The Crystal Structure of 5-Amino-6-chloro-4-nitro-2-(β-D-ribofuranosyl)-2*H*-pyridazin-3-one

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The nucleoside, 5-amino-6-chloro-4-nitro-2-β-D-ribofuranosyl-2*H*-pyridazin-3-one (**4**), was synthesized as part of a study to prepare potential chemotherapeutic compounds. An X-ray crystal structure study of the compound was initiated in order to substantiate its formula and to examine its conformation and absolute configuration. The structure was determined using direct methods and refined to an R of 0.020 (R_w = 0.024). The compound has a glycosyl torsion angle of 71.2°. The structure contains three intermolecular hydrogen bonds and one intramolecular hydrogen bond.

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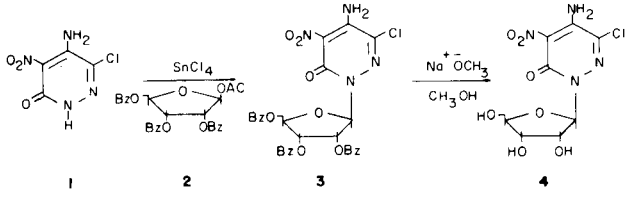
In a search to prepare new nucleosides with chemotherapeutic activity, an analog of the active nucleoside 6-azacytidine (2) was synthesized. It has been attempted to correlate the activity of this compound with various structural parameters such as the glycosyl torsion angle denoted as χ. Donohue and Trueblood (3) have pointed out that the most probable values of this torsion angle range between -15° and +75°, termed *anti*, and between 165° and 255° termed *syn*. In many biologically active *ortho* azanucleosides, such as 6-azacytidine, this torsion angle has been found to have a "high *anti*" value, that is, intermediate between the *anti* and *syn* region having a range from about 76° to 110° (4). It has been proposed that such values are possible in *ortho* azanucleosides because the *ortho* nitrogen has replaced a C-H group in that position and the lack of the H atom allows relatively free rotation about the glycosidic bond.

Scheme



The nucleoside of choice was **4**, a compound in which the 3-nitrogen atom of 6-azacytidine was replaced with a carbon atom substituted with a nitro group. This would allow one to evaluate the effect of replacing a ring nitrogen atom with a C-NO_2 group. Sidgwick (5) proposed that such a substitution would leave the electronic structure of the ring unchanged.

The synthesis for the nucleoside is given in the scheme. The treatment of the pyridazine base **1** (6) with one equivalent of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribo-

Table 1
Physical and Crystal Data

monoclinic, C2	
Z	= 4
a	= 20.4166 (43) Å
b	= 8.7239 (11) Å
c	= 6.6753 (10) Å
β	= 91.293 (15) °
V	= 1188.6 (3) Å^3
D_x	= 1.803 g-cm^{-3}
D_m	= 1.783 g-cm^{-3}
μ	= 3.77 cm^{-1}
T	= 173 °K
F(000)	= 664

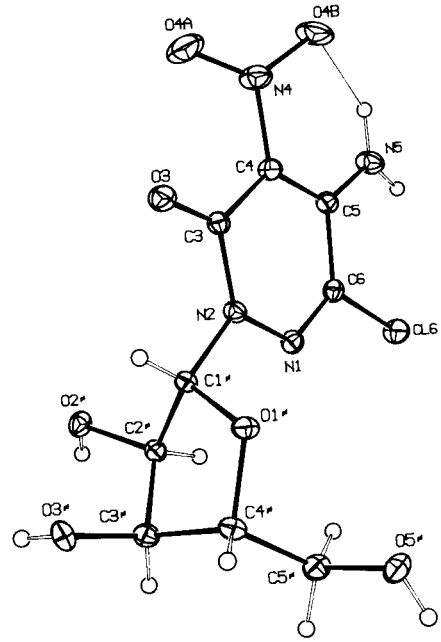


Figure 1. The conformation of the title compound with atom labels.

