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Synthesis of reactive nucleic acid analogues and their application for the study of structure and functions of biopolymers

I E Kanevskii, S A Kuznetsova

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Abstract. Data on the synthesis of reactive derivatives of nucleic acid analogues and their application for the study of structure and functions of biopolymers are generalised. The main types of such analogues including photoactivated reagents containing azidoaryl, halogeno, and thiol groups, psoralen and its derivatives, platinum-based reagents, and nucleic acid analogues containing substituted pyrophosphate or acyl phosphate internucleotide groups are presented. The mechanisms of interaction of these compounds with proteins and nucleic acids are considered. The prospects for the *in vivo* application of reactive nucleic acids in various systems are discussed. The bibliography includes 76 references.

I. Introduction

Synthetic fragments of nucleic acids containing modified units are widely employed in solving practical problems of molecular biology, biotechnology, and medicine. Derivatives of nucleic acids containing reactive groups are of special interest. These compounds can form covalent bonds with the reactive groups of biopolymers and are promising tools for the study of the topography of active centres of proteins, their functional features, and molecular mechanisms of DNA – protein recognition. The interest in these compounds has been considerably increased in recent years owing to the development of sense biotechnology. The main attention is concentrated on the search for new active groups and elaboration of methods for their directed introduction into nucleic acids.

This review describes the main types of reactive analogues of nucleic acids, *viz*., photoactivated reagents, platinum-based reagents, and new promising analogues of nucleic acids containing substituted pyrophosphate or acyl phosphate internucleotide groups. The use of reactive nucleic acids in the analysis of proteins and nucleic acids and prospects for their further application in various systems *in vivo* are surveyed. A large class of alkylating reagents first proposed by Grineva and Knorre in the late 60's is beyond the scope of this review because this material has been considered rather comprehensively in recent reviews (see, *e.g*., Refs 1, 2). For the same reason, reagents with redox mechanisms of action [1,2] have been omitted from consideration.

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II. Photoactivated nucleic acid analogues

In recent years, reactive nucleic acids containing photoactivated groups have gained wide acceptance as helpful tools for directed modification of proteins and nucleic acids and for the investigation of the structure and functions of these biopolymers. Normally, groups that are activated upon irradiation with light of wavelength > 300 nm are employed. This excludes the possibility of other photochemical transformations of the biopolymers themselves.

Among photoactivated compounds, nucleic acid analogues carrying azidoaryl, halogeno, or mercapto groups and various psoralen derivatives are the most popular.

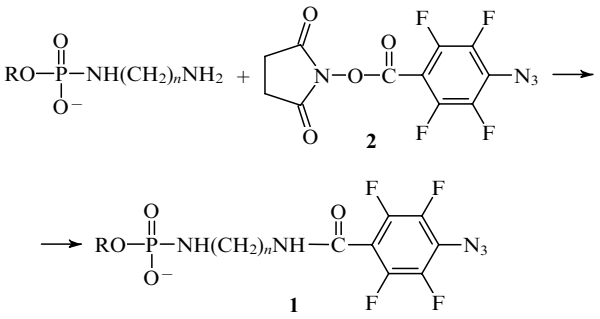
1. Nucleic acid derivatives containing the azido group

Reactive azido derivatives of oligonucleotides include synthetic oligonucleotides that are modified at the sugar-phosphate backbone or at heterocyclic bases by reagents containing the azido group. Irradiation converts them into reactive intermediates (nitrenes), which can further react with reactive groups of proteins and nucleic acids.

a. Introduction of azidoaryl groups into nucleic acids

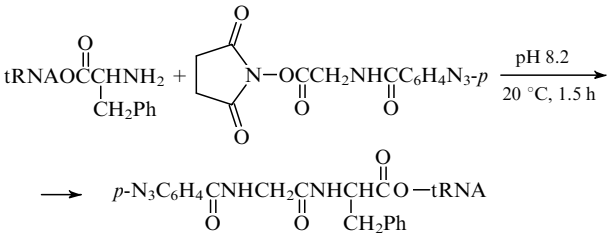
Azidoaryl groups are introduced into synthetic oligonucleotides either in the course of their synthesis with the use of modified nucleotide components [3] or by postsynthetic modification of oligonucleotides by various aryl azide derivatives. The most popular reagents contain *p*-azidophenyl, 2-nitro-5-azidophenyl, and azidoperfluorophenyl groups,[4,5] or azidoproflavin.[6]

Synthesis of azido derivatives of oligonucleotides makes use of the reactive groups inherent or incorporated into the oligonucleotides. The latter kind of groups involves aliphatic amino and thiol groups. Thus, an oligodeoxyribonucleotide azidoperfluorophenyl derivative **1** was obtained by acylation of the terminal amino group that had been introduced into the oligonucleotide using *p*-azidotetrafluorobenzoic acid *N*-hydroxysuccinimide ester **2**.[5]



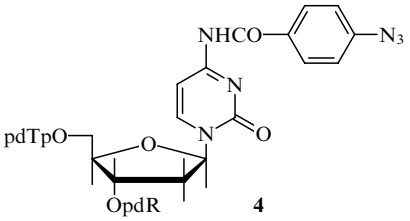
R is the oligodeoxyribonucleotide residue, $n = 2$ or 6.

The azido derivative of *E. coli* $tRNA^{Phe}$ **3** was obtained by acylation of the amino group of the phenylalanine residue that had been introduced into tRNA with *N*-(*p*-azidobenzoyl)glycine *N*-hydroxysuccinimide ester.[7]



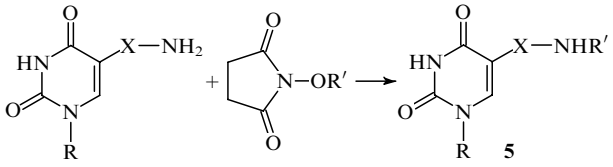
3 (95%)

A similar approach was used to introduce the azido group at the exocyclic amino group of cytidine. *p*-Azidobenzoic acid *N*-hydroxysuccinimide ester was used as the acylating reagent. This reaction gave the pentanucleotide azido derivative **4**.[8]

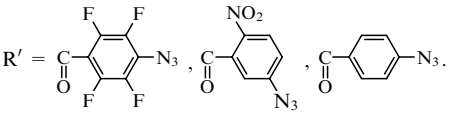


R — TAG

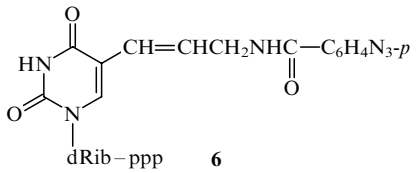
Various azido derivatives of the oligonucleotide d(UCCACTT) **5** were obtained by acylation of the amino group attached through a spacer to C(5) of the terminal deoxyuridine residue with *p*-azidotetrafluoro-, 5-azido-2-nitro-, or *p*-azidobenzoic acids *N*-hydroxysuccinimide esters.[4,9]



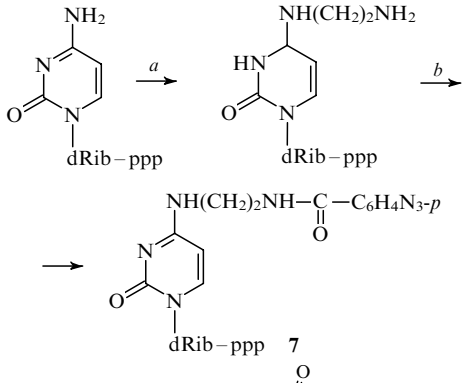
R = d(CCACTT); X = CH_2, $CH_2OCH_2CH_2$, $CH_2NHCOCH_2CH_2$;

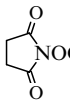


5-[3-(*p*-Azidobenzoyl)aminoprop-1-enyl]deoxyuridine 5′-triphosphate (**6**) was used to incorporate the azido group into the $tRNA^{Tyr}$ SUP4 gene. This compound was obtained by the reaction of 5-(3-aminoprop-1-enyl)deoxyuridine 5′-triphosphate with *p*-azidobenzoic acid *N*-hydroxysuccinimide ester (DMF, pH 8.5, 20 °C, 3 h in the dark).[10] This was followed by incorporation of compound **6** into DNA using DNA polymerase T4. The DNA thus modified was used for probing DNA – protein contacts in a specific transcription complex comprising the modified DNA, RNA polymerase III, and several transcription factors.



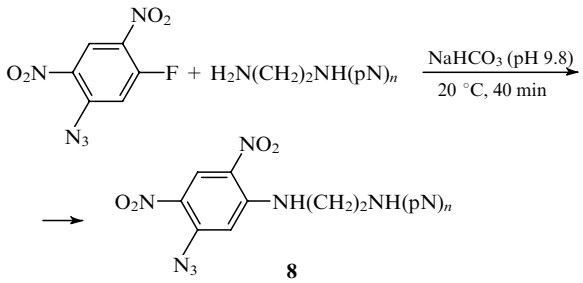
Recently, a novel azido-containing reagent 4-[2-(*p*-azidobenzoyl)aminoethyl]deoxycytidine 5′-triphosphate (**7**) has been proposed for this purpose.[11] This compound was also obtained using azidobenzoic acid *N*-hydroxysuccinimide ester.



(*a*) $H_2N(CH_2)_2NH_2$, $NaHSO_3$; (*b*) , pH 8.5, 3 h, 20 °C.

The first step of this synthesis included the hydrogen sulfite-catalysed transamination of the amino group of deoxycytidine 5′-triphosphate with ethylenediamine and isolation of the reaction product by column chromatography. The reaction of 4-(2-aminoethyl)deoxycytidine 5′-triphosphate with a 10-fold excess of *p*-azidobenzoic acid *N*-hydroxysuccinimide ester was conducted at reduced illumination. The target product **7** was also isolated by column chromatography and analysed by TLC. Compound **7** was introduced into a specific region of the $tRNA^{Tyr}$ SUP4 gene. The modified DNA was used for the probing of DNA – protein contacts in the transcription complex.

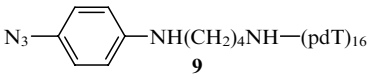
The reaction of 5-fluoro-2,4-dinitrophenylazide with the amino group of the ethylenediamine spacer attached at the terminal phosphate group of oligoribonucleotides gave photoactivated analogues of mRNA **8**, which represent oligoribonucleotide derivatives of various lengths having an arylazido group at the 5′-end of the oligoribonucleotide fragment.[12]



pN = pA, $n = 5$; pN = pU, $n = 4$, 7, and 8.

The reagents **8** were used for affinity modification of the 5′-region of the mRNA-binding centre of the *E. coli* ribosome.

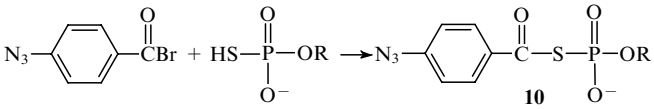
To study the localisation of the protein environment of DNA in chromatin, Kobets *et al.*[13] have used a photoactivated derivative of hexadecathymidylate **9**, in which the *p*-azidoaniline residue was linked to the 5′-terminal phosphate group through a 1,4-butanediamine spacer.



9

Compound **9** was obtained by phosphorylation of 4-(*p*-azidoanilino)butylamine with the oligonucleotide *N*-methylimidazolide. Earlier, Godovikova *et al.*[14] have studied the ability of amino acid residues to undergo modification by oligonucleotide derivatives in which the *p*-azidoaniline fragment was linked to the terminal phosphate group of the oligonucleotide directly rather than through a spacer. It was found that these reagents yield *p*-benzoquinonimide derivatives on irradiation and subsequent reaction with amino acids results in splitting of the reagent from the oligonucleotide. On the contrary, modification of proteins with reagents of the type **9** occurs specifically to give covalently bound nucleic acid – protein complexes.[13]

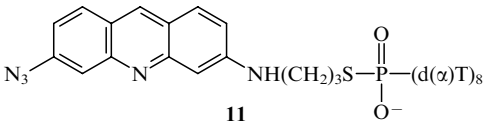
In addition to arylation and aroylation of aliphatic amino groups, alkylation or acylation of a thiol group that had been introduced into oligonucleotides is also employed for the preparation of azido derivatives of oligonucleotides using the corresponding halide derivatives of aryl azides. Thus acylation of the 5′-terminal thiolphosphate of oligothymidylate with *p*-azidobenzoyl bromide gives the *p*-azidobenzoyl derivative **10**.[15]



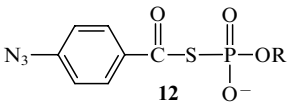
10

R is the oligothymidylate residue.

