Russian Chemical Reviews **68** (11) 967 – 982 (1999) © 1999 Russian Academy of Sciences and Turpion Ltd

UDC 547.963.3 : 577.113.6

Site-specific photosensitised modification of nucleic acids with biradical and electrophilic reagents

M I Dobrikov

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Abstract. Photosensitised oxygen-independent chemical modification of nucleic acids including free-radical cycloaddition and electrophilic addition (type III photodynamic effect) are considered. The main attention is given to site-specific photomodification. New approaches to highly specific affinity modification of nucleic acids, *viz*., sensitised photomodification with binary photoreagent – sensitiser systems and two-photon photosensitised reactions, are described. The prospects for application of electrophilic photoreagents for *in vivo* modification of nucleic acids are discussed. The bibliography includes 119 references.

I. Introduction

The development of methods for targeted chemical modification of nucleic acids (NA) at definite nucleotide sequences is of great importance for the study of cellular processes connected with gene expression,[1,2] design of gene-addressed biologically active substances [3] and the development of new technologies based on the use of oligonucleotide chips and microelements for new generations of computers.[4]

The targeted modification of NA is carried out with affinity reagents which can bind specifically to definite nucleotide sequences and contain reactive groups bringing about NA modification upon binding. Fragments of antibiotics,[5,6] oligonucleotides [3,7,8] and their derivatives [2,9] are used for the recognition of definite nucleotide sequences. Compounds generating free radicals,[3,10] alkylating reagents [3,8] and photoreactive compounds [2,3,7,11] are used as the affinity reagents.

Chemically reactive oligonucleotide derivatives can react with different cell components, which results in undesirable toxicity and considerable expenditure of reagents in side reactions. Photoreactive derivatives possess considerable advantages, *viz*., they are nontoxic in the dark and their photoactivation is easily controlled. Considerable experience has been gained in the last few years in the area of synthesis, studies of physicochemical properties, biological application of novel reagents containing photoreactive groups and the determination of the structure of NA photomodification products under the action of these reagents.

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Received 13 April 1999
Uspekhi Khimii **68** (11) 1062 – 1079 (1999); translated by R L Birnova

The design of DNA-cleaving reagents which are chemically stable and can be photoactivated is a topical problem. These reagents are helpful tools in the study of DNA structure when used as photonucleases, photofootprinting reagents and potential photoreagents in the phototherapy of cancer and various viral infections.[11 – 13]

Photosensitised NA modification reactions are commonly referred to as photoinduced reactions occurring at wavelengths greater than the intrinsic absorption of NA.[14] These reactions involve three main types,[15,16] *viz*., photooxidation with radical species or by photoinduced electron transfer (type I photodynamic effect), photooxidation with singlet oxygen (type II photodynamic effect) and oxygen-independent free-radical and electrophilic addition (type III photodynamic effect).

The classification of photosensitised reactions of NA is problematic and any description of them is ambiguous, for photochemical reactions are numerous, they occur in parallel and in low quantum yields. Their study is complicated by the fact that the same photomodification end products are formed by different mechanisms.[7]

This review is devoted to the analysis of the most important photosensitised, oxygen-independent free-radical and electrophilic addition reactions. One-photon [17,18] and two-photon [19,20] photochemical reactions of NA induced by far UV light (190 – 300 nm), photooxidation of DNA with singlet oxygen [3,7,21] and radical species [22 – 24] as well as oxidation caused by photoinduced electron transfer [11,25,26] have been studied in sufficiently great detail and will not be considered in this review.

II. Free-radical cycloaddition and electrophilic addition photoreactions of nucleic acids

Several types of oxygen-independent photosensitised chemical modifications of NA are known,[14,16] *e.g.,* free-radical photocycloaddition to the double bonds of bases,[27 – 45] photoinduced alkylation,[46 – 58] arylation,[59 – 63] acylation [27,64] and other electrophilic addition reactions. Modification of NA with highly electrophilic species formed during photochemical reactions is especially attractive, since the electrophilic species manifest pronounced selectivity with respect to nucleophilic bases, such as guanine and adenine. In turn, photocycloaddition reactions occur with high selectivity at the double bonds of the pyrimidine bases.[27]

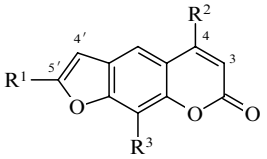
1. Free-radical photocycloaddition

Excitation of most ketones and thioketones gives rise to biradical species which initiate radical cycloaddition reactions involving the double bonds of the heterocyclic bases of NA. Psoralen and

thiopyrimidine derivatives are the most popular and efficient photoreagents of this type.

a. Photomodification of nucleic acids with psoralen derivatives

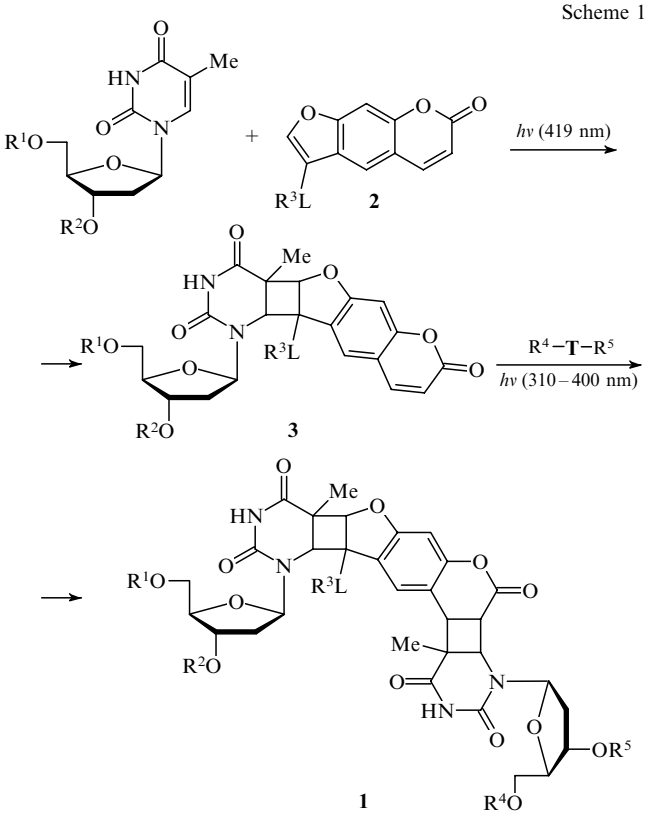
Psoralens (7*H*-furo[3,2-*g*][1]benzopyran-7-ones) are tricyclic aromatic compounds, which have a planar structure and can intercalate between base pairs of double-stranded DNA (ds-DNA). They contain two photoreactive double bonds, *viz*., C(3)=C(4) in the pyrone ring and C(4′)=C(5′) in the furan ring.



R^1, R^2, R^3 = H, Alk.

Irradiation of DNA duplexes at 310–420 nm in the presence of psoralens results in covalent binding of the latter to pyrimidine bases of DNA (preferentially, to thymine) at the C(5)=C(6) bond to give cyclobutane adducts (Scheme 1). Photoaddition reaction of psoralens to cytidine occurs 15-fold more slowly than that to thymidine.[1] Psoralens are bifunctional photoreagents; they can cross-link both DNA strands, especially when intercalating in the 5′-TpA-3′ sequence.[1,28–38] 5′-TpA/3′-ApT fragments are present in signal sequences of many genes as potential targets for psoralen-containing oligonucleotides.

Scheme 1



L is $CH_2NH(CH_2)_2NH$-p, R^1-R^5 are fragments of oligonucleotide chains.

Using molecular modelling methods, it was shown[1] that psoralen incorporates between the nucleotides of a target in two different ways, *viz*., between nucleosides N_{n+1} and N_n or between nucleosides N_n and N_{n-1}, where N_n is the nucleoside complementary to the 5′-terminal nucleoside of the psoralen conjugate. Spatial structure of the reaction centre which is the most favourable for photoaddition, is realised in the former case. The incorporation of psoralen is especially efficient where thymidine residues occupy positions $n+1$ and $n-1$, and adenosine residues occupy position n of the target. In this case, the reaction occurs simultaneously in both directions. The yield of the cross-linked product of the type **1** amounts to 95%.

In recent years, psoralen-containing oligonucleotide derivatives have been used mostly for photomodification of ds-DNA.

Studies of Gunther *et al*.[29] have shown that the 16-membered conjugate Pso-$CH_2NH(CH_2)_2$NH-p-5′-TTTTCTTTTCCCCC-CT **2** forms a triplex with DNA regions of HIV-1 containing homopurine–homopyrimidine sequences. Psoralen intercalates into ds-DNA thus increasing the melting temperature of the triplex from 20 to 29 °C. Irradiation with UV light at wavelengths > 310 nm yields mono- and bis-adducts **3** and **1**, which is confirmed by their electrophoretic mobility. The addition of the furan ring of psoralen to the C(5)=C(6) bond of thymine next to the 5′-end of the homopurine sequence of the DNA-target gives the cyclobutane adduct **3**. This is followed by photochemical cycloaddition of the pyrone ring of the adduct **3** to the C(5)=C(6) bond of 3′-terminal thymine in the homopyrimidine chain, resulting in the bisadduct **1**.

The DNA region of HIV-1 contains two sequences which can serve as targets for the conjugate **2**. Both sequences are involved in cross-linkage with high efficiency. Besides, at higher exposures and higher concentrations of the oligonucleotide reagent **2**, cross-linking occurs in the regions where the 5′-terminal 8-membered fragment (T_4CT_3) of the conjugate **2** binds to ds-DNA. The reason for such modification is that the sequence $(pC)_6$ of the conjugate **2** cannot be fully protonated under physiological conditions, and the overall specificity of the 16-membered conjugate **2** is determined by the fragment (T_4CT_4). Substitution of $(pG)_6$ for $(pC)_6$ results in photomodification at only two specific sequences.[29]

The dependence of the length of the oligonucleotide address on the degree of modification of target ds-DNA *in vitro* was studied.[29] It was shown that 10- and 16-membered psoralen-containing oligonucleotide conjugates were effective in sequence-specific photocross-linking of mouse ds-DNA, while an 8-membered oligonucleotide reagent had little effect. Binding of the 10-membered conjugate to ds-DNA occurred rapidly (in less than 10 min).

A conjugate of 5-alkoxypsoralen and 5′-pTTTTC*C*TC*T-C*C*C*TC*T, where C* is 5-methylcytosine residue introduced for increasing the triplex stability, was used to inhibit the expression of the interleukin 2 α-chain receptor gene.[30] The conjugate bound to the promoter homopurine–homopyrimidine sequence to yield a triplex. The mutant form of the promoter containing a transversion of three nucleotides in the centre of the homopurine–homopyrimidine sequence was used as a control. The conjugate did not bind to the mutant promoter.

Irradiation with UV light (365 nm) was accompanied by photocycloaddition of the psoralen 3,4- and 4′,5′-double bonds to the C(5)=C(6) bonds of the sequence 5′-TpA-3′ at the duplex–triplex junction, resulting in the bisadduct **1**. The formation of a covalently bound triplex inhibited transcription *in vitro*, while transcription of the mutant form of the promoter was not inhibited. After *in vitro* photomodification of DNA, the photocross-linked triplex was transfected into human HeLa cells. The irradiated mutant promoter was transcribed with the same efficiency as the nonirradiated one; the activity of the irradiated promoter was inhibited by 80% (according to the photocross-linkage yield).

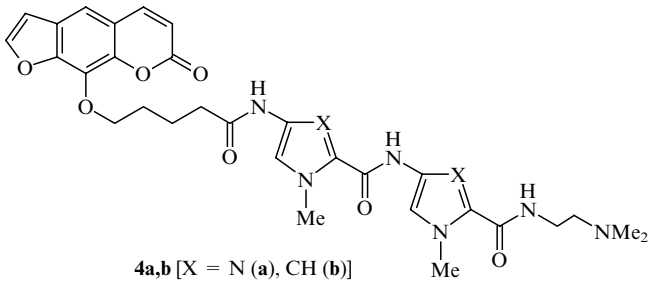
The formation of photoadducts in a triplex of a bacteriophage λ ds-DNA with a conjugate 4′-hydroxymethyl-4,5′,8′-trimethyl-Pso-pAGGAAGGGGG was most extensively studied by Gasparro *et al*.[31] The quantum yield (φ) of the monoadduct **3** was 0.0065, while that of the bisadduct **1** was 5 times as great. The formation of the bisadduct **1** implies that the C(4′)=C(5′) bond of the furan ring of psoralen reacted first and then the C(3)=C(4) bond of the pyrone ring. Otherwise, the bisadduct is not formed. It was therefore proposed to carry out the irradiation in a stepwise manner, *viz*., first, with visible light (419–447 nm) and then with UV light (> 310 nm). The efficiency of photomodification upon irradiation with visible light was 400 times lower than with UV

light, but in this case the monoadduct **3** is formed at the T166 residue (yield 40%). Subsequent UV irradiation gives the bisadduct **1** (yield 62%) and monoadducts (yields at pyrimidine and purine residues were 9% and 22%, respectively). In this two-step irradiation, the total degree of photomodification of ds-DNA reached 95%.

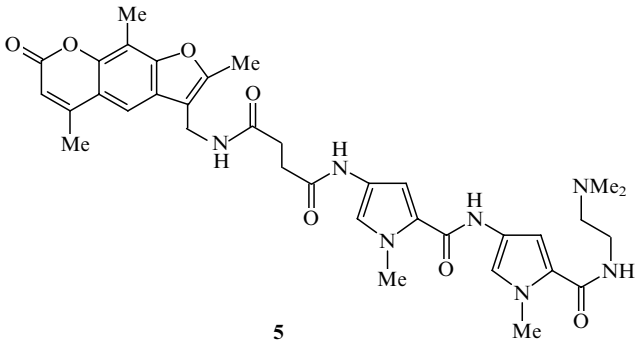
Irradiation with visible light (419 nm, 30 min) of ds-DNA within a complex with a triplex-forming psoralen conjugate **2** yielded the monoadduct **3** in 87% yield. Further irradiation with UV light for 5 min resulted in the bisadduct **1** (in up to 92% yield).[31] A conjugate 4′-hydroxymethyl-Pso-4,5′,8-trimethyl-pAGGAAGGGGG was used for site-specific injury of the supF-gene incorporated into vectors of *E.coli* phage λ[32] and virus SV40.[33] It was shown that the formation and photocross-linking of the triplex can also occur *in vivo*.[34]

Inhibition of gene expression with a conjugate 5-hydroxy-Pso-pTC*TTC*TTC*C*TC*T-C*TT, where C* is 5-methylcytosine, in transfected HeLa cells is reported.[38] After UV irradiation (> 310 nm) of the triplex *in vitro,* the amplification of a DNA fragment was inhibited by 95%. Plasmid pCH110 was photomodified and incorporated into HeLa cells. The activity of the gene of the incorporated β-galactosidase 5 h after transfection was 5%.

