Translated from *Uspekhi Khimii*, **54**, 1420-1447 (1985) U.D.C. 577.112.4

Reactive Derivatives of Nucleic Acids and Their Components as Affinity Reagents

D.G.Knorre and V.V.Vlasov

The review is devoted to derivatives of nucleic acids and their components—nuclectides, nucleoside triphosphates, and oligonucleotides carrying reactive groups. Such derivatives are important tools for the investigation of protein-nucleic acid interactions and the functional topography of complex protein and nucleoprotein structures and can give rise to the prospect of being able to influence in a highly selective manner living organisms, including the nucleic acids and the nucleoproteins of the genetic apparatus. The review considers the principal groups of such reagents, the methods of their synthesis, and their properties which determine the possibility of their use for the selective (affinity) modification of biopolymers. The general principles of the construction of affinity reagents and their applications are analysed in relation to nucleotide affinity reagents. The bibliography includes 121 references.

CONTENTS

I. Introduction 836
II. Certain physicochemical characteristics of the affinity modification process 837
III. The principal problems solved by the method of affinity modification and the general principles governing the construction and use of affinity reagents 839
IV. Reactive derivatives of nucleosides and nucleoside mono-, di-, and tri-phosphates 843
V. Reactive derivatives of oligonucleotides 846
VI. Reactive derivatives of nucleic acids 847

I. INTRODUCTION

Among the inexhaustible variety of proteins in living nature, a large group of proteins possess the ability to interact specifically with nucleotides and nucleic acids having a definite structure. The substrates or effectors for hundreds of enzymes with different specificities are nucleotides, nucleoside di- and tri-phosphates, and nucleotide coenzymes. Virtually all the proteins and the nucleoproteins ensuring the storage, replication, and application of hereditary information in cells and viruses participate in selective interaction with nucleic acids. These are the enzymes of the DNA replication system, enzymes for the transcription and subsequent processing of the RNA formed, the repair enzymes ensuring the removal of damaged sections of DNA and their regeneration, and all the macromolecular components involved in the biosynthesis of proteins, including aminoacyl-tRNA-synthetases, ribosomes, and numerous translation factors. The study of the mechanism and, in the first place, the structural principles of the selective interaction of nucleic acids and nucleotides with proteins, frequently referred to as the protein–nucleic acid recognition, is one of the essential tasks of molecular biology and bio-organic chemistry.

The selective interaction between nucleic acids is of no less cardinal importance, although perhaps not in terms of quite so many aspects, in ensuring the operation of the key processes involved in vital activity. It is sufficient to recall that the specific interaction of the complementary nucleotide sequences constitutes the basis of the double helix structure of DNA established by Watson and Crick.[1]

One of the most effective methods for the study of the specific interactions of biopolymers with low-molecular-weight or polymeric partners (ligands) is chemical modification of biopolymers by means of reagents which retain the principal structural features of a specific ligand and hence the affinity for the polymer and at the same time carry a reactive group capable of chemical interaction with the biopolymer under conditions close to those which really obtain in its functioning. Such reagents have been called affinity reagents and the method itself is usually referred to as affinity labelling or affinity modification.[2,3] In the complex of a biopolymer with an affinity reagent the chemical reaction with the biopolymer takes place in the vicinity of the region of specific interaction (recognition), the ligand being covalently fixed in this region. The fragment of the affinity reagent ensuring a specific affinity for the biopolymer directs the reagent to a specific section on the surface of the protein molecule or to a specific sequence of nucleotides in the nucleic acid and therefore constitutes a kind of address which helps the reacting group to find its target. Affinity reagents can therefore be referred to also as addressed reagents.

Reactive derivatives of nucleic acids and their components are therefore important instruments for the investigation of the protein–nucleic acid recognition and the interaction between nucleic acids. These derivatives also lead to the prospect of being able to act in a highly selective manner on many key biochemical and molecular-genetic processes in living organisms.

This review is devoted to the chemistry of addressed nucleotide reagents, containing as the address nucleotides, nucleoside triphosphates, oligonucleotides, and nucleic acids, the methods of their synthesis, and their properties important for their use in affinity modification. Its aim is not to give a systematic account of all the numerous applications of the reagents for the solution of problems in biochemistry, molecular and cell biology, and medicine. These applications constitute merely individual, most thoroughly demonstrated and important examples. To a large extent the review is based on the studies carried out in the Biochemical Division of the Novosibirsk Institute of Organic Chemistry, Siberian Division of the USSR Academy of Sciences, which has now been transformed into the Novosibirsk Institute of Bio-organic Chemistry.

Reactive derivatives of nucleic acids and their components constitute one of the most systematically investigated groups of affinity reagents. The main principles of their construction, the problems arising in the use of such derivatives, and the ways in which they can be solved have therefore been elucidated in a particularly clear-cut manner in the course of their investigation. A special Section of the review is therefore devoted to the general questions of affinity modification in relation to addressed nucleotide reagents.

II. CERTAIN PHYSICOCHEMICAL CHARACTERISTICS OF THE AFFINITY MODIFICATION PROCESS

According to the main idea of the method of affinity modification, the reaction of a biopolymer P proceeds in a complex with an affinity reagent attached via a system of non-covalent interactions in the region where the biopolymer recognises a certain natural ligand L. The formation of the complex PL can be detected either by a number of physicochemical methods or from the manifestation of the biological function due to the interaction of P with L. In most of the examples below we are dealing with enzyme activity, a quantitative characteristic of which is the rate of the enzyme-catalysed reaction. This rate is proportional to the concentration of the enzyme–substrate complex or, in the case of a reaction involving two substrates, the concentration of the complex of the enzyme with both substrates. The recognition region is in this case part of the active centre of the enzyme.

The concentration of the complex PL is given by

$$[PL]=K[L]P_t/(1+K[L]),$$

where K is the constant for the association of P with L and P_t the total concentration of the biopolymer.

The affinity reagent, constructed on the basis of a natural ligand L and containing a reactive group X, can be represented by L–X. This reagent competes with L for the recognition region. In the presence of both compounds, the concentrations of the complexes of P with L and L–X are given by

$$[PL]=K[L]P_t/(1+K[L]+K_x[L-X]),$$
$$[P\cdot L-X]=K_x[L-X]P_t/(1+K[L]+K_x[L-X]),$$

where K_x is the constant for the association of P with L–X.

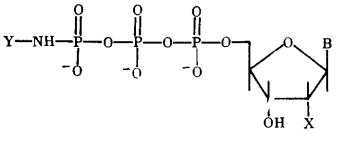
If a quantitative characteristic of a biological function F is proportional to the concentration of the complex, then, in the general case, where the complex P.L–X is also functionally active, we have

$$F=\alpha[PL]+\alpha_x[P\cdot L-X],$$

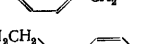
where α and α_x are proportionality coefficients. For an enzymic reaction, these characteristics are the rate constants for the conversion of the complexes PL and P.L–X into the enzyme–reaction product complex. If $\alpha_x = 0$, i.e. the complex with the reagent is functionally inactive, then L–X behaves as a competing inhibitor in relation to L.

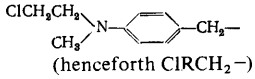
For the systems in which reactive derivatives of nucleic acids and their components have been investigated, several instances of the functional activity of affinity reagents are known.

Derivatives of nucleoside 5′-triphosphates, in the first place compounds (I, a–f), have found extensive applications in the study of nucleic acid polymerases:

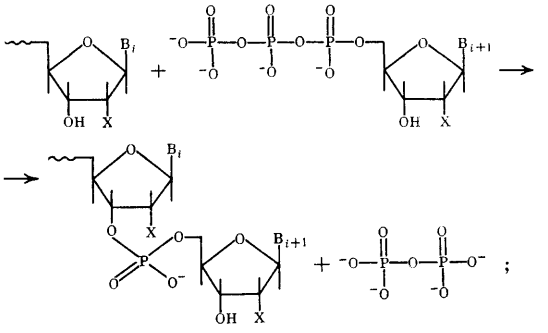


(Ia) B = adenine
(Ib) B = guanine
} X=OH, Y= 

(Ic) B = thymine X=H, Y= 

(Id) B = adenine, X = H
(Ie) B = thymine, X = H
(If) B = adenine, X = OH
} Y = 
(henceforth ClRCH$_2$–)

Nucleic acid polymerases catalyse the synthesis of DNA molecules (DNA polymerases) or RNA (RNA polymerases) by transferring the nucleotide residue from nucleoside 5′-triphosphate to the growing polynucleotide chain:



X = H for DNA polymerases;
X = OH for RNA polymerases.

Henceforth in the review we shall employ widely the nomenclature adopted in the nucleic acid chemistry and approved by the International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry (IUB).[4] According to this nomenclature, the orthophosphoric acid residue is designated by p and the nucleoside residue by the initial letter of its name. The symbol p to the left of that of the nucleoside means that the phosphate is linked to the 5′-hydroxy-group, while p on the right means that the phosphate is linked to the 3′-hydroxy-group of ribose or deoxyribose. In the case of deoxyribonucleosides, the letter d is placed before the nucleoside symbol. In this formulation the reaction catalysed by polymerases is represented as follows:

$$\sim pN^i + pppN^{i+1} \xrightarrow[\text{(RNA polymerase)}]{} \sim pN^ipN^{i+1} + pp \quad ; \qquad (1)$$
$$\sim pdN^i + pppdN^{i+1} \xrightarrow[\text{(DNA polymerase)}]{} \sim pdN^ipdN^{i+1} + pp \; .$$

The most important feature of nucleic acid polymerases is their dependence on the template nucleic acid, RNA or DNA. Accordingly, one distinguishes RNA-dependent and DNA-dependent polymerases. The template nucleic acid promotes, at each stage of chain propagation, the selection of a fragment for incorporation in the growing chain of one of four monomers, complementary to the template nucleotide located in the decoding section of the enzyme. Since after the addition of a new monomeric fragment, the template shifts by one unit relative to the decoding section and the selection is carried out by the next template nucleotide, the nucleic acid is synthesised with the nucleotide sequence complementary to the template nucleic acid.

Studies carried out with three nucleic acid polymerases from *Escherichia coli* showed that the γ-amides of nucleoside triphosphates, in particular the reactive derivatives (Ia) and (Ib), are substrates of the DNA-dependent RNA polymerase.[5,6] On the other hand compounds (Ic) and (Id) are effective affinity reagents for a DNA-dependent DNA polymerase (DNA polymerase I)[7] and the RNA-dependent DNA polymerase.[8] The γ-amide of dTTP (Ic) was not found to possess substrate properties in relation to the DNA polymerase I. It has been

shown in relation to the γ-anilide of adenosine 5′-triphosphates† that not the pyrophosphate but the pyrophosphate anilide [*N*-phenylpyrophosphorimidate?] is liberated in the synthesis of RNA from this analogue.

The key enzymes in the biosynthesis of proteins are aminoacyl-tRNA-synthetases. Each of them catalyses the addition of a specific aminoacid to the corresponding transport RNA by forming an ester linkage between the aminoacid carboxyl and the OH group at the 3′-end of RNA. The process is coupled with the hydrolysis of ATP to adenosine 5′-monophosphate (AMP) and pyrophosphate:

$$RCH(NH_3^+)COO^- + pppA + tRNA - OH \rightleftarrows tRNA{-}OCOCH(NH_3^+)R + pA + pp \quad (2)$$

The reagent (Ia) is a competitive inhibitor for a broad spectrum of aminoacyl-tRNA-synthetases and cannot replace ATP as the substrate.[9–11] However, in the case of leucyl-tRNA-synthetases isolated from the chloroplasts and cytoplasm of *Euglena gracilis*,[12] compound (Ia) proved to be a substrate capable of replacing ATP in reaction (2).

The biosynthesis of protein takes place on ribosomes and is programmed by the template (information) RNA (mRNA). The aminoacid sequence in the protein synthesised is programmed in the mRNA by a sequence of trinucleotides (codons) which select from a mixture of aminoacyl-tRNAs one possessing an anticodon specifically recognised by the corresponding codon. The corresponding aminoacid is thereby selected. For example, polyuridylic acid $(pU)_n$ promotes the selection of phenylalanyl-tRNA with the anticodon –pGpApA–.

