Chemical Research in Toxicology

JUNE 2000
VOLUME 13, NUMBER 6

Communications

Predicted Exocyclic Amino Group Alkylation of 2′-Deoxyadenosine and 2′-Deoxyguanosine by the Isopropyl Cation

Patrick Blans and James C. Fishbein*

Department of Chemistry and Biochemistry, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, Maryland 21250

Received March 10, 2000

Diisopropyltriazene in aqueous 10% acetonitrile (pH 7.0 ± 0.4) yields N^6-isopropyl-2′-deoxyAdo as the predominant product and N^2-isopropyl-2′-deoxyGuo in yields comparable with the O^6 adduct in reactions with 2′-deoxyAdo and 2′-deoxyGuo, respectively. These observations are inconsistent with what is expected on the basis of the regnant hypothesis concerning factors that determine atom site selectivity in diazonium ion-mediated alkylations. An alternative explanation based on the fleeting existence of the reactive intermediates involved is consistent with these observations.

Introduction

Diazonium ions and carbocations mediate the DNA base alkylating activity of mutagenic and carcinogenic nitrosamines, nitrosamides, triazenes, and compounds with related structure (*1*−*6*). This alkylating activity is generally believed to be responsible for their deleterious biological activities. In the simplest cases, the activities of compounds that give rise to the methyl and ethyldiazonium ions are believed to be manifest by the formation of O^6-alkylGua and O^4-alkylThy adducts (*7*−*9*).

The factors that give rise to the atom site selectivity of diazonium ion-mediated alkylations have been of significant interest because the biologically active oxygen adducts are typically minor products (*10*−*14*). The regnant notion in this matter holds that the "S_N1/S_N2 character" of the substitutions and the hardness or softness of electrophiles and nucleophilic atoms are the major determinants, at least in consideration of purine adducts. In this framework, for guanine base, exocyclic oxygen methylation is more extensive, at the expense of endocyclic N7 methylation, with the methyldiazonium ion than with methyl methanesulfonate because of the greater "S_N1 character" of the diazonium ion substitution. The absence of N^2 adducts from either electrophile is ascribed to the softness of this site, relative to the "harder" O^6 atom. With the ethyldiazonium, purportedly a harder cation, a more "S_N1-like" substitution shifts a larger proportion of the products to the exocyclic O^6, again with no N^2 alkylation reported. The isopropyldiazonium ion has not been studied, though it has explicitly been predicted, on the basis of greater hardness, to yield more O^6 adduct and, presumably, no N^2 adduct (*13*). In the case of the adenine base, the exocyclic N^6 atom is the "soft" site. Neither methyl- nor ethyldiazonium ion alkylates N^6.

In contrast, we predicted in the case of secondary (*sec*-) carbocations arising from *sec*-diazonium ions that the

10.1021/tx000059j CCC: $19.00
Published on Web 05/06/2000

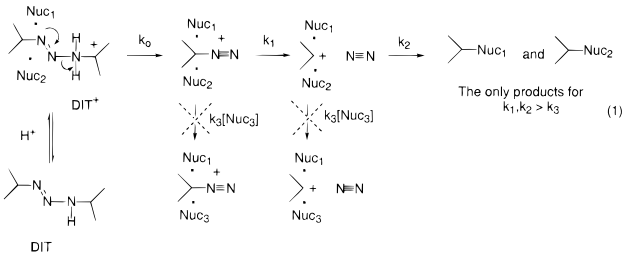
Table 1. Yields of Isopropylated Bases in the Reaction of Diisopropyltriazene with 2′-DeoxyAdo and 2′-DeoxyGuo in Aqueous Solutions of 10 vol % Acetonitrile[a]

2′-deoxyAdo reaction product	%	2′-deoxyGuo reaction product	%
1-isopropylAde	<0.002	1-isopropylGua	sna[b]
3-isopropylAde	<0.003	N^2-isopropylGua	0.025 (±0.003)
N^6-isopropylAde	0.022 (±0.001)	3-isopropylGua	0.008 (±0.001)
7-isopropylAde	0.009 (±0.001)	O^6-isopropylGua	0.077 (±0.001)
		7-isopropylGua	sna[b]