Penetration of psoralen derivatives through hydrophobic cell membranes and their resistance to nuclease hydrolysis could be enhanced if psoralen was coupled to distamycin-like imidazole (**4a**) and pyrrole (**4b** and **5**) derivatives.[35–37]



4a,b [X = N (**a**), CH (**b**)]



5

The imidazole analogues of distamycin **4a** can deliver psoralen to GC-rich DNA regions, while the pyrrole analogues **4b** and **5**, to AT-rich DNA regions. The imidazole analogue **4a** photomodifies DNA 10 times more efficiently than the original psoralen. The efficiency of photomodification of ds-DNA with the pyrrole analogue **4b** is 1000 times greater than that with psoralen and 100 times greater than that with the imidazole analogue.[1]

These data suggest that psoralen conjugates are promising reagents for photomodification of NA *in vivo*. High phototoxicity of psoralens as regards modification of ds-DNA is determined by the formation of bisadducts **1**.

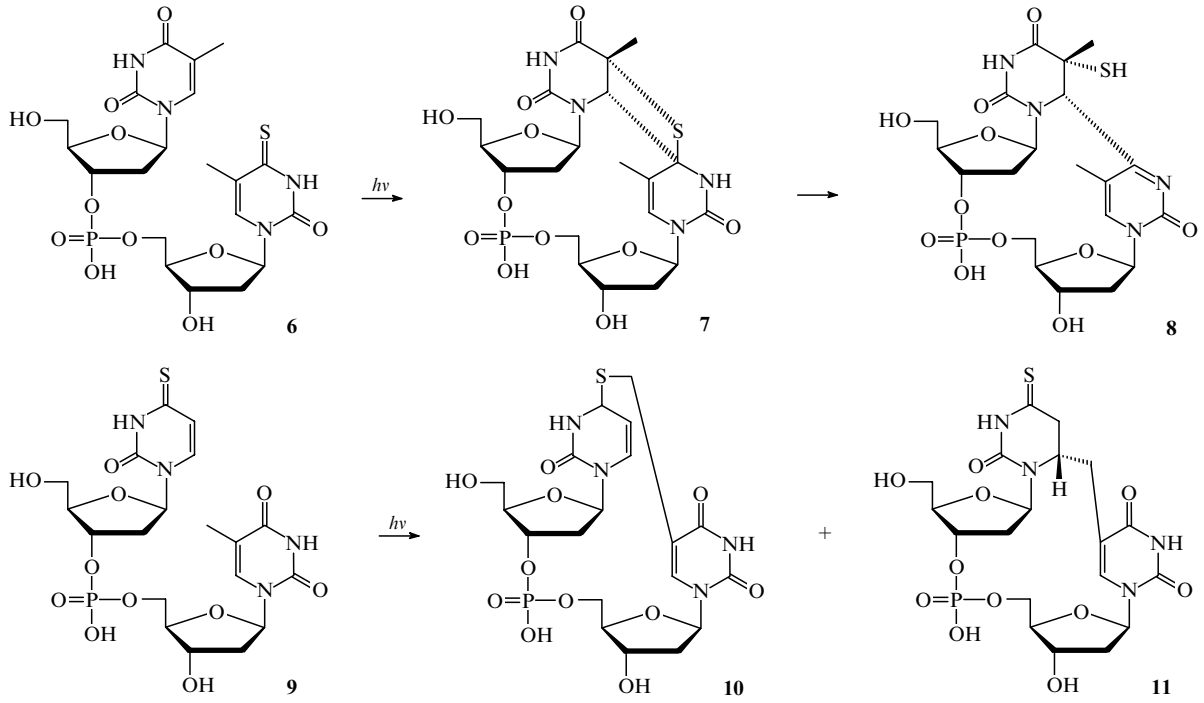
b. Photomodification of nucleic acids with 4-thiopyrimidine derivatives

Along with psoralens, thiopyrimidine nucleotides, *e.g.*, 4-thiouridine (s^4U) and 4-thiothymidine (s^4T), are used as photoreagents able to undergo free-radical cycloaddition. The presence of a sulfur atom instead of an oxygen atom at position 4 causes a shift of the absorption spectrum towards the long-wave region (λ = 330 nm) and the appearance of new photochemical properties.[39,40]

Irradiation of thiopyrimidines with UV light (330 nm) leads to the population of the excited state S_2 which is rapidly (within 5 ps) and in high quantum yield ($\varphi = 0.9$) converted into state T_1. Photolysis of d(s^4U) in water in the presence of oxygen yields dU.[40]

Thiopyrimidine nucleotides can form covalent adducts with all natural nucleic bases, but the quantum yield of the photoreaction with pyrimidine bases is higher than that with purines. Irradiation of the dinucleotide dTp(s^4U) (**6**) gives two stable products **7** and **8** (quantum yield 0.02) (Scheme 2). It is assumed

Scheme 2



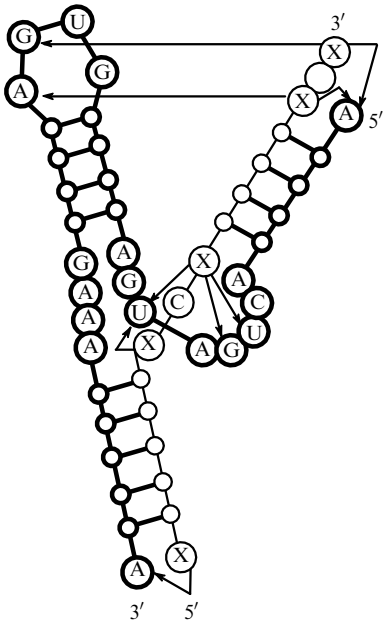


Figure 1. The structure of a ribozyme complex (bold-type) containing an RNA fragment.

that the [2 + 2]-cycloaddition of thiopyrimidine involving the excited C = S bond to the C(5) = C(6) bond of a pyrimidine results in the adduct **7** and subsequent chemical reactions yield the final product **8**.[40]

Irradiation of the dinucleotide d(s^{4}U)pT (**9**) afforded the adducts **10** and **11** (Scheme 2). In all probability, they are formed due to hydrogen abstraction from the methyl group of thymine by photoexcited thiouridine followed by recombination of radical species.[40]

A significant advantage of thiopyrimidines as photoreagents is that the van der Waals radius of the sulfur atom is by only 0.45 Å larger than that of the oxygen atom. This does not affect the structure of oligo- and polynucleotides with incorporated thiopyrimidine residues.[39] The photochemical properties of thiopyrimidines were most completely employed in the study of the tertiary structure and mechanism of action of ribozymes (Fig. 1).[39–45] Thiouridine residues were introduced into the RNA substrate at positions X. Irradiation of the ribozyme complex with RNA was accompanied by the formation of cross-links (indicated by arrows in Fig. 1). The photoaffinity modification data suggest that several alternative conformations appear in the ribozyme complex with RNA in different stages of a catalytic cycle of RNA hydrolysis.[40–42]

2. Photoinduced alkylation of DNA

Numerous photochemical methods for the generation of electrophilic species that alkylate DNA have been described.[27,46–58] These methods involve the preparation of azacycloheptatetraene *via* an intermediate singlet nitrene by photodecomposition of aromatic azides,[46–52] generation of quinolylmethylcarbocations by heterolytic photodecomposition of quinolylmethylisothiouronium salts,[53] preparation of quinonemethide by photoenolisation of 5- and 2-methylnaphthoquinone derivatives[54–57] and generation of oxonium cations from monothioacetals by photoinduced electron transfer.[58]

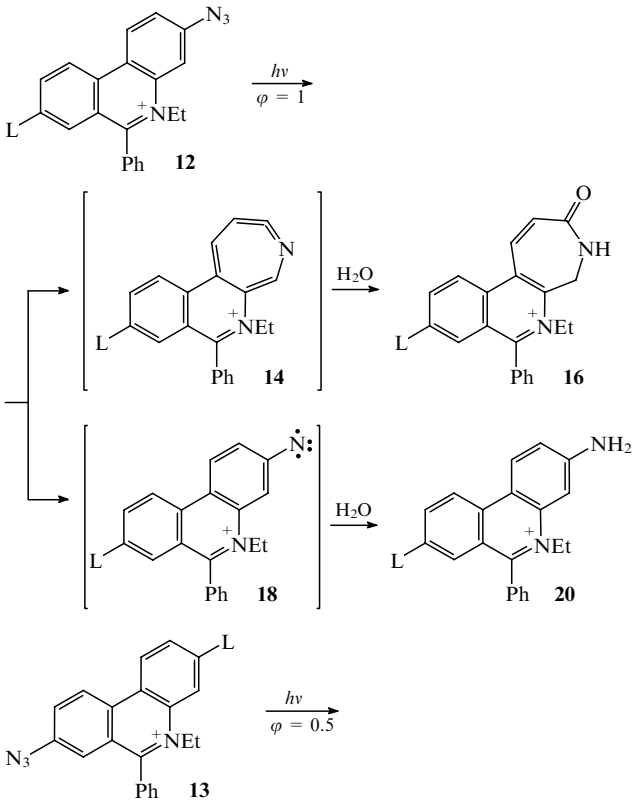
a. Photomodification of nucleic acids by aromatic azides

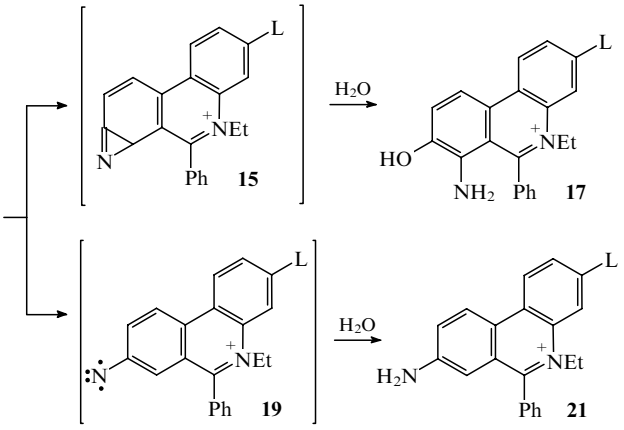
Oligonucleotide derivatives of aromatic azides are widely used in photomodification of DNA[46,47,50–52] and RNA[48,49] owing to their high photoreactivity and accessibility.

Conformational changes of a *p*-azidophenacyl 5′-thiophosphate derivative of ribozyme L-21 Sca I were studied in different stages of binding and catalytic cleavage of an RNA-substrate.[48,49] Irradiation of the photoreactive ribozyme derivative with UV light (312 nm) in the absence of the RNA-substrate gives covalent adducts at the A88\A89\A90 residues (yield 12%). The photomodification affected the A114\A115 residues of ribozyme (yield 34%) within the complex with the fragment GGCCCU-CUAAAAA where ribozyme hydrolysed the phosphodiester bond between the uridine and adenine residues; in the absence of Mg^{2+} cations, the C102\A103\A104 residues were photomodified in 38% yield.[48] The data obtained made it possible to establish the position of the ribozyme internal guide sequence (IGS) containing the *p*-azidophenacyl residue in different stages of the catalytic cycle of RNA hydrolysis.[48,49]

Ethidium derivatives **12** and **13** proved to be the most efficient photoreagents for DNA modification.[50,51] In all probability, this is associated with the ability of these residues to intercalate into DNA and with the photochemical properties of aromatic azides. The photolysis products of the azides **12** and **13** in water have been studied.[51] This reaction yields intermediate electrophilic species, *viz*., the azacycloheptatetraene **14** and the aziridine **15**; which add water to give the adducts **16** and **17**. In both cases, the triplet nitrenes **18** and **19** were formed, which underwent nonproductive decomposition as regards NA photomodification, *viz*., reduction to the amines **20** and **21**, respectively.

Formally, photolysis of the azide **13** in the presence of nucleophiles should give arylation products of the type **17**; however, these products have not been obtained and this azide is considered for convenience of comparison.





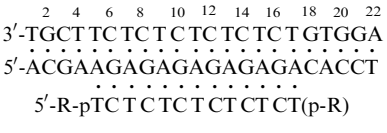
L = $NHCO(CH_2)_2NH_2$.

Heptanucleotide pCCAAACA conjugates with the azides **12** and **13** linked through 5′-phosphate were used for site-specific photomodification of the ^{32}P-labelled complementary octanucleotide 5′-*pTGTTTGGC.[51] The introduction of azidoethidium residues strongly stabilises the complementary complexes by increasing their melting temperature by 20–30 °C.



Irradiation of these duplexes with UV light (320 nm) results in covalent adducts in 40% and 60% yields, respectively. After treatment with piperidine which results in the cleavage of the DNA chain at the modified bases, the total degree of the octanucleotide photomodification increased up to 70% and 80%, respectively. The results of piperidine-catalysed cleavage led the authors to conclude that modification predominantly affected the C8 residue and, to a lesser degree, the G7 residue (indicated by arrows). The formation of up to 10 different adducts (many of which are unstable) was established by HPLC, but the attempts to isolate these adducts and to determine their structure were unsuccessful.

The oligonucleotides p(TC)$_6$Tp containing the azides **12** and **13** at their terminal phosphates were used for photomodification of ds-DNA in the triplex **22**.[50]



22

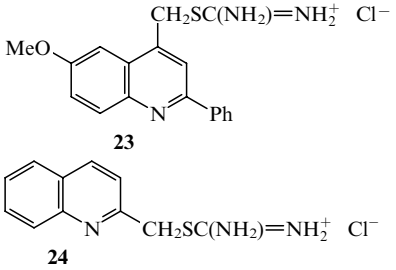
Irradiation of the triplex **22** (320–420 nm) containing the conjugate 5′-(**12**)p(TC)$_6$T as a photoreagent resulted in spontaneous cleavage of the purine-rich chain at the residue G3 (the degree of photomodification was 15%) as well as in the formation of covalent adducts with this residue (yield 30%). The pyrimidine-rich chain was photomodified to a far lesser degree; this was accompanied by the formation of covalent adducts with the residue T4 (yield 10%).

