# Synthesis of fluorescein-labeled oligonucleotides bearing a tag in position 2′ of modified adenosine and arabinoadenosine

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Oligonucleotide conjugates containing fluorescein residues in the sugar-phosphate backbone were synthesized by the standard solid-phase phosphoramidite method using phosphoramidites of 9-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-2-methoxalylamino-β-D-ribofuranosyl]- and 9-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-2-methoxalylamino-β-D-arabinofuranosyl]-$N^6$-benzoyladenine. The relative efficiency of the oligonucleotide synthesis with modified phosphoramidites was estimated.

**Key words:** 2′- modified nucleosides, reactive linker group, oligonucleotide synthesis.

Nowadays, research in molecular biology, genetic diagnostics, and medicine cannot be conceived without the use of synthetic, in particular, modified oligonucleotides. Functional groups can be introduced into the heterocyclic bases, the internucleotide phosphate group, or the ribose fragment. As regards retention of the structure and properties of oligonucleotide complexes, the C(5) atom (see Ref. 1) of pyrimidine, the C(8) atom (see Ref. 2) of natural, or the C(7) atom (see Ref. 3) of 7-deazapurine nucleosides are the sites of choice for the introduction of substituents. The introduction of substituents in the internucleotide phosphate group creates a chiral center and, hence, requires separation of stereoisomers. The modification at position 2′ of nucleosides appears promising.[4] This modification induces minor distortions in the structure of oligonucleotide complexes and allows one to prepare nucleosides with C(2′)-*exo*- and C(2′)-*endo*-conformations of the carbohydrate residue and to orient the substituent to either minor of major groove of the NA duplex helix.[5] A drawback of this modification is the presence of substituents adjacent to the phosphoramidite group, which in some cases increases the time necessary for the condensation and decreases the yields, especially in the case of bulky substituents.[6,7]

"Active linker groups" based on oxalamides have been proposed for the post-synthetic modification of oligonucleotides at position 2′ of cytidine[8] and uridine.[9] Since the substituents are introduced into an oligonucleotide after it has been synthesized, this method provides a route to a broad range of derivatives containing aliphatic amino groups. The use of di- and polyamines in this step extends this method to the introduction of functional substituents into oligonucleotides.[10]

The present work continues a series of studies dealing with the development of methods for the synthesis of oligonucleotide conjugates using modified nucleoside units bearing reactive linker group.[8,11,12] Our aim was to prepare 9-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-2-methoxalylamino-β-D-ribofuranosyl]- and 9-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-2-methoxalylamino-β-D-arabinofuranosyl]-$N^6$-benzoyladenine phosphoramidites and to compare the degree of inclusion of modified arabino and ribo monomers into oligonucleotides using the synthesis of 18-mers bearing fluorescent markers as examples.

## Results and Discussion

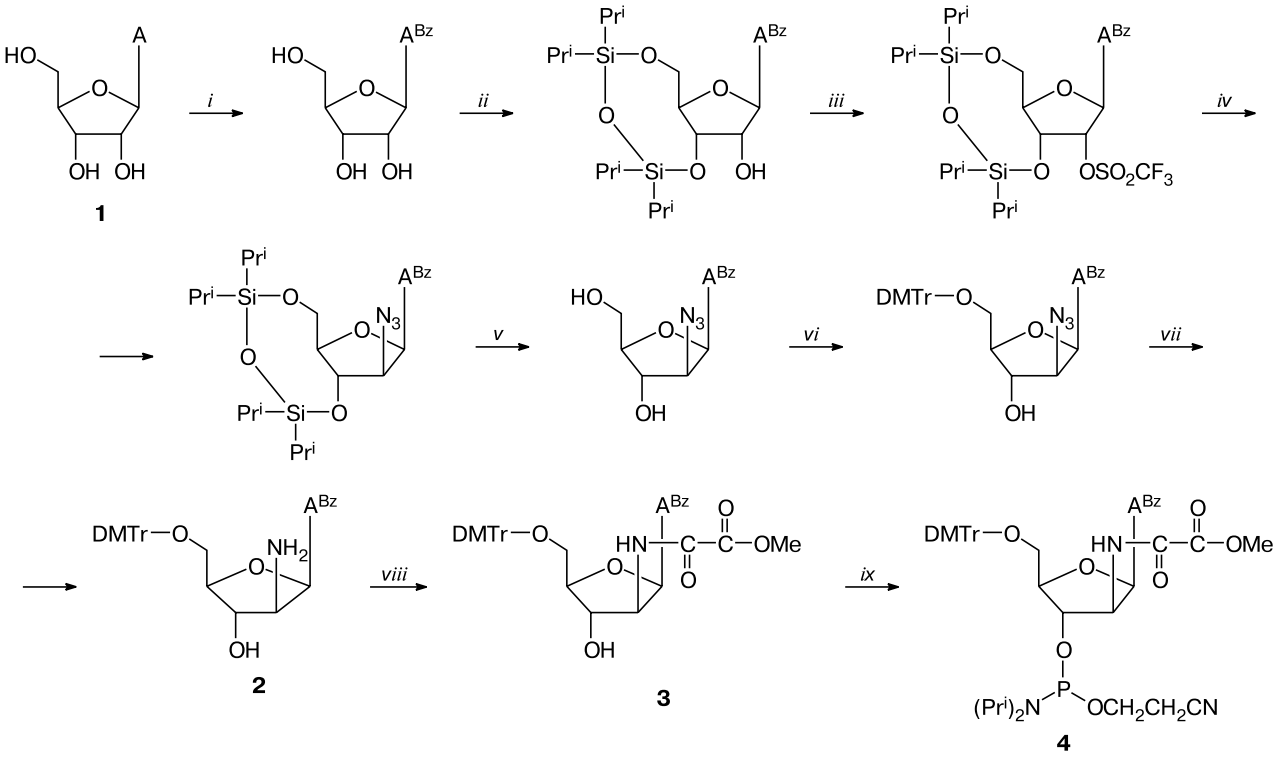
We chose 2′-amino-2′-deoxyarabinoadenosine and 2′-amino-2′-deoxyadenosine derivatives as the starting compounds for the introduction of *N*-methoxalyl groups into the N(2′)-position of purine nucleosides.

