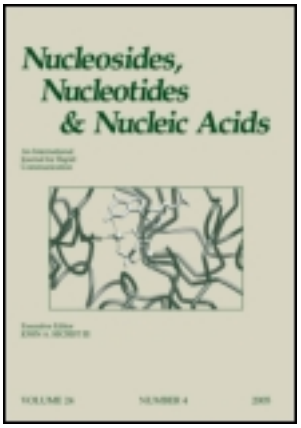

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5-Substituted Pyrimidine L-2′-Deoxyribonucleosides: Synthetic, Quantum Chemical, and NMR Studies

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5-SUBSTITUTED PYRIMIDINE L-2′-DEOXYRIBONUCLEOSIDES: SYNTHETIC, QUANTUM CHEMICAL, AND NMR STUDIES

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□ *As a part of an ongoing medicinal chemistry, we report here the synthesis and structure evaluation of 1-(2-deoxy-3,5-di-O-acetylpentofuranosyl)-5-[(3-methyl-5-oxo-1-phenyl-4,5-dihydro-4H-pyrazol-4-ylidene) pyrimidine-2,4(1H,3H)-dione* ***5*** *and 5-[bis(3-methyl-5-oxo-1-phenyl-4,5-dihydro-4H-pyrazol-4-yl)methyl-1-(2-deoxy-3,5-di-O-acetylpentofuranosyl)pyrimidine-2,4(1H,3H)-dione* ***6*** *derived from 3′,5′-di-O-acetyl-5-formyl-2′-deoxy-β-L-uridine* ***1****. Base hydrolysis of compounds* ***1*** *and* ***6*** *furnished their deacetylated analogues in good yields, whereas hydrolysis of* ***5*** *was troublesome. Structural features of these molecules are discussed by NMR spectra analyses and density functional theory quantum chemical calculations. The newly synthesized L-analogues show no significant activity against vaccinia and cowpox viruses.*

Keywords L-nucleoside; tautomerism; NMR; DFT calculations

INTRODUCTION

Drug discovery in antiviral chemotherapy has provided effective treatments for various viral infections. In particular, nucleoside analogues have been the cornerstone of antiviral treatments for several decades. In the search for new, safe, and effective agents within this class, L-nucleoside enantiomers of the natural D-nucleosides represent an immense breakthrough.[1] As a general feature, these compounds with an unnatural configuration confer in most cases lower toxicity and higher metabolic stability compared with the natural D-counterparts. As a result of our recent study, which showed that several novel 5-substituted pyrimidine 2′-deoxyribonucleosides **I** have

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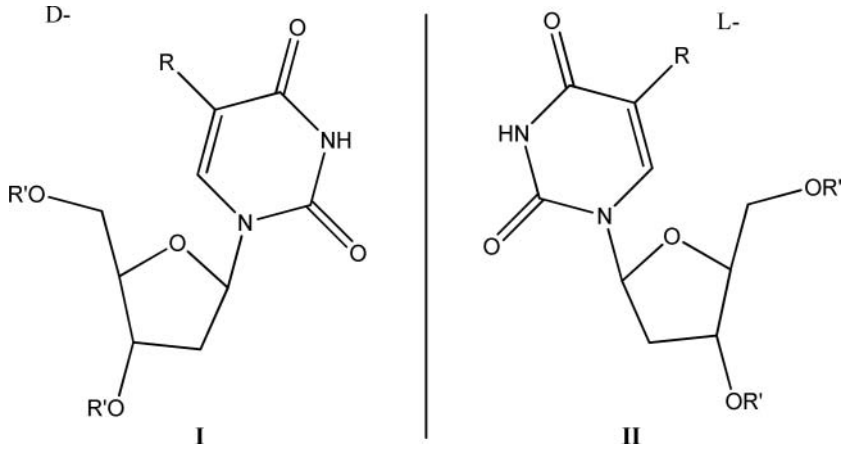
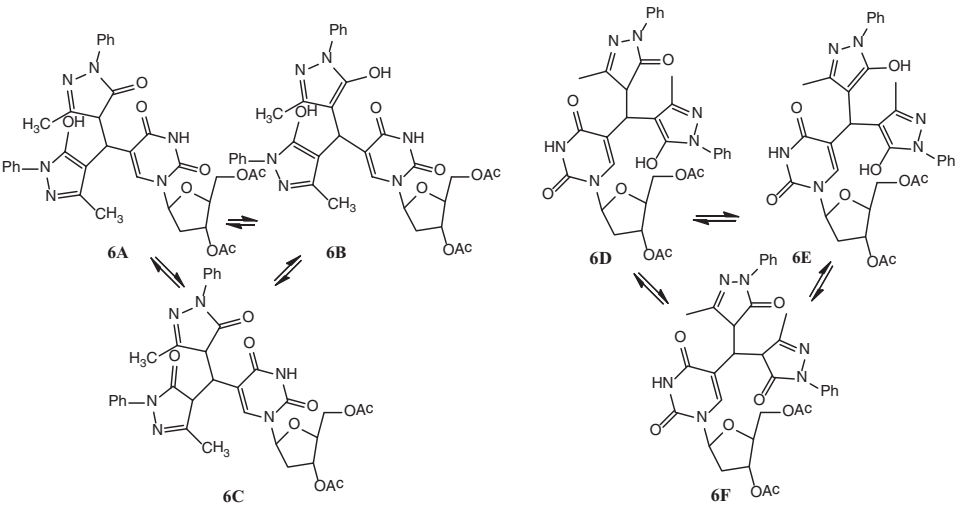


