Quantum Chemical Characterization of the Reactions of Guanine with the Phenylnitrenium Ion

Jerry M. Parks,[†,‡] George P. Ford,[†,§] and Christopher J. Cramer*[,||]

Department of Chemistry, Southern Methodist University, Dallas, Texas 75275-0314, and Department of Chemistry and Supercomputer Institute, University of Minnesota, 207 Pleasant St. SE, Minneapolis, Minnesota 55455-0431

cramer@chem.umn.edu

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Density functional calculations at the B3LYP/6-311+G(2d,p)//pBP/DN* level predict all cationic adducts combining guanine, at either its N2, O6, N7, or C8 positions, with phenylnitrenium ion, at either its N, 2, or 4 positions, to be lower in energy than the separated reactants. This relative stability of all adducts is preserved after addition of aqueous solvation free energies computed at the SM2 level, although some leveling of the adduct relative energies one to another is predicted. Cations having the lowest relative energies in solution correspond structurally to those adducts most commonly found when guanine reacts with larger, biologically relevant nitrenium ions in vitro and in vivo. One of these, the N−C8 adduct, is stabilized both by a rearomatized phenyl ring and by the operation of an anomeric effect not found in any of the others. On the basis of energetic analysis, direct conversion of an N−N7 cation to an N−C8 cation according to a previously proposed mechanism is unlikely; however, an alternative rearrangement converting a 2-N7 cation to an N−C8 cation via the intermediacy of a five-membered ring may be operative in nitrenium ions with aromatic frameworks better able than phenyl to stabilize endocyclic cationic charge.

Introduction

A common feature of chemical mutagens and carcinogens is their ability to covalently modify DNA base sites. Key mutations in specific genes, especially those controlling cell proliferation and tumor suppression, are believed to greatly increase the likelihood of eventual tumor formation.[1] The expectation that the nature of the initial DNA−carcinogen adduct has an important bearing on the propensity of the compound to lead to such mutations has motivated many careful in vitro and in vivo studies of this process. Of the three major classes of chemical carcinogens, (i) alkylating agents, (ii) polycyclic aromatic hydrocarbons, and (iii) aromatic amines and nitro compounds, the latter are by far the most complex in this respect.[2]

These compounds do not react with DNA directly but are first oxidatively converted to highly reactive arylnitrenium ions, $ArNR^+$. The kinds and amounts of adducts observed depend on the structures of both the aryl and the R groups. In many cases the dominant adduct isolated in vivo is a guanine C8 derivative, although this is frequently accompanied by smaller amounts of other adducts (see Figure 1 for purine numbering convention). The adduct arising from attack of the exocyclic guanine amino group on the arylamine ring is one such common secondary product.[3−6] Thus, for example, degradation of the DNA extracted from animals[7,8] or bacterial cells[9]

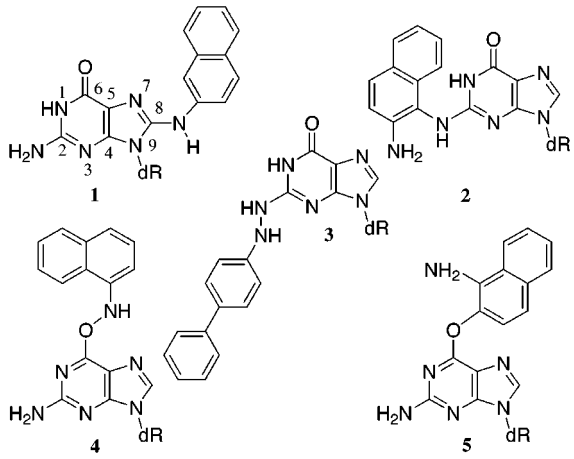

Figure 1. Guanine−nitrenium ion adducts isolated under various experimental conditions.

[†] Southern Methodist University.

[‡] Current address: Department of Chemistry, Duke University, Durham, NC 27706.

[§] Deceased.

[||] University of Minnesota.

(1) Josephy, P. D. *Molecular Toxicology*; Oxford University Press: New York, 1997; p 281.

(2) *DNA Adducts, IARC Scientific Publication No. 25*; Hemminki, K., Dipple, A., Shuker, D. E. G., Kadlubar, F. F., Segerbäck, D., Bartsch, H., Eds.; International Agency for Research on Cancer: Lyon, 1994.

(3) Kadlubar, F. F. In *DNA Adducts, IARC Scientific Publication No. 25*; Hemminki, K., Dipple, A., Shuker, D. E. G., Kadlubar, F. F., Segerbäck, D., Bartsch, H., Eds.; International Agency for Research on Cancer: Lyon, 1994; p 199.

(4) Segerbäck, D.; Bartsch, H. In *DNA Adducts, IARC Scientific Publication No. 25*; Hemminki, K., Dipple, A., Shuker, D. E. G., Kadlubar, F. F., Segerbäck, D., Bartsch, H., Eds.; International Agency for Research on Cancer: Lyon, 1994; p 217.

(5) Beland, F. A.; Marques, M. M. In *DNA Adducts, IARC Scientific Publication No. 25*; Hemminki, K., Dipple, A., Shuker, D. E. G., Kadlubar, F. F., Segerbäck, D., Bartsch, H., Eds.; International Agency for Research on Cancer: Lyon, 1994; p 229.

(6) Delclos, K. B.; Kadlubar, F. F. In *Comprehensive Toxicology*; Bowen, G. T., Fischer, S. M., Eds.; Elsevier: Oxford, 1997; Vol. 12, p 141.

(7) Kadlubar, F. F.; Anson, J. F.; Dooley, K. L.; Beland, F. A. *Carcinogenesis* **1981**, *2*, 467.

