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Alkylation of Nucleic Acids by a Model Quinone Methide

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Abstract: Quinone methides and related electrophiles represent a common class of intermediates that form during metabolism of drugs and xenobiotics and may lead to DNA alkylation. The intrinsic reactivity of these species has now been characterized using a stable model compound, *O*-(*tert*-butyldimethylsilyl)-2-bromo-methylphenol, designed to generate an *o*-quinone methide in the presence of fluoride. The resulting deoxynucleoside adducts were assigned unambiguously through use of two-dimensional NMR and, in particular, heteronuclear multiple-bond connectivity (HMBC). Both purines, dG and dA, reacted at their *exo*-amino groups. In contrast, dC had previously been shown to react at its cyclic N3 position [Rokita, S. E.; Yang, J.; Pande, P.; Greenberg, W. A. *J. Org. Chem.* **1997**, *62*, 3010−3012], and the relatively nonnucleophilic T remained inert under all conditions examined. Surprisingly, the efficiency of cytosine modification exceeded that of adenine and guanine by more than 10-fold in competition studies with the deoxymononucleosides. Reaction of all residues was suppressed in duplex DNA, but none was affected more than cytosine (>3600-fold). Guanine consequently emerged as the predominant target in duplex DNA in accord with the selectivity of most natural products forming quinone methide-like species. These general observations may then in part reflect the ability of the *exo*-amino group of guanine to maintain its reactivity most effectively from nucleoside to helical DNA.

Introduction

Structural characterization of products formed by DNA alkylation provides the molecular basis for subsequent drug design and genotoxic evaluation. Initial studies on alkylation relied on simple reagents such as diazoalkanes,[1] dialkyl sulfates,[2] and alkyl halides[3] which helped to identify the most nucleophilic sites of deoxynucleotides and DNA. Considerable attention has since been directed to a range of synthetic and natural drugs as well as environmental toxins and their metabolites.[4] Typically, the specificity of modification has depended on the reaction pathway and the reagent's ability to associate selectively with a particular nucleotide sequence or conformation. Almost every nitrogen and oxygen of the pyrimidine and purine bases can be alkylated under some condition (Figure 1). For example, hard electrophiles generated by *N*-alkyl-*N*-nitrosourea react preferentially at cytosine O^2, guanine O^6, and to a lesser extent thymine O^2 and O^4.[5] In contrast, softer electrophiles such as

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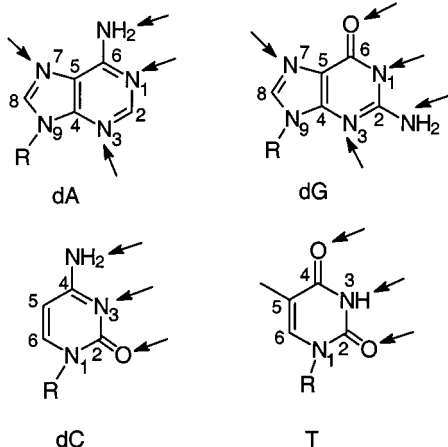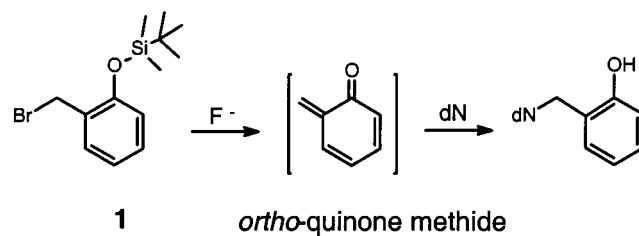
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Figure 1. Alkylation sites on purine and pyrimidine nucleobases (R = 2′-deoxyribose).

dimethyl sulfate and methyl iodide primarily modify nitrogen nucleophiles and in particular guanine N7 and adenine N1.[5] More complex reagents and natural products are additionally affected by their association with, and in some cases activation by, a specific site of DNA. Aziridinyl-cytidine conjugates,[6] cyclopropylpyrroloindole derivatives,[7–9] neocarzinostatin,[10] and most recently, ecteinascidin[11] best illustrate the high selectivity achieved through localized activation.

