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Bromoacetophenone-Based Photonucleases: Photoinduced Cleavage of DNA by 4′-Bromoacetophenone–Pyrrolecarboxamide Conjugates

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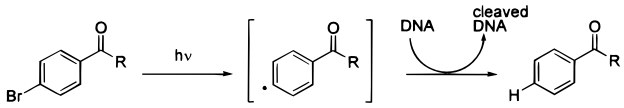
ABSTRACT



4′-Bromoacetophenone derivatives which upon excitation can generate monophenyl radicals capable of hydrogen atom abstraction were investigated as photoinducible DNA cleaving agents. Pyrrolecarboxamide-conjugated 4′-bromoacetophenones were synthesized, and their DNA cleaving activities and sequence selectivities were determined.

The design and synthesis of DNA cleaving agents are goals of considerable basic and applied significance in chemistry, biology, and medicine.[1] Beginning in 1985, the structures of a new and now substantial family of highly potent DNA cleaving agents represented in part by neocarzinostatin, dynemicin, and calicheamicin began to emerge. Collectively, these agents share a polyunsaturated nine- or ten-membered ring core, commonly a cyclic enediyne, which undergoes cycloaromatization to produce a highly reactive aryl or styryl diradical, the intermediate which abstracts hydrogen atoms from DNA initiating its cleavage. The novel structures and activities of these agents attracted much synthetic interest from our and other laboratories,[2,3] although the complexity of these agents and even simpler analogues often required lengthy sequences involving unstable intermediates. As a consequence, we and others have initiated efforts to identify simpler, more stable, and synthetically more accessible precursors to aryl and vinyl radicals, the key intermediates

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required for DNA cleavage.[4,5] Recently, we reported that simple, commercially available benzotriazoles when combined with a DNA recognition subunit can upon photoactivation cleave DNA in a potent and selective fashion.[5] We have now examined the utility of haloarenes as radical progenitors for DNA cleavage and report herein that these readily available compounds upon excitation[6] are effective DNA cleaving agents.

Our studies are based on the observation that excitation of a haloarene can lead to homolytic cleavage of a carbon−halogen bond, thereby generating a phenyl radical,[7] potentially capable of causing single-stranded lesions to DNA. By attaching a suitable haloarene to a DNA recognition element, it was expected that this radical generation could be localized to specific sites on DNA.[8] As a first test of this concept for DNA cleaving agents, we elected to use 4′-bromoacetophenone derivatives linked to synthetic oligopeptides, pyrrolecarboximides,[9] as shown in Figure 1.

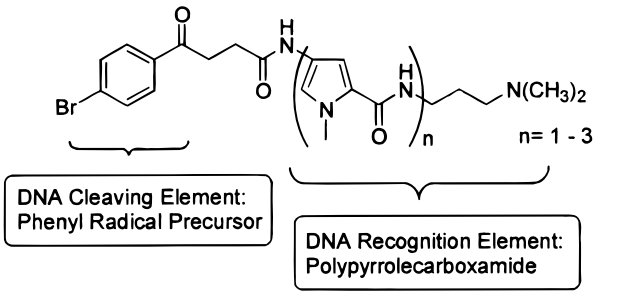
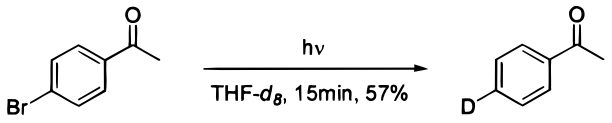


Figure 1. 4′-Bromoacetophenone−pyrrolecarboxamide conjugates as photoinducible DNA cleaving agents.

