New phosphoramidite derivatives for the preparation of oligonucleotides containing a hydrazide group in any specified position of the oligonucleotide chain

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A new versatile method for the preparation of oligonucleotides containing hydrazide groups in any position of the oligonucleotide chain by standard phosphoramidite automated oligonucleotide synthesis is proposed. The method is based on the use of a series of new modified components for oligonucleotide synthesis. An original protecting group for the hydrazide group is proposed. The presence of the hydrazide group in the obtained oligonucleotides and its high reactivity were demonstrated by the reaction with 4-methoxybenzaldehyde in solution.

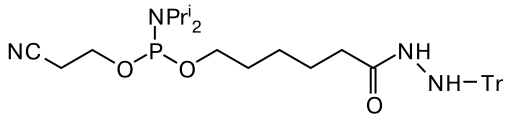
Key words: modified oligonucleotides, hydrazide group, oligonucleotide synthesis, phosphoramidite derivatives, conjugation.

Synthetic modified oligonucleotides are now widely used in bioorganic chemistry, molecular biology, and bioengineering. Of particular interest are oligonucleotides bearing reactive groups not encountered in natural oligonucleotides, which extends the scope of their applicability, as this allows immobilization on various surfaces and conjugation with molecules of different nature.[1—10]

A topical task of bioorganic chemistry is the development of efficient methods for the preparation of such oligonucleotides bearing reactive groups. The hydrazide group is one of these, which imparts valuable properties to the oligonucleotide. First of all, this is the possibility of efficient synthesis of conjugates with molecules and surfaces containing aldehyde groups to give *N*-acylhydrazones. The reaction requires no activating reagents; it occurs rapidly under mild conditions and gives products in high yields.[11—15] This method has a number of advantages over the reaction of amines with aldehydes, which yields Schiff´s bases.[11] The latter are less stable than hydrazones, and additional reduction for the formation of more stable secondary amines is often required.[11,13,14]

The application of oligonucleotides containing modifications in the middle of the chain can give conjugates in which both oligonucleotide ends are free for further modification, for example, for radioactive labeling, for incorporation of such oligonucleotides into extended NA molecules, for covalent binding of complementary and branched oligonucleotide structures, for example, tris-oligonucleotides,[16—19] and for other purposes.

Methods for preparation of hydrazide-containing oligonucleotides have not been adequately developed. The existing methods are not versatile and suffer from some drawbacks.[11—15,20] Oligonucleotides containing the hydrazide group at the 5´- or 3´-end are commercially avaible from Metabion.[13] Their synthesis is based on phosphoramidite derivative **1** developed by the Nanogen Recognomics company.



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However, 3´-modified oligomers can be obtained only provided that 5´-*O*-phosphoramidite nucleoside derivatives are used in the automated synthesis. Their cost is rather high, while the addition efficiency is lower than that of 3´-*O*-phosphoramidite derivatives. This limits the applicability of compound **1** for the synthesis of 3´-modified oligonucleotides.

The transformation of the ester group into hydrazide on treatment with hydrazine was used to prepare 3´-modified oligonucleotides.[12] This approach is rather effective; however, it cannot claim for being versatile, because the

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Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 11, pp. 2585—2595, November, 2005.

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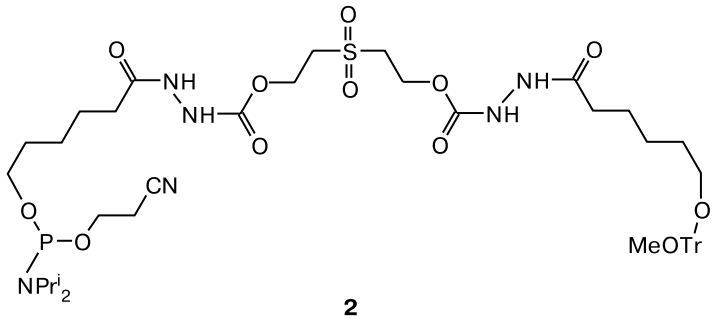

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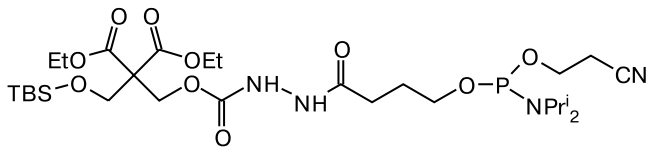
post-synthetic procedure based on the hydrazinolysis[21] is accompanied by the formation of large amounts of by-products due to modification of *N*-benzoylcytosine residues.[21] Therefore, hydrazinolysis can be used[21—23] only in combination with special labile protecting groups. For example, a protecting group more labile than the benzoyl group is used for the exocyclic amino group of cytidine, which limits the applicability of the method. In addition, after removal of the protecting group, the oligonucleotide may still be unstable against such a strong nucleophile as hydrazine.[24]

Recently, we proposed to use phosphoramidite derivative **2** based on bis(2-hydroxyethyl) sulfone for the introduction of the hydrazide group in the ends of the oligonucleotide chain.[11]

This compound allows one to synthesize 5′- and 3′-modified oligomers and oligonucleotides containing hydrazide groups in both ends.[11]

Note that if the hydrazide group is unprotected, there is a probability of undesirable formation of an amide from the hydrazide under the action of ammonia. This reaction proceeds at a noticeable rate under standard conditions of post-synthetic treatment of oligonucleotides (25—33% aqueous ammonia at 55 °C)[11] both in the case of unprotected hydrazide and hydrazide that is deprotected during treatment with ammonia. The elimination of bis(2-hydroxyethyl) sulfone takes place in 5 h, while complete removal of protecting groups from the oligonucleotide requires 16 h (see Refs 11 and 25). Thus, the hydrazide function is liberated before the treatment with ammonia is over. The use of highly labile protection for exocyclic groups of heterocyclic bases[21,26—32] allows one to remove these groups under mild conditions in which the hydrazide function remains intact. However, derivatives with these protecting groups are rather expensive.



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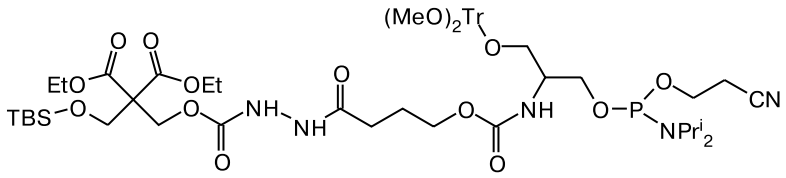

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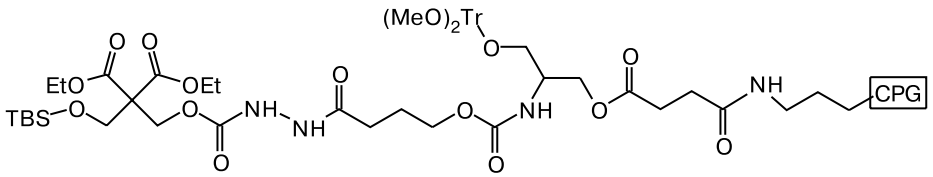

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Note. CPG is the polymeric support based on controlled pore glass, TBS = Bu^tMe_2Si—.

This communication describes a new versatile method for the preparation of deliberately modified hydrazide-containing oligonucleotides based on a unified procedure, that completely eliminates the formation of by-products during deprotection. The method implies the use of an original protecting group for the hydrazide function and its selective removal after treatment with ammonia. We developed new hydrazide-containing phosphoramidite derivatives **3** and **4** and a modified polymeric support **5**, which permit the preparation of oligonucleotides containing a hydrazide function at the 5′-end of the chain, inside the chain, and at the 3′-end.

Results and Discussion

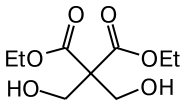
In the development of new hydrazide-containing phosphoramidite derivatives **3** and **4**, and modified polymeric support **5**, the attention was concentrated on the nature of the protecting group for the hydrazide function. This group was selected considering the following:

— the protecting group should be completely stable during the oligonucleotide synthesis with conventionally protected phosphoramidite derivatives and a standard synthetic protocol;[3,31,32]

— the group should be stable during the standard post-synthetic treatment with ammonia (16 h at 55 °C);

— the group should be selectively removed under conditions where the hydrazide group remains intact.

