ε 2,700), 305 (4,800), 316 (4,800), 354 (6,600), 370 (7,500).

^1H NMR (Me_2SO-d_6): δ 4.86 (d, 1, J = 6.9 Hz, $C_{1'}$-H), 11.28, 12.45, 13.83 (3s, 3, 3 ring NH), and other sugar protons.

Analysis :		C	H	N	S
	Calcd for $C_{10}H_{12}N_4O_5S$:	40.00	4.03	18.65	10.67
	Found:	39.78	4.28	18.91	10.46

5,7-Dichloro-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]-pyrimidine (61a). Method 1. To a solution of 59 (2.05 g, 5 mmol) in anhydrous THF (40 ml) was added NaH (60% dispersion in oil, 0.60 g, 15 mmol) and stirred at room temperature for 30 min. Phenylphosphonic dichloride (15 ml, 25 mmol) was added in one lot and stirring was continued for 30 min. The solvent was evaporated under reduced pressure on a steam bath, the residue was stirred at 160°C for 2 hours and then poured into crushed ice (200 g) with stirring. After stirring of 30 min. the aqueous solution was extracted with

ethyl acetate (3 x 150 ml). The combined organic phase was washed repeatedly with cold water till the pH of 6. After drying over anhydrous Na_2SO_4, the ethyl acetate was evaporated and the residue was purified on a silica gel column (2 x 30 cm) using $CHCl_3$:acetone (20:1, v/v) as the solvent, to yield 1.8 g (81%) of pure 61a, mp 57-60°C.

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:acetone, 20:1, v/v

Infrared (KBr) : δ 1740 (OAc), 3240 (NH) cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 260 nm (ε 20,100), 309 (29,500);

λ_{max} (pH 7) 270 nm (ε 18,800), 310 (17,400);

λ_{max} (pH 11) 278 nm (ε 23,200), 335 (11,600).

^1H NMR ($CDCl_3$) : δ 2.10, 2.20, 2.30 (3s, 9, 3 $OCOCH_3$), 5.40 (d, 1, J = 6.0 Hz, $C_{1'}H$), 9.80 (s, 1, N_1-H), and other sugar protons.

		C	H	N	Cl
Analysis :	Calcd for $C_{16}H_{16}Cl_2N_4O_7$:	42.97	3.61	12.53	15.85
	Found:	43.11	4.41	12.24	15.75

Method 2. A mixture of 59 (4.10 g, 10 mmol) and freshly distilled $POCl_3$ (150 ml) was heated under reflux for 48 hours, and then evaporated down to ~25 ml before it was poured into ice-water (250 ml) with stirring. After stirring for 20 min, the aqueous solution was extracted with chloroform (3 x 200 ml) and the combined organic phase was worked up as described in Method 1. The product isolated by this procedure was found to be identical with 61a prepared by Method 1. Yield 1.80 g (40%), mp 58-60°C.

5,7-Dichloro-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine (61b). To a solution of 61a (0.45 g, 1 mmol) in dioxane (20 ml) was added 10% NaOH solution (1.2 ml) in water and stirred at room temperature for 3 hours. The

reaction mixture was neutralized with Dowex-50 (H^+) resin. The resin was removed by filtration. Evaporation of the filtrate and purification of the residue on a silica gel column using $CHCl_3$:MeOH (9:1, v/v) gave 61b, which was crystallized from water to yield 0.15 g (46%), mp 128-129°C (dec.).

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 9:1, v/v

Infrared (KBr) : ν 3200-3400 (OH, NH) cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 260 nm (ε 9,300), 309 (18,900);

λ_{max} (pH 7) 270 nm (ε 5,100), 315 (8,800);

λ_{max} (pH 11) 278 nm (ε 20,900), 345 (12,800).

Analysis :		C	H	N	Cl
	Calcd for $C_{10}H_{10}Cl_2N_4O_4 \cdot \frac{1}{2}H_2O$:	36.38	3.35	16.96	21.47
	Found:	36.12	3.55	17.09	21.63

7-Amino-5-chloro-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine (5-Chloroformycin, 62). A mixture of 61a (0.9 g, 2 mmol) and liquid NH_3 (25 ml) was stirred in a pressure reaction vessel at room temperature for 15 hours. The NH_3 was allowed to evaporate and the residue was crystallized from water with the aid of decolorizing carbon to yield 0.54 g (89%) of the title compound, mp 156°C.

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 700, 760, 890, 940, 1040, 1095, 1230, 1350, 1400, 1445, 1510, 1550, 1600, 1650, 2930 and 3150-3320 cm^{-1}.

Ultraviolet : λ_{max} (pH 1 and 7) 230 nm, sh (ε 7,800), 297 (9,000);

λ_{max} (pH 11) 237 nm (ε 17,500), 305 (6,500).

^{1}H NMR (Me_2SO-d_6): δ 4.90 (d, 1, J = 8.0 Hz, C_1'H), 7.92 (br s, 2, NH_2), and other sugar protons.

		C	H	N	Cl
Analysis :	Calcd for $C_{10}H_{12}ClN_5O_4 \cdot \frac{1}{2}H_2O$:	38.78	4.23	22.61	11.44
	Found:	38.82	4.41	22.64	11.60

3-Methylpyrazolo[4,3-d]pyrimidin-7(6H)-one (69). 4-Amino-3-methylpyrazole-5-carboxamide [55] (68, 5 g, 35.7 mmol) was boiled with formamide (30 ml) for 3 hours. To the warm solution was added water (90 ml) and the solution was placed in the refrigerator overnight. The precipitated solid was collected by filtration and crystallized from water to yield 3.1 g (58%), mp > 320°C [Lit.[55] mp > 330°C (dec.)].

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 700, 720, 780, 870, 920, 985, 1020, 1145, 1175, 1270, 1380, 1515, 1580, 1670, 1680, 2860-3180 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 281 nm (ε 6,600);

λ_{max} (pH 7) 281 nm (ε 7,100);

λ_{max} (pH 11) 287 nm (ε 8,400).

		C	H	N
Analysis :	Calcd for $C_6H_6N_4O$:	48.00	4.03	37.31
	Found:	47.82	4.22	37.28

6-Amino-s-triazolo[3,4-f]-as-triazine-8(7H)-thione (83). To a solution of 6-amino-s-triazolo[3,4-f]-as-triazin-8(7H)-one (72, 5.0 g, 32.9 mmol) in dry pyridine (150 ml) was added purified P_2S_5 (20 g, 90 mmol) and the mixture was heated under reflux for 6 hours. After cooling to room temperature, the

solvent was evaporated and the residue was poured into crushed ice (100 g) and stirred for an hour. After heating the aqueous mixture on a steam bath for 2 hours, the solid that separated was collected by filtration, washed with cold water (until the pH was neutral), followed by ethanol (2 x 50 ml) and dissolved in water (50 ml) containing NaOH. The clear solution was decolorized with Norit, filtered and the filtrate was acidified with glacial acetic acid to pH 4. The yellow solid was collected, washed with cold water (2 x 25 ml), followed by ethanol (3 x 25 ml) and dried to yield 2.24 g (40.6%) of the title compound, mp > 300°C.

Chromatography : Absorbent - silica gel

Solvent - CH_3CN:0.1 M NH_4Cl, 7:3, v/v

Infrared (KBr) : Major bands - ν 850, 980, 1075, 1250, 1300, 1370, 1500, 1570, 1640, 1680, 2800, 2950, 3120 and 3300 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 225 nm (ε 11,300), 335 (6,500);

λ_{max} (pH 7) 224 nm (ε 8,800), 328 (6,600);

λ_{max} (pH 11) 225 nm (ε 9,600), 328 (7,000).

		C	H	N	S
Analysis :	Calcd for $C_4H_4N_6S$:	28.56	2.39	49.97	19.06
	Found:	28.57	2.64	49.66	18.93

6-Amino-8-benzylthio-s-triazolo[3,4-f]-as-triazine (84). A mixture of 83 (2.68 g, 16 mmol), sodium carbonate (2.03 g, 19.2 mmol) and benzylbromide (2.24 ml, 19 mmol) in dry DMF (5 ml) was heated at 40°C and stirred for 3 hours. The mixture was poured into ice-water (50 ml) and adjusted to pH 4 with glacial acetic acid. The aqueous solution was extracted with chloroform (2 x 75 ml) and the combined organic phase was washed with water (20 ml) before it was dried over anhydrous Na_2SO_4. Removal of the solvent gave a

residue, which was purified on a silica gel column (40 g) using $CHCl_3$:MeOH (96:4, v/v) as the solvent. Appropriate fractions were pooled, solvent evaporated and the homogeneous solid was crystallized from chloroform to afford 2.26 g (55%) of the title compound, mp 176°C.

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 96:4, v/v

Infrared (KBr) : Major bands - ν 600, 670, 830, 950, 1050, 1252, 1350, 1365, 1457, 1482, 1575, 1630 and 3050-3400 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 224 nm (ε 20,200), 283 (8,000);

λ_{max} (pH 7) 224 nm (ε 21,700), 281 (9,300);

λ_{max} (pH 11) 223 nm (ε 21,300), 281 (8,300).

Analysis :	C	H	N	S
Calcd for $C_{11}H_{10}N_6S$:	51.14	3.90	32.53	12.41
Found:	50.97	3.84	32.43	12.45

5-Bromo-6-azauracil (85). To a suspension of 6-azauracil (3.6 g, 31.8 mmol) in water (75 ml) was added liquid bromine (3 ml), and the mixture was stirred at room temperature for 48 hours. The solid that separated was collected by filtration, washed with water (3 x 25 ml) and air-dried to yield 3.6 g of the material. Evaporation of the filtrates gave additional 1.0 g material. Crystallization of the combined solids from water gave 4.0 g (65.5%) of the title compound, mp 232-234°C [Lit.[59] mp 232-234°C].

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 630, 740, 840, 990, 1015, 1080, 1105, 1130, 1270, 1330, 1400, 1560, 1590, 1680 and 2800-3220 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 279 nm (ε 6,000);

λ_{max} (pH 7) 293 nm (ε 4,600);

λ_{max} (pH 11) 283 nm (ε 4,000).

		C	H	N	Br
Analysis :	Calcd for $C_3H_2BrN_3O_2$:	18.80	1.04	21.90	41.66
	Found:	18.68	1.23	21.94	41.83

5-Hydrazino-6-azauracil (86). A mixture of 85 (1.92 g, 10 mmol) and hydrazine (97%, 0.62 g, 20 mmol) in water (15 ml) was heated in a teflon lined steel bomb at 140-145°C for 4 hours. The bomb was cooled in ice and opened. The crystalline solid that deposited was collected, washed with cold water (3 x 25 ml) and recrystallized from large excess of water to yield 0.85 g (65%) of the title compound, mp 248-250°C [Lit.[60] mp 235°C].

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 665, 730, 775, 840, 880, 1030, 1125, 1175, 1295, 1315, 1420, 1450, 1530, 1600, 1670, 1700 and 2830-3480 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 271 nm (ε 3,900);

λ_{max} (pH 7) 292 nm (ε 3,800);

λ_{max} (pH 11) 271 nm, sh (ε 2,400).