Analogously polyadenylic acid $(pA)_n$ promotes the selection of the ribosomal lysyl-tRNA. The selected aminoacyl-tRNA reacts with the growing polypeptide chain, present on the ribosome in the form of peptidyl-tRNA, in the following manner:

$$\begin{aligned} &NH_3^+CH(R^1)CO-NHCH(R^2)CO-\ldots NHCH(R^i)CO-tRNA^i + \\ &+NH_3^+CH(R^{i+1})CO-tRNA^{i+1} \rightarrow NH_3^+CH(R^1)CO-\ldots \\ &-NHCH(R^i)CO-NHCH(R^{i+1})CO-tRNA^{i+1}+tRNA^i. \end{aligned} \quad (3)$$

In the presence of polyuridylic acid and even of its short fragments down to $(pU)_3$, phenylalanyl-tRNA is therefore bound to the ribosome. In the presence of polyadenylic acid, Lys-tRNA is bound to the ribosome. It has been found that the alkylating derivatives of oligonucleotides $(pN)_n$, carrying the *N*-2-chloroethyl-*N*-methylaminophenyl fragment ClR either at the 5′- or the 3′-end also stimulate the binding to the ribosome of the aminoacyl-tRNA coded by them, i.e. the introduction of a reactive group in the 3′- or 5′-position does not disturb the functional activity of the oligonucleotide.[13–15]

Thus the affinity reagents L–X either function together with the ligand L or behave as competitive inhibitors of the function performed by the biopolymer. Naturally, reversible inhibition must be studied under conditions where the chemical activity of L–X, resulting in the covalent binding of L–X to the recognition region of the biopolymer, is not manifested. This can be readily achieved in the case of photoactive reagents, for example the reagents (Ia) and (Ib), which react under ultraviolet irradiation, which converts the azide, chemically inert under physiological conditions, into the highly reactive nitrene biradical. For this, it is sufficient to investigate the functional activity of P in the presence of L and L–X in the dark. Otherwise, it is necessary to employ fairly rapid tests for functional activity carried out over a period significantly shorter than the time of the chemical reaction of L–X in the complex with P. For certain reagents it is possible to convert the reactive derivative into an inactive derivative with a very similar structure. For example, having kept the reagents (Id) and (If) in water for a time sufficient to hydrolyse the C–Cl bond, it is possible to obtain from $ClRCH_2NHpppN$ the derivative $HORCH_2NHpppN$ similar in structure and hence in affinity to the biopolymer, to investigate its inhibiting activity as a function of the polymer being studied, and hence to estimate its affinity for the biopolymer.

The rate of the affinity modification is

$$v = k[P{\cdot}L-X] = \frac{kK_x[L-X]P_t}{1+K[L]+K_x[L-X]},$$

where k is the rate constant for the reaction of L–X with P in the complex P.L–X.

When the affinity reagent (and the natural ligand when the latter has been introduced into the reaction mixture) are present in a sufficient excess relative to the polymer and do not undergo side reactions, we have

$$v = \frac{kK_x[L-X]_0P_t}{1+k[L]_0+K_x[L-X]_0},$$

where $[L]_0$ and $[L{-}X]_0$ are the initial concentrations. Since the total concentration of the biopolymer decreases in affinity modification, we have

$$P_t = (P_t)_0 - [L-X-P],$$

where $(P_t)_0$ is the initial concentration of the biopolymer and L–X–P is the product of the affinity modification. In this case the process is a pseudofirst-order reaction:

$$v = \frac{d[L-X-P]}{dt} = \frac{kK_x[L-X]_0}{1+K[L]_0+K_x[L-X]_0}\{(P_t)_0 - [L-X-P]\}.$$

It follows from this expression that the dependence of the apparent rate constant on the concentration of the affinity reagent is hyperbolic and the natural ligand inhibits the affinity modification process (it is usually said that it protects the polymer against affinity modification). Both these findings are important criteria, whose fulfillment shows that the process investigated proceeds via the affinity modification mechanism.

If the addressed section of the reagent is fixed in the recognition region as a result of the affinity modification, the interaction of the natural ligand L with the biopolymer P is significantly hindered or becomes altogether impossible, i.e. the biopolymer becomes functionally inactive. Assuming that the functional activity of the biopolymer, retained at a specific instant, is proportional to the concentration of the unmodified biopolymer, it is possible to follow the course of the affinity modification from the inactivation of the biopolymer, which is described by a kinetic equation for a pseudo-first-order reaction in the presence of a sufficient excess of L–X in relation to the polymer:

$$-\frac{dF}{dt} = k_{app}F = \{kK_x[L-X]_0/(1+K_x[L-X]_0\}F.$$

All the equations presented were derived on the assumption that L–X is consumed only in the reaction with the polymer in the complex P.L–X. If the reagent has been taken in excess relative to P, the reaction proceeds until the polymer has been completely consumed. However, in fact, sufficiently active groups X can as a rule react also with water, with the buffer components of the medium, and also with the biopolymer itself without preliminary complex formation, including its sections which have no direct bearing on the recognition region. The group X then reacts and loses its reactivity, while the address remains unaltered, i.e. the affinity for the

†Adenosine 5′-triphosphate (ATP) is the substrate or effector in hundreds of different enzymic reactions and its reactive derivatives and analogues are widely used in the study of the corresponding enzymes.

biopolymer is retained. The reaction product L–R becomes an inhibitor of the affinity modification with a constant K_r, which is the constant for the association of L–R with P. A kinetic analysis of this process has been carried out[16] on the assumption that the reagent has been taken in significant excess. The equation of the kinetic curve in the general case where $K_r = K_x$ is

$$[L-X-P]/(P_t)_0 = 1 - \left[\frac{1+K_x[L-X]_0}{1+K_r[L-X]_0-(K_r-K_x)[L-X]_0\exp(-k_0 t)}\right]^{\frac{\gamma K_x}{K_r-K_x}}.$$

In this case the reaction does not go to completion, i.e. the degree of conversion of P into L–X–P does not reach unity even in the limit:

$$\{[L-X-P]/(P_t)_0\}_{t=\infty} = 1 - \left(\frac{1+K_x[L-X]_0}{1+K_r[L-X]_0}\right)^{\frac{\gamma K_x}{K_r-K_x}}, \quad (4)$$

where k_0 is the pseudofirst-order rate constant for the reaction of L–X in solution with water and other components and $k_0\gamma$ is the rate constant for the conversion of the complex P.L–X into the affinity modification product. The degree of conversion at the end of the reaction can be measured with a sufficient accuracy even when the affinity modification is rapid and it is difficult to obtain the kinetic characteristics of the process. Evidently, in a suitable range of concentrations, it is possible to obtain from Eqn.(4) the association constants K_x and K_r as well as the quantity γ, which are present in Eqn.(4) as independent parameters.

The kinetics of the affinity modification become complicated still further when the interaction of P with L–X is a complex reaction proceeding via the formation of active intermediate species. This process has been considered by Knorre and co-workers,[17,18] such consideration being outside the framework of this review.

III. THE PRINCIPAL PROBLEMS SOLVED BY THE METHOD OF AFFINITY MODIFICATION AND THE GENERAL PRINCIPLES OF THE CONSTRUCTION AND USE OF AFFINITY REAGENTS

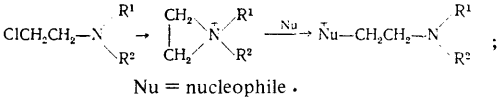
The nature of the reactive group of the affinity reagent, the method of introducing it into a natural ligand or its analogue, the carrier of affinity for the biopolymer (address), and the method used to carry out the affinity modification are to a large extent determined by the problem which has to be solved with the aid of the given reagent. Therefore, before proceeding to a systematic description of the different types of reactive derivatives of nucleic acids and their components, we shall consider the principal problems in the solution of which the method of affinity modification is used and the requirements arising from this which must be met by the affinity reagents and by the procedures employed for the affinity modification.

We shall begin with the question of the investigation of the region of the specific interaction of the biopolymer with the ligand with the aid of a reactive derivative of the ligand.

In its most simplified form, this approach presupposes that the addressing component of the reactant is fairly rigidly fixed in the recognition region via non-covalent interactions, after which the biopolymer is attacked by the reacting group and the reagent is covalently fixed in the same region. For a biopolymer with a known primary structure, it is then determined which particular aminoacid or nucleotide residue has been chemically modified. The monomeric fragment located in the recognition region or in its immediate vicinity is thereby found. If the three-dimensional structure of the biopolymer is known and data or fairly reliable ideas about the conformation of the reagent are available, it is possible to determine the entire region in which the biopolymer interacts with the address.

Clearly the rigid fixation of the address should sharply limit the region of contact between the biopolymer and the reacting group and hence the region in which chemical modification can occur. For it to actually occur, it is necessary that in this region there should be at least one group capable, in principle, of chemical interaction with the reagent employed. This is more probable the less selective the reacting group.

From this point of view, among heterolytic processes the most favourable are the reactions of strong and only weakly selective electrophiles with numerous nucleophilic centres of proteins and nucleic acids. The latter include the imidazole rings of the histidine residues, the ε-amino-groups of the lysine residues, the carboxy-groups of the aspartate and glutamate residues, the hydroxy-groups of the serine, threonine, and tyrosine residues, the SH groups of the cysteine residues, the carbonyl oxygen of the peptide bonds and glutamine and asparagine residues, the terminal amino- and carboxy-groups of the polypeptide chains, many positions in the heterocyclic bases of nucleic acids, and the phosphodiester internucleotide and terminal phosphomonoester groups of the polynucleotide chains. Among the reacting groups of this type in affinity reagents based on nucleic acids and their components, the moderately reactive CH_2ICO and CH_2BrCO groups and the aromatic and aliphatic 2-chloroethylamino-groups have found the greatest applications at the present time. The former react via a direct mechanism and the latter via the intermediate formation of highly electrophilic ethyleneimmonium cations:

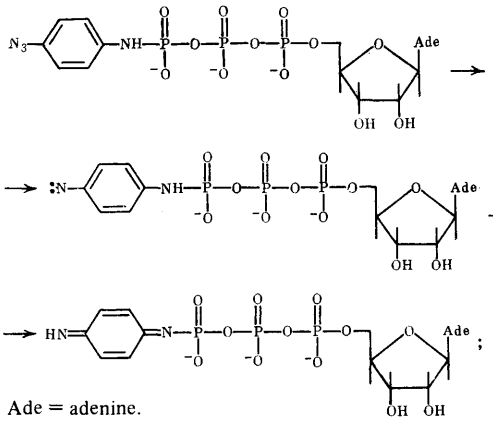


Nu = nucleophile.

For aromatic 2-chloroethylamines, the first stage is rate-limiting[19] and the intermediate cations are extremely reactive and do not accumulate in measurable concentrations, reacting almost instantaneously with nucleophiles, including water, which is invariably present when biopolymers undergo affinity modification. Their reactivity in relation to nucleophiles has come to be characterised by the competition factor, which is defined as the ratio of the rate of reaction with the nucleophile to the rate of reaction with water divided by the concentration of the nucleophile.[20]

Pt^{2+} derivatives have also been proposed recently as the reacting groups in affinity reagents for attack on nucleophilic centres.[21,22]

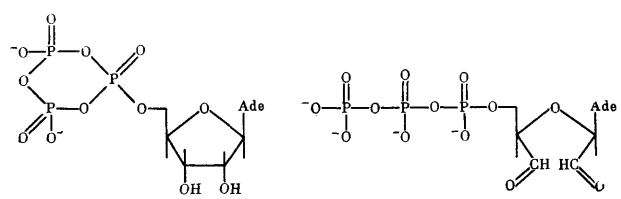
According to data obtained in anhydrous model systems, the singlet nitrene R–N:, capable of being inserted in X–H bonds, including C–H bonds, is even less selective. Derivatives of aryl azides, which generate nitrenes on irradiation with ultraviolet light, have therefore found extensive applications as affinity reagents. The potentially singlet nitrene can modify even the side groups in hydrophobic aminoacids–alanine, valine, leucine, isoleucine, phenylalanine, and proline. However, recent investigations of the behaviour of nitrenes in aqueous solution have shown that rapid secondary reactions, which depend significantly on the nature of other groups in the same benzene ring, are characteristic of these species.[23–24] Thus it has been established that compound (Ia) namely the γ-p-azidoanilide of ATP, is rapidly converted into a quinone imine imide on irradiation of its aqueous solution with ultraviolet light.[25]



Ade = adenine.

