Syntheses of 6-Amino-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]-1,3-oxazin-4-one, an Isostere of Oxanosine, and the Guanosine Analog 6-Amino-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one *via* Ring Closure of Pyrazole-5-thioureido Intermediates

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Received September 18, 1989

6-Amino-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]-1,3-oxazin-4-one (**4**), an isostere of the nucleoside antibiotic oxanosine has been synthesized from ethyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrazole-4-carboxylate (**6**). Treatment of **6** with ethoxycarbonyl isothiocyanate in acetone gave the 5-thioureido derivative **7**, which on methylation with methyl iodide afforded ethyl 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-[(*N'*-ethoxycarbonyl-*S*-methylisothiocarbamoyl)amino]pyrazole-4-carboxylate (**8**). Ring closure of **8** under alkaline media furnished 6-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]-1,3-oxazin-4-one (**10**), which on deisopropylidenation afforded **4** in good yield. 6-Amino-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**5**) has also been synthesized from the AICA riboside congener 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrazole-4-carboxamide (**12**). Treatment of **12** with benzoyl isothiocyanate, and subsequent methylation of the reaction product with methyl iodide gave 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-[(*N'*-benzoyl-*S*-methylisothiocarbamoyl)amino]pyrazole-4-carboxamide (**15**). Base mediated cyclization of **15** gave 6-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**14**). Deisopropylidenation of **14** with aqueous trifluoroacetic acid afforded **5** in good yield. Compound **4** was devoid of any significant antiviral or antitumor activity in culture.

J. Heterocyclic Chem., **27**, 787 (1990).

Oxanosine (**1a**) is a novel nucleoside antibiotic isolated recently [1] from the culture broth of *Streptomyces capreolus* MG265-CF3, and characterized as 5-amino-3-(β-D-ribofuranosyl)imidazo[4,5-*d*]-1,3-oxazin-7-one by X-ray crystallographic studies [2]. This nucleoside inhibited the growth of HeLa cells in culture (IC_{50} of 32 μg/ml). It suppressed the growth of L1210 leukemia in mice [1] and was relatively nontoxic. Oxanosine also inhibited the growth of *Escherichia coli* K-12 on peptone agar and this antibacterial activity was antagonized by guanosine [1]. This nucleoside antibiotic was effective in inhibiting the growth of rat kidney cells infected with a temperature-sensitive mutant of Rous sarcoma virus at a permissive temperature of 33° than at a nonpermissive temperature of 39° [3]. The mode of antitumor [4] and antiviral [5] actions of oxanosine have been studied. It is shown that oxanosine is a competitive inhibitor of GMP synthetase (E.C.6.3.5.2) with a Ki value of 7.4×10^{-4} [4]. This interesting and potent biological activity, coupled with its biogenetic relationship with guanosine, resulted in the multistep synthesis of oxanosine [6] and 2'-deoxyoxanosine (**1b**) [7]. 2'-Deoxyoxanosine exhibited a stronger antineoplastic activity (IC_{50} of 0.15 μg/l) than oxanosine (0.53 μg/l) [7].

In animal studies, oxanosine was found to degrade enzymatically (hydrolysis of the oxazinone ring) to yield the bioinactive products [1,2]. In an effort to prevent this enzymatic hydrolysis, Niitsuma and co-workers [8] have prepared 3-deazaoxanosine (**2**). Although 3-deazaoxanosine was resistant to the hydrolytic enzyme of mouse serum, it

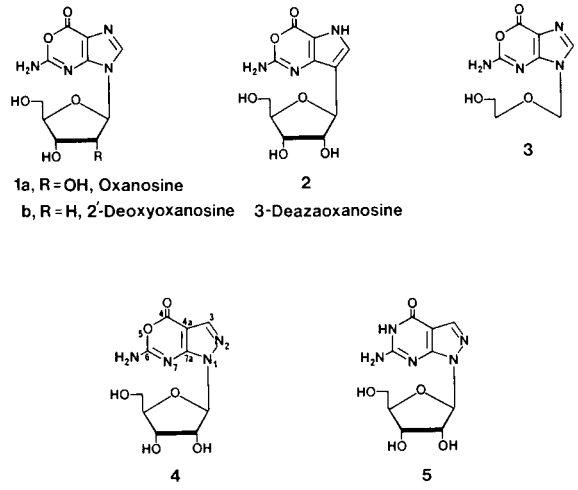


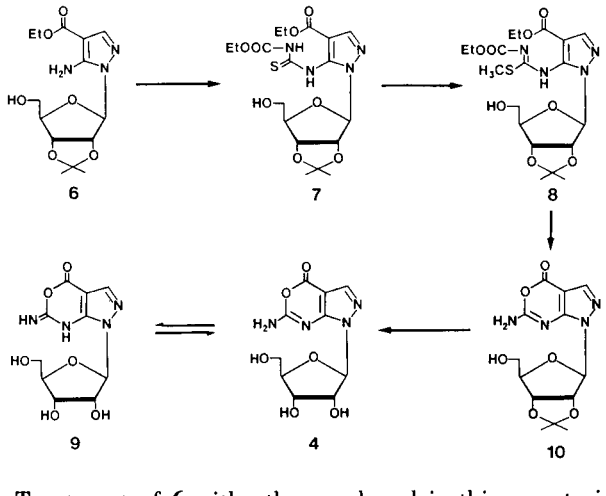
Figure 1

was much less active than oxanosine as an antitumor agent [8]. Recently, the acyclovir congener of oxanosine {5-amino-3-[(2-hydroxyethoxy)methyl]-3*H*-imidazo[4,5-*d*]-1,3-oxazin-7-one, **3**} was prepared [9], but no biological activity has been reported. In view of these observations, it is of interest to modify the imidazo[4,5-*d*]-1,3-oxazine moiety of oxanosine into pyrazolo[3,4-*d*]-1,3-oxazine ring, since several pyrazolo[3,4-*d*]pyrimidine ribonucleosides exhibit significant biological activity [10-12]. We have now synthesized 6-amino-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]-1,3-oxazin-4-one (**4**), an isostere of oxanosine, in the hope that it might be metabolically more stable and selective in

