Supplementary Information

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

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Identification of bands in Figure 4:

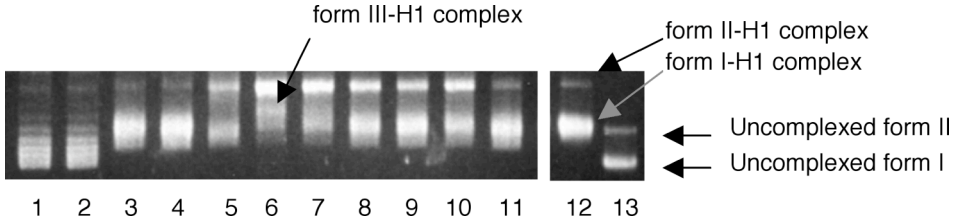


Fig. S1 Comparison of markers (lanes 12 and 13) with Figure 4 (lanes 1-11), the cleavage of pUC19 DNA (102 μM/bp DNA and 0.23 mg/mL histone H1 in 10% DMSO/10 mM Tris buffer, pH 8) by **9**. Lanes 1 and 2, DNA alone; lanes 3 and 4, DNA and histone; lanes 5-11, DNA, histone, and **9** (50, 50, 25, 13, 6.3, 3.1, and 1.5 μM, respectively). The samples in lanes 1, 4, and 5 were incubated in ambient light for 20 minutes, and the mixtures in lanes 2, 3 and 6-11 were irradiated with Pyrex-filtered light from a 450 W medium pressure mercury arc lamp for 20 minutes. The markers were prepared from another sample of commercial pUC19 containing forms I and II DNA: lane 13, DNA alone; and lane 12, DNA and histone.[1]

Quantitation of bands in Figures 2 and 4. The bands in the scanned gel were quantitated with the NIH ImageJ program[2] to give the following relative band intensities:

Figure 2

lane	Form I	Form II	Form III
3	100.0	0.0	0.0
4	0.0	16.0	17.4
5	0.0	17.9	16.2
6	0.0	21.7	15.8
7	0.0	25.2	15.1
8	0.0	34.3	10.9
9	0.0	52.3	9.9
10	0.0	68.5	6.9
11	0.0	80.1	5.1
12	27.2	61.1	0.0
13	66.1	32.9	0.0
14	83.5	15.9	0

Figure 4

lane	Free form I DNA	H1-complexed DNA		
		Form I	Form II	Form III
1	100.0	0.0	0.0	0.0
2	100.0	0.0	0.0	0.0
3	0.0	100.0	0.0	0.0
4	0.0	100.0	0.0	0.0
5	0.0	66.2	33.8	0.0
6	0.0	0.0	65.0	35.0
7	0.0	51.7	48.3	0.0
8	0.0	64.6	35.4	0.0
9	0.0	72.6	27.4	0.0
10	0.0	83.4	16.6	0.0
11	0.0	89.6	10.4	0.0

General. $PdPhI(Ph_3)_2$ was prepared according to a literature procedure.[2] The reagents 4-iodobenzoic acid, DCC, EDC, NHS, $W(CO)_6$, NaCp, $ZnCl_2$, and CH_2Cl_2 were used as purchased. THF was distilled from sodium benzophenone ketyl immediately prior to use.

Preparation of the succinimide ester of 4-iodobenzoic acid 7. 4-Iodobenzoic acid (0.2566 g, 1.035 mmol) and *N*-hydroxysuccinimide (0.1179 g, 1.024 mmol) were placed in an oven dried three-necked round bottom flask that was then fitted with a reflux condenser. The system was flushed with nitrogen and dry dichloromethane (25 mL) was added, followed by EDC (0.1973 g, 1.032 mmol). The mixture was heated at reflux for 2 hours. After the mixture cooled, it was extracted with a saturated sodium bicarbonate solution (2 x 15 mL) and with water (3 x 15 mL). The organic layer was dried with magnesium sulfate and then filtered. The solvent was removed from the filtrate by rotary evaporation, resulting in a white solid (0.3531 g, 100%); ^{1}H NMR (400 MHz, C_3D_6O) δ 2.96 (s, 4H), 7.89 (d, J = 8.4 Hz, 2H), 8.01 (d, J = 8.8 Hz, 2H); [Lit.[3] ^{1}H NMR (300, $CDCl_3$) δ2.89 (s, 4H), 7.75 (d, J = 8.24 Hz, 2H), 7.82 (d, J = 8.24 Hz, 2H)].