This compound is a powerful electrophile which reacts with imidazole, morpholine, and mercaptoethanol, but its selectivity is significantly higher than that of nitrenes.

For reagent (Ia) as an example, it is seen that, apart from the address, which in the given instance is the ATP residue, and the reacting group, it contains the connecting phenylamide fragment (spacer). For a rigid fixation of the address, the modification takes place in this instance outside the recognition region. Such reagents have come to be called exoreagents. For the reacting group to come into contact with the active centre, it is desirable that it be part of the composition of the address itself, i.e. that an endoreagent be employed. Naturally, this can be done only at the expense of some distortion of part of the address, having made sure that such distortion does not alter sharply the affinity for the biopolymer. Adenosine 5'-trimetaphosphate (II) (ATMP) or the product of the oxidation of ATP with periodatate at the *cis*-diol group (oATP) may serve as examples of endoreagents.



ATMP oATP

The former is a powerful phosphorylating reagent and, as a result of the modification, the ATP residue is directly bound to the nucleophilic centre of the biopolymer. The latter is capable of forming Schiff bases with the ε-amino-groups of lysine residues via the aldehyde groups.

A number of attempts at direct photochemical cross-linking of nucleic acids and their components in complexes with biopolymers have been described. By virtue of the short life of the excited state, one may expect that modification takes place directly in the region of the interaction of the chromophore with the biopolymer. However, the nature of the products formed and their stability, including their stability under conditions necessary for the subsequent identification and localisation of the modified fragment, have so far been little investigated and it is as yet impossible to assess the likely usefulness of this approach for the determination of the recognition region.

As already mentioned, the idea of the rigid fixation of the address is not itself non-controversial. Any recognition is ensured by a multipoint interaction. Less populated states with interaction via an incomplete set of points should therefore exist in the biopolymer–ligand complex together with the state in which all the contact points are employed. As a result of this, the reacting group can acquire a significant mobility. Biopolymers are also not rigid molecules and comprise different conformations in different proportions, as shown by the ability of many biopolymers to undergo specific conformational transitions. The biopolymer–ligand complex can therefore be represented by a multiplicity of states with different mutual orientations of the reacting group and the reactive sections of the biopolymer. The probability of affinity modification is determined not only by the population of a particular state but also by the extent to which this state is favourable for chemical reaction. Therefore, as a result of the modification of the biopolymer by the affinity reagent, the address may be fixed in a state which differs significantly from the functionally significant state and can actually be displaced from the recognition region, as shown schematically in Fig. 1.

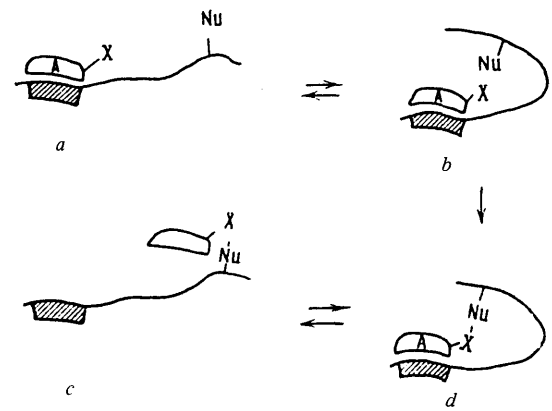


Figure 1. Schematic representation of the modification of a biopolymer at the nucleophilic centre by means of an Nu reagent consisting of the address A and the electrophile X, which leads to the fixation of the address outside the recognition region (shaded part): *a*) predominant conformation of the biopolymer–reagent complex; *b*) conformation present in a small proportion but having a favourable mutual disposition of the reacting groups; *c*) the same conformation after the reaction; *d*) predominant conformation of the modification product.

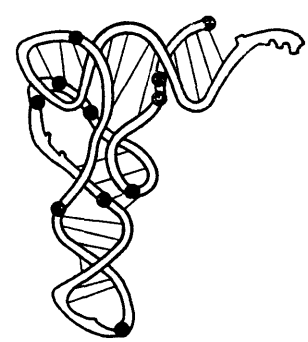


Figure 2. The positions in the structure of phenylalanine tRNA from *E. coli* of the guanosine residues (filled circles) through which groups capable of photoactivation are introduced statistically in the preparation of the azido-tRNA affinity reagent.[37]

It follows from all the foregoing features that even small changes both in the recognition region of the biopolymer and in its immediate environment as well as in the structure of the reagent can exert a drastic influence on the process and the result of the affinity modification. We shall present several examples.

As already stated, compound (Ia) is a competitive inhibitor of aminoacyl-tRNA synthetase from *E.coli*[9] and tryptophanyl-t-RNA synthetase from the ox pancreas.[10] In both cases ultraviolet irradiation of the complex of compound (Ia) with the enzyme entails the covalent addition of the reagent to the enzyme. However, whereas in the latter case it is accompanied by the inactivation of the enzyme, the addition of up to 20 residues of the reagent to phenylalanyl-tRNA synthetase does not lead to the loss of kinetic activity, i.e. the address remains fixed outside the ATP recognition region. The replacement of the *p*-azidoaniline residue in compound (Ia) by the *p*-azido-*N*-methylbenzylamine residue gives rise to a reagent which adds photochemically to phenylalanyl-tRNA synthetase and inactivates the enzyme.[26]

On irradiation with ultraviolet light, the reagents (Ie) and (Id) inactivate the RNA-dependent DNA polymerase and DNA polymerase(I) from *E.coli*. However, in order to obtain the same level of inactivation of the enzyme a concentration of the reagent lower by a factor of 50 is required in the first case compared with the second case.

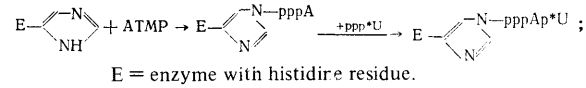
The reagent (If) has been investigated as an affinity reagent in relation to yeast hexokinase—the key enzyme in carbohydrate metabolism, catalysing the transfer of the orthophosphate residue from ATP to the 6-hydroxy-group of glucose. Depending on the pH of the medium, the enzyme exists in the monomeric or dimeric form. It has been found that compound (If) is not a competitive inhibitor of the monomeric hexokinase and accordingly does not inactivate the active centre of the enzyme. On the other hand, it has an affinity for the dimeric form of hexokinase comparable to that of ATP and inhibits this enzyme irreversibly.[27] It is of interest to note that an analogous derivative of AMP, namely $ClRCH_2NHpA$, inhibits irreversibly the monomeric form of hexokinase via the affinity modification mechanism, but does not act on its dimeric form.[28]

Thus the same reagent can behave very differently in relation to biopolymers fulfilling isotypical functions or in relation to different states of the same biopolymer. On the other hand, the examples presented have shown that, by introducing certain changes into the structure of the address of the spacer fragments, it is usually possible to find a suitable affinity reagent for the biopolymer investigated.

Together with a change in the structure, it is possible to influence the results of the affinity modification by altering the method by which it is carried out. A procedure has been proposed[29] which can find fairly wide-scale applications in the affinity modification of enzymes catalysing two- and three-substrate reactions. The initial stage of the reaction catalysed by the RNA polymerase from *E.coli* has been investigated and it was found that in the presence of the substrates pppA and pppU in conjunction with the template employed (a fragment of the T7 bacteriophage DNA) the enzyme catalysed the reaction

$$pppA + pppU \rightarrow pppApU + pp\ .$$

The use of ATMP as the affinity reagent can lead to its non-specific modification by virtue of its high reactivity. However, when ATMP was added to the enzyme, the subsequent addition of the second unit of the polynucleotide chain is possible only when the PPPA fragment is bound in the region of the active centre of the enzyme. When non-radioactive ATMP and UTP labelled with ^{32}P in the α-position in the triphosphate fragment are used, the radioactive label is found only in the section corresponding to the active centre of the enzyme. It has been shown that the addition is to a histidine residue:[29]



E = enzyme with histidine residue.

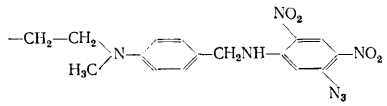
All that has been said above demonstrates that the synthesis and application of affinity reagents, suitable for the location of individual sections of monomers in the recognition region, is by no means the trivial task it might have appeared to be on the basis of a simplified model of affinity modification. It is therefore not surprising that only a limited number of reliably interpreted results have so far been obtained on these lines.

The problem of elucidating the functional significance of individual subunits in multisubunit structures with the aid of affinity modification appears much simpler. In this case it is sufficient that the reaction should have occurred within the limit of the subunit which interacts with the address. The greatest number of studies of this kind have been carried out with ribosomes and in particular with the ribosomes from *E.coli*, for which the primary structure of all the RNA molecules constituting the ribosome (ribosomal RNA or rRNA) and all the 53 proteins are now known. The identification of the regions corresponding to the binding of mRNA, namely two tRNA molecules carrying the peptide and aminoacyl residues (the P and A sites in ribosomes), as well as the region catalysing the transfer of the peptide residue to the aminoacyl-tRNA, the so-called peptidyltransferase centre, is of primary interest. Since the region where the codon–anticodon interaction takes place is of greatest importance for the mRNA recognition region, reactive derivatives of oligonucleotides containing one or several codons are widely used. Since the modifying proteins and rRNA fragments are identified by electrophoresis on the basis of the determination of electrophoretic mobilities, it is important that the large oligonucleotide or polynucleotide should be removed before identifying the modified component. For this purpose, it is convenient to employ reagents from which the address can be split off under fairly mild conditions. The oligoribonucleotide derivatives $ClRCH_2NH(pN)_n$ and $(pN)_n > CHRCl$, mentioned in the previous Section, in which the alkylating fragment is joined to the address via either a phosphoramide or an acetal linkage, satisfy this condition particularly well. Both types of linkage are fairly stable under the conditions of affinity modification but readily hydrolyse in a weakly acid medium.

In many cases the region of the interaction of the polymeric address with a complex subunit structure is fairly extensive. In particular, the regions of the interaction of mRNA and tRNA with a ribosome embrace a number of components of the ribosome, both rRNA and proteins. In order to identify this region by the traditional procedure based on the use of an individual compound as the affinity reagent, it is necessary to have a set of reagents with different positions of the reacting group relative to the address. Thus, in a study of the region in which mRNA is bound to ribosomes from *E.coli* and from rat liver[14,30–32] with the aid of alkylating derivatives of oligouridylates, it was found that both the distribution of the label between rRNA and the proteins and the sets of the modified proteins differ sharply depending on the end (3′ or 5′) of the oligonucleotide address to which the *N*-2-chloroethyl-*N*-methylaminophenyl fragment is linked.

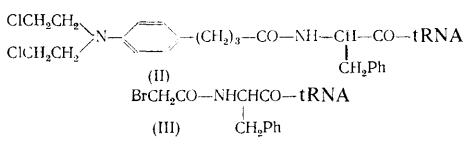
The synthesis of a large set of derivatives with different fixed positions of the reactive groups is fairly laborious for a polymeric address, for example, tRNA. It is therefore convenient to use as reagents for the first stage of the investigation derivatives in which the active groups are scattered statistically throughout the polymer molecule. The number

of such groups in each individual tRNA molecule should be small, not more than 2–3, in order not to disturb the ability of tRNA to interact specificially with the ribosome. tRNA derivatives containing fragments capable of being photoactivated have been synthesised for this purpose:



They are bound covalently to numerous tRNA guanine residues (Fig.2).[33] When 1–2 such residues are present on the molecules of the phenylalanine tRNA from *E.coli*, the latter retain the ability to bind phenylalanine enzymically and the resulting phenylalanyl-tRNA is bound selectively to the ribosome in the presence of poly-U. There derivatives (azido-tRNA) have found extensive applications in the study of the regions in which tRNA is bound to the ribosome in various functional states of the latter.[34,35]

The problem of the region corresponding to the interaction of phenylalanine tRNA with phenylalanyl-tRNA synthetase from *E.coli* has also been solved with the aid of azido-tRNA.[33] The above enzyme consists of two types of subunits—α and β. The problem was initially investigated with the aid of alkylating derivatives of tRNA carrying reactive groups on the aminoacyl fragment:



At first sight contradictory results were obtained: the first reagent alkylated predominantly the α-subunit[36] while the second alkylated mainly the β-subunit.[37] The use of azido-tRNA showed that, when the complex of the enzyme with the above derivatives is irradiated, tRNA adds predominantly to the β-subunit.[33] The fact that, for a substituent of small size at the α-amino group, the β-subunit is also alkylated and that after an increase in the size of the substituent mainly the α-subunit is alkylated permitted the additional conclusion that the acceptor end of tRNA in the complex with the enzyme is located near the region where the subunits come into contact.