its biological activity. Moreover, 6-aminoallopurinol ribonucleoside (6-amino-1-β-D-ribofuranosyl-1*H*-pyrazolo-[3,4-*d*]pyrimidin-4(5*H*)-one, **5**), prepared and reported from our laboratory [13], has shown significant antiparasitic activity in culture [14]. The previous preparation of **5** involved the direct glycosylation of the trimethylsilyl derivative of 4,6-dichloropyrazolo[3,4-*d*]pyrimidine [15], which usually gives a mixture of N-1 and N-2 glycosylated products. The separation of these positional isomers is sometimes tedious and cumbersome. We, therefore, developed a convenient and large scale synthesis of **5** by the ring annulation of 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrazole-4-carboxamide (**12**). Thus, in this paper we describe a facile synthesis of the oxanosine analog (**4**) and the structurally related guanosine analog (**5**).

The synthesis of 6-amino-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]-1,3-oxazin-4-one (**4**) was accomplished using the viable starting material ethyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrazole-4-carboxylate (**6**). The nucleoside **6** was prepared as reported by Schmidt and coworkers [16]. Although Schmidt *et al.* claimed to have obtained **6** in 49% yield by the reaction of 1-deoxy-1-hydrazinyl-2,3-*O*-isopropylidene-D-ribose with ethyl (ethoxymethylene)cyanoacetate in methanol at room temperature, this reaction, in our hands, did not give more than 15% yield of **6**. However, when the reaction was carried out in boiling ethanol, a 55% yield of analytically pure **6** was obtained. The ^{1}H nmr spectrum of **6** revealed a $\Delta\delta$ value of 0.17 ppm for the isopropylidene methyl signals indicating β-configuration [17]. Attempts to form the 1,3-oxazine ring from **5** by direct cyanation of the 5-amino function with cyanogen bromide and ring closure under strongly alkaline conditions, as described for the synthesis of 3-deazaoxanosine [8] was unsuccessful. Therefore, we approached the synthesis of **4** *via* the 5-thiourido derivative (**7**).

Scheme 1



Treatment of **6** with ethoxycarbonyl isothiocyanate in dry acetone at reflux temperature gave a complex reaction mixture from which the desired ethyl 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-[(*N'*-ethoxycarbonylthiocarbamoyl)amino]pyrazole-4-carboxylate (**7**) was isolated in a 33% yield. Methylation of **7** with methyl iodide in 0.1 *N* aqueous sodium hydroxide gave the methylated product ethyl 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-[(*N'*-ethoxycarbonyl-*S*-methylisothiocarbamoyl)amino]pyrazole-4-carboxylate (**8**), which was isolated in a 93% yield after silica gel column chromatography. Cyclization of **8** with boiling 0.5 *N* aqueous sodium hydroxide furnished a compound identified as 6-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]-1,3-oxazin-4-one (**10**). However, the isolated yield of **10** was only 27%. Deisopropylidenation of **10** with 15% aqueous acetic acid at room temperature afforded the target nucleoside 6-amino-1-(β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]-1,3-oxazin-4-one (**4**) in a 63% yield.

The oxanosine analog (**4**) is capable of existing in four different tautomeric forms. In order to assign the most probable structure of **4**, we first measured the ^{1}H nmr spectrum of the precursor **10** in DMSO-d_6. It was observed that, when a spectrum was run on a freshly prepared solution, **10** exists predominantly as the C-6 amino tautomer, exhibiting a sharp singlet at δ 6.58 ppm with an integral of two protons for the primary amino group. However, after an extended period of time (4-5 hours) at 20° in DMSO-d_6, compound **10** tautomerizes to the thermodynamically more stable imino isomer and exhibits two broad singlets at δ 12.25 ppm and 13.72 ppm, which are assigned for the C-6 imino and N_7H protons. Compound **4** also exists as a C-6 imino tautomer **9**, as confirmed by ^{1}H nmr, which revealed two N*H* signals resonating at δ 12.22 ppm and 13.65 ppm. We further confirmed the tautomerization of **10** by comparing ^{13}C nmr in DMSO-d_6 of **10** with that of **4**. A significant downfield shift of C-6 signal at δ 151.44 ppm for **10** was observed. The C-6 signal of **4** resonates at δ 157.10 ppm, indicating nucleoside **4** exists as C-6 imino isomer. Further, the uv spectrum of freshly prepared solution of **10** was compared with the uv spectrum of **4**. It was observed that **10** has the λ max of 285 nm, 284 nm, and 269 nm at *p*H 1, 7, and 11, respectively, (which are very similar to those reported [1] for oxanosine), while in the case of **4** the observed λ max was 289 nm, 291 nm and 290 nm. These observations further confirmed that compound **4** exists as an C-6 imino tautomer. The possibility of existence of the remaining two tautomers of **4**, *viz.* 4-hydroxy-6-imino-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]-1,3-oxazine and 6-imino-1-(β-D-ribofuranosyl)-1*H*, 2*H*-pyrazolo-[3,4-*d*]-1,3-oxazin-4-one, was ruled out on the basis of the fact that there was no C-4 hydroxyl signal observed in ^{1}H nmr of **4**. Further, the ^{13}C nmr spectra showed that C-4 chemical shift of **4** (δ 175.43) is not similar to that of **5** (δ 158.76) (Table 1), due primarily to the presence of a neighboring cyclic keto group. Since the ^{13}C chemical shift of

C-3 of **4** (δ 137.15) is very similar to that of **5** (δ 136.20), the probability of the hydrogen being residing on N-2 was excluded. From these ^{1}H nmr, ^{13}C nmr and uv spectral analysis, it was concluded that, in freshly prepared solution compound **10** exists predominantly as the amino tautomer, while the oxanosine analog **4** exists as the C-6 imino tautomer as shown in structure **9**. Such an amino-imino tautomerism in the case of 5-aza-7-deazaguanosine series has recently been documented in the literature [18].