		C	H	N
Analysis :	Calcd for $C_3H_3N_3O_2$:	25.18	3.52	48.94
	Found:	25.43	3.64	49.23

s-Triazolo[3,4-f]-as-triazine-6,8(5H,7H)-dione (87). A mixture of 86 (2.0 g, 14 mmol) and 97% formic acid (10 ml) was heated under reflux for 48 hours. Formic acid was evaporated to dryness, the residue triturated with

ethanol (3 x 25 ml) and finally crystallized from water to yield 1.20 g (56%), mp > 300°C.

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 705, 840, 880, 920, 960, 1025, 1055, 1120, 1165, 1285, 1340, 1385, 1410, 1435, 1565, 1610, 1710 and 2800-3520 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 235 nm, sh (ε 2,300);

λ_{max} (pH 7) 232 nm, sh (ε 2,000), 280 (1,700);

λ_{max} (pH 11) 243 nm, sh (ε 2,500), 280 (1,700).

Analysis :		C	H	N
	Calcd for $C_4H_3N_5O_2 \cdot H_2O$:	28.07	2.94	40.93
	Found:	28.12	2.85	40.69

s-Triazolo[4,3-c]pyrimidin-5(1H)-one (90).

Step I. 6-Hydrazinopyrimidin-2(1H)-one (89). Cytosine (1.5 g, 0.013 mol) was combined with hydrazine hydrate (5.0 g, 85%) and heated at 80°C for an hour. After cooling, the solid that separated was collected by filtration, washed with cold water (2 x 25 ml), followed by ethanol (2 x 25 ml) and crystallized from water to yield 0.63 g (37%), mp > 310°C (dec.) [Lit.[62] mp > 300°C].

Step II. s-Triazolo[4,3-c]pyrimidin-5(1H)-one (90). A mixture of 89 (0.5 g, 3.96 mmol) and formic acid (97%, 5 ml) was heated under reflux for 3 hours. The reaction mixture was evaporated to dryness, the residue washed with cold water (3 x 25 ml) followed by ethanol (2 x 25 ml) and crystallized from water to yield 0.49 g (90%) of the title compound, mp > 300°C.

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (HBr) : Major bands - ν 640, 810, 920, 945, 990, 1135, 1180, 1195, 1255, 1280, 1345, 1355, 1400, 1480, 1570, 1625, 1730, 1740 and 2750-3120 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 247 nm (ε 5,900), 263 (6,700);
λ_{max} (pH 7) 247 nm (ε 5,900), 262 (6,300);
λ_{max} (pH 11) 254 nm (ε 5,400), 277 (6,100).

Analysis :		C	H	N
	Calcd for $C_5H_4N_4O$:	44.12	2.96	41.16
	Found:	44.01	3.18	41.24

7-Chloro-s-triazolo[4,3-c]pyrimidin-5(1H)-one (94). 4-Chloro-6-hydrazinopyrimidin-2(1H)-one (95, 3.0 g, 18.75 mmol) was mixed with formic acid (97%, 5 ml) and heated under reflux for 16 hours. After cooling to ~10°C, the crystalline material that separated was collected by filtration, washed with cold water (2 x 20 ml), followed by ethanol (2 x 20 ml) and air-dried. It was crystallized from water to yield 1.3 g (40.8%) of the title compound, mp > 240°C (dec.).

Chromatography : Absorbent - silica gel
Solvent - EtOAc:H_2O:acetone:MeOH, 14:1:1:1, v/v

Infrared (KBr) : Major bands - ν 670, 690, 740, 910, 1140, 1165, 1250, 1290, 1320, 1360, 1440, 1480, 1500, 1530, 1590, 1740, 2920, 3100 and 3440 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 269 nm (ε 8,100), 275 (7,900);
λ_{max} (pH 7) 262 nm (ε 7,100), 280 (9,200);
λ_{max} (pH 11) 262 nm (ε 7,200), 280 (9,400).

Analysis :		C	H	N	Cl
	Calcd for $C_5H_3ClN_4O$:	35.21	1.77	32.85	20.79
	Found:	34.91	1.99	32.61	20.51

7-Amino-s-triazolo[4,3-c]pyrimidin-5(1H)-one (97). A mixture of 4-amino-6-hydrazinopyrimidin-2(1H)-one (96, 4.23 g, 30 mmol) and triethyl orthoformate (150 ml) in methanol (500 ml) was stirred at room temperature for 16 hours. The solvent was evaporated and the residue was purified on a silica gel column using EtOAc:H_2O:n-PrOH (4:2:1, upper phase) as the solvent to yield 2.4 g (53%) of the title compound, mp > 300°C.

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 690, 750, 830, 975, 1005, 1135, 1175, 1235, 1330, 1410, 1460, 1515, 1570, 1610, 1660, 1700, 1750 and 2800-3360 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 255 nm (ε 3,200), 286 (7,000);

λ_{max} (pH 7) 285 nm (ε 4,700);

λ_{max} (pH 11) 278 nm (ε 5,100).

		C	H	N
Analysis :	Calcd for $C_5H_5N_5O \cdot \frac{1}{4}H_2O$:	38.58	3.56	45.00
	Found:	38.45	3.52	44.76

1-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-s-triazolo[1,5-a]-s-triazine-5,7-dione (99a). To a suspension of 3-amino-4-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,2,4-triazole (98, 5.0 g, 10 mmol)[64] in anhydrous tetrahydrofuran (150 ml), a solution of chlorocarbonylisocyanate (1.05 g, 10 mmol) in dry tetrahydrofuran (10 ml) was added dropwise with stirring. After 15 min. an additional 0.2 g (2 mmol) of chlorocarbonylisocyanate was added and stirring was continued for another 15 min. Solvent was evaporated under reduced pressure and the residual foam was triturated with anhydrous ether (2 x 50 ml) to obtain homogenous solid, yield 5.85 g (98%); mp > 105°C (dec.).

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:acetone, 8:2, v/v

Infrared (KBr) : Major bands - ν 705, 1020, 1065, 1090, 1110, 1170, 1260, 1445, 1550, 1610, 1720, 1760 and 2970-3430 cm^{-1}.

Ultraviolet : λ_{max} (MeOH) 227 nm (ε 51,600), 268 sh (3,000).

		C	H	N
Analysis :	Calcd for $C_{30}H_{23}N_5O_9$:	60.30	3.88	11.72
	Found:	60.08	3.72	11.56

1-β-D-Ribofuranosyl-s-triazolo[1,5-a]-s-triazine-5,7-dione (99b). To a solution of 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-s-triazolo[1,5-a]-s-triazine-5,7-dione (99a), 3.0 g, 5 mmol) in anhydrous methanol (200 ml) was added sodium methoxide until the pH of the solution was between 8 and 9. The reaction mixture was stirred at room temperature overnight. The solution was neutralized with Dowex-50 (H^+) resin, the resin was removed by filtration and washed with hot water several times. The combined filtrate and washings were evaporated *in vacuo* and the residue was triturated with ether (5 x 50 ml). The ether insoluble solid was crystallized from aqueous methanol as white micro-needles, yield 1.0 g (75%); mp 216-217°C.

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 690, 740, 760, 790, 830, 875, 950, 1030, 1060, 1090, 1110, 1160, 1220, 1285, 1385, 1450, 1540, 1600, 1670, 1745, 3280, 3360 and 3500 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 221 nm (ε 7,800), 238 (6,500);

λ_{max} (pH 7) 223 nm (ε 8,400), 238 (6,800);

λ_{max} (pH 11) 227 nm (ε 8,700).

			C	H	N
Analysis	:	Calcd for $C_9H_{11}N_5O_6$:	37.90	3.89	24.55
		Found:	37.81	3.83	24.49

Pyrrolo[3,2-c]pyridin-4(5H)-one (106). To a stirred suspension of N_1-benzylpyrrolo[3,2-c]pyridin-4(5H)-one [68] (107, 7.0 g) in liquid NH_3 (300 ml) was added a total of 2.4 g of sodium, in small portions, during 25-30 min., by which time the characteristic deep-blue color of the sodium persists. At this point, the blue color was discharged by careful addition of NH_4Cl (7.0 g), and the NH_3 is allowed to evaporate to dryness under a stream of nitrogen. The solid residue was dissolved in water (75 ml) and the insoluble impurities removed by filtration and the clear filtrate was stored in the refrigerator overnight. The crystalline material that separated was collected by filtration and recrystallized from water to yield 3.5 g (66%) of 2,7-dideazaallopurinol, 106, mp 234°C (dec.) [Lit.[68] mp 245-247°C].

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 735, 745, 775, 780, 810, 880, 900, 1050, 1095, 1135, 1200, 1235, 1250, 1330, 1415, 1480, 1580, 1610, 1640 and 2860-3280 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 265 nm (ε 8,300);

λ_{max} (pH 7 and 11) 263 nm (ε 11,000).

			C	H	N
Analysis	:	Calcd for $C_7H_6N_2O$:	62.68	4.51	20.88
		Found:	62.43	4.48	20.95

Pyrrolo[3,2-c]pyridine-4,6(5H,7H)-dione (3,7-Dideazaxanthine, 112). 3-Ethoxycarbonylpyrrole-2-acetamide[70] (113, 2.12 g, 10.8 mmol) and 95% EtOH (12 ml) was heated to 80°C at which time a clear solution formed. Then, 10% aqueous NaOH (13 ml) was added. Immediately the color started becoming pink. After 10 min. the reaction mixture was cooled in ice for an hour, acidified with 20% HCl, and cooled again in an ice-bath for 2 hours. The tiny pink crystalline solid that separated was collected by filtration and recrystallized from glacial acetic acid as buff-colored needles of 112, yield 1.37 g (84%), mp > 300°C [Lit.[70] mp > 300°C].

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 6:1, v/v

Infrared (KBr) : Major bands - ν 660, 750, 850, 925, 1060, 1120, 1175, 1200, 1260, 1320, 1360, 1490, 1670, 2880, 3080, 3200 and 3300 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 242 nm (ε 4,700), 287 (3,200);

λ_{max} (pH 7) 237 nm (ε 3,800), 287 (4,500);

λ_{max} (pH 11) 268 nm (ε 7,200), 304 (5,300).

Analysis :	C	H	N
Calcd for $C_7H_6N_2O_2$:	56.00	4.03	18.66
Found:	55.90	3.99	18.22

4,6-Dichloropyrrolo[3,2-c]pyridine (111). Compound 112 (3.0 g, 20 mmol) was combined with phenylphosphoric dichloride (8.7 ml, 60 mmol) and the resulting dark mixture was heated at 160°C for 3 hours. After cooling to room temperature, the contents were poured into ice-water (200 ml). The semi-solid obtained by decanding the water was triturated with 1 N NaOH (100 ml). The resulting insoluble solid was collected by filtration, washed with cold water

(2 x 50 ml), air-dried and crystallized from ethyl acetate to yield 1.5 g (40.1%), mp 244-247°C (dec.) [Lit.[72] mp 258°C].

