Synthesis of 4,6-Disubstituted-7-β-D-ribofuranosyl- and Arabinofuranosylpyrazolo[3,4-*d*]pyrimidines and Certain Related Ribonucleosides

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Several disubstituted pyrazolo[3,4-*d*]pyrimidine, pyrazolo[1,5-*a*]pyrimidine and thiazolo[4,5-*d*]pyrimidine ribonucleosides have been prepared as congeners of uridine and cytidine. Glycosylation of the trimethylsilyl (TMS) derivative of pyrazolo[3,4-*d*]pyrimidine-4,6(1*H*,5*H*,7*H*)-dione (**4**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**5**) in the presence of TMS triflate afforded 7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo-[3,4-*d*]pyrimidine-4,6(1*H*,5*H*)-dione (**6**). Debenzoylation of **6** gave the uridine analog 7-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4,6(1*H*,5*H*)-dione (**3**), identical with 7-ribofuranosyloxoallopurinol reported earlier. Thiation of **6** gave **7**, which on debenzoylation afforded 7-β-D-ribofuranosyl-6-oxopyrazolo[3,4-*d*]pyrimidine-4(1*H*,5*H*)-thione (**8**). Ammonolysis of **7** at elevated temperature gave a low yield of the cytidine analog 4-amino-7-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-6(1*H*)-one (**11**). Chlorination of **6**, followed by ammonolysis, furnished an alternate route to **11**. A similar glycosylation of TMS-**4** with 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride (**12**) gave mainly the N7-glycosylated product **13**, which on debenzylation provided 7-β-D-arabinofuranosylpyrazolo[3,4-*d*]pyrimidine-4,6(1*H*,5*H*)-dione (**14**). 4-Amino-7-β-D-arabinofuranosylpyrazolo[3,4-*d*]pyrimidin-6(1*H*)-one (**19**) was prepared from **13** *via* the C4-pyridinium chloride intermediate **17**. Condensation of the TMS derivatives of 7-hydroxy- (**20**) or 7-aminopyrazolo[1,5-*a*]pyrimidin-5(4*H*)-one (**23**) with **5** in the presence of TMS triflate gave the corresponding blocked nucleosides **21** and **24**, respectively, which on deprotection afforded 7-hydroxy- **22** and 7-amino-4-β-D-ribofuranosylpyrazolo[1,5-*a*]pyrimidin-5-one (**25**), respectively. Similarly, starting either from 2-chloro (**26**) or 2-aminothiazolo[4,5-*d*]pyrimidine-5,7-(4*H*,6*H*)-dione (**29**), 2-amino-4-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-5,7(6*H*)-dione (**28**) has been prepared. The structure of **25** was confirmed by single crystal X-ray diffraction studies.

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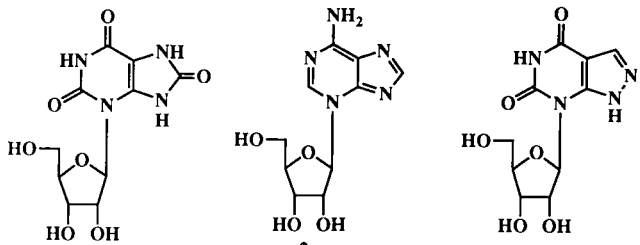
Our interest in 3-glycosylated purine derivatives has been outlined in an earlier publication [1]. This interest has been stimulated by the isolation and characterization [2,3] of 3-β-D-ribofuranosyluric acid (**1**) from bovine erythrocytes and by the observation of interesting biological properties [4-7] of synthetic 3-β-D-ribofuranosyladenine (isoadenosine, **2**). Isoadenosine has been found [6] to inhibit the growth of various tumor cell lines, both *in vitro* and *in vivo*, as well as to show significant activity against adeno III virus in culture. This interest has been further stimulated by the isolation [8] of 7-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4,6(1*H*,5*H*)-dione (oxoallopurinol ribofuranoside, **3**) from the urine of patients treated with allopurinol, and the report [9] that **3**, presumably as the corresponding 5'-monophosphate, inhibits the *de novo* pyrimidine biosynthesis. These findings prompted the synthesis and study of a number of other purine nucleoside analogs in our [1,10] and other [11-15] laboratories.

Figure 1



1
3-β-D-Ribofuranosyl-uric acid

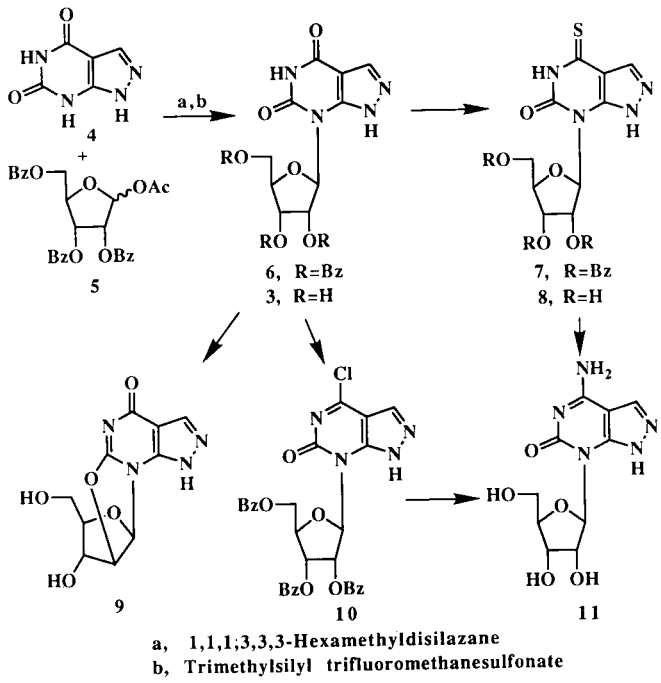
2
Isoadenosine

3
Oxoallopurinol ribofuranoside

As a further extension of our investigations in this area, in this communication we now report the synthesis of several 4,6-disubstituted-7-β-D-ribofuranosyl- and arabinofuranosylpyrazolo[3,4-*d*]pyrimidines which are structurally related to uridine and cytidine. Also prepared are the uridine and cytidine congeners in the pyrazolo[1,5-*a*]pyrimidine ring system. The synthesis of the uridine analog (**3**) in

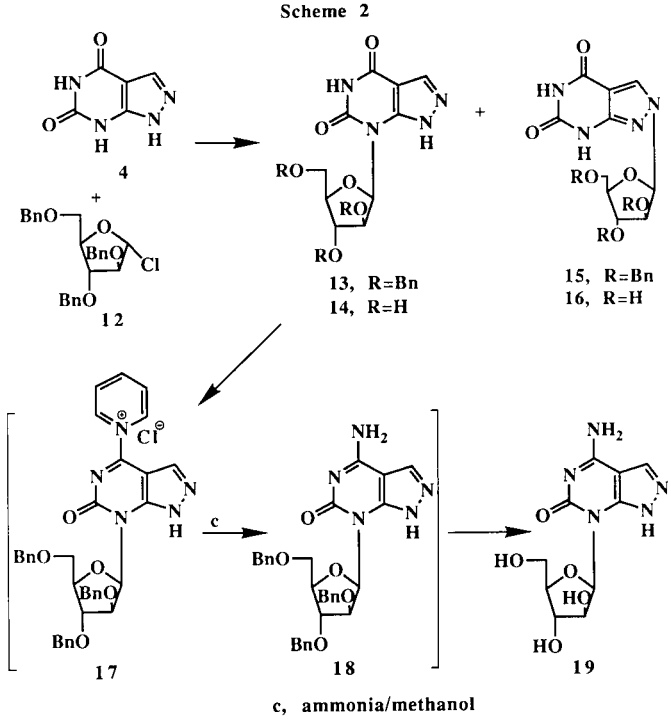
the pyrazolo[3,4-*d*]pyrimidine ring system has been accomplished by the trimethylsilyl procedure [16]. The silylation of pyrazolo[3,4-*d*]pyrimidine-4,6(1*H*,5*H*,7*H*)-dione (**4**) [17] was accomplished with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) in the presence of a catalytic amount of ammonium sulfate in dry pyridine. The tristrimethylsilyl derivative thus obtained was condensed with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**5**) in 1,2-dichloroethane using 1.44 molar equivalent [18] of trimethylsilyl trifluoromethanesulfonate (TMS triflate) at room temperature. A clean reaction was observed and the reaction product was isolated by crystallization from ethyl acetate to obtain 7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine-4,6(1*H*,5*H*)-dione (**6**) in a 66% yield. No other nucleoside product was detected in the reaction mixture by tlc procedures (silica gel using 2% methanol in dichloromethane as the eluent). A removal of the protecting benzoyl groups of the glycon moiety of **6** with methanolic ammonia (saturated at 0°) at room temperature furnished 7-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4,6(1*H*,5*H*)-dione (**3**). Compound **3** was found to be identical (mp, ir, uv) with 7-ribofuranosyloxoallopurinol prepared by chemical [19], as well as enzymatic [8] procedures.

