Molecular and Crystal Structure of O^4-Methyluridine. Reaction Coordinates for an Incipient Nucleophilic Attack Seen by Short Intermolecular Sugar–Base Interactions

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Abstract: Base-alkylated nucleosides are mutagenic in that they are capable of inducing base mispairing. Here we report the first structure of an *O*-alkylated pyrimidine nucleoside, O^4-methyluridine ($C_{10}H_{14}O_6N_2$). The compound crystallizes with two independent molecules per asymmetric unit in the orthorhombic space group D_2^4-$P2_12_12_1$ with unit cell parameters a = 4.986 (1) Å, b = 19.463 (1) Å, c = 23.453 (1) Å, V = 2275 Å^3, Z = 8, D_c = 1.507 g cm^{-3}, D_m = 1.51 g cm^{-3}. The structure was solved by direct methods and refined by full-matrix least-squares technique to a final R index of 0.028 (R_w = 0.035) using 1916 intensities with $I > 1.5\sigma(I)$. Molecules A and B exhibit the favored ribofuranosyl and glycosyl conformations: the $^3T_2$ sugar pucker (P_A = 7.6 (3)°, τ_{mA} = 37.5 (2)°, P_B = 11.4 (3)°, τ_{mB} = 42.0 (2)°) and the anti conformation about the glycosyl bond C(1′)–N(1) (X_A = 9.4 (3)° and X_B = 10.9 (3)°). The conformations about the exocyclic C(4′)–C(5′) bond are gauche$^+$ (ψ_A = 56.1 (3)° and ψ_B = 52.7 (3)°). Methylation profoundly affects the geometric and electronic properties of the pyrimidine ring such that they more closely resemble those of cytosine than diketo uracil. The close approach of the O(4′) oxygen atom of a neighboring sugar to the base of molecule A represents the reaction coordinates for an incipient nucleophilic attack by O(4′) on the base C(2) atom, which results in pyramidization at the C(2) atom and distortion of the pyrimidine ring to a twist boat conformation. Further significant changes are induced in the bond orders about the C(7)–O(4)–C(4)–C(5)–C(6) fragment. Methylation precludes base–base hydrogen bonds, and only base–sugar and sugar–sugar hydrogen bonds are found. In both molecules the keto O(2) atoms are involved in hydrogen bonding. The hydrogen bond preferences exhibited by O^4-methyluridine suggest that only certain pairing schemes with guanosine are possible. For the O^4-methyluridine to engage in these pairings, the C(7) methyl group has to be rotated about 80° from the observed syn-periplanar orientation.

In 1969 Loveless identified the alkylation product deoxy-O^6-methylguanosine and postulated that this alkylated base could induce mutations by permitting base mispairing due to the deprotonation of N(1).[1] Subsequent studies by Singer revealed that the carcinogenic *N*-nitroso alkylating agents were also capable of alkylating all three pyrimidine oxygens (O(2) and O(4) of uridine or thymidine and O^2 of cytidine).[2] Methylation of the O(4) of uridine was shown to be mutagenic by template activity experiments in which templates of poly(ribouridine) containing O^4-methyluridine allowed the incorporation of GMP and CMP but not AMP or UMP into the poly(A) chain.[3] The deprotonation of N(3) and the steric effects of the methyl group were cited as the cause for this partially specific mutagenesis.

Recently, Hruska and Blonski completed ^{1}H and ^{13}C NMR studies on a series of uracil ribonucleosides that were methylated at the O(2), N(3), O(4), and C(5) positions of the pyrimidine ring.[4] Of specific interest was how alkylation affected the stereochemical and electronic properties of the modified nucleosides. Their conformational results for O^4-methyluridine revealed that the molecule displays an anti conformation about C(1′)–N(1) and significant preferences for the C(3′) endo sugar pucker and the gauche$^+$ conformation around the C(4′)–C(5′) bond. Furthermore, the J(5–6) proton coupling constant indicated that the O-alkylated uridines displayed a decreased π-bond order of the C(5)–C(6) bond as compared to the diketo uracils.

In this work we have carried out the crystal structure determination of O^4-methyluridine to gain a detailed insight into the electronic perturbations caused by methylation. The two independent molecules found in the asymmetric unit exhibit the favored conformations for pyrimidine nucleosides; the anti glycosyl conformation, the C(3′) endo sugar pucker, and the gauche$^+$ conformation about the C(4′)–C(5′) bond. The pyrimidine bases, however, display significant differences in their electronic and geomtric properties that can be attributed to stacking (monopole-induced dipole) interactions between the pyrimidine base of A and the O(4′) oxygen atom of the ribofuranosyl ring. Methylation of O(4), which results in deprotonation of the N(3) nitrogen atom, modifies the hydrogen-bonding properties of the uracil base. Base pairing between O^4-methyluridine and adenosine or cytidine can at best involve only one hydrogen bond. However, base pairing between O^4-methyluridine and guanosine allows two hydrogen bonds. Two possible O^4meU–G pairing schemes are discussed.

Experimental Section

Crystals of O^4-methyluridine were obtained by slow evaporation from an ethanol solution, and one with dimensions 0.25 mm × 0.15 mm × 0.15 mm was selected for data collection. Oscillation and Weissenberg pictures indicated an orthorhombic lattice with the space group $P2_12_12_1$. The cell parameters, which were refined by a least-squares algorithm using 25 automatically centered reflections, were a = 4.986 (1) Å, b = 19.463 (1) Å, c = 23.453 (1) Å, V = 2275 Å^3, and Z = 8. Intensity data were collected on an Enraf-Nonius CAD4 diffractometer using Ni-filtered Cu Kα radiation (λ = 1.5418 Å) up to a 2θ limit of 140° by the ω-2θ scan mode. Of the 2534 independent reflections collected, 1916 reflections with $I/\sigma(I)$ greater than 1.5 were used for structural analysis. Four reflections monitored during data collection revealed approximately 8% crystal decay and the appropriate correction was applied. An absorption correction employing an empirical ψ curve and also Lorentz and polarization corrections were applied to the intensities.

