Synthesis and properties of oligodeoxyribonucleotides containing 2′-*O*-(2,3-dihydroxypropyl)- and 2′-*O*-(2-oxoethyl)arabinouridine residues*

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2′-*O*-(2,3-Dihydroxypropyl)arabinouridine-containing oligodeoxyribonucleotides were synthesized starting from a new modified nucleoside, *viz.*, 2′-*O*-(2,3-dihydroxypropyl)arabinouridine, and the corresponding 3′-phosphoramidite. Oxidation of these oligodeoxyribonucleotides with sodium periodate afforded oligonucleotides containing 2′-*O*-(2-oxoethyl)arabinouridine residues. Subsequent modification of the aldehyde-containing oligonucleotides involved the reactions with 9-hydrazinoacridine and *N*-aminooxyacetyl peptide and reductive amination by 4-(1-pyrenyl)butyrohydrazide and biotin hydrazide. Thermal stabilities of duplexes of modified oligodeoxyribonucleotides with complementary oligodeoxyribonucleotides are slightly lower than those of natural duplexes. Duplexes with complementary oligoribonucleotides are substantially destabilized.

Key words: modified oligonucleotides, duplexes.

Oligonucleotides including modified oligonucleotides find wide application in various fields of molecular biology. They are used as primers for DNA sequencing and amplification, for the determination of DNA primary structures by hybridization, and also as potential therapeutics.[2,3] In particular, modified oligonucleotides are employed for the preparation of conjugates with various compounds, such as peptides, oligosaccharides, and fluorescent dyes for studying the behavior of nucleic acids *in vitro* or *in vivo*. Affinity modification of DNA-binding proteins is used to study the structures and functioning of the active sites of the latter.[4,5]

Conjugation of fluorophores or electrochemically active compounds to oligonucleotides enables the use of such derivatives in the design of DNA detection systems by hybridization.[6—8] Marker groups substantially increase sensitivity of DNA detection. Many conjugates of oligonucleotides with peptides, carbohydrates, and lipophilic compounds more easily penetrate cell membranes than native nucleic acids, and this fact can be used in antisense and antigene biotechnology.[9—11]

Covalent attachment of proteins to nucleic acids is the method of choice for solving a series of problems. First and foremost, it allows identification of the amino acids interacting with particular regions of nucleic acids in nucleic acid—protein complexes. In studies of multiprotein complexes formed during the replication, translation, *etc.*, one can determine a protein component interacting with a region of nucleic acid recognition[12] and examine cell extracts with the aim of revealing proteins that selectively interact with particular nucleotide sequences. This approach is also applicable in the SELEX technology for the design of nucleic acid aptamers that irreversibly bind to proteins.[13,14] In recent years, considerable progress has been achieved in crystallization of covalent nucleic acid—protein complexes and elucidation of their structures by X-ray diffraction.[15]

Earlier, we have demonstrated that 2′-*O*-(2-oxoethyl)uridine-containing DNA duplexes can successfully be used for the affinity modification of DNA-binding proteins, such as transcription factor NF-κB[16] and methyl transferase SsoII.[17] The reactive aldehyde group can selectively react with the adjacent ε-amino group of lysine in proteins under conditions of reductive amination.

Modifications of a ribose residue at position 2 generally cause only small distortions in the oligonucleotide structure and allow the preparation of nucleosides containing carbohydrate residues in both the C(2′)-*endo* ($^2T_3$) and C(2′)-*exo* conformations ($^3T_2$) and direction of the substituent into either the minor or major groove of

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the nucleic acid duplex.[18] In 2′-deoxy-2′-fluoro- and 2′-*O*-alkylribonucleotides, the sugar residue adopts the stable C(3′)-*endo* conformation ($^3T_2$), the alkyl groups protruding into the minor groove of the duplex.[19—21] On the contrary, stabilization of the C(2′)-*endo* conformation is typical of 2′-*S*-alkyl-2′-thio- and 2′-amino-2′-deoxyribonucleotides.[22,23] To prepare nucleosides in a required conformation, one can not only vary the nature of the 2′-substituent but also use the *arabino* epimer. It has been demonstrated[24,25] that arabino- and 2′-*O*-alkylarabinooligonucleotides adopt the C(2′)-*endo* conformation and give B-form duplexes with DNAs. In this case, 2′-substituents protrude into the major groove of the DNA duplex. At the same time, the insertion of a 2′-*O*-methylarabinonucleotide unit into the DNA duplex leads to a slight decrease in its melting point compared to the nonmodified duplex.[26,27]

The aim of the present study was to introduce a modified unit containing the 2′-aldehyde group into a DNA duplex. The substituent would be expected to protrude into the major groove of the DNA duplex and cause no noticeable distortions of its local structure. We chose 2′-*O*-(2-oxoethyl)arabinouridine as a modified nucleoside.

When interacting with a DNA duplex, many DNA-binding proteins recognize a particular nucleotide sequence and bind to this sequence without unwinding the double helix. By comparing the results of covalent attachment of a protein to 2′-*O*-(2-oxoethyl)uridine- and 2′-*O*-(2-oxoethyl)arabinouridine-containing DNA duplexes, one can determine whether the reactive lysine residue is located on the side of the major or minor groove. Study by NMR spectroscopy demonstrated that sterically hindered 2′-*O*-alkylarabinonucleotides produce no substantial distortions of the DNA B form.[25]

