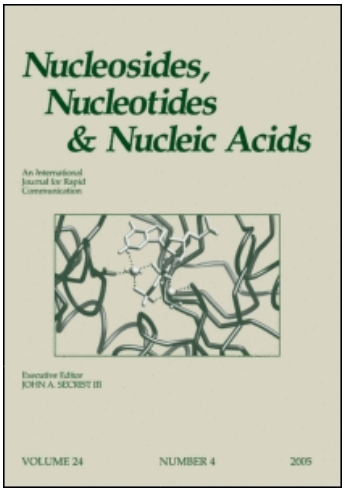

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Structural Features of 2',3'-Dideoxy-2',3'-Didehydrocytidine, A Potent Inhibitor of the HIV (AIDS) Virus

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STRUCTURAL FEATURES OF 2',3'-DIDEOXY-2',3'-DIDEHYDROCYTIDINE, A POTENT INHIBITOR OF THE HIV (AIDS) VIRUS[1]

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ABSTRACT: The structure and conformation of 2',3'-dideoxy-2',3'-didehydrocytidine (2',3'-dideoxycytidin-2'-ene, d4C), a potent inhibitor of the human immunodeficiency virus, was determined by X-ray crystallography. The nucleoside crystallizes in the orthorhombic space group $P2_12_12_1$ with cell dimensions a = 8.603(1), b = 9.038(1), c = 25.831(2) Å and with two independent molecules in the asymmetric unit (Z = 8). Atomic parameters were refined by full-matrix least squares to a final value of R = 0.033 for 2258 observed reflections. The molecules are quite flexible: in molecule A the glycosyl torsion angle (χ_{CN}) is 61.3° and the $-CH_2OH$ side chain is in the gauche* orientation while in molecule B χ_{CN} = 19.8° and the side chain is trans. The five-membered rings are slightly puckered (~0.1 Å), O4' being endo in molecule A and exo in molecule B. A mechanism is proposed for the known instability of 2',3'-unsaturated nucleosides.

Various 2',3'-dideoxyribonucleosides and 2',3'-dideoxy-2',3'-didehydroribonucleosides are potent inhibitors of the human immunodeficiency virus (HIV-1), a virus which is responsible for a highly lethal viral infection — acquired immunodeficiency syndrome (AIDS). Several reviews have appeared recently concerned with evaluation of various compounds for potential therapy of AIDS.[2-6] 2',3'-Dideoxy-2',3'-didehydrocytidine (2',3'-dideoxycytidin-2'-ene, d4C) was first synthesized by Horwitz et al.,[7] and subsequently by Lin et al.,[8] by a different route. This compound and other 2',3'-unsaturated and 2',3'-saturated pyrimidine nucleoside analogs are very potent inhibitors of HIV-1 and other retroviruses in a variety of cell culture systems.[6,8-16] Purine nucleosides unsaturated in the 2',3' position have also been synthesized.[17]

The 2',3'-dideoxy- and 2',3'-dideoxy-2',3'-didehydropyrimidine nucleosides are phosphorylated to the mono-, di- and triphosphates by cellular enzymes.[18-24] Balzarini et al.[9] studied the enzyme kinetics and stability of d4C and the corresponding unsaturated thymidine analog (d4T). They found that 2',3'-unsaturated nucleosides are markedly less stable, due to spontaneous degradation, than the corresponding 2',3'-saturated nucleosides.

The triphosphate analogs of 2',3'-saturated and 2',3'-unsaturated pyrimidine nucleosides exert a potent inhibitory effect against HIV-1 and other retroviral reverse transcriptases relative to cellular DNA polymerases,[25-30] and in addition may be incorporated terminally into DNA and thereby prevent further DNA chain elongation.

Because of the importance of d4C as a potential drug for therapy of AIDS, based on its potent activity against the AIDS virus in several in vitro systems, it is important to obtain as much information as possible about its physical and chemical properties. This is the first crystal structure determination of a nucleoside with an unsaturated five-membered ring. Our results, together with those we obtained about the structures of other anti-AIDS nucleosides, viz. 3'-azido-3'-deoxythymidine (AZT)[31] and 2',3'-dideoxycytidine (d2C, DDC),[32] will, we hope, facilitate the design of more efficient and less toxic anti-AIDS drugs.

