Supplementary Material

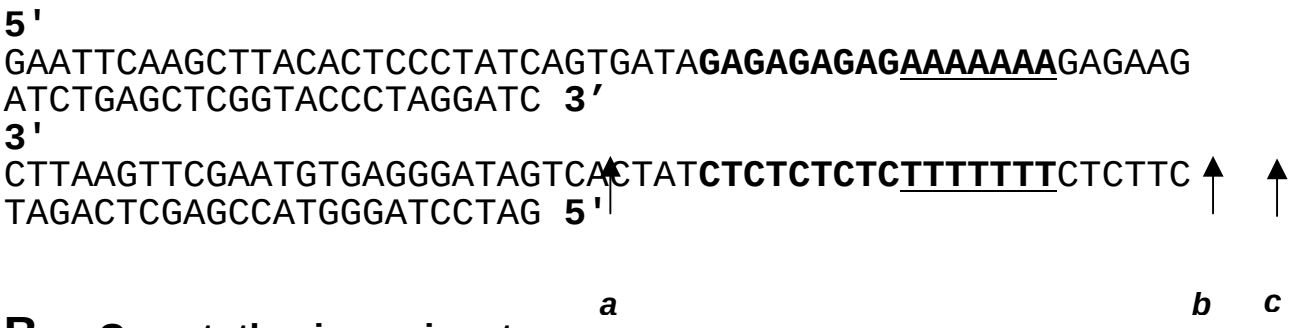
Spatial organization of topoisomerase I-mediated DNA cleavage induced by camptothecin-oligonucleotides conjugates

Paola B. Arimondo, Stéphane Angenault, Ludovic Halby, Alexandre Boutorine, Frédéric Schmidt, Claude Monneret, Thérèse Garestier, Jian-Sheng Sun, Christian Bailly and Claude Hélène

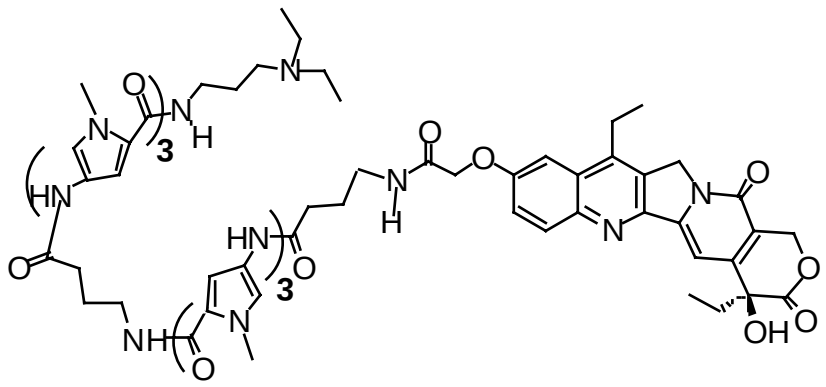
In addition, as control, we used a hairpin polyamide ligand, N,N-dimethyl-N'{1-methyl-4-[1-methyl-4-[1-methyl-4-[4-{[1-methyl-4-[1-methyl-4[1-methyl-4-(4-aminobutyryl)aminopyrrol-2-carbonyl]aminopyrrol-2-carbonyl]aminopyrrol-2-carbonyl]}aminobutyryl]aminopyrrol-2-carbonyl]aminopyrrol-2-carbonyl]aminopyrrol-2-carbonyl}propylendiamine, hereafter designated MGB (Figure S1) that binds in the minor groove of DNA. Similarly to the TFO, once covalently linked to 10-carboxyCPT, this molecule was capable of driving topoisomerase I-mediated DNA cleavage specifically at its binding site (1). Also when attached to compound ***2***, according to the procedures previously described (1), the MGB conjugate induced sequence-specific cleavage at site *b* near the binding site of the ligand (Figure S2). Cleavage experiments (Figure S3) on the two short duplexes resulted in directed cleavage by the conjugate only on the oligopyrimidine strand of the duplex and upstream of the binding site (sites $\mathbf{b}^{\mathbf{Y3'}}$ and ***4'***, lane 4). The free MGB did not induce specific cleavage (lane 5).

