Synthesis of Certain 3-Alkoxy-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidines Structurally Related to Adenosine, Inosine and Guanosine [1,2]

Jack D. Anderson†, and N. Kent Dalley

Department of Chemistry, Brigham Young University,
Provo, Utah 84602

Ganapathi R. Revankar* and Roland K. Robins

Nucleic Acid Research Institute, 3300 Hyland Avenue,
Costa Mesa, California 92626

Received April 7, 1986

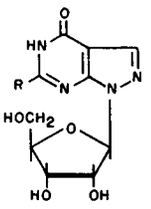
Several 3-alkoxysubstituted pyrazolo[3,4-*d*]pyrimidine ribonucleosides structurally related to adenosine, inosine and guanosine have been prepared by the direct glycosylation of preformed aglycon precursor containing a 3-alkoxy substituent. Ring closure of 5(3)-amino-3(5)-ethoxypyrazole-4-carboxamide (**6b**) with either formamide or potassium ethyl xanthate gave 3-ethoxyallopurinol (**7b**) and 3-ethoxy-6-thioxopyrazolo[3,4-*d*]-pyrimidin-4(5*H*,7*H*)-one (**10**), respectively. Methylation of **10** gave the corresponding 6-methylthio derivative **15**. Similar ring annulation of 5(3)-methoxypyrazole-4-carboxamide (**6a**) with formamide afforded 3-methoxyallopurinol (**7a**). Treatment of 5(3)-amino-3(5)-methoxypyrazole-4-carbonitrile (**5a**) with formamidine acetate furnished 4-amino-3-methoxypyrazolo[3,4-*d*]pyrimidine (**4**). High-temperature glycosylation of **7b** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose in the presence of boron trifluoride etherate gave a 2:1 mixture of N-1 and N-2 glycosyl blocked nucleosides **11b** and **13b**. Deprotection of **11b** and **13b** with sodium methoxide gave 3-ethoxy-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**12b**) and the corresponding N-2 glycosyl isomer **14b**, respectively. Similar glycosylation of either **4** or **7a**, and subsequent debenzoylation gave exclusively 4-amino-3-methoxy-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**9**) and 3-methoxy-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4-(5*H*)-one (**12a**), respectively. The structural assignment of **12a** was made on the basis of single-crystal X-ray analysis. Application of this general glycosylation procedure to **15** gave the corresponding N-1 glycosyl derivative **16** as the sole product, which on debenzoylation afforded 3-ethoxy-6-(methylthio)-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**17**). Oxidation of **16** and subsequent ammonolysis furnished the guanosine analog 6-amino-3-ethoxy-1-β-D-ribofuranosylpyrazolo[3,4-*d*]-pyrimidin-4(5*H*)-one (**19**). Similarly, starting from 3-methoxy-4,6-bis(methylthio)pyrazolo[3,4-*d*]pyrimidine (**20**), 6-amino-3-methoxy-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**23**) was prepared.

J. Heterocyclic Chem., **23**, 1869 (1986).

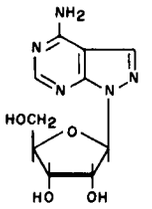
Recent reports from this laboratory have described the synthesis of certain ribonucleosides of the pyrazolo[3,4-*d*]-pyrimidine ring system [3-7] having substituents at C-3, C-4 and C-6 positions. The rationale behind preparing such compounds has been outlined in an earlier publication [5]. 1-ß-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (allopurinol ribonucleoside, **1**) inhibits the growth of the pathogenic protozoan *Leishmania* at concentrations that have little or no effect on mammalian cells [8-10]. This selectivity appears to be due to the differences in purine salvage pathways in the host and parasite. Since most parasites lack *de novo* purine biosynthesis [10-15], these organisms are dependent on the salvage pathway for purine nucleoside metabolism and will accept certain structurally similar pyrazolo[3,4-*d*]pyrimidines instead of purines [10]. Both *Leishmania* [16] and *Trypanosoma* [17] species have a unique enzyme nucleoside phosphotransferase that converts allopurinol ribonucleoside to its 5'-monophosphate [8,18], whereas mammalian cells are either ineffective or less effective in phosphorylation of **1**. Sequential conversion of allopurinol ribonucleoside 5'-phosphate by the parasite enzymes adenylosuccinate synthetase and succino-AMP lyase to the cytotoxic metabolite 5'-phosphate of 4-aminopyrazolo[3,4-*d*]pyrimidine ribonucleoside (**3**) and its incorporation into the cellular RNA of the parasite (as the 5'-triphosphate), is proposed to be the mechanism of action.

Recently, 4-aminopyrazolo[3,4-*d*]pyrimidine (4-APP) has been shown to be effective in the treatment of experimental Chagas disease in mice [19,20], whereas the ribonucleoside derivatives of allopurinol (**1**) and 4-APP (**3**) were shown to be several-fold more active than allopurinol against promastigotes of the isolates of American *Leishmania brazilienses, Leishmaina mexicana* [21] and *Leishmania tropica* [9] *in vitro*. The guanine analogue 6-aminopyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (6-aminoallopurinol) has also recently been shown to be significantly active against *Trypanosoma cruzi* epimastigotes *in vitro* [20]. We have reported a convenient synthesis of 6-amino-1-ß-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**2**) [3] and several 3-substituted allopurinol, as well as 6-aminoallopurinol ribonucleosides [5,7]. Introduction of certain substituents, such as bromo, cyano or carbamoyl function at position 3 of pyrazolo[3,4-*d*]pyrimidine ribonucleosides

led to significant increase of antitumor [22-24], antiviral [7] and antiparasitic [20] activity. As a part of our ongoing synthetic program, we have now prepared several 3-alkoxy derivatives of **1**, **2** and **3**. The introduction of an alkoxy group could provide significant increase in electron density of the heterocyclic ring since it would appear from previous studies [5] that there is in general good bulk tolerance for inhibitory activity (enzyme binding) relative to position 3.



