Chemistry of the 2,5-Didehydropyridine Biradical: Computational, Kinetic, and Trapping Studies toward Drug Design

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Abstract: A combined computation, kinetic, and trapping study of the 2,5-didehydropyridine biradical finds the hydrogen abstraction reaction to be tunable by protonation. The observation of small amounts of pyridine products in the thermolysis of a *C,N*-dialkynylimine, or azaenediyne, only when the reaction occurs in the presence of moderate amounts of protic acid, is consistent with qualitative theoretical predictions as well as ab initio calculations at the CASSCF and CASMP2 levels. The implication of these findings for a more selective antitumor agent is discussed.

Introduction

We report a combined computational, kinetic, and trapping study of the reactions of the *C,N*-dialkynylimines,[1] or azaenediynes, with an emphasis on the delineation of the conditions under which the chemistry of an intermediate 1,4-arene biradical might direct us toward a DNA-damaging agent with greater selectivity against tumor cells as compared to *p*-benzyne. The latter biradical occurs in the activated form of the enediyne natural products[2] such as calicheamicin, dynemicin, and esperamicin and has been implicated as the key structural element conferring cytotoxicity to the compounds in Scheme 1.[3]

These natural products, as well as their more recently discovered cousins, e.g. kedarcidin,[4] C-1027,[5] and namenamicin,[6] were promising candidates as chemotherapeutic agents. The compounds displayed high activity against a number of tumor cell lines,[7] both in vitro and in vivo, but unfortunately also showed unacceptable toxic side effects in both animal and human trials.[8] Further development of this family of compounds as drugs requires, accordingly, a way to engineer greater selectivity for tumor cells into the compounds. Attempts to achieve this end through immunoconjugates of the calicheamicin chromophore to a monoclonal antibody[9] have not been entirely successful.

In the present study, the desired end is approached differently through a modification to the enediyne "warhead" common to all the natural products so as to generate a modified biradical intermediate whose DNA-damaging ability would be enhanced for tumor cells.[10] The operational model is a qualitative quantum chemical picture of reactivity patterns in singlet carbenes[11] and biradicals, on which basis we had conducted kinetic studies in solution on the hydrogen abstraction rates for substituted *p*-benzyne biradicals.[12] The prediction of slow hydrogen abstraction by singlet biradicals was experimentally confirmed in that work, leading us to the present prediction that biradicals based on a 2,5-didehydropyridine nucleus could show pH-dependent hydrogen abstraction ability (Scheme 2).

The present computational, kinetic, and trapping study, and accompanying synthetic project, seeks to verify the prediction that the 2,5-didehydropyridine biradical does abstract hydrogens more readily at the low pH values and, hence, can serve as the prototype for a DNA-damaging agent more selective for tumor cells.

Experimental Section

The synthesis of *C,N*-dialkynylimines, **1a** and **1b**, was done according to a route which closely resembles the synthesis published recently by David and Kerwin.[1] Alkynylation of the tosyl ester of a *C*-alkynyloxime by an alkynyl cuprate reagent proceeds without Beckmann rearrangement, as reported by Wuerthwein and Weigman[13] for alkynylations of similar oxime esters, most likely through a free radical coupling mechanism. Kinetic runs on the tandem ring closure/ring opening of the azaenediynes were performed for **1b**. Dilute samples of **1b** in *n*-heptane (0.14 mM) were thermolyzed in a constant temperature oil bath (temperature stability ± 0.2 °C), removed, and

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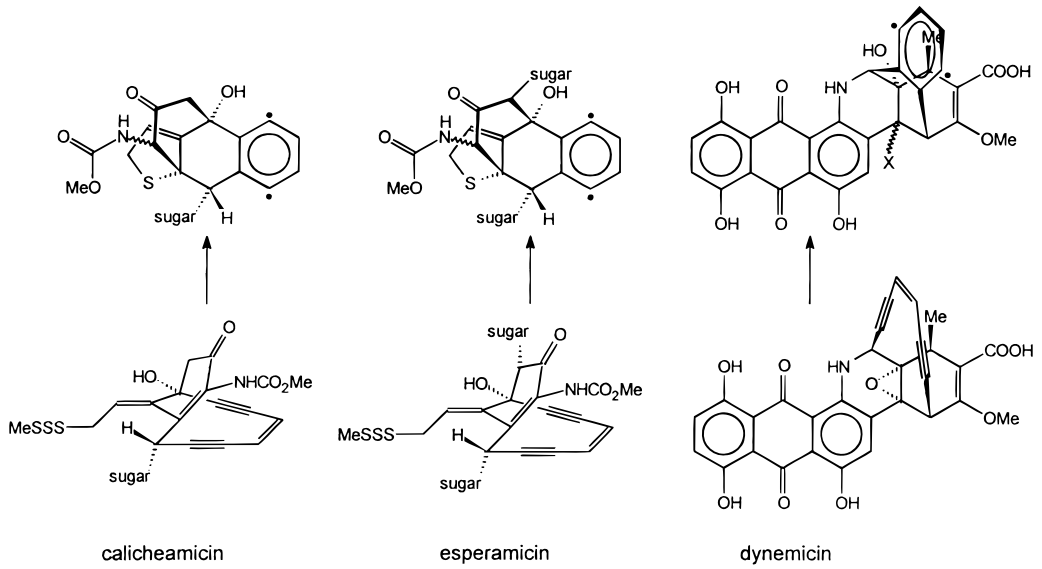
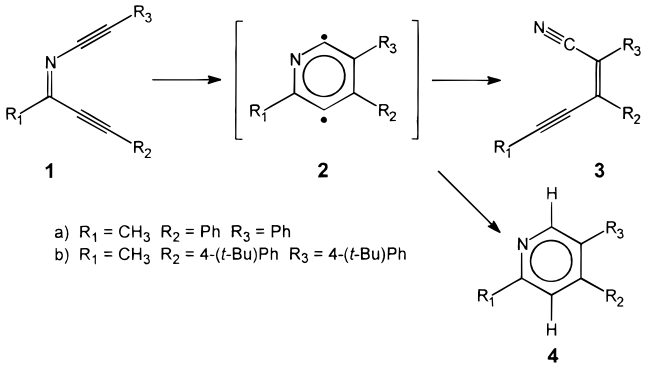
Scheme 1



Scheme 2



assayed by HPLC (Merck LaChrome, LiChrospher Si-60, 250/4 5 μm column, UV detector fixed at 337 nm). The rearrangement was clean and first-order, yielding 24 points at five temperatures over a 42 °C range. These were fitted to the Arrhenius equation, yielding a linear fit (correlation coefficient: 0.996) from which the activation parameters were extracted in the ordinary way. The crystalline solid **1b** was used for the Arrhenius plot, instead of **1a**, an oily liquid, because of its greater ease in purification and handling.

Trapping of the a putative biradical intermediate at room temperature and ~100 °C was done by thermolysis of dilute (~2 mM) solutions of **1a** in sealed vials containing diisopropyl ether (solvent and hydrogen atom donor), 2-fluoropyridine (solvent and buffer), and HBF_4 (protic acid) in varying proportions. The diisopropyl ether and fluoropyridine, typically in a 1:1 volume-to-volume ratio, serve as the reaction medium. After each reaction, the solutions were subjected to a minimal workup (only enough to protect the GC/MS), consisting of neutralization with solid K_2CO_3 and filtration through a short silica plug, prior to GC/MS analysis (Fisons MD800, DB11 capillary column). Authentic samples of 2-methyl-4,5-diphenylpyridine (**4a**), 1-cyano-1,2-diphenylpent-1-en-3-yne (**3a**), phenylacetonitrile (**5**), and 1-phenylbut-1-yn-3-one (**6**) (which are, respectively, the products of hydrogen abstraction by an intermediate biradical, the cyclization/ring-opening product, and the two hydrolysis products of **1a**) were prepared to validate the GC/MS identification of each compound by both retention time and mass spectrometric fragmentation pattern. The azaenediynes themselves, when run through the GC/MS, are indistinguishable from the product nitriles, presumably because they rearrange rapidly in the 230 °C GC injector port. Deuterium abstraction experiments were conducted in the same manner, but with diisopropyl ether-d_{14} as the solvent.