Yet another problem in the solution of which affinity reagents can find extensive applications is that of the determination of the primary structure of large polymer molecules, for example, DNA. An important stage in the determination of the primary structure is the selective cleavage of the large polymer molecule into fragments of moderate length. In relation to DNA, this problem is solved with the aid of special restriction enzymes, which selectively cleave DNA in the vicinity of specific nucleotide sequences. At the same time, there are many chemical methods for the cleavage of DNA. For example, alkylation of the purine bases leads to their elimination, which makes it possible to cleave DNA under alkaline conditions in the depurinated sections. DNA is also cleaved in the presence of Fe^{2+} ions and O_2. For this purpose Fe^{2+} should be chelated, which is achieved either with the aid of special antibiotics, for example, bleomycin, or with the aid of model chelating agents, such as ethylenediamine-tetra-acetic acid.[38] The cleavage of DNA with the aid of dyes on irradiation by a 337 nm laser having a high irradiation density has been described recently.[39] The cleavage takes place as a result of the absorption by the dye of two quanta and the subsequent radiationless excitation energy transfer to the σ-bonds of the ribose phosphate skeleton.

The addition of the corresponding reactive groups to oligonucleotides of specific structure leads to the prospect for the selective cleavage of DNA and RNA in the vicinity of sections complementary to the addressing oligonucleotides. Fig.3 presents the results obtained when a DNA fragment is treated by an addressed alkylating reagent complementary to the single-stranded section of this fragment.[40]

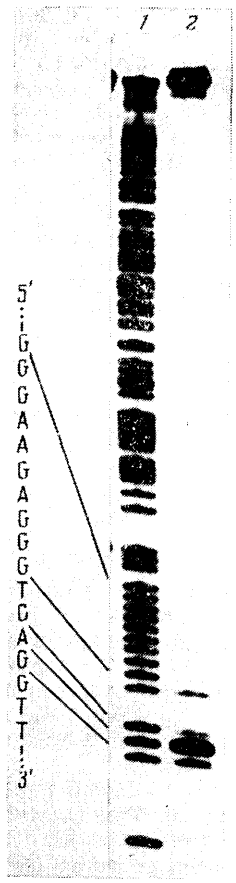


Figure 3. Results of the electrophoretic analysis in a polyacrylamide gel (autoradiograph) of the products of the cleavage of the 303-nucleotide single-stranded DNA fragment labelled at the 3'-end with the ^{32}P radioisotope after the complementarily addressed alkylation with the reagent $ClRCH_2$. .NHd(pTpGpApCpCpCpTpCpTpTpCpCpCpA) which is complemetary to the sequence of the fragment TpGpGpGpApApGp. ApGpGpGpTpCpA and after treatment with piperidine in order to achieve cleavage at the alkylated purines: *1*) products of the partial degradation of the fragment at the purine nucleotides (marker for the determination of the cleavage sites); *2*) products of the degradation of the fragment obtained with the aid of the complementarily addressed modification.[40]

In all the cases described above we were dealing with the use of affinity reagents as research instruments. However, another application of these reagents has begun to develop vigorously, namely the application involving specific action on biological processes. We shall consider this aspect in

relation to the approaches whereby nucleic acids, i.e. the genetic apparatus, can be specifically acted upon.

It is known that chemical influences on nucleic acids have found extensive applications for the preparation of modified forms of living organisms resulting from a change in the DNA structure (mutations). Such influences on nucleic acids are used in oncology for the suppression of the multiplication of malignant tumour cells, in certain special situations for the suppression of the immune response by the organism, for example, for the prevention of the rejection of surgically transplanted kidneys, and to regulate the multiplication of viruses in viral infections. In all the cases described one is dealing with relatively unselective reagents, which make an impact on all nucleic acids. Thus, in chemical mutagenesis the changes occur statistically in all the genes, generating numerous, as a rule unfavourable or even lethal, changes in the genetic apparatus. However, when one is dealing with micro-organisms or plants, the few mutations which impart to the organism a feature which is of interest from the practical point of view can be discovered by subsequent selection. Such selection can lead to the creation of new forms of plants and micro-organisms. When chemical reagents acting on nucleic acids, namely derivatives of 2-chloroethylamine, *cis*-dichlorodiammineplatinum, and others are used, in some cases it is possible to select doses which effectively arrest the growth of the tumour but are not yet lethal for the human organism as a whole. In those few cases where this can be achieved, chemotherapy makes it possible to prolong life and sometimes also to effect a radical cure of the patient.

The prospect of increasing the selectivity of such reagents and in the ideal case of creating reagents which would suppress the multiplication of viruses or cancer cells without harming healthy cells and would also suppress the multiplication of the immunocompetent cells (T-lymphocytes) which are designed to reject transplanted organs but would not act on cells fulfilling other numerous protective functions in the living organism is attractive. One of the imaginable pathways leading to the fulfillment of this aim involves the creation of complementarily addressed reagents on the basis of oligonucleotides or nucleic acids complementary to the affected target. This problem was formulated in a general form for the first time by Summerton.[41]

In order to realise fully this prospect, it is necessary to solve a number of new problems. In the first place, we are dealing with the creation of reagents acting on nucleic acids but at the same time possessing components having the same chemical composition and acting as the address. It is necessary to prevent to the maximum possible extent the action of the reacting group on the address itself, particularly in the numerous preliminary operations. One of the possible procedures involves the application of reacting groups whose reactivity is regulated in some way. It has been suggested[42] that the *N*-2-chloroethylalkylaminophenyl group, having the formyl residue in the *para*-position in the benzene ring, be used for this purpose. As a result of the strong electron-accepting effect of the formyl group, the alkylating properties of the 2-chloroethylamino-group are weak. This makes it possible to obtain nucleic acid derivatives of interest to the experimenter virtually without any risk of alkylating the address. Directly before using the reagent, it is possible to reduce the formyl group to the hydroxymethyl group, which increases by two orders of magnitude the reactivity of the *p*-*N*-2-chloroethyl-*N*-methylaminophenyl residue.

If we think of the future employment of complementarily addressed reagents as medical preparations or for the induction of mutations in living organisms but not in isolated DNA molecules, it is necessary to take into account, in the synthesis of the reagents, the possibility of the degradation of the address by nucleases and the necessity for the reagent to cross the outer and inner membranes of the target cells.

Studies on affinity modification involving living objects are in the very initial stage. In relation to the alkylation of derivatives of oligothymidylates with one uridine residue at the 3′-end, carrying at this end the alkylating groups $(dTp)_nUCHRCl$, it has been shown that they modify the complementary polyadenyl fragments in the cells of the Krebs ascites carcinoma, such fragments being usually located at the 3′-end of the mRNA of higher organisms.[43] At the same time it has been shown that the address is preserved and the high selectivity of the alkylation reaction has been demonstrated: the degree of modification of the fragments of poly-A exceeded by two orders of magnitude the degree of modification of the remaining nucleic acids in the cells. In order to increase their ability to permeate through the membrane, oligonucleotides with ethylated internucleotide phosphates $[dTp(C_2H_5)]_nUCHRCl$ were used.[44] The penetration into the cell was then significantly greater than in the case of the non-ethylated negatively charged address. The first successful data concerning the possibility of the effective modification of viral nucleic acids in cells and specific mRNA of the cells were obtained in experiments designed to suppress the development of the influenza virus in the cells of chicken fibroblasts[45] and to suppress the synthesis of immunoglobulins in the tumour cells of mouse myeloma.[46]

IV. REACTIVE DERIVATIVES OF NUCLEOSIDES AND NUCLEOSIDE MONO-, DI-, AND TRI-PHOSPHATES

Nucleosides and nucleoside mono, di-, and tri-phosphates are the simplest compounds containing chemical structures characteristic of nucleic acids: the heterocyclic bases, ribose or deoxyribose, and phosphoric acid residues. In order to obtain affinity reagents, it is possible to introduce reactive groups into each of these structures.

Reactive groups are frequently introduced into heterocyclic bases by their halogenation and subsequent substitution of the halogenated compounds. The widely employed derivatives of purine nucleoside polyphosphates having the azido-group in the 8-position as well as derivatives of purine nucleoside polyphosphates having SH or azido-groups in the 6-position may serve as examples of derivatives obtained in this way.

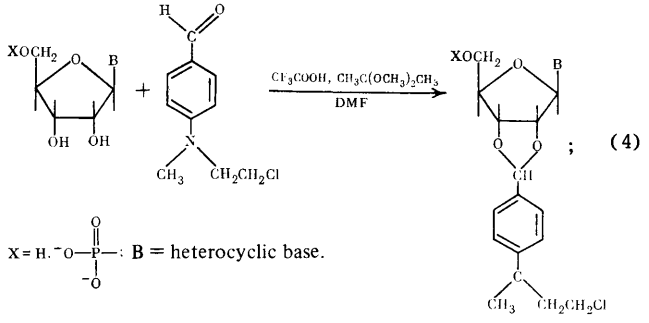
Such derivatives are affinity reagents of the endo-type, since the most specific fragments of the structure of nucleoside polyphosphates, recognised by enzymes, are their heterocyclic bases. A second approach to the introduction of reactive groups into heterocyclic bases leads to the formation of reagents of the exo-type and is based on the application of heterobifunctional reagents containing two groups with different chemical properties. One of the groups of the reagent ensures its addition to the heterocyclic base, while the other correspondingly becomes the reactive group of the affinity reagent obtained. Thus photoactivatable and arylating affinity reagents have been obtained by acylating the amino-groups of purine nucleoside polyphosphates by the halide of the corresponding substituted carboxylic acids.[47]

In the studies of the properties of derivatives of nucleoside polyphosphates having reactive groups in the heterocyclic bases it was observed that as a rule they differ sharply from the normal substrate in terms of their ability to interact with enzymes. For example, the analogue of guanosine triphosphate with the 4-azidobenzyl residue in the amino-group could not be used for the affinity modification of the enzyme adenylate cyclase, since its affinity for this enzyme was too low.[47] This is not surprising, because it is known that heterocyclic

bases play the main role in ensuring the specificity of such interactions. The introduction of substituents into heterocyclic bases should influence their interaction with enzymes and can influence their conformational state. Thus the 8-azido-derivatives of purine nucleoside polyphosphates mentioned above have the *anti*- and not the *syn*-conformation, in contrast to the normal substrates.[48] The synthesis and properties of derivatives of nucleoside polyphosphates having reactive groups in the heterocyclic bases have been recently considered in a number of reviews[48–50] and will not be discussed here.

