Demonstration and Definition of the Noncovalent Binding Selectivity of Agents Related to CC-1065 by an Affinity Cleavage Agent: Noncovalent Binding Coincidental with Alkylation

Dale L. Boger,* Jiacheng Zhou and Hui Cai

Department of Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, U.S.A.

Abstract—A study of the DNA cleavage efficiency and selectivity of CDPI$_3$–EDTA (4), an affinity cleavage agent based on the structure of CC-1065, is described. The studies with 4 provide direct evidence of AT-rich noncovalent binding coincidental with all DNA alkylation sites observed with (+)- or ent-(−)-CC-1065. Copyright © 1996 Elsevier Science Ltd

Introduction

(+)-CC-1065 (1)[1] is the initial member of a class of exceptionally potent antitumor antibiotics now including the duocarmycins[2–9] that derive their biological properties through a sequence selective alkylation of DNA.[10–20] Extensive studies, including the unusual observation of the unnatural enantiomer alkylation of duplex DNA, have defined the characteristics of this DNA alkylation reaction, which has been shown to proceed by reversible, stereoelectronically-controlled adenine N3 addition to the least substituted carbon of the activated cyclopropane at five base-pair AT-rich sites in the minor groove.[21–32] Since the disclosure of CC-1065 extensive efforts have been devoted to determining the origin of its DNA alkylation selectivity, to establish the link between DNA alkylation and the ensuing biological effects[33] and to define the fundamental principles underlying the relationships between its structure, functional reactivity and biological properties.
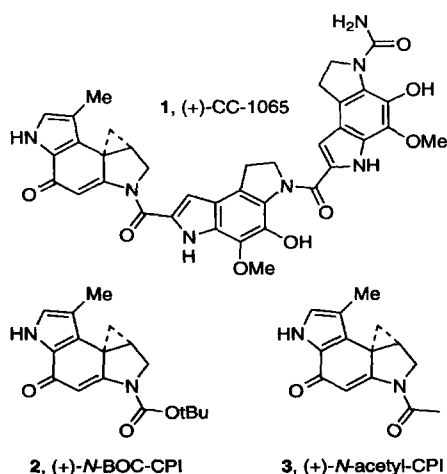
the DNA alkylation selectivity have emerged. One that attributes the selectivity to kinetic reactivity at the preferred alkylation sites[17–22] and our own proposal, where the preferential noncovalent binding selectivity restricts the accessible sites of DNA alkylation.[10–16,26,27] The former has been suggested to result from sequence-dependent catalysis through C4 carbonyl protonation by a strategically placed phosphate in the DNA backbone[20–22] or preferential alkylation at junctions of bent DNA,[34–36] while the latter has been attributed to preferential noncovalent binding[37,38] and subsequent alkylation within the narrower, deeper minor groove of AT-rich DNA. Central to the different interpretations have been the perceived similarities[21,22] or distinctions[26,27] in the alkylation selectivities of simple derivatives of the alkylation subunit [i.e., N-BOC-CPI or N-acetyl-CPI (2 and 3), and (+)-CC-1065 itself]. The former proposal is based on the premise that 1–3 alkylate the same sites and that the nature of the alkylation reaction controls the selectivity irrespective of noncovalent binding.[21,22] In contrast, the latter proposal requires that the AT-rich noncovalent binding selectivity of CC-1065 and its accessibility to the adenine N3 sites that accompanies deep penetration into the AT-rich minor groove controls the alkylation sequence selectivity. Notably, this latter model is supported by the experimentally observed AT-rich noncovalent binding selectivity of the agents,[37,38] which nicely accommodates the reverse and offset 5 base-pair AT-rich alkylation selectivity of the natural and unnatural enantiomers[27] and requires that 1 and 2–3 exhibit distinct alkylation selectivities.[26,27] Two key studies have demonstrated further that the former proposals cannot be accurate and that the latter noncovalent binding model accounts for the observed DNA alkylation selectivity.[39,40] In particular, the demonstration of a switch in the inherent enantiomeric DNA alkylation selectivity that accompanies the simple reversal of the agent orientation observed with the extended versus reversed analogues of duocarmycin SA

1, (+)-CC-1065

2, (+)-N-BOC-CPI

3, (+)-N-acetyl-CPI

In these studies, two distinct proposals for the origin of

and CC-1065 illustrates that the same fundamental recognition features are operative for both the natural and unnatural enantiomers and that the AT-rich noncovalent binding selectivity and the depth of minor groove penetration surrounding the alkylation site control the alkylation selectivity.[40]

Complementary to these studies, herein we detail a study of the affinity cleavage agent **4**.[41] These studies serve to reinforce the preceding studies, which demonstrated a preferential AT-rich noncovalent binding selectivity through measurement of binding constants[37,38] and provide a further direct characterization of noncovalent binding coincidental with all DNA alkylation sites observed with CC-1065.
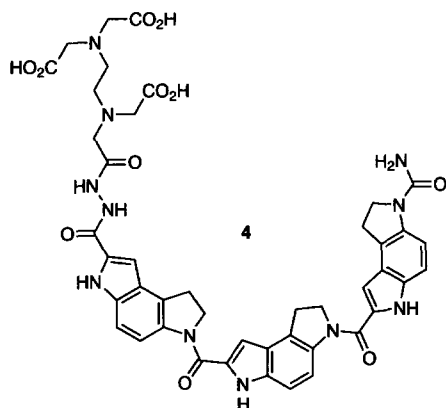
Results and Discussion

DNA cleavage properties of 4

DNA cleavage by Fe^{II}–**4** was first assessed with supercoiled ΦX174 DNA. In these studies, the Fe^{II} complex of **4** was found to produce both single and double strand cleavage in the presence of O_2 and 2-mercaptoethanol at concentrations substantially more efficiently than background iron itself. Typical results are summarized in Table 1. The agent alone in the absence of iron did not cause DNA cleavage.

