# Aqueous Molecular Dynamics Simulations of Eight Modified 2′,3′-Dideoxypyrimidine Nucleosides

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**Abstract**: In order to study the effects of solvent (water) on the conformational properties of (modified) nucleosides and in order to investigate the way electronegative substituents favor certain sugar puckering modes, aqueous molecular dynamics simulations on eight modified 2′,3′-dideoxypyrimidine nucleosides were performed. The three principal conformational parameters of those nucleosides (*i.e.* torsion angles $\gamma$ and $\chi$ and the pseudorotation phase angle $P$) were examined during a 300-ps run of molecular dynamics using the *AMBER* force field. The attention was focused on the impact of the *gauche* effect of electronegative substituents on the preferred sugar puckering mode which was thought to be an important factor for influencing the overall conformational preferences and thus the activity. The results from the calculations, however, do not bring forward the *gauche* effect of electronegative substituents as a conclusive element for conformational preferences of the sugar ring. Interconversion between the N- and the S-type sugar conformations occurs rapidly and frequently for any C2′- or C3′-substituent. The study of the torsion angles $\chi$ and $\gamma$ (*anti* and *+sc* orientation, respectively) does not reveal additional conformational information to solid-state data.

## Introduction

2′,3′-Dideoxypyrimidine nucleosides are among the most potent and selective antihuman immunodeficiency virus (HIV) compounds.[1] In particular 2′,3′-dideoxynucleosides with a C3′-fluoro or C3′-azido substituent are the most prominent anti-HIV agents.[2] With the furanose ring pucker assumed to be of constant amplitude, the conformation of nucleosides can be described by three major parameters: the *N*-glycosidic bond torsion angle $\chi$, the torsion angle $\gamma$ around the exocyclic C4′–C5′ bond, and the pseudorotation phase angle $P$.[3] A very interesting hypothesis was formulated concerning the influence of C3′-substituents on the conformational behavior of nucleosides.[4,5] It states that due to the *gauche* effect (the preference shown by certain electronegative atom combinations for *gauche* rather than for *trans* staggered conformations) certain C3′-substituents should stabilize an extreme S-type sugar conformation ($P = \pm 210°$). In such conformations the *+sc* orientation of O5′ is destabilized with increasing access to the *ap* orientation, which may be more favorable for anti-HIV activity via pronounced reactivity for phosphorylation.[6] Intracellular phosphorylation of nucleosides to their 5′-triphosphate form is necessary indeed to obtain active compounds. The significance of the *gauche* effect involving C3′-substitution is evident, noticing the fact that, in nuleosides, C3′-substituents, if in an axial position, are approximately *gauche* to the sugar O4′ atom. Consequently, C3′-substituents having a strong *gauche* effect with O4′ (such as –F and –OH[7] or $-N_3$ (the azide N1′ nitrogen is also quite negatively charged, due to the predominance of the $-N^{-}-N^{+}\equiv N$ resonance form[8])) will tend to stabilize the S-type sugar conformation in which the C3′-substituents are maximally axial. A recent conformational analysis of substituent effects on the sugar puckering mode in relation to the anti-HIV activity of 2′,3′-dideoxypyrimidine nucleosides, however, revealed that solid-state conformations characterized by $P$, $\chi$, and $\gamma$ do not provide decisive information for predicting possible anti-HIV activity.[9] The same study also showed that any rationalization of the activity or inactivity of nucleosides in terms of the *gauche* effect of electronegative substituents on the furanose ring conformation could not be found by using the semiempirical quantum chemical AM1 method. In the present study the effects of solvent (water) on the conformational properties of (modified) nucleosides and the way electronegative substituents favor certain sugar puckering modes were examined using molecular dynamics simulations. Unfortunately, the *AMBER* force field (a part of the molecular modeling package *Discover*[10]) which was used to perform the molecular dynamics simulations is parameterized neither for the fluorine atom nor for the nitrogen atoms in an azido group. This handicap was overcome by choosing appropriate substituents on both the C2′- and C3′-positions. Table I gives an overview of the eight modified nucleoside analogues which were included in the study. With these selected structures it was possible to examine the influence of both an electronegative substituent (O in –OH and $-OCH_3$) and a nonelectronegative substituent (C in $-CH_3$) in both the C3′- and C2′-positions. To complete the study, the unsubstituted C2′- and C3′-positions (–H) and also the C2′–OH and C3′–OH substitutions were included. The partial atomic charges of the modified riboses used in the molecular mechanics force field *AMBER* were obtained by fitting the partial charges to the electrostatic potentials as calculated by the *ab initio* program *GAUSSIAN 80-USCF*.[11,12] Since there is a *gauche* term included for the O–C–C–O dihedral angle in the AMBER force field,

