ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2012, Vol. 38, No. 6, pp. 625–638. © Pleiades Publishing, Ltd., 2012.
Original Russian Text © M.S. Kupryushkin, D.V. Pyshnyi, 2012, published in Bioorganicheskaya Khimiya, 2012, Vol. 38, No. 6, pp. 706–720.

Oligonucleotide Derivatives in Nucleic Acid Hybridization Analysis. III. Synthesis and Investigation of Properties of Oligonucleotides, Bearing Bifunctional Non-Nucleotide Insertion

M. S. Kupryushkin and D. V. Pyshnyi[1]

Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, pr. Akademika Lavrent'eva 8, Novosibirsk, 630090 Russia

e-mail: pyshnyi@niboch.nsc.ru

Received December 19, 2011; in final form, January 20, 2012

Abstract—Non-nucleotide phosporamidites were synthetized, having a branching backbone with different positions for functional groups. Phosphoramidite monomers obtained contain intercalator moiety, 6-chloro-2-methoxyacridine, and additional hydroxyl residue protected with dimethoxytrityl group or with the *tert*-butyldimethylsilyl group for post-synthetic modification. Oligothymidilates containing one or more modified units in different positions of the sequence were synthesized. The melting point and thermodynamic parameters of the formation of complementary duplexes formed by modified oligonucleotides were defined (change in enthalpy and entropy). The introduction of intercalating residue causes a significant stabilization of DNA duplexes. It is shown that the efficiency of the fluorescence of acridine residue in the oligonucleotide conjugate significantly changes upon hybridization with DNA.

Keywords: branched DNA, non-nucleotide insertion, modified oligonucleotides, fluorescence probe, thermal stability

DOI: 10.1134/S106816201206009X

INTRODUCTION

The derivatives of oligonucleotides are widely used as molecular tools in modern studies of living systems and molecular diagnostics. At present, automatic synthesis is a versatile method for obtaining fragments of nucleic acids (NA). The vast majority of the modifications are introduced into the synthesized oligonucleotide chain in the process of building with the application of appropriately functionalized phosphoramidite synthons. Many of the functional groups are introduced into the synthetic NA in the non-nucleotide inserts. The range of derivatives of this type is broad: molecular probes in detection systems of point mutations [1–3], dendritic structures and other supramolecular structures [4, 5], gene-directed or antisense agents [6–11], derived precursors for further functionalization and/or modification of the oligonucleotide structure requiring the "mild" reaction conditions [12–16].

A wide range of commercially available synthons was developed for obtaining the oligonucleotides bearing various groups at the ends as well as within the chain. Versatile use of phosphoramidite monomers, providing an opportunity for the insertion of functional residues in the inner part of the oligonucleotide is obvious. These monomers contain two reactive centers for incorporation of the residues in the synthesized chain and additional groups (one or more) to realize the possibility of the branching phosphodiester backbone and/or introduction into the inner part of the oligonucleotide derivative of the desired residue with a given functionality. The application of the branched skeleton of a non-nucleotide insertion based on the substituted saturated hydrocarbons leads to the existence of chiral centers in the structure of such a link [17–22]. However, for example, in [23] it was proved that differences in the spatial orientation of an additional group consisting of an oligonucleotide derivative cause different hybridization properties of the corresponding enantiomer derivatives. The use of racemic insertion is possible, but often functionally justified, as synthons derived from optically pure isomers are expensive.

The disadvantages associated with the presence of a chiral center in the insertion can be avoided by creating the hydrocarbon structure bearing identical substituents or domains [3, 4, 24]. Obtaining the achiral non-nucleotide synthons, whose properties do not depend on the nature of the structure of their substitu-

Abbreviations: CEP-2, cyanoethoxy diisopropylaminophosphinyl; DMAP, 4-*N*,*N*-dimethylaminopyridine; DIPEA, diisopropylethylamine; DMTr, dimethoxytrityl; TBDMS, *tret*-butyldimethylsilyl; TEA, triethylamine; Tz, 1H-tetrazole. The prefix "d" in the notation of oligodeoxyribonucleotides is omitted.

[1] Corresponding author: phone: +7(383)363-51-51; e-mail: pyshnyi@niboch.nsc.ru. Communication II see [1].

ents (functional groups), is possible using the benzene ring and its analogues as a center of backbone branching for insertion [25–27]. Structures containing nitrogen atoms as centers of backbone branching are a fundamentally different type of three or more achiral functionalized monomers [2, 28, 29].

The aim of this study is to analyze the possibility of creating the achiral multifunctional non-nucleotide insertion (two functions for the introduction in the oligonucleotide chain and two additional functions) that is suitable for automatic synthesis of NA derivatives and has a relatively small size. The insertion prototype must meet the following requirements: 1) the possibility of introducing it into the oligonucleotide sequence in the standard procedures for automated DNA synthesis; 2) the absence of chiral centers to exclude the possibility of ambiguous orientation of residues in the modified DNA duplexes; 3) the presence of a group for additional modifications of the oligonucleotide chain; and 4) the presence of a group that compensates for the "disturbing" effect of non-nucleotide insertion in the carbohydrate–phosphate backbone.

The availability of synthons of this type largely determines the prospects for the development of a number of approaches aimed at creating the gene-directed compounds, NAzymes, structural blocks for NA-nanoarchitectonics. The establishment of such structures may facilitate the formation of complex oligonucleotide constructs with spatially contiguous fragments containing different functional groups.

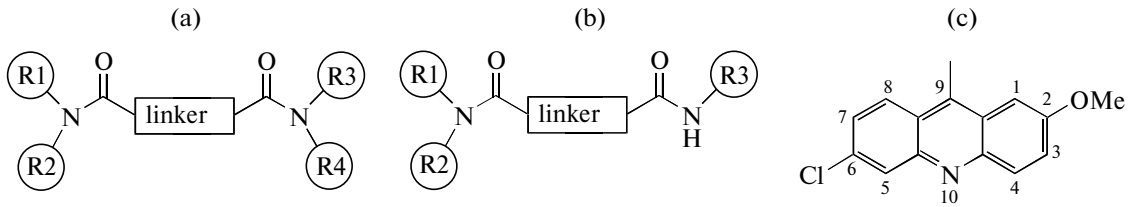
RESULTS AND DISCUSSION

Design of Multi-Functional Non-Nucleotide Insertion

In accordance with the objectives of the work, the phosphoramidite synthon of achiral non-nucleotide insertion must contain four blocks: two groups to ensure its inclusion in the growing chain of oligonucleotide during automatic synthesis, and the two functional groups, one of which is able to stabilize the duplex structure, while the other provides the ability for further modification of the oligonucleotide.

Aliphatic amines, which enable the formation of branching centers due to the presence of nitrogen atoms in them, were chosen as the basis of the branched backbone of non-nucleotide insertion devoid of chiral centers [2, 28, 29]. A variety of commercially available amines and amino alcohols, the relative simplicity and efficiency of their transformation within protocols used in organic and bioorganic chemistry, make this approach useful. For example, diethanolamine, *N*-(2-hydroxyethyl) ethylenediamine and other similar aliphatic amines and amino alcohols can serve as convenient small blocks for the introduction of branching points. Ethylene fragments located between the heteroatoms (N and/or O), apparently, are the lowest possible linker fragments separating the reaction and other functional centers in phosphoramidite synthons. The secondary amino group of these compounds may act as articulation points of individual elements created for the branched backbone. The application of residues of dicarboxylic acids, a variety of precursors which is widely available, is reasonable as the linkers that connect the functionalized block of insertion. Preparation of amides from secondary amines at the articulation points would eliminate the availability of key sites (in the form of tertiary amines) in the final structure of the synthon.

The presented strategy of forming the synthon of insertion is universal, involving the carrying out of a limited set of standard reactions: introduction of protective groups, phosphitylation of hydroxy components, functionalization of primary amine groups and formation of an amide bond. At the same time, a variety of tetra- (Scheme 1a) and tri-functional monomers (Scheme 1b) can be obtained by combining a set of blocks used.



Scheme 1. Schematic representation of the structure of the tetra- and tri-functional monomers and the residue structure of duplex-stabilizing agent used.

We have previously described in detail the effect of various non-nucleotide insertions based on the oligoethyleneglycol and oligomethylenediols on the complexing properties of the "bridge" oligonucleotides [30]. Influence of insertion based on diethylene glycol [31], which is comparable in linear dimensions to diethanolamine, a basic element of insertion we created is sufficiently completely described. Since the introduction of non-nucleotide fragments in the carbohydrate–phosphate backbone of oligonucleotide markedly destabilizes the duplex structure formed by them, we chose the residue of substituted acridine, 6-chloro-2-metoxy acridine, intercalating into the double helix (Scheme 1c) as an important additional function of the developed insertion. The employ-

