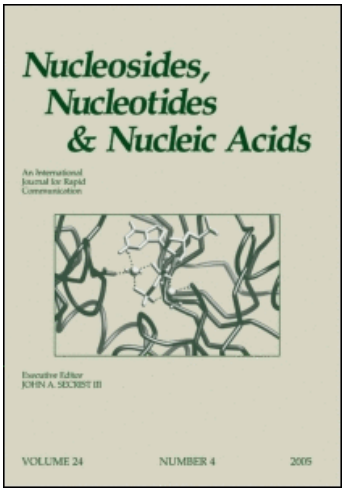

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Conformational Studies on Nucleosides with Furanose Ring Modifications. 1.

Shashidhar N. Rao[a]
[a] Searle Research and Development, Skokie, IL

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CONFORMATIONAL STUDIES ON NUCLEOSIDES WITH FURANOSE RING MODIFICATIONS. 1.

Shashidhar N. Rao
Searle Research and Development,
4901 Searle Parkway,
Skokie, IL 60077.

Abstract

Conformational energy calculations have been presented on adenine and thymine nucleosides in which the furanose ring is replaced by 2',3'-dideoxy-2',3'-didehydrofuran using molecular mechanics and conformational analysis. Conformational energies have been evaluated using the MM2 and AMBER94 force field parameters at two different dielectric constants. The results are presented in terms of isoenergy contours in the conformational space of the glycosidic (χ) and C4'-C5' (γ) bonds torsions. In general, the χ-γ interrelationships exhibit similarities with the corresponding plots for unmodified nucleosides and nucleotides, reported previously. Consistency of the calculated preferred conformations with the X-ray data is sensitive to the force field employed.

Introduction

Modified nucleosides with both purine and pyrimidine bases have been the subject of many recent investigations as potential anti-viral agents[1-10]. In this light, extensive structure-activity relationships (SAR) and pharmacological profiles of modified nucleosides as anti-HIV agents, inhibitors of hepatitis B virus replication, adenylate cyclase inhibitors, anti-tumor agents etc. have been reported[1-11]. Some modified nucleosides (e.g. 2',3'-dideoxy-2',3'-didehydrothymidine; D4T) have unique biological properties rendering them less toxic than AZT and hence viable candidates for clinical trials as anti-HIV agents. Pharmacokinetic studies on D4T have shown that this compound is well absorbed and is predominantly eliminated unchanged, in addition to having penetration through the blood brain barrier in mice[12].

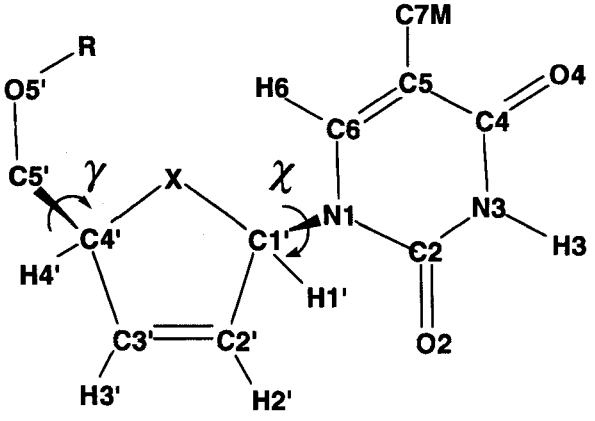
Despite extensive biological and chemical studies on furanose-modified nucleosides, structural information on them has been confined to a limited amount of X-ray crystallographic data on a few modified nucleosides[11,13-16]. A few detailed theoretical investigations on base-modified nucleosides have been previously reported[16-21]. These include a conformational analysis of D4T using the TRIPOS force field[16]. In addition, pseudoreceptor models have been built to rationalize the activities of modified nucleosides as anti-viral agents and inhibitors of thymidine kinase[22]. In this light, it would be interesting to examine the the role of various furanose ring modifications on conformational interdependencies in modified nucleosides. Specifically, the conformational preferences of the nitrogenous base orientations relative to the rest of the molecule could be of interest in outlining strategies for the design of additional nucleoside modifications. As a part of a continuing effort to study conformational preferences in modified nucleotides and nucleosides, this paper presents conformational energy calculations on 2',3'-dideoxy-2',3'-didehydrothymidine (D4T) and 2',3'-dideoxy-2',3'-didehydroadenosine (D4A) in the conformational space of the glycosidic (χ) and C4'-C5' (γ) bond torsions. The findings of the investigations are qualitatively consistent with the single crystal X-ray data on D4T and D4A and will hopefully provide guidelines for further design of modified nucleosides. Also, the results of the calculations are sensitive to the presence or absence of a methyl group on the C5' hydroxyl moiety as well as the force field employed.

Methods

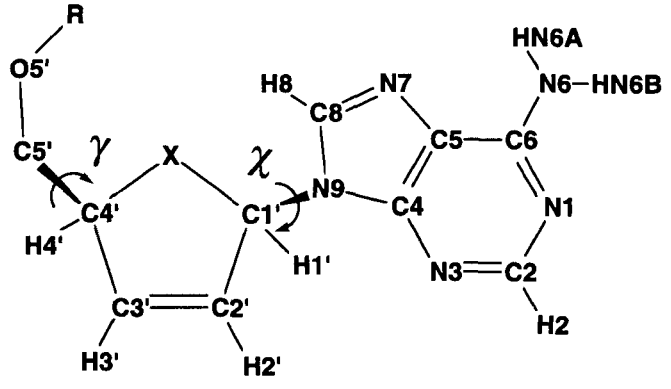
Preliminary models of **1** - **2** (illustrated below schematically) were built using MacroModel (v5.0) and energy refined with the MM2 force field[23]. The nomenclature adopted for atoms and torsions is as described in reference 24. Multiple conformations of **1** - **2** were generated by varying the torsions χ (O1'-C1'-N1-C6) and γ (C3'-C4'-C5'-O5') (schematically illustrated below) at an interval of 10°. The resultant collections of 1296 conformations were energy refined using the BatchMin module[12] by varying all degrees of freedom except for χ, γ. These torsions were constrained to their starting values with a weight of 1000 kcal/mole-degree. In the simulations, both the MM2 and AMBER94[25] force fields were employed.

The partial atomic charges on the atoms of **1** and **2** (APPENDIX 1) were obtained by fitting to the *ab initio* electrostatic potential surfaces (calculated using Gaussian94 with the RHF/6-31G* basis sets). Two dielectric constants, 1.0 and 4.0 were employed in the calculations. These correspond respectively to a vacuum environment and a polar

environment (as inside a protein, or a polar solvent). These dielectric constants have 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[26].