Analogously, the reaction of 3-azido-6-(3-bromopropylamino)acridine[6] or *p*-azidobenzoyl bromide[16] with the terminal thiolphosphate group of oligothymidylate yielded azidoproflavin (**11**) or *p*-azidobenzoyl (**12**) derivatives of oligonucleotides, respectively.



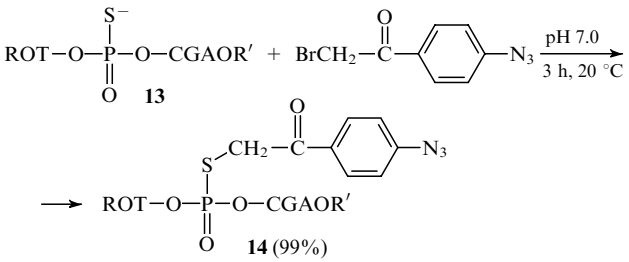
11



12

R is the oligodeoxyribonucleotide residue.

p-Azidophenacyl bromide[17] was also used for modification of the oligonucleotide **13** containing an internucleotide thiolphosphate group that had been introduced in the oligonucleotide synthesis.[17]

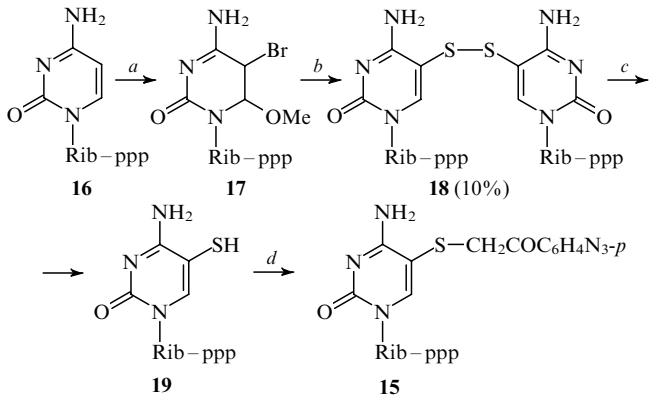


13 **14** (99%)

R and R′ are fragments of the oligonucleotide sequence.

The azido group can be incorporated through a thiol group into a heterocyclic base as well. Thus 5-[(4-azidophenacyl)thio]-cytidine 5′-triphosphate (**15**) has been synthesized according to Scheme 1.[18] All the reactions were carried out in the dark or under reduced illumination.

Scheme 1



16 **17** **18** (10%) **19** **15**

(*a*) MeOBr, 4 °C, 12 – 24 h; (*b*) Na_2S_2, 4 °C, 5 h; 20 °C, 48 h; (*c*) $NaBH_4$, H_2O, 0 °C, 3 h; (*d*) $BrCH_2COC_6H_4N_3$-*p*, DMSO, 4 °C, 12 h.

Treatment of cytidine 5′-triphosphate **16** with methyl hypobromite gave a bromomethoxy derivative **17**. The reaction product of compound **17** with sodium disulfide was isolated by extraction and column chromatography. The yield of 5,5-dithiobis(cytidine 5′-triphosphate) **18** was 10%. Such a low yield was attributed to the negative charge of both reacting molecules.[18] An alternative route to the disulfide from a nucleoside was also attempted.[18] In this case, the yield of the reaction products was much higher. Then, the 2′- and 3′-OH groups of ribose were protected and the 5′-OH group of the disulfide derivative was phosphorylated. The final stage of this synthesis was more complicated and labourious due to a greater number of purification steps and more complex isolation procedure of intermediate compounds, which resulted in nearly the same yield of 5,5″-dithiobis(cytidine 5′-triphosphate) **18**. 5-Mercaptocytidine 5′-triphosphate **19** was obtained from compound **18** by reduction with sodium borohydride. The reaction mixture was treated with *p*-azidophenacyl bromide in DMSO. The target product **15** was isolated by extraction and column chromatography (80% yield). The azido derivative of the nucleoside triphosphate **15** was introduced into various positions of RNA in the course of polymerase synthesis.

Thus, the methods available allow one to incorporate the azidoaryl groups both into heterocyclic bases of nucleic acids and the sugar-phosphate backbone. They involve acylation of aliphatic amino groups of nucleic acids with *N*-hydroxysuccinimide esters of azidobenzoic acid and its derivatives or alkylation (acylation) of a thiol group that had been introduced into nucleic acids using the corresponding azidoaryl halides. As a rule, these reactions occur with high efficiency (the product yield is 80% – 99%). However, whereas the former route makes use of the exocyclic amino groups of heterocyclic bases of nucleic acids for the incorporation of the azido groups, in the case of azidoaryl halides an additional step is required , *viz*., the incorporation of a thiol group into nucleic acids. This may complicate the procedure and reduce the yields of the target products.

b. Cross-coupling of nucleic acid derivatives containing the azido groups with biopolymers

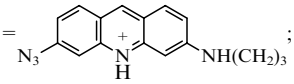
On irradiation with light (photolysis), compounds containing azidoaryl groups generate highly reactive short-lived radicals, *viz*., nitrenes. In the singlet state, the lifetime of nitrenes depends on the nature of substituents in the aromatic nucleus and varies from nano- to microseconds.[19] This state of nitrene radicals is very unstable: they either undergo intramolecular rearrangements to yield various reactive intermediates or react with neighbouring reactive groups. In the latter case, intermolecular covalent bonds may be formed. Using azidophenyl derivatives as examples, it has been shown that introduction of fluorine atoms or nitro groups into the benzene ring decreases the probability of intramolecular

rearrangements and simultaneously increases the probability of nitrene involvement in intermolecular reactions.[20]

For the first time, azido derivatives of oligonucleotides have been used for the study of the formation of cross-coupled DNA duplexes **20** from a 27-membered matrix oligonucleotide and a complementary octa(α-thymidylate) carrying an azidoproflavin residue linked with the 5′-thiolphosphate group of the α-anomer through a spacer arm.[6]

5′d(....^{5}TGAGTAAAAAAAATGAGT^{22}....)
X – Sp[α]d(TT T TT T TT)

20

X =  ; Sp is the 5′-thiolphosphate residue.

UV irradiation of oligonucleotides constituting the system **20** ($\lambda > 300$ nm) in 0.1 M NaCl resulted in cross-linking. The photoaddition product was treated with an alkali, which resulted in selective cleavage at modification sites. The residues T^{9} and T^{18} (1 : 3) of the DNA template were found to be the main sites of this cleavage. This ratio was rationalised by postulating parallel and antiparallel orientations of the oligonucleotide probe during the formation of a complementary complex or the existence of a triplex structure. Further experiments showed that the second hypothesis was more probable, since octa(α-thymidylate) in 0.1 M NaCl can form ternary complexes with the template.

Photomodification of the pentadecanucleotide **21** and the hexadecanucleotide **22** with the oligonucleotide derivatives **23**–**28** carrying the azido group at various positions of the nucleotide probe has been carried out by Levina *et al.*[21]

5′ TAAGTGGAGTTTGGC **21**

5′ AGAAAGTGAGTGTATC **22**

3′ TTCACCTp – X **23**

3′ CTTTCACU – X^{1} **24**

3′ X – pCAAACCG **25**

3′ X – pAAACCG **26**

X^{1}
|
3′ TUCACCT **27**

X^{1}
|
3′ CACUCAC **28**

X = $HN(CH_2)_3NHCOC_6H_4N_3$-*p*, X^{1} = $CH_2O(CH_2)_2NHCOC_6H_4N_3$-*p*.

The oligonucleotide derivatives employed contained the azido group attached through a spacer to the 3′(5′)-terminal phosphates (reagents **23**, **25**, and **26**) or to the C(5) atom of a terminal deoxyuridine (reagent **24**) or deoxyuridine inside the chain (reagents **27** and **28**).[21] The reagents complementary to the adjacent sites of the target were also used in pairs: tandems of reagents **23** + **25** and **23** + **26**. UV irradiation at λ = 303–365 nm yielded products of covalent addition of one or two (in the case of tandems) reagents to the target. The efficiency of photoaddition of reagents **23** and **26** containing the azido group (at the 5′- or 3′-ends, respectively) to the pentadecanucleotide was approximately the same, and the yields of the photoaddition products were 70%. However, the use of a tandem of these reagents increases the efficiency of photomodification up to 80%. The modification predominantly affects the G^{9} residue of the oligonucleotide **21**, which is not involved in the formation of the complementary complex. The adduct yield decreases to 55% and 51%, respectively, when reagents **27** and **28** containing the uridine azido derivative inside the chain are used. The decrease in the photoreaction efficiency is apparently due to the involvement of the modified base in a complementary reaction. This is confirmed by the fact that the efficiency of photoaddition in the case of reagent **24** having an azidonucleotide at its 5′-end reaches 69% and modification affects predominantly the G^{7} residue of the oligonucleotide **22**, whereas the G^{8} residue is modified in a lesser degree.

Highly reactive nitrenes generated from the azido derivatives of oligonucleotides upon photolysis can instantaneously react with the neighbouring reactive groups as well as with amino acid residues of a DNA – protein complex.

Investigation of the structure of the transcription factor TFIIIC of RNA polymerase III was carried out using a genomic DNA sequence of yeast $tRNA^{Tyr}$ SUP4 containing one or several azido derivatives of deoxyuridine **6**.[10] A complex of modified DNA with the protein was irradiated with UV light (λ = 254 nm) for 5 min. Analysis of photoaddition products allowed one to identify four subunits of TFIIIC and to establish their position relative to SUP4 in the DNA – protein complex. This study was extended by using a genomic DNA sequence SUP4 containing azido derivatives of cytidine **7**.[11] In contrast with the previous example, a non-purified protein fraction was used for modification. Analysis of photoaddition products revealed a protein with molecular mass of 40 kDa, which could not be detected when purified TFIIIC has been used. It was assumed that this protein is a component of the transcription complex.

Affinity modification of the 5′-region of the mRNA-binding centre of the *E. coli* ribosome was studied using *N*-[2-(2,4-dinitro-5-azidophenyl)aminoethyl]-5′-phosphoramidites of oligouridylates **8**.[12] It was found that compounds **8** (N = U, n = 4, 7, and 8) stimulate binding of code-specific aminoacyl-tRNA with the ribosome. Upon UV irradiation of the ribosome – reagent **8** – tRNA ternary complex ($\lambda > 350$ nm), covalent binding of the reagent **8** with the ribosome occurred. Up to 10% of the reagent **8** in the ternary complex was cross-linked with the ribosomal proteins of the 30S and 50S subunits. It was found that in the 30S subunit modification affected proteins S3, S4, S9, S11, S12, S14, S17, S19, and S20. In the 50S subunit, it was proteins L2, L13, L16, L27, L32, and L33 that were modified. The array of the modified proteins strongly depended on the length of the oligonucleotide fragment of the reagent and on the binding of the tRNA molecule to the site A of the ribosome.

By virtue of their ability to form stable covalent bonds with the reactive groups of proteins, azido derivatives of oligonucleotides are successfully employed for identifying amino acid residues in the active centres of DNA-recognising proteins. Thus identification of the amino acid residues of the restriction endonuclease *Taq*I that reacts with the recognition site was made using the photoreagent **13** containing the azido group just at the *Taq*I cleavage site.[17] The complementary complex 'oligonucleotide probe **13** – template DNA' was UV-irradiated (λ = 306 nm, 5 min) following 5-min incubation with *Taq*I. The efficiency of formation of the covalently bound DNA – protein complex was 15% – 20%. An analysis of photoaddition products allowed one to identify the amino acid residue involved in the formation of a covalent bond with the oligonucleotide probe as Tyr161.

Modified fragments of RNA containing 5-(4-azidophenacyl)thiocytidine residues at the 3′-end or in the middle of the chain were used for photoaffinity modification of T7 and *E. coli* RNA polymerases.[18] The complexes of modified RNA with T7 and *E. coli* RNA polymerases were UV-irradiated (λ = 302 nm) for 2 – 4 min at 20 °C. An analysis of photoaddition products revealed RNA-binding domains in the corresponding RNA polymerases.

In recent years, azido derivatives of oligonucleotides have been employed for modification of the proteins of chromatin. Thus reagent **9** was used to study structural rearrangements in chromatin during the cell cycle.[13] To this end, the spectra of proteins modified in the nuclei isolated from *HeLa* cells in two different phases of the cell cycle, *viz*., the S-phase and in the region

of transition from the G_1 to the S-phase (G_1/S), were compared. Modification of proteins in the nuclei isolated in the S-phase of the cell cycle gave five nuclear proteins with molecular masses 160, 87, 72, 59, and 46 kDa and was specific. The spectrum of the modified proteins changed on going from the S-phase to the G_1/S interface. In particular, the 160 kDa protein was not modified. The results obtained by other authors [13] confirm the idea that photoactivated oligonucleotides are promising for studies of the structure and functions of chromatin.

