

Supporting Information

for

Hairpin DNA Sequences Bound Strongly by Bleomycin Exhibit Enhanced Double-Strand Cleavage

Basab Roy and Sidney M. Hecht*

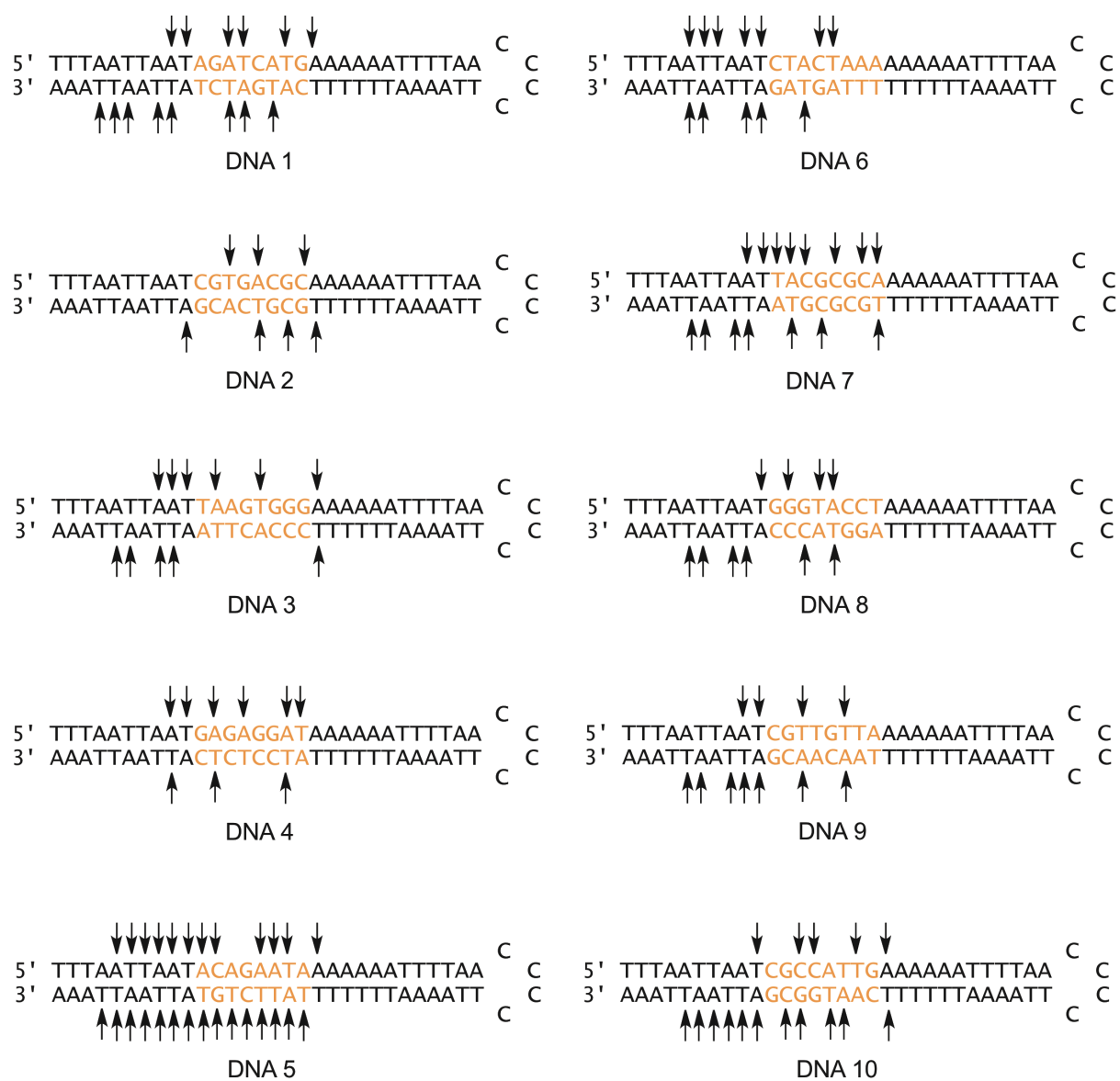


Figure S1. Sites of BLM-mediated damage for the ten hairpin DNAs studied.¹⁵

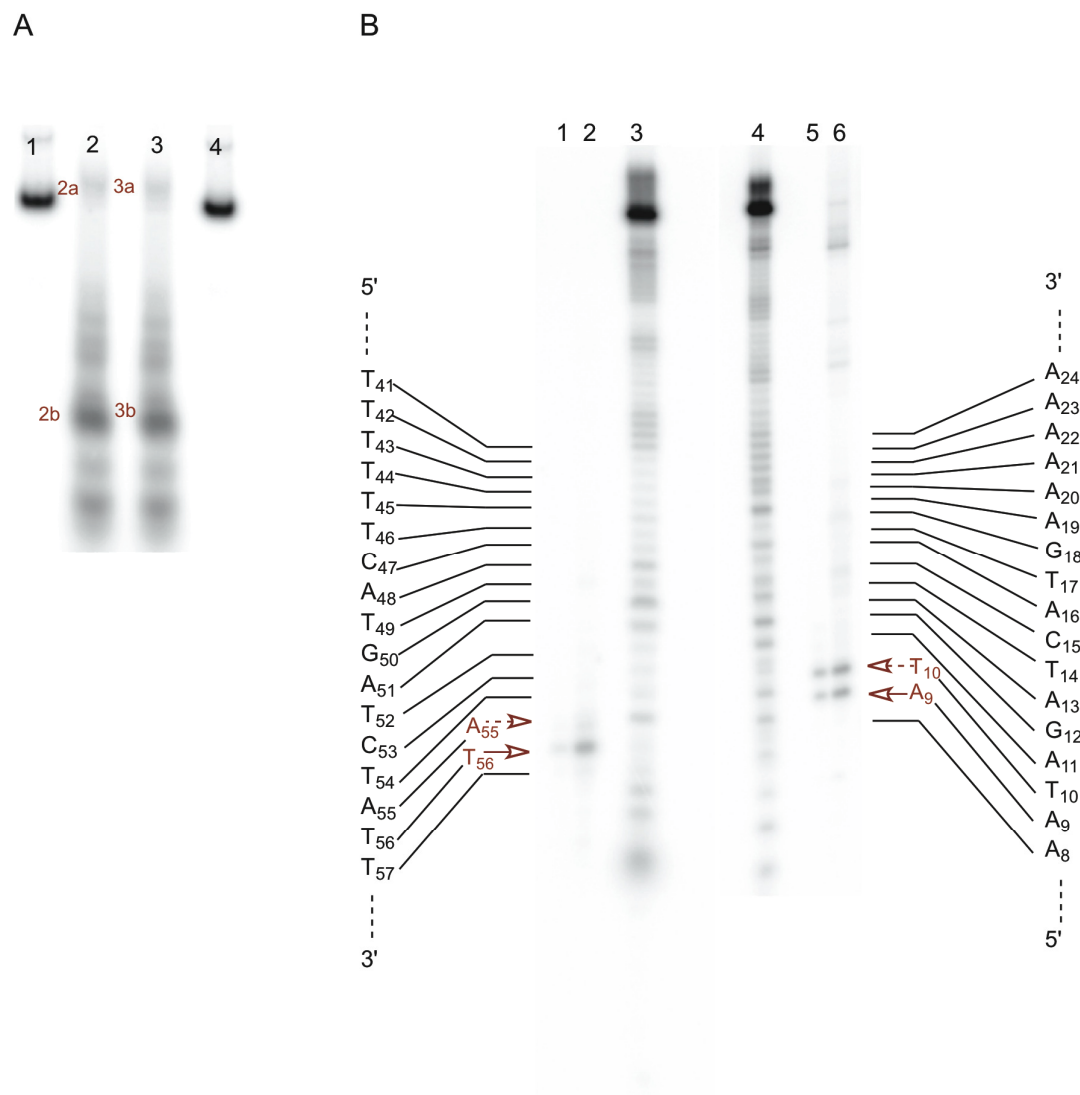


Figure S2. Analysis of Fe•bleomycin induced double-strand cleavage sites on hairpin DNA **1**. (A) Double-strand cleavage of [3'-³²P]-end labeled (lanes 1 and 2) and [5'-³²P]-end labeled (lanes 3 and 4) 64-nucleotide hairpin DNA **1** by bleomycin A₅. Lane 1, [3'-³²P]-end labeled DNA alone; lane 2, 5 μM Fe(II)•BLM A₅; lane 3, 5 μM Fe(II)•BLM A₅; lane 4, [5'-³²P]-end labeled DNA alone. (B) Sequencing gel analysis of bleomycin induced double-strand cleavage sites on [3'-³²P]-end labeled (lanes 1-3) and [5'-³²P]-end labeled (lanes 4-6) DNA **1**. Each lane (except lanes 3 and 4) corresponds to a numbered cleavage band, shown in (A). Lane 1, band 2b; lane 2, band 2a; lane 3, Maxam-Gilbert G+A sequencing lane of [3'-³²P]-end labeled DNA **1**; lane 4, Maxam-Gilbert G+A sequencing lane of [5'-³²P]-end labeled DNA **1**; lane 5, band 3b; lane 6, band 3a.

