Ab initio base-pairing energies of an oxidized thymine product, 5-formyluracil, with standard DNA bases at the BSSE-free DFT and MP2 theory levels†

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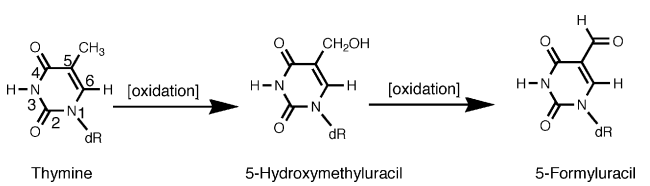
Oxidation of the thymine methyl group produces two stable products, non-mutagenic 5-hydroxymethyluracil and highly mutagenic 5-formyluracil. We have calculated the interaction energy of base-pair formation involving 5-formyluracil bound to the natural DNA bases adenine (A), cytosine (C), guanine (G), and thymine (T), and discuss the effects of the 5-formyl group with respect to similar base-pairs containing uracil, 5-hydroxyuracil, thymine (5-methyluracil), and 5-hydroxycytosine. The interaction geometries and energies were calculated four ways: (a) using density functional theory (DFT) without basis set super-position error (BSSE) corrections, (b) using DFT with BSSE correction of geometries and energies, (c) using Møller–Plesset second order perturbation theory (MP2) without BSSE correction, and (d) using MP2 with BSSE geometry and energy correction. All calculations used the 6-311G(d,p) basis set. Notably, we find that the A:5-formyluracil base-pair is more stable than the precursor A:T base-pair. The relative order of base-pair stabilities is A:5-Fo-U > G:5-Fo-U > C:5-Fo-U > T:5-Fo-U.

Introduction

DNA undergoes constant damage due to various environmental factors, such as ionizing radiations and reactive oxygen species that cause numerous covalent, irreversible modifications to the bases or the deoxyribose sugars in DNA.[1,2] Some of these modifications miscode or block DNA replication and lead to a variety of diseases including cancer and diseases of aging.[3,4] During oxidation, pyrimidine bases are frequently modified at the 5th position. Oxidation of the thymine methyl group yields 5-hydroxymethyluracil (5-Hm-U) and 5-formyl uracil (5-Fo-U)[5] (Scheme 1). Several lines of evidence show that 5-Hm-U doesn't miscode or block DNA replication, and exhibits normal base-pairing properties.[6,7] 5-Hm-U pairs only with adenine during DNA replication.[8] Therefore, 5-Hm-U is an innocuous lesion. In contrast 5-Fo-U residues miscode at very high frequency.[9,10] The high mutagenic potential of 5-Fo-U is attributed to the strong electron-withdrawing formyl group that would reduce the electron density in the pyrimidine ring and would render the glycosidic bond weaker. Besides, the highly reactive –CHO group can interact with DNA-binding proteins and potentially interfere with their functions. Bacterial and mammalian cells contain multiple repair enzymes to excise 5-Fo-U from their respective DNA.[11]



Scheme 1 Products of thymine oxidation.

Oxidation of thymine to 5-Fo-U had been shown to promote the misincorporation of guanine and decreases the incorporation of adenine during DNA replication.[12] Furthermore, the primer terminus containing the 5-Fo-U:G pair was more readily extended than that containing a T:G pair. During DNA synthesis using DNA polymerase I Kf, 5-Fo-dUTP could be substituted for dCTP, and the substitution efficiency increased with increasing pH. The pH-dependent ability of 5-Fo-U in the template to incorporate dGTP, and the ability to substitute dCTP in DNA synthesis, suggested the presence of an ionized form of 5-Fo-U. The pK_a of the 5-Fo-UTP is determined to be 8.6.[13] So, when pH > pK_a, the ionized form of 5-Fo-U will be the predominant form, and it would form a Watson–Crick type base-pair with G, while the keto form (when pH < pK_a) would form a wobble base-pair. However, under physiological conditions, the keto form of 5-Fo-U would be the predominant form.

