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Photoinduced DNA Cleavage by Cyclopentadienyl Metal Complexes Conjugated to DNA Recognition Elements

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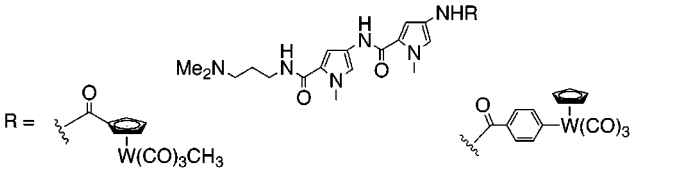
ABSTRACT



DNA recognition elements have been attached to $CpW(CO)_3CH_3$ and $CpW(CO)_3Ph$, which produce methyl and phenyl radicals that cleave DNA upon photolysis. The inclusion of binding moieties in 3 increases the efficiency but not the selectivity of strand scission over that seen in the simple unfunctionalized complex, while 11 cleaves preferentially at T sites within AT-rich tracts.

The identification of new compounds for the modification of nucleic acids is an important goal in chemistry, biology, and medicine, as shown by recent intense efforts aimed toward the enediynes and other organic radical sources,[1] cisplatin and similar complexes,[2] and bleomycin and other redox-active coordination complexes.[3] These agents represent a variety of active species and mechanisms of action, including nucleotide base functionalization[4] and hydrogen atom abstraction by organic or oxygen-centered radicals,[5] and each modification pathway has proven antibiotic and anticancer activity. Aside from their potential as chemotherapeutic agents, molecules that modify oligonucleotides are useful tools for the elucidation and manipulation of the primary, secondary, and tertiary structure of DNA[6] and for investigating the behavior of other molecules as they interact with oligonucleotides.[7] Particularly advantageous for all of

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(1) For lead references, see: Smith, A. L.; Nicolaou, K. C. *J. Med. Chem.* **1996**, *39*, 2103−2117. For some recent examples, see: Kabayashi, S.; Reddy, R. S.; Sugiura, Y.; Sasaki, D.; Miyagawa, N.; Hirama, M. *J. Am. Chem. Soc.* **2001**, *123*, 2887−2888. Weinstein, D. S.; Nicolaou, K. C. *J. Chem. Soc., Perkin Trans. 1* **1999**, 545−558.

(2) For some recent examples, see: Burstyn, J. N.; Heiger-Bernays, W. J.; Cohen, S. M. Lippard, S. J. *Nucleic Acids Res.* **2000**, *28*, 4237−4243. Williams, K. M.; Cerasino, L.; Natile, G.; Marzilli, L. G. *J. Am. Chem. Soc.* **2000**, *122*, 8021−8030.

(3) For lead references, see: Abraham, A. T.; Zhou, X.; Hecht, S. M. *J. Am. Chem,. Soc.* **2001**, *123*, 5167−5175. Fu, P. K.-L.; Bradley P. M.; Turro, C. *Inorg. Chem.* **2001**, *40*, 2476−2477. Ananias, D. C.; Long, E. C. *J. Am. Chem. Soc.* **2000**, *122*, 10460−10461. Melvin, M. S.; Tomlinson, J. T.; Saluta, G. R.; Kucera, G. L.; Lindquist, N.; Manderville, R. A. *J. Am. Chem. Soc.* **2000**, *122*, 6333−6334. Hoehn, S. T.; Junker, H.-D.; Bunt, R. C. Turner, C. J.: Stubbe, J. *Biochemistry* **2001**, *40*, 5894−5905. Boger, D. L.; Cai, H. *Angew. Chem., Int. Ed.* **1999**, *38*, 448−476. Oyoshi, T.; Sugiyama, H. *J. Am. Chem. Soc.* **2000**, *122*, 6313−6314. Tullius, T. D. In *Comprehensive Supramolecular Chemistry*, Suslick, K. S., Ed.; Elsevier: Oxford, 1996.

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(6) For example, see: Schuster, G. B. *Acc. Chem. Res.* **2000**, *33*, 253−260.

