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TOWARDS THE DEVELOPMENT OF NON-ENEDIYNE APPROACHES FOR MIMICKING ENEDIYNE CHEMISTRY: DESIGN, SYNTHESIS AND ACTIVITY OF A 1,4-BISDIAZONIUM COMPOUND

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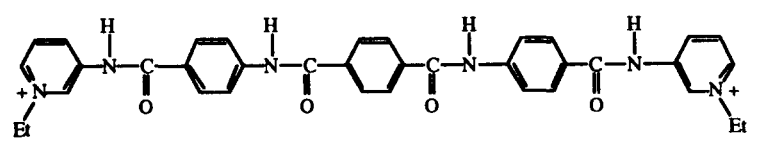
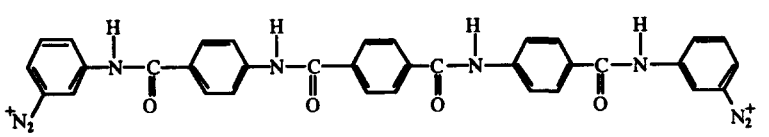
SUMMARY: 1,4-bisdiazonium compounds, which may be precursors of aryl 1,4-diradicals, have the potential to mimic the DNA cleaving activity of the enediyne antibiotics. To this end, the ability to generate and activate 1,4-bisdiazonium compounds in good yield (e.g., **3** to **4**, 72%) and to use them (e.g., **2**) for DNA cleavage are demonstrated.

The reaction of aromatic diazonium compounds with cuprous halides, the Sandmeyer reaction, is believed to proceed via aryl radicals.[1] We, therefore, envisioned that, like the aryl radicals generated by the enediyne natural products,[2] these may also have the capability to induce strand scission in DNA. Indeed, we recently reported[3] that **1**, a bisdiazonium compound structurally similar to the d(A·T)-specific minor groove binding drug **NSC-101327**[4] and possessing the two diazonium groups on different aryl rings, efficiently cleaved supercoiled DNA in the presence of cuprous chloride. Reported herein are our preliminary findings on the suitability of 1,4-bisdiazonium compounds, the potential precursors of aryl 1,4-diradicals, to mimic the DNA cleaving activity of the enediyne anticancer antibiotics.[2]

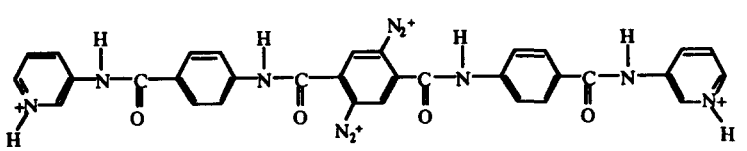
NSC-101327



1

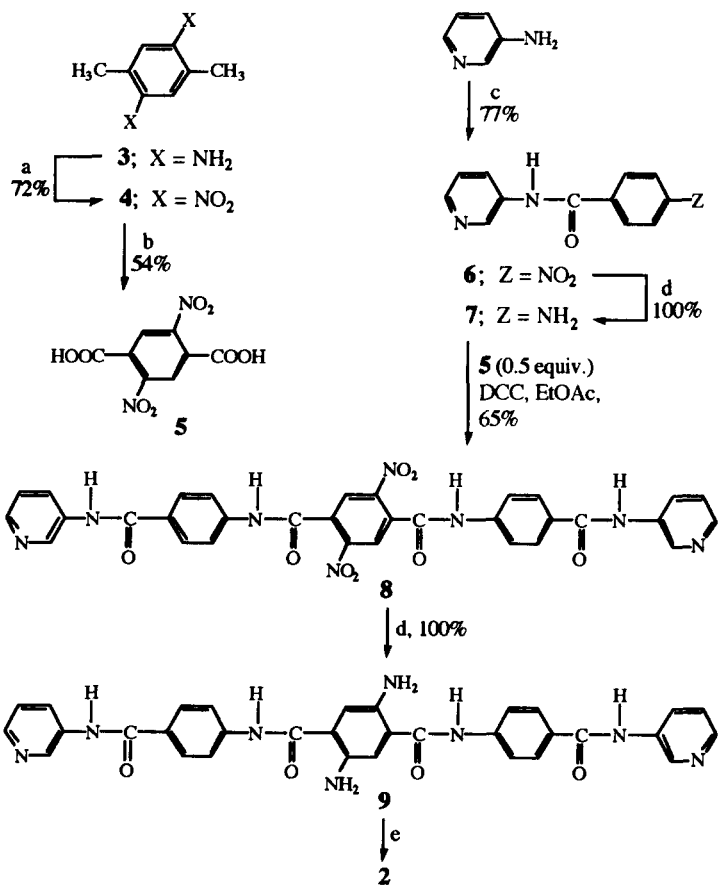


2



The 1,4-bisdiazonium compound **2**, which is also structurally related to **NSC-101327**,[4] but possessing the two diazonium groups on the same aryl ring, was envisioned as our target molecule. Its synthesis, as summarized in