FIGURE 1 Structures of biologically active 5-substituted pyrimidine D-2′-deoxyribonucleosides **I** and the title L-nucleosides **II**.

potent in vitro antiviral activity against two representative orthopox, vaccinia, and cowpox viruses,[2–6] herein we report the synthesis of some of their L-analogues **II** (Figure 1).

The building block unit for the present study, namely 3′,5′-di-acetyl-5-formyl-2′-deoxy-L-uridine **1**, was successfully prepared in good yield by the following protocol: (1) Treating L-thymidine **2** with acetic anhydride to afford 3′,5′-di-*O*-acety-L-thymidine **3**; (2) oxidation of **3** with potassium peroxysulfate ($K_2S_2O_8$) in the presence of $CuSO_4{\cdot}5H_2O$ and 2,6-lutidine in aqueous acetonitrile to give **1** (see Scheme 1).



SCHEME 1

Condensation of **1** with equimolar of 1-phenyl-3-methyl-2-pyrazolin-5-one **4** afforded a mixture of compounds **5** and **6** in 3:2 mole ratio. Of the four isomeric forms theoretically possible, the spectral data of **5** indicated that it exists solely in one form A. Its ^{13}C NMR in chloroform showed 22 signals only, which correlated well with all its proton signals in the Heteronuclear Multiple Bond Correlation (HMQC) spectrum. The preferred conformation of **5** was determined from the nuclear Overhauser effect (NOE) data measurement and by analysis of the nuclear Overhauser effect spectroscopy (NOESY) spectrum, which confirmed interactions between the methyl group and the substituted vinyl proton, as well as the H6 and the methylene protons on C5′ of sugar moiety. This result is in agreement with the previously reported result for the D-analogue.

Because an accurate knowledge of the conformational properties of these nucleosides would be an important help for the interpretation of drug–target interactions, in the present work, conformational preference of **5** is supported by molecular modeling using the density functional theory (DFT) in gas phase and solution.

According to difference on the calculated total energy it is found that the isomer **5A** (anti orientation) is more stable than isomer (syn orientation) **5B** by 2.513 kcal/mol (Table 1), and that can be related to the presence of C-H···O type of hydrogen bond, namely (C_{11}-H_{43}···O_{22}) (Figure 2). The calculations show that the H-bond is stronger in isomer **5A** relative to isomer **5B** because its bond length is shorter by 0.026 Å, its bond angle is smaller, and the O_{22} atom is more planar (Table 2). Calculation of natural population analysis (Table 3) shows that the electrons accumulate on O_{22} in isomer **5A** (-0.663 e$^-$) more than isomer **5B** (-0.632 e$^-$) and the H_{43} was found to be more deficient in electrons in isomer **5B** (0.298 e$^-$) relative to isomer **5A** (0.292 e$^-$). A simple calculation of the force of attraction between the two atoms forming H-bond (H_{43} and O_{22}) in the two isomers according to Coulomb's law shows that the force of attraction between the two atoms in isomer **5A** is more than isomer **5B** by 7.128×10^{-11} N. There is also another C-H···O H-bond, which is formed in two isomers, namely C_{11}-H_{45}···O_{12} in isomer **5B** and C_{11}-H_{45}···O_{22} in isomer **5A**. Although the two H-bonds are fairly planar, the latter is found to be shorter by 0.134 Å.

TABLE 1 B3LYP/6–31G(d,p) calculated total energies of structures **5A–5D**

Compound	Total energy (a.u.)
5A	−1749.30928102
5B	−1749.30527692
5C	−1749.28662385
5D	−1749.28693448

