# Evidence for C-1′ Hydrogen Abstraction from Modified Oligonucleotides by Fe·Bleomycin

Robert J. Duff, Erik de Vroom, Annemiek Geluk, and Sidney M. Hecht*

*Departments of Chemistry and Biology*
*University of Virginia*
*Charlottesville, Virginia 22901*

Gijs A. van der Marel and Jacques H. van Boom

*Department of Organic Chemistry*
*University of Leiden*
*Leiden, The Netherlands*

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The bleomycins (BLMs) are antitumor antibiotics employed clinically for the treatment of several malignancies.[1] Fe(II)·BLM can mediate oxidative degradation of DNA[2] and RNA[3] in a sequence selective fashion; it seems likely that damage of one or both of these macromolecules contributes importantly to the observed antitumor effects of the bleomycins.[2,4]

Oxidative destruction of both DNA[2,5] and RNA[3b,d] by activated Fe·BLM, which is believed to be a high-valent metal–oxo complex,[2] involves abstraction of H from C-4′ of (deoxy)ribose. Analogous abstraction of H from C-1′ has been postulated for DNA,[6] and later for the DNA strand of an RNA–DNA heteroduplex.[7] While reasonable chemically, no evidence has been obtained for DNA degradation by this pathway. Recently, Absalon et al.[8] have demonstrated convincingly that degradation of the DNA strand of an RNA–DNA heteroduplex formed from two homopolymers does not involve abstraction of C-1′ H. Presently, we describe the degradation of two self-complementary oligodeoxynucleotides (Figure 1) containing a single ribo or ara nucleoside at a position susceptible to oxidative degradation by Fe(II)·BLM.[3b,9,10] In contrast to all results obtained to date with other substrates, these modified octanucleotides were found to afford products that can be envisioned as resulting from abstraction of C-1′ H, in addition to those produced from abstraction of C-4′ H.

The oligonucleotides d(CGCT$_3$A$_3$GCG)[9] and d(GCG-TAGCG)[10] are efficient, site-selective substrates for Fe·BLM. As anticipated, the products resulted predominantly from oxidative destruction of the sugar moieties in cytidine-3 and cytidine-7; these included 2′-deoxycytidylyl(3′→5′)(2′-deoxyguanosine 3′-(phosphoro-2′′-*O*-glycolate)) (CpGpCH$_2$COOH), which presumably formed by initial abstraction of C-4′ H from cytidine$_3$.[2,5,9] The same product was obtained upon treatment of C$_3$-ribo and C$_3$-ara CGCTAGCG and constitutes one line of evidence for the nature of the chemical products formed from RNA-containing oligonucleotides by Fe·BLM.[3b] In the belief that these modified oligonucleotides might possess altered conformations at cytidine-3 conducive to Fe·BLM-mediated abstraction of C-1′ H,[11] the reaction mixtures were analyzed for products that might result from this initial event.