[a] Values are the averages of duplicate determinations in three different experiments. [b] Standard not available.

nucleophilic character of the atoms would be unimportant in alkylation site selectivity (*15*). This prediction was based on the conclusion that *sec*-diazonium ions form *sec*-carbocations, and the *sec*-carbocations react indiscriminately with adjacent nucleophiles, in both cases with rate constants that are faster than that of diffusion. This conclusion was based on the lack of discrimination between nucleophiles with very different nucleophilicities in alkylation by benzylic and *sec*-carbocations, the observation of products derived from "internal return" of fragments of the diazonium ion precursor in the products of alkylation, and the observation that ratios of even solvent-derived products in mixed solvents were not controlled by solvent nucleophilicities.

The consequence is further illustrated in a mechanism for decomposition of diisopropyltriazene (DIT) that is summarized in eq 1[1] (*16*−*18*).



The rate constants for *sec*-diazonium ion dissociation (k_1) to carbocation and subsequent reaction (k_2) of isopropyl carbocation with adjacent (even solvent) nucleophiles (Nuc_1 and Nuc_2) are faster than that of diffusion (k_3) to another nucleophile (Nuc_3) that is required for any significant selectivity. Thus, the products are constrained by indiscriminate reaction with nucleophiles in the solvation shell of the protonated triazene (DIT^+) at the time it decomposes, and not by the ability of electrophiles to diffuse among and select between nucleophiles on the basis of nucleophilicity. Because the association constants for association of DIT^+ with different atom sites are expected to be similar, the formation of simple alkyl adducts of the exocyclic amino groups of purines was anticipated.

We report here the formation of N^6-isopropyl-2′-deoxyAdo as the predominant product and N^2-isopropyl-2′-deoxyGuo in yields comparable with that of the O^6 adduct in the reactions of diisopropyltriazene with 2′-deoxyAdo and 2′-deoxyGuo, respectively.

Experimental Section

Materials. [8-^{14}C]-2′-DeoxyAdo and [8-^{3}H]-2′-deoxyGuo were purchased from Moravek Biochemicals Inc. (Brea, CA). 1,3-Diisopropyltriazene (DIT) was a gift from C. J. Michejda (Frederick Cancer Research Center, Frederick, MD). Isopropyl methanesulfonate, 3-, N^6-, and 7-isopropylAde, N^2- and 3-isopropylGua, and O^6-isopropylGua were prepared by literature methods (*19*−*21*). O^6-Isopropyl-2′-deoxyGuo was prepared by O^6-alkylation of N^2-, 3′-*O*-, or 5′-*O*-triacetyl-2′-deoxyGuo (*22*−*24*).

Methods. (1) General Procedures for Reaction of DIT with Nucleosides. The experiments were carried out by mixing, using a stopped-flow apparatus [$t_{1/2} \sim 2$ s (*18*)], an acetonitrile solution of DIT with 10 volume equivalents of a buffered [0.15 M phosphate (pH 7.0 ± 0.4)] aqueous solution containing the radioactive nucleoside. The reaction mixture was stirred at room temperature for 30 min. The final DIT concentration was 0.08 M. After completion of the reactions, the mixtures were subjected to hydrolysis in 0.1 M HCl at 80 °C for 45 min and neutralized.

(2) Analysis of Isopropylpurine Adducts. To aliquots of the neutralized acid were added synthetic standards. The solution was chromatographed on a Waters HPLC system using a Phenomenex 5 μm C18 Luna column and methanol/water [0.01 M phosphate buffer (pH 5.8)] gradients with UV/vis diode array and radiochemical detectors in sequence. The inefficiency of the alkylation resulted in a large "off-scale" radioactive signal for unadducted purines that eluted between 16 and 30 min. The identity and amount of the products of the reaction were determined from the radioactive peaks which coeluted with the UV peaks of the standards. Two different sets of chromatography conditions were used for a single sample. In each case, the radioactive peaks eluted with the same standard under each condition. Doubling of reaction or acid hydrolysis times altered the yields of products by less than ±12%.