Drugs and reagents that form a quinone methide or related intermediate are most often found to modify the *exo*-amino group (N[2]) of guanine.[12] Model studies have also described the potential reactivity of the *exo*-amino group (N[6]) of adenine,[13] and activity of a pyrrolizidine suggested that the cyclic nitrogen (N3) rather than the exocyclic nitrogen (N[4]) of cytosine was the target of a quinone methide-like intermediate.[14] In contrast, relatively stable quinone methide intermediates formed during

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Scheme 1

1   *ortho*-quinone methide

oxidation of the preservative BHT (2,6-di-*tert*-butyl-4-methylphenol) react with many nitrogen nucleophiles of DNA although the exocyclic purine amines remain the major site of modification.[15]

Our laboratory has applied quinone methide and related electrophilic intermediates to investigations of both enzyme catalysis and DNA modification. Such species were designed to form under alternative control of local pH,[16] irradiation (λ > 330 nm),[17,18] reduction,[17] and fluoride.[19,20] Efforts have most recently centered on the potential of target DNA itself to promote formation of quinone methides.[21] Since associative (S_N2) and dissociative (S_N1 and related ionic) pathways of DNA alkylation typically yield different products,[5,22] our mechanistic analysis has begun with product characterization. A preliminary note on the model reactivity of dC presented the first unambiguous connectivity between this pyrimidine and a quinone methide adduct (Scheme 1).[23] Structural assignments for the related products of dA and dG are now reported below. The sites of alkylation are also shown to remain constant for residues in their deoxynucleoside, single-stranded deoxyoligonucleotide, and duplex deoxypolynucleotide forms. Only the relative yield of each product is affected by the nature of the target DNA. Guanine modification predominates in duplex DNA, although cytosine unexpectedly exhibits the greatest intrinsic reactivity of the deoxymononucleosides.

Results and Discussion

Characterization of Deoxynucleotide Adducts. A series of silyl phenol derivatives had previously been developed in our laboratory as quinone methide precursors for DNA cross-linking[20] and oligonucleotide-directed alkylation (Scheme 1).[19,21] In each case, desilylation was proposed to initiate reaction, and no spontaneous benzylic substitution was expected. Indeed, the nucleosides (0.1–0.5 mM) were not detectably modified after incubation with *O*-(*tert*-butyldimethylsilyl)-2-bromomethylphenol (**1**, 0.1–100 mM) for 24 h at 37–50 °C in aqueous DMF or acetonitrile. Furthermore, the weakly nucleophilic residue, T, did not even react in the added presence of potassium fluoride (500 mM). The remaining residues, dC,[23] dA, and dG, were

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only alkylated in the combined presence of **1** and fluoride, thus confirming a role for a quinone methide intermediate. Likewise, the structures of the purine adducts are also consistent with quinone methide reaction. As described below, these adducts were characterized through a series of 2-dimensional NMR experiments that culminated with direct connectivities between the electrophile and deoxynucleoside as observed by heteronuclear multiple-bond connectivity (HMBC).[24]

Quinone Methide Adduct of dA. Reaction of dA was first examined in an aqueous DMF solution of **1** and potassium fluoride at 25 °C. Within 30 min, dA was converted to a single product as detected by analytical thin-layer chromatography (silica). All attempts to purify this product failed due to its extreme lability. Preliminary investigation suggests that its decomposition regenerated unmodified dA. Surprisingly, an alternative and stable adduct of dA was formed when the reaction mixture above was maintained for 14 h at 50 °C instead of 25 °C. The resulting adduct was isolated from silica gel chromatography in 43% yield without problem and found to be stable under neutral conditions. This same adduct was generated from reaction with single- and double-stranded DNA at 37 °C (see below) and therefore subject to complete NMR characterization. Further studies on the unstable product are in progress to learn the significance of this transient species that would otherwise remain undetectable in assays relying on enzymatic hydrolysis of DNA or most other methods currently used for identifying polynucleotide adducts.

The products of dA alkylation at N1, N3, N7, or N^6 are all distinguishable through HMBC methods since each would exhibit a unique connectivity between the benzylic protons and purine carbons. As a prerequisite to such analysis, the ^1H and ^{13}C spectra of the stable dA adduct formed at 50 °C were fully assigned. The signals associated with the deoxyribose moiety were identified through comparison with its parent, dA. Both heteronuclear multiple-quantum coherence (HMQC)[25] (positions 12, 13, 14, 15) and HMBC (positions 10 and 11, see Figure 2) experiments were used to assign the phenolic appendage in analogy to that applied for the dC adduct (summarized in Table 1; see also, Supporting Information).[23] The corresponding phenolic moieties attached to both dC and dA exhibited ^{13}C chemical shifts within 2.7 ppm of each other and ^1H chemical shifts within 0.14 ppm.

Two of the purine carbons (C4 and C8) were identified by their long-range interactions with H1′ using HMBC, and differentiated by direct connectivity of C8 to H8 using HMQC (Supporting Information). Similarly, C2 was assigned by its direct connectivity to H2. The two quaternary carbons C5 and C6 were distinguished through long-range coupling to H8 and H2, respectively. The benzylic protons that were critical for establishing the adduct linkage were initially obscured in routine ^1H spectra by trace quantities of solvent protons. However, HMQC experiments indicated that both the benzylic ($-CH_2-$) and H4′ (ribose) signals were present within the dominant solvent signal. The nonexchangeable signals were subsequently revealed after cooling the sample to 229 K, which caused a downfield shift of the solvent signal. At this low temperature, the benzylic ^1H resonance resolved into two signals that suggested restricted rotation between solution conformers.

