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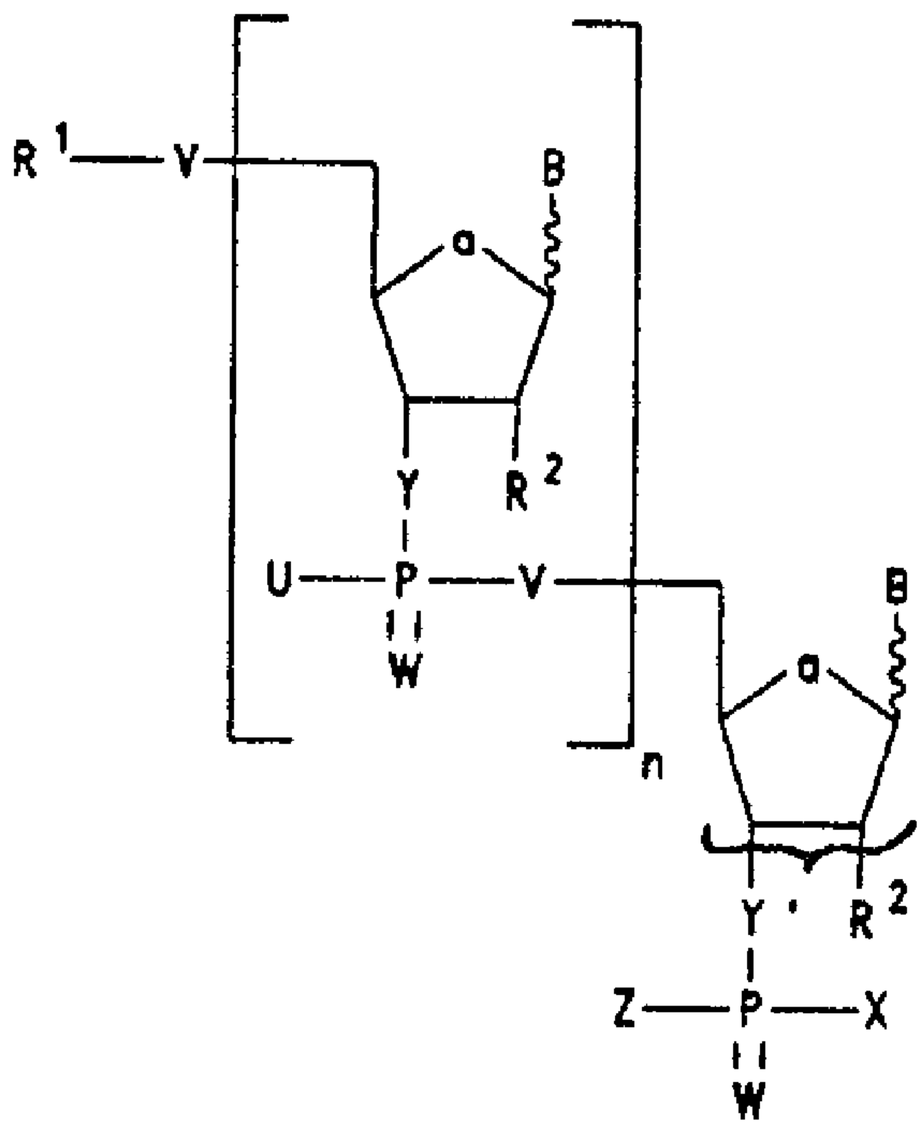


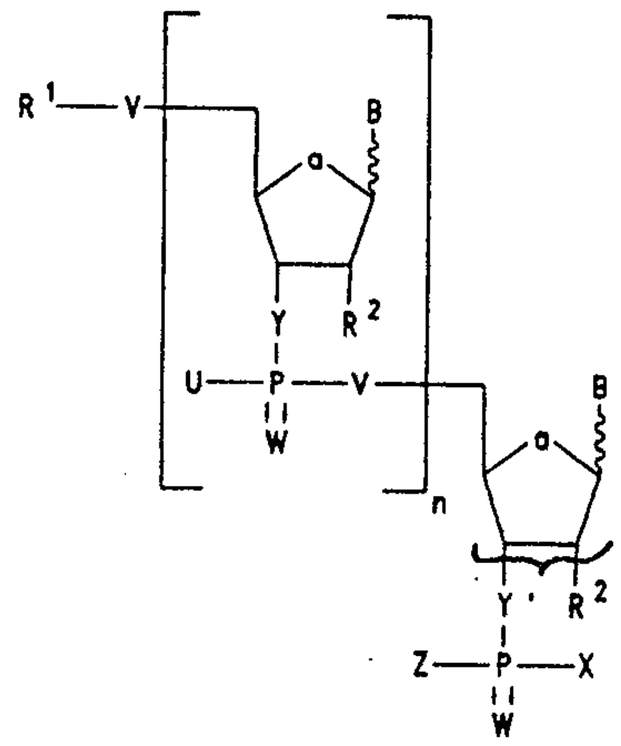
(I)

(57) Abrégé/Abstract:
The invention relates to compounds of the formula I (see formula I) where R^1 is H, alkyl, acyl, aryl or a phosphate residue; R^2 is H, OH, alkoxy, NH_2 or halogen; B is a base customary in nucleotide chemistry; a is O or CH_2; n is an integer from 1 to 100; W = O, S or Se; V = O, S or NH; Y = O, S, NH or CH_2; Y' = O, S, NH or alkylene; X = OH or SH; U = OH, SH, SeH, alkyl, aryl or amine and Z = OH, SH, SeH, an optionally substituted radical from the group comprising alkyl, aryl, heteroaryl, alkoxy or amino, or a group which favors intracellular uptake or serves as the label of a DNA probe or attacks the target nucleic acid during hybridization, where if Z = OH, SH, CH_3 or OC_2H_5, at least one of the groups X, Y, Y', V or W is not OH or O or R^1 is not H; a process for their preparation and their use as inhibitors of gene expression, as probes for detecting nucleic acids and as aids in molecular biology.

Abstract of the disclosure

Oligonucleotide analogs, their preparation and use

The invention relates to compounds of the formula I



(I)

where R^1 is H, alkyl, acyl, aryl or a phosphate residue; R^2 is H, OH, alkoxy, NH_2 or halogen; B is a base customary in nucleotide chemistry; a is O or CH_2; n is an integer from 1 to 100; W = O, S or Se; V = O, S or NH; Y = O, S, NH or CH_2; Y′ = O, S, NH or alkylene; X = OH or SH; U = OH, SH, SeH, alkyl, aryl or amine and Z = OH, SH, SeH, an optionally substituted radical from the group comprising alkyl, aryl, heteroaryl, alkoxy or amino, or a group which favors intracellular uptake or serves as the label of a DNA probe or attacks the target nucleic acid during hybridization, where if Z = OH, SH, CH_3 or OC_2H_5, at least one of the groups X, Y, Y′, V or W is not OH or O or R^1 is not H; a process for their preparation and their use as inhibitors of gene expression, as probes for detecting nucleic acids and as aids in molecular biology.