5-Fo-U can form base-pairs with all four DNA bases. However, whether the formation of all such base-pairs occurs *in vivo* is not known. Melting temperatures, obtained from UV-monitored thermal melting curves of 5-Fo-U containing DNA duplexes, were in the order of: A > G > T > C.[14] The DNA repair enzyme, XPC-HR23B recognizes and excises 5-Fo-U lesions from DNA. This enzyme is shown to bind very strongly to 5-Fo-U:C, moderately to 5-Fo-U:G and 5-Fo-U:T pairs, and very weakly to the 5-Fo-U:A pairs, in DNA duplexes.[15] The excision activity was also found to follow the same order, suggesting that the efficiency of the DNA repair enzyme depends on the instability of the 5-Fo-U base-pair.

Quantum mechanical research into DNA base-pair and base stacking interaction energies have been the subject of several excellent reviews,[16–18] and previous work described the relative energies of 5-Fo-U in the *cis- vs. trans*-configurations.[19,20] Recently,[21,22] we described the base-pairing interactions of uracil and

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5-hydroxy-uracil (5-OH-U) with the standard DNA bases, and here we describe the base-pairing interactions of 5-Fo-U and compare these with our previous results for U and 5-OH-U base-pairs.

Results and discussion

Among the standard DNA bases, 5-Fo-U forms base-pairs with A, G and C with similar energies of −13.5, −13.3 and −13.0 kcal mol^{-1}, respectively, determined at the BSSE-free MP2 level of theory (Table 1). Although the 5-Fo-U base-pairs formed with A and G are within 0.2 kcal mol^{-1} of each other at three levels of theory, the energies differ by 1.3 kcal mol^{-1} at the BSSE-contaminated MP2 level of theory. It must be noted that the base-pairs formed with cytosine are twisted nearly 40 degrees, and thus they would be much less stable in the context of a DNA duplex than these calculations would suggest. Both conformations of the T:5-Fo-U base-pairs are significantly weaker, with interaction energies of −10.3 kcal mol^{-1} (conformation 1) and −10.1 kcal mol^{-1} (conformation 2).

When adenine base-pairs with uracil or a derivative, the adenine forms a hydrogen bond with the uracil O4 oxygen (Fig. 1). Comparing the base-pairs formed by adenine with either uracil, 5-Fo-U or 5-OH-U (Fig. 1), the calculations show that the 5-hydroxy substitution and the 5-formyl substitution stabilize the base-pair, by 0.4 and 0.6 kcal mol^{-1}, respectively, *versus* the isoenergetic U- or T-containing base-pairs (Table 1). Thus, the A:5-Fo-U base-pair is more stable than the parental A:T ("A:5-methyl-uracil") base-pair from which it was produced. This has clear implications regarding its stability and therefore its ability to lead to mutations upon replication. However, it has been suggested that the glycosidic bond of 5-Fo-U is significantly less stable (148 day half-life under physiological conditions[19]) than that of uracil, due to the presence of the strong electron-withdrawing formyl group at the 5th position. Therefore, depurination may remove this damaged base from the DNA over several months. Although the substitution site is two bonds distant from the glycosidic bond, the present calculations indicate a consistently larger glycosidic bond (0.0016 to 0.0045 Å) in free or base-paired 5-Fo-U (see ESI†) compared to uracil and 5-OH-U, and this is consistent with the suggestion that the 5-Fo-U glycosidic bond is weaker and more labile than the same bond in U or 5-OH-U.

Table 1 Hydrogen bond strengths[a] (kcal mol^{-1}) of 5-formyluracil, 5-hydroxyuracil and uracil bases paired with standard DNA bases and comparison to standard Watson–Crick (WC) GC and AT base-pairs

Base-pair	ΔE^{DFT}	$\Delta E^{DFT,BSSE}$	ΔE^{MP2}	$\Delta E^{MP2,BSSE}$
G : C WC[b]	−28.3	−27.8	−28.0	−24.9
G : 5-OH-C	−28.2	−27.8	−27.8	−24.7
G : 5-OH-U[b]	−16.9	−15.8	−18.0	−14.9
G : U[b]	−15.4	−14.1	−16.5	−13.4
G : 5-Fo-U	−15.3	−14.1	−16.3	−13.3
A : 5-Fo-U	−15.4	−13.9	−17.6	−13.5
A : 5-OH-U[b]	−15.2	−13.4	−17.5	−13.3
A : U[b]	−15.2	−13.2	−17.2	−12.9
A : T WC[b]	−15.1	−13.1	−17.2	−12.9
C : 5-Fo-U	−14.6	−13.4	−16.2	−13.1
C : 5-OH-U[b]	−13.4	−12.1	−15.4	−12.2
C : U[b]	−13.7	−12.4	−15.3	−12.0
T : 5-OH-U(2)[b]	−13.2	−11.5	−14.4	−10.9
T : 5-Fo-U(2)	−12.2	−10.6	−13.6	−10.1
T : U(2)[b]	−12.1	−10.2	−13.3	−9.8
T : 5-Fo-U(1)	−12.4	−10.9	−13.7	−10.3
T : 5-OH-U(1)[b]	−12.5	−10.6	−13.4	−10.2
T : U(1)[b]	−12.3	−10.5	−13.4	−9.9

