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Supplementary Materials

Sequence-Specific Targeting of Duplex DNA Using Camptothecin-Triple Helix Forming Oligonucleotide Conjugate and Topoisomerase I

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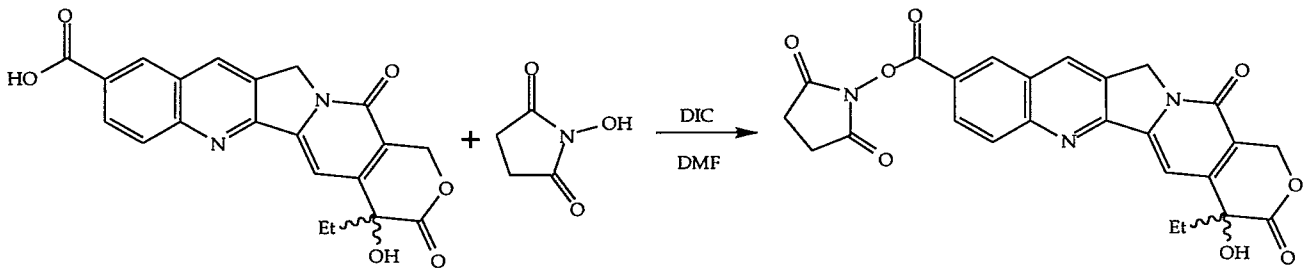
Synthesis of TFO-Camptothecin Conjugate **3**



10-Carboxycamptothecin (**1**)

10-carboxycamptothecin[8] (40 mg; 0.1 mmole) was dissolved in DMF (1.5 ml) and reacted with N-hydroxysuccinimide (12 mg; 0.1 mmole) in the presence of N,N-diisopropylcarbodiimide (DIC) (23 mg; 1.1 mmole). The solution was stirred at 20 °C for 5 hrs. then diluted with DMF (2.5 ml). This solution was used directly for conjugation to the free aliphatic amino group on TFO **2**.