(3) Controls for Analytical Methods. (i) Reactions of 2′-DeoxyAdo. Control experiments involving the incubation of 2′-deoxyAdo with buffer, isopropylamine, and 2-propanol and subsequent acid hydrolysis gave some unidentified signals that did not obviate quantitation of the known adducts. 1-IsopropylAde was indirectly identified by the conversion of 1-isopropylAde to N^6-isopropylAde under basic conditions (Dimroth rearrangement) (*25*, *26*). After reaction of 2′-deoxyAdo with DIT, the nucleoside was subjected to a basic hydrolysis (pH 13, for 24 or 48 h at 40 °C) followed by acid hydrolysis, as described above. A control experiment involved isopropylation of 2′-deoxyAdo by isopropyl methanesulfonate. The reaction was carried out over 24 h and involved three additions of a 400-fold molar excess of alkylating agent with intervening additions of NaOH to maintain the pH. The reaction after each addition was essentially complete within 5 h as indicated by the absence of a pH change in the last 0.5 h of this reaction period.

(4) Reactions of 2′-DeoxyGuo. Acid hydrolysis of authentic O^6-isopropyl-2′-deoxyGuo in the presence of all reaction components except diisopropyltriazene indicated a >95% yield of the O^6-isopropylGua.

Results

The quantitative summary of the adduction in aqueous solution of 2′-deoxyAdo and 2′-deoxyGuo by diisopropyltriazene is included in Table 1. The data were derived from HPLC analysis of products of reactions with radiolabeled nucleosides, after acid hydrolysis to the bases. Typical product profiles are shown in Figures 1 and 2. The upper panels of each figure were generated from UV absorbance, and the lower panels were the output of a radiochemical detector. The UV signals are the result of doping the reaction mixes after reaction with nonradiolabeled standards.

The major product in reactions of 2′-deoxyAdo with diisopropyltriazene was N^6-isopropylAde, indicated by the coelution of the UV standard (upper panel) with the radioactivity (lower panel). Elution positions for 7- and 3-isopropylAde are indicated by the UV trace in the upper panel of Figure 1. An upper limit for 1-isopropylAde was

[1] An alternative to eq 1 entails formation of the carbocation directly from DIT^+ (*18*), but in either case, both *sec*-diazonium ions and *sec*-carbocations react faster than diffusion (*15*).

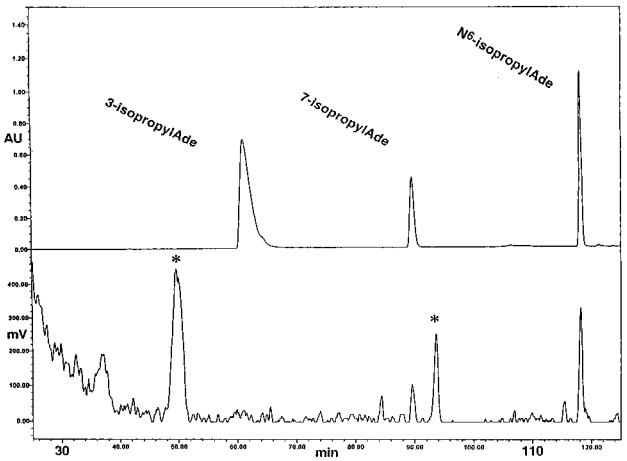


Figure 1. Chromatograms indicating UV absorption (top) and radioactivity (bottom) signals from the reaction (pH 7 ± 0.4, aqueous 10% acetonitrile) of [8-^{14}C]-2′-deoxyAdo with 1,3-diisopropyltriazene followed by acid hydrolysis and addition of 3-, 7-, and N^6-isopropylAde standards. The peaks marked with an asterisk were identified in similar amounts in control experiments which lacked triazene but contained buffer, nucleoside, 2-propanol, and isopropylamine at concentrations equal to that found at the normal reaction end point.