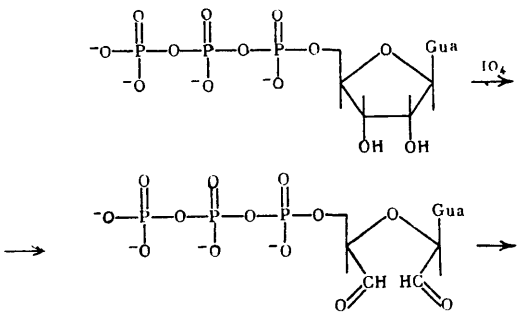
The principal method for the modification of ribose and deoxyribose involves the acylation of their hydroxy-groups. A number of derivatives with sulphonylating and aryl azide groups in the 2′(3′)-positions in ribose have been obtained by acylating adenosine polyphosphates.[49] Acylation of adenosine and subsequent separation of the product yielded derivatives with alkylating or sulphonylating groups in the 5′- or 2′(3′)-positions in ribose.[51,52]

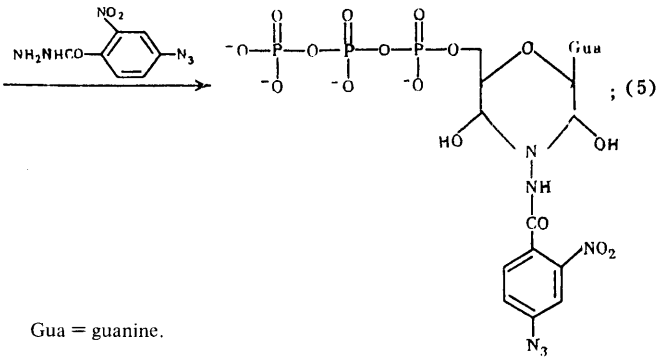
A method has been developed for the introduction of the alkylating fragment at the 2′,3′-cis-diol group. For this purpose, the nucleoside or nucleotide is treated in anhydrous dimethylformamide with 4-(*N*-2-chloroethyl-*N*-methylamino)-benzaldehyde in the presence of CF_3COOH and dimethoxypropane. Derivatives of uridine, adenosine, cytidine, and all four 5′-nucleotides have been obtained in this way.[53–56]



However, they have been tested as affinity reagents only at the level of oligo- and poly-nucleotides and will be discussed in the next Section.

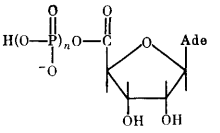
The oxidation of nucleosides and their polyphosphates by periodate leads to the cleavage of the furanose ring at the 2′,3′-diol group with formation of a reactive centre—the dialdehyde group. The latter can itself interact reversibly with the amino-groups of proteins via the formation of Schiff bases. The reduction of Schiff bases with tetrahydroborates leads to the irreversible binding of the reagents to the amino-derivatives. The nucleoside polyphosphates oxidised at the ribose residue have been used for the affinity modification of many enzymes.[49] The reaction of the ribose-oxidised guanosine triphosphate with a bifunctional hydrazide reagent yielded a derivative with an aryl azide group:[57]





Gua = guanine.

Derivatives of adenosine mono- and tri-phosphates, capable of reacting with the nucleophilic groups of proteins, have been obtained by phosphorylating adenosine oxidised at the 5′-carbon atom:[58,59]



$n = 1, 3$.

Reactive groups can be introduced into the structure of nucleoside polyphosphates by replacing the entire phosphate group or part of it. Many reagents of this type have been described in reviews[48–50] and many of them have been used as affinity reagents. However, numerous facts indicate that this method of introducing reactive groups into the structure of nucleoside polyphosphates is non-optimum, since the decrease of the overall negative charge of the molecule has a significant influence on its interaction with enzymes. Evidently the most rational procedure for the synthesis of affinity reagents derived from nucleoside polyphosphates involves the introduction of the reactive groups into their terminal phosphates. Derivatives of nucleoside triphosphates, which are the substrates of hundreds of enzymic reactions and which have reactive groups at the γ-phosphate residue, are of greatest interest. It is to be expected that the ability of the molecule as a whole to undergo specific interactions is least disturbed in such derivatives, because neither the base nor the carbohydrate component of the molecule have been affected and only the charge of the triphosphate group has been reduced by unity.[60] The vast majority of enzymic reactions with participation of nucleoside triphosphates represent either phosphate transfer or the transfer of the nucleotide residue to an acceptor, so that the triphosphate group should be located directly in the active centre of the enzyme near the acceptor binding centre. For this reason, reactive γ-derivatives of nucleoside triphosphates can be used also for the investigation of the acceptor binding sections.

In order to obtain derivatives of nucleoside 5′-triphosphates containing different substituents bound to the γ-phosphate, a simple general method, based on their conversion, with the aid of carbodiimides, into nucleoside 5′-trimetaphosphates, has been developed. The reaction can proceed both in an anhydrous medium using dicyclohexylcarbodiimide and in an aqueous solution using a water-soluble carbodiimide. The resulting nucleoside 5′-trimetaphosphate readily reacts with amines to form the corresponding γ-amides. Examples of compounds obtained in this way are compounds (I, a–f).[61–63]

In conformity with general considerations, it has been found that such γ-amides exhibit an affinity for the majority of the enzymes investigated in the class of hydrolases,

phosphotransferases, and synthetases and, as already mentioned, in some cases can serve as the substrates of these enzymes (see Section II). A systematic account of the data published up to 1980 has been given in the review of Grachev et al.[64] Since that time, the range of enzymes and protein factors investigated has expanded.

It has been shown that the derivative (If) is an affinity reagent in relation to the enzyme catalysing the reversible phosphorolysis of the terminal group in pCpCpA, which is a property characteristic of all transport RNA (ATP, CTP: tRNA nucleotidyl transferase):[65]

$$\text{tRNA-pXpCpCpA + pyrophosphate (pp)} \rightleftarrows \text{tRNA-pXpCpC + pppA,} \quad (5)$$

$$\begin{aligned}&\text{tRNA-pXpCpC + pp} \rightleftarrows \text{tRNA-pXpC + pppC,}\\ &\text{tRNA-pXpC + pp} \rightleftarrows \text{tRNA-pX + pppC;}\\ &\text{X = A, C, G, or T.}\end{aligned} \quad (6)$$

It is also an affinity reagent in relation to acetylcoenzyme A carboxylase, catalysing the conversion of acetyl-CoA into malonyl-CoA:[66]

$$\text{ATP} + HCO_3^- + CH_3CO\text{-CoA} \rightarrow \text{ADP} + p + {}^-OOC-CH_2-CO\text{-CoA},$$

and being of key importance for the biosynthesis of aliphatic acids and in relation to the Na^+, K^+-dependent ATPase, which plays the main role in the transmembrane transport of sodium and potassium ions.[67] It has been established that compound (Ib), a photoactive derivative of GTP, effects the affinity modification of the GTP-dependent protein initiation factor involved in the initial stage of the biosynthesis of proteins in eukaryotic organisms. Among the three subunits of the factor, the γ-subunit is modified exclusively.[68] It has been demonstrated in relation to creatine kinase, an important enzyme in bioenergetic processes in mice, catalysing the transfer of phosphate from creatine phosphate to ADP and supplementing thereby the store of mouse ATP, i.e.

$$^-OOC-CH_2-N(CH_3)-\underset{\underset{NH}{\|}}{C}-NH-\underset{\underset{^-O}{|}}{\overset{\overset{O}{\|}}{P}}-O^- + \text{ppA} \rightarrow {}^-OOC-CH_2-N(CH_3)-\underset{\underset{NH}{\|}}{C}-NH_2 + \text{pppA},$$

that not only the reactive derivatives with an aromatic spacer of the type (I, a–f) but also derivatives of aliphatic 2-chloroethylamines, for example

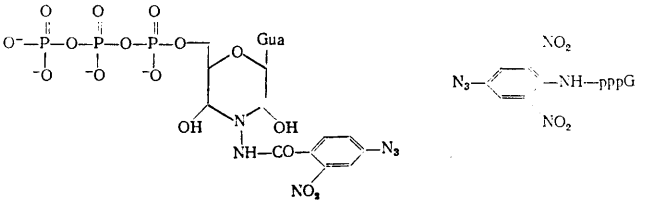
$$ClCH_2CH_2\diagdown \atop H_3C\diagup N\text{—pppA}$$

are effective affinity reagents in relation to ATP-dependent enzymes.[69]

As already mentioned in Section III, the nucleoside 5′-trimetaphosphates are themselves affinity reagents. Apart from the RNA polymerase already mentioned, this has been demonstrated in relation to nucleotide-tRNA transferase[65] and phenylalanyl-tRNA synthetase from *E. coli*.[70]

The molecule of nucleotides are fairly large and the region of contact between the proteins "recognising" them can be fairly extensive. The data obtained with the aid of reagents without reactive groups in different parts of the molecule can therefore supplement significantly one another.

As an example, one may quote studies carried out with the protein elongation factor EF-G, which, combined in the complex with GTP, promotes the migration to the P site of the molecule of peptidyl-tRNA elongated by one aminoacid, formed on a ribosome via reaction (3) and present initially in the A site (such migration is necessary to make possible the start of the next peptide chain elongation cycle with selection of the next aminoacyl-tRNA molecule). In order that the EF-G factor should dissociate from the ribosome after such migration, it is necessary that the GTP molecule be hydrolysed to GDP and orthophosphate. Such activity is indeed characteristic of the complex of the ribosome and EF-G. However, in order to establish whether the corresponding catalytic centre is located on the ribosome or on the factor itself, it is important to determine where the GTP binding region is located. Experiments on affinity modification carried out with derivatives having reactive groups on the triphosphate fragment and on the ribosome residue have shown in both cases that the factor and not the ribosome is affinity labelled, i.e. that throughout most of its extent GTP is bound precisely to the factor:[57,71]



As a second example one may quote data obtained for ATP(CTP):tTNA-nucleotidyl-transferase. As can be seen from Schemes 5 and 6, this enzyme catalyses at least two reactions—the transfer of the CMP residue from CTP to the molecules of tRNA with an incomplete pCpC fragment and the transfer of AMP from ATP to tRNA with a complete pCpC fragment. It has not been established whether the enzyme has two different centres in order to effect these two reactions or whether all the events are played out at a single centre whose specificity changes depending on the nature of the tRNA end fragment. The use of periodate-oxidised ATP and CTP, namely oATP and oCTP, containing the dialdehyde groups in the modified ribose fragment, has shown that both compounds inactivate the enzyme. However, the employment of oATP leads predominantly to the inactivation of the enzyme in reaction (5), while oCTP inactivates reaction (6). These data show that CTP and ATP are located at least partially in different regions.[72]

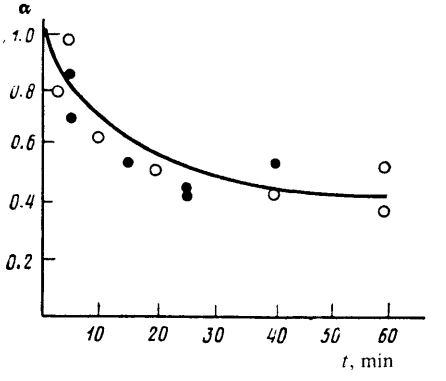


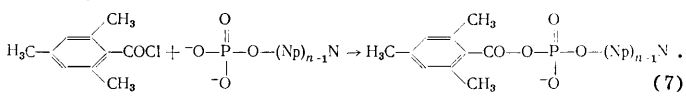
Figure 4. Kinetic curve for the inactivation of the tRNA-nucleotidyltransferase in the presence of 10^{-3} M reagent (If) at 37 °C: filled circles—residual activity determined from the incorporation of [^{3}H]AMP; open circles—ditto determined from the incorporation of [^{3}H]CMP in tRNA.[65]

On the other hand, studies carried out with the aid of the derivative (If) having an alkylating group bound to the γ-phosphate, and also with the aid of ATMP and CTMP have shown that the modification of the enzyme suppresses both activities to the same extent. As an example, Fig. 4 presents

the results of a study of the kinetics of the enzyme inactivation by the derivative (If) measured on the basis of the incorporation in tRNA of labelled AMP and CMP from which the pCpCpA end has been partly removed. Evidently, within the limits of experimental error, the data obtained by means of both tests agree. This result shows that, when derivatives with a reactive group on the triphosphate fragment are used, the group which is equally important for reactions (5) and (6) is attacked.[65]

