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Chemistry of the Alkali-Labile Lesion Formed from Iron(II) Bleomycin and d(CGCTTTAAAGCG)[†]

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ABSTRACT: Two sets of products are formed from DNA upon treatment with Fe(II)·bleomycin + O_2. One set, which is believed to derive from a C-4′ hydroperoxy derivative of the DNA deoxyribose moiety, includes the four possible base propenals, as well as DNA oligomers having deoxynucleoside 3′-(phosphoro-2″-*O*-glycolates) at their 3′-termini. The other set of products consists of free bases and alkali-labile lesions, the latter of which had not previously been characterized structurally. By use of the self-complementary dodecanucleotide d(CGCTTTAAAGCG) having a site modified by Fe·bleomycin three nucleotides from the 5′-end, it has been possible to characterize the alkali-labile product as a C-4′ hydroxyapurinic acid. When the bleomycin-treated dodecanucleotide was treated with agents that effected decomposition of the alkali-labile lesion, products of the form CpGpx were obtained, and these proved useful for structural characterization of the alkali-labile lesion. Treatment with alkali produced CpGpx, where x was 2,4-dihydroxycyclopentenone. Alternatively, treatment with hydrazine provided a pyridazine derivative, and aqueous alkylamines led to formation of CpGp itself. The structures of all dinucleotides produced from the alkali-labile lesion were verified by direct comparison with authentic synthetic samples.

The bleomycins are antitumor antibiotics in use clinically for the treatment of certain neoplasms (Carter, 1978; Crooke, 1978; Umezawa, 1979). Bleomycin A_2 is the major constituent of the mixture of bleomycins employed in the clinic. The therapeutic effects of bleomycin are believed to be expressed at the level of DNA degradation; this process has been studied in cell-free systems and shown to proceed in the presence of any of several redox-active metal ions and O_2 (Ishida & Takahashi, 1975; Sausville et al., 1978; Ehrenfeld et al., 1984, 1985, 1987; Burger et al., 1984; Suzuki et al., 1985; Kuwahara et al., 1985) or *hν* (Sugiura et al., 1982; Chang & Meares, 1982, 1984).

The products of DNA-mediated strand scission have been shown to include base propenals (Burger et al., 1980; Giloni et al., 1981) and free bases (Burger et al., 1982; Wu et al., 1983), as well as oligonucleotides having the four possible nucleoside 3′-(phosphoro-2″-*O*-glycolates) at their 3′-termini (Giloni et al., 1981; Murugesan et al., 1985).

It has been known for some time that bleomycin also produces an alkali-labile lesion, subsequent base treatment of which results in DNA strand scission (Hecht, 1979). Structural elucidation of this lesion proved challenging, but was ultimately accomplished following base treatment of the alkali-labile lesion (Sugiyama, 1985c); more recently the proposed structure was verified following reduction of the lesion with $NaBH_4$ and subsequent digestion with P_1 nuclease and alkaline phosphatase (Rabow et al., 1986).

Presently, we describe fully the experiments that permitted identification (Sugiyama et al., 1985c) of the structures of the Fe·BLM[1]-derived alkali-labile lesion and its base-induced decomposition product. Also described are additional chemical transformations of the alkali-labile lesion that help to secure

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[1] Abbreviations: BLM, bleomycin; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran; HPLC, high-pressure liquid chromatography; DTT, dithiothreitol; ATP, adenosine 5′-triphosphate; TLC, thin-layer chromatography; EDTA, ethylenediaminetetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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the connectivity of carbon atoms in the assigned structure and which can control the mode of decomposition of the lesion to produce a product identical with those that arise from the Maxam–Gilbert sequencing procedure (Maxam & Gilbert, 1977, 1980).

EXPERIMENTAL PROCEDURES

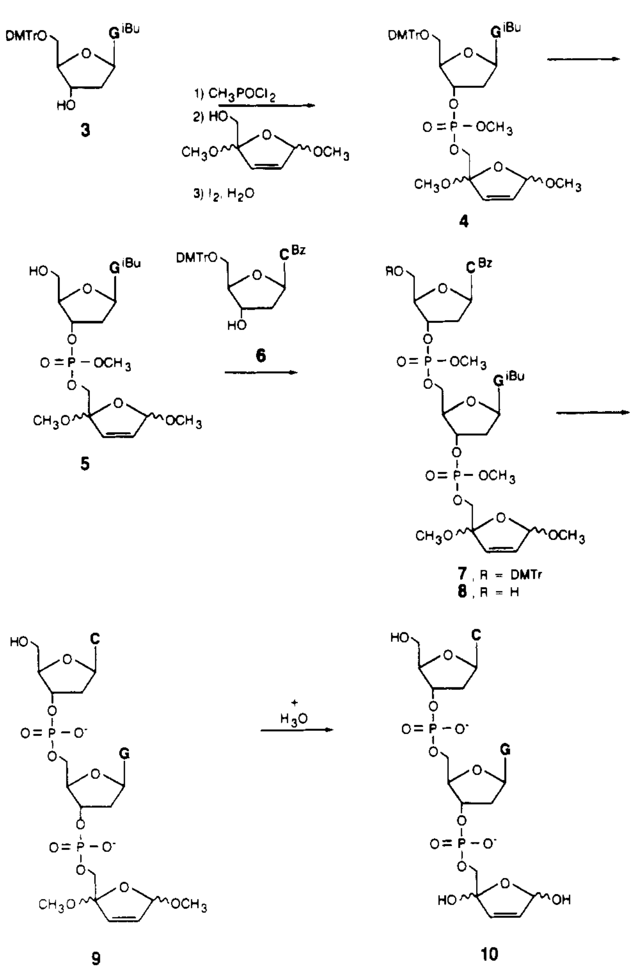
Materials

Blenoxane was obtained from Bristol Laboratories through the courtesy of Dr. William Bradner and was fractionated as described to provide bleomycin A_2 (Chen et al., 1977; Oppenheimer et al., 1979). Decarbamoylbleomycin A_2 was obtained as described (Sugiyama et al., 1985a). Nucleosides **3** and **6** were purchased from Sigma Chemical Co. T4 polynucleotide kinase (unit definition: 1 unit catalyzes the transfer of 1 nmol of the terminal phosphate from ATP to the 5′-OH terminus of micrococcal nuclease treated DNA in 30 min at 37 °C) was obtained from Bethesda Research Laboratories. Calf intestine alkaline phosphatase (unit definition: 1 unit hydrolyzes 1 μmol of *p*-nitrophenyl phosphate in 1 min at 37 °C) was purchased from Boehringer Mannheim. The dodecanucleotide 5′-d(CGCTTTAAAGCG) was prepared and 5′-^{32}P end-labeled as described (Murugesan et al., 1985). Flash chromatography was carried out on Macherey-Nagel silica gel 60 (230–400 mesh). TLC was carried out on silica gel 60 plates manufactured by E. Merck (aluminum support, 0.2 mm; glass support, 0.25 and 0.5 mm). HPLC analysis was carried out on a 25-cm Du Pont Zorbax Sax anion-exchange column or on a Rainin Microsorb C_{18} Short-One column.