Methyl (4-*tert*-Butylphenyl)ethynyl Ketone.[14] A 9.28 g portion of 4-*tert*-butylphenylacetylene (58.7 mmol) was dissolved in 100 mL of freshly distilled THF and cooled to −78 °C. Then, 36.5 mL of 1.6 N *n*-butyllithium in hexane (58.4 mmol) was added slowly. The solution was stirred for 90 min, after which 7.3 mL of $BF_3{\cdot}Et_2O$ (8.2 g, 58.4 mmol) was added dropwise. A cold solution (−78 °C) of 8.8 g of acetic anhydride in 10 mL of THF was injected in one portion. The resulting mixture was stirred for 15 min. The reaction was quenched with 200 mL of 1 N NaOH solution and extracted with hexane. The resulting turbid organic layer was filtered through Celite, dried over $MgSO_4$, and isolated by vacuum distillation (0.05 Torr/88 °C). Yield: 5.43 g, 27.2 mmol, 47%. Mp: 33−35 °C. IR (CCl_4): 2966 s, 2906 m, 2203 s, 1676 s, 1504 m, 1358 m, 1282 m, 1157 m, 1106 m, 1018 m. ^{1}H NMR (200 MHz, $CDCl_3$): 7.55−7.34 (m, 4H), 2.45 (s, 3H), 1.33 (s, 9H). ^{13}C NMR (50 MHz, $CDCl_3$): 184.2, 154.1, 132.6, 125.3, 116.4, 90.6, 87.7, 34.6, 32.3, 30.6. MS (EI): 200 [M^+; 22], 185 [$M^+ - 15$; 100], 170 [$M^+ - 30$; 10], 157 [$M^+ - 43$; 18], 115 [$M^+ - 85$; 20]. HRMS (EI): 200, exact mass found 200.1201, calcd for $C_{14}H_{16}O$, 200.120 115. R_f (hexane/ethyl acetate, 4/1): 0.35.

Methyl (4-*tert*-Butylphenyl)ethynyl Ketoxime. Methyl (4-*tert*-butylphenyl)ethynyl ketone (5.43 g, 27.2 mmol), 2.10 g of $NH_2OH{\cdot}$HCl, and 5.00 g of sodium acetate were suspended in 50 mL of ethanol and stirred vigorously. The reaction was followed by TLC and stopped after 2 h, after the ketone had fully reacted. The mixture was extracted with water and then hexane/ethyl acetate, 4/1, and the organic layer was dried over Na_2SO_4. The crude material was separated by column chromatography (5.8 cm, 520 g of silica, hexane/ethyl acetate, 6/1). Note that the oxime with the OH syn to the acetylenic linkage rapidly cyclizes; hence only the anti isomer is isolated. Yield: 2.09 g, 9.70 mmol, 36%. IR (KBr): 3300 b, 3969 s, 2222 w, 1603 w, 1503 m, 1024 m. ^{1}H NMR (200 MHz, $CDCl_3$): 8.44 (s, 1H), 7.50−7.38 (m, 4H), 2.16 (s, 3H), 1.32 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): 153.4, 139.8, 132.2, 125.7, 118.7, 100.9, 79.8, 35.0, 31.2, 20.6. MS (EI): 215 [M^+; 35], 200 [$M^+ - 15$; 100], 184 [$M^+ - 31$; 20], 170 [$M^+ - 45$; 20]. HRMS (EI): 200, exact mass found 200.1075, calcd for $C_{13}H_{14}NO$ 200.107 539. R_f (hexane/ethyl acetate, 6/1): 0.375.

Methyl (4-*tert*-Butylphenyl)ethynyl Ketoxime Tosylate. The oxime (2.09 g, 9.70 mmol) was dissolved in an ice-cold mixture of pyridine and dichloromethane, 2.12 g of tosyl chloride was added, and the reaction was monitored by TLC. After 5 h the solvents were removed, and the reaction mixture was consecutively extracted with 400 mL of ethyl acetate and washed with 100 mL of 0.2 M HCl, 100 mL of saturated $NaHCO_3$ solution, and 100 mL of brine. The organic layer was dried with $MgSO_4$, and the solvent was removed. The recrystallization from hexane/dichloromethane yielded 1.96 g (55%)

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of crystals. Mp: 79−82 °C. IR (CCl_4): 2968 s, 2870 m, 2234 m, 2191 m, 1598 m, 1387 s, 1180 s, 1096 m, 1023 m. ^{1}H NMR (200 MHz, $CDCl_3$): 7.92 (d, J = 7.88, 2H), 7.5−7.3 (m, 6H), 2.47 (s, 3H), 2.18 (s, 3H), 1.35 (s, 9H). ^{13}C NMR (50 MHz, $CDCl_3$): 154.2, 150.0, 145.0, 132.8, 132.4, 129.6, 128.9, 125.6, 117.5, 103.3, 78.9, 34.9, 30.9, 21.6, 20.7. MS (EI): 369 [M^+; 6], 354 [$M^+ - 15$; 11], 313 [$M^+ - 56$; 7], 199 [$M^+ - 170$; 25], 184 [$M^+ - 185$; 100]. HRMS (EI): 369, exact mass found 369.1389, calcd for $C_{21}H_{23}NO_3S$ 369.139 864.

Copper(I) (4-*tert*-Butylphenyl)acetylide.[15] Nitrogen was bubbled through 250 mL of a concentrated aqueous ammonia solution for 30 min, after which 10.7 g of CuI was added. An oxygen-free ethanolic solution of 9.2 g of 4-*tert*-butylphenylacetylene was cannulated into the well-stirred copper salt solution. A yellow precipitate formed. The resulting suspension was filtered, and the yellow solid was washed consecutively with 200 mL of water, 200 mL of ethanol, 200 mL of hexane, 200 mL of ethanol, and 100 mL of water. After the mixture was dried (0.01 Torr) at room temperature, 9.20 g of copper acetylide was obtained. Yield: 9.20 g, 43.4 mmol, 80%.

1,6-Bis(4-*tert*-butylphenyl)-3-aza-4-methylhex-3-ene-1,5-diyne.[1,13] Compound **1b** was prepared, starting with a suspension of 0.55 g of copper acetylide (2.6 mmol) in THF, to which was added a solution of 0.91 g of 4-*tert*-butylphenylacetylene (5.8 mmol) and 3.2 mL of butyllithium solution (1.6 M in hexane) in THF at 0 °C. When the mixture became clear, it was added dropwise at −28 °C to a solution of 1.91 g of methyl (4-*tert*-butylphenyl)ethynyl ketoxime tosylate in 150 mL of THF. The mixture was allowed to warm to room temperature over 12 h, and the reaction was quenched with saturated aqueous NH_4Cl. The mixture was diluted with ethyl acetate, the layers were separated, and the organic solvents were removed. The residue was suspended in a mixture of hexane/ethyl acetate, 4/1, and filtered through silica. The resulting raw material was purified twice by column chromatography: (1) 400 g of silica, 5.8 cm, hexane/ethyl acetate, 15/1; (2) 600 g of silica, 5.8 cm, hexane/dichloromethane, 4/1 gradually to 2/1, yielding a yellow solid consisting of the cis and trans isomers, which were not separable by column chromatography or HPLC. Yield: 0.250 g, 0.63 mmol, 12.2%. Mp: 126−128 °C. IR (CCl_4): 2966 s, 2905 w, 2858 w, 2213 m, 2174 m, 1500 w, 1552 m. ^{1}H NMR (200 MHz, $CDCl_3$): 7.53−7.35 (m, 8H), 2.50, 2.42 (2 s, 3H), 1.332, 1.327 (2 s, 18H). ^{13}C NMR (125 MHz, $CDCl_3$): 164.3, 160.9, 153.9, 153.6, 151.5, 151.3, 132.5, 132.4, 131.3, 131.1, 125.7, 125.6, 125.4, 125.35, 121.5, 121.3, 118.2, 118.1, 100.2, 100.15, 95.7, 92.6, 91.9, 89.9, 89.7, 86.0, 35.05, 35.0, 34.8, 31.21, 31.19, 31.1, 26.7, 25.5. MS (EI): 355 [M^+; 90], 340 [$M^+ - 15$; 100], 284 [$M^+ - 71$; 33], 242 [$M^+ - 113$; 80]. Anal. Calcd for $C_{26}H_{29}N$ (355.52): C, 87.84; H, 8.22; N, 3.94. Found: C, 88.11; H, 8.22; N, 4.00. UV (heptane): 195.5 (4.8), 251.5 (4.5), 335.5 (4.4).