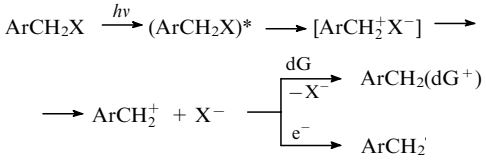
With the 3′-(**12**)p(TC)$_6$T derivative as a photoreagent, the total degree of photomodification of both chains did not exceed 10%.

Irradiation of the triplex **22** with the 5′-(**13**)p(TC)$_6$T reagent resulted in highly selective photomodification of the pyrimidine-rich chain. No direct cleavage was observed in this case; the covalent adducts (yield 35%) and the product of modification of the residue T4 were detected using treatment with piperidine.

b. Photomodification of DNA with benzylisothiouronium salts

A study of photolysis of a series of benzyl compounds of the type $ArCH_2X$ capable of generating carbocations revealed that the quinolyl-2(4)-methylisothiouronium salts **23** and **24** manifested the highest photoreactivity. Their irradiation with UV light (350 nm) in water gave the carbocations $ArCH_2^+$ (yield 90%, quantum yield 0.09).[53]





dG is deoxyguanosine.

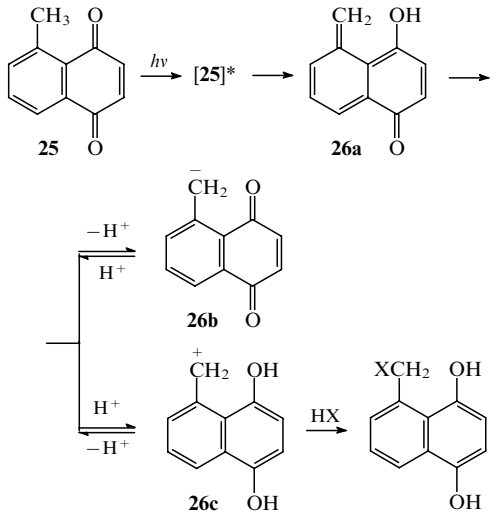
Photomodification of ds-DNA with the salts **23** and **24** resulted in insignificant cleavage of the target independent of its sequence. Most probably, the reaction occurs as hydrogen abstraction from deoxyribose by a benzyl radical ($ArCH_2^{\cdot}$) formed simultaneously with subsequent oxygen-dependent cleavage of the DNA-target. After treatment with piperidine, the degree of DNA modification increased 10-fold due to G-specific cleavage. It is assumed that quinoline residues intercalate into DNA in such a way that the positively charged isothiouronium residues of the reagents **23** and **24** are located on the duplex surface. Alkylation of guanine residues with a photoinduced quinolylmethylcarbocation at position N(7) is considered to be the most probable mechanism of G-specific modification. The quantum yield of photoalkylation product formation is rather low [$\varphi = (5-6) \times 10^{-6}$]; the yield of adducts with respect to the photoreagent does not exceed 1%.[53] Because of low efficiency of photoalkylation, these photoreagents have not found wide application.

c. Photoalkylation of nucleic acids with methylnaphthoquinones

Modification of single-stranded DNA (ss-DNA) with an oligonucleotide derivative of 5-methyl-1,4-naphthoquinone upon irradiation with UV light (> 345 nm) is the best studied example of photoinduced alkylation of NA.[54–56] Unlike photomodification with the isothiouronium salts **23** and **24**, the products of oxidative cleavage of the DNA-target were not found and the alkylation product was exclusively formed in ~ 20% yield. Neither piperidine (DNA cleavage at the sites of bases alkylated at the nitrogen atoms of a heterocycle), nor sodium borohydride [cleavage at the sites of pyrimidine bases with a saturated C(5)–C(6) bond] effected cleavage of the DNA-target. Pre-irradiation of the photoreagent resulted in its complete inactivation due to the photodegradation of the substituted naphthoquinone.[54] It was shown that photogenerated quinonemethides alkylate G and C residues at the exocyclic amino groups. Such adducts are more resistant to alkaline hydrolysis of DNA than the ordinary alkylation products.[56]

The efficiency of photoalkylation of DNA with oligonucleotide derivatives of 3-(1,4-naphthoquinonyl)thiopropionic acid with substituents at positions 2, 5 and 6 has been studied.[55,56] 5-Methyl-substituted naphthoquinones proved to be the most photoreactive agents, the degree of alkylation of ss-DNA regions reached 27%. The yields of products of DNA alkylation with 2- and 6-methylnaphthoquinones did not exceed 6%–8%.

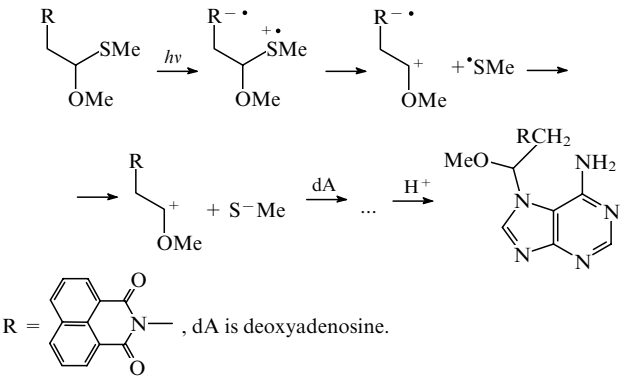
The mechanism of photoalkylation of DNA with 5-methylnaphthoquinone (**25**) in aqueous solutions has not been studied until very recently.[57] Upon photoexcitation, the naphthoquinone **25** is converted into 4-hydroxy-5-methylidenenaphthalen-1(5*H*)-one (**26a**), which can be deprotonated to give the naphthoquinone-5-carbanion (**26b**) or protonated to give the corresponding carbocation **26c**. The reaction of the carbocation **26c** with nucleophiles (HX) is the limiting step of photoinduced alkylation.



Photolysis under physiological conditions results predominantly in the carbanion **26b** ($pK_a = 6.5$); the corresponding value for the carbocation **26c** is 1.1. This fact is the main reason for the low efficiency of DNA photoalkylation with methylnaphthoquinones.

d. Photomodification of nucleic acids with monothioacetals

Irradiation of 1,8-naphthalimidoacetaldehyde monothioacetals with UV light (> 330 nm) generates S-centred radical cations due to intramolecular electron transfer to the photoexcited naphthalimide. The radical cation dissociates into a carbocation and an alkylthio radical, which is rapidly reduced by the naphthalimide radical anion.[58]



The carbocation reacts with nucleophiles (*e.g.*, the reaction with MeOH in the absence of oxygen occurs with a 98% yield and a quantum yield of 0.038) to give alkylation products.

1,8-Naphthalimidoacetaldehyde monothioacetal was irradiated with UV light (366 nm) in the presence of a triplet sensitiser (acetophenone); in this case, 99% of the light was absorbed by acetophenone. It was thus demonstrated that the electron transfer occurs *via* the triplet state of naphthalimide.

Irradiation of supercoiled DNA of pBR 322 with UV light (365 nm) in the presence of this monothioacetal resulted in its conversion into cyclic DNA after heating. Adenine alkylated at position N(7) was the main DNA photomodification product.[58]

Thus, several types of electrophilic photoreagents are currently known which can be used for photoalkylation of NA upon irradiation with proximal UV light (320 – 400 nm). However, low efficiency of these reagents and the lack of photoreactivity in the visible region of the spectrum restricts their application for the *in vivo* photomodification of NA.

3. Photoarylation of DNA

Ruthenium(II) polypyridyl complexes photosensitise the formation of single-strand breaks in ds-DNA in low quantum yields [$\varphi = (1-7) \times 10^{-6}$].[59,60] The mechanism of cleavage was investigated with short 5′-labelled oligonucleotides, which were irradiated with visible light (436 nm) together with a $Ru(TAP)_3$ complex, where TAP is 1,4,5,8-tetraazaphenanthrene. It was found that the main photosensitised reaction is not the cleavage of oligonucleotide chains, but rather the formation of covalent adducts of the Ru(II) complex with the oligonucleotide.[59,61] The yield of adducts in the $Ru(bipy)_nL_{3-n}$ series, where L = TAP or HAT, bipy is 2,2′-bipyridyl and HAT is 1,4,5,8,9,12-hexaazatriphenylene was rather high for $n = 0,1$, while no covalent adducts were formed for $n = 2,3$.

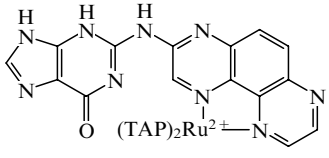
With an increase in n, the photochemical properties of Ru(II) and Os(II) complexes change, which is manifested in a bathochromic shift of absorption and fluorescence maxima and a decrease in the reducing potential (E^*_{red}) of the excited state (Table 1). Covalent adducts with the DNA-target are formed only in the case of Ru(II) and Os(II) complexes the E^*_{red} values of which exceed the redox potential of guanosine monophosphate ($E_{ox} = 0.92$ V).

Table 1. Photochemical properties of Ru(II) and Os(II) complexes: the absorption (λ_{max}) and fluorescence maxima (λ_{fl}); the values of the reduction potential (E^*_{red}) of photoexcited triplet states and the possibility of formation of covalent adducts with DNA.[61]

Complexes	λ_{max} /nm	λ_{fl} /nm	E^*_{red} /V	Adducts
$Ru(HAT)_3$	440	596	1.46	+
$Ru(bipy)(HAT)_2$	472	661	1.12	+
$Ru(bipy)_2(HAT)$	484	742	0.83	–
$Ru(TAP)_3$	437	608	1.32	+
$Ru(bipy)(TAP)_2$	465	649	1.06	+
$Ru(bipy)_2(TAP)$	484	714	0.86	–
$[Ru(bipy)_2]_2HAT$	572	800	1.03	+
$[Ru(phen)_2]_2HAT$	564	820	1.02	+
$Os(TAP)_3$	–	–	1.11	+

The bimetallic complexes $[Ru(bipy)_2]_2HAT$ and $[Ru(phen)_2]_2HAT$ (where phen is *o*-phenanthroline) attract substantial interest as electrophilic photoreagents, since they also form covalent adducts with DNA; their absorption maxima show bathochromic shifts by about 100 nm relative to monometallic complexes.

The covalent adduct formed by photomodification of ds-DNA with the $Ru(TAP)_3$ complex was isolated and characterised. The exocyclic 2-amino group of guanine was covalently bound to the C(2) position of TAP. Photomodification of DNA with $Ru(bpz)_3$ (bpz is 2,2′-bipyridazinyl), $Ru(TAP)_2bipy$,[61] $[Ru(phen)_2]_2HAT$[60] and $Os(TAP)_3$[59] complexes gave similar photoadducts.



Although the Ru(II) polypyridyl complexes intercalate into ds-DNA through the major groove, the formation of covalent adducts takes place in the minor groove.

The mechanism of photosensitised arylation of DNA with Ru(II) complexes has not been finally elucidated; however, it is

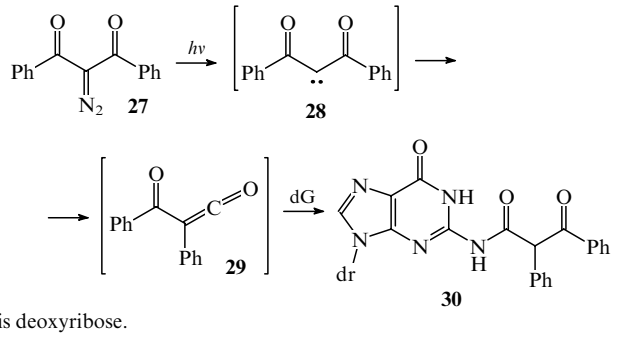
believed that the photoinduced electron transfer from the guanine residue to the excited ligand (TAP*) is followed by proton transfer from the radical cation $G^{+\bullet}$ to the radical anion $TAP^{-\bullet}$ as well as by recombination of radicals and oxidative rearomatisation of the $TAPH_2$ residue.[59,61]

Oligonucleotide conjugates of Ru(II) polypyridyl complexes were used to study photoinduced electron transfer,[62,63] but experimental support for complementarily addressed photomodification of DNA by these derivatives is lacking.

It should be noted that metal polypyridyl complexes open up new opportunities for the design of photoreagents that can be employed for the *in vivo* modification of NA. This is due to the fact that the properties of such complexes can be varied within a very wide range by changing the central metal atom and the ligands.

4. Photoacylation of DNA

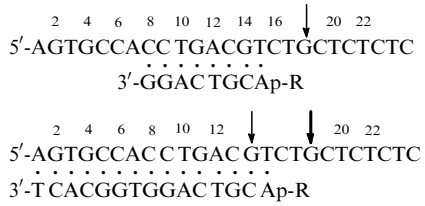
It is known that photoinduced dissociation of dibenzoyldiazomethane (**27**) results in dibenzoylcarbene (**28**), which is converted into the electrophilic ketene **29** capable of reacting with nucleophiles following the Wolff rearrangement.[27,64] Thus its photolysis (366 nm) in the presence of 3′,5′-di-*O*-(*tert*-butyldiphenylsilyl)-2′-deoxyguanosine gives a guanosine derivative **30** with an acylated exocyclic amino group.[27]



dr is deoxyribose.

Irradiation of dibenzoyldiazomethane (**27**) together with ss-DNA in the presence of the singlet oxygen trap, NaN_3, gave covalent adducts. Treatment of the latter with piperidine is accompanied by G-specific cleavage owing to the reaction with the ketene **29** and insignificant nonspecific cleavage due to hydrogen abstraction from deoxyribose by the carbene **28**.[27]

Site-specific photomodification of a 25-membered fragment of ss-DNA with 8- and 15-membered complementary oligonucleotide conjugates having a substituent (R) at the 5′-terminal phosphate in the presence of NaN_3 results exclusively in covalent adducts in 32% and 47% yields, respectively.



R = 4-(PhC(O)C(=N_2)CO)$C_6H_4CH_2OC(O)NH(CH_2)_6NH$.