Methods

2,5-Dimethoxy-2,5-dihydrofurfuryl Methyl 5′-O-(Dimethoxytrityl)-N-isobutyryl-2′-deoxy-3′-guanylate (**4**). To a solution of 0.4 mL of dry collidine and 1.6 mL of freshly distilled tetrahydrofuran was added 70 μL (0.46 mmol) of methyl phosphorodichloridite at −78 °C under N_2. This solution was treated dropwise under N_2 with a cold solution containing 305 mg (0.49 mmol) of 5′-*O*-(dimethoxytrityl)-*N*-isobutyryl-2′-deoxyguanosine (**3**) in 2.5 mL of THF. The addition was carried out over a period of 1 h, after which time the combined solution was stirred at −78 °C under N_2 for an additional 20 min. To this solution was added dropwise under N_2 a cold solution containing 81 mg (0.51 mmol) of 2,5-dimethoxy-2,5-dihydrofurfuryl alcohol (Achmatowicz et al., 1971) in 3 mL of THF. The addition was carried out over a period of 1 h; the combined solution was then stirred at −78 °C under N_2 for 15 min. The reaction mixture was then treated with a solution consisting of 150 mg (0.6 mmol) of iodine in 0.33 mL of H_2O, 0.33 mL of pyridine, and 0.33 mL of THF. After an additional 10 min at 25 °C, the reaction mixture was concentrated under diminished pressure, and the residue was dissolved in $CHCl_3$ and washed with 1% aqueous $NaHSO_3$ and H_2O. The organic phase was dried (Na_2SO_4) and concentrated, and the residue was purified by flash chromatography (Still et al., 1978) on a 5-g silica gel column; elution was with 5% ethanol in ethyl acetate. 2,5-Dimethoxy-2,5-dihydrofurfuryl methyl 5′-*O*-(dimethoxytrityl)-*N*-isobutyryl-2′-deoxy-3′-guanylate (**4**) was obtained as a colorless foam: yield 202 mg (47%); $\lambda_{max}^{CH_3OH}$ 231 and 260 nm; (partial) ^{1}H NMR [$CDCl_3$, $(CH_3)_4Si$] δ 0.80–1.15 (m, 6), 3.67 (s, 6), 5.20–6.20 (m, 4), 6.50–6.80 (m, 4), 6.80–7.45 (m, 9), and 7.67 (s, 1).

2,5-Dimethoxy-2,5-dihydrofurfuryl Methyl N-Isobutyryl-2′-deoxy-3′-guanylate (**5**). A solution of 180 mg (0.2 mmol) of 2,5-dimethoxy-2,5-dihydrofurfuryl methyl 5′-*O*-(dimethoxytrityl)-*N*-isobutyryl-2′-deoxy-3′-guanylate (**4**) in 12 mL of 80% aqueous acetic acid was stirred at 25 °C for 1 h. The solution was concentrated under diminished pressure, and the residue was treated with portions of water and again concentrated. The resulting residue was purified by flash chromatography on a 5-g silica gel column. Elution with 5% CH_3OH in $CHCl_3$ provided 2,5-dimethoxy-2,5-dihydrofurfuryl methyl *N*-isobutyryl-2′-deoxy-3′-guanylate (**5**) as a colorless foam: yield 92 mg (80%); $\lambda_{max}^{CH_3OH}$ 260 nm; (partial) ^{1}H NMR [$CDCl_3$, $(CH_3)_4Si$] δ 1.22 (d, 6), 3.00–3.50 (m, 6), 5.15–6.25 (m, 4), and 7.82 (s, 1); FAB mass spectrum (positive ion) *m/z* 574 $(M + H)^+$, (negative ion) *m/z* 572 $(M - H)^-$.

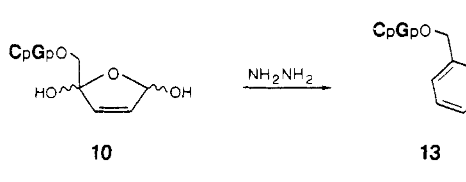


2,5-Dimethoxy-2,5-dihydrofurfuryl Methyl N-Benzoyl-5′-O-(dimethoxytrityl)-2′-deoxy-P-methylcytidylyl-(3′→5′)-N-isobutyryl-2′-deoxy-3′-guanylate (**7**). A solution containing 23 μL (0.16 mmol) of methyl phosphorodichloridite in 0.5 mL of dry THF and 0.14 mL of collidine under N_2 was cooled to −78 °C and treated with 104 mg (0.164 mmol) of *N*-benzoyl-5′-*O*-(dimethoxytrityl)-2′-deoxycytidine (**6**) in 0.9 mL of dry THF over a period of 30 min. The solution was stirred at −78 °C for 10 min and then treated with 78 mg (0.136 mmol) of 2,5-dimethoxy-2,5-dihydrofurfuryl methyl *N*-isobutyryl-2′-deoxy-3′-guanylate (**5**) in 1 mL of THF over a period of 25 min. The reaction mixture was stirred at −78 °C for 30 min and then at 25 °C for 15 min. The reaction mixture was then treated with a solution consisting of 50 mg (0.2 mmol) of iodine in 0.33 mL of H_2O, 0.33 mL of pyridine, and 0.33 mL of THF. After an additional 10 min at 25 °C, the reaction mixture was concentrated and the residue was dissolved in 20 mL of ethyl acetate and washed with 10 mL of 5% aqueous $NaHSO_3$. The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on a 5-g silica gel column; elution was with 5–15% ethanol in ethyl acetate. Dinucleotide **7** was obtained as a white solid, yield 56 mg (32%). The crude product was used directly for detritylation.

2,5-Dimethoxy-2,5-dihydrofurfuryl Methyl N-Benzoyl-2′-deoxy-P-methylcytidylyl-(3′→5′)-N-isobutyryl-2′-deoxy-3′-guanylate (**8**). A solution of 70 mg (55 μmol) of dinucleotide **7** in 5 mL of 80% aqueous acetic acid was maintained at 25 °C for 1 h. The solution was concentrated under diminished pressure, and the residue was treated with 5 mL of water and concentrated again. The residue was purified by flash chromatography on a 5-g silica gel column; elution was with $CHCl_3$ and then with 5–10% CH_3OH in $CHCl_3$. 2,5-Dimethoxy-2,5-dihydrofurfuryl methyl *N*-benzoyl-2′-deoxy-*P*-methylcytidylyl-(3′→5′)-*N*-isobutyryl-2′-deoxy-3′-guanylate (**8**) was obtained as a colorless foam: yield 40 mg (78%); $\lambda_{max}^{CH_3OH}$ 235, 260 and 287 (sh) nm; (partial) ^1H NMR [$CDCl_3$, $(CH_3)_4Si$] δ 1.00–1.30 (d, 6), 3.05–3.25 (m, 3), 3.25–3.52 (m, 3), 3.52–3.90 (m, 6), and 7.30–8.50 (m, 7).

3′-(2,5-Dimethoxy-2,5-dihydrofurfuryl) 2′-Deoxycytidylyl-(3′→5′)-2′-deoxy-3′-guanylate (**9**). Dinucleotide **8** (39 mg, 40 μmol) was dissolved in 2 mL of a 1:1:2 thiophenol–triethylamine–dioxane solution and stirred at 25 °C for 1.5 h. Concentration of the solution under diminished pressure afforded a white solid that was partitioned between water and benzene. The aqueous layer was concentrated, and the colorless residue was dissolved in 2 mL of concentrated NH_4OH, sealed in a glass tube, and heated at 50 °C for 12 h. The cooled solution was concentrated, and the residue was purified by flash chromatography on a 10-g silica gel column. Elution with 7:1:2 2-propanol–NH_4OH–H_2O afforded 3′-(2,5-dimethoxy-2,5-dihydrofurfuryl) 2′-deoxycytidylyl-(3′→5′)-2′-deoxy-3′-guanylate (**9**) as a white solid: yield 20 mg (64%); $\lambda_{max}^{CH_3OH}$ 253 and 273 (sh) nm; λ_{min} 222 nm; ^1H NMR (D_2O) δ 1.47 (m, 1), 2.25 (m, 1), 2.55 (m, 1), 2.78 (m, 1), 2.95–3.12 (m, 3), 3.25–3.43 (m, 3), 3.59 (s, 2), 3.65–4.10 (m, 5), 4.32 (s, 1), 4.50 (s, 1), 4.90 (s, 1), 5.48 (d, 1), 5.89 (d, 1, J = 7 Hz), 5.95–6.25 (m, 4), 7.53 (d, 1, J = 7 Hz), and 7.99 (s, 1); FAB mass spectrum (positive ion) m/z 779 $(M + H)^+$, (negative ion) m/z 777 $(M - H)^-$.

