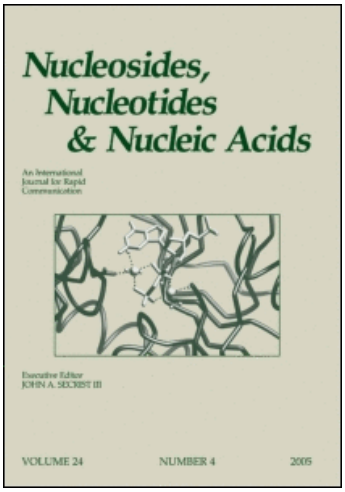

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Conformational Studies on Deoxyribonucleosides of C6-Substituted Pyrimidines

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CONFORMATIONAL STUDIES ON DEOXYRIBONUCLEOSIDES OF C6-SUBSTITUTED PYRIMIDINES

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Abstract

Conformational energy calculations have been presented on 6-methyl-2'-deoxyuridine using molecular mechanics and conformational analysis. The results are presented in terms of isoenergy contours in the conformational space of the glycosidic (χ) and C4'-C5' (γ) bonds torsions, for three commonly found furanose geometry, C2' endo, C3' endo and O1'endo. The χ-γ interrelationships are very similar to those previously calculated for unmodified nucleosides and nucleotides.

Introduction

Modulation of gene expression by antisense oligonucleotides has been recently recognized to be a potential target for drug discovery in anti-cancer and anti-viral therapy[1-3]. Numerous nucleotide-based antisense drug candidates have been synthesized and shown to possess desirable *in vitro* hybridization properties (affinity to the targeted nucleic acid)[4-6]. However, many of them suffer from the disadvantage of susceptibility to nucleolytic cleavage by nucleases and are consequently unsuitable as viable therapeutic targets[1,7-8]. The strategies aimed at overcoming the problem of nucleolytic cleavage have focused primarily on chemical modifications to oligonucleotide backbone. For example, modified nucleotides with methylphosphonates, phosphoramidates and phosphorothioates instead of the backbone phosphate have been shown to possess significant resistance to nuclease degradation[1,9].

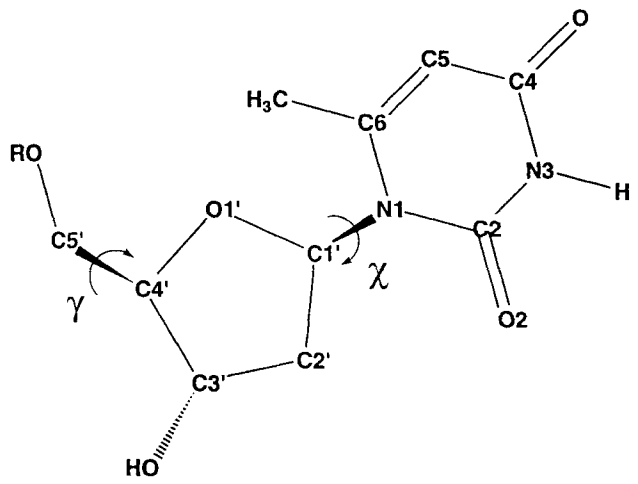
Recently, antisense oligonuclotides have been reported[10] with the base modified nucleotides as an additional strategy in the development of nuclease resistant drug

candidates with desirable antisense properties (binding affinity and the ability to support RNase H mediated RNA degradation). Specifically, nucleosides with modified pyrimidines (such as 6-azathymidine, 6-methyl-2'-deoxyuridine) have been included into antisense oligonucleotides[10]. Such oligonucleotides have been shown to possess enhanced nuclease resistance, reasonable binding to complementary DNA and RNA fragments and form heteroduplexes with RNA that support RNase H mediated cleavage[10].

In light of their potential biological significance, it is of interest to determine the structural attributes of nucleosides with modified bases. This paper reports the results of a set of semi-exhaustive conformational analyses on a C6-methylated pyrimidine nucleoside (**1**), using molecular mechanics methods. An analog of **1** in which the C5' methoxy is replaced by a hydroxyl group (**2**), has also been investigated to understand the significance, if any, of intramolecular hydrogen bonding interactions. It is found that as in the case of unmodified nucleosides and nucleotides, the glycosidic torsion (χ) is influenced both by the furanose geometry and the torsion about the C4'-C5' bond[11] (γ). The interrelationship between χ and γ in the C6-methylated nucleosides is not significantly affected in the presence of the C5' hydroxyl instead of the methoxy group for all the sugar geometries investigated.