In both cases, treatment of photomodification products with piperidine caused cleavage of the DNA-target at the residue G18 located in the single-stranded fragment of the duplexes; in the case of the 15-membered reagent, additional cleavage occurred at the residue G14 (indicated by arrows). The greater part of the adducts is not cleaved with piperidine. The total degree of modification after treatment with piperidine increased up to 33% and 56%, respectively. The formation of piperidine-labile adducts is due to the acylation at the N(7) position of guanine, whereas that of piperidine-stable adducts, by the acylation at the exocyclic amino group.[27]

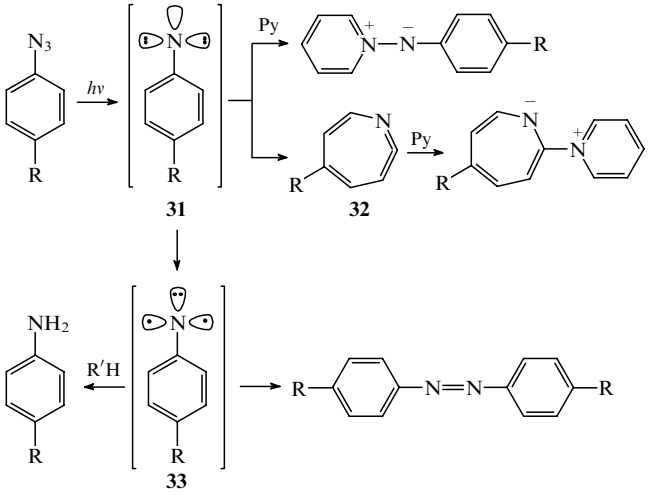
Thus, substituted diazo ketones are promising reagents for G-specific photoacylation of NA.

5. Other electrophilic addition reactions

A series of 8-alkoxy-5-azidopsoralens were synthesised with the aim of integrating the photochemical properties of aromatic azides and psoralens and investigating their ability in the photomodification of NA. Irradiation of azidopsoralens in acetonitrile with UV light (351 nm) affords a triplet nitrene and an azacycloheptatetraene, whereas irradiation in water yields exclusively a triplet nitrene. The reactivity of these species is insufficient for efficient photomodification of DNA. Being a triplet sensitiser, psoralen favours the formation of a triplet nitrene. Such a detrimental effect of the psoralen residue on the photochemistry of the azido group is the reason for the inability of pBR322 DNA to undergo cleavage with 8-alkoxy-5-azidopsoralens upon UV irradiation.[65]

Oligonucleotide reagents containing aromatic azido groups were also fairly inefficient in the photomodification of NA due to numerous side reactions. In the case of aromatic azides, the reaction does not occur *via* the highly reactive singlet nitrene **31** but rather *via* the much less reactive azacycloheptatetraene **32** formed from the nitrene **31** by rapid intramolecular ring expansion. Besides, nitro-, cyano-, formyl-, acyl- and, particularly, amino-substituents in aromatic azides accelerate intersystem crossing into a more stable but less reactive triplet nitrene **33** upon irradiation.[52] The nitrene **33** is usually consumed in radical dimerisation reactions to give azo derivatives or is reduced to the corresponding aniline without formation of NA modification products (Scheme 3).

Scheme 3



Perfluoroaromatic azides represent a more promising class of photoreagents, since their photodissociation predominantly results in the singlet nitrene **31**.[52,66–68] The presence of fluorine atoms in *o*-positions relative to the azido group decreases the rate of azacycloheptatetraene formation 1700-fold.[66] In addition, the acceptor fluorine atoms increase the electrophilicity of the singlet nitrene 10-fold[67] and reactivities of the triplet nitrene and the azacycloheptatetraene, 1000 and 10 000-fold, respectively,[68] in comparison with nonfluorinated derivatives.

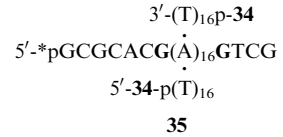
Dobrikov *et al.*[46,47] studied the effect of the aryl azide structure on the efficiency of site-specific photomodification of DNA by comparing the reactivities of oligonucleotide derivatives of 4-azido-, 5-azido-2-nitro- and 4-azidotetrafluorobenzamides. Upon irradiation with UV light, these compounds generate different species, *viz*., azacycloheptatetraene **32**, the triplet (**33**) and the singlet (**31**) nitrenes, respectively; the yields of covalent adducts in site-specific photomodification were 5%, 15% and 70%, respectively.[67] The reactivity of the species **32** and **33** is insufficient for modification of NA. The specificity of photomodification of DNA-targets containing T, C, A or G residues opposite to the photoreagent was studied with an example of an

oligonucleotide reagent containing a perfluoroaryl azide residue **34** coupled through 5′-phosphate. Treatment of modification products with piperidine showed that the degree of modification of these residues was ~ 50%, the largest degree of modification being 40%, 45%, 45% and 65%, respectively.[46,47,69] The reactions of the singlet nitrene **31** with the nitrogen atoms of the pyridine type of the heterocycles result in N-amination (Scheme 3). Photomodification of ss-DNA is most probably accompanied by electrophilic addition at position N(7) of guanine. The use of two oligonucleotide reagents complementary to the neighbouring regions of the DNA-target and containing two perfluoroaryl azide residues **34** at the contact site made it possible to increase the yield of covalent photomodification adducts to 80%.[70]

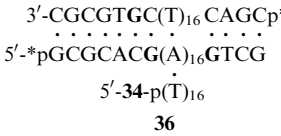
A highly efficient site-specific photomodification of 27-membered fragments of ss- and ds-DNA with a 4-azidotetrafluorobenzamide derivative of $p(T)_{16}$ has been carried out.[71,72]



34 (n = 2, 3)



35



36

Photomodification of ss-DNA within the triplex **35** occurred mainly at the bases G7 and G24 (bold-typed); the yields of covalent adducts amounted to 77%. Residues G7 of A-rich and G-22 of T-rich strands (the yields of covalent adducts were 37% and 16%, respectively) were the main sites of photomodification of ds-DNA within the triplet **36**. After piperidine treatment, the total degree of modification of the triplex **36** increased up to 50% in A-rich and up to 42% in T-rich strands due to noncovalent modification at pyrimidine bases.[71,72]

The effects of *p*-substituents in perfluoroaromatic azides on the efficiency of site-specific photomodification of ss-DNA was also studied. The increase in the conjugation chain in substituted perfluoroaryl azides accelerates the intersystem crossing of the singlet nitrene into the triplet one and decreases the efficiency of DNA photomodification. Studies with pyridines as the chemical trap for the singlet nitrene **31** revealed that the yield of covalent adducts with DNA correlates with the yield of pyridine ylides in the photolysis of perfluoroaryl azides in 6 M pyridine. It was assumed that the electrophilic addition of the singlet nitrene to the nitrogen atoms of the pyridine type in NA bases is the most feasible mechanism of covalent adduct formation.[73]

The efficiency of site-specific photomodification (irradiation with UV light, $\lambda = 303-370$ nm, irradiation power 0.5 mW cm^{-2}, 5 min, 4 °C, pH 7.0) of ss-RNA and ss-DNA fragments (5 mmol litre^{-1}) with oligonucleotide conjugates of perfluoroaryl azide **34** (50 mmol litre^{-1}) was compared by Levina *et al.*[74]

Reagent	Yield of covalent adducts for targets (%)	
	5′-d(TGTTTGGC)	5′-UGUUUGGC
5′-**34**-pd(AAACA)	~5	~5
5′-**34**-pd(CAAACA)	65	30
5′-**34**-pd(CCAAACA)	75	55

The efficiency of photomodification increases with an increase in the length of the oligonucleotide address. Photomodification of RNA occurs less efficiently than that of DNA, which most probably depends on the structures and stabilities of duplexes. Nevertheless, 6–7-membered ribonucleotide conjugates carrying a perfluoroaryl azide residue **34** at the 5′-terminal phosphate were used to study the decoding centre of human 80S ribosomes.[75] Irradiation of a ribosome–tRNA–photoreactive mRNA analogue complex with UV light (270–380 nm) resulted in binding of the analogue to the 40S subunit (45%–55%) and 18S sRNA (8%–22%). Only one base, *viz.*, the residue G1207, underwent photomodification within sRNA.

Kazantsev *et al.*[76] studied the kinetics of site-specific photomodification of 26-membered ss-DNA with oligonucleotide conjugates of perfluoroaryl azide **34**. Irradiation with UV light (> 300 nm) resulted in the formation of covalent adducts; upon treatment with piperidine, cleavage (predominantly, at residue G19 and, to a lesser degree, at residues G17, A18 and G20) (indicated by arrows) occurred.



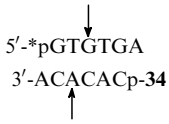
The kinetic data shown in Table 2 suggest that the efficiency of photomodification with 5′-*p*-azidotetrafluorobenzamide conjugates is not very high. The maximum degree of photomodification with a 11-membered conjugate was higher than that with 6- and 8-membered ones due to the higher value of the constant K_x. Irradiation of the conjugates in the absence of a DNA target resulted in photodeactivation and an approximately 200-fold decrease in affinity for the target.[76]

Table 2. The association constants (K_x) of the conjugates with the target, the efficiency of photomodification within the duplexes (γ_{ef}) and maximum degrees of photomodifications (PZ) at 37 °C.

Photoreagent	K_x /mol litre^{-1}	γ_{ef}	PZ (%)
CTTACCCTTCTp-**34**	8.4×10^6	0.2	70
ACCCTTCTp-**34**	1.1×10^5	0.33	55
ACCCTTp-**34**	1×10^5	0.27	40

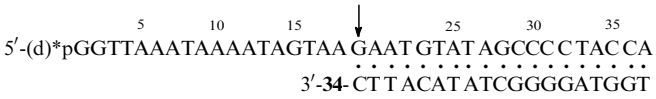
Note. The concentrations of a DNA-target and the conjugates are 10 nmol litre^{-1} and 60 mmol litre^{-1}, respectively (see Ref. 76).

Site-specific photomodification of a ss-DNA fragment with an oligonucleotide conjugate containing the azide **34** was carried out in order to elucidate the possible reasons for such photodeactivation.[77]



Irradiation of a labelled reagent in the absence of the target also resulted in a partial loss of its affinity for the target; treatment with piperidine resulted in cleavage at all the bases of the address, the total degree of cleavage was 44%. Irradiation with an excess of a target and subsequent hydrolysis revealed that the modification of the address occurred at the residue A4 (yield 29%), *i.e.*, the formation of the duplex did not prevent the modification of the address. With a 5000-fold excess of the conjugate, photomodification affected the residue G3 of the target localised in the double-stranded fragment. The maximum yield of photomodification reaches 80%; γ_{eff} is close to unity.[77]

Site-specific photomodification of a 37-membered DNA fragment with an oligonucleotide reagent carrying a perfluoroaryl azide residue at 3′-phosphate in complexes with human immunodeficiency virus reverse transcriptase (HIV RT)[78,79] or with DNA-polymerase α has been carried out.[80]



Irradiation of these complexes with UV light (> 300 nm) has led to cross-linking of the primer to matrices with 60% [78, 79] and 70% [80] efficiency. Treatment with piperidine resulted in cleavage of the target at the residue G19 which is located opposite the photoreagent incorporated into the primer.

Thus, by virtue of high reactivity of the species formed upon irradiation of perfluorinated azides, these have found wide application in photomodification of NA. The main disadvantage of these photoreagents is the lack of noticeable photoreactivity in the visible region of the spectrum, which restricts their application in complex biological systems.

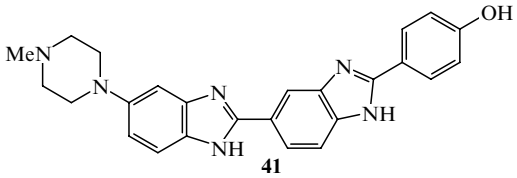
III. Sensitised photomodification of DNA with binary systems

1. Photomodification of DNA with two-component (sensitiser – photoreagent) systems

Irradiation of DNA containing 5-bromo- or 5-iododeoxyuridine residues with UV light (302 – 313 nm) induces numerous photochemical reactions, *viz*., modification of bases, cross-linking, single- and double-strand breaks, *etc*. (Scheme 4).[23, 81 – 88]

The mechanism of formation of single-strand breaks initiated by photolysis of halogenated pyrimidines was studied in detail for oligonucleotides **37** containing 5′-A^{Br}U sequences.[23, 81, 82] Irradiation with UV light (302 nm) resulted in photoinduced electron transfer from adenine to the excited triplet state of bromouridine ^{3}(^{Br}U)* with the formation of a radical cation of adenine Ade$^{+\bullet}$, and a radical anion of uridine (compound **38**). This is followed by elimination of Br$^-$ from the radical anion; the 5-uracil radical formed abstracts hydrogen from the adjacent C(1′) atom to give the carbocation **39**.[81] Addition of water to the C(1′) atom of the carbocation **39** results in the 1′-hydroxy derivative **40**. Apurinisation of compound **40** and subsequent reactions bring about cleavage of the DNA chain. The structure of stable DNA cleavage products has been considered in a review of Pratviel *et al*.[81] Similar reactions take place in self-complementary duplexes of the type 5′-d(GCA^{Br}UGC)$_2$ containing 5′-A^{Br}U sequences.[83]

Irradiation (313 nm) of DNA containing 5-d^{I}U fragments results in alkali-dependent single-strand cleavage with the efficiency 0.5; its mechanism is somewhat different.[84] The photoreaction occurs *via* the homolytic cleavage of the C(5)—I bond of uridine. The 5-uracyl radical formed competitively abstracts hydrogen atoms from the C(1′) or C(2′) atoms of deoxyribose on its 5′-side.[81] The efficiency of analogous cleavage induced by irradiation with UV light (λ = 365 nm) in the presence of Hoechst 33258 bisbenzoimidazole **41** reaches 0.9. Under these conditions, the initial rate of sensitised photomodification is 4 – 5 times greater than the rate of photomodification in the absence of bisbenzoimidazole. Binding of compound **41** to DNA occurs in the minor groove, where it strongly absorbs UV light (365 nm, 23 000 mol^{-1} litre cm^{-1}) and undergoes excitation. The electron transfer from bisbenzoimidazole **41** to 5-d^{I}U induces elimination of I$^-$ and the formation of an uracil radical; reactions of the latter bring about photomodification of DNA.[84] Similar results were obtained in the photo-induced cleavage of DNA containing 5-d^{Br}U fragments sensitised to long-wave UV light with bisbenzoimidazole **41**.[85]



41

It is believed that bisbenzoimidazole dyes bind preferentially to (AT)$_4$- and (AT$_3$)A(5-^{Br}U) sequences of DNA [86] and thus ensure high efficiency of electron transfer to the adjacent residues of halogenated pyrimidines.