FIGURES

A Target duplex:

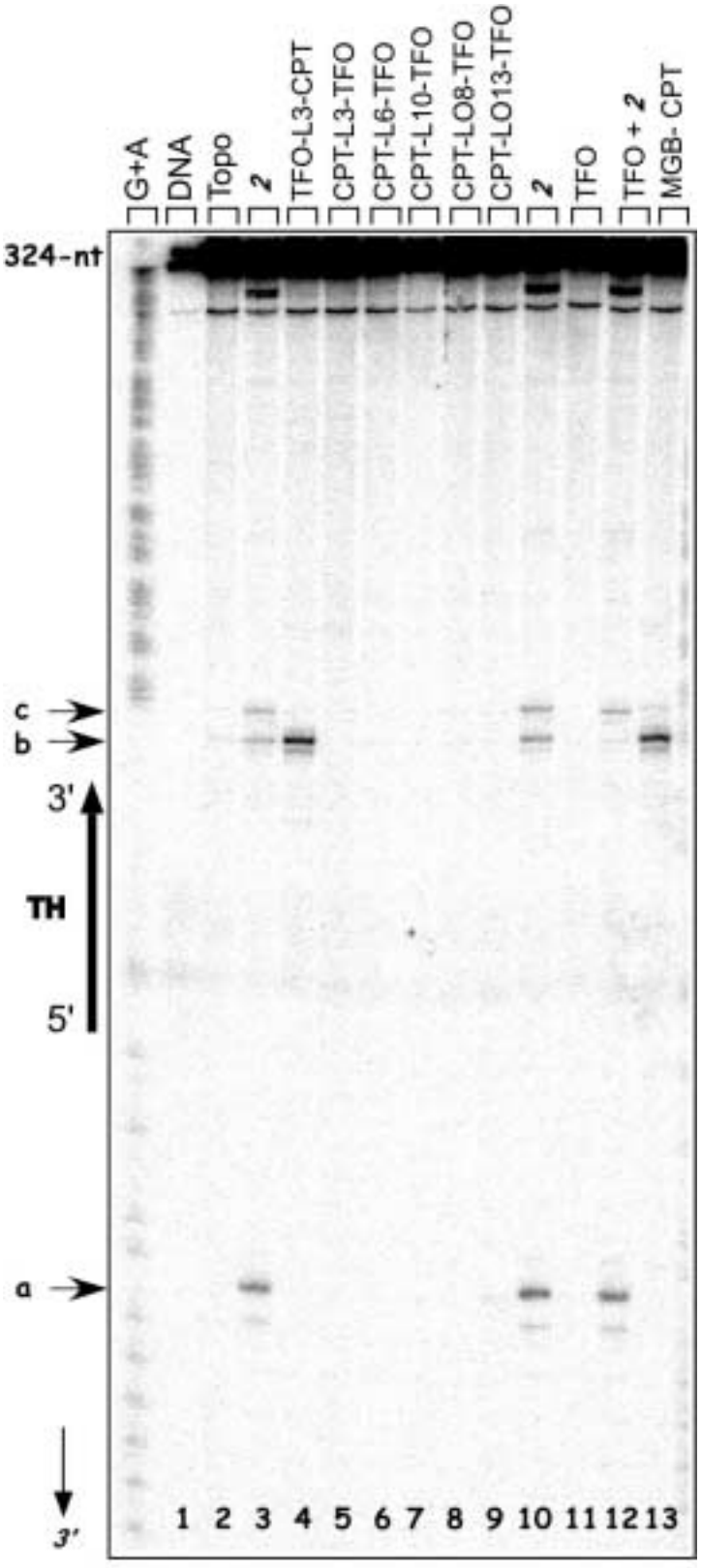


B Camptothecin conjugate:



MGB-CPT

Fig. S1. A) Sequence of the DNA target used in this study. The binding site for the hairpin polyamide MGB is underlined. **B)** Structure of the MGB-camptothecin conjugates used in this study. 7-Ethyl-10-hydroxycamptothecin acetic acid was attached through N-succinimide activation to the terminal amino group of the linker attached to the extremity of the MGB as described in (2).



Arimondo et al. Figure S2

Fig. S2. Sequence analysis of the Topo I-mediated cleavage products on the 324-bp target duplex, as described in Figure 3. Lane 1, duplex, incubated with Topoisomerase I (lane 2) and in the presence of 5 μM of ***2*** (lane 3 and 10), 0.5 μM TFO-L3-CPT (lane 4), 0.5 μM CPT-L3-TFO (lane 5), 0.5 μM CPT-L6-TFO (lane 6), 0.5 μM CPT-L10-TFO (lane 7), 0.5 μM CPT-LO8-TFO (lane 8), 0.5 μM CPT-LO13-TFO (lane 9), 5 μM TFO (lane 11), 5 μM TFO and ***2*** (lane 12), and 1 μM MGB-CPT (lane 13).

