Russian Chemical Reviews **80** (5) 429 – 452 (2011) © 2011 Russian Academy of Sciences and Turpion Ltd

DOI 10.1070/RC2011v080n05ABEH004188

Synthesis and properties of carbohydrate – phosphate backbone-modified oligonucleotide analogues and nucleic acid mimetics

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Abstract. Advances in the synthesis of oligo(deoxy)ribonucleotide analogues and nucleic acid mimetics made in the last decade are summarized. Attention is focused on new methods for the synthesis of derivatives with a modified ribose – phosphate backbone (phosphorothioate, boranophosphate, and nucleoside phosphonate derivatives) and derivatives devoid of the phosphate group. Among nucleic acid mimetics, conformationally restricted modified peptide nucleic acids, including those bearing a negative or positive charge, and morpholino oligomers are considered. Advantages and drawbacks of the main types of analogues as regards the complexity of the synthesis and the possibility of their application as antisense agents or reagents for hybridization analysis are compared. The bibliography includes 282 references.

I. Introduction

Advances in molecular biology at the end of the 20th century resulted in the wide use of oligodeoxyribo- and oligoribonucleotides and their derivatives as efficient investigation tools. In medical practice, the application of oligonucleotides is currently limited mainly to the purposes of the diagnosis. However, attempts to design drugs for the correction of expression of particular genes are still made. As a result of the progress achieved in the 1980s, synthetic chemists faced two challenges. First, there is a need in efficient preparative methods for the synthesis of oligonucleotides in amounts sufficient for their practical use. Second, there is a necessity to find synthetic analogues free of drawbacks inherent to natural nucleic acids (NA), such as low cell membrane permeability and fast *in vivo* enzymatic degradation.[1, 2]

Along with the development of methods for the synthesis of natural oligodeoxyribo- and oligoribonucleotides,[3] chemists made efforts to prepare modified oligomers, which, on the one hand, would retain the ability to maintain complementary interactions between heterocyclic bases and, on the other hand, would acquire new properties, for example, the electroneutrality (for the penetration through lipid membranes) or resistance to nucleases. Copolymers of polyvinyl alcohols,[4] polyacrylate,[5] or polyacrylamide[6] and uracil, adenine, cytosine, or hypoxanthine were synthesized and characterized. More recently, short oligodeoxynucleotide analogues containing carbonate, carbamate, oxyacetic ester, or oxyacetamide linkages between nucleosides instead of the phosphate group have been synthesized and investigated.[7]

In the early 1990s, the most probable mechanisms of the therapeutic action of oligonucleotides were determined. These are the cleavage of complementary template RNA with RNase H, the blockage of RNA processing, RNA reverse transcription or translation through the formation of stable heteroduplexes, and the regulation of DNA transcription by triplexes or through the insertion into complementary DNA duplexes. Other practical applications of oligonucleotides and their synthetic analogues in molecular biology studies are extensively investigated.[8] The following main requirements on physicochemical properties of these compounds were formulated: stability in biological media and in cells and the ability to penetrate cell membranes and form stable complementary complexes with nucleic acids.[9, 10]

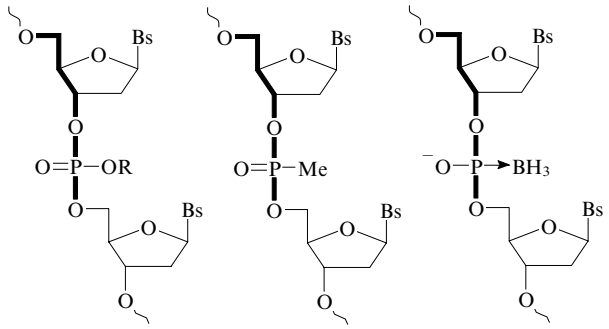
The required physicochemical properties of oligonucleotide analogues (OA) can be achieved primarily by modifying the sugar – phosphate backbone of oligomers. Phosphotriester† (**1**),[11] methylphosphonate (**2**),[12] borano-

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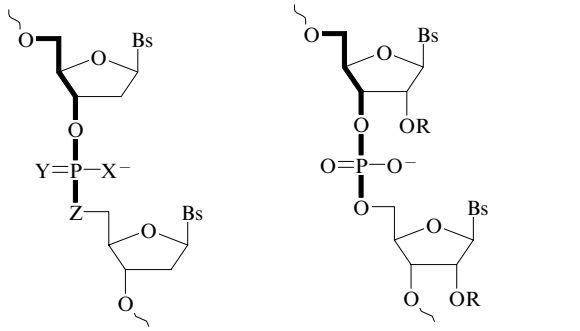
Received 10 October 2010
Uspekhi Khimii **80** (5) 452 – 476 (2011); translated by T N Safinova

† Hereinafter, the bond sequence in modified moieties is indicated by half-thick lines, Bs stands for the purine or pyrimidine base.

phosphate (**3**),[13] phosphoromono(di)thioate (**4**),[14] and 2′-O-substituted (**5**)[15] oligonucleotides, as well as other related compounds, which are currently assigned to first- and second-generation OA,[16] were synthesized.



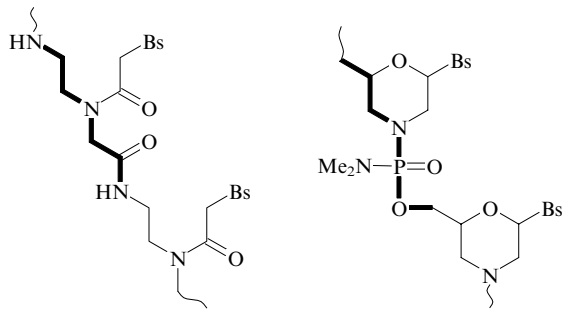
1 (R = Me, Et...) **2** **3**



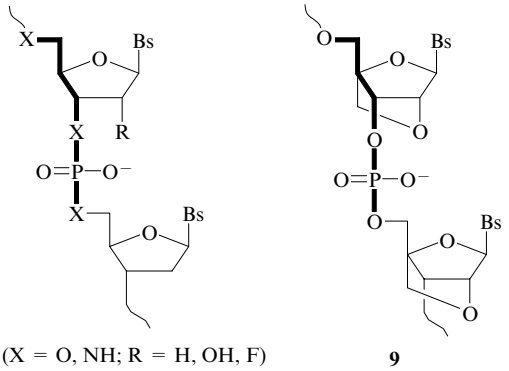
4 (X, Y, Z = O, S) **5** (R = H, Me, F, OCH_2CH_2OMe)

Advances in the use of first- and second-generation OA in the antisense approach to the gene expression regulation are summarized in the monograph.[17]

More recently, third-generation analogues, such as peptide nucleic acids (PNA, **6**), as well as morpholino (**7**), 3′(5′)-*N*-phosphoramidate (**8**), and conformationally restricted (**9**) oligonucleotides, have been synthesized.



6 **7**



8 (X = O, NH; R = H, OH, F) **9**

Some of the resulting oligomers (for example, macromolecules **6** and **7**) do not contain the sugar–phosphate backbone and retain only the sequence of heterocyclic bases, which can be involved in complementary interactions and are linked by a particular (regular) number of covalent bonds. It is more correct to refer to such oligomers as nucleic acid mimetics. The data on the synthesis and properties of third-generation OA and nucleic acid mimetics characterized up to the early 21st century are considered in the reviews.[18–23]

In spite of a large number of the already available various derivatives, in which the sugar–phosphate backbone of natural oligo(deoxy)ribonucleotides was modified, the synthesis of these compounds continues to attract interest. This is to a large extent due to the high level of the development of molecular biology and, as a consequence, an extension of the range of problems for the solution of which OA may be useful.[24–28] Examples of the application of these compounds in molecular biology and medicine are presented in the recent monograph.[29]

The present review summarizes the data obtained primarily in the last decade and concerning the design, synthesis, and properties of ribose–phosphate backbone-modified oligonucleotide analogues and nucleic acid mimetics. Attention is focused on new methods for the synthesis (including preparative) of these compounds, as well as on their physicochemical and biological properties. In some cases, these properties are unique, for example, some of these compounds combine excellent hybridization and discrimination characteristics in complexes with nucleic acids with high solubility in water and biological stability, as well as the ability to penetrate cell membranes. It should be noted that approaches to the solution of the problems associated with the application of natural and modified oligonucleotides in medicine and, particularly, the problems associated with the delivery of therapeutic oligonucleotide agents to the sites of action in cells are extensively studied. One of approaches involves the design of oligo(deoxy)ribonucleotide conjugates with different carriers, such as peptides, lipids, or nanoparticles.[30–33] However, oligo(deoxy)-ribonucleotides modified in such a way are beyond the scope of the present review.

II. Oligonucleotide analogues with modified internucleoside linkages

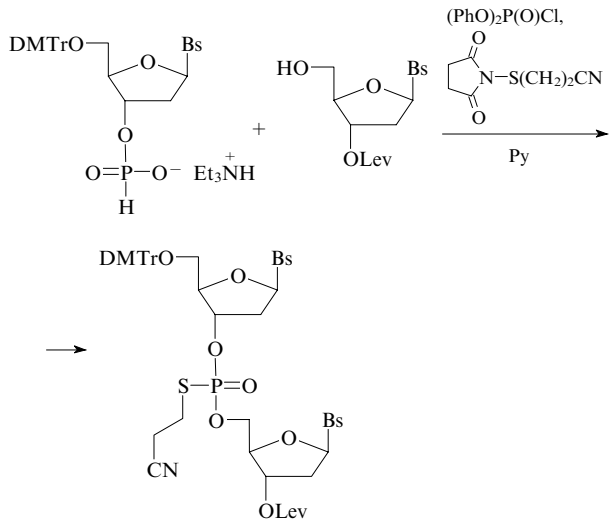
1. Negatively charged analogues

Nowadays, more than 40 antisense oligonucleotides and their analogues are at different stages of preclinical and clinical trials, more than a half of them belonging to phosphorothioates **4** (Y = S; X = Z = O).[27] Since there is a need in large amounts (up to hundreds of kilograms[34]) of phosphorothioate oligonucleotide analogues, investigations on the optimization of methods for their synthesis are underway. In the review,[3] it was mentioned that, in spite of advances in solid-phase synthesis of phosphorothioate OA,[35] a combination of methods for the synthesis in solution and on solid support will be apparently optimal for the production of these derivatives in preparative amounts.

The drug Vitravene™, which is the only oligodeoxynucleotide approved by the US Food and Drug Administration (FDA), is a 21-mer phosphorothioate. This compound was synthesized[34] in solution by the modified H-phospho-

nate method. In the first step, four different protected trinucleotides were synthesized. To improve the stability of the internucleoside H-phosphonate diester group and increase the yield of intermediate di- and trimers, the scheme of the four-component condensation was developed (Scheme 1).

Scheme 1



DMTr is 4,4′-dimethyltrityl, Lev is $C(O)(CH_2)_2Ac$, Py is pyridine.

The oxidation of P–H bonds in the course of condensation with the simultaneous protection of the resulting phosphothiodiester group substantially simplified the synthesis of dimers and trimers in high yields characteristic of the synthesis of oligo(deoxy)ribonucleotides on a solid support without the use of a large excess of the nucleotide component. As the oligomer chain is further increased with the use of trinucleotide units, the yields of the products are 92%–94% per step up to the pentadecamer and 88%–89% for the 18- and 21-mer analogues. The authors of the cited study mentioned that the above-described protocol, including the condensation, deprotection, and purification of phosphorothioate oligodeoxyribonucleotides, can easily be scaled up and optimized, which is of great practical importance.

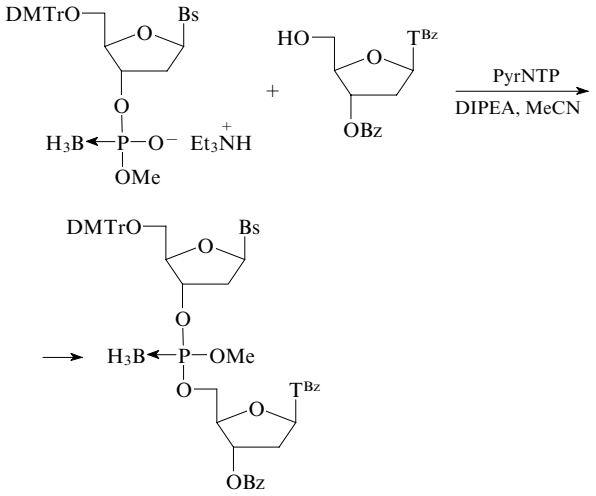
Phenylacetyl disulfide,[35] 3-amino-1,2,4-dithiazole-5-thione,[36] and *N*,*N*-dimethyl thiuram disulfide [37] were suggested for use as new reactants for the oxidation with the aim of increasing the yield and purity of the final products for the automated solid-phase synthesis of phosphorothioates by the phosphoramidite method. Besides, a new procedure was developed for the attachment of the purine nucleoside to a support,[38] and a new versatile linkage for the solid-phase synthesis was described.[39]

The phosphorus atom in phosphorothioate OA is chiral and the properties of individual diastereomers of these compounds are substantially different.[40] Recently, a new method has been developed for the stereospecific formation of phosphorothioate internucleoside linkages in oligodeoxyribonucleotides, which made it possible to exclude the separation of diastereomers of phosphorothioates.[41] This method is based on the intramolecular catalysis by the 4-methoxy-1-oxido-2-picolyl group protecting the phosphate moiety. Earlier, this approach has been successfully used in the phosphotriester method for the synthesis of natural oligodeoxyribonucleotides.[42] In the study,[43] a similar approach was applied to the solid-phase synthesis of natural and 2′-modified oligoribonucleotides, including the stereospecific synthesis of their phosphorothioate analogues. Recent advances in the stereospecific synthesis of phosphorothioate OA are summarized in the review.[44]

It should be noted that phosphorodithioate OA **4** (X = Y = S, Z = O) containing unesterified sulfur atoms have been synthesized rather long ago, and their properties are well known,[45] whereas 5′-S-phosphorodithioates **4** (Z = S) containing one of the sulfur atom as a linkage group have been described only recently.[46] Polish researchers synthesized dithymidine and cytidine thymidine dinucleotides **4** (X = Z = S, Y = O; commonly denoted as d[N*p*(*s*)*s*T)] and separated their diastereomers. The stereochemistry of individual diastereomers was proposed [46] based on the empirical rules [47] for the assignment of the absolute configuration at phosphorus in phosphorothioate derivatives. Tetra- and decathymidylates containing a modified linkage in the middle of the chain were synthesized.

