Russian Chemical Reviews **73** (7) 701 – 733 (2004) © 2004 Russian Academy of Sciences and Turpion Ltd

DOI 10.1070/RC2004v073n07ABEH000835

Nucleosides and oligonucleotides containing 2′-reactive groups: synthesis and applications

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Abstract. The known methods for the synthesis of 2′-functionalised nucleosides, oligonucleotides and their conjugates are systematised. The applications of 2′-modified nucleosides and oligonucleotides are discussed. The bibliography includes 451 references.

I. Introduction

At present, the use of synthetic (including modified) oligonucleotides is unavoidable for progress in molecular biology and medicine. The interest in oligonucleotides and their derivatives as potential therapeutics able to influence, in one way or another, intracellular transformations of nucleic acids (NA) has been steadily increasing in the past decade. In addition, oligonucleotides are applied in antisense [1] and anti-gene [2] biotechnologies. The use of NA-based ribozymes [3] and aptamers [4] as well as RNA interference [5, 6] are no less promising trends.

Treatment of fragments of native NA with various chemical reagents usually results in statistical intrachain or terminal modifications. Incorporation of a modified fragment into a predetermined position of the oligomeric chain in the course of oligonucleotide synthesis is a more versatile approach which enables the introduction of reactive groups into heterocyclic bases, internucleotide phosphate groups or carbohydrate fragments. Obviously, nucleic acids should preserve their ability for complementary interactions. In this context, modification of functional groups involved in complementary interactions is undesirable, since it causes significant destabilisation of an NA duplex formed by the modified oligomer and complementary NA template.[7, 8] The C(5) atom of pyrimidine [9, 10] and the C(7) [11] or the C(8) atom [12] of purine nucleosides are sites of choice for the modification of heterocyclic bases, primarily because of minimum disturbances in the structures of the modified NA fragments obtained. The introduction of substituents into internucleotide phosphate groups results in the apperance of chiral centres and, as a consequence, the existence of two stereoisomers. Modification at the C(2′) atom of nucleosides seems to be especially promising, since the disturbances in the structures of DNA duplexes upon incorporation of thus modified fragments into the oligonucleotide structure are minimum. Besides, the introduction of reactive groups into the 2′-position allows the synthesis of nucleosides with both C(2′)-*exo*- and C(2′)-*endo*-conformations of the carbohydrate residue, directing the substituent to both minor and major grooves of the NA duplex.[13] The synthesis of 2′-modified oligonucleotides is reduced to the preparation of nucleoside phosphoramidite derivatives containing protected reactive groups with subsequent incorporation of the unit formed into the oligonucleotide chain by automated synthesis.

This review deals with the analysis of published papers devoted to the synthesis of nucleosides containing a reactive group in the 2′-position; this group is linked to the C(2′) atom either directly or through various linkers. Main attention is given to derivatives which can be incorporated into the oligonucleotide chain. The groups, which differ in reactivity from that of the functional groups of the natural nucleosides, will be referred to as the reactive groups. Here we do not consider modified nucleosides containing a single or a double C—C bond at the C(2′) atom, since these nucleosides, with only few exceptions, are not potential objects for conjugation with other compounds and incorporation into oligonucleotides.

The application of nucleosides and oligonucleotides containing reactive groups at the C(2′) atom is also discussed.

II. Synthesis of modified nucleosides containing reactive groups in the 2′-position

Numerous methods for the synthesis of nucleosides containing nucleophilic (amino or thio groups) and electrophilic (carboxy, aldehyde, *etc.*) groups in the 2′-position are known to date.

1. Synthesis of nucleosides containing nucleophilic groups

a. Synthesis of 2′-amino-2′-deoxynucleosides

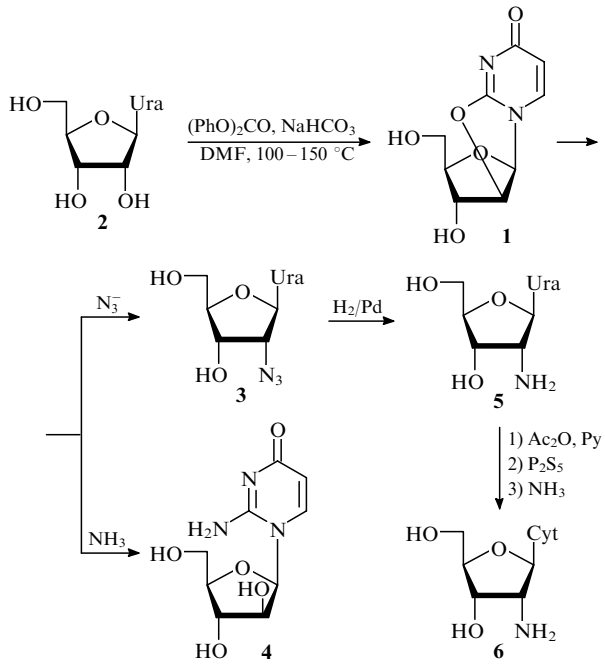
Nucleophilic substitution at the C(2′) atom of activated nucleoside derivatives under the action of the azide ion and subsequent reduction of the azide to the amine is the most popular approach to the introduction of the amino group into the 2′-position of a nucleoside. The synthesis of nucleosides containing an azido group in the sugar moiety has been reviewed.[14] Here we shall consider only those papers which were not cited in that review [14] or were considered only briefly.

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Received 19 December 2003
Uspekhi Khimii **73** (7) 757 – 791 (2004); translated by R L Birnova

$O^2,2'$-Anhydrouridine (**1**) is most frequently used as an activated derivative for the synthesis of pyrimidine 2′-amino-2′-deoxyribonucleosides. The anhydronucleoside **1** can be prepared by different methods among which the reaction of uridine (**2**) with diphenyl carbonate in the presence of sodium hydrogencarbonate as a base is the most popular.[15,16] The reaction of compound **1** with a weak nucleophile (*e.g.*, the azide ion) yields the 2′-substituted nucleoside **3**. If a stronger nucleophilic agent, *e.g.*, ammonia, is used, the reaction involves the heterocyclic base and yields arabinoisocytidine (**4**).

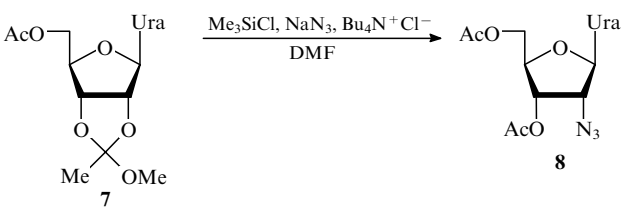
The reduction of 2′-azido-2′-deoxyuridine (**3**) was used for the preparation of 2′-amino-2′-deoxyuridine (**5**) in 50% yield.[17] Subsequent modification of the heterocyclic base in compound **5** yields the analogous cytidine derivative **6**.



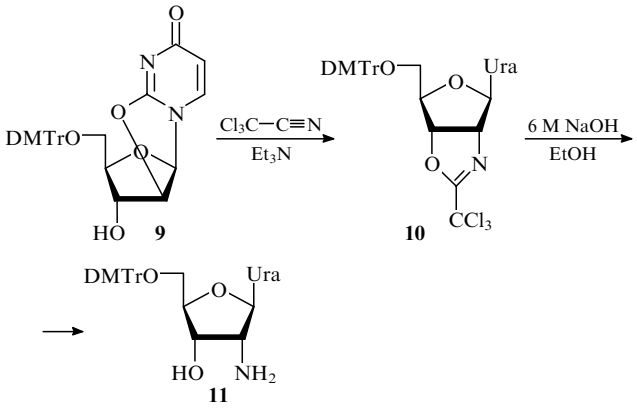
A similar procedure for the synthesis of pyrimidine 2′-amino-2′-deoxyribonucleosides starting from 5′-*O*-trityl- (see Ref. 18) and 3′,5′-di-*O*-benzoyl-$O^2,2'$-anhydrouridine was developed later.[19] In the latter case, partial deprotection of the 3′-hydroxy group took place.

The yield of compound **3** can be increased to 70% through *in situ* generation of lithium azide in the reaction of lithium fluoride with trimethylsilyl azide.[20,21] The addition of tetramethylethylenediamine to the reaction mixture favours the solubilisation of lithium azide in DMF due to its complexation with lithium ions, which increases the yield of the azido derivative **3** up to 80%. A similar effect was observed when a system Me_3SiN_3 – KF – 18-crown-6 was used.[20]

5′-*O*-Acetyl-2′,3′-*O*-(1-methoxyethylidene)uridine (**7**) was used as a starting compound in the synthesis of protected azidouridine **8**.[22] The generation of anhydrouridine occurs *in situ*. Nucleophilic substitution under the action of sodium azide in the presence of Me_3SiCl and Bu_4NCl results in compound **8** in 80% yield.

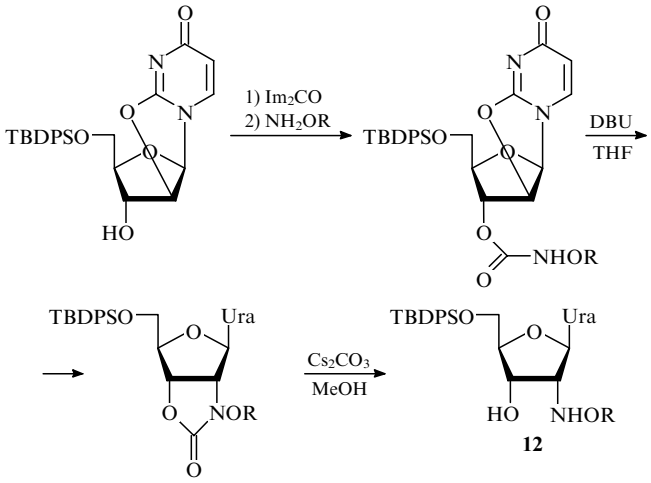


An alternative approach to the synthesis of 2′-amino-2′-deoxyribonucleosides has been developed.[23] The reaction of 5′-protected anhydrouridine **9** with trichloroacetonitrile affords trichloroacetimidate **10**; its subsequent hydrolysis with an ethanolic alkali yields the target product, *viz.*, 2′-amino-2′-deoxyuridine **11**.

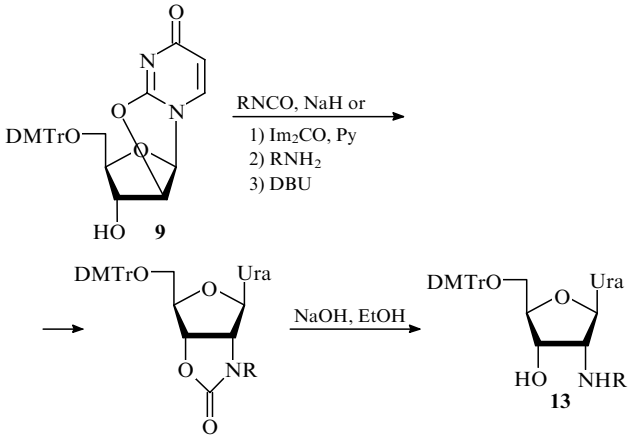


DMTr = $(4\text{-MeOC}_6H_4)_2PhC$.

This methodology gives access to pyrimidine 2′-alkoxyaminonucleosides **12**[24,25] and 2′-alkylaminonucleosides **13**[26,27] as well as 2′-hydroxyaminonucleosides.[28–30] The use of the *tert*-butyldiphenylsilyl protecting group for the 5′-hydroxy function allows the preparation of easily crystallisable products.

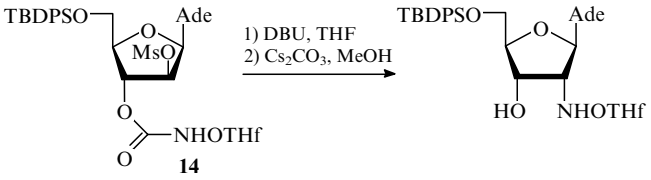


TBDPS = Bu^tPh_2Si; Im is imidazolyl; R = Me, Et, Bn, $(CH_2)_2OH$; DBU is 1,8-diazabicyclo[5.4.0]undec-7-ene.



R = Et, $(CH_2)_7Me$, 2-$PyCH_2$, 2-$Py(CH_2)_2$.

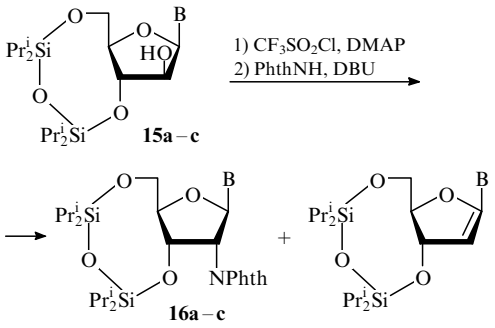
5′-*O*-*tert*-Butyldiphenylsilyl-2′-tetrahydrofuranyloxyamino-2′-deoxyadenosine was prepared starting from mesylated arabinoadenosine **14** using a similar approach.[29]



14

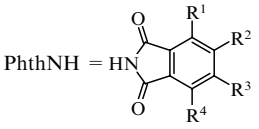
Ms = $MeSO_2$, THf is tetrahydrofuranyl.

Recently, Beigelman and co-workers [31] have developed an efficient scheme for the synthesis of protected 2′-amino-2′-deoxyribonucleosides. The starting arabinonucleosides **15a**–**c** with the tetraisopropyldisiloxane-1,3-diyl (TIPDS) [32] protecting group for 3′- and 5′-OH-groups were activated by treatment with trifluoromethanesulfonyl chloride. Nucleophilic substitution of the triflate group in the derivatives formed by treatment with phthalimides containing various substituents in the benzene ring resulted in 2′-phthalimido-2′-deoxynucleosides **16a**–**c** in 35%–70% yields. 4,5-Dichlorophthalimide proved to be the nucleophile of choice; its application did not result in the formation of side products such as 2′-deoxy-1′,2′-didehydronucleosides. This is a preparative and versatile method for the synthesis of purine and pyrimidine nucleosides.



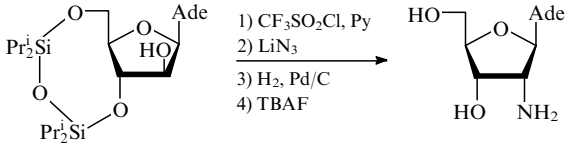
15a–**c** → **16a**–**c** +

B = Ura (**a**), Ade^{Bz} (**b**), Cyt^{Ac} (**c**); DMAP = 4-Me_2NPy;



R^1	R^2	R^3	R^4
H	H	H	H
Cl	Cl	Cl	Cl
H	Cl	Cl	H
NO_2	H	H	H
H	NO_2	H	H

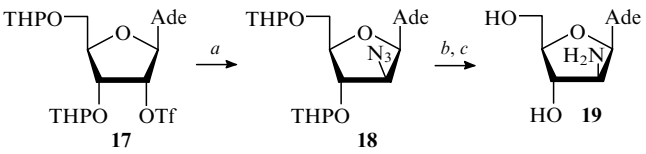
Realisation of a similar method has been described.[33,34] In this case, the triflate of the protected arabinoadenosine obtained in the first step was introduced in the reaction with lithium azide with subsequent reduction of the azido group.



TBAF = $Bu_4N^+F^-$.

The nucleophilic substitution of 2′-*O*-sulfonyl groups in ribonucleoside derivatives, which also occurs with reversal of the configuration at the C(2′)-atom, is used for the synthesis of 2′-amino-2′-deoxyarabinonucleosides. Of this type of nucleoside, purine nucleosides are synthetically more accessible.

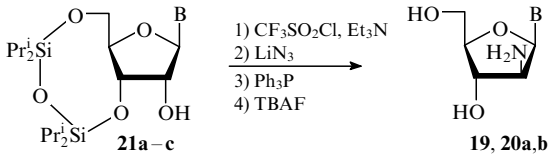
Starting from 3′,5′-di-*O*-tetrahydropyranyl-2′-*O*-trifluoromethylsulfonyladenosine (**17**), 2′-azido-2′-deoxy-β-D-arabinoadenosine **18** was prepared, which was reduced to the target nucleoside **19** after deprotection.[35]



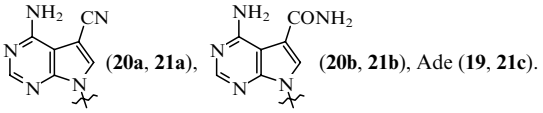
17 **18**

THP is tetrahydropyranyl, Tf = CF_3SO_2.
(*a*) NaN_3, (*b*) H^+, (*c*) Ph_3P, H_2O.

The same strategy was used in the synthesis of 2′-amino-2′-deoxy-β-D-arabinoadenosine (**19**) [36] and its analogues **20a**,**b** [37] from protected ribonucleosides **21a**–**c**.



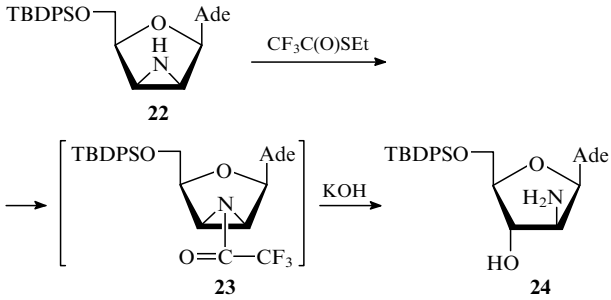
21a–**c** → **19**, **20a**,**b**



(**20a**, **21a**), (**20b**, **21b**), Ade (**19**, **21c**).

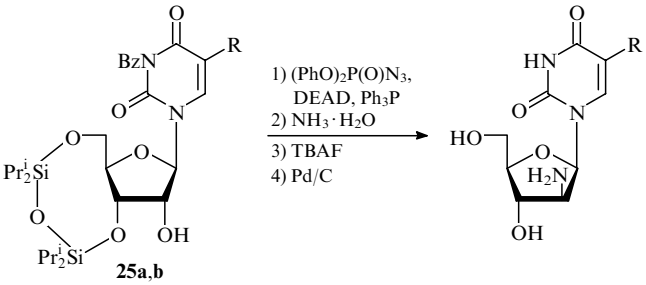
It should be noted that the glycosidic bond in the 2′-*O*-tosyl adenosine derivative is cleaved under the action of nucleophilic agents in DMF resulting in the liberation of adenine.[38] The nucleophilic substitution of the tosyloxy group under the action of NaN_3 in hexamethylphosphoramide has been carried out, however, with low yields [46% and 11% for 2′-*O*-(toluene-*p*-sulfonyl)adenosine and -guanosine, respectively].[39]

A scheme for the preparation of 2′-amino-2′-deoxyarabinoadenosine from the β-D-lyxoadenosine derivative **22** has been proposed.[40] The opening of the aziridine ring in the *N*-trifluoroacetyl derivative **23** occurred in a virtually regioselective manner; the yield of compound **24** was 49%.



22 → **23** → **24**

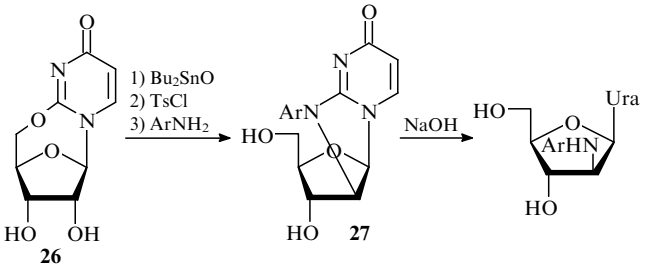
Pyrimidine 2′-amino-2′-deoxyarabinonucleosides are synthetically less accessible and were prepared for the first time as late as 1989 using the reaction of protected ribouridine **25a** and ribothymidine **25b** with diphenyl phosphorazidate in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine with subsequent reduction of the azido group. The presence of a benzoyl protecting group at the N^3-atom of the heterocyclic base is necessary for preventing the closure of the O^2,2′-anhydro ring, which yields 2′-amino derivatives with a ribo-configuration.[41,42]



25a,**b**

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

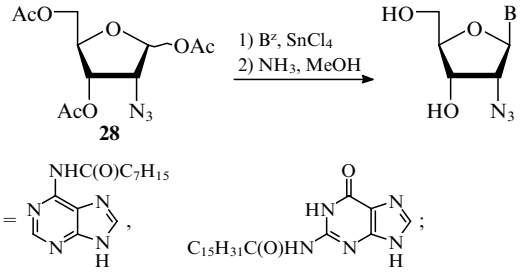
An original scheme for the synthesis of 2′-arylamino-2′-deoxy-β-D-arabinouridines from the O^2,5′-anhydroderivative **26** has been developed.[43] Selective tosylation of the 2′-hydroxy group and subsequent treatment with aromatic amines yielded tricyclic compounds **27**, which were further hydrolysed with aqueous alkali. If aliphatic amines were used, the intermediate tricyclic compounds underwent degradation.



Ts = 4-$MeC_6H_4SO_2$; Ar = Ph, 4-MeC_6H_4.

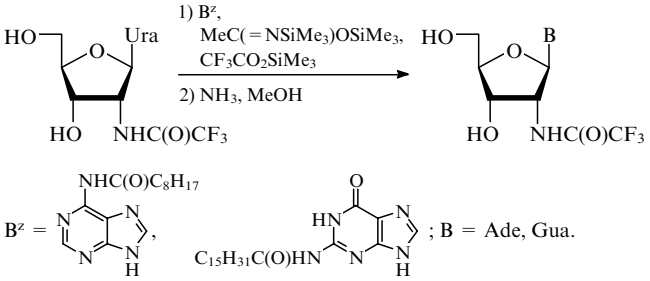
The synthesis of 2′-amino-2′-deoxyadenosine from 9-(2,3-anhydro-D-lyxofuranosyl)adenine has been described.[44]

In all the methods for the synthesis of 2′-amino-2′-deoxynucleosides, the amino group was introduced using modification of activated derivatives of preformed nucleosides. An alternative strategy consists of glycosylation of heterocyclic bases by premodified sugars. First attempts to synthesise 2′-amino-2′-deoxyderivatives of purine nucleosides starting from D-altrose[45] and glucosamine[46] were unsuccessful. Glycosylation of purines by protected 2-azido-2-deoxyribose **28** was more successful; however, a mixture of α and β-anomers of the target 2′-azido-2′-deoxyribonucleosides was obtained (7- and 9-isomers are formed in the case of guanosine).[47]



B = Ade (38%), Gua (21%).

Later, purine 2′-amino-2′-deoxynucleosides were synthesised by transglycosylation of protected adenine and guanine with 2′-trifluoroacetamido-2′-deoxyuridine.[48]



; B = Ade, Gua.

