and the Robert A. Welch Foundation for generous financial support.

Supplementary Material Available: Tables of bond distances, bond angles, atomic coordinates, and thermal parameters for **3** (6 pages); listings of observed and calculated structure factors for **3** (6 pages). Ordering information is given on any current masthead page.

Novel Interstrand Cross-Links Induced by the Antitumor Antibiotic Carzinophilin/Azinomycin B

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Carzinophilin is an antitumor antibiotic isolated from the culture broth of *Streptomyces sahachiroi*,[1] whose structure determination has remained elusive for over 30 years.[2,3] Comparison of carzinophilin with azinomycin B, a recently[4] isolated metabolite from *Streptomyces griseofuscus* S42227, confirms that they are the same compound.[5] Earlier studies concluded that carzinophilin caused significant DNA damage both in vitro and in vivo as a result of cross-link formation.[6] We wish to report that bifunctional alkylation by carzinophilin[7] affords interstrand cross-links in the major groove between G and purine residues two bases removed in duplexed DNA fragments containing the sequences

5′-GNT-3′	5′-GNC-3′
3′-CNA-5′	3′-CNG-5′

We reached this conclusion by the rational design of synthetic oligonucleotides containing the bases inosine and 7-deazaguanine at the target alkylation sites.

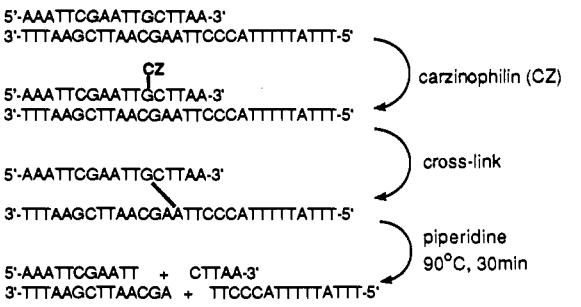
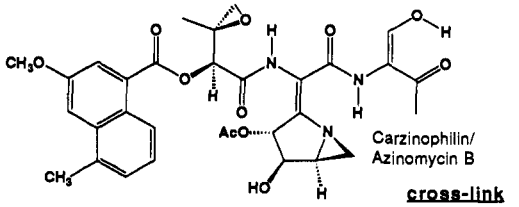
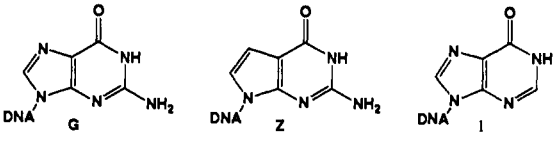


Figure 1.

Table I. Relative Efficiency of Interstrand Cross-Link Formation of Duplex DNAs **1**–**10** Following Incubation with Carzinophilin/Azinomycin B (* Denotes Trace). All Duplexes Are Identical Except for Variations in the Highlighted Three Base Pair Sequence



	cross-link	
1.	5'-AAATTCGAATT GCT TAA-3' (12 13 14) 3'-TTTAAGCTTAA CGA ATTCCCATTTTTATTT-5' (19 18 17)	++
2.	5'-AAATTCGAATT ZCT TAA-3' 3'-TTTAAGCTTAA CGA ATTCCCATTTTTATTT-5'	0
3.	5'-AAATTCGAATT GCT TAA-3' 3'-TTTAAGCTTAA CZA ATTCCCATTTTTATTT-5'	+
4.	5'-AAATTCGAATT ICT TAA-3' 3'-TTTAAGCTTAA CGA ATTCCCATTTTTATTT-5'	+
5.	5'-AAATTCGAATT GCT TAA-3' 3'-TTTAAGCTTAA CIA ATTCCCATTTTTATTT-5'	++
6.	5'-AAATTCGAATT GAT TAA-3' 3'-TTTAAGCTTAA CTA ATTCCCATTTTTATTT-5'	0*
7.	5'-AAATTCGAATT GTT TAA-3' 3'-TTTAAGCTTAA CAA ATTCCCATTTTTATTT-5'	0*
8.	5'-AAATTCGAATT GCC TAA-3' 3'-TTTAAGCTTAA CGG ATTCCCATTTTTATTT-5'	++
9.	5'-AAATTCGAATT GAC TAA-3' 3'-TTTAAGCTTAA CTG ATTCCCATTTTTATTT-5'	+
10.	5'-AAATTCGAATT GCA TAA-3' 3'-TTTAAGCTTAA CGT ATTCCCATTTTTATTT-5'	0*