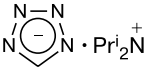
9-[2-Amino-2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-arabinofuranosyl]-$N^6$-benzoyladenine (2′-amino-2′-deoxyarabinoadenosine) was synthesized as shown in Scheme 1. Starting from adenosine **1**, we carried out a series of transformations proposed previously[13] with some modifications aimed at increasing the yields of the target products. For example, removal of the disiloxane protection in step *v* was performed by pyridinium hydrofluoride.[14] Unlike the original procedure, azido group re-

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 9, pp. 1618—1624, September, 2006.

1066-5285/06/5509-1677 

**Scheme 1**



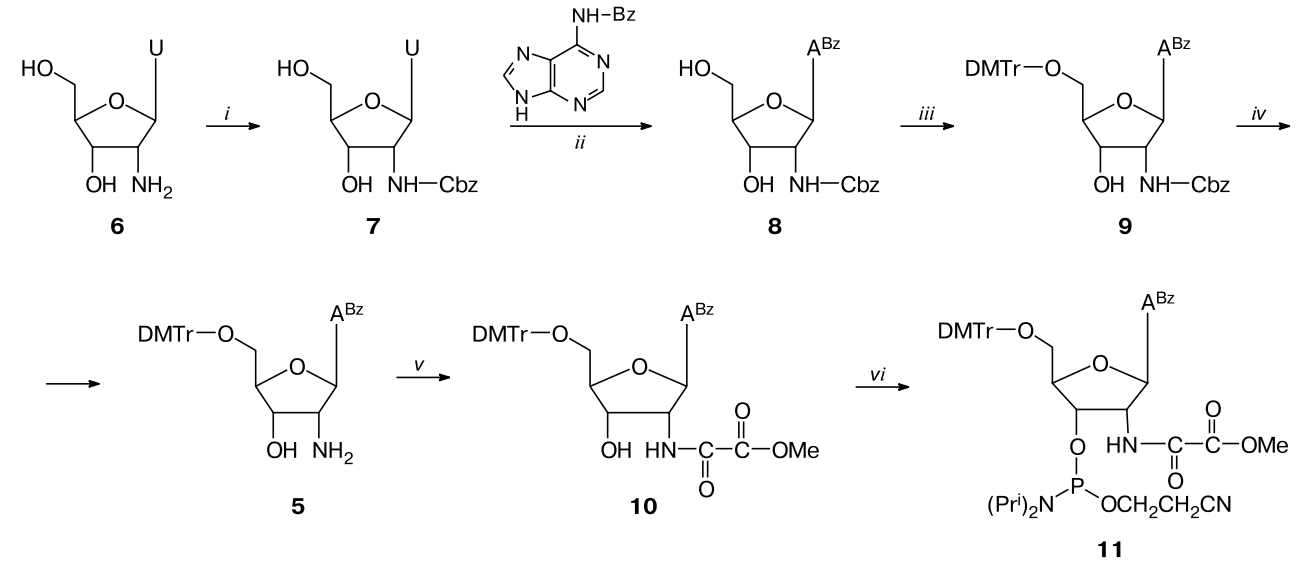
*i*. $Me_3SiCl$, BzCl, $NH_3$ (aq.); *ii*. $ClSi(Pr^i)_2OSi(Pr^i)_2Cl$; *iii*. $(CF_3SO_2)_2O$, $CH_2Cl_2$—pyridine (10 : 1); *iv*. $NaN_3$, DMF; *v*. 4.48 *M* HF in a pyridine—water mixture (5 : 1); *vi*. DMTrCl, pyridine; *vii*. $H_2$/Pd (C), MeOH; *viii*. MeOC(O)C(O)OMe, $Et_3N$, MeOH; *ix*. $((Pr^i)_2N)_2PO(CH_2)_2CN$,  $\cdot Pr^i_2\overset{+}{N}H_2$, $CH_2Cl_2$.

duction and tritylation were carried out in the reverse order. This modification excludes the introduction of a dimethoxytrityl group into the N(2´) position used in the original synthetic protocol. The replacement of the azido group reduction with triphenylphosphine by catalytic hydrogenation increased the product yield in this step from 50 to 98%. Thus, 9-[2-amino-2-deoxy-5-*O*-(4,4´-dimethoxytrityl)-β-D-arabinofuranosyl]-$N^6$-benzoyladenine (**2**) was prepared in ~30% yield in five steps starting from readily available adenosine **1**. The introduction of the methoxalyl group (**2**→**3**) and the synthesis of the target phosphoramidite **4** were carried out according to the described procedures.[8]

Despite the fact that a number of methods for the synthesis of 2´-amino-2´-deoxyadenosine have been reported, the preparation of 9-[2-amino-2-deoxy-5-*O*-(4,4´-dimethoxytrityl)-β-D-ribofuranosyl]-$N^6$-benzoyladenine **5**, *i.e.*, the starting compound for the introduction of a reactive group into position 2´, is much more challenging than that for the arabino analog. The synthetic routes described in the literature imply either the use of expensive arabinoadenosine as the starting compound[15,16] or a multistep synthesis *via* 8,2´-anhydro-9-β-D-arabinofuranosyladenine resulting in a low product yield.[17] An alternative approach consists of the transglycosylation/glycosylation reaction.[18,19] In this case, the yields of 2´-amino-2´-deoxyadenosine were about 30%.