[a] All geometries and energies were optimized at the theory level indicated using the 6-311G(d,p) basis set. DFT was performed with B3LYP. BSSE geometry optimizations used the counterpoise method. The BSSE-free optimized interaction energies include deformation energies, and they are therefore equal to the complexation energies of the dimers. For the BSSE-contaminated calculations (ΔE^{DFT} and ΔE^{EMP2}), neither the geometry nor the energy have been corrected for BSSE. For the BSSE-free calculations ($\Delta E^{DFT,BSSE}$ and $\Delta E^{EMP2,BSSE}$), both the geometries and the energies have been corrected for BSSE. Thermal and zero point energy corrections were not made. [b] Values reproduced from ref. 22—reproduced by permission of the Royal Society of Chemistry.

Comparing the base-pairs formed by guanine with either uracil or 5-Fo-U (Fig. 1), it is interesting to note that the electron withdrawing effects of the formyl group have a very modest effect on the base-pair energy (Table 1). The energies of these two base-pairs differ by only 0.2 kcal mol^{-1} at the BSSE-contaminated MP2 level. In contrast, an electron donating 5-hydroxy substituent stabilizes this base-pair by 1.6 kcal mol^{-1}. We previously[21,22] rationalized this stabilization as arising from the formation of an internal hydrogen bond between the uracil 5-OH proton and the O4 oxygen for base-pairs in which the O4 oxygen is not involved in a base-pairing hydrogen bond. Such a bond would pull electron density towards the O4 oxygen and away from the C4 carbon, and therefore also from the neighboring N3 and H3 atoms, making them more electrophilic. The O4 oxygen is only 2.11 Å from the hydroxyl proton in 5-OH-U, but it is 2.59 Å from the aldehydic proton in 5-Fo-U and the aldehyde proton, not being attached to a heteroatom, cannot form hydrogen bonds. For comparison, we calculated the energy of the G:5-OH-C base-pair, and found that this base-pair is 0.2 kcal mol^{-1} less stable than the Watson–Crick GC base-pair. Because cytosine contains an amino group at the C4 position rather than a carbonyl group, the hydroxyl proton of 5-OH-C is rotated nearly 70 degrees out of the base-pair plane to avoid steric interaction with the cytosine amino protons. Because of this rotation, the hydroxyl proton of 5-OH-C cannot form an internal hydrogen bond and thus no stabilization is observed.

In both of the T:5-Fo-U base-pairs, the interaction energy is slightly stabilized compared to the corresponding T:U base-pair. In the T:5-Fo-U(1) conformation, the interaction energy (−10.3 kcal mol^{-1}), is 0.4 kcal mol^{-1} more stable than the T:U(1) base-pair. In the T:5-Fo-U(2) base-pair, the interaction energy (−10.1 kcal mol^{-1}) is 0.3 kcal mol^{-1} more stable than the T:U(2) base-pair. For both uracil and 5-formyl uracil pairing with T, conformation 1 is preferred. In contrast, conformation 2 is preferred by 0.7 kcal mol^{-1} when the uracil has a 5-hydroxy substitution. The conformation 2 base-pairing pattern, using the O2 oxygen and the H3 proton of the uracil, is similar to that of pairing with G, and so the same internal hydrogen bonding interactions suggested for the stability enhancement of the G:5-OH-U base-pair pertains to the T:5-OH-U(2) base-pair as well. This effect is not present for the base-pairs involving thymine in the other conformation, and the energies of the T:U, T:5-OH-U and T:5-Fo-U base-pairs, −9.9, −10.2 and −10.3 kcal mol^{-1}, respectively, reflect this.