Overall, the dA adduct exhibited ^{13}C chemical shifts within 1 ppm of those of its parent nucleoside except for C6. This carbon uniquely exhibited a moderate shift (1.8 ppm), and this
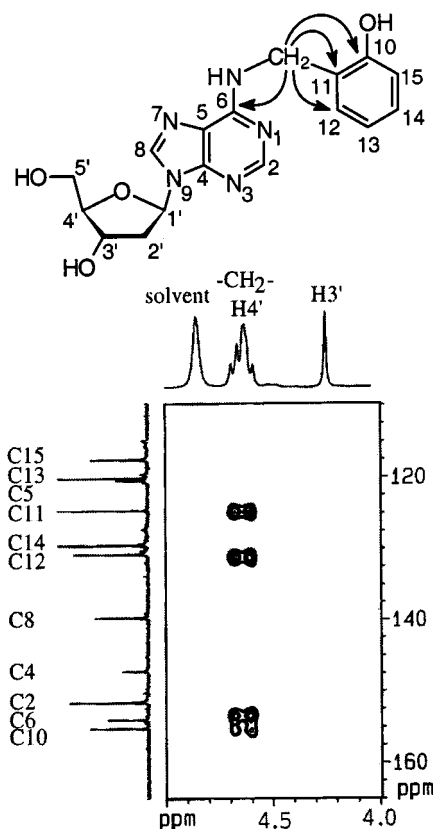

Figure 2. HMBC of dA adduct.

Table 1. ^1H and ^{13}C NMR Data for Purine Adducts Formed by Reaction of **1** in the Presence of Fluoride

position	dA adduct		dG adduct	
	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
2	8.34	151.5		159.2
4		147.2		150.4
5		120.5		117.7
6		153.9		155.3
8	7.93	139.7	7.89	138.3
$-CH_2-$	4.64	42.6	5.22	39.6
10		155.2		155.5
11		124.6		123.3
12	7.25	130.7	7.22	130.8
13	6.87	120.1	6.73	121.5
14	7.18	129.4	7.06	130.4
15	6.91	117.4	6.79	116.2

first suggested attachment of the electrophile at dA N^6. In contrast, modification of dA N1 would have perturbed the chemical shift of the purine carbons C2, C4, C5, and C6 by 3.6–7.5 ppm.[26] Formation of the dA N^6 adduct was further supported by its 14 nm bathochromic shift in ultraviolet absorption (273 nm) relative to its parent.[2a] Such a shift does not result from equivalent alkylation of dA N1.[2a] HMBC protocols provided the most definitive evidence for connectivity between dA N^6 and the benzylic position ($-CH_2-$) (Figure 2). As expected, long-range coupling was detected between the benzylic protons and the phenolic carbons C10, C11, and C12, but coupling was also apparent between these protons and purine C6. Only alkylation of N^6 would yield this connectivity. An alternative reaction at N1 would yield coupling between the benzylic protons and both C2 and C6. This was not observed for the dA adduct formed above. Finally, modification at dA

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N^6 is most consistent with the known tendency of quinone methides to react with the *exo*-amino group of purines, although most examples still derive from reaction of guanine.[12,13,15]

Quinone Methide Adduct of dG. The *exo*-amino group (N^2) of dG is the critical target of mitomycin- and anthracycline-dependent alkylation of duplex DNA which can form via quinone methide-like intermediates.[12] However, these natural products contain a rich array of functional groups that may in part control reaction through their preassociation with DNA.[27] Few studies had previously focused on the nature of a simple quinone methide, and consequently the intrinsic selectivity of this electrophile remained ambiguous. Alkylation of dG by **1** and potassium fluoride in aqueous DMF generated a single nucleotide adduct that was isolated after silica gel chromatography in a 31% yield. The ultraviolet absorbance maximum of this product (263 nm) had shifted 11 nm relative to that of dG (252 nm). Such a shift is consistent with alkylation at N^2 or N3 of dG.[28] In comparison, alkylation of N1, N7, and O^6 does not generally affect the absorbance maximum.[28]

