Electronic Supplementary Information

An ESR and DFT study of Hydration of the 2′-Deoxyuridine-5-yl Radical: Possible Hydroxyl Radical Intermediate

Lidia Chomicz[b], Alex Petrovici[a], Ian Archbold[a], Amitava Adhikary[a], Anil Kumar[a], Michael D. Sevilla[a,], Janusz Rak[b,]**

[a]Department of Chemistry, Oakland University, Rochester, Michigan 48309, USA
E-mail: sevilla@oakland.edu

[b]Department of Chemistry, University of Gdansk, 80-308 Gdansk, Poland
E-mail: janusz.rak@ug.edu.pl

*Authors for correspondence

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ESR experimental details

The solutions of BrdU (4-12 mg/mL, Sigma-Aldrich) in H_2O or in D_2O containing 7.5 M LiCl, bubbled with gaseous nitrogen to remove oxygen from the samples, were drawn into a 4 mm quartz tube and cooled down to 77 K in liquid nitrogen to form a clear transparent glass. Such prepared samples were then irradiated with Co-60 γ-source to doses of 480-500 Gy. These solutions also contained potassium ferrocyanide ($K_4[Fe(CN_6]•3H_2O$, Mallinckrodt) in order to scavenge holes and eliminate the possibility that the $Cl_2•^-$ formed upon γ-irradiation of the aqueous glass contributes to radical formation in BrdU via annealing. Under the above-described experimental conditions, the primary products formed in the 77 K glass resulted exclusively from prehydrated electron attachment[1] to the solute. After irradiation, an ESR spectrum at 77K was taken in dark (since $5BrdU•^-$ is photoreactive[2]). ESR spectra were recorded using an E-9 Century Series ESR spectrometer equipped with dual cavities[2]. Next, the samples were annealed stepwise for 15 to 20 min to temperatures ranging from 135 K to 165 K at 5 K intervals; annealing softened the glass and allowed for secondary reactions. After each annealing step, the samples were again cooled to 77 K and their ESR spectra were recorded.

DFT calculation details

The conformational search for reasonable structures of the water···2′-deoxyuridinyl radical and hydroxyl radical···uridine complexes have been limited to the geometries justified by the type of the considered reaction. Although many conformations between 2′-deoxy uridine and water exist (for instance, for the water···uracil dimer four low energy minima resulted from the conformational search limited to the geometries stabilized by the maximal number of hydrogen bonds were reported[3]) only these structures which are present in the considered reaction entrance channel are important. Therefore, we considered only such geometries where the water molecule or hydroxyl radical was placed in a close vicinity to the molecular center (C5 or C6) with which they react. Due to this approach, we were able to consider only a limited number of geometries and still obtain sound results. For each transition state, we confirmed that it is connected to the corresponding substrate and product geometries by carrying out the IRC calculations.[4]

ESR spectra in D_2O

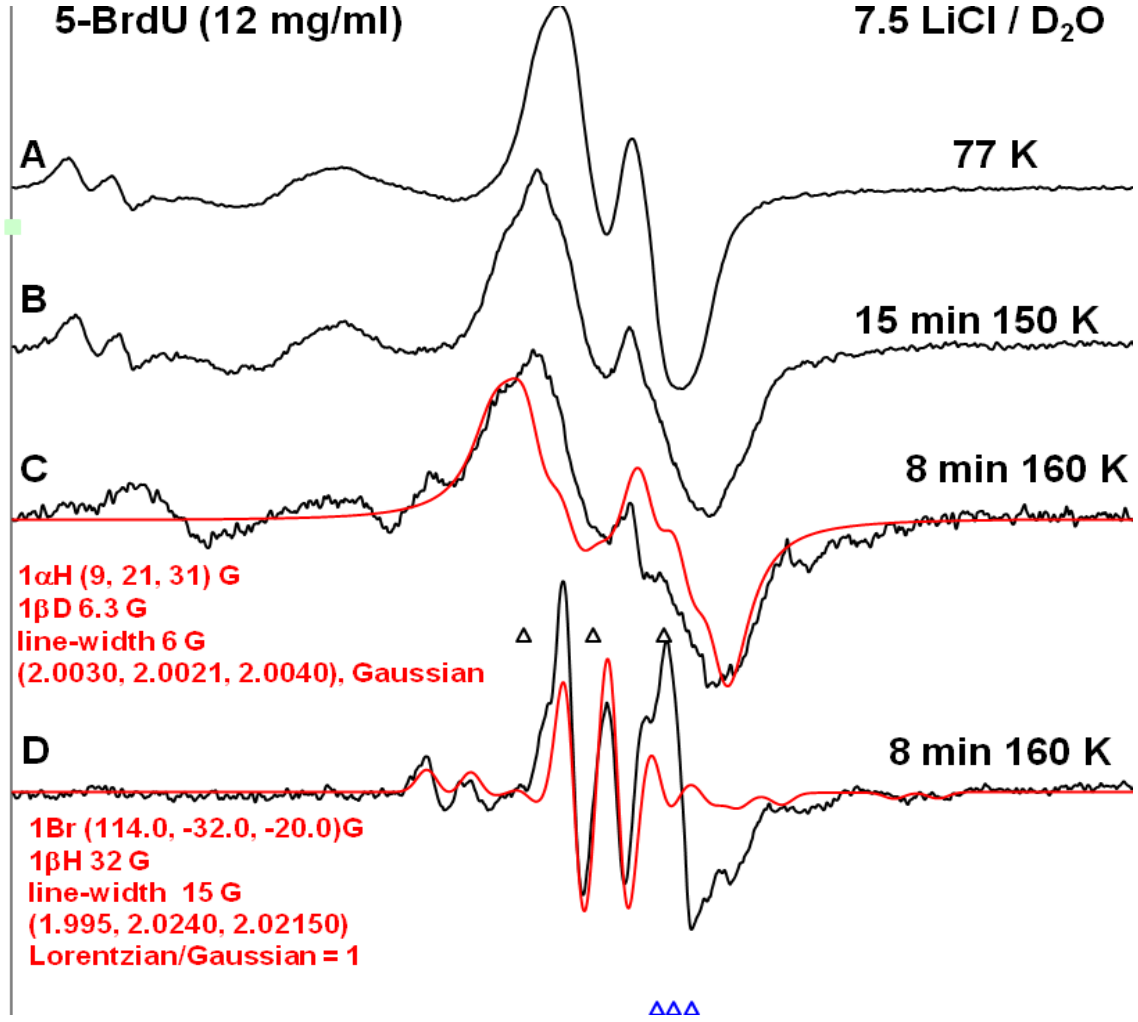


Figure S1. ESR spectra of matched samples of BrdU in 7.5 M LiCl in D_2O with $K_4Fe(CN)_6$ in excess as hole scavenger (A) immediately after γ-irradiation (absorbed dose = 500 Gy) and shows the anion radical of BrdU. The two low field lines are from Cl_2^{-}•. Spectra (B)-(D) were obtained on progressive annealing to temperatures and times shown. All spectra were recorded at 77 K. Figure (B) shows the formation of the dU-5-yl radical and (C) shows the formation of the 5-UDOD• radical in addition to the deuteration of the anion radical forming 6-D-5BrU• shown by the two low field lines. The simulated (red) 5-UDOD• spectrum (C) obtained using one anisotropic α-proton and one isotropic β-deuteron HFCC values is superimposed on the experimentally recorded spectra for comparison. (D) shows the same

sample shown in (C) with a larger field scan width of 1000G and increased modulation from 3G in C to 6G in D and power from 0.020 mW in C to 0.2 mW in D. The simulated (red) spectrum for the 6-D-5BrU• spectrum (D) was obtained employing one anisotropic Br-atom and one isotropic β-proton HFCC value. This spectrum is superimposed on the experimentally recorded spectrum for comparison. The broad component at g =2.005 in D is that of 5-UDOD• shown in (C) but broadened from changing conditions. Note that figures (A)-(C) are at the same conditions (220G total spectral scan) whereas figure (D) is approximately 1000G in total width. The three triangular field markers are individually separated by 13.09 G with the central marker at g=2.0056. The lower set of markers applies to figure D only.

