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Highly Efficient DNA Interstrand Crosslinking Induced by an Antitumor Antibiotic, Carzinophilin

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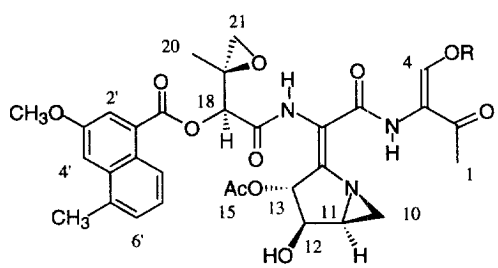
Abstract: The alkylation of d(TAGCTA)2 by an antitumor antibiotic, carzinophilin, and its 4-methyl derivative was investigated. It was found that both carzinophilin (**1a**) and 4-*O*-methylcarzinophilin (**1b**) react with d(TAGCTA)2 to provide the corresponding monoadducts and the interstrand crosslinked adducts. Heating the crosslinked adduct of **1b** provided a stable base adduct which consists of a 1:1:1 ratio of adenine, guanine and 4-*O*-methylcarzinophilin. The structure of the base adduct was consistent with double crosslinks between guanine and the epoxide moiety and between adenine and the aziridine moiety. HPLC analysis indicates that the first alkylation occurs at the aziridine moiety with adenine N7 and the second crosslinking proceeds highly efficiently between the epoxide moiety and guanine N7.

The antitumor antibiotic, carzinophilin (**1a**), was isolated from *Streptomyces sahchiroi* by Hata *et al.* in 1954.[1] Its prominent cytotoxicity is suggested to be derived from the putative interstrand crosslinking ability of the aziridine and epoxide moieties of carzinophilin.[2] This unprecedented structure and function make this agent an attractive target for synthetic efforts; however, no total synthesis of the agent has been reported.[3] Recently, Armstrong *et al.* demonstrated that interstrand crosslinking occurs at N7 of the purine base at the 5'-GNC-3' or 5'-GNT-3' sequences in the duplex DNA fragment.[4] However, the detailed chemistry of the crosslinking has not been elucidated. In the present study, we investigated the alkylation of self-complementary oligodeoxynucleotide (ODN) d(TAGCTA)2 by carzinophilin. It was demonstrated that (i) the first alkylation step occurs between the aziridine moiety and adenine N7 and (ii) the highly efficient second crosslinking proceeds between the epoxide moiety and guanine N7.



Carzinophilin (**1a**)

1a: R = H
1b: R = CH_3

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A reaction mixture containing **1a** and d(TAGCTA)2 in neutral buffered solution was incubated at 0 ℃ and the progress of the reaction was monitored by HPLC. Figure 1 shows the HPLC profile of the reaction mixture after 20 h incubation, indicating the formation of major and minor products eluted at 16 and 27 min, respectively. Ion spray MS of the HPLC peaks suggests that the major and the minor products were the crosslinked adduct (**3a**) and monoalkylated adduct (**2a**), respectively.[5] In fact, the isolated adduct **2a** was found to react further with d(TAGCTA) to produce **3a** quantitatively. These results clearly indicate that the second crosslinking step proceeds quite efficiently. Upon heating at 90 ℃ for 5 min, the isolated **2a** was degraded to the abasic site-containing ODN **4**, whereas the isolated **3a** produced **4** and **5** in a 1: 1 ratio (Scheme 1).[6]

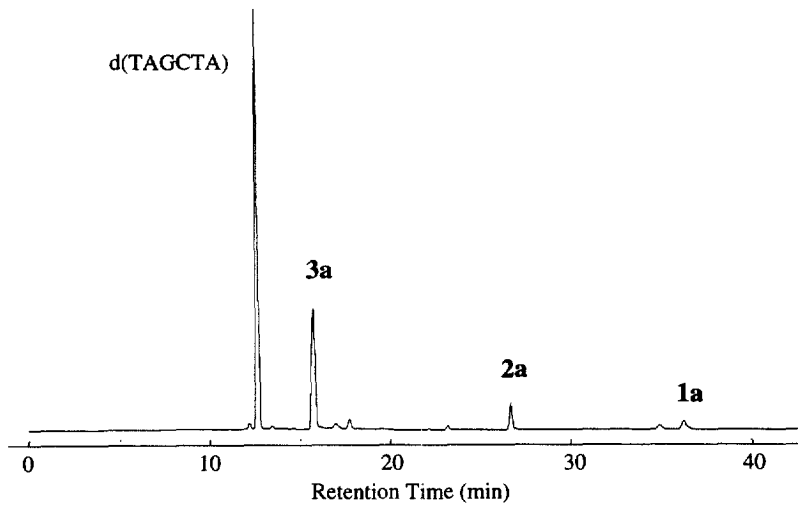
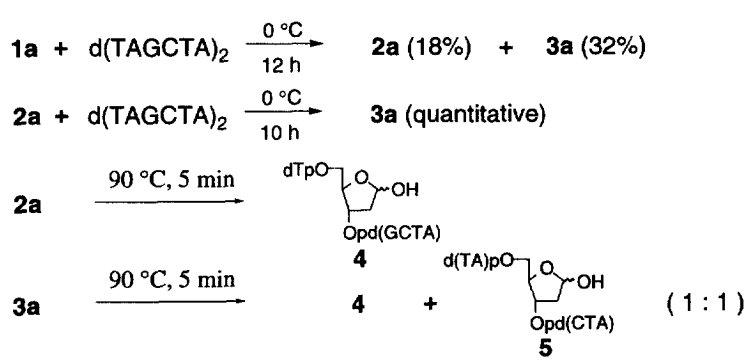