Preparation of succinimide ester 8. Tungsten hexacarbonyl (0.3066 g 0.8712 mmol) was placed in an oven dried three-necked round bottom flask that was fitted with a reflux condenser. The system was flushed with nitrogen, and dry THF (10 mL) was added, followed by sodium cyclopentadienide (0.52 mL of a 0.5 M solution, 1.04 mmol). The mixture was heated at reflux for 18 hours. After the reaction mixture cooled to room temperature, zinc chloride (1.3 mL of a 0.7 M solution, 0.910 mmol) was added; and the mixture was stirred for 2 hours. Succinimide ester **7** (0.1512 g, 0.4381 mmol) was placed in a separate oven dried three-necked round bottom flask, which was fitted with a dry addition funnel containing $PdPhI(PPh_3)_2$ (0.0278 g, 0.0333 mmol). The flask was flushed with nitrogen, and dry THF (10 mL) was added. The solution of $CpW(CO)_3ZnCl$ was transferred via cannula to the flask contining **1**, and the Pd catalyst was immediately added to the reaction. The mixture was stirred for 2.5 hours, after which the solvent was evaporated. Purification was achieved by column chromatography on silica with an ethyl acetate:hexane solution (2:3) as the eluent. This gave a yellow solid (0.2415 g, 71%); ^{1}H NMR (400 MHz, C_3D_6O) δ 2.93 (s, 4H), 5.91 (s, 5H), 7.62 (d, J = 8.8 Hz, 2H), 7.95 (d, J = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, acetone-d_6) δ 26.4, 94.6, 121.6, 128.388, 146.1, 148.0, 163.6, 170.8,

220.7, 228.5; IR (neat) 2023 WC≡O), 1911 (WC≡O), 1764 (C=O), 1737 (C=O), 1579 (asymmetric N-O) 1363 (symmetric N-O) cm^{-1}; ESI MS *m/z* (rel intensity) 554 (18, $^{186}W\text{-}MH^+$), 552 (17, $^{184}W\text{-}MH^+$), 551 (9, $^{183}W\text{-}MH^+$), 550 (15, $^{182}W\text{-}MH^+$).

Preparation of spermine derivative 9. Succinimide ester **8** (0.1382 g, 0.2508 mmol) was placed in an oven dried two-necked round bottom flask fitted with a reflux condenser, and the system was then flushed with nitrogen. Dry dichloromethane (25 mL) was added, followed by spermine (0.0253 g, 0.1250 mmol). The mixture was heated at reflux for 18 hours. After the mixture cooled it was extracted with a saturated sodium bicarbonate solution (3 x 15 mL). The organic layer was dried with sodium sulfate and then filtered, and the solvent was evaporated from the filtrate. The residue was dissolved in a minimal amount of dichloromethane and the product was precipitated by the addition of hexanes. After the mixture was filtered, the product was washed from the filter using dichloromethane. The solvent was evaporated, resulting in a yellow solid (0.1064 g, 79%); ^{1}H NMR (400 MHz, CD_3OD) δ 1.58 (br s, 4H), 1.81 (m, 4H), 2.63 (br s, 4H), 2.68 (t, *J* = 6.8 Hz, 4H), 3.42 (t, *J* = 6.8 Hz, 4H), 5.70 (s, 10H), 7.39 (d, *J* = 8.4 Hz, 4H), 7.77 (d, *J* = 8.4 Hz, 4H); ^{13}C NMR (100 MHz, acetone-d_6) δ 28.3, 30.7, 39.0, 48.1, 50.1, 94.3, 126.9, 131.8, 134.1, 147.4, 168.0, 220.7, 229.6; IR (neat) 2012 WC≡O), 1903 (WC≡O), 1629 (C=O), 1540 (N-H) cm^{-1}; FAB HRMS Calcd for $C_{40}H_{42}N_4O_8W_2Li$ ($M+Li^+$): *m/z*= 1081.2182. Found: 1081.2170.

