Modeling the Action of an Antitumor Drug: A Density Functional Theory Study of the Mechanism of Tirapazamine

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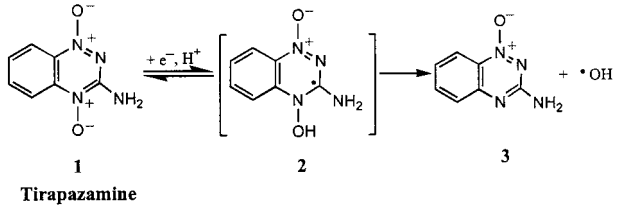
Abstract: Density functional theory methods are employed to investigate experimentally proposed mechanisms by which the antitumor drug tirapazamine may react with a DNA sugar-C_1' radical to give the sugar derivative deoxyribonolactone, with concomitant DNA strand breakage. For the previously proposed minor pathway, ionization of the sugar-C_1' radical by tirapazamine, the calculated ionization energy, and the electron affinity of the models of the sugar-C_1' radical of DNA and tirapazamine suggest that tirapazamine must be protonated to be able to oxidize the sugar-C_1' radical. The preferred mechanism for reaction of tirapazamine with a sugar-C_1' radical, in agreement with experimental observations, is found to proceed by direct attack of an *N*-oxide oxygen of tirapazamine at the sugar-C_1' position, followed by homolytic cleavage of the N−O bond of the drug moiety. Possible alternative mechanisms are also investigated.

Introduction

A unique feature of many cancerous tumors is the existence of hypoxic regions, that is, regions of oxygen-poor cells.[1−3] Such cells are often quite resistant to more conventional forms of antitumor treatment such as radiotherapy and chemotherapy.[4] Consequently, there has been considerable effort to identify potential antitumor drugs that specifically target such cells. One such class of potential hypoxia-specific drugs is the benzotriazine di-*N*-oxides of which a particularly promising candidate is 3-amino-1,2,4-benzotriazine-1,4-dioxide,[5−7] or tirapazamine (shown in Scheme 1).

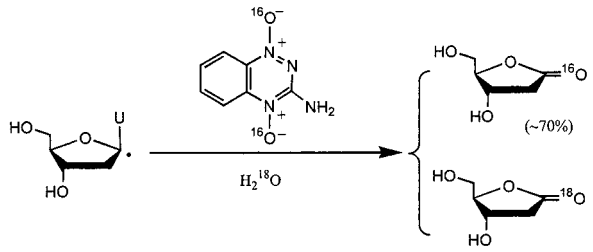
Tirapazamine has been the subject of extensive experimental investigations,[8−11] and has been shown to derive its biological activity from its ability to cause DNA cleavage in hypoxic tumor cells. It is known that in vivo, under hypoxic conditions, tirapazamine may undergo an enzymatic one-electron reduction to form the activated intermediate **2**, see Scheme 1. It has been proposed that **2** may abstract hydrogen directly from DNA.[7] However, it has been found that **2** decomposes to **3** releasing a hydroxyl radical, and it has been suggested[11] that both **2** and •OH are involved in DNA cleavage. In either case, a sugar-C_1' radical is formed which then reacts further to give the corresponding deoxyribonolactone (Scheme 2) and the DNA strand is cleaved. However, radical-mediated DNA cleavage depends on molecular oxygen.[12] Thus, the involvement of **2** and •OH does not explain the remarkable selectivity of tirapazamine toward hypoxic tumor cells.

Scheme 1. Schematic Illustration of the Activation of Tirapazamine by One-Electron Enzymatic Reduction



1 Tirapazamine **2** **3**

Scheme 2. Schematic Illustration of the Product Distribution for the Reaction of Tirapazamine with a Sugar-C_1' DNA Radical



Experimentally, it has been suggested[8,10] that for DNA exposed to γ-radiolysis, the presence of tirapazamine increases the amount of strand cleavage. Hence, it has been proposed that tirapazamine may act as a molecular oxygen surrogate or mimic in radical-mediated DNA damage reactions. Hwang et al.[10] have performed a systematic experimental study of the reaction of tirapazamine with sugar-C_1' DNA radicals. From oxygen isotope labeled product analysis of the final deoxyribonolactone it was found that the reaction proceeds predominantly by direct transfer of an *N*-oxide oxygen, in particular the O_4 oxygen, of the tirapazamine moiety to the sugar-C_1' radical. Only a minor