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Description

Oligonucleotide analogs, their preparation and use

The present invention relates to novel oligonucleotide analogs with useful physical, biological and pharmacological properties and a process for their preparation. Their application relates to their use as inhibitors of gene expression (antisense oligonucleotides, ribozymes, sense oligonucleotides and triplex-forming oligonucleotides), as probes for detecting nucleic acids and as aids in molecular biology. Oligonucleotides are being used to an increasing extent as inhibitors of gene expression (G. Zon, Pharmaceutical Research 5, 539 (1988); J. S. Cohen, Topics in Molecular and Structural Biology 12 (1989) Macmillan Press; C. Helene and J.J. Toulme, Biochimica et Biophysica Acta 1049, 99 (1990); E. Uhlmann and A. Peyman, Chemical Reviews 90, 543 (1990)). Antisense oligonucleotides are nucleic acid fragments whose base sequence is complementary to an mRNA which is to be inhibited. This target mRNA can be of cellular, viral or other pathogenic origin. Suitable cellular target sequences are, for example, those of receptors, enzymes, immuno- modulators, ion channels or oncogenes. The inhibition of viral replication using antisense oligonucleotides has been described, for example, for RSV (Rous sarcoma virus), HSV-1 and -2 (herpes simplex virus type I and II), HIV (human immunodeficiency virus) and influenza viruses. In this context oligonucleotides are employed which are complementary to the viral nucleic acid. By contrast, the sequences of sense oligonucleotides are designed in such a way that these oligonucleotides bind ("capture") nucleic acid-binding proteins or nucleic acid-processing enzymes, for example, and thereby inhibit their biological activity (Helene, 1990). Viral targets which can be

mentioned here as examples are reverse transcriptase, DNA polymerase and transactivator proteins. Triplex-forming oligonucleotides generally have DNA as their target, and after binding to this DNA form a triple helical structure. While generally the processing (splicing etc.) of mRNA and its translation into protein are inhibited using antisense oligonucleotides, triplex-forming oligonucleotides inhibit the transcription or replication of DNA (Helene et al., 1990, Uhlmann and Peyman, 1990). However, it is also possible to bind single-stranded nucleic acids in a first hybridization with an antisense oligonucleotide, with the formation of a double strand which then forms a triplex structure with a triplex-forming oligonucleotide in a second hybridization. In this case the antisense and triplex binding regions can be contained either in two separate oligonucleotides or in one oligonucleotide. The so-called ribozymes, which destroy the target RNA as a result of their ribonuclease activity (J.J. Rossi and N. Sarver, TIBTECH 8, 179 (1990)), represent a further application of synthetic oligonucleotides.

Suitably labeled nucleic acid fragments are employed in DNA diagnostic investigation as so-called DNA probes for specific hybridization to a nucleic acid which is to be detected. Here, the specific formation of the new double strand is followed using labeling which preferably is not radioactive. In this way, genetic and malignant diseases, and diseases caused by viruses or other pathogens, can be detected.

In their naturally occurring form, oligonucleotides are little, or not at all, suited for the majority of the said applications. They have to be chemically modified so that they are suitable for the specific requirements. In order that oligonucleotides can be employed in biological systems, for example for inhibition of viral replication, they must fulfil the following preconditions:

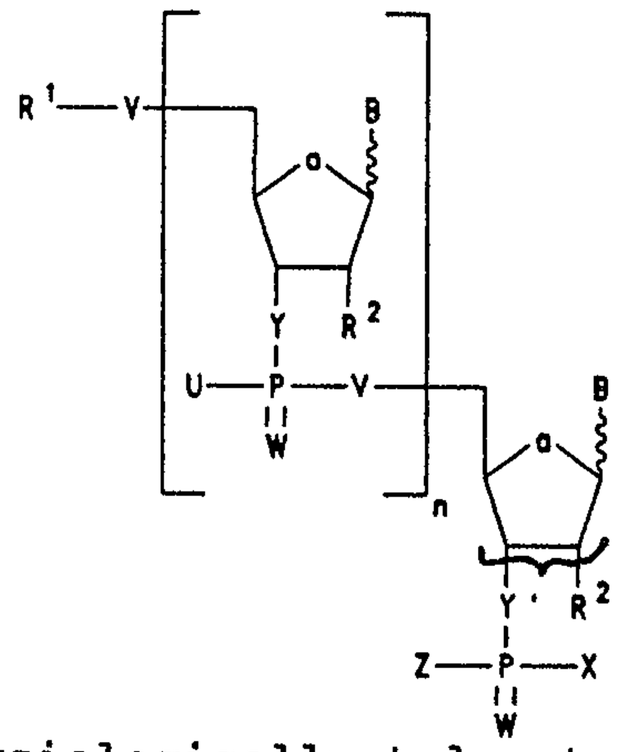
1. They must possess a sufficiently high degree of stability under in vivo conditions, that is in serum as well as intracellularly.
2. They must be able to pass through the cell and nuclear membranes.
3. They must bind to their target nucleic acid in a base-specific manner under physiological conditions in order to exert the inhibitory effect.

These preconditions are not essential for DNA probes; however, these oligonucleotides must be derivatized in a manner which permits detection, for example by fluorescence, chemiluminescence, colorimetry or specific staining, (Beck and Köster, Anal. Chem. 62, 2258 (1990)).

Chemical alteration of the oligonucleotides usually takes place by altering the phosphate backbone, the ribose unit or the nucleotide bases in an appropriate manner (Cohen, 1989; Uhlmann and Peyman, 1990). A further method, which is frequently employed, is the preparation of oligonucleotide 5′-conjugates by reacting the 5′-hydroxyl group with appropriate phosphorylation reagents. Oligonucleotides which are only modified at the 5′-end have the disadvantage that they are degraded in serum. If, on the other hand, all the internucleotide phosphate radicals are altered, the properties of the oligonucleotides are often drastically changed. For example, the solubility of the methylphosphonate oligonucleotides in aqueous medium is diminished, as is their ability to hybridize. Phosphorothioate oligonucleotides have non-specific effects, so that, for example, homooligomers are also active against viruses.

The object is, therefore, to prepare oligonucleotide analogs with specific activity, increased serum stability and good solubility.

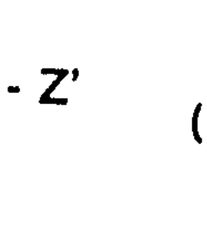
The invention relates to oligonucleotide analogs of the formula I



(I)

and their physiologically tolerated salts, where

R^1 is hydrogen, C_1-C_{18}-alkyl, preferably C_1-C_6-alkyl, C_2-C_{18}-alkenyl, C_2-C_{18}-alkynyl, C_2-C_{18}-alkylcarbonyl, C_3-C_{19}-alkenylcarbonyl, C_3-C_{19}-alkynylcarbonyl, C_6-C_{20}-aryl, (C_6-C_{14})-aryl-(C_1-C_8)-alkyl, or a radical of the formula II



(II);

R^2 is hydrogen, hydroxyl, C_1-C_{18}-alkoxy, halogen, azido or NH_2;

B is a conventional base in nucleotide chemistry, for example natural bases such as adenine, cytosine, guanine and thymine or unnatural bases such as purine, 2,6-diaminopurine, 7-deazaadenine, 7-deazaguanine, N^4N^4-ethanocytosine, N^6N^6-ethano-2,6-diaminopurine, pseudoisocytosine;

a is oxy or methylene;

n is an integer from 1 to 100, preferably 10 to 40;

W is oxo, thioxo or selenoxo;

V is oxy, thio or imino;

Y is oxy, thio, imino or methylene;

Y′ is oxy, thio, imino, $(CH_2)_m$ or $V(CH_2)_m$, where

m is an integer from 1 to 18, preferably from 1 to 6;

X is hydroxyl or mercapto;

U is hydroxyl, mercapto, SeH, C_1-C_{18}-alkoxy, preferably C_1-C_6-alkoxy, C_1-C_{18}-alkyl, preferably C_1-C_6-alkyl, C_6-C_{20}-aryl, (C_6-C_{14})-aryl-(C_1-C_8)-alkyl, NHR^3, NR^3R^4 or a radical of the formula III $(OCH_2CH_2)_pO(CH_2)_qCH_2R^{11}$ (III), where

R^3 is C_1-C_{18}-alkyl, preferably C_1-C_8-alkyl, C_6-C_{20}-aryl, (C_6-C_{14})-aryl-(C_1-C_8)-alkyl, $-(CH_2)_c-[NH(CH_2)_c]_d-NR^{12}R^{12}$, where c is an integer from 2 to 6 and d is an integer from 0 to 6, and each R^{12} independently of the other is hydrogen or C_1-C_6-alkyl or C_1-C_4-alkoxy-C_1-C_6-alkyl, preferably methoxyethyl;

