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Methods for the synthesis of oligonucleotides containing reactive electrophilic groups

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Abstract. The published data on the methods of chemical synthesis of oligonucleotides containing 5′- and 3′-terminal or internal carboxy or aldehyde functional groups are considered. The known methods are systematised and their advantages and disadvantages are discussed. The bibliography includes 65 references.

I. Introduction

At present, modified oligonucleotides are widely employed in molecular biology for the study of regularities of nucleic – protein recognition, in antisense biotechnology as well as in the design of therapeutic drugs for the treatment of viral and hereditary diseases. Successful solution of these tasks requires the synthesis of oligonucleotide conjugates with various compounds, *e.g.*, reporter molecules, intercalators, biologically active peptides and enzymes possessing unique properties. In this context, the preparation of single- or double-stranded DNA molecules containing functional groups differing from those of natural DNA in reactivity is of special importance. The introduction of new reactive groups into the oligonucleotide chain allows successful synthesis of hybrid molecules.

Numerous methods for the synthesis of oligonucleotides containing nucleophilic groups (*e.g.*, amino or thio groups) are known;[1] their incorporation is usually achieved using commercially available reagents. In contrast, the preparation of oligonucleotides containing reactive electrophilic groups (*e.g.,* carboxy or aldehyde groups) is rather problematic. The body of literature on this subject is rather scarce.† We believe that the analysis and systematisation of the available data can give an impetus to the development of methods for the synthesis of oligonucleotides with electrophilic groups.

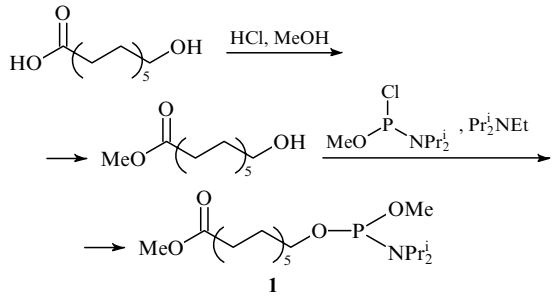
Carboxy or aldehyde functional groups can be introduced at any predetermined position of a synthetic DNA fragment, *viz.*, as the 5′- or 3′-end group or within the oligonucleotide chain. It is the position that has been chosen as the basis for the classification of methods for the synthesis of modified oligonucleotides.

In all the studies reviewed herein, oligonucleotides were prepared by the automated solid-phase phosphoramidite method using a standard protocol.[1,2] The synthesis of oligonucleotides containing a dialdehyde group is not considered here, being detailed in another review.[3] Standard procedures for the isolation of modified oligonucleotides (reversed-phase, ion-exchange HPLC, polyacrylamide gel electrophoresis, *etc.*) and the methods used to confirm the structures of intermediate and target products also fall beyond the scope of this review, since in the vast majority of studies the correct analysis using NMR spectroscopy and mass spectrometry has been carried out.

II. Synthesis of oligonucleotides containing a 5′-terminal carboxy or aldehyde group

The carboxy group is commonly incorporated at the 5′-end of the oligonucleotide using a modified non-nucleoside unit attached to the oligonucleotide chain in the final step of the automated synthesis. It is desirable to protect the carboxy group in the course of solid-phase synthesis by esterification.

A method for the synthesis of carboxy-containing oligonucleotides using a phosphoramidite derivative of methyl 12-hydroxydodecanoate **1** synthesised as shown in the scheme below has been described.[4]



1

The attachment of phosphoramidite **1** to the oligonucleotide chain was carried out in accordance with a standard protocol of

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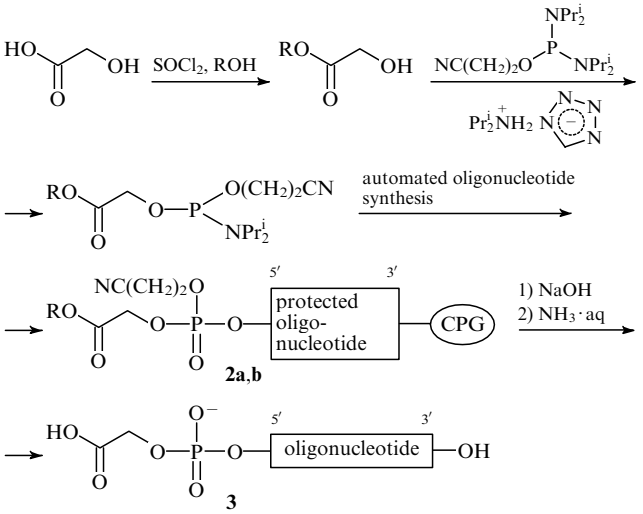
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† Several papers describing the preparation of 5′-carboxy- and 5′-aldehyde-containing oligonucleotides have recently been surveyed in brief in a review [1] devoted to the synthesis of various derivatives of nucleic acid fragments.

automated oligonucleotide synthesis. The 5′-ester group was hydrolysed with aqueous trimethylamine ($Me_3N : H_2O = 3 : 1$, 37 °C, 48 h) with subsequent deprotection with ammonia to yield the target oligonucleotide. This reaction sequence was used because treatment of an ester with a concentrated aqueous solution of ammonia yields the corresponding amide.

The reactivity of the carboxy group incorporated was confirmed in the reaction with amines in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (DEC).[4] At the concentration of the amine of 5×10^{-3} mol litre^{-1} and pH 4.0, *N*-acylurea is formed. One possible reason is the protonation of the free amino group of the nucleophile in acidic media. Attempts to avoid the formation of a side product by increasing the concentration of the amine up to 0.1–0.5 mol litre^{-1} or by increasing the pH to 5.5–6.7 failed. This problem was overcome by adding imidazole to the reaction mixture: this reacts with *O*-acylisourea to give an active intermediate, *viz*., acid imidazolide, which reacts with the amine. The rapid formation of imidazolide prevents $O \rightarrow N$-acyl migration. This approach allowed the preparation of conjugates of 5′-carboxy-containing oligonucleotides with amines in quantitative yields.[4]

A method for the synthesis of modified oligonucleotides **2a**,**b** containing ester groups at the 5′-termini has been described.[5] Postsynthetic treatment of these compounds successively with 0.1 M NaOH (4 h, 20 °C) and concentrated aqueous ammonia (7 h, 55 °C) afforded the 5′-carboxy-containing oligomer **3**.



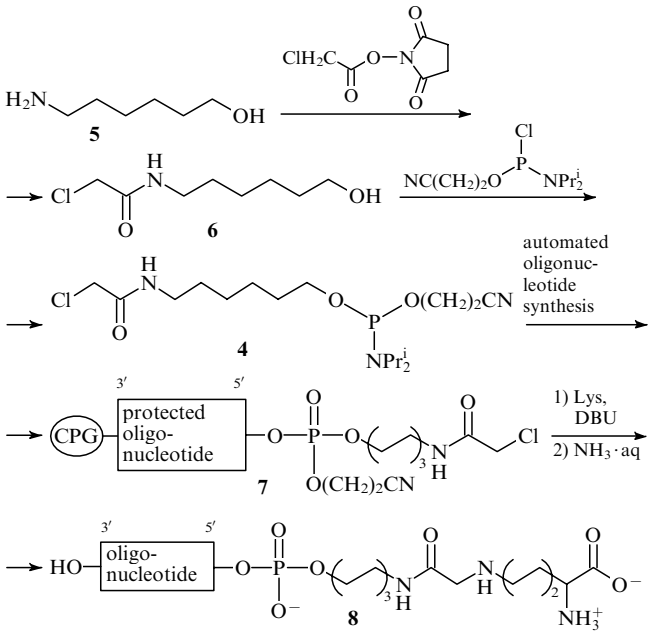
2a,**b**

3

R = $Cl(CH_2)_2$ (**2a**), CF_3CH_2 (**2b**); CPG is controlled pore glass.

A similar procedure was proposed by Guzaev *et al*.[6] for the synthesis of 5′-carboxy-containing oligonucleotides. In this case, automated oligonucleotide synthesis included incorporation of a non-nucleoside phosphoramidite **4** prepared by treatment of 6-aminohexanol (**5**) with *N*-chloroacetoxysuccinimide with subsequent phosphitylation of compound **6**. It was found[6] that an attempt to synthesise a bromoacetamide analogue of phosphoramidite **4** gave products of the Michaelis–Arbuzov reaction as deduced from ^{31}P NMR spectra. The presence of both nucleophilic and electrophilic reaction centres in the target phosphoramidite **4** makes the latter storage-unstable; therefore, compound **4** was introduced into an automated oligonucleotide synthesis immediately after its preparation.

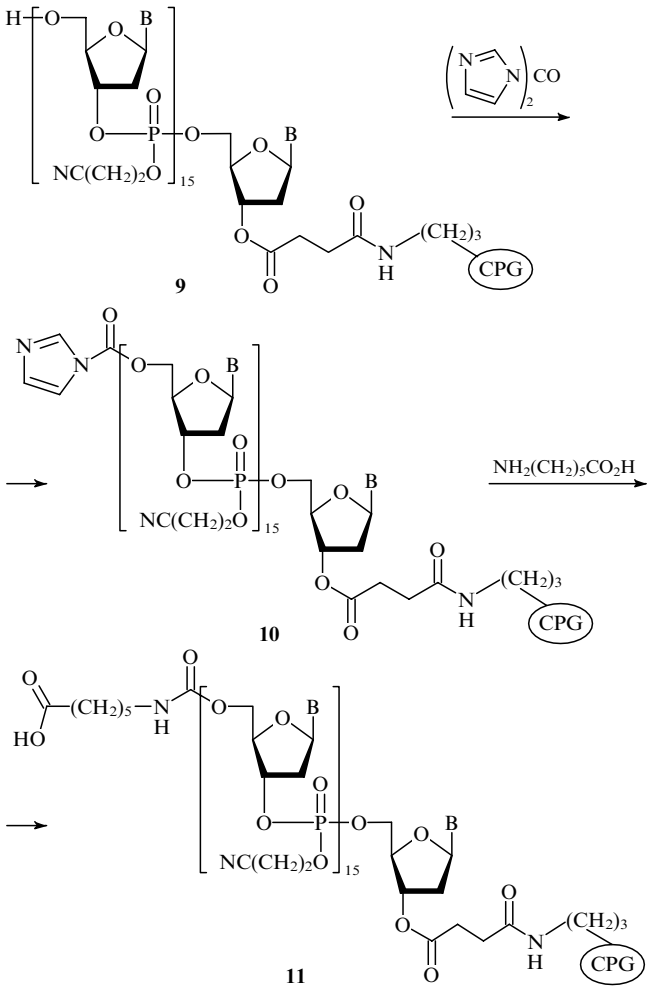
Postsynthetic treatment of model oligonucleotides **7** with lysine in the presence of a base, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), and then with concentrated aqueous ammonia gave oligomers **8** with 5′-terminal carboxy groups. It is noteworthy that the derivatives **7** can be used in the synthesis of oligonucleotides containing various functional groups.



5 **6** **4** **7** **8**

A method for the synthesis of oligonucleotides with 5′-terminal carboxy groups involving introduction of the carboxy groups after completion of the automated oligonucleotide synthesis has been described.[7]

In this case, the 5′-terminal hydroxy group in the oligonucleotide **9** was activated with 1,1′-carbonyldiimidazole and the derivative **10** reacted with 6-aminohexanoic acid in pyridine to yield the carboxy-containing oligonucleotide **11**.

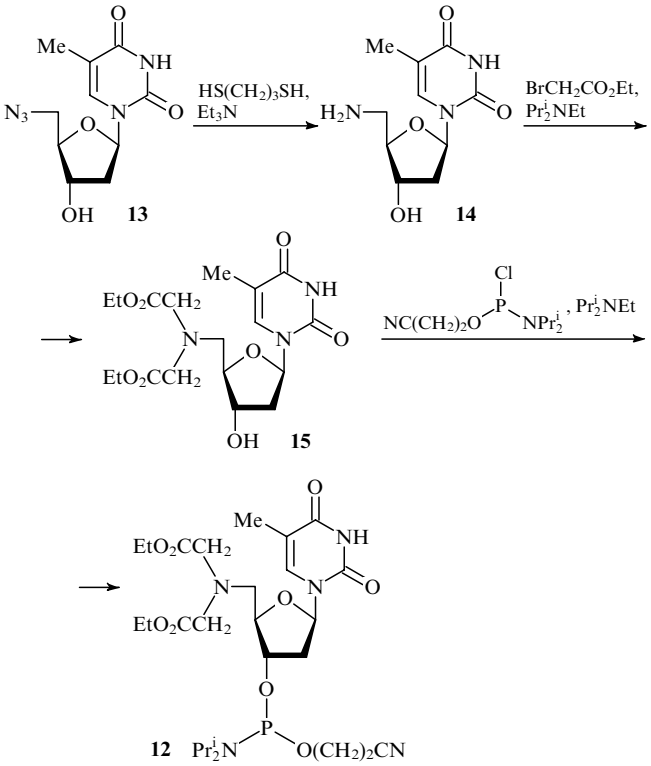


9 **10** **11**

B is a heterocyclic base.

After detachment of compound **11** from the solid phase and deprotection of the functional groups, an intercalator (daunomycin) was attached.[7]

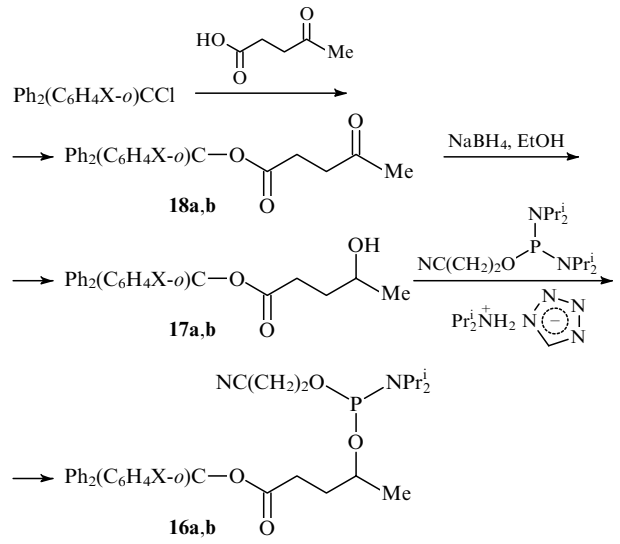
An original approach to the synthesis of modified oligonucleotides containing an iminodicarboxylic acid residue at the 5′-end has been developed.[8] The phosphoramidite derivative **12** was synthesised starting from 5′-azido-5′-deoxythymidine (**13**). Reduction of the azide with propane-1,3-dithiol gave nucleoside **14** with a 5′-group; alkylation of the latter with ethyl bromoacetate and subsequent phosphitylation of the derivative **15** afforded 5′-modified phosphoramidite **12**. The resulting compound was introduced into a standard automated oligonucleotide synthesis; after its completion, the ester was hydrolysed with aqueous sodium hydroxide.



The method for the synthesis of oligonucleotides with 5′-terminal carboxy groups described in study [9] is based on the use of the modified phosphoramidite derivative **16a** which was introduced into the automated oligonucleotide synthesis in the final step. The 2-chlorotrityl and trityl groups commonly employed in peptide synthesis, which can easily be eliminated by mild acid treatment,[10] were used for the carboxy protection.