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 6:1, v/v

Infrared (KBr) : Major bands - ν 715, 755, 825, 880, 955, 1070, 1100, 1180, 1275, 1310, 1385, 1430, 1485, 1540, 1590, 3150 and 3180 cm^{-1}.

Ultraviolet : λ_{max} (pH 1, 7 and 11) 273 nm (ε 4,800), 287 sh (3,800).

		C	H	N
Analysis :	Calcd for $C_7H_4Cl_2N_2$:	44.95	2.16	14.98
	Found:	45.20	2.49	14.68

6-Aminoimidazo[4,5-c]pyridin-4(5H)-one (3-Deazaguanine, 116). A mixture of 5(4)-cyanomethylimidazole-4(5)-carboxamide[76] (15.0 g, 100 mmol) and 10% aqueous sodium carbonate (150 ml) was heated under reflux for 4 hours. After cooling to room temperature, the reaction mixture was neutralized to pH 6 with concentrated HCl, and allowed to stand at 4°C for 24 hours. The solid that deposited was collected by filtration, washed well with ice-water (5 x 10 ml), air-dried and crystallized from water to yield 7.5 g (50%) of 3-deazaguanine, mp > 320°C [Lit.[76] mp > 350°C].

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : ν 1665 (C=O), 2950-3400 (NH_2) cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 274 nm (ε 11,500), 312 (6,500);

λ_{max} (pH 7) 262 nm (ε 10,500), 298 (8,100);

λ_{max} (pH 11) 262 nm (ε 10,000), 298 (7,800).

		C	H	N
Analysis :	Calcd for $C_6H_6N_4O$:	48.00	4.03	37.31
	Found:	48.08	4.21	37.27

6-Amino-3-β-D-ribofuranosylimidazo[4,5-c]pyridin-4(5H)-one (7-Ribosyl-3-deazaguanine, 119).

Step I. Methyl 4-cyanomethyl-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-imidazole-5-carboxylate (118). Methyl 5(4)-cyanomethylimidazole-4(5)-carboxylate[76] (114, 25 g, 0.151 mol) was refluxed under anhydrous conditions for 12 hours with HMDS (300 ml) and $(NH_4)_2SO_4$ (0.5 g). The excess HMDS was removed by distillation under reduced pressure providing the TMS derivative as a yellowish brown oil. The oil was dissolved in dry 1,2-dichloroethane (800 ml). 1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (28, 76.5 g, 0.151 mol) was added to the solution followed by addition of $SnCl_4$ (12.7 ml, 0.109 mol) in one portion and the reaction mixture was stirred at room temperature for 24 hours before it was poured into a 5% $NaHCO_3$ solution (3 lit.). The mixture was filtered through a Celite pad and extracted with $CHCl_3$ (3 x 1000 ml), and the combined, dried ($MgSO_4$) extracts were evaporated to dryness. Column chromatography [~25 g of silica gel packed in benzene-ethyl acetate (1:1) to 1.0 g of crude product and eluted with benzene-ethyl acetate (1:1)] provided, as the first isomer off the column, methyl 4-cyanomethyl-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)imidazole-5-carboxylate (118) in 34.5% yield, as white foam.[76]

^1H NMR (Me_2SO-d_6): δ 3.78 (s, 3, CH_3), 4.20 (s, 2, CH_2), 6.8 (d, 1, J = 2 Hz, $C_1'H$), 8.43 (s, 1, C_2H), and other sugar protons.

		C	H	N
Analysis :	Calcd for $C_{33}H_{27}N_3O_9$:	65.02	4.43	6.90
	Found:	65.18	4.53	6.94

Further elution of the column with benzene-ethyl acetate (1:1) provided methyl 5-cyanomethyl-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)imidazole-4-carboxylate (117) in a 29.5% yield.

Step II. 6-Amino-3-β-D-ribofuranosylimidazo[4,5-c]pyridin-4(5H)-one (119). A mixture of 118 (6.09 g, 10 mmol) and liquid NH_3 (25 ml) was placed in a steel bomb (45 ml). The bomb was three-quarters submerged in a steam bath and heated for 2 hours. The NH_3 was allowed to evaporate at room temperature, and the residue was subjected to a vacuum overnight. The residue was triturated with MeOH (250 ml) and then crystallized from water (charcoal) to provide the title compound as white needles, 2.5 g (80%), mp 210°C (dec.) [Lit.[76] mp 210°C (dec.)].

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 690, 720, 780, 860, 900, 980, 1060, 1090, 1130, 1190, 1250, 1290, 1320, 1345, 1380, 1415, 1460, 1515, 1560, 1615, 1635, 1665 and 2680-3430 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 277 nm (ε 9,200), 317 (4,700);

λ_{max} (pH 7) 257 nm (ε 4,800), 317 (5,600);

λ_{max} (pH 11) 257 nm (ε 5,100), 316 (5,400).

^1H NMR (Me_2SO-d_6): δ 5.42 (s, 2, NH_2), 5.61 (s, 1, C_7H), 6.24 (d, 1, J = 5 Hz, $C_{1'}H$), 8.34 (s, 1, C_2H), 10.73 (s, 1, NH), and other sugar protons.

			C	H	N
Analysis	:	Calcd for $C_{11}H_{14}N_4O_5$:	46.80	4.99	19.85
		Found:	46.50	5.22	19.61

2-Thiopyrrolo[2,3-d]pyrimidine-4(1H,3H)-dione (125). Ring annulation of 2-thio-5-(2,2-diethoxyethyl)pyrimidine-4(1H,3H)-dione (124) in the presence of 0.2 N HCl, according to the procedure of Davoll[78] gave the title compound in a 80% yield, mp > 300°C [Lit.[78] mp > 300°C].

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 685, 730, 895, 1070, 1130, 1200, 1300, 1375, 1430, 1480, 1580, 1650, 2900, 3010, 3120 and 3280 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 238 nm (ε 8,100), 294 (10,700);

λ_{max} (pH 7) 224 nm (ε 10,500), 284 (11,800);

λ_{max} (pH 11) 224 nm (ε 10,500), 284 (11,600).

			C	H	N	S
Analysis	:	Calcd for $C_6H_5N_3OS$:	43.10	3.01	25.13	19.18
		Found:	42.87	3.04	24.87	18.92

2-Methylthiopyrrolo[2,3-d]pyrimidin-4(3H)-one (126). To a solution of 125 (3.34 g, 20 mmol) in 0.1 N NaOH (50 ml) was added CH_3I (5 ml) and the mixture was stirred at room temperature for 3 hours. The mixture was evaporated to dryness and the residue was dissolved in water (100 ml). The aqueous solution was neutralized with Dowex-50 (H^+) resin, stirred for 30 min. and the resin was removed by filtration. Evaporation of the filtrate and crystallization of the residue from aqueous ethanol gave 3.0 g (83.1%) of the title compound as white needles, mp 265-266°C (dec.) [Lit.[78] mp 265-267°C (dec.)].

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 690, 725, 750, 825, 885, 950, 960, 1080, 1125, 1210, 1280, 1400, 1550, 1660, 2900, 3010, 3080, 3240 and 3400 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 217 nm (ε 11,400), 269 (9,000);

λ_{max} (pH 7) 216 nm (ε 11,900), 269 (8,500);

λ_{max} (pH 11) 226 nm (ε 10,600), 271 (7,200).

		C	H	N	S
Analysis :	Calcd for $C_7H_7N_3OS$:	46.39	3.89	23.18	17.69
	Found:	46.04	3.82	22.98	17.46

Pyrrolo[2,3-d]pyrimidin-4(3H)-one (7-Deazahypoxanthine, 121). To a solution of 126 (3.62 g, 20 mmol) in 15% NH_4OH (150 ml) and ethanol (25 ml) was added Raney nickel (10 g), and the mixture was heated under reflux for 1 hour. The mixture was filtered through a Celite pad and the filtrate evaporated to dryness. The residue was dissolved in water (50 ml) and neutralized with Dowex-50 (H^+) resin. The resin was removed by filtration and the filtrate was evaporated to dryness. The residue was crystallized from water to yield 1.40 g (82.3%) of 121, mp > 300°C [Lit.[78] mp 338-340°C].

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 6:1, v/v

Infrared (KBr) : Major bands - ν 720, 800, 880, 1110, 1215, 1290, 1320, 1360, 1415, 1460, 1500, 1555, 1660, 2860, 2920, 2980 and 3090 cm^{-1}.

Ultraviolet : λ_{max} (pH 1 and 7) 261 nm (ε 8,400);

λ_{max} (pH 11) 264 nm (ε 8,900).

		C	H	N
Analysis :	Calcd for $C_6H_5N_3O \cdot \frac{1}{2}H_2O$:	49.99	4.19	29.15
	Found:	49.97	3.96	29.47

4-Chloropyrrolo[2,3-d]pyrimidine (122). Chlorination of 121 with $POCl_3$ in the presence of N,N-dimethylaniline, according to the procedure of Lupke and Seela[79] gave the title compound in a 71% yield, mp 195°C (dec.) [Lit.[79] mp 178°C].

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:acetone, 8:2, v/v

Infrared (KBr) : Major bands - ν 755, 830, 850, 860, 920, 980, 1215, 1225, 1270, 1360, 1430, 1445, 1490, 1550, 1600, 2830, 2980 and 3120 cm^{-1}.

Ultraviolet : λ_{max} (MeOH) 220 nm (ε 22,000), 274 (3,600), 281 sh (3,500).

		C	H	N	Cl
Analysis :	Calcd for $C_6H_4ClN_3$:	46.93	2.63	27.36	23.09
	Found:	46.84	2.73	27.45	22.92

4-Methoxypyrrolo[2,3-d]pyrimidine (123). Compound 122 (3.0 g, 20 mmol) was dissolved in dry methanol (125 ml) and sodium methoxide (3.3 g, 60 mmol) was added. The mixture was heated under reflux with the exclusion of moisture for 2 days. The solution was concentrated to ~30 ml and water was added. The resulting solid was collected by filtration and crystallized from aqueous methanol to yield colorless needles, 2.0 g (66.6%), mp 207-209°C [Lit.[79] mp 214°C].

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:acetone, 4:1, v/v

Infrared (KBr) : Major bands - ν 740, 830, 870, 900, 955, 1045, 1090, 1130, 1150, 1260, 1310, 1345, 1400, 1445, 1470, 1570, 1590, 1880 and 2840-3200 cm^{-1}.

Ultraviolet : λ_{max} (MeOH) 214 nm (ε 19,400), 262 (6,100).

