ORGANIC LETTERS

2008
Vol. 10, No. 11
2127−2130

A Novel DNA Hairpin Substrate for Bleomycin

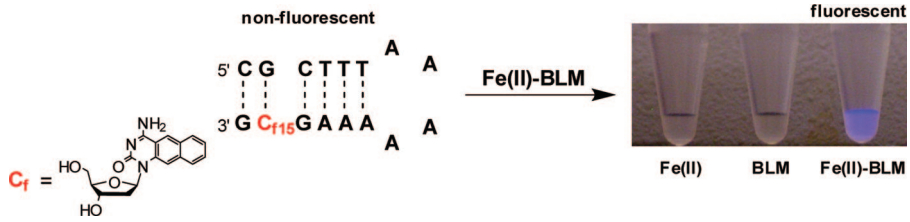
Yoshitsugu Akiyama,[†] Qian Ma,[†] Erin Edgar,[‡] Andrei Laikhter,[‡] and Sidney M. Hecht*,[†]

Departments of Chemistry and Biology, University of Virginia, Charlottesville, Virginia 22904, and Integrated DNA Technologies, 1710 Commercial Park, Coralville, Iowa 52241

sidhecht@virginia.edu

Received March 3, 2008

ABSTRACT



A 16-nucleotide DNA hairpin containing 4-aminobenzo[*g*]quinazoline-2-one 2′-deoxyribose at position 15 has been prepared and found to lack significant fluorescence. When treated with Fe(II)·BLM, the hairpin was found to undergo oxidative transformation selectively at position 15. The predominant fluorescent product was characterized and quantified. The pro-fluorescent DNA hairpin was used as a substrate for 15 bleomycin congeners, and the results were compared with those obtained following cleavage of a radiolabeled DNA duplex and PAGE analysis.

The bleomycins (BLMs) are glycopeptide-derived antitumor antibiotics.[1] BLM exhibits sequence selective DNA strand scission, predominantly at 5′-GC-3′ and 5′-GT-3′ sequences in the presence of O_2 and metal ions such as Fe or Cu,[2] and also shape-selective cleavage of some RNAs.[3] $tRNA_3^{Lys}$ has been shown to be especially susceptible to cleavage by Fe(II)•BLM and may plausibly represent a therapeutically relevant locus for the drug.[4] DNA and RNA degradation by BLM depends critically on several facets of its structure, which is organized into discrete structural domains, including DNA binding, metal binding and linker domains, and a disaccharide moiety and C-terminal substituent (Figure 1). The individual structural elements in BLM control metal binding, O_2 binding and activation, selective DNA or RNA

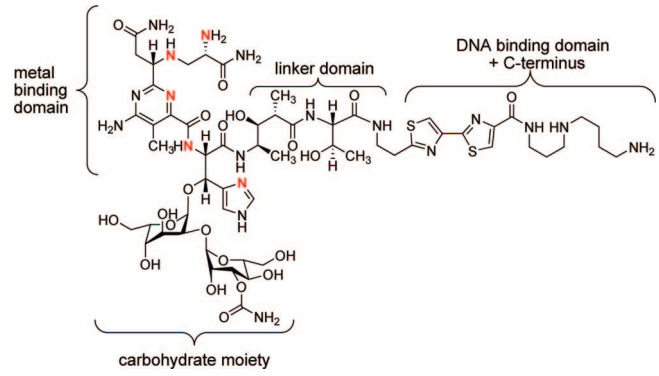


Figure 1. Structure of bleomycin A_5 (BLM A_5) including the nitrogen atoms (red) believed to coordinate the metal ion cofactor.

[†] University of Virginia.
[‡] Integrated DNA Technologies.

(1) (a) *Bleomycin Chemotherapy*; Sikic, B. I., Rozencweig, M., Carter, S. K., Eds.; Academic Press: Orlando, FL, 1985. (b) Hecht, S. M. *J. Nat. Prod.* **2000**, *63*, 158. (c) Chen, J.; Stubbe, J. *Nat. Rev. Cancer* **2005**, *5*, 102.

(2) (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*, 2746. (c) Ehrenfeld, G. M.; Shipley, J. B.; Heimbrook, D. C.; Sugiyama, H.; Long, E. C.; van Boom, J. H.; van der Marel, G. A.; Oppenheimer, N. J.; Hecht, S. M. *Biochemistry* **1987**, *26*, 931.

