Supplementary Material

Organometallic Photonucleases: Synthesis and DNA-Cleavage Studies of Cyclopentadienyl Metal-Substituted Dendrimers Designed to Increase Double-Strand Scission

Allison L. Hurley and Debra L. Mohler*

Department of Chemistry, Emory University, 1515 Pierce Drive, Atlanta GA 30322

Experimental Details:

General. Spermine (**1**) and DAB-Am-X (X=4, 8, and 16) dendrimers were purchased from Aldrich and used without further purification. [η^5-((Succinimidooxy)carbonyl)cyclopentadienyl]methyltricarbonyltungsten (**2**) was prepared according to literature procedure. Anhydrous methylene chloride was purchased from Aldrich and used without further purification. Triethylamine was distilled from calcium hydride prior to use. NMR spectra were obtained with a Varian FT-NMR spectrometer operating at either 300 or 400 MHz.

Substituted spermine derivative 3. Spermine (0.010 g, 0.051 mmol) was dissolved in dry CH_2Cl_2 (4 mL) and the solution was cooled to -40 ^oC. Tungsten complex **2** (0.050 g, 0.102 mmol) was dissolved in dry CH_2Cl_2 and was added via cannula at -40 ^oC to the spermine solution. The reaction mixture was allowed to warm slowly to 25 ^oC over 3 hours. After stirring for one additional hour at room temperature, the reaction mixture was washed with saturated aqueous $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried with anhydrous Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified by precipitation from chloroform/hexane to give a yellow solid (0.028 g, 0.027 mmol): 58% yield; IR (NaCl) 2012, 1908, 1652, 1540 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 5.98 (t, *J* = 2 Hz, 4H), 5.67 (t, *J* = 2 Hz, 4H), 3.39 (t, *J* = 6.4 Hz, 4H), 2.83 (t, *J* = 6.8 Hz, 4H), 2.80 (t, *J* = 6.4 Hz, 4H), 1.85 (m, 4H), 1.71 (t, *J* = 6.4 Hz, 4H), 0.46 (s, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 229.5, 216.9, 165.9, 101.5, 95.0, 93.1, 49.5, 47.0, 37.9, 29.5, 27.1, -32.2; FAB HRMS *m/z* calcd. for MH^+: 951.1787. Found 951.1777.

General Procedure for preparation of $CpW(CO)_3CH_3$-substituted DAB-Am-X dendrimers. The appropriate dendrimer was placed in a two-neck round bottom flask and the system was thoroughly purged with argon. Dry CH_2Cl_2 (8 mL) was added followed by dry Et_3N (0.025 mL). Succinimide ester **2** was added to the dendrimer solution while argon was bubbled through the solution. After 16 hours of stirring at room temperature under an argon atmosphere, the reacton mixture was washed with saturated aqueous $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried with anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by precipitation from chloroform/hexane or acetone/hexane.

DAB-Am-4-[CONHCpW(CO)$_3$CH$_3$]$_4$ (5). The general procedure was employed using dendrimer **4** (0.012 g, 0.038 mmol) and tungsten complex **2** (0.075 g, 0.153 mmol). The crude product was purified by precipitation from chloroform/hexane to give a yellow solid (0.044 g, 0.024 mmol): 64% yield; IR (NaCl) 2014, 1917, 1636, 1551 cm^{-1}; ^1H NMR (400 MHz, acetone- d_6): δ 8.02 (br t, 4H), 6.14 (t, *J* = 2 Hz, 8H), 5.71 (t, *J* = 2 Hz, 8H), 3.40 (br q, 8H), 2.59 (br m, 12H), 1.78 (br m, 8H), 1.56 (br s, 4H), 0.47 (s, 12H); ^{13}C NMR (100 MHz, acetone-d_6): δ 230.0, 217.2, 163.2, 103.4, 93.8, 93.2, 54.4, 52.2, 38.7, 27.7, 25.2, -31.9; FAB HRMS *m/z* calcd. for MH^+: 1813.2495. Found 1813.2490.

DAB-Am-8-[CONHCpW(CO)$_3$CH$_3$]$_8$ (6). The DAB-Am-8 dendrimer (0.015 g, 0.019 mmol) and the succinimide ester **2** (0.75g, 0.153 mmol) were combined according to the general procedure. The crude product was purified by precipitation from acetone/hexane to give a yellow solid (0.050 g, 0.013 mmol): 70% yield; IR (NaCl) 2013, 1909, 1653, 1636, 1541 cm^{-1}; ^1H NMR (400 MHz, acetone-d_6): δ 8.17 (br t, 8H), 6.21 (t, *J* = 2 Hz, 16H), 5.71 (t, *J* = 2 Hz, 16H), 3.41 (br t, 16H), 2.40–2.82 (br m, 36H), 1.74 (br m, 28H), 0.49 (s, 24H); ^{13}C NMR (100 MHz, acetone-d_6): δ 230.0, 217.3, 163.3, 103.4, 93.9, 93.3, 52.8, 52.4, 38.8, 28.3, -31.7; ESI-MS *m/z* (rel. intensity) 3765.4 (100, M^+), 1883.6 (65, MH^{2+}), 1256.2 (47, MH^{3+}).

