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C3-Spacer-containing circular oligonucleotides as inhibitors of human topoisomerase I

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ABSTRACT

Some dumbbell-shaped circular oligonucleotides containing internal C3-spacers and Topo I-binding sites were designed and synthesized which displayed high inhibitory efficiency on the activity of human Topo I as well as resisted the degradation by some DNA repair enzymes.

DNA topoisomerase I (Topo I) catalyzes topological rearrangements of DNA through sequential single-stranded breakage, strand free rotation and further rejoining of phosphodiester backbone of DNA, which removes knots and catenanes generated by replication, transcription and recombination inside cells.[1] This nuclear enzyme has been found to be highly abundant in fast proliferating tumor cells than in normal tissue[2] and identified as a molecular target of some anticancer agents such as camptothecins, indolocarbazoles and indenoisoquinolines.[3] Besides these quinoline alkaloids and other organic compounds[4] that were identified as inhibitors of human Topo I in the past, certain oligonucleotides were designed previously in our lab that displayed high potency on the activity of this DNA relaxing enzyme.[5] However, due to their possession of mismatched base pairs,[5] the previously designed oligonucleotide inhibitors could be vulnerable to the degradation by some DNA repair enzymes. With the aim of developing oligonucleotide inhibitors that could resist the hydrolysis by mismatch and nick-resolving DNA repair enzymes, C3-spacer ($-CH_2-CH_2-CH_2-$) modifications[6] have been introduced into certain oligonucleotide structures during our recent investigations. Herein, we report that some of the newly designed C3-spacer-containing oligonucleotides in our studies not only exhibit high efficiency on the activities of human Topo I but also resist the degradation by a certain mismatch and nick-resolving DNA repair enzyme.

Figure 1 shows a dumbbell-shaped circular oligonucleotide[7] (Oligonucleotide 1) designed during our recent studies, which possesses two 'extraordinarily thermostable hairpins'[8] at both termini of its duplex structure. It is anticipated that introduction of these two hairpins to Oligonucleotide 1 could lead to an increase of thermal stability of the dumbbell-shaped structure and prevent itself from hydrolysis by exonucleases.[8] In addition, it has been well established in the past that during the strand scission and religation process, Topo I forms transitorily a covalent bond with the 3′ end of DNA fragment and holds the 5′ end of the second fragment through physical interaction.[9] Taking advantage of the fragile interacting fashion between Topo I and the resultant 5′ end of DNA, a C3-spacer was introduced into the dumbbell-shaped structure near Topo I cutting site of Oligonucleotide 1 in order to generate an irreversible inhibition to the nuclear enzyme. It was our expectation that once Topo I would bind to Oligonucleotide 1 and further cause a strand scission at its cutting site (Fig. 2),[10] the religation reaction between the cut fragments might not readily take place. This could happen because the presence of C3-spacer in the dumbbell-shaped structure might lead to a free dissociation of the corresponding trinucleotide (5′ GGA, see Fig. 2) from Topo I and its complementary stretch, thus leading to chemically irreversible damage to the enzyme.[11,12] In addition, it was anticipated during our early investigation that Oligonucleotide 1 could resist the hydrolysis by some mismatch- and nick-resolving DNA repair enzymes since introduction of C3-space would not lead to any base mismatches or open 5′ and 3′ ends in the dumbbell-shaped structures.

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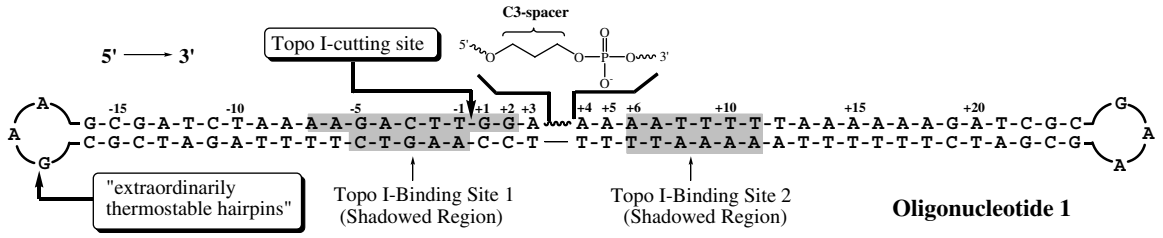


Figure 1. Schematic representation of a C3-spacer-containing dumbbell-shaped oligonucleotide.

