

Review

# A Visual Compendium of Principal Modifications within the Nucleic Acid Sugar Phosphate Backbone

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**Abstract:** Nucleic acid chemistry is a huge research area that has received new impetus due to the recent explosive success of oligonucleotide therapy. In order for an oligonucleotide to become clinically effective, its monomeric parts are subjected to modifications. Although a large number of redesigned natural nucleic acids have been proposed in recent years, the vast majority of them are combinations of simple modifications proposed over the past 50 years. This review is devoted to the main modifications of the sugar phosphate backbone of natural nucleic acids known to date. Here, we propose a systematization of existing knowledge about modifications of nucleic acid monomers and an acceptable classification from the point of view of chemical logic. The visual representation is intended to inspire researchers to create a new type of modification or an original combination of known modifications that will produce unique oligonucleotides with valuable characteristics.

**Keywords:** artificial nucleic acid; antisense; oligonucleotide; chemical modification; synthetic biology



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## 1. Introduction

Natural nucleic acids, DNA and RNA, form the basis of all living beings. These molecules are not only involved in the storage, transmission, and reproduction of information but also perform various, sometimes quite complex, functions from the catalysis of chemical reactions to the control of gene expression [1]. The formation of complex three-dimensional structures due to complementary interactions of nitrogenous bases is a fundamental property of these natural polymers. The prospect of influencing processes determined by this property gave impetus to the development of oligonucleotide chemistry, from its methodological aspects, synthetic schemes, and automated protocols to its wide range of applications.

The study on the structure and properties of nucleic acids allowed one to decode the human genome and understand the nature of many hereditary diseases. Oligonucleotide sequences capable of interacting with natural nucleic acids have enormous potential as means for studying biological systems and processes, diagnostic tools, and new biomedical products. However, the use of oligonucleotides based on natural nucleic acids has its own limitations, including insufficient resistance to enzymatic degradation in biological media caused by the action of nucleases that cleave phosphodiester bonds, low bioavailability, and the joint use of specific delivery systems [2]. To solve these problems and impart the required properties to polymers, the structural unit of natural nucleic acids is subjected to modification, and this subsequently led to the formation of a new direction of research [3].

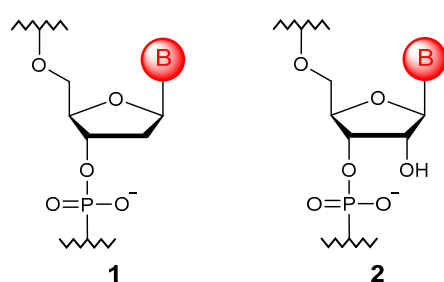
Modified oligonucleotides, or artificial genetic polymers, have become an essential instrument in such therapeutic approaches as RNA interference [4], aptamer binding [5], and CpG oligonucleotide immunostimulation [6]. CRISPR/Cas9 genome editing has gained enormous popularity with the application of modified guide RNAs [7,8]. However, the most fruitful approach, which resulted in the first FDA-approved oligonucleotide drug, is the antisense oligonucleotide (ASO) principle [9]. While the therapeutic effect of ASOs

is primarily associated with the cleavage of target mRNA by the RNase H enzyme, these short-strand nucleotide analogs can block mRNA processing or translation upon binding, form triple helices with DNA, prevent splicing or destabilize pre-mRNA, and attach to miRNAs, thus inhibiting transcription and affecting gene expression. The ASO strategy extensively recruits nucleic acid modifications to increase efficacy, enzymatic stability, and decrease the immune response and off-target toxicity of the developed oligoes [10].

This review is aimed at systematizing information about the currently known principal structural modifications of the sugar phosphate backbone of natural nucleic acids. These modifications, either alone or in combination, can impart artificial polymers with the desired properties and level out the drawbacks of oligonucleotide chains to provide wider experimental and medical applications.

## 2. Natural Nucleic Acids and Modification Directions

Nucleic acids (NAs) are biological polymers whose monomer units are nucleotides (Figure 1). Nucleotides have a similar structure in both DNA (1) and RNA (2): a nitrogenous base, a pentose sugar, and a phosphate group. While the RNA monomer is based on  $\beta$ -D-ribose, the DNA chain is built on  $\beta$ -D-deoxyribose units linked by phosphodiester bonds. The pentose ring, through a glycosidic bond, carries one of five possible nitrogenous bases, with three (adenine, guanine, and cytosine) being common to DNA and RNA and the other two found only in a specific NA (thymine in DNA; uracil in RNA). These five nitrogenous bases are usually called major nucleobases since many naturally occurring modified ribonucleosides can be found in tRNA, rRNA, mRNA, and lncRNA molecules [11].



**Figure 1.** The structure of DNA (1) and RNA (2) monomer units.

The finite set of nitrogenous bases, the instability under the action of nucleases, and specific physicochemical characteristics of natural nucleic acids have inspired researchers to search and develop analogs with certain properties that allow one to more fully utilize the inexhaustible potential of these unique biomolecules for both biomedical and research purposes. Over 50 years of intensive research in the field of oligonucleotide chemistry has made it possible not only to artificially synthesize natural nucleic acid oligomers but also to develop synthetic approaches for modifying their main structural elements. The proposed review is an attempt to systematize knowledge about the nucleotide analogs synthesized to date and identify and classify the basic modifications affecting the sugar and phosphate moieties in oligomers. This review does not discuss modified nitrogenous bases and their artificial analogues; achievements in this area of research can be found in the following papers [12,13].

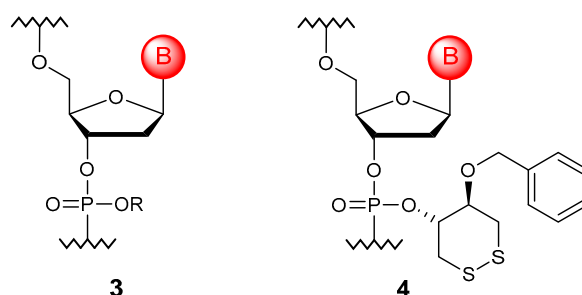
## 3. Phosphate Modifications

The anionic nature and poor metabolic stability of the natural phosphodiester group are two main problems when using oligonucleotides as therapeutic agents. In this regard, modification of the phosphodiester group has historically become the first modification aimed at both increasing resistance to nucleases and overcoming biological barriers such as cell membranes. The types of modifications given below are used specifically for constructing oligomeric chains; however, there are also modifications that allow crosslinking oligomers to each other. Such modified NAs, such as borono-analogs [14], can formally be

considered carrying a substitution for the phosphodiester bond, but the functional purpose of this modification is different.

### 3.1. Phosphotriesters

The main purpose of esterification (Figure 2) is to remove the negative charge from the nucleic acid backbone. It is believed that such derivatives (3) can hybridize with natural NAs, forming more stable duplexes due to the elimination of electrostatic repulsion [15]. To date, not only ethers, such as methyl, ethyl, isopropyl, and others [16,17], but ether modifications carrying functional groups [18], polyethylene glycol chains [19], fluorescent labels [20], and hydrophobic and cationic substituents [21] have been obtained. However, such a modification acquired its main practical significance in the context of delivering oligonucleotides into the cell: prodrug-type modifications (4) that impart not only resistance to the action of enzymes but also increase uptake by the cell were developed [22–24].