X= O and R = H ; **1a**
X= O and R = CH_3 ; **1b**



X= O and R = H ; **2a**
X= O and R = CH_3 ; **2b**

The results of the conformational energy calculations on the modified nucleosides are shown in terms of energy plots in the (χ,γ) space, wherein isoenergy contours (1 through 5 kcal/mole) are drawn at 1 kcal/mole intervals (FIGS 1 through 6). Of these, FIGS 1 through 4 correspond to energy calculations done with the MM2 force field within MacroModel, while FIGS 5 and 6 correspond to energy calculations with the AMBER94 force field. In the case of calculations with MM2 force field, plots are shown for both the

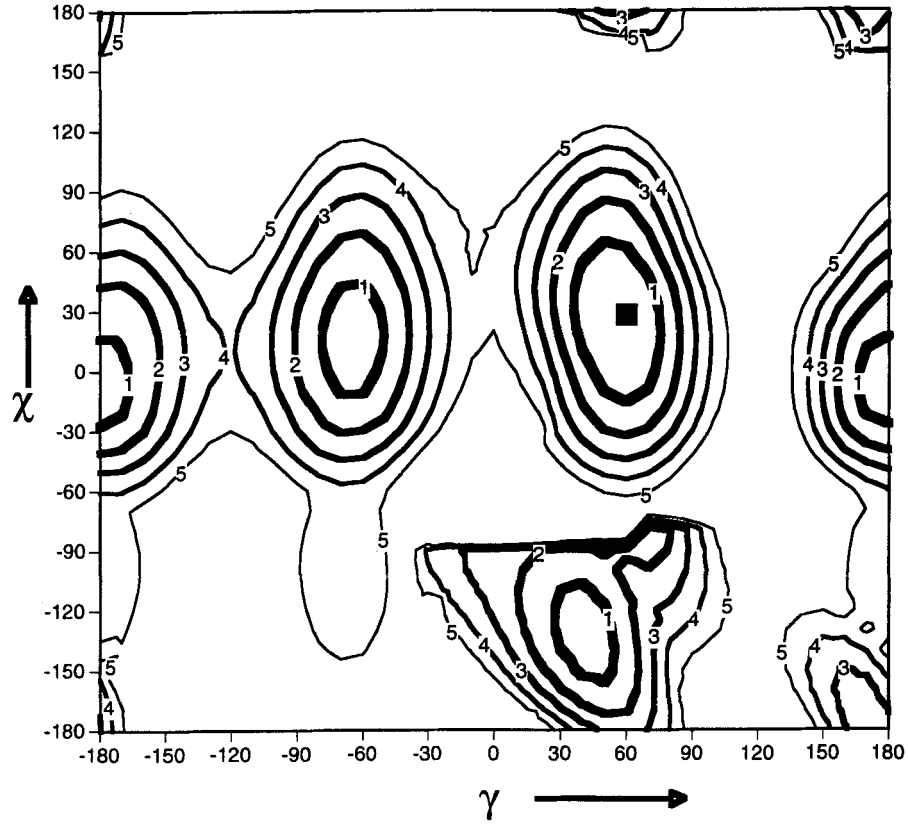


FIG. 1a. Conformational energy map of **1a** in the (χ,γ) space (Dielectric of 1.0 and MM2 force field). Minima are listed in TABLE 1a.

TABLE 1a : Energy minima of **1a** in the conformational space (χ,γ) (FIG. 1a ; ε =1 ; MM2).

Minimum Number	γ (°)	χ (°)	Relative Energy (Kcal/mole)
M1	60.0	30.0	0.00
M2	180.0	-10.0	0.44
M3	40.0	-130.0	0.54
M4	-60.0	20.0	0.56
M5	170.0	-170.0	2.37

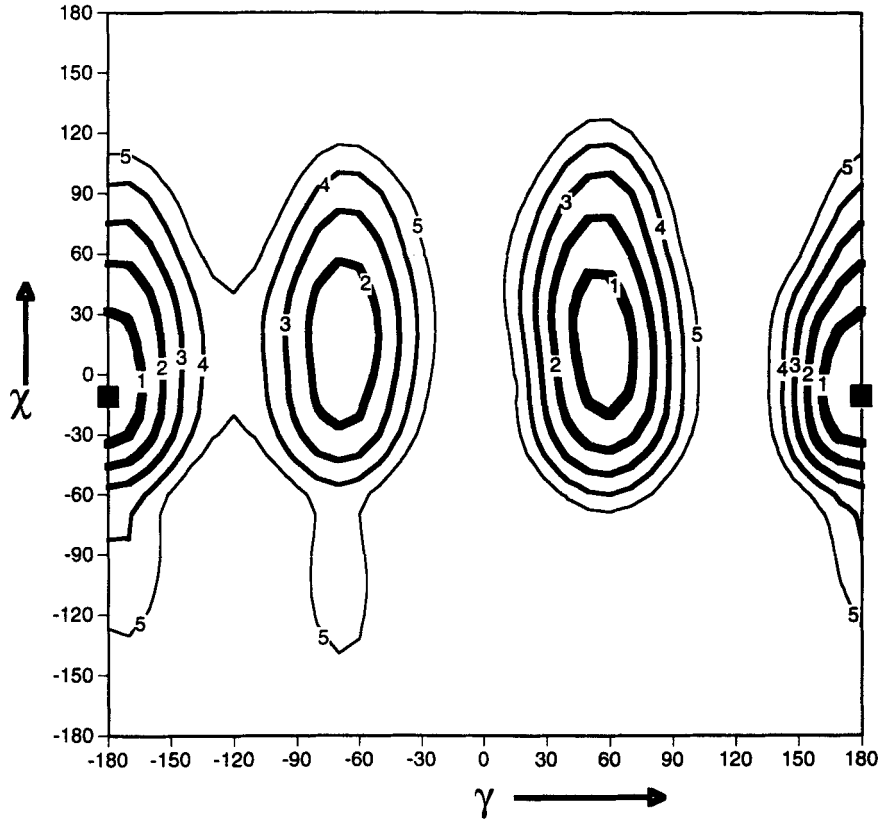


FIG. 1b. Conformational energy map of **1b** in the (χ,γ) space (Dielectric of 1.0 and MM2 force field). Minima are listed in TABLE 1b.

TABLE 1b : Energy minima of **1b** in the conformational space (χ,γ) (FIG. 1b ; ε =1 ; MM2).

Minimum Number	γ (°)	χ (°)	Relative Energy (Kcal/mole)
M1	180.0	-10.0	0.00
M2	60.0	20.0	0.41
M3	-70.0	20.0	1.26

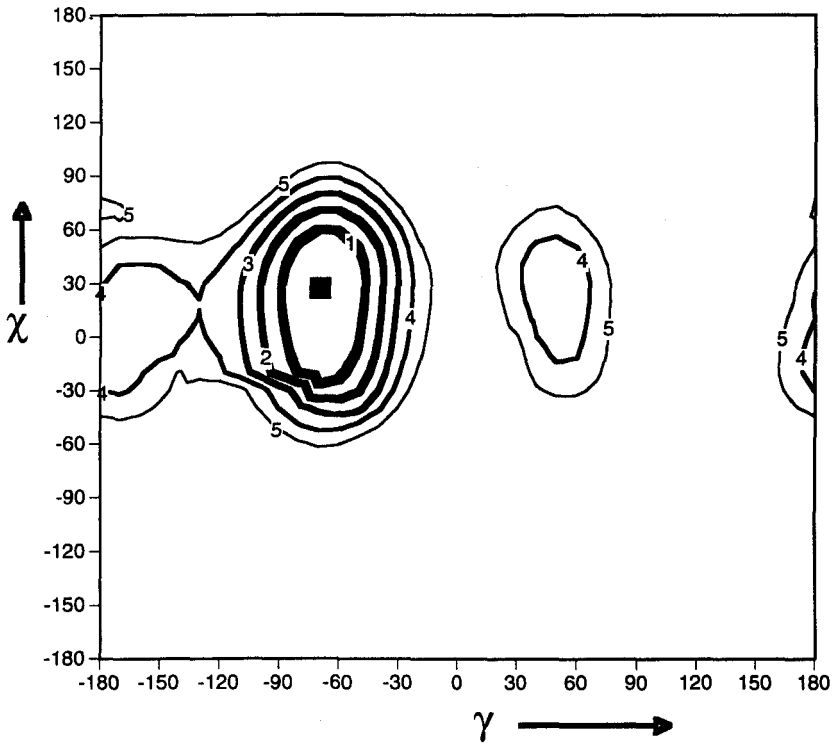


FIG. 2a. Conformational energy map of **2a** in the (χ,γ) space (Dielectric of 1.0 and MM2 force field). Minima are listed in TABLE 2a.

