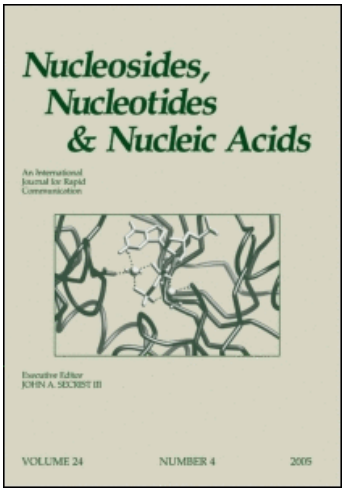

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A New Approach to Potentiate Site-Specific Hybridization: A set of Hydrophobic Heterobifunctional Short Oligodeoxyribonucleotides

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A NEW APPROACH TO POTENTIATE SITE-SPECIFIC HYBRIDIZATION: A SET OF HYDROPHOBIC HETEROBIFUNCTIONAL SHORT OLIGODEOXYRIBONUCLEOTIDES

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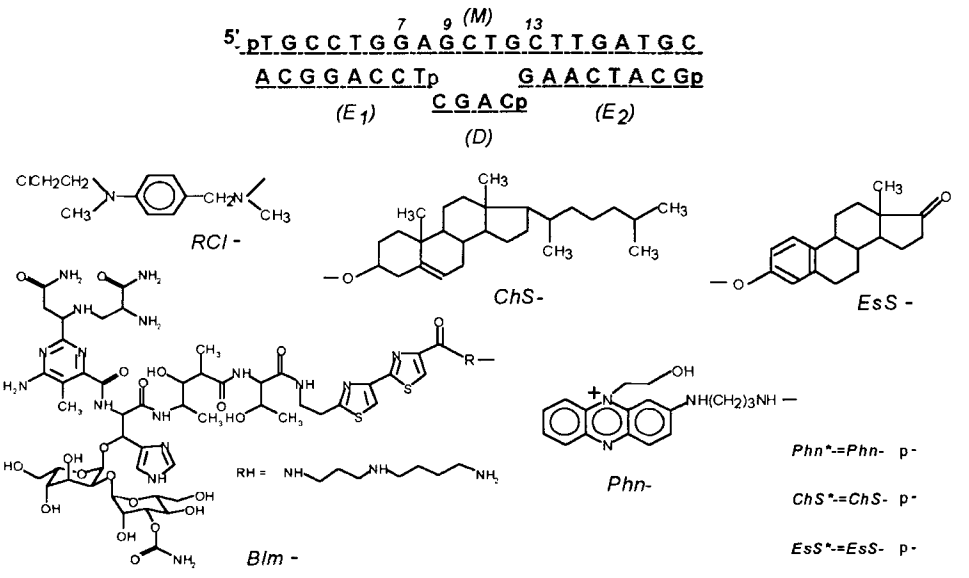
Abstract An effective approach to enhance the short oligonucleotide reagent to attack ssDNA based on the use of a set of short oligonucleotide heterobifunctional derivatives bearing steroid residues is proposed.

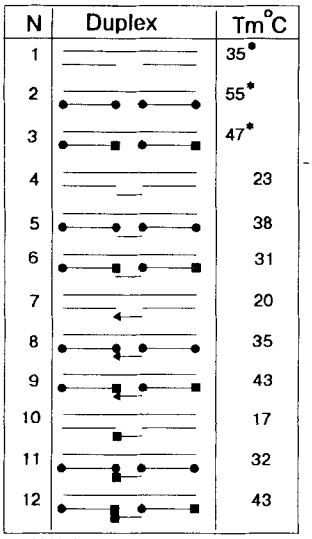
We have shown before that an alkylating reagent of short oligonucleotide - tetranucleotide - can modify effectively and site-specifically ssDNA fragment in the presence of effectors, these being diphenazinium derivatives of short oligonucleotides[1,2]. To improve the cell penetration of oligonucleotides, their hydrophobicity should be increased by attachment of steroid residues[3] . The goal of this work was to investigate the site-specific interaction between ssDNA and the tandemic set of short oligodeoxyribonucleotide derivatives - reagent and effectors -containing steroid residues.



