Design of a Cyclopropyl Quinone Methide Reductive Alkylating Agent

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Described herein is the formation of a cyclopropyl quinone methide species by leaving group elimination from an indole-based hydroquinone. This species is structurally related to the A-ring of CC-1065 and is a nucleophile trap. In addition, the cyclopropyl quinone methide species can trap or eliminate a proton. Unlike the A-ring of CC-1065, stereoelectronic factors favor opening of the fused cyclopropane ring so as to result in ring expansion. Also described herein is the utility of ^{13}C NMR spectroscopy in following the fate of the cyclopropyl quinone methide species. Finally, the cyclopropyl quinone methide species reversibly alkylates the guanine N(7) position of DNA, resulting in cytotoxicity.

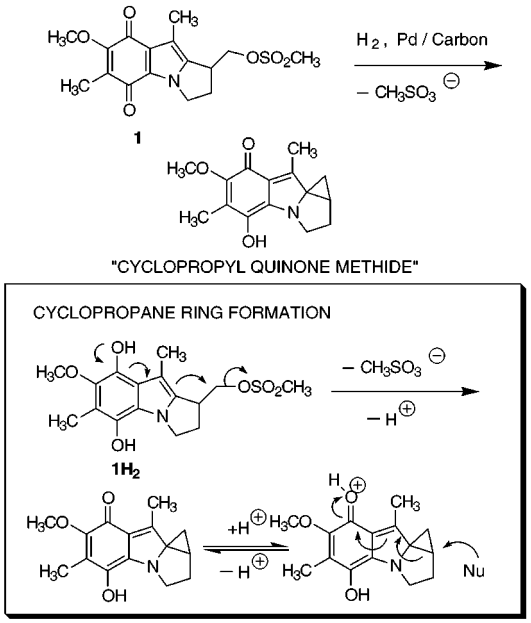
Introduction

Quinone methide chemistry is of general interest since many naturally occurring quinones[1,2] and synthetic analogues[3−8] can form the quinone methide species upon two-electron reduction and leaving-group elimination. Quinone methide species can alkylate DNA,[1,2] and the design of these reactive species is therefore relevent to the design of new antitumor agents.[9−11] We wondered if a cyclopropyl quinone methide could form upon reductive activation of a quinone as illustrated in Scheme 1 for **1**. Elimination of the methanesulfonate leaving group from reduced **1** (**1H$_2$**) would afford a cyclopropane derivative as illustrated in the inset of Scheme 1.

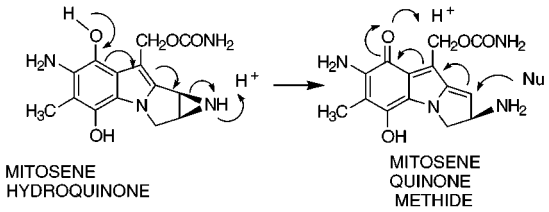
The inspiration for the cyclopropyl quinone methide design came from the mitosenes[12−14] and the A-ring of CC-1065,[15] Schemes 2 and 3. As illustrated in Scheme 2, the mitosene hydroquinone affords the quinone methide by elimination of the protonated aziridinyl nitrogen. Compound **1H$_2$** is a mitosene-like hydroquinone that can eliminate the leaving group only by formation of a cyclopropane ring. The mitosene quinone methide is an alkylating agent of DNA, and the cyclopropyl quinone methide should do likewise based on reactivity of the A-ring of CC-1065, Scheme 3. Carbonyl oxygen protonation of the A-ring prompts nucleophile attack at the least-substituted carbon of the cyclopropane ring. Furthermore, natural products found in bracken[16,17] are well-known precedents of cyclopropane-based alkylating agents.

Previous studies in this laboratory explored the feasibility of cyclopropyl quinone methide formation from

Scheme 1



Scheme 2



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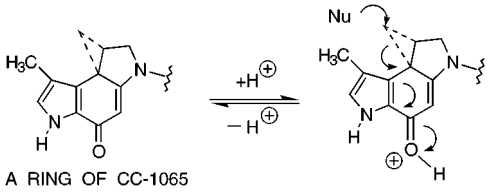
Scheme 3



A RING OF CC-1065

quinazoline-[18] and benzimidazole-based[19] systems. These systems merely underwent elimination of the leaving group from a tautomer intermediate to afford an alkene. In contrast, the indole derivative **1H$_2$** cannot tautomerize in the same way, and therefore, the postulated cyclopropane derivative can form in solution. This paper discusses the preparation, fate, DNA alkylation properties, and cytotoxicity of the cyclopropyl quinone methide species shown in Scheme 1. Also discussed is the utility of ^{13}C NMR spectroscopy in the elucidation of the cyclopropyl quinone methide rearrangements and DNA alkylation chemistry.

Synthesis of 1. This synthesis started with an indole **2** previously prepared in the laboratory,[20] Scheme 4. The desired cyano derivative **3** was prepared in one step from **2** employing the tosylmethyl isocyanide anion[21,22] followed by aqueous workup. The nitrile was then converted to the ester **4** by alcoholysis in methanol/HCl.[23] Selective reduction of the ester of **4** to the hydroxymethyl derivative **5** was carried out with lithium aluminum hydride. Catalytic reduction of the nitro group to an amine followed by Fremy oxidation[24] afforded **6**, which was treated with methanesulfonyl chloride/pyridine to afford **1**. To prepare the ^{13}C analogues required for the hydrolysis and DNA alkylation studies, the cyano analogue **3** was exchanged with Na^{13}CN in DMSO. After one such exchange, incorporation of ^{13}C in **3** was about 50%.

Solvolysis Studies of 1H$_2$. These studies provided evidence for the formation of the cyclopropyl quinone methide species shown in Scheme 1. Product studies revealed that this reactive species has three possible fates: a prototropic shift resulting in alkene ring formation, a prototropic shift resulting in alkane ring formation, and nucleophile trapping. These reactions, with some exceptions, resulted in ring expansion to afford a six-membered ring.

The first step in the solvolytic study was to determine the presence of reduction side products when **1** is converted to **1H$_2$** in the presence of 5% Pd on carbon and hydrogen. When an aqueous buffer was the solvent, reduction of **1** followed by immediate oxidative workup afforded only **1**. When methanol was the solvent, a trace amount of **7** (Scheme 5) was obtained along with **1**. Therefore, the reduction step itself did not form any of the observed aqueous solvolysis products, but this step may be responsible for at least one of the methanol solvolysis products.

