

DEVELOPMENT OF NEW DNA-BINDING AND CLEAVING MOLECULES DESIGN, SYNTHESIS AND ACTIVITY OF A BISDIAZONIUM SALT

Dev P. Arya, Philip M. Warner,* and David J. Jebaratnam*
Department of Chemistry, Northeastern University, Boston, MA 02115

SUMMARY: The bisdiazonium compound **1** has been shown to cleave DNA at concentrations as low as 0.1 μ M. Evidence is presented to indicate that **1** is binding to the minor groove of DNA, and that binding may be responsible for its effectiveness as a DNA cleaving reagent.

Since the discovery of the enediyne anticancer antibiotics, research in this area has focused primarily on the design and synthesis of simple enediyne structures that can generate benzenoid diradicals via the Bergman cyclization.¹ The use of non-enediyne precursors for the generation of benzenoid diradicals, however, are rather rare.² In this communication we report the design, synthesis, and activity of a bisdiazonium salt. We show that such molecules are available through simple synthetic transformations, and that they may be designed to bind and cleave duplex DNA under a variety of conditions.

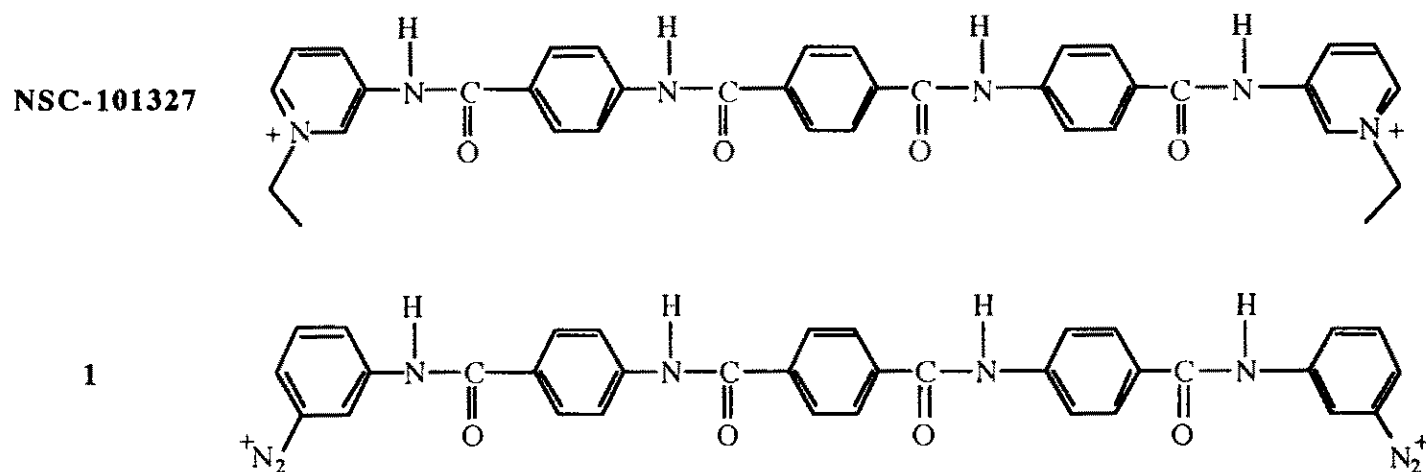
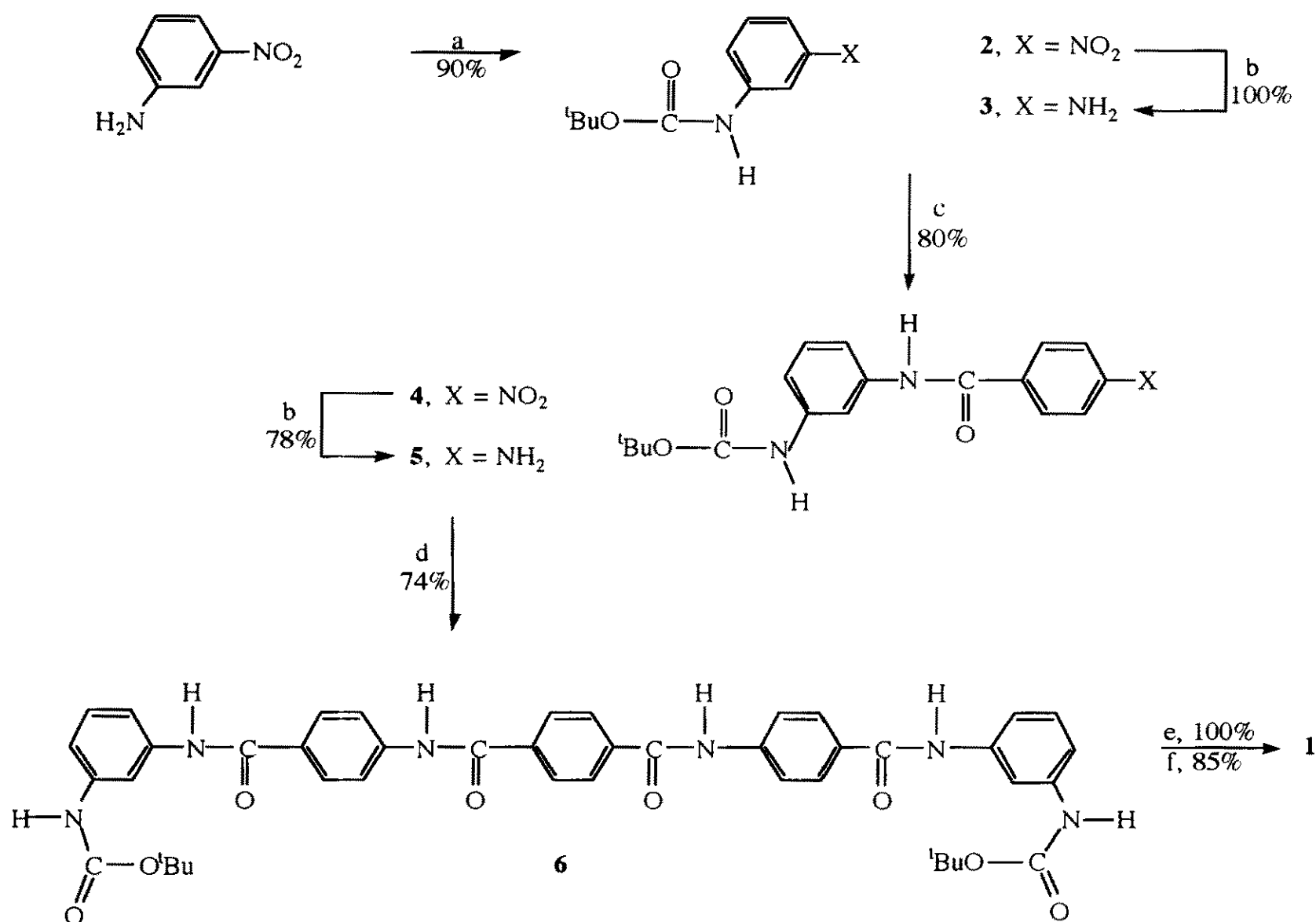