Diethyl 2,2-bis(hydroxymethyl)malonate (**6**) used previously for the synthesis of phosphorylated oligonucleotides served as the key compound.[33]



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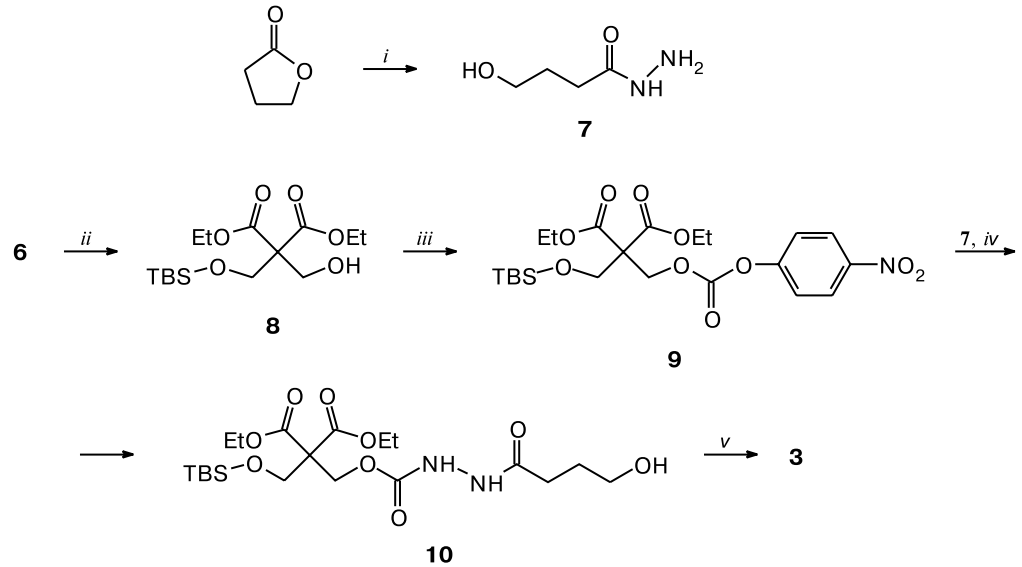
The protecting group based on this ester is stable in the presence of bases if both hydroxy groups are protected. However, it can be cleaved under mild basic conditions if at least one of the hydroxy groups is free. Thus, one of the hydroxy groups should be bound to the hydrazide function, while the other should be protected; this will make the overall structure stable during treatment with ammonia.

The *tert*-butyldimethylsilyl (TBS) group was chosen as the *O*-protecting group in compound **6**. This group is used in the oligoribonucleotide synthesis for the protection of 2′-hydroxy groups[34] and, hence, fully complies with the above requirements. Indeed, it completely withstands the conditions of both the oligonucleotide synthesis and the post-synthetic treatment and can be selectively removed on treatment with tetrabutylammonium fluoride under mild conditions. A specific feature of the post-synthetic treatment in the preparation of the modified oligonucleotide is that the first ammonia treatment of the immobilized oligonucleotide is carried out with ethanolic rather than aqueous solutions of ammonia. It was shown[34] that aqueous ammonia may partly remove the TBS group, which is inadmissible at this stage.

Compound **3** was prepared to be used in the synthesis of 5′-modified oligonucleotides. 4-Hydroxybutyrohydrazide (**7**) was taken as the starting compound (Scheme 1).

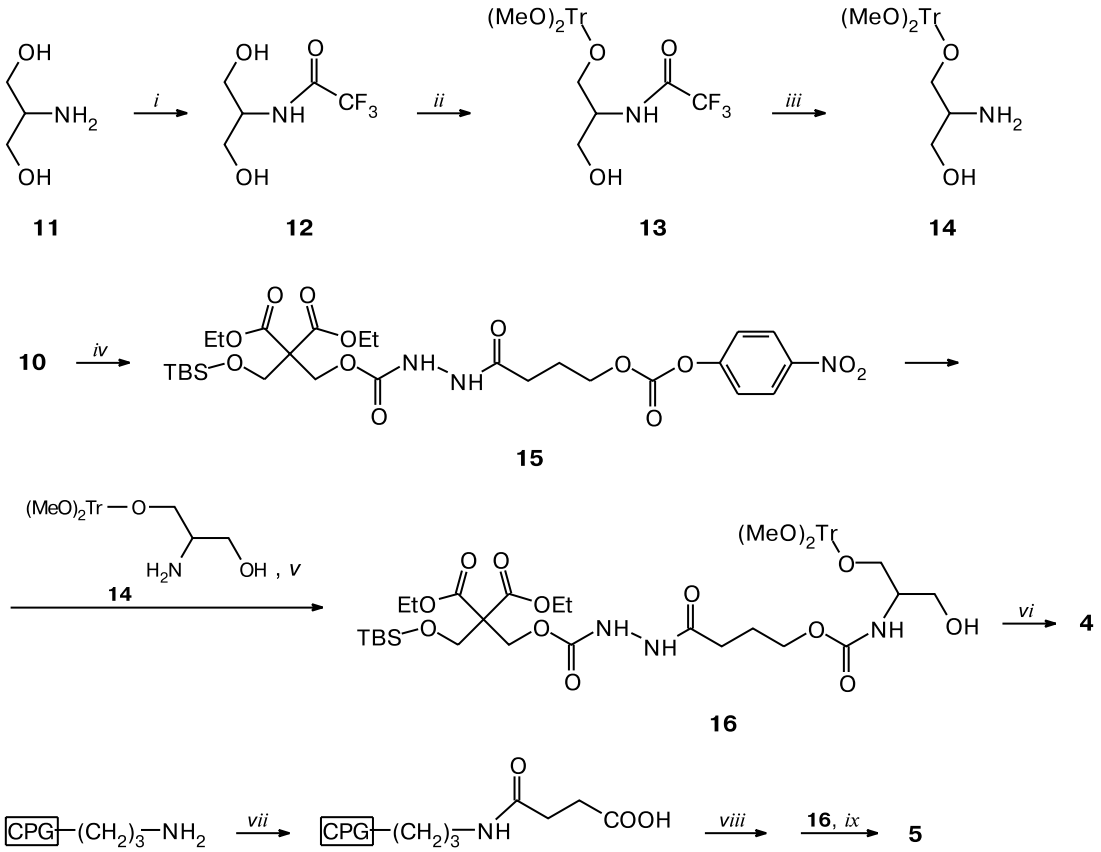
Compound **4** and the polymeric support **5** were synthesized starting from 2-aminopropane-1,3-diol (**11**) (Scheme 2); thus, the distance between two phosphate

Scheme 1



Reagents and conditions: *i*. N_2H_4, EtOH; *ii*. Bu^tMe_2SiCl(TBS—Cl), DMAP, Py, CH_2Cl_2; *iii*. 4-$O_2NC_6H_4OCOCl$, DMAP, Py, CH_2Cl_2; *iv*. DMAP, DMF, Py; *v*. $P(OCH_2CH_2CN)(NPr^i_2)_2$, $Pr^i_2NH \cdot CH_2N_4$, CH_2Cl_2.

Scheme 2



Reagents and conditions: *i*. CF_3COOEt, Py, CH_2Cl_2; *ii*. $(MeO)_2TrCl$, Py, CH_2Cl_2; *iii*. NH_4OH, EtOH; *iv*. 4-$O_2NC_6H_4OCOCl$, Py, DMAP, CH_2Cl_2; *v*. Py, DMAP, CH_2Cl_2; *vi*. $P(OCH_2CH_2CN)(NPr^i_2)_2$, $Pr^i_2NH \cdot CH_2N_4$, CH_2Cl_2; *vii*. succinic anhydride, DMAP, Py; *viii*. Me_3SiCl, Py; *ix*. 2,4,6-$Pr^i_3C_6H_2SO_2Cl$, *N*-methylimidazole.

groups existing in natural nucleosides may be retained in the modified oligonucleotides.[5,35–39] The incorporation of 2-aminopropane-1,3-diol-based units in the oligonucleotide results in the formation of a diastereomer mixture; however, for most purposes, this is not crucial.[5,35–39]

Compounds **3** and **4** were characterized by ^1H and ^{31}P NMR spectroscopy and mass spectrometry.

Using the obtained phosphoramidite derivatives **3** and **4** and the modified polymeric support **5**, we synthesized a set of oligodeoxyribonucleotides with 5′-terminal, intrastrand, and 3′-terminal modifications (Table 1).

The oligonucleotide synthesis was carried out using the phosphoramidite derivatives of nucleosides with conventional protecting groups according to a standard phosphoramidite protocol.

At the stage of incorporation of modified units **3** and **4**, the condensation time was increased to 5 min. Initially, we repeated treatment with derivatives **3** and **4** twice; however, the incorporation efficiency is rather high even after a single treatment. The efficiency of the attachment of the modified units was at least 90% in the case of treatment and about 98% in the case of repeated treatment.

