Characterization of the Low Mobility Product. Because a complete characterization of the formed products was deemed essential to an understanding of the degradation of the Dickerson-Drew dodecamer by Fe(II)•BLM A_2, the anomalous low mobility band seen in Figure 2 was studied in detail. The stability of this product with respect to alkali treatment, both before and after reaction with sodium borohydride, is shown (Supporting Information, Figure 1). Lane 2 shows the products of the reactions of 500 μM Fe(II)•BLM A_2 with 12 μM DNA duplex. In this experiment, the higher BLM concentration employed allowed the weaker cleavage sites at C_3 and T_8 to be observed readily. In addition to the appearance of the expected phosphate and hydroxycyclopentenone-terminating products derived from abasic sites, treatment of the mixture with 1 M piperidine subsequent to reaction with Fe•BLM caused the disappearance of the low mobility product (lanes 3 and 4). However, if the Fe•BLM reaction was treated with $NaBH_4$ prior to reaction, the low mobility band persisted (lanes 5 and 6). This reductively stabilized product could be isolated and purified from the reaction mixture by preparative PAGE (lane 7). Treatment of this purified product with piperidine is shown in lanes 8 and 9. This material was largely refractory to alkali; piperidine treatment produced only a small amount of degradation product, which comigrated with the phosphate-terminating fragment resulting from the alkali treatment of the abasic lesion at cytidine$_{11}$. A second treatment of this purified product with sodium borohydride afforded a material that was essentially completely refractory to piperidine-induced degradation (lanes 10 and 11), suggesting that the first borohydride reaction had not proceeded with 100% efficiency.

In order to characterize the nature of this $NaBH_4$-stabilized modified oligomer further, it was prepared and isolated on a larger scale. After isolation, it was subjected to enzymatic digestion and HPLC analysis. The HPLC traces, measured at 260 nm, are shown (Supporting Information, Figure 2). Digestion of unmodified dodecanucleotide with deoxyribonuclease I, nuclease P1, and calf intestinal phosphatase yielded the four nucleoside products which, under the HPLC conditions employed, eluted in the order deoxycytidine, deoxyguanosine, deoxythymidine, and deoxyadenosine, as determined by coinjection with authentic standards. Peak integration, after correction for extinction coefficients, confirmed that these deoxynucleosides were present in a 4:4:2:2 ratio. Similar analysis of the modified oligomer yielded, in addition to the four nucleosides, an additional product **1** which eluted between deoxycytidine and deoxyguanosine. The UV spectrum of this product indicated that it contained guanosine (data not shown). Further, peak integrations indicated that the four normal nucleosides (C, G, T and A, respectively) were present in the ratio ~3:3:2:2, while peak **1** accounted for one guanosine. Notably, detection at 292 nm, the absorbance maximum for bleomycin, showed that **1** did not contain a covalent BLM - DNA crosslink (data not shown). Further digestion of this mixture with snake venom phosphodiesterase, an enzyme which mediates the hydrolysis of phosphodiester bonds and release of 5'-dNMP's, eliminated peak **1**; only the four nucleosides were observed. Peak integration showed that the fourth deoxyguanosine was recovered, but the fourth deoxycytidine remained absent (C:G:T:A ~ 3:4:2:2). The conversion of dGMP (the product of the phosphodiesterase reaction) to deoxyguanosine presumably occurred via the agency of the alkaline phosphatase still in solution. In a separate experiment it was shown that if product **1** was purified by HPLC and then treated with phosphodiesterase, dGMP was produced (data not shown). The inferred transformations are outlined as Scheme 1 of the Supporting Information.

These experimental observations paralleled those of Rabow et al. in their study of the alkali-labile lesion produced during the BLM-mediated degradation of the hexamer d(CGCGCG).[16] In that study, it was shown unequivocally that their product **1** resulted from abasic site formation. The abasic site in the oligonucleotide was not recognized by DNase 1, nuclease P1, or alkaline phosphatase. However, this product was a substrate for snake venom phosphodiesterase, which removed the sugar fragment. The chemistry of the digestion analysis, as reported by Rabow et al. and utilized here, was identical with that outlined Scheme 1 of the

Supporting Information. Thus the low mobility product (Figure 2) is not a covalent BLM – DNA crosslink, but rather an oligonucleotide containing an abasic site.

The evidence also indicated that this abasic lesion occurred at cytidine$_{11}$. The electrophoretic analysis in Figure 1 (Supporting Information) showed that the small amount of degradation that did occur upon treatment of the isolated product with piperidine yielded a fragment that comigrated with a cytidine$_{11}$ cleavage product terminating with a 3'-phosphate moiety. This conclusion was supported further by Maxam-Gilbert sequence analysis of the modified, 5'-^{32}P end labeled oligonucleotide. These polyacrylamide gels exhibited no anomalous mobilities for any band corresponding to fragments shorter than 11 nucleotides.

