# 2-Alkynyl-*N*-propargyl Pyridinium Salts: Pyridinium-Based Heterocyclic Skipped Aza-Enediynes that Cleave DNA by Deoxyribosyl Hydrogen-Atom Abstraction and Guanine Oxidation

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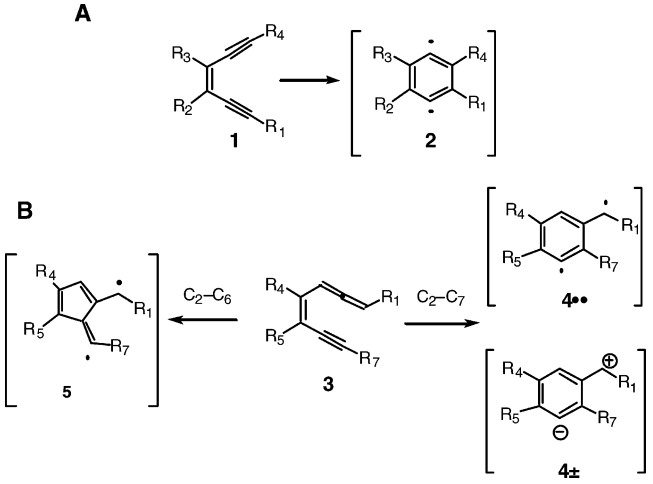
ABSTRACT: Diradical-generating cyclizations such as the enediyne Bergman cyclization and the enyne allene Myers−Saito cyclization have been exploited by nature in the mechanism of DNA cleavage by a series of potent antitumor antibiotics. Alternative diradical-generating cyclizations have been proposed in the design of selective antitumor agents; however, little information is available concerning the utility of these alternative cyclizations in radical-based DNA cleavage chemistry. One such alternative diradical-generating cyclization, the aza-Myers−Saito cyclization of aza-enyne allenes that are derived from base-promoted isomerization of skipped aza-enediynes, has been recently reported. Here, we report the synthesis and DNA cleavage chemistry of a series of pyridinium skipped aza-enediynes (2-alkynyl-*N*-propargyl pyridinium salts). Efficient DNA cleavage requires the presence of the skipped aza-enediyne functionality, and optimal DNA cleavage occurs at basic pH. Within this series of compounds, the analogue bearing a *p*-methoxyphenyl group on the pyridinium 2-alkyne substituents was found to be the most effective DNA cleavage agent, displaying significant supercoiled DNA-nicking activity at concentrations as low as 1 $\mu$M. Detailed studies of this analogue show that DNA cleavage occurs through 4′-hydrogen-atom abstraction from the DNA backbone and oxidation of guanine bases. This is the first report of enediyne-like radical-based DNA cleavage by an agent designed to undergo an alternative diradical-generating cyclization.

The enediyne Bergman cyclization (**1** → **2**, Scheme 1A) continues to generate a great deal of interest because of its fundamental role in the mechanism of DNA cleavage by natural and designed anticancer agents (*1*−*3*). The *p*-benzyne diradical intermediates (**2**) generated by this thermal cyclizations are capable of abstracting hydrogen atoms from the deoxyribose backbone of DNA, leading to DNA strand cleavage and ultimately cell death (*4*). Inspired by the proposed enyne cumulene cyclization of neocarzinostatin, Myers and Saito independently proposed an analogous diradical-generating cyclization of enyne allenes (**3**→ **4**••, Scheme 1) (*5*). The Myers−Saito cyclization also gives rise to products corresponding to the trapping of an ionic intermediate, formulated as the zwitterion **4**±, particularly when these cyclizations are carried out in polar solvents such as methanol (Scheme 1) (*5*, *6*). A different diradical-generating $C_2-C_6$ cyclization of enyne allenes (**3** → **5**) was first reported by Schmittel et al. (*7*). Enyne allenes that have been designed to undergo either Schmittel (*8*, *9*) or Myers−Saito cyclizations (*10*, *11*) have been shown to cleave DNA. In the case of enyne allenes that undergo Myers−Saito cyclizations, G-selective DNA cleavage has been observed, which has been proposed to arise either from electrophilic attack on the DNA by the allene **3** or by the zwitterionic form of the cyclization intermediate **4**± (*12*, *13*).

Scheme 1: Bergman (A), Myers−Saito (B, $C_2-C_7$), and Schmittel (B, $C_2-C_6$) Diradical-Generating Cyclizations




Diradical-generating cyclizations analogous to those shown in Scheme 1 have been proposed for DNA cleavage applications of hetero-enediynes or hetero-enyne allenes (*14*−*21*). These heteroatom variants of the Bergman and Myers−Saito cyclizations may have advantages over the all-carbon systems in the design of DNA cleavage agents because of their inherently lower cyclization barriers (*14*, *18*) and the possibility of modulating the reactivity of the resultant diradical through protonation in the acidic tumor environment (*22*). When some of these heteroatom variant

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Scheme 2: Aza-Myers−Saito Cyclization of an Aza-Enyne Allene Derived from a Skipped Aza-Enediyne

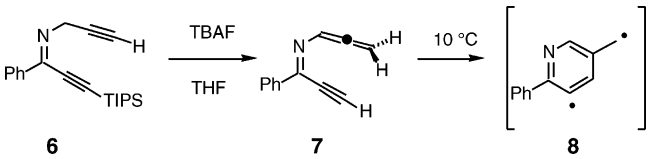
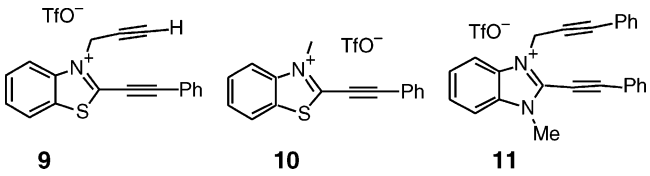



Chart 1: Bezothiazolium and Benzimidazolium Skipped Aza-Enediynes




cyclizations are allowed to proceed in organic solvents, products corresponding to the trapping of diradical intermediates are observed (*18*, *23*−*28*). However, the ability of hetero-enediynes or hetero-enyne allenes to effect DNA cleavage in aqueous solution has not been widely demonstrated, and in the few cases where DNA cleavage has been observed, it is not clear that this proceeds through hydrogen-atom abstraction or by other means (*19*−*21*).

We recently reported aza-Myers−Saito cyclization of a *C*-alkynyl-*N*-propargyl imines (skipped aza-enediynes) **6** to α-5-didehydropicoline diradical **8** through the intermediate aza-enyne allene **7** (Scheme 2). The aza-enyne allene **7** could not be purified, because cyclization occurs readily at subambient temperatures, leading to products of hydrogen-atom abstraction by **8** and, in methanol, products corresponding to the trapping of the corresponding closed-shell zwitterion.

To apply this aza-Myers cyclization to the design of potential DNA cleavage agents, both the potential hydrolytic lability and the stereochemical isomerization of the imine double bond were addressed by incorporation of this moiety into a heteroaromatic ring. We have previously reported the preparation of two different series of such heterocyclic skipped aza-enediynes (*20*, *21*). The benzothiazolium series, exemplified by **9** (Chart 1), serve as electrophiles, leading to DNA adducts that undergo guanosine-specific cleavage after piperidine/heat treatment (*20*). In this series, the presence of a skipped aza-enediyne functionality is not required for the G-selective cleavage activity; the *N*-methyl benzothiazolium triflate **10** is as effective as **9** (*21*). In contrast, the benzimidazole skipped aza-enediynes, exemplified by **11**, form DNA adducts leading to cytosine-selective DNA cleavage, particularly under acidic conditions (*20*). The difference in the covalent DNA interactions in the benzothiazolium and benzimidazolium series demonstrates that the identity of the heterocyclic system can have a pronounced effect on the DNA cleavage chemistry; however, in neither case is there evidence for DNA cleavage involving hydrogen-atom abstraction from the DNA backbone, as would be expected for an aza-Myers-type cyclization process.

Here, we report the synthesis of DNA cleavage chemistry of pyridinium-based skipped aza-enediynes. We show that, for this heterocyclic system, efficient DNA cleavage requires the presence of the skipped aza-enediyne functionality and that optimal DNA cleavage is observed for an analogue bearing a *p*-methoxyphenyl group on the pyridinium 2-alkyne substituent. Detailed studies of this analogue show that DNA cleavage occurs through hydrogen-atom abstraction from the DNA backbone and oxidation of guanine bases. To the best of our knowledge, this is the first report of radical-based DNA cleavage by compounds designed to undergo diradical-generating cyclization reactions akin to the Bergman, Myers−Saito, or Schmittel reactions.

## EXPERIMENTAL PROCEDURES

*General Procedures*. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Ether and tetrahydrofuran were distilled from sodium/benzophenone immediately prior to use. Dichloromethane, dichloroethane, and pyridine were distilled from calcium hydride immediately prior to use. Triethylamine and *N*,*N*-diisopropylethylamine were distilled from calcium hydride and stored over potassium hydroxide pellets under an atmosphere of argon. All reactions were performed under argon in oven-dried glassware.

Unless otherwise noted, NMR spectra were determined in $CDCl_3$ on a spectrometer operating at 300 MHz ($^1$H) and 75 MHz ($^{13}$C). Unless otherwise noted, mass spectra were obtained by chemical ionization using methane as the ionizing gas.

*General Procedures: Sonogashira Couplings with 2-Bromopyridine.* A 250 mL three-necked flask equipped with a condenser was purged with argon and quickly fitted with a U-shaped adapter connected to a 5 mL round-bottomed flask charged with $Pd(PPh_3)_2Cl_2$ (0.57 g, 0.81 mmol). CuI (0.095 g, 0.50 mmol), triethylamine (60 mL), trimethylsilyl acetylene (3.06 g, 30.5 mmol), and 2-bromopyridine (3.97 g, 25.1 mmol) were placed into the reaction flask. The reaction was initiated by the addition of the palladium catalyst, and the reaction mixture was heated under reflux for 18 h. The reaction mixture was filtered through activated alumina, which was subsequently washed with EtOAc. The combined filtrate was washed with saturated $NaHCO_3$, water, and brine. The solvent was dried ($Na_2SO_4$) and evaporated, and the 2-alkynyl pyridine product was purified by Kugelrohr distillation or flash chromatography.