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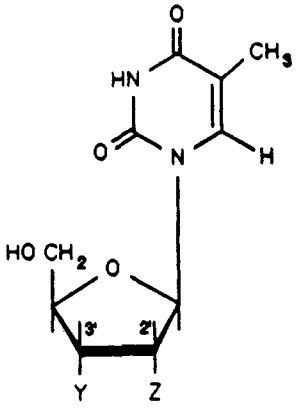
Table I. Summary of the Modified Nucleosides Examined by Molecular Dynamics Using the AMBER Force Field




| Y | Z | compound name | abbreviated name |
|---|---|---|---|
| OH | H | thymidine | Thd |
| $OCH_3$ | H | 3′-deoxy,3′-methoxythymidine | C3′-methoxy-dThd |
| $CH_3$ | H | 3′-deoxy,3′-methylthymidine | C3′-methyl-dThd |
| H | OH | 3′-deoxy-5-methyluridine | C2′-OH-dThd |
| H | $OCH_3$ | 3′-deoxy-2′-methoxythymidine | C2′-methoxy-dThd |
| H | $CH_3$ | 3′-deoxy-2′-methylthymidine | C2′-methyl-dThd |
| H | H | 3′-deoxythymidine | dThd |
| OH | OH | 5-methyluridine | C2′-OH,C3′-OH-dThd |

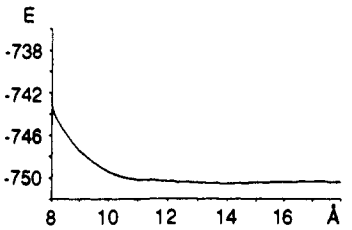



**Figure 1.** Total nonbonded energy $E$ (kcal mol$^{-1}$; van der Waals and Coulombic) for the solvated thymidine molecule as a function of the energy cutoff distance (Å).

clear differences should be demonstrated between electronegative and nonelectronegative C2′- and/or C3′-substituents.

## Results and Discussion

All eight structures were soaked in a cubic water box with a length of 16 Å. After 1000 cycles of conjugate gradients minimization to remove initial strain, the systems were equilibrated with 20 ps of molecular dynamics at 310 K (37 °C) and 1 atm (isobaric–isothermal ensemble) using temperature and pressure relaxation times of 0.1 ps. The compressibility factor $C$ was defaultly set to $10^{-5}$ bar$^{-1}$. Initial velocities were taken from a Maxwell-Boltzmann distribution for the target temperature. The simulations were then continued for 280 ps at 310 K and 1 atm with data collection every 0.2 ps. Consistent with the program parameterization for the use of water, a dielectric constant $\epsilon$ = 1 was applied. On the basis of Figure 1, which displays the nonbonded energy dependence on the cutoff distance, a cutoff distance of 12 Å was used to compromise between reliability of the model and computational cost of the simulations. *Discover* uses a fifth-order polynomial as a switching function to smoothly turn off the interactions. The size of the switching window is 1.5 Å. The nonbonded pair list was updated every 20 steps (20 fs). All the molecular dynamics calculations were performed on a Silicon Graphics 4D/35 workstation using the *AMBER* force field. The calculations took about 460 CPU hours per simulation. The total energy converged for all eight compounds during the molecular dynamics simulations (Figure 2), indicating an equilibrium in the system between potential and kinetic energy. The

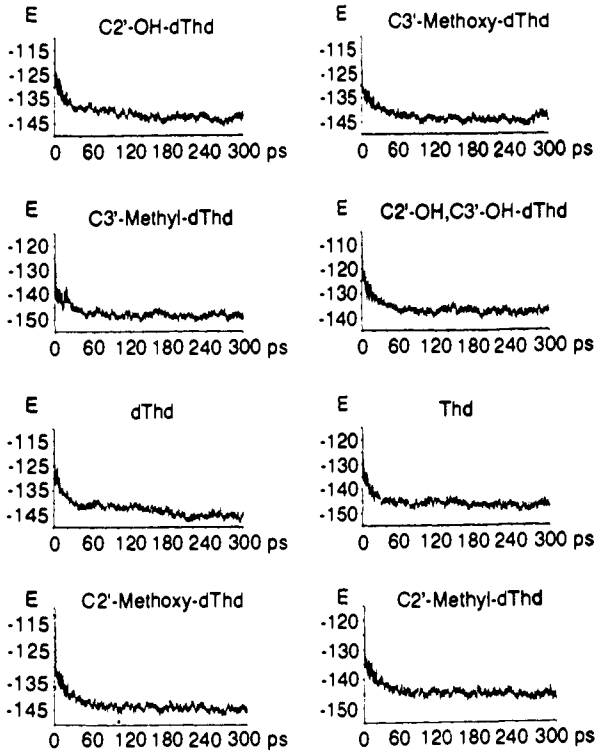



**Figure 2.** Calculated total energy $E$ (kcal mol$^{-1}$ 10$^{-1}$) as a function of time (300 ps) for the eight selected nucleosides.