Wide application of azido derivatives of nucleic acids in the studies of nucleic acid – nucleic acid and nucleic acid – protein interactions is indicated by the existence of reproducible protocols for the synthesis and their high reactivity. However, in some cases the high reactivity of nitrenes photogenerated from the azido derivatives may decrease the specificity of the reactions and thus imposes certain limitations on the application of azidoaryl derivatives of nucleic acids in structure – function studies of biopolymers.

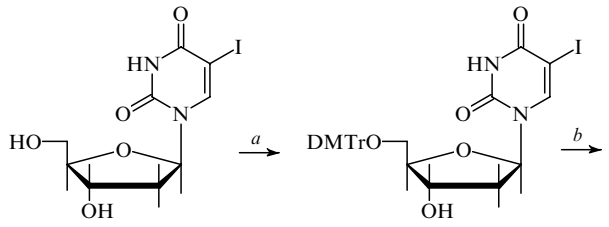
2. Derivatives of nucleic acids containing halogen or mercapto groups

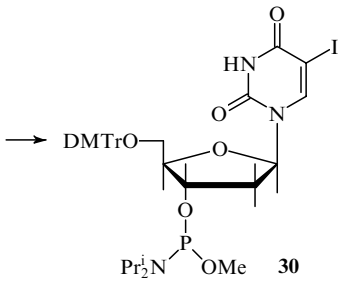
In recent years, photoactivated oligonucleotide derivatives with bromo, iodo, or mercapto groups have been widely employed for the study of nucleic acid – protein or nucleic acid – nucleic acid interactions. The interest in these compounds is due to their ability to generate free-radical intermediates that can form covalent bonds with electron-donor groups of proteins and nucleic acids on irradiation with long-wave UV light. Reactions according to radical mechanisms are also possible, but only in the case where the reacting groups are at a distance of several angström.[22] This circumstance makes possible the use of oligonucleotide halogeno and thiol derivatives for identifying direct nucleic acid – nucleic acid or nucleic acid – protein contacts.

a. Synthesis of halogeno and mercapto derivatives of oligonucleotides

Oligonucleotide derivatives carrying halogeno or mercapto groups are usually obtained by chemical or enzymic syntheses using modified nucleotide components, such as 4-mercapto or 5-bromo(iodo) uridine or cytidine. DNA derivatives containing halogeno or mercapto groups are obtained by solid-phase phosphoramidite synthesis with bromo-, iodo-, or mercapto-deoxyribonucleoside phosphoramidites as monomeric units. Thus, 5-bromo-2′-deoxyuridine (5-BrdU) derivatives of DNA that included a recognition site for the transcription factor NF-kB were obtained by Liu *et al.*[23] The modified uridine was introduced into various positions of DNA by solid-phase synthesis with 5-BrdU phosphoramidites.

The 5-iodo-2′-deoxyuridine derivative of the deoxyribonucleotide GCTCACCGAA – IdU – GC **29** was synthesised in a similar way [24] from compound **30** obtained in quantitative yield according to the following scheme:



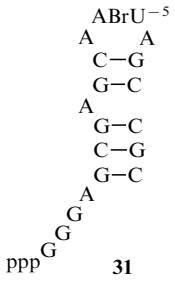


30

(*a*) DMTrCl = $(p\text{-MeOC}_6H_4)_2C(Ph)Cl$, Py; (*b*) $(Pr^i_2N)_2P(Cl)OMe$, Pr^i_2NEt, CH_2Cl_2, 20 °C, 10 min.

It should be noted that the high sensitivity of aromatic iodides to nucleophilic substitution prevents deprotection of internucleotide phosphates and heterocyclic bases by conventional treatment with NH_4OH at 55 °C. Removal of protective groups leading to the oligomer **29** could be carried out by treatment with NH_4OH for 48 h at room temperature. Under these conditions, no nucleophilic substitution of iodine in the aromatic nucleus occurred, while phosphate groups and heterocyclic bases were completely deprotected.

Halogeno or mercapto derivatives of oligoribonucleotides are also obtained using modified nucleoside triphosphates as substrates for T7 RNA polymerase in the polymerase-catalysed synthesis of RNA. Thus a 19-membered oligoribonucleotide **31** containing 5-bromouridine (5-BrU) at position (−5) was used to identify amino acid residues in the site of binding of the bacteriophage R17 membrane protein with genomic RNA.[25] The synthesis of compound **31** was carried out using 5-bromouridine 5′-triphosphate by a T7 RNA polymerase-catalysed synthesis on a synthetic DNA template.



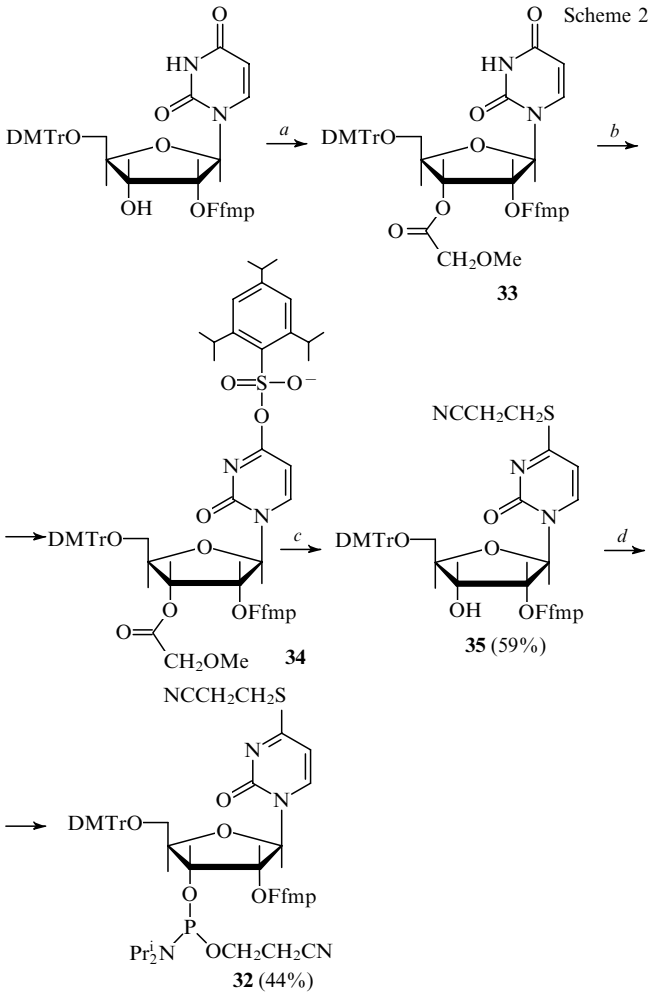
31

The same approach was used to obtain oligoribonucleotides with a nucleotide sequence analogous to that of compound **31** but with U or C 5-iodo derivatives at position (−5).[26]

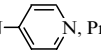
A method for the synthesis of 4-(2-cyanoethyl)thiouridine 32 phosphoramidite has been proposed (Scheme 2).[27] This compound was further used in the chemical synthesis of modified RNA. The 2′-OH group of ribose was protected by a 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl group. The mercapto group of uridine was protected as a 2-cyanoethyl derivative.

The reaction of 2′-*O*-[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]-5′-*O*-dimethoxytrityluridine with methoxyacetic anhydride in dry pyridine gave the derivative **33** in quantitative yield. To obtain the uridine derivative **35** with a cyanoethyl group at position 4, compound **33** was treated with 2,4,6-triisopropylbenzenesulfonyl chloride, after which the sulfonate **34** was introduced into reaction with 3-mercaptopropionitrile. The resulting compound was treated with ammonia in methanol to deprotect the 3′-hydroxy group of ribose. Phosphitylation of the 3′-OH group of the protected ribonucleoside **35** was carried out with 2-cyanoethyl bis(diisopropylphosphoramidite). After purification, compound **32** was obtained in 44% yield and further used in a solid-phase phosphoramidite synthesis to obtain photoactivated derivatives

Scheme 2



(*a*) $(MeOCH_2CO)_2$, Py, 20 °C, 24 h; (*b*) 2,4,6-$Pr^i_3C_6H_2SO_2Cl$,

Me_2N–, Pr^i_2NEt, 20 °C, 12 h; (*c*) 1. $HS(CH_2)_2CN$, MeCN,

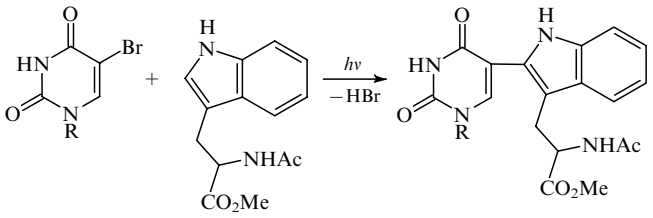
Pr^i_2NEt, 20 °C, 12 h; 2. NH_3, MeOH, 20 °C, 9 h;

(*d*) $(Pr^i_2N)_2PO(CH_2)_2CN$, CH_2Cl_2, , 20 °C, 48 h.

of RNA containing 4-mercaptouridine in different positions of the recognition sites of the HIV Rev protein and the bacteriophage MS2 membrane protein.

b. The use of oligonucleotide halogeno and mercapto derivatives for cross-linking to proteins and nucleic acids

As has been mentioned above, UV irradiation of bromo(iodo) or mercapto derivatives of oligonucleotides generates reactive intermediates, which interact with reactive groups of proteins and nucleic acids. Using an oligonucleotide derivative containing 5-BrU as an example, it was shown that the mechanism of photoactivated reaction of halogeno derivatives with the protein amino groups depends on the wavelength.[28] Upon UV irradiation with $\lambda = 308$ nm, the 5-BrU molecule passes from the low-energy singlet state to the reactive triplet state n,π^* responsible for the binding of BrU–DNA with the associated protein, presumably through the initial electron transfer. The intermolecular covalent binding of BrU with amino acid residues, such as cysteine, glutathione, or tryptophan, is accompanied by elimination of HBr.



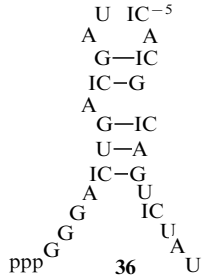
R is the oligonucleotide residue.

Upon UV irradiation with $\lambda = 254$ nm, the 5-BrU molecule passes into a high-energy singlet state π,π^*, which results in the cleavage of the C–Br bond to yield free radicals $U^{\bullet}$ and a Br atom. The highly reactive radical $U^{\bullet}$ either reacts with the reactive groups of the nearest environment (in particular, with the formation of a covalent bond with the amino groups of a protein) or passes into the triplet state, $^3\pi,\pi^*$.[29]

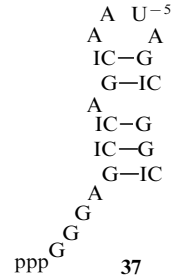
Synthetic analogues of the promoter region of DNA containing several 5-BrdU residues were used to study covalently bound complexes of a series of transcription factors of the albumin gene (HNF1, APF, vAPF, α1TEB, HPI) with the promoter regions of this gene.[30] Yet another aim of this study was to identify the polypeptide chains involved in the formation of a vAPF–APF complex and the nucleotide residues involved in the interaction with the protein. Complexes of DNA derivatives with the proteins were irradiated at $\lambda = 321$ nm. An analysis of cross-linking products revealed that the DNA–protein contact sites were distributed asymmetrically on a pseudopalyndromic recognition site: the nucleotides crucial for the recognition were located in the middle of the pseudopalyndromic sequence but the recognition site had other contacts of the protein with several nucleotides.

In order to investigate the formation of cross-linked complexes of a membrane protein with an operator of bacteriophage R17 transcription (R17 RNA) and to identify the amino acid residue responsible for the formation of the cross-linkage, Willis *et al.*[25] proposed to use a synthetic analogue of R17 RNA containing a 5-BrU residue at position (−5) (compound **30**). Its complex with the membrane protein was irradiated at $\lambda = 308$ nm for 5 min after which the protein concentration was increased and the complex was irradiated for an additional 5 min. The efficiency of formation of the cross-linked complex was 22%. The amino acid residue involved in this process was identified as Tyr85. It was found that cross-linking did not occur if Tyr85 was substituted by Ser.[25] These results are in good agreement with the above-described mechanism of the photoinduced reaction of oligonucleotides containing 5-BrU with the amino acid residues of the protein according to which 5-BrU derivatives cross-link only with aromatic amino acids upon irradiation at $\lambda = 308$ nm.[29]

Earlier, it was found[31] that oligonucleotides containing 5-IU residues are more efficient reagents for cross-linking to proteins than those containing 5-BrU. The efficiency of covalent photoaddition of iodine-containing reagents is usually 3 to 5 times higher than that of their bromo analogues. Therefore, the 5-BrU residue at position (−5) of compound **30** was substituted by the 5-IC residue.[26] Five additional 5-IC residues were introduced into various positions of RNA **36** in order to demonstrate the specificity of formation of the cross-linked complex.