Scheme 2

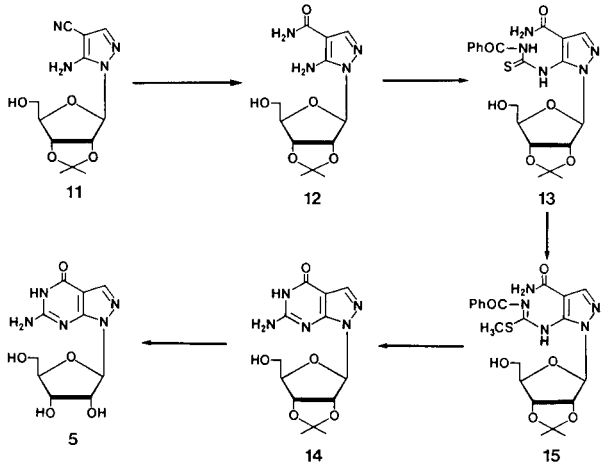


Table 1

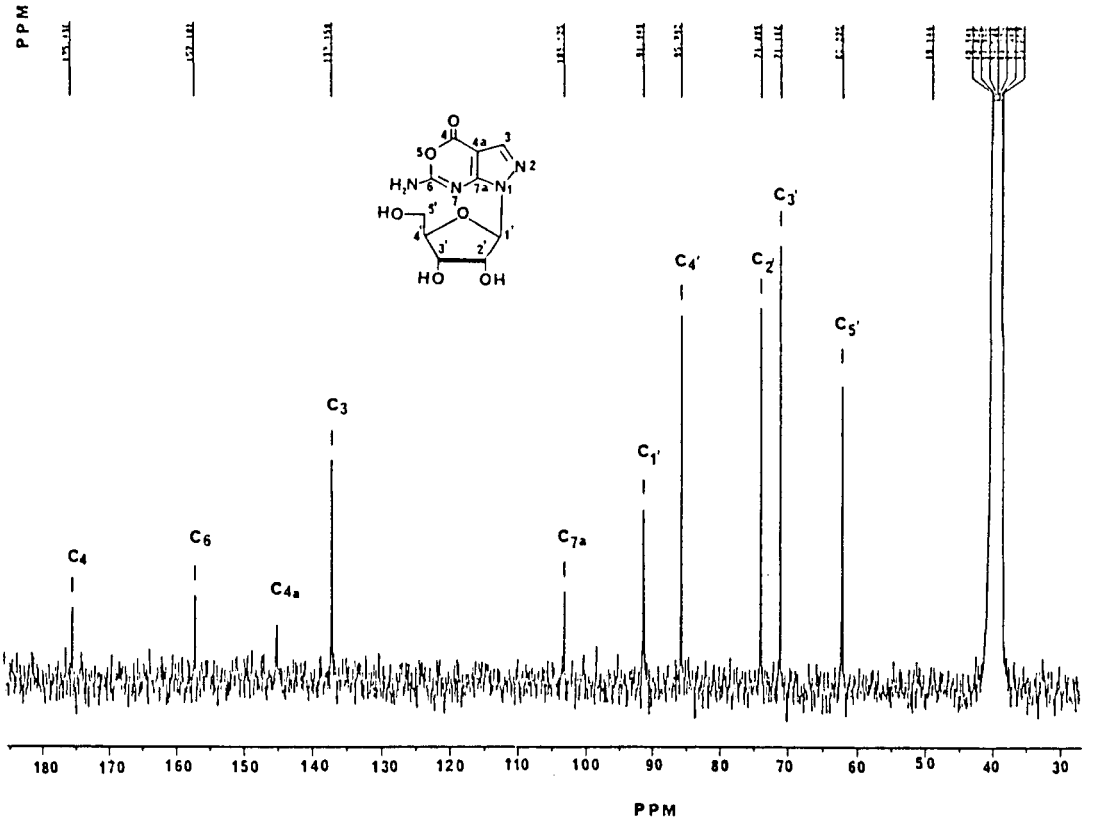
^{13}C NMR Chemical Shifts of **4** and **5** in DMSO-d_6 at 25.3 MHz (expressed in δ values using TMS as internal standard)

Carbon	Compound **4**	Compound **5**
3	137.15	136.20
4	175.43	158.76
6	157.10	156.53
4a	145.44	155.26
7a	103.12	100.15
1'	91.34	88.00
2'	74.00	73.40
3'	71.16	71.21
4'	85.78	85.19
5'	62.22	62.75

The synthesis of 6-amino-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**5**) was accomplished using the readily available 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrazole-4-carboxamide (**12**) *via* the conventional 5-thiourido intermediate [19-21]. Although compound **12** was synthesized by Schmidt and co-workers [16,22] employing the *N*-formyl/benzoyl derivative of 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrazole-4-carboxamide, the reported yields (40-67%) are comparatively low. In the present study we elected to use 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrazole-4-carbonitrile (**11**) [23] as the precursor, which on oxidative hydrolysis with ammonium hydroxide and hydrogen peroxide gave a 97% yield of **12** as crystalline material. The key intermediate 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl-5-[(*N'*-benzoylthiocarbamoyl)amino]pyrazole-4-carboxamide (**13**) was prepared in a 75% yield by the direct condensation of **12** with benzoyl isothiocyanate in acetone at reflux temperature. Compound **13** was purified on a silica gel column and isolated as crystalline material of mp 130-131°. Methylation of **13** with methyl iodide in 0.1 *N* aqueous sodium hydroxide furnished an almost quantitative yield of the corresponding methylthio derivative **15** as crystalline material. When a methanolic solution of compound **15** was boiled with 2 *N* aqueous sodium hydroxide solution, a clean reaction was observed and the ring closed product 6-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**14**) was obtained in a 80% yield after neutralization of the reaction mixture with 2 *N* hydrochloric acid. This facile ring closure indicated that it is not necessary to go through the 5-*N'*-benzoylguanidino intermediate, as reported by Yamazaki *et al.* [19] in the case of corresponding imidazole series, to obtain guanosine. Isopropyl group removal from **14** with aqueous trifluoroacetic acid at room temperature gave the target nucleoside **5** as needles in an 80% yield. Compound **5** was found to be identical in all respects with 6-amino-1-(β-D-ribofuranosyl)-1*H*-pyrazolo-[3,4-*d*]pyrimidin-4(5*H*)-one reported previously from our laboratory [13].