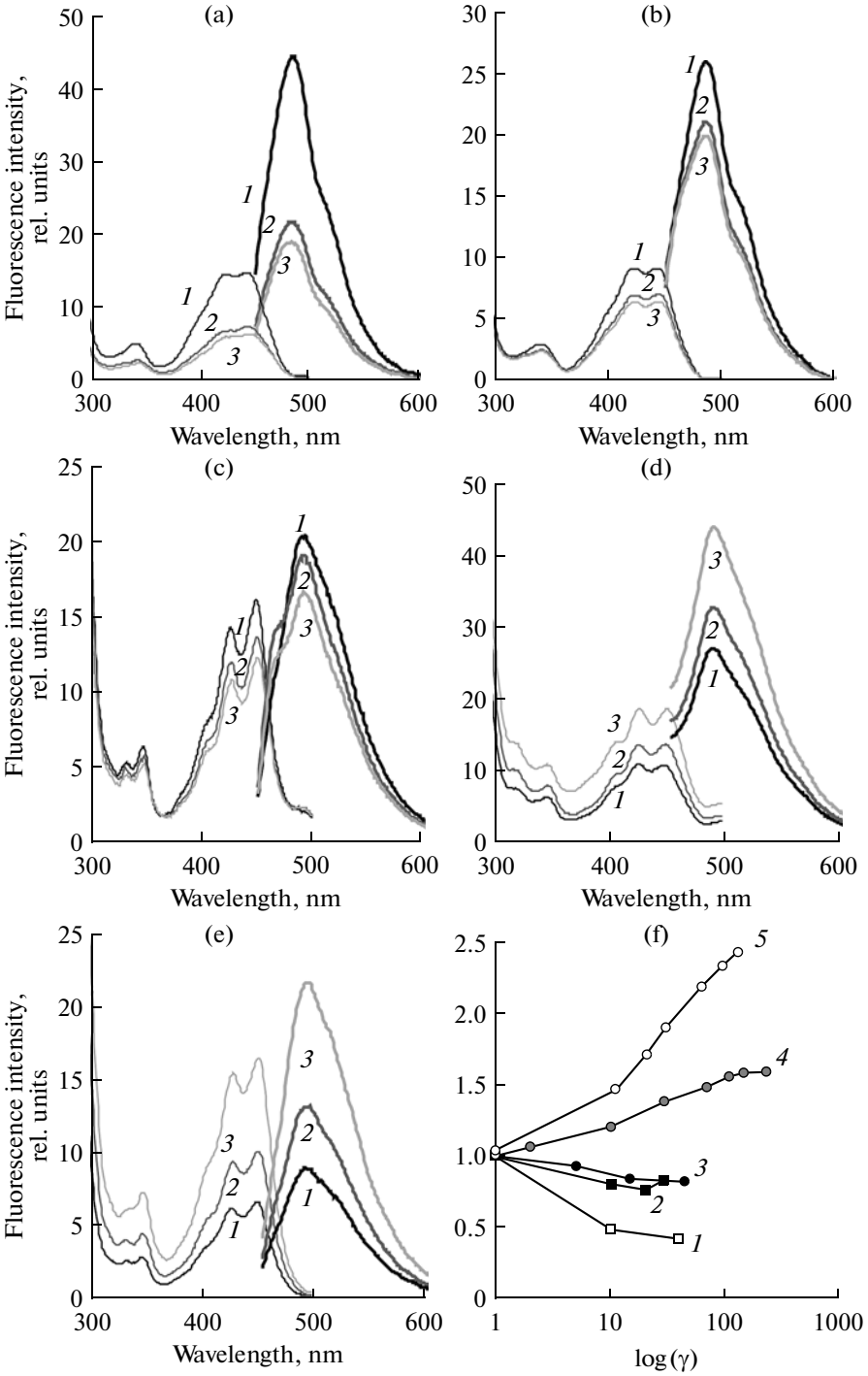


Fig. 1. Fluorescence spectra of (a–e) of acridine-containing oligothymidylates in the absence and presence of the complementary strand and the corresponding curves of fluorometric titration (f). a, $[T_3ZT_3] = 2 \times 10^{-6}$M, γ: 0 (*1*), 10 (*2*), 40 (*3*); b, $[ZT_6] = 10^{-6}$ M, γ: 0 (*1*), 10 (*2*), 20 (*3*), c, $[YT_6] = 10^{-6}$ M, γ: 0 (*1*), 5 (*2*) and 45 (*3*); d, $[Y_2T_6] = 8.8 \times 10^{-7}$ M, γ: 0 (*1*), 10 (*2*), 230 (*3*), e, $[YT_6YT] = 1.2 \times 10^{-6}$ M, γ: 0 (*1*), 11 (*2*), 130 (*3*), where γ is the excess of the matrix CA_6C with respect to the oligothymidylate derivative ($[CA_6C]$ / [oligothymidylate]); f, titration curves of the ZT_6 (*1*), T_3ZT_3 (*2*), Y_2T_6 (*3*), YT_6YT (*4*) and YT_6 (*5*). Buffer solution: 1 M NaCl, 0.01 M sodium phosphate (pH 7.3), 0.1 mM Na_2EDTA. (a–e). The excitation spectra ($\lambda_{em} = 550$ nm) (thin lines on the left side of panels a–f); emission spectra ($\lambda_{ex} = 400$ nm) (bold lines on the right).

ment of this residue as an effective duplex-stabilizing agent is described in detail in the literature [32–35].

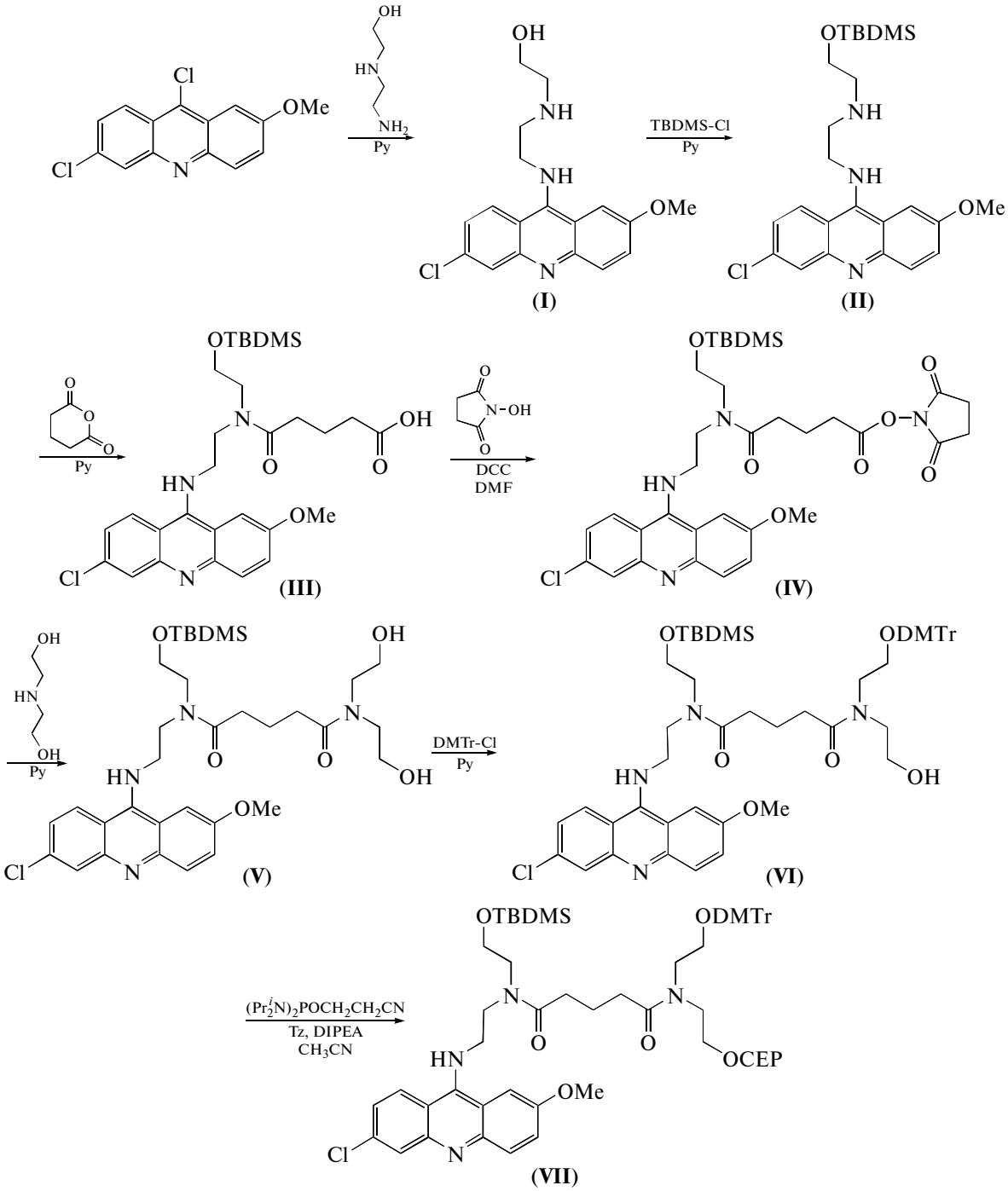
An additional function of insertion is the ability a post-synthetic modification of the synthesized oligonucleotide. To do this, the created insertion must contain a hydroxyl group blocked by the residue "orthogonal" to the 4,4'-dimethoxy trityl group (DMTr) commonly used in the automatic synthesis. For exam-

ple, *tert*-butyl dimethyl silyl group (TBDMS), which is selectively removed in the presence of fluoride ions, meets this requirement [36].

Considering the aforesaid, we have chosen diethanolamine and *N*-(2-hydroxyethyl) ethylenediamine as the fragments of backbone insertion, ensuring the presence of required hydroxyl and amino functions in the structure of the non-nucleotide synthon. To join the functionalized blocks of backbone the residue of glutaric acid, which provides a spatial separation of bulk residues in synthons, was chosen as the linker group.

Synthesis of Acridine-Containing Non-Nucleotide Insertions

Synthesis of the first non-nucleotide multifunctional phosphoramidite synthon was performed according to Scheme 2.