Kinetic Isotope Effect discussion

Another argument supporting our conclusions comes from the kinetic isotope effect (KIE) observed in formation of the hydrate from the reaction of 5-U-yl• and water. As indicated by Figure S1, the reaction of 5-U-yl• with heavy water is greatly suppressed. The KIE[5] calculated for the elementary reaction leading to the hydroxyl radical amounts to $8x10^1$ at 150 K, which corresponds to the increase of the barrier in D_2O by 1.31 kcal/mol and fully accounts for the observed effect. We also have calculated the kinetics of water addition to the 5-U-yl• based on the calculated free energy of activation of 12.1 kcal/mol and found a rate of $8x10^4$ s^{-1}. Early experimental pulse radiolysis work on electron reactions with 5-bromouracil suggested the 5-U-yl radical underwent first order hydrolysis reaction with water with a 15 to 20 μsec lifetime.[6] This corresponds to about a rate constant of ca. $5x10^4$ which is in excellent agreement with the calculated value.

Optimized structures of substrates and transition states leading to "hydrate" radicals.

In Figure S2, the geometries and activation free energies for the formation of 5-UHOH• and 6-UHOH• are shown. The hydroxyl radical may attack the C5 and C6 uracil centers approaching them from the both sides of the uracil plane. Therefore, four transition states were considered (see Figure S2). Note that in accordance with the pulse radiolysis experimental observation that OH-radical attack is predominantly (ca. 80%) at C5 of uracil (see ref. 16), the activation barriers are found to be smaller for the OH radical attack on the C5 site from either side of the uracil ring than the corresponding OH-radical attack on the C6 site. Comparing barrier difference for the formation of the respective C5 vs. C6 adducts from different sides of the uracil base plane amounts to differences of 0.56 and 2.35 kcal/mol (see Figure S2 in which the absolute values for all the 4 compared barriers are given). Using these differences one can calculate, employing the Arrhenuis equation, that at 160 K (our temperature of annealing) formation of 5-UHOH• should be faster than formation of 6-UHOH• by 5.8 to 1625 times depending on the approach of the OH. Moreover, the substrate conformation giving the kinetic barriers differing by 0.56 kcal/mol is more stable than the other one by ca. 1.4 kcal/mol in the free enthalpy scale. These results suggest that the ESR spectrum shown in Figure 1D (main text) is dominated by the 5-UHOH• and the small admixture of doublet may partially result from the 6-UHOH•. Note that according to the Wegscheider principle, the ratio of equilibrium concentrations of 5-UHOH• to 6-UHOH• should be equal to the ratio of the appropriate rate constants and for the reaction pair corresponding to the more stable substrate (see the upper panel of Figure S2) it amounts to 5.8. This in reasonable agreement with 70% of quartet measured in the experiment when it is recalled that the H5 equatorial conformation of the 5-UHOH• should be partially responsible for the observed doublet, and therefore, only a fraction of 30% doublet results from 6-UHOH•.

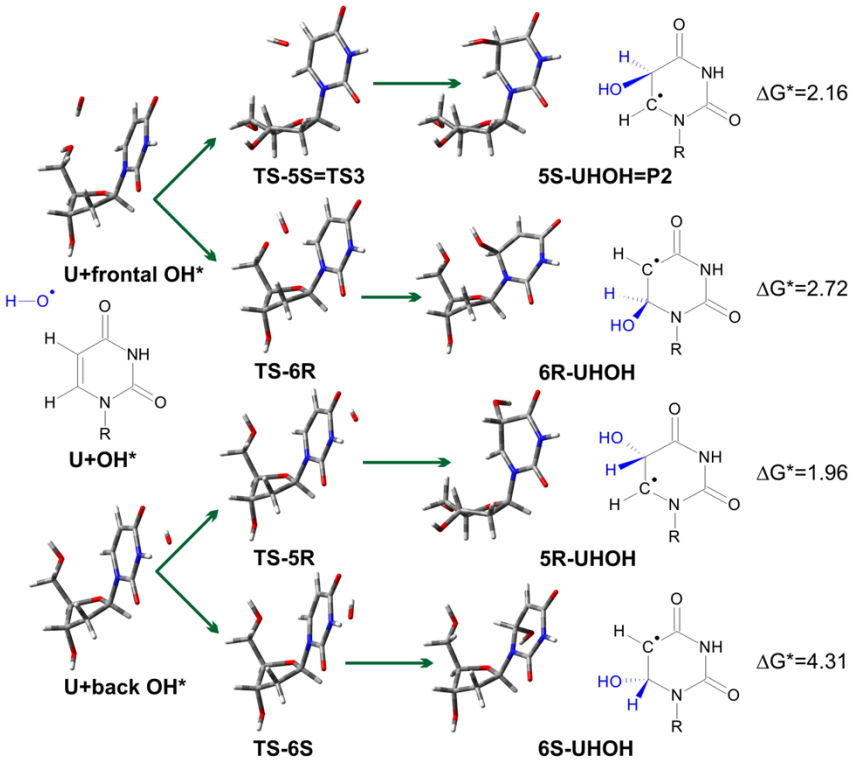
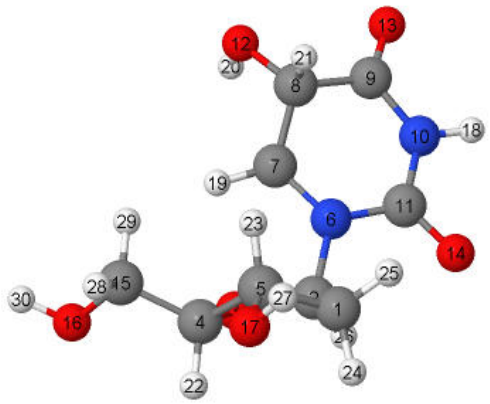


Figure S2. The attack of hydroxyl radical on uridine - the respective activation barriers given in the free enthalpy scale (kcal/mol).

----------C5-OH Adduct S stereoisomers----------

TE = -911.306292954 A.U.

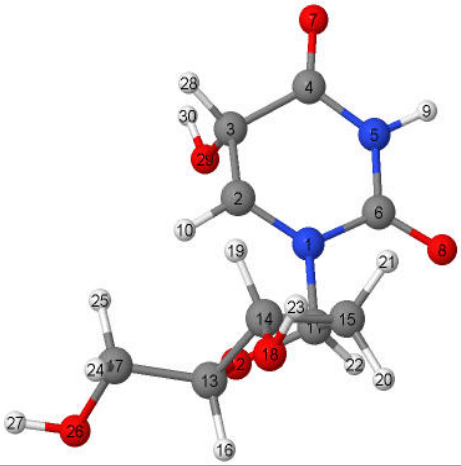


S-form-H21-axial conformer

Jmol

Atom	A_{iso} (G)	A_{aniso} (G)		
		xx	yy	zz
H19	-20.51	-11.29	-0.56	11.85
H21	38.56	-1.89	-0.59	2.48

TE = -911.306963455 A.U.