1,6-Diphenyl-3-aza-4-methylhex-3-ene-1,5-diyne. Compound **1a** was prepared analogously to **1b** and gave analytical data identical with those reported in ref 1.

2-Methyl-3,4-diphenylpiperidine.[16] 4-Oxo-1,2-diphenylpentanecarbonitrile was prepared, following procedure of El Bouz et al.[17] Then 5.0 g of the cyano ketone was dissolved in ethanol/THF and degassed by sparging with argon. $PtO_2 \cdot H_2O$ (100 mg) was added to the solution, which was kept under 8 atm of hydrogen for 17 h at 80 °C. The catalyst was destroyed by adding dichloromethane, the mixture was filtered, and the basic compounds were transferred to the water layer by adding 250 mL of 1 N HCl and ether. The water layer was reextracted with 500 mL of 1 N NaOH and ether. The organic layer was dried with Na_2SO_4 and evaporated. An analytical sample was distilled (0.5 Torr, 180 °C). The sample contained all eight possible stereoisomers. ^{1}H NMR (200 MHz, $CDCl_3$): 7.22−6.80 (m, 10H), 3.58−2.80 (m, 4H), 2.20−1.40 (m, 4H), 1.40−1.10 (4d, 3H).

2-Methyl-4,5-diphenylpyridine.[18] Compound **4a** was prepared from 2-methyl-3,4-diphenylpiperidine. Then 500 mg of the mixture of the isomeric piperidines was mixed with 100 mg of Pd/C (10% Pd) and heated to 240 °C for 30 min, followed by 300 °C for 10 min. The mixture was allowed to cool to room temperature and extracted with 25 mL of ethanol. The solvent was removed, and the crude product was dissolved in ether. A 20 mL portion of 1 N HCl in ether was added to precipitate the hydrochlorides. The solution was decanted, and the insoluble salts were dissolved in ethanol. After ether was added, the solution became turbid. The mixture was cooled to −78 °C and allowed to warm to room temperature overnight. The resulting crystals were the pure hydrochloride of **4a**. The hydrochloride was extracted with 25 mL of 1 N NaOH and ether, the organic layer was dried over Na_2SO_4, and the solvent was removed. The pyridine was a waxy, white solid with no detectable melting point. IR (CCl_4): 3060 m, 3027 m, 2961 m, 2926 m, 1947 w, 1877 w, 1807 w, 1754 w, 1590 s, 1475 s, 1438 m, 1381 m, 1262 s. ^{1}H NMR (300 MHz, $CDCl_3$): 8.51 (s, 1H), 7.28−7.22 (m, 6H), 7.21 (s, 1H), 7.16−7.10 (m, 4H), 2.63 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 157.6, 150.5, 148.4, 139.1, 138.0, 133.4, 130.1, 129.5, 128.4, 128.0, 127.3, 124.4, 111.2, 23.3. MS (EI): 245 [M^+; 100], 244 [$M^+ - H$; 65], 230 [$M^+ - 15$; 22], 215 [$M^+ - 30$; 8], 202 [$M^+ - 45$; 15]. HRMS (EI): 245, exact mass found 245.1205, calcd for $C_{18}H_{15}N$ 245.120 450. Mp [**4a**]·HCl: 178−181 °C.

(*Z*)-1-Cyano-2,3-bis(4-*tert*-butylphenyl)pent-1-en-3-yne. Compound **3b** was prepared by heating 17.1 mg of **1b** in 3 mL of heptane at 98 °C for 7 h. The solvent was removed, and the product was purified by preparative TLC (hexane/ethyl acetate, 25/1). Yield: 13.3 mg, 78%. IR (CCl_4): 2965 s, 2869 m, 2226 m, 2208 m, 1734 m, 1605 m, 1462 m, 1364 m, 1267 m, 1109 m, 1018 m, 838 m. ^{1}H NMR (200 MHz, $CDCl_3$): 7.26−7.12 (m, 8H), 2.18 (s, 3H), 1.29 (s, 9H), 1.28 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): 152.6, 152.1, 137.3, 133.0, 129.1, 129.06, 125.5, 125.2, 119.8, 117.0, 98.0, 80.5, 79.6, 34.75, 34.7, 31.2, 31.1, 5.1. MS (EI): 355 [M^+; 90], 340 [$M^+ - 15$; 100], 284 [$M^+ - 71$; 33], 242 [$M^+ - 113$; 80]. HRMS (EI): 355, exact mass found 355.2299, calcd for $C_{26}H_{29}N$ 355.230 000. UV (pentane): 202 (4.7), 239 (4.5), 324 (4.4).

(*Z*)-1-Cyano-2,3-diphenylpent-1-en-3-yne.[1] Compound **3a** was prepared analogously to **3b** by heating a sample of **1a** in heptane at 120 °C for 6 h. After cooling, the solvent was removed. The residue was purified by preparative TLC (hexane/ethyl acetate, 25/1). To remove traces of **4a**, the crude product was extracted five times with 10 mL of 0.1 N HCl and ethyl acetate. The organic layer was dried and evaporated. The purity of **3a** was checked by GC/MS. Analytical data matched those in ref 1.

2-Iodopropane-d_7. A 22.5 g portion of 2-propanol-d_8 (d > 99%) was added to 3.5 g of red phosphorus. The flask was fitted with a 25 mL dropping funnel filled with 42 g of iodine on top of which there was a condenser. The alcohol was heated to reflux, and small portions of the collected alcoholic iodine solution were dropped into the flask. After 4 h, the addition was finished, and the mixture was heated to reflux for 12 h. The product was purified by distillation through a 20 cm Vigreux column. Yield: 40.40 g, 228.2 mmol, 70%. Bp: 87.5 °C. IR (CCl_4): 2236 m, 2212 m, 2122 m, 2059 m, 1148 m, 987 s, 977 s. ^{1}H NMR (300 MHz, $CDCl_3$): 4.3 (C2-*H*, I = 14.7), 1.8 (C1−H, I = 28.9). MS: 177 [M^+; 32.2, *d*7], 176 [M^+; 0.75, *d*6], 159 [$M^+ - CD_3$; 0.77], 127 [I^+; 16.6], 50 [$M^+ - I$; 32.2, 100, *d*7], 49 [$M^+ - I$; 2.4, *d*6]. An isotopic purity of 99.7% was determined by GC/MS. The deuteration grades at C1 (99.7%) and at C2 (99.5%) were determined by ^{1}H NMR.

Diisopropyl Ether-d_{14}.[19] Silver nitrate (23.5 g) was dissolved in water from which silver hydroxide was precipitated with 200 mL of 5 N NaOH solution. The precipitate was washed with water and mixed with 20 g of 2-iodopropane-d_7. The mixture was refluxed for 5 h and then distilled. Careful distillation over metallic sodium produced the pure ether. Yield: 0.76 g, 13.1 mmol, 12%. Bp: 61 °C. IR (CCl_4): 2228 s, 2147 m, 2094 m, 1214 m, 1159 s, 1089 s, 1048 s, 964 m. ^{1}H NMR (200 MHz, $CDCl_3$): 3.55 (C2-*H*, I = 24.5), 1.08 (C1-*H*, I = 44.3). MS: 116 [M^+; 0.4, *d*14], 98 [$M^+ - CD_3$; 19.8, *d*11], 97 [$M^+ - CD_3$; 0.77, *d*10], 66 [$M^+ - C_3D_7$; 7, *d*11], 58 [M^{2+}], 50 [C_3D_7; 100], 49 [C_3D_6H; 4.78]. An overall isotopic purity of 99.7% was determined

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by GC/MS. The levels of deuteration at C1 (99.7%) and C2 (99.5%) were determined by ^{1}H NMR.

