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Selective targeting of DNA for cleavage within DNA–histone assemblies by a spermine–$[CpW(CO)_3Ph]_2$ conjugate†

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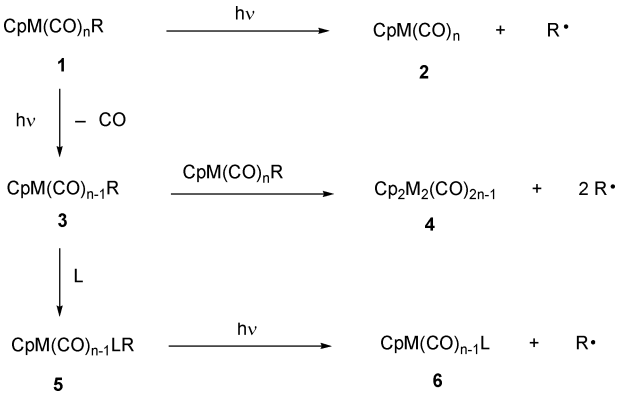
In contrast to the histone-modifying action of other complexes of the type $CpML_nR$, the compound obtained by linking the phenyl rings of two $CpW(CO)_3Ph$ moieties to the DNA-binding agent spermine selectively cleaves DNA in DNA–histone assemblies.

Due to their proven antibiotic and antitumor activity, molecules that modify nucleic acids are important tools in chemistry, biology, and medicine. Of particular note in the latter area are compounds that cause the double-strand cleavage of DNA,[1] since such lesions are more difficult than single-strand events for the cellular machinery to repair.[2] Molecules that modify oligonucleotides are also useful for the study of primary, secondary, and tertiary structure of DNA[3] and for the elucidation of the binding modes of other molecules with nucleic acids.[4] In all of these applications, the design of systems that incorporate triggering mechanisms[5] for producing the active species provides the potential ability to target specific substrates and structures. For example, we have previously employed the photolysis of organometallic species of the type $CpM(CO)_nR$ to generate carbon-centered radicals that cause single- and/or double-strand cleavage of plasmid DNA.[6]

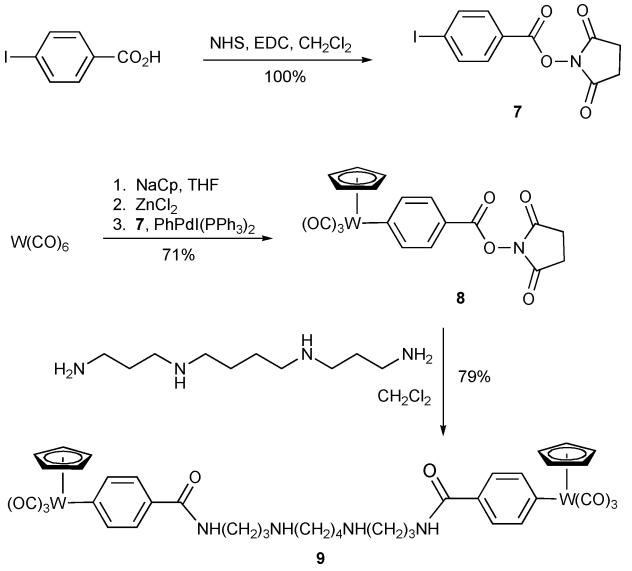
Although radicals show potential as tools for the elucidation of secondary and tertiary DNA structure, both carbon-centered and hydroxy radicals are known to modify histone proteins, causing either protein–DNA crosslinking[7] or the dissociation of protein–DNA assemblies.[8] One approach to circumvent this undesired reactivity is to attach DNA recognition elements to a triggerable radical precursor, thus producing the radical in close proximity to DNA. Therefore, we now report the synthesis and DNA-cleaving behavior of compound **3**, which is obtained by linking the phenyl rings of two $CpW(CO)_3Ph$ moieties to the DNA-binding agent spermine.[9,10] Importantly, this molecule selectively cleaves DNA in DNA–histone assemblies without giving biomolecular dissociation.

Because spermine is fully protonated at physiological pH, it is cationic and binds to anionic DNA *via* electrostatic forces;[11] and this interaction is at most only slightly sequence-selective,[12] although its exact manner is unclear. Compound **3** was expected to bind and to cleave DNA with the same lack of specificity upon photolysis at wavelengths greater than 300 nm, conditions which are known to produce carbon-centered radicals in $CpM(CO)_nR$ complexes, the general route for which is shown (Scheme 1).[13] It is generally accepted that the primary photoprocess for complexes of the formula **1**, in which M = W or Fe and R = CH_3 or C_6H_5, involves loss of carbon monoxide (to give **3**), which may be accompanied by homolysis of the metal–methyl or metal–aryl bond to yield the metal-based radical **2** along with methyl or phenyl radical. However, radical formation may occur by multiple pathways, as has been suggested for the photolysis of $CpW(CO)_3CH_3$, the only complex whose photochemistry has been extensively studied. In this case, it has been proposed that $CpW(CO)_2CH_3$ (**3**) reacts with another molecule of starting material to produce the metal–metal bonded species **4** and two methyl radicals. Furthermore, it has been demonstrated that the 16-electron species $CpW(CO)_2CH_3$ (**3**) can coordinate a variety of ligands (*e.g.*, L = PPh_3, CH_3CN, THF, or H_2O); and when $CpW(CO)_2(PPh_3)CH_3$ (either purified or produced *in situ* during the photolysis of **1** in the presence of PPh_3) is photolyzed, methyl radicals are formed. It is such carbon-centered radicals that have been implicated as the active species leading to DNA strand scission.[6] The attachment of spermine to the phenyl ring of $CpW(CO)_3Ph$ (instead of the Cp moiety) is necessary to avoid diffusible radicals, which reduce selectivity.[14]