Table 2

Atomic Positions as Fractional Coordinates
(X 10^5; H Atoms X 10^4) and Isotropic Thermal Parameters
(X 10^4; H Atoms X 10^3). U's for Non-hydrogen Atoms
are Equivalent Isotropic Parameters

Atom	X	Y	Z	U
N(1)	106460(6)	76228(17)	25508(18)	125(3)
N(2)	110940(6)	87534(16)	26192(18)	115(3)
C(3)	109625(7)	103317(19)	25569(21)	118(4)
C(4)	102635(8)	106757(18)	25116(22)	134(4)
C(5)	97787(7)	95444(20)	24512(21)	123(4)
C(6)	100335(7)	79769(19)	24730(21)	121(4)
C(1')	117738(7)	82029(18)	26864(21)	105(4)
C(2')	118881(7)	69256(18)	42094(21)	112(4)
C(3')	124748(7)	61002(17)	33161(21)	127(4)
C(4')	122867(7)	61355(18)	10942(21)	127(4)
O(1')	119330(5)	75734(14)	7845(15)	126(3)
O(3)	114155(5)	112508(14)	25215(17)	162(3)
N(4)	100768(7)	122900(17)	25375(24)	197(4)
O(4A)	104590(7)	132229(17)	32724(25)	323(4)
O(4B)	95274(6)	126134(17)	18072(23)	270(4)
N(5)	91309(6)	97448(19)	24141(21)	175(4)
Cl(6)	94899(2)	64750(0)	23777(6)	171(1)
O(2')	119973(5)	75544(15)	61526(16)	143(3)
O(3')	130695(5)	69639(15)	35998(18)	153(3)
C(5')	118632(8)	47948(19)	4079(22)	146(4)
O(5')	117965(5)	48847(14)	-17345(16)	158(3)
H(5A)	8979(11)	10703(32)	2443(33)	22(6)
H(5B)	8865(14)	8926(39)	2475(39)	37(7)
H(1'A)	12013(9)	8955(27)	3010(27)	6(4)
H(2'A)	11503(10)	6282(29)	4203(30)	16(5)
H(3'A)	12512(10)	5091(29)	3787(32)	17(5)
H(4'A)	12666(9)	6173(24)	201(28)	9(4)
H(2')	11927(13)	6800(35)	6854(39)	33(7)
H(3')	13153(11)	6935(29)	4773(38)	20(5)
H(5'A)	11419(11)	4807(30)	949(32)	20(5)
H(5'B)	12073(10)	3846(28)	727(30)	15(5)
H(5')	11800(12)	3993(36)	-2154(38)	29(6)

furanose (**2**) in the presence of 2 molar equivalents of anhydrous stannic chloride afforded the blocked nucleoside **3**. This nucleoside formation is similar to the procedure of Saneyoshi and Satoh (7). Deblocking was achieved with sodium methoxide in methanol to provide the deblocked nucleoside **4** (8). The nucleoside was shown to be the β-anomer by X-ray diffraction.

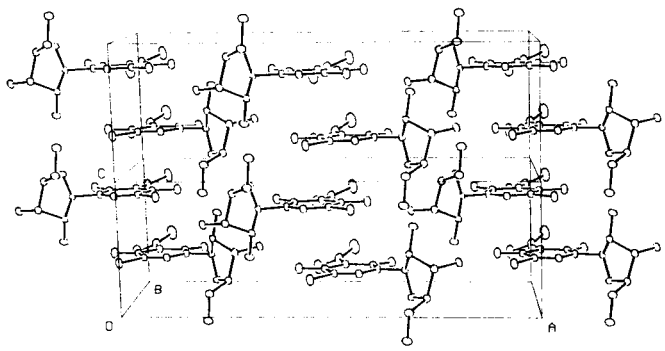


Figure 2. The packing diagram of the nucleoside viewed along the b axis. The figure shows the expected planarity of the base ring.

EXPERIMENTAL

5-Amino-2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-6-chloro-4-nitro-2*H*-pyridazin-3-one (**3**).

Compound **1** (1 g, 5.25 mmoles) was suspended in a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**2**) (2.65 g, 5.25 mmoles) in 250 ml of dry acetonitrile. Stannic chloride (1.21 ml, 10.5 mmoles) was added to the mixture and stirred for 24 hours at room temperature (the solution become clear in about one hour); tlc indicated the formation of the nucleoside **3**. The acetonitrile was removed *in vacuo* keeping the temperature of the water bath below 40°. The resulting syrup was dissolved in dry chloroform and poured into a solution of sodium bicarbonate. The suspension was then filtered through celite. The chloroform layer was separated, dried with anhydrous sodium sulfate and removed *in vacuo* to give a syrup. The syrup was chromatographed on a column of silica gel (60-200 mesh) using toluene:ethyl acetate (3:1) as eluent. The spot corresponding to the nucleoside was collected and the solvent removed *in vacuo* to give 1.7 g of a yellow foam (51%); ^{1}H nmr (deuteriochloroform): δ 6.6 (d, 1H, J = 4.0 Hz, H-1'), 8.5 (broad s, 2H, NH_2), and other sugar protons.