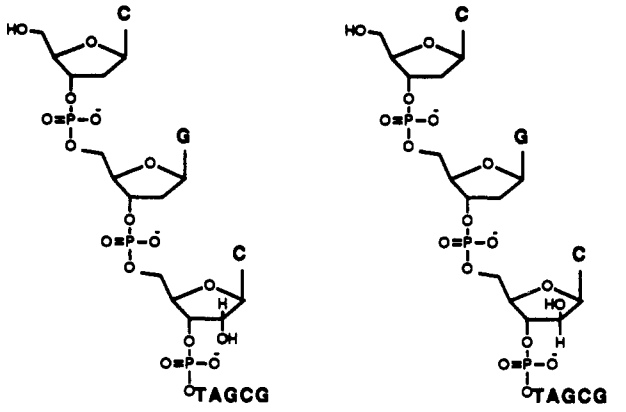
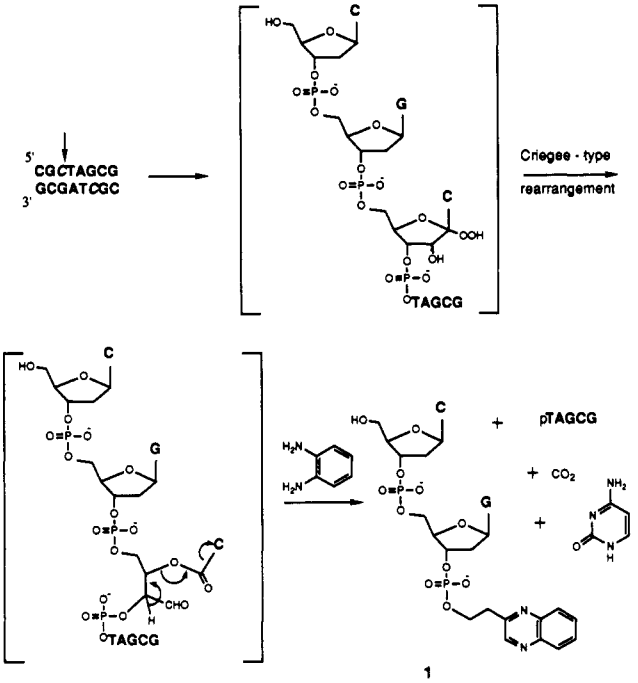



**Figure 1.** Structures of two self-complementary oligonucleotides employed as substrates for Fe(II)·BLM $A_2$.

**Scheme I.** Products Formed from Substrate Octanucleotides following Putative Oxidation at C-1′ by Fe(II)·BLM $A_2$




(1) (a) Umezawa, H. In *Bleomycin: Current Status and New Developments*; Carter, S. K., Crooke, S. T., Umezawa, H., Eds.; Academic Press: New York, 1978; pp 15–20. (b) Sugiura, Y.; Takita, T.; Umezawa, H. *Met. Ions Biol. Syst.* **1985**, *19*, 81.

(2) (a) Hecht, S. M. *Acc. Chem. Res.* **1986**, *19*, 83. (b) Kozarich, J. W.; Stubbe, J. *Chem. Rev.* **1987**, *87*, 1107.

(3) (a) Magliozzo, R. S.; Peisach, J.; Cirolo, M. R. *Mol. Pharmacol.* **1989**, *35*, 428. (b) Carter, B. J.; de Vroom, E.; Long, E. C.; van der Marel, G. A.; van Boom, J. H.; Hecht, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9373. (c) Carter, B. J.; Reddy, K. S.; Hecht, S. M. *Tetrahedron* **1991**, *47*, 2463. (d) Holmes, C. E.; Carter, B. J.; Hecht, S. M. *Biochemistry*, in press.

(4) (a) Ishida, R.; Takahashi, T. *Biochem. Biophys. Res. Commun.* **1975**, *66*, 1432. (b) Sausville, E. A.; Stein, R. W.; Peisach, J.; Horwitz, S. B. *Biochemistry* **1978**, *17*, 2740. (c) Sausville, E. A.; Stein, R. W.; Peisach, J.; Horwitz, S. B. *Biochemistry* **1978**, *17*, 2746.

(5) (a) Wu, J. C.; Kozarich, J. W.; Stubbe, J. *J. Biol. Chem.* **1983**, *258*, 4694. (b) Wu, J. C.; Kozarich, J. W.; Stubbe, J. *Biochemistry* **1985**, *24*, 7562. (c) Kozarich, J. W.; Worth, L., Jr.; Frank, B. L.; Christner, D. F.; Vanderwall, D. E.; Stubbe, J. *Science* **1989**, *245*, 1396.

(6) Hecht, S. M. In *Bleomycin, Chemical, Biochemical and Biological Aspects*; Hecht, S. M., Ed.; Springer-Verlag: New York, 1979; pp 1–23.

(7) Krishnamoorthy, C. R.; Vanderwall, D. E.; Kozarich, J. W.; Stubbe, J. *J. Am. Chem. Soc.* **1988**, *110*, 2008.

(8) Absalon, M. J.; Krishnamoorthy, C. R.; McGall, G.; Kozarich, J. W.; Stubbe, J. *Nucleic Acids Res.* **1992**, *20*, 4179.

