

Electronic Supplementary Information (ESI) service

SITE-SPECIFIC OXIDATIVE CLEAVAGE OF DNA BY METALLOSALEN-DNA CONJUGATES

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General Procedures.

T4 Polynucleotide kinase was obtained from US Biochemical, and [γ - 32 P]-ATP (7000 Ci/mmol) was purchased from ICN. Radioactive bands in polyacrylamide gels were analyzed as described [paper ref. 7]. Tris(hydroxymethyl)amino methane (Tris) was purchased as a molecular biology reagent from Sigma. Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry was performed on a PerSeptive Biosystems, Inc. (Foster City, CA), Voyager-DETM PRO BiospectrometryTM Workstation MALDI-TOF mass spectrometer.

DNA synthesis and purification.

Unmodified DNA oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, IA) and purified by reverse-phase HPLC (RP-HPLC). Preparation of metallosalen-DNA conjugate **1** was described previously [paper ref. 7].

Guanosine DMS sequencing reactions.

Radiolabeled **2** (20 fmol) in 52.6 mM Tris pH 8.0, 1.05 mM EDTA pH 8.0 (volume of 9.5 μ L) was mixed with dimethyl sulfate (DMS, 0.5 μ L) and incubated at 4 °C for 5 min. Modified DNA was recovered by isopropanol precipitation, redissolved in 15:1 water: piperidine (10 μ L, freshly diluted), and incubated at 95 °C for 30 min. DNA fragments were recovered by ethanol precipitation and analyzed by 20% denaturing PAGE (Figure S1).

Metallosalen-DNA mediated oxidative DNA cleavage.

A second Ni metallosalen-DNA conjugate (**9**) was synthesized as described [paper ref. 7] and was evaluated for oxidative DNA cleavage of complementary strands (**10**, Figure S2). DNA sequences flanking the metallosalen site were varied (compare to **1**) to ensure the sequence independence of metallosalen-mediated DNA cleavage of complementary strands.

DNA cleavage mediated by metallosalen-DNA **9**.

A mixture of **9** and **10** (each at 4 μ M) with radiolabeled substrate (40 fmol) in 25 mM Tris pH 7.5 and 150 mM NaCl, was heated at 95 °C for 10 min and then cooled to RT over 2 h. A solution of MMPP (4.5 mM) was added so its final concentration was 900 μ M. Each DNA strand was present in a final concentration of 2 μ M. This solution was incubated at 37 °C for 30 min. The DNA fragments were recovered by precipitation with isopropanol. The oligonucleotides were redissolved in 15:1 water: piperidine (10 μ L, freshly diluted) and incubated at 95 °C for 30 min. The DNA fragments were recovered by precipitation with ethanol and analyzed by 20% denaturing PAGE (Figure S3) and MALDI-TOF MS (Table S1).