Methods

Preliminary models of **1** and **2** (FIG. 1) were built using MacroModel (v3.5) and energy refined with the MM2 force field[12]. This force field was employed throughout the investigations, unless stated otherwise. The nomenclature adopted for describing the atom names and torsion angles is as described in reference 11. Multiple conformations of **1** and **2** were generated for three furanose ring geometries : C2' endo, C3' endo and O1' endo. The endocyclic torsion angles corresponding to these three puckers are listed in TABLE 1, while their bond lengths and bond angles are the same as in ref. 11. In each case, the torsions χ and γ (FIG. 1) were varied at 10° interval. The resultant collections of 1296 conformations were energy refined using the BatchMin module[12] by varying all degree of freedom except for χ, γ and the torsions defining the sugar pucker. The torsions χ and γ were constrained to their starting values with a weight of 1000 kcal/mole-degree, while the endocyclic torsions corresponding to the sugar pucker were constrained with a weight of 300 kcal/mole-degree. In this manner, the sugar puckers were allowed to be somewhat flexible within their respective conformational domains (viz. C2' endo, C3' endo and O1' endo), since ***one of the goals*** of this investigation was to study the sensitivity of the χ–γ relationship as a function of the furanose geometry.



R = CH_3 ; 1 R= H ; 2

FIG. 1. Schematic illustration of the compounds **1** and **2** investigated in this study. The hydrogens on carbon atoms are not shown.

TABLE 1 : Endocyclic torsion angles (in degrees) in the furanose sugar of 2'-deoxy-6-methyluridine.

Pucker	C1'-C2'	C2'-C3'	C3'-C4'	C4'-O1'	C1'-O1'
C2' endo	33.3	-34.8	24.6	-3.9	-18.6
O1' endo	24.1	0.1	-23.9	39.6	-40.1
C3' endo	-25.3	37.1	-37.5	22.4	2.1

The partial atomic charges on the atoms of **1** and **2** (TABLE 2) were obtained by fitting to the *ab initio* electrostatic potential surfaces (calculated using Gaussian92 with the RHF/6-31G* basis sets). A dielectric of 4.0 was employed throughout the calculations. This dielectric has been previously used by numerous theoretical investigations on the structure and conformations of nucleic acid constituents and the results obtained therein are qualitatively consistent with experimental data[10].

The results of the conformational energy calculations on the modified nucleosides are shown in terms of energy plots in the (χ,γ) space, illustrated in FIGS. 2 and 3,

TABLE 2 : Atom names and their partial atomic charges (in e.s.u) calculated by fitting to the *ab initio* electrostatic potential surface obtained by using the 6-31G* basis set.

Molecule 1		Molecule 2	
Atom	Charge	Atom	Charge
O5'	-0.0648	O5'	-0.4705
O5'-methyl	-0.0630	HO5'	0.3443
C5'	-0.3334	C5'	-0.1938
H5'A	0.0931	H5'A	0.0716
H5'B	0.1065	H5'B	0.0898
C4'	0.4272	C4'	0.3366
H4'	0.0442	H4'	0.0532
O1'	-0.3722	O1'	-0.3275
C1'	0.3732	C1'	0.4258
H1'	0.0646	H1'	0.0547
N1	-0.3480	N1	-0.4105
C2	0.8650	C2	0.9019
O2	-0.6888	O2	-0.6937
N3	-0.7899	N3	-0.8027
H3	0.4461	H3	0.4467
C4	1.0450	C4	1.0501
O4	-0.7090	O4	-0.7097
C5	-0.8563	C5	-0.8562
H5	0.2933	H5	0.2918
C6	0.4922	C6	0.5169
C6-methyl	0.0086	C6-methyl	0.0011
C2'	-0.3418	C2'	-0.4397
H2'A	0.0797	H2'A	0.1266
H2'B	0.1306	H2'B	0.1421
C3'	0.1350	C3'	0.2456
H3'	0.0763	H3'	0.0661
O3'	-0.6726	O3'	-0.6996
HO3'	0.4329	HO3'	0.4389

respectively, for **1** and **2**. Isoenergy contours (1 through 6 kcal/mole) are drawn in the (χ,γ) space at 1 kcal/mole intervals. The thickest contour corresponds to 1 kcal/mole relative to global minimum and the value increases as the thickness decreases. Thus the thinnest contour corresponds to 6 kcal/mole. The energy minima are listed in TABLES 3 and 4, and are called M1 (global minimum) and M2, M3, etc. (secondary minima) in this paper. Only the global minima are marked (X) in the (χ,γ) maps.

