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DNA Cleavage by Aromatic Amines

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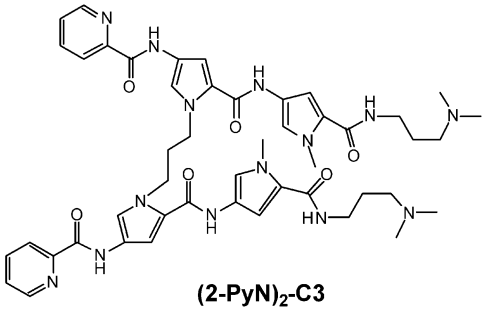
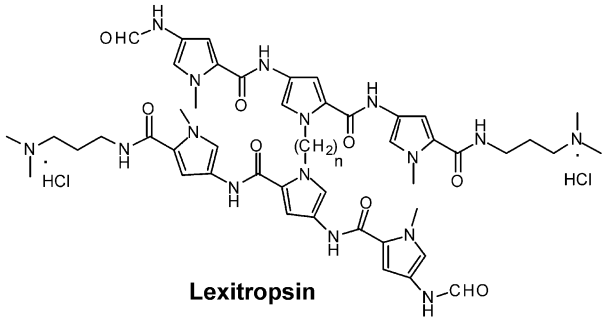
Abstract—A series of aryl amines was found to induce cleavage of DNA. Subsequent refinement led to an efficient family of dimeric derivatives capable of cleavage at low concentration. Initial investigations suggest this is an unprecedented mode of DNA cleavage, which may be ultimately applied to the development of sequence-specific agents.

DNA cleavage by natural or designed molecules has long been recognized as a potentially valuable way to affect cell proliferation, forming the basis of a number of effective cancer chemotherapeutics.[1] Hydrolysis of the phosphate linkage of and electrophilic alkylation of its base pairs are two of the most obvious approaches for DNA degradation. Another paradigm, popularized by minor groove binders including the enediyne antibiotics, and intercalators such as the anthraquinones, is to initiate cleavage via attack on the deoxyribose moiety. The former case involves attack by carbon-centered, non-diffusible radicals,[2] whereas the latter involves the production of oxygen-centered radicals (e.g., $HO^{\bullet}$).[3] We have shown that bisdiazonium ions structurally similar to the d(A/T) specific minor groove binding drug NSC-101327 can cleave supercoiled DNA at low concentrations.[4] These cleavages were initiated either thermally or photochemically. In the former case, it was advantageous, although not necessary, to add metal ions. In light of the discoveries of both Dervan and Lown that dimeric derivatives of distamycin were effective as minor groove binders [e.g., $(2\text{-PyN})_2\text{-C}_3$[5] and the bis-Lexitropsins[6]] we have also synthesized dimeric amines that can be converted to dimeric diazonium ions; these also proved to be effective cleavers.[7] Prompted by this discovery, we wished to undertake a systematic study of such triarylamide-bridged amines, since (i) they are readily prepared, in contrast to comparable distamycin type templates and (ii) variation of functionality can be conducted at ease. Our synthetic route to second generation analogues called for systematic variation of aryl substituents, to enable us to study the relative contribution of electron modulating functionality to the activity of the diazonium ions. Accordingly, triaryl amides **2a**–**c** were produced, using sequential coupling methods (Scheme 1).[8]

The original plan called for diazotization of **2** (isoamyl nitrite, acetic acid, 0 °C) and subsequent investigation of DNA cleaving ability. Though this indeed proved