Structural characterization of the alkylated product followed the same method as that applied to the dC[23] and dA adducts. Again, signals attributed to the deoxyribose group of the product were identified through comparison with the parent dG. [1]H and ^{13}C assignments for the phenolic ring were also based on HMQC and HMBC experiments as before (Table 1; see also, Supporting Information). The corresponding chemical shifts of this ring were determined independently for the dA and dG adducts but differed by no more than 0.14 ppm for the ^{1}H signals and 1.4 ppm for the ^{13}C signals. The sole nonexchangeable proton on the purine, H8, exhibited long-range coupling to C5 and C4, and the anomeric proton, H1′, exhibited equivalent coupling to C4 and C8 (Supporting Information). No connectivities within the purine system distinguished C2 and C6, but their chemical shifts are most consistent with N^2 rather than O^6 alkylation. Modification of guanine O^6 significantly perturbs the ^{13}C resonances of C2, C4, C5, and C6 (>2.7 ppm),[29] whereas modification of guanine N^2 would likely perturb only C2 at most. Indeed, reaction between **1**, fluoride, and dG resulted in a 5.5 ppm shift in the resonance of C2, and the remaining purine carbons C4, C5, C6, and C8 shifted less than 1.6 ppm.

The benzylic protons connecting dG with the phenol group were shifted downfield relative to their counterparts in the dA adduct (Table 1) and distinct from trace solvent protons. This allowed for HMBC experiments to be performed under ambient temperature instead of 229 K as required for the dA adduct. Long-range coupling was observed from the benzylic protons ($-CH_2-$) to C10, C11, C12, and, most significantly, C2 of dG (Figure 3). The ^{13}C NMR does not resolve C6 and C10 sufficiently to exclude possible coupling between C6 and the benzylic protons. However, such coupling would necessitate alkylation of N1 or O^6 which is not compatible with the ^{13}C chemical shifts.[29] Reaction at the *exo*-amino group, dG N^2, uniquely satisfies the coupling data in addition to the alkylation-induced perturbations of the ^{13}C spectrum and ultraviolet absorbance. Therefore, the intermediate generated by the deprotection of **1** expressed the same intrinsic specificity as the quinone methide-like intermediates generated by anticancer antibiotics such as mitomycin and menogaril.[12]
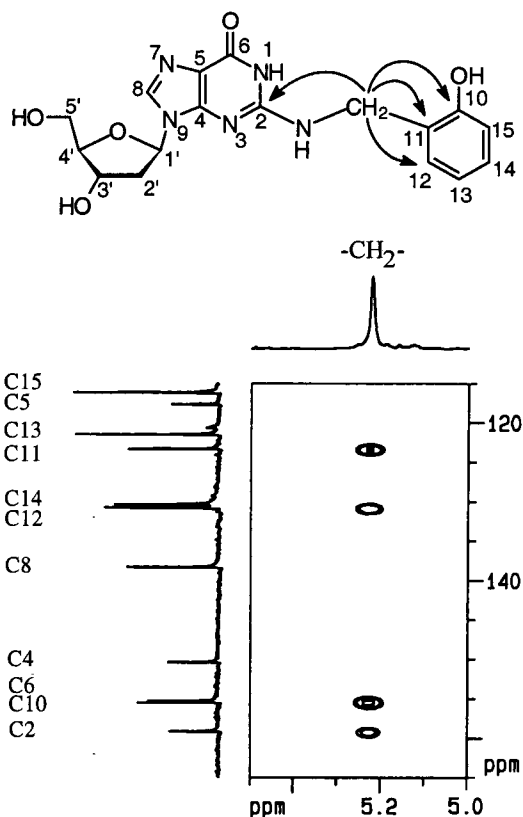

Figure 3. HMBC of dG adduct.

Relative Reactivity of Pyrimidines and Purines at the Deoxynucleoside Level. The structural characterization above helped to identify the likely path of nucleoside modification with **1** as well as generate standards necessary for examining alkylation selectivity within DNA. Modification of deoxypoly-nucleotides may be variably affected by the innate reactivity of the individual residues as well as by the steric, geometric, and electronic characteristics of their helical orientation in duplex DNA.[5] The specificity of secondary reactions such as cross-linking may also be profoundly influenced by the site of initial alkylation.[30] For example, *O*-(*tert*-butyldimethylsilyl)-2,6-bis-(bromomethyl)phenol, a bifunctional analogue of **1**, expressed a selectivity for cross-linking at 5′...d(CG)...3′ that may have reflected control by one or both alkylation events.[20] Since **1** is only competent for monoalkylation, its specificity was used to characterize the initial process of DNA modification.