Among first- and second-generation OA, boranophosphate OA **3** have attracted great attention in recent years because these compounds can be used in boron neutron capture therapy for cancer treatment.[48,49] Earlier, these compounds have been synthesized by the phosphoramidite and H-phosphonate methods; however, the oxidation of intermediate phosphite in the presence of borane afforded a large number of by-products.[50] In the study,[51] the phosphotriester approach was used for the synthesis of boranophosphate OA (Scheme 2). In this case, the borane group is involved in the phosphorylating agent.

Scheme 2



T^{Bz} is 3-benzoylthymidine, PyrNTP is 3-nitro-1,2,4-triazol-1-yl-tris-(pyrrolidin-1-yl)phosphonium hexafluorophosphate,
DIPEA is diisopropylethylamine.

Japanese researchers recommended also additional protecting groups for thymidine and deoxyguanosine and optimized the deprotection procedure. During further improvement of the procedure, this research group performed the solid-phase synthesis of boranophosphate deoxyoligomers by the phosphotriester method. The developed procedures were then used for the synthesis of oligoribonucleotides.[52,53] It should be noted that boranophosphate OA can serve as precursors of H-phosphonates, because the borane group is easily removed in the presence of Lewis acids. The further oxidation of such H-phospho-

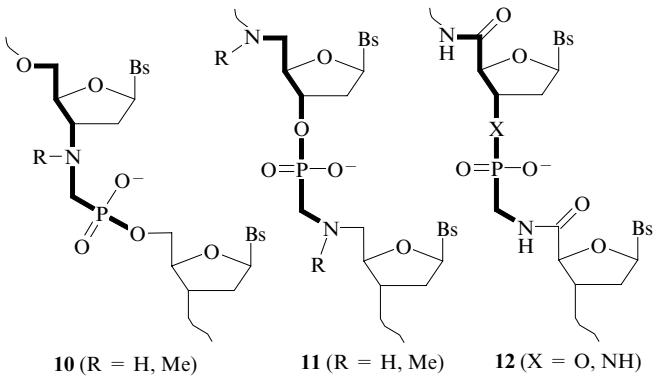
nates affords other oligonucleotide derivatives substituted at the phosphorus atom, such as phosphotriesters, phosphorothioates, and phosphoramidates.[54]

Boranophosphate OA **3** also contains a chiral phosphorus atom. Hence, the development of stereospecific methods for the synthesis of individual diastereomers and investigation of their properties are important problems. Recently, two methods have been developed for the stereocontrolled formation of the boranophosphate linkage by the stereospecific reaction of the optically pure 3′-oxazaphospholidine derivative of a nucleoside with a hydroxy-containing component in the presence of a weakly nucleophilic acid catalyst. One method involves the stepwise introduction of borane (phosphoramidite synthesis).[55] In another method, borane is introduced in the last step by boronation of optically pure intermediates having H-phosphonate internucleoside linkages.[56]

The method for the synthesis of phosphoramidate OA **8** of the deoxyribo and ribo series was developed in the 1990s based on the corresponding phosphoramidite derivatives of nucleosides and was performed in the 5′→3′ direction.[21] It was found that the introduction of 3′-N−P−5′-O linkages into oligo(deoxy)ribonucleotides leads to substantial stabilization of the corresponding complementary complexes, particularly in the case of oligoribo derivatives (R = OH).[57] Nowadays, phosphoramidates OA[58] and their thio analogues[59] are extensively studied as specific telomerase inhibitors with the aim of suppressing tumour growth. The data on the use of phosphoramidate OA **8** as broad-spectrum antitumour agents are summarized in the review.[60]

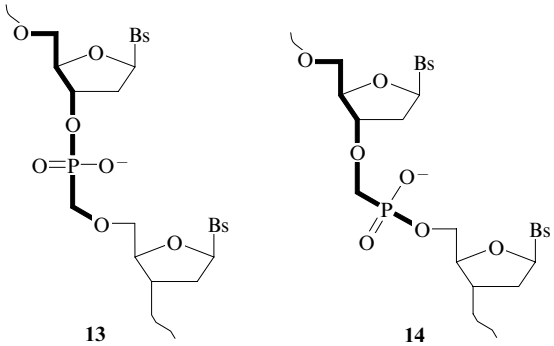
In addition to the well-studied first- and second-generation OA, numerous compounds containing phosphonic groups instead of the phosphoric acid linkage between (deoxy)ribonucleosides in natural nucleic acids were synthesized. As opposed to first-generation methylphosphonate analogues **2**, these compounds retain the negatively charged ribose–phosphate backbone of oligomers, resulting in an increase in their solubility in water, and these compounds are resistant to nucleases and generally do not contain additional diastereomeric centres. These oligomers can be synthesized from monomers by the well-known methods based on the condensation of natural nucleotides.

For example, new 3′- and 5′-aminomethylphosphonic oligomers **10** and **11** and 5′-carboxamidomethylphosphonic thymidine oligomers **12** were synthesized[61] by the triester condensation using intramolecular nucleophilic catalysis.[42]



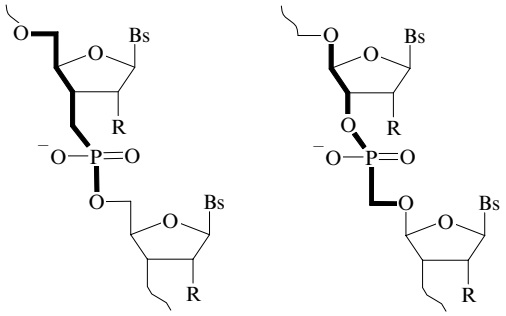
10 (R = H, Me) **11** (R = H, Me) **12** (X = O, NH)

A versatile scheme was developed for the synthesis of 5′-O- and 3′-O-phosphonomethylene derivatives **13** and **14** of all four deoxyribonucleotides.[62] The triester condensation was proposed for the synthesis of oligomers. To perform this synthesis, the 4-methoxy-1-oxido-2-picolyl protecting group was introduced into the phosphonic acid group.



13 **14**

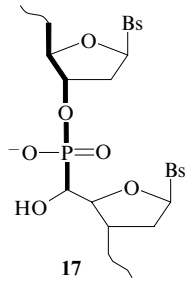
In addition to nucleoside phosphonate analogues with the repeating sugar–phosphate backbone consisting of seven covalent bonds (compounds **10**–**14**), oligomers **15** and **16** with a sequence of six covalent bonds were documented.[63]



15 (R = H, OH) **16** (R = H, OH)

In the study,[63] OA **15** were synthesized with the use of 2′-substituted 3′-C-methylene-H-phosphonate monomers. Apparently, the introduction of the methylene group into oligomers **5** will enhance enzyme stability and improve bioaccessibility of 2′-substituted analogues**.** The key step of the synthesis involves the coupling of 3′-iodomethyl derivatives with a phosphinic acid salt by the Arbuzov reaction. Then nucleoside 3′-methylene-H-phosphonates were transformed into the corresponding phosphonamidites in four steps. Oligomers **15**, which were synthesized with the use of 3′-C-methylene-H-phosphonates and contained several modified units, have a high affinity for complementary RNA and are resistant to nucleases. It was expected[63] that the use of phosphonamidites in the solid-phase method would increase the efficiency of the synthesis of modified oligonucleotides.

Czech researchers[64,65] synthesized monomers and the corresponding 5′-α-(OH)C-methylene-containing oligomers **17** with the repeating unit consisting of five covalent bonds.



17

However, in the general case a decrease in the length of the repeating unit to five bonds leads to destabilization of complementary complexes.

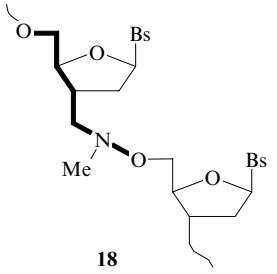
In further investigations, this research group focused their efforts on the development of a method for the synthesis of monomers and oligomers of nucleoside phosphonic acids **16**. The internucleoside linkage was attached to the carbon atom at position 4′ of deoxyribose in two possible epimeric configurations. L-*Threo*- and D-*erythro*-2′,5′-dideoxy-4′-phosphonomethoxythymidines[66] and oligomers **16** based on these compounds were synthesized. However, the hybridization properties of new oligothymidylates were worse than those of natural compounds.[67] Studies on the design of new promising nucleoside phosphonate oligomers, including those of the ribo, xylo, and arabino series, with the use of this approach are underway.[68–71]

On the whole, it should be noted that the synthesis of monomers for nucleoside phosphonate OA involves many steps and is cumbersome. Oligomers **15** isosteric with natural nucleic acids seem to be the most promising nucleoside phosphonate OA although 3′-iodomethyl nucleosides serving as key compounds for the synthesis of monomers in these processes are difficult to synthesize.

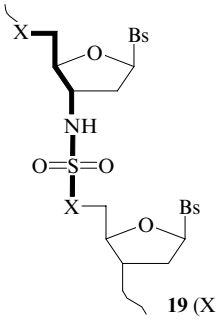
2. Nonionic analogues of oligonucleotides

As has already been mentioned in early studies with nucleic acids and their analogues, due to the polyanionic nature of nucleic acids the latter cannot penetrate cell membranes without assistance of special receptor proteins, whereas uncharged analogues easily penetrate into cells, for example, *via* diffusion.[72] As a consequence, uncharged OA, such as methylphosphonates **2** or phosphotriesters **1**, have come to the attention of chemists long ago. However, because of the low stability of complementary complexes of these compounds and the presence of an additional diastereomeric centre, these compounds were of little use in biological studies. In recent years, new types of uncharged OA devoid of additional chiral atoms and containing different internucleoside linkages have been synthesized.

Dimers with a methylene-*N*-methylimine internucleoside linkage were synthesized,[73,74] and these compounds were used for the preparation of oligomers **18** of the deoxyribo and ribo series. The thermal stability of complementary duplexes containing several modified linkages in one of the chains was investigated, and the structures of the complementary DNA/RNA dodecamer duplex with one modified linkage were studied in detail by NMR spectroscopy. Based on the results of these studies, it was concluded that the formation of DNA/RNA duplexes with the participation of modified oligomers is a favourable process.



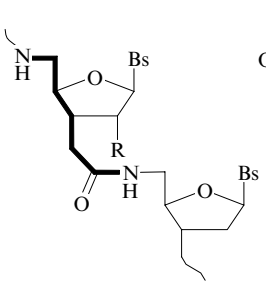
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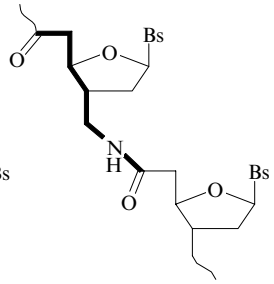
19 (X = O, NH)

Sulfamide and sulfamate derivatives of the dimers T*s*T and 2′-F-U*s*T and deoxyoligomers **19** having modified internucleoside linkages (up to five modified units) were described.[75–77] Since complementary complexes containing such linkages proved to be thermally stable, sulfamate derivatives deserve further investigation.

After the discovery of the special role of RNA oligomers in the regulation of gene expression,[78] chemists have paid great attention to the synthesis of modified RNA derivatives. Along with the earlier prepared OA of the deoxy series **20** and **21** (R = H) containing the methylenecarboxamide group instead of the phosphodiester linkage,[79] dimers of the ribonucleosides adenosine and uridine **20** were synthesized (R = OH).[80]



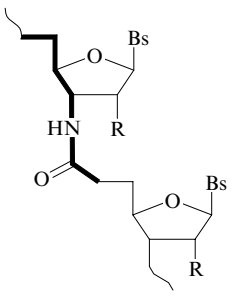
20 (R = H, OH)



21

3′-C-Methylenecarboxylated uridine and adenosine are produced by the reaction of 3′-keto derivatives of nucleosides with [(ethoxycarbonyl)methylene]triphenylphosphorane followed by the stereoselective reduction of the double bond. 3′-C-Methylenecarboxy-5′-N-amide dimers were synthesized by the condensation of carboxylated monomers with 5′-aminonucleosides in the presence of dicyclohexylcarbodiimide (DCC) or through the formation of activated *p*-nitrophenyl ethers.

The conditions of the reaction of keto derivatives of nucleosides with [(ethoxycarbonyl)methylene]triphenylphosphorane described in the study[80] were further improved in the study,[81] where all four 3′-modified ribonucleosides were prepared. This research group synthesized also the modified ribothymidine thymidine dimer, its phosphoramidite derivative, and a series of oligodeoxynucleotides containing one modified internucleotide linkage.[82] The synthesis of monomers for the preparation of OA **22** having the internucleotide C(3′)–NH–C(O)–CH_2–C(5′) linkage was documented.[83]



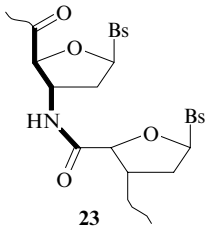
22 (R = H, OH)

A general enantioselective method was developed for the synthesis of protected 5′-azido-3′-carboxymethyl ribonucleosides for the preparation of methylene-carboxamide

RNA analogues starting from simple achiral molecules (not nucleosides).[84]

It should be noted that in the general case the design of OA containing the methylene-carboxamide internucleoside linkage isosteric with natural nucleic acids requires considerable synthetic efforts. As a rule, six covalent bonds in the repeating motif (indicated by semibold lines in the formulas) of isosteric oligomers **18**–**22** ensure the best modelling of the parameters of natural complementary complexes and lead to an increase in thermal stability of complementary duplexes.

