ORGANIC LETTERS

2008
Vol. 10, No. 10
1889−1892

Gas-Phase Reactivity of Aromatic σ,σ-Biradicals toward Dinucleoside Phosphates

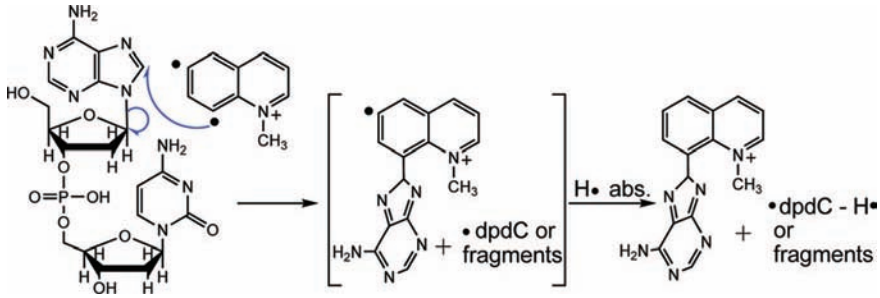
Linan Yang, John J. Nash, Michael J. Yurkovich, Zhicheng Jin, Nelson R. Vinueza, and Hilkka I. Kenttämaa*

Department of Chemistry, Purdue University, West Lafayette, Indiana 47907

hilkka@purdue.edu

Received February 11, 2008

ABSTRACT



In order to improve the understanding of the interactions of aromatic σ,σ-biradicals with DNA, the reactivity of three isomeric σ,σ-biradicals toward four dinucleoside phosphates was studied in a mass spectrometer. The dinucleoside phosphates were evaporated into the mass spectrometer by using laser-induced acoustic desorption (LIAD). The results demonstrate that the structure of the σ,σ-biradical and the base sequence of the dinucleoside phosphate can have a major influence on these reactions.

Aromatic σ,σ-biradicals with a 1,4-relationship between the radical sites are the reactive intermediates of the most potent antitumor antibiotics found thus far, the enediyne class of nonhydrolytic DNA cleavers.[1] Unfortunately, the clinical use of these drugs is hindered by their high cytotoxicity. Molecular level knowledge of the key process, the reaction of the σ,σ-biradical intermediate with DNA, could facilitate the rational design of less toxic synthetic DNA-cleaving agents. These agents might not be limited to those whose action is based on the naturally occurring 1,4-biradical intermediates and analogues but may also include "unnatural" biradicals. However, experimental studies of the reactions of biradicals with DNA are extremely challenging due to the very short lifetimes of the biradicals in solution and the complexity of DNA.

Intrinsic chemical properties of highly reactive molecules can be studied by using experiments carried out in high vacuum. Previously, we have examined the gas-phase reactivity of selected σ-monoradicals[2] and σ,σ-biradicals[3] toward individual DNA components, such as riboses and nucleobases, by using Fourier-transform Ion Cyclotron Resonance (FT-ICR) mass spectrometry and the distonic ion approach[4] (i.e., by affixing a charged moiety to the biradical

(1) See, for example: (a) Nicolaou, K. C.; Dai, W.-M. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1387. (b) Nicolaou, K. C.; Dai, W.-M.; Tsay, S.-C.; Estevez, V. A.; Wrasidlo, W. *Science* **1992**, *256*, 1172. (c) Borders, D. B.; Doyle, T. W. Eds. *Enediyne Antibiotics as Antitumor Agents*; Marcel Dekker: New York, 1995. (d) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089. (e) Wenk, H. H.; Winkler, M.; Sander, W. *Angew. Chem., Int. Ed.* **2003**, *42*, 502.

(2) Liu, J.; Petzold, C. J.; Ramírez-Arizmendi, L. E.; Pérez, J.; Kenttämaa, H. I. *J. Am. Chem. Soc.* **2005**, *127*, 12758.

(3) (a) Adeuya, A.; Yang, L.; Amegayibor, F. S.; Nash, J. J.; Kenttämaa, H. I. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 1325. (b) Yang, L. Ph.D. Thesis, Purdue University, 2007.

(4) See, for example: (a) Chyall, L. J.; Kenttämaa, H. I. *J. Am. Chem. Soc.* **1994**, *116*, 3135. (b) Smith, R. L.; Kenttämaa, H. I. *J. Am. Chem. Soc.* **1995**, *117*, 1393. (c) Petucci, C.; Nyman, M.; Guler, L.; Kenttämaa, H. I. *J. Am. Chem. Soc.* **2002**, *124*, 4108. (d) Amegayibor, F. S.; Nash, J. J.; Lee, A. S.; Thoen, J.; Petzold, C. J.; Kenttämaa, H. I. *J. Am. Chem. Soc.* **2002**, *124*, 12066.

