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Reactivity of Substituted Charged Phenyl Radicals toward Components of Nucleic Acids

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Abstract: Reactions of differently substituted phenyl radicals with components of nucleic acids have been investigated in the gas phase. A positively charged group located meta with respect to the radical site was employed to allow manipulation of the radicals in a Fourier-transform ion cyclotron resonance mass spectrometer. All of these electrophilic radicals react with sugars via exclusive hydrogen atom abstraction, with adenine and uracil almost exclusively via addition (likely at the C8 and C5 carbons, respectively), and with the nucleoside thymidine by hydrogen atom abstraction and addition at C5 in the base moiety (followed by elimination of $^{\bullet}CH_3$). These findings parallel the reactivity of the phenyl radical with components of nucleic acids in solution, except that the selectivity for addition is different. Like HO$^{\bullet}$, the electrophilic charged phenyl radicals appear to favor addition to the C5-end of the C5−C6 double bond of thymine and thymidine, whereas the phenyl radical preferentially adds to C6. The charged phenyl radicals do not predominantly add to thymine, as the neutral phenyl radical and HO$^{\bullet}$, but mainly react by hydrogen atom abstraction from the methyl group (some addition to C5 in the base followed by loss of $^{\bullet}CH_3$ also occurs). Adenine appears to be the preferred target among the nucleobases, while uracil is the least favored. A systematic increase in the electrophilicity of the radicals by modification of the radicals' structures was found to facilitate all reactions, but the addition even more than hydrogen atom abstraction. Therefore, the least reactive radicals are most selective toward hydrogen atom abstraction, while the most reactive radicals also efficiently add to the base. Traditional enthalpy arguments do not rationalize the rate variations. Instead, the rates reflect the radicals' electron affinities used as a measure for their ability to polarize the transition state of each reaction.

Introduction

Degradation of DNA via radical attack plays an important role in various biological processes, including chemotherapy and carcinogenesis.[1] Among the various radicals involved in DNA damage, the hydroxyl radical (HO$^{\bullet}$) has been the most scrutinized due to its high abundance in human cells, high reactivity, and hazardous potential.[2,3] Its chemistry, and that of some other electrophilic radicals (e.g., *tert*-BuO$^{\bullet}$, HS$^{\bullet}$), is known to be dominated by addition to the nucleobases (predominant addition to C5 of the C5−C6 carbon−carbon double bond of pyrimidine bases and to C4, C5, or C8 in purine bases),[3−5] although hydrogen abstraction from sugars has also been reported.[3]

Some antitumor drugs, such as those of the enediyne type, generate aromatic σ,σ-biradicals that are believed to cleave double-stranded DNA via direct hydrogen atom abstraction from the sugar moiety.[6] Benzoyl peroxide and some other compounds are metabolized to aromatic monoradicals (i.e., benzoyloxyl and phenyl) that may cause DNA damage through both hydrogen atom abstraction and addition pathways.[7] However, a detailed mechanistic understanding of these and related processes is limited.[7−10]

To the best of our knowledge, only one study has been reported in the literature that specifically focuses on the

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reactivity of phenyl radicals toward DNA components (phenyl, 4-methyl-, and 4-methoxyphenyl; a few studies[11] have appeared on reactions of arenediazonium ions, but the role of the free phenyl radical in these reactions is unclear).[9b] The authors concluded that phenyl radicals abstract hydrogen atoms from several sites in sugars but they display somewhat different selectivity than HO•. The phenyl radicals preferentially add to the C5−C6 double bond of cytosine, uracil, and thymine, and their derivatives, and this is the overall dominant reaction. The addition occurs preferentially to C6 instead of C5 (the ratio of attack at C5 vs C6 for thymine is 0.3:1), although addition to C5 predominates for the electrophilic[2,3] HO•. This selectivity was rationalized by the slightly nucleophilic character of the phenyl radical.[9b] However, no rationale was provided for the observed preferential addition to C5 of uracil and uracil-containing nucleosides and nucleotides (at pH 7.4; at pH < 2, addition occurs at C6),[9b] a site also favored by the electrophilic HO•. The phenyl radicals were reported to undergo addition to a base in nucleosides, but also to abstract hydrogen atoms to yield sugar-derived radicals at larger amounts than found for HO•. Also this finding was rationalized[9b] by the phenyl radicals' nucleophilic character, which was suggested to lead to faster attack at the electrophilic sites in the sugar moiety. In contrast to the reactivity observed for the pyrimidine bases and their derivatives, no signals were detected for the products of the purine bases and nucleosides (except for adenosine 5′-triphosphate, but the detected products could not be identified). Others have reported the formation of 8-aryl adducts for adenine and guanine and their derivatives upon exposure to arenediazonium ions in solution.[11] However, whether these products are formed from the free phenyl radicals or the diazonium ions was not unambiguously demonstrated.[9]

The fundamental factors that control the reactivity of phenyl radicals toward biomolecules were not evaluated in the above studies. In fact, even the study of reactions with simple organic substrates has been limited to a few phenyl radicals carrying substituents only in the para-position.[11,12] However, a better understanding of the structural features and other factors that control the reactivity of phenyl radicals toward DNA and its components could greatly benefit the rational design of synthetic DNA cleavers and hence facilitate the development of more efficient and less toxic pharmaceuticals.

Gas-phase studies provide an opportunity to examine the intrinsic (solvent-free) reactivity of radical intermediates without the perturbation of solvation effects or competing reactions with substances other than the substrates of interest. In our earlier studies, we have employed Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR) to examine the intrinsic reactivity of phenyl radicals toward various simple organic substrates. The experimental approach is based on the placement of a chemically inert charged group (often pyridinium) into the radical to allow its manipulation in the mass spectrometer.[13] Addition of neutral groups into the radical allows for the evaluation of substituent effects on the radical's reactivity. This approach has been employed to demonstrate that electron-withdrawing groups in positively charged phenyl radicals enhance their rates of hydrogen atom abstraction from simple organic hydrogen atom donors,[14] thiomethyl abstraction from dimethyl disulfide,[15a] iodine abstraction from allyl iodide,[15b] and addition to *tert*-butylisocyanide[15a] and to simple aromatic substrates.[16] These findings were rationalized by an increased polarization of the transition state associated with atom abstraction and addition.[15,16] This type of structure/reactivity information for biological radical reactions would be valuable because it could allow "tuning" of the radicals' reactivity and selectivity toward different sites in DNA. We report here the first gas-phase investigation of substituent, enthalpic, and polar effects on the reactivity of differently substituted phenyl radicals toward various nucleic acid components.