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exposed to *N*-hydroxy-2-aminonaphthalene—a precursor of the 2-naphthylnitrenium ion—yields modified deoxynucleosides **1** and **2** (Figure 1). The same adducts are found when DNA is treated in vitro with *N*-hydroxy-2-aminonaphthalene under mildly acidic conditions.[10]

N-Hydroxy-4-aminobiphenyl also reacts with DNA both in vivo and in vitro to give a guanine C8 adduct analogous to **1** as the major product. In this case the minor competing reaction of the guanine amino group occurs at the nitrenium nitrogen, generating hydrazine **3** as a minor product.[9] Adducts of the latter kind have also been detected in the reactions of benzidine, but seem to be unique to these aryl nuclei.[11] *N*-Hydroxy-1-aminonaphthalene reacts with DNA in vivo and in vitro in yet a different way. Here, only minor amounts of the guanine C8 adduct are formed.[12] The two principal adducts **4** and **5** involve substitution at the guanine O^6-position.[13]

The rationalization of the complex regiochemistry in the interactions of carcinogenic electrophiles with nucleic acid base sites is an important, and largely unrealized, goal in carcinogenesis research. The potential value of quantum chemical calculations in this connection was eloquently argued by Scribner a quarter of a century ago.[14] Since that time a number of useful generalizations based on qualitative ideas of molecular orbital theory have been made.[15−17] However, successful a priori prediction of nucleic acid base/carcinogen adduct distribution have so far been limited to a few simple alkylating agents using MNDO semiempirical molecular orbital calculations.[18−20] The regiochemical problem is inherently more complex for arylnitrenium ions. Most simple alkylation reactions take place through a single electrophilic center. The reaction regiochemistry therefore relates only to alternative base sites. For the arylnitrenium ions the regiochemical outcome is greatly complicated by the possibility of reactions at either the nitrenium ion nitrogen or one of several ring positions. Second, the relative reactivities of individual base sites toward alkylating agents are qualitatively similar regardless of whether the reaction takes place on the deoxynucleoside, with RNA, or with single- or double-stranded DNA.[21] The greater size and complexity of the nitrenium ion electrophiles make it far more likely that the observed regiochemistry will be influenced by the secondary and tertiary structure of the nucleic acid substrate. Some preliminary experimental work has begun to appear addressing the reactivity of isolated and helical nucleosides with arylnitrenium ions.[22−25]

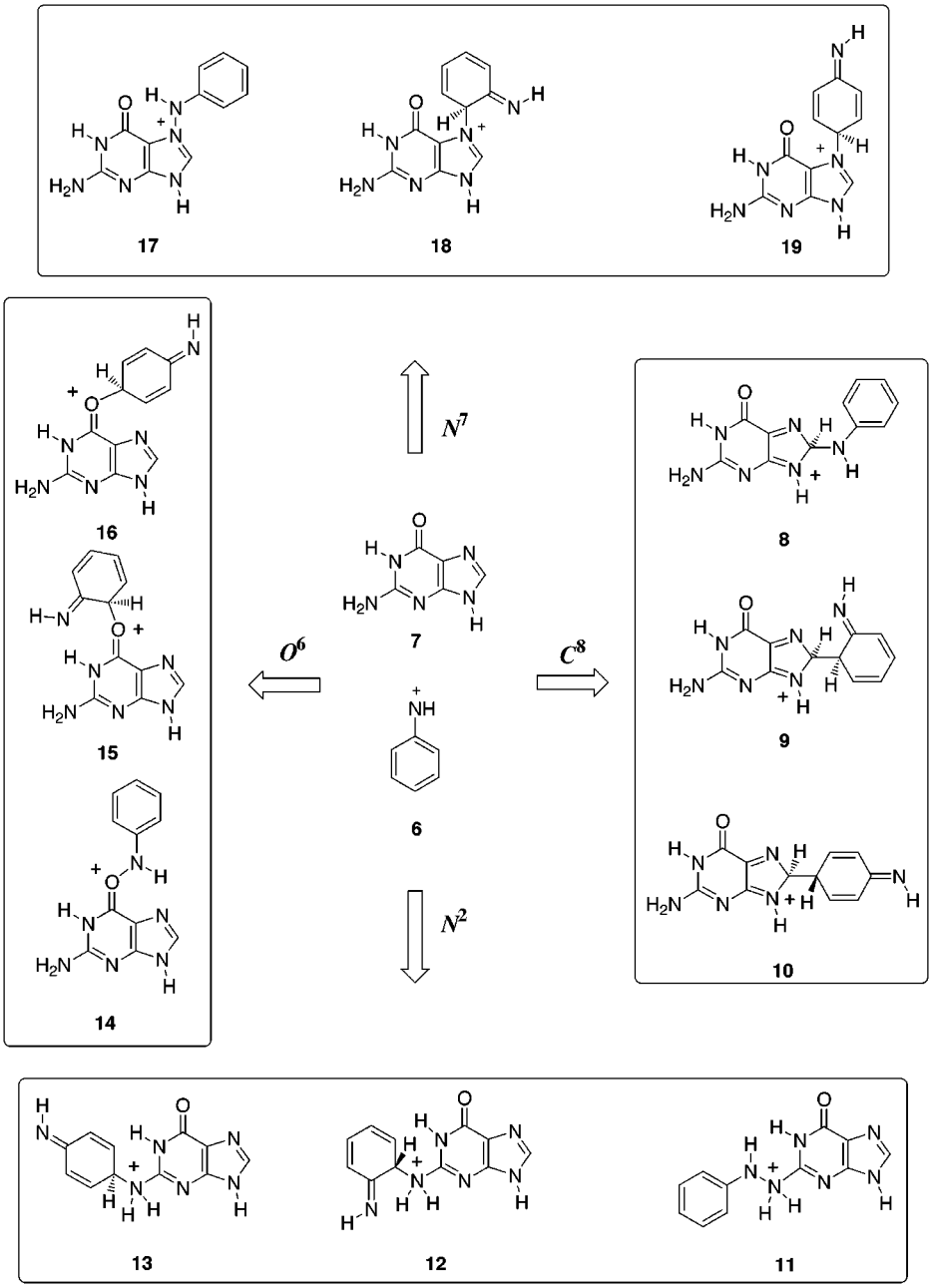
In this work, we examine whether there is a correlation between the most prevalent observed nitrenium ion adducts and the thermodynamic stability of the initial products of electrophilic attack leading to those adducts. Although aniline is generally regarded as a weak carcinogen[26] (the most potent carcinogenic precursors of arylnitrenium ions are those having fused or bridged aryl rings) the phenylnitrenium ion **6** provides a convenient starting point for theoretical study. The possible adducts with deoxyguanosine residues in nucleosides and nucleic acids, studied here as derivatives of the parent guanine **7**, are illustrated in Scheme 1. Of these, only products ostensibly derived from **8** have so far been observed for precursors of **6**.[27] However, analogues of all but **9** have been observed in reactions that are reasonably formulated as passing through arylnitrenium intermediates of one kind or another.