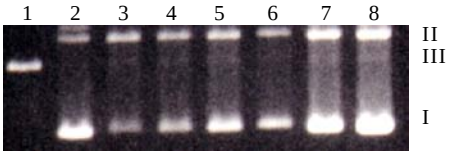
DAB-Am-16-[CONHCpW(CO)$_3$CH$_3$]$_{16}$ (7). Following the general procedure, DAB-Am-16 (0.016g, 0.0096 mmol) and tungsten complex **2** (0.075g, 0.153 mmol) were combined. The crude product was purified by precipitation from acetone/hexane to give a yellow solid (0.046g, 0.006 mmol): 63% yield; IR (NaCl) 2014, 1915, 1640, 1549 cm^{-1}; ^{1}H NMR (400 MHz, acetone-d$_6$): δ 8.24 (br s, 16H), 6.22 (br s, 32H), 5.71 (br s, 32H), 3.41 (br s, 32H), 2.36-3.12 (br m, 84H), 1.74 (br s, 60H), 0.49 (s, 48H); ^{13}C NMR (100 MHz, acetone-d$_6$): δ 230.0, 217.3, 163.4, 103.4, 93.9, 93.3, 53.0, 52.4, 39.0, 28.4, -31.6.

DNA Cleavage Studies–General. Purified, deionized water was obtained by filtration with a four cartridge Barnstead E-Pure apparatus and was used for all aqueous reactions and dilutions. Plasmid pBR322 DNA (4361 bp) was obtained from New England Biolabs. High Strength Analytical Grade Agarose was purchased from Bio-Rad. Gel electrophoresis was carried out with 1% 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 0.20% bromophenol and 0.20% xylene cyanol FF.

Plasmid relaxation assays. A DMSO solution was made of the compound of interest and serial dilutions were made. The appropriate DMSO solution was added to a 1.5 mL plastic centrifuge tube containing 9 times the volume of a solution containing 66.6 μM/bp DNA (pBR322) in 20 mM tris-HCl reaction buffer pH 8 (final concentration = 60.0 μM/bp). The tubes were then strapped to the outside of a water-jacketed reaction vessel for a Hanovia photolysis apparatus with a Pyrex filter and irradiated with light from a 450 W medium pressure mercury arc lamp for 20 minutes. After the irradiation and precipitation of the DNA (see below), 10 μL of the tris-HCl reaction buffer and 5 μL of loading buffer were added to each tube and the contents of the tube were loaded onto a 1% agarose gel and electrophoresed for 12 h at 30 V. The gel was then stained in a diltue solution of ethidium bromide (~0.5 μg/mL) for 10 minutes and then destained with water. The DNA was visualized with UV light and photographed using a Polaroid DS34 camera with black and white Polaroid 667 film.

Precipitation methods:
Method A: Irradiated samples were diluted 3:4 with an aqueous solution of NaOAc (3 M), $MgCl_2$ (0.1 M), and EDTA (0.25 M), and the resulting solution was diluted 1:3 with absolute EtOH. After cooling at -20 °C for one hour, the precipitate was isolated by centrifugation (12,000 rpm) at 4 °C for 20 min. The supernatant was removed, and 5 μL NaOAc (3 M, pH 5) and 150 μL absolute EtOH were added. The mixture was cooled at -20 °C for 4 h after which the precipitate was again isolated by centrifugation and removal of the supernatant.
Method B: Irradiated samples were diluted 2:3 with 1% SDS and incubated at room temperature for 1 h. After dilution (1:11) with aqueous NaOAc (3M) and addition of two times the total volume of absolute EtOH, the mixture was chilled at -20 °C for 30 min and then on dry ice for 20 min. After centrifugation at 4 °C and 12,000 rpm for 20 min, the supernatant was removed.

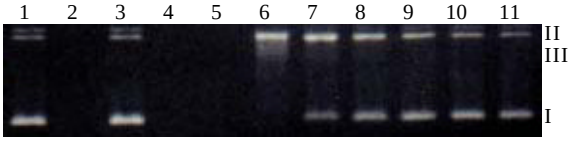
Photolysis of spermine derivative 3 in the presence of pBR322 DNA



lane	1*	2	3	4	5	6	7	8
Concentration of **3** (μM)	0	0	15	12.5	10.0	7.5	5.0	2.5
Molecules/bp	0	0	0.25	0.21	0.17	0.13	0.083	0.042
Equivalents CpW(CO)$_3$CH$_3$	0	0	0.50	0.42	0.34	0.26	0.166	0.084
Irradiation time (min)	0	0	20	20	20	20	20	20

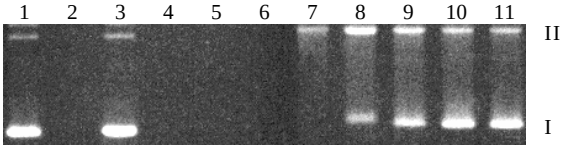
*form III marker

Photolysis of spermine derivative 3 and pBR322 DNA (without DNA precipitation)



