Supporting Information

Metal Cation Complexation and Activation of Reversed CPyI Analogues of CC-1065 and Duocarmycin SA: Partitioning the Effects of Binding and Catalysis

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DNA Alkylation Studies. General procedures, the preparation of singly 5' end-labeled double-stranded DNA, the agent binding studies, gel electrophoresis, and autoradiography were conducted following procedures described in full detail elsewhere.[22] Eppendorf tubes containing 5' ^{32}P-end-labeled DNA (4.5 μL, w794 or w836 DNA) in 10 mM Tris buffer (pH 7.5) were treated with the agent in DMSO (0.5 μL at the specified concentration). The solution was mixed by vortexing, subjected to brief centrifugation, and incubated at the specified temperature for 30 min. If specified, the incubations were then treated with the zinc bis(acetylacetonate) in DMSO (0.5 μL at the specified concentration), mixed by vortexing, subjected to brief centrifugation, and incubated at the specified temperature for the specified time. The covalently modified DNA was separated from unbound agent by EtOH precipitation. EtOH precipitation was carried out by adding *t*RNA (1 μL of a 10 μg/μL solution), 3 M NaOAc (0.1 volume) and –20 °C EtOH (2.5 volumes). The solutions were mixed and chilled at –78 °C for 0.5 h or longer. The DNA was reduced to a pellet by centrifugation at 4 °C for 30 min. The pellet was dried in vacuum and resuspended in 5 μL TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5). The solution was warmed at 100 °C for 30 min to induce cleavage at the alkylation sites, allowed to cool to 25 °C and centrifuged. Formamide dye (5 μL) was added to the supernatent. Prior to electrophoresis, the samples were denatured by warming at 100 °C for 5 min, centrifuged, and placed in an ice bath. The supernatent was loaded directly onto the gel. Sanger dideoxynucleotide sequencing reactions were run as standards adjacent to the reaction samples. Polyacrylamide gel electrophoresis (PAGE) was run on an 8% sequencing gel under denaturing conditions (8 M urea) in TBE buffer (100 mM Tris, 100 mM boric acid, 0.2 mM Na_3EDTA) and was followed by autoradiography.

Relative Alkylation Rates. Following the procedure detailed above, Eppendorf tubes containing 5' end-labeled DNA (4.5 μL) in 10 mM Tris buffer (pH 7.5) were treated with agents in DMSO (0.5 μL), mixed, and incubated at 25 °C for 5, 15, 30 min, 1, 3, 10, or 24 h, respectively. Subsequent isolation of the alkylated DNA by EtOH precipitation, resuspension in TE buffer (5 μL, pH 7.5), thermolysis (30 min, 100 °C), subsequent PAGE, and autoradiography were conducted as detailed above. Relative rates were derived from the slopes of plots of % integrated optical density (%IOD) of the w794 (5' A<u>A</u>TTT or 5' AATT<u>A</u>) or w836 (5' AAAAAA) high affinity alkylation site cleavage bands versus time. Representative lanes are presented in Figure S3, plots of %IOD are shown in Figure S4, and relative and absolute rates are presented in Table 1 and Supporting Information Table 1.