The starting phosphoramidites **16a**,**b** were obtained by phosphitylation of 2-chlorotrityl (**17a**) and trityl (**17b**) esters of 4-hydroxyvaleric acid (formed upon reduction of the corresponding oxo derivatives **18a**,**b** with sodium borohydride) with 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite. Since the total yield of compound **16a** markedly exceeded that of compound **16b** and taking into account the higher stability of the 2-chlorotrityl group under the conditions used in the automated procedure, modified oligonucleotides **19** containing 5′-terminal carboxy groups were prepared from the phosphoramidite derivative **16a**.

The 5′-terminal 2-chlorotrityl protective group was removed under standard conditions. The carboxy group in the oligonucleotide **19** formed was activated using 1-hydroxybenzotriazole (HOBT) and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium (HBTU) hexafluorophosphate (40 min, 35 °C). The activated oligonucleotide was used [9] in solid-phase syntheses of a series of conjugates of modified oligonucleotides containing various nucleophilic components including fluorescent labels and short-chain peptides (Scheme 1).



The introduction of an aldehyde group at the 5′-end of an oligonucleotide has been described.[4] The 5′-terminal ester group of a synthetic oligomer **20** was deprotected with aqueous trimethylamine, and the resulting compound **21** was introduced into a reaction with 3-aminopropane-1,2-diol in the presence of DEC and imidazole to give the vicinal diol **22**. The latter is a precursor of the aldehyde which can be formed upon oxidation of the diol **22** with sodium periodate (Scheme 2).

It was recommended [4] that the oxidation of the vicinal diol **22** should be performed immediately before further modification of the oligonucleotide **23**, since the aldehyde group reacts with the amino groups of the heterocyclic bases. The reactivity of the aldehyde function was demonstrated by the reaction of the oligonucleotide **23** with biotin hydrazide with subsequent reduction of the acylhydrazone formed with sodium cyanoborohydride.

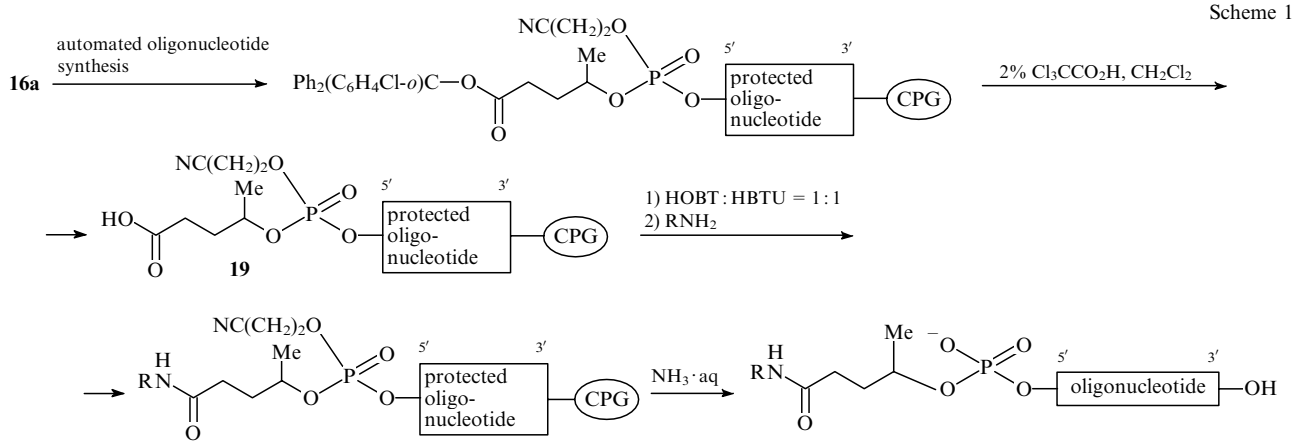
The introduction of the aldehyde group into the 5′-end of the oligonucleotide can be effected using an alternative approach which does not require any preliminary synthesis of a carboxy-containing oligomer. For this purpose, a phosphoramidite derivative of an aldehyde-containing nucleoside (or a non-nucleoside unit) or its precursor is incorporated inside the oligonucleotide chain in the final step of the automated synthesis. Obviously, in the former case, the carbonyl group must be protected. Such protection is necessary in order to avoid side reactions during the synthesis and postsynthetic treatments. Only one protective group, *viz*., the 1,3-bis(*m*-chlorophenyl)imidazolidine group, meets all the requirements for protective groups in automated oligonucleotide synthesis,[11] however, this group is not used very often.

The problems related to the side reactions of aldehyde groups can be overcome by introducing a precursor of an aldehyde-containing fragment, *e.g*., vicinal diol, instead of an aldehyde-containing fragment, into the oligonucleotide chain. The phosphoramidite derivative **24** was synthesised [12] in order to obtain oligonucleotides with 5′-terminal aldehyde groups.

Phosphoramidite **24** was synthesised from the alcohol **25** as the starting compound. The latter was converted into *tert*-butyldimethylsilyl ether **26** which was further oxidised into the vicinal diol **27** using catalytic amounts of osmium tetraoxide in the presence of *N*-methylmorpholine *N*-oxide. After benzoylation of the diol **27**, *tert*-butyldimethylsilyl protection was removed with tetrabutylammonium fluoride followed by phosphitylation of compound **28**.

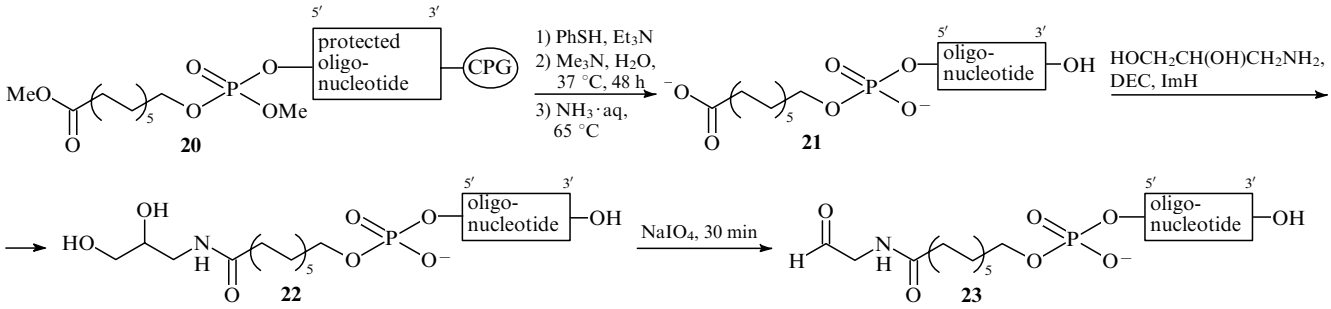
The resulting phosphoramidite derivative **24** was introduced into an automated oligonucleotide synthesis; after its completion, protective groups were removed with concentrated aqueous ammonia. The oxidation of the *vic*-diol groups in the resulting

Scheme 1



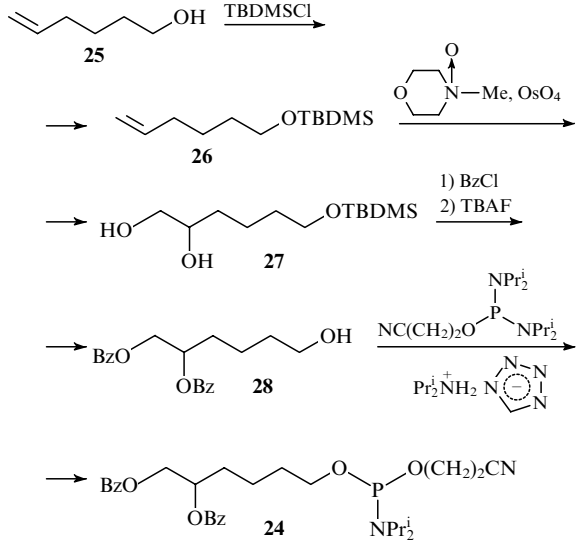
R is amine, amino acid, peptide residue

Scheme 2



ImH is imidazole.

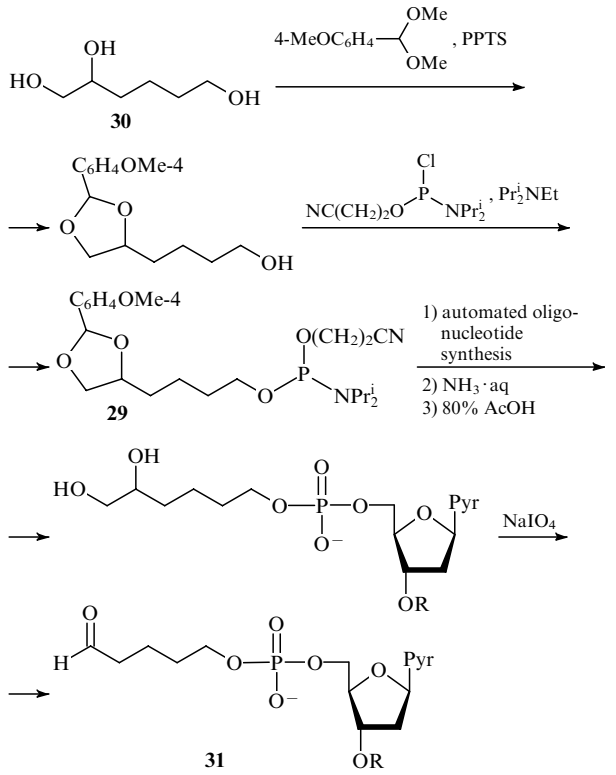
oligomers with sodium periodate was carried out as described previously,[4] *i.e.,* immediately before further transformation of the aldehyde function.[12]



TBDMS = Bu^tMe_2Si, TAF = $Bu^n_4N^+F^-$.

The method for the synthesis of oligonucleotides with 5′-terminal aldehyde groups proposed by Forget *et al.*[13] also entails the introduction of modified phosphoramidite **29** in the final step of the automated synthesis. It is noted that the two-step synthesis of compound **29** is a simpler and more convenient procedure than that described elsewhere.[12]

The *vic*-diol fragment of commercially available hexane-1,2,6-triol (**30**) was selectively blocked with the 4-methoxybenzylidene protective group under the action of 4-methoxybenzaldehyde dimethylacetal in the presence of catalytic amounts of pyridinium *p*-toluenesulfonate (PPTS). This protective group is stable under conditions of the automated oligonucleotide synthesis and is deblocked with 80% aqueous acetic acid in 1 h. In addition, the

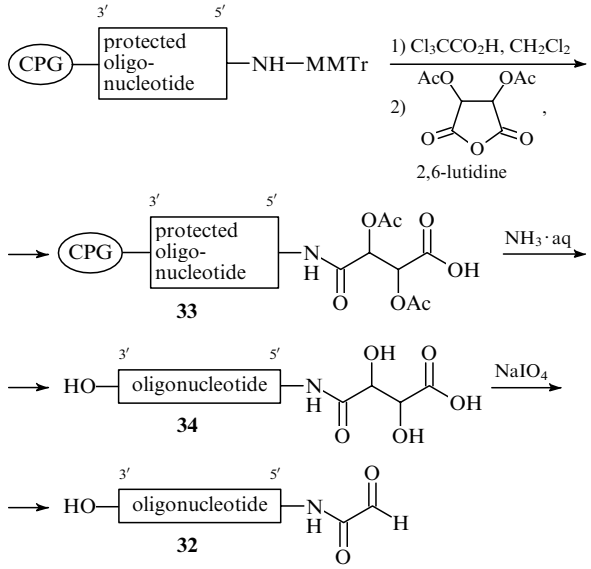


Pyr is pyrimidine heterocyclic base,
R is a fragment of the oligonucleotide chain.

hydrophobic nature of this protective group allows one to use it as an analogue of the dimethoxytrityl group in the isolation of target oligomers by reversed-phase HPLC. In the final step, treatment of deprotected oligonucleotides with a 50-fold excess of sodium periodate (30 min, 20 °C) gave the modified oligonucleotides **31** containing an aldehyde group at the 5′-end. A series of conjugates of oligonucleotides **31** with peptides [13] and sugars [14] containing aminooxy groups have been synthesised.

An original procedure for the synthesis of oligonucleotides **32** containing 5′-terminal glyoxylic amide has been developed.[15] This functional group reacts with hydrazines, hydroxylamines and ω-aminothiols without any activation, but unlike the aldehyde group, it is storage-stable.

In the synthesis of modified oligonucleotides **32,** a commercially available phosphoramidite with a linker containing a protected amino group was incorporated into the oligomeric chain in the final step of the automated synthesis. Monomethoxytrityl protection was removed with trichloroacetic acid in dichloromethane; the free amino group was acylated with diacetyltartaric anhydride in the presence of lutidine. The acetyl groups in the modified oligonucleotide **33** formed were removed by treatment with concentrated aqueous ammonia; the amide bond was resistant to ammonolysis. The oxidative cleavage of the vicinal diol **34** with sodium periodate (1.7 equiv. of $NaIO_4$ pH 6.6) yielded modified oligomers **32** with 5′-terminal glyoxylamide groups which were further used in the synthesis of oligonucleotide–peptide conjugates.

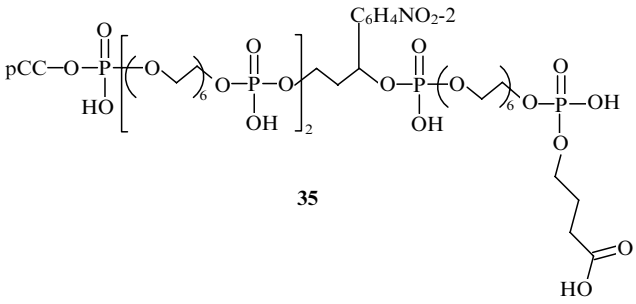


MMTr = (4-$MeOC_6H_4$)Ph_2C.

III. Synthesis of oligonucleotides containing a 3′-terminal carboxy or aldehyde group

At present, the synthesis of oligonucleotides with a 3′-terminal carboxy or aldehyde group usually entails incorporation of an additional non-nucleoside unit. Two different approaches to the synthesis of oligonucleotides modified at the 3′-end are known. In one of them, a fragment containing a protected hydroxy group is immobilised on a polymeric support. After completion of oligonucleotide synthesis, a reactive electrophilic group is formed at the 3′-end of the oligonucleotide as a result of chemical or photolytic cleavage of the covalent bond between the polymer and the linker. An alternative approach consists in the introduction of a carboxy or a carbonyl group after detachment of the synthesised oligonucleotide from the polymeric support. On the whole, the methods for the synthesis of oligonucleotides with 3′-terminal reactive electrophilic groups have a significant disadvantage, *viz.*, in both cases, the principle of incorporation of the functional group excludes further solid-phase reactions with the nucleophilic components.

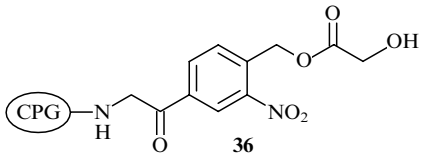
The dinucleotide **35** containing a photolabile *o*-nitrophenyl group and a carboxy group at the 3′-end, which was further used for the attachment of amines, has been synthesised.[16]



After termination of the solid-phase synthesis, the dinucleotide immobilised on the polymer through an ester bond was treated with a 33% aqueous solution of triethylamine (60 °C, 12 h) to detach it from the support and then with concentrated aqueous ammonia to remove the protective groups. The terminal carboxy group can be used for the attachment of reporter molecules to the oligonucleotide; these can selectively be removed from the support owing to the presence of a photolabile fragment.