Scheme 1, calls for the coupling of **5** and **7**. Although a lengthy literature procedure[5] was available for the synthesis of **5**, we sought to obtain it in a single step from commercially available 2,5-dimethyl-1,4-phenylenediamine **3**. However, all our attempts to oxidize the amino groups, including procedures for simultaneous oxidation[6] of the amino and methyl groups, led to unidentifiable product mixtures. Disappointed with these results, we then turned to the Sandmeyer process of replacing diazonium groups by nitrite anions.[1b,7] Although literature hinted that concentrated mineral acids may be required for the bisdiazotization of 1,4-phenylenediamines[1b,c] and that the corresponding 1,4-bisdiazonium may, in some cases, be isolated as tetrafluoroborate salts,[8] their conversion to the corresponding dinitro compounds has never been reported. With 2,5-dimethyl-1,4-phenylenediamine **3**, however, we have been unable to isolate the bisdiazonium tetrafluoroborate salt, nor to obtain the desired dinitro compound **4** (Scheme 1) using *in situ* procedures in concentrated mineral acids. The latter may not be that surprising because concentrated mineral acids have been known to promote unfavorable side reactions.[1c] Gratifyingly, after some experimental work, we found that an *in situ* treatment of **3** with isoamyl nitrite (2.2 equiv., acetic acid, 25°C, 30 min.)[9] followed by reaction at 60°C with an aqueous solution containing excess $NaNO_2$, $NaHCO_3$ and $CuSO_4$ yields **4** (72%).[10] Since the formation of **4** may be viewed as indirect evidence for *in situ* generation and activation of the corresponding 1,4-bisdiazonium compound, it appears that we are not restricted to concentrated mineral acids for the bisdiazotization of 1,4-phenylenediamines and that the above diazotization condition (2.2 equiv. isoamyl nitrite, acetic acid, 25°C, 30 min.),[9] which we have successfully utilized with **1**,[3] may also be suitable for DNA cleavage studies involving 1,4-bisdiazonium compounds (e.g., **2**).



Scheme 1: Synthesis of **2**. Reagents and conditions. a) 2.2 equiv. isoamyl nitrite, acetic acid, 25°C, 30 min., followed by excess $NaNO_2$, $NaHCO_3$, $CuSO_4$ in H_2O, 60°C, 20 min., 72%; b) CrO_3, H_2SO_4, 25°C, 1h, 54%; c) 1 equiv. 4-nitrobenzoyl chloride, pyridine, 80°C, 12 h, 77%; d) 3 atm. H_2, 10% Pd-C, DMF-ethanol, 25°C, 5 h, 100%; e) bisdiazotization (*in situ*) using 2.2 equiv. isoamyl nitrite, acetic acid, 25°C, 30 min.

The remainder of the synthesis, as outlined in Scheme 1,[11] proceeded in a straight forward manner. The chromium trioxide[5] oxidation of **4** gave **5** (54%). The synthesis of **7**, the second component needed for coupling, was accomplished in 2 steps; coupling of 3-aminopyridine with 4-nitrobenzoyl chloride (pyridine, reflux, 12 h, 77%) followed by catalytic hydrogenation (3 atm. H_2, 10% Pd-C, ethanol, 25°C, 5 h, 100%). Heating of **7** with 0.5 equiv. of **5** in ethyl acetate in the presence of dicyclohexylcarbodiimide (DCC) afforded **8** (65%). Reduction of the nitro groups (3 atm. H_2, 10% Pd-C, ethanol, 25°C, 5 h, 100%) followed by diazotization (2.2 equiv. isoamyl nitrite, acetic acid, 25°C, 30 min.)[9] completes the synthesis of **2**.

The ability of **2**, generated *in situ* from **9** (Scheme 1), to cleave DNA was investigated using the supercoiled pBR322 (Form I) DNA. Similar to our previous studies,[3] we examined several reducing agents (Fe^{2+}, I^-, and Cu^+) and optimized conditions for efficient DNA cleavage. Cuprous chloride works the best and, as revealed by control experiments (Figure 1), DNA cleavage occurs only when **2** and cuprous chloride are present.

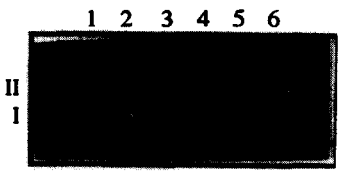


Figure 1: Cleavage of pBR322 supercoiled DNA by **2**. All cleavage reactions were performed in the dark at 25°C for 1 h. Reaction vials contained 714 ng of pBR322 supercoiled DNA (64 nM) in a 10 mM Tris-HCl buffer, pH = 7.6, containing 5 mM NaCl and 0.1 mM EDTA. Electrophoresis was conducted at 50V (2 h) on a 0.7% agarose gel, and stained with ethidium bromide after electrophoresis. Lane 1, control DNA; Lane 2, DNA + CuCl (200 μM); Lane 3, DNA + **2** (100 μM); Lane 4, DNA + **2** (10 μM) + CuCl (20 μM); Lane 5, DNA + **2** (50 μM) + CuCl (100 μM); Lane 6, DNA + **2** (100 μM) + CuCl (200 μM). Form I - supercoiled DNA and Form II - relaxed DNA (single-strand cleavage).