1, R = H
2, R = NH_2

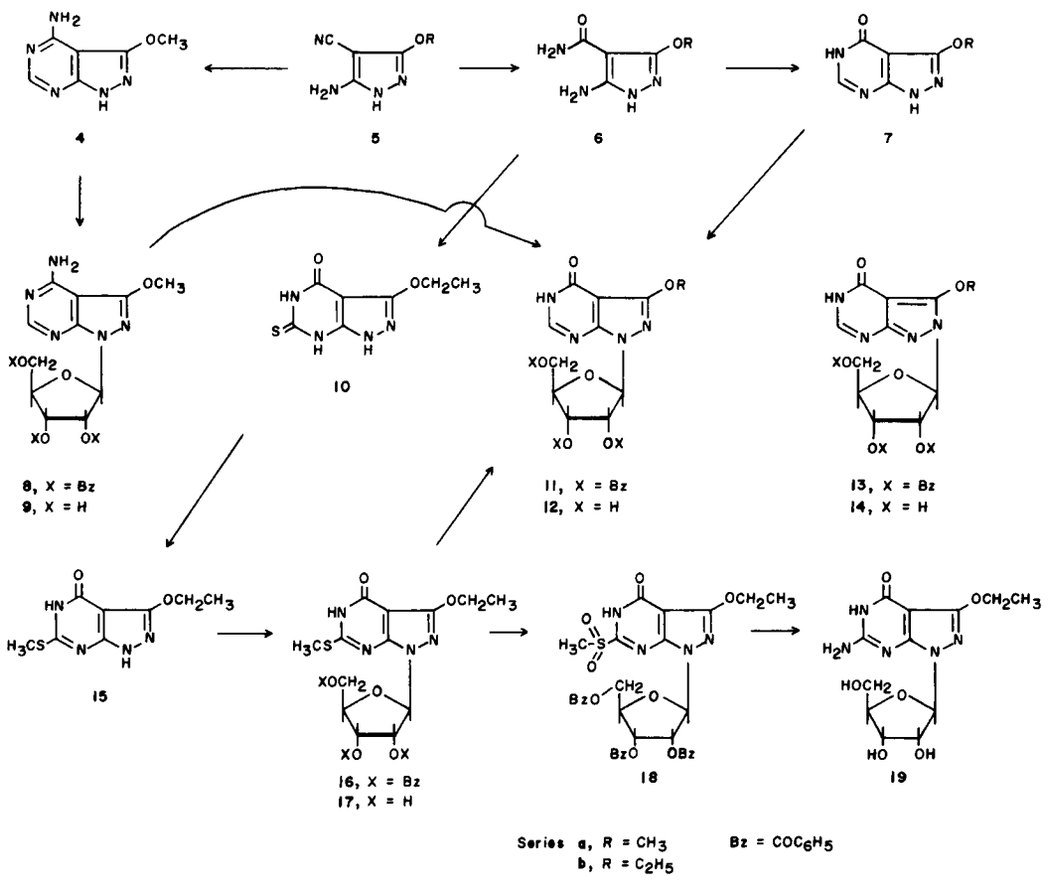


3

The synthesis of such 3-alkoxypyrazolo[3,4-*d*]pyrimidine ribonucleosides seemed propitious by the direct glycosylation of preformed aglycon possessing a 3-alkoxy substituent (Scheme I). For the preparation of 3-ethoxypyrazolo-[3,4-*d*]pyrimidin-4(5*H*)-one (**7b**), 5(3)-amino-3(5)-ethoxypyrazole-4-carboxamide (**6b**) was found to be a viable starting material. Acid hydrolysis of 5(3)-amino-3(5)-ethoxypyrazole-4-carbonitrile (**5b**) [25] with concentrated sulfuric acid at room temperature gave **6b**. Ring closure of **6b** with formamide at reflux temperature furnished 3-ethoxyallopurinol (**7b**). The assignment of the pyrazolo[3,4-*d*]pyrimidine structure was based on elemental analysis and ^1H nmr studies. An additional aromatic proton resonance at δ 7.92 ppm due to C_6H and the absence of a primary amino group resonance at δ 5.96 ppm was observed for **7b**, which was present in the starting pyrazole **6b**. Furthermore, the uv spectrum of **7b** exhibited the characteristic large bathochromic shift, which is expected due to the annulation of the pyrimidone moiety to the pyrazole ring. Treatment of **6b** with potassium ethyl xanthate in dimethylformamide at reflux temperature, according to the general procedure of Yamazaki and coworkers [26] gave the ring closed product 3-ethoxy-6-thioxopyrazolo[3,4-*d*]pyrimidin-4(5*H*,7*H*)-one (**10**), which on subsequent methylation with methyl iodide under alkaline conditions furnished the key intermediate 3-ethoxy-6-(methylthio)pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**15**).

A similar procedure was employed to obtain 3-methoxypyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**7a**). Treatment of

Scheme I



1,1-dicyanoketene-2,2-dimethyl acetyl [25] with hydrazine hydrate (85%) readily gave 5(3)-amino-3(5)-methoxypyrazole-4-carbonitrile (**5a**). As for **6b**, acid hydrolysis of the nitrile function of **5a** afforded 5(3)-amino-3(5)-methoxypyrazole-4-carboxamide (**6a**), which on further ring closure with formamide furnished **7a**. When compound **5a** was heated under reflux with triethyl orthoformate and subsequent treatment of the reaction product with methanolic ammonia at 90°, 4-amino-3-methoxypyrazolo[3,4-*d*]pyrimidine (**4**) was formed, which was isolated in 57% yield. Ring closure of **5a** with formamidine acetate at 130° provided yet another convenient method for the preparation of **4**.

The glycosylation studies with these 3-alkoxysubstituted pyrazolo[3,4-*d*]pyrimidines were next undertaken. Although a number of procedures for the glycosylation of pyrazolo[3,4-*d*]pyrimidine ring system are reported in the literature [27], the high-temperature glycosylation method, developed and recently reported from our laboratory [5], appears to be the method of choice. The elevated temperature normally employed in this procedure favors the formation of the desired thermodynamically more stable N-1 glycosyl isomer. Thus, direct glycosylation of **7b** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose in the presence of the catalyst boron trifluoride etherate in a boiling polar aprotic solvent such as nitromethane gave approximately a 2:1 mixture of isomeric blocked nucleosides. The mixture of isomers was separated on a flash silica gel column and identified as 3-ethoxy-1-(2,3,5-tri-*O*-benzoyl-ß-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**11b**, 38%) and the corresponding N-2 glycosyl isomer **13b** (20%), a distribution favorable for our specific needs. No formation of other isomeric nucleosides [28] was observed. Deprotection of the individual isomers **11b** and **13b** with 1*N* sodium methoxide in methanol provided an excellent yield of 3-ethoxy-1-ß-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**12b**) and the corresponding N-2 glycosyl isomer **14b**, respectively. The uv spectrum of the major isomer **12b** was very similar to that of **7b**, whereas **14b** exhibited a bathochromic shift relative to **7b**, which is indicative of N-2 glycosyl attachment [3,29]. In the ^{1}H nmr spectrum, the signal for the anomeric proton of the N-1 isomer **12b** appeared further downfield with a larger coupling constant than for the corresponding N-2 isomer **14b**. This observation is also consistent with other similar reports of pyrazolo[3,4-*d*]pyrimidine ribonucleosides [3,29].

A similar high-temperature glycosylation of 3-methoxyallopurinol (**7a**) gave almost exclusively the desired 3-methoxy-1-(2,3,5-tri-*O*-benzoyl-ß-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**11a**) as crystalline material (51%). The reaction was essentially complete within 20 minutes and longer reaction times did not improve the yield of **11a**. Careful investigation furnished chromatographic evidence of the formation of another nucleoside material in a very minor amount (<3%); presumably the positional N-2 isomer. No attempt was made to isolate this minor product. Debenzoylation of **11a** with sodium methoxide in methanol gave the inosine analog 3-methoxy-1-ß-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (3-methoxyallopurinol ribonucleoside, **12a**). The absolute structural assignment of **12a** was made on the basis of single-crystal X-ray crystallographic studies.

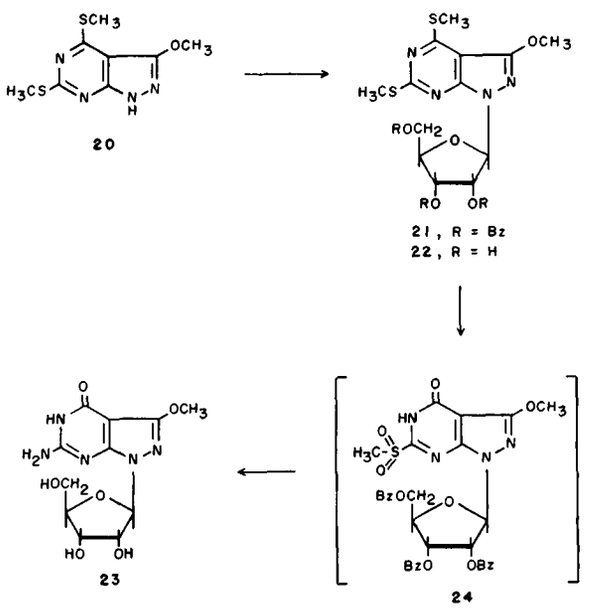
The other heterocycle that was employed for glycosylation studies was 4-amino-3-methoxypyrazolo[3,4-*d*]pyrimidine (**4**). Glycosylation of **4** with the fully protected sugar in the presence of boron trifluoride etherate in boiling nitromethane gave a 77% yield of 4-amino-3-methoxy-1-(2,3,5-tri-*O*-benzoyl-ß-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**8**) as the only isolable isomer. Removal of the benzoyl blocking groups using sodium methoxide in methanol gave nearly a quantitative yield of the adenosine analog 4-amino-3-methoxy-1-ß-D-ribofuranosylpyrazolo[3,4-*d*]-pyrimidine (**9**). The uv absorption spectrum of **9** was very similar to that of **4** indicating N-1 glycosyl attachment. Moreover, deamination of **9** with aqueous nitrous acid gave **12a**, thus confirming the structural assignment of **9**.

In an effort to develop a synthetic procedure that would lead to 3-alkoxy substituted guanosine analogs **19** and **23** the use of **15** and **20** for the glycosylation studies was investigated. Application of the general high-temperature glycosylation procedure to **15** using boron trifluoride etherate in dry nitromethane furnished crystalline 3-ethoxy-6-(methylthio)-1-(2,3,5-tri-*O*-benzoyl-ß-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**16**) as the sole product (Scheme I), which was isolated in 61% yield. Formation of other nucleosidic products was not observed in this reaction. The structural proof for **16** was obtained by dethiation with Raney nickel. The product obtained from dethiation reaction was identical to **11b**, prepared by direct synthesis. Methoxide ion catalyzed debenzoylation of **16** provided an excellent yield of 3-ethoxy-6-(methylthio)-1-ß-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**17**). Oxidation of **16** with *m*-chloroperoxybenzoic acid in anhydrous dichloroethane readily gave the corresponding methyl sulfonyl derivative **18**. The appearance of two strong absorption bands at 1100 and 1260 cm^{-1} in the ir spectrum indicated that oxidation of the sulfur had indeed occurred. Also in the ^{1}H nmr (DMSO-d$_6$) of **18**, all the protons, except benzoyl, C$_4$-*H* and C$_5$-*H*$_2$, were shifted downfield as compared to those of **16**. The SO_2CH_3 protons had a considerable shift of 0.98 ppm, whereas the anomeric proton of **18** shifted by 0.20 ppm. This downfield shift in **18** would be expected due to the sulfonyl group, and similar shifts have been observed with other methylthio substituted pyrazolo[3,4-*d*]pyrimidine nucleosides [7]. Treatment of **18** with liquid ammonia at 90° for 18 hours gave a 29%

yield of one of the desired guanosine analogs 6-amino-3-ethoxy-1-ß-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4-(5*H*)-one (**19**).