R^4 is C_1-C_{18}-alkyl, preferably C_1-C_8-alkyl and particularly preferably C_1-C_4-alkyl, C_6-C_{20}-aryl or (C_6-C_{10})-aryl-(C_1-C_8)-alkyl, or, in the case of NR^3R^4, is, together with R^3 and the nitrogen atom carrying them, a 5-6-membered heterocyclic ring, which can additionally contain a further hetero atom selected from the group comprising O, S, N,

p is an integer from 1 to 100, preferably 3 to 20 and particularly preferably 3 to 8,

q is an integer from 0 to 22, preferably 0 to 15,

R^{11} is hydrogen or a functional group such as hydroxyl, amino, NHR^{13}, COOH, $CONH_2$, $COOR^{12}$ or halogen, where R^{12} is C_1-C_4-alkyl, preferably methyl;

Z = Z′ are hydroxyl, mercapto, SeH, C_1-C_{22}-alkoxy, preferably C_6-C_{18}-alkoxy, $-O-(CH_2)_b-NR^{12}R^{13}$, where b is an integer from 1 to 6, and R^{13} is C_1-C_6-alkyl or R^{12} and R^{13}, together with the nitrogen atom carrying them, form a 3-6-membered ring, C_1-C_{18}-alkyl, preferably C_1-C_8-alkyl, C_6-C_{20}-aryl, (C_6-C_{14})-aryl-(C_1-C_8)-alkyl, preferably (C_6-C_{10})-aryl-(C_1-C_4)-alkyl, (C_6-C_{14})-aryl-(C_1-C_8)-alkoxy, preferably (C_6-C_{10})-aryl-(C_1-C_4)-alkoxy, where aryl includes heteroaryl, and aryl is optionally substituted by 1, 2 or 3 identical or different radicals selected from the group comprising

carboxyl, amino, nitro, C_1-C_4-alkylamino, C_1-C_6-alkoxy, hydroxyl, halogen and cyano, C_1-C_{18}-alkylmercapto, NHR^3, NR^3R^4, a radical of the formula III or a group which favors intracellular uptake or serves as the label for a DNA probe, or, during hybridization of the oligonucleotide analog to the target nucleic acid, attacks the latter with binding, crosslinking or cleavage,

and

the curved bracket indicates that R^2 and the neighboring phosphoryl residue can be located in the 2′- and 3′-position or else the opposite way round in the 3′- and 2′-position,

where each nucleotide can be present in its D- or L-configuration and the base B can be located in the α- or β-position, with the proviso that, if Z = hydroxyl, mercapto, methyl or ethoxy, at least one of the groups X, Y, Y′, V and W is not hydroxyl, oxy or oxo, or R^1 is not hydrogen.

Preferred are oligonucleotide analogs of the formula I and their physiologically tolerated salts, where the base B is located in the β-position, the nucleotides are present in the D-configuration, R^2 is located in the 2′-position and a is oxy.

Particularly preferred are oligonucleotide analogs of the formula I, where

R^1 is hydrogen, C_1-C_6-alkyl, in particular methyl, or a radical of the formula II;

R^2 is hydrogen or hydroxyl, in particular hydrogen;

n is an integer from 10 to 40, in particular 12 to 30;

m is an integer from 1 to 6, in particular 1;

U is hydroxyl, mercapto, C_1-C_6-alkoxy, C_1-C_6-alkyl, NR^3R^4 or NHR^3, in particular hydroxyl or C_1-C_6-alkyl, where

R^3 is C_1-C_8-alkyl, preferably C_1-C_4-alkyl, or methoxyethyl, and B, W, V, Y, Y', X and Z have the abovementioned meaning.

Especially preferred are oligonucleotide analogs of the formula I, where V, Y' and Y have the meaning of oxy. Additionally particularly preferred are oligonucleotide analogs of the formula I, where V, Y, Y' and W have the meaning of oxy or oxo.

Very particularly preferred are oligonucleotide analogs of the formula I, where V, Y, Y', W and U have the meaning of oxy, oxo or hydroxyl.

Furthermore, oligonucleotide analogs of the formula I are preferred, where R^1 is hydrogen.

Especially preferred are oligonucleotide analogs of the formula I, where U, V, W, X, Y' and Y have the meaning of oxy, oxo or hydroxyl and R^1 is hydrogen.

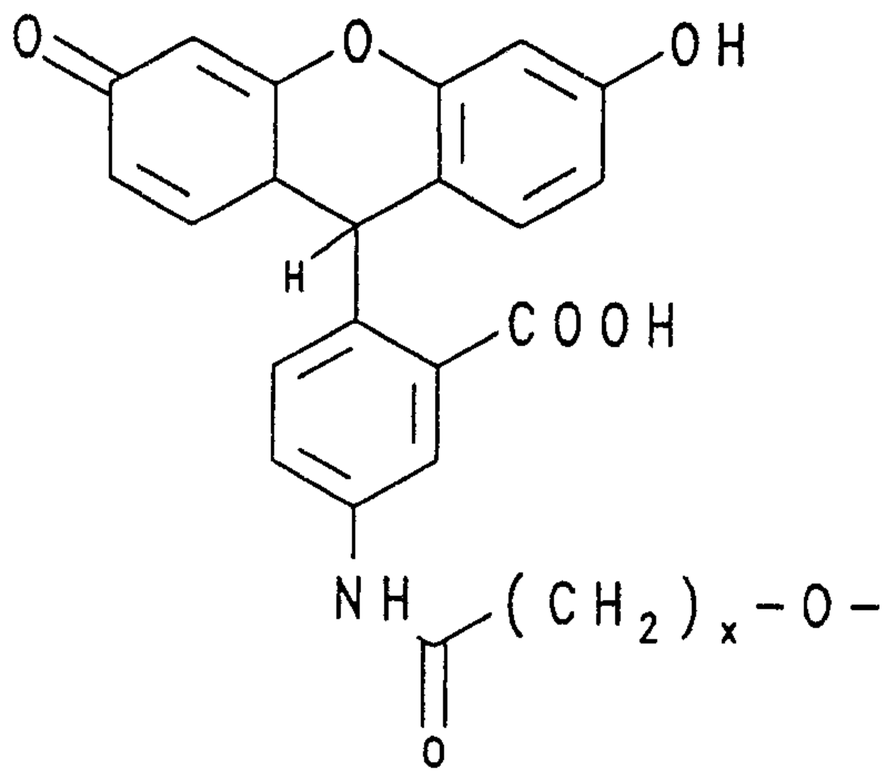
The residues which occur repeatedly, such as R^2, B, a, W, V, Y, U, R^3, R^4, p, q and Z, can, independently of each other, have identical or different meanings, i.e. each V, for example, is, independently of the others, oxy, thio or imino.

Halogen is preferably fluorine, chlorine or bromine.