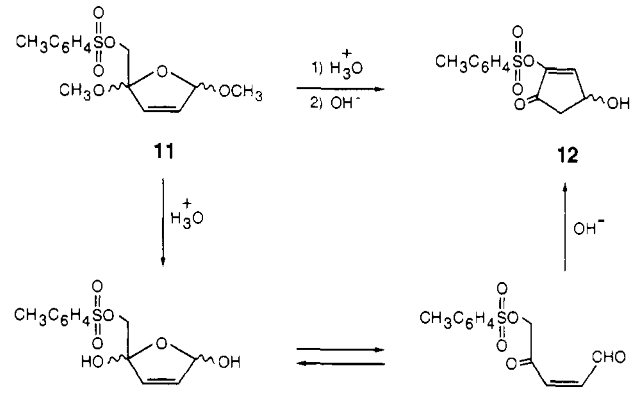
3′-(2,5-Dihydroxy-2,5-dihydrofurfuryl) 2′-Deoxycytidylyl-(3′→5′)-2′-deoxy-3′-guanylate (**10**). Dinucleotide **9** (0.56 A_{260} unit, ~40 μg) was dissolved in 200 μL of 0.1 N HCl and maintained at 25 °C for 30 min. HPLC showed disappearance of starting material and appearance of a single new peak (Figure 1); assuming similar response factors, the conversion proceeded essentially quantitatively. After incubation in 0.1 N HCl at 37 °C, HPLC analysis indicated that 51% of the material initially formed was still present after 1 h; 18% was present after 3 h. The product exhibited somewhat greater stability in 0.1 N formic acid.



3′-(3-Hydroxy-5-oxo-1-cyclopenten-1-yl) 2′-Deoxycytidylyl-(3′→5′)-2′-deoxy-3′-guanylate (**1**). An aliquot (20 μL) of the acid hydrolysate derived from **9** (vide supra) was treated with 4 μL of 1 N NaOH and then heated at 60 °C for 2 min. The reaction mixture was neutralized with 2 μL of 1 N formic acid and then analyzed by HPLC (Figure 2). Two isomers of 3′-(3-hydroxy-5-oxo-1-cyclopenten-1-yl) 2′-deoxycytidylyl-(3′→5′)-2′-deoxy-3′-guanylate (**1**) were formed in apparently equal amounts and in an overall yield of ~70%.

The conversion **9** → **10** → **1** was repeated with 4 mg of dinucleotide **9**. This material was dissolved in 2 mL of 0.1 N HCl and maintained at 25 °C for 1 h. The reaction mixture was then treated with 400 μL of 1 N NaOH and heated at 60 °C for 5 min. The reaction mixture was neutralized with aqueous formic acid, and the product was isolated by lyophilization. Dinucleotides **1** were isolated as a yellow syrup by HPLC: yield 1.0 mg; λ_{max} 250 and 271 (sh) nm; FAB mass spectrum (positive ion) m/z 733 $(M + H)^+$, (negative ion) m/z 731 $(M - H)^-$. See Figure 3 and Table I for ^1H NMR spectral data.

1-O-(2,5-Dimethoxy-2,5-dihydrofurfuryl) p-Toluenesulfonate (**11**). A solution containing 1 g (6.3 mmol) of 2,5-dimethoxy-2,5-dihydrofurfuryl alcohol (Achmatowicz et al., 1971) in 5 mL of pyridine was treated with 1.2 g (6.3 mmol) of *p*-toluenesulfonyl chloride. The reaction mixture was stirred at 25 °C overnight and then treated with water and extracted with ethyl acetate (3 × 50 mL). The organic layer was dried (Na_2SO_4) and concentrated to afford 1.9 g of crude product. Crystallization from ethyl acetate provided 1-*O*-(2,5-dimethoxy-2,5-dihydrofurfuryl) *p*-toluenesulfonate (**11**) as colorless microneedles: yield 1.0 g (50%); mp 48–50 °C dec; ^1H NMR [$CDCl_3$, $(CH_3)_4Si$] δ 2.43 (s, 3), 3.14 (s, 3), 3.43 (s, 3), 3.97 (d, 1, J = 11 Hz), 4.14 (d, 1, J = 11 Hz), 5.39 (s, 1), 5.86 (d, 1, J = 6 Hz), 6.12 (d, 1, J = 6 Hz), 7.34 (d, 2, J = 8 Hz), and 7.78 (d, 2, J = 8 Hz); mass spectrum m/z 315 $(M + H)^+$. This compound proved to be very unstable when stored under ambient conditions.

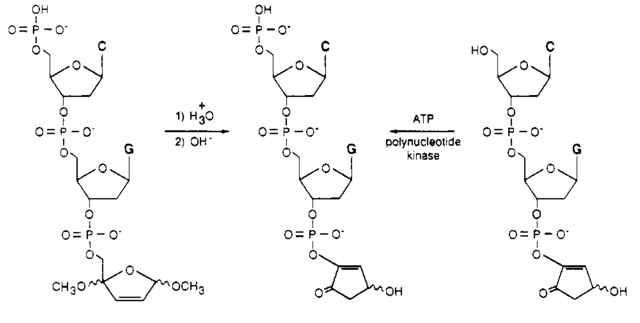


2,4-Dihydroxycyclopentenone 2-O-p-Toluenesulfonate (**12**). 1-*O*-(2,5-Dimethoxy-2,5-dihydrofurfuryl) *p*-toluenesulfonate (5 mg, 16 μmol) was dissolved in 2 mL of 88% formic acid and stirred at 25 °C for 1 h. The reaction mixture was concentrated to dryness under diminished pressure, and the residue was dissolved in 50 mL of an alkaline solution (pH ~9) containing 0.1 M Na_2CO_3 and 0.1 M $CH_3NH_2 \cdot HCl$ and maintained at 25 °C for 2 h. The reaction mixture was extracted with ethyl acetate, and the organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by silica gel TLC, developing with 1:1 hexane–ethyl acetate, and then by HPLC on a C_{18} reverse-phase column, eluting with 40:60 CH_3OH–H_2O. 2,4-Dihydroxycyclopentenone 2-*O*-*p*-toluenesulfonate (**12**) was isolated as a colorless syrup: yield 1.5 mg (35%); mass spectrum m/z 269 $(M + 1)^+$. See Table I for ^1H NMR spectral data.

3′-(3-Pyridazinylmethyl) 2′-Deoxycytidylyl-(3′→5′)-2′-deoxy-3′-guanylate (**13**). Dinucleotide **9** (2 mg, 2.6 μmol) was hydrolyzed to **10** in 0.1 N HCl at 25 °C. The formation of **10** was verified by reverse-phase HPLC and by the conversion of a sample of **10** (obtained in the same fashion in a parallel experiment) to dinucleotide **1** upon treatment with NaOH at 90 °C.

The solution containing **10** was neutralized and treated with 0.1 M $NH_2NH_2 \cdot HCl$ (pH ~8). The resulting solution was maintained at 25 °C for 15 min, and the product was purified by reverse-phase HPLC. 3′-(3-Pyridazinylmethyl) 2′-deoxycytidylyl-(3′→5′)-2′-deoxy-3′-guanylate (**13**) was isolated as a white solid: yield 1.0 mg (53%); λ_{max} 250 and 270 (sh) nm; ^{1}H NMR (D_2O) δ 1.52 (m, 1), 2.18 (m, 1), 2.46 (m, 1), 2.68 (m, 1), 3.47 (m, 2), 3.90 (m, 3), 4.17 (br s, 1), 4.43 (m, 1), 4.89 (m, 1), 5.10 (d, 2, J = 8.5 Hz), 5.81 (d, 1, J = 7.5 Hz), 5.93 (t, 1), 5.99 (t, 1), 7.42 (d, 1, J = 7.5 Hz), 7.71 (m, 1), 7.83 (d, 1), 7.87 (s, 1), and 8.96 (d, 1); FAB mass spectrum (positive ion) m/z 729 $(M + H)^+$, (negative ion) m/z 727 $(M - H)^-$.