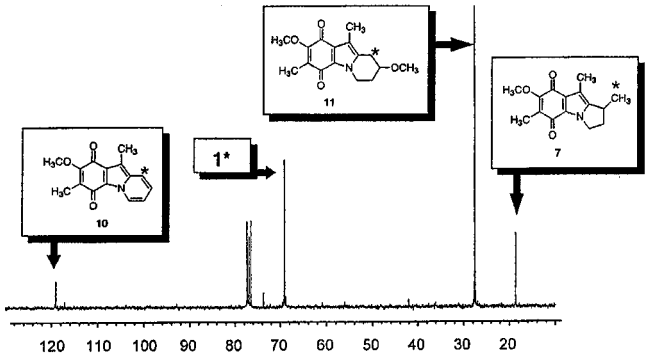


Figure 1. ^{13}C NMR spectrum of a $CDCl_3$ solution of the reaction mixture obtained from the incubation of **1H$_2$** in anaerobic methanol at 30 °C for 24 h.

Reduction of **1** in methanol followed by anaerobic incubation for 24 h and then aerobic workup afforded the five products shown in Scheme 5 along with unreacted **1** for a material balance of 80%.

The solvolysis reaction was also carried out with ^{13}C-labeled **1*** and a ^{13}C NMR spectrum obtained for the crude organic extract. This ^{13}C NMR spectrum, shown in Figure 1, reveals the presence of **10**, along with **9** as a slightly upfield resonance, as well as **1**, **11**, and **7**. The trace product **8** was not observed in the ^{13}C NMR spectrum. When the ^{13}C NMR spectrum was not noise decoupled, **7** appeared as a quartet (J = 127 Hz), **11** appeared as a triplet of doublets (J = 129, 4 Hz), **10** appeared as a doublet of doublets (J = 166, 8 Hz), and **1** appeared as a triplet of doublets (J = 151, 5 Hz). These ^{13}C assignments were confirmed by product isolation from the crude mixture and spectral identification; see the Experimental Section. The mass balance is 80%, and therefore, more products must be present. A close inspection of the ^{13}C NMR spectrum reveals the presence of a resonance at 74 ppm that may be the result of methanol trapping without ring expansion. In the nucleophile trapping experiments described below, we did observe the presence of **6**, which is the result of water trapping without ring expansion.

The ^{13}C NMR spectrum of Figure 1 shows that product analysis of cyclopropyl quinone methide rearrangements can be readily evaluated from the number of resonances (the number of solvolysis products) as well as from the chemical shifts and splitting patterns.

The origins of the products shown in Scheme 5 are discussed below in conjunction with Schemes 6 and 7.

Prototropic shifts in the cyclopropyl quinone methide to afford a fused alkene or alkane ring are illustrated in Scheme 6. One of the prototropic shift products, hydroquinone **12**, will be oxidized to **9** upon aerobic workup. Further oxidation of **9** to **10** should readily occur during aerobic workup because **10** is stabilized by aromaticity. In contrast, the prototropic shift to give the alkane ring results in formation of the quinone species directly without aerobic oxidation. Thus, anaerobic reaction mixtures containing **1H$_2$** become orange due to the formation of **7** and **8**. Trapping of the cyclopropyl quinone methide species by methanol affords the ring-expanded hydroquinone **13**, which is converted to **11** upon oxidative workup, Scheme 7. Nucleophile trapping by the cyclopropyl quinone methide species is largely accompanied by ring expansion. In contrast, the A-ring of CC-1065 traps nucleophiles without any ring expan-

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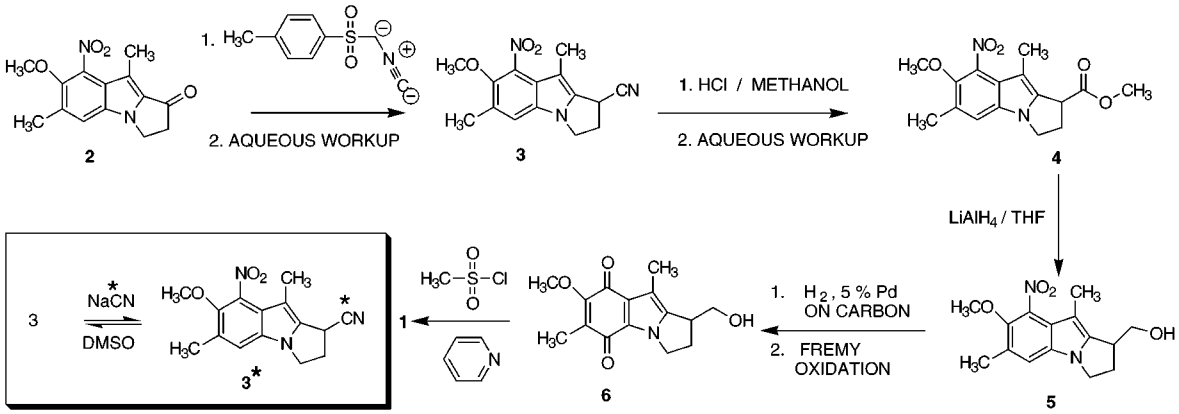
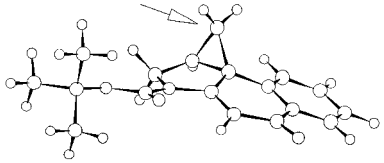
Scheme 4





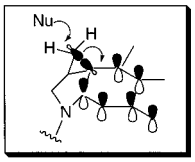
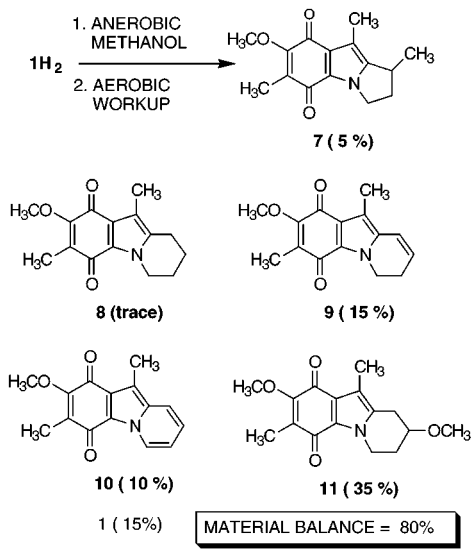


Figure 2. Minimized structures (Insight II using steepest descent algorithm) of a derivative of the A-ring of CC-1065[25] showing the preferred site of nucleophile attack with an arrow. The inset shows the breaking bond interacting with the π-system.