Scheme 1 Photochemical production of carbon-centered radicals in the photolysis of $CpM(CO)_nR$ complexes.

The synthesis of **9** was straightforward (Scheme 2), beginning with the preparation of the succinimide ester **7** from 4-iodobenzoic acid. Subsequent treatment of this activated ester



Scheme 2 Synthesis of **9**.

† Electronic supplementary information (ESI) available: Full experimental details. See http://dx.doi.org/10.1039/b504248h

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with the zinc salt of $CpW(CO)_3$ anion yielded the substituted phenyl tungsten complex **8**, which then reacted with spermine to produce the desired compound **9**.

The DNA-cleaving activity of compound **9** was assessed initially 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). Thus, after the photolysis of spermine derivative **9** through a Pyrex filter in the presence of pBR322 DNA, a small amount of a 1% SDS solution was added to the reaction mixtures, in order to reduce the aggregration of DNA that is known to occur with spermine itself.[15] Analysis by agarose gel electrophoresis (Fig. 1) showed a band corresponding to form III DNA and resulting from nonrandom double-strand cleavage[16] at compound concentrations greater than 9 μM (lanes 4–6) or 0.30 molecules bp^{-1}, a value lower than that reported for light-induced double strand cleavage of DNA by the natural enediyne dynemicin (0.75 molecules bp^{-1}).[17] Additionally, form II DNA arising from single-strand cleavage occurred at ratios as low as 0.038 molecules bp^{-1} (lane 7), representing a 39-fold improvement (per radical center) in single-strand cleaving ability over the simple complex $CpW(CO)_3CH_3$, which causes single-strand scission at 1.5 molecules bp^{-1}.[6] One control experiment demonstrated that the organometallic compound was necessary for cleavage (lane 3); however, the other (lane 2) showed that high intensity light was not required to effect strand scission. This latter observation suggested that ambient light was sufficient to activate **9**, a surprising result, based on previous work with other substituted or unsubstituted $CpM(CO)_nR$ complexes.

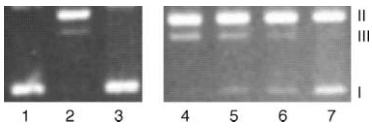

Fig. 1 Cleavage of pBR322 DNA (30 μM bp^{-1} in 10% DMSO/10 mM Tris buffer, pH 8) by **9**. Lanes 1 and 3, DNA alone; lanes 2 and 4–7, DNA and **9** (22, 14, 11, 9, and 7.2 μM, respectively). Samples in lanes 1 and 2 were incubated on the benchtop, and those in lanes 3–7 were irradiated with Pyrex-filtered light from a 450 W medium pressure mercury arc lamp for 20 minutes.

To further probe the use of ordinary room light in inducing DNA cleavage by **9**, pBR322 DNA was incubated with varying concentrations of the compound on the bench; and two control samples were prepared and incubated in the dark (Fig. 2). In these experiments, photolysis with room light produced form II DNA at concentrations above 1.1 μM of **9** (lane 9); and the linearization of DNA was observed at concentrations higher than 7.2 μM (lane 8), presumably resulting from the accumulation of single-strand damage.[16] Control experiments showed that both the presence of **9** (lane 3) and room light (lane 2) were required for strand scission.

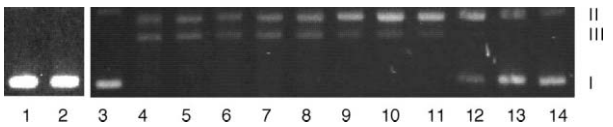

Fig. 2 Cleavage of pBR322 DNA (30 μM bp^{-1} in 10% DMSO/10 mM Tris buffer, pH 8) by **9** with activation by ambient light. Lanes 1 and 3, DNA alone; lanes 2 and 4–14, DNA and **9** (22, 22, 18, 14, 11, 9, 7.2, 5.8, 4.6, 2.3, 1.15 and 0.58 μM, respectively). The reaction mixtures in lanes 1 and 2 were mixed and allowed to sit in the dark for 20 minutes; those in all other lanes were exposed to ambient light for 20 minutes.