DNA cleavage studies-general. The plasmid DNA, pBR 322 or pUC19 (2686 bp), was purchased from New England Biolabs. Histone H1 (Sigma designation: IIIS) was purchased from Sigma. Purified, deionized water was obtained by filtration with a four cartridge Barnstead E-Pure apparatus and was used for all aqueous reactions and dilutions. High strength analytical grade agarose was purchased from Bio-Rad. Gel electrophoresis was performed with agarose gels and 90 mM TBE buffer in a Gibco BRL Horizon 20:25 electrophoresis apparatus. The concentrated loading buffer for agarose gels consisted of 35% (w/v) sucrose solution containing 0.2% bromophenol and 0.2% xylene cyanol FF.

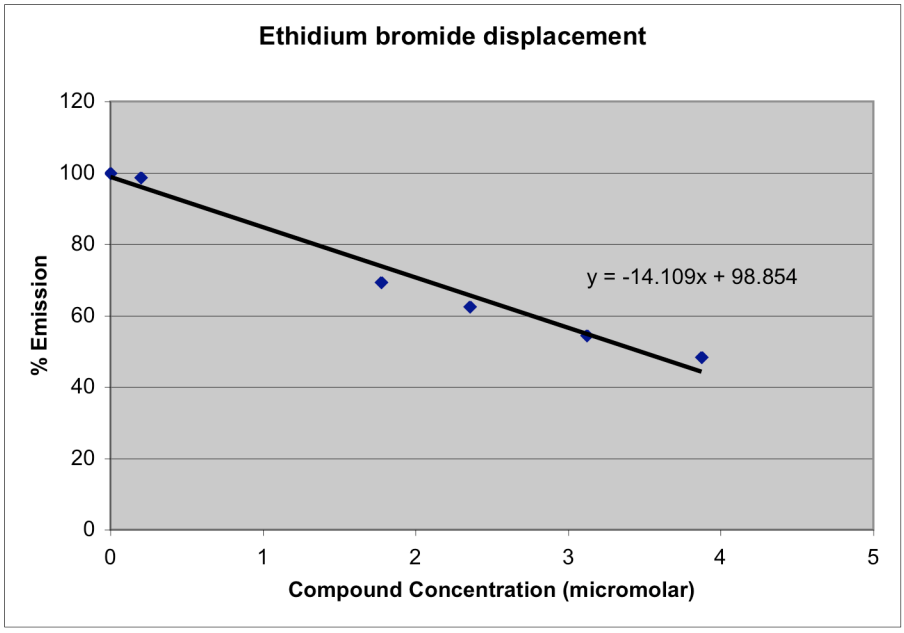
DNA cleavage reaction mixtures. The spermine derivative was dissolved into DMSO and serial dilutions were made to achieve the desired concentrations. To 18.0 μL portions of the DNA solution (33.3 μM/bp in plastic microcentrifuge tubes was added 2.0 μL of the appropriate concentration of the spermine derivative solution. The tubes were then either incubated at room temperature on the benchtop in ambient light or strapped to a water-jacketed reaction vessel with a Pyrex filter and irradiated for 20 minutes with light from a 450 W medium pressure mercury arc lamp. True dark controls were prepared, incubated, and loaded onto the gel in a darkened room by red light. After irradiation/incubation, 1 μL of 1% SDS solution and 5 μL of loading buffer were added; and the samples were loaded on 1.0 % agarose gel and electrophoresed for 12 hours at 30 V. The gels were stained in a dilute solution of ethidium bromide (~0.5 μg/mL) for a minute and then destained with water for 15 minutes. The DNA was visualized with UV light and photographed using a Polaroid DS34 camera with black and white Polaroid 667 film.