36



37

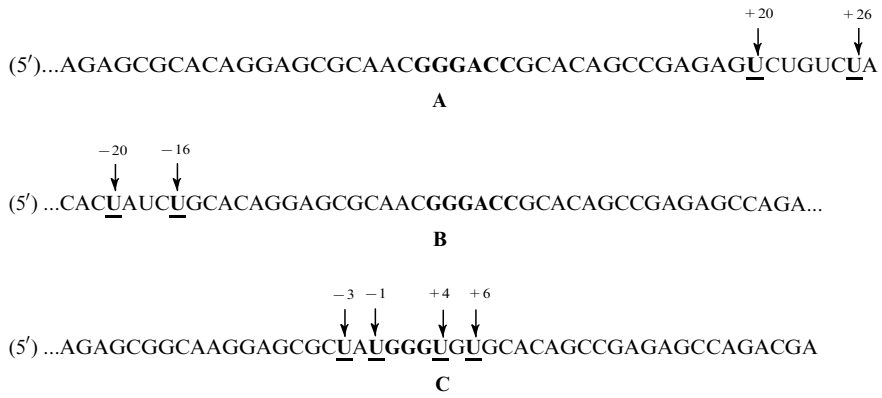


Figure 1. Model mRNA containing 4-mercaptouridine residues: A — mRNA 8′; B — mRNA 9′; C — mRNA 10′. The encoding sequences are bold-faced, and modified bases are underlined.

A complex of the membrane protein with compound **36** was irradiated with UV light (λ = 325 nm) for 2 h. The efficiency of cross-linking was 75% – 95%. It is noteworthy that the formation of the cross-linked complex was accompanied by partial cleavage of the RNA chain. When an analogous RNA devoid of the 5-IC residue at position (−5) (compound **37**) was used, the cross-linked RNA – protein complex was not formed.

On irradiation at $\lambda > 300$ nm, oligonucleotides containing 4-mercaptouridine reacted by a free radical mechanism and cross-linked through reactive groups with proteins and nucleic acids.[32] In the case of nucleic acids, the cross-linking was the most efficient between the adjacent uridine or adenine residues. This makes it possible to use mercapto derivatives of oligonucleotides for the studies of both nucleic acid – protein and nucleic acid – nucleic acid contacts. Thus a series of model mRNAs containing 4-mercaptouridine residues in various positions were synthesised to study cross-linking between mRNA and 18S RNA within a complex with the 80S ribosome (Fig. 1).[33] The synthetic mRNAs contained encoding sequences for glycine and/or tryptophan (GGG and ACC, respectively).

The mRNA – 80S ribosome complex was irradiated with UV light (λ = 320 – 365 nm) in the presence and in the absence of $tRNA^{Gly}$ and/or $tRNA^{Thr}$. The efficiency of formation of a cross-linked mRNA – 18S RNA complex depended on the presence of tRNA only for the mRNA 9′ derivative and was 20% in the presence of tRNA and 40% in its absence. The efficiency of cross-linking was 8% and 14% for mRNA 8′ and mRNA 10 derivatives, respectively. It was shown that a covalent bond is formed with U630 and U1111/A1112 of the 80S ribosome 18S rRNA.

Synthetic nucleic acid analogues having reactive thiol groups are widely employed in the studies of the structure and function of ribosomes. Thus a series of model RNAs have been synthesised to study the sites of binding of 5S RNA with the 50S subunit of the ribosome[34] in which 18% of uridine residues were statistically substituted by 4-mercaptouridine residues. The 5S RNA – ribosome 50S subunit complex was irradiated at λ = 300 nm for 10 min. The yield of the product of cross-linking of 5S RNA to the 23S ribosomal RNA was more than 75%. The structures of the products formed were not established, although it was shown that U89 of 5S RNA and U2477 of 23S RNA of 50S ribosomal subunits were cross-linked.

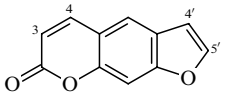
Thus, the indisputable advantage of halogeno and mercapto derivatives of nucleic acids is their high reactivity and the ability to operate at a zero distance, which makes possible the probing of direct nucleic acid – protein contacts. At the same time, the high reactivity of these intermediates may be the cause of nonspecific modification of proteins and other cell components and their partial destruction.[26] Moreover, the need for additional activation with light of definite wavelengths restricts the application of halogeno and mercapto derivatives of nucleic acids *in vivo*.

3. Psoralen derivatives of nucleic acids

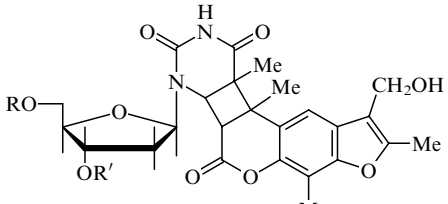
Nucleic acids that contain psoralen residues or its derivatives belong to the most efficient, selective photoreagents that are widely employed for affinity modification and structural analysis of nucleic acids.

a. Introduction of psoralen into oligonucleotides

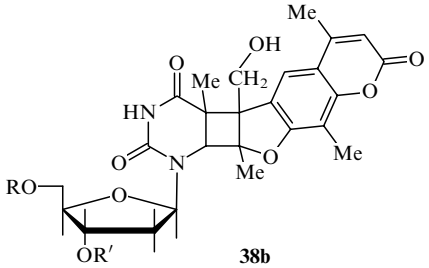
Psoralens (7*H*-furo[3,2-*g*][1]benzopyran-7-ones)† are aromatic compounds with three linearly fused rings of α-pyrone, benzene, and furan. They contain two photoactivated double bonds, C(3) = C(4) in the pyrone ring and C(4′) = C(5′) in the furan ring.



Irradiation with long-wave UV light (λ = 300 – 365 nm) can result in cycloaddition of both these bonds to the C(5) = C(6) bond of thymidine or uridine to form a cyclobutane ring. Thus psoralen derivatives **38a** and **38b** of a dodecanucleotide containing only one thymidine residue were obtained.[35,36]



38a



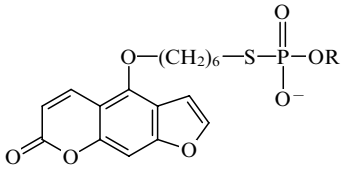
38b

R = d(GAAGC); R′ = d(ACGAGC).

Photochemical addition of psoralen gives reactive derivatives only if the C(4′) = C(5′) bond of the furan ring is involved in the reaction with the thymidine residue. In the case of thymidine cycloaddition to the C(3) = C(4) bond of the pyrone ring, the psoralen derivative formed is incapable of further photochemical

† Hereinafter the names used in the original publications are given.

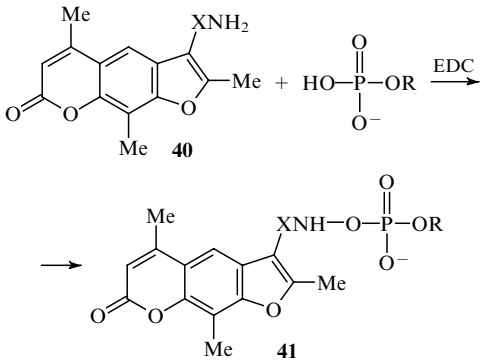
addition.[37] Therefore, the introduction of psoralen into terminal phosphate groups of oligonucleotides has now become an especially widespread procedure, for it allows modifications that involve the reactive centres of the psoralen molecule. Thus a psoralen derivative of a 16-membered oligonucleotide **39** has been synthesised by alkylation of its 5′-terminal thiolphosphate group with 5-(ω-iodohexyloxy)psoralen.[38]



39

R = TTTTCTTTTCCCCCCT.

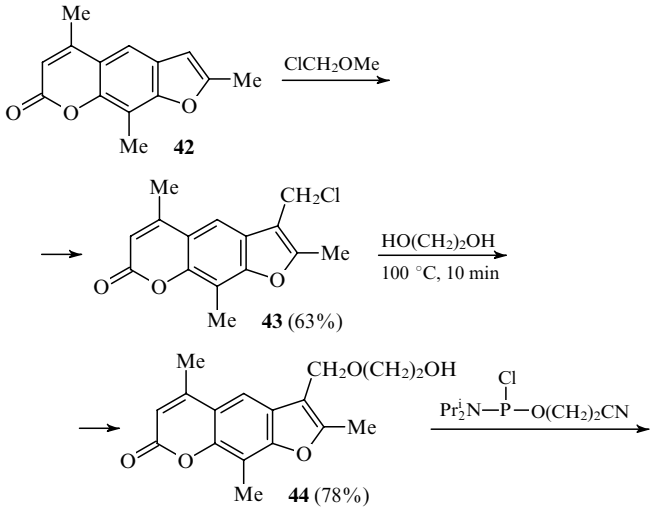
4′-Aminoalkylamino-4,5′,8-trimethylpsoralen **40** was attached to the 5′-phosphate group of a methylphosphonate analogue of the oligonucleotide using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (CDI) as the activating reagent.[39]

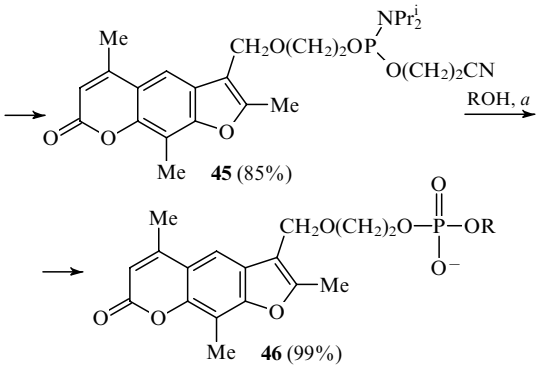


X = NH–CH_2; NH–$(CH_2)_n$–$NHCH_2$, n = 2, 4;
R is a methylphosphonate analogue of the oligodeoxyribonucleotide;
EDC is EtN=C=N$(CH_2)_2CH_2NMe_2$.

The reaction was performed in a lutidine hydrochloride buffer (pH 7.5) for 14 h at 20 °C. The yield of compound **41** was 85%.

The attachment of psoralen to the 5′-end of an oligonucleotide can also be carried out in the solid-phase oligonucleotide synthesis. Thus a 4,5′,8-triethylpsoralen residue **42** was incorporated into the oligonucleotide by phosphoramidite synthesis using psoralen β-cyanoethyl-*N*,*N*-diisopropylphosphoramidite **45** at the final stage.[40]

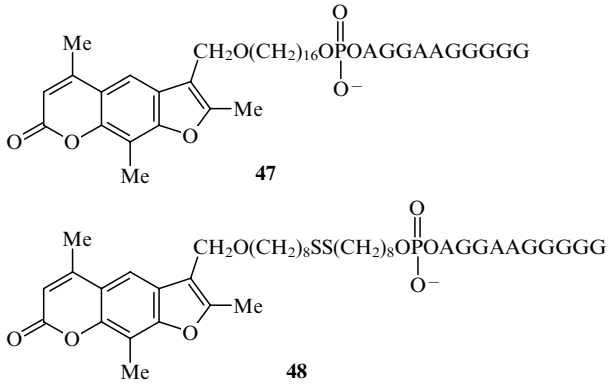




(*a*) 1. tetrazole; 2. I_2; 3. NH_3, 55 °C, 6 h;
R = d(TAGCCGCTATCGGTTAGT).

The first step of this synthesis consisted in chloromethylation of 4,5′,8-trimethylpsoralen **42** with chloromethyl methyl ether. Then, a linker group was introduced into the resulting compound **43**. Phosphitylation of 4′-(2-hydroxyethoxymethyl)-4,5′,8-trimethylpsoralen **44** with β-cyanoethyl-*N*,*N*-diisopropylamidophosphochloridite was carried out in the presence of ethyldiisopropylamine for 30 min at 20 °C. The oligonucleotide psoralen derivative **46** was obtained on an automated DNA synthesiser using a standard phosphoramidite protocol. The psoralen phosphoramidite **45** was attached to the 5′-end of the oligonucleotide at the last stage of this synthesis. Oxidation and deprotection with concentrated ammonia gave 5′-psoralen derivative **46** in 99% yield.