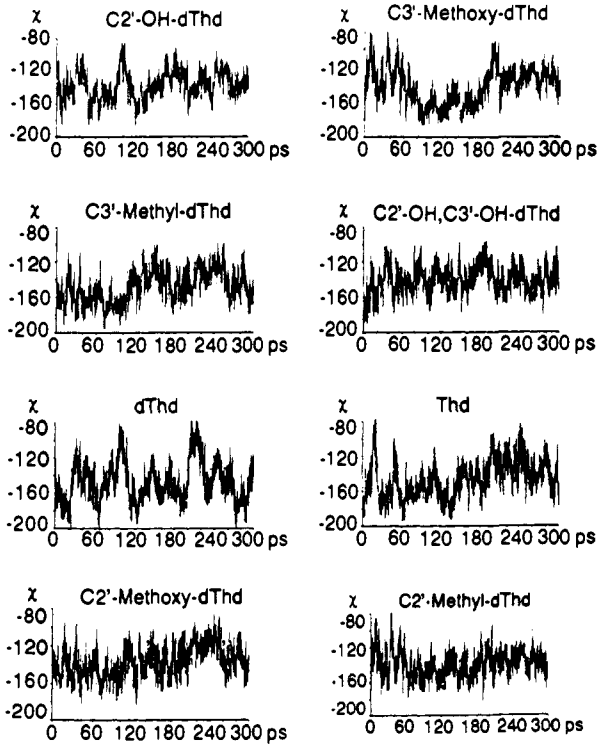



**Figure 3.** Torsion angle χ as a function of time (300 ps) for the eight selected nucleosides.

averaged values for the temperature and pressure were well constrained about their target values (310 K and 1 atm; data not shown).

**Torsion Angle χ.** Figure 3 plots the course of the *N*-glycosidic bond torsion angle χ for the eight modified nucleosides. In all eight structures an *anti* orientation is observed. The values of χ averaged over 300 ps are listed in Table II.

**Torsion Angle γ.** Figure 4 plots the course of the exocyclic torsion angles γ. In all structures, except 3′-deoxythymidine

**Table II.** Averaged Values of $\chi$, $\gamma$, and $\Psi_m$ (Esd's in Parentheses) of the Eight Modified Nucleosides along with the Percent of Time the Furanose Spends in Different Sugar Puckering Modes

| abbreviated name | averaged values $\chi$ | $\gamma$ | $\Psi_m$ | time (%) C3′-*endo* | O4′-*endo* | C2′-*endo* |
|---|---|---|---|---|---|---|
| Thd | −147(21) | 59(10) | 40(6) | 17.2 | 35.4 | 47.4 |
| C3′-methoxy-dThd | −142(20) | 57(11) | 41(6) | 14.6 | 23.1 | 62.3 |
| C3′-methyl-dThd | −148(17) | 60(10) | 40(6) | 54.3 | 22.2 | 23.5 |
| C2′-OH-dThd | −137(17) | 59(11) | 40(6) | 52.4 | 32.8 | 14.8 |
| C2′-methoxy-dThd | −136(16) | 57(9) | 41(6) | 50.6 | 21.8 | 27.6 |
| C2′-methyl-dThd | −139(15) | 58(11) | 40(6) | 35.3 | 18.5 | 46.2 |
| dThd | −147(23) | 59(12) | 41(7) | 36.3 | 52.4 | 11.3 |
| C2′-OH,C3′-OH-dThd | −141(16) | 56(10) | 40(6) | 16.9 | 17.6 | 65.5 |