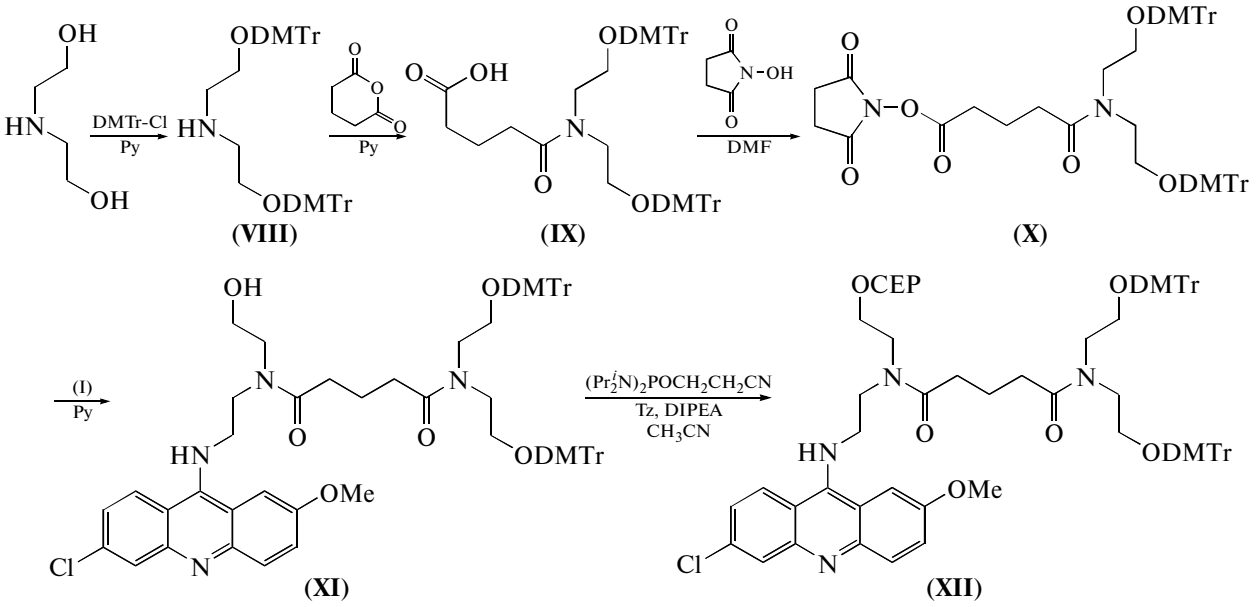


Scheme 2. Synthesis bifunctionalized synthon of non-nucleotide insertion (**VII**).

Initially, the addition of an agent capable of stabilizing the duplex to the *N*-(2-hydroxyethyl) ethylenediamine was carried out with the formation of amino alcohol (**I**, yield 73%). It was found that a substitution reaction of the chlorine atom in position 9 of the acridine derivative by the primary amino group of the amino alcohol is carried out in the selected conditions. The hydroxyl group of the compound was blocked with a silyl group by reaction with *tert*-butyl dimethyl chlorosilane (yield of compound (**II**) was 95%). This position of the synthesized insertion can be further used for further modification of the main chain of the oligonucleotide construct. Branching of the backbone was carried out by the treatment of glutaric anhydride derivative (**I**), resulting in a derivative (**III**) (yield 80%) was obtained. Carboxylic acid (**III**) was converted to an activated ester (**IV**) in the presence of *N*-hydroxysuccinimide and a condensing agent (DCC) and used it in the reaction with the second part of the amine backbone–diethanolamine.

In our chosen conditions, this reaction proceeded selectively leading to the formation of an amide (**V**), even in the presence of free hydroxyl groups. The yield after two stages of activation and condensation was 60%. After the standard procedures dimethoxytritylation (yield (**VI**) 37%) and phosphilation the desired product (**VII**) was obtained in 76% yield (Scheme 2). The transformation of the diol (**V**) in the (**VI**) derivative turned out to be one of the least effective synthetic steps in this scheme, due to the accumulation of the corresponding di-*O*-substituted by-product during the reaction. This partly explains the low total yield of the desired product (**VII**) (9.4%).

It should be noted that the strategy of gradual assembly of structures of the required synthon presented in Scheme 2 can be implemented in block form. We tested the possibility of using a block synthesis for obtaining an alternative synthon of non-nucleotide insertion containing a smaller number of functional groups (Scheme 3).



Scheme 3. Obtaining the synthon of non-nucleotide insertion of simplified structure (**XII**).

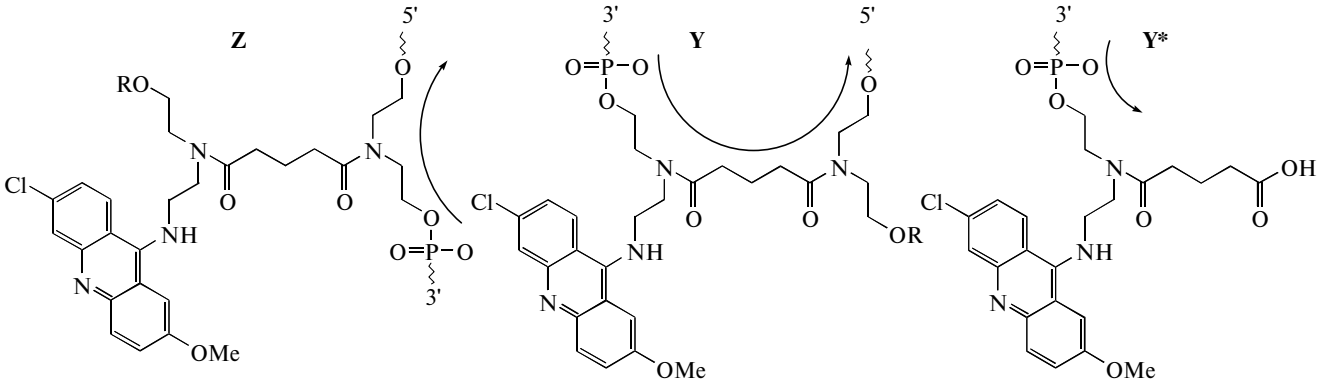
In this case, dimethoxytritylation of diethanolamine (compound **VIII**), acylation of the amino diol substituted with glutaric anhydride (compound **IX**), activation of the carboxyl group by *N*-hydroxysuccinimide in the presence of DCC (compound **X**), followed by acylation of acridine derivative (**I**) with a compound (**XI**) were carried out sequentially. The yield of compound (**XI**) in the four stages was 48%. The phosphitylation reaction of the hydroxyl group in the resulting alcohol led to an alternative synthon (**XII**) with an 85% yield. Thus, the total yield in the implementation of a simplified five-step scheme for obtaining the synthon was about 40%. So, we have developed schemes of receiving a non-nucleotide insertion on the basis of substituted aminoethanols and tested various approaches to obtaining phosporamidites of non-nucleotide insertions contained bearing acridine residue.

It should be noted that the principal moments that define the prospects of using such non-nucleotide synthons are the stability of the oligonucleotide derivatives bearing a developed non-nucleotide insertion in the synthesis and the deprotection of oligonucleotides, and the preservation of the ability to form duplexes with complementary sequences of NA.

Table 1. Results of mass spectrometric analysis and electrophoretic mobility of selected products of synthesis of modified oligonucleotides

Desired product	Identified product	*m/z* (calculation/exper.)	μ#
$\mathbf{Z}T_6$	$\mathbf{Z}T_6$–[H^+]	2485.5/2483.7	0.7
$T_3\mathbf{Z}T_3$	$T_3\mathbf{Z}T_3$ + [H^+]	2485.5/2484.5	0.7
$(T_3)_2\mathbf{Z^2}T_3$	$(T_3)_2\mathbf{Z^2}T_3$–[H^+]	3283.4/3277.7	0.75
	$T_3(T_2)\mathbf{Z^2}T_3$–[H^+]	2979.6/2975.4	0.74
	$T_3(T)\mathbf{Z^2}T_3$–[H^+]	2675.4/2670.5	0.72
	$T_3\mathbf{Z^1}T_3$–[H^+]	2371.2/2366.2	0.71
$\mathbf{Y}T_6$	$\mathbf{Y}T_6$–[H^+]	2372.2/2371.4	0.7
	pT_6 – [H^+]	1843.2/1841.2	0.97
$(\mathbf{Y})_2\mathbf{Y^1}T_6$	pT_6–[H^+]	1843.2/1841.3	0.97
	$\mathbf{Y^*}T_6$–[H^+]	2284.1/2283.6	0.85
	$\mathbf{Y}T_6$–[H^+]	2371.2/2370.6	0.7
	$\mathbf{Y}_2T_6$–[H^+]	2980.3/2978.5	0.63
$(T_5)_2\mathbf{Y^1}T$	$T_5\mathbf{Y}T$ + [Li^+]	2379.2/2379.1	0.76
	$\mathbf{Y^*}T$–[H^+]	763.1/764.3	0.52
$(T_3)_2\mathbf{Y^1}T_3$	pT_3–[H^+]	929.6/928.6	1.15
	$\mathbf{Y^*}T_3$–[H^+]	1731.5/1372.3	0.57
	$T_3\mathbf{Y}T_3$–[H^+]	2371.2/2372.0	0.76
$(\mathbf{Y}T_6)_2\mathbf{Y^1}T$	$\mathbf{Y}T_6\mathbf{Y}T$+[H^+]	3286.4/3287.5	–
	$\mathbf{Y^*}T$–[H^+]	763.1/764.3	–
$((T_2)_2\mathbf{Y^1}T_2)_2\mathbf{Y^1}T_2$	$\mathbf{Y^*}T_2$–[H^+]	1067.3/1068.3	–

Note: **Z** and **Y** (**Y***) are non-nucleotide units introduced with the compounds **(VII)** and **(XII)**, respectively.



where R = –TBDMS (**Z**), –OH (**Z^1**, **Y**), either the phosphodiester derivative residue (**Z^2**, **Y^1**). The arrow shows the direction of synthesis of the oligonucleotide sequence on the polymer support. In this case, the conventional 3'- and 5'-positions of non-nucleotide insertion mean the place of articulation with the nucleotide or other non-nucleotide units of chain (5'- and 3'-, respectively), μ# is the relative electrophoretic mobility in 20% denaturing polyacrylamide gel (8 M urea, pH 8.3) calculated with respect to the electrophoretic mobility of bromophenol blue.