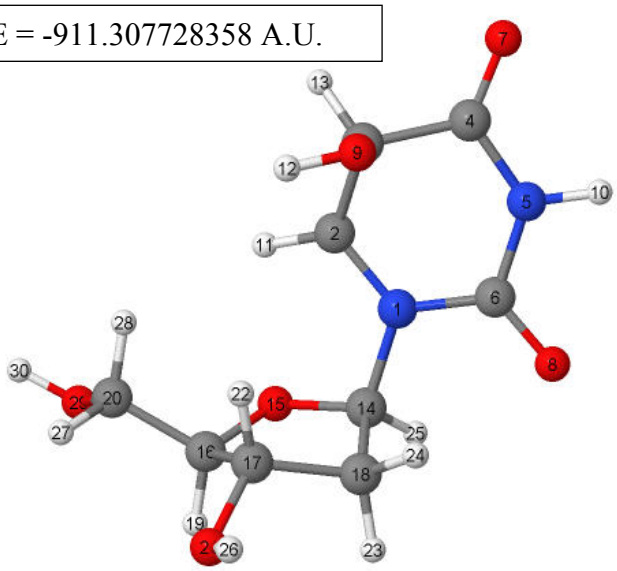


S-form-H28-equatorial conformer

Atom	A_{iso} (G)	A_{aniso} (G)		
		Xx	Yy	zz
H10	-19.37	-10.83	-0.90	11.73
H28	8.57	-2.22	-1.18	3.40

----------C5-OH Adduct R stereoisomers----------

TE = -911.307728358 A.U.

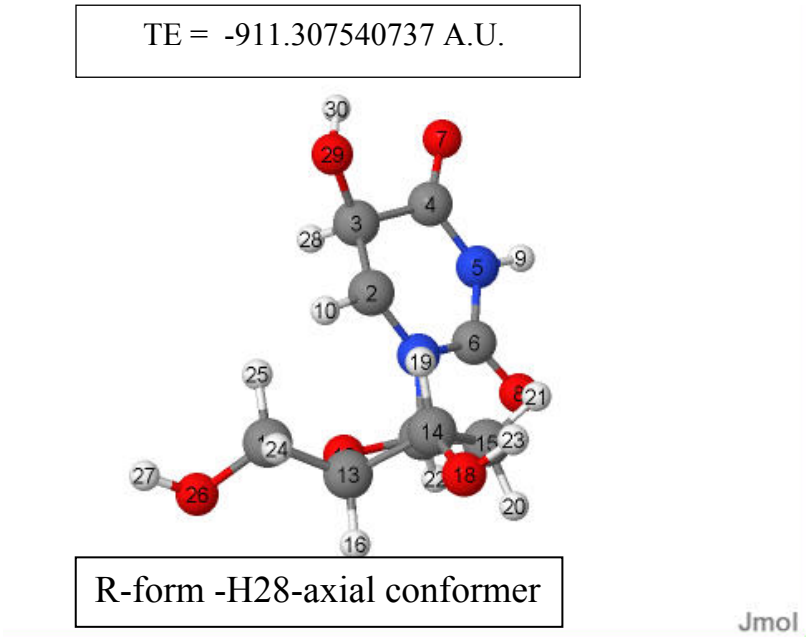


Jmol

R-form-H13-equatorial conformer

Atom	A_{iso} (G)	A_{aniso} (G)		
		Xx	yy	zz
H11	-21.53	-10.95	-0.50	11.45
H13	0.79	-2.05	-1.13	3.19

TE = -911.307540737 A.U.

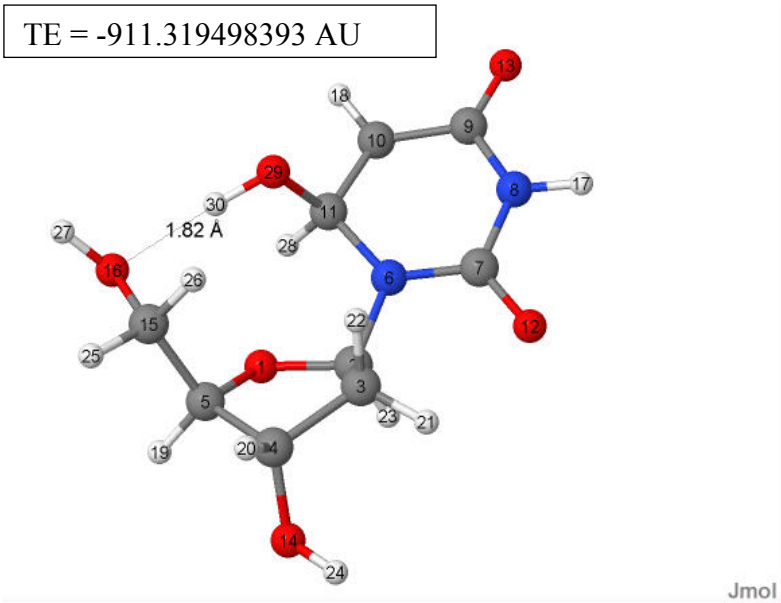


R-form -H28-axial conformer

Atom	A_{iso} (G)	A_{aniso} (G)		
		xx	yy	zz
H10	-15.0	-11.15	-1.06	12.21
H28	35.07	-1.90	-0.63	2.54

---------C6-OH---Adduct R stereoisomer with H-bond----------

TE = -911.319498393 AU

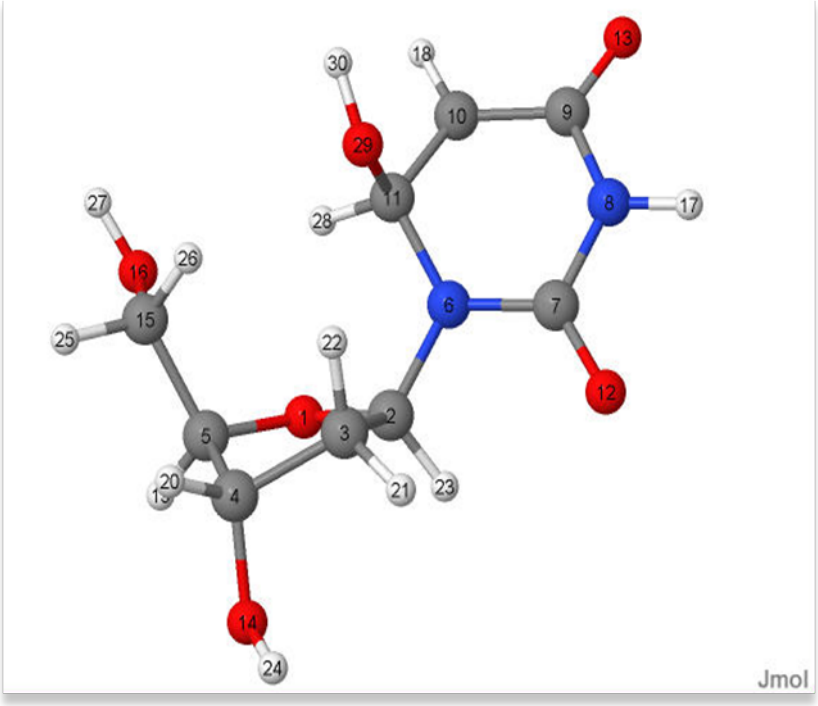


R-form -H28- axial conformer (Mixed)
Internal Hydrogen bonding present

Atom	A_{iso} (G)	A_{aniso} (G)		
		Xx	yy	zz
H18	-23.68	-11.25	-1.47	12.72
H28	18.66	-2.06	-1.17	3.23

----------C6-OH---Adduct R stereoisomer ---without H-Bond--------

TE = -911.314778808 A.U.