Structure Determination and Refinement. The structure was solved by application of the multisolution tangent formula technique using the

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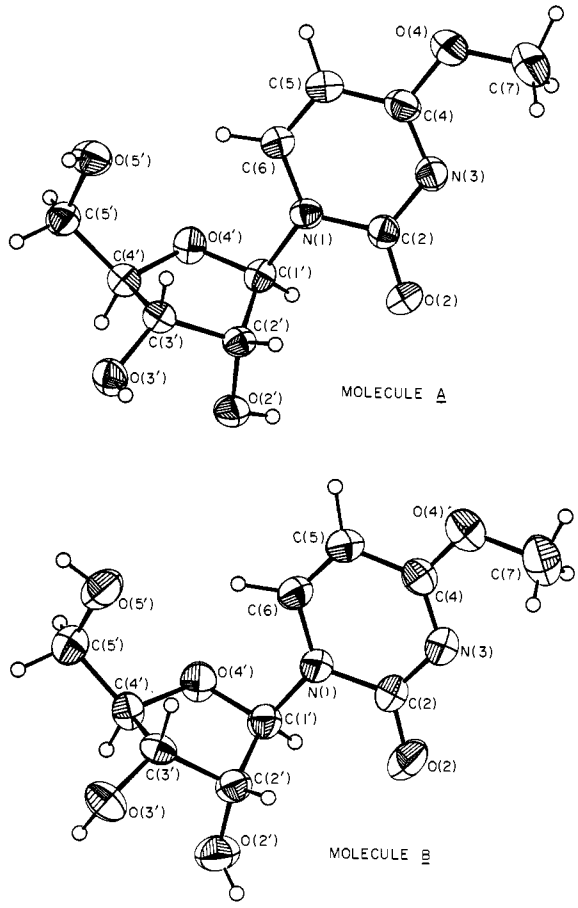


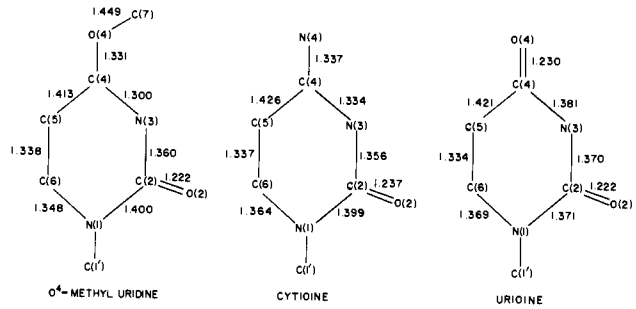
Figure 1. ORTEP drawings of the independent molecules *A* and *B* of O^4-methyluridine. The non-hydrogen atoms are represented by 50% probability ellipsoids while the hydrogen atoms are drawn as spheres of arbitrary size.

computer program MULTAN.[5] The *E* map computed for the phase set having the highest figure of merit and the lowest residual revealed all 18 non-hydrogen atoms of the independent molecules. The initial *R* index from the *E* map was 0.195, and subsequent block-diagonal least-squares refinement employing anisotropic temperature factors reduced the residual to 0.064. Difference Fourier syntheses revealed all 28 hydrogen atoms, and additional cycles of block-diagonal least-squares refinement using anisotropic thermal parameters for the non-hydrogen atoms and isotropic thermal parameters for the hydrogen atoms resulted in convergence. Since there were significant differences in the pyrimidine base geometries, two rounds of full-matrix least-squares refinement were carried out on the non-hydrogen atoms to ensure that the contributions from the off-diagonal terms were minimal. No changes resulted from this refinement.

A modified counting statistics weighting scheme was used in which $w = 1/[\sigma^2(F) + (0.02F_o)^2]$.[6] The final *R* index was 0.028 and the R_w value[7] was 0.035. The average shift/error ratios for the atomic parameters were 0.002 for the non-hydrogen atoms, with a maximum value of 0.016 for C(7)-A and 0.023 for the hydrogen atoms. Scattering factors for the oxygen, nitrogen, and carbon atoms were taken from Cromer and Waber[8] and those for the hydrogen atoms were from Stewart, Davidson, and Simpson.[9]

Conformational Energy Calculations. Energy calculations employing semiempirical potential functions[10,11] were carried out to determine the accessible conformations about the C(4)–O(4) bond for O^4-methyluridine using the X-ray coordinates of molecule B. The total potential energy (V_{tot}) is the sum of van der Waals interactions (V_{nb}), the electrostatic interactions (V_{es}), and the torsional potential for rotation about single bonds (V_t): $V_{tot} = V_{nb} + V_{es} + V_t$. V_{nb} are approximated by using the Lennard-Jones potential function: $V_{nb}(r) = -A_{ij}/r_{ij}^6 + B_{ij}/r_{ij}^{12}$. The parameters A_{ij} are evaluated from the atomic polarizabilities and effective number of electrons by use of the Slater–Kirkwood equation,[12] and B_{ij} are chosen such that the Lennard-Jones potential function displays a minimum when $r_{ij} = r_i^0 + r_j^0 + 0.2$ Å, where r_i^0 and r_j^0 are the van der Waals radii of the interacting pair of atoms.[13] V_{es} are measured in the monopole approximation employing the expression $V_{es} = 332e_ie_j/\epsilon r_{ij}$, where e_i and e_j are the magnitudes of the partial electronic charges[14,15] and ϵ is the effective dielectric constant, which was set to 4.[13] $V_t = V_0/2(1 + 3\cos\phi)$, which assumes a threefold potential function about the C(4)–O(4) bond, was used assuming a rotational barrier of 1.2 kcal/mol for C(4)–O(4).[10]

Chart I



Results and Discussion

ORTEP[16] drawings of both independent molecules indicating the atom numbering and overall molecular conformation are given in Figure 1. The fractional positional and isotropic thermal parameters for all atoms are presented in Table I.[17]

Ribose Geometry and Conformation. The ribose sugar rings of both independent molecules display very similar bond lengths. The largest difference is in the C(4′)–O(4′) bond, with a value of (1.451 (3) Å) in molecule A and 1.440 (3) Å in molecule B. The difference is 0.011 Å or 3.7σ. The bond angles are also similar with the exception of the O(3′)–C(3′)–C(4′) angle, which is 6.4° greater in molecule A (Table II). All non-hydrogen bond lengths and bond angles are listed in Table II.