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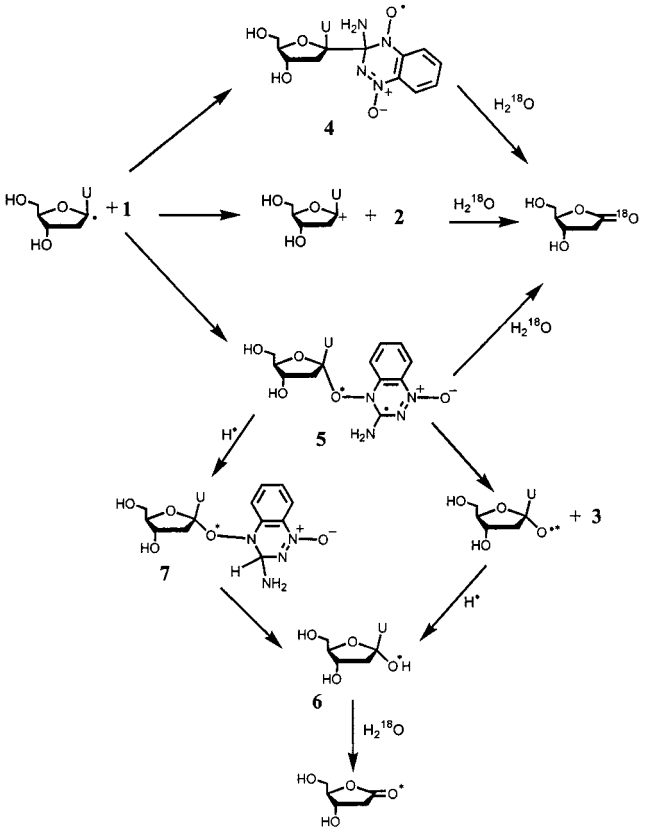
Scheme 3. Schematic Illustration of the Experimentally Proposed Mechanisms for the Reaction of the Sugar-C_1' Radical with Tirapazamine



amount was derived from direct attack of H_2O at the sugar-C_1' position (Scheme 2).

For the observed predominant direct oxygen transfer, Hwang et al.[10] proposed the formation of the sugar-drug covalent adduct intermediate **5**, which may then react further by two possible mechanisms (see Scheme 3). In one mechanism, the cross-linked N−O bond of intermediate **5** cleaves to produce an alkoxyl radical. Subsequent reduction of the alkoxyl radical forms the sugar-derived species **6** which can then hydrolyze to produce the corresponding deoxyribonolactone which leads to cleavage of the DNA strand. In the alternative mechanism, an initial reduction of **5** occurs to give the neutral intermediate **7**. The cross-linked N−O bond then breaks, and hydrogen transfer to the oxygen of the now cleaved N−O bond gives the sugar-derived species **6** which, as described above, can then react further to give deoxyribonolactone and DNA strand cleavage. However, experiments do not differentiate between the two proposed reaction mechanisms.

For the minor pathway, Hwang et al.[10] suggested that it may indicate an electron-transfer mechanism by which the sugar-C_1' radical activates tirapazamine, i.e., via formation of a sugar-C_1' cation intermediate. However, the exact mechanisms by which such an electron transfer may occur were unclear. It has been suggested[8] that the minor pathway may proceed via the carbon−carbon covalent adduct intermediate **4**, or may possibly occur via the carbon−oxygen covalent adduct intermediate **5** (see Scheme 3).[10]

The reliability and computational efficiency of DFT methods offers an attractive theoretical approach to gain insight into large biological systems. Numerous biological radicals derived from the DNA bases,[13a−d] sugar moiety,[13e,14] and amino acids[15] have been extensively studied using density functional theory. In the present paper, density functional theory methods have been used to investigate the possible mechanisms by which tirapazamine may act as a surrogate for molecular oxygen in radical-mediated DNA cleavage. At the levels of theory employed in the present study, the reactions of 2′-deoxyuridin-1′-yl radical with tirapazamine were modeled by **8** and **9**, respectively (Figure 1).

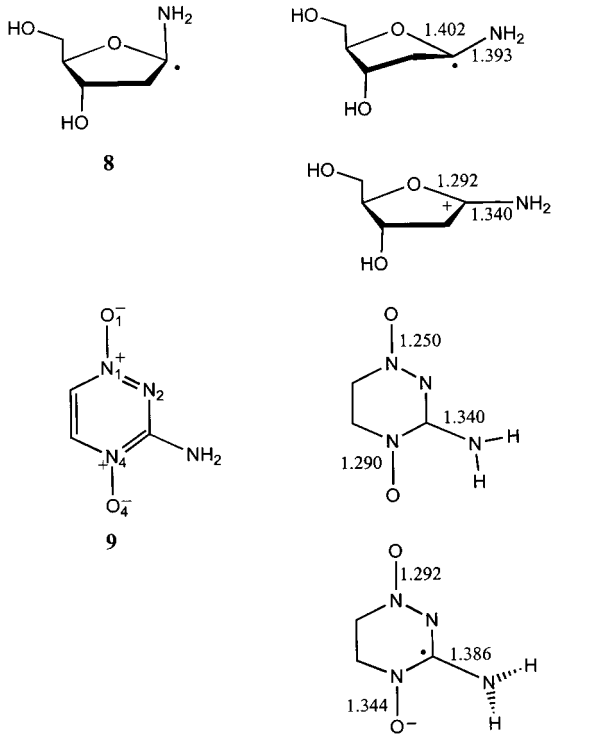


Figure 1. The model systems **8** and **9** used in place of 2′-deoxyuridin-1′-yl radical and tirapazamine, respectively. Selected optimized parameters of the neutral and cationic forms of **8** and the neutral and anionic forms of **9** are also shown.