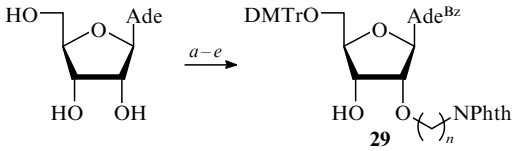
The condensation of 3-*O*-acetyl-2-azido-5-*O*-benzoyl-2-deoxy-D-arabinofuranosyl chloride, prepared from D-glucose by a multistep synthesis, with silylated cytosine afforded 1-(2-azido-2-deoxy-β-D-arabinofuranosyl)cytosine in 37% yield. This reaction was also accompanied by the formation of the α-anomer (yield 10%).[41]

Some examples are known of chemoenzymatic synthesis of modified nucleosides which combines classical methods of organic synthesis with the use of enzymes[49] and cell cultures.[50] Thus 2′-amino-2′-deoxyadenosine is produced by the bacteria *Actinomadura*.[51,52] Transglycosylation of hypoxanthine[53] and adenine[54] by 2′-amino-2′-deoxyuridine in a cell culture of *Ervinia herbicola* with a degree of conversion of up to 50% has been carried out. 2′-Amino-2′-deoxy-β-D-arabinoadenosine was converted into the corresponding inosine derivative under the action of adenosine deaminase.[55]

Synthesis of 2′-*O*-aminoalkylnucleosides

The preparation of 2′-*O*-aminoalkylnucleosides can be regarded as a special case of alkylation of the 2′-hydroxy group. The methods for the synthesis of 2′-*O*-alkylnucleosides have been reviewed.[56] In this Section, special attention will be given to the introduction and protection of the amino group.

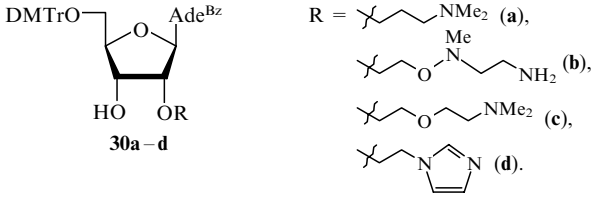
2′-*O*-Aminoalkyl nucleosides **29** were first obtained in 1991 by Cook and co-workers[57] Free adenosine was alkylated with phthalimidoalkyl bromides in the presence of sodium hydride.[57–61]



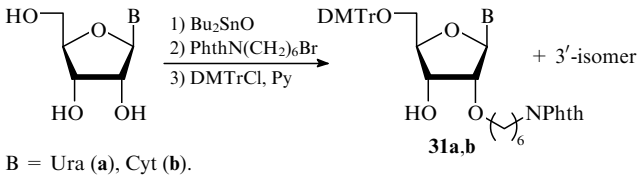
(*a*) $PhthN(CH_2)_nBr$, NaH, DMF; (*b*) Me_3SiCl, Py; (*c*) BzCl; (*d*) NH_4OH; (*e*) DMTrCl, Py; *n* = 2–6.

This reaction yields a mixture of 2′- and 3′-isomers which can be separated only after benzoylation of the exocyclic amino group of adenine and tritylation of the 5′-hydroxy group. Although the total yields of nucleosides **29** are low (10%–20%), the advantage of this method is that it does not require additional protection/deprotection steps and eliminates multiple chromatography of intermediates. In addition, the aminoalkyl group is introduced in one step, while the phthaloyl protecting group is stable under the conditions of the oligonucleotide synthesis and is removed by standard post-synthetic treatments with concentrated aqueous ammonia on heating. Trifluoroacetyl[62] or 2-cyanoethoxycarbonyl groups can be used instead of the phthaloyl protecting group.[63]

Nucleosides **30a–d** in which the amino groups are linked to the O(2′) atom through more complex linkers have also been used in oligonucleotide synthesis.[64]



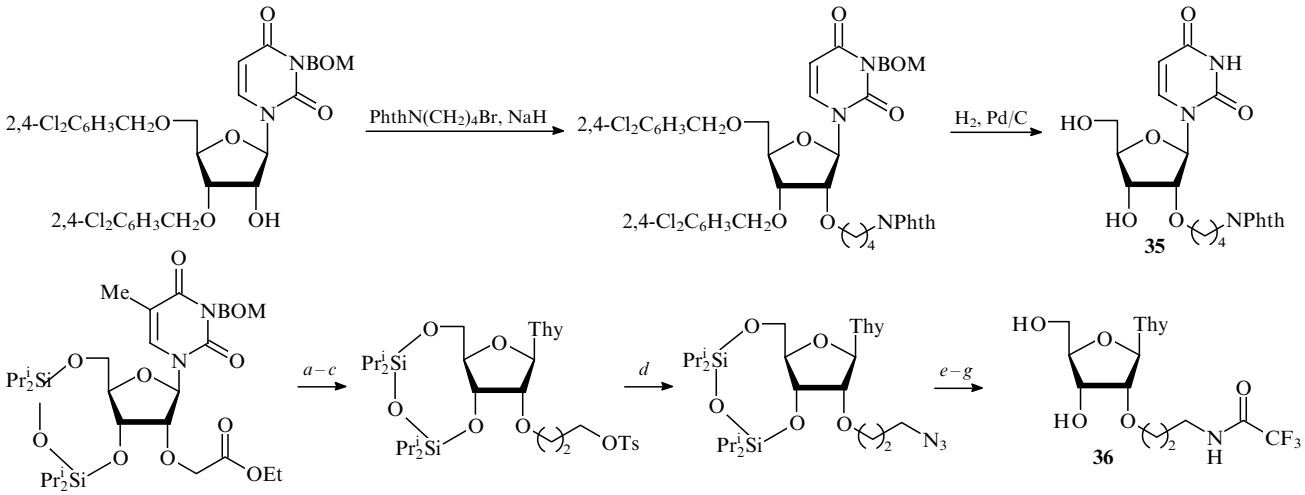
Unfortunately, direct alkylation of non-protected nucleosides is possible only in the case of adenosine and its analogues. Metal-directed alkylation of the *cis*-diol group permits one to increase the total yield of pyrimidine nucleosides **31a**,**b** to 46% (no base alkylation is observed), but the regiospecificity of the substitution is still low (a mixture of 2′- and 3′-isomers is formed in a 55 : 45 ratio).[65]



B = Ura (**a**), Cyt (**b**).

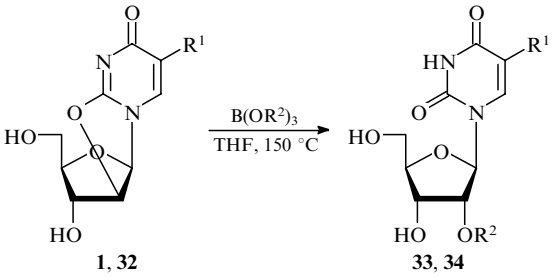
A method for the alkylation of O^2,2′-anhydrouridine **1** and thymidine **32** by boric acid esters has been developed.[63,66–69] The yields of compounds **33** and **34** are 15%–20%. The opening of the

Scheme 1



BOM = CH_2OBn; (*a*) $LiBH_4$, MeOH, THF; (*b*) TsCl, Et_3N, DMAP, CH_2Cl_2; (*c*) H_2, Pd/C; (*d*) NaN_3, DMF; (*e*) $SnCl_2$, MeOH; (*f*) $(CF_3CO)_2O$, Py; (*g*) TBAF, THF.

ring in compound **32** was achieved by treatment with phthalimidoethanol in the presence of titanium tetraisopropoxide.[70]

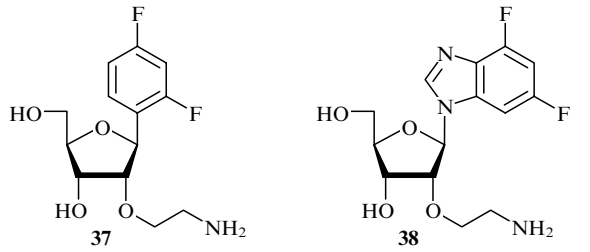


1, **32** **33**, **34**

1, **33**: R^1 = H; **32**, **34**: R^1 = Me; $R^2 = (CH_2)_n$NPhth; n = 2, 3.

A more standard version of the synthesis of 2′-*O*-aminoalkylnucleosides **35** and **36** based on protection of all the reaction centres with the exception of that to be modified has been published.[71,72] Here, all the synthetic steps are high-yielding, but the total yield is only 10%–25% due to the large number of steps (Scheme 1).

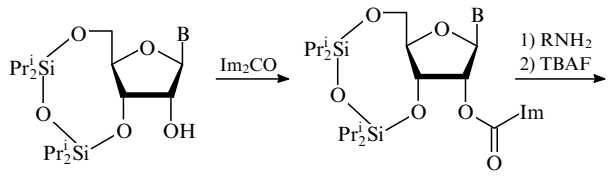
A similar synthesis of modified glycosides **37** and **38** with non-native aglycons has been carried out.[73]



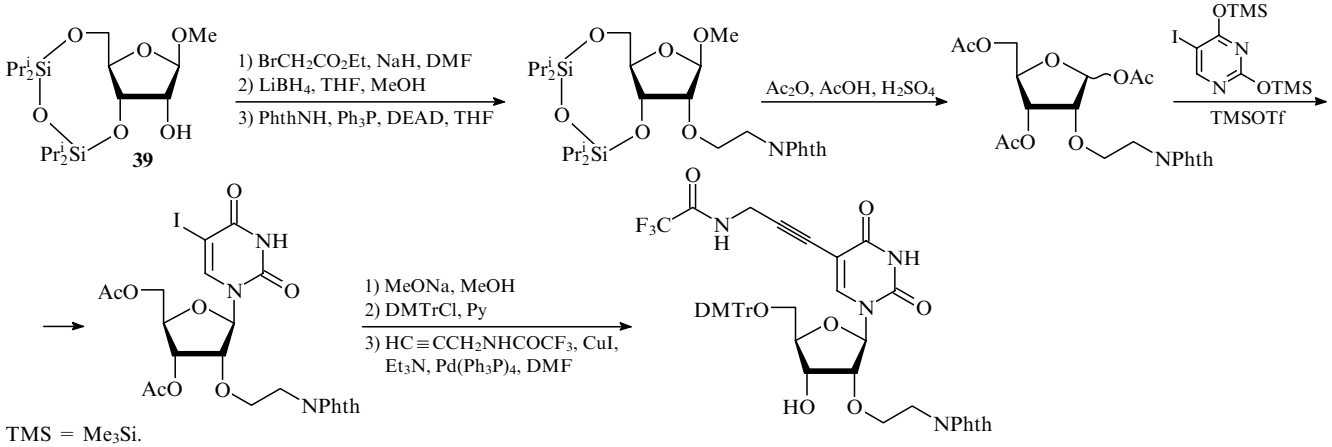
37 **38**

Sollogoub *et al.*[74] have performed alkylation of the protected riboside **39** with subsequent introduction of an amino group under conditions of the Mitsunobu reaction; glycosylation was carried out in the later steps of the synthesis (Scheme 2).

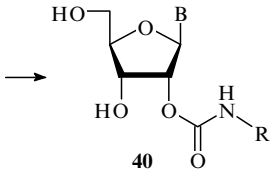
The aminoalkyl groups were attached to the O(2′) atom through a carbamoyl linker.[75–77] This approach provides high yields of the aminoalkylated derivatives **40**.



Scheme 2



TMS = Me_3Si.

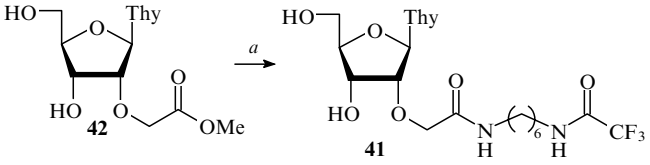


40

B = Cyt^{Bz}: R = $(CH_2)_6NHC(O)CF_3$;
B = Ura: R = $(CH_2)_2NPhth$, $CH_2(CH_2CH_2O)_3(CH_2)_2NH_2$, $CH_2(CH_2CH_2NH)_2(CH_2)_2NH_2$, $(CH_2)_3NH(CH_2)_2NH_2$, Leu-PheNH_2.

The carbamate of 3′,5′-protected arabinouridine was prepared in a similar way.[78]

Keller and Haner[79] have synthesised *N*-acylated 2′-*O*-(9-amino-2-oxo-3-azanonyl)thymidine **41** starting from the nucleoside **42** containing a glycolic acid fragment in the 2′-position.



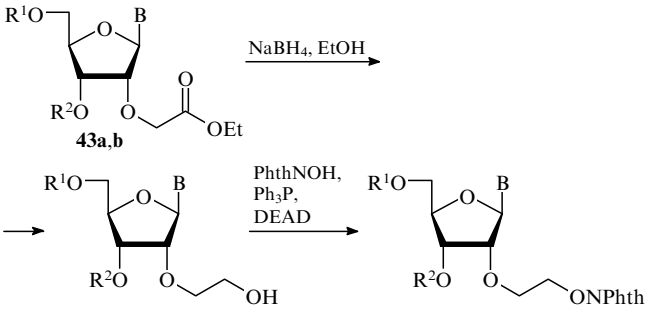
42 **41**

(*a*) $H_2N(CH_2)_6NHC(O)CF_3$.

c. Synthesis of nucleosides containing an aminooxy group

Treatment of nucleosides with *N*-hydroxyphthalimide under conditions of the Mitsunobu reaction is an optimum method for the introduction of an aminooxy group.[80] This method is well developed and the phthaloyl protecting group meets all the requirements for the automated oligonucleotide synthesis.

A possible approach to the introduction of the aminooxyethyl group into the 2′-position of protected thymidine **43a** and adenosine **43b** has been described.[81,82]

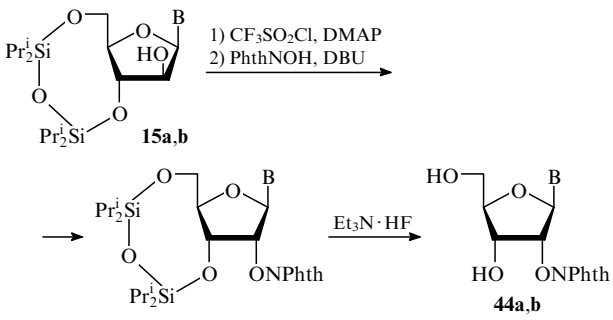


43a,**b**

B = Thy: $R^1 = R^2 = 2,4\text{-}Cl_2C_6H_3CH_2$ (**a**);
B = Ade: $R^1 - R^2 = (Pr^i_2Si)_2O$ (**b**).

Recently, a shorter route to the synthesis of pyrimidine 2′-*O*-(2-hydroxyethyl)nucleosides by alkylation of O^2,2′-anhydronucleosides by boric acid esters has been proposed.[83–85] The nucleosides thus prepared are intermediate compounds in the synthesis of protected 2′-*O*-(2-aminooxyethyl)nucleosides.

In an attempt to introduce the phthalimido group directly into the 2′-position of 3′,5′-*O*-protected nucleosides, the yields of the target products did not exceed 10% because of the low reactivity of the secondary hydroxy group under conditions of the Mitsunobu reaction. Nevertheless, the desired products **44a**,**b** were prepared by substitution of the 2′-*O*-trifluoromethylsulfonyl group in the arabinouridine derivatives **15a** and the arabinoadenosine derivatives **15b**.[86] This reaction occurs with inversion of the configuration of the C(2′) atom.



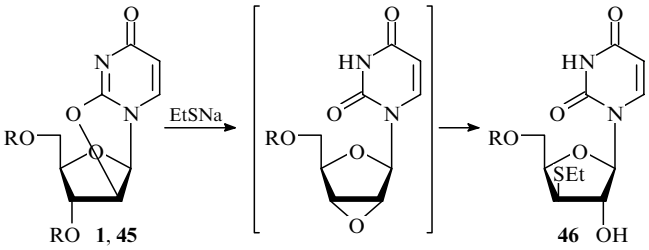
15a,**b** **44a**,**b**

B = Ura (**a**), Ade (**b**).

d. Synthesis of nucleosides containing a thiol group

In this subsection, we shall consider the synthesis of 2′-thionucleosides and 2′-*O*-mercaptoalkylnucleosides. A review[87] devoted to the synthesis of various thionucleosides has been published recently; however, methods for the synthesis of C(2′)-derivatives are not considered there.

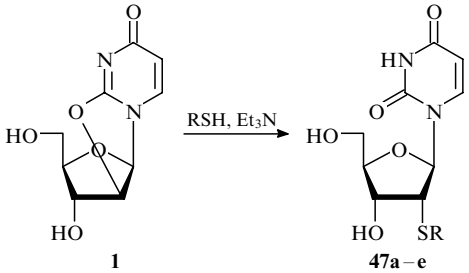
The first attempts to carry out the reaction of free O^2,2′-anhydrouridine **1**[88] or its protected derivative **45**[89] with sodium ethanethiolate resulted in 3′-*S*-ethylthio-3′-deoxyxylouridine **46** as the main product.



1, **45** **46**

1: R = H; **45**: R = Ac.

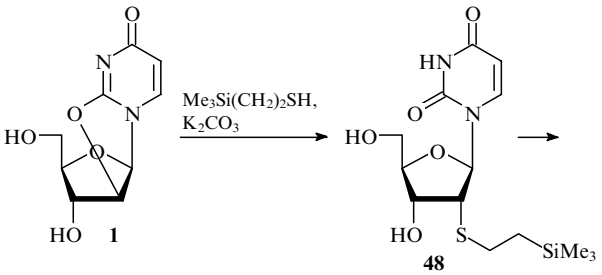
Later, it was found that preparation of the nucleoside 2′-thioderivatives **47a**–**e** by nucleophilic substitution at the C(2′) atom of the anhydronucleoside **1** requires the use of weaker nucleophiles, *e.g.*, thioacetic acid[90,91] and various thiols[92] and that the reaction was carried out in the presence of triethylamine or N^1,N^1,N^3,N^3-tetramethylguanidine. This approach was repeatedly used later.[93–96]



1 **47a**–**e**

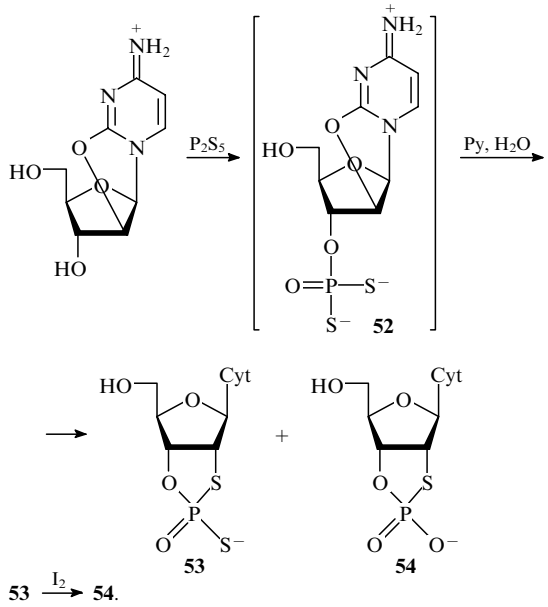
R = Ac (**a**), Me (**b**), Et (**c**), Bn (**d**), 4-$MeOC_6H_4$ (**e**).

Recently, it was proposed that 2-(trimethylsilyl)ethanethiol in the presence of potassium carbonate can be used for the synthesis of pyrimidine 2′-thionucleosides.[97] The uridine **48** thus modified can be easily converted into the mixed disulfide **49** and the analogous cytidine derivatives **50** and **51**. The high cost of reagents is the main disadvantage of this method. An attempt to deprotect the thiol group in compound **48** with tetrabutylammonium fluoride yielded a complex mixture of reaction products.



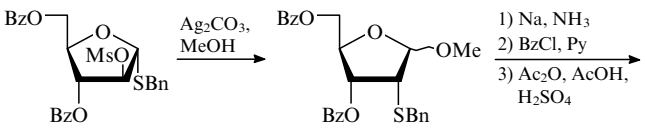
(*a*) Ac_2O, Py; (*b*) , $POCl_3$, Et_3N; (*c*) NH_3.

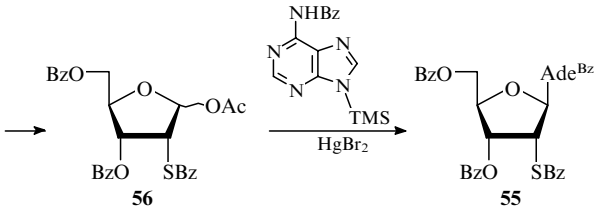
2′-Thiocytidine derivatives can be prepared not only by transamination of uridine,[93,97] but also by opening of the O^2,2′-anhydrocytidine ring.[98] An intramolecular attack of the 3′-*O*-dithiophosphate group at the C(2′) atom of substituted O^2,2′-anhydrocytidine **52** has been described.[98] This yields a mixture of 2′,3′-cyclodithiophosphate **53** and 2′,3′-cyclothiophosphate **54** which can be prepared from compound **53** by treatment with iodine.



53 $\xrightarrow{I_2}$ **54**.

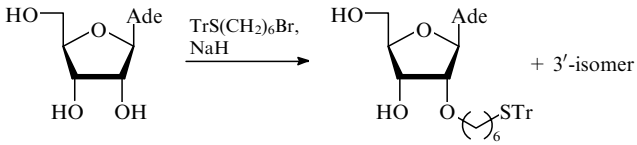
The synthesis of 2′-benzoylthioadenosine **55** by glycosylation of protected adenine with the ribose thioanalogue **56** has been described.[99]



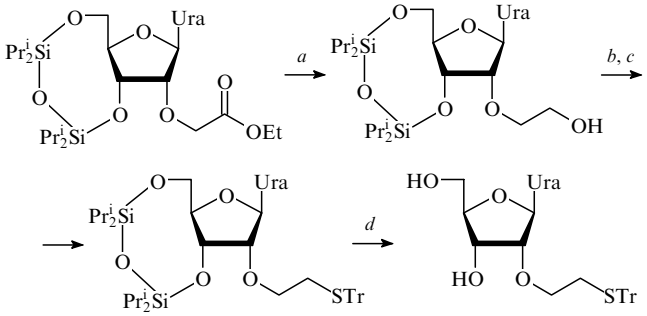


Although 2′-thionucleosides were first described about 25 years ago, they came into the routine practice of oligonucleotide synthesis only very recently.[93,100]

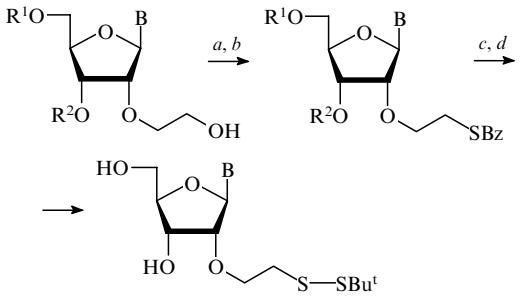
Two strategies are feasible for the synthesis of 2′-*O*-mercaptoalkylnucleosides, *viz*., the reaction of nucleosides with alkyl halides containing a protected thiol group,[101–103] or the preparation of 2′-*O*-hydroxyalkyl nucleosides with subsequent conversion of the hydroxy group into the thiol group.[104–107]



Tr = Ph_3C.



(*a*) $NaBH_4$, MeOH, Bu^tOH; (*b*) Ph_3P, CBr_4, MeCN; (*c*) TrSH, DBU, C_6H_6; (*d*) TBAF, THF.



