[S2-1] *[4/21/2000 (Fri) 09:10~09:50/Room B112, B1 Floor, Bldg 26]*

Photocleavage of DNA by 4'-Bromoacetophenone-Pyrrolecarboxamide Conjugates

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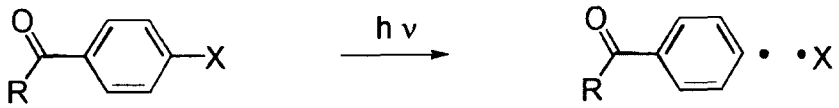
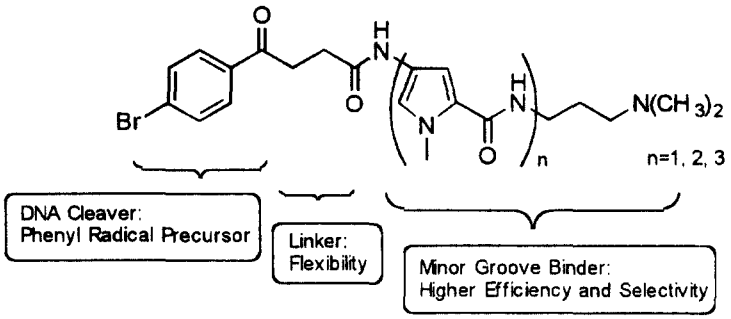
The design and synthesis of DNA cleaving molecules are currently a topic of intense research investigations.[1] Especially, enediyne anticancer antibiotics in this area have been focused primarily on the design and syntheses of simple enediyne structures which can be mimic their mechanistic feature.[2] Regarding enediyne family as activatable DNA cleaving agents, such complex structures might not be needed but rather that any aryl or vinyl radical would be capable of causing hydrogen atom abstraction reaction from deoxyribose, which initiated the scission of DNA.[3] However, few efforts have been directed at the investigation of simple phenyl radical species as DNA cleaving agents. Recently, our group has been investigated simple photoactivatable DNA cleaving agents represented by benzotriazoles which could be readily prepared and exhibit potent and selective DNA cleaving activity.[4] Concerning with other simple and potent carbon centered radical precursors, halogenated organic compounds are of interest as photoinducible DNA cleaving agents since irradiation can generate carbon centered radicals by homolysis of carbon-halogen bond.[5]

Therein, we report the design, synthesis, and activity of mono carbon centered radical precursor, halophenyl ketone, as a photoinducible DNA cleaver, which has lower triplet energy than that of plain halobenzene and can be generate a reactive phenyl radical by the photoinduced C-X bond cleavage.

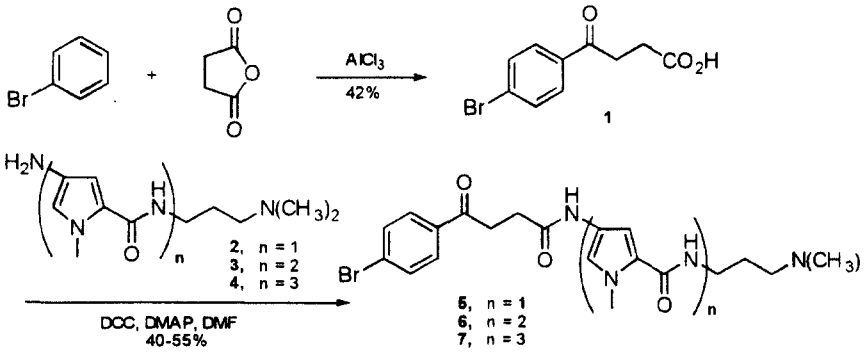


In addition, there are various approaches on the development of sequence selective DNA cleaving agent by conjugates of DNA cleaver and recognition elements.[6] Since naturally occurring oligopeptides such as netropsin and distamycin bind to DNA in the minor groove of the helix,[7] here the phenyl radical generating species has been attached to a pyrrole polyamide as synthetic oligopeptides in order to increase the DNA cleaving ability and DNA sequence selectivity. The DNA cleaving moiety and minor groove binders are connected by methylene linker to allow flexibility on this molecule as shown below.



The photolytic behavior of bromoacetophenone was investigated under several different conditions using a medium pressure mercury arc lamp equipped with a Pyrex filter. The homolytic cleavage of the carbon-halogen bond can be explained by the state interconversions as simple internal electron transfers from the π^*orbital to the C-X σ^* orbital (π to σ electron transfer), this being the actual electronic change required for σ radical formation.[8] In all cases, the debrominated product, acetophenone, was the major product. The fact this reaction proceeds by a radical process was confirmed by photolysis of 4`-bromoacetophenone in THF-d_8 leading to the corresponding deuterated acetophenone induced from homolysis of C-Br bond.