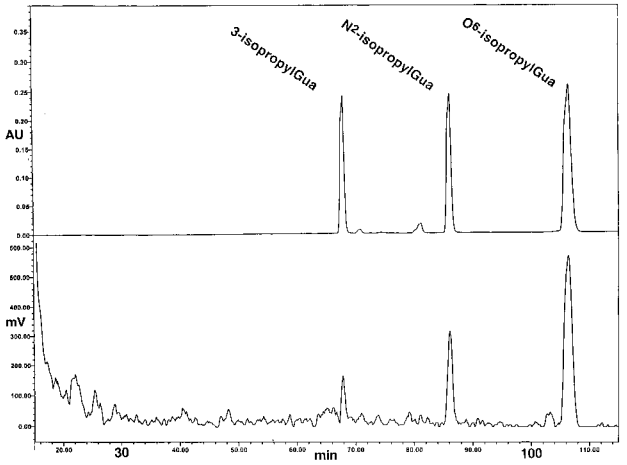


Figure 2. Chromatograms indicating UV absorption (top) and radioactivity (bottom) signals from the reaction (pH 7 ± 0.4, aqueous 10% acetonitrile) of [8-^{3}H]-2′-deoxyGuo with 1,3-diisopropyltriazene followed by acid hydrolysis and addition of 3-, N^2-, and O^6-isopropylGua standards. Control experiments similar to those described in the legend of Figure 1 were devoid of signals in this region.

determined indirectly (see Methods) by initial base hydrolysis after triazene reaction to convert the 1-isopropyl-2′-deoxyAdo to N^6-isopropyl-2′-deoxyAdo. Comparison of the yield of N^6-isopropylAde from the subsequent acid hydrolysate of this reaction with that from a portion of the reaction mix not subjected to base hydrolysis showed a less than 10% increase in the amount of N^6 adduct.

A control experiment (see Methods) was undertaken in which isopropyl methanesulfonate was reacted with 2′-deoxyAdo, and the results indicate that less than 10% of the 1-isopropylAde formed in this reaction rearranges to N^6-isopropylAde over a 3 h incubation at neutral pH. Acid hydrolysis of a portion of the reaction mixture after reaction for 24 h indicated that the yields of 3-, N^6-, and 7-isopropylAde adducts were 0.11, 0.30, and 0.27%, respectively. Additional incubation (3 h) of the remaining unhydrolyzed material, followed by acid hydrolysis of a portion, produced an increase in the amount of N^6-isopropylAde of less than 12%. Base hydrolysis of the unhydrolyzed material remaining after the additional 3 h incubation period, followed by acid hydrolysis, revealed a 104% increase in the magnitude of the signal for N^6-isopropylAde, indicating a 0.32% yield of 1-isopropyl-2′-deoxyAdo. Thus, over the 3 h incubation period, a maximum of 10% [=12%/(104% + 12%)] of 1-isopropyl-2′-deoxyAdo was converted to N^6-isopropyl-2′-deoxyAdo.

The major product detected in the reaction of triazene with 2′-deoxyGuo, after acid hydrolysis, was O^6-isopropylGua, with N^2-isopropylGua being a third less abundant. Neither 7- nor 1-isopropylGua standards were available for determining the elution times of these adducts by UV detection.