B = Ura, Cyt^{Bz}, $Gua^{Bu^iC(O)}$; $R^1 - R^2 = (Pr^i_2Si)_2O$, $R^1 = R^2 = Bu^tMe_2Si$.
(*a*) $MeSO_2Cl$, Py, CH_2Cl_2; (*b*) BzSH, Et_3N, DMF; (*c*) HF, MeCN; (*d*) 1-(*tert*-butylthio)hydrazine-1,2-dicarboxymorpholide, LiOH, MeOH, THF.

Moreover, target 2′-*O*-mercaptoalkyl nucleosides can be prepared *via* intermediate bromoalkyl nucleosides.[108]

2. Synthesis of nucleosides containing electrophilic groups

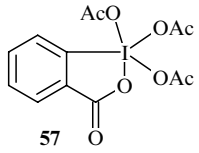
In contrast to nucleophilic groups, electrophilic groups (with the exception of monosubstituted phosphate and keto groups) cannot be directly introduced at the C(2′) atom of the nucleoside. This is due to the fact that such nucleosides are highly unstable and prone to β-elimination. Therefore, the use of linkers is the only possible approach to the attachment of electrophilic groups at the C(2′) atom of the nucleoside. A recently published review [109] devoted to the preparation of modified oligonucleotides containing electrophilic groups describes the synthesis of nucleosides containing

aldehyde and carboxy groups. Here we thought it expedient to highlight some aspects of the problem not touched upon in the above-cited review.

a. Synthesis of 2′-oxonucleosides

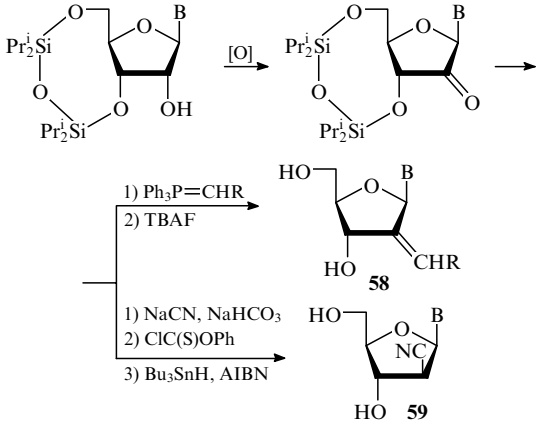
It has long been thought that 2′-oxo derivatives of nucleosides are highly unstable and undergo decomposition due to the high lability of the *N*-glycosidic bond.[110] Nevertheless, Robins and co-workers[111,112] succeeded in the oxidation of 3′,5′-protected uridine by CrO_3. Later, this approach was used by other authors.[113,114] Matsuda *et al.*[115–120] have performed the synthesis of protected 2′-oxonucleosides using the Swern oxidation by the oxalyl chloride–DMSO–triethylamine system.[121,122] This reaction requires protection of the heterocyclic base.[123,124] A recently published paper[125] deals with a comparison of various acylating reagents used in the Swern oxidation of 3′,5′-protected cytidine. It was shown that the use of trifluoroacetic anhydride provides the maximum yield of 2′-oxocytidine.

In 1990, Robins *et al.*[126] used the Dess–Martin 'periodinane' {1,1,1-triacetoxybenzo[*d*]-1,2-iodoxol-3(1*H*)-one} **57** for the oxidation of the 2′-hydroxy group.[127] At present, this is the method of choice,[128,129] since the reaction is selective and the product formed can be used in subsequent transformations without additional purification.



57

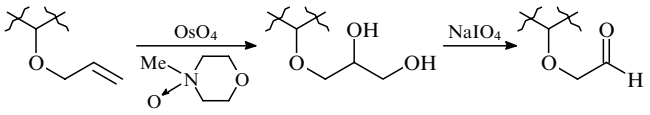
2′-Oxonucleosides are further used in the synthesis of the 2′-deoxy-2′-methylidene derivatives **58** by the Wittig reaction[130–133] or of the cyano derivatives **59**.[134,135] The modified nucleosides **58** and **59** are extensively employed in the therapy of cancer and viral diseases.[136,137] However, the introduction of these derivatives into the oligonucleotide chain results in significant destabilisation of the NA duplex;[138] therefore, their use in the synthesis of modified oligonucleotides is hardly justified.



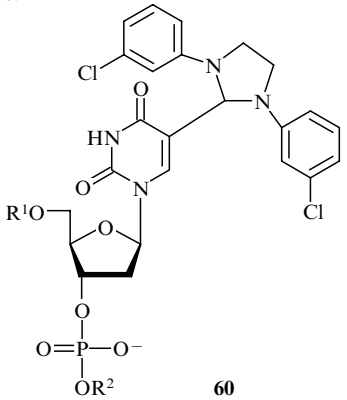
B is a free or protected heterocyclic base,
R = Alk.

b. Synthesis of nucleosides containing an aldehyde group

Synthesis of nucleosides containing an aldehyde group in the 2′-position is usually carried out by oxidative cleavage of their precursors (*vic*-diol derivatives) by sodium periodate.



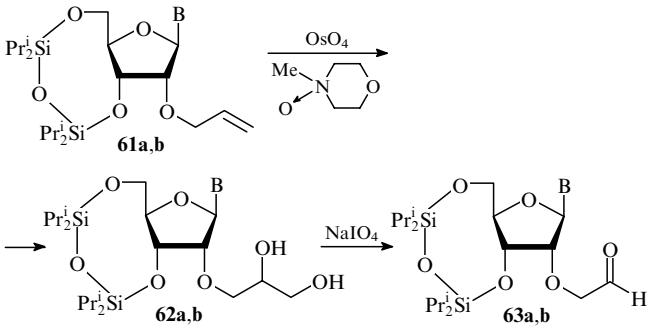
The choice of this strategy is determined by a number of factors. First, the oxidation of the vinyl group to the aldehyde group occurs under milder conditions than that of the primary hydroxy group. Second, if a nucleoside to be introduced into the oligonucleotide chain already contains an aldehyde group, the latter should be protected to prevent its reaction with the amino groups of heterocyclic bases and phosphorus(III) derivatives and oxidation by iodine. Unfortunately, no suitable protecting group has been found so far. Thus the *N*,*N*′-bis(3-chlorophenyl)imidazolidine protecting group[139] recommended for the protection of the aldehyde group of 5-formyl-2′-deoxyuridine has not found wide application, because its removal requires long-term treatment of the oligonucleotide **60** with 80% acetic acid, which leads to the degradation of even the most stable homothymidine oligonucleotides.



60

R^1 and R^2 are fragments of an oligodeoxyribonucleotide chain.

Studies by Glick and co-workers[105,106] who first performed the synthesis of the 2′-*O*-allyl pyrimidine nucleoside derivatives **61a**,**b**, their subsequent oxidation to the *vic*-diols **62a**,**b** and then cleavage to the aldehydes **63a**,**b**, represent an example of this methodology.

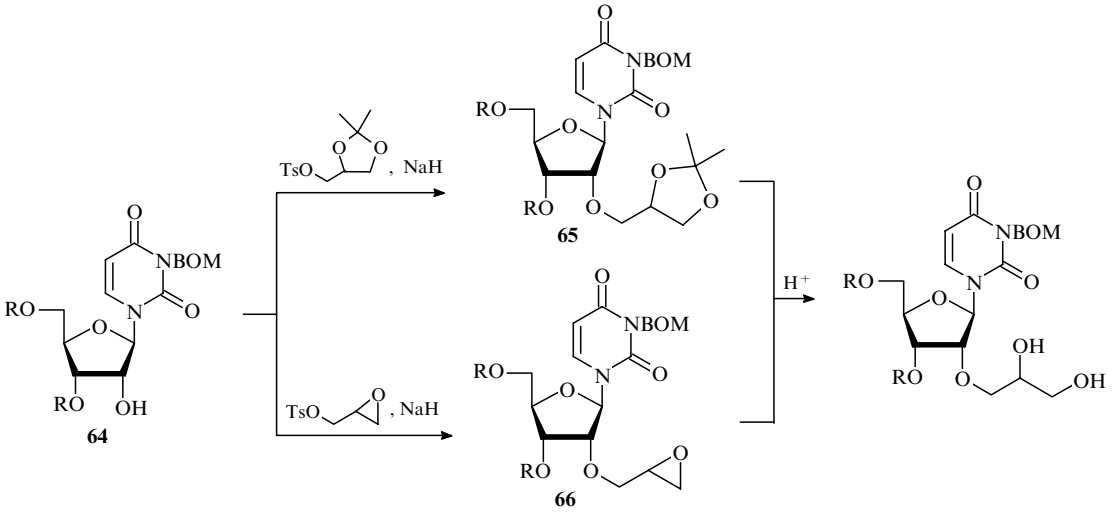


B = Ura (**a**), Cyt^{Bz} (**b**).

A similar approach was used in other studies[140,141] with the only exception that the *vic*-diol group of protected uridine **62a** was benzoylated into a 3′-phosphoroamidite derivative, which was then used in automated oligonucleotide synthesis. The aldehyde group was generated at the oligonucleotide level.

Martin[71] proposed the use of isopropylideneglycerol and glycidol toluene-*p*-sulfonates for the alkylation of the 2′-hydroxy group of the protected nucleoside **64**. The *vic*-diol fragment was generated upon acid hydrolysis of the isopropylidene protecting group in the modified uridine **65** or of the oxirane **66** (Scheme 3).

Scheme 3



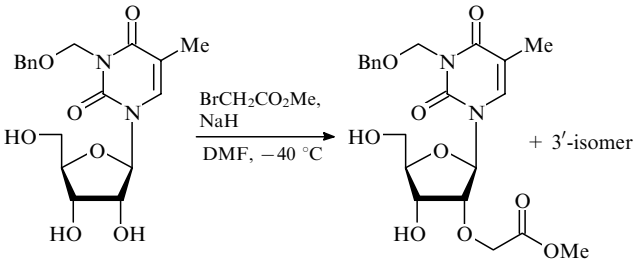
R = 2,4-$Cl_2C_6H_3CH_2$.

Mention should also be made of the recently published reviews [142–144] devoted to the synthesis and applications of nucleoside and oligonucleotide dialdehyde derivatives.

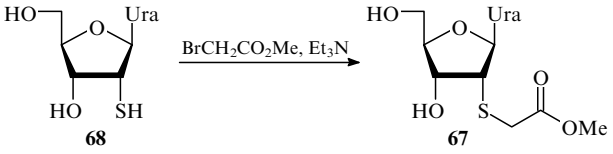
c. Synthesis of nucleosides containing a carboxy group

Studies devoted to the synthesis of 2′-*O*-alkoxycarbonyl methylnucleosides are generalised in the review.[109] Here, we shall consider only those publications which have not been included in that review [109] or demand additional discussion.

Alkylation of 3-*N*-(benzyloxymethyl)ribothymidine containing free hydroxy groups [145] occurred with high regiospecificity (the isomer ratio 2′ : 3′ was 9 : 1) (however, the yield of the 2′-isomer is not indicated).



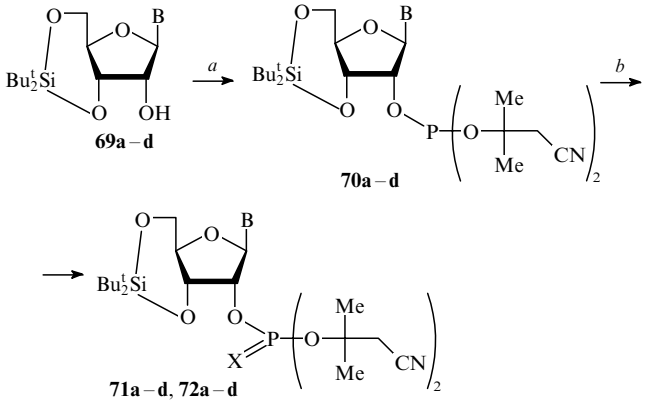
The preparation of 2′-methoxycarbonylmethylthio-2′-deoxyuridine **67** has been described.[94,95] The alkylation of the nonprotected derivative **68** by methyl bromoacetate partly involved the N(3) atom of the heterocyclic base.

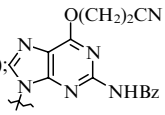


According to literature data,[146] the 2′-*S*-alkyl group is exposed to the major groove of the DNA duplex contrary to the 2′-*O*-alkyl group, which is exposed to the minor groove.[147] The difference in the arrangement of reactive groups in the NA duplexes allows preparation of conjugates with different properties from modified oligonucleotides and other molecules.

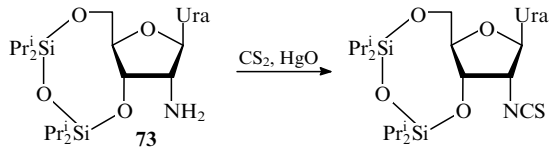
d. Synthesis of nucleosides containing other electrophilic groups

Studies by Sekine *et al.*[148,149] describe the synthesis of nucleosides and oligonucleotides containing a 2′-phosphate or a 2′-thiophosphate group. The 3′- and 5′-protected nucleosides **69a–d** were converted into the trivalent phosphorus derivatives **70a–d**; their subsequent oxidation with *tert*-butyl hydrogen peroxide or sulfur yielded 2′-phosphates **71a–d** and thiophosphates **72a–d**, respectively.



B = Ura^{Bz} (**a**), $Cyt^{Bu^tC(O)}$ (**b**), Ade^{Bz} (**c**);  (**d**);
71a–d: X = O; **72a–d**: X = S;
(*a*) $Pr^i_2NP(OCMe_2CH_2CN)_2$, TetrH; (*b*) Bu^tO_2H or S_8;
TetrH is tetrazole.

The conversion of the 2′-amino group of modified uridine **73** into the isothiocyanate group, which can further be introduced into reactions with various nucleophiles, has been described.[150]



These data suggest that the methodology for synthesis of modified nucleosides containing free or protected reactive groups in the 2-position of the carbohydrate fragment is fairly well developed. The former are used for the preparation of conjugates with various compounds, whereas the latter are employed for the preparation of protected phosphoramidite derivatives for oligonucleotide synthesis.

III. Synthesis of conjugates based on 2′-modified nucleosides and oligonucleotides

Two, different in principle, approaches to the synthesis of NA, *viz*., chemical and enzymatic, are known. The overwhelming majority of 2′-modified oligonucleotides have been synthesised using the chemical approach. The latter implies initial preparation of protected nucleoside derivatives for automatic oligonucleotide synthesis. Natural $3' \rightarrow 5'$ internucleotide bonds are usually formed using 3′-phosphoramidite nucleoside derivatives, although some exceptions are possible. The dimethoxytrityl protecting group is widely used for the protection of the 5′-hydroxy group, whereas amino groups of heterocyclic bases are protected by groups that are labile in alkaline media. This approach enables the synthesis of oligomers containing up to 80 – 100 nucleotides. NA with longer chains are synthesised using chemical [151] or enzymatic [152] ligation.

The use of the enzymatic approach, which entails the incorporation of nucleoside 5′-triphosphates into NA using DNA and RNA polymerases, is confined to 2′-amino-2′-deoxy- [153] and 2′-alkoxyaminonucleosides.[154, 155] In addition, the substrate properties of pyrimidine 2′-amino-2′-deoxynucleoside triphosphates are inferior to the natural nucleoside triphosphates.[156]

Oligonucleotide 2′-conjugates can be prepared using three different approaches. The first of them includes the preparation of nucleoside conjugates with other compounds and their subsequent introduction into the automated oligonucleotide synthesis. The stability of phosphoramidite derivatives of conjugates in the course of the oligonucleotide synthesis and post-synthetic treatments is a prerequisite. The possibility of selective conjugation and no need for post-synthetic modifications of oligonucleotides are considered as advantages of this method. However, this requires considerable amounts of modified nucleosides, which significantly restricts the potential of this method.

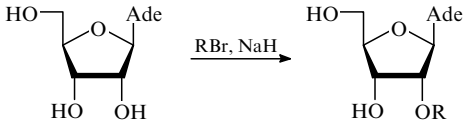
The second approach consists of the conjugation of 2′-modified oligonucleotides immobilised on a polymeric support. To achieve this, a modified fragment containing a 2′-protected reactive group is incorporated into the oligonucleotide chain in the course of solid-phase chemical synthesis. The protecting group in this fragment should retain stability throughout the oligonucleotide synthesis and be selectively removed on its termination without any effect on the newly formed bonds in the protected oligonucleotide. 2′-Hydroxy and amino groups are protected either by the allyloxycarbonyl group, which is removed by palladium(0) complexes, or by the fluorenylmethoxycarbonyl group, which is removed upon treatment with morpholine or piperidine. The levulinoyl and phthaloyl groups are used for the protection of hydroxy and amino groups, respectively, and are removed by hydrazinolysis. Fluorenylmethyl protection, which is removed by treatment with morpholine or piperidine, is used for the carboxy group. Various photolabile protecting groups are also used for this purpose. The use of *tert*-butyldimethylsilyl and *tert*-butyldiphenylsilyl protecting groups is limited to automated synthesis on polystyrene carriers, since controlled-pore glass (CPG) support is destroyed upon removal of protecting groups under the action of the fluoride anion.

Conjugation is carried out after deprotection of the reactive group. Oligonucleotide conjugates immobilised on a polymeric support are washed to remove excess reagents, deprotected, and the product is split from the solid phase. The conjugation is usually carried out in anhydrous organic solvents, especially in the case of conjugates with hydrophobic compounds that are poorly soluble in aqueous and aqueous-organic media. Selective removal of 2-cyanoethyl protecting groups from phosphate groups by treatment with morpholine increases the hydrophilicity of the protected oligonucleotide and favours its conjugation in aqueous – organic media. The fact that protection of heterocyclic bases increases the lability of the *N*-glycosidic bond should also be taken into consideration.

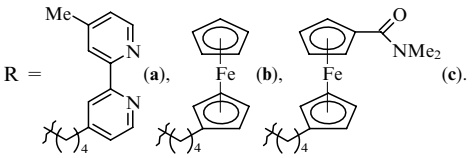
The third approach entails the synthesis of oligonucleotide conjugates in solution. In this case, the protection of the reactive group in the 2′-position should be stable under conditions of oligonucleotide synthesis and be easily removed in the course of standard post-synthetic treatments. Side reactions with the imino and amino groups of heterocyclic bases, *e.g*., during the amide bond formation in compounds containing carboxy groups, can occur, which is a limitation of this method.

1. Synthesis of nucleoside 2′-conjugates

Three different approaches to the synthesis of nucleoside 2′-conjugates with various compounds are possible. The first of them entails direct attachment of various molecules to the 2′-hydroxy groups of nucleosides with formation of ether,[56] carbamate [76] or phosphotriester [157] bonds. The second approach consists of preliminary attachment, to the C(2′)-atom, of a linker containing an amino [57] or a carboxy group,[79] which is then made to react with another compound to form an amide bond. Acylation of 2′-amino-2′-deoxynucleosides is yet another route.

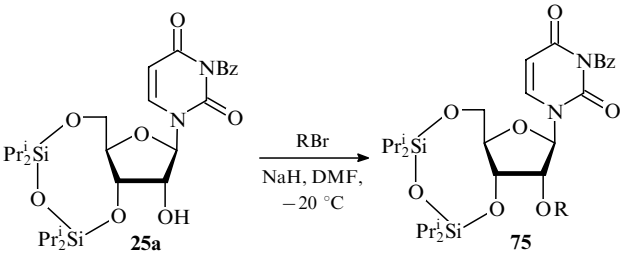


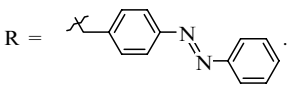
74a – c



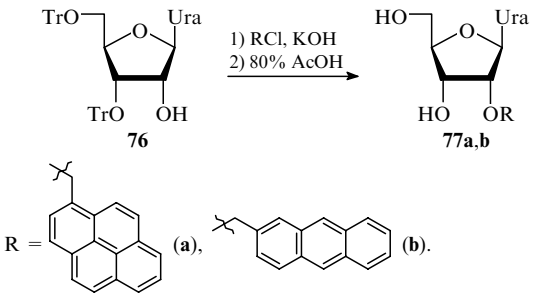
The preparation of the adenosine derivatives **74a – c** containing 2,2′-bipyridine and ferrocene residues linked to the O(2′) atom through a butylene bridge has been described.[158 – 160] The alkylation of adenosine occurs regiospecifically, the yields of 3′-derivatives are insignificant.

A similar alkylation procedure was used [161] for the introduction of an azobenzene fragment at the O(2′)-atom of protected uridine **25a**. The resulting derivative **75** was deprotected and incorporated into an oligonucleotide.

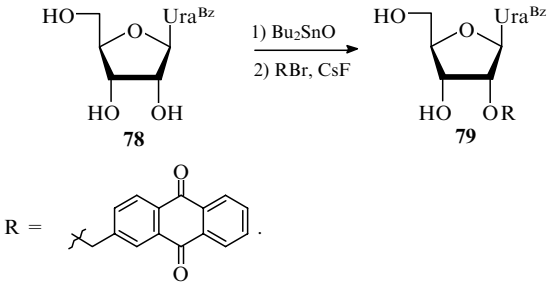




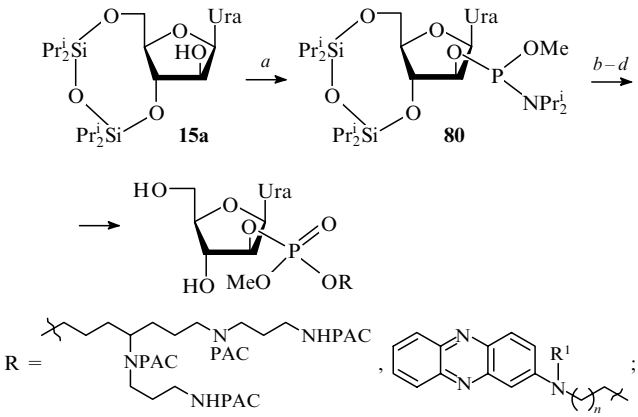
The alkylation of 3′,5′-protected uridine **76** by substituted methyl chlorides in the presence of potassium hydroxide was used for the introduction of polyaromatic molecules into the carbohydrate fragment of the nucleoside.[162, 163] It is of note that the heterocyclic base was not affected, compounds **77a**,**b** being the only reaction products.



The metal-directed alkylation of uridine has been described.[164, 165] Prior to the reaction, the *vic*-diol system of N^3-benzoyluridine (**78**) was activated by dibutyltin oxide and the intermediate formed was introduced into the reaction with 2-bromomethylanthraquinone. The addition of caesium fluoride to the reaction mixture increases the proportion of the 2′-isomer **79**.