In cells cultured on 5-d^{Br}U-containing media, one 5-d^{Br}U residue per ~ 200 nucleotides is incorporated into DNA. Irradiation of cells with UV light (365 nm) in the presence of bisbenzoimidazole **41** induces the formation of protein – nucleic acid cross-links as well as single- and double-strand breaks of DNA and modification of bases,[87, 88] resulting in multiple aberrations of chromosomes and cell death. The death of cells irradiated in the presence of the photoreagent 5-d^{Br}U and the sensitiser **41** occurs 30 000 times more efficiently than in the case of UV-irradiated native cells and 20 times more efficiently than in the case of 5-d^{Br}U-containing cells irradiated in the absence of the sensitiser.[87]

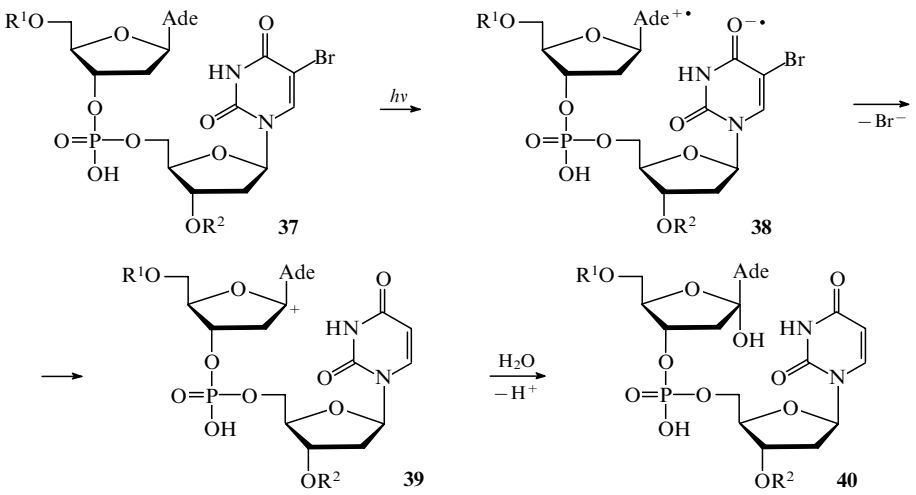
The use of two-component sensitiser – photoreagent systems has made it possible to combine high reactivity of the photoreagent with spectral-luminescent properties of the sensitiser.

2. Sensitised photomodification of DNA with binary systems of oligonucleotide reagents

For the majority of photoreactive derivatives, light absorption and dark reactions within a complementary complex with a DNA-target and in solution occur at the same rate. At best, this results in nonproductive decomposition of the photoreagent in solution and triggers undesirable side reactions.

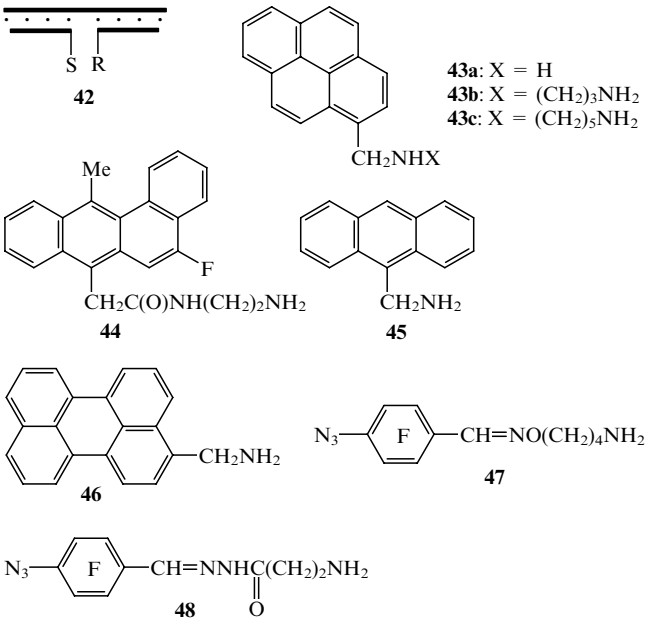
However, the specificity of oligonucleotide derivatives is insufficient for modification of nucleic acids *in vivo*, since under physiological conditions (37 °C) stable complexes are formed at

Scheme 4



the oligonucleotide lengths of 12–13 bases. Oligonucleotides having the lengths of 18–20 bases, which is necessary for highly specific recognition of unique nucleotide sequences within the DNA structure, can form numerous imperfect complexes with incompletely complementary sequences.[89]

To overcome these drawbacks, the separation of light absorption and photomodification was suggested. For this purpose, binary systems of the oligonucleotide conjugates **42** complementary to the adjacent sequences of a DNA-target and containing chemically inert groups, *viz*., a sensitiser (S) and a photoreagent (R), have been developed. The groups S and R being brought together within the structure of the complementary complex form a photoreactive centre which can be activated by irradiation with long-wave UV or visible light due to a non-radiative transfer of the electron excitation energy from the sensitiser to the photoreagent. The thus excited photoreagent induces photomodification of only that DNA-target which is an integral part of the complementary complex.[90–100] The lengths of both nucleotide addresses (both were decanucleotides) were chosen so that the melting temperature of the duplexes was ~ 37 °C; such short oligonucleotides cannot form imperfect duplexes with incomplete complementary sequences under physiological conditions.[89]



Pyren-1-yl-methylamines (**43a–c**),[89,94–96] *N*-(5-fluoro-12-methylbenzo[*a*]anthracen-7-ylacetyl)ethylenediamine (**44**),[90–93] anthracen-9-ylmethylamine (**45**)[98] and perylen-1-ylmethylamine (**46**)[97,99,101,102] derivatives were used as sensitisers, whereas perfluoroaromatic azides (*p*-azidotetrafluorobenzamides **34**,[94,95] *O*-(4-aminobutyl)-*p*-azidotetrafluorobenzaldoxime (**47**)[96] and *N*-(4-azidotetrafluorobenzylidene)-*N*′-(3-aminopropionyl)hydrazine (**48**)[89–93,101,102] were used as photoreagents.

Several sensitisation mechanisms, *e.g*., singlet–singlet energy transfer from a photoexcited sensitiser to a photoreagent,[89–94,98] a two-quantum triplet–triplet energy transfer[95,96] and photoinduced electron transfer from a photoreagent to an excited sensitiser,[97,99,101,102] have been suggested.

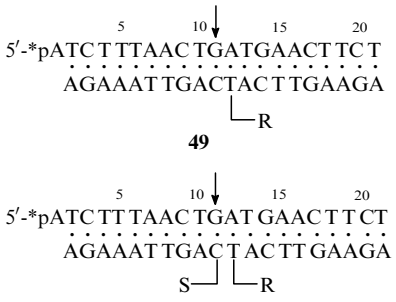
a. Singlet–singlet energy transfer

Singlet–singlet energy transfer occurs provided the energy of the 0,0-transition between the lowest vibrational levels of the ground and the first electronically excited states in the sensitiser molecule is greater than the energy of the corresponding transition in the photoreagent R molecule and the emission spectrum of the excited sensitiser (S*) overlaps with the absorption spectrum of the photoreagent R.[103]

The energy of the 0,0-transition in aromatic azides is ~ 70 kcal mol^{-1} and their weak $n\pi^*$-absorption is observed at 450 nm.[104] Derivatives of polyaromatic hydrocarbons, *viz*., pyrene **43**, benzoanthracene **44** and anthracene **45**, have high quantum yields of fluorescence and low Stokes shifts (*i.e*., the minimum energy losses in photoexcitation and subsequent fluorescence). The energies of 0,0-transitions in unsubstituted pyrene, benzoanthracene and anthracene are equal to 76,[104] 72[105] and 73 kcal mol^{-1} (see Ref. 104) and exceed those of 0,0-transitions in the azides **34**, **47** and **48**. Thus, the fluorescence spectra of compounds **43–45** overlap with the absorption spectra of these azides.[91]

The efficiency of the energy transfer strongly depends on the distance between the sensitiser and the photoreagent and at commonly used micromolar concentrations of oligonucleotide derivatives the energy transfer from the sensitiser to the photoreagent does not occur in solution.

UV irradiation (365–390 nm) of ss-DNA complexes with an oligonucleotide conjugate containing a photoreagent in the absence (complex **49**) and in the presence (complex **50**) of a sensitiser results in covalent adducts. The azide **34** was used as a photoreagent (R), while the pyrene derivatives **43a–c** were used as sensitisers (S). Treatment of reaction products with piperidine showed that direct (within the complex **49**) and sensitised (within the complex **50**) photomodification affects the residue G11.[89]



The initial rate and efficiency of direct (within the complex **49**) and sensitised (within the complex **50**) photomodification of the target depended on the localisation of the photoreagent and the sensitiser on the 3′- or 5′-phosphates as well as on the length of the linker between the sensitiser and the oligonucleotide address. Oligonucleotide derivatives containing a photoreagent at 3′-phosphate appeared to be more efficient, *viz*., the yield of covalent adducts amounted to 70%. The rate of photomodification sensitised to UV light (365–390 nm) was 100–1500 times higher than that of direct complementarily addressed modification and decreased with an increase in the linker length in the series of the pyrenyl derivatives **43a–c**.[94]

Photomodification of ss-DNA in the complexes **49** and **50** with the oligonucleotide azide derivative **48** has been carried out. At equimolar ratios of conjugate and target, the largest yields of covalent adducts of direct photomodification within the complex **49** (Fig. 2, curve *1*) and of photomodificataion sensitised with the oligonucleotide benzoanthracene derivative **44** within the complex **50** (Fig. 2, curve *2*) are identical (~ 30%), but the initial rate of the sensitisation reaction is 120 times greater than that of direct photomodification. The observed increase in the rate of photomodification is due to efficient energy transfer from the photoexcited sensitiser S* to the photoreagent R. With a 50-fold excess of oligonucleotide conjugates (curve *3*), the initial rate of sensitised photomodification was not practically changed in comparison with the rate of sensitised modification represented by the curve *2*, but the yield of covalent adducts increases up to 72%. The yield of direct photomodification products increases insignificantly under these conditions.[90,93]

To account for these results, the dependence of the yields of covalent adducts was studied with a deficit of the sensitiser and an excess of the photoreagent on the concentration ratio of the

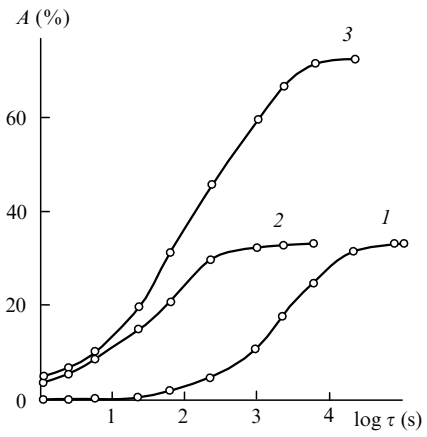


Figure 2. The yield (*A*) of covalent adducts of an oligonucleotide conjugate containing an azide residue **48** to a complementary DNA-target upon irradiation with UV light (365–390 nm) of the complexes **49** (*1*) and **50** (*2*, *3*); concentrations/ μmol litre^{-1}; oligonucleotide conjugates, 50 (*1*–*3*), DNA-target, 50 (*1*, *2*), 1 (*3*).

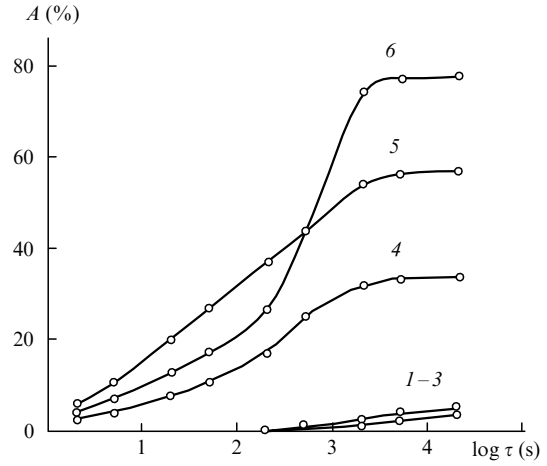


Figure 4. The temperature dependence of the yields of the covalent adducts (*A*) upon photomodification of a mixture of M13BMR4 ss-DNA restricts within the complexes **49** (*1*–*3*) and **50** (*4*–*6*) upon irradiation with visible light (400–480 nm)/ °C: (*1*, *4*) 4; (*2*, *5*) 20; (*3*, *6*) 37.

sensitiser to the DNA-target (Fig. 3). In the absence of the catalytic effect of the sensitiser it should be linear as in the case of irradiation under such conditions (10 min, 4 °C) where the components of the complex **50** are not exchanged for the solution components (curve *1*). With an increase in the irradiation time and in temperature, each sensitiser molecule initiates the modification of several target molecules (curves *2* and *3*). Thus with a 100-fold deficit of the sensitiser (irradiation at 42 °C), each sensitiser molecule induces photomodification of up to 24 target molecules.[93]

The specificity of direct and sensitised photomodification of a natural DNA-target was studied.[91,92] A mixture of 18 fragments of ss-DNA of M13BMR4 was obtained by enzymatic hydrolysis. One of these fragments having the length of ~150 bases contained a sequence-target complementary to oligonucleotide addresses (similar to the complexes **49** and **50**); while other fragments did not contain this sequence. The results of direct and sensitised photomodification of a mixture of DNA restricts with an oligonucleotide conjugate carrying an azide residue **48** at ^{32}P-labelled 5′-phosphate are shown in Fig. 4.

