Pergamon

Tetrahedron Letters, Vol. 38, No. 47, pp. 8169-8172, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)10249-0

Nucleotide C4' Radical Fragmentation is Base-Dependent

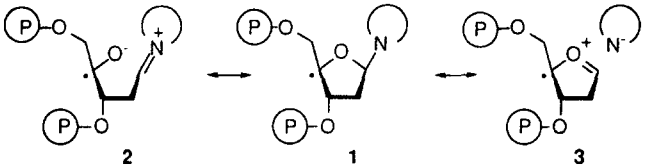
David Crich* and Xue-Sheng Mo

Department of Chemistry, University of Illinois at Chicago, 845 West Taylor Street, Chicago, IL 60607-7061

Abstract: Nucleotide C4' radicals undergo radical ionic fragmentation with expulsion of the 3'-phosphate giving a 3',4'-radical cation. With a series of mononucleotides it is shown that the rate of this fragmentation is a function of the base (T ~ G > ABz > A ~ C) with the least basic, less-hydrogen bonded nucleotides fragmenting more rapidly in aqueous methanol. © 1997 Elsevier Science Ltd.

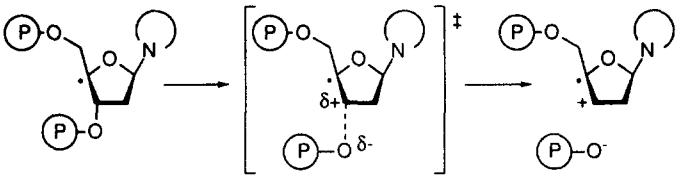
In oligonucleotide chemistry and biochemistry the base, carbohydrate, and phosphate moieties are most often considered as separate entities.[1,2] Recent work from this laboratory, which demonstrated the profound, retarding effect of the 2'-substituent in *ribo*-nucleotides on the fragmentation of 4'-radicals,[3] alerted us to the possible effect of the base on the chemistry of deoxyribonucleotide based 4'-radicals. Such C4'-radicals are central to the degradation of oligonucleotides by the bleomycin[4,5] and enediyne[6-8] classes of antitumor antibiotic. Although there has been considerable work of late on the elucidation of the mechanism of strand scission following hydrogen atom abstraction from a C4'-site,[4,9] the possibility of the rate of one or more of the steps being affected by the individual base has not been addressed.

We hypothesized that a nucleotide 4'-radical might reasonably be represented as a hybrid of the resonance forms **1**, **2**, and **3** (Scheme 1). This being the case, it is apparent that the base interacts directly through hyperconjugation with the unpaired electron at the 4'-position. It is also apparent that the relative contributions of forms **1**, **2**, and **3** will depend on the base with **1** being more important for those bases better able to support positive charge, and **3** for those more capable of sustaining negative charge. A further prediction is that, for a fixed given base, the relative importance of **1**, **2** and **3** will depend on the environment, that is on the extent to which the base is involved in hydrogen bonding (as a net donor or acceptor) and, thus, its incorporation in single, double, or triple helices.



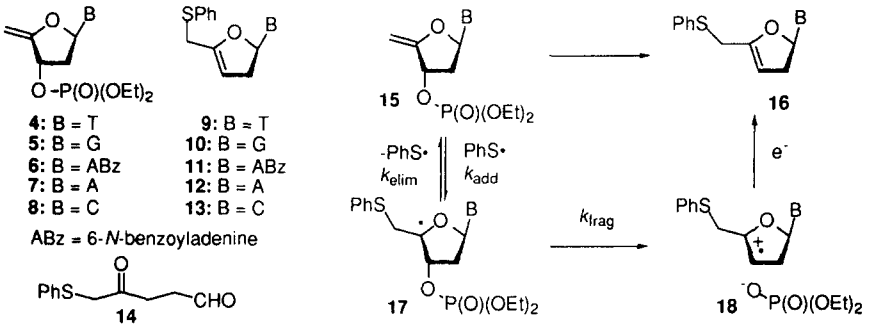
Scheme 1

Recognition of the interaction (**1** ↔ **2** ↔ **3**) leads to the hypothesis that any reaction of a C4'-radical proceeding through a polarized transition state will be affected to some extent by the nature of the base and the environment. Thus, it is likely that the rate of radical-ionic fragmentation (Scheme 2) of nucleotide C4' radicals[3,10] will be a function of the base and its hydrogen bonding pattern. Here, we outline our initial results which show that the rate of such radical-ionic fragmentation reactions is indeed a function of the base.