EXPERIMENTAL

Crystals of d4C, $C_9H_{11}N_3O_3$, belong to the orthorhombic space group $P2_12_12_1$, and the cell dimensions are a = 8.603(1), b = 9.038(1), c = 25.831(2) Å. Three-dimensional X-ray intensity data were measured on a CAD4 diffractometer with Cu Kα radiation. Of the 2385 unique reflections ($2\theta \leq 150°$), 2260 (95%) had intensities $\geq 3\sigma(I)$ and were considered observed. The intensities were corrected for Lorentz and polarization factors; absorption corrections were unnecessary (μ = 8.5 cm^{-1}).

There are two independent molecules in the asymmetric unit (Z = 8). The crystal structure was determined by direct methods.[33] All hydrogen atoms were located on difference Fourier maps. Atomic parameters were refined by full-matrix least squares with anisotropic temperature factors for non-hydrogen atoms. The refinement converged

TABLE 1. Final atomic parameters and their standard deviations

Atom	x	y	z	B_{eq}/B
N 1A	0.0036(2)	0.6293(2)	0.76999(7)	3.60(7)
C 2A	0.1653(2)	0.6397(2)	0.77291(8)	3.31(8)
O 2A	0.2399(2)	0.6598(2)	0.73272(6)	4.53(7)
N 3A	0.2358(2)	0.6293(2)	0.81951(7)	3.65(7)
C 4A	0.1530(3)	0.5983(3)	0.86120(9)	3.75(8)
N 4A	0.2261(3)	0.5866(3)	0.90656(9)	5.10(11)
C 5A	-0.0127(3)	0.5804(3)	0.85909(9)	4.52(11)
C 6A	-0.0815(3)	0.5996(3)	0.81318(9)	4.15(9)
C 1'A	-0.0747(3)	0.6605(3)	0.72065(9)	3.92(9)
C 2'A	-0.1854(4)	0.5410(3)	0.70393(10)	4.83(12)
C 3'A	-0.3228(4)	0.5986(3)	0.69614(10)	4.60(10)
C 4'A	-0.3233(3)	0.7595(3)	0.70719(10)	4.18(9)
O 4'A	-0.1672(2)	0.7875(2)	0.72610(7)	4.20(7)
C 5'A	-0.4407(3)	0.8140(4)	0.74600(13)	5.13(13)
O 5'A	-0.4384(2)	0.7307(3)	0.79193(9)	5.70(10)
N 1B	0.5476(2)	0.2220(2)	-0.03066(7)	3.73(7)
C 2B	0.5515(3)	0.0685(3)	-0.02588(9)	3.91(9)
O 2B	0.5065(3)	0.0121(2)	0.01505(7)	5.14(8)
N 3B	0.6041(3)	-0.0132(2)	-0.06603(9)	4.29(8)
C 4B	0.6624(3)	0.0533(3)	-0.10785(9)	3.80(8)
N 4B	0.7167(4)	-0.0305(3)	-0.14623(9)	5.05(11)
C 5B	0.6662(3)	0.2101(3)	-0.11264(8)	3.93(9)
C 6B	0.6037(3)	0.2898(3)	-0.07390(9)	3.83(9)
C 1'B	0.4791(3)	0.3086(3)	0.01323(9)	4.48(10)
C 2'B	0.5928(4)	0.3363(4)	0.05605(9)	5.49(13)
C 3'B	0.6170(5)	0.4777(4)	0.06109(11)	6.12(16)
C 4'B	0.5277(4)	0.5632(3)	0.02085(11)	5.24(12)
O 4'B	0.4361(2)	0.4491(2)	-0.00465(7)	4.79(8)
C 5'B	0.6328(4)	0.6402(4)	-0.01735(12)	5.40(13)
O 5'B	0.5462(4)	0.6862(3)	-0.06139(10)	6.84(12)
H N41A	0.174(5)	0.540(5)	0.9326(17)	7.3(10)
H N42A	0.349(8)	0.619(7)	0.9026(22)	12.9(17)
H 5A	-0.070(4)	0.555(4)	0.8864(14)	6.2(8)
H 6A	-0.188(4)	0.595(4)	0.8077(11)	4.7(6)
H 1'A	0.004(3)	0.676(3)	0.6966(10)	4.1(6)
H 2'A	-0.150(4)	0.456(4)	0.6988(12)	5.5(8)
H 3'A	-0.411(4)	0.536(4)	0.6833(13)	5.5(8)
H 4'A	-0.334(4)	0.819(3)	0.6774(11)	4.4(6)
H 5'A	-0.422(4)	0.918(4)	0.7565(13)	5.8(8)
H 5"A	-0.538(5)	0.828(5)	0.7314(14)	7.2(9)
H O5'A	-0.533(6)	0.721(5)	0.8004(17)	8.2(12)
H N41B	0.741(4)	0.010(4)	-0.1785(13)	5.1(7)
H N42B	0.683(5)	-0.138(5)	-0.1498(15)	8.1(11)
H 5B	0.708(4)	0.255(3)	-0.1448(11)	4.3(6)
H 6B	0.597(4)	0.387(4)	-0.0752(11)	4.9(7)
H 1'B	0.390(4)	0.239(4)	0.0256(11)	4.6(6)
H 2'B	0.650(5)	0.243(4)	0.0766(14)	6.3(8)
H 3'B	0.683(5)	0.548(4)	0.0872(16)	7.9(10)
H 4'B	0.448(4)	0.627(4)	0.0367(10)	4.8(6)
H 5'B	0.686(4)	0.722(4)	-0.0024(14)	5.6(7)
H 5"B	0.709(4)	0.562(4)	-0.0299(13)	6.0(8)
H O5'B	0.556(5)	0.765(5)	-0.0664(15)	7.5(11)