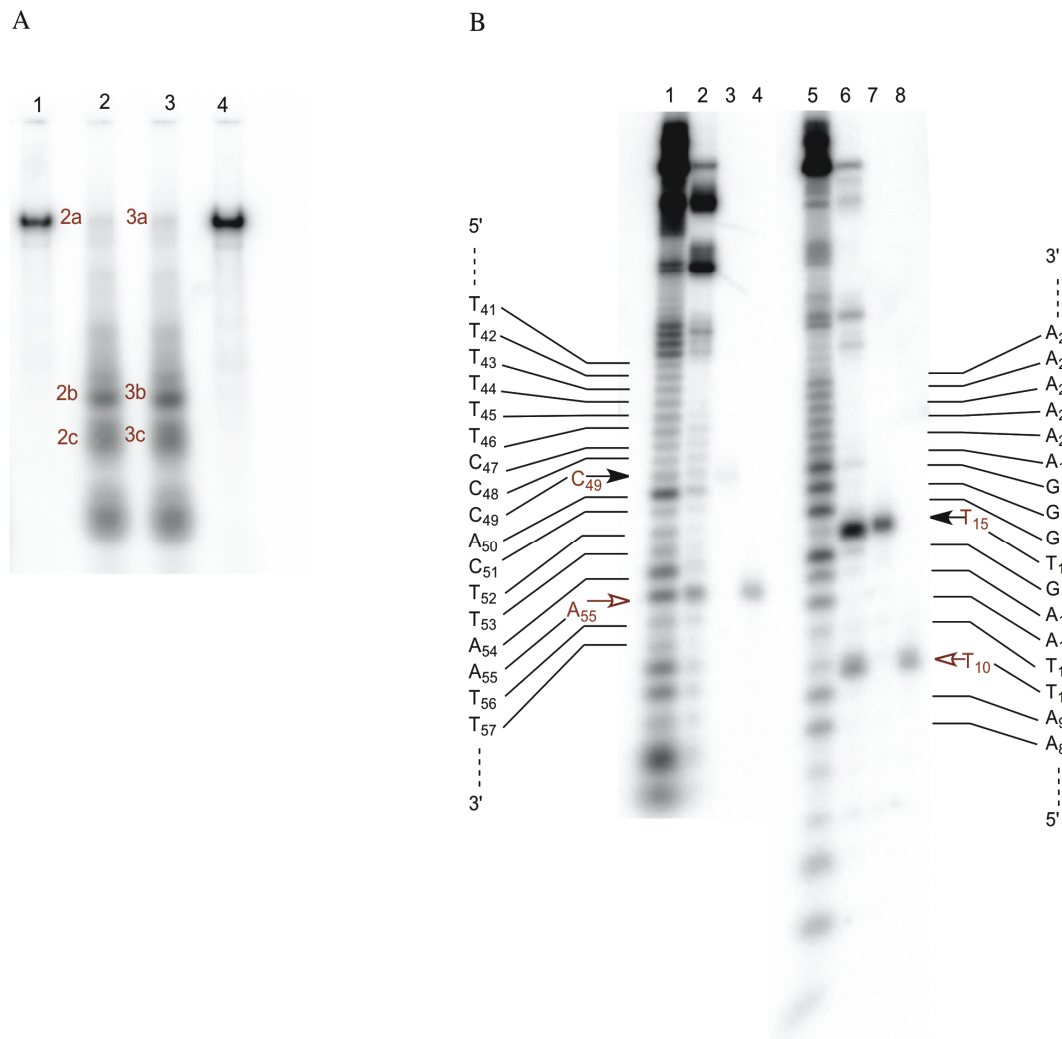


Figure S3. Analysis of Fe•bleomycin induced double-strand cleavage sites on hairpin DNA **3**. (A) Double strand cleavage of [3'-³²P]-end labeled (lanes 1 and 2) and [5'-³²P]-end labeled (lanes 3 and 4) 64-nucleotide hairpin DNA **3** by bleomycin A₅. Lane 1, [3'-³²P]-end labeled DNA alone; lane 2, 5 μM Fe(II)•BLM A₅; lane 3, 5 μM Fe(II)•BLM A₅; lane 4, [5'-³²P]-end labeled DNA alone. (B) Sequencing gel analysis of bleomycin induced double-strand cleavage sites on [3'-³²P]-end labeled (lanes 1-4) and [5'-³²P]-end labeled (lanes 5-8) DNA **3**. Each lane (except lanes 1 and 5) corresponds to a numbered cleavage band, shown in (A). Lane 1, Maxam-Gilbert G+A sequencing lane of [3'-³²P]-end labeled DNA **3**; lane 2, band 2a; lane 3, band 2b; lane 4, band 2c; Lane 5, Maxam-Gilbert G+A sequencing lane of [5'-³²P]-end labeled DNA **3**; lane 6, band 3a; lane 7, band 3b; lane 8, band 3c.