The ratio of double-strand to single strand cleavage was determined in a kinetic study of the generation of Form II and Form III ΦX174 DNA and typical results are illustrated in Figure 1. The DNA cleavage reaction exhibited initial fast kinetics in the first 2 min and a slower subsequent rate which may reflect conversion to a less active or inactive form of the agent or metal reactivation kinetics. We assumed a Poisson distribution for the formation of single- and double-strand cleavage to calculate the average number of such events per DNA molecule using the Freifelder–Trumbo equation.[42] The data for the first 2–5 min could be fitted to a linear equation and provided a 1:30 ratio of double to single strand cleavage events. A theoretical ratio of 1:100 would be expected if the random accumulation of single strand cleavages within the 5386 base pairs of ΦX174 DNA produces the linear

Table 1. Cleavage of supercoiled ΦX174 RFI DNA by Fe^{II}–4[a]

| Concentrations (μM)		% Form		
4	Fe^{II}	I	II	III
0	0	95.0	5.0	0
50	50	0	84.0	16.0
40	40	0	87.6	12.4
20	20	11.5	84.7	3.8
10	10	15.4	81.4	3.2
5	5	34.0	66.0	0
1	1	47.6	52.4	0
0	1	87.2	12.8	0
0	5	78.0	22.0	0
0	10	76.9	23.1	0
0	20	44.7	53.5	1.8

[a]Direct fluorescence quantitation of the DNA in the presence of ethidium bromide was conducted using a Millipore BioImage 60S RFLP system visualized on a UV (312 nm) transilluminator taking into account the relative fluorescence intensities of Forms I–III ΦX174 DNA (Form II and Form III fluorescence intensities are 0.7 times that of Form I).

A.
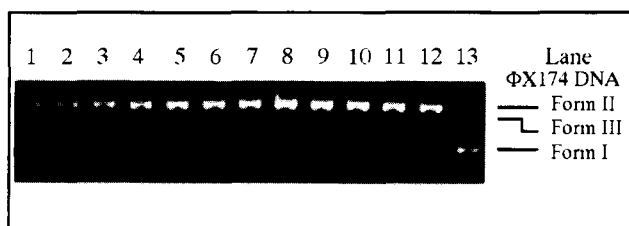
B.
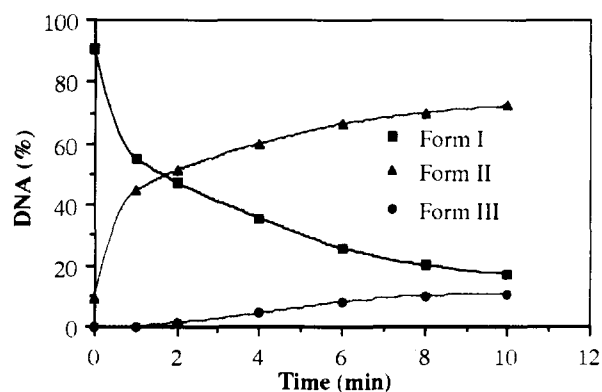
Figure 1. (A) Agarose gel illustrating the kinetics of supercoiled ΦX174 DNA cleavage by Fe^{II}–4 (50 μM). Reaction solutions contained 0.25 μg supercoiled ΦX174 DNA (1.4×10^{-8} M) in 50 mM Tris–HCl buffer (pH 8.0) containing 10 mM 2-mercaptoethanol. The DNA cleavage reactions were run at 37 °C and electrophoresis was conducted at 50 V for 2.5 h on a 1.0% agarose gel containing 0.1 μg/mL ethidium bromide. Lanes 1–12, extent of the reaction at time = 1, 2, 4, 6, 8, 10, 12, 15, 18, 20, 30 and 40 min; lane 13, control ΦX174 DNA, 95% Form I, 5% Form II. (B) The percentage of Form I–III DNA present at each time point taking into account the relative fluorescence intensities (Form II and Form III fluorescence intensities are 0.7 times that of Form I).
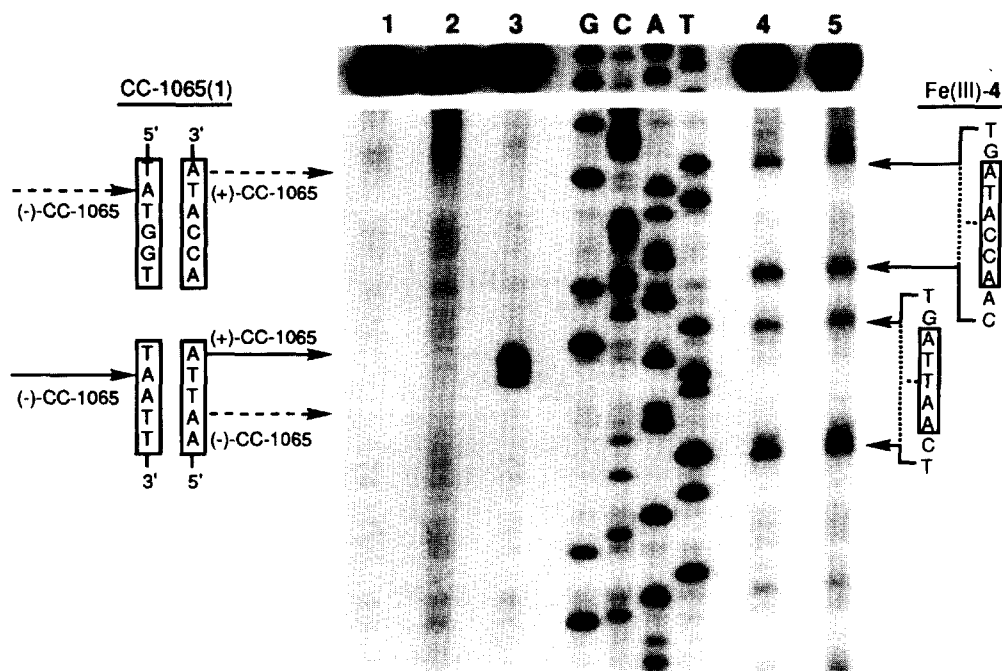
Figure 2. Cleavage of 5' ³²P-end-labeled double-strand DNA (SV40 DNA fragment, 156 base-pairs, nucleotide no. 5239–150, clone w794) by (+)-CC-1065 (**1**) and Fe^{III}–**4**, for clarity only a 40–50 bp AT-rich region is shown. Thermally-induced DNA cleavage by (+)-CC-1065 (**1**) at its alkylation sites was conducted by warming the covalently modified DNA solution in TE buffer (pH 7.5) at 100 °C for 30 min. The DNA cleavage reactions of Fe^{II}–**4** were initiated by addition of 50% aqueous H_2O_2 after mixing the Fe^{III}–**4** complex with the DNA in 10 mM phosphate (K_2HPO_4–KH_2PO_4)/10 mM KCl buffer (pH 7.0). The DNA cleavage reactions were run for 10 min at 4 °C before being quenched with the addition of 50% aqueous glycerol. The electrophoresis was conducted at 1100 V (5.5 h) on an 8% denaturing PAGE and visualized by autoradiography. Lane 1, control DNA; lane 2, 50 μM Fe^{III} control; lane 3, 10⁻⁶ M (+)-CC-1065 (**1**); G, C, A and T sequencing reactions; lane 4, 20 μM Fe^{III}–**4**, lane 5, 50 μM Fe^{III}–**4**.