TABLE 2a : Energy minima of **2a** in the conformational space (χ,γ) (FIG. 2a ; ε =1 ; MM2).

Minimum Number	γ (°)	χ (°)	Relative Energy (Kcal/mole)
M1	-70.0	30.0	0.00
M2	50.0	30.0	3.29
M3	-170.0	10.0	3.46

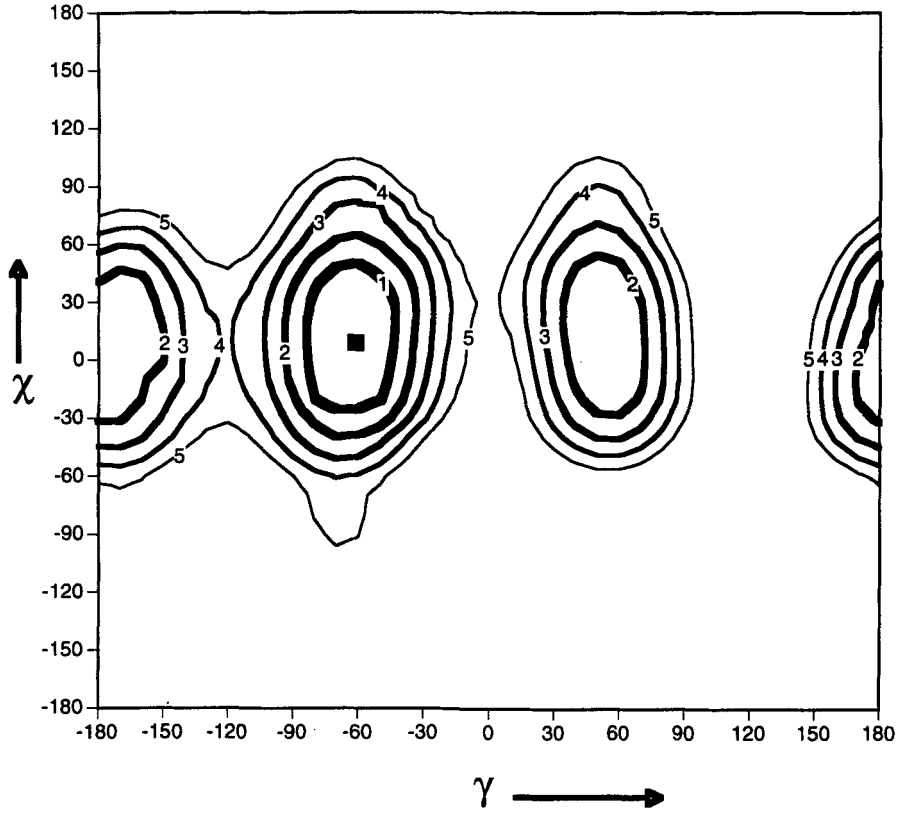


FIG. 2b. Conformational energy map of **2b** in the (χ,γ) space (Dielectric of 1.0 and MM2 force field). Minima are listed in TABLE 2b.

TABLE 2b : Energy minima of **2b** in the conformational space (χ,γ) (FIG. 2b ; ε =1 ; MM2).

Minimum Number	γ (°)	χ (°)	Relative Energy (Kcal/mole)
M1	-60.0	10.0	0.00
M2	50.0	20.0	1.02
M3	-170.0	20.0	1.19

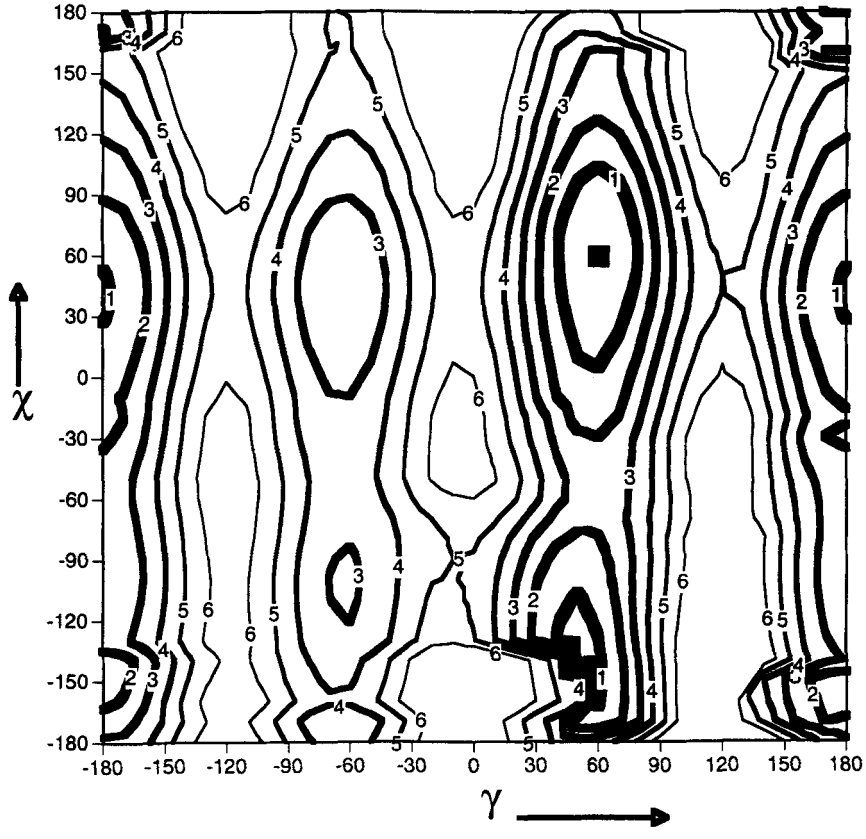


FIG. 3a. Conformational energy map of **1a** in the (χ,γ) space (Dielectric of 4.0 and MM2 force field). Minima are listed in TABLE 3a.

TABLE 3a : Energy minima of **1a** in the conformational space (χ,γ) (FIG. 3a ; ε =4 ; MM2).