Scheme 1



The synthesis of the cytidine analog 4-amino-7-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-6(1*H*)-one (**11**) was accomplished by transformation of the hydroxyl function of **6**. The reaction of **6** with phosphorus pentasulfide in dioxane at reflux temperature proceeded smoothly to give 7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-6-oxopyrazolo[3,4-*d*]pyrimidine-4(1*H*,5*H*)-thione (**7**), which was isolated by silica gel column chromatography in a 68% yield. Deprotection of **7** with methanolic ammonia at room temperature furnished a 84% yield of 7-β-D-ribofuranosyl-6-oxopyrazolo[3,4-*d*]pyrimidine-4(1*H*,5*H*)-thione (**8**). The direct nucleophilic displacement of the thio function of **7** by ammonia at elevated temperature resulted in a low yield of the desired cytidine analog **11**. This prompted us to investigate the alternate route to **11** *via* the chloro intermediate **10**. Thus treatment of **6** with tetraethylammonium chloride, *N*,*N*-diethylaniline and phosphorus oxychloride at reflux temperature gave presumably 4-chloro-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-6(1*H*)-one (**10**) which, when reacted with methanolic ammonia gave a 45% yield of crystalline **11**.

Scheme 2



The preparation of 7-β-D-arabinofuranosylpyrazolo-[3,4-*d*]pyrimidine-4,6(1*H*,5*H*)-dione (**14**) was envisioned to be possible *via* the ring opening of the 2',6-anhydronucleoside **9**. The preparation of crystalline 7-β-D-ribofuranosyl-2',6-anhydropyrazolo[3,4-*d*]pyrimidin-4(1*H*)-one (**9**) was accomplished in excellent yield by the treatment of **3** with diphenyl carbonate and sodium hydrogen carbonate in hexamethylphosphoramide at 150° for 30 minutes. However, our attempts to open the anhydro linkage under various alkaline conditions were unsuccessful. Thus, in an effort to prepare the desired **14**, the direct glycosylation of **4** with the protected α-halogenose **12** was considered. 2,3,5-Tri-*O*-benzyl-α-D-arabinofuranosyl chloride (**12**) was prepared as reported [20] and reacted with the tristrimethylsilyl derivative of **4** in 1,2-dichloroethane in the presence of 1.44 molar equivalent of tin(IV) chloride at room tem-

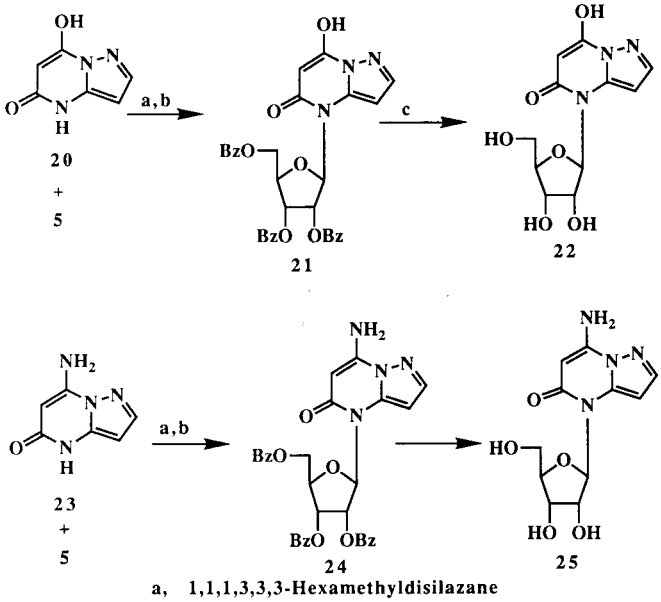
perature. A complex reaction mixture was obtained from which crystalline 7-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)pyrazolo[3,4-*d*]pyrimidine-4,6(1*H*,5*H*)-dione (**13**) was isolated in a 33% yield. A small amount (2.3%) of 2-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dione (**15**) was also isolated. Debenzylation of **13** with palladium hydroxide in ethanol in the presence of cyclohexene (as hydrogen source) at reflux temperature gave 7-β-D-arabinofuranosylpyrazolo[3,4-*d*]-pyrimidine-4,6(1*H*,5*H*)-dione (**14**). The ultraviolet absorption spectrum of **14** was essentially identical to that of 7-ribofuranosyloxoallopurinol (**3**), thereby proving the site of glycosylation in **14** as N7. However, a similar debenzylation of **15** with palladium hydroxide gave an intractable reaction mixture from which the isolation of the desired 2-β-D-arabinofuranosylpyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,-7*H*)-dione (**16**) was rather difficult. In order to circumvent this difficulty, compound **15** was debenzylated with boron trichloride at -78° to afford crystalline **16** in a 50% yield. Although the site of glycosylation in **16** is presumed to be N2, the anomeric configuration of both **14** and **16** was assigned as β on the basis of $J_{1',2'}$ coupling constants (5.3 Hz and 5.2 Hz, respectively) observed for the anomeric proton in the ^{1}H nmr spectra, which are within the region of 3.5-8.0 Hz, expected for a vicinal, *cis* arrangement of the $C_{1'}$ and $C_{2'}$ protons [21].

The synthesis of 4-amino-7-β-D-arabinofuranosylpyrazolo[3,4-*d*]pyrimidin-6(1*H*)-one (**19**) was accomplished by manipulation of the functional groups in **13**. Thus, pyridine assisted phosphorylation [22] of **13** with phosphorus oxychloride gave the reactive intermediate 7-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-6(1*H*)-oxopyrazolo[3,4-*d*]-pyrimidin-4-yl-pyridinium chloride (**17**), which on sequential treatment with methanolic ammonia and palladium hydroxide/cyclohexene furnished **19** in an overall yield of 76%. The ultraviolet absorption spectrum of **19** (in *p*H 1, 7 and 11) was essentially similar to that of **11**, lending support to the assigned structure of **19**.

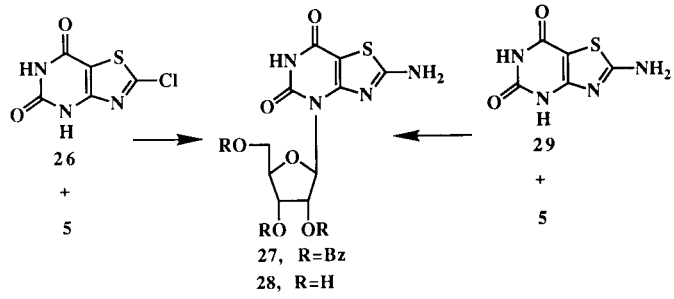
The preparation of the uridine and cytidine analogs in the pyrazolo[1,5-*a*]pyrimidine ring system was next considered by the direct glycosylation of the corresponding aglycons. The aglycons 7-hydroxypyrazolo[1,5-*a*]pyrimidin-5(4*H*)-one (**20**) and 7-aminopyrazolo[1,5-*a*]pyrimidin-5(4*H*)-one (**23**) were prepared as reported by Makisumi [23]. Silylation of **20** and **23** with excess of HMDS in pyridine in the presence of catalytic amount of ammonium sulfate gave the corresponding trimethylsilyl (TMS) derivatives. Glycosylation of the TMS derivative of **20** with **5** in 1,2-dichloroethane in the presence of TMS triflate afforded a single nucleoside product, which was isolated in a 55% yield by silica gel column chromatography procedures and identified as 4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-7-hydroxypyrazolo[1,5-*a*]pyrimidin-5-one (**21**). Conventional debenzoylation of **21** with methanolic ammonia at room temperature furnished the uridine analog 4-β-D-ribofuranosyl-7-hydroxypyrazolo[1,5-*a*]pyrimidin-5-one (**22**). A similar glycosylation of the TMS derivative of **23** with **5**, followed by debenzoylation of the condensation product gave the cytidine analog 7-amino-4-β-D-ribofuranosylpyrazolo[1,5-*a*]pyrimidin-5-one (**25**) in good yield.