*2-Trimethylsilanylethynylpyridine (**16b**)* (*29*). Kugelrohr distillation afforded 2.62 g (58% yield). $^1$H NMR $\delta$: 0.27 (s, 9 H), 7.20 (ddd, 1 H, $J$ = 7.8, 3.6, 1.2 Hz), 7.43 (ddd, 1 H, $J$ = 7.8, 1.8, 1.2 Hz), 7.62 (ddd, 1 H, $J$ = 7.8, 7.8, 1.8 Hz), 8.54 (ddd, 1 H, $J$ = 3.6, 1.8, 1.8 Hz). $^{13}$C NMR $\delta$: 0.7, 94.3, 103.3, 122.6, 126.8, 135.7, 142.6, 149.4. MS *m*/*z*: 176 ($MH^+$). HRMS *m*/*z*: calcd for $C_{10}H_{13}NSi$, 176.0896; found, 176.0891.

*2-[(Triisopropylsilanyl)ethynyl]pyridine (**16c**)*. Kugelrohr distillation afforded 2.58 g (34% yield). $^1$H NMR $\delta$: 1.09−1.16 (m, 21 H), 7.21(ddd, 1 H, $J$ = 7.5, 4.8, 1.2 Hz), 7.46 (ddd, 1 H, $J$ = 7.8, 1.2, 0.9 Hz), 7.62 (ddd, 1 H, $J$ = 7.5, 7.5, 1.8 Hz), 8.58 (ddd, 1 H, $J$ = 4.8, 1.8, 0.9 Hz). $^{13}$C NMR $\delta$: 11.3, 18.5, 91.8, 105.6, 122.8, 127.6, 136.0, 143.1, 149.6. MS *m*/*z*: 260 ($MH^+$). HRMS *m*/*z*: calcd for $C_{16}H_{25}NSi$, 260.1834; found, 260.1835.

*2-(Phenylethynyl)pyridine (**16d**)* (*30*). Purification by flash chromatography (siliga gel, 10% EtOAc in hexane) afforded 0.54 g (30%) of **16d**. $^1$H NMR $\delta$: 7.21 (ddd, 1 H, $J$ = 6.5, 4.8, 1.2 Hz), 7.24−7.35 (m, 3 H), 7.50 (ddd, 1 H, $J$ = 6.7, 1.1, 1.1 Hz), 7.56−7.59 (m, 2 H), 7.64 (ddd, 1 H, $J$ = 7.7,

7.7, 1.8 Hz), 8.59 (ddd, 1 H, $J = 4.8, 1.8, 1.1$ Hz). $^{13}$C NMR $\delta$: 88.6, 89.2, 122.2, 122.7, 127.1, 128.3, 128.9, 132.0, 136.1, 143.4, 150.0. MS *m*/*z*: 180 ($MH^+$). HRMS *m*/*z*: calcd for $C_{14}H_9N$, 180.0813; found, 180.0810.

*2-(4-Trifluoromethylphenylethynyl)pyridine (**16e**).* Purification by flash chromatography (silica gel, 60% EtOAc in hexane) afforded 1.37 g (19%) of **16e**. $^1$H NMR (500 MHz) $\delta$: 7.25 (ddd, 1 H, $J = 5.8, 4.8, 1.0$ Hz), 7.52−7.54 (m, 1 H), 7.59−7.61 (m, 2 H), 7.67−7.70 (m, 3 H), 8.62 (ddd, 1 H, $J = 4.8, 1.8, 1$ Hz). $^{13}$C NMR (125 MHz) $\delta$: 87.4, 90.6, 123.8 (q, $CF_3$, $J_{CF} = 272$ Hz), 123.2, 125.3 (q, $J_{CF} = 4$ Hz), 126.1, 127.3, 130.6 (q, $J_{CF} = 32$ Hz), 132.2, 136.2, 142.8, 150.2. MS *m*/*z*: 248 ($MH^+$). HRMS *m*/*z*: calcd for $C_{14}H_8F_3N$, 248.0687; found, 248.0680.

*2-(4-Methoxyphenylethynyl)pyridine (**16f**).* Kugelrohr distillation afforded 0.37 g (25% yield). $^1$H NMR $\delta$: 3.78 (s, 3 H), 6.85 (dd, 2 H, $J = 6.9, 2.1$ Hz), 7.17 (ddd, 1 H, $J = 7.5, 4.8, 1.2$ Hz), 7.43−7.52 (m, 3 H), 7.61 (ddd, 1 H, $J = 7.5, 7.5, 1.8$ Hz), 8.55 (ddd, 1 H, $J = 4.8, 1.7, 0.9$ Hz). $^{13}$C NMR $\delta$: 55.2, 87.5, 89.4, 114.0, 114.2, 122.3, 126.8, 133.5, 136.0, 143.7, 149.9, 160.1. MS *m*/*z*: 210 ($MH^+$). HRMS *m*/*z*: calcd for $C_{14}H_{11}NO$, 210.0919; found, 210.0927.

*2-(3,4,5-Trimethoxylphenylethynyl)pyridine (**16g**).* Purification by flash chromatography (silica gel, 10% EtOAc in hexane) afforded 0.53 g (63% yield) of **16g**. $^1$H NMR $\delta$: 3.85 (s, 9 H), 6.83 (s, 2 H), 7.21 (ddd, 1 H, $J = 7.8, 4.8, 1.2$ Hz), 7.49 (d, 1 H, $J = 7.8$ Hz), 7.67 (ddd, 1 H, $J = 7.8, 7.8, 1.8$ Hz), 8.59 (d, 1 H, $J = 4.8$ Hz). $^{13}$C NMR $\delta$: 56.1, 60.9, 87.7, 89.4,109.2, 117.1, 122.7, 126.9, 139.2, 139.3, 143.3, 150.0, 153.0. MS *m*/*z*: 270 ($MH^+$). HRMS *m*/*z*: calcd for $C_{16}H_{15}NO_3$, 270.1130; found, 270.1128.

*4-Methyl-2-phenylethynylpyridine (**16h**).* A mixture of 4-methyl-2-(trifluoromethanesulfonyl)oxypyridine (1.20 g, 4.9 mmol), prepared according to the method of Savage and co-workers (*32*), tetrahydrofuran (15 mL), *N*,*N*-diisopropylethylamine (2.6 mL), and phenylacetylene (0.76 g, 7.5 mmol) was combined with tri(dibenzylideneacetone)dipalladium-(0) (0.23 g, 0.25 mmol) and tri-*o*-tolulylphosphine (0.03 g, 0.99 mmol) using a modification of the general procedure above. Evaporation of the solvent followed by flash column chromatography (silica gel, 20% EtOAc in hexane) afforded 0.47 g (50% yield) of **16h** as a dark brown oil. $^1$H NMR $\delta$: 2.29 (s, 3 H, $CH_3$), 7.00 (ddd, 1 H, $J = 5.1, 1.5, 0.6$), 7.29−7.32 (m, 4 H), 7.54−7.57 (m, 2 H), 8.42 (d, 1 H, $J = 5.1$). $^{13}$C NMR $\delta$: 20.7, 88.6, 88.7, 122.3, 123.7, 127.9, 128.2, 128.8, 131.9, 143.1, 147.2, 149.6. MS *m*/*z*: 194 ($MH^+$). HRMS *m*/*z*: calcd for $C_{14}H_{11}N$, 194.0970; found, 194.0971.

*2-(2-Phenethyl)pyridine (**14**).* Into a pressure tested, glass reaction vessel was placed 2-(2-phenylethynyl)pyridine **16d** (0.2 g, 1.12 mmol), ethanol (75 mL), and 10% palladium on an activated carbon catalyst (0.02 mg). Hydrogenation was performed in a Parr apparatus under 40 psi hydrogen for 3 h. The reaction mixture was filtered through Celite, which was then washed with methanol. The combined filtrate was concentrated to afford 0.195 g of **14** (96% yield) as a brown oil. $^1$H NMR $\delta$: 3.07−3.15 (m, 4 H), 7.07−7.13 (m, 2 H), 7.18−7.33 (m, 5 H), 7.54 (ddd, $J = 7.8, 7.8, 1.8, 1$ H), 8.58−8.60 (m, 1 H). $^{13}$C NMR $\delta$: 35.8, 40.0, 121.0, 122.8, 125.8, 128.1, 128.3, 136.1, 141.3, 149.0, 160.9. MS *m*/*z*: 184 ($MH^+$). HRMS *m*/*z*: calcd for $C_{13}H_{13}N$, 184.1126; found, 184.1121.

*General Procedure: Alkylation of Pyridines with Propargyl Triflate or Methyl Triflate.* Prop-2-ynyl-2-trifluoromethansulfonate (0.48 g, 2.6 mmol) prepared according to method of Vedejs and co-workers (*33*) was added to a 25 mL pear-shaped flask containing ether (9 mL) chilled at −42 °C. In a separate 25 mL pear-shaped flask, the pyridine, dissolved in ether (9 mL) and cooled to −42 °C, was added dropwise via cannula to the prop-2-trifluoromethansulfonate solution or to a solution of methyl triflate in ether. After the reaction mixture was stirred for 2 h, it was filtered with a fritted glass funnel and washed with cold ether to obtain a solid. If necessary, this solid was recrystallized to afford analytically pure material.

*2-Ethynyl-1-prop-2-ynylpyridinium Triflate (**17a**).* Obtained as a pink solid (0.6 g, 42% yield). mp: 120−122 °C. $^1$H NMR (acetone-$d_6$, 500 MHz) $\delta$: 3.62 (t, 1 H, $J = 2.6$ Hz), 5.42 (s, 1 H), 5.86 (d,1 H, $J = 2.5$ Hz), 8.38 (dd, 1 H, $J = 6.6, 7.8$ Hz), 8.50 (d, 1 H, $J = 7.8$), 8.85 (dd, 1 H, $J = 7.8, 7.8$ Hz), 9.47 (d, $J = 6.6$, 1 H). $^{13}$C NMR (acetone-$d_6$, 125 MHz) $\delta$: 50.7, 74.1, 74.5, 81.6, 99.0, 122.3 (q, $CF_3$, $J = 321$ Hz), 129.4, 134.1, 137.3, 147.2, 147.7. MS (FAB) *m*/*z*: 290 ($MH^+$), 142. HRMS (FAB) *m*/*z*: calcd for $C_{11}H_9F_3NO_3S$, 290.0099; found, 290.0106. Anal. Calcd for $C_{11}H_8F_3NO_3S^+/3H_2O$: C, 44.45; H, 2.94; N, 4.71; S, 11.01. Found: C, 44.55; H, 2.71; N, 4.76; S, 10.79.