The relative efficiency of deoxynucleoside alkylation in the presence of **1** and fluoride was first implied by the variable yields of adducts obtained during synthesis (dC > dG ≈ dA ≫ T). However, the intrinsic selectivity was also determined by examining their competitive reaction directly. An equimolar mixture of T, dC, dG, and dA (0.5 mM each) was incubated with excess **1** and potassium fluoride for 24 h at 37 °C in phosphate buffer pH 7 and 30% acetonitrile. The resulting products were then separated and quantified using C-18 reverse-phase chromatography (Figure 4A). Under these reaction conditions, the intermediate quinone methide was primarily quenched by water to form the major product, hydroxymeth-ylphenol, and only a fraction of the deoxynucleotides were alkylated concurrently. A small single-stranded deoxyoligo-nucleotide, 5′-d(ACGTCAGGTGGCACT), was similarly incu-bated and then subject to enzymatic digestion prior to chro-

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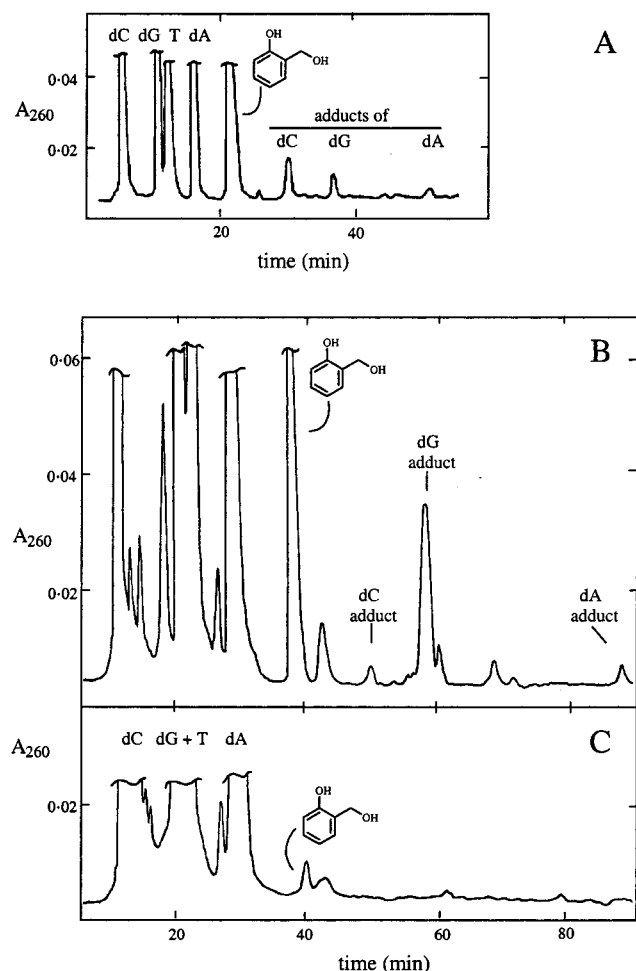
Figure 4. Reverse-phase chromatographic analysis of purine and pyrimidine alkylation induced by **1** and KF. (A) The product profile generated from an equimolar mixture of dC, dG, T, and dA was characterized directly after reaction using a linear gradient of acetonitrile (60 min) and (B) the corresponding profile from calf thymus DNA was generated after enzymatic digestion and use of two sequential and linear gradients of acetonitrile (109 min) (see Methods for details). (C) Reaction and analysis of calf thymus DNA and **1** was repeated in the absence of fluoride as a control to measure the extent of spontaneous reaction and to identify contaminants within the DNA.

Table 2. Relative Reactivity of Each Residue of DNA in the Presence of **1** and Fluoride[a]

	relative reactivity			
target	dC	dG	dA	T
dN equimolar	6200	520	210	—[b]
single strand	310	38	8.4	—[b]
duplex DNA	1.7	19	1.0	—[c]

[a] Product formation was quantified by integrating the A_{260} of chromatograms such as those illustrated in Figure 4 and normalizing for the relative ϵ_{260}[41] and abundance of each deoxynucleoside (dN). The single strand deoxyoligonucleotide contained 3 dA, 4 dC, 5 dG, and 3 T; duplex DNA (calf thymus) contained 42% C + G and 58% A + T.[43] [b] Not detectable and no products were left unassigned. [c] Not determined since no standards were produced under model conditions.

