A Pyrimidine Nucleoside Constrained in the Syn Form. Structure and Conformation of 6-Methyl-2′-deoxyuridine[1]

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Abstract: The three-dimensional structure of 6-methyl-2′-deoxyuridine (m^6dU) was determined by X-ray crystallography. The crystals belong to the orthorhombic space group $P2_12_12_1$ and the cell dimensions are a = 20.189 (2), b = 8.203 (3), and c = 6.591 (1) Å. Intensity data were measured with a diffractometer and the structure was solved by direct methods. Least-squares refinement, which included all hydrogen atoms, converged at R = 4.4%. The conformation about the glycosyl bond is syn (χ_{CN} = 242.3°), imposed by the bulky methyl group at C(6). The deoxyribose ring has the somewhat unusual C(1′)exo–C(2′)endo pucker ($_1T^2$). The $-CH_2OH$ side chain adopts the gauche–gauche (g^+) conformation and it is hydrogen bonded to the pyrimidine base via an intramolecular O(5′)–H···O(2) bond. The bases are stacked, and the distance between adjacent rings is 3.28 Å. A discussion of the solution conformation of m^6dU is given in the light of the crystal data.

Nucleosides and nucleotides can adopt either the syn or the anti conformation about the glycosyl bond.[3] There is no definite preference for either conformation when the base is a purine. Consequently, both syn and anti conformers have been found in crystal structures, the latter being more common.[4] In solution the two conformations exist in an equilibrium, and attempts to determine this equilibrium have included both CD spectroscopy, the results of which are rather uncertain,[5] and NMR spectroscopy, which also fails to yield unequivocal results.[6] However, if there is a very bulky substituent at C(8), only the syn conformation is possible; we recently determined the crystal structure of a purine nucleoside which was unequivocally constrained in the syn form.[7]

The situation is somewhat different when the base is a pyrimidine. We are aware of only four pyrimidine nucleosides and two cyclic nucleotides the crystal structure of which revealed a syn conformation: both 6-methyluridine[8] (m^6U)[9] and orotidine[10] have substituents at C(6), but there is no such substituent in s^4U,[11] in $flac_2^{3',5'}dU$,[12] in sodium 2′,3′-CMP,[13] or in BUMP.[14] There is no doubt that the anti conformers of pyrimidine nucleosides which are unsubstituted at the 6 position of the base are more stable, but this does not preclude the existence of a syn–anti equilibrium in solution, particularly in nonpolar solvents.[15] In view of the scarcity of crystallographic data on syn pyrimidine nucleosides (no details have been published on the orotidine structure), it seemed desirable to carry out an X-ray analysis of m^6dU (which favors the syn conformation in solution[16]) in order to examine what structural features are associated with the syn conformation.

The discovery[17] of syn deoxyguanosine units in left-handed double-helical DNA provides a new impetus for study of the conformational consequences of syn nucleosides. There exists some evidence for the presence of syn uridine units in oligonucleotides.[18] This study of m^6dU, the first syn pyrimidine nucleoside with an unsubstituted deoxyribose ring, provides some insight into the stereochemical changes elicited by syn pyrimidine bases.

Experimental Section

6-Methyl-2′-deoxyuridine, $C_{10}H_{14}N_2O_5$, was prepared, starting with 2-amino-β-D-arabinofuro[1′,2′:4,5]oxazoline, according to Holy,[19] and crystallized from ethanol–acetonitrile (80:20 v/v). Precession photographs established the space group uniquely as $P2_12_12_1$. A crystal measuring 0.25 × 0.50 × 0.60 mm, mounted along the c axis on a card-controlled Picker diffractometer, provided the following data: a = 20.189 (2) Å, b = 8.203 (3) Å, c = 6.591 (1) Å, V = 1091.5 Å^3, d_x = 1.47 g cm^{-3}, d_m = 1.45 g cm^{-3} (flotation in chlorobenzene–bromobenzene), Z = 4 (20 °C, Cu $K\alpha_1$, λ 1.540 56 Å; Cu $K\alpha_2$, λ 1.544 39 Å), $F(000)$ = 512, μ(Cu $K\alpha$) = 9.70 cm^{-1}.

The moving-crystal/moving-counter method ($\theta/2\theta$ scan) was used to collect the intensity data. Reflections with net counts <100 or <8% of background were categorized as unobserved. There were 1111 unique reflections accessible to the diffractometer ($2\theta \leq 130°$) of which 986 had intensities above threshold values. The intensities were corrected for Lorentz and polarization factors; absorption corrections were considered unnecessary.