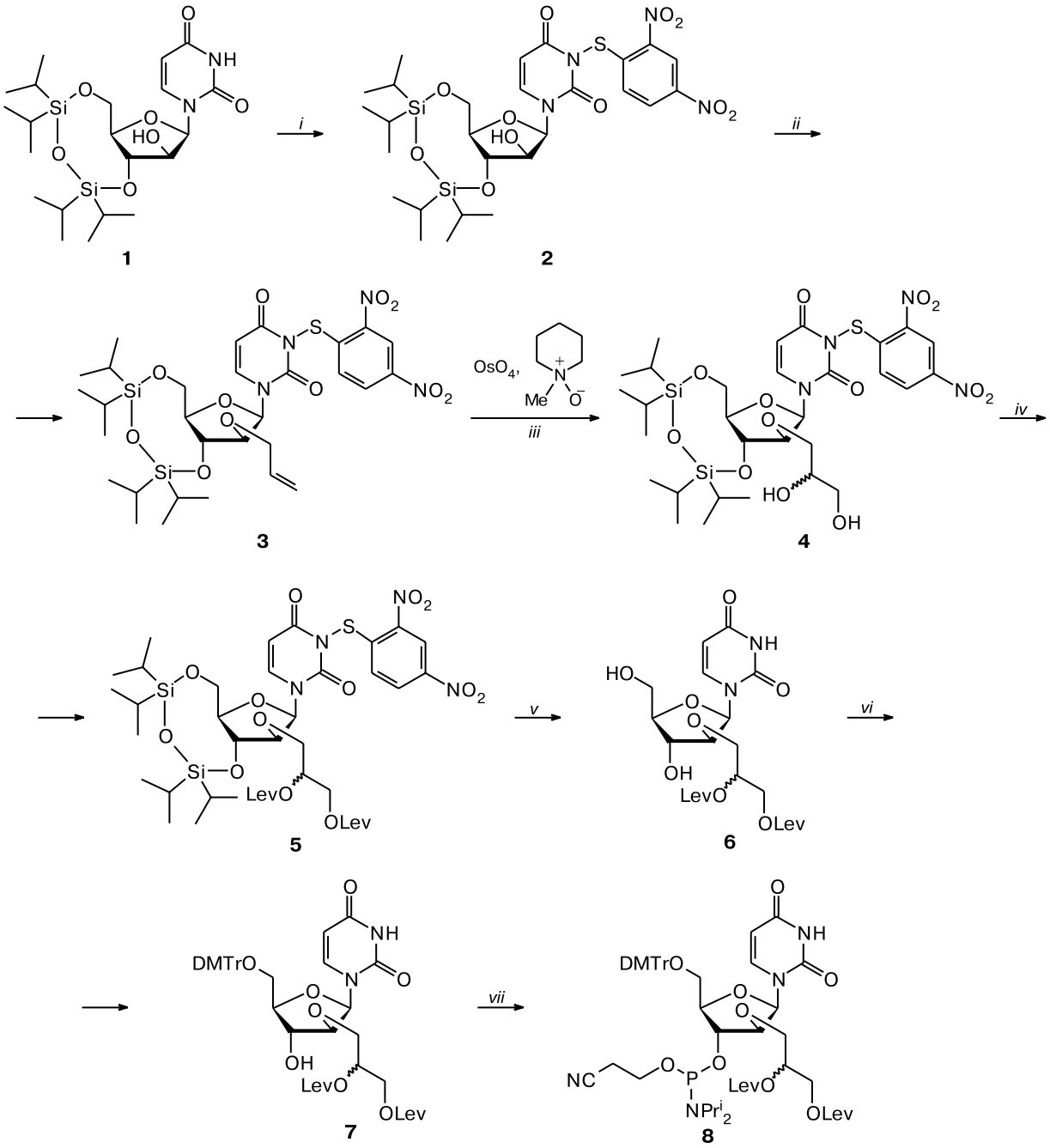
We prepared 2′-*O*-(2-oxoethyl)oligonucleotides using conventional periodate oxidation of the 1,2-diol group in the corresponding precursor.[28,29] To retain the C(2′)-*endo* conformation ($^2T_3$) of the carbohydrate fragment, 2′-*O*- or 2′-*S*-alkyl derivatives may be used. Since sulfides are oxidized with sodium periodate to give sulfoxides,[30] we used a 2′-*O*-alkylarabino derivative. Synthesis of 2′-modified oligonucleotides involves the preparation of a phosphoramidite of a modified nucleoside containing the protected 1,2-diol group and automated synthesis followed by removal of the protecting groups.

Results and Discussion

The synthesis of 1-[2-*O*-(2,3-dihydroxypropyl)-β-D-arabinofuranosyl]uracil derivatives is presented in Scheme 1. The starting compound **1**[31] was transformed into the *N*(3)-(2,4-dinitrophenylsulfenyl) derivative with transient trimethylsilylation of the 2′-OH group.[32] Allylation of *N*(3)-protected arabinouridine **2** was carried out in the presence of 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BDDDP).[33] Hydroxylation of the double bond of the allyl group of nucleoside **3** with osmium tetraoxide in the presence of *N*-methylmorpholine *N*-oxide[34] was carried out under the optimum conditions found in our earlier investigation.[35] Under these conditions, the 5,6-double bond of the heterocyclic base remains intact, which made it possible to prepare the target product **4** in high yield. The diol group in compound **4** was protected by acylation with levulinic anhydride in the presence of DMAP. Levulinic anhydride was generated from levulinic acid in the presence of *N*,*N*′-dicyclohexylcarbodiimide immediately before the reaction. The levulinoyl protecting group was chosen because it can be selectively removed with hydrazinium acetate. This makes it possible to subsequently generate the free aldehyde group in a completely protected oligonucleotide immobilized on a polymer support and perform solid-phase reactions, for example, in the preparation of combinatorial libraries and for the coupling with hydrophobic compounds.[36] Attempts to remove the silyl or dinitrophenylsulfenyl protecting groups in compound **5** by the reaction with Bu^n_4NF in THF[37] led to removal of the levulinoyl protecting groups as well. Therefore, we used triethylamine trihydrofluoride as a milder desilylating agent.[38] The synthesis of 5′-*O*-(4,4′-dimethoxytrityl) derivative **7** followed by its 3′-phosphitylation was carried out according to standard procedures.[39] The phosphitylation time was increased to 2 h, which was associated with steric hindrance of the 3′-hydroxy group in compound **7**.

Phosphoramidite derivative **8** was used in the automated oligonucleotide synthesis. In the step of coupling of the modified unit, the reaction time was increased to 15 min, and the condensation was carried out twice. Products of the oligonucleotide synthesis were cleaved from the polymer support and all alkali-labile protecting groups were removed with concentrated aqueous ammonia at 55 °C. The structures of analogs of dodecadeoxyribonucleotide **I** containing one or two 2′-*O*-(2,3-dihydroxypropyl)arabinouridine residues, *viz.*, dodecadeoxyribonucleotides **II—IV**, are given in Table 1. Oligodeoxyribonucleotides **I—IV** are complementary to the region located in the TAR hairpin structure of HIV-1 mRNA, which will allow one to study the effect of the attached molecules (nonradioactive labels, peptide fragments) on specific binding of oligodeoxyribonucleotides to a target and on the ability to inhibit Tat-dependent transcription of HIV-1 DNA *in vitro*.[40] 5′-Dimethoxytrityl derivatives of modified oligonucleotides **I—IV** were isolated by reversed-phase HPLC. The purity of detritylation products was monitored by ion-pair reversed-phase HPLC. The oligonucleotides were characterized by matrix-assisted laser desorption ionization time-of-flight (MALDI TOF) mass spectrometry (Table 2).