The 20-mer oligonucleotide-target (*M*) was modified by alkylating and cleaving (bleomycin A_5) reagents on the basis of tetranucleotide (*D*) and its steroid derivatives.

The modification was carried on in the presence of effectors, these flanking the reagents and being octanucleotides (E_1 and E_2) or their derivatives containing both steroid and phenazinium or two phenazinium residues at terminal phosphates.

TABLE 1. Tm of duplexes of target *M* and tetranucleotide D and its derivatives EsS*-D and ChS*-D in the presence of effector pairs



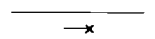
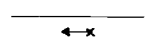
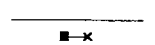
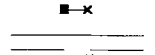
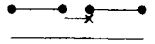
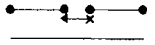
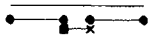
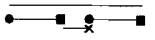
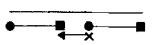
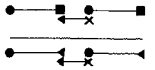
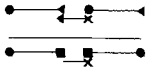
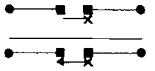
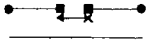
0.1M NaCl, 0.1 M sodium cacodylate (pH 7.4), 0.001 M EDTA, concentration of each component - 1.3×10^{-5} M
●- *Phn* ■- *ChS* ◄ - *EsS*
* - Tm of duplexes of *M* and octanucleotides and their derivatives.

The steroid residues were attached to tetra- and octanucleotides to increase their hydrophobicity and cell penetration. We used cholesterol (***ChS***) and estrone (***EsS***) as hydrophobic groups because they have different bulky and hydrophobic properties. The phenazinium residues (***Phn***) were linked to the effectors to increase their hybridizational properties[4]. The DNA-modification by an oligonucleotide reagent depends on stability of their duplexes. The attachment of the bulky hydrophobic groups to oligonucleotides may influence their hybridization properties and stability of tandemic duplexes. Therefore, Tm of duplexes formed by DNA-target and tetranucleotide (*M* + *D*) and its steroid derivatives (*M* + ***ChS****-D*, *M* + ***EsS****-D*) were at first found and the effector influence on their stability was investigated.

The data obtained (TABLE 1) show that octanucleotides and their derivatives formed stable complexes with DNA-target (dupl.1-3). The tetranucleotide *D* and its derivatives ***ChS****-D* and ***EsS****-D* which normally did not form complexes with target *M* (Tm < 7°C) did so in the presence of the effectors.

The strongest complexes examined were formed by the tetranucleotide derivatives ***ChS****-D* or ***EsS****-D* in the presence of the effectors containing both phenazinium and steroid (cholesteryl β-alanine) residues (dupl.9 and 12, Tm 43°C). This duplex stabilization may be produced by the hydrophobic interaction between the contiguous steroid residues of oligonucleotide derivatives: ***Phn****-E_1-**ChS*** and ***ChS****-D* or ***EsS****-D*. A similar stabilization has also been shown previously [5,6]. In contrast, the effector influence on the capability of tetranucleotide *D* to form duplex with DNA-target correlated to the hybridizational properties of the octanucleotides and their derivatives. The strongest complex of tetranucleotide *D* with the target *M* was produced with the diphenazinium derivatives (dupl.5, Tm 38°C), which formed themselves with target the most stable complex (Tm 55°C).

TABLE 2.Modification of target (% and site) by the reagents in the presence of effector pairs.

N	Duplex	% and site* a) X = *RCl*	b) X = *Bln*
13		trace	<10
14		trace	trace
15		0	0
16		5 (G9)	-
17		44 (G9)	35 (C10),10
18		20 (G9)	23 (C10), 8
19		6 (G9)	trace
20		24 (G9), 2 (G7)	21 (G7), 14
21		21 (G9), 3(G7)	25 (C10), 13
22		37 (G9), 4(G7)	11 (G7), 13
23		31 (G9), 3 (G7)	20 (C10), 9
24		0	0
25		0	trace
26		0	21 (C10), 24
27		trace	18 (C13), 17

X = *RCl* : 0,1 M NaCl, 0.01 M Tris-HCl (pH 7.2), 0.001 M EDTA, [*M*]=5 x 10^{-7} M, [reagent]=10^{-5} M, [effector]=10^{-5} M. *- after piperidine treatment.
X = *Bln* : 0,2 M LiCl, 0.01 M Tris-HCl (pH 7.5), [Fe^{2+}] = 5 x 10^{-5} M, [HSC_2H_4OH] = 0.05 M, [*M*]=5 x 10^{-7} M, [reagent]=2 x 10^{-6} M,[effector]= 10^{-5} M. *- without piperidine treatment.