DNA assuming that it requires two sequential cleavages within 15 base pairs on the complementary strands. Experimentally, Fe^{II} alone produced a ratio of 1 : 98. Thus, Fe^{II}–**4** was only slightly better than Fe^{II} itself, suggesting that few of the multiple cleavages may be related by a single binding event.

In addition, the binding of CDPI$_3$ to DNA was not inhibited by linkage with EDTA. Binding of CDPI$_3$–EDTA versus CDPI$_3$ with poly(dA)–poly(dT) resulted in a larger stabilization of the duplex toward thermal denaturization, $Δ_m$ = + 20 °C and + 13 °C, respectively.

The selectivity of DNA cleavage by **4** was examined within w794 DNA and its complement w836 DNA[39] by monitoring strand cleavage of singly ³²P 5'-end-labeled double-strand DNA after exposure to Fe^{III}–**4** followed by activation with H_2O_2[43] in 10 mM phosphate buffer (pH 7.0, 10 mM KCl, 4 °C, 10 min). Thus, incubation of the labeled duplex DNA with Fe^{III}–**4** in the presence of excess H_2O_2 led to sequence selective cleavage of the DNA. The reactions were quenched by the addition of 50% aqueous glycerol. Removal of the agent by EtOH precipitation of the DNA, resuspension of the treated DNA in aqueous buffer and high-resolution polyacrylamide gel electrophoresis (PAGE) of the resultant DNA under denaturing conditions adjacent to Sanger sequencing reactions permitted the identification of the sites of DNA cleavage. A range of reaction conditions were examined and the DNA cleavage efficiencies and the clarity of the sequence selectivity

proved to be sensitive to the reaction time, temperature, pH and unusually dependent upon the choice of buffer. Reactions conducted in 10 mM or 25 mM Tris–HCl (pH 7 or 8) proved better than those conducted in TE buffer (pH 8), but not nearly as effective as those conducted in 10 mM phosphate buffer (pH 7, 10 mM KCl, 4 °C, 10 min). Similar observations have been made with the Fe^{III} complexes of bleomycin A$_2$ activated for DNA cleavage with H_2O_2.[44] Use of lower concentrations of the phosphate buffer (2.5 versus 10 mM), the use of pH 5.7 or 8.0 versus 7.0 and the omission of added 10 mM KCl resulted in reduced DNA cleavage efficiencies and a lower resolution of the cleavage selectivity. The clarity of the DNA cleavage selectivity as well as its efficiency also proved to be dependent upon the conditions of activation of the Fe^{III} complex. Both increased steadily as the concentration of H_2O_2 was increased from 300 μM to 5 mM and finally to 1.5 M. Presumably this is derived from the fast activation kinetics and although little experimental difference was observed between 5 mM and 1.5 M H_2O_2, the results were less satisfactory below 5 mM H_2O_2. At the optimal concentrations of H_2O_2, the reactions could be conducted over short reaction times at low temperature (4 °C, 10 min). Finally, more dependable results were obtained when the Fe^{III}–**4** complex was freshly prepared and preformed with subsequent dilution to the desired concentrations prior to addition to the DNA, rather than sequential additions of **4** and Fe^{III} directly to the buffered DNA solutions. Subsequent preincubation

with the DNA was preformed for 10 min prior to activation of the DNA cleavage reaction with the addition of aqueous H_2O_2. Although little difference was observed for the DNA cleavage efficiency using a 10 min versus 1 min preincubation period, the clarity of DNA cleavage sequence selectivity did improve. Alternative efforts to employ the Fe^{II} complex of 4 in 50 mM Tris–HCl buffer (pH 8) containing 10 mM 2-mercaptoethanol in the presence of O_2 led to DNA cleavage at concentrations well below background Fe^{II}, but the results were less satisfactory. Analogous observations have been made in the study of O_2 versus H_2O_2 activation of Fe–bleomycin complexes by Burger,[45] Hecht,[46] and ourselves,[44] and are comparable with the observations made in the detailed mechanistic study of the reaction of Fe^{III}–EDTA with H_2O_2.[47]

Under the optimized conditions, a surprisingly selective and well defined cleavage of DNA by Fe^{III}–4 was observed and a representative illustration of this may be found in Figure 2. Within the region of w794 DNA illustrated in Figure 2, a set of cleavages bracketing the five base AT-rich site, 5'-AATTA, were observable at 0.02–50 μM Fe^{III}–4 and those bracketing the additional three base AT-rich site were detectable only at the higher concentrations of 1–50 μM Fe^{III}–4. The cleavage sites appear in pairs and correspond to two orientations of the agent within a binding site providing nearly equally intense cleavage at both ends.