The structure was determined by the multisolution approach to direct methods, and the atomic parameters were refined by block-diagonal least squares. All scattering factors were taken from the "International Tables for X-ray Crystallography"[20] and the oxygen curve was corrected for anomalous dispersion. All hydrogen atoms were located on difference Fourier maps and their coordinates and isotropic temperature parameters were refined. Throughout the refinement the function $\sum w(|F_o| - |F_c|)^2$ was minimized and a factor of 0.8 applied to all shifts. The following weighting scheme was used during the final stages: $w = w_1w_2$, where $w_1 = 1$ for $|F_o| \leq 8$, $w_1 = 8/|F_o|$ for $|F_o| > 8$; $w_2 = \sin^2\theta/0.3$ for $\sin^2\theta < 0.3$ and $w_2 = 1$ for $\sin^2\theta \geq 0.3$. This weighting scheme made the average

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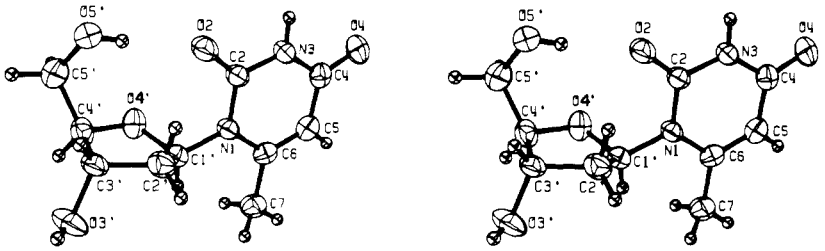

Figure 1. Stereoscopic view of m^{6}dU; the thermal ellipsoids correspond to 50% probability.

Table I. Final Atomic Parameters and Their Standard Deviations

A. Nonhydrogen Atoms[a]

atom	x	y	z	B_{eqv}
N(1)	3145(1)	3369(3)	4193(4)	2.54(9)
C(2)	3556(1)	4722(3)	4198(5)	2.67(10)
O(2)	4160(1)	4636(3)	4186(5)	3.74(9)
N(3)	3253(1)	6208(3)	4206(4)	2.70(9)
C(4)	2581(1)	6508(4)	4253(5)	2.87(11)
O(4)	2379(1)	7912(3)	4296(4)	4.05(9)
C(5)	2185(1)	5053(4)	4231(5)	3.04(11)
C(6)	2455(1)	3557(4)	4217(5)	2.70(9)
C(7)	2035(1)	2048(4)	4211(6)	3.64(13)
C(1')	3462(1)	1757(3)	4242(6)	2.96(12)
C(2')	3937(2)	1368(4)	2490(5)	3.43(13)
C(3')	4412(1)	181(4)	3448(5)	2.99(12)
O(3')	4216(1)	−1486(3)	3161(5)	4.55(12)
C(4')	4380(1)	529(4)	5729(6)	3.28(12)
O(4')	3845(1)	1645(3)	6036(3)	3.41(9)
C(5')	5005(2)	1312(5)	6584(6)	4.14(15)
O(5')	5180(1)	2745(3)	5500(4)	3.69(9)

B. Hydrogen Atoms[b]

atom	x	y	z	B
H(3)	355(2)	701(5)	413(7)	3.9(0.8)
H(5)	172(2)	513(5)	444(6)	3.7(0.8)
H(71)	212(2)	118(6)	313(7)	4.6(0.9)
H(72)	212(2)	150(5)	539(6)	3.1(0.7)
H(73)	160(2)	220(6)	411(8)	5.1(1.0)
H(1')	310(2)	105(4)	447(6)	3.1(0.7)
H(2')	420(2)	240(4)	209(6)	3.4(0.7)
H(2'')	371(2)	104(5)	131(7)	4.0(0.9)
H(3')	483(1)	42(4)	289(5)	1.8(0.6)
H(O3')	440(3)	−188(7)	220(9)	6.8(1.2)
H(4')	429(2)	−52(5)	638(6)	3.8(0.8)
H(5')	489(2)	142(6)	805(7)	5.1(1.0)
H(5'')	542(2)	53(6)	653(8)	5.8(1.1)
H(O5')	483(2)	326(5)	537(7)	4.2(0.9)

[a] All coordinates were multiplied by 10^4. [b] All coordinates were multiplied by 10^3.

values of $w(\Delta F^2)$ independent of $|F_o|$ and $\sin^2\theta$. After the final cycle the average parameter shift equaled 0.15σ and the largest 0.61σ. The conventional residual index R is 0.044 and the weighted index R' is 0.057 for 1084 reflections, including those unobserved ones for which $|F_o| < |F_c|$ and excluding nine strong ones which suffered from extinction. A final difference Fourier map showed no significant features. The final atomic coordinates and the equivalent isotropic B values are given in Table I.