Analysis :		C	H	N
	Calcd for $C_7H_7N_3O$:	56.37	4.73	28.17
	Found:	56.33	4.82	28.29

Pyrrolo[3,2-d]pyrimidine-2,4(1H,3H)-dione (128). A solution of 2-phenylpyrimido[5,4-c]pyridazine-6,8(7H)-dione [80] (127, 0.39 g, 1.6 mmol) in glacial acetic acid (10 ml) was hydrogenated over 10% Pd/C at room temperature at atmospheric pressure for 5 hours. After filtration through Celite, the clear solution was evaporated and the residue was triturated with a small amount of ethanol. The solid that separated was collected by filtration and crystallized from methanol to yield 0.163 g (62%) of the title compound, mp > 300°C [Lit.[80] mp > 300°C].

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 680, 735, 770, 840, 890, 1010, 1050, 1130, 1140, 1280, 1370, 1395, 1440, 1555, 1650, 1680, 2920, 3020 and 3180 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 267 nm (ε 10,800);

λ_{max} (pH 7) 267 nm (ε 12,200);

λ_{max} (pH 11) 265 nm (ε 10,500).

^1H NMR (Me_2SO-d_6): δ 5.83 (t, 1, C_7H), 7.13 (t, 1, C_6H), 10.55, 10.73 and 11.81 (3s, 3, ring NH).

			C	H	N
Analysis	:	Calcd for $C_6H_5N_3O_2$:	47.68	3.34	27.81
		Found:	47.47	3.45	27.61

2,4-Dichloro[3,2-d]pyrimidine (129). A suspension of the sodium salt of 128 (2.0 g, 10.25 mmol) in phenylphosphonium dichloride (12.0 g, 7.75 ml, 61.5 mmol) was heated at 170-180°C for 3 hours. The reaction mixture was poured while still hot into crushed ice (200 g) with vigorous stirring. The aqueous solution was extracted with ethyl acetate (3 x 150 ml), and the organic phase was washed with saturated aqueous $NaHCO_3$ solution (2 x 50 ml), followed by water (2 x 50 ml) and then dried over Na_2SO_4. Evaporation of the solvent and crystallization of the residue from 50% aqueous methanol gave 1.7 g (88%) of the title compound, mp 223°C [Lit.[82] mp 224°C].

Chromatography : Absorbent - silica gel

Solvent - toluene:ethyl acetate, 5:1, v/v

Infrared (KBr) : Major bands - ν 740, 780, 860, 890, 980, 1110, 1175, 1235, 1280, 1390, 1445, 1475, 1515, 1530, 1610, 2870, 3040 and 3160 cm^{-1}.

Ultraviolet : λ_{max} (MeOH) 224 nm (ε 30,900), 279 (7,800).

			C	H	N	Cl
Analysis	:	Calcd for $C_6H_3Cl_2N_3$:	38.39	1.61	22.34	37.72
		Found:	38.42	1.90	22.52	37.89

6-Amino-9-(2-deoxy-β-D-ribo-hexopyranosyl)purine (135). Liquid NH_3 (50 ml) was added to a stainless steel bomb containing 6-chloro-9-(2-deoxy-3,4,6-tri-O-p-nitrobenzoyl-β-D-ribo-hexopyranosyl)purine (6.42 g, 8.58 mmol). The closed vessel was heated on a steam bath for 12 hours. After cooling and venting the bomb, cold MeOH (50 ml) was added, and the solvents evaporated.

The residue was dissolved in water and the aqueous solution was extracted with ether (8 x 200 ml) before it was evaporated to dryness. The residue was crystallized from 90% aqueous ethanol to yield 1.83 g (76%) of the title compound, mp 232°C.

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 680, 740, 900, 975, 1010, 1050, 1060, 1240, 1290, 1325, 1410, 1440, 1480, 1590, 1635, 2900, 3010, 3200 and 3320 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 256 nm (ε 14,500);

λ_{max} (pH 7) 259 nm (ε 14,500);

λ_{max} (pH 11) 259 nm (ε 15,000).

		C	H	N
Analysis :	Calcd for $C_{11}H_{15}N_5O_4$:	46.97	5.37	24.90
	Found:	46.98	5.34	24.91

9-(2-Deoxy-β-D-ribo-hexopyranosyl)hypoxanthine (134). To a solution of 135 (0.10 g, 0.36 mmol) in water (1.5 ml), cooled to 0°C, was added glacial acetic acid (0.2 ml). Sodium nitrite (0.17 g) was added, little at a time, the flask sealed with parafilm and the contents of the flask allowed to warm to room temperature. After stirring for 2 days, the reaction mixture was diluted with water (15 ml) and evaporated to dryness. Co-evaporation of the residue with water (2 x 25 ml), and finally crystallization from water gave white needles of the target compound, 70 mg (68%), mp 184-185°C.

Chromatography : Absorbent - silica gel

Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 690, 750, 780, 910, 1070, 1130, 1220, 1340, 1370, 1410, 1450, 1490, 1550, 1590, 1690, 2920, 3030 and 3400 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 248.5 nm (ε 11,900);
λ_{max} (pH 7) 248 nm (ε 11,600);
λ_{max} (pH 11) 252 nm (ε 12,900).

		C	H	N
Analysis :	Calcd for $C_{11}H_{14}N_4O_5 \cdot \frac{1}{2}H_2O$:	45.36	5.19	19.24
	Found:	45.61	5.21	19.21

Neplanocin D (137). To a solution of neplanocin A (40 mg) in water (3 ml) containing $NaNO_2$ (105 mg) was added glacial acetic acid (0.17 ml) and heated with stirring at 48°C for 2 hours. The reaction mixture was evaporated to dryness and the residue was purified by reverse phase HPLC techniques using aqueous CH_3CN as the solvent. The fractions containing the homogenous product were pooled and lyophilized to yield 10 mg of the title compound, mp 207-207.5°C.

Chromatography : Absorbent - silica gel
Solvent - EtOAc:H_2O:n-PrOH, 4:2:1, upper phase

Infrared (KBr) : Major bands - ν 690, 780, 850, 1040, 1110, 1210, 1300, 1390, 1445, 1510, 1540, 1580, 1680, 2920, 3250, 3360 and 3400 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 249 nm (ε 11,200);
λ_{max} (pH 7) 249 nm (ε 12,000);
λ_{max} (pH 11) 254 nm (ε 12,700).

		C	H	N
Analysis :	Calcd for $C_{11}H_{12}N_4O_4$:	50.00	4.57	21.20
	Found:	49.91	4.68	21.34

2-Methylthio-7-phenylpyrazolo[1,5-a]-s-triazin-4(3H)-one (140). A solution of 7-phenyl-2-thiopyrazolo[1,5-a]-s-triazin-4(1H,3H)-one [87] (139, 7.32 g, 30 mmol) in ethanol (100 ml) containing sodium hydroxide (2.4 g) in 50 ml of water, was stirred at room temperature while methyl iodide (4.25 g, 30 mmol) was added dropwise. After 10 minutes, the addition was completed and the sodium salt of the product began to precipitate. The reaction mixture was stirred an additional 30 min. and the salt was separated by filtration. The salt was dissolved in a minimum amount of water and acidified by the addition of 2 N H_2SO_4. The precipitated product was collected by filtration and crystallized from ethanol to give 3.6 g (47%) of the title compound as off-white crystals, mp 249°C [Lit.[87] mp 249-251°C].

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 6:1, v/v

Infrared (KBr) : Major bands - ν 775, 1145, 1240, 1340, 1400, 1450, 1465, 1580, 1600, 1740, 2960, 3140 and 3500 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 257 nm (ε 14,200);

λ_{max} (pH 7) 255 nm (ε 14,700);

λ_{max} (pH 11) 255 nm (ε 15,200).

		C	H	N	S
Analysis :	Calcd for $C_{12}H_{10}N_4OS$:	55.79	3.90	21.69	12.41
	Found:	55.86	3.79	21.73	12.52

5-Amino-6-methyl-2-methylthiopyrimidin-4(3H)-one (142). To a solution of 6-methyl-2-methylthio-5-phenylazopyrimidin-4-one (141, 10.75 g, 38 mmol) in 1 N NaOH (300 ml) was added sodium dithionate (19.9 g, 114 mmol), with stirring at 65-70°C, over a period of 30 min. After complete addition, stirring was continued for 30 min. To the hot solution, decolorizing carbon (3 g) was

added, heated again for 5 min. and filtered through a Celite pad. Concentration of the filtrate to ~25 ml, and cooling to 5°C, deposited brown solid, which was collected by filtration and crystallized from ethanol to yield 3.0 g (46%) of 142, mp 242-243°C.

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 6:1, v/v

Infrared (KBr) : Major bands - ν 755, 928, 960, 980, 1200, 1255, 1300, 1410, 1435, 1465, 1590, 1640, 2800, 3350 and 3450 cm^{-1}.

Ultraviolet : λ_{max} (pH 1) 230 nm (ε 5,900), 265 sh (2,900), 291 sh (2,700);

λ_{max} (pH 7) 230 nm (ε 4,600), 290 (4,800);

λ_{max} (pH 11) 265 nm (ε 4,500), 285 sh (4,000).

Analysis :	C	H	N	S
Calcd for $C_6H_9N_3OS$:	42.08	5.29	24.54	18.72
Found:	41.87	5.29	34.34	18.92

2-Methylthiopyrimido[4,5-d]-1,2,3-triazin-4(3H)-one N,-oxide (143). Compound 142 (2.0 g, 11.7 mmol) was dissolved in 1 N NaOH solution (60 ml) containing $NaNO_2$ (2.0 g) and the mixture was cooled to 0°C. 2.5 N HCl (120 ml) was added dropwise over a period of 30 min. After the addition was over, stirring was continued at room temperature for an hour, during which time a thick precipitate was formed. The precipitate was collected by filtration, washed with cold water (3 x 25 ml) followed by cold ethanol (2 x 25 ml). The product was crystallized from aqueous ethanol to yield 1.72 g (69%) of the title compound, mp 182°C (dec.).

Chromatography : Absorbent - silica gel

Solvent - $CHCl_3$:MeOH, 6:1, v/v

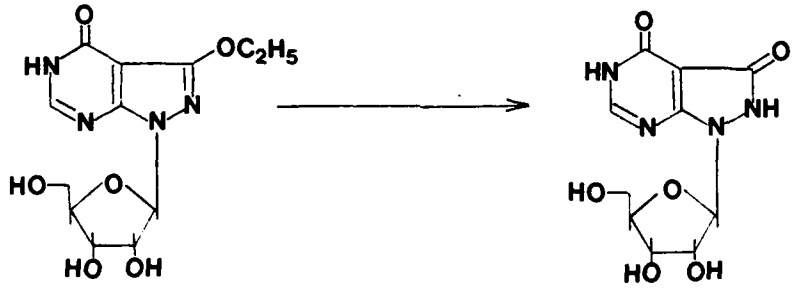
Infrared (KBr) : Major bands - ν 690, 860, 965, 1150, 1225, 1240, 1320, 1360, 1440, 1510, 1535, 1705, 2900, 3050 and 3400 cm^{-1}.

^1H NMR (Me_2SO-d_6): δ 2.50 (s, 3, SCH_3), 8.66 (s, 1, C_8H).