Scheme 1



i. 1) Me_3SiCl, pyridine, 2) DnbsCl, pyridine, 3) aqueous NH_3, pyridine; *ii*. $H_2C{=}CHCH_2Br$, BDDDP, MeCN; *iii*. Aqueous acetone; *iv*. $(MeCOCH_2CH_2CO)_2O$, DMAP, pyridine—CH_2Cl_2; *v*. $Et_3N(HF)_3$, THF; *vi*. DMTrCl, pyridine; *vii*. $Pr^i{}_2NP(Cl)OCH_2CH_2CN$, $EtNPr^i{}_2$, CH_2Cl_2.

Investigation of the effect of the modified units on the stability of duplexes with complementary DNA and RNA fragments (see Table 1) revealed a slight decrease in the melting point for complementary DNA and a more substantial decrease in the melting point for the duplex with RNA. Recall that we used a uridine derivative, whereas a single substitution of 2′-deoxyuridine for thymidine in DNA leads to a decrease in the melting temperature of the duplex by 0.5 °C.[41] Our results are in good agreement with the data obtained earlier[26,27] for the modified oligonucleotide containing the 1-(2-*O*-methyl-β-D-arabinofuranosyl)thymine residues where the melting tem-

Table 1. Thermal stability of duplexes formed by 2′-modified oligonucleotides with complementary DNA and RNA

Oligonucleotide[a]	$T_m \pm 0.5$/°C	
	TGAGCCTGGGAG	r(UGAGCCUGGGAG)
CTCCCAGGCTCA (**I**)	56.0	58.6
CTCCCAGGCUCA (**II**)	52.9[b] (53.1)[c]	49.5[b] (48.7)[c]
CUCCCAGGCTCA (**III**)	50.3[b] (55.2)[c]	42.3[b] (49.5)[c]
CUCCCAGGCUCA (**IV**)	49.1[b] (47.0)[c]	40.9[b] (41.0)[c]

[a] U is a modified unit.
[b] The melting point of the duplex in which U is 2′-*O*-(2,3-dihydroxypropyl)arabinouridine.
[c] The melting point of the duplex in which U is 2′-*O*-(2-oxoethyl)arabinouridine.

Table 2. Yields and mass-spectrometric data (MALDI-TOF) for 2′-modified oligonucleotides and related conjugates

Conjugated moiety	Yield (%)			MS, *m/z*, found / calculated for $[M + H]^+$		
	II′	**III′**	**IV′**	**II′**	**III′**	**IV′**
OH*	—	—	—	3643.4	3643.4	3719.4
				3645.1	3641.3	3718.3
Biotin hydrazide	74	79	69	3853.7	3853.7	4140.0
				3853.9	3855.1	4142.1
4-(Pyren-1-yl)butyrohydrazide	82	—	74	3897.3	—	4228.1
				3893.0		4223.5
9-Hydrazinoacridine	84	85	62	3802.6	3802.6	4036.8
				3800.9	3803.0	4038.1
H_2NOCH_2CO-DPGYIGSR-NH_2	65	—	50	4528.3	—	5490.3
				4531.0		5489.2

* OH are oligonucleotides containing 2′-*O*-(2,3-dihydroxypropyl)arabinouridine.

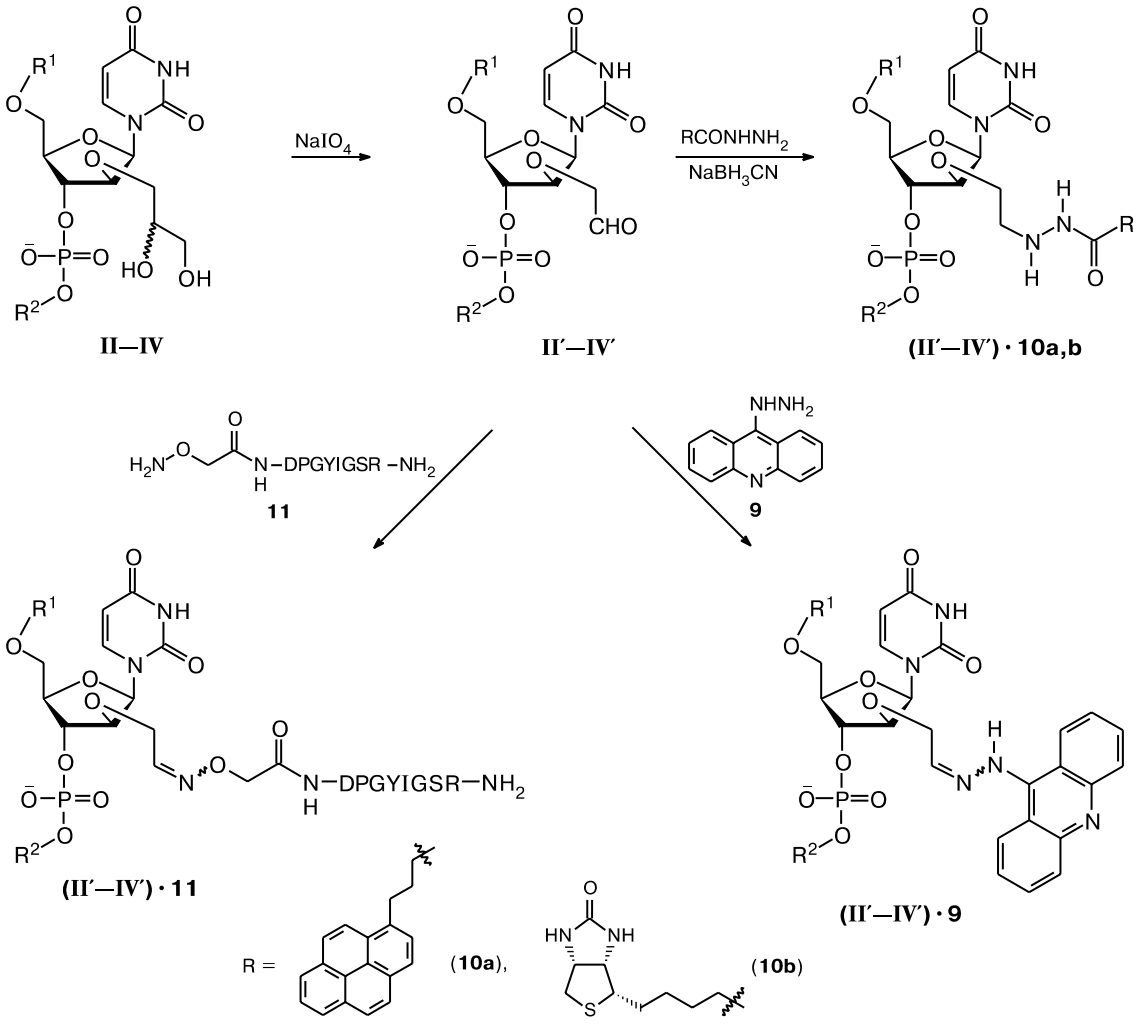
perature of the duplexes with complementary DNA and RNA fragments decreased by 3.0 and 4.5 °C, respectively. Earlier, it has been demonstrated[24] that 2′-*O*-methylarabinonucleosides adopt the C(2′)-*endo* conformation and thus their introduction into oligodeoxyribonucleotides causes almost no changes in the local structure, and the resulting duplexes with DNA have the classical B form. On the contrary, hybrid DNA-RNA duplexes are structurally similar to the A form due to conformational lability of DNA.[42] The bulky 2′-*O*-alkyl substituent in arabinouridine rather rigidly fixes a local conformation of the oligonucleotide and, correspondingly, hinders the conformational transition of the modified duplex (B → A). It should also be taken into account that the 2,3-dihydroxypropyl group can hinder the formation of a duplex with RNA due to both steric effects and partial displacement of water molecules associated with the modified oligonucleotide.