Experimental Section

All experiments were performed using a Finnigan model FTMS 2001 Fourier-transform ion cyclotron resonance mass spectrometer (FT-ICR).[14] This instrument contains a differentially pumped dual cell aligned within the magnetic field of a 3.0 T superconducting magnet. Two Edwards diffusion pumps (800 L/s), each backed by an Alcatel 2012 mechanical pump, were employed to maintain a nominal base pressure of $<10^{-9}$ Torr. Samples were used as received from the manufacturer.

Charged phenyl radicals (**a**−**e**) were generated by using procedures reported elsewhere.[13−17] The precursors for radicals **a**−**d** were 1,3-diiodobenzene, 1,3-dichloro-5-iodobenzene, 3,5-dibromonitrobenzene, and bromobenzene, respectively. These reagents were added into one side of the dual cell at a nominal pressure of 6.0×10^{-8} Torr (measured by an ionization gauge) through a Varian leak valve or a heated solids probe. Pyridine (3-fluoropyridine for **c**; our previous studies[13−15] have demonstrated that introduction of F into the pyridinium ring does not affect reaction products or reaction rates; 3-iodopyridine was used for **d**) was introduced at the same nominal pressure into the same cell by using a batch inlet system equipped with a variable leak valve. The mixture was ionized for approximately 30 ms by an electron beam (30 eV electron energy, 6 μA emission current). The resulting substituted benzene radical cation was allowed to react with pyridine for 2 s, which leads to ipso substitution[18] of one of the halogen atoms. Ionized methanol was used to protonate 3-iodopyridine, the precursor for radical **e**.

Transfer of the substituted halobenzene ions into the other side of the dual cell was carried out by grounding the conductance limit plate for approximately 175 μs. The ions were isolated by ejecting all other ions via the application of a series of stored-waveform inverse Fourier transform (SWIFT)[19] excitation pulses to the plates of the cell. The

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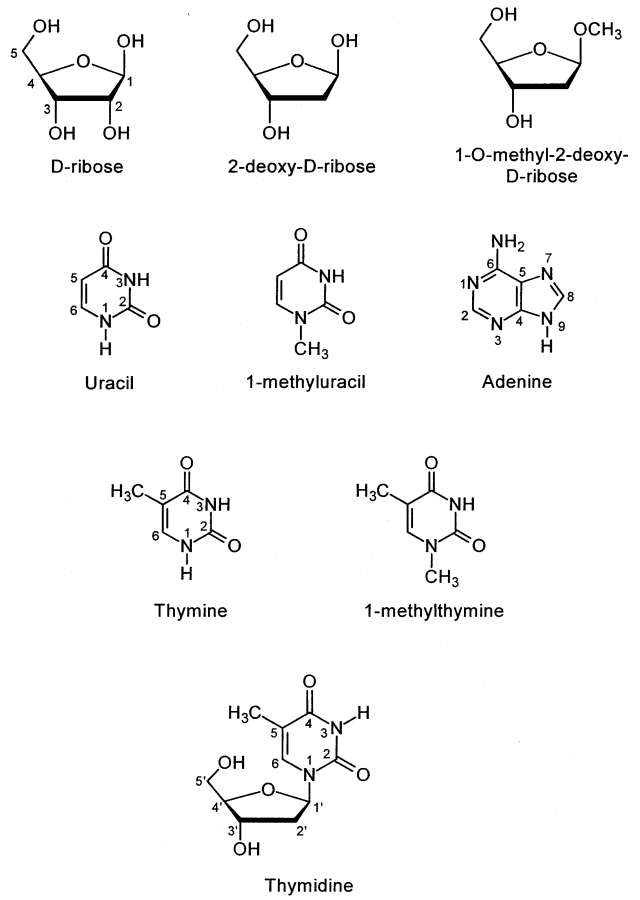
desired charged phenyl radicals were formed by homolytically cleaving a carbon−iodo or a carbon−nitro bond. This was accomplished by using sustained off-resonance irradiated collision-activated dissociation (SORI-CAD),[20] carried out by introducing argon into the cell via a pulsed valve assembly[21] (maximum nominal pressure in the cell $\sim 1 \times 10^{-5}$ Torr). The ions were accelerated by exciting them continuously for 1 s at a frequency 1 kHz higher than their cyclotron frequency and were activated via collisions with argon. The ions were allowed to cool for 400 ms via IR emission and by collisions with the neutral biomolecules (introduced via a heated solids probe) present in the cell.

The charged phenyl radicals were isolated by ejecting unwanted ions from the cell with a series of SWIFT pulses and were allowed to react with the neutral biomolecules for a variable period of time (1−180 s). The neutral reagents were introduced into the ultrahigh vacuum region via a heated solids probe. After initial pressure variations, a stable pressure of the biomolecules was eventually reached in the cell, thus enabling rate measurements. Chirp excitation (124 Vp-p amplitude, 2.7 MHz bandwidth, and sweep rate of 3200 Hz/μs) was employed for detection of the ions. All spectra are the average of 15−20 scans that were collected as 64k data points and subjected to one zero-fill before Fourier transformation. Primary products were identified on the basis of their fixed relative abundances at short reaction times.

Reactions studied under the above conditions follow pseudo-first-order kinetics, which allows one to obtain the second-order reaction rate constants (k_{exp}) from a semilogarithmic plot of the relative abundance of the reactant ions versus time. The corresponding collision rate constants (k_{coll}) were estimated by using a parameterized trajectory theory.[22] The efficiency of each reaction (i.e., the fraction of collisions that lead to a reaction) is given by k_{exp}/k_{coll}. The rate constant measurements are estimated to have an accuracy of ±50%, with a precision better than ±10%. The greatest uncertainty arises from measurement of the pressure in the cell. The pressure readings of the ion gauges were corrected for their sensitivity toward each neutral biomolecule[23] and for the pressure gradient between the cell and the ion gauge. The latter correction factor was obtained by measuring rates of highly exothermic, barrierless reactions (i.e., electron transfer to the CS_2 radical cation or proton transfer from protonated acetone) assumed to occur at collision rate for the biomolecules.

All calculations reported in this work were carried out by using the Gaussian 98 revision A.7 suite of programs.[24] The geometries of most of the molecules were fully optimized, and the zero-point vibrational energies (ZPVE) were calculated at the B3LYP/6-31G(d) level of theory. All structures correspond to stationary points on their potential energy surfaces and possess no imaginary frequencies. The vibrational energies were scaled by a factor of 0.9804 to account for the overestimation of the frequencies by this method. For the calculation of the vertical thermochemical values, the molecules' geometries obtained at the B3LYP/6-31+G(d) level of theory were directly used in single-point calculations of their ionized forms.