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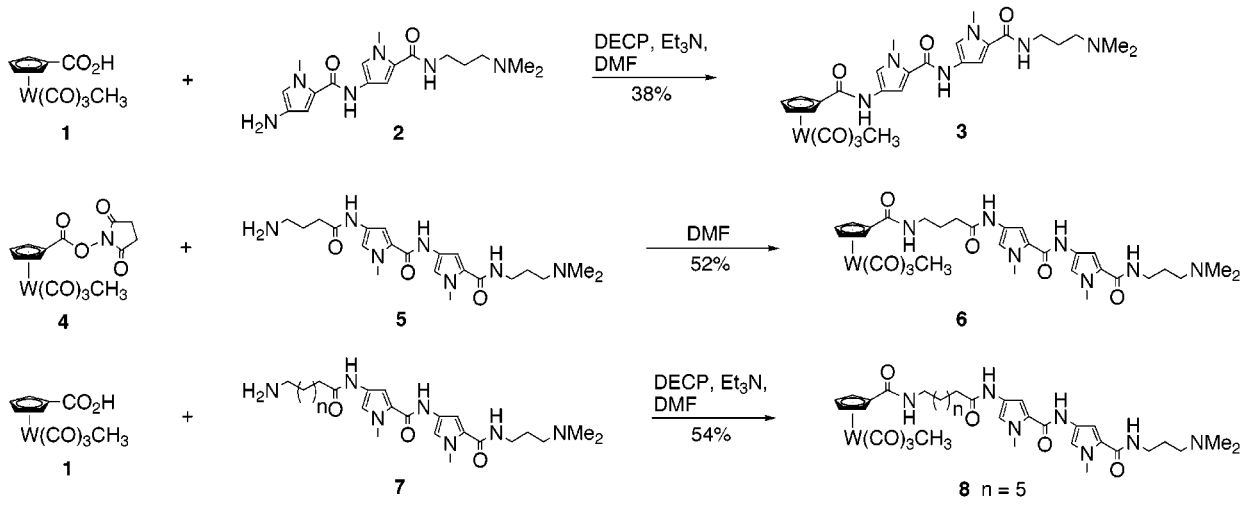
Scheme 1



these applications are devices that contain a triggering mechanism,[8] because of their potential ability to target specific substrates or substructures. Toward this end, we recently reported[9] the single-strand cleavage of plasmid DNA by the photolysis of $CpW(CO)_3CH_3$ at 1.5 molecules/bp.

Although the activity of the simple tungsten complex is remarkable considering its lack of recognition elements, its selectivity and efficiency should be increased by conjugation to a DNA binding component.[10−12] This would facilitate association with DNA prior to the photolytic production of a methyl radical, which is presumed to be the primary active species. The recognition element could be chosen to select certain sequences to function as part of a chemical nuclease,[13] and a variety of such agents is available. Of these, designed analogues of the minor groove binders netropsin and distamycin[14] are especially attractive because they can be readily synthesized on a solid support and can be programmed to recognize virtually any DNA sequence.[15] Therefore, we now describe the preparation and DNA cleaving behavior of $CpW(CO)_3CH_3$ and $CpW(CO)_3Ph$ conjugated to netropsin analogues.

The preparation of the conjugates **3**, **6**, and **8** was accomplished in a straightforward manner (Scheme 1). Either the carboxylic acid **1** or its succinimide ester **4**[16] was coupled under standard conditions to the amines **2**,[17] **5**,[18] or **7**. These compounds allow the comparison of the effects of the variation in tether length on DNA cleavage behavior.

The DNA cleaving activity of each of the new complexes was initially assessed using a plasmid relaxation assay to monitor the conversion of circular supercoiled DNA (form I) to relaxed circular (form II) and linear DNA (form III). Each of the compounds was photolyzed through a Pyrex filter in the presence of pBR322 DNA, and the results are shown in Figure 1. Quantitation[19] of the bands in these gels indicated that form II DNA resulting from single strand cleavage was present at complex concentrations of 1.4, 5.6, and 5.6 μM for **3** (a, lane 11), **6** (b, lane 9), and **8** (c, lane 9), respectively. Additionally, form III DNA (presumably arising from random, proximal single strand cuts) was observed for the photolysis of **3** and **6** at 45 and 180 μM, respectively. These values correspond to ratios of 1.5 and 6.0 molecules/bp and are within an order of magnitude of the ratio reported for

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(13) However, this system does not fit the strictest definition of a nuclease, because it is not catalytic in cleaving agent.

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(19) Densitometry was accomplished with the NIH ImageJ software program. The amount of supercoiled DNA was multiplied by a factor of 1.22 to account for reduced ethidium bromide intercalation into the form I plasmid DNA.