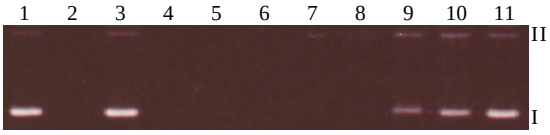
lane	1	2	3	4	5	6	7	8	9	10	11
Concentration of **3** (μM)	0	166	0	166	83	42	21	10	5.2	2.6	1.3
Molecules/bp	0	2.8	0	2.8	1.4	0.69	0.35	0.17	0.09	0.04	0.02
Equivalents $CpW(CO)_3CH_3$	0	5.6	0	5.6	2.8	1.4	0.69	0.35	0.17	0.09	0.04
Irradiation time (min)	0	0	20	20	20	20	20	20	20	20	20

Photolysis of DAB-Am-8-$[CONHCpW(CO)_3CH_3]_8$ (6) in the presence of pBR322 DNA.



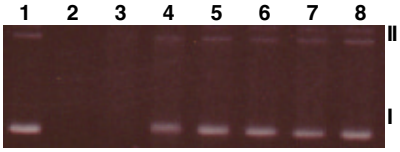
lane	1	2	3	4	5	6	7	8	9	10	11
Concentration of **6** (μM)	0	166	0	166	83	42	21	10	5.2	2.6	1.3
Molecules/bp	0	2.8	0	2.8	1.4	0.69	0.35	0.17	0.09	0.04	0.02
Equivalents $CpW(CO)_3CH_3$	0	22.4	0	22.4	11.2	5.6	2.8	1.4	0.69	0.35	0.17
Irradiation time (min)	0	0	20	20	20	20	20	20	20	20	20

Photolysis of DAB-Am-16-$[CONHCpW(CO)_3CH_3]_{16}$ (7) in the presence of pBR322 DNA.



lane	1	2	3	4	5	6	7	8	9	10	11
Concentration of **7** (μM)	0	166	0	166	83	42	21	10	5.2	2.6	1.3
Molecules/bp	0	2.8	0	2.8	1.4	0.69	0.35	0.17	0.09	0.04	0.02
Equivalents $CpW(CO)_3CH_3$	0	44.8	0	44.8	22.4	11.2	5.6	2.8	1.4	0.69	0.35
Irradiation time (min)	0	0	20	20	20	20	20	20	20	20	20

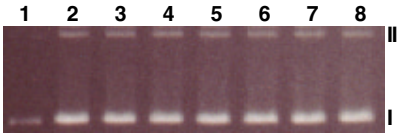
Treatment of pBR322 DNA with spermine derivative 3 without photolysis.



lane	1	2	3	4	5	6	7	8
Concentration of **3** (μM)	0	166	83	42	21	10	5.2	2.6
Molecules/bp	0	2.8	1.4	0.69	0.35	0.17	0.09	0.04

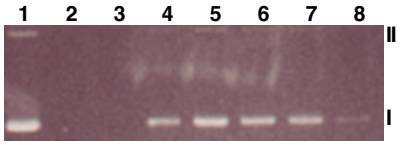
Equivalents $CpW(CO)_3CH_3$	0	5.6	2.8	1.4	0.69	.035	.017	0.09

Treatment of pBR322 DNA with DAB-Am-4-[CONHCpW(CO)$_3$CH$_3$]$_4$ (5) without photolysis.



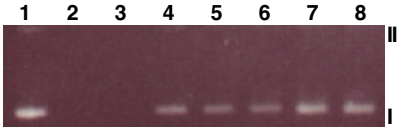
lane	1	2	3	4	5	6	7	8
Concentration of **5** (μM)	0	166	83	42	21	10	5.2	2.6
Molecules/bp	0	2.8	1.4	0.69	0.35	0.17	0.09	0.04
Equivalents $CpW(CO)_3CH_3$	0	11.2	5.6	2.8	1.4	0.69	0.35	0.17

Treatment of pBR322 DNA with DAB-Am-8-[CONHCpW(CO)$_3$CH$_3$]$_8$ (6) without photolysis.



lane	1	2	3	4	5	6	7	8
Concentration of **6** (μM)	0	166	83	42	21	10	5.2	2.6
Molecules/bp	0	2.8	1.4	0.69	0.35	0.17	0.09	0.04
Equivalents $CpW(CO)_3CH_3$	0	22.4	11.2	5.6	2.8	1.4	0.69	0.35

Treatment of pBR322 DNA with DAB-Am-16-[CONHCpW(CO)$_3$CH$_3$]$_{16}$ (7) without photolysis.



lane	1	2	3	4	5	6	7	8
Concentration of **7** (μM)	0	166	83	42	21	10	5.2	2.6
Molecules/bp	0	2.8	1.4	0.69	0.35	0.17	0.09	0.04
Equivalents $CpW(CO)_3CH_3$	0	44.8	22.4	11.2	5.6	2.8	1.4	0.69