Scheme 2

The experimental design follows that used in our earlier ribonucleotide/deoxyribonucleotide comparison,[3] and is an adaptation of the method of Giese and co-workers.[11] The five substrates **4** - **8** were prepared and isolated by adaptations of standard means. Each was dissolved (0.05 M), individually in 5mm NMR tubes, in CD_3OD/D_2O (10/1) and treated with thiophenol (5 molar equivalents). After degassing, di-*tert*-butyl peroxalate (10 mol %) was added and the tubes incubated at 40 °C. As determined by ^1H and ^{31}P-NMR spectroscopy, each substrate was converted smoothly to the products **9** - **13**, respectively, and diethyl phosphate. The fragmentation products **9** - **13** were readily identified in the crude reaction mixtures by ^1H-NMR spectroscopy but only **11** was sufficiently stable to enable isolation by preparative thin layer chromatography on silica gel, with the others undergoing hydrolysis to the known[12] aldehydo-ketone **14**.[13] The formation of the glycals **9** - **13** is readily rationalized by reversible addition of the PhS• radical to the exocyclic glycal **15** which generates the C4' radical **17**. This then suffers radical-ionic fragmentation to **18**. Single electron transfer from thiophenol or thiophenate finally affords the observed product **16** (Scheme 3).



Scheme 3

A series of competition reactions were conducted, under the same conditions of concentration and temperature as the initial experiments, in which **5**, **6**, and **7** were allowed to compete with **4** for reaction with PhS• and subsequent fragmentation. The ratio of **5**, **6**, or **7**/**4**, as determined by ^1H-NMR spectroscopy, was then plotted as a function of the percentage conversion of **4** (Figure 1). A second, similar series was then carried out in which **5**, **6**, and **7** were pitted against **8** for reaction with PhS• leading to the results depicted in Figure 2.[14] The reactivity sequence **4** ~ **5** > **6** > **7** is readily derived from Figure 1, and **5** > **6** > **7** ~ **8** from Figure 2. Combining the two sequences gives **4** ~ **5** > **6** > **7** ~ **8** which translates into T ~ G > ABz > A ~ C and the conclusion that the rate of this reaction is indeed a distinct function of the base.

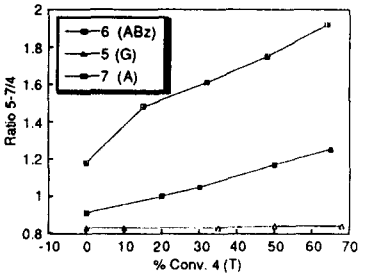


Figure 1

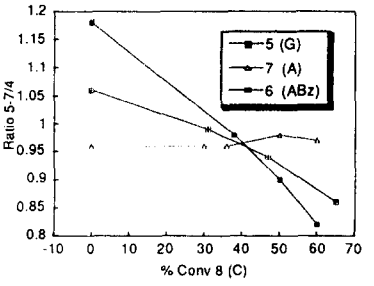
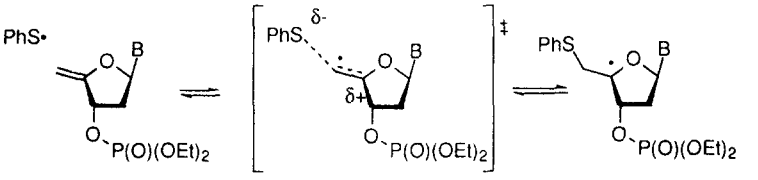


Figure 2

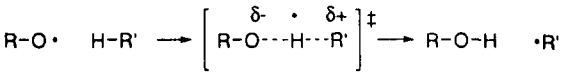
The observed reactivity pattern best correlates with basicity. Under the conditions of the experiment the much more basic **7** and **8**, if not fully protonated, will be net hydrogen bond acceptors and accordingly charged δ+, whereas the distinctly non-basic **4** and **5** will carry a much smaller, if any, partial positive charge. This in turn renders **7** and **8** much less able to support resonance structure **2** and more able to sustain **3**. An increased contribution from **3** to the resonance hybrid will destabilize the polarized transition state for fragmentation illustrated in Scheme 2 and so retard the overall reaction rate.[15] Benzoylation of **7** gives **6** and renders the heterocycle less basic, reduces the contribution of **3** to the resonance hybrid, and leads to the observed acceleration in fragmentation rate.

It could be argued that the observed trend derives from a shift in the **15/17** equilibrium or in the corresponding rates of addition and elimination of PhS• (k_{add} and k_{elim}, respectively, Scheme 3). Certainly, the transition state for addition/elimination of PhS• will be polarized (Scheme 4), in a similar sense to the radical-ionic fragmentation. However, it is evident that such transition state polarization will be much more substantial in the radical-ionic fragmentation leading to the fully charged radical cation, than in the addition/elimination of PhS• which proceeds without overall separation of charge. The observed effect will therefore be predominantly a function of a change in the fragmentation rate constant k_{frag} (Scheme 3). All in all, it is clear from the above experiments that the base has a definite effect on the reactions of nucleotide C4' radicals. It is also clear from the comparison of **6** and **7**, that changing the environment of a given base will have an affect on the chemistry of the radical.