3′-(2,5-Dimethoxy-2,5-dihydrofurfuryl) 5′-Phospho-2′-deoxycytidylyl-(3′→5′)-2′-deoxy-3′-guanylate (**14**). A reaction mixture (200-μL total volume) containing 0.2 mg (0.3 μmol) of dinucleotide **9**, 10 mM ATP, 10 mM $MgCl_2$, 5 mM DTT, 0.1 mM spermidine, 0.1 mM EDTA, and 20 units of T4 polynucleotide kinase in 50 mM Tris-HCl buffer, pH 7.6, was incubated at 37 °C for 5 h. The progress of the reaction was monitored by silica gel TLC. The product was purified by TLC on silica gel; development was with 7:2:2 2-propanol–NH_4OH–H_2O (R_f = 0.3). Dinucleotide 5′-phosphate **14** was obtained as a colorless oil: yield 2.3 A_{260} units (90%); λ_{max} (pH 7) 251 nm.



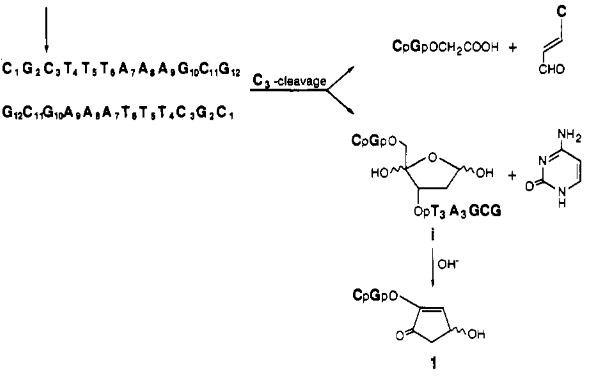
14 **2** **1**

3′-(3-Hydroxy-5-oxo-1-cyclopentenyl) 5′-Phospho-2′-deoxycytidylyl-(3′→5′)-2′-deoxy-3′-guanylate (**2**). *Method A.* To 25 μg of dinucleotide 5′-phosphate **14** was added 250 μL of 0.1 N HCl. The reaction mixture was maintained at 25 °C for 1 h and then treated with 50 μL of 1 N NaOH and heated at 60 °C for 2 min. After neutralization with formic acid, the reaction mixture was analyzed by HPLC.

Method B. A reaction mixture (150-μL total volume) containing 0.1 mg (0.15 μmol) of dinucleotide **1**, 20 mM ATP, 10 mM $MgCl_2$, 5 mM DTT, 0.1 mM spermidine, 0.1 mM EDTA, and 10 units of T4 polynucleotide kinase in 50 mM Tris-HCl buffer, pH 7.6, was incubated at 37 °C for 12 h. The appearance of the desired dinucleotide 5′-phosphate was monitored by HPLC. The product was purified by TLC on silica gel; development was with 7:2:2 2-propanol–NH_4OH–H_2O (R_f = 0.3). Dinucleotide 5′-phosphate **2** was obtained as a colorless oil: yield 1.1 A_{260} units (~90%); λ_{max} 250 and 271 (sh) nm. HPLC analysis indicated that the samples prepared by methods A and B had identical chromatographic properties.

Transformation of 3′-(2,5-Dihydroxy-2,5-dihydrofurfuryl) 2′-Deoxycytidylyl-(3′→5′)-2′-deoxy-3′-guanylate (**10**) *in Alkali and n-Butylamine.* Dinucleotide **9** (0.02 A_{260} unit) was hydrolyzed in 200 μL of 0.1 N HCl at 25 °C for 2 h; HPLC analysis indicated the formation of dinucleotide **10** (cf. Figure 1). A 3-μL aliquot was treated with 0.2 N NaOH in a total volume of 20 μL at 90 °C for 10 min. The reaction mixture was adjusted to pH ~8 with 1 N CH_3COOH and then treated with 2.5 units of calf intestine alkaline phosphatase at 25 °C for 15 min. The reaction products were analyzed by reverse-phase HPLC and found to consist of dinucleotide **1** (61%) and CpG (20%).

Scheme I: Products Resulting from Fe·BLM-Mediated Degradation of d(CGCTTTAAAGCG) at Cytidine-3



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Another 3-μL aliquot of the acid hydrolysate was treated with 0.2 N *n*-butylamine in a total volume of 20 μL at 90 °C for 10 min. The reaction mixture was adjusted to pH 8 with 1 N CH_3COOH and then treated with 2.5 units of calf intestine alkaline phosphatase at 25 °C for 15 min. HPLC analysis indicated quantitative conversion to CpG (Figure 8).

Detection of Dinucleotides **1**, **13**, *and CpG(p) following Digestion of d(CGCTTTAAAGCG) with Fe(II)·BLM A_2.* Reaction mixtures (100-μL total volume) contained 50 mM sodium cacodylate, pH 7.0, d(CGCTTTAAAGCG) (1 mM final nucleotide concentration), and 0.5 mM Fe(II)·BLM A_2 or Fe(II)·decarbamoyl-BLM A_2. The reaction was initiated by the addition of freshly prepared $Fe^{II}(NH_4)_2(SO_4)_2$, and the reaction mixture was incubated at 0 °C for 15 min.

For detection of dinucleotide **1**, the reaction mixture was treated with 0.2 N NaOH (final concentration) at 90 °C for 10 min. HPLC analysis indicated the formation of 2.8 μM dinucleotide **1** and 4.1 μM CpGp (the latter detectable as CpG after alkaline phosphatase treatment).

For detection of CpG, the reaction mixture was treated with 0.2 N *n*-butylamine at 90 °C for 10 min, adjusted to pH 8 with 1 N CH_3COOH, and then treated with 2.5 units of calf intestine alkaline phosphatase at 25 °C for 15 min. HPLC analysis (Figure 9) indicated the formation of 7 μM CpG.

For detection of dinucleotide **13**, the reaction mixture was treated with 5 μL of 1 M NH_2NH_2·HCl (pH ~8), and the combined solution was maintained at 0 °C for 15 min prior to HPLC analysis. Approximately 7 μM dinucleotide **13** was found (Figure 7).

RESULTS

To permit structural analysis of the alkali-labile lesion formed concomitant with DNA degradation by Fe(II)·BLM, we have employed the self-complementary DNA oligonucleotide d($^{5'}$CGCTTTAAAGCG$^{3'}$), which has been shown to contain a bleomycin cleavage site at cytidine-3 (Sugiyama et al., 1985b). It was anticipated that subsequent base-induced cleavage of the formed alkali-labile lesion at position 3 would leave a dinucleotide of the form CpGpx, which would be amenable to structure elucidation (Schemes I and II).

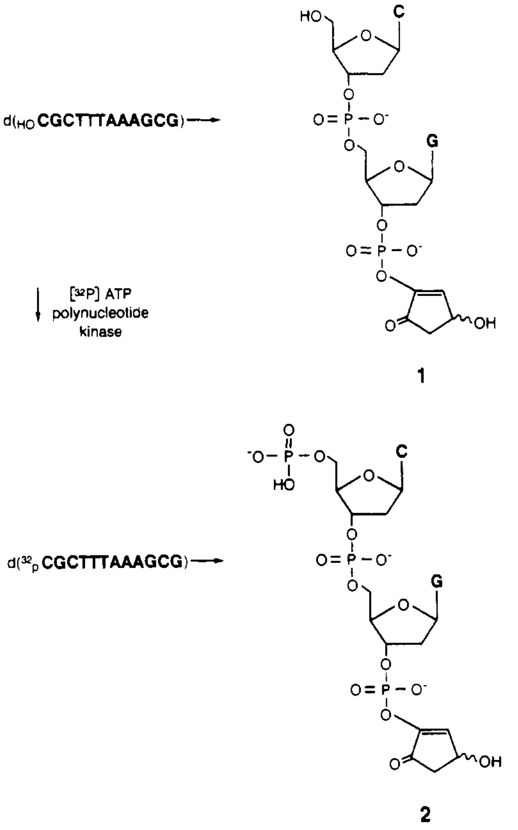
Several nucleotide derivatives were prepared to facilitate a study of the structure and chemistry of the alkali-labile lesion resulting from the degradation of this DNA oligomer with Fe(II)·BLM A_2. The key synthetic intermediate was dinucleotide **9**, which was prepared from 2,5-dimethoxy-2,5-dihydrofurfuryl alcohol (Achmatowicz et al., 1971) and commercially available 5′-*O*-(dimethoxytrityl)-*N*-isobutyryl-2′-deoxyguanosine (**3**) in ~6% overall yield (**3** → **4** → **5** → **7**