Table 3

Distances (Å), Angles (°) and Torsion Angles (°) in Base Ring

1	2	3	4	1-2	1-2-3	1-2-3-4
N(1)	N(2)	C(3)	C(4)	1.345(2)	126.08(12)	3.07(20)
N(2)	C(3)	C(4)	C(5)	1.403(2)	112.89(13)	-2.54(20)
C(3)	C(4)	C(5)	C(6)	1.458(2)	123.20(15)	1.03(21)
C(4)	C(5)	C(6)	N(1)	1.398(2)	114.09(13)	.35(21)
C(5)	C(6)	N(1)	N(2)	1.463(2)	124.70(15)	.03(21)
C(6)	N(1)	N(2)	C(3)	1.288(2)	118.97(14)	-1.95(21)
O(3)	C(3)	N(2)	N(1)	1.225(2)	119.93(13)	-176.05(13)
O(3)	C(3)	C(4)	C(5)		127.17(15)	176.51(15)
O(3)	C(3)	N(2)	C(1')			1.34(20)
O(3)	C(3)	C(4)	N(4)			-3.66(23)
N(4)	C(4)	C(3)	N(2)	1.459(2)	117.02(14)	177.29(13)
N(4)	C(4)	C(5)	C(6)		119.79(14)	-178.80(13)
N(4)	C(4)	C(5)	N(5)			-.09(24)
O(4A)	N(4)	O(4B)		1.222(2)	124.57(16)	
O(4A)	N(4)	C(4)	C(3)		118.86(14)	-25.16(22)
O(4A)	N(4)	C(4)	C(5)			154.67(16)
O(4B)	N(4)	C(4)	C(3)	1.245(2)	116.57(14)	155.35(15)
O(4B)	N(4)	C(4)	C(5)			-24.82(22)
N(5)	C(5)	C(4)	C(3)	1.334(2)	127.54(16)	179.73(14)
N(5)	C(5)	C(6)	N(1)		118.36(15)	-178.48(14)
N(5)	C(5)	C(6)	Cl(6)			1.84(19)
Cl(6)	C(6)	C(5)	C(4)	1.717(2)	118.90(11)	-179.33(11)
Cl(6)	C(6)	N(1)	N(2)		116.40(13)	179.72(10)
C(4)	C(3)	N(2)	C(1')			-179.54(12)
C(6)	N(1)	N(2)	C(1')			-179.49(12)
C(3)	N(2)	C(1')	C(2')		120.12(12)	135.53(13)
C(3)	N(2)	C(1')	O(1')			-106.48(14)
N(1)	N(2)	C(1')	C(2')		113.75(13)	-46.78(16)
N(1)	N(2)	C(1')	O(1')			71.22(15)

Distances (Å), Angles (°) and Torsion Angles (°) in Sugar Moiety

C(1')	C(2')	C(3')	C(4')	1.523(2)	101.17(11)	-40.54(13)
C(2')	C(3')	C(4')	O(1')	1.530(2)	100.80(11)	33.55(14)
C(3')	C(4')	O(1')	C(1')	1.524(2)	105.56(11)	-12.81(14)
C(4')	O(1')	C(1')	C(2')	1.460(2)	109.06(11)	-13.79(14)
O(1')	C(1')	C(2')	C(3')	1.427(2)	106.12(12)	34.42(14)
O(2')	C(2')	C(1')	O(1')	1.421(2)	110.21(12)	157.04(11)
O(2')	C(2')	C(3')	C(4')		115.42(11)	-159.47(12)
O(2')	C(2')	C(3')	O(3')			-44.87(17)
O(3')	C(3')	C(2')	C(1')	1.438(2)	111.60(12)	74.05(14)
O(3')	C(3')	C(4')	O(1')		108.13(12)	-83.63(13)
O(3')	C(3')	C(4')	C(5')			155.48(12)
C(5')	C(4')	C(3')	C(2')	1.519(2)	114.03(12)	-87.34(14)
C(5')	C(4')	O(1')	C(1')		110.03(11)	110.65(13)
O(5')	C(5')	C(4')	C(3')	1.436(2)	107.39(12)	-172.95(12)
O(5')	C(5')	C(4')	O(1')			68.68(15)
N(2)	C(1')	C(2')	C(3')	1.468(2)	112.97(12)	154.33(12)
N(2)	C(1')	O(1')	C(4')		109.43(11)	-135.98(12)

Anal. Calcd. for $C_{30}H_{23}ClN_4O_{10}$ (634.99): C, 56.75; H, 3.65; Cl, 5.58; N, 8.82. Found: C, 56.69; H, 3.86; Cl, 5.77; N, 8.76.