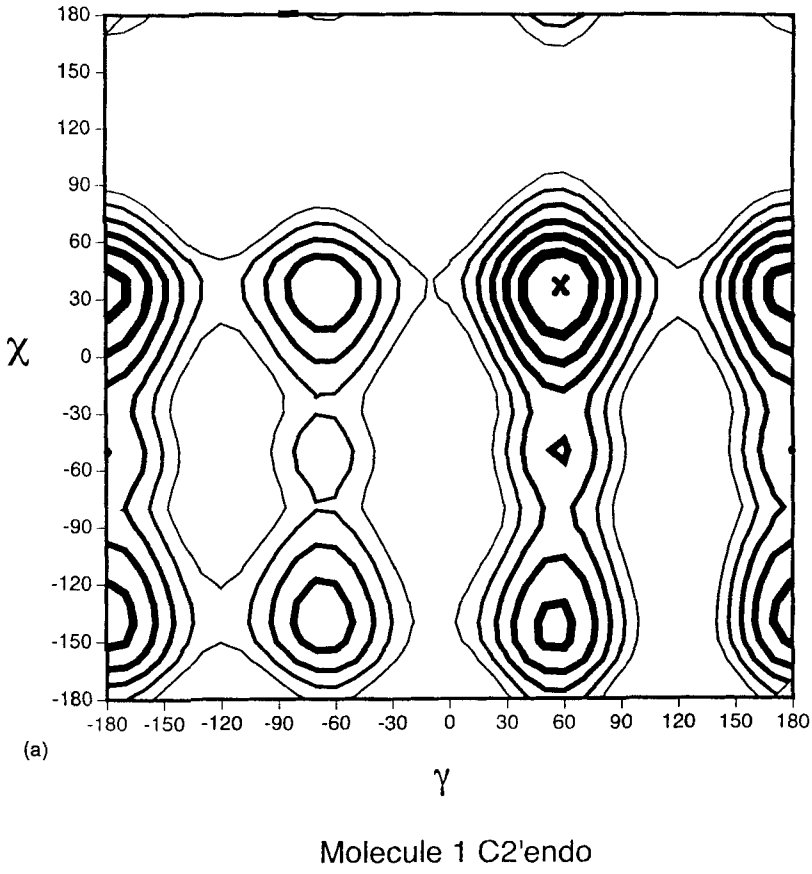


FIG. 2. Conformational energy plot for **1** in the χ–γ space corresponding to the C2' endo (a), O1' endo (b) and C3' endo (c) furanose geometry.

(continued)

Results

FIGS. 2a through 2c illustrate the conformational energy plots of **1** in its (χ,γ) space, corresponding to the C2' endo, O1' endo and C3' endo puckers of the furanose ring. The global and secondary energy minima in these plots are listed in TABLES 3a through 3c. In FIG. 2a, the global minimum (M1) is found at (χ,γ) = (30°,60°) and corresponds to a structure with *anti* conformation of the base and *gauche*$^+$ conformation about C4'-C5'. This conformational combination is predominantly observed in the crystal structures of oligonucleotides and other nucleic acid constituents. Secondary minima with *anti* orientation of 6-methyluracil are also observed for γ = 180° and -60°, but are destabilized by