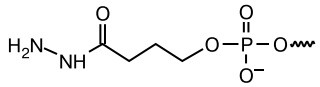
The sequence of post-synthetic treatments of the modified oligonucleotides was dictated by their structural features. Scheme 3 shows the post-synthetic procedure for a modified oligonucleotide containing a residue of compound **4**. The base-labile protecting groups were removed by treatment with a solution of ammonia in anhydrous ethanol,[34] which is a conventional procedure for oligoribonucleotides with standard protecting groups (16 h, 55 °C).[40–42]

We attempted to select milder conditions for the removal of protecting groups and tested a saturated solution of ammonia in anhydrous MeOH (60 h, 20 °C or 16 h, 55 °C) and a 2 *M* solution of ammonia in anhydrous EtOH (60 h, 20 °C (see Ref. 32) or 16 h, 55 °C). The standard treatment with 25% aqueous ammonia (16 h, 55 °C) was used as the reference. The reaction mixtures were analyzed by reversed-phase HPLC (see, for example, Fig. 1, *a*). It was found that all the conditions mentioned ensure the quantitative removal of the protecting groups

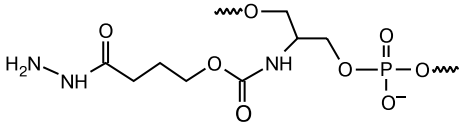
Table 1. Hydrazide-containing oligonucleotides and their nonmodified analogs

Oligonucleotide	Primary structure (5′→3′)	MALDI-TOF mass spectra, $[M - H]^-$	
		Found / Calculated	Molecular formula
I	tgg tag ccg cta gat	4607.005 4608.12	$C_{147}H_{184}N_{57}O_{89}P_{14}$
II	atc tag cgg cta cca	4535.971 4537.14	$C_{145}H_{183}N_{56}O_{87}P_{14}$
III	tgg tag ccg cta gat **Y3**	4904.208 4905.67	$C_{155}H_{200}N_{60}O_{96}P_{15}$
IV	atc tag cgg cta cca **Y3**	4833.173 4834.83	$C_{153}H_{199}N_{59}O_{94}P_{15}$
V	**Y1** tgg tag ccg cta gat	4787.104 4788.25	$C_{151}H_{193}N_{59}O_{93}P_{15}$
VI	**Y1** atc tag cgg cta cca	4716.070 4717.48	$C_{149}H_{192}N_{58}O_{91}P_{15}$
VII	**Y1** tgg tag ccg cta gat **Y3**	5084.307 5085.12	$C_{159}H_{209}N_{62}O_{100}P_{16}$
VIII	**Y1** atc tag cgg cta cca **Y3**	5013.271 5014.85	$C_{157}H_{208}N_{61}O_{98}P_{16}$
IX	tgg tag ccg c**Y2**a gat	4600.015 4602.16	$C_{145}H_{187}N_{58}O_{89}P_{14}$
		5262.856[a] 5265.47[a]	$C_{182}H_{233}N_{58}O_{98}P_{14}Si$[a]
X	atc t**Y2**g cgg cta cca	4519.966 4521.04	$C_{143}H_{187}N_{54}O_{89}P_{14}$
XI	**Y2** tgg tag ccg c**Y2**a gat	4897.217 4898.39	$C_{153}H_{203}N_{61}O_{96}P_{15}$
XII	atc t**Y2**g cgg cta cca **Y3**	4817.168 4817.96	$C_{151}H_{203}N_{57}O_{96}P_{15}$
XIII	tgg **Y2**ag ccg c**Y2**a gat	4593.024 4594.27	$C_{143}H_{190}N_{59}O_{89}P_{14}$
XIV[b]	tgg tag ccg cta gat **Y3A**	5022.340 5023.27	$C_{163}H_{206}N_{60}O_{97}P_{15}$
XV[b]	**Y1A** tgg tag ccg cta gat	4905.237 4905.98	$C_{159}H_{199}N_{59}O_{94}P_{15}$
XVI[b]	tgg tag ccg c**Y2A**a gat	4718.147 4719.09	$C_{153}H_{193}N_{58}O_{90}P_{14}$

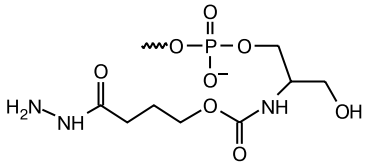
Structure of the units **Y1—Y3**



Y1



Y2



Y3

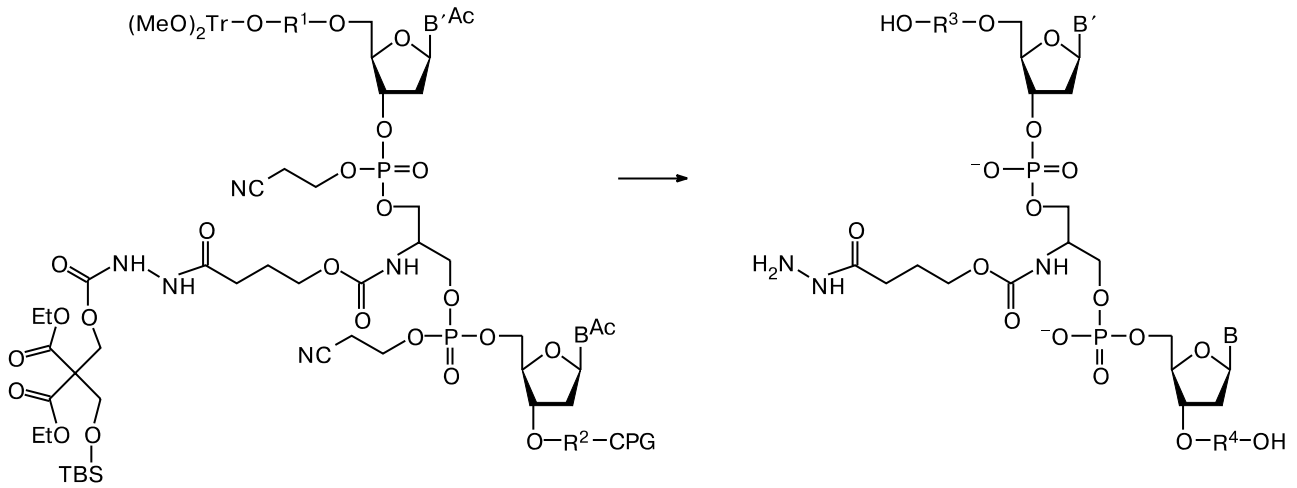
[a] For oligonucleotides **IX**, the calculated and found masses of the oligonucleotides with 5′-dimethoxytrityl group after treatment with a concentrated solution of ammonia in anhydrous ethanol are also given.

[b] Oligonucleotides **XIV—XVI** are anisaldehyde conjugates of oligonucleotides **III**, **V**, and **IX**, respectively. **Y1A**, **Y2A**, **Y3A** are anisaldehyde adducts of units **Y1**, **Y2**, **Y3**, respectively.

from nonmodified oligodeoxyribonucleotides. Subsequently, modified oligonucleotides were deprotected by treatment with a saturated solution of ammonia in anhydrous ethanol for 17 h at 55 °C (see Scheme 3).

After treatment with ammonia, modified oligonucleotides were isolated by reversed-phase HPLC. Their structures were confirmed by MALDI-TOF mass spectrometry. The spectra contained peaks with m/z corresponding to the $[M - H]^-$ and $[M - H - (MeO)_2Tr]^-$ ions (see, for example, Fig. 2, *a*). The dimethoxytrityl group was removed by treatment with 80% AcOH (the same procedure was applied when using derivative **5**). Then oligonucleotides were treated with a solution of Bu_4NF in anhydrous THF, which resulted in elimination of the *tert*-butyldimethylsilyl group (see Scheme 3); after that, hydrazide was liberated under mild alkaline conditions. To this end, oligonucleotides were treated with 12.5% aqueous ammonia for 15 min at room temperature (see Scheme 3). Free oligonucleotides were analyzed and isolated by reversed-phase HPLC. This revealed a major peak, which always comprised at least 80% (see, for example, Fig. 1, *b*). The structures of the target hydrazide-containing oligonucleotides were confirmed by MALDI-TOF mass spectrometry (see Table 1); the spectra exhibited peaks corresponding to $[M - H]^-$ ions (see, for example, Fig. 2, *b*).