Computational Methods and Nomenclature. Unless otherwise stated, molecular geometries were fully optimized with no geometrical constraints. All transition state structures were identified by the presence of one imaginary frequency in analytic computations of the molecular force constants. Many of the molecules described herein can exist as multiple conformers. However, we approximate their population-averaged free energies using the electronic energy of a single conformer. That structure was chosen in the following manner. First, we carried out conformational analysis at the AM1[28] level, systematically searching about all rotatable bonds using an automated search algorithm. The four or five lowest energy structures at this level of theory were then reoptimized at the pBP/DN* level, this being a perturbative implementation[29] of a model using the exchange and correlation functionals of Becke[30] and Perdew,[31] respectively, and a numerical basis set, DN*,[32,33] that is designed to mimic the polarized split-valence Gaussian basis set 6-31G(d). The lowest energy structure from this level was chosen for subsequent use, and a single-point calculation at the B3LYP level of theory[34−36] using the 6-311+G(2d,p) basis set[33] was carried out for it.

To model aqueous solvation effects, Solvation Model 2 (SM2)[37,38] calculations were carried out for the lowest energy gas-phase species. The pBP/DN* geometries were

(8) Dooley, K. L.; Beland, F. A.; Bucci, T. J.; Kadlubar, F. F. *Cancer Res.* **1984**, *44*, 1172.
(9) Beland, F. A.; Beranek, D. T.; Dooley, K. L.; Heflich, R. H.; Kadlubar, F. F. *Environ. Hlth. Perspect.* **1983**, *49*, 125.
(10) Kadlubar, F. F.; Unruh, L. E.; Beland, F. A.; Straub, K. M.; Evans, F. E. *Carcinogenesis* **1980**, *1*, 139.
(11) Yamazoe, Y.; Zenser, T. V.; Miller, D. W.; Kadlubar, F. F. *Carcinogenesis* **1988**, *9*, 1635.
(12) Murofishi, Y.; Hashimoto, Y.; Sudo, K.; Okamoto, T. *Chem. Pharm. Bull.* **1981**, *29*, 2730.
(13) Kadlubar, F. F.; Miller, J. A.; Miller, E. C. *Cancer Res.* **1978**, *38*, 3628.
(14) Scribner, J. D. *J. Natl. Cancer Inst.* **1975**, *55*, 1035.
(15) Dipple, A.; Moschel, R. C.; Hudgins, W. R. *Drug Metab. Rev.* **1982**, *13*, 249.
(16) Dipple, A.; Moschel, R. C. *Prog. Clin. Biol. Res.* **1990**, *340A*, 71.
(17) Dipple, A. *Carcinogenesis* **1995**, *16*, 437.
(18) Ford, G. P.; Scribner, J. D. *J. Am. Chem. Soc.* **1983**, *105*, 349.
(19) Ford, G. P.; Scribner, J. D. *Chem. Res. Toxicol.* **1990**, *3*, 219.
(20) Ford, G. P. *J. Mol. Struct. (THEOCHEM)* **1997**, *401*, 253.
(21) Singer, B. *Nucleic Acids Res.* **1975**, *15*, 219.
(22) McClelland, R. A.; Kahley, M. J.; Davidse, P. A. *J. Phys. Org. Chem.* **1996**, *9*, 355.
(23) McLelland, R. A.; Gadosy, T. A.; Ren, D. *Can. J. Chem.* **1998**, *76*, 1327.
(24) Novak, M.; Kennedy, S. A. *J. Phys. Org. Chem.* **1998**, *11*, 71.
(25) De Riccardis, F.; Bonala, R. R.; Johnson, F. *J. Am. Chem. Soc.* **1999**, *121*, 10453.
(26) Sax, N. I. *Cancer Causing Chemicals*; Van Nostrand Reinhold: New York, 1981.
(27) Marques, M. M.; Mourato, L. L. G.; Santos, M. A.; Beland, F. A. *Chem. Res. Toxicol.* **1996**, *9*, 99.
(28) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
(29) St-Amant, A. *Rev. Comput. Chem.* **1996**, *7*, 217.
(30) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098.
(31) Perdew, J. P. *Phys. Rev. B* **1986**, *33*, 8822.
(32) *SPARTAN version 5;* Wavefunction, Inc.: Irvine, CA, 1998.
(33) Hehre, W. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory;* Wiley: New York, 1986.
(34) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
(35) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
(36) Stephens, P. J.; Devlin, F. J.; Ashwar, C. S.; Bak, K. L.; Taylor, P. R.; Frisch, M. J. In *Chemical Applications of Density Functional Theory*; Laird, B. B., Ross, R. B., Ziegler, T., Eds.; ACS Symposium Series; Vol. 629; American Chemical Society: Washington, DC, 1996; p 105.
(37) Cramer, C. J.; Truhlar, D. G. *Science* **1992**, *256*, 213.
(38) Cramer, C. J.; Truhlar, D. G. *J. Comput.-Aid. Mol. Des.* **1992**, *6*, 629.