Computational Methods

All geometry optimizations were performed with the B3LYP hybrid density functional in conjunction with the 6-31G(d) basis set using the GAUSSIAN 98 suite of programs.[16] The B3LYP functional is a combination of Becke's three-parameter hybrid exchange functional,[17] as implemented in GAUSSIAN 98,[18] and the Lee−Yang−Parr cor-

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relation functional.[19] Harmonic vibrational frequencies and zero-point vibrational energy (ZPVE) corrections were calculated at the same level of theory. Relative energies were obtained by performing single-point calculations at the B3LYP level in conjunction with the 6-311G(d,p) basis set using the above optimized geometries and with inclusion of the appropriate scaled[20] ZPVE, i.e., B3LYP/6-311G(d,p)//B3LYP/6-31G(d) + ZPVE.

For all open- and closed-shell systems, the unrestricted (UB3LYP) and restricted (RB3LYP) B3LYP procedures were used, respectively. The symbols U and R have been neglected for simplicity. All energies are in kJ mol^{-1}, unless otherwise specified. The optimized structures of all species encountered in this present study are given in Table S1 of the Supporting Information.

Results and Discussion

Oxidation of the Sugar-C_1' Radical by Tirapazamine. Hwang et al.[10] suggested that the sugar-C_1' radical may be oxidized by tirapazamine to form the sugar-C_1' cation. The feasibility of electron transfer from the sugar-C_1' radical to tirapazamine was probed by comparing the calculated ionization energy of **8** and the calculated electron affinity of **9**.

The optimized structures of the sugar-C_1' neutral radical **8** and its cation, and of the neutral drug **9** and its anion, are shown in Figure 1. The sugar-C_1' radical center of **8** is pyramidal by approximately 36°, while the sugar-C_1' center of the corresponding cation is planar. The shortened C−N and C−O bonds in the cation indicate the presence of strong π-electron delocalization across the O−C−N moiety. The optimized structure of **9** is a conjugated system with an almost planar structure. In the corresponding anion, however, O_4 protrudes out of the plane of the six-membered 1,2,4-triazine ring by approximately 4° while the amino group is pyramidal. In both neutral **9** and the anion, the two N−O bonds differ with the N_1-O_1 bond having relatively more double bond character than the N_4-O_4 bond.

The calculated ionization potential for the sugar-C_1' radical **8** is 459.9 kJ mol^{-1}, while the calculated electron affinity of **9** is just 60.7 kJ mol^{-1}. Hence, due to the very large difference between the ionization potential of **8** and electron affinity of **9** we conclude that it is unlikely that the sugar-C_1' radical can be oxidized by tirapazamine alone. The effect of protonation on the ability of tirapazamine to oxidize the sugar radical was investigated by comparing the electron affinity of protonated **9** with the ionization potential of **8**. We note that the calculated proton affinity (PA) of the O_4 site of **9** is quite large, approximately 896.4 kJ mol^{-1}, suggesting that tirapazamine may be easily protonated. The calculated electron affinity of protonated **9** is 588.4 kJ mol^{-1}. Comparison with the calculated ionization potential (459.9 kJ mol^{-1}) for the sugar-C_1' radical **8** suggests that protonated tirapazamine is more likely to be able to oxidize the sugar-C_1' radical to produce the corresponding cation.

We note, however, that the present calculations are performed without inclusion of solvent effects; they are gas-phase calculations. An experimental study[21] of the pK_a values of tirapazamine-related *N*-oxide species suggested that, in fact, tirapazamine may not be protonated under physiological conditions.

Formation of Deoxyribonolactone via a C−C Covalent Adduct Intermediate. Possible reaction mechanisms by which the deoxyribonolactone moiety may be formed via some covalent adduct intermediate were also examined. The triazine ring of tirapazamine possesses several possible sites at which the sugar-C_1' radical may attack and form a covalently cross-linked product. We began by examining the mechanism for formation of a sugar-C_1'−triazine-C_3 cross-linked intermediate.

