Experimental Section.

General Information: Elemental analyses were carried out by Atlantic Microlab Inc. High-resolution mass spectral analyses were carried out by Mass Consortium, San Diego, California. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AVANCE 300DMX (300 MHz) spectrometer. Chemical shifts are given in parts per million (ppm) relative to tetramethylsilane (TMS) and coupling constants ($J$) are reported in hertz (Hz). Absorption measurements were made on a Hitachi U-2001 UV-vis spectrophotometer. Thin layer chromatography (TLC) was carried out on, either, Analtech UV254 silica gel, or Merck aluminum oxide 60 F254 plates. Flash chromatography was performed using silica-gel Kieselgel 60, 230-400 mesh, or Al$_2$O$_3$ (Brockman Activity I). Distilled, deionized water from a Milli-Q system was used for all aqueous solutions and manipulations. Other solvents were purified and dried according to standard procedures. Agarose gel electrophoresis was carried out in 40 mM Tris-acetate buffer (pH 8.0), containing 5 mM EDTA. Agarose gel loading buffer: 40 mM Tris-acetate (pH 8.0), 5 mM EDTA, 40% glycerol, 0.3% bromophenol blue.

Scheme Used for the Synthesis of Prodigiosin Analog, 3 and 4.

3: $X = NH$  
4: $X = S$
5-(5-ethyl-1H-pyrrol-2-ylmethylene)-4-methoxy-1,5-dihydropyrrol-2-one (5). To a DMSO solution (20 mL) of 4-methoxy-3-pyrrolin-2-one (1.47 g, 13.0 mmole) and 5-ethyl-1H-pyrrole-2-carboxaldehyde (0.800 g, 6.5 mmole) was added 2N NaOH (20 mL) under argon. The solution was stirred at 60 °C for 18 h and then poured into H₂O (50 mL) and extracted with CHCl₃ (3 x 20 mL). The combined organic fractions were dried (MgSO₄), filtered, and evaporated to dryness under reduced pressure. The crude material was purified by flash chromatography on silica gel eluting with 20:80 hexane:ethyl acetate to give 5 as a yellow solid: yield 1.48 g (97%); ¹H NMR (CDCl₃) δ 10.89 (bs, 1H), 10.37 (bs, 1H), 6.39 (m, 1H), 6.34 (s, 1H), 6.01 (m, 1H), 5.11 (s, 1H), 3.92 (s, 3H), 2.80 (q, J = 7.6, 2H), 1.36 (t, J = 7.6, 3H); ¹³C NMR (CDCl₃) δ 173.6, 168.4, 142.5, 126.1, 123.4, 117.8, 107.4, 103.1, 90.5, 58.6, 21.7, 14.1. Anal. Calcd. for C₁₂H₁₄N₂O₂: C, 65.56; H, 6.51; N, 12.74. Found: C, 65.68; H, 6.47; N 12.70.

2-trifluoromethanesulfonyloxy-4-methoxy-5-[(5-ethyl-2H-pyrrol-2-ylidene)methyl]-1H-pyrrole (6). Trifluoromethanesulfonic anhydride (146.9 mg, 0.521 mmole) was added over 30 min via a syringe pump to a solution of 5 (61.0 mg, 0.260 mmole) dissolved in CH₂Cl₂ (10 mL) at 0 °C under argon. Following 1 h at 0 °C, the solution was poured into 2% NaHCO₃ (100 mL) and extracted with CHCl₃ (3 x 20 mL). The combined organic extracts were dried (Na₂SO₄), filtered, and evaporated to dryness under diminished pressure. The crude material was purified by flash chromatography on silica gel eluting with 50:50 hexane:ethyl acetate to give 6 as a yellow solid: yield 89.7 mg (94%); ¹H NMR (CDCl₃) δ 10.83 (bs, 1H), 7.00 (s, 1H), 6.65 (m, 1H), 6.04 (m, 1H), 5.34 (s, 1H), 3.85 (s, 3H), 2.71 (q, J = 7.6, 2H), 1.29 (t, J = 7.6, 3H); ¹³C NMR (CDCl₃) δ 168.3, 161.4, 147.0, 147.0, 128.8, 123.5, 122.5, 121.2, 116.9, 110.0, 87.5, 21.9, 13.0. Anal. Calcd. for C₁₃H₁₃F₃N₂O₄S: C, 44.57; H, 3.74; N 8.00. Found: C, 45.00; H, 3.79; N, 7.90.
5-(5-ethyl-pyrrol-2-ylidenemethyl)-4-methoxy-1H,1’H-[2,2’] bipyrrrole (3). To a solution of 6 (67.4 mg, 0.184 mmole) in 1,4-dioxane under argon was added in series 1-BOC-pyrrole-2-boronic acid (Frontier Scientific Inc., 155.3 mg, 0.736 mmole), potassium carbonate (203.4 mg, 1.47 mmole), and tetrakis(triphenylphosphine) palladium (8.2 mg, 0.0092 mmole). The resulting mixture was heated to 70 °C and allowed to stir for 6 h. The solution was then poured into H₂O (100 mL) and extracted with CHCl₃ (3 x 20 mL). The combined organic extracts were dried (Na₂SO₄), filtered, and evaporated to dryness under reduced pressure. The crude material was purified by flash chromatography on Al₂O₃ eluting with 99:1 ethyl acetate:MeOH to give 3 as a dark red solid: yield 16.2 mg (33%); UV-vis (MeOH-HCl) λ_max = 527 nm (ε = 100,824 M⁻¹cm⁻¹); ^1H NMR (CDCl₃) δ 6.81 (s, 1H), 6.65 (m, 2H), 6.46 (m, 1H), 6.10 (m, 1H), 6.00 (s, 1H), 5.84 (m, 1H), 3.90 (s, 3H), 2.20 (q, J = 7.6, 2H), 0.95 (t, J = 7.6, 3H); ES-MS (positive ionization): [M + H]^+ = 268.1. HRMS calcd for m/z [M + H]^+ 268.1450, found 268.1453.