$(2\text{-PyN})_2\text{-C3}$



Lexitropsin

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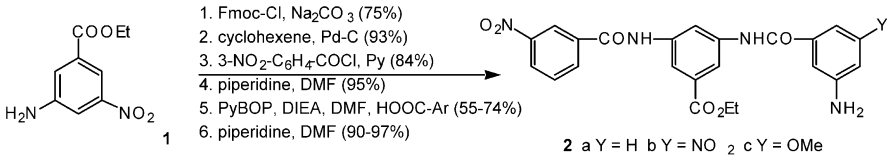
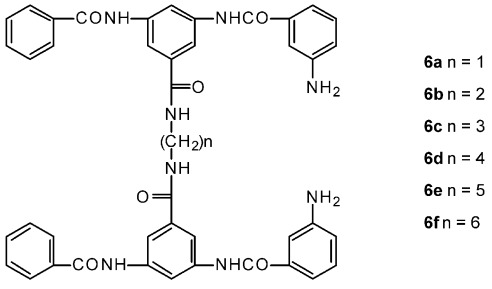


Scheme 1. Synthesis of substituted triarylamides.

successful, to our surprise, during our model studies it was in fact revealed that **2a–c** *themselves* were capable of unwinding ΦXI74 DNA, resulting in substantial conversion to R_fII form within 12 h at 37 °C. Preliminary analysis showed that the nature of the appendage Y influenced the efficiency of the cleavage, with the apparent trend **2a** > **2c** > **2b** warranting further investigation (Table 1, entries 2–5). Prolonged exposure resulted in conversion to R_fIII DNA, and subsequent degradation. With these intriguing data in hand, we elected to follow our initial plan in the form of dimeric structures related to the (2-PyN)$_2$-C$_3$ and bis-Lexitropsin class, using modifications of the existing route (Scheme 2). The aryl amines **5** proved an order of magnitude more active at DNA cleavage, and showed some selectivity within the series, particularly on extended exposure (Table 1, entries 6–11). As had been observed by Dervan and Lown, the nature of the tether length between the two binding entities can be expected to play a role in the binding and therefore cleavage events. Accordingly, based on **5a**, further analogues were prepared with variable tether lengths, using variations on the synthetic procedures adopted in Scheme 2. The analogues, **6a–f** all showed appreciable cleavage, but **6a** and **6c** were most effective (Table 1, entries 12–17). These observations are significant, suggesting that designed *amines* are clearly capable of inducing efficient DNA cleavage at certain concentrations.

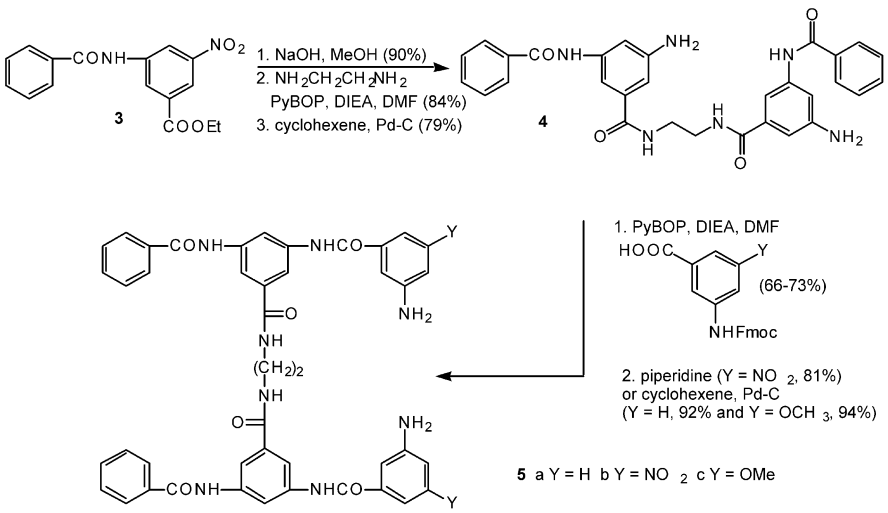
With the results in hand, a series of mechanistic probes was designed; firstly, it was determined that inhibition of DNA cleavage by **6c** was only effective using >1000-fold excess of the minor groove binder distamycin A (entries 18–20).[9] This is in stark contrast to earlier

Table 1. DNA cleavage induced by arylamines[a]