Scheme 1



kept frozen for these calculations. We adopt the following notation for the various levels of theory:

$$\text{AM1} = \text{AM1}$$

$$\text{pBP} = \text{pBP/DN*}$$

$$\text{B3LYP} = \text{B3LYP/6-311+G(2d,p)//pBP}$$

$$\text{B3LYP + SM2} = \text{B3LYP} + (\text{SM2//pBP} - \text{AM1//pBP})$$

where the term in parentheses in the final expression defines the SM2 solvation free energy for the pBP geometry.

B3LYP calculations were carried out using the Gaussian 94 program suite.[39] Calculations at all other levels of theory were accomplished using the Spartan version 5.0 molecular modeling package.[32]

To facilitate comparison, we will refer to the various guanine/phenylnitrenium ion adducts not only by their number in Scheme 1, but also by the two positions at which they are joined. For **6**, possible attachment points are the exocyclic N and ring positions 2 and 4. For **7**, the possible attachment points are N2, O6, N7, and C8. Thus, for example, 2-C8 refers to the product of electrophilic attack by phenylnitrenium C2 on the C8 position of guanine, i.e., **9**.

Results and Discussion

We begin with an analysis of the relative energetics of the different condensation products, both in the gas phase and in aqueous solution, focusing not only on the chem-

(39) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T. A.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Peng, C. Y.; Ayala, P. A.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 94 RevE.2;* Gaussian Inc.: Pittsburgh, PA, 1995.

Table 1. Energies (kcal mol^{-1}) of Separated 6 and 7 and All Adducts Relative to 8[a]

species	AM1	pBP	B3LYP	B3LYP+SM2
6 + **7**	46.4	51.5	47.2	36.1
8, N−C8	0.0	0.0	0.0	0.0
9, 2-C8	8.3	17.8	17.2	21.1
10, 4-C8	19.9	22.3	21.6	21.9
11, N−N2	33.4	30.7	32.2	23.7
12/**12***, 2-N2	26.0	11.2[b]	13.2[b]	2.4[b]
13, 4-N2	32.0	37.6	38.3	33.3
14, N−O6	27.8	18.9	19.6	22.6
15/**15***, 2-O6	10.8	4.1[b]	7.9[b]	6.7[b]
16, 4-O6	15.6	14.5	14.1	17.8
17, N−N7	4.7	−1.5	−1.5	−3.5
18, 2-N7	0.8	0.5	0.5	1.2
19, 4-N7	5.6	1.9	2.1	5.6

[a] Absolute AM1 (kcal mol^{-1}), and pBP and B3LYP (E_h) energies: **6**, 246.9, −286.743 51, −286.746 79; **7**, 48.8, −542.771 96, −542.722 69; **8**, 249.3, −829.597 50, −829.544 64. All other absolute energies may be computed therefrom. The SM2 solvation free energy for **8** is −65.1 kcal mol^{-1}. [b] Energy of adduct following spontaneous proton shift.

istry but also on the relative utility of the lower levels of electronic structure theory as compared to the B3LYP level. We then examine two possible nitrenium ion adduct rearrangements that have been proposed in the literature to determine whether they are likely to be energetically feasible.

Table 1 lists the gas-phase energies for infinitely separated guanine and phenylnitrenium ion and for all of the various adducts in Scheme 1 relative to the most commonly found experimental adduct, **8**. The comparison is made for all three levels of gas-phase theory. Figure 2 presents the relative energies from the highest level of gas-phase theory, B3LYP, in a histogram-like format. Table 1 also contains relative energies in aqueous solution at the B3LYP+SM2 level.

In the case of adducts **12** and **15**, the structures drawn in Scheme 1 proved to be unstable with respect to (barrierless) proton transfers to the original nitrenium nitrogen atom. These tautomerizations are illustrated in Figure 3. Estimates of the driving forces behind each rearrangement were obtained by constraining the otherwise broken N−H bond to remain fixed at 1.055 Å (a value derived from consideration of other higher energy conformers that *were* stable to tautomerization, although their higher energy made them uninteresting from a chemical point of view). Partial optimizations subject to this constraint led to structures for **12** and **15** that were roughly 15 and 5 kcal mol^{-1} higher in energy, respectively, at either the pBP or the B3LYP level of theory. Subsequent discussion will distinguish between the pre- and post-tautomerization structures by appending an asterisk to the relevant name when the latter is indicated.

Trends in Gas-Phase Relative Energies. All adducts are computed to be lower in energy than separated **6** and **7**. In the gas phase this result is by no means surprising insofar as the system bears a positive charge. It is possible that stable ion−molecule complexes exist that are lower in energy than some of the highest energy adducts, but we did not attempt to find them. For the lowest energy adducts, on the other hand, given the degree to which charge becomes increasingly delocalized along the condensation pathway, it seems unlikely that such complexes would intervene in what would otherwise be a barrierless addition.

It is apparent from inspection of Table 1 that the least favorable mode of addition is that involving electrophilic attack at N2 of guanine when there is no base to which to transfer a proton from the developing ammonium ion. In products **11**, **12**, and **13**, the charged position, being quaternary, cannot be stabilized by resonance delocalization. This is presumably the reason for the substantially higher energies of these adducts (using the AM1 level as a best estimate for **12**, since it is reasonably accurate for **11** and **13**, and higher levels of theory are not available owing to the spontaneous tautomerization described above).