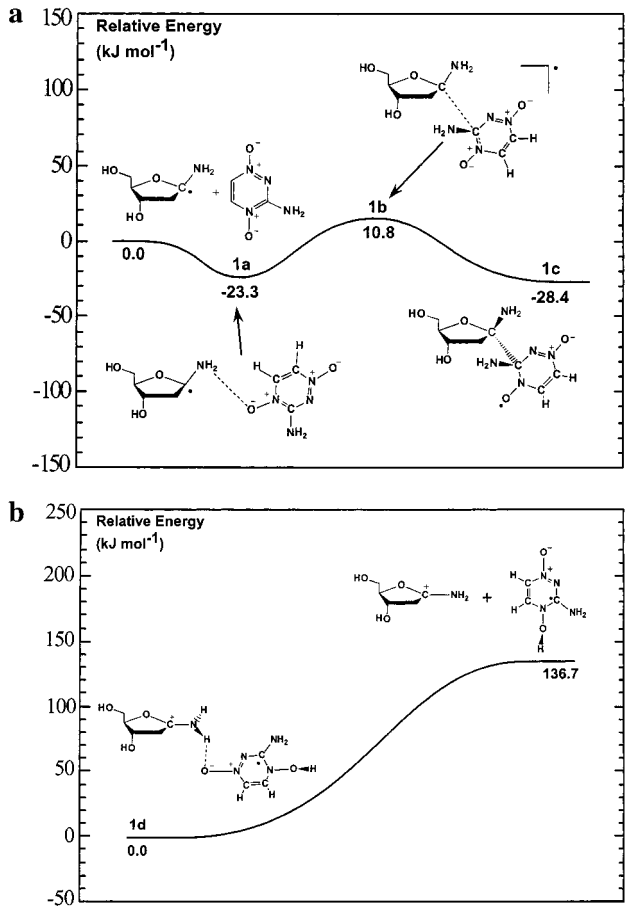

Figure 2. Schematic energy profile for the formation of (a) the carbon−carbon cross-linked product and (b) the sugar-C_1 cation via decomposition of the complex **1c**.

The addition of the drug (**9**) to the sugar-C_1' radical (**8**) leads to the formation of the hydrogen-bonded complex **1a**, lying 23.3 kJ mol^{-1} lower in energy than the initial reactants (Figure 2a). The C−C cross-linked product **1c** can then be formed by attack of the sugar-C_1' radical at the C_3 position of the 1,2,4-triazine ring via transition structure (TS) **1b**, at a cost of 34.1 kJ mol^{-1}. It should be noted that TS **1b** lies higher in energy than the initial reactants by 10.8 kJ mol^{-1}. The product **1c**, which is effectively a model of species **4** in Scheme 3, lies 28.4 kJ mol^{-1} lower in energy than the initial reactants, and the radical center is now localized on the O_4 *N*-oxide oxygen.

All attempts to locate possible transition structures for cleavage of the C−C bond of **1c** by hydrolysis, i.e., by attack of H_2O at the sugar-C_1' or triazine-C_3 positions or by addition across the $C_1'-C_3$ bond, were unsuccessful. An alternative possible mechanism is that the C−C cross-link bond may break heterolytically to give the corresponding sugar cation and 3-amino-1,2,4-triazine radical anion. However, as described above, the triazine moiety has a relatively low electron affinity while that of its protonated derivative is considerably higher. Thus, we decided to examine the effects of protonation on the C−C covalent adduct intermediate **1c**, more specifically protonation at the O_4 site.

Upon protonation, **1c** was calculated to dissociate without a barrier to form the complex **1d**, which is illustrated schemati-

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cally in Figure 2b. Complex **1d** is an intermolecularly hydrogen-bonded complex between the sugar-C_1' cation and what can be thought of as the protonated derivative of the 3-amino-1,2,4-triazine anion. In other words, protonation of the C−C covalent adduct **1c** at O_4 induces an intramolecular redox reaction, with an electron transfer from the sugar to the drug moiety and cleavage of the sugar-C_1'−drug-C_3 bond.

Energetically, the dissociation of **1c** to give **1d** upon addition of a proton at the O_4 position is favored by approximately 1133.1 kJ mol^{-1} (not shown), indicating a large proton affinity for **1c**. We note that at our present level of theory, the proton affinity of H_2O is calculated to be approximately 700 kJ mol^{-1}. Thus, the considerably larger proton affinity of **1c** suggests that once formed it may be easily protonated with little or no barrier to give **1d**. As mentioned above, the present calculations neglect the effects of solvation. However, from an experimental study[21] of the pK_a values of tirapazamine-related radical species, the radicals were more likely to be protonated under physiological conditions than their closed-shell parents. Furthermore, they suggested that the observed values may be due to protonation of the *N*-oxide oxygen rather than at a nitrogen. The dissociation of complex **1d** to the sugar-C_1' cation and drug derivative (see Figure 2b) is calculated to require approximately 136.7 kJ mol^{-1}. The inclusion of solvent effects would stabilize to a greater extent the dissociated products than **1d**, thus giving a lower dissociation energy. The above results suggest that the decomposition of **1c** may be an alternative *indirect* oxidation reaction pathway for the generation of the sugar-C_1' cation.

The resulting fate of the sugar-C_1 cation is still a matter of much discussion.[22,23] Hence, possible reactions by which the sugar-C_1 cation may react directly with H_2O to give the resulting deoxyribonolactone were also investigated.