(3) (a) 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. (b) Holmes, C. E.; Carter, B. J.; Hecht, S. M. *Biochemistry* **1993**, *32*, 4293. (c) Morgan, M. A.; Hecht, S. M. *Biochemistry* **1994**, *33*, 10286. Hecht, S. M. *Bioconjugate Chem.* **1994**, *5*, 513.

10.1021/ol800445x CCC: $40.75
Published on Web 04/30/2008

binding, H atom abstraction and subsequent oxidative transformation of the nucleic acid substrates. Accordingly, the design of bleomycin congeners with altered and improved properties requires a systematic approach and significant effort. Several groups have worked to elucidate the relationship between chemical structure and functions of BLM by the use of (semi)synthetic approaches.[5] An important milestone in efforts to prepare BLMs with altered properties in nucleic acid binding and degradation was realized with the solid-phase synthesis of BLM congeners.[6] In addition to greatly facilitating the preparation of single BLMs, the analogues could be prepared on solid supports compatible with on-bead assay of the fully deprotected BLMs.[6] Solid-phase synthesis has also permitted the elaboration of a combinatorial library of 108 BLM analogues,[7] the properties of which have recently been described.[8] The library contained potent (deglyco)BLM analogues that cleave DNA to a greater extent than the corresponding parent (deglyco)BLM. In addition, some BLM analogues in the library exhibited an altered selectivity of DNA binding and cleavage.[8] It is anticipated that larger libraries may afford access to bleomycin congeners exhibiting potentially useful properties, e.g., the ability to cleave individual cellular RNAs specifically.

While the synthesis of BLM libraries on solid supports is readily accomplished and the elaborated BLMs can be assayed for DNA cleavage while still attached to the synthesis beads, there is a key unresolved issue for the evaluation of larger BLM libraries. The library whose synthesis and evaluation has been described[7,8] was a parallel library, i.e., containing compounds in individual containers. While this strategy has important advantages, it also requires that each of the several assays employed for characterization of the new analogues be carried out individually on each member of the library.

To facilitate the evaluation of much larger numbers of BLM analogues, it is anticipated that future studies may involve mix-and-split libraries.[9] This would result in a large number of beads, each containing multiple copies of a single BLM congener. Such libraries can be assayed en masse, e.g., by the use of appropriate molecular beacons, potentially permitting visual identification of beads containing BLM analogues that are particularly proficient in a specific assay. We previously described a DNA molecular beacon that can be used for this purpose.[10]

The molecular beacon initially described employed fluorescence resonance energy transfer (FRET). Kinetics[10] and visualization[6b,c] of BLM-mediated DNA cleavage were achieved using a 16-nucleotide (nt) hairpin DNA (5′-$CGCT_3A_7GCG$-3′) having a fluorescence-quencher pair at the 5′- and 3′-ends.[10] While this oligonucleotide permitted the characterization of individual BLMs, it was not cleaved with an efficiency comparable to unmodified DNA,[10] owing presumably to the interaction of the attached fluorescein or dabcyl moieties with the sites on the DNA−substrate cleaved by BLM. Thus, the characterization of libraries using this beacon may be idiosyncratic, rather than reflecting the intrinsic properties of individual BLM analogues.

Presently, we report the site-specific incorporation of a fluorescent nucleobase (N_f) into 16-nt hairpin DNAs at BLM cleavage sites (hairpin DNA-N_f) and the strong enhancement in fluorescence caused by BLM-mediated DNA strand scission. Several types of N_f have been incorporated into DNA as probes of nucleic acid structure, dynamics, and interactions.[11,12] To detect cleavage of DNA by BLM based on the use of a DNA containing a fluorescent nucleobase, we focused on the position of insertion and selection of N_f. In our previous study on the cleavage of a 16-nt hairpin DNA, it was found that cleavage occurred at cytidine$_{15}$ for Fe(II)•BLM.[10] As regards the choice of N_f, we sought a nucleobase having a compact ring structure because a larger fluorophore-nucleobase[13] or fluorophore-linked base[14] seemed more likely to affect the sequence recognition of BLM due to steric effects. Certain benzo[*g*]quinazoline heterocycles have exhibited strong fluorescence emission with favorable quantum yields in buffered aqueous solution at neutral pH.[15]

(4) (a) Abraham, A. T.; Lin, J.-J.; Newton, D. L.; Rybak, S.; Hecht, S. M. *Chem. Biol.* **2003**, *10*, 45. (b) Tao, Z.-F.; Konishi, K.; Keith, G.; Hecht, S. M. *J. Am. Chem. Soc.* **2006**, *128*, 14806.