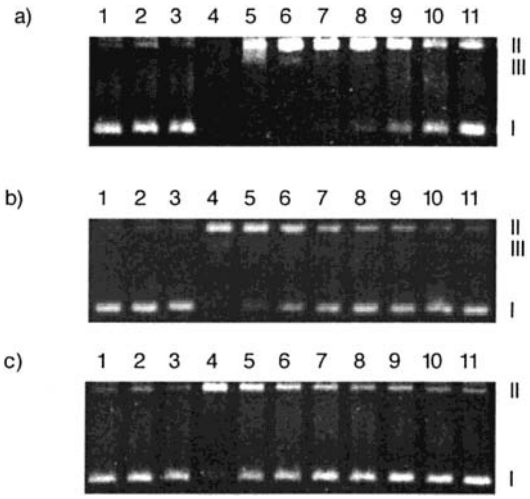


Figure 1. Photoinduced cleavage of pBR322 DNA (30 μM/bp in 10% DMSO/10 mM Tris buffer, pH 8) by hybrid compounds **3** (a), **6** (b), and **8** (c). Lane 1, DNA alone; lane 2, DNA alone, irradiated; lane 3, DNA + complex (180 μM), no irradiation; lanes 4 through 11, DNA + complex (180, 90, 45, 22.5, 11.3, 5.6, 2.8, and 1.4 μM, respectively). Reactions in lanes 4−11 were irradiated with Pyrex-filtered light from a 450-W medium-pressure mercury arc lamp for 20 min.

the photoinduced cleavage of DNA by the natural enediyne dynemicin (0.75 molecules/bp).[20] In all cases, control experiments (lanes 2 and 3) show that both light and the complex are necessary for strand scission to occur. Additionally, cleavage by **3** was inhibited in the presence of excess cysteine, a general radical trap (Figure S2, Supporting Information), a finding that is in line with our previous implication of a carbon-centered radical in the mechanistic pathway leading to strand scission by $CpW(CO)_3CH_3$.[9]

From these experiments, it is apparent that the attachment of minor groove binders increases the efficiency of single-strand cleavage 10- to 30-fold over that of the simple complex (which cleaves in a single-stranded manner at 45 μM);[9] this potency depends on the distance between the complex and recognition element. Indeed, for the hybrid molecule **3**, in which the netropsin analogue is connected directly to the Cp ring, the DNA was completely and reproducibly obliterated at 180 μM (a, lane 4).

To investigate the sequence selectivity of the DNA-binding and -cleaving behavior of one of the new compounds,

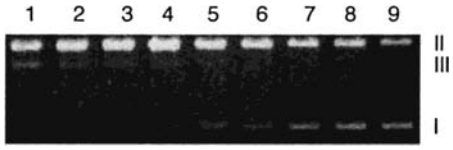


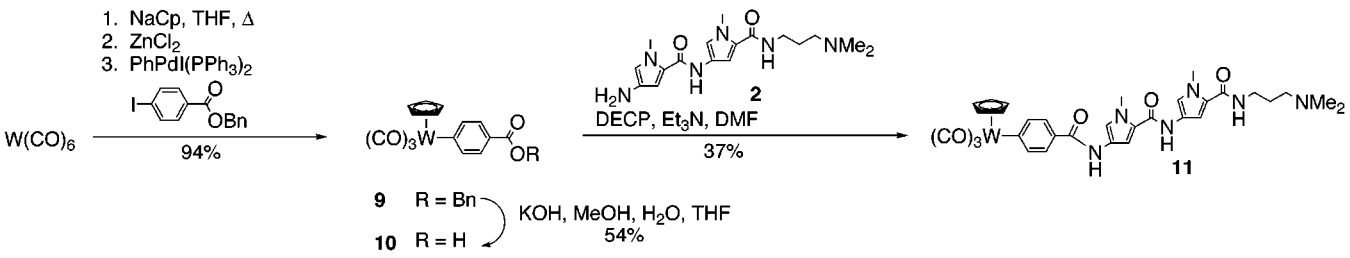
Figure 2. Photoinduced cleavage of pBR322 DNA (60 μM/bp in 10% DMSO/10 mM Tris buffer, pH 8) by hybrid compound **11**. Lanes 1 through 9, DNA + complex (360, 180, 90, 45, 22.5, 11.3, 5.6, 2.8, and 1.4 μM, respectively). Reactions in all lanes were irradiated for 20 min.