Evidence that the glycosylation of this ring system had occurred at the N4 position was obtained by comparing the ^{1}H nmr spectra of **22** and **25** with those of several related heterocycles [24]. It has been observed that *N*-methylation in pyrazolo[1,5-*a*]pyrimidine ring system results in an increase in the coupling constant between protons on neighboring carbons [24]. In the case of N1 methyl compound, the $H_{2,3}$ coupling constants are of the order of 3.5 Hz, whereas N4 methylation results in smaller coupling of 2.0 Hz [25,26]. Since ribosylation or methylation is expected to produce similar inductive effects, and the fact that the observed $J_{H_{2,3}}$ in **22** and **25** is <2.0 Hz leads to the conclusion that N4 is the site of glycosylation. In addition, a direct glycosylation on a nitrogen adjacent to a bridgehead nitrogen has not been documented in the literature

Scheme 3



Scheme 4



[24]. The anomeric configuration of **22** and **25** were assigned as β on the basis of ^{1}H nmr data. The ^{1}H nmr spectra of **22** and **25** in DMSO-d_6 revealed the anomeric doublet centered around δ 6.0 with a small coupling constant ($J_{1',2'}$ = 4.5 to 4.9 Hz), which is within the acceptable limits for β-ribonucleosides [27,28]. Finally, the structure of **25** was unequivocally established by single crystal X-ray diffraction studies.

An analogous glycosylation of the TMS derivative of 2-chlorothiazolo[4,5-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (**26**) [29] with **5** resulted in the formation of an unexpected condensation product, which was isolated and subsequently characterized as 2-amino-4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidine-5,7(6*H*)-dione (**27**). It is of particular interest to note that during the silylation reaction, the liberated ammonia nucleophilically displaced the reactive chloro group at position 2 to give the corresponding 2-amino derivative. A similar glycosylation of preformed 2-aminothiazolo[4,5-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (**29**) [29] also gave an excellent yield of **27**, which was found to be identical in all respects (mp, mixture mp, tlc, ir, uv and ^{1}H nmr) with the sample obtained as above from **26**. Debenzoylation of **27** with methanolic ammonia at room temperature furnished crystalline 2-amino-4-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-5,7(6*H*)-dione (**28**) in a 81% yield. The assignment of the site of glycosylation for **28** was based primarily on uv spectra in which **28** displayed absorption maxima at longer wavelength (bathochromic shift) in *p*H 1 buffer compared to the aglycon **29** [10]. A relatively small coupling constant ($J_{1',2'}$ = 4.2 Hz) for the anomeric proton of **28** indicated the β-anomeric configuration [28].

Single Crystal X-Ray Diffraction Analysis of Compound **25**.

Table 1

Crystal and Experimental Data for Compound **25**

A. Crystal Data

Empirical formula	$C_{11}H_{14}N_4O_5$
Formula weight	282.26
Crystal color, habit	colorless, prism
Crystal dimensions (mm)	0.40 x 0.20 x 0.15
Crystal system	orthorhombic
Number of reflections used for unit cell determination (2θ range)	25 (73.2 - 80.0°)
Omega scan peak width at half-height	0.38
Lattice parameters:	
	a = 8.547(1)Å
	b = 20.546(1)Å
	c = 6.672(2)Å
	v = 1171.7(3)Å^3
Space group	$P2_12_12_1$
Z value	4
D_{calc}	1.600 g/cm^3
F_{000}	592 (electrons)
μ(CuKα)	10.46cm^{-1}

B. Intensity Measurements

Diffractometer	Rigaku AFC5R
Radiation	CuKα (λ=1.54178Å)
Temperature	23°C
Attenuators	Zr foil (factors: 3.6, 12.3, 44.7)
Take-off angle	6.0°

A colorless prism crystal (from methanol:dichloromethane) of compound **25** having approximate dimensions of 0.40 x 0.20 x 0.15 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC5R diffractometer with graphite monochromated CuKα radiation and a 12KW rotating anode generator. A summary of crystal and experimental data, as well as intensity measurements is given in Table 1.

Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 25 carefully centered reflections in the range $73.19 < 2\theta < 79.97°$ correspond to an orthorhombic cell with dimensions: a = 8.547 (1) Å; b = 20.546 (1) Å; c = 6.672 (2) Å; and V = 1171.7 (3) Å^3.

The data were collected at a temperature of 23 ± 1° using the ω-2θ scan technique to a maximum 2θ value of 120.1°. Omega scans of several intense reflections, made prior to data collection, had an average width at half-height of 0.38° with a take-off angle of 6.0°. Scans of (1.26 + 0.30 tan θ)° were made at a speed of 32.0°/min (in omega). The weak reflections [$I < 10.0\sigma(I)$] were rescanned (maximum of 3 rescans) and the counts were accumulated to assure good counting statistics. Stationary background counts were recorded on each side of the reflection. The ratio of peak counting time to background counting time was 2:1. The diameter of the incident beam collimator was 0.5 mm and the crystal to detector distance was 400.0 mm.

A total of 1065 reflections was collected. The intensities of three representative reflections which were measured after every 150 reflections remained constant throughout data collection indicating crystal and electronic stability (no decay correction was applied).

The linear absorption coefficient for CoKα is 10.5 cm^{-1}. Azimuthal scans of several reflections indicated no need for an absorption correction. The data were corrected for Lorentz and polarization effects. A correction for secondary extinction was applied (coefficient = 0.32744E-05).

Structure Solution and Refinement.

The structure was solved by direct methods [30,31]. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included in difference map positions. The final cycle of full-matrix least-squares refinement was based on 865 observed reflections [$I > 3.00\sigma(I)$] and 182 variable parameters and converged (largest parameter shift was 0.00 times its esd) with unweighted and weighted agreement factors of:

$R = \Sigma||Fo| - |Fc||/\Sigma|Fo| = 0.036$

$R_w = [(\Sigma w(|Fo| - |Fc|)^2/\Sigma w Fo^2)]^{1/2} = 0.042$

The standard deviation of an observation of unit weight was 2.08. The weighting scheme was based on counting statistics and included a factor (p = 0.02) to downweight

Table 2

Hydrogen Bonding Interactions in the Crystal Lattice of Compound **25**

	A-H, Å	H...B, Å	A...B, Å	A-H...B
O5'-O5'H...O3'	1.07	2.05	2.873(5)	132
O3'-O3'H...O5	1.05	1.70	2.731(5)	165
O2'-O2'H...O5'	1.06	1.77	2.762(5)	154
N9-N9HA...O2'	0.98	2.30	2.951(5)	123
N9-N9HB...O3'	0.95	2.22	3.101(5)	153

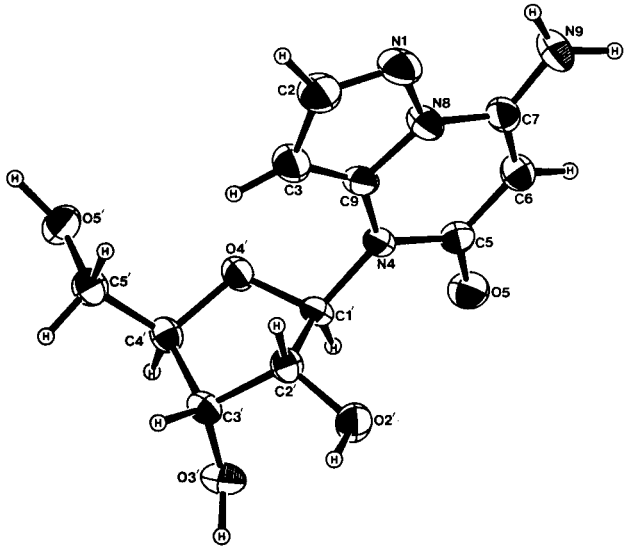


Figure 2. Perspective drawing of **25** showing atom labeling.

the intense reflections. Plots of $\Sigma w(|Fo| - |Fc|)^2$ versus $|Fo|$, reflection order in data collection, sin θ/λ, and various classes of indices showed no unusual trends. The maximum and minimun peaks on the final difference Fourier map corresponded to 0.19 and -0.26 $e^-/Å^3$, respectively.