Discussion

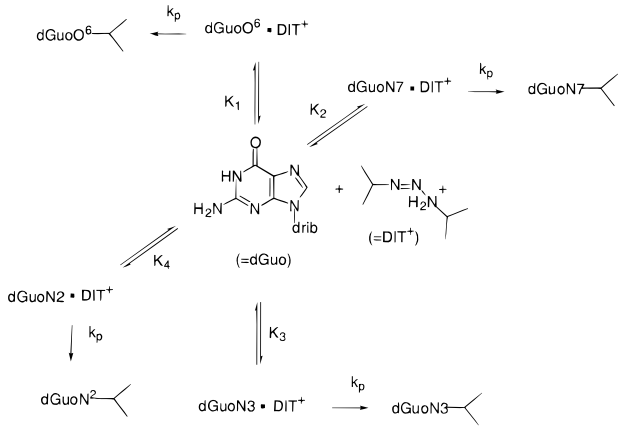
Isopropylation of Purine Exocyclic Amino Groups. To our knowledge, the data summarized in Table 1, which demonstrate the binding of a diazonium ion-derived alkyl fragment to the exocyclic amino groups of purines, are unprecedented in reactions with nucleosides or DNA. The data in Table 1 indicate that in the reaction with 2′-deoxyAdo, the N^6-isopropyl product is predominant, with the yield of the 7-isopropyl adduct being ∼45% of this, and upper limits for the 1 and 3 adducts being 7−10-fold smaller than that of the N^6 adduct. In the case of the reaction with 2′-deoxyGuo, the yield of the N^2 adduct is $^1/_3$ of that of the O^6 adduct, while the yield of the 3 adduct is 10-fold lower than that of the O^6 adduct. The yield of the N^2 adduct relative to that of the putatively mutagenic O^6 adduct is remarkable. By comparison, in reactions of the ethyl diazonium ion with DNA, the N^2 adduct was not detected while the O^6 adduct yield was more than 20 times that of the least prevalent quantifiable adduct, 3-Cyt (*10*).

The N^6-isopropylAde that formed does not arise from the Dimroth rearrangement of an initial 1-isopropyl-2′-deoxyAdo. A control experiment (Results) shows that 1-isopropyl-2′-deoxyAdo rearranges to the N^6 isomer to an extent of <10%, over 3 h at pH 7. The data in Table 1 for the triazene reaction involve only 30 min of reaction. Because the yield of 1-isopropylAde is <10% of that of N^6-isopropylAde in this reaction, the yield of N^6-isopropylAde resulting from rearrangement is negligible, <1% of the observed yield of the N^6 adduct.

The substantial relative yields of N^6-isopropylAde and N^2-isopropylGua (Table 1) disprove the regnant hypothesis (13) that the isopropyldiazonium ion should not alkylate the "soft" exocyclic purine nitrogen atoms.

An Alternative Hypothesis. The very short lifetimes of the *sec*-diazonium ions and carbocations, and the indiscriminate alkylation by the latter, account for the observed alkylation of exocyclic amino groups (*15*, *27*, *28*). This high reactivity means that the distribution of adducts is determined mainly by the equilibrium constants for formation of encounter complexes, as indicated in Scheme 1. In Scheme 1, the association of the diazonium ion precursor, the triazenium cation (DIT$^+$), with various sites of 2′-deoxyGuo (dGuo) is indicated by the equilibria (K_{1-4}), and the products that are formed (indicated by the isopropyl groups bonded to numbered atoms) arise from the k_p steps (condensed versions of the general eq 1). The rate-limiting step in product formation is DIT$^+$ bond cleavage, and the rate of this process is

Scheme 1



independent of atom nucleophilicity (*16*−*18*). The carbocations subsequently formed in the encounter complexes in Scheme 1 select between alkylation of the specific base nucleophilic atoms with which they are associated and alkylation of surrounding solvent molecules. There is considerable evidence that cations of this type do not discriminate in bond formation between nucleophiles with very different nucleophilicities (*15*, *27*, *28*). Therefore, the yield of adducted nucleoside from any of the encounter complexes is likely to be similar among the different complexes. The yield of any particular adduct, and therefore the product distribution as a whole, is thus controlled mainly by differences in the amounts of each association complex, i.e., differences in K_{1-4}.