Scheme 1



The peptide-linked bromoacetophenone was synthesized as shown in Scheme I. 3-(4'-bromobenzoyl)propanoic acid was prepared by the Friedel-Crafts succinoylation of bromobenzene and coupled with pyrrole polyamide **2-4**, prepared from *N*-methyl pyrrole according to Shibuya's method.[9]

The DNA cleaving activities of compounds **5-7** 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 bromoactophenones were irradiated at various concentrations for 30 min in the presence of ϕX174RFI DNA (30μM/bp) in 1:9 DMSO:Tris buffer (20mM, pH 7.5). All compounds tested exhibited the DNA cleaving activities. Bromoacetophenone formed form III at 10 mM with complete disappearance of form I (data not shown). As we expected from previous study, this DNA cleaving activity was remarkably enhanced by DNA binding moiety, pyrrole polyamide, depending on the number of pyrrole unit as well as the concentrations of the compounds.

The concentration of the pyrrole polyamide linked bromoacetophenone was ranged to 3 to 200 μM to compare their DNA cleaving activity. Form III DNA was observed at 30 μM concentration of distamycin type analog 7. The 3 μM reaction of distamycin analog gave higher activity even than those of the 20 μM netropsin analog with complete disappearance of form I at concentrations above 20 μM. The DNA cleaving activity of acetophenone analog which has no bromine substituent on the phenyl ring was tested. At high concentration (1mM) of acetophenone analog, the cleaving activity was barely recognizable, emphasizing the role of the phenyl bromide as a phenyl radical precursor.

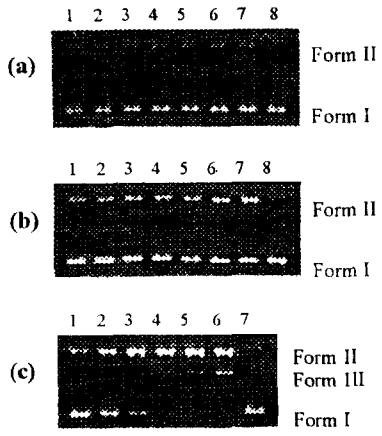


Figure 1. Light induced cleavage of DNA by peptide linked 4'-bromoacetophenones **5**, **6** and **7**. Supercoiled DNA (ϕX174RF) runs at position I, nicked DNA at position II, and linear DNA at position III. Unless otherwise indicated, all DNA cleavage reactions were irradiated with Pyrex-filtered light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

The inhibitory effect of radical scavengers was also examined to confirm the attribution of carbon centered radical to DNA cleaving activity. TEMPO, known as a carbon centered radical scavenger, and sodium benzoate, a hydroxyl radical scavenger, were added into the reaction mixtures for the DNA cleavage assay. The DNA cleaving activity of compound 7 was decreased as the concentration of TEMPO was increased while its activity was not affected by sodium benzoate (data not shown).

Figure 2. Autoradiogram of 8% denaturing gel 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 450W medium pressure mercury arc lamp for 30min at 25°C. The cleaving 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 10μM, 50μM, and 200μM, respectively; lanes 6-8, DNA + **6** at concentrations of 10μM, 50μM, and 200μM, respectively; lanes 9-12, DNA + 7 at concentrations of 5μM, 15μM, 50μM, and 100μM, respectively; lane 13, DNA + 4'-bromoacetophenone (500μM).

The cleavage selectivity of the peptide linked bormoacetophenone 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.[10] As expected for a cleaving agent bound to a distamycin or netropsin analog, the cleavage intensities are the highest in AT-rich regions of the DNA. A significant cleaving site is marked to the right of the autoradiogram shown in Figure 2.

The autoradiogram shown in Figure 2 was quantified by densitometry, and this data was used to construct histograms for the DNA cleavage observed in the lower regions of the autoradiograms. 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 leading to the highest affinity of distamycin type analog 7 to bind to DNA and cleave it.

The use of bromoacetophenone species as phenyl radical precursors thus offers a lead into the design of new DNA cleaving agents. Bromoacetophenone derivatives possessing netropsin or distamycin-type minor groove binders showed potential DNA cleaving activity and sequence selectivity at micromolar concentrations of the reagents. Future studies may benefit from modification and design of the simple molecules to cleave DNA possessing the sequence selectivity.

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