(5) (a) Kittaka, A.; Sugano, Y.; Otsuka, M.; Ohno, M. *Tetrahedron* **1988**, *44*, 2811. (b) Kittaka, A.; Sugano, Y.; Otsuka, M.; Ohno, M. *Tetrahedron* **1988**, *44*, 2821. (c) Hamamichi, N.; Natrajan, A.; Hecht, S. M. *J. Am. Chem. Soc.* **1992**, *114*, 6278. (d) Boger, D. L.; Menezes, R. F.; Honda, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 273. (e) Kane, S.; Natrajan, A.; Hecht, S. M. *J. Biol. Chem.* **1994**, *269*, 10899. (f) Boger, D. L.; Teramoto, S.; Honda, T.; Zhou, J. *J. Am. Chem. Soc.* **1995**, *117*, 7338. (g) Boger, D. L.; Teramoto, S.; Zhou, J. *J. Am. Chem. Soc.* **1995**, *117*, 7344. (h) Boger, D. L.; Ramsey, T. M.; Cai, H.; Hoehn, S. T.; Kozarich, J. W.; Stubbe, J. *J. Am. Chem. Soc.* **1998**, *120*, 53. (i) Boger, D. L.; Ramsey, T. M.; Cai, H.; Hoehn, S. T.; Stubbe, J. *J. Am. Chem. Soc.* **1998**, *120*, 9139. (j) Boger, D. L.; Menezes, R. F.; Cai, H.; Hoehn, S. T.; Stubbe, J. *J. Am. Chem. Soc.* **1998**, *120*, 9149. (k) Katano, K.; An, H.; Aoyagi, Y.; Overhand, M.; Sucheck, S. J.; Stevens, W. C.; Hess, C. D.; Zhou, X.; Hecht, S. M. *J. Am. Chem. Soc.* **1998**, *120*, 11285. (l) Zuber, G.; Quada, J. C.; Hecht, S. M. *J. Am. Chem. Soc.* **1998**, *120*, 9368. (m) Zou, Y.; Fahmi, N. E.; Vialas, C.; Miller, G. M.; Hecht, S. M. *J. Am. Chem. Soc.* **2002**, *124*, 9476.

(6) (a) Leitheiser, C. J.; Rishel, M. J.; Wu, X.; Hecht, S. M. *Org. Lett.* **2000**, *2*, 3397. (b) Tao, Z.-F.; Leitheiser, C. J.; Smith, K. L.; Hashimoto, S.; Hecht, S. M. *Bioconjugate Chem.* **2002**, *13*, 426. (c) Smith, K. L.; Tao, Z.-F.; Hashimoto, S.; Leitheiser, C. J.; Wu, X.; Hecht, S. M. *Org. Lett.* **2002**, *4*, 1079. (d) Thomas, C. J.; Chizhov, A. O.; Leitheiser, C. J.; Rishel, M. J.; Konishi, K.; Tao, Z.-F.; Hecht, S. M. *J. Am. Chem. Soc.* **2002**, *124*, 12926.

(7) (a) Leitheiser, C. J.; Smith, K. L.; Rishel, M. J.; Hashimoto, S.; Konishi, K.; Thomas, C. J.; Li, C.; McCormick, M. M.; Hecht, S. M. *J. Am. Chem. Soc.* **2003**, *125*, 8218. (b) Leitheiser, C. J.; Hecht, S. M. *Curr. Opin. Drug Discovery Dev.* **2003**, *6*, 827.

(8) Ma, Q.; Xu, Z.; Schroeder, B. R.; Sun, W.; Wei, F.; Hashimoto, S.; Konishi, K.; Leitheiser, C. J.; Hecht, S. M. *J. Am. Chem. Soc.* **2007**, *129*, 12439.