We used a reported[20] protocol for the synthesis of 2´-amino-2´-deoxyadenosine from 2´-amino-2´-deoxyuridine **6** and adapted it for the subsequent preparation of phosphoramidite (Scheme 2). Product **7** formed upon *N*-acylation of compound **6** was subjected to transglycosylation (step *ii*), and the trimethylsilyl protection was removed by treatment with a 1 *M* solution of tetrabutylammonium fluoride in dioxane.[21] The introduction of the dimethoxytrityl protection at the 5´-hydroxy group of 2´-aminoadenosine derivative **8** furnished compound **9** in an overall yield of 25% based on the starting 2´-aminouridine **6**. The benzyloxycarbonyl protection was removed by catalytic hydrogenation on a Pd catalyst in dioxane and then the methoxalyl group was introduced (→**10**) and phosphitylation was carried out (→**11**). The phosphoramidites thus obtained were used for the

**Scheme 2**



*i*. CbzCl, $NaHCO_3$, $H_2O$, pH 9; *ii*. 1,2-dichloroethane, bis(trimethylsilyl)acetamide, $CF_3SO_2OSiMe_3$, 1 *M* $Bu_4NF$ in 1,4-dioxane; *iii*. DMTrCl, DMAP, pyridine; *iv*. $H_2$/5% Pd/C, 1,4-dioxane; *v*. MeOC(O)C(O)OMe, $Et_3N$, MeOH; *vi*. $[(Pr^i)_2N]_2PO(CH_2)_2CN$,  , $CH_2Cl_2$.

synthesis of oligonucleotide derivatives bearing fluorescent tags.*

Fluorescein was chosen as the fluorescent marker for the oligonucleotide probe; this was introduced into the 11th residue of an oligonucleotide using either monomer **4** or monomer **11** (Scheme 3, Table 1). The quencher (BHQ)** was attached to the 3′-end of oligonucleotides (ON) using a properly modified solid support.

The nonmodified oligonucleotide ON-1 and oligonucleotides containing modified ribo (ON-5, $ON^A$-6) and arabino units (ON-3, $ON^A$-4)*** in the 11th residue were synthesized in parallel on a multichannel oligonucleotide synthesizer, which enabled comparison of the introduction efficiency for the modified monomers (see Table 1).

The attachment of a modified unit required an increase the condensation time from 40 s (the "conventional" synthesis) to 30 min. After completion of the synthesis, the oligonucleotide attached to the resin was first treated with ethylenediamine and then with concentrated ammonia to split off the oligonucleotide from the resin and to remove the protecting groups. Treatment of the obtained oligonucleotides $ON^A$-4 and $ON^A$-6 with an excess of fluorescein isothiocyanate (FITC) affords oligonucleotides ON-4 and ON-6.

It can be seen from the data presented in Table 1 that the yields of oligonucleotides synthesized with monomer **11** are much lower than those with monomer **4**. Figure 1 shows the electrophoregram of the reaction mixtures containing oligonucleotides ON-1 to ON-3, $ON^A$-4, ON-5, and $ON^A$-6. The oligonucleotide conjugates containing BHQ (like BHQ—fluorescein) show a characteristic color before staining of the gel. This allows easy identification of the bands corresponding to the target conjugates.

According to the electrophoregram, the reaction mixtures of oligonucleotides $ON^A$-4 and $ON^A$-6 contain shorter oligonucleotides. In the synthesis of $ON^A$-6, a minor product whose mass spectrum corresponded to the oligonucleotide 5′-GCCGATT-BHQ-3′, was isolated by preparative electrophoresis. Presumably, the lower efficiency of incorporation of monomer **11** is due to greater steric shielding from the oxalamide group located in position 2′ of the ribose fragment than in the arabino analog. Of the two oligonucleotides corresponding to a full-size oligonucleotide, only the compound isolated from the upper band did react with FITC, giving rise to conjugate ON-6. Since only one component of the reaction

* The synthesized oligonucleotides were used as Taq-man-probes for the detection of a C677T point mutation by the Real-Time PCR technique. The sequence 5′-TGTCTGCGGGA*GCCGATT-3′ was selected taking into account the requirements specified for the design of this type of probes.