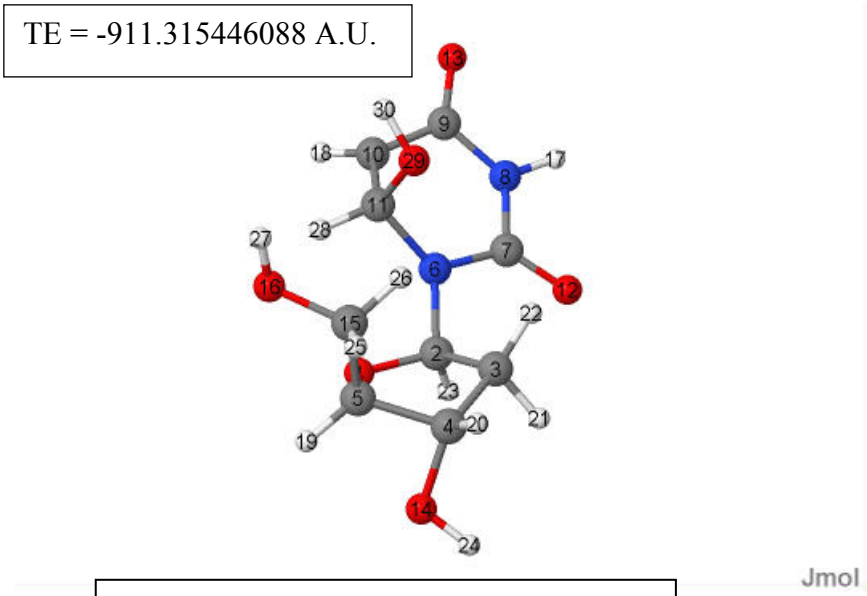


R-form –H28- equatorial conformer
Starting geometry was H28 axial. Thus only the equatorial form is stable when internal H-bonding is prevented by constraining 30-29-11-10 dihedral.

Atom	A_{iso} (G)	A_{aniso} (G)		
		Xx	yy	zz
H18	-23.84	-11.39	-1.39	12.78
H28	4.09	-2.04	-1.25	3.29

----------C6-OH---Adduct R stereoisomer ---without H-Bond--------

TE = -911.315446088 A.U.

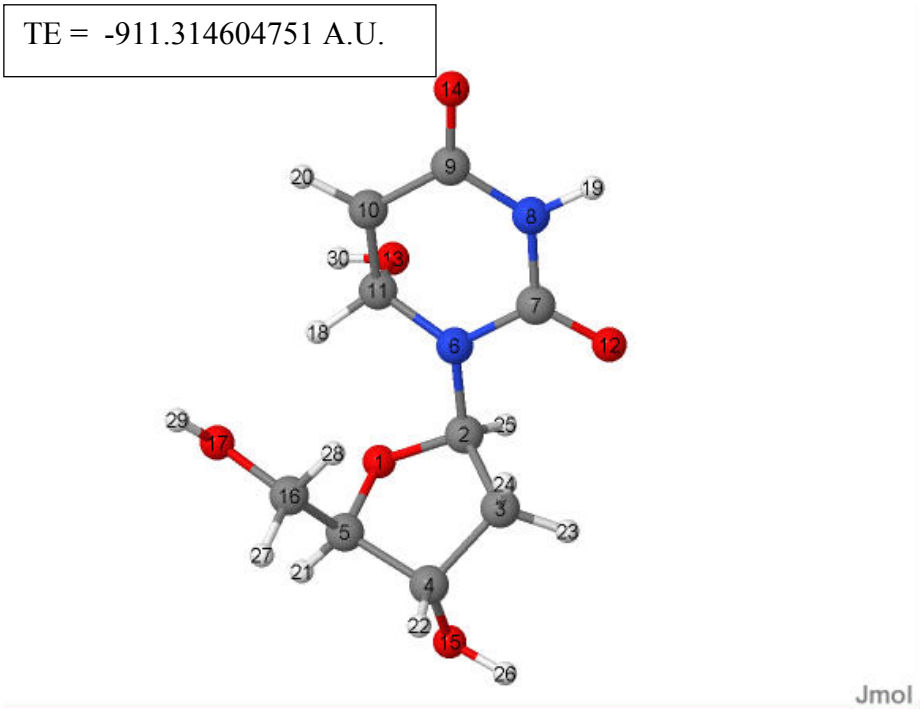


R-form -H28- equatorial conformer

Atom	A_{iso} (G)	A_{aniso} (G)		
		xx	yy	zz
H18	-23.67	-11.35	-1.42	12.77
H28	3.78	-2.05	-1.22	3.26

----------C6-OH Adduct S stereoisomer without H-bond----------

TE = -911.314604751 A.U.

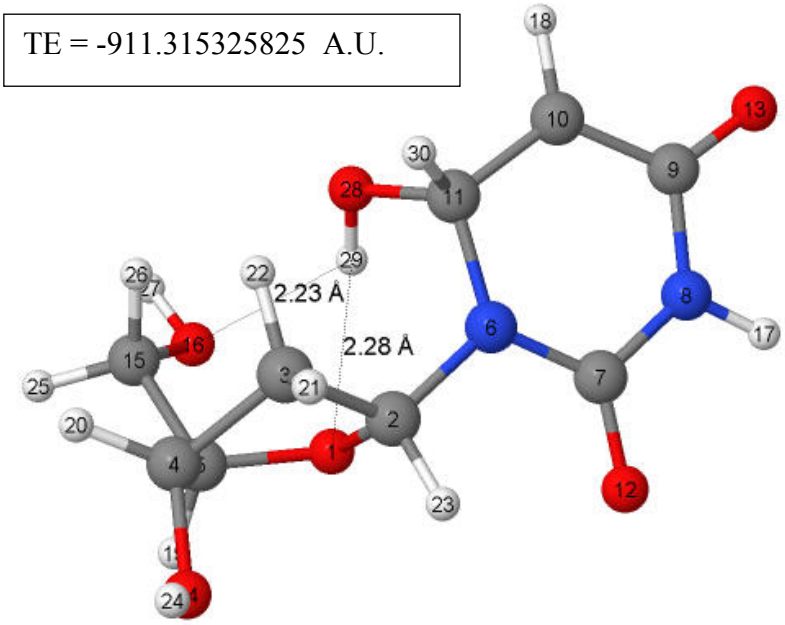


S-form –H18- equatorial conformer

Atom	A_{iso} (G)	A_{aniso} (G)		
		xx	yy	zz
H18	4.37	-2.05	-1.22	3.27
H20	-23.44	-11.29	-1.43	12.73

----------C6-OH Adduct S stereoisomer with H-bond----------

TE = -911.315325825 A.U.

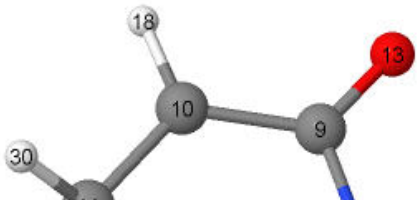


Jmol

S-form –H30- mixed conformer (Starting geometry H30 axial)
Internal Hydrogen bonding present

Atom	A_{iso} (G)	A_{aniso} (G)		
		xx	yy	zz
H18	-23.62	-11.21	-1.47	12.68
H30	16.97	-2.14	-1.13	3.27

----------C6-OH Adduct S stereoisomer without H-bond--------

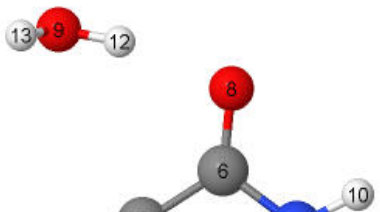


TE = -911.312436649 A.U.