The 1,2-diol group at position 2′ of a carbohydrate fragment of oligonucleotides **II—IV** was oxidized to the aldehyde group with a ~1000-fold excess of sodium periodate in a weakly acidic medium (Scheme 2). Periodate oxidation afforded modified aldehyde-containing oligonucleotides **II′—IV′**.

Then we synthesized conjugates of oligonucleotides **II′—IV′** with 9-hydrazinoacridine (**9**), 4-(pyren-1-yl)butyrohydrazide (**10a**) (fluorescent labels), biotin hydrazide (**10b**) (marker group), and *N*-aminooxyacetyl peptide (**11**) (see Scheme 2). Peptide **11** is an *N*-modified fragment of the B1 domain of laminin (926—933)[43] and ensures binding to several types of receptors on the cell surface.

The reactions were carried out in 50% aqueous DMSO at pH 4.5—5.0. The results of HPLC analysis of the conjugate of oligonucleotide **II′** with 4-(pyren-1-yl)butyrohydrazide (**10a**) (Fig. 1, *a*) and the results of mass-spectrometric analysis confirmed the formation of the conjugate **II′·10a** (Fig. 1, *b*). It should be noted that the conjugates of the modified oligonucleotides with aromatic hydrazine (**II′·9—IV′·9**) and *N*-aminooxy peptide (**II′·11—IV′·11**), unlike conjugates with hydrazides, are stable under the reaction conditions and under condi-

Scheme 2



R^1, R^2 are deoxyribonucleotides

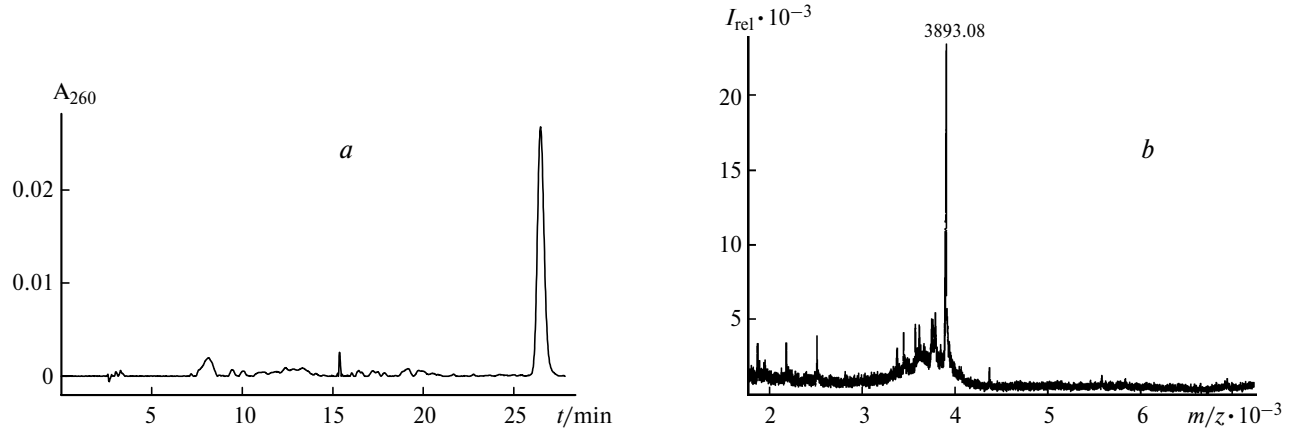


Fig. 1. (*a*) HPLC analysis of the reaction mixture after the reaction of oligonucleotide **II´** with 4-(pyren-1-yl)butyrohydrazide (**II´ · 10a**) and (*b*) the MALDI TOF mass spectrum of conjugate **II´ · 10a**.

tions of ion-pair reversed-phase HPLC and do not require reduction. This fact agrees well with the literature data.[44] The conjugates of compounds **9—11** were isolated by reversed-phase HPLC and analyzed by ion-pair reversed-phase HPLC and MALDI TOF mass spectrometry (see Table 2).

To summarize, we synthesized a new modified arabinouridine derivative containing the 1,2-diol group at position 2′ of the ribose, 1-[2-*O*-(2,3-dihydroxypropyl)-β-D-arabinofuranosyl]uracil, and its 3′-phosphoramidite. Two new types of modified oligonucleotides containing the 1,2-diol or aldehyde group at position 2′ of the arabinouridine residue were synthesized. The reactivity of the aldehyde group in the arabinonucleoside is virtually identical to that of the aldehyde group in the ribonucleoside.[45] Duplexes based on modified oligonucleotides can be used for affinity modification of DNA-binding proteins because the introduction of 2′-*O*-(2-oxoethyl)arabinouridine into the oligomer chain causes no essential changes in local structures of nucleic acids.