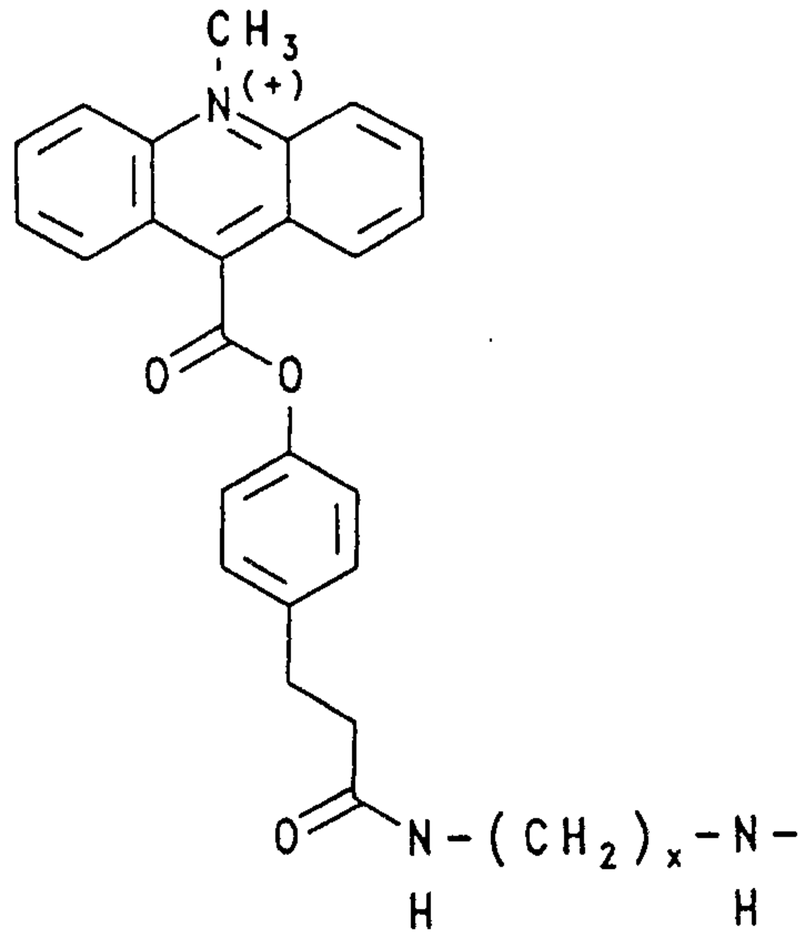
Heteroaryl is understood to mean the radical of a monocyclic or bicyclic (C_3-C_9)-heteroaromatic, which contains one or two N atoms and/or an S or an O atom in the ring system.

Examples of groups which favor intercellular uptake are various lipophilic radicals such as $-O-(CH_2)_x-CH_3$, where x is an integer from 6-18, $-O-(CH_2)_n-CH=CH-(CH_2)_m-CH_3$, where n and m are independently of each other an integer from 6 to 12, $-O-(CH_2CH_2O)_4-(CH_2)_9-CH_3$, $-O-(CH_2CH_2O)_8-(CH_2)_{13}-CH_3$ and $-O-(CH_2CH_2O)_7-(CH_2)_{15}-CH_3$, and also steroid residues, such as cholesteryl, and conjugates which make use of natural carrier systems, such as bile acid, folic acid, 2-(N-alkyl, N-alkoxy)-aminoanthraquinone and conjugates of

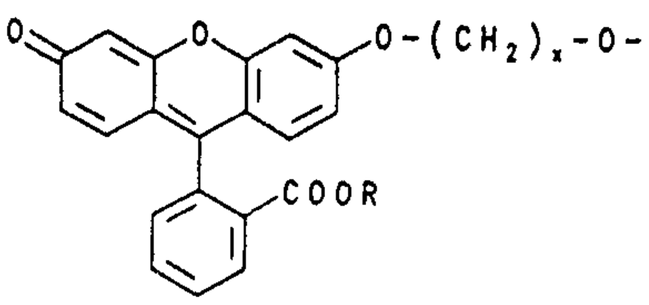
mannose and peptides of the corresponding receptors, which lead to receptor-mediated endocytosis of the oligonucleotides, such as EGF (epidermal growth factor), bradykinin and PDGF (platelet derived growth factor). Labeling groups are understood to mean fluorescent groups, for example of dansyl (= N-dimethyl-1-amino-naphthyl-5-sulfonyl) derivatives, fluorescein derivatives or coumarin derivatives, or chemiluminescent groups, for example of acridine derivatives, as well as the digoxigenin system, which is detectable by ELISA, the biotin group, which is detectable by the biotin/avidin system, or linker arms with functional groups which allow a subsequent derivatization with detectable reporter groups, for example an aminoalkyl linker, which is reacted with an acridinium active ester to form the chemiluminescent probe. Typical labeling groups are:



Fluorescein derivative
(x = 2-18, preferably 4-6)



Acridinium ester



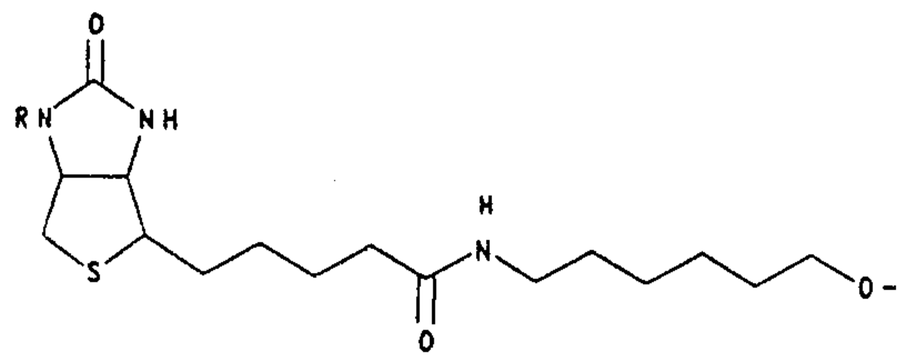
x = 2-18, preferably 4

R = H or C_1-C_4-alkyl

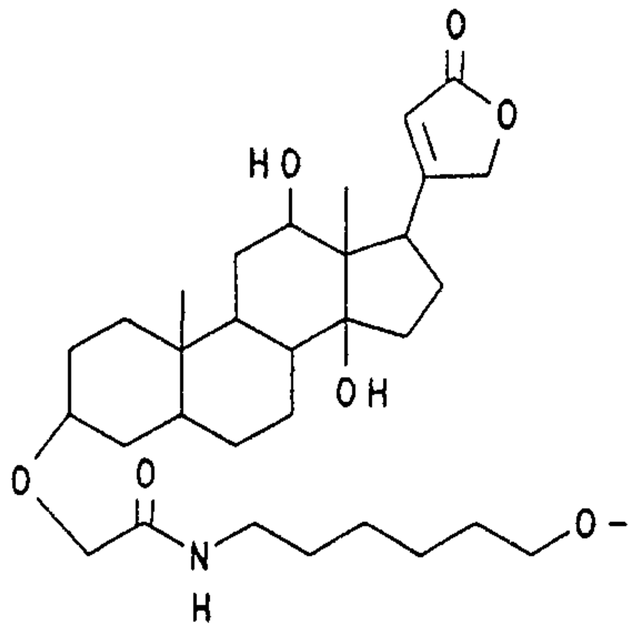
(= "fluorescein" for x = 4 and R = CH_3)

Fluorescein derivative

R = H or amino-protective group

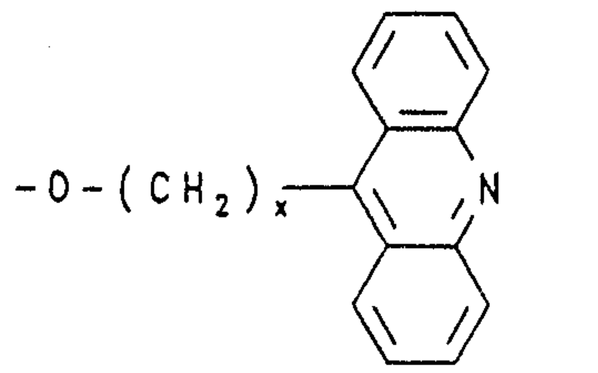


Biotin conjugate (= "biotin" for R = Fmoc)