For the synthesis of 6-amino-3-methoxy-1-ß-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**23**), 3-methoxy-4,6-bis(methylthio)pyrazolo[3,4-*d*]pyrimidine (**20**) was found to be a convenient starting material (Scheme II). Treatment of **5a** with carbon disulfide in pyridine, followed by methylation of the reaction product with methyl iodide in alkaline conditions gave **20** in good yields. Analogous ring closure of 3-aminopyrazole-4-carbonitrile with carbon disulfide to obtain fused pyrimidinedithiones and subsequent rearrangement by 1*N* sodium hydroxide to yield pyrazolo[3,4-*d*]pyrimidinedithione has been documented in the literature [30-32]. High-temperature glycosylation of **20** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose furnished 3-methoxy-4,6-bis(methylthio)-1-(2,3,5-tri-*O*-benzoyl-ß-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**21**) as the sole nucleoside product. Deacetylation of **21** with sodium methoxide in methanol afforded 3-methoxy-4,6-bis(methylthio)-1-ß-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**22**). Oxidation of **21** with *m*-chloroperoxybenzoic acid and subsequent ammonolysis of the oxidized product **24** gave **23**. The uv absorption spectrum of **23** was similar to that of **19**, thus confirming the structural assignment of **23**.

Scheme II



In conclusion, use of the high-temperature glycosylation procedure for the synthesis of certain 3-alkoxy-1-ß-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidines proved to be rather successful.

Single Crystal X-Ray Diffraction Analysis of **12a**.

Slow crystallization of 3-methoxy-1-ß-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**12a**) from methanol gave X-ray quality crystals. A suitable single crystal of dimension 0.30 mm × 0.25 mm × 0.20 mm was mounted on

Table I

Positional (× 10^4) and thermal (× 10^3) parameters for compound **12a**. (Values in parenthesis are e.s.d.'s for the parameters)

Atom	x	y	z	Ueq
N7	4002(6)	−2090(2)	5497(2)	44(1)
C6	4321(8)	−2929(3)	5724(2)	47(1)
HC6	3245	−3379	5546	56 [a]
N5	6019(7)	−3230(2)	6188(2)	46(1)
HN5	6059	−3874	6302	53 [a]
C4	7726(8)	−2678(2)	6517(2)	42(1)
OC4	9165(7)	−3007(2)	6945(2)	62(1)
C4A	7425(7)	−1736(2)	6278(2)	35(1)
C3	8616(8)	−897(2)	6387(2)	38(1)
OC3	10470(5)	−806(2)	6836(2)	49(1)
C7	11377(8)	107(3)	6912(3)	49(1)
H1C7	12815	120	7180	59 [a]
H2C7	10280	478	7180	59 [a]
H3C7	11585	342	6402	59 [a]
N2	7675(6)	−219(2)	5996(2)	36(1)
N1	5787(5)	−609(2)	5638(2)	36(1)
C7A	5619(7)	−1518(2)	5787(2)	33(1)
C1'	4536(6)	−127(2)	5045(2)	31(1)
HC1'	2990	−350	5078	37 [a]
C2'	4516(7)	919(2)	5154(2)	32(1)
HC2'	4714	1135	5670	35 [a]
O2'	2363(4)	1249(2)	4883(2)	39(1)
HO2'	2090(82)	1825(3)	5071(25)	65(14) [a]
C3'	6440(7)	1213(2)	4613(2)	30(1)
HC3'	7929	1177	4846	34 [a]
O3'	6381(5)	2156(2)	4397(2)	40(1)
HO3'	5194	2229	4022	73(16)
C4'	6138(7)	551(2)	3944(2)	32(1)
HC4'	5015	802	3600	38 [a]
C5'	8164(7)	409(3)	3414(2)	40(1)
H1C5'	8462	976	3152	49 [a]
H2C5'	7761	−55	3046	49 [a]
O5'	10206(5)	133(2)	3789(2)	46(1)
HO5'	10771(84)	505(29)	4043(25)	70(14) [a]
O1'	5541(4)	−303(1)	4314(2)	38(1)
CM	6425(11)	2375(5)	6916(4)	91(3)
H1CM	5441	2439	6478	108 [a]
H2CM	6018	2824	7295	108 [a]
H3CM	6252	1772	7129	108 [a]
OCM	8650(8)	2469(3)	6775 (2)	91 (3)
HOCM	9222	2573	7256	191(35) [a]

Ueq is defined as one-third of the trace of the orthogonalised Uij tensor.
[a] Numbers are normal isotropic U values.

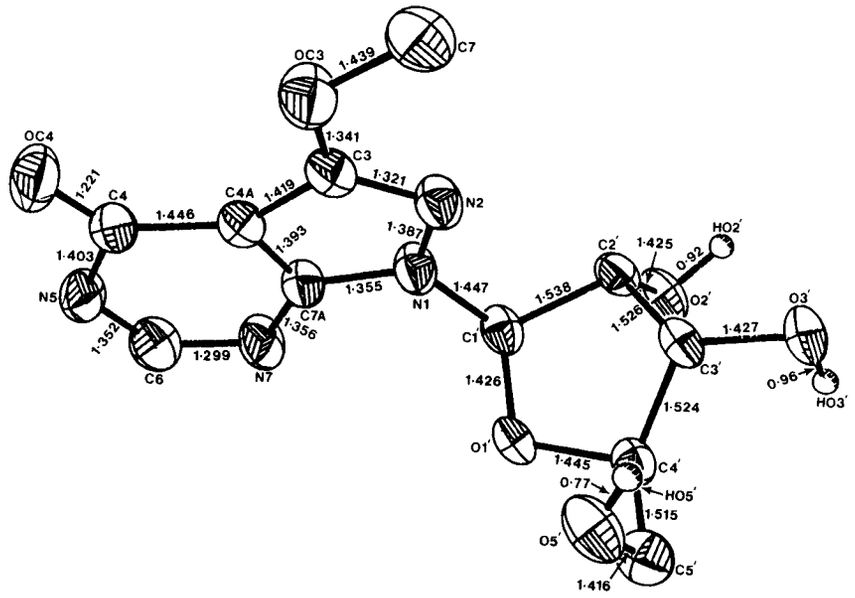


Figure I. Computer drawing of compound **12a**. Uncertainties for bonds involving non-hydrogen atoms range from 0.004 to 0.006 Å while uncertainties on O-H bonds are about 0.03 Å. Hydrogens bonded to carbon and nitrogen atoms and also the atoms of the methanol molecule are not included in the drawing.

a Nicolet P3 autodiffractometer, and the diffraction data were collected utilizing graphite monochromated Mo Kα radiation (λ = 0.71073 Å). The compound crystallized in the orthorhombic space group $P2_12_12_1$ with lattice parameters: a = 5.830(3) Å, b = 14.584(11) Å, c = 17.439(17) Å and α = ß = γ = 90°. The volume of the unit cell was 1483(2) Å^3 with Z = 4. Single crystal data was collected using a θ-2θ variable speed scan technique to a sin θ/λ limit of 0.70. A total of 2603 reflections were collected. The data was merged to 2498 unique reflections, of which 1729 were considered observed as F > 2.5(F). The structure was solved using direct methods. An early difference map revealed a molecule of methanol as solvent in the crystal structure. The structure was refined by a cascading blocked least-squares procedure to a final R value of 0.066 and R_w = 0.059. Weights were based on counting statistics. All non-hydrogen atoms were refined anisotropically. Positions for hydrogen atoms bonded to carbon and nitrogen atoms were calculated based on bond geometry. The methoxy group on C-3 and of the methanol molecule were refined as rigid bodies. The hydrogen atoms of C-2'-OH, C-3'-OH and C-5'-OH of the glycon and of the alcohol hydrogen of the methanol molecule were located in difference maps and were refined isotropically. All calculations and computer drawing of the structure were made using the program package SHELXTL [33]. Scattering factors of the atoms were obtained from the International Tables of X-ray Crystallography [34].