Experimental

We used the following reagents: 3′-(2-cyanoethyl-*N,N*-diisopropyl)phosphoramidites of 5′-*O*-dimethoxytrityl-2′-deoxyribonucleosides, 5-*S*-ethylthiotetrazole (Glen Research, USA), 1-(β-D-arabinofuranosyl)uracil (Sigma, USA), β-cyanoethyl *N,N*-diisopropylphosphoramidochloridite, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, 2,4-dinitrobenzenesulfenyl chloride, triethylamine, chlorotrimethylsilane, 4-(*N,N*-dimethylamino)pyridine, 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BDDDP), allyl bromide, DMSO (Fluka, USA), *N*-methylmorpholine *N*-oxide, osmium tetraoxide, triethylamine trihydrofluoride, tetrabutylammonium fluoride, 4,4′-dimethoxytrityl chloride, *N,N*-diisopropylethylamine, levulinic acid, *N,N′*-dicyclohexylcarbodiimide, sodium periodate, 9-hydrazinoacridine, sodium cyanoborohydride, lithium perchlorate (Aldrich, USA), 4-(1-pyrenyl)butyrohydrazide, biotin hydrazide (Pierce, USA), sodium hydrocarbonate, sodium carbonate, sodium sulfate, sodium chloride, sodium acetate, 30% aqueous ammonia, ethanol, acetic acid, dichloromethane, pyridine, tetrahydrofuran, acetone (Reakhim, Russia), and acetonitrile (Cryochrom, Russia). The peptide *N*-aminooxyacetyl-DPGYIGSR-amide was synthesized according to a known procedure.[45] Thin-layer chromatography was carried out on Kieselgel 60 F_{254} plates (Merck, Germany). Column chromatography was performed on silica gel 33—70 (BDH, UK). The ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker DRX-500 instrument in $CDCl_3$ and DMSO-d_6, using residual $CHCl_3$ and DMSO, respectively, as the internal standards. The ^{31}P NMR spectra were measured with H_3PO_4 as the external standard. The MALDI TOF mass spectra were obtained on a Voyager DE instrument (PerSeptive Biosystems, USA). Solutions of 2,4,6-trihydroxyacetophenone (10 mg mL^{-1} in methanol) or 2,5-dihydroxybenzoic acid (10 mg mL^{-1} in methanol) were used as matrices in mass-spectrometric analysis. A freshly prepared mixture of solutions of 2,6-dihydroxyacetophenone (40 mg mL^{-1} in methanol) and ammonium hydrocitrate (80 mg mL^{-1} in water) (1 : 1, v/v) was used for oligonucleotides. Elemental analysis was carried out on a Thermosinigan CHNS-Analyzer/EA1112 instrument. The absorbance and UV spectra were recorded on a Varian Cary 50 single-beam spectrophotometer (USA) in quartz cells with a 1 cm optical path length.

1-[3,5-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]-3-(2,4-dinitrophenylsulfenyl)uracil (2). Compound **1** (2.85 g, 5.9 mmol), which was prepared according to a known procedure,[30] was dried by coevaporation with pyridine (3×10 mL) and dissolved in anhydrous pyridine (90 mL). Then Me_3SiCl (17.7 mmol, 7.2 mL) was added with stirring at room temperature under argon. After 30 min (TLC control; $CHCl_3$—EtOH, 9 : 1, R_f 0.8), 2,4-dinitrobenzenesulfenyl chloride (DnbsCl) (9.4 mmol, 2.2 g) was added to the reaction mixture. After 2 h (TLC control; $CHCl_3$—EtOH, 95 : 5, R_f 0.5), a concentrated ammonia solution (8.8 mL) and water (12.3 mL) were added. The completeness of detrimethylsilylation was monitored by TLC ($CHCl_3$—EtOH, 9 : 1, R_f 0.5). After 30 min, the reaction mixture was concentrated and the residue (oil) was dissolved in EtOAc (100 mL). The solution was washed with a saturated aqueous $NaHCO_3$ solution (50 mL) and water (2×50 mL) and dried with anhydrous Na_2SO_4. The solvent was removed, the residue was dissolved in a minimum volume of chloroform, and hexane (100 mL) was added. The precipitate of bis(2,4-dinitrophenyl) disulfide that formed was filtered off. The target nucleoside was isolated from the filtrate by column chromatography (gradient of ethyl acetate in benzene 0→20%). The yield of compound **2** was 2.68 g (67%).

Found (%): C, 47.71; H, 5.37; N, 8.21; S, 4.81. $C_{27}H_{40}N_4O_{11}SSi_2$. Calculated (%): C, 47.35; H, 5.89; N, 8.18; S, 4.68. ^{1}H NMR ($CDCl_3$), δ: 8.78 (s, 1 H, $H_{Ar}(3)$); 8.26 (m, 1 H, Ar); 7.89 (d, 1 H, H(6), $J_{5,6}$ = 6.3 Hz); 7.79 (m, 1 H, Ar); 5.97 (m, 1 H, H(1′)); 5.67 (d, 1 H, H(5)); 4.78—4.50 (m, 2 H, H(3′), H(4′)); 4.01 (br.d, 2 H, H(5′)); 3.55 (m, 1 H, H(2′)); 0.8—1.0 (m, 28 H, Pr^i). MS: found 682.0; calculated for $[M + H]^+$ 685.9.

1-[3,5-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2-*O*-allyl-β-D-arabinofuranosyl]-3-(2,4-dinitrophenylsulfenyl)uracil (3). Compound **2** (2.68 g, 3.9 mmol) was dried by coevaporation with anhydrous toluene (3×10 mL) and the residue was dissolved in anhydrous MeCN (40 mL). 2-*tert*-Butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (1.6 mL, 5.2 mmol) and allyl bromide (0.6 mL, 6.5 mmol) were added with stirring. The reaction was carried out at ~20 °C for 2 h. The completeness of the reaction was monitored by TLC ($CHCl_3$—EtOH, 97 : 3, R_f 0.71). The solvent was removed, the residue (oil) was dissolved in benzene, and the target compound was isolated by column chromatography (gradient of ethyl acetate in benzene 0→15%). The yield of compound **3** was 2.3 g (81%).

Found (%): C, 49.87; H, 5.76; N, 7.81; S, 4.36. $C_{30}H_{44}N_4O_{11}SSi_2$. Calculated (%): C, 49.71; H, 6.12; N, 7.73; S, 4.42. ^{1}H NMR ($CDCl_3$), δ: 8.82 (s, 1 H, $H_{Ar}(3)$); 8.23 (d, 1 H, $H_{Ar}(5)$, $J_{HAr(5),HAr(6)}$ = 2.3 Hz); 7.75 (d, 1 H, H(6), $J_{5,6}$ = 8.0 Hz); 7.55 (d, 1 H, $H_{Ar}(6)$); 6.17 (d, 1 H, H(5)); 6.05 (d, 1 H, H(1′), $J_{1'2'}$ = 3.0 Hz); 5.81 (m, 3 H, —CH_2—C<u>H</u>=C<u>H</u>$_2$); 5.20 (dd, 1 H, H(3′), $J_{2',3'}$ = 3.5 Hz, $J_{3',4'}$ = 0.5 Hz); 4.55 (d, 2 H, H(5′), $J_{4',5'}$ = 3.6 Hz); 4.35 (dd, 2 H, —C<u>H</u>$_2$—CH=CH_2, $J_{1'',2''}$ = 4.9 Hz, $J_{1''_a,1''_b}$ = 12.1 Hz); 4.15 (m, 1 H, H(4′)); 3.75 (dd, 1 H,

H(2′)); 1.00—1.13 (m, 28 H, Pr^i). MS: found 721.1; calculated for $[M + H]^+$ 725.9.

