Thermodynamic versus Kinetic Products of DNA Alkylation as Modeled by Reaction of Deoxyadenosine

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Abstract: Alkylating agents that react through highly electrophilic quinone methide intermediates often express a specificity for the weakly nucleophilic exocyclic amines of deoxyguanosine (dG N^2) and deoxyadenosine (dA N^6) in DNA. Investigations now indicate that the most nucleophilic site of dA (N1) preferentially, but reversibly, conjugates to a model *ortho*-quinone methide. Ultimately, the thermodynamically stable dA N^6 isomer accumulates by trapping the quinone methide that is transiently regenerated from collapse of the dA N1 adduct. Alternative conversions of the dA N1 to the dA N^6 derivative by a Dimroth rearrangement or other intramolecular processes are not competitive under neutral conditions, as demonstrated by studies with [6-^{15}N]-dA. Both a model quinone methide precursor and its dA N1 adduct yield a similar profile of deoxynucleoside products when treated with an equimolar mixture of dC, dA, dG, and T. Consequently, the most readily observed products of DNA modification resulting from reversible reactions may reflect thermodynamic rather than kinetic selectivity.

Introduction

Describing the exact structure of a DNA adduct formed by alkylation frequently serves as the initial step toward evaluating its potential therapeutic or deleterious properties. This approach is typically applied to each alkylating agent, because our ability to predict reaction efficiency and specificity remains inadequate. Competition between various nucleophiles of DNA and the surrounding medium is essentially controlled by the basic principles of accessibility, electrostatics, proximity, and intrinsic reactivity. The first two of these are dictated by the helical arrangement of residues within duplex DNA. Proximity in turn is most often guided by noncovalent preassociation resulting from selective recognition of a nucleotide sequence or structure. Such interactions are also not easily anticipated for new classes of compounds, although abundant information on a few systems has led to the creation of a wide variety of reagents for site specific alkylation and cross-linking.[1] Data on intrinsic reactivity, the final and perhaps most fundamental principle, often appear least transferable between various reactants because chemical mechanisms affecting product formation remain obscure. Detection and characterization of a kinetic product of deoxyadenosine (dA) alkylation now helps to reconcile the apparent contradiction between the strength of nucleophiles in DNA and their propensity for addition to a model quinone methide.

Most every heteroatom of DNA exhibits at least some nucleophilic character, and each may be variably targeted for alkylation depending on the nature of the electrophile and the reaction conditions. Our current understanding of this process derives from extensive empirical[2,3] and theoretical[4,5] investigations. In general, transition-state characteristics are extrapolated back from profiles of isolated products. The general influence of hard and soft electrophiles as manifest in the distribution of O- to N-alkylated products has been evident since the earliest work on simple alkylating agents based on dialkyl sulfates, nitrosoureas, and diazoniums.[2] Many additional parameters have become equally apparent from study of other electrophiles that predominantly target the weak nucleophiles of DNA. Even very subtle changes in reactant structure can profoundly influence the distribution of alkylation products, as illustrated by extensive studies on benzyl halides and styrene oxides. Modification at the highly nucleophilic N7 of guanine typically prevails under conditions favoring direct displacement, whereas modification at the weakly nucleophilic N^2 of guanine typically prevails under conditions favoring more ionic transition states.[6,7] The N^2 of guanine is also the predominant site of modification for metabolites of environmental toxins such as benzo[*a*]pyrene,[8] mitomycin C,[9] and certain pyrrole derivatives.[10]

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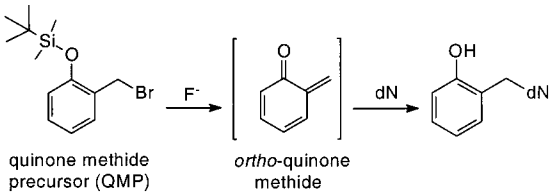
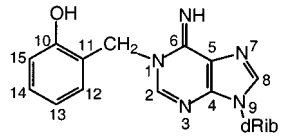
Scheme 1



The target selectivity of quinone methides and related intermediates may seem a bit anomalous because these highly reactive electrophiles generally yield products of alkylation at the *exo*-amino groups of guanine (N^2) and adenine (N^6) rather than at the more nucleophilic sites such as guanine N7 and adenine N1.[10−17] These results are even more surprising, since a strong dependence on nucleophilicity is evident for their reaction with amino acids.[18,19] Various explanations have been presented to rationalize the selectivity observed with DNA, but none have been especially satisfying. In particular, most extrapolations attempt to draw conclusions from product profiles that assume a series of irreversible processes, and yet, quinone methide addition has the potential to be reversible.[14,19−21] In such cases, the relative stability of competing derivatives rather than the relative energy of their transition states may determine the final distribution of products. The origins of reaction selectivity may concurrently be obscured by intramolecular rearrangement of certain initial products, as illustrated by the ability of N1-alkyl dA to undergo a Dimroth rearrangement to form its more stable N^6-alkyl analogue.[7,22−28] Accordingly, we have utilized our well described quinone methide model system (Scheme 1)[16,17,29] to determine the basis for the selectivity expressed by dA. The thermodynamic stability of the individual adducts rather than the kinetics of their formation appears to control the profile of detectable products. This same principle likely dominates the profiles of deoxyguanosine (dG) and deoxycytosine (dC) adducts as well and should provide an important perspective for modification of nucleic acids in general.