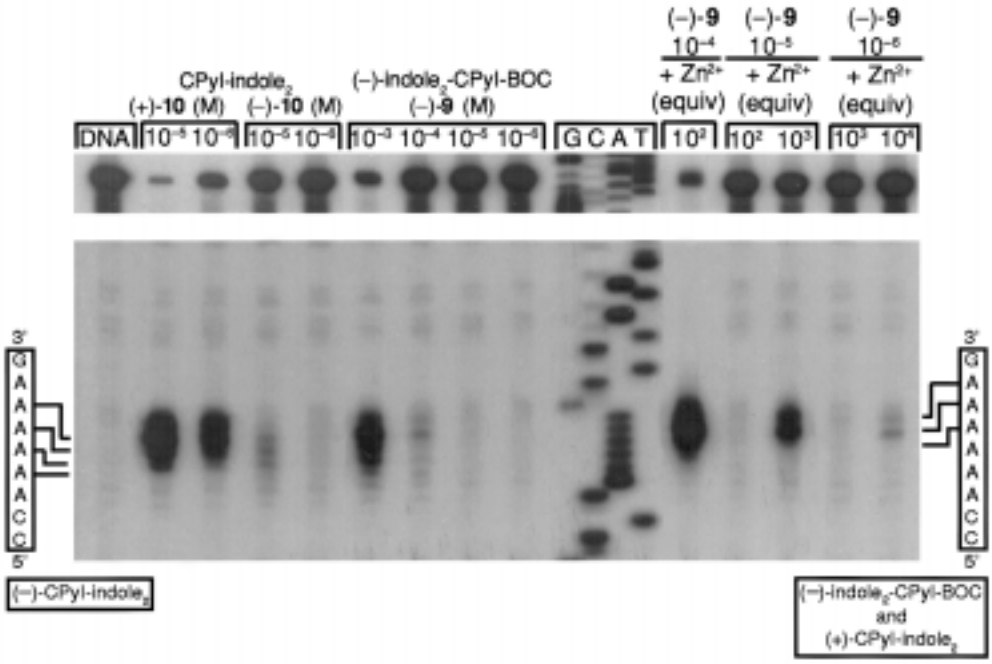


Figure S1

Figure S1. Thermally-induced strand cleavage of w836 DNA (146 bp, nucleotide nos. 5289–91); DNA–agent incubation at 25 °C for 24 h, removal of unbound agent and 30 min of thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography; lane 1, control DNA; lanes 2–3, (+)-CPyI-indole$_2$ (**10**, 1×10^{-5} and 1×10^{-6} M); lanes 4–5, (−)-CPyI-indole$_2$ (**10**, 1×10^{-5} and 1×10^{-6} M); lanes 6–9, (−)-indole$_2$-CPyI-BOC (**9**, 1×10^{-3} to 1×10^{-6} M); lanes 10–13, Sanger G, C, A and T sequencing reactions; lane 14, (−)-indole$_2$-CPyI-BOC (**9**, 1×10^{-4} M) with Zn(acac)$_2$ (100 equiv); lanes 15–16, (−)-indole$_2$-CPyI-BOC (**9**, 1×10^{-5} M) with Zn(acac)$_2$ (100 and 1000 equiv); lanes 17–18, (−)-indole$_2$-CPyI-BOC (**9**, 1×10^{-6} M) with Zn(acac)$_2$ (1000 and 10^4 equiv).