Both ribofuranose rings are found in the $^3T_2$ twist conformation,[18] where the phase angles of pseudorotation (*P*)[19] are 7.6 (3)° for molecule A and 11.4 (3)° for molecule B. The amplitudes of pseudorotation (τ_m) are 37.5 (2)° and 42.0 (2)° for molecules A and B, respectively, and reveal significant flattening of the ribofuranosyl ring of A as compared to that of B. The conformation about the exocyclic C(4′)–C(5′) bond is the preferred gauche$^+$,[20] where the values for A and B are 56.1 (3)° and 52.7 (3)°, respectively. A list of other pertinent torsion angles is given in Table III.

Glycosyl Geometry and Conformation. The glycosidic bond, N(1)–C(1′), is 1.481 (4) Å in molecule A and 1.490 (3) Å in molecule B and is similar to that reported for uridine (average 1.490 Å).[21] The bond angle N(1)–C(1′)–O(4′) is identical (within

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Table I. Atomic Parameters for O^4-Methyluridine[a]

atom	x	y	z	B_{eq}[b] or B, Å^2
N(1)-A	9858 (4)	103 (1)	5090 (1)	2.51 (3)
C(2)-A	8043 (5)	−78 (1)	4659 (1)	2.72 (6)
O(2)-A	8072 (4)	256 (1)	4216 (1)	3.45 (3)
N(3)-A	6388 (4)	−624 (1)	4750 (1)	3.07 (3)
C(4)-A	6431 (5)	−923 (1)	5245 (1)	3.28 (6)
O(4)-A	4871 (4)	−1472 (1)	5334 (1)	4.64 (3)
C(7)-A	3232 (7)	−1701 (1)	4863 (1)	5.36 (10)
C(5)-A	8045 (7)	−722 (1)	5705 (1)	3.80 (7)
C(6)-A	9799 (6)	−210 (1)	5603 (1)	3.11 (6)
C(1')-A	11893 (5)	623 (1)	4931 (1)	2.54 (6)
C(2')-A	10773 (5)	1352 (1)	4874 (1)	2.45 (6)
O(2')-A	12320 (4)	1724 (1)	4473 (1)	3.33 (3)
C(3')-A	11300 (5)	1640 (1)	5466 (1)	2.54 (6)
O(3')-A	11526 (4)	2363 (1)	5499 (1)	3.41 (3)
C(4')-A	13977 (5)	1316 (1)	5623 (1)	2.61 (6)
O(4')-A	13910 (3)	647 (1)	5350 (1)	2.86 (2)
C(5')-A	14587 (6)	1214 (1)	6247 (1)	3.08 (6)
O(5')-A	12558 (4)	818 (1)	6516 (1)	3.71 (3)
N(1)-B	9648 (5)	2581 (1)	7783 (1)	3.19 (3)
C(2)-B	9203 (6)	1893 (1)	7642 (1)	3.89 (7)
O(2)-B	10553 (5)	1646 (1)	7260 (1)	6.22 (7)
N(3)-B	7300 (5)	1534 (1)	7929 (1)	3.98 (7)
C(4)-B	5958 (6)	1838 (1)	8332 (1)	3.68 (7)
O(4)-B	4018 (5)	1510 (1)	8605 (1)	4.86 (6)
C(7)-B	3439 (8)	807 (2)	8436 (1)	5.50 (11)
C(5)-B	6419 (6)	2531 (1)	8501 (1)	3.93 (7)
C(6)-B	8253 (7)	2880 (1)	8208 (1)	3.51 (7)
C(1')-B	11760 (5)	2937 (1)	7444 (1)	3.09 (6)
C(2')-B	10784 (6)	3102 (1)	6843 (1)	3.14 (6)
O(2')-B	13067 (4)	3135 (1)	6482 (1)	4.36 (6)
C(3')-B	9603 (5)	3812 (1)	6935 (1)	2.80 (6)
O(3')-B	9089 (4)	4183 (1)	6432 (1)	3.93 (6)
C(4')-B	11667 (5)	4129 (1)	7335 (1)	3.04 (6)
O(4')-B	12387 (4)	3567 (1)	7704 (1)	3.49 (3)
C(5')-B	10749 (6)	4729 (1)	7688 (1)	3.90 (7)
O(5')-B	8384 (5)	4549 (1)	7984 (1)	5.15 (6)
H7(1)-A	242 (9)	−214 (2)	500 (2)	10.4 (12)
H7(2)-A	199 (9)	−129 (2)	473 (2)	9.8 (11)
H7(3)-A	435 (8)	−181 (2)	453 (1)	8.6 (11)
H5-A	794 (6)	−98 (1)	608 (1)	3.5 (6)
H6-A	1106 (5)	−5 (1)	589 (1)	3.5 (6)
H1'-A	1266 (6)	47 (1)	457 (1)	2.9 (6)
H2'-A	888 (4)	134 (1)	479 (1)	1.1 (4)
H2'(O)-A	1247 (6)	148 (1)	416 (1)	5.0 (7)
H3'-A	982 (5)	148 (1)	573 (1)	3.0 (5)
H3'(O)-A	1010 (6)	255 (1)	545 (1)	5.0 (7)
H4'-A	1552 (4)	160 (1)	544 (1)	1.2 (4)
H5'(1)-A	1478 (5)	166 (1)	643 (1)	1.9 (5)
H5'(2)-A	1641 (6)	95 (1)	627 (1)	4.1 (7)
H5'(O)-A	1155 (6)	109 (1)	675 (1)	3.4 (6)
H7(1)-B	309 (7)	81 (1)	806 (1)	5.6 (8)
H7(2)-B	171 (8)	67 (2)	868 (1)	8.3 (10)
H7(3)-B	524 (9)	57 (2)	850 (1)	9.0 (11)
H5-B	530 (6)	270 (1)	885 (1)	3.5 (6)
H6-B	857 (6)	339 (1)	822 (1)	4.3 (6)
H1'-B	1338 (5)	264 (1)	745 (1)	3.3 (6)
H2'-B	952 (5)	279 (1)	671 (1)	2.2 (5)
H2'(O)-B	1273 (7)	291 (1)	618 (1)	5.5 (8)
H3'-B	785 (5)	377 (1)	716 (1)	3.0 (5)
H3'(O)-B	1064 (6)	436 (1)	627 (1)	4.9 (7)
H4'-B	1327 (5)	425 (1)	713 (1)	3.0 (5)
H5'(1)-B	1226 (6)	485 (1)	796 (1)	3.8 (6)
H5'(2)-B	1051 (6)	514 (1)	742 (1)	4.4 (6)
H5'(O)-B	799 (8)	493 (2)	813 (1)	7.0 (9)