Preparation of Model Oligonucleotide Derivatives

The possibility of embedding the derived insertion in oligonucleotides was tested, for example, in the automatic synthesis of a series of oligodeoxyribonucleic thymidylate derivatives. Modified residue was introduced as the 5'-end of the chain, and in various positions inside the oligomers. The introduction of the insertion was performed with a modified protocol for oligodeoxyribonucleotide synthesis: increased condensation time (up to 5 min) and the concentration of non-nucleotide monomers increased to 0.1 M. The use of synthons **(VII)** and **(XII)**, should lead, respectively, to the presence of "**Z**" and "**Y**" residues within the target oligonucleotide derivatives (Table 1).

Bearing in mind that the presence of substituted acridine residue in the insertions may require the post-synthetic deprotection of oligonucleotides, under mild conditions, different variants of the removal of protective groups and oligonucleotide from the polymeric carrier were tested. According to the analysis by RP HPLC, treatment oligomers for more than 3 h in concentrated aqueous ammonia at room temperature resulted in a noticeable degradation of the product. As alternative conditions were tested: 1) 0.4 M NaOH in

methanol, 2) ethanolamine in water (1 : 1), 3) a saturated solution of ammonia in methanol, 4) 0.05 M K_2CO_3 in methanol, 5) 1,8-diazabicyclo [5.4.0]undec-7-ene in methanol (1 : 10 by volume), and 6) *tert*-butylamine in methanol–water (1 : 1 : 2).

It was shown that the first three options, as well as the standard way of deprotection by aqueous ammonia, leads to significant degradation of acridine-containing products until their complete destruction in aqueous ethanolamine. The accumulation of the oligonucleotides lacking the intercalating fragment and the corresponding acridine derivative was recorded in all cases. Options 4 and 5 are allowed after the deprotection within 5 h to obtain the expected product with satisfactory yields, but further exposure of the oligonucleotide under these conditions led to the accumulation of its degradation products. All tested variants and the corresponding storage times were chosen in such a way as to ensure complete cleavage of nucleotides from the polymeric carrier. The lowest level of degradation of the desired product, even after incubation for 24 h, was observed when using the *tert*-butylamine solution in aqueous methanol, which was chosen as the optimal deblocking solution.

The homogeneity of the products obtained was confirmed by electrophoretic analysis in denaturing conditions (20% polyacrylamide, 8 M urea). The main product of synthesis of each oligonucleotide derivative and the most common by-products isolated by chromatography were analyzed by TOF mass spectrometry (MALDI-TOF). The list of characterized synthesis products of modified oligothymidylates and a number of typical by-products are presented in Table 1.

The data RP-HPLC and mass spectrometry show that the application of insertion developed allows one to obtain only oligonucleotides with terminal modifications with satisfactory yields (40–50%). In the case of modification of the chain inside, the observed yield of target products did not exceed 30%. It was found that among the products of automatic synthesis of modified oligonucleotides there is a significant amount of 5'-phosphorylated oligonucleotides, for example, $\mathbf{pT_6}$ during the synthesis of $\mathbf{YT_6}$, and $\mathbf{T_6}$ is present only in trace amounts. This indicates the effective addition of a non-nucleotide unit to the growing chain, but as a result of further reactions the synthesized product is subjected to degradation at the place of introduction of the non-nucleotide insertion. In the case where non-nucleotide insertion is not a terminal unit, a number of acridine-containing by-products were found other than those mentioned 5'-phosphorylated oligonucleotides in reaction mixtures.

Considering the specifics of removal of *tert*-butyl dimethyl silyl protection (treatment of tetrabutylammonium fluoride), oligonucleotide $\mathbf{T_3ZT_3}$ on the polystyrene support was synthesized to test the possibility of using an additional hydroxyl group of compound (**VII**). It was shown that after selective removal of silyl protection and attempts to introduce an additional trithymidilate fragment the yield of the desired branching product ($\mathbf{(T_3)_2Z^2T_3}$, see note to Table 1) was extremely low. Along with an oligonucleotide $\mathbf{(T_3)_2Z^2T_3}$ according to the mass spectrometric analysis the short oligonucleotides containing one or two additional trithymidilate links in the side chain ($\mathbf{T_3(T_2)Z^2T_3}$, $\mathbf{T_3(T)Z^2T_3}$) were recorded among the products of synthesis of the branched derivative. The yield of branched oligonucleotides was less than 10% of the total oligonucleotide fractions, apparently due to the steric hindrances created by neighboring hydroxyl group with additional bulky substituents (acridine derivative).

We have synthesized a full-size structure only when it was introduced at the 5'-end of oligonucleotide ($\mathbf{YT_6}$) of the planned derivatives of oligonucleotides with insertion **Y**-type structure. We should also note that this insertion involves a parallel insertion of two identical units at the stages following the introduction of the non-nucleotide unit in the synthesized nucleotide chain. Attempts to obtain derivatives with a branched target oligonucleotides were unproductive. No increase in the number of nucleotide synthons supplied or increase in the time of condensation allow for enhancing the yield of branched products.

In all cases, the oligonucleotides in which the building of the chain after the introduction insertion took place in only one of a pair of the available hydroxyl residues in the unlocked non-nucleotide unit: $\mathbf{Y_2T_6}$ instead of the planned sequence $\mathbf{(Y)_2Y^1T_6}$ (see notes in Table 1), $\mathbf{T_5YT}$ instead $\mathbf{(T_5)_2Y^1T}$, etc. corresponded to the isolated product with the maximum molecular weight in the reaction mixtures. The most likely causes reducing the efficiency of the resulting synthon are steric constraints arising from contact of phosphoramidites with the hydroxyl groups of the unlocked non-nucleotide insertion and/or rapid degradation of the product of this reaction in the automatic synthesis.

In addition to the by-products shown above (absence of condensation on one of the hydroxyl groups), the products resulting from degradation of the built-in non-nucleotide unit were found. For example, the by-products with the degraded backbone of insertion **Y*** were obtained in the synthesis of oligonucleotides with an internal localization of the non-nucleotide unit for the separation of reaction mixtures (Table 1). According to mass spectrometric analysis, the structure of such a unit presumably corresponds to carboxylic acid residue. It is important to note that during the transition from the preparation of derivatives $\mathbf{(Y)_2Y^1T_6}$ and $\mathbf{(T_3)_2Y^1T_3}$ to the synthesis of compounds $\mathbf{(YT_6)_2Y^1T}$ and $\mathbf{((T_2)_2Y^1T_2)_2Y^1T_2}$ with a large number of units after the first injection of non-nucleotide unit, the content of by-products having in their composition degraded **Y***-structure in the post-synthetic mixtures increases. At the same time, these deg-

Table 2. Thermodynamic parameters of formation and melting points of DNA/DNA complexes of native oligonucleotides and their acridine-containing derivatives

Compound	$\Delta S°$, cal/(mol K)	$\Delta H°$, kcal/mol	$\Delta G°(37°C)$, kcal/mol	T^*_{melt}, °C
T_6	–117.6	–38.8	–2.4	6.8
$T_3\hat{}T_3$	–114.0**	–35.4**	0.0**	–9.5**
T_3ZT_3	–113.8	–39.6	–4.3	20.5
ZT_6	–144.5	–50.1	–5.2	29.1
YT_6	–149.8	–51.2	–4.8	26.7
Y_2T_6	–145.5	–49.9	–4.8	26.6
YT_6YT	–161.0	–56.1	–6.1	35.5
T_5YT	–171.0	–57.9	–4.9	28.8

Note: ^ is non-nucleotide insertion based on the phosphodiester diethylene glycol; * the melting point was calculated for the total concentration of oligonucleotide chains, taken in stoichiometric ratio equal to (1×10^{-4} M) in buffer 1 M NaCl, 10 mM sodium phosphate (pH 7.3), 0.1 mM Na_2EDTA; ** thermodynamic parameters and magnitude of the melting point of the complex were calculated by the method described in [31].

radation products were detected in the synthesis of a derivative with a 5'-terminal insertion **YT_6** (data not shown). The experiments conducted on the stability of the already isolated oligonucleotide derivatives (**T_3ZT_3**, **ZT_6**, **YT_6YT**) with repeated exposure of mixture of *tert*-butylamine in aqueous methanol, showed no degradation.

Summarizing the data obtained, it can be concluded that the partial degradation (or hidden modification) of oligomer chain in the case of application of **Y**-type synthon occurs during the automatic synthesis of the oligomer.

Hybridization Properties of Model Oligonucleotides

The impact of the proposed acridine-containing insertion on the efficiency of complexation of oligonucleotide derivatives obtained was characterized by thermal denaturation of model duplexes formed with the participation of the target oligonucleotide **CA_6C** (**M**). To ensure the uniqueness of the binding of derivatives with the DNA target, the hexaadenylate fragment was flanked by cytidine residues.

The values of melting temperatures of complexes and thermodynamic parameters of their formation are presented in Table 2. Analysis of the data showed that the introduction of proposed non-nucleotide insertions, as in [32–35], in all cases leads to a significant stabilization of duplexes formed. The presence of one acridine residue increases the melting point of hexanucleotide complex by the value from 13.7 to 22.8°C. Introduction of the insertion at the end of the chain or the position of the penultimate unit of hexathymidylate has a more pronounced stabilization of the short complex compared to the case of localization in the central part of duplex structure. The presence of acridine residue fully compensates of destabilizing effect resulted from perturbation of the regular structure of the carbohydrate-phosphate backbone paired with its extension. The stability of the **T_3ZT_3/M** complex is higher than the stability of the corresponding unmodified complex, and, according to the calculations, much greater than the stability of the duplex with non-nucleotide insertion on the basis of phosphodiester diethylene glycol, which causes an almost equal increase in the contour length of the oligonucleotide backbone. Apparently, the acridine residue, effectively interacting with the surrounding base pairs, provides the observed effects.

The maximum stabilizing effect allows the presence of two intercalating residues in the duplex, and only in case of modification of the terminal (3' and 5') sections. A pair of **Y**-type insertions successively introduced at the 5'-end (**Y_2T_6/M** complex) has no additional stabilizing effect in comparison with a single insertion of the same type (Table 2).