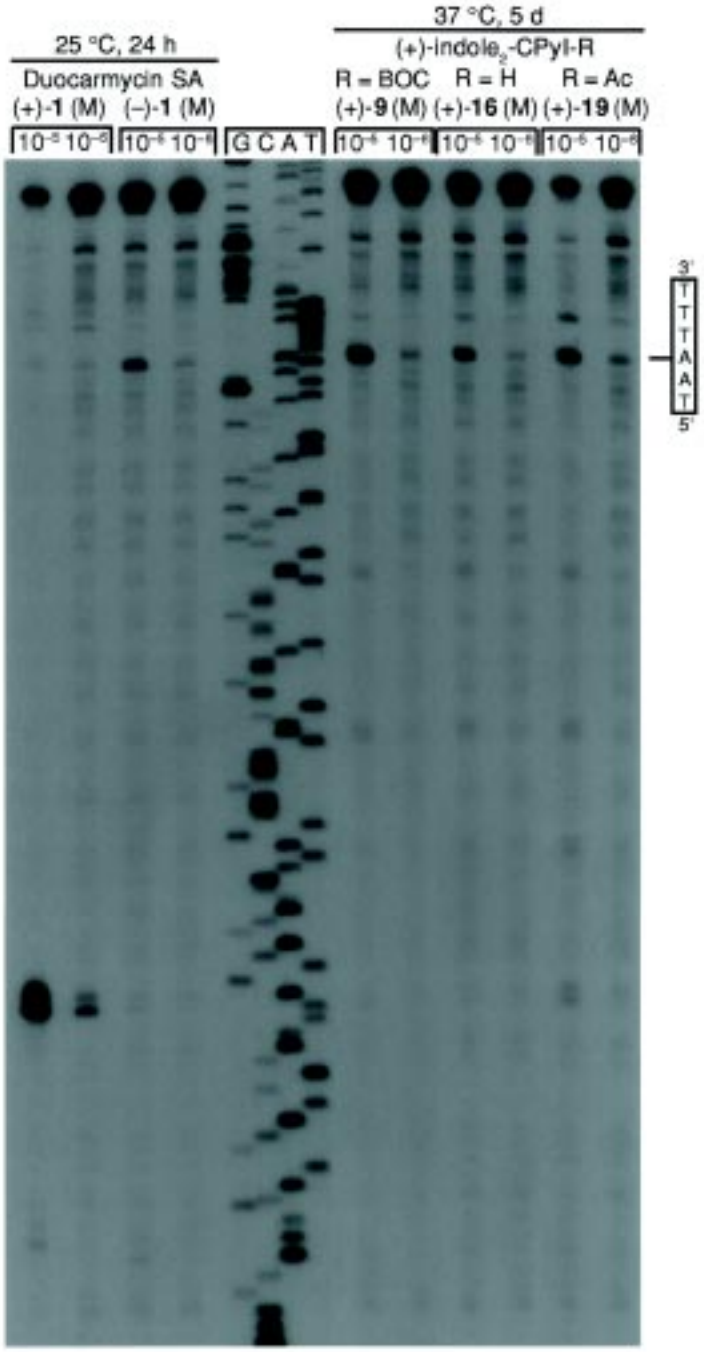


Figure S2

Figure S2. Thermally-induced strand cleavage of w794 DNA (SV40 DNA segment, 144 bp, nucleotide nos. 138–5238); DNA–agent incubation at 25 °C (24 h, duocarmycin SA) or 37 °C (5 d, indole$_2$-CPyI-R), removal of unbound agent and 30 min of thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography; lanes 1–2, (+)-duocarmycin SA (**1**, 1×10^{-5} and 1×10^{-6} M); lanes 3-4, (-)-duocarmycin SA (**1**, 1×10^{-5} and 1×10^{-6} M); lanes 5–8, Sanger G, C, A and T sequencing reactions; lanes 9–10, (+)-indole$_2$-CPyI-BOC (**9**, 1×10^{-5} and 1×10^{-6} M); lanes 11–12, (+)-indole$_2$-CPyI-H (**16**, 1×10^{-5} and 1×10^{-6} M); lanes 13–14, (+)-indole$_2$-CPyI-Ac (**19**, 1×10^{-5} and 1×10^{-6} M).