In summary, the results presented here clearly show that 1,4-bisdiazonium compounds may be generated and activated in good yield and that, in the presence of reducing agents, they may be used to induce strand scission in DNA. Currently, our efforts are focused studying the differences and similarities between the enediyne[2,12] and 1,4-bisdiazonium approaches to generating aryl 1,4-diradicals, and these will be reported in due course.

Acknowledgment. We thank the National Science Foundation (CHE-9406469) for support of this work.

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10. A typical procedure is as follows: Isoamyl nitrite (20 mL, 16.2 mmol, 2.2 equiv.) was added dropwise to a room temperature solution of 2,5-dimethyl-1,4-phenylenediamine **3** (1.0 g, 7.4 mmol) in 10 mL acetic acid and 40 mL of water. After 30 min., the reaction mixture was poured, as quickly as possible, to a 60°C solution containing 20g $NaNO_2$, 8g $NaHCO_3$, 4g $CuSO_4$ in 80 mL of water. *Caution:* Frothing occurs on addition of the bis-diazonium salt solution; the reaction was stirred vigorously until frothing ceases (*ca.* 20 min.). After this period, the reaction mixture was allowed to cool to room temperature and, then, kept in a refrigerator overnight. The fluffy brown precipitate was filtered, washed successively with water and 1N HCl, and chromatographed on a silica gel column (hexane : ethyl acetate = 92 : 8, R_f = 0.4) to obtain **4** (1.04 g, 72%).
^1H NMR (d_6-DMSO): δ 8.1 (s, 2H), 2.6 (s, 6H); ^{13}C (d_6-DMSO): δ 150.2, 132.0, 128.0, 19.2.
Mass Spec.(*m/z*): 196 (molecular ion), 179 (78%), 133 (28%), 106 (24%), 91 (26%); mp 138°C (Lit.[5] 142°C).

11. All new compounds gave consistent spectral/analytical data. **5** ^1H NMR (d_6-acetone): δ 11.90 (2H, br, CO_2H), 8.50 (2H, s); ^{13}C (d_6-acetone): δ 163.5, 150.8, 131.0, 127.1. **6** ^1H NMR (d_6-DMSO): δ 10.77 (1H, s, N-H), 8.94 (1H, d, J = 2.04 Hz), 8.40 (2H, d, J = 8.82 Hz), 8.35 (2H, m), 8.20 (2H, d, J = 8.82 Hz), 7.43 (1H, dd, J = 4.77, 8.25 Hz); ^{13}C (d_6-DMSO): δ 164.2, 149.2, 144.9, 142.0, 139.8, 135.3, 129.2, 127.4, 123.5. **7** ^1H NMR (d_6-DMSO): δ 9.96 (1H, s, NH), 8.90 (1H, d, J = 2.49 Hz), 8.25 (1H, dd, J = 1.47, 4.77 Hz), 8.18 (1H, td, J = 1.47, 8.34 Hz), 7.74 (2H, d, J = 8.67 Hz), 7.35 (1H, dd, J = 4.77, 8.34 Hz), 6.62 (2H, d, J = 8.70 Hz), 5.83 (2H, br, NH_2); ^{13}C (d_6-DMSO): δ 165.6, 152.4, 143.7, 141.7, 136.4, 129.5, 126.9, 123.3, 120.3, 112.5. **8** ^1H NMR (d_6-DMSO): δ 11.59 (2H, s, NH), 11.37 (2H, s, NH), 9.45 (2H, s), 8.86 (2H, d, J = 7.53 Hz), 8.72 (2H, s), 8.64 (2H, d, J = 4.98 Hz), 8.19 (4H, d, J = 7.2 Hz), 8.02 (2H, dd, J = 4.98, 7.53 Hz), 7.89 (4H, d, J = 7.2 Hz); ^{13}C (d_6-DMSO): δ 165.4, 161.6, 148.3, 141.9, 138.0, 134.7, 133.7, 129.2, 128.7, 126.53, 126.49, 125.9, 119.24, 119.11. **9** ^1H NMR (d_6-DMSO): δ 10.84 (2H, s, NH), 10.67 (2H, s, NH), 9.10 (2H, s), 8.35 (4H, d, J = 8.1 Hz), 8.10 (4H, d, J = 8.1 Hz), 7.95 (4H, m), 7.49 (2H, m), 7.39 (2H, s), 5.76 (4H, s, NH_2); ^{13}C (d_6-DMSO): δ 166.4, 162.2, 143.2, 142.4, 140.7, 136.5, 128.90, 128.71, 128.63, 128.54, 128.50, 128.39, 124.0, 119.4.

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