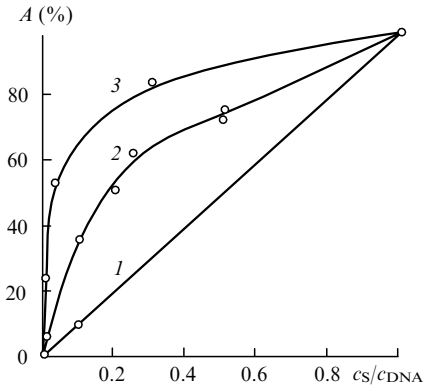


Figure 3. The dependence of the relative yields (*A*) of the covalent adducts of sensitised photomodification within the complex **50** on the concentration ratio of the sensitiser (c_S) and DNA-target (c_{DNA}) upon irradiation with UV light (365–390 nm). The yield of adducts at an equimolar c_s/c_{DNA} ratio is taken as 100%. The concentration of the DNA-target and the oligonucleotide photoreagent is 50 μmol litre^{-1}. The irradiation was performed for 10 min, 4 °C (*1*); 90 min, 42 °C (*2*) and 300 min, 42 °C (*3*).

An excess of noncomplementary DNA fragments has practically no effect on γ_{eff} of sensitised modification (curve *6*) in comparison with modification within the model complex **50**. The efficiency of direct photomodification of a mixture of restricts is noticeably decreased (curves *1*–*3*) in comparison with γ_{eff} for the complex **49**, apparently due to the competition with partially complementary sequences of different fragments of M13 DNA. The efficiency and rate of direct photomodification weakly depend on temperature (curves *1*–*3*). In the case of sensitised photomodification, its efficiency increases sharply with an increase in temperature from 4 to 37 °C (curves *4*–*6*). In this case, the rate of the reaction increases first (curves *4*, *5*) and then decreases 2-fold as the melting temperature of the complex has been reached (curve *6*).

Such a temperature dependence is due to the ability of the single-stranded fragment to form hairpins near the modification site; at low temperatures, the target sequence becomes inaccessible for oligonucleotide conjugates. The accessibility of the target sequence and the efficiency of sensitised photomodification increase with an increase in temperature, which causes the unwinding of the structured sites of ss-DNA.

At 37 °C, the initial rate of sensitised photomodification is four orders of magnitude higher (curve *6*), and the degree of modification is 60 times greater than in the case of a direct reaction (curve *1*). At the same temperature, the level of nonspecific modification of fragments of M13 DNA containing no complementary insert is ~1.5 times lower for the photosensitised reaction than for the direct one.[92] It was found that the selectivity of sensitised photomodification determined as a ratio, γ_{eff}, of specific to nonspecific modification is 90 times greater than that of the direct reaction.[91,92]

Modifications predominantly affect the G residues, the *PZ* value being dependent on their number in the $(pG)_n$-sequence of DNA-targets at the modification site. The yields of covalent adducts of direct modification at $n = 1$, 2 and 4 are 33%–41%, 68%–72% and 82%–85%, respectively, whereas those of photosensitised modification products are 62%–68%,[89,98] 75%–82%[94,98] and 98%–99%,[98] respectively.

The photomodification of a DNA-target (5′-*pATGGATT-CTTGAAGGGGACCGCATT) with a binary system of conjugates complementary to the underlined regions and containing anthracene **45** and azide **48** residues has been carried out. The results of treatment of the reaction products with piperidine show that photomodification predominantly affected the residues G15 and G16 and, to a lesser degree, the residues G14 and G17.[98] This finding made it possible to establish the structure of the binary

system of oligonucleotide conjugates with the DNA-target (data from two-dimensional ^{1}H NMR).[100] The probabilities of the contacts of perfluoroaryl azide residues with bases G14 – G17 of the DNA-target were as follows: G14 : G15 : G16 : G17 = 12 : 40 : 40 : 8.[98]

b. Photoinduced electron transfer

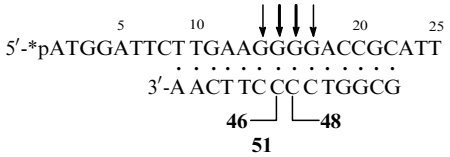
In the foregoing section, it was demonstrated that the use of binary systems of oligonucleotide conjugates increases the initial rate of photosensitised photomodification of ss-DNA (100 – 10 000-fold in comparison with the rate of direct photomodification) and the efficiency of the specific reaction (by nearly two orders of magnitude). However, sensitisation due to singlet – singlet energy transfer does not fully prevent nonspecific modification, as is the case with long-chain ($\geqslant$ 7500 bases) sequences. The occurrence of nonspecific reactions is due to the following. A conjugate containing a photoreagent binds to those complementary sequences of a DNA-target which have no adjacent sequences complementary to the conjugate containing a sensitiser.[90]

In order to prevent nonspecific modification in the formation of imperfect complexes, each of the groups in the photoreactive centre should be inactive under irradiation conditions.

With this in mind, Dobrikov *et al.*[97,99] developed a binary system of oligonucleotide derivatives of the sensitiser **46** and the photoreagent **48** complementary to the neighbouring regions of a DNA-target within the complex **50**; this system can be activated with visible light (440 – 580 nm).

The excitation energy ($E_{0,0}$) of the perylene derivative **46**, which was chosen as a sensitiser, is less (64 kcal mol^{-1}) [104] than the energy of the 0,0-transition in aromatic azides, but perylene is capable of sensitising their photodecomposition due to photoinduced electron transfer from the azido group to the photoexcited sensitiser.[104]

Figure 5 shows the results of direct photomodification of a DNA-target within the complex **49** upon irradiation with visible light (420 – 580 nm) (curve *1*) and photomodification within the complex **50** sensitised with oligonucleotide derivatives of benzoanthracene **44** (curve *2*) and perylene **46** (curve *3*). The initial rate of perylene-sensitised photomodification is nearly 300 000 times higher and that of benzoanthracene-sensitised reactions is nearly 10 times higher in comparison to direct photomodification. Irradiation of the complex **49** with UV light (365 – 390 nm) results in adducts (yield $\sim$ 30%) which are cleaved by piperidine at the guanosine residue G14 of the DNA-target (marked by an arrow). The visible light-induced photomodification within the complex **50** with a conjugate of perylene **46** gives covalent adducts (yield $\sim$ 70%) cleaved at the same guanosine residue, which is most likely due to electron transfer from the lone electron pair of the nitrogen atom in the azido group of the photoreagent to the photoexcited perylene.[97,104] The perylene residue generates a radical anion which is rapidly oxidised by atmospheric oxygen to the corresponding 1,12-perylenequinone.[97] This is accompanied by a simultaneous formation of a radical cation from perfluoroaryl azide, which after a series of dark conversions induces DNA photomodification similar to that triggered by the singlet nitrene.



Sensitised photomodification (450 – 580 nm, 15 min) of a DNA sequence from the promoter region of the multiple drug resistance gene (MDR-1) containing four G residues in the region of the photoreactive centre with a binary system of oligonucleotide conjugates (complex **51**) occurs with nearly quantitative yield.[101,102] Treatment of the reaction products with piperidine revealed that modification affected predominantly residues G15 and G16 and, to a lesser degree, residues G14 and G17. Photomodification did not take place in the absence of the sensitiser.

Thus, binary systems of oligonucleotide reagents containing perylene **46** and perfluoroaryl azide **48** residues can be used for quantitative photomodification of a DNA-target upon irradiation with visible light in the blue-green region of the spectrum (450 – 580 nm) where both components of the system are singly practically inactive.

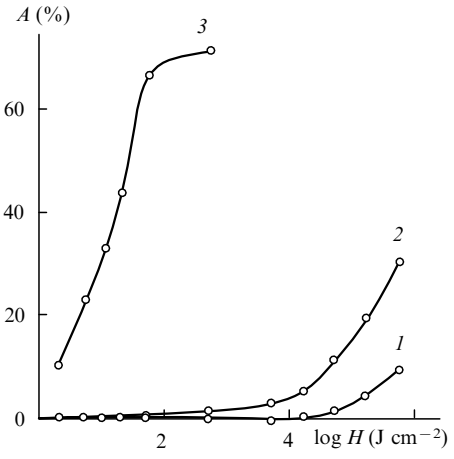


Figure 5. The dependence of the yields of the covalent adducts of DNA photomodification in the complexes **49** (*1*) and **50** (*2*, *3*) upon irradiation with visible light on exposure (*H*). The concentration of the DNA target is 1 μmol litre^{-1}, that of oligonucleotide conjugates is 50 μmol litre^{-1}. The irradiation was performed at the wavelengths/ nm: 420 – 580 (*1*), 450 – 580 (*2*, *3*). The oligonucleotide conjugate containing a benzoanthracene **44** (*2*) or perylene **46** (*3*) residue was used as a sensitiser.

IV. Two-quantum photosensitised modification of DNA

The $^{\bullet}$OH radical and singlet $^1O_2$ are the main reagents for nucleic acid photooxidation and possess high toxicity. It was therefore proposed to use photoreagents, which do not form active diffusing species. The chemical activity of photoreagents can be triggered by two-quantum excitation of higher electronic states.[106,107] The progress in picosecond laser technologies has made it possible to conduct multiquantum reactions. Two-quantum excitation can be mediated by two different mechanisms, *viz.*, resonant two-quantum excitation and nonresonant two-photon excitation.

The former consists in a consecutive absorption of two photons *via* a real intermediate state of the absorbing species. This is populated in the absorption of the first quantum and is a starting point for the absorption of the second quantum. The real excited state has a definite lifetime (normally, 10^{-4} – 10^{-9} s). Photochemical modification of DNA can be performed both from higher singlet and triplet excited states.[15,106,108]

The second mechanism, *viz.*, nonresonant two-quantum excitation, does not involve any real intermediate state. In this case, the second photon will be absorbed simultaneously with the first one, *i.e.*, within $\sim 10^{-15}$ s. Two-quantum (especially, nonresonant) excitation requires high light intensities ($I \geqslant 3 \times 10^{14}$ W m^{-2}).[108]

Summation of light quantum energy needed for a photochemical reaction to take place is an important feature of two-quantum excitation mechanisms. Yet another peculiarity of two-quantum reactions is their correspondence to spectroscopic selection rules. If a photosensitiser molecule has a centre of symmetry, the absorption of each light quantum changes the parity of the wave function. Hence, two-quantum excitation can generate reactive states which are forbidden in single-quantum excitation.[108,109]

1. Two-quantum nonresonant excitation

Two-quantum excitation is a promising field for practical application of known photoreagents, since it allows one to trigger photochemical reactions by irradiation with red and infrared light instead of ultraviolet and violet light. IR irradiation (750–1000 nm) can penetrate deeply into tissues, is not absorbed by cell components and does not induce cell injuries. The specificity of its action can thus be significantly increased.[108]

Psoralens belong to the most promising candidates for photoreagents, but their spectral photosensitivity was limited by the range of 320–436 nm.[108,110] Considerable progress was achieved in the past few years in overcoming this obstacle. Thus irradiation of 8-methoxy-, 4′-amino-4,5,8-trimethyl-[109] and 4′-hydroxymethyl-4,5′,8-trimethylpsoralens[111] with a titanium-sapphire laser (730 nm) was accompanied by nonresonant two-quantum excitation and anti-Stokes fluorescence at 452 nm the intensity of which showed a quadratic dependence on the peak power of the laser.[108] The same photochemical reactions were triggered as in the case of single-quantum activation of psoralens with UV light (365 nm).

Photobiological properties of psoralen in two-quantum excitation were studied *in vivo* in inactivation of *Salmonella typhimurium* His-operon. Short-pulse ($\tau \sim 0.2$ ns), high-flux laser irradiation ($I = 3 \times 10^{14}$ W m^{-2}) in the absence of psoralen was characterised by low mutagenicity; in the presence of psoralen, laser irradiation (730 nm) fully suppressed the expression of the His-operon due to the formation of covalent psoralen bisadducts with ds-DNA.[108]

2. Two-quantum resonant excitation

Two-quantum resonant excitation consists in sequential absorption of two quanta of light, resulting in both higher excited singlet states (S_n):

$$S_0 \xrightarrow{h\nu} S_1 \xrightarrow{h\nu} S_n,$$

and higher excited triplet states (T_n):

$$S_0 \xrightarrow{h\nu} S_1 \longrightarrow T_1 \xrightarrow{h\nu} T_n.$$

a. Two-quantum resonant singlet–singlet excitation

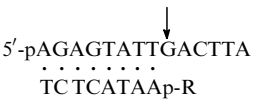
Photoexcitation generates primarily singlet states, S_n, characterised by higher energy but several orders of magnitude shorter lifetimes than the corresponding triplet states. Thus the lifetime of state S_n is less than 10^{-11} s, while that of state S_1 is usually 10^{-8} s. The appropriate conditions for bimolecular reactions from states S_n are attained in the case where the sensitiser forms a donor–acceptor complex with the molecule-target in the ground state or an exciplex in an excited state.[111]

For realisation of this mechanism, the photoreagents should have a low yield of intersystem crossing (ISC) into the triplet state and the corresponding $S_1 \rightarrow S_n$ conversions. Some polymethine dyes ($\varphi_{ISC} \leqslant 0.03$), tetraazaporphyrins ($\varphi_{ISC} \leqslant 0.15$), Mg^{2+}- and Zn^{2+}-phthalocyanines (MPC^{2+}, M = Mg, Zn) ($\varphi_{ISC} \leqslant 0.06$), *etc.*, meet these requirements.[106,112]

Nonlinear absorption of $MgPC^{2+}$ ($\varepsilon_{681} = 2.2 \times 10^5$, $\varphi_{fl} = 0.6$, $\tau_{fl} = 5$ ns) was observed in pulse ($\tau = 1.6$ ns) laser excitation (680 nm) with a pulse density of $> 10^{24}$ Einstein cm^{-2} s^{-1}. The yield of two-quantum excited molecules reached $\sim 100\%$ at the pulse density of 10^{27} Einstein cm^{-2} s^{-1}. The absorption spectrum of $^*MgPC^{2+}$ from state S_1 has a long-wave maximum at $\lambda = 710$ nm. Simultaneous irradiation of $MgPC^{2+}$ with light of two different wavelengths ($\lambda = 680$ and 710 nm) results in efficient population of state S_n ($\tau \sim 15$ ps), which is insufficient for the normal course of bimolecular reactions but sufficient for a photoinduced electron transfer which is completed within 0.3 ps.[106]

Laser-induced two-quantum photomodification of DNA sensitised by different intercalating dyes among which ethidium bromide proved to be the most efficient one has been studied.[113–115]

Site-specific two-quantum photomodification of ss- and ds-DNA with oligonucleotide conjugates of ethidium derivatives **20** and **21** has been carried out. Irradiation with a nitrogen laser (337 nm, $I = 150$ W m^{-2}) results in stepwise two-quantum excitation of the chromophore intercalated into DNA with subsequent modification of the target. The energy of two quanta was sufficient for initiating different photomodification reactions including the cleavage of phosphodiester bonds.[113,114] The focussed laser irradiation of the duplex



resulted in a spontaneous cleavage of the DNA-target at the residue G9 (yield $\sim 10\%$) and the formation of covalent adducts (yields 20%–40%).