Incubation of carzinophilin (11 °C, pH 7, 24 h) with duplex DNA **1** (Table I), ^{32}P-end-labeled at the 5′ terminus of the long or the short strand[8] followed by denaturing polyacrylamide gel electrophoresis (PAGE) analysis, afforded bands of higher molecular weight (lower gel mobility), which were identified as

(1) Hata, T.; Koga, F.; Sano, Y.; Kanamori, K.; Matsumae, A.; Sugawara, R.; Shima, T.; Ito, S.; Tomizawa, S. *J. Antibiot., Ser. A* **1954**, *7*, 107. All experiments were conducted with carzinophilin from *S. sahachiroi*. The structure of carzinophilin shown in this paper was proposed by Nakashima for azinomycin B (ref 4).

(2) Initial decomposition fragments: Onda, M.; Konda, Y.; Omura, S.; Hata, T. *Chem. Pharm. Bull.* **1971**, *19*, 2013. For other structures, see: Lown, J. W.; Hanstock, C. C. *J. Am. Chem. Soc.* **1982**, *104*, 3213. Onda, M.; Konda, Y.; Hatano, A.; Hata, T.; Omura, S. *J. Am. Chem. Soc.* **1983**, *105*, 6311. Onda, M.; Konda, Y.; Hatano, A.; Hata, T.; Omura, S. *Chem. Pharm. Bull.* **1984**, *32*, 2995.

(3) Synthetic approaches to CZ have generated partial fragments which correlate well with spectral data of the natural product, although ultimate confirmation of the structure will have to await total synthesis or X-ray analysis. For initial studies, see: Shibuya, M. *Tetrahedron Lett.* **1983**, *24*, 1175. Garner, P. *Tetrahedron Lett.* **1984**, *25*, 5855. Garner, P.; Park, J. M.; Rotello, V. *Tetrahedron Lett.* **1985**, *26*, 3299. More recently, partial fragments containing the epoxide of structure **1** have been synthesized: Shibuya, M.; Terauchi, H. *Tetrahedron Lett.* **1987**, *28*, 2619. Ando, K.; Yamada, T.; Shibuya, M. *Heterocycles* **1989**, *29*, 2209. England, P.; Chun, K.-H.; Moran, E. J.; Armstrong, R. W. *Tetrahedron Lett.* **1990**, *31*, 2669. Moran, E. J.; Armstrong, R. W. *Tetrahedron Lett.* **1991**, *32*, 3807.

(4) Yokoi, K.; Nagaoka, K.; Nakashima, T. *Chem. Pharm. Bull.* **1986**, *34*, 4554. Ishizeki, S.; Ohtsuka, M.; Irinoda, K.; Kukita, K.-I.; Nagaoka, K.; Nakashima, T. *J. Antibiot.* **1987**, *40*, 60. Nagaoka, K.; Matsumoto, M.; Oono, J.; Yokoi, K.; Ishizeki, S.; Nakashima, T. *J. Antibiot.* **1986**, *34*, 1527.

(5) Armstrong, R. W.; Salvati, M. E.; Moran, E. J. Submitted for publication.

(6) Terawaki, A.; Greenberg, J. *Biochim. Biophys. Acta* **1966**, *119*, 59. Terawaki, A.; Greenberg, J. *Nature* **1966**, *209*, 481. Lown, J. W.; Begleiter, A.; Johnson, D.; Morgan, A. R. *Can. J. Biochem.* **1976**, *54*, 110.

(7) Formation of identical cross-links were observed for the drug derived from both *S. sahachiroi* (carzinophilin) and *S. griseofuscus* (azinomycin).