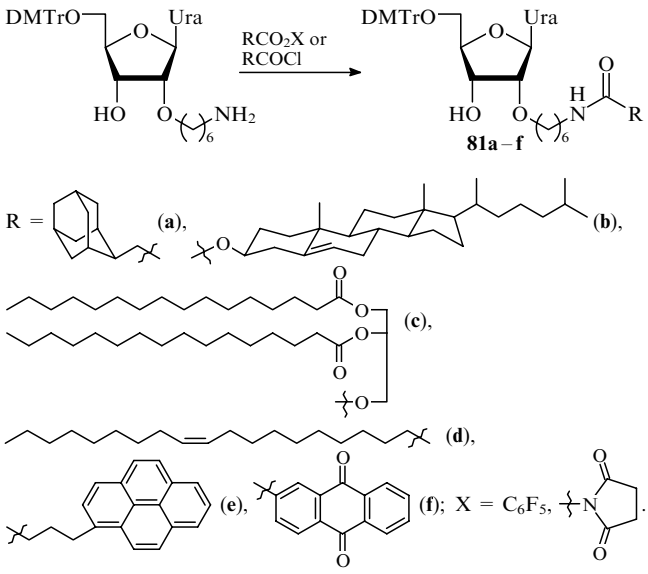


Chattopadhyaya and co-workers[166, 167] have developed a simple and convenient scheme for the attachment of a spermine C-homologue to a nucleoside through a 2′-phosphate group. The 2′-phosphoramidite **80** formed from the protected arabinouridine **15a** was made to react with the hydroxy derivative of the spermine analogue with subsequent oxidation of the phosphorus-containing fragment and deprotection of the hydroxy groups. In these studies, spermine was considered as a cationic constituent able to increase the stability of the NA duplex due to interaction with the phosphate groups of the opposite chain. Similarly, phenazine derivatives were attached to the O(2′)-atom of arabinouridine through a phosphate group.[157]

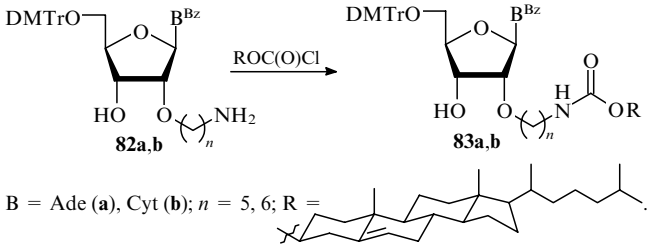


PAC = $PhOCH_2C(O)$; R^1 = PAC, Me; n = 2, 4, 6.
(*a*) $Pr^i_2NP(Cl)OMe$, Pr^i_2NEt; (*b*) ROH, TetrH;
(*c*) I_2, THF, Py, H_2O; (*d*) TBAF.

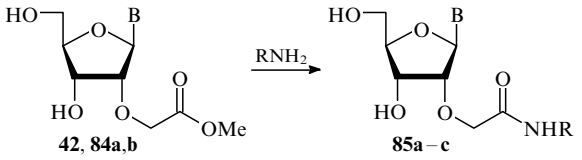
Yet another approach to the synthesis of nucleoside 2′-conjugates has been developed by Cook and co-workers.[168, 169] This included the introduction of fragments of various compounds into the 2′-position of uridine through an aminohexyl linker. This method was used in the synthesis, from one modified nucleoside, of hydrophobic derivatives **81a**–**d**[168, 169] as well as compounds with fluorescent (**81e**[170]) and electrochemical (**81f**[171]) labels suitable for incorporation into the oligonucleotide chain.



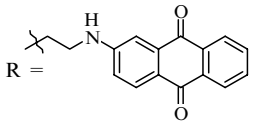
Cholesterol was attached to the modified nucleosides **82a**,**b**.[172] The resulting derivatives **83a**,**b** were introduced into a standard automated oligonucleotide synthesis. The presence of a cholesterol residue in the modified oligonucleotides strongly affects their pharmacodynamic properties.



Keller and Haner[79, 173] have carried out reactions of 2′-*O*-(methoxycarbonylmethyl)nucleosides **42** and **84a**,**b** with 2-[(2-aminoethyl)amino]anthraquinone, octylamine and 6-(*N*,*N*-dimethylamino)hexylamine. The resulting nucleoside conjugates **85a**–**c** were first converted into phosphoramidite derivatives and then incorporated into the oligonucleotide chain. The presence of such modifications in oligonucleotide molecules increases the thermal stabilities of the respective NA duplexes in comparison with native analogues.



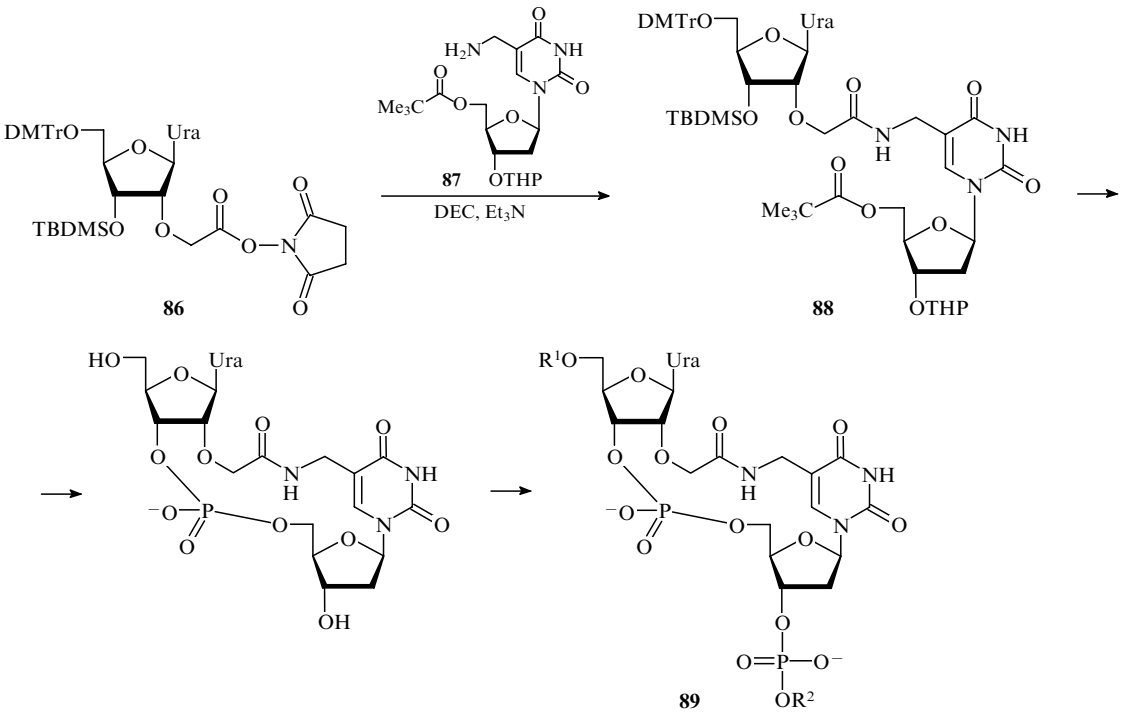
B = Cyt^{Bz} (**84a**, **85a**), Ade^{Bz} (**84b**, **85b**), Thy (**42**, **85c**);



Starting from protected uridine **86** containing an activated 2′-carboxy group and protected 5-aminomethyl-2′-deoxyuridine **87**, Sekine and co-workers[174, 175] have synthesised the dinucleoside **88;** this was converted into a dinucleoside phosphate having a fixed conformation[174] and incorporated into the oligonucleotide **89** (Scheme 4).[175]

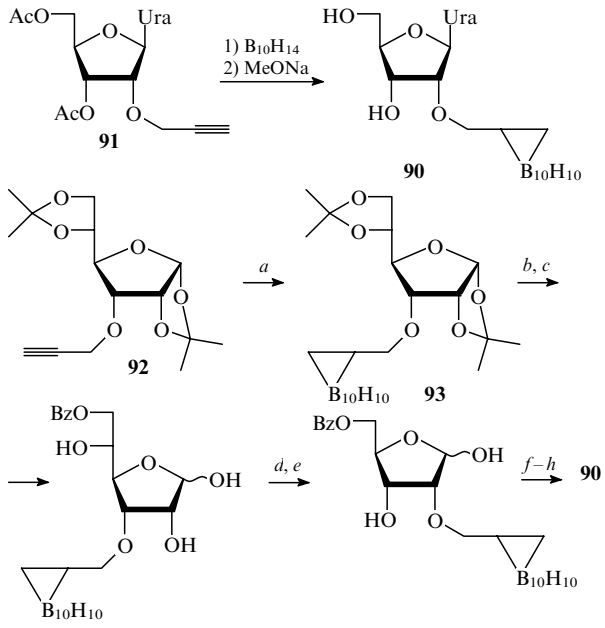
The synthesis of 2′-*O*-carboranylmethyluridine **90** starting from the propargyluridine derivative **91**[176, 177] or protected allofuranose **92** has been carried out.[178] In the latter case, the attach-

Scheme 4



TBDMS = Bu^{t}Me$_2$Si; DEC = Me$_2$N(CH$_2$)$_3$N=C=NEt; R^1 and R^2 are fragments of an oligonucleotide chain.

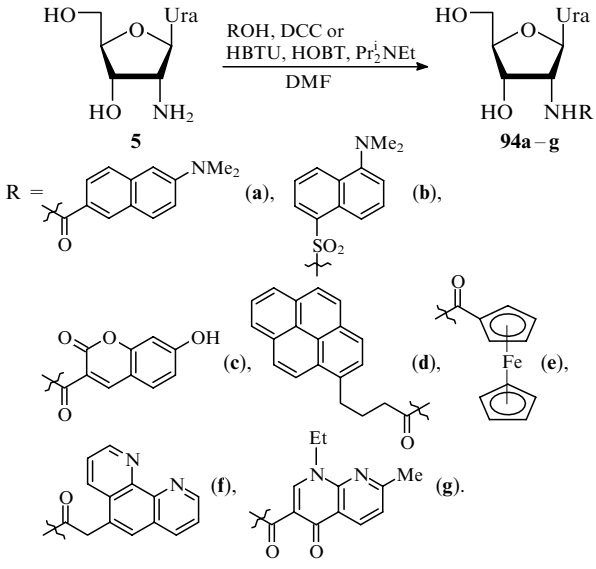
ment of decaborane to the carbohydrate **92** gave first the 3′-isomer **93**; after removal of isopropylidene protecting groups and cleavage of the *cis*-diol system, this was transformed into a ribofuranose derivative and used for glycosylation.



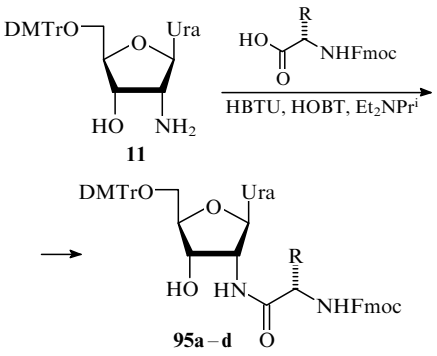
(*a*) $B_{10}H_{14}$; (*b*) H^+; (*c*) BzCl; (*d*) $NaIO_4$; (*e*) Py, H_2O, Δ; (*f*) Ac_2O, Py; (*g*) Ura^{TMS}, TMSOTf; (*h*) MeONa.

Carboranyl nucleosides are used in boron neutron-capture therapy of malignant tumours.[179,180] *In vivo* studies have shown[181] that the nucleoside **90** effectively penetrates into cells, but is not phosphorylated. The intracellular boron concentration varies from 5 to 35 μg g^{-1}, *i.e.*, is sufficient for the implementation of efficient boron neutron-capture therapy. Further studies have shown[182] that the incorporation of the nucleoside **90** into the oligonucleotide chain significantly decreases the stability of the DNA duplex, most probably due to the formation of a negatively charged *nido*-form of carborane under the action of ammonia upon standard post-synthetic treatments.

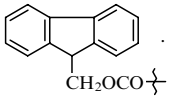
Synthesis of conjugates based on 2′-amino-2′-deoxyuridine **5** included the acylation of the amino group with acids activated by dicyclohexylcarbodiimide (DCC) or a mixture of 1-hydroxybenzotriazole (HOBT) with *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium (HBTU) hexafluorophosphate. This approach was used for the introduction, into the 2′-position of the nucleoside, of various fluorescent labels, such as 6-dimethylamino-2-naphthalene (**94a**),[183,184] the dansyl group (**94b**),[185] coumarin (**94c**)[186] and pyrene (**94d**),[187] of the electrochemical label ferrocene (**94e**),[188–190] of phenanthroline (**94f**)[191] (chemical nuclease in the presence of multicharged metal ions) and of nalidixic acid **94g**;[192] the attachment of the latter to the 3′-end of an oligonucleotide significantly increases the thermal stability of the duplex formed.



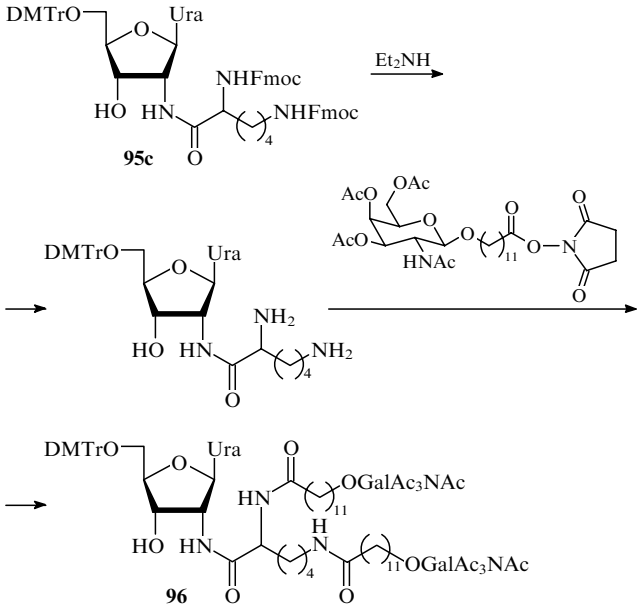
Protected 2′-amino-2′-deoxyuridine **11** was acylated with amino acids.[193] The derivatives **95a–d** obtained were converted into phosphoramidites and incorporated into ribozymes to increase catalytic activity and stability against nucleases.



R = Me (**a**), Bn (**b**), $(CH_2)_4NHFmoc$ (**c**), CH_2CO_2Bn (**d**);

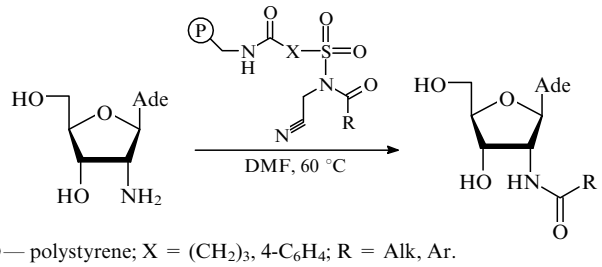
Fmoc = 

The protected 2′-amino-2′-deoxyuridine **95c** acylated with *N*-protected lysine served as a basis for the preparation of the bisconjugate **96** containing peracetylgalactosamine residues.[194] Unfortunately, subsequent reactions of the nucleoside phosphoramidite derivative **96** were strongly hampered due to steric hindrances.



Acylation of free and protected 2′-amino-2′-deoxyadenosine with various aromatic acids in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide has been described.[34, 195]

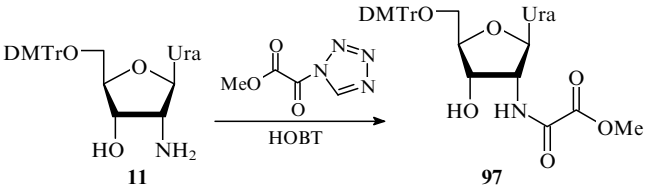
An unusual version of the acylation of 2′-amino-2′-deoxyadenosine has been proposed.[196] Here, the role of acylating agents is played by activated *N*-sulfo-*N*-cyanomethylamides of carboxylic acids immobilised on a solid phase (safety catch linker).[197] Aminomethylated polystyrene containing 4-substituted benzoic or butyric acid residues were used as polymeric supports. The yields in this reaction are more than 95%.



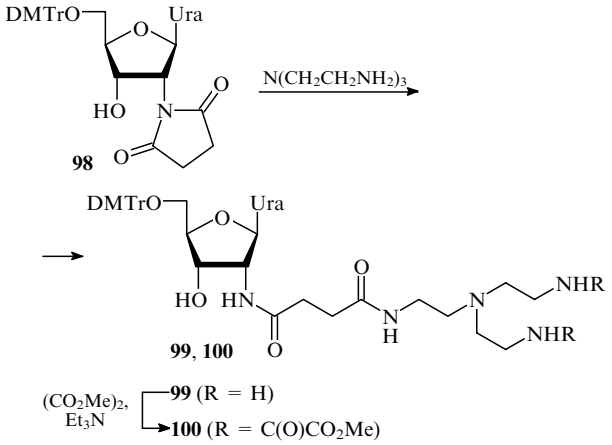
Ⓟ — polystyrene; X = $(CH_2)_3$, 4-C_6H_4; R = Alk, Ar.

This approach was used for the preparation of various 2′-aroyl derivatives of N^6-substituted 2′-amino-2′-deoxyadenosine and 2′,5′-diamino-2′,5′-dideoxyadenosine.[198–201]

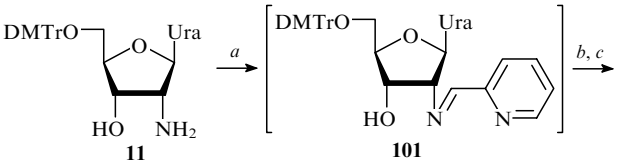
An original procedure for the synthesis of the nucleoside **97** having a methoxalylamido fragment in the 2′-position by the reaction of protected 2′-amino-2′-deoxyuridine **11** with methoxalyltetrazole has been developed by Polushin.[202]

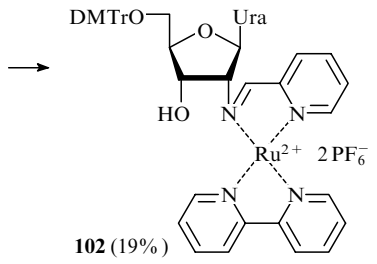


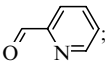
Later[203] 2′-succinimido-2′-deoxyuridine **98** and the dendrimers **99**, **100**[204] based on it were synthesised.



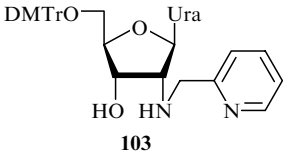
The imino derivative **101** synthesised starting from 2′-amino-2′-deoxyuridine **11** reacted *in situ* with bis(bipyridine)ruthenium(II) chloride.[205, 206] The resulting nucleoside **102** was converted into a phosphoramidite derivative and incorporated into the oligonucleotide chain; however, the yield in the condensation step was extremely low.[27] The yield in the immobilisation of the nucleoside **102** on a polymeric support using a succinate linker was comparable to those for non-modified nucleosides.[206] The complex **102** within the oligonucleotide is stable under the conditions of the post-synthetic treatments with saturated ammonia. Derivatives of the type **102** are the optimum reagents for modification of 3′- (see Ref. 206) and 5′-ends[207] of oligonucleotides, since they cause minimum changes in the structure of the DNA duplex.





(*a*) ; (*b*) $Ru(bpy)_2Cl_2$; (*c*) KPF_6; bpy is 2,2′-bipyridine.

The nucleoside **103** containing a picolylamino group in the 2′-position has recently been synthesised.[27] The introduction of such a modified fragment into the oligonucleotide chain decreases the thermal stability of the DNA duplex by 2–4 °C. These oligonucleotides can also be used for ruthenium chelation.



103

2. Solid-phase synthesis of oligonucleotide conjugates

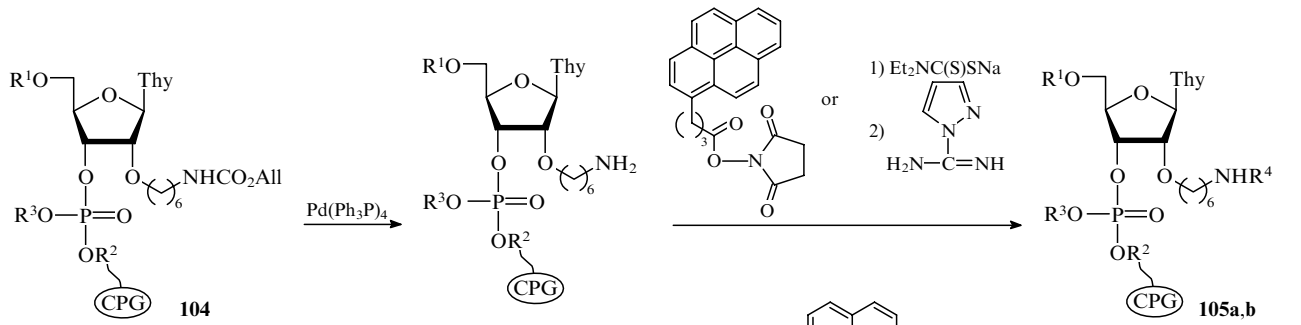
The publications on this subject are few in number, although in some cases, *e.g.*, for incorporation of hydrophobic molecules, this method is the optimum and is the only possible solution for the construction of combinatorial libraries. The term 'combinatorial libraries' is used to encompass a set of conjugates containing identical cores (*e.g.*, oligonucleotides), but differing in the fragments of molecules attached to them. It is of note that such sets of conjugates are prepared simultaneously, *e.g.*, by treatment of an oligonucleotide containing the amino group with a mixture of activated carboxylic acids with subsequent separation of the reaction products. Combinatorial oligonucleotide libraries are extensively used in bioorganic chemistry and molecular biology, particularly, in the search for compounds that are highly resistant to nucleases.[208]

An alternative approach to the synthesis of conjugates based on immobilised oligonucleotides **104** containing a protected 2′-*O*-(6-aminohexyl)nucleoside has been described by Manoharan and co-workers [209] Selective removal of the allyloxycarbonyl protecting group by a palladium(0) complex and subsequent reaction of pyrenylbutyric acid ester with *N*-hydroxysuccinimide give compound **105a.** The internucleotide bonds and protecting groups of heterocyclic bases and internucleotide phosphates remained intact. Later, the same authors [210] carried out the synthesis of oligonucleotides **105b** containing a guanidine group attached to a 2′-*O*-aminohexyl linker (Scheme 5).

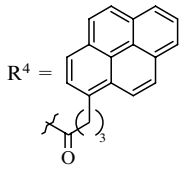
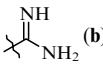
A recently published paper [211] describes the synthesis of the oligonucleotide conjugates **106a**,**b** with 3′-terminal 2′-acylamido groups. A nucleoside with an *N*-protected phenylalanylamide residue in the 2′-position was immobilised on a polymeric support through a standard succinate linker. The allyloxycarbonyl protecting group in compound **107** was selectively removed and the immobilised nucleoside was acylated and introduced into the standard oligonucleotide synthesis (Scheme 6).