		C	H	N	S
Analysis :	Calcd for $C_6H_4N_5O_2S$:	34.28	1.91	33.32	15.24
	Found:	33.99	2.23	33.29	15.18

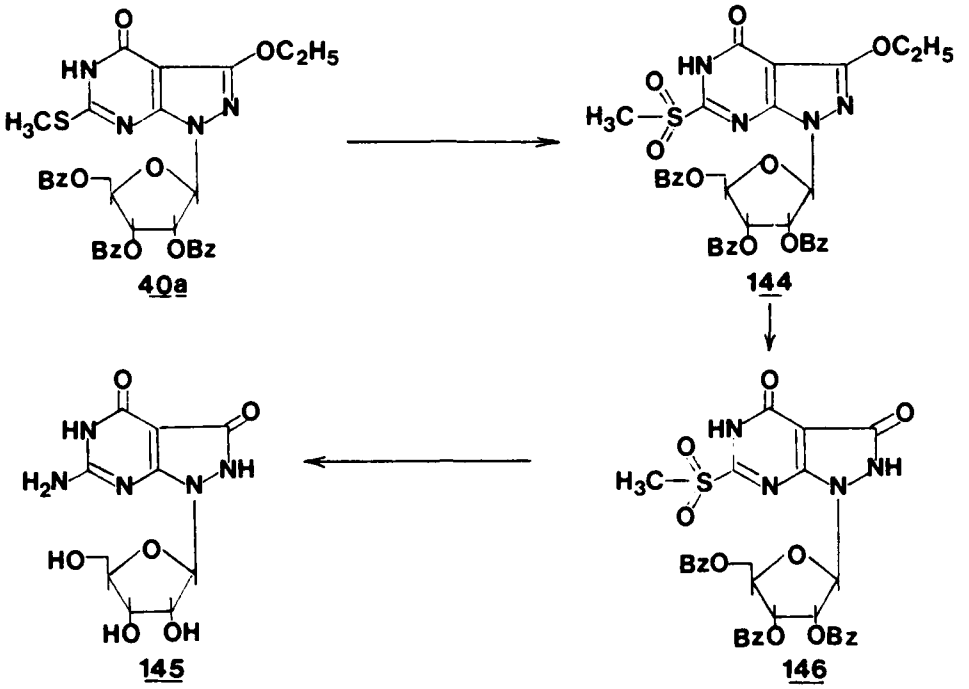
IV. WORK IN PROGRESS

Efforts are under way to prepare 3-oxoallopurinol ribonucleoside (31) from 39, and also 3-oxo-6-aminoallopurinol ribonucleoside (145) from 40a, which has been recently prepared in our laboratory. Ether cleavage of 39 with



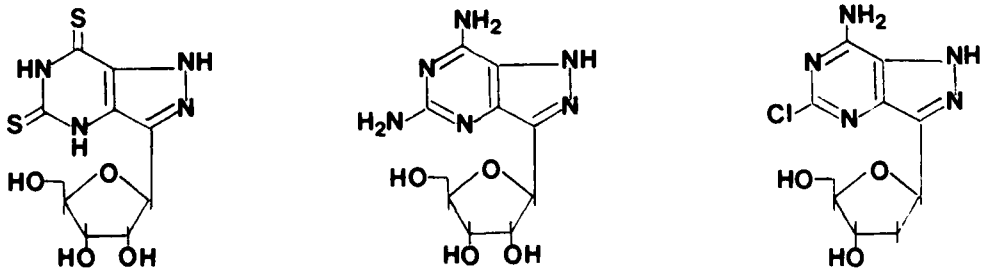
39 31

trimethylsilyl iodide, under carefully controlled conditions, is expected to yield the desired 3-oxoallopurinol ribonucleoside (31). Oxidation of 3-ethoxy-6-methylthio-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrazolo-[3,4-d]pyrimidin-4(5H)-one (40a) with m-chloroperoxybenzoic acid in CH_2Cl_2 should give the corresponding methylsulfonyl derivative 144. When compound 144 is allowed to react with trimethylsilyl iodide, ether cleavage should occur to give 146, which on treatment with liquid NH_3 at 90°C for several hours is expected to yield the guanosine analog 145.



40a 144 145 146

The breakthrough in formycin chemistry reported from our laboratory [48] has opened up new vistas of obtaining hitherto inaccessible 5,7-disubstituted pyrazolo[4,3-d]pyrimidine ribonucleosides. Functional group manipulation of triacetyloxoformycin B (59) led to the preparation of a small amount of biologically significant 3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine-5,7-(4H,6H)-dithione (65), and 5-aminoformycin (66b). Efforts are now under way



65 66b 147

to prepare larger amounts of 65 and 66b. A four-step deoxygenation procedure using phenoxythiocarbonylation [88-90] of the 2'-hydroxy group of 5-chloro-

AD-A164 470 NEW INOSINE AND GUANOSINE ANALOGS AS INHIBITORS OF PARASITIC INFECTIONS(U) BRIGHAM YOUNG UNIV PROVO UT R K ROBINS ET AL. 30 NOV 85 DAMD17-82-C-2224 2/2

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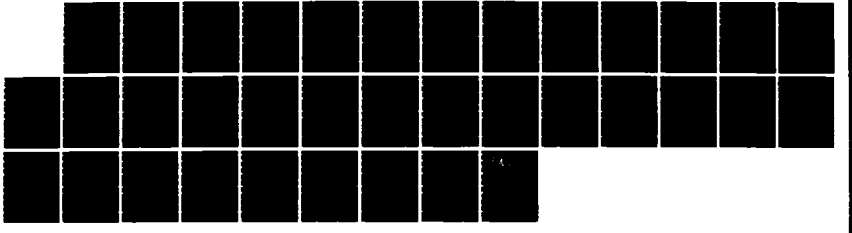
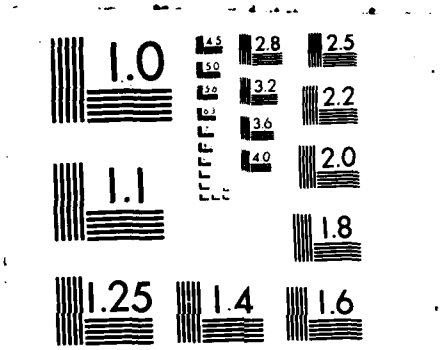
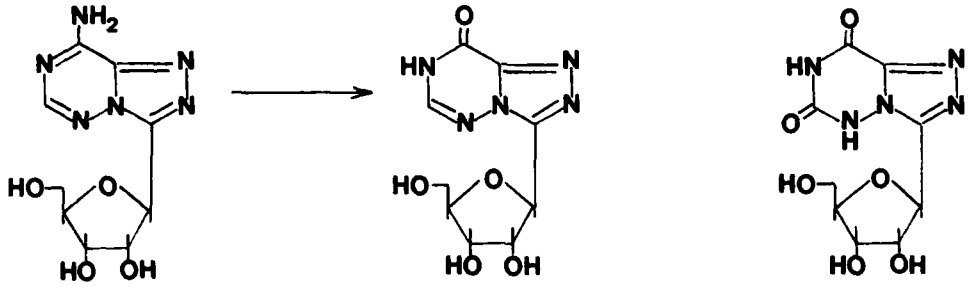




formycin (62) to obtain 7-amino-5-chloro-3-(2-deoxy-β-D-erythro-pentofuranosyl)pyrazolo[4,3-d]pyrimidine (147) is in progress.

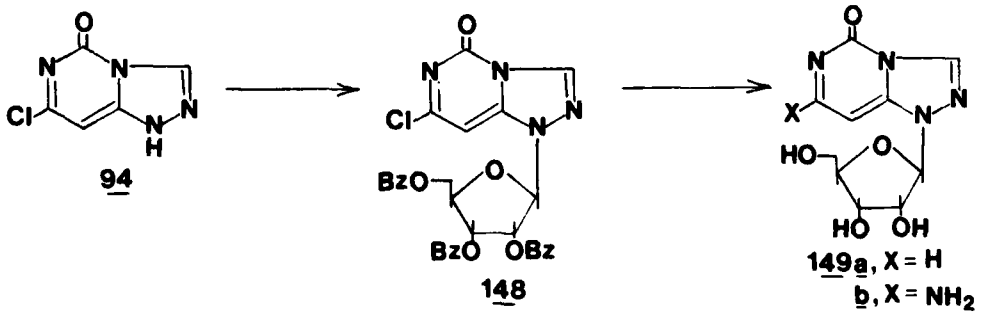
In a preliminary study, we were able to prepare the adenosine analog in the s-triazolo[3,4-f]-as-triazine ring system (80a). We are in the process of



80a 80b 82

optimising the experimental conditions to prepare 80a from 8-methylthio-3-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-s-triazolo[3,4-f]-as-triazine (81), on a preparative scale, so that this adenosine analog (80a) could be studied in animals in greater detail. Nitrous acid deamination of 80a should yield the formycin B analog 80b. Similarly, the synthesis of the oxoformycin B analog, 3-β-D-ribofuranosyl-s-triazolo[3,4-f]-as-triazine-6,8(5H,7H)-dione (82) from 79 is under active investigation.

We have recently initiated the glycosylation studies with 7-chloro-s-triazolo[4,3-c]pyrimidin-5(1H)-one (94, BL-05599) to obtain the inosine and guanosine analogs in this ring system with a bridgehead nitrogen atom.



94 148 149a, X = H
b, X = NH2

Glycosylation of the trimethylsilyl derivative of 94 with a protected sugar, or treatment of unsilylated 94 with a protected sugar under high temperature

glycosylation conditions reported recently from our laboratory,[44] should give 7-chloro-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-s-triazolo[4,3-c]pyrimidin-5-one (148). Catalytic dehalogenation of 148 with Pd/C, followed by debenzoylation should provide the desired inosine analog 149a. Treatment of 148 with $MeOH/NH_3$ at elevated temperature and pressure should furnish the guanosine analog 149b. It is also planned to prepare the 5'-monophosphates of 149a and 149b to see if there is any therapeutic advantage of the nucleotide form of the drug.

V. SUMMARY OF ANTIPARASITIC TESTING DATA ON COMPOUNDS SUBMITTED TO WALTER REED ARMY INSTITUTE OF RESEARCH (WRAIR) UNDER THE SUBJECT CONTRACT

The Department of Parasitology, Walter Reed Army Institute of Research, Washington, D.C., under the direction of the Contracting Officer Technical Representative Mr. H. A. Musallam, supplied us with the following antiparasitic screening data.