DNA Structure. As Altona and coworkers have shown,[24] scalar coupling constants of the deoxyribose hydrogens can be used to determine sugar pucker states and the equilibrium between the north (near C3'-endo, where C3'-endo is defined as having a pseudorotation phase angle P = 18°) and south (near C2'-endo, where P = 162°) conformations. In the case of the free dodecanucleotide, which has previously been shown to adopt a B-form conformation in solution,[15d,e,25-27] measurements of the deoxyribose *J* couplings[25] are consistent with a relatively small range of pseudorotation phase angles (P = 90 - 180°); there was at most a modest contribution from sugars adopting a north conformation. Further, the DQF-COSY spectra of the free dodecanucleotide[27] indicates that only the terminal C_1 and G_{12} residues exhibit H2"-H3' cross peaks and strong H3'-H4' cross peaks, a feature characteristic of significant C3'-endo character. This observation can thus be used to constrain the pseudorotation phase angles of these residues to the range (P = 72 - 100°), which represents a structure that is primarily B-form but which also contains some 3'-endo character. It also includes the O4'-endo sugar pucker (P = 90°).[27] Since no other H2"-H3' couplings were observed, the pseudorotation phase angles of all other residues are constrained to a range (P = 110 - 162°), which includes C2'-endo and C1'-exo conformations (where C1'-exo is defined as P = 126°), as well as twisted forms of both.

Examination of the DQF-COSY and NOESY spectra of the Zn•BLM A_2–d(CGCGAATTCGCG)$_2$ complex showed that the dodecanucleotide retained its B-form structure upon complexation with Zn(II)•BLM. The DQF-COSY spectra indicated that only the terminal bases had H2"-H3' cross peaks (not shown), as observed for the free duplex. Further, the H1'-H2' cross peaks were stronger than the H1'-H2" cross peaks (Supporting Information, Figure 3). This result is consistent with $J_{1'-2'}$ being greater than $J_{1'-2"}$, a common feature of B-form duplexes.[25] In the NOESY spectra, all H8/H6 protons displayed cross peaks to their own sugar H1', H2', and H2" protons, as well as to the H1', H2', and H2" protons of the flanking 5' nucleotide residue. As required for a B-form duplex, the NOE cross peak from the H8/H6 base proton to its own H2' sugar proton tended to be much stronger than the NOE cross peak to the H2' proton on the flanking 5' sugar.[28] The opposite would be expected for an A-form helix with C3'-endo sugar puckers.[28] These features were used to constrain the sugar conformations during the molecular dynamics calculations. For the terminal residues (G_{12} and C_1), P was constrained to a range (P = 72 - 100°) that included the O4' sugar pucker (P = 90°), through analogy with the free dodecanucleotide. All other residues were constrained to a wide range (P = 110 - 162°) that included the C2'-endo (P = 162°) and the C1'-exo (P = 126°) conformations. These values were converted into ranges for the individual sugar torsion angles, v , using the relationship $v_j = T_m\cos[P + 144(j-2)]$, where T_m is 35 °C and j is 0-4.[24]

Experimental

BLM-Mediated DNA Cleavage Reactions. The DNA substrates used in this study are shown in Figure 3. Annealing of the duplex oligonucleotides was carried out in 200 μL of 20 mM sodium cacodylate, pH 6.0, containing 40 or 50 mM NaCl and 10 μM concentrations of each of the labeled and unlabeled DNA strands. The solution was heated to ~75 °C and allowed to cool slowly (6 - 8 h) to 4 °C. This procedure yielded a final duplex concentration of 10 μM. For a typical BLM reaction, 1 μL of this solution was dissolved in 10 μL (total volume) of 10

mM sodium cacodylate, pH 7.4, containing various concentrations of Fe(II)•BLM A_2 as described in the text and figure legends. Fe^{2+} and BLM were added separately but simultaneously from concentrated stock solutions. Reactions were allowed to proceed for 15 - 30 min on ice. Alkali treatment was effected as described in the figure legends using either 0.2 M n-butylamine (90 °C, 10 min) or 1 M piperidine (90 °C, 30 min). All products were then precipitated by the addition of 15 - 20 volumes of a 2% $LiClO_4$ solution in acetone, followed by washing with acetone, then 95% ethanol.

For experiments in which sodium borohydride treatment was employed, products of the bleomycin reactions were first precipitated by addition of 15-20 volumes of a 2% $LiClO_4$ solution in acetone. The DNA pellet was then redissolved in 40 μL of a solution containing 0.5 M $NaBH_4$, 0.5 M Tris Cl buffer, pH 8.0, and 1 μg of sonicated calf thymus DNA. This solution was allowed to react at room temperature for 1 h. The reaction was quenched with 10 μL of glacial acetic acid, and the products were precipitated with three volumes of ethanol.

Annealing reactions for the hairpin sequences were carried out using only labeled oligomer in order to maintain low concentrations that favor hairpin formation over bulged duplex.[48] Solutions containing approximately $5x10^6$ cpm (~ 2 pmol) of the labeled hairpin oligonucleotide in 100 μL of 50 mM sodium cacodylate, pH 6.5, containing 10 mM NaCl were heated to 95 °C and allowed to cool slowly to room temperature. Reactions of these substrates with BLM were then carried out as described above.

All products were analyzed by 20% sequencing PAGE and visualized either by autoradiography or phosphorimager analysis. Phosphorimager data was analyzed using Imagequant software. Quantitation was effected by determining the total integrated peak volumes in rectangles drawn around the bands of interest, after correction for background.