Table 1. ^{1}H and ^{13}C NMR Data (DMF-d_7) of dA and the dA N1 Adduct



	dA N1 adduct		dA	
position	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
2	8.73	148.5	8.40	153.1
4		143.4		149.8
5		122.9		120.6
6		155.7		157.3
8	8.33	139.7	8.19	140.5
CH_2	5.26	48.2		
10		158.3		
11		124.2		
12	7.63	131.9		
13	6.81	119.8		
14	7.20	130.9		
15	6.82	119.2		

Results

Quinone Methide Alkylation at the N1 Position of dA. Initial application of our quinone methide precursor (QMP) demonstrated that stable adducts of dC and dG were efficiently generated under ambient conditions.[16,17,29] A highly labile adduct of dA was also formed under these conditions, but its analysis was temporarily supplanted by discovery of an isomeric and stable N^6 adduct. This alternative species formed at higher temperatures (50 °C) and became a focus of interest after it was also isolated from reaction with duplex DNA.[16] In contrast, the labile deoxynucleoside adduct rapidly reverted to dA in methanol after silica gel chromatography and would not be stable enough to persist through the procedures for DNA analysis. Alternative methods of isolating and storing this derivative have since been pursued because of suspicion that it might be the N-1 adduct of dA. Reverse phase (C-18) chromatography using a gradient of acetonitrile in aqueous triethylammonium acetate (pH 4) was found to be most convenient and provided the labile product in a 75% yield based on the parent dA. Furthermore, this material was sufficiently stable in DMF ($t_{1/2} \sim 96$ h) rather than CH_3OH ($t_{1/2} \sim 6$ h) to allow for its characterization and storage.

NMR analysis of the labile product was performed in analogy to that used earlier to identify the dA N^6 adduct.[16] First, the ^{1}H resonances were assigned by their chemical shifts and multiplicities, and the ^{13}C resonances were subsequently determined by their diagnostic connectivity to geminal and proximal protons as detected by HMQC (heteronuclear multiple-quantum coherence)[30] and HMBC (heteronuclear multiple-bond correlation),[31] respectively (Table 1). All of the ^{13}C chemical shifts of the product's purine ring other than C8 are significantly perturbed ($\geq$1.6 ppm) relative to their corresponding signals in dA. This would be expected for modification of dA at N1 and is distinct

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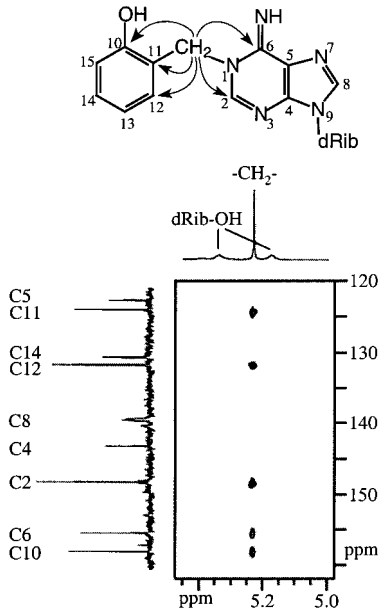


Figure 1. HMBC of the dA N1 adduct **1** in DMF-d_7.

from linkage at N^6 for which only the C6 signal is shifted by more than 1 ppm.[16,32] The absorbance maximum of the new derivative (260 nm, pH 4) is also consistent with an N1 adduct, because modification at N7 or N^6 would result in a 7−14 nm bathochromic shift relative to that of dA.[33]

While ^{13}C chemical shifts and UV absorbance data are suggestive of modification at dA N1, unambiguous assignment of the structure was established by HMBC long-range correlations between the benzylic protons and the purine carbons (Figure 1). Connectivities were observed between these protons and both C2 and C6 of dA. The additional connectivities associated with the phenolic carbons C10, C11, and C12 are intrinsic to QMP and independent of adduct structure. All data ascribed to the purine are uniquely satisfied by the anticipated properties of an N1 adduct. None of the competing nucleophiles of dA, N3, N7, or N^6 would exhibit dual correlation to the C2 and C6 positions.

The proficiency of forming the N1 adduct of dA under mild conditions broadened the possible origins of the N^6 adduct isolated from reaction with DNA. Perhaps these derivatives formed independently and competitively, or perhaps these adducts were related through an isomerization mechanism. The latter process was at first considered probable because of extensive precedence for a Dimroth rearrangement that converts N1 alkylated derivatives to their N^6 isomers (Scheme 2).[7,22−28] To explore this and alternative mechanisms, the N1 adduct of dA was resynthesized using QMP and [6-^{15}N]-dA. As expected, the ^1H NMR of the ^{15}N-product showed the presence of a doublet ($J = 66$ Hz) for the *exo*-imino proton (7.35 ppm) (Figure 2). This assignment was verified by the corresponding doublet (164.8 ppm) with the same coupling constant observed by ^{15}N NMR. Both chemical shifts and the ^1J (N, H) coupling constant are consistent with a terminal sp^2-hybridized ^{15}N resonance with a single attached proton.[34,35] The unusual peak shape of the ^1H resonance, evident for both the ^{14}N and ^{15}N derivatives, suggests