** Black hole quencher.

*** $ON^A$-4 and $ON^A$-6 are precursor oligonucleotides for the synthesis of conjugates ON-4 and ON-6.

**Scheme 3**

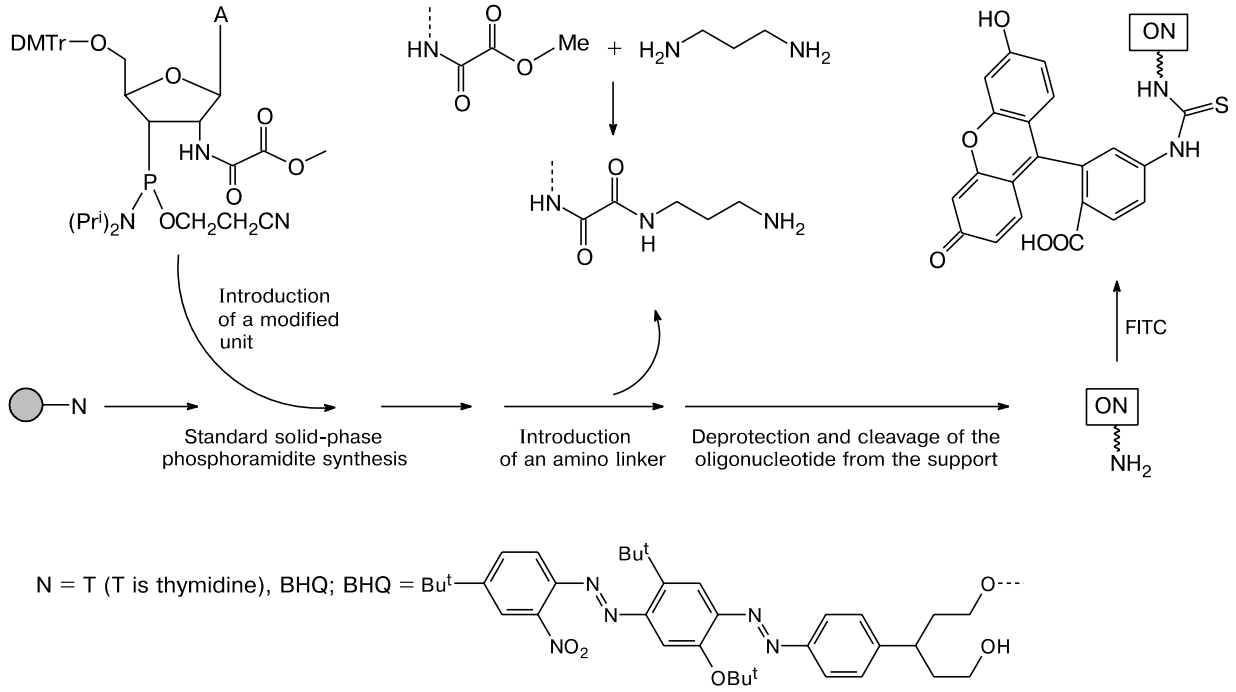



**Table 1.** Structure and mass spectrometric data of the synthesized oligonucleotides

| Oligonicleotide[a] | Mass spectrum | | Yield[c] /nmol |
|---|---|---|---|
| | Calculated | Found[b] | |
| TGTCTGCGGGAGCCGATT (ON-1) | 5544.0 | 5548.0 | 43.3 |
| TGTCTGCGGGAGCCGATT-[BHQ] (ON-2) | 6224.3 | 6225.0 (6144.1) | 8.7 |
| TGTCTGCGGG[$A^4$]GCCGATT (ON-3) | 5673.0 | 5674.3 | 39.0 |
| TGTCTGCGGG[$A^{4\text{-FAM}}$]GCCGATT-[BHQ] (ON-4) | 6742.4 | 6244.1 (6664.0; 6269.7) | 12.4 |
| TGTCTGCGGG[$A^{11}$]GCCGATT (ON-5) | 5673.0 | 5674.8 | 6.9 |
| TGTCTGCGGG[$A^{11\text{-FAM}}$]GCCGATT-[BHQ] (ON-6) | 6742.4 | 6743.9 (6663.0; 6268.2) | 2.9 |

[a] $A^4$($A^{11}$), $A^{4\text{-FAM}}$($A^{11\text{-FAM}}$) are nucleotide units introduced by phosphoramidites **4** (or **11**) and containing a fluorescent marker (FAM). [b] The most intensive peaks are given in parentheses.
[c] The yield was calculated from the optical density of oligonucleotide solutions at 260 nm with allowance for $\varepsilon_{260}$ of FAM and BHQ. For ON-4 and ON-6 conjugates, the averaged data of two syntheses are presented.

mixture reacted with FITC, no additional purification of conjugate $ON^A$-6 was subsequently carried out.

It is worth noting that in the mass spectra of all BHQ-containing oligonucleotide conjugates, the target compounds are responsible for minor peaks. The masses of the major peaks are lower than those calculated theoretically by 78—80 units (or 474—476 units in the case of ON-4 and ON-6). This fact is attributable to the substantial destruction of the dyes during recording of the mass spectra.

The electronic absorption spectra of the synthesized oligonucleotide conjugates ON-2, $ON^A$-4, and $ON^A$-6 exhibited a broad band with an absorption peak at 535 nm (BHQ), while in the case of oligonucleotides ON-4 and ON-6, bands at 494 nm (fluorescein) and 535 nm (a shoulder, BHQ) were observed.

In view of the more complicated synthetic protocol and less effective incorporation of monomer **11** into oligonucleotides, its use is reasonable only if an oligonucleotide with strictly specified structural parameters is required.