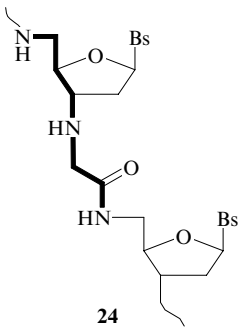
The synthesis of carboxamide oligomers of thymidine **23** composed of so-called nucleoside β-amino acids as monomer units was documented.[85]



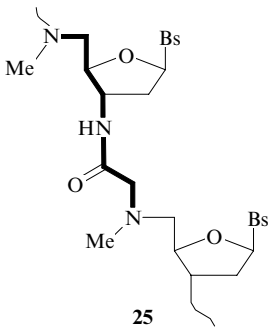
23

Nucleoside β-amino acids in a carbohydrate component contain the carboxy and amino groups in the β position with respect to each other. Oligomers based on such nucleosides contain a shortened repeating motif consisting of four covalent bonds. Nucleoside β-amino acids can easily be synthesized by oxidation of protected 3′-aminonucleosides.[86] It was found[85] that the parameters of the helical conformation of complementary complexes of oligomers **23** differ from those observed earlier. This fact can be used for the design of biopolymers with an unusual macromolecular structure.

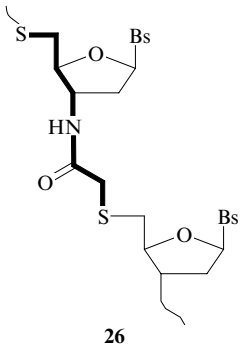
As was mentioned in the study,[87] the repeating motif composed of six covalent bonds in the carbohydrate–phosphate backbone of natural nucleic acids can sometimes be increased by one bond without loss of specificity of the complementary binding and a substantial decrease in thermal stability of the complexes. This is why, various carboxamide OA containing seven covalent bonds, *viz*., glycinamide (**24**), *N*-methylglycinamide (**25**), thioacetamide (**26**), and carboxamidoglycine (**27**, R = H) oligomers, were prepared along with carboxamide OA **20**–**22**.



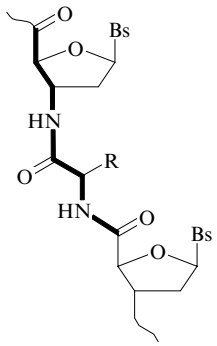
24



25



26



27 [R = H, $(CH_2)_4NH_2$, $(CH_2)_2SMe$...]

This class of compounds have attracted interest because deep transformations of natural (deoxy)ribonucleosides are usually not required for the synthesis of their monomers.

Oligomers of thymidine **24** linked through glycinamide linkages were documented.[88] The starting monomers were synthesized by alkylation of the corresponding protected 5′,3′-dideoxydiaminonucleosides with bromoacetic acid. It should be noted that the nucleoside phosphonate and glycinamide oligomers were synthesized according to the same solid-phase synthesis protocol with the activation of both phosphonic and carboxylic acid residues in the presence of triisopropylbenzenesulfo(nitro)triazolide and 1-methylimidazole. It was found that complementary complexes of oligothymidylate analogues **24** having glycinamide linkages form more stable complexes with complementary oligoadenylates than their analogues **12** containing phosphonic linkages. In all cases, OA–RNA complexes proved to be more stable than OA–DNA complexes.

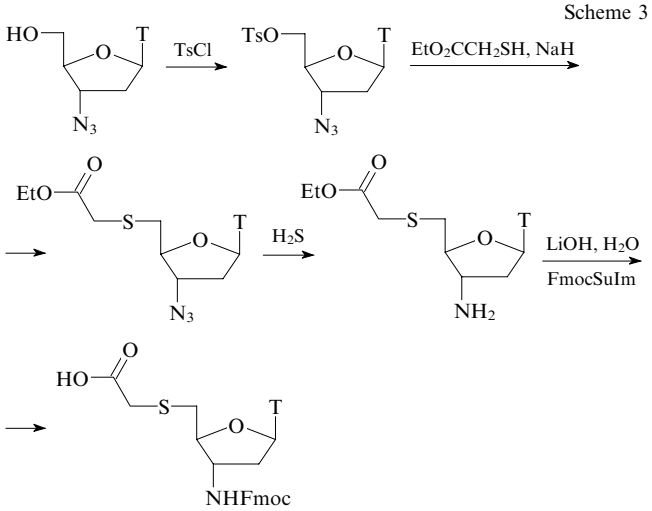
More recently, oligodeoxynucleotide analogues **25** containing the isomeric glycinamide internucleoside linkage were synthesized.[89] The alkylation of the 5′-N-methylamino-substituted monomer with ethyl bromoacetate afforded a compound containing the tertiary amino group, which does not require protection in the oligonucleotide synthesis.[90] The presence of such modified internucleoside linkages leads to a decrease in thermal stability of complementary complexes with natural oligodeoxyribonucleotides; however, the thermal stability of complexes with oligoribonucleotides was not investigated.

Recently, Indian researchers have described a new type of OA, *viz*., compounds **26** containing the thioacetamide group between nucleosides.[91] The thymidine monomer was synthesized in high yield in several steps starting from 3′-azidothymidine (Scheme 3).

Octamers containing from one to eight modified units along with peptide nucleic acid fragments were synthesized by methods of peptide chemistry.[91] Oligomers **26** containing thioacetamide internucleoside linkages form stable complementary complexes with RNA, whereas complexes with DNA were not observed.

Chimeric octadecaoligodeoxyribonucleotides containing one or two thioacetamide internucleoside linkages were described in the study.[92] It was noted that in this case the formation of complementary DNA–RNA complexes is more favourable compared to DNA–DNA complexes. It was hypothesized that the use of isomeric thioacetamide linkages in the thymidine monomer having a *lyxo* configuration can lead to an additional increase in the selectivity of thioacetamide OA with respect to RNA.[93] The detailed

Scheme 3



TsCl is *p*-toluenesulfonyl chloride, Fmoc-Su-Im is fluorenylmethoxycarbonyl *N*-succinimidyl carbonate.

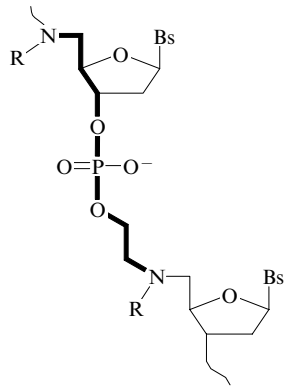
study of the structures and thermodynamic parameters of complementary DNA–DNA tetradecamer duplexes containing up to three thioacetamide internucleoside linkages revealed factors responsible for their low thermal stability.[94] The use of thioacetamide OA **26** as molecules specifically recognizing RNA holds great promise; however, only thymidine derivatives were synthesized.

As mentioned above, nucleoside β-amino acids are easily accessible and can be used for the synthesis of peptide-like oligomers **23**.[85] Nevertheless, it is necessary to increase the size of the repeating motif composed of four covalent bonds in these oligomers to form stable complementary complexes with natural nucleic acids. It was suggested [95] that an additional linkage group consisting of natural α-amino acids, such as proline, sarcosine, methionine, or lysine (oligomers **27**) be introduced into oligomers. Thymidine-containing modified octamers were synthesized. All resulting OA form more stable complementary complexes with RNA than with DNA. Further studies of the influence of the stereochemistry of α-amino acids used as linkages between nucleoside β-amino acids showed that the configuration of the linkage is responsible for the strength of stacking interactions of heterocyclic bases in OA **27**. The latter, in turn, has an effect on the stability of their complementary complexes and selectivity of the binding to DNA or RNA.[96] In the study,[97] it was also found that the selectivity of the binding of carboxamide OA to RNA depends on the stereochemistry of the internucleoside linkage if it contains an additional chiral carbon atom.

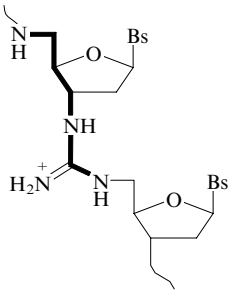
On the whole, it should be noted that among nonionic oligonucleotide analogues considered in this section, thioacetamide OA **26** are the most promising in terms of the simplicity of the synthesis and the efficiency of the formation of complementary complexes, although data only on the synthesis of oligomers of thymidine were published. The exclusive selectivity of the binding to RNA is an interesting fact.[91]

3. Positively charged oligonucleotide analogues

As the body of data on interactions of natural and modified oligonucleotides with cells continues to grow, additional evidence becomes available that the introduction of cationic groups into oligonucleotide molecules not only leads to stabilization of complementary complexes but also assists in the penetration of OA into cells.[98] Consequently, OA positively charged under physiological conditions are considered as promising agents for antisense therapy. Both amino groups protonated at physiological pH, for example, primary or secondary amino groups in a linkage (oligomers **28**),[99] and cationic groups, for example, guanidinium groups (oligomers **29**),[100] can serve as sources of positive charges.



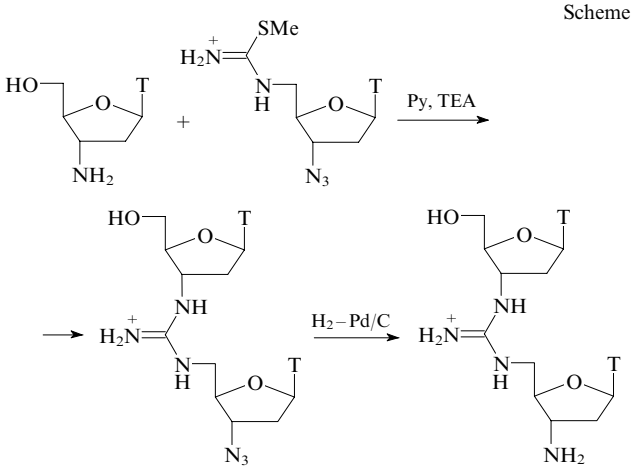
28 (R = H, Me)



29

The synthesis of the thymidine dimer (Scheme 4) containing the guanidinium group as an internucleoside linkage was documented.[100] A two-step increase in the length of the chain accompanied by reduction of the 3′-azido group after each condensation was proposed as a method for the synthesis of such oligomers.

Scheme 4

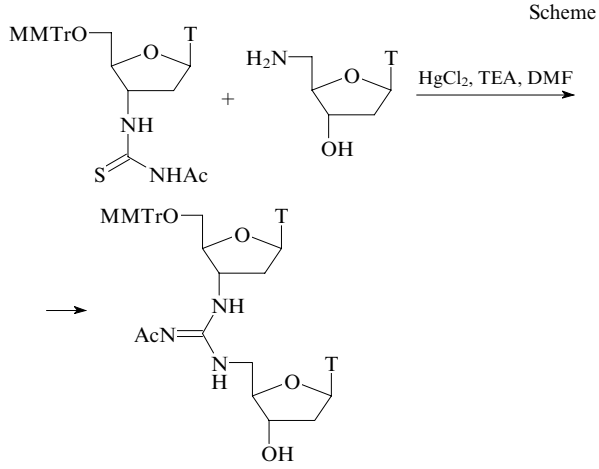


TEA is triethylamine.

To synthesize chimeric oligodeoxyribonucleotides containing up to three guanidinium moieties, the scheme of the synthesis of the thymidine dimer (Scheme 5) was modified [101] because the protection of the guanidinium group is required in the standard phosphoramidite protocol for oligonucleotide synthesis.

The condensation of the thymidine derivative of acetylthiourea and an amine component in the presence of $HgCl_2$ occurred through the intermediate formation of carbodiimide. The reaction afforded a dimer acylated at the guanidinium group, which can be used in the oligonucleotide synthesis after phosphitylation. It should be noted that the condensation occurs more rapidly and under milder

Scheme 5



MMTr is 4-monomethoxytrityl.

conditions in the presence of mercury salts compared to the reaction in the absence of these salts (2 h at room temperature *versus* 40 h at 100 °C). This scheme of the condensation accompanied by the activation of the thiourea derivative with mercury(II) chloride proved to be convenient also in the solid-phase synthesis of modified oligomers in the 5′→3′ direction.

The synthesis of guanidinium thymidine octamers was described in the study.[102] The inclusion of up to four uncharged internucleoside units composed of urea residues into guanidinium oligomers **29** made it possible to perform the fine tuning of the efficiency and specificity of the binding of polycationic oligomers **29** to a complementary DNA sequence. More recently, the authors synthesized thymidine and deoxyadenosine monomers containing the 3′-(*N*-acyl)thiourea group. In addition, the solid-phase synthesis of chimeric oligomers in the 3′→5′ direction was adapted with the use of porous glass as a support, and thymidine- and adenosine-containing hexamers were prepared.[103]

Guanidinium OA **29** containing all four heterocyclic bases were synthesized from deoxyguanosine[104] and deoxycytidine monomers.[105] More recently, this research group has characterized in detail the structures of complementary complexes of chimeric OA containing guanidinium groups using different physicochemical methods.[106–108] It was shown that these OA form much more stable complementary complexes than natural oligo(deoxy)ribonucleotides without loss of specificity.[107]

It should be noted that the diversity of modifications of internucleoside linkages is not limited to the types of OA containing modified negatively charged, nonionic, and positively charged internucleoside linkages considered in Section II. The studies on the synthesis of nonionic OA having sulfide, sulfoxide, sulfonic,[109,110] phosphonoacetyl,[111] phosphonoformyl,[112] and formacetal[113,114] internucleoside linkages deserve mention. These and some other OA are considered in the recent review.[115] As a rule, the preparation of analogues without phosphate groups requires more efforts both for the synthesis of monomers and further assembly of homogeneous or chimeric oligomers. Investigations on the design and synthesis of various OA having modified internucleoside linkages are underway. Extensive research on the structures of complementary complexes of such OA provides a theoretical basis and, probably, allows the prediction of optimal structures necessary for performing particular functions by modified oligomers.[116]

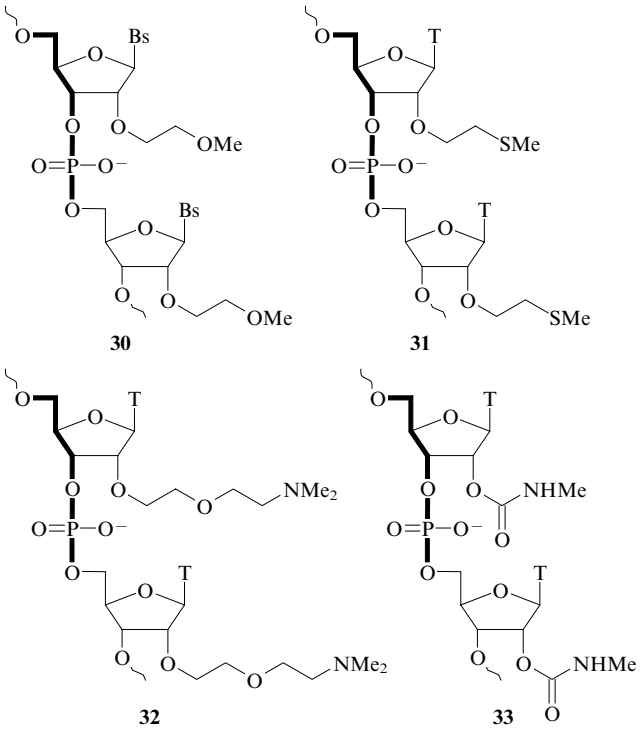
III. Carbohydrate-modified oligonucleotide analogues

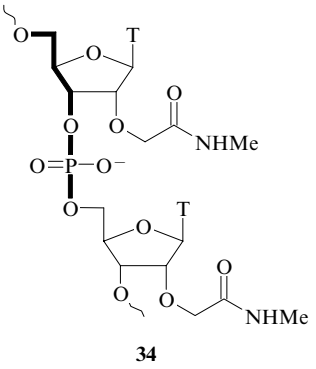
In the previous section, we considered OA containing a modified linkage between the virtually unaltered nucleosides involved in natural nucleic acids. Since our classification is somewhat arbitrary (for example, we considered compounds, in which not only the linkage but also the position 2′ of ribose were modified[53]), we believed that the nature of the linkage between monomers is the main factor determining the structure of oligomers and their complementary complexes. In this section, we deal with OA, in which carbohydrate-modified nucleosides are linked by phosphoric acid moieties, as in natural nucleic acids. 2′-O-Modified oligoribonucleotides **5**[117,118] and conformationally restricted OA **9**[119] are the most important representatives of such analogues.