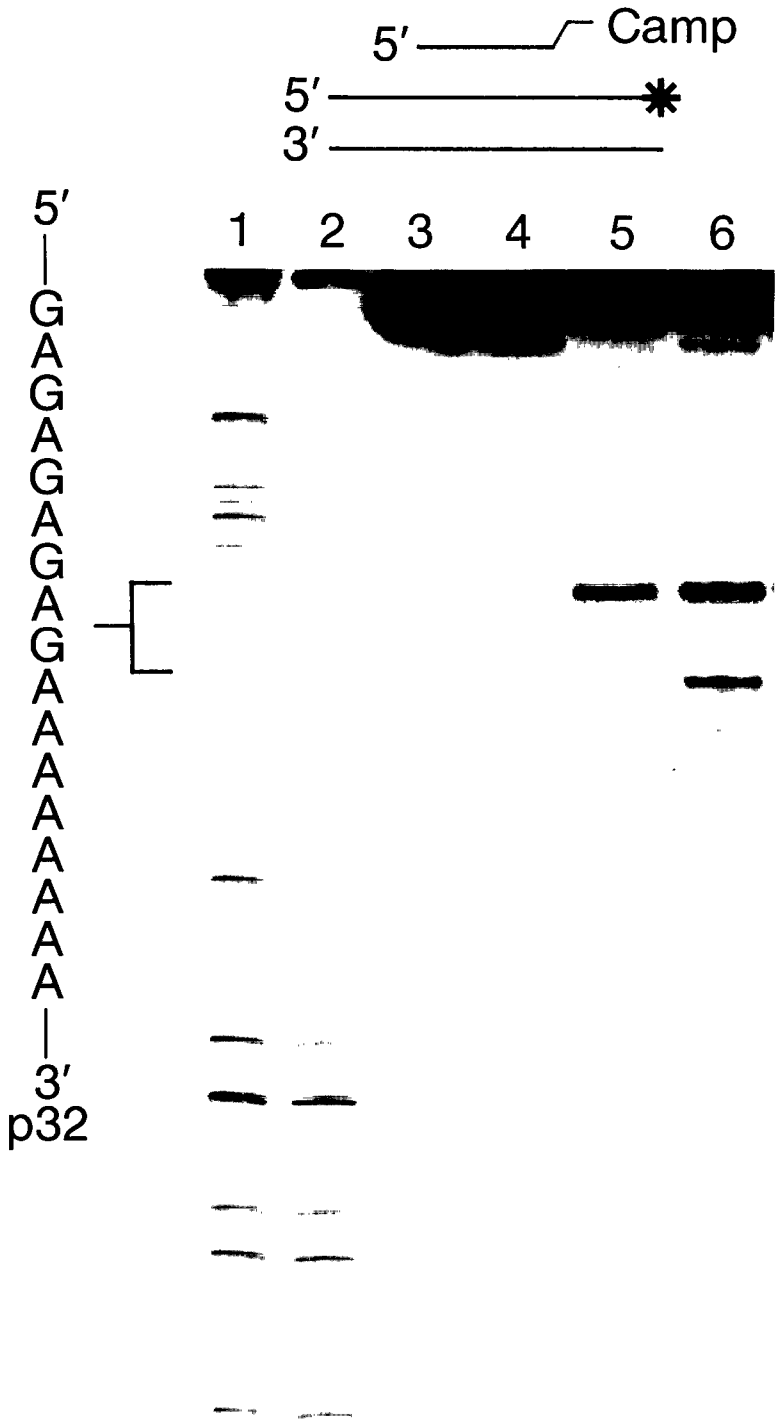
TFO **2**, 5'CUCUCUCUCUUUUUUUX, was synthesized by automated methods using an H-phosphonate method[9] on 1 μmole scale based on the loading of the controlled pore glass polymer support from Clontech. The TFO was release from the polymer support and freed of protecting groups by treatment with concentrated ammonia at 55 °C for 16 hrs. Following evaporation under reduced pressure, the TFO was dissolved in 100 μl of H_2O saturated with triethylamine (TEA) and reevaporated. This was done to remove ammonia which could subsequently quench the active ester. The TFO was then dissolved in 80 μl H_2O and TEA (20 μl), then 100 μl of the activated camptothecin DMF solution, ~25 μmole, (shown above) was added and the solution was stirred at 20 °C. After 2 hrs., 50 μl more of the activated camptothecin solution was added. After 2 hrs., the reaction mixture was

evaporated under reduced pressure, dissolved in 400 µl H_2O and extracted 6 times with 10% HOAc/10% 2-propanol/80% CH_2Cl_2 to remove unreacted camptothecin. The aqueous solution was evaporated under reduced pressure and purified by 20% denaturing polyacrylamide electrophoresis. There was a distinct mobility difference between the starting material, TFO **2**, and the product, TFO **3**, with TFO **3** migrating at a slower rate.

Trapping of cleavable complexes on the duplex target 3' end radiolabeled on the top strand.

The experiments were performed in an analogous manner to those described in the main text for the bottom strand labeling study. The only difference was the location of the radiolabel as noted. The results for the top strand labeling are shown in Figure 3. Lanes 1-6 designations are as stated in the Figure 2 legend of the main text.

Lanes 4, 5, and 6 required overexposure to reveal Topo I-mediated cleavage sites. The primary cleavage site in the camptothecin only reaction (lane 6) is <1% of the total by densitometry analysis. This same weak cleavage site was observed with the TFO camptothecin conjugate **3** and Topo I (lane 5) and more weakly with the non-conjugated TFO **2** and Topo I (lane 4). It appears that merely a triple helix-duplex junction without any camptothecin can result in inefficient top strand targeting. This inefficient targeting may be a general property of triple helix-duplex junctions or it may be an exception because this site is also susceptible to camptothecin mediated cleavage. Confirmation will require the examination of other triple helix-duplex junctions with different sequence contexts.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3′
1 2 3 4 5 6
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p32