1-[3,5-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2-*O*-(2,3-dihydroxypropyl)-β-D-arabinofuranosyl]-3-(2,4-dinitrophenylsulfenyl)uracil (4). Compound **3** (2.3 g, 3.19 mmol) was dissolved in acetone (35 mL). A solution of *N*-methylmorpholine *N*-oxide (0.56 g, 4.8 mmol) in water (7 mL) and a solution of osmium tetraoxide (4 mg) in acetone (1 mL) were added with stirring to the reaction mixture at room temperature. The reaction was monitored by TLC ($CHCl_3$—EtOH, 95 : 5, R_f 0.2). After 3 days, a saturated $Na_2S_2O_3$ solution (1 mL) was added. The reaction mixture was diluted with EtOAc and washed with a saturated $NaHCO_3$ solution (50 mL) and water (2×10 mL). The organic phase was dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. The target product was isolated by column chromatography (gradient of ethanol in chloroform 0→15%). The yield of compound **4** was 2.1 g (90%).

Found (%): C, 47.77; H, 5.66; N, 7.44; S, 4.26. $C_{30}H_{46}N_4O_{13}SSi_2$. Calculated (%): C, 47.48; H, 6.11; N, 7.38; S, 4.22. ^{1}H NMR ($CDCl_3$), δ: 8.78 (s, 1 H, $H_{Ar}(3)$); 8.20 (d, 1 H, $H_{Ar}(5)$, $J_{HAr(5),HAr(6)}$ = 2.0 Hz); 7.70 (d, 1 H, H(6), $J_{5,6}$ = 6.9 Hz); 7.31 (d, 1 H, $H_{Ar}(6)$); 6.10 (m, 1 H, H(1′)); 5.71 (d, 1 H, H(5)); 4.35 (m, 1 H, H(3′)); 4.13 (m, 2 H, H(5′)); 4.00 (m, 1 H, H(4′)); 3.73 (d, 1 H, H(2′), $J_{1',2'}$ = 3.0 Hz); 3.51 (br.d, 2 H, $C\underline{H}_2$—CH(OH)—CH_2OH); 3.31 (m, 3 H, CH_2—$C\underline{H}$(OH)—$C\underline{H}_2$OH); 3.08 (m, 2 H, O<u>H</u>); 1.21—0.95 (m, 28 H, Pr^i). MS: found 756.2; calculated for $[M + H]^+$ 760.0.

1-[3,5-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2-*O*-(2,3-dilevulinoyloxypropyl)-β-D-arabinofuranosyl]-3-(2,4-dinitrophenylsulfenyl)uracil (5). Levulinic acid (0.5 mL, 5.5 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (0.5 g, 2.8 mmol) in dioxane (30 mL) were vigorously stirred for 2 h. *N*,*N*′-Dicyclohexylurea that precipitated was filtered off and the resulting solution of levulinic anhydride was added to a solution of compound **4** (2.1 g, 2.8 mmol) in anhydrous pyridine (20 mL). Then a catalytic amount (5 mg) of 4-(*N*,*N*-dimethylamino)pyridine was added. After 18 h (the reaction was monitored by TLC; $CHCl_3$—EtOH, 95 : 5, R_f 0.3), the reaction mixture was concentrated *in vacuo*, and the residue (oil) was dissolved in EtOAc (50 mL). The solution was washed with a saturated $NaHCO_3$ solution (50 mL) and water (2×50 mL) and dried with anhydrous Na_2SO_4. Target product **5** was isolated by column chromatography (gradient of ethanol in chloroform 0→10%) in a yield of 1.6 g (60%).

Found (%): C, 50.57; H, 5.76; N, 5.74; S, 3.29. $C_{40}H_{58}N_4O_{17}SSi_2$. Calculated (%): C, 50.30; H, 6.12; N, 5.87; S, 3.36. ^{1}H NMR ($CDCl_3$), δ: 8.89 (s, 1 H, $H_{Ar}(3)$); 8.28 (d, 1 H, $H_{Ar}(5)$, $J_{HAr(5),HAr(6)}$ = 3.0 Hz); 7.70 (d, 1 H, H(6), $J_{5,6}$ = 7.4 Hz); 7.55 (d, 1 H, $H_{Ar}(6)$); 6.28 (d, 1 H, H(1′)); 6.20 (d, 1 H, H(5)): 5.79 (m, 1 H, CH_2—$C\underline{H}$(OLev)—CH_2OLev); 5.58 (m, 1 H, H(3′)); 4.47 (m, 2 H, H(5′)); 4.11 (m, 2 H, CH_2—CH(OLev)—$C\underline{H}_2$OLev); 3.80 (m, 2 H, $C\underline{H}_2$—CH(OLev)—CH_2OLev); 3.55 (m, 1 H, H(4′)); 3.39 (d, 1 H, H(2′), $J_{1',2'}$ = 3.6 Hz): 2.75 (m, 4 H, OCO—$C\underline{H}_2$—CH_2—COMe); 2.65 (m, 4 H, OCO—CH_2—$C\underline{H}_2$—COMe); 2.10 (s, 6 H, CO—$C\underline{H}_3$); 1.68—0.95 (m, 28 H, Pr^i). MS: found 952.7; calculated for $[M + H]^+$ 956.2.

1-[2-*O*-(2,3-Dilevulinoyloxypropyl)-β-D-arabinofuranosyl]uracil (6). Triethylamine trihydrofluoride (5.3 mmol, 1 mL) was added with stirring to a solution of compound **5** (1.6 g, 1.68 mmol) in anhydrous THF (10 mL) at ~20 °C, and the reaction mixture was stirred for 3 h (TLC control; $CHCl_3$—EtOH, 9 : 1, R_f 0.1). Column chromatography (gradient of ethanol in chloroform 0→15%) afforded compound **6** in a yield of 0.73 g (85%).