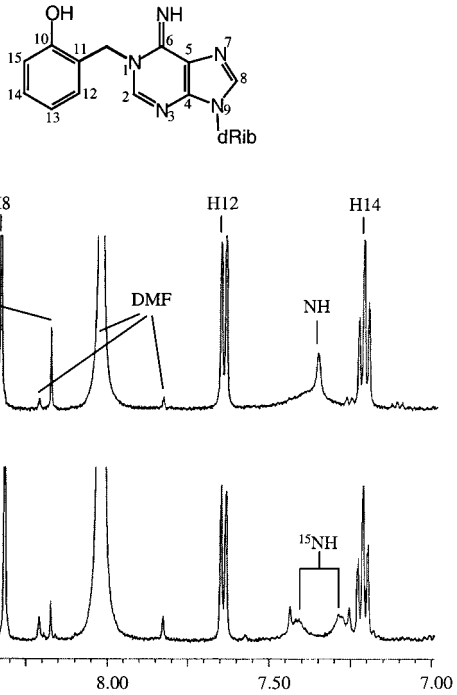
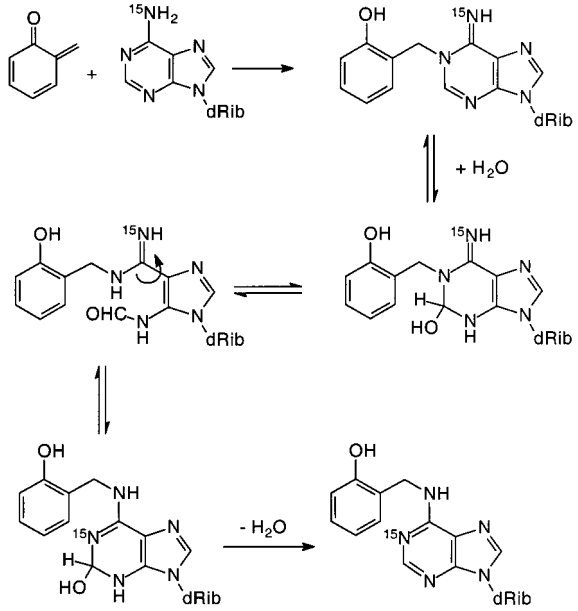


Figure 2. Selected region of the ^1H NMR spectra of (A) the N1 adduct of unlabeled dA and (B) the N1 adduct of [6-^{15}N]-dA.

Scheme 2



the formation of two solution conformers that may be analogous to those detected previously for the related N^6 adduct.[16] The [^{15}N]-labeled material generated here was particularly helpful in characterizing the pH-dependent consumption of the dA N1 adduct and distinguishing intra- and intermolecular transfer by isotope dilution experiments described in a later section.

Direct Alkylation versus Dimroth Rearrangement in Formation of the dA N^6 Adduct. [6-^{15}N]-dA was at first submitted to conditions similar to those used previously to generate the N^6 adduct with QMP (aq DMF, 50 °C, 14 h).[16] Direct alkylation would link the exocyclic ^{15}N with the benzylic position of the transient electrophile, whereas a Dimroth rearrangement would place ^{15}N in the N1 position of the purine ring and a benzylic substituted ^{14}N in the exocyclic position (Scheme 2). NMR analysis of the resulting product indicated

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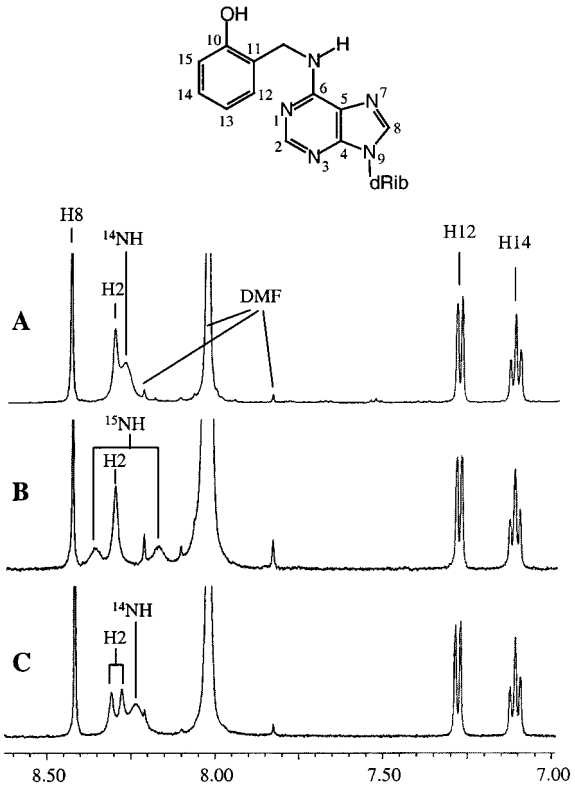

Figure 3. Selected region of the ^{1}H NMR spectra of (A) the N^6 adduct of unlabeled dA, (B) the N^6 adduct of [6-^{15}N]-dA, and (C) the N^6 adduct of [1-^{15}N]-dA.

that reaction occurred directly at the exocyclic ^{15}N, and no evidence for rearrangement was detected. The proton signal associated with the exocyclic amine (8.25 ppm) was split into a doublet by the presence of ^{15}N and exhibited a large coupling constant (94 Hz) (Figure 3A,B). This value is characteristic of one bond coupling ($\sim$ 90 Hz) and much larger than that expected for a two bond coupling indicative of a possible Dimroth product ($\sim$20 Hz).[23,28,35] ^{15}N NMR also confirmed the large coupling constant of the ^{15}N signal. In addition, its chemical shift (89.6 ppm) is consistent with a [6-^{15}N]-alkylamine derivative of dA[36] and expected for direct alkylation of the exocyclic N^6 position. At least under the conditions used to prepare the nucleoside standards of DNA alkylation, the N1 and N^6 adducts of dA are not related through a Dimroth rearrangement.