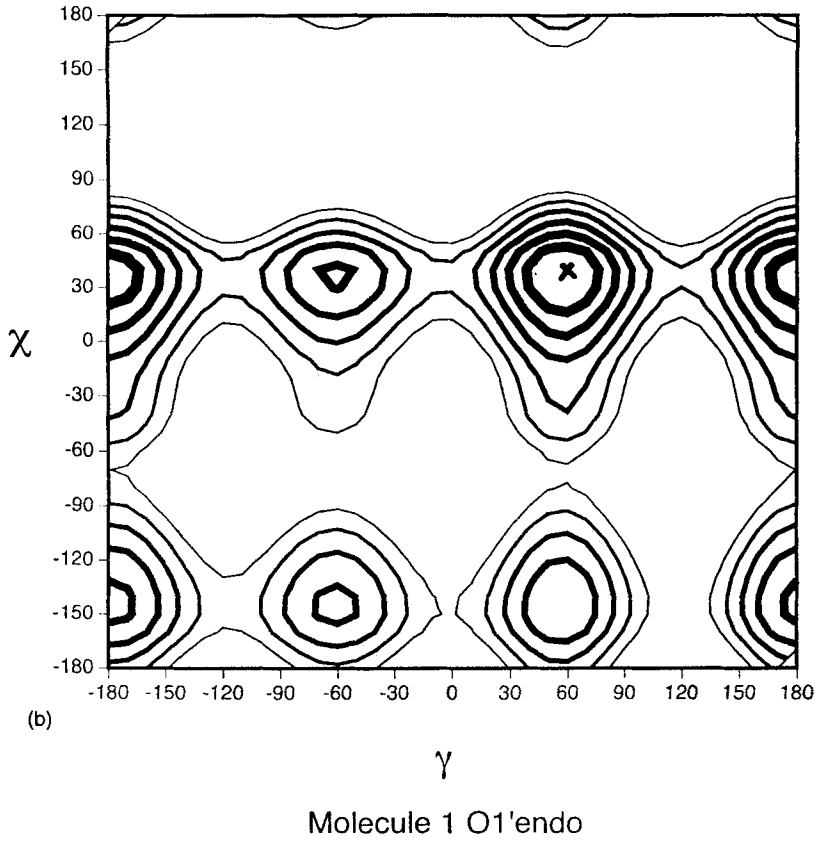


FIG. 2. Continued

0.5 and 2.5 kcal/mole, respectively, relative to the global minimum conformation (TABLE 3a). The minima corresponding to the *syn* conformation of the base are destabilized by at least 1.3 kcal/mole relative to M1. The high *anti* conformations (χ = 110° to 160°) are energetically significantly (> 6 kcal/mole) destabilized relative to M1. The energy barriers to conformational transitions between M1 and the secondary minima in normal *anti* region of the glycosidic torsion (M2 and M3) are at least 5 kcal/mole. The corresponding barriers for transitions to minima with the *syn* conformation of the base are either around 3.5 kcal/mole (minimum M4) or greater than 6 kcal/mole (M5 and M6). The 5 kcal/mole contour encloses about 40% of the total (χ,γ) conformational space.

The O1' endo geometry of the furanose ring of **1** does not cause any significant shifts in the position of the global minimum (M1) and secondary minima from those observed for