10.1021/ol800312g CCC: $40.75
Published on Web 04/23/2008

Table 1. Reactions of (Bi)radicals **a**−**d** with Selected Dinucleoside Phosphates

	a	b	c	d
dApdG	no reaction observed	no reaction observed	A[a] abs (44%)[b] G[c] abs (43%) G-H• abs (13%)	H• abs (46%) A-H• abs (19%) A abs (11%) G-H• abs (12%) G abs (12%)
dApdC	no reaction observed	no reaction observed	A abs (53%) C[d] abs (28%) C-HNCO abs (19%)	H• abs (60%) C abs (14%) A-H• abs (14%) A abs (12%)
dCpdT	no reaction observed	no reaction observed	C abs (73%) C-HNCO abs (27%)	H• abs (88%) abs (12%)
dTpdT	no reaction observed	no reaction observed	no reaction observed	H• abs (100%)

[a] A = adenine. [b] Relative product abundances are given in parentheses. [c] G = guanine. [d] C = cytosine.

of interest to permit its mass spectrometric manipulation and analysis). These studies demonstrated that charged gas-phase mono- and biradicals yield the same products as has been reported for analogous neutral mono- and biradicals in solution.

Singlet−triplet gap (S−T gap; the energy difference between the lowest energy singlet and triplet states) and electron affinity (EA; the energy released upon addition of an electron to the radical site) are two important factors that control the reactivities of the singlet biradicals discussed above. As the S−T gap increases, *radical* reactivity decreases due to the need to partially uncouple the biradical electrons in the transition state.[3,5] Hence, σ,σ-biradicals with large S−T gaps are usually either unreactive or attack riboses and nucleobases via nonradical pathways.[3,6] On the other hand, the polarity of the transition state for a radical reaction depends on the biradical's EA. Hence, biradicals with a larger EA tend to react faster. A large EA may even counterbalance the reduction in radical reactivity caused by a large S−T gap.[3]

Recently, we demonstrated that laser-induced acoustic desorption (LIAD) can be used to evaporate thermally labile biomolecules into the gas phase as intact neutral molecules.[7] This technique allows us to extend our biradical studies to dinucleoside phosphates, and hence to start exploring the factors that influence biradicals' reactions with molecules containing all the three DNA components (base, sugar and phosphate moieties). The results presented here demonstrate that the structure of the σ,σ-biradical and the base sequence of the dinucleoside phosphate can have a major influence on these reactions.

Reactions of selected dinucleoside phosphates (Table 1; obtained from Sigma-Aldrich) with three isomeric σ,σ-biradicals, *N*-methyl-5,8-didehydroisoquinolinium cation (**a**, a *p*-benzyne analogue), *N*-methyl-5,7-didehydroquinolinium cation (**b**, a *m*-benzyne analogue), *N*-methyl-6,8-didehydroquinolinium cation (**c**, another *m*-benzyne analogue), and a related monoradical (**d**; Figure 1), were examined in a 3 T

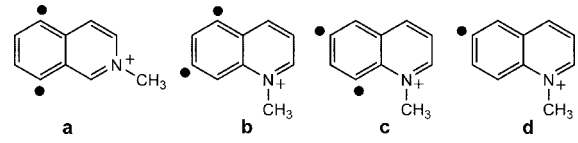


Figure 1. Bi- and monoradicals studied.

Nicolet model FTMS 2000 dual-cell FT-ICR mass spectrometer.[8] Biradicals **b** and **c** have been generated previously.[3] Biradical **a** was generated in an analogous manner from 5-iodo-8-nitroisoquinoline synthesized according to a literature method.[9] These three biradicals were selected because they can be generated in high abundances. In order to avoid the possibility of proton transfer reactions (the dinucleoside phosphates are highly basic), the (bi)radicals were *N*-methylated in this study. It should be noted that even though the charged site in these (bi)radicals is chemically unreactive, it nevertheless does influence the observed reactions via inductive effects. This issue is addressed in detail in the literature.[10,11]

The *p*-benzyne analogue **a** (calculated S−T gap: −5.0 kcal/mol[12]) does not yield observable products upon interac-

(5) (a) Schottelius, M. J.; Chen, P. *J. Am. Chem. Soc.* **1996**, *118*, 4896. (b) Logan, C. F.; Chen, P. *J. Am. Chem. Soc.* **1996**, *118*, 2113. (c) Hoffner, J.; Schottelius, M. J.; Feichtinger, D.; Chen, P. *J. Am. Chem. Soc.* **1998**, *120*, 376. (d) Roth, W. R.; Hopf, H.; Wasser, T.; Zimmermann, H.; Werner, C. *Liebigs Ann.* **1996**, 1691.