The efficiency of spontaneous cleavage of the DNA-target showed a quadratic dependence on the irradiation power density. After piperidine-induced cleavage, the total degree of modification of the DNA-target increased due to cryptic modification and reached 90%. Unfocussed laser irradiation (2 W m^{-2}) of the duplex gave exclusively covalent adducts (yield 40%).[115]

The mechanism of two-quantum cleavage is not finally elucidated yet. However, it was shown that an addition of chemical traps of radicals and $^1O_2$ did not decrease substantially the efficiency of spontaneous cleavage. The majority of breaks are at a distance of not more than one base from the ethidium residue. Consequently, the migration radius of two-quantum excitation along the DNA chain does not exceed 4–5 Å.[113,114]

The attachment of ethidium **20** to the heptanucleotide 5′-ACAAACCp caused an increase in the melting temperature of the duplex with the complementary octanucleotide 5′-pTGTTTGGC from 23 to 50 °C. Irradiation of the duplex with a nitrogen laser (337 nm, $\tau = 8$ ns, $I = 1200$ W m^{-2}, 4 h) up to complete photodegradation of the ethidium residue in the case of the 5′-ACAAACCp-**20** conjugate gives rise to covalent adducts (yield 40%). Their treatment with piperidine induced the cleavage at the residues G6 and G7 and increased the degree of injury of the target to 65% due to cryptic modification.[50] Irradiation of the octanucleotide 5′-pTGTTTGGC duplex with the 5′-ACAA-ACCp-**21** conjugate yields 55% of covalent adducts and 5% of direct cleavage products. Treatment with piperidine induces cleavage at the same residues (G6 and G7) (total yield 70%).

Irradiation of a ds-DNA triplex with the 5′-$(TC)_6$T derivative of ethidium **20** results in a direct cleavage (yield 10%) of the polypurine chain at residues G(−1), A(0), A(1) and G(2) relative to the position of ethidium within the triplex. This was not accompanied by any noticeable photomodification of the polypyrimidine chain.[50]

The type of injuries and noticeable specificity of photomodification with respect to the electron-donor purine bases is consistent with the following mechanism. In the case of nanosecond duration of the laser pulse, the triplet state cannot be formed and a two-quantum excitation passes into state S_n and is accompanied by ionisation and formation of an ethidium radical cation and a free electron. The former causes the electron abstraction from the purine bases of DNA or hydrogen atoms from deoxyribose. The quantum yield of ethidium bromide-sensitised photodegradation of DNA is very low ($\varphi = 5 \times 10^{-8}$) due to a reverse electron transfer; however, in the presence of the chemical trap of electrons, *viz.*, methyl viologen, it increases by an order of magnitude.[116]

b. Two-quantum resonant triplet–triplet excitation

Binary systems of oligonucleotide reagents open up broad opportunities for the application of different mechanisms of two-quantum sensitisation in site-specific photomodification of

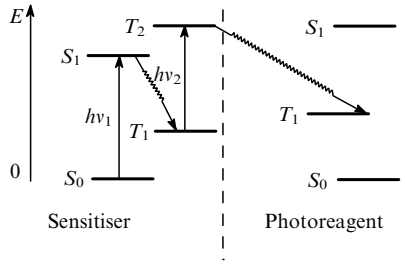


Figure 6. The distribution pattern of the ground and excited states of pyrene **43a** and perfluoroarylazide **34**.

DNA. Sensitised photomodification was carried out by irradiation with UV and visible light (365 – 580 nm) simultaneously of a complex of a DNA-target with a binary system of oligonucleotide conjugates **50** containing pyrene **43a** and azide **34** residues. This was accompanied by a sixfold increase (in comparison with irradiation at 365 – 390 nm) in modification rate and a change of its directionality from the guanine residue G11 to the thymine residue T13.[95] It was assumed that these conditions favour two-quantum triplet – triplet sensitisation of the azide **34** with the pyrene **43a** (its mechanism is shown in Fig. 6).

Pyrene has rather high quantum yield of intersystem crossing into the triplet state ($\varphi_{ISC} = 0.37$),[117] but the energy of the triplet level of pyrene [$E(T_1) = 48.7$ kcal mol^{-1}][118] is much lower than that of aromatic azides [$E(T_1) \approx 68$ kcal mol^{-1}].[104] Consequently, one-quantum triplet sensitisation of the azide **34** with pyrene is impossible in this case.

However, pyrene is characterised by intense triplet – triplet absorption in the visible region. The lifetime of the triplet state (T_1) is rather high ($\tau \approx 0.5$ s) and pyrene, being in this state, can absorb the second quantum [in this case, of visible light ($h\nu_2$)] in order to pass to higher triplet levels (Fig. 6). The energy of the excited triplet level of pyrene [$E(T_2) = 77.8$ kcal mol^{-1}][118] markedly exceeds the energy of the triplet state of the azide **34**. As a result, the two-quantum triplet – triplet transfer of energy from the sensitiser to the T_1-level of the photoreagent is thermodynamically allowed and should occur at a diffusionally controlled rate. The azide **34** excited by a triplet – triplet energy transfer further dissociates into nitrogen and the triplet nitrene **33**. The latter causes photomodification of the DNA-target with the formation of products characteristic of this biradical species.

Figure 7 presents the logarithmic dependences of the initial rates of accumulation of covalent adducts in the complexes **49** and **50** on the light flux intensity upon irradiation at various wavelengths. In these coordinates, the slope of the straight line

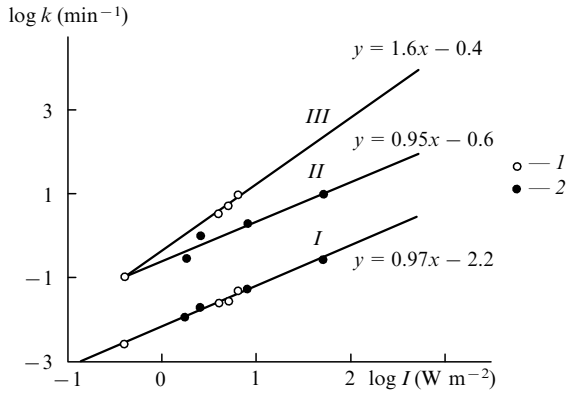
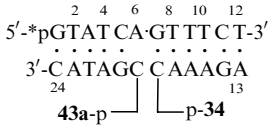


Figure 7. The dependence of the initial rate constant (k) of direct (I) and sensitised (II, III) photomodification of DNA on the light flux intensity (I) within the irradiation range/ nm: 365 – 440 (*1*), 365 – 390 (*2*).

corresponds to the reaction order with respect to light. It can be seen that under any irradiation conditions, the slope of the photomodification straight line is close to unity (straight line I). The slope of the straight line for sensitised photomodification upon irradiation with UV light (365 – 390 nm) is also close to unity (straight line II), whereas upon simultaneous irradiation with UV and visible light (365 – 580 nm) it is equal to 1.6 (curve III). This suggests that such irradiation conditions favour two-quantum DNA photomodification which occurs in parallel with one-quantum one.

The critical distance for a two-quantum triplet – triplet energy transfer is equal to 5 – 6 Å, which is 2 – 3 times less than that for one-quantum energy transfer.[119] The occurrence of two-quantum sensitisation within the complementary complex **50** suggests that the sensitiser and photoreagent residues can approach each other to a distance of up to 6 Å, at least at the moment of light quantum absorption. This was confirmed in 2D NMR studies of the structure of a complex of a binary system with a DNA-target.



The pyrene residue **43a** stabilises the complex by increasing the melting temperature by 7 °C due to hydrophobic interactions with heterocyclic bases. It is localised on the side of the small groove and reacts with the residues T4 – C5 – A6 – G7 of the DNA-target. The photoreagent occupies a position on the side of the major groove near the residue G7. The distance between the centres of pyrene and azide residues is equal to 8 – 9 Å. This convergence of the sensitiser and the photoreagent ensures highly efficient transfer.[100] Irradiation of the duplex results in the target photomodification at the residue G7.

Thus, the results presented above demonstrate high prospects of two-quantum methods for NA photomodification, since they allow the use of light of longer wavelengths and ensure a control over the reactivity of photoreagents under variable irradiation conditions.

V. The prospects for the use of photoreagents for targeted modification of nucleic acids *in vivo*

The mechanisms of photosensitised reactions (modification and cleavage of nucleic acids by the majority of photoreagents) considered in this review have not been finally elucidated. A more detailed knowledge of these mechanisms can be extremely useful for elaboration of novel, more efficient reagents capable of direct cleavage of phosphodiester bonds.

High specificity of photomodification is the most obvious advantage of oligonucleotide conjugates of psoralens and perfluoroaryl azides as photoreagents. Apart from the presence of an address, this is due to the absence of diffusing reactive species and the selectivity toward definite heterocyclic bases (residues T and U in the case of psoralens and residues G in the case of perfluoro azides).

Sensitised photomodification of NA with binary systems of oligonucleotide conjugates combines favourably the advantages of physical (*viz*., the catalytic effect of the sensitiser) and chemical sensitisation (*viz*., the lack of freely diffusing reactive species). Yet another merit of this approach is that the photoreactive centre is formed from two inert elements only after independent recognition of an extended sequence of the DNA target by two oligonucleotide addresses.

Two-quantum sensitisation has a number of advantages as regards the development of efficient approaches to targeted modification of nucleic acids *in vivo*. Excitation of reagents with two quanta of long-wave light allows one to increase considerably the specificity of sensitised photomodification of a DNA-target by inhibiting one-quantum side reactions, while a change in the

directionality of photomodification in the two-quantum sensitisation can be more useful in the study of biological complexes than with one-quantum sensitisation.

The use of nonresonant two-quantum excitation makes it possible to initiate photochemical reactions by irradiation with red and IR light but will hardly increase the specificity of photomodification. At such high peak intensities, all cell components which absorb in the UV region at a double frequency will be excited in a two-quantum mode. In this respect, two-quantum resonant excitation at two different wavelengths seems to be a more promising approach. In this case, only compounds the absorption of which is close to that of photosensitisers in both ground and excited states will be excited together with them, but the probability of this reaction is rather low.

The results described in the review suggest that a combination of binary systems of oligonucleotide conjugates with two-quantum excitation methods is the most helpful tool for selective photomodification of nucleic acids *in vivo*.

This review has been partially supported by the project 'Leading Research Schools' (Grant No. 96-15-97732).