Scheme 1



Results and Discussion

Gas-phase reactions of components of nucleic acids, including D-ribose, 2-deoxy-D-ribose, uracil, thymine, adenine, and thymidine (Scheme 1), were examined with several phenyl radicals that each carry a pyridinium group for mass spectrometric manipulation. The generation of the radicals (**a**−**e**; **c** carries a 3-fluoropyridinium charge site) is shown in Schemes 2 and 3.[13−17] The radicals were isolated from other charged molecules and transferred into a clean reaction chamber for reactivity studies. The results obtained for each different type of a substrate are discussed below.

Sugars. All of the charged phenyl radicals react with D-ribose, 2-deoxy-D-ribose, and 1-*O*-methyl-2-deoxy-D-ribose exclusively via hydrogen atom abstraction (Table 1). This finding is in qualitative agreement with results obtained in solution for the reactions of the phenyl radical[9] and 4-methoxyphenyl radical[9b] with D-ribose[9] and 2-deoxy-D-ribose.[9b]

Addition of an electron-withdrawing group (i.e., Cl, Br) to the meta-position with respect to the radical site in the charged phenyl radical **a** slightly enhances its reactivity toward each of the sugars examined. In the most pronounced case, radical **a** abstracts a hydrogen atom from 1-*O*-methyl-2-deoxy-D-ribose at an efficiency of 11%, whereas the bromo-substituted radical **c** reacts in the same fashion at an efficiency of 20% (Table 1). A further increase in the electron deficiency of the radical, caused by placement of the radical site in the same ring that contains the charge (radicals **d** and **e**), drastically enhances the efficiency of hydrogen atom abstraction. For example, radicals **d** and **e** react with 1-*O*-methyl-2-deoxy-D-ribose at efficiencies

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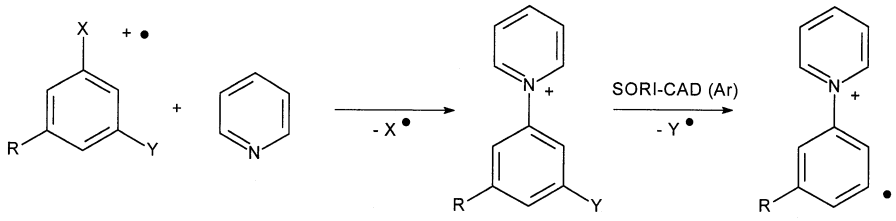
Scheme 2



a: R = H, X = I, Y = I
b: R = Cl, X = Cl, Y = I
c: R = Br, X = Br, Y = Br
(F-pyridine instead of pyridine)

Scheme 3

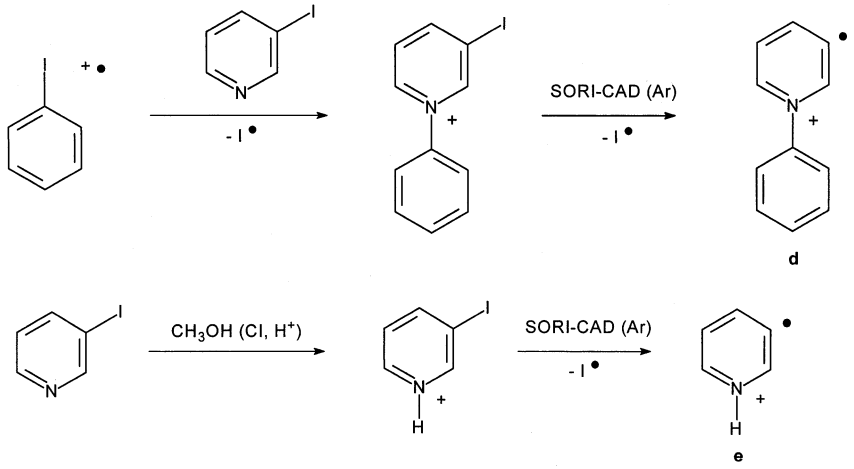
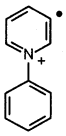


Table 1. Product Distributions and Reaction Efficiencies[a] Measured for the Reactions of Radicals **a**−**e** with Sugars

R =	Ribose IE_v = 9.1 eV[b]	2-deoxy-D-ribose IE_v = 9.0 eV[b]	1-O-methyl-2-deoxy-D-ribose IE_v = 9.0 eV[b]
a H	H• Abstraction (100%) Eff. = 4%	H• Abstraction (100%) Eff. = 6%	H• Abstraction (100%) Eff. = 11%
b Cl	H• Abstraction (100%) Eff. = 6%	H• Abstraction (100%) Eff. = 9%	H• Abstraction (100%) Eff. = 19%
c Br[c]	H• Abstraction (100%) Eff. = 7%	H• Abstraction (100%) Eff. = 8%	H• Abstraction (100%) Eff. = 20%
d 	H• Abstraction (100%) Eff. = 32%	H• Abstraction (100%) Eff. = 32%	H• Abstraction (100%) Eff. = 47%
e 	H• Abstraction (100%) Eff. = 53%	H• Abstraction (100%) Eff. = 54%	H• Abstraction (100%) Eff. = 61%

[a] Reaction efficiency (Eff.) = second-order reaction rate constant/collision rate constant (k_{exp}/k_{coll}). [b] B3LYP/6-31G(d) level of theory. [c] The charged group is 3-fluoropyridine instead of pyridine.

of 47 and 61%, respectively. The results reveal the reactivity pattern **a** (R = H) < **b** (Cl) ≈ **c** (Br) < **d** < **e**. This finding is in agreement with results obtained on the reactions of these radicals with simple hydrogen atom donors, such as tetrahydrofuran.[14b] In this earlier work, we found that the polarity of the H• abstraction transition state (polar effects) was an

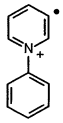
important factor controlling the reactivity of the radicals toward simple organic hydrogen atom donors. To test whether the same rationale applies to sugar molecules, the enthalpic and polar factors associated with these reactions were evaluated, as described below.