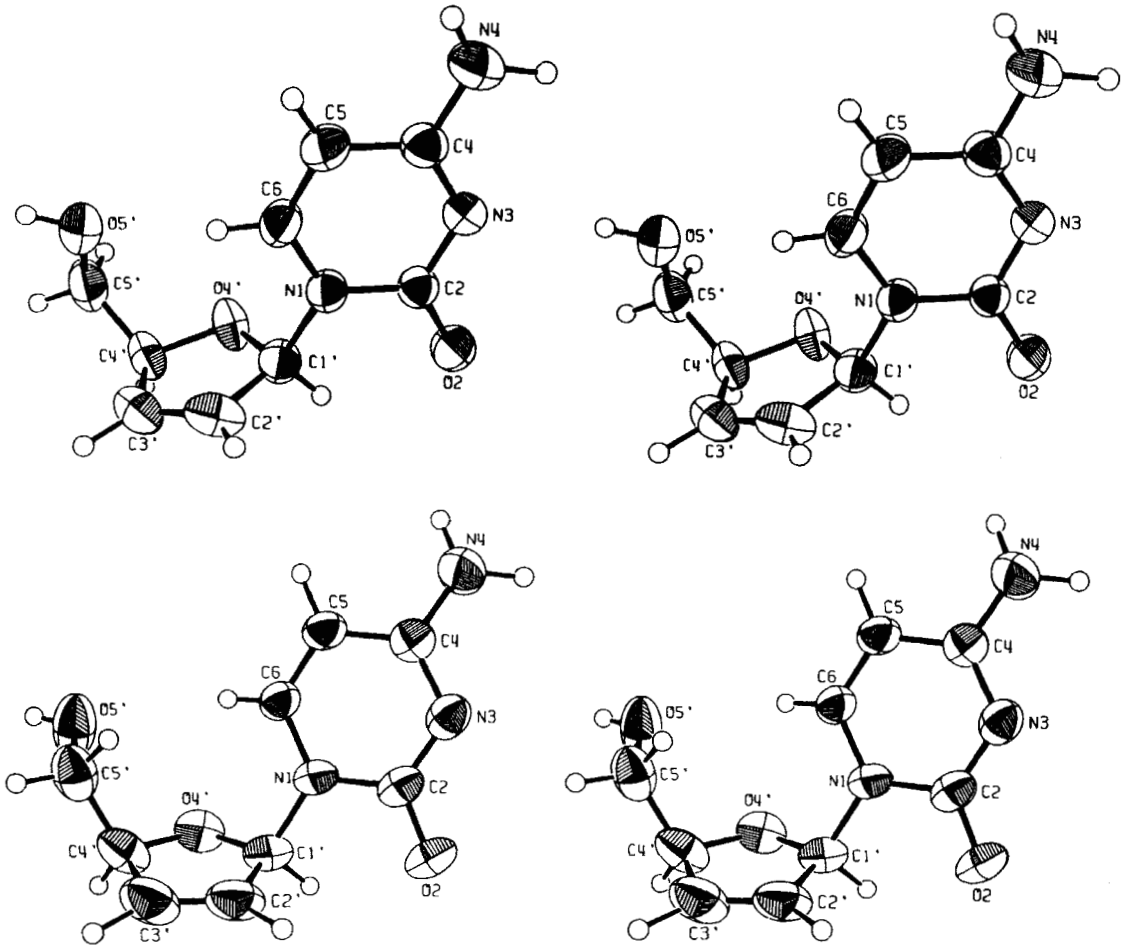


FIG. 1. Stereoscopic views of molecule A (top) and molecule B (bottom) of d4C.