(9) Geysen, H. M.; Schoenen, F.; Wagner, D.; Wagner, R. *Nat. Rev. Drug Dis.* **2003**, *2*, 222.

(10) Hashimoto, S.; Wang, B.; Hecht, S. M. *J. Am. Chem. Soc.* **2001**, *123*, 7437.

(11) Marti, A.; Jockusch, S.; Li, Z.; Ju, J.; Turro, N. J. *Nucleic Acids Res.* **2006**, *34*, e50.

(12) (a) Wojczewski, C.; Stolze, K.; Engels, J. W. *Synlett.* **1999**, 1667. (b) Hawkins, M. E. *Cell Biochem. Biophys.* **2001**, *34*, 257. (c) Rist, M. J.; Marino, J. P. *Curr. Org. Chem.* **2002**, *6*, 775. (d) Okamoto, A.; Saito, Y.; Saito, I. *J. Photochem. Photobiol. C: Photochem. Rev.* **2005**, *6*, 108. (e) Tor, Y. Ed. *Tetrahedron* **2007**, *63*, 3415.

(13) (a) Coleman, R. S.; Madaras, M. L. *J. Org. Chem.* **1998**, *63*, 5700. (b) Matray, T. J.; Kool, E. T. *Nature* **1999**, *399*, 704. (c) Strassler, C.; Davis, N. E.; Kool, E. T. *Helv. Chim. Acta* **1999**, *82*, 2160.

(14) (a) Netzel, T. L.; Zhao, M.; Nafisik, K.; Headrick, J.; Sigman, M. S.; Eaton, B. E. *J. Am. Chem. Soc.* **1995**, *117*, 9119. (b) Seela, F.; Zulauf, M.; Sauer, M.; Deimel, M. *Helv. Chim. Acta* **2000**, *83*, 910. (c) Kerr, C. E.; Mitchell, C. D.; Headrick, J.; Eaton, B. E.; Netzel, T. L *J. Phys. Chem. B* **2000**, *104*, 1637. (d) Seela, F.; Feiling, E.; Gross, J.; Hillenkamp, F.; Ramzaeva, N.; Rosemeyer, H.; Zulauf, M. *J. Biotechnol.* **2001**, *86*, 269. (e) Hurley, D. J.; Seaman, S. E.; Mazura, J. C.; Tor, Y. *Org. Lett.* **2002**, *4*, 2305. (f) McKeen, C. M.; Brown, L. J.; Nicol, J. T.; Mellor, J. M.; Brown, T. *Org. Biomol. Chem.* **2003**, *1*, 2267.

(15) (a) Godde, F.; Toulme, J. J.; Moreau, S. *Biochemistry* **1998**, *37*, 13765. (b) Godde, F.; Toulme, J. J.; Moreau, S. *Nucleic Acids Res.* **2000**, *28*, 2977.

Effective quenching of intrinsic fluorescence by duplex formation containing 4-aminobenzo[*g*]quinazoline-2-one 2′-*O*-CH_3 riboside was also observed.[15b] Consequently, 4-aminobenzo[*g*]quinazoline-2-one 2′-deoxyribonucleoside (C_f) was chosen for insertion into a 16-nt hairpin DNA at C_{15} (hairpin DNA-C_{f15}) in lieu of cytidine (Figure 2).

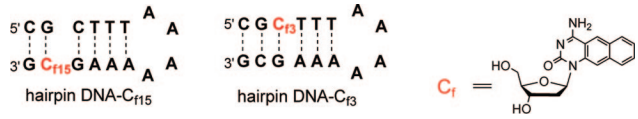
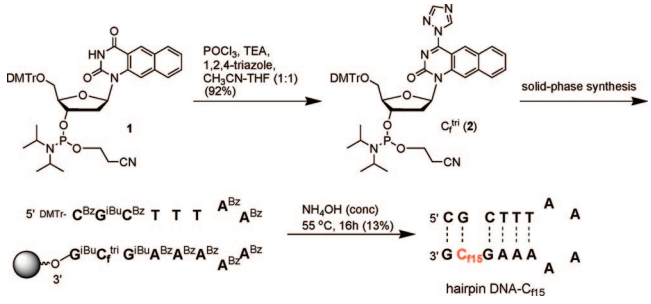


Figure 2. Chemical structures of hairpin DNA-C_{f15} and hairpin DNA-C_{f3} having the 2′-deoxyriboside of 4-aminobenzo[*g*]quinazoline-2-one (C_f) at positions 15 and 3, respectively.