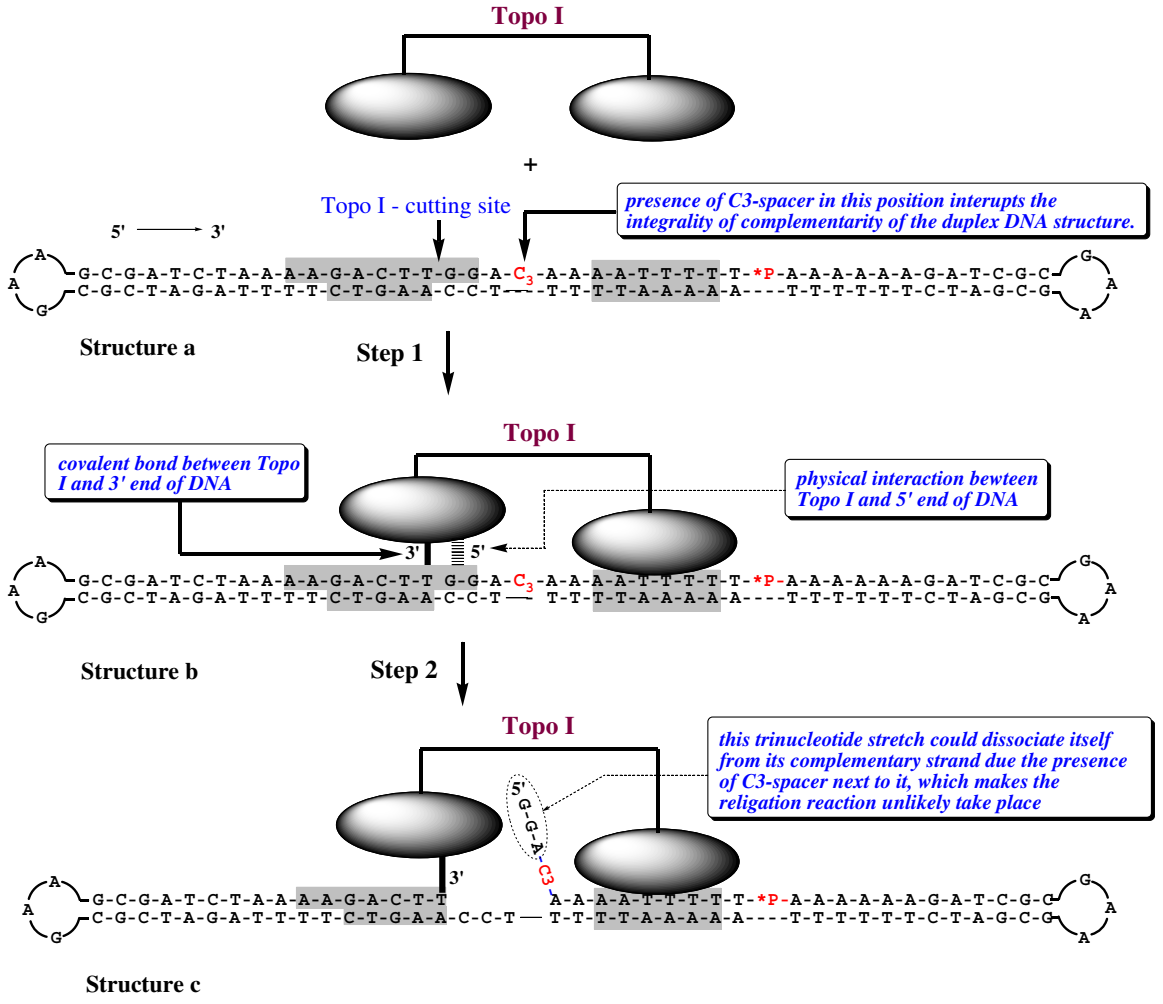
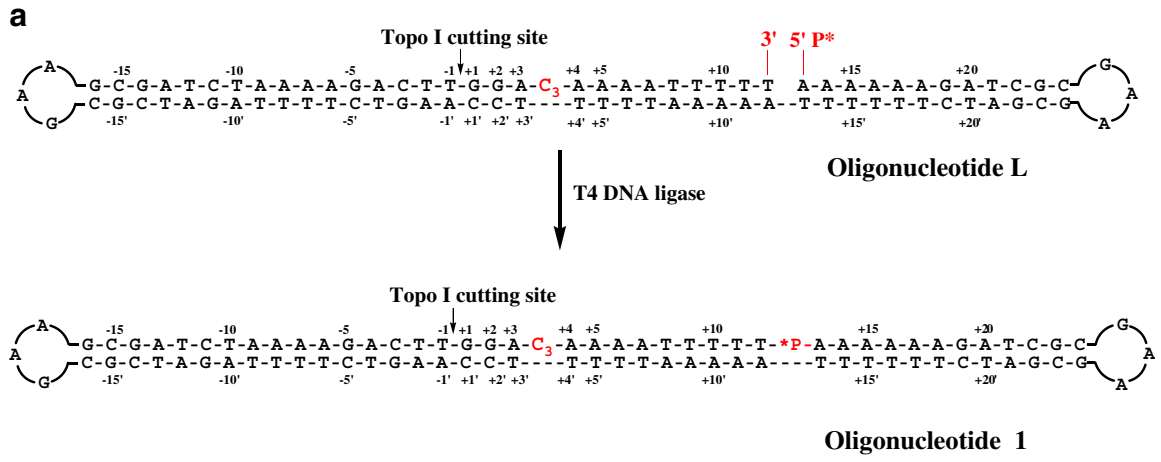