[a] Fractional positional parameters are multiplied by 10^4 for non-hydrogen atoms and 10^3 for hydrogen atoms. [b] $B_{eq} = {}^4/_3 \Sigma_i \Sigma_j B_{ij} a_i \cdot a_j$.

1.5σ) in both molecules (average 109.6 (2)°); however, the N(1)–C(1')–C(2') bond angle shows a significant difference, where the value in A (113.9 (2)°) is 2.5° greater than in B (111.4 (2)°). The conformation about the glycosyl bond is anti for both molecules, with χ_A = 9.4 (3)° and χ_B = 10.9 (3)°.

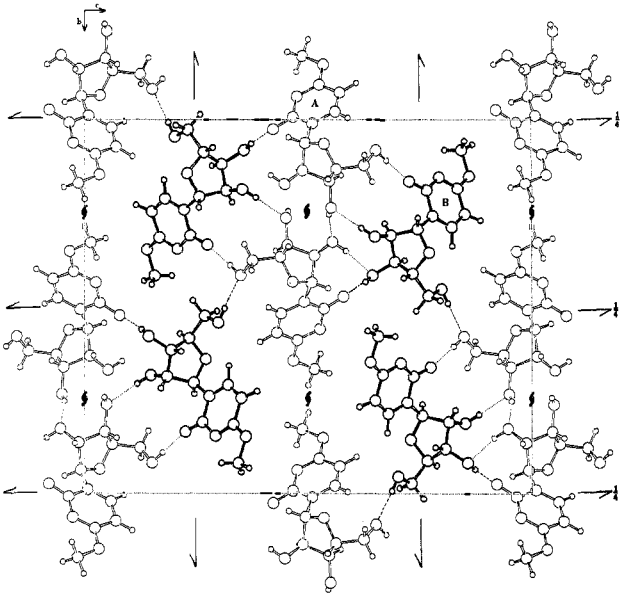

Figure 2. Crystal packing diagram viewed down the *a* axis. Molecule A (nine molecules displayed) is drawn with light bonds while molecule B (four molecules displayed) is drawn with dark bonds. The molecules labeled A and B are those corresponding to the coordinates given in Table I.

Base Geometry, Planarity, and Conformation. The bond lengths and bond angles of the bases generally display similar values; however, significant differences are observed (Table II). The C(4)–O(4) bond of molecule A (1.338 (3) Å) is 0.014 Å longer than the corresponding bond of molecule B (1.324 (4) Å), whereas the O(4)–C(7) bond is 0.008 Å shorter in A (1.445 (4) Å) than in B (1.453 (4) Å). Larger differences are seen in the C(4)–C(5) and C(5)–C(6) bond lengths. The C(4)–C(5) bond lengths found for molecule A (1.402 (4) Å) is 0.022 Å smaller than that found for molecule B (1.424 (3) Å), whereas the C(5)–C(6) bond of molecule A (1.347 (4) Å) is 0.017 Å longer than the corresponding bond in molecule B (1.330 (4) Å). The averaged values for the pyrimidine ring of molecules A and B are shown. Five of the six bond lengths, with the exception of C(4)–C(5), are closer to the values of cytidine[22] than diketo uridine[21] (Chart I).[23] Of particular interest is the C(5)–C(6) bond, (average 1.338 Å), which by its decreased π-bond order reflected by cytosine-like character (average 1.337 Å) of the modified base in NMR studies.[4] Additional differences between the molecules are observed for the bond angles about C(4). The N(3)–C(4)–C(5) bond angle in molecule A (124.9 (3)°) is 1.5° larger than that in molecule B (123.4 (3)°) while the N(3)–C(4)–O(4) angle is 1.3° larger in B (120.5 (3)°) than in A (119.2 (3)°). These geometric differences between molecules A and B are attributed to the distortions of base A caused by short intermolecular contacts (see below).