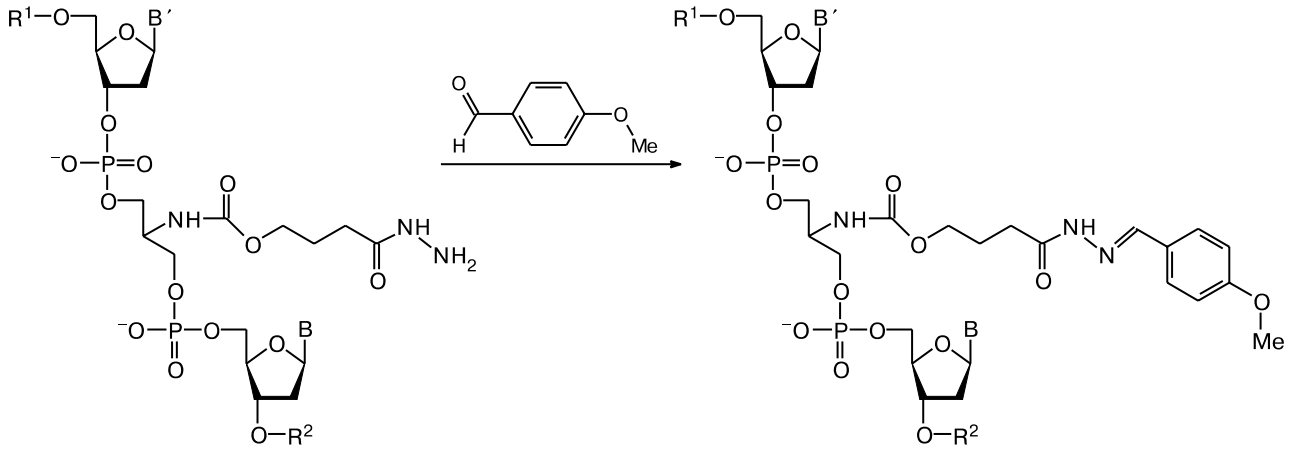
Scheme 3



R^1, R^2 are fragments of protected oligonucleotide; R^3, R^4 are fragments of the deprotected oligonucleotide; B, B′ are the nucleic base residues.

Reagents and conditions: 1) NH_3/EtOH, 17 h, 55 °C; 2) 80% AcOH, 30 min, 20 °C; 3) $Bu_4N^+F^-$, THF, 18 h, 20 °C; 4) 12.5% NH_4OH, 15 min, 20 °C.

Scheme 4



R^1, R^2 are oligonucleotide chain fragments.

Oligonucleotides containing a hydrazide function reacted with anisaldehyde (Scheme 4). The yield of the conjugates was as high as 60% (the reaction conditions were not optimized); they had longer retention times in the reversed-phase HPLC. The adduct of oligonucleotide **IX** (see Table 1) with anisaldehyde was isolated by reversed-phase HPLC (Fig. 3), its structure being confirmed by MALDI-TOF mass spectrometry; the spectrum displayed a peak corresponding to the $[M - H]^-$ ion (see Fig. 2, *c*).

Thus, the method we proposed for the synthesis of phosphoramidites **3—5** as the starting compounds for the preparation of oligodeoxynucleotides provides a facile route to modified oligonucleotides containing hydrazide functions in any specified positions of the oligomer, *i.e.*, at the 3′- and 5′-ends and within the chain. This is attained using the standard automated phosphoramidite oligonucleotide synthesis. The preparation of oligonucleotides containing several hydrazide functions in the same chain is also possible.

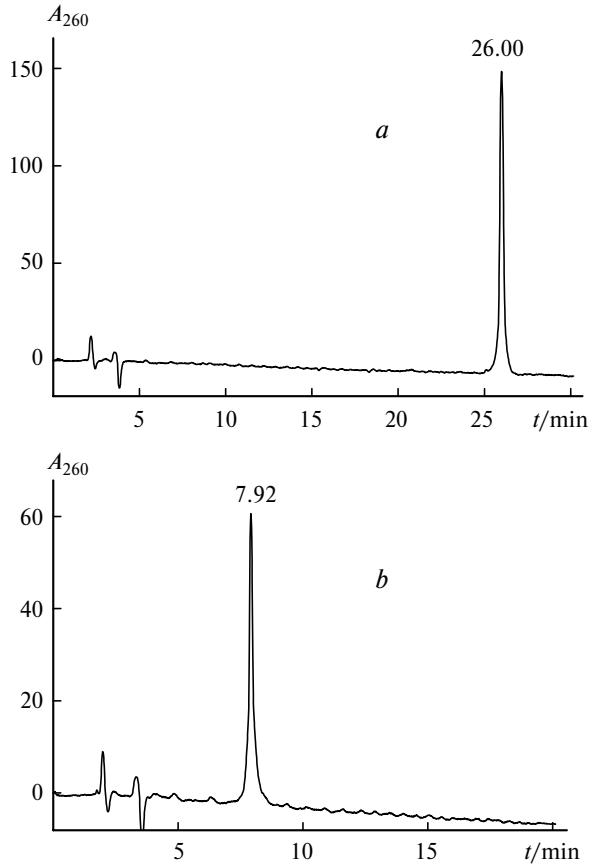


Fig. 1. Reversed-phase HPLC analysis of the 5′-dimethoxytrityl group-containing hydrazide derivative of oligonucleotide **IX** isolated from the reaction mixture after treatment of the polymeric support with a concentrated solution of ammonia in anhydrous ethanol (*a*); after deprotection procedure (*b*). The conditions of analysis are presented in the Experimental.

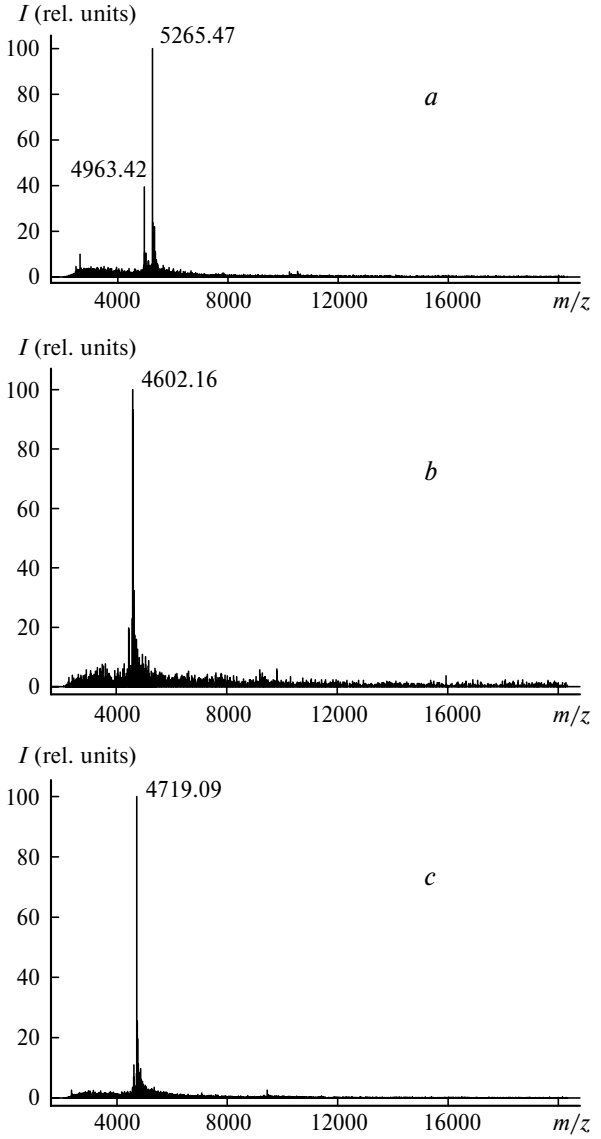


Fig. 2. MALDI-TOF mass-spectrometric analysis of oligonucleotide **IX** with a 5′-dimethoxytrityl group after treatment with a concentrated solution of ammonia in anhydrous ethanol (calculated: 5262.856, $[M - H]^-$; found: 5265.47) (*a*); oligonucleotide **IX** isolated after completion of the deprotection procedure (calculated: 4600.015, $[M - H]^-$; found: 4602.16) (*b*); conjugate of oligonucleotide **IX** with anisaldehyde (calculated: 4718.147, $[M - H]^-$; found: 4719.09) (*c*). The conditions of analysis are presented in the Experimental.

Experimental

Commercial reagents (Acros Organics, Aldrich, Bayer, Fluka, Merck, Pharmacia Biotech, and Sigma) were used. All anhydrous solvents were distilled prior to use. Unless stated otherwise, all the reactions were carried out with stirring under dry nitrogen at room temperature. The reaction mixtures were concentrated, solutions of the residues in CH_2Cl_2 were washed with brine (×2), dried with Na_2SO_4, concentrated, and chromatographed on a column with SiO_2 Kieselgel 60 (230—400 mesh) (Merck) using dichloromethane— ethanol mixtures as eluents.

Thin-layer chromatography was performed on Kieselgel 60 F_{254} plates (Merck) in the following solvent systems: *A*, dichloromethane; *B*, dichloromethane—ethanol (95 : 5); *C*, dichloromethane—ethanol (9 : 1); *D*, dichloromethane—ethanol (85 : 15); and *E*, dichloromethane—triethylamine (99 : 1).

The optical absorption and UV spectra of oligonucleotide solutions were recorded on a Varian Cary 1E spectrophotometer in a quartz cell with an optical path of 1 mm.

^{1}H NMR spectra were recorded at 200.131 MHz on a Bruker DPX-200 instrument; the ^{1}H NMR spectra of phosphoramidite derivatives **3** and **4** were measured at 400.131 MHz and the ^{31}P NMR spectra, at 161.992 MHz on a Bruker DRX-400 instrument.