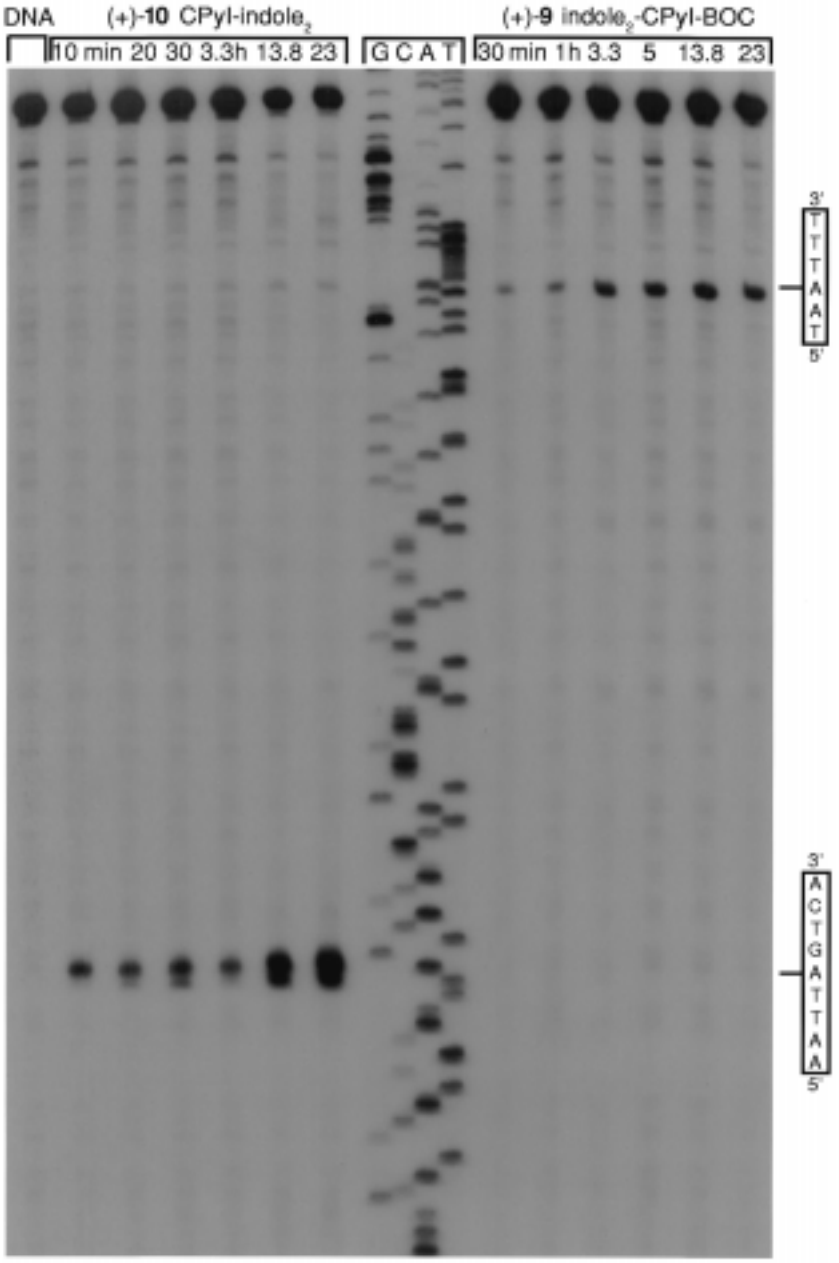
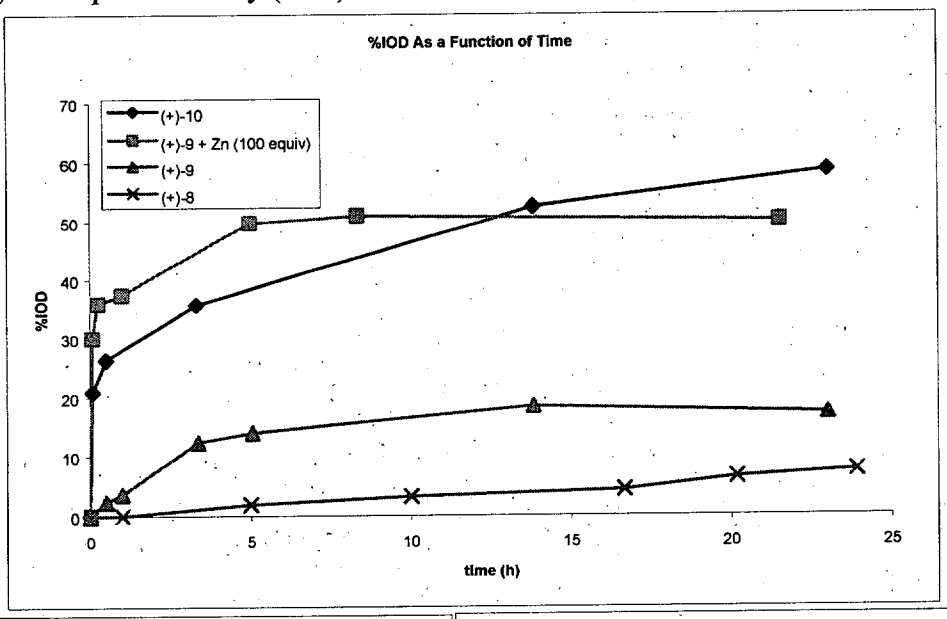