Minimum Number	γ (°)	χ (°)	Relative Energy (Kcal/mole)
M1	60.0	60.0	0.00
M2	50.0	-140.0	0.62
M3	180.0	40.0	0.89
M4	180.0	-140.0	1.25
M5	-60.0	40.0	2.09
M6	-60.0	-100.0	2.86

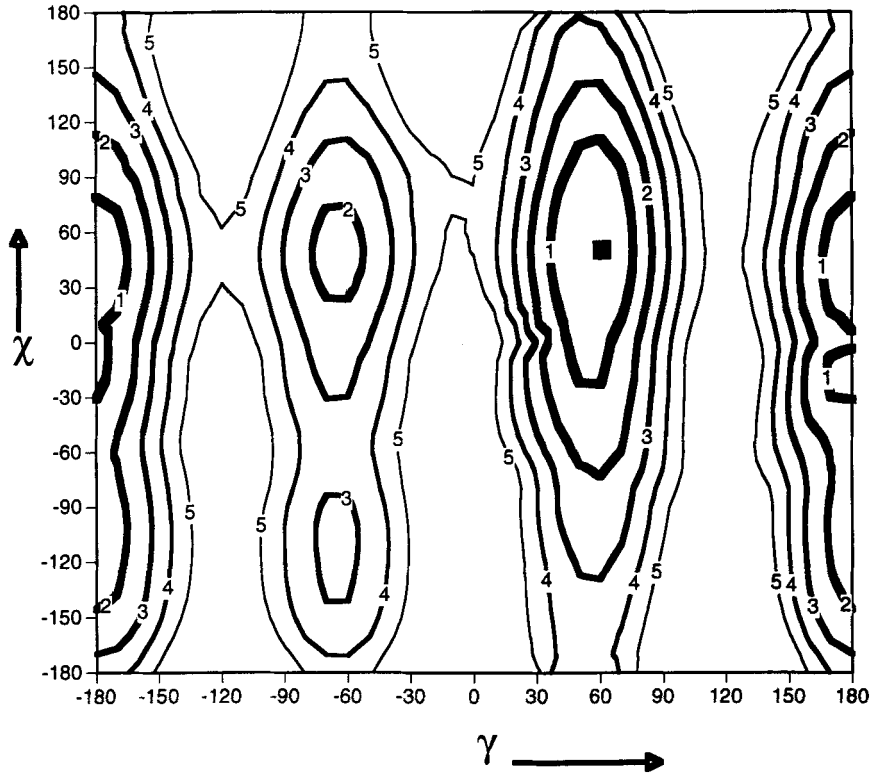


FIG. 3b. Conformational energy map of **1b** in the (χ,γ) space (Dielectric of 4.0 and MM2 force field). Minima are listed in TABLE 3b.

TABLE 3b : Energy minima of **1b** in the conformational space (χ,γ) (FIG. 3b ; ε =4 ; MM2).

Minimum Number	γ (°)	χ (°)	Relative Energy (Kcal/mole)
M1	60.0	50.0	0.00
M2	180.0	40.0	0.40
M3	-60.0	50.0	1.67
M4	-70.0	-110.0	2.76

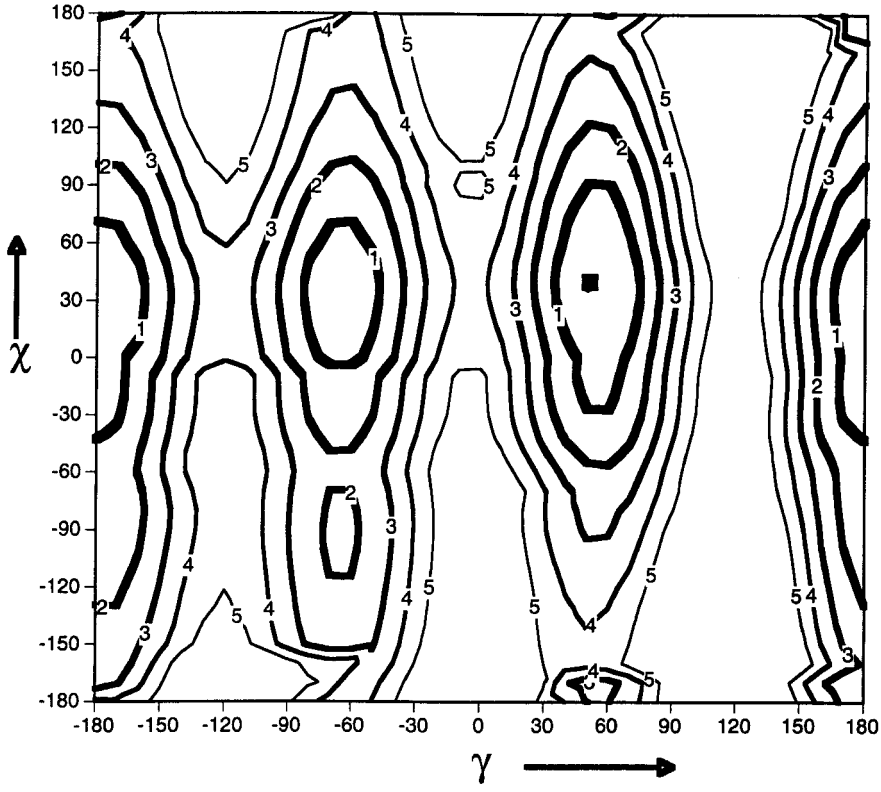


FIG. 4a. Conformational energy map of **2a** in the (χ,γ) space (Dielectric of 4.0 and MM2 force field). Minima are listed in TABLE 4a.

TABLE 4a : Energy minima of **2a** in the conformational space (χ,γ) (FIG. 4a ; ε =4 ; MM2).

Minimum Number	γ (°)	χ (°)	Relative Energy (Kcal/mole)
M1	50.0	40.0	0.00
M2	180.0	30.0	0.18
M3	-60.0	40.0	0.36
M4	-60.0	-90.0	1.84

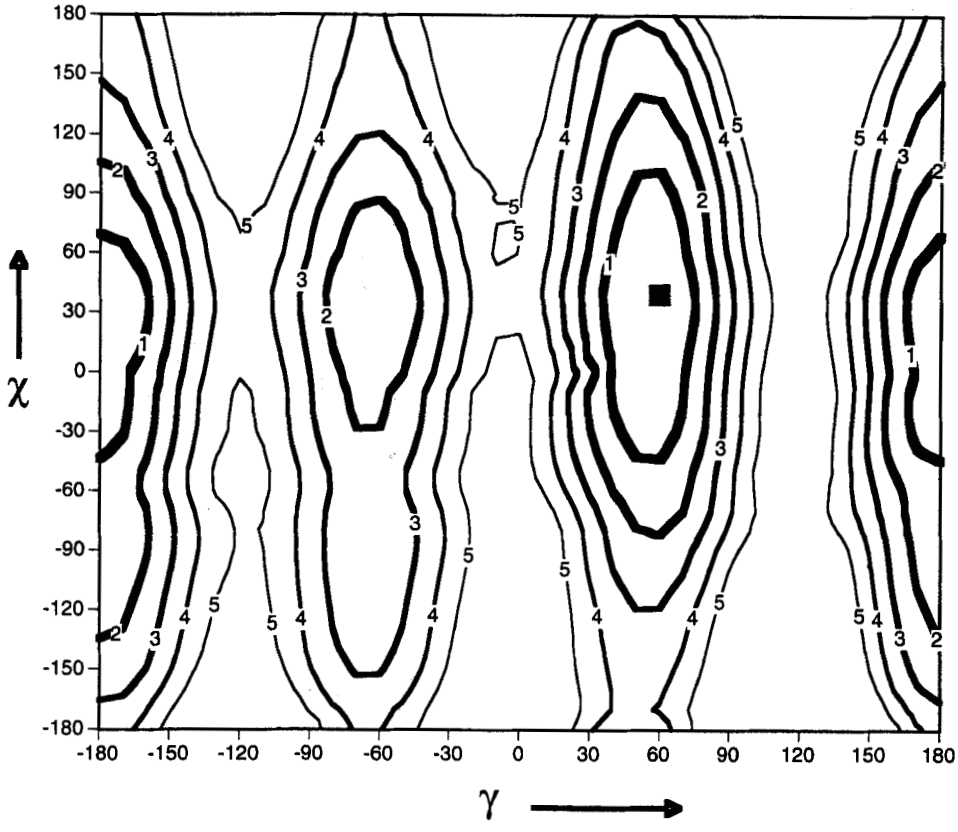


FIG. 4b. Conformational energy map of **2b** in the (χ,γ) space (Dielectric of 4.0 and MM2 force field). Minima are listed in TABLE 4b.