(8) Duplex **1** was designed to probe the competitive formation of G-to-G cross-links involving G residues which are either 3′ or 5′ diagonally-substituted. To facilitate the identification of potential cross-links, DNA strands of differing lengths were chosen since only interstrand cross-links should coelute when either strand is separately radiolabeled. This procedure readily differentiates between intrastrand reactions or strand modifications (multiple alkylations) which might also result in bands of decreased mobility by denaturing PAGE analysis. Complementary DNA strands were synthesized on an Applied Biosystems Model 381A synthesizer and were purified as detritylated oligomers using an Axxi-Chrom ODS column (10–15% CH_3CN gradient in a 0.1 M triethylammonium acetate solution at pH 7).

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cross-linked DNA.[9] The desired bands were cut from the gel, isolated by electroelution, treated with piperidine (90 °C, 30 min), and resubmitted to PAGE analysis. Cross-linked material 5'-end-labeled on the shorter strand afforded a major cut at the guanine at position 12 (G_{12}) when compared to a Maxam and Gilbert G-specific lane.[10] Likewise, cross-linked material 5'-end-labeled on the longer strand afforded cleavage at the A_{17} residue, corresponding to a three base pair bisalkylation between G and A residues in the sequence

5'-GCT-3'
3'-CGA-5'

Conversion of isolated cross-link to a major cleavage site on each strand suggests that the covalent bond formation occurs at the piperidine-labile locations.[11,12]

Inosine (I) and the modified nucleoside 7-deazaguanine (Z) were synthetically incorporated to evaluate potential base alkylation sites. Substitution of the G_{12} residue involved in the G-to-A cross-link in **1** with 7-deazaguanine **2** resulted in no cross-link formation. No piperidine-sensitive scission was detected at this base even in single-stranded experiments. Cross-links were generated upon replacement of G_{18} with 7-deazaguanine **3**, but were not observed with either T (**6**) or A (**7**) substitution at this base. Substitution of inosine for either the G_{12} or G_{18} residue afforded cross-links (**4**, **5**). However, yields were lower for **4**, consistent with the decreased nucleophilicity of the N7 atom of inosine.[13] Substitution of the A_{17} residue with G afforded G-to-G bisalkylations in the sequences

5'-GCC-3'	5'-GAC-3'
3'-CGG-5'	3'-CTG-5'
8	**9**

Attempts to induce G-to-G cross-links involving adjacent base pairs failed (**10**).[14]

All G residues in either *single* strand of **1** are alkylated by carzinophilin at various levels of efficiency, whereas all A residues (including A_{17}) are inert (data not shown). Given the differences in nucleophilicity of N7 of A and G, these results support a model for cross-link formation in which the first alkylation at G_{12} directs the second alkylation at A_{17} (Figure 1). The reaction at the usually inert A_{17} residue is thus template directed, and the kinetics of alkylation are governed by the high concentration of electrophile at the second alkylation site. For **8** and **9**, G-to-G cross-links could lead to a mixture of diastereomeric products. Within this framework, involvement of N7 of G_{12} is supported by the results obtained with the modified duplexes **2**–**5**.[15] The greater propensity for depurination at the A_{17} residue (vs G_{12}) after cross-link formation in **1** is consistent with the lower stability of N7 alkylations at this base.[16] Finally, formation of cross-links in **1**, **3**–**5**, **8**, and **9** at purine residues located in the 5' direction of the three base pair sequence leads us to the preliminary conclusion that this novel natural product reacts in the major groove. Further characterization of the drug–DNA complex will be determined when suitable amounts of material become available.

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Supplementary Material Available: Experimental details and polyacrylamide gels showing cross-linking experiments for DNAs **1**–**10** (7 pages). Ordering information is given on any current masthead page.

(9) DNA–CZ complexes were treated with 0.2 N NaOH in order to prevent depurination of cross-links when submitted to PAGE analysis. Cross-linked DNA which had not been exposed to base (obtained in low yield) could be electroeluted from gel and converted to material of identical migration to that which had been treated with NaOH prior to gel analysis. Stabilization of N7 alkylations under similar conditions has been previously reported: Kohn, K. W.; Spears, C. L. *Biochim. Biophys. Acta* **1967**, *145*, 743. For a recent demonstration of nitrogen mustard cross-link stabilization using base to elucidate the novel 5'-GXC-3' sequence selectivity of mechloroethylenamine, see: Ojwang, J. O.; Grueneberg, D. A.; Loechler, E. L. *Cancer Res.* **1989**, 6529.

