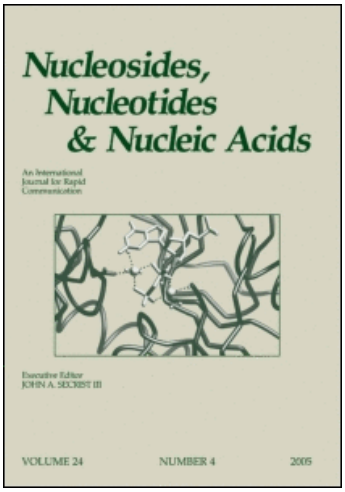

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Steroid and N-(2-Hydroxyethyl)phenazinium Oligodeoxynucleotides

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STEROID AND N-(2-HYDROXYETHYL)PHENAZINIUM OLIGODEOXYNUCLEOTIDES

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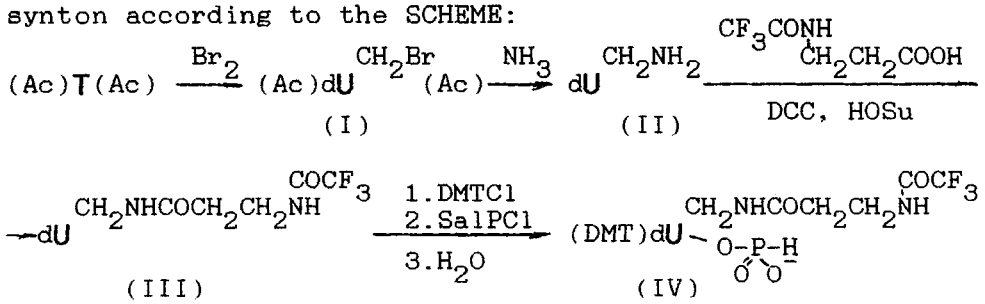
*Abstract.*Oligonucleotide derivatives carrying steroid and N-(2-hydroxyethyl)phenazinium (Phn) residues were synthesized, and tested for the ability to form complexes.

It was shown elsewhere that an N-(2-hydroxyethyl)phenazinium residue (Phn) stabilizes the corresponding complementary duplexes[1], while a steroid residue enhances permeability into cells[2]. These groups were attached to the terminal[1,2] or internucleotide phosphates[3]; steroids were introduced only in blocked oligonucleotides.

Here we describe a method for the synthesis of oligonucleotide derivatives carrying a Phn residue at C-5-modified thymine and a method for the coupling of a steroid residue to the 5'-phosphate of deblocked oligonucleotides; the ability of the synthesized derivatives to form complementary complexes was assayed.

To couple the modified thymidine residue to the oligonucleotide, we synthesized the corresponding H-phosphonate synton according to the SCHEME:



DMTCl, dimethoxytritylchloride; DCC, dicyclohexylcarbodiimide; HOSu, N-hydroxysuccynimide; SalPCl, salicylchlorophosphite

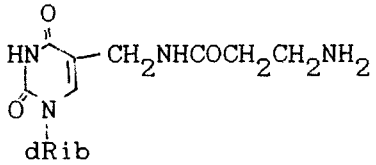
Compound I was prepared by bromination of the blocked thymidine[4]. PMR-spectra of compound III revealed CH_2-group protons (δ=4.3 and 2.5 ppm) in addition to deoxyuridine protons. H-phosphonate of the modified deoxyuridine (IV) was obtained according to the routine method[5]. Using standard H-phosphonate monomers and IV and Victoria-6M synthesizer[6] the following hexadeoxynucleotides were obtained:

TTCCCA

$dU^{NH_2}TCCCA$ (V)

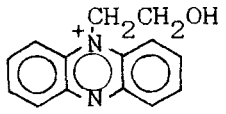
$TdU^{NH_2}CCCA$ (VI)

dU^{NH_2} =



Complete phosphodiesterase digestion gave dU^{NH_2}, T, C, A for V and TdU^{NH_2}, C, A for VI in correlation with the expected ones. Phn residue was coupled to the amino groups of the modified d-uridine of the deblocked and isolated oligonucleotides under the condition described[1]. For example,

$TdU^{NH_2}CCCA \longrightarrow TdU^{NHPhn}CCCA$ Phn=



Quantitative yield of Phn derivatives was achieved in 10 min. The absorbance peaks (237, 268, 290 (shoulder), 400 and 530 nm) were observed in electron spectra of the RPC-purified compounds in agreement with the available data[1]. The ability of the obtained hexanucleotides to form complexes was tested. Table 1 shows that if Phn is in heterocyclic base, T_m of the duplex rises but not as drastically as in the case of Phn introduced to the 5′-phosphate.

Recently the cholesterol esters of oligonucleotides (ChS-pN_n) have been synthesized by phosphotriester method[2]. Here we elaborated a method for introduction of cholesterol

TABLE 1

T_m °C of the complexes TGAATGGGAAGA · X

X	T_m	X	T_m
TTCCCA	16	dU^{NHPhn}TCCCA	25
PhnNHCH$_2$NH-pTTCCCA	34	TdU^{NHPhn}CCCA	22

Oligonucleotide concentration was equal to $2 \cdot 10^{-5}$ M in a buffer: 0.16M NaCl, 0.02M Na_2HPO_4, 0.1mM EDTA, pH 7.4

TABLE 2

T_m° C of X Y complexes

X	Y	T_m	X	Y	T_m
pT_{10}	Y_1	32	pTGACCCTCTTCCCA	Y_2	50
ChS-pT_{10}	Y_1	35	ChS-pTGACCCTCTTCCCA	Y_2	54
pT_{10}p-TS	Y_1	29	TTCCCA	Y_3	16
pT_{10}p-ES	Y_1	30	ChS-pTTCCCA	Y_3	23
pT_{10}p-ChS	Y_1	29	ChSNH ~ pTTCCCA	Y_3	20
Phn ~NH-pT_{10}p-ChS	Y_1	39			

ES, ergosterol; TS, testosterone; ChS, cholesterol; Phn NH-, Phn-NH(CH$_2$)$_3$NH-; Y_1, pA_{16}; Y_2, TTGAATGGGAAGAGGGTCAGGTT; Y_3, TGAATGGGAAGA; melting condition as indicated in TABLE 1.

residue (ChS) to deblocked oligonucleotides. Cholesterol ester of β-alanine, ChS-OCOCH$_2$CH$_2$NH$_2$ (ChS ~ NH$_2$), was synthesized and coupled to the activated oligonucleotide:

$$pN_n \xrightarrow[2.\text{ChS}^{\sim}\ NH_2]{1.\ Ph_3P,\ Py_2S_2,\ MeIm} \text{ChS}^{\sim}\text{NHp}N_n$$

A number of oligonucleotides up to 16 units were synthesized with a 70-80% yield. RPC-purification revealed high

hydrophobicity of the products (elution in 80% CH_3CN), which is in agreement with the data on cholesterol esters of oligonucleotides described earlier[2].

TABLE 2 presents the data on the effect of steroid on the oligonucleotide ability to form complexes. It follows that this ability does not, in fact, depend on the nature of steroid.

Introduction of a steroid residue to the 3′-phosphate of oligonucleotide slightly decreases stability of the complexes; in the case of the 5′-cholesterol oligonucleotide derivatives, even some stabilization of the duplex is observed irrespective of the mode of attechment of the ChS-residue (P-O or P-N bond). Phn attached to the 5′-phosphate of the 3′-cholesterol ester of decathymidylate caused a considerable increase in T_m of the corresponding duplex.

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