For the remaining three sites of guanine attack considered here—O6, N7, and C8—additional effects beyond the particular site can influence the relative energetics significantly. Thus, for example, the 2-C8 and 4-C8 adducts **9** and **10** are predicted to be quite high in relative energy, consistent with the observation that formation of these adducts destroys the aromaticity of the imidazole ring of guanine by making the C8 position quaternary. However, the N−C8 adduct **8**, which also sacrifices imidazole aromaticity, is predicted to be more stable than all other adducts with the exception of **17**. The roughly 20 kcal mol^{-1} of additional stability this molecule possesses compared to **9** and **10** must be attributable in part to the aromaticity reestablished in the nitrenium ion benzene ring after condensation to the nitrenium ion nitrogen. This energetic effect is presumably even larger than the observed difference, but is offset slightly by the expected difference in energetics for the "typical" C−C vs C−N single bonds that differentiate adducts **8**, **9**, and **10**.

In the O6 and N7 adducts, the analogous bond energy differences are considerably larger: N−N and N−O bonds, like those that would be formed following electrophilic attack by the nitrenium nitrogen, are significantly weaker than the corresponding C−N and C−O bonds that would be formed from condensation involving the aromatic ring of **7**, so the benefits of recovered aromaticity are substantially reduced. Thus, although N−N7 **17** is indeed the lowest energy adduct, the margin separating it from its 2- and 4-analogues is much smaller than is the situation for C8 adducts. In the O6 adducts, the N−O bond is sufficiently weak that **15** and **16** are both lower in energy than **14**. In the case of **15**, at the AM1 level it is stabilized by a strong hydrogen bond from H(N1) to the adduct imine nitrogen—at the DFT level, that proton spontaneously transfers to give the more stable tautomer **15***. Hydrogen bonds are found in selected other structures, but appear to play less of a role in stabilization since geometric constraints typically prevent them from attaining anything approaching a preferred linear arrangement.

The surprisingly large stability of **8** merits additional comment. We note that **8** is unique in permitting the possibility for anomeric stabilization of the charge by delocalization of the lone pair density on the formerly nitrenium nitrogen into an accepting σ^*_{C-N} orbital (Figure 4).[40] Geometric analysis of the minimum energy structure suggests this is an important effect. The exocyclic C−N bond, at 1.422 Å, is very short for a single

(40) Cramer, C. J. In *The Encyclopedia of Computational Chemistry*; Schleyer, P. v. R., Allinger, N. L., Clark, T., Gasteiger, J., Kollman, P. A., Schaefer3, H. F., Schreiner, P. R., Eds.; John Wiley & Sons: Chichester, 1998; p 1294.

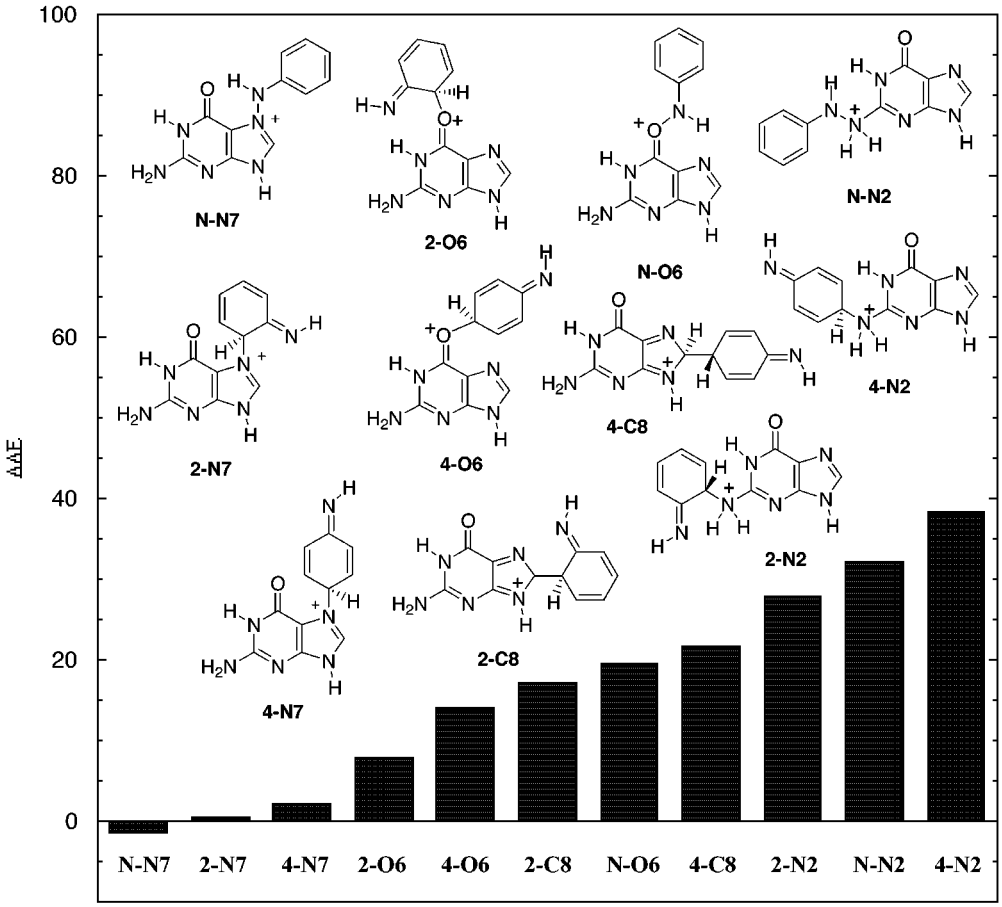


Figure 2. Energies of different adducts relative to N−C8 at the B3LYP level.