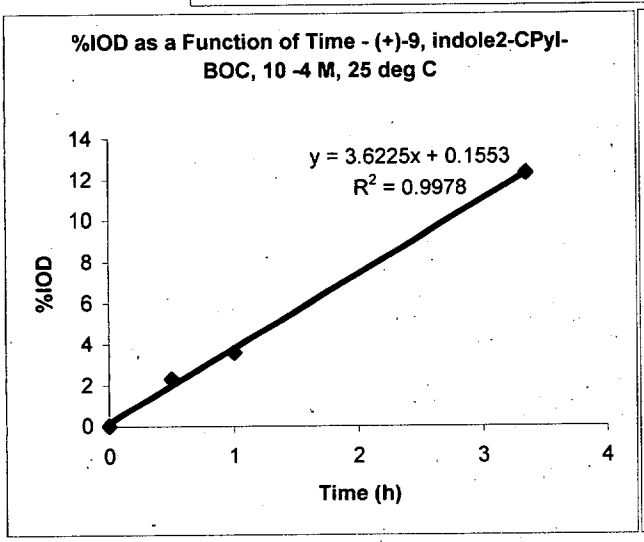
Figure S3

Figure S3. Representative lanes from a time course study (10 min to 23 h, 25 °C, 1×10^{-4} M agent) of the w794 DNA alkylation by the extended and reversed agents highlighting their relative DNA alkylation rates. Lane 1, control DNA; lanes 2–7, (+)-CPyI-indole$_2$ (**10**, 10, 20, 30 min, 3.3, 13.8, 23 h); lanes 8–11, Sanger G, C, A and T sequencing reactions; lanes 12–17, (+)-indole$_2$-CPyI-BOC (**9**, 30 min, 1, 3.3, 5, 13.8, 23 h).

Compound	Absolute Rate[a]	Relative Rate
10, (+)-CPyI-indole$_2$	879	1
9, (+)-indole$_2$-CPyI-BOC	1.42	0.0016
9, (−)-indole$_2$-CPyI-BOC	0.216	0.00024
19, (+)-indole$_2$-CPyI-Ac	3.58	0.0040
19, (−)-indole$_2$-CPyI-Ac	1.12	0.0013
16, (+)-indole$_2$-CPyI-H	0.540	0.00061
16, (−)-indole$_2$-CPyI-H	0.176	0.00020
8, (+)-*N*-BOC-CPyI	0.044	0.00005
8, (+)-*N*-BOC-CPyI + Zn	9.53	0.011
9, (+)-indole$_2$-CPyI-BOC + Zn	1220	1.4

[a] Averaged rates (h^{-1}) of DNA alkylation within w794 and its complement w836 as determined by plotting % integrated optical density (IOD) as a function of time (see below).





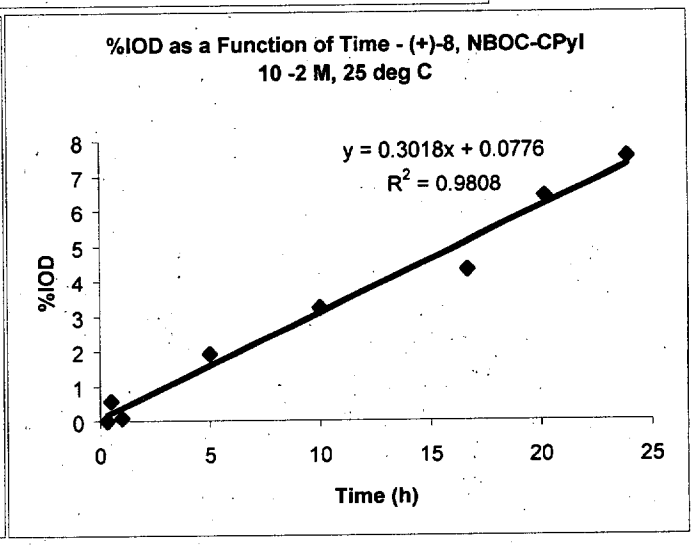


Figure S4. Representative plots of %IOD of 5' end-labeled DNA derived from the alkylation and thermally-induced cleavage at the w794 DNA high affinity alkylation site, 5'-d(AATTA)-3', for (+)-CPyI-indole$_2$ (**10**), (+)-indole$_2$-CPyI-BOC (**9**) in the absence and presence of Zn(acac)$_2$, and (+)-*N*-BOC-CPyI (**8**) as a function of time. Absolute rates were determined by taking the slope of the linear regions of these plots.