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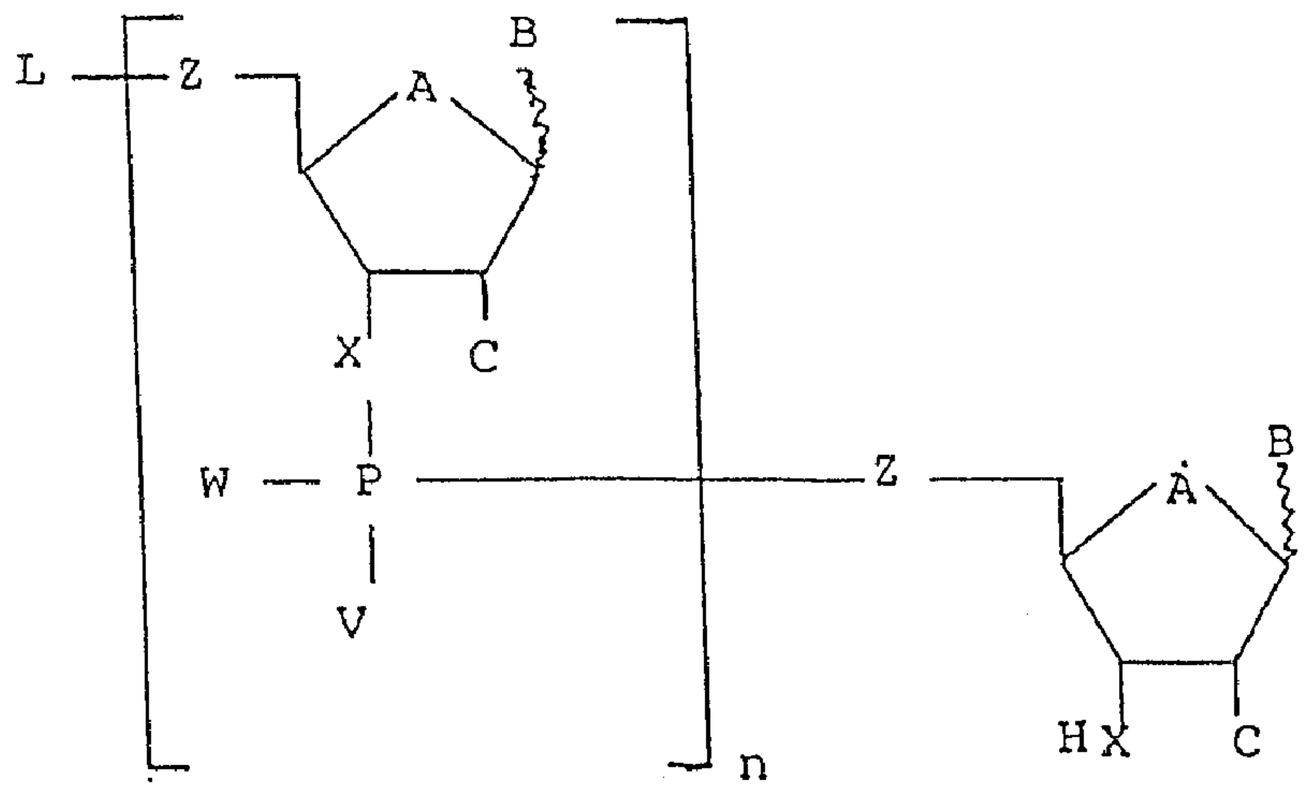
(54) **2'-O-ALKYLNUCLEOTIDES AINSI QUE POLYMERES QUI CONTIENNENT DE CES NUCLEOTIDES**

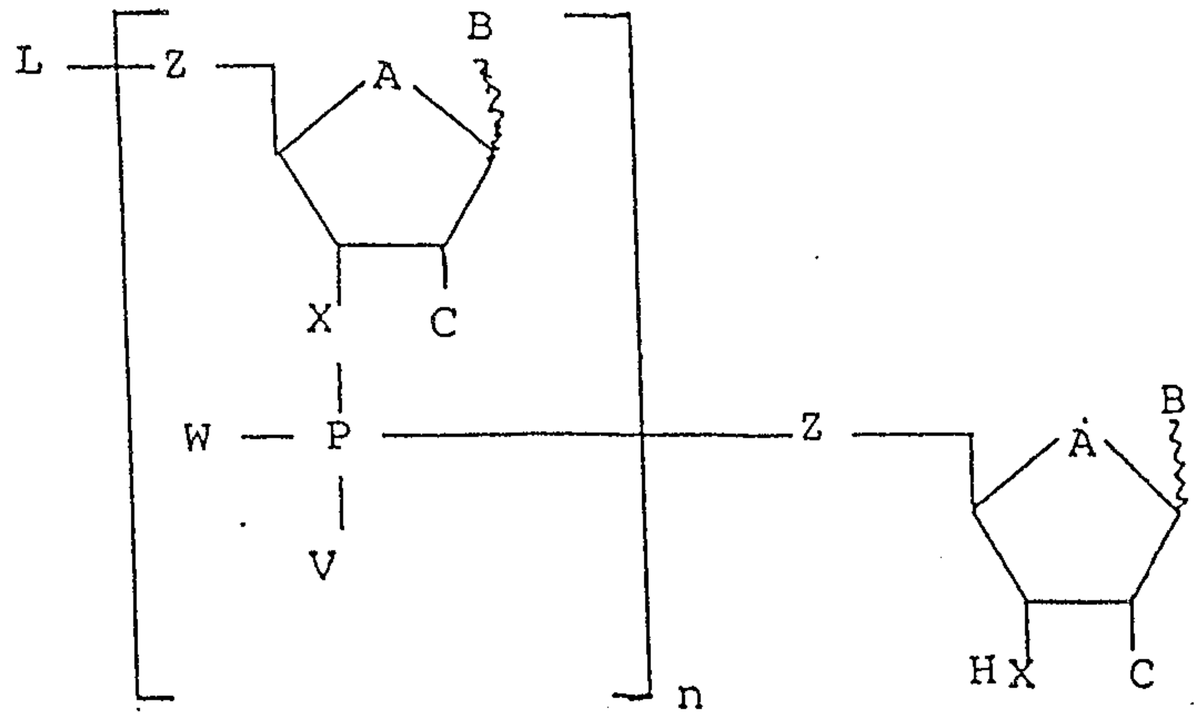
(54) **2'-O-ALKYLNUCLEOTIDES AS WELL AS POLYMERS WHICH CONTAIN SUCH NUCLEOTIDES**