## Experimental

The following chemicals and equipment were used: uridine, diphenyl carbonate, dimethyl oxalate, 2-cyanoethyl-*N*,*N*,*N*´,*N*´-tetraisopropyl phosphoramidite, fluorescein isothiocyanate,

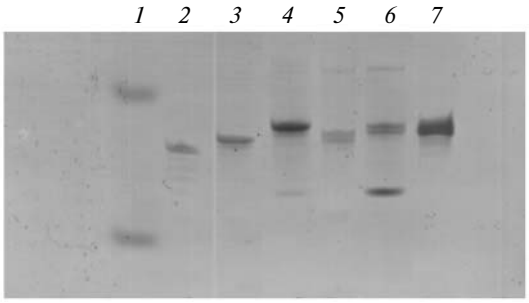



**Fig. 1.** Electrophoregram of the reaction mixtures of oligonucleotides: (*1*) tracking dyes; *2* control oligonucleotide 5′-TGTCTGCGGGAGCCGATT-3′ (ON-1); (*3*) oligonucleotide containing 2′-methoxalylamino-2′-deoxyarabinoadenosine 5′-TGTCTGCGGG[$A^4$]GCCGATT-3′ (ON-3); (*4*) oligonucleotide containing 2′-methoxalylamino-2′-deoxyarabinoadenosine and BHQ 5′-TGTCTGCGGG[$A^4$]GCCGATT-[BHQ]-3′ ($ON^A$-4); (*5*) oligonucleotide containing 2′-methoxalylamino-2′-deoxyriboadenosine 5′-TGTCTGCGGG[$A^{11}$]GCCGATT-3′ (ON-5); (*6*) oligonucleotide containing 2′-methoxalylamino-2′-deoxyriboadenosine and BHQ 5′-TGTCTGCGGG[$A^{11}$]GCCGATT-[BHQ]-3′ ($ON^A$-6); (*7*) control oligonucleotide 5′-TGTCTGCGGGAGCCGATT – [BHQ]-3′ (ON-2).

5% Pd/C (Aldrich, USA), trimethylsilyl chloride (Fluka, Switzerland), 4,4′-dimethoxytrityl chloride, 1,2,4-triazole (Chem-IMPEX, USA), phosphorus oxychloride, benzyloxycarbonyl chloride, bis(trimethylsilyl)acetamide, trimethylsilyl trifluoromethanesulfonate (Merck, Germany), sodium azide (Serva, Germany). The solvents and other commercial chemicals manufactured in Russia (chemically pure grade, analytically pure grade, or extra pure grade) were purified, if required, by standard procedures.[22] HPLC analysis of reaction mixtures was carried out on a Milichrom 4 chromatograph (Russia) on a 2×50 mm column with Lichrosorb RP–18, 10 μm (Merck), gradient elution with 0 → 40% acetonitrile in water at a rate of 100 μL $min^{-1}$. Column chromatography was performed on Kieselgel 60 (63—100 μm, Merck). Gradient elution with methanol in chloroform (0 → 20%) was used. The TLC-Alufolien Kieselgel 60 $F_{254}$ plates (Merck, Germany) were used for TLC. The electronic absorption spectra of oligonucleotide conjugates were recorded on a Hewlett Packard 8453 spectrometer (USA) in water. NMR spectra were measured on Bruker WP-200-SY and Bruker AM-400 spectrometers in $CDCl_3$, $D_2O$, or $(CD_3)_2CO$. For $^1H$ NMR spectra, $Me_4Si$ was used as the internal standard and in the case of $^{31}P$ NMR spectra, external 85% $H_3PO$ in $D_2O$ was employed. The signals were assigned on the basis of analysis of NMR spectra of related compounds.[8,11–13,20] Mass spectra were run on a Bruker REFLEX III MALDI TOF mass spectrometer (Bruker Analytical Systems, Inc.).

The 18-mer oligonucleotides were synthesized on a DNA Synthesizer ASM-800 (Biosset, Russia) by the standard phosphoramidite protocol. The conjugates containing a fluorophor and a quencher were synthesized using the solid support with BHQ kindly provided by A. N. Sinyakov (Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the RAS). When introducing the modified monomers **4** or **11**, the condensation time was increased to 30 min. Oligonucleotides were purified on Poly-Pak$^{TM}$ cartridges (Glen Res Co. USA) according to manufacturer′s protocol. Oligonucleotides ON-1 to ON-6 were analyzed by HPLC and in 20% denaturing polyacrylamide gel. Bromophenol Blue and Xylene Cyanol in 70% formamide were used as tracking dyes. The nucleotide material in the gel was visualized by a Stains-all solution (Sigma).

### *Synthesis of modified monomers*

**9-[2-Amino-2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-arabinofuranosyl]-$N^6$-benzoyladenine (2)** was prepared by the modified procedure (see Results and Discussion). The spectroscopic characteristics of the product corresponded to published data.[13]