**Figure 2.** General structure of the phosphotriester monomer unit (3) and selected phosphotriester modification (4).

Interestingly, the phosphotriester modification can be obtained by reducing borane phosphonate oligonucleotides with metal ions [25]. In addition, the elementary units themselves can be used as esterification agents; thus, branched oligonucleotides could be obtained [26].

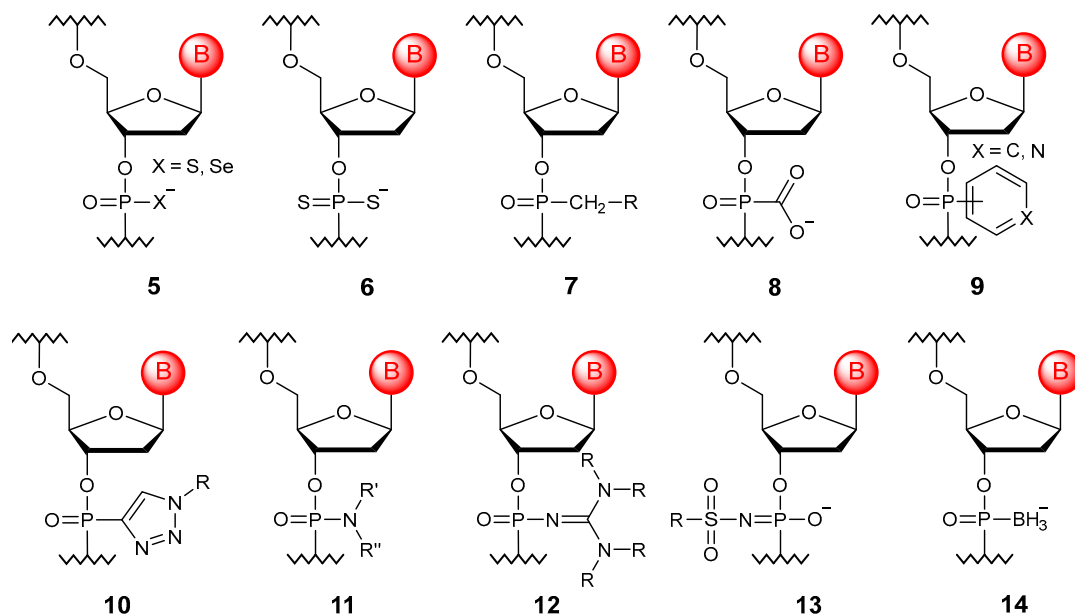
### 3.2. Substitution of Non-Bridging Oxygen Atoms

The non-bridging oxygen atom can be replaced with sulfur or selenium to obtain phosphorothioate [27] and selenoate [28] monomers (5), which allows oligonucleotides with such modifications to impart resistance to nucleases (Figure 3). Despite the frequent representation as =S, it was shown that sulfur does not form a double bond [29], so cationic oligonucleotides could be obtained [30]. Due to the retention of a negative charge on each internucleotide function, phosphorothioate derivatives are the closest analogues of natural oligonucleotides. However, such a substitution leads to chirality on phosphorus, and, therefore, approaches to the stereoselective synthesis of such derivatives have been developed [31,32].

If both non-bridging oxygens are replaced by sulfur, phosphorodithioate monomers (6) are obtained [33]. Such compounds are not chiral and are absolutely resistant to the action of all known nucleases. However, they are not widely used in the development of potential drugs since such a substitution results in the nonspecific binding to partially complementary DNA as well as to proteins. Most often, phosphorodithioate oligonucleotides are used in the design of aptamer libraries [34].

Another significant modification is the substitution of oxygen with carbon (7), primarily the methyl group ( $R = H$ ) [35]. Oligonucleotides containing such nonionic bonds are resistant to degradation by cellular nucleases and are consumed by cells unchanged. At the same time, alkylphosphonates can be obtained enzymatically [36]. Alkyl can be used to attach some functional groups to phosphorus. So, to reduce the total negative charge, cationic units, such as alkylaminophosphonates [37,38], can be introduced into the oligomer structure. On the other hand, since the loss of the negative charge leads to poor solubility and the aggregation of oligomers in aqueous solutions, negatively charged

modifications of non-bridging oxygen that remain resistant to nucleases were developed. The carboxyl group can be linked to phosphorus through a methylene bridge, such as in phosphonoacetates [39], or directly attached to the phosphorus atom to obtain phosphonoformates (8) [40]. In the form of neutral esters, these modified oligonucleotides are consumed by cells through passive diffusion.



**Figure 3.** Structures of DNA monomer units with non-bridging oxygen modifications (5–14).

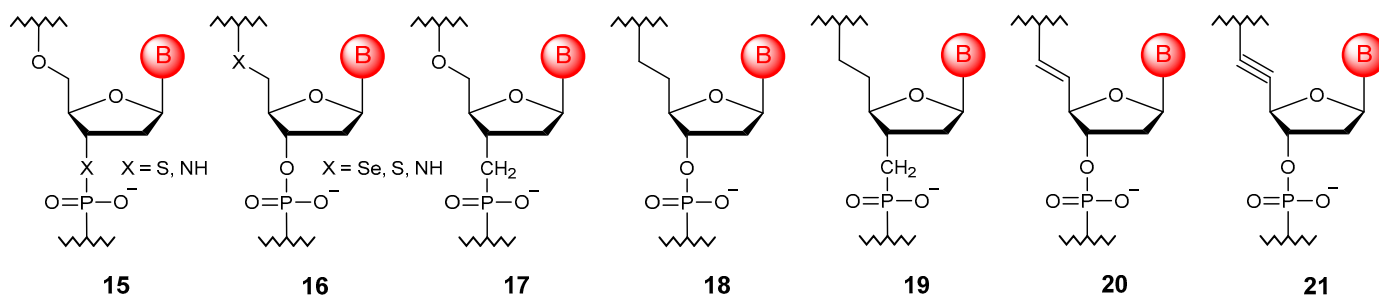
In addition, other derivatives, such as phenylphosphonates and pyridylphosphonates (9) [41,42], as well as triazolylphosphonates (10), were synthesized. The latter are obtained from alkynylphosphonates via the click reaction [43]. The binding of phosphorus to the aromatic system makes oligonucleotides even more resistant to nucleases, probably due to their greater steric hindrance.

However, phosphoramidates (11) are the most widespread modified oligonucleotides. The non-bridging oxygen atom has been substituted with nitrogen to obtain various derivatives, starting from alkyl phosphoramidates [44], triazinyl phosphoramidates [45], and phosphoramidates with cationic substituents [46], including those containing guanidine groups [47]. Although phosphoryl guanidines (12) [48] are structurally close to phosphoramidates, the prospects of combining these oligonucleotides with phosphorothioate modifications for different therapeutic applications were repeatedly noted, while at least three stereo-regular mixed backbone phosphorothioate-phosphoryl guanidine oligonucleotides entered clinical trials in 2021 [49]. Another practically valuable group of modifications that should be mentioned here is substituted sulfonyl phosphoramidates (13), among which mesyl phosphoramidates ( $\text{R} = \text{CH}_3$ ) turned out to be promising for replacing phosphorothioate groups in ASOs [50]. Such modified oligonucleotides have shown significant advantages over commonly used phosphorothioates in their affinity to RNA, nuclease stability, and specificity of antisense action [51].