Bergman Cyclization Kinetics. A 100 μL portion of **1b** in heptane (0.14 mM) was pipetted into a 3 mL screw cap vial. The vial was thermolyzed in a constant-temperature oil bath (Haake F3, ±0.2 °C). After thermolysis, the vial was cooled in water, and 20 μL was injected into the HPLC. The substances **1b** and **3b** were monitored at a fixed wavelength of 337 nm. The cyclization was investigated at five different temperatures: 82.1, 92.0, 105.0, 114.5, and 124.4 °C. At each temperature three to six samples were taken. The reaction rates were determined by dividing the initial integrals of **1b** by the obtained integrals after a certain time. The 24 resulting points were fitted to the Arrhenius equation, yielding a linear fit with a correlation of 0.996. An activation energy of 23.1 ± 1.5 kcal/mol and an *A*-factor $\log_{10} A$ of 9.7 ± 0.9 were obtained. This sample size requires a *t*-value of 2, leading to the reported error bond of 2σ.

Trapping Procedure. A typical experiment was performed as follows: Due to the hydrolysis of the azaenediyne at higher temperatures and low pH values, the reaction solutions were prepared in a glovebox. A sample of **1a** was freshly purified by preparative TLC and checked by GC/MS prior to use. **1a** (1.3 mg) was dissolved in 1.3 mL of distilled (Na, benzophenone) diisopropyl ether. Then 100 μL of this solution was pipetted into a 3 mL screw cap vial, to which were added 100 μL of distilled (from CaH_2) 2-fluoropyridine and 10 μL of a 0.1 M HBF_4·Et_2O solution in diisopropyl ether with a few drops of THF. The solution was thermolyzed at 97 °C for 31 min. The solution was allowed to cool, and K_2CO_3 was added. The mixture was filtered through a silica plug with ethyl acetate and concentrated to about 100 μL prior to injection into the GC/MS for analysis.

Ab initio calculations were performed with Gaussian 94[20] on either IBM RS/6000-590 or DEC Alpha AXP 8400 5/300 workstations. Energies along the ring-closure/ring-opening reaction coordinate for the parent azaenediyne were computed at the CASSCF(6×6)/6-31G* level for CASSCF(6×6)/3-21G-optimized geometries obtained in the following fashion. The geometry specification was made such that the CC and CN bond lengths for the two bonds being made or broken could be fixed. Fixing both bond lengths to a large enough value led to the localization of the six orbitals needed for the CASSCF (two NBMO's and two pairs of σ and σ* orbitals along the bonds being made or broken). A localized active space along the reaction coordinate was maintained by stepping the bond lengths back in small increments while allowing the remaining degrees of freedom to relax. Once the minima and transition states were located by this procedure, unconstrained optimizations to refine the geometries were performed. All stationary points were then checked with frequency calculations to confirm that they had either zero or one negative force constants. Dynamic correlation effects were then included with the CASMP2 method. Comparable calculations[21] for *p*-benzyne at a variety of levels have appeared in the last several years.

A two-dimensional potential surface for the hydrogen abstraction reaction between the biradical and methanol was obtained similarly. The procedure is essentially identical with that used previously by Logan and Chen[22] to compare the abstraction of hydrogen from methanol by *p*-benzyne to that by the phenyl radical. The present surface extends that comparison to the 2,5-didehydropyridine biradical. In this case, abstraction by both "ends" of the biradical was considered, the two "ends" no longer being degenerate. A 4 × 4 active space for the CASSCF and CASMP2 calculations was found by setting the distances from the hydrogen being abstracted to both the biradical and the incipient hydroxylmethyl radical large. This led again to localization of the four orbitals in the active space (the two biradical NBMO's and the methanol σ_{CH} and σ^*_{CH}). The two-dimensional potential surface was then generated by stepping the two bond lengths over a fine grid while simultaneously allowing all other degrees of freedom to relax (no symmetry constraints). The points used to generate the final contours were CASMP2/6-31G** single-point energies computed at CASSCF/3-21G geometries with the 4 × 4 active space.

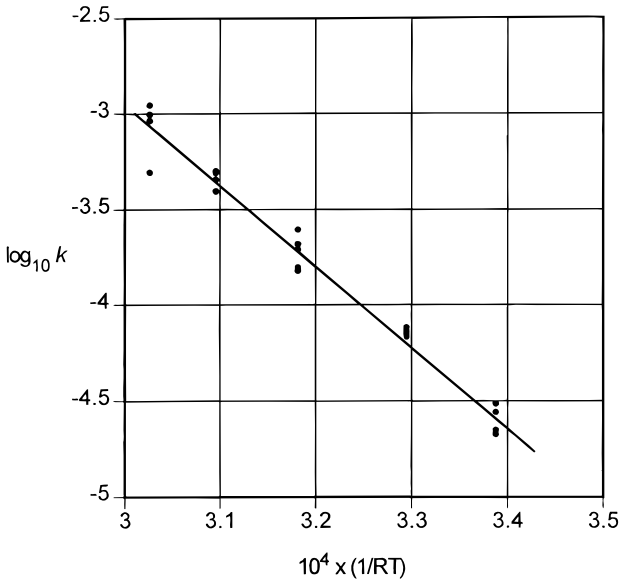

Figure 1. Arrhenius plot of the logarithmic rate of disappearance of **1b** versus inverse temperature. Twenty-four points at five temperatures (82.0, 92.0, 105.0, 115.5, and 124.4 ± 0.2 °C) yield the activation parameters: $\log_{10} A = 9.7 \pm 0.9$ and $E_a = 23.1 \pm 1.5$ kcal/mol. Error bounds are quoted as 2σ.

Results

The overall yield for the synthesis is low, but sufficient quantities for mechanistic studies are nevertheless readily obtained from inexpensive starting materials. Arrhenius activation parameters for the Bergman cyclization of **1b** to the 6-methyl-3,4-bis(4-*tert*-butylphenyl)-2,5-didehydropyridine biradical **2b** were extracted from the linear fit shown in Figure 1, giving $\log A = 9.7 \pm 0.9$ and $E_a = 23.1 \pm 1.5$ kcal/mol with the error bounds reported as 2σ. These figures are entirely consistent with the earlier qualitative report for the cyclization by David and Kerwin.[1]

For the right combination of conditions, listed in Table 1, we observe the product of hydrogen abstraction reactions by the didehydropyridine biradical **2a** in trapping experiments. Under each set of conditions, the GC/MS trace was searched for the characteristic mass spectra of 2-methyl-4,5-diphenylpyridine (**4a**), 1-cyano-1,2-diphenylpent-1-en-3-yne (**3a**), phenylacetonitrile (**5**), and 1-phenylbut-1-yn-3-one (**6**), which are, as noted previously, the products of hydrogen abstraction by an intermediate biradical, the cyclization/ring-opening product, and the two hydrolysis products of **1a**.

In the absence of added acid, no hydrogen abstraction products could be found in the 100 °C thermolysis of **1a**. As expected, **3a** was formed in high yield, again in agreement with the observations of David and Kerwin.[1] With a small amount of acid (<1 molar equiv relative to azaenediyne), there was

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Table 1. Representative Trapping Results in the Thermolysis of **1a** under a Variety of Conditions[a]

substrate	 (buffer)	$HBF_4 \cdot Et_2O$ (100 mM in diisopropyl ether)	conditions	identifiable products by GC/MS
1a, 1.0 mg/mL	100 μL	100 μL (25 equiv)	100 °C/1 h	−
1a, 1.0 mg/mL	100 μL	10 μL (2.5 equiv)	100 °C/1 h	**3a**, **4a**, **5a**, **6a** + other products
1a, 1.0 mg/mL	100 μL	1 μL (0.25 equiv)	100 °C/1 h	**3a**
1a, 1.0 mg/mL	100 μL	−	100 °C/1 h	**3a**
1a, 1.0 mg/mL	100 μL	10 μL (2.5 equiv)	25 °C/1 h	**3a**
1a, 1.0 mg/mL	−	10 μL (2.5 equiv)	100 °C/1 h	traces of several products
3a, 1.0 mg/mL	100 μL	10 μL (2.5 equiv)	100 °C/1 h	**3a**
4a, 1.0 mg/mL	100 μL	10 μL (2.5 equiv)	100 °C/1 h	**4a**
4a, 1.0 mg/mL	100 μL	100 μL (25 equiv)	100 °C/1 h	−

[a] Because of the presumed isomerization of **1a** to **3a** in the GC/MS injector port, any residual **1a** appears as **3a** on the column. The donor solvent, DIPE, is diisopropyl ether (100 μL).

