Triazole Photonucleases: A New Family of Light Activatable DNA Cleaving Agents

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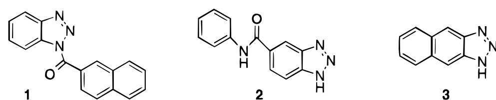
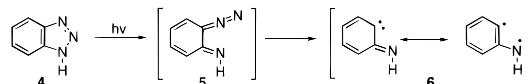
Esperamicin, calicheamicin, dynemicin, neocarzinostatin, and related DNA cleaving agents have attracted considerable interest in recent years due in part to their highly potent antitumor activity, novel mode of action, and potential service as reagents in nucleic acid research.[1,2] Common to all of these agents is their ability to undergo inducible cycloaromatization to an aryl or indenyl diradical which abstracts hydrogens from proximate deoxyribosyl sites, leading to DNA scission.[1−3] Efforts to synthesize these natural products or superior analogs have progressed impressively, resulting in a number of imaginative strategies for the assembly of the enediyne precursors of the DNA-damaging diradicals.[4] In contrast, relatively little effort has been directed at the investigation of simple aryl (mono) radicals or related species as nucleic acid cleaving agents, even though such intermediates are readily prepared and exhibit similar reactivity to aryl diradicals,[5] being implicated in the mechanism of action of the above agents.[6] Several years ago, we started studies directed at the development of conceptually new approaches to radical-based DNA cleaving agents and describe below our initial investigation of a novel family of activatable DNA cleaving agents represented by triazoles **1−3**.

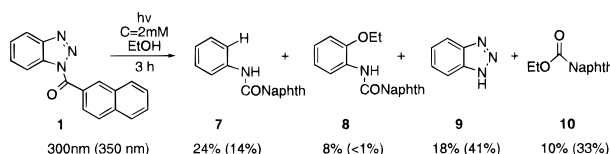


Our initial studies were guided by the mechanistic and operational advantages offered by photoinducible radical formation.[7] Illustrative of this point, commercially available benzotriazole (**4**) has been shown to be transformed photochemically at 77 K into the spectroscopically observable intermediate **6**.[8] This reaction is proposed to proceed from the lowest excited singlet state of **4** (π,π^*), leading initially to a detectable azoimine **5**. **5** is stable only at low temperature and converts thermally or photochemically to **6**. Singlet **6** can react directly or undergo intersystem crossing to the triplet which is capable of hydrogen abstraction and thereby of serving as a potential agent for DNA cleavage.

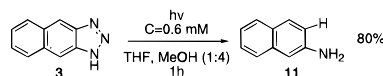


Agents **1−3**, incorporating several structural and chromophore variations of interest, were selected for cleavage studies. 1-(2-Naphthoyl)benzotriazole (**1**) was formed quantitatively by treatment of **4** with Et_3N and 2-naphthoyl chloride. The amidobenzotriazole **2** was obtained through a 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide-mediated coupling of benzotriazole-5-carboxylic acid and aniline in the presence of 1-hydroxybenzotriazole in DMF (91%). Finally, naphthotriazole **3** was synthesized from 2,3-diaminonaphthalene by published procedures.[9]

As a test of its competency as a radical generator, triazole **1** was photolyzed in ethanol at 300 and 350 nm. Products **7** and **8** were obtained, in accord with studies on benzotriazole itself,[8] along with solvolysis products **9** and **10**. When the photolysis



of **1** was carried out in CD_3OD, deuterium incorporation (75%) was observed. Photolysis of **3** produced 2-aminonaphthalene (**11**, 80% isolated yield). Deuterium incorporation (>90%) was observed when this reaction was carried out in THF-d_8. Photolysis of benzotriazole **2** in methanol or acetonitrile also produced a photoextrusion product, 4-aminobenzanilide.



The ability of these molecules to cleave DNA was determined by their effectiveness in converting circular supercoiled DNA (form I) to circular relaxed DNA (form II) and linear DNA (form III). For this purpose, triazoles **1−3** (Figure 1) were irradiated

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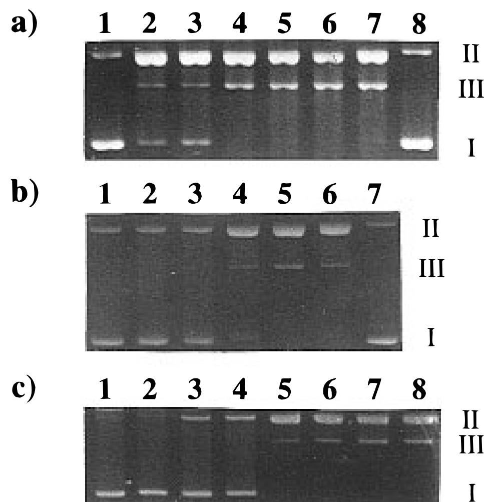