The base-pair formed between cytosine and 5-Fo-U is significantly more stable than those formed between cytosine and uracil or 5-OH-U at all levels of theory used. At the BSSE-free MP2

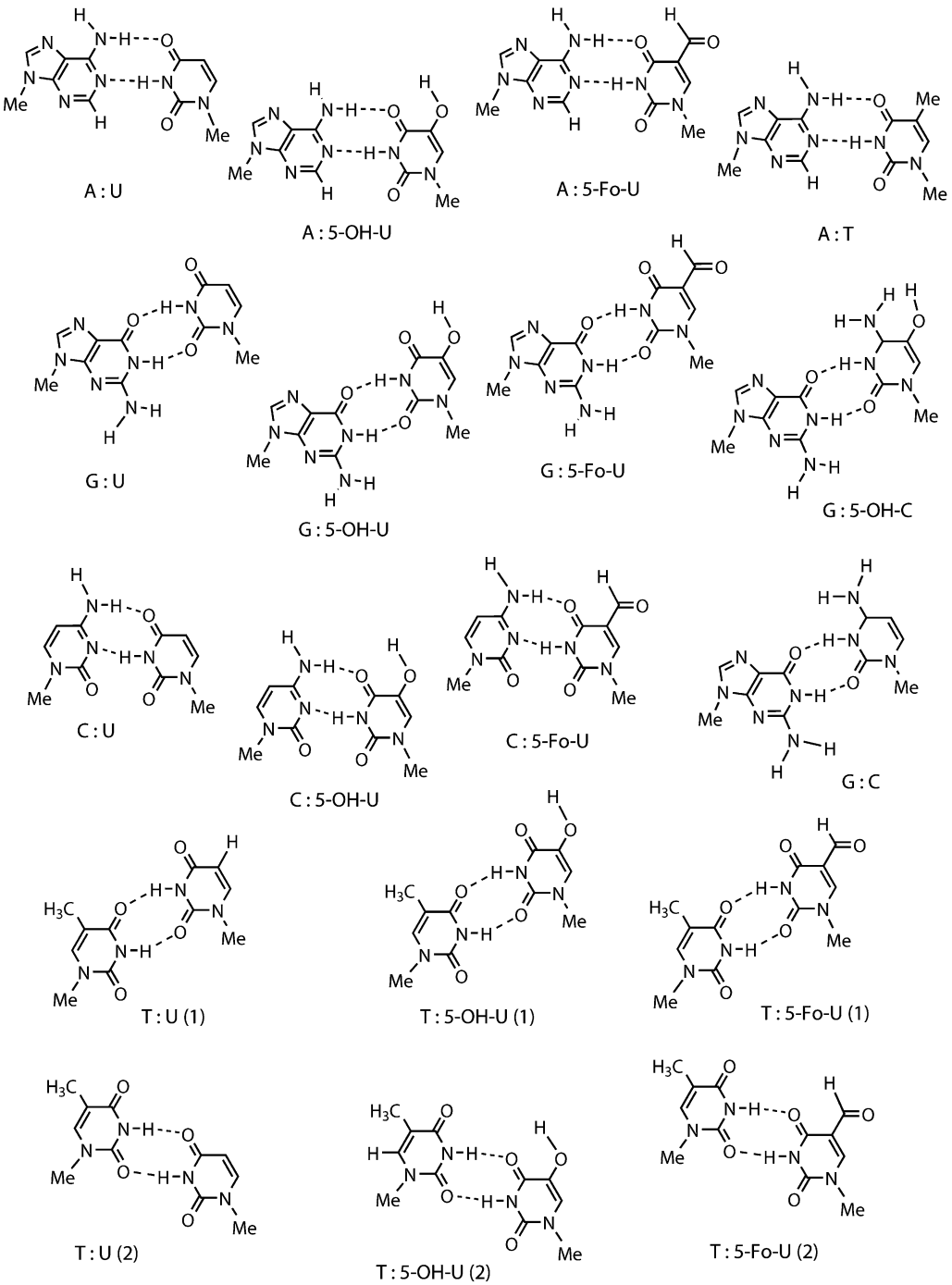

Fig. 1 Base-pairs incorporating uracil (U), 5-formyl uracil (5-Fo-U), and 5-hydroxy uracil (5-OH-U).

theory level, the energy of the C:5-Fo-U base-pair (−13.0) is 1.0 and 0.8 kcal mol^{-1} more stable than those of either the C:U (−12.0) or the C:5-OH-U (−12.2) base-pairs.