A similar strategy has been used to obtain derivatives of oligonucleotides **47** and **48**.[41]



Compound **48** with a disulfide bond, was synthesised using a mercaptoalkyl phosphoramidite that was introduced into an automated oligonucleotide synthesis prior to the attachment of the psoralen derivative.

Thus, psoralen derivatives of nucleic acids can be obtained both by photochemical cycloaddition of psoralen to the heterocyclic bases of nucleic acids and by chemical attachment of psoralen derivatives to terminal phosphate groups of nucleic acids. The latter approach has become more popular because the ability of psoralen for subsequent photochemical addition is fully preserved in this case. Moreover, photochemical modification of heterocyclic bases of nucleic acids with psoralen makes the *N*-glycosidic bond more labile and can sometimes result in the destruction of the polynucleotide chain.

b. Cross-linking of psoralen-containing oligonucleotides to biopolymers

Psoralen-containing oligonucleotides are mostly used for directed modification of nucleic acids. A detailed study of their cross-linking to complementary sites of nucleic acids has been carried out by Shi.[35] To this end, the dodecanucleotide GAAGCT*ACGAGC derivatives containing a psoralen residue fused to thymidine both by its pyrone and furan rings (compounds **38a** and **38b**,

respectively) were used. Modified oligonucleotides **38a** and **38b** were hybridised with a complementary template and irradiated with monochromatic light of various wavelengths. It was shown that irradiation of the nucleic acid – oligonucleotide **38a** complex with light in a broad range of wavelengths resulted in the predominant splitting of the 4′-hydroxymethyl-4,5′,8-trimethylpsoralen residue. In this case, the efficiency of cross-linking between complementary oligonucleotides was extremely low. With irradiation of the complementary nucleic acid – oligonucleotide **38b** complex at $\lambda < 313$ nm, both splitting of the psoralen residue and simultaneous formation of a covalent oligonucleotide – nucleic acid bond occur. Irradiation of the latter complex with $\lambda > 313$ nm results in highly efficient formation of a cross-linked complex, and psoralen is not split off. These results are in good agreement with other data on the stability of the psoralen monoadduct with thymidine. It was shown that thymidine fused with psoralen at the furan ring **38b** was more resistant to irradiation at $\lambda < 313$ nm than the compound of the type **38a** with the pyrone ring fusion. Introduction of a psoralen derivative of thymidine into an oligonucleotide only slightly increases the monoadduct resistance to photocleavage.[42]

Psoralen-containing oligonucleotides have been used in studies of DNA transcription with the RNA polymerase of *E. coli*.[36] 4′-Hydroxymethyl-4,5′,8-trimethylpsoralen was introduced into one of the chains of a 140-membered DNA duplex containing a promoter of *E. coli* RNA polymerase. Upon irradiation at $\lambda = 320-380$ nm, psoralen fused with a particular thymidine residue in one of the duplex chains reacted with the closely situated thymidine residue of the complementary chain. The yield of the cross-linked DNA duplex was 80%. It was shown that transcription of this duplex was blocked at a distance of one nucleotide from the site of cross-linking.

Methylphosphonate analogues of oligonucleotides having psoralen derivatives at their 5′-ends and linked to the phosphate group of the oligonucleotide through various spacer groups were used for attachment to two 35-membered deoxyribonucleotides.[39]

3′ d(...GTAT T TAT T TT TT TTAATCA...)
Ps – X – pd (AA TAAAAAAAAT)

49

3′ d(...GATTAAAAAAAATAAATACGGT...)
Ps – X – pd (AT T T TTT T TAT)

50

X = $NHCH_2$, $NH(CH_2)_nNHCH_2$, $n = 2, 4$;
Ps is the 4,5′,8-trimethylpsoralen residue. The fragments of oligonucleotides containing internucleotide methylphosphonate groups are underlined.

The cross-linked complex was obtained by irradiation of the duplexes **49** and **50** with light of $\lambda = 365$ nm at 4 °C for 20 min. The yield of the photoaddition products strongly depended on the length of the spacer arm and reached maximum values (75% and 80% for systems **49** and **50**, respectively) at $n = 4$. Studies of the temperature dependence of the photoaddition reaction revealed that the efficiency of cross-linkage of DNA duplexes was determined by the stability of the complementary complex formed. Irradiation of photoaddition products at $\lambda = 254$ nm led to the cleavage of the cross-linked DNA duplexes and recovery of the original compounds. It was thus concluded that cross-linking of modified oligonucleotides is a result of formation of a cyclobutane ring between the C(5) – C(6) bond of thymine and the C(3) – C(4) bond of the pyrone ring of psoralen. The adduct with the furan ring of psoralen is not formed due to steric hindrances.

Psoralen attached to the terminal phosphate group of an oligonucleotide through C(5) retains its capacity to form a bisadduct. Therefore, this type of psoralen-containing oligonucleotides within a triplex can form a ternary cross-linked oligonucleotide complex. The structure of the triplex is stabilised by intercalation of psoralen residues into a double helix of DNA. Thus psoralen-containing, 16-membered homopyrimidine oligonucleotide **39** was used for cross-linking to a double-helical region of DNA of the human immunodeficiency provirus **51**[38] (the site where the formation of a triplex structure takes place is boldfaced).

5′ CCACTTTTT**AAAAGAAAAGGGGGGA**CTGG
AA**TTT TCTTT TCCC CC CT**

51

The sequence **51** contains a recognition site for the restriction endonuclease *Dra*I (TTTAAA) overlapping with two or three nucleotides in the formation of a triplex with the oligonucleotide **39**. Plasmids pLTR and pBT1 containing one or two copies of sequence **51**, respectively, were incubated with the oligonucleotide **39** and irradiated with UV light ($\lambda > 310$ nm). The photomodified plasmids were used as substrates for the restriction enzyme *Dra*I. It was found that photomodification with the psoralen derivative **39** selectively inhibited the enzymic splitting at the site overlapping with the triplex structure.

The ability of psoralen-containing oligonucleotides to form cross-linked triplex complexes has been used for site-specific insertion of mutations into animal cells in order to investigate their repair mechanism.[41] To this end, the frequency and spectrum of mutations induced by various psoralen-containing oligonucleotides in several cell lines with various repair disturbances, *viz*., *xeroderma pigmentosum* variant (XPV) and *xeroderma pigmentosum* group A (XPA), were compared with the corresponding parameters of normal and *Fanconi animia* (FA) cells. In these studies, compounds **47** and **48** were used, which form triplexes with the 167 – 176 region of the *sup*F gene. It is noteworthy that the psoralen residue of compound **48** is linked with the oligonucleotide through a disulfide bond, which can be cleaved by dithiothreitol; this allows one to study mutagenesis in the absence of the site-directed oligonucleotide part of the probe. The triplex structure of compound **47** (or **48**) with the *sup*F gene in the vector pSP189 was irradiated with long-wave UV or visible light. UV irradiation gave a cross-linked triplex in 65% yield, while upon irradiation with visible light the yield did not exceed 1%. The cross-linkage was formed between T166 and T167 residues. The DNA vector thus modified was transformed into the appropriate cultures of human cells. Analysis of mutagenesis in the line XPV revealed an unusually high frequency of mutations (more than 30%) with a unique spectrum that differed from those of XPA, FA, and normal cells. These data led Raha *et al*.[41] to conclude that this approach is useful both for the description of repair defects in XPV cells and other genetic analyses of human cells.

The reactive oligonucleotide **46**, modified at the 5′-end with 4′-hydroxyethoxymethyl-4,5′,8-trimethylpsoralen, was used to obtain a cross-linked DNA duplex within the complementary complex **52**. This DNA duplex was obtained in 75% yield upon UV irradiation of the complex **52** at $\lambda = 350$ nm at 20 °C.[40]

X – pTAGCCGC TATC GGTTAGT
GCCATC GGCGATAGCCAATCA

52

X is 4′-hydroxyethoxymethyl-4,5′,8-trimethylpsoralen.

The glycosidic bond of the nucleotide involved in cross-linking of psoralen-containing oligonucleotides to nucleic acids becomes more labile. This property has been exploited in site-specific cleavage of DNA.[35] The cleavage site was mostly determined by the length of the spacer arm between the psoralen residue and the oligonucleotide end.

Thus, owing to their ability for selective cycloaddition to thymidine or uridine residues, psoralen-containing nucleic acids are widely employed for the study of structure and function of nucleic acids. The possibility of formation of bisadducts makes them helpful tools for the study of mechanisms of formation of triplex structures.

III. Platinum-containing nucleic acid analogues

Such compounds as *cis*- (cisplatin, *cis*-**53**) and *trans*-diamminedichloroplatinum(II) (*trans*-**53**) and its analogues are widely used for the synthesis of Pt-containing oligonucleotides.



cis-**53** *trans*-**53**

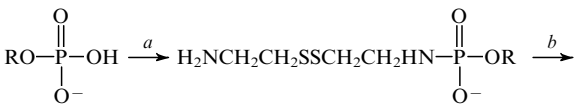
As is known, cisplatin-derived reagents, unlike *trans*-isomers, have wide therapeutic applications as efficient antitumour drugs.[43] A great number of investigations have been recently devoted to the study of peculiarities of interaction of *cis*- and *trans*-isomers of diamminedichloroplatinum(II) with two-helical sequences of DNA.

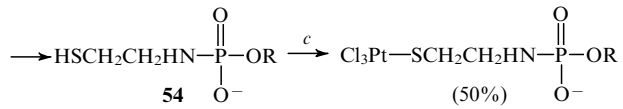
When bifunctional platinum derivatives, such as *cis*- and *trans*-**53** interact with DNA duplexes, they can form covalent bonds both between the neighbouring bases of the same chain and between spatially close bases in the complementary chains of a duplex. When *cis*-**53** is used, cross-linking occurs between two neighbouring guanine residues or an adenine and guanine of the same chain and, to a lesser degree, between two guanine residues of the same chain separated by one nucleotide or between closely arranged guanine residues of the complementary chains. *Trans*-**53** induces cross-linking mostly between the closely arranged guanine and cytidine residues of the complementary chain and, to a lesser degree, between two guanine residues of the same chain separated by one nucleotide. The difference in the mode of interaction of *cis*- and *trans*-stereoisomers of diamminedichloroplatinum(II) with DNA results in the change in the conformation of the modified DNA duplexes. This, in turn, affects the binding of these duplexes with DNA-recognising proteins.[44] Recently, it has been demonstrated that some proteins of nucleic acid metabolism can efficiently recognise DNA with cross-links induced by *cis*-**53**.[44,45] On the other hand, the DNA cross-linked by both stereoisomers is recognised by these proteins far less effectively.[44,45]

These results suggest that the differences in the conformations of native cellular DNA resulting from the interactions of *cis*- and *trans*-isomers of diamminedichloroplatinum(II) play a crucial role in the therapeutic efficiency of these compounds as antitumour drugs.

1. Methods for introduction of platinum reagents into oligonucleotides

Derivatives of diaminodichloroplatinum(II) are bifunctional reagents, which can interact with nucleophiles. Both nucleophilic centres of residues A, G, and C and the thio groups incorporated into oligonucleotides can be used for modification of oligonucleotides by these reagents. Prior to the introduction of an aliphatic thio group into a 16-membered oligonucleotide, the latter was treated with cystamine in the presence of *N*-methylimidazole and water-soluble carbodiimide and the resulting compound was treated with dithiothreitol.[46] Reaction of compound **54** with K_2PtCl_4 was carried out in an aqueous medium at pH 7–8 and gave a reactive nucleotide derivative with a spacer 2-thioethylamino group at the 5′-end. The synthesis was performed according to the following scheme:





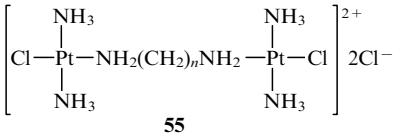
54 (50%)

R is oligonucleotide residue;

(*a*) $[H_2N(CH_2)_2S]_2$, , EDC; (*b*) $[HSCH_2CH(OH)]_2$;

(*c*) K_2PtCl_4, pH 7–8, 20 °C, 2 h.

The binuclear Pt complex **55** was used for modification of oligonucleotides **56**–**59** carrying a 5′-terminal or an internucleotide thiophosphate group.[47]



55

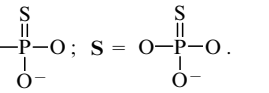
n = 4, 5, 6.