(9) (a) Sugiyama, H.; Kilkuskie, R. E.; Chang, L.-H.; Ma, L.-T.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1986**, *108*, 3852. (b) Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *Biochemistry* **1988**, *27*, 58 and references therein.

(10) Van Atta, R. B.; Long, E. C.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1989**, *111*, 2722.

(11) As noted previously,[7] opening of the minor groove to give a duplex having more A-form character leads to greater accessibility in the minor groove of C-1′ H as compared with C-4′ H. In fact, substitution of ara-C for dC is known to destabilize local B-form conformation (Gao, Y.-G.; van der Marel, G. A.; van Boom, J. H.; Wang, A. H.-J. *Biochemistry* **1991**, *30*, 9922); alteration of conformation has been noted specifically for the ara-C-containing octanucleotide studied here (Pieters, J. M. L.; de Vroom, E.; van der Marel, G. A.; van Boom, J. H.; Koning, T. M. G.; Kaptein, R.; Altona, C. *Biochemistry* **1990**, *29*, 788).

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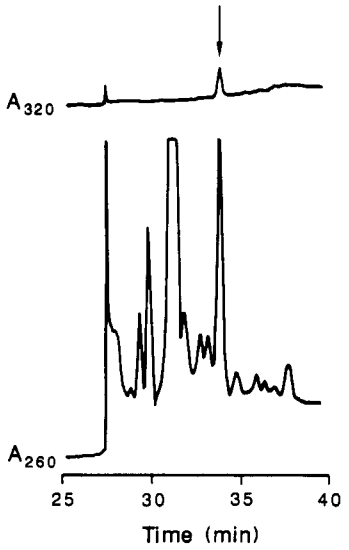


**Figure 2.** HPLC analysis of Fe(II)·BLM-treated $C_3$-ara CGCTAGCG, following additional incubation with 1,2-diaminobenzene. Separation was achieved on an Alltech Lichrosorb $C_{18}$ column (10 $\mu$M); elution was with aqueous 0.1 M $NH_4OAc$ containing increasing amounts of $CH_3CN$ (0–20 min, 0.1 M $NH_4OAc$; 20–22 min, linear gradient, 0 → 6% $CH_3CN$ in 0.1 M $NH_4OAc$; 22–32 min, 6 → 15% $CH_3CN$ in 0.1 M $NH_4OAc$; 32–50 min, 15% $CH_3CN$) at a flow rate of 2 mL/min. The eluate was monitored at 260 and 320 nm; authentic dinucleotide **1** coeluted with the peak at 33.9 min (see arrow). The small $A_{320}$ peak at 27.5 min was an artifact due to the gradient maker.

As outlined in Scheme I, initial formation of a C-1′ ribose radical might be expected to lead to the formation of a C-1′ hydroperoxynucleotide intermediate, in analogy with transformations noted for the putative C-4′ radical.[2,5,9] As illustrated, Criegee rearrangement of the C-1′ hydroperoxide would not lead directly to strand scission; accordingly, 1,2-diaminobenzene was added to effect conversion of the envisioned intermediate to 2′-deoxycytidylyl(3′→5′)(2′-deoxyguanosine 3′-(phosphoro-2″-*O*-(hydroxyethyl)-2-quinoxaline)) (**1**).

Following treatment of $C_3$-ara CGCTAGCG with Fe(II)·BLM $A_2$ in the presence of 1,2-diaminobenzene,[12] the reaction mixture was analyzed by reversed-phase HPLC (Figure 2). An authentic, synthetic sample of dinucleotide **1**[13] was found to have an absorption maximum at 320 nm ($\epsilon$ 5900) in addition to the characteristic absorption at shorter wavelength, so the HPLC eluate was monitored at both 260 and 320 nm. As shown in Figure 2, a product absorbing at 260 and 320 nm eluted at 33.9 min; this material had the same UV spectrum as authentic **1**. Repetition of the experiment using [$^{14}$C]-1,2-diaminobenzene (14.7 Ci/mol) afforded a peak containing radioactive material coincident with the $A_{320}$ peak.[14] The same results were obtained when $C_3$-ribo CGCTAGCG was treated with Fe(II)·BLM $A_2$ and then with 1,2-diaminobenzene, although significantly less **1** was obtained.