pH Dependent Decomposition of the dA N1 Adduct. The fate of the N1 adduct was next examined because it appeared to form most readily under mild conditions but neither persisted nor converted directly to its more stable N^6 derivative. Solutions of the N1 adduct (7 mM) were incubated at 37 °C in 30% aq DMF, pH 4.0, 7.5, 10.0, and 13.0, and monitored over time using reverse-phase C-18 HPLC. Only a minor fraction of this N1 alkylated derivative ($\sim$ 5%) hydrolyzed under the acidic conditions over 10 h, and all loss generated an equivalent quantity of dA. In contrast, the N1 derivative was much less stable under mild alkaline conditions. Approximately 95% of this derivative hydrolyzed to dA within 4 h (pH 7.5 and 10.0). Interestingly, an additional 2% appeared instead to form the N^6 adduct. Isomerization to the N^6 adduct dominated rapid consumption of the initial N1 adduct at pH 13. Within 2 h, 95% of this N1 adduct was converted to its N^6 isomer, and no competing hydrolysis to form dA was evident.

Despite the lack of Dimroth rearrangement observed during synthesis of the N^6 standard above, conversion of the N1 to N^6 adduct at high pH was reminiscent of the known promotion of this rearrangement under alkaline conditions.[26] Accordingly, the N1 adduct of [6-^{15}N]-dA described earlier was isomerized to its N^6 derivative at pH 13, isolated by HPLC and characterized by NMR. In this case, the proton signal split by ^{15}N corresponded to the purine H2 (8.29 ppm), and its coupling constant (16 Hz) typified a two bond ^{1}H$-^{15}$N coupling (Figure 3C).[27,28,37] Again, these assignments were supported by complementary ^{15}N NMR analysis revealing a doublet (J = 16 Hz) at 229 ppm. This ^{15}N chemical shift is typical of a nonterminal sp^2 hybridized ^{15}N such as the N1 position of dA.[27,37] These results are most easily explained by formation of the N^6 adduct of [1-^{15}N]-dA through a Dimroth rearrangement. Consequently, alkylation of dA by the model quinone methide generates the N1 adduct as its kinetic product which then undergoes a Dimroth rearrangement under alkaline conditions. The absence of this rearrangement under neutral conditions suggested that an alternative intra- or intermolecular transfer of the quinone methide equivalent is likely responsible for generating the thermodynamically favored N^6 derivative identified previously from reaction of DNA.[16]

Intra- versus Intermolecular Isomerization of the N1 Adduct of [6-^{15}N]-dA. Isotope dilution experiments using the N1 adduct of [6-^{15}N]-dA in the presence of excess unlabeled dA provided a convenient method for detecting additional mechanisms by which the N1 adduct might still contribute to the formation of its N^6 derivative even at a pH below $\sim$13 (Scheme 4). The associative nature of an intramolecular isomerization would dictate complete retention of ^{15}N in the final alkylation product, while dissociative and intermolecular processes would allow for loss of this label. One equivalent of the N1 adduct of [6-^{15}N]-dA was consequently submitted along with 10 equivalents of unlabeled dA to the conditions used initially for generating the synthetic standard of the N^6 adduct (50% aq DMF, $\sim$ 1 M KF, 50 °C, 14 h). Although potassium fluoride was not necessary to initiate the reaction, it was added to maintain reaction conditions consistent with those needed for inducing the original conversion of QMP. The N^6 adduct did indeed form during this incubation and was isolated by HPLC in a 50% yield based on the initial N1 adduct. Mass spectral analysis of this product (FAB) detected only 9 $\pm$ 1% of the original ^{15}N label (>99%) that was present in the parent N1 adduct.

This result suggests that a statistical equilibration was established between the labeled and unlabeled dA which in turn supports a fully dissociative regeneration of the reactive quinone methide intermediate. Thus, the N1 adduct of dA serves as a source for this electrophilic intermediate in a manner similar to that previously illustrated for the desilylated QMP.[16,17,29] In addition, nucleophilic reaction of dA N^6 is highly competitive with that of water in order to maintain a 50% yield of its N^6 adduct in the presence of $\sim$40 mol excess of water. Such a slow addition of water to an *ortho*-quinone methide has recently been established through comparative studies with O-, N-, and S-nucleophiles.[19] Finally, the ^{15}N/^{14}N ratio observed here would also be consistent with an associative S_N2 displacement of the dA N1 moiety by the N^6 position of a competing dA. However, the lack of detectable benzylic displacement from the *O*-silyl protected QMP and its *O*-methyl derivative would discount this alternative.[1g,16,38]