1. 2′-Modified oligonucleotide analogues

Compounds **5** containing 2′-modified (primarily, 2′-O-alkylated) nucleoside units belong to so-called second-generation antisense oligonucleotide analogues. Both the corresponding monomers and oligomers can be rather easily synthesized. Besides, these OA are characterized by high resistance to nucleases and a high affinity for complementary RNA. They are widely used in clinical trials of antisense therapy drugs.[27,120]

In recent years, in addition to classical 2′-*O*-methyl and 2′-*O*-(2-methoxy)ethyl derivatives **30**, 2-methylthioethyl (**31**),[121] protonated 2′-*O*-2-[(2-*N*,*N*-dimethylamino)ethoxy]-ethyl (**32**),[122] 2′-*O*-(*N*-methyl)carbamate (**33**), 2′-*O*-[(2-(*N*-methylamino)-2-oxo]ethyl (**34**)[123,124] analogues and other compounds have been synthesized.



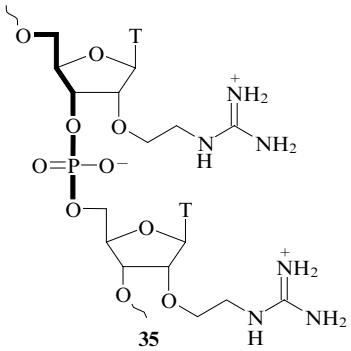


34

It is known that the nature and properties of substituents (hydrophobicity, electronegativity, volume, *etc.*) at the carbon atom in position 2′ of ribose have a strong effect on the stability of complementary complexes of modified oligonucleotides. It was found[125] that the presence of 2′-*O*-carbamate nucleosides in a modified oligonucleotide (oligomers **33**) leads to destabilization of the complementary complex, whereas the introduction of one additional methylene group into a substituent in position 2′ (oligomers **34**) results in an increase in the melting point of the duplex with RNA. Investigations of the structures of duplexes of self-complementary modified decamers by X-ray diffraction revealed conformational restrictions in the case of carbamate derivatives.

More recently, this research group has carried out a systematic study of the influence of the nature of the 2′-O-substituent on the structure and stability of complementary duplexes. Ten new modified 2′-O-alkylated nucleosides containing hydrophobic or hydrophilic residues or residues protonated under physiological conditions were synthesized starting from ribothymidine.[126] With the purpose of obtaining more precise X-ray diffraction data, synthetic protocols were developed to introduce the selenium atom into 2′-modified nucleosides instead of the oxygen atom of the hydroxy group.[127]

It was found[128] that the presence of the protonated amino group results in an increase in thermal stability of complementary complexes of 2′-O-modified oligonucleotides with DNA and RNA. More recently, these authors have synthesized 2′-O-modified nucleosides containing the cationic guanidinium group, *viz.*, compounds **35**.[129]



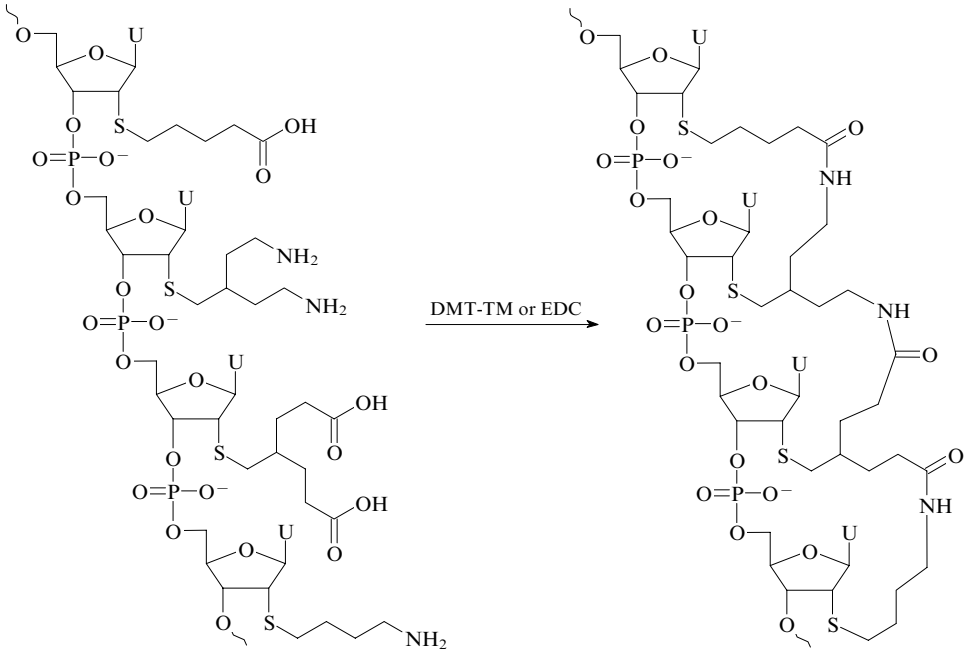
35

To use modified monomers in the standard phosphoramidite protocol of the solid-phase oligonucleotide synthesis, the new *N*-(2-cyanoethoxy)carbonyl protecting group for the guanidinium moiety was used and the conditions of deprotection of oligonucleotides were optimized.[129]

Since various 2′-O-modified OA can be rather easily synthesized, these compounds are extensively studied not only as antisense agents[130] but also for the application in other areas, such as the synthesis of oligonucleotide–peptide conjugates,[131] aptamers,[132] and nanomaterials for the construction of molecular networks and blocks for the design of molecular machines.[133, 134]

The so-called nylon-DNA were described in the study.[135] To form regular amide bonds, 2′-S-substituted

Scheme 6



DMT-TM is 4-methyl-(4,6-dimethoxy-1,3,5-triazin-2-yl)morpholinium chloride, EDC is 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide.

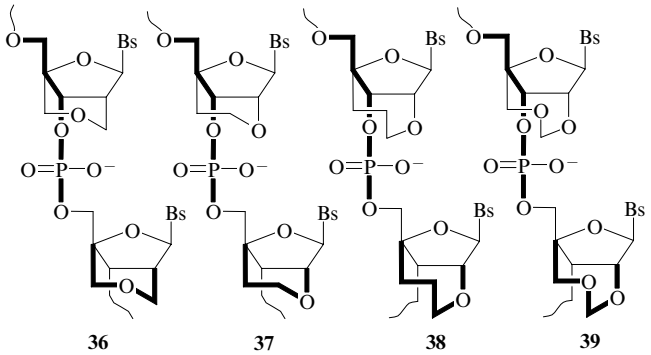
uridine monomers containing linkages with one(two) amino or one(two) carboxy groups were introduced into oligothymidylate in such a way as to obtain the desired structure in the subsequent intramolecular condensation in an aqueous solution (Scheme 6).

More recently, these researchers have developed the above-described approach and performed the assembly of nylon-DNA in a complex with a complementary oligonucleotide. They studied in detail the thermodynamic characteristics of the resulting constructs. It was found that the formation of the amide bond (covalent macrocycle closure) in nylon-DNA substantially increases the affinity of these compounds for DNA and RNA oligomers, whereas precursor oligomers non-closed in a loop (see Scheme 6) do not have such properties.[136]

2. Conformationally restricted oligonucleotide analogues

In the 1990s, the search for new oligonucleotide analogues for antisense therapy preferably forming complexes with RNA culminated in the design of so-called locked nucleic acids (LNA) **9** belonging to third-generation oligonucleotide analogues. Due to high thermal stability of complementary complexes with natural nucleic acids[137] and commercial availability, compounds **9** have found wide use as tools for antisense technologies.[22] Along with phosphorothioate oligonucleotide analogues, LNA **9** are currently under preclinical and clinical evaluation as agents *via* the antisense principle.[27] According to the physicochemical data, high thermal stability of complementary LNA complexes is determined by the frozen conformation of the carbohydrate moiety.[138]

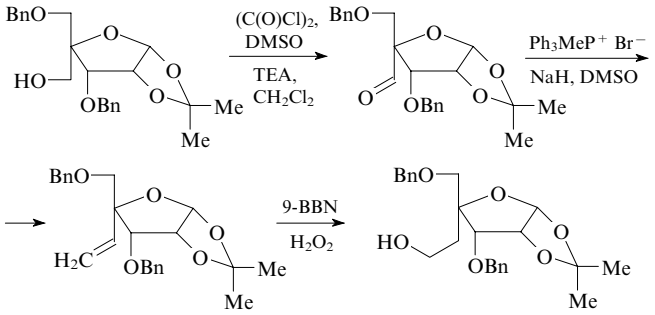
The search for oligonucleotide analogues possessing such properties was continued later on. Conformationally restricted OA containing C(4′)–C(2′) bridges composed of four (oligomers **36** and **37**)[139–141] and five covalent bonds (oligomers **38** and **39**) were synthesized.[142, 143] These compounds are called bridged nucleic acids (BNA).



36 **37** **38** **39**

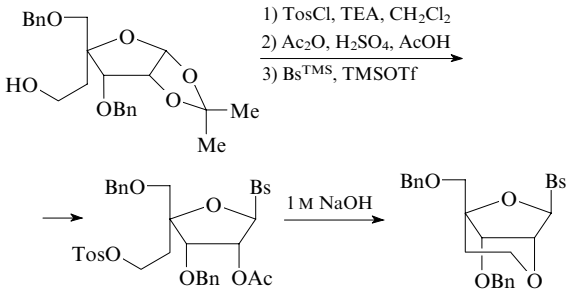
The synthesis of four modified nucleosides having oxyethylene bridges, *viz.*, 2′-O,4′-C-ethylene-bridged nucleic acids **37** (ENA), and oxypropylene-bridged thymidine nucleoside **38** was documented.[142] To produce ENA monomer **37**, the pentofuranose derivative containing a long chain at the C(4′) atom was synthesized in several steps {Swern oxidation, Wittig reaction, reduction of alkene in the presence of 9-borobicyclo[3.3.1]nonane (9-BBN) followed by oxidation with hydrogen peroxide} starting from protected 4′-hydroxymethylpentofuranose (Scheme 7).

Scheme 7



Then the product was transformed into the target nucleoside by conventional methods used in nucleoside chemistry (Scheme 8).

Scheme 8



Bs^{TMS} is a silylated base, TMSOTf is trimethylsilyl triflate.

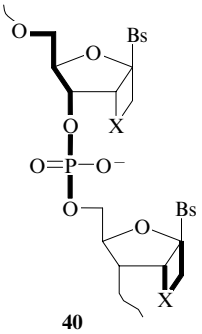
The replacement of benzyl protecting groups by the dimethoxytrityl group followed by phosphitylation afforded phosphoramidite of the target ENA nucleoside, which was used in the routine oligonucleotide synthesis protocol according to the phosphoramidite method. A thymidine nucleoside having an oxypropylene linkage was synthesized in a similar way.

Japanese researchers performed a comprehensive study of complementary complexes (duplexes and triplexes) formed by OA composed of a different number of ENA-modified units by various physicochemical methods.[144, 145] It was found that oxyethylene nucleosides in analogues **37** are in the 3′-*endo* conformation, like oxymethylene nucleosides in LNA analogues **9**. However, the available slight conformational differences result in that complementary triplexes can be formed with fully modified ENA analogues, as opposed to fully modified LNA analogues. The synthesis of phosphorothioate and phosphoramidate ENA derivatives of oligonucleotides was documented.[146] Analogues of ethylene-bridged nucleic acids were successfully used as antisense agents in different model systems[147–149] and *in vivo*,[150] as well as in the analysis of the polymorphism in allels.[151] The detailed data on the properties and applications of ENA analogues are summarized in the reviews.[152, 153]

Along with BNA **36** and **37** containing the six-membered ring between the C(4′) and C(2′) atoms that restricts the conformation of nucleosides, OA **38** and **39** with the oxygen-containing seven-membered ring were synthesized.[142, 143] The introduction of modified oxypropylene units (oligomers **38**) into OA does not lead to additional stabilization of complementary complexes, whereas BNA **39** containing the formacetal group form complementary complexes, which are thermally more stable than natural oligo-

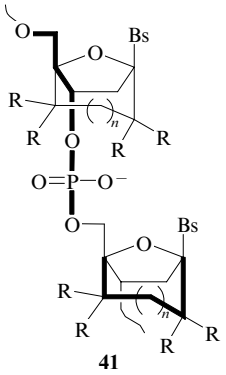
nucleotides. It was speculated [143] that an increase in the hydrophilicity of the linkage has a positive effect on the affinity of OA for complementary DNA and RNA sequences.