Table 2. Absolute Energies and Singlet−Triplet Splittings from ab Initio Calculations at the CASSCF and CASMP2 Levels for the *p*-Benzyne, 2,5-Didehydropyridine, and 2,5-Didehydropyridinium Biradicals

			
CASSCF(6×6)6-31G* singlet (hartrees)	−229.4235532	−245.4234846	−245.7745936
CASSCF(6×6)6-31G* triplet, vertical	−229.4209942	−245.4134308	−245.7710849
CASSCF(6×6)6-31G* triplet, adiabatic	−229.4217876	−245.4170952	−245.7720467
CASSCF(6×6)6-31G* ΔE_{ST} (kcal/mol)	1.6 (vertical) 1.1 (adiabatic)	6.3 (vertical) 4.0 (adiabatic)	2.2 (vertical) 1.6 (adiabatic)
CASMP2(6×6)6-31G* singlet (hartrees)	−230.0963386	−246.1206479	−246.4582237
CASMP2(6×6)6-31G* triplet, vertical	−230.0929887	−246.1075771	−246.4540073
CASMP2(6×6)6-31G* triplet, adiabatic	−230.0952040	−246.1135411	−246.4568636
CASMP2(6×6)6-31G* ΔE_{ST} (kcal/mol)	2.1 (vertical) 0.7 (adiabatic)	8.2 (vertical) 4.5 (adiabatic)	2.7 (vertical) 0.9 (adiabatic)

little change. Although very large excesses of acid (>10 equiv) destroyed **1a** as well as **4a**, the thermolysis of **1a** with intermediate amounts of acid (e.g. 2.5 equiv) resulted, aside from the production of **3a**, in the reproducible production of the trapping product, 2-methyl-4,5-diphenylpyridine (**4a**) in small, but clearly detectable, quantities. A rough estimate of the combined yield for **3a** and **4a** is the order of a percent, with a **4a**/**3a** ratio of ∼1:20. A large amount of hydrolysis products, plus polymeric material, accounted for most of the material. If the 2-fluoropyridine buffer was omitted, the overall yield of identifiable products dropped to the limits of detection by GC/MS, which presumably happens because the strong acid protonates the azaenediyne itself under those conditions, leading to irreversible loss of material prior to cyclization. A search for **4a**-d_2 in the same reaction conducted with perdeuterated diisopropyl ether was inconclusive. The results reported here comprise the first observation of a hydrogen abstraction reaction by a 2,5-didehydropyridine-type biradical. The conditions under which hydrogen abstraction occurs had been predicted, as discussed below, on the basis of ab initio calculations and a simple model for biradical reactivity.

Control experiments, in which either **3a** or **4a** were prepared in the same solution for which hydrogen abstraction products were observed, and then thermolyzed at 100 °C for 1 h prior to GC/MS analysis, found both products to be stable to the reaction conditions. Importantly, the ring-opening product, 1-cyano-1,2-diphenylpent-1-en-3-yne (**3a**), under the reaction conditions, gave rise to neither **4a** nor the range of other products from **1a**, establishing that the key pyridine product could not have arisen from an acid-induced rearrangement of the ring-opening product. Interestingly, samples of **1a**, stored at 4 or 25 °C as dilute solutions in hydrogen-containing solvents, for periods of 6 months showed traces of **4a**, according to GC/MS analysis.

Ab initio calculations were performed to probe three properties of the title biradical. The vertical and adiabatic singlet−triplet gaps of the 2,5-didehydropyridine biradical, the 2,5-didehydropyridinium biradical, and *p*-benzyne (as a reference) were found at the CASSCF(6×6)/6-31G* and CASMP2(6×6)/6-31G* levels and are listed in Table 2.

An experimentally measured value for *p*-benzyne[23] of 3.8 ± 0.5 kcal shows that the present calculations slightly overestimate the stability of the metastable triplet relative to the singlet ground state. Nevertheless, the trend is clear that incorporation of a nitrogen into the ring increases the singlet−triplet splitting of the biradical by several kcal/mol relative to *p*-benzyne at the same level of theory. Moreover, protonation of that nitrogen reduces the singlet−triplet splitting back to a value comparable to that in the hydrocarbon system.[10]

One-dimensional relaxed potential surfaces for the tandem Bergman cyclization/ring-opening reaction of the all-hydrocarbon system (for reference), the parent azaenediyne, and its N-protonated version are shown in Figures 2−4.

The reference surface for hex-3-ene-1,5-diyne in Figure 2 agrees reasonably well with the original Bergman estimate[24] and recent experimental redeterminations by Roth[25] and Squires.[26] The CASMP2 calculation clearly places the biradical too low in energy, relative to its closed-shell precursor; the CASSCF

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(26) Wenthold, P. G.; Squires, R. R. *J. Am. Chem. Soc.* **1994**, *116*, 6961.

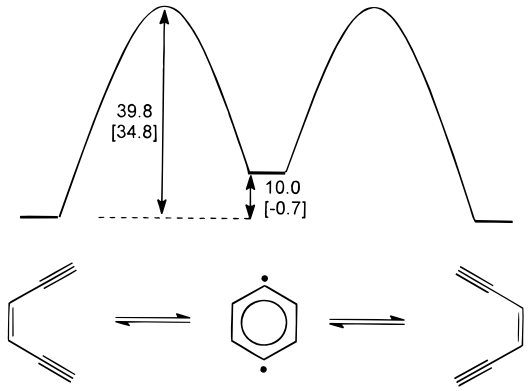

Figure 2. Computed one-dimensional reaction coordinate for the parent Bergman cyclization. Energy differences in kcal/mol at the CASSCF-(6×6)/6-31G* and CASMP2(6×6)/6-31G* (numbers in brackets) levels of theory.

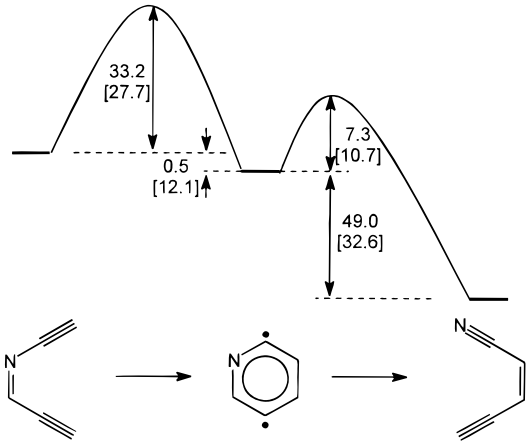

Figure 3. Computed one-dimensional reaction coordinate for the Bergman cyclization of an unsubstituted azaenediyne. Energy differences in kcal/mol at the CASSCF(6×6)/6-31G* and CASMP2(6×6)/6-31G* (numbers in brackets) levels of theory.