5-(5-ethyl-pyrrol-2-ylidenemethyl)-4-methoxy-2-thiophenyl-pyrrole (4). To a solution of 6 (135.5 mg, 0.370 mmole) in 1,4-dioxane under argon was added in series thiophene-2-boronic acid (Aldrich, 189.3 mg, 1.48 mmole), potassium carbonate (408.9 mg, 2.96 mmol), and tetrakis(triphenylphosphine) palladium (16.49 mg, 0.0185 mmole). The resulting mixture was heated to 90 °C and allowed to stir for 6 h. The solution was then poured into H₂O (100 mL) and extracted with CHCl₃ (3 x 20 mL). The combined organic fractions were dried (Na₂SO₄), filtered, and concentrated under reduced pressure. The crude material was purified by flash chromatography on Al₂O₃ eluting with 99:1 ethyl acetate:MeOH to give 4 as a dark orange solid: yield 36.1 mg (34%); UV-vis (MeOH-HCl) λ_max = 512 nm (ε = 62,170 M⁻¹cm⁻¹); ^1H NMR (CDCl₃) δ 7.39 (m, 1H), 7.32 (m, 1H), 7.03 (m, 1H), 6.79 (s, 1H), 6.50 (m, 1H), 5.95 (m, 1H),
5.89 (s, 1H), 3.82 (s, 3H), 2.71 (q, J = 7.6, 2H), 1.31 (t, J = 7.6, 3H); ES-MS (positive ionization) 

**Cell Culture:** HL-60 cells, a model of human promyelocytic leukemia, were maintained in 
RPMI-1640 (Gibco BRL, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS). 
The cells were kept in a humidified atmosphere of 5% CO2-95% air at 37 °C. Cell doubling time 
was ca. 24 h under these culture conditions and the cells tested negative for mycoplasma 
contamination. Cell numbers were measured with a Coulter Counter (Coulter Electronics, Inc., 
Hialeah, FL). Clonogenicity assays were performed with cells growing in log phase.

**Soft Agar Clonogenicity Assay:** Clonogenic survival assays were used to assess the cytotoxicity 
of prodigiosin (1) and the synthetic analogs 3 and 4 in HL-60 cancer cells. Stock solutions of 1, 
3 and 4 (1 mM) were prepared in DMSO. HL-60 cells (45,000 in 1.5 mL of RPMI-1640 media 
supplemented with 10% FBS) were plated in 9.6 cm² culture dishes. Eighteen hours after 
plating, cells were exposed to various concentrations of 1 (2, 5, 10, and 15 µM), 3 (2, 5, 10, and 
20 µM), and 4 (5, 10, 20, and 50 µM). Colony formation was assessed 7-10 days later, at which 
time colonies (>50 cells) were scored using an inverted light microscope. Results were based on 
three independent experiments assayed in quadruplicate.

**Relaxation of Supercoiled Plasmid DNA by CuProd:** Reaction mixtures (20 mL total volume) 
contained either 400 ng or 800 ng of supercoiled DNA (Form I), 10 mM MOPS buffer (pH 7.4) 
and various concentrations of 1 with equimolar Cu(OAc)₂. Reaction mixtures were incubated for 
various times at 37 °C, and then quenched by the addition of 4 µL loading buffer. Samples were 
loaded onto a 1% agarose gel containing ethidium bromide (1 µg/mL). The gel was run at 110 V 
for 2 h and visualized by UV illumination.
Figure S1. Effect of NaCl on the relaxation of Form I DNA by CuProd (10 μM). Reaction mixtures (20 μL total volume) contained 400 ng of Form I DNA and were carried out at 37 °C for 30 min in 10 mM MOPS buffer pH 7.4 containing 10 vol. % CH3CN. Lane 1, DNA alone; lane 2, + 10 μM 1, lane 3 + 10 μM Cu(II); lane 4, + Cu•prodigiosin; lanes 5-8, + 12.5, 25, 50 and 100 mM NaCl, respectively.

Figure S2. DNA composition as a function of reaction time with CuProd (30 μM). Diamonds represent fraction of Form I DNA; triangles fraction of Form II DNA; squares fraction of Form III DNA. Fractions are calculated based on the total intensity of the DNA control lane, with the nicked fractions corrected for nicked DNA present prior to reaction.
Figure S3. The number of ds-breaks (n2) as a function of CuProd concentration. Reactions were carried out as described in the caption below Figure 1. The concentration of NaCl was 25 mM/10 μM CuProd.

Figure S4. Structure-activity relationships in copper-mediated DNA cleavage by prodigiosin. Reaction mixtures (20 μL total volume) contained 400 ng of Form I DNA in 10 mM MOPS buffer, pH 7.4, 25 mM NaCl, 10 vol% CH3CN, and were incubated at 37 °C for 30 min. Lane 1, DNA alone; lane 2, + 20 μM 1; lane 3, + 20 μM 3; lane 4, + 20 μM 4; lane 5, + 20 μM Cu(OAc)2; lane 6, + 20 μM CuProd; lane 7, + 20 μM Cu(3); lane 8, + 20 μM Cu(4).