Results and Discussion

Deoxyribose Moiety. As can be seen in Figure 1, the deoxyribose ring adopts the C(1')exo–C(2')endo ($_1T^2$) conformation. The pseudorotational parameters[21] are $P = 139.4°$ and $\tau_m = 31.3°$. This pucker is just outside the usual ranges (P = 0–36 and 144–180°) for nucleosides and nucleotides,[4b] and it also differs from other pyrimidine nucleosides in syn conformation (see below). We have recently shown that deoxyribose rings in nucleosides can adopt unusual conformations, both in crystalline state and in solution.[22,23] In order to ascertain whether any distortions of the deoxyribose ring are necessary to accommodate the syn conformation, we can compare its geometry (Figure 2) with that in hm^{5}dU, where the sugar ring has the similar C(1')exo ($_1E$) conformation ($P = 129.0°$).[23] Four of the five bonds in the ring are identical within experimental error while C(1')–C(2') is significantly longer (by 0.027 Å) in the present structure. However, a bond length of 1.535 Å is quite normal, and the difference is probably attributable to differences in thermal vibration. Four of the endocyclic bond angles agree within 1°, but the one at C(2') is significantly smaller (by 1.9°) than in the previous structure. Again, however, an angle of 102.6° is not unusual for 2'-deoxyribose rings.[24] The largest deviations are in the exocyclic bond angles O(3')–C(3')–C(4') and N(1)–C(1')–O(4'), but the latter angle is abnormally small in hm^{5}dU owing to an unusual C(6)–H···O(4') hydrogen bond.[23] Another similarity between this structure and that of hm^{5}dU is the unusual flattening of the deoxyribose ring. The puckering amplitudes (τ_m) are 31.3° in m^{6}dU and 32.1° in hm^{5}dU. While there may be a correlation between this flattening and the unusual conformation of the deoxyribose rings in these two structures, additional relevant X-ray analyses would be necessary to establish it.

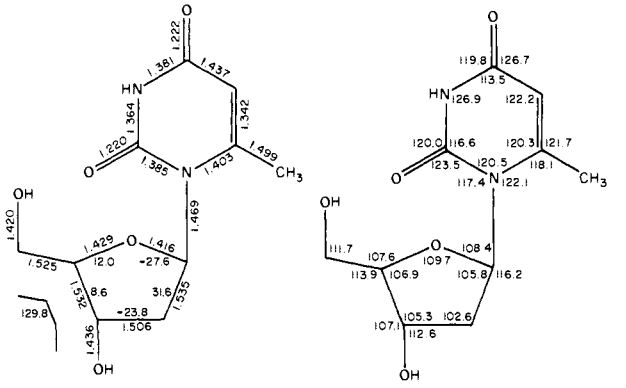

Figure 2. Left: bond distances (Å) and torsion angles (deg). The estimated standard deviations are 0.003–0.005 Å and 0.3–0.4°, respectively. Right: bond angles (deg). The esd's are 0.2–0.3°.

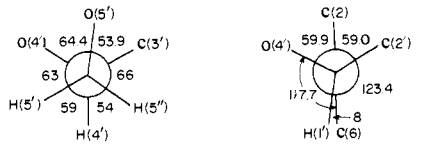

Figure 3. Newman projections along (left) the C(4')–C(5') bond and (right) the N(1)–C(1') bond.

As shown in the Newman projection (Figure 3), the conformation of the –CH_2OH side chain is gauche–gauche (g^+). This is the most commonly observed conformation,[4a] and in the present case it is stabilized by an intramolecular O(5')–H···O(2) hydrogen bond (see below).