(6) (a) Nelson, E. D.; Artau, A.; Price, J. M.; Kenttämaa, H. I. *J. Am. Chem. Soc.* **2000**, *122*, 8781. (b) Nelson, E. D.; Artau, A.; Price, J. M.; Tichy, S. E.; Jing, L.; Kenttämaa, H. I. *J. Phys. Chem. A* **2001**, *105*, 10155.

(7) (a) Pérez, J.; Ramírez-Arizmendi, L. E.; Petzold, D. J.; Guler, L. P.; Nelson, E. D.; Kenttämaa, H. I. *Int. J. Mass Spectrom.* **2000**, *198*, 173. (b) Petzold, D. J.; Ramírez-Arizmendi, L. E.; Heidbrink, J. L.; Pérez, J.; Kenttämaa, H. I. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 192.

(8) (a) Campbell, J. L.; Fiddler, M. N.; Crawford, K. E.; Gqamana, P. P.; Kenttämaa, H. I. *Anal. Chem.* **2005**, *77*, 4020. (b) Crawford, K. E.; Campbell, J. L.; Fiddler, M. N.; Duan, P.; Qian, K.; Gorbaty, M. L.; Kenttämaa, H. I. *Anal. Chem.* **2005**, *77*, 7916.

(9) Gamage, S. A.; Spicer, J. A.; Rewcastle, G. W.; Milton, J.; Sohal, S.; Dangerfield, W.; Mistry, P.; Vicker, N.; Charlton, P. A.; Denny, W. A. *J. Med. Chem.* **2002**, *45*, 740.

(10) Petzold, C. J.; Nelson, E. D.; Lardin, H. A.; Kenttämaa, H. I. *J. Phys. Chem. A* **2002**, *106*, 9767.

tion with any of the dinucleoside phosphates studied here. This finding is not entirely unexpected since this *p*-benzyne is unreactive toward riboses and reacts slowly via adduct formation with thymine, cytosine, and adenine.[3] Further, the reactivity of **a** may be influenced by ring-opening (retro-Bergman) within the ion−molecule complex or even before the formation of this complex. This possibility is currently under investigation.

The *m*-benzyne analogues **b** and **c** have larger (calculated) S−T gaps (−18.8 kcal/mol[12] and −16.3 kcal/mol,[12] respectively) and smaller (calculated) EAs (5.03 eV[13] and 5.24 eV,[13] respectively) than their *p*-benzyne isomer (EA: 5.59 eV[14]). Hence, they are expected to be even less reactive than **a**. Indeed, no reaction products were observed for **b**. However, **c** reacts by base abstraction with most of the dinucleoside phosphates (Table 1) even though its (calculated) S–T gap and EA are similar to those of **b**. Earlier studies have indicated that the reactivity of *m*-benzyne analogues toward simple organic substrates is affected by not only the magnitudes of the S−T gap and EA but also the dehydrocarbon atom separation.[15] A larger dehydrocarbon atom separation makes it energetically easier to uncouple the (singlet) biradical electrons in the transition state for radical reactions. Molecular orbital calculations (UBLYP/cc-pVDZ[16]) on the potential energy surface (Figure 2) for

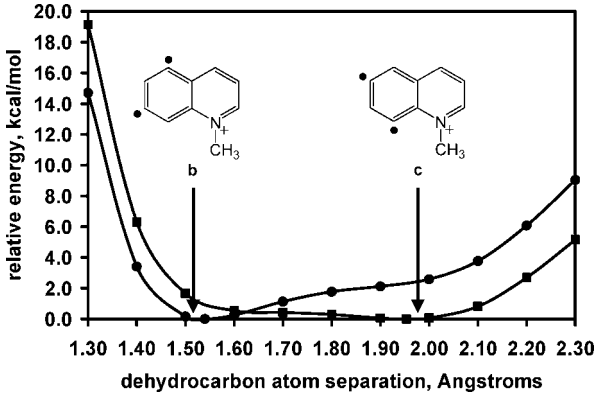
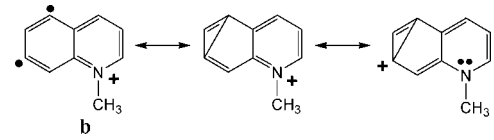


Figure 2. Relative energy versus dehydrocarbon atom separation for the isomeric *m*-benzyne analogues **b** and **c**.

dehydrocarbon atom separation for **c** yield a minimum energy structure with a dehydrocarbon atom separation of about 2.0 Å (Figure 2), which is larger than that of other similar *m*-benzynes. In particular, similar UBLYP/cc-pVDZ calculations for **b** show an energetic preference for a "closed", tricyclic structure (over an "open" biradical structure; Figure 2), although the tricyclic and open structures differ in energy by only about 2.0 kcal/mol.