5′ **Sp**CCTTTTCCTTCCCTTTT **56**

5′ CACAATTCS CACACAAAC **57**

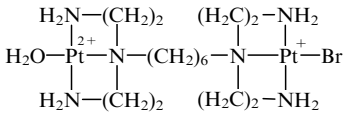
5′ **Sp**CACAATTCCCACAAC **58**

5′ **Sp**GCTCACAATTCCACAC **59**



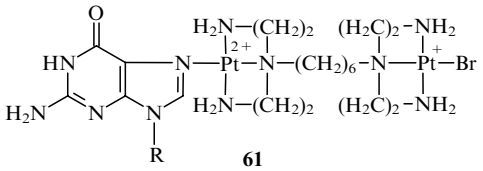
The reaction between modified oligonucleotides **56**–**59** and compound **55** was carried out in an aqueous medium in the presence of KBH_4 for 90 or 60 min at 37 °C. In the former case, the Pt-containing oligonucleotides were used for cross-linking to the complementary fragments of human immunodeficiency virus DNA without preliminary isolation from the reaction mixture. In the latter case, the products were isolated by dialysis.

An analogous binuclear complex **60** containing two Pt atoms with different ligands and differing strongly in their reactivity has been proposed for direct modification of heterocyclic bases of oligonucleotides.[48]



60

Compound **60** binds with the greatest efficiency with the N(7) atom of the guanine base through its Pt^{2+} moiety; this reaction gives adducts of the type **61** in virtually quantitative yields (H_2O, pH 5.0, 4 h, 20 °C).

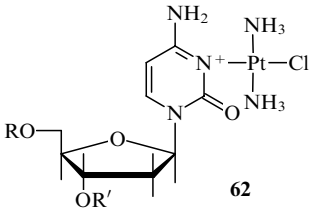


61

The nucleophilic centres of adenine and cytosine residues can also undergo modification.

In some cases, where it was necessary to obtain oligonucleotide monoadducts with *trans*-diamminedichloroplatinum, the latter were treated with $AgNO_3$ in the presence of $NaClO_4$ (prior to the synthesis or directly in the reaction mixture containing oligonucleotides) (see, for example, Refs 49, 50). This reaction is conducted in an acetate buffer (pH 3.6) at 37 °C for 15–30 min. As a result, diamminedichloroplatinum is converted into aquadiamminechloroplatinum $[Pt(NH_3)_2Cl(H_2O)]^+$. The latter selectively modifies the guanine residues of an oligonucleotide sequence. Platinum-containing oligonucleotides are isolated by

ion-exchange chromatography. However, when *cis*-aquahydroxydiammineplatinum (*cis*[$(NH_3)_2Pt(OH)(H_2O)$]NO_3 is used, modification affects all of the G, C, and A residues of the oligonucleotide. In this case, the covalent bond can also be formed between the nucleotides of the same chain. Reagents with one platinum residue can be prepared from oligothymidylates containing only one G, C, or A residue. Thus treatment of oligothymidylate having a single cytidine residue with a 10-fold excess of *cis*-aquahydroxydiammineplatinum nitrate [51] gave compound **62**. The degree of conversion of the original oligonucleotide was 97% – 99%:



62

R = d(TT), R′ = TTTTTTT.

The reaction was conducted in an aqueous medium (pH 7.0) for 24 h at 30 °C; the degree of conversion of the original compound and the homogeneity of the final product were estimated by microcolumn chromatography. Compound **62** was used for modification of DNA polymerase α of human placenta.

Thus, modern methods allow one to incorporate platinum both into heterocyclic bases and into a predetermined position of the sugar-phosphate backbone of nucleic acids. In the latter case, the synthesis of platinum-containing derivatives must be preceded by introduction of mercapto groups into the sugar-phosphate backbone.

2. Cross-linking of Pt-containing oligonucleotides with biopolymers

Bifunctional Pt-containing compounds are often used to obtain cross-linked nucleic acid – nucleic acid or nucleic acid – protein complexes.[52, 53] The synthesis of platinum-containing oligonucleotides makes it possible to increase considerably the efficiency and selectivity of modification of biopolymers by these reagents.

Cross-linking of Pt-containing oligonucleotides with biopolymers can be carried out in two ways: (1) by direct reaction of Pt-containing oligonucleotides with complementary sites of nucleic acids or DNA-recognising proteins and (2) by treatment of a preformed complex of a biopolymer with an oligonucleotide probe containing nucleophilic SH or NH_2 groups at predetermined positions with bifunctional Pt reagents. Thus treatment of a complementary complex **63** formed by a 322-membered DNA target and a 16-membered oligonucleotide probe carrying a cysteamine residue at its 5′-end with K_2PtCl_4 resulted in cross-linking. The efficiency of cross-linking varied from 10% to 50% depending on K_2PtCl_4 concentration.[46]

(5′) Xpd(CACAATTCC ACACAAC)
(3′) d(....ACGAGTGTTAAGGTGTGT TGTA....)

63

X = $HSCH_2CH_2NH$

The reaction involved the attachment of Pt coordinated with three chloride ions to the 5′-SH group of the oligonucleotide probe and subsequent modification of the DNA target.

A similar approach was used by Chu and Orgel [54] who carried out affinity modification of the transcription factors CREB and JUN using platinum-containing oligonucleotides. To this end, six internucleotide thiophosphate groups have been introduced into each CRE and TRE recognition site for the transcription factors CREB and JUN in covalently closed DNA duplexes. The complexes of oligonucleotide probes with the corresponding proteins were treated either with K_2PtCl_4 or with *trans*- or *cis*-diamminedichloroplatinum(II). The efficiency of cross-linking between oligonucleotides and proteins with K_2PtCl_4 or *trans*-**53** was from 30% to 50% depending on the reagent concentration. Treatment of a DNA – protein complex with *cis*-**53** did not result in cross-linking. The use of analogous probes devoid of internucleotide thiophosphate groups resulted in several-fold lower efficiency of cross-linking. Presumably, this method [54] can be employed for the isolation of DNA-recognising proteins.

A Pt-containing nonanucleotide **61** (R = TCCGCCTTT) was used for modification of a complementary hexanucleotide GGCGGA within a two-helical complex **64**.[48] The yield of the cross-linked complex was 80%.

XBr
(5′) TCCGCCTTT
(3′) AGGCGG

64

Unlike psoralen derivatives, treatment of guanine residues of template oligonucleotides with platinum-containing reagents favours stabilisation of the glycosidic bond. An analysis of products of partial scission of the template at purine bases allows one to identify particular guanine residues that have been modified. It was found that one of the two 5-terminal guanine residues of the template was involved in cross-linking.[48]

A detailed study of cross-linking of oligonucleotides modified with the reagent **55** at the 5′-terminal or at internucleotide thiophosphate groups to complementary fragments of DNA was carried out by Gruff and Orgel.[47] The reaction was studied both within DNA duplexes and within ternary complexes using oligonucleotides **56** – **59** and **65** – **68**.

19 20
TTTAAAAGGGAAGGAAAA**GG**TAAAGAC

65

CAGAAATGGAAAAGGAAGGGAAAATTT

66

AAATTTTCCCTTCCTTTTCCATTTCTG

67

TCGTATGTTGTGGGAATTGTGAGCGGATAACAATTT

68

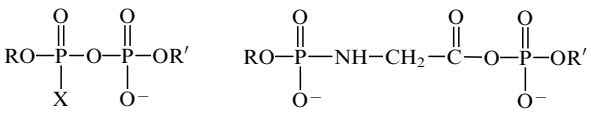
Using duplexes **65** – **56** as an example, it was shown that cross-linking was the most efficient (64% yield) when the reagent **55** ($n = 6$) was used. This reagent was chosen for subsequent experiments. The reaction was carried out for 60 min at 37 °C in the presence of $NaClO_4$. Modification affected residues G19 and G20 of the oligonucleotide **65** (marked bold). Under the same conditions, the efficiency of cross-linking in duplexes **58** – **68** and **59** – **68** was much lower (46% and 41%, respectively), which was attributed to the formation of intrachain cross-links in oligonucleotides **58** and **59** containing purine bases.[47] In the case of the oligonucleotide **57**, the nucleotide composition of which is identical with that of the oligonucleotide **58** but which contains a platinum reagent inside the chain (because the thiophosphate group is present in the middle of the sugar-phosphate backbone), the efficiency of cross-linking decreased to 37%. This low efficiency may be due to the involvement of the modified base in complementary interaction. A ternary complex was formed by oligonucleotides **66** and **67** and an analogue of a Pt-containing oligonucleotide **56** in which all cytidine residues had been substituted by 5-methylcytidine (oligonucleotide **69**). Such a substitution significantly stabilised the triplex structure. The reaction carried out at 25 °C for 60 min resulted in cross-linking of the oligonucleotide **69** with the chain **66** enriched with purine nucleotides and with 53% – 68% efficiency. It was shown that the efficiency of this reaction strongly depended on the stability of the triplex structure. Thus a rise in temperature up to 37 °C sharply decreased the yield of cross-linked products. Replacement of the oligonucleotide probe **69** by **56** also decreased the yield down to several per cent.

As can be seen from the above data, modification of DNA with derivatives of *cis*-diamminedichloroplatinum (*cis*-**53**) is of particular interest. Yet, attempts were made to obtain protein–nucleic acid complexes in which DNA molecules were modified by *trans*-diamminedichloroplatinum (*trans*-**53**). The main problem remains to establish the reason for poor recognition of DNA modified with *trans*-**53** by DNA-binding proteins. As can be evidenced from a detailed study carried out by Brabec and Leng,[49] modification with *trans*-**53** decreases the thermal stability of modified DNA duplexes and significantly distorts the geometry of the double helix. Apparently, these conformational effects account for low yields of proteins cross-linked with DNA modified by *trans*-**53**.

Thus, affinity modification of biopolymers by platinum-containing reagents can be achieved both by direct treatment of biopolymers with Pt-containing nucleic acids and by treatment of preformed nucleic acid–nucleic acid or nucleic acid–protein complexes with Pt-containing reagents. In the latter case, nucleic acid derivatives containing SH or NH_2 groups are mainly used. The attention of investigators is mainly focussed on the elucidation of mechanisms of interaction of Pt-containing nucleic acid derivatives with biopolymers and of the effect of this interaction on the functional properties of the latter. We believe that further developments in this field will enlarge the scope of applications of Pt-containing nucleic acids and will open up new possibilities for their therapeutic applications in various systems *in vivo*.

IV. Reactive nucleic acids containing substituted pyrophosphate and acyl phosphate groups

A new type of oligonucleotide derivatives containing reactive groups in the sugar-phosphate backbone was developed and used for affinity modification of proteins at the Laboratory of Nucleic Acid Chemistry of the Moscow State University in the late 80's.[55–57] This type of compounds contains substituted internucleotide pyrophosphate (**70**) or acyl phosphate (**71**) groups.



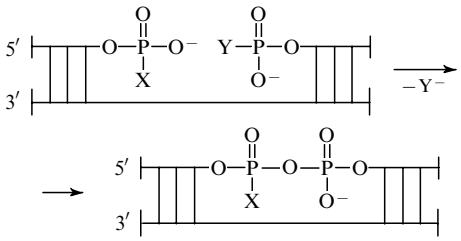
70 **71**

R,R′ are the oligonucleotide residues; X is the residue of an aliphatic alcohol or an amine.

The distinctive features of this class of compounds are high stability in aqueous solutions in the absence of strong nucleophiles, the ability to react with nucleophilic groups of a protein at a zero distance under conditions close to physiological ones, and the fact that they can be used without additional activation. Thus, these compounds are promising tools for the study of structure and functions of nucleic acid-recognising proteins and for their affinity modification. In addition, they can find application in sense biotechnology and medicine as therapeutic preparations, particularly in the treatment of genetic and oncological diseases and AIDS.

1. Directed introduction of substituted pyrophosphate and acyl phosphate groups into oligonucleotides

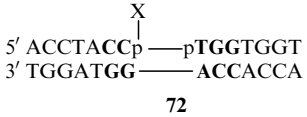
Synthesis of nucleic acid analogues containing substituted reactive pyrophosphate groups at predetermined positions of the sugar-phosphate backbone is based on chemical ligation, which consists in condensation of two oligonucleotides the ends of which are converged on a complementary template. One of these oligonucleotides bears an alkoxy or an alkylamino group in the 3′(5′)-terminal phosphate, while the 5′(3′)-end of the other oligonucleotide is phosphorylated.[55,58] The reaction proceeds according to the following scheme:



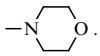
X is the alkoxy or alkylamino group;
Y is the residue of the coupling reagent.