5-Amino-6-chloro-4-nitro-2-(β-D-ribofuranosyl)-2*H*-pyridazin-3-one (**4**).

Compound **3** (5 g, 7.87 mmoles), 0.5 g of dry sodium methoxide and 60 ml of dry methanol were refluxed for 3 hours. Dowex 50W-X8 cation exchange resin (hydrogen form) was added slowly until *p*H 7 was reached. The Dowex resin was filtered and the methanol removed *in vacuo* to give a solid. Crystallization from water-ethanol gave 1.65 g (65%), mp 209-211°; ir (potassium bromide): 1300, 1530 cm^{-1} (NO_2), 1650 cm^{-1} (C=O), 3320 cm^{-1} (OH and NH_2); ^1H nmr (DMSO-d_6): δ 6.12 (d, 1H, J = 3.0, H-1') 8.31 (broad s, 2H, NH_2), and other sugar protons.

Anal. Calcd. for $C_9H_{11}ClN_4O_7$ (322.66): C, 33.50; H, 3.44; Cl, 10.99; N, 17.36. Found: C, 33.42; H, 3.19; Cl, 10.98; N, 17.56.

Data Collection.

The compound crystallized as pale yellow crystals in the monoclinic space group C2 with four molecules per unit cell. Accurate lattice parameters (Table 1) were determined by least-squares refinement of 45 2Θ angles ($25° < 2\Theta < 42°$, λ = .71069 Å, T = 173° K) measured on a Syntex P2, diffractometer. Systematic extinctions (hkℓ, h+k odd) were determined by preliminary intensity measurements.

Intensities for 1461 unique reflections ($2\Theta < 55°$) were measured at a temperature of 173° K by an ω-scan procedure (scan width, 1.5°; scan speed 2° min^{-1}) on the diffractometer. Backgrounds were counted one degree before and one degree after the $K_{\bar{\alpha}}$ peak, the total background counting time being equal to the total scan time. Four check reflections (004, 040, 600, 224) measured every 96 reflections indicated that the

Table 4

Least-Squares Data for the Base Ring

Atoms used for the calculation of the plane of the base	Deviations of the atoms from the plane of the base (Å)
N(1)	0.0025(12)
N(2)	-0.0113(12)
C(3)	0.0163(14)
C(4)	-0.0085(15)
C(5)	-0.0029(15)
C(6)	-0.0061(14)
exocyclic atoms adjacent to the base	
O(3)	0.0726(11)
N(4)	-0.0438(16)
N(5)	-0.0287(14)
Cl(6)	0.0314(14)
C(1)	-0.0026(14)

crystal suffered some decomposition during the data collection. Corrections for this and Lorentz and polarization effects were made.

Solution of the Structure.

Eleven atoms of the base rings were located in the E-map generated from the most consistent set of phases from MULTAN 78 (9). The remaining non-hydrogen atoms were located in a diffenence Fourier. Following the refinement of all positional and anisotropic thermal parameters of the non-hydrogen atoms (R = 0.040) another difference Fourier map was calculated. It was possible to locate all the hydrogen atoms in this map; subsequently the positions and isotropic thermal parameters of these atoms were refined. Refinement of all parameters based on 1421 reflections having $I < 3\sigma(I)$ and weight for F values calculated after the method of Stout and Jensen (10) yielded R = .0218, R_w = .0330 and a "goodness of fit" $\Sigma w\Delta^2/(m-n)^{1/2}$, of 1.546 (m = 1421, n = 233).

The final refinement cycles with $w_{hkl} = 1/(\sigma(F_{hkl}) + \Delta F_{hkl}/5.12)^2$ gave R = .0203, R_w = .0245 and a "goodness of fit" of 1.000 (11). Changes in the parameters were less than four percent of their errors. The scattering Simpson (12). The scattering factor tables for all other atoms and the anomalous dispersion corrections for Cl were taken from the International Tables for X-ray Crystallography (13).

Positional parameters and isotropic (or equivalent) thermal parameters of all atoms are given in Table 2. In as much as the nucleoside crystallizes in a non-centrosymmetric space group, an attempt was made to determine its absolute configuration. The reported configuration refined to an R value of 0.02026 while its enantiomorph (obtained by reversing the sign on all positional parameters) refined to an R value of 0.02100. Hamilton's R factor ratio indicates that we may accept at the 0.995 level the hypothesis that the absolute configuration is the reported structure (14).