V. REACTIVE DERIVATIVES OF OLIGONUCLEOTIDES

The first reactive derivatives of oligonucleotides were obtained by Grineva and co-workers in connection with the method for the complementarily addressed modification of nucleic acids which they developed.[73,74] The alkylating derivatives of oligonucleotides with a reactive group at the 3′-end were synthesised by reaction (4) which in broad outline proved to be suitable in this case also. The only significant modification on passing from monomers to short oligomers and longer oligonucleotides was the employment of Cetavlon salts of oligonucleotides in order to improve their solubility in dimethylformamide.[75] The method has also been applied to the more readily available deoxyribo-oligonucleotides with a 3′-terminal ribonucleotide fragment.[76] The reaction of *p*-(*N*-2-chloroethyl-*N*-methylamino)benzylamine ($ClRCH_2NH_2$) with the 5′-terminal phosphate group of the oligonucleotide in the presence of condensing agents has been developed for the synthesis of derivatives with an alkylating group at the 5′-end. The condensing reagents used were initially diphenyl phosphorochloridate[77] and later mesitoyl chloride, which leads to the formation of a mixed anhydride between the 5′-terminal phosphate and mesitylenecarboxylic acid:[78]



(7)

Treatment of the resulting mixed anhydride with the amine $ClRCH_2NH_2$ leads to the formation of the required product. When ribo-oligonucleotides are employed, these condensing agents have been shown[79–81] to produce intramolecular cyclic phosphotriesters with participation of the 2′-hydroxy-group, which hydrolyse in water with the partial cleavage of the internucleotide bonds and the isomerisation of the natural internucleotide 3′ → 5′ bond to the 2′ → 5′ bond. Recently such derivatives have been obtained predominantly using as the condensing agent a mixture of triphenylphosphine and dipyridyl disulphide,[30] which does not attack the internucleotide bond in the presence of highly basic amines.[82]

Many important characteristics of the complementarily addressed modification have been established with the aid of these reagents. It has been shown in relation to the reaction of ribosomal RNA from *E.coli*[83] that alkylation of the latter by derivatives of the oligoadenilates $(pA)_nCHRCl$ proceeds as a typical intracomplex reaction—the degree of modification of the ribosomes as a function of the reagent concentration reaches a plateau and does not increase on further increase of the excess of the reagent. In the presence of a small excess of the reagent, about 90% of the latter is consumed in the alkylation of the ribosomes, although, according to the competition factor for the intermediate ethyleneimmonium cations, this quantity should have been a fraction per cent if the reaction proceeded without the preliminary complex formation.[83] The composition of the alkylated bases changes sharply. Whereas the alkylation of tRNA by the analogous derivatives of uridine of the methyl ester of UMP results in the predominant or even exclusive alkylation of the guanine residues,[84,85] the reaction in the complex, where the direction of attack is determined primarily not by the relative reactivities of the bases but by their accessibility to the attack by the alkylating group, fairly rigidly fixed by the address, results in the alkylation of adenine, guanine, and cytosine to comparable extents.[86] Analogous results have been obtained in the study of the alkylation of DNA.[87,88] It has been shown in relation to the reaction of yeast valine tRNA (Fig.5) with the reagent dCpdGprACHRCl, which interacts with the C–G fragments of tRNA, that the fourth, reckoning from the complex formation section, residues in the direction of the 5′-end of the target molecule are alkylated.[89] Later these data were confirmed on a synthetic model:

(5′) d(pXpXpCpCpTpGpTpTpTpGpGpC) (3′)
(3′) ClRHC < rAd(CpApApApCpCpGpT) (5′)

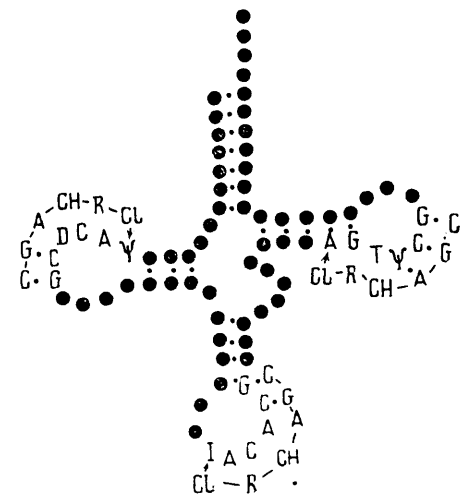


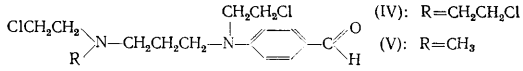
Figure 5. Specific alkylation by the complementarily addressed reagent dCpdGprACHRCl in the structure of the yeast valine tRNA;[89] the letters denote only the nucleotides in the reagent binding regions.

Here and in the next analogous scheme the symbol d is placed before the entire deoxyoligonucleotide sequence, rA denotes adenosine ribonucleoside, and the 5′- and 3′-ends of the two antiparallel complementary chains are indicated in brackets. In all the cases investigated only the d(pXpX) fragment was alkylated in the complex.[90]

Alkylation of DNA by oligonucleotide derivatives with subsequent cleavage at the alkylation points has been proposed as a general method for the selective cleavage of DNA at sections adjoining the nucleotide sequences complementary to the address.[91]

Several other methods for the introduction of reactive groups into the terminal fragments of oligonucleotides have been described. Thus, periodate oxidation followed by treatment with *N*-(4-azidobenzoyl)glycylhydrazide[92] and acylation of the *cis*-diol group with the imidazolide of *p*-azidobenzoic acid[93] have been used for the introduction of the photoactive arylazido-group at the 3′-terminal *cis*-diol.

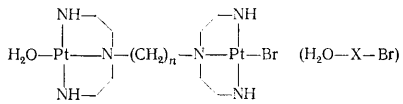
The introduction of an alkylating group into an oligonucleotide at the 5′-end via the alkylation with a trifunctional agent (IV),[42] in which the reactivity of one of the 2-chloroethylamino-groups is controllable, has been described:



(IV): R=CH_2CH_2Cl
(V): R=CH_3

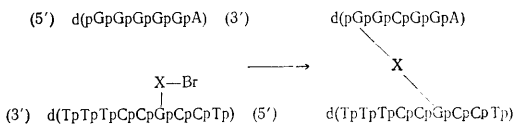
The phosphate is alkylated by the reactive aliphatic group (the second C—Cl bond is then hydrolysed) and the reactivity of the aromatic 2-chloroethylamino-group in the resulting derivative is then increased by reducing the formyl group to the hydroxymethyl group with sodium tetrahydroborate.[94]

The bifunctional platinum derivative[22]



(H_2O—X—Br)

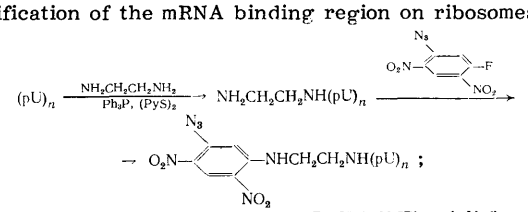
has been suggested for the introduction of reactive residues into the inner sections of an oligonucleotide. Water is readily substituted by the guanine residue in the oligonucleotide functioning as the address. The Pt—Br bond is significantly stronger and reacts with nucleic acid bases only under the conditions of a stable contact generated by a complementary complex. Complementarily addressed modification in a complex has been achieved in this way and, among the four guanine groups of the target oligonucleotide, only one, located in the position within the complex most favourable for the reaction, proved to have been modified:



Derivatives of oligonucleotides having the dimethylaminonaphthalenesulphonyl (dansyl, DNS) residue have been obtained. It has been shown in relation to the derivative DNS-$NHCH_2CH_2(pT)_9$ [95] that, in the two-quantum excitation by means of a laser having a wavelength of 337 nm, the polyadenylic acid complementary to the address is cleaved in the presence of this reagent, but polyuridylic and polycytidylic acids are not cleaved under these conditions.

The addition of a chelating fragment, namely ethylenediaminetetra-acetic acid, to an oligothymidylate has also been achieved. In the presence of Fe^{2+} and O_2 this derivative cleaves the complementary polyadenylate and, to a significantly lesser extent, non-complementary polynucleotides.[96]

In many cases a special group, by means of which it is possible to carry out a highly selective reaction with a heterobifunctional reagent containing the future reactive fragment of the addressed reagent, is introduced beforehand to reactive oligonucleotide derivatives in the course of their synthesis. Aliphatic or aromatic amino-groups, which can be introduced either at the terminal phosphate[97–99] or into the heterocyclic base, for example, in the synthesis of an oligonucleotide containing 5-aminouracil instead of one of the uracil residues,[100] are used for this purpose. As an example, one may quote derivatives employed for the affinity modification of the mRNA binding region on ribosomes:



NH_2Ph—pApUpG + $(BrCH_2CO)_2O$ → $BrCH_2CONHPh$—pApUpG ;

UpUpUp(5-NH_2)U + ICH_2COCl → UpUpUp (5-ICH_2CONH)U .

Derivatives in which the terminal phosphate has been replaced by the phosphorothioate residue have been proposed for the same purpose. In particular, derivatives of deoxyoligoribonucleotides have been obtained in this way by alkylating phosphorothioates with reagents (IV) or (V) with subsequent activation of the aromatic 2-chloroethylamino-group via its reduction with tetrahydroborate.[101,102]

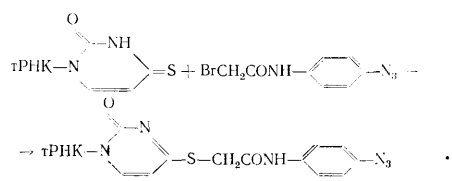
A novel method of synthesis of reactive derivatives of oligoribonucleotides has been proposed.[6,103] It is based on the substrate activity of γ-derivatives of nucleoside 5′-triphosphates in a reaction catalysed by RNA polymerase. In the synthesis of RNA on a DNA template using instead of the natural substrate its derivative with a reactive group, a product retaining this group at the 5′-end has been obtained:

$$N_3PhNHpppA \xrightarrow{pppU} N_3PhNHpppApU \xrightarrow{pppC} N_3PhNHpppApUpC \rightarrow \ldots$$

In certain cases reactive derivatives can be obtained by the appropriate treatment of the complex of the oligonucleotide or its derivative with the biopolymer. Thus the affinity modification of the mRNA binding region has been achieved[104] by treating the complex of the ribosomes of *E.coli* with the phenylalanine tRNA and $pp(pU)_7$ with water-soluble carbodiimide, which converts the terminal triphosphate group into a trimetaphosphate group.

VI. REACTIVE DERIVATIVES OF NUCLEIC ACIDS

The greatest number of reactive derivatives have now been described for transfer RNA in connection with the study of their interaction with ribosomes and aminoacyl-tRNA synthetases. A list of the derivatives synthesised can be found in other reviews.[105,106] Their greatest number has been obtained by the addition of the corresponding heterobifunctional reagents directly to the *cis*-diol group, for example, via reaction (4),[107] or with preliminary oxidation by periodate, and via the α-amino-group of aminoacyl tRNA. Compounds (II) and (III) are examples of such derivatives. The first affinity modification of ribosomes has been achieved with the aid of compound (II), first described by Grachev and Ryvkin,[108] and information has been obtained about the location of the peptidyl-transferase centre.[109] The selective addition of bifunctional reagents to certain so-called minor bases, which are present in large amounts in tRNA, has also been employed. Thus the presence of 4-thiouridine in the 8-position relative to the 5′-end is characteristic of the tRNA from *E.coli* and the 4-thiouridine group can be selectively alkylated by a reagent with an aryl azide group:[9]



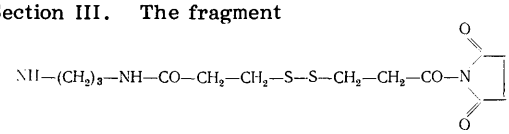
The method of direct photo cross-linking has also been used in the study of the interaction of tRNA with aminoacyl-tRNA synthetases.[110] A detailed analysis of the data obtained by this method can be found in the paper of Remy and co-workers in Knorre's monograph.[3] It must be emphasised that ultraviolet irradiation in the region of 260 nm damages significantly nucleic acids and proteins and the results obtained by the method of direct photo cross-linking must be treated with much caution. The method is undoubtedly attractive because of its simplicity and the absence of any perturbations to the ligand before the instant of elimination and the possibility of carrying out the process very rapidly using powerful light

pulses. However, as already stated, a detailed preliminary study of the reactions occurring and of the properties of the photo cross-linking products formed is required.

