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A SINGLE PRECURSOR APPROACH TO NEW DNA CLEAVING AND CROSSLINKING AGENTS

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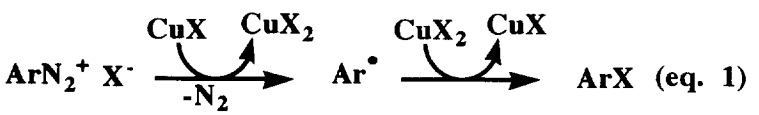
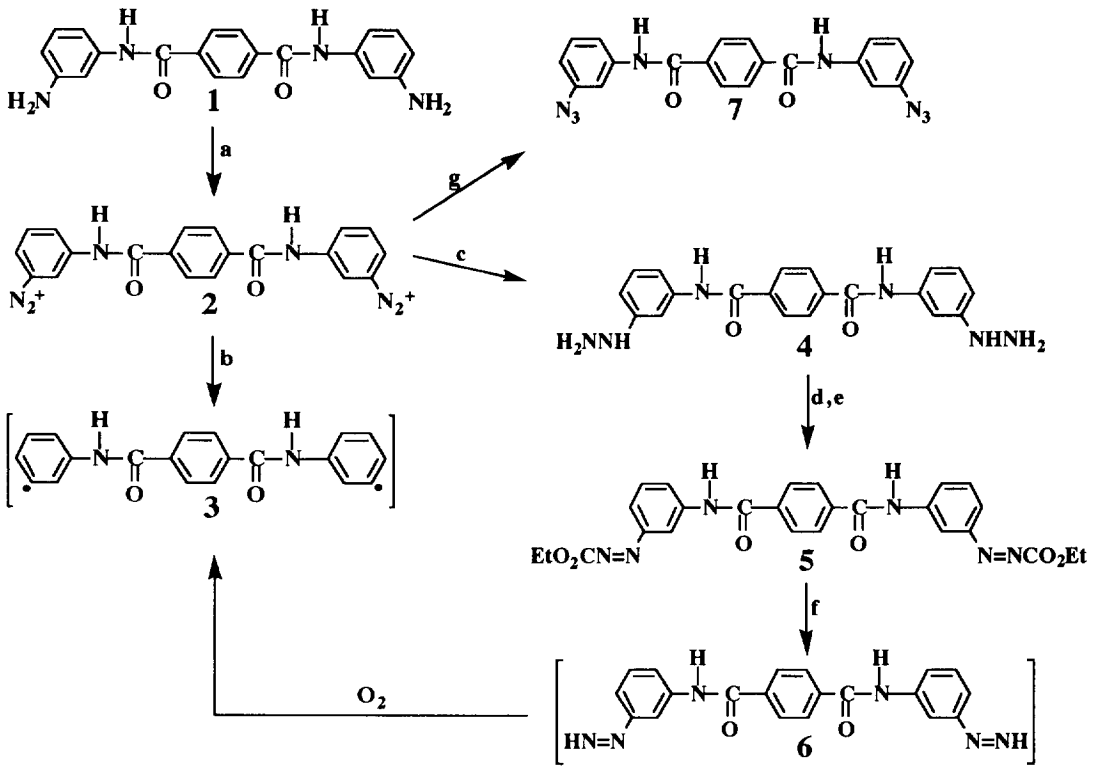
ABSTRACT: It is demonstrated that aryl diamine **1**, designed after a d(A·T) specific minor groove binding agent, can serve as a precursor for the synthesis of two DNA cleaving (bisdiazonium **2** and bisazoester **5**) and one crosslinking (bisazide **7**) agents and, to this end, the present strategy is introduced as a convergent approach for the development of DNA targeted anticancer drugs.