Table I: ^{1}H NMR Chemical Shift Data for 2,4-Dihydroxycyclopentenone Derivatives **1** and **12**[a]

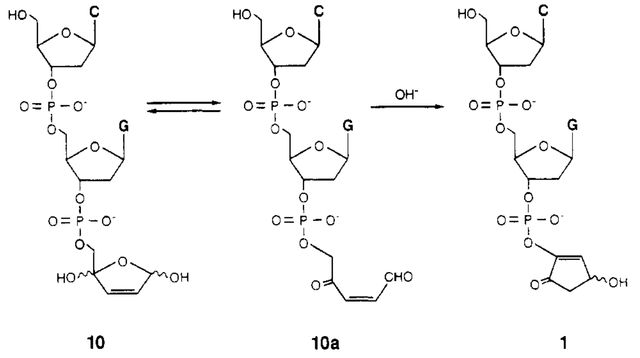
	protons (δ)								
compound	aromatic	H-1′	H-2′	H-3′	H-4′	H-5′	H-3″	H-4″	H-5″
1, dCp-	5.84 (H-5), 7.48 (H-6)	5.93	1.55, 2.19	4.44	3.91	3.48			
1, -pdGp	7.91 (H-8)	6.09	2.52, 2.87	4.96	4.23	3.91			
hydroxycyclopentenone							6.77	4.86	2.19, 2.87
12, $CH_3C_6H_4$-	2.46 (CH_3), 7.37, 7.86 (C_6H_4)								
hydroxycyclopentenone							7.30	5.01	2.30, 2.82

[a] Obtained at 360 MHz in D_2O (**1**) or $CDCl_3$ (**12**).

Scheme II: Dinucleotide Analogues Resulting from the Degradation of d(CGCTTTAAAGCG) and d(pCGCTTTAAAGCG) with Fe·BLM and Then with Alkali



Scheme III: Formation of Diastereomeric Dinucleotides **1** from **10** (**10a**)



→ **8** → **9**). Compound **9** was characterized by positive and negative ion FAB mass spectrometry and by analysis of its 360-MHz ^{1}H NMR spectrum.

Treatment of dinucleotide **9** with 0.1 N HCl at room temperature effected its efficient conversion to putative dinucleotide **10** (Figure 1). Although **10** proved to be unstable, as anticipated, it could be converted readily to stable deviatives amenable to characterization, from which its intermediacy was inferred. For example, treatment of **10** ($\rightleftharpoons$**10a**) with alkali at elevated temperature (60 or 90 °C for 2–10 min) resulted

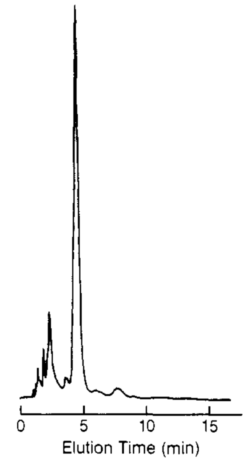


FIGURE 1: Analysis of dinucleotide **10** by reverse-phase HPLC. The sample was analyzed on a Rainin Microsorb Short-One C_{18} column (monitored by A_{254}), eluting with 0.2 M ammonium formate containing 2.5% acetonitrile, at a flow rate of 1.5 mL/min.

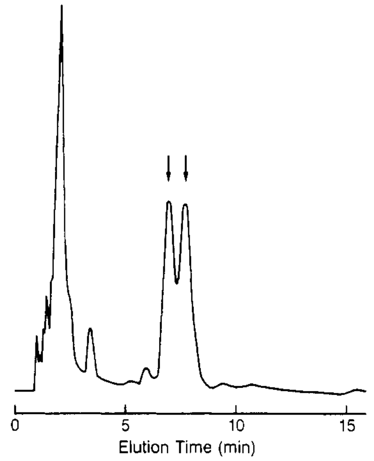


FIGURE 2: Analysis of diastereomeric dinucleotides **1** by reverse-phase HPLC; the analysis was carried out as described in the legend to Figure 1.

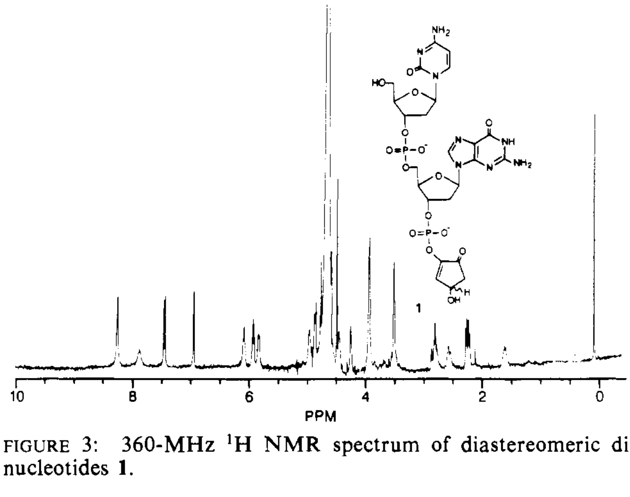


FIGURE 3: 360-MHz ^{1}H NMR spectrum of diastereomeric dinucleotides **1**.

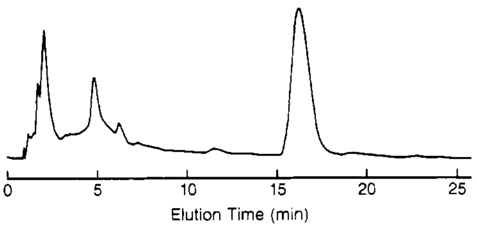


FIGURE 4: Analysis of synthetic dinucleotide **13** by reverse-phase HPLC. HPLC analysis was carried out as described in the legend to Figure 1. Dinucleotide **13** eluted at 16.5 min under these conditions, well separated from the other DNA degradation products.

in the formation of dinucleotide **1** in ~70% yield (Scheme III). Dinucleotide **1** was partially resolved into its diastereomeric components by reverse-phase HPLC (Figure 2). The mixture of diastereomers was characterized by its UV, 360-MHz ^{1}H NMR (Figure 3, Table I) and FAB mass spectra. Complete assignment of resonances in the ^{1}H NMR spectrum of **1** was facilitated by analysis of the ^{1}H NMR spectrum of the structurally simpler model compound 2,4-dihydroxycyclopentenone 2-*O*-tosylate (**12**). Dinucleotide **10** was also converted to a pyridazine derivative (**13**) by brief treatment with hydrazine. Dinucleotide **13** was shown to be stable to the conditions employed here for derivatization of BLM-damaged DNA, including heat, aqueous mineral acid and base, and *n*-butylamine (vide infra); its characterization included HPLC analysis (Figure 4), positive and negative ion FAB mass spectrometry, and high-field ^{1}H NMR spectroscopy. The NMR spectrum could be assigned completely, including the resonances corresponding to the pyridazine moiety itself [δ 5.10 (d, 2, $J = 8.5$ Hz, C^3), 7.83 (d, 1, C-4), 7.71 (m, 1, C-5), and 8.96 (d, 1, C-6)].

Also prepared for comparative purposes (Scheme II) was dinucleotide **2**, the 3′-phosphate derivative of **1**. This compound was obtained both by enzymatic phosphorylation of **1** with T4 polynucleotide kinase and by phosphorylation of **9** prior to treatment with acid and base (i.e., **9** → **14** → **2**). That both pathways provided the same product was verified by HPLC analysis under several conditions.