The ^{13}C nmr spectrum (chemical shifts measured in DMSO-d_6 at 25.3 MHz) of 6-amino-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]-1,3-oxazin-4-one (**4**) is comparable with that of 6-amino-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]-pyrimidin-4(5*H*)-one (**5**) (Table 1). This reveals that the chemical shifts of the C-3, C-6, C-2', C-3', C-4' and C-5' of **4** which appears at δ 137.15, 157.10, 74.00, 71.16, 85.78 and 62.22 ppm, respectively, were similar to the chemical shifts of C-3, C-6, C-2', C-3', C-4' and C-5' of **5** and appeared at δ 136.20, 156.53, 73.40, 71.21, 85.19 and 62.75 ppm (Figure 2). The C-4 of **4** resonates at δ 175.43 ppm, further downfield, as compared to δ 158.76, C-4 of **5** presumably due to an influence of oxygen in the oxazine ring. Moreover, C-7a and C-1' of **4** appeared at δ 103.12 and 91.34 ppm, slightly higher than that of C-7a and C-1' of **5**. A similarity in ^{13}C-chemical shifts of **4** with **5** further explains the unequivocal assignment of (**4**) as 6-amino-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]-1,3-oxazin-4-one. A similar pattern in ^{13}C nmr spectrum was observed in case of oxanosine and guanosine [1].

Compound **4** was evaluated *in vitro* for its ability to inhibit the growth of L1210 leukemia, WI-L2 and CCRF-CEM (for antitumor effects), as well as against HSV-1, para-3, VV and Cox B-1 viruses (for antiviral effects). However, nucleoside **4** did not exhibit any significant biological effect in these systems.


PPM
C4
C6
C4a
C3
C7a
C1'
C4'
C2'
C3'
C5'
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30
PPM

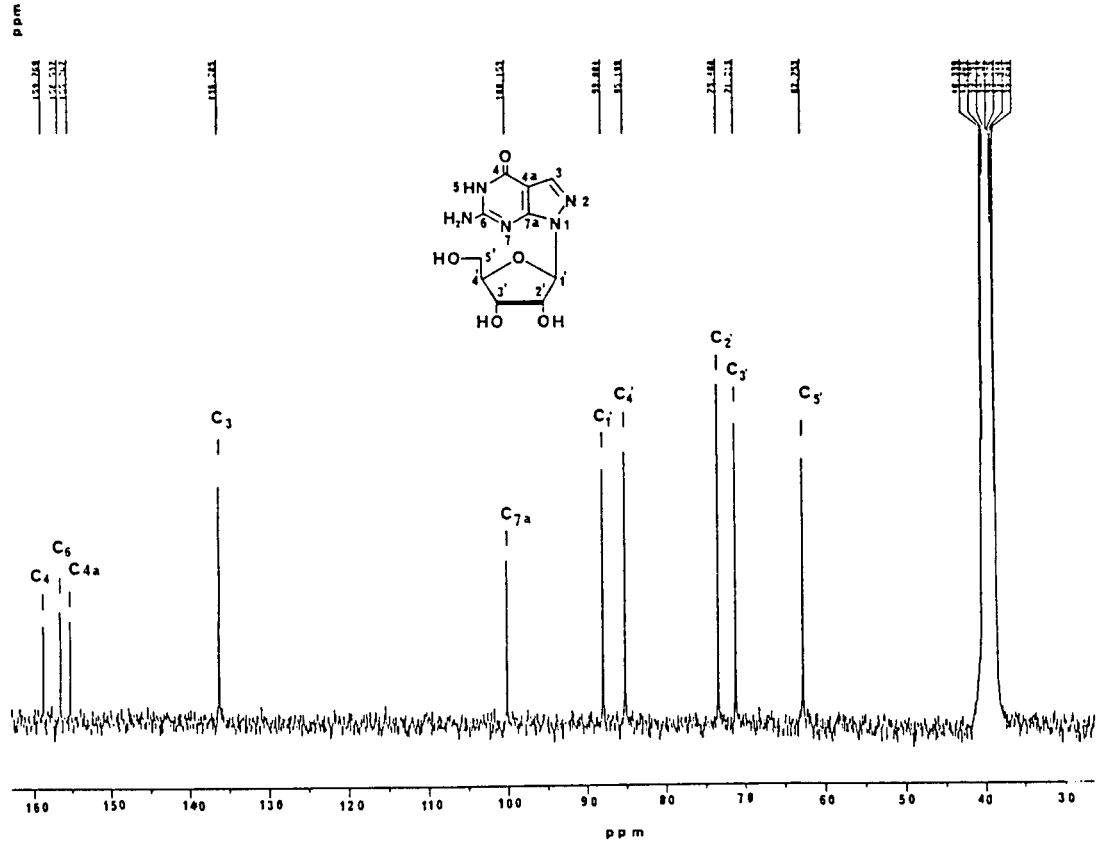

ppm
C4
C6
C4a
C3
C7a
C1'
C4'
C2'
C3'
C5'
150 140 130 120 110 100 90 80 70 60 50 40 30
ppm

Figure 2

In conclusion, we have successfully synthesized 6-amino-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]-1,3-oxazin-4-one (**4**), an isostere of the naturally occurring nucleoside antibiotic oxanosine. Also, a facile and high-yield synthetic pathway has been developed for the preparation of 6-amino-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**5**).