The pyrimidine ring atoms of molecule A exhibit large displacements from its least-squares plane (Table IV). The average deviation for the six ring atoms is |0.022 (11) Å|, with a maximum deviation of 0.037 (3) Å for C(2). The exocyclic base atoms, O(2), O(4), and C(7), as well as C(1') display even greater average displacements, |0.151 (44) Å|. On the other hand, the pyrimidine ring of molecule B is flat (average displacement |0.008 (5) Å|). Here the substituent atom C(1') is in the plane of the base while the keto O(2) atom is displaced by 0.027 (3) Å. The methoxy group atoms, O(4) and C(7), deviate the farthest from the plane (−0.075 (3) Å and −0.135 (5) Å, respectively).

The C(7) methyl group of both molecules assumes the synperiplanar conformation in which the N(3)–C(4)–O(4)–C(7)

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(23) The average standard deviations for the averaged bond lengths of O^4-methyluridine, cytidine, and diketo uridine are 0.004, 0.004, and 0.005 Å, respectively.

Table II. Bond Lengths (Å) and Angles (deg) (Non-hydrogen Atoms Only)

bond	A	B	angle	A	B
N(1)-C(2)	1.402 (3)	1.397 (3)	N(1)-C(2)-O(2)	118.1 (3)	117.6 (3)
C(2)-O(2)	1.226 (3)	1.219 (3)	N(1)-C(2)-N(3)	118.3 (2)	119.2 (3)
C(2)-N(3)	1.362 (3)	1.357 (3)	O(2)-C(2)-N(3)	123.6 (2)	123.2 (2)
N(3)-C(4)	1.299 (3)	1.300 (3)	C(2)-N(3)-C(4)	118.7 (2)	119.1 (3)
C(4)-O(4)	1.338 (3)	1.324 (4)	N(3)-C(4)-O(4)	119.2 (2)	120.5 (3)
O(4)-C(7)	1.445 (4)	1.453 (4)	N(3)-C(4)-C(5)	124.9 (3)	124.4 (3)
C(4)-C(5)	1.402 (4)	1.424 (3)	O(4)-C(4)-C(5)	115.9 (3)	116.1 (3)
C(5)-C(6)	1.347 (4)	1.330 (4)	C(4)-O(4)-C(7)	117.1 (2)	117.8 (3)
C(6)-N(1)	1.349 (3)	1.348 (4)	C(4)-C(5)-C(6)	116.3 (3)	116.8 (3)
N(1)-C(1′)	1.481 (3)	1.490 (3)	C(5)-C(6)-N(1)	120.4 (3)	121.1 (3)
C(1′)-O(4′)	1.407 (3)	1.405 (3)	C(6)-N(1)-C(2)	121.0 (2)	120.5 (3)
C(1′)-C(2′)	1.531 (3)	1.525 (3)	C(6)-N(1)-C(1′)	123.2 (2)	124.0 (2)
C(2′)-O(2′)	1.415 (3)	1.420 (3)	C(1′)-N(1)-C(2)	115.6 (2)	115.6 (2)
C(2′)-C(3′)	1.520 (3)	1.517 (3)	N(1)-C(1′)-O(4′)	109.7 (2)	109.4 (2)
C(3′)-O(3′)	1.414 (3)	1.407 (3)	N(1)-C(1′)-C(2′)	113.9 (2)	111.4 (2)
C(3′)-C(4′)	1.521 (3)	1.523 (3)	O(4′)-C(1′)-C(2′)	106.9 (2)	106.8 (2)
C(4′)-O(4′)	1.451 (3)	1.440 (3)	C(1′)-C(2′)-O(2′)	109.5 (2)	107.7 (3)
C(4′)-C(5′)	1.508 (3)	1.503 (3)	C(1′)-C(2′)-C(3′)	101.5 (2)	100.6 (2)
C(5′)-O(5′)	1.420 (3)	1.412 (4)	O(2′)-C(2′)-C(3′)	108.9 (2)	110.8 (2)
			C(2′)-C(3′)-O(3′)	115.5 (2)	114.8 (2)
			C(2′)-C(3′)-C(4′)	102.7 (2)	101.2 (2)
			O(3′)-C(3′)-C(4′)	109.2 (2)	115.6 (2)
			C(3′)-C(4′)-O(4′)	104.2 (2)	103.4 (2)
			C(3′)-C(4′)-C(5′)	117.8 (2)	116.6 (2)
			C(5′)-C(4′)-O(4′)	108.3 (2)	109.6 (2)
			C(4′)-C(5′)-O(5′)	111.0 (2)	109.4 (2)
			C(4′)-O(4′)-C(1′)	110.8 (2)	110.3 (2)

Table III. Selected Torsion Angles (deg)

	A	B
O(4′)-C(1′)-N(1)-C(6) (χ_{CN})	9.4 (3)	10.9 (3)
C(4′)-O(4′)-C(1′)-C(2′) (τ_0)	7.1 (3)	5.1 (3)
O(4′)-C(1′)-C(2′)-C(3′) (τ_1)	−27.5 (3)	−29.0 (3)
C(1′)-C(2′)-C(3′)-C(4′) (τ_2)	36.4 (3)	40.3 (3)
C(2′)-C(3′)-C(4′)-O(4′) (τ_3)	−33.2 (3)	−38.4 (3)
C(3′)-C(4′)-O(4′)-C(1′) (τ_4)	16.5 (3)	21.1 (3)
C(3′)-C(4′)-C(5′)-O(5′) (ψ)	56.1 (3)	52.7 (3)
O(4′)-C(4′)-C(5′)-O(5′)	−61.7 (3)	−64.2 (3)
C(7)-O(4)-C(4)-N(3)	0.4 (3)	0.4 (3)
C(7)-O(4)-C(4)-C(5)	−178.6 (3)	178.5 (4)