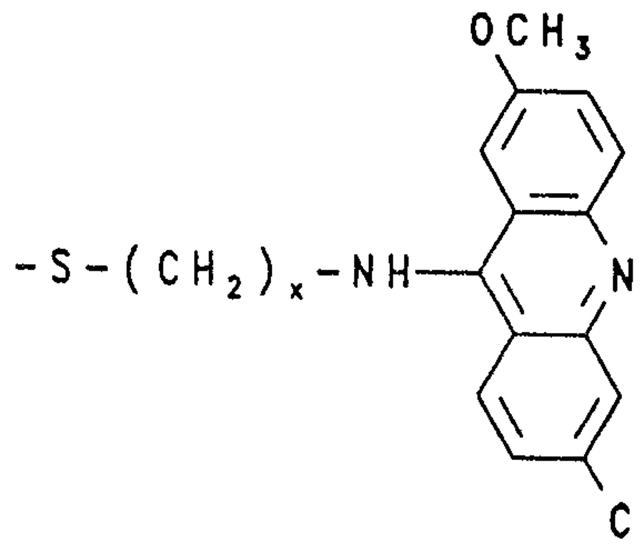


Digoxigenin conjugate

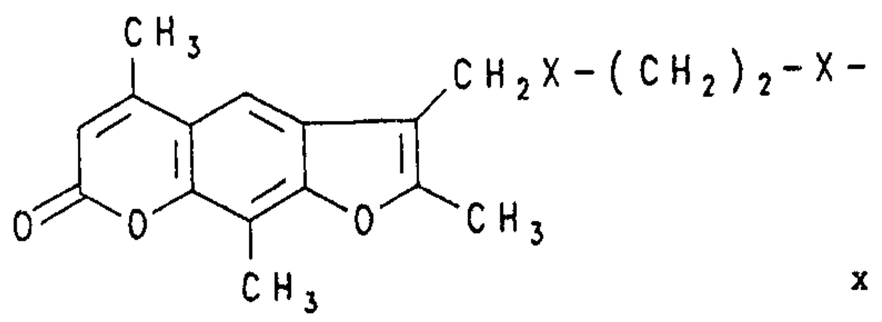
Oligonucleotide analogs which bind to nucleic acids or intercalate and/or cleave or crosslink contain, for example, acridine, psoralen, phenanthridine, naphthoquinone, daunomycin or chloroethylaminoaryl conjugates. Typical intercalating and crosslinking residues are:



Acridine derivative x = 2 - 12, preferably 4

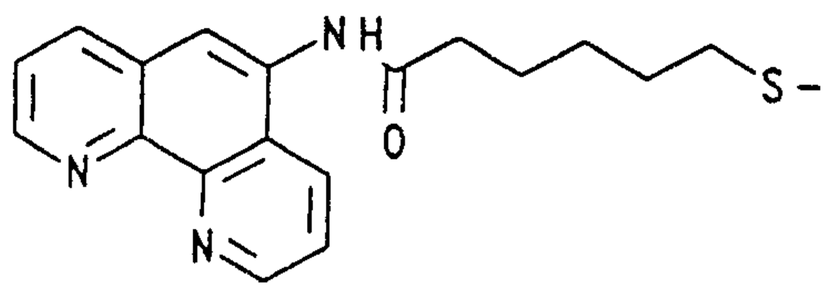


x = 2-12, preferably 4

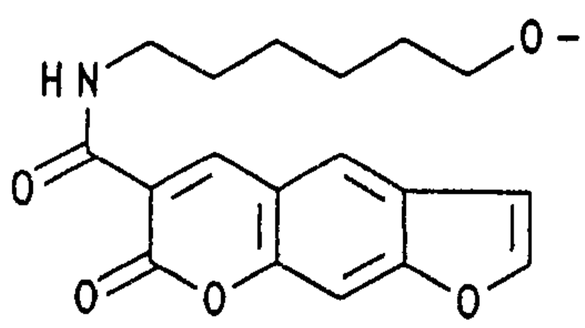


X = -NH or -O-

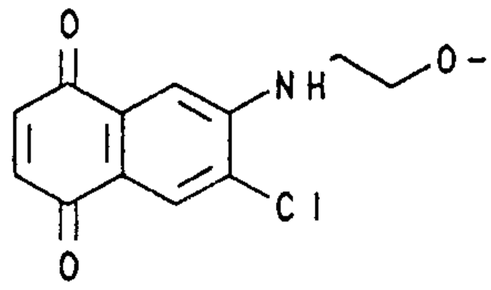
Trimethylpsoralen conjugate (= "psoralen" for X = O)



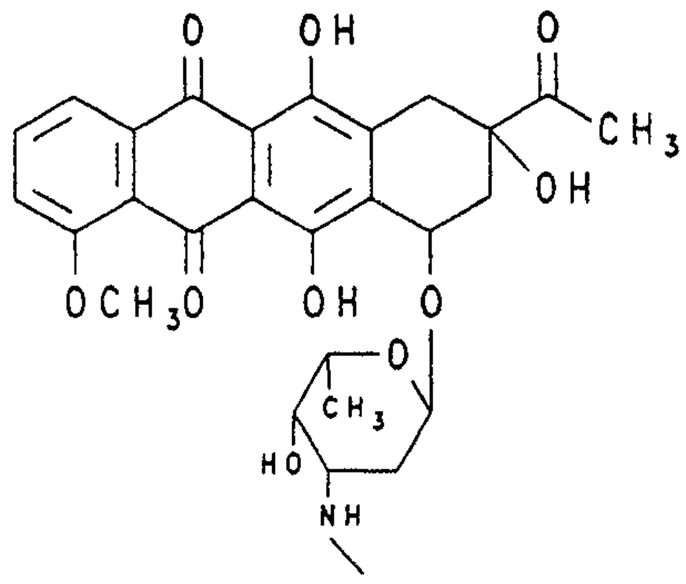
Phenanthroline conjugate



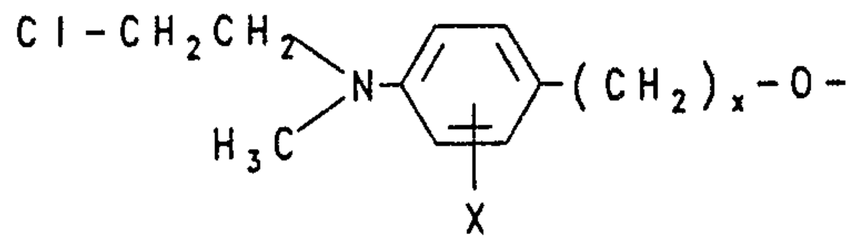
Psoralen conjugate



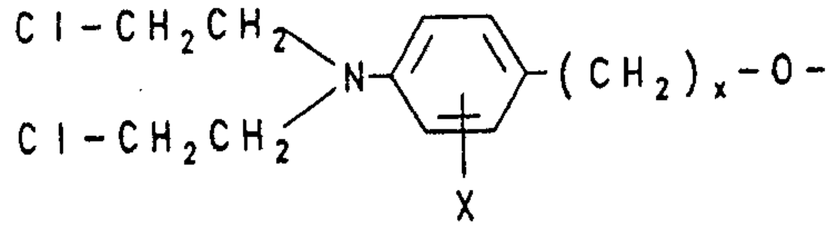
Naphthoquinone conjugate



Daunomycin derivative



x = 1-18, X = alkyl, halogen, NO_2, CN, -C-R



x = 1-18, X = alkyl, halogen, NO_2, CN, -C-R-

The morpholinyl and the imidazolidinyl radicals may be mentioned as examples of NR^3R^4 groups in which R^3 and R^4, together with the nitrogen atom carrying them, form a 5- to 6-membered heterocyclic ring, which additionally contains a further hetero atom.

The invention is not limited to α- and β-D- or L-ribofuranosides, α- and β-D- or L-deoxyribofuranosides and corresponding carbocyclic 5-membered ring analogs, but is also valid for oligonucleotide analogs which are composed of other sugar components, for example ring-expanded and ring-contracted sugars, acyclic sugar derivatives or suitable sugar derivatives of another type. Furthermore, the invention is not limited to the derivatives of the phosphate radical which are cited by way of example in formula I, but also relates to the known dephospho derivatives.

As for the synthesis of biological oligonucleotides, the preparation of oligonucleotide analogs of the formula I takes place in solution or preferably on a solid phase, optionally with the aid of an automatic synthesis apparatus.