*1-Prop-2-ynyl-2-trimethylsilanylethynylpyridinium Triflate (**17b**).* Recrystallized from chloroform/hexane to afford 0.40 g (64% yield) of **17b** as white plates. mp: 65 °C (dec). $^1$H NMR (500 MHz) $\delta$: 0.31 (s, 9 H), 2.78 (t, 1 H, $J = 2.5$ Hz), 5.58 (d, 2 H, $J = 2.5$ Hz), 8.02 (d, 1 H, $J = 7.9$ Hz), 8.08 (dd, 1 H, $J = 7.9, 6.9$ Hz), 8.53 (dd, 1 H, $J = 7.9, 7.9$ Hz), 9.20 (d, 1 H, $J = 7.9$ Hz). $^{13}$C NMR $\delta$: −1.1, 49.8, 73.3, 79.5, 92.7, 119.2, 120.6 (q, $CF_3$, $J = 320$ Hz), 127.8, 132.1, 136.6, 146.2, 146.7. MS (FAB) *m*/*z*: 364 ($MH^+$), 214, 176. HRMS(FAB) *m*/*z*: calcd for $C_{13}H_{16}NSi$, 214.105203; found, 214.103528. Anal. Calcd for $C_{14}H_{16}F_3NO_3SSi^+/3H_2O$: C, 45.52; H, 4.55; N, 3.79; S, 8.68. Found: C, 45.74; H, 4.30; N, 3.83; S, 8.28.

*1-Prop-2-ynyl-2-[(triisopropylsilanyl)ethynyl]pyridinium Triflate (**17c**).* Recrystallization from chloroform/hexane yielded 0.17 g (34% yield) of **17b** as white flakes. mp: 123−124 °C. $^1$H NMR $\delta$: 1.12−1.15 (m, 18 H), 1.18−1.29 (m, 3 H), 2.73 (t, 1 H, $J = 2.7$ Hz), 5.62 (d, 2 H, $J = 1.5$ Hz), 8.03 (ddd, 1 H, $J = 7.8, 1.2$), 8.09 (ddd, 1 H, $J = 7.8, 6.6, 1.2$), 8.52 (dd, 1 H, $J = 7.8, 7.8$), 9.25 (d, 1 H, $J = 6.6$). $^{13}$C NMR $\delta$: 11.1, 18.4, 49.9, 73.5, 79.4, 94.6, 117.4, 127.9, 132.4, 136.7, 146.0, 147.2. MS *m*/*z*: 448 ($MH^+$), 298, 260. HRMS *m*/*z*: calcd for $C_{20}H_{29}F_3NO_3SSi$, 448.1590; found, 448.1611. Anal. Calcd for $C_{20}H_{28}F_3NO_3SSi$: C, 53.67; H, 6.31; N, 3.13; S, 7.16. Found: C, 53.63; H, 6.33; N, 3.06; S, 7.09.

*2-Phenylethynyl-1-prop-2-ynylpyridinium Triflate (**17d**).* Obtained as a gray solid (0.152 g, 73%). mp: 106−107 °C. $^1$H NMR [dimethyl sulfoxide (DMSO)-$d_6$][1] $\delta$: 4.05 (t, 1 H, $J = 2.4$), 5.74 (d, 2 H, $J = 2.4$), 7.56−7.85 (m, 3 H), 7.84 (d, 2 H, $J = 6.6$), 8.17 (ddd, 1 H, $J = 7.8, 6.3, 1.5$), 8.45 (dd, 1 H, $J = 7.8, 1.5$), 8.68 (ddd, 1 H, $J = 7.8, 7.8, 1.5$),

[1] Abbreviations: DMSO, dimethyl sulfoxide; DTT, dithiothreitol; EDTA, *N*,*N*,*N*′,*N*′-ethylenediaminetetraacetic acid; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; TBE, Tris−borate−EDTA; Tris, *N*,*N*,*N*-tris(hydroxymethyl)aminomethane.

9.26 (d, 1 H, $J = 6.3$). $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$: 49.4, 75.1, 80.1, 81.4, 106.55, 118.9, 127.4, 129.2, 132.0, 132.1, 132.6, 136.4, 146.2, 146.1. MS *m/z*: 368 ($MH^+$), 218. HRMS *m/z*: calcd for $C_{17}H_{12}F_3NO_3S$, 368.0568; found, 368.0573. Anal. Calcd for $C_{17}H_{12}F_3NO_3S$: C, 55.58; H, 3.29; N, 3.81; S, 8.73. Found: C, 55.69; H, 3.13; N, 3.67; S, 8.36.

*1-Prop-2-ynyl-2-(4-trifluoromethylphenylethynyl)-pyridinium Triflate (**17e**).* Recrystallization from chloroform/ether afforded 0.54 g of **17e** as a white powder (49% yield). mp: 151−153 °C (dec). $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 4.08 (t, 1 H, $J = 2.5$), 5.80 (d, 2 H, $J = 2.5$), 7.99 (d, 2 H, $J = 8.0$ Hz), 8.08 (d, 2 H, $J = 8.0$ Hz), 8.25 (ddd, 1 H, $J =$ 7.7, 6.0, 1.5 Hz), 8.53 (dd, 1 H, $J = 7.7$, 1.5 Hz), 8.74 (ddd, 1 H, $J = 7.7$, 7.7,1.0 Hz), 9.32 (dd, 1 H, $J = 6.0$, 0.5). $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$: 49.5, 75.0., 81.5, 81.6, 103.9, 123.0 (q, $CF_3$, $J_{CF} = 321$ Hz), 124.7 (q, $J_{CF} = 271$ Hz), 123.2, 126.0 (q, $J_{CF} = 4$ Hz), 127.2, 131.0 (q, $J_{CF} = 32$ Hz), 132.5, 133.4, 135.8, 146.3, 146.4. MS (FAB) *m/z*: 286 ($C_{17}H_{11}F_3N^+$). HRMS (FAB) *m/z*: calcd for $C_{17}H_{11}F_3N$, 286.0844; found, 286.0845. Anal. Calcd for $C_{18}H_{11}F_6NO_3S^+/3H_2O$: C, 49.98; H, 2.66; N, 3.17. Found: C, 48.93; H, 2.55; N, 3.11.

*2-(4-Methoxyphenylethynyl)-1-prop-2-ynylpyridinium Triflate (**17f**).* Recrystallization from methylene chloride/hexane afforded **17f** as green crystals (0.19 g, 68% yield). mp: 142−145 °C (dec). $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$: 3.51 (t, 1 H, $J = 2.5$), 3.89 (s, 3 H), 5.69 (d, 2 H, $J = 2.5$), 7.07−7.09 (m, 2 H), 7.74−7.77 (m, 2 H), 8.03 (ddd, 1 H, $J = 8$, 6, 1), 8.28 (dd, 1 H, $J = 7.0$, 1.0 Hz), 8.57 (ddd, 1 H, $J = 8.0$, 8.0, 1.0 Hz), 9.16 (dd, 1 H, $J = 6.0$, 1.0 Hz). $^{13}$C NMR ($CD_3OD$, 125 MHz) $\delta$: 50.5, 75.0, 80.47, 81.3, 111.4, 112.0, 115.6, 117.4, 132.9, 135.1, 136.0, 139.6, 146.4, 146.9, 164.4. MS *m/z*: 398 ($MH^+$), 210. HRMS *m/z*: calcd for $C_{18}H_{15}F_3NO_4S$, 398.0674; found, 398.0678. Anal. Calcd for $C_{18}H_{14}F_3NO_4S$: C, 54.41; H, 3.55; N, 3.52. Found: C, 54.01; H, 3.18; N, 3.50.

*1-Prop-2-ynyl-2-(3,4,5-trimethoxyphenylethynyl)pyridinium Triflate (**17g**).* Recrystallization from chloroform afforded 0.23 g (54% yield) of bright yellow needles. mp: 162−163 °C (dec). $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$: 3.58 (t, 1 H, $J = 2.7$ Hz), 3.84 (s, 3 H), 3.89 (s, 6 H), 5.74 (d, 2 H, $J = 2.7$ Hz), 7.14 (s, 2 H), 8.08 (ddd, 1 H, $J = 8.0$, 6.3, 1.5 Hz), 8.34 (dd, 1 H, $J = 8.0$, 1.5 Hz), 8.61 (ddd, 1 H, $J = 8.0$, 8.0, 1.0 Hz), 9.20 (dd, 1 H, $J = 6.3$, 1.0 Hz). $^{13}$C NMR ($CD_3OD$, 125 MHz) $\delta$: 50.7, 57.0, 61.3, 75.0, 80.0, 81.5, 110.5, 111.5, 115.2, 127.8, 133.2, 139.3, 143.4, 146.5, 147.0, 155.0. MS *m/z*: 308 ($C_{19}H_{18}NO_3^+$). HRMS *m/z*: calcd for $C_{19}H_{18}NO_3$, 308.1287; found, 308.1291. Anal. Calcd for $C_{20}H_{18}F_3NO_6S$: C, 52.51; H, 3.97; N, 3.06; S, 7.01. Found: C, 52.50; H, 3.97; N, 2.98; S, 7.37.

*4-Methyl-2-phenylethynyl-1-prop-2-ynylpyridinium Triflate (**17h**).* Recrystallization from chloroform/ether afforded 0.20 g of **17h** as an off-white powder (67% yield). mp: 165−167 °C. $^1$H NMR (500 MHz) $\delta$: 2.67 (s, 3 H), 3.49 (t, 1 H, $J = 2.5$), 5.64 (d, 2 H, $J = 2.5$), 7.54 (dddd, 2 H, $J = 7.5$, 7.5, 1.5, 1.5), 7.50 (dddd, 1 H, $J = 7.5$, 7.5, 1.5, 1.5), 7.78−7.80 (m, 2 H), 7.91 (dd, 1 H, $J = 6.5$, 1.5), 8.21 (d, 1 H, $J = 1.5$), 8.99 (d, 1 H, $J = 6.5$). $^{13}$C NMR (125 MHz) $\delta$: 20.7, 48.7, 73.9, 79.2, 79.8, 107.2, 119.4, 128.0, 129.0, 131.8, 132.5, 132.6, 136.9, 144.4, 160.8. MS *m/z*: 382 ($MH^+$), 232. HRMS *m/z*: calcd for $C_{18}H_{15}F_3NO_3S$, 382.0725; found, 382.0717. Anal. Calcd for $C_{18}H_{14}F_3NO_3S$: C, 56.69; H, 3.70; N, 3.67. Found: C, 56.18; H, 3.32; N, 3.61.