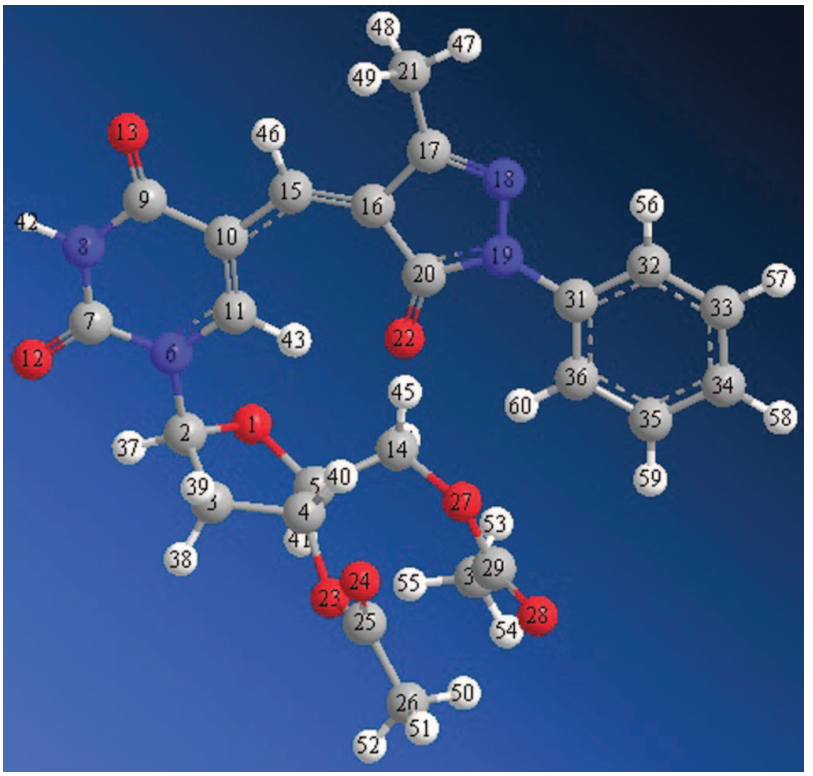


FIGURE 2 B3LYP/6–31G(d,p) optimized geometry for **5A** with atom number scheme (Color figure available online).

In the case of the other two isomers **5D** (syn orientation) and **5C** (anti orientation), the DFT calculations show that these are less stable than isomer **5A** or **5B** due to lack of H-bond, C_{11}-$H_{43}\cdots O_{22}$, which is present in isomers **5A** and **5B**. The isomer **5D** is found to be less stable than isomer **5B** by

TABLE 2 B3LYP/6–31G(d,p) calculated bond lengths (Å), bond angles (degree), and dihedral angles (degree) concerning the H-bonds formed in structures **5A–5D**

	Compound			
	5A	**5B**	**5C**	**5D**
$H_{43}\cdots O_{22}$	1.841	1.867		
$H_{45}\cdots O_{12}$		2.370		2.360
$H_{45}\cdots O_{22}$	2.236			
C_{11}-$H_{43}\cdots O_{22}$	111.56	149.21		
C_{14}-$H_{45}\cdots O_{12}$		132.55		134.15
C_{14}-$H_{45}\cdots O_{22}$	142.56			
N_6-C_{11}-$H_{43}\cdots O_{22}$	179.1	175.3		
O_{27}-C_{14}-$H_{45}\cdots O_{12}$		168.6		166.0
O_{27}-C_{14}-$H_{45}\cdots O_{22}$	119.34			

TABLE 3 B3LYP/6–31G(d,p) calculated charges using the natural population analysis scheme on centers concerning the H-bonds formed in the structures **5A–5D**

	Compound			
Center	**A**	**B**	**C**	**D**
N_6	−0.44204	−0.43783	−0.44986	−0.44340
C_{11}	0.11090	0.10738	0.07271	0.07876
O_{12}	−0.59328	−0.61116	−0.59735	−0.61527
C_{14}	−0.15151	−0.15922	−0.14612	−0.15835
O_{22}	−0.66291	−0.63250	−0.57918	−0.57587
O_{27}	−0.55355	−0.56188	−0.55558	−0.56437
H_{43}	0.29152	0.29841	0.25615	0.25791
H_{45}	0.28290	0.27360	0.25772	0.27208

11.510 kcal/mol. The calculations show that isomer **5D** is more stable than isomer **5C** by 0.195 kcal/mol. That can be ascribed to the presence of the other H-bond, C_{11}-$H_{45}\cdots O_{12}$ in isomer **5D**; in contrast, its analogues C_{11}-$H_{45}\cdots O_{22}$ cannot be formed in isomer **5C**. So the net conclusion drawn from the DFT/B3LYP/6–3-1G (d,p) calculations on isomers is that the order of stability of the four isomers is **5A** > **5B** ≫ **5D** > **5C**.

On the other hand, the ^{1}H NMR spectrum of **6** in chloroform showed an isomeric mixture. The presence of two tautomers in solution was confirmed by the presence of pair-wise $CH_{1'}$ at δ6.17, δ6.35 ppm. These two forms were assigned as **6A** and **6B** (syn orientations) and are in 0.33:0.67 ratio (see experimental part) by combination of 1D COSY and 2D HMQC spectra. This observation was also supported by DFT calculations, and the rotation of the nucleobase about the glycosidic bond relative to the sugar moiety was also considered. According to the total energy of each isomer in the gas phase calculations it is found that the order of stabilization is **6B** > **6A** > **6C** (syn orientations). Isomer **6B** is more stable than isomer **6A** by 0.075 kcal/mol, and it is more stable than isomer **6C** by 1.412 kcal/mol. The stability of isomer **6B** over **6A** or **6C** can be attributed to the presence of two H-bonds between the uracil oxygen atom and hydroxyl groups (Figure 3).