A computer drawing showing structural formula, conformation, atom labels and interatomic bond distances of compound **12a** is shown in Figure I. The asymmetric unit of the structure contains a molecule of methanol but was not included in the figure. The hydrogens of the aglycon moiety are also omitted for clarity. The positional and thermal parameters of the atoms of the nucleoside are listed in Table I. The result of this structure determination study confirmed that the nucleoside **12a** exists in the ß-anomeric configuration and the site of glycosyl attachment is N-1. The ribose moiety and the aglycon 3-methoxyallopurinol exists is the *anti* conformation. The torsion angles about the glycosidic bond have the following values: N2-N1-C1'-C2' is −31.9° and N2-N1-C1'-O1' is 87.9°. The aglycon portion of **12a** is planar with the largest deviation of any atom from the least-squares plane being 0.033 Å for N2.

The glycon moiety is in the C3' endo (^{3}E) configuration. All of the alcoholic hydrogen atoms of the carbohydrate, N5H of the heterocycle and the alcoholic hydrogen of the methanol molecule are involved in intermolecular hydrogen bonds and this data is summarized in Table II. The methanol oxygen is involved in two rather strong hydrogen bonds and forms the link between two symmetry related nucleoside molecules. There are no intramolecular hydrogen bonds.

Table II

Hydrogen bond data. Numbers in parenthesis are e.s.d. values

D.....H.....A	H.....A Å	O.....A Å	O-H.....A(°)	Translation of A
N5 HN5 O5′	1.910(4) [a]	2.816(4)	156.5(1) [a]	$-\frac{1}{2}+x, -\frac{1}{2}-y, 1-z$
O2′ HO3′ O3′	1.800(39)	2.703(3)	169(4)	$-\frac{1}{2}+x, -\frac{1}{2}-y, 1-z$
O3′ HO3′ OM	1.714(5) [a]	2.648(5)	164.0(2) [a]	$-\frac{1}{2}+x, \frac{1}{2}-y, 1-z$
O5′ HO5′ O2′	2.047(44)	2.805(4)	167(5)	$1+x, y, z$
OM HOM OC4	1.883(3) [a]	2.648(5) [a]	141.8(3)	$2-x, \frac{1}{2}+y, 1\frac{1}{2}-z$

[a] e.s.d values are unreasonably low because the positional parameters of the hydrogen were not refined. D, donor atom; H, hydrogen atom; A, acceptor atom.

EXPERIMENTAL

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 water as indicated by elemental analysis were verified by ^{1}H 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 Galbraith Laboratories, Inc., Knoxville, Tennessee, and Robertson Laboratory, Florham Park, New Jersey. Thin-layer chromatography (tlc) was run on silica gel 60 F-254 plates (EM Reagents). E. Merck silica gel (230-400 mesh) was used for flash column chromatography. All solvents used were reagent grade. Detection of nucleoside components on tlc was by uv light and with 10% sulfuric acid in methanol spray followed by heating. Evaporations were carried out under reduced pressure with the bath temperature below 30°.

5(3)-Amino-3(5)-methoxypyrazole-4-carbonitrile (**5a**).

To a rapidly stirred suspension of 1,1-dicyanoketene-2,2-dimethyl acetyl [25] (2.80 g, 20.3 mmoles) in water (50 ml) was added hydrazine hydrate (85%, 1.0 ml, 20.6 mmoles). A clear solution was obtained and an exothermic reaction was noticed. Upon cooling the reaction mixture the product crystallized. Recrystallization of the product from water gave analytically pure **5a** as colorless needles, 2.30 g (82%), mp 160-161°; ir (potassium bromide): ν 2200 (C≡N), 3300-3400 (NH_2) cm^{-1}; uv: λ max (*p*H 1) 221 nm (ϵ 7,100); λ max (*p*H 7) 221 nm (ϵ 7,600); λ max (*p*H 11) 223 nm (ϵ 8,300); ^{1}H nmr (DMSO-d_6): δ 3.72 (s, 3, OCH_3), 6.30 (s, 2, NH_2, exchanged with deuterium oxide), 10.98 (s, 1, N*H*).

Anal. Calcd. for $C_5H_6N_4O$: C, 43.48; H, 4.38; N, 40.56. Found: C, 43.35; H, 4.36; N, 40.30.

4-Amino-3-methoxypyrazolo[3,4-*d*]pyrimidine (**4**). Method A.

To a solution of **5a** (6.25 g, 45 mmoles) in triethyl orthoformate (100 ml) was added molecular sieves (4A, 20 g) and the mixture was heated under reflux for 4 hours. The reaction mixture was filtered and the filtrate evaporated to dryness. The residue was coevaporated with dry toluene (3 × 50 ml) and triturated with anhydrous ethyl ether. The resulting white solid was collected by filtration, placed in a steel bomb with methanolic ammonia (saturated at 0°, 100 ml) and heated at 90° for 18 hours. After cooling, the reaction mixture was evaporated to dryness and the residue was crystallized from aqueous ethanol to yield 4.25 g (57%) of **4**, mp 258°; ir (potassium bromide): ν 3190-3430 (NH, NH_2) cm^{-1}; uv: λ max (*p*H 1) 228 nm (ϵ 22,000); λ max (*p*H 7), 244 nm (ϵ 6,300), 276 (4,000); λ max (*p*H 11) 276 nm (ϵ 3,800); ^{1}H nmr (DMSO-d_6): δ 3.90 (s, 3, OCH_3), 7.0 (br s, 2, NH_2, exchanged with deuterium oxide), 8.04 (s, 1, C_6H), 12.37 (br s, 1, ring N*H*).

Anal. Calcd. for $C_6H_7N_5O$: C, 43.64; H, 4.27; N, 42.40. Found: C, 43.48; H, 4.35; N, 42.48.

Method B.

Compound **5a** (6.24 g, 45 mmoles) and formamidine acetate (6.25 g, 60 mmoles) were fused at 130° for 15 minutes. After cooling to room temperature, the hardened solid was dissolved in hot aqueous methanol, treated with decolorizing carbon (Norit A) and filtered. Upon cooling, the crystalline product that separated was collected by filtration and dried to yield 5.30 g (74%) of **4**. This product was identical in all respects to **4** prepared by Method A.

4-Amino-3-methoxy-1-(2,3,5-tri-*O*-benzoyl-ß-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**8**).

A mixture of **4** (1.27 g, 7.7 mmoles) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (5.80 g, 11.5 mmoles) in dry nitromethane (150 ml) was brought to reflux temperature whereupon freshly distilled boron trifluoride etherate (1.46 ml, 11.6 mmoles) was added through the condenser by syringe. The reaction was monitored by tlc (chloroform:acetone, 8:2, v/v). After 40 minutes an additional charge of the sugar (1.50 g, 2.9 mmoles) and the catalyst (0.5 ml, 3.9 mmoles) was added to the refluxing reaction mixture. After 90 minutes of total reaction time, the solution was cooled and evaporated to dryness. The residue was dissolved in ethyl acetate (200 ml) and washed with saturated aqueous sodium bicarbonate solution (2 × 100 ml), followed by water (2 × 100 ml). After drying over anhydrous sodium sulfate, the solvent was evaporated to dryness to yield an amber colored foam, which was purified on a flash silica gel column using chloroform:methanol (40:1, v/v) as the eluent. A second chromatography over flash silica gel column using hexane:ethyl acetate (1:1, v/v) gave analytically pure **8** as colorless foam, 3.60 g (77%); mp 96-101°; ir (potassium bromide): ν 1720 (C=O), 3260-3480 (NH_2) cm^{-1}; uv: λ max (*p*H 1) 224 nm (ϵ 50,000), 260 (6,700); λ max (*p*H 7) 230 nm (ϵ 31,000), 269 (17,000); λ max (*p*H 11) 225 nm (ϵ 40,800), 268 (15,800); ^{1}H nmr (DMSO-d_6): δ 3.84 (s, 3, OCH_3), 6.58 (d, 1, $C_1{}'H$, J = 3.7 Hz), 7.43-7.94 (m, 17, NH_2 and benzoyl aromatics), 8.15 (s, 1, C_6H), and other sugar protons.

Anal. Calcd. for $C_{32}H_{27}N_5O_8$: C, 63.05; H, 4.46; N, 11.49. Found: C, 63.09; H, 4.53; N, 11.44.

4-Amino-3-methoxy-1-ß-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**9**).

A solution of compound **8** (2.60 g, 4.27 mmoles) in dry methanol (100 ml) was adjusted to *p*H 9 with 1*N* sodium methoxide in methanol. After stirring overnight at room temperature, the reaction mixture was neutralized with Dowex-50 (H^+) resin. The resin was removed by filtration and the filtrate was evaporated down to a volume of 20 ml whereupon the product crystallized. After cooling, the crystalline product was collected and recystallized from aqueous methanol to obtain 1.19 g (94%) of the title compound as colorless prisms, mp 149-150° (sinters), 185-187°; ir (potasium bromide): ν 3200-3450 (NH_2) cm^{-1}; uv: λ max (*p*H 1) 230 nm (ϵ 19,900); λ max (*p*H 7 and 11) 248 nm (ϵ 4,300), 278 (3,600); ^{1}H nmr (DMSO-d_6): δ 3.90 (s, 3, OCH_3), 5.98 (d, 1, $C_1{}'H$, J = 4.4 Hz), 6.90-7.50 (br s, 2, NH_2), 8.13 (s, 1, C_6H), and other sugar protons.