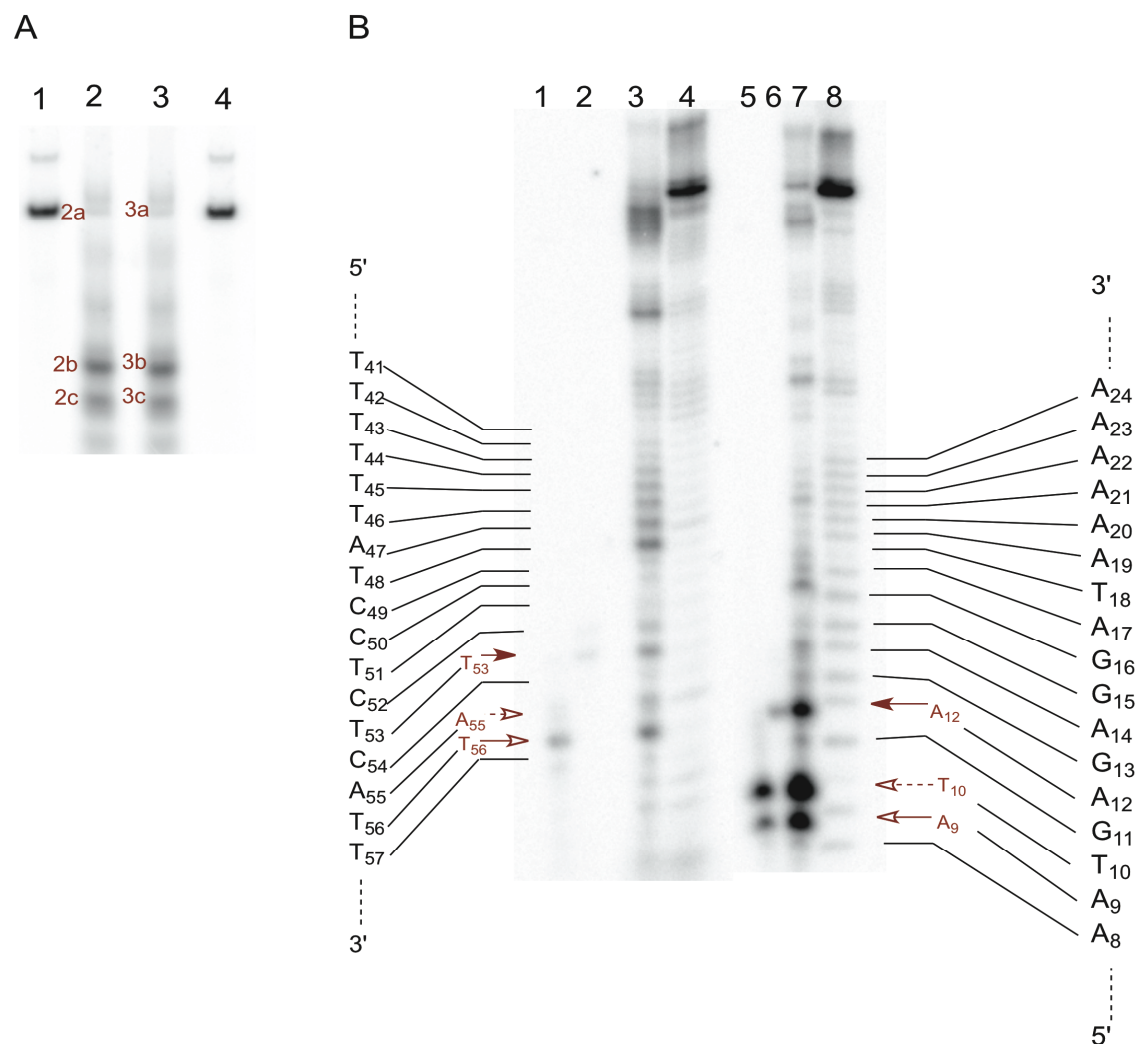


Figure S4. Analysis of Fe•bleomycin induced double-strand cleavage sites on hairpin DNA **4**. (A) Double strand cleavage of [3'-³²P]-end labeled (lanes 1 and 2) and [5'-³²P]-end labeled (lanes 3 and 4) 64-nucleotide hairpin DNA **4** by bleomycin A₅. Lane 1, [3'-³²P]-end labeled DNA alone; lane 2, 5 μM Fe(II)•BLM A₅; lane 3, 5 μM Fe(II)•BLM A₅; lane 4, [5'-³²P]-end labeled DNA alone. (B) Sequencing gel analysis of bleomycin induced double-strand cleavage sites on [3'-³²P]-end labeled (lanes 1-4) and [5'-³²P]-end labeled (lanes 5-8) DNA **4**. Each lane (except lanes 4 and 8) corresponds to a numbered cleavage band, shown in (A). Lane 1, band 2c; lane 2, band 2b; lane 3, band 2a; lane 4, Maxam-Gilbert G+A sequencing lane of [3'-³²P]-end labeled DNA **4**; lane 5, band 3c; lane 6, band 3b; lane 7, band 3a; lane 8, Maxam-Gilbert G+A sequencing lane of [5'-³²P]-end labeled DNA **4**.