*2-Ethynyl-1-methylpyridinium Triflate (**18a**).* Obtained as a pink solid (0.5 g, 90% yield). mp: 140 °C (dec). $^1$H NMR (acetone-$d_6$, 500 MHz) $\delta$: 4.62 (s, 3 H), 5.31 (s, 1 H), 8.22 (dd, 1 H, $J = 7.0$, 6.0 Hz), 8.38 (d, 1 H, $J = 7.0$ Hz), 8.71 (ddd, 1 H, $J = 7.0$, 7.0, 1.3 Hz), 9.21 (d, 1 H, $J = 6.0$ Hz). $^{13}$C NMR (acetone-$d_6$, 125 MHz) $\delta$: 48.4, 74.5, 97.7, 122.3 (q, $CF_3$, $J = 321$ Hz), 128.6, 133.3, 138.0, 146.3, 148.7. MS (CI) *m/z*: 118 ($C_8H_8N^+$). HRMS (CI) *m/z*: calcd for $C_8H_8N$, 118.0657; found, 118.0654.

*1-Methyl-2-phenylethynylpyridinium Triflate (**18d**).* Recrystallization from dichloromethane/ether afforded 0.05 g (24% yield) of **18d** as an light greenish-white solid. $^1$H NMR (DMSO-$d_6$) $\delta$: 4.45 (s, 1 H), 5.73 (s, 3 H), 7.56−7.59 (m, 3 H), 7.82 (d, 2 H, $J = 7.0$ Hz), 8.10 (dd, 1 H, $J = 6.0$, 6.0 Hz), 8.33 (d, 1 H, $J = 7.0$ Hz), 8.57 (dd, 1 H, $J = 6.0$, 6.0 Hz), 9.10 (d, 1 H, $J = 5.0$ Hz). $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$: 47.3, 80.3, 105.2, 119.0, 126.7, 129.2, 131.4, 131.7, 132.5, 137.0, 144.8, 147.3. MS *m/z*: 194 ($C_{14}H_{12}N^+$). HRMS *m/z*: calcd for $C_{14}H_{12}N$, 194.0970; found, 194.0972.

*1-Methyl-2-(4-trifluoromethylphenylethynyl)pyridinium Triflate (**18e**).* Obtained as an off-white solid (0.3 g, 98% yield). mp: 143−144 °C (dec). $^1$H NMR (DMSO-$d_6$) $\delta$: 4.47 (s, 3 H), 7.95 (d, 1 H, $J = 8.2$ Hz), 8.05 (d, 1 H, $J = 8.2$ Hz), 8.14 (ddd, 1 H, $J = 7.6$, 6.1, 1.5 Hz), 8.43 (dd, 1 H, $J =$ 6.9, 1.2 Hz), 8.61 (dd, 1 H, $J = 6.9$ Hz), 9.14 (d, 1 H, $J =$ 6.1 Hz). $^{13}$C NMR (DMSO-$d_6$) $\delta$: 47.4, 81.8, 102.6, 124.5 (q, $J_{CF} = 270$ Hz), 120.0 (q, $CF_3$, $J_{CF} = 320$ Hz), 123.3, 125.5 (q, $J_{CF} = 4$ Hz), 127.3, 130.8 (q, $J_{CF} = 32$ Hz), 131.8, 133.3, 136.5, 144.9, 147.5. MS (CI) *m/z*: 262 ($C_{15}H_{11}F_3N^+$). HRMS (CI) *m/z*: calcd for $C_{15}H_{11}F_3N$, 262.0844; found, 262.0852.

*2-(4-Methoxyphenylethynyl)-1-methylpyridinium Triflate (**18f**).* Obtained as a white solid (0.3 g, 98% yield). mp: 172 °C (dec). $^1$H NMR (400 MHz) $\delta$: 3.88 (s, 3 H), 4.48 (s, 3 H), 7.06 (ddd, 2 H, $J = 6.8$, 2.4, 2.4 Hz), 7.72 (ddd, 2 H, $J =$ 6.8, 2.4, 2.4 Hz), 7.94 (ddd, 1 H, $J = 7.6$, 7.6, 1.2 Hz), 8.22 (dd, 1 H, $J = 8.4$, 1.2 Hz), 8.49 (dd, 1 H, $J = 7.6$, 1.2 Hz), 8.91 (d, 1 H, $J = 6.0$ Hz). $^{13}$C NMR $\delta$: 48.0, 56.2, 80.5, 109.8, 112.1, 115.9, 123.9, 127.0, 132.4, 135.8, 145.7, 147.8, 164.1. MS *m/z*: 224 ($C_{15}H_{14}NO^+$). HRMS *m/z*: calcd for $C_{15}H_{14}NO$, 224.1075; found, 224.1075.

*1-Prop-2-ynylpyridinium Triflate (**19**).* Obtained as a white powder (1.2 g, 88% yield). mp: 53−54 °C (dec). $^1$H NMR (DMSO-$d_6$) $\delta$: 4.04 (t, 1 H, $J = 2.6$ Hz), 5.62 (d, 2 H, $J =$ 2.6 Hz), 8.20 (dd, 1 H, $J = 7.5$, 6.4 Hz), 8.66 (dddd, 1 H, $J =$ 7.5, 7.5, 1.3, 1.3 Hz), 9.11 (dd, 1 H, $J = 6.4$, 1.3 Hz). $^{13}$C NMR (125 MHz) $\delta$: 49.9, 75.5, 81.4, 120.0 (q, $CF_3$, $J_{CF} =$ 320 Hz), 128.3, 144.4, 146.6. MS *m/z*: 118 ($C_8H_8N^+$). HRMS *m/z*: calcd for $C_8H_8N$, 118.0657; found, 118.0660.

*2-Phenethyl-1-prop-2-ynylpyridinium Triflate (**20**).* Obtained as a beige solid (0.09 g, 5% yield). mp: 106−107 °C. $^1$H NMR $\delta$: 3.22 (t, 2 H, $J = 7.8$ Hz), 3.53 (t, 1 H, $J =$ 2.6 Hz), 3.56 (t, 2 H, $J = 7.8$ Hz), 5.52 (d, 2 H, $J = 2.6$ Hz), 7.23−7.34 (m, 5 H), 7.96−8.01 (m, 2 H), 8.47−8.53 (m, 1 H), 9.09 (d, 1 H, $J = 6.0$ Hz). $^{13}$C NMR $\delta$: 34.6, 35.1, 48.6, 75.3, 81.5, 127.4, 128.0, 129.6, 129.9, 130.7, 140.1, 146.5, 147.5, 159.6. MS *m/z*: 222 ($C_{16}H_{16}N^+$), 184. HRMS *m/z*: calcd for $C_{16}H_{16}N$, 222.1283; found, 222.1284. Anal. Calcd for $C_{17}H_{16}F_3NO_3S$: C, 54.98; H, 4.34; N, 3.77; S, 8.63. Found: C, 54.75; H, 4.19; N, 3.66; S, 8.33.

*1-Prop-2-ynyl-2-trifluoromethylpyridinium Triflate (**21**).* Obtained as a white powder (0.6 g, 37% yield). mp: 87−88 °C (dec). $^1$H NMR $\delta$: 3.68 (t, 1 H, $J$ = 2.7 Hz), 5.75 (d, 2 H, $J$ = 2.7 Hz), 8.53 (dd, 1 H, $J$ = 7.0, 7.0 Hz), 8.71 (dd, 1 H, $J$ = 6.6, 1.5 Hz), 8.94 (dd, 1 H, $J$ = 7.0, 7.0 Hz), 9.66 (dd, 1 H, $J$ = 6.0, 0.9 Hz). $^{13}$C NMR ($CDCl_3$, 125 MHz) $\delta$: 50.8, 73.9, 83.0, 120.9 (q, $CF_3$, $J_{CF}$ = 320 Hz), 122.0 (q, $CF_3$, $J_{CF}$ = 273 Hz), 129.3 (d, $J_{CF}$ = 5 Hz), 133.4, 141.2 (d, $J_{CF}$ = 36 Hz), 150.4, 150.6. MS *m*/*z*: 336 ($MH^+$), 186 ($C_9H_7F_3N^+$). HRMS *m*/*z*: calcd for $C_{10}H_7F_6NO_3S$, 336.0129; found, 336.0137.