However, solid phase synthesis of oligonucleotides with a phosphate or phosphate ester radical at the 3′-end is not possible by the standard phosphoramidite chemistry of Caruthers (M.D. Matteucci and M.H. Caruthers, J. Am. Chem. Soc. 103, 3185 (1981)), since the first nucleotide unit is bound to the solid support via the 3′-hydroxyl group and for this reason oligonucleotides with a 3′-hydroxyl group always result from these syntheses. Various processes based on the solid-phase method have been described, which processes, however, are all laborious and often cannot be used for preparing derivatives such as phosphate esters or alkylphosphonates (R. Eritja et al., Tetrahedron Lett. 32, 1511 (1991); P. Kumar et al., Tetrahedron Lett. 32, 967 (1991); W. T.

Markiewicz and T.K. Wyrzykiewicz, Phosphorus, Sulfur and Silicon 51/52, 374 (1990); E. Felder et al., Tetrahedron Lett. 25, 3967 (1984); R. Lohrmann and J. Ruth, DNA 3, 122 (1984)).

The invention therefore relates to a process for preparing oligonucleotide analogs of the formula I, where

a) a nucleotide unit with a 3′(2′)-terminal phosphorus-(V) grouping and a free 5′-hydroxyl or mercapto group is reacted with a further nucleotide unit with a phosphorus(III) or phosphorus(V) grouping in the 3′ position, or its activated derivatives,
or

b) the oligonucleotide analog is constructed with fragments in a similar manner,

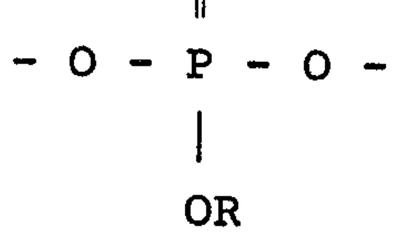
and protective groups, which have been temporarily introduced in the oligonucleotides obtained according to (a) or (b) in order to protect other functions, are removed and the oligonucleotide analogs of the formula I thus obtained are, where appropriate, converted into their physiologically tolerated salt.

Employed as starting component for the solid-phase synthesis is a solid support of the formula IV

$$D\text{-}X'\text{-}CH_2CH_2\text{-}S(O)_x\text{-}CH_2CH_2\text{-}A\text{-}T \qquad (IV),$$

where

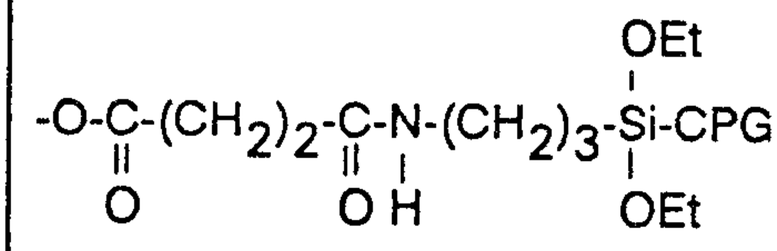
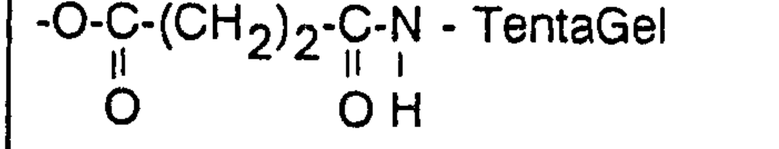
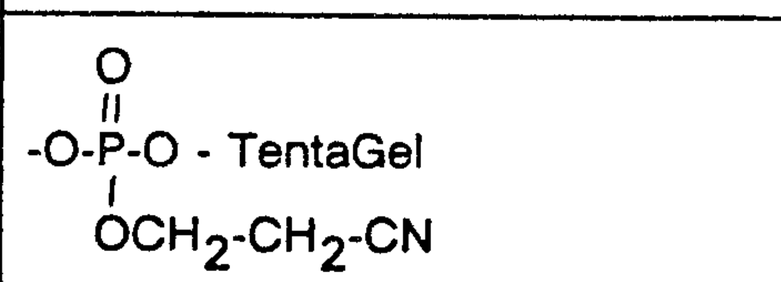
A is a linker arm, which, for example, is a residue of a dicarboxylic acid, a diol, an alkylamine, a dicarboxylic acid monoalkylamide, an acid amide or a phosphate of the formula



where R is = hydrogen or C_1-C_6-alkyl which is optionally substituted by -CN, preferably methyl or 2-cyanoethyl, T is a solid support, for example of materials such as CPG (controlled pore glass), silica gel or an organic resin such as polystyrene (PS) or a graft copolymer of PS and polyethylene glycol (POE), which is modified in the side chain by functional groups such as hydroxyl, amino, halogen or COOH,

D is a protective group which can be removed without cleaving the linker arm A and the X'-CH_2CH_2-$S(O)_x$-CH_2CH_2- radical (see Bioorg. Chem. 14 (1986) 274-325), such as 4-methoxytetrahydropyranyl and dimethoxytrityl, preferably dimethoxytrityl, x is an integer zero, 1 or 2 and X' is oxy or thio.

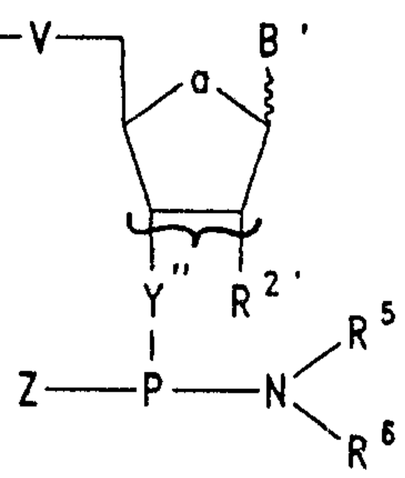
The linker arm A, which connects the solid support T to the sulfur-containing radical by a chemical bond (amide, ester inter alia) (Damka et al., Nucleic Acids Res. 18, 3813 (1990)), is preferably a succinic acid residue (O-C(O)-CH_2CH_2-C(O)-), an oxalic acid residue (O-C(O)-C(O)-), an alkylamine, preferably LCAA (long chain alkylamine), or polyethylene glycol. A succinic acid residue is particularly preferred. In particular cases, for example in combination with substituents which do not withstand lengthy treatment with ammonia, more labile linkers such as the oxalyl linker are advantageous. The preparation of solid supports of the formulae IV a-c is described in Example 1.

Träger	D	X'	x	A-T
IVa	DMTr	O	2	
IVb	DMTr	O	2	
Träger	D	X'	x	A-T
IVc	DMTr	O	0	

The solid-phase synthesis can take place according to the phosphate triester method, the H-phosphonate method or the phosphoramidite method, preferably according to the phosphoramidite method (E. Sonveaux, Bioorg. Chem. 14, 274 (1986)). The protective group D is always first of all removed from the support of the formula IV, preferably by an acid, for example trichloroacetic acid in methylene chloride. In the case of the phosphoramidite method, the support of the formula IV' thus obtained

$$HX'-CH_2-CH_2-S(O)_x-CH_2CH_2-A-T \qquad (IV'),$$

where x, X', A and T have the abovementioned meaning, is condensed in the presence of a weak acid such as tetrazole with a nucleoside phosphoramidite of the formula V



(V)

where

R is a protective group which can be removed under mild conditions, such as 4-methoxytetrahydropyranyl or dimethoxytrityl,

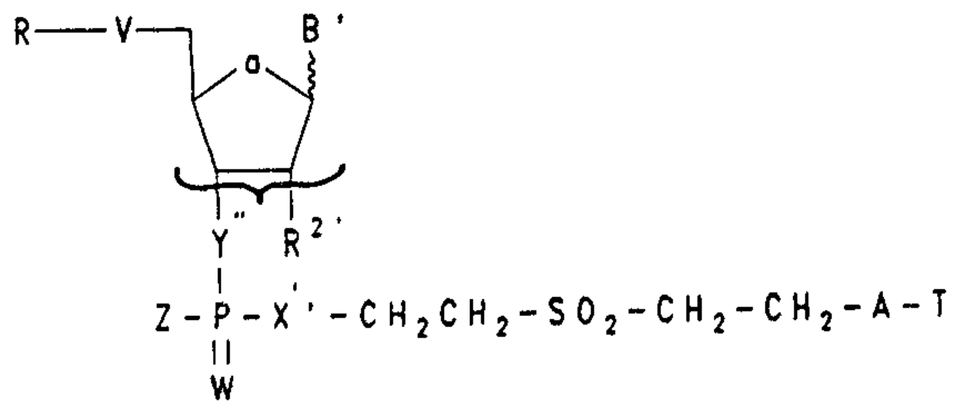
$R^{2'}$ is hydrogen, C_1-C_{18}-alkoxy, halogen or a protected hydroxyl or amino group and

R^5 and R^6 independently of each other are C_1-C_{12}-alkyl, or both residues together form a 5 to 6-membered ring,

Y'' is oxy, thio or $(CH_2)_m$, and

a, m, V and Z have the abovementioned meaning.

