Supplemental Information

Sequence-Selective Interaction of the Minor-Groove Interstrand Cross-Linking Agent SJG-136 with Naked and Cellular DNA: Footprinting and Enzyme Inhibition Studies

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LEGENDS

SI-1 (Relating to Figure 4)

Analysis of footprint intensities (from the footprinting gel shown in **Figure 4**) for the interaction of SJG-136 (**3**) with the MS2 T7 sequence at 3, 10 and 30 μM dose levels. Top Panel = nucleotides 22-119 of the MS2 fragment (from footprinting of forward-labeled MS2); Bottom Panel = nucleotides 123-233 of the MS2 fragment (from footprinting of reverse-labeled MS2).

SI-2 (Relating to Figures 5 to 7)

Analysis of the intensity of each T-stop was carried out by first measuring the 'raw' intensity of each band using ImageQuant v1.2. Then, assuming 100% efficiency of incorporation of radiolabeled cytidine into each fragment, normalised intensities were obtained by dividing the 'raw' intensities of each band by the number of cytidine residues in the corresponding transcript (taken from the sequence).

SI-3

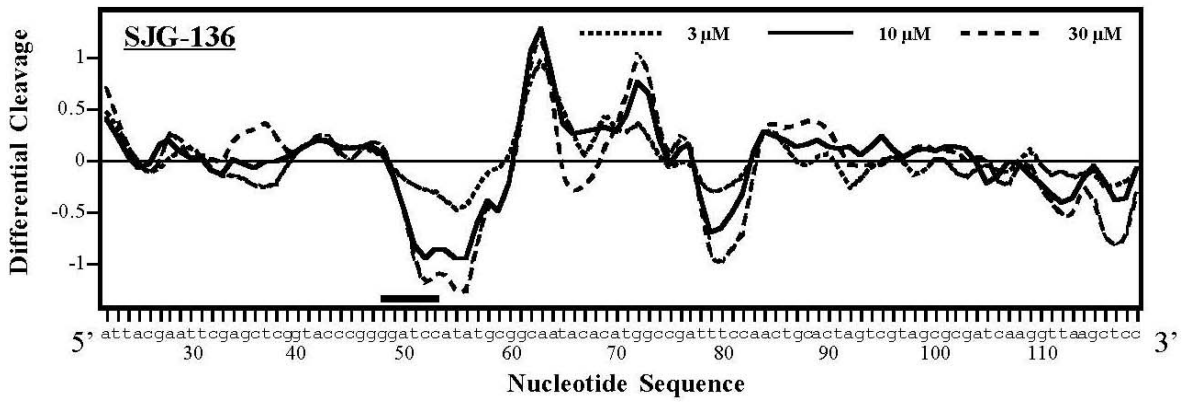
T-stop assay showing the concentration-dependent effect of GD113 (**4**) on the inhibition of T7 RNA polymerase at concentrations ranging from 0.5 to 50 μM. Although GD113 is a PBD dimer, it is constructed from C7/C7'-linked PBD units and so is unable to form effective interstrand DNA cross-links due to its geometrical shape. Thus the stop sites at 5'-^{192}TCT^{194}-3' and 5'-^{127}TCC^{129}-3' (i.e., 5'-AGA-3' and 5'-GGA-3', respectively, on the lower strand) observed between 15-50 μM are thought to be due to mono-covalent adducts.

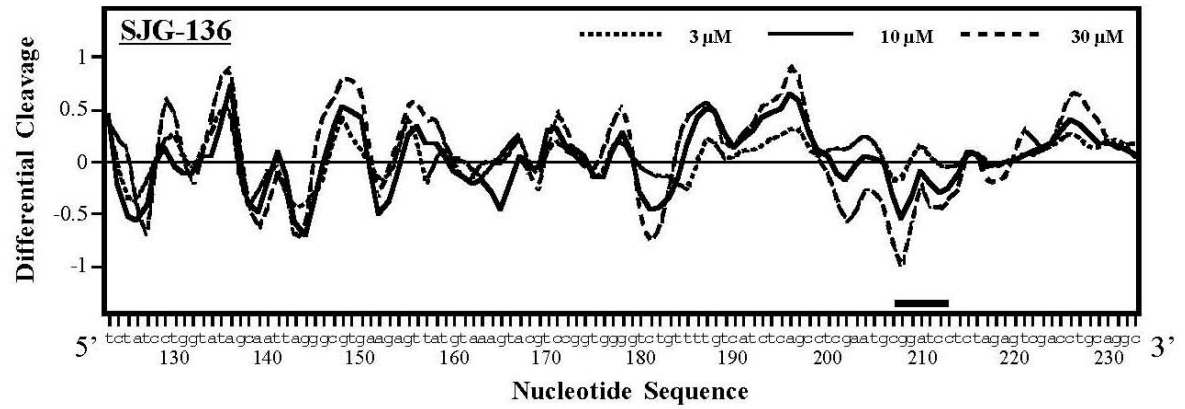
SI-4 (Relating to Figure 9B)

Analysis of footprint intensities (from the footprinting gel shown in **Figure 9B**) for the interaction of SJG-136 (**3**) with the pGL3-C plasmid at 1, 3, and 10 μM dose levels. The prominent interstrand cross-linking sequence at 5'-AGATCT-3' is co-incidental with the *Bgl*II cleavage site.