(57) Oligonucleotides having the general formula, (see above formula) in which B denotes an arbitrary nucleobase, A equals 0 or CH_2; X or Z equals O, S, NH or denotes CH_2 whereby X and Z can be the same or different, V and W denote O, S, Se, NH_2 or an alkyloxy residue, or OH or SH whereby V and W can be the same or different in a monomer unit and L is a H atom or a partner of a binding pair and C equals -O-R and R is an alkyl group with at least 1 C atom which may be modified if desired, or it denotes an alkenyl or alkinyl group with at least 2 C atoms which may be modified if desired, whereby the modification consists of a substitution by one or several halogen, cyano, carboxy, hydroxy, nitro or/and mercapto residues, and n is an arbitrary whole number, are stable antisense probes which bind specifically. Such oligonucleotides and polynucleotides may be used for the regulation of gene expression and as pharmaceutical agents. They are synthesized from the corresponding 2'-substituted monomers according to well-known methods, preferably on a solid phase.

Abstract

Oligonucleotides having the general formula,



in which
B denotes an arbitrary nucleobase,
A equals 0 or CH_2;
X or Z equals O, S, NH or denotes CH_2 whereby X and Z can be the same or different,
V and W denote O, S, Se, NH_2 or an alkyloxy residue, or OH or SH whereby V and W can be the same or different in a monomer unit and
L is a H atom or a partner of a binding pair and
C equals -O-R and R is an alkyl group with at least 1 C atom which may be modified if desired, or it denotes an alkenyl or alkinyl group with at least 2 C atoms which may be modified if desired, whereby the modification consists of a substitution by one or several halogen, cyano, carboxy, hydroxy, nitro or/and mercapto residues, and
n is an arbitrary whole number, are stable antisense probes which bind specifically. Such oligonucleotides and polynucleotides may be used for the regulation of

gene expression and as pharmaceutical agents. They are synthesized from the corresponding 2'-substituted monomers according to well-known methods, preferably on a solid phase.

Description

The invention concerns new nucleotide monomers as well as oligonucleotides and polynucleotides which contain such monomers, a process for their production and their use for regulating gene expression as antisense probes and as pharmaceutical agents.

Antisense oligonucleotides and polynucleotides are known to one skilled in the art and are described in summary for example in "Spektrum der Wissenschaft" (1990), pages 70 to 77. These are understood as the nucleotides which are complementary to the actual gene and have a sequence with the opposite orientation. Such antisense molecules act on gene expression in a regulatory manner and in so doing play an important role in determining whether a hereditary sequence coded in a gene is translated into a protein. In this process the separation of the two DNA strands is triggered by a short RNA chain, the so-called primer, which first opens up the DNA double helix and hybridizes with the origin of replication. It has been shown that the gene expression does not only depend on the concentration of these primer molecules but on their ratio to the antisense RNA. In this manner it is therefore possible to specifically switch predetermined genes on and off and thus to regulate the entire cell function. Thus it has for example already been achieved to make cells which have been malignantly transformed by means of a polyoma virus appear healthy by introducing expression vectors for an antisense RNA against src into these polyoma transformed cells. By this means these cells loose their cancerogenic characteristics. Similar results with this antisense technique have already been achieved on the oncogenes fos, ras and sys. Moreover it

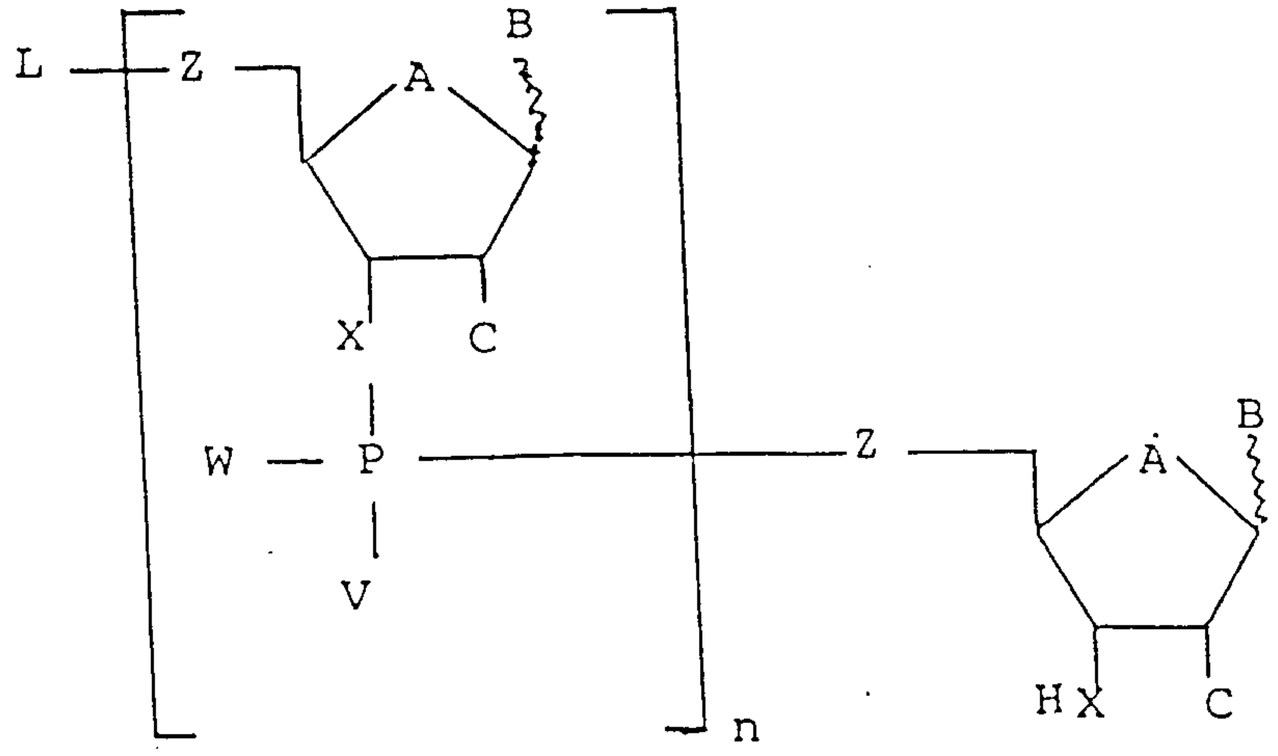
has already even been possible to inhibit infections by herpes viruses, influenza viruses and the HIV virus in tissue cultures using antisense oligonucleotides. Already with the aid of biotinylated antisense oligonucleotides it has also been possible to investigate the formation and action of the splicing complex in more detail (S. Barabino, B. Sproat et al., The EMBO Journal 8, 4171-4178 (1989)).