Secondary diazonium ions and the subsequent carbocations formed outside the contact distance of the atom sites, or even formed in contact with one atom site, survive for an insufficient amount of time to diffuse up to, or among, and select between base nucleophilic atoms which of necessity involves encounter of, and thus capture by, solvent (*15*, *27*). This type of reaction involving intermediates that react with solvent molecules faster than diffusion has been referred to as a "pre-association" mechanism because reactions with molecules other than solvent must occur by complex formation that precedes formation of the reactive intermediate formation (*15*).

The observed preferential alkylation of exocyclic sites (N^6 of 2′-deoxyAdo and O^6 and N^2 of 2′-deoxyGuo) is possibly due to sterically more favorable association or the incursion of π-cation (triazenium ion) complexes that could conceivably favor exocyclic atom alkylation. The possible involvement of such complexes in aromatic alkylations by diazonium ions has been recently suggested (*27*). Differences in the association constants in Scheme 1 that would give rise to the selectivity observed for the data in Table 1 require differences between the various complexes in interaction free energies of a little more than 1 kcal/mol.

Generality and Importance. A wide variety of simple diazonium ions can be anticipated to give rise to exocyclic amino adducts. Among the known nitrosamines and triazenes that are carcinogenic and mutagenic are a number that give rise to *sec*-diazonium ions and thus *sec*-carbocations (*1*, *2*, *4*, *29*). Notably, P450 enzyme hydroxylation of a number of the tobacco specific nitrosamines is known, or likely, to occur at sites that give rise to *sec*-diazonium ions (*30*). On the basis of the results presented here, exocyclic amino group alkylation is to be anticipated in these cases. Beyond this, all nitrosamines that give rise to *primary* diazonium ions can give rise to such adducts. This conclusion is based on the fact that most primary diazonium ions more complex than the ethyl diazonium ion decompose with extensive, 20−50%, skeletal rearrangement (*28*) to yield *sec*-carbocations whose atom site selectivity will likewise be governed by association equilibria analogous to that in Scheme 1, where the diffusionally equilibrated primary diazonium ion replaces DIT^+ in Scheme 1. Such rearrangement has been explored with respect to 7- versus O^6-2′-deoxyGuo alkylation, but exocyclic amino group alkylation has not been investigated (*31*−*37*).

The biological significance of small exocyclic amino−alkyl adducts is unknown.

Acknowledgment. This work was supported by NCI Grant RO1 CA52881. We are most grateful to Dr. C. J. Michejda (Frederick Cancer Research Center) for providing the diisopropyltriazene used in these experiments.