In contrast to most studies with affinity cleavage agents,[43,48] the reaction is remarkably clean when conducted using the optimized conditions providing predominantly a single cleavage site at each end of the binding site rather than a cluster of cleavage sites characteristic of a diffusible oxidant. This is illustrated nicely for the set of cleavage sites that bracket the AT-rich five base-pair site 5'-AATTA. This sequence constitutes a high-affinity alkylation site for (+)-CC-1065 (5'-AATTA) with alkylation of the 3'-adenine and agent binding in the 3'→5' direction across the five base-pair AT-rich site (Fig. 3). It also constitutes a minor alkylation site for the unnatural enantiomer of CC-1065 (5'-AATTA) with alkylation of the second 5' adenine, agent binding in the reverse 5'→3' direction, and with the agent binding covering the full five base-pair AT-rich site starting at the first 5' adenine and extending over the adenine alkylation site, as well as the following three 3' bases (Fig. 3). A detailed discussion of this reverse and offset AT-rich alkylation selectivity of the enantiomeric agents has been described elsewhere.[27] The complementary sequence in the partner strand fails to react competitively with (+)-CC-1065, but contains a high affinity alkylation site for ent-(−)-CC-1065. Bracketing this sequence are two cleavage sites for 4, 5'-TCAAT-TAGT, which are two bases removed from each end of the five base-pair AT-rich binding and alkylation site. This corresponds to cleavage from both bound orienta-
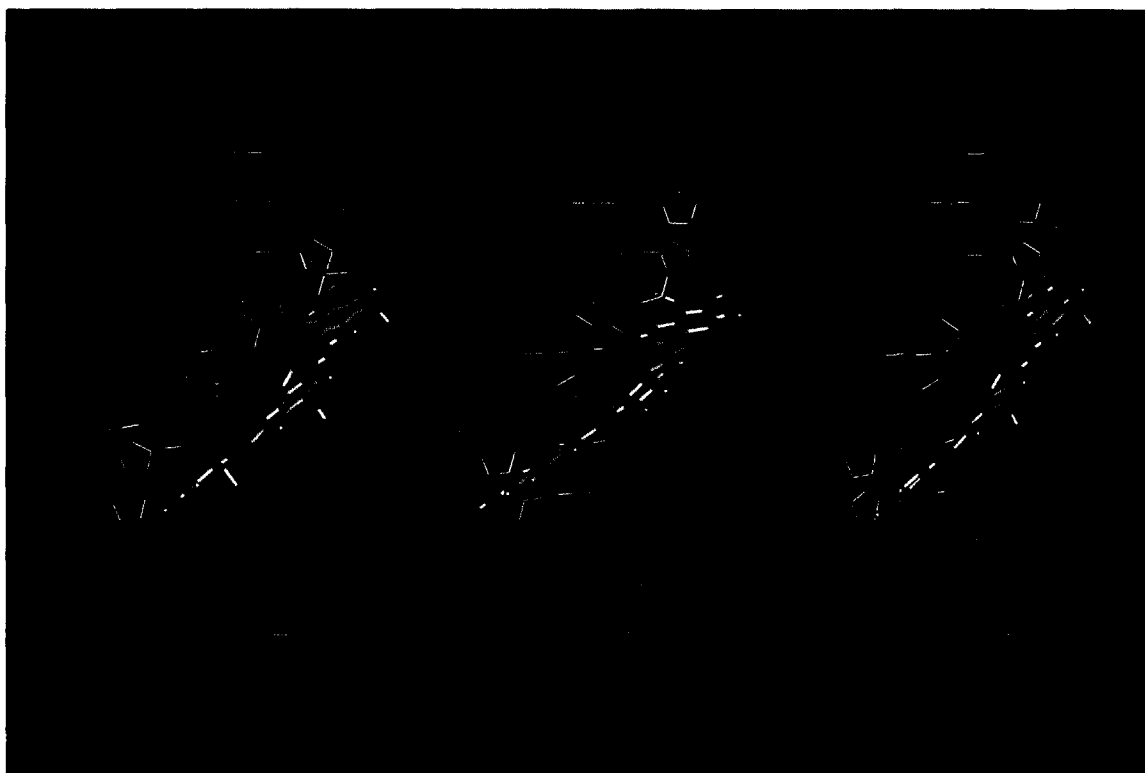
Figure 3. Comparison stick models of (+)-CC-1065 and ent-(−)-CC-1065 alkylation at the high affinity site within w794 DNA: duplex 5'-(CTCAATTAGTC). The (+)-CC-1065 binding extends in the 3'→5' direction (blue strand) from the adenine N3 alkylation site across the five base site 5'-AATTA (left). The ent-(−)-CC-1065 binding at its minor alkylation site extends in the reverse 5'→3' direction (blue strand) from the adenine N3 alkylation site across the five base site 5'-AATTA (middle). The ent-(−)-CC-1065 binding at its major alkylation site on the complementary strand extends in the 5'→3' direction (red strand) from the adenine N3 alkylation site across the five base site 5'-TAATT (right). The model complexes were generated with MacroModel (AMBER force field supplemented with agent parameters).
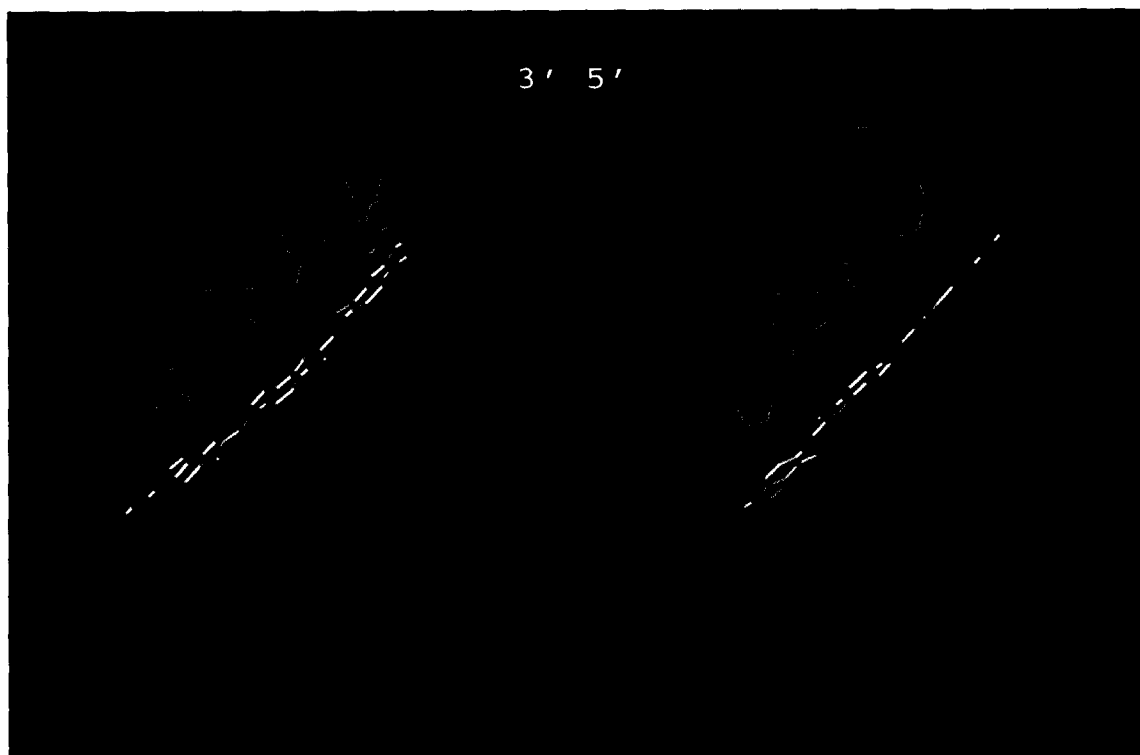
Figure 4. Stick models illustrating the two bound orientations of Fe^{III}–4 within the w794 high affinity binding site: duplex 5'-(C<u>T</u>CAATTAG<u>T</u>C). In both instances, the CDPI$_3$ binding spans the five-base AT-rich site 5'-AATTA. The Fe^{III} chelation subunit extends in the minor groove from this binding site approximately 1–2 base pairs in either direction; the Fe^{III} atom is illustrated with a green ball. Expanded views of this region are shown in Figure 5 and highlight the positioning of the Fe^{III} relative to the C4'–H abstraction sites (Fig. 6) that lead to DNA cleavage. The model complexes were generated with MacroModel (AMBER force field supplemented with agent parameters including X-ray coordinates taken from RbFe(OH$_2$)EDTA–H$_2$O, ref 50).

tions of **4** within the five base-pair AT-rich site, 5'-AATTA, and models of both these bound orientations and their respective cleavage sites are shown in Figures 4 and 5.