Figure 2. Diagrammatic illustration of anticipated inhibitory mechanisms of a C3-spacer-containing oligonucleotide (Oligonucleotide 1) on the activities of human topoisomerase I in our studies. When Oligonucleotide 1 is incubated with Topo I (Step 1), this nuclear enzyme is expected to bind to the oligonucleotide and subsequently form a covalent bond with the newly generated 3′ end of DNA and to hold the 5′ end through physical interaction (Structure b). The presence of C3-spacer near the newly generated 5′ end will interrupt the integrity of complementarity of the local duplex structure, which could consequently allow the trinucleotide of 5′ G-G-A- to dissociate freely from its complementary tract (Structure c). This free dissociation of 5′ G-G-A- from its complementary sequence could make the religation reaction impossible, thus leading an irreversible damage to the enzyme.

Oligonucleotide 1 was accordingly synthesized during our investigations through ligation reactions of a C3-spacer-containing linear 86-mer precursor (Oligonucleotide L) catalyzed by T4 DNA ligase (Fig. 3b). Circularity of the backbone of the formed Oligonucleotide 1 was further confirmed via hydrolysis using T7 exonuclease (Fig. 4). The inhibitory effect of Oligonucleotide 1 on the activity of human Topo I was examined through determining the efficiency of relaxation reaction of pBR322 catalyzed by this DNA relaxing enzyme in the presence of our newly designed dumbbell-shaped oligonucleotides. As shown in Figure 5, the ratio of relaxed forms to non-relaxed forms of pBR322 decreased with the increase of concentration of Oligonucleotide 1 (Fig. 5b) and the obtained IC_{50} value for Oligonucleotide 1 was 33 nM (Fig. 7)[13] under our reaction conditions. As a control experiment, the inhibitory effect of Oligonucleotide 2 was also examined in our studies, which possesses the same sequence as that of Oligonucleotide 1 except for the absence of a C3-spacer in its duplex structure. As shown in Figure 5c, this non-C3-spacer-containing oligonucleotide displayed much low inhibitory efficiency and gave rise to an IC_{50} value as high as ~1.2 μM. The observed difference in inhibitory



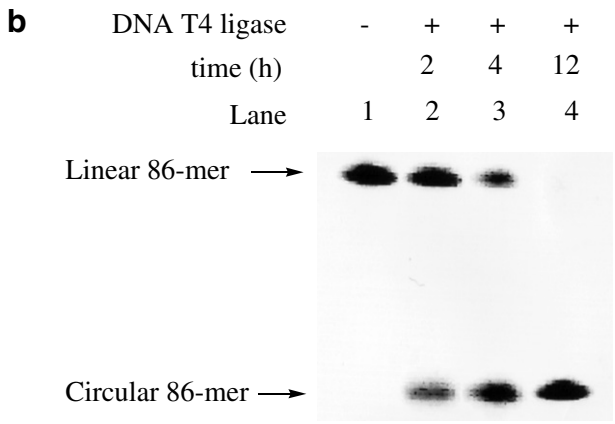


Figure 3. Synthesis and analysis of formation of Oligonucleotide 1. (a) Diagrammatic illustration of synthetic route toward Oligonucleotide 1 from its linear precursor (Oligonucleotide L). (b) Polyacrylamide gel electrophoretic analysis of formation of Oligonucleotide 1. Lane 1, linear precursor (Oligonucleotide L) alone; lanes 2–4, ligation reaction of Oligonucleotide L catalyzed by T4 DNA ligase lasting for 2, 4, and 12 h respectively.