However, the antisense oligonucleotides and polynucleotides known up to now have the disadvantage that after being introduced into an intact cell they are attacked and degraded by RNA- and DNA-specific nucleases which leads to a loss in their activity. Thus it has already been attempted to inhibit the degradation of polynucleotides and oligoribonucleotides by nucleases by means of 2'-O-methyl substitution (B. Sproat et al., Nucleic Acids Research 17 (1989), 3373-3386).

This invention seeks to provide new oligonucleotides and polynucleotides which are resistant to attack by nucleases and which bind with improved specificity to a complementary nucleotide strand.

It has now been surprisingly found that this can be achieved by substituting the 2' position with an alkyloxy group having at least two carbon atoms.

The invention therefore concerns nucleopolymers based on 2'-O-alkylnucleotides having the general formula II



in which

B represents an arbitrary derivative known to one skilled in the art of any nucleoside base and in particular an adenin-9-yl (A), a cytosin-1-yl (C), a guanin-9-yl (G), a uracil-1-yl (U), a hypoxanthin-9-yl (I) or a thymin-1-yl group (T). Of the adenine derivatives the 2-aminoadenin-9-yl residue is preferred. It is expedient that one or several of the nucleoside bases carry a substituent L which facilitates attachment to particular parts of the cell or to enzymes or also to suitable chromatographic material. Such affinity substituents are known to one skilled in the art.

A denotes an O atom or a CH_2 group,

X or Z represent an O atom, a S atom, a NH or CH_2 group and can be the same or different,

V and W represent an O, S or Se atom or represent an -OH, -SH, $-NH_2$, alkyl or alkyloxy group.

Preferred alkyl and alkyloxy groups have 1 to 4 carbon atoms and are in particular $-CH_3$, C_2H_5 and/or OCH_3 or

OC_2H_5. In a monomer unit in the oligonucleotides and polynucleotide according to the present invention V and W can be the same or different.
L represents an H atom or a partner of a binding pair.

C denotes a group having the general formula -O-R in which R is an alkyl group with at least 2 C atom which may modified if desired, or an alkenyl or alkinyl group with at least 2 C atoms which may be modified if desired, whereby the modification consists of a substitution by one or several halogen, cyano, carboxy, hydroxy, nitro or/and mercapto residues. The alkyl group preferably has 3 to 6 carbon atoms and in particular 3 or 4 carbon atoms. Examples of particularly suitable alkyl groups are propyl and butyl, however, modified alkyl groups such as cyanomethyl are also preferred. Alkenyl chains are particularly preferred and in particular alk-2-enyl residues of which in turn an allyl residue is preferred. The propargyl residue may be mentioned as example of an alkinyl group.

Preferred polymers according to the present invention have one or several of the monomer units previously described, if desired in combination with other monomer units in which -O-R equals -O-allyl, A equals O, X equals O, Z equals O, W equals O and V equals OH and the C1 carbon atom of the sugar is in the ß-configuration. In a further preferred embodiment the oligonucleotides and polynucleotides according to the present invention have a 3'-deoxyribonucleoside at their 3' end which inhibits attack by 3' exonucleases and additionally impedes degradation by these enzymes.

By incorporating a partner of a binding pair, e.g. selected from the pairs antibody/antigen or

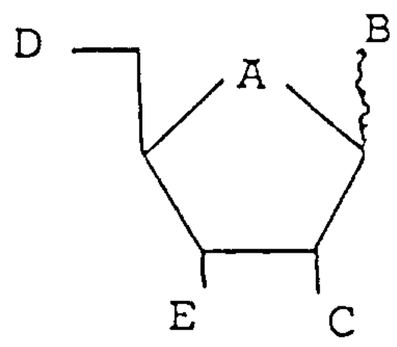
biotin/avidin or streptavidin, preferably biotin or a dinitrophenyl residue into the polymer according to the present invention it is possible to immobilize the nucleotide polymer according to the present invention and to carry out an affinity chromatography of proteins, nucleic acids and/or protein/nucleic acid complexes which bind to the immobilized nucleotide polymer.

A feature of the oligonucleotides according to the present invention is their excellent hybridization to nucleic acids that have a corresponding target sequence which is complementary to them and that they are particularly inert towards degradation by nucleases which is why they have a high biological half-life in living cells. Moreover, compared to the state of the art they have a reduced non-specific binding to nucleic acid binding proteins.

However, the invention also concerns nucleotide monomers which are suitable for the synthesis of the oligonucleotides and polynucleotides according to the present invention and which have in the 2' position of the sugar moeity an alkyloxy, alkenyloxy or alkinyloxy residue with at least one carbon atom which, if desired, is modified by one or several halogen, cyano, carboxy, hydroxy, nitro or/and mercapto residues.

Particularly preferred residues are O-alk-2-enyl residues and in particular O-allyl residues. Such a

monomer usually has the general formula I



in which A, B and C have the meanings defined above and D and E are reactive groups capable of forming 3'-5' internucleotide bonds or denote a $-PO_4H_2$, $-P_2O_7H_3$ or $-P_3O_{10}H_4$ group. Such groups are known to one skilled in the art and are for example described in B. Sproat et al., Nucleic Acids Research 18 (1990), 41-49 as well as comprehensively in E.L. Winnacker, "Gene und Klone", VCH Verlagsgesellschaft mbH, Weinheim (Germany) (1985), in particular pages 44 to 49 and in Froehler/Matteucci, Tetrahedron Lett. (1986), p. 469-472. The OH group is particularly preferred as the reactive group. The $-PO_4H_2$, $-P_2O_7H_3$ and the $-P_3O_{10}H_4$ group are also preferred as D and/or E. These mono-, di- or triphosphates, or salts of the compounds having the general formula I can be preferably incorporated as 5'-triphosphates into growing nucleic acid chains for example enzymatically using DNA/RNA polymerases (c.f. e.g. Random priming, Anal. Biochem. 132 (1983) 6-13, Nick translation, J. Mol. Biol. 113 (1977) 237-251). Using the reactive mononucleotides according to the present invention it is possible to produce in a known way the oligonucleotides and polynucleotides which are also according to the present invention in particular on a solid phase. The production of such polynucleotides from the corresponding mononucleotides is known to one skilled in the art and is for example also described in

more detail in the above-mentioned literature references. The invention therefore also concerns a process for the production of oligonucleotides and polynucleotides using the nucleotide monomers according to the present invention.