Degradation of the dodecanucleotides d(CGCTTTAAAGCG) and d(pCGCTTTAAAGCG) was carried out with 83 μM concentrations of the oligomers and 250 μM "activated" Fe·BLM A$_2$ (Sugiyama et al., 1985b) over a period of 15 min. Subsequent treatment with 0.2 N NaOH at 90 °C for several minutes effected cleavage of the alkali-labile lesions; the products of these reactions were analyzed by anion-exchange and C$_{18}$ reverse-phase HPLC in comparison with authentic synthetic samples. As shown in Figure 5, degradation of d(CGCTTTAAAGCG) with Fe·BLM A$_2$ and alkali gave several products, one of which comigrated with authentic 3′-(3-hydroxy-5-oxo-1-cyclopenten-1-yl) 2′-deoxy-5′-*O*-phosphonocytidylyl-(3′→5′)-2′-deoxy-3′-guanylate (**1**) when analyzed by anion-exchange HPLC. Although the diastereomers of **1** could not be separated by anion-exchange HPLC, their partial resolution was possible on a C$_{18}$ reverse-phase HPLC column; as illustrated previously (Sugiyama et al., 1985c) these two diastereomers comigrated with authentic synthetic samples. Because the primary site of d(CGCTTTAAAGCG) degradation by Fe·BLM A$_2$ is cytidine-11, rather than cytidine-3, we also effected degradation of this dodecanucleotide with a bleomycin congener whose preferred site of oligonucleotide degradation was cytidine-3 (Sugiyama et al., 1986); in this way a better yield of diastereomers **1** was anticipated (cf. Scheme I). As shown in Figure 6, HPLC analysis of a sample of d(CGCTTTAAAGCG) digested successively with Fe(II)·decarbamoyl-BLM A$_2$ and alkali gave greatly enhanced peaks (cf. Figure 1; Sugiyama et al., 1985c) having the same chromatographic properties as the authentic diastereomers of **1**. Degradation of 5′-^{32}P-labeled d(pCGCTTTAAAGCG) with Fe(II)·BLM A$_2$ and alkali provided 5′-^{32}P end-labeled material that comigrated with authentic dinucleotide **2** when analyzed by anion-exchange HPLC (data not shown).

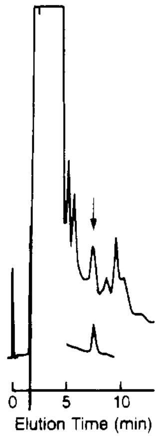


FIGURE 5: Analysis of the products formed from d(CGCTTTAAAGCG) following successive treatments with Fe(II)·BLM A$_2$ and alkali. The arrow indicates the position of elution of a peak that comigrated with authentic dinucleotide **1** (inset) when analyzed by anion-exchange HPLC. Sample analysis was carried out on a 25-cm Du Pont Zorbax Sax anion-exchange column; elution was with 0.05 M ammonium phosphate buffer, pH 4.5, at a flow rate of 2 mL/min (monitored at 254 nm).

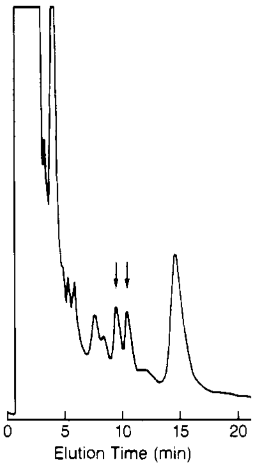


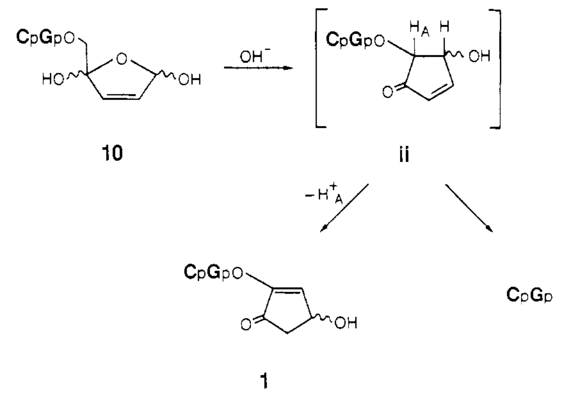
FIGURE 6: Analysis of the products formed from d(CGCTTTAAAGCG) following successive treatments with Fe(II)·decarbamoyl-BLM A$_2$ and alkali. The peaks indicated by the arrows comigrated with authentic diastereomeric dinucleotides **1** when analyzed by reverse-phase HPLC (under the same conditions as indicated in the legend to Figure 1).

Also investigated was the formation of a dinucleotide identical with **13** upon treatment of d(CGCTTTAAAGCG) successively with Fe(II)·BLM A$_2$ and hydrazine. As shown in Figure 7, this treatment resulted in the formation of a product that comigrated with authentic dinucleotide **13**.

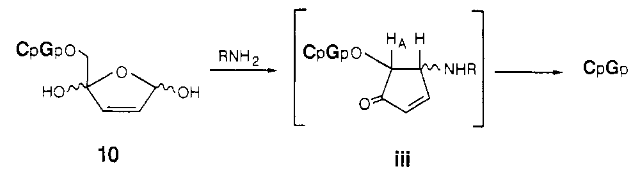
It was found that, in addition to dinucleotide **1**, treatment of **10** with alkali at high temperature produced CpGp as a reaction product in low yield; repetition of the experiment several times demonstrated considerable variability in the amount of CpGp produced. Mechanistically, this material could arise as shown in Scheme IV by tautomeric rearrangement of intermediate ii, with concomitant elimination of CpGp. To test this hypothesis, we employed *n*-butylamine, rather than alkali, for the decomposition of the alkali-labile lesion in the belief that a modified intermediate (iii, Scheme

FIGURE 7: Analysis of the products formed from d(CGCTTTAAAGCG) following successive treatments with Fe(II)·BLM A_2 and hydrazine. The sample was analyzed on a reverse-phase HPLC column as described in the legend to Figure 1; the product that eluted at 15 min (arrow) was shown to comigrate with authentic dinucleotide **13**.

Scheme IV: Possible Mechanism for the Observed Formation of Dinucleotide **1** and CpGp from Dinucleotide **10**



Scheme V: Formation of CpGp from Dinucleotide **10** via Putative Intermediate iii



V) would lead primarily to CpGp. As shown in Figure 8, treatment of dinucleotide **10** with 0.2 N *n*-butylamine at 90 °C for 10 min effected its quantitative conversion to CpGp; the latter was detected as CpG following digestion with calf intestine alkaline phosphatase. Application of this chemistry to Fe(II)·BLM A_2 treated d(CGCTTTAAAGCG) resulted in the formation of CpGp at the expense of dinucleotide **1** (Figure 9; Scheme VI).

The transformations studied to characterize the alkali-labile lesion i formed at cytidine-3 of d(CGCTTTAAAGCG) are summarized in Scheme VI.

DISCUSSION

Bleomycin-mediated DNA degradation results in the formation of a number of products, whose identity has been studied in several laboratories. These include free bases and base propenals derived from each of the four constituent nucleotides of DNA. Because they are formed as monomeric, easily separable products of low molecular weight from all natural and synthetic DNA substrates, these species were identified fairly readily. Characterization of the bases was accomplished by direct comparison with authentic samples (Kuramochi et al., 1981; Burger et al., 1982; Wu et al., 1983); the structures of the base propenals were established both by comparison with synthetic samples (Giloni et al., 1981; Johnson et al., 1984, Murugesan et al., 1985) and by acid-catalyzed

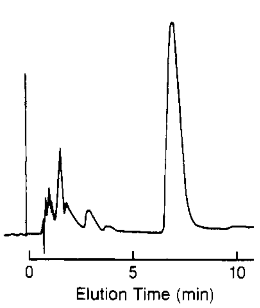


FIGURE 8: Conversion of dinucleotide **10** to CpGp via the agency of *n*-butylamine. The product was analyzed by reverse-phase HPLC (Rainin Microsorb Short-One C_{18} column, 0.2 M ammonium formate, pH 7.0, containing 1.5% CH_3CN, at a flow rate of 1.5 mL/min) following conversion to CpG.