Pyrimidine Moiety. All bond lengths in the pyrimidine moiety are normal except N(1)–C(6), which is relatively long, reflecting the substitution at C(6). Substitution at C(5) or C(6) also affects the endocyclic bond angles at these atoms. When both atoms are

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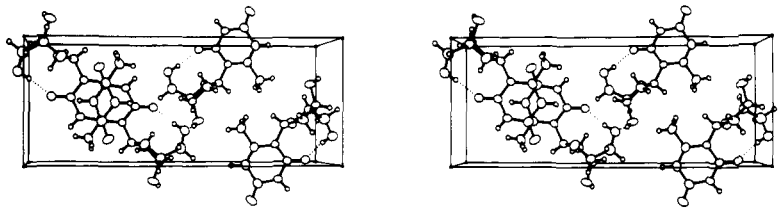

Figure 4. Stereoscopic view along z of the contents of a unit cell. The directions of the axes are $x\rightarrow$ and $y\uparrow$. Dotted lines indicate hydrogen bonds.

unsubstituted, as in uridine[25] or in lyxouracil,[26] the angle at C(6) is 3–4° larger than the one at C(5). With a methyl substituent at C(5), as in 5-methyluridine[27] or in thymidine,[28] that difference increases to about 7°. However, when the methyl group is attached to C(6), as in this structure or in 6-methyluridine,[8] the angle at C(6) is *smaller*, by 1–2°, than the one at C(5).

Another interesting relationship is that between the exocyclic angles C(1′)–N(1)–C(2) and C(1′)–N(1)–C(6). In most pyrimidine nucleosides the latter angle is 5–8° larger than the former.[24-29] When the conformation about the glycosyl bond is syn rather than anti, that difference may decrease,[8] or it may disappear.[11-13] In the present structure there is a difference of 4.7° which we attribute to the intramolecular O(5′)–H···O(2) hydrogen bond. In the two independent molecules of m^{6}U the difference between these angles is marginally larger in the one with the same hydrogen bond (3.3 vs. 1.9°). In our recent structure analysis of hm^{5}dU,[23] we pointed out the correlation between the intramolecular C(6)–H···O(4′) hydrogen bond and the equal exocyclic bond angles at N(1). It appears, therefore, that the syn conformation distorts the exocyclic bond angles at N(1), even if the conformation of the sugar ring is C(2′)endo,[8] but this distortion can be partly or completely canceled by intramolecular hydrogen bonding.

The six atoms of the pyrimidine ring are completely coplanar (Table II) and so are O(2) and the methyl carbon C(7). Only C(1′) and O(4) deviate from this plane. The dihedral angle between this plane and the mean plane through the five sugar ring atoms is 85.0°.

Conformation about the Glycosyl Bond. The torsion angle O(4′)–C(1′)–N(1)–C(6) about the glycosyl bond (χ_{CN}) is 242.3° (Figure 3), corresponding to a syn conformation. This conformation is imposed by the methyl substituent at C(6). Even with a C(1′)exo sugar pucker, which maximizes the distance between the methyl group and the sugar ring, an anti conformation would bring one of the methyl hydrogens to within <2 Å of both H(2′) and O(4′), clearly an impossible situation. As it is, we found the distance between H(2′) and O(4′) to be rather short (2.30 Å). Thus, there can be no syn–anti equilibrium in solution for m^{6}dU, making it a useful nucleoside analogue for establishment of NMR parameters for the syn form.[16]

As shown in Table III, the range of the glycosyl torsion angles is remarkably narrow, only 12° if one excludes s^{4}U. Both Saenger[8,30] and Sundaralingam[31] have discussed the correlation between the syn conformation and the sugar ring pucker. Saenger's original view[8] that strain and distortion of bond lengths and angles can be avoided only if the sugar conformation is C(2′)endo was later extended to include the C(3′)endo–C(4′)exo conformation.[30] Reviewing the crystal structures of some syn nucleosides, Yathindra and Sundaralingam[31] concluded that the syn conformation tends to shift the phase angle of the sugar ring in the direction of the O(4′)endo pucker (P = 90°). In Table III we show the conformational parameters, which we calculated from published torsion angles, of several pyrimidine nucleosides and analogues in syn conformation. It should be pointed out that the O(4′)endo conformation in sodium 2′,3′-CMP is to some extent imposed by the phosphodiester ring which is fused to the ribose ring. Such fusion to five-membered or smaller rings usually results in an envelope conformation with the atom opposite the common bond being puckered.[32,33] If we, therefore, disregard sodium 2′,3′-CMP, we see that in s^{4}U, flac$_2^{3',5'}$dU, and m^{6}dU the phase angle is closer to 90° than usual, while in m$_2$-ribo-L and in m^{6}U the ring conformation is C(2′)endo. We may also point out that, of the five molecules in Table III with type S conformation,[21] four are within a fairly narrow range of P (150.7–169.1°) but m^{6}dU is outside that range. Thus, pending additional X-ray studies of pyrimidine nucleosides in the syn form, one may conclude that the ring conformations in such nucleosides are not as restricted as previously assumed, and that the energy barrier between the C(2′)endo and C(3′)endo conformations may not be too high.