Molecules that cleave and/or crosslink DNA are potential anticancer drugs.[1] Here, we describe the identification of three new classes of compounds: *bisdiazonium* and *bisazoester* for DNA cleavage and *bisazide* for interstrand DNA crosslinking. In addition, we also show that all the three compounds can be synthesized from a single precursor, namely the corresponding aryl diamine, thus introducing a convergent approach for the development of DNA targeted anticancer drugs. The aryl diamine **1** (Scheme 1),[2] designed after the d(A·T) specific minor groove binding drug **NSC-101327**,[3,4] herein serves as the model precursor to illustrate the principles of our work.



The development of bisdiazonium **2** (Scheme 1) as a DNA cleaving agent was inspired by the Sandmeyer reaction (eq. 1). These reactions, which are high yielding and catalytic with respect to cuprous salts, are believed to proceed via aryl radicals;[5] in the case of **2**, the aryl diradical **3** (Scheme 1). We envisioned that, like the aryl radicals generated by the enediyne anticancer antibiotics,[6] diradical **3** may also be used to induce strand scission in DNA provided (i) its trapping by cupric salts (CuX_2), the second step in eq. 1, is slower than hydrogen atom abstraction from the sugar-phosphate backbone of DNA, and (ii) the conditions for the synthesis and activation of bisdiazonium **2** are compatible with the DNA duplex structure. Since our design[4] insures DNA binding capability for bisdiazonium **2**, similar to the known **NSC-101327**,[3] the diradical **3** (generated from **2**) may also be expected to remain on the DNA duplex. In such a case, hydrogen atom abstraction from the sugar-phosphate backbone, which may be viewed as "intramolecular", can be faster than the intermolecular trapping of diradical **3** by cupric salts. Thus, the first requirement may potentially be satisfied.



Scheme 1. Reagents and conditions. a) *In situ* bisdiazotization using 2 equiv. isoamyl nitrite, acetic acid, 25°C, 30 min.; b) *In situ* treatment with cuprous chloride, 1 h; c) $SnCl_2$, HCl, 25°C, 6 h, 40%; d) $ClCO_2CH_2CH_3$, pyridine, 25°C, 1 h, 85%; e) N-bromosuccinimide, pyridine, 25°C, 20 min., 52%; f) Tris-HCl buffer (pH = 7.2) and 50% THF, 12 h; g) NaN_3, acetic acid, 25°C, 1 h, 65%.

The synthesis of bisdiazonium **2** from aryl diamine **1**, however, requires strongly acidic media that are not compatible with DNA duplex. A potential solution might be the isolation of the corresponding bisdiazonium tetrafluoroborate salt,[7] and its subsequent utilization in appropriate aqueous buffers. But higher water solubility, rendered by the two positively charged diazonium units, did not permit precipitation of the tetrafluoroborate salt. Gratifyingly, however, an *in situ* diazotization procedure (2 equiv. isoamyl nitrite, acetic acid, 25°C, 30 min.)[8] proved to be suitable, as the duplex structures of DNA are compatible with the weaker acetic acid. Thus, the second requirement is also satisfied. Similar to our previous studies,[4] we examined a variety of reducing agents to activate bisdiazonium **2** for DNA cleavage. Cuprous chloride activation worked the best, the Form I band of ΦX174 DNA (250 ng, 35 nM) disappeared completely in the presence of 0.1 μM **2** and 0.2 μM cuprous chloride (Figure 1, lane 4), providing an activity *ca.* 800-fold better than that of the simple benzene diazonium tetrafluoroborate salt (data not shown).

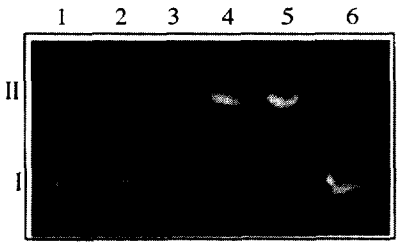


Figure 1. Cleavage of ΦX174 supercoiled DNA by bisdiazonium **2**. All reactions, which were run for 1 h at 25°C, contained 250 ng of ΦX174 supercoiled DNA (35 nM) in 40 mM Tris-acetate buffer, pH = 8.2. Electrophoresis was conducted at 50V (3.0 h) on a 0.7% agarose gel stained with ethidium bromide. Lane 1, DNA + CuCl (1 μM); Lane 2, DNA + **2** (0.5 μM); Lane 3, DNA + **2** (0.1 μM) + $Fe(NH_4)_2SO_4$ (0.2 μM); Lane 4, DNA + **2** (0.1 μM) + CuCl (0.2 μM); Lane 5, DNA + **2** (0.5 μM) + CuCl (1 μM); Lane 6, control DNA.