It should be noted that the synthesis of various modified nucleosides, including those containing conformationally restricted bridges, has attracted great attention due to antiviral activity of these compounds.[154] In the recent review,[155] methods for the synthesis and conformations of some of such bridged nucleosides are considered in detail. Certain nucleosides containing a covalent linkage between positions 1′ and 2′ (for example, 1′-2′-oxetanes [156] and 1′-2′-azetidines [157]) were used for the synthesis of modified oligomers **40**. In addition to bicyclic nucleosides containing a heteroatom (oxygen or nitrogen) in the bridging moiety, OA **41** containing saturated or unsaturated carbocyclic moieties,[158] including those with different substituents,[159] were prepared.



40

X = O, NH.



41

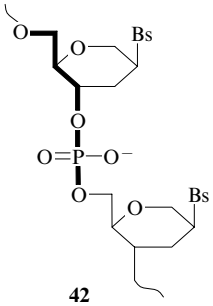
R = H, Me, OH; n = 0, 1.

It was shown [159] that these compounds are more stable in biological media than LNA and ENA analogues. A new method was developed for the closure of the carbocycle by intramolecular free-radical cyclization with the purpose of synthesizing the corresponding monomers of conformationally restricted carbocyclic OA **41** (carbo-LNA and carbo-ENA). Earlier, the ion cyclization of tosylate esters has been used for this purpose (see Scheme 8).

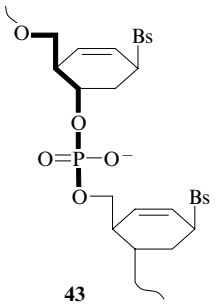
The influence of the configuration, the size, and the nature (hydrophobicity–hydrophilicity) of substituents at asymmetric carbon atoms in the carbocyclic bridge of BNA on the stability of complementary duplexes, the activation of RNase H, and the stability of phosphodiester linkages in solution and physiological media was studied in detail.[160] Based on these data, 2 of 52 carbo-LNA and carbo-ENA analogues were chosen. These compounds form thermally stable complementary complexes with RNA, are resistant to nucleases, and can stimulate RNase H activity. In the authors' opinion, it is due to these characteristics that these oligomers are ideal candidates for use as agents for antisense therapy or siRNA therapy (siRNA are short interfering RNA).[160]

It should be noted that, in spite of the fact that the synthesis of modified bridged nucleosides involves many steps (15–18), it is important that these nucleosides can be used for the preparation of oligomers by efficient modern methods. Besides, due to high thermal stability of complementary complexes with the participation of such oligonucleotides, trials of various BNA as antisense agents are very popular. The latest advances in this area are summarized in the review.[161]

It should be noted that natural and modified nucleic acids can be the synthesized not only by chemical methods but also by the enzymatic process (with DNA and RNA polymerases). In the latter case, triphosphates of the corresponding nucleosides are used as the starting compounds.[162] The substrate properties of triphosphates of non-natural nucleosides in the enzymatic template-dependent polymerization, the antiviral activity of modified triphosphates, and the hybridization properties of the corresponding OA are analyzed in the review.[162] Compounds containing six-membered rings, such as tetrahydropyran (**42**) [163] and cyclohexene (**43**),[164] as the carbohydrate moiety were investigated.



42



43

Nucleosides of the L series containing α-L-threose as the carbohydrate moiety were mentioned as promising compounds for the synthesis of OA. Nucleoside derivatives containing tetrahydropyran in the L configuration [165] and L isomers of cyclohexenyl derivatives were synthesized for further investigations of oligomers of the L series.[166] The thermal stability of complementary complexes formed by OA **42** and **43** of the L series with both natural nucleic acids and related compounds of the D series were systematically studied.[167, 168]

IV. Nucleic acid mimetics

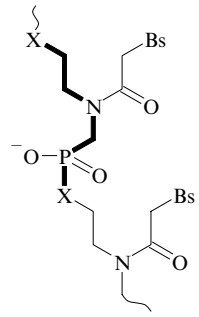
In the previous sections, we considered OA containing either a modified internucleoside linkage or a modified carbohydrate moiety of the nucleoside. Oligonucleotide analogues containing both modified moieties are also known. In these compounds, only the sequence of heterocyclic bases of natural nucleic acids, which can be involved in complementary interactions and are separated by the same (or regular) number of covalent bonds, remains unchanged.

These analogues include peptide nucleic acids **6**, morpholino analogues **7**, and some other oligomers referred to as nucleic acid mimetics. Peptide nucleic acids **6** are most unusual among these compounds because they do not contain a cyclic moiety as part of their backbone and have glycine rather than a phosphoric acid moiety as a linkage. Nevertheless, PNA **6** have a high affinity for complementary natural nucleic acid sequences and are resistant to enzymes (nucleases, proteases, *etc.*). The synthesis and unique properties of PNA are considered in the reviews.[18, 169, 170] In the last decade, the following new types of PNA were synthesized: negatively charged,[171] conformationally restricted,[172] and protonated, or cationic, PNA.[173] These modifications are aimed at increasing the solubility of PNA in water, improving the penetration into cells, gaining structural order of modified oligomers, and

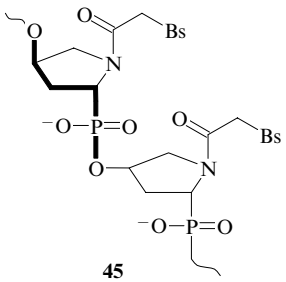
enhancing the selectivity of the PNA – DNA – RNA binding.

1. Negatively charged peptide nucleic acids

Negatively charged PNA **44** belong to oligomers, in which the carboxamide group in the glycine linkage is replaced by a phosphonate or phosphonamide group.[174]



44 (X = O, NH)

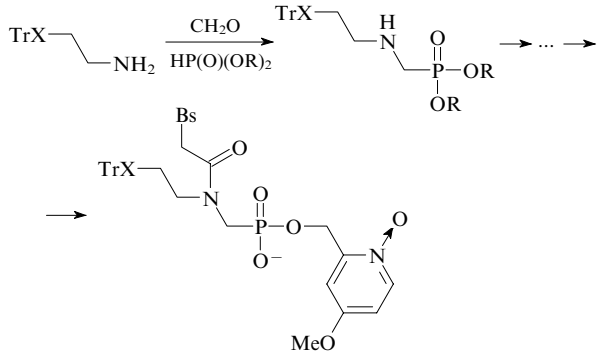


45

Compounds **44** do not formally belong to peptide nucleic acids because a carboxamide bond is absent in the linkage between monomer units. In the study,[174] these oligomers were referred to as phosphonate analogues of PNA (phosphono-PNA). Later on, this term was applied also to conformationally restricted structural analogues of phosphonate-containing PNA **45**. In these compounds, the aminoethylglycine moiety of the linkage between monomer units is covalently closed to form the N-containing heterocycle (pyrrolidine), and the heterocyclic base is attached *via* an acetamide group (as opposed to phosphonate nucleic acid analogues **10** – **17** considered in Section II).

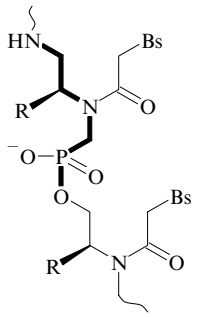
The synthesis of protected phosphonate monomers of phosphono-PNA was described.[174] The key step of the synthesis is the formation of derivatives of phosphonic acid by the Kabachnik – Fields reaction (Scheme 9).

Scheme 9



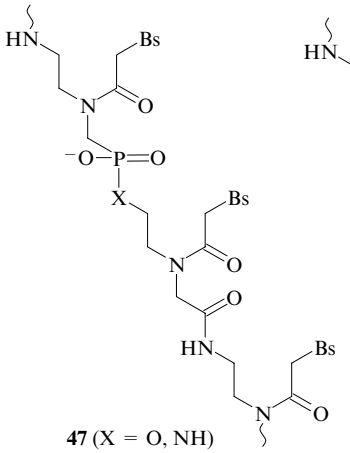
TrX = MMTrNH or DMTrO; R = Ar or Alk.

In the latter study, the order of the addition of heterocyclic bases was varied, and the dependence of the rate of condensation of monomers on the nature of the protecting group of phosphonic acid was investigated. It was found that the 4-methoxy-1-oxido-2-picolyl protecting group, being an efficient intramolecular catalyst, leads to a substantial increase in the rate of condensation resulting in the formation of phosphonodiester and phosphonomonoester monoamide bonds in the presence of triisopropylbenzenesulfo(nitro)triazolide.[42] The same procedure was used for the synthesis of monomers for side-chain substituted oligomers **46** starting from (*R*)-(−)-2-amino-1-butanol and L-serine methyl ether [175] or other natural amino acids,[176] as well as for the synthesis of all possible optical isomers for the preparation of conformationally restricted 1-acetyl-4-hydroxypyrrolidine-2-phosphono-PNA **45**.[177]

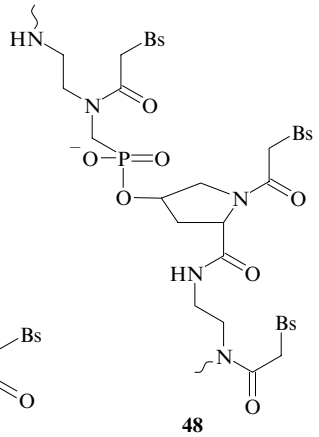


46 (R = Me, Et, ...)

Chimeric oligomers **47** consisting of phosphono-PNA and PNA monomers were synthesized [176, 178] by the solid-phase method.



47 (X = O, NH)

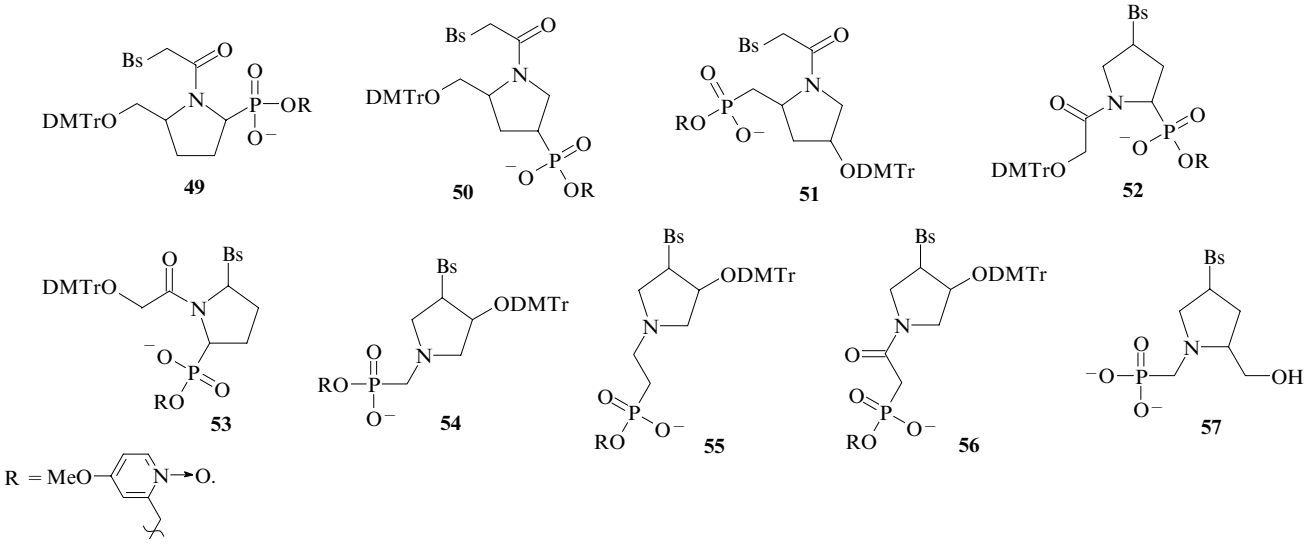


48

It was expected that these oligomers would retain high affinity for complementary nucleic acids and would be better soluble in water than PNA. The conditions of the deprotection and condensation were optimized depending on the type of the monomer (PNA, phosphono-PNA) and the type of the linkage that is formed in the condensation (phosphonoester, phosphonamide, or carboxamide linkages). Among chimeric oligomers synthesized with the use of optically active phosphono monomers, oligomers **48** based on *trans*-4-hydroxy-L-proline form the most stable complexes with complementary natural nucleic acid sequences.[179] Phosphonohydroxyproline-containing nucleic acid mimetics and chimeric oligomers based on these compounds were successfully used as tools for hybridization analysis,[180 – 182] the messenger RNA isolation,[183] and the gene expression regulation.[171, 184]

Later on, new types of conformationally restricted pyrrolidine-based phosphono nucleic acids were synthesized.[185] In these compounds, the hydroxy group, the heterocyclic base, and the phosphonic acid moiety are attached to different atoms of the pyrrolidine ring.

The synthesis of nucleic acid mimetics based on monomers **49** – **53** was documented.[185]



Czech researchers[186] synthesized all diastereomers of 3,4-hydroxypyrrolidine-based nucleoside analogues (compounds **54**–**56**) starting from L- or D-tartaric acid.