Table II. Least-Squares Planes and Deviations of Atoms from Them[a]

plane 1[b]		plane 2[b]	
atom	Δ, Å[c]	atom	Δ, Å[c]
N(1)	0.000	C(2′)	0.037
C(2)	−0.004	C(3′)	−0.047
N(3)	0.006	C(4′)	0.055
C(4)	−0.008	O(4′)	−0.016
C(5)	0.005	C(1′)*	−0.428
C(6)	0.000	C(5′)*	1.363
C(1′)*	−0.049		
O(2)*	−0.009		
O(4)*	−0.024		
C(7)*	0.003		
O(5′)*	−0.908		
H(O5′)*	−0.81		

[a] Atoms marked with an asterisk were not included in the calculation of the plane. [b] Plane 1: $0.0105X - 0.0075Y + 0.9999Z - 2.8089 = 0$. Plane 2: $0.6805X + 0.7316Y - 0.0400Z - 6.1278 = 0$. [c] Esd's are 0.002–0.004 Å for nonhydrogen atoms.

Hydrogen Bonding and Packing. All three available protons participate in hydrogen bonds. The scheme may be represented as follows: N(3)–H···O(3′)–H···O(5′)–H···O(2). The geometry of these bonds is given in Table IV. As mentioned above, the O(5′)–H···O(2) bond is intramolecular. Table III shows that this bond occurs in four of the seven cases in which it can occur. In contrast to Saenger,[8] we tend to agree with Pitha[34] and with Rabczenko et al.,[15] who concluded that this internal hydrogen bond may lead to an appreciable stabilization of the syn conformer, particularly in nonpolar solvents. Such a hydrogen bond may well be responsible for stabilizing both independent molecules of m$_2$-ribo-L in the syn conformation, even though the molecule could also exist in the anti form.[35] It is worth noting that both s^{4}U and sodium 2′,3′-CMP, where this bond was not observed,

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Table III. Conformational Parameters for Pyrimidine Nucleosides in Syn Conformation[a]

structure[b]	P	τ_m	χ_{CN}	O(5')-H···O(2)	ref
$s^4U \cdot 1.5H_2O$	34.6 (^3_4T)	40.2	272.9	no	11
$flac_2^{3',5'}dU$	38.5 (^3_4T)	34.5	251.7	impossible	12
BUMP	49.6 ($_4T^3$)	46.7	251.4	impossible	14
Na 2',3'-CMP·$4H_2O$	81.2 ($^0T_4$)	36.1	242.9	no	13
m^6dU	139.4 ($_1T^2$)	31.3	242.3	yes	this work
m_2-ribo-L, A	163.3 (^2E)	37.8	252.1	yes	30
B	150.7 ($^2T_1$)	41.5	240.8	yes	
m^6U, A	158.8 (^2E)	35.4	250.9	yes	8
B	169.1 ($^2T_3$)	36.5	252.7	no	

[a] The values of P, τ_m (ref 21), and χ_{CN} are in degrees. [b] See note 9 for abbreviations.

Table IV. Distances and Angles for Hydrogen Bonds

	distances, Å			angles, deg	
D-H···A	D···A	H···A	H···A_{cor}	D-H···A	H-D···A
N(3)-H···O(3') (x, 1 + y, z)	2.798	1.94	1.79	163	12
O(3')-H···O(5') (1 − x, $-^1/_2 + y$, $^1/_2 - z$)	2.776	1.99	1.84	164	11
O(5')-H···O(2) (x, y, z)	2.721	1.93	1.79	160	14

crystallize as hydrates. The strength of this bond in the m^6dU structure is indicated by the relatively short corrected H···O distance.[36]

The packing of the molecules in the crystal is shown in Figures 4 and 5. The crystal structure of m^6dU is remarkably similar to, almost isomorphous with, that of m^6U. This would indicate that the difference between the conformations of the sugar rings cannot be attributed to packing forces. As in m^6U, there are distinct hydrophobic and hydrophilic regions. The former consists of the pyrimidine rings which are stacked perpendicular to the c axis. The distance between adjacent rings is 3.28 Å and the dihedral angle between their planes is 1.5°. O(2) extends into the hydrophilic region which is formed by the deoxyribose moiety.