Then we examined the ^{32}P-end labeled target ***M*** modification by reagents on the basis of the tetranucleotide and its steroid derivatives containing at the 5'-end phosphate as reactive groups either alkylating amine (***RCl***) or cleaving antibiotic bleomycin A_5 (***Blm***)[7] in the presence of different sets of effector pairs at 37°C. There were practically no products of interaction between 20-mer ***M*** and both types of reagents of tetranucleotide and its derivatives without the effectors (dupl.13-15).

The effect of the diphenazinium derivatives ***Phn*-E_1-Phn*** and ***Phn*-E_2-Phn*** was at first evaluated on the target ***M*** attack by reagents. In this case, the effector influence on a target modification by alkylating reagents as well as bleomycin ones were similar. The highest level of alkylation as well as cleavage of the target ***M*** was produced by the reagent on the basis of tetranucleotide ***D-RCl*** (44%) and ***D-Blm*** (45%) (dupl.17) in accordance with thermal denaturation data obtained. The attachment of the estrone residues to the reagents resulted in a decrease of modification levels by 1,5-2 fold. The poor target modification by cholesterol reagents ***ChS*-D-RCl*** and ***ChS*-D-Blm*** may be caused by spatial difficulties because of bulky cholesterol. Furthermore, it should be noted that the native octanucleotides were not helpful for modification. When used an alkylating reagent ***EsS*-D-RCl,*** the highest level of target modification took place in the presence of the effectors bearing steroid residues at 5'-end phosphates when steroid residues of reagent ***EsS*-D-RCl*** and effector ***Phn*-E_1-*St*** (*St*=***ChS**** or ***EsS****) became contiguous to each other (dupl.. 22a, 23a). The cholesterol effector pair ***Phn*-E_1-ChS*** and ***Phn*-E_2-ChS*** was most beneficial (dupl.22a). Nevertheless, a vicinity of the alkylating group of reagent ***EsS*-D-RCl*** and steroid residue of ***St*-E_2-Phn*** was fatal for modification DNA (dupl.24a-27a). In all cases, the target alkylation resulted in the modification of only one base G9, practically. Thus, for an alkylation DNA-target the set of short oligonucleotide derivatives ***Phn*-E_1-ChS + EsS*-D-RCl + Phn*-E_2-ChS*** was the most optimal set.

The DNA cleavage was realized by reagents containing bleomycin group (***D-Blm*** and ***EsS*-D-Blm***) by a more intricate way. It may be due to the properties of the bleomycin: larger

bulk and hydrophobicity in comparison with the alkylating residue and, moreover, it is able to bind with DNA. The data obtained indicate that estrone effector pair was more beneficial than cholesterol one (dupl. 20b-23b). The attachment of the estrone residue to reagent, which causes an additional hydrophobic interaction between reagent and effector ***Phn*-E_1-*St***, somewhat decreased the target modification. However, the vicinity of residues, ***Bln*** of reagent and ***EsS*** of effector ***EsS*-E_2-*Phn***, significantly improved the level of target modification in the contrast with DNA alkylation under the same conditions (dupl.26,27). Change of the set of reagent and effectors resulted in change of the main site of DNA cleavage.

Despite some differences of DNA-target modification by the reagent containing either alkylating or bleomycin residues we can infer that the reagent on the basis of short oligonucleotides is capable to successfully attack DNA target even at 37^{o}C in the presence of the effectors flanking it. The oligonucleotides containing not only polyaromatic groups but and steroid residues may be used as effectors.

Thus, the proposed set of bifunctional derivatives of short oligonucleotides bearing steroid residues (reagent and effectors) seems beneficial for use them as therapeutic drugs. The components of the set could have capability for successful cell penetration due to steroid residues, nuclease resistance due to groups masking terminal phosphates, and therefore, could attack intracellular DNA.

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