References

(1) Lijinsky, W. (1992) *Chemistry and Biology of N-nitroso Compounds*, Cambridge University Press, Cambridge, U.K.
(2) Loeppky, R. N., and Michejda, C. J. (1994) *Nitrosamines and Related N-Nitroso Compounds*, American Chemical Society, Washington, DC.
(3) Searle, C. E. (1984) *Chemical Carcinogens*, 2nd ed., American Chemical Society, Washington, DC.
(4) Michejda, C. J., Smith, R. H., and Koepke, M. B. K. (1994) DNA Alkylation by Triazenes and Related Compounds. *IARC Sci. Publ.* **125**, 323−337.
(5) Kroeger-Koepke, M. B., Smith, R. H., Jr., Goodnow, E. A., Brashears, J., Kratz, L., Andrews, A. W., Alword, W. G., and Michejda, C. J. (1991) 1,3-Dialkyltriazenes: Reactive Intermediates and DNA Alkylation. *Chem. Res. Toxicol.* **4**, 334−340.
(6) Smith, R. H., Jr., Wladkowski, B. D., Mehl, A. F., Cleveland, M. J., Rudrow, E. A., Chmurny, G. N., and Michejda, C. J. (1989) 1,3-Dialkyltriazenes: Tautomeric Equilibria and Rates and Products of Decomposition. *J. Org. Chem.* **54**, 1036−1042.
(7) Loveless, A. (1969) Possible Relevance of O6-Alkylation of Deoxyguanosine to the Mutagenicity and Carcinogenicity of Nitrosamines and Nitrosamides. *Nature* **223**, 206−207.
(8) Singer, B., Spengler, S. J., Fraenkel-Conrat, H., and Kusmierek, J. T. (1986) O4-Methyl, Ethyl, or Isopropyl Substituents on Thymidine in poly(dA-dT) All Lead to Transitions Upon Replication. *Proc. Natl. Acad. Sci. U.S.A.* **83**, 28−32.
(9) Horsfall, M. J., Gordon, A. J., Burns, P. A., Zielenska, M., and Glickman, B. W. (1990) Mutational Specificity by Alkylating Agents and the Influence of DNA Repair. *Environ. Mol. Mutagen.* **15**, 107−122.
(10) Lawley, P. D. (1984) Carcinogenesis by Alkylating Agents. In *Chemical Carcinogens, Vol. 1* (Searle, C. E., Ed.) Vol. 182, pp 325−484, American Chemical Society, Washington, DC.
(11) Moschel, R. C., Hudgins, W. R., and Dipple, A. (1986) Reactivity Effects on Site Selectivity in Nucleoside Aralkylation: A Model for the Factors Influencing the Sites of Carcinogen-Nucleic Acid Interactions. *J. Org. Chem.* **51**, 4180−4185.
(12) Dipple, A. (1988) Reactive Metabolites of Carcinogens and Their Interactions with DNA. In *Chemical Carcinogens, Activation Mechanism, Structural and Electronic Factors, and Reactivity* (Politzer, P., and Martin, F. J., Eds.) pp 32−62, Elsevier, New York.
(13) Dipple, A. (1995) DNA Adducts of Chemical Carcinogens. *Carcinogenesis* **16**, 437−441.
(14) Loechler, E. L. (1994) A Violation of the Swain-Scott Principle, and *Not* S_N1 versus S_N2 Reaction Mechanisms, Explains Why Carcinogenic Alkylating Agents Can Form Different Proportions of Adducts at Oxygen versus Nitrogen in DNA. *Chem. Res. Toxicol.* **7**, 277−280.
(15) Finneman, J. I., and Fishbein, J. C. (1995) Mechanism of Benzyl Transfer in the Decay of (*E*)-Arylmethanediazoates and Aryldiazomethanes in Aqueous Solutions. *J. Am. Chem. Soc.* **117**, 4228−4239.
(16) Jones, C. J., Kelly, M. A., Sinnott, M. L., and Smith, P. J. (1980) Unimolecular Heterolysis of a Nitrogen−Nitrogen Bond. *J. Chem. Soc., Chem. Commun.*, 322−323.