In many cases, selective cleavage of the oligonucleotide from the polymeric support without removal of all protective groups acquires special importance in the synthesis of nucleotide–peptide conjugates. To this end, Greenberg *et al.*[17–19] used polymeric supports modified with photolabile groups.

Initially, in the synthesis of carboxy-containing oligonucleotides, a method was applied in which the first nucleoside fragment was attached to the polymer **36** through a photolabile *o*-nitrobenzyl linker. In the final step of the synthesis, the oligonucleotides were split from the polymer by irradiation with light with the wavelength ~ 400 nm. The yields of the modified oligomers did not exceed 62%, which can be attributed to the destruction of the target product upon irradiation.[17]

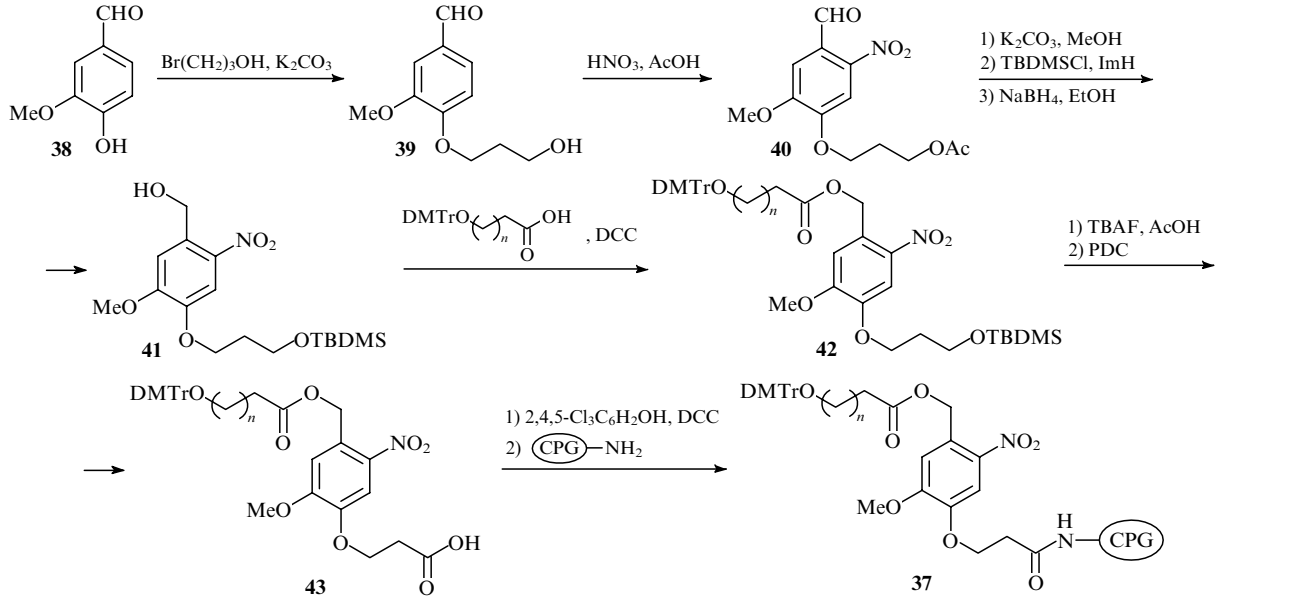


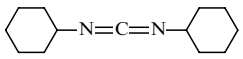
Polymers **37** with linkers of variable lengths between the first nucleoside fragment and the photolabile group were used for the synthesis of oligonucleotides, the time of photolysis was thereby reduced.[18] Vaniline (**38**) was used as a starting compound in the synthesis of the polymeric support **37** (Scheme 3). In the first step, vaniline was alkylated with 3-bromopropanol after which the intermediate **39** was subjected to nitration in glacial acetic acid accompanied by simultaneous *O*-acetylation to form the derivative **40**. Removal of the acetyl and introduction of the *tert*-butyldimethylsilyl protective group and further reduction of the formyl group to the hydroxymethyl group gave substituted benzyl alcohol **41**. Condensation of compound **41** with the corresponding protected hydroxycarboxylic acid in the presence of dicyclohexylcarbodiimide yielded the derivative **42**, one of the protected hydroxy groups in which was converted into a carboxy group as a result of selective deprotection and subsequent oxidation with pyridinium dichromate.

Immobilisation of the resulting compound **43** on a polymeric support was carried out using activated trichlorophenyl esters in the final step of the synthesis. To minimise the formation of side products, the remaining amino groups of the polymer were acetylated.

The use of the polymeric support **37** made it possible to obtain the target oligonucleotides with 3′-terminal carboxy groups in quantitative yields. The yield of a modified oligonucleotide was

Scheme 3



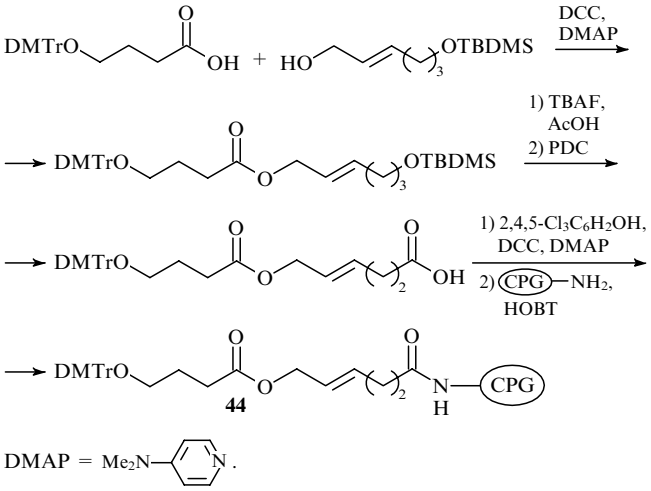
DMTr = $(4\text{-MeOC}_6\text{H}_4)_2\text{PhC}$, n = 1–4, PDC is pyridinium dichromate, DCC =  .

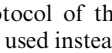
defined as the ratio of the amount of the modified oligomer obtained upon photolysis and deprotection with concentrated aqueous ammonia to the amount of the oligomer prepared by treatment with ammonia without preliminary irradiation.

The oligonucleotides prepared were further employed in the synthesis of conjugates with peptides and fluorescent labels without removal of protective groups.[19] The reactions were carried out in organic media; the carboxy groups were activated with a HOBT–HBTU mixture (HBTU : HOBT : peptide = 1 : 1 : 5) in the presence of diisopropylethylamine. The yields of the conjugates were 85%–100%.

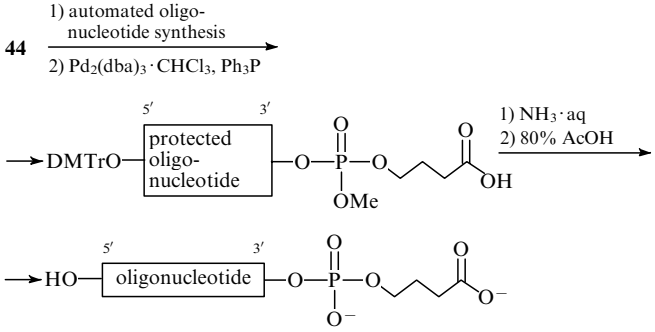
It was proposed[20] that polymeric support **44** with a linker containing an allylic-type ester selectively cleavable under the action of a palladium catalyst[21] would be used in the synthesis of oligonucleotides with 3′-terminal carboxy groups. This allows the removal of the modified oligonucleotide from the polymeric support without deprotection of the functional groups.

The synthesis of the modified polymer **44** was carried out as shown in the scheme below.



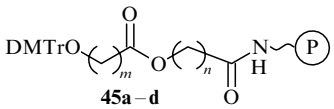
DMAP =  .

The use of the polymeric support **44** requires a change in the standard protocol of the automated synthesis. Thus *tert*-butyl peroxide was used instead of iodine in a pyridine–water mixture, since this results in iodination of the double bond in the oxidation of internucleotide phosphite groups.[20] Moreover, the methyl group was used instead of the β-cyanoethyl group to protect the internucleotide phosphate groups. The reason is that during the cleavage of an ester bond between the oligonucleotide and the polymeric support under the action of tris(dibenzylideneacetone)-dipalladium(0) and triphenylphosphine in a n-butylamine–1.2 M formic acid mixture, the β-cyanoethyl protective group is cleaved completely at 55 °C in 1 h, whereas removal of the methyl group was less significant under identical conditions (10% in 7 h). The same method was used for the synthesis of a series of modified oligonucleotides.[20]

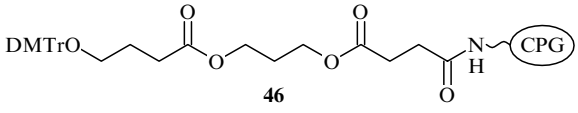


dba is dibenzylideneacetone.

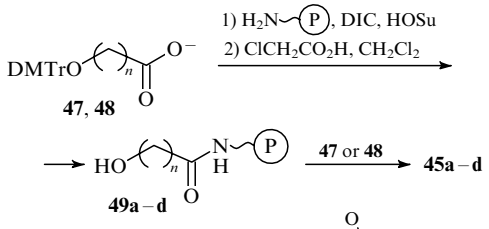
Since functional groups are introduced at the 3′-end of the oligonucleotide owing to the cleavage of the ester bond between the solid phase and the oligomer, the requirements of the polymeric supports are very strict as regards the stabilities and reactivities of the ester fragments. A series of homologous polymers **45a–d** and **46** have been designed in a search for a polymeric support which meets better these requirements.[22,23]

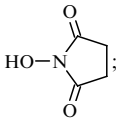


a: P = CPG, n = 3, m = 3;
b: P = CPG, n = 1, m = 3;
c: P = CPG, n = 1, m = 1;
d: P = PS (PS is polystyrene), n = 1, m = 1.

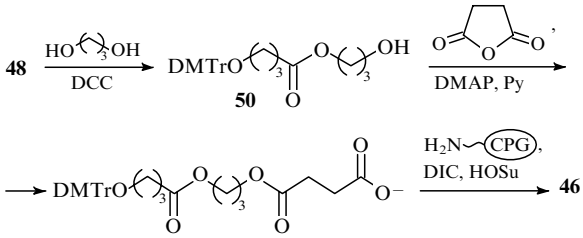


The polymeric supports **45a–d** were prepared by the reaction of glycolic (**47**) and 4-hydroxybutyric acids (**48**) containing a dimethoxytrityl protective group with the amino groups of the polymer in the presence of *N*,*N*′-diisopropylcarbodiimide and *N*-hydroxysuccinimide. After detritylation, the polymers **49a–c** were introduced into a reaction with protected acids **47** and **48** in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride and catalytic amounts of *N*-methylimidazole. When polystyrene was used as a starting support, the activation of the carboxy group in compound **47** was carried out in the presence of carbodiimide and *N*-hydroxysuccinimide.

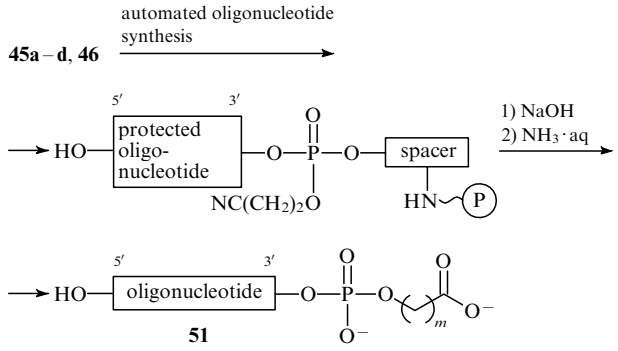


DIC = $Pr^iN=C=NPr^i$, HOSu = ;
P = CPG (**49a–c**), PS (**49d**);
n = 1 (**47**, **49b–d**), 3 (**48**, **49a**).

The modified polymeric support **46** was prepared by esterification of the protected 4-hydroxybutyric acid **48** with propane-1,3-diol in the presence of DCC resulting in compound **50** which was further acylated with succinic anhydride and immobilised on a solid phase using the carbodiimide method.



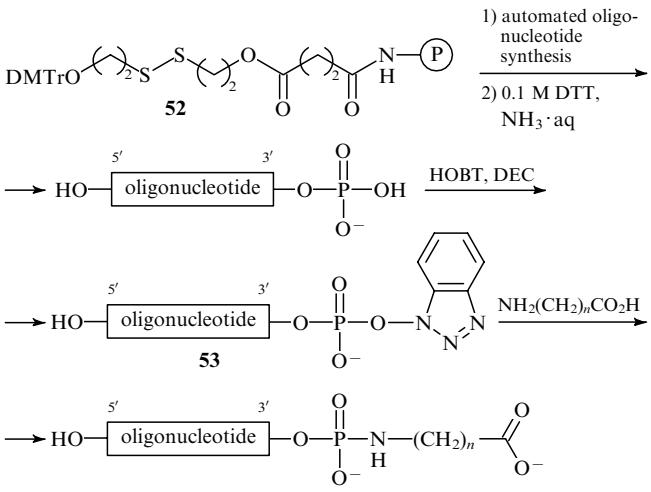
The polymeric supports **45a–d** and **46** were used in the synthesis of oligonucleotides **51** containing a 3′-terminal carboxy group using the phosphoramidite approach. Cleavage of the oligomers from the solid phase and deprotection of internucleotide phosphates were performed by treatment with a 0.1 M aqueous solution of sodium hydroxide or 0.5 M DBU in H_2O; deprotection of heterocyclic bases was achieved by subsequent treatment with concentrated aqueous ammonia.



m = 1, 3.

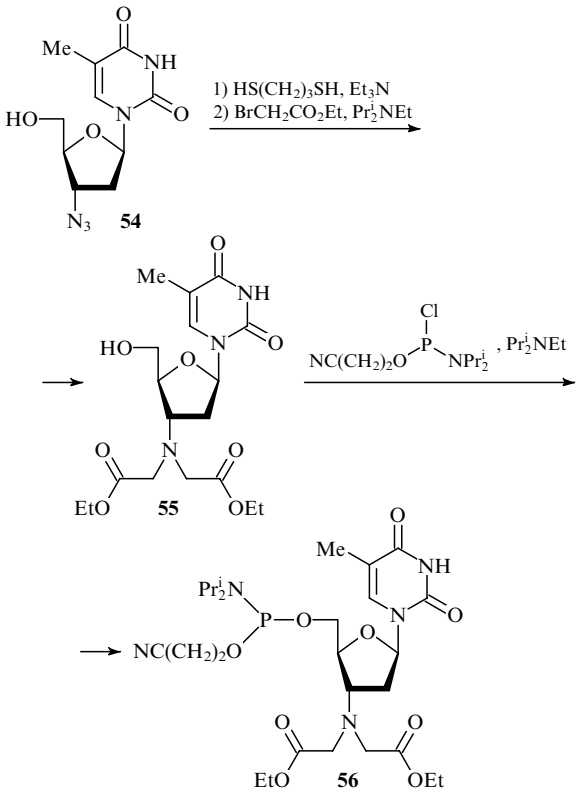
Conjugates of modified oligonucleotides **51** with α,ω-diaminoalkanes were prepared by treatment of oligomers immobilised on modified supports with the corresponding amino component. However, the action of these reagents on oligodeoxyribonucleotides is accompanied by transamination of *N*-protected cytidine residues. Therefore, prior to the synthesis [23] benzoyl protective groups of the heterocyclic bases (cytosine and adenine) were selectively removed with hydrazine in acetic acid. Under these conditions, oligonucleotides remained bound to the polymeric supports.