The calculation of the population of each isomer shows that in gas phase using the total energy the population of **6A**:**6B**:**6C** is 44.65:50.67:4.67. The population of **6A**:**6B**:**6C** in chloroform solution was found to be 16.10:83.25:0.65 using the total free energy of solvation calculated using the CPCM//B3LYP/6–31G(d,p) method and 17.48:81.32:1.20 when using the total energy in solution.

The same trend of stability was found in the three sets of rotomers **6E** > **6D** > **6F** (antiorientations) with populations of 94.36%, 3.45%, and 2.19%, respectively, in the gas phase and 99.80%, 0.19%, and 0.01% in solution using the total free energy of solvation calculated using the

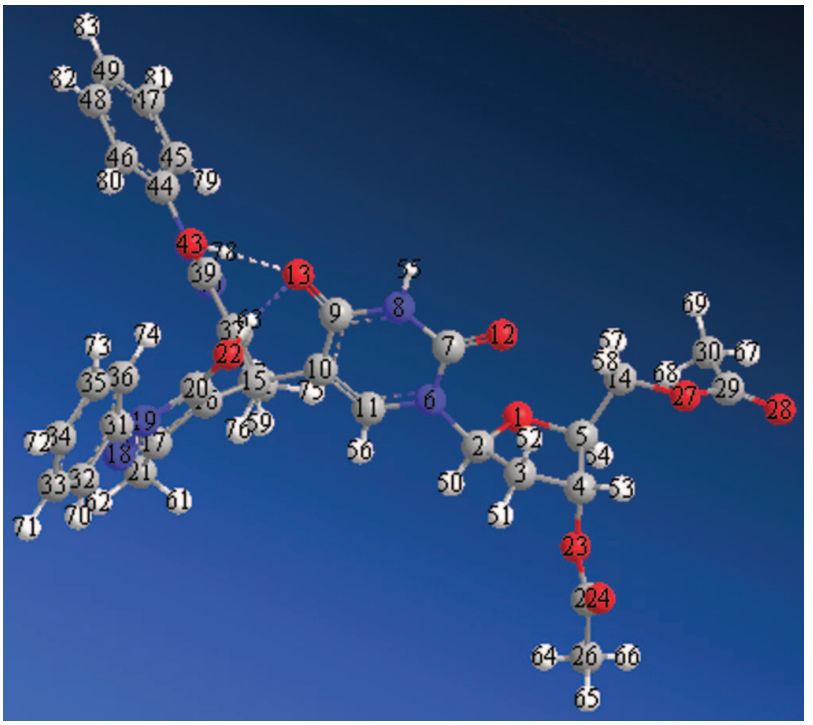


FIGURE 3 B3LYP/6–31G(d,p) optimized geometry for **6B** with atom number scheme (Color figure available online).

CPCM//B3LYP/6–31G(d,p) method. When using the total energy in solution, the population was found to be 0.38% of isomer **6D**, 99.61% of isomer **6E** (Figure 4), and 0.01% of isomer **6F**.

It can be seen from the examination of Table 4 that the syn conformers are more stable than their corresponding antiforms in the gas phase and in solution. Table 5 shows the intramolecular H-bonds computed at the B3LYP/6–31G(d,p) level.

Because the deacetylated D-analogues of the above-prepared compounds were found to be more active biologically, based on our previously reported

TABLE 4 B3LYP/6–31G(d,p) calculated total energies of isomers **6A–6F**

Isomer	Total Energy (a.u.)	Total energy in solution (a.u.)	Total free energy in solvation (a.u.)
6A	−2321.696438	−2321.733452	−2321.696840
6D	−2321.693561	−2321.728732	−2321.689235
6B	−2321.696558	−2321.734904	−2321.698391
6E	−2321.696684	−2321.733996	−2321.695143
6C	−2321.694307	−2321.730922	−2321.693809
6F	−2321.693131	−2321.725440	−2321.685592