Yet another approach to the attachment of an amino group to the 3′-end of an oligonucleotide entails the use of a 5′-phosphor-

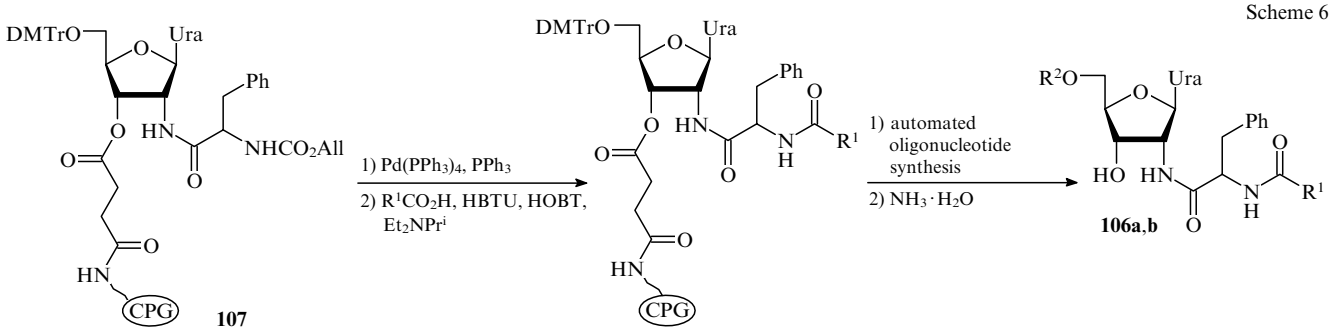
Scheme 5

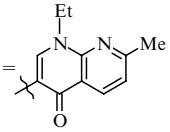
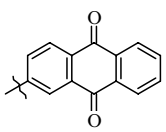


R^1 and R^2 are fragments of a protected oligonucleotide chain; $R^3 = (CH_2)_2CN$; CPG is controlled-pore glass.

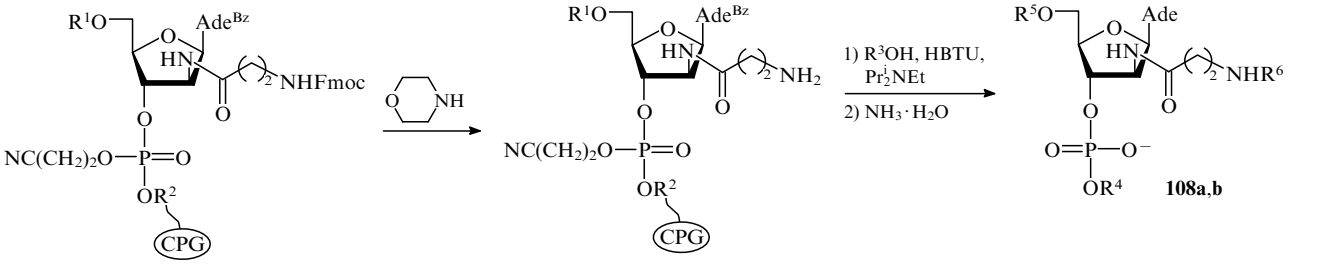
R^4 =  (**a**),  (**b**);

Scheme 6



R^1 =  (**a**),  (**b**); R^2 is a fragment of an oligonucleotide chain.

Scheme 7



R^1 and R^2 are fragments of a protected oligonucleotide chain; R^3 = –Gly-LeuNHFmoc, –Gly-Phe-D-Ala-TyrNHFmoc; R^4 and R^5 are fragments of a deprotected oligonucleotide chain; R^6 = –Gly-LeuNH$_2$ (**a**), –Gly-Phe-D-Ala-TyrNH$_2$ (**b**).

amidite derivative of a modified nucleoside that is incorporated into the oligonucleotide chain by reversed (5′→ 3′) automated oligonucleotide synthesis.[211] High yields in the *N*-acylation step enable the construction of a combinatorial library and investigation of the resistance of 3′-oligonucleotide conjugates against nucleases.[208]

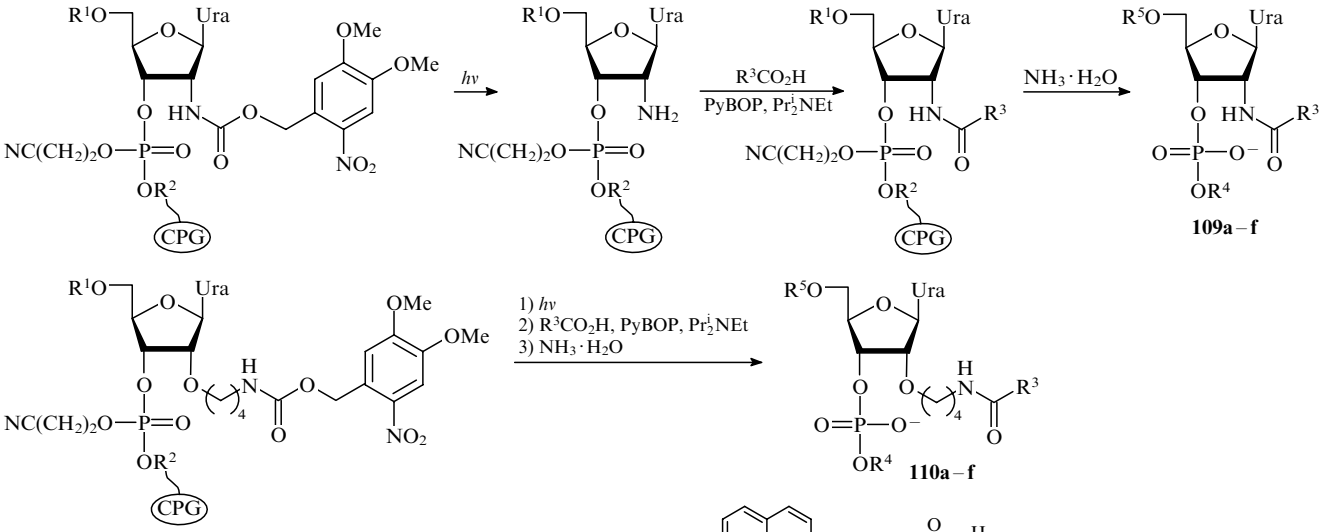
Synthesis of peptide–oligonucleotide conjugates has been described by Oretskaya and co-workers[212] The protected amino group of 2′-(β-alanyl)amido-2′-deoxyarabinoadenosine incorporated into oligonucleotides was selectively deprotected by morpholine or piperidine. Subsequent acylation with *N*-protected peptides and removal of protecting groups afforded the target conjugates **108a**,**b** with a degree of conversion of 80%–90% (Scheme 7).

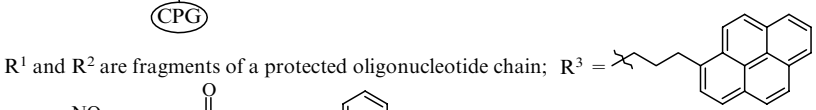
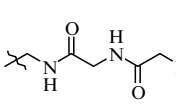
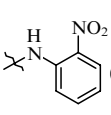
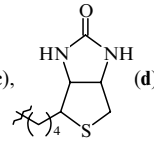
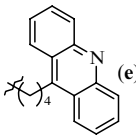
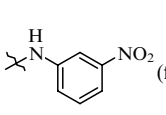
Hwang and Greenberg[213, 214] have recommended the use of photo-removable 4,5-dimethoxy-2-nitrobenzyloxycarbonyl protection for the 2′-amino group of modified nucleosides. The reactivities of 2′-amino-2′-deoxy- and 2′-*O*-(4-aminobutyl)uridine groups of oligonucleotides in acylation reactions have been studied. In some cases, the yields of the products **109** and **110** were rather low (*e.g.*, the yield of the oligonucleotide–peptide conjugate **110b** was 30%) (Scheme 8).

Manoharan and co-workers[215] used phthaloyl protection (which is selectively removable by hydrazinolysis) of aminooxy groups of modified nucleosides. The aminooxy group is extremely reactive with respect to carbonyl compounds. This type of conjugation is extensively used in peptide chemistry.[216] The oximes thus prepared are stable over a broad pH range and can be reduced to *N*,*O*-disubstituted hydroxylamines which are even more stable under drastic conditions (Scheme 9).

Sawai and co-workers[94, 95] have proposed to simultaneously remove protecting groups and convert the ester group in a modified fragment into an amide group. In this way, dinucleoside monophosphates were prepared containing non-substituted (**111a**), *N*-alkyl (**111b**), *N*-aminoalkyl (**111c**) carbamoyl groups and a dendrimer (**111d**) in the 2′-position. It is of note that the yields of the target products were smaller in the case of more complex amines. When *N*-protected adenosine, cytidine and guanosine were used instead of thymidine and uridine, side transamination occurred.

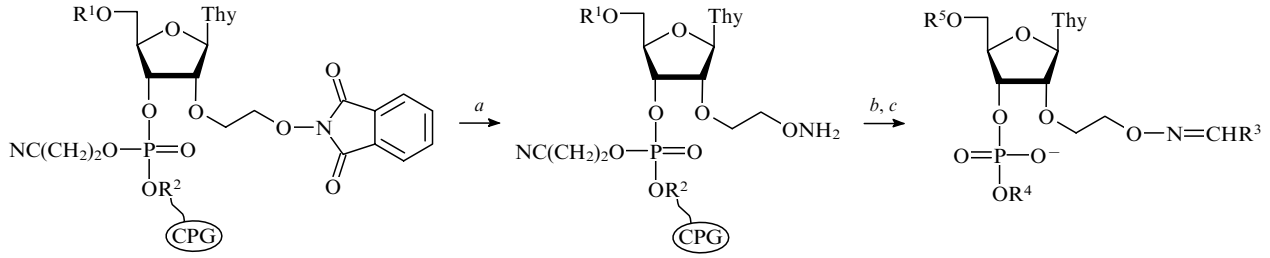
Scheme 8



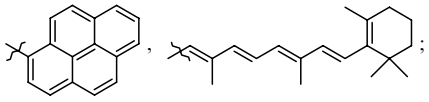
R^1 and R^2 are fragments of a protected oligonucleotide chain; R^3 =  (**a**),  (**b**),  (**c**),  (**d**),  (**e**),  (**f**); R^4 and R^5 are fragments of a deprotected oligonucleotide chain;

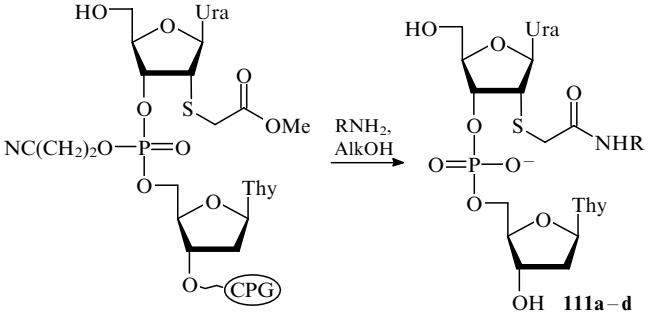
PyBOP is benzotriazol-1-yloxytris(pyrollidino)phosphonium hexafluorophosphate.

Scheme 9



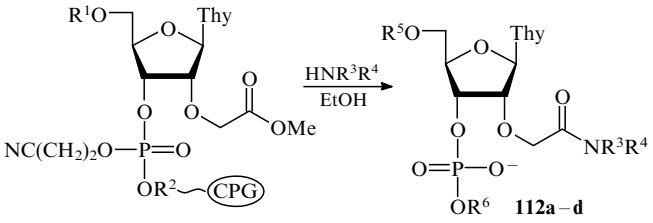
(*a*) $N_2H_4 \cdot H_2O$, Py, AcOH; (*b*) R^3CHO, DMF or MeCN; (*c*) $NH_3 \cdot H_2O$.

R^1 and R^2 are fragments of a protected oligonucleotide chain; R^3 = 
R^4 and R^5 are fragments of a deprotected oligonucleotide chain.



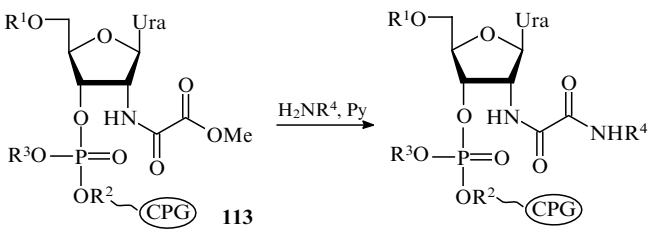
R = H (**a**), $(CH_2)_6Me$ (**b**), $(CH_2)_2NH_2$ (**c**), $(CH_2)_2N(CH_2CH_2NH_2)_2$ (**d**); Alk = Me, Et.

This methodology was further developed by Manoharan and co-workers [145] If primary amines were used, high yields of compounds **112a**–**c** were achieved within 24 h at room temperature, but the yield of compound **112d** in the reaction with dimethylamine was as low as 50%; at higher temperature, side products were formed.



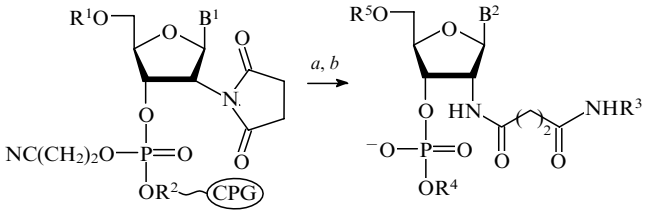
R^1 and R^2 are fragments of a protected oligonucleotide chain;
R^3 = H: R^4 = H (**a**), Me (**b**), $(CH_2)_2NMe_2$ (**c**); R^3 = R^4 = Me (**d**);
R^5 and R^6 are fragments of a deprotected oligonucleotide chain.

Polushin [202] has prepared a series of oligonucleotides **113** containing a methoxalylamido group in the 2′-position. This group is more electrophilic in comparison with ordinary ester group, which permits one to carry out reactions with amines under milder conditions without simultaneous splitting of the oligonucleotide from the polymer.



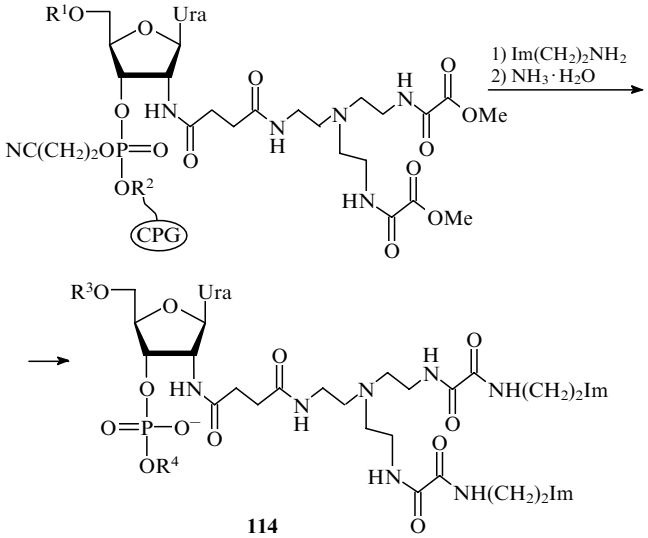
R^1 and R^2 are fragments of a protected oligonucleotide chain;
$R^3 = (CH_2)_2CN$; $R^4 = (CH_2)_{12}NH_2$.

In 2′-succinimido derivatives of 2′-deoxyuridine and -cytidine, the five-membered imide ring is opened upon reactions with various amines. [203, 204, 217]



B^1 = Ura, Cyt^{Bz}; B^2 = Ura, Cyt; R^1 and R^2 are fragments of a protected oligonucleotide chain; R^3 = Me, $(CH_2)_2OH$, $(CH_2)_6OH$, $(CH_2)_3NMe_2$, $(CH_2)_n$Im; n = 2, 3; R^4 and R^5 are fragments of a deprotected oligonucleotide chain; (*a*) R^3NH_2; (*b*) $NH_3 \cdot H_2O$.

The same authors [204, 217] have described the synthesis of dendrimeric derivatives based on 2′-succinimido-2′-deoxyuridine incorporated into the oligonucleotide chain. The attachment of branched polyamines to a modified nucleoside fragment was followed by acylation of free amino groups with methoxalyltetrazole. Treatment of the dendrimer formed with histamine affords the conjugate **114** which is a potential chemical nuclease.

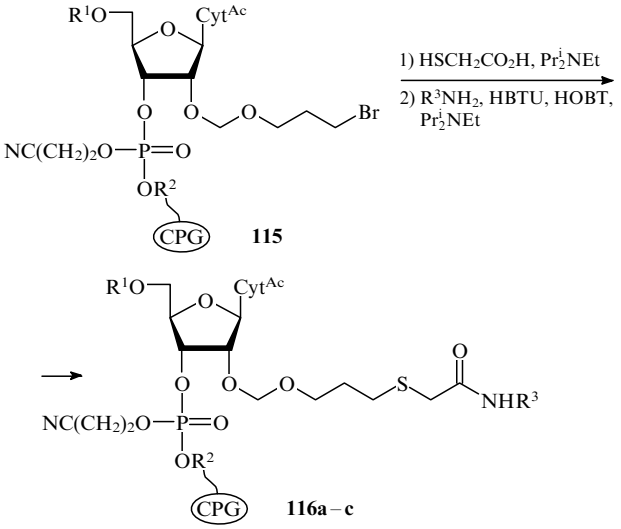


114

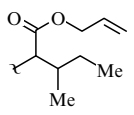
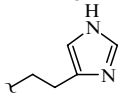
R^1 and R^2 are fragments of a protected oligonucleotide chain;
R^3 and R^4 are fragments of a deprotected oligonucleotide chain.

The incorporation of a bromoalkyl group into an oligonucleotide enables conjugation with nucleophiles, such as thiols and sodium azide. [108, 218] Thus the preparation of oligonucleotide conjugates with amines included initial synthesis of modified

oligonucleotides **115** immobilised on a polymeric support containing a ω-bromoalkyl group at the O(2′) atom.[219] These were treated with thioglycolic acid in the presence of a base. After the activation of the carboxy group in the acids formed, the latter reacted with various amines to yield compounds **116a–c**.



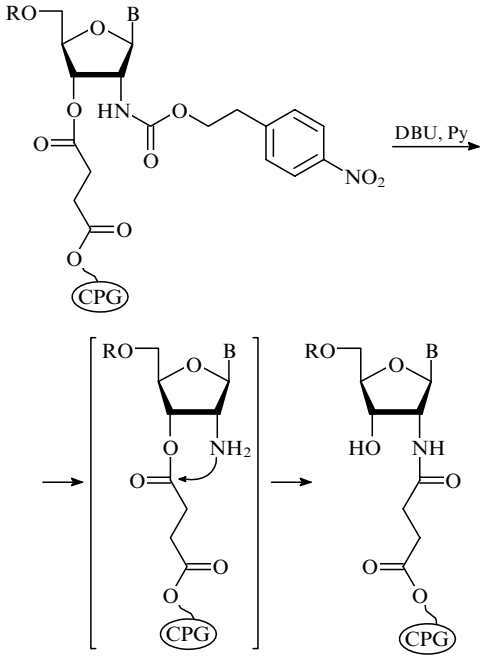
R[1] and R[2] are fragments of a protected oligonucleotide chain;

R[3] =  (**a**), Me (**b**),  (**c**).

3. Solution synthesis of oligonucleotide conjugates

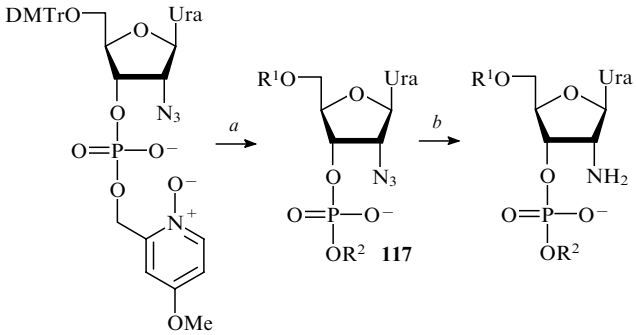
Oligonucleotides containing pyrimidine 2′-amino-2′-deoxyribonucleoside fragments have been especially extensively used for conjugation in solution. The amino group in the 2′-position has pK_a = 6.2,[220] which was confirmed by ^{1}H NMR (see Ref. 221) and ^{13}C NMR (see Ref. 222) spectroscopic data. This enables its selective acylation at appropriate pH precluding the involvement of amino groups of heterocyclic bases with pK_a from 3 to 5. The significant decrease in the melting temperature of the duplexes formed by 2′-amino-2′-deoxyribooligonucleotides with non-modified NA is the main disadvantage of this modification. Thus the melting temperature of a 15-membered duplex decreases by 2.7–13.8 °C depending on the structure of the duplex and the position of the modified fragment in the chain.[223]

The choice of a protecting group for the amino group of a modified fragment for the incorporation of 2′-amino-2′-deoxyribonucleosides into the oligonucleotide chain presents a problem. The use of trifluoroacetyl protection for this purpose was suggested, but the condensation of a commercially available 2′-trifluoroacetamido-2′-deoxyuridine phosphoramidite with a growing oligonucleotide chain occurs in a relatively low yield (~ 90% in 10 min),[224] which can be attributed to the high electronegativity of the trifluoroacetyl protecting group. This problem can be overcome by increasing the condensation time;[225] however, side reactions thus occur, which diminish the total yield of the oligonucleotide.[226] The trifluoroacetyl protecting group was also used for the protection of the amino group of 2′-amino-2′-deoxyguanosine.[227] The fluorenylmethoxycarbonyl protecting group meets all the requirements of oligonucleotide synthesis, but the thus protected 2′-amino-2′-deoxyuridine phosphoramidite derivative was unstable in an acetonitrile solution and was destroyed even in a solid state at −20 °C.[224, 228] Nevertheless, this group was used for protection.[229] The use of the phthalimide group was also proposed;[224] the latter was introduced with high yields and could be completely removed with standard post-synthetic treatments, but in this case the condensation efficiency could be increased by only 3%–5%. The use of the 2-(4-nitrophenyl)ethoxycarbonyl protecting group[230, 231] can hardly be called successful, since its removal demands preliminary 12-h treatment of the protected oligonucleotide with a 1 M solution of DBU in pyridine. The thus protected 2′-amino-2′-deoxynucleoside cannot be immobilised on a polymeric support using a succinate linker due to intramolecular $O \rightarrow N$ migration of the acyl group upon deprotection. The stability of the amide bond in the 2′-succinamide formed prevents the removal of the modified oligonucleotide from the polymeric support.



R is a fragment of a protected oligonucleotide chain;
B is a protected heterocyclic base.

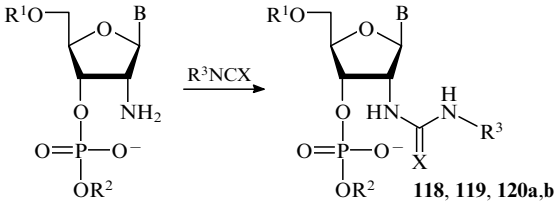
A new approach, in principle, to the synthesis of oligonucleotides with 2′-amino-2′-deoxyribonucleoside fragments has been developed by Polushin *et al.*[232] The oligonucleotides **117** containing a 2′-azido-2′-deoxyuridine fragment were prepared using the phosphotriester method. The azido group was further reduced to the amino group by treatment with triphenylphosphine or tris(2-carboxyethyl)phosphine (TCEP) in the presence of water. However, this approach has not received wide application, since the solid-phase phosphotriester method is no longer used in oligonucleotide synthesis due to the lengthy reaction cycle and lower yields in the condensation step in comparison with the phosphoramidite method. Nevertheless, today it is the only approach which enables the synthesis of oligonucleotides containing 2′-amino-2′-deoxyribonucleoside fragments in the middle of the chain.