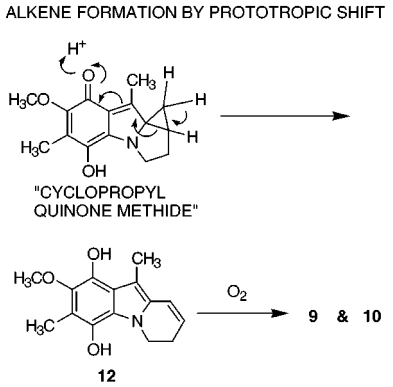
Scheme 5



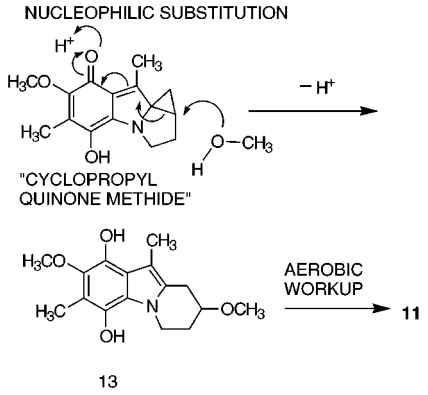
sion.[15,25] Comparison of the models (Insight II using steepest descent algorithm) of a derivative of the A-ring of CC-1065 from Boger and Johnson[25] with that of the cyclopropyl quinone methide revealed that both systems are under the same stereoelectronic control, Figures 2 and 3.

Nucleophilic attack of the CC-1065 A-ring at the least substituted carbon, indicated with the arrow in Figure 2, and subsequent bond breaking results in good overlap of the developing p-orbital with the π-system, inset of Figure 2. In contrast, attack at the more substituted

Scheme 6



Scheme 7



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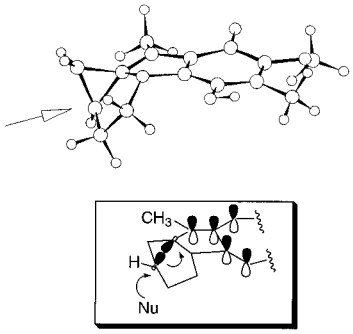
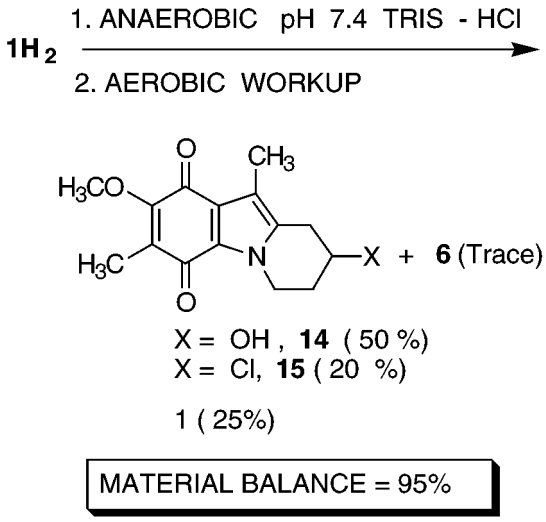


Figure 3. Minimized structures (Insight II using steepest descent algorithm) of the cyclopropyl quinone methide with an arrow showing the preferred site of nucleophile. The inset shows the breaking bond interacting with the π-system.

Scheme 8



carbon would result in p-orbital development orthogonal to the π-system. For the largest degree of overlap, the breaking bond should be nearly perpendicular to the π-system plane. Nucleophilic attack of the cyclopropyl quinone methide at the more substituted carbon, indicated with the arrow in Figure 3, results in excellent p-orbital overlap since the breaking bond is out of the π-system plane, inset of Figure 3. In fact, the breaking cyclopropane bond of the A-ring of CC-1065 is 45° out of this plane[25] and that of the cyclopropyl quinone methide is nearly the same. As a result of favorable stereoelectronic control, the cyclopropyl quinone methide species rapidly undergoes ring-expansion reactions without any detectable buildup.

Reaction conditions that favor nucleophile trapping are illustrated in Scheme 8. Incubation of **1H$_2$** in an anaerobic aqueous buffer (pH 7.4 Tris−HCl) followed by aerobic workup afforded **14** and **15**, along with unreacted **1**, as the major isolated products. The same solvolysis reaction with ^{13}C-labeled **1** was carried out, and a ^{13}C NMR spectrum of the unseparated products was obtained, Figure 4.

This ^{13}C NMR spectrum clearly shows the presence of the ring-expansion products **14** and **15** (upfield chemical shifts) along with the non-ring-expanded products **1** and **6** (downfield chemical shifts). The presence of **6** was not detected in the isolation study because it was a trace product. Its formation could arise by an S_N2 attack of water at the least hindered position of the cyclocopropyl

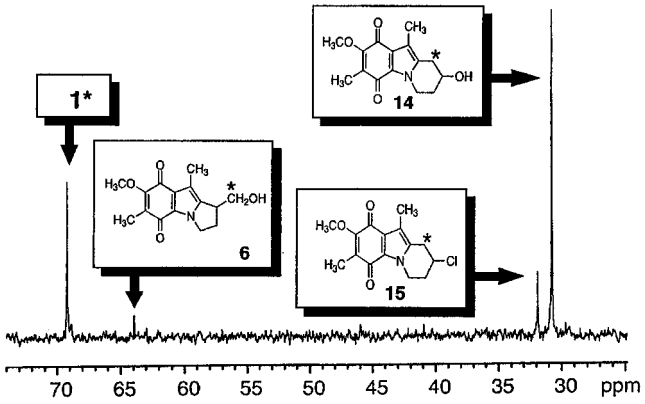


Figure 4. ^{13}C NMR spectrum of a $CDCl_3$ solution of the reaction mixture obtained from the incubation of **1H$_2$** in 0.05 M pH 7.4 tris buffer at 30 °C for 24 h.