The 3′-terminal carboxy group was introduced into the oligonucleotide following termination of the automated synthesis.[7] The cleavage of the target product containing a 3′-terminal phosphate group from the modified polymer **52** occurred under the action of 0.1 M dithiothreitol (DTT) in concentrated aqueous ammonia as a result of cleavage of the disulfide bond and subsequent intramolecular attack of the sulfide ion formed at the carbon atom. The carboxy group was introduced by treatment of the activated deprotected 3′-phosphorylated oligonucleotide **53** with ω-aminocarboxylic acid.

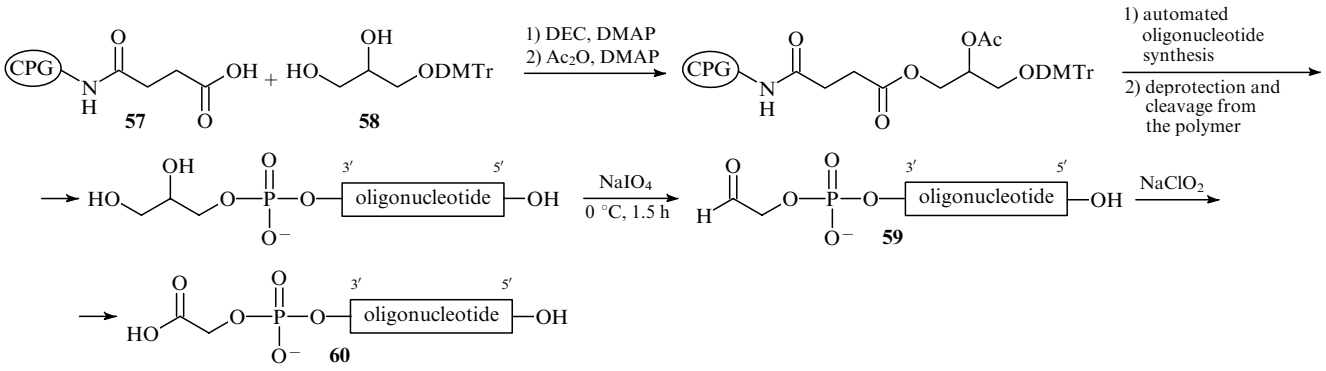


(P)—NH— aminopropylfractosyl-500, *n* = 5, 10.

The synthesis of modified oligonucleotides containing a 3′-terminal iminodiacetic acid residue has been described.[8] These compounds can bind metal ions which stabilise the function of certain proteins. 3′-Azido-3′-deoxythymidine (**54**) as a starting nucleoside component was further converted into 3′-amino-3′-deoxythymidine and alkylated with two equivalents of ethyl



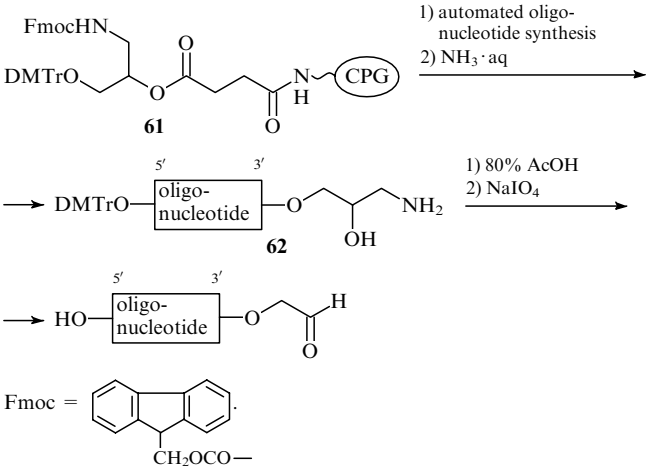
Scheme 4



bromoacetate. The resulting product **55** was converted into a modified phosphoramidite derivative **56** which was introduced into the oligonucleotide synthesis in the final step. The elongation of the chain was performed from the 5′-end of the oligomer to its 3′-end. On termination of the synthesis, the carboxy groups of the target oligonucleotides were deblocked with an aqueous solution of sodium hydroxide.

The synthesis of oligonucleotides containing 3′-terminal aldehyde groups has been described.[24] The polymeric support **57** was treated with monosubstituted glycerol **58** and the modified polymeric support with a linker was introduced into the automated oligonucleotide synthesis. After deprotection and cleavage from the polymer, the oligonucleotide containing a fragment with a vicinal diol group was oxidised with sodium periodate to yield modified oligomers **59**. Treatment of oligonucleotides **59** with sodium chlorite gave oligonucleotides **60** with 3′-terminal carboxy groups (Scheme 4).

An original approach[25] to the introduction of aldehyde groups into the 3′-termini of oligonucleotides based on the use of a commercially available polymeric support **61** in an automated synthesis[26] has been proposed.



Ammonolysis of the ester bond between the polymer and the synthetic oligonucleotide yields the oligomer **62** containing a 3′-terminal α-aminoalcohol fragment. Subsequent removal of the dimethoxytrityl group and oxidative cleavage with sodium periodate results in oligonucleotides with 3′-terminal aldehyde groups.

Conjugates of synthetic oligonucleotides with aminooxy derivatives of some peptides have been prepared.[25] The reactions were carried out in aqueous solutions in the presence of a threefold excess of aminooxy components (pH 4.5, 2 h). The yields of the conjugates were ~ 50%.

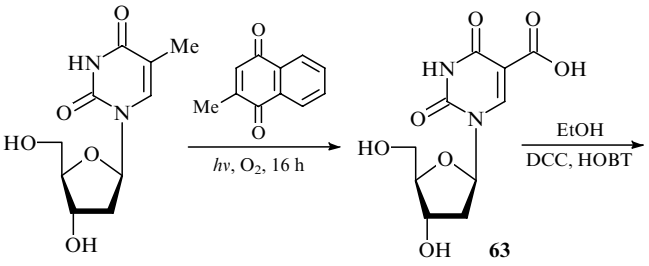
IV. Synthesis of oligonucleotides containing an internal carboxy or aldehyde group

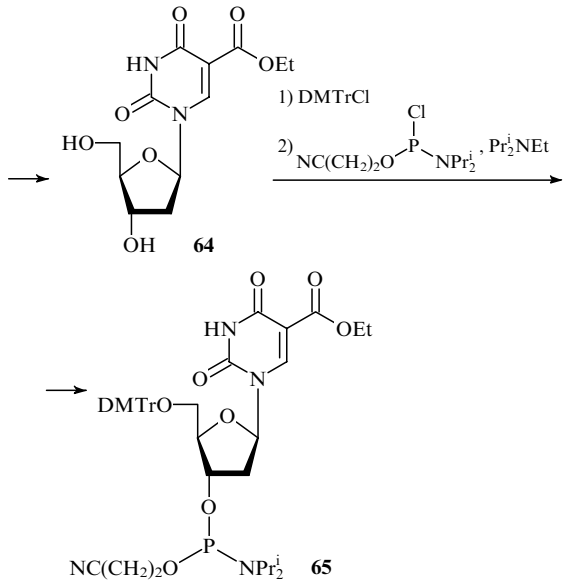
Oligonucleotides with electrophilic groups within the chain are especially preferred in molecular biology studies. This type of modification seems to hold great promise, since it leaves the ends of the oligomeric chain free, which allows incorporation of a radioactive or fluorescent label at the 5′-end and further transformations of the functional group on the polymeric support provided this can selectively be deprotected. A reactive group can be introduced at any predetermined position of the oligonucleotide chain, *viz*., at a heterocyclic base, at the 2′-position of the carbohydrate fragment or the internucleotide phosphate group. The presence of a reactive functional group in the oligomeric chain influences the stability of DNA duplexes formed by modified oligomers and complementary NA matrices as can be judged from the change in the melting temperatures of such duplexes in comparison with the melting temperatures of non-modified analogues.

1. Oligonucleotides containing a carboxy or aldehyde group in the heterocyclic base

The preparation of oligonucleotides containing a reactive electrophilic group in the heterocyclic base entails the introduction of a modified phosphoramidite derivative, with a carboxy or aldehyde group either blocked or formed upon postsynthetic treatment of the oligomer, into the automated oligonucleotide synthesis. The methyl group of thymidine is modified especially easily. Modified oligonucleotides containing 5-carboxy-2′-deoxyuridine **63** have been synthesised.[27]

Substitution of the carboxy group for the methyl group of thymidine was carried out by oxidation with 2-methyl-1,4-naphthoquinone (menadione) in the air under irradiation (350 nm, 16 h). Esterification of the carboxy group in compound **63** was carried out in dry ethanol in the presence of carbodiimide and 1-hydroxybenzotriazole without preliminary protection of the hydroxy groups of the carbohydrate residue. The resulting compound **64** was converted into the phosphoramidite derivative **65**, which was further incorporated into the oligonucleotide chain in the automated synthesis. The hydrolysis of the ethyl ester of 5-carboxy-2′-deoxyuridine with simultaneous removal of the remaining protective groups and cleavage of the target product

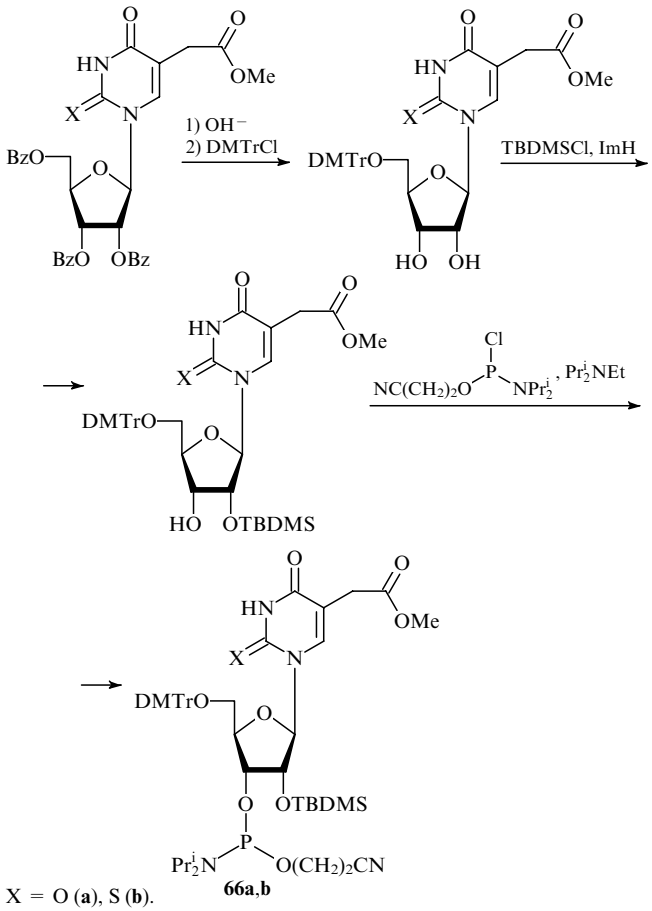




from the solid phase were performed by treatment with 0.2 M sodium hydroxide in methanol.

To investigate the relationship between the structure and biological properties of the anticodon domain of *E. coli* $tRNA^{Lys}$, Davis *et al.* [28,29] synthesised modified RNA fragments containing substituents with carboxy groups in position 5 of uridine or N^6 of adenosine.

The synthesis of modified oligoribonucleotides containing 5-methoxycarbonylmethyluridine or 2-thio-5-methoxycarbonylmethyluridine has been described.[28] The starting nucleosides for the synthesis of phosphoramidites **66a**,**b** were prepared by the Vorbruggen glycosylation [30] of the corresponding disilylated 5-methoxycarbonyluracil and 5-methoxycarbonylmethyl-2-thiouracil derivatives with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose. Subsequent transformations were carried out by standard procedures used in the synthesis of ribonucleoside phosphoramidite derivatives.



X = O (**a**), S (**b**).

Compounds **66a**,**b** were incorporated into the oligoribonucleotide chain. The oligomers synthesised were deprotected with 10% DBU in methanol (12 h, 25 °C) and $Et_3N \cdot 3HF$; interestingly, treatment with DBU was accompanied by deprotection of the carboxy group. The structures of the modified oligoribonucleotides were confirmed by mass spectrometry and ^1H NMR spectroscopy. The authors [28] call special attention to the fact that incorporation of the 5-methoxycarbonylmethyluridine and 2-thio-5-methoxycarbonylmethyluridine residues into the RNA fragment did not virtually affect the thermal stability of the RNA hairpin.

The same group of American investigators reported the synthesis of oligoribonucleotides containing a modified nucleoside **67**.[29] The target phosphoramidite derivative **68** containing protected hydroxy and carboxy groups was synthesised starting from adenosine (**69**) (Scheme 5). Acetylated adenosine reacted with ethyl chlorocarbonate to give the nucleoside **70**. Simultaneous introduction of carboxy and secondary hydroxy groups into compound **70** was effected by treatment with L-threonine. Trimethylsilylethyl and *tert*-butyldimethylsilyl protective groups, which can be removed with $Et_3N \cdot 3HF$ after termination of the automated synthesis, were selected for the protection of the incorporated functional groups.

The phosphoramidite derivative **68** was used [29] for the synthesis of the modified oligoribonucleotide sequence of the *E. coli* $tRNA^{Lys}$ fragment.

An interesting procedure for the synthesis of oligodeoxyribonucleotides containing 5-methoxycarbonylmethyluridine residues has been described.[31,32] Condensation of dimethyl-2-bromomethyl fumarate (**72**) and arabinoaminooxazoline **73** synthesised from D-arabinose under the action of cyanamide and 6 M aqueous ammonia (Scheme 6) is the key step in the synthesis of the modified phosphoramidite derivative of 5-methoxycarbonylmethyl-2-deoxyuridine **71**. Treatment of O^2,2′-anhydro-5-methoxycarbonylmethyluridine formed (**74**) with acetic anhydride in boiling acetic acid results in the opening of the anhydrocycle. Acetyl protective groups are removed by treatment with methanolic ammonia. The resulting modified nucleoside **75** can be used as a starting compound in the oligoribonucleotide synthesis.