In analogy with related studies,[43] the DNA cleavage reaction is most likely derived from C4'-H or C5'-H abstraction from the deoxyribose backbone by the activated Fe^{III} complex or a liberated free radical (HO·). Given the clarity and single site reaction of the DNA cleavage reactions with Fe^{III}–4 and the modeled proximity of the Fe^{III} center to the reacting centers, it is tempting to suggest that it may be the intermediate perferryl complex or an iron-oxo intermediate that is directly responsible for the H-abstraction reaction rather than a diffusable oxidant. Importantly, the minor groove location of the agent is apparent from the offset nature of the complementary strand cleavage sites (Fig. 6).[43]

Similarly, all additional sites of alkylation by either (+)-CC-1065 or ent-(−)-CC-1065, including their minor sites, were found within regions bracketed by cleavage sites for **4**. The low affinity and less preferred alkylation sites for **1** occur within shorter AT-rich sequences that also coincide with lower affinity binding sites observed with Fe^{III}–4. To a first approximation, the high affinity alkylation sites for either (+)- or ent-(−)-CC-1065 all lie in high affinity binding sites detected with Fe^{III}–4 at low concentrations (0.02–1

μM). Similarly, the lower affinity alkylation sites lie in shorter AT-rich sequences that require higher concentrations of Fe^{III}–4 (1–50 μM) for detection or contain a competitive high affinity alkylation site on the partner strand of the duplex DNA. For example, the sequence 5'-CATAG illustrated in Figure 2, which is detected only at the higher concentrations of Fe^{III}–4 (1–50 μM), incorporates a minor alkylation site for both (+)-CC-1065 (5'-CCAT<u>A</u>) and ent-(−)-CC-1065 (complementary strand 5'-<u>TA</u>TGG). Notably, no major or minor alkylation sites for CC-1065 were detected that lie outside a binding site observed for **4** and to a first approximation the relative alkylation efficiency parallels the binding affinity detected with Fe^{III}–4.

However, while noncovalent binding is a prerequisite for observation of DNA alkylation and to a first approximation dictates the relative alkylation efficiency, it does not insure covalent bond formation. Rather, spacial and stereoelectronic features of the ensuing reaction must be satisfied within a binding site for observation of DNA alkylation. For CC-1065, a classic example of this phenomenon is found in the palindromic sequence 5'-AATT. When bracketed by G–C base pairs, both strands of this sequence are alkylated by ent-(−)-CC-1065, but they fail to react with (+)-CC-1065. Noncovalent binding of ent-(−)-CC-1065 in either orientation within this duplex places the activated cyclopropane in a proper orientation and directed toward the appropriate strand

to alkylate the site 5'-AATT with agent binding in the 5'→3' direction relative to the alkylated strand covering the site 5'-AATTN. In contrast, the natural enantiomer binding in either orientation directs the activated cyclopropane only toward the 3' T on either strand, precluding any opportunity for adenine N3
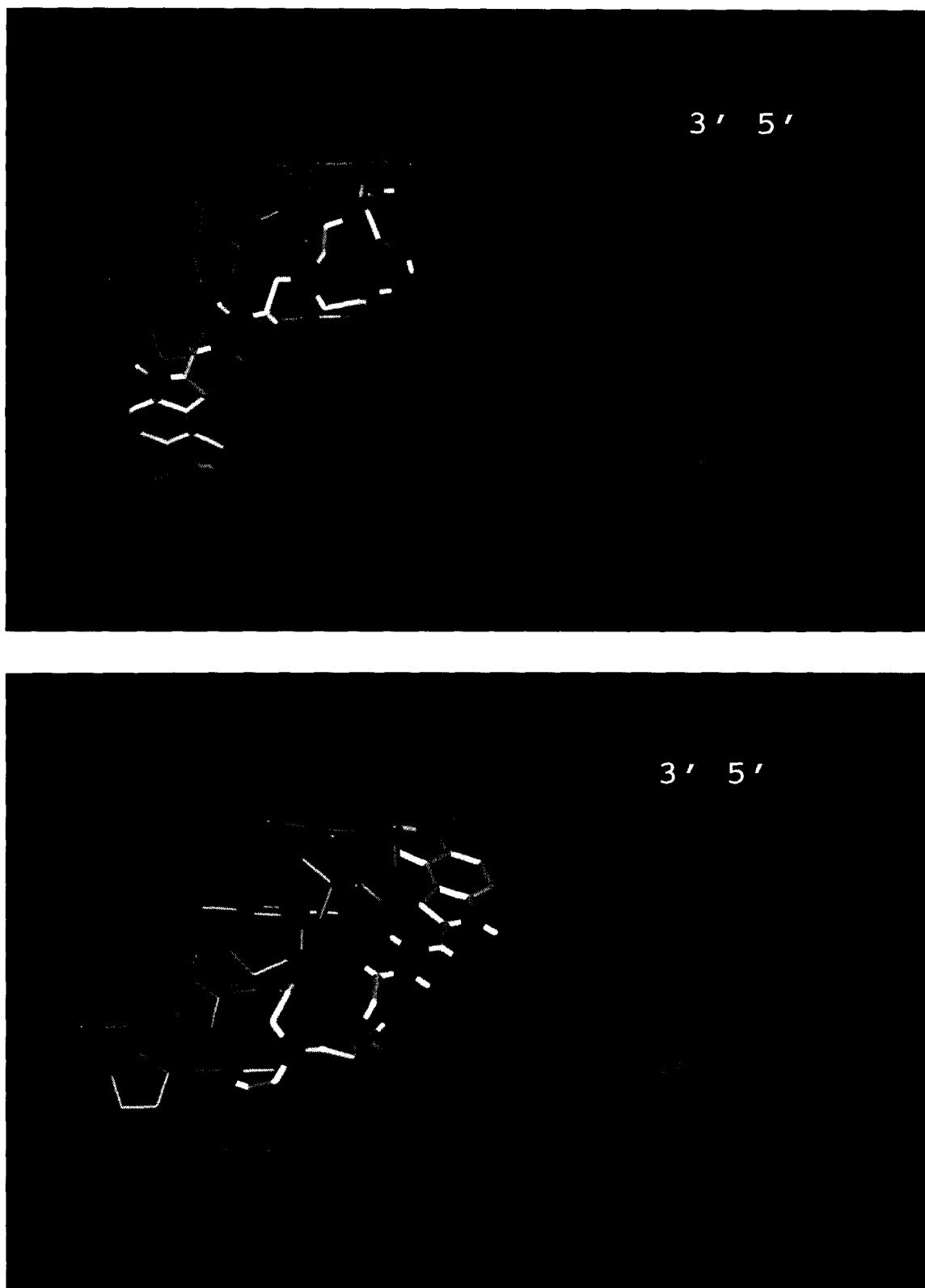
Figure 5. Expansion of the Fe^{III} region (green ball) of the stick models shown in Figure 4 illustrating the origin of the DNA cleavage sites (5'-CTCAATTAGTC, blue strand) and (5'-GACTAATTGAG, red strand), related to the central high-affinity binding site (blue: 5'-AATTA 5'-TAATT: red) within w794 DNA. The locations of the required C4'—H atom abstraction sites that lead to DNA cleavage are shown in green. Flexibility in this terminus of the agent would allow it to swivel from side to side to approach either site even more closely.