matographic analysis. Finally, calf thymus DNA was examined under equivalent conditions, although no products other than hydroxymethylphenol were evident until the DNA concentration was increased 10-fold to a final concentration of 20 mM (nt) (Figure 4B). DNA alkylation in every case required the presence of fluoride to remove the silyl protecting group from **1** and allow for generation of the *o*-quinone methide. When fluoride was
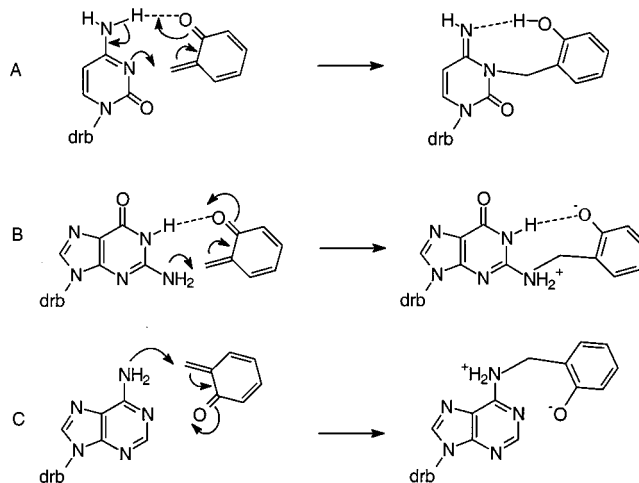

Figure 5. Possible reactive orientations between an *o*-quinone methide and (A) dC, (B) dG, and (C) dA.

excluded from the reaction mixture (Figure 4C), no alkylated products were observed and only a trace of hydroxymethylphenol was apparent. Thus, an alternative mechanism of reaction involving direct displacement of the benzyl bromide by the pyrimidines or purines is unlikely.

The nucleophilicity of DNA has been described by numerous laboratories using a wide variety of reagents, but rarely has cytosine exhibited greatest reactivity.[5] In part, this could be due to the instability of some dC N3 adducts.[15] However, the quinone methide generated by **1** modified dC over 10-fold more frequently than dG or dA (Table 2). Another example of this interesting preference for dC appears to involve a quinone methide-like intermediate as well, in this case one generated by a pyrrolizidine alkaloid.[14] Such a specificity is not unique to this class of intermediates. Cytosine dC N3 is also the dominant target of alkylation by styrene oxide,[31] although neither the epoxide nor the alkaloid express more than a mild preference for dC N3 relative to the nucleophilic sites of dG and dA.

Product distribution can often change dramatically in response to quite subtle variations in electrophile structure as illustrated by reaction of guanosine with a series of styrene oxides alternatively containing electron donating and withdrawing substituents.[22d] Similarly, benzylation of dC can preferentially form either N3- or N4-adducts depending on the electronic characteristics of the aromatic ring.[22b] DNA modification has also demonstrated sensitivity to the nature of low molecular weight quinone methides. The reactivity of these intermediates is known to be enhanced by solvation, hydrogen bonding, and protonation of their carbonyl moiety.[32,33] Conversely, substituents that shield the carbonyl from such interactions diminish their reactivity.[15,34] This effect is well illustrated by the remarkable stability of the *p*-quinone methide ($t_{1/2} = 51$ min, pH 7.4) formed by oxidation of 2,6-di-*tert*-butyl-4-methylphenol and the contrasting lability of its hydroxylated derivative capable of forming an intramolecular hydrogen bond to the carbonyl ($t_{1/2} = 6.7$ min, pH 7.4).[32b] Both intermediates generate numerous products with DNA and the most reactive analogue

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forms the greatest number of adducts including multiple derivatives of dG and dC.[15]

The *o*-quinone methide generated from **1** is expected to be even more reactive than the alkyl-substituted analogues above,[32b,34] and therefore its greater selectivity in nucleoside modification was not anticipated. However, our results can still be rationalized by the electrophile's general sensitivity to protonation and its unique ortho geometry. Activation of the quinone methide can be envisioned from hydrogen bonding between the *exo*-amino group of dC and the carbonyl group of the quinone methide (Figure 5A). This would also minimize charge development during alkylation since the hydrogen-bonded proton could transfer directly to the nascent phenolate product. The para derivative above cannot associate with dC in a similar manner and reacts only weakly with this nucleoside relative to dG and dA.[15] Likewise, transient interaction between the *o*-quinone methide and dG could provide hydrogen bonding but little proton transfer (Figure 5B), and a complex with dA could provide neither stabilizing feature (Figure 5C). Related intramolecular proton transfers have already been invoked to explain the facile formation of quinone methides from 1-aminomethyl-2-naphthol[35] and *o*- (but not *p*-) methoxymethylphenol.[36] Although such interactions help to explain the high reactivity of dC, they could not be used to predict modification at dG N^2 or dA N^6 over their respective N3 or N1 positions, alternatives that might also benefit from intramolecular proton transfer. Hence, no one property of the electrophile or nucleophile fully controls the distribution of products.