EXPERIMENTAL

Melting points (uncorrected) were determined in a Thomas-Hoover capillary melting-point apparatus. Elemental analyses were performed by Robertson Laboratory, Madison, NJ. The presence of water as indicated by elemental analysis was verified by ^{1}H nmr spectroscopy. Thin layer chromatography (tlc) was performed on plates of silica gel 60F-254 (EM Reagents). Silica gel (E. Merck; 230-400 mesh) was used for flash column chromatography. All solvents used were regent grade. Detection of nucleoside components in tlc was by uv light, and with 10% sulfuric acid in methanol spray followed by heating. Evaporations were conducted under diminished pressure with the bath temperature below 30°. Infrared (ir) spectra were recorded in potassium bromide with a Perkin-Elmer 1420 spectrophotometer and ultraviolet spectra (uv) were recorded on a Beckman DU-50 spectrophotometer. Nuclear magnetic resonance (^{1}H nmr) spectra were recorded at 300 MHz with an IBM NR/300 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as the internal standard (key: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad).

Ethyl 5-Amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrazole-4-carboxylate (**6**).

A solution of 1-deoxy-1-hydrazinyl-2,3-*O*-isopropylidene-D-ribose [23] (102 g, 544 mmoles) in absolute ethanol (750 ml) was purged with dry argon for 30 minutes. To this solution, ethyl (ethoxymethylene)cyanoacetate (91.93 g, 537 mmoles) dissolved in absolute ethanol (400 ml) was added portionwise over a period of 1 hour at 0°. The reaction mixture was stirred at 0° for 30 minutes and an additional 30 minutes at room temperature, before it was refluxed for 18 hours. The reaction mixture was evaporated to dryness and the residue was purified on a flash silica gel column using hexane:ethyl acetate (7:3, v/v) as the eluent to give 98 g (55%) of **6** as a light yellow syrup; ^{1}H nmr (DMSO-d_6): δ 1.25 (t, 3 H, $COOCH_2CH_3$), 1.30 and 1.48 (2s, 6 H, isopropylidene), 3.38 (m, 2 H, $C_{5'}H$), 4.08 (m, 1 H, $C_{4'}H$), 4.19 (q, 2 H, $COOCH_2CH_3$), 4.83 (q, 1 H, $C_{3'}H$), 4.92 (t, 1 H, $C_{5'}OH$), 5.23 (d, 1 H, $C_{2'}H$) and 7.54 (s, 1 H, C_3H).

This product was found to be identical with the one prepared as described by Schmidt *et al.* [16].

Ethyl 1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-5-[(*N'*-ethoxycarbonylthiocarbamoyl)amino]pyrazole-4-carboxylate (**7**).

To a stirred solution of **6** (8.0 g, 24.44 mmoles) in anhydrous acetone (200 ml), ethoxycarbonyl isothiocyanate (3.25 g, 24.78 mmoles) in anhydrous acetone (25 ml) was added portionwise over a period of 10 minutes and the reaction mixture was refluxed for 48 hours. The reaction mixture was evaporated to dryness and the residue was dissolved in methanol (~25 ml), adsorbed onto silica gel (~60 g) and loaded on the top of a prepacked flash silica gel column in hexane and eluted with hexane:ethyl acetate, (8:2, v/v). The fractions having R_f of 0.48 (dichloromethane:ethyl acetate, 7:3, v/v) were pooled and evaporated to give a white foam, which on crystallization from hexane/ethyl acetate gave 3.6 g (33%) of **7** as a white crystalline product, mp 82-84°; ir: ν max 1210 (C=S), 1720 (C=O), 3200-3400 (NH, OH) cm^{-1}; uv (*p*H 1): λ max 265 nm (ε 6,700), 213 (9,500); (*p*H 7): λ max 266 nm (sh, ε 5,700), 211 (13,800); (*p*H 11): λ max 252 nm (sh, ε 7,500); ^{1}H nmr (DMSO-d_6): δ 1.30 (m, 6 H, 2 $COOCH_2CH_3$), 1.31 and 1.46 (2s, 6 H, isopropylidene), 3.41 (m, 2 H, $C_{5'}H$), 4.28 (m, 4 H, 2 $COOCH_2CH_3$), 4.88 (m, 2 H, $C_{4'}H$ and $C_{3'}H$), 5.26 (d, 1 H, $C_{2'}H$), 5.90 (s, 1 H, $C_{1'}H$), 8.00 (s, 1 H, C_3H), 11.19 and 11.61 [2 br s, 2 H, N*H*(CS) and N*H*(CO)].

Anal. Calcd. for $C_{18}H_{26}N_4O_8S\cdot\frac{1}{2}H_2O$ (467.48): C, 46.24; H, 5.82; N, 11.98; S, 6.85. Found: C, 46.28; H, 5.86; N, 12.02; S, 6.86.

Ethyl 1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-5-[*N'*-ethoxycarbonyl-*S*-methylisothiocarbamoyl)amino]pyrazole-4-carboxylate (**8**).

To a stirred solution of **7** (10.0 g, 21.81 mmoles) in methanol (50 ml) containing 0.1 *N* aqueous sodium hydroxide (150 ml) was added methyl iodide (3.61 g, 24.90 mmoles). The reaction mixture was stirred at room temperature for 2 hours and then evaporated to dryness. The residue was dissolved in water (150 ml) and extracted with dichloromethane (3 × 60 ml). The combined organic phase was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography using dichloromethane:methanol (95:5, v/v) as the eluent to give 9.6 g (93%) of **8** as a white foam, mp 110-112°; ir: ν max 1700 (N-CO), 1750 (C=O, ester), 3200-3400 (NH, OH) cm^{-1}; uv (*p*H 1): λ max 244 nm (sh, ε 10,800), 218 (16,400); (*p*H 7): λ max 248 nm (sh, ε 10,100), 215 (19,500); (*p*H 11): λ max 248 nm (ε 8,100); ^{1}H nmr (DMSO-d_6): δ 1.21 (m, 6 H, 2 $COOCH_2CH_3$), 1.29 and 1.45 (2s, 6 H, isopropylidene), 2.36 (s, 3 H, SCH_3), 3.5 (m, 2 H, $C_{5'}H$), 4.11 (m, 5 H, $C_{4'}H$ and 2 $COOCH_2CH_3$), 4.85 (q, 2 H, $C_{3'}H$ and $C_{5'}OH$), 5.15 (d, 1 H, $C_{2'}H$), 5.74 (s, 1 H, $C_{1'}H$), 7.79 (s, 1 H, C_3H) and 10.36 (1 br s, 1 H, N*H*).