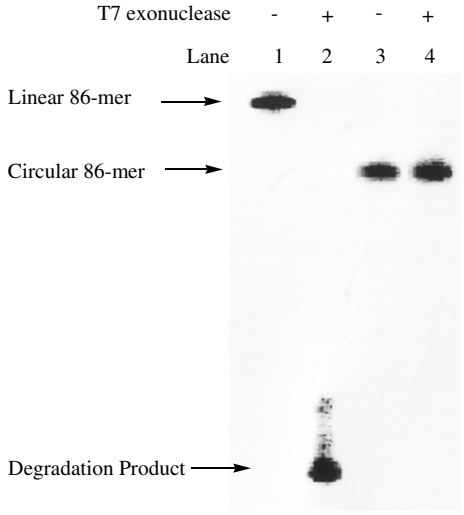


Figure 4. Polyacrylamide gel electrophoretic confirmation of circularity of Oligonucleotide 1 in its backbone. Lane 1, ^{32}P labeled 5′ phosphorylated 86-mer linear precursor (Oligonucleotide L) alone; lane 2, ^{32}P labeled 5′ phosphorylated 86-mer Oligonucleotide L in the presence of T7 exonuclease; lane 3, Oligonucleotide 1 alone; lane 4, Oligonucleotide 1 in the presence of T7 exonuclease.

efficiency between Oligonucleotide 1 and Oligonucleotide 2 could be taken as an indication that C3-spacer modification indeed plays a crucial role in the inhibitory action on the activity of Topo I as originally designed (Fig. 2).

To maintain the integrity of its genome and normal functioning, cell adopts intricate enzymatic systems for detecting and resolving DNA damages generated by environmental factors and normal metabolic processes.[14] A number of DNA repair enzymes such as those that recognize mismatch base pairs and nicked sites[11] have been identified in the past. One of our major intentions in placing a C3-spacer into Oligonucleotide 1 is to prevent its degradation by DNA repair proteins for taking place as neither mismatch base pairs nor nicked sites will be generated upon introduction of this modification. As model studies, T7 endonuclease I, a DNA repair protein that removes mismatch base pairs and nicked sites from duplex DNA sequences, was selected during our investigations. This DNA repair protein was accordingly allowed to react with a mismatch-containing dumbbell-shaped structure (Oligonucleotide 3),[15] which was consequently hydrolyzed into low molecular weight fragments within 1 h (lane 4 in Fig. 6). However, when Oligonucleotide 1 (C3-spacer-containing dumbbell-shaped structure) and Oligonucleotide 2 (regular dumbbell-shaped structure without modification) were incubated with T7 endonuclease I, no degradation product was generated (lane 6 and lane 2). These observations indicate that our newly designed C3-spacer-containing oligonucleotides could indeed resist DNA repair protein as expected.

With the purpose of determining the correlation between position of C3-spacers in the dumbbell-shaped structures and inhibitory efficiency of human Topo I, additional oligonucleotides containing C3-spacers at certain different locations were subsequently synthesized (Table 2) and examined. As shown in Table