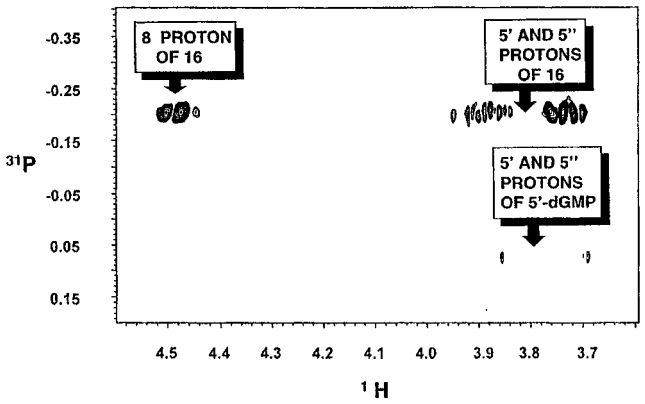


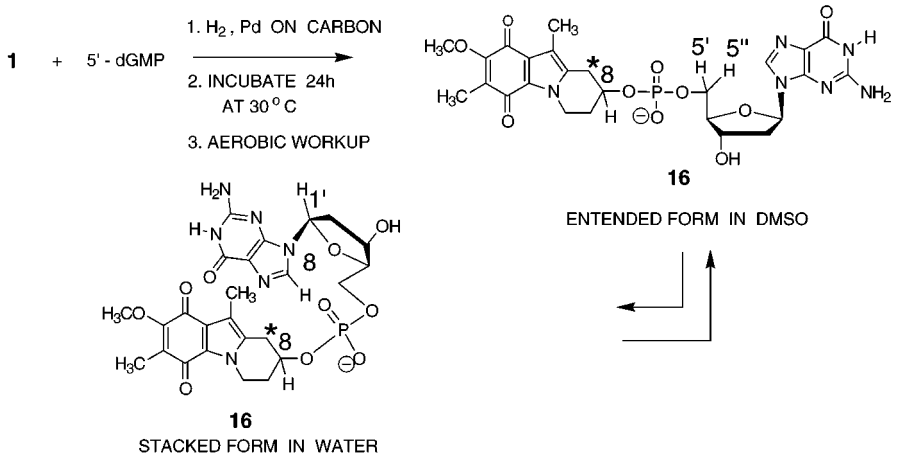
Figure 5. ^{1}H-detected ^{1}H−^{31}P correlation spectrum of **16** in DMSO-d_6 with $J = 20$ Hz. The spectrum also shows 5′-dGMP is present as a trace impurity.

quinone methide. Alternatively, **6** could have formed as a result of hydrolytic removal of the methanesulfonyl group of **1**. However, incubation studies of **1** in aqueous buffers, in the absence of reductive activation, revealed the absence of **6**. The findings cited above show that the ring expansion of the cyclopropyl quinone methide is the major nucleophile-trapping process.

The ring-expansion process is synthetically useful under the following conditions: Compound **14** can be obtained as the major product in 51−65% yield from **1** by reduction in aqueous buffer and incubation for 24 h at 30 °C followed by aerobic workup. The alkene product **9** was obtained as the minor product from these reactions; see the Experimental Section for details. A low yield of acetate trapping product was observed when an acetate buffer was employed. Similarly compound **15** can be obtained in high yield by the same procedure when the aqueous buffer is $\mu = 1.0$ with KCl.

Reductive Alkylation of Biological Nucleophiles. Biological nucleophiles studied as substrates for the cyclopropyl quinone methide include 5′-dGMP and DNAs. Reductive alkylation of 5′-dGMP at pH 7.4 afforded the phosphate adduct **16** shown in Scheme 9 as the sole product. The phosphate group is dianionic at this pH and readily traps the reactive species arising from the protonated cyclopropyl quinone methide. At pH 4.6, 5′-dGMP exists as the less nucleophilic phosphate monoanion and reductive alkylation by **1** does not occur, even at the reactive N(7) position.

Scheme 9



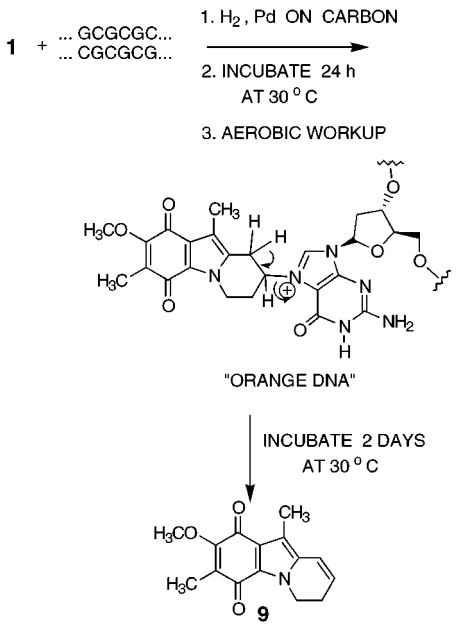
Confirmation of **16** as the correct structure of the reductive alkylation product of **1** with 5′-dGMP was arrived at from the NMR studies described below. Proton NMR shows one or two sets of resonances depending if DMSO-d_6 or D_2O are the respective solvents; see the Experimental Section. These results are due to stacking of the rings in water in contrast to the presence of the extended form in DMSO-d_6, Scheme 9.

Since racemic **1** and enantiomerically pure 5′-dGMP are employed in these studies, two diastereomers of **16** will result from phosphate alkylation. Stacking of the heterocyclic rings in water will bring the stereocenters close together, resulting in two sets of some proton resonances. For example, **16** shows one anomeric (C1′) triplet in DMSO-d_6 but two such triplets in D_2O. This stacking phenomenon was also observed with the 5′-dAMP PBI phosphate adducts.[26] Alternatively, the proton NMR results observed with **16** can be explained by preferential stacking of only one of the diastereomers.

Evidence for the formation of a phosphate adduct was obtained from a $^1H-^{31}P$ correlation spectrum[27] of **16**, Figure 3. This spectrum clearly shows the coupling of phosphate to the 5″ and 5′ protons of the 5′-dGMP part of **16** as well as the coupling of phosphate to the 8-proton of the pyridoindole ring of **16**. In contrast, 5′-dGMP, which happened to be present as a trace impurity in **16**, shows phosphate coupling only to the 5″ and 5′ protons. When ^{13}C-labeled **1** was employed for the reductive alkylation of 5′-dGMP, **16*** possessed a ^{13}C NMR chemical shift of 28.7 ppm that was split into a triplet with $J = 129$ Hz; see the structures in Scheme 9 for the location of the label. The ^{13}C NMR results indicate that nucleophile trapping was accompanied by ring expansion.