Formation of Deoxyribonolactone by Reaction of H_2O with the Sugar-C_1' Cation. For our model system, the addition of a single water molecule to the sugar-C_1' cation results in the formation of complex **1e**, lying lower in energy by 47.1 kJ mol^{-1} (Figure 3a). The hydrolyzed product **1g** is formed by the addition of the water across the C−N bond via TS **1f**, at a cost of approximately 178.6 kJ mol^{-1}. We note that **1g** lies 19.8 kJ mol^{-1} higher in energy than the initial reactants. The high barrier for the hydrolysis of the sugar-C_1' cation by a single water is probably due in part to the need to form a tight four-membered ring in TS **1f**. Thus, we also considered the reaction of the sugar-C_1' cation with two H_2O molecules, as one may act as a base to enhance the nucleophilicity of the other, or may assist in stabilizing transition structures or intermediates.

Upon addition of two H_2O to the sugar-C_1' cation, they are able to form complex **1h** (Figure 3b), which lies lower in energy than the initial reactants by approximately 127.3 kJ mol^{-1}. In complex **1h**, the two water molecules have formed a hydrogen-bond bridge between the amine group at C_1' and with the −OH group at C_3'. Addition of a water across the C−N bond assisted by another water is then able to proceed via TS **1i**, with a barrier of 115.6 kJ mol^{-1}. The final complex formed, **1j**, lies approximately 50.2 kJ mol^{-1} lower in energy than the initial reactants. In TS **1i** it can be seen that one H_2O moiety acts as a proton donor to the amine N, while concomitantly acting as a proton acceptor from the H_2O moiety that is attacking the C_1' center (see Figure 3b). It should also be noted that TS **1i** lies 11.7 kJ mol^{-1} lower in energy than the initial reactants. Thus,

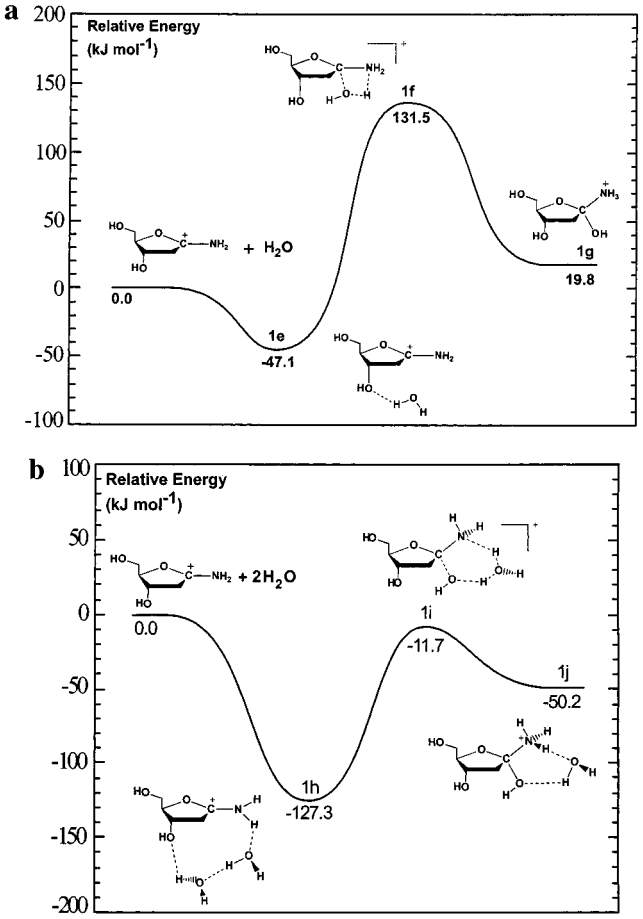

Figure 3. Schematic energy profiles for the reaction of the sugar-C_1 cation with (a) one water molecule and (b) two water molecules.

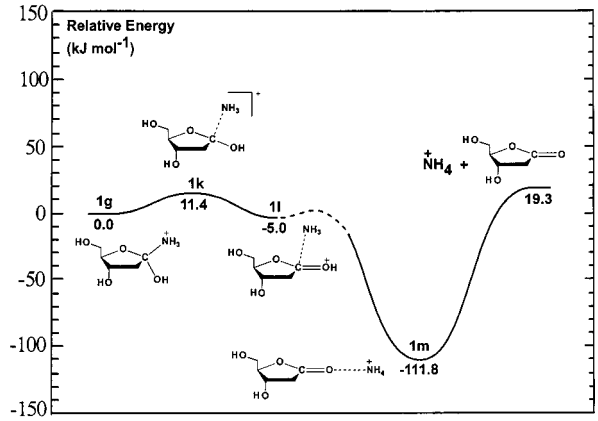

Figure 4. Schematic energy profile for formation of deoxyribonolactone from the protonated sugar-C_1 hydroxylated derivative.

it is possible for complex **1j** to be formed directly from the initial reactants via TS **1i**, without passing through **1h**. Comparison of the above energetics of the mechanisms for hydrolysis by one and two H_2O molecules also provides a very clear example of the effect of explicitly including greater solvent effects for some processes. To simplify the calculations performed to determine the fate of complex **1j**, the complexed H_2O moiety in **1j** was neglected, i.e., only reactions of **1g** were considered (see Figure 4).