It should be noted that the observed thermodynamic effect of the introduction of **Z**- and **Y**-type insertions is associated with a pronounced increase in the enthalpy of complex formation, which indicates the implementation of stacking interactions of acridine residue with DNA bases [37]. The minimum enthalpy contribution from the introduction of insertion was observed when it is introduced into the central part of the duplex (**T_3ZT_3**). In this case, the stabilizing effect is associated with the presence of the modified residue in the structure of the positive charge arising from protonation of the heterocyclic nitrogen atom in the acridine residue [36].

The resulting thermodynamic data fully confirm the high efficiency of 6-chloro-2-methoxyacridine residue as an agent for stabilizing the duplex structure, and are in good agreement with the previously described properties of various acridine-containing oligonucleotides [32–35].

Fluorescent Properties of Acridine-Containing Derivatives of Oligonucleotides

The change of fluorescence efficiency of the residue(s) of the dye upon complexation of oligonucleotide derivatives is well known. The formation of the

double helix with the labeled components can lead both to an increase in the fluorescence of dyes, such as the presence of ethidium residues [38] and its suppression, as described, in particular, for the pyrene residues [39]. The presence of such features in the molecular probes is often used to develop highly sensitive test systems to identify a specific NA-target and local violation of their structure [2].

It has been previously described, that we use intercalating residue that changes its fluorescent properties in hybridization with the NA [40]. In the course of this work, the properties of the obtained acridine-containing oligonucleotides were characterized. It was found that the fluorescence of acridine residue in the formation of a duplex structure can both increase and decrease. Double quenching (by a factor of 2.3) occurs upon complexation of oligonucleotide with an internal insertion ($\mathbf{T_3ZT_3}$) (Fig. 1a). At the same time the terminal insertion of intercalator hardly changes their characteristics, the transition to the double-stranded state of the oligonucleotides of $\mathbf{ZT_6}$ (Fig. 1b) and $\mathbf{YT_6}$ (Fig. 1c) leads only to a slight decrease, by a factor of 1.3, in the efficiency of fluorescence. The presence of two acridine residues has the opposite effect: the binding of oligonucleotides $\mathbf{Y_2T_6}$ (Fig. 1d), and $\mathbf{YT_6YT}$ (Fig. 1e) with the complementary sequence in both cases is associated with the buildup of fluorescence by a factor of 1.6 and 2.4 times, respectively. We note that more pronounced changes are observed when the dye residues are separated in the structure of an oligonucleotide derivative.

Thus, the intercalating residue in the insertion can play not only the role of a stabilizing agent duplex, but also act as a sensor of transition of conjugates from the free to the bound state (Figs. 1a–1e), which significantly increases the prospects of using multifunctional units on receipt of new DNA structures.

CONCLUSIONS

By optimization of the conditions of a seven-step synthesis and isolation of intermediate products at each stage, the phosphoramidite synthon (**VII**) for compact non-nucleotide insertion was obtained with a satisfactory yield. Transition to the block method instead of a continuous method of synthesis will increase the efficiency of synthesis of the non-nucleotide synthon, and this is demonstrated by the example of obtaining phosphoramidite (**XII**). Thus, we proposed ways to obtain phosphoramidite synthons, potentially allowing the introduction of the branching unit in the synthesized oligonucleotide chain and, in addition to this, carrying a residue that has a stabilizing influence in the formation of the duplex.

It was found that the synthons (**VII**) and (**XII**) effectively incorporated into the growing chain end of the oligomer, but lead to a drop in the reaction yield of subsequent nucleotide residues. These data indicate that detritylated hydroxyl groups of non-nucleotide insertion are poorly accessible for the addition of phosphoramidites to them and, moreover, seem to contribute to the degradation of non-nucleotide insertion to the oligonucleotide chain of the subsequent units during the addition (established for **Y**-type insertion). In the latter case, the accumulation of oligomers bearing terminal monophosphoester residue is recorded, which may be the result of degradation of the built-in chain of the non-nucleotide unit during the subsequent cycle(s) of addition of additional units. This conclusion is supported by the fact that the oligothymidylates containing acridine residue, pre-allocated and bearing the insertion, are stable both at the end and in the inner part of the chain when kept in conditions of post-synthetic deprotection.

The establishment of the causes of accumulation of products of the phosphodiester backbone cleavage requires additional analysis beyond the scope of this work. However, it should be noted that there were no similar features in the analysis of publications devoted to the development and application of non-nucleotide insertions based on the diethanolamine. For example, when creating the pyrene-containing insertions, the backbone of which is the branched amide based on the diethanolamine, *N*-acylated with various diaminocarboxylic acids, the authors avoid discussion of the effectiveness of inclusion of phosphoramidites they developed in the oligonucleotide chain and stability analysis of the derivatives when removing them from the substrate and deprotection [2].

The presented data suggest the existence of objective problems in the creation and application of compact functionalized synthons involving branching of the phosphodiester backbone of oligonucleotides. Nevertheless, our findings indicate that the designed non-nucleotide insertions introduced in the carbohydrate–phosphate backbone at least partially perform the planned features. The acridine derivative residue, as expected, increases the thermal stability of duplexes with the complementary target, changing the efficiency of the intrinsic fluorescence relative to the unbound state. The branched oligonucleotides were not synthesized in sufficient quantities to conduct studies of their physico-chemical properties due to the low efficiency of incorporation of the nucleotide unit by means of the additional HO-function of the created **Z**-and **Y**-type insertions. This situation requires further study and should be taken into account when designing similar non-nucleotide synthons. We consider an orthogonal protecting group of additional HO-components and the linker fragment connecting the disubstituted diethanol amine residues as the most promising components for variations in the structure of the multifunctional and compact synthons considered.

EXPERIMENTAL

The reagents used in this work were as follows: phosphoramidites (GlenResearch, United States), 2-cyanoethyl-*N*,*N*,*N*',*N*'-tetraisopropylphosphoramidite (LMH, ICBFM SB RAS), 6,9-dichloro-2-methoxyacridine, glutaric anhydride, diethanolamine, dicyclohexylcarbodiimide, Stains-all (Aldrich, United States), *N*-(2-hydroxyethyl)ethylenediamine, *tert*-butyldimethylsilyl chloride, imidazole, 4-*N*,*N*-dimethylaminopyridine, *N*-hydroxysuccinimide, triethylamine, *N*-methylimidazole (Fluka, Switzerland), 4,4'-dimethoxytrityl chloride (ChemGenes, United States), urea, tetrabutylammonium fluoride (Merck, Germany), acrylamide (DiaM, Russia), tetrazole (Acros, United States), ethyl diisopropyl amine, bromophenol blue, xilencianol FF (Sigma, United States), reagents and solvents of chemically pure and analytical pure grades. The dehydration of solvents was performed by standard methods with further aging over molecular sieves and calcium hydride.

The TLC plates were DC-Alufolien Kieselgel 60 F_{254} (Merck, Germany); the solvent system are ethanol–triethylamine, 95 : 5 (A), dichloromethane–ethanol–triethylamine, 69 : 30 : 1 (B), ethanol (C); ethyl acetate–triethylamine, 95 : 5 (D); toluene–ethyl acetate–triethylamine, 50 : 49 : 1 (E); dichloromethane–ethanol, 97.5 : 2.5 (F); ethyl acetate–triethylamine, 99 : 1 (G).

For column adsorption chromatography the column Kieselgel 60 (Merck, Germany) with volume of 100 mL filled to 70% of sorbent was used in all cases.

For evaporation and concentration of aqueous and organic solutions Rotavapor R200, Rotavapor RE120 (Buchi, Switzerland) and Concentrator 5301 (Eppendorf, Germany) were used at a pressure of 10–15 mm Hg and a temperature of up to 40°C.

Mass spectrometry (MALDI-TOF) analysis of the investigated compounds was carried out on a Reflex-III or Autoflex-III devices (Bruker Daltonics, Germany).

^{1}H, ^{13}C and ^{31}P NMR spectra (δ, ppm, *J*, Hz) were recorded on a Bruker AV 300/400 spectrometer at 300, 400 and 160 MHz, respectively, relative to an external standard of tetramethylsilane (^{1}H) and H_3PO_4 (^{31}P) in 3–5% solution of DMSO-d_6. The processing of the analytical results was performed using SPINWORKS. The spectra were recorded at the Center for the Collective Use of the Analysis of Organic Compounds and Materials NIOC SB RAS.

***N*-(2-hydroxyethyl)-*N*'-(6-chloro-2-methoxyacridine-9-yl)ethylenediamine (I).** A mixture of 3 g (10.78 mmol) of 6,9-dichloro-2-methoxyacridine and 11 mL (11.3 g, 107.8 mmol) *N*-(2-hydroxyethyl) ethylenediamine was evaporated with abs. pyridine (2 × 15 mL), dissolved in 120 mL of abs. pyridine and incubated 20 h at 70°C. The reaction mixture was kept for 12 h at 5°C. The crystals were filtered and washed with cold abs. pyridine, dried to constant weight. The yield of (**I**) was 2.72 g (73%), R_f 0.5 (A). ^{1}H NMR δ: 2.61 (2 H, t, *J* 5.7, –NH–C**H$_2$**–CH$_2$–OH); 2.88 (2H, t, *J* 6.2, Acr–NH–CH$_2$–C**H$_2$**–NH–); 3.44 (2 H, t, *J* 5.7, 5–NH–CH$_2$–C**H$_2$**–OH); 3.79 (2 H, t, *J* 6.2, Acr–NH–C**H$_2$**–CH$_2$–NH–); 3.94 (3 H, s, OC**H$_3$**); 7.32 (1H, dd, J_1 9.l, J_2 2.1, H7), 7.41 (1H, dd, J_1 9.3, J_2 2.5, H3), 7.62 (1H, s, H1), 7.82 (1 H, d, *J* 9.3, H5); 7.86 (1H, s, H4), 8.36 (1H, d, *J* 9.3, H8). ^{13}C NMR δ: 49.58, 49.67, 51.62, 55.89, 60.67, 101.23, 115.28, 117.73, 122.94, 124.39, 126.9, 133.79, 148.13, 150.88, 155.37. Mass spectrometry: *m/z* 346.4 $[M + H]^+$. Calculated 346.8 ($C_{18}H_{20}N_3O_2Cl$).