Relative Reactivity of Pyrimidines and Purines at the Single-Stranded Deoxyoligonucleotide and Duplex Deoxypolynucleotide Level. Both pyrimidine and purine reactivity is suppressed within the model deoxyoligonucleotide (Table 2). This type of effect is often associated with electrostatic repulsion between anionic reagents and the anionic phosphodiester linkage,[37] but the quinone methide intermediate is neutral and should be relatively insensitive to charge. The decrease in reactivity is more likely caused by steric effects derived from the ensemble of condensed structures formed by single-stranded DNA.[38]

The well-described characteristics of standard B-helical DNA are sufficient to explain its variable inhibition of duplex polynucleotide alkylation. The N3 of cytosine is protected from reactants since it forms the interior hydrogen bond of three such bonds formed between cytosine and guanine. Not surprisingly, modification of cytosine N3 within duplex DNA was suppressed by more than 3600-fold (Table 2). Even this low basal reactivity probably depended on transient dissociation of the dC-dG pairing. Interestingly, a dC-dC mismatch in duplex DNA was recently shown to be the major product of cross-linking (N3 to N3) by the nitrogen mustard mechlorethamine that ordinarily exhibits a very high specificity for dG-dG cross-linking (N7 to N7).[39]

The target sites of dG (N^2) and dA (N^6) also participate in hydrogen bonding, but both maintain some exposure to the

minor and major grooves, respectively. If the accessible surface of these groups[40] were the only criteria for reaction, then alkylation at dG N^2 would have been inhibited to a greater extent than alkylation at dA N^6. However, the converse was observed. Modification of adenine decreased 210-fold from its deoxynucleoside to B-helical derivative whereas a decrease of only 27-fold was equivalently noted for guanine (Table 2). Perhaps the loss of guanine reactivity was moderated by the ability of the relatively hydrophobic minor groove to establish a local high concentration of **1** and its electrophilic intermediate in the vicinity of guanine N^2.

The overall suppression of cytosine and adenine reaction in duplex DNA consequently results in a 10-fold preference for modification of duplex DNA at guanine N^2. Essentially all anticancer antibiotics and related compounds that react through a quinone methide-like intermediate specifically alkylate this same minor groove site,[12] yet this is not the only target of electrophilic reaction in the minor groove. For example, cyclopropylpyrroloindole derivatives modify adenine N3 through a local activation process that does not involve quinone methide generation.[7−9] Accordingly, the selectivity of a quinone methide may then reflect the general characteristics of this electrophile as well as the specific nature of its preassociation with DNA. For mitomycin alkylation and cross-linking, the minor groove purine amine serves as both the recognition element and reaction target as most recently demonstrated through comparative studies on the reactivity of guanine and 2,6-diaminopurine within duplex DNA.[27c]

Experimental Section

Materials. Chemicals and solvents were purchased from Fisher, Aldrich, and Sigma and used without further purification. Silica gel for column chromatography (230−400 mesh) was purchased from EM Sciences. Alkaline phosphatase and phosphodiesterase I were purchased from Sigma. Calf thymus DNA was also purchased from Sigma and purified with phenol−chloroform extraction and ethanol precipitation prior to use. The deoxyoligonucleotide, 5′-d(ACGTCAGGTGGCACT), was purchased from Keystone Labs. All aqueous solutions were made with water purified by a standard filtration system to yield a resistivity of 17.8−18 MΩ.

General Methods. All ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX 500 instrument. Signals are reported in parts per million (ppm) and coupling constants (*J*) in Hz. Ultraviolet absorbance spectra were recorded with a Perkin-Elmer *λ*-5 spectrophotometer. High-resolution mass spectra were obtained with a VG Analytical ZAB instrument for fast ion bombardment (UC−Riverside Mass Spectrometry Facility). Reaction products were separated and quantified analytically by reverse-phase C-18 (ultracarb, Phenomenex) chromatography using a Varian 5000 HPLC and a Varian 2050 variable-wavelength detector.

2′-Deoxyadenosine Adduct. 2′-Deoxyadenosine (0.38 g, 1.5 mmol) and *O*-(*tert*-butyldimethylsilyl)-2-bromomethylphenol[23] (0.68 g, 2.2 mmol) were dissolved in 1 mL of DMF and combined with an aqueous solution of KF (2.64 M, 1.5 mL). The reaction was heated at 50 °C for 14 h, cooled, and then directly subjected to silica gel flash chromatography (CHCl₃:CH₃OH, 4:1) to yield the dA N^6 adduct (0.23 g, 43% yield). ^{1}H NMR (500 MHz, 95% CDCl₃ and 5% methanol-*d₄*, 229 K) *δ* 2.34 (m, 1H), 2.85 (m, 1H), 3.77 (m, 1H), 3.92 (m, 1H), 4.16 (s, 1H), 4.64 (m, 3H), 6.32 (m, 1H), 6.87 (m, 1H), 6.91 (m, 1H), 7.18 (m, 1H), 7.25 (m,1H), 7.93 (s, 1H), 8.34 (s, 1H). ^{13}C NMR (126 MHz, 95% CDCl₃ and 5% methanol-*d₄*, 296 K) *δ* 40.6 (2C), 62.8, 72.1, 87.1, 89.0, 117.4, 120.1, 120.4, 124.6, 129.4, 130.7, 139.6, 147.3, 151.6, 153.9, 155.2. HRMS (FAB, glycerol) *m/z*: 358.1509 (M + H⁺). Calcd for C₁₇H₁₉O₄N₅ (M + H⁺): 358.1515.