alkylation. This is due merely to the spatial relationship of the reacting partners and is not the consequence of the absence of selective catalysis for reaction. In fact, the recent demonstration that alternative electrophiles may substitute for the activated cyclopropane[39] and

that the dissimilar agents $(+)$-DSA–CDPI$_2$ and ent-$(-)$-CDPI$_2$–DSA exhibit identical DNA alkylation selectivities[40] suggest that the spatial and stereoelectronic requirements of the reaction are unusually flexible.

Conclusions

The behavior of CC-1065 has proven analogous to that described for N-bromoacetyldistamycin,[49] where selective adenine N3 alkylation was observed within available noncovalent binding sites. CC-1065 DNA alkylation was observed only within noncovalent binding sites defined by Fe$^{\mathrm{III}}$–4 demonstrating that noncovalent binding is a prerequisite for and intimately associated with the observation of DNA alkylation and to a first approximation that the relative cleavage efficiency observed with Fe$^{\mathrm{III}}$–4 was found to parallel the relative alkylation efficiency of $(+)$- and/or ent-$(-)$-CC-1065.

Experimental

Thermally-induced strand cleavage of 5′ ^{32}P end-labeled double-strand DNA following alkylation by $(+)$-CC-1065 (1): DNA alkylation selectivity

A 9 μL solution of the 5′ end-labeled w794 or w83b DNA[39] in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) was treated with a 1 μL solution of $(+)$-CC-1065 (1, 10^{-6} M) in DMSO. The solution was mixed by vortexing the solution and brief centrifugation and subsequently incubated at 4 °C for 24 h. The covalently-modified DNA was separated from unbound agent by EtOH precipitation of the DNA and resuspended in TE buffer (10 μL, pH 7.5). The solution of DNA in an Eppendorf tube sealed with parafilm was warmed at 100 °C for 30 min to induce cleavage at the alkylation sites, allowed to cool to 25 °C and centrifuged. Formamide dye (0.03% xylene cyanol FF, 0.03% bromophenol blue, 8.7% Na$_2$EDTA, 250 mM) was added (5 μL) to the supernatant. Prior to electrophoresis, the sample was denatured by warming at 100 °C for 5 min, placed in an ice-bath, and centrifuged and the supernatant (2.8 μL) was loaded directly onto the gel. Sanger dideoxynucleotide sequencing reactions were run as standards adjacent to the reaction samples. Polyacrylamide gel electrophoresis (PAGE) was run on an 8% sequencing gel under denaturing conditions (8 M urea) in TBE buffer (100 mM Tris, 100 mM boric acid, 0.2 mM Na$_2$EDTA) followed by autoradiography.

5′ ^{32}P End-labeled double-strand DNA cleavage by Fe$^{\mathrm{III}}$–4: sequence selectivity study

All reactions were run with freshly prepared Fe$^{\mathrm{III}}$ complexes. The Fe$^{\mathrm{III}}$–4 complexes were prepared by first combining 4 μL of a 2000 μM solution of 4 in DMSO with 4 μL of a freshly prepared equimolar aqueous FeCl$_3$ solution and subsequent dilution to the desired 10 × concentrations (200 μM and 500 μM) prior
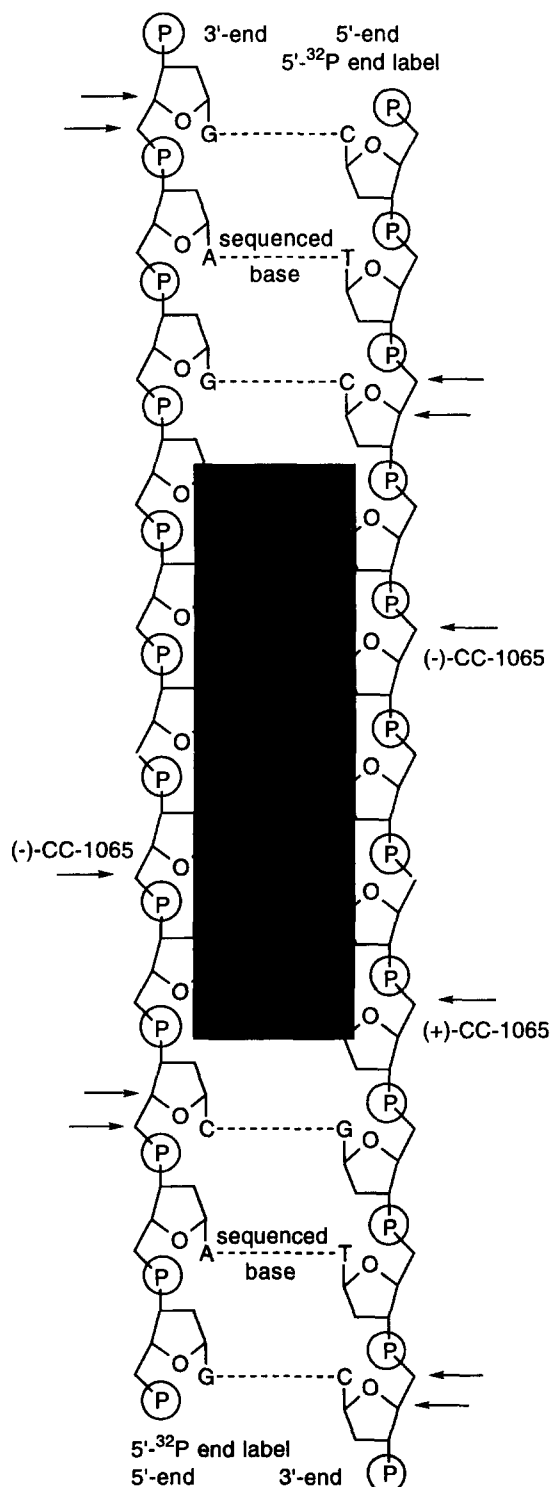
Figure 6. Model illustrating the relationship between the observed sequenced cleavage sites for Fe$^{\mathrm{III}}$–4, its C4′—H and C5′—H abstraction sites, the five base-pair AT-rich noncovalent binding site and the $(+)$- and ent-$(-)$-CC-1065 alkylation sites shown in Figures 3–5 for the w794 high-affinity site.