The alternating DNA polymers, poly[dG-dC]·poly[dG-dC] and poly[dA-dT]·poly[dA-dT] were treated with reduced **1** in anaerobic pH 7.4 tris buffer. The A−T alternating polymer was not reductively alkylated since the DNA isolated after the reaction had no visible spectrum; in contrast, the G−C alternating polymer was reductively alkylated to afford "orange DNA" upon aerobic workup. A visible spectrum of 2.6 mg of this DNA in water revealed that 2.4% of the G−C base pairs were alkylated, based on the $\lambda_{max} = 499$ and $\epsilon = 2000$ for the hydrolysis product **14** and the molecular weight of 663

Scheme 10



D for the GC base pair. The visible λ_{max} values for the alkylated DNA were 364 and 486 nm, which are blue shifted compared to **14** (374 and 499 nm) and **15** (376 and 490 nm). The explanation for this observation is that alkylation occurred to form an N(7) adduct of a guanine base as illustrated in Scheme 10.

The electron-deficient quarternary nitrogen of the adduct would result in the observed spectral shifts. This observation and the complete selectivity for G−C base pairs supports the formation of N(7)−guanine adducts. In fact, cyclopropane-based alkylating agents related to the bracken ultimate carcinogen[17] also react exclusively with the N(7) of guanine. When the "orange DNA" was subjected to mild hydrolysis at 30 °C for 2 days, a red precipitate was observed. The precipitate was collected as a pellet and analyzed by mass spectrometry, which indicated that the adduct was eliminated from DNA as **9**.

Cytotoxicity. The reversible nature of G−C reductive alkylation by **1** suggested that it would possess low cytoxicity but significant cytostatic effects. Thus, the log LC_{50} in the National Cancer Institutes GO-cell-line screen (concentration needed to bring about 50% cell kill)[28] possessed a medium value of −4.25. In contrast, the log

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GI_{50} (concentration needed to bring about 50% growth inhibition) was −5.51 and the medium log TGI (total growth inhibition) was −4.71 in the same screen. The cytostatic effect of **1** was principally observed in the renal cancer and melanoma panels, while other cancer types (leukemia, nonsmall cell lung cancer, colon, CNS, ovarian, prostrate, and breast) showed a higher value than the medium values (i.e., higher concentrations and therefore less active). "COMPARE" analysis[29] revealed that the TGI pattern in the 60-cell-line screen were substantially like that for mitomycin C, correlating coefficient = 0.634. This observation could be due to the reductive activation requirement of both agents. Mitomycin C is activated by the cellular enzyme DT-diaphorase[30,31] as are many other quinone-based antitumor agents.[32,33] The differential selectivity of **1** for melanoma and renal cancer is very likely related to the presence of DT-diaphorase in these cell lines.[34] The PBI reductive alkylating agents previously developed in this laboratory are activated by DT-diaphorase and likewise show a high level of activity against melanoma and renal cancer.

Conclusions

Evidence is provided via product studies that a cyclopropyl quinone methide species can form in solution and trap or lose a proton as well as trap a nucleophile. These reactions usually occur with ring expansion to afford a fused six-membered ring product. The present study also shows the utility of ^{13}C enrichment in studying the rearrangement products. By counting the number of ^{13}C NMR resonances, the number of unique products can be determined. Furthermore, the chemical shifts provide insight into the structure of these adducts. For example, the ^{13}C NMR study of the anaerobic hydrolysis of the cyclopropyl quinone methide (Figure 2) revealed the presence of a trace amount of **6**, which was the result of nucleophilic attack at the least substituted cyclopropane carbon.

The ring-expansion reactions described herein are synthetically useful under specific conditions (see the Experimental Section) and provide good yields of the hydroxy (**14**) and chloro (**15**) derivatives. This synthetic methodology represents a new approach to functionalized 6,7,8,9-tetrahydropyrido[1,2-*a*]indoles, which are of interest in the context of bioactive natural product synthesis.[35]

The low cytotoxicity of **1** is attributed to the reversibility of guanine N(7) reductive alkylation. The quarternized nitrogen readily eliminates to afford a stable six-membered ring alkene **9**, which is conjugated to an aromatic system. Nevertheless, **1** does show a great deal of differential toxicity due to its bioactivation presumably by DT-diaphorase.

Experimental Section

All analytically pure compounds were dried under high vacuum in a drying pistol heated with refluxing methanol. Fremy salt was purchased from Aldrich, stored over calcium chloride desiccant in a refrigerator, and used within 1 month. Some compounds contained fractional amounts of water of crystallization that was determined from the elemental analyses found. Elemental analyses were run at Atlantic Microlab, Inc., Norcross, GA. Uncorrected melting points and decomposition points were determined with a Mel-Temp apparatus. All TLC was run with silica gel plates with fluorescent indicator, employing a variety of solvents. IR spectra were taken as KBr pellets or thin films; the strongest IR absorbances are reported. ^1H and ^{13}C NMR spectra were obtained on a 300 MHz spectrometer, and chemical shifts are reported relative to TMS. The NMR peak assignments of some compounds were assigned on the basis of two-dimensional experiments and ^{13}C labeling, and these compounds (**14** and **15**) have peak assignments.

1-Cyano-2,3-dihydro-6,9-dimethyl-7-methoxy-8-nitro-1*H*-pyrrolo[1,2-*a*]indole(3). To a stirred solution of 500 mg (1.8 mmol) of **2** and 850 mg (4.4 mmol) of tosylmethyl isocyanide in 5 mL of dry dimethoxyethane, cooled at 0 °C, was slowly added a sodium ethoxide solution freshly prepared by reacting 120 mg (5 mmol) of sodium with a solution consisting of 2 mL of dry ethanol and 3 mL of dry dimethoxyethane. After the reaction mixture was stirred for 1 h at 0 °C and then 1 h at room temperature, water was added and the resulting mixture was extracted 3× with 50 mL portions of dichloromethane. The organic extracts were dried over Na_2SO_4 and then concentrated to a residue, which was purified by flash chromatography on silica gel using chloroform as the eluant. The isolated product was recrystallized from chloroform/hexane: 220 mg (42%) yield; mp 156−158 °C; TLC (chloroform/methanol 95:5) R_f = 0.66; IR (KBr pellet) 3015, 2965, 2936, 2245, 1532, 1481, 1445, 1372, 1267, 1233, 1151, 1104, 1023 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.14 (1H, s), 4.23 and 4.08 (3H, 2m), 3.87 (3H, s) 3.01 (2H, m), 2.43 and 2.19 (6H, 2s); MS (EI mode) *m*/*z* 285 (M^+) 268 (M^+ − OH), 252, 237, 223, 212, 209. Anal. Calcd for $C_{15}H_{15}O_3N_3$: C, 63.11; H, 5.30; N, 14.72. Found: C, 63.03; H, 5.35; N, 14.66.