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

5(3)-Amino-3(5)-methoxypyrazole-4-carboxamide (**6a**).

To a cooled (10°), concentrated sulfuric acid (15 ml) was added finely powdered **5a** (1.50 g, 10.8 mmoles) with stirring so that the temperature did not rise above 25°. The solution was stirred at room temperature for 4 hours and then poured with stirring into a mixture of water (50 ml) and ice (50 g). The aqueous solution was neutralized with 50% aqueous sodium hydroxide solution, keeping the temperature below 20°. The precipitated sodium sulfate was removed by filtration, the filtrate adjusted to *p*H 7 and evaporated to dryness. The residue was purified by continuous extraction with ethyl acetate and crystallized from water to yield 1.50 g (88%) of **6a**, mp 242-244°; ir (potassium bromide): ν 1650 (C=O), 3300-3450 (NH_2) cm^{-1}; uv: λ max (*p*H 1) 226 nm (ε 7,500); λ max (*p*H 7) 230 nm (ε 6,300); λ max (*p*H 11) 232 nm (ε 7,500); ^1H nmr (DMSO-d_6): δ 3.95 (s, 3, OCH_3), 6.13 (s, 2, NH_2, exchanged with deuterium oxide), 10.87 (s, 1, ring N*H*).

Anal. Calcd. for $C_5H_8N_4O_2$: C, 38.46; H, 5.16; N, 35.88. Found: C, 38.50; H, 5.20; N, 35.59.

5(3)-Amino-3(5)-ethoxypyrazole-4-carboxamide (**6b**).

5(3)-Amino-3(5)-ethoxypyrazole-4-carbonitrile [25] (**5b**, 3.0 g, 19.7 mmoles) was slowly added to concentrated sulfuric acid (15 ml). The temperature of the reaction mixture was kept below 25° by cooling with an ice bath. After stirring for one hour at room temperature the reaction mixture was poured over ice (50 g) and neutralized with aqueous sodium hydroxide solution (50%). After extraction with ethyl acetate (9 × 75 ml), the combined organic layer was dried (sodium sulfate) and evaporated to yield 1.90 g of **6b**. The remaining aqueous solution was treated with ethyl acetate in a liquid-liquid extractor yielding an additional 0.90 g of the product. Crystallization of the combined solids from water gave 2.64 g (79%) of the title compound; mp 197-198°; ir (potassium bromide): ν 1650 (C=O), 3300-3430 (NH_2) cm^{-1}; uv: λ max (*p*H 1) 225 nm (ε 9,400); λ max (*p*H 7) 231 nm (ε 8,800); λ max (*p*H 11) 233 nm (ε 8,700); ^1H nmr (DMSO-d_6): δ 1.23-1.39 (t, 3, OCH_2CH_3), 4.05-4.28 (m, 2, OCH_2CH_3), 5.96 (s, 2, NH_2), 6.72 and 6.11 (2 br s, 2, $CONH_2$), 10.83 (s, 1, ring N*H*).

Anal. Calcd. for $C_6H_{10}N_4O_2$: C, 42.35; H, 5.92; N, 32.92. Found: C, 42.13; H, 5.86; N, 32.68.

3-Methoxypyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**7a**). Method A.

Compound **6a** (0.25 , 1.6 mmoles) was heated in formamide (15 ml) with stirring at 180° for 20 minutes. The solution was allowed to cool to room temperature whereupon water (10 ml) and trifluoroacetic acid (1 ml) were added and the mixture boiled until about 5 ml of solvent remained. Upon cooling a precipitate formed, which was collected by filtration and dissolved in hot water containing 5% dimethyl formamide. On cooling the title compound crystallized. Recrystallization of the product from aqueous methanol gave **7a** as colorless needles, 0.10 g (38%); mp 263-264°; ir (potassium bromide): ν 1700 and 1740 (C=O), 1070 (C-O-C) cm^{-1}; uv: λ max (*p*H 1 and 7) 218 nm (ε 22,400), 243 sh (5,100); λ max (*p*H 11) 223 nm (ε 14,100), 265 (4,000); ^1H nmr (DMSO-d_6): δ 3.90 (s, 3, OCH_3), 7.92 (s, 1, C_6H), 11.85 and 12.73 (2, br s, 2, N_1H and N_5H).

Anal. Calcd. for $C_6H_6N_4O_2$: C, 43.38; H, 3.64; N, 33.72. Found: C, 43.20; H, 3.65; N, 33.43.

Method B.

A mixture of **6a** (2.75 g, 17.6 mmoles), formamidine acetate (5.0 g, 48 mmoles) and formamide (8 ml) was fused to a clear melt by heating at 150° for 10 minutes. Upon cooling the product that separated was collected and worked up with DMF/water as in Method A to yield 1.84 g (63%) of product, which was identical in all respects to **7a** prepared by Method A.

3-Ethoxypyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**7b**).

Compound **6b** (2.30 g, 13.5 mmoles) and formamide (23 ml) were heated in an open beaker at 185° for 30 minutes. The solution was allowed to cool to 50°, 4*N* acetic acid (100 ml) was added and the mixture was reheated to boil the solvent down to a volume of 25 ml. Crystallization occurred upon cooling. The product was collected by filtration, washed with cold water (2 × 10 ml) and recrystallized from water containing a few drops of dimethyl formamide to yield 1.10 g (76%) of the title compound; mp 274-275°; ir (potassium bromide): ν 1650 (C=O) 1070 (C-O-C) cm^{-1}; uv: λ max (*p*H 1 and 7) 217 nm (ε 14,300), 260 sh (2,500); λ max (*p*H 11) 223 nm (ε 9,500), 268 (2,800); ^1H nmr (DMSO-d_6): δ 1.25-1.40 (t, 3, OCH_2CH_3), 4.14-4.38 (m, 2, OCH_2CH_3), 7.92 (s, 1, C_6H), 11.85 and 12.87 (2 br s, 2, N_1H and N_5H).

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

3-Methoxy-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**11a**).

A mixture of **7a** (3.95 g, 23.8 mmoles) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (18.0 g, 37.7 mmoles) in anhydrous nitromethane (200 ml) was brought to reflux temperature, whereupon freshly distilled boron trifluoride etherate (4.5 ml, 35.6 mmoles) was added through the condenser by syringe. The reaction mixture was stirred at reflux temperature for 20 minutes and then cooled to room temperature. Following evaporation of the solvent, the brown glassy residue was purified by flash chromatography over silica gel using dichloromethane:acetone (10:1, v/v) as an eluent. Appropriate homogeneous fractions were pooled and evaporated to give an amber colored syrup. The residual syrup was dissolved in methanol (100 ml), sonicated and allowed to stand in the refrigerator overnight. The crystalline product that deposited was collected by filtration and recrystallized from methanol to yield 7.33 g (51%) of the title compound; mp 230-231°; ir (potassium bromide): ν 1690 (C=O), 1720 (C=O of esters) cm^{-1}; uv: λ max (*p*H 1 and 7) 226 nm (ε 48,000), 260 sh (23,800); λ max (*p*H 11) 265 nm (ε 17,000); ^1H nmr (DMSO-d_6): δ 3.81 (s, 3, OCH_3), 6.56 (d, 1, C_1H, J = 2.7 Hz), 7.37-8.10 (m, 15, benzoyl aromatics), 12.24 (br s, 1, ring N*H*).

Anal. Calcd. for $C_{32}H_{26}N_4O_9$: C, 62.95; H, 4.29; N, 9.18. Found: C, 62.86; H, 4.33; N, 9.08.

3-Methoxy-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**12a**).

Method A.

To a solution of **11a** (2.80 g, 4.6 mmoles) in absolute methanol (100 ml) was added 2*N* sodium methoxide in methanol until a *p*H of 9 was reached. The solution was stirred overnight at room temperature, followed by neutralization with Dowex-50 (H^+) resin. The resin was removed by filtration and the filtrate evaporated to dryness. The residue was triturated with dry ethyl ether and the solid that separated was collected by filtration. Crystallization of the solid from methanol gave colorless needles, 1.27 g (93%), mp 204-205°; ir (potassium bromide): ν 1690 (C=O) cm^{-1}; uv: λ max (*p*H 1) 219 nm (ε 24,500), 260 sh (3,500); λ max (*p*H 7) 219 nm (ε 21,000), 260 sh (3,300); λ max (*p*H 11), 273 nm (ε 4,700); ^1H nmr (DMSO-d_6): δ 3.90 (s, 3, OCH_3), 5.96 (d, 1, C_1H, J = 4.1 Hz), 8.04 (s, 1, C_6H), 12.12 (br s, 1, N_5H), and other sugar protons.