Characterization of the Stabilized Abasic Lesion. Large scale preparation of oligonucleotides containing the reductively stabilized lesion were carried out as follows, using high concentrations of Fe^{2+} and BLM relative to available O_2 to facilitate selective formation of the abasic lesion.[3,7] An annealed solution of the dodecanucleotide, prepared as described above but without any radiolabel, was dissolved in 500 μL (total volume) of a solution that contained 4 mM each of Fe^{2+} and BLM A_2 in 10 mM Na cacodylate, pH 7.4; the final duplex concentration was 100 μM. The reaction was incubated on ice for 30 min, followed by precipitation of the DNA by the addition of 50 μL of 3 M sodium acetate, pH 5.5, and 2 mL of ice cold ethanol. The resulting DNA pellet was washed once with ethanol, then redissolved in 200 μL of H_2O. A 100-μL aliquot of 0.5 M $NaBH_4$ in 0.5 M Tris Cl, pH 8.0, was added, and the solution was incubated for 20 - 30 min at room temperature. Another 100-μL aliquot of a freshly prepared borohydride solution was added, and the incubation was continued for an additional 20 min. The solution was then neutralized with 100 μL of glacial acetic acid, and the DNA was precipitated by addition of 1.5 mL of ice cold ethanol. After washing once with 70% ethanol, the oligomer containing the lesion was purified by 20% preparative denaturing (8 M urea) PAGE.

Digestion analyses were carried out by incubating ~0.5 - 3 A_{260} units of oligomer sequentially with nuclease P1, calf intestinal phosphatase, and then snake venom phosphodiesterase, as indicated. The DNA was first incubated with 30 units of nuclease P1 for 12 h at 37 °C in 100 μL of 50 mM sodium acetate, pH 5.5, containing 10 mM $MgCl_2$. After 12 h, 10 μL of 1 M Tris Cl, pH 9.0, and 8 - 10 units of phosphatase were added. The incubation was continued for an additional 4 h. The digested material was then either subjected to HPLC analysis or digested further with 0.02 unit of snake venom phosphodiesterase for an additional 3 h before HPLC analysis. C_{18} reversed phase HPLC analysis of the digested products was carried out on a Vydac 218TP1010 column using a 30-min linear gradient elution of 0 to 10 % acetonitrile in 0.1 M NH_4OAc, pH 6.5.

Table 1. ^{1}H NMR chemical shifts (ppm) of Zn•BLM A_2 and with equimolar d(CGCGAATTCGCG)$_2$ (+ DNA) at 15 °C, 40 mM NaCl, pH 7.0.

Residue	Zn•BLM A_2	+ DNA	Δδ
Ala α	3.72	3,81	+0.09
Ala β	2.49	2.54	+0.05
Ala β'	3.36	3.37	+0.01
Ala NH	4.32	n.d.[a]	
Pro α	2.87	2.87	0.00
Pro α'	3.24	3.31	+0.07
Pro β	4.50	4.60	+0.10
Pyr Me	2.38	2.45	+0.07
Pyr NH_2	7.02	7.08	+0.06
His α	4.85	4.91	+0.06
His β	5.20	5.25	+0.05
His 2	8.04	8.09	+0.05
His 4	7.31	7.36	+0.05
Val α	1.95	1.77	-0.18
Val β	3.42	3.48	+0.06
Val γ	3.62	3.66	+0.04
Val αMe	0.98	0.96	-0.02
Val γMe	0.93	0.96	+0.03
Val NH	7.48	7.50	+0.02

Table 1. Continued

Residue	Zn•BLM A_2	+ DNA	Δδ
Thr α	4.10	4.19	+0.09
Thr β	4.00	4.10	+0.10
Thr Me	1.02	1.07	+0.05
Thr NH	7.96	7.71	-0.25
Bit α	3.22	3.07	-0.15
Bit β	3.57	3.50	-0.07
Bit 5	8.21	n.d.	
Bit 5'	8.04	n.d.	
Bit NH	8.30	8.27	-0.03
Gul 1	5.31	5.35	+0.04
Gul 2	4.06	4.10	+0.04
Gul 3	4.03	n.d.	
Gul 4	3.71	n.d.	
Gul 5	3.85	n.d.	
Gul 6,6'	3.61, 3.69	n.d.	
Man 1	4.89	4.95	+0.06
Man 2	4.09	n.d.	
Man 3	4.03	n.d.	
Man 4	3.67	n.d.	
Man 5	3.70	n.d.	

Residue	Zn•BLM A_2	+ DNA	Δδ
Man 6,6'	3.79, 3.97	n.d.	
Sul NH	8.90	8.58	-0.32
Sul α	3.38	3.37	-0.01
Sul β	2.14	2.12	-0.02
Sul γ	3.60	3.50	-0.10
Sul Me_2^+	2.88	2.91	+0.03

[a] n.d.; not determined

Table 2. ^{1}H NMR Chemical Shifts (ppm) of the DNA Resonances in the Zn•BLM A_2 - d(CGCGAATTCGCG)$_2$ Complex at 15 °C, 40 mM NaCl, pH 7.0.