Enthalpic Effects. Differences in the thermodynamic driving force for the reactions discussed above might induce variations in transition state energies that explain the reaction efficiency trends discussed above (Hammond postulate, Bell−Evans−Polanyi principle, Marcus theory).[25] Therefore, the exothermicity associated with abstraction of a hydrogen atom was examined by calculating the enthalpy changes for hydrogen atom abstraction by each radical from the sugars at the B3LYP/6-31G(d)+ZPVE level of theory. Our calculations suggest that hydrogen atom abstraction from all of the carbons in the sugars is highly exothermic (16−24 kcal/mol). For example, for radical **a**, abstraction of a hydrogen atom from positions 1, 2, 3, 4, and 5 in D-ribose (Scheme 1) was estimated to be exothermic by 17−21 kcal/mol. Therefore, the radicals are likely to abstract hydrogen atoms from multiple sites, but possibly not to the same extent from each site. Indeed, solution experiments suggest that although the phenyl and hydroxyl radicals attack several (if not all) of the different C−H bonds, they do so to different extents and form different isomer mixtures of the product sugar radicals.[3,9,26]

Because abstraction of a hydrogen atom from many different sites in the sugars is highly exothermic, and the exothermicities differ by less than 4 kcal/mol for a given radical, only values for the abstraction of a hydrogen atom from position 1 in the sugars are reported in Table 2. These exothermicities do not correlate with the observed trend in reaction efficiencies. Furthermore, they show very little sensitivity to any change in the radical's structure (i.e., substitution on the radical). For example, hydrogen atom abstraction from D-ribose is calculated to be 21 and 22 kcal/mol exothermic for the *N*-phenyl-3-dehydropyridinium radical **d** and the 3-dehydropyridinium radical **e**, respectively (Table 2). This difference in exothermicities cannot account for the significant difference in reaction efficiencies observed for these two radicals (i.e., 32 and 53% for radicals **d** and **e**, respectively; Table 1). The same applies to the other reactions studied.

The exothermicity for hydrogen abstraction from *all* positions in 1-*O*-methyl-2-deoxy-D-ribose is estimated to be higher by 1−5 kcal/mol than those for D-ribose and 2-deoxy-D-ribose (Table 2 shows calculations for the C1 position). This finding is likely to explain why 1-*O*-methyl-2-deoxy-D-ribose reacts faster than D-ribose and 2-deoxy-D-ribose (Table 1). In addition, 1-*O*-methyl-2-deoxy-D-ribose has an additional site of attack, the methyl group attached to the 1-oxygen. Abstraction of a hydrogen atom from this position is also very exothermic (17 kcal/mol for radical **a**; B3LYP/6-31G(d)+ZPVE) and may be contributing to the observed rate enhancement as compared to the other sugars.

Table 2. Reaction Exothermicities (kcal/mol) Estimated (B3LYP/6-31G(d)+ZPVE) for Hydrogen Atom Abstraction from the C1′-Position in Sugars by the Charged Phenyl Radicals **a**−**e**

		D-Ribose	2-deoxy-D-ribose	1-O-methyl-2-deoxy-D-ribose
a	**R = H**	17.7	17.8	20.5
b	**R = Cl**	17.8	17.9	20.6
c	**R = Br**[a]	17.7	17.8	20.4
d		20.7	20.8	23.5
e		21.7	21.8	24.4

[a] This radical carries a 3-fluoropyridinium charge site instead of a pyridinium.

Polar Factors. Increasing the polarization of a transition state of an atom abstraction reaction of a radical can decrease its energy. This has been known for years, and models have been developed to understand polar effects in radical reactions.[25] Based on these models, reactions involving electrophilic and nucleophilic radicals are likely to be influenced by "ionic resonance structures" that can contribute to the electronic structure of the transition state.[25] For electrophilic radicals, such as those reported here, the energy of the most relevant ionic configuration is lowered by increasing the vertical electron affinity (EA_v) of the radical or by decreasing the vertical ionization energy (IE_v) of the substrate.[25]

The vertical electron affinities of the charged phenyl radicals **a**−**e** were calculated previously.[14b,16] The observed electron affinity ordering **a** (R = H) < **b** (Cl) ≈ **c** (Br) < **d** < **e** (the EAs are 4.87,[13b] 5.11,[16] 5.12,[16] 5.78,[13b] and 6.12 eV,[14b] respectively, at the B3LYP/6-31+G(d) level of theory) parallels the trend in the reaction efficiencies described above (Figure 1). For example, radicals **a** and **e** are estimated to have the lowest (4.86 eV) and highest (6.12 eV) EA_v, respectively. These radicals also display the lowest and the highest reaction efficiencies, respectively. Therefore, just as was reported for simple hydrogen atom donors,[14b] the observed reactivity trends among the different radicals toward sugars can be rationalized by polar effects. It follows that an increase in the electrophilicity of a phenyl radical, for example, via addition of electron-withdrawing groups, should enhance its ability to abstract a hydrogen atom from the sugar moiety of DNA.

Nucleobases. Hydrogen atom abstraction and/or addition reactions are the predominant pathways observed (Table 3) for reactions of the charged phenyl radicals with the individual nucleobases of the base pairs uracil−adenine (U−A) and

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(26) Schuchmann, M. N.; von Sonntag, C. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1958.

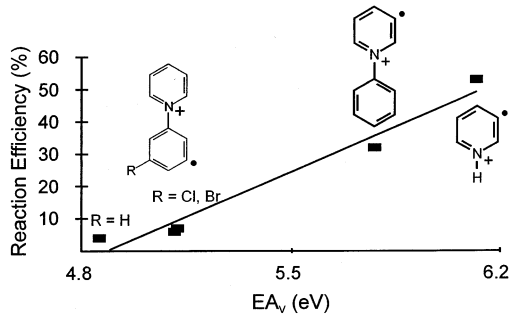


Figure 1. Hydrogen atom abstraction efficiencies measured for the reactions of radicals **a**−**e** with D-ribose versus calculated vertical electron affinities (EA_v) of the radicals. A straight line has been drawn through the data points to guide the eye, but this is not meant to imply a linear correlation between the efficiencies and electron affinities.

thymine−adenine (T−A), as well as 1-methyluracil and 1-methylthymine.[27] The inability to evaporate guanine without thermal decomposition prohibited reactivity studies with the base pair cytosine−guanine (C−G).

Uracil, 1-Methyluracil, and Adenine. The charged radicals **a**−**e** react with uracil, 1-methyluracil, and adenine almost exclusively via addition, forming a stable adduct and/or adduct that has lost a hydrogen atom by spontaneous fragmentation (Table 3). A detectable amount of hydrogen atom abstraction also occurs. Because of the high proton affinity of adenine (225.3 kcal/mol[28]), only proton transfer was observed when this base was allowed to react with the acidic radical **e**.