Table IV. Deviation (Å) from the Least-Squares Planes of the Pyrimidine Rings

	A	B		A	B
N(1)[b]	−0.027 (2)	−0.009 (3)	O(2)	0.106 (3)	0.027 (3)
C(2)[b]	0.037 (3)	0.010 (3)	O(4)	−0.108 (3)	−0.075 (3)
N(3)[b]	−0.012 (3)	0.001 (3)	C(7)	−0.196 (4)	−0.137 (5)
C(4)[b]	−0.022 (3)	−0.013 (3)	C(1′)	−0.194 (3)	−0.005 (4)
C(5)[b]	0.031 (3)	0.014 (3)			
C(6)[b]	−0.006 (3)	−0.004 (3)			

[a] Equations of the least-squares planes employing unit weights are as follows: molecule A, $-0.689X + 0.647Y + 0.328Z = 0.69$; molecule B, $0.693X - 0.292Y + 0.659Z = 13.91$. [b] Atoms included in the calculation of the least-squares plane.

torsion angle is 0.4 (3)° for both A and B. The distances between the N(3) nitrogen atoms and the two closest methyl protons are 2.55 (4) Å and 2.57 (4) Å for molecule A and 2.55 (3) Å and 2.54 (3) Å for molecule B.

Hydrogen Bonding and Molecular Packing. A crystal packing diagram illustrating the six hydrogen bonds found in the structure (Table V) is shown in Figure 2. The independent molecules are anchored in the asymmetric unit by hydrogen bonds between the keto oxygen atom O(2)-B and the O(5′)-A proton (2.577 (3) Å) and the hydroxyl oxygen atom O(3′)-A and the O(2′)-B proton (2.857 (3) Å). The four remaining hydrogen bonds are formed between neighboring molecules and produce a network of bonds that stabilizes the crystal lattice (Table V). Of these four, molecule A, which forms a hydrogen bond to a screw-related molecule, acts as the proton acceptor three times. Molecule B, on the other hand, does not form any hydrogen bonds to its screw-related neighbor and acts as a proton acceptor only once.

Hydrophobic interactions are also prevalent in the molecular packing of the independent molecules (Figure 2 and Table VI). A hydrophobic pocket is formed between two adjacent methyl groups of molecule A and the neighboring H(5) protons of molecule B. Furthermore, these methyl carbon atoms are in favorable van der Waals contact with the O(4)-B atoms of neighboring molecules (3.178 (3) Å). Similarly, the C(7) atom of molecule B has favorable van der Waals contact with the neighboring oxygen atom O(2)-A (3.264 (3) Å). Whereas the methoxy group of molecule A is not involved in stacking interactions, the methoxy group of molecule B does exhibit some stacking interactions with its translationally related base. However, these interactions are relatively weak with the exception of the O(4)–C(2) contact (3.379 (3) Å and Table VI). Stacking interactions between the pyrimidine bases and their translationally related ribofuranosyl ring oxygen atoms are discussed below.

Effects of Methylation and Intermolecular Sugar–Base Stacking. The presence of the two independent molecules offers an opportunity to study not only the effects of methylation on the electronic and stereochemical properties of uridine but also the effects of intermolecular stacking on these properties. Molecule B displays only weak intermolecular contacts between the pyrimidine ring and its translationally related ribosyl ring oxygen atom

Table V. Hydrogen Bond Lengths (Å) and Angles (deg)

A-H···B	B (*xyz*)	A-H	H···B	A···B	A-H-·B
O(2′)A-H···O(3′)B	3 (001)	0.88 (2)	2.06 (2)	2.898 (3)	159 (2)
O(3′)A-H···O(2′)A	3 (−101)	0.81 (3)	1.99 (2)	2.749 (3)	157 (3)
O(5′)A-H···O(2)B	1 (000)	0.91 (2)	1.69 (2)	2.577 (3)	164 (2)
O(2′)B-H···O(3′)A	1 (000)	0.85 (2)	2.01 (2)	2.857 (3)	174 (3)
O(3′)B-H···O(2)A	3 (001)	0.93 (3)	1.82 (3)	2.729 (3)	164 (2)
O(5′)B-H···O(5′)A	4 (201)	0.84 (4)	1.94 (4)	2.774 (3)	174 (4)

Table VI. Shorter Intermolecular Contacts (Å)

O(4')A···C(2)A	2.977 (3)	O(4')B···C(6)B	3.426 (3)
O(4')A···N(3)A	3.103 (3)	C(7)A···O(4)B	3.178 (3)
O(4')A···N(1)A	3.207 (3)	C(7)B···O(2)A	3.264 (3)
O(4')A···C(4)A	3.313 (3)	O(4)B···C(2)B	3.379 (3)
O(4')A···C(6)A	3.412 (3)	C(7)B···O(2)B	3.513 (3)
O(4')A···C(5)A	3.470 (3)	C(7)B···C(2)B	3.520 (3)
O(4')B···C(5)B	3.406 (3)		

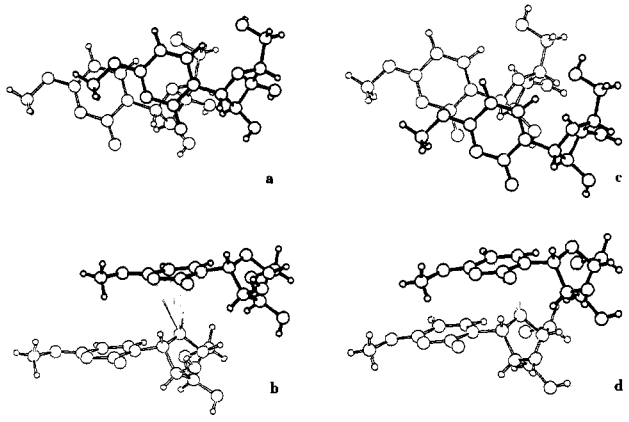

Figure 3. Projections perpendicular (a) and parallel (b) to the base plane displaying the intermolecular stacking between molecule A and an adjacent ribose ring oxygen atom. The close approach of the sugar ring O(4') atom to the base C(2) atom represents the reaction coordinates for an incipient nucleophilic attack (see text). Projections c and d display similar orientations for molecule B. The dashed lines represent intermolecular contacts between the O(4') oxygen atoms and the bases from 2.9 to 3.5 Å (see Table IV).