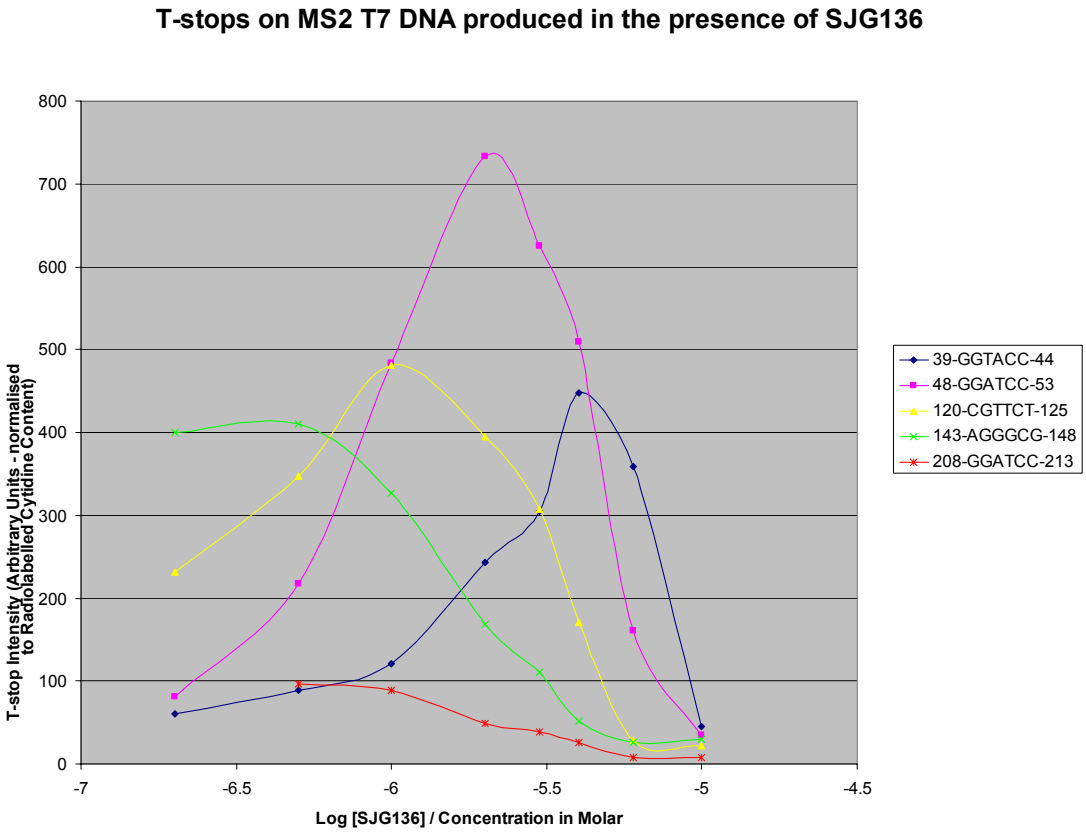
†This work was partly supported by Cancer Research UK (C180/A1060, SP1938/0402, SP1938/0201, SP1938/0301 and SP2000/0402 to DET, and C2259/A3083 to JAH) and Yorkshire Cancer Research (to TCJ).