Since DNA binding and cleavage mediated by BLM is achieved from the minor groove of DNA,[16] projection of the benzo[*g*]quinazoline heterocycle in the DNA duplex into the major groove was anticipated not to affect DNA binding and cleavage by Fe(II)•BLM. Accordingly, it was hoped that hairpin DNA-C_{f15} would exhibit efficient quenching of benzo[*g*]quinazoline fluorescence due to formation of the base-paired duplex, while strong fluorescence emission would result from release of C_f derivatives following treatment with Fe(II)•BLM analogues. Because we wish to identify BLM analogues having altered DNA cleavage properties, and have noted previously that some BLM congeners exhibit altered strand selectivity of DNA cleavage,[17] a second hairpin DNA containing the fluorescent nucleobase in lieu of cytidine$_3$ (hairpin DNA-C_{f3}) was also prepared (Figure 2).

To incorporate C_f into the 16-nt hairpin DNAs, triazolide phosphoramidite **2** was prepared from benzo[*g*]quinazoline-2,4-(3H)-dione phosphoramidite **1**[15a] (Scheme 1). We found

Scheme 1. Synthesis of Hairpin DNA-C_{f15}



that the triazolide moiety could be introduced into the uridine analogues at the phosphoramidite level and converted to the requisite cytidine derivative concomitant with the deblocking of the formed oligonucleotide by treatment with NH_4OH, according to synthesis procedures for 4-triazolothymidine or uridine derivatives.[18] This mode of conversion into the C_f derivative via the triazolide phosphoramidite obviated the need for the additional transformations that had been employed for the 2′-*O*-CH_3 ribonucleoside of 4-aminobenzo[*g*]quinazoline-2-one.[15b] The conversion of **1** into triazolide phosphoramidite **2** was accomplished in 92% yield.[19] Hairpin DNA-C_{f15} was synthesized by standard automated DNA synthesis protocols (1 μmol scale). The overall and average stepwise coupling yields were determined to be 91.4% and 99.4%, respectively. Deprotected and purified hairpin DNA-C_{f15} was obtained as a colorless powder in 13% yield (1 μmol scale). Hairpin DNA-C_{f3} was prepared analogously.

The fluorescence emission of hairpin DNA-C_{f15} following treatment with Fe(II)•BLM was monitored by fluorescence spectroscopy. Figure 3 shows the emission spectrum of 0.72

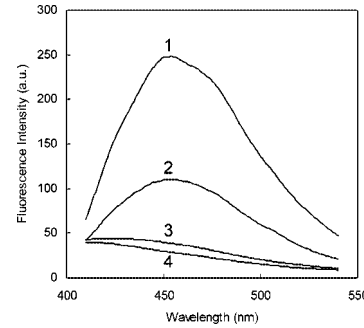


Figure 3. Emission spectra of hairpin DNA-C_{f15} (0.72 μM) treated with 2.5 μM Fe(II)•BLM A_5 (curve 1) and 2.5 μM BLM A_5 (curve 4) after 30 min (λ_{ex} 310 nm) at 25 °C in 10 mM Na cacodylate buffer, pH 7.0, with 100 mM NaCl. The emission spectra of hairpin DNA-C_{f3} treated with Fe(II)•BLM A_5 (curve 2) and BLM A_5 (curve 3) under the same conditions are also shown.

μM hairpin DNA-C_{f15} following treatment with 2.5 μM Fe(II)•BLM A_5. The spectrum exhibited emission enhancement (maximum λ_{em} 455 nm) when excited at 310 nm. In addition, an 8-fold enhancement of fluorescence emission was observed relative to the hairpin DNA-C_{f15} treated with BLM A_5 in the absence of Fe^{2+} (as a control); this was similar to the fluorescence enhancement observed with our previous DNA beacon.[10] This indicates that BLM can recognize and cleave a DNA containing the non-natural fluorescent nucleobase C_f, releasing C_f derivatives. As expected, hairpin DNA-C_{f15} itself exhibited little fluorescence when excited at 310 nm. As anticipated based on earlier studies with DNA duplexes of similar sequence,[17,20] analogous treatment of hairpin DNA-C_{f3} with Fe(II)•BLM A_5 produced less fluorescence enhancement (Figure 3).