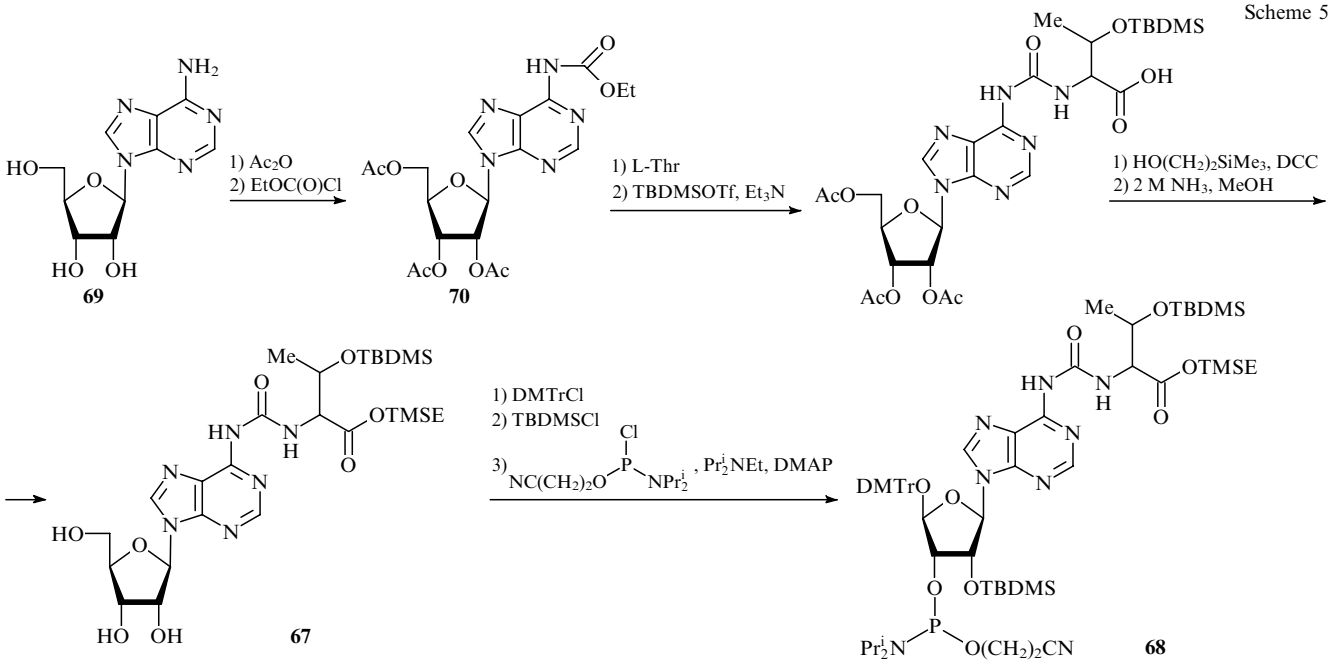
The opening of the anhydrocycle in compound **74** under the action of hydrogen bromide in trifluoroacetic acid or of acetyl bromide in acetonitrile results in 2′-bromo-2′-deoxy-3′,5′-di-*O*-acetyl-5-methoxycarbonylmethyluridine (**76**); reduction of the latter with hydrogen over palladium or with tributyltin hydride in the presence of azoisobutyronitrile affords compound **77**. Further transformations of nucleoside **77** into phosphoramidite **71** were carried out using standard methods.

The phosphoramidite derivative **71** was used [32] in the synthesis of oligonucleotides containing substituents with protected carboxy groups in position 5 of the heterocyclic base. The presence of a modified fragment in the oligonucleotide chain was confirmed by HPLC of products of enzymatic hydrolysis by snake venom phosphodiesterase and alkaline phosphatase.

These oligonucleotides immobilised on a solid phase were treated with 50% solutions of diamines in ethanol at room temperature to give the corresponding amides. However, it was shown [32] that the 5-methoxycarbonylmethyl group (methyl ester) in position 5 is not very reactive: the reaction time with diamines is 12 h.

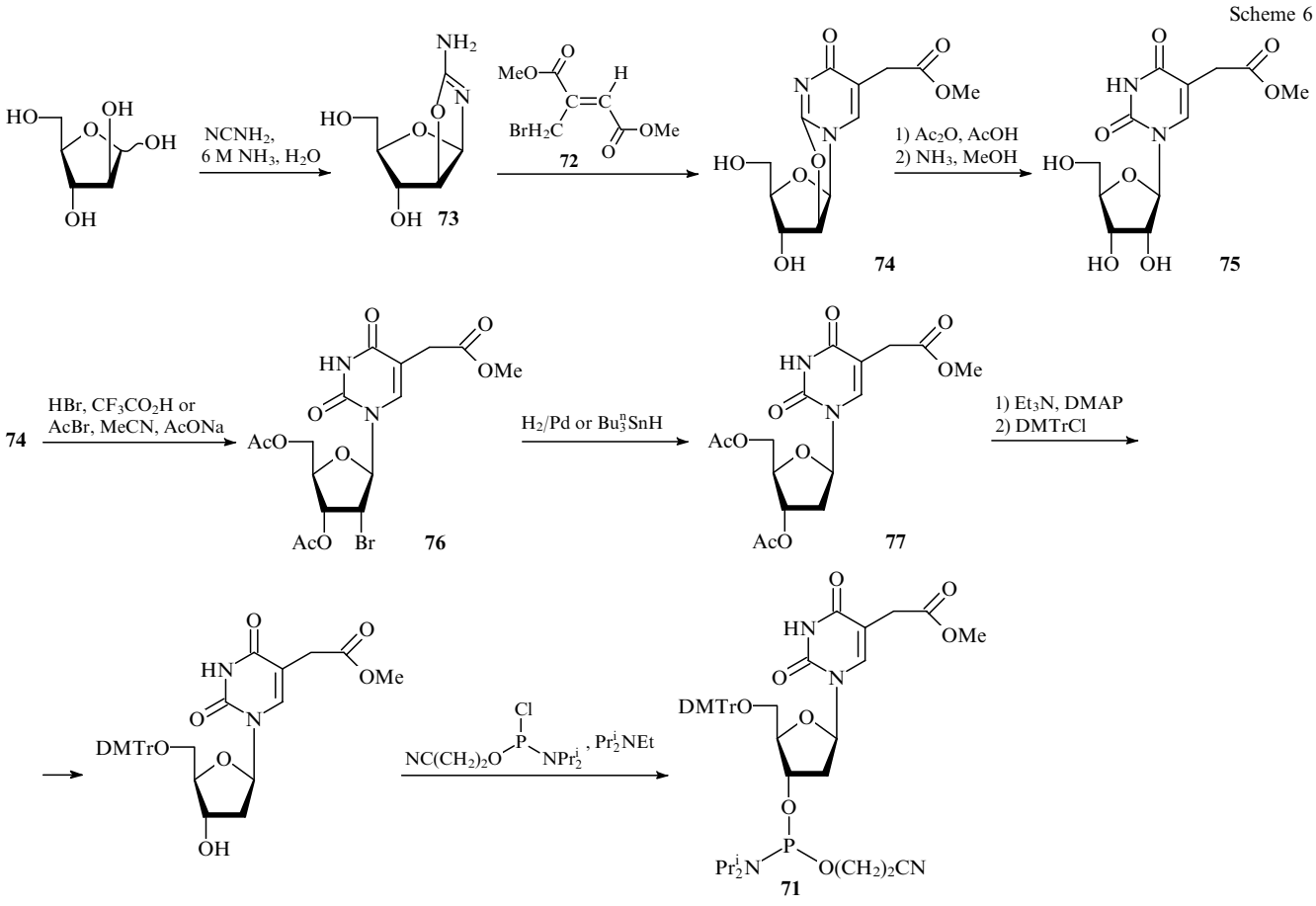
In order to reduce the reaction time with diamines, the same group of Japanese investigators used modified oligonucleotides in which the carboxy group of the substituent at C(5) of the heterocyclic base was protected as a cyanomethyl ester.[33] The target phosphoramidite **78** was prepared from the diacetyl derivative **77**. The key stage in this synthesis is esterification of the free carboxy group of nucleoside **79** with bromoacetonitrile in the

Scheme 5

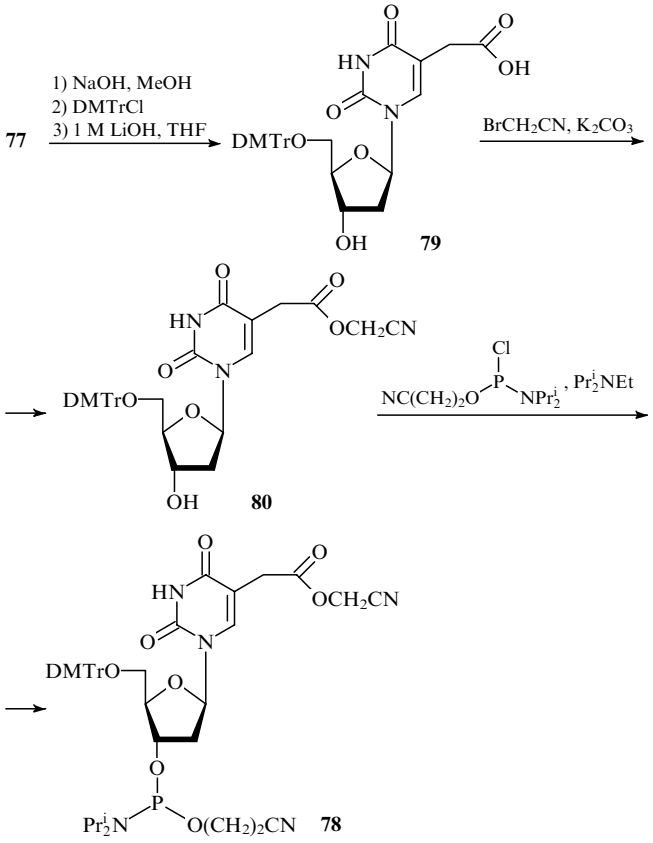


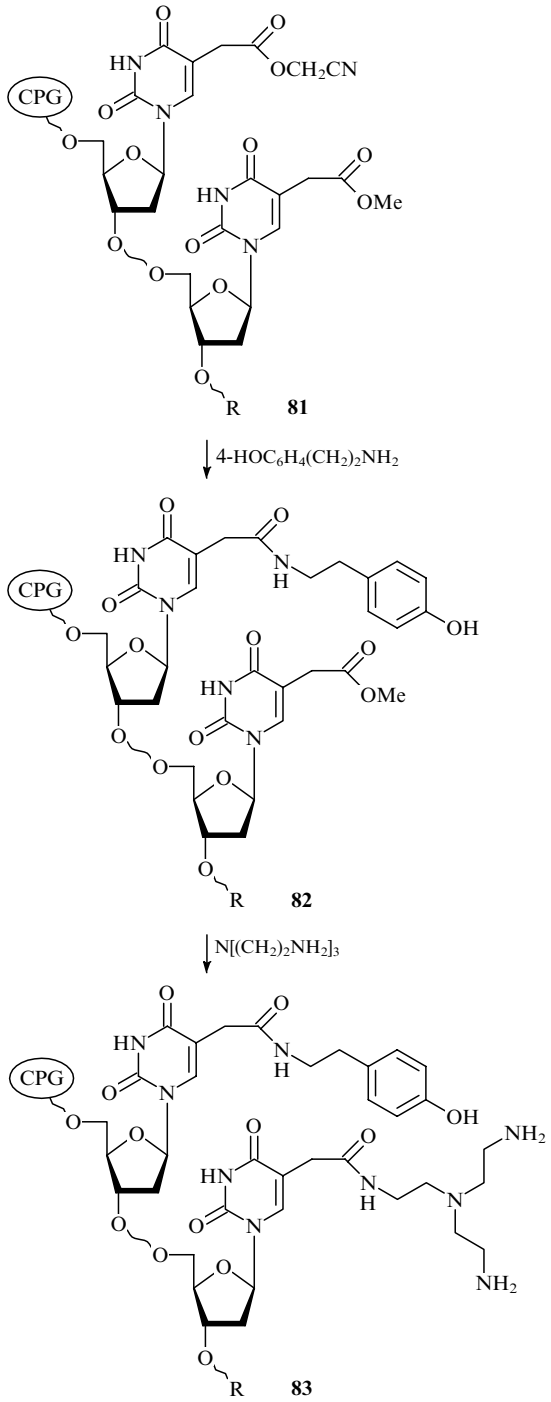
Thr = $NH_2CH(CO_2H)CH(OH)Me$, Tf = CF_3SO_2, TMSE = $Me_3Si(CH_2)_2$.

Scheme 6



presence of potassium carbonate in hexamethylphosphoric triamide (HMPA). The yield of the product **80** was 70%. The increase in the reaction time and the use of chloroacetonitrile and triethylamine decreased the yield of the ester **80** to 30% due to the formation of the N^3-cyanomethyl derivative.





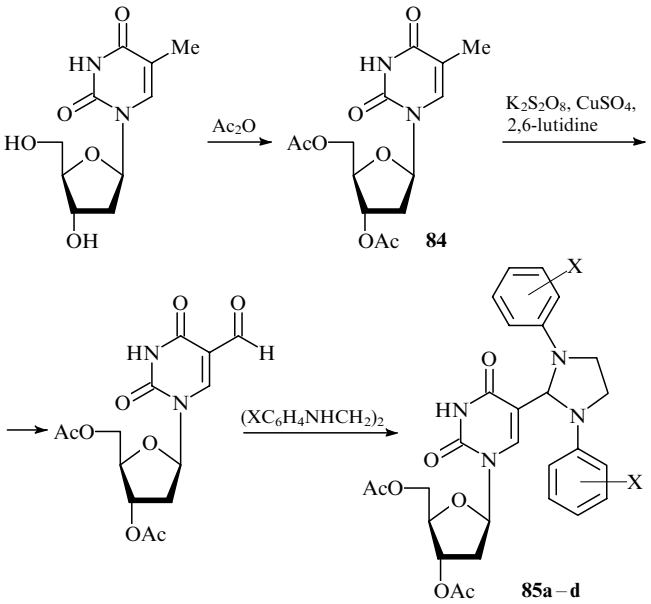
R is a fragment of a protected oligonucleotide chain.

The strategy of synthesis of oligomers with a cyanomethylated carboxy group within the chain is similar to that described in study.[32] The presence of a modified unit was confirmed by enzymatic hydrolysis with nuclease P1 and alkaline phosphatase. Aminolysis of the resulting oligonucleotides showed that the cyanomethyl ester is more reactive than the methyl ester and the use of this particular type of oligonucleotides allows the reduction of the reaction time to 1 h, whereas the concentration of the amino component can be decreased to 0.6–1.0 mol litre^{-1}. However, the reaction with tyramine was over only in five days, which can be attributed to poor solubility of this compound in organic solvents and low pK_a of its amino group. It was found that the addition of catalytic amounts of triazole to the reaction mixture significantly increases the rate of aminolysis.

In the synthesis of oligonucleotide conjugates with two different amino components, advantage was taken of the difference in the reactivities of methyl and cyanomethyl esters.[34] To this end, the phosphoramidite derivatives **71** and **78** were employed in the synthesis of oligonucleotides **81** containing both 5-methoxycarbonyl and 5-cyanomethoxycarbonylmethyluridine residues.

The incorporation of two different functional groups was carried out in the following way. First, the oligonucleotide **81** immobilised on a solid phase was treated with tyramine in the presence of triazole (20 °C, 3 days); in this case, tyramine reacted selectively with the cyanomethyl ester. After washing of the polymer, tris(2-aminoethyl)amine (50% ethanolic solution) was added to the oligonucleotide **82** and complete aminolysis resulted in the derivative **83**. The presence of two different amide groups was confirmed by enzymatic hydrolysis of the isolated oligonucleotide under the action of snake venom phosphodiesterase, alkaline phosphatase and nuclease P1.