Methods based on the selective excitation of certain specific chromophores present in tRNA as minor components and absorbing in the long-wavelength ultraviolet part of the spectrum, sufficiently remote from the region corresponding to absorption by the remaining bases and proteins, are nowadays more attractive. An example of such a group is 4-thiouridine already mentioned. It has been shown[9] that irradiation of phenylalanine tRNA from *E.coli* in a complex with phenylalanyl-tRNA synthetase leads to the cross-linking of these two macromolecules. According to the data of Ofengand and Liou,[111] the tRNA containing in the 5'-position in the codon a uridine derivative (5-carboxymethoxyuridine), in a ternary complex with a ribosome and mRNA, is cross-linked on ultraviolet irradiation with the pRNA of the small subunit of the ribosome. The irradiation of ribosomes in a complex with yeast phenylalanine tRNA and polyuridilic acid leads to the cross-linking of the latter with the nucleoside wybutosine adjoining the anticodon from the 3'-end.[112]

As already stated in Section III, many problems of the protein–nucleic acid recognition and of the specific action on nucleic acids can be solved using derivatives of polynucleotides and nucleic acids in which reactive groups have been introduced statistically. Polynucleotides of this type can be obtained by copolymerisation with monomers containing chemically reactive groups. Thus the copolymerisation of ADP with 8-azido-ADP and of inosine 5'-diphosphate with 8-azidoinosine diphosphate has been described.[113] The copolymers obtained were used for the affinity modification of the RNA polymerase from *E.coli*.

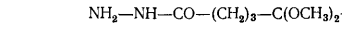
Derivatives of phenylalanine tRNA from *E.coli* with statistically attached aryl azide groups have already been described in Section III. The fragment



with acylating activity has been introduced statistically[114] into accessible (not involved in the formation of the secondary structure) cytosine residues of the initiator methionine tRNA from *E.coli*. The derivative obtained was used for the affinity modification of methionyl-tRNA synthetase from the same source. The presence of the S–S bridge makes it possible to split off tRNA from the protein by treatment with thiols after the affinity modification. 4-Thiouracil residues, formed from cytosine residues on treatment of tRNA with hydrogen sulphide, have been introduced statistically into the same tRNA by treatment with hydrogen sulphide. The photoaffinity modification of ribosomes has been achieved with the aid of such tRNA.[115]

The use of the T7 phage mRNA, containing alkylating groups introduced by treating the mRNA with reagent (IV), for specific mutagenesis involving specific genes has been described.[116,117] Analogous derivatives have been obtained from the DNA fragment containing the gene imparting resistance to tetracycline.[118] These reagents have been used for the specific induction of mutations in the pBR 322 plasmid, widely used in gene engineering studies. Although all the experiments have been carried out with isolated phage and plasmid DNA, it is evident that this approach leads to prospects for mutagenesis in living organisms, directed to specific genes, as well as to the prospect for the blocking of the operation of specific genes. The statistical character of the distribution of the reactive groups in the solution of such problems is especially significant, since it is not known beforehand into which points within the gene it is necessary to introduce modifications in order to obtain interesting mutations or in order to switch off its operation.

The initial idea has been realised in a study[119] in which the cytosine residues in the T7 phage mRNA were modified by the reagent



After the introduction of the reagent at the cytosine groups of the address, it was activated by acid treatment, which converts the ketal into a ketone, enhancing thereby the electrophilic character of the C–Br bond. The statistical introduction of aldehyde groups into DNA forming part of the composition of chromatin was achieved by treatment with dimethyl sulphate and subsequent splitting off of 7-methylguanine groups. The aldehyde groups readily form Schiff bases with the amino-groups of the chromatin proteins in contact with them. Treatment with sodium tetrahydroborate leads to the formation of stable alkylamines, i.e. to the covalent binding of DNA to the proteins. Analysis of the reaction products make it possible to obtain important information about the structural organisation of nucleosomes—the nucleoprotein complexes in which the DNA in the cells of higher organisms is packed.[120]

---o0o---

The data presented in the review show that organic and bio-organic chemistry have armed investigators with a large arsenal of fine chemical instruments for the study of the problem of the protein–nucleic acid recognition and the development of fundamentally new approaches to influencing the genetic apparatus in living organisms. The number of reactive derivatives of nucleic acids and their components known at the present time is so large that the present authors have been unable to describe or even list all such derivatives and were forced to confine themselves to the description of the principal types. On the other hand, the methodology of the application of these instruments to the purpose of research still requires profound physicochemical and biochemical investigations. The authors believe that the most acute problems include the study of the mechanisms of the reactions used for the affinity modification of biopolymers and also the study by modern physical methods of the structures of the complexes of biopolymers with affinity reagents and the structures of the products formed. Such investigations have now become possible in relation to derivatives not only of nucleotides but also of those of oligonucleotides, which is a result of impressive advances in the field of their chemical synthesis.

Complexes of derivatives of oligonucleotides with complementary oligonucleotides and nucleic acids are unique models for the study of the general characteristics of their intracomplex reactions, modelling in their specificity natural enzymic reactions. In fact a new field of bio-organic chemistry, namely the organic chemistry of nucleic acid duplexes, has already begun to form.[121]

The possibility of obtaining oligonucleotides and their derivatives in amounts running into tens and hundreds of milligrams makes it possible to begin systematic studies on such derivatives of biologically active substances. The possibility of establishing the primary structure of virtually any nucleic acids ensures the specific synthesis of oligo- and poly-nucleotide addresses for the most varied sections of the genetic apparatus in living organisms and the development on this basis of methods whereby biological objects can be influenced, which are superior in their selectivity to other chemical methods and approach, in terms of the level of their selectivity, the most sophisticated antibiotics and

enzymes. The authors hope that this review will attract the attention of chemists involved in the most varied disciplines (organic and inorganic chemists, physical chemists, and analytical chemists) to this new vigorously developing field of science, which has extremely attractive prospects.

REFERENCES

1. J.D.Watson and F.H.Crick, Nature, 1953, **171**, 737.
2. W.B.Jakoby and M.Wilchek (Editors), "Methods in Enzymology", Acad.Press, New York–London–San Francisco, 1977, Vol.56.
3. D.G.Knorre (Editor), "Afinnaya Modifikatsiya Biopolymerov" (Affinity Modification of Biopolymers), Izd.Nauka, Novosibirsk, 1983.
4. "Biochemical Nomenclature and Related Documents", Spottiswoode Ballantyne Press, London, Colchester and Beccles, 1978.
5. M.A.Grachev, E.F.Zaichikov, A.G.Pletnev, and V.V.Starikov, Mol.Biol., 1980, **14**, 575.
6. E.D.Sverdlov, S.A.Tsarev, N.N.Modyanov, V.M.Lipkin, M.A.Grachev, E.F.Zaichikov, and A.G.Pletnev, Bioorg.Khim., 1978, **4**, 1278.
7. V.N.Buneva, N.V.Kudryashova, V.A.Kurbatov, and A.G.Romashchenko, Biokhimiya, 1978, **43**, 2261.
8. V.N.Buneva, T.V.Demidova, D.G.Knorre, N.V.Kudryashova, A.G.Romashchenko, and M.G.Staroobrazova, Mol.Biol., 1980, **14**, 1080.
9. V.G.Budker, D.G.Knorre, V.V.Kravchenko, O.I.Lavrik, K.A.Nevinsky, and N.M.Teplova, FEBS Letters, 1974, **49**, 159.
10. V.Z.Akhverdyan, L.L.Kiselev, D.G.Knorre, O.I.Lavrik, and G.A.Nevinskii, Mol.Biol., 1977, **113**, 475.
11. N.A.Bulychev, O.I.Lavrik, and G.A.Nevinskii, Mol.Biol., 1980, **14**, 558.
12. R.Krauspe and O.I.Lavrik, Europ.J.Biochem., 1983, **132**, 545.
13. V.G.Budker, D.G.Knorre, and S.N.Yasnova, Mol. Biol., 1972, **6**, 581.
14. O.I.Gimautdinova, G.G.Karpova, D.G.Knorre, and N.D.Kobetz, Nucl.Acids Res., 1981, **9**, 3465.
15. V.G.Budker, N.I.Grineva, N.D.Kobets, T.S.Lomakina, and S.P.Maev, Biokhimiya, 1978, **43**, 761.
16. D.G.Knorre and T.A.Chimitova, FEBS Letters, 1981, **131**, 249.
17. D.G.Knorre and T.A.Chimitova, Mol.Biol., 1978, **12**, 814.
18. I.I.Gorshkova and D.G.Knorre, Bioorg.Khim., 1980, **6**, 230.
19. W.C.J.Ross, "Biological Alkylating Agents" (Translated into Russian), Izd.Meditsina, Moscow, 1964.
20. N.M.Emanuel' and D.G.Knorre, "Kurs Khimicheskoi Kinetiki" (Textbook of Chemical Kinetics), Izd.Vys. Shkola, Moscow, 1984, p.274.
21. V.V.Vlasov and S.A.Kazakov, Bioorg.Khim., 1982, **8**, 499.
22. V.V.Vlassov, V.V.Gorn, E.M.Ivanova, S.A.Kazakov, and S.V.Mamaev, FEBS Letters, 1983, **162**, 286.
23. T.S.Gall, N.P.Gritsan, S.D.Myzina, N.M.Bazhin, and E.V.Nemtseva, Dokl.Akad.Nauk SSSR, 1983, **273**, 883.
24. P.E.Nielson and O.Buchardt, Photochem.Photobiol., 1982, **35**, 317.
25. A.G.Badashkeyeva, T.S.Gall, E.V.Efimova, D.G.Knorre, A.V.Lebedev, and S.D.Mysina, FEBS Letters, 1983, **155**, 263.
26. V.N.Ankilova, D.G.Knorre, V.V.Kravchenko, O.I.Lavrik, and G.A.Nevinsky, FEBS Letters, 1975, **60**, 172.
27. V.N.Buneva, D.G.Knorre, and I.O.Pacha, Biokhimiya, 1980, **45**, 1004.
28. V.N.Buneva, E.Yu.Dobrikova, D.G.Knorre, I.O.Pacha, and T.A.Chimitova, FEBS Letters, 1981, **135**, 159.
29. M.A.Grachev and A.A.Mustaev, FEBS Letters, 1982, **137**, 89.
30. O.I.Gimautdinova, G.G.Karpova, and N.A.Kozyreva, Mol.Biol., 1982, **16**, 752.
31. J.Stahl and N.D.Kobetz, FEBS Letters, 1981, **123**, 269.
32. J.Stahl and N.D.Kobetz, Mol.Biol., 1984, **9**, 219.
33. V.V.Vlasov, O.I.Lavrik, S.V.Mamaev, V.E.Chizhikov, and S.N.Khodyreva, Mol.Biol., 1980, **14**, 531.
34. C.C.Karpova, Mol.Biol., 1984, **18**, 1194.
35. G.T.Babkina, E.V.Bausk, D.M.Graifer, G.G.Karpova, and N.B.Matasova, FEBS Letters, 1984, **170**, 290.
36. M.Baltzinger, F.Fasiolo, and P.Remy, Europ.J. Biochem., 1979, **97**, 481.
37. O.I.Lavrik and S.N.Khodyreva, Biokhimiya, 1979, **44**, 570.
38. S.-H.Chiou, J.Biochem.(Japan), 1984, **96**, 1307.
39. M.I.Stockmann, Phys.Letters, 1980, **76A**, 191.
40. E.B.Brosalina, V.V.Vlasov, I.V.Kutyavin, and S.V.Mamaev, Dokl.Akad.Nauk SSSR (in the press).
41. J.Summerton, J.Theor.Biol., 1979, **78**, 77.
42. A.A.Gall', V.A.Kurbatov, A.A.Mustaev, and G.V.Shishkin, Izv.Sibir.Otd.Akad.Nauk SSSR, Ser. Khim.Nauk, 1979, **2**, 99.
43. E.M.Ivanova, G.G.Karpova, D.G.Knorre, V.S.Popova, A.S.Rait, and L.E.Stefanovich, Mol. Biol., 1984, **18**, 613.
44. V.F.Zarytova, E.M.Ivanova, G.G.Karpova, D.G.Knorre, N.P.Pichko, and A.S.Rait, Bioorg. Khim., 1981, **7**, 1512.
45. V.V.Vlasov, V.V.Gorn, I.V.Kutyavin, L.V.Yurchenko, N.K.Sharova, and A.G.Bukrinskaya, Mol. Genet., Mikrobiol.i Virusol., 1984(11), 36.
46. V.V.Vlasov, A.A.Godovikov, V.F.Zarytova, E.M.Ivanova, D.G.Knorre, and I.V.Kutyavin, Dokl. Akad.Nauk SSSR, 1984, **276**, 1263.
47. T.Pfeuffer and F.Eckstein, FEBS Letters, 1976, **67**, 354.
48. R.F.Colman, Ann.Rev.Biochem., 1983, **52**, 67.
49. N.I.Sokolova and S.S.Tret'yakova, Bioorg.Khim., 1983, **9**, 1157.
50. K.H.Scheit, "Nucleotide Analogs: Synthesis and Biological Function", John Wiley and Sons, New York, 1980.
51. P.K.Pal, R.J.Reischer, W.J.Wechter, and R.F.Colman, J.Biol.Chem., 1978, **253**, 6644.
52. R.F.Colman, P.K.Pal, and J.L.Wyatt, in "Methods in Enzymology" (Edited by W.B.Jacoby and M.Wilchek), Acad.Press, New York–London, 1977, **46**, 240.
53. A.M.Belikova and N.I.Grineva, Izv.Sibir.Otd.Akad. Nauk SSSR, Ser.Khim.Nauk, 1966, **3**, 79.
54. N.I.Grineva, V.F.Zarytova, and D.G.Knorre, Zhur. Obshch.Khim., 1970, **40**, 215.
55. A.M.Belikova and N.I.Grineva, Izv.Sibir.Otd.Akad. Nauk SSSR, Ser.Khim.Nauk, 1971, **5**, 119.
56. A.G.Ven'yaminova and N.I.Grineva, Izv.Sibir.Otd. Akad.Nauk SSSR, Ser.Khim.Nauk, 1971, **4**, 111.

