Supplementary Information for:

Synthesis and DNA Cleavage Activity of A Bifunctional Intercalator-Linked Copper(II) Macrocycle

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Experimental Details

Reagents

Type III-A agarose, and the N-[2-hydroxyethyl]piperazine-N'[2-ethanesulfonic acid] (HEPES), were purchased from Sigma Chemical Co. Water was purified by passage through a Millipore purification system to a resistance of 18 MΩ and sterilized by autoclave. All other chemicals were purchased from Aldrich and used without further purification.

Chemicals

Acridine orange was purchased from Aldrich and was purified according to the literature procedure. All other chemicals from Aldrich were used without further purification. Acetonitrile were distilled from CaH_2. HBr was distilled over red phosphorous prior to use.

Instrumentation and Analytical Methods

For pH determinations an Orion Research digital ion analyzer model 611 equipped with a Ross semimicro temperature compensation electrode was utilized and the temperature was regulated by a circulating water bath (Lauda MT). Ethidium-stained agarose gels were imaged on a Molecular Dynamics FluorImager 575 equipped with a 610 nm long pass filter. ^{1}H NMR spectra were obtained on Bruker WM-300 NMR spectrometers. DMSO-d_6 were used as solvents. ^{1}H NMR spectra were referenced with respect to DMSO-d_6. Mass spectrometry (FABMS, MALDI-MS) was performed by the University of Wisconsin-Madison, Department of Chemistry Mass Spectrometry Facility. Elemental analysis was performed by Galbraith Laboratory.

Supercoiled Plasmid DNA Preparation

The supercoiled plasmid, pBluescript II ks(-) was purchased from Stratagene Cloning Systems. The DNA was transformed into DH5α bacterial cells via electroporation, and the

transformed bacteria were cultured in medium containing ampicillin. The supercoiled DNA was harvested according to standard procedures and purified using the Plasmid Mega Kit purification system from Qiagen.

Aerobic DNA Cleavage Studies

All manipulations with ligand **1** and DNA were carried out under scrupulous exclusion of visible light in a sealed laboratory under safe light illumination. The metal complexes **2**-M were prepared by mixing equimolar amounts of metal salts ($MnBr_2$, $Co(NO_3)_2$, $CuBr_2$, or $Zn(NO_3)_2$) and ligand **1** in water. Solutions (50 μL total volume) containing 0.05 mg cm^{-3} supercoiled plasmid DNA (25 nM in supercoiled DNA or 150 μM in phosphate units) were incubated in sterile 0.5 mL microfuge tubes with varying concentrations of **2**-M at 50°C. The pH was maintained at 7.8 with 40 mM HEPES or HEPPSO. Each reaction tube was covered in aluminum foil to prevent exposure to light. Addition of the ligand to the reaction mixtures, and all sample preparation and subsequent handling were done under safe light conditions. The reactions were quenched by ethanol precipitation and if necessary, the tubes were stored at -20 °C until analyzed by agarose gel electrophoresis. All experiments were performed at least in triplicate.

Anaerobic DNA Cleavage Studies

All manipulations with ligand **1** and DNA were carried out under scrupulous exclusion of visible light in a sealed laboratory under safe light illumination. Reaction mixtures were prepared in a nitrogen-filled Plexiglas glove box in microfuge tubes with o-ring sealed caps. HEPES buffers and deoxygenated water, were prepared by vacuum filtration through 0.22 μM cellulose acetate filtration units from Corning. The buffers and deoxygenated water were stored under a nitrogen atmosphere prior to use. Solutions (50 μL total volume) containing 0.05 mg

cm^{-3} supercoiled plasmid DNA (25 nM in supercoiled DNA or 150 μM in phosphate units) were incubated in sterile 0.5 mL microfuge tubes with varying concentrations of **2**-Cu at 50 °C. The pH was maintained at 7.8 with 40 mM HEPES. The reaction tubes were transferred to an argon-filled vacuum desiccator, covered in aluminum foil and incubated in the sealed desiccator at 50 °C. The reaction mixtures were quenched by ethanol precipitation or quenched by cooling to 0 °C; bromophenol blue and xylene cyanol were added to each reaction tube and all samples were stored at -20 °C until analyzed by agarose gel electrophoresis. All experiments were performed at least in triplicate.