S-form –H30- mixed conformer (starting geometry H30 equatorial)

Atom	A_{iso} (G)	A_{aniso} (G)		
		xx	yy	zz
H18	-23.58	-11.21	-1.43	12.63
H30	18.24	-2.06	-1.10	3.16

Water Complex with Uracil C5 Radical

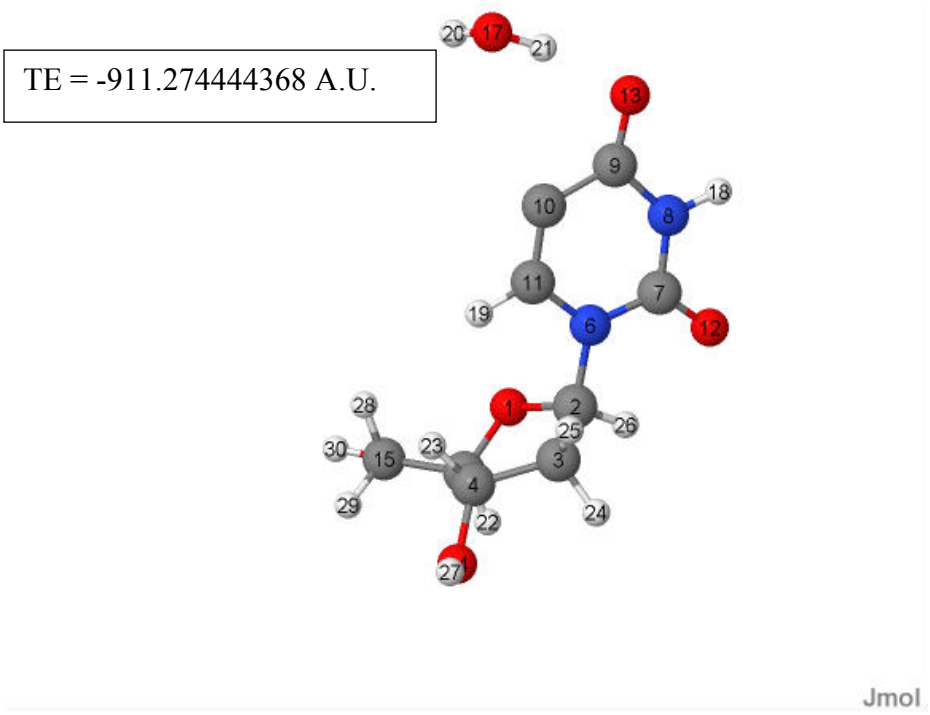


TE = -911.274416224 A.U.

Atom	A_{iso} (G)	A_{aniso} (G)		
		Xx	yy	zz
H10	5.81	-1.11	-0.74	1.86
H11	19.23	-2.28	-1.99	4.27

Water Complex with Uracil C5 Radical (C10 in figure below)

TE = -911.274444368 A.U.



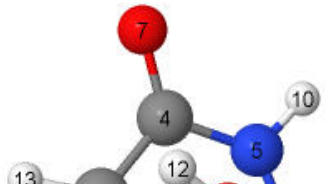
Atom	A_{iso} (G)	A_{aniso} (G)		
		xx	yy	Zz
H18	5.81	-1.11	-0.74	1.85
H19	19.25	-2.28	-2.00	4.28

P1 Species form by Water addition to C5 Radical (C10 in figure below)

TE = -911.295181022 A.U.

Atom	A_{iso} (G)	A_{aniso} (G)		
		xx	yy	Zz
H18	-3.73	-2.68	-1.03	3.70
H19	-15.82	-8.61	-0.01	8.62

OH Radical Complex formed after Hydrogen abstraction from water by the Uracil-C5-yl radical



TE = -911.275857288 A.U.

Atom	A_{iso} (G)	A_{aniso} (G)		
		xx	yy	Zz
H12	-23.53	-23.95	-7.81	31.75
H13	-1.44	-1.62	-0.54	2.16

XYZ coordinates of the structures presented in Figures 2 and S2.

E – electronic energy [Hartree]

G –free enthalpy [Hartree]

Path A: U* E=-911.274444368 G=-911.087902

C	0.000000	0.000000	0.000000
N	0.000000	0.000000	1.382374
C	1.185232	0.000000	2.103602
N	2.336468	0.017742	1.331524
C	2.440618	0.032685	-0.054459
C	1.146033	0.020742	-0.681597
C	-1.277979	-0.053441	2.097300
O	-1.959191	-1.212292	1.646805
C	-3.352026	-0.925598	1.543962
C	-3.407154	0.530932	1.086415
C	-2.205693	1.145748	1.817141
O	-4.656466	1.075881	1.450177
C	-4.000972	-1.891354	0.585496
O	-4.024885	-3.175351	1.186449
O	1.236469	-0.007645	3.321622
O	3.536913	0.053017	-0.605104
O	2.899231	0.115751	-3.373464
H	3.205293	0.022781	1.858609
H	-0.977623	-0.030715	-0.471656
H	2.569348	-0.766947	-3.574380
H	3.235969	0.067331	-2.461333
H	-3.829567	-1.005574	2.532686
H	-3.275722	0.577913	-0.003923
H	-2.555232	1.570975	2.761227
H	-1.701511	1.930907	1.250419
H	-1.019547	-0.140802	3.152813
H	-4.784192	1.916506	0.994283
H	-3.429317	-1.903084	-0.352439
H	-5.017003	-1.530752	0.378047
H	-4.424542	-3.801546	0.571581

TS1 E=-911.233484052 G=-911.043572

C	-1.736474	-1.465823	0.577745
N	-0.712267	-0.793821	-0.041148
C	-0.954233	0.303605	-0.899013
C	-2.219907	0.840106	-0.953979
C	-3.245356	0.356536	-0.093076
N	-3.008096	-0.936081	0.360266
C	0.658492	-1.209944	0.214510
C	1.499260	-1.456695	-1.052448
C	2.486642	-0.286328	-1.088197
C	2.600355	0.080643	0.390690
O	1.306849	-0.163788	0.932244
C	2.986803	1.519524	0.625230
O	3.213182	1.707219	2.012819

O	3.771039	-0.614036	-1.575546
O	-2.403462	2.488037	-0.905773
O	-4.257586	0.993518	0.235574
O	-1.592058	-2.462796	1.278710
H	-3.748143	-1.389770	0.885185
H	-0.146683	0.557475	-1.574710
H	-1.767909	2.886047	-0.278513
H	-3.310152	2.578408	-0.522692
H	3.342052	-0.583425	0.861392
H	2.077413	0.566860	-1.648024
H	2.072639	-2.380048	-0.932741
H	0.882338	-1.546449	-1.948797
H	0.588591	-2.102238	0.836083
H	3.741076	-0.668931	-2.538297
H	2.174627	2.166238	0.265081
H	3.894328	1.730453	0.044363
H	3.485151	2.619729	2.164847

P1 E=-911.295181022 G=-911.104375

C	0.000000	0.000000	0.000000
N	0.000000	0.000000	1.414999
C	1.157217	0.000000	2.164610
N	2.327127	0.014295	1.437126
C	2.384498	-0.062429	0.050763
C	1.217830	-0.058771	-0.668309
C	-1.276454	-0.016426	2.101350
O	-1.932451	-1.244874	1.784708
C	-3.319793	-1.006426	1.584700
C	-3.387401	0.388725	0.965608
C	-2.238602	1.112270	1.675133
O	-4.668332	0.933362	1.207718
C	-3.903410	-2.085253	0.707666
O	-3.940261	-3.297600	1.443622
O	1.178228	0.003896	3.392659
O	3.632765	-0.125554	-0.454577
O	1.333919	-0.067786	-2.039985
H	3.186462	0.005423	1.971121
H	-0.958186	-0.036044	-0.496582
H	0.772483	-0.760228	-2.413874
H	3.563654	-0.182615	-1.420565
H	-3.849998	-0.988677	2.550402
H	-3.205187	0.319142	-0.116388
H	-2.640425	1.608863	2.562366
H	-1.739575	1.857914	1.052487
H	-1.050001	0.021368	3.166280
H	-4.780448	1.731120	0.677021
H	-3.279894	-2.186606	-0.191313
H	-4.912635	-1.773486	0.408325
H	-4.312101	-3.990895	0.886191