Figure 1. HPLC profile of **1a**-treated d(TAGCTA)2 after incubation at 0 ℃ for 20 h. The reaction mixture (total volume 100 μL) containing 50 μM of **1a**, 10 % (v/v) methanol and an 83 μM strand concentration of d(TAGCTA) in 50 mM Na cacodylate (pH 7.0) was incubated at 0 ℃ for 20 h. The reaction mixture was analyzed by HPLC on a Cosmosil 5C18 column (4.6 x 150 mm) detected at 254 nm; elution was with 0.05 M ammonium formate containing 0-40 % acetonitrile, with a linear gradient for 40 min at a flow rate of 1.0 mL/min.

Scheme 1



Unfortunately, heating **3a** gave a cluster of peaks with a longer retention time on HPLC, suggesting the decomposition of the putative base adduct under the conditions. Further attempts to characterize **2a** and **3a** were unsuccessful. We reasoned that the decomposition of base adduct **3a** is probably due to the thermal instability of the enolic 4-hydroxyl group of the adduct **3a**.[2b] Thus, we employed *O*-methylated derivative **1b** in place of **1a** to further examine the reaction with d(TAGCTA)2. HPLC analysis of the reaction mixture of **1b** with d(TAGCTA)2 indicated that the formation of mono adduct **2b** and crosslinked adducts **3b** occurred in a similar manner as the reaction of **1a**.[8] The isolated **2b** reacted further with d(TAGCTA) to produce **3b** quantitatively. Upon heating, **3b** gave abasic site-containing ODNs **4** and **5** together with the formation of a discrete peak (**6**) at a longer retention time on HPLC. The product **6** was isolated by HPLC and subjected to spectroscopic analysis. The ^{1}H NMR and ion spray MS indicated that the product is a 1:1:1 adduct of adenine, guanine and **1b**.[9] However, the substitution pattern at the ring opening of the aziridine and the epoxide moieties was not elucidated due to the lack of the NOEs between the purine bases and the reacted site of carzinophilin. Since a putative base adduct should have diol mono-ester functionality, base hydrolysis and subsequent $NaIO_4$ oxidation of **6** were then conducted. The treatment of **6** with hot alkaline (90 ℃, 5 min) and subsequent oxidation with $NaIO_4$ resulted in a formation of 7-(2-oxopropyl)guanine (**7**) in 80% yield. The authentic sample of **7** was prepared from deoxyguanosine by a reported method.[10] These results suggest that the N7 of guanine is alkylated by the epoxide moiety of **1b** and thus the structure was tentatively assigned as **6** (Scheme 2).

Scheme 2

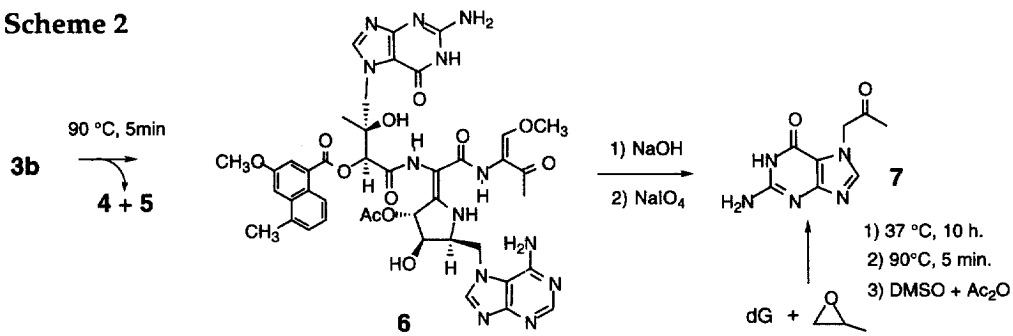


Figure 2 shows the time course of the product formation in the reaction of **1b** with d(TAGCTA)2 at 0 ℃. It is noteworthy that no formation of a monoadduct with the epoxide moiety was observed on HPLC. The results suggest that the conformational change induced by the first aziridine alkylation accelerates the second crosslinking step with the epoxide moiety. These results indicate that the crosslinking proceeds via a two-step mechanism in which adenine N7 first reacts with the aziridine moiety and subsequently guanine N7 crosslinks with the epoxide moiety as shown in Scheme 3.