Product Analysis and Quantitation for DNA Cleavage Reactions

The extent of supercoiled DNA cleavage was determined via densitometric analysis of ethidium bromide-containing agarose gels. Samples of DNA were loaded under safe light conditions and the gels were run in the dark. Plasmid cleavage products were separated on 0.8% ethidium bromide-containing agarose gel in 0.5x TBE buffer at 140 volts for 2.5 hours. Fluorescence imaging and analysis with the program ImageQuaNT version 4.1 (the volume quantitation method) was used to determine the amount of DNA cleavage. Background fluorescence was determined by reference to a lane containing no DNA. To account for the decreased ability of ethidium bromide to intercalate into supercoiled DNA (form I) versus nicked DNA (form II) and linear DNA (form III) a correction factor of 1.42 was utilized. The relative amounts of the different forms of DNA were determined by dividing the fluorescence intensity of each band by the sum of fluorescence intensities for all bands in that lane. The total percent of DNA cleaved was calculated as {total % cleaved = [(% form II) + 2(% form III)]} since there must be at least two cleavage events to go from supercoiled to linear DNA.

Preparation of New Compounds

3,6-Bis(dimethylamino)-10-(6-[1,4,7-triazacyclo[5.2.1.0$^{4.10}$]decane]hexyl) acridinium iodide, (6). A portion of 1,4,7-triazacyclo[5.2.1.0$^{4.10}$]decane (**5**), (0.562 g, 4.0 mmol) was dissolved in 30 mL of dry CH_3CN. This solution was allowed to stir for 30 min under N_2. A portion of 10-(6-Bromohexyl)-3,6-bis(dimethylamino)acridinium iodide, **4,** (0.450 g, 0.808 mmol) dissolved in 20 mL of CH_3CN was added over a period of one hour through an addition funnel. The reaction mixture stirred at room temperature for 2 days. The bright orange product crystallized out upon removing ~60 % of the solvent. The solid was washed with acetonitrile and ether. (0.520 mg, 87% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.65 (s, 1H), 7.81 (d, 2H), 7.25 (d, 2H), 6.65 (s, 2H), 5.50 (s, 1H), 4.75 (m, 2H), 3.60 (m, 6H), 3.39 (s, 12H), 3.29 (s, 8H), 1.85 (m, 4H), 1.60 (m, 2H), 1.45 (m, 2H). FABMS [M] 488.3, [M+Br]$^+$ 567.2

3,6-Bis(dimethylamino)-10-(6-[1,4,7-triazacyclononane]hexyl) acridinium bromide, [AO-(CH$_2$)$_6$-([9]aneN$_3$)]Br, (1). A portion of (**6**) (0.430 g, 0.618 mmol) was dissolved in 45 mL of H_2O and heated to reflux for 3 hours. NaOH (0.247g) was added to the reaction and allowed to reflux for another 4 hours. The solution was then cooled to room temperature and extracted with 6 x 30 mL of $CHCl_3$. The combine organic layers dried over Na_2SO_4 and concentrated via rotary to afford a brownish-red oil. H_2O (5 mL) was added, followed by 20 mL of concentrated HBr. Diethyl ether was layered above, allowing the product to crystallize out at 4 °C. The product was filtered and dried to afford bright-orange power. (0.220 mg, 39% yield) ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.90 (s, 1H), 8.22 (d, 2H), 7.95 (d, 2H), 6.70 (s, 2H), 4.75 (m, 2H), 3.60 (m, 2H), 3.50 (s, 4H) 3.39 (s, 12H), 3.29 (s, 8H), 1.90 (m, 2H), 1.60 (m, 2H), 1.45 (m, 2H)**.** FABMS [M]$^+$ 477.5. Found: C 36.1%, H 5.8%, N 8.8%, Br 43.4%. Calc. for $C_{29}H_{45}N_6Br$•5HBr: C 36.2%, H 5.8%, N 8.7%, Br 41.5%.