	NH	NH_2	H6/H8	H5/H2	H1'	H2'	H2"	H3'	H4'	H5',5"
C_1			7.61	5.82	5.70	1.95	2.40	4.70	4.07	3.74
G_2	13.05	6.89	7.93		5.87	2.63	2.71	4.96	4.34	3.98
C_3		8.40, 6.47	2.28	5.36	5.50	1.86	2.26	4.80	4.11	
G_4	12.68		7.86		5.47	2.65	2.77	4.99	4.29	
A_5			8.11	7.19	5.98	2.68	2.94	5.06	4.45	4.19
A_6			8.13	7.62	6.16	2.56	2.94	5.02	4.47	4.26
T_7	13.71		7.14	1.21	5.93	2.01	2.56	4.84	4.20	
T_8	13.87		7.38	1.51	6.11	2.17	2.56	4.91	4.21	
C_9		8.42, 6.59	7.45	5.59	5.69	2.04	2.42	4.89	4.16	
G_{10}	12.91		7.92		5.81	2.66	2.66	4.99	4.37	
C_{11}		8.42, 6.59	7.33	5.43	5.73	1.89	2.32	4.82	4.12	
G_{12}			7.93		6.06	2.64	2.36	4.66	4.11	4.03

Table 3. Backbone Torsion Angles[a] (°) Used in the Restrained Molecular Dynamics Calculations.

Torsion Angle	Torsion Angle Values	Range
α	-60	30
β	180	50
γ	60	30
ε	180	50
ζ	-85	50

[a]Backbone torsion angles are defined as: P(i) - α - O5' - β - C5' γ - C4' - δ - C3' - ε - O3' - ζ - P(i + 1).

Table 4. Hydrogen Bond Distance (Å) Restraints Used in the Restrained Molecular Dynamics Calculations.

Atom 1	Atom 2	Distance	Range
C1'	C1' (across each base pair)	10.87	0.20
A-T base pairs			
N1	N3	2.80	0.1
N6	O4	2.80	0.1
C-G base pairs			
N3	N1	2.80	0.1
N4	O6	2.70	0.1
O2	N2	2.80	0.1