Separately, it is worth mentioning the boranophosphate modification (14) [52], which can be considered a functional hybrid of phosphate, phosphorothioate, and methylphosphonate fragments. In such oligonucleotides, the borane group is isoelectronic to O and S, and the internucleotide group is negatively charged like the phosphodiester group in native and phosphorothioate polymers. The boranophosphate group is also isostructural with the diester moiety of nuclease-resistant methylphosphonates. In addition, oligonucleotides modified in this way can be used as precursors to introduce other substitutions of one or both non-bridging oxygen atoms [53].

### 3.3. Substitution of Bridging Oxygen Atoms

Another type of phosphate group modification is the replacement of bridging oxygens at positions 3' (15) or 5' (16) with selenium, sulfur, or nitrogen atoms to produce their corresponding selenophosphates [54], thiophosphates [55], and phosphoramidates [56,57]. The main advantage of such substitutions is an increase in the resistance to nucleases without the appearance of a chiral center (Figure 4). An interesting fact is that the unnatural 3'-N-P bond can be formed naturally by a modified DNA polymerase [58].



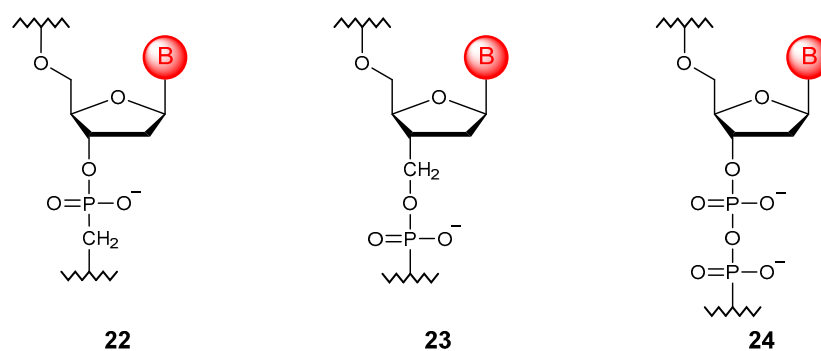
**Figure 4.** Structures of DNA monomer units with bridging oxygen modifications (15–21).

One can also replace the oxygen atom with carbon. In this case, it is also possible to synthesize both 3'-methylene phosphonate (17) [59] and 5'-methylene phosphonate oligomers (18) [60], while the replacement of both atoms allows one to obtain bismethylene phosphinates (19) [61]. Other possible carbon substitutions of the bridging oxygen atom are represented by vinyl phosphonate oligonucleotides (20), for which it is possible to vary the configuration due to E/Z isomerism [62], as well as alkynyl phosphinate oligonucleotides (21), which allow one to improve the duplex-stabilizing properties due to the leveling of protons in position 6' repelling the nitrogenous base [63]. However, formally, these substitutions already affect the sugar residue.

In practice, it is quite rare to find only one modification; most often, combinations of bridged and non-bridged substitutions are used. The most common oligonucleotides are thiophosphoramidate ones [64].

### 3.4. Phosphate Linkage Extension

The phosphate unit can be elongated by adding the methylene group to the chain (Figure 5). In this way, it is possible to obtain methylene phosphonate derivatives (22) and (23), which have significant resistance to nucleases but retain the ability to form duplexes with natural NAs [65,66]. Another method of extension is to repeat the phosphoester unit. Such fully modified diphosphodiester oligomers (24) retain the ability to undergo complementary interactions and exhibit significant stability [67].



**Figure 5.** Structures of phosphate-extended DNA monomer units (22–24).

#### 4. Sugar Linking Backbone Modifications

Modifications in this section affect both the phosphate group and the sugar residue. Oligonucleotides modified in this way most often do not retain their 3' and 5' oxygen atoms, the presence of which is determined by the structure of ribofuranose. However, it is generally accepted to consider changes of this kind as internucleotide linkage modifications.

A large number of works are devoted to the development and use of deoxyribose and ribose backbone cross-links that are alternatives to the phosphate group. When making such substitutions, they are primarily guided by the total length, which should be close to the native one to preserve the possibility of complementary interactions, as well as by the useful physicochemical parameters of the proposed crosslinking. All such modifications can be divided into acyclic and cyclic modifications.