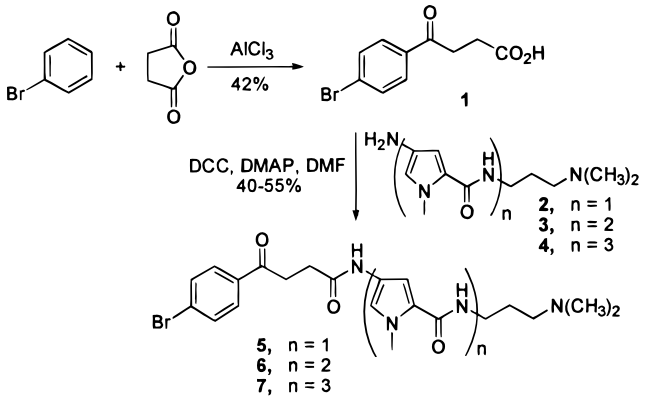
As a reference point for this study, we investigated the ability of simple haloacetophenones to abstract hydrogen atoms upon excitation, a required event for DNA cleavage. The photolytic behavior of 4′-bromoacetophenone is representative. The photolyses were performed by using a medium-pressure mercury arc lamp equipped with a Pyrex filter under anaerobic conditions. THF was used as solvent and as a DNA surrogate. In all cases, the debrominated product, acetophenone, was the major compound produced. In accord with the intermediacy of an aryl radical, when the photolysis of 4′-bromoacetophenone was conducted in THF-d_8, the monodeuterated product (Scheme 1)was obtained in 15 min in 57% yield along with unreacted starting material (22%).

Scheme 1



Having established the utility of 4′-bromoacetophenone as an aryl radical progenitor, we next sought to attach this subunit to a series of pyrrolecarboxamide-based DNA minor groove binders. The pyrrole-linked 4′-bromoacetophenone conjugates were synthesized as shown in Scheme 2. 3-(4′-Bromobenzoyl)propanoic acid was prepared by the Friedel−Crafts succinoylation of bromobenzene and coupled with pyrrole polyamides **2**−**4**, prepared from *N*-methylpyrrole

Scheme 2



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according to Shibuya's method[10] to obtain 4′-bromoacetophenone−pyrrolecarboxamide conjugates **5**−**7**.

The DNA cleaving activities of compounds **5**−**7** (Figure 2) were determined by monitoring their effectiveness in converting circular supercoiled DNA (form I) to circular relaxed DNA (form II) and linear DNA (form III). 4′-Bromoacetophenone and pyrrole polyamide-linked 4′-bromoactophenones were irradiated at various concentrations for 30 min in the presence of ϕX174RFI DNA (30 μM/bp) in 1:9 DMSO:Tris buffer (20 mM, pH 7.5) under aerobic conditions. All conjugates tested caused DNA cleavage. The cleavage efficiency was substrate and concentration dependent. For calibration, 4′-bromoacetophenone itself formed form III DNA with complete disappearance of form I but only at high concentrations (10 mM, data not shown).

