Supporting Information

DNA Alkylation Properties of Yatakemycin

Jay P. Parrish,[†] David B. Kastrinsky,[†] Scott E. Wolkenberg,[†] Yasuhiro Igarashi,[‡] and Dale L. Boger[*†]

Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA and Biotechnology Research Center, Toyama Perfectural University 5180, Kurokawa, Kosugi, Toyama 939-0398, Japan

[†]The Scripps Research Institute

[‡]Toyama Perfectural University

DNA Alkylation Studies: Selectivity and Efficiency. Eppendorf tubes containing singly ^{32}P 5′-end-labeled double-stranded DNA[12] (9 μL) in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) were treated with the agents in DMSO (1 μL at the specified concentrations). The solutions were mixed by vortexing and brief centrifugation and subsequently incubated at 4 °C or 25 °C for 24–96 h. The covalently modified DNA was separated from unbound agent by EtOH precipitation of the DNA. The EtOH precipitations were carried out by adding *t*-RNA as a carrier (1 μL, 10 μg/μL), 3 M NaOAc (0.1 volume) and –20 °C EtOH (2.5 volumes). The solutions were mixed and chilled at –78 °C in a REVCO freezer for 1 h or longer. The DNA was reduced to a pellet

by centrifugation at 4 °C for 15 min and washed with a –20 °C 70% EtOH (in TE containing 0.2 M NaCl). The pellets were dried in a Savant Speed Vac concentrator and resuspended in TE buffer (10 μL). The solutions of alkylated DNA were warmed at 100 °C for 30 min to induce cleavage at the adenine N3 alkylation sites. After brief centrifugation, formamide dye solution (5 μL) was added. Prior to electrophoresis, the samples were denatured by warming at 100 °C for 5 min, placed in an ice bath, centrifuged briefly, and the supernatant (2.8 μL) was loaded onto a gel. Sanger dideoxynucleotide sequencing reactions were run as standards adjacent to the agent treated DNA reaction samples. Polyacrylamide gel electrophoresis (PAGE) was run on an 8% sequencing gel under denaturing conditions (19:1 acrylamide: *N*,*N*-methylenebisacrylamide, 8 M urea) in TBE buffer (100 mM Tris, 100 mM boric acid, 0.2 mM Na_2EDTA). PAGE was pre-run for 30 min with formamide dye solution prior to loading the samples. Autoradiography of dried gels was carried out at –78 °C using Kodak X-Omat AR film and a Picker Spectra^{TM} intensifying screen.

Relative Rates of DNA Alkylation. Following the procedure detailed above, Eppendorf tubes containing 5′ end-labeled w836 DNA (9 μL) in TE buffer (pH 7.5) were treated with the compounds to be compared (1 μL, 10^{-5} M in DMSO). At these concentrations (10^{-4}–10^{-6} M), the rate of DNA alkylation was independent of compound concentration indicating saturated binding. The solutions were mixed and incubated at 25 °C for various increments of time (1–360 min, (e.g., for **1**) or 1, 3, 6, 12, 24, 48, and 72 h (e.g., for $CDPI_2$-DSA)). Subsequent isolation of the alkylated DNA by EtOH precipitation, resuspension in TE buffer (10 μL, pH 7.5), thermolysis (30 min, 100 °C), concurrent PAGE, and autoradiography were conducted as detailed above. The relative

rate for the alkylation at high affinity sites were derived from the slopes of the plots of percent integrated optical density (IOD) of the remaining unreacted DNA band versus time. Triplicate runs provided comparable qualitative results and ±10% absolute values.