The observation of significant amounts of stable addition products in the gas phase (a stable adduct accounts for 65−94% of the product distribution for uracil and 1-methyluracil; 21−28% for adenine) suggests that the adducts formed upon reaction of the charged phenyl radicals with these nucleobases are long-lived enough to undergo cooling via emission of photons[29] (collisional stabilization is unlikely to play a role in the stabilization process due to the low pressures used in the experiments). Similar findings have been reported for charged phenyl radicals upon reaction with simple aromatic substrates.[16] The formation of resonance stabilized radicals upon addition to the phenyl ring, and the relatively large size of the adducts, rationalize the characteristic behavior of these adducts to rapidly stabilize by emission of light. In fact, the rate-determining step in the formation of stable adducts between charged phenyl radicals and simple aromatic substrates is the addition step rather than the light emission process, as indicated by the fact that the second-order reaction rate constants measured for formation of the stable adducts correlate with the transition state energies for addition.[16]

The outcome of the competition between hydrogen atom abstraction and addition to uracil, 1-methyluracil, and adenine by the charged phenyl radicals reflects the calculated large differences in the exothermicities of the reactions. For example, while abstraction of a hydrogen atom from the N1-position of uracil (calculated to be the most favored site) by radical **a** was estimated to be 17.5 kcal/mol exothermic (B3LYP/6-31G(d) + ZPVE), addition of this radical to the C5-position of uracil is even more exothermic ($\Delta H_{rxn} = -30.7$ kcal/mol). Addition is the dominant reaction. Similarly, the predominant addition to 1-methyluracil and to adenine by radical **a** is explained by a thermodynamic driving force that is at least 12 kcal/mol greater than that for H$^\bullet$ abstraction.

The site-selectivity of attack of the charged phenyl radicals could not be unambiguously determined on the basis of experimental data. Therefore, the energetics associated with addition of the radicals **a** and **d** to several sites in uracil, 1-methyluracil, and adenine were examined computationally (B3LYP/6-31G(d) + ZPVE). For radical **a**, additions to the C5- and C6-positions in uracil were estimated to be 30.7 and 26.1 kcal/mol exothermic, respectively. Addition to other sites in uracil (e.g., N3-site) is less favored by at least 15 kcal/mol. Analogous results were obtained for 1-methyluracil. Therefore, the thermodynamically favored site of addition of charged phenyl radicals to uracil and 1-methyluracil is concluded to be the C5-position of the C5−C6 alkenic bond. Transition state energies calculated for addition to C5 and C6 of uracil by a simple analogue of the radicals studied here, 3-dehydroanilinium ion, reflect the reaction exothermicities (the transition state for addition to C5 is lower in energy than that for addition to C6 by 3.4 kcal/mol). Similarly, the transition states for addition of HO$^\bullet$ and phenyl radical to C5 are calculated to be energetically more favorable than those to C6 by 3.9 and 1.4 kcal/mol, respectively. These results are in agreement with previous reports[2,3,9b] that these radicals predominantly add to the C5-carbon in uracil.

For adenine, addition to the C8-site is calculated to be the thermodynamically preferred channel (Scheme 4), although addition to C2 is close in energy (for **a**, addition is 33.2 and 29.7 kcal/mol exothermic for the C8- and C2-positions, respectively). The observed predominant fragmentation of the adenine adduct by hydrogen atom loss demonstrates that either C2 or C8, or both, are favored addition sites.

Polar effects have been reported to play a rate-controlling role in many addition reactions involving neutral radicals in solution[12f,25e,f,30] and charged phenyl radicals in the gas phase.[16] This is also likely to be the case for the addition reactions observed here because the reaction efficiencies for addition increase with the electron deficiency of the attacking radical. For example, radical **a** adds to adenine at an efficiency of only ~2%, whereas radical **d** adds at ~50% efficiency (Table 3). Thermodynamic effects may be also contributing to the rate difference between these two radicals because addition of radical **d** to adenine is about 5 kcal/mol more exothermic than that of radical **a** (38.6 vs 33.2 kcal/mol, respectively; B3LYP/6-31G(d)+ZPVE).

The addition barrier is expected to be lowered due to polar effects upon a decrease in the ionization energy of the substrate.[25] On the basis of the IE_v values listed in Table 3, the reaction efficiencies among the three nucleobases that predomi-

(27) The methylated bases of uracil and thymine were used to better model nucleobases in nucleic acids, because the N1-position of these nucleobases is not attached to a hydrogen, but instead to a carbon atom of 2-deoxy-D-ribose.

(28) Mallard, W. G., Linstrom, P. J., Eds. *NIST Chemistry WebBook, NIST Standard Reference Database Number 69*; National Institute of Standards and Technology: Gaithersburg, MD, March 1998; http://webbook.nist.gov.

(29) (a) Dunbar, R. C. *Mass Spectrom. Rev.* **1992**, *11*, 309. (b) Ho, Y. P.; Dunbar, R. C. *J. Phys. Chem.* **1993**, *97*, 11474. (c) Dunbar, R. C. *Int. J. Mass Spectrom. Ion Processes* **1997**, *160*, 1. (d) Cheng, Y. W.; Dunbar, R. C. *J. Phys. Chem.* **1995**, *99*, 10802. (e) Ryzhov, V.; Dunbar, R. C. *Int. J. Mass Spectrom. Ion Processes* **1997**, *167*/*168*, 627.

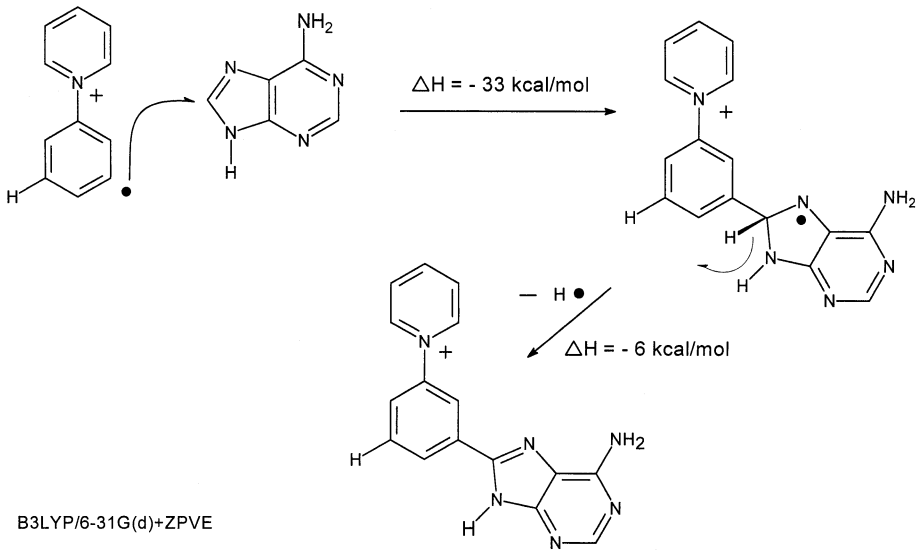
(30) (a) Roberts, B. P.; Diart, V. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1761. (b) Radom, L.; Wong, M. W.; Pross, A. *J. Phys. Chem. A* **1998**, *102*, 2237. (c) Wong, M. W.; Pross, A.; Radom, L. *J. Am. Chem. Soc.* **1994**, *116*, 6284. (d) Wong, M. W.; Pross, A.; Radom, L. *J. Am. Chem. Soc.* **1994**, *116*, 11938.