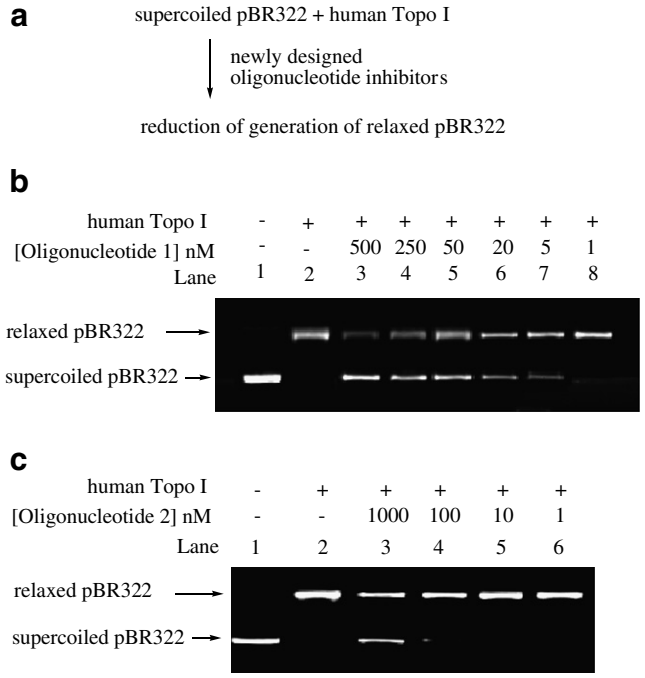


Figure 5. Studies of inhibitory effect of our newly designed oligonucleotides on the activity of human Topo I. (a) Schematic illustration of human Topo I-catalyzed DNA relaxation reaction that is inhibited by our newly designed oligonucleotides. (b) Agarose gel electrophoretic analysis of inhibitory effect of Oligonucleotide 1 on human Topo I. (c) Agarose gel electrophoretic analysis of inhibitory effect of Oligonucleotide 2 on human Topo I. Concentrations of Oligonucleotide 1 in lanes 2–8 in (b) were 0, 500, 250, 50, 25, 5 and 1 nM, respectively, while the sample in lane 1 is pBR322 alone. Concentrations of Oligonucleotide 2 in lanes 2–6 in (c) were 0, 1, 10, 100 and 1000 nM, respectively, and the sample in lane 1 is pBR322 alone.

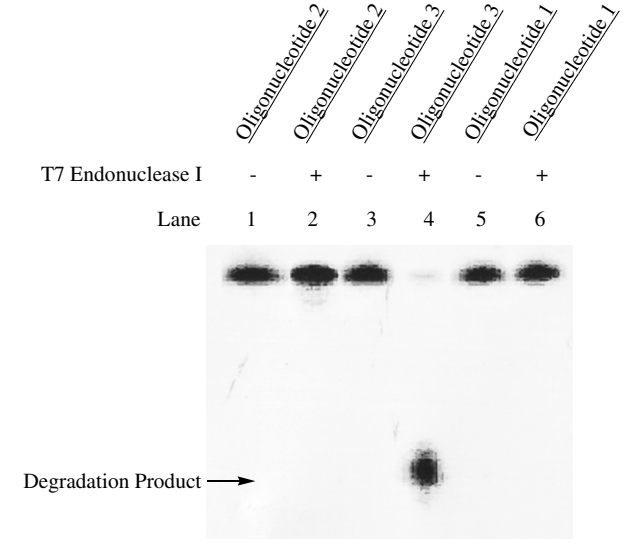


Figure 6. Polyacrylamide gel electrophoretic analysis of hydrolytic products of Oligonucleotide 1, 2, and 3 generated by T7 endonuclease I. Solutions containing 50 mM NaCl, 10 mM Tris–HCl (pH 7.9), 10 mM $MgCl_2$, 1 mM Dithiothreitol, 10 U of T7 endonuclease I and ^{32}P-labeled oligonucleotides (Oligonucleotide 1, 2, or 3) were incubated at 37 °C for 1 h. The hydrolytic reaction products were further analyzed using polyacrylamide gel electrophoresis (15%). Lane 1, 86-mer circular Oligonucleotide 2 alone; lane 2, 86-mer circular Oligonucleotide 2 in the presence of T7 endonuclease I; lane 3, 86-mer circular Oligonucleotide 3 alone; lane 4, 86-mer circular Oligonucleotide 3 in the presence of T7 endonuclease; lane 5, 86-mer circular Oligonucleotide 1 alone; lane 6, 86-mer circular Oligonucleotide 3 in the presence of T7 endonuclease I.