Results presented in Table 1 show that the 5-Fo-U:C base-pair is more stable than the 5-Fo-U:T pair, at all levels of theory. However, melting temperatures[14] and relative excision activities by DNA repair enzymes[15] suggest otherwise. Our *ab initio* calculations were done on isolated base-pairs while the experiments were done on DNA duplexes. Our results indicate that the 5-Fo-U:C base-pair will have significantly higher propeller twist (*ca.* 40°) than the other base-pairs, and hence will cause significant disruption of base stacking within the duplex DNA. Therefore, it is reasonable to expect that the presence of the 5-Fo-U:C base-pair would destabilize the DNA duplex more than the presence of the 5-Fo-U:T pair would do.

Effect of BSSE corrections

Among the DFT calculations without BSSE geometry corrections, the over-estimations in base-pair stability are nucleotide

Table 2 Hydrogen bond distances of structures shown in Fig. 1 calculated at different levels of theory

	DFT		DFT (BSSE-free)		MP2		MP2 (BSSE-free)	
Base-pair	r_1/Å	r_2/Å	r_1/Å	r_2/Å	r_1/Å	r_2/Å	r_1/Å	r_2/Å
G : C WC[a,b]	*1.77	1.91	*1.79	1.94	*1.78	1.89	*1.86	1.98
G : 5-OH-U[b]	*1.79	*1.80	*1.81	*1.83	*1.77	*1.79	*1.86	*1.88
G : 5-Fo-U	*1.79	*1.84	*1.82	*1.87	*1.81	*1.82	*1.89	*1.91
G : 5-OH-C[a]	*1.77	*1.90	*1.79	1.94	*1.79	1.89	*1.87	1.97
G : U[b]	*1.81	*1.83	*1.84	*1.86	*1.79	*1.84	*1.87	*1.94
A : 5-Fo-U	1.80	*1.94	1.84	*1.96	1.78	*1.97	1.87	*2.03
A : 5-OH-U[b]	1.80	*1.96	1.84	*1.98	1.77	*1.98	1.87	*2.04
A : U[b]	1.82	*1.93	1.87	*1.94	1.80	*1.95	1.90	*2.01
A : T WC[b]	1.82	*1.93	1.87	*1.94	1.80	*1.95	1.90	*2.01
C : 5-Fo-U	*1.87	1.91	*1.89	1.96	1.85	*1.90	1.97	*1.98
C : 5-OH-U[b]	*1.88	1.94	*1.91	1.99	1.85	*1.90	1.99	*1.99
C : U[b]	*1.84	1.95	*1.87	2.01	*1.87	1.88	*1.95	2.01
T : 5-Fo-U(2)	*1.83	*1.90	*1.87	*1.94	*1.83	*1.89	*1.92	*1.99
T : 5-OH-U(2)[b]	*1.82	*1.85	*1.86	*1.89	*1.82	*1.84	*1.91	*1.94
T : U(2)[b]	*1.86	*1.87	*1.90	*1.90	*1.86	*1.86	*1.95	*1.95
T : 5-Fo-U(1)	*1.82	*1.90	*1.85	*1.93	*1.81	*1.88	*1.91	*1.99
T : 5-OH-U(1)[b]	*1.84	*1.89	*1.88	*1.92	*1.83	*1.88	*1.92	*1.98
T : U(1)[b]	*1.85	*1.87	*1.89	*1.90	*1.84	*1.86	*1.94	*1.96

* Indicates a hydrogen bond involving a carbonyl group.[a] $r3 = 1.92$, 1.94, 1.93 and 2.01* at the DFT, DFT (BSSE-free), MP2 and MP2 (BSSE-free) levels, respectively. [b] Values taken from ref. 22—reproduced by permission of the Royal Society of Chemistry.

dependent. For the base-pairs involving C:5-X-U (X = H, OH, CHO) the BSSE ranges from 1.2 to 1.3 kcal mol^{-1}, while the overestimations for the analogous A, G and T base-pairs range from 1.5 to 2.0, 1.1 to 1.3, and 1.5 to 1.9 kcal mol^{-1}, respectively. Thus, the BSSE error is generally smaller for the G and C bases when bound to uracil or a 5-substituted uracil, and the BSSE error is about 50% larger for the nucleotides A or T when bound to uracil or a 5-substituted uracil. However, the smaller BSSE error is observed for the G:5-OH-C and GC Watson–Crick base-pairs, which have BSSE energy errors of only 0.4 and 0.5 kcal mol^{-1}, respectively.