Table 3. Efficiencies[a] and Products[b] Measured for Reactions of Charged Radicals **a**−**e** with Nucleobases

radical	uracil $IE_v = 9.15$ eV[c]	1-methyluracil $IE_v = 8.78$ eV[c]	adenine $IE_v = 8.02$ eV[c]	thymine $IE_v = 8.72$ eV[c]	1-methylthymine $IE_v = 8.41$ eV[c]
a	H• abstraction (10%) adduct-H• (3%) adduct (87%) Eff. = 0.05%	H• abstraction (1%) adduct-H• (6%) adduct (93%) Eff. = 1%	H• abstraction (14%) adduct-H• (65%) adduct (21%) Eff. = 2%	H• abstraction (85%) adduct-CH_3• (15%) Eff. = 2%	H• abstraction (74%) adduct-CH_3• (26%) Eff. = 3%
b	H• abstraction (8%) adduct-H• (5%) adduct (87%) Eff. = 0.2%	H• abstraction (1%) adduct-H• (5%) adduct (94%) Eff. = 3%	H• abstraction (5%) adduct-H• (72%) adduct (23%) Eff. = 5%	H• abstraction (76%) adduct-CH_3• (24%) Eff. = 4%	H• abstraction (69%) adduct-CH_3• (31%) Eff. = 11%
c[d]	H• abstraction (7%) adduct-H• (5%) adduct (88%) Eff. = 0.2%	H• abstraction (1%) adduct-H• (6%) adduct (93%) Eff. = 4%	H• abstraction (4%) adduct-H• (74%) adduct (22%) Eff. = 6%	H• abstraction (75%) adduct-CH_3• (25%) Eff. = 4%	H• abstraction (69%) adduct-CH_3• (31%) Eff. = 11%
d	H• abstraction (5%) adduct-H• (17%) adduct (78%) Eff. = 2%	H• abstraction (2%) adduct-H• (33%) adduct (65%) Eff. = 13%	H• abstraction (6%) adduct-H• (66%) adduct (28%) Eff. = 53%	H• abstraction (70%) adduct-CH_3• (30%) Eff. = 22%	H• abstraction (63%) adduct-CH_3• (37%) Eff. = 45%
e	H• abstraction (4%) adduct-H• (69%) adduct (27%) Eff. = 5%	not examined	H^+ transfer	H• abstraction (74%) adduct-CH_3• (26%) Eff. = 26%	H• abstraction (69%) adduct-CH_3• (31%) Eff. = 48%

[a] Reaction efficiency (Eff.) = k_{exp}/k_{coll}. [b] Relative product abundances are given in parentheses. [c] B3LYP/6-31G(d). [d] This radical carries a 3-fluoropyridinium charge site.

Scheme 4



nantly undergo addition reactions, adenine, 1-methyluracil, and uracil, are expected to follow the order adenine (IE_v = 8.02 eV) > 1-methyluracil (IE_v = 8.78 eV) > uracil (IE_v = 9.15 eV). Indeed, the observed reactivity ordering matches the expected trend, thus providing further evidence in support of the critical role that polar factors play in controlling these biologically important reactions. It can be concluded that biological substrates with lower oxidation potentials are likely to be more vulnerable toward radical damage.

Thymine and 1-Methylthymine. The preferred pathway for reaction of the charged phenyl radicals with thymine and 1-methylthymine is hydrogen atom abstraction from the C5-methyl group (Scheme 5a; Table 3). This reaction has been reported also for the hydroxyl radical in solution (although to a minor extent),[3,31] and it predominates the reactions of $O^{-\bullet}$, $H_2NCOCH_2^{\bullet}$, and $CysSO_2OO^{\bullet}$.[3,32,33] Molecular orbital calculations suggest that the hydrogen atom abstraction by the charged phenyl radicals from the C5-methyl group is a highly exothermic process (at least 26 kcal/mol for reactions of all radicals with thymine; B3LYP/6-31G(d)+ZPVE). Some hydrogen abstraction also occurs from N1 in thymine, thermodynamically the second-most favored site (Figure 2). This reaction was revealed by the examination of the reactions of radicals **a** and **b** with CD_3-thymine. About 87% deuterium abstraction and 13% hydrogen abstraction was observed for this substrate.

Thymine and 1-methylthymine do not form stable adducts with the charged phenyl radicals. Instead, these adducts spontaneously fragment by the loss of a methyl radical. Elimination of $CD_3^{\bullet}$ was observed for CD_3-thymine. This addition/elimination reaction must involve initial attack by the charged phenyl radicals to the C5-site of the C5−C6 double

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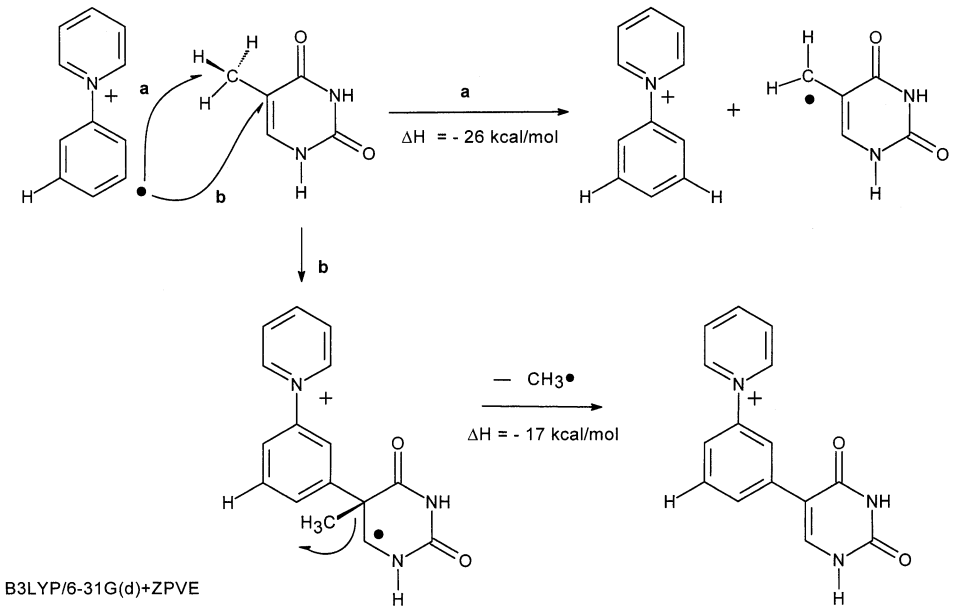
(33) Razskazovskii, Y.; Sevilla, D. *Int. J. Radiat. Biol.* **1996**, *69*, 75.