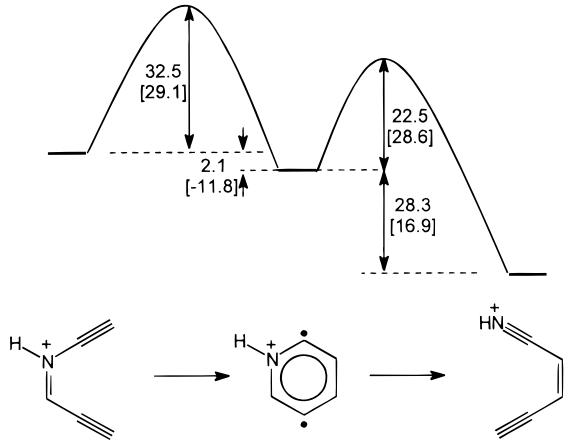

Figure 4. Computed one-dimensional reaction coordinate for the Bergman cyclization of an unsubstituted protonated azaenediyne. Energy differences in kcal/mol at the CASSCF(6×6)/6-31G* and CASMP2-(6×6)/6-31G* (numbers in brackets) levels of theory.

calculation does very well with the biradical, but overestimates the activation energy by about 10 kcal/mol. This systematic error—reasonable ΔE but E_a too high—presumably carries over into the didehydropyridine surfaces as well. The surfaces for the unprotonated and protonated forms of the didehydropyridine biradical are strongly asymmetric, with the product nitrile lying substantially below the initial azaenediyne. Significantly,

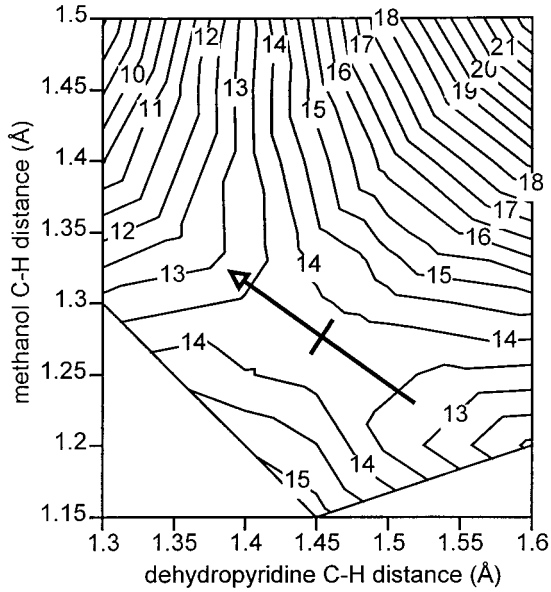

Figure 5. Computed contour plot of the two-dimensional surface for the hydrogen abstraction reaction of 2,5-didehydropyridine and methanol in the region around the transition state. The two coordinates are the respective C−H bond lengths from the abstracted hydrogen to the biradical and the donor. The energies along the contours are given in kcal/mol relative to the asymptotic limit of large separation (10 Å) in the reactant channel (not shown). The transition vector is indicated, with the transition state at the crossbar.

protonation hardly affects the activation barrier for the initial Bergman cyclization, but markedly affects the barrier for ring opening of the intermediate biradical. While the precise numbers will probably change somewhat at a higher level of theory, the trend, as discussed below, is unlikely to be altered in any significant way.

A two-dimensional potential surface for hydrogen abstraction from methanol by an unsubstituted 2,5-didehydropyridine is shown in Figure 5.

The two coordinates are the distance from the hydrogen atom to carbons on either the biradical or the incipient hydroxymethyl radical. The transition state is slightly earlier than for the comparable reaction by *p*-benzyne, occurring when the bond lengths from the transferring hydrogen to the biradical, and the hydroxymethyl moiety, are 1.45 and 1.28 Å, as compared to 1.375 and 1.275 Å for *p*-benzyne.[22] The computed activation energy is 13.8 kcal/mol, with the approach of the methanol to the end of the didehydropyridine away from the nitrogen. The 4 × 4 active space at the transition-state geometry is shown in Figure 6.

Approach to the other end gave a slightly higher activation energy and was therefore not considered further. The activation energy, in this case, is 4.3 kcal/mol higher than that computed for the same reaction of *p*-benzyne at the same level of theory.[22] Computation of potential surfaces for the 2,5-didehydropyridium biradical was less successful. We found the ion−dipole interaction[27] to be so large that it completely dominated the approach of the protonated biradical to the methanol moiety. In solution, the purely electrostatic interaction would be largely screened out by the solvent dielectric, which unfortunately is

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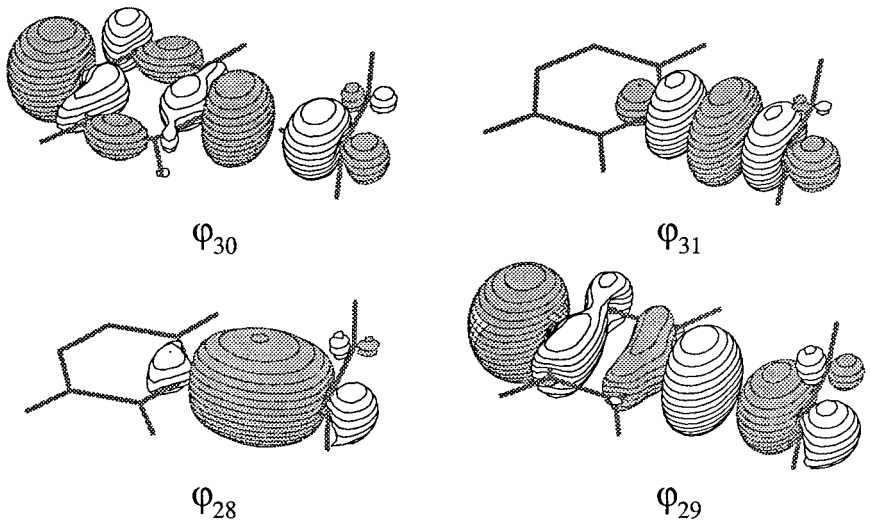

Figure 6. Orbitals in the 4 × 4 active space for the CASSCF and CASMP2 calculations of the hydrogen abstraction reaction by the 2,5-didehydropyridine biradical and methanol.

not included in the present computational model. Without explicit inclusion of solvent, presumably through a SCRF procedure,[28] the surfaces for the ionic reaction are skewed beyond usefulness and are therefore not further considered in this study.

Geometries for all stationary points along the one-dimensional potential surfaces, as well as absolute energies for the single-point ab initio calculations that go into the two-dimensional surfaces, are included in the Supporting Information.

Discussion

The ready availability of the *C,N*-dialkynylimines, albeit in low overall yield, makes mechanistic studies feasible. As had been reported by David and Kerwin,[1] the compounds show a half-life of ∼40−50 min at 110 °C, presumably undergoing ring closure/ring opening in rapid succession to generate the observed nitrile product. Attempts reported in that work to trap an intermediate biradical, by either hydrogen transfer from a suitable donor, or by intramolecular addition to an unsaturated moiety, were unsuccessful, leading to the suggestion that perhaps the biradical was not on the reaction coordinate at all.

The present report constitutes the continuation of the ongoing project[10−12,22] whereby we have explored structural effects on the hydrogen abstraction rates by singlet biradicals. Extending a qualitative thermochemical model for predicting the heats of formation for singlet carbenes and biradicals,[11] we had predicted that singlet biradicals would abstract hydrogens more slowly than comparable monoradicals. The prediction was experimentally confirmed for the 9,10-didehydroanthracene biradical,[12] for which the hydrogen abstraction rate from 2-propanol was found to be reduced by 2−3 orders of magnitude relative to the phenyl or 9-anthryl radicals. A comparable rate reduction was subsequently found for the parent *p*-benzyne biradical,[29] as well, in a trapping study of its hydrogen abstraction reactions with methanol. Briefly stated, the model predicts that a "noninteracting" biradical is best approximated by the triplet, even if the biradical has a singlet ground state. For a singlet ground-state 1,4-arene biradical to engage in radical-like chemistry, e.g. hydrogen abstraction, it must go back through a curve-crossing on the potential surface which adds an extra increment in the activation energy that scales with the singlet−triplet gap. To state the same result in another way, the singlet lies below the triplet because it is stabilized, not because the triplet is destabilized. Accordingly, one must pay back that stabilization energy to reach a transition state where the two electrons in the NBMO's have been effectively uncoupled. The particular mechanism for stabilization (relative to additivity or homodesmotic reaction estimates) for the present 1,4-arene biradicals is through-bond coupling between the two nominal NBMO's, as has been discussed extensively by the groups of Hoffmann[30] and Paddon-Row.[31] The *p*-benzyne biradical was, in fact, one of the original examples presented by Hoffmann to demonstrate the effect. In a further step, one can surmise that an increase in the electron density in the intervening σ-bonds could increase the through-bond coupling and hence the singlet−triplet splitting. Conversely, decreased electron density could decrease the coupling and reduce the singlet−triplet splitting.[10] The in-plane lone pair of the 2,5-didehydropyridine biradical lies antiperiplanar to the σ-bonds coupling the NBMO's and therefore could donate electron density. However, when the nitrogen is protonated, the effect is reversed. This qualitative reasoning is confirmed by the ab initio computed singlet−triplet gaps, where the didehydropyridine, with its lone pair, shows a much larger singlet−triplet gap than *p*-benzyne. One infers, then, that the unprotonated 2,5-didehydropyridine biradical should abstract hydrogens much more slowly than *p*-benzyne and more slowly than the protonated species. The ab initio calculations support this contention as well, with the increase in the computed activation energy for the didehydropyridine biradical commensurate with the increase in singlet−triplet splitting. Protonation, on the other hand, should both reduce the singlet−triplet splitting and restore the rate of hydrogen abstraction to almost the original *p*-benzyne value. The absolute values for the computed E_a for hydrogen abstraction appear to be slightly