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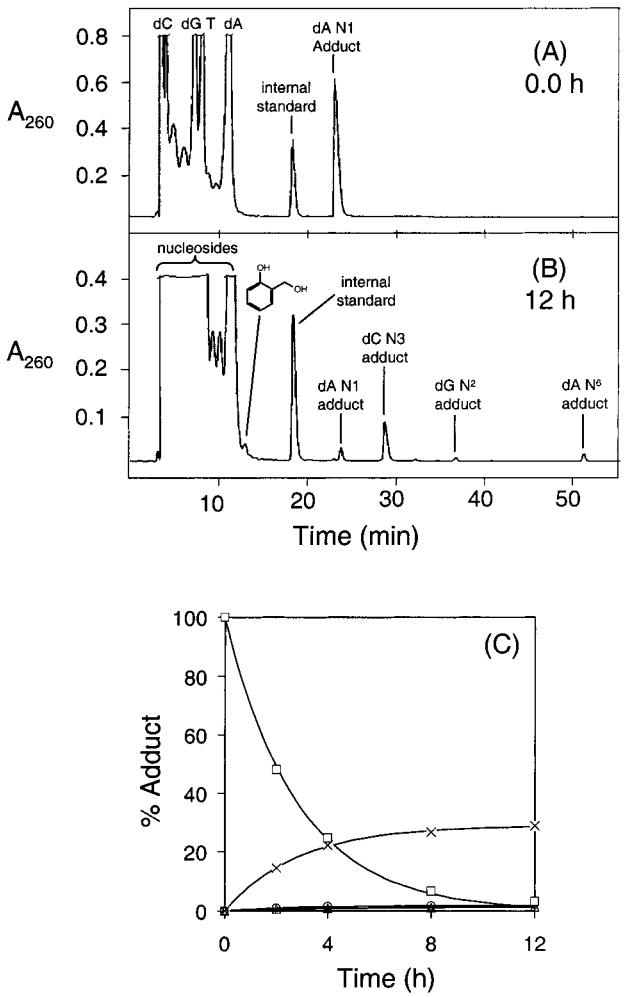

Figure 4. Quinone methide transfer from a transient nucleoside adduct. The dA N1 adduct was incubated at 37 °C with excess dC, dG, dA and T in the presence of an internal standard (phenol). Products were separated and quantified by reverse-phase (C-18) chromatography as illustrated after (A) 0 h and (B) 12 h. Consumption of the dA N1 adduct (□) and formation of the dC N3 (×), dG N^2 (△), and dA N^6 (○) adducts were monitored over time and fit by nonlinear regression to first-order processes as represented by the solid lines.

Quinone Methide Transfer from the N1 Adduct of dA to Other Deoxynucleosides of DNA. A logical extension of the intermolecular isomerization observed above would predict equivalent transfer of the quinone methide moiety from the N1 position of dA to all other nucleophilic sites on DNA that are already known to form adducts with this intermediate.[16,17] Such a transfer would begin to illustrate the diversity of pathways available for reagents or drugs that may act reversibly to modify DNA. The relative reactivity of each nucleoside had previously been examined by competition experiments in the presence of QMP, and these determinations were now repeated in the alternative presence of the dA N1 adduct. HPLC analysis (Figure 4) indicated that more than 95% of this initial N1 adduct was consumed within 12 h (30% aq acetonitrile, pH 7, 37 °C), and three stable deoxynucleoside adducts were generated concurrently in a combined yield of 30%. These products, the N^6 derivative of dA, the N^2 derivative of dG, and the N3 derivative of dC, correspond to the same major adducts formed with QMP.[16,17] Their relative abundance (1.3:1.0:19, respectively) once again illustrates the exceptional reactivity of dC that had been suggested by earlier studies with QMP.[16] As expected, no product associated with T was detected in these or related experiments.[16] Little of the remaining quinone methide equivalent was isolated as its hydrolysis product. Instead, it may have formed insoluble dimers, trimers, and higher molecular weight species as previously described for related structures.[39,40]

Discussion

Investigations based on an inducible system for generating a transient *ortho*-quinone methide (QMP) were originally used to differentiate the reactivity expressed by individual deoxynucleosides and that expressed collectively by duplex DNA.[16,17] The type of products identified by these studies, and particularly those formed by alkylation of the exocyclic amines of dG (N^2) and dA (N^6), were emblematic of DNA modification induced by a wide variety of quinone methide-like intermediates.[9,11,12,14,15] However, the origins of the dA adduct were sufficiently ambiguous to prompt additional analysis in hopes of gaining valuable insights into the apparent specificity of quinone methides for weak rather than strong nucleophiles. These efforts began with the successful isolation and characterization of an unstable product of dA alkylation that had been observed only transiently while first preparing a synthetic standard of the dA N^6 adduct.[16] This species has now been identified as the isomeric N1 adduct of dA through a variety of NMR experiments and most notably from the $^1H-^{13}C$ correlations observed by HMBC (Figure 1). This observation consequently demonstrates that strong nucleophiles of DNA are capable of reacting with the transient *ortho*-quinone methide model, even though their products may not be generally isolable after long incubations.

Potential conversion of the N1 adduct to its N^6 derivative was made possible through a Dimroth rearrangement, although the efficiency of this process is highly dependent on reaction conditions and adduct structure (Scheme 2).[26] This rearrangement also often requires alkaline conditions. Still, N1 adducts formed by 2-bromoethanol[41] and butadiene monoxide[42] both rearrange to their N^6 derivatives under neutral conditions. Such conditions also generate dA N^6 adducts of styrene oxide resulting from rearrangement of transient N1 analogues as well as from direct reaction with N^6.[24,28,43] In contrast, the N^6 adduct of dA formed with QMP in aqueous DMF did not originate from sequential N1 alkylation and intramolecular rearrangement. Retention of an ^{15}N label in the N^6 position of the dA adduct indicated direct reaction between the quinone methide and this weakly nucleophilic site of dA (Scheme 3, Figure 3B). A Dimroth rearrangement was only observed after the N1 adduct was incubated at pH 13 (Figure 3C). At lower pH (7.5 and 10), the N1 adduct merely hydrolyzed to reform dA in the absence of excess deoxynucleosides or other nucleophilic compounds. This result was the first to suggest that addition to the quinone methide was reversible. In this case, the dA N1 moiety would then act both as a strong nucleophile for coupling to the quinone methide and as a good leaving group for regenerating this same intermediate. Hence, an equilibrium may be established rapidly between the quinone methide and its kinetic product, the N1 adduct of dA. Ultimately, this process would dissipate as the quinone methide was slowly consumed by irreversible formation