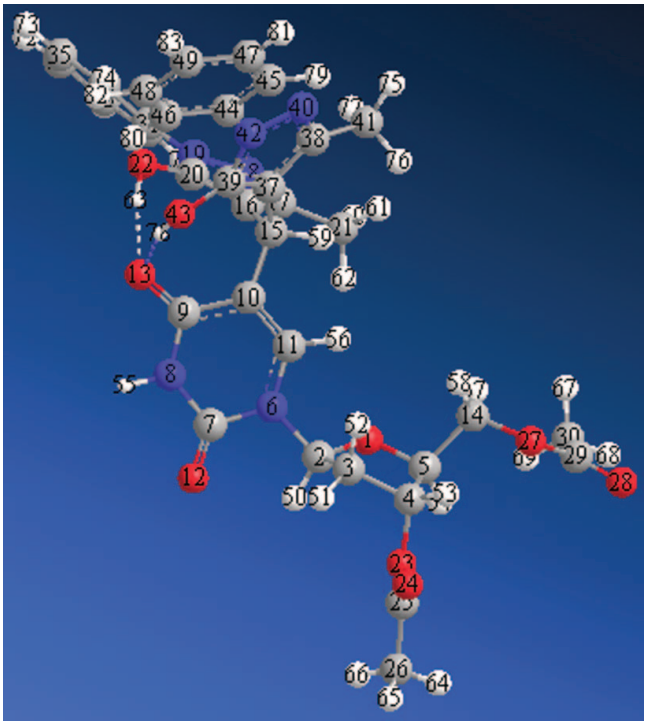


FIGURE 4 B3LYP/6–31G(d,p) optimized geometry for **6E** with atom number scheme (Color figure available online).

work, we have decided to prepare compound **7** using the following approach. First, the formyl group in **1** was protected as dimethyl acetal **8**, which was then treated with NaOMe/MeOH and AcOH/H_2O sequentially to give **7** (see Scheme 4). Also, compound **9** was prepared via mild base hydrolysis

TABLE 5 B3LYP/6–31G(d,p) calculated H-bonds bond lengths (Å) formed in isomers **6A–6F**

	Isomer					
	6A	**6D**	**6B**	**6E**	**6C**	**6F**
$H_{83}\cdots O_{43}$	1.770					
$H_{83}\cdots O_{13}$	2.535					
$H_{77}\cdots O_{22}$		1.783				
$H_{77}\cdots O_{13}$		2.469				
$H_{63}\cdots O_{13}$			1.744	1.762		
$H_{78}\cdots O_{13}$			1.765	1.735		
$CH_{75}\cdots O_{13}$					2.127	
$CH_{60}\cdots O_{43}$					2.238	
$CH_{60}\cdots O_{13}$						2.225
$CH_{75}\cdots O_{22}$						2.175
$CH_{50}\cdots O_{12}$		2.227		2.314		2.263
$CH_{52}\cdots O_{12}$			2.316			
$CH_{53}\cdots O_{12}$	2.446				2.440	

TABLE 6 B3LYP/6–31G(d,p) calculated gas phase total energies and solvation-free energies of **9A** and **9B** isomers

Isomer	Total energy (a.u.)	Total free energy of solvation (a.u.)
9A syn	−2016.372897	−2016.388382
9B anti	−2016.370376	−2016.384524

of its corresponding **6** and its NMR spectra showed that it exists in one tautomeric form **9A** only in dimethyl sulfoxide solution (see Experimental section). On the other hand, compound **5** was found to be very sensitive to base hydrolysis and the workup of the reaction mixture was tedious.

According to the total energy of each isomer in the gas phase calculations, it is found that the order of stabilization is **9A** (syn orientation) > **9B** (antiorientation) (Table 6). The syn-isomer is more stable than the anti-isomer by 1.582 kcal/mol. The stability of syn-isomer over anti-isomer can be attributed to the presence of additional two H-bonds formed in the syn-isomer. The syn-isomer has a hydroxyl group oriented in a direction suitable for H-bond formation with the uracil and the sugar ring oxygen atoms (Figure 5, Table 7). The calculated charges using the natural population analysis are summarized in Table 8. The calculation of population of each isomer shows that in gas phase using the total energy, the population will be 6.48% of anti-isomer and 93.52% of syn-isomer. The population in dimethyl sulfoxide (DMSO) solvent was found to be 1.65% of anti-isomer and 98.35% of syn-isomer using the total free energy of solvation calculated using the CPCM//B3LYP/6–31G(d,p) method.

The newly synthesized L-analogues show no significant activity against vaccinia and cowpox viruses. At this point, it seems that the D-configuration is crucial for exhibiting the antiviral activity.

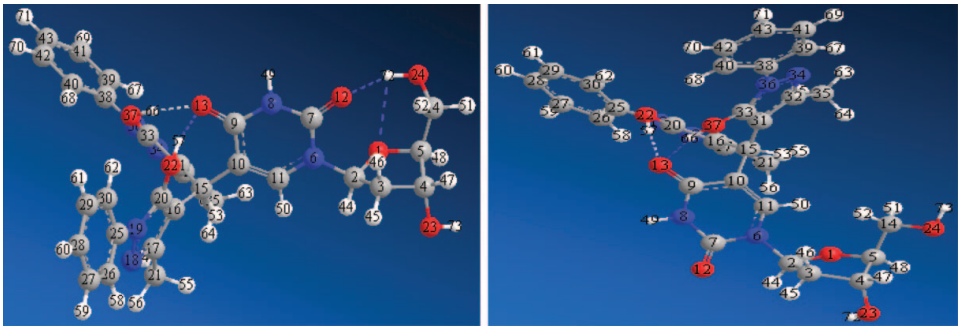


FIGURE 5 B3LYP/6–31G(d,p) optimized geometry for **9A** and **9B** with atom number scheme (Color figure available online).

