Tetrahedron Letters, Vol. 34, No 49, pp. 7823-7826, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

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

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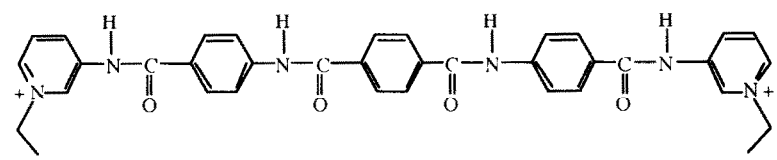
SUMMARY: The bisdiazonium compound **1** has been shown to cleave DNA at concentrations as low as 0 1 μM Evidence is presented to indicate that **1** is binding to the minor groove of DNA, and that binding may be responsible for its effectiveness as a DNA cleaving reagent

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

NSC-101327



1

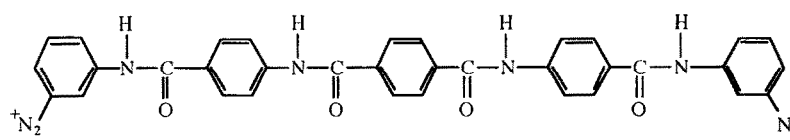
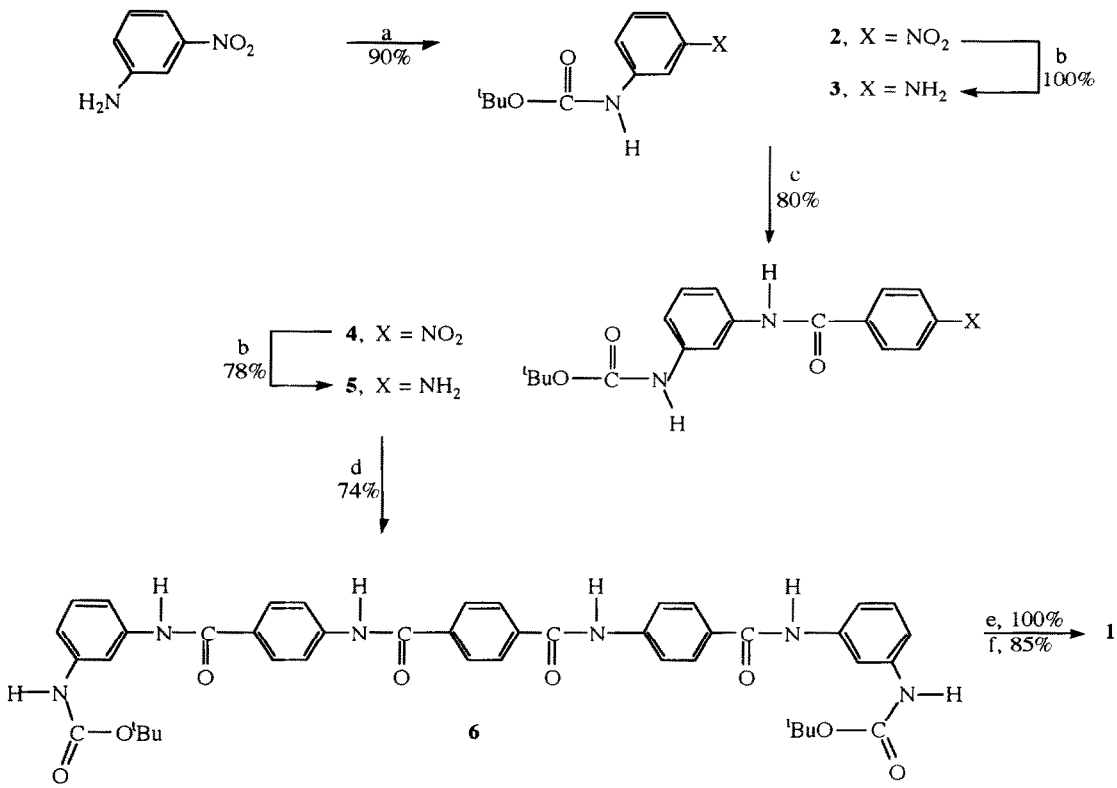


Figure 1: The d(A·T)-specific, minor groove binding drug **NSC-101327** and the designed bisdiazonium compound **1**

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*-butyloxycarbonyl (BOC) group[5] and hydrogenated (3 atm H_2, 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 (CF_3COOH, 25°C), followed by diazotization (isoamyl nitrite[6] 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 H_2, 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 ΦX174 (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 ΦX174 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 ΦX174 supercoiled DNA by **1** Solutions contained 0 25 ng of ΦX174 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 ΦX174 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 + hυ + **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)

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4. All new compounds gave consistent spectral and analytical data. Selected ^{1}H NMR data for new compounds (in d_6-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 8Hz), 5.62 (2H, s, NH_2), 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)

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