**9-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-2-methoxalylamino-β-D-arabinofuranosyl]-$N^6$-benzoyladenine (3).** A solution of nucleoside **2** (555 mg, 0.825 mmol) in a mixture of $Et_3N$ (750 μL, 5.4 mmol) and MeOH (10 mL) was added dropwise with stirring to a solution of dimethyl oxalate (640 mg, 5.4 mmol) in MeOH (5 mL). The reaction mixture was stirred for 14 h at ~20 °C, the end of the reaction being detected by TLC: $R_f$ of the product 0.29, for **2**, $R_f$ 0.20 ($CH_2Cl_2$—MeOH—$Et_3N$, 10 : 0.5 : 0.1). The reaction mixture was concentrated to 3—4 mL, and hexane was added. The precipitate was chromatographed on a column with silica gel in a MeOH—$CHCl_3$ gradient (0→10%) with 1% $Et_3N$. The fractions containing the target product was concentrated, the residue was dissolved in $CHCl_3$ (2 mL), and the product was precipitated with hexane (20 mL). Drying *in vacuo* gave 321 mg (52%) of compound **3** as a white powder. $^1H$ NMR (acetone-$d_6$), δ: 3.40 (m, 2 H, H(5′)); 3.58 (s, 3 H, OMe); 3.71 (s, 6 H, OMe (Ar)); 4.15 (m, 1 H, H(4′)); 4.93 (m, 1 H, H(3′)); 5.10 (m, 1 H, H(2′)); 6.58 (d, 1 H, H(1′), $J$ = 8 Hz); 6.75 (m, 4 H, (Ar)); 7.54—7.16 (m, 12 H, 3 H($COC_6H_5$), 9 H(Ar)); 8.12 (d, 2 H, $COC_6H_5$, $J$ = 8 Hz); 8.44 (s, 1 H, H(2)); 8.53 (s, 1 H, H(8)). MS (MALDI-TOF), *m/z*: 759.27 [M + H]; 781.26 [M + Na]. $C_{41}H_{38}N_6O_9$. Calculated: M = 758.27.

**9-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-2-methoxalylamino-3-(2-cyanoethoxy-*N,N*-diisopropylaminophosphino)-β-D-arabinofuranosyl]-$N^6$-benzoyladenine (4)** was prepared by the standard procedure from nucleoside **3** (240 mg, 0.32 mmol). The reaction time was 4 h. Yield 143 mg (47%). $R_f$ 0.45 ($CH_2Cl_2$—MeOH—$Et_3N$, 10 : 0.5 : 0.1). $^{31}P$ NMR (acetone-$d_6$), δ: 150.9; 150.2. MS (MALDI-TOF), *m/z*: 959.3707 [M + H]. $C_{50}H_{55}N_8O_{10}P$. Calculated: M = 958.3778.

**1-(2-Amino-2-deoxy-β-D-ribofuranosyl)uracil (6)** was prepared by a published procedure.[8]

**1-[2-(*N*-Benzyloxycarbonyl)amino-2-deoxy-β-D-ribofuranosyl]uracil (7)** was prepared by a known procedure.[23] Sodium hydrogencarbonate (0.46 g, 5.43 mmol) was added with stirring to a solution of compound **6** (0.56 g, 2.27 mmol) in water (23 mL), the solution was heated to 50 °C (pH 9), and benzyloxycarbonyl chloride (0.45 mL, 3.23 mmol) was added in 50 μL portions over a period of 10 min. The mixture was vigorously stirred for 1 h (TLC monitoring: $R_f$ of the product 0.44, for **6**, $R_f$ 0.01 ($CH_2Cl_2$—MeOH, 9 : 1)). After the starting compound disappeared from the reaction mixture, the mixture was extracted with 3×10 mL of ether, and citric acid was added to the aqueous layer to pH 5. The product was extracted from water with a $CHCl_3$—THF mixture (4 : 1) (10 mL portions) until the

target product was no longer detected in the organic phase. The combined organic layer was dried with anhydrous $Na_2SO_4$. Yield 0.45 g (52%), foam. The spectroscopic characteristics of the product corresponded to published data.[20]

**9-[2-(*N*-Benzyloxycarbonyl)amino-2-deoxy-β-D-furanosyl]-$N^6$-benzoyladenine (8)** was prepared by a known procedure[20] from compound **7** (134 mg, 0.356 mmol) with 1,2-dichloroethane as the solvent. After completion of transglycosylation, the reaction mixture was concentrated, and the residue was dissolved in a 1 *M* solution of tetrabutylammonium fluoride in dioxane and stirred for 1 h at room temperature. The reaction mixture was concentrated, and the residue was dissolved in $CHCl_3$ and chromatographed on silica gel. The product was eluted with a MeOH—$CHCl_3$ gradient mixture (0→10%). The target fractions with $R_f$ 0.75 (EtOAc—MeOH, 8 : 2) were concentrated. The resulting compound **8** was used in the next step without additional purification

**9-[2-(*N*-Benzyloxycarbonyl)amino-2-deoxy-5-*O*-(4,4´-dimethoxytrityl)-β-D-ribofuranosyl]-$N^6$-benzoyladenine (9).** Compound **8** obtained in the preceding step was concentrated with dry pyridine (15 mL) to 1 mL. 4-Dimethylaminopyridine (5 mg) and 4,4´-dimethoxytrityl chloride ($DMT_2Cl$) (117 mg, 0.35 mmol) were added and the mixture was stirred for 16 h. The reaction mixture was concentrated, dissolved in $CHCl_3$ containing 0.1% pyridine, and washed with water, a 5% solution of $NaHCO_3$, and again water. The organic layer was dried with $Na_2SO_4$ and concentrated to 5 mL, and hexane (50 mL) was added. The product was chromatographed on a column with silica gel using chloroform containing 0.1% pyridine as the eluent. The target fractions with $R_f$ 0.38 ($CHCl_3$—MeOH, 9 : 1) were concentrated and precipitated with hexane. Yield 70 mg (25%) based on 1-(2-amino-2-deoxy-β-D-ribofuranosyl)uracil (**6**). $^1$H NMR ($CD_3OD$), δ: 3.5 (m, 2 H, H(5´)); 3.75 (s, 6 H, OMe(Ar)); 4.28 (m, 1 H, H(4´)); 4.50 (m, 1 H, H(3´)); 5.00 (s, 2 H, C(O)O$\underline{CH_2}C_6H_5$); 5.3 (dd, 1 H, H(2´), $J_{2´,1´}$ = 2 Hz, $J_{2´,3´}$ = 7 Hz); 6.25 (d, 1 H, H(1´), *J* = 4 Hz); 6.84 (m, 4 H, (Ar)); 7.72—7.26 (m, 17 H, 3 H ($COC_6H_5$), 9 H (Ar), 5 H (C(O)OCH$_2$C$_6\underline{H_5}$)); 8.11 (d, 2 H, $COC_6H_5$, *J* = 4 Hz); 8.51 (s, 1 H, H(2)); 8.62 (s, 1 H, H(8)).