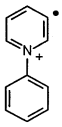
Scheme 5



bond (Scheme 5b). Any addition to the C6-carbon should have yielded a stable adduct as occurs for the isomeric 1-methyluracil (same IE) at the same rate as the addition/elimination reaction for thymine (Table 3). This selectivity parallels that reported for a few other electrophilic radicals in solution (e.g., HO•, *t*-BuO•).[3,5a] On the other hand, the nucleophilic phenyl radical, as well as $HOCH_2^•$ and $HOCH(CH_3)^•$ (and some other nucleophilic radicals), have been reported to favor attack at the C6-position in solution.[3,9] In agreement with these findings, we calculate a lower-energy transition state for addition of the 3-dehydroanilinium radical to C5 than C6 (by 3.4 kcal/mol), but a higher-energy transition state for the phenyl radical (by 1.1 kcal/mol). This differing selectivity of different types of radicals has been rationalized in the literature by preferred addition of nucleophilic radicals to the more electron-poor end, and electrophilic radicals to the more electron-rich end, of the C5−C6 double bond. However, this rationale is challenged by the recent finding that electrophilic thiyl radicals add to C6 of thymine.[34]

As for the reactions of the charged radicals with sugars, uracil, and adenine, increasing the electron deficiency of the radical enhances its overall reactivity toward thymine and 1-methylthymine (Table 3). The rates for both hydrogen atom abstraction and addition increase (Table 4). For example, radical **a** abstracts a hydrogen atom from thymine at an efficiency that is about 10% (1.7%) of the efficiency (15.4%) measured for radical **d**. The addition channel follows the same trend. Radical **d** adds 22 times faster to thymine than does radical **a**. Similarly, hydrogen abstraction efficiency increases by a factor of 10, but addition efficiency increases almost by a factor of 30 for reaction of radicals **a** and **d** with adenine. These results suggest that the addition barrier may be more sensitive to changes in the radical's polarity than the hydrogen abstraction barrier, in agreement with earlier findings made on simpler reacting systems.[16] Molecular orbital calculations (B3LYP/6-31G(d)+ZPVE) show that the

Table 4. Absolute Reaction Efficiencies[a] for the Reactions of Radicals **a**−**e** with Thymine and 1-Methylthymine

		Thymine	1-methylthymine
a	R = H	H• Abstraction: 1.7% Adduct-CH_3• : 0.3% Overall = 2%	H• Abstraction: 2.2% Adduct-CH_3• : 0.8% Overall = 3%
b	R = Cl	H• Abstraction: 3.0% Adduct-CH_3• : 1.0% Overall = 4%	H• Abstraction: 7.6% Adduct-CH_3• : 3.4% Overall = 11%
c	R = Br[b]	H• Abstraction: 3.0% Adduct-CH_3• : 1.0% Overall = 4%	H• Abstraction: 7.6% Adduct-CH_3• : 3.4% Overall = 11%
d		H• Abstraction: 15.4% Adduct-CH_3• : 6.6% Overall = 22%	H• Abstraction:28.3% Adduct-CH_3• :16.7% Overall = 45%
e		H• Abstraction:19.2% Adduct-CH_3• : 6.8% Overall = 26%	H• Abstraction: 33.1% Adduct-CH_3• : 14.9% Overall = 48%

[a] Absolute reaction efficiency = product branching ratio × overall reaction efficiency. [b] This radical carries a 3-fluoropyridinium charge site.

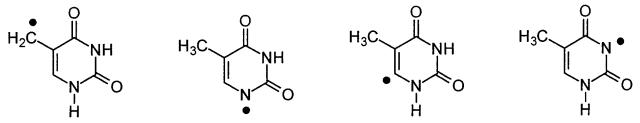


Figure 2. Relative enthalpy changes (kcal/mol) associated with hydrogen atom abstraction from thymine by the *N*-3-dehydrophenylpyridinium radical **a** (B3LYP/6-31G(d)+ZPVE). Abstraction of a hydrogen atom from the C5-methyl group is favored.

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Table 5. Reaction Products and Absolute Reaction Efficiencies[a] (in Parentheses) Measured for the Reactions of Radicals **a**−**d** with Thymidine

		Thymidine (IE_v = 8.99 eV)
a	**R = H**	H• Abstraction = 93% (10.2%) Adduct-CH_3• = 7% (0.8%) Overall = 11%
b	**R = Cl**	H• Abstraction = 78% (21.1%) Adduct-CH_3• = 22% (5.9%) Overall = 27%
c	**R = Br**[b]	H• Abstraction = 77% (22.3%) Adduct-CH_3• = 23% (6.7%) Overall = 29%
d		H• Abstraction = 63% (58.0%) Adduct-CH_3• = 37% (34.0%) Overall = 92%

[a] Absolute reaction efficiency (given in parentheses next to the branching ratios) = product branching ratio × overall reaction efficiency. [b] This radical carries a 3-fluoropyridinium charge site.

exothermicity associated with addition by radical **d** (−28.6 kcal/mol) to thymine is greater than that of radical **a** (−25.6 kcal/mol) by ∼3 kcal/mol. Therefore, the reaction of radical **d** is not only associated with more favorable polar effects, but also with a slightly greater thermodynamic driving force.

Methylation of the N1-position of thymine was found to increase both hydrogen atom abstraction and addition efficiencies (Table 4). For example, thymine reacts with radical **d** at efficiencies of 15 and 7% for hydrogen abstraction and addition, respectively, whereas 1-methylthymine undergoes the same reactions at efficiencies of 28 and 17%, respectively. Computational results suggest that the radicals attack the same sites of both bases (i.e., hydrogen abstraction from the C5-methyl group, and addition to C5 of the double bond) and that methylation only slightly affects the reaction exothermicity for both pathways. For example, the enthalpy change for abstraction of a hydrogen atom from thymine and 1-methylthymine by radical **d** was estimated to be −29 and −24 kcal/mol, respectively. On the basis of thermodynamics, thymine should react faster than 1-methylthymine. However, as mentioned above, this is not what was observed. The reactivity ordering of these two bases can be rationalized by their different ionization energies (Table 3). The IE_v ordering complements the observed reactivity, 1-methylthymine (IE_v = 8.41 eV) > thymine (IE_v = 8.72 eV). Hence, polar effects prevail over enthalpic effects in this case.