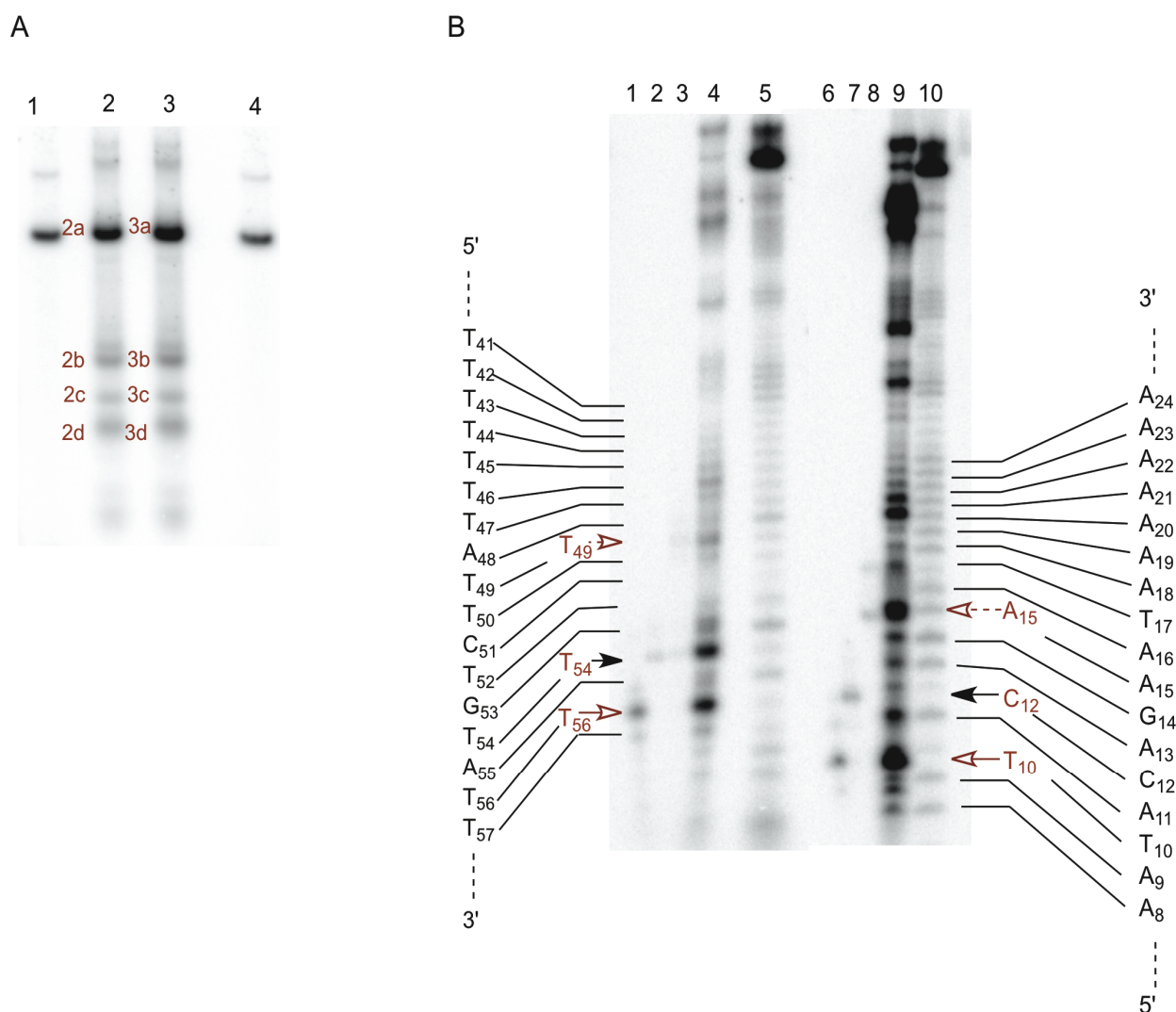


Figure S5. Analysis of Fe•bleomycin induced double-strand cleavage sites on hairpin DNA **5**. (A) Double strand cleavage of [3'-³²P]-end labeled (lanes 1 and 2) and [5'-³²P]-end labeled (lanes 3 and 4) 64-nucleotide hairpin DNA **5** by bleomycin A₅. Lane 1, [3'-³²P]-end labeled DNA alone; lane 2, 5 μM Fe(II)•BLM A₅; lane 3, 5 μM Fe(II)•BLM A₅; lane 4, [5'-³²P]-end labeled DNA alone. (B) Sequencing gel analysis of bleomycin induced double-strand cleavage sites on [3'-³²P]-end labeled (lanes 1-5) and [5'-³²P]-end labeled (lanes 6-10) DNA **5**. Each lane (except lanes 5 and 10) corresponds to a numbered cleavage band, shown in (A). Lane 1, band 2d; lane 2, band 2c; lane 3, band 2b; lane 4, band 2a; lane 5, Maxam-Gilbert G+A sequencing lane of [3'-³²P]-end labeled DNA **5**; lane 6, band 3d; lane 7, band 3c; lane 8, band 3b; lane 9, band 3a; Lane 10, Maxam-Gilbert G+A sequencing lane of [5'-³²P]-end labeled DNA **5**.