TABLE 4b : Energy minima of **2b** in the conformational space (χ,γ) (FIG. 4b ; ε =4 ; MM2).

Minimum Number	γ (°)	χ (°)	Relative Energy (Kcal/mole)
M1	60.0	40.0	0.00
M2	180.0	30.0	0.17
M3	-60.0	40.0	1.18

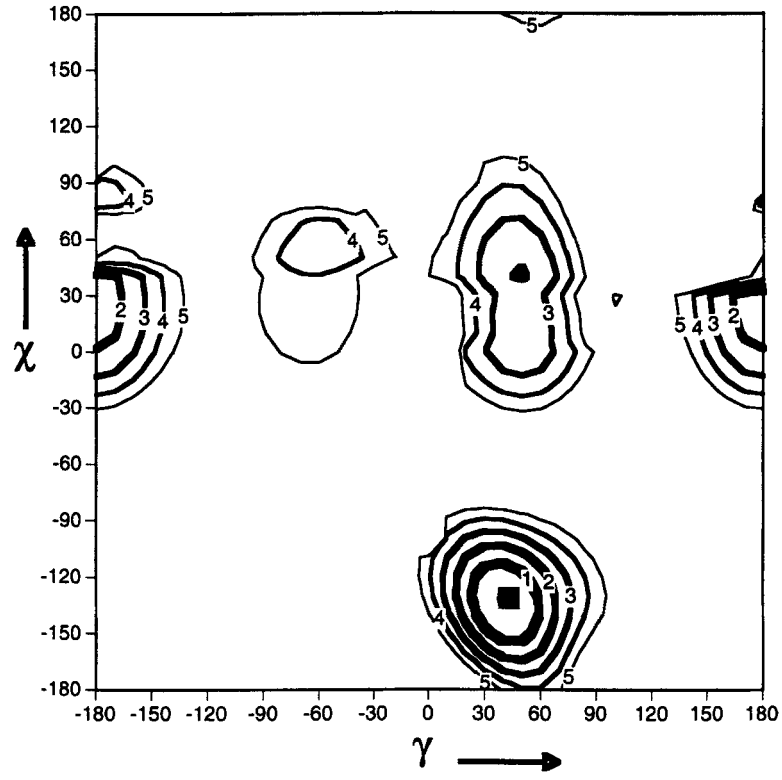


FIG. 5a. Conformational energy map of **1a** in the (χ,γ) space (Dielectric of 1.0 and AMBER94 force field). Minima are listed in TABLE 5a.

TABLE 5a : Energy minima of **1a** in the conformational space (χ,γ) (FIG. 5a ; ε =1 ; AMBER4).

Minimum Number	γ (°)	χ (°)	Relative Energy (Kcal/mole)
M1	40.0	-130.0	0.00
M2	180.0	30.0	1.48
M3	50.0	40.0	1.92
M4	-60.0	50.0	3.19

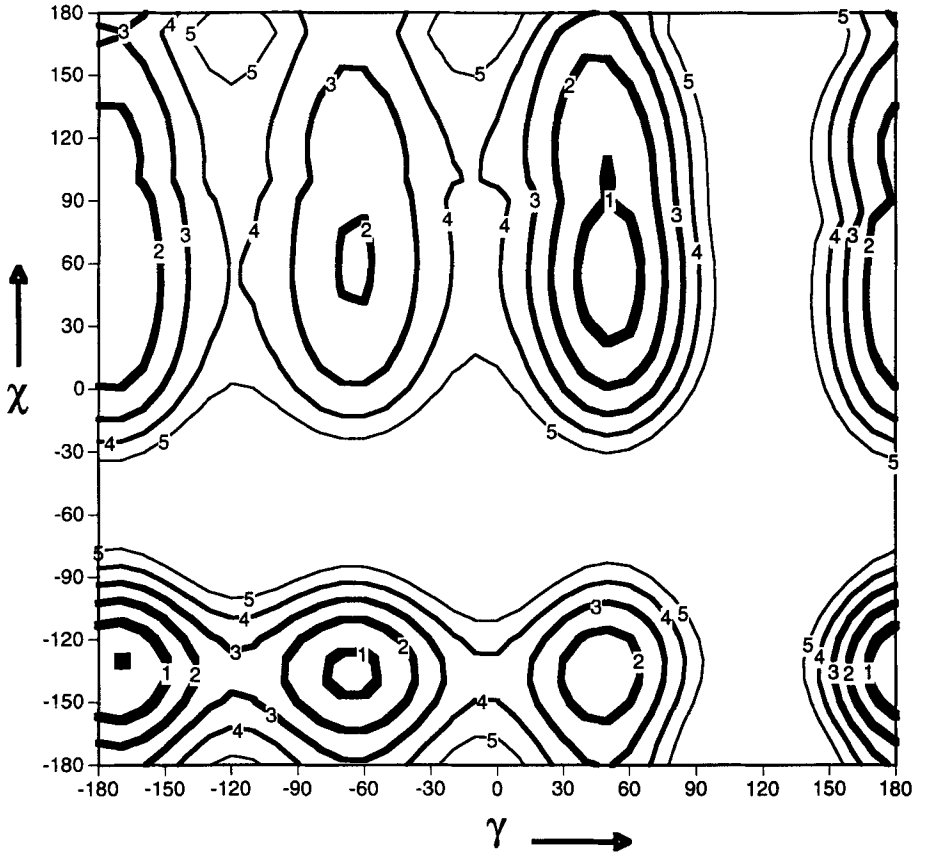


FIG. 5b. Conformational energy map of **1a** in the (χ,γ) space (Dielectric of 4.0 and AMBER94 force field). Minima are listed in TABLE 5b.

TABLE 5b : Energy minima of **1a** in the conformational space (χ,γ) (FIG. 5b ; ε =4 ; AMBER4).