**9-[2-Amino-2-deoxy-5-*O*-(4,4´-dimethoxytrityl)-β-D-ribofuranosyl]-$N^6$-benzoyladenine (5).** Compound **9** (403 mg, 0.5 mmol) was hydrogenated under atmospheric pressure in the presence of 5% Pd/C catalyst (50 mg) and 30% $NH_3$(aq.) (0.5 mL) in dioxane (20 mL) for 12 h. The catalyst was filtered off and washed with dioxane (3×10), and the combined dioxane solution was concentrated. The yield of the product formed as a foam was 290 mg (87%). $R_f$ 0.19 ($CHCl_3$—MeOH, 9 : 1). $^1$H NMR ($CD_3OD$), δ: 3.5 (m, 2 H, H(5´)); 3.76 (s, 6 H, OMe); 4.34 (m, 1 H, H(4´)); 4.5 (m, 1 H, H(3´)); 4.62 (m, 1 H, H(2´)); 6.20 (d, 1 H, H(1´), *J* = 4 Hz); 6.84 (m, 4 H, (Ar)); 7.72—7.20 (m, 12 H, 3 H ($COC_6H_5$), 9 H (Ar)); 8.11 (d, 2 H, $COC_6H_5$, *J* = 4 Hz); 8.51 (s, 1 H, H(2)); 8.62 (s, 1 H, H(8)). MS (MALDI-TOF), *m/z*: 673.23 [M + H]; 694.93 [M + Na]. $C_{38}H_{36}N_6O_6$. Calculated: M = 672.7290.

**9-[2-Deoxy-2-methoxalylamino-5-*O*-(4,4´-dimethoxytrityl)-β-D-ribofuranosyl]-$N^6$-benzoyladenine (10)** was synthesized from nucleoside **5** (124 mg, 0.184 mmol) by a procedure described for compound **3**. A white powder, yield 60 mg (44%). $R_f$ 0.47 ($CHCl_3$—MeOH, 9 : 1). $^1$H NMR (acetone-$d_6$), δ: 3.50 (m, 2 H, H(5´)); 3.76 (s, 3 H, OMe); 3.79 (s, 6 H, OMe(Ar)); 4.36 (m, 1 H, H(4´)); 4.73 (m, 1 H, H(3´)); 5.55 (m, 1 H, H(2´)); 6.37 (d, 1 H, H(1´), *J* = 8 Hz); 6.85 (m, 4 H, (Ar)); 7.65—7.19 (m, 12 H, 3 H ($COC_6H_5$), 9 H (Ar)); 8.12 (d, 2 H, $COC_6H_5$, *J* = 8 Hz); 8.42 (s, 1 H, H(2)); 8.54 (s, 1 H, H(8)). MS (MALDI-TOF), *m/z* 759.26 [M + H]. $C_{41}H_{38}N_6O_9$. Calculated: M = 758.27.

**9-[2-Deoxy-2-methoxalylamino-5-*O*-(4,4´-dimethoxytrityl)-3-(2-cyanoethoxy-*N,N*-diisopropylaminophosphino)-β-D-ribofuranosyl]-$N^6$-benzoyladenine (11)** was prepared by a standard procedure from nucleoside **10** (50 mg, 0.065 mmol). The reaction time was 3 h. Yield 47 mg (75%). $R_f$ 0.62 ($CHCl_3$—MeOH, 9 : 1). $^{31}$P NMR (acetone-$d_6$), δ: 152.53; 152.41. MS (MALDI-TOF), *m/z*: 959.3724 [M + H]. $C_{50}H_{55}N_8O_{10}P$. Calculated: M = 958.3778.

### *Synthesis of oligonucleotide conjugates*

After completion of the solid-phase synthesis, the resin with attached oligonucleotides ON-3, ON$^A$-4, ON-5, and ON$^A$-6 was transferred into tubes, treated with 200 μL of ethylenediamine, and kept for 2 h at 30 °C. Complete cleavage of the oligonucleotides from the resin and removal of the protecting groups were performed by adding concentrated aqueous ammonia (0.5 mL) followed by stirring of the reaction mixture for 24 h at 30 °C. After preliminary purification of the reaction mixtures on a Poly-Pak$^{TM}$ cartridge according to manufacturer´s recommendations, the solutions of oligonucleotides were concentrated to 100 μL and treated with a 8% solution of $LiClO_4$ in acetone (1 mL). The precipitated lithium salts of oligonucleotides containing an amine linker group in the 11th residue were dissolved in the minimum volume of water (20—50 μL). A 1/10 volume of a 1 *M* solution of $NaHCO_3$ and a saturated solution of fluorescein isothiocyanate (FITC) were added (in five portions over a period of 12 h at 30 °C, 20-fold molar excess of FITC in DMF). The oligonucleotide conjugates were purified by gel filtration on Sephadex G-10 (a 2×50 mm column), elution with 10 *mM* Tris-HCl, 1 *mM* EDTA, pH 7.4. The purified conjugates were reprecipitated with a 2% solution of $LiClO_4$ in acetone.