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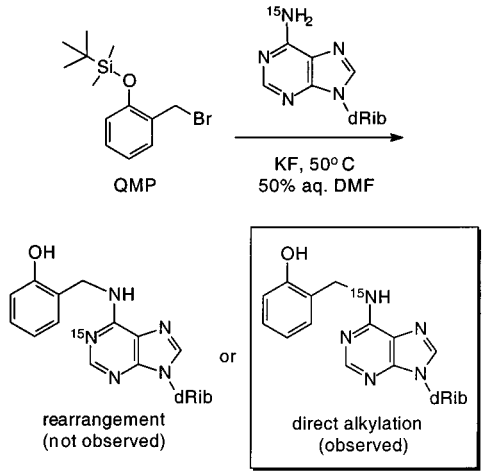
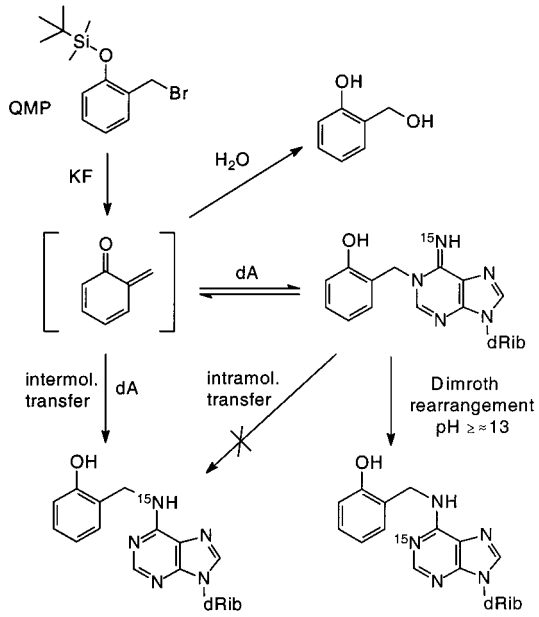
Scheme 3



Scheme 4



of thermodynamic products such as hydroxymethylphenol, the N^6 adduct of dA, and other stable derivatives of QMP (Scheme 4).

The general selectivity for reactions of DNA with the quinone methide and related intermediates may also reflect the thermodynamic stability rather than kinetic efficiency of product formation. In this case, the principles of hard and soft nucleophiles and electrophiles are not appropriate for rationalizing the commonly observed dominance of dG N^2 and dA N^6 modification in duplex DNA. Initial addition to quinone methides most likely exhibits a strong dependence on nucleophilicity[19] and may mimic the hierarchy of dimethyl sulfate-dependent alkylation via S_N2 substitution (dG N7 > dA N1 > dC N3).[2] Such a product profile would typically be obscured by decomposition of the unstable adducts that would serve to release the most potent nucleophiles and reform the quinone methide intermediate as illustrated with dA (Scheme 4).

The surprisingly high selectivity for modification of dC N3 originally observed at the nucleoside level with QMP was initially ascribed to a specific proton transfer from dC to the quinone methide[16] on the basis of its known sensitivity to protonation.[12,44] However, the balance of nucleophilicity and acidity for each site undergoing modification might also explain this product profile, if the reversibility of reaction is considered. Alkylation at dG N7 and dA N1 would likely form most readily because of the strong nucleophilicity of these sites. However, subsequent elimination of these nucleosides from their adducts would proceed equally well because of the high acidity of dG N7 and dA N1 (pK_a of 2.5 and 3.8, respectively).[45] The N3 position of dC is less nucleophilic and acidic (pK_a of 4.3).[45] Accordingly, dC may then trap the quinone methide less efficiently yet still dominate the profile by forming a persistent adduct. Adducts of the exocyclic amines of dG (N^2) and dA (N^6) are also stable and accumulate more slowly as expected from their even weaker nucleophilicity and acidity. These latter products ultimately prevail over the dC adduct for reaction of duplex DNA on account of the added steric and electrostatic properties of its helical structure.[4,16]

The nucleoside model system used in this investigation also demonstrates the general potential for purine adducts to act as effective sources and carriers of quinone methide equivalents (Figure 4). Although the role of the N1 position of dA is quite evident from reaction at the nucleoside level, equivalent activity in duplex DNA is likely to be severely inhibited by the constraints of base pairing. However, complete shielding of this position is not expected because at least a limited reactivity of comparable positions, dG N1 and dC N3, can be observed through formation of their stable quinone methide adducts in DNA.[16,17] Furthermore, dG N7 remains quite accessible within the major groove of duplex DNA and may represent the most significant target and carrier of the quinone methide. Transient formation of dG N7 adducts has previously been detected from reaction of dG with two related *para*-quinone methide derivatives, and in one example, alternative N1 and N^2 adducts were generated as the N7 adduct decomposed.[12] Low levels of the dG N7 adduct have also been detected in a competitive assay with QMP and the four deoxynucleotides, dG, dC, dA, and T.[17] Again, trace quantities of this species should not imply a low reactivity of dG N7 but rather a high lability of its corresponding adduct. Two paths of decomposition are available to this species. One regenerates the unmodified DNA and releases the quinone methide for further alkylation, and the other forms an abasic site and releases the stable N7 adduct of guanine. This latter derivative has also been isolated from reaction of DNA with *ortho*- and *para*-quinone methides.[12,17]