An analysis of the geometries of **b** and **c** indicates that the preference for a tricyclic structure for **b** is a result of a significant contribution by the resonance structures shown in Scheme 1. The tricyclic resonance structure on far right

Scheme 1. Important Resonance Structures of **b**



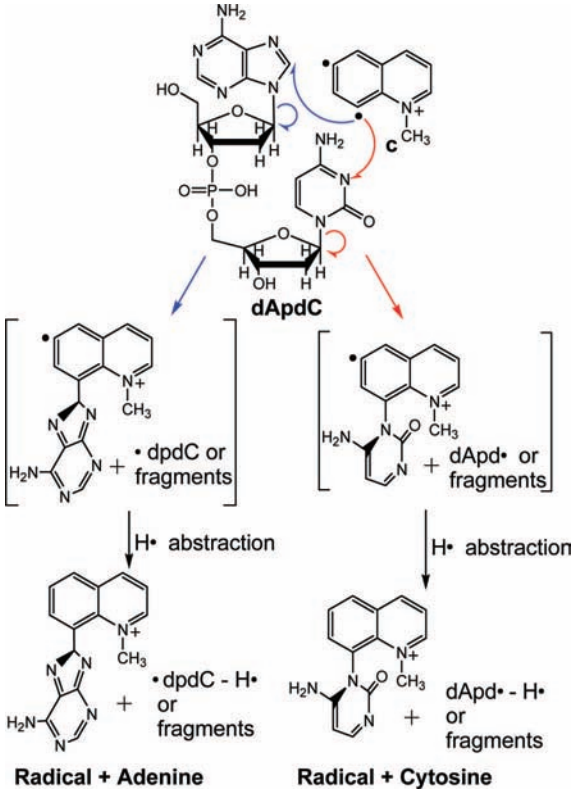
permits greater charge delocalization away from the nitrogen atom into a formally aromatic cyclopropenium ion. This delocalization is not possible for **c**, i.e., an analogous resonance structure for **c** does not exist.

Biradical **c** attacks dinucleoside phosphates (Table 1) via abstraction of a guanine molecule (actually, the nucleobase radical and a hydrogen atom), guanyl radical, adenine molecule, cytosine molecule, or cytosine-HNCO (actually, a cytosine molecule that has lost HNCO either during or after abstraction), whereas the related monoradical **d** undergoes predominant hydrogen atom abstraction (Table 1). No evidence was obtained for thymine or sugar attack by **c**. This reactivity can be partially explained based on the reactions of biradical **c** with the individual DNA components. This biradical reacts with cytosine (efficiency, or the fraction of collisions that lead to reaction products: 25%) and adenine (efficiency: 31%) much faster than with either 2-deoxyribose (efficiency: 3.6%) or thymine (efficiency: 0.5%). Biradical **c** attacks 2-deoxyribose via both hydrogen atom abstraction (60%) and addition (40%), and nucleobases via predominant addition (thymine: 18% hydrogen atom abstraction and 82% addition; cytosine: 1% hydrogen atom abstraction and 99% addition; adenine: 100% addition).

A possible mechanism for the reaction of biradical **c** with dApdC is shown in Scheme 2. Previous studies of the reactions of charged phenyl monoradicals with cytosine and adenine suggest that N-3 in cytosine and C-8 in adenine are the most favored addition sites.[17,18] There is no reason to believe that this is not true also for biradical **c**. The initially formed nucleobase adducts of **c** are likely to undergo a nucleobase−sugar bond cleavage since this occurs upon reactions of monoradicals with dinucleoside phosphates.[2] Dissociation of the product complex leads to the observed nucleobase radical abstraction product. In some cases, the unquenched radical site of **c** abstracts a hydrogen atom from the neutral product (or its fragments) before the collision