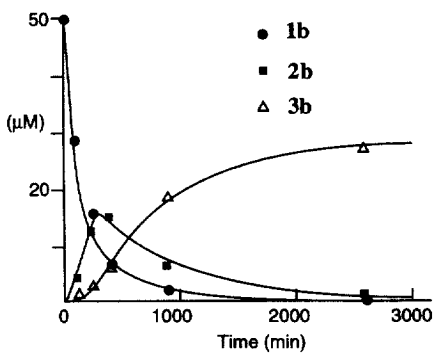
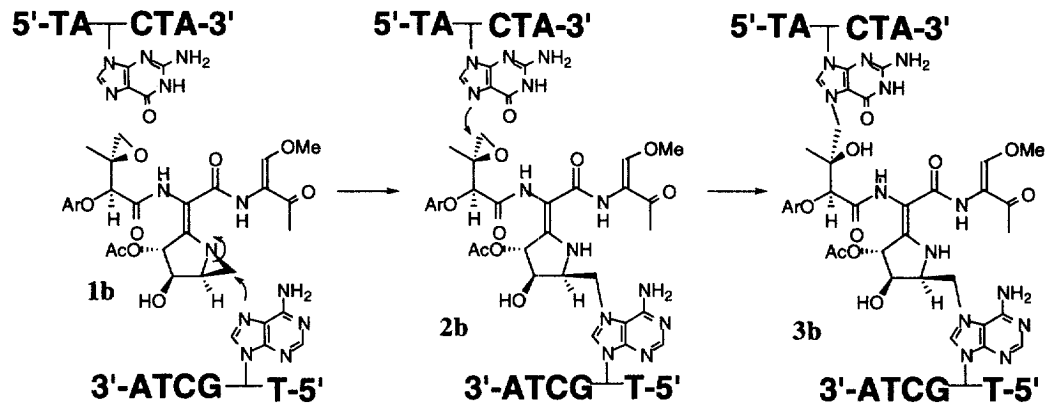


Figure 2. Time-dependent product distribution in the reaction of **1b** with d(TAGCTA)$_2$ at 0 °C.

The present study revealed that the aziridine moiety of carzinophlin first alkylates purine N7 to provide a mono adduct which facilitates an efficient second crosslinking. A detailed understanding of the molecular basis for the DNA alkylation by carzinophilin would provide useful information for the design of an efficient synthetic interstrand crosslinking agent with potent cytotoxic activity such as disrupting replication and transcription.

Scheme 3



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2. Carzinophilin is identical with azinomycin B which was isolated from cultures of *Streptomyces griseofuscus*: (a) Nagaoka, K.; Mastumoto, M.; Ono, J.; Yokoi, K.; Ishizeki, S.; Nakashima, T. *J. Antibiot.* **1986**, *39*, 1527. (b) Yokoi, K.; Nagoka, K.; Nakashima, T. *Chem. Pharm. Bull.* **1986**, *34*, 4554. (c) Moran, E. J.; Armstrong, R. W. *Tetrahedron Lett.* **1991**, *32*, 3807.
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4. Armstrong, R. W.; Salvati, M. E.; Nguyen, M. *J. Am, Chem, Soc.* **1992**, *114*, 3144.
5. Ion spray MS; **2a** obs 2414.2 (negative), calc. 2414.9; **3a** obs 4204.8 (negative), calc 4206.1.
6. Ion spray MS; **4** obs 1658.4 (negative), calc. 1658.1. **5** obs 1674.2 (negative), calc 1674.1. The structures of **4** and **5** were further confirmed by NaBH4 reduction and subsequent enzymatic digestion as described previously.[7]
7. (a) Sugiyama, H.; Fujiwara, T.; Ura, A.; Tashiro, T.; Yamamoto, K.; Kawanishi, S.; Saito, I. *Chem. Res. Toxicology* **1994**, *7*, 673. (b) Sugiyama, H.; Hosoda, M.; Saito, I.; Asai, A.; Saito, H. *Tetrahedron Lett.* **1990**, *31*, 7197.
8. Ion spray MS; **2b** obs 2428.2 (negative), calc. 2428.9; **3b** obs 4219.4 (negative), calc 4220.1.
9. Ion spray MS; **6** obs 923.5 (positive), 923.5 (negative) calc. 923.9.
10. Solomon, J. J.; Mukai, F.; Fedyk, J.; Segal, A. *Chem.-Biol. Interactions* **1988**, *67*, 275. **7**: ^{1}H NMR ($CDCl_3$) δ 10.78 (brs, 1H, 1-NH), 7.78 (s, 1H, 8), 6.18 (s, 2H, 2-NH_2), 5.16 (s, 2H, CH_2), 2.18 (s, 3H, CH_3); FABMS (positive ion) m/z 208 [M+1].
11. Carzinophilin was obtained from Kyowa Hakko through the courtesy of Drs. Akira Asai and Hirofumi Nakano. This work was supported by a Grant-in-Aid for Scientific Research on Priority Research from the Ministry of Education, Sports and Culture, Japan.