***N*-[2-(*tert*-Butyldimethylsilyloxy)ethyl]-*N*'-6-chloro-2-methoxyacridine-9-yl]ethylenediamine (II).** A mixture of 1 g (2.9 mmol) of compound (**I**) and 0.99 g (14.5 mmol) of dimethylaminopyridine was evaporated with abs. pyridine (10 mL), dissolved in 40 mL of abs. pyridine. 2.18 g (14.5 mmol) TBDMS-Cl was added, and held for 1 h at 50°C, then the reaction mixture was evaporated to the oily condition, dissolved in 10 mL of ethanol, and 600 mL of 0.1 M NaOH was added up to complete precipitation. The precipitate was filtered, washed with cool bidistilled water, dried to constant weight. The yield compound (**II**) was 1.33 g (95%), R_f 0.9 (A). ^{1}H NMR δ: –0.04 (6 H, s, $(CH_3)_2$Si), 0.79 (9 H, s, $(CH_3)_3$CSi); 2.61 (2H, t, *J* 5.8, –NH–C**H$_2$**–CH$_2$–O–); 2.88 (2 H, t, *J* 6.1, Acr–NH–CH$_2$–C**H$_2$**–NH–); 3.57 (2 H, t, *J* 5.8, –NH–CH$_2$–C**H$_2$**–O–); 3.79 (2 H, t, *J* 6.1, Acr–NH–C**H$_2$**–CH$_2$–NH–); 3.93 (3 H, s, OC**H$_3$**), 7.25–8.41 (6 H, Ar). Mass spectrometry: *m/z* 460.7 $[M + H]^+$. Calculated 461.1 ($C_{18}H_{20}N_3O_2Cl$).

4-{*N*-[2-*tert*-Butyldimethylsilyloxy)ethyl]-*N*-[2-(6-chloro-2-methoxyacridine-9-ylamine)ethyl]carbamoyl} butanoic acid (III). A mixture of 1.33 g (2.9 mmol) of compound (**II**) and 0.32 g (2.9 mmol) DMAP were evaporated with abs. pyridine (2 × 5 mL), dissolved in 30 mL of abs. pyridine. 1.65 g (14.5 mmol) of glutaric anhydride was added, and held for 1 h at room temperature. Further, the reaction mixture was evaporated to the oily condition, dissolved in 10 mL of ethanol, and 600 mL of 1 M HCl was added to complete precipitation. The precipitate was filtered and washed with cold distilled water, and dried to constant weight. The yield compound (**III**) was 1.32 g (80%), R_f 0.8 (B). ^{1}H NMR, δ: –0.04 (6 H, s, $(CH_3)_2$Si); 0.77 (9 H, s, $(CH_3)_3$CSi); 1.73 (2 H, m, C(O)–CH$_2$–C**H$_2$**–CH$_2$–COOH); 2.23 (2 H, m, C(O)–C**H$_2$**–CH$_2$–CH$_2$–COOH); 2.34 (2 H, m, C(O)–CH$_2$–CH$_2$–C**H$_2$**–COOH); 3.39 (2 H, t, –NH–C**H$_2$**–CH$_2$–O–, *J* 5.3), 3.67 (2 H, t, *J* 5.3, –NH–CH$_2$–C**H$_2$**–O–); 3.7 (2 H, t, *J* 5.9, Acr–NH–CH$_2$–C**H$_2$**–NH–); 3.9–4.0 (5 H, t, Acr–NH–C**H$_2$**–CH$_2$–NH–, OCH$_3$); 7.25–8.41 (6 H, Ar). ^{13}C NMR, δ: –5.25, 18.10, 20.44, 25.95, 31.91, 33.22, 46.01, 49.14, 50.27, 56.42, 59.21, 60.8, 101.53, 117.67, 120.94, 123.39, 127.45, 134.5, 139.09, 155.46, 156.31, 174.45, 175.57. Mass spectrometry: *m/z* 574.84 $[M + H]^+$. Calculated 575.2 ($C_{29}H_{40}N_3O_5ClSi$).

***N*-[2-*tert*-Butyldimethylsilyloxy)ethyl]-*N*-[2-(6-chloro-2-methoxyacridine-9-ylamine)ethyl]-*N'*,*N'*-bis [2-hydroxyethyl]glutaramide (V)**. A mixture of 1.32 g (2.3 mmol) of compound (**III**) and 0.79 g (4.6 mmol) *N*-hydroxy succinimide was evaporated with abs. dimethylformamide (10 mL), dissolved in 40 mL of abs. dimethylformamide, 1.42 g (4.6 mmol) DCC was added, and reaction mixture was held for 6 h at room temperature. Control: TLC, R_f (ref) 0.3, R_f (product) 0.6 (system C). Dicyclohexylurea was filtered off, 0.84 g (6.9 mmol) of DMAP and 4.4 mL (4.8 g, 46 mmol) of diethanolamine were added to the reaction mixture and the mixture was kept for 6 h at room temperature. The reaction mixture was filtered from the dicyclohexylurea remnants, and evaporated to the oily condition. 200 mL of CH_2Cl_2 was added and washed with 0.05 M NaOH (3 × 200 mL). The organic phase was dried over anhydrous Na_2SO_4, concentrated by evaporation to oil and dissolved in 10 mL of CH_2Cl_2. Further purification was performed by column chromatography using a step gradient: 200 mL of dichloromethane/triethylamine mixture (99 : 1), 150 mL dichloromethane/ethanol/triethylamine mixture (94 : 5 : 1) 160 mL of dichloromethane/ethanol/triethylamine mixture (84 : 15 : 1), 200 mL of dichloromethane/ethanol/triethylamine mixture (69 : 30 : 1). The fractions containing the product were evaporated, and the substance dried to constant weight. The compound yield (**V**) was 0.92 g (60%), R_f 0.45 (C). Mass spectrometry: m/z 662.0 $[M + H]^+$. Calculated 662.3 ($C_{29}H_{40}N_3O_5ClSi$).

***N*-[2-*tert*-Butyldimethylsilyloxy)ethyl]-*N*-[2-(6-chloro-2-methoxyacridine-9-ylamine)ethyl]-*N'*-[2-(4,4'-dimethoxytrityloxy)ethyl]-*N'*-[2-hydroxyethyl]glu-taramide (VI).** A solution of compound (**V**) (0.92 g, 1.38 mmol) in abs. pyridine (10 mL) was evaporated, the remnant was dissolved in 30 mL of abs. pyridine. 0.57 g (1.68 mmol). DMTr-Cl was added, and incubated for 3 h at room temperature. The reaction mixture was evaporated, the oily residue was dissolved in 150 mL CH_2Cl_2 and washed with a saturated solution of NaCl, containing 5% $NaHCO_3$ (3 × 200 mL). The organic phase was dried over anhydrous Na_2SO_4, evaporated to oil with toluene and dissolved in 10 mL of toluene. The reaction product was purified by column chromatography using a linear gradient of ethanol concentration (0–7%) in toluene/triethylamine mixture (99 : 1), the total volume of eluent of 600 mL). The fractions containing the product were evaporated, and dissolved in 6 mL of ethanol. 300 mL of 0.1 M NaOH was added up to complete precipitation. The precipitate was filtered, washed with bidistilled water, and dried to constant weight. The yield compound (**VI**) was 0.49 g (37%), R_f 0.3 (D). ^{1}H NMR, δ: –0.07 (6 H, s, $(CH_3)_2Si$); 0.75 (9 H, s, $(CH_3)_3CSi$); 1.67 (2 H, m, *J* 7.1, C(O)–CH_2–CH_2–CH_2–C(O)); 2.20–2.40 (4 H, m, *J* 7.1, C(O)–CH_2–CH_2–CH_2–C(O)); 3.0–3.1 (2 H, m, –CH_2–CH_2–ODMTr); 3.36–3.65 (14 H, m, –CH_2–CH_2–); 3.71 (6 H, s, OCH_3), 3.93 (3 H, s, OCH_3); 6.8–8.4 (19 H, aromatics). ^{13}C NMR, δ: –5.73, 17.67, 20.62, 25.55, 31.63, 31.93, 45.52, 46.54, 47.80, 47.96, 49.66, 50.34, 54.93, 55.49, 58.71, 59.06, 60.73, 61.56, 85.39, 100.01, 113.08, 122.24, 124.11, 126.53, 127.1, 127.56, 127.7, 129.52, 133.29, 135.47, 144.69, 148.33, 149.86, 155.17, 157.97, 171.89, 174.04.