57. A.S.Girshovich, V.A.Pozdnyakov, and Yu.A.Ovchinnikov, Dokl.Akad.Nauk SSSR, 1974, **219**, 481.
58. A.Hampton, P.J.Harper, T.Sasaki, and P.Howgate, in "Methods in Enzymology" (Edited by W.B.Jacoby and M.Wilchek), Acad.Press, New York–London, 1977, **46**, 302.
59. A.Hampton, P.J.Harper, T.Sasaki, P.Howgate, and R.K.Preston, J.Biochem., 1979, **10**, 259.
60. G.T.Babkina, V.L.Knorre, D.G.Knorre, and O.I.Lavrik, Dokl.Akad.Nauk SSSR, 1974, **216**, 1165.
61. G.T.Babkina, V.S.Zarytova, and D.G.Knorre, Bioorg.Khim., 1975, **1**, 611.
62. V.F.Zarytova, D.G.Knorre, V.A.Kurbatov, A.V.Lebedev, V.V.Samukov, and G.V.Shishkin, Bioorg.Khim., 1975, **1**, 793.
63. D.G.Knorre, V.A.Kurbatov, and V.V.Samukov, FEBS Letters, 1976, **70**, 105.
64. M.A.Grachev, D.G.Knorre, and O.I.Lavrik, in "Soviet Scientific Reviews, Section D, Biology Reviews", Vol.2, (Edited by V.P.Skulachev, OPA, Amsterdam, 1981, p.107.
65. V.N.Buneva, I.I.Gorshkova, D.G.Knorre, and I.O.Pacha, Dokl.Akad.Nauk SSSR, 1982, **267**, 1492.
66. V.N.Buneva, A.G.Rabinkov, I.A.Yatsenko, R.E.Libinzon, and E.V.Goryachenkova, 1984, **277**, 1256.
67. K.N.Dzhandzhugazyan, N.N.Modyanov, and A.A.Mustaev, Biol.Membrany, 1984, **1**, 823.
68. T.V.Kurzchalia, U.A.Bommer, G.T.Babkina, and G.G.Karpova, FEBS Letters, 1984, **175**, 313.
69. G.A.Nevinskii, M.G.Gazaryants, and O.I.Lavrik, Bioorg.Khim., 1984, **10**, 656.
70. S.N.Khodyreva, E.A.Sycheva, V.N.Ankilova, and O.I.Lavrik, Mol.Biol., 1984, **18**, 1316.
71. A.S.Girshovich, V.A.Pozdnyakov, and Yu.A.Ovchinnikov, Europ.J.Biochem., 1976, **69**, 321.
72. P.Masiakovski and M.P.Deutscher, J.Biol.Chem., 1980, **255**, 11240.
73. A.M.Belikova, V.F.Zarytova, and N.I.Grineva, Tetrahedron Letters, 1967(37), 3557.
74. N.I.Grineva, V.F.Zarytova, and D.G.Knorre, Izv. Sibir.Otd.Akad.Nauk SSSR, Ser.Khim.Nauk, 1968, **5**, 118.
75. V.K.Rait, G.G.Karpova, and N.I.Grineva, Bioorg. Khim., 1977, **3**, 31.
76. V.F.Zarytova, N.I.Sokolova, N.I.Grineva, D.G.Knorre, and Z.A.Shabarova, Izv.Sibir.Otd. Akad.Nauk SSSR, Ser.Khim.Nauk, 1968, **6**, 102.
77. N.I.Grineva and T.S.Lomakina, Zhur.Obshch. Khim., 1972, **42**, 1630.
78. O.I.Gimautdinova, N.I.Grineva, G.G.Karpova, T.S.Lomakina, and E.L.Shelpakova, Bioorg.Khim., 1978, **4**, 917.
79. V.G.Budker, V.F.Zarytova, D.G.Knorre, N.D.Kobets, and O.I.Ryazankina, Bioorg.Khim., 1977, **3**, 618.
80. V.L.Drutsa, V.F.Zarytova, D.G.Knorre, A.V.Lebedev, N.I.Sokolova, and Z.A.Shabarova, Dokl.Akad.Nauk SSSR, 1977, **233**, 595.
81. V.F.Zarytova, V.K.Rait, and T.S.Chernikova, Bioorg.Khim., 1977, **3**, 1626.
82. G.F.Mishenina, V.V.Samukov, and T.N.Shubina, Bioorg.Khim., 1979, **5**, 886.
83. N.I.Grineva and G.G.Karpova, Mol.Biol., 1974, **8**, 832.
84. A.M.Belikova and N.I.Grineva, Izv.Sibir.Otd. Akad.Nauk SSSR, Ser.Khim.Nauk, 1971, **5**, 119.
85. V.V.Vlassov, N.I.Grineva, and D.G.Knorre, FEBS Letters, 1972, **20**, 66.
86. N.I.Grineva and G.G.Karpova, Bioorg.Khim., 1975, **1**, 588.
87. N.I.Grineva and S.D.Myzina, Mol.Biol., 1972, **9**, 502.
88. N.I.Grineva, G.G.Karpova, S.D.Myzina, and A.N.Chemasova, Bioorg.Khim., 1975, **1**, 1707.
89. N.I.Grineva, G.G.Karpova, L.M.Kuznetzova, T.V.Venkstern, and A.A.Bayev, Nucleic.Acids Res., 1977, **4**, 1609.
90. V.V.Gorn, G.G.Karpova, D.G.Knorre, I.V.Kutyavin, and N.P.Pichko, Dokl.Akad.Nauk SSSR, 1983, **270**, 613.
91. N.I.Grineva, Vestnik Akad.Med.Nauk SSSR, 1972(2), 83.
92. H.Towbin and D.Elson, Nucleic Acids Res., 1978, **5**, 3389.
93. M.B.Gottikh, M.G.Ivanovskaya, V.P.Veiko, and Z.A.Shabarova, Bioorg.Khim., 1981, **7**, 1310.
94. S.I.Oshevskii, M.A.Grachev, and A.A.Mustaev, Bioorg.Khim., 1983, **9**, 958.
95. N.V.Bulychev, A.V.Lebedev, L.Z.Benimetskaya, A.L.Kozionov, Yu.E.Nesterikhin, S.Yu.Novozhilov, S.G.Rautian, and M.I.Shtokman, Bioorg.Khim., 1984, **10**, 520.
96. A.S.Boutorin, V.V.Vlassov, S.A.Kazakov, I.V.Kutyavin, and M.A.Podymonogin, FEBS Letters, 1984, **172**, 43.
97. M.A.Prokof'ev and Z.A.Shabarova, Mol.Biol., 1978, **12**, 245.
98. O.Pongs and E.Lanka, Proc.Nat.Acad.Sci.USA, 1975, **72**, 1505.
99. O.I.Gimautdinova, M.A.Zenkova, G.G.Karpova, and L.M.Podust, Mol.Biol., 1984, **18**, 907.
100. R.Luhrmann, U.Schwarz, and H.G.Gassen, FEBS Letters, 1973, **32**, 55.
101. S.I.Oschevski, Ind., 1982, **143**, 119.
102. V.V.Vlasov, A.A.Gall', A.A.Godovikov, V.F.Zarytova, I.V.Kutyavin, I.P.Motovilova, and G.V.Shishkin, Dokl.Akad.Nauk SSSR, 1984, **274**, 1244.
103. M.A.Grachev and A.A.Mustaev, FEBS Letters, 1982, **137**, 89.
104. O.I.Gimautdinova, G.G.Karpova, N.I.Komarova, and S.B.Frolova, Bioorg.Khim., 1985, **11**, 499.
105. E.Kuechler and J.Ofengand, in "Transfer RNA: Structure, Properties and Recognition", Cold Spring Harbor, 1979, p.413.
106. D.G.Knorre and O.I.Lavrik, in "Theory and Practice in Affinity Techniques" (Edited by R.V.Sundaram and F.Eckstein, Acad.Press, London–New York–San Francisco, 1978, p.169.
107. V.V.Vlasov, N.I.Grineva, and G.G.Karpova, Mol. Biol., 1974, **8**, 752.
108. M.A.Grachev and M.I.Ryvkin, Nucleic Acids Res., 1975, **2**, 1237.
109. E.S.Bochkareva, V.G.Budker, A.S.Girschovich, D.G.Knorre, and N.M.Teplova, FEBS Letters, 1971, **19**, 121.
110. G.P.Budzik, S.S.M.Lam, H.J.P.Schoemaker, and P.R.Schimmel, J.Biol.Chem., 1975, **250**, 4433.
111. J.Ofengand and R.Liou, Biochemistry, 1980, **21**, 4814.
112. A.J.M.Matzke, A.Barta, and E.Kuechler, J.Biochem. (Japan), 1980, **112**, 169.
113. I.L.Cartwright and D.W.Hutchinson, Nucleic Acids Res., 1980, **8**, 1675.
114. D.Valenzuela, O.Leon, and L.D.H.Schulman, Biochem.Biophys.Res.Comm., 1984, **119**, 677.
115. D.Barritault, R.H.Buckingham, A.Favre, and G.Thomas, Biochimie, 1981, **63**, 587.

116. G.L.Dianov, L.P.Ovchinnikova, E.N.Voronina, E.B.Kokoza, and R.I.Salganik, Dokl.Akad.Nauk SSSR, 1979, **248**, 465.
117. R.I.Salganik, G.L.Dianov, L.P.Ovchinnikova, E.N.Voronina, E.B.Kokoza, and A.V.Mazin, Proc. Nat.Acad.Sci.USA, 1980, **77**, 2796.
118. R.I.Salganik, A.V.Mazin, G.L.Dianov, and L.P.Ovchinnikova, Dokl.Akad.Nauk SSSR, 1984, **274**, 197.
119. J.Summerton and P.A.Bartlett, J.Mol.Biol., 1978, **122**, 145.
120. A.D.Mirzabekov, V.V.Shick, A.V.Belyavsky, and S.G.Bavykin, Proc.Nat.Acad.Sci. USA, 1978, **75**, 4184.
121. D.G.Knorre and Z.A.Shabarova, Bioorg.Khim., 1984, **10**, 129.

Novosibirsk Institute of
Bio-organic Chemistry,
Siberian Division of the
USSR Academy of Sciences