Neutral atom scattering factors were taken from Cromer and Waber [32]. Anomalous dispersion effects were included in Fcalc [33]; the values for $\Delta f'$ and $\Delta f''$ were those of Cromer [34]. All calculations were performed using the TEXSAN [35] crystallographic software package of Molecular Structure Corporation, The Woodlands, Texas 77381.

Table 3

Selected Torsion Angles (°) in Compound **25**

(1)	(2)	(3)	(4)	Angle	(1)	(2)	(3)	(4)	Angle
O5'	C5'	C4'	O4'	63.0(5)	N8	C7	C6	C5	-0.9(8)
O5'	C5'	C4'	C3'	-178.3(4)	N1	N8	C7	C6	-176.8(5)
N4	C1'	04'	C4'	-158.0(3)	N1	N8	C9	C3	0.2(7)
N4	C1'	C2'	C3'	162.7(4)	N1	C2	C3	C9	0.3(8)
N4	C1'	C2'	C2'H	40.0	C4'	O4'	C1'	C1'H	87.0
N4	C5	C6	C7	-0.6(8)	C4'	C5'	O5'	O5'H	-170.0
N4	C9	N8	N1	-178.0(4)	C1'	O4'	C4'	C4'H	-98.0
N4	C9	N8	C7	-7.2(8)	C1'	N4	C5	C6	-176.7(4)
N4	C9	C3	C2	177.5(6)	C1'	C2'	O2'	O2'H	178.0
N4	C9	C3	C3H	-5.0	C1'	C2'	C3'	C3'H	-153.0
N8	N1	C2	C3	-0.2(7)	C6	C7	N9	N9HB	174.0
N8	N1	C2	C2H	-175.0	C7	N8	N1	C2	174.9(6)
N8	C7	N9	N9HA	70.0	C9	N4	C1'	C1'H	167.0
N8	C7	N9	N9HB	-6.0	C9	C3	C2	C2H	174.0

Table 4

Positional parameters and B (eq) for compound **25**

Atom	x	y	z	B(eq)
O5'	0.8060(4)	0.6947(2)	-0.1692(5)	3.1(2)
O4'	0.5576(4)	0.6720(1)	0.0923(5)	2.4(1)
O3'	0.5889(4)	0.7904(1)	0.3969(5)	3.0(1)
O2'	0.5284(4)	0.6765(2)	0.6264(5)	3.0(2)
O5	0.1583(3)	0.6399(1)	0.2662(6)	3.1(1)
N4	0.4055(4)	0.5957(2)	0.2652(6)	2.1(2)
N9	0.2265(5)	0.4090(2)	0.2663(7)	3.3(2)
N8	0.4300(4)	0.4819(2)	0.2595(7)	2.6(2)
N1	0.5368(5)	0.4321(2)	0.2471(8)	3.4(2)
C5'	0.8282(6)	0.7006(2)	0.0411(7)	2.6(2)
C4'	0.6741(6)	0.7223(2)	0.1288(7)	2.2(2)
C3'	0.6780(6)	0.7332(2)	0.3576(7)	2.2(2)
C2'	0.5983(5)	0.6716(2)	0.4336(7)	2.2(2)
C1'	0.4751(5)	0.6601(2)	0.2749(7)	2.0(2)
C5	0.2400(5)	0.5897(2)	0.2665(8)	2.4(2)
C6	0.1783(5)	0.5259(2)	0.2676(8)	2.6(2)
C7	0.2709(5)	0.4715(2)	0.2655(8)	2.7(2)
C2	0.6710(6)	0.4631(2)	0.228(1)	3.6(2)
C3	0.6563(5)	0.5310(2)	0.227(1)	3.3(2)
C9	0.4999(5)	0.5414(2)	0.2482(8)	2.4(2)
O5'H	0.8982	0.6718	-0.2520	2.0
C5'HA	0.8764	0.6510	0.1005	2.0
C5'HB	0.9019	0.7377	0.0820	2.0
C4'H	0.6323	0.7693	0.0819	2.0
C3'H	0.8049	0.7339	0.4158	2.0
O3'H	0.6287	0.8110	0.5320	2.0
C2'H	0.6732	0.6307	0.4479	2.0
O2'H	0.6137	0.6831	0.7398	2.0
C1'H	0.3873	0.6978	0.2540	2.0
C6H	0.0598	0.5258	0.2577	2.0
N9HA	0.1153	0.4013	0.2937	2.0
N9HB	0.3057	0.3768	0.2506	2.0
C2H	0.7737	0.4348	0.2031	4.2
C3H	0.7358	0.5662	0.2084	3.9

Table 5

Intramolecular Distances (Å) Involving the Hydrogen Atoms in **25**

atom	atom	distance	atom	atom	distance
O5'	O5'H	1.071	C4'	C4'H	1.076
O3'	O3'H	1.053	C3'	C3'H	1.153
O2'	O2'H	1.060	C2'	C2'H	1.060
N9	N9HA	0.981	C1'	C1'H	1.087
N9	N9HB	0.952	C6	C6H	1.015
C5'	C5'HA	1.169	C2	C2H	1.066
C5'	C5'HB	1.025	C3	C3H	1.000

Intramolecular Distances (Å) Involving the Nonhydrogen Atoms in **25**

atom	atom	distance	atom	atom	distance
O5'	C5'	1.421(6)	N8	C7	1.377(6)
O4'	C4'	1.456(5)	N8	C9	1.364(5)
O4'	C1'	1.429(5)	N1	C2	1.318(6)
O3'	C3'	1.424(5)	C5'	C4	1.509(6)
O2'	C2'	1.422(6)	C4'	C3'	1.543(6)
O5	C5	1.245(5)	C3'	C2'	1.525(6)
N4	C1'	1.452(5)	C2'	C1'	1.512(6)
N4	C5	1.420(5)	C5	C6	1.414(6)
N4	C9	1.381(5)	C6	C7	1.370(6)
N9	C7	1.338(5)	C2	C3	1.402(6)
N8	N1	1.374(5)	C3	C9	1.361(6)

Estimated stardard deviations are given in parentheses.

Figure 2 illustrates the molecular conformation of compound **25**. Probable hydrogen bonding interactions in the crystal lattice of **25** are listed in Table 2. Selected torsion angles (Table 3) and positional parameters (Table 4) for compound **25** are also given. Intramolecular distances (Table 5) and bond angles (Table 6) involving the hydro-

Table 6

Intramolecular Bond Angles (°) Involving the Hydrogen Atoms in Compound 25

atom	atom	atom	angle	atom	atom	atom	angle
C5'	O5'	O5'H	116.63	C4'	C3'	C3'H	110.82
C3'	O3'	O3'H	108.48	C2'	C3'	C3'H	108.58
C2'	O2'	O2'H	111.50	O2'	C2'	C2'H	103.22
C7	N9	N9HA	115.50	C3'	C2'	C2'H	114.70
C7	N9	N9HB	117.65	C1'	C2'	C2'H	111.13
N9H	N9	N9HB	126.67	O4'	C1'	C1'H	96.28
O5'	C5'	C5'HA	107.88	N4	C1'	C1'H	111.11
O5'	C5'	C5'HB	114.11	C2'	C1'	C1'H	117.38
C4'	C5'	C5'HA	115.72	C5	C6	C6H	111.96
C4'	C5'	C5'HB	102.35	C7	C6	C6H	125.03
C5'HA	C5'	C5'HB	109.96	N1	C2	C2H	117.96
O4'	C4'	C4'H	111.17	C3	C2	C2H	127.97
C5'	C4'	C4'H	116.28	C2	C3	C3H	131.33
C3'	C4'	C4'H	99.44	C9	C3	C3H	124.49
O3'	C3'	C3'H	115.52				