Figure 1. Light-induced cleavage of DNA by triazole derivatives. Supercoiled DNA runs at position I, nicked DNA at position II, and linear DNA at position III. Unless otherwise indicated, reactions in experiments a and c were irradiated with Pyrex-filtered light from a 450 W medium-pressure mercury arc lamp; reactions in experiment b were irradiated with light (300 nm) from a Rayonet photochemical reactor. (a) Cleavage activity of compound **1**. Lane 1, ϕX174 RFI DNA control (30 μM/bp); lanes 2−7, DNA + **1** at concentrations of 60 μM, 130 μM, 180 μM, 260 μM, 370 μM, and 1.2 mM, respectively; lane 8, DNA + **1** (1.2 mM), no *hν*. (b) Cleavage activity of compound **2**. Lane 1, ϕX174 RFI DNA control (30 μM/bp); lanes 2−6, DNA + **2** at concentrations of 25, 50, 200, 400, and 800 μM; lane 7, DNA + **2** (800 μM), no *hν*. (c) Cleavage activity of compound **3**. Lane 1, pBR322 DNA control (30 μM/bp); lane 2, DNA + **3** (1 mM), no *hν*; lanes 3−8, DNA + **3** at concentrations of 15 μM, 30 μM, 150 μM, 300 μM, 750 μM, and 1 mM.

at various concentrations for 30 min in the presence of either pBR322 plasmid or ϕX174 RFI DNA (30 μM/bp) in 1:9 DMSO: Tris buffer (20 mM, pH 7.5). In all cases, only the combination of triazole and light produced single- and double-strand cuts in the DNA [(a) lanes 2−7, (b) lanes 2−6, (c) lanes 3−8]. At the lowest concentrations of triazole, residual form I DNA is observed, along with a substantial amount of form II DNA.[10] At higher concentrations, the amount of damaged DNA increases linearly as the amount of intact DNA decreases, with complete disappearance of form I at concentrations above 150 μM. Form III DNA, the result of double-strand cleavage, appears as a product in the reactions with **1**−**3** at 60, 200, and 150 μM, respectively.

The cleavage selectivity of triazole **1** was determined by sequencing analyses of the DNA cleavage products (Figure 2) obtained when **1** (75 μM to 1.5 mM) was photolyzed in the presence of a 5′-^{32}P-labeled 516 base pair restriction fragment from pBR322. The resulting cleavage pattern is concentration independent. Comparison with the Maxam−Gilbert G markers

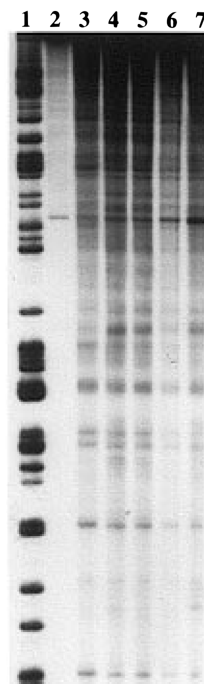

Figure 2. Autoradiogram of a 12% denaturing polyacrylamide gel showing cleavage of 5′-^{32}P end-labeled EcoRI/RsaI restriction fragment from pBR322 by **1.** All reactions were irradiated with Pyrex-filtered light for 30 min. Lane 1, Maxam−Gilbert G reaction; lane 2, DNA + **1** (1.5 mM), no *hν*; lanes 3−7, DNA + **1** at concentrations of 1.5 mM, 750 μM, 300 μM, 150 μM, and 75 μM, respectively.

(lane 1)[11] reveals high selectivity for guanine residues, especially at −GG− sites. Longer irradiation times produce more intense cleavage bands, but not additional cleavage at new sites.

The above studies show that readily prepared triazoles can be induced to undergo photoextrusion under conditions required for DNA cleavage, producing intermediates capable of hydrogen abstraction. When these photolyses are conducted in the presence of plasmid DNA, single- and double-strand cleavages are observed. The cleavage pattern is selective for guanine residues, indicating a remarkable intrinsic cleavage selectivity for agents not otherwise equipped with DNA recognition elements. This cleavage could arise from a σ,π-diradical functioning like a phenyl radical in a hydrogen abstraction process. Equally plausible alternative pathways involve carbene insertion, carbene alkylation, cleavage by azoimines, or photoinduced electron transfer, the last consistent with the selectivity observed for other photoelectron transfer agents.[12] Further studies of this new class of cleaving agents are in progress.

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(10) In a separate experiment, **1** was shown to induce single-strand cleavage at concentrations as low as 3 μM.

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