The C_1'−NH_3 bond of **1g** may be cleaved via TS **1k** with a barrier of just 11.4 kJ mol^{-1} to give the complex **1l**, lower in energy than **1g** by 5 kJ mol^{-1}. The leaving NH_3 group may then abstract a proton from the C_1'−OH group to give the

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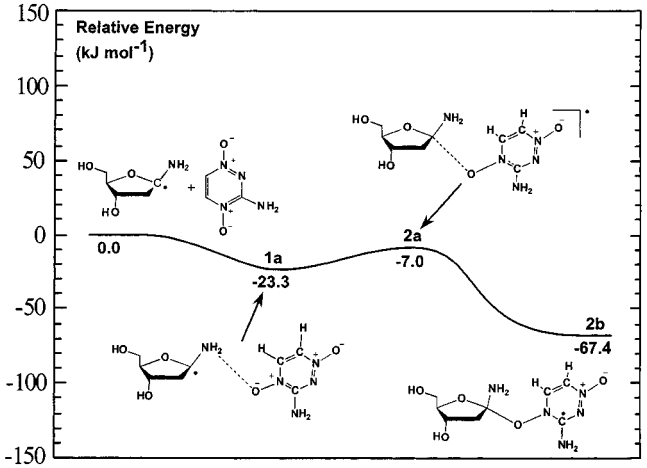

Figure 5. Schematic energy profile for the formation of the carbon−oxygen cross-linked intermediate.

deoxyribonolactone···NH_4^+ complex **1m**, lying 111.8 kJ mol^{-1} lower in energy than **1g**. At the present level of theory, no transition structure interconnecting **1l** and **1m** could be located (as indicated by the hashed line in Figure 4). However, we note that in **1j** the NH_3 moiety is only weakly complexed to the sugar-C_1' center ($r(C_1'\cdots NH_3) = 2.702$ Å) and is almost perpendicular to the $-OH^+$ group ($\angle N-C_1'-OH = 90.8°$). Thus, it is most likely that any transition structure interconnecting **1l** and **1m** will have quite small barriers (Figure 4). This may also explain the difficulty in locating the transition structure.

The present calculations show the reaction of our model system cation with one and two water moieties. In DNA itself, the reactions may differ due to the presence of more solvent and the nature of the nucleobase. Such calculations are not practical at the present level of theory. However, the end result of the reaction of the cation with water will be the same: formation of the deoxyribonolactone.

Formation of Deoxyribonolactone via a C−O Covalent Adduct Intermediate. The predominant mechanism for formation of the deoxyribonolactone moiety was proposed to occur via direct attack, and transfer, of an *N*-oxide oxygen of tirapazamine, see Introduction (Scheme 3). In particular, it was proposed to occur by formation of a bond between the sugar-C_1' radical and tirapazamine via O_4, **5**, which could then react further to eventually give deoxyribonolactone. However, no such covalent adduct intermediate was experimentally observed and furthermore, the exact mechanisms by which it may eventually form deoxyribonolactone were unclear. We began by examining the mechanism for formation of the proposed $C_1'-O_4$ covalent adduct intermediate.

As mentioned previously, when the sugar-C_1' radical and drug interact they can form the intermolecularly hydrogen-bonded complex **1a** lying 23.3 kJ mol^{-1} lower in energy (Figure 5). The O_4 *N*-oxide oxygen can then attack the sugar-C_1' radical site via TS **2a**, at a cost of just 16.3 kJ mol^{-1}, to give the $C_1'-O_4$ covalent adduct intermediate **2b** which lies considerably lower in energy by approximately 67.4 kJ mol^{-1}. Importantly, TS **2a** lies 17.8 kJ mol^{-1} lower in energy than TS **1b**, the transition structure for formation of the C−C covalent adduct intermediate **1c** (cf. Figure 2a). Furthermore, TS **2a** lies 7.0 kJ mol^{-1} *lower* in energy than the initial reactants. Thus, it is possible that intermediate **2b** may form directly from the initial reactants.