Figure 1: The d(A·T)-specific, minor groove binding drug NSC-101327 and the designed bisdiazonium compound **1**

The d(A·T)-specific, minor groove binding drug NSC-101327 (Figure 1) served as a model for the design of our bisdiazonium compound **1**.³ Its central part, which has been suggested to be involved in specific hydrogen bonding to dA·dT pairs, was retained in **1**. However the two pyridinium units, which are believed to be responsible for water

solubility and interaction with the negatively charged phosphodiester backbone, were replaced by two diazonium units capable of functioning in the same way. Thus, it is reasonable to assume that **1** will be similar to **NSC-101327** in its interaction with duplex DNA.

The synthesis of **1** is described in Scheme 1.⁴ The amine function of the commercially available 3-nitroaniline was protected with the *tert*-butoxycarbonyl (BOC) group⁵ and hydrogenated (3 atm H₂, 10% Pd-C) to obtain **3** (90%). This, on treatment with 4-nitrobenzoyl chloride (80%), followed by hydrogenation as before (78%), yielded **5**. Heating **5** with 0.5 equiv of terephthaloyl chloride in pyridine afforded **6** (74%). Deprotection (CF₃COOH, 25°C), followed by diazotization (isoamyl nitrite⁶ in acetic acid), completed the synthesis of **1**.