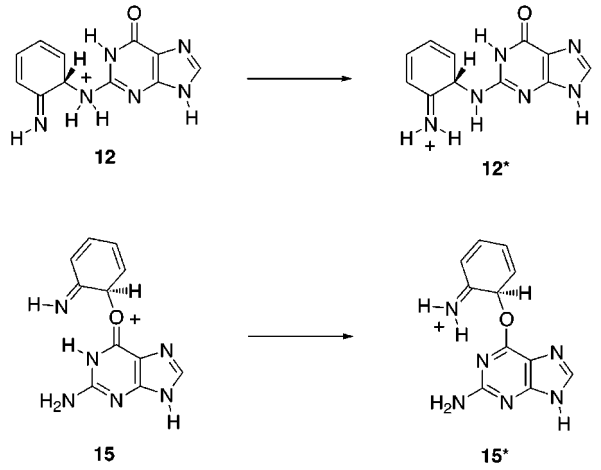


Figure 3. Tautomerizations of **12** and **15** predicted to occur spontaneously at the pBP level of theory.

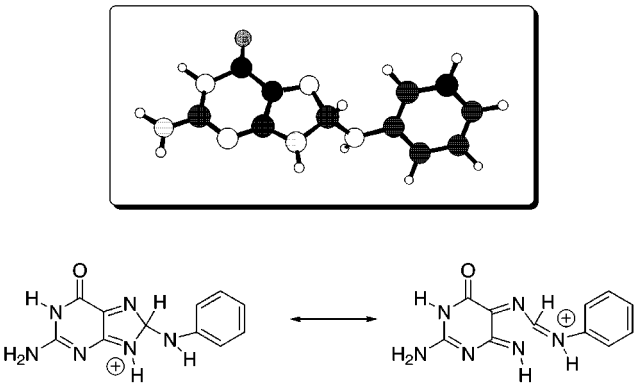


Figure 4. The optimized structure at the pBP level, inset, is consistent with the indicated anomeric stabilization of **8**.

bond, while the accepting endocyclic bond is quite long, at 1.495 Å (cf. 1.467 Å for the other endocyclic C−N bond in the five-membered ring); this is consistent with the large contribution of a formal double-bond/no-bond resonance structure to the molecular character. In addition, the sum of the valence bond angles about the donor nitrogen atom is 351.9 deg; such a strong displacement toward planarity for an atom otherwise expected to be pyramidal is another strong indication of anomeric stabilization. Finally, the torsion angle about the exocyclic C−N bond is essentially that which maximizes the overlap necessary for anomeric stabilization. So, while there is no simple way to quantify the magnitude of the anomeric stabilization as it contributes to the overall stability of this isomer, its large geometric manifestation argues for its importance in an energetic sense as well.

Indeed, the unique anomeric interaction in **8** may be responsible for the curiously high degree of nitrenium N-alkylation exhibited by guanine. Most simple nucleophiles, e.g., methoxide or azide, preferentially substitute either the 2 or 4 positions of the aromatic ring in arylnitrenium ions. In these cases, N-substitution fails to introduce an anomeric stabilization, and bond-strength considerations presumably dominate the reactivity, thus mitigating against N-substitution.

Trends in Relative Energies in Solution. Relative to adduct **8** (the arbitrary zero of energy), the effects of

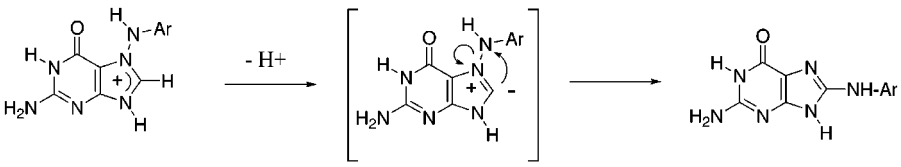


Figure 5. Proposed ylide rearrangement path from N−N7 to N−C8 product.

aqueous solvation are most notable in providing significant stabilization to N2 adducts; lesser effects are seen for other isomers. Overall, the influence of solvation is to stabilize those isomers having more concentrated charge relative to those with more delocalized charge. Thus, the N2 adducts **11** and **13** are formally quaternary ammonium cations, and such localized charge makes them 5 to 8 kcal mol^{-1} better solvated than, say, **8**, which can be drawn as a number of different charge localized resonance structures. Still more resonance structures can be drawn for O6 adducts, and these are among the least well solvated species owing to the more diffuse nature of their positive charge.

A separate consideration in the magnitude of the solvation free energies is the exposure of the group(s) carrying charge. Thus, the best solvated species is **12***. In this isomer, the charge is reasonably concentrated in the iminium functionality and it is moreover highly exposed to the surrounding solvent. Isomer **15*** enjoys similarly favorable solvation. Other isomers show fairly small perturbations in their energies relative to **8** as a result of solvation, the differential delocalization and exposure effects either being small in magnitude or being of roughly equal magnitude and opposite sign.

It is important to remember that the influence of a surrounding DNA double helix need not by any means be like the predicted influence of a homogeneous high-dielectric solvent like water. Nevertheless, it is useful to understand the fundamentals of solvation as it affects relative adduct energies.

Possible Rearrangement Pathways. The trends in relative energies for different adducts examined here tracks qualitatively well with those species isolated in the majority of experimental studies, which is an interesting and provocative result. A key exception to this trend, though, are the low energies of N7 adducts, which are only rarely seen experimentally. Of course, it must be borne in mind that there is no a priori requirement that the relative cation product energies precisely parallel those of the rate-determining transition states for the processes leading to their formation. However, the fact that there *is* some qualitative agreement along these lines suggests that either the product distribution reflects an overall thermodynamic control, or that there is a linear-free-energy-like relationship between activation free energies, if there are any, and product energies. In the absence of activation barriers (i.e., if the condensations proceed at the diffusion limit), the correlation between qualitative branching ratios and cation product energies must simply be accepted at face value.