to addition of the w794 or w836 DNA to achieve their final concentrations of 20 and 50 μM. Each of the Fe^{III} complex solutions (2 μL) were treated with 7 μL of a buffered DNA solution containing the 5' ^{32}P-end-labeled w794 or w836 DNA in 10 mM phosphate buffer (KH_2PO_4, pH 7.0) containing 10 mM KCl and the mixture was incubated at 25 °C for 10 min. The final concentrations of the agents employed in the study were 50 μM Fe^{III} control and 20 or 50 μM Fe^{III}–4. The DNA cleavage reactions were initiated by adding 1 μL of 50% aqueous H_2O_2 (1.5 M final concentration). The DNA reaction solutions were incubated at 4 °C for 10 min. The reactions were quenched with the addition of 1 μL of 50% aqueous glycerol followed by EtOH precipitation and isolation of the DNA. The DNA was resuspended in 7 μL of TE buffer (pH 8.0) and formamide dye (5 μL) was added to the supernatant. Prior to electrophoresis, the samples were warmed at 100 °C for 5 min, placed in an ice bath, centrifuged, and the supernatant (2.8 μL) was loaded onto the gel. Sanger dideoxynucleotide sequencing reactions were run as standards adjacent to the agent-treated DNA. Gel electrophoresis was conducted using a denaturing 8% sequencing gel (19:1 acrylamide:N,N-methylenebis-acrylamide, 8 M urea) at 1100 V for 5.5 h. Formamide dye contained xylene cyanol FF (0.03%), bromophenol blue (0.3%) and aqueous Na_2EDTA (8.7%, 250 mM). Electrophoresis running buffer (TBE) contained Tris base (100 mM), boric acid (100 mM) and Na_2EDTA–H_2O (0.2 mM). Gels were prerun for 30 min with formamide dye prior to loading the samples. Autoradiography of the dried gel was carried out at −78 °C using Kodak X-Omat AR film and a Picker spectra intensifying screen.

Acknowledgment

References

1. Chidester, C. G.; Krueger, W. C.; Mizsak, S. A.; Duchamp, D. J.; Martin, D. G. *J. Am. Chem. Soc.* **1981**, *103*, 7629.

2. Ichimura, M.; Ogawa, T.; Takahashi, K.; Kobayashi, E.; Kawamoto, I.; Yasuzawa, T.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1990**, *43*, 1037.

3. Ichimura, M.; Ogawa, T.; Katsumata, S.; Takahashi, K.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1991**, *44*, 1045.

4. Takahashi, I.; Takahashi, K.; Ichimura, M.; Morimoto, M.; Asano, K.; Kawamoto, I.; Tomita, F.; Nakano, H. *J. Antibiot.* **1988**, *41*, 1915.

5. Yasuzawa, T.; Iida, T.; Muroi, K.; Ichimura, M.; Takahashi, K.; Sano, H. *Chem. Pharm. Bull.* **1988**, *36*, 3728.

6. Ogawa, T.; Ichimura, M.; Katsumata, S.; Morimoto, M.; Takahashi, K. *J. Antibiot.* **1989**, *42*, 1299.

7. Ichimura, M.; Muroi, K.; Asano, K.; Kawamoto, I.; Tomita, F.; Morimoto, M.; Nakano, H. *J. Antibiot.* **1988**, *41*, 1285.

8. Ohba, K.; Watabe, H.; Sasaki, T.; Takeuchi, Y.; Kodama, Y.; Nakazawa, T.; Yamamoto, H.; Shomura, T.; Sezaki, M.; Kondo, S. *J. Antibiot.* **1988**, *41*, 1515.

9. Ishii, S.; Nagasawa, M.; Kariya, Y.; Yamamoto, H.; Inouye, S.; Kondo, S. *J. Antibiot.* **1989**, *42*, 1713.

10. Boger, D. L.; Johnson, D. S. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 3642.

11. Boger, D. L. *Acc. Chem. Res.* **1995**, *28*, 20.

12. Boger, D. L. In *Advances in Heterocyclic Natural Products Synthesis*; Pearson, W. H., Ed.; JAI: Greenwich, Connecticut, 1992; Vol. 2, p 1.

13. Boger, D. L. *Chemtracts: Org. Chem.* **1991**, *4*, 329.

14. Boger, D. L. In *Heterocycles in Bioorganic Chemistry*; Bergman, J.; van der Plas, H. C.; Simonyl, M., Eds.; Royal Society of Chemistry: Cambridge, 1991; p 103.

15. Boger, D. L. *Proc. Robert A. Welch Found. Conf. Chem. Res., XXXV. Chem. Front. Med.* **1991**, *35*, 137.

16. Coleman, R. S.; Boger, D. L. In *Studies in Natural Products Chemistry*; Rahman, A.-u.-, Ed.; Elsevier: Amsterdam, 1989; Vol. 3, p 301.

17. Hurley, L. H.; Draves, P. H. In *Molecular Aspects of Anticancer Drug-DNA Interactions*; Neidle, S.; Waring, M., Eds.; CRC: Ann Arbor, 1993; Vol. 1, p 89.