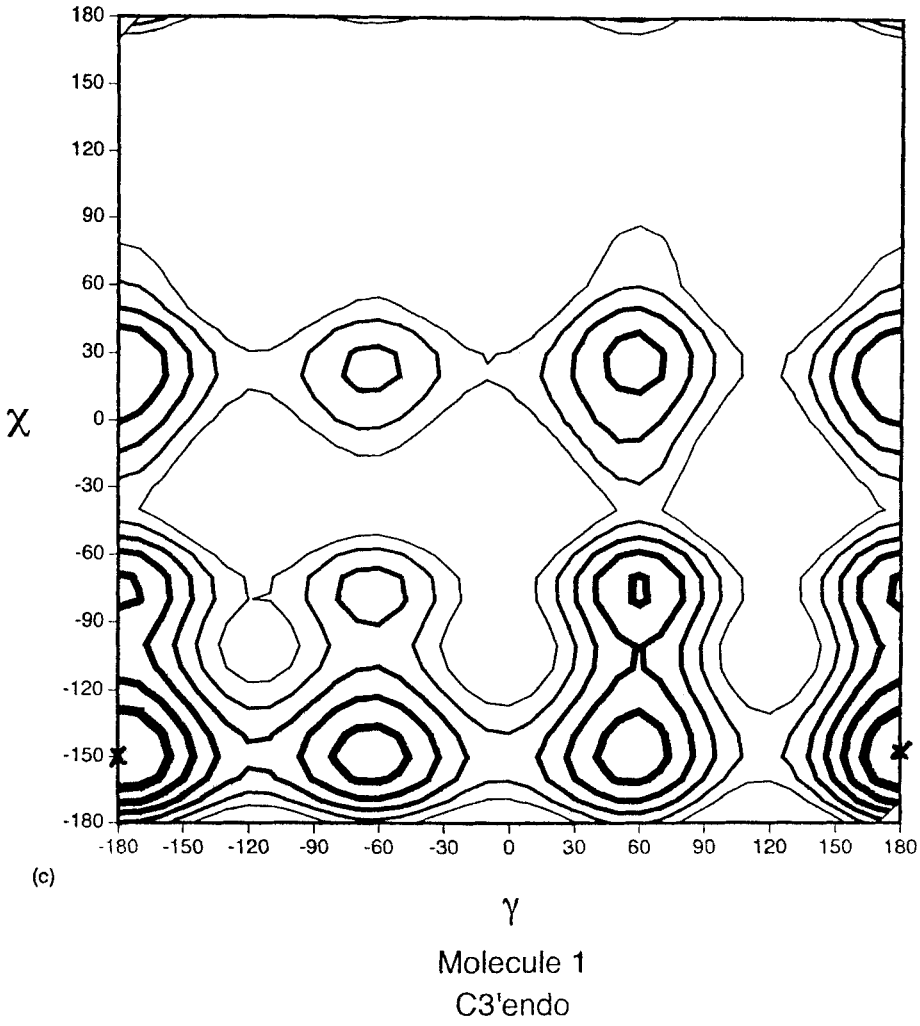


FIG. 2. Continued

the C2' endo geometry (TABLE 3b). Secondary minima with *anti* orientation of the 6-methyl uracil for γ = 180° and -60° are destabilized by about 0.5 and 2.5 kcal/mole, respectively. For the *syn* orientation of the pyrimidine, the minimum at γ = 180° (M4) is energetically the least expensive (1.8 kcal/mole relative to M1). The barriers to conformational transitions between M1 and the secondary minima are at least 4 kcal/mole for the *anti* region of χ and are higher than 6 kcal/mole for transitions to minima in the *syn* region of χ.

TABLE 3a : Energy minima of **1** in the conformational space (χ,γ) for the C2' endo furanose geometry (FIG. 2a). The energy of the global minimum is -131.5 kcal/mole.

Minimum Number	γ (deg)	χ (deg)	Relative Energy (Kcal/mole)
1	60.0	40.0	0.00
2	180.0	30.0	0.60
3	180.0	-140.0	1.55
4	60.0	-140.0	1.76
5	-70.0	40.0	2.20
6	-70.0	-140.0	2.41

TABLE 3b : Energy minima of **1** in the conformational space (χ,γ) for the O1' endo furanose geometry (FIG. 2b). The energy of the global minimum is -128.6 kcal/mole.

Minimum Number	γ (deg)	χ (deg)	Relative Energy (Kcal/mole)
1	60.0	40.0	0.00
2	180.0	40.0	0.41
3	180.0	-140.0	1.75
4	-60.0	40.0	1.84
5	60.0	-150.0	2.11
6	-60.0	-150.0	2.75

TABLE 3c : Energy minima of **1** in the conformational space (χ,γ) for the C3' endo furanose geometry (FIG. 2c). The energy of the global minimum is -127.9 kcal/mole.

Minimum Number	γ (deg)	χ (deg)	Relative Energy (Kcal/mole)
1	180.0	-150.0	0.00
2	60.0	-150.0	1.41
3	-60.0	-150.0	1.46
4	180.0	-80.0	1.81
5	60.0	-80.0	1.90
6	180.0	20.0	2.03
7	60.0	30.0	2.59
8	-60.0	-80.0	3.52
9	-70.0	20.0	3.78

In FIG. 2c (corresponding to the C3' endo furanose geometry), the global minimum occurs for (*syn, trans*) combination of (χ,γ). Eight secondary minima, all destabilized by more than 1 kcal/mole relative to M1, are observed (TABLE 3c). As in the case of the map for the C2' endo sugar geometry, here too, the high *anti* conformation is energetically forbidden. The minima corresponding to (*anti, gauche*$^+$) and (*anti, trans*) combinations of (χ,γ) are destabilized by 2.6 and 2.5 kcal/mole, respectively, relative to M1. A feature not observed in FIG. 2a is the occurrence of minima corresponding to $\chi \sim -70°$ to -80° (low *anti*). Two of these (M7 and M8) are destabilized by less than 2 kcal/mole relative to M1. With the exception of M8, the barriers to conformational transitions from the global minimum to all other secondary minima are at least 5 kcal/mole. In the case of M8, the barrier is around 2.4 kcal/mole.