***N*-[2-*tert*-Butyldimethylsilyloxy)ethyl]-*N*-[2-(6-chloro-2-methoxyacridine-9-ylamine)ethyl]-*N'*-[2-(4,4'-dimethoxytrityloxy)ethyl]-*N'*-[2-(2-cyano-ethyloxy-*N*,*N*-diisopropylphosphoramiditeoxy)ethyl]glutaramide (VII).** A solution of compound (**VI**) 0.14 g (0.15 mmol) in abs. acetonitrile (2 × 5 mL) was evaporated and the residue was dissolved in 2 mL of abs. acetonitrile. A solution of 0.025 g (0.35 mmol) of tetrazole, 0.16 mL (0.12 g, 0.9 mmol) DIPEA, 0.13 mL (0.12 g, 0.38 mmol) of 2-cyanoethyl-*N*,*N*,*N'*,*N'*-tetraisopropyl phosphoramidite in 0.72 mL of abs. acetonitrile was kept for 30 min. To the resulting solution was added dropwise a solution of compound (**VI**) for 2 min, and incubated for 1 h at room temperature. The reaction mixture was evaporated to 3/4 volume; 100 mL of CH_2Cl_2 was added and washed with 0.3 M KH_2PO_4 (3 × 150 mL). The organic phase was dried with anhydrous Na_2SO_4, evaporated to dryness, dissolved in 5 mL of ethanol and placed in 300 mL of 0.3 M KH_2PO_4. The precipitate was filtered, washed with cold bidistilled water, and dried to constant weight. The yield of compound (**VII**) was 0.12 g (76%), R_f 0.6 (D). ^{1}H NMR, δ: –0.07 (6 H, s, $(CH_3)_2Si$); 0.76 (9 H, s, $(CH_3)_3CSi$); 1.0–1.25 (12 H, m, $(CH_3)_2CH$–); 1.68 (2 H, m, *J* 7.1, –C(O)–CH_2–CH_2–CH_2–C(O)–); 2.33 (4 H, m, *J* 7.1, C(O)–CH_2–CH_2–CH_2–C(O)); 2.71 (2 H, t, –CH_2CN); 3.04–3.16 (2 H, m, –CH_2–CH_2–ODMTr); 3.33–3.68 (18 H, m, –CH_2–CH_2–, –CH_2–CH_2–CN, $(CH_3)_2CH$–); 3.71 (6 H, s, –OCH_3), 3.94 (3 H, s, –OCH_3) 6.8–8.4 (19 H, aromatics). ^{31}P NMR, δ: 149.93, 150.11.

***N*,*N*-Bis[2-(4,4'-dimethoxytrityloxy)ethyl]amine (VIII).** A solution of diethanolamine 0.52 g (5 mmol) in abs. pyridine (2 × 15 mL) was evaporated, dissolved in 20 mL of abs. pyridine. 1.4 g (11 mmol) DMTr-Cl was added, and incubated for 3 h at room temperature. R_f 0.5 (E). Further, we used the reaction mixture without isolation of the product.

4-{*N*,*N*-Bis[2-(4,4'-dimethoxytrityloxy)ethyl]carbamoyl}butanoic acid (IX). To the reaction mixture of compound (**VIII**) was added 1.70 g (15 mmol) of glutaric anhydride. The reaction was kept overnight at room temperature. The reaction mixture was evaporated to the oily condition. 200 mL of CH_2Cl_2 was added, and extracted with a saturated solution of NaCl, containing 5% $NaHCO_3$ (3 × 200 mL). The organic phase was dried over Na_2SO_4, and evaporated to oil. R_f 0.1 (E). The product was used in the subsequent reaction without further purification.

***N*-[2-Hydroxyethyl]-*N*-[2-(6-chloro-2-methoxyacridine-9-ylamine)ethyl]-*N'*,*N'*-bis[2-(4,4'-dimethoxytrityloxy)ethyl]glutaramide (XI).** Tenth of the reaction mix-

ture of the compound (**IX**) (0.5 mmol) and 0.07 g (0.58 mmol) *N*-hydroxysuccinimide was evaporated with abs. dimethylformamide (10 mL) dissolved in 20 mL of abs. dimethylformamide. 0.21 g (1 mmol) of DCC was added, and the reaction mixture was kept overnight at room temperature. Control: TLC, R_f 0.6 (F). After filtration from dicyclohexylurea in the reaction mixture was added 0.13 g (10 mmol) DMAP and 0.25 g (0.72 mmol) of compound (**I**) and incubated for 6 hours at room temperature. The reaction mixture was filtered from the dicyclohexylurea, and evaporated to the oily condition. 200 mL of CH_2Cl_2 was added, and extracted with 0.05 M NaOH (3 × 200 mL). The organic phase was dried with anhydrous Na_2SO_4, evaporated to oil and dissolved in 6 mL of toluene. Further purification was performed by column chromatography using a step gradient: 200 mL of toluene/triethylamine mixture (99 : 1), 400 mL of toluene/ethanol/triethylamine mixture (89 : 10 : 1). The fractions containing the product were evaporated; the substance was dried to constant weight. The yield of compound (**XI**) was 0.32 g (48%), R_f 0.2 (A). ^{13}C NMR, δ: 20.80, 31.84, 45.42, 46.81, 48.01, 48.45, 54.97, 55.53, 59.10, 61.57, 85.51, 100.51, 106.7, 113.15, 123.90, 125.21, 126.59, 127.18, 127.60, 128.20, 129.58, 133.40, 135.56, 144.77, 149.20, 149.98, 153.97, 155.15, 156.34, 158.02, 172.05, 174.02.

***N*,*N*-Bis[2-(4,4'-dimethoxytrityloxy)ethyl]-*N'*-[2-(2-cyanoethyloxy-*N*,*N*-diisopropyl diisopropylphosphoramiditeoxy)ethyl]-*N'*-[2-(6-chloro-2-methoxyacridine-9-ylamine)ethyl]glutaramide (XII).** A solution of compound (**XI**) 0.20 g (0.17 mmol) in abs. acetonitrile (2 × 5 mL) was evaporated, the residue was dissolved in 2 mL of abs. acetonitrile. A solution of 0.03 g (0.4 mmol) of tetrazole, 0.18 mL (0.13 g, 1 mmol) DIPEA, 0.16 mL (0.13 g, 0.43 mmol) of 2-cyanoethyl-*N*,*N*,*N'*,*N'*-tetraisopropyl phosphoramidite in 0.84 mL of abs. acetonitrile was kept for 30 min. To the resulting solution was added dropwise over 2 min a solution of compound (**XI**) and incubated for 1 h at room temperature. The reaction mixture was evaporated to the 3/4 volume. 100 mL of CH_2Cl_2 was added, and the extraction was carried out with 0.3 M KH_2PO_4 (3 × 150 mL). The organic phase was dried with anhydrous Na_2SO_4, evaporated to oil with toluene and dissolved in 4 mL of toluene. Purification was performed by flash chromatography, 200 mL of toluene/ethyl acetate/triethylamine mixture (50 : 49 : 1). After evaporation of acetonitrile, the product was dried to constant weight. The yield compound (**XII**) was 0.15 g (85%), R_f 0.6 (G). ^{13}C NMR, δ: 19.81, 20.69, 21.93, 24.25, 31.96, 42.31, 45.48, 46.63, 48.02, 48.53, 54.95, 55.51, 57.98, 61.67, 85.51, 100.01, 113.10, 113.70, 116.35, 118.81, 122.23, 124.10, 126.56, 127.22, 127.60, 129.57, 133.30, 135.54, 144.77, 145.83, 148.44, 149.80, 155.21, 158.02, 171.93, 174.07. ^{31}P NMR, δ: 146.95, 148.05.

Oligodeoxyribonucleotide derivatives were synthesized on an automated DNA synthesizer ASM-800 (Biosset, Russia) using standard synthons (Glen Research, United States) for phosphoramidite protocol. The resulting synthons (**VII** and **XII**) were used as 0.1 M solutions in acetonitrile. At the stage of condensation of the synthesized insertions, the volumes of solutions of activator (tetrazole 0.5 M) and the monomer supplied (from 20 to 80 μL), as well as the condensation time (from 1 to 5 min) compared with the standard method were increased. Removal of the polymer and deprotection of the protecting groups of nitrogenous bases of the synthesized oligonucleotides were carried out in a standard way, as well as through various methods of "soft", "very soft" and "fast" deprotection listed on the site of Glen Research, United States. For the same purposes, in the case of modified oligonucleotide solution, the *tert*-butylamine was used in a methanol/water mixture (1 : 1 : 2) for 5 h at 37°C.

Removal of *tert*-butyldimethylsilyl protection in the synthesis of branched oligonucleotides was produced by keeping the polymer with a synthesized sequence in a 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran for 1 h.

To analyze the homogeneity of the intermediate products of synthesis of the desired non-nucleotide insertions, the analytical RP HPLC was carried out on a Milichrome A02 chromatograph (EcoNova, Russia) using a column (2 × 75 mm) with a sorbent Prontosil-120-5-C18 (EcoNova, Russia) and the concentration gradient of acetonitrile (0–90%) in 0.05 M aqueous solution $LiClO_4$ (flow rate of 150 mcl/min, the thermostat temperature of 50°C). Detection was performed at four wavelengths: 300, 330, 350 and 360 nm.

The modified oligonucleotides were isolated by reverse-phase chromatography on a Agilent 1100 instrument in a concentration gradient of acetonitrile (0–80%) in a solution of 0.05 M solution $LiClO_4$ for 30 min, flow rate of 1.5 mL/min using a column (4.6 × 150 mm) with a sorbent Eclipse XDB-C8 (5 mcm) (Agilent, United States), as well as on a Agilent 1200 series instrument in a gradient of acetonitrile (0–75%) in 0.02 M solution of triethyl ammonium acetate for 30 minutes, the rate of 1.5 mL/min and a column (4.6 × 250 mm) with sorbent Zorbax SB-C18 (5 mcm). The absorption spectra were recorded at wavelengths from 220 to 500 nm. Oligonucleotide material was precipitated by a 10-fold volume of 2% solution of $LiClO_4$ in ether/acetone mixture (3 : 7). The supernatant was separated after centrifugation; the precipitate was washed with acetone (2 × 1 mL) and dried at 2–4 mmHg.

The calculation of the concentration of oligonucleotide derivatives was carried out, assuming molar absorption coefficients of the modified oligonucleotides, at 260 nm as the sum of the molar absorption coefficients of the unmodified oligonucleotides and the value of 23000 $M^{-1}\,cm^{-1}$ for the acridine-containing derivative. The ε_{260} value of the acridine derivative

was obtained by measuring the optical absorption of the solution of (**I**) in water with a known concentration. The absorption spectra of aqueous solutions were recorded on a UV-2100 spectrophotometer (Shimadzu, Japan).