Minimum Number	γ (°)	χ (°)	Relative Energy (Kcal/mole)
M1	-170.0	-130.0	0.00
M2	50.0	50.0	0.46
M3	-70.0	-140.0	0.79
M4	-170.0	50.0	1.08
M5	50.0	-140.0	1.35
M6	-60.0	60.0	1.90

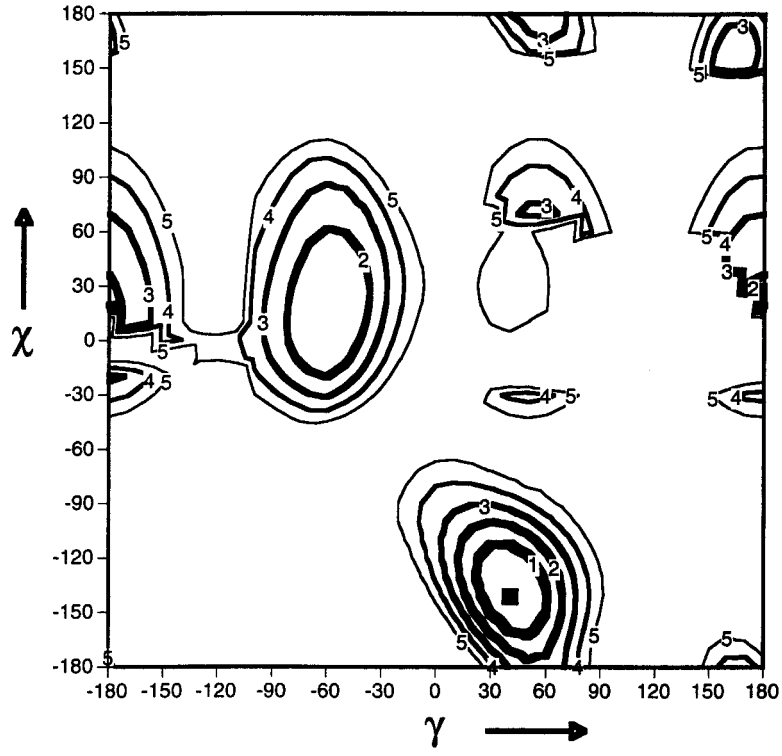


FIG. 6a. Conformational energy map of **2a** in the (χ,γ) space (Dielectric of 1.0 and AMBER94 force field) Minima are listed in TABLE 6a.

TABLE 6a : Energy minima of **2a** in the conformational space (χ,γ) (FIG. 6a ; ε =1 ; AMBER4).

Minimum Number	γ (°)	χ (°)	Relative Energy (Kcal/mole)
M1	40.0	-140.0	0.00
M2	-60.0	10.0	1.05
M3	180.0	20.0	1.79
M4	160.0	150.0	2.23
M5	180.0	-20.0	2.76
M6	50.0	70.0	2.79

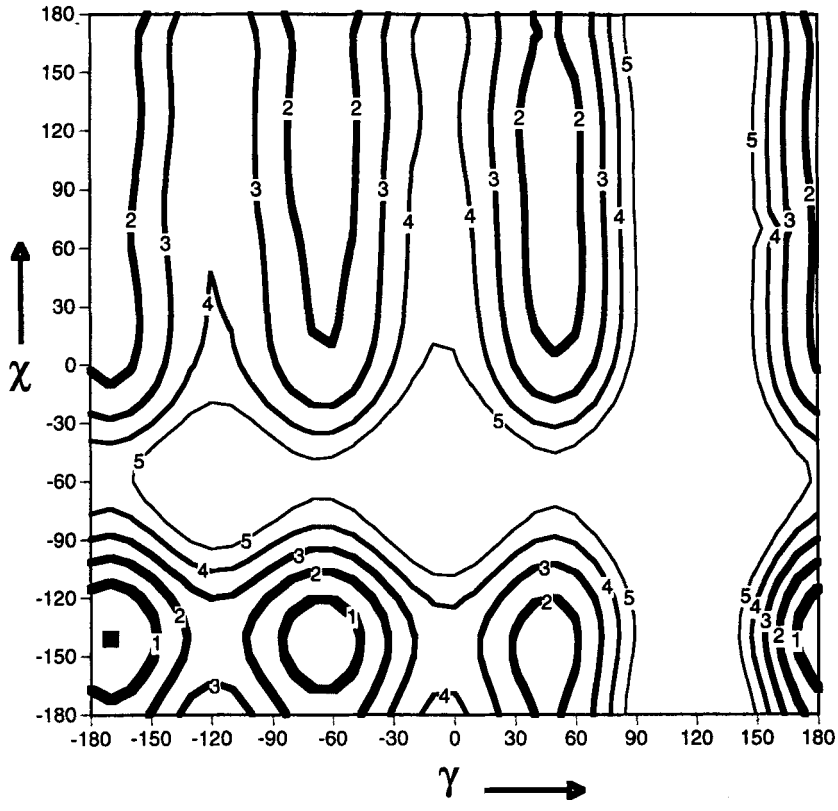


FIG. 6b. Conformational energy map of **2a** in the (χ,γ) space (Dielectric of 4.0 and AMBER94 force field). Minima are listed in TABLE 6b

TABLE 6b : Energy minima of **2a** in the conformational space (χ,γ) (FIG. 6b ; ε =4 ; AMBER4).

Minimum Number	γ (deg)	χ (deg)	Relative Energy (Kcal/mole)
M1	-170.0	-140.0	0.00
M2	-70.0	-140.0	0.40
M3	50.0	120.0	1.46

dielectric constants of 1 (FIGS 1a, 1b, 2a and 2b) and 4 (FIGS 3a, 3b, 4a and 4b) for all the four molecules. The AMBER94 calculations are reported only for **1a** and **2a** at both the dielectric values. In all these plots, the thickest contour corresponds to 1 kcal/mole relative to global minimum and the relative energy value increases as the thickness decreases. Thus the thinnest contour corresponds to 5 kcal/mole. The energy minima are listed in TABLES 1 and 2. Only the global minima are marked by dark solid squares (■) in the (χ,γ) maps. It should be noted that the terms "global minimum" and "local minimum" are used in the context of a specific conformational energy plot rather than in an absolute sense.

Results

FIGS. 1a and 1b illustrate the conformational energy plots of the modified nucleosides **1a** and **1b**, in their (χ,γ) space, for the dielectric constant of 1.0. The global and secondary energy minima in these plots are listed in TABLES 1a & 1b. In FIG. 1a, 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 **1a** are also observed for γ = 180° and -60° and are destabilized by less than 0.6 kcal/mole relative to the global minimum conformation (TABLE 1a). The minimum corresponding to the *syn* conformation of the thymine base is destabilized by around 0.55 kcal/mole relative to M1. This conformation corresponds to a structure in which the hydroxyl group at C5' forms an intramolecular (O-H...O) hydrogen bond with the O2 of the base. The high *anti* conformations (χ = 110° to 160°) are energetically destabilized relative to M1 by at least 5 kcal/mole for all values of γ. 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 4 kcal/mole. The corresponding barrier for transitions to the minimum with the *syn* conformation of the base is greater than 6 kcal/mole. It should be noted that values of χ in the range (-90° to -70°) and γ in the range (-30° to 90°) result in structures which have severe short contacts between the hydroxymethyl group attached at C4' and the O2 of the pyrimidine base. The 5 kcal/mole contour encloses about 65% of the total (χ,γ) conformational space.

FIGS. 1b illustrates the effect of protecting the C5' hydroxyl group with a methyl moiety, which would prevent intramolecular hydrogen bonding with the thymine O2, on the conformational energy plot in the (χ,γ) space. Firstly, the global minimum occurs for

an extended conformation about the C4'-C5' bond (γ =180°), although the glycosidic torsion still lies in the *anti* region (TABLE 1b).