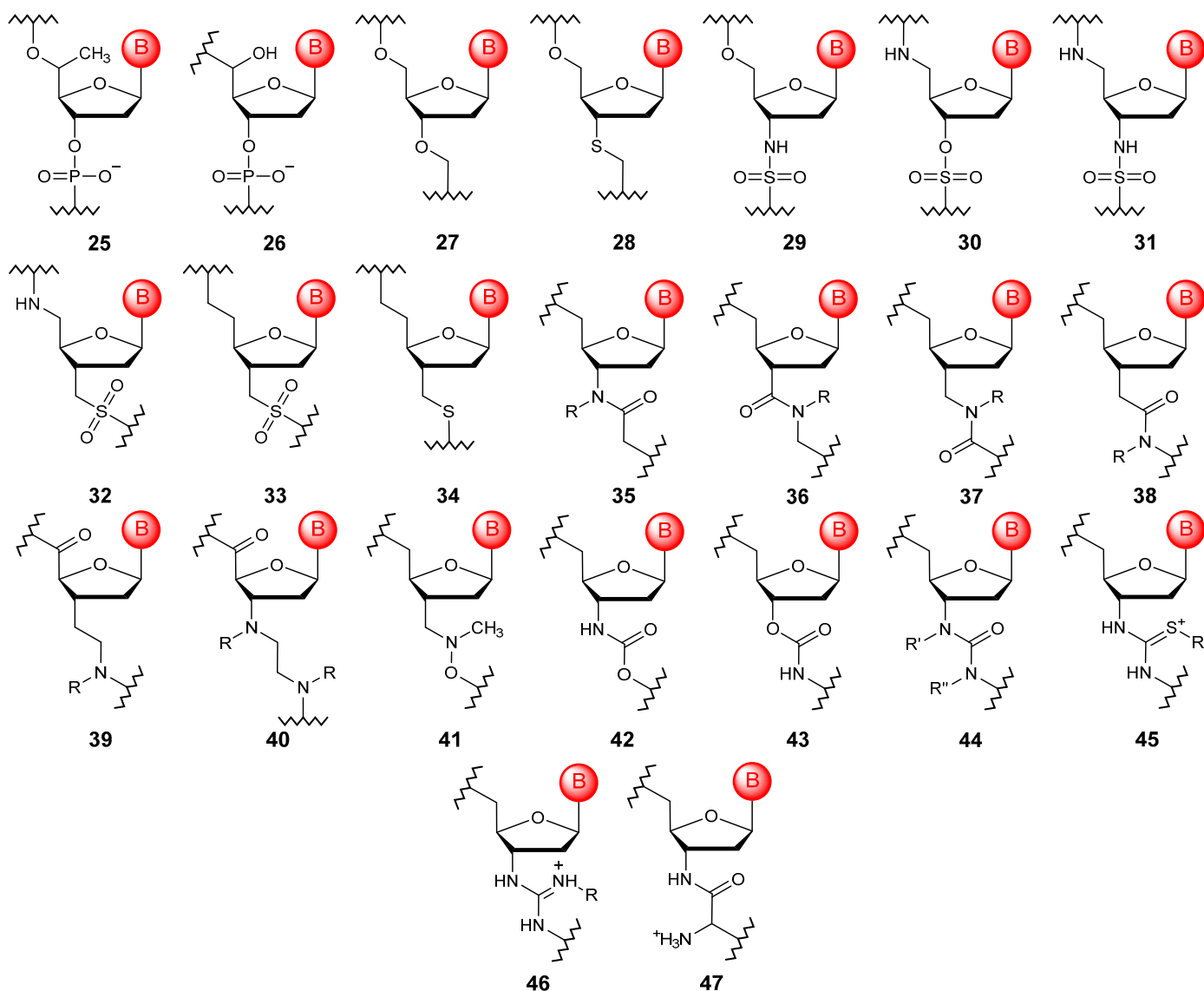
##### 4.1. Acyclic Linkages

Firstly, the introduction of a substituent to the carbon atom at position 5' should be considered an alternative linkage (Figure 6). In particular, the introduction of the methyl substituent (25) allows one to obtain modified oligonucleotides with characteristics more suitable for use in antisense therapy, although it leads to the appearance of a chiral center on the carbon atom [68,69]. To date, the introduction of other substituents has been described, but their use is limited to terminal positions in small interfering RNAs [70]. Minimal modification of the natural phosphate linkage allows the production of  $\alpha$ -hydroxyphosphonate oligonucleotides (26), which have marked differences in hybridization properties depending on the configuration of the 5' carbon atom [71].

The formal replacement of the phosphorus atom and two non-bridging oxygen atoms with a carbon atom allows the formation of oligonucleotides with formacetal linkage (27) [72]. Such a minor structural change can be easily introduced into the polymer without compromising the helical structure and the stability of duplexes and triplexes. The increased affinity of formacetal oligonucleotides to complementary RNA fragments can be further increased by substituting the 3'-bridging oxygen with a sulfur atom when forming a thioacetal linkage (28) [73]. Based on the replacement of the phosphorus atom with a sulfur atom, a variety of modified oligonucleotides with sulfamate (29, 30) [74,75], sulfamide (31) [76,77], sulfonamide (32) [78], sulfonic (33), and dimethylene sulfide (34) bridging groups [79], often having interesting characteristics, have been obtained. However, when creating modified polymers, amide fragments (35–40) are most often used as alternative acyclic linkages, allowing the internucleotide crosslinking to be configured in different ways [80–83].

An interesting non-phosphorus type of linear cross-linking is the methylene(methylimine) modification (41) [84]. This modification has many advantages, such as an achiral and neutral backbone, high affinity for RNA, and significant resistance to nucleases. Other acyclic linkages include carbamate variants (42–43), which can be found in oligonucleotides with locked units [85] and urea derivatives (44) [86], although these are considered too short to adopt the preferred oligonucleotide conformation in the duplex.

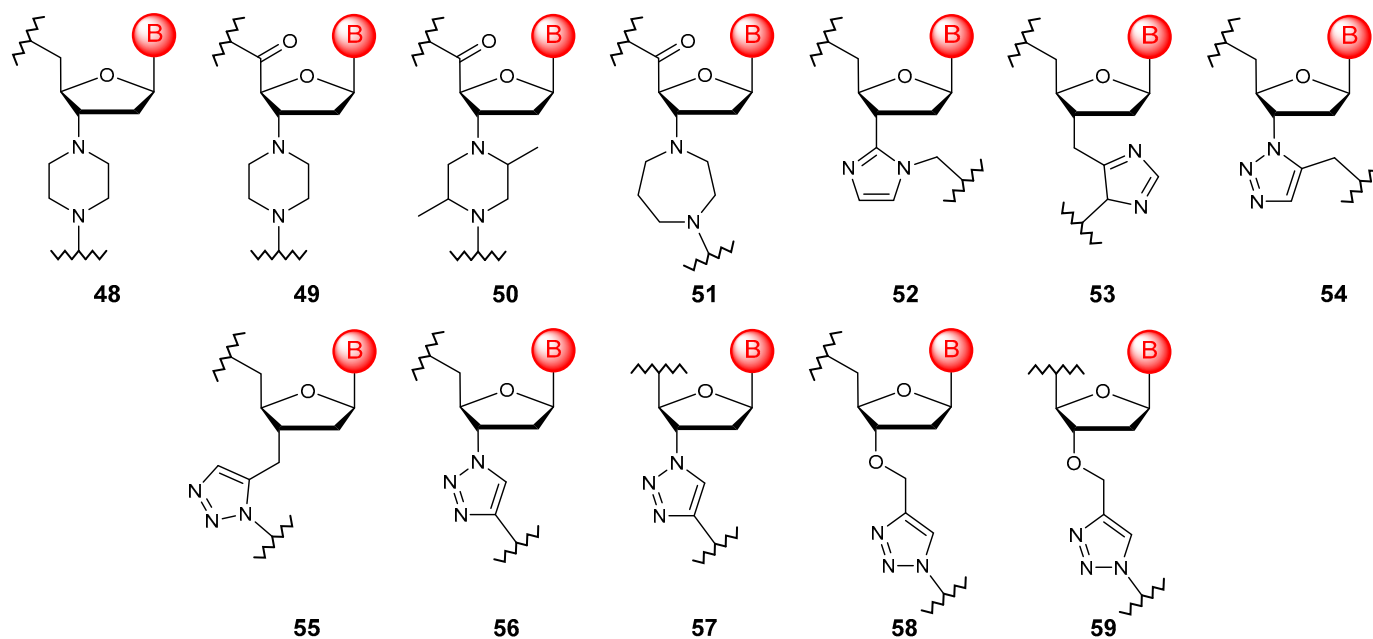
A complete replacement of the negatively charged phosphodiester bond is also possible with alternative positively charged structural motifs. The introduction of thiourea (45) [87] or guanidinium (46) [88] linkages into the structure of oligonucleotides, which are positively charged at physiological pH, leads to the formation of zwitterionic or even oligocationic backbone structures, and this significantly improves the absorption of such polymeric structures. Another positively charged modification of oligomers that touches on the internucleotide bond is the obtaining of nucleosyl amino acids (47), which can significantly increase the stability of the final polymers in biological media, such as blood plasma [89].



**Figure 6.** Structures of DNA monomer units with acyclic linkages (25–47).

#### 4.2. Cyclic Linkages

To date, there are not many examples of cross-linking nucleoside structures into a polymer using cyclic fragments (Figure 7). However, the use of such heterocyclic systems, such as piperazine (48–50) [90], homopiperazine (51) [80], imidazole (52, 53) [91], and triazole (54, 55) [92], for these purposes can be classified as such modifications. The last modification is interesting because, with the development of click chemistry, the number of options and the ease of introducing such internucleoside cross-linking have increased significantly (56–59) [93,94]. The introduction of a heterocyclic motif into the linkage structure does not provide any benefit in the stability, binding ability, and resistance of the final polymers. However, triazole linkages are still being used, in particular, in the synthesis of oligomers from locked units [95], precisely because of the development of such a synthetic approach. Moreover, the biocompatibility of 1,5-disubstituted triazole linkages with DNA polymerases have been revealed recently [96].