Positive-ion mass spectra (electrospray ionization, ESI) were obtained on a Bruker Esquire LC 00153 mass spectrometer.

The negative ion MALDI-TOF mass spectra of oligonucleotides were recorded on Voyager DE-RP (Perseptive Biosystems) and Bruker Daltonics Autoflex instruments. The samples were exposed to the radiation of a nitrogen laser with a wavelength of 337 nm. The laser desorption-generated ions were energetically

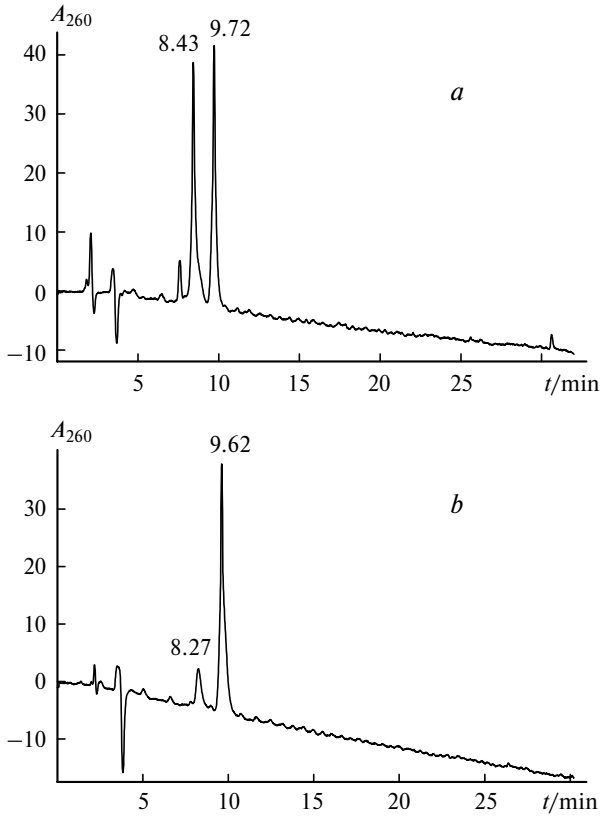


Fig. 3. Reversed-phase HPLC analysis of the reaction mixture during the synthesis of conjugate of the hydrazide-containing oligonucleotide **IX** with anisaldehyde (*a*); the isolated conjugate (*b*). The conditions of analysis are presented in the Experimental.

stabilized during the extraction period of 300 ns and then directed through a linear TOF mass analyzer with a voltage of 25 or 20 kV (for the Daltonics Autoflex instrument). 3-Hydroxypyridine-2-carboxylic acid in a mixture of acetonitrile and water (1 : 1) was used as the matrix.

Analysis of the reaction mixtures and isolation of the target modified oligonucleotides were carried out by reversed-phase HPLC on Kontron chromatographs (Kontron Instruments) using 4×250 mm CC 250/4 Nucleodur 100-5 C18 ec columns (Macherey-Nagel), particle size 7 μm, elution rate 1 mL min^{-1} (~20 °C). For analysis and isolation of oligonucleotides containing a dimethoxytrityl group, a gradient of acetonitrile (5—35%) in a 0.1 *M* aqueous solution of ammonium hydrogencarbonate (30 min, 1% per min) was used. In other cases, a gradient of acetonitrile (5—20%, 0.5% per min, 30 min) was used.

4-Hydroxybutyrohydrazide (7). γ-Butyrolactone (15.37 mL, 200 mmol) was dissolved in ethanol (200 mL), and a 35% aqueous solution of hydrazine (18.31 mL, 200 mmol) was added. The reaction mixture was refluxed for 20 h and cooled, the solvents were evaporated *in vacuo*, and the residue was dried *in vacuo*. Yield 22.9 g (96.8%). The product was used without further purification. ^{1}H NMR (D_2O), δ: 1.81 (m, 2 H, $C\underline{H}_2CH_2OH$); 2.27 (t, 2 H, $C\underline{H}_2(CH_2)_2OH$, J = 7.6 Hz); 3.59 (t, 2 H, $C\underline{H}_2OH$, J = 6.4 Hz). MS, found: m/z 119.2 $[M + H]^+$. $C_4H_{10}N_2O_2$. Calculated: [M + H] = 119.134.

2-Trifluoroacetamidopropane-1,3-diol (12). Anhydrous pyridine (10 mL) was twice added to, and distilled from, aminopropane-1,3-diol (**11**) (3.64 g, 40 mmol) and the residue was dissolved in a mixture of anhydrous pyridine (20 mL) and anhydrous CH_2Cl_2 (20 mL). Ethyl trifluoroacetate (9.52 mL, 80 mmol) was added with stirring. After 24 h, the solvents and the remaining CF_3CO_2Et were evaporated. Anhydrous benzene (20 mL) and then anhydrous CH_2Cl_2 (20 mL) were added to, and distilled from, the residue . The resulting compound **12** was used without further purification. Yield 7.26 g (97.0%), R_f 0.2 (*C*). ^{1}H NMR ($CDCl_3$), δ: 3.93 (m, 5 H, CH_2CHCH_2). MS, found: m/z 187.9 $[M + H]^+$. $C_5H_8F_3NO_3$. Calculated: [M + H] = 188.117.

1-*O*-(4,4′-Dimethoxytrityl)-2-trifluoroacetamidopropane-1,3-diol (13). A solution of 4,4′-dimethoxytrityl chloride (8.47 g, 25 mmol) in anhydrous CH_2Cl_2 (100 mL) was slowly added dropwise with stirring to a solution of compound **12** (7.26 g, 38.8 mmol) in anhydrous pyridine (150 mL). After 1.5 h, the reaction mixture was concentrated, the residue was dissolved in CH_2Cl_2 (200 mL), and the solution was washed with a saturated aqueous solution of $NaHCO_3$, brine, and water. The organic phase was separated, dried for 2 h with anhydrous sodium sulfate, and concentrated. The residue was chromatographed on a column with SiO_2 using solvent systems *A* and *B*. The yield was 3.38 g (27.6%), R_f 0.6 (*B*). ^{1}H NMR (DMSO-d_6), δ: 3.11 (m, 2 H, $CHC\underline{H}_2OH$); 3.53 (m, 3 H, $TrOC\underline{H}_2C\underline{H}$); 3.79 (s, 6 H, $C\underline{H}_3O$); 6.93 (d, 4 H, C_6H_4, J = 8.9 Hz); 7.28 (d, 4 H, C_6H_4); 7.40 (m, 5 H, C_6H_5). MS, found: m/z 488.4 $[M + H]^+$. $C_{26}H_{27}F_3NO_5$. Calculated: $[M + H]^+$ = 490.184.

2-Amino-1-*O*-(4,4′-dimethoxytrityl)propane-1,3-diol (14). A 33% aqueous solution of ammonia (100 mL) was added to a solution of compound **13** (3.0 g, 6.13 mmol) in ethanol (100 mL). After 2 h at 55 °C and for 48 h at room temperature, the reaction mixture was concentrated, and the residue (oil) was dissolved in CH_2Cl_2 (100 mL). The solution was washed with a saturated aqueous solution of $NaHCO_3$ (100 mL) and brine, dried for 2 h with anhydrous sodium sulfate, concentrated, and chromatographed on a column with SiO_2 with solvent systems *A*—*D* as eluents. Yield 1.55 g (64.3%), R_f 0.2 (*C*). ^{1}H NMR (DMSO-d_6), δ: 2.89 (m, 3 H, $C\underline{H}C\underline{H}_2OH$); 3.34 (m, 2 H, $TrOC\underline{H}_2$); 3.74 (s, 6 H, $C\underline{H}_3O$); 6.89 (d, 4 H, C_6H_4, J = 8.7 Hz); 7.25 (d, 4 H, C_6H_4); 7.38 (m, 5 H, C_6H_5). MS, found: m/z 416.2 $[M + Na]^+$. $C_{24}H_{27}NO_4$. Calculated: [M + Na] = 416.465.