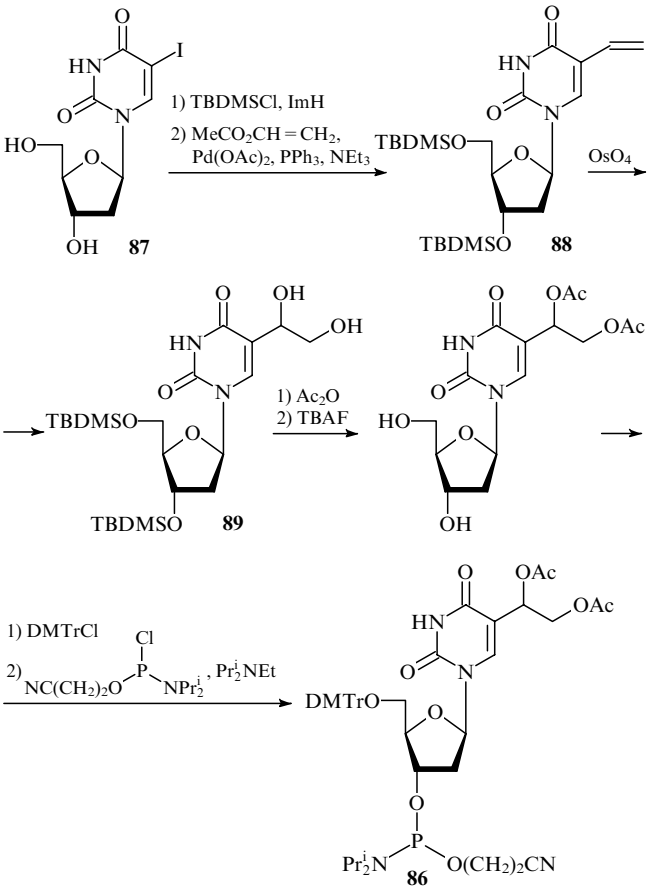
The methods for the synthesis of oligonucleotides containing an aldehyde group in the heterocyclic base usually entail mild oxidation of the methyl group of thymine. Thus the synthesis of modified oligomers containing 5-formyl-2′-deoxyuridine residues has been described.[11,35] The key step in this synthesis was the oxidation of the methyl group of protected thymidine **84** to the formyl group under the action of potassium persulfate in the presence of copper sulfate and 2,6-lutidine (yield 52%). It was recommended to use the 1,3-diphenylimidazolidine protective group, which is stable under alkaline conditions and is removed by treatment with an acid, for protection of the aldehyde group.[36,37] By introducing various electron-withdrawing substituents into the benzene rings of *N*,*N*′-bis(phenyl)ethylenediamine, one can vary the pK of the amino groups and thus control the ease of cleavage of the protective group under the action of acids.



X = H (**a**), 4-F (**b**), 4-Cl (**c**), 3-Cl (**d**).

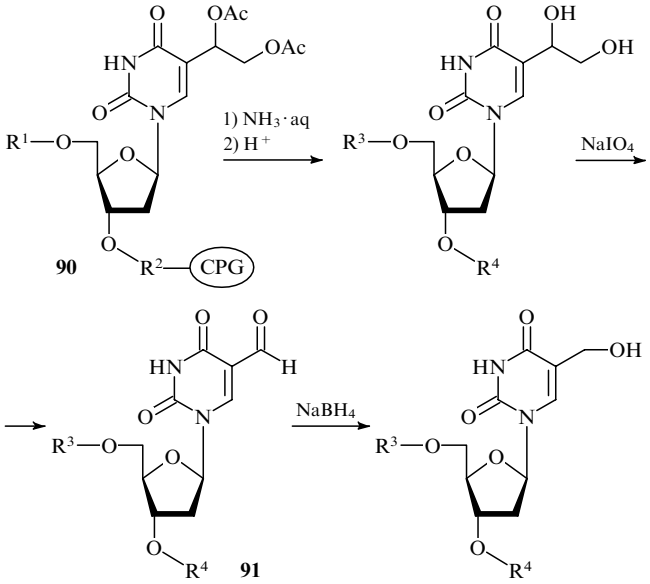
The stabilities of modified nucleosides **85a**–**d** in acidic media have been investigated. It was shown that the diphenylimidazolidine protective groups do not withstand conditions of the cleavage of the 5′-dimethoxytrityl protective group (3% trichloroacetic acid in dichloromethane, 25 °C). A decrease in the concentration of trichloroacetic acid to 1% helps avoid the deprotection of the introduced carbonyl group in compound **85d**, which has been chosen for the synthesis of the phosphoramidite derivative and subsequent oligonucleotide synthesis. Bis(3-chlorophenyl)-imidazolidine protection was removed in the final step using 80% aqueous acetic acid. The presence of an aldehyde group in modified oligonucleotides was confirmed by enzymatic hydrolysis with a mixture of snake venom phosphodiesterase and alkaline phosphatase. An alternative approach to the synthesis of oligonucleotides containing 5-formyl-2′-deoxyuridine residues has been described.[38] In this case, the phosphoramidite derivative of the precursor compound **86** was introduced into the automated nucleotide synthesis. The carbonyl group was generated by post-synthetic treatment of the oligonucleotide fragment containing a *vic*-diol group, with sodium periodate.

Scheme 7



5-Iodo-2′-deoxyuridine (**87**), which was further converted into the vinyl derivative **88** by reaction with vinyl acetate in the presence of catalytic amounts of palladium acetate and triphenylphosphine (Scheme 7), has been used as a starting compound for the synthesis of the phosphoramidite **86**.[38] The vinyl fragment was oxidised with osmium tetraoxide in the presence of *N*-methylmorpholine *N*-oxide and the *vic*-diol group in position 5 of compound **89** was protected by acetyl groups. Phosphoramidite **86** was introduced into the automated oligonucleotide synthesis.

The resulting modified oligomers **90** were treated with concentrated aqueous ammonia to remove acetyl protective groups and to achieve detachment from the solid phase and then the *vic*-diol group was oxidised with sodium periodate (50 equiv. of $NaIO_4$, 1 min, 0 °C). Compound **91** was reduced with sodium borohydride, which served as an indirect evidence for the internal position of the carbonyl group in the oligonucleotide.

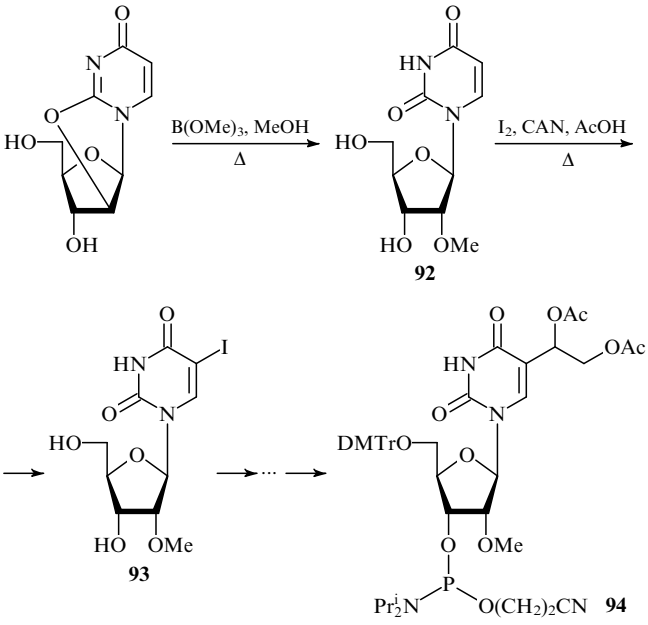


R^1 and R^2 are fragments of a protected oligonucleotide chain;
R^3 and R^4 are fragments of a deprotected oligonucleotide chain.

The effect of the aldehyde group incorporated into a heterocyclic base on the thermal stabilities of duplexes formed from the oligomer **91** and its complementary natural oligonucleotide, has been studied.[38] It was shown that the presence of a formyl group decreases the melting temperatures of the heterogeneous 12- and 6-membered duplexes by 4.1 °C and 5.8 °C, respectively, in comparison with the non-modified analogues.

The phosphoramidite derivative **86** was also used in the synthesis of an oligonucleotide sequence containing a recognition site for the transcription factor NFκb.[39] It is known[40] that 5-formyluridine binds covalently to the ε-amino group of lysine. Compound **86** was synthesised according to a scheme similar to that described in [38] except that the vinyl fragment was introduced into the derivative **87** using tributylvinyltin in the presence of catalytic amounts of the $Pd(MeCN)_2Cl_2$ complex instead of vinyl acetate, which made it possible to increase the yield of compound **88** from 68% to 80%.[39]

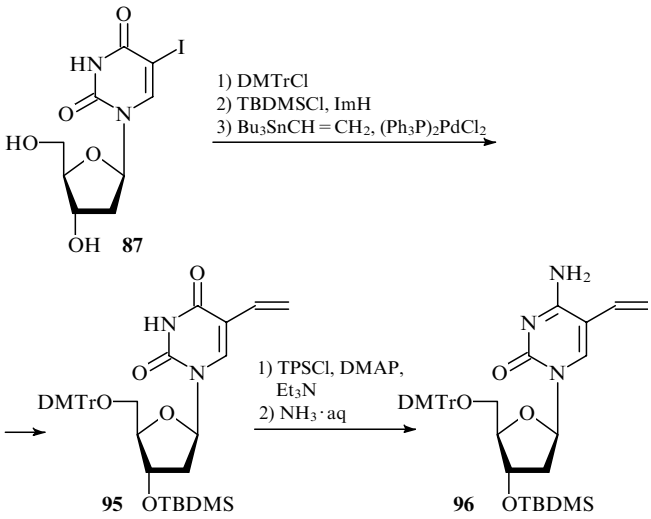
Oligomers containing 5-formyl-2′-*O*-methyluridine residues were synthesised in order to investigate the covalent binding of modified oligonucleotides to the transcription factor NFκb.[39] Commercially available O^2,2′-anhydrouridine was used as a starting compound in this synthesis; the anhydro ring was opened with trimethylborate. Iodination of the resulting derivative **92** afforded modified nucleoside **93**, which was further converted into the phosphoramidite derivative **94**.



CAN is cerium ammonium nitrate.

After termination of the automated oligonucleotide synthesis and complete deprotection of the functional groups, the *vic*-diol group was oxidised with sodium periodate. It was shown [39] that the introduction of a formyl group did not virtually affect the binding of the modified oligomer to the transcription factor.

The above-described approach was also used in the synthesis of oligonucleotides containing 5-formyl-2′-deoxycytidine.[41] The vinyl residue was introduced into the C(5) position of the heterocyclic base **87** by treatment of the protected nucleoside with a mixture of tributylvinyltin and bis(triphenylphosphine)dichloropalladium, which made it possible to markedly reduce the reaction time (from 16 to 1.5 h) and to increase the yield of the target product **95** to 80%. The modified 2′-deoxycytidine **96** was prepared from the protected uridine **95** by activation of the



TPSCl = 2,4,6-$Pr^i_3C_6H_2SO_2Cl$.

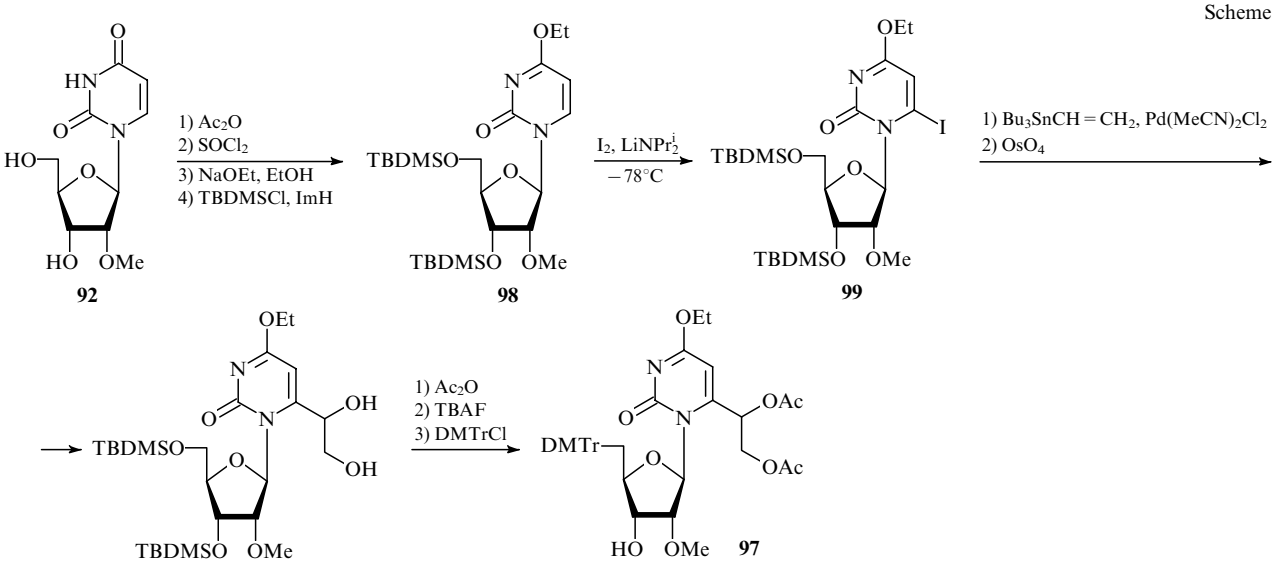
4-oxo group with 2,4,6-triisopropylbenzenesulfonyl chloride and subsequent interaction of the intermediate compound with ammonia. In compound **96**, the vinyl group was oxidised, the exocyclic amino group and the hydroxy groups of the *vic*-diol formed were simultaneously protected and the 3′-hydroxy group was selectively deprotected. The nucleoside formed was used for the preparation of the corresponding phosphoramidite derivative incorporated into the oligonucleotide chain.

After deprotection of the oligonucleotides and oxidative cleavage of the *vic*-diol group with sodium periodate, some physicochemical properties of the synthetic oligomers, *e.g.*, pK_a of the 5-formyl-2′-deoxycytidine residue and the melting temperatures of the DNA duplexes formed by the modified oligonucleotides and the complementary NA matrices, were established. It was shown [41] that the introduction of the carbonyl function had virtually no effect on the thermal stability of the modified duplex.