Anal. Calcd. for $C_{11}H_{14}N_4O_6 \cdot CH_3OH$: C, 43.64; H, 5.49; N, 16.96. Found: C, 43.58; H, 5.32; N, 17.07.

Method B.

To a solution of **9** (0.59 g, 2 mmoles) in glacial acetic acid (10 ml) was added a saturated aqueous solution of sodium nitrite (2.0 ml) dropwise, and the mixture was heated at 80-85° for several days. The solvent was evaporated to dryness and the residue containing multiple of compounds was purified on a silica gel preparative tlc plate using chloroform:methanol (6:1, v/v) as the developer solvent. The appropriate band was scraped off and eluted with the above solvent system. Evaporation of the solvent and crystallization of the residue from methanol gave 0.11 g (19%) of the title compound; mp 204-205°. This material was found to be identical with the one prepared by Method A.

3-Ethoxy-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**11b**) and 3-Ethoxy-2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**13b**).

Method A.

In the same manner as for **11a**, direct glycosylation of **7b** (2.75 g, 15.3

mmoles) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (12.20 g, 24.2 mmoles) in anhydrous nitromethane (150 ml) in the presence of boron trifluoride etherate (2.9 ml, 23 mmoles) at reflux temperature for 3 hours gave a mixture of **11b** and **13b**. Chromatography of the mixture on a flash silica gel column using dichloromethane:acetone (10:1, v/v) gave pure isomers. The N-1 isomer **11b** eluted first from the column. The product was crystallized from ethyl acetate containing a few drops of methanol to yield 3.60 g (38%) of **11b**, mp 184-186°; ir (potassium bromide): ν 1690 (C=O), 1720 (C=O of esters) cm^{-1}; uv: λ max (*p*H 1 and 7) 230 nm (ϵ 22,200), 273 sh (7,500); λ max (*p*H 11) 230 nm (ϵ 24,700), 273 sh (6,200); ^1H nmr (DMSO-d_6): δ 1.14-1.26 (m, 3, OCH_2CH_3), 4.10-4.30 (m, 2, OCH_2CH_3), 6.40 (d, 1, $C_1'H$, J = 2.5 Hz), 7.38-8.04 (m, 16, benzoyl aromatics and C_6H), and other sugar protons.

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

Subsequent fractions eluted from the column contained pure N-2 isomer **13b**. Evaporation of the homogeneous fractions gave 1.90 g (20%) of amber colored syrup. Without characterization the syrup was used for debenzoylation reaction.

Method B.

Compound **16** (1.60 g, 2.4 mmoles) was combined with Raney nickel (W-4, 7.0 g) in absolute ethanol (75 ml) and the mixture refluxed for 2 hours at which time tlc (silica gel, benzene:ethyl acetate, 4:1, as the solvent) indicated the reaction to be complete. The cooled reaction mixture was filtered through a Celite pad, washed with hot ethanol, and the combined filtrates evaporated to dryness. Crystallization of the residue from ethyl acetate gave 1.02 g (68%) of **11b**, which was found to be identical with the one prepared by Method A.

3-Ethoxy-1-ß-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**12b**).

To a solution of **11b** (3.20 g, 5.1 mmoles) in anhydrous methanol (100 ml) was added 1*N* sodium methoxide in methanol until a *p*H of 9 was reached. After stirring overnight at ambient temperature under anhydrous conditions the reaction mixture was neutralized with Dowex-50 (H^+) resin, filtered and the filtrate evaporated to dryness. Trituration of the residue with anhydrous ethyl ether gave a white precipitate, which was crystallized from aqueous ethanol to yield 1.40 g (85%) of **12b** as colorless needles; mp 158-159°; ir (potassium bromide): ν 1670 and 1710 (C=O) cm^{-1}; uv: λ max (*p*H 1 and 7) 219 nm (ϵ 21,200), 260 (3,700); λ max (*p*H 11) 274 nm (ϵ 4,800); ^1H nmr (DMSO-d_6): δ 1.26-1.40 (t, 3, OCH_2CH_3), 4.15-4.38 (m, 2, OCH_2CH_3), 5.94 (d, 1, $C_1'H$, J = 5.4 Hz), 8.0 (s, 1, C_6H), and other sugar protons.

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

3-Ethoxy-2-ß-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**14b**).

Compound **13b** (3.80 g, 6.1 mmoles) was treated in a like manner as for **11b** with 1*N* sodium methoxide in methanol to yield 1.70 g (90%) of the title compound as colorless needles (water); mp 189-191°; ir (potassium bromide): ν 1690 (C=O) cm^{-1}; uv: λ max (*p*H 1 and 7) 219 nm (ϵ 18,000), 247 sh (6,500); λ max (*p*H 11) 227 nm (ϵ 11,500), 250 (6,100), 287 (5,900); ^1H nmr (DMSO-d_6): δ 1.24-1.40 (t, 3, OCH_2CH_3), 4.14-4.35 (m, 2, OCH_2CH_3), 5.74 (d, 1, $C_1'H$, J = 4.5 Hz), 7.83 (s, 1, C_6H), and other sugar protons.

Anal. Calcd. for $C_{12}H_{16}N_4O_6 \cdot \frac{1}{2}H_2O$: C, 44.86; H, 5.33; N, 17.44. Found: C, 45.12; H, 5.30; N, 17.65.

3-Ethoxy-6-thioxopyrazolo[3,4-*d*]pyrimidin-4(5*H*,7*H*)-one (**10**).

A mixture of **6b** (3.50 g, 20.6 mmoles) and potassium ethyl xanthate (11.53 g, 72 mmoles) in dimethyl formamide (75 ml) was heated under reflux for 3 hours. The reaction mixture was cooled (5-10°) for one hour in an ice bath whereupon the solid which separated was collected by filtration and washed with cold methanol (2 × 25 ml). The solid was dissovled in 2*N* sodium hydroxide solution, heated to 70° and acidified (*p*H 4) with glacial acetic acid. The flocculent colorless precipitate was collected, washed with water (2 × 25 ml) and dried to yield 2.83 g (65%) of **10**, mp >315°; ir (potassium bromide): ν 1180 (C=S), 1600 and 1680 (C=O) cm^{-1}; uv: λ max (*p*H 1) 218 nm (ϵ 9,700), 283 (15,600); λ max (*p*H 7) 243 nm (ϵ 8,500), 285 (13,800); λ max (*p*H 11) 246 nm (ϵ 9,300), 285 (12,700); ^1H nmr (DMSO-d_6): δ 1.26-1.44 (t, 3, OCH_2CH_3), 4.20-4.50 (m, 2, OCH_2CH_3), 11.85 (s, 1, N_1H), 12.91 (br s, 2, N_5H and N_7H).

Anal. Calcd. for $C_7H_8N_4O_2S \cdot \frac{1}{2}H_2O$: C, 38.00; H, 4.10; N, 25.32; S, 14.49. Found: C, 38.01; H, 3.86; N, 25.03; S, 14.35.

3-Ethoxy-6-(methylthio)pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**15**).

To a solution of **10** (2.50 g, 11.8 mmoles) in 1*N* sodium hydroxide (150 ml) was added methyl iodide (0.73 ml, 11.8 mmoles) with efficient stirring. After stirring for 18 hours at room temperature, the reaction mixture was filtered and the filtrate was acidified (*p*H 4) with glacial acetic acid. The colorless precipitate that separated was collected by filtration, air dried and crystallized from aqueous ethanol to yield 1.90 g (74%) of **15**, mp 248-250°; ir (potassium bromide): ν 1620 (C=O), 2860-3200 (NH) cm^{-1}; uv: λ max (*p*H 1) 233 nm (ϵ 13,900), 267 (9,000); λ max (*p*H 7) 233 nm (ϵ 16,300), 260 (10,000); λ max (*p*H 11) 236 nm (ϵ 19,000), 259 (11,300); ^1H nmr (DMSO-d_6): δ 1.17-1.33 (t, 3, OCH_2CH_3), 4.0-4.30 (m, 2, OCH_2CH_3), 2.49 (s, 3, SCH_3), 12.15 (br s, 1, N_1H), 12.55 (br s, 1, N_5H).

Anal. Calcd. for $C_8H_{10}N_4O_2S$: C, 42.47; H, 4.45; N, 24.76; S, 14.17. Found: C, 42.51; H, 4.46; N, 24.75; S, 13.91.

3-Ethoxy-6-(methylthio)-1-(2,3,5-tri-*O*-benzoyl-ß-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**16**).