Diethyl 2-*tert*-butyl(dimethyl)silyloxymethyl-2-hydroxymethylmalonate (8). Anhydrous pyridine (20 mL) was twice added to, and distilled from, diethyl 2,2-bis(hydroxymethyl)malonate (**6**) (22.02 g, 100 mmol) and the residue was dissolved in a mixture of anhydrous pyridine (50 mL) and anhydrous CH_2Cl_2 (50 mL). This solution was added dropwise to a stirred solution of *tert*-butyl(dimethyl)silyl chloride (7.54 g, 50 mmol) and DMAP (610 mg, 5 mmol) in a mixture of anhydrous pyridine (50 mL) and anhydrous CH_2Cl_2 (50 mL), and the mixture was stirred for 48 h. The usual workup gave compound **8**, yield 13.20 g (79.0%), R_f 0.5 (*A*). ^{1}H NMR ($CDCl_3$), δ: 0.05 (s, 6 H, $(CH_3)_2Si$); 0.85 (s, 9 H, $(CH_3)_3CSi$); 1.27 (t, 6 H, $C\underline{H}_3CH_2$, J = 7.2 Hz); 4.06 (s, 2 H, CH_2OSi); 4.11 (s, 2 H, $C\underline{H}_2OH$); 4.22 (q, 4 H, $CH_3C\underline{H}_2O$). MS, found: m/z 335.3 $[M + H]^+$. $C_{15}H_{30}O_6Si$. Calculated: [M + H] = 335.481.

Diethyl 2-*tert*-butyl(dimethyl)silyloxymethyl-2-(*p*-nitrophenoxycarbonyl)oxymethylmalonate (9). Anhydrous pyridine

(2.67 mL, 33 mmol) and then monohydroxy compound **8** (10.03 g, 30 mmol) were added with stirring to a solution of *p*-nitrophenyl chloroformate (6.35 g, 31.5 mmol) and DMAP (366 mg, 10 mmol) in anhydrous CH_2Cl_2 (200 mL). After 2.5 h, the precipitate was filtered off and the filtrate was concentrated. The usual workup gave compound **9**, yield 10.95 g (73.0%), R_f 0.7 (*A*). ^{1}H NMR ($CDCl_3$), δ: 0.05 (s, 6 H, $(CH_3)_2Si$); 0.86 (s, 9 H, $(CH_3)_3CSi$); 1.27 (t, 6 H, $C\underline{H}_3CH_2$, J = 7.1 Hz); 4.13 (s, 2 H, CH_2OSi); 4.23 (q, 4 H, $CH_3C\underline{H}_2O$); 4.81 (s, 2 H, CH_2OCO); 7.35 (d, 2 H, C_6H_4, J = 9.2 Hz); 8.27 (d, 2 H, C_6H_4). MS, found: m/z 500.3 $[M + H]^+$. $C_{22}H_{33}NO_{10}Si$. Calculated: [M + H] = 500.584.

Diethyl 2-*tert*-butyl(dimethyl)silyloxymethyl-2-[2-(4-hydroxybutyryl)hydrazino]carbonyloxymethylmalonate (10). 4-Hydroxybutyrohydrazide (**7**) (2.6 g, 22 mmol) was dissolved in anhydrous DMF (100 mL); *p*-nitrophenyl carbonate **9** (10.94 g, 21.9 mmol), anhydrous pyridine (1.618 mL, 20 mmol), and DMAP (244 mg, 2 mmol) were added. After 16 h, the solvent was evaporated. After the usual workup of the reaction mixture, the residue was chromatographed on a column with SiO_2 using solvent systems *A*—*C* for elution to give compound **10**, yield 6.34 g (66.3%), R_f 0.5 (*C*). ^{1}H NMR ($CDCl_3$), δ: 0.03 (s, 6 H, $(CH_3)_2Si$); 0.84 (s, 9 H, $(CH_3)_3CSi$); 1.24 (t, 6 H, $C\underline{H}_3CH_2$, J = 7.2 Hz); 1.89 (m, 2 H, $C\underline{H}_2CH_2OH$); 2.38 (t, 2 H, $C\underline{H}_2(CH_2)_2OH$, J = 6.8 Hz); 3.70 (t, 2 H, $CH_2C\underline{H}_2OH$, J = 6.1 Hz); 4.05 (s, 2 H, CH_2OSi); 4.19 (q, 4 H, $CH_3C\underline{H}_2O$); 4.65 (s, 2 H, CH_2OCO). MS, found: m/z 479.3 $[M + H]^+$. $C_{20}H_{38}N_2O_9Si$. Calculated: [M + H] = 479.609.

Diethyl 2-*tert*-butyl(dimethyl)silyloxymethyl-2-(2-{4-[(diisopropylamino)(2-cyanoethoxy)phosphinoxy]butyryl}hydrazino)carbonyloxymethylmalonate (3). Anhydrous MeCN (5 mL) was twice added to, and distilled from, compound **10** (263 mg, 0.55 mmol) and diisopropylamine tetrazolide (113 mg, 0.66 mmol), and the residue was dissolved in anhydrous CH_2Cl_2 (10 mL). 2-Cyanoethyl bis(*N*,*N*´-diisopropyl) phosphorodiamidite (420 μL, 1.32 mmol) was added dropwise with stirring. After 30 min, the reaction mixture was washed with cooled brine, the organic phase was dried for 2 h with anhydrous sodium sulfate and concentrated, and the residue was dried for 24 h *in vacuo*. Cold anhydrous pentane was added, and the mixture was kept at 4 °C for 16 h. The supernatant was decanted, and the residue was dried *in vacuo* for 48 h and used in the oligonucleotide synthesis without further purification. The yield of compound **3** was 351 mg (94.0%), R_f 0.7 (*C*). ^{1}H NMR ($CDCl_3$), δ: 0.03 (s, 6 H, $(CH_3)_2Si$); 0.84 (s, 9 H, $(CH_3)_3CSi$); 1.18 (m, 12 H, $((C\underline{H}_3)_2CH)_2N$); 1.26 (t, 6 H, $C\underline{H}_3CH_2$, J = 6.0 Hz); 1.96 (m, 2 H, $CH_2C\underline{H}_2CH_2OP$); 2.36 (m, 2 H, $C\underline{H}_2(CH_2)_2OP$); 2.75 (t, 2 H, CH_2CN, J = 6.0 Hz); 3.53 (m, 2 H, $C\underline{H}_2CH_2CN$); 3.60 (m, 2 H, $((CH_3)_2C\underline{H})_2N$); 3.79 (m, 2 H, $(CH_2)_2C\underline{H}_2OP$); 4.05 (s, 2 H, $C\underline{H}_2OSi$); 4.18 (q, 4 H, $CH_3C\underline{H}_2O$); 4.65 (s, 2 H, $C\underline{H}_2OCO$). ^{31}P: 147.82. MS, found: m/z 679.1 $[M + H]^+$. $C_{29}H_{55}N_4O_{10}PSi$. Calculated: [M + H] = 679.827.

Diethyl 2-*tert*-butyl(dimethyl)silyloxymethyl-2-{2-[4-(*p*-nitrophenoxycarbonyl)oxybutyryl]hydrazino} carbonyloxymethylmalonate (15). Compound **10** (6.44 g, 10 mmol) was dissolved in anhydrous CH_2Cl_2 (55 mL). Anhydrous pyridine (970 μL, 12 mmol), then a solution of 4-$O_2NC_6H_4OCOCl$ (2.22 g, 11 mmol) in anhydrous CH_2Cl_2 (20 mL), and DMAP (122 mg, 1 mmol) were added. After 2 h, the reaction mixture was worked-up in a usual way to give compound **15**, yield 4.67 g (72.5%). R_f 0.7 (*B*). ^{1}H NMR ($CDCl_3$), δ: 0.02 (s, 6 H, $(CH_3)_2Si$); 0.83 (s, 9 H, $(CH_3)_3CSi$); 1.23 (t, 6 H, $C\underline{H}_3CH_2$, J = 7.2 Hz); 2.13 (m, 2 H, $C\underline{H}_2CH_2OH$); 2.40 (t, 2 H, $C\underline{H}_2(CH_2)_2OCO$, J = 7.1 Hz); 4.04 (s, 2 H, CH_2OSi); 4.18 (q, 4 H, $CH_3C\underline{H}_2O$); 4.36 (t, 2 H, $CH_2C\underline{H}_2OCO$, J = 6.2 Hz); 4.65 (s, 2 H, CH_2OCO); 7.38 (d, 2 H, C_6H_4, J = 9.4 Hz); 8.27 (d, 2 H, C_6H_4). MS, found: m/z 644.4 $[M + H]^+$. $C_{27}H_{41}N_3O_{13}Si$. Calculated: [M + H] = 644.712.