Scheme 1. Synthesis of **1**. Reagents and conditions: a) di-*tert*-butyl dicarbonate, pyridine, 80°C, 12 h, 90%; b) 3 atm H₂, 10% Pd-C, ethanol, 25°C, 6 h; c) 1 equiv 4-nitrobenzoyl chloride, pyridine, 80°C, 12 h, 80%; d) 0.5 equiv terephthaloyl chloride, pyridine, 80°C, 12 h, 74%; e) trifluoroacetic acid, 25°C, 2 h, 100%; f) 2 equiv isoamyl nitrite, acetic acid, 25°C, 15 min, 85%.

We first investigated the capability of **1** to undergo a double Sandmeyer reaction. Reaction with isoamyl nitrite in acetic acid, followed by the addition of potassium iodide produced the corresponding diiodide in 85% yield. Although gratifying, given the mechanistic uncertainty of the process,⁷ a successful Sandmeyer reaction does not demonstrate the intermediacy of a diradical. It only shows that **1** might be capable of generating an intermediate or intermediates that cleave DNA.

The ability of **1** to cleave DNA was investigated using the supercoiled Φ X174 (Form I) of DNA (Figure 2). We performed several experiments to determine optimum condition(s) for efficient DNA cleavage, and the results were probed by electrophoresis. Of the reducing agents examined (Fe^{2+} , I^- , and Cu^+), cuprous salts produced the best results (data not shown). In addition, using such controlled experiments as DNA alone (lane 1), DNA + CuCl (lane 2), DNA + **1** (lane 3), DNA + **1** + CuCl (lanes 4 and 5), and DNA + **1** + CuCl + distamycin A (lane 7), we have shown that DNA cleavage occurs only when both components (i.e., **1** and cuprous chloride) are present. In addition, we have also found that photolytic activation of **1** leads to DNA cleavage (lane 6).⁸ Under both conditions, the Form I band of a solution containing 0.25 μg Φ X174 DNA (14 nM) disappeared completely in the presence of 0.1 μM **1** and 0.3 μM cuprous chloride or light. This activity is comparable to those of the most efficient enediyne mimics reported to date.¹

We have also found that the addition of distamycin A, a d(A·T) - specific minor groove binder that is known to displace **NSC-101327** from duplex DNA,³ suppressed the DNA cleavage (lane 7). This is a clear indication that, under our experimental conditions, **1** is most likely binding to the minor groove of DNA, and that binding may be contributing to the effectiveness of DNA cleavage. Since cuprous salts and light are believed to generate benzenoid diradicals and dications, respectively, from bisdiazonium compounds,⁷ we also expect their DNA cleavage mechanisms to be different. Such mechanistic questions as well as the analysis of the interaction of DNA with **1** are currently underway in our laboratories, and these will be reported in due course.



Figure 2. Cleavage of Φ X174 supercoiled DNA by **1**. Solutions contained 0.25 ng of Φ X174 supercoiled DNA (14 nM) in 40 mM Tris-acetate, pH = 8.2 containing 1 mM of EDTA. Unless indicated, all DNA cleavage reactions were run for 1 h at 25°C, and electrophoresis was conducted at 50V (3.0 h) on a 0.7% agarose gel in the presence of ethidium bromide. (From left to right) lane 1, control Φ X174 DNA, lane 2, DNA + CuCl (3 μM), lane 3, DNA + **1** (1 μM), lane 4, DNA + CuCl (3 μM) + **1** (1 μM); lane 5, DNA + CuCl (0.3 μM) + **1** (0.1 μM), lane 6, DNA + $h\nu$ + **1** (0.1 μM), 1 min, lane 7, DNA + CuCl (3 μM) + **1** (1 μM) + distamycin A (100 μM). Form I - supercoiled DNA and Form II - relaxed DNA (single-strand cleavage).