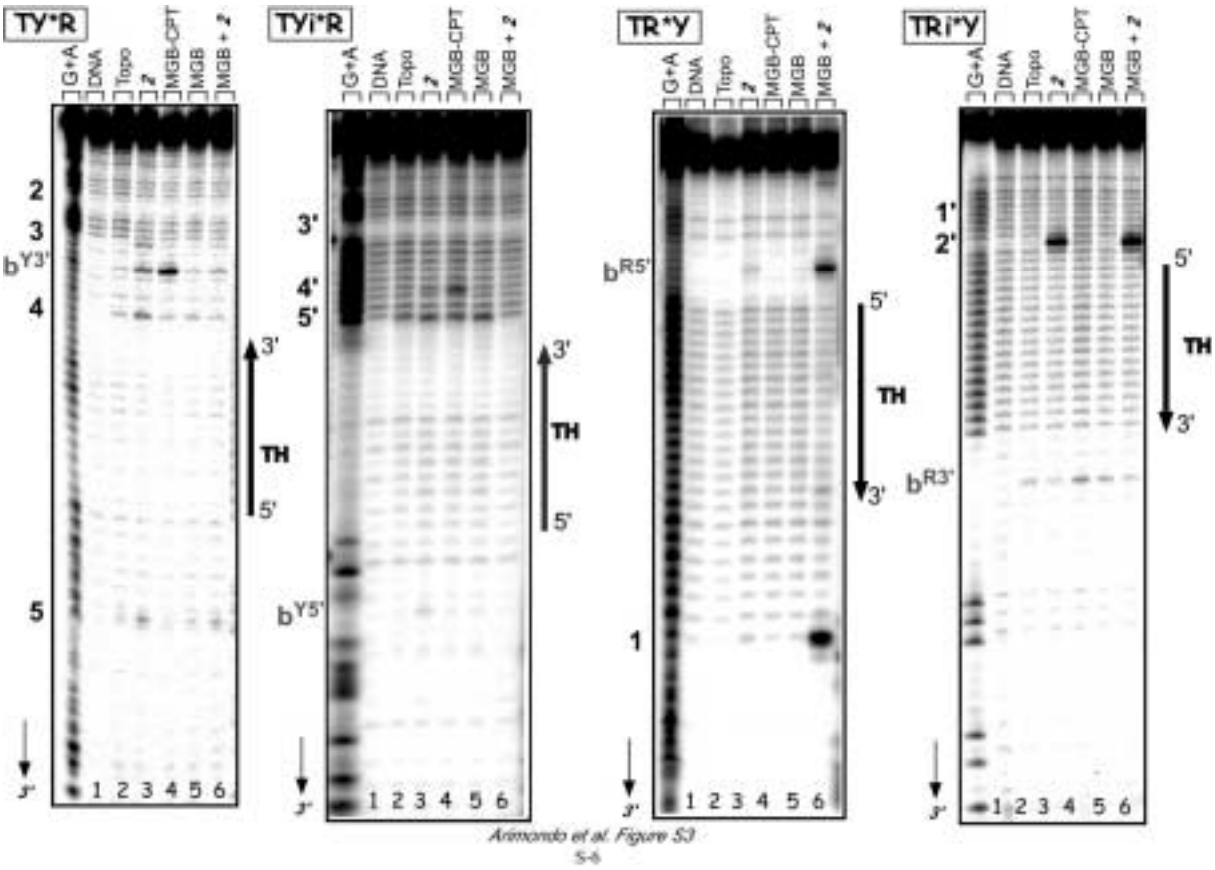


Fig. S3. Positioning of Topoisomerase I in the presence of MGB conjugate. Sequence analysis of the Topo I-mediated cleavage products on each 3'-end radiolabeled (50 nM) strand of the 59-bp duplexes. Adenine/guanine-specific Maxam-Gilbert chemical cleavage reactions were used as markers. The positions of the cleavage sites are indicated on each

strand according to the nomenclature defined in figure 4. The triple helix region is indicated as its orientation; radiolabeled strands are indicated by asterisks.

Lane 1, target duplex; duplex incubated with Topoisomerase I (10 units) (lane 2) and in the presence of 5 μM ***2*** (lane 3); 1 μM MGB-CPT (lane 4); 1 μM MGB (lane 5); 1 μM MGB + 5 μM ***2*** (lane 6).

REFERENCES

1. Arimondo, P. B., Bailly, C., Boutorine, A., Ryabinin, V., Syniakov, A., Sun, J. S., Garestier, T. and Hélène, C. (2001) Directing Topoisomerase I-mediated DNA Cleavage to Specific Sites by Camptothecin tethered to Minor and Major Groove Ligands. *Angewandte Chem. Int. Ed.*, **40,** 3045-3048.

2. Grimm, G., Boutorine, A. and Hélène, C. (2000) Rapid Routes of Synthesis of Oligonucleotide Conjugates from Non-Protected Oligonucleotides and Ligands Possessing Different Nucleophilic or Electrophilic Functional Groups. *Nucleosides Nucleotides Nucleic Acids*, **19**, 1943-1965.