While the development of bisdiazonium **2** was gratifying, we reasoned that its instability and the need to use non-physiological conditions for its generation and activation would make it a less attractive agent for *in vivo* applications. Therefore, inspired by the mechanistic work of Kosower and coworkers,[9] we initiated research on bisazoester **5** (Scheme 1). In keeping with our theme, it also comes from bisdiazonium **2** (and hence aryl diamine **1**), via a three step sequence: reduction to give **4** ($SnCl_2$, HCl, 25°C, 6 h, 40%),[10] followed by acylation ($ClCO_2CH_2CH_3$, pyridine, 25°C, 1 h, 85%)[9b] and oxidation (N-bromosuccinimide, pyridine, 25°C, 20 min., 52%).[11] Based on the mechanistic proposals of Kosower and coworkers (Scheme 1),[9] we anticipated that **5** would undergo hydrolysis (in neutral or slightly basic aqueous solutions) and decarboxylation to produce unstable bisdiazene **6** which, in the presence of oxygen, would decompose to generate the DNA cleaving diradical **3**. Indeed, as indicated by the gel (Figure 2), bisazoester **5** induced DNA cleavage in an aqueous buffer (pH = 7.2). Compared to bisdiazonium **2**, however, it is somewhat less effective as indicated by lane 5 (Figure 1) *vs.* lane 4 (Figure 2). In addition, it is also less soluble in aqueous buffers and, as such, necessitated the addition of tetrahydrofuran (THF) to the reaction buffer. We believe that both of these differences may be attributed to the positively charged diazonium groups present in **2**, which are capable of aiding water solubility and increasing affinity to DNA by their interaction with the negatively charged phosphodiester backbone. Nevertheless, the present results provide the first example of the ability of an azoester to mediate strand scission in DNA.

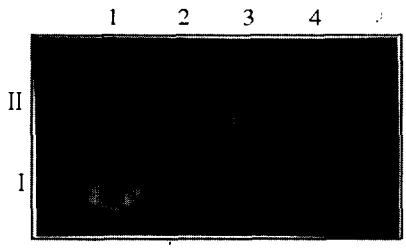


Figure 2. Cleavage of ΦX174 supercoiled DNA by bisazoester **5**. Reaction samples contained varying amounts

of **5** (see below), 250 ng (35 nM) of ΦX174 in 10 mM Tris-HCl buffer (pH = 7.2) and 50% THF. All cleavage reactions were run for 12 h at 25°C. Electrophoresis was conducted at 50V (3.0 h) on a 0.7% agarose gel stained with ethidium bromide. Lane 1, control DNA; Lane 2, DNA + **5** (50 μM); Lane 3, DNA + **5** (5 μM); Lane 4, DNA + **5** (0.5 μM).

Additionally, we envisioned that bisazide **7** (Scheme 1) is another compound that may be synthesized from bisdiazonium **2** (and hence from aryl diamine **1**).[12] Like bisazoester **5**, it also has the advantage of chemical stability. When photolyzed,[13] however, it can potentially lead to permanent, enzymatically irreparable DNA crosslinking; thus, bisazide **7** is a potential anticancer drug. To this end, bisdiazonium **2** was converted to bisazide **7** (NaN_3, acetic acid, 25°C, 1 h, 65%)[12] and photolyzed in the presence of the following oligonucleotide duplex, d(CGCGAATGCGC)·d(GCGCATTCGCG).[14] Indeed, as shown by the denaturing polyacrylamide gel (Figure 3), interstrand crosslinking occurs and it requires activation of **7** by room (visible) or 254 nm UV light. Although aryl azides have long been used for photoaffinity labeling[15] and, more recently, for studying nucleic acid-protein interactions[16] and tertiary structures in RNA,[17] the potential utility of aryl bisazides for crosslinking DNA and/or RNA duplexes has never been exploited. Our results, presented here with bisazide **7**, are indeed the first in this regard and this, we believe, should encourage further research on the design of bisazide-based agents for interstrand DNA crosslinking.[13,18]