Electrophoretic analysis was performed in 20% polyacrylamide gel under denaturing conditions (acrylamide: *N*,*N*'-methylene bisacrylamide (30 : 1), 8 M urea, 89 mM Tris-borate, pH 8.3, 2 mM Na_2-EDTA) in TBE buffer (89 mM Tris-borate, pH 8.3, 2 mM Na_2EDTA) at a voltage of 50 V/cm. The application of oligonucleotide samples on the gel was performed in a solution of 8 M urea containing 0.05% xilencianol FF and 0.05% bromophenol blue.

The results of electrophoretic separation in 20% polyacrylamide gel under denaturing conditions were visualized by staining with Stains-all.

Thermal denaturation of oligonucleotide duplexes was carried out in a buffer of 1 M NaCl, 0.01 M sodium phosphate (pH 7.3), 0.1 mM Na_2EDTA, at total concentrations of oligonucleotide components, taken in stoichiometric ratio, from 10^{-5} M to 2×10^{-4} M on an apparatus with a temperature-controlled optical-cuvette-based UV detector of liquid chromatograph Milichrome (Nauchpribor, Russia). Recording of the melting curves in the multiwave mode (at least four wavelengths in the range 240–300 nm) was carried out with step-by-step automatic switching of the monochromator. The integration time of the signal at each wavelength did not exceed 1.2 s. The rate of heating of samples did not exceed 0.7–1°C/min. As the temperature sensor we use a copper-constantan thermocouple calibrated with an absolute accuracy of ±0.1°C, which was connected to a Shch-1516 digital voltmeter (Russia).

Thermodynamic parameters of formation of the independent complexes were determined by the optimization method of the melting curves in the two-state model with sloping baselines using SIMPLEX (Ivanov A.V. ILP SB RAS).

Measurements of fluorescence were performed on a SFM 25 (Kontron, Italy) or Saga Eclipse EL06113032 spectrofluorimeters (Varian, United States).

In the measurement of fluorescence the buffer solution of 1 M NaCl, 0.01 M sodium phosphate (pH 7.3), 0.1 mM Na_2EDTA, containing modified oligonucleotides at a concentration of 5×10^{-7}–5×10^{-6} M was used. Portions of 1–5 mL of the concentrated (1 mM) solution of oligonucleotide–matrix CA_6C (**M**) were added to the cuvette containing modified oligonucleotides in buffer at 6°C.

The emission spectra were recorded between 450 and 700 nm under excitation of the sample at a wavelength of 400 nm, the excitation spectra were recorded during irradiation with light having a wavelength from 200 nm to 500 nm with emission detection at 550 nm.

ACKNOWLEDGMENTS

The authors thank A.A. Lomzov and V.V. Koval (ICBFM SB RAS) for the analysis of the thermal stability of oligonucleotide duplexes and for the recording of mass spectra, respectively. This work was supported by the RFBR (Project no. 10-04-01492-a), Program of the Russian Academy of Sciences Presidium "Molecular and cell biology" and partially by an interdisciplinary grant from the Siberian Branch of the Russian Academy of Sciences and the Ministry of Education and Science of the Russian Federation.

REFERENCES

1. Dmitrienko, E.V., Khomyakova, E.A., Pyshnaya, I.A., Bragin, A.G., Vedernikov, V.E., and Pyshnyi, D.V., *Russ. J. Bioorg. Chem.*, 2010, vol. 36, pp. 734–745.
2. Okamoto, A., Ichiba, T., and Saito, I., *J. Am. Chem. Soc.,* 2004, vol. 126, pp. 8364–8365.
3. Yamana, K., Iwai, T., Ohtani, Y., Sato, S., Nakamura, M., and Nakano, H., *Bioconjugate Chem.*, 2002, vol. 13, pp. 1266–1273.
4. Shchepinov, M.S., Udalova, I.A., Bridgman, A.J., and Southern, E.M., *Nucleic Acids Res.*, 1997, vol. 25, pp. 4447–4454.
5. Shchepinov, M.S., Kalim, U.M., Elder, J.K., Frank-Kamenetskii, M.D., and Southern, E.M., *Nucleic Acids Res.*, 1997, vol. 27, pp. 3035–3041.
6. Bashkin, J.K., Xie, J., Daniher, A.T., Sampath, U., and Kao, L.F., *Org. Chem.*, 1996, vol. 61, pp. 2314–2321.
7. Inoue, H., Furukawa, T., Shimizu, M., Tamura, T., Matsui, M., and Ohtsuka, E., *Chem. Commun.*, 1999, vol. 1, pp. 45–46.
8. Kuzuya, A., Mizoguchi, R., Morisawa, F., Machida, K., and Komiyama, M., *J. Am. Chem. Soc.*, 2002, vol. 124, pp. 6887–6894.
9. Daniher, A.T. and Bashkin, J.K., *Chem. Commun.*, 1998, vol. 10, pp. 1077–1078.
10. Putnam, W.C. and Bashkin, J.K., *Chem. Commun.*, 2000, vol. 9, pp. 767–768.
11. Jamil, A., Zubin, E.M., and Stetsenko, D.A., *Nucleic Acids Symp. Ser.,* 2008, vol. 52, pp. 719–720.
12. Nelson, P.S., Sherman-Gold, R., and Leon, R., *Nucleic Acids Res.*, 1989, vol. 17, pp. 7179–7186.
13. Nelson, P.S., Kent, M., and Muthini, S., *Nucleic Acids Res.,* 1992, vol. 20, pp. 6253–6259.
14. Raddatz, S., Mueller-Ibeler, J., Kluge, J., and Schweitzer, M., *Nucleic Acid Res.*, 2002, vol. 30, pp. 4793–4802.
15. Tona, R. and Haner, R., *Bioconjugate Chem.*, 2005, vol. 16, pp. 837–842.
16. Polushin, N.N., *Nucleic Acids Res.*, 2000, vol. 28, pp. 3125–3133.
17. Antsypovich, S.I., Oretskaya, T.S., and Kedrovski, G., *Russ. Chem. Bull.*, 2005, vol. 54, pp. 2585–2595.
18. Shi, Y., Machida, K., Kuzuya, A., and Komiyama, M., *Bioconjugate Chem.*, 2005, vol. 16, pp. 306–311.
19. Fukui, K. and Tanaka, K., *Nucleic Acids Res.*, 1996, vol. 24, pp. 3962–3967.

20. Kuzuya, A., Mizoguchi, R., Morisawa, F., Machida, K., and Komiyama, M., *J. Am. Chem. Soc.*, 2002, vol. 124, pp. 6887–6894.

21. Kim, S.J., Bang, E., and Kim, B.H., *Synlett*, 2003, vol. 12, pp. 1838–1840.

22. Katajisto, J., Heinonen, P., and Lonnberg, H., *Org. Chem.*, 2004, vol. 69, pp. 7609–7615.

23. Kashida, H., Liang, X., and Asanuma, H., *Curr. Org. Chem.*, 2009, vol. 13, pp. 1065–1084.

24. Guzaev, A., Salo, H., Azhayev, A., and Lonnberg, H., *Bioconjugate Chem.*, 1996, vol. 7, pp. 240–248.

25. Suzuki, Y., Otomo, T., Ozaki, H., and Sawai, H., *Nucleic Acids Symp. Ser.*, 2000, vol. 44, pp. 125–126.

26. Utagawa, E., Ohkubo, A., Sekine, M., and Seio, K., *Org. Chem.*, 2007, vol. 72, pp. 8259–8266.

27. Zimmerman, J., Cebulla, M.P.J., Mönninghff, S., and von Kiedrowski, G., *Angew. Chem., Int. Ed. Engl.*, 2008, vol. 47, pp. 3626–3630.

28. Lin, K. and Matteucci, M., *Nucleic Acids Res.*, 1991, vol. 19, pp. 3111–3114.

29. Lin, K. and Matteucci, M., US Patent No. 5414077, 1995.

30. Pyshnyi, D.V., Lomzov, A.A., Pyshnaya, I.A., and Ivanova, E.M., *J. Biomol. Struct. Dynam.*, 2006, vol. 23, pp. 567–580.

31. Lomzov, A.A., Pyshnaya, I.A., Ivanova, E.M., and Pyshnyi, D.V., *Dokl. Biochem. Biophys.*, 2006, vol. 409, pp. 211–215.

32. Asseline, U., Toulme, F., Thuong, N.T., Delarue, M., Montenay-Garestier, T., and Helene, C., *EMBO J.*, 1984, vol. 3, pp. 795–800.

33. Asseline, U., Thuong, N.T., and Helene, C., *J. Biol. Chem.*, 1985, vol. 260, pp. 8936–8941.

34. Fukui, K., Morimoto, M., Segawa, H., Tanaka, K., and Shimidzu, T., *Bioconjugate Chem.*, 1996, vol. 7, pp. 349–355.

35. Kuzuya, A., Machida, K., Mizoguchi, R., and Komiyama, M., *Bioconjugate Chem.*, 2002, vol. 13, pp. 365–369.

36. Corey, E.J. and Venkateswarlu, A., *J. Am. Chem. Soc.*, 1972, vol. 94, pp. 6190–6191.

37. Pyshnyi, D.V., Lokhov, S.G., Sil'nikov, V.N., Shishkin, G.V., Ivanova, E.M., and Zarytova, V.F., *Bioorg. Khim.*, 1999, vol. 25, pp. 40–55.

38. Kelley, S.O., Holmin, R.E., Stemp, E.D.A., and Barton, J.K., *J. Am. Chem. Soc.*, 1997, vol. 119, pp. 9861–9870.

39. Christensen, U.B. and Pedersen, E.B., *Nucleic Acids Res.*, 2002, vol. 30, pp. 4918–4925.

40. Asseline, U., Delarue, M., Lancelot, G., Toulme, F., Thuong, N.T., Montenay-Garestier, T., and Helene, C., *Proc. Natl. Acad. Sci. USA*, 1984, vol. 81, pp. 3297–3301.