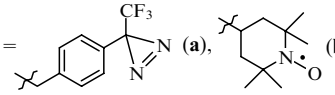
(*a*) is oligonucleotide synthesis; (*b*) Ph_3P or TCEP, H_2O, MeCN;
R[1] and R[2] are fragments of an oligonucleotide chain.

Synthesis of conjugates based on oligonucleotides containing 2′-amino groups is carried out using *N*-acylation in a borate, phosphate or carbonate buffer at pH 8–8.5. Under these con-

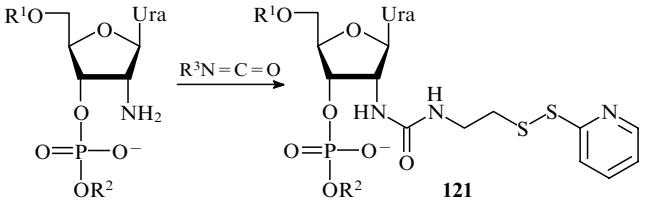
ditions, the amino groups of heterocyclic bases are protonated, which allows selective involvement of the 2′-amino group of the modified fragment. Activated carboxylic acids, isocyanates or isothiocyanates are used as acylating reagents. This approach was used for the preparation of conjugates (**118**–**120a**,**b**) with fluorescein isothiocyanate,[233–235] rhodamine 5-isocyanate,[222] a photochemically active diazirine derivative **120a**[236] and a spin label **120b**.[237]



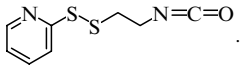
R^1 and R^2 are fragments of an oligonucleotide chain;
118: R^3 is fluorescein, X = S, B = Ura;
119: R^3 is rhodamine, X = O, B = Cyt.

120: R^3 =  (**a**), (**b**); X = O, S; B = Ura.

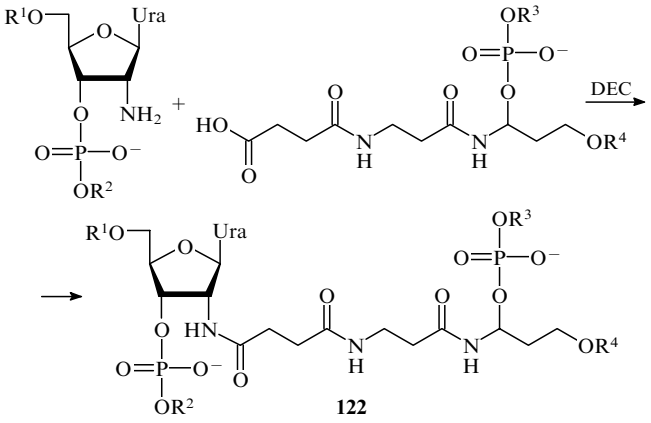
Sigurdsson *et al.*[238–240] have prepared the conjugates **121** by the reaction of a 2′-amino-2′-deoxyuridine fragment of an oligonucleotide with 2-isothiocyanatoethyl 2-pyridyl disulfide.

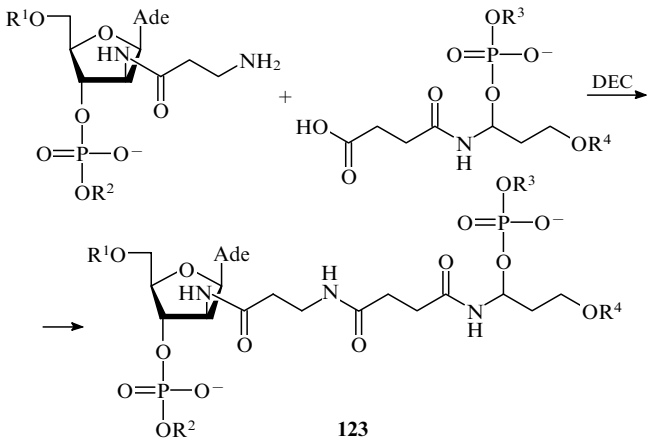


R^1 and R^2 are fragments of an oligonucleotide chain;

$R^3N{=}C{=}O$ = .

The oligonucleotides containing 2′-amino-2′-deoxyuridine[241–244] or 2′-(β-alanylamido-2′-deoxyarabinoadenosine[245,246] were used by Shabarova *et al.* in the synthesis of covalently bound duplexes **122** and **123**. In this case, a nonnucleoside fragment containing a carboxy group at the end of an extended linker was introduced into the complementary chain.



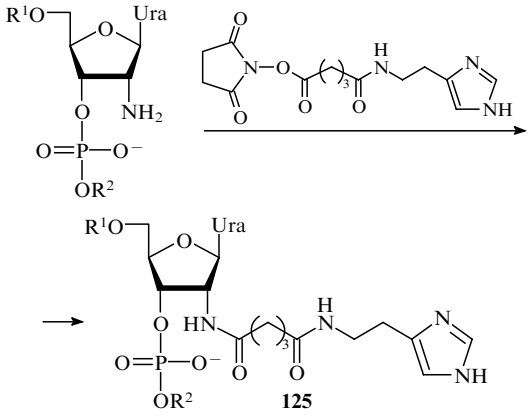


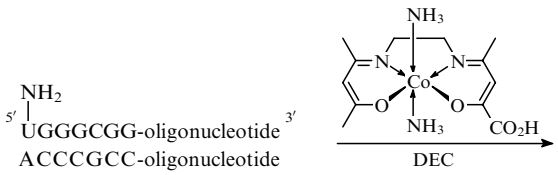
R^1, R^2, R^3 and R^4 are fragments of oligonucleotide chains.

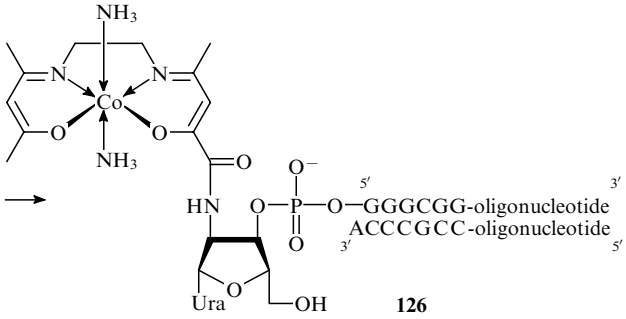
Oligonucleotides **124**, where the chain units are linked by amide bonds in addition to phosphodiester bonds through linkers attached to the C(2′) atoms, have been synthesised.[247] The spatial proximity of carboxy and amino groups determined the high regioselectivity of this reaction, the yield of the target product **124** being 78%; the formation of side products was not observed (Scheme 10).

Substituted carboxylic acid esters of *N*-hydroxysuccinimide are often used as activated acylating agents. Silverman and Cech[248] have performed the acylation of oligonucleotides containing 2′-amino-2′-deoxyuridine using pyrenylacetic and pyrenylbutyric acid esters of *N*-hydroxysuccinimide; Weeks and coworkers[249–251] used biotinylbutyric ester for this purpose.

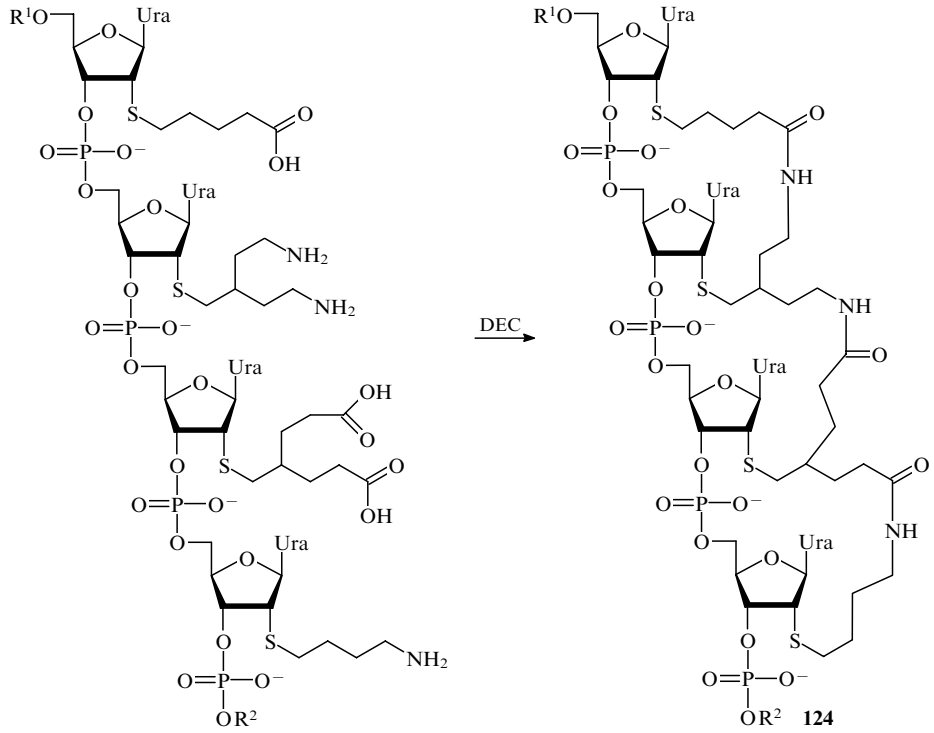
Conjugates of modified oligonucleotides containing 2′-amino-2′-deoxyuridine with histamine **125**[252] and the cobalt complex **126** have been synthesised.[253] In the latter case, the acylation was







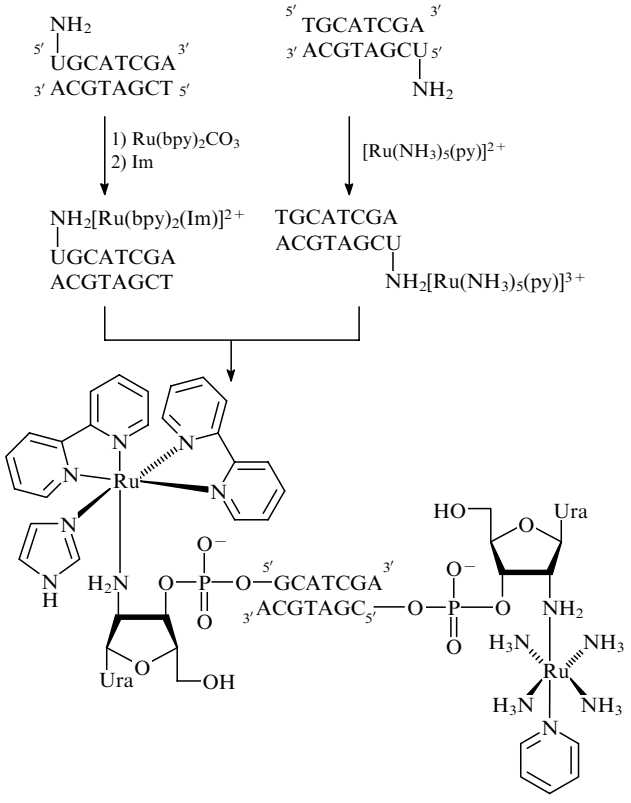
Scheme 10



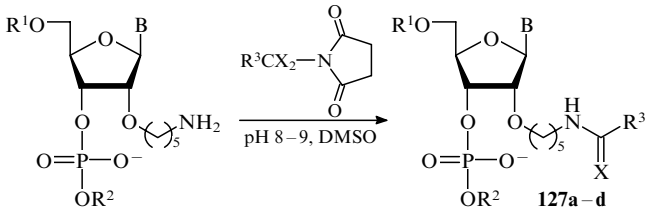
R^1 and R^2 are fragments of an oligonucleotide chain.

performed in the duplex in order to prevent the interaction of the carboxy group of the ligand with the amino groups of the heterocyclic bases.

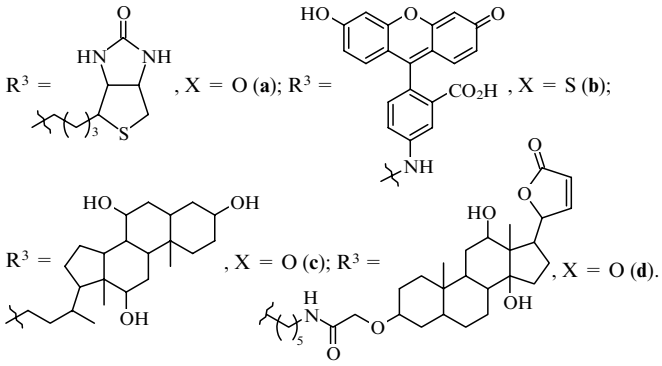
Studies by Meade *et al.*[254, 255] are devoted to the synthesis of ruthenium complexes of 2′-amino-2′-deoxyribooligonucleotides. Complementary oligonucleotides containing 2′-amino-2′-deoxyuridine at the 5′-ends were modified by two ruthenium complexes differing in degree of oxidation. The complex formation was carried out within the DNA duplexes to prevent interaction of the ruthenium complexes with exocyclic amino groups of the heterocyclic bases. The modified DNA duplex was used for the study of photoinduced electron transfer along the DNA chain which plays an essential role in the damage and repair of DNA.[256, 257]



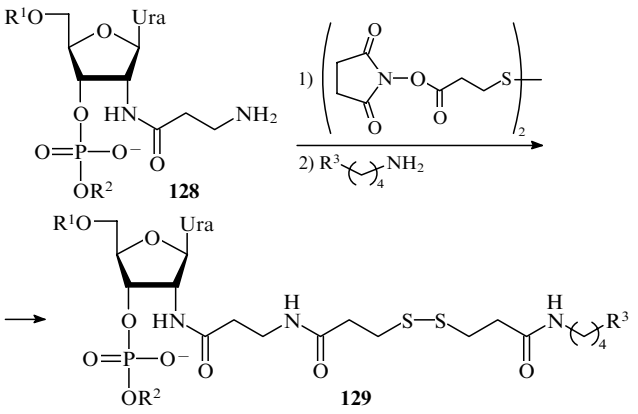
A series of conjugates **127a**–**d** were prepared by reactions of modified oligonucleotides containing 2′-*O*-(5-aminopentyl)uridine[65] and -adenosine[57] with various *N*-hydroxysuccinimide esters.



R^1 and R^2 are fragments of an oligonucleotide chain; B = Ade, Ura;

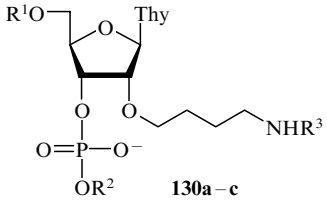


Acylation of the oligoribonucleotide **128** containing 2′-(β-alanylamido)-2′-deoxyuridine with a bifunctional reagent [3,3′-dithiobis(propionic) acid ester of *N*-hydroxysuccinimide] eventually allowed preparation of conjugate **129** containing a peptide linked to the C(2′) atom through a disulfide linker.[258] The disulfide bond can be cleaved by thiols, which simplifies the mass-spectrometric analysis of the conjugates with peptides.

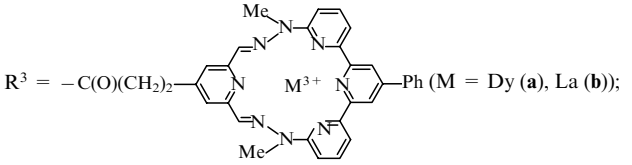


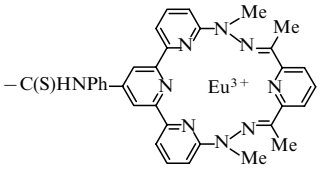
R^1 and R^2 are fragments of an oligonucleotide chain; R^3 is a fragment of the Tat-protein.

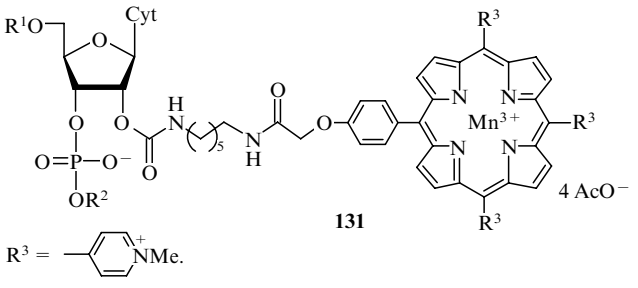
Conjugates of oligonucleotides containing 2′-*O*-(4-aminobutyl)ribothymidine with lanthanide complexes **130a**–**c**,[259] and conjugates of oligonucleotides containing 2′-*O*-(6-aminohexylcarbamoyl)cytidine linked with a porphyrin manganese complex (**131**) were synthesised in a similar way.[75]



R^1 and R^2 are fragments of an oligonucleotide chain;

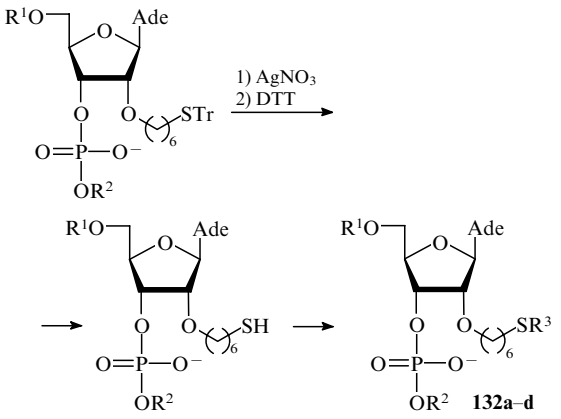




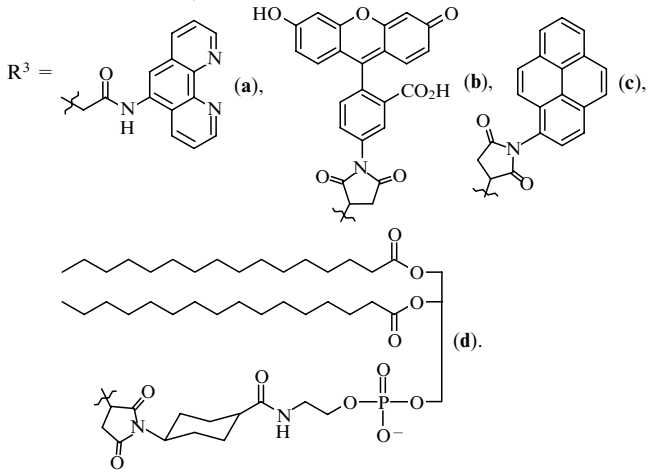


The presence, in modified oligonucleotides, of a thiol group, which can be involved in the formation of disulfide bonds, thioethers and thioesters offers broad opportunities for conjugation.[260]

2′-*O*-(6-Mercaptohexyl)adenosine incorporated into the oligomeric chain was converted into the conjugates **132a**–**d** using *S*-alkylation by iodoacetamidophenanthroline or addition of maleimide derivatives of fluorescein, pyrene and a phospholipid.

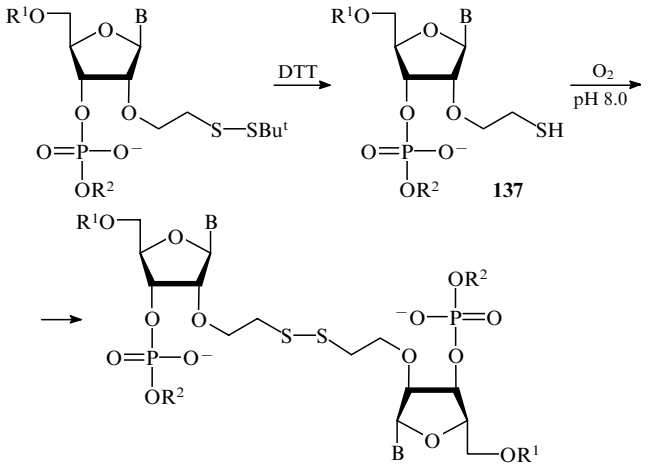


R^1 and R^2 are fragments of an oligonucleotide chain; DTT is dithiothreitol;



Sigurdsson *et al.*[238,240] have succeeded in synthesising cross-linked duplexes **133** and conjugates with the fluorescent label bimane **134**[239] starting from oligonucleotides **135** containing a thiol group as well as conjugates with glutathione **136** based on oligomers **121** containing an activated thiol group (Scheme 11).[239]

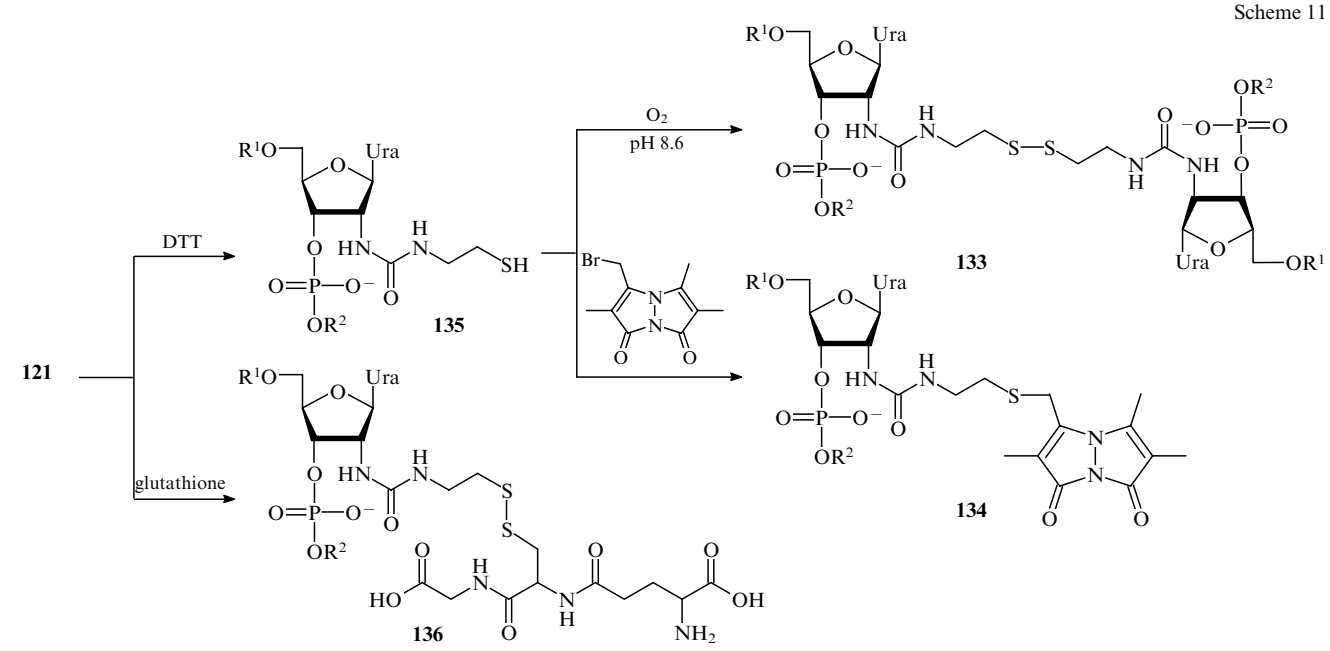
Covalent ‘homocross-linking’ of thiols **137** has been carried out; the duplexes formed were used for the study of the spatial structure of $tRNA^{Phe}$.[105,106]



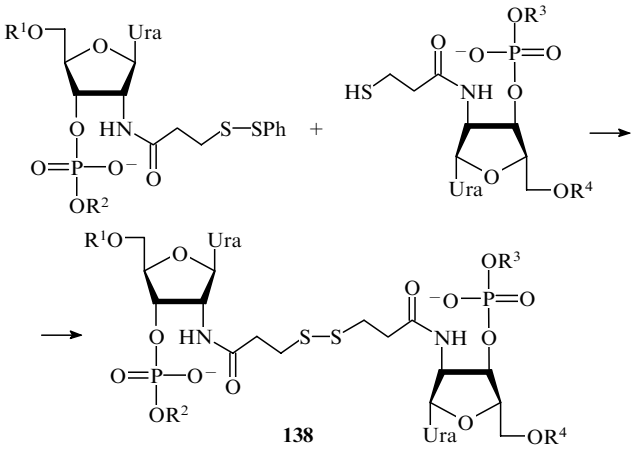
R^1 and R^2 are fragments of $tRNA^{Phe}$; B = Ura, Cyt.