18. Warpehoski, M. A. In *Advances in DNA Sequence Specific Agents*; Hurley, L. H., Ed.; JAI: Greenwich, 1992; Vol. 1, p 217.

19. Warpehoski, M. A.; McGovren, J. P.; Mitchell, M. A.; Hurley, L. H. In *Molecular Basis of Specificity in Nucleic Acid–Drug Interactions*; Pullman, B.; Jortner, J., Eds.; Kluwer: Netherlands, 1990; p 531.

20. Warpehoski, M. A.; Hurley, L. H. *Chem. Res. Toxicol.* **1988**, *1*, 315.

21. Hurley, L. H.; Lee, C.-S.; McGovren, J. P.; Warpehoski, M. A.; Mitchell, M. A.; Kelly, R. C.; Aristoff, P. A. *Biochemistry* **1988**, *27*, 3886.

22. Hurley, L. H.; Warpehoski, M. A.; Lee, C.-S.; McGovren, J. P.; Scahill, T. A.; Kelly, R. C.; Mitchell, M. A.; Wienienski, N. A.; Gebhard, I.; Johnson, P. D.; Bradford, V. S. *J. Am. Chem. Soc.* **1990**, *112*, 4633.

23. Warpehoski, M. A.; Harper, D. E.; Mitchell, M. A.; Monroe, T. J. *Biochemistry* **1992**, *31*, 2502.

24. Hurley, L. H.; Reynolds, V. L.; Swenson, D. H.; Petzold, G. L.; Scahill, T. A. *Science* **1984**, *226*, 843.

25. Reynolds, V. L.; Molineaux, I. J.; Kaplan, D. J.; Swenson, D. H.; Hurley, L. H. *Biochemistry* **1985**, *24*, 6228.

26. Boger, D. L.; Coleman, R. S.; Invergo, B. J.; Sakya, S. M.; Ishizaki, T.; Munk, S. A.; Zarrinmayeh, H.; Kitos, P. A.; Thompson, S. C. *J. Am. Chem. Soc.* **1990**, *112*, 4623; Boger, D. L.; Coleman, R. S. *J. Am. Chem. Soc.* **1988**, *110*, 1321, 4796.

27. Boger, D. L.; Johnson, D. S.; Yun, W.; Tarby, C. M. *Bioorg. Med. Chem.* **1994**, *2*, 115.

28. Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Kitos, P. A.; Suntornwat, O. *J. Org. Chem.* **1990**, *55*, 4499.

29. Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *J. Am. Chem. Soc.* **1990**, *112*, 8961.

30. Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H. *J. Am. Chem. Soc.* **1991**, *113*, 6645.

31. Boger, D. L.; Yun, W. *J. Am. Chem. Soc.* **1993**, *115*, 9872.

32. Boger, D. L.; Johnson, D. S.; Yun, W. *J. Am. Chem. Soc.* **1994**, *116*, 1635.

33. Boger, D. L.; Johnson, D. S.; Wrasidlo, W. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 631.

34. Sun, D.; Lin, C. H.; Hurley, L. H. *Biochemistry* **1993**, *32*, 4437.

35. Lin, C. H.; Hill, G. C.; Hurley, L. H. *Chem. Res. Toxicol.* **1992**, *5*, 167.

36. Lin, C. H.; Beale, J. M.; Hurley, L. H. *Biochemistry* **1991**, *30*, 3597.

37. Boger, D. L.; Invergo, B. J.; Coleman, R. S.; Zarrinmayeh, H.; Kitos, P. A.; Thompson, S. C.; Leong, T.; McLaughlin, L. W. *Chem.-Biol. Interact.* **1990**, *73*, 29.

38. Boger, D. L.; Sakya, S. M. *J. Org. Chem.* **1992**, *57*, 1277; Boger, D. L.; Coleman, R. S.; Invergo, B. J. *J. Org. Chem.* **1987**, *52*, 1521.

39. Boger, D. L.; Munk, S. A.; Zarrinmayeh, H. *J. Am. Chem. Soc.* **1991**, *113*, 3980; Boger, D. L.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1431; Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, 2661.

40. Boger, D. L. Johnson, D. S. *J. Am. Chem. Soc.* **1995**, *117*, 1443.

41. Boger, D. L.; Zhou, J. *J. Org. Chem.* **1993**, *8*, 3018.

42. Freifelder, D.; Trumbo, B. *Biopolymers* **1969**, *7*, 681.

43. Dervan, P. B. *Science* **1986**, *232*, 464; Van Dyke, M. W.; Hertzberg, R. P.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 5470; Youngquist, R. S.; Dervan, P. B. *Proc. Natl.*

Acad. Sci. U.S.A. **1985**, *82*, 2565; Schultz, P. G.; Taylor, J. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 6861; Hertzberg, R. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 313; Youngquist, R. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1985**, *107*, 5528; Schultz, P. G.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 6834; Taylor, J. S.; Schultz, P. G.; Dervan, P. B. *Tetrahedron* **1984**, *40*, 457; Schultz, P. G.; Dervan, P. B. *J. Biomol. Struc. Dyn.* **1984**, *1*, 1133; Wade, W. S.; Mrksich, M.; Dervan, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 8783; Distefano, M. D.; Dervan, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 11006; Mrksich, M.; Dervan, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 2572; Mrksich, M.; Dervan, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 9892.

44. Boger, D. L.; Honda, T.; Menezes, R. F.; Colletti, S. L. *J. Am. Chem. Soc.* **1994**, *116*, 5631; Boger, D. L.; Teramoto, S.; Honda, T.; Zhou, J. *J. Am. Chem. Soc.* **1995**, *117*, 7338; Boger, D. L.; Teramoto, S.; Zhou, J. *J. Am. Chem. Soc.* **1995**, *117*, 7344.

45. Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1981**, *256*, 11636; Burger, R. M.; Tian, G.; Drlica, K. *J. Am. Chem. Soc.* **1995**, *117*, 1167.

46. Natrajan, A.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1990**, *112*, 3997.

47. Bull, C.; McClune, G. J.; Fee, J. A. *J. Am. Chem. Soc.* **1983**, *105*, 5290.

48. Gravert, D. J.; Griffin, J. H. *J. Org. Chem.* **1993**, *58*, 820.

49. Baker, B. F.; Dervan, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 2700; Baker, B. F.; Dervan, P. B. *J. Am. Chem. Soc.* **1985**, *107*, 8266.

50. Hoard, J. L.; Lind, M.; Silverton, J. V. *J. Am. Chem. Soc.* **1961**, *83*, 2770.