Compound **15** (2.20 g, 9.7 mmoles) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (7.40 g, 14.7 mmoles) were reacted in the presence of boron trifluoride etherate (1.86 ml, 14.7 mmoles) in dry nitromethane (100 ml) in the same manner as described for the preparation of **11b**. The product was purified on a flash silica gel column using hexane:ethyl acetate (1:1, v/v) as the eluent, and crystallized from ethyl acetate:ether mixture to yield 3.90 g (61%) of **16**, mp 213-214°; ir (potassium bromide): ν 1730 (C=O) cm^{-1}; uv: λ max (*p*H 1) 238 nm (ϵ 32,500), 280 (16,400); λ max (*p*H 7) 241 nm (ϵ 26,800), 280 (15,100); λ max (*p*H 11) 232 nm (ϵ 25,100), 263 (9,400); ^1H nmr (DMSO-d_6): δ 1.30-1.50 (t, 3, OCH_2CH_3), 2.50 (s, 3, SCH_3), 4.10-4.30 (m, 2, OCH_2CH_3), 6.40 (d, 1, $C_1'H$, J = 2.3 Hz), 7.30-7.80 (m, 15, benzoyl aromatics), 12.30 (2 br s, 1, N_5H), and other sugar protons.

Anal. Calcd. for $C_{34}H_{30}N_4O_9S$: C, 60.89; H, 4.51; N, 8.35; S, 4.78. Found: C, 60.83; H, 4.45; N, 8.41; S, 5.07.

3-Ethoxy-6-(methylthio)-1-ß-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**17**).

In the same manner as for **12b**, the title compound was prepared using **16** (0.90 g, 1.3 mmoles) and 1*N* sodium methoxide in methanol (50 ml). The product was crystallized from water to yield 0.37 g (77%) of **17**, mp 214-215°; ir (potassium bromide): ν 1070 (C-O-C), 1670 (C=O), 3360 (OH) cm^{-1}; uv: λ max (*p*H 1) 234 nm (ϵ 14,800), 275 (8,400); λ max (*p*H 7) 234 nm (ϵ 15,700), 274 (8,900); λ max (*p*H 11) 238 nm (ϵ 15,200), 261 (9,300); ^1H nmr (DMSO-d_6): δ 1.26-1.44 (t, 3, OCH_2CH_3), 2.45 (s, 3, SCH_3), 4.08-4.26 (m, 2, OCH_2CH_3), 5.98 (d, 1, $C_1'H$, J = 4.6 Hz), 12.38 (s, 1, N_5H), and other sugar protons.

Anal. Calcd. for $C_{13}H_{16}N_4O_6S$: C, 43.57; H, 5.06; N, 15.63; S, 8.95. Found: C, 43.40; H, 5.00; N, 15.61; S, 8.82.

3-Ethoxy-6-(methylsulfonyl)-1-(2,3,5-tri-*O*-benzoyl-ß-D-ribofuranosyl)pyrazolo(3,4-*d*)pyrimidin-4(5*H*)-one (**18**).

A solution of **16** (0.60 g, 0.9 mmoles) and *m*-chloroperoxybenzoic acid (0.46 g, 2.7 mmoles) in anhydrous dichloromethane (75 ml) was stirred at room temperature for 48 hours at which time the solvent was evaporated to dryness. The residue was dissolved with heating in methanol (25 ml) and the insoluble material was removed by filtration. On cooling the filtrate overnight, the title compound crystallized as long needles, 0.40 g (64%), mp 136-138°; ir (potassium bromide): ν 1100 and 1260 (SO_2CH_3), 1720 (C=O) cm^{-1}; uv: λ max (*p*H 1) 222 nm (ϵ 49,000), 270 (19,600); λ max (*p*H 7) 220 nm (ϵ 42,100), 270 (9,100); λ max (*p*H 11) 216 nm (ϵ 49,100), 270 (10,500); ^1H nmr (DMSO-d_6): δ 1.25-1.45 (t, 3, OCH_2CH_3), 3.48 (s, 3,

SO_2CH_3), 4.10-4.28 (m, 2, OCH_2CH_3), 6.60 (d, 1, $C_1'H$, J = 4.5 Hz), 7.44-8.05 (m, 15, benzoyl aromatics), and other sugar protons.

Anal. Calcd. for $C_{34}H_{30}N_4O_{11}S$: C, 58.12; H, 4.30; N, 7.97; S, 4.56. Found: C, 57.99; H, 4.39; N, 7.92; S, 4.77.

6-Amino-3-ethoxy-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**19**).

A mixture of **18** (0.56 g, 0.8 mmole) and liquid ammonia (25 ml) was heated in a sealed steel reaction vessel at 90° for 18 hours. After cooling, the ammonia was allowed to evaporate. The residue was triturated with boiling benzene (3 × 25 ml) and the benzene insoluble solid was dissolved in hot methanol (20 ml). Further purification by flash silica gel column using chloroform:methanol (8:2, v/v), and crystallization from methanol gave 75 mg (29%) of the title compound; mp 205-207°; ir (potassium bromide): ν 1700 (C=O), 3200-3450 (OH, NH_2) cm^{-1}; uv: λ max (*p*H 1) 252 nm (ε 15,700); λ max (*p*H 7) 254 nm (ε 25,800); λ max (*p*H 11) 254 nm (ε 16,300); ^1H nmr (DMSO-d_6): δ 1.23-1.38 (t, 3, OCH_2CH_3), 4.05-4.25 (m, 2, OCH_2CH_3), 5.80 (d, 1, $C_1'H$, J = 4.5 Hz), 6.65 (br s, 2, NH_2, exchanged with deuterium oxide), 10.15 (br s, 1, N_5H), and other sugar protons.

Anal. Calcd. for $C_{12}H_{17}N_5O_6 \cdot ½\ CH_3OH$: C, 43.73; H, 5.57; N, 20.40. Found: C, 44.07; H, 5.42; N, 20.40.

3-Methoxy-4,6-bis(methylthio)pyrazolo[3,4-*d*]pyrimidine (**20**).

A solution of **5a** in pyridine:carbon disulfide (1:1, 100 ml) was heated under reflux for 5 hours. After cooling to room temperature, the solid that deposited was collected by filtration and washed with methanol (2 × 25 ml). A solution of the solid in 1*N* sodium hydroxide was heated at 80° and reprecipitated by adding glacial acetic acid (to *p*H 4.5). The resulting solid (10.0 g) was collected, suspended in water (500 ml) and dissolved by adding 1*N* sodium hydroxide solution (60 ml). Methyl iodide (5.87 ml, 94.3 mmoles) was added dropwise to the solution and stirred rapidly at room temperature for 3 hours. After cooling in an ice bath, the solid that separated was collected by filtration and crystallized from methanol/-*N*,*N*-dimethylformamide to yield 9.80 g (86%) of **20** as light yellow needles, mp 232-233°; ir (potassium bromide): ν 1220 (C-SCH_3) cm^{-1}; uv: λ max (*p*H 1) 255 nm (ε 11,200), 295 (8,000); λ max (*p*H 7) 255 nm (ε 8,400), 294 (5,800); λ max (*p*H 11) 255 nm (ε 15,700), 299 (7,900); ^1H nmr (DMSO-d_6): δ 2.52 and 2.56 (2s, 6, 2 SCH_3), 3.95 (s, 3, OCH_3), 12.75 (br s, 1 ring N*H*).

Anal. Calcd. for $C_8H_{10}N_4OS_2$: C, 39.65; H, 4.16; N, 23.12; S, 26.46. Found: C, 39.55; H, 4.29; N, 23.12; S, 26.18.

3-Methoxy-4,6-bis(methylthio)-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**21**).

Compound **20** (1.0 g, 4.1 mmoles) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (3.10 g, 6.1 mmoles) were reacted in the presence of boron trifluoride etherate (0.78 ml, 6.2 mmoles) in dry nitromethane (60 ml) in the same manner as described for the preparation of **11b**. The product was purified on a flash silica gel column using toluene:ethyl acetate (25:1, v/v) as the eluent and crystallized from ethyl acetate:ether mixture to yield 1.27 g (45%) of **21**, mp 87-90°; ir (potassium bromide): ν 1225 (C-SCH_3), 1725 (C=O) cm^{-1}; uv: λ max (methanol) 230 nm (ε 64,900), 258 (43,900), 295 (15,100); ^1H nmr (DMSO-d_6): δ 2.48 and 2.49 (2s, 6, 2SCH_3), 3.86 (s, 3, OCH_3), 6.60 (d, 1, $C_1'H$, J = 2.5 Hz), 7.50-7.87 (m, 15, benzoyl aromatics), and other sugar protons.

Anal. Calcd. for $C_{34}H_{30}N_4O_8S_2$: C, 59.46; H, 4.40; N, 8.16; S, 9.34. Found: C, 59.60; H, 4.40; N, 7.97; S, 9.30.