Found (%): C, 51.27; H, 5.96; N, 5.44. $C_{22}H_{30}N_2O_{12}$. Calculated (%): C, 51.36; H, 5.88; N, 5.45. ^{1}H NMR (DMSO-d_6), δ: 10.61 (s, 1 H, H(3)); 8.11 (d, 1 H, H(6), $J_{5,6}$ = 8.1 Hz); 6.12 (m, 1 H, H(1′)); 5.89 (d, 1 H, H(5)); 4.45 (m, 1 H, —CH_2—$C\underline{H}$(OLev)—CH_2OLev); 4.38 (d, 1 H, H(2′), $J_{1',2'}$ = 4.1 Hz); 4.37 (m, 2 H, CH_2—CH(OLev)—$C\underline{H}_2$OLev); 4.05 (m, 1 H, H(3′)); 3.84 (m, 1 H, H(4′)); 3.76 (m, 2 H, H(5′)); 2.78 (m, 2 H, $C\underline{H}_2$—CH(OLev)—CH_2OLev); 2.65—2.48 (m, 8 H, CH_2—CH(O<u>Lev</u>)—CH_2O<u>Lev</u>); 2.18 (s, 6 H, CO—$C\underline{H}_3$). MS: found 513.9; calculated for $[M + H]^+$ 514.5.

1-[2-*O*-(2,3-Dilevulinoyloxypropyl)-5-*O*-(4,4′-dimethoxytrityl)-β-D-arabinofuranosyl]uracil (7). Compound **6** (0.73 g, 1.42 mmol) was dried by coevaporation *in vacuo* with anhydrous pyridine (3×10 mL) and dissolved in anhydrous pyridine (25 mL). 4,4′-Dimethoxytrityl chloride (1.7 mmol, 0.63 g) was added with stirring at ~20 °C (TLC control; $CHCl_3$—EtOH, 95 : 5, R_f 0.4). After 2 h, excess dimethoxytrityl chloride was quenched by the addition of MeOH (5 mL) and the reaction mixture was concentrated. The residue (oil) was dissolved in $CHCl_3$ (100 mL) and washed with a saturated $NaHCO_3$ solution (2×50 mL) and water (2×50 mL). Standard work-up and column chromatography (gradient of ethanol in chloroform 0→10%) afforded compound **7** in a yield of 1.1 g (95%).

Found (%): C, 63.17; H, 5.96; N, 3.44. $C_{43}H_{48}N_2O_{14}$. Calculated (%): C, 63.23; H, 5.92; N, 3.43. ^{1}H NMR ($CDCl_3$), δ: 10.21 (s, 1 H, H(3)); 7.68 (d, 1 H, H(6), $J_{5,6}$ = 7.8 Hz); 7.45—6.88 (m, 13 H, DMTr); 6.19 (d, 1 H, H(1′), $J_{1',2'}$ = 2.8 Hz); 5.56 (d, 1 H, H(5)); 5.27 (s, 1 H, C(3′)—O<u>H</u>); 4.45 (m, 1 H, CH_2—$C\underline{H}$(OLev)—CH_2OLev); 4.37 (m, 2 H, CH_2—CH(OLev)—$C\underline{H}_2$OLev); 4.25 (dd, 1 H, H(3′), $J_{2',3'}$ = 3.2 Hz, $J_{3',4'}$ = 0.2 Hz); 4.03 (dd, 1 H, H(2′)); 3.80 (s, 6 H, O$C\underline{H}_3$); 3.54 (m, 1 H, H(4′)); 3.45 (d, 2 H, $H_a(5')$, $J_{H(4'),H_a(5')}$ = 2.4 Hz); 3.45 (d, 2 H, $H_b(5')$, $J_{H(4'),H_b(5')}$ = 10.4 Hz); 2.78 (m, 2 H, $C\underline{H}_2$—CH(OLev)—CH_2OLev); 2.65—2.48 (m, 8 H, CH_2—CH(O<u>Lev</u>)—CH_2O<u>Lev</u>); 2.18 (s, 6 H, CO—$C\underline{H}_3$). MS: found 816.2; calculated for $[M + H]^+$ 817.8.

1-{2-*O*-(2,3-Dilevulinoyloxypropyl)-3-*O*-[(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphino]-5-*O*-(4,4′-dimethoxytrityl)-β-D-arabinofuranosyl}uracil (8). β-Cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.18 mL, 2.7 mmol) was added dropwise to a solution of compound **7** (1.1 g, 1.35 mmol), which was thoroughly predried, and *N*,*N*-diisopropylethylamine (0.27 mL, 2.7 mmol) in anhydrous dichloromethane (20 mL) at ~20 °C. After 2 h (TLC control; CH_2Cl_2—Et_3N, 98 : 2, R_f 0.4), the reaction mixture was diluted with dichloromethane (15 mL) and washed with a cold saturated NaCl solution (2×25 mL). After standard work-up, target phosphoramidite **8** was isolated by column chromatography (gradient of methanol in dichloromethane containing 0.5% of triethylamine 0→3%) in a yield of 0.9 g (70%).

^{1}H NMR ($CDCl_3$), δ: 10.01 (s, 1 H, H(3)); 7.68 (d, 1 H, H(6), $J_{5,6}$ = 7.2 Hz); 7.45—7.20, 6.85—6.73 (m, 13 H, DMTr); 6.23 (d, 1 H, H(1′), $J_{1',2'}$ = 3.0 Hz); 5.58 (d, 1 H, H(5)); 5.32 (m, 1 H, $CH_2C\underline{H}$); 4.45—4.28 (m, 2 H, H(2′), $CHC\underline{H}_aH_b$); 4.17—4.12 (m, 2 H, H(4′), $CHC\underline{H}_a\underline{H}_b$); 3.89—3.79 (m, 2 H, H(3′), $C\underline{H}_2CH$); 3.73 (s, 6 H, DMTr); 3.52 (dd, 1 H, $H_a(5')$,

$J_{H(4'),H_a(5')}$ = 6.0 Hz, $J_{H_a(5'),H_b(5')}$ = 12.1 Hz); 3.65 (m, 4 H, C<u>H</u>$(CH_3)_2$, $POCH_2$); 3.47 (dd, 1 H, $H_b(5')$, $J_{H(4'),H_b(5')}$ = 10.0 Hz); 2.78 (m, 4 H, $CO_2C\underline{H}_2$); 2.66 (m, 4 H, C<u>H</u>$_2$CO); 2.54 (t, 2 H, C<u>H</u>$_2$CN); 2.15 (s, 6 H, $COCH_3$); 1.10 (m, 12 H, C<u>H</u>$_3$CH). ^{13}C NMR ($CDCl_3$), δ: 206.63 (2 C, C=O, Lev); 172.41 (2 C, OC=O, Lev); 162.59 (1 C, C(4)); 158.67 (2 C, DMTr); 150.54 (1 C, C(2)); 144.43 (1 C, DMTr); 140.45 (1 C, C(6)); 132.28, 130.19, 128.29, 127.96, 127.12, 117.74 (11 C, DMTr); 117.62 (1 C, CN); 113.22, 112.11 (4 C, DMTr); 103.05 (1 C, C(5)); 100.04 (1 C, C(1′)); 87.16 (1 C, C(4′)); 86.47 (1 C, C(2′)); 84.64 (1 C, C(3′)); 83.04 (1 C, DMTr); 68.27 (1 C, C(2′)); 65.49 (1 C, C(1′′)); 62.27 (1 C, C(5′)); 62.04 (1 C, C(3′)); 59.92 (1 C, $POCH_2$); 55.32 (2 C, DMTr); 42.79 (1 C, PNCH); 37.51 (2 C, <u>C</u>H_2COCH_3); 29.92 (2 C, $CO_2\underline{C}H_2$); 29.76 (2 C, CO<u>C</u>H_3); 26.11 (4 C, CH_3, Pr^i); 20.26 (1 C, $CH_2\underline{C}N$). ^{31}P NMR ($CDCl_3$), δ: 150.40, 151.73. MS: found 1016.1; calculated for $[M + H]^+$ 1018.1.