It should be noted that monomers **54** and **55** contain a tertiary nitrogen atom, which can be protonated, when being involved in oligomers, under physiological pH. Protected adenine nucleotide analogues **54**–**56**, which were constructed based on 3,4-hydroxypyrrolidine, were used for the synthesis of chimeric oligomers containing both natural and modified nucleotides.[187] The conformations of nucleoside and nucleotide analogues **56** and **57** in aqueous solutions were extensively studied by the NMR method.[188, 189] The synthesis and properties of phosphonate analogues of PNA, particularly of conformationally restricted oligomers, are considered in the recent review.[190]

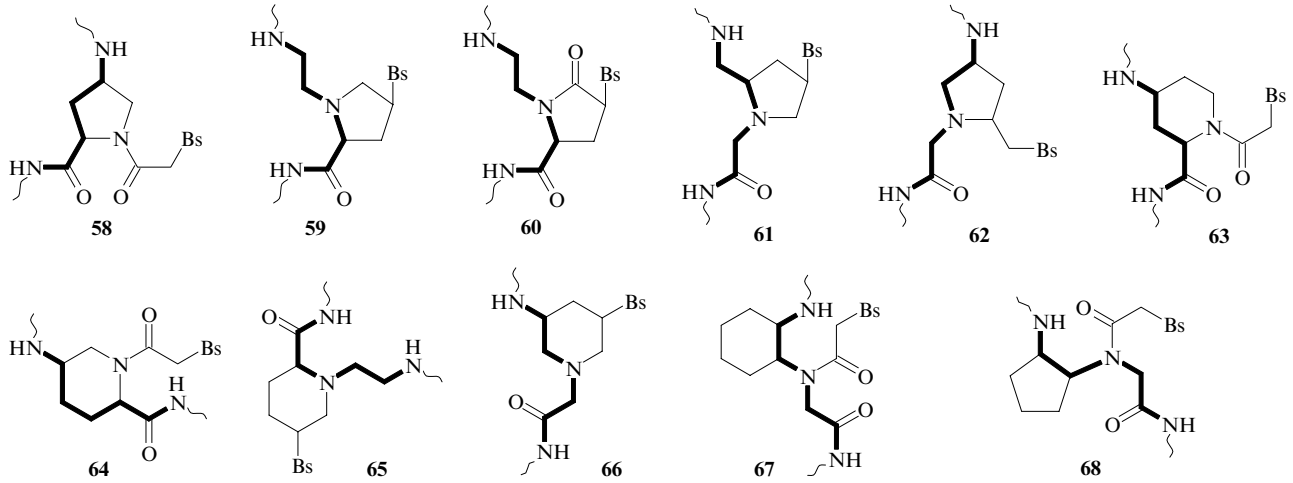
It should be noted that the design of monomers for the synthesis of negatively charged phosphonate analogues of PNA requires considerable efforts. However, the effective idea of the use of the protecting group of the phosphonate moiety for intramolecular catalysis[174] of the condensation made it possible to prepare oligomers of different length. Chimeric oligomers **48** containing *trans*-1-acetyl-4-hydroxy-L-proline units and oligomers **45** based on (2*S*,4*R*)-1-acetyl-4-hydroxypyrrolidine-2-phosphonic acid proved to be highly efficient antisense agents.[185]

2. Conformationally restricted peptide nucleic acids, including protonated analogues

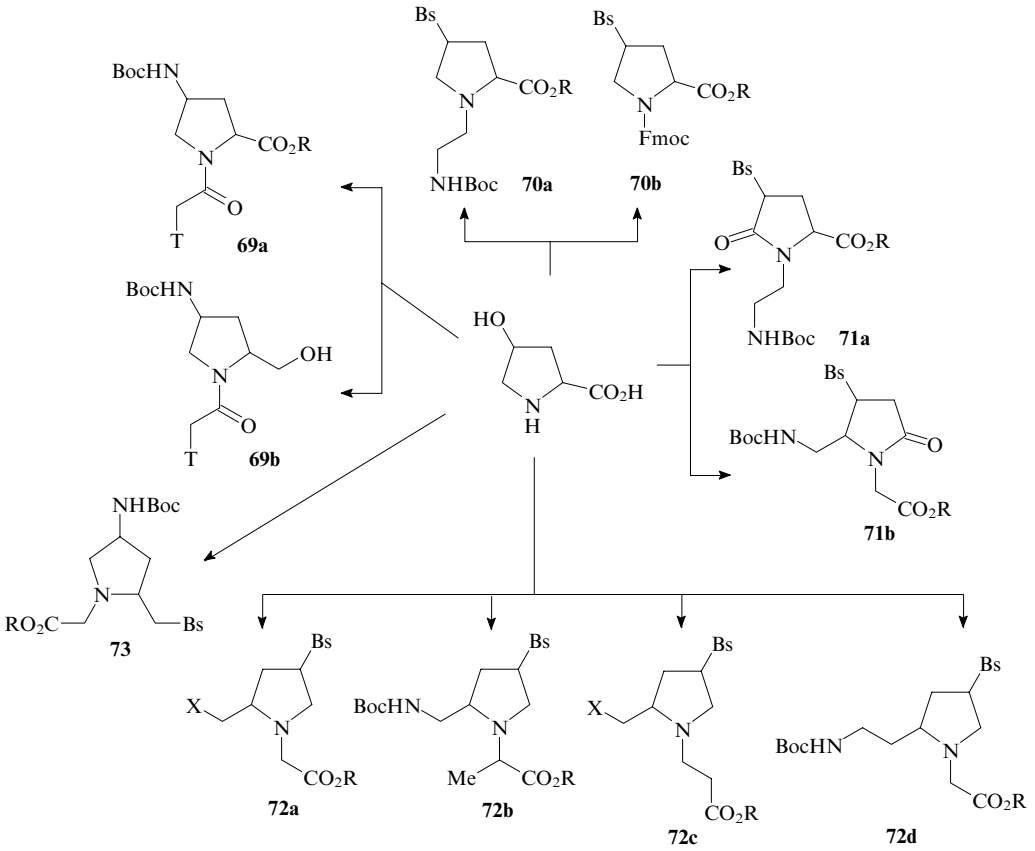
It is known that PNA, having a high affinity for complementary DNA/RNA sequences, can form almost equally stable complementary complexes with both parallel and antiparallel strands.[191] To improve the selectivity of PNA with respect both to binding and to the preferred formation of complementary complexes with DNA or RNA, it is necessary to restrict the flexibility of the PNA molecule.[192] Over several years, different research groups made attempts to introduce chiral moieties into the PNA structure or restrict the conformational flexibility of the oligomer chain by the closure of the aminoethylglycine backbone of PNA to form a ring in different ways.[193] Main types of conformationally restricted PNA analogues **58**–**68** are considered in the reviews.[172, 194]

Since both neutral oligomers (**58**, **60**, **63**, **64**, **67**, **68**) and oligomers protonated at physiological pH (**59**, **61**, **62**, **65**, **66**) are known, we will not consider protonated conformationally restricted PNA in a separate section. As a rule, these PNA can be synthesized in a similar way by different methods of peptide chemistry.[18]

Modified PNA oligomers **58**–**62** are based on the five-membered pyrrolidine ring. These compounds were synthesized starting from 4-hydroxyproline, which contains all



Scheme 10



R = Me, Et; Boc is *tert*-butoxycarbonyl; X = BocNH, DMTrO.

functional groups necessary for further transformations.[172] In the study,[195] D-*trans*- and L-*trans*-proline derivatives of thymine **69a** were synthesized by methods developed earlier (Scheme 10).

The introduction of even one chiral monomer into PNA led to the stabilization mainly of complementary PNA duplexes in a particular orientation; however, the thermal stability of the complexes was substantially lower. In the study,[196] it was hypothesized that this is due to a decrease in the length of the repeating motif in the backbone of pyrrolidine-based oligomer **58** to five covalent bonds as opposed to usual six covalent bonds in the ribose phosphate backbone of natural nucleic acids, as well as to the aminoglycine moiety in PNA. Pyrrolidine carbamate PNA analogues were proposed for the solution of this problem.[197] The synthesis of oligomers from thymine monomers **69b** (2*S*,4*S* configuration) linked by carbamate bonds (see Scheme 10) was documented. However, the melting points of the corresponding complementary triplexes were lower than those of unmodified PNA.

Additional stabilation of complementary triplexes of conformationally restricted PNA compared to PNA with the retention of the specificity of the binding was achieved by interchanging the heterocyclic base and the linkage and replacing the acetate moiety in the linkage with an aminoethyl group. The resulting molecule contains the tertiary nitrogen atom protonated at physiological pH (see Scheme 10, compound **70a**).[198] Later on, this research group synthesized derivatives **70a** having the 2*S*,4*S* and 2*R*,4*S* configurations (two of the four possible diastereomers) and containing not only thymine but also other heterocyclic bases, as well as guanine at position 7.[199,200] Heterocyclic bases were introduced by alkylation with hydroxyproline mesylate or by the Mitsunobu reaction depending on the type of the heterocyclic base. It was found that the stability of complementary PNA chimeric duplexes containing conformationally restricted units of oligomer **70a** depends both on the nature of the heterocyclic base and the stereochemistry of the modified PNA fragment. Chinese researchers synthesized a similar monomer having the 2*S*,4*S* configuration and adenine-containing oligomer **59**.[201]

It was found[202] that thymine-containing decamer **59** based on substituted pyrrolidine **70a** having the 2*R*,4*R* configuration forms a complementary complex with poly-A, whereas it does not interact with poly-dA at all. The reassuring data on the selectivity of the binding to RNA gave impetus to further investigations. This research group synthesized monomers **70b** (see Scheme 10), which served as components for the synthesis of oligomers with the use of various β-amino acids as linkages between pyrrolidine moieties.[203] The use of chiral β-amino acids results in the formation of an additional asymmetric centre in the molecules. It was shown that the use of 1-aminopyrrolidine-(2*R*)-carboxylic acid as a linkage provides the selectivity of the binding of thymine-containing oligomers to DNA rather than to RNA.[204] To study in more depth the influence of the configuration and the nature of the linkage on the complementary binding of nucleic acid mimetics, thymine oligomers containing 2-aminocyclopentanecarboxylic acid (all possible isomers) as the linkage were prepared.[205] Only the *S*,*S* configuration of the linkage provides the highly specific and efficient formation of complementary duplexes. More recently, similar oligomers containing all heterocyclic

bases have been synthesized, and it was confirmed that these compounds can specifically bind to DNA in an antiparallel orientation.[206]

Monomers **71a** (see Scheme 10) having the 2*S*,4*S* configuration and oligomers **60** based on these monomers were synthesized by the selective oxidation of N-alkylated pyrrolidine in the presence of the $RuCl_3 - NaIO_4$ system.[207, 208] In this case, conformationally restricted PNA bind preferably to DNA compared to RNA. If amino and carboxy groups are attached to the pyrrolidine ring, as in compound **71b** (the reverse binding, the 3*S*,5*R* configuration), the corresponding adenine-containing oligomer binds preferably to RNA.[209] It should be noted that pyrrolidinone oligomers **60** do not contain nitrogen atoms protonated at physiological pH, which, in particular, leads to low solubility of these compounds in water.

Monomers **72a** for oligomers **61** were proposed by three research groups[210–212] (2*S*,4*S*, 2*R*,4*R*, and 2*R*,4*S* configurations). English researchers used such monomers (2*R*,4*R* configuration) for the synthesis of a thymine-containing pentamer, which exhibited the unusual kinetic selectivity of the binding to RNA.[213] However, an adenine-containing pentamer showed this effect only in a slight degree.[214] Later, oligomers **61** containing all four heterocyclic bases were synthesized with the use of the Fmoc[215] or Boc protection,[216] the Boc group being more efficient. However, heterooligomers do not exhibit the selectivity of the binding of complementary strands, as well as of the binding to RNA or DNA.

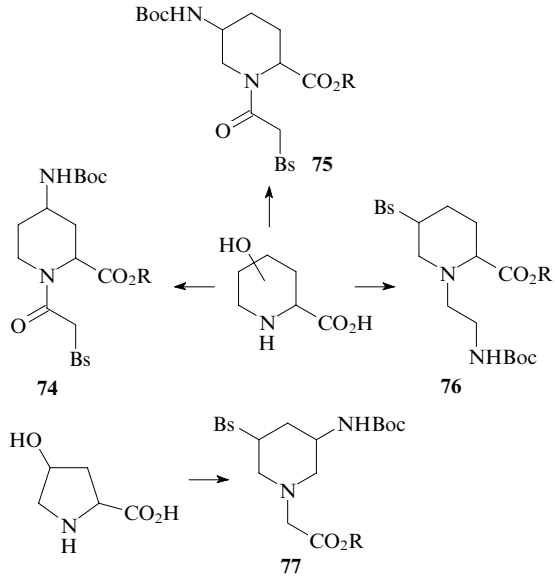
To achieve the fine tuning of the conformation of complementary complexes, an additional chiral centre was introduced into the linkage (see Scheme 10, compound **72b**),[217] as in the above-considered analogues described in the studies.[204, 205] In addition, the linkage was increased by one methylene group to prepare compounds **72c**,**d** (2*R*,4*R* configuration).[218] In the case of compound **72d**, the preferred RNA binding of modified PNA was achieved.

Chimeric oligomers containing natural nucleotides and pyrrolidine moieties **72a** (the pyrrolidine derivative has the 2*S*,4*S* or 2*R*,4*R* configuration, the combination of protecting groups was adapted to the phosphoramidite method for the synthesis of DNA or PNA) do not form thermally stable complementary complexes with DNA or RNA.[211] The introduction of an additional methylene group into the linkage (monomers **72b**, the 2*S*,4*S* configuration) eliminates the effect of destabilization of complexes of chimeric oligomers upon the inclusion of monomers **72b** both into DNA[219] and PNA, the formation of the complex with RNA being preferable.[220]

The influence of the stereochemistry of the pyrrolidine moiety in **73** (see Scheme 10) and the sequence of chimeric PNA **62** on the stability and the preferable orientation of complementary duplexes was investigated in detail.[221] In the case of thymine-containing monomers, all four possible diastereomers of **73** were synthesized; for other heterocyclic bases, 2*R*,4*S* isomers were obtained. An additional methylene group between the pyrrolidine ring and the heterocyclic base should provide higher conformational flexibility of the heterocycle upon the formation of complementary complexes. It was found that the diastereomerism has opposite effects on the thermal stability of complementary duplexes and triplexes. The introduction of 4*R* diastereomers stabilizes the duplex, whereas the introduction of 4*S* diastereomers results in its destabilization. In the case of RNA complexes, the binding in the antiparallel orientation to compounds containing the pyrrolidine moiety in the *S*,*S* or *R*,*R* configuration is preferable. Compounds having the 2*R*,4*S* configuration do not show selectivity of the binding to DNA or RNA.

Simultaneously with the study of pyrrolidine PNA, Indian researchers synthesized[222] modified PNA monomers **74**–**77** containing the piperidine ring (Scheme 11) for the purpose of studying oligomers **63**–**66**.

Scheme 11



R = Me, Et.

It was expected that the introduction of the methylene linkage into aminopropylglycine PNA resulting in the formation of the nitrogen-containing six-membered ring would compensate high conformational flexibility of this type of nucleic acid mimetics. In the study,[222] the 2*S*,4*S* diastereomer of thymine monomer **74** was prepared starting from protected 4-hydroxypipecolinic acid, and then compound **74** was used for the synthesis of modified PNA **63**. However, the introduction of this monomer into PNA destabilizes the complementary triplex.