Intramolecular Bond angles (°) Involving the Nonhydrogen Atoms in Compound 25.

atom	atom	atom	angle	atom	atom	atom	angle
C4	O4'	C1'	108.4(3)	C3'	C2'	C1'	102.0(4)
C1'	N4	C5	119.1(4)	O4'	C1'	N4	108.6(3)
C1'	N4	C9	120.0(4)	O4'	C1'	C2'	103.1(3)
C5	N4	C9	120.8(4)	N4	C1'	C2'	117.3(4)
N1	N8	C7	122.8(4)	O6	C5	N4	119.2(4)
N1	N8	C9	112.0(3)	O6	C5	C6	124.0(4)
C7	N8	C9	125.0(4)	N4	C5	C6	116.9(4)
N8	N1	C2	103.0(3)	C5	C6	C7	122.8(4)
O5'	C5'	C4'	106.9(4)	N9	C7	N8	115.4(4)
O4'	C4'	C5'	108.8(3)	N9	C7	C6	128.2(4)
O4'	C4'	C3'	106.5(4)	N8	C7	C6	116.3(4)
C5'	C4'	C3'	114.1(5)	N1	C2	C3	113.8(4)
O3'	C3'	C4'	106.9(4)	C2	C3	C9	104.1(4)
O3'	C3'	C2'	112.6(4)	N4	C9	N8	117.7(4)
C4'	C3'	C2'	101.5(4)	N4	C9	C3	135.1(4)
O2'	C2'	C3'	115.4(4)	N8	C9	C3	107.2(4)
O2'	C2'	C1'	110.6(4)				

Estimated standard deviations are given in parentheses.

gen atoms in **25** are also listed. The structural determination confirms that compound **25** is an N4-glycosylated product with the β-anomeric configuration. Further details of the X-ray structural determinations of compound **25** and certain related nucleosides will be published elsewhere [36].

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 solvent 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 reagent 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).

7-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine-4,6(1*H*,5*H*)-dione (**6**).

A mixture of dry pyrazolo[3,4-*d*]pyrimidine-4,6(1*H*,5*H*,7*H*)-dione [17] (**4**, 3.04 g, 20 mmoles), ammonium sulfate (50 mg), 1,1,1,-3,3,3-hexamethyldisilazane (HMDS, 10 ml) and anhydrous pyridine (6.6 ml) was heated under reflux for 3 hours. Excess HMDS and pyridine were removed by distillation and the residue was subjected to a high vacuum for several hours. The dry residue was dissolved in anhydrous 1,2-dichloroethane (180 ml) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**5**, 11.08 g, 22 mmoles) was added. The mixture was cooled to 0° and trimethylsilyl trifluoromethanesulfonate (TMS triflate, 5.4 ml, 1.44 equivalents) was added dropwise with stirring. The solution was gradually warmed up to room temperature and stirred for an additional 6 hours. Methanol (6 ml) was added and after stirring for 30 minutes at room temperature, the solvents were evaporated. The residue was dissolved in ethyl acetate (300 ml) and the organic layer washed successively with saturated aqueous sodium hydrogen carbonate (2 x 75 ml), water (2 x 75 ml), saturated aqueous sodium chloride (50 ml) and dried over anhydrous sodium sulfate. After evaporation of the solvent, the solid thus obtained was crystallized from ethyl acetate to yield 7.8 g (66%) of the title compound as white needles, mp 255-256°; ir: ν max 1720 (C=O), 3100-3450 (NH) cm^{-1}; uv (*p*H 1): λ max 234 nm (ε 20,600); (*p*H 7): λ max 234 nm (ε 24,500); (*p*H 11): λ max 231 nm (ε 28,700); ^{1}H nmr (DMSO-d_6): δ 4.51-4.76 (m, 3 H, $C_{4'}H$ and $C_{5'}CH_2$), 6.18 (dd, 1 H, $C_{3'}H$), 6.35 (dd, 1 H, $C_{2'}H$), 6.52 (d, 1 H, J = 2.7 Hz, $C_{1'}H$), 7.36-7.94 (m, 15 H, 3 OBz), 8.54 (s, 1 H, C_3H), 11.28 (br s, 1 H, N_1H) and 13.73 (br s, 1 H, N_5H).

Anal. Calcd. for $C_{31}H_{24}N_4O_9$: C, 62.41; H, 4.06; N, 9.39. Found: C, 62.34; H, 3.97; N, 9.22.

7-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4,6(1*H*,5*H*)-dione (**3**).

A solution of **6** (5.0 g, 8.3 mmoles) in methanolic ammonia (50 ml, saturated at 0°) was stirred at room temperature for 18 hours in a pressure bottle. After the evaporation of methanol, the residue was triturated with warm benzene (3 x 25 ml) and crystallized from water to give 2.1 g (88%) of **3**, mp 241-243° (lit [19] mp 241°); ir: ν max 1680 (C=O), 3100-3450 (OH, NH) cm^{-1}; uv (*p*H 1): λ max 253 nm (ε 6,950); (*p*H 7): λ max 252 nm (ε 7,100); (*p*H 11): λ max 263 nm (ε 9,600); ^{1}H nmr (DMSO-d_6): δ 3.61 (m, 2 H, $C_{5'}CH_2$), 3.63 (m, 1 H, $C_{4'}H$), 4.10 (m, 1 H, $C_{3'}H$), 4.77 (m, 1 H, $C_{2'}H$), 5.02-5.21 (m, 3 H, $C_{2'}OH$, $C_{3'}OH$ and $C_{5'}OH$), 6.09 (d, 1 H, J = 6.0 Hz, $C_{1'}H$), 8.45 (s, 1H, C_3H), 11.11 (br s, 1 H, N_1H) and 13.67 (br s, 1 H, N_5H).

Anal. Calcd. for $C_{10}H_{12}N_4O_6$: C, 42.25; H, 4.25; N, 19.71. Found: C, 41.94; H, 4.04; N, 19.62.

7-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-6-oxopyrazolo[3,4-*d*]-pyrimidine-4(1*H*,5*H*)-thione (**7**).

To a suspension of **6** (2.0 g, 3.3 mmoles) in dry dioxane (70 ml) was added purified phosphorus pentasulfide (1.76 g, 3.9 mmoles) and the reaction mixture was heated under reflux for 3 hours with the exclusion of moisture. Excess dioxane was evaporated and the residual syrup poured on crushed ice (~100 g) with vigorous stirring. After stirring for 1 hour, chloroform (200 ml) was added and the organic layer was separated, washed successively with water (2 x 50 ml), saturated aqueous sodium chloride solution (50 ml), dried over anhydrous sodium sulfate and evaporated to dryness. The residue was purified by chromatography on a

silica gel column (3 x 25 cm) using 2% methanol in chloroform as eluent, to afford 1.4 g (68%) of **7** as a yellow amorphous powder, mp 206-208°; ir: ν max 1100 (C=S), 1680 (C=O), 3260 (NH) cm^{-1}; uv (*p*H 1): λ max 233 nm (ε 25,600), 349 (29,300); (*p*H 7): λ max 232 nm (ε 21,800), 349 (28,700); (*p*H 11): λ max 230 nm (ε 21,300), 343 (25,400); ^1H nmr (DMSO-d_6): δ 4.70-4.74 (m, 3 H, $C_{4'}H$ and $C_{5'}CH_2$), 6.07 (m, 1 H, $C_{3'}H$), 6.32 (dd, 1 H, $C_{2'}H$), 6.50 (d, 1 H, J = 2.4 Hz, $C_{1'}H$), 7.48-8.01 (m, 16 H, C_3H and 3 OBz), 12.11 (s, 1H, N_1H) and 12.38 (s, 1 H, N_5H).

Anal. Calcd. for $C_{31}H_{24}N_4SO_8$: C, 60.78; H, 3.95; N, 9.14; S, 5.23. Found: C, 60.53; H, 4.21; N, 9.23; S, 5.01.