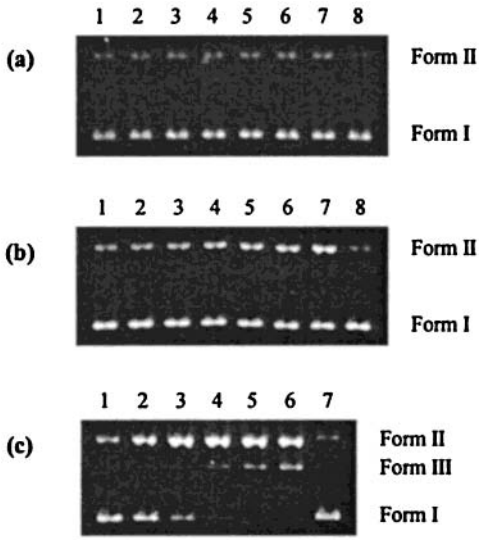

Figure 2. Light-induced cleavage of DNA by pyrrole-linked 4′-bromoacetophenones **5**, **6**, and **7**. Supercoiled DNA (ϕX174RF) runs at position I, nicked DNA at position II, and linear DNA at position III. Unless otherwise indicated, all DNA cleavage reactions were irradiated with Pyrex-filtered light from a 450 W medium-pressure mercury arc lamp for 30 min at 25 °C. (a) Lane 1−7, DNA (30 μM/bp) + **5** at concentrations of 3, 10, 20, 30, 50, 100, and 200 μM, respectively; lane 8, control ϕX174RF DNA + **5** (200 μM), no *hν*. (b) Lane 1−7, DNA (30 μM/bp) + **6** at concentrations of 3, 10, 20, 30, 50, 100, and 200 μM, respectively; lane 8, control ϕX174RF DNA + **6** (200 μM), no *hν*. (c) Lane 1−6, DNA (30 μM/bp) + **7** at concentrations of 3, 10, 20, 30, 50, and 100 μM, respectively; lane 7, control ϕX174RF DNA + **7** (200 μM), no *hν*.

As expected from design considerations, the efficiency of DNA cleavage was strongly influenced by the DNA binding moiety as well as the concentration of the test compound. Upon irradiation, the monopyrrole linked agent **5** produced detectable formation of form II DNA in a concentration dependent fashion over the range of 3−200 μM. The dipyrrole-linked system **6** produced more pronounced cleavage than **5** over the same concentration range as would be expected from its stronger association with DNA. In accord with this finding, the tripyrrole analogue **7** proved to be even more effective, producing even form III DNA at a concentration of 30 μM. The 3 μM reaction of distamycin type analogue **7** gave higher activity than even those observed at 10-fold higher concentrations of the netropsin type analogue **6** with complete disappearance of form I at concentrations above 20 μM. The DNA cleaving activity of the corresponding desbromoacetophenone analogue of **5** was also tested as a control. Even at high concentrations (1 mM), this analogue produced barely detectable DNA cleavage in accord with the crucial role of the bromide substituent in the cleavage process.

The inhibitory effects of radical scavengers on the cleavage process were also examined with TEMPO, a known carbon-centered radical scavenger, and sodium benzoate, a hydroxyl radical scavenger. When TEMPO was added to the reaction mixture, the DNA cleaving activity of compound **7** decreased as the concentration of TEMPO increased while its activity was not affected by sodium benzoate as shown in Figure 3.

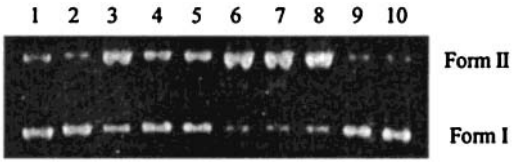

Figure 3. The effect of radical scavenger on light-induced cleavage of DNA by peptide-linked 4′-bromoacetophenone **7**. Supercoiled DNA (ϕX174RF) runs at position I, nicked DNA at position II, and linear DNA at position III. Unless otherwise indicated, all DNA cleavage reactions were irradiated with Pyrex-filtered light from a 450 W medium-pressure mercury arc lamp for 30 min at 25 °C. Lane 1, control ϕX174RF DNA (30 μM/bp); lanes 2, DNA + TEMPO (200 mM); lanes 3−5, DNA + **7** (15 μM) + TEMPO at concentrations of 2, 100, and 200 mM, respectively; lanes 6−8, DNA + **7** (15 μM) + sodium benzoate at concentrations of 2, 100, and 200 mM, respectively; lane 9, control DNA + sodium benzoate (200 mM); lane 10, DNA + **7** (15 μM), no *hν*.

The cleavage selectivity of the peptide-linked 4′-bromoacetophenone derivatives was determined by sequencing analyses of the DNA cleavage products obtained when compounds **5**−**7** were photolyzed in the presence of a 3′-^{32}P labeled 517 base pair restriction fragment from pBR322.[11] As expected for a cleaving agent bound to a distamycin (**7**) or netropsin (**6**) analogue, the cleavage intensities are the highest in AT-rich regions of the DNA as shown in Figure 4. A significant cleavage site is marked to the right of the autoradiogram.