REFERENCES

- 1 For reviews, see: (a) Nicolaou, K. C., Dai, W.-M. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1387, (b) Goldberg, I. H. *Acc. Chem. Res.* **1991**, *24*, 191; (c) Lee, M. D., Ellestad, G. A., Borders, D. B. *Acc. Chem. Res.* **1991**, *24*, 235, (d) Nicolaou, K. C., Smith, A. L. *Acc. Chem. Res.* **1992**, *25*, 497 (e) Dedon, P. C., Goldberg, I. H. in C. L. Propst and T. J. Perun (Ed.) *Nucleic Acid Targeted Drug Design*, Marcel Dekker, Inc., New York, **1992**, 475.
- 2 (a) Griffiths, J.; Murphy, J. A. *J. Chem. Soc., Chem. Commun.* **1992**, 24, (b) Bregant, T. M., Groppe, J., Little, R. D. *Division of Organic Chemistry Abstract #414, The 206th American Chemical Society National Meeting*, Chicago, Illinois, August 22-27, 1993.
- 3 (a) Burckhardt, G.; Zimmer, Ch.; Baguley, B. *J. Biomolec. Str. Dyn.* **1987**, *4*, 813, (b) Luck, G.; Reinert, K.-E.; Baguley, B., Zimmer, Ch. *J. Biomolec. Str. Dyn.* **1987**, *4*, 1079
4. All new compounds gave consistent spectral and analytical data. Selected ¹H NMR data for new compounds (in d₆-DMSO). **2** δ 9.91 (1H, s, NH), 7.77 (1H, d, J = 7.8 Hz), 7.74 (1H, pseudo t, J = 6.9 Hz), 7.71 (1H, s), 7.68 (1H, d, J = 6.9 Hz), 1.79 (9H, s), **3** δ 9.09 (1H, s, NH), 6.84 (1H, dd, J = 7.8, 7.2 Hz), 6.81 (1H, s), 6.54 (1H, d, J = 7.2 Hz), 6.17 (1H, ddd, J = 7.8, 2.1, 0.9 Hz), 1.53 (9H, s), **4** δ 10.66 (1H, s, NH), 9.42 (1H, s, NH), 8.39 (2H, d, J = 8.1 Hz), 8.18 (2H, d, J = 8.1 Hz), 7.70 (1H, d, J = 6.9 Hz), 7.68 (1H, s), 7.41 (pseudo t, J = 6.9 Hz), 6.98 (1H, d, J = 6.6 Hz), 1.56 (9H, s), **5** δ 9.88 (1H, s, NH), 9.80 (1H, s, NH), 7.87 (2H, d, J = 7.8 Hz), 7.81 (1H, s), 7.35 (2H, m), 6.84 (1H, d, J = 7.2 Hz), 6.60 (2H, d, J = 7.8 Hz), 5.62 (2H, s, NH₂), 1.41 (9H, s), **6** δ 10.60 (2H, s, NH), 10.19 (2H, s, NH), 10.01 (2H, s, NH), 8.18 (4H, s), 8.00 (8H, m), 7.80 (2H, d, J = 6.9 Hz), 7.70 (2H, s), 7.35 (2H, pseudo t, J = 6.6 Hz), 6.92 (2H, d, J = 6.6 Hz), 1.50 (18H, s); **1** (as bis-trifluoroacetate salt) δ 10.74 (2H, s, NH), 10.46 (2H, s, NH), 8.19 (4H, s), 8.03 (10H, m), 7.70 (2H, d, J = 7.2 Hz), 7.45 (2H, pseudo t, J = 7.8 Hz), 7.08 (2H, d, J = 7.8 Hz)
- 5 Greene, T. W. *Protective Groups in Organic Synthesis*: Wiley, New York, 1981
- 6 Takamura, N.; Mizoguchi, T.; Koga, K.; Yamada, S. *Tetrahedron* **1975**, *31*, 227
- 7 (a) Patai, S. (Ed.) *The Chemistry of Diazonium and Diazo Groups*, Wiley, New York, 1978, Part I and II, (b) Galli, C. *Chem. Rev.* **1988**, *88*, 765
8. For a similar activation of diazonium salts, see Behr, J. P. *J. Chem. Soc., Chem. Commun.* **1989**, 101

(Received in USA 13 August 1993; accepted 29 September 1993)