Oligonucleotide alkyl esters used in this reaction are obtained by postsynthetic modification of 3′-phosphorylated oligonucleotide with alcohols mediated by coupling reagents[59] or in the course of automated oligonucleotide synthesis.[60] Oligonucleotide amides are usually obtained by postsynthetic modification of 3′-phosphorylated oligonucleotides by amines in the presence of coupling reagents.[59]

Chemical ligation was used to obtain oligodeoxyribonucleotides containing *O*-methyl- or *N*-alkyl-substituted pyrophosphate groups.[55] The experiments were carried out with system **72** having a recognition site for the restriction-modification enzymes *Eco*RII, *Sso*II, and *Mva*I (boldfaced).

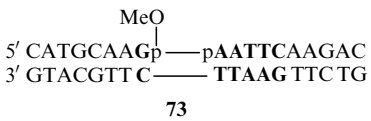


72

X = OEt, $NH(CH_2)_3Me$, $NHCH_2COOEt$, .

Activation of the phosphate group for the synthesis of compounds with a substituted pyrophosphate group was carried out with CDI or *N*-hydroxybenzotriazole esters. The reaction proceeds with the greatest efficiency when CDI is used as the coupling reagent. The yields of chemical ligation products are 35%–80% depending on the nature of the non-nucleotide substituent. With triazole esters, the yields decreased down to 10%–15%.

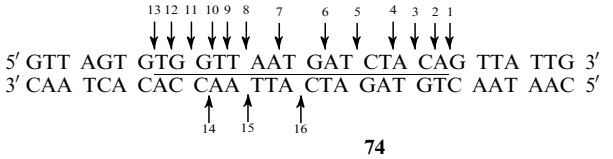
Reactive DNA duplexes **73** containing an *O*-methyl-substituted pyrophosphate group in the recognition site of the restriction-modification enzymes *Eco*RI and *Rsr*I[56] (boldfaced) were obtained in a similar way.



73

In this case, a methoxy group was used as a non-nucleotide substituent, because it induces minimum distortions in the geometry of the double helix. This is important for the subsequent investigation of the interaction of modified DNA duplexes with proteins. The modified oligonucleotide containing an *O*-methyl-substituted pyrophosphate group was obtained in 25%–30% yield.

A series of DNA duplexes **74** containing an *O*-methyl-substituted pyrophosphate group in various positions of the HNF-1 recognition site have been synthesised to probe DNA contacts with the transcription factor HNF-1.[61] (The recognition site HNF-1 is marked with a straight line; the positions of modified groups are indicated by arrows).

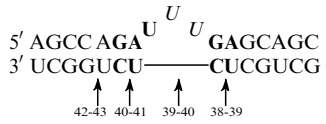


74

The reactive group was introduced into the upper and the lower chains of the duplexes by chemical ligation. Each modified duplex had only one substituted pyrophosphate group. The average yield of chemical ligation products was 15%.

Chemical ligation was also used to introduce a substituted pyrophosphate group into DNA duplexes of different lengths and compositions.[58, 62]

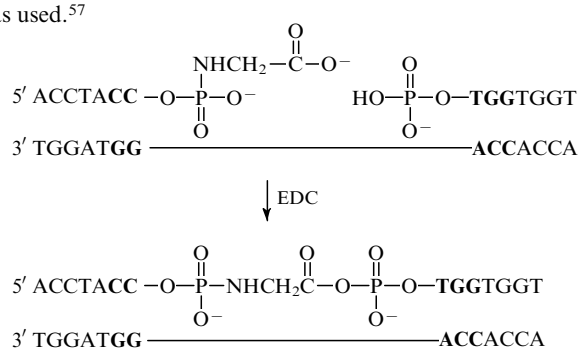
Introduction of substituted pyrophosphate groups into RNA was first described by Naryshkin *et al.* who studied the interaction of TAR RNA with the Tat-protein of HIV-1.[63] These authors synthesised a series of RNA duplexes **75** containing a reactive group in various positions of the recognition site of the boldfaced Tat-protein (the position of the reactive group is marked by arrows).



75

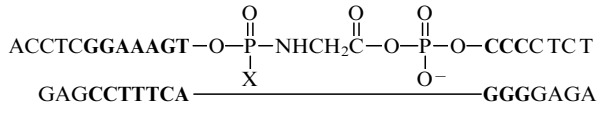
Since the efficiency of chemical ligation of oligoribonucleotides is extremely low (1% – 2%), oligoribonucleotides containing 2′-deoxyuridine *O*-alkylphosphate at their 3′-ends were used to introduce a substituted pyrophosphate group into RNA duplexes. The ligation was performed on an oligodeoxyribonucleotide template. Then the modified chain was isolated, and an equimolar amount of a complementary oligoribonucleotide was added to obtain RNA duplexes. Chemical ligation products were obtained in 35% – 55% yields.

Analogously, an internucleotide acyl phosphate group was introduced into the duplex structure by chemical ligation. In this case a modified oligonucleotide with a glycine residue at the 3′-end was used.[57]



The modified oligonucleotide containing a glycine residue was obtained by direct condensation of the heptanucleotide ACCTACCp with glycine ethyl ester in the presence of CDI followed by saponification of the ester bond. Condensation of oligonucleotides within the DNA duplex was also carried out in the presence of CDI. The yield of the chemical ligation product was 24%.

This method was also used to obtain the DNA duplex **76** containing a reactive acyl phosphate group in the recognition site of NF-kB (the kB site is boldfaced).[64]

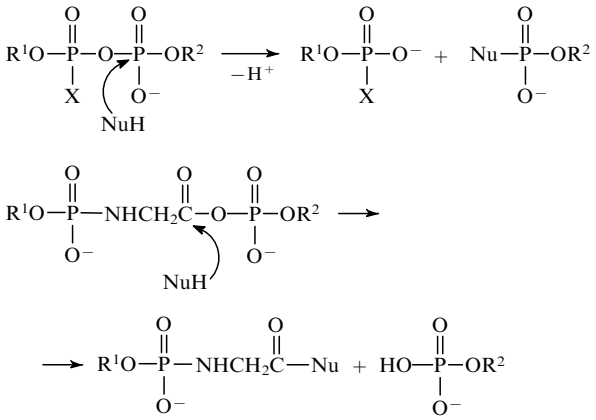


76

The starting oligonucleotide modified at the 3′-terminal phosphate group with a glycine residue was obtained using *N*-hydroxybenzotriazole esters.[59]

2. Affinity modification of nucleic acid-specific proteins by nucleic acid analogues containing substituted internucleotide pyrophosphate or acyl phosphate groups

Investigation of the properties of reactive nucleic acids containing substituted pyrophosphate or acyl phosphate groups revealed that the modified group of these compounds is stable in aqueous buffers in the absence of strong nucleophiles at pH 6.00 – 8.75. At the same time, it is easily and quantitatively cleaved by primary and secondary amines, including nucleophilic amino acids of a protein. The cleavage of the modified group is accompanied by a transfer of the nucleic acid fragment onto the nucleophile.[55, 57]



X is an alkoxy or alkylamino group; R^1,R^2 are the oligonucleotide residues.

By virtue of their ability to form covalent bonds with nucleophilic amino acid residues of nucleic acid-recognising proteins, these compounds are successfully used for the study of enzymes of nucleic acid metabolism as well as for affinity modification and probing of binding sites of transcription factors. Thus the reactive DNA duplex **73** was used for affinity modification of the restriction-modification enzymes R.*Eco*RI, R.*Rsr*I, M.*Eco*RI, and M.*Rsr*I.[56] The protein complex with a modified substrate was incubated for 20 – 24 h at 25 – 37 °C. The maximum yields of cross-linked complexes (40% and 30%, respectively) were observed for methyl transferases M.*Eco*RI and M.*Rsr*I. It was found that it is the phosphoramide covalent bond that is formed between the oligonucleotide residue and the protein.

Affinity modification of the restriction endonuclease *Eco*RI (R.*Eco*RI) by the reactive DNA duplex **72** has been carried out by Shabarova *et al.*[65] The duplex **72** containing an *O*-ethyl-substituted pyrophosphate group in the recognition site of R.*Eco*RI was incubated with the enzyme for 18 h at 37 °C. The reaction was carried out in an aqueous buffer containing *N*-methylimidazole because this reagent catalyses the reactions of nucleophilic substitution in aqueous media.[66] The efficiency of cross-linking of the modified duplex **72** with R.*Eco*RI in the presence of *N*-methylimidazole was 15%. At the same time, the yield of the cross-linking product in a buffer devoid of *N*-methylimidazole did not exceed 1%. This confirms the catalytic role of *N*-methylimidazole in nucleophilic substitution reactions of oligonucleotides in aqueous media.[56]

The active centre of the transcription factor HNF-1 was probed[61] using a series of DNA duplexes containing an *O*-methyl-substituted pyrophosphate group in various positions (1 – 16) of the HNF-1 recognition site. These studies were carried out both with a gene-engineered protein, which represented a DNA-binding domain of HNF-1, and with proteins isolated from nuclear extracts of rat liver. Modified substrates were incubated with the protein or a nuclear extract at 4 °C or 20 °C for 10 min or 12 h. This reaction yielded cross-linked complexes both for the DNA-binding domain of HNF-1 and the HNF-1 of the nuclear extract. Cross-linking occurred only if a DNA duplex contained a reactive group in position 10. The efficiency of cross-linking was

40% of the non-covalent binding. It has been shown for the first time that cross-linking of a modified substrate with HNF-1 isolated from a nuclear extract occurred in a specific manner and was not accompanied by modification of other components of the nuclear extract. This finding holds much promise for the application of oligonucleotides with substituted pyrophosphate groups in various *in vivo* systems and for creation on their basis of new generations of therapeutic agents.

Compounds with a substituted pyrophosphate group were also used for establishing the contact site for the transcription factor NF-kB and DNA.[58]

RNA duplexes containing substituted pyrophosphate groups can also be used as reactive derivatives for affinity modification of biopolymers. The interaction of RNA duplexes TAR **75** with the Tat-protein and the Tat-peptide (37–72) of HIV-1 has been studied.[63] The RNA–protein complex was incubated for 18–24 h at 10 °C. Cross-linking with the Tat-peptide (37–72) was especially efficient when an RNA duplex containing a modified group in position 38–39 was used. The product yield was 50%. Analysis of the complex revealed that Lys51 of the main region of the Tat-peptide is involved in the formation of a covalent bond.

A first report concerning the successful application of a DNA duplex containing an acyl phosphate group for affinity modification of the p50 subunit of NF-kB has been published recently.[64] The DNA duplex **76** containing a reactive acyl phosphate group in position 6 of the kB region was used for cross-linking with the protein. The efficiency of cross-linking was 10%. Acyl phosphates are acylating reagents.[57] The group formed as a result of their interaction with nucleophilic amino acids of a protein is carboxamide, which unlike the phosphoramidate is relatively stable in acidic media. The application of nucleic acid analogues with an acyl phosphate group for affinity modification of DNA-recognising proteins may significantly facilitate both the isolation of protein–nucleic acid complexes and subsequent identification of amino acids that react with the reactive group.

Thus, reactive nucleic acids containing substituted pyrophosphate or acyl phosphate groups are successfully employed for the study of nucleic acid–protein interactions. It should be noted, however, that introduction of these groups into the sugar-phosphate backbone of nucleic acids increases the length of the internucleotide bond, which, in turn, may decrease the efficiency of its binding with the protein.[61] This problem can be overcome through the use of nucleic acid duplexes containing reactive groups at other positions of the protein binding site.[61]

V. Prospects for the application of reactive oligonucleotides for directed *in vivo* modification of biopolymers

One of the most interesting applications of reactive oligonucleotides is the design on their basis of antiviral and antitumour drugs the effects of which are due to inhibition of gene expression in viral or oncogenic cells. The ability of reactive oligonucleotides to covalently bind to proteins of nucleic acid metabolism may suppress their activity and thus control the expression of the genetic material. It was found [67] that human $tRNA_3^{Lys}$ containing 4-mercaptouridine residues efficiently interacts with HIV reverse transcriptase to form a cross-linked complex. This interaction results in the block of a region in reverse transcriptase that is responsible for the initiation of reverse transcription of HIV.