Automated oligodeoxyribonucleotide synthesis was carried out on an Applied Biosystems 394A synthesizer (USA) according to a standard protocol using commercial reagents and solvents. Controlled pore glass (CPG-500) containing an immobilized nucleoside was used as the polymer support. The specific loading of the polymer with the first monomer unit was 25—30 μmol g^{-1}. 3′-Phosphoramidites of natural 2′-deoxyribonucleosides were used as 0.1 *M* solutions in anhydrous acetonitrile. The concentrations of the 3′-phosphoramidite derivative of arabinouridine **8** in acetonitrile was 0.15 mol L^{-1}. The reaction time in the step of coupling of the modified monomer was increased to 15 min, and condensation was carried out twice. In this step, the degree of conversion was approximately 95%, which is slightly lower than that for 3′-phosphoramidites of natural 2′-deoxyribonucleosides. Oligodeoxyribonucleotides were deprotected and liberated from the polymer support with a concentrated aqueous ammonia solution at 55 °C for 18 h, the solutions of the 2′-modified oligonucleotides were concentrated *in vacuo*, and the target products were isolated by ion-pair reversed-phase HPLC. The oligonucleotides were desalted by five consecutive evaporations with 50% aqueous ethanol and treated with 80% aqueous acetic acid (1 mL) at ~20 °C for 30 min. Then the acetic acid was evaporated, and 50% aqueous ethanol was added and distilled off three times. The residue was dissolved in water and the resulting oligodeoxyribonucleotides were analyzed by ion-pair reversed-phase HPLC.[46]

Analysis and isolation of oligonucleotides. After completion of the reactions, the mixtures of oligonucleotides were analyzed and the products (after their isolation) were tested for purity by ion-pair reversed-phase HPLC on a Waters chromatograph (USA) equipped with a 4×250 mm column packed with Diasorb-130-$C16_T$ (particle size, 7 μm). The conditions of analytical separation: the column temperature was 45 °C, the flow rate was 1 mL min^{-1}. The eluent: 48 *mM* potassium phosphate buffer, pH 7.0, containing 2 *mM* tetra-*n*-butylammonium dihydrophosphate. The gradient of acetonitrile concentration: 5—20.4% (1 min); 20.4—21.6% (1 min); 21.6—23.2% (3 min); 23.2—24.4% (5 min); 24.4—25.6% (10 min). Oligodeoxyribonucleotides and their conjugates were isolated by reversed-phase HPLC on a Tracor chromatograph (Netherlands) equipped with a 4×250 mm column packed with Diasorb-130-$C16_T$ (particle size, 7 μm). The conditions of separation: the column temperature was 45 °C, the flow rate was 1 mL min^{-1}. The eluent: 0.1 *M* ammonium acetate; the gradient of acetonitrile concentration was 0—40% (80 min).

Synthesis of modified 2′-aldehyde-containing oligonucleotides II′—IV′. Modified 2′-*O*-(2,3-dihydroxypropyl)arabinouridine-containing oligonucleotides (0.5 OE_{260}) were dissolved in a 0.1 *M* acetate buffer, pH 4.6 (10 μL). Then a 5 *mM* sodium periodate solution (5 μL) was added, the reaction mixture was kept at ~20 °C for 1 h, and a 2 *M* acetate buffer (20 μL) was added. The oligonucleotide material was twice precipitated with ethanol and washed with ethanol (200 μL).

Synthesis of conjugates of 2′-aldehyde-containing oligonucleotides II′—IV′ with hydrazides. Modified aldehyde-containing oligonucleotides (0.5 OE_{260}) were dissolved in a 0.4 *M* acetate buffer, pH 4.6 (10 μL). Then a 20 *mM* hydrazide solution in DMSO (1 μL) was added. After 1 h, a 0.5 *M* $NaBH_3CN$ solution (5 μL) was added at ~20 °C, the reaction mixture was kept for 2 h, and then a 4 *M* NaOAc solution (5 μL) and ethanol (200 μL) were added. The conjugate that precipitated was reprecipitated with ethanol and washed with ethanol (200 μL). The target compounds were dissolved in a deionized water (50 μL) and analyzed by ion-pair reversed-phase HPLC.

Synthesis of conjugates of 2′-aldehyde-containing oligonucleotides II′—IV′ with 9-hydrazinoacridine and *N*-aminooxyacetyl peptide. Modified aldehyde-containing oligonucleotides (0.5 OE_{260}) were dissolved in a 0.4 *M* acetate buffer, pH 4.6 (10 μL). Then a 20 *mM* solution of 9-hydrazinoacridine or *N*-aminooxy peptide in DMSO (1 μL) was added, the reaction mixture was kept at ~20 °C for 1 h, and a 4 *M* sodium acetate solution (5 μL) and ethanol (200 μL) were added. The precipitate of the conjugate was reprecipitated with ethanol and washed with ethanol (200 μL). The target compounds were dissolved in deionized water (50 μL) and analyzed by ion-pair reversed-phase HPLC.

Study of thermal stability of duplexes. The temperature dependences of UV absorption of nucleic acid duplexes were measured on a Varian Cary 50 single-beam spectrophotometer (USA) at a wavelength of 260 nm, the rate of temperature increase was 0.5 K min^{-1} in a temperature range of 5—85 °C followed by a decrease in the temperature with a rate of 0.5 K min^{-1} in the same temperature range in temperature-controlled Pye Unicam quartz cells (UK) with an optical path length of 1 cm. Thermal stability of the duplexes was studied in a phosphate buffer (100 *mM* NaCl, 10 *mM* Na_2HPO_4, 1 *mM* EDTA, pH 7.0) at a duplex concentration of 4 μmol L^{-1}.

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