The conjugates **138** containing a disulfide bond were formed in the thiol–disulfide exchange reaction.[261]

Scheme 11

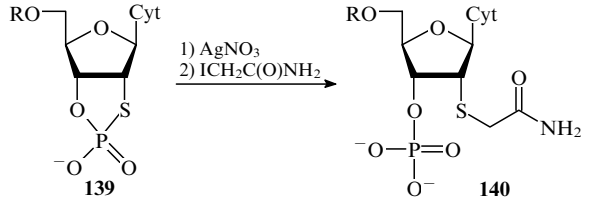


R^1 and R^2 are fragments of an oligonucleotide chain.



R^1, R^2, R^3 and R^4 are fragments of an oligonucleotide chain.

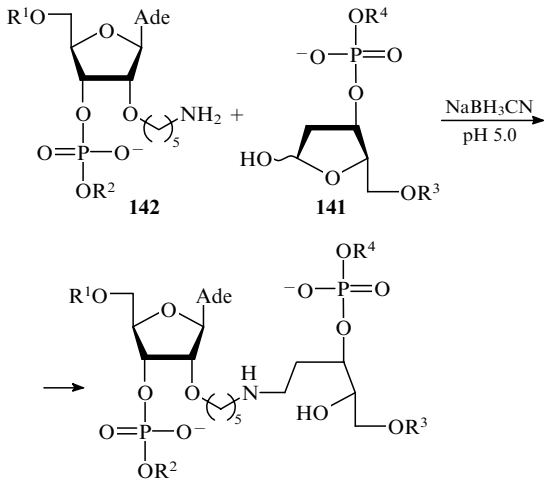
Hamm and Piccirilly [262] have synthesised oligonucleotides **139** containing 2′-*S*,3′-*O*-cyclophosphate at the 3′-end. The free 2′-thiol group was generated by treatment of the oligomers **139** with silver nitrate; subsequent reaction with iodoacetamide yielded the derivatives **140**. This approach can be used for modification of 2′-thionucleosides localised in the middle of the oligonucleotide chain.



R is a fragment of an oligonucleotide chain.

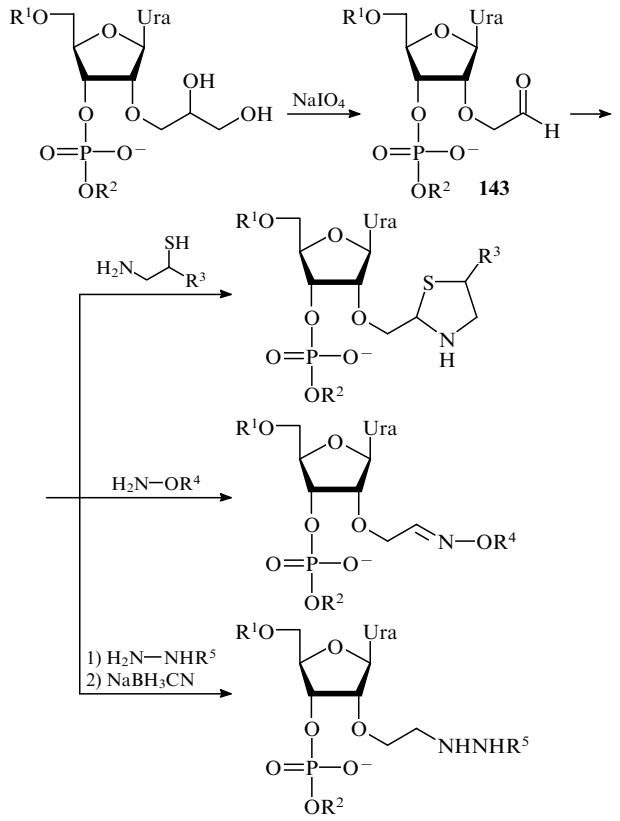
No formation of side 1′,2′-episulfides was observed, which occurs in the case of 2′-thionucleotides in an alkaline medium.[263,264] Presumably, this reaction involves silver thiolate rather than a free thio group. It is of note that 2′-thiooligonucleotides are more stable in alkaline media than non-modified oligoribonucleotides.

Cook and co-workers [265–267] have prepared a cross-linked duplex by reductive amination of an AP site in the oligonucleotide **141** with an aminoalkyl substituent at the O(2′) atom of adenosine **142** in the opposite chain.

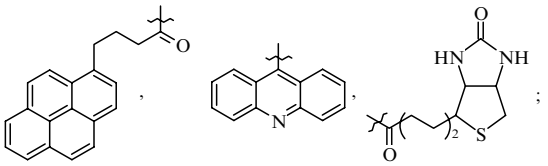


R^1, R^2, R^3 and R^4 are fragments of an oligonucleotide chain.

The addition of a series of 1,2-aminothiols, *O*-alkylhydroxylamines and hydrazides to oligonucleotides **143** containing an acetaldehyde residue at the O(2′) atom [268] yielded conjugates with peptides, pyrene, acridine and biotin. The hydrazones synthesised were unstable at neutral pH, which required their additional reduction to hydrazines with sodium cyanoborohydride. It is of note that this is the first example of simultaneous addition of several peptide residues to an oligonucleotide molecule owing to incorporation of several modified fragments into the chain.

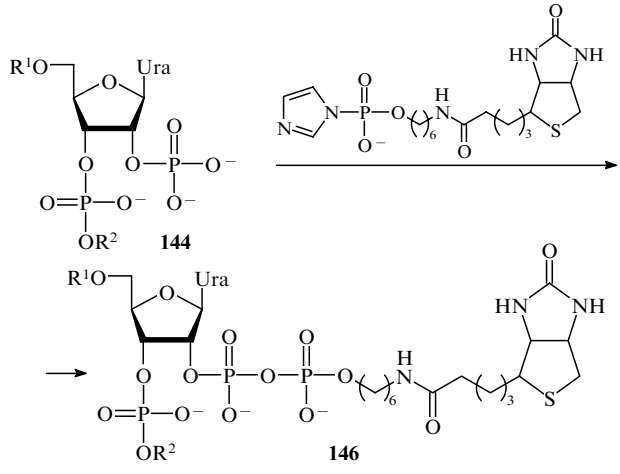


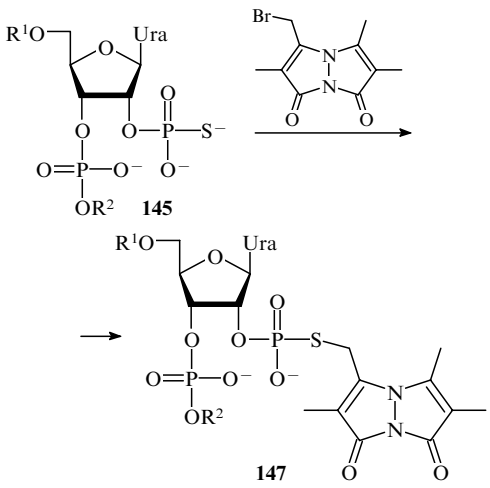
R^1 and R^2 are fragments of an oligonucleotide chain; R^3 = C(O)NHCH$_2$CO$_2$H, C(O)—CFSRAYPTPLRSKKT—OH, C(O)—GYGPKKKRKVGG—OH,



R^4 = CH$_2$C(O)–DPGYIGSR–OH;
R^5 = C(O)(CH$_2$)$_2$C(O)–DPGYIGSR–OH.

Sekine *et al.*[148] used oligonucleotides **144** and **145** containing 2′-phosphate and -thiophosphate groups for the introduction of biotin and biman to give the conjugates **146** and **147** containing the pyrophosphate and thiophosphate bonds, respectively. Later, it was shown[269] that the 2′-phosphate and -thiophosphate groups are easily and selectively removed on heating. Studies of the mechanism of this reaction have shown that the presence of a neighbouring 3′-phosphate group favours 2′-dephosphorylation.





R^1 and R^2 are fragments of an oligonucleotide chain.

IV. The use of nucleosides, nucleotides and oligonucleotides containing reactive groups in the 2′-position

1. The use of nucleosides and nucleotides

2′-Modified nucleosides and nucleotides can find application as selective inhibitors of certain enzymes. To this end, a group able to react with amino acid residues in the active site of the enzyme and thus to irreversibly inhibit it is introduced into a nucleoside. This approach is effective in a case where the structure of the enzyme active site and the mechanisms of its functioning have been studied in detail.

The use of 5′-diphosphates of pyrimidine 2′-thio- and 2′-thiocyanatonucleosides for the inhibition of ribonucleoside diphosphate reductase has been suggested.[270–272] This enzyme plays a key role in the biosynthesis of 2′-deoxyribonucleosides from ribonucleosides by catalysing the reduction of ribose as a constituent of ribonucleoside diphosphates to 2-deoxyribose. This reaction requires two hydrogen atoms which are generated by thioredoxin, a reduced protein cofactor containing two free SH groups in the cysteine residues. The reduction of ribose is accompanied by the oxidation of the cysteine residues; these conditions favour the conversion of thioredoxin into a disulfide form. It was suggested[271,273] that 2′-thio- and 2′-thiocyanate nucleoside diphosphate derivatives react competitively with the cysteine residues in the active centre of the enzyme and thus induce its inhibition. Besides, 2′-methylidene-2′-deoxyribonucleosides were used for the inhibition of ribonucleoside diphosphate reductase.[274]

2′-Hydroxyamino-2′-deoxyribonucleosides able to generate free radicals manifested high anti-fibrosacroma activity *in vitro* and *in vivo*.[28–30] It is of interest that 3′-isomers of these nucleosides inhibit RNA synthesis and suppress tumour growth in mice.

2′-Acylamino-2′-deoxyadenosine derivatives containing aromatic acid residues[195,275–277] manifested high inhibiting activity towards glyceraldehyde-3-phosphate dehydrogenase from *Trypanosoma brucei* which is the cause of sleeping sickness.[278] Effective inhibition was induced even by submicromolar concentrations of the nucleosides. X-ray diffraction analysis has shown[279] that the binding of N^6,2′-modified adenosine to glyceraldehyde-3-phosphate dehydrogenase significantly changes the enzyme structure.

2. The use of 2′-modified oligonucleotides and their conjugates

a. Antisense and anti-gene biotechnologies

The main trend over the past 15–20 years was the use of modified oligonucleotides for suppression of gene expression by their interactions with cell DNA and RNA. This is achieved using antisense and anti-gene approaches and RNAi.

The antisense mechanism of oligonucleotide action implies their intracellular complexation with mRNA to yield hybrid duplexes which prevent translation due to the inhibition of mRNA binding to ribosomes,[280,281] reverse transcription [282] or processing of mRNA.[283,284] In some cases, such hybrid duplexes act as substrates for the RNAase H,[281] which catalyses hydrolysis of a ribonucleic component, and undergo 'decapitation' of the 5′-end of the RNA molecule,[285] sequence-specific cleavage of RNA [3,286–289] or covalent binding of the oligonucleotide to RNA.[290] The most recent achievements in antisense biotechnology have been described in the review.[291]

In contrast, the anti-gene mechanism entails either direct binding of a modified oligonucleotide to genomic DNA and formation of a triplex on a complementary sequence [2,292] or unwinding of the double helix and formation of a heterogeneous duplex or D-loop.[293] This results in the inhibition of transcription of the corresponding genes and an increase in the susceptibility of the DNA site to nucleases [294] and chemical reagents.[295–298] Both antisense and anti-gene mechanisms are based on enhanced affinities of modified oligonucleotides for the complementary sequence in comparison with their native counterparts.

The phenomenon of RNA interference was discovered in 1998;[299] it consists of the degradation of mRNA induced by a double-helical RNA, which is complementary to a certain region of the former, and, as a consequence, of inhibition of gene expression. Initially, extended RNA duplexes (300–1000 bp) were used for this purpose, but later it was shown [300,301] that RNA interference was induced by shorter (21–23 bp) duplexes. A strong intracellular effect is observed upon introduction of even very few copies of RNA duplexes into the cell, which can be due to their functioning in a catalytic mode. A salient advantage of short RNA duplexes is that in mammalian cells they do not elicit non-specific immune response, which involves interferon production and cell death as in the case of extended RNA duplexes. For this reason, short RNA duplexes are considered to be efficient selective inhibitors of expression of individual genes and promising candidates for medicinal drugs.

Unfortunately, even minor modifications of RNA, *e.g.*, substitution of inosine for guanosine,[302] introduction of 2′-*O*-methylated nucleotides [303] or addition of a non-complementary base pair,[304] completely abolish RNA interference. The introduction of 2′-amino-2′-deoxyuridine or 2′-fluoro-2′-deoxyuridine into the sense chain of an RNA duplex only slightly inhibits the RNA interference, whereas modification of an antisense chain is crucial.[302] Nevertheless, it was shown that modification of two 3′-terminal nucleotides is possible.[303] Studies of RNA interference in mammalian cells have demonstrated [305] that the introduction of 2′-fluoro-2′-deoxyuridine and conformationally protected 2′-*O*-4′-*C*-methylidene nucleosides into RNA duplexes has no effect on the efficiency of RNA interference. The activities of single-helical, 22-membered RNA (the so-called miRNA [306,307]) are being investigated; their mechanism of action is similar to RNA interference.[5,6,308,309]

A recently developed SELEX procedure [4,310,311] enables the selection of NA molecules with predetermined properties from combinatorial libraries containing up to 10^{25} individual molecules.[312,313] The choice of target NA molecules is based on their ability to form stable complexes with various proteins and their low-molecular-mass analogues. As a rule, these properties are inherent in RNA molecules containing 40–100 nucleotide units, termed as 'aptamers'. The introduction of modified nucleosides into the structure of aptamers [314] increases their conformational mobilities and resistance to nucleases. The structures of modified aptamers can be established using tandem mass spectrometry combined with ESI-MS electrospray ionisation.[315,316] SELEX is also used for the analysis of ribozyme libraries.[317]

The use of oligonucleotides as therapeutic drugs requires that they possess high resistance to nucleases, can efficiently be delivered to the cell, selectively bound to the targets and are free of side effects. Low cost and availability of modified oligonucleotides are no less important. Thus, the construction of efficient antisense and anti-gene agents demands simultaneous solution of many problems.

It has been shown [318,319] that inside the cell oligonucleotides are predominantly hydrolysed under the action of exogenous nucleases. Today, the problem of stability of oligonucleotides may be considered to be solved owing to the use of 2′-*O*-methylated nucleotides, conformationally locked nucleic acids [320,321] and various 3′-terminal modifications, which significantly increase the resistance of NA against nucleases.

The problem of targeted delivery of oligonucleotides to cells is much more complicated. Its solution is difficult due to the lack of clear understanding of the mechanisms of oligonucleotide penetration across cell membranes, which involves either non-specific adsorption on the surface of the cell membrane and subsequent diffusion or receptor-mediated endocytosis. In this respect, covalent [260,322] and non-covalent [323] oligonucleotide conjugates with peptides present considerable interest. No less attention is given to the use of liposomes as vehicles for targeted delivery of oligonucleotides, *e.g.*, anionic and neutral,[324,325] cationic,[326] pH-sensitive,[327] fusogenic (*i.e.*, able to penetrate through cell membranes),[328] folate receptor-targeted liposomes [329] and immunoliposomes.[330] Of interest are oligonucleotide glycoconjugates in which receptor-mediated endocytosis is effected through lectin receptors.[331] The intracellular distribution of these conjugates between the cytosol and the nucleus is determined by their interaction with intracellular lectins. Hydrophobic nanoparticles [332] and polyamidoamine dendrimers [333] are also used as cargos for oligonucleotide delivery. The transmembrane transport of thiophosphorylated oligonucleotides [334] and of hydrophobic oligonucleotide conjugates [64] has also been discussed.

The design of antisense analogues of nucleic acids allows modification of both the sugar–phosphate backbone and heterocyclic bases. 2′-*O*-Alkyloligonucleotides containing reactive groups and their conjugates with other compounds are especially widely used along with other 2′-modified oligonucleotides in antisense biotechnology. Thus it has been found [268] that the attachment of 10–15-membered peptides to the O(2) atom of the carbohydrate fragment through a linker virtually does not affect the binding of the antisense conjugate to TAR RNA. Moreover, the solution of quite a number of practical tasks demands the introduction of more complex groups into the 2-position of the carbohydrate fragment of NA. The synthesis of 2′-*O*-{2-[2-(*N*,*N*-dimethylamino)ethoxy]ethyl}oligonucleotides is an example.[69] The introduction of such a modification into the oligomer structure increases the thermal stability of duplexes with RNA due to the *gauche*-effect,[71] while the presence of a positive charge increases its resistance against nucleases.[335]

3′-Phosphoramidite derivatives of 2′-*O*-methylnucleosides are commercially available, which determines the wide application of 2′-*O*-methyl oligonucleotides with internucleotide thiophosphate and phosphodiester bonds in antisense biotechnology. High *in vitro* [336] and *in vivo* activities of such oligomers have been demonstrated in experiments on various cell lines [337–341] and in mice.[342] One of the most popular approaches [343] entails the synthesis of a chimeric oligonucleotide which is made up of 2′-*O*-alkylribonucleosides and 2′-deoxyribonucleosides linked through internucleotide thiophosphate bonds in the middle of the chain. RNA duplexes with such oligonucleotides serve as substrates for RNAase H. It should be noted that the simultaneous presence of thiophosphate groups and 2′-*O*-methylnucleotide units results in additive inhibition of gene expression.[340] A similar approach entails the activation of RNAase P.[341] This enzyme splits the 3′-terminal fragment of RNA within the duplex,[344] which occurs more selectively than traditional RNAase H.[345]

2′-*O*-Methoxyethylated RNAs are thought to be the most promising among other 2′-modified oligonucleotides.[346] It has been shown [347] that such oligomers penetrate more easily across cell membranes, are stable in blood plasma [348] and specifically inhibit gene expression.[349]

In addition, 2′-*O*-methoxyethylated RNAs are effective inhibitors of telomerase.[350] Moreover, the inhibition of 3′-polyadenylation of pre-mRNA by such oligonucleotides is the reason for the increased stability of mRNA and, correspondingly, for the hyperexpression of proteins.[351]

2′-*O*-Alkylated oligonucleotides have also found wide application in anti-gene biotechnology. It was shown that 2′-*O*-methyloligonucleotides form kinetically and thermodynamically more stable triplexes in comparison with native oligonucleotides,[352] which is due to the pre-organisation of the oligonucleotide structure, [*i.e.*, a more favourable C(3′)-*endo*-, C(2′)-*exo*-conformation of the carbohydrate fragment] and van der Waals interactions between the 2′-*O*-methyl groups and purine bases.[353] Conformationally fixed 2′-*O*,4′-*C*-methylidene oligonucleotides confer even higher stabilities on the triplexes under conditions close to physiological,[354] the best results being observed in the case of 2′-*O*-(2-aminoethyl)oligonucleotides.[355–357] NMR spectroscopic studies have shown that in this case the increased stability of the triplex is due to electrostatic interactions of the charged amino groups of the pyrimidine oligonucleotide with the phosphate groups of the opposite purine chain of DNA.[70, 358] The so-called *gauche*-effect,[359] which determines the magnitude of the torsion angles in the system X–C–C–Y (X and Y are electronegative substituents), contributes significantly to the stabilities of the triplexes. The presence of a *gauche*$^+$-effect for the 2′-*O*-aminoethyl group determines the conformation which is optimum for the interaction with the phosphate group of the opposite strand; this favours the pre-organisation of the oligonucleotide. The presence of a charged amino group is yet another factor which determines the stabilities of the triplexes at low concentrations of Mg^{2+} close to physiological;[357] this is not typical of triplex-forming oligonucleotides.[360] It should be noted that the incorporation of a large number of modified units[356] alleviates the inhibition of gene expression *in vivo*. It was suggested[357] that this is due to the increased hydrophobicity of the oligonucleotide owing to the partial neutralisation of the negative charges of phosphate groups and, as a consequence, its adsorption on cell membranes, hydrophobic proteins, *etc*. The decrease in the intracellular concentration of a modified oligonucleotide also diminishes its anti-gene effect. Recently, it has been shown[361] that such modified oligonucleotides are promising for site-specific mutagenesis *in vivo*. The incorporation of 2′-*O*-aminoethyl-5-propargylaminopyrimidine nucleosides into oligonucleotides[362] exerted an additive effect caused by the interaction of two amino groups, which provided further stabilisation of the triplexes with DNA.

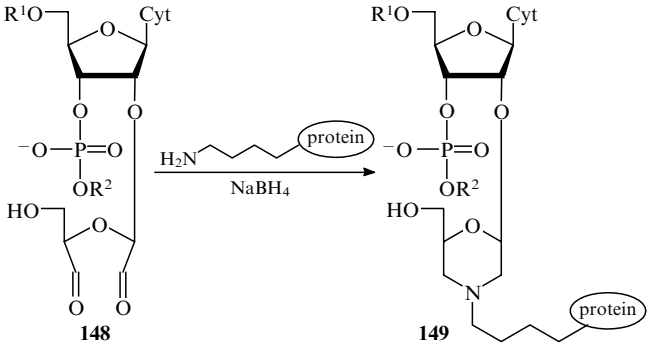
b. Studies of DNA – protein interactions

The second line of the use of modified oligonucleotides is the study of protein – nucleic acid interactions. This includes, in particular, covalent binding of nucleic acids to proteins.[142, 143, 363] The most significant information about the structures of NA – protein conjugates can be obtained by X-ray diffraction analysis,[364] however, it is not always possible to isolate sufficient amounts of specific protein complexes with DNA and/or bring about their crystallisation. Besides, the results can be wrong due to differences in the conformations of macromolecules in solution or in crystalline form. NMR analysis of the structures of NA – protein complexes is an alternative approach.[365] However, the data obtained by NMR spectroscopy are often difficult to interpret. Site-specific mutagenesis[366, 367] and chemical modification of proteins are potent methods for the study of DNA – protein interactions, however, their application demands preliminary identification of a specific site in the protein molecule responsible for the interaction with NA. The use of the 'footprinting' technique allows the identification of DNA sequences recognised by DNA-binding proteins,[368] but cannot be used for the identification of specific protein sites interacting with NA. Covalent attachment of proteins to modified nucleic acids containing reactive groups not only allows the identification of the protein site interacting with NA, but sometimes enables the determination of the amino acid responsible for the binding.[363, 369] The changes in the NA structure are minimal in this case.