Diethyl 2-*tert*-butyl(dimethyl)silyloxymethyl-2-[2-(4-{*N*-[1-(4,4´-dimethoxy)trityloxy-3-hydroxypropan-2-yl]carbamoyloxy}butyryl)hydrazino]carbonyloxymethylmalonate (16). Compound **15** (2.49 g, 3.86 mmol), anhydrous pyridine (344 μL, 4.25 mmol), and DMAP (47 mg, 0.386 mmol) were added to a solution of 2-amino-1-*O*-(dimethoxytrityl)propane-1,3-diol (**14**) (1.52 g, 3.86 mmol) in anhydrous CH_2Cl_2 (25 mL), and the mixture was stirred for 16 h. The usual workup gave 2.16 g (62.4%) of compound **16**, R_f 0.6 (*C*). ^{1}H NMR (DMSO-d_6), δ: 0.02 (s, 6 H, $(CH_3)_2Si$); 0.81 (s, 9 H, $(CH_3)_3CSi$); 1.16 (t, 6 H, $C\underline{H}_3CH_2$, J = 6.9 Hz); 1.78 (m, 2 H, $C\underline{H}_2CH_2CONHNH$); 2.38 (m, 2 H, $C\underline{H}_2CONHNH$); 2.95 (m, 2 H, $C\underline{H}_2OH$); 3.48 (t, 2 H, $C\underline{H}_2(CH_2)_2CONHNH$, J = 5.3 Hz); 3.73 (s, 6 H, CH_3O); 3.94 (s, 2 H, CH_2OSi); 3.95 (m, 2 H, $TrOC\underline{H}_2$); 4.14 (q, 4 H, $CH_3C\underline{H}_2O$); 4.40 (s, 2 H, $C\underline{H}_2OCONHNH$); 4.57 (m, 1 H, $TrOCH_2C\underline{H}$); 6.87 (d, 4 H, C_6H_4, J = 8.8 Hz); 7.23 (d, 4 H, C_6H_4); 7.34 (m, 5 H, C_6H_5). MS, found: m/z 898.7 $[M + H]^+$. $C_{45}H_{63}N_3O_{14}Si$. Calculated: [M + H] = 899.079.

Diethyl 2-*tert*-butyl(dimethyl)silyloxymethyl-2-{2-[4-(*N*-{1-(4,4´-dimethoxy)trityloxy-3-[(diisopropylamino)(2-cyanoethoxy)phosphinoxy]propan-2-yl}carbamoyloxy)butyryl]hydrazino}carbonyloxymethylmalonate (4). Diisopropylamine tetrazolide (154 mg, 0.9 mmol) was added to compound **16** (674 mg, 0.75 mmol), anhydrous MeCN (10 mL) was twice added to, and distilled from, the mixture, and the residue was dissolved in anhydrous CH_2Cl_2 (15 mL). 2-Cyanoethyl bis(*N*,*N*-diisopropyl) phosphorodiamidite (571 μL, 1.8 mmol) was added with stirring. After 25 min, the reaction mixture was worked-up in the usual way, as described for compound **3** (dried *in vacuo* for 16 h). The yield of compound **4** was 824 mg (91.0%). R_f 0.75 (*B*). The resulting compound **4** was used in the oligonucleotide synthesis without further purification. ^{1}H NMR ($CDCl_3$), δ: 0.03 (s, 6 H, $(CH_3)_2Si$); 0.84 (s, 9 H, $(CH_3)_3CSi$); 1.18 (m, 12 H, $((C\underline{H}_3)_2CH)_2N$); 1.26 (t, 6 H, $C\underline{H}_3CH_2$, J = 6.3 Hz); 1.94 (m, 2 H, $C\underline{H}_2CH_2CONHNH$); 2.22 (t, 2 H, $C\underline{H}_2CONHNH$, J = 6.6 Hz); 2.64 (m, 2 H, $CHC\underline{H}_2OP$); 2.74 (t, 2 H, CH_2CN, J = 6.0 Hz); 3.53 (m, 4 H, $C\underline{H}_2CH_2CN$; $C\underline{H}_2(CH_2)_2CONHNH$); 3.69 (m, 2 H, $((CH_3)_2C\underline{H})_2N$); 3.78 (s, 6 H, CH_3O); 4.00 (m, 1 H, $TrOCH_2C\underline{H}$); 4.05 (s, 2 H, CH_2OSi); 4.12 (m, 2 H, $TrOC\underline{H}_2$); 4.18 (q, 4 H, $CH_3C\underline{H}_2O$); 4.65 (s, 2 H, $C\underline{H}_2OCONHNH$); 6.81 (d, 4 H, C_6H_4, J = 9.0 Hz); 7.26 (d, 4 H, C_6H_4); 7.30—7.40 (m, 5 H, C_6H_5). ^{31}P: 148.46; 148.55. MS, found: m/z 1120.7 $[M + Na]^+$. $C_{54}H_{80}N_5O_{15}PSi$. Calculated: [M + Na] = 1121.287.

Immobilization of compound 16 on the polymeric support. Aminopropyl-CPG (80—120 mesh, pore size 700 Å) with amino group loading of 44 μmol g^{-1} (500 mg, 22 μmol based on amino groups) was suspended in pyridine (3 mL), and succinic anhydride (220 mg, 2.2 mmol) and DMAP (10.75 mg, 88 μmol) were added. The polymeric support was degassed and stirred for 12 h, then the polymer was washed on a filter with pyridine (5×10 mL), ethanol (5×10 mL), diethyl ether (5×10 mL), and dried *in vacuo*. After that, the polymeric support was suspended in pyridine (2 mL), trimethylchlorosilane (400 μL, 3.13 mmol)

was added, and the suspension was degassed and stirred for 16 h. The support was separated, washed on the filter with pyridine (5×10 mL), ethanol (5×10 mL), and diethyl ether (5×10 mL), and dried *in vacuo*. Then the polymeric support was suspended in pyridine (1 mL), and 2,4,6-triisopropylbenzenesulfonyl chloride (60 mg, 200 μmol) and *N*-methylimidazole (46.6 μL, 600 μmol) were added. The mixture was degassed and stirred for 30 min. Then compound **16** (79 mg, 88 μmol) and, after 2 h, methanol (5 mL) were added, and the mixture was stirred for 16 h. Then the polymeric support was separated, washed on the filter with pyridine (5×10 mL), ethanol (5×10 mL), and diethyl ether (5×10 mL), and dried *in vacuo*.

The resulting specific loading of the polymeric support by modified units was determined by spectrophotometry. The modified polymeric support (1 mg) was treated with a 3% solution of CF_3COOH (50 μL) in 50% aqueous CH_3CN. The reaction mixture was centrifuged, the supernatant was withdrawn, and the polymeric support was washed twice with 50% aqueous CH_3CN (425 μL). The washings were separated from the polymeric support by centrifugation, and combined with the supernatant. The absorbance of the dimethoxytrityl cation present in the solution was measured in the visible region at 478 nm. The absorbance thus found was used to calculate the amount of the dimethoxytrityl cation. The specific loading of the polymeric support with the modified units was taken to be equal to the determined amount of eliminated dimethoxytrityl cation. The specific loading of the polymeric support with compound **16** was about 20 μmol g^{-1}.

Oligonucleotide synthesis (general procedure). Modified oligonucleotides (and their natural analogs) were synthesized on a Gene Assembler automated DNA synthesizer (Pharmacia Biotech); the phosphoramidite protocol of the oligonucleotide synthesis was used. The scale of the synthesis was 1.3 μmol. The condensation was activated by 4,5-dicyanoimidazole. The concentration of the modified phosphoramidite derivatives **3** and **4** was 0.12 mol L^{-1}; the time of the condensation step was increased to 5 min. For the synthesis of 3′-modified oligonucleotides, polymeric support **5** (65 mg) was used.

Post-synthetic treatment of modified oligonucleotides (general procedure). The polymeric support with the immobilized modified oligonucleotide was treated with a saturated solution of ammonia in anhydrous ethanol at 55 °C. After 17 h, the polymer was separated and washed with water (3×500 μL), the aqueous solutions were combined and concentrated *in vacuo* (using a Speed Vac Plus 110A vacuum concentrator (Savant)). The reaction mixtures were analyzed and the target products were isolated by HPLC.

The isolated oligonucleotides containing a dimethoxytrityl group were treated with 80% AcOH (500 μL) at ~20 °C; after 30 min, 50% aqueous methanol (500 μL) was added, the resulting solution was concentrated to dryness, and 50% aqueous methanol (500 μL) was twice added and distilled off.

Then the oligonucleotide samples were treated with a 1 *M* solution of Bu_4NF in anhydrous THF (130 μL). After 18 h, a 2 *M* aqueous solution of triethylammonium acetate, pH 7.0 (130 μL) was added, and the solution was diluted with water to 1 mL and desalinated using NAP-5 or NAP-10 columns (Pharmacia Biotech). The resulting solution was concentrated *in vacuo* to 100 μL, and a 25% aqueous solution of ammonia (100 μL) was added. After 15 min, the solution was concentrated to dryness and dissolved in water (1 mL). The structures of the target oligonucleotides were confirmed by MALDI-TOF mass spectrometry (see Table 1).