Possible reactions of the C−O covalent adduct intermediate **2b** were then examined. In particular, we examined formation

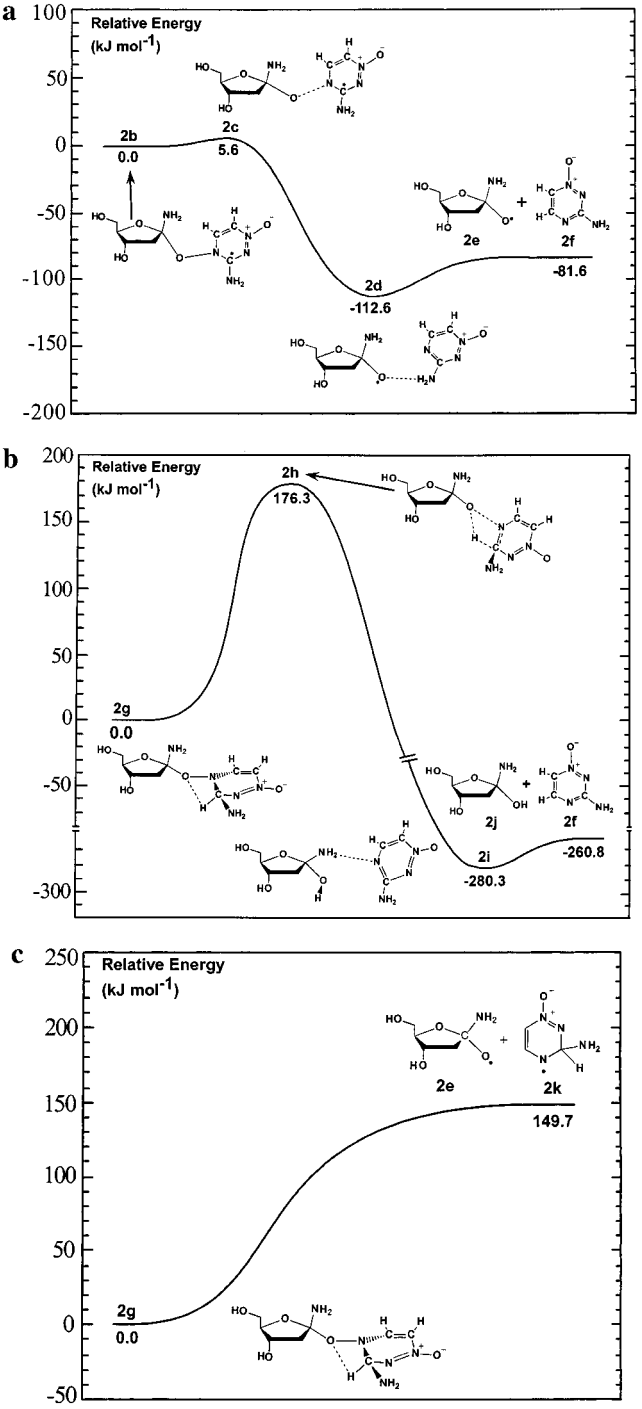

Figure 6. Schematic energy profile for (a) mechanism A, (b) mechanism B (part A), and (c) mechanism B (part B) (see text).

of the deoxyribonolactone moiety via homolytic cleavage of the O_4-N_4 bond (mechanism A) and via addition of hydrogen ($H^\bullet$) to the drug-C_3 radical site of **2b** (mechanism B). These two mechanisms are the same as those proposed by Hwang et al. (see Scheme 3).[10] In addition, considering our previous findings for the C−C covalent adduct intermediate **1c**, we also examined the effects of protonation on **2b** (mechanism C).

The schematic energy profile obtained for mechanism A is shown in Figure 6a. The O_4-N_4 bond of **2b** may cleave homolytically via TS **2c** at a cost of just 5.6 kJ mol^{-1}, to give complex **2d**, lying lower in energy than **2b** by 112.6 kJ mol^{-1}. Complex **2d** is an intermolecularly hydrogen-bonded complex between the alkoxyl radical derivative (**2e**) of the sugar-C_1' radical and the drug metabolite (**2f**). The small barrier to

dissociation of **2c** may suggest why the C−O covalent adduct intermediate **5** (see Scheme 3) was not observed experimentally. We note that **2f** corresponds to our model equivalent of **3** (see Scheme 1). It requires just 31.0 kJ mol^{-1} for **2d** to dissociate completely to **2e** and **2f**.

The calculated hydrogen affinity of the alkoxyl radical **2e** is quite large, approximately 420.5 kJ mol^{-1}. This suggests that such a radical species may relatively easily obtain a hydrogen to form the sugar-C_1 hydroxylated species, **6** in Scheme 3. Once the hydroxylated species is formed it may then react further to give the corresponding deoxyribonolactone, with concomitant cleavage of the DNA backbone.