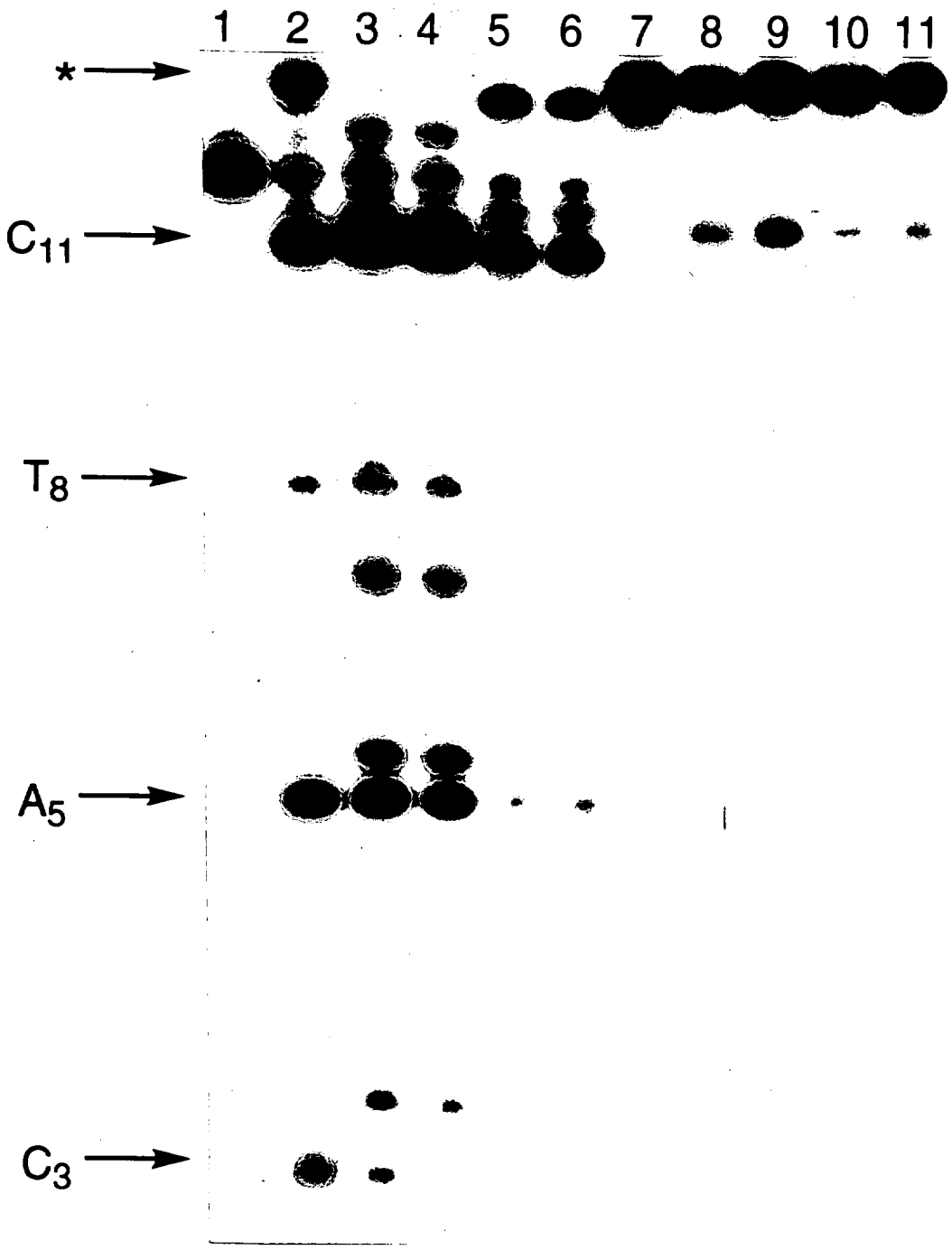


Figure 1. Polyacrylamide gel electrophoretic analysis of the reductive stabilization of the lesion (*) produced upon reaction of Fe•BLM with the dodecanucleotide duplex d(GCGCAATTCGCG)$_2$. Lane 1, DNA control; lane 2, 500 μM Fe(II)•BLM A_2 + 12 μM duplex; lanes 3 and 4, treatment of the Fe•BLM reaction mixture with 1 M piperidine; lanes 5 and 6, treatment of the Fe•BLM reaction mixture with $NaBH_4$, followed by piperidine; lane 7, purified, $NaBH_4$-stabilized lesion (*); lanes 8 and 9, treatment of the purified lesion with piperidine; lanes 10 and 11, treatment of the purified lesion with $NaBH_4$, followed by piperidine.

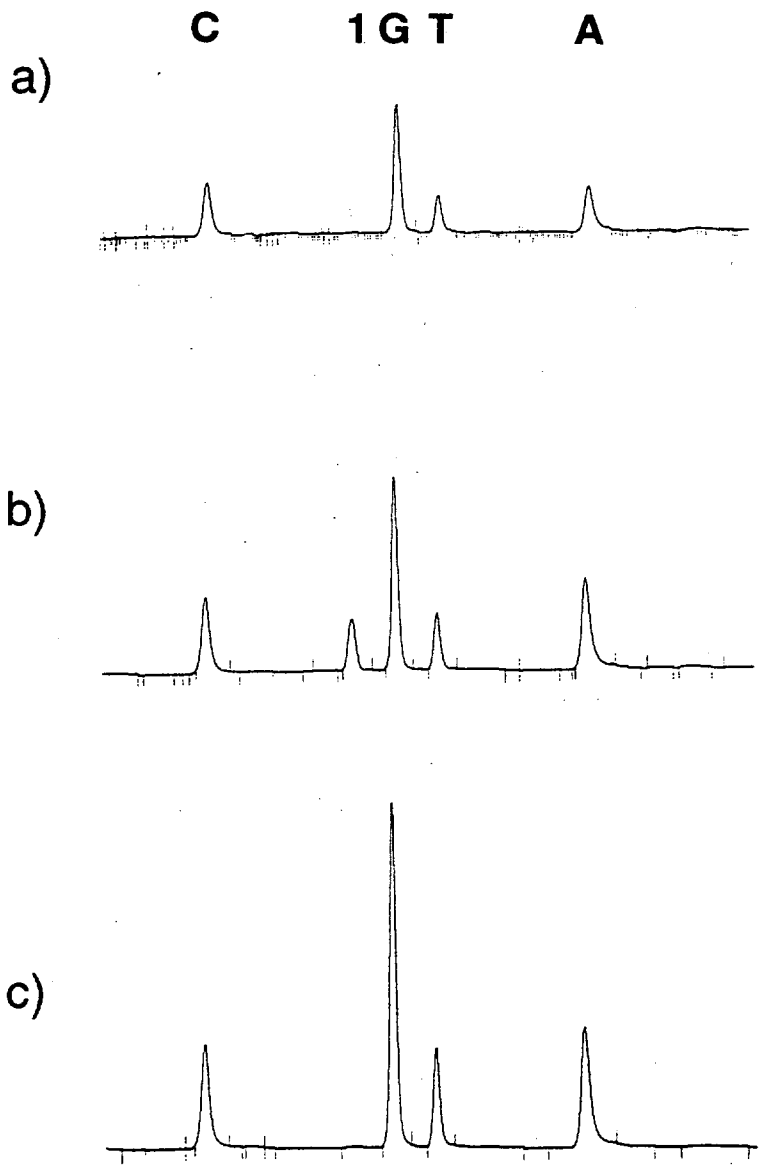


Figure 2. Enzymatic digestion and HPLC analysis of the Fe(II)•BLM-modified oligonucleotide containing the lesion (*). (a) Unmodified dodecanucleotide digested with DNase 1, nuclease P1 and alkaline phosphatase; (b) lesion (*) digested under the same conditions; (c) incubation product from (b), after further treatment with snake venom phosphodiesterase.