The following methodology was used:

Antileishmanial Evaluation: Human macrophage cultures were derived from the monocytes of the peripheral blood of normal human volunteers by methods previously described.[91] After being infected with amastigotes of Leishmania tropica WR 401 (NIH 173), infected macrophage cultures in 1.0 ml of culture medium were exposed to a constant dose of a drug for 6 days. The culture medium used was RPMI-1640 (GIBCO Laboratories, Grand Island, NY) containing 10% heat-inactivated fetal calf serum (GIBCO Laboratories), penicillin (50 U/ml), and streptomycin (50 µg/ml). After 6 days the number of amastigotes per 100 macrophages in control (non-drug treated) cultures and experimental cultures was determined in counting 100 to 200 Giesma-stained macrophages in each culture. The number of macrophages per culture was estimated by counting 20 representative fields for each culture. In initial experiments, drug

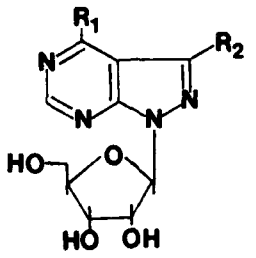
doses of 0.01 to 1.0 μM were employed. Generally, the drug dosage was increased in subsequent experiments until macrophage toxicity (see below) or a dose of at least 70 μM was achieved.

Enumeration of Data: The number of Leishmania amastigotes per 100 macrophages surviving in drug-treated cultures was expressed as a percentage of the number in simultaneously cultivated controls. The concentration of drug calculated to eliminate 50% of amastigotes compared to controls (the 50% effective dose [ED_{50}]) was determined by nonlinear regression analysis[92] of the results of each experiment. For drugs for which the dose-response curve was so flat that statistical analysis could not be performed, the ED_{50} was estimated by inspection of the data.

The 50% effective doses (ED_{50}) for the elimination of Leishmania amastigotes from infected macrophages exposed to several pyrazolo[3,4-d]pyrimidine nucleosides synthesized are listed in Table I. The estimated ED_{50} for 1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-thione (R_1=SH, R_2=H, thiopurinol riboside) is similar to that for allopurinol riboside. No more than 60% of organisms were eliminated by allopurinol riboside or thiopurinol riboside at the highest concentrations tested (72 to 290 μM). 3-Aminoallopurinol riboside (BK-48455) and the nitroso compound have similar ED_{50}s. However, 3-bromoallopurinol riboside (BK-15661) was found to be more active (ED_{50} > 15 μM) than allopurinol riboside against L. tropica in vitro.

A systematic investigation of the antileishmanial activity of purine analogs synthesized during this study has recently been published.[26] In addition to these compounds, certain purine analogs and formycin derivatives synthesized in our laboratory exhibited significant antitrypanosomal activity in vitro (Table II) and in vivo (Table III and Table IV). Table V shows comparative antiparasitic activity of formycin B and certain purine analogs.

Table I. *In Vitro* Antileishmanial Activity of Certain 1-β-D-Ribofuranosylpyrazolo[3,4-d]pyrimidine



R_1	R_2	ED_{50}(μM)
OH	H	76-190
SH	H	72
OH	Br	> 15
OH	NH_2	>177
2-Nitroso-1-β-D-ribofuranosylpyrazolo-[3,4-d]pyrimidin-3,4(5H)-dione		>160

Thioformycin B has been shown to have significant activity against L. donovani in vitro (87% suppression). 3-Deazaguanosine (BK-17405) was more active than allopurinol or allopurinol riboside against L. tropica in vitro (ED_{50} of 3.6 μM), and has shown significant activity against L. donovani in animals (76% suppression), suggesting that guanosine derivatives may have potential as antiparasitic agents. 2-Methylinosine (BK-48428) had an ED_{50} of 0.21 μM. The heterocycle 3-cyanoallopurinol (BK-49818) was found to be significantly active against trypanosomes (ED_{50} of 0.39 μM) and malaria in vitro. Selenoformycin B was more active than thioformycin B, but less active than formycin B against L. tropica promastigotes in vitro with an ED_{50} of 0.2 μM. Although the EC_{50} (concentration of drug that inhibits the growth rate of cells by 50%) value of allopurinol riboside is similar to that of formycin B

(7.5 μM) for T. cruzi epimastigotes, the observed EC_{50} value of 1-methylformycin B is 0.6 μM. More of 1-methylformycin B is made available for further studies. However, by far the most active nucleoside which showed in vitro antitrypanosomal activity continues to be 2-methylinosine (BK-48428), suggesting further in vivo studies with BK-48428.

Sinefungin, a naturally-occurring antifungal nucleoside antibiotic, is one of the most potent growth inhibitors against Leishmania, but is quite toxic to humans. A recent data (supplied by Mr. H. A. Musallam) indicates that oxoformycin B (60, BK-74731) at 20 μM in combination with sinefungin (5 nm) showed a 33.22% increase in toxicity above the expected additive effect (Table VI). It has been suggested by Dr. Linda Nolan, of Department of Microbiology, University of Massachusetts, that a combination of sinefungin and the compounds in Table VII could provide a rational approach to chemotherapy minimizing toxicity to the host.

Table II. In Vitro Antitrypanosomal Activity of Certain Purine Analogs

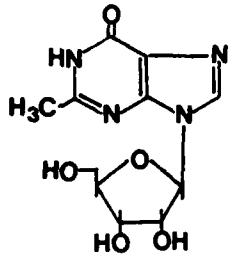
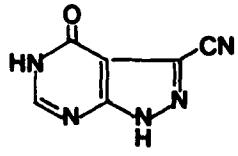
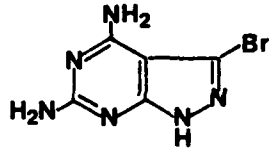
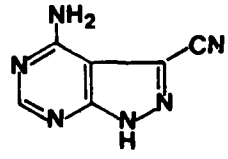
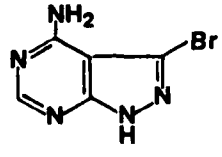
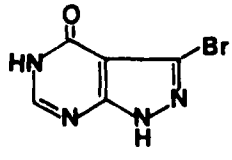
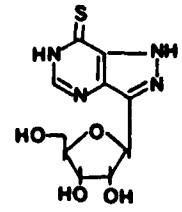
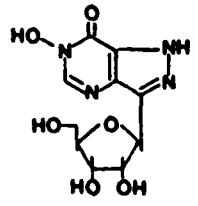
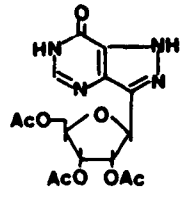
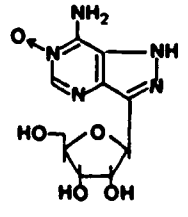
Compound	WRAIR No.	ED_{50} (μM)
	BK-48428	0.21
	BK-49818	0.39
	BK-62964	1.56
	BK-49827	2.03
	BK-17272	3.91
	BK-02593	4.51

Table III. RECENT IN VIVO ANTITRYPANOSOMIASIS DATA

COMPOUND	ARMY NO.	BYU NO.	PARASITE	DOSE mg/kg	NO. OF ANIMALS	CURE
	BK-63005	RV-407	T-Rhod.	13.3	5	5
	BK-50900	SI-69	T-Rhod.	424	5	4
	BK-48384	RA-250	T-Rhod.	13.3	5	3
	BK-48482	SI-12	T.Rhod.	26.5	5	3

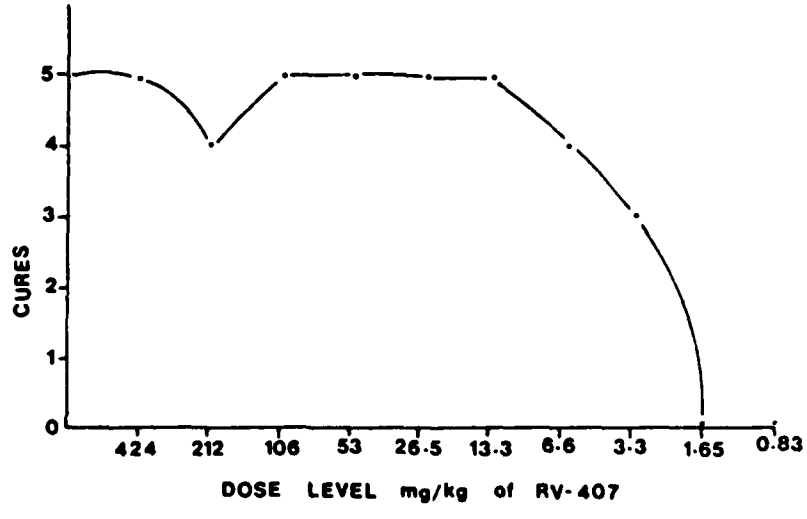


Table IV. RECENT IN VIVO TRYPANOSOMA RHODESIENSE DATA

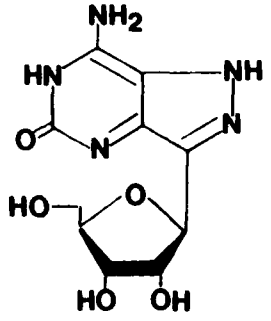
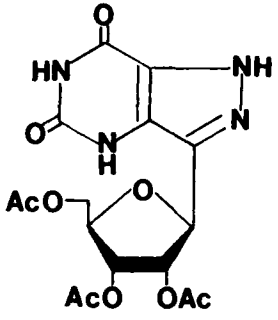
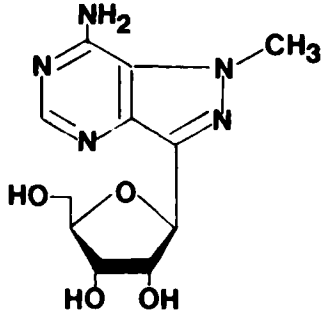
Compound	ARMY No.	BYU No.	Dose, mg/kg	No.of animals	Cure
	BK-71338	SI-246	26.5 53.0 106.0 212.0 424.0	5 5 5 5 5	2 3 5 5 5
	BK-95196	SI-256	424.0	5	1
	BK-71329	SI-50	424.0	5	1

Table V

Comparative Antiparasitic Activity of Formycin B and Certain Purine Analogs

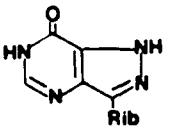
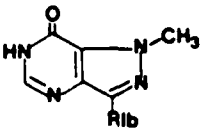
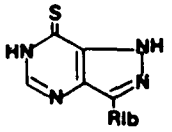
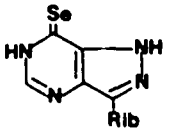
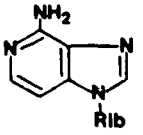
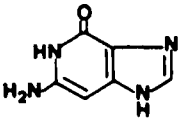
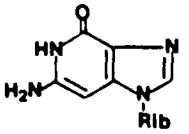
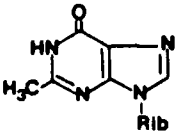
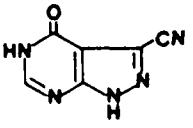
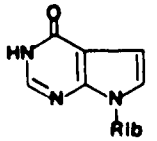
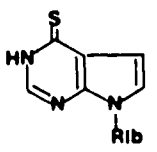
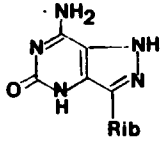
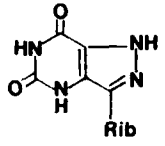
	In Vitro, ED_{50} μM			In Vivo
	L. tropica	T. cruzi	Malaria	L. donovani
	0.04			
		0.6		
	3.6			87% suppression
	0.2			
	>72			
	>134	2.99		
	3.6			76% suppression
		0.21		
		0.39	+	
	0.2			
	>18			

Table VI

Compound	% Inhibition Alone	% Inhibition with 5nm Sinefungin	Predicted Inhibition	% Increase in Expected Toxicity
Sinefungin 5 nM	19.71	-	-	-
9-Deazainosine 1 µm	5.66	19.71	25.66	Less 5.95
Formycin B 0.1 µm	56.71	85.64	76.42	9.22
Oxoformycin B 20 µm BK74731	27.99	80.92	47.70	33.22
Oxoformycin A 4 µm BK71338	66.04	85.53	85.75	same
Allopurinol riboside 50 µm	37.73	61.63	57.44	4.19

% Inhibition was determined at 72 hours.