TABLE 7 B3LYP/6–31G(d,p) calculated bond lengths (Å), bond angles (degree), and dihedral angles (degree) concerning the H-bonds formed in anti- and syn-isomers

	9 Isomer	
	Anti	Syn
$H_{66}\cdots O_{13}$	1.737	1.756
$H_{57}\cdots O_{13}$	1.749	1.738
$H_{72}\cdots O_{12}$		2.359
$H_{72}\cdots O_{1}$		2.456
O_{22}-$H_{57}\cdots O_{13}$	165.86	165.74
O_{37}-$H_{66}\cdots O_{13}$	167.13	167.68
O_{24}-$H_{72}\cdots O_{12}$		135.73
O_{24}-$H_{72}\cdots O_{1}$		97.88
C_9-O_{13}-$H_{66}\cdots O_{37}$	15.9	25.5
C_9-O_{13}-$H_{57}\cdots O_{22}$	28.9	13.0
C_7-O_{12}-$H_{72}\cdots O_{24}$		134.6

EXPERIMENTAL

Melting points were recorded with a Barnstead 1201D electrothermal melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400 MHz spectrometer. The chemical shifts are reported in parts per million (δ) and signals are quoted as s (singlet, d (doublet), t (triplet), m (multiplet), dd (doublet of doublets), and ddd (doublet of doublets of doublets). The mass spectra were performed on a HP 1100 MSD spectrometer at the HT Laboratories, San Diego. The High Resolution Mass Spectra (HRMS) were performed on a JEOL HX 110 A spectrometer at the Department of Chemistry, University of Arizona.

TABLE 8 B3LYP/6–31G(d,p) calculated charges using the natural population analysis scheme on centers concerning the H-bonds formed in anti- and syn-isomers

	Isomer			
	Gas phase		Solution phase	
Center	Anti	Syn	Anti	Syn
O_1	−0.59530	−0.58411	−0.60082	−0.60185
C_7	0.82970	0.84237	0.84473	0.85261
C_9	0.68794	0.69196	0.69020	0.69441
O_{12}	−0.61608	−0.63372	−0.64738	−0.65509
O_{13}	−0.70477	−0.69780	−0.72414	−0.72056
O_{22}	−0.72288	−0.72168	−0.73073	−0.72557
O_{24}	−0.75829	−0.75198	−0.79251	−0.78216
O_{37}	−0.72394	−0.72206	−0.72704	−0.73033
H_{57}	0.52695	0.51847	0.53057	0.52419
H_{66}	0.51968	0.52616	0.52543	0.53006
H_{72}	0.47505	0.49285	0.51007	0.50246

3′-5′-di-O-Acetyl-β-L-thymidine 3

Acetic anhydride (52 mL) was added to a mixture of β-L-thymidine (4.84 g, 20 mmol) and 4-(dimethylamino)pyridine (0.124 g). The reaction mixture was stirred for 48 hours at room temperature, then rotary evaporated. The traces of acetic anhydride were removed by adding ethanol 25 × 3 mL and rotary evaporated. The product was recrystallized from water. m.p. 127–128°C; yield 5.87 g. ^{1}H NMR ($CDCl_3$) δ: 1.87 (s, 3H, $CH_3C(5)$), 2.06, 2.07 (2s, 6H, $2CH_3CO$), 2.13 (m, 1H, $H_aC(2')$), 2.41 (ddd, J = 1.6, 5.6, 14.4 Hz, 1H, $H_bC(2')$), 4.19 (m, 1H, HC(4′)), 4.28 (dd, J = 3.2, 12.2 Hz, 1H, $H_aC(5')$), 4.32 (dd, J = 4.4, 12.2 Hz, 1H, $H_bC(5')$), 5.17 (m, 1H, HC(3′)), 6.28 (dd, J = 5.6, 8.40 Hz, 1H, HC(1′)), 7.24 (s, 1H, HC(6)), 9.91 (s, 1H, NH). FAB HRMS: $(MH)^+$ found m/e 327.1207. $C_{14}H_{18}N_2O_7$ requires m/e 327.1192.