(10) Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499.

(11) Alkylations (<20%) were also detected by piperidine treatment of isolated cross-linked DNA at the G residues of the second potential cross-link site

5'-CG-3'
3'-GC-5'

Non-piperidine-labile alkylations could be difficult to detect using the base treatment strategy. However, PAGE analysis of a 3'-end-labeled isolated cross-link suggests no molecular weight change of fragments obtained upon piperidine treatment.

(12) Specific base (G and A) and groove (major) locations of the cross-links were independently confirmed by subjecting the isolated cross-linked duplexes to PAGE analysis after treatment with the chemical nuclease reagent (1,10-phenanthroline)copper (OP-Cu): Sigman, D. S.; Graham, D. R.; DAurora, V.; Stern, A. M. *J. Biol. Chem.* **1979**, *254*, 12269. Ferrous EDTA has also been used to confirm cross-link location in other systems: Weidner, M. F.; Millard, J. T.; Hopkins, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 9270. Millard, J. T.; Weidner, M. F.; Raucher, S.; Hopkins, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 3637.

(13) Belikova, A. M.; Grineva, N. I.; Karpova, G. G. *Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk* **1972**, *4*, 110. Suzuki, Y. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 2469.

(14) Reaction of carzinophilin with **10** afforded only G monoalkylations, precluding adjacent base pair cross-links. However, formation of cross-links in **8** could involve both adjacent and three base pair G residues.

(15) The absence of alkylation or cross-links for **2** can be interpreted as resulting from a modification in the electron density of the N3 atom as a consequence of replacement of N7 with carbon, precluding alkylation at the N3 position. The N7 nitrogen could also be crucial to binding or activation of the drug–DNA complex.

(16) Drinkwater, N. R.; Miller, E. C.; Miller, J. A. *Biochemistry* **1980**, *19*, 5087.

A Variable-Temperature Study of the Quenching of Benzophenone Triplet by Oxygen: Involvement of Exciplexes

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Throughout the last three decades, the chemistry of the high-energy triplet states of aromatic ketones has occupied the attention of photochemists.[1] Benzophenone (Bp) is typical of these species, and its relatively high T_1 energy (69 kcal/mol) causes it to be used as a photosensitizer for other molecular T_1 states. With energy acceptors such as aromatic hydrocarbons, it is generally agreed that the energy transfer proceeds with 100% efficiency. This is decidedly not the case, however, when molecular oxygen is the energy acceptor;[2] the fraction of triplet Bp(T_1) quenching processes that lead to singlet oxygen formation (S_Δ) is close to 0.3.[2c] This nonunity value of S_Δ is not confined to the case of benzophenone triplets; very few singlet oxygen sensitizers show $S_\Delta = 1$.[3] This variance possibly arises in part from the variety of the electron

(1) Many instances can be found in the following: Turro, N. J. *Modern Molecular Photochemistry*; Benjamin/Cummings: Menlo Park, CA, 1978.

(2) (a) Gorman, A. A.; Lovering, G.; Rodgers, M. A. J. *J. Am. Chem. Soc.* **1978**, *100*, 4527. (b) Garner, A.; Wilkinson, F. *Singlet Oxygen*; Ranby, B., Rabek, J. F., Eds.; Wiley: New York, 1978; p 48. (c) Gorman, A. A.; Hamblett, I.; Rodgers, M. A. J. *J. Am. Chem. Soc.* **1984**, *106*, 4679.

(3) For comprehensive tables of S_Δ values, see: (a) Saltiel, J.; Atwater, B. W. *Advances in Photochemistry*; Volman, D. H., Hammond, G. S., Gollnick, K., Eds.; Wiley: New York, 1988; Vol. 14, p 1. (b) Gorman, A. A.; Rodgers, M. A. J. *Handbook of Organic Photochemistry*; Scaiano, J. C., Ed.; CRC Press: Boca Raton, FL, 1989; Vol. II, p 229.

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