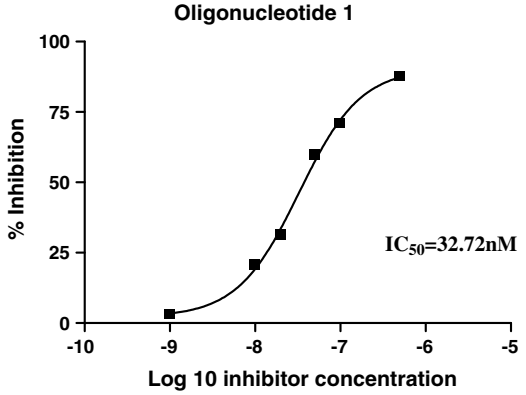


Figure 7. Correlations between concentration of Oligonucleotide 1 and its percent inhibition on topoisomerase I activity. Percentage of relaxation was defined as the ratio of band density of relaxed DNA over those of relaxed DNA plus supercoiled DNA [relaxed DNA/ (relaxed DNA + supercoil DNA)][13] while (100% – percentage of relaxation) was taken as the percent inhibition of topoisomerase I activity by Oligonucleotide 1. The DNA bands were quantified using Gel Documentation System (G:Box HR, Syngnene, Cambridge, UK) equipped with Gene Tools Software.

Table 1
Inhibitory efficiency (IC_{50}) of some C3-spacer-containing oligonucleotides on the activity of human Topo I[a]

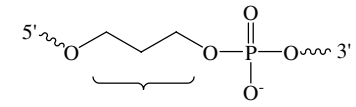
Names of oligonucleotides	IC_{50} (nM)
Oligonucleotide 4 (C3-spacer between G_{+1} to G_{+2})	89
Oligonucleotide 5 (C3-spacer between G_{+2} to A_{+3})	52
Oligonucleotide 1 (C3-spacer between A_{+3} to A_{+4})	33
Oligonucleotide 6 (C3-spacer between A_{+4} to A_{+5})	63
Oligonucleotide 7 (C3-spacer between A_{+5} to A_{+6})	93

[a] See Table 2 for detailed sequences of these oligonucleotides.

1, when a C3-spacer appeared near the Topo I-cutting site, the inhibitory efficiencies of the corresponding oligonucleotides decreased progressively (IC_{50} of Oligonucleotide 4 = 89 nM; IC_{50} of Oligonucleotide 5 = 52 nM while IC_{50} of Oligonucleotide 1 = 33 nM). This happened most likely because a C3-spacer, when it occurred near the binding site of Topo I, could decrease the binding affinity of the oligonucleotides toward this nuclear enzyme. On the other hand, when a C3-spacer emerged far off from Topo I-cutting site, the corresponding oligonucleotides displayed low inhibitory efficiency as well (IC_{50} of Oligonucleotide 6 = 63 nM; IC_{50} of Oligonucleotide 7 = 93 nM) as compared to Oligonucleotide 1 (IC_{50} = 33 nM). These decreases of inhibitory efficiency could be caused by that when these C3-spacers are located far away from the Topo I-cutting site, they will be in a position close to the second binding site of Topo I (Fig. 1), which could consequently prevent the binding of this nuclear enzyme to the corresponding oligonucleotides.

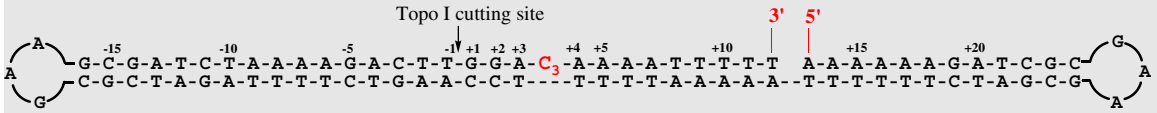
In conclusion, some C3-spacer-containing dumbbell-shaped oligonucleotides were designed and synthesized in our lab recently, which exhibited high inhibitory efficiency on the activity of human Topo I (e.g., IC_{50} of Oligonucleotide 1 = 33 nM). Moreover, these newly designed oligonucleotides resisted the hydrolysis by a certain mismatched base pair- and nick sites-resolving DNA repair protein, which could consequently enhance their chance of survival in cellular environments. It is our hope that the outcomes of the current studies could provide useful information for designing new oligonucleotide-based inhibitors of human Topo I in the future.