The conformational energy plots in the (χ,γ) space of **2** for the three sugar puckers are qualitatively similar to the corresponding plots of **1**. Hence, only the plot for the C2' endo sugar is illustrated graphically (FIG. 3), with the corresponding minima listed in TABLE 4. The energy barriers to various conformational transitions are also similar. The plots in FIGs. 2a and 3 differ in the following manner : (1) the secondary minimum in the (*syn,*

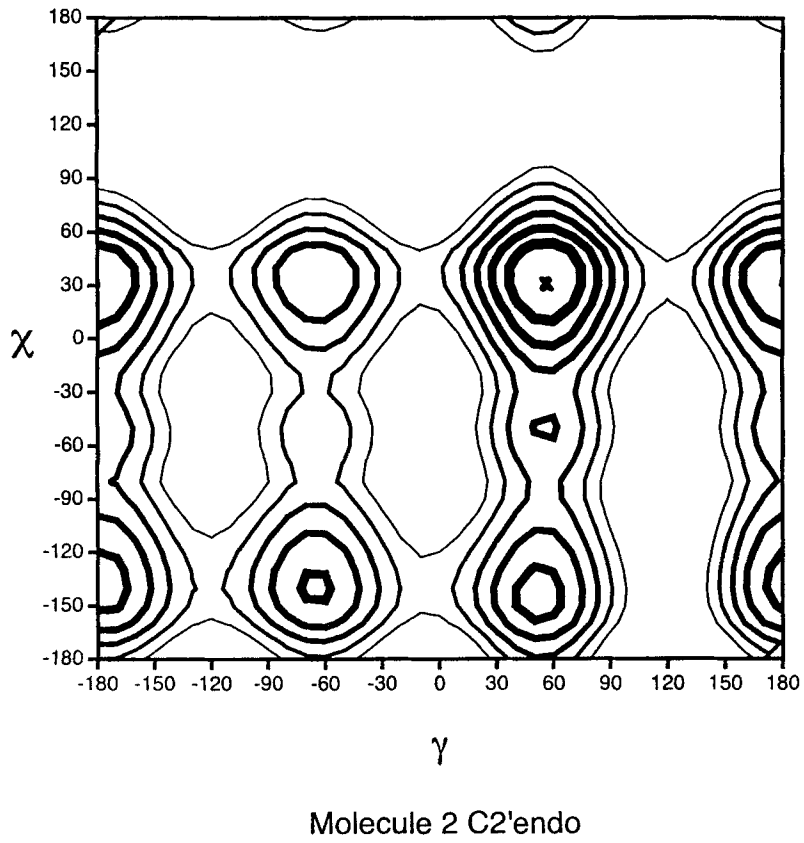


FIG. 3. Conformational energy plot for **2** in the χ–γ space corresponding to the C2' endo furanose geometry.

gauche$^-$) region of the (χ,γ) space in FIG. 3 is destabilized by 1.8 kcal/mole, while the corresponding destabilization in FIG. 2a is 2.5 kcal/mole; (2) the 6 kcal/mole contour occupies a larger area of the (χ,γ) space in FIG. 3 than in FIG. 2a.

Discussion

Conformational energy calculations using molecular mechanics methods on 6-methyl uridine nucleoside and nucleotide analogs have been carried out to understand the energetic preferences of the torsions about the glycosidic and C4'-C5' bonds and furanose puckering. As in the case of unsubstituted uridine nucleotides and nucleosides, here too, the (*anti, gauche*$^+$) combination of (χ,γ) is energetically the most preferred for 6-methyl-2'-

TABLE 4 : Energy minima of **2** in the conformational space (χ,γ) for the C2' endo furanose geometry (FIG. 3).