Anal. Calcd. for $C_{19}H_{28}N_4O_8S$ (472.50): C, 48.29; H, 5.97; N, 11.86; S, 6.71. Found: C, 48.35; H, 5.65; N, 11.76; S, 6.71.

6-Amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-pyrazolo-[3,4-*d*]-1,3-oxazin-4-one (**10**).

To a solution of **8** (3.5 g, 7.40 mmoles) in ethanol (15 ml) was added 0.5 *N* aqueous sodium hydroxide (125 ml) and the mixture was refluxed for 30 minutes. The reaction mixture was cooled in an ice bath, neutralized (*p*H 7) with 0.5 *N* aqueous hydrochloric acid at 0° and evaporated to dryness. The residue was dissolved in methanol (~25 ml) and adsorbed onto silica gel (~40 g) and placed on the top of a prepacked silica gel column in dichloromethane. The column was eluted with dichloromethane:methanol (97:3, v/v) and the appropriate fractions were pooled and evaporated to give 0.65 g (27%) of **10** as a white foam, mp 85-86°; ir: ν max 1695 (C=O), 3400-3440 (OH, NH_2) cm^{-1}; uv (*p*H 1): λ max 285 nm (ε 10,300), 242 (10,200), 213 (18,800); (*p*H 7): λ max 284 nm (ε 10,100), 238 (10,300), 210 (21,400); (*p*H 11): λ max 269 nm (ε 10,700); 237 (10,900); ^{1}H nmr (DMSO-d_6): δ 1.32 and 1.50 (2s, 6 H, isopropylidene), 3.42 (m, 2 H, $C_{5'}H$), 4.14 (q, 1 H, $C_{4'}H$), 4.88 (m, 2 H, $C_{3'}H$ and $C_{5'}OH$), 5.30 (d, 1 H, $C_{2'}H$), 6.50 (s, 1 H, $C_{1'}H$), 8.05 (s, 1 H, C_3H), 12.25 and 13.72 (2 br s, 2 H, 2 N*H*).

Anal. Calcd. for $C_{13}H_{16}N_4O_6\cdot\frac{1}{2}H_2O$ (333.29): C, 46.83; H, 5.14; N, 16.81. Found: C, 46.71; H, 5.01; N, 17.01.

6-Amino-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]-1,3-oxazin-4-one (**4**).

A solution of **10** (0.125 g, 0.385 mmole) in methanol (4 ml) was treated with 15% aqueous acetic acid (25 ml) and the reaction mixture was stirred at room temperature for seven days. The reaction mixture was evaporated to dryness and the residue was co-evaporated with methanol (3 × 15 ml). The residue was dissolved in methanol (~ 10 ml), adsorbed onto silica gel (~ 3 g) and loaded on the top of a dry pre-packed silica gel column and eluted with dichloromethane:methanol (8:2, v/v). The appropriate homogeneous fractions were pooled and evaporated to give 0.069 g (63%) of **4** as a white powder. An analytical sample was obtained by crystallization of the pure compound from methanol/dichloromethane, mp 142-144°; ir: ν max 1700 (C=O), 3300-3400 (OH, NH) cm^{-1}; uv (*p*H 1): λ max 289 nm (ε 20,100), 239 (7,000); (*p*H 7): λ max 291 nm (ε 18,500), 234 (9,500); (*p*H 11): λ max 290 nm (ε 18,400), 235 (9,000); ^1H nmr (DMSO-d_6): δ 3.60 (m, 2 H, $C_{5'}H$), 3.93 (q, 1 H, $C_{4'}H$), 4.14 (br s, 2 H, $C_{3'}H$ and $C_{5'}OH$), 4.42 (s, 1 H, $C_{2'}H$), 5.14 (br s, 1 H, $C_{3'}OH$), 5.41 (br s, 1 H, $C_{2'}OH$), 6.13 (d, 1 H, $J_{1',2'}$ = 4.11 Hz, $C_{1'}H$), 8.01 (s, 1 H, C_3H), 12.22 and 13.65 (2 br s, 2 H, 2 NH).

Anal. Calcd. for $C_{10}H_{12}N_4O_6 \cdot 2H_2O$ (320.25): C, 37.47; H, 5.00; N, 17.48. Found: C, 37.70; H, 4.91; N, 17.31.

5-Amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrazole-4-carboxamide (**12**).

To a solution of 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrazole-4-carbonitrile (**11**) [23] (10.0 g, 35.68 mmoles) in methanol (60 ml) and water (20 ml) was added concentrated ammonium hydroxide (170 ml) and 30% hydrogen peroxide (80 ml). The mixture was stirred in a steel bomb at room temperature for 18 hours. The steel bomb was opened carefully at 0° and the volatile products were evaporated to dryness. The residue thus obtained was co-evaporated with ethanol (3 × 80 ml), washed with ether and crystallized from methanol/dichloromethane to give 10.36 g (97%) of **12** as a white crystalline compound, mp 180-181° (Lit [22] mp 181°); ir: ν max 1660 ($CONH_2$), 3200-3400 (OH, NH_2) cm^{-1}; uv (*p*H 1): λ max 254 nm (sh, ε 6,500), 235 (sh, 6,400); (*p*H 7): λ max 253 nm (ε 7,400), 236 (7,400); (*p*H 11): λ max 252 nm (ε 7,500), 235 (7,300); ^1H nmr (DMSO-d_6): δ 1.30 and 1.48 (2s, 6 H, isopropylidene), 3.43 (m, 2 H, $C_{5'}CH_2$), 4.08 (t, 1 H, $C_{4'}H$), 4.84 (d, 1 H, $C_{3'}H$), 4.95 (br s, 1 H, $C_{5'}OH$), 5.22 (d, 1 H, $C_{2'}H$), 6.01 (s, 1 H, $C_{1'}H$), 6.58 (br s, 2 H, NH_2), 6.82 and 7.40 (2 br s, 2 H, $CONH_2$) and 7.73 (s, 1 H, C_3H).