3-Methoxy-4,6-bis(methylthio)-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**22**).

In the same manner as for **12a**, the title compound was prepared using **21** (1.51 g, 2.2 mmoles) and 1*N* sodium methoxide in methanol (50 ml). The product was crystallized from water to yield 0.73 g (89%) of the title compound, mp 164-166°; ir (potassium bromide): ν 1230 (C-SCH_3), 3350 (OH) cm^{-1}; uv: λ max (*p*H 1), 258 nm (ε 38,300), 297 (15,500); λ max (*p*H 7) 258 nm (ε 40,300), 296 (16,000); λ max (*p*H 11) 257 nm (ε 40,600) 296 (16,200); ^1H nmr (DMSO-d_6): δ 2.57 and 2.59 (2s, 6, 2SCH_3), 3.97 (s, 3, OCH_3), 6.02 (d, 1, $C_1'H$, J = 2.5 Hz), and other sugar protons.

Anal. Calcd. for $C_{13}H_{16}N_4O_5S_2$: C, 41.70; H, 4.85; N, 14.96; S, 17.12. Found: C, 41.40; H, 4.62; N, 14.89; S, 17.03.

6-Amino-3-methoxy-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**23**).

A solution of **21** (2.42 g, 3.5 mmoles) and *m*-chloroperoxybenzoic acid (2.44 g, 14.3 mmoles) in anhydrous dichloromethane (100 ml) was stirred at room temperature for 18 hours. The reaction mixture was filtered to remove the precipitated *m*-chlorobenzoic acid and the filtrate evaporated to dryness. The residue was found to be a mixture of two components, mainly the methylsulfone (**24**) and minor amounts of the methylsulfoxide. This mixture (0.78 g) and liquid ammonia (40 ml) was heated in a steel reaction vessel at 90° for 18 hours. After cooling, the ammonia was allowed to evaporate. The residue was triturated with boiling toluene (2 × 30 ml) and the toluene insoluble solid was dissolved in hot methanol (15 ml). Further purification by flash silica gel column using chloroform:-methanol (6:1, v/v) as the eluent and crystallization from methanol gave 0.15 g (14% overall yield) of the title compound as colorless needles, mp 241-242°; ir (potassium bromide): ν 1690 (C=O), 3170-3330 (OH, NH_2) cm^{-1}; uv: λ max (*p*H 1) 253 nm (ε 7,700); λ max (*p*H 7) 256 nm (ε 8,500); λ max (*p*H 11) 255 nm (ε 9,000); ^1H nmr (DMSO-d_6): δ 3.82 (s, 3, OCH_3), 5.79 (d, 1, $C_1'H$, J = 2.0 Hz), 6.69 (br s, 2, NH_2, exchanged with deuterium oxide), 10.43 (br s, 1, N_5H), and other sugar protons.

Anal. Calcd. for $C_{11}H_{15}N_5O_6 \cdot ¾H_2O$: C, 40.43; H, 5.09; N, 21.43. Found: C, 40.71; H, 5.17; N, 21.08.

REFERENCES AND NOTES

† Current address: Nucleic Acid Research Institute, 3300 Hyland Avenue, Costa Mesa, CA 92626.

* To whom correspondence should be addressed.

[1] This work is taken, in part, from the Ph.D. Dissertation of J. D. A., Brigham Young University, 1985.

[2] This investigation was supported in part by U. S. Army Medical Research and Development Command Contract DAMD 17-82-C-2224 to Brigham Young University. This is contribution Number 1782 to the U. S. Drug Development Program on Antiparasitic Drugs.

[3] H. B. Cottam, G. R. Revankar and R. K. Robins, *Nucleic Acids Res.*, **11**, 871 (1983).

[4] B. G. Ugarkar, H. B. Cottam, P. A. McKernan, R. K. Robins and G. R. Revankar, *J. Med. Chem.*, **27**, 1026 (1984).

[5] 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 (1984).

[6] Z. Kazimierczuk, H. B. Cottam, G. R. Revankar and R. K. Robins, *J. Am. Chem. Soc.*, **106**, 6379 (1984).

[7] C. R. Petrie, H. B. Cottam, P. A. McKernan, R. K. Robins and G. R. Revankar, *J. Med. Chem.*, **28**, 1010 (1985).

[8] D. J. Nelson, S. W. LaFon, J. V. Tuttle, W. H. Miller, R. L. Miller, T. A. Krenitsky, G. B. Elion, R. L. Berens and J. J. Marr, *J. Biol. Chem.*, **254**, 11544 (1979).

[9] J. D. Berman, L. S. Lee, R. K. Robins and G. R. Revankar, *Antimicrob. Agents Chemother.*, **24**, 233 (1983).

[10] J. J. Marr, R. L. Berens, N. K. Cohn, D. J. Nelson and R. S. Klein, *Antimicrob. Agents Chemother.*, **25**, 292 (1984).

[11] W. Trager, *Ciba Symp.*, **20**, 225 (1974).

[12] J. J. Marr, D. J. Nelson and R. L. Berens, *Biochim. Biophys. Acta*, **544**, 360 (1978).

[13] W. E. Gutteridge and N. Gaborek, *Int. J. Biochem.*, **10**, 415 (1979).

[14] C. R. Ceron, R. A. Caldas, C. F. Feliz, M. H. Mundim and I. Roitman, *J. Protozool.*, **26**, 479 (1979).

[15] R. L. Berens, J. J. Marr, S. W. LaFon and D. J. Nelson, *J. Mol. Biochem. Parasitol.*, **3**, 187 (1981).

[16] D. J. Nelson, C. J. L. Bugge, G. B. Elion, R. L. Berens and J. J. Marr, *J. Biol. Chem.*, **254**, 3959 (1979).

[17] J. J. Marr, R. L. Berens and D. J. Nelson, *Science*, **201**, 1018

(1978).

[18] J. J. Marr, R. L. Berens, D. J. Nelson, T. A. Krenitsky, T. Spector, S. W. LaFon and G. B. Elion, *Biochem. Pharmacol.*, **31**, 143 (1982).

[19] J. L. Avila, A. Avila, E. Munoz and H. Monzon, *Exp. Parasitol.*, **56**, 236 (1983).

[20] J. L. Avila, *Interciencia*, **8**, 405 (1983).

[21] J. L. Avila and M. A. Casanova, *Antimicrob. Agents Chemother.*, **22**, 380 (1982).

[22] G. A. Bhat, J.-L. G. Montero, R. P. Panzica, L. L. Wotring and L. B. Townsend, *J. Med. Chem.*, **24** 1165 (1981).

[23] S. M. Hecht, R. B. Frye, D. Werner, T. Fukui and S. D. Hawrelak, *Biochemistry*, **15**, 1005 (1976).

[24] R. P. Panzica, G. A. Bhat, R. A. Earl, J.-L. G. Montero, L. W. Roti Roti and L. B. Townsend, in "Chemistry and Biology of Nucleosides and Nucleotides", R. E. Harmon, R. K. Robins and L. B. Townsend, eds, Academic Press, New York, 1978, pp 121-134.

[25] W. J. Middleton and V. A. Engelhardt, *J. Am. Chem. Soc.*, **80**, 2829 (1958).

[26] A. Yamazaki, I. Kumashiro and T. Takenishi, *J. Org. Chem.*, **32**, 3032 (1967).

[27] G. R. Revankar and R. K. Robins, in "Chemistry of Nucleosides and Nucleotides," Vol. II, L. B. Townsend, ed, Plenum Publishing Corp., New York, N. Y., in press.

[28] H. Vorbruggen, K. Krolikiewicz and B. Bennua, *Chem. Ber.*, **114**, 1234 (1981).

[29] F. W. Lichtenthaler and E. Cuny, *Chem. Ber.*, **114**, 1610 (1981).

[30] E. C. Taylor, R. N. Warrener and A. McKillop, *Angew. Chem., Int. Ed. Engl.*, **5**, 309 (1966).

[31] Yu. N. Bulychev, I. A. Korbukh and M. N. Preobrazhenskaya, *Khim. Geterotsikl. Soedin.*, 536 (1981).

[32] Yu. N. Bulychev, I. A. Korbukh and M. N. Preobrazhenskaya, *Khim. Geterotsikl. Soedin.*, 253 (1984).

[33] G. M. Sheldrick, "SHELXTL. An Integrated System for Solving, Refining and Displaying Crystal Structures from Diffraction Data", 4th Revision, University of Gottingen, Federal Republic of Germany, 1983.

[34] International Tables for X-Ray Crystallography, Vol **4**, J. A. Ibers and W. C. Hamilton, eds, Kynoch Press, Birmingham, England, 1974, p 99.