To characterize the formed products, HPLC analysis of

(16) (a) Kuwahara, J.; Sugiura, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 2459. (b) Bailly, C.; Waring, M. J. *J. Am. Chem. Soc.* **1995**, *117*, 7311.

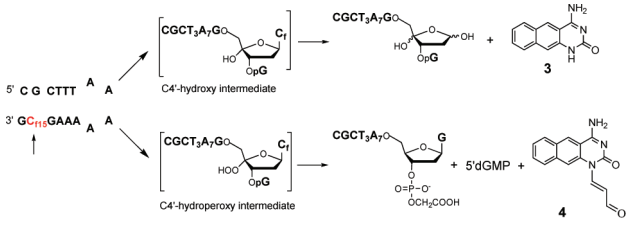
(17) Sugiyama, H.; Kilkuskie, R. E.; Chang, L.-H.; Ma, L.-T.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1986**, *108*, 3852.

(18) (a) Webb, T. R.; Matteucci, M. K. *Nucleic Acids Res.* **1986**, *14*, 7661. (b) Cowart, M.; Gibson, K. J.; Allen, D. J.; Benkovic, S. J. *Biochemistry* **1989**, *28*, 1975. (c) Xu, Y.-Z.; Zheng, Q.; Swann, P. F. *J. Org. Chem.* **1992**, *57*, 3839. (d) Chirakul, P.; Sigurdsson, S. T. *Org. Lett.* **2003**, *5*, 917. (e) Jin, S.; Miduturu, C. V.; McKinney, D. C.; Silverman, S. K. *J. Org. Chem.* **2005**, *70*, 4284.

(19) The conversion of **1** to **2** was 98% based on the peak areas of the signals from the H-5 protons of **1** (8.73 ppm) and **2** (8.31 ppm).

hairpin DNA-C_{f15} was carried out after treatment with Fe(II)·BLM A_5. It is known that cleavage of DNA by BLM is achieved both through C4′-OH and C4′-OOH intermediates,[1b,c] which can be quantified by C_{18} reversed-phase HPLC using oligonucleotides having sequences similar to those employed here.[17,20] As shown in Scheme 2, cleavage

Scheme 2. Cleavage Products Resulting from Treatment of Hairpin DNA-C_{f15} with Fe(II)·BLM



of hairpin DNA-C_{f15} at cytidine$_{15}$ would result in the release of fluorescent cytosine (fCytosine, **3**) and fluorescent cytosine propenal (fCytosine propenal, **4**). The formation of **3** and **4** would be readily distinguished from the products released upon cleavage at cytidine$_3$ (i.e., cytosine and cytosine propenal) and consistent with cleavage of the ribose moiety at C_{15} via C4′-OH and C4′-OOH intermediates, respectively. In fact, degradation of hairpin DNA-C_{f15} by Fe(II)·BLM A_5 was accompanied by the release of two fluorescent compounds when monitored chromatographically. By comparison with authentic synthetic fCytosine (**3**) (Scheme 1, Supporting Information), it was found that one of the fluorescent products comigrated with authentic synthetic fCytosine.[21] Quantification of the cleavage of hairpin DNA-C_{f15} by Fe(II)·BLM indicated that the majority of cleavage (78%) occurred at C_{15}, with the remainder at C_3. This was in quite good agreement with earlier studies that employed DNA duplexes of similar sequence.[17,20] The total of cleavage events at C_3 and C_{15} was also not significantly less than that observed for the unmodified duplexes. In both these regards, hairpin DNA-C_{f15} is clearly better than the DNA beacon reported initially.[10] Interestingly, the ratio of products formed using the two pathways shown in Scheme 2 was substantially different at the two sites of cleavage (i.e., C_3 vs C_{15}). This type of difference in degradation chemistry at two sites in one DNA−substrate has been documented previously.[22] The hairpin DNA-C_{f3} was degraded with even greater efficiency by Fe(II)·BLM A_5 (Table S1, Supporting Information).[23]