Anal. Calcd. for $C_{12}H_{18}N_4O_5 \cdot \frac{1}{2}H_2O$ (307.29): C, 46.88; H, 6.23; N, 18.23. Found: C, 46.87; H, 6.10; N, 18.22.

1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-5-[(*N'*-benzoylthiocarbamoyl)amino]pyrazole-4-carboxamide (**13**).

To a stirred solution of **12** (10.0 g, 33.52 mmoles) in anhydrous acetone (200 ml) was added a solution of benzoyl isothiocyanate (6.55 g, 40.13 mmoles) in anhydrous acetone (50 ml), portionwise over a period of 30 minutes and the mixture was refluxed for 6 hours. The reaction mixture was evaporated to dryness and co-evaporated with anhydrous acetone (3 × 35 ml). The residue on purification by flash chromatography using hexane:ethyl acetate (6:4, v/v) as the eluent and crystallization from dichloromethane/hexane gave 11.58 g (75%) of **13**, mp 130-131°; ir: ν max 1220 (C=S), 1670 ($CONH_2$), 1600 (COC_6H_5) cm^{-1}; uv (*p*H 1): λ max 277 nm (sh, ε 12,700), 247 (18,300); (*p*H 7): λ max 240 nm (ε 23,200); (*p*H 11): λ max 237 nm (ε 23,700); ^1H nmr (DMSO-d_6): δ 1.32 and 1.45 (2s, 6 H, isopropylidene), 3.36 (m, 2 H, $C_{5'}CH_2$), 4.08 (t, 1 H, $C_{4'}H$), 4.86 (d, 1 H, $C_{3'}H$), 4.89 (d, 1 H, $C_{5'}OH$), 5.30 (d, 1 H, $C_{2'}H$), 5.96 (s, 1 H, $C_{1'}H$), 7.19 and 7.53 (2 br s, 2 H, $CONH_2$), 7.55-8.04 (m, 6 H, C_3H and COC_6H_5), 12.04 and 12.15 [2 br s, 2 H, C(S)NH, C(O)NH].

Anal. Calcd. for $C_{20}H_{23}N_5O_6S \cdot H_2O$ (479.43): C, 50.10; H, 5.25; N, 14.60; S, 6.68. Found: C, 50.38; H, 5.31; N, 14.59; S, 6.61.

1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-5-[*N'*-benzoyl-*S*-methylisocarbamoyl)amino]pyrazole-4-carboxamide (**15**).

To a solution of **13** (9.5 g, 20.59 mmoles) in methanol (45 ml) was added 0.1 *N* aqueous sodium hydroxide (30 ml) and to this mixture, methyl iodide (3.4 g, 24.17 mmoles) was added portionwise over a period of 15 minutes. The reaction mixture was stirred at room temperature for 2.5 hours and then neutralized (*p*H 7) with glacial acetic acid. The reaction mixture was extracted with dichloromethane (2 × 50 ml) and the combined organic phase was washed with water (3 × 150 ml), dried over anhydrous sodium sulfate and evaporated to dryness. The residue on purification by flash chromatography using dichloromethane:methanol (9:1, v/v) as the eluent gave 9.35 g (96%) of pure **15** as a white foam. Analytical sample was obtained by crystallization of pure product from dichloromethane/hexane, mp 110-112°; ir: ν max 1615 (C=O, benzoyl), 1650 (C=O, amide) cm^{-1}; uv (*p*H 1): λ max 298 nm (sh, ε 4,300), 245 (18,800); (*p*H 7): λ max 287 nm (sh, ε 5,800), 243 (18,800), 218 (16,500); (*p*H 11): λ max 266 nm (sh, 12,100), 225 (sh, 18,200); ^1H nmr (DMSO-d_6): δ 1.30 and 1.48 (2s, 6 H, isopropylidene), 2.46 (s, 3 H, SCH_3), 3.48 (m, 2 H, $C_{5'}CH_2$), 4.12 (m, 1 H, $C_{4'}H$), 4.91 (m, 2 H, $C_{3'}H$ and $C_{5'}OH$), 5.16 (d, 1 H, $C_{2'}H$), 5.92 (s, 1 H, $C_{1'}H$), 7.25 and 7.57 (2 br s, 2 H, $CONH_2$, one overlaps with benzoyl signal, exchangeable with deuterium oxide), 7.44-7.77 (m, 5 H, COC_6H_5), 7.89 (s, 1 H, C_3H) and 11.36 (br s, 1 H, NH).

Anal. Calcd. for $C_{21}H_{25}N_5O_6S$ (475.52): C, 53.04; H, 5.30; N, 14.73; S, 6.74. Found: C, 52.79; H, 5.31; N, 14.50; S, 6.52.

6-Amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-pyrazolo-[3,4-*d*]pyrimidin-4(5*H*)-one (**14**).

To a solution of **15** (9.0 g, 18.92 mmoles) in methanol (15 ml) was added 2 *N* aqueous sodium hydroxide (180 ml) and the mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature, neutralized (*p*H 7) with 2 *N* aqueous hydrochloric acid and evaporated to dryness. The residue was dissolved in methanol (~ 25 ml), adsorbed onto silica gel and loaded on the top of a pre-packed dry silica gel column. The column was eluted with dichloromethane:methanol (9:1, v/v), the appropriate homogeneous fractions were pooled and evaporated to dryness to give 4.86 g (80%) of **14** as a white powder. An analytical sample was obtained by crystallization of the pure product from methanol/dichloromethane, mp 210-211°; ir: ν max 1680 (C=O), 3180-3360 (OH, NH_2) cm^{-1}; uv (*p*H 1): λ max 252 nm (ε 14,600), 210 (22,600); (*p*H 7): λ max 253 nm (ε 15,300), 210 (23,600); (*p*H 11): λ max 264 nm (ε 11,400), 218 (25,800); ^1H nmr (DMSO-d_6): δ 1.31 and 1.48 (2s, 6 H, isopropylidene), 3.54 (m, 2 H, $C_{5'}CH_2$), 4.12 (m, 1 H, $C_{4'}H$), 4.96 (m, 2 H, $C_{3'}H$ and $C_{5'}OH$), 5.21 (q, 1 H, $C_{2'}H$), 6.07 (d, 1 H, J = 1.02 Hz, $C_{1'}H$), 7.88 (s, 1 H, C_3H) and 10.68 (br s, 1 H, NH).