Finally the invention also concerns the use of the oligonucleotides and polynucleotides obtained according to the present invention as antisense probes and as pharmaceutical agents in particular as pharmaceutical agents for the treatment of cells infected with viruses such as e.g. the herpes, influenza or the AIDS pathogen as well as for the regulation of gene expression.

The invention is elucidated in more detail by the following examples.

E x a m p l e 1

2'-O-ally-oligoribonucleotides and 2'-O-methyl-oligoribonucleotides, each having an identical sequence, were produced by the phosphoramidite method according to the process described by B. Sproat, B. Beijer and A. Iribarren in Nucleic Acids Research, Vol. 18 (1990), 41-49. Subsequently both probes labelled with ^{32}P- phosphate at their 5' end were incubated with a nuclear extract which was obtained from Hela cells as described by A. Lamond et al. in Cell, Vol. 58 (1989), 383-390. Both probes were then subjected to gel chromatography. It turns out that the 2'-O-allyl-oligonucleotides according to the present invention have an extraordinarily high specific binding activity and a negligible non-specific binding activity in comparison

with the 2'-O-Me-oligonucleotides which are part of the state of the art.

The nucleotide sequence was

5'-AIAACAIAUACUACACUUIA

It binds to human U2 RNA.

E x a m p l e 2

Various nucleases were added to the 2'-O-allyl-oligoribonucleotides produced according to example 1 and their sensitivity to enzymatic degradation was determined. In comparison to normal non-modified RNA with an identical sequence it turned out that on digestion with pancreatic RNase A, RNase CL-3, RNase T1, RNase T2 and RNase U2 the 2'-O-allyl-oligonucleotides according to the present invention are completely resistant to enzymatic attack by nucleases whereas in contrast natural RNA is completely degraded in all cases.

E x a m p l e 3

3',5'-O-(tetraisopropyldisiloxan-1,3-diyl)-2-chloro-6-(2,6-dichlorophenoxy)purine riboside (A) was synthesized as described by Sproat, B.S., Beijer, B. and Iribarren, A., Nucleic Acids Research, 1990, 18, 41-49. The compound A obtained in this way was then allylated as described below.

Synthesis of 3',5'-O-(tetraisopropyldisiloxan-1,3-diyl)-2'-O-allyl-2-chloro-6-(2,6-dichlorophenoxy)purine riboside (B):

Tris(dibenzylidene-acetone)dipalladium (O) (174 mg, 0.19 mmol) and 1,4-bis (diphenylphosphine)butane (324 mg, 0.76 mmol) were suspended in dry tetrahydrofuran (50 ml). A solution of compound A (13.11 g, 19 mmol) and allylethylcarbonate (4.95 g, 38 mmol) in 50 ml dry tetrahydrofuran was added and the mixture was heated for 30 minutes under reflux. Silica gel t.l.c. in petroleum ether/ethyl acetate (2:1 v/v) showed a complete reaction with a single UV-positive spot of R_f 0.54 (compound A has an R_f 0.41). The solvent was removed in a vacuum and a red syrup remained which was dissolved in petroleum ether/ethyl acetate (9:2 v/v) and the solution was filtered in order to remove the insoluble Pd-phosphine complex. The product was purified by preparative liquid chromatography on silica gel and eluted with petroleum ether/ethyl acetate (9:2 v/v). The pure compound B was obtained in this manner in the form of a light yellow foam (12.4 g, 89.4 %). ^{13}C NMR spectrum ($CDCl_3$)δ: 158.12 (C6), 153.13 and 152.54 (C-2 and C-4), 144.66 (phenyl C-1), 142.43 (C-8), 133.75 (-CH = of allyl), 128.77 (phenyl C-2 and C-6), 128.52 (phenyl C-3 and C-5), 127.12 (phenyl C-4), 120.51 (C-5), 117.0 (= CH_2 of allyl), 88.26 (C-1'), 81.16 (C-4'), 80.6 (C-2'), 71-4 (O-CH_2- of allyl), 69.54 (C-3'), 59.61 (C-5'), 17.19-16.63 (isopropyl CH_3s), 13.16, 12.70 and 12.29 p.p.m. (isopropyl CHs).

Compound B was converted further via various steps into the corresponding nucleotide monomer, namely 5'-O-dimethoxytrityl-N^2-dimethylaminomethylidene-2'-O-allylguanosine-3'-O-(2-cyano-ethyl-N,N-diisopropylphosphoramidite) using the method described in Nucleic Acids Research, 1990, 18, 41-49.

Monomers were converted into polymers as described in Nucleic Acids Research, 1989, 17, 3373-3386.

E x a m p l e 4

Synthesis of 3',5'-O-(tetraisopropyldisiloxan-1,3-diyl)-2'-O-propargyl-4-O-(2,6-dichlorophenyl)uridine (C):

3',5'-O-(tetraisopropyldisiloxan-1,3-diyl)-4-O-(2,6-dichlorophenyl)uridine (18.95 g, 30 mmol) was dried in a vacuum by evaporation of acetonitrile. The remaining foam was dissolved in anhydrous acetonitrile (50 ml), a 80 % by weight solution of propargyl bromide in toluene (3.56 ml, 33 mmol) followed by 2-tert.-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (9.55 ml, 33 mmol) were added while stirring and excluding humidity. Silica gel t.l.c. in hexane/ethyl acetate (2:1 v/v) showed a complete reaction after 5 hours with a UV-absorbing product spot of R_f 0.38. The solvent was removed in a vacuum leaving behind a cream-coloured foam. The product was purified by preparative liquid chromatography using petroleum ether/dichloromethane/ethyl acetate (8:2:1 by volume) as an eluant. The pure product C was obtained as a white foam (10.7 g, 53.3 %). ^{13}C NMR spectrum ($CDCl_3$) δ: 169.80 (C-4), 154.60 (C-2), 144.76 (phenyl C-1), 144.41 (C-6), 128.69 (phenyl C-2 and C-6), 128.65 (phenyl C-3 and C-5), 127.08 (phenyl C-4), 93.80 (C-5), 89.80 (C-1'), 81.70 (C-2'), 80.34 (C-4'), 79.54 (-C≡ of propargyl), 74.63 (≡CH of propargyl), 67.63 (C-3'), 59.37 (C-5'), 58.01 (OCH_2 propargyl), 17.36, 17.21, 16.90 and 16.73 (isopropyl CH_{3s}), 13.36, 12.92, 12.84 and 12.28 p.p.m. (isopropyl CH_s).