**Figure 7.** Structures of DNA monomer units with cyclic linkages (48–59).

## 5. Sugar Modifications

A reason for studying artificial NAs is to develop modified oligonucleotides with improved chemical and biological properties that allow them to function more efficiently than DNA/RNA in biomedical, biotechnological, and nanotechnological applications. The stability of natural oligonucleotide duplexes, as well as the formation of stable and functional protein–oligonucleotide complexes, is determined primarily by the conformation and dynamics of the sugar moiety. Thus, the ribofuranose modification in NAs is a widely used method for manipulating the activity of natural polymers.

### 5.1. Esterification of Free Hydroxy Groups in RNA

Currently, RNA-based drugs are being actively developed. However, RNA is inherently unstable, potentially immunogenic, and requires the use of special targeted delivery systems. The sensitivity of RNA to RNases is determined by the presence of the 2'-hydroxy group in the sugar ring, and therefore, modifications of the ribose fragment were primarily aimed at replacing this position.

The simplest modification of RNA is methylation at the oxygen atom of the 2'-hydroxy group (60), while a similar modification occurs in nature [97]. Oligonucleotides with the 2'-O-methyl modification have increased resistance to nucleases and affinity to target RNA, as well as a reduced immune response (Figure 8). Many ether modifications of the 2'-hydroxy group have also been proposed, including alkyl modifications (61) [98], those containing functional groups such as 2'-O-allylic (62) [99], 2'-O-cyanoethyl (63) [100], and 2'-O-acetalester (64) [101], positively charged groups that could compensate for the negative charge of the phosphate residue, for example, amine (65–68) [102–105] and guanidine (69) [106], as well as clickable modifications (70, 71) [107]. In this case, the most successful turned out to be the 2'-O-methoxyethyl modification (72), which allows improving therapeutically significant properties, namely increasing the affinity to RNA, resistance to nucleases, and thermal stability of the complexes [108]. This modification can be found in a number of FDA-approved oligonucleotide drugs [109].



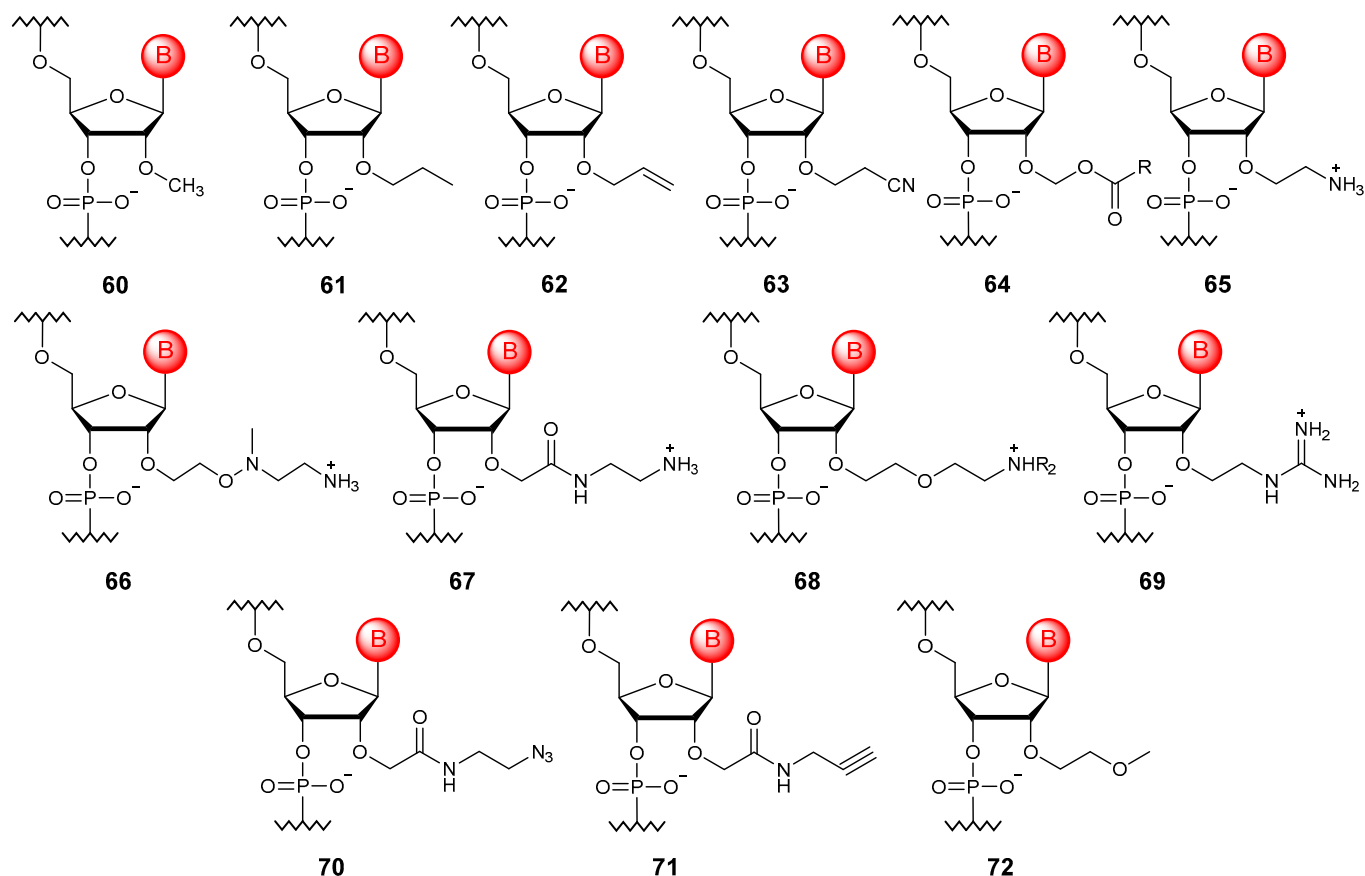


Figure 8. Structures of 2'-O-esterified RNA monomer units (60–72).

### 5.2. The Introduction of Substituents into the Ribose Ring

Although the natural DNA molecule is more stable than RNA due to the absence of the 2'-hydroxy group in the ribose ring, its stability is still not sufficient for clinical use. In this regard, it was proposed to introduce substituents, primarily those with high electronegativity, into this position (Figure 9) to increase not only stability but also affinity to RNA due to conformational factors [110].