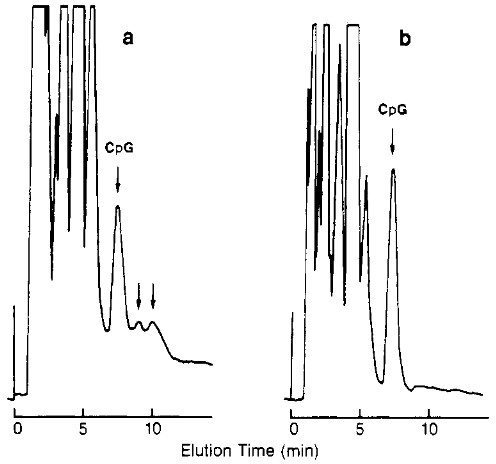
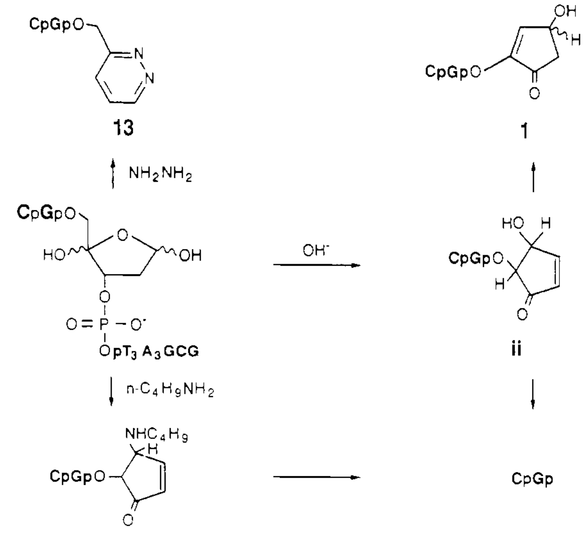


FIGURE 9: Analysis of the products formed from d(CGCTTTAAAGCG) following successive treatments with Fe(II)·BLM A_2 and either 0.2 N NaOH (panel a) or 0.2 N *n*-butylamine (panel b) at 90 °C for 10 min. Analysis by reverse-phase HPLC (see legend to Figure 8) indicated the formation of CpGp (analyzed as CpG following treatment with alkaline phosphatase; 7.2-min retention time) and diastereomeric dinucleotides **1** (arrows in panel a; retention times 8.6 and 9.6 min).

Scheme VI: Derivation of Derivatives **13**, **1**, and CpGp from the Alkaline-Labile Product Formed at Cytidine-3 of d(CGCTTTAAAGCG)



decomposition to free bases and malondialdehyde, the latter of which was detected as its thiobarbituric acid (Burger et al., 1980) and phenylhydrazine (Giloni et al., 1981) adducts. Murugesan et al. (1985) prepared the cis and trans isomers of 3-(adenin-9′-yl)propenal and 3-(thymin-1′-yl)propenal and demonstrated that the trans isomers were the exclusive products of Fe(II)·BLM-mediated DNA strand scission.

Also formed in parallel with free bases and base propenals were oligonucleotides of altered structure. Because these alterations occurred at a number of sites within any sizable DNA substrate, involved more than one type of chemical modification of DNA at any given site, and provided oligomeric products of varying size and abundance that were difficult to separate and analyze, these products proved more difficult to characterize initially. One type of product, whose formation involved DNA strand scission and the concomitant formation of a base propenal, was shown to be an oligomer having a 3′-(phosphoro-2″-*O*-glycolate) at its 3′-terminus. The structure of this species was deduced initially by use of substrate DNA radiolabeled at specific positions in the deoxyribose moiety and by the observation that further treatment of BLM-digested oligonucleotides with appropriate enzymes or strong acid effected the release of glycolic acid (Giloni et al., 1981). More direct evidence was later obtained by sequential treatments of DNA with Fe(II)·BLM and λ exonuclease, which produced the four possible nucleotide 3′-(phosphoro-2″-*O*-glycolate) derivatives; these were shown to be identical with authentic synthetic samples (Murugesan et al., 1985). Finally, by use of oligonucleotides of defined structure having a limited number of bleomycin cleavage sites, it has been possible to effect reasonably efficient conversion directly to dinucleotides terminating with a 3′-(phosphoro-2″-*O*-glycolate) moiety; the products have subsequently been characterized by spectral means and by comparison with authentic synthetic samples (Uesugi et al., 1984; Murugesan et al., 1985).

One additional Fe(II)·BLM-mediated alteration of DNA structure has been noted (Hecht, 1979; Burger et al., 1982; Wu et al., 1983; Sugiyama et al., 1985c), namely, the alkali-labile lesion that accompanies the formation of free base. This alteration per se does not result in DNA strand scission, but does produce a chemically sensitive lesion whose manipulation affords complicated mixtures of products, some of which are also unstable. Therefore, its structural characterization proved quite difficult. Successful resolution of the problem was achieved by the use of the self-complementary oligonucleotide d(CGCTTTAAAGCG), which is modified by Fe(II)·BLM predominantly at the double-stranded $^{5'}$-GC-$^{3'}$ site (Sugiyama et al., 1985b, 1986). The formed alkali-labile lesions were treated with base, yielding as a major reaction product a dinucleotide of the form CpGpx; it was anticipated that structure elucidation of this dinucleotide would permit inferences to be drawn concerning the nature of the alkali-labile lesion itself. In the belief that the alkali-labile lesion might result from C-4′ hydroxylation of the ribose moiety in DNA, which should lead to the observed release of free base and generation of a C-4′ hydroxyapurinic acid (i, Scheme I), we prepared dinucleotide derivatives of the form CpGpx whose formation from the putative C-4′ hydroxyapurinic acid intermediate could be envisioned under alkaline conditions. In this fashion, the product derived from the alkali-labile lesion at cytidine-3 was shown to be identical with diastereomeric dinucleotides **1** (Sugiyama et al., 1985c). Because the formation of **1** from the alkali-labile lesion must involve a rearrangement of the apurinic acid moiety, we have carried out additional chemical transformations to help clarify the mode of formation of **1** and the structure of the alkali-labile lesion from which it derives.

Since the base-catalyzed decomposition of alkali-labile lesion i (Scheme I) might reasonably be thought to involve initial elimination of the 3′-phosphate ester, dinucleotide **10** was prepared as described (vide supra) and characterized by HPLC (Figure 1). Synthesis of this dinucleotide permitted direct verification of its anticipated instability in neutral, aqueous solution; the ready conversion of **10** to **1** under alkaline conditions (~70% yield) provided a sample of diastereomeric dinucleotides **1** for analysis of Fe(II)·BLM-mediated events. Consistent with our earlier report (Sugiyama et al., 1985c), the formation of **1** from d(CGCTTTAAAGCG) as a consequence of successive treatments with Fe(II)·BLM A_2 and base was verified by anion-exchange (Figure 5) and reverse-phase HPLC (Figure 9a). Fe(II)·decarbamoyl-BLM A_2, which cleaves d(CGCTTTAAAGCG) predominantly at cytidine-3 (Sugiyama et al., 1986), also produced **1** in much greater amounts, as expected (Figure 6). Further, Fe(II)·BLM A_2 mediated degradation of d(pCGCTTTAAAGCG) provided products identical with diastereomic dinucleotides **2** (i.e., with the 5′-phosphates of **1**). Degradation of d-(CGCTTTAAAGCG) in the presence of Fe·BLM and ascorbate (Sugiyama et al., 1986) resulted in greater amounts of oligonucleotide degradation but did not alter the nature of the products formed.