Attention to kinetic and thermodynamic selectivity will no doubt enhance our ability to predict modification of DNA by many reactive intermediates. The importance of this principle is becoming increasingly evident, as illustrated in recent studies on mitomycin C, ecteinascidin 743, and pyrrolizidine derivatives that identified a diversity of initial products not previously anticipated.[40,46] Such considerations should not be limited to alkylation, because, for example, carcinogenic arylamines can form dG N7 adducts that isomerize to their more stable derivatives linked through dG C8.[47] Finally, the ability of a transient intermediate to form metastable products essentially extends its effective lifetime and creates a reservoir of reactive equivalents. Reversible addition of certain amino acids and

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nucleosides to quinone methides may thus provide a method of dispersing an otherwise short-lived reactant throughout a biological system.[19] Recent investigations have confirmed this possibility elegantly by demonstrating that purine, pyrimidine, and amino acid adducts of malondialdehyde are more potent sources of malondialdehyde in vitro and in vivo than malondialdehyde itself.[48]

Experimental Section

General Materials and Methods. Chemicals and solvents were purchased from Fisher, Aldrich, and Sigma and used without further purification unless otherwise specified. Deuterated solvents for NMR spectroscopy were purchased from Cambridge Isotope Laboratories. All aqueous solutions were made with water purified by standard filtration to yield a resistivity of 18.0 MΩ. ^{1}H, ^{13}C, and ^{15}N NMR spectra (DMF-d_7) were recorded on an AMX 500 spectrometer (^{1}H, 500.13 MHz; ^{13}C, 125.77 MHz; ^{15}N, 50.70 MHz). Chemical shifts (δ) are reported in parts per million (ppm) relative to the standard values for DMF (^{1}H, ^{13}C) or to liquid NH_3 (^{15}N, 0 ppm) by an external standard of $^{15}NH_4{}^{15}NO_3$.[49] Coupling constants (J) are reported in hertz (Hz). Low resolution (LRMS) and high resolution (HRMS) mass spectra were determined on a VG7070E instrument using fast atom bombardment. Isotopic ratios (^{15}N/^{14}N) were determined using the mean of five measurements for each sample and control.[50] UV absorption spectra were obtained on a Hewlett-Packard 8453 UV−vis spectrophotometer. Reverse phase C-18 chromatography was performed on analytical (Varian Microsorb-MV C-18, 300 Å particle size, 250 mm × 4.6 mm) and semipreparative (Alltech Econosphere C-18, 10 μm particle size, 250 mm × 10 mm) columns using a Jasco PU-980 HPLC equipped with a Jasco MD-1510 UV−vis multiwavelength scanning detector. A solvent gradient of 3% acetonitrile in 49 mM triethylammmonium acetate, pH 4.0, to 25% acetonitrile in 38 mM triethylammonium acetate, pH 4, over 66 min (1 mL/min) was used for product analysis, and a solvent gradient of 5% acetonitrile in 48 mM triethylammmonium acetate, pH 4.0, to 30% acetonitrile in 35 mM triethylammonium acetate, pH 4, over 30 min (5 mL/min) was used for product isolation.

N1 Adduct of dA. 2′-Deoxyadenosine (6 mg, 20 μmol) and *O*-(*tert*-butyldimethylsilyl)-2-(bromomethyl)phenol (QMP, 11 mg, 37 μmol)[29] were dissolved in DMF (50 μL) and combined with an aqueous solution of KF (2.64 M, 50 μL). The reaction was stirred at 25 °C for 30 min and then directly subjected to preparative purification by HPLC to yield the desired adduct (6 mg, 75% yield). ^{1}H NMR δ 2.41 (m, 1H), 2.90 (m, 1H), 3.66 (m, 1H), 3.72 (m, 1H), 3.99 (m, 1H), 4.55 (m, 1H), 5.15 (bs, OH), 5.26 (s, 2H), 5.48 (bs, OH), 6.36 (t, 1H, J = 7.5), 6.81 (t, 1H, J = 7.5), 6.82 (d, 1H, J = 7.5), 7.20 (td, 1H, J = 7.5, 1.7), 7.35 (bs, NH), 7.63 (dd, 1H, J = 7.5, 1.7), 8.33 (s, 1H), 8.73 (s, 1H). ^{13}C NMR δ 41.1, 48.2, 62.9, 72.0, 85.2, 89.4, 119.2, 119.8, 122.9, 124.2, 130.9, 131.9, 139.7, 143.4, 148.5, 155.7, 158.3. HRMS (FAB, glycerol) m/z 358.1520 (M + H$^+$); calcd for $C_{17}H_{20}N_5O_4$ (M + H$^+$), 358.1515.

N1 Adduct of [6-^{15}N]-dA. [6-^{15}N]-2′-Deoxyadenosine (9 mg, 30 μmol)[37] and QMP (16 mg, 53 μmol) were dissolved in DMF (75 μL) and combined with an aqueous solution of KF (2.64 M, 75 μL). The reaction was stirred at 25 °C for 30 min and then directly subjected to preparative purification by HPLC to yield the desired adduct ^{15}N-labeled adduct (6 mg, 50% yield). ^{1}H NMR δ 2.41 (m, 1H), 2.90 (m, 1H), 3.66 (m, 1H), 3.72 (m, 1H), 3.99 (m, 1H), 4.55 (m, 1H), 5.25 (s, 2H), 6.36 (t, 1H, J = 7), 6.81 (t, J = 7.5, 1H), 6.82 (d, 1H, J = 7.5), 7.20 (td, 1H, J = 7.5, 1.7), 7.35 (d, ^{15}NH, J = 66), 7.64 (dd, 1H, J = 7.5, 1.7), 8.32 (s, 1H), 8.72 (s, 1H). ^{15}N NMR δ 164.8 (d, ^{15}NH, J = 66). HRMS (FAB, glycerol) m/z 359.1495 (M + H$^+$); calcd for $C_{17}H_{20}N_4{}^{15}N_1O_4$ (M + H$^+$), 359.1486. Isotope ratio analysis by LRMS (FAB) showed >99% ^{15}N in the product.