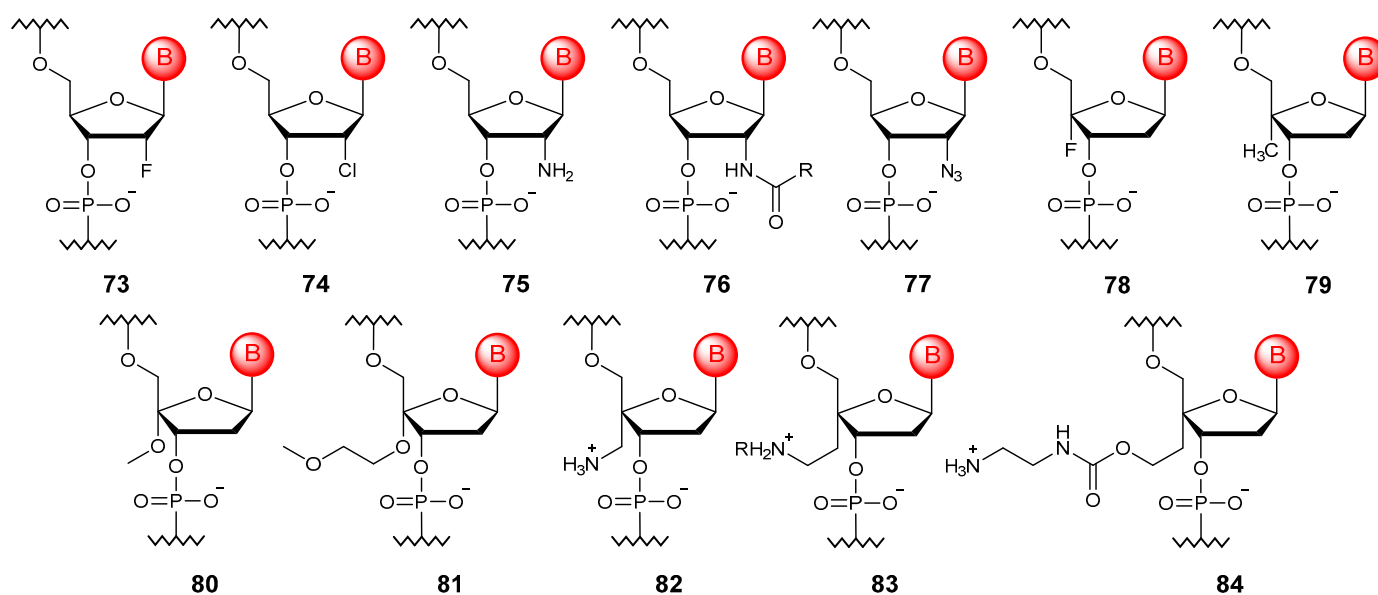


Figure 9. Structures of 2'- and 4'-substituted DNA monomer units (73–84).

Halogen atoms, in particular, fluorine (73) [111] or chlorine (74) [112], can be introduced into the ribose ring. Such halogen-modified oligonucleotides allow one to increase resistance to nucleases while also suppressing immune stimulation. The 2'-F modification is also often used to finetune the RNA-cleaving activity of modified ASOs [113]. Electron-donating substituents, namely the amino group and its derivatives (75, 76) [114], can also be introduced into the 2' position of the ribose ring, which stimulates a more DNA-preferred configuration of ribose. In this case, the introduction of azide (77) [115] is the 2' modification with the widest range of applications. Such modified oligonucleotides can be further functionalized with dyes or biotin and, like other 2'-substituted ones, be synthesized by modified DNA polymerases [112].

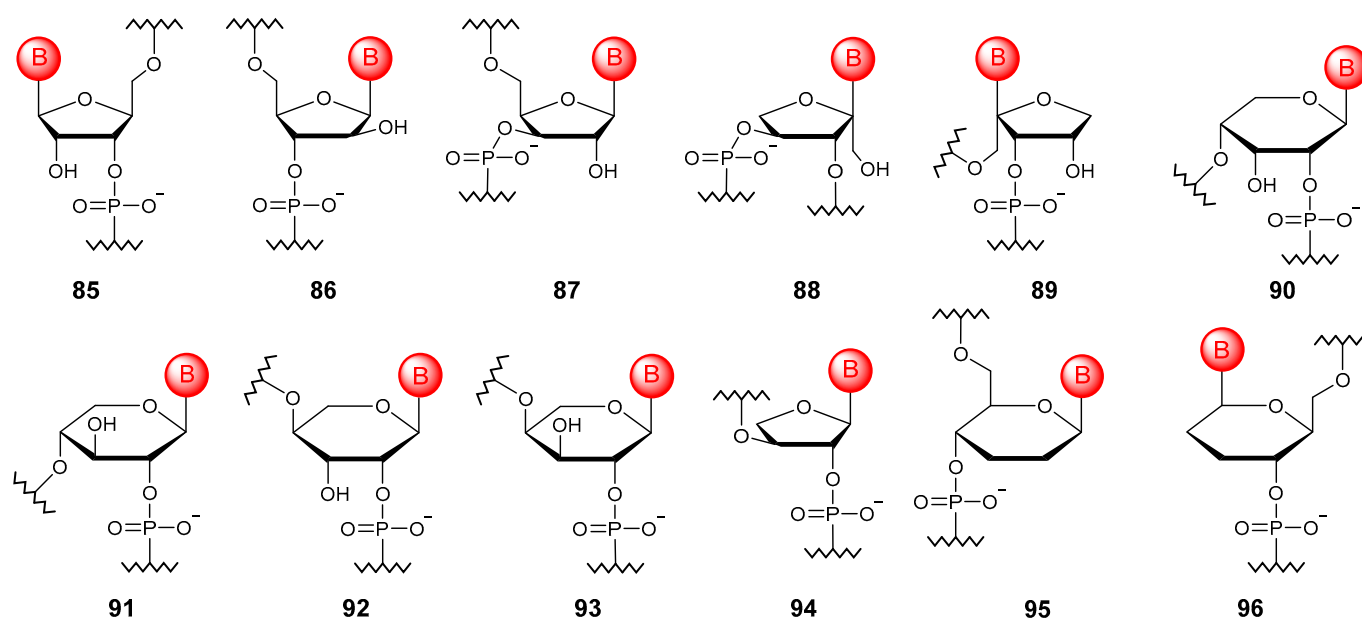
A number of 4' modifications of the ribose ring have also been described in the literature; these include the introduction of fluorine (78) [116], methyl (79), alkoxy groups (80, 81) [117], and amino alkyl substitutions (82–84) [118]. Oligonucleotides modified in this way represent DNA constructs that mimic the behavior of RNA. However, such modifications are most often used in combination with 2' modifications for more precise conformational adjustments of the ribose ring [119,120].

### 5.3. Alternative Sugar Moieties

A drastic way to change the geometry and conformational mobility of the sugar moiety is to replace the ribose ring with alternative cyclic and acyclic fragments. Such modifications are aimed at modulating specific interactions and properties of oligonucleotides, including the stability and recognition ability.

#### 5.3.1. Ribose Isomers

The natural structure of RNA is formed by  $\beta$ -D-ribofuranose, a cyclic form of D-ribose. If the enantiomer is used instead, that is, L-ribose (Figure 10), then the so-called spiegelmers (85) could be obtained. Such oligomers are non-immunogenic and practically resistant to enzymatic degradation; they do not interact with natural NAs but are widely used as aptamers [121]. It is also possible to obtain oligomers based on other isomers of ribose in the furanose form, including arabinose (86) [122] and xylose (87) [123], while the latter has an orthogonal pairing system.



**Figure 10.** Structures of NA monomer units based on ribose isomers (85–96) and other sugars (94–96).

Oligonucleotide chains can also be constructed using isomeric ketopentoses. Thus, nucleic acids based on  $\alpha$ -L-xylulofuranose (88) and  $\beta$ -L-ribulofuranose (89), although they

do not exhibit pairing ability, but the point introduction of such elementary units allows one to enhance the orthogonal base pairing properties of the chimeric oligonucleotide [124].