This work was supported by the Russian Foundation for Basic Research (Projects No. 05-04-48985 and No. 04-04-48566) and the Russian Academy of Sciences (Fundamental Research Program of the RAS "Biosphere Origin and Evolution").

## References

1. A. F. Nasonov and G. A. Korshunova, *Usp. Khim.*, 1999, **68**, 532 [*Russ. Chem. Rev.*, 1999, **68**, 532 (Engl. Transl.)].
2. M.T. Tierney and M. W. Grinstaff, *Org. Lett.*, 2000, **2**, 3413.
3. H. Rosemeyer, N. Ramzaeva, E. M. Becker, E. Feiling, and F. Seela, *Bioconjug. Chem.*, 2002, **13**, 1274.
4. T. S. Zatsepin, E. A. Romanova, and T. S. Oretskaya, *Usp. Khim.*, 2004, **73**, 757 [*Russ. Chem. Rev.*, 2004, **73**, 701 (Engl. Transl.)].
5. M. Manoharan, *Biochim. Biophys. Acta*, 1999, **1489**, 117.
6. H. Ozaki, S. Momiyama, K. Yokotsuka, and H. Sawai, *Tetrahedron Lett.*, 2001, **42**, 677.
7. K. Yamana, T. Mitsui, H. Hayashi, and H. Nakano, *Tetrahedron Lett.*, 1997, **38**, 5815.

8. S. V. Vasileva, T. V. Abramova, T. M. Ivanova, G. V. Shishkin, and V. N. Silnikov, *Bioorgan. Khim.*, 2004, **30**, 264 [*Russ. J. Bioorg. Chem.*, 2004, **30**, 234 (Engl. Transl.)].
9. N. N. Polushin, *Tetrahedron Lett.*, 1996, **37**, 3231.
10. N. A. Lebedeva, E. A. Mal´tseva, I. Yu. Garipova, S. V. Vasileva, E. A. Belousova, I. O. Petruseva, N. I. Rechkunova, V. N. Silnikov, and O. I. Lavrik, *Biokhimiya*, 2004, **69**, 260 [*Biochemistry (Moscow)*, 2004, **69**, 208 (Engl. Transl.)].
11. T. V. Abramova, S. V. Vasileva, T. M. Ivanova, G. V. Shishkin, and V. N. Silnikov, *Bioorgan. Khim.*, 2004, **30**, 254 [*Russ. J. Bioorg. Chem.*, 2004, **30**, 224 (Engl. Transl.)].
12. S. V. Vasilyeva, T. V. Abramova, and V. N. Silnikov, *Nucleosides Nucleotides, Nucleic Acids*, 2004, **23**, 993.
13. E. M. Zubin, S. I. Antsypovich, T. S. Oretskaya, E. A. Romanova, E. M. Volkov, V. N. Tashlitsky, N. G. Dolinnaya, and Z. A. Shabarova, *Nucleosides Nucleotides*, 1998, **17**, 425.
14. P. Godzina, K. Adrych-Rozek, and W. T. Markiewicz, *Nucleosides Nucleotides,* 1999, **18**, 2397.
15. S. Van Calenbergh, E. Van Den Eeckhout, P. Herdewijn, A. De Bruyn, C. Verlinde, W. G. J. Hol, M. Callens, A. Van Aerschot, and J. Rozensky, *Helv. Chem. Acta*, 1994, **77**, 631.
16. M. J. Robins, S. D. Hawrelak, E. A. Hernandez, and S. F. Wnuk, *Nucleosides Nucleotides*, 1992, **11**, 821.
17. M. Ikehara, T.Maruyama, H.Miki, and Y. Takatsuka, *Chem. Pharm. Bull.*, 1977, **25**, 754.
18. J. B. Hobbs and F. Eckstein, *J. Org. Chem.*, 1977, **42**, 714.
19. M. Imazawa and F. Eckstein, *J. Org. Chem.*, 1979, **44**, 2039.
20. Yu. A. Sharkin, M. V. Yas´ko, A. Yu. Skoblov, and L. A. Aleksandrova, *Bioopgan. Khim.*, 1996, **22**, 297 [*Russ. J. Bioorg. Chem.*, 1996, **22**, 253 (Engl. Transl.)].
21. R. Kierzek, *Nucleosides Nucleotides*, 1985, **4**, 641.
22. A. J. Gordon and R. A. Ford, *The Chemist´s Companion. A Handbook of Practical Data, Techniques, and References*, Wiley-Interscience, New York, 1972.
23. A. A. Gershkovich and V. K. Kiberev, *Sintez peptidov. Reagenty i metody* [*Peptide Synthesis. Reagents and Methods*] Naukova dumka, Kiev, 1987 (in Russian).

*Received November 15, 2005;*
*in revised form August 29, 2006*