Radical Scavenging Studies. The DNA and spermine derivative solutions were made as described above, and the appropriate amounts of cysteine and TEMPO were dissolved in H_2O. The reaction mixtures were prepared by combining 1.0 μl of the solution of **9**, 1.0 μl of the cysteine or TEMPO solution (or H_2O), and 18.0 μl of the DNA solution.

DNA cleavage within DNA-histone assemblies. DNA and histone concentrations in all reactions were 0.102 μM/bp and 0.23 μg/μL, respectively. The spermine derivative was dissolved into DMSO at a concentration of 1 mM and serial dilutions were made to 0.015 mM. Reaction mixtures were prepared by adding 0.5 μL of the appropriate concentration of the spermine derivative solution to 4.5 μL of the free DNA solution or the histone/DNA assembly solution in plastic microcentrifuge tubes. The tubes were strapped to a water-jacketed reaction vessel and irradiated for 20 minutes with light from a 450 W medium pressure mercury arc lamp. After irradiation, 1 μL of loading buffer was added and then the samples were loaded on 1.5 % agarose gel and electrophoresed for 4.5 hours at 70 V. The gels were stained, visualized, and photographed as described above.

Ethidium bromide displacement assays. Fluorescence intensity was determined using a Turner Model 430 Spectrofluorometer with a Xenon lamp at an excitation wavelength of 540 nm and emission wavelength of 590 nm. Ultrapure ethidium bromide was purchased from Gibco BRL and was dissolved in 20 mM tris-HCl reaction buffer (pH 8) . Poly[d(AT)•(d(AT)] (ε_{260} = 6600 $M^{-1}cm^{-1}bp^{-1}$) was purchased from Sigma and dissolved in 20 mM tris HCl, 100 mM NaCl (pH 8) buffer. The exact DNA concentration was determined using a Cary 50 UV spectrophotometer.

To a 3 mL optical glass cell of 10 mm pathlength was added 36.8 μL of poly[d(AT)•(d(AT)] solution (0.325 mM), 30.0 μL of ethidium bromide solution (125 μM), and 2.93 mL of tris-HCl reaction buffer (pH 8). The fluorescence of this solution (4.0 μM in bp DNA, 1.26 μM ethidium bromide) was determined and then aliquots (5 – 20 μL) of compound **9** (180 μM in DMSO) were added. The fluorescence intensity was recorded after the addition of each aliquot. The addition of the tungsten solution was repeated until the fluorescence intensity decreased to approximately 20% of its original value. A plot relating % fluorescence intensity to concentration of tungsten compound was constructed, and the apparent binding constant (K_{app}) was calculated from the following equation: $K_{EtBr}[EtBr] = K_{app}[\mathbf{9}]$, where [**9**] is the concentration of **9** at 50% decrease in fluorescence and $K_{EtBr} = 9.5 \times 10^6\ M^{-1}$. The following graph gave $K_{app} = 3.4 \times 10^6\ M^{-1}$.



1. We gratefully acknowledge Trey Maddox for running the gel showing the markers.
2. Densitometry was accomplished with the NIH ImageJ software program, available at http://rsb.info.nih.gov/ij/download.html. The amount of supercoiled DNA was *not* multiplied by a factor of 1.22 (in the experiments involving histone H1 only) to account for reduced ethidium bromide intercalation into the form I plasmid DNA, because the extent to which H1 binding reduces ethidium bromide intercalation has not been determined.
3. Fitton, P.; Rick, E. A. *J. Organometal. Chem.* **1971**, *28*, 287-291.
4. Khalaj, A.; Beiki, D.; Rafiee, H.; Najafi, R. *J. Labelled Cpd. Radiopharm.* **2001**, *44*, 235-240.