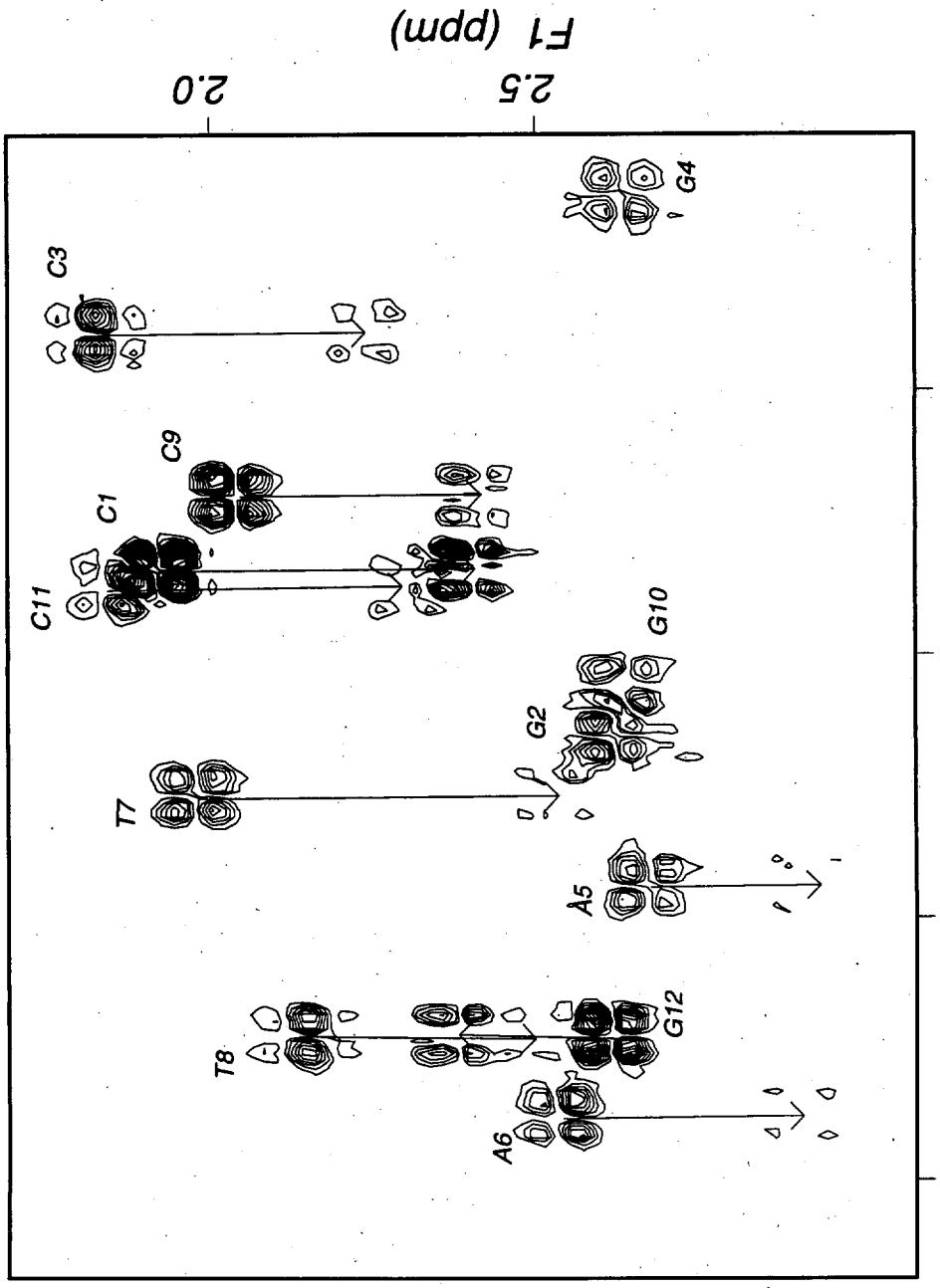


Figure 3. H1' - H2',H2" cross peak region of the DQF-COSY spectrum of the Zn(II)•BLM A_2 - d(GCGCAATTCGCG)$_2$ complex in D_2O at 15 °C. Cross peaks between H1' and H2' are indicated by residue. The arrows illustrate the extension of the assignment from 2' protons to 2" protons. Except for the 3'-terminal G_{12} residue, 2' protons resonated upfield relative to 2" protons of the same residue.