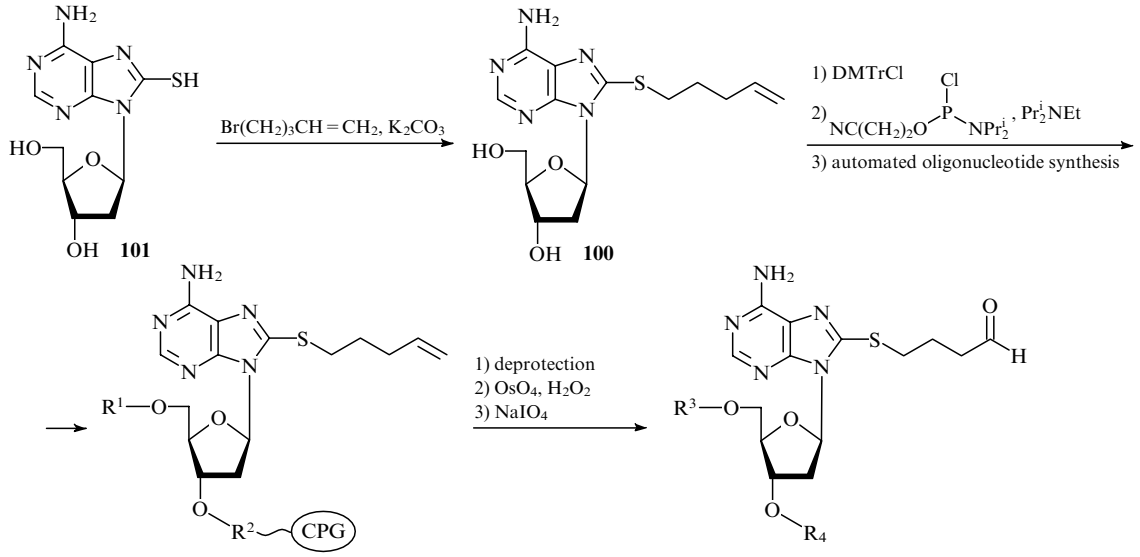
Oligomers containing 6-formyl-2′-*O*-methylcytidine fragments were synthesised in order to investigate the DNA-binding domain of the protooncogen *c-myb*.[42] Since 6-(1,2-diacetoxyethyl)-2′-deoxyuridine is unstable even at room temperature,[38] its protected analogue, *viz*., 1-6-(1,2-diacetoxyethyl)-[4-ethoxy-2-oxo-1,2-dihydropyrimidin-1-yl]-2-*O*-methyl-5-*O*-dimethoxytrityl-β-D-ribofuranoside (**97**), was used as a precursor of 6-formyl-2′-*O*-methylcytidine.[42]

2′-*O*-Methyluridine (**92**), in which the acylation of the hydroxy groups of the carbohydrate fragment was followed by the activation of the 4-oxo group with thionyl chloride (Scheme 8), was used

Scheme 8



Scheme 9



R^1 and R^2 are fragments of a protected oligonucleotide chain; R^3 and R^4 are fragments of a deprotected oligonucleotide chain.

as a starting compound in the synthesis of nucleoside **97.** Subsequent treatment of the protected 2′-*O*-methyluridine product with sodium ethoxide in ethanol and *tert*-butyldimethylsilyl chloride yielded the derivative **98**. Treatment of nucleoside **98** with iodine in the presence of lithium diisopropylamide at −78 °C afforded the corresponding protected 6-iodouridine **99**. The introduction of a vinyl fragment into the derivative **99** and its subsequent oxidation with osmium tetraoxide in the presence of *N*-methylmorpholine *N*-oxide was carried out as described previously. [38]

After acetylation of hydroxy groups, removal of *tert*-butyldimethylsilyl protection and introduction of dimethoxytrityl protective groups, compound **97** was used for the preparation of the corresponding phosphoramidite derivative which was incorporated into the oligonucleotide chain by the automated synthesis. Standard postsynthetic treatments and oxidation with sodium periodate afforded target oligomers containing 6-formyl-2′-*O*-methylcytidine.

It was shown [42] that the introduction of one modified fragment decreased the melting temperature of a 23-membered DNA duplex in comparison with its natural analogue by 6 °C.

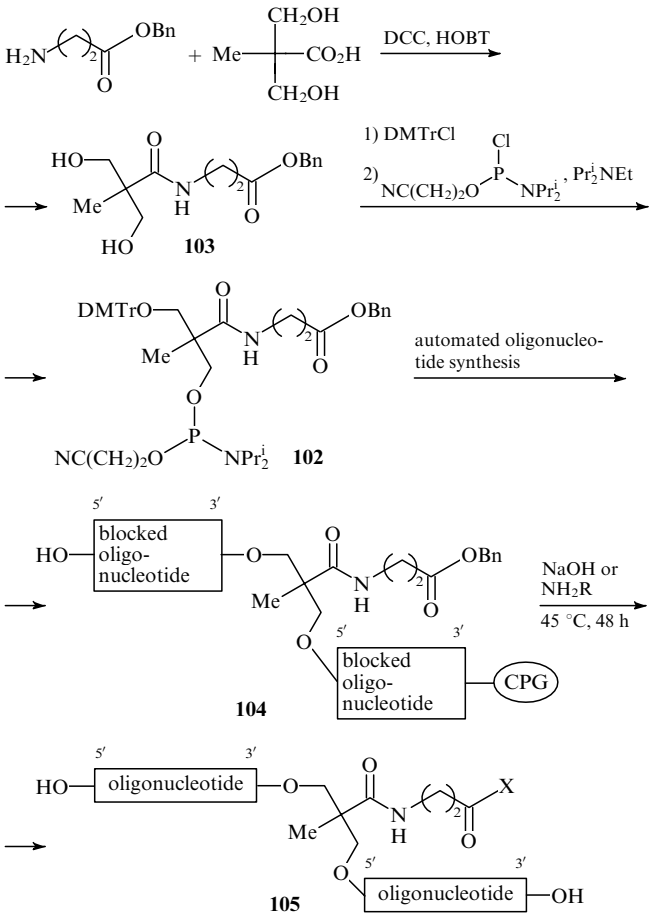
An original approach to the synthesis of oligonucleotides containing an aldehyde group in position 8 of deoxyadenosine has been described.[43] This approach involves the introduction of the phosphoramidite derivative of compound **100** containing a pent-4-enylthio group and prepared starting from thiol **101**, into an automated synthesis (Scheme 9).[44] The oxidation of the double bond of the pent-4-enyl residue was carried out after termination of the synthesis of the oligonucleotide with osmium tetraoxide in the presence of hydrogen peroxide and *N*-methylmorpholine *N*-oxide in the dark at 25 °C. These conditions excluded the destruction of the modified oligomer and the unwanted oxidation of sulfur.

The *vic*-diol group was converted into the aldehyde group by oxidation with sodium periodate (100 equiv.); the presence of this group was confirmed by reactions of the modified oligonucleotide with aminooxy compounds and ^{1}H NMR spectroscopic data.

Conjugates of modified oligonucleotides with fluorescent labels have been prepared.[45] It was shown that the introduction of one heterocyclic base with an aldehyde group localised in a C(3) linker decreases the melting temperature of an 11-mer duplex by 14 °C.

2. Oligonucleotides containing a carboxy or aldehyde group in a non-nucleotide fragment

Endo *et al.*[46] have prepared a non-nucleoside phosphoramidite **102** containing a protected carboxy group which can be incorporated into the chain or be attached to the 5′-end of the oligonucleotide. The key step in the synthesis of compound **102** is condensation of benzyl 4-aminobutyrate with 2,2-bis(hydroxymethyl)propionic acid in the presence of DCC and HOBT resulting in compound **103**. Phosphoramidite **102**, which was further used for the synthesis of model oligonucleotides **104** containing an

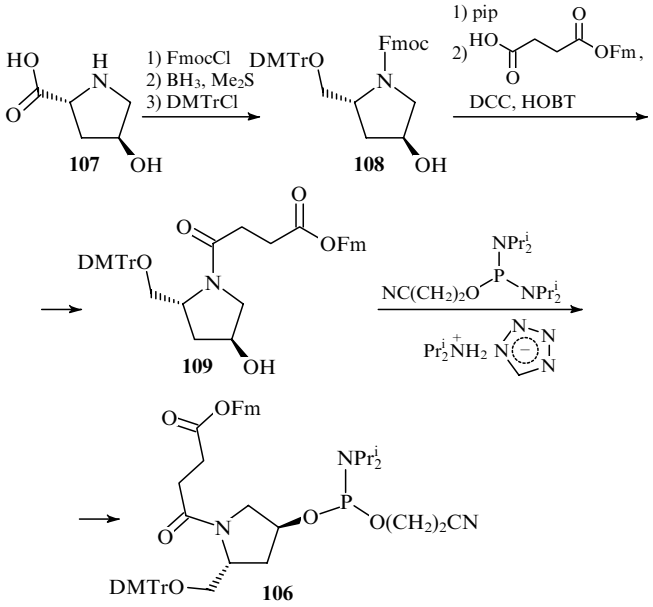


X = OH or NHR.

internal protected carboxy group, was obtained by protection of one primary hydroxy group of the diol **103** with an equimolar amount of dimethoxytrityl chloride and subsequent phosphitylation of the tritylation product. Conjugates of modified oligomers **104** with various amines and diamines were synthesised using the solid-phase method. It was shown [46] that treatment of the oligonucleotide **104** immobilised on a polymeric support with a solution of alkyl amine in an organic solvent results in the quantitative formation of the conjugate **105** (X = NHR).

An alternative procedure for the introduction of a protected carboxy group into the oligonucleotide was proposed by Hebert *et al.*[47] who synthesised model dinucleoside phosphates from the phosphoramidite derivative **106**.

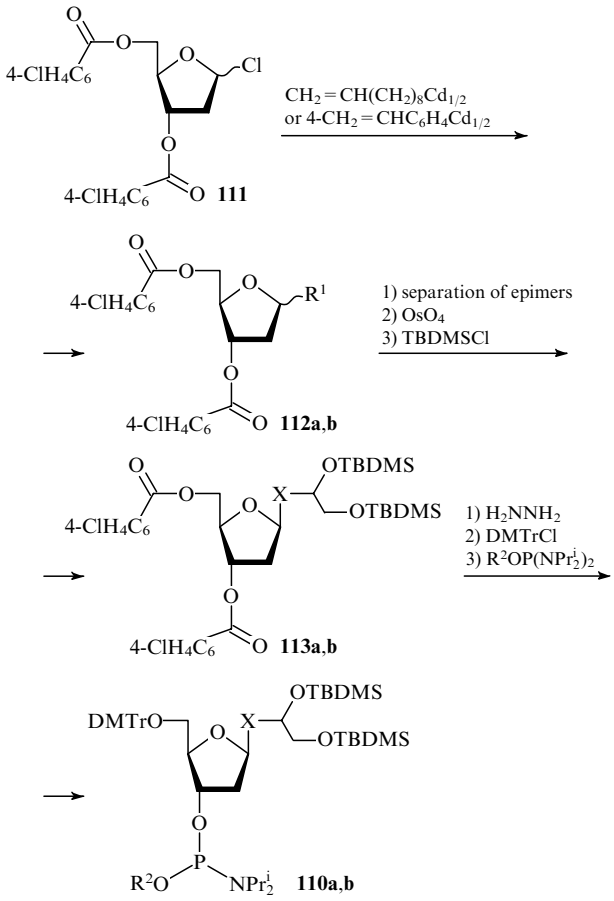
The amino and carboxy groups in 4-hydroxy-L-proline (**107**) were protected, which has been used as a starting compound in the synthesis of phosphoramidite **106**; deprotection of the amino group in the resulting compound **108** with piperidine and subsequent acylation with fluorenylmethyl hydrogen succinate in the presence of DCC and HOBT led to the amide **109**. The derivative **109** was then phosphitylated with the formation of the target phosphoramidite **106** which was employed in the oligonucleotide synthesis. The yield for the step of attachment of the modified fragment was 97%.



Fm is fluorenylmethyl; pip is piperidine.

The use of other acylating reagents for the treatment of compound **108** with a deprotected amino group has made it possible [47] to prepare dinucleoside phosphates containing various functional groups.

The introduction of an aldehyde group into the oligonucleotide chain was carried out by a group of Belgian investigators [48, 49] who used non-nucleoside phosphoramidites **110a**,**b** containing a *vic*-diol fragment for automated synthesis. The phosphoramidite derivative **110a** was prepared from commercially available deoxyribofuranosyl chloride **111**. The introduction of the dec-9-enyl fragment was carried out under the action of the corresponding organocadmium compound [50] with subsequent separation of the epimers **112a** (*R* : *S* = 65 : 35). Despite the fairly high yield of the *R*-epimer, further modification was performed on the *S*-epimer, since its configuration corresponded to the β-configuration of the anomeric centre of natural nucleosides. As a consequence, modified oligonucleotides synthesised with the use of the *S*-epimer can form stable duplexes with mRNA. The *S*-epimer **112a** was oxidised with osmium tetraoxide in the presence of *N*-methylmorpholine *N*-oxide; the hydroxy groups in the resulting *vic*-diol derivative were protected as *tert*-butyldimethylsilyl ethers eventually resulting in the derivative **113a**.



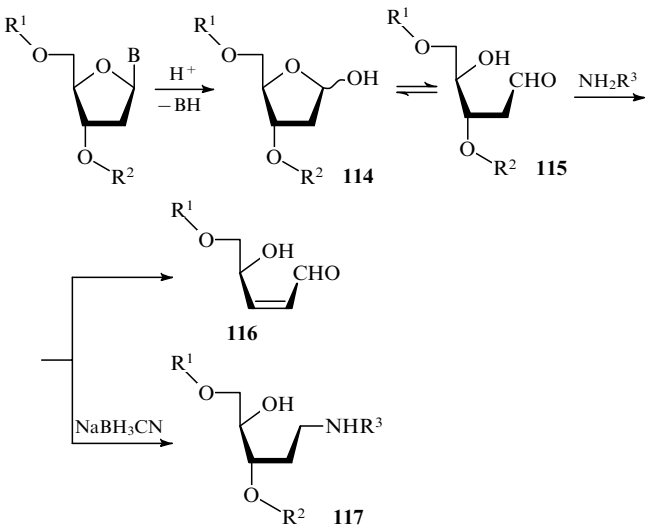
R^1 = $(CH_2)_8CH=CH_2$ (**112a**), $C_6H_4CH=CH_2$-4 (**112b**);
R^2 = $(CH_2)_2CN$ (**110a**), Me (**110b**);
X = $(CH_2)_8$ (**110a**, **113a**), 4-C_6H_4 (**110b**, **113b**).

Compound **113b** was prepared in a similar way. In this compound, the protected *vic*-diol group is located in the *para*-position of the benzene ring, which was used as an analogue of the heterocyclic base of the nucleoside. Removal of *p*-chlorobenzoyl protection and selective incorporation of dimethoxytrityl protective groups with subsequent phosphitylation of compounds **113a**,**b** gave the phosphoramidite derivatives **110a**,**b**, which were introduced into the automated oligonucleotide synthesis.

After deprotection of the modified oligonucleotides, the *vic*-diol groups were oxidised with sodium periodate. The carbonyl-containing oligonucleotides were used [48, 49] for the synthesis of conjugates with biotin and fluorescent labels.

3. Oligonucleotides containing an aldehyde group in the apurine site

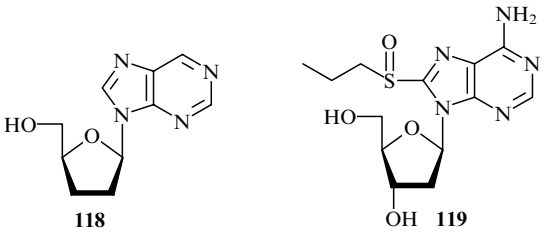
The formation of the apurine (AP) site is a simpler and, therefore, more popular procedure for the introduction of the aldehyde group into the oligonucleotide chain. Elimination of the purine base from the oligonucleotide occurs under the action of strong acids (HCl, HCOOH). In the oligomeric chain, the apurine fragment exists as three tautomers, *viz.*, α- and β-anomeric forms of deoxyribofuranose **114** (1 : 1) and the aldehyde-containing structure **115** (~ 1%).[51, 52] Depending on the nature and concentration of the amine, its addition to the AP-containing oligonucleotide leads to either the scission of the chain due to β-elimination of 3′-phosphate and the formation of the derivative **116**,[51, 53–55] or the addition of the corresponding amine and the formation of the Schiff base which is reduced to the conjugate **117** upon treatment with sodium cyanoborohydride (pH 5.0).[51, 52, 56, 57]



B is purine heterocyclic base;
R^1 and R^2 are fragments of an oligonucleotide chain.