Reversibility Studies. General procedures, the preparation of singly 5′-end-labeled DNA (w836), the experimental setup, reaction conditions (temperature = 25, 37, and 50 °C, pH = 6, 7.4, and 8.4, time = 1, 2, 4, and 8 days), gel eletrophoresis, and autoradiography were conducted according to procedures described in full detail.[8]

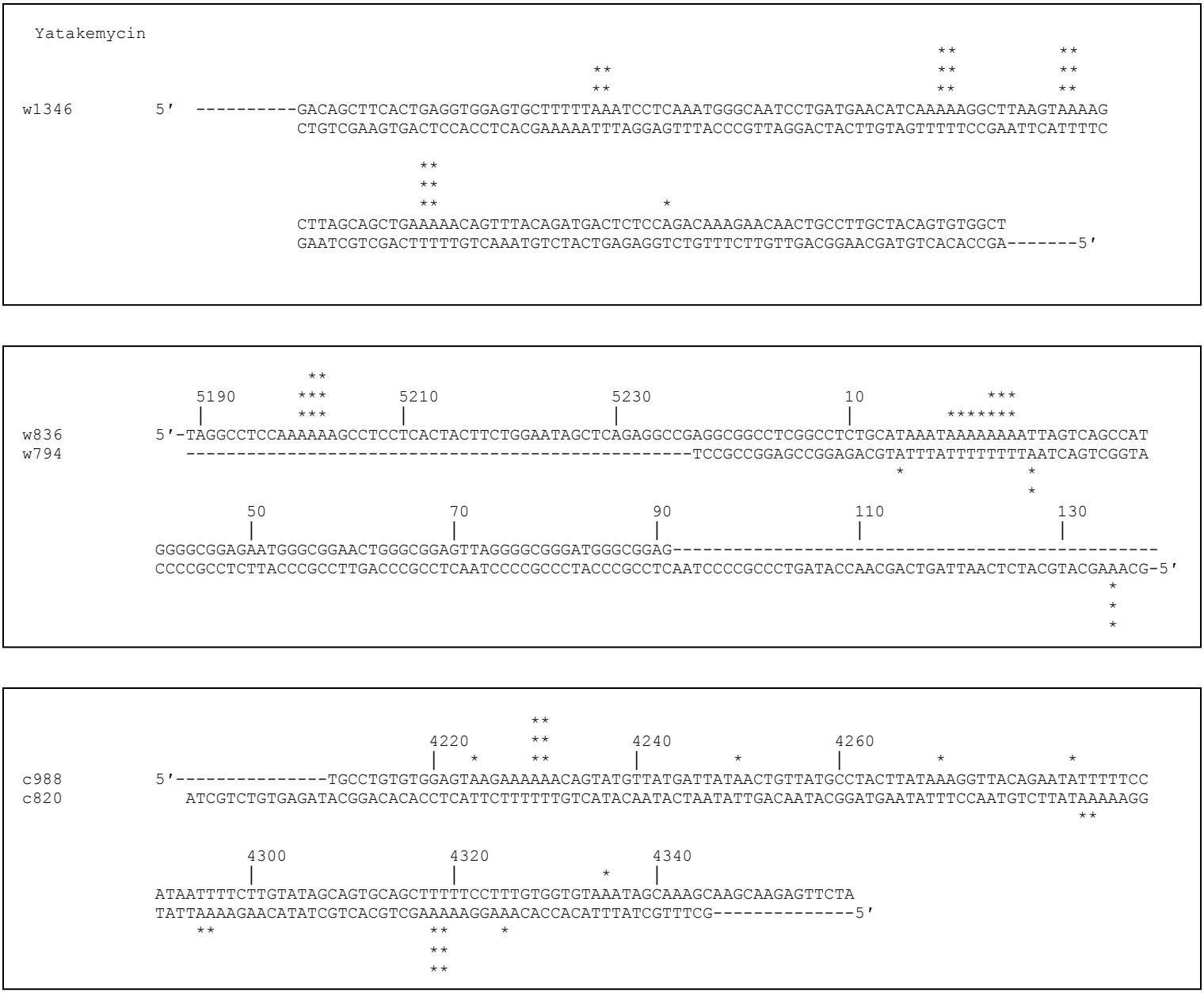

Figure S1. Alkylation sites for yatakemycin, the number of * indicate the efficiency (intensity) of alkylation at the indicated site.