Path B: U* E=-911.274416224 G=-911.087633

N	-2.728363	-1.318890	0.430644
C	-1.408163	-1.738955	0.382338
N	-0.499999	-0.763367	-0.002967
C	-0.909267	0.524496	-0.294258
C	-2.198683	0.859712	-0.234376
C	-3.238924	-0.057648	0.147436
O	-1.095315	-2.885076	0.655957
O	-4.441378	0.170721	0.233894
O	-4.666503	2.912714	-0.475696
H	-3.394916	-2.033125	0.710382
H	-0.119560	1.222796	-0.554909
H	-4.710638	1.968548	-0.242142
H	-4.479506	2.936981	-1.420586
C	0.928075	-1.088136	-0.050027
O	1.588005	-0.192563	0.828766
C	2.840101	0.182923	0.259573
C	2.571021	0.243828	-1.243066
C	1.570223	-0.903131	-1.439510
H	3.593829	-0.595532	0.454222
C	3.295935	1.492315	0.851326
O	3.791518	0.081365	-1.931620
H	2.118866	1.213403	-1.495707
H	2.125378	-1.803953	-1.712732
H	0.825204	-0.708683	-2.213510
H	1.006655	-2.114156	0.310091
H	4.150953	1.849960	0.262759
H	2.479835	2.223461	0.773313
O	3.659739	1.273853	2.204196
H	3.940281	2.111021	2.592015
H	3.671712	0.324543	-2.857513

TS2 E=-911.250815280 G=-911.068391

N	-2.878144	-1.190340	0.405423
C	-1.579768	-1.665007	0.343432
N	-0.631791	-0.720736	-0.030917
C	-0.979957	0.587528	-0.291932
C	-2.252806	0.998970	-0.216249
C	-3.325539	0.099186	0.147337
O	-1.311698	-2.827177	0.594899
O	-4.510548	0.396519	0.231369
O	-3.462055	3.049648	-0.578240
H	-3.575777	-1.878894	0.672150
H	-0.157437	1.248392	-0.545850
H	-4.263675	2.539513	-0.343812
H	-2.644330	2.187968	-0.448322
C	0.779924	-1.108858	-0.091489

O	1.479759	-0.282947	0.824005
C	2.754892	0.042179	0.275288
C	2.497071	0.182279	-1.224198
C	1.433860	-0.897119	-1.471768
H	3.460694	-0.787061	0.438207
C	3.283672	1.295387	0.924811
O	3.709823	-0.019293	-1.916280
H	2.101297	1.185598	-1.436354
H	1.937611	-1.814588	-1.786250
H	0.702688	-0.625968	-2.236107
H	0.810348	-2.151384	0.225231
H	4.159319	1.629253	0.353087
H	2.510489	2.074271	0.879011
O	3.630398	0.995968	2.266642
H	3.967656	1.794685	2.688821
H	3.607132	0.266725	-2.831900

U+OH* E= -911.275857288 G=-911.087402

N	0.763822	0.163954	0.710471
C	0.824859	-1.095072	0.185250
C	1.980132	-1.654416	-0.250972
C	3.219124	-0.907574	-0.149050
N	3.066394	0.355810	0.419211
C	1.891981	0.961247	0.804640
O	4.326395	-1.294682	-0.502379
O	1.846295	2.114768	1.207704
O	1.209170	0.096817	-2.180301
H	3.898541	0.932188	0.491947
H	-0.119605	-1.623971	0.155711
H	1.449714	-0.568574	-2.853921
H	2.003807	-2.658873	-0.649880
C	-0.527719	0.833235	1.031265
O	-1.505854	-0.157988	1.220130
C	-2.566866	-0.010993	0.263012
C	-1.948278	0.787080	-0.880856
C	-1.004900	1.720150	-0.121721
H	-3.376813	0.579934	0.714065
C	-3.102535	-1.361269	-0.141162
O	-2.977795	1.434383	-1.596033
H	-1.375558	0.119896	-1.541804
H	-1.583045	2.560331	0.275432
H	-0.176947	2.101412	-0.721440
H	-0.357475	1.381749	1.957986
H	-2.613038	1.806495	-2.408159
H	-3.792055	-1.208531	-0.981900
H	-2.277526	-2.002497	-0.480323
O	-3.767873	-1.931265	0.972624
H	-4.128993	-2.788503	0.718120

TS3 E=-911.273660399 G= -911.083965

N	0.716273	0.337688	0.620820
C	0.822194	-0.986233	0.361196
C	2.004688	-1.562526	-0.027198
C	3.233667	-0.777922	0.020371
N	3.021841	0.567078	0.307404
C	1.822614	1.183491	0.578916
O	4.360663	-1.205306	-0.170243
O	1.724653	2.381620	0.782865
O	1.693404	-0.913213	-2.064153
H	3.835697	1.173607	0.314591
H	-0.095277	-1.554044	0.451332
H	1.233798	-1.718889	-2.357701
H	2.083862	-2.632522	-0.159351
C	-0.593776	1.002365	0.903143
O	-1.534788	0.010273	1.213330
C	-2.599166	-0.001761	0.246258
C	-2.021410	0.702860	-0.977808
C	-1.115307	1.747710	-0.324798
H	-3.438738	0.591041	0.635537
C	-3.060497	-1.414533	-0.008609
O	-3.081705	1.231076	-1.741937
H	-1.429556	-0.006590	-1.575550
H	-1.730277	2.593789	-0.002935
H	-0.312882	2.113939	-0.966396
H	-0.420837	1.647567	1.765032
H	-2.746168	1.510681	-2.602354
H	-3.766249	-1.387581	-0.849462
H	-2.204102	-2.040320	-0.294477
O	-3.679007	-1.902839	1.168771
H	-3.999863	-2.797647	1.006270

P2 E=-911.307728358 G= -911.115049

N	0.693227	0.404777	0.549832
C	0.802795	-0.954681	0.360824
C	1.974374	-1.463270	-0.387246
C	3.233875	-0.707225	0.009287
N	3.013978	0.612390	0.330124
C	1.782366	1.237521	0.514535
O	4.349149	-1.191118	-0.008599
O	1.702765	2.450877	0.666506
O	1.875600	-1.221356	-1.802199
H	3.824852	1.208620	0.460901
H	-0.036672	-1.566123	0.649886
H	1.088812	-1.679291	-2.129801
H	2.132409	-2.525747	-0.187411
C	-0.611841	1.068444	0.839036
O	-1.541703	0.087711	1.219629

C	-2.602828	-0.012493	0.256468
C	-2.062005	0.660055	-1.002582
C	-1.174723	1.752959	-0.405819
H	-3.467240	0.560280	0.621109
C	-3.004621	-1.453029	0.060978
O	-3.148727	1.129891	-1.769965
H	-1.461761	-0.057084	-1.581931
H	-1.808582	2.593473	-0.105594
H	-0.392081	2.115977	-1.073260
H	-0.430730	1.754976	1.666703
H	-2.831435	1.393552	-2.642167
H	-3.715564	-1.491722	-0.775129
H	-2.121720	-2.049859	-0.204694
O	-3.596546	-1.920167	1.261140
H	-3.879368	-2.833604	1.136705