Table VII

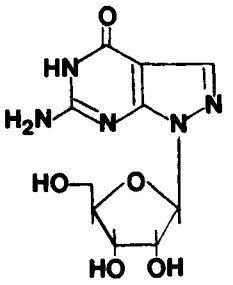
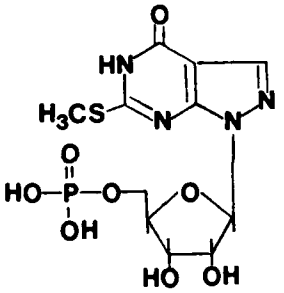
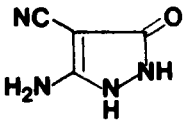
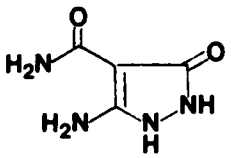
Compound	Structure	Concentration	% Inhibition Alone	% Inhibition When Added Together
Oxoformycin A BK71338		20 µm	78.6	
		30 µm	80.9	
		50 µm	83.4	
Oxoformycin B BK74731		20 µm	37.7	
		30 µm	46.0	
		50 µm	52.8	
Both Compounds Together		20 µm		78.0
		30 µm		80.0
		50 µm		82.6

% Inhibition determined at 72 hours.

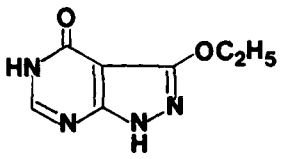
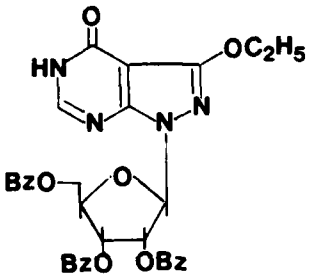
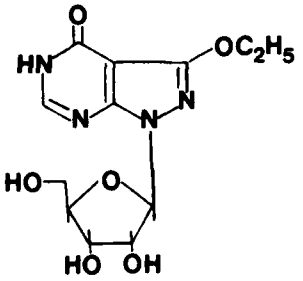
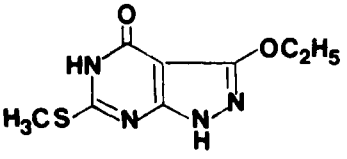
VI. LIST OF COMPOUNDS SUBMITTED TO WALTER REED ARMY INSTITUTE OF RESEARCH (WRAIR) FROM FEBRUARY 1, 1984 TO JUNE 30, 1985

During the progress report period, February 1, 1984 through June 30, 1985, the following fifty-five (55) compounds were prepared and submitted to the Contracting Officer's Technical Representative, Department of Medicinal Chemistry, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D. C., for antiparasitic evaluation. The chemical structure of each of these compounds is shown below:

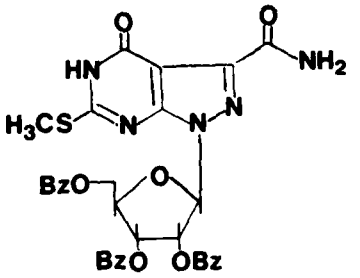
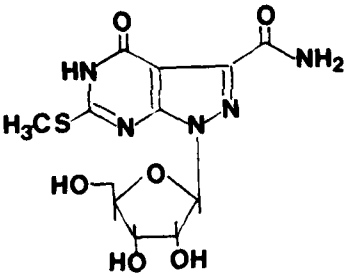
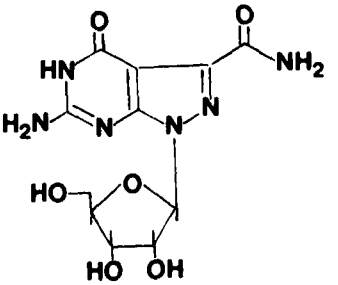
NO.	COMPOUND	QUANTITY	BYU NO.	WRAIR NO.	REF.
1.	1-β-D-Ribofuranosylpyrazolo[3,4-d]-pyrimidin-4(5H)-one	5.00 g	SI-21		38
2.	6-Chloro-4-methoxypyrazolo[3,4-d]-pyrimidine	1.20 g	RE-588	BK-74446	40
3.	6-Aminopyrazolo[3,4-d]pyrimidin-4(1H,5H)-one	2.00 g	RE-465		40

NO.	COMPOUND	QUANTITY	BYU NO.	WRAIR NO.	REF.
4.	6-Amino-1-β-D-ribofuranosylpyrazolo-[3,4-d]pyrimidin-4(5H)-one 	1.00 g	RE-502	BK-48464	38
5.	6-Methylthio-1-β-D-ribofuranosyl-[3,4-d]pyrimidin-4(5H)-one 5'-phosphate 	0.25 g	SH-292		p.63
6.	5-Amino-4-cyanopyrazol-3(1H,2H)-one 	1.50 g	SH-358		p.49
7.	5-Amino-4-carbamoylpyrazol-3(1H,2H)-one 	1.50 g	SH-350		p.50

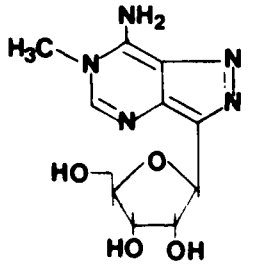
NO.	COMPOUND	QUANTITY	BYU NO.	WRAIR NO.	REF.
8.	Pyrazolo[3,4-d]pyrimidine-3,4-(2H,5H)-dione	2.00 g	SH-357	BK-98768	p.51
9.	4-Aminopyrazolo[3,4-d]pyrimidin-3(1H,2H)-one	1.10 g	SH-387		p.52
10.	4-Amino-3-methoxypyrazolo[3,4-d]-pyrimidine	1.70 g	SH-381	BL-05580	p.52
11.	4-Amino-3-methoxy-1-β-D-ribofuran-osylpyrazolo[3,4-d]pyrimidine	1.50 g	SH-389		p.53

NO.	COMPOUND	QUANTITY	BYU NO.	WRAIR NO.	REF.
12.	3-Ethoxypyrazolo[3,4-d]pyrimidin-4(5H)-one 	1.50 g	SH-321		p.54
13.	3-Ethoxy-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-d]-pyrimidin-4(5H)-one 	1.40 g	SH-331		p.56
14.	3-Ethoxy-1-β-D-ribofuranosyl-pyrazolo[3,4-d]pyrimidin-4(5H)-one 	0.90 g	SH-333		p.57
15.	3-Ethoxy-6-methylthiopyrazolo-[3,4-d]pyrimidin-4(5H)-one 	1.80 g	SH-296		p.55

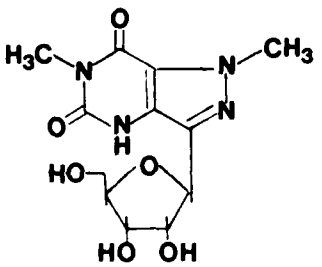
NO.	COMPOUND	QUANTITY	BYU NO.	WRAIR NO.	REF.
16.	3-Ethoxy-6-methylthio-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-pyrazolo[3,4-d]pyrimidin-4(5H)-one	1.90 g	SH-304		p.57
17.	3-Ethoxy-6-methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]-pyrimidin-4(5H)-one	1.70 g	SH-303		p.58
18.	6-Thiopyrazolo[3,4-d]pyrimidin-4,6(5H,7H)-dione-3-carboxamide	2.00 g	SM-57	BK-74526	p.59
19.	6-Methylthio-4(5H)-oxopyrazolo-[3,4-d]pyrimidine-3-carboxamide	1.50 g	SB-191	BK-74428	p.60

NO.	COMPOUND	QUANTITY	BYU NO.	WRAIR NO.	REF.
20.	6-Methylthio-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-4(5H)-oxopyrazolo[3,4-d]-pyrimidine-3-carboxamide 	1.70 g	SB-192		p.60
21.	6-Methylthio-1-β-D-ribofuranosyl-4(5H)-oxopyrazolo[3,4-d]-pyrimidine-3-carboxamide 	1.60 g	SB-276		p.61
22.	6-Amino-1-β-D-ribofuranosyl-4(5H)-oxopyrazolo[3,4-d]pyrimidine-3-carboxamide 	1.40 g	SB-204	BK-74455	p.62

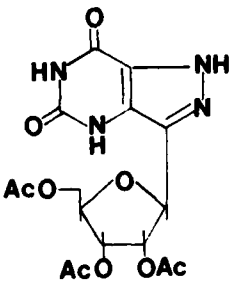
NO.	COMPOUND	QUANTITY	BYU NO.	WRAIR NO.	REF.
23.	7-Amino-6-methyl-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine	1.00 g	SI-315	BK-74419	47

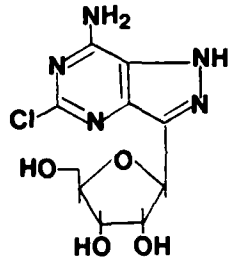
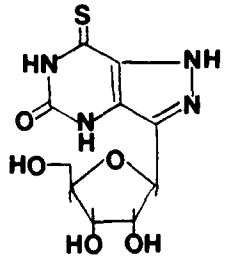
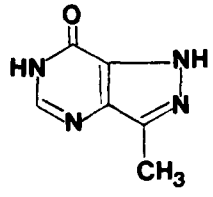
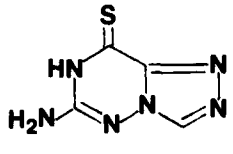


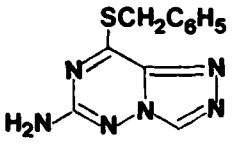
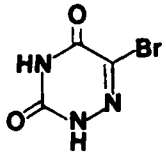
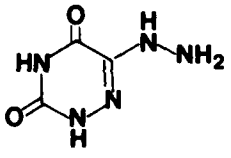
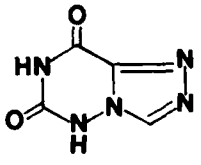
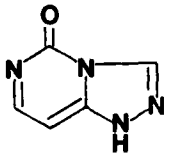
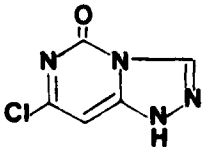
24.	1,6-Dimethyl-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine-5,7(4H)-dione	0.25 g	SI-317	BK-74393	48