at $\underline{R}$ = 0.033 and $\underline{R}_w$ = 0.048 for 2258 observed reflections ($\underline{w} = 8.0/|\underline{F}_o|$ for $|\underline{F}_o| > 8.0$). Two strong reflections (004 and 201) suffered from secondary extinction and were given zero weights. The coordinates and temperature parameters are listed in Table 1. A list of structure factors is available from the first author.

RESULTS AND DISCUSSION

Stereoscopic views of the two independent molecules are presented in Fig. 1. Bond lengths, bond angles and torsion angles are shown in

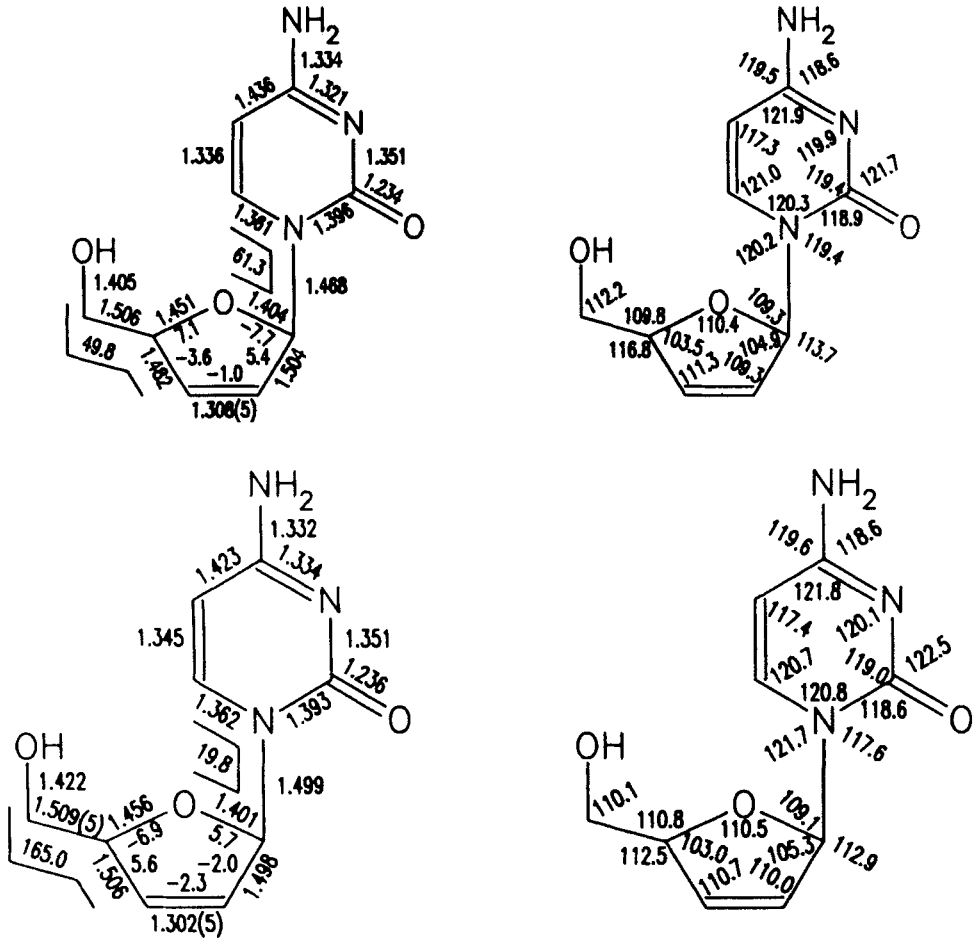


FIG. 2. Bond lengths (in Å), torsion angles (in deg), and bond angles (in deg) in molecules A (top) and B (bottom); unless otherwise indicated, their estimated standard deviations are 0.003–0.004 Å, 0.3–0.4°, and 0.2–0.3°, respectively.