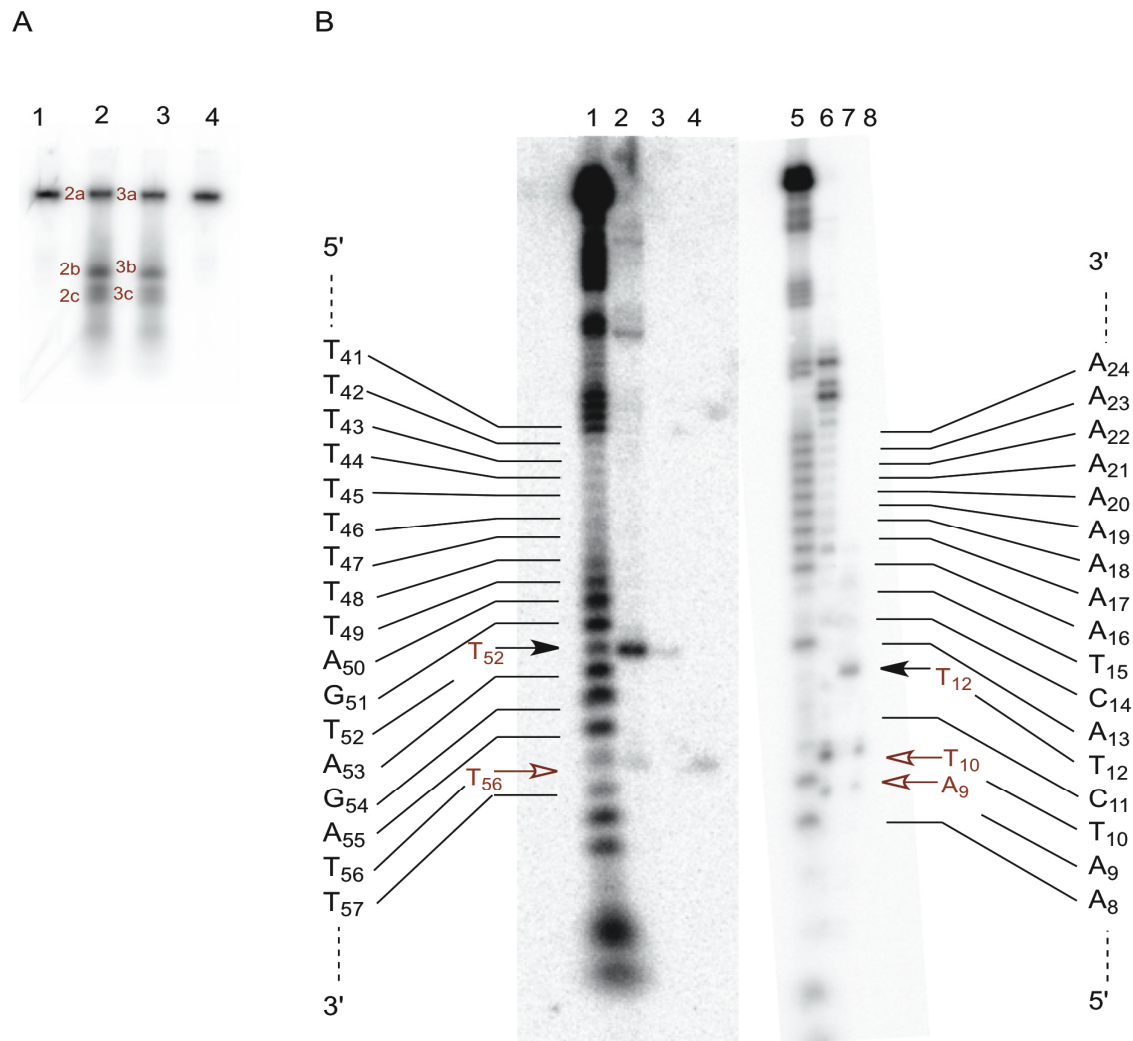


Figure S6. Analysis of Fe•bleomycin induced double-strand cleavage sites on hairpin DNA **6**. (A) Double strand cleavage of [3'-³²P]-end labeled (lanes 1 and 2) and [5'-³²P]-end labeled (lanes 3 and 4) 64-nucleotide hairpin DNA **6** by bleomycin A₅. Lane 1, [3'-³²P]-end labeled DNA alone; lane 2, 5 μM Fe(II)•BLM A₅; lane 3, 5 μM Fe(II)•BLM A₅; lane 4, [5'-³²P]-end labeled DNA alone. (B) Sequencing gel analysis of bleomycin induced double-strand cleavage sites on [3'-³²P]-end labeled (lanes 1-4) and [5'-³²P]-end labeled (lanes 5-8) DNA **6**. Each lane (except lanes 1 and 5) corresponds to a numbered cleavage band, shown in (A). Lane 1, Maxam-Gilbert G+A sequencing lane of [3'-³²P]-end labeled DNA **6**; lane 2, band 2a; lane 3, band 2b; lane 4, band 2c; lane 5, Maxam-Gilbert G+A sequencing lane of [5'-³²P]-end labeled DNA **6**; lane 6, band 3a; lane 7, band 3b; lane 8, band 3c.