3′,5′-di-O-Acetyl-5-formyl-2′-deoxy-β-L-uridine 1

Acetonitrile solution (55 mL) containing 3′-5′-di-O-acetyl-β-L-thymidine (5 g, 15.3 mmol) and 2,6-lutidine (6.1 mL) under Argon atmosphere was added to a clear blue solution of $K_2S_2O_8$ (8.3 g, 30.7 mmol) and $CuSO_4 \cdot 5H_2O$ (1.5 g) in water (55 mL) at 65°C. The mixture was stirred at 65–70°C and monitored by TLC (EtOAc: n-C_6H_{14} 2:1) until the disappearance of the starting material (ca ∼3 hours). The mixture was concentrated to half of the initial volume and the remaining solution was extracted with EtOAc. The organic layer was washed with water. The aqueous layers were combined and back-extracted with $CHCl_3$. The organic layers were combined, dried over anhydrous Na_2SO_4, and evaporated in vacuum. The residue was purified by flash chromatography (EtOAc: n-C_6H_{14} 2:1) to afford **1**; m.p. 140–141°C (1.58 g). ^{1}H NMR ($CDCl_3$) δ: 2.12, 2.21 (2s, 6H, 2 CH_3CO), 2.26 (m, 1H, $H_aC(2')$), 2.61 (ddd, J = 2.8, 3.2, 14.0 Hz, 1H,, $H_bC(2')$), 4.36 (m, 3H, HC(4′), and $H_2C(5')$), 5.17 (m, 1H, HC(3′)), 6.33 (t, J = 6.80 Hz, 1H, HC(1′)), 8.49 (s, 1H, HC(6)), 9.12 (s, 1H, NH), 10.02 (s, 1H, CHO). ^{13}C NMR ($CDCl_3$) δ: 20.90, 21.07, 39.01, 63.80, 74.19, 83.33, 86.31, 111.95, 1444.55, 149.38, 161.73, 170.51, 170.71, 185.96. FAB HRMS: $(MH)^+$, found 341.0986. $C_{14}H_{16}N_2O_8$ requires m/e 341.0985.

Reaction of Compound 1 with 3-methyl-1-phenyl-pyridazolin-5-one 4

Solution of 3-methyl-1-phenyl-pyrazolin-5-one (87 mg, 0.50 mmol) was added drop-wise over a period of 3 hours with stirring to a warm solution of compound **1** (170 mg, 0.50 mmol) in anhydrous ethanol (3 mL) at 45°C. The reaction mixture was left at this temperature for 7 hours, then at room temperature overnight. After evaporation to dryness, the residue was purified

by flash chromatography (EtOAc: n-C_6H_{14} 4:1) to afford 3′,5′-di-O-Acetyl-5-((3-methyl-5-oxo-1-phenyl-1H-pyrazol-4(5H)-ylidene)methyl)-2′-deoxy-β-L-uridine **5**; m.p. 238–240°C (15 mg); ^{1}H NMR ($CDCl_3$) δ: 1.97 (s, 3H, CH_3), 2.14, 2.34 (2s, 6H, 2 CH_3CO), 2.58 (m, 2H, $H_2C(2')$), 4.47 (m, 1H, HC(4′)), 4.46 (dd, J = 5.4, 11.9 Hz, 1H, $H_aC(5')$), 4.54 (dd, J = 4.8, 11.9 Hz, 1H, $H_bC(5')$), 5.39 (m, 1H, HC(3′)), 6.37 (t, J = 6.0 Hz, 1H, HC(1′)), 7.19 (tt, J = 1.2, 7.6 Hz, 1H, p-Ph), 7.41 (t, J = 8.0 Hz, 2H, m-Ph), 7.68 (s, 1H, HC =), 7.91 (dd, J = 1.2, 8.0 Hz, 2H, o-Ph), 9.27 (s, 1H, HC(6)), 10.92 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 13.34, 20.92, 21.16, 37.74, 64.01, 74.37, 83.15, 86.64, 109.01, 119.45, 125.38, 126.57, 129.06, 136.27, 138.32, 148.47, 149.37, 151.08, 161.95, 162.70, 170.43, 170.63. FAB HRMS: $(MH)^+$, found m/e 497.1675. $C_{24}H_{24}N_4O_8$ requires m/e 497.1672.

3′,5′-di-O-Acetyl-5-((5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)methyl)-2′-deoxy-β-L-uridine **6**; m.p. 148–150°C (15 mg); ^{1}H NMR ($CDCl_3$) δ: 1.84, 1.95, 2.01, 2.04, 2.06, 2.12, 2.18, 2.25, 2.32, 2.38, 2.55 (s, s, m, s, m, s, s, s, s, m, m, 14.67 H, CH_3 groups of two forms A and B, $H_2C(2')$ and $H_2C(2'')$), 3.40 (bs, 0.67H, HC(1″) form A), 4.10 (m, 2H, $H_2C(5')$), 4.33 (m, 0.33H, HC(4′) form B), 4.49 (m, 0.67H, HC(4′) form A), 4.78 (bs, 0.33H, HC(1″) form B), 5.15, 5.37 (2m 2:1, 1H, HC(3′) forms A and B, 6.17, 6.35 (2t 2:1, 1H, HC(1′)), 7.10–7.91 (m, 11H, ArH and HC(6)), 9.63, 9.80, 10.03, 10.89 (4bs, 2.33H, NH and OHs); ^{13}C NMR ($CDCl_3$) δ: 11.91, 12.13, 13.32, 17.21, 20.74, 20.93, 21.15, 21.11, 26.28, 37.07, 37.70, 43.33, 63.62, 64.06, 74.39, 74.66, 82.52, 83.13, 86.45, 86.77, 108.98, 114.58, 119.18, 119.48, 121.34, 121.48, 125.36, 125.40, 126.32, 126.45, 129.06, 129.11, 129.18, 136.41, 136.94, 137.38, 137.67, 138.18, 138.31, 147.11, 147.35, 148.53, 149.43, 150.23, 151.10, 156.64, 162.05, 162.72, 163.82, 170.40, 170.46, 170.56, 170.73, 170.91; FAB HRMS: $(MH)^+$, found m/e 671.2436. $C_{34}H_{35}N_6O_9$ requires m/e 671.2466.