The autoradiogram shown in Figure 4 was quantified by densitometry, and these data were used to construct histograms for the DNA cleavage observed in the lower regions

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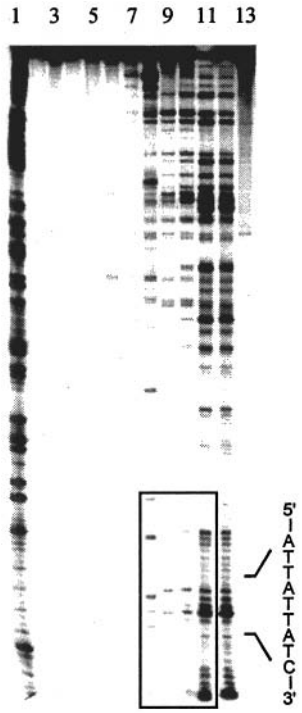

Figure 4. Autoradiogram of 8% denaturing polyacrylamide gel showing cleavage of 3′-^{32}P end-labeled 517 base pair restriction fragment (EcoRI/RsaI) from pBR322 by peptide-linked bromoacetophenone **5**, **6**, and **7**. All reactions were irradiated with Pyrex-filtered light from a 450 W medium-pressure mercury arc lamp for 30 min at 25 °C. The cleavage site is shown to the right of the autoradiogram. Lane 1, Maxam−Gilbert G reaction; lane 2, DNA control; lanes 3−5, DNA + **5** at concentrations of 10, 50, and 200 μM, respectively; lanes 6−8, DNA + **6** at concentrations of 10, 50, and 200 μM, respectively; lanes 9−12, DNA + **7** at concentrations of 5, 15, 50, and 100 μM, respectively; lane 13, DNA + 4′-bromoacetophenone (500 μM).

of the autoradiograms (Figure 5). The histograms show that compounds **6** and **7** produce cleavage within and adjacent to sites of multiple contiguous AT base pairs, and the cleavage pattern remains at high concentration. The cleavage assay and sequencing of these compounds showed remarkable correlation between chain lengths and activities with the highest activity for the distamycin type analogue **7**.

In summary, these studies show that 4′-bromoacetophenone derivatives upon irradiation can function as DNA cleaving agents putatively through the generation and reaction of phenyl radicals. Upon conjugation to suitable DNA recognition elements, these derivatives cleave DNA at the

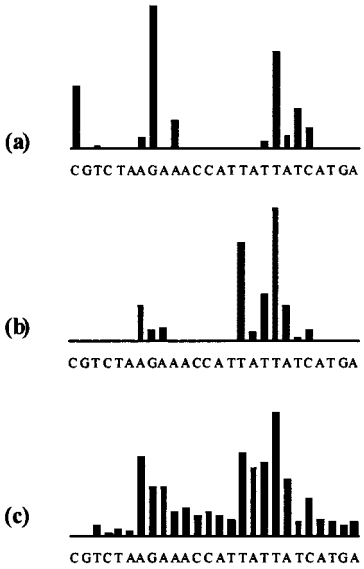

Figure 5. Histograms of DNA cleavage sites by the pyrrole-linked 4′-bromoacetophenone analogues. The relative extent of cleavage was estimated from the densitometric scans of the lower regions of the autoradiograms shown in Figure 3, and the height of the bar represents relative cleavage intensity at the indicated bases: (a) 200 μM of compound **6**, (b and c) compound **7** at concentrations of 15 and 50 μM, respectively.

sites targeted by the recognition subunit. This work provides a new class of DNA cleaving agents that are notably easier to prepare and significantly more stable than enediyne systems. In addition, the use of light to turn these agents "on" offers unique *external* control over the initiation of the cleavage process relative to bimolecular activations required with other agents. More generally, given the number of photochemical and thermal methods to generate phenyl and vinyl radicals, it is expected that this strategy could be extended to a variety of other radical progenitor systems.

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Supporting Information Available: Details of synthesis and characterization of the listed compounds and experiments for cleavage of DNA and its assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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