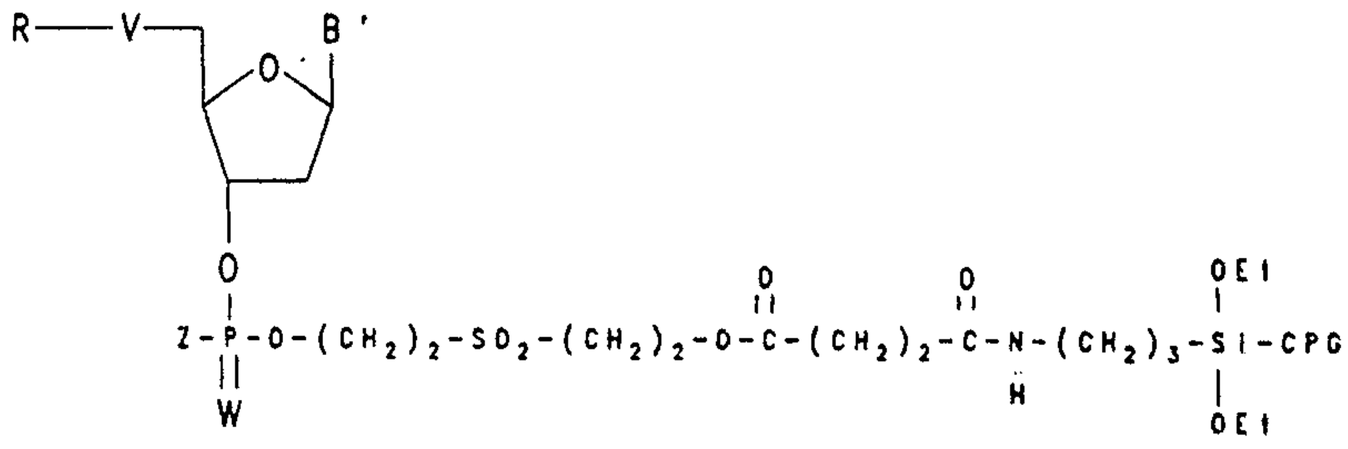
Subsequently, the support thus obtained is oxidized in a manner known per se with iodine water (W = O) or with TETD (tetraethylthiuram disulfide) or elemental sulfur (W = S) or with selenium (W = Se) to form the derivatized support of the formula VII



(VII)

where

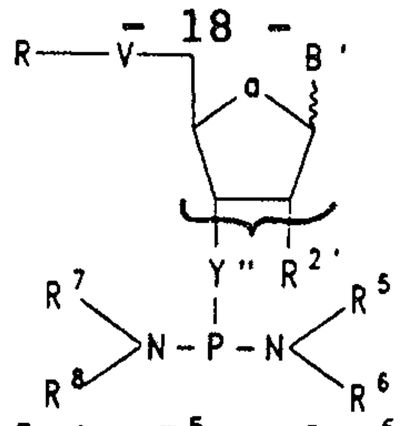
R, V, B', $R^{2'}$, Z, X', W, Y'', A and T have the abovementioned meaning. Supports of the formula VIIa



(VIIa)

are preferably prepared.

The phosphoramidite of the formula V can be obtained, for example, from the bisamidite of the formula VI

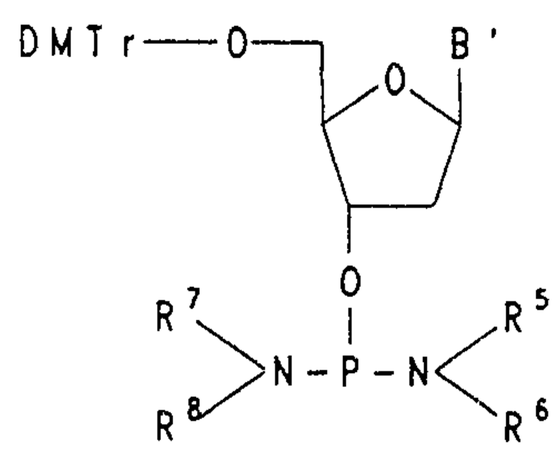


(VI)

where

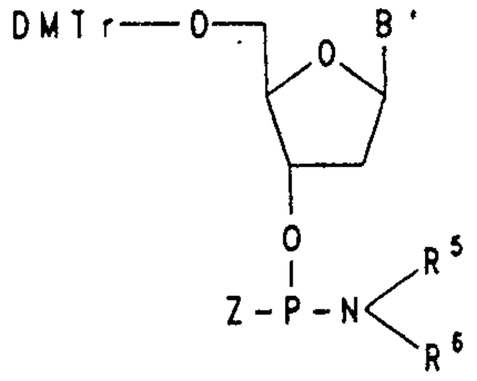
R^7 and R^8 are identical to R^5 and R^6 and

a, R, V, B', $R^{2'}$, Y'', R^5 and R^6 have the abovementioned meaning, by reaction with the corresponding alcohol or thioalcohol using tetrazole catalysis (Example 2, Method A), if Z is = alkoxy or alkylmercapto (J.E. Marugg et al., Tetrahedron Lett. 27, 2271 (1986). Preferred bis-amidites are those of the formula VIa



VIa)

In this way the amidites of the formulae VIII a-m were prepared, for example,



(VIII)

where

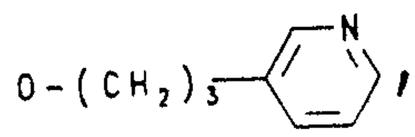
R^5 and R^6 have the abovementioned meaning, Z has the meaning of

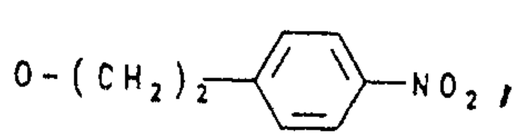
a) $O-CH_2CH_3$,

b) $O-i-C_3H_7$,

c) $O-n-C_6H_{13}$

d) $O-n-C_{18}H_{37}$,

e) ,

f) ,

g-k) a residue of the formula III (R^{11} = H), where in the case of

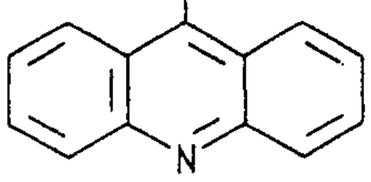
g) p = 3 and q = 0,

h) p = 4 and q = 9,

i) p = 5 and q = 4 and in the case of

k) p = 8 and q = 13,

p) CH_3 $(CH_2)_4$-O-

m) 

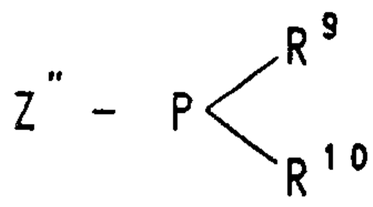
and B′ is

$Cyt^{i\text{-}Bu}$ in the case of a), c) and d),

Thy in the case of b) and p) and

Cyt^{Bz} in the case of e) - k) and m).