Changes in the physicochemical properties of individual oligonucleotide fragments can be caused by even minor disturbances in the geometry of the molecule; the effects of modification can be determined by kinetic methods.[370]

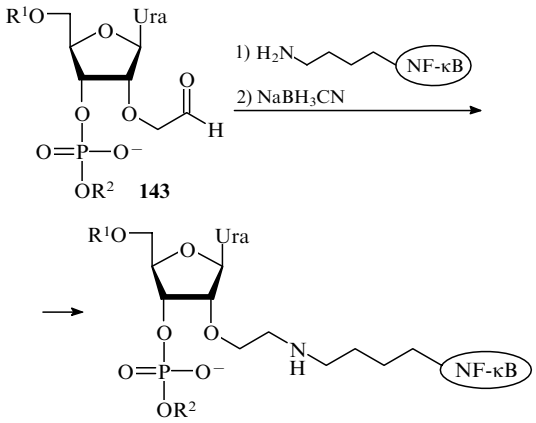
The interaction of a fragment of the Tat-protein with C(2′)-modified RNA has been studied.[258] It was shown that the conjugate **129** (see above) is formed in the reaction of the activated ester at the C(2′) atom of Urd(23) with the ε-amino group of Lys(51).

Oligonucleotides **148** containing a dialdehyde group in the 2′-position were used for the study of protein-NA interactions with restriction endonuclease *Eco*RII, DNA-methyltransferases *Eco*RII,[371] *Mva*I (see Refs 372–374) and T7 RNA polymerase.[373, 375] The use of modified oligonucleotides permits one to inhibit the enzyme activity without any effect on the ability of enzymes to bind the NA substrate. The interaction of oligonucleotides **148** with enzymes occurs in a selective manner and involves the ε-amino groups of lysine. The yields of the conjugates **149** are 3% – 5%.



R^1 and R^2 are fragments of an oligonucleotide chain.

The covalent binding of modified oligonucleotides **143** containing 2′-*O*-(2-oxoethyl)uridine to the transcription factor NF-κB has been described.[376] This modification seems to be more preferable than the dialdehyde group, since its small size favours its interaction with the ε-amino groups of lysine closest to DNA.

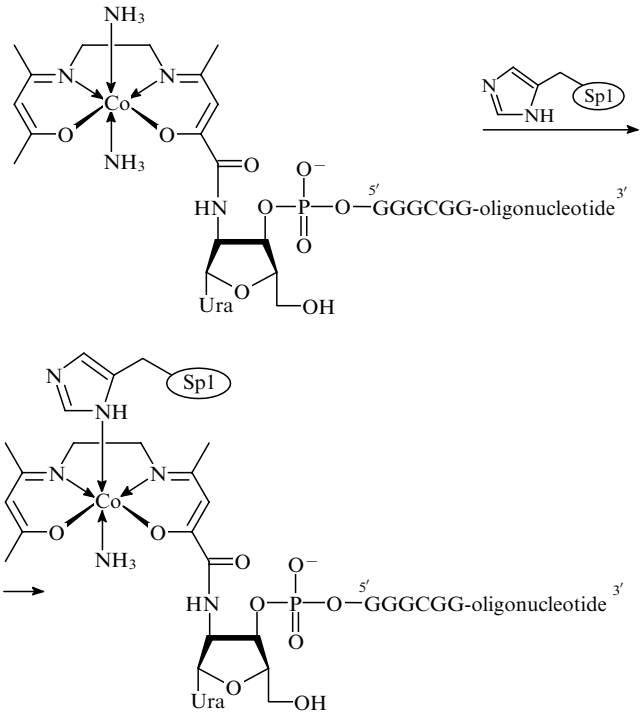


R^1 and R^2 are fragments of an oligonucleotide chain.

The covalently bound duplexes **122** and **123**[241–244] are extremely resistant against nucleases. Their interactions with the transcription factor NF-κB have been studied; the efficiency of the binding of the modified duplexes to the protein was higher than of native duplexes.[377] For a more detailed description of the synthesis and applications of covalently bound nucleic acids see Ref. 378.

Oligonucleotides **89** containing intrachain cross-links in the neighbouring nucleotide units [174, 175] have a chain kink at the modification site and structurally correspond to the U-turn of tRNA. These oligonucleotides can be used for the study of RNA – protein interactions.

It was suggested [253] that the transcription factor Sp1 could be selectively inhibited using irreversible binding of side histidine groups in the protein molecule to the cobalt atom of a metal complex covalently linked to the oligonucleotide.



Ribonucleic acids containing 2′-amino-2′-deoxyribonucleotide fragments were repeatedly used as aptamers possessing higher binding constants to various proteins and receptors than native nucleic acids. The proteolytic enzyme elastase from human neutrophils,[379] the vascular endothelium growth factor,[380] the fibroblast growth factor,[381] the protein Rev HIV-1,[382] the human γ-interferon,[383] *N*-methylmesoporphyrin (a low-molecular-mass analogue of heme-containing proteins),[384, 385] as well as insulin [386] and acetylcholine receptors [387] served as the objects of this study. After incorporation of the 2′-amino group into RNA, the ability to form hydrogen bonds remains. In addition, the presence of such modifications has only marginal effect on the local structure of RNA molecules because of the similarity of the van der Waals radii of the amino and hydroxy groups.

Oligonucleotides containing 2′-amino-2′-deoxyribonucleotide fragments were also used in the study of the role of 2′-hydroxy groups in RNA – protein interactions. The interactions of DNA with ribonuclease H1 from *E.coli*,[388] of RNA with the bacteriophage MS2 membrane protein [389, 390] and uracyl-DNA glycosylase [391, 392] have been studied. Aurup *et al.*[393] have synthesised mRNA from 2′-amino-2′-deoxyribonucleoside-5′-triphosphates and demonstrated that such an mRNA cannot be translated *in vitro* and *in vivo*.

c. Study of the structures of nucleic acids

The fluorescent properties of oligonucleotides containing a methylpyrene residue at the O(2′) atom [162] have been studied.[394 – 396] It was shown that the pyrene residue intercalates between the heterocyclic bases in a duplex with DNA as can be evidenced from the shift in its fluorescence maximum. In contrast, duplexes with RNA are characterised by strongly enhanced emission, which suggests the localisation of the pyrene residue outside the duplex. These conclusions were confirmed by the data from the thermal stabilities of the duplexes and CD spectra. In addition, the fluorescence characteristics of pyrene-containing oligonucleotides appeared to be highly sensitive to the presence of mismatches.[396] These oligonucleotides can be used in structural analysis of RNA.[397 – 399]

The emission characteristics of modified oligonucleotides where pyrene was attached to the C(2′) atom of uridine through an extended linker [170] were studied in relation to the nature of the 3′-flanking nucleotide and the presence of a complementary chain, which made it possible to determine the electron transfer energies and conformational mobilities of heterocyclic bases in various single- and double-stranded structures.

Silverman *et al.*[187, 248, 400] have used a series of modified oligonucleotides with different lengths of linkers between the pyrene residue and the C(2′) atom in order to study intron folding of the *Tetrahymena* group I P4 – P6 domain. The folding was accompanied by an increase in fluorescence intensity, which suggested changes in the environment around the modified nucleotide. It was demonstrated that the folding of RNA occurs in different ways depending on the ionic strength and pH of the solution.

The use of oligonucleotides containing 2′-*O*-(*N*-pyrenylmethylcarbamoyl)uridine has been proposed recently.[78] The spatial approximation of modified nucleosides in the complementary chains results in the formation of a pyrene excimer in the major groove of a DNA duplex.[170] The use of a linker with low conformational mobility has made it possible to prepare derivatives possessing strong fluorescence.

Nakano and co-workers [183 – 186, 235] have published a series of papers devoted to the synthesis of oligonucleotides containing various fluorescent labels at the C(2′) atom. Hybridisation of oligonucleotides containing a 4-(*N*,*N*-dimethylamino)phenylsulfamide group with a complementary DNA chain was accompanied by an increase in fluorescence intensity.[184, 185] The fluorescent properties of oligonucleotide duplexes containing fluorophore pairs (dansyl – fluorescein and coumarin — fluorescein) in the opposite chains have been studied.[186, 235] Unfortunately, in the former case the quantum yield of the energy transfer was too low, which excludes the use of such oligonucleotides for fluorescence resonance energy transfer (FRET). The quantum yield for the coumarin – fluorescein pair amounted to 25%; the quenching of the fluorescence of the energy donor was observed only in the case where the acceptor was conjugated with a complementary oligonucleotide.

Electrochemical detection of the DNA structure is a promising approach to the study of mononucleotide polymorphism.[401 – 403] A series of patents [404, 405] and papers [159, 406, 407] devoted to different applications of modified oligonucleotides containing 2′-*O*-ferrocenylalkylnucleoside fragments for the electrochemical detection of mononucleotide polymorphism in DNA fragments have been published. DNA chips and devices based on them are now commercially available (eSensor™, eSensor™ 4800 Reader, Motorola Clinical Micro Sensors, USA). For a more detailed description of the synthesis and properties of ferrocene-containing oligonucleotides see Ref. 403.

The introduction of the anthraquinone residue at the C(2′) atom [164] increases the thermal stabilities of duplexes of modified oligonucleotides with DNA and RNA.[408] ^{1}H NMR spectroscopic studies have shown [409] that the anthraquinone residue linked to the C(2′) atom intercalates between heterocyclic base pairs. This property was used in the study of electron transfer mechanisms along the DNA chain.[165] In further studies, oligonucleotides containing 2′-*O*-anthraquinonylmethyluridine were used in development of a novel type of DNA sensor.[410, 411] The efficiency of the electron transfer was shown to increase drastically upon formation of a duplex between the anthraquinone-containing oligonucleotide immobilised on an electrode and the NA target.

Whittemore *et al.*[171, 412] have used oligonucleotides containing an anthraquinone residue attached through a 2′-*O*-aminohexyl linker for electrochemical detection of complementary NA mole-

cules on the electrode surface with the help of cyclic voltammetry. The use of a dropping mercury electrode [171] did not reveal any steady dependence of the voltammetric characteristics on the concentration of the NA under study, which can be attributed to the non-specific adsorption of the oligonucleotide on the mercury surface. In further studies,[412] an oligonucleotide containing a 5′-terminal thiol group was immobilised on the surface of a gold electrode. To minimise non-specific adsorption, the electrode surface was treated with 2-mercaptoethanol after which a complementary oligonucleotide containing an anthraquinone residue was added and the detection was begun. If is of interest that the UV spectroscopic data exclude intercalation of the anthraquinone residue between heterocyclic base pairs.[412]

Oligonucleotides containing a ruthenium complex at the C(2′) atom [206] were suggested as models for the study of electron transfer along the DNA chain. However, it was found that the oxidation of the guanosine residue within the duplex occurs in very low yields.

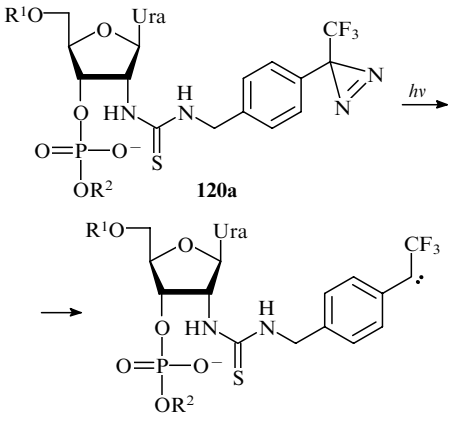
Recently published reviews are devoted to the use of thiol-containing oligoribonucleotides for the study of structures and functions of ribozymes [413] and the three-dimensional structure of RNA.[414, 415]

Sigurdsson *et al.*[238] unambiguously chose one of the two tertiary structures of a ribozyme containing covalently bound domains which have earlier been deduced from X-ray diffraction analysis data. This approach was further used to prove proximity of two domains of a hairpin ribozyme.[416]

A study of the formation of disulfide cross-links between modified oligonucleotide chains [261] has made it possible to estimate the rates of the thermal motions of structural elements of the ribozyme *Nhe*I. In further studies, the switch-over of the nuclease activity of ribozyme HHα10 to a ligating activity could be achieved through the use of similar modifications.[417, 418] A considerable increase in the ligation rate, which was not accompanied by changes in hydrolysis rates, was attributed to a sharp decrease in the thermal motions of the ribozyme domains,[417] structural changes in the catalytic site of the enzyme [418] and, as a consequence, in arrangement of the 2′-hydroxy and 3′-phosphate groups of the substrate.

Glick and co-workers [105, 106] have used disulfide bond formation between thiol-containing oligonucleotides for the study of the spatial structure of $tRNA^{Phe}$. It was found that the effects of covalent cross-links on the three-dimensional structure of tRNA are minimum. The kinetics and thermodynamics of the folding of $tRNA^{Phe}$ upon cleavage of disulfide cross-links due to thiol–disulfide exchange were studied.[419]

Modified 5S rRNA **120a**, which contains a photochemically active aryl(trifluoromethyl)diazirine group,[236] was used in the study of the spatial environment of 5S rRNA within the ribosome.



R^1 and R^2 are fragments of an oligoribonucleotide chain.

Upon UV irradiation, diazirine is converted into a highly reactive carbene which attacks even aliphatic C—H-bonds.[420] An analysis of the reaction products has made it possible to establish several contacts between 5S rRNA and 23S rRNA.

Buchmueller *et al.*[421, 422] have used oligoribonucleotides containing 2′-(4-azido-2-hydroxybenzamido)-2′-deoxyuridine for detecting non-equilibrium structures formed upon folding of RNA.

An interesting application for oligonucleotides containing pyrimidine 2′-amino-2′-deoxyribonucleosides has been proposed by Weeks and co-workers [249–251] who have demonstrated that the acylation rate of the 2′-amino group depends on the conformational mobility of the modified nucleotide fragment. This is maximum for a single-stranded NA and minimum for an ideal duplex. The kinetic and thermodynamic parameters of the acylation with the biotinylbutyric acid *N*-hydroxysuccinimide ester were established for all possible nucleotide pairs in DNA–DNA and DNA–RNA duplexes.[250, 251] The data obtained were used in further studies of the local structure of $tRNA^{Asp}$ (see Ref. 249) and detection of mononucleotide polymorphism.[251] It was shown in particular that the acylation rate of a 2′-amino-2′-deoxyribonucleoside incorporated into the anticodon loop of $tRNA^{Asp}$ (see Ref. 249) exceeds that of nucleosides involved in complementary interactions or representing elements of the tertiary structure.

It has been shown [423] that the acylation rate of the 2′-amino group is determined not only by the local conformational mobilities of oligonucleotides, but also by the chemical nature of the vicinal groups in the 3′-position. The reaction rate drops drastically in the presence of a phosphotriester group in the 3′-position of the 3′,5′-cyclophosphate group or upon substitution of threofuranose for the ribose residue. This suggests the involvement of the 3′-phosphate or the 3′-hydroxy group in the catalysis of the acylation reaction. The results obtained and the data from computer simulation prompted a conclusion on a possible catalytic role of the 2′-hydroxy group in the formation of a peptide bond in the peptidyl site of the ribosome.

Sigurdsson *et al.*[424, 425] have studied the effects of metal ions on the structure and interactions of modified TAR RNA with different fragments of the Tat-protein using RNA carrying a spin label in the 2′-position.[237] ESR studies have shown that the substitution of sodium ions for calcium ions does not influence the structure of TAR RNA, but weakens the interaction of a fragment of a mutant Tat-protein with definite nucleotide fragments. The introduction of several spin labels into the TAR RNA [426] enabled the investigation into the interactions of the latter with dyes, intercalators and antibiotics and the determination of binding sites for these molecules. In further studies, this modification was used for measuring the distances in double-helical RNA using ESR spectroscopy.[427]

d. Ribozymes

Ribozymes are short-chain RNA, which catalyse the sequence-specific cleavage of RNA, ligation and some other processes.[3] The hammerhead ribozyme is the best studied.[428–430] In this Section, we consider ribozymes that incorporate nucleosides containing reactive groups in the 2′-position.

The incorporation of 2′-amino-2′-deoxyribonucleosides,[228, 431–437] 2′-thionucleosides,[433, 435] 2′-aminooxynucleosides,[86, 433, 435] 2′-*O*-methyl- and 2′-*O*-allylnucleosides [438] into definite positions of the ribozyme significantly increases the resistance of RNA against nucleases with simultaneous preservation of, or a small decrease in, the catalytic activity. Similar results were obtained for the DNAzyme.[439]

However, it was found [440] that the catalytic activity of a ribozyme decreases significantly as a result of incorporation of certain substituents into the 2′-position. This effect does not depend on the size of the group incorporated, but rather is determined by the nature of the bond at the C(2′) atom. Some modifications, *e.g.*, 2′-acylamido and 2′-alkylcarbamoyl groups presumably prevent conformational changes in the ribozyme molecule in the catalytic act. This is confirmed by the data [441] on the lack of catalytic activity of a hammerhead ribozyme contain-

ing 2′-acetamido groups and the data of Beigelman *et al.*[193] on a substantial decrease in the catalytic activity upon acylation of 2′-amino-2′-deoxyuridine with amino acids (alanine, phenylalanine, lysine and asparagine).

According to Hamm *et al.*,[442] cleavage of an RNA substrate containing 2′-thiocytidine instead of native cytidine by the ribozyme dHH16 proceeds *via* an intermediate 1′,2′-episulfide by a mechanism similar to hydrolysis in an alkaline medium.[263, 264] Under these conditions, the cleavage rate of modified RNA decreases by two orders of magnitude in comparison with native oligoribonucleotides. Further studies of the properties of oligonucleotides containing 2′-thiocytidine units revealed[443] that this reaction requires the presence of manganese ions. Moreover, it does not occur in the absence of oxygen or in the presence of free radical scavengers in the reaction mixture.

The incorporation of several 2′-thiocytidine-5′-phosphate units into the ribozyme fully suppressed its catalytic activity.[444]

Schwans *et al.*[445] have synthesised a modified *Tetrahymena* group I intron P4–P6 domain using α-thiotriphosphates of 2′-thionucleosides. Isolation of RNAs, which differed from the native fragment in gel-electrophoretic mobilities, and subsequent cleavage of the thiophosphate groups in the modified RNAs by iodine has made it possible to determine the positions of 2′-hydroxy groups in the domain essential for its folding.

Stromberg *et al.*[446–448] have studied self-splicing of the intron IA2 of pre-mRNA formed during the expression of the gene *nrdB* of the bacteriophage T4. Attachment of guanosine to the 5′-end of the intron occurs, which was accompanied by cleavage of RNA with subsequent elimination of the guanosine residue from the intron sequence in the presence of metal ions. When 2′-amino-2′-deoxyguanosine was used as a cofactor instead of guanosine, the splicing rate decreased 20–25-fold. An analysis of the dependence of the cleavage rate of guanosine on pH, concentration and the nature of bivalent metal ions has made it possible to reveal the interactions of metal ions with 2′-hydroxy and 2′-amino groups. Similar studies of catalytic activities of *Tetrahymena* RNA have shown[449, 450] that the 2′-amino group and the bivalent metal ion compete for the same 5′-phosphate group in the active site of the ribozyme.

The modified ribozymes currently available represent complex structures which contain simultaneously 2′-*O*-methyl nucleosides, 2′-amino-2′-deoxyuridine, 2′-fluoro-2′-deoxynucleosides and thiophosphate internucleotide bonds.[451] The introduction of bulky substituents into the 2′-position significantly decreased the ribozyme activity;[229, 440] therefore, the use of such constructions seems to hold little promise.

V. Conclusion

At present, the synthesis of oligonucleotides containing reactive groups in the 2′-position and of their conjugates is a promising approach to targeted changes in the physicochemical and substrate properties of nucleic acid fragments. Among modified nucleosides, 2′-amino-2′-deoxyribonucleosides are especially popular. These can easily be incorporated into NA by chemical or enzymatic methods and then selectively acylated. This circumstance has determined the commercial availability of the pyrimidine 2′-amino-2′-deoxyribonucleoside phosphoramidite derivatives and 5′-triphosphates (TriLink BioTech, USA).

The methodology of preparation of modified oligonucleotides where the molecules or reactive groups are linked to the C(2′) or O(2′) atom has been developed fairly well. A vast number of publications are devoted to the synthesis of oligonucleotides containing nucleophilic groups, since in the majority of cases the corresponding phosphoramidite derivatives are commercially available.

Conjugates containing an amide bond are prepared by activation of the carboxy group using methods originally developed for peptide synthesis where the concentration of a compound containing a carboxy group exceeds that of the amino-containing component by an order of magnitude. It therefore seems expedient to perform the reactions of oligonucleotides bearing amino groups with an excess of carboxyl-containing compounds. Moreover, the presence of an amino group in the oligonucleotide chain fits well into the protocol of oligonucleotide synthesis, while the carboxy group needs to be deblocked prior to standard post-synthetic treatments.

Modified oligonucleotides where the thiol groups are attached to the O(2′) atom through various linkers are readily accessible and are used for the preparation of conjugates with a disulfide bond.

The reason for the rare employment of oligonucleotides containing an aldehyde or an oxo group is unclear. The synthesis and applications of aldehyde-containing oligonucleotides have been documented only in the past decade.[109] A commercially available modified polymeric carrier (Glen Research) for the synthesis of oligonucleotides with an aldehyde group at the 3′-end, has become popular. A 4-formylbenzoic activated ester designed for the incorporation of the aldehyde group into oligonucleotides containing an aliphatic amino group has recently become available (Trilink Biotech, USA).

These data altogether suggest that the aforementioned methods for the preparation of conjugates in solution and on polymeric supports are nearly equally efficient. In choosing a method for conjugation in solution, special attention should be given to the solubility of the reaction components and selectivity of the reaction, while the substances to be attached to the oligonucleotide should be easily separated from the reaction products by HPLC or gel electrophoresis. In the case of a solid-phase version, the bond formed and the molecule attached should retain stability under the conditions of post-synthetic treatments. The conjugation of nucleosides is less important but nevertheless promising in the synthesis of compounds carrying various reporter groups.

Analysis of the publications devoted to the use of 2′-modified oligonucleotides has demonstrated significant progress in many fields of molecular biology, antisense and anti-gene biotechnology and enzymology.

The authors thank Drs. A Yu Tomashevsky (University of Virginia, Charlottesville, VA, USA), O Yu Smirnova (German Cancer Research Centre, Heidelberg, Germany) and D A Stetsenko (Medical Research Council, Cambridge, UK) for their valuable assistance in the preparation of this review for publication. This review has been written within the framework of the Programme ‘Universities of Russia’ (Project No. UR.05.03.010) and with the financial support of the Wellcome Trust (Grant No. CRIG 069419) and the Russian Foundation for Basic Research (Project No. 03-04-48957).

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