7-β-D-Ribofuranosyl-6-oxopyrazolo[3,4-*d*]pyrimidine-4(1*H*,5*H*)-thione (**8**).

In a similar manner as described for **3**, debenzoylation of **7** (1.0 g, 1.63 mmoles) with methanolic ammonia (40 ml) gave 0.41 g (84%, crystallized from ethanol) of **8**, mp 204-205°; ir: ν max 1100 (C=S), 3100-3500 (OH, NH) cm^{-1}; uv (*p*H 1): λ max 258 nm (ε 8,200), 324 (18,400); (*p*H 7): λ max 257 nm (ε 8,800), 323 (19,500); (*p*H 11): λ max 283 nm (ε 12,700), 320 (18,500); ^1H nmr (DMSO-d_6): δ 3.41 (m, 2 H, $C_{5'}CH_2$), 3.84 (m, 1 H, $C_{4'}H$), 4.10 (m, 1 H, $C_{3'}H$), 4.77 (m, 1 H, $C_{2'}H$), 4.87-5.26 (m, 3 H, $C_{2'}OH$, $C_{3'}OH$ and $C_{5'}OH$), 6.08 (d, 1 H, J = 6.0 Hz, $C_{1'}H$), 8.53 (s, 1 H, C_3H), 12.42 (s, 1 H, N_1H) and 13.82 (br s, 1 H, N_5H).

Anal. Calcd. for $C_{10}H_{12}N_4SO_5$: C, 39.99; H, 4.03; N, 18.65; S, 10.67. Found: C, 39.68; H, 4.14; N, 18.47; S, 10.69.

7-β-D-Ribofuranosyl-2',6-anhydropyrazolo[3,4-*d*]pyrimidin-4(1*H*)-one (**9**).

To a solution of **3** (0.57 g, 2 mmoles) and diphenyl carbonate (0.54 g, 2.5 mmoles) in hexamethylphosphoramide (3 ml) was added sodium hydrogen carbonate (26 mg) and the reaction mixture was heated at 150° for 30 minutes. The reaction mixture was cooled to room temperature and then poured into water (20 ml). The aqueous layer was washed with chloroform (3 x 10 ml) and evaporated to dryness. The residue was crystallized from aqueous ethanol to afford 0.37 g (70%) of **9**, mp 298° dec; ir: ν max 1680 (C=O), 3150-3490 (OH, NH) cm^{-1}; uv (*p*H 1): λ max 239 nm (ε 5,600), 251 (6,000); (*p*H 7): λ max 239 nm (ε 6,700), 251 (7,000); (*p*H 11): λ max 258 nm (ε 7,900); ^1H nmr (DMSO-d_6): δ 4.11 (m, 2 H, $C_{5'}CH_2$), 4.47-4.63 (m, 3 H, $C_{2'}H$, $C_{3'}H$ and $C_{4'}H$), 5.38 (br s, 1 H, $C_{3'}OH$), 5.64 (br s, 1 H, $C_{5'}OH$), 6.05 (s, 1 H, $C_{1'}H$), 7.95 (s, 1 H, C_3H) and 11.25 (br s, 1 H, N_1H).

Anal. Calcd. for $C_{10}H_{10}N_4O_5 \cdot 1/5H_2O$: C, 44.62; H, 3.88; N, 20.76. Found: C, 44.85; H, 3.62; N, 20.53.

4-Amino-7-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-6(1*H*)-one (**11**). Method A.

A mixture of **6** (2.38 g, 4.0 mmoles), tetraethylammonium chloride hydrate (2.64 g, 8.0 mmoles), *N*,*N*-diethylaniline (0.13 ml, 8.08 mmoles) and freshly distilled phosphorus oxychloride (20 ml) was heated under a nitrogen atmosphere in a round bottom flask placed in an oil bath at 100° for 30 minutes. The reaction mixture was allowed to cool to room temperature and excess phosphorus oxychloride was evaporated. The residue was dissolved in chloroform (100 ml), poured onto crushed ice (~100 g) and stirred vigorously for 1 hour. The aqueous phase was washed with chloroform (3 x 50 ml). The combined organic extracts were washed successively with cold 1N hydrochloric acid (50 ml), cold saturated aqueous sodium hydrogen carbonate (2 x 40 ml), cold water (2 x 40 ml), dried over anhydrous sodium sulfate and evaporated to yield **10**. The residual **10** was dissolved in methanolic ammonia (30 ml, saturated at 0°) and the solution was stirred overnight at room temperature in a pressure bottle. Methanol was evaporated and the residue was purified by chromatography on a silica gel column (3 x 25 cm) using ethyl acetate: acetone: methanol: water (5:1:1:1, v/v) as the eluent to yield a colorless amorphous solid. Crystallization of the solid from aqueous ethanol gave 0.50 g (44.5%) of **11**, mp 228-230°; ir: ν max 1680 (C=O), 3200-3500 (OH, NH, NH_2) cm^{-1}; uv (*p*H 1): λ max 270.5 nm (ε 8,200); (*p*H 7): λ max 250 nm (ε 13,000); (*p*H 11): λ max 269.5 nm (ε 11,900); ^1H nmr (DMSO-d_6): δ 3.55 (m, 2 H, $C_{5'}CH_2$), 3.64 (m, 1 H, $C_{4'}H$), 4.07 (m, 1 H, $C_{3'}H$), 4.65 (m, 1 H, $C_{2'}H$), 4.95-5.08 (m, 3H, $C_{2'}OH$, $C_{3'}OH$ and $C_{5'}OH$), 6.19 (d, 1 H, J = 6.4 Hz, $C_{1'}H$), 7.85 (s, 2 H, NH_2), 8.13 (s, 1H, C_3H) and 13.0 (br s, 1 H, N_1H).

Anal. Calcd. for $C_{10}H_{13}N_5O_5 \cdot H_2O$: C, 39.86; H, 5.02; N, 23.24. Found: C, 39.57; H, 5.08; N, 23.03.

Method B.

A suspension of **7** (1.0 g, 1.63 mmoles) in liquid ammonia (10 ml) was heated at 80° in a sealed pressure vessel for 24 hours. After cooling to -40°, the reaction vessel was opened and ammonia was allowed to evaporate. The residue was triturated several times with ether. The ether insoluble residue was purified on a silica gel column (2 x 25 cm) using ethyl acetate: acetone: methanol: water (5:1:1:1, v/v) as the eluent to yield 90 mg of pure **11**, mp 227-229°. This product was found to be identical in all respects (mixture mp, tlc, ir, uv and ^1H nmr) to that obtained by Method A.

7-(2,3,5-Tri-*O*-benzyl-β-D-arabinofuranosyl)pyrazolo[3,4-*d*]pyrimidine-4,6(1*H*,5*H*)-dione (**13**) and 2-(2,3,5-Tri-*O*-benzyl-β-D-arabinofuranosyl)pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dione (**15**).

A mixture of dry **4** (7.0 g, 46 mmoles), ammonium sulfate (150 mg), HMDS (60 ml) and anhydrous pyridine (15 ml) was heated under reflux overnight. Excess HMDS and pyridine were removed by distillation and the residue was subjected to a high vacuum for several hours. A solution of 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride [20] (**12**) [prepared from 2,3,5-tri-*O*-benzyl-1-O-(*p*-nitrobenzoyl)-D-arabinose (28.0 g, 49.2 mmoles)], in anhydrous 1,2-dichloroethane (300 ml) was added with stirring to the above silyl derivative. The mixture was cooled to 0° and tin(IV) chloride (2 ml, 1.44 molar equivalents) was added dropwise under argon atmosphere. The reaction mixture was allowed to warm gradually to room temperature and the stirring continued for additional 10 hours (a total reaction time of 16 hours). The solvent was evaporated, the residue dissolved in ethyl acetate (250 ml), and washed with saturated aqueous sodium hydrogen carbonate solution (100 ml). The emulsion thus formed was filtered through a Celite pad, the organic layer separated, dried over anhydrous sodium sulfate and evaporated to dryness. The residual glass thus obtained was purified by chromatography over silica gel column (3.5 x 40 cm) using 5% methanol in dichloromethane as the eluent. The following two nucleosides were isolated in the order listed: 7-(2,3,5-Tri-*O*-benzyl-β-D-arabinofuranosyl)pyrazolo[3,4-*d*]pyrimidine-4,6(1*H*,5*H*)-dione (**13**). Crystallized from a mixture of dichloromethane: cyclohexane: ethanol to yield 8.5 g (33%) as colorless needles, mp 145-146°; ir: ν max 1690, 1710 (C=O), 3200-3400 (NH) cm^{-1}; uv (methanol): λ max 256 nm (ε 9,400); ^1H nmr (DMSO-d_6): δ 6.65 (d, 1H, J = 6.0 Hz, $C_{1'}H$), 8.43 (s, 1 H, C_3H), 10.98 (s, 1 H, N*H*), 13.52 (s, 1 H, N*H*) and other sugar protons.