*Supercoiled DNA Cleavage Assay.* The DNA cleavage efficiency of these pyridinium salts was determined by incubation with aqueous solutions of supercoiled ΦX174 plasmid DNA. The supercoiled DNA was diluted to 50 $\mu$M base pairs in 50 mM *N*,*N*,*N*-tris(hydroxymethyl)aminomethane (Tris) buffer at pH 7 or 8. The reaction mixtures containing the compound and 13% (v/v) DMSO were incubated for 24 h at 37 °C (unless otherwise noted). DNA products were separated by agarose gel electrophoresis [1× Tris−borate−*N*,*N*,*N*′,*N*′-ethylenediaminetetraacetic acid (EDTA) (TBE) at 90 V for 1 h], stained with ethidium bromide (0.25 $\mu$g/mL), and the images were analyzed using a fluorimager with ImageQuant software. After we corrected for the differential staining ability of the different forms of DNA (*34*), the percent cleavage was determined using eq 1.

$$\text{percent cleavage} = \frac{(2[\text{form III}]) + [\text{form II}]}{(2[\text{form III}] + [\text{form II}] + [\text{form I}])} \times 100 \quad (1)$$

Normalized DNA cleavage at each drug concentration was calculated using eq 2. The values of normalized percent cleavage of DNA presented in Table 2 represent the mean ± standard deviation from at least three determinations.

$$\text{normalized percent cleavage} = \frac{\text{percent cleavage (drug)} - \text{percent cleavage (control)}}{100 - \text{percent cleavage (control)}} \quad (2)$$

*DNA Oligonucleotide Cleavage Assay.* Two 5′ singly end-labeled DNA oligonucleotides were used for DNA cleavage assays. A 14 base-pair oligonucleotide was prepared by 5′ labeling of d(GATAATGGATAAGC) with [$\gamma$-$^{32}$P]ATP (Amersham) using T4 polynucleotide kinase. The labeled DNA was purified by elution through a Bio-Rad Biospin column and annealed with an excess of unlabeled d(GCTTATCCATTATC). The resulting duplex was purified by preparative polyacrylamide gel electrophoresis (PAGE). A 228 base-pair DNA fragment was prepared as previous described (*20*). Briefly, 40 pmol of the L151 [d(CGGCATCAGAGCAGTTGTA)] or R378 [d(AACGTCGTGACTGGGAAAAC)] primer was 5′-labeled with [$\gamma$-$^{32}$P]ATP using T4 polynucleotide kinase and was purified by elution through a Biospin column. The purified radiolabeled primer was then used directly in a polymerase chain reaction (PCR) reaction by adding 40 pmol of the reverse unlabeled primer R378 (or L151), 10 ng of pUC19 DNA template, 5 $\mu$L of 10× buffer (100 mM Tris-HCl, 15 mM $MgCl_2$, and 500 mM KCl at pH 8.3), 0.2 mM dNTPs, and 2.5 units of Taq DNA polymerase (Roche) and adjusting with $H_2O$ to 50 $\mu$L. PCR was then performed (initial denaturation at 95 °C for 5 min; the cycling conditions were as follows: 95 °C for 15 s, 55 °C for 30 s, and 72 °C for 1 min, for 25 cycles, and then final extension was done at 72 °C for another 5 min). The PCR product was purified with a Microcon PCR spin column (Millipore), which separated the PCR product from its primers, the dNTPs, and the polymerase.

Unless otherwise noted, DNA cleavage reactions were carried out in 20 $\mu$L reaction mixtures consisting of 25,000 cpm of 5′-radiolabeled DNA fragments, drug, 5% DMSO, and 10 mM Tris-HCl buffer (pH 7) with or without 1 $\mu$g of calf thymus DNA. The samples were incubated at 37 °C for 12 h. The reaction mixtures were diluted with 100 $\mu$L of precipitation buffer (10 $\mu$g of glycogen and 0.45 M sodium acetate at pH 5.2), extracted with phenol/chloroform, and precipitated with ethanol. The pellets were washed twice with 70% ethanol. One set of samples was subjected to heat treatment with 10% piperidine at 95 °C for 15 min. The samples were then dissolved in formamide loading dye and analyzed by 8% denaturing PAGE. The images were visualized by PhosphoImager (Molecular Dynamics model 445 SI).

*8-Oxo-deoxyguanosine (8-Oxo-dG) Analysis.* A 120 $\mu$L reaction mixture consisting of calf thymus DNA (approximately 3.6 mM base pairs) in 5% DMSO in 50 mM Tris buffer (pH 7) with or without **17f** (5 mM) was incubated at 37 °C for 2 h in the dark. The reaction mixture was extracted with phenol/chloroform, and the DNA was precipitated with ethanol. The pellets were washed with 70% ethanol (3×) and lyophilized. The DNA was enzymatically digested with nuclease P1 (2 mM NaOAc at pH 4.5 and 0.1 mM $ZnCl_2$) and alkaline phosphatase (80 mM Tris buffer at pH 9.0). The digested mixture was directly analyzed by high-performance liquid chromatography (HPLC) [Beckman Ultrasphere ODS column (4.6 × 2500 mm) with isocratic elution (4 mM citric acid, 8 mM ammonium acetate, and 20 mg/L EDTA at pH 4.0 with 5% MeOH] using a UV dector (254 and 280 nm) and a four-channel electrochemical detector (0, +150, +290, and +380 mV). The mole ratio of 8-oxo-dG, whose concentration was determined coulometrically versus a standard curve of authentic material, to deoxyguanosine (dG), whose concentration was determined from the absorbance at 260 nm versus a standard curve of authentic material, was calculated. Oxidative damage was expressed as the ratio of 8-oxo-dG to $10^5$ molecules of dG.

## RESULTS AND DISCUSSION

*Synthesis of Pyridinium-Based Skipped Aza-Enediynes.* A series of pyridine-based skipped aza-enediynes **17a**−**h** were synthesized by Sonogashira coupling (*30*, *33*) of 2-bromopyridines **12a** or **12h** with a variety of terminal acetylenes to afford the 2-alkynyl pyridines **16a**−**h**, which were subjected to alkylation with propargyl triflate (*36*) to afford the skipped aza-enediynes (Scheme 3 and Table 1). A number of related pyridinium compounds were also prepared by propargylation of pyridines lacking a 2-alkynyl substituent (**19**−**21**, Scheme 3) and by methylation of selected 2-alkynyl pyridines (**18a**, **18d**, and **18e**, Scheme 3 and Table 1). All final pyridinium triflate salts were isolated as hygroscopic solids that were purified by either washing with cold ether or recrystallization. These salts were characterized by $^1$H and $^{13}$C NMR, low-

Scheme 3: Synthesis of Pyridinium Skipped Aza-Enediynes

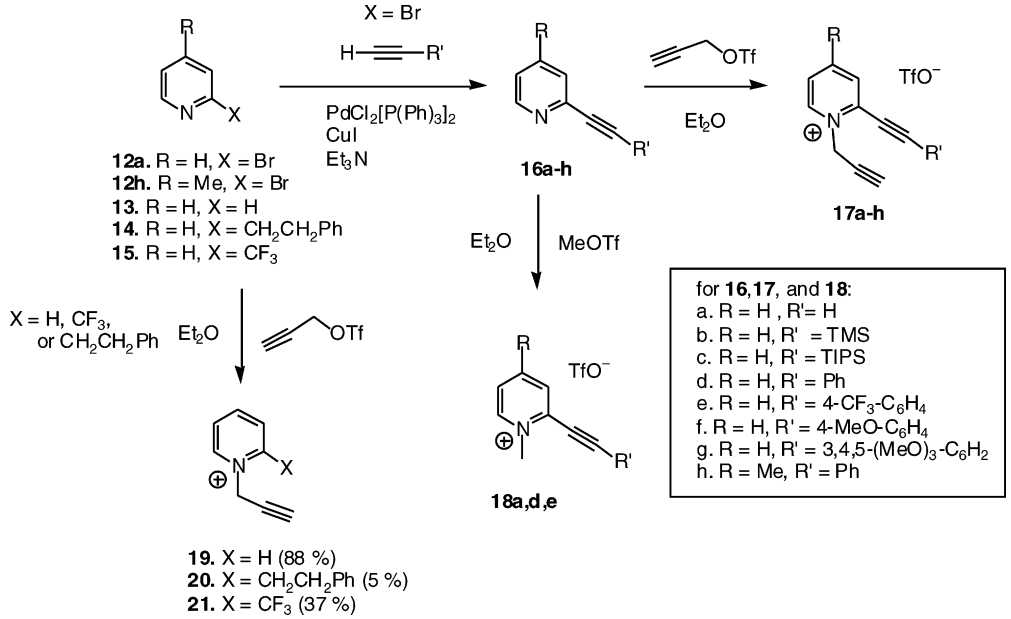



Table 1: Preparation of *N*-Propargyl-2-alkynyl Pridinium Triflates and Related Compounds

| series | yield of **16**[a] (%) | yield of **17**[a] (%) | yield of **18**[a] (%) |
|---|---|---|---|
| **a** | *b* | 42 | 90 |
| **b** | 58 | 64 | na[c] |
| **c** | 34 | 34 | na[c] |
| **d** | 30 | 72 | 97 |
| **e** | 19 | 49 | 98 |
| **f** | 25 | 68 | na[c] |
| **g** | 63 | 54 | na[c] |
| **h** | 50 | 66 | na[c] |

[a] Refer to Scheme 3. [b] **16a** was obtained commercially. [c] Not applicable.

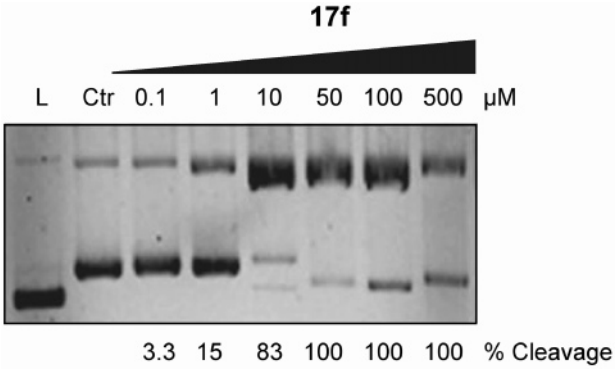



FIGURE 1: Supercoiled DNA cleavage by **17f**. Supercoiled ΦX174 phage DNA (50 $\mu$M base pairs) was incubated alone (lane Ctr) or with 0.1, 1, 10, 50, 100, and 500 $\mu$M **17f** in 50 mM Tris buffer at pH 8 and 13% (v/v) DMSO for 12 h at 37 °C and analyzed by gel electrophoresis (1% agarose, ethidium bromide stain). Lane L is linear DNA. The normalized percent DNA cleavage is shown below each lane.

and high-resolution mass spectrometry, and elemental analysis, all of which were consistent with the proposed structures.

*Supercoiled DNA Cleavage Studies*. An initial evaluation of the DNA cleavage ability of the pyridinium skipped aza-enediynes and related compounds was carried out using supercoiled DNA cleavage assays. It was expected that the propargyl/allene isomerization required for subsequent aza-Myers−Saito cyclization of these skipped aza-enediynes would occur more readily at higher pH; therefore, DNA cleavage assays were performed at both pH 7 and 8. After agarose gel electrophoresis, the amounts of supercoiled, nicked relaxed, and linear DNA products were determined; a representative gel is shown for compound **17f** (Figure 1). To quantify the DNA cleavage activity of these compounds, at least three individual experiments for each compound at each pH were performed and results are reported as the average $EC_{25}$, the concentration of compounds required to produce 25% normalized DNA cleavage (Table 2).