Minimum Number	γ (deg)	χ (deg)	Relative Energy (Kcal/mole)
1	60.0	30.0	0.00
2	180.0	30.0	0.97
3	180.0	-140.0	1.58
4	50.0	-150.0	1.60
5	-60.0	-140.0	1.86
6	-60.0	30.0	2.07

deoxyuridine, when the furanose geometry is C2' endo and O1' endo. Further, the *syn* conformation of the modified base is generally destabilized by at least 1- 2 kcal/mole, except when the sugar puckering is C3' endo, in which case the (*syn, trans*) combination of (χ,γ) corresponds to the energetically most preferred structure.

The presence of a hydroxyl or a methoxy group at C5', does not significantly influence the positions of the minima in the (χ,γ) space for either of the three sugar puckerings. Intuitively, the *syn* conformation of the base may deemed to be more stable when the hydroxyl group replaces methoxy at C5', because of the potential for intramolecular hydrogen bonding between HO5' and O2 in the pyrimidine. An examination of the models shows that these two atoms are involved in hydrogen bonding interactions for all the three sugar puckerings with their distances varying from 1.8 to 2.0 Å (FIG. 4). However, these interactions are somewhat offset by electrostatic repulsion between O2 and O1' which are separated by about 2.5 Å when the sugar geometries are C2' endo and O1' endo. This may contribute to a relative destabilization of the *syn* conformation of **2** (for these two sugar puckers), whose global minimum is found in the *anti* region of the glycosidic torsion.

The global minimum for the C3' endo pucker lies in the *syn* region of the glycosidic torsion in both **1** and **2**. This preference is similar to that in unmodified nucleosides and

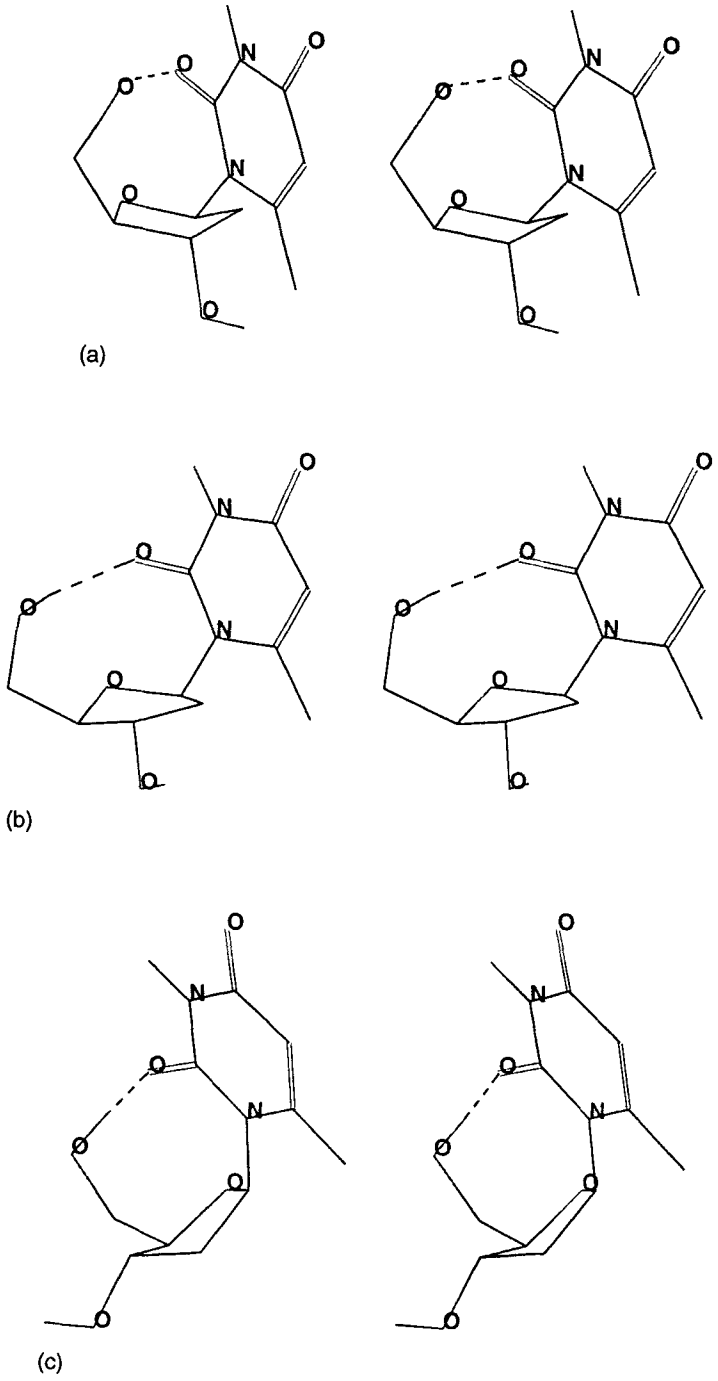


FIG. 4. Computer graphic illustration of **2** with *syn* orientation of 6-methyluracil and (a) C2' endo, (b) C3' endo and (c) O1' endo deoxyribose pucker.