The rate of acid hydrolysis of the *N*-glycosidic bond, which depends on pH, plays a crucial role in the formation of the apurine site. It is known[58] that among other natural nucleosides, the highest apurinisation rate (7.3×10^{-2} min^{-1}) is manifested by 2′-deoxyadenosine. The half-transformation time upon treatment of 2′-deoxyadenosine with 30 mM HCl at 37 °C is 9.5 min. Most frequently, apurinisation of an oligonucleotide is effected by treatment with 80% formic acid (20 °C, 30 min) or 0.2 M hydrochloric acid (37 °C, 90 min).[56]

To avoid drastic acid conditions for the formation of the apurine site, it was proposed[51] to use the artificial adenosine analogue, dideoxynebularin (**118**).



The apurinisation rate constant of this unit within the oligonucleotide chain upon treatment with 30 mM HCl (37 °C, 13 min) is 23.7×10^{-2} min^{-1}, the half-transformation time is 2.9 min. However, the absence of a 3′-hydroxy group in the structure of the monomeric fragment **118** makes impossible its use for the formation of the intrachain AP site.

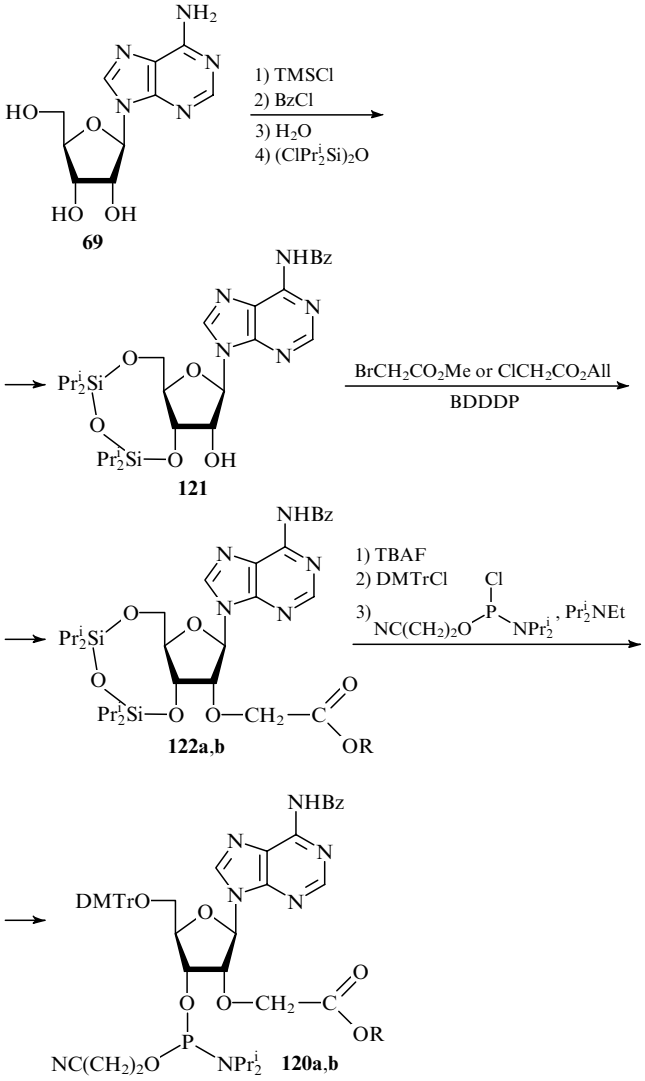
Synthesis of oligonucleotides containing 8-propylsulfinyl-2′-deoxyadenosine residues **119** obtained by the oxidation of the corresponding sulfide[44] has been described.[57] Apurinisation of this unit was carried out at pH 3 (65 °C), which was followed by the preparation of modified oligonucleotide conjugates with fluorescent labels.

4. Oligonucleotides containing a carboxy or aldehyde group in the 2′-position of the carbohydrate fragment

In the majority of cases, modification of the 2′-position does not result in any significant destabilisation of the DNA duplex. It should be noted, however, that the synthesis of oligonucleotides containing a reactive group in the carbohydrate residue is the most tedious procedure, which requires the selection of an orthogonal system of protective groups.

Obviously, the 2′-carboxy group should be blocked in the course of the automated synthesis. The methyl ester commonly used for this purpose is removed with sodium hydroxide in H_2O or methanol.

Modified oligonucleotides containing 2′-*O*-carboxymethyl groups protected with methyl or allyl esters have been synthesised.[59] The latter can selectively be removed by treatment with a Pd(0) complex in the presence of morpholine which facilitates further transformations of the carboxymethyl group on solid supports in organic media. Modified phosphoramidite derivatives **120a**,**b** were synthesised starting from adenosine (**69**). The key step in this synthesis was the alkylation of the 2′-position of the carbohydrate residue of protected adenosine **121** with methyl bromoacetate or with allyl chloroacetate in the presence of a strong organic base, *e.g.*, *tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BDDDP).[60] The use of alkali metal hydrides as a base[61,62] has several disadvantages, *e.g.*, formation of side products, fairly low yields (⩽18%), *etc.*, and significantly restricts the selection of protective groups. Alkylation in the presence of BDDDP leads to the formation of the target compounds **122a**,**b** in 35%–40% yields. Deprotection of 3′- and 5′-hydroxy groups, selective introduction of dimethoxytrityl protection and further phosphitylation of nucleosides **122a**,**b** yielded the phosphoramidite derivatives **120a**,**b**, which were further incorporated into the oligonucleotide chain in the course of the automated synthesis.

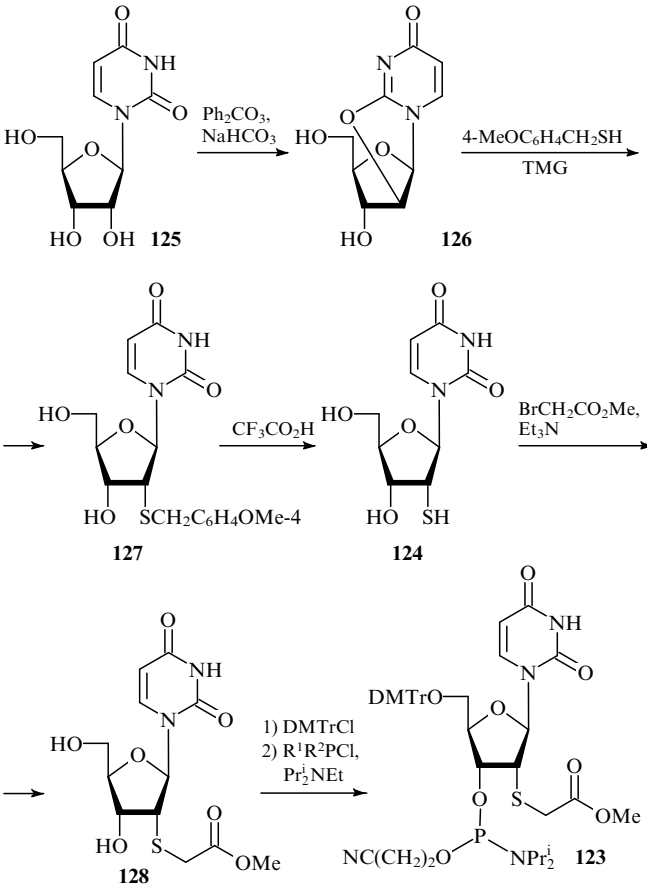


R = Me (**a**), All (**b**).

On completion of the synthesis, methyl ester is saponified with 2 M sodium hydroxide (20 °C, 30 min), while allyl ester is selectively removed by treatment with tetrakis(triphenylphosphine)-palladium in the presence of morpholine. The reactivity of the incorporated 2′-*O*-carboxy group was confirmed by reaction with

ethylenediamine in aqueous solution in the presence of water-soluble carbodiimide.

An original approach to the preparation of oligonucleotides containing a 2′-carboxymethylthio group has been developed.[63] The phosphoramidite derivative of 2′-methoxycarbonylmethylthio-2′-deoxyuridine **123** obtained from 2′-mercapto-2′-deoxyuridine (**124**) was introduced into the automated oligonucleotide synthesis.



The synthesis of 2′-mercapto-2′-deoxyuridine (**124**) from uridine **125** was carried out *via* the cyclic nucleoside **126** using the opening of the anhydro ring with 4-methoxytoluenethiol in the presence of tetramethylguanidine with subsequent treatment of the derivative **127** with trifluoroacetic acid. The alkylation of compound **124** was carried out using methyl bromoacetate without any preliminary protection of the heterocyclic base and the carbohydrate residue. The yield of 2′-methoxycarbonylmethylthio-2′-deoxyuridine (**128**) was 73%; the reaction was accompanied by the formation of trace amounts of the side product, *viz*., the N^3-alkylated nucleoside.[63]

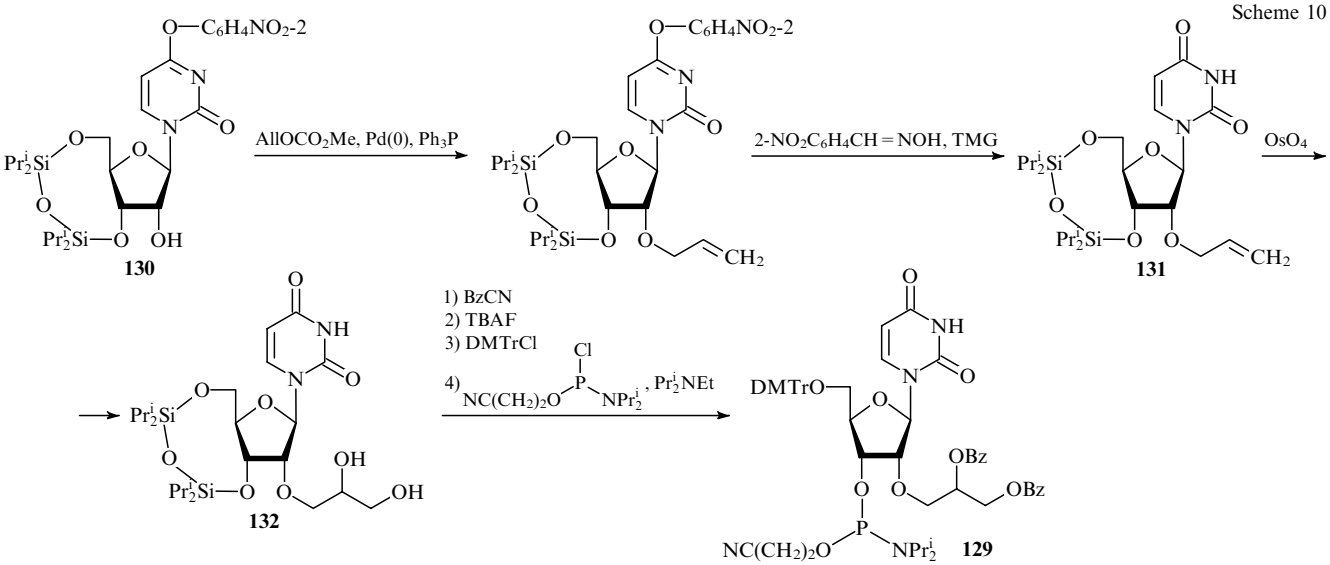
The yield of the model dinucleoside phosphate dU*pT (dU* is a modified deoxyuridine fragment) during the introduction of the modified fragment **123** was 60% – 70%. This fact is attributed to the steric hindrances due to the presence of a bulky substituent in the 2′-position. The solid-phase method was used in the synthesis of conjugates of the model compound dU*pT with aliphatic amines (yield 50%).

Syntheses of 15-mer 2′-modified oligonucleotides with heterogeneous composition have been carried out; the presence of the modified unit was confirmed by enzymatic hydrolysis with a mixture of snake venom phosphodiesterase and alkaline phosphatase.

The synthesis of oligonucleotides containing an aldehyde group in the 2′-position of the carbohydrate residue has been developed.[59,64] In this synthesis, the phosphoramidite derivative **129** containing a protected *vic*-diol fragment was used as a modified unit. The key steps in the synthesis of compound **129** include alkylation of the 2′-position of the protected uridine **130** with allyl methyl carbonate in the presence of Pd(0) and triphenylphosphine and subsequent oxidation of compound **131** with osmium tetraoxide in the presence of *N*-methylmorpholine *N*-oxide to give the derivative **132**. These authors have selected the conditions where the oxidation of 2′-*O*-allylic ether occurred selectively, without the involvement of the double bond of the heterocyclic base. The hydroxy groups of the *vic*-diol fragment of the modified uridine **132** were protected as benzoates (Scheme 10).

The phosphoramidite derivative **129** was used in the synthesis of modified oligonucleotides. The benzoyl protective groups of the 2′-*vic*-diol fragment were removed by standard postsynthetic treatments; the aldehyde function was generated by treatment with sodium periodate. Model conjugates of modified oligonucleotides with 2,4-dinitrophenylhydrazine, methyl ester of methionine[64] and some compounds containing aminooxy, hydrazino, hydrazido or 1,2-aminothio groups have been prepared.[65]

Scheme 10



V. Conclusion

At present, the preparation of oligonucleotide conjugates with various molecules holds great promise for directed changes in physicochemical and substrate properties of nucleic acid fragments, which is a prerequisite for their application in molecular biological and clinical studies. Synthesis of conjugates wherein the reactive group of the oligonucleotide reacts selectively with the ligand, whereas other functional groups of the reacting molecules remain unaffected, seems to be an optimum procedure for the synthesis of conjugates. Such reactions include interaction of carboxy or aldehyde groups of modified oligonucleotides with amino compounds.

The formation of conjugates with amide bonds requires the activation of the carboxy group. The choice of an activating reagent depends, in particular, on the composition of the reaction medium. In aqueous solutions, water-soluble carbodiimide is most commonly used, while the syntheses performed in organic media utilise preferably condensation reagents based on phosphonium or uronium salts. Each of these variants has disadvantages of its own. In the former case, heterocyclic fragments of nucleic acids react with carbodiimide involving the polarised double bond C=N. In the latter case, conjugation in organic media often poses the problem of solubility of the reacting molecules. For this reason, very often the ligand molecule is added to an oligonucleotide immobilised on an insoluble polymeric support. Further treatment of the conjugate with ammonia or alkali aimed at the cleavage of the bond between the oligonucleotide and the polymer and deprotection of functional groups may lead to the destruction of the non-nucleotide fragment of the hybrid molecule.

In the case of oligonucleotides containing an aldehyde group, additional activation of the electrophilic group is unnecessary and the reaction can be carried out in water-organic media, which helps overcome the problem of solubility of the interacting molecules. The possibility of using hydrazines, hydrazides, hydroxylamines and compounds containing 1,2-aminothiol groups along with amines for the synthesis of conjugates is an indisputable advantage of oligonucleotides containing aldehyde groups. Therefore, the use of modified oligonucleotides with aldehyde groups is synthetically more preferable as can be judged from the increasing number of publications on this subject.

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