compound **3** was photolyzed in the presence of a 3′-^{32}P end-labeled restriction fragment (Figure S1). DNA-footprinting experiments with methidiumpropyl-EDTA-iron(II), dithiothreitol, and dioxygen[21] followed by high-resolution denaturing polyacrylamide gel electrophoresis revealed the preferential recognition of AT stretches of at least four base pairs, in accord with previous investigations of the primary binding sites of netropsin and distamycin.[22] Conjugate **3** gives an apparent binding constant, K_{app}, that is approximately 10-fold lower than that of distamycin[18] (1.11×10^6 vs 10^7, respectively), as measured by ethidium bromide displacement.[23] Despite the AT selectivity observed in the binding of these agents, virtually no sequence preference was seen for strand scission upon photoactivation. The nonspecificity of strand scission is most likely the result of the combination of the lower reactivity (relative to phenyl or hydroxyl radical) of the putative active species (methyl radical) with its diffusibility.

Therefore, a functionalized metal complex, which generated a radical that remained attached to the recognition element, was prepared (Scheme 2). The benzyl ester protected phenyltungsten compound **9** was synthesized by published methodology[24] from tungsten hexacarbonyl and the protected aryl iodide.[25] A number of other protecting groups (TMSE, MOM, TBS) were investigated; however, none could be removed without destroying the organometallic species. After several sets of standard debenzylation conditions led only to hydrogenolysis of the tungsten−carbon bond in **9**, a mild saponification reaction finally produced **10**. This acid was then coupled to netropsin derivative **2** by previously described methods to give **11**.

In cleaving plasmid DNA (Figure 2), the nondiffusible radical precursor **11** was as efficient as **3**, as determined by

Scheme 2



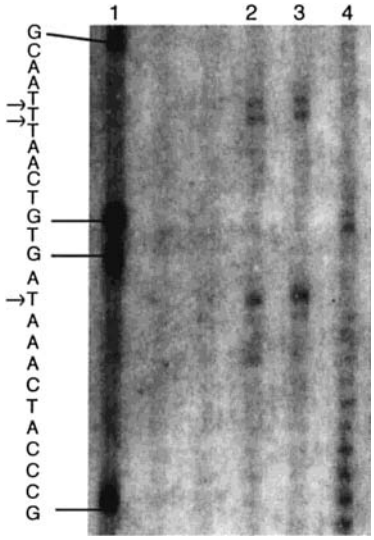


Figure 3. Autoradiogram of a 10% denaturing polyacrylamide gel for binding and photoinduced cleavage of the 3′-^{32}P-end-labeled 167 bp restriction fragment (*Eco*RI/*Rsa*I) of pBR322 DNA/calf thymus DNA (94 μM/bp in 10% DMSO/10 mM Tris buffer, pH 8) by **11**. Lane 1, Maxam−Gilbert G reaction; lanes 2 and 3, DNA + complex (90 and 45 μM, respectively), irradiated; lane 4, MPE-EDTA/Fe(II)/DTT/O_2 footprinting of DNA + complex (360 μM). Reactions in lanes 2 and 3 were irradiated for 20 min.

the lowest concentration required to effect cleavage. Single strand scission was observed at 0.047 molecules/bp (2.8 μM, lane 8); double strand cleavage, again arising from random proximal single strand cuts, was seen at 0.75 molecules/bp (180 μM, lane 4). Once more, both light and complex were required to give strand scission (Figure S3).

As with **3**, netropsin conjugate **11** bound DNA at AT-rich sequences (Figure 3, lane 4) with a $K_{app} = 1.00 \times 10^6$. However, unlike **3**, the nondiffusible radical produced by photolysis of **11** cleaved DNA in a sequence-specific manner (lanes 2 and 3). Thus, strand scission (indicated by arrows) occurred primarily at T residues within the binding site. This selectivity differs from that of a netropsin−benzotriazole conjugate, which cleaves at G residues on the 5′ end of binding sites.[11] Compound **11** also shows different specificity than a netropsin−trimethylenemethane conjugate, which cuts at varying A and T residues within AT-rich regions.[12]

In summary, the attachment of a minor groove binding moiety to $CpW(CO)_3CH_3$ results in increased strand scission of DNA; however, this process does not show significant sequence selectivity, most likely because the photogenerated methyl radical can diffuse from the binding site. In contrast, localized damage was observed for **11**, which produces a nondiffusible phenyl radical.

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Supporting Information Available: Plasmid assay gel quantitation data, autoradiogram for **3**, experimental and synthetic details, and characterization data for compounds **3**, **6**, **8**, and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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