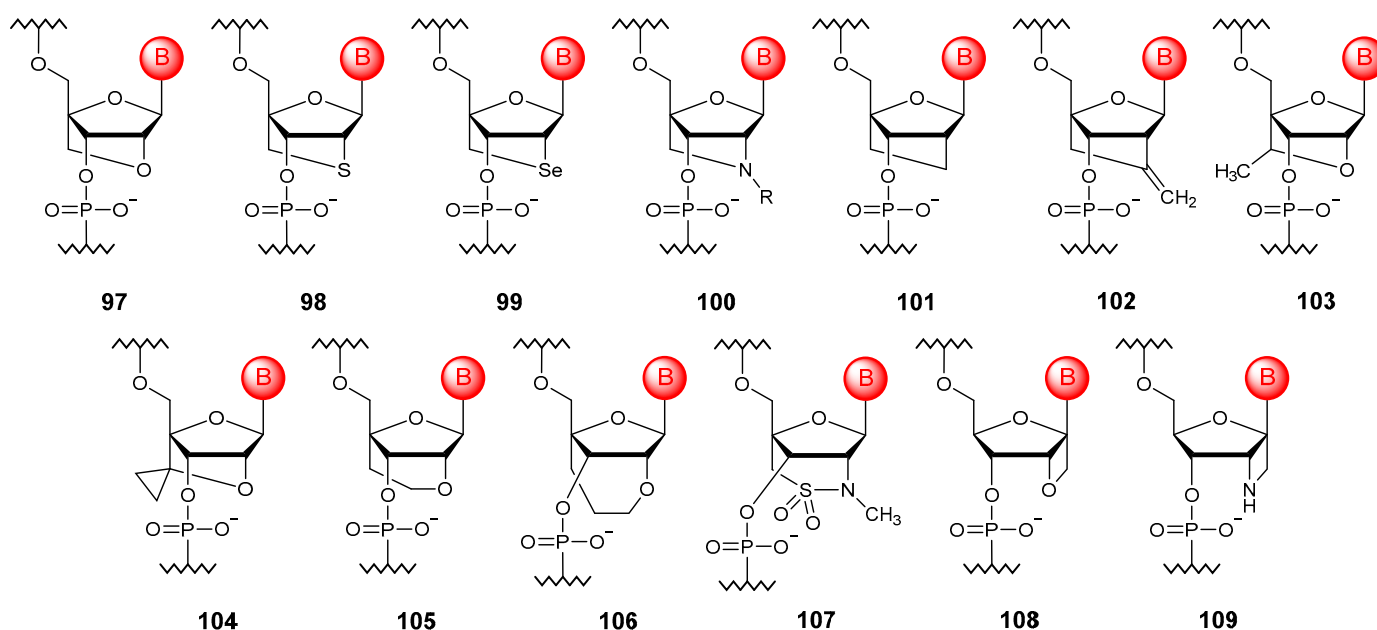
If we consider the pyranose form, it is possible to obtain oligonucleotides based on ribose (90), xylose (91), lyxose (92), and arabinose (93), which have pairing abilities [125].

### 5.3.2. Other Sugars

Other sugars such as threose and glucose can be used to construct the oligonucleotide chain (Figure 10). The threose NA (94) obtained in the first case is the simplest of all potential alternatives to natural NAs of the oligonucleotide type, capable of pairing with DNA and RNA and having greater hydrolytic stability compared with RNA [126]. Homo-DNA derived from 2',3'-dideoxy- $\beta$ -D-glucopyranose (95) does not tend to form helical structures [127], while  $\alpha$ -homo-DNA (96) can interact with RNA to form parallel duplexes [128].

### 5.4. Bridged Ribose Ring Modifications

To increase the affinity to RNA, a modification of the ribose ring consisting of a methylene bridge between 2'-oxygen and 4'-carbon (Figure 11) was proposed by two independent scientific groups [129,130]. This new type of oligonucleotide was called locked nucleic acids (97). The main idea of such a modification is to reduce the conformational mobility of ribose and increase the local organization of the carbohydrate-phosphate backbone, which leads to the formation of stronger duplexes with DNA and RNA [131,132].



**Figure 11.** Structures of bridged NA monomer units (97–109).

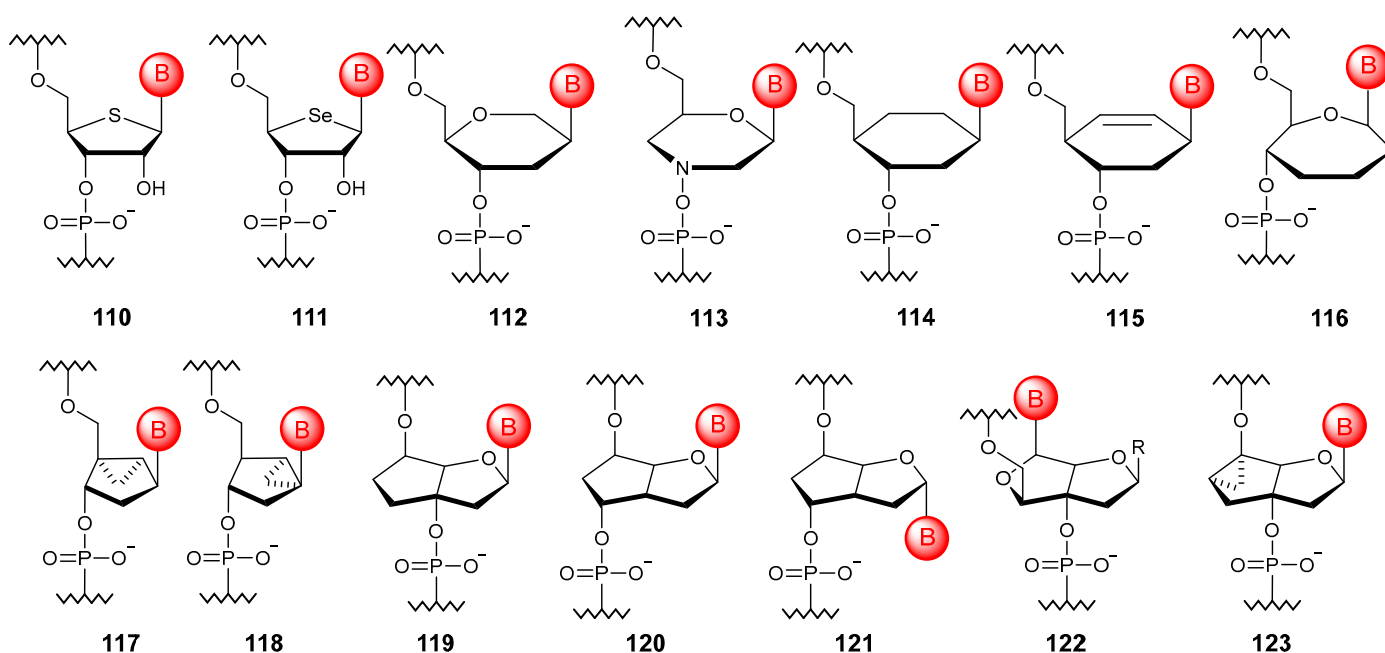
To date, various oligonucleotide modifications that form a 2',4'-bridge of different lengths and compositions have been proposed; these are usually referred to as 2',4'-bridged NAs. In comparison with the initially proposed structure, the linking chain of which consisted of two atoms, carbon and oxygen, oligonucleotides with an atom of sulfur (98) [133], selenium (99) [134], nitrogen [135], including a substituted one (100) [136–140], and also simply carbon (101) [141] and methylene substituted carbon (102) [142] instead of the original heteroatom were developed. In addition, modifications with a substituent (103) [143] and a heterocyclic fragment (104) [144] within the bridge, as well as oligonucleotides with three-atom (105) [145] and four-atom bridged modifications (106) [146], including with various heteroatoms (107) [147], have been obtained. The whole variety of structures of such modified NAs obtained to date can be found in the review [148].