The alkali-labile lesion was characterized further by treatment of i (Scheme I) with hydrazine, which effected its facile (0 °C, 15 min) conversion to a product identical with dinucleotide **13** in quantitative yield. The ready formation of this pyridazine derivative is consistent with the behavior expected of an alkali-labile lesion having structure i. Further, since formation of this derivative is not envisioned as involving rearrangement of the deoxyribose moiety, it helps to establish the connectivity of carbon atoms in the alkali-labile lesion. Further support for the connectivity of atoms, and for the structure of the alkali-labile lesion itself, derives from the recent report of Rabow et al. (1986), who characterized the alkali-labile lesion derived from cytidine-5 in d(CGCGCG) following additional reduction with sodium borohydride and digestion with P_1 nuclease and alkaline phosphatase.

Analysis of Fe·BLM-treated 5′-^{32}P end-labeled DNA on polyacrylamide gels has been observed to give multiple bands at each nucleotide site cleaved (D'Andrea & Haseltine, 1978; Kross et al., 1982). The DNA fragments that produce these bands were thought to terminate with functionalities other than 3′-phosphates as none comigrated with DNA fragments produced by the Maxam–Gilbert technique (D'Andrea & Haseltine, 1978; Takeshita et al., 1978), the latter of which do terminate with 3′-phosphate groups (Maxam & Gilbert, 1977). Interestingly, we found that successive treatments of 5′-^{32}P end-labeled DNA with Fe·BLM and alkali resulted in the appearance of bands that did comigrate with Maxam–Gilbert DNA fragments, suggesting the production of oligomers having 3′-phosphates from alkali-labile lesions (H. Sugiyama and S. M. Hecht, unpublished results).

In an effort to define the chemistry that led to production of these new fragments, we studied the alkai-mediated decomposition of dinucleotide **10**. Alkali treatment of **10** was found to produce a product that comigrated with CpGp in low and variable yields (Scheme IV). To enhance the yield of CpGp and provide additional evidence for its derivation from a cyclopentenone intermediate, we incubated samples of **10** with amines in the belief that the formed intermediate (iii, Scheme V) would decompose selectively to CpGp. It was observed that incubation of **10** in aqueous *n*-butylamine (90 °C, 10 min) effected its conversion to CpGp in essentially quantitative yield. The same transformation was noted when piperidine or ammonium hydroxide was employed, albeit in slightly lower (~80%) yield.

That dinucleotide **1** and CpGp were both produced from alkali-labile lesion i (Scheme I) was further supported by an experiment in which samples of Fe(II)·BLM-digested d-(CGCTTTAAAGCG) were further treated with 0.2 N NaOH (Figure 9a) or 0.2 N *n*-butylamine (Figure 9b). As shown, significant amounts of product that comigrated with CpGp were produced under both conditions in this experiment, but *n*-butylamine clearly produced CpGp in enhanced yield at the expense of diastereomeric dinucleotides **1**. Although it is an obvious consequence of the chemistry illustrated here, it may nonetheless be worth noting that nucleophilic substituents in macromolecules that interact with DNA (e.g., nuclear proteins) could similarly enhance BLM-mediated DNA cleavage via reaction with alkali-labile lesions. The present findings suggest that this type of process could also result in structural modification of the DNA binding proteins and conceivably in cross-linking of the proteins to DNA (cf. Schemes IV–VI).

The intermediacy of ii in the production of dinucleotide **1** from the alkali-labile lesion i (Schemes I, IV, and VI) is well precedented chemically (Büchi et al., 1971; Stork et al., 1975; Floyd, 1978) and provides support for the overall mechanistic scheme presented. The use of an alkylamine to control the mode of collapse of ii (iii) as shown in Scheme IV is novel and suggests some interesting opportunities for the manipulation of nucleic acid structure. In this context, it may be noted that a number of workers have suggested the use of BLM as a DNA sequencing agent. While the sequence selectivity of BLM itself may not be great enough to permit its use as a primary determinant of sequence, the use of alkylamines for the decomposition of the alkali-labile lesion produces a product identical with that formed during Maxam–Gilbert sequencing (Maxam & Gilbert, 1977, 1980) and should facilitate the use of BLM, or modified bleomycins, for analysis of DNA structure.

It is interesting that both sets of products resulting from Fe·BLM-mediated DNA degradation appear to involve initial modification of the C-4′ position of deoxyribose in DNA. One set of products, leading to DNA strand scission and the formation of base propenals and oligonucleotides terminating with 3′-(phosphoro-2″-*O*-glycolate) moieties, can be envisioned as arising from a C-4′ hydroperoxy derivative of deoxyribose (Hecht, 1986a,b). The other products, investigated in detail here, are the alkali-labile lesion and free bases; these would seem to be derived from C-4′ hydroxy derivatives of deoxyribose. The possibility that these two sets of products both derive from some common DNA intermediate has been explored in some detail. Evidence in support of this possibility includes the observed O_2 dependence of product formation (Burger et al., 1982; Wu et al., 1985a) and studies that have employed a synthetic analogue of DNA radiolabeled with ^3H at C-4′ of deoxyribose (Wu et al., 1983, 1985a,b). The possibility that some component of product formation may also be a function of the existence of more than one form of activated bleomycin also seems worthy of consideration, especially in view of the report (Ekimoto et al., 1985) that the ability of bleomycin to mediate lipid peroxidation is altered as a function of time following bleomycin activation.

As noted above, both sets of products formed from DNA by Fe·BLM involve the net removal of C-4′ H from deoxyribose. If C–H bond breakage is mediated directly by activated Fe·BLM, then it would be logical to expect the putative "metal-oxo" center (Hecht, 1986a,b) to reside in proximity to C-4′ H during the process of bond breakage, i.e., in the minor groove of DNA.

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Registry No. **1** (diastereomer 1), 111137-63-4; **1** (diastereomer 2), 111137-64-5; **2**, 111026-32-5; **3**, 107514-63-6; **4**, 111026-33-6; **5**, 111026-34-7; **6**, 67219-55-0; **7**, 111059-93-9; **8**, 111059-94-0; **9**, 96914-13-5; **10**, 96914-12-4; **11**, 111026-35-8; **12**, 111026-36-9; **13**, 111026-37-0; **14**, 111026-38-1; decarbamoyl-BLM, 106064-23-7; CpG(p), 7600-33-1; 5′-d(CGCTTTAAAGCG), 97920-47-3; 5′-^{32}P-d(CGCTTTAAAGCG), 111026-39-2; $MePOCl_2$, 3279-26-3; NH_2Bu, 109-73-9; NH_2NH_2, 302-01-2; spermidine, 124-20-9; 2,5-dimethoxy-2,5-dihydrofurfuryl alcohol, 19969-71-2.

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Sequence-Influenced Interactions of Oligoacridines with DNA Detected by Retarded Gel Electrophoretic Migrations[†]

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ABSTRACT: We have found that di-, tri-, tetra-, and hexa-9-acridinylamines are so efficiently associated with DNA during electrophoresis in polyacrylamide or agarose gels that they retard its migration. The retardation is roughly proportional to the reagent to base pair ratio, and the magnitude of the retardation indicates that a combined charge neutralization/helix extension mechanism is mainly responsible for the effect. Furthermore, DNA sequence dependent differences are observed. Thus, the pUC 19 restriction fragments (*Hae*III or *Alu*I), which in the native state comigrate upon gel electrophoretic analysis, could be separated in the presence of a diacridine, and specific DNA fragments responded differently to different diacridines. These results suggest that the effect also is due to a contribution from the DNA conformation and that the DNA conformation dynamics are influenced differently upon binding of different diacridines. We foresee three applications of this observation: (1) in analytical gel electrophoretic separation of otherwise comigrating DNA molecules, (2) in studies of polyintercalator–DNA interaction, and (3) in measurements of polyintercalator-induced DNA unwinding.

The development and DNA-binding properties of polyintercalating reagents have attracted much attention (Wakelin, 1986) due to the potentials of such compounds as chemotherapeutics (Atwell et al., 1986) as well as to the general interest in DNA–ligand (not least protein) interactions.

Anomalous electrophoretic migration of DNA fragments in polyacrylamide gels has recently been demonstrated as a sensitive measure of altered DNA conformations such as DNA

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