5-Formyl-2′-deoxyuridine 7

In the presence of Amberlite IR-120 (200 mg), 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine (**1**, 510 mg, 1.5 mmol) in 25 mL of anhydrous methanol were refluxed with stirring for 4 hours. The mixture was filtered to remove the Amberlite resin and the filtrate was concentrated. The residue was purified through silica gel column chromatography with a mixture of EtOAc and Hexane (4:1, v/v) as eluant to give a colorless solid (210 mg). The intermediate **8** was dissolved in 5 mL of anhydrous methanol, and 3.3 mL of NaOMe/MeOH solution (0.5 M) was added. The mixture was stirred at ambient temperature for 3 hours. At completion, the mixture was concentrated under reduced pressure. To this residue was added 4 mL of AcOH and 1.5 mL of water. The mixture was then stirred at 50°C overnight. At

completion, the solvent was removed under vacuum. The residue was purified through column chromatography (chloroform–methanol, 4:1) to give **7** (100 mg). m.p. 157∼158°C; ^{1}H NMR (DMSO-d_6) δ: 2.51∼2.56 (m, 2H, H2′), 3.53∼3.63 (m, 2H, H5′), 383∼3.85 (m, 1H, H4′), 4.21∼4.24 (m, 1H, H3′), 5.23 (bs, 2H, 2OH), 6.08 (t, 1H, H1′), 8.68 (s, 1H, H6), 9.74 (s, 1H, CHO). ^{13}C NMR (DMSO-d_6) δ: 41.32, 61.40, 70.53, 86.54, 88.62, 111.38, 147.75, 150.46, 162.73, 186.96. FAB HRMS: $(MH)^+$, found m/e 257.0782. $C_{10}H_{13}N_2O_6$ requires m/e 257.0774.

5-((5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)methyl)-2′-deoxy-β-L-uridine 9

A mixture of compound (120 mg, 0.18 mmol) and K_2CO_3 (74 mg, 0.54 mmole) in anhydrous methanol (5 ml) was stirred at room temperature and monitored by TLC ($CHCl_3$:MeOH 4:1). After 1 hour the reaction mixture was filtered off and the solvent was evaporated in vacuum to afford 9 (30 mg). m.p. 181–183°C; ^{1}H NMR (DMSO-d_6) δ: 1.96∼2.13 (m, 2H, H2′), 2.07, 2.14 (2s, 6H, $2CH_3$),3.21 (d, 1H, OH3′), 3.46∼3.55 (m, 2H, H5′), 3.68∼3.75 (m, 1H, H4′), 3.79 (bm, 1H, OH5′), 4.21∼4.35 (m, 1H, H3′), 4.62 (s, 1H, CH), 4.85, 5.25 (2bs, 2H, 2OH), 6.15 (t, 1H, H1′), 7.00∼7.04, 7.26∼7.30, 7.84∼7.91 (3m, 10H, ArH), 8.09 (s, 1H, H6), 11.13 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 13.68, 26.01, 62.83, 71.38, 84.34, 88.13, 101.71, 102.03, 118.39, 119.83, 119.90, 123.69, 128.92, 128.95, 135.80, 141.26, 141.36, 146.09, 146.53, 150.85, 157.38, 158.23, 163.59. FAB HRMS: $(MH)^+$, found m/e 587.2250. $C_{30}H_{31}N_6O_7$ requires m/e 587.2254.

METHOD OF CALCULATIONS

To estimate the population of the isomers in the mixture at thermodynamic equilibrium, each structure of the isomers was optimized at DFT/B3LYP/6–31G(d,p) level in gas phase using the Gaussian 03 package.[7]

This method and the basic set were found to give accurate molecular geometries.[8] The solvated species were calculated with the CPCM continuum solvation method.[9,10] For each isomer, a single point in the solvent was done. The total energies and the Gibbs free energies of solvation were calculated. Isomer populations were calculated from the following equations:

$$n_i = \exp\left(-\frac{\Delta G_i}{RT}\right),$$

$$\Delta G_i = G_i - G_j,$$

$$N = n_A + n_B + n_C,$$

$$\%_i = \frac{n_i}{N} \times 100,$$

where n_i is the ratio number of isomers of type i and j, ΔG_i is the energy difference of isomers i and j; j is an isomer chosen arbitrarily; R is the gas constant (1.987 cal mol^{-1} K^{-1}), and T is the absolute temperature (289.15 K).

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