At the DFT theory level, geometry corrections for BSSE had a larger effect on the hydrogen bond lengths that utilize a nitrogen atom as the proton acceptor than do hydrogen bonds in which a carbonyl group serves as the proton acceptor (Table 2). In the base-pairs with either A or C pairing with a uracil derivative, the hydrogen bond between the uracil H3 proton and the A or C nitrogen atom electron donor is generally lengthened by 0.04–0.05 Å, while the remaining hydrogen bond is only lengthened by 0.01 to 0.03 Å.

Among the BSSE-contaminated MP2 calculations, the BSSE energy errors are considerably larger than those observed for the DFT calculations. The largest energy contaminations were observed in the A:5-X-U (X = H, OH, CHO) base-pairs, where the errors ranged from 4.1 to 4.3 kcal mol^{-1}. For the analogous base-pairs involving 5-X-U paired with C, G or T, the errors ranged from 3.1 to 3.3, 3.0 to 3.1 and 3.2 to 3.5 kcal mol^{-1}, respectively. The G:C and G:5-OH-C base-pairs had BSSE energy contaminations of 3.1 kcal mol^{-1}, similar to those in the G:5-X-U base-pairs. This contrasts considerably with the DFT calculations in which the G:C and G:5-OH-C base-pairs had relatively low BSSE energy contaminations.

Geometry changes from the BSSE corrections at the MP2 theory level provided larger increases (~0.06 to 0.14 Å) to the hydrogen bond lengths (Table 2). In general, the hydrogen bond length increases are roughly about twice as large in the MP2 calculations as they are at the DFT level of theory.

Experimental

All calculations were performed with GAUSSIAN 98[23] using the 6-311G(d,p) basis set and methods previously described.[22] Geometry and energy corrections for BSSE used the Counterpoise Correction method of Boys and Bernardi[24] implemented in GAUSSIAN 98 as outlined by Simon, Duran and Dannenberg.[25] Two orientations are possible for the formyl group of 5-Fo-U. The formyl oxygen can be either *cis* or *trans* to the keto group at the 4th position (Fig. 1). The *trans* rotamer was previously shown to be more stable than the *cis* isomer,[19,20] and our results agree, indicating that the *trans* rotamer is favored by −5.5 and −4.4 kcal mol^{-1} at the DFT and MP2 theory levels, respectively, compared to the *cis* rotamer. Therefore, all base-pair calculations reported here were carried out using the *trans* isomer of 5-Fo-U.

A medium quality basis set was used due to the extensive time requirement of obtaining BSSE-free geometries. Šponer and Hobza estimated[26] that the dispersion error due to the use of a moderate-sized basis set, rather than a complete basis set, is approximately −2.0 to −3.0 kcal mol^{-1}, and their more recent results[27] bear this out. The method used here, however, provides for more accurate geometries and the BSSE corrections are more significant (up to −4.3 kcal mol^{-1}) than the error due to an incomplete basis set.

Conclusions

The thymine oxidation product 5-Fo-U can form stable base-pairs with the standard DNA bases with decreasing stability in the order A:5-Fo-U > G:5-Fo-U > C:5-Fo-U > T:5-Fo-U. However, in the C:5-Fo-U base-pair, the bases are twisted about 40 degrees relative to one another, and so in the context of a DNA duplex,

one could expect this pair base to be much less stable than these calculations on isolated bases would suggest. The A:5-Fo-U base-pair is 0.6 kcal mol^{-1} more stable than the precursor A:T base-pair from which it might arise. Thus, a 5-Fo-U-deoxyribose-triphosphate preferentially binds opposite an A and thus does not lead to mutations upon later replication at that site.