A 1',2'-bridging modification (108), allowing conformational restriction of the ribose ring, has also been described in the literature [149]. Although it does not offer significant advantages over unmodified oligonucleotides, further replacement of the 2'-oxygen atom with nitrogen (109) allows one to increase the affinity of NAs modified in this way to RNA [150].

## 5.5. Substitution with Non-Sugar Cycles

### 5.5.1. Monocyclic Substitution

The formal replacement of the ring oxygen atom with sulfur or selenium (Figure 12) allows one to obtain the so-called thioRNA (110) [151] and selenoRNA (111) [152]. Oligonucleotides built on such artificial heterocyclic fragments have an increased ability to form duplexes with RNA and high resistance to endonucleases, despite the presence of 2'-OH groups. Another cyclic system widely used to construct the oligonucleotide chain is 1,5-anhydrohexitol. Despite the fact that all four isomers have been synthesized to date, the most studied and practically used are  $\beta$ -D-hexitol nucleic acids (112), which have a pronounced ability to hybridize with natural NAs, especially with RNA [153]. If the ribose ring is replaced by a morpholine system, then the nucleotides modified in this way are morpholino nucleic acids (113) [154].



**Figure 12.** Structures of NA monomer units based on alternative cyclic motifs (110–123).

The transition to a six-membered all-carbon scaffold was carried out in cyclohexanyl NAs, among which only the D-isomer (114) is capable of hybridizing with natural NAs [155]. An unsaturated modification of such oligonucleotides, called cyclohexenyl NAs (115), has the ability to undergo conformational adaptation when incorporated into a natural DNA chain [156]. There are also oligonucleotides based on a seven-membered ring, such as oxepane NAs (116), which are able to associate with their respective complementary RNA strands [157].

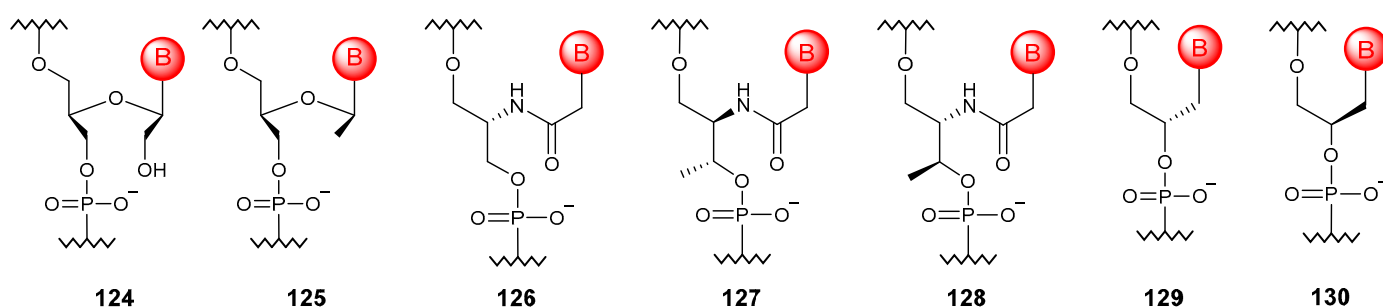
### 5.5.2. Bicyclic and Tricyclic Substitutions

Bicyclic artificial NAs include methanocarbacyclic oligonucleotides (117, 118) [158]. The fact that the bicyclo[3.1.0]hexyl scaffold effectively mimics the 3'-endo structure of the sugar moiety by locking the conformation of the cyclopentane ring with cyclopropyl fragment allows the modified oligonucleotides to exhibit increased affinity to RNA and stability to nuclease degradation. Formal ring closure through 3' and 5' carbon atoms of

the ribose ring in natural NAs allows one to obtain the so-called bicyclo-DNA (**119**) [159], which has increased affinity to complementary RNA and nuclease resistance. To date, the modification with a displaced phosphodiester linkage (**120**) [160], as well as its anomeric variant (**121**) [161], has been developed. Using formal ring closure between the 2' oxygen atom and the 3' carbon of the arabinose ring, bicyclic oligonucleotides (**122**) with functional groups facing the major groove were proposed [162]. There are also oligomers built on a tricyclic fragment (**123**), which show some potential in the treatment of genetic disorders [163].

### 5.6. Acyclic Substitutions

The formal “cleavage” of the bond between 2' and 3' carbon atoms of the ribose ring produces an acyclic analog of RNA (Figure 13), unlocked NAs (**124**) capable of modulating the stability of NA duplexes and base-pairing specificity [164]. Further “cleavage” of the 2'-CH<sub>2</sub>OH group results in the so-called flexible NAs (**125**) that weakly hybridize with DNA [165].



**Figure 13.** Structures of simplified NA monomer units (**124**–**130**).

If 2-amino-1,3-propanediol is used instead of ribose, it is possible to obtain serinol NAs (**126**), which form highly stable heteroduplexes with complementary DNA and RNA sequences [166]. The chirality of such oligomers depends only on the sequence, since the enantiomer is an oligomer with the reverse sequence. A nucleic acid based on L-threoninol (**127**) instead of ribose can bind to DNA and RNA, while the D-isomer (**128**) is not capable of such interactions [167]. Among the glycol NAs (**129**, **130**), only oligonucleotides in the S configuration are capable of hybridizing with RNA [168].

## 6. Alternative Scaffolds for Nitrogenous Bases

The synthetic possibilities for the formation of alternative scaffolds bearing nitrogenous bases are virtually limitless. The development and study of hybridization properties of so-called peptide NAs built both on the classic aminoethyl glycine motif [169], as well as on the basis of dipeptide structures [170] and conformationally constrained analogs [171], has outgrown into a separate research area. In addition, combinations of the modifications described above allow one to obtain new types of oligonucleotides. Along with locked NAs, morpholine-containing oligonucleotides have become mainstream in recent decades, among which phosphorodiamidate analogs are the most commercially successful and can be found in four FDA-approved drugs [109]. At the same time, a huge number of combinations have not yet been translated into reality, and perhaps some of them will lead to the emergence of unique properties of oligonucleotides that could manage diseases and pathologies for which classical drugs are ineffective.

## 7. Conclusions

Artificial NAs are an important tool for realizing the inexhaustible potential of natural NAs. Modified nucleotides have now achieved such progress that they have become an important class of therapeutic drugs. Chemical modifications are needed both to impart stability, resistance, and specificity compared with natural NAs, as well as other properties

necessary for their use as drugs. This review provides basic information that allows the unprepared reader to understand the variety of possible modifications of the sugar phosphate backbone of natural NAs.

The proposed classification is not just a listing of modifications but an attempt to systematize the existing knowledge in oceanic oligonucleotide chemistry from the point of view of chemical (structural) logic. It should be noted that, in reality, it is almost impossible to find oligonucleotides based on a single modification. Most often, combinations of modifications, as well as the point inclusion of modified nucleotide units and their combinations in the oligonucleotide, are used. When designing new oligonucleotides, it is necessary to focus on modern achievements but be based on principal modifications, which will introduce a rational grain into the process of obtaining the desired characteristics. We believe that the classification presented in this review, accompanied by appropriate visualization, will inspire researchers to create oligonucleotides with new types of modifications or an original combination of known modifications, which, in the future, can become promising drugs or diagnostic tools.

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