The reaction of hydrazide-containing oligonucleotides with anisaldehyde (general procedure). The desalinated aqueous solution of the hydrazide-containing oligonucleotide (content of the oligonucleotide material 5.0 nmol) was concentrated to dryness and then dissolved in 0.1 *M* MES buffer (pH 5.0—5.3) (10 μL). A 1 *M* solution of 4-methoxybenzaldehyde in DMF (4 μL) was added. After 30 or 60 min at 30 or 37 °C, the solution was diluted to 500 μL with a 1 m*M* aqueous solution of ammonium acetate, and the oligonucleotides were desalted using 1 m*M* aqueous ammonium acetate for elution. Then the solution was concentrated *in vacuo* to 400 μL, and the reaction mixtures were analyzed by reversed-phase HPLC. The oligonucleotide and anisaldehyde conjugates were isolated by HPLC under the same conditions. The fractions containing conjugates were collected and concentrated *in vacuo* to dryness, and the residue was dissolved in water (400 μL) and analyzed by HPLC (see, for example, Fig. 3) and MALDI-TOF mass spectrometry (see Table 1).

S. I. Antsypovich is grateful to the Alexander von Humboldt Foundation (Germany) for the research fellowship. This work was partially supported by the program "Universities of Russia" (Project No. 05.02.547).

References

1. J. Goodchild, *Bioconjugate Chem.*, 1990, **1**, 165.
2. M. Gallo, J. M. Montserrat, and A. M. Iribarren, *Braz. J. Med. Biol. Res.*, 2003, **36**, 143.
3. *Oligonucleotide Synthesis: Methods and Applications (Methods in Molecular Biology)*, Ed. P. Herdewijn, Humana Press, Totowa New Jersey, 2004, 435 p.
4. S. Verma and F. Eckstein, *Annu. Rev. Biochem.*, 1998, **67**, 99.
5. S. I. Antsypovich and T. S. Oretskaya, *Usp. Khim.*, 1998, **67**, 274 [*Russ. Chem. Rev.*, 1998, **67**, 245 (Engl. Transl.)].
6. I. Lebedeva and C. A. Stein, *Annu. Rev. Pharmacol. Toxicol.*, 2001, **41**, 403.
7. C. H. Tung and S. Stein, *Bioconjugate Chem.*, 2000, **11**, 605.
8. S. T. Crooke, *Antisense Drug Technology Principles, Strategies and Applications*, Ed. S. T. Crooke, Marcel Dekker Inc., New York, 2001, 916 p.
9. P. Couvreur and C. Malvy, *Pharmaceutical Aspects of Oligonucleotides*, Eds P. Couvreur and C. Malvy, Taylor and Francis, London, 1999, 321 p.
10. T. S. Zatsepin, D. A. Stetsenko, M. J. Gait, and T. S. Oretskaya, *Bioconjugate Chem.*, 2005, **16**, 471.
11. S. I. Antsypovich and G. von Kiedrowski, *Nucleosides, Nucleotides and Nucleic Acids*, 2005, **24**, 211.
12. K. Achilles and G. von Kiedrowski, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 1229.
13. S. Raddatz, J. Mueller-Ibeler, J. Kluge, L. Wass, G. Burdinski, J. R. Havens, T. J. Onofrey, D. Wang, and M. Schweitzer, *Nucleic Acids Res.*, 2002, **30**, 4793.
14. S. S. Ghosh, P. M. Kao, and D. Y. Kwoh, *Anal. Biochem.*, 1989, **178**, 43.
15. H. Strobel, L. Dugue, P. Marliere, and S. Pochet, *Nucleic Acids Res.*, 2002, **30**, 1869.

16. G. von Kiedrowski, L.-H. Eckardt, K. Naumann, W. M. Pankau, M. Reimold, and M. Rein, *Pure Appl. Chem.*, 2003, **75**, 609.
17. L. H. Eckardt, K. Naumann, W. M. Pankau, M. Rein, M. Schweitzer, N. Windhab, and G. von Kiedrowski, *Nature*, 2002, **420**, 286.
18. L. Eckard, K. Naumann, W. M. Pankau, and G. von Kiedrowski, *12th International Symposium on Supramolecular Chemistry (ISSC-XII) (Eilat, Israel, October 2002)*, *Abstrs.*, Eilat, Israel, 2002, O—47.
19. M. Scheffler, A. Dorenbeck, S. Jordan, M. W´stefeld, and G. von Kiedrowski, *Angew. Chem., Int. Ed. Engl.*, 1999, **38**, 3311.
20. E. M. Zubin, D. A. Stetsenko, T. S. Oretskaya, and M. J. Gait, *Nucleosides, Nucleotides and Nucleic Acids*, 2003, **22**, 1375.
21. N. N. Polushin, A. M. Morocho, B. Chen, and J. S. Cohen, *Nucleic Acids Res.*, 1994, **22**, 639.
22. N. N. Polushin, I. N. Pashkova, O. G. Chakhmakhcheva, V. A. Efimov, *Bioorgan. Khim.*, 1993, **19**, 318 [*Russ. J. Bioorg. Chem.*, 1993, **19** (Engl. Transl.)].
23. N. N. Polushin, I. N. Pashkova, and V. A. Efimov, *Nucleic Acids Res. Symp. Ser.*, 1991, **24**, 49.
24. Z. A. Shabarova and A. A. Bogdanov, *Advanced Organic Chemistry of Nucleic Acids*, VCH Verlagsgesellschaft, Weinheim, 1994, 588 c.
25. T. Horn and M. S. Urdea, *Tetrahedron Lett.*, 1986, **27**, 4705.
26. J. C. Schulhof, D. Molko, and R. Teoule, *Nucleic Acids Res.*, 1987, **15**, 397.
27. B. Uznanski, A. Grajkowski, and A. Wilk, *Nucleic Acids Res.*, 1989, **17**, 4863.
28. N. D. Sinha, P. Davis, N. Usman, J. Perez, R. Hodge, J. Kremsky, and R. A. Casale, *Biochimie*, 1993, **75**, 13.
29. N. D. Sinha, D. P. Michaud, S. K. Roy, and R. A. Casale, *Nucleic Acids Res.*, 1994, **22**, 3119.
30. E. Sonveaux, *Protecting Groups in Oligonucleotide Synthesis.* in *Methods in Molecular Biology: Protocols for Oligonucleotide Conjugates and Analogs*, Ed. S. Agrawal, Humana Press, Totowa New Jersey, 1993, **26**, 516 p.
31. M. J. Damha and K. K. Ogilvie, *Oligoribonucleotide Synthesis*, in *Methods in Molecular Biology: Protocols for Oligonucleotides and Analogs*, Ed. S. Agrawal, Humana Press, Totowa New Jersey, 1993, **20**, 1.
32. S. N. Mikhailov, J. Rozenski, E. V. Efimtseva, R. Busson, A. Van Aerschot, and P. Herdewijn, *Nucleic Acids Res.*, 2002, **30**, 1124.
33. A. Guzaev, H. Salo, A. Azhayev, and H. Lonnberg, *Tetrahedron*, 1995, **51**, 9375.
34. S. Muller, J. Wolf, and S. A. Ivanov, *Curr. Org. Synthesis*, 2004, **1**, 293.
35. S. I. Antsypovich, E. A. Kubareva, V. N. Tashlitsky, T. S. Oretskaya, and Z. A. Shabarova, *Eur. J. Biochem.*, 1998, **255**, 414.
36. S. I. Antsypovich, T. S. Oretskaya, E. A. Romanova, E. M. Volkov, V. N. Tashlitsky, M. Vasser, and Z. A. Shabarova, *FEBS Lett.*, 1996, **378**, 224.
37. S. I. Antsypovich, T. S. Oretskaya, E. A. Romanova, E. M. Volkov, V. N. Tashlitskii, M. Vasser, Z. A. Shabarova, *Bioorgan. Khim.*, 1996, **22**, 264 [*Russ. J. Bioorg. Chem.*, 1996, **22**, 224 (Engl. Transl.)].
38. S. I. Antsypovich, T. S. Oretskaya, E. M. Volkov, E. A. Romanova, V. N. Tashlitsky, M. Blumenfeld, and Z. A. Shabarova, *Nucleosides Nucleotides*, 1996, **15**, 923.
39. S. I. Antsypovich, E. M. Volkov, T. S. Oretskaya, E. A. Romanova, V. N. Tashlitskii, M. Blyumenfel´d, Z. A. Shabarova, *Bioorgan. Khim.*, 1995, **21**, 774 [*Russ. J. Bioorg. Chem.*, 1995, **21**, 669 (Engl. Transl.)].
40. J. Stawinski, R. Stromberg, M. Thelin, and E. Westman, *Nucleic Acids Res.*, 1988, **16**, 9285.
41. T. F. Wu, K. K. Ogilvie, and R. T. Pon, *Nucleic Acids Res.*, 1989, **17**, 3501.
42. S. A. Scaringe, C. Francklyn, and N. Usman, *Nucleic Acids Res.*, 1990, **18**, 5433.

Received April 22, 2005;
in revised form June 27, 2005