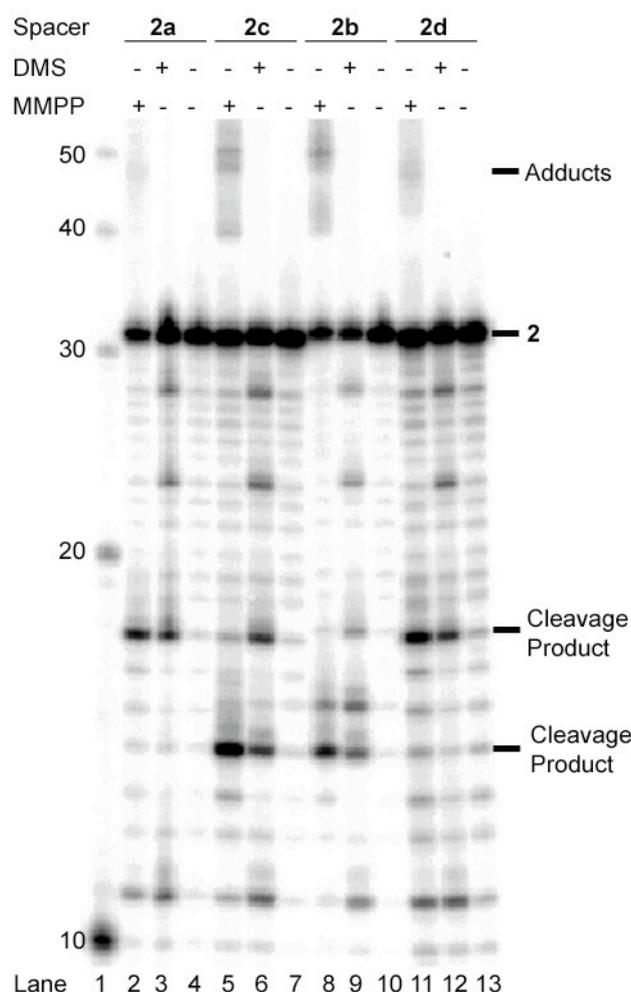


Figure S1. Gel electrophoresis assay of **2a–d** oxidative cleavage by Ni-salen-DNA **1** with piperidine treatment. 5'-radiolabeled **2** was hybridized to Ni-salen-DNA **1** and treated at 37 °C for 30 min. All reactions were completed by incubation with 600 mM piperidine for 30 min at 95 °C. Cleavage products were analyzed by 20% denaturing PAGE. Lane 1: 10 bp marker. Lanes 2-13, grouped in three-lane sets: first lane oxidation reaction, second-DMS dG sequencing, third-no oxidant. Lanes 2-4, **2a** (AT); lanes 5-7, **2c** (GC); lanes 8-10, **2b** (GG); lanes 11-13, **2d** (TT).

Figure S2. Sequences for metallosalen-DNA conjugate **9** cleavage of **10**. Where XX = TT (**10a**) or GG (**10b**).

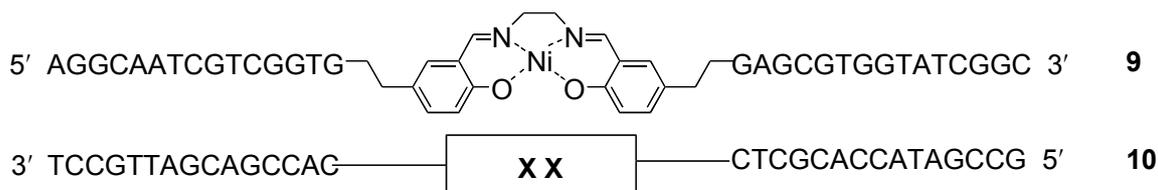


Figure S3. Gel electrophoresis assay of **9** cleavage of **10a** and **10b** using MMPP with dG DMS sequencing.

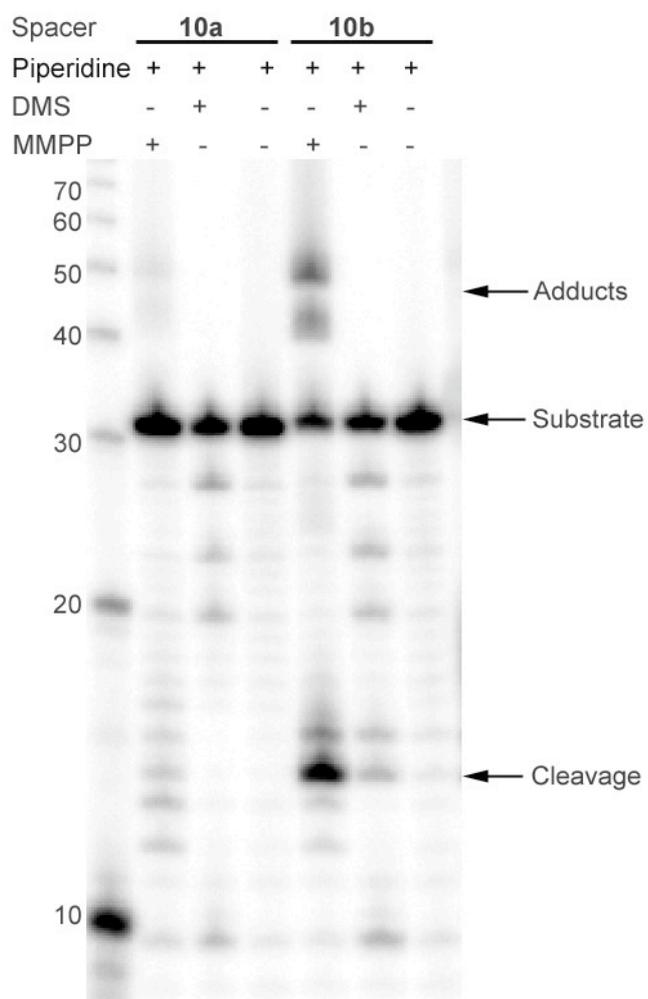


Table S1. MALDI-TOF MS characterization of cleavage products of **10b**. p = phosphate.

Strand	Fragment	[M] calc'd	[M] found
10b	5'- GCC GAT ACC ACG CTC G-p-3'	4908.12	-
	5'-p- CAC CGA CGA TTG CCT-3'	4593.94	4593.87
	5'- GCC GAT ACC ACG CTC-p-3'	4578.92	4578.95
	5'-p-GCA CCG ACG ATT GCC T-3'	4923.14	4924.14