Of particular interest is the application of reactive oligonucleotide derivatives in various *in vivo* systems. The first studies in this area revealed a number of limitations related to weak penetrability of these reagents through the cellular membrane and their enhanced sensitivity with respect to cellular exonucleases. Besides, nonspecific modification of functional components of various cell structures by these reagents also presents some problem.[68] The permeability problem can be circumvented through the use of hydrophobic oligonucleotide derivatives (see Ref. 69). Several approaches are now used to stabilise DNA fragments in the cell, such as construction of covalently closed dumbbell-like DNA duplexes,[70] cyclic DNAs,[71] and DNA duplexes with covalently linked chains.[72]

A serious limitation for the *in vivo* application of a vast number of reactive oligonucleotides is that an additional treatment (UV irradiation, heating, chemical reagents) are required for their activation. Therefore, the most promising reagents for *in vivo* application are apparently platinum derivatives and reactive nucleic acids containing substituted pyrophosphate or acyl phosphate groups. These compounds do not require additional activation; the mechanisms of their interaction with nucleic acid-recognising proteins have been studied in sufficiently great detail. Besides, they have already been used in nuclear cell extracts.[54, 61] It should be noted, however, that modification of proteins with platinum derivatives of oligonucleotides in nuclear extracts is not always specific.[54] Besides, this procedure is inefficient unless mercapto derivatives of nucleic acids are used. At the same time, it has been shown [61] that modification of the transcription factor HNF-1 in nuclear extracts of rat liver is highly specific and efficient. This circumstance gives every reason to believe that such compounds are promising for *in vivo* applications. In order to increase their resistance to cell exonucleases, it may be necessary to change their structure, *e.g.*, to design on their basis covalently closed DNA duplexes, or to synthesise DNA duplexes with covalently linked chains. The methods of their synthesis have already been developed [61, 73–75] and the first encouraging results of the application of structures with substituted pyrophopsphate groups for affinity modification of the proteins of nucleic acid metabolism have been obtained.[76]

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References

1. D G Knorre, V F Zarytova, A G Badashkeeva, O S Fedorova, in *Biotekhnologiya* (*Itogi Nauki i Tekhniki*) [Biotechnology (Advances in Science and Engineering Series) (Moscow: Izd. VINITI, 1991) Vol. 37, p. 1
2. D G Knorre, V V Vlasov, V F Zarytova, A V Lebedev, O S Fedorova, in *Design and Targeted Reactions of Oligonucleotide Derivatives* (Boca Raton, FL: CRC Press, 1994) p. 366
3. L A Sylvers, J Wower *Bioconjug. Chem.* **4** 411 (1993)
4. D R Tabatadze, M I Dobrikov, A S Levina, T A Prikhodko, G V Shishkin, V F Zarytova *Nucl. Acids Symp. Ser.* **24** 247 (1991)
5. M I Dobrikov, V F Zarytova, N I Komarova, A S Levina, S G Lokhov, T A Prikhod'ko, G V Shishkin, D R Tabatadze, M M Zaalishvili *Bioorg. Khim.* **18** 540 (1992) [a]
6. T Le Doan, L Perrouault, D Praseuth, N Habhoub, J-L Decout, N T Thuong, J Lhomme, C Helene *Nucl. Acids Res.* **15** 7749 (1987)
7. P Mitchell, K Stade, M Osswald, R Brimacombe *Nucl. Acids Res.* **21** 887 (1993)
8. M G Ivanovskaya, N I Sokolova, Z A Shabarova *Bioorg. Khim.* **5** 35 (1979) [a]
9. V F Zarytova, A S Levina, L M Khalimskaya, Z A Sergeeva *Bioorg. Khim.* **18** 640 (1992) [a]
10. B Bartholomew, G A Kassavetis, B R Braun, E P Geiduschek *Embo J.* **9** 2197 (1990)
11. B J Lannutti, J Persinger, B Bartholomew *Biochemistry* **35** 9821 (1996)
12. O I Gimautdinova, M A Zenkova, G G Karpova, L M Podust *Mol. Biol.* **18** 907 (1984) [b]
13. N D Kobets, A V Gorozhankin, T S Godovikova, V N Sil'nikov, D G Knorre *Dokl. Akad. Nauk* **349** 822 (1996) [c]
14. T S Godovikova, M V Berezovskii, D G Knorre *Bioorg. Khim.* **21** 858 (1995) [a]

15. D Praseuth, M Chassignol, M Takasugi, T Le Doan, N T Thuong, C Helene *J. Mol. Biol.* **196** 939 (1987)
16. A S Boutorin, T Le Doan, J P Battioni, D Mansuy, D Dupre, C Helene *Bioconjug. Chem.* **1** 350 (1990)
17. A N Mayer, F Barany *Gene* **153** 1 (1995)
18. M M Hanna, Y Zhang, J C Reidling, M J Thomas, J Jou *Nucl. Acids Res.* **21** 2073 (1993)
19. A Reizer, H M Wagner, in *The Chemistry of the Azido Group* (Ed. S Patai) (New York: Interscience, 1971) p. 441
20. T-Y Liang, G B Schuster *J. Am. Chem. Soc.* **109** 7803 (1987)
21. A S Levina, D R Tabatadze, V F Zarytova, M I Dobrikov, V N Gorn, L M Khalimskaya *Bioorg. Khim.* **20** 21 (1994) [a]
22. Y L Dubreuil, A Expert-Bezancon, A Favre *Nucl. Acids Res.* **19** 3653 (1991)
23. J Liu, M Sodeoka, W S Lane, G L Verdine *Proc. Natl. Acad. Sci. USA* **91** 908 (1994)
24. R D Sheardy, N C Seeman *J. Org. Chem.* **51** 4301 (1986)
25. M C Willis, K A LeCuyer, K M Meisenheimer, O C Uhlenbeck, T H Koch *Nucl. Acids Res.* **22** 4947 (1994)
26. K M Meisenheimer, P L Meisenheimer, M C Willis, T H Koch *Nucl. Acids Res.* **24** 981 (1996)
27. A McGregor, M V Rao, G Duckworth, P G Stockley, B A Connolly *Nucl. Acids Res.* **24** 3173 (1996)
28. T M Dietz, R J von Trebra, B J Swanson, T H Koch *J. Am. Chem. Soc.* **109** 1793 (1987)
29. T M Dietz, T H Koch *Photochem. Photobiol.* **46** 971 (1987)
30. S Cereghini, M Blumenfeld, M Yaniv *Genes Develop.* **2** 957 (1988)
31. M C Willis, B J Hicke, O C Uhlenbeck, T R Cech, T H Koch *Science* **262** 1255 (1993)
32. O Dontsova, A Kopylov, R Brimacombe *Embo J.* **10** 2613 (1991)
33. D M Graifer, D I Juzumiene, P Wollenzien, G G Karpova *Biochemistry* **33** 3878 (1994)
34. O Dontsova, V Tshkov, S Dokudovskaya, A Bogdanov, T Doring, J Rinke-Appel, S Thamm, B Greuer, R Brimacombe *Proc. Natl. Acad. Sci. USA* **91** 4125 (1994)
35. Y B Shi, J E Hearst *Biochemistry* **26** 3792 (1987)
36. Y B Shi, V Gamper, J E Hearst *Nucl. Acids Res.* **15** 6843 (1987)
37. D Kanne, K Straub, J E Hearst, H Rapoport *J. Am. Chem. Soc.* **104** 6754 (1982)
38. C Giovannangeli, N T Thuong, C Helene *Nucl. Acids Res.* **20** 4275 (1992)
39. B L Lee, A Murakami, K R Blake, S B Lin, P S Miller *Biochemistry* **27** 3197 (1988)
40. U Pieles, U Englisch *Nucl. Acids Res.* **17** 285 (1989)
41. M Raha, G Wang, M M Seidman, P M Glazer *Proc. Natl. Acad. Sci. USA* **93** 2941 (1996)
42. Y B Shi, J E Hearst *Biochemistry* **26** 3786 (1987)
43. A L Pinto, S J Lippard *Biochim. Biophys. Acta.* **780** 167 (1985)
44. J Kasparkova, V Brabec *Biochemistry* **34** 12379 (1995)
45. B A Donahue, M Augot, S F Bellon, D K Treiber, J H Toney, S J Lippard, J M Essigmann *Biochemistry* **29** 5872 (1990)
46. B C Chu, L E Orgel *Nucl. Acids Res.* **17** 4783 (1989)
47. E S Gruff, L E Orgel *Nucl. Acids Res.* **19** 6849 (1991)
48. V V Vlassov, V V Gorn, E M Ivanova, S A Kazakov, S V Mamaev *FEBS Lett.* **162** 286 (1983)
49. V Brabec, M Leng *Proc. Natl. Acad. Sci. USA* **90** 5345 (1993)
50. M Boudvillain, R Dalbies, C Aussourd, M Leng *Nucl. Acids Res.* **23** 2381 (1995)
51. G A Nevinskii, V N Podust, A S Levina, O V Khalabuda, O I Lavrik *Bioorg. Khim.* **12** 357 (1986) [a]
52. F Oshita, A Eastman *Oncol. Res.* **5** 111 (1993)
53. V Brabec, V Kleinwachter, J L Butour, N P Johnson *Biophys. Chem.* **35** 129 (1990)
54. B C Chu, L E Orgel *Nucl. Acids Res.* **20** 2497 (1992)
55. S A Kuznetsova, M G Ivanovskaya, Z A Shabarova *Bioorg. Khim.* **16** 219 (1990) [a]
56. A A Purmal, Z A Shabarova, R I Gumport *Nucl. Acids Res.* **20** 3713 (1992)
57. S A Kuznetsova, M G Ivanovskaya, Z A Shabarova *Bioorg. Khim.* **17** 1633 (1991) [a]
58. I A Kozlov, E A Kubareva, M G Ivanovskaya, Z A Shabarova *Mol. Biol.* **31** 63 (1997) [b]
59. M G Ivanovskaya, M B Gottikh, Z A Shabarova *Nucleosides Nucleotides* **6** 913 (1987)
60. N A Naryshkin, M G Ivanovskaya, T S Oretskaya, E M Volkov, M Dzh Geit, Z A Shabarova *Bioorg. Khim.* **22** 691 (1996) [a]
61. S A Kuznetsova, C Catherine, U Edgardo, I Elias, M Vasseur, M Blumenfeld, Z A Shabarova *Nucl. Acids Res.* **24** 4783 (1996)
62. E A Kubareva, O A Fedorova, M B Gottikh, H Tanaka, C Malvy, Z A Shabarova *FEBS Lett.* **381** 35 (1996)
63. N A Naryshkin, M A Farrow, M G Ivanovskaya, T S Oretskaya, Z A Shabarova, M J Gait *Biochemistry* **36** 3496 (1997)
64. M G Ivanovskaya, I A Kozlov, E A Kubareva, E A Taran, T V Sarazev, Z A Shabarova *Mol. Biol.* **31** 435 (1997) [b]
65. Z A Shabarova, G Ya Sheflyan, S A Kuznetsova, E A Kubareva, O H Sysoev, M G Ivanovskaya, E S Gromova *Bioorg. Khim.* **20** 413 (1994) [a]
66. Z A Shabarova, M G Ivanovskaya, M G Isaguliants *FEBS Lett.* **154** 288 (1983)
67. Y Mishima, J A Steitz *Embo J.* **14** 2679 (1995)
68. D E Wilman, T A Connors in *Molecular Aspects of Anti-Cancer Drug Action* (Weinheim: Verlag Chemie, 1983)
69. A S Boutorin, L V Gus'kova, E M Ivanova, N D Kobetz, V F Zarytova, A S Ryte, L V Yurchenko, V V Vlassov *FEBS Lett.* **254** 129 (1989)
70. B C F Chu, L E Orgel *Nucl. Acids Res.* **20** 5857 (1992)
71. N G Dolinnaya, M Blumenfeld, I N Merenkova, T S Oretskaya, N F Krynetskaya, M G Ivanovskaya, M Vasseur, Z A Shabarova *Nucl. Acids Res.* **21** 5403 (1993)
72. S I Antsypovich, T S Oretskaya *Usp. Khim.* **67** 274 (1998) [*Russ. Chem. Rev.* **67** 247 (1998)
73. S A Kuznetsova, I E Kanevskii, T S Oretskaya, Z A Shabarova *Bioorg. Khim.* **22** 532 (1996) [a]
74. I E Kanevskii, S A Kuznetsova, E M Volkov, Z A Shabarova *Mol. Biol.* **30** 1144 (1996) [b]
75. S A Kuznetsova, I E Kanevskii, T S Oretskaya, Z A Shabarova *Nucleosides Nucleotides* **22** 1231 (1996)
76. I E Kanevskii, Candidate Thesis in Chemical Sciences, Moscow State University, Moscow, 1996

[a] — *Russ. J. Bioorg. Chem. (Engl. Transl.)*
[b] — *Mol. Biol. (Engl. Transl.)*
[c] — *Dokl. Chem. Technol., Dokl. Chem. (Engl. Transl.)*