Were there to be barriers to condensation, and were the products predicted from kinetic control based on these barriers to be different than those predicted from the thermodynamics of the products, then the experimentally observed product mixtures would require some sort of mechanism for conversion of the initially formed kinetic products to the thermodynamic ones. The most obvious such path is, of course, product fragmentation and recondensation until equilibrium is reached, but this hardly seems likely given the fairly high energy of the separated reactants compared to most of the products, i.e., condensation is effectively irreversible. In certain instances, however, plausible intramolecular rearrangement pathways are available that might conceivably interconvert products. We examine two of those here.

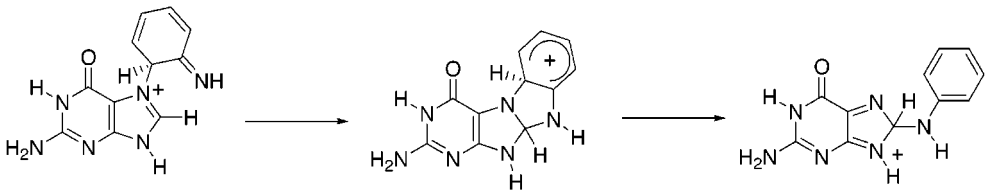
In 1992 Humphreys et al.[41] proposed that initial C8 adduct formation was unlikely insofar as this carbon would be expected to be a weaker nucleophile than the adjacent N7. To arrive at C8-substituted products, they suggested a multistep pathway in which initial adduct formation takes place at N7, resulting in a postulated increase in the acidity of the C8 proton. Subsequent deprotonation at C8 yields an ylide intermediate that may undergo a 1,2-shift of the arylamine functionality so as to rearrange to the more biologically prevalent C8 adduct (Figure 5).

Humphreys et al.[41] noted as an alternative to the ylide 1,2-shift pathway a nucleophilic attack by the arylamine nitrogen on C8 to form a diaziridine intermediate, followed by N−N bond scission to deliver **8**, but discounted the likelihood of such a process given the significant strain expected in the three-membered ring. Kennedy et al.,[42] considering the same product interconversion, also discounted the likelihood of a diaziridine intermediate, but pointed out that a 1,2-shift in the initial cation was not necessarily unreasonable given the resonance delocalization available to product **8**.

Given the similar energies of **8** and **17** in both the gas phase and solution, we chose to examine the direct cationic 1,2-rearrangement process. However, all attempts to generate a reaction coordinate (e.g., by either forcing ring closure in increments of 0.1 Å, or elongating the existing bond by similar increments) ultimately led only to species having energies in excess of separated products without the intervention of any discernible transition state structure(s). We take this as indication that the only direct pathway interconverting **8** and **17** is dissociation/recombination. We have not examined the same rearrangement in the ylide owing to the very large uncertainty that would be associated with calculating the pK_a of the C8 proton in **17**.

An alternative route to **8** that derives from rearrangement of an initial N7 product is available starting from **18**. As illustrated in Figure 6, closure of **18** to create a resonance-stabilized diazolidine cation **20** may be followed by C−N bond scission to generate **8** (note that an ylide analogue of this process without the intermediacy of the five-membered ring is also possible, but we do not examine it here, again because the predicted pK_a of the C8 proton in **18** would not be expected to be particularly

(41) Humphreys, W. G.; Kadlubar, F. F.; Guengerich, F. P. *Proc. Natl. Acad. Sci., USA* **1992**, *89*, 8278.
(42) Kennedy, S. A.; Novak, M.; Kolb, B. A. *J. Am. Chem. Soc.* **1997**, *119*, 7654.



18 **20** **8**

Figure 6. Proposed rearrangement path from 2-N7 to N−C8 product.

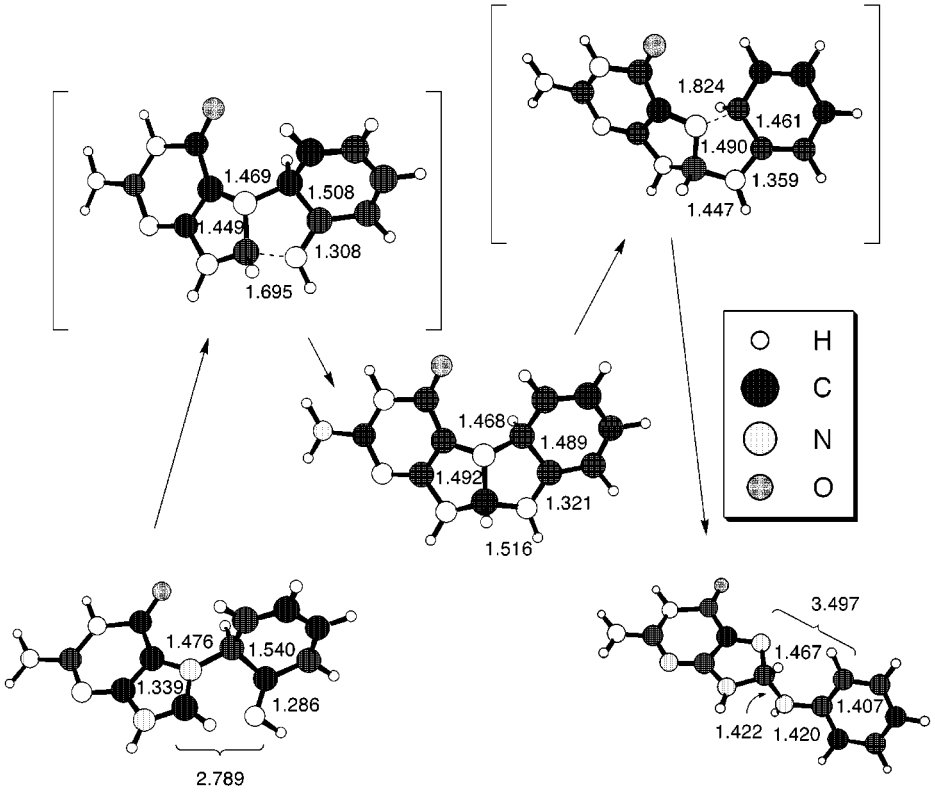


Figure 7. Structures of stationary points at the pBP level for the five-membered ring rearrangement process. Bond distances (ang) in that ring are provided.