SI-1

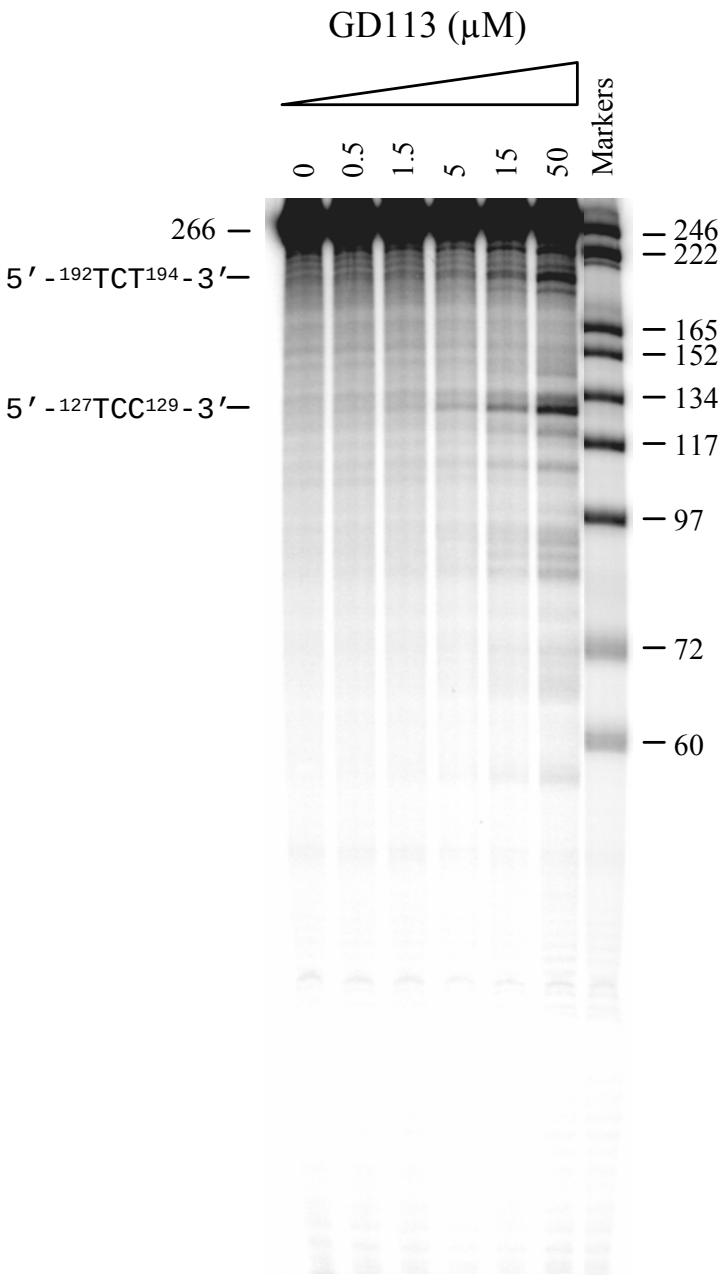

SJG-136
3 µM
10 µM
30 µM
Differential Cleavage
1
0.5
0
-0.5
-1
5' attacgaattcgagctcggtacccggggatccatatgcggcaatacacatggccgatttccaactgcactagtcgtagcgcgatcaaggttaagctcc 3'
30
40
50
60
70
80
90
100
110
Nucleotide Sequence


SJG-136
3 µM
10 µM
30 µM
Differential Cleavage
1
0.5
0
-0.5
-1
5' tctatcctggtatagcaattagggcgtgaagagttatgtaaagtacgtccggtgggtctgttttgtcatctcagcctcgaatgcggatcctctagagtcgacctgcaggc 3'
130
140
150
160
170
180
190
200
210
220
230
Nucleotide Sequence

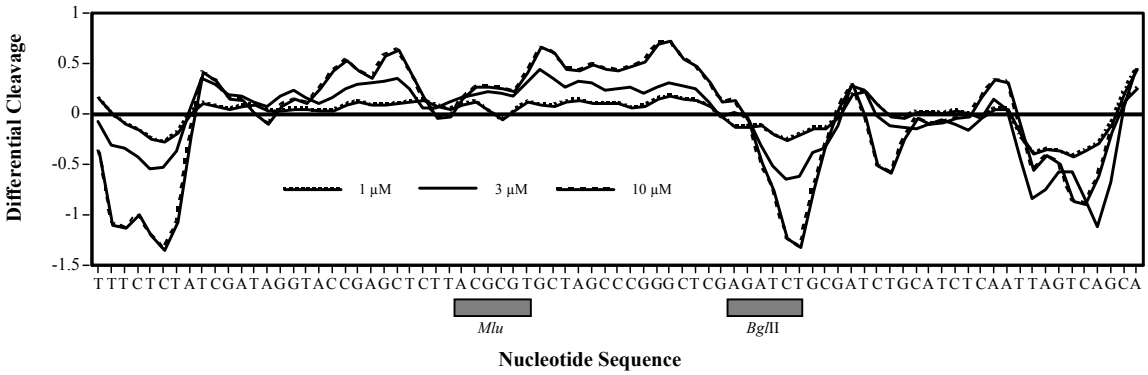
SI-2


T-stops on MS2 T7 DNA produced in the presence of SJG136
T-stop Intensity (Arbitrary Units - normalised to Radiolabelled Cytidine Content)
800
700
600
500
400
300
200
100
0
-7
-6.5
-6
-5.5
-5
-4.5
Log [SJG136] / Concentration in Molar
39-GGTACC-44
48-GGATCC-53
120-CGTTCT-125
143-AGGGCG-148
208-GGATCC-213

SI-3


GD113 (μM)
0
0.5
1.5
5
15
50
Markers
266
5′-192TCT194-3′
5′-127TCC129-3′
246
222
165
152
134
117
97
72
60

SI-4


Differential Cleavage
1
0.5
0
-0.5
-1
-1.5
1 µM
3 µM
10 µM
T TT C T CT AT C GA T AG GT AC C GA GC T C TT T A C GC G T GC T AG C C C GG G C T C GA GA T C T GC GA T C T GC A T C T C AA T T AG T C AG C A
Mlu
BglII
Nucleotide Sequence