Comparison with NMR Data. Some comment on the solution conformation of m^6dU seems warranted in the light of our crystallographic data. ^1H NMR data have revealed a preference for the syn conformation in m^6dU[16,37] and its 3'- and 5'-monophosphate derivatives.[16] (The m^6dU moiety retains this conformation when incorporated into pyrimidine–pyrimidine and purine–pyrimidine dideoxynucleoside monophosphates.[38]) Thus in both the solid and solution states the 2-keto oxygen is available for intramolecular hydrogen bonding with the 5'-hydroxyl group. Such a hydrogen bond can of course result only if the molecule is oriented gauche–gauche (g^+) about the C(4')–C(5') bond. In the crystal state the g^+ orientation is attained and the O(5')–H···O(2) hydrogen bond is formed. In aqueous solution the presence of the syn base leads to a destabilization of the g^+ conformer and the gauche–trans (t) orientation is now the dominant conformation about the C(4')–C(5') bond.[16] Thus, the hydrogen bond cannot be important in polar solutions, presumably owing to preferential interactions involving solvent molecules.

NMR studies have shown that the pucker of the 2'-deoxyfuranose ring is affected by the syn pyrimidine base.[16,37] The magnitudes of the cis H–H couplings, $J_{1'2''}$ and $J_{2'3'}$, were found to be about 1.5 Hz larger in the m^6dU derivatives than in the corresponding dT derivatives which favor the anti conformation. On this basis it was concluded that, in the m^6dU series, the H–H torsion angles about the C(1')–C(2') and C(2')–C(3') bonds, time averaged over the N⇌S blend, were closer to the eclipsed (τ = 0°) form. One might be tempted to attribute these changes solely to a general flattening of the ring (decrease in τ_m). However, a decrease in τ_m is expected to lead to an increase in the sum $J_{1'2'} + J_{3'4'}$. No such general trend was evident in dT and m^6dU and their derivatives. It seems likely, therefore, that changes in P as well as in τ_m are occurring as a result of anti to syn transition.

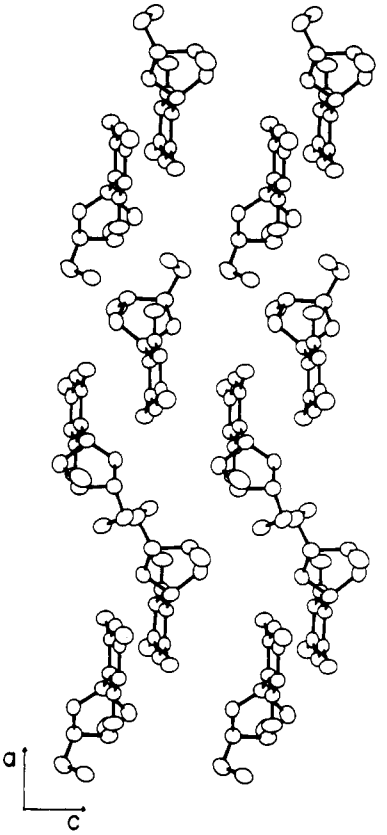

Figure 5. Projection of the crystal structure showing the stacking of bases.

A comparison of the crystallographic data of dT and m^6dU can serve as a starting point for understanding the behavior of deoxyribose rings in solution: in m^6dU P = 139.4° ($_1T^2$), τ_m = 31.3°; in dT[28] P = 187.5° ($_3T^2$), τ_m = 38.2°. Thus, with a change from the anti thymine base to the syn 6-methyluracil base, the ring remains in the S domain, but is pseudorotated by 48.1° toward the O(4')endo (P = 90°) mode. Furthermore, the ring is significantly flattened ($\Delta\tau_m$ = −6.9°) by the syn base. It seems reasonable to suggest, based on other correlations between solid- and solution-state geometries,[39] that a syn base will have a similar effect in solution on a deoxyribose ring with an S type of pucker, viz., a flattening and a pseudorotation within the S range toward O endo. The increases in the cis $J_{1'2''}$ and $J_{2'3'}$ couplings of m^6dU

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relative to dT are consistent with such changes in an S type of pucker.[40] However, there seem to be no steric interactions in a syn pyrimidine nucleoside which are serious enough to preclude an N type of pucker, and thus in general one should expect an N⇌S interconversion in solution for syn as well as anti molecules. It is important to note that our crystallographic data provide no information about the influence of syn pyrimidine bases on a deoxyribose ring with an N-type pucker. Such information can only be obtained from a comparison of syn and anti 2'-deoxyribosides crystallized in the N range. There are many examples of anti,N conformations,[21] but none of syn,N. A more detailed evaluation of the solution conformations should await such data.