reliable). In this instance, intermediate **20** is predicted to be more stable than separated reactants, having a predicted energy of 22 kcal mol^{-1} relative to **8** in aqueous solution. The transition state structures corresponding to initial ring closure and final ring cleavage also have energies somewhat below separated reactants, 28 and 31 kcal mol^{-1} relative to **8**, respectively, but the relative energy of the rate-determining second TS structure is sufficiently high that this unimolecular rearrangement would be expected to be slow at biological temperatures. Structures for the various stationary points along the rearrangement pathway are provided in Figure 7. The bond lengths in the five-membered ring are entirely consistent with the formalism invoked by the line structures in Figure 6.

At least for aniline, then, the energetics suggest that C8 products are more likely to derive from direct substitution of the C8 position, and not from rearrangement, in analogy with recent laser flash photolysis results of McClelland et al.,[43] which have been interpreted to support direct formation of a C8 product with the 2-fluorenylnitrenium ion. However, in cases where the intermediate cation analogue of **20** may be more highly stabilized by additional delocalization (e.g., in a biphenyl- or naphthylnitrenium[44]), the five-membered ring rearrangement pathway could be more energetically accessible and account for some N7 to C8 product transformation. Such rearrangement might partially explain the general failure to observe N7 products experimentally, even though they are predicted to be especially stable as primary adducts with phenylnitrenium as noted above.

Comparison of Theoretical Models. Although phenylnitrenium is one of the smallest possible aromatic nitrenium ions, nevertheless, the molecules studied here are rather large from a practical computational standpoint. As such, the identification of efficient levels of theory that may prove useful for at least qualitative purposes is a worthwhile effort. Of the three levels of theory presented thus far, the two DFT levels are clearly the most theoretically complete, and they give results that are fairly similar. Taking the B3LYP gas-phase energies relative to **8** as a standard, the mean unsigned error in the pBP results for the remaining 12 entries in

(43) McClelland, R. A.; Ahmad, A.; Dicks, A. P.; Licence, V. E. *J. Am. Chem. Soc.* **1999**, *121*, 3303.

(44) Falvey, D. E. Unpublished results.

Table 1 is 1.2 kcal mol^{-1}. The largest differences between the two levels of theory occur for separated reactants, and for the rearranged products **12*** and **15***. Thus, at least for these cationic species, the faster pure DFT method with a modestly sized basis set is a useful substitute for the B3LYP level.

Interestingly, the semiempirical AM1 level also has some semiquantitative utility. The same comparison above, using B3LYP energies for constrained structures **12** and **15** to compare to their unrearranged AM1 analogues, gives a mean unsigned error of 3.6 kcal mol^{-1}. With the exception of **9**, most of the larger errors are associated with very high energy structures, which are of minor chemical interest in any case; the source of the error in **9** is not obvious. The performance of AM1 in this regard is better than the considerably more expensive HF/3-21G and HF/6-31G* levels of theory, both of which we examined but whose results are not presented here because of their limited utility.

This analysis suggests that AM1 will continue to be useful in studies of nitrenium ion reactivity, at least as a first step in prioritizing further study of low-energy vs high-energy processes. Of equal importance, the utility of the AM1 energies suggests that the AM1 charge distributions are probably reasonable. As these charge distributions are used in computing SM2 solvation free energies, such support for their likely quality bolsters confidence in the aqueous solution results determined as B3LYP+SM2.

Conclusions

Both in the gas phase and in aqueous solution, all cationic adducts combining guanine and phenylnitrenium ion at either N2, O6, N7, or C8 of the former and the N, 2, or 4 positions of the latter are predicted to be lower in energy than the separated reactants. Aqueous solvation effects level the relative energies of different adducts, since in the gas phase delocalized charge is favored, while in the condensed phase adducts having greater charge concentration are better solvated. Those adducts having the lowest relative energies in aqueous solution are analogues of those adducts most commonly found experimentally when guanine reacts with larger, biologically relevant nitrenium ions, except for N7 adducts, which are predicted to be very stable but are only rarely isolated.

The stability of the N−C8 adduct is rationalized as deriving from its having a rearomatized phenyl ring, but also from the operation of an anomeric effect not present in any of the other adducts. This unique interaction potentially also rationalizes the preference exhibited by nucleophiles other than guanine reacting with arylnitrenium ions to substitute the aromatic ring rather than nitrogen, since in most of those cases N-substitution introduces no such anomeric interaction.

Examination of 2 rearrangement pathways from N7 adducts to the N−C8 adduct suggest that direct conversion of an N−N7 cation to an N−C8 cation is unlikely to take place, except possibly as a dissociation/reassociation process, but a five-membered ring-closure/ring-opening process converting 2-N7 cation to N−C8 cation may be energetically accessible in nitrenium ions having significant charge stabilizing potential within their aromatic frameworks. This observation may partly explain why, as noted above, isolation of N7 adducts is rare.

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Supporting Information Available: Optimized pBP/DN* geometries for structures **8**−**20**. This material is available free of charge via the Internet at http://pubs.acs.org.

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