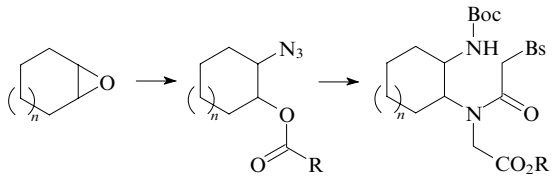
Later, 2,5-substituted derivatives (2*S*,5*R*)-**75** were prepared for the synthesis of modified PNA **64**.[223] In the general case, the introduction of this type of monomers into PNA led to certain stabilization or a slight destabilization of triplexes. The influence of the introduction of monomers **74** and **75** into PNA on the complementary interactions with DNA or RNA in duplexes was investigated in detail.[223] In this case, a decrease in the melting point of the complexes was observed, and there were no favourable orientations for the complementary binding.

Scheme 11 shows yet another design of modified monomers for conformationally restricted PNA, *viz*., compound **76** (2*S*,5*R* and 2*R*,5*S* configurations).[224, 225] Chimeric PNA **65** containing monomers **76**, as a rule, form more stable complementary complexes. Oligomers **66** were synthesized also with the use of monomers **77** (3*S*,5*S* and 3*R*,5*R*)[226, 227] derived from 4-hydroxyproline (see Scheme 11). Monomers **77** introduced into the PNA oligomer additionally stabilize the complementary triplex.

The parameters of possible complementary complexes formed by modified nucleic acids with six-membered rings (cyclohexane, cyclohexene, or tetrahydropyran) instead of

furanose were analyzed in the review.[228] Based on the data on the three-dimensional structures of complementary triplexes and duplexes, it was expected[229] that the introduction of modified monomers, which contain the saturated six-membered carbocycle (oligomers **67**) with the 1,2-arrangement of substituents in the *cis* orientation, into PNA could lead to different thermal stability of complementary complexes with DNA and with RNA. The corresponding monomers **78** (1*S*,2*R* or 1*R*,2*S* configuration) were synthesized starting from 1,2-epoxycyclohexane (Scheme 12). The key step of the synthesis involves the enantioselective enzymatic hydrolysis of the racemate of 1-acyloxy-2-azidocyclohexane.

Scheme 12



78: $n = 1$;
79: $n = 0$.

R = Me, Et.

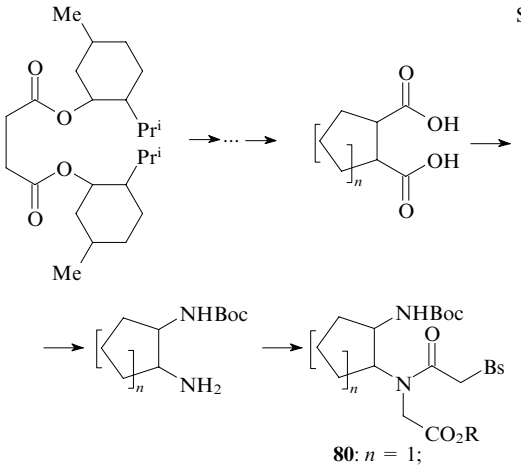
To study the three-dimensional structures of complementary complexes in more detail, modified PNA monomer **79** containing the five-membered carbocycle (1*S*,2*R* or 1*R*,2*S* configuration) was synthesized from 1,2-epoxycyclopentane according to a similar scheme.[230] Chimeric PNA oligomers containing *cis*-cyclohexyl- (**78**) and *cis*-cyclopentylcarbocyclic modified PNA monomers (**79**) were investigated. It was found that, in spite of a certain destabilization, oligothymine PNA containing modified cyclohexyl monomer **78** in the 1*S*,2*R* configuration binds preferably to RNA.[231] In the case of hetero-containing‡ PNA sequences, the introduction of monomers **78** having both the 1*S*,2*R* and 1*R*,2*S* configurations led to a considerable discrimination of the DNA/RNA binding.[232] As opposed to *cis*-cyclohexyl analogues **78**, the introduction of *cis*-cyclopentyl-containing monomers **79** into oligothymine PNA led to a substantial stabilization of complementary complexes.[233] The introduction of monomers *cis*-**79** into hetero-containing PNA sequences resulted in stabilization of complementary complexes; however, no noticeable discrimination of the DNA/RNA binding was observed.[234]

Another research group described carbocyclic monomer **80** containing a five-membered ring and substituents in the *trans* orientation (1*S*,2*S* configuration) (Scheme 13).[235]

(−)-Dimenthyl succinate was used as the starting compound in the synthesis of monomer **80**. The key steps of the synthesis involve the asymmetric alkylation of the starting compound with propane-1,3-diol ditosylate resulting in the construction of the cyclopentane ring followed by the separation of diastereomers and the formation of mono-Boc-protected 1,2-diaminocyclopentane. The method for the synthesis of mono-Boc-protected 1,2-diaminocyclopentane was substantially improved later on, and the corresponding cytosine-, adenine-, and guanine-containing monomers were prepared.[236] More recently, carbocyclic

‡ Hetero-containing sequences are sequences consisting of different heterocyclic bases.

Scheme 13



80: $n = 1$;
81: $n = 0$.

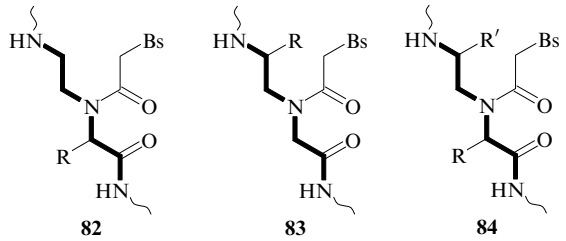
R = Me, Et.

monomer **81** containing the propane ring and substituents in the *trans* orientation (1*S*,2*S* configuration) was synthesized according to the same scheme.[237] The inclusion of monomers **80** containing the 1,2-substituted five-membered ring (1*S*,2*S* configuration) into PNA led to stabilization of complementary PNA/DNA duplexes, triplexes, and quadruplexes.[238] In the study,[239] it was shown that the introduction of a modified monomer into the PNA probe sequence led to an increase in the sensitivity and selectivity of the DNA detection, which made it possible to decrease the length of oligomers necessary for the detection.[240]

To summarize this section, it should be noted that due to extensive investigation of conformationally restricted PNA, tens of new various compounds were designed. In addition to the above-considered monomers, let us mention monomers containing the azetidine ring[241] and 1,1-substituted cyclopentane derivatives.[242] Unfortunately, most of modifications led to destabilization of complementary complexes. Nevertheless, in some cases either the discrimination of the PNA/DNA/RNA binding or an increase in the thermal stability of complementary complexes was achieved. In spite of certain interesting results, the complexity of the synthesis of monomers is a limitation to the wide use of conformationally restricted PNA. In recent studies[243,244] great attention has been paid to oligomers constructed from simple monomers, whose synthesis is not cumbersome, as well as to analogues prepared by the template-dependent enzymatic synthesis from triphosphates of modified monomers or PNA assembled on a template.

3. Other substituted, including positively charged, peptide nucleic acid derivatives

As mentioned above, in the absence of complementary oligomers, PNA do not have an ordered structure in solution.[170] One of the possible ways of increasing the thermal stability of complementary complexes and enhancing the specificity of the binding is to impart a helical conformation to a PNA oligomer by introducing substituents into the backbone with the simultaneous creation of chiral centres. The replacement can be performed in the glycine moiety (α-substitution, oligomers **82**), the aminoethyl moiety (γ-substitution, oligomers **83**), and in both positions (oligomers **84**).[193]

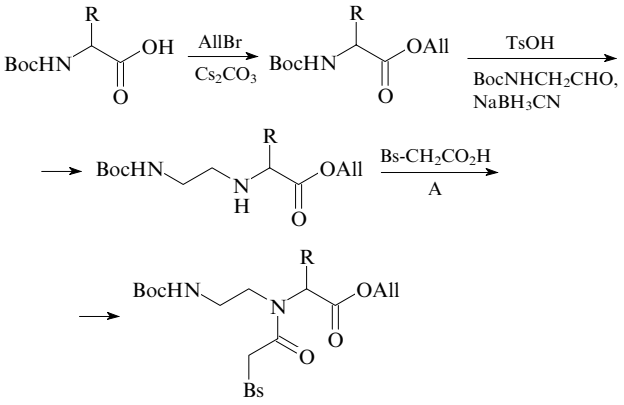


82 **83** **84**

R, R′ = Bu^{i}, $(CH_2)_4NH_2$, $(CH_2)_2CO_2H$, $(CH_2)_4NHC(NH_2)NH_2^+$, CH_2OH, *etc.*

Italian and Danish researchers performed a systematic investigation of substituted PNA. Chimeric PNA **82** containing chiral α-substituted thymine monomers were described in the study.[245] In this study, monomers were synthesized with the use of leucine, lysine, aspartic acid, and glutamic acid of the L and D series instead of glycine (Scheme 14).

Scheme 14



R = Bu^{t}, *etc.*; Bs-CH_2CO_2H is a carboxylated base; A is a condensing agent.

It was found that in the general case, the D configuration of amino acids is favourable for the formation of the right-handed helix of modified PNA in solution and, consequently, for the formation of complementary complexes with right-handed natural nucleic acids, whereas the L configuration substantially destabilizes complexes. The structure of the complementary complex of modified PNA containing three sequential D-lysine residues was studied in detail by X-ray diffraction and circular dichroism (CD) spectroscopy.[246,247] The synthesis of thymine-, adenine-, and cytosine-containing α-substituted D-lysine monomers was performed in the study.[248] D-Lysine residues facilitate exclusively the antiparallel orientation of complementary hetero-PNA complexes. It was shown[249,250] that D-lysine-containing analogues exhibit higher specificity in the hybridization analysis compared to PNA and DNA.

The so-called submonomer strategy can be used to increase the optical purity of modified PNA. In this strategy, a heterocyclic base is introduced after the addition of a versatile modified unit to the oligomer chain that grows during the solid-phase synthesis.[251] To additionally enhance the structuring of modified PNA, these authors synthesized γ-lysine-substituted derivative **83** and α,γ-lysine-substituted derivatives **84** containing two chiral centres.[252]

The step of reductive amination in the synthesis of γ-substituted derivatives (see Scheme 14) was performed with the use of lysine-substituted aldehyde, which was prepared by the reduction of the corresponding *N*-methoxy-*N*-methylamide, instead of Boc-substituted aminoacetaldehyde. Heterocyclic bases were introduced after the inclusion of a submonomer into the growing oligomer chain. As opposed to α-substituted PNA, the formation of complementary complexes with γ-substituted derivatives is favoured by the L configuration of the lysine residue. In the case of α-substituted D-lysine-containing modified PNA, it was shown that short oligomers **82** consisting of 2–4 monomer units with D-lysine residues can be efficiently inserted into the DNA double helix.[253] This research group obtained interesting results providing evidence that short oligomers (decamers) can be used as efficient tools for hybridization analysis.[254,255]

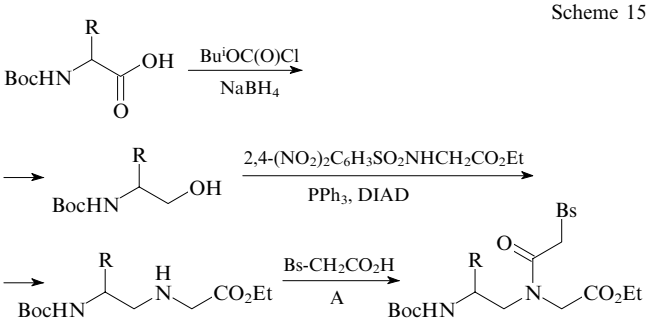
α-Substituted PNA **82** containing the guanidine residue were proposed for the use in the synthesis of cationic oligomers.[256] The synthesis of these compounds according to Scheme 14 was performed using arginine instead of glycine as the linkage. Oligothymine guanidine-containing decamer **82** specifically binds to DNA and efficiently penetrates into cells.[256] Later on, this research group synthesized compounds containing other heterocyclic bases, as well as compounds containing the arginine residue not only in the natural L configuration but also in the D configuration.[257] It was found that chimeric PNA composed of alternating PNA monomers and monomers bearing D-arginine residues specifically bind to RNA and penetrate into cells. Similar results were obtained for complexes with DNA in the case of heterosequences.[258] More recently, it has been shown that chimeric antisense oligomers composed of alternating PNA monomers and monomers bearing D-arginine residues specifically suppress the human E-caderin gene transcription and are less toxic than analogous PNA oligomers conjugated to arginine residues.[259]

Along with the above-considered α- and γ-substituted cationic and protonated derivatives, γ-substituted compounds **83** containing simple side substituents were synthesized. Modified PNA containing L-serine and L-alanine residues were prepared.[260] It was expected that the formation of hydrogen bonds by the hydroxy group of the side substituent in aqueous solutions would facilitate the ordering of the three-dimensional structures of chiral PNA. Aldehyde required for the synthesis was prepared according to the conventional scheme by the reduction of the corresponding *N*-methoxy-*N*-methylamide from the protected serine derivative. The detailed NMR spectroscopic study of the conformation of the modified alanine CT-PNA dimer in solution showed that the introduction of substituents in the L configuration at the γ position facilitates the formation of right-handed helical structures. The introduction of such modified monomers leads to an increase in the melting point of complementary PNA/DNA and PNA/RNA duplexes. It was found that γ-substituted PNA oligomers that have a right-handed helical structure in solution can be inserted into the DNA double helix.[261–263]

A new generation of guanidine-PNA was described.[264] As opposed to the compounds prepared earlier with the use of D-arginine in such a way that the substituent is attached at the α position (oligomers **82**, see Scheme 14),[257] new analogues were synthesized with the use of L-amino acid, and the substituent was finally attached at the γ position (oligomers **83**). In the study,[264] the backbone of the monomer was constructed by the Mitsunobu reaction instead of the reductive amination with the corresponding aldehyde. The condensation of the corresponding alcohol with a

protected glycine derivative leads to an increase in the optical purity of the reaction product (Scheme 15).