XYZ coordinates for Figure S2 structures. Note that:

U+ frontal OH* = **U+OH*** from Figure 2
TS3-5S = **TS3** from Figure 2
5S-UHOH = **P2** from Figure 2

TS-6R E=-911.2756256 G=-911.083063

C	1.587111	-1.445771	-0.165960
N	0.641656	-0.478117	-0.476334
C	0.944371	0.848112	-0.472266
C	2.249752	1.290893	-0.352993
C	3.309411	0.338946	-0.085230
N	2.875114	-0.982125	-0.013240
C	-0.766035	-0.949710	-0.561210
C	-1.384915	-1.205366	0.820367
C	-2.811421	-0.679563	0.646790
C	-2.595757	0.483188	-0.315572
O	-1.535083	0.045282	-1.181898
C	-2.219559	1.770715	0.410430
O	-1.720258	2.757602	-0.476886
O	-3.647552	-1.617797	-0.011537
O	0.940612	1.231458	1.585489
O	4.491841	0.614861	0.069470
O	1.295370	-2.624356	-0.047480
H	3.571849	-1.688796	0.199509
H	0.132152	1.514213	-0.746497
H	2.494734	2.339628	-0.447114
H	-3.471320	0.639751	-0.953504
H	-3.256406	-0.352412	1.593590
H	-1.355391	-2.260875	1.094868
H	-0.849085	-0.626784	1.579534
H	-0.744490	-1.842355	-1.188137

H	-3.767343	-2.386842	0.559785
H	-3.095069	2.134648	0.962632
H	-1.411591	1.580840	1.124868
H	-2.416471	3.016297	-1.093938
H	1.073849	2.189331	1.698011

6R-UHOH E=-911.3154458 G=-911.119821

O	-1.408123	0.063123	-1.129192
C	-0.680345	-0.921902	-0.424714
C	-1.454731	-1.196635	0.872496
C	-2.874689	-0.762996	0.508379
C	-2.608640	0.412459	-0.427919
N	0.694862	-0.434540	-0.233422
C	1.667614	-1.383145	-0.088336
N	2.970730	-0.910320	0.037867
C	3.387126	0.396283	-0.162040
C	2.310924	1.356552	-0.269156
C	0.921881	0.949365	0.097884
O	1.444595	-2.587103	-0.093325
O	4.579253	0.682373	-0.226942
O	-3.555809	-1.750799	-0.251446
C	-2.442409	1.720286	0.327318
O	-1.993840	2.701675	-0.594040
O	0.749143	1.094591	1.514604
H	3.686102	-1.625453	0.106805
H	0.176614	1.547742	-0.433069
H	2.552024	2.391679	-0.471566
H	-3.398316	0.511461	-1.178418
H	-3.464767	-0.467555	1.383569
H	-1.396238	-2.246660	1.166841
H	-1.057602	-0.581582	1.684222
H	-0.595580	-1.818220	-1.041907
H	-3.685390	-2.533893	0.298045
H	-3.410937	1.996605	0.762754
H	-1.716774	1.594282	1.142902
H	-1.961136	3.556165	-0.148047
H	0.730729	2.036959	1.735994

U+back OH* E=-911.2770415 G=-911.087794

C	-1.383604	-1.406815	-0.164918
N	-0.511016	-0.373532	0.126889
C	-0.908227	0.932832	0.093646
C	-2.167919	1.300952	-0.254830
C	-3.140326	0.288374	-0.618074
N	-2.646675	-1.013714	-0.541554
C	0.857782	-0.787873	0.550352
C	1.674360	-1.365185	-0.618523
C	3.029068	-0.663598	-0.481584

C	2.639361	0.663930	0.157523
O	1.548119	0.330336	1.031888
C	2.231072	1.714315	-0.860778
O	1.736868	2.832892	-0.143495
O	3.883233	-1.326351	0.436708
O	-4.297312	0.487743	-0.968110
O	-1.044581	-2.580741	-0.104480
O	-2.740009	0.495626	2.166272
H	-0.144432	1.655718	0.365836
H	-3.289956	-1.765858	-0.765633
H	-2.459801	2.341526	-0.285759
H	3.437337	1.054047	0.794709
H	3.525530	-0.525723	-1.448754
H	1.761060	-2.450566	-0.554988
H	1.202237	-1.116590	-1.573361
H	0.718915	-1.502402	1.364334
H	4.125615	-2.187575	0.074103
H	3.109258	1.981389	-1.460410
H	1.460747	1.316982	-1.539179
H	1.546830	3.546393	-0.764585
H	-2.569446	1.385759	2.529177

TS-5R E=-911.2756689 G=-911.084665

O	1.566764	0.372143	1.030209
C	0.895692	-0.776401	0.601571
C	1.697702	-1.372536	-0.567618
C	3.040242	-0.640038	-0.479080
C	2.635223	0.699554	0.124310
N	-0.494002	-0.404771	0.194743
C	-1.346614	-1.473155	-0.063386
N	-2.618362	-1.126546	-0.454570
C	-3.137492	0.158250	-0.592837
C	-2.208957	1.206426	-0.188602
C	-0.912221	0.882502	0.118292
O	-0.973562	-2.630597	0.036146
O	-2.867146	0.907979	1.881232
O	-4.272902	0.327691	-1.008788
O	3.923464	-1.256513	0.443339
C	2.184734	1.707773	-0.918765
O	1.668156	2.832393	-0.227166
H	-0.163624	1.636659	0.345264
H	-3.231952	-1.902183	-0.682743
H	-2.507272	2.236908	-0.321210
H	3.435724	1.126462	0.733852
H	3.515200	-0.523743	-1.459658
H	1.807020	-2.453594	-0.473930
H	1.201979	-1.161595	-1.519598
H	0.786559	-1.466567	1.440425
H	4.179502	-2.122584	0.102229

H	3.047632	1.981160	-1.537264
H	1.417602	1.269824	-1.575517
H	1.458215	3.525391	-0.864918
H	-2.608074	1.794051	2.188770

5R-UHOH E=-911.3062930 G=-911.113999

C	-1.347921	-1.812881	0.283788
C	-0.685622	-1.037643	-0.854344
O	-1.531734	0.041380	-1.156120
C	-2.627549	0.105779	-0.229269
C	-2.180538	-0.728957	0.967860
N	0.649494	-0.505280	-0.446444
C	0.856628	0.831788	-0.169746
C	2.048829	1.227082	0.633934
C	3.252756	0.352630	0.270089
N	2.930487	-0.922565	-0.132894
C	1.664668	-1.422357	-0.441020
O	2.339691	2.594150	0.515163
O	4.403918	0.724171	0.370113
O	1.501804	-2.607008	-0.709032
C	-2.955609	1.539433	0.105190
O	-3.456647	2.169626	-1.061407
O	-3.323240	-1.196840	1.649696
H	3.693844	-1.579267	-0.259918
H	0.011508	1.494431	-0.269805
H	2.465163	2.808190	-0.420194
H	1.875881	1.058499	1.711240
H	-3.506139	-0.370971	-0.686732
H	-1.553772	-0.119022	1.635249
H	-2.026940	-2.564109	-0.132316
H	-0.622853	-2.306445	0.931767
H	-0.513555	-1.640697	-1.746923
H	-3.055238	-1.580427	2.493627
H	-3.705614	1.531604	0.907449
H	-2.055872	2.049035	0.475481
H	-3.690480	3.081013	-0.849766