All of the pyridinium skipped aza-enediynes examined effect significant single-stranded DNA nicking at concentrations in the low micromolar range (Table 2) (*37*). With the exception of the two silyl-substituted analogues **17b** and **17c**, the DNA cleavage by the pyridinium skipped aza-enediynes is between 1.5 and 3.8 times more efficient at pH 8 than pH 7. Examination of the series **17d**−**f**, in which substitution on the 2-phenylethynyl moiety is varied, demonstrates a modest electronic effect on the DNA cleavage activity. The electron-donating *p*-methyoxyphenyl-substituted analogue **17f** is the most active DNA cleavage agent in this series, followed by the phenyl analogue **17d**, which is slightly more active than the *p*-trifluoromethylphenyl analogue **17e**. However, increasing the number of methoxy groups from one to three, as in 3,4,5-trimethoxyphenyl analogue **17g**, does not result in increased DNA cleavage activity over **17f**. The origin of these substituent effects may involve a balancing of electronic effects or a steric preference about the 2-ethynyl substituent. In either case, the *p*-methoxyphenyl analogue **17f** is not only the most effected DNA cleavage agent within this pyridinium series, it is also a more effective DNA cleavage agent than any of the previously reported heterocyclic skipped aza-enediynes (*20*, *21*).

The DNA cleavage results summarized in Table 2 demonstrate that the skipped aza-enediyne functionality is generally required for efficient DNA cleavage activity. With two exceptions, none of the compounds that lack the skipped aza-enediyne functionality demonstrates any appreciable DNA cleavage activity. The *N*-methyl-2-ethynyl pyridinium

Table 2: Cleavage of Supercoiled DNA by Pyridinium Skipped Aza-Enediynes and Related Compounds

| compound | pH 7 $EC_{25}$ ($\mu$M)[a] | pH 8 $EC_{25}$ ($\mu$M)[a] |
|---|---|---|
| **16e** | nd[b] | >100 |
| **16f** | nd[b] | >100 |
| **17b** | 4.6 ± 1.5 | 9.8 ± 2.3 |
| **17c** | 23.0 ± 5.2 | 23.5 ± 6.5 |
| **17d** | 15.5 ± 1.5 | 10.5 ± 1.5 |
| **17e** | 21.5 ± 8.5 | 11.5 ± 1.5 |
| **17f** | 6.8 ± 1.7 | 1.8 ± 1.0 |
| **17g** | 28.0 ± 3.0 | 16.1 ± 1.2 |
| **17h** | 16.5 ± 0.5 | 6.5 ± 0.4 |
| **18a** | 37.8 ± 5.2 | 39.0 ± 3.0 |
| **18d** | >100 | >100 |
| **18e** | >100 | >100 |
| **19** | nd[b] | >100 |
| **20** | >100 | >100 |
| **21** | 39.5 ± 3.5 | 44.3 ± 7.3 |

[a] Concentration of the compound required to effect 25% normalized DNA cleavage, expressed as the average of at least three assays ± standard deviation. [b] Not determined.

salt **18a** shows weak DNA cleavage activity, as does the *N*-propargyl-2-trifluoromethyl pyridinium salt **21**. None of the other *N*-methyl pyridinium salts (**18d** and **18e**) or *N*-propargyl pyridinium salts (**19** and **20)** examined cleaved DNA to any appreciable degree at concentrations up to 100 $\mu$M, the highest concentration examined.

The propensity of 2-ethynyl pyridinium salts to undergo nucleophilic addition has been noted (*38*). We found that the *N*-propargyl-2-ethynyl compound **17a** was unstable in nucleophilic solvents such as methanol, which precluded its evaluation in these DNA cleavage assays. On the basis of these observations, it is likely that the *N*-methyl-2-ethynyl pyridinium triflate **18a** acts as an electrophile toward DNA, leading to DNA cleavage in this assay. The 2-trifloromethyl-*N*-propargyl pyridinium salt **21** also displays DNA cleavage activity, although only at concentrations approximately twice as high as the weakest DNA cleaving skipped aza-enediyne. *N*-Propargyl pyridinium salts can also undergo nucleophilic attack (*39*). To probe the nature of the supercoiled DNA cleavage because of **17f** and **21** in more detail, inhibitor studies were carried out. While the DNA cleavage by both **17f** and **21** were inhibited by equimolar dithiothreitol (DTT) (35 and 70% inhibition, respectively), only the DNA cleavage by **21** was inhibited by $NaN_3$ and KBr (data not shown). The sensitivity of **21** to the presence of nucleophiles such as KBr may indicate that the DNA cleavage by this compound proceeds through a different mechanism than that because of the skipped aza-enediyne **17f**.

*Pyridinium Skipped Aza-Enediyne-Mediated DNA Cleavage Leads to Sequence-Independent, Frank-Strand Breaks and G-Selective Cleavage after Piperidine/Heat Treatment.* The pyridinium salt **17f** demonstrates the most pronounced, pH-dependent supercoiled DNA cleavage activity of the heterocyclic skipped aza-enediynes examined; therefore, this compound was selected for further studies. The sequence preference and nature of the DNA cleavage was examined using 229 base-pair 5′-$^{32}$P-labeled duplex DNA oligonucleotide, prepared as previously described (*20*). Incubation of the labeled DNA with **17f** in Tris buffer at pH 7 for 16 h at 37 °C followed by gel electrophoresis of the DNA products reveals a sequence-independent DNA cleavage that does not require postincubation piperidine/heat treatment (Figure 2A). DNA cleavage reactions that were treated with 10% piperidine at 95 °C for 15 min prior to electrophoresis show, in addition to an enhancement in the nonsequence selective cleavage, a more pronounced, G-selective cleavage pattern (Figure 2B). Under these conditions, the sequence-independent, frank DNA cleavage by **17f** is significant at concentrations of 50 $\mu$M and higher, whereas concentrations of **17f** as low as 1 $\mu$M result in significant DNA cleavage after piperidine/heat treatment. The frank DNA strand cleavage by **17f** occurs slowly over a period of hours; significant frank DNA cleavage is seen after 1−2 h, with a maximum in frank DNA cleavage after 12 h in the presence of 250 $\mu$M **17f** (data not shown). As expected, compound **16d**, which does not cleave supercoiled DNA (see above), also does not produce any DNA cleavage products when incubated with the 5′-labeled DNA duplex oligonucleotide (data not shown).

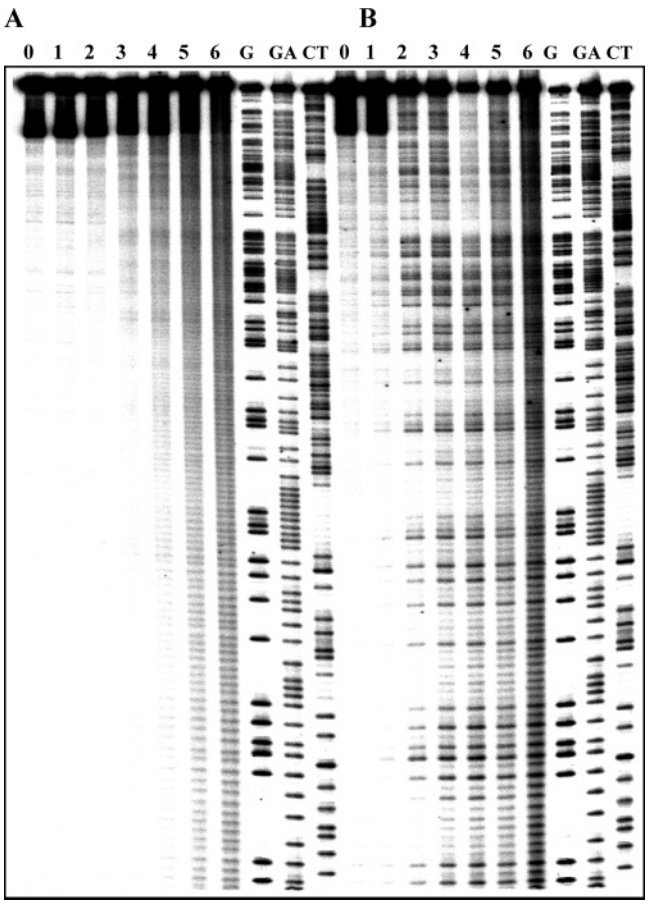



FIGURE 2: Cleavage of 5′-labeled DNA by pyridinium triflate **17f** leads to sequence-independent, frank-strand scission and G-selective cleavage after piperidine/heat treatment. Singly 5′-$^{32}$P-labeled double-stranded DNA (L151, 229 bp) was incubated with 0, 1, 10, 50, 100, 250, and 500 $\mu$M **17f** (lanes 0−6) in 50 mM Tris buffer at pH 7 and at 37 °C for 16 h. (A) Samples were subjected to phenol/chloroform extraction and EtOH precipitation, followed by gel electrophoresis. (B) Samples were treated the same as in A but heated at 90 °C for 15 min in 10% piperidine before gel electrophoresis. Lanes G, GA, and CT are Maxam−Gilbert sequencing reaction products.

The DNA cleavage because of **17f** is not light-dependent. Separate DNA cleavage reactions were carried out in the presence of **17f** (50 $\mu$M) in the dark and under irradiation with a sun lamp for 2 h. As shown in Figure 3, the DNA cleavage proceeds equally well under both conditions.

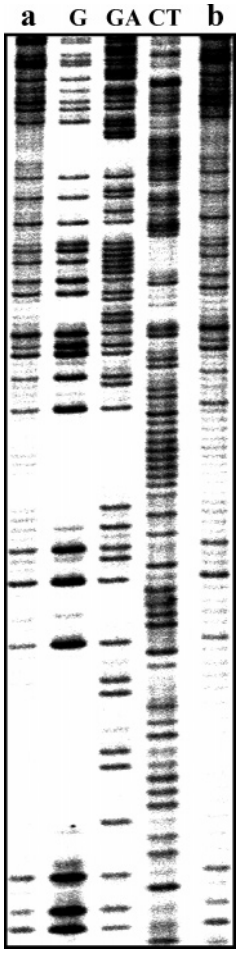



FIGURE 3: DNA cleavage because of **17f** does not require light. Singly 5′-$^{32}$P-labeled double-stranded DNA (L151, 229 bp) was incubated with 50 $\mu$M **17f** in 50 mM Tris buffer at pH 7 for 2 h in the dark (lane a) or with irradiation using a sun lamp (65 W, 25 cm in distance) (lane b). Incubation mixtures, which contained 1 $\mu$g of calf thymus DNA, were subjected to phenol/chloroform extraction, ethanol precipitation, and 10% piperidine at 95 °C for 15 min prior to electrophoresis. Lanes G, GA, and CT are Maxam−Gilbert sequencing lanes.