Scheme 15



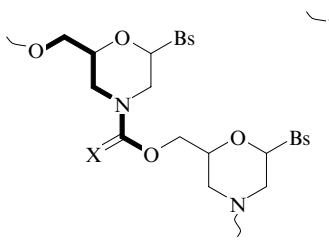
R = $(CH_2)_4NHC(NHCbz)(NCbz)$ (Cbz is benzyloxycarbonyl); DIAD is diisopropylazidodicarboxylate.

The introduction of the guanidine moiety at the γ position led to a substantial increase in the thermal stability of the complementary complexes compared to the earlier studied compounds containing the guanidine substituent in the α position.

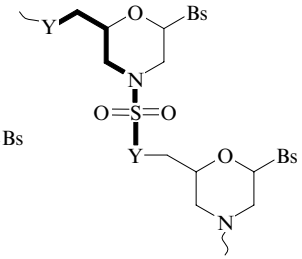
To summarize, the synthesis of monomers of chiral PNA **82**–**84** is facile, and the synthesis of oligomers is analogous to that of PNA. Nucleic acid mimetics show great promise in the hybridization analysis and the regulation of gene expression.[255, 259, 265] The introduction of side substituents containing different functional groups into PNA provides additional possibilities for the binding of reporter (signaling) compounds to OA [265] and for the solution of other problems, for example, the problem of t chemical ligation of PNA oligomers.[266]

4. Morpholino oligomers

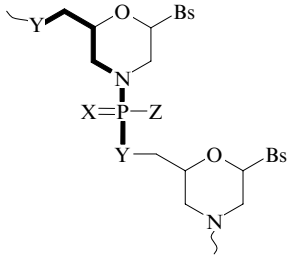
Among nucleic acid mimetics, morpholine analogues of oligonucleotides **7** are most advantageously employed in the regulation of gene expression. Initially, US researchers used oligomers containing different linkages between monomer units, such as (thio)carbamate (**85**), sulfamate, sulfamide (**86**), and phosphoryl (**87**) linkages.[267]



85 (X = O, S)



86 (Y = O, NH, NMe)

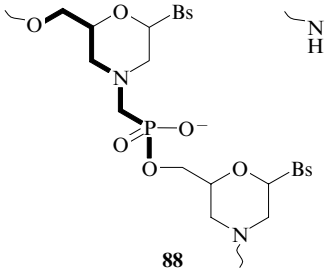


87 (X = O, S; Y = O, NH, NMe; Z = $NAlk_2$).

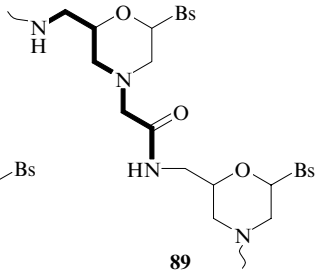
However, later on dimethylaminomorpholinophosphate derivatives **7** were recognized as most useful compounds in terms of the efficiency of the synthesis, solubility in water, and the ability to form complementary complexes with natural nucleic acid in spite of the fact that these compounds contain an additional chiral phosphorus atom, due to which this type of oligomers usually exist as racemic mixtures of 2^n diastereomers, where n is the number of phosphate groups.[268]

Recent advances in the use of morpholino nucleic acid mimetics as antisense agents are summarized in the reviews.[269, 270] Morpholino OA are particularly successful tools for the regulation of gene expression in the course of the embryonic development, mainly *Danio rerio*, of various organisms.[271] Certain antisense morpholine OA are currently under clinical trials.[27]

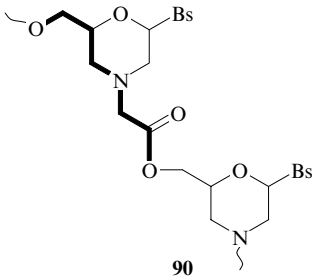
Dimethylaminomorpholinophosphate OA **7** are commercially available. Studies on the development of morpholino OA containing other linkages between monomers were relatively scarce in the last decade. The synthesis of morpholino monomers and the corresponding achiral oligomers **88**–**90** containing methylenephosphonate, methylenecarboxamide, or methylenecarboxylate linkages between monomers was documented.[272]



88



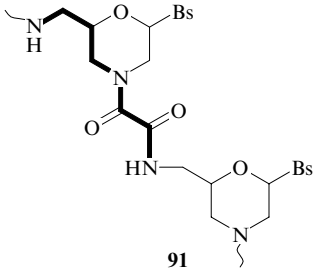
89



90

It was found that the presence of a phosphonate bond leads to a substantial destabilization of complementary complexes with DNA, whereas the introduction of carboxamide or carboxylate groups results in an increase in the melting point of complementary complexes or only in a slight decrease in stability compared to natural compounds.

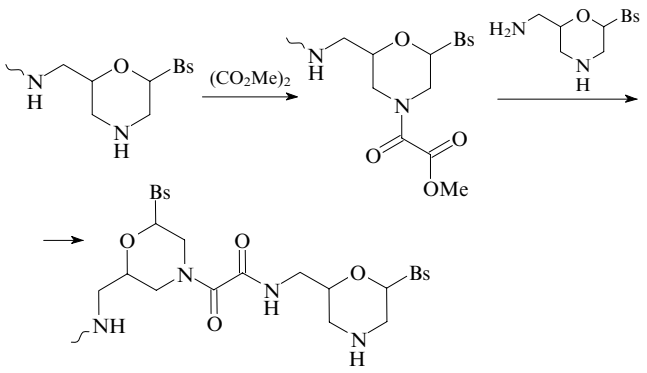
A new type of morpholino nucleic acid mimetics **91** containing the oxalyl diamide linkage was described.[273]



91

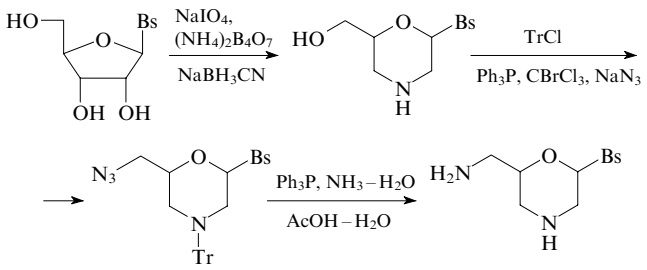
It is interesting that these oligomers can be synthesized without protection of amino groups of heterocyclic bases (Scheme 16). This fact substantially simplifies the procedure for the preparation of oligomers.

Scheme 16



Recently, a simplified scheme has been developed for the synthesis of aminomethylmorpholino monomers starting from non-protected ribonucleosides (Scheme 17).

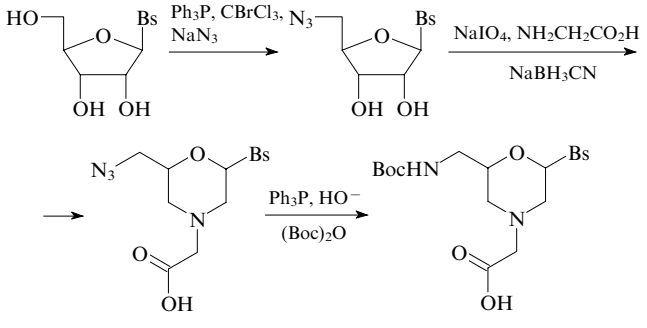
Scheme 17



Bs = U, A, C, G.

All for Boc-protected 2-aminomethyl-4-carboxymethylmorpholino monomers necessary for the synthesis of oligomers **89** (Scheme 18) were also synthesized.[274]

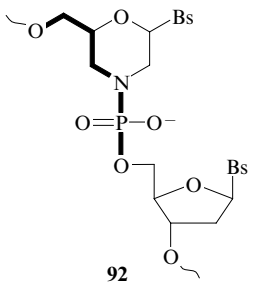
Scheme 18



Bs = U, A^{Bz} (6-*N*-benzoyladenine), C^{Bz} (4-*N*-benzoylcytosine), G^{Bu^i} (2-*N*-isobutyrylguanine).

The authors of the study[274] intend to continue research on the synthesis of methylenecarboxamide morpholino OA.

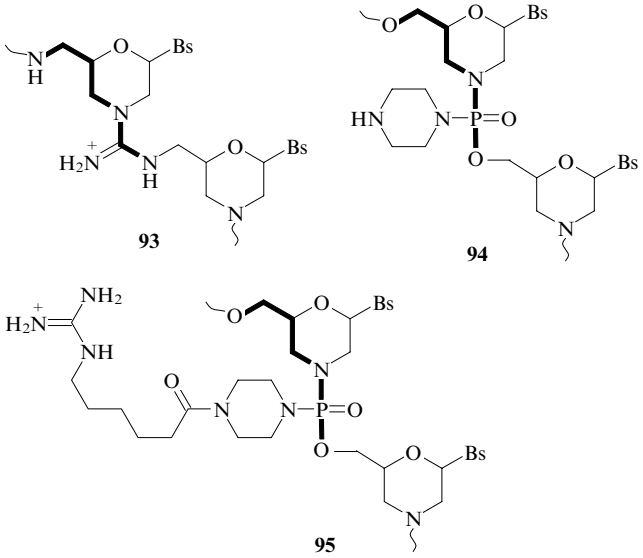
It is known that monoesterified phosphoric acid monoamides are easily hydrolyzed in weakly acidic conditions (pH 4.0). Nevertheless, Chinese researchers have recently prepared chimeric oligodeoxyribonucleotides **92** containing morpholinophosphate units.[275]



92

A modified unit was introduced into molecules according to the routine phosphoramidite method with the use of a 2-cyanoethyl-*N*,*N*-diisopropyl-*N*-morpholino phosphoramidite derivative. Chimeric oligodeoxyribonucleotides are stable in aqueous solutions at pH > 7.0. Later on, these researchers prepared chimeric oligoribonucleotides and used them to study RNA interference in mammalian cells.[276]

Recently, new positively charged morpholino nucleic acid mimetics **93**–**95** protonated at physiological pH have been synthesized.



93 **94**

95

Chimeric oligodeoxyribonucleotides **93** containing one modified morpholine unit with a guanidinium linkage were described.[277] It should be noted that the introduction of a modified unit at the 3′ end of the oligomer leads to a slight stabilization of the complementary duplexes, whereas the introduction of the unit at the centre of the oligomer results in a substantial decrease in stability. However, modified complementary triplexes are much more stable at pH 6.0–7.0 than non-modified triplexes. Another research group synthesized piperazine- (**94**)[278] and guanidine-containing cationic (**95**)[279] morpholino oligomers, which are protonated at physiological pH. Guanidine-containing nucleic acid mimetics **95** more effectively inhibit *in vitro* and *in vivo* growth of the bacteria *Escherichia coli* compared to protonated piperazine-containing compounds **94**. The conjugation of morpholino nucleic acid mimetics **94** and **95** to membrane-penetrating peptides contributes to the efficiency of the inhibition.

Taking into account that morpholino nucleic acid mimetics are easy to synthesize and that these compounds have found wide use in molecular biology and medicine,[280] it can be expected that studies on the synthesis of these analogues will be continued in the nearest future.

V. Conclusions

The present review summarizes main advances in the design of OA optimized primarily for the use as antisense agents for the regulation of gene expression and genetic information analysis. Only a few attempts were made to perform a direct experimental comparison of the antisense action of particular modification of oligonucleotides using cell culture model systems (see, for example, Refs 281 and 282). For instance, an international research team studied the influence of 21 types of chemical modifications on the activity of siRNA duplexes in the suppression of eGFP expression in the HeLa cell culture model system.[282] A total of 2160 duplexes were investigated, and 134 siRNA analogues, which are more active than natural siRNA, were identified, among which only 26 analogues did not impair cell survival. The authors stated the rules for the chemical functionalization favourable for an increase in the activity of modified interfering RNA and a decrease in their cell toxicity. Evidently, the mechanism of RNA interference is only one of the possible mechanisms of gene expression regulation. It is difficult to imagine how much experimental work will be required for the direct experimental examination of other approaches, for the comparison of the results, for the passing from model systems of the eGFP expression to real problems and living organisms. Besides, the optimal structures of OA and nucleic acid mimetics will be apparently changed depending on the problem at hand.

The variety of problems faced by molecular biologists is determined by the need in different types of chemical structures of OA. The diversity of the already available derivatives is limited only by the imagination and experience of synthetic chemists. Oligonucleotide analogues considered in Section II, particularly compounds **18**–**27** and **29**, which do not contain an internucleoside phosphate group (non-ionic and positively charged OA), are more complex from the synthetic point of view than compounds described in Section III. In the latter case, the problems of the synthesis of oligomers are associated mainly with the development of efficient routes to required monomers in sufficient amounts. To deliver negatively charged OA to cells, additional special means are required.[31–34] This substantially interferes with the use *in vivo* of these analogues. Nucleic acid mimetics considered in Section IV generally require great efforts both in the synthesis of monomers and the assembly of monomers to an oligomer sequence. These efforts are justifiable only if these oligomers are much more efficient (at least by a factor of tens or hundreds) than other types of analogues. Further direct comparative investigations on the application of OA and nucleic acid mimetics for the regulation of gene expression and diagnostics may help in formulating criteria of the efficiency of action.

In our opinion, the data on DNA/RNA therapy available in the scientific literature (5000–7000 publications per year according to the PubMed database during 2005–2008)[27] do not allow one to make an unambiguous decision in favour not only of a particular structure but even in favour of a particular type of oligonucleotide analogues. Nevertheless, in terms of the possible use of OA and nucleic acid mimetics in therapy of infectious and hereditary diseases, one of the possible criteria for the oligomer structure will be the cost and benefits of future drug production. This is why only compounds which can be produced on an industrial scale reached the stage of clinical trials. Morpholino nucleic acid mimetics belong to the most efficient oligomers in experiments on the regulation of gene expression.[270] The synthesis of monomers for these analogues is facile, and the starting compounds (ribonucleosides) are available. In our opinion, efforts aimed at the design of new types of morpholino OA may be fruitful.

This review has been written with the financial support of the Federal Programme 'Leading Scientific Schools' (Grant NSh-3185.2010.4) and the Integration Interdisciplinary Project of the Siberian Branch of the Russian Academy of Sciences No. 88.

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