(11) Price, J. M.; Nizzi, K. R.; Campbell, J. L.; Kenttämaa, H. I. *J. Am. Chem. Soc.* **2003**, *125*, 131.
(12) CASPT2/cc-pVDZ//MCSCF/cc-pVDZ level of theory.
(13) UBLYP/aug-cc-pVDZ//MCSCF/cc-pVDZ level of theory.
(14) UBLYP/aug-cc-pVDZ//UBLYP/cc-pVDZ level of theory.
(15) (a) Johnson, W. T. G.; Cramer, C. J. *J. Am. Chem. Soc.* **2001**, *123*, 923. (b) Johnson, W. T. G.; Cramer, C. J. *J. Phys. Org. Chem.* **2001**, *14*, 597. (c) Nash, J. J.; Nizzi, K.; Adeuya, A.; Yurkovich, M. J.; Cramer, C. J.; Kenttämaa, H. I. *J. Am. Chem. Soc.* **2005**, *127*, 5760.
(16) The (U)BLYP functional provides the best agreement with highly correlated levels of theory for the structures of *m*-benzynes: (a) Winkler, M.; Sander, W. *J. Phys. Chem. A* **2001**, *105*, 10422. (b) Winkler, M.; Cakir, B.; Sander, W. *J. Am. Chem. Soc.* **2004**, *126*, 6135. (c) See also: Schreiner, P.; R.; Navarro-Vazuez, A.; Prall, M. *Acc. Chem. Res.* **2005**, *38*, 29.
(17) Ramirez-Arizmendi, L. E.; Heidbrink, J. L.; Guler, L. P.; Kenttämaa, H. I. *J. Am. Chem. Soc.* **2003**, *125*, 2272.
(18) Huang, Y.; Kenttämaa, H. I. *J. Am. Chem. Soc.* **2003**, *125*, 987.

Scheme 2. Possible Mechanism for Biradical Attack



complex dissociates, yielding the base abstraction product. This mechanism is different from that proposed for the reactions of DNA-bound biradicals generated from enediyne drugs because the drug intermediates are oriented in a specific way after intercalation or binding with DNA, which limits their modes of reactivity.[1]

The results indicate that thymine is the nucleobase least susceptible to (bi)radical attack, followed by cytosine and the purine bases, adenine and guanine. These findings are in agreement with what would be expected on the basis of polar effects. An increase in the vertical ionization energy (IE) of the substrate (and decrease in the vertical EA of the radical) is expected to increase the transition state energy in a radical addition reaction.[19] Among the four nucleobases, thymine has the highest vertical IE (9.20 eV), followed by cytosine (8.94 eV), adenine (8.48 eV), and guanine (8.24 eV).[20]

In summary, the results presented here demonstrate that the structure of a σ,σ-biradical has a major effect on its reactivity toward dinucleoside phosphates. The S−T gap and EA are known to be important factors that control the reactivity of these singlet biradicals toward DNA components.[3] However, the dehydrocarbon atom separation in *m*-benzyne analogues can be equally important. Since the reactivity of *m*-benzyne analogues can be altered by relatively simple structural changes,[15c] these species may provide promising "warheads" for synthetic antitumor agents. The generation of such biradicals in solution is currently under investigation in our laboratories. The structure of the dinucleoside phosphate also has a strong influence on its reactions with (bi)radicals. Among the DNA bases, thymine was found to be least susceptible to (bi)radical attack, possibly due to its high IE. Further, cytosine is the only base that undergoes skeletal fragmentation (loss of HNCO) upon biradical (but not monoradical) attack. Interestingly, the same fragmentation has been reported for collision-activated dissociation of a cytosine adduct of an enediyne.[21]

Acknowledgment. We thank the National Institutes of Health for financial support.

Supporting Information Available: Tables of Cartesian coordinates, zero-point vibrational energies, and 298 K thermal contributions for the (bi)radicals studied here. This material is available free of charge via the Internet at http://pubs.acs.org.

OL800312G

(19) Clarke, J. S.; Rypkema, H. A.; Kroll, J. H.; Donahue, N. M.; Anderson, J. G. *J. Phys. Chem. A* **2000**, *104*, 4458.

(20) Lias, S. G.; Levin, R. D.; Kafafi, S. A. In Ion Energetics Data. In *NIST Chemistry WebBook, NIST Standard Reference Database Number 69*; Linstrom, P. J., Mallard, W. G., Eds.; National Institute of Standards and Technology: Gaithersburg, MD, June 2005.

(21) Sherman, C. L.; Pierce, S. E.; Brodbelt, J. S.; Tuesuwan, B.; Kerwin, S. M. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 1342.