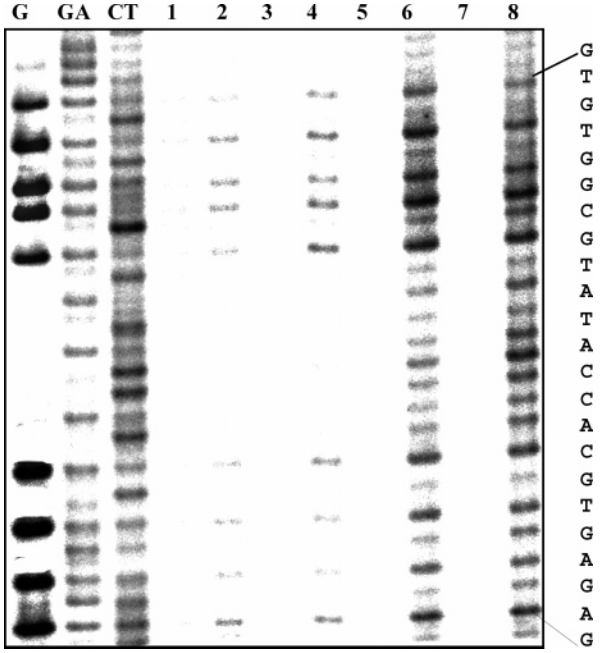



FIGURE 4: DNA cleavage by **17f** is pH-dependent. Double-stranded, 5′-labeled DNA (L151, 229 bp) was incubated with 50 $\mu$M **17f** in pH 6 and 10 mM sodium phosphate buffer or pH 7, 8, or 9 and 10 mM Tris buffer at 37 °C for 2 h. DNA reaction samples were extracted with phenol/chloroform, subjected to ethanol precipitation, and treated with 10% piperidine (95 °C, 15 min) prior to electrophoresis. Lanes 1, 3, 5, and 7 are DNA-incubated in the absence of **17f** at pH 6, 7, 8, and 9, respectively. Lanes 2, 4, 6, and 8 are DNA-incubated with **17f** at pH 6, 7, 8, and 9, respectively. Lanes G, GA, and CT are Maxam−Gilbert sequencing reaction products.

Similarly, no difference was noted when supercoiled DNA cleavage assays were carried out in the dark versus under ambient light conditions (data not shown).

The pH dependence of the cleavage of duplex DNA oligonucleotides was investigated by carrying out incubations of 5′-radiolabeled DNA with 50 $\mu$M **17f** in phosphate buffer (pH 6) and Tris buffer (pH 7, 8, and 9), followed by piperidine/heat treatment and electrophoresis. As shown in Figure 4, the DNA cleavage was most pronounced at pH 9, and at both pH 8 and 9, cleavage at all sites was observed, with enhanced cleavage at all G residues. At lower pH, weaker, G-selective cleavage was observed. These results are in qualitative accordance with the supercoiled DNA cleavage studies, in which **17f** was 3.8 times more effective at pH 8 compared to pH 7 (see above). The isomerization of *N*-propargylic imines to the corresponding allenes requires basic conditions (*14*). The pH dependence of the DNA cleavage by **17f** may similarly reflect a requirement for isomerization of the propargylic substituent prior to the cleavage event. The nature of the pH-dependent DNA cleavage exhibited by **17f** is distinct from that observed for the benzothiazolium salt **10** and the benzimidazolium skipped aza-enediyne **11**. The guanosine-selective DNA cleavage by **10** is pH-independent between pH 6 and 9 (*21*), and the benzimidazole **11** causes cytosine-selective cleavage at pH 6 and 7 but not pH 8 and above (*20*).

*G-Selective Cleavage Is Due to Singlet-Oxygen-Independent Guanine Oxidation.* Several possible origins of the G-selective DNA cleavage by **17f** observed after piperidine/heat treatment were explored. As has been observed with 2-alkynyl benzothiazolium salts (*21*), selective alkylation of guanine residues in DNA can lead to stable adducts that undergo cleavage upon treatment with hot piperidine, in analogy to the Maxam−Gilbert G-selective cleavage reaction with dimethyl sulfate. In the case of the 2-alkynyl benzothiazolium triflate **10**, these adducts can be observed as low-mobility electrophoretic bands (*21*). A 5′-$^{32}$P-labeled 14 base-pair duplex was incubated with 500 $\mu$M **10** or **17f** at 37 °C, for 2 h, and the DNA products were immediately subjected to gel electrophoresis (Figure 5). While the incubations with **10** afford a band migrating more slowly than the unmodified, labeled DNA (lane 1 in Figure 5), no such band is observed in the case of **17f**. These results indicate that **17f** does not form an adduct with the DNA or, if an adduct is formed, it cannot be resolved by gel electrophoresis under these conditions. This later possibility is unlikely, given the ready detection by gel electrophoresis of adducts formed between short duplex oligonucleotides and the related compounds **10** (Figure 5) and **11** (*20*).

Guanine is the most easily oxidized DNA base, and a number of oxidants, such as singlet oxygen, lead to G-selective cleavage of DNA (*40*, *41*). In addition to the lack

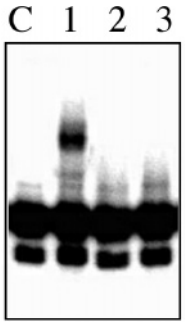



FIGURE 5: Pyridinium skipped aza-enediyne **17f** does not form a stable adduct with DNA. A 5′-$^{32}$P-labeled 14-mer duplex was incubated with 500 $\mu$M **10** in 10 mM sodium phosphate buffer at pH 6 (lane 1) or 500 $\mu$M **17f** in 10 mM sodium phosphate buffer at pH 6 (lane 2) or pH 8 (lane 3) and 37 °C for 2 h, and the products were analyzed by electrophoresis. No low-mobility adduct is observed in the case of **17f**.

of evidence for a photochemical mechanism of DNA cleavage (see above), the possible involvement of singlet oxygen in the G-selective DNA cleavage by **17f** is excluded by the observation of equal DNA cleavage efficiency in $D_2O$ and $H_2O$ (*42*, *43*) (data not shown). However, there is evidence that the G-selective cleavage by **17f** proceeds through guanine oxidation. Incubation of calf thymus DNA with **17f** followed by enzymatic hydrolysis and analysis by HPLC with electrochemical detection reveal the formation of 8-oxo-dG ($5.7 \pm 0.4/10^5$ mol of dG). The ratio of 8-oxo-dG/dG in DNA incubated in the presence of **17f** is over 100 times greater than the ratio of DNA incubated in the absence of **17f** ($0.046 \pm 0.006/10^5$ mol of dG).

*Frank DNA Strand Scission Is Due to Deoxyribosyl Hydrogen-Atom Abstraction.* The sequence-independent, frank-strand scission of DNA by **17f** is similar to that observed by the hydroxyl radical (*44*). A difference between the DNA cleavage because of **17f** and that because of hydroxyl radicals is the sensitivity of the later to specific cosolvents. The rate of reaction between the hydroxyl radical and DMSO (*45*) is over 300 times faster than that for acetonitrile (*46*). The addition of 5% of DMSO as a cosolvent in hydroxyl radical DNA cleavage reactions causes near complete inhibition of the DNA cleavage, when compared to the cleavage in the presence of 5% acetonitrile (Figure 6). In contrast to this very pronounced inhibitory effect of DMSO on the DNA cleavage because of hydroxyl radicals, there is only a very subtle effect of DMSO cosolvent on the DNA cleavage because of **17f** (Figure 6).

The ability of the minor-groove binding DNA ligand distamycin A to inhibit the DNA cleavage by **17f** was also investigated. The 229 bp, 5′-labeled DNA oligonucleotide was incubated with **17f** (500 $\mu$M) in phosphate buffer at pH 8 for 2 h at 37 °C in the absence or presence of distamycin A (1−500 $\mu$M), and the DNA cleavage products were resolved by PAGE after piperidine/heat treatment. Under these conditions, the DNA cleavage by **17f** is predominantly sequence-independent in the absence of distamycin A (lane 1 in Figure 7). In the presence of distamycin A, the DNA cleavage because of **17f** is selectively inhibited at sites on the 229-bp-labeled DNA oligonucleotide containing four or more consecutive AT base pairs (lanes 2−4 in Figure 7). The observation of a distamycin A "footprint" indicates that attack by **17f** occurs from the minor groove face (*47*) of the DNA double helix.

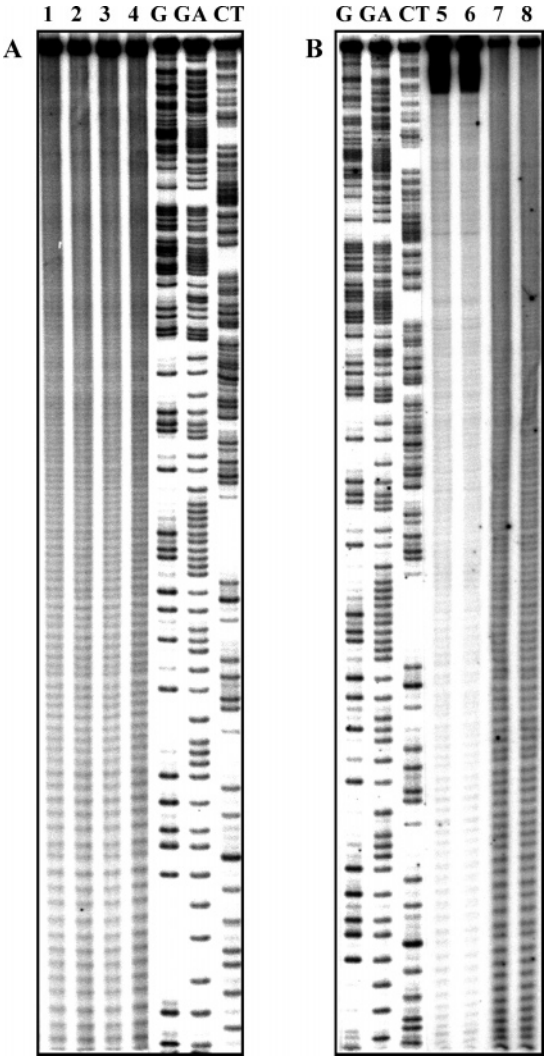



FIGURE 6: DNA cleavage because of **17f** is relatively insensitive to DMSO. (A) 5′-Labeled double-stranded DNA (L151, 229 bp) was incubated with 500 $\mu$M **17f** in 50 mM Tris buffer at pH 8 and 37 °C for 12 h in the presence of either 5% DMSO (lanes 1 and 3) or 5% acetonitrile (lanes 2 and 4). (B) 5′-Labeled double-stranded DNA (L151, 229 bp) was subjected to a hydroxyl radical generated by the Fenton reaction [0.05% $H_2O_2$, 1.5 mM L-ascorbic acid, 15 $\mu$M $Fe(NH_4)_2(SO_4)_2$, and 0.3 mM EDTA] in 50 mM sodium phosphate at pH 8 and room temperature for 2 min in the presence of either 5% DMSO (lanes 5 and 6) or 5% acetonitrile (lanes 7 and 8).