(Figure 3c,d), in which the closest contacts are O(4') to C(5) (3.402 (3) Å) and to C(6) (3.426 (3) Å). Thus the electron density distribution and the geometry of the base are influenced primarily by the methylation of O(4), which results in the strongly conjugated O(2)=C(2)—N(3)=C(4)—O(4) fragment. Molecule A, on the other hand, has strong intermolecular contacts between the pyrimidine ring and its translationally related ribofuranosyl ring oxygen atoms (Figure 3a,b), where the distances between the O(4') atom and the C(2), N(3), and N(1) atoms are 2.977 (3) Å, 3.103 (3) Å, and 3.207 (3) Å, respectively. The remaining pyrimidine ring atoms also stack with O(4') but these interactions are weaker (Table VI). Molecule A also displays a strongly conjugated O(2)=C(2)—N(3)=C(4)—O(4) fragment due to the methylation of O(4). It is interesting that large bonding differences between A and B are observed for the C(7)–O(4)–C(4)–C(5)–C(6) fragment, which is opposite the >C(2)=O(2) carbonyl group. These differences appear to result from the monopole-induced dipole interactions involving the ribosyl O(4') oxygen atom of molecule A and a neighboring base, which result in delocalization of π-electrons from the C(5)–C(6) bond into the C(4)–C(5) bond. As a consequence of this shift the local electron density about the C(4) carbon atom is increased, forcing the C(4)–O(4) bond to transfer some electron density into the O-(4)–C(7) bond. The results of these correlated shifts are the decreased π-bond order of the C(5)–C(6) and C(4)–O(4) bonds and the increased π-bond orders of the C(4)–C(5) and possibly O(4)–C(7) bonds.

Similar monopole-induced dipole interactions appear to occur in the dinucleoside monophosphate UpA.[24] There, the uracil base of the second independent molecule (2) has very short intermolecular contacts between the O(4') oxygen atom and the neighboring N(3) (2.899 Å) and C(2) (2.919 Å) atoms, whereas the other independent molecule (1) exhibits weaker contacts between the O(4') and C(6) (3.412 Å) and N(1) (3.201 Å) atoms. As in O^4-methyluridine the C(4)–C(5) (1.413 (8) Å) and C(5)–C(6) (1.368 (8) Å) bond lengths of molecule 2 are longer and shorter

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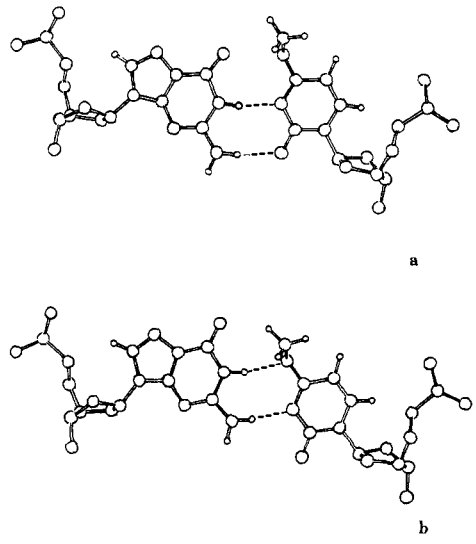

Figure 4. Possible base-pairing schemes between O^4-methyluridine and guanosine with (a) Watson–Crick hydrogen bonding and (b) non-Watson–Crick hydrogen bonding. Note that the N(3)–C(4)–O(4)–C(7) torsion angle, ϕ, has been rotated 110° from the syn-periplanar conformation. The methoxy group must be rotated ±80° to expose the N(3) ring nitrogen atom for participation in base-pairing interactions. The anti-periplanar conformation for the methoxy group would appear to be the favored position from many crystal structures of methoxy compounds (see text).

than the corresponding bonds of molecule 1, 1.400 (7) Å and 1.330 (7) Å, respectively. Although the absolute values must be viewed with caution, the trend is clearly the same. Other structures of uridine in which two independent molecules are found are scarce. The above observations may be contrasted with uridine[21] and 6-methyluridine,[25] which also crystallize with two independent molecules per asymmetric unit but show no sugar–base stacking interactions and therefore no differences in the C(4)–C(5) or C(5)–C(6) bond lengths of the independent molecules.

Reaction Coordination for an Incipient Nucleophilic Attack. Another effect of stacking of the O(4') oxygen atom with the base is to increase the nonplanarity of the uracil ring. This distortion (and pyramidization at the C(2) atom) is caused primarily by the strong C(2)–O(4') interaction in which the C(2) atom is pulled 0.037 (3) Å from the least-squares plane of the base (Table IV). The deviation of the C(2) carbon atom from the N(3)–N(1)–O(2) plane (0.010 (3) Å) and the O(4')–C(2)–O(2) angle (102.6(3)°) are similar to those values reported for other crystal structures in which a nucleophile is in close contact with a carbonyl carbon atom.[26,27] This interaction provides information on the reaction coordinates for a nucleophilic attack on the base C(2) atom. Such a mode of attack may be envisaged by the O(2') oxygen atom of a pyrimidine arabinonucleoside on the C(2) carbonyl carbon in the formation of the corresponding 2,2' anhydro cyclic analogue. It may be mentioned that the participation of the carbonyl oxygen atom O(2)-A in hydrogen bonding cannot be responsible for the ring distortion since molecular B also exhibits this hdyrogen bonding and yet its pyrimidine ring is planar.