Compound C could be converted via various steps into cytidine and uridine monomers for the production of solid phase polymers.

E x a m p l e 5

Synthesis of 3',5'-O-(tetraisopropyldisiloxan-1,3-diyl)-2'-O-cyanomethyl-4-O-(2,6-dichlorophenyl)uridine (D):

The alkylation was carried out analogous to example 4 using bromoacetonitrile instead of propargylbromide. The title compound was obtained as a white foam in 55 % yield, R_f 0.51 on silica gel t.l.c. in petroleum ether/ethyl acetate (1:1 v/v). ^{13}C NMR spectrum ($CDCl_3$) δ: 169.91 (C-4), 154.57 (C-2), 144.54 (phenyl C-1), 143.96 (C-6), 128.69 (phenyl C-2 and C-6), 128.60 (phenyl C-3 and C-5), 127.12 (phenyl C-4), 115.72 (CN of cyanomethyl), 94.10 (C-5), 89.17 (C-1'), 82.34 (C-2'), 81.60 (C-4'), 67.27 (C-3'), 59.06 (C-5'), 55.82 (CH_2 of cyanomethyl), 17.20, 17.08, 16.80 and 16.61 (isopropyl CH_{3s}), 13.23, 12.70 and 12.24 p.p.m. (isopropyl CH_s).

Ex a m p l e 6

2'-O-propylation is best carried out by 2'-O-allylation followed by reduction of the allyl group. 2'-O-butylation is best carried out by 2'-O-crotylation (using crotylbromide and the process as described in example 4) followed by a reduction of the crotyl group.

E x a m p l e 7

3',5'-O-(tetraisopropyldisiloxan-1,3-diyl)-4-O-(2,6-dichlorophenyl)-uridine

14.65 g (60 mmol) dried uridine is dissolved in 150 ml anhydrous pyridine and the solution is cooled in an ice bath. A solution of 21 g (67 mmol) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in 10 ml dichloromethane is added to this over 15 minutes while stirring and excluding humidity. After the addition is completed, the mixture is stirred for a further 3 hours at room temperature; afterwards the complete conversion into a product of R_f 0.58 is observed in a thin layer chromatogram (silica gel; mobile solvent chloroform/ethanol 9:1). The reaction is stopped by addition of 5 ml methanol and the mixture is evaporated in a vacuum. The residue is taken up in 200 ml dichloromethane and extracted twice with 200 ml 1 mol/l sodium bicarbonate solution in each case. The organic phase is dried over Na_2SO_4, filtered and evaporated in a vacuum. The residue is twice coevaporated in a vacuum with 25 ml toluene in each case whereafter a white foamy residue results. This is dissolved in 200 ml anhydrous 1,2-dichloroethane and 42 ml triethylamine (300 mmol) and 22.5 ml chlorotrimethylsilane (180 mmol) are added while stirring and excluding humidity. After a reaction time of 30 minutes a thin layer chromatogram (silica gel; petroleum ether/ethyl acetate 2:1) shows a complete conversion with a spot of R_f 0.39. The reaction mixture is poured into 500 ml 1 mol/l sodium bicarbonate solution while stirring vigorously, the organic phase is separated and dried over Na_2SO_4. It is evaporated in a vacuum after filtration and the residue is coevaporated twice with 25 ml dry toluene in each case. The 2'-O-

trimethylsilyl derivative obtained in this way is dissolved in 300 ml anhydrous dichloromethane and 42 ml triethylamine (300 mmol), 19.5 g 2-mesitylenesulphonyl chloride (90 mmol) and 1.8 g 4-dimethylaminopyridine (15 mmol) are added while stirring and excluding humidity. A complete conversion to a product with a R_f 0.63 is observed in TLC (silica gel; petroleum ether/ethyl acetate 2:1) after a reaction time of 30 minutes. 1.35 g 1,4-diazabicyclo[2.2.2]octane (12 mmol) and 19.6 g 2,6-dichlorophenol (120 mmol) are added to the reaction solution and it is stirred for 2 hours at room temperature. After this time the conversion is complete as a TLC in petroleum ether/ethyl acetate 2:1 on silica gel shows (R_f 0.56). The reaction mixture is stirred into 500 ml 1 mol/l Na bicarbonate solution, the organic phase is separated, dried over Na_2SO_4, filtered and evaporated in a vacuum. 2'-O-trimethylsilylether is obtained as an oily, viscous residue. The syrup is dissolved in 300 ml dichloromethane and a solution of 28.5 g p-toluene sulfonic acid monohydrate (150 mmol) in 100 ml tetrahydrofuran is added to this while stirring. After 2.5 minutes 28 ml triethylamine is added in order to neutralize the acid. Afterwards the reaction solution is poured into 500 ml 1 mol/l Na bicarbonate solution while stirring vigorously. The organic phase is separated, dried over Na_2SO_4 and the solvent is distilled off in a vacuum. The TLC (silica gel; petroleum ether/ethyl acetate 1:1) shows a spot with a R_f 0.59 of 2,6-dichlorophenyl-2-mesitylenesulfonate and a further spot with a R_f 0.41 of the desired product. The crude product is purified in several portions by preparative chromatography on silica gel using petroleum ether/ethyl acetate (2:1) as the eluant. After evaporating the fractions, 25.6 g corresponding to 67.5 % of the theoretical yield is obtained as the pure final product.