Anal. Calcd. for $C_{31}H_{30}N_4O_6$: C, 67.13; H, 5.40; N, 10.10. Found: C, 67.21; H, 5.27; N, 10.28.

2-(2,3,5-Tri-*O*-benzyl-β-D-arabinofuranosyl)pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dione (**15**) was obtained as white needles after crystallization from a mixture of ethyl acetate and cyclohexane, yield 0.60 g (2.3%), mp 150-151°; ir: ν max 1680, 1710 (C=O), 3060-3300 (NH) cm^{-1}; uv (methanol): λ max 237 nm (ε 6,200), 262 (8,500); ^1H nmr (DMSO-d_6): δ 6.22 (d, 1 H, J = 5.7 Hz, $C_{1'}H$), 8.43 (s, 1 H, C_3H), 10.78 (s, 1 H, N*H*), 11.42 (s, 1 H, N*H*) and other sugar protons.

Anal. Calcd. for $C_{31}H_{30}N_4O_6$: C, 67.13; H, 5.40; N, 10.10. Found: C, 66.97; H, 5.24; N, 9.94.

7-β-D-Arabinofuranosylpyrazolo[3,4-*d*]pyrimidine-4,6(1*H*,5*H*)-dione (**14**).

To a solution of **13** (2.0 g, 3.6 mmoles) in absolute ethanol (50 ml) were added cyclohexene (20 ml) and palladium hydroxide (1.5 g, 20% on carbon) and the mixture was heated under reflux for 17 hours. The reaction mixture was cooled, filtered through a Celite pad and washed with hot methanol (5 x 50 ml). The combined filtrate and washings were evaporated to dryness and the residue was crystallized from aqueous ethanol to yield 0.60 g (59%) of **14** as needles, mp 233-235°; ir: ν max 1680, 1715 (C=O), 2800-3500 (OH, NH) cm^{-1}; uv (*p*H 1): λ max 254 nm (ε 6,800); (*p*H 7): λ max 252 nm (ε 6,900); (*p*H 11): λ max 265 nm (ε 9,500); ^1H nmr (DMSO-d_6): δ 6.34 (d, 1 H, J = 5.3 Hz, $C_{1'}H$), 8.15 (s, 1 H, C_3H), 11.03 (br s, 1 H, N*H*), 13.55 (br s, 1 H, N*H*) and other sugar protons.

Anal. Calcd. for $C_{10}H_{12}N_4O_6$: C, 42.25; H, 4.25; N, 19.71. Found: C, 42.30; H, 4.09; N, 19.80.

2-β-D-Arabinofuranosylpyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dione (**16**).

To a stirred solution of **15** (1.1 g, 2 mmoles) in dry dichloromethane (40 ml) at -78° was added boron trichloride (1*M* solution in dichloromethane, 35 ml, 35 mmoles) during 15 minutes. The reaction mixture was stirred at -78° for 2 hours and then at -20° for 3 hours. A mixture of methanol:dichloromethane (40 ml, 1:1, v/v) was added to the mixture, which was then stirred at 0° for 30 minutes, diluted with additional amount of methanol (50 ml) and neutralized with concentrated ammonium hydroxide keeping the temperature below 10°. The reaction mixture was filtered and the filtrate was evaporated to a colorless solid. The solid was dissolved in methanol, adsorbed onto silica gel (10 g), and the mixture was evaporated to dryness. The dried silica gel was placed on top of a flash silica gel column (4 x 20 cm) packed in dichloromethane. The column was eluted with dichloromethane→methanol gradient to give the desired product, which was crystallized from aqueous ethanol to yield 0.28 g (50%) as white needles, mp 251-253°; ir: ν max 1670, 1710 (C=O), 2840-3440 (OH, NH) cm^{-1}; uv (*p*H 1 and 7): λ max 261 nm (ε 5,400); (*p*H 11): λ max 283 nm (ε 3,900), 287 (3,900); ^1H nmr (DMSO-d_6): δ 5.84 (d, 1 H, J = 5.2 Hz, $C_{1'}H$), 8.40 (s, 1 H, C_3H), 10.80 (br s, 1 H, N*H*), 11.20 (br s, 1 H, N*H*) and other sugar protons.

Anal. Calcd. for $C_{10}H_{12}N_4O_6$: C, 42.25; H, 4.25; N, 19.71. Found: C, 42.22; H, 4.26; N, 19.44.

4-Amino-7-β-D-arabinofuranosylpyrazolo[3,4-*d*]pyrimidin-6(1*H*)-one (**19**).

To a solution of **13** (0.80 g, 1.44 mmoles) in dry pyridine (8 ml) was added phosphorus oxychloride (0.25 ml) and the mixture was stirred at room temperature for 2 days. Excess pyridine and phosphorus oxychloride were evaporated to yield **17**. The crude **17** was dissolved in methanolic ammonia (200 ml, saturated at 0°) and the solution was stirred at room temperature for 18 hours in a pressure bottle. Methanol was removed by evaporation and the residue was chromatographed over a silica gel column (2.5 x 25 cm) using 5% methanol in dichloromethane as the solvent. The appropriate homogeneous fractions were pooled and evaporated to yield 0.62 g (75%) of **18** as gum.

The gummy **18** (0.60 g) was dissolved in absolute ethanol (50 ml) to which were added cyclohexene (20 ml) and palladium hydroxide (0.60 g, 20% on carbon) and heated under reflux for 17 hours with the exclusion of moisture. After cooling the reaction mixture to room temperature, it was filtered, decolorized with carbon and evaporated to dryness. The residue was crystallized from ethanol to yield 0.24 g (76%) of **19** as needles, mp 210-212°; ir: ν max 1650 (C=O), 3000-3600 (OH, NH, NH_2) cm^{-1}; uv (*p*H 1): λ max 269 nm (ε 7,000); (*p*H 7): λ max 249 nm (ε 10,700); (*p*H 11): λ max 270 nm (ε 12,200); ^1H nmr (DMSO-d_6): δ 6.33 (d, 1 H, J = 5.5 Hz, $C_{1'}H$), 7.64 (br s, 2 H, NH_2), 7.89 (s, 1 H, C_2H), 12.7 (s, 1 H, N*H*) and other sugar protons.

Anal. Calcd. for $C_{10}H_{13}N_5O_5 \cdot 1/4C_2H_5OH$: C, 42.79; H, 4.96; N, 23.76. Found: C, 42.91; H, 4.64; N, 23.43.

4-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-7-hydroxypyrazolo[1,5-*a*]pyrimidin-5-one (**21**).

In a similar manner as described for **6**, silylation of 7-hydroxypyrazolo[1,5-*a*]pyrimidin-5(4*H*)-one [23] (**20**, 3.0 g, 19.8 mmoles) with HMDS (30 ml), in the presence of ammonium sulfate (0.10 g), and subsequent glycosylation with **5** (10.0 g, 19.8 mmoles) in the presence of TMS triflate (5 ml) gave the crude reaction product. Purification of the crude product by chromatography on a silica gel column (4 x 40 cm) using a gradient of dichloromethane→ethanol gave 6.5 g (55%) of **21** as crystalline material, mp 78-80°; ir: ν max 1715 (C=O), 3200-3350 (OH) cm^{-1}; uv (methanol): λ max 278 nm (ε 3,700).