Discussion.

The structure of the nucleoside and the atoms labels are shown in Figure 1. The bond lengths and angles and torsion angles are listed in Table 3.

The title compound is of interest because it is an *ortho*-azanucleoside. Several such compounds have been shown to have biological activity and structural studies have been initiated to attempt to determine a structure-function relationship. The compounds studied include both aza-pyrimidines [for example, 6-azacytidine (15) and 3-deaza-6-azauridine (4)] and 8-azapurines [for example, 8-azaadenosine (16) and formycin (17)]. Characteristics common to all these compounds are a glycosyl torsion angle in the "high *anti*" region, that is intermediate between the *anti* and *syn* region, and a rather short glycosidic bond. In all cases above except formycin, the glycosidic bond is a N-C bond while in formycin it is a C-C bond. In the title compound

Table 5

Bond Lengths (Å) in the Ribose Moiety for Title Compound **4**, 6-Azacytidine (**5**) and 3-Deaza-6-azauridine (**6**)

	4	**5**	**6**
C(1')-C(2')	1.523	1.537	1.507
C(2')-C(3')	1.530	1.530	1.528
C(3')-C(4')	1.524	1.522	1.526
C(4')-O(1')	1.460	1.440	1.446
O(1')-C(1')	1.427	1.427	1.429
C(2')-O(2')	1.421	1.421	1.411
C(3')-O(3')	1.438	1.415	1.426
C(4')-C(5')	1.519	1.511	1.507
C(5')-O(5')	1.436	1.428	1.415

Table 6

Hydrogen Bonding Data for the Nucleoside

1—2---3	1---3(Å)	2---3(Å)	1-2---3(°)
	Intermolecular		
O(2')-H(2')...O(5')	2.759(2)	1.937(30)	174(3)
O(3')-H(3')...O(3)	2.842(2)	2.075(25)	160(2)
O(5')-H(5')...O(3')	2.853(2)	2.039(30)	169(2)
	Intramolecular		
N(5)-H(5A)...O(4B)	2.644(2)	2.059(26)	124(2)

these same features are present as the χ torsion angle of 71.2° is at the upper portion of the *anti* range and the glycosidic bond length is 1.468(2) Å. It is probable that these parameters are present because of the absence of a hydrogen atom on the *ortho* atom (in contrast to the situation with an *ortho* C-H group) which reduces the energy barrier to rotation about the C-N bond and also allows rather close contacts between the *ortho* nitrogen atoms and the ribose. In this structure the N(1) - C(2') and N(1) - H(2'A) interatomic distances are 2.811(3) and 2.361(21) Å, respectively.

Several of the bond lengths (see Table 3) in the base ring are longer than normal. This is particularly true for N(2) - C(3), C(4) - C(5) and C(5) -C(6) which are all about 0.04 Å longer than chemically similar bonds in other nucleosides. These bonds are affected by the electron withdrawing properties of the adjacent functional groups, namely a carbonyl oxygen atom, a NO_2 group and a chlorine atom. The base ring atoms are essentially planar as is shown in Figure 2. The deviations of the ring atoms from the least-squares plane calculated for these six atoms are listed in Table 4. Also included in Table 4 are the deviations of the exocyclic atoms from that plane.

Bond lengths in the ribose moiety are normal. Comparisons of the similar bond lengths in 6-azacytidine, and 3-deaza-6-azauridine, are listed in Table 5. The sugar pucker is $^2T_3$ (18) and the conformation about C(4') C(5') is gt (19). Both of these conformational parameters are common in such nucleosides.

The structure includes one intramolecular and three intermolecular hydrogen bonds. Data for these interactions are listed in Table 6. The effect of the hydrogen bonding involving O(4B) can be seen in the N(4) -O(4B) bond length which is nearly 0.02 Å longer than the N(4) - O(4A) bond. All alcohol hydrogen atoms of the ribose moiety are involved in intermolecular hydrogen bonding. The only hydrogen atom available for hydrogen bonding that is not involved as such is H(5B) of the $-NH_2$ group of the base.

The structure of this compound has many similarities to the biologically active 6-azacytidine and it was expected that some correlations between activity and structure would be suggested from the study. However, compound **4** has been shown to be inactive in *in vitro* L-1210 screening (20).

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