For the proposed[10] alternative, mechanism B, the calculated hydrogen affinity for the drug-C_3 centered radical **2b** is just 241.3 kJ mol^{-1}. This is quite a low hydrogen affinity on account of the enhanced stability of the ring-carbon centered radical. Furthermore, it is significantly lower than that of the alkoxyl radical **2e**. This suggests that such a process may encounter a considerable energy barrier, as predicted by the empirical relationships of Evans−Polanyi and Semenov.[24] For completeness, however, we considered the reactions that the hydrogenated derivative **7** (see Scheme 3) may undergo to give the deoxyribonolactone moiety. The addition of hydrogen (H$^\bullet$) to the drug-C_3 radical center of **2b** gives **2g** in Figure 6b. The schematic energy profiles obtained for further reactions of **2g** are shown in Figure 6b (part A of mechanism B) for the formation of a sugar-C_1' hydroxylated derivative **2j**, and in Figure 6c (part B of mechanism B) for the formation of the sugar alkoxyl radical **2e**. In Figure 6b, an intermolecularly hydrogen-bonded complex **2i** between the sugar-C_1' hydroxylated derivative (**2j**) and **2f** can form, lying lower in energy by 280.3 kJ mol^{-1}! However, this reaction proceeds via TS **2h** at a considerable cost of 176.3 kJ mol^{-1}. Thus, even if **2g** were formed, it is unlikely that such a reaction will occur. Alternatively, the O_4—N_4 bond of **2g** may undergo homolytic cleavage at a cost of 149.7 kJ mol^{-1} to give the alkoxyl radical (**2e**) and the radical drug derivative **2k** (Figure 6c). While this alternative mechanism still requires a considerable amount of energy, it is significantly less than that required for formation of **2i** from **2g**. The alkoxyl radical formed can then react as described previously to eventually form deoxyribonolactone.

The protonation of **2b** (mechanism C) can conceivably occur at several sites on the drug moiety, in particular at the O_1 or O_4 positions. Protonation at the bridging O_4 position results in cleavage of the O_4—N_4 bond to give the sugar-C_1' hydroxylated derivative **2j**, while protonation at the O_1 position results in a shortening of the O_4−N_4 bond and a lengthening of the sugar-C_1'−O_4 bond of **2b**, i.e., emphasizes the sugar-drug nature of **2b**. As might be expected, the calculated proton affinity of O_1 (1029.0 kJ mol^{-1}) is higher than that of the bridging O_4 (975.6 kJ mol^{-1}). Thus, while protonation of **2b** is not expected to enhance the ability of tirapazamine to transfer an oxygen to the sugar-C_1' radical, it is not expected to inhibit or lead to radically different reaction mechanisms than those already considered above.

(24) See, for example: Laidler, K. J. *Chemical Kinetics*, 3rd ed.; HarperCollins Publishers: New York, 1987; p 71.

Conclusions

Possible mechanisms by which tirapazamine may react with DNA sugar-C_1' radicals to form the sugar derivative deoxyribonolactone with concomitant cleavage of the DNA strand have been investigated by use of the B3LYP density functional theory method.

Experimentally proposed mechanisms involving ionization of the sugar-C_1' radical by tirapazamine were investigated. The present results predict the electron affinity of tirapazamine to be considerably lower than the ionization energy of the sugar-C_1' radical. Thus, it is unlikely that the sugar-C_1' radical can be oxidized by tirapazamine. However, the electron affinity of the protonated form of tirapazamine is estimated to be greater than the ionization energy of the sugar-C_1' radical. Hence, protonation of tirapazamine may be a prerequisite for it to oxidize the sugar-C_1' radical.

The proposed formation of a sugar-C_1'−drug-C_3 covalent adduct intermediate **4** is found to occur with a modest barrier. Protonation of the drug moiety of **4** is predicted to result in heterolytic cleavage of the C−C bond, without a barrier, to give the sugar-C_1' cation.

In both of the above mechanisms, the resulting sugar-C_1' cation will react with solution H_2O to give deoxyribonolactone with inclusion of an oxygen from H_2O. At the present level of theory, we are unable to distinguish which mechanism may be responsible for the experimentally observed minor pathway: the conversion of a sugar-C_1' radical to deoxyribonolactone by incorporation of an oxygen from a solution H_2O molecule. It is possible that both mechanisms occur to different degrees.

We find that the energetically preferred pathway, in agreement with the experimental observations, occurs via direct attachment of the O_4 *N*-oxide oxygen of tirapazamine at the sugar-C_1' radical center. This is predicted to be able to occur without a barrier. The transfer of the oxygen O_4 can then be completed by homolytic cleavage of the O_4—N_4 bond, which proceeds with a very small barrier of less than 6 kJ mol^{-1}. The resulting sugar-alkoxyl radical derivative can then relatively easily obtain a hydrogen to form the sugar-C_1' hydroxylated derivative, which can then react further to give the deoxyribonolactone. The present calculations suggest this to be the preferred mechanism for formation of the experimentally observed major product: formation of the deoxyribonolactone by incorporation of an *N*-oxide oxygen. The experimentally proposed alternative, involving addition of H$^\bullet$ to the drug-C_3 position of the sugar-C_1'−O_4-drug intermediate **5**, is predicted to not occur due to the low hydrogen affinity of **5**.

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Supporting Information Available: Archive entries of the B3LYP/6-31G(d) optimized structures (Table S1) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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