N^6 Adduct of [6-^{15}N]-dA.[16] [6-^{15}N]-2′-Deoxyadenosine (6 mg, 20 μmol)[37] and QMP (11 mg, 37 μmol) were dissolved in DMF (50 μL) and combined with an aqueous solution of KF (2.64 M, 50 μL). The reaction was stirred at 50 °C for 14 h, cooled, and then directly subjected to preparative purification by HPLC to yield the desired ^{15}N-labeled adduct (1 mg, 13% yield). ^{1}H NMR δ 2.38 (m, 1H), 2.86 (m, 1H), 3.67 (m, 1H), 3.76 (m, 1H), 4.03 (m, 1H), 4.58 (m, 1H), 4.76 (s, 2H), 5.42 (d, OH, J = 4.0), 5.46 (t, OH, J = 5.1), 6.49 (dd, 1H, J = 6.0, 8.0), 6.77 (t, 1H, J = 8.0), 6.89 (dd, 1H, J = 8.0, 0.9), 7.11 (td, 1H, J = 8.0, 0.9), 7.28 (dd, 1H, J = 8.0, 0.9), 8.25 (d, ^{15}NH, J = 94.2), 8.29 (s, 1H), 8.42 (s, 1H), 10.49 (bs, ArOH). ^{15}N NMR δ 89.6 (d, ^{15}NH, J = 94). HRMS (FAB, glycerol) m/z 359.1499 (M + H$^+$); calcd for $C_{17}H_{20}N_4{}^{15}N_1O_4$ (M + H$^+$), 359.1486.

pH Dependent Stability of the N1 Adduct of dA. Aq DMF (30%) solutions of the dA adduct (7 mM) in 70 mM buffer (pH 4, sodium acetate; pH 7.5, potassium phosphate; pH 10, sodium carbonate; and pH 13, potassium phosphate) were incubated at 37 °C. Aliquots were then analyzed by HPLC at the indicated times (pH 4, 2 and 10 h; pH 7.5, 2 and 4 h; pH 10 and 13, 2 h).

N^6 Adduct of [1-^{15}N]-dA Formed by Dimroth Rearrangement. The N1 adduct of [6-^{15}N]-dA (2 mg, 6 μmol) was dissolved in aq potassium phosphate (800 μL, pH 13, 30% DMF) and incubated at 37 °C for 2 h. The mixture was then dried under reduced pressure, redissolved in methanol (100 μL), and directly isolated by preparative HPLC to yield the rearranged adduct (1 mg, 50% yield). ^{1}H NMR δ 2.39 (m, 1H), 2.85 (m, 1H), 3.67 (m, 1H), 3.76 (m, 1H), 4.03 (m, 1H), 4.58 (m, 1H), 4.77 (s, 2H), 5.42 (bs, 2H, OH), 6.49 (dd, 1H, J = 6.0, 8.0), 6.77 (t, 1H, J = 8.0), 6.89 (dd, 1H, J = 8.0, 0.9), 7.11 (td, 1H, J = 8.0, 0.9), 7.28 (dd, 1H, J = 8.0, 0.9), 8.23 (bs, NH), 8.29 (d, 1H, J = 16.0), 8.42 (s, 1H), 10.43 (bs, ArOH). ^{15}N NMR δ 227.1 (d, ^{15}N^1, J = 16). HRMS (FAB, glycerol) m/z 359.1497 (M + H$^+$); calcd for $C_{17}H_{20}N_4{}^{15}N_1O_4$ (M + H$^+$), 359.1486.

Isotope Dilution during Intermolecular Isomerization from the N1 to N^6 Adduct of dA. The N1 adduct of [6-^{15}N]-dA (2 mg, 6 μmol) and dA (15 mg, 60 μmol) were dissolved in DMF (40 μL) and combined with an aqueous solution of KF (2.64 M, 40 μL). The reaction mixture was incubated at 50 °C for 14 h and then subjected directly to preparative HPLC to yield the partially ^{15}N-enriched N^6 adduct of dA (1 mg, 50%). ^{1}H NMR data were consistent with a standard sample of the N^6 adduct of dA.[16] Isotope ratio analysis by LRMS (FAB) showed 9% ^{15}N in the product.

Competitive Alkylation of Deoxynucleosides by Quinone Methide Transfer from the N1 Adduct of dA. The N1 adduct of dA (1.6 mM), each deoxynucleoside (dG, dC, dA, and T) (4 mM), and phenol (internal standard, 4 mM) were dissolved in aq potassium phosphate, pH 7 (160 mM, 1 mL, 30% CH_3CN), and incubated for 24 h (37 °C). Aliquots were then removed at the indicated times (Figure 4C) and analyzed by HPLC. Each product was normalized with respect to the ϵ_{260} values of their parent deoxynucleoside.[45] The reported product distribution represents the average of two such determinations (± 6%).

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Supporting Information Available: Full HMQC and HMBC of the dA N1 adduct (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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