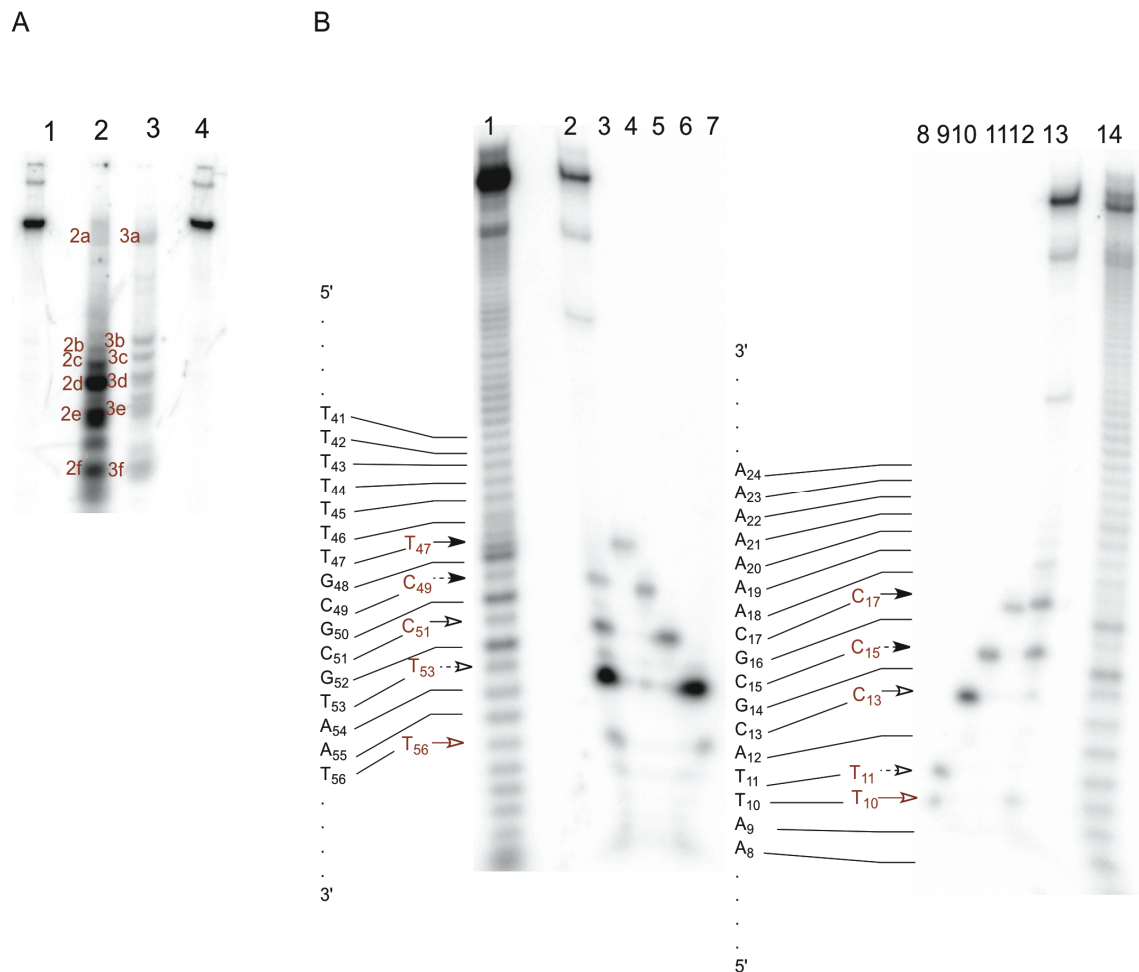


Figure S7. Analysis of Fe•bleomycin induced double-strand cleavage sites on hairpin DNA 7. (A) Double strand cleavage of $[3'-^{32}\text{P}]$ -end labeled (lanes 1 and 2) and $[5'-^{32}\text{P}]$ -end labeled (lanes 3 and 4) 64-nucleotide hairpin DNA 7 by bleomycin A₅. Lane 1, $[3'-^{32}\text{P}]$ -end labeled DNA alone; lane 2, 5 μM Fe(II)•BLM A₅; lane 3, 5 μM Fe(II)•BLM A₅; lane 4, $[5'-^{32}\text{P}]$ -end labeled DNA alone. (B) Sequencing gel analysis of bleomycin induced double-strand cleavage sites on $[3'-^{32}\text{P}]$ -end labeled (lanes 1-7) and $[5'-^{32}\text{P}]$ -end labeled (lanes 8-14) DNA 7. Each lane (except lanes 1 and 14) corresponds to a numbered cleavage band, shown in (A). Lane 1, Maxam-Gilbert G+A sequencing lane of $[3'-^{32}\text{P}]$ -end labeled DNA 7; lane 2, band 2a; lane 3, band 2b; lane 4, band 2c; lane 5, band 2d; lane 6, band 2e; lane 7, band 2f; Lane 8, band 3f; lane 9, band 3e; lane 10, band 3d; lane 11, band 3c; lane 12, band 3b; lane 13, band 3a; lane 14, Maxam-Gilbert G+A sequencing lane of $[5'-^{32}\text{P}]$ -end labeled DNA 7.