Methyl 2,3-Dihydro-6,9-dimethyl-7-methoxy-8-nitro-1*H*-pyrrolo[1,2-*a*]indole-1-carboxylate (4). To a mixture of 500 mg (1.75 mmol) of **3** in 30 mL of methanol at −20 to −40 °C was added hydrogen chloride gas until saturated. The resulting mixture was stoppered and stored in the refrigerator overnight. The reaction mixture was concentrated to a residue, to which was added water, and the resulting mixture was extracted 3× with 50 mL portions of chloroform. The organic layer was dried (Na_2SO_4) and then concentrated to a residue, which was purified by flash chromatography on silica gel using chloroform as the eluant. The isolated product was recrystallized from chloroform to afford **4** as a yellow crystalline solid that was washed with hexane: 390 mg (70%) yield; mp 109−111 °C, TLC (chloroform/ethyl acetate 90:10) R_f = 0.82; (KBr pellet) 2957, 2918, 1744, 1520, 1447, 1371, 1296, 1258, 1194, 1167, 1030 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.11 (1H, s), 4.2 and 4.0 (3H, 2m), 3.85 (3H, s), 3.73 (3H, s), 2.9 and 2.8 (2H, 2m), 2.42 and 2.11 (6H, s); MS (EI mode) *m*/*z* 318 (M^+), 301 (M^+ − OH), 288, 270, 259 (M^+ − CH_3OCO), 242, 227, 212, 198. Anal. Calcd for $C_{16}H_{18}N_2O_5$: C, 60.37; H, 5.70; N, 8.80. Found: C, 60.49; H, 5.71; N, 8.87.

2,3-Dihydro-6,9-dimethyl-1-(hydroxymethyl)-7-methoxy-8-nitro-1*H*-pyrrolo[1,2-*a*]indole (5). To a stirred solution of 200 mg (5 mmol) of LAH in 10 mL of dry THF, cooled to −20 °C, was added 260 mg (0.82 mmol) of **4** in 20 mL of dry THF over 10 min. The reaction was then stirred for 10 min at −20 °C followed by the addition of 5 mL of ethyl acetate. After 5 min, the reaction was filtered through Celite and concentrated to a residue, which was combined with 50 mL of water and extracted 3× with 40 mL portions of chloroform. The chloroform extracts were dried over Na_2SO_4 and concentrated to a residue, which was recrystallized from chloroform/hexane to afford **5** as a yellow crystalline solid: 146 mg (80%)

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yield; mp 131−133 °C; TLC (chloroform/ethyl acetate 90:10); R_f = 0.27; IR (KBr pellet) 3333, 2944, 1422, 1460, 1367, 1325, 1240, 1151, 1028 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.10 (1H, s), 4.03 (2H, m), 3.86 (3H, s), 3.86 (2H, t), 2.77 and 2.55 (2H, 2m) 2.42 and 2.13 (6H, 2s); MS (EI mode) *m/z* 290 (M^+), 273 ($M^+ - OH$), 259 ($M^+ - CH_2OH$), 242, 227, 213, 198, 184. Anal. Calcd for $C_{16}H_{18}N_2O_5$: C, 62.06; H, 6.21; N, 9.65. Found: C, 61.96; H, 6.24; N, 9.56.

2,3-Dihydro-6,9-dimethyl-1-(hydroxymethyl)-7-methoxy-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione (6) was prepared by the following two-step process.

The product obtained above, 80 mg (0.27 mmol) of **5**, was reduced for 6 h in methanol under 50 psi H_2 employing 35 mg of 5% Pd on carbon as catalyst. After complete reduction, the catalyst was removed by filtration through Celite and the filtrate concentrated to a residue. No attempt was made to purify the reactive amino alcohol product.

This product was dissolved in 10 mL of water containing 200 mg of monobasic potassium phosphate. To this solution was added a solution consisting of 750 mg of Fremy's salt in 30 mL of water containing 250 mg of monobasic potassium phosphate. The reaction mixture was stirred at room temperature for 18 h and then extracted 5× with 30 mL portions of chloroform. The dried (Na_2SO_4) extracts were concentrated to a red solid, which was recrystallized from chloroform/hexane: 46 mg (61%) yield; mp 138−140 °C; TLC (chloroform/methanol 95:5) R_f = 0.27; IR (KBr pellet) 3406, 2953, 2922, 1661, 1624, 1493, 1366, 1321, 1283, 1215, 1117, 1051, 999 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.12 (2H, m), 3.93 (3H, s), 3.84 (2H, m), 3.33 (1H, m, C(1) proton), 2.65 and 2.51 (2H, 2m), 2.28 and 1.90 (6H, 2s); MS (EI mode) *m/z* 275 (M^+), 260, 244, 229, 214, 201. Anal. Calcd for $C_{15}H_{17}NO_4 \cdot 0.25\ H_2O$: C, 64.40; H, 6.26; N, 5.0. Found: C, 64.38; H, 6.36; N, 4.91.

2,3-Dihydro-6,9-dimethyl-1-[(methanesulfonoxy)methyl]-7-methoxy-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione (1). To a stirred solution of 200 mg (0.73 mmol) of **6** in 1.6 mL of dry pyridine, cooled at 0 °C, was slowly added 0.6 mL (7.75 mmol) of methanesulfonyl chloride. After being stirred for 20 min at 0 °C and 4 h at room temperature, the reaction mixture was diluted with 40 mL chloroform, and 10 mL of water was then added dropwise to decompose the excess methanesulfonyl chloride. The chloroform layer was washed consecutively with 2 N hydrochloric acid, 5% sodium bicarbonate, and finally water. The chloroform layer was dried over Na_2SO_4 and then concentrated to an orange solid, which was recrystallized from chloroform/hexane: 195 mg (76%) yield; mp 117−119 °C; TLC (chloroform/methanol 95:5) R_f = 0.59; IR (KBr Pellet) 3437, 3030, 2930, 1661, 1643, 1606, 1481, 1343, 1219, 1175, 1107, 997 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.35 and 4.25 (4H, 2m), 3.99 (3H, s), 3.57 (1H, m), 2.99 (3H, s), 2.80 and 2.48 (2H, 2m), 2.31 and 1.94 (6H, 2s); MS (EI mode) *m/z* 353 (M^+), 338 ($M^+ - CH_3$), 323, 257, 244, 239, 227, 214, 201. Anal. Calcd for $C_{16}H_{19}NO_6S \cdot 0.25H_2O$: C, 53.30; H, 5.50; N, 3.96. Found: C, 53.29; H, 5.34; N, 3.84.