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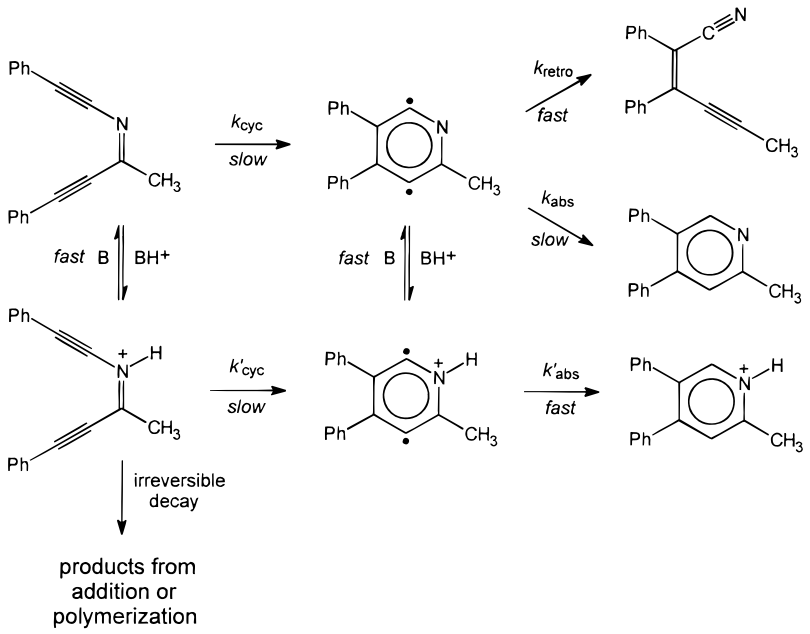
(29) Roth, W. R.; Hopf, H.; Wasser, T.; Zimmermann, H.; Werner, C. *Liebigs Ann.* **1996**, 1691.

(30) Hoffmann, R.; Iwamura, A.; Hehre, W. J. *J. Am. Chem. Soc.* **1968**, *90*, 1499. Hoffmann, R. *Acc. Chem. Res* **1971**, *4*, 1.

(31) A recent review of experimental and theoretical work on through-bond coupling may be found in the following: Paddon-Row, M. N.; Jordan, K. D. In *Modern Models of Bonding and Delocalization*; Liebman, J. F., Greenberg, A., Eds.; VCH Publishers: New York, 1988; Chapter 3.

Scheme 3



high, but the *difference* in activation energies implied by the computed singlet−triplet gaps suggests, nevertheless, a rate difference of perhaps 2 orders of magnitude, triggered by protonation. This should appear as a pH-dependence for the hydrogen abstraction products obtained by thermolysis of the azaenediyne precursors to the biradicals.

A quick examination of the trapping results confirms the prediction that the hydrogen abstraction reaction product, i.e., 2-methyl-4,5-diphenylpyridine (**4a**), is formed when the azaenediyne **1a** is thermolyzed in the presence of a moderate excess of acid. The low combined yield of **3a** and **4a** in the acid-containing runs can be assigned, based on the control experiments, to the irreversible loss of **1a** to hydrolysis or polymerization in competition with Bergman cyclization. The important experimental observable here, however, is not the absolute yield of products but rather the ratio of **4a** to **3a**, which is zero (no **4a**) in the absence of added acid and approximately 1:20 for the runs with 2.5 equiv of HBF_4. The absence of any trace of hydrogen abstraction by the unprotonated azaenediyne in the 100 °C thermolyses is conveniently explained by two separate effects, the relative magnitudes of which remain yet to be investigated. The first of the two is the effect of protonation upon hydrogen abstraction rate by the biradical, as discussed above. Working in the same direction is the second effect, a lifetime effect, which can be understood by comparing Figures 3 and 4. Whereas the parent hydrocarbon undergoes a degenerate Bergman cyclization reversibly, the "tilted" potential surface for the azaenediyne makes the retro-Bergman ring opening to the nitrile irreversible with an activation barrier of only ∼7 kcal/mol. The decreased macroscopic half-life for the azaenediyne relative to the parent hydrocarbon, commented upon by David and Kerwin,[1] arises because the disappearance of the azaenediyne reflects the kinetics of rate-limiting cyclization, while that of the original Bergman enediyne reflects the rate of bimolecular reactions of the biradical. Examination of Figure 4 shows, however, that protonation dramatically increases the ring-opening barrier for the biradical. The altered energetic ordering of the azaenediyne, the biradical, and the product nitrile upon protonation is, in hindsight, obvious. One should note that the basicity of the 2,5-didehydropyridine biradical should not be too different from that of the comparable pyridine, meaning that pK_a for the conjugate acid would be ∼5.5−6.5. On the basis of the known basicity of *N*-alkylimines,[32] and the known base-weakening effects of electronegative N-substituents (heteroatoms, sp^2- or sp-hybridized carbon) in the analogous amidines, the azaenediyne should be a much weaker base than the biradical by an estimated 4−5 pK_a units. The nitrile should be the weakest base of all the structures. Upon protonation, the less basic compounds will be raised in energy relative to the more basic ones, which is exactly what is depicted in Figure 4. The transition states should also move in the direction predicted by the Hammond postulate. The net result is that the barrier for the irreversible deactivation of the biradical by a retro-Bergman reaction is substantially raised by protonation.

To manipulate the extent of hydrogen abstraction by modulating pH, one needs to explicitly consider the proton-transfer equilibria. It would be a gross oversimplification to say that one simply needs to add acid to protonate the biradical and thus suppress ring opening. Proton-transfer reactions between electronegative heteroatoms in a hydrogen-bonding solvent are exceedingly fast, with exothermic proton transfers occurring at or near the diffusion limit.[33] At any given pH, deprotonation of the 2,5-didehydropyridinium biradical (itself slow to ring open) in a fast preequilibrium would lead to a irreversible decay via the fast ring opening of the unprotonated biradical. The observed rate for ring opening for the protonated biradical would be, therefore, that for the unprotonated species, weighted by the equilibrium fraction of unprotonated biradical (Scheme 3).

This leads to an estimate for the conditions under which hydrogen abstraction by 2,5-didehydropyridine can occur, in competition with ring opening. For argument's sake, take Arrhenius parameters for ring-opening of the unprotonated biradical to be $A = 10^{12}$ s^{-1} and $E_a = 7$ kcal/mol. For hydrogen abstraction by the unprotonated biradical, take $A = 10^8$ M^{-1} s^{-1} and $E_a = 6$ kcal/mol. For the protonated biradical, assume the same preexponential, but a lower activation energy, $E_a = 4$

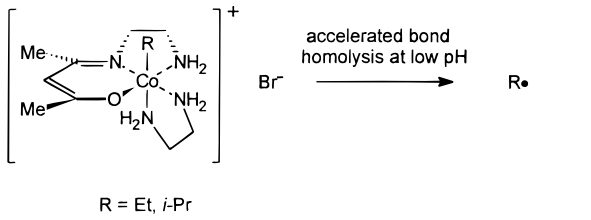
(32) Cordes, E. H.; Jencks, W. P. *J. Am. Chem. Soc.* **1963**, *85*, 2843.