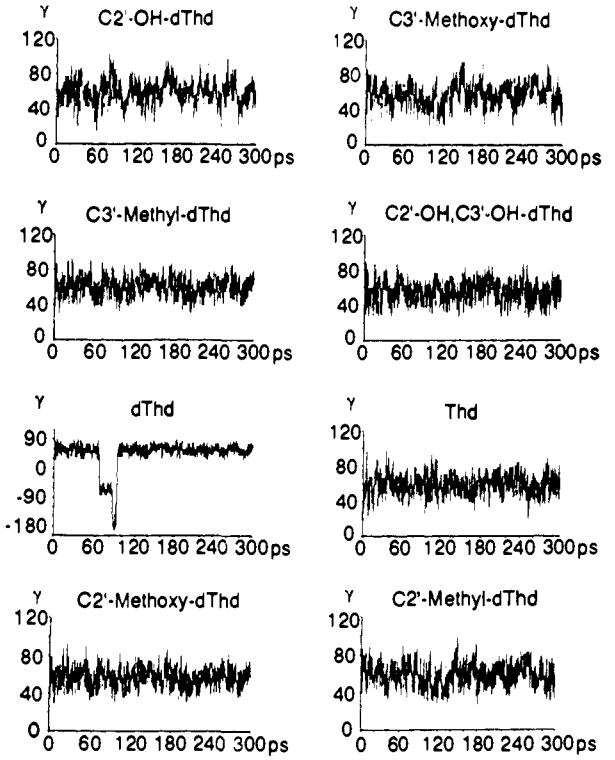



**Figure 4.** Torsion angle $\gamma$ as a function of time (300 ps) for the eight selected nucleosides.

(dThd), $\gamma$ is locked in the +*sc* conformation. Although the barrier for adopting the two other conformations in which all substituents are in staggered positions (−*sc* and *ap*) is rather small (±4 kcal mol$^{-1}$),[13] the +*sc* orientation is predominant during the whole molecular dynamics simulation process. Stabilization due to the intramolecular hydrogen bond (O5′···H-C6) favors the +*sc* orientation.[14] Values for the hydrogen-bond geometries during the simulations are listed in Table III. 3′-Deoxythymidine, with no C2′- nor C3′-substituents, is the most flexible one of the eight modified nucleosides studied here. The latter is expressed by the fact that $\gamma$ is able to jump for 35 ps from the basic +*sc* orientation to the −*sc* and *ap* orientations (see Figure 4). The values of $\gamma$ averaged over 300 ps are listed in Table II.

**Pseudorotation Phase Angle *P*.** Most of our attention is focused on this parameter because it should elucidate the part of the different C2′- and/or C3′-substituents in the preferred sugar puckering mode. Figure 5 plots the course of the pseudorotation phase angles *P* for the eight modified nucleosides. Table II lists the time-averaged values of the maximum out-of-plane pucker

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**Table III.** Averaged Values of the Hydrogen-Bond Geometries (Esd's in Parentheses) of the Eight Modified Nucleosides

| abbreviated name | O5′···H–C6 distance (Å) | O5′···H–C6 angle (deg) |
|---|---|---|
| Thd | 3.38(0.43) | 136(12) |
| C3′-methoxy-dThd | 2.98(0.42) | 145(11) |
| C3′-methyl-dThd | 3.15(0.40) | 135(10) |
| C2′-OH-dThd | 3.26(0.41) | 137(12) |
| C2′-methoxy-dThd | 3.03(0.40) | 141(11) |
| C2′-methyl-dThd | 2.99(0.41) | 142(11) |
| dThd[a] | 3.37(0.46) | 137(13) |
| C2′-OH,C3′-OH-dThd | 2.99(0.41) | 142(10) |

[a] Averaged values during the time $\gamma$ is in the +*sc* orientation.