Preparation of ^{13}C-Labeled 3. To 200 mg (0.7 mmol) of **3** and 101 mg (2.02 mmol) of $Na^{13}CN$ was added 10 mL of dry DMSO under an N_2 atmosphere. The reaction was stirred for 30 min at room temperature and then for 4 h at 45 °C while maintaining a nitrogen atmosphere. The completed reaction was diluted with water and extracted 3× with 20 mL portions of chloroform. The chloroform extracts were washed 5× with 30 mL portions of water and then were dried (Na_2SO_4). The dried extracts were concentrated to a residue and purified by flash chromatography on silica gel using chloroform as the eluant. The isolated **3*** was recrystallized from chloroform/hexane: 120 mg (60%) yield. Incorporation of ^{13}C in the cyanide group was 50% by mass spectrometry; ^{13}C NMR ($CDCl_3$) δ 117.8.

Hydrolysis in Anaerobic Methanol. To a mixture consisting of 21 mg (0.06 mmol) of **1** and 5 mg of Pd on carbon was added 10 mL of methanol. The mixture was then deaerated with argon for 30 min, followed by bubbling hydrogen gas for 5 min, and finally bubbling with argon for 30 min to remove the excess hydrogen. The reaction mixture was incubated at 30 °C for 24 h and then combined with air and stirred for 1 h. The catalyst was filtered off, and the filtrate was concentrated to red solid, which was subjected to preparative silica gel thin-layer chromatographic separation using chloroform as the eluant. The physical properties of products are provided below:

2,3-Dihydro-1,6,9-trimethyl-7-methoxy-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione (7): 0.8 mg (5%) yield; TLC (chloroform), R_f = 0.36; ^{1}H NMR ($CDCl_3$) δ 4.27 and 4.10 (2H, 2m), 3.22 (1H, m), 2.71 and 2.11 (2H, 2m), 2.28 and 1.94 (6H, 2s); MS (El mode) *m/z* 259 ($M^+ - CH_3$), 241, 230, 216.

3,10-Dimethyl-2-methoxy-6,7,8,9-tetrahydropyrido[1,2-*a*]indole-1,4-dione (8): trace yield; TLC (chloroform), R_f = 0.36; ^{1}H NMR ($CDCl_3$) δ 4.30 (2H, t, J = 6 Hz), 3.97 (3H, s), 2.69 (2H, t, J = 6 Hz), 2.2 and 1.94 (6H, 2s), 1.95 and 1.84 (4H, 2m); MS (EI mode) *m/z* 259 (M^+), 244 ($M^+ - CH_3$), 241, 230, 216.

3,10-Dimethyl-2-methoxy-8,9-dihydropyrido[1,2-*a*]indole-1,4-dione (9): 2.3 mg (15%) yield; TLC (chloroform), R_f = 0.47; ^{1}H NMR ($CDCl_3$) δ 6.48 (1H, doublet of triplet, J = 9.9, 1.5 Hz), 6.0 (1H, doublet of triplet, J = 9.9, 4.2 Hz), 4.74 (2H, t, J = 7.2 Hz), 3.99 (3H, s), 2.51 (2H, m), 2.29 and 1.95 (6H, 2s); MS (EI mode) *m/z* 257 (M^+), 242, 228, 214.

3,10-Dimethyl-2-methoxypyrido[1,2-*a*]indole-1,4-dione (10): 1.5 mg (10%) yield; TLC (chloroform) R_f = 0.35; ^{1}H NMR ($CDCl_3$) δ 9.4 (1H, d, J = 7.2 Hz), 7.57 (1H, d, J = 7.2 Hz, 7.09 (1H, t, J = 7.2 Hz), 6.56 (1H, t, J = 7.2 Hz), 3.99 (3H, s), 2.58 and 2.06 (6H, 2s); MS (EI mode) *m/z* 255 (M^+), 240 ($M^+ - CH_3$), 226, 212.

3,10-Dimethyl-2,8-dimethoxy-6,7,8,9-tetrahydropyrido[1,2-*a*]indole-1,4-dione (11): 6 mg (35%) yield; mp 127−129 °C; TLC (chloroform) R_f = 0.34; IR (KBr pellet) 3553, 3481, 3414 (hydrate bands), 1655, 1631, 1500, 1431, 1367, 1300, 1195, 1115 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.38 (2H, m), 3.97 and 3.41 (6H, 2s), 3.78 (1H, m), 2.91 (1H, dd, J = 16.7 Hz, 4.8 Hz), 2.78 (1H, dd, J = 16.7, 4.8 Hz), 2.20 and 1.95 (6H, 2s), 2.1 (2H, m); MS (EI mode) *m/z* 289 (M^+), 274 ($M^+ - CH_3$), 257 ($M^+ - CH_3OH$), 244, 231, 218. Anal. Calcd for $C_{16}H_{19}NO_4 \cdot 0.05H_2O$: C, 66.23; H, 6.58; N, 4.82. Found: C, 66.15; H, 6.59; N, 4.71.

Hydrolysis of 1H$_2$ in Anaerobic Aqueous Buffer. A solution consisting of 2 mL of DMSO and 21 mg (0.06 mmol) of **1** was added to 8 mL of 0.05 M pH 7.4 Tris buffer containing 1 M KCl. To this solution was added 5 mg of 5% Pd on carbon, and the mixture was then deaerated with argon for 30 min, followed by bubbling hydrogen gas for15 min, and finally bubbling with argon for 30 min to remove the excess hydrogen. The reaction mixture was incubated at 30 °C for 24 h and then opened to the air. The catalyst was filtered off and the filtrate extracted 3× with 20 mL portions of chloroform. The extracts were dried (Na_2SO_4) and concentrated to a red solid, which was subjected to preparative silica gel thin-layer chromatographic separation using chloroform/methanol (95:5) as eluant. The physical properties of hydrolysis products **14** and **15** are provided below:

3,10-Dimethyl-8-hydroxy-2-methoxy-6,7,8,9-tetrahydropyrido[1,2-*a*]indole-1,4-dione (14): 8 mg (50%) yield; mp 152−153 °C; TLC (chloroform/methanol 95:5) R_f = 0.29; IR (KBr pellet) 2414, 2930, 1659, 1626, 1500, 1433, 1368, 1327, 1208, 1154, 1109, 1001 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.51 and 4.36 (3H, m, C(6) proton and C(8)-diastereomeric methylene), 3.96 (3H, s, methoxy), 2.99 (1H, dd, J = 17, 4.7 Hz, C(9)-diastereomeric methylene), 2.71 (1H, dd, J = 17, 5.9 Hz, C(9)-diastereomeric methylene), 2.11 (2H, m, C(7)-diastereomeric methylene), 2.21 and 1.94 (6H, 2s, 3,10-dimethyl); MS (EI mode) *m/z* 275 (M^+), 260 ($M^+ - CH_3$), 257 ($M^+ - H_2O$), 244, 232, 216, 204. Anal. Calcd for $C_{15}H_{17}NO_4 \cdot 0.1H_2O$: C, 65.45; H, 6.18; N, 5.09. Found: C, 65.16; H, 6.27; N, 4.99.