Quantification of products formed from d(CGCTAGCG), $C_3$-ara CGCTAGCG, and $C_3$-ribo CGCTAGCG was carried out by HPLC analysis using authentic synthetic standards having known response factors.[14] Treatment of 250 $\mu$M oligonucleotide with 400 $\mu$M Fe(II)·BLM $A_2$ gave comparable amounts of products (114–120 $\mu$M) in each case, although the ratio of products derived from cytidine-3 and cytidine-7 varied substantially.[15] For $C_3$-ara CGCTAGCG, dinucleotide **1** constituted 58% of the products derived from degradation of cytidine-3; the comparable figure for $C_3$-ribo CGCTAGCG was ~10%.

The detection of dinucleotide **1** in the foregoing experiments provides strong suggestive evidence for the degradation of $C_3$-ribo and $C_3$-ara CGCTAGCG via a pathway involving initial abstraction of C-1′ H from the susceptible sugar moiety of each oligonucleotide.[17] Thus, in common with other DNA-cleaving agents whose mode of DNA degradation can change according to the substrate employed,[18] Fe·BLM would seem to be capable of abstracting either C-4′ H or C-1′ H from appropriate substrate oligonucleotides.

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(12) Reaction mixtures (50-$\mu$L total volume) contained 250 $\mu$M octanucleotide (2 mM nucleotide concentration), 0.4 mM BLM $A_2$, and 0.8 mM $Fe^{II}(NH_4)_2(SO_4)_2$ in 100 mM sodium cacodylate, pH 7.0. Reactions were initiated by $Fe^{2+}$ addition and incubated ($O_2$ bubbling) at 0 °C for 30 min. Following treatment with 25 $\mu$L of 1:1 EtOH–$H_2O$ containing 0.5 M 1,2-diaminobenzene, the combined solution was heated at 55 °C for 30 min. The cooled solution was extracted with ether and concentrated prior to HPLC analysis.

(13) The synthesis of dinucleotide **1** was carried out in analogy with the synthesis of CpGpCH$_2$COOH (Murugesan, N.; Xu, C.; Ehrenfeld, G. M.; Sugiyama, H.; Kilkuskie, R. E.; Rodriguez, L. O.; Chang, L.-H.; Hecht, S. M. *Biochemistry* **1985**, *24*, 5735); 2-(2-hydroxyethyl)quinoxaline was prepared as described (Elina, A. S.; Tsyrul'nikova, L. G. *Zh. Obshch. Khim.* **1964**, *34*, 2077). Characterization of dinucleotide **1** included analysis of its UV, $^1$H-NMR, and FAB mass spectra.

(14) Dinucleotide **1** was also detected (30.2-min elution time) on an Alltech Econosphere $C_{18}$ column (3 $\mu$m); elution was with 0.1 M $NH_4OAc$, pH 6.8; flow rate 1.4 mL/min. This column was employed for quantification of key products.[9]

(15) The ratio of cleavage at the two GC sites has been shown to change as the structure of the BLM congener[9a] or oligonucleotide substrate[16] was varied.

(16) (a) Gold, B.; Dange, V.; Moore, M. A.; Eastman, A.; van der Marel, G. A.; van Boom, J. H.; Hecht, S. M. *J. Am. Chem. Soc.* **1988**, *110*, 2347. (b) Long, E. C.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1990**, *112*, 5272.

(17) Although less likely, we cannot presently exclude the possibility that dinucleotide **1** arose from an oxidative process involving C-2′ of the sugar moiety of cytidine-3.

(18) See, e.g.: (a) Goldberg, I. H. *Acc. Chem. Res.* **1991**, *24*, 191. (b) Kappen, L. S.; Goldberg, I. H. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6706.