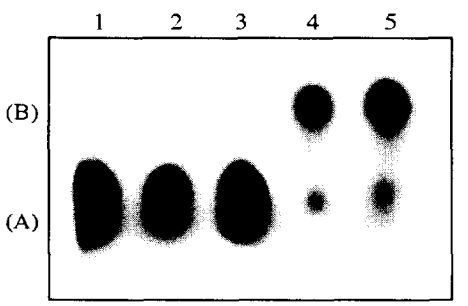


Figure 3. Cross-linking of an oligonucleotide duplex by bisazide **7**. Oligonucleotide duplex was formed by annealing the [5′-^{32}P] end-labeled d(CGCGAATGCGC) (1 μg, 25 nM) with its complementary, unlabeled strand d(GCGCATTCGCG) in a pH = 7.8 buffer containing 20 mM Tris-acetate, 1 mM EDTA, and 50 mM NaCl. Polyacrylamide (20%) denaturing gel was run at 450V. Lane 1, oligonucleotide; Lane 2, oligonucleotide exposed to UV light (254 nm, 1-2 min.); Lane 3, oligonucleotide + bisazide **7** (400 μM) in dark; Lane 4, oligonucleotide + bisazide **7** (400 μM) exposed to room (visible) light for 12 h; Lane 5, oligonucleotide + bisazide **7** (400 μM) exposed to UV light (254 nm, 1-2 min.). Band (A) is labeled oligonucleotide, and the slower migrating band (B) corresponds to the product of interstrand crosslinking.

In summary, we have described three classes of compounds: (i) *bisdiazonium* **2**, activated by cuprous salts, for DNA cleavage, (ii) *bisazoester* **5**, functioning under physiological conditions, for DNA cleavage and (iii) *bisazide* **7**, activated by light, for interstrand DNA crosslinking. Perhaps an important point of interest is that,

even though the activation of the two functional groups present in each of these molecules may be sequential and need not occur simultaneously, bisazide **7** led to efficient interstrand crosslinking whereas bisdiazonium **2** and bisazoester **5** did not produce any double-stranded cleavage (indicated by the absence of Form III band in Figures 1 and 2). This observation is currently under investigation with the use of analogs possessing these functional groups at different positions. Regardless, the fact that all three can be accessed from a single precursor, namely the corresponding aryl diamine **1**, makes our approach to DNA targeted drugs rather convergent. In addition, our findings on **5** and **7** are the first examples of the ability of azoester functionality and aryl bisazide molecules to cleave and crosslink, respectively, duplex DNAs. Lastly, the fact that azoester compounds are capable of functioning under physiological conditions is also encouraging with regard their potential therapeutic utility.

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2. Reaction of terephthaloyl chloride (0.5 equiv.) and 3-nitroaniline in refluxing pyridine for 24 h, followed by hydrogenation (3 atm. H_2, 10% Pd-C, ethanol, 12 h) afforded the aryl diamine **1**. ^{1}H NMR (d_6-DMSO, 300 MHz): δ 10.21 (2H, br. s), 8.09 (4H, s), 7.13 (2H, pseudo t, J = 1.5 Hz), 7.0 (2H, dd, J = 7.8, 8.1 Hz), 6.90 (2H, d, J = 8.1 Hz), 6.33 (2H, d, J = 7.8 Hz), 5.25 (4H, br. s). IR (nujol): cm^{-1} 3430 and 3340 (-NH_2), 3238 and 3190 (amide N-H), 3000-2828 (C-H stretch), 1630 (C=O, amide I band), 1600 (N-H bend, amide II band).
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