R_f value (silica gel; petroleum ether/ethyl acetate 2:1) 0.23

^{13}C NMR spectrum ($CDCl_3$) δ: 169.82 (C-4), 154.70 (C-2), 144.94 (C-6), 144.77 (phenyl-C-1), 128.92 (phenyl-C-2 and C-6), 128.71 (phenyl C-3 and C-5), 127.11 (phenyl C-4), 94.05 (C-5), 92.22 (C-1'), 82.01 (C-4'), 74.88 (C-2'), 68.89 (C-3'), 60.35 (C-5'), 17.40 - 16.85 (isopropyl-CH_3's), 13.34, 12.91, 12.83 and 12.48 p.p.m. (isopropyl-CH's).

E x a m p l e 8

3',5'-O-(tetraisopropyldisilocan-1,3-diyl)-2'-O-allyl-4-O-(2,6-dichlorophenyl)uridine

Tris(dibenzylidene-acetone)dipalladium(0) (0.183 g, 0.2 mmol) and 1,4-bis(diphenylphosphino)butane (0.341 g, 0.8 mmol) are suspended in dry tetrahydrofuran (40 ml) under an argon atmosphere. A solution of the compound produced according to example 7 (12.63 g, 20 mmol) and allylethylcarbonate (5.2 g, 40 mmol) in dry tetrahydrofuran (60 ml) are added and the mixture is heated for 30 minutes unter reflux. Thin layer chromatography (silica gel, mobile solvent petroleum ether/ethyl acetate, 2:1, v/v) is used to check that the reaction has run to completion. The reaction product is shown by a new band having a R_f value of 0.48. After cooling the mixture is filtered and the solvent is removed in a vacuum. The reaction product is purified by preparative chromatography on silica gel with 3 % ethyl acetate in dichloromethane as the mobile solvent.

After evaporating the fractions, 11 g (81.9 % of the theoretical yield) of the final product is obtained.

R_f value (silica gel thin layer chromatography; petroleum ether/ethyl acetate 2:1): 0.51

^{13}C NMR specturm ($CDCl_3$) δ: 169.61 (C-4), 154.49 (C-2), 144.64 (phenyl C-1), 144.37 (C-6), 134.29 (allyl CH), 128.75 (phenyl C-2 and C-6), 128.51 (phenyl C-3 and C-5), 126.93 (phenyl C-4), 116.85 (allyl = CH_2), 93.50 (C-5), 89.94 (C-1'), 81.64 (C-2'), 80.40 (C-4'), 70.90 (O-CH_2 of allyl), 67.49 (C-3'), 59.34 (C-5'), 17.24, 17.10, 16.79 and 16.63 (isopropyl CH_3s), 13.21, 12.83, 12.70 and 12.30 p.p.m. (isopropyl CHs).

E x a m p l e 9

2'-O-allyl-4-O-(2,6-dichlorophenyl)uridine

5.5 g (8.19 mmol) of the compound produced according to example 8 is dissolved in 20 ml dry tetrahydrofuran and 1.1 mol/l tetrabutylammonium fluoride in 18 ml tetrahydrofuran is added while stirring. The reaction is completed after 5 minutes as shown by a thin layer chromatogram on silica gel using ethanol/chloroform (5 : 95 v/v) as the mobile solvent (R_f: 0.22).

The reaction is stopped with pyridine/methanol/water (50 ml, 3:1:1 v/v) and the solution is applied while stirring to the pyridine form of Dowex 50 Wx4-200 (Trade Mark) resin (30 g suspended in pyridine/methanol/-water 50 ml 3:1:1 v/v). The mixture is stirred for 20 minutes, the resin is filtered off and washed with the above-mentioned solvent (3 x 50 ml).
The combined filtrates and washing

solutions are evaporated in a vacuum to dryness, taken up in toluene and evaporated again. The crude product is purified in 3 portions by preparative chromatography on silica gel with 6 % ethanol in chloroform as the eluting agent. After evaporating the fractions in a vacuum, ethanol and pyridine residues are removed by addition of toluene and again evaporating in a vacuum at 45°C. After evaporation 2.91 g (82.9 % of the theoretical yield) of the pure final product is obtained.

R_f value (silica gel; ethanol/chloroform 1:4): 0.57

^{13}C NMR spectrum ($CDCl_3$) δ: 169.74 (C-4); 155.28 (C-2), 146.20 (C-6), 144.42 (phenyl C-1), 133.54 (allyl CH), 128.67 (phenyl C-2 and C-6), 128.57 (phenyl C-3 and C-5), 127.13 (phenyl C-4), 117.98 (allyl = CH_2), 94.41 (C-5), 89.60 (C-1'), 84.54 (C-4'), 81.01 (C-2'), 71.10 (allyl CH_2O), 67.43 (C-3') and 59.55 p.p.m. (C-5').

E x a m p l e 10

2'-O-allyl-uridine

2.91 g (6.79 mmol) of the compound prepared according to example 9 is dissolved in 20 ml dry acetonitrile. 2.82 g (16.98 mmol) 2-nitrobenzaldoxime and 1.76 g (15.28 mmol) 1,1,3,3-tetramethylguanidine in 20 ml dry acetonitrile are added and the mixture is stirred for 18 hours at room temperature. A thin layer chromatogram on silica gel using ethanol/chloroform (1:4 v/v) as the mobile solvent shows that the reaction has run to completion (R_f 0.37). The solvent is removed by evaporation in a vacuum and the remaining residue is dissolved in 100 ml dichloromethane and the product is extracted with 100 ml

water. The aqueous phase is washed with 100 ml dichloromethane and subsequently with 100 ml diethyl ether. The slightly yellow aqueous phase is subsequently stirred for 5 minutes with the pyridine form of Dowex 50 Wx4-200 resin (25 g). The resin is removed by filtration and the turbid filtrate is washed twice with 50 ml dichloromethane and subsequently with 100 ml ether. The aqueous phase is evaporated in a vacuum. Traces of water are removed by addition of methanol and tetrahydrofuran and subsequently evaporation. The desired compound is crystallized from methanol, filtered off and washed with ether and dried. 1.83 g (94 % of theory) of 2'-O-allyluridine is obtained.

R_f value (silica gel; ethanol/chloroform 1:4 v/v): 0.39

^{13}C NMR spectrum (pyridine - d_5) δ: 164.48 (C-4), 151.72 (C-2), 140.76 (C-6), 135.11 (CH of allyl), 116.96 (allyl = CH_2), 102.17 (C-5), 88.26 (C-1'), 85.72 (C-4'), 82.57 (C-2'), 71.38 (allyl CH_2O), 69.41 (C-3') and 60.75 p.p.m. (C-5').