Entry	Amine concn (μM)		Time (h)	Additive	R_fI (%)	R_fII (%)	R_fIII (%)
1	—	—	12	—	90	10	0
2	**2a**	1.0	12	—	1	99	0
3	**2b**	1.0	12	—	44	56	0
4	**2c**	1.0	12	—	11	89	0
5	**2a**	1.0	48	—	0	75	25
6	**5a**	0.1	24	—	35	65	0
7	**5b**	0.1	24	—	36	64	0
8	**5c**	0.1	24	—	34	66	0
9	**5a**	0.1	48	—	7	93	0
10	**5b**	0.1	48	—	10	90	0
11	**5c**	0.1	48	—	0	99	1
12	**6a**	0.1	24	—	0	100	0
13	**6c**	0.1	24	—	24	76	0
14	**6d**	0.1	24	—	37	63	0
15	**6e**	0.1	24	—	35	65	0
16	**6f**	0.1	24	—	38	62	0
17	**6c**	0.1	48	—	1	99	0
18	**6c**	0.1	24	10 μM DA[b]	25	75	0
19	**6c**	0.1	24	100 μM DA	65	35	0
20	**6c**	0.1	24	1000 μM DA	95	5	0
21	**6c**	0.1	12	10 μM[c] $M^{2+/3+}$	27	73	0
22	**6c**	0.1	12	Ar/N_2	26	74	0

[a]All incubations conducted with amines (6 μL at indicated concn) and ΦX174 bacteriophage DNA (6 μL of 0.14 μM) in Tris–acetate–EDTA buffer in the absence of light at pH 8.2/37 °C unless noted. Gels electrophoresed on agarose (0.7%) at 80 V/2.5 h, stained (ethidium bromide), then subjected to scanning densitometry.
[b]DA, distamycin A.
[c]10 mM aliquots of metal chlorides used.



Scheme 2. Preparation of dimeric polyamines.

results obtained with simple diamines and diazonium ions,[4,7] and, though speculative, may point to an alternative mode of binding and recognition to that expected for polyamides of this general class.

One possibility might be the involvement of trace metal cations, and to investigate this, analogous cleavage experiments were conducted in the presence of 100-fold excess of Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, Ni^{2+}, or Zn^{2+} salts. In all cases, using a 24 h incubation period, no change (relative to control) was observed (entry 21). We were also concerned regarding the possible participation of free radical chain reactions in the cleavage process. To address this issue, cleavage induced by **6c** was evaluated in the presence and absence of a 10-fold excess of oxygen radical scavengers including KI, DABCO, Tiron, and catalase. No change relative to control was observed, nor was a difference found when cleavage was conducted under simulated deoxygenated conditions, implying that a radical species is not involved (entry 22).

A remaining possibility is that some form of nucleophilic attack mechanism involving the arylamines is responsible, and to investigate this further, cleavage was examined over a wide pH range. Significantly, cleavage induced by **6c** was muted at pH > 8.2, yet it was enhanced at pH 7.3 (80% R_fII/24 h) and pH 6.5 (90% R_fII/24 h), relative to control (entry 13). At still lower pH, uncatalyzed cleavage competes, preventing accurate controls from being developed. However, it is evident that when the aniline group(s) is partially protonated ($pK_b \sim 9$), cleavage is enhanced, which may imply some form of proton-transfer mechanism for the initiation of cleavage. This would constitute a new mode of DNA cleavage, and the structure–activity correlations observed suggest a degree of control may be attainable. Ongoing research, involving the use of restriction fragments, in concert with in-depth binding analyses,[10] and NMR studies are designed to shed further light on the process.[6,11]

An unprecedented method of DNA cleavage via readily accessible arylamines has been revealed. Application of the process to specific DNA sequences and microenvironments, together with kinetic analysis promises to lead to new possibilities in the design of antiproliferatives and chemical nucleases.

Acknowledgements

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140.6, 140.9, 150.2, 167.1, 167.9, 168.1; $C_{45}H_{40}N_8O_6$ requires: C, 68.52, H, 5.11; found: C, 68.88, H, 4.98.
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