Anal. Calcd. for $C_{13}H_{17}N_5O_5 \cdot \frac{1}{4}H_2O$ (327.81): C, 47.63; H, 5.38; N, 21.36. Found: C, 47.67; H, 5.18; N, 21.00.

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

A solution of 6-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**14**, 0.3 g, 0.92 mmole) in trifluoroacetic acid (3 ml) and water (1 ml) was stirred at room temperature for 30 minutes. The reaction mixture was evaporated to dryness and co-evaporated with ethanol (3 × 10 ml). The residue was dissolved in methanol (~5 ml), adsorbed onto silica gel (~5 g) and placed on the top of a silica gel column packed in ethyl acetate. The column was eluted with ethyl acetate:water:1-propanol (4:2:1, upper phase) and the appropriate homogeneous fractions were pooled and evaporated to dryness. The residue on crystallization from water gave 0.21 g (80%) of **5** as white needles, mp 262-263° (Lit [13] mp 263°); ir: ν max 1680 (C=O), 3340-3520 (NH_2, OH) cm^{-1}; uv (*p*H 1): λ max 252 nm (ε 13,400); (*p*H 7): λ max 252 nm (ε 14,200); (*p*H 11): λ max 263 nm (ε 11,100); ^1H nmr (DMSO-d_6): δ 3.57 (m, 2 H, $C_{5'}CH_2$), 3.86 (q, 1 H, $C_{4'}H$), 4.16 (q, 1 H, $C_{3'}H$), 4.48 (q, 1 H, $C_{2'}H$), 4.76 (q, 1 H, $C_{5'}OH$), 5.06 (d, 1 H, $C_{3'}OH$), 5.33 (d, 1 H, $C_{2'}OH$), 5.87 (d, 1 H, J = 4.35 Hz, $C_{1'}H$), 6.72 (br s, 2 H, NH_2), 7.84 (s, 1 H, C_3H) and 10.63 (br s, 1 H, NH).

Anal. Calcd. for $C_{10}H_{13}N_5O_5$ (283.24): C, 42.40; H, 4.62; N, 24.73. Found: C, 42.67; H, 4.80; N, 24.48.

Acknowledgement.

We wish to thank Ms. Sandy Young for the meticulous and superb preparation of this manuscript.

REFERENCES AND NOTES

[1] N. Shimada, N. Yagisawa, H. Nagasawa, T. Takita, M. Hamada, T. Takeuchi and H. Umezawa, *J. Antibiot.*, **34**, 1216 (1981).

[2] H. Nakamura, N. Yagisawa, N. Shimada, T. Takita, H. Umezawa and Y. Iitaka, *J. Antibiot.*, **34**, 1219 (1981).

[3] Y. Uehara, M. Hasagawa, M. Hori and H. Umezawa, *Biochem. J.*, **232**, 825 (1985).

[4] N. Yagisawa, N. Shimada, T. Takita, M. Ishizuki, T. Takeuchi and H. Umezawa, *J. Antibiot.*, **35**, 755 (1982).

[5] Y. Uehara, M. Hasegawa, M. Hori and H. Umezawa, *Cancer. Res.*, **45**, 5230 (1985).

[6] N. Yagisawa, T. Takita and H. Umezawa, *Tetrahedron Letters*, **24**, 931 (1983).

[7] K. Kato, N. Yagisawa, N. Shimada, M. Hamada, T. Takita, K. Maeda and H. Umezawa, *J. Antibiot.*, **37**, 941 (1984).

[8] S. Niitsuma, K. Kato, T. Takita and H. Umezawa, *Tetrahedron Letters*, **26**, 5785 (1985).

[9] H. Matsumoto, C. Kaneki, T. Mori and Y. Mizuno, *Chem. Pharm. Bull.*, **37**, 229 (1989).

[10] 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), and references cited therein.

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

[12] K. Ramasamy, B. S. Sharma, W. B. Jolley, R. K. Robins and G. R. Revankar, *J. Med. Chem.*, **32**, 1905 (1989).

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

[14] J. L. Avila, T. Rojas, A. Avila, M. A. Polegre and R. K. Robins, *Antimicrob. Agents Chemother.*, **31**, 447 (1987).

[15] R. K. Robins, *J. Am. Chem. Soc.*, **79**, 6407 (1957).

[16] R. R. Schmidt, W. Guilliard and J. Karg, *Chem. Ber.*, **110**, 2445 (1977).

[17] J.-L. Imbach, J.-L. Barascut, B. L. Kam, B. Ryner, C. Tamby and C. Tapiero, *J. Heterocyclic Chem.*, **10**, 1069 (1973).

[18] H. Rosemeyer and F. Seela, *J. Org. Chem.*, **52**, 5136 (1987).

[19] A. Yamazaki, I. Kumashiro and T. Takenishi, *J. Org. Chem.*, **32**, 1825 (1967).

[20] A. Yamazaki, M. Okutsu and Y. Yamada, *Nucleic Acids Res.*, **3**, 251 (1976).

[21] A. Yamazaki and M. Okutsu, *J. Heterocyclic Chem.*, **15**, 353 (1978).

[22] R. R. Schmidt, J. Karg and W. Guilliard, *Angew. Chem., Ed. Engl.*, **14**, 64 (1975).

[23] O. L. Acevedo, S. H. Krawczyk and L. B. Townsend, *J. Org. Chem.*, **51**, 1050 (1986).