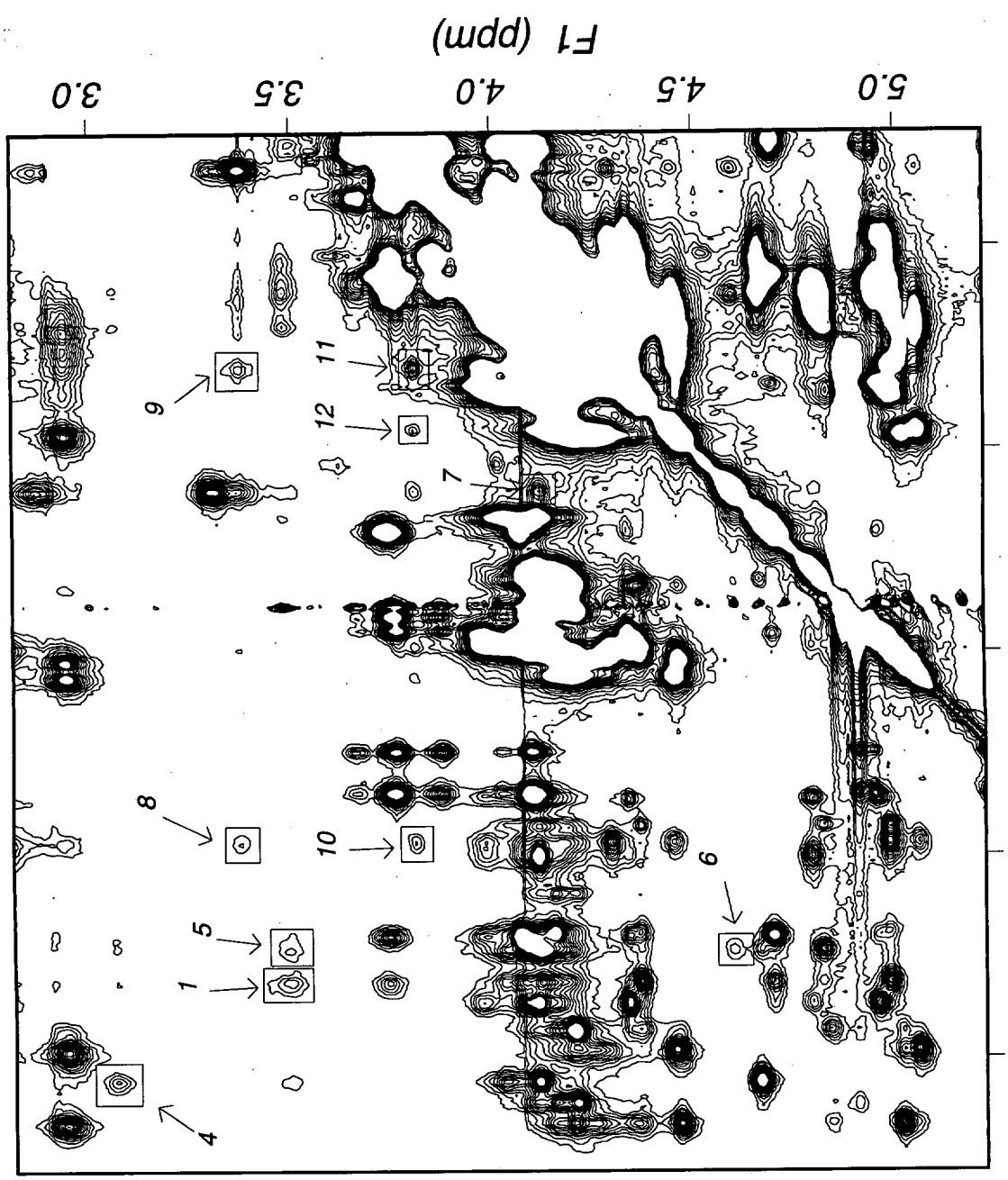


Figure 4. Expanded NOESY ^{1}H NMR spectrum of the Zn(II)•BLM A_2–d(CGCGAATTCGCG)$_2$ complex in D_2O at 15 °C, τ_{mix} = 300 ms. Intermolecular BLM-DNA cross peaks are denoted by arrows and numbers. Assignments: 1, Sul α - C_1H5; 4, Bit α - G_{12}H1'; 5, Bit β - C_{11}H1'; 6, Pro β - C_{11}H1'; 7, Pro β - C_{11}H4'; 8, Ala β' - G_4H1'; 9, Ala β' - G_4H4'; 10, Ala α - G_4H1'; 11, Ala α - G_4H4'; 12, Ala α - A_5H4'.