8-Chloro-3,10-dimethyl-2-methoxy-6,7,8,9-tetrahydropyrido[1,2-*a*]indole-1,4-dione (15): 2 mg (20%) yield; mp 169−171 °C; TLC (chloroform/methanol 95:5) R_f = 0.70; IR (KBr pellet) 3448, 2938, 1659, 1628, 1499, 1431, 1387, 1327, 1300, 1244, 1206, 1125, 1099 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.50 (3H, m, C(6) proton and C(8)-diastereomeric methylene), 3.98 (3H, s, methoxy), 3.21 (1H, dd, J = 17, 4.7 Hz, C(9)-diastereomeric methylene), 3.05 (1H, dd, J = 17 Hz, 5.9 Hz, C(9)-

diasteromeric methylene), 2.33 (2H, m, C(7)-diastereomeric methylene), 2.21 and 1.95 (6H, 2s, 3,10-dimethyl); MS (EI mode) *m*/*z* 295 (M^+), 278 ($M^+ - CH_3$) 275, ($M^+ - H_2O$) 264, 258, 250, 244, 214, 200. Anal. Calcd for $C_{15}H_{16}ClNO_3 \cdot 0.7H_2O$: C, 58.80; H, 5.68; N, 4.57. Found: C, 58.82; H, 5.31; N, 4.58.

Preparation of the 5′-dGMP Adduct (16). To a solution of 237 mg (0.56 mmol) of the disodium salt of 5′-dGMP·$3H_2O$ in 50 mL of 0.05 M pH 7.4 Tris−HCl, containing 25 mg of 5% Pd on carbon, was added a solution of 51 mg (0.14 mmol) of **1** in 5 mL of DMSO. The mixture was deaerated with argon for 30 min and then purged with H_2 gas until the reaction mixture became colorless, about 10 min. The excess H_2 was then removed by purging with argon for 10 min. The reduced reaction mixture was incubated at 30 °C for 24 h and then opened to the air. The reaction mixture was centrifuged at 12000*g* for 20 min, and the supernatant was extracted 3× with chloroform to remove hydrolysis products. Chromatographic analysis of the hydrolysis products provided 1.5 mg (4%) of **15**, 9 mg (23%) of **14**, and 5 mg (10%) of **1**. The aqueous phase was orange in color and contained **16** along with unreacted 5′-dGMP. Concentration of the aqueous phase to complete dryness (no DMSO remaining) under high vacuum was followed by dissolution in ∼1 mL of H_2O and then placement on a 100 g Baker Phenyl reversed-phase column prepared with water. The 5′-dGMP moved somewhat faster than **16** while eluting with water. Collection of the orange band and then concentration to a dry residue was followed by chromatographic separation on a smaller (20 g) Baker Phenyl reversed-phase column. The aqueous product band was concentrated to dryness and dissolved in 1 mL of water. The small amount of column residue was removed by centrifugation (12000*g* 10 min) and the supernant lyophilized to a red solid: 15.2 mg (17%) yield; TLC (1-butanol/acetic acid/water 5:2:3) $R_f = 0.32$; ^{1}HMR (dimethyl-d_6 sulfoxide) δ 7.90 (1H, s,), 7.19 (1H, bs), 6.64 and 6.57 (2H, 2bs), 6.11 (1H, t, $J = 7$ Hz, C-1′ proton), 5.50 (1H, bs), 4.469 (2H, m), 4.30 and 4.20 (2H, 2m), 3.85 (3H, s), 3.80 (1H, s), 3.74 (2H, m), 2.78 (1H, m), 2.54 and 2.19 (2H, m), 2.10 (1H, m), 2.05 and 1.84 (6H, 2s), 2.01 (2H, m); ^{1}H NMR (D_2O) δ 7.85 and 7.74 (1H, 2s), 6.16 and 6.05 (1H, 2t, $J = 6.5$ Hz), 4.65 (1H, m, 4.37 (1H, m), 4.10 (2H, m), 4.05 (1H, m), 3.73 and 3.71 (3H, 2s), 2.77 (2H, m), 2.76 and 2.54 (2H, 2m), 2.53 and 2.30 (2H, 2m), 1.78 and 1.75 (6H, 2s), 1.93 (2H, m); ^{31}P NMR (DMSO-d_6) δ −0.2; ^{31}P NMR (D_2O) δ −0.65.

Alternating DNAs Treated with Reduced 1. The reaction mixture consisted of the following components: 2.6 mg of poly[dG-dC]·poly[dG-dC] or poly[dA-dT]·poly[dA-dT] (6×10^{-6} mol of base pairs) dissolved in 2 mL of 0.05 M pH 7.4 Tris buffer, 2.6 mg (6×10^{-6} moles) of **1** dissolved in 250 μL of dimethyl sulfoxide and 2.6 mg of 5% Pd on carbon. The combination of these components, deaeration, and catalytic reduction were carried out as described under hydrolysis of **1H$_2$**. The anaerobic incubation of the reaction was carried out at 30 °C for 24 h. The completed reaction was opened to the air and filtered through Celite to remove the catalyst. The filtrate was extracted three times with 50 mL portions of chloroform to remove hydrolysis products. The aqueous layer was adjusted to 0.3 M sodium acetate with 3.0 M pH 5.1 sodium acetate stock and then diluted with three volumes of ethanol. This mixture was chilled at −20 °C for 24 h and then centrifuged at 12000*g* for 15 min. The DNA pellet was washed by suspending in ethanol and centrifuging. Weight of the vacuum-dried DNA pellet was 2.5−3.0 mg.

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