2′-Deoxyguanosine Adduct (3). 2′-Deoxyguanosine (0.13 g, 0.5 mmol) and *O*-(*tert*-butyldimethylsilyl)-2-bromomethylphenol[23] (0.23 g,

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0.7 mmol) were dissolved in 5 mL of DMF by warming the mixture to 37 °C. Reaction was initiated by adding KF (2.64 M, 0.9 mL), and then the resulting solution was maintained at 37 °C for 2 h. Solvent was removed under high vacuum, and the remaining residue was subjected to silica gel flash chromatography (CH_2Cl_2:MeOH, 10:1) to yield the dG N^2 adduct (58 mg, 31% yield). ^{1}H NMR (500 MHz, methanol-d_4) δ 2.27 (m, 1H), 2.63 (m, 1H), 3.58 (m, 2H), 3.92 (m, 1H), 4.45 (m, 1H), 5.22 (s, 2H), 6.18 (t, 1H, $J = 6.5$), 6.73 (t, 1H, $J = 7.5$), 6.79 (d, 1H, $J = 8.1$), 7.06 (t, 1H, $J = 7.3$), 7.22 (d, 1H, $J = 7.7$), 7.89 (s, 1H). ^{13}C NMR (126 MHz, methanol-d_4) δ 39.6, 41.3, 63.4, 72.8, 85.8, 89.3, 116.2, 117.7, 121.5, 123.3, 130.4, 130.8, 138.3, 150.4, 155.3, 155.5, 159.2. HRMS (FAB, glycerol) m/z: 374.1442 (M + H$^+$). Calcd for $C_{17}H_{19}O_5N_5$ (M + H$^+$): 374.1464.

Competitive Alkylation of Deoxynucleosides. Reactions were initiated by adding an acetonitrile solution of **1** (30 μL) to an aqueous solution of dC, dG, dA, T, KF, and potassium phosphate, pH 7 (70 μL). The resulting mixture of **1** (100 mM), each dN (0.5 mM), KF (500 mM), and buffer (10 mM) was incubated for 24 h (37 °C) and then applied directly to reverse-phase C-18 HPLC. A solvent gradient of 3% acetonitrile in 49 mM triethylammonium acetate, pH 4.0, to 25% acetonitrile in 38 mM triethylammonium acetate, pH 4.0, over 66 min (1 mL/min) was used to separate the starting materials and products. Detection and quantification of these materials were based on absorbance at 260 nm and normalized with respect to the individual ϵ_{260} values.[41] The alkylated products were assumed to have ϵ_{260} values proportionate to their parent deoxynucleoside.

Alkylation of Single- and Double-Stranded DNA. The deoxyoligonucleotide, 5′-d(ACGTCAGGTGGCACT) (2 mM in nt) and calf thymus DNA (20 mM in nt) were independently alkylated under conditions identical to those used above (100 mM **1**, 500 mM KF, 10 mM potassium phosphate, pH 7). After reaction (24 h, 37 °C), samples were additionally dialyzed (MW cutoff 1000) against water overnight to remove salts and low molecular weight organic compounds. The deoxyoligonucleotide solutions were then lyophilized to dryness, redissolved in triethylammonium acetate (100 μL, 100 mM, pH 10.0), and hydrolyzed with alkaline phosphatase (1 unit) and phosphodiesterase I (0.027 unit).[42] The enzyme digestion was neutralized after 5 h (37 °C) by addition of aqueous acetic acid (5 μL, 5.6 mM) and then analyzed by HPLC as described above. The calf thymus DNA solution was treated equivalently except additional alkaline phosphatase (10 units) and phosphodiesterase (0.27 unit) were used in a 24 h incubation. To separate the deoxynucleoside adducts from background material associated with calf thymus DNA, some alteration of the HPLC gradient protocol was necessary (3% acetonitrile in 49 mM triethylammonium acetate, pH 4.0, to 11% acetonitrile in 45 mM triethylammonium acetate, pH 4.0, over 24 min (1 mL/min), followed by 11% to 25% acetonitrile in 38 mM triethylammonium acetate, pH 4.0, over the next 85 min).

Supporting Information Available: Full HMQC and HMBC spectra of dA and dG adducts (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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