Table 2
Sequences and modifications of oligonucleotides prepared during our investigations

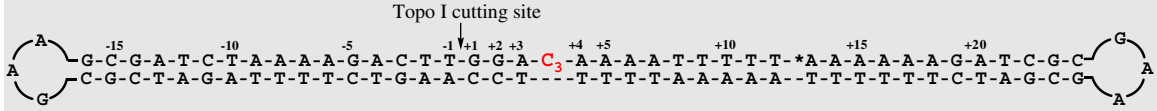


b C3-spacer

Oligonucleotide L (linear precursor)

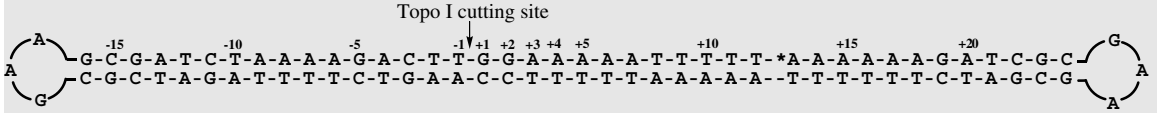


Oligonucleotide 1 (C3-spacer-containing circular oligonucleotide)

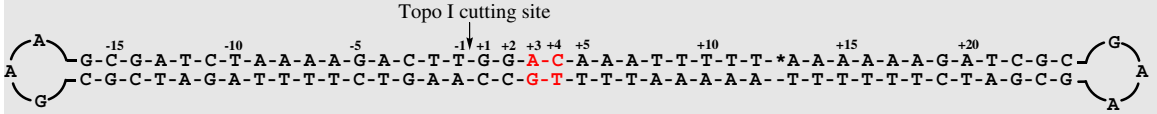


C3-spacer is positioned between A_{+3} and A_{+4}

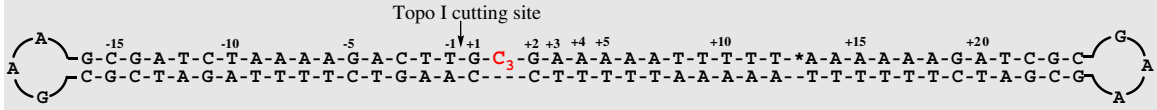
Oligonucleotide 2 (Circular oligonucleotide containing no C3-spacer modification)



Oligonucleotide 3 (Mismatch-containing circular oligonucleotide)

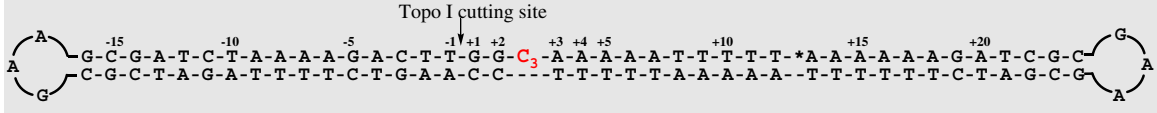


Oligonucleotide 4 (C3-spacer-containing circular oligonucleotide)



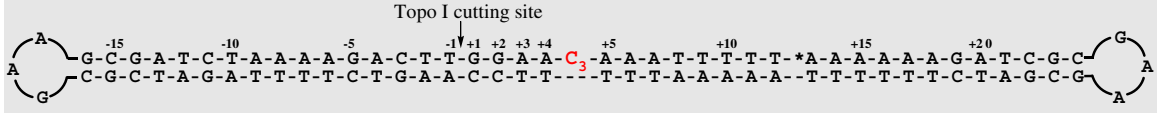
C3-spacer is positioned between A_{+1} and A_{+2}

Oligonucleotide 5 (C3-spacer-containing circular oligonucleotide)



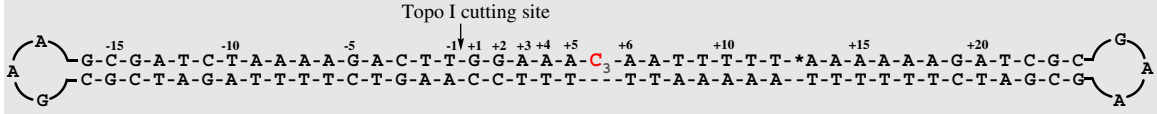
C3-spacer is positioned between A_{+2} and A_{+3}

Oligonucleotide 6 (C3-spacer-containing circular oligonucleotide)



C3-spacer is positioned between A_{+4} and A_{+5}

Oligonucleotide 7 (C3-spacer-containing circular oligonucleotide)



C3-spacer is positioned between A_{+5} and A_{+6}

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.04.076.

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