As in FIG. 1a, secondary minima do occur for χ values in the anti region. However, the minimum corresponding to γ =-60° is destabilized relative to the global minimum by more than 1 kcal/mole. The most noticeable difference between the energy plots in FIGS 1a and 1b occurs in the *syn* region of the glycosidic torsion, which is energetically highly destabilized in the latter because of severe stereochemical contacts between the OCH3 moiety at C5' and O2 of the pyrimidine. This feature would also be present if the moiety attached to O5' is a phosphate or a phosphonate.

FIGS. 2a and 2b illustrate the conformational energy plots of the modified nucleosides **2a** and **2b**, in their (χ,γ) space, for the dielectric constant of 1.0. The global and secondary energy minima in these plots are listed in TABLES 2a & 2b.

In FIG. 2a, the global minimum (M1) is found at (χ,γ) = (30°,-70°) and corresponds to a structure with *anti* conformation of the base and *gauche*$^-$ conformation about C4'-C5'. Two secondary minima occur at (χ,γ) = (30°,50°) and (10°,-170°) and are destabilized relative to the global minimum by 3.3 and 3.5 kcal/mole, respectively. In FIG. 2b, the global minimum (M1) occurs at (χ,γ) = (10°,-60°). However, this plot is characterized by two secondary minima both corresponding to the *anti* conformation of the purine (TABLE 2b) and are destabilized by about 1.0 & 1.2 kcal/mole.

In the (χ,γ) plots for both **2a** and **2b**, structures with glycosidic torsion in the high *anti* and *syn* regions are energetically destabilized relative to the respective global minima by more than 6 kcal/mole.This is due both to repulsive electrostatic interactions and stereochemical contacts the six membered ring of the purine makes with the substitutents at C5'.

The effects of a higher dielectric constant (4.0) on the conformational energy maps of **1** & **2** in the (χ,γ) space are illustrated in FIGS 3a, 3b, 4a and 4b. It is noted that the positions of the principal minima (global and secondary minima within 2 kcal/mole of the former) are not significantly affected in these plots. However, the relative de-emphasis on electrostatic interaction energies resulting from a higher dielectric constant do allow for larger fragment of conformational space to be occupied by 5 kcal/mole contour. This demonstrates the significance of electrostatic interactions in the stabilization of various minima with the dielectric constant of 1.0. The differences

between the plots at the two dielectric constants are most noticeable for the molecules with adenine (**2a** and **2b**).

In the (χ,γ) plots for **2a** and **2b** at the dielectric constant of 1.0 (FIGS 2a and 2b), the area occupied by the 5 kcal/mole contour in the region defined by ($0° < \gamma < 90°$; $-60° < \chi < 90°$) is smaller in the case of **2a** which has a hydroxyl group at C5' than in the case of **2b** which has a hydroxy methyl moiety at C5'. The polar nature of the former combined with the dielectric of 1.0 may render this region relatively more destabilized than in the latter. This is supported by the fact that the corresponding differences in the plots at the dielectric constant of 4.0 are smaller due to greater de-emphasis on the electrostatic interactions.

FIGS. 5a and 6a show the conformational energy plots in the (χ,γ) space of **1a** and **2a** wherein the energies are calculated at a dielectric constant of 1.0 using the AMBER94 force field[13]. The corresponding plots for the dielectric of 4.0 are shown in FIGS. 5b and 6b. In FIG. 5a, the global minimum occurs at the (χ,γ) conformational combination of (-130°, 40°) corresponding to a *syn* conformation of the base and a *gauche*$^+$ orientation about C4'-C5'. This structure is stabilized by favorable electrostatic and hydrogen bonding interactions between the C5' hydroxyl and the O2 of thymine. Three secondary minima in the *anti* region of the conformational space are noted (TABLE 5a) and correspond to the three standard orientations about the C4'-C5' bond. Of these, the minimum corresponding to an extended *trans* conformation about C4'-C5' has the lowest energy while the secondary minimum in the (*anti*, *gauche*$^+$) region of the conformational space is destabilized by 2.5 kcal/mole. In addition, a secondary minimum destabilized by 3.5 kcal/mole is located in the high *anti* region for the *trans* C4'-C5' conformation. The barriers to conformational transitions between the global minimum and the secondary minima are at least 6 kcal/mole and thus higher than those calculated using the MM2 force field (FIG. 1a). The energy plot in FIG. 5b has its global minimum at the (χ,γ) conformational combination of (-130°, 190°) and has five secondary minima with *anti* and *syn* orientations of the glycosidic bond (TABLE 5b). The 5 kcal/mole contour occupies around 75% of the total conformational space. Unlike in the case of FIG. 5a, the barriers to conformational transitions between the global minimum and the secondary minima are around 2 to 3 kcal/mole.

In the (χ,γ) conformational energy plot of **2a** the global minimum occurs at the (χ,γ) conformational combination of (-140°, 40°) corresponding to the syn conformation of the purine (FIG. 6a). Two secondary minimum with relative energies of less than 2

kcal/mole occur in the (*anti, gauche*$^-$) and (*anti, trans*) regions of the conformational space. All the other secondary minima are destabilized by more than 2 kcal/mole (TABLE 6a). In the corresponding plot for the dielectric of 4.0 (FIG. 6b), the global minimum occurs at the conformational combination of (-140°, -170°), while two secondary minima, destabilized by about 0.4 and 1.5 kcal/mole are observed (TABLE 6b). No secondary minima are observed for the *anti* orientation of the glycosidic bond. The barriers to conformational transitions between the global minimum and the secondary minima are similar to those found in the corresponding energy plots for D4A.

Discussion

Conformational energy calculations using molecular mechanics methods on 2',3'-dideoxy-2',3'-didehydrothymidine (D4T) and 2',3'-dideoxy-2',3'-didehydroadenosine (D4A). have been carried out to understand the energetic preferences of the torsions about the glycosidic and C4'-C5' bonds. In the case of D4T, the (*anti, gauche*$^+$) and (*anti, trans*) combinations of (χ,γ) lead to the energetically most preferred structures, depending on whether the O5' can participate in an intramolecular hydrogen bond (with O2) or not. Further, the *syn* conformation of the pyrimidine base corresponds to an energetically preferred structure when O5' is uncapped and is in a position to hydrogen bond with O2. However, this conformation leads to an energetically highly destabilized structure when O5' is capped with a methyl group.

The calculated energetic preferences of D4T are similar to those of the unmodified thymidine nucleoside in which the furan ring is more flexible. In the latter, the energetically most favorable structure has an *anti* conformation of the glycosidic bond, while the syn conformation is relatively destabilized. The five-membered ring in D4T has a geometry very similar to either the O1' endo or the O1' exo sugar ring pucker of the unmodified nucleoside, although it should be noted that in the latter the C2' and C3' atoms are sp3 hybridized unlike in D4T, where they are sp2 hybridized. The rigidity of the double bond with no pseudo-axial and pseudo-equatorial hydrogens at the C2' and C3' atoms make it feasible for the *syn* conformation of the base to lead to an energetically favored structure when a hydroxyl group is present at C5'. The latter forms an intramolecular O-H...O hydrogen bond with heteroatom separation distances of around 2.9 Å and O-H...O angles of around 175°.