(33) Proton-transfer reactions in aqueous solution between nitrogen or oxygen bases are very fast, even diffusion-controlled, when the pK_a difference is not too large. Protonation should therefore be faster than hydrogen abstraction by the biradical. Maskill, H. *The Physical Basis of Organic Chemistry*; Oxford University Press: Oxford, 1985.

kcal/mol,[34] for hydrogen abstraction. One should note here that that observation of traces of hydrogen abstraction product **4a** in long-term-stored samples of **1a** kept at 4 °C ($t_{1/2}$ = 51 months for Bergman cyclization) or 25 °C ($t_{1/2}$ = 3.6 months) suggests that both E_a and A are lower for hydrogen abstraction by the biradical than for ring opening. In other words, hydrogen abstraction is favored enthalpically but disfavored entropically, which is just what reasonable guesses based on literature precedents suggest.[34] Now, assume the ΔpK_a between the didehydropyridinium and a buffer (in this case 2-fluoropyridine[35]) to be 6 units, with respective concentrations of biradical (total protonated and unprotonated), buffer, and hydrogen donor solvent at 10^{-5}, 5, and 5 M, respectively, and a temperature of 100 °C. As noted above, we ignore the rate for direct ring opening of the protonated biradical in this "straw-man" calculation. As one increases the amount of added (strong) acid from zero to 0.25 equiv (relative to biradical) to 2.5 equiv, and on to 25 equiv, the ratio of protonated to unprotonated biradical goes from 0 to 0.19 to 3.5 to 48. Accordingly, the ratio of ring-opening product to hydrogen abstraction product falls from 523 to 141 to 10 to 0.75, with the qualitative effects of higher abstraction rate by the protonated biradical, and its higher steady-state concentration, both contributing comparably to the overall effect. This rough estimate is completely consistent with the experimental observations. One can rationalize the difficulty in observing deuterium abstraction by considering that the abstraction reaction, with its large primary kinetic isotope effect, must compete against a ring opening that has no isotope effect. Reduction of the ratio of abstraction product to ring-opening product by perhaps 1 order of magnitude would make unambiguous detection problematic.

How does this become a concept for rational drug design? There have been several modified enediynes reported, where a pendent group enhances molecular recognition or provides a trigger for Bergman cyclization[36] to improve selectivity for tumor cells. However, none of the previous approaches sought to tailor the actual hydrogen abstraction rates to improve selectivity. Greater toxicity of a modified enediyne compound in tumor cells versus normal cells might be achievable if intracellular pH could be used as a trigger for increased reactivity of a biradical. A microphysiological handle for the selection of tumor over normal cells derives from the demonstrated ability of hyperglycemia and/or certain existing drugs, e.g., amiloride, nigericin, and hydralazine, to preferentially lower the intracellular pH in tumor cells. Administration of amiloride or nigericin at dosages where no discernible effect is found in normal cells drops the intracellular pH in a wide range of tumor cell types[37−40] from 7.2 to between 6.2 and 6.6. More striking is the effect of hyperglycemia, which has been shown to cause a further acidification[41] to a remarkably low intracellular pH of 5.5. Moderate hypoxia also contributes in the same direction. In 1989, Tannock[42] suggested selective acidification as a means to target tumor cells, but the chemical arsenal lacked the necessary tools needed for the construction of a pH-dependent cytotoxin. Since then, there have been some attempts, notably by Tietze[43] and Shibuya[44] and their co-workers, who synthesized acid-labile prodrugs which hydrolyze rapidly at pH 6.2 to yield toxic products. Even more promising is the very recent report, by Vol'pin and co-workers,[45] of a pH-triggered source of reactive free radicals (under physiological conditions) modeled on the alkylcobalamins (Scheme 4).

Scheme 4



The rate of alkyl radical release from the alkylcobalt(III) complex showed an sigmoidal dependence on pH between 4.0 and 8.0 with a (modest) 3-fold increase in the rate of radical production as the pH dropped from 7.5 to 6.5 (aqueous phosphate buffer, 20 °C). The chemical studies were immediately followed by trials of the cobalt complex in rats with Guerin carcinoma, mice with Ascit-L1210 leukemia, and rats with sarcoma 45. While details differed in each case, the overall trend was surprisingly good. Administration of between 20 and 100 mg/kg of the cobalt complex, accompanied by glucose infusion to induce acidification (reported to be 0.90 pH units), resulted in large tumor growth delays and mean increases in life span from 23 to 61%. Complete regression was observed in up to 40% of the test animals. The effect in the absence of hyperglycemia was much less pronounced. The complex also significantly enhanced the effectiveness of both radiotherapy and chemotherapeutic agents such as *cis*-platin, as evidenced by further increases in life expectancy.

The azaenediynes **1a** and **1b**, and the corresponding 2,5-didehydropyridine biradicals **2a** and **2b**, prepared for this

(34) The assumed log A and E_a for both the Bergman cyclization and the hydrogen abstractions are reasonable compared to the measured values for the hydrocarbon cases in ref 29. These "straw-man" values do not necessarily match the ab initio calculated ones, but are meant to represent a reasonable guess at the real values given the systematic errors in the computed values. In addition, the activation energy for hydrogen abstraction from diisopropyl ether is undoubtedly lower than that from methanol by a few kcal/mol.

(35) The pK_a of 2-fluoropyridium has been variously reported to be between −0.44 and 1.41, depending on the solvent. The more positive value pertains to nonaqueous solution. Brown, H. C.; McDaniel, D. H. *J. Am. Chem. Soc.* **1955**, *77*, 3752. Clark, H. R.; Beth, L. D.; Burton, R. M.; Garrett, D. L.; Miller, A. L.; Muscio, O. J., Jr. *J. Org. Chem*. **1981**, *46*, 4363. Abraham, M. H.; Duce, P. P.; Prior, D. V.; Barratt, D. G.; Morris, J. J.; Taylor, P. J. *J. Chem. Soc., Perkin Trans. 2* **1989**, 1355.

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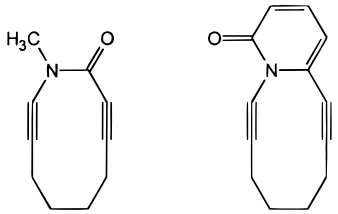
Scheme 5



mechanistic study, are *not* the ideal candidates for drug development, although their chemistry does lay out the conditions that a real candidate would have to fulfill. The azaenediynes **1a** and **1b** themselves are too labile with respect to hydrolysis, and the pK_a values for the didehydropyridinium biradicals need to be adjusted by substitution. Moreover, without inclusion of the azaenediyne moiety into a strained macrocycle[46] to lower the activation energy for Bergman cyclization (without much affecting the retro-Bergman and hydrogen abstraction), the temperatures needed for biradical formation are 50−70 °C higher than physiological. Promising structures under study include those shown in Scheme 5.

Nevertheless, with the conditions defined, the challenge now becomes the selection and synthesis of a biradical precursor which can go into in vivo testing. If the high cytotoxicity of the natural products could be preferentially displayed in tumor cells, then one might achieve good therapeutic results with less severe side effects than has been seen for natural enediyne compounds. This work is underway.

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Conclusion

We report the experimental observation of hydrogen abstraction by a 2,5-didehydropyridine biradical, formed by Bergman cyclization of an azaenediyne. The qualitative reactivity of the intermediate biradical agrees with theoretical predictions, and is furthermore tunable by protonation. The enhanced yield of hydrogen abstraction products at lower pH would be a potential basis for the construction of an antitumor agent based on radical-induced damage to cellular components by the title biradical.

Supporting Information Available: Geometries for all stationary points along the one-dimensional potential surfaces and absolute energies for the single-point ab initio calculations that go into the two-dimensional surfaces (48 pages). See any current masthead page for ordering and Internet access instructions.

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