Nucleoside. Among the nucleosides, only thymidine can be thermally desorbed into the FT-ICR without decomposition (if heated slowly). Therefore, only its reactions with the radicals were examined. Thymidine is basic enough to abstract a proton from radical **e** (proton affinity = 226.6 kcal/mol[20]), and this was the only reaction observed for this radical. Radicals **a**−**d** react with thymidine predominantly by abstracting a hydrogen atom from the sugar and/or base moiety (Table 5). Our experimental conditions do not allow us to unambiguously determine the site-selectivity of attack. However, several observations suggest that the radicals attack both the sugar and the base moieties. First, similar to thymine and 1-methylthymine, addition followed by elimination of a methyl radical was observed, suggesting radical attack at the C5-carbon in the base moiety of the nucleoside. Second, a greater extent of hydrogen atom abstraction was observed for reactions of radicals **a**−**c** with thymidine as compared to thymine and 1-methylthymine (Tables 3 and 5), suggesting abstraction of hydrogen atoms not only from the base but also from the sugar moiety. Finally, thymidine was found to react more efficiently than any of the other substrates studied (Tables 1, 3, and 5), including the individual sugars and nucleobases. This result is readily rationalized if both the sugar and the base moiety of the nucleoside are susceptible to radical attack.

The above results suggest that some hydrogen abstraction is occurring from the C5-methyl group in the base moiety of the nucleoside. This would result in the formation of the 2′-deoxy-5-methyleneuridin-5-yl radical, a species that has received recent scrutiny because of its known involvement in DNA damage.[35] This species has been shown to undergo hydrogen atom abstraction from hydrogen atom donors.[35g] Hence, it has been suggested that the 2′-deoxy-5-methyleneuridin-5-yl radical may be able to transfer damage to a sugar of an adjacent nucleotide and thus cause DNA cleavage.[35g] Our gas-phase results demonstrate that phenyl radicals are indeed capable of generating this species.

Increase in the electron deficiency of the radicals enhances the overall reactivity (i.e., the efficiencies of hydrogen abstraction and addition reactions increase). For example, the *N*-(3-dehydrophenyl)pyridinium radical **a** reacts by hydrogen abstraction at an efficiency of 10% (93% × 0.11 in Table 5), whereas the *N*-phenyl-3-dehydropyridinium radical **d** does so nearly 6 times as fast (58%). With respect to addition, an even greater extent of rate enhancement was observed. For example, radical **d** adds to thymidine 42 times faster than does radical **a**. These results suggest that the capacity of phenyl radicals to attack thymidine (and perhaps DNA) can be enhanced by increasing their electrophilicity.

Conclusions

The work reported here represents the first direct comparison of the reactivity of phenyl radicals of varying electrophilicities toward the sugar and base moieties of nucleic acids in the gas phase. The radicals were observed to undergo direct hydrogen atom abstraction from sugars, in qualitative agreement with the results reported[6c,9b] for the neutral phenyl radical and HO• in

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solution. Increasing the electron deficiency of the radical site (e.g., by adding electron-withdrawing groups to the meta-position with respect to the radical site) was found to facilitate the reaction. Differences in the thermodynamic driving force cannot rationalize the extent of rate enhancements, as the reaction exothermicities were found to be nearly independent of substitution on the radical. However, as observed previously for simple hydrogen atom donors,[14b] the rate enhancements among the radicals follow the trends in their electron affinities, which reflect their ability to stabilize the transition state via polar effects. Hence, a phenyl radical's ability to abstract a hydrogen atom from 2-deoxy-D-ribose in DNA can probably be facilitated by increasing its electrophilicity.

However, an increase in a phenyl radical's electrophilicity also leads to enhanced reactivity toward the nucleobases. Addition was found to be the predominant pathway in the reactions of the charged phenyl radicals with adenine and uracil, just as for $HO^{\bullet}$ in solution.[6c] C8 is calculated to be the thermodynamically favored site for addition to adenine by the radicals studied. The experimental results are in agreement with this addition site, but they do not unambiguously prove it. The radicals preferentially add to the C5-carbon in pyrimidines, in agreement with the results obtained for the $HO^{\bullet}$ radical.[6c] This finding supports the notion that C5 in pyrimidines is the likely site of addition for electrophilic radicals in general. The preference[9b] of the slightly nucleophilic phenyl radical to add to the C6 carbon in thymine is also in agreement with this thinking. However, the reported[9b] attack by the phenyl radical at the C5-carbon in uracil deviates from the above trend.

Surprisingly, hydrogen atom abstraction dominates the reactions of the electrophilic phenyl radicals with thymine and thymidine, although this reaction has not been reported for the neutral phenyl radical,[9b] and it is slow or absent for $HO^{\bullet}$.[6c,9b] The difference in reactivity among the different bases can be rationalized by the fact that, in thymine and thymidine, hydrogen atom abstraction from the C5-methyl group is highly exothermic and hence can compete efficiently with addition to the base. Increasing the electron deficiency of the radicals (as reflected by increasing electron affinity) was found to enhance the rates of both hydrogen atom abstraction and addition reactions. These results indicate that the reactivity of electrophilic phenyl radicals toward both sugar and base components of DNA, and hence their biological activity (i.e., ability to cleave DNA), can be enhanced through an increase in their electron deficiency.

The results obtained here confirm the earlier proposal[9b] that phenyl radicals can damage DNA directly via hydrogen atom abstraction from the sugar moiety, in addition to indirect damage via attack to the nucleobase. The findings further indicate that the reactivity and selectivity of aromatic σ-radicals toward DNA components are strongly dependent on the polarity of the reacting system. The less reactive, less electrophilic phenyl radicals are more selective toward hydrogen atom abstraction from the sugar moiety, while the more reactive radicals also cause substantial base damage. The sensitivity of the radical reactions to polar effects further suggest that biological substrates with low oxidation potentials (e.g., adenine) are likely to be most vulnerable toward radical damage.

Finally, we suggest that the increase in hydrogen abstraction ability observed[36] earlier upon protonation of some didehydropyridines, studied as desirable active moieties for new synthetic antitumor drugs, is at least partially due to polar effects and not solely due to changes in the biradicals' singlet−triplet splittings as proposed.[36] The possibility of pH control over the biological activity of basic phenyl mono- and biradicals encourages the continuing assessment of such species, especially because tumor cells are known to be relatively acidic.

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