References

1. S I Antsypovich, T S Oretskaya *Usp. Khim.* **67** 274 (1998) [*Russ. Chem. Rev.* **67** 245 (1998)]
2. C Helene, J-J Toulme *Biochim. Biophys. Acta* **1049** 99 (1990)
3. D G Knorre, V V Vlassov, V F Zarytova, A V Lebedev, O S Fedorova *Design and Targeted Reactions of Oligonucleotide Derivatives* (Boca Raton, FL: CRC Press, 1994)
4. E Braun, Y Eichen, U Sivan, G Ben-Yoseph *Nature (London)* **391** 775 (1998)
5. H P Spielmann, P A Fagan, T M Bregant, R D Little, D E Wemmer *Nucl. Acids Res.* **23** 1576 (1995)
6. R Kuroda, H Satoh, M Shinomiya, T Watanabe, M Otsuka *Nucl. Acids Res.* **23** 1524 (1995)
7. A S Boutorine, D Brault, M Takasugi, O Delgado, C Helene *J. Am. Chem. Soc.* **118** 9469 (1996)
8. V V Vlassov *The Lock and Key Principle* (Ed. J-P Behr) (New York: Wiley, 1994) p. 89
9. B Armitage, T H Koch, H Frydenlund, H Orum, H-G Batz, G B Schuster *Nucl. Acids Res.* **25** 4674 (1997)
10. D S Sigman, A Mazunder, D M Perrin *Chem. Rev.* **93** 2295 (1993)
11. I Siato, M Takayama, H Sugiyama, T Nakamura *NATO ASI Ser.* **479** 163 (1996)
12. D G Knorre *Bioorg. Khim.* **23** 3 (1997) [a]
13. D S Sigman *Biochemistry* **29** 9098 (1990)
14. G Laustriat *Biochimie* **68** 771 (1986)
15. R P Wayne *Principles and Applications of Photochemistry* (Oxford, New York, Tokyo: Oxford University Press, 1988)
16. J Cadet, M Berger, C Decarroz, J R Wagner, J E Van Lier, Y M Ginot, P Vigny *Biochimie* **68** 813 (1986)
17. R V Bensasson, E J Land, E J Truscott *Flash Photolysis and Pulse Radiolysis. Contributions to the Chemistry of Biology and Medicine* (Oxford, New York, Toronto: Pergamon Press, 1983)
18. N K Kochetkov, E I Budovskii, E D Sverdlov, N A Simukova, M F Turchinskii, V N Shibaev *Organicheskaya Khimiya Nukleinovykh Kislot* (Organic Chemistry of Nucleic Acids) (Moscow: Khimiya, 1970) p. 615
19. D N Nikogosyan *Laser Picosecond Spectroscopy and Photochemistry of Biomolecules* (Ed. V S Letokhov) (Bristol: Hilger, 1987) p. 212
20. H Gorner, M Wala, D Schulte-Frohlinde *Photochem. Photobiol.* **55** 173 (1992)
21. D Magda, R A Miller, M Wright, J L Sessler, B L Iverson, P I Sanson *NATO ASI Ser.* **479** 337 (1996)
22. D G Knorre, O S Fedorova, E I Frolova *Usp. Khim.* **62** 70 (1993) [*Russ. Chem. Rev.* **62** 65 (1993)]
23. J C Quada Jr, M J Levy, S M Hecht *J. Am. Chem. Soc.* **115** 12171 (1993)
24. I H Holdberg *Acc. Chem. Res.* **24** 191 (1991)
25. D A Dunn, V H Lin, I E Kochevar *Biochemistry* **31** 16620 (1992)
26. D B Hall, J K Barton *J. Am. Chem. Soc.* **119** 5045 (1997)
27. K Nakatani, J Shirai, S Sando, I Saito *J. Am. Chem. Soc.* **119** 7626 (1997)
28. B L Lee, K R Blake, P S Miller *Nucl. Acids Res.* **16** 10681 (1988)
29. E J Gunter, P A Havre, F P Gasparro, P M Glazer *Photochem. Photobiol.* **63** 207 (1996)
30. C Giovannangeli, N T Thuong, C Helene *Nucl. Acids Res.* **20** 4275 (1992)
31. F P Gasparro, P A Havre, G A Olack, E J Gunther, P M Glazer *Nucl. Acids Res.* **22** 2845 (1994)
32. P A Havre, E J Gunther, F P Gasparro, P M Glazer *Proc. Natl. Acad. Sci. USA* **90** 7879 (1993)
33. P A Havre, P M Glazer *J. Virol.* **67** 7324 (1993)
34. M Grigoriev, D Praseuth, A L Guieysse, P Robin, N T Thuong, C Helene, A Harel-Bellan *Proc. Natl. Acad. Sci. USA* **90** 3501 (1993)
35. J A Hartley, S R McAdam, S Das, M C Roldan, M K Haskell, M Lee *Anti-Cancer Drug Des.* **9** 181 (1994)
36. K E Rao, G Gosselen, D Mrani, C Perigaut, J L Imbach, C Bailly, J P Henichart, P Colson, C Houssier, J W Lown *Anti-Cancer Drug Des.* **9** 221 (1994)
37. C Bailly, J B Chaires *Bioconjugate Chem.* **9** 513 (1998)
38. G Delons, J-P Clarenc, B Lebleu, J-P Leonetti *J. Biol. Chem.* **269** 16 933 (1994)
39. K M Meisenheimer, T H Koch *Critical Rev. Biochem. Mol. Biol.* **32** 101 (1997)
40. A Favre, C Saintome, J-L Fourrey, P Clivio, P Laugaa *J. Photochem. Photobiol., B Biol.* **42** 109 (1998)
41. P Laugaa, A Woisard, J-L Fourray, A Favre *C. R. Hebd. Seances Acad. Sci., Life Sci.* **318** 307 (1995)
42. H W Pley, K M Flaherty, D B McKay *Nature (London)* **372** 68 (1994)
43. A Woisard, J-L Fourrey, A Favre *J. Mol. Biol.* **239** 366 (1994)
44. W G Scoott, J B Murray, J Arnold, B L Stoddard, A Klug *Science* **274** 2065 (1996)
45. C Bravo, F Lescure, P Laugaa, J-L Fourrey, A Favre *Nucl. Acids Res.* **24** 1351 (1996)
46. M I Dobrikov, V F Zarytova, N I Komarova, A S Levina, S A Lokhov, T A Prikhod'ko, G V Shishkin, D R Tabatadze, M M Zaalishvili *Bioorg. Khim.* **18** 540 (1992) [a]
47. A S Levina, D R Tabatadze, M I Dobrikov, G V Shishkin, L M Khalimskaya, V F Zarytova *Antisense Nucl. Acid Drug Develop.* **6** 119 (1996)
48. J-F Wang, W D Downs, T R Cech *Science* **260** 504 (1993)
49. J-F Wang, T R Cech *J. Am. Chem. Soc.* **116** 4178 (1994)
50. A A Koshkin, K Yu Kropachev, S V Mamaev, N V Bulychev, S G Lokhov, V V Vlassov, A V Lebedev *J. Mol. Recogn.* **7** 177 (1994)
51. N P Gritsan, A A Koshkin, A Yu Denisov, Yu Ya Markushin, E V Cherepanova, A V Lebedev *J. Photochem. Photobiol., B Biol.* **37** 40 (1997)
52. J Brunner *Annu. Rev. Biochem.* **62** 483 (1993)
53. U Henriksen, C Larsen, G Karup, C Jeppesen, P E Nielsen, O Buchardt *Photochem. Photobiol.* **53** 299 (1991)
54. M Chatterjee, S E Rokita *J. Am. Chem. Soc.* **112** 6397 (1990)
55. M Chatterjee, S E Rokita *J. Am. Chem. Soc.* **113** 5116 (1991)
56. M Chatterjee, S E Rokita *J. Am. Chem. Soc.* **116** 1690 (1994)
57. Y Chiang, A J Kresge, B Hellrung, P Schunemann, J Wirz *Helv. Chim. Acta* **80** 1106 (1997)
58. I Saito, M Takayama, T Sakurai *J. Am. Chem. Soc.* **116** 2653 (1994)
59. J M Kelly, M M Feeney, L Jacquet, A K-D Mesmaeker, J-P Lecomte *Pure Appl. Chem.* **69** 767 (1997)
60. A K-D Mesmaeker, I Ortmans, O V Gijte, W Bannwarth, in *The 30th International Conference on Coordination Chemistry (Abstracts of Reports), Kyoto, 1994* p. 68
61. C Mousheron, A K-D Mesmaeker, J M Kelly *J. Photochem. Photobiol., B Biol.* **40** 91 (1997)
62. C J Murphy, M R Arkin, Y Jenkins, N D Ghatlia, H S Bossmann, N J Turro, J K Barton *Science* **262** 1025 (1993)
63. D B Hall, R E Holmlin, J K Barton *Nature (London)* **382** 731 (1996)
64. K Nakatani, J Shirai, R Tamaki, I Saito *Tetrahedron Lett.* **36** 5363 (1995)
65. T Chen, J Michalak, M S Platz *Photochem. Photobiol.* **61** 600 (1995)
66. R Poe, K Schnapp, M J T Young, J Grayzar, M S Platz *J. Am. Chem. Soc.* **114** 5054 (1992)
67. G B Schuster, M S Platz *Adv. Photochem.* **17** 69 (1992)

68. K A Schnapp, R Poe, E Leyva, N Soundararajan, M S Platz *Bioconjugate Chem*. **4** 172 (1993)
69. M I Dobrikov, V V Gorn, V F Zarytova, A S Levina, T A Prikhod'ko, G V Shishkin, D R Tabatadze, M M Zaalishvili *Bioorg. Khim*. **18** 1190 (1992) [a]
70. A S Levina, D R Tabatadze, V F Zarytova, M I Dobrikov, V V Gorn, L M Khalimskaya *Bioorg. Khim*. **20** 21 (1994) [a]
71. A S Levina, D R Tabatadze, V F Zarytova, M I Dobrikov, V V Gorn, S A Lokhov *Bioorg. Khim*. **20** 30 (1994) [a]
72. A S Levina, D R Tabatadze, M I Dobrikov, G V Shishkin, V F Zarytova *Antisense Nucl. Acid Drug Develop*. **6** 127 (1996)
73. M I Dobrikov, R Yu Dudko, A S Levina, L M Khalimskaya, G V Shishkin *Bioorg. Khim*. **22** 200 (1996) [a]
74. A S Levina, M V Berezovsky, A G Veniaminova, M I Dobrikov, M N Repkova, V F Zarytova *Biochimie* **75** 25 (1993)
75. K N Bulygin, D M Graifer, M N Repkova, I A Smolenskaya, A G Veniaminova, G G Karpova *RNA* **3** 1480 (1997)
76. A V Kazantsev, G A Maksakova, O S Fedorova *Bioorg. Khim*. **21** 767 (1995) [a]
77. V V Koval', G A Maksakova, O S Fedorova *Bioorg. Khim*. **23** 266 (1997) [a]
78. S V Doronin, M I Dobrikov, M Buckle, P Roux, H Buc, O I Lavrik *FEBS Lett*. **354** 200 (1994)
79. M I Dobrikov, S V Doronin, I V Safronov, G V Shishkin *Khimiya v Interesakh Ustoichivogo Razvitiya* **2** 605 (1994) [b]
80. M I Dobrikov, S V Doronin, O I Lavrik *FEBS Lett*. **313** 31 (1992)
81. G Pratviel, J Bernadou, B Meunier *Angew. Chem., Int. Ed. Engl*. **34** 746 (1995)
82. H Sugiyama, Y Tsutsumi, K Fujimoto, I Saito *J. Am. Chem. Soc*. **115** 4443 (1993)
83. H Sugiyama, Y Tsutsumi, I Saito *J. Am. Chem. Soc*. **112** 6720 (1990)
84. R O Rahn, H G Sellin *Photochem. Photobiol*. **55** 309 (1992)
85. B S Rosenstein, R B Setlow, F E Ahmed *Photochem. Photobiol*. **31** 215 (1980)
86. T Hard, P Fan, D R Kearns *Photochem. Photobiol*. **51** 77 (1990)
87. C L Limoli, J P Day, J F Ward, W F Morgan *Photochem. Photobiol*. **67** 233 (1998)
88. C L Limoli, J F Ward *Radiat. Res*. **138** 312 (1994)
89. M I Dobrikov, S A Gaidamakov, A A Koshkin, N P Luk'yanchuk, G V Shishkin, V V Vlassov *Dokl. Akad. Nauk* **344** 122 (1995) [c]
90. V V Vlassov, M I Dobrikov, S A Gaidamakov, E K Gaidamakova, T I Gainutdinov, A A Koshkin *NATO ASI Ser*. **479** 195 (1996)
91. M I Dobrikov, S A Gaidamakov, A A Koshkin, G V Shishkin, V V Vlassov *Dokl. Akad. Nauk* **351** 547 (1996) [c]
92. M I Dobrikov, S A Gaidamakov, A A Koshkin, V V Vlassov *Dokl. Akad. Nauk* **351** 687 (1996) [c]
93. M I Dobrikov, S A Gaidamakov, E K Gaidamakova, T I Gainutdinov, A A Koshkin, V V Vlassov *Antisense Nucl. Acid Drug Develop*. **7** 309 (1997)
94. M I Dobrikov, S A Gaidamakov, A A Koshkin, T I Gainutdinov, N P Luk'yanchuk, G V Shishkin, V V Vlassov *Bioorg. Khim*. **23** 191 (1997) [a]
95. M I Dobrikov, S A Gaidamakov, A A Koshkin, T I Gainutdinov, N P Luk'yanchuk, G V Shishkin, V V Vlassov *Bioorg. Khim*. **23** 553 (1997) [a]
96. M I Dobrikov, S A Gaidamakov, G V Shishkin, V V Vlassov *Bioorg. Khim*. **24** 831 (1998) [a]
97. M I Dobrikov, S A Gaidamakov, T I Gainutdinov, E D Tenetova, G V Shishkin, V V Vlassov *Bioorg. Khim*. **25** 31 (1999) [a]
98. M I Dobrikov, S A Gaidamakov, T I Gainutdinov, T M Ivanova, V V Vlassov *Bioorg. Khim*. **25** 137 (1999) [a]
99. M I Dobrikov, S A Gaidamakov, T I Gainutdinov, E D Tenetova, G V Shishkin, V V Vlassov *Dokl. Akad. Nauk* **358** 403 (1998) [c]
100. E V Bichenkova, D S Marks, S G Lokhov, M I Dobrikov, V V Vlassov, K T Douglas *J. Biomol. Struct. Dyn*. **15** 307 (1997)
101. M I Dobrikov, T I Gainutdinov, V V Vlassov, in *The XIIIth International Round Table 'Nucleosides, Nucleotides and their Biological Applications' (Abstracts of Reports), Montpellier, 1998* p. 332
102. M I Dobrikov, T I Gainutdinov, V V Vlassov *Nucleosides Nucleotides* **18** 1517 (1999)
103. M Ya Mel'nikov, V A Smirnov *Fotokhimiya Organicheskikh Radikalov* (Photochemistry of Organic Radicals) (Moscow: Moscow State University, 1994) p. 90
104. L J Leyshon, A Reiser *J. Chem. Soc., Faraday Trans. 2* **11** 1918 (1972)
105. H A Sharifian, C-H Puin, F-B Jiang, S-M Perk *J. Photochem*. **30** 229 (1985)
106. K Teuchner, A Pfarrherr, H Stiel, W Freyer, D Leupold *Photochem. Photobiol*. **57** 465 (1993)
107. D Leupold, W Freyer *J. Photochem. Photobiol., B Biol*. **12** 311 (1992)
108. W G Fisher, W P Partridge Jr, C Dees, E A Wachter *Photochem. Photobiol*. **66** 141 (1997)
109. C R Lambert, H Stiel, D Leupold, M C Lynch, I E Kochevar *Photochem. Photobiol*. **63** 154 (1996)
110. D H Oh, R J Stanley, M Lin, W K Hoeffler, S G Boxer, M W Berns, E A Bauer *Photochem. Photobiol*. **65** 91 (1997)
111. G O Bekker, A V El'tsov *Vvedenie v Fotokhimiyu Organicheskikh Soedinenii* (Introduction to Photochemistry of Organic Compounds) (Leningrad: Khimiya, 1976)
112. D Leopold, I E Kochevar *Photochem. Photobiol*. **66** 562 (1997)
113. N V Bulychev, Doctoral Thesis in Chemical Sciences, Novosibirsk Institute of Bioorganic Chemistry, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, 1991
114. L Z Benimetskaya, N V Bulychev, A L Kozionov, A A Koshkin, A A Lebedev, S Yu Novozhilov, M I Shtokman *Bioorg. Khim*. **14** 48 (1988) [a]
115. L Z Benimetskaya, N V Bulychev, A L Kozionov, A V Lebedev, S Yu Novozhilov, M I Stockmann *Biopolymers* **28** 1129 (1989)
116. Z Shaquiri, E Keskinova, A Spassky, D Angelov *Photochem. Photobiol*. **65** 517 (1997)
117. F Wilkinson *Organic Molecular Photophysics* Vol. 2 (Eds J B Birks, J London) (New York: Willey, 1975) p. 95
118. H Labhart, W Heinzermann *Organic Molecular Photophysics* Vol. 1 (Eds J B Birks, J London) (New York: Willey, 1973) p. 297
119. V L Ermolaev, E N Bodunov, E B Sveshnikova, T A Shakhverdov, in *Bezyzluchatel'nyi Perenos Energii Elektronnogo Vozbuzhdeniya* (Nonradiative Transfer of Energy of Electron Excitation) (Leningrad: Nauka, 1977) p. 94

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