(17) Jones, C. J., Kelly, M. A., Sinnott, M. L., Smith, P. J., and Tzotzos, G. T. (1982) Pathways for the Decomposition of Alkylaryltriazines in Aqueous Solution. *J. Chem. Soc., Perkin Trans. 2*, 1655−1664.
(18) Smith, R. R., Denlinger, C. L., Kupper, R., Mehl, A. F., and Michejda, C. J. (1986) Decomposition of 1,3-Dialkyltriazenes in Aqueous Buffers: Kinetic and Mechanistic Studies. *J. Am. Chem. Soc.* **108**, 3726−3730.
(19) Lawley, P. D., Orr, D. J., and Jarman, M. (1975) Isolation and Identification of Products from Alkylation of Nucleic Acids: Ethyl- and Isopropyl-Purines. *Biochem. J.* **145**, 73−84.
(20) Balsiger, R. W., and Montgomery, J. A. (1960) Synthesis of Potential Anticancer Agents. XXV. Preparation of 6-Alkoxy-2-Aminopurines. *J. Org. Chem.* **25**, 1573−1575.
(21) Williams, H. R., and Mosher, H. S. (1954) Peroxides. I. *n*-Alkyl Hydroperoxides. *J. Am. Chem. Soc.* **76**, 2984−2987.
(22) Gaffney, B. L., Marky, L. A., and Jones, R. A. (1984) Synthesis and Characterization of a Set of Four Dodecadeoxyribonucleosides Containing O6-Methylguanine Opposite Adenine, Cytosine, Guanine and Thymine. *Biochemistry* **23**, 5686−5691.
(23) Mitsunobu, O. (1980) The Use of Diethyl Azodicarboxylate and Triphenylphosphine in Synthesis and Transformation of Natural Products. *Synthesis* **1**, 1−28.
(24) Mag, M., and Engels, J. W. (1988) Synthesis and structure assignments of amide protected nucleosides and their use as phosphoramidites in deoxyoligonucleotide synthesis. *Nucleic Acids Res.* **16**, 3525−3543.
(25) Fugii, T., and Itaya, T. (1998) The Dimroth Rearrangement in the Adenine Series: A Review Updated. *Heterocycles* **48**, 359−390.
(26) Itaya, T., Tanaka, F., and Fugii, T. (1972) Purines VI. Rate Study and Mechanism of the Dimroth Rearrangement of 1-Alkoxy-9-alkyladenines and 1-Alkyl-9-methyladenines. *Tetrahedron* **28**, 535−547.
(27) Pezacki, J. P., Shukla, D., Lusztyk, J., and Warkentin, J. (1999) Lifetimes of Dialkylcarbocations Derived from Alkanediazonium Ions in Solution: Cyclohexadienyl Cations as Kinetic Probes for Cation Reactivity. *J. Am. Chem. Soc.* **121**, 6589−6598.
(28) Zollinger, H. (1995) *Diazo Chemistry II. Aliphatic, Inorganic and Organometallic Compounds*, pp 241−304, VCH, Weinheim, Germany.
(29) Preussmann, R., and Eisenbrand, G. (1984) N-Nitroso Carcinogens in the Environment. In *Chemical Carcinogens, Vol. 2* (Searle, C. S., Ed.) Vol. 182, pp 829−868, American Chemical Society, Washington, DC.
(30) Hecht, S. S. (1998) Biochemistry, Biology, and Carcinogenicity of Tobacco-Specific N-Nitrosamines. *Chem. Res. Toxicol.* **11**, 559−603.
(31) Scribner, J. D., and Ford, G. P. (1982) *n*-Propyldiazonium Ion Alkylates O^6 of Guanine with Rearrangement, but Alkylates N-7 without Rearrangement. *Cancer Lett.* **16**, 51−56.
(32) Ford, G. P., and Scribner, J. D. (1983) Theoretical Study of Gas-Phase Methylation and Ethylation by Diazonium Ions and Rationalization of Some Aspects of DNA Reactivity. *J. Am. Chem. Soc.* **105**, 349−354.
(33) Ford, G. P. (1986) Unimolecular Dissociation of Primary Alkanediazonium Ions. Ab Initio and Semi-empirical Molecular Orbital Calculations. *J. Am. Chem. Soc.* **108**, 5104−5108.
(34) Ford, G. P., and Smith, C. T. (1987) Unexpected Bond Order Relationships in Extremely "Loose" S_N2 Transition States. *J. Chem. Soc., Chem. Commun.*, 44−45.
(35) Morimoto, K., Tanaka, A., and Yamaha, T. (1983) Reaction of 1-*n*-propyl-1-nitrosourea with DNA *in vitro*. *Carcinogenesis* **4**, 1455−1458.
(36) Ortlieb, H., and Kliehues, P. (1980) Reaction of *N*-*n*-butyl-*N*-nitrosourea with DNA in vitro. *Carcinogenesis* **1**, 849−854.
(37) Saffhill, R. (1984) In vitro Reaction of *N*-*n*-Butyl-*N*-nitrosourea and *n*-Butylmethanesulphonate With Guanine and Thymine Bases of DNA. *Carcinogenesis* **5**, 621−625.

TX000059J