nucleotides[11]. It should be noted that in an oligonucleotide, the above described hydrogen bonding interactions are absent, leading effectively to the stabilization of the *anti* conformation, as exemplified by **1**. It may be pointed out that the conformational features described for **1** and **2** are equally applicable to nucleosides and nucleotides of 5,6-dimethylthymine, previously used in the synthesis of antisense oligonucleotides[10] since the 5-methyl group does not directly influence the glycosidic conformation or its relationship with the C4'-C5' torsion.

An important feature of the present study deals with the force field employed to carry out the molecular mechanics calculations. Previous studies on nucleic acid constituents have typically employed the AMBER and CHARMm force fields and semi-empirical approaches to derive models consistent with experimental data[14]. As in those studies, here too, the MacroModel implementation of MM2 (MM2*) has also been validated against the well-known conformational preferences in nucleosides and nucleotides (Rao and Balaji, unpublished data). For example, the *syn* conformation about the glycosidic bond in the pyrimidine nucleosides is shown to be energetically less favored over the more commonly observed *anti* conformation[11]. The energy differences between these two conformations for the C2' endo sugar and a *gauche*$^+$ conformation about C4'-C5' ($\gamma \sim 60°$) vary between 1.5 to 1.8 kcal/mole using the MM2* force field, while the corresponding differences are found to be between 1.7 to 2.0 kcal/mole when the AMBER force field is employed. The details of the computational protocol used to demonstrate the applicability of MM2* to nucleosides and nucleotides of adenine, guanine, thymine, uridine and cytosine in deriving models consistent with experimental data, will be published elsewhere.

To the best of my knowledge, no crystal structure analyses of C6-substituted pyrimidine nucleosides have been reported. However, the crystal structure of a nucleoside with a C6-substituted pyrimidine as part of a tricyclic base has been recently reported[13]. In this compound, the substituent at C5' is a hydroxyl group and the C2' and C3' atoms of the furanose ring are tied through an isopropylidene ring. O1' endo sugar pucker and the *syn* conformation of the modified base were observed in the crystal structure. The tricyclic bulk across the C5 and C6 of the pyrimidine caused a significant destabilization of the *anti* conformation due to severe stereochemical short contacts and consequently led to the stabilization of the *syn* conformation.

Based on solution (NMR) studies of oligonucleotides containing 6-substituted pyrimidines, Sanghvi *et. al.*[10] have reported a *syn* conformation for the glycosidic torsion in the 2'-deoxy-6-methyluridine. They have argued that the *anti* conformation would be destabilized in such a compound. The calculations presented here clearly demonstrate that incorporating

the conformational flexibility about the sugar ring, it is feasible to stabilize the *anti* orientation of the modified base. Preference for C3' endo pucker in ribofuranose may cause the orientation of the modified pyrimidine to be changed either to low *anti* or to *syn*.

Conclusion

Conformational energy calculations on a 6-methyluridine nucleoside and a related nucleotide analog indicate that their conformational preferences are similar to those of the corresponding unmodified pyrimidine. The preferred combination for the glycosidic and C4'-C5' bond conformations is calculated to be (*anti, gauche*$^+$) for the C2' endo and O1' endo puckers of the furanose. For the C3' endo sugar, the energetically favored combination is (*syn,trans*). The salient features obtained in these investigations are consistent with the crystal structure of a highly constrained pyrimidine analog in which the furanose ring is also constrained through a cyclic methylethylidene moiety. While the NMR observed *syn* conformation is calculated to be one of the energetically favored structures, it is not the global minimum. Thus its occurrence in solution may be attributed to solvent effects not explicitly taken into considerations.

Acknowledgments

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