Base Pairing between O^4-Methyluracil (Thymine) and Guanine. The methylation of the keto O(4) oxygen atom restricts the possible pairing schemes for O^4-methyluracil and O^4-methylthymine. In 1977 Abbott and Saffhill postulated a guanine–O^4-methylthymine mispair in the standard cis glycosyl configuration (where the glycosyl C(1')–N bonds are on the same side as in the Watson–Crick pairs) in which hydrogen bonds are formed between the N(3) nitrogen and the keto O(2) oxygen atoms of the modified base and the N(2) and N(3) nitrogen atoms of guanine.[28] Neither the O(4) methoxy nor the O(6) keto oxygen

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atoms was involved in hydrogen bonding. A similar scheme for a guanine-O^4-methyluracil mispair has also been proposed.[4] In order to form this pair the torsion angle ϕ [N(3)-C(4)-O(4)-C(7)] must be rotated at least ±80° (see Figure 4a) from the syn-periplanar conformation to alleviate steric clash between the C(7) methyl group and the guanine O(6) atom, particularly. Crystal structure determinations of and ab initio quantum mechanical calculations on many compounds containing monomethoxyphenyl moieties have shown that the planar conformations ($\phi = \pm 180°$) are easily accommodated by allowing the two methyl protons to straddle the ortho ring protons, thus avoiding unfavorable proton-proton interactions.[29] It is likely that the methoxy group will assume the anti-periplanar conformation in the guanine-O^4-methyluracil pair as an in-plane methoxy group allows maximum conjugation between the aromatic π electrons and the lone-pair electrons of O(4). However, the methoxy group of a guanine-O^4-methylthymine base pair must take an electronically less favorable (out of plane) conformation to avoid a collision between the C(5) and C(7) methyl groups that arises when ϕ approaches 180°. A second base-pairing model (Figure 4b) requiring a "wobble" of the pyrimidine base and the involvement of the O(4) atom is not as likely since the methoxy O(4) oxygen atom is expected to be less favored than the keto O(2) oxygen atom in hydrogen bonding.

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Summary

The conformations of the two independent molecules of O^4-methyluridine are similar. They display the C(3') endo sugar pucker, anti disposition of the base, and gauche$^+$ conformation about the C(4')-C(5') bond. However, the geometries of the pyrimidine bases show some striking differences. The pyrimidine ring of molecule A is in a twist-boat conformation, while that of molecule B is flat. Bonding differences are seen in the C(7)-O(4)-C(4)-C(5)-C(6) half of the base. These differences are attributed to the monopole-induced dipole interactions between the ribose ring O(4') oxygen atom and a neighboring pyrimidine base of A.

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Supplementary Material Available: Tables of anisotropic thermal parameters and observed and calculated structure factors (9 pages). Ordering information is given on any current masthead page.

An Examination of Relaxation Reagents for Conformational Analysis of Peptides in Aqueous Solution

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Abstract: Second-order rate constants have been measured for proton spin-lattice relaxation of protons of peptides and model amides in water, catalyzed by 2,2,6,6-tetramethylpiperidinyl-1-oxy (Tempo) and the *N*-(2-hydroxyethyl)ethylenediaminetriacetic acid (HEEDTA) complexes of Gd^{3+} and in some cases Cr^{3+}. Examined were 2-azacyclononanone, 2-azacyclohexanone, Ac-Pro-NHMe, Ac-Sar-NHMe, cyclo(His-Asp), and cyclo(Gly-Pro-D-Gln)$_2$. The effectiveness of the nitroxyl as a relaxation reagent in water reflects primarily the degree of exposure of the observed proton to the external environment, as previously assumed, although there is some evidence of CONH···O-N< association in dimethyl sulfoxide. In contrast, the Gd^{3+} complex shows affinity for carbonyl oxygen and can be used to distinguish cis from trans isomers at peptide bonds to proline and N-alkyl amino acid residues. The specificity of the Gd complex is enhanced in methanol and lost in dimethyl sulfoxide. CrHEEDTA was observed to show some selective effects not readily explained. HEEDTA complexes of Eu^{3+}, Ho^{3+}, and Dy^{3+} were also prepared.

The effectiveness of paramagnetic species in inducing nuclear magnetic relaxation depends on distance, so that paramagnetic cosolutes can be used to determine, by relaxation effects, the periphery of a folded chain molecule. For this kind of study of peptides we have preferred nitroxyls[1-3] as the paramagnetic species, and we have hesitated to use transition metal or lanthanide ions because chelation with less stable conformations of the peptides could distort the interpretation of differential relaxation effects. However, chelation by peptide might be less likely when the solvent is water and the ion is already associated with a good complexing agent. Therefore, we compared the specificities of a nitroxyl, 2,2,6,6-tetramethylpiperidinyl-1-oxy (Tempo), and a neutral lanthanide complex, gadolinium *N*-(2-hydroxyethyl)ethylenediaminetriacetate (HEEDTA), in inducing spin-lattice relaxation of protons of several peptide models in aqueous solutions. The quantities measured were the second-order rate constants for relaxation catalyzed by the reagent. For *N*-acetylsarcosine *N*-methylamide we explored the dependence of the relaxation rate constants on peptide concentration in water, and we used the gadolinium complex in methanol and dimethyl sulfoxide as well. We also report some results with the HEEDTA complex of chromium(III).

Experimental Section

Materials. *N*-(2-Hydroxyethyl)ethylenediaminetriacetic acid (HEEDTA), 2,2,6,6-tetramethylpiperidinyl-1-oxy (Tempo), 2-azacyclononanone, 2-azacyclohexanone (2-piperidone), and dysprosium, europium, gadolinium, and holmium oxides were obtained from Aldrich. Cyclo(His-Asp)[4] and cyclo(Gly-Pro-D-Gln)$_2$[5] have been reported previously.

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