E x a m p l e 11

2'-O-allyl-uridine-5'-monophosphate

1.42 g 2'-O-allyl-uridine (5.0 mmol) is phosphorylated according to the method of Yoshikawa et al. (1967) Tetrahedron Lett. 50, 5065. The yield was 980 mg after chromatographic purification.

E x a m p l e 12

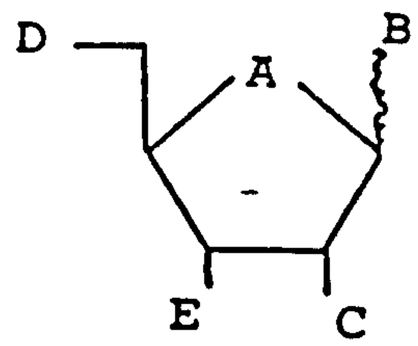
2'-O-allyl-uridine-5'-diphosphate and -5'-triphosphate

In order to produce di- and triphosphates, 365 mg (1 mmol) of each of the monophosphates was reacted in each case with orthophosphoric acid and pyrophosphoric acid according to the method of Hoard and Ott (1965) J. Am. Chem. Soc. 87, 1785. The yields were 220 mg diphosphate and 140 mg triphosphate.

All 5'-phosphates were characterized by means of elementary analysis, electrophoresis and ^{31}P-NMR spectroscopy.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A nucleotide analogue of the general formula I,



in which

B is an arbitrary nucleobase,

A is an O atom or CH_2;

C is a group -O-R in which R is an alk-2-enyl or alkynyl group, unsubstituted or substituted with one or more substituents, which may be the same or different, selected from halogen, cyano, carboxy, hydroxy, nitro and mercapto, and

D and E denote reactive groups capable of forming 3'-5' internucleotide bonds or denote a $-PO_4H_2$, $-P_2O_7H_3$ or $-P_3O_{10}H_4$ group.

2. A nucleotide as claimed in claim 1, wherein the nucleobase B is a adenin-9-yl, cytosin-1-yl, guanin-9-yl, uracil-1-yl, hypoxanthin-9-yl or thymin-1-yl residue.

3. A nucleotide as claimed in claim 1, wherein the nucleobase B is a 2-aminoadenin-9-yl residue.

4. A nucleotide as claimed in claim 1, wherein R is an alk-2-enyl residue.

5. A nucleotide as claimed in claim 2, wherein R is an alk-2-enyl residue.

6. A nucleotide as claimed in claim 3, wherein R is an alk-2-enyl residue.

7. A nucleotide as claimed in claim 1, wherein R is an allyl residue.

8. A nucleotide as claimed in claim 2, wherein R is an allyl residue.

9. A nucleotide as claimed in claim 3, wherein R is an allyl residue.

10. A nucleotide as claimed in claim 1, wherein D and E are selected from -OH, $-PO_4H_2$, $-P_2O_7H_3$ and $-P_3O_{10}H_4$.

11. A process for the production of polynucleotides and oligonucleotides comprising: reacting at least one nucleotide as claimed in claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, with another nucleotide.

12. A process as claimed in claim 11, wherein said reacting is on a solid phase.

13. A nucleotide polymer or oligomer obtainable by reacting reactive mononucleotides wherein said reactive nucleotides contain at least one nucleotide analogue as claimed in claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

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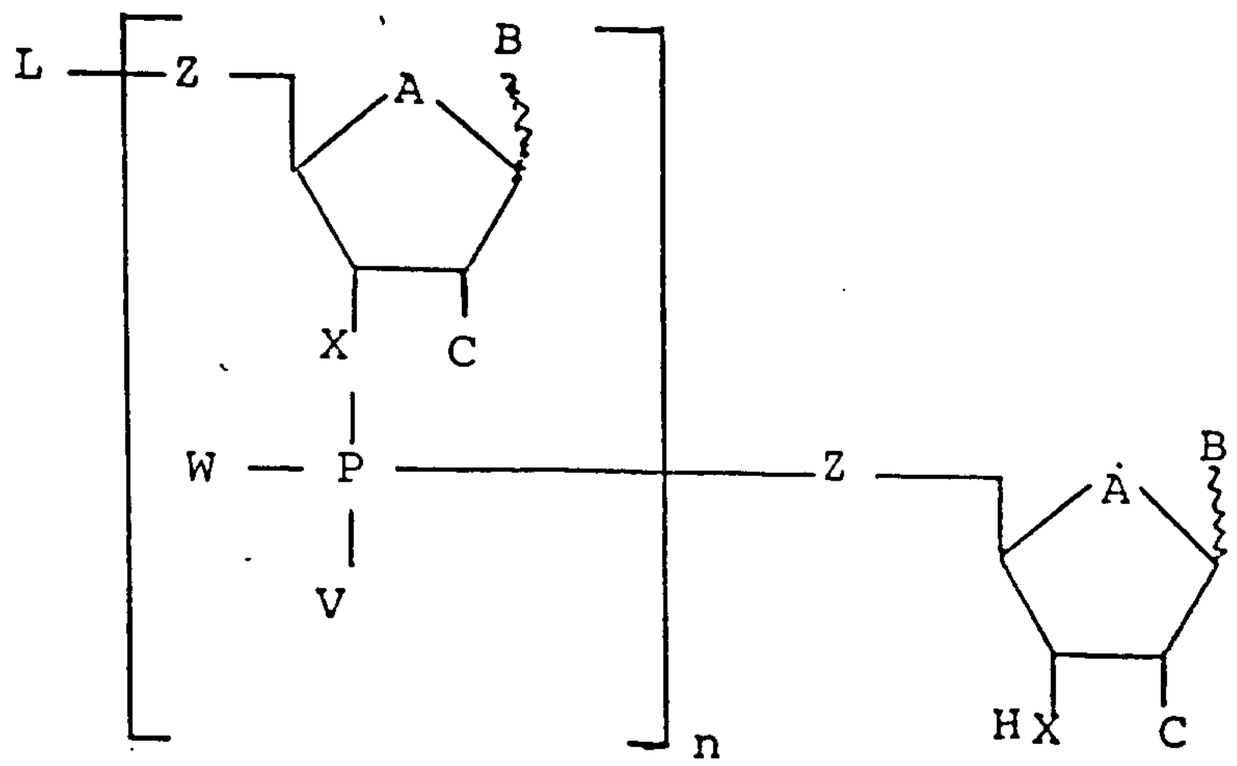
14. A polymer or oligomer of claim 13, wherein said reacting is on a solid phase.