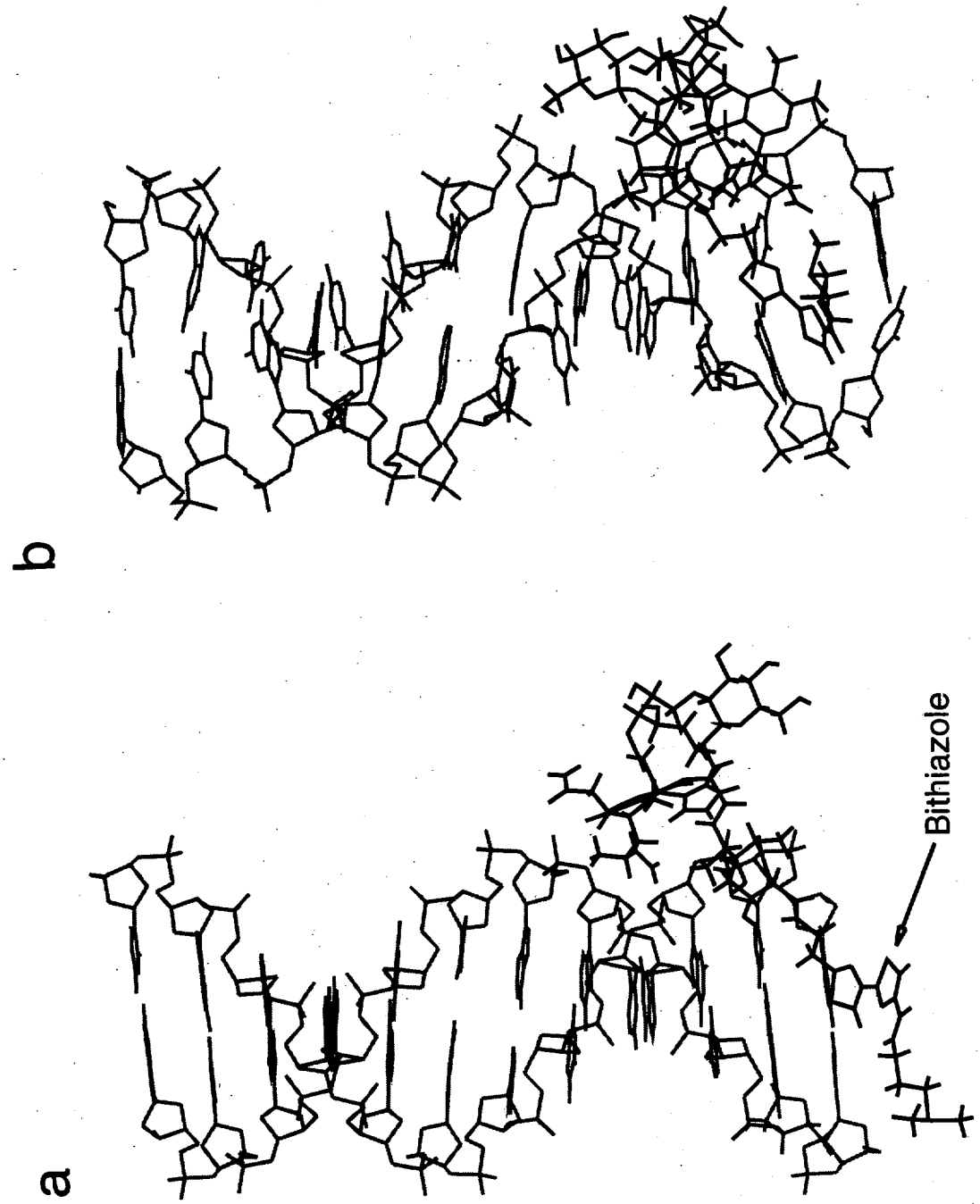
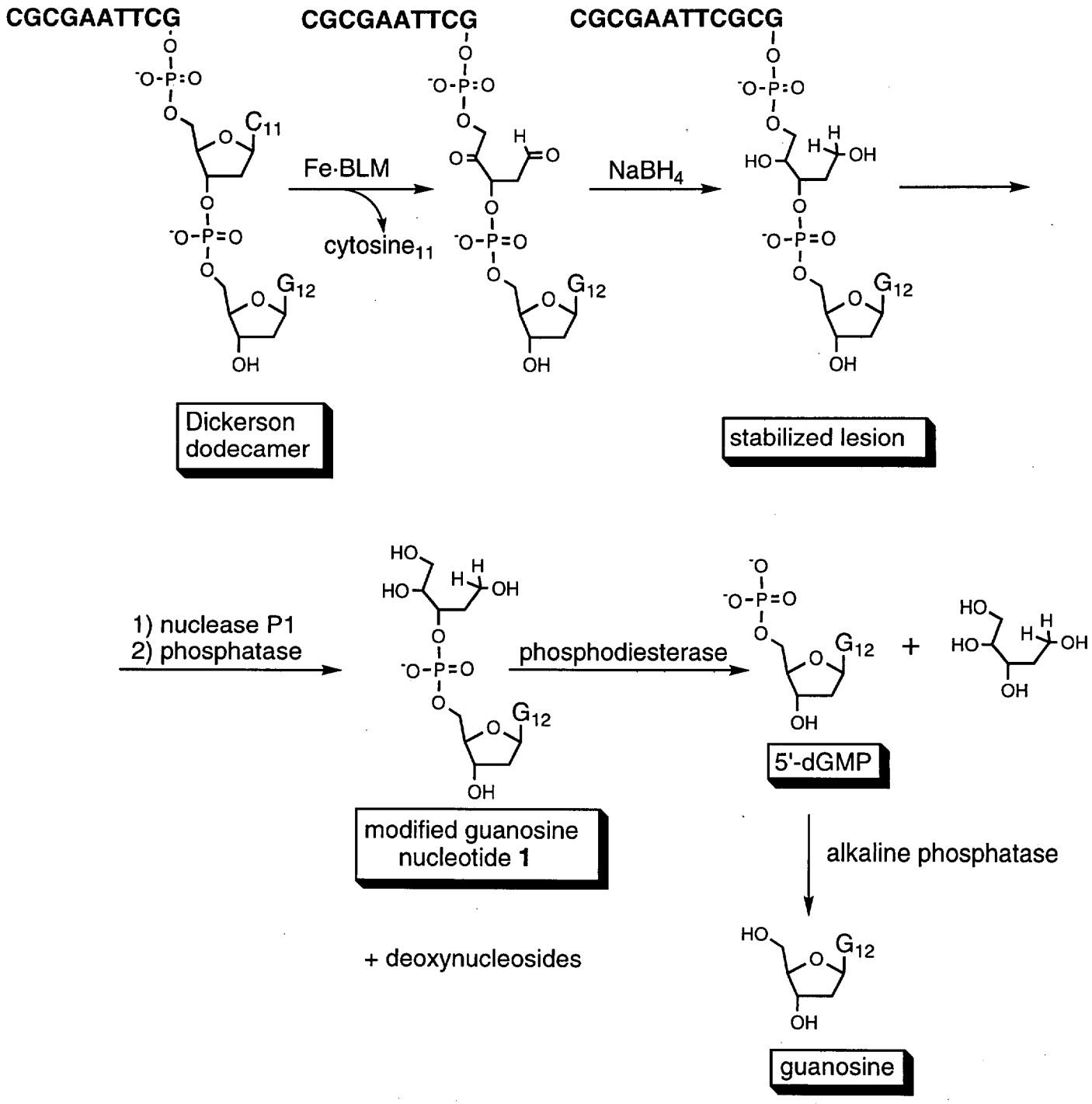


Figure 5. Stuctures highlighting alternative orientations of dodecanucleotide binding by the bithiazole moiety of BLM. Structure a, bithiazole is present in a "*trans*" orientation and is stacked at the end of the duplex. Structure b, bithiazole is present in a "*cis*" orientation and is partially inserted between the last two base pairs of the duplex.



Legend to Scheme

Scheme 1. Biochemical analysis of the $NaBH_4$-stabilized alkali-labile lesion formed during the reaction of Fe(II)•BLM with $d(CGCGAATTCGCG)_2$.