In contrast to deoxyribonucleosides, there is no agreement between NMR and crystallographic results for ribonucleosides. In the solid state the conformation of uridine is anti,C(3')endo[25] and that of 6-methyluridine is syn,C(2')endo.[8] On the other hand, NMR data for the latter suggest a shift away from the C(2')endo conformer.[42] Thus, the correlation between the sugar pucker and the syn conformation about the glycosyl bond remains unclear.

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Supplementary Material Available: Anisotropic temperature parameters and a listing of observed and calculated structure factors (6 pages). Ordering information is given on any current masthead page.

(40) We do not mean to imply that the type S pucker of a syn deoxyriboside in solution is exactly the same as in the crystal structure of m^6dU. The comparison of the solution and solid-state geometries is complicated by the presence of the intramolecular O(5')-H···O(2) hydrogen bond which is present in the solid but not in aqueous solution. Undoubtedly the formation of this bond places some constraints on the sugar which are reflected in the values of P and τ_m. Relevant in this regard are the crystal data for m^6U (Table III). A significant difference in P (10.3°), though not in τ_m (1.1°), is noted between the A form which has a hydrogen bond and the B form which does not. It is likely that the more flexible deoxyribose ring[21,39,41] will be even more distorted by an O(5')-H···O(2) bond. Unfortunately, the only crystal data for syn pyrimidine deoxyribosides other than m^6dU are those of the highly substituted $flac_2^{3',5'}dU$ (Table III) and thus no meaningful comments on the influence of hydrogen bonding on deoxyribose puckering can be made at this time.

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Determination of the Lifetime of Singlet Oxygen in D_2O Using 9,10-Anthracenedipropionic Acid, a Water-Soluble Probe

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Abstract: A water-soluble singlet oxygen monitor has been used in time-resolved laser photolysis experiments. The disodium salt of 9,10-anthracenedipropionic acid (ADPA) is bleached to an endoperoxide on reaction with singlet oxygen. This change in absorbance was followed by kinetic spectrometry. Singlet oxygen was formed by laser excitation of methylene blue, and the resulting decay of ADPA was first order. Measurement of the rate constant for ADPA bleaching in D_2O as a function of ADPA concentration yielded a line whose slope, $(8.2 \pm 0.5) \times 10^7$ L mol^{-1} s^{-1}, is the bimolecular rate constant for quenching of singlet oxygen by ADPA. The intercept of this line, $(1.9 \pm 0.1) \times 10^4$ s^{-1}, corresponds to the rate constant for natural decay of singlet oxygen in D_2O. The resulting value for τ_Δ, 53 ± 3 μs, substantiates the previously determined values for the singlet oxygen lifetime in D_2O solutions containing ionic surfactant micelles. ADPA shows itself to be a convenient and specific monitor for detecting the presence and decay of singlet oxygen in aqueous systems. To demonstrate this further, the rate constant for singlet oxygen quenching by histidine in D_2O has been determined, using ADPA, to be $(6.1 \pm 0.5) \times 10^7$ L mol^{-1} s^{-1}, a value which is in good agreement with those previously determined using the furan DPBF as singlet oxygen monitor, both in surfactant solution and in alcohol-water mixtures.

The combined discoveries of Khan and Kasha[2a] and of Foote and Wexler,[2b] which implicated an excited state of oxygen in dye-sensitized photooxidations, led to a rapidly growing area of research. Since then, a large number of researchers have been actively involved in the investigation of the reactivity of singlet oxygen toward organic substrates, and more recently examination of its possible role in biological systems.[3] To a large degree, the efforts in the biochemical area have been hampered by the rather limited methods of monitoring the presence and activity of singlet oxygen, especially where water is the major component of the solvent.

In condensed phases the natural lifetime of the $O_2^*(^1\Delta_g)$ state is governed by solvent deactivation via electronic-vibrational energy transfer.[4] In most common polar and nonpolar liquids

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