Anal. Calcd. for $C_{32}H_{25}N_3O_9$: C, 64.54; H, 4.23; N, 7.06. Found: C, 64.75; H, 4.49; N, 6.73.

4-β-D-Ribofuranosyl-7-hydroxypyrazolo[1,5-*a*]pyrimidin-5-one (**22**).

In a similar manner as described for **3**, debenzoylation of **21** (1.30 g, 2.2 mmoles) with methanolic ammonia (100 ml) gave 0.43 g (70%) of the title product, mp 180-182° (sintering at 125°); ir: ν max 1690 (C=O), 3100-3500 (OH) cm^{-1}; uv (*p*H 1): λ max 275 nm (ε 5,100), 277 (5,100); (*p*H 7): λ max 225 nm (ε 11,400), 274 (14,800); (*p*H 11): λ max 225 nm (ε 15,800), 275 (12,700); ^1H nmr (DMSO-d_6): δ 5.03 (s, 1 H, C_6H), 5.98 (d, 1 H, J = 4.5 Hz, $C_{1'}H$), 6.0 (d, 1 H, J = 1.38 Hz, C_3H), 7.47 (d, 1 H, J = 1.44 Hz, C_2H) and other sugar protons.

Anal. Calcd. for $C_{11}H_{13}N_3O_6 \cdot H_2O$: C, 43.85; H, 5.01; N, 13.94. Found: C, 44.03; H, 5.36; N, 13.75.

7-Amino-4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[1,5-*a*]-pyrimidin-5-one (**24**).

In a similar manner as described for **6**, silylation of 7-aminopyrazolo[1,5-*a*]pyrimidin-5(4*H*)-one [23] (**23**, 2.0 g, 13.3 mmoles) with HMDS (20 ml) in pyridine (10 ml), in the presence of ammonium sulfate (0.15 g), and subsequent glycosylation with **5** (6.8

g, 13.5 mmoles) in the presence of TMS triflate (3.8 ml) gave the crude reaction product. Purification of the crude product by chromatography on a silica gel column (2.5 x 40 cm) using 2.5% ethanol in dichloromethane as the eluent gave 6.2 g (78%) of **24** as a colorless glass, mp 113-115°; ir: ν max 1710 (C=O), 3340-3440 (NH_2) cm^{-1}; uv (methanol): λ max 227 nm (ε 61,100), 276 (21,800).

Anal. Calcd. for $C_{32}H_{26}N_4O_8 \cdot 0.3H_2O$: C, 64.06; H, 4.46; N, 9.33. Found: C, 63.84; H, 4.29; N, 9.25.

7-Amino-4-β-D-ribofuranosylpyrazolo[1,5-*a*]pyrimidin-5-one (**25**).

In a similar manner as described for **3**, debenzoylation of **24** (3.5 g, 5.9 mmoles) with methanolic ammonia (200 ml) gave 1.4 g (84%) of **25**, after crystallization from methanol, mp 215-216°; ir: ν max 1660 (C=O), 3100-3500 (OH, NH_2) cm^{-1}; uv (*p*H 1 and 11): λ max 279 nm (ε 13,400); (*p*H 7): λ max 278 nm (ε 13,300); ^{1}H nmr (DMSO-d_6): δ 4.92 (s, 1 H, C_6H), 6.13 (d, 1 H, J = 4.9 Hz, $C_{1'}H$), 6.4 (d, 1 H, J = 2.0 Hz, C_3H), 7.79 (d, 1 H, J = 1.8 Hz, C_2H) and other sugar protons.

Anal. Calcd. for $C_{11}H_{14}N_4O_5$: C, 46.81; H, 5.00; N, 19.85. Found: C, 46.93; H, 4.90; N, 19.62.

2-Amino-4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazolo[2,5-*d*]-pyrimidine-5,7(6*H*)-dione (**27**). Method A.

A mixture of 2-chlorothiazolo[4,5-*d*]pyrimidine-5,7(4*H*,6*H*)-dione [29] (**26**, 1.34 g, 6.6 mmoles), HMDS (20 ml), ammonium sulfate (30 mg) and dry pyridine (1 ml) was heated under reflux in a nitrogen atmosphere for 3 hours. After the removal of solvents, the residue was taken up in anhydrous acetonitrile (50 ml) and the carbohydrate **5** (4.0 g, 7.9 mmoles) was added. The reaction mixture was cooled to 0° and TMS triflate (1.8 ml, 1.44 equivalents) was gradually added with stirring. The mixture was allowed to warm to room temperature and stirred overnight. Methanol (3 ml) was added and the reaction mixture evaporated to dryness. The residue was dissolved in dichloromethane (100 ml) and washed successively with saturated aqueous sodium hydrogen carbonate (2 x 25 ml), water (2 x 50 ml), saturated aqueous sodium chloride (25 ml), dried over anhydrous sodium sulfate and evaporated to dryness. Purification of the crude product on a silica gel column (2 x 25 cm) using a 3% methanol in dichloromethane as the eluent gave 1.02 g (25%) of pure **27** as light yellow foam. An analytical sample was obtained by crystallization from ethanol, mp 150-152°; ir: ν max 1720 (C=O), 3100-3450 (NH, NH_2) cm^{-1}; uv (*p*H 1): λ max 233 nm (ε 44,700), 312 (12,400); (*p*H 7): λ max 233 nm (ε 44,800), 310 (13,100); (*p*H 11): λ max 233 nm (ε 43,600), 305 (9,700); ^{1}H nmr (DMSO-d_6): δ 4.52-4.74 (m, 3 H, $C_{4'}H$ and $C_{5'}H_2$), 6.22 (dd, 1 H, $C_{3'}H$), 6.24 (d, 1 H, $C_{2'}H$), 6.52 (d, 1 H, J = 2.1 Hz, $C_{1'}H$), 7.36-7.98 (m, 15 H, 3 OBz), 8.60 (s, 2 H, NH_2) and 11.43 (s, 1 H, N_6H).

Anal. Calcd. for $C_{31}H_{24}N_4SO_9$: C, 59.23; H, 3.84; N, 8.91; S, 5.10. Found: C, 58.99; H, 3.59; N, 8.78; S, 5.10.

Method B.

A mixture of 2-aminothiazolo[4,5-*d*]pyrimidine-5,7(4*H*,6*H*)-dione [29] (**29**, 0.6 g, 3.25 mmoles), ammonium sulfate (15 mg), pyridine (0.5 ml) and HMDS (10 ml) was heated under reflux for 3 hours. After the removal of solvents, the residue was taken up in dry acetonitrile (25 ml) and **5** (2.0 g, 3.96 mmoles) was added. The reaction mixture was cooled to 0° and TMS triflate (0.9 ml) was added with stirring. The reaction mixture was allowed to warm to room temperature and stirred overnight. After workup, as described in Method A, the product was crystallized from ethanol to yield 1.68 g (82%) of **27**, mp 152-154°. The product was found to be identical in all respects (mixture mp, tlc, ir, uv and ^{1}H nmr) to **27** prepared by Method A.

2-Amino-4-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-5,7(6*H*)-dione (**28**).

In a similar manner as described for **3**, debenzoylation of **27** (1.23 g, 1.95 mmoles) with methanolic ammonia at room temperature gave 0.50 g (81.0%) of the title compound. It was crystallized from water as needles, mp 290-292° dec; ir: ν max 1620 (C=O), 3200-3500 (OH, NH, NH_2) cm^{-1}; uv (*p*H 1): λ max 221 nm (ε 18,000), 308 (12,900); (*p*H 7): λ max 220 nm (ε 20,300), 308 (14,100); (*p*H 11): λ max 304 nm (ε 12,900); ^{1}H nmr (DMSO-d_6): δ 3.43-3.71 (m, 3 H, $C_{4'}H$ and $C_{5'}CH_2$), 6.11 (d, 1 H, J = 4.2 Hz, $C_{1'}H$), 8.48 (s, 2 H, NH_2), 11.15 (br s, 1 H, N_6H) and other sugar protons.

Anal. Calcd. for $C_{10}H_{12}N_4SO_6$: C, 37.97; H, 3.82; N, 17.71; S, 10.13. Found: C, 37.93; H, 3.75; N, 17.52; S, 10.37.

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