To more closely examine the nature of the DNA products formed in the presence of **17f**, a 5′-labeled, 14 base-pair duplex oligonucleotide was incubated with 500 $\mu$M **17f** for 14 h at 37 °C and the DNA products were analyzed by high-resolution PAGE, both before and after piperidine/heat treatment. As a control, the DNA products formed by treating the labeled DNA with hydroxyl radicals were also analyzed. Hydroxyl radicals (*45*) and other radical-based DNA cleavage agents (*1*, *2*, *48*) that abstract the 4′-hydrogen atoms from the minor groove of DNA lead to the formation of 3′-phosphoglycolate-terminated DNA fragments. As shown in Figure 8, the expected 3′-phosphate-terminated and 3′-phosphoglycolate-terminated DNA products of hydroxyl radical DNA cleavage are resolved as a doublet of bands at each cleavage site, with the less abundant 3′-phosphoglycolate-terminated products migrating slightly faster than the more abundant 3′-phosphate-terminated products, which are

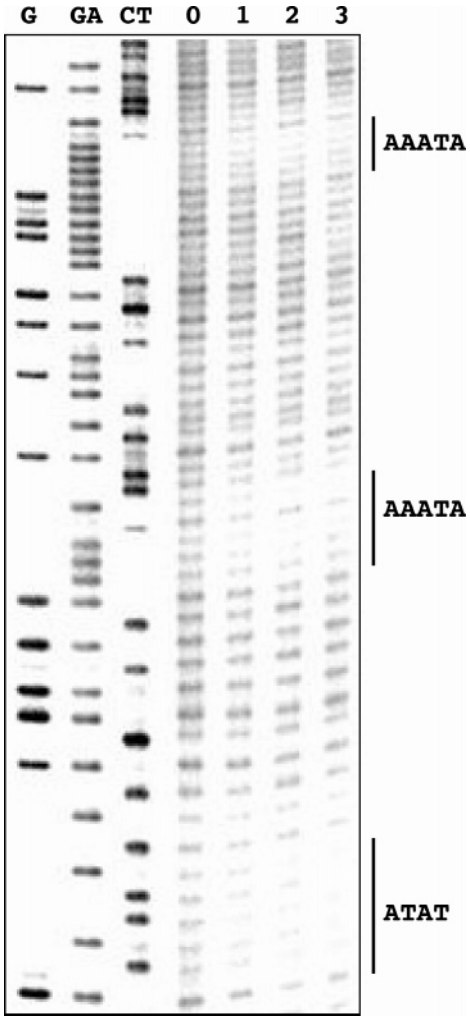



FIGURE 7: Distamycin inhibits the cleavage of DNA by **17f** by blocking access to the minor groove. Double-stranded, 5′-labeled DNA (L151, 229 bp) was incubated in the presence of various concentrations of distamycin A (0, 1, 10, and 100 $\mu$M, lanes 0−3) in 50 mM phosphate buffer at pH 8 and 37 °C for 15 min, followed by the addition of **17f**. After an additional 2 h, the DNA was purified by phenol/chloroform extraction and ethanol precipitation, followed by treatment with 10% piperidine (95 °C, 15 min) and electrophoresis. The sequence to the right of the figure corresponds to regions of protection from **17f**-mediated DNA cleavage in the presence of distamycin A.

identical to the products of Maxam−Gilbert sequencing reactions. A comparison of the products of hydroxyl radical cleavage to those produced by **17f** prior to piperidine/heat treatment (lane 1 in Figure 8) demonstrates that **17f** also affords 3′-phosphglycolate-terminated DNA cleavage products, although, in the case of **17f**, the ratio of the 3′-phosphoglycolate-terminated to 3′-phosphate-terminated products is lower than in the case of the hydroxyl radical. After piperidine/heat treatment of the DNA treated with **17f**, additional cleavage affording 3′-phosphate-terminated products is observed at all cleavage sites but especially at G residues (lane 2 in Figure 8). While the presence of 3′-phosphoglycolate-terminated DNA cleavage products is commensurate with deoxyribose 4′-hydrogen-atom abstraction (*48*), it is possible that other sites of deoxyribose hydrogen-atom abstraction are involved in the DNA cleavage by **17f**. Attack at other hydrogen atom sites in the minor groove of DNA, including the deoxyribose 5′ position, leads to 3′-phosphate-terminated products (*48*, *49*). The deoxyribose 4′ and 5′ hydrogens line the edge of the DNA minor groove, where negative-charge density is highest (*50*). It is possible that cationic **17f** or the corresponding aza-enyne allene forms weak, sequence-independent electrostatic associations with DNA that may help position it for hydrogen-atom abstraction at these positions.

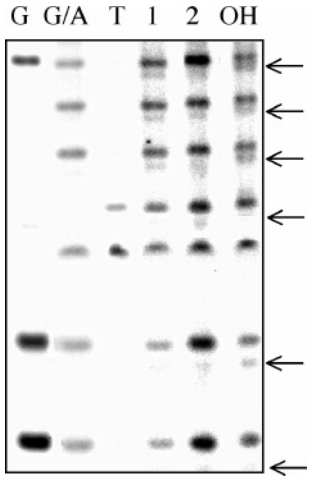



FIGURE 8: Pyridinium triflate **17f** cleavage of DNA produces 3′-phosphoglycolate-terminated products. A 5′-labeled 14-mer duplex oligonucleotide was incubated with **17f** (500 $\mu$M) in 50 mM Tris buffer at pH 7 for 14 h at 37 °C, and the DNA cleavage products were compared to those generated by the hydroxyl radical [0.05% $H_2O_2$, 1.5 mM ascorbate, 15 $\mu$M $Fe(NH_4)_2(SO_4)_2$, and 0.3 mM EDTA for 2 min at room temperature]. Lane 1, **17f**. Lane 2, **17f** followed by piperidine/heat treatment. Lane OH, hydroxyl radical. Lanes G, GA, and T are Maxam−Gilbert sequencing lanes.

The above results show that the pyridinium skipped aza-enediyne **17f** cleaves DNA through two pathways. The observation of sequence-independent, frank-strand scission, distamycin footprints, and 3′-phosphoglycolate products all indicate a radical-based DNA cleavage mechanism involving deoxyribose hydrogen-atom abstraction. The lack of inhibition by DMSO and the ratio of 3′-phosphoglycolate-terminated/3′-phosphate-terminated products observed with **17f** indicate that diffusible hydroxyl radicals are not involved in the DNA hydrogen-atom abstraction. Although the identity of the radicals (or diradicals) responsible for the DNA hydrogen-atom abstraction in the presence of **17f** is not known, certain observations point to a role of aza-Myers or aza-Schmittel cyclization in this process. Efficient DNA cleavage requires both the presence of the skipped aza-enediyne moiety and basic pH, which may induce the isomerization of the skipped aza-enediyne to the aza-enyne allene required for diradical-generating cyclization. Attempts to identify the trapping products of this cyclization have not been successful because of the complexity of reaction mixtures when **17f** is incubated under DNA cleavage reaction conditions (*51*).

The second pathway of DNA cleavage by **17f** leads to G-selective cleavage products after piperidine/heat treatment. The inability to observe DNA adducts with **17f** and the observation of 8-oxo-dG products indicate that this G-selective cleavage proceeds through DNA oxidation. This DNA oxidation does not require light and is not mediated by singlet oxygen. On the basis of the DMSO inhibition results, it is also unlikely that this oxidation is mediated by hydroxyl radicals. One possible, unifying mechanism to account for both the sequence-independent, frank DNA

strand scission and the G-selective DNA cleavage by **17f** involves guanine H8 hydrogen-atom abstraction or oxidation by a species derived from **17f** after aza-Myers or aza-Schmittel cyclization (*40*). However, the details of the mechanism for DNA oxidation by **17f** are not clear, and alternative processes that do not involve cyclization cannot be excluded at this time.

## CONCLUSIONS

In summary, we have reported the synthesis of a series of *N*-propargyl-2-alkynylpyridinium triflates as pyridinium-based skipped aza-enediynes that may undergo base-promoted isomerization and cyclization to diradicals capable of cleaving DNA. The efficient DNA cleavage by these pyridinium salts requires the presence of the skipped aza-enediyne functionality and basic pH but does not require light. Detailed analysis of the DNA cleavage by one member of this class has shown that two pathways for DNA cleavage exist. One pathway leads to sequence-independent, frank DNA strand scission through deoxyribose 4′-hydrogen-atom abstraction. A second pathway leads to G-selective DNA cleavage after piperidine/heat treatment. This second pathway involves DNA oxidation and the formation of 8-oxo-dG.

Radical-based DNA cleavage agents based on enediyne or enyne allene diradical-generating cyclizations are known; however, this is the first example in which a heteroatom variant diradical-generating cyclization has been employed in the design of a DNA cleavage agent that demonstrates radical-based DNA cleavage products. The ease of preparation of these pyridinium skipped aza-enediynes makes them attractive for further elaboration and optimization through, for example, the incorporation of DNA-recognition elements.

## ACKNOWLEDGMENT

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