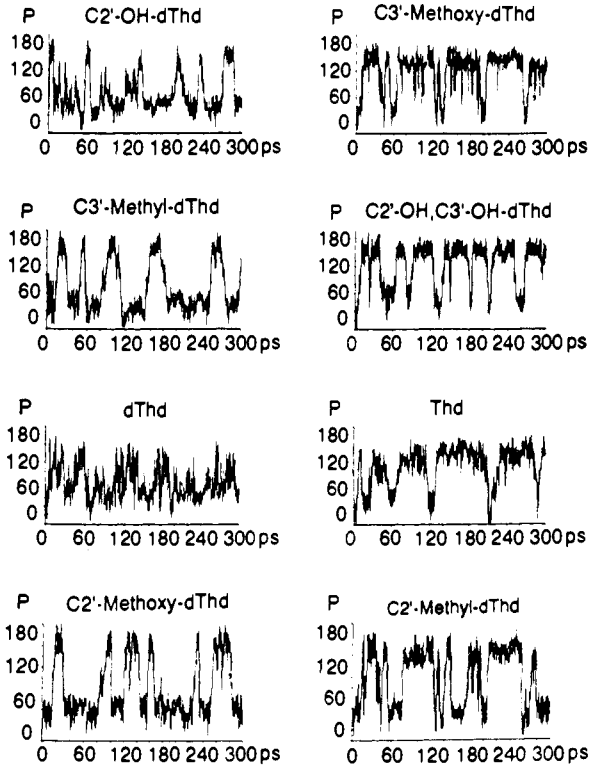


**Figure 5.** Pseudorotation phase angle *P* as a function of time (300 ps) for the eight selected nucleosides.

$\Psi_m$ and the percentages of time the sugar pucker is found in the C3′-*endo* conformation (N) ($P < 54°$), in the O4′-endo conformation ($54 \le P \le 126°$), or in the C2′-*endo* conformation (S) ($P > 126°$). The O4′-*exo* conformation (*P* about 270°) is not included because all transitions between the N- and the S-type sugar conformations passed via the energetically lower O4′-*endo* intermediate. Forgetting about the intermediate O4′-*endo* form and dividing the sugar puckering mode into only two categories, N-type ($P \le 90°$) and S-type ($P > 90°$), we see very clearly the role of the different C2′- and/or C3′-substituents. From Figure 6 we learn that electronegative substituents such as –OH and –$OCH_3$, in both the C2′- and C3′-positions, favor the axial position of the substituent. This is due to the *gauche* effect which is accounted for in the *AMBER* force field and leads to an N-type sugar conformation for electronegative C2′-substituents and to an S-type sugar conformation for electronegative C3′-substituents. C2′- or C3′-methyl substitution has the inverse effect on the sugar puckering mode, but the differences (especially for C2′-methyl-dThd) are less pronounced. For 3′-deoxythymidine (dThd), the C3′-*endo* conformation (N) is favored, but from Table II and Figure 5 we learn that the sugar pucker is very unstable and that it spends more than half of the time (52.4%) in the intermediate O4′-*endo* conformation. This suggests extreme flexibility for

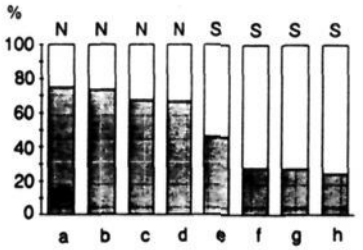


**Figure 6.** Representation of the percent N-type (hatched) and S-type (blank) sugar conformations during the 300 ps of molecular dynamics simulation for all eight nucleosides: a, C2′-OH-dThd; b, dThd; c, C3′-methyl-dThd; d, C2′-methoxy-dThd; e, C2′-methyl-dThd; f, Thd; g, C2′-OH,C3′-OH-dThd; h, C3′-methoxy-dThd.

dThd. C2′-OH,C3′-OH-dThd (5-methyluridine), which has an electronegative substituent (–OH) in both the C2′- and C3′-positions, has a preference for the S-type sugar conformation. This is in agreement with many X-ray data for C5-modified uridines (*e.g.* 5-aminouridine[15] and 5-chlorouridine[16]). 5-Methyluridine itself, however, exhibits an N-type sugar conformation.[17]

It should be stressed that whatever the substituents on the C2′- or C3′-positions are, interconversion between the N- and the S-type sugar conformations takes place about every 20 ps and it takes only about 2 ps to repucker. The same repuckering time was also found for $tRNA^{Phe}$.[18] In conclusion, interconversion for all eight modified nucleosides takes place frequently and rapidly. None of the eight structures shows any extreme S-type ($P \pm 210°$) sugar conformation, and by this, the exocyclic torsion angle $\gamma$ is kept in the $+sc$ orientation. This $\gamma$ $+sc$ orientation is linked to the $\chi$ *anti* orientation by the formation of an intramolecular O5′···H–C6 hydrogen bond. In general, aqueous molecular dynamics simulations show that electronegative substituents do favor an axial position on the sugar ring, but they cannot prevent the sugar ring from repuckering.

## Conclusions

The aqueous molecular dynamics simulations of eight modified 2′,3′-dideoxypyrimidine nucleosides reveal information about the conformation which is threefold: (1) the torsion angle $\chi$ is found exclusively in the *anti* orientation, (2) the torsion angle $\gamma$ adopts preferentially the $+sc$ orientation hereby helped by the stabilizing effect of the intramolecular hydrogen bond, and (3) electronegative C2′- and C3′-substituents favor an axial position due to the *gauche* effect which is accounted for in the force field. But nevertheless, the furanose sugar is able to repucker rapidly and frequently between C3′-*endo* (N) and C2′-*endo* (S) via the O4′-*endo* intermediate.

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