Acknowledgements

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References

1 L. H. Breimer, *Mol. Carcinog.*, 1990, **3**, 188–197.
2 E. C. Friedberg, G. C. Walker and W. Siede, *DNA Repair and Mutagenesis*, American Society for Microbiology, Washington, DC, 1995.
3 B. N. Ames, L. S. Gold and W. C. Willet, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**, 5258–5265.
4 J. H. J. Hoeijmakers, *Nature*, 2001, **411**, 366–374.
5 E. Mullaart, P. H. M. Lohman, F. Brendes and J. Vijg, *Mutat. Res.*, 1990, **237**, 189–210.
6 S. Bjelland, L. Eide, R. W. Time, R. Stote, I. Eftedal, G. Volden and E. Seeberg, *Biochemistry*, 1995, **34**, 14758–14764.
7 L. J. Mi, E. Mahl, W. Chaung and R. J. Boorstein, *Mutat. Res.*, 1997, **374**, 287–295.
8 D. D. Levy and G. W. Teebor, *Nucleic Acids Res.*, 1991, **19**, 3337–3343.
9 A. Klungland, R. Paulsen, V. Rolseth, Y. Yamada, Y. Ueno, P. Wiik, A. Matsuda, E. Seeberg and S. Bjelland, *Toxicol. Lett.*, 2001, **119**, 71–78.
10 S. Bjelland, H. Anensen, I. Knaevelsrud and E. Seeberg, *Mutat. Res.*, 2001, **486**, 147–154.
11 Q. M. Zhang, I. Miyabe, Y. Matsumoto, K. Kino, H. Sugiyama and S. Yonei, *J. Biol. Chem.*, 2000, **275**, 35471–35477.
12 A. Masaoka, H. Terato, M. Kobayashi, Y. Ohyama and H. Ide, *J. Biol. Chem.*, 2001, **276**, 16501–16510.
13 M. Yoshida, K. Makino, H. Morita, H. Terato, Y. Ohyama and H. Ide, *Nucleic Acids Res.*, 1997, **25**, 1570–1577.
14 H. Sugiyama, S. Matsuda, K. Kino, Q. M. Zhang, S. Yonei and I. Saito, *Tetrahedron Lett.*, 1996, **37**, 9067–9070.
15 K. Kino, Y. Shimizu, K. Sugasawa, H. Sugiyama and F. Hanaoka, *Biochemistry*, 2004, **43**, 2682–2687.
16 P. Hobza and J. Šponer, *Chem. Rev.*, 1999, **99**, 3247–3276.
17 E. T. Kool, J. C. Morales and K. M. Guckian, *Angew. Chem., Int. Ed.*, 2000, **39**, 990–1009.
18 J. Šponer, J. Leszczynski and P. Hobza, *Biopolymers*, 2001/2002, **61**, 3–31.
19 D. K. Rogstad, J. Heo, N. Vaidehi, W. A. Goddard, A. Burdzy and L. C. Sowers, *Biochemistry*, 2004, **43**, 5688–5697.
20 C. J. LaFrancois, Y. H. Jang, T. Cagin, W. A. Goddard and L. C. Sowers, *Chem. Res. Toxicol.*, 2000, **13**, 462–470.
21 V. Thiviyanathan, A. Somasunderam, D. E. Volk and D. G. Gorenstein, *Chem. Commun.*, 2005, (3), 400–402.
22 D. E. Volk, V. Thiviyanathan, A. Somasunderam and D. G. Gorenstein, *Org. Biomol. Chem.*, 2006, **4**, 1741–1745.
23 M. J. Frisch, G. W. Trucks, H. B. Schegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, P. Salvador, J. J. Dannenberg, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Lui, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. G. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gomalez, M. Head-Gordon, E. S. Replogle and J. A. Pople, *GAUSSIAN 98 (Revision A.11)*, Gaussian, Inc., Pittsburgh, PA, 2001.
24 S. F. Boys and F. Bernardi, *Mol. Phys.*, 1970, **19**, 553–566.
25 S. Simon, M. Duran and J. J. Dannenberg, *J. Chem. Phys.*, 1996, **105**, 11024–11031.
26 J. Šponer and P. Hobza, *J. Phys. Chem.*, 2000, **104**, 4592–4597.
27 J. Šponer, P. Jurečka and P. Hobza, *J. Am. Chem. Soc.*, 2004, **126**, 10142–10151.