Fig. 2. To our knowledge, this is the first crystal structure determination of a 2,'3'-unsaturated nucleoside analog. For this reason, it is of interest to examine the geometrical features of the d4C molecules and compare them with those of nucleosides with saturated furanose rings. The fortuitous presence of two independent molecules provides additional information about flexibility.

The bond lengths and bond angles in the pyrimidine moieties of the two molecules are in good agreement with one another and with previously observed values.[34] As usual, the rings are not exactly planar; C2 deviates most from the least-squares plane: 0.027(3) Å in molecule A and 0.029(3) Å in molecule B. On the other hand, the glycosyl torsion angles differ significantly: 61.3° in molecule A and 19.8° in molecule B. The former angle would be normal in ribo- or deoxyribonucleosides with sugar rings in type S (C2' endo) conformations while the latter is correlated to type N (C3' endo) puckers. These correlations are attributable to non-bonded interactions between H6 and the carbohydrate moiety.[35] The diversity observed in d4C reflects the absence of such interactions. In contrast to puckered ribose or deoxyribose rings, the dihydrofuran ring in d4C is almost flat, and the hydrogen atoms which normally interact with the aglycon, H2' and H3', are in the plane of the ring and pointing away from the pyrimidine. The only interaction remaining is that between N1-C6 and C1'-O4'. In molecule B these bonds are almost eclipsed, causing a significant elongation of the glycosyl bond (N1-C1'). The same correlation between glycosyl torsion angles and bond lengths was observed in the two molecules of 3'-azido-3'-deoxythymidine (AZT).[31]

As indicated by the endocylic torsion angles (Fig. 2), the five-membered rings are not exactly planar. In molecule A, O4' is 0.108(4) Å above the least-squares plane through the other four atoms (on the same side as the pyrimidine ring, i.e. O4' endo) while in molecule B, O4' is displaced by 0.090(5) Å below that plane (O4' exo). In the absence of pseudorotation, a description of these rings in terms of pseudorotational parameters is not meaningful. For the record, however, $P = 97.4°$, $\tau_m = 7.8°$ for molecule A; $P = 250.7°$, $\tau_m = 7.0°$ for molecule B.

The conformational flexibility is also evident in the $-CH_2OH$ side chain conformation. In molecule A we found a gauche$^+$ rotamer while in molecule B the conformation is trans. The former rotamer is often stabilized by a C6-H···O5' hydrogen bond [35], but the O4' endo pucker of the five-membered ring forces the proton donor and acceptor too far apart. On the other hand, a gauche$^+$ conformation with an O4' exo pucker would bring those two atoms too close together. Consequently,

TABLE 2. Distances and angles for hydrogen bonds

			Distances (Å)		Angles (deg)
D	A	at	D···A	H···A_{corr}	D–H···A
N4A–H1···O2B	$-\frac{1}{2}+x$, $\frac{1}{2}-y$, $1-z$		2.910(3)	1.91	160(4)
N4A–H2···O5'B	x, y, $1+z$		3.013(4)	2.08	148(5)
O5'B–H···N3B	x, $1+y$, z		2.765(3)	1.81	167(4)
N4B–H1···O2A	$1-x$, $\frac{1}{2}+y$, $\frac{1}{2}-z$		2.844(3)	1.86	157(3)
N4B–H2···O5'A	$1+x$, $-1+y$, $-1+z$		2.998(3)	2.17	135(4)
O5'A–H···N3A	$-1+x$, y, z		3.033(3)	2.09	163(4)