Scheme 4

Hydrogen atom abstraction by electrophilic radicals, such as alkoxyl radicals, also involves polarized transition states in which a partial positive charge resides on the developing radical (Scheme 5).[16-20] It is likely, therefore, that hydrogen abstraction from nucleotide C4' sites by 1,4-arenediyls, as with the endiynes, or by the still ill-defined activated bleomycin species [21] will be also base and environment susceptible.



Scheme 5

The present experiments may have important ramifications in the design of free-radical based antitumor antibiotics where it would now seem sensible not only to aim for sequence-selective binding but also to target the most efficiently cleaved base. The present results may also help resolve a current literature controversy. In their investigation of Fe.bleomycin with double stranded DNA, Stubbe and co-workers find that their results are best interpreted in terms of the quenching of the C4' radical by oxygen before any fragmentation of the type discussed here.[4] On the other hand, Giese et al, working with unambiguously generated C4' radicals in single stranded DNA, see fragmentation before quenching with oxygen.[9] It is possible that this dichotomy simply reflects the use of different bases in very different environments by the two groups. Experiments designed to quantify the present observations are currently underway and will be reported on in due course.

Acknowledgment. We thank the NIH and NSF for partial support of this work.

References

(1) *Bioorganic Chemistry: Nucleic Acids*; Hecht, S. M., Ed.; OUP: New York, 1996.
(2) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.
(3) Crich, D.; Mo, X.-S. *J. Am. Chem. Soc.* **1997**, *119*, 249-250.
(4) Stubbe, J.; Kozarich, J. W.; Wu, W.; Vanderwall, D. E. *Acc. Chem. Res.* **1996**, *29*, 322-330.
(5) Hecht, S. M. In *DNA and RNA Cleavers and Chemotherapy of Cancer and Viral Diseases*; Meunier, B., Ed.; Kluwer Academic: Dordrecht, 1996, p 77-89.
(6) Christner, D. F.; Frank, B. L.; Kozarich, J. W.; Stubbe, J.; Golik, J.; Doyle, T. W.; Rosenberg, I. E.; Krishnan, B. *J. Am. Chem. Soc.* **1992**, *114*, 8763-8767.
(7) Kappen, L. S.; Goldberg, I. H.; Stubbe, J. *Biochemistry* **1991**, *30*, 2034-2042.
(8) Hangeland, J. J.; De Voss, J. J.; Heath, J. A.; Townsend, C. A. *J. Am. Chem. Soc.* **1992**, *114*, 9200-9202.
(9) Giese, B.; Beyrich-Graf, X.; Erdmann, P.; Petretta, M.; Schwitter, U. *Chem. Biol.* **1995**, *2*, 367-375.
(10) Giese, B.; Beyrich-Graf, X.; Burger, J.; Kesselheim, C.; Senn, M.; Schafer, T. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1742-1743.
(11) Giese, B.; Burger, J.; Kang, T. W.; Kesselheim, C.; Wittmer, T. *J. Am. Chem. Soc.* **1992**, *114*, 7322-7324.
(12) Molander, G. A.; Eastwood, P. R. *J. Org. Chem.* **1995**, *60*, 8382-8393.
(13) The instability of **9** has previously been noted by Giese.[11]
(14) Direct competition of **4** with **8** was not possible due to insufficient spectral resolution.
(15) In the absense of hydrogen bonding, the ability of a particular base to support the resonance form **2**, and so accelerate fragmentation, might reasonably be expected to be a function of the electron density, and so ionization potential of the base: no such correlation is observed. A recent compilation of experimental and theoretical ionization potentials: Hutter, M.; Clark, T. *J. Am. Chem. Soc.* **1996**, *118*, 7574-7577.
(16) Paul, V.; Roberts, B. P.; Willis, C. R. *J. Chem. Soc., Perkin Trans. 2* **1989**, 1953.
(17) Roberts, B. P. *J. Chem. Soc., Perkin Trans. 2* **1996**, 2719-2725.
(18) Busfield, W. K.; Grice, I. D.; Jenkins, I. D. *J. Chem. Soc., Perkin Trans. 2* **1994**, 1079-1086.
(19) Busfield, W. K.; Grice, I. D.; Jenkins, I. D.; Monteiro, M. J. *J. Chem. Soc., Perkin Trans. 2* **1994**, 1071-1077.
(20) Brunckova, J.; Crich, D. *Tetrahedron* **1995**, *51*, 11945-11952.
(21) The last characterized intermediate is a ferric peroxide complex: Sam, J. W.; Tang, X. J.; Peisach, J. *J. Am. Chem. Soc.* **1994**, *116*, 5250-5256.

(*Received in USA* 11 *September* 1997; *revised* 19 *September* 1997; *accepted* 22 *September* 1997)