TS-6S E=-911.2729351 G=-911.080924

C	1.651407	-1.430542	-0.534096
C	0.836899	-0.773014	0.594503
O	1.524771	0.376720	0.992904
C	2.556140	0.693000	0.042168
C	2.977546	-0.662142	-0.512736
N	-0.534388	-0.389455	0.135092
C	-0.983152	0.893354	0.210147
C	-2.266828	1.233685	-0.176772
C	-3.216421	0.191113	-0.510181
N	-2.687937	-1.095306	-0.436658

C	-1.397290	-1.447381	-0.109208
O	-1.876493	0.875939	2.098608
O	-1.026545	-2.608757	-0.050200
O	-4.384325	0.367296	-0.832538
C	2.045222	1.640273	-1.029669
O	1.554897	2.797905	-0.373555
O	3.901784	-1.215251	0.410336
H	-0.225106	1.643659	0.405880
H	-3.315018	-1.867990	-0.635619
H	-2.584241	2.266422	-0.213351
H	3.361885	1.168291	0.607136
H	3.424474	-0.578341	-1.509708
H	1.791611	-2.498312	-0.362069
H	1.144477	-1.305749	-1.494759
H	0.698153	-1.422483	1.461777
H	4.163986	-2.092353	0.103921
H	2.871602	1.885780	-1.707014
H	1.248342	1.159843	-1.617796
H	1.300977	3.451698	-1.036189
H	-2.098723	1.801984	2.299374

6S-UHOH E=-911.3171008 G=-911.121786

C	1.535414	-1.466650	-0.605465
C	0.748181	-0.809038	0.539491
O	1.472945	0.337547	0.931865
C	2.532012	0.605425	-0.001003
C	2.897014	-0.767431	-0.552384
N	-0.616195	-0.386476	0.133179
C	-1.017647	0.982393	0.439882
C	-2.382330	1.271395	-0.083664
C	-3.330621	0.222186	-0.379082
N	-2.826911	-1.061292	-0.273109
C	-1.501222	-1.408845	-0.034290
O	-1.164917	-2.588265	-0.017208
O	-4.501618	0.425428	-0.689893
C	2.086467	1.573922	-1.082336
O	1.664134	2.766197	-0.440901
O	3.770313	-1.370219	0.390400
O	-1.038912	1.221044	1.835770
H	-0.294811	1.658169	-0.023844
H	-3.455293	-1.834641	-0.458833
H	-2.718914	2.297947	-0.135893
H	3.352337	1.041637	0.575173
H	3.372128	-0.702988	-1.537793
H	1.620166	-2.545947	-0.471917
H	1.037576	-1.279021	-1.560839
H	0.649786	-1.465978	1.408451
H	3.991941	-2.259409	0.087042
H	2.930441	1.762930	-1.756579

H	1.265552	1.136181	-1.670451
H	1.448726	3.425383	-1.111729
H	-0.117054	1.346121	2.109570

Discussion of DFT results presented above for OH-radical addition to dU

For OH-radical addition to C5, both the R and S stereoisomers are formed in either R or S stereoisomers, each with equatorial OH and axial OH conformers that are found as stationary states of nearly equal energy. The equatorial OH addition to C5 gives couplings for the C6 alpha proton of -20.5 G, -15 G (S isomer, R isomer) and C5 beta proton 38.6 G, 35 G (S isomer, R isomer) which are in good agreement with those found experimentally (-21 and 41 G) for the quartet observed. The axial OH addition to C5 gives couplings of -19.4, 8.6 (S isomer) -21.5 G and 0.8 G (R isomer) which would result in a doublet spectrum. Since all of these isomers are of near equal energy, each of these isomers should contribute to the experimentally observed ESR spectrum.

Further DFT calculations were performed for the less probable OH addition to C6 which again has R and S isomers with possible axial and equatorial forms for the C6-OH adduct radical. The results show the axial OH conformation is found to be most stable placing the C6H proton in the equatorial position for three of the four cases (if internal hydrogen bonding is prevented). This results in a doublet spectrum only from ca. -23 G owing to the C5 alpha proton and a small C5 beta proton in three of the four cases. In one case, two proton couplings near 20 G are predicted which is not in keeping with experiment. A similar result is found (two protons near 20 G) when internal hydrogen bonding is allowed which again could not explain the experimental result. Thus, only the C5-OH-radical adduct is predicted to form the quartet spectrum found by experiment. Some C6-OH-radical adduct would form as well as some C5-OH-radical adduct would also be produced with OH in the axial position and each of these radicals is expected to yield mainly a doublet spectrum that would be superimposed on the quartet. The experimentally obtained ESR spectrum does suggest about 30% doublet and 70% quartet.

Effects of the rotation of the OH group in the P2 radical (OH axial):

This section shows that the rotation of the OH group in the P2 type radical does not influence our conclusions. We have calculated the rotational profile for the 5S species. Three minima were identified; see Figure R1 below.

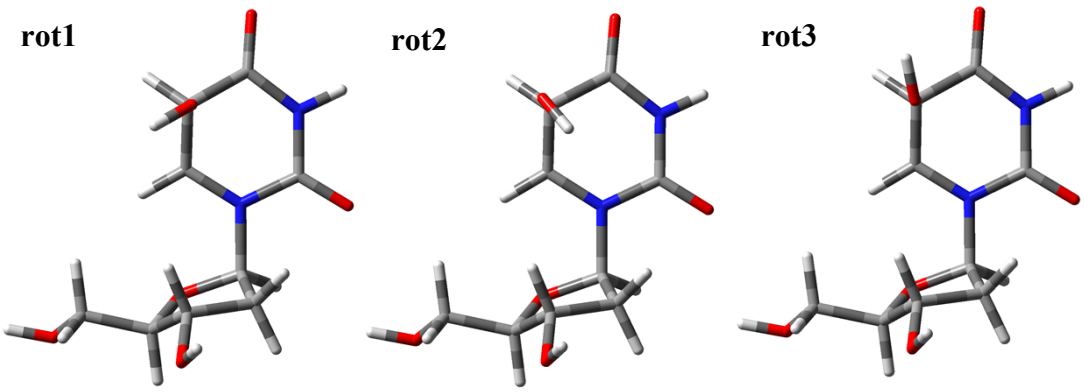


Figure R1. P2 rotamers; **rot1** is the most stable one.

The rotamer rot1 (see Figure R1) shown in the paper (see P2 in Figure 2) is the most stable one and the two other, rot 2 and rot 3, are less stable with respect to rot 1 by 0.04 and 0.43 kcal/mol (in electronic energy scale; see Table R1). Thus, as expected, such small energy differences only marginally influence the averaged reaction energies. Similarly, as indicated by the couplings gathered in Table R1 (see below), the ESR spectral parameters could only slightly be affected by the presence of the less stable rotamers.

Table R1. The relative stabilities (ΔE) and the isotropic and anisotropic couplings calculated at the M06-2x/6-31++G(d,p) level, aqueous solution (PCM model) for the P2 rotamers.

Rotamer	Θ[a] [°]	ΔE [kcal/mol]	Atom	A_{iso} [G]	A_{aniso} [G]		
					xx	yy	zz
rot1	64.0	0.00	H(C5)	-21.53	-10.95	-0.50	11.45
			H(C6)	0.79	-2.05	-1.13	3.19
rot2	165.6	0.43	H(C5)	-21.53	-10.93	-0.61	11.54
			H(C6)	5.14	-2.06	-1.11	3.16
rot3	-45.9	0.04	H(C5)	-21.93	-11.10	-0.61	11.71
			H(C6)	3.26	-2.12	-1.19	-3.30

[a]Θ=H(OH)-O5-C5-H5

Supplementary references

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