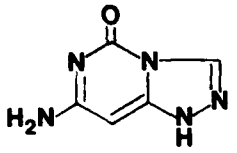
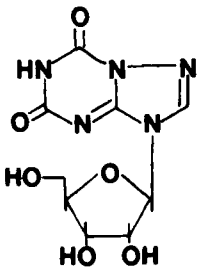
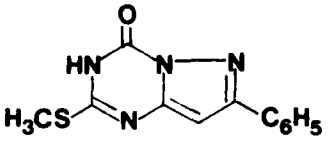
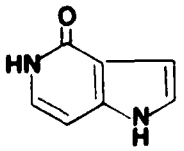
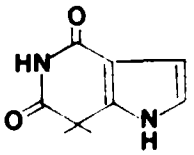


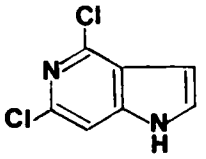
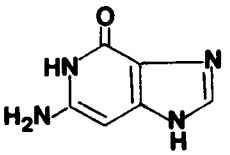
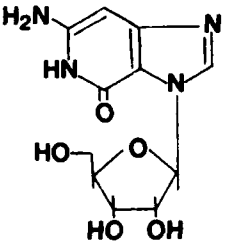
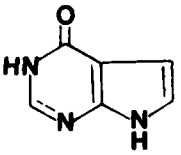
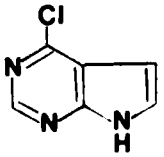
25.	3-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidine-5,7(4H,6H)-dione	1.40 g	SI-256	BK-95196	p.67

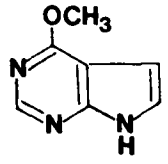
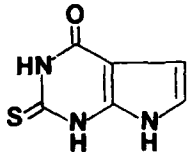
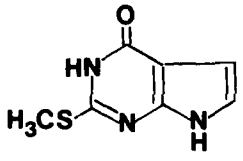
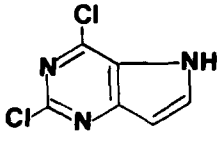
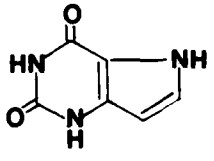


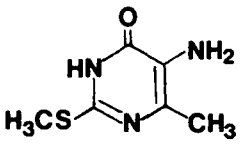
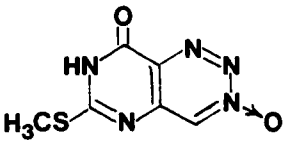
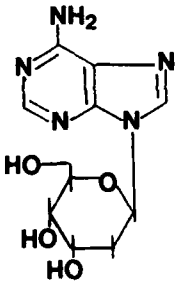
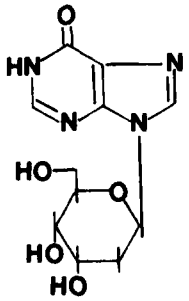
NO.	COMPOUND	QUANTITY	BYU NO.	WRAIR NO.	REF.
26.	7-Amino-5-chloro-3-β-D-ribofurano-sylpyrazolo[4,3-d]pyrimidine 	1.00 g	SI-366		p.71
27.	7-Thio-3-β-D-ribofuranosylpyrazolo-[4,3-d]pyrimidine-5(4H,6H)-dione 	1.00 g	SI-400	BK-98811	p.67
28.	3-Methylpyrazolo[4,3-d]pyrimidin-7(6H)-one 	1.10 g	RA-305		p.72
29.	6-Amino-s-triazolo[3,4-f]-as-triazine-8(7H)-thione 	1.30 g	YS-24		p.72

NO.	COMPOUND	QUANTITY	BYU NO.	WRAIR NO.	REF.
30.	6-Amino-8-benzylthio-s-triazolo-[3,4-f]-as-triazine 	1.50 g	YS-31	-	p.73
31.	5-Bromo-6-azauracil 	1.50 g	SI-334	BK-74437	59
32.	5-Hydrazino-6-azauracil 	0.80 g	SI-341	BK-74400	60
33.	s-Triazolo[3,4-f]-as-triazine-6,8(5H,7H)-dione 	1.75 g	SI-347		p.75
34.	s-Triazolo[4,3-c]pyrimidin-5(1H)-one 	2.00 g	RE-647		p.76
35.	7-Chloro-s-triazolo[4,3-c]-pyrimidin-5(1H)-one 	1.00 g	RE-694	BL-05599	p.77

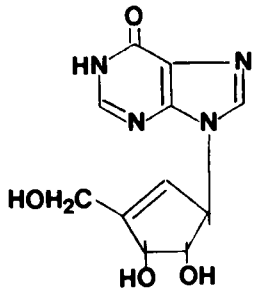
NO.	COMPOUND	QUANTITY	BYU NO.	WRAIR NO.	REF.
36.	7-Amino-s-triazolo[4,3-c]-pyrimidin-5(1H)-one 	1.30 g	RE-636		p.78
37.	1-β-D-Ribofuranosyl-s-triazolo-[1,5-a]-s-triazine-5,7-dione 	2.00 g	SI-129		p.79
38.	2-Methylthio-7-phenylpyrazolo-[1,5-a]-s-triazin-4(3H)-one 	1.80 g	YS-51		87
39.	Pyrrolo[3,2-c]pyridin-4(5H)-one 	2.00 g	RE-678	BK-98786	68
40.	Pyrrolo[3,2-c]pyridine-4,6-(5H,7H)-dione 	0.80 g	Re-615		70

NO.	COMPOUND	QUANTITY	BYU NO.	WRAIR NO.	REF.
41.	4,6-Dichloropyrrolo[3,2-c]-pyridine 	1.00 g	RE-618	BK-74508	72
42.	6-Aminoimidazo[4,5-c]pyridin-4-(5H)-one 	2.50 g	RA-163A		76
43.	6-Amino-3-β-D-ribofuranosyl-imidazo[4,5-c]pyridin-4(5H)-one 	1.60 g	SN-177		76
44.	Pyrrolo[2,3-d]pyrimidin-4(3H)-one 	2.00 g	RV-485	BK-74517	78
45.	4-Chloropyrrolo[2,3-d]pyrimidine 	2.00 g	RV-564	BK-98795	79

NO.	COMPOUND	QUANTITY	BYU NO.	WRAIR NO.	REF.
46.	4-Methoxypyrrolo[2,3-_]pyrimidine 	1.30 g	RE-677	BK-98802	79
47.	2-Thiopyrrolo[2,3-d]pyrimidine-4(1H,3H)-dione 	2.00 g	RV-577	BL-01500	78
48.	2-Methylthiopyrrolo[2,3-d]-pyrimidin-4(3H)-one 	2.00 g	RV-578	BL-01519	78
49.	2,4-Dichloropyrrolo[3,2-d]-pyrimidine 	2.00 g	ST-10	BK-98777	82
50.	Pyrrolo[3,2-d]pyrimidine-2,4-(1H,3H)-dione 	1.90 g	RA-309		80

NO.	COMPOUND	QUANTITY	BYU NO.	WRAIR NO.	REF.
51.	5-Amino-6-methyl-2-methylthio-pyrimidin-4(3H)-one 	1.80 g	YS-12		p.92
52.	2-Methylthiopyrimido[4,5-d]-1,2,3-triazin-4(3H)-one N7-oxide 	1.80 g	YS-13		p.93
53.	6-Amino-9-(2-deoxy-β-D-ribo-hexo-pyranosyl)purine 	1.80 g	RJ-160	BL-01484	p.89
54.	9-(2-Deoxy-β-D-ribo-hexopyranosyl)-hypoxanthine 	1.80 g	RJ-263	BL-01493	p.90

NO.	COMPOUND	QUANTITY	BYU NO.	WRAIR NO.	REF.
55.	Neplanocin D	1.00 g	SB-327	BL-05606	p.91



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VIII. **STAFFING**

Contract No. DAMD17-82-C-2224

During the report period the following personnel have been engaged in the work on the contract:

Name	Effort	
Roland K. Robins, Ph.D. Principal Investigator	10%	Sept. 1, 1984 to Dec. 31, 1984 January 1, 1985 to June 30, 1985 No compensation
Ganapathi R. Revankar, Ph.D. Co-Investigator	40%	Feb. 1, 1984 to June 30, 1985
Bheemarao G. Ugarkar, Ph.D. Postdoctoral Research Fellow	100%	Feb. 1, 1984 to Jan. 31, 1985
Yogesh Sanghvi, Ph.D. Postdoctoral Research Fellow	100%	Feb. 1, 1985 to June 30, 1985
Howard B. Cottam, Ph.D. Postdoctoral Research Fellow	50%	Feb. 1, 1984 to June 30, 1985
A. David Adams, B. S. Technician	40%	Feb. 1, 1984 to June 30, 1985
Jack Anderson, B. S. Graduate Research Assistant	50%	Sept. 1, 1984 to June 30, 1985
L. Mark Lee, B. S. Graduate Research Assistant	50%	Jan. 1, 1985 to June 30, 1985
L. Dee Nord, B. S. Graduate Research Assistant	50%	Jan. 1, 1985 to June 30, 1985
Evan S. Whitaker Lab Technician	50%	April 1, 1985 to June 30, 1985

Date: July 10, 1985

IX. APPENDIX

The following papers have been published from the work supported by Contract DAMD17-82-C-2224

1. Synthesis and Antiviral/Antitumor Activities of Certain Pyrazolo-[3,4-d]pyrimidine-4(5H)-selone Nucleosides and Related Compounds.
   B. G. Ugarkar, H. B. Cottam, P. A. McKernan, R. K. Robins and G. R. Revankar, J. Med. Chem., 27, 1026-1030 (1984).

2. Synthesis and Biological Activity of Certain 3,4-Disubstituted Pyrazolo[3,4-d]pyrimidine Nucleosides.
   H. B. Cottam, C. R. Petrie, P. A. McKernan, R. J. Goebel, N. K. Dalley, R. B. Davidson, R. K. Robins and G. R. Revankar, J. Med. Chem.,27, 1119-1127 (1984).

3. Synthesis of 1-Methyl-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidin-7-(6H)-selone and Certain Related Nucleosides and Nucleotides.
   B. G. Ugarkar, R. K. Robins and G. R. Revankar, Nucleosides Nucleotides, 3, 233-244 (1984).

4. A Simple Oxidation of Formycin to Oxoformycin and Oxoformycin B. Synthesis of 6-Methyloxoformycin - A C-Nucleoside Analog of Doridosine.
   B. G. Ugarkar, G. R. Revankar and R. K. Robins
   J. Heterocycl. Chem., 21, 1865-1870 (1984).

5. Synthesis and Biological Activity of 6-Azacadeguomycin and Certain 3,4,6-Trisubstituted Pyrazolo[3,4-d]pyrimidine Ribonucleosides.
   C. R. Petrie, H. B. Cottam, P. A. McKernan, R. K. Robins and G. R. Revankar, J. Med. Chem., 28, 1010 (1985).

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