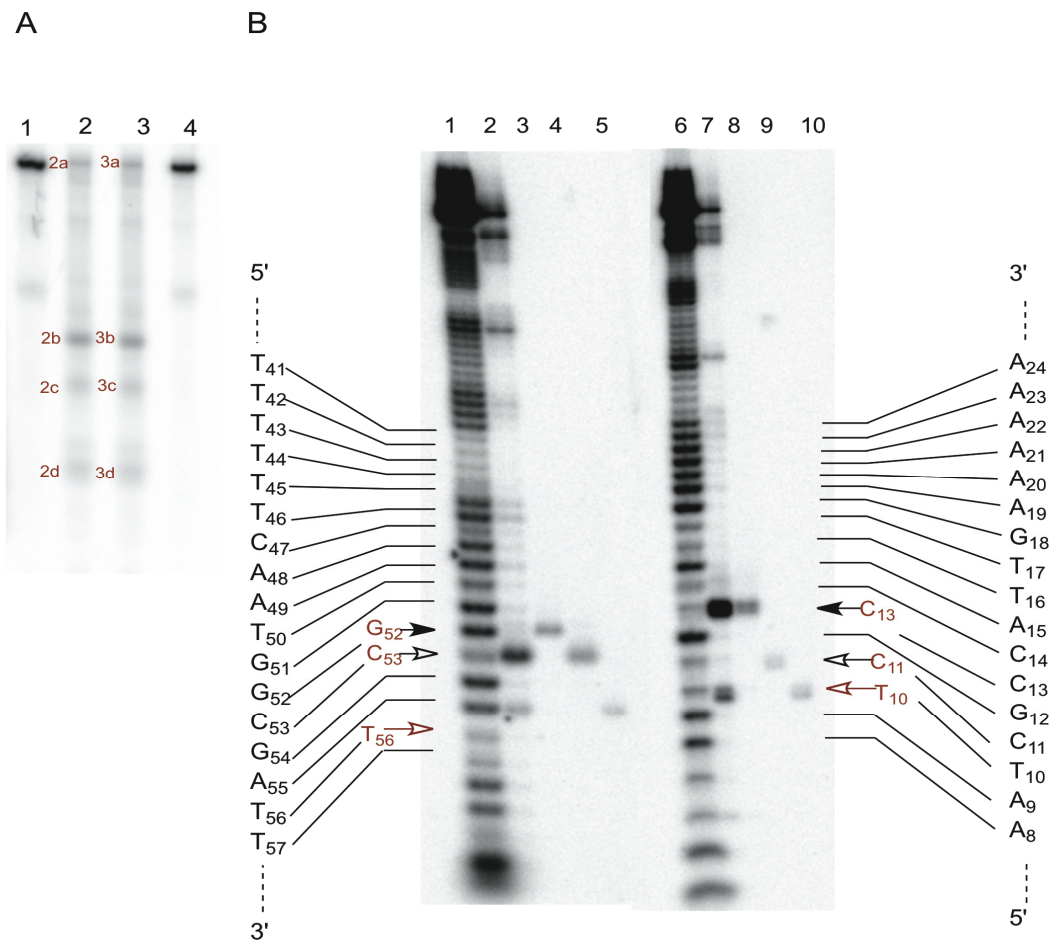


Figure S8. Analysis of Fe•bleomycin induced double-strand cleavage sites on hairpin DNA **10**. (A) Double strand cleavage of [3'-³²P]-end labeled (lanes 1 and 2) and [5'-³²P]-end labeled (lanes 3 and 4) 64-nucleotide hairpin DNA **10** by bleomycin A₅. Lane 1, [3'-³²P]-end labeled DNA alone; lane 2, 5 μM Fe(II)•BLM A₅; lane 3, 5 μM Fe(II)•BLM A₅; lane 4, [5'-³²P]-end labeled DNA alone. (B) Sequencing gel analysis of bleomycin induced double-strand cleavage sites on [3'-³²P]-end labeled (lanes 1-5) and [5'-³²P]-end labeled (lanes 6-10) DNA **10**. Each lane (except lanes 1 and 6) corresponds to a numbered cleavage band, shown in (A). Lane 1, Maxam-Gilbert G+A sequencing lane of [3'-³²P]-end labeled DNA **10**; lane 2, band 2a; lane 3, band 2b; lane 4, band 2c; lane 5, band 2d; lane 6, Maxam-Gilbert G+A sequencing lane of [5'-³²P]-end labeled DNA **10**; lane 7, band 3a; lane 8, band 3b; lane 9, band 3c; Lane 10, band 3d.