Thus, ambient and high intensity irradiation show similar efficiencies in causing single-strand cleavage; but in contrast to the previous experiments, none of the ambient light activated cleavage mixtures contained all three forms of DNA, indicating that true double-strand scission does not occur under these conditions. The most likely cause for this phenomenon is that double-strand events require the absorption of two photons (one for each organometallic moiety, assuming a quantum yield of 1 for the production of each radical[18]) within the period of time that **9** is associated with a particular stretch of DNA. While ethidium bromide displacement assays[19] show that **9** complexes DNA with an apparent binding constant $K_{app} = 3.4 \times 10^6\ M^{-1}$, apparently only high intensity irradiation is able to overcome the low quantum efficiency of radical formation to produce two radical centers within the residence time of **9** at a given site in DNA, leading to the concurrent damaging of both strands of the duplex.

Additional evidence for the lack of diffusion of the radicals produced in the photolysis of **9** was provided by studies in which radical scavengers were added to the reaction mixtures before photolysis (Fig. 3). When 100 or 10 equivalents (lanes 3 and 4, respectively) of cysteine, which can function as a general radical trap,[20] were present in the reaction mixture, similar amounts of cleavage were observed as in a control reaction lane (lane 2). Likewise, 2,2,6,6-tetramethyl-1-piperdinyloxyl (TEMPO), a stable nitroxide which traps carbon-centered radicals,[21] but not those on oxygen, also appeared not to suppress strand scission by **9** (lanes 7 and 8 *vs.* lane 6). If the radical species responsible for DNA cleavage were diffusible, both cysteine and TEMPO would be expected to inhibit the formation of nicked DNA, as was observed previously in the case of the unsubstituted complex $CpW(CO)_3Ph$.[6]

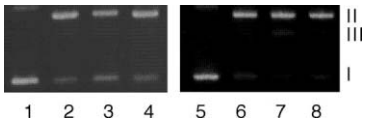

Fig. 3 Effect of radical scavengers on the cleavage of pBR322 DNA (30 μM bp^{-1} in 5% DMSO/10 mM Tris buffer, pH 8) by **9**. Lanes 1 and 5, DNA alone; lanes 2 and 6, DNA and **9** (6.0 μM); lanes 3 and 4, DNA, **9** (6.0 μM), and cysteine (600 and 60 μM, respectively) lanes 7 and 8, DNA, **9** (6.0 μM), and TEMPO (600 and 60 μM, respectively). Samples in lanes 1 and 5 were incubated on the benchtop, and those in lanes 2–4 and 6–8 were irradiated with Pyrex-filtered light from a 450 W medium-pressure mercury arc lamp for 20 minutes.

After the demonstration of the basic DNA-cleaving behavior of **9**, its capacity to damage DNA selectively within DNA/H1 assemblies was investigated. Thus, **9** was irradiated in the presence of the supramolecular complex of pUC19 DNA with calf thymus histone H1, and the results were analyzed by gel electophoresis (Fig. 4). A band corresponding to the form III DNA-H1 assembly was observed at compound concentrations greater than 50 μM (lane 6) and single-strand cleavage of complexed DNA[22] occurred at concentrations of **9** greater than 3.1 μM (lane 10). Again, both the presence of **9** and high intensity light were required to achieve significant strand scission (lanes 4 and 5, respectively), although ambient light caused some DNA cleavage (lane 5). Importantly, in none of the reactions was uncomplexed DNA observed, suggesting a much higher level of DNA cleavage than histone modification.

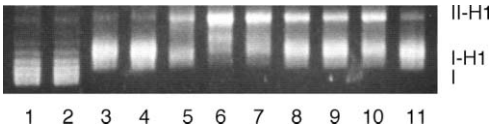

Fig. 4 Cleavage of pUC19 DNA (102 μM bp^{-1} DNA and 0.23 mg mL^{-1} histone H1 in 10% DMSO/10 mM Tris buffer, pH 8) by **9**. Lanes 1 and 2, DNA alone; lanes 3 and 4, DNA and histone; lanes 5–11, DNA, histone, and **9** (50, 50, 25, 13, 6.3, 3.1, and 1.5 μM, respectively). Mixtures in lanes 2, 4 and 6–11 were irradiated with Pyrex-filtered light from a 450 W medium pressure mercury arc lamp for 20 minutes.[22]

In conclusion, the spermine–$[CpW(CO)_3Ph]_2$ conjugate **3** has demonstrated the ability to cause both double- and single-strand breaks at low compound : DNA ratios in purified DNA. While

high intensity light is necessary for true double strand cleavage, and ambient light is sufficient for cutting only one strand. Additionally, **9** gives a high degree of nucleic acid cleavage in DNA–histone assemblies without causing dissociation of the biomolecular complex. Thus, the attachment of a suitably charged DNA recognition element to a cleaving agent provides a method to cleave DNA selectively within DNA–histone assemblies by exploiting the differences in charge between the two biomolecules.

Acknowledgements

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