As the new hairpin DNA was prepared to facilitate the rapid evaluation of BLM combinatorial libraries, it seemed important to demonstrate that the results obtained were not dissimilar to those obtained using more laborious techniques. Accordingly, DNA cleavage efficiency by fluorescence emission and its correlation with results obtained by gel electrophoresis was determined for 15 BLM congeners (Figure S1, Supporting Information) known to exhibit quite different DNA degradation efficiencies. The cleavage of hairpin DNA-C_{f15} by the BLM analogues confirmed that small differences in structure between the analogues could strongly affect the resulting fluorescence intensity (Figure S2, Supporting Information). BLM A_5 exhibited the strongest fluorescent enhancement of the analogues studied. With the exception of isoBLM A_2, the analogues of BLM A_5 differing in the nature of the carbohydrates such as tallysomycin A, decarbamoyl BLM A_2, and L-gulose BLM A_5 afforded diminished fluorescence intensity compared to that of BLM A_5.[24] As is clear from Figure S2 (Supporting Information), several of the BLM analogues tested (e.g., phleomyin, BLM BAPP, decarbamoyl BLM A_5, decarbamoyl BLM A_2, and iso BLM A_2) produced significant enhancement of fluorescence from hairpin DNA-C_{f15}. In comparison, other BLMs produced minimal DNA cleavage under the conditions employed. To evaluate whether or not the fluorescence intensities produced from hairpin DNA-C_{f15} correlated with those that would have been obtained by routinely used DNA cleavage assays, a 5′-^{32}P end labeled 16-nucleotide hairpin DNA lacking C_f was treated with the same 15 Fe(II)·BLMs. Fe(II)·BLM A_5 was also the most potent in this assay (Figure S3, Supporting Information). Critically, the results of polyacrylamide gel analysis generally showed quite similar trends in efficiencies of cleavage activity for most BLMs as that determined by fluorescence emission, indicating that hairpin DNA-C_{f15} can likely be used to characterize BLMs produced as members of BLM combinatorial libraries. Any BLM congener producing cleavage at C_{15}, or a significant alteration of hairpin DNA ternary structure as a result of cleavage elsewhere, should be detectable using this new substrate.

Acknowledgment. This work was supported by NIH Research Grant No. CA76297, awarded by the National Cancer Institute.

Supporting Information Available: Experimental procedures for the synthesis and characterization of C_f phosphoramidite (**2**), fCytosine (**3**), and hairpin DNA-C_{f15} as well as experimental details of the BLM-mediated DNA cleavage. This material is available free of charge via the Internet at http://pubs.acs.org.

OL800445X

(20) (a) Sugiyama, H.; Kilkuskie, R. E.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1985**, *107*, 7765. (b) Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 4104. (c) Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *Biochemistry* **1988**, *27*, 58.

(21) Moreover, mass spectrometric analysis of **3** collected by C_{18} reversed-phase HPLC had *m/z* 212.2, in good agreement with that determined for authentic synthetic fCytosine (*m/z* 211.6).

(22) Long, E. C.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1990**, *112*, 5272.

(23) While the ratio of cleavage events at C_3 vs C_{15} was essentially identical with that observed for hairpin DNA-C_{f15}, the actual chemistry of degradation at the two sites was actually reversed from that found for hairpin DNA-C_{f15}. Thus the presence of C_f within the duplex structure influences the specific chemistry of degradation at a given site, but not the total degradation events (which is the process intended to be monitored by the use of this sensor).

(24) Although the exact function(s) of the carbohydrate moiety in BLM are incompletely understood, this result supports earlier observations concerning the importance of the sugars in BLM.[6d,25]

(25) (a) Sugiyama, H.; Ehrenfeld, G. M.; Shipley, J. B.; Kilkuskie, R. E.; Chang, L.-H.; Hecht, S. M. *J. Nat. Prod.* **1985**, *48*, 869. (b) Boger, D. L.; Teramoto, S.; Zhou, J. *J. Am. Chem. Soc.* **1995**, *117*, 7344. (c) Choudhury, A. K.; Tao, Z.-F.; Hecht, S. M. *Org. Lett.* **2001**, *3*, 1291. (d) Brahim, S.; Prévotat, L.; Yatouji, S.; Mérino, D.; Cortier, M.; Rébé, C.; Micheau, O.; Kenani, A.; Bettaieb, A. *Biochem. Pharmacol.* **2007**, *74*, 1445.