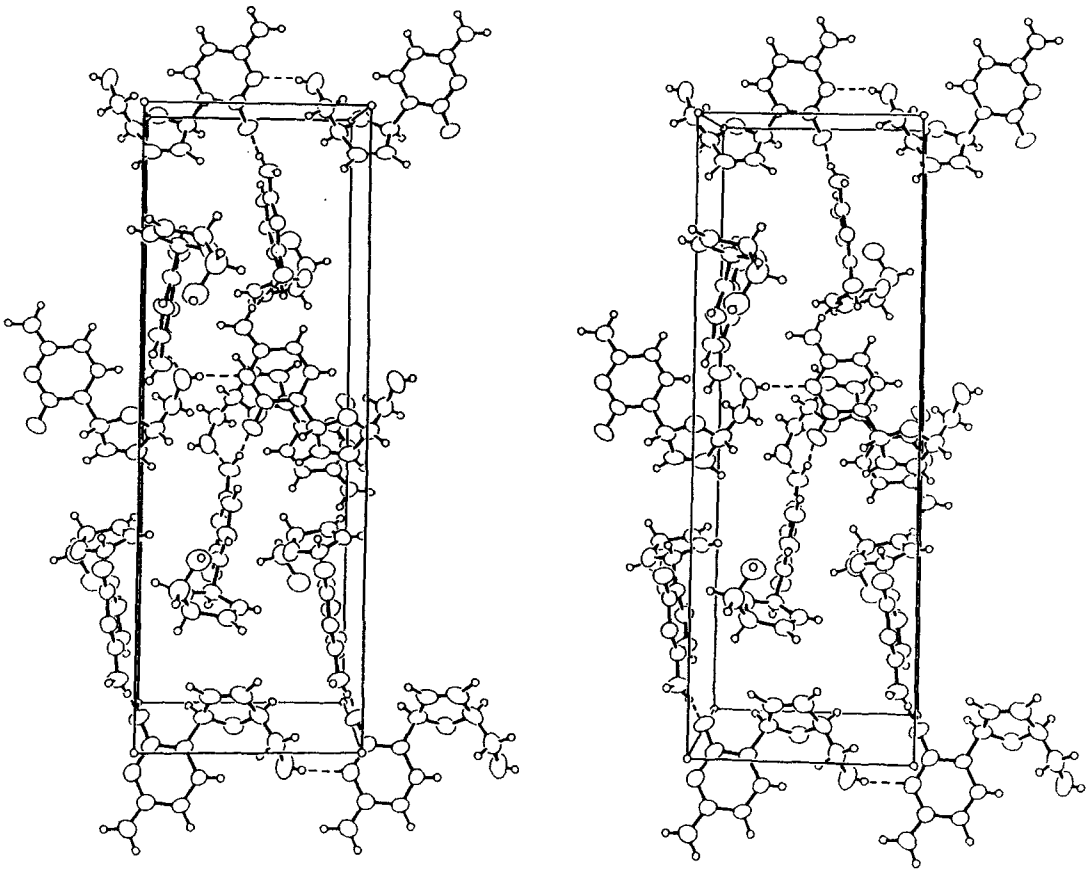


FIG. 3. Stereoscopic view of the molecular packing in the unit cell. Some hydrogen bonds are indicated by dashed lines. The directions of the axes are x up, y →, z ↑. In molecules A the pyrimidine rings are approx. perpendicular to y, in molecules B they are approx. perpendicular to x.

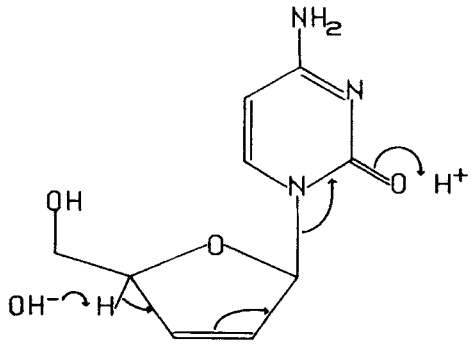
we observe the trans conformation in molecule B. Each of these two rotamers is favored by the gauche effect.[35]

In the crystal, the molecules are held together by an extensive network of hydrogen bonds which can be represented schematically as follows:

```
N4A-HN41A···O2B          N4B-HN41B···O2A
|                        |
HN42A···O5'B-H···N3B     HN42B···O5'A-H···N3A
```

The relevant distances and angles are listed in Table 2. The corrected H···A distances were obtained after normalizing O-H and N-H bond lengths to their nominal values of 0.97 and 1.04 Å, respectively. As shown in the packing diagram (Fig. 3), these hydrogen bonds form chains of molecules extending along each of the three crystal axes.

In both molecules, the temperature parameters of the atoms in the five-membered ring are substantially higher than those of the cytosine atoms. The largest displacements are exhibited by C2' and C3', a surprising result in view of these atoms being double-bonded. It is possible that the increased mobility of these atoms reflects the previously mentioned lability of 2',3'-dideoxy-2',3'-didehydronucleosides.[9] A mechanism which would explain the observed release of free bases is shown on the next page.



This hydrolysis could also take place, albeit much more slowly, in a crystal exposed to a somewhat humid atmosphere.

Our study has shown that 2',3'-unsaturated nucleosides can be quite flexible: they can adopt substantially different conformations

about the glycosyl bond, different puckers of the dihydrofuran ring, and different side chain rotamers. This flexibility may well be necessary for d4C to act as a substrate to cellular kinases.

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