In D4A, the energetically most preferred structures have combinations of (χ,γ) in the (*anti, gauche*) region with conformations corresponding to the *syn* glycosidic orientations

destabilized by more than 5 kcal/mole. These preferences are independent of whether the O5' hydroxyl is capped or not, since the adenine base does not present a functionality for intramolecular hydrogen bond of the type seen in D4T, in a sterically feasible manner. The occurrence of minima (albeit higher in energy by around 1.5 to 2.5 kcal/mole relative to the global minimum) with *syn* glycosidic orientations in the (χ,γ) conformational energy plots at higher dielectric is indicative of the role of the electrostatics in their destabilization.

The calculations carried out in these investigations demonstrate considerable differences in the profiles of energetic preferences for the two force fields employed in conjunction with the charge model described earlier. Specifically, the electrostatic interactions seem to play a significantly more dominant role in the treatment by the AMBER94 force field. The low energy preferences of D4A and D4T shift from the anti orientation of the glycosidic torsion in the case of MM2 force field treatment to the syn orientation of the glycosidic torsion in the AMBER94 force field treatment. This is attributed to the stabilization of the latter conformation via intramolecular hydrogen bond between the C5'hydroxyl and either the O2 of the pyrimidine or the N3 of purine. The fact that syn orientation of the glycosidic bond is preferred in the AMBER94 force field treatment for either modified nucleoside even at a higher dielectric is suggestive of the level of overemphasis on electrostatic interactions.

How do the results of these calculations compare with the available crystal structure information ? The X-ray crystallographic studies on D4T[14] demonstrate glycosidic torsions of 62° and 6° in two different geometries with *gauche*$^+$ conformations about C4'-C5' (γ ~ 54° and 61°). Thus, it is gratifying to note that these conformational combinations of the glycosidic and C4'-C5' torsions lie within 1 kcal/mole of the global minimum conformation in FIG. 1a, particularly in light of the fact that the conformational energy calculations have been carried out without explicit consideration of crystal packing forces. By contrast, the X-ray crystal structure of D4A (in which the C5' hydroxyl is uncapped) demonstrated a *trans* conformation about C4'-C5' together with the anti orientation of the purine base. This conformational combination is predicted to be energetically destabilized relative to the most preferred structure by at least 3 kcal/mole at the dielectric of 1.0 (FIG. 2a) and by about 1 kcal/mole at the dielectric of 4.0 (FIG. 2c). This apparent qualitative inconsistency may be rationalized by the role of the crystal packing forces (including intermolecular hydrogen bonding interactions) not considered in the presented calculations. The occurrence of high anti conformations in the crystal structure of D4T reported in reference 14 is inconsistent with the high energy

for that conformer in FIG 1a but is consistent with the energetic preferences calculated at the higher dielectric of 4.0. The calculated *syn* glycosidic conformational preferences in D4T and D4A on the basis of the AMBER94 force field are qualitatively *entirely* inconsistent with the observed X-ray crystallographic data on these compounds.

A comparison of the results of the present investigations on the conformational preferences of D4T to those previously reported[14] brings forth the following points of similarities and differences. First, it must be pointed out that the previous studies have employed the TRIPOS force field with a distance dependent dielectric of 4r (where r is the interatomic distance) in order to obtain models consistent with the X-ray crystal structure enviroment. By contrast, the present study has employed the MM2 force field implementation in MacroModel with two different constant dielectric values of 1 and 4. It is noteworthy that the TRIPOS force field does not account for oxygen lone pair interactions as does the molecular mechanics force field employed in the context of MM2. Furthermore, the charge model used in the previous study derived charges from the AM1 electrostatic potentials while *ab initio* methods have been employed in the present study. The global minimum and the secondary minima occur in similar positions for the *anti* conformation of the glycosidic bond in the (χ,γ) conformational energy plots in reference 14 and in FIG. 1a. However, in the previous study[14], the minimum corresponding to the *syn* glycosidic conformation occurs for the *trans* conformation of C4'-C5' bond, by contrast with FIG. 1a where it occurs for the *gauche*$^+$ conformation. The two investigations also show similarities in the calculated energetic preferences of the high *anti* conformation of the glycosidic torsion where some of the reported crystal structures occur. However, in the present study, the energy plot corresponding to the dielectric of 4.0 illustrated in FIG. 3a needs to be compared with the previously reported (χ,γ) conformational energy plot. Thus, it is clear that in contrast to the previous report, a constant dielectric of either 1 or 4 can account for the X-ray data just as satisfactorily as a distance dependent dielectric (4r) which is rarely employed in the conformational analyses of nucleic acid constituents and their derivatives.

Conclusion

Conformational energy calculations on 2',3'-dideoxy-2',3'-didehydrothymidine and 2',3'-dideoxy-2',3'-didehydroadenosine indicate that their conformational preferences, calculated using the MM2 force field, are generally similar to those of the corresponding nucleosides with a flexible furanose. The preferred combination for the glycosidic and C4'-C5' bond conformations is calculated to be (*anti, gauche*$^+$) for the pyrimidine

compound, while the corresponding combination is (*anti, gauche*$^-$) for the adenine containing compound. The salient features obtained in these investigations are mostly qualitatively consistent with the crystal structures of the modified nucleosides. Low energy conformers calculated using the AMBER94 force field demonstrate a *syn* conformation about the glycosidic bond and are qualitatively inconsistent with the observed X-ray data.

APPENDIX 1 : 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.

Atom	1a	1b	Atom	2a	2b
O5'	-0.462	-0.074	O5'	-0.471	-0.256
RO5'	0.335	0.072	RO5'	0.414	0.122
C5'	-0.063	-0.254	C5'	0.072	0.023
H5'A	0.048	0.078	H5'A	0.026	0.049
H5'B	0.039	0.078	H5'B	0.026	0.031
C4'	0.468	0.492	C4'	0.423	0.456
H4'	-0.012	0.003	H4'	0.009	-0.002
O1'	-0.321	-0.318	O1'	-0.311	-0.374
C1'	0.273	0.232	C1'	0.318	0.332
H1'	0.088	0.107	H1'	0.072	0.085
N1	-0.181	-0.121	N9	-0.120	-0.134
C2	0.794	0.761	C8	0.234	0.226
O2	-0.693	-0.691	H8	0.142	0.148
N3	-0.686	-0.646	N7	-0.632	-0.627
H3	0.431	0.424	C5	0.087	0.077
C4	0.813	0.765	C6	0.696	0.690
O4	-0.668	-0.661	N6	-0.936	-0.928
C5	-0.120	-0.043	HN6A	0.441	0.438
C5-methyl	-0.017	-0.030	HN6B	0.441	0.438
C6	-0.104	-0.183	N1	-0.750	-0.747
H6	0.232	0.228	C2	0.442	0.433
			H2	0.149	0.152
			N3	-0.748	-0.751
C2'	-0.225	-0.251	C2'	-0.384	-0.299
H2'	0.186	0.202	H2'	0.232	0.212
C3'	-0.364	-0.391	C3'	-0.337	-0.353
H3'	0.210	0.220	H3'	0.212	0.202

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