15. A polymer or oligomer of nucleotide analogues with the formula II



in which:

X or Z	are O, S, NH or CH_2 wherein X and Z can be the same or different in a monomer unit;
V and W	are O, S, Se, NH_2, an alkyl or alkyloxy residue, or OH or SH wherein V and W can be the same or different in a monomer unit;
L	is an H atom or a partner of a binding pair selected from antibody/antigen, biotin/-avidin, biotin/streptavidin or a dinitro-phenyl residue;
B, A and C	are as defined in claim 1; and
n	is a whole number greater than 1.

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16. A polymer or oligomer as claimed in claim 15, wherein L is a biotin residue.

17. A polymer or oligomer as claimed in claim 13, having a 3'-deoxyribonucleoside at a 3'-terminal end.

18. A polymer or oligomer as claimed in claim 14, having a 3'-deoxyribonucleoside at a 3'-terminal end.

19. A polymer or oligomer as claimed in claim 15 or 16, having a 3'-deoxyribonucleoside at a 3'-terminal end.

20. Use of a polymer or oligomer as claimed in claim 13, as an antisense probe for inhibiting gene expression.

21. Use of a polymer or oligomer as claimed in claim 14, 15, 16, 17 or 18, as an antisense probe for inhibiting gene expression.

22. Use of a polymer or oligomer of claim 13, as a pharmaceutical agent.

23. Use of a polymer or oligomer of claim 14, 15, 16, 17 or 18, as a pharmaceutical agent.

24. Use of a polymer or oligomer of claim 13, as an antiviral agent.

25. Use of a polymer or oligomer of claim 14, 15, 16, 17 or 18, as an antiviral agent.

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26. A polymer or oligomer of claim 13, for use in the treatment of cells infected with viruses.

27. A polymer or oligomer of claim 14, 15, 16, 17 or 18, for use in the treatment of herpes, influenza or AIDS.

28. A polymer or oligomer of claim 13, for use in regulation of gene expression.

29. A polymer or oligomer of claim 14, 15, 16, 17 or 18, for use in regulation of gene expression.

30. Use of a nucleotide as defined in claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, in the production of polynucleotides and oligonucleotides.

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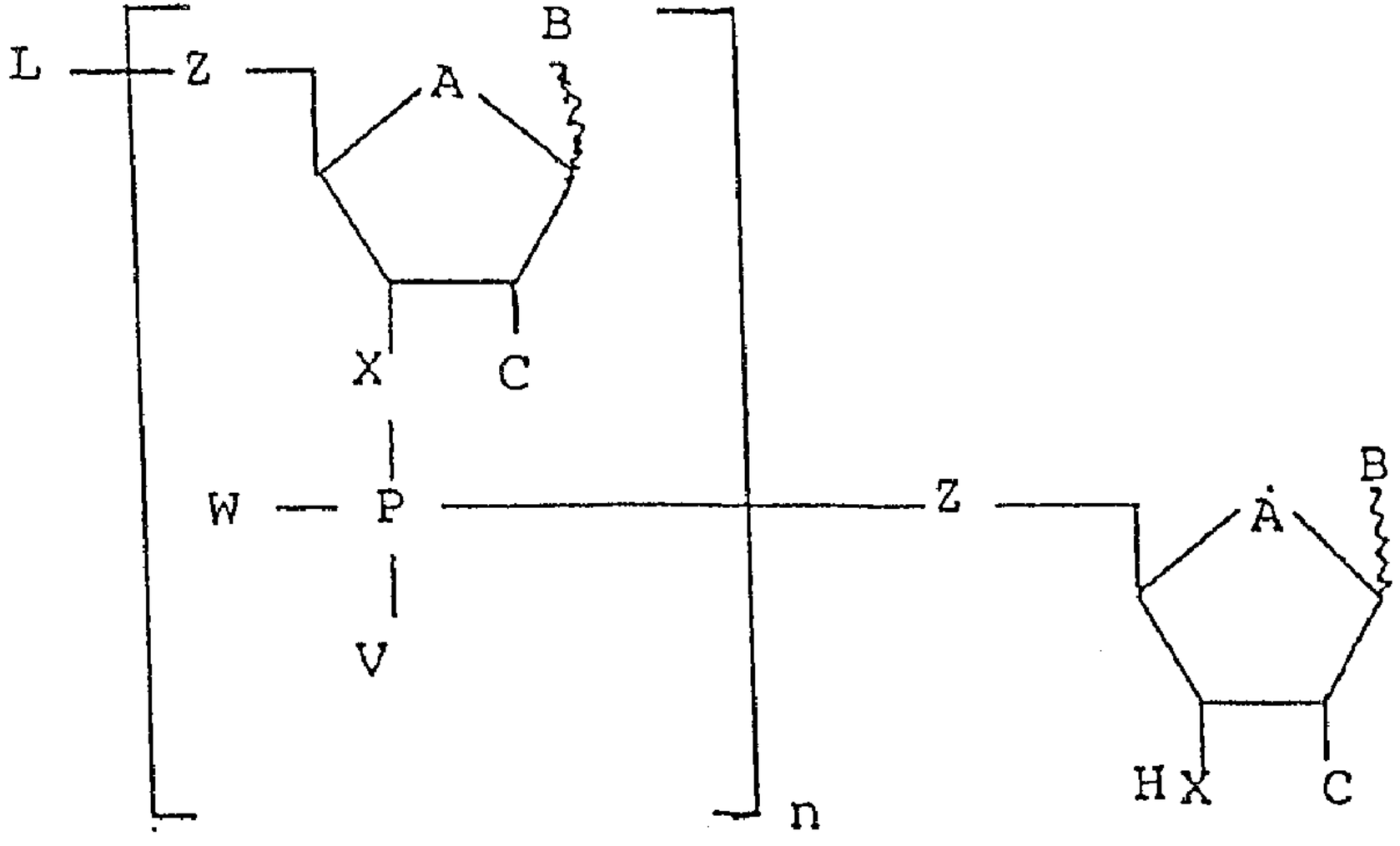
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