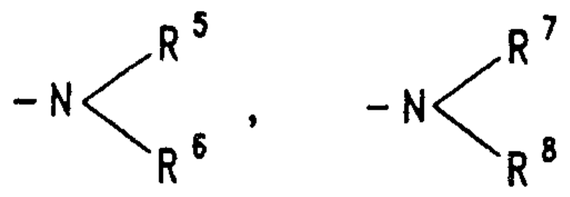
An alternative method for loading the support is the reaction of the phosphitylation reagent of the formula IX



(IX),

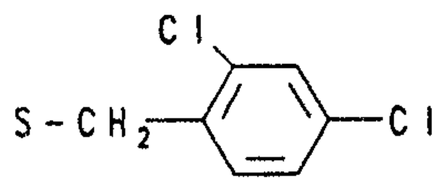
where

R^9 and R^{10} are, independently of each other, Cl, or Z′′, where Z′′ is = Z, with the proviso that hydroxyl, mercapto and SeH must be present as protected derivatives,



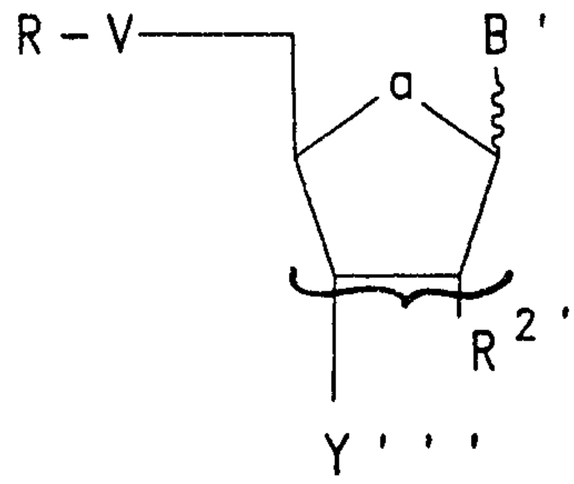
for example as O-CH_2CH_2-CN, O-CH_3, S-CH_2CH_2CN, X′-CH_2CH_2-$S(O)_{x'}$-CH_2CH_2-X′-D

or



, preferably as X′-CH_2CH_2-$S(O)_{x'}$-CH_2CH_2-X′-DMTr, where x′ is an integer zero or 1, in particular as O-CH_2CH_2-S-CH_2CH_2-O-DMTr, and R^5, R^6, R^7, R^8, X′, DMTr and D have the above-mentioned meaning, with a nucleoside with a free 3′(2′)-

group of the formula X



(X)

where

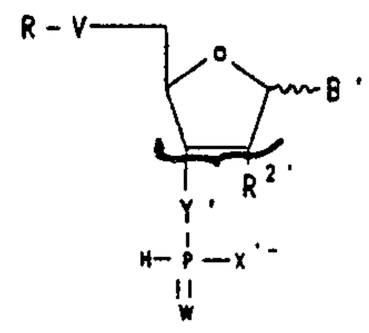
Y''' is oxy or thio and V, B' and R have the abovementioned meaning, and subsequent condensation of the compound thus obtained onto the support of the formula IV' in the presence of a condensing agent, such as tetrazole (for R^9, R^{10} = NR^5R^6 or NR^7R^8) or diisopropylamine (for R^9, R^{10} = Cl); this often represents the quicker method (Example 3, method B). Subsequent oxidation with iodine water or sulfur or selenium then leads to the compound of the formula VIIa. The protective group R can now be removed and the oligonucleotide synthesis continued in a known manner. At the end of the synthesis, the protective groups are removed in a known manner from the support-bound oligonucleotide analog thus obtained, and the oligonucleotide analog of the formula I according to the invention is then cleaved off the support.

If the synthesis was concluded in the last cycle with a unit of the formula V, an oligonucleotide analog of the formula I (R^1 = H) is obtained with a 5'-hydroxyl group and a phosphorus-containing conjugation at the 3'-end. If, on the other hand, a phosphorylating reagent, for example of the formula IX, where R^9 is = Z'', is employed in the last condensation step, an oligonucleotide analog of the formula I with R^1 = formula II, which possesses a phosphate-containing substitution at both the 3'- and 5'-ends, then results from the synthesis.

The preparation of oligonucleotides with a 3'-terminal phosphoramidate group is, for example, possible by reaction of the support of the formula IV' (x =

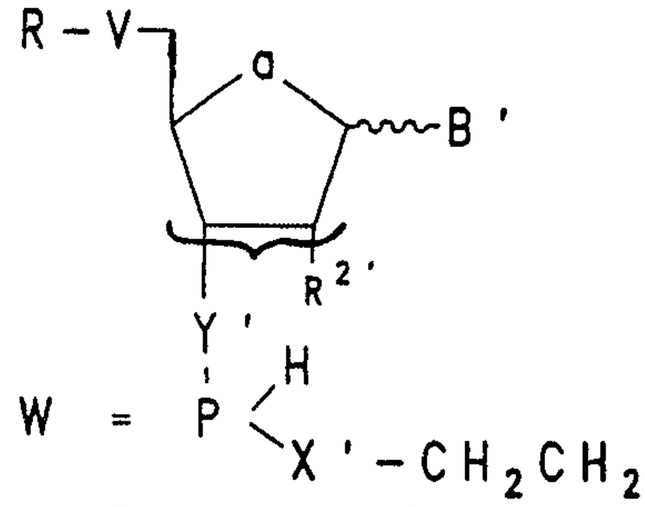
0) with the monomeric methoxyphosphoramidite of the formula V (Z = O-CH_3) in the presence of tetrazole, if the oxidation is carried out, as described in Jäger et al. (Biochemistry 27, 7237 (1988), with iodine/H_2NR^3 or HNR^3R^4, where R^3 and R^4 have the abovementioned meaning.

In certain cases (Z=NHR^3, NR^3R^4, O, S or Se) the introduction of the group Z can also take place by the H-phosphonate method, in which a nucleoside H-phosphonate of the formula XI



(XI)

where R, V, a, B′, Y′, X′ and W have the abovementioned meaning, is initially reacted with a support of the formula IV′ in the presence of a condensing agent such as pivaloyl or adamantoyl chloride and a base such as pyridine. The H-phosphonate diester formed, of the formula VII′

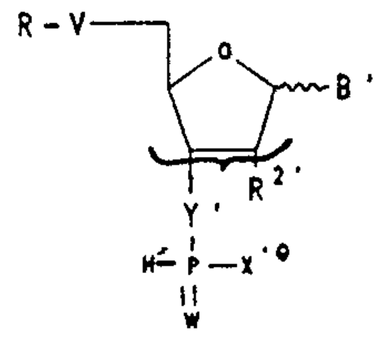


(VII′)

is then subjected to an oxidative phosphoramidation (B. Froehler, Tetrahedron Lett. 27, 5575 (1986)) or to oxidation with iodine water, sulfur or selenium. In this way an oligonucleotide with a 3′-terminal cholesteryl group can be prepared starting from, for example, VII′ (x = 0), with a cholesteryloxycarbonyl-aminoalkylamine in the presence of carbon tetrachloride. By oxidative amidation with 2-methoxyethylamine, oligonucleotides with a 3′-O-(2′-methoxyethyl)-phosphoramidate residue are

obtained, for example. Subsequent chain construction takes place in a known manner according to the phosphoramidite, H-phosphonate or triester methods.

The preparation of oligonucleotide analogs of the formula I is also possible using the triester method, where the hydroxyl group of the support of the formula IV' is reacted with a protected phosphate diester of the formula XII



(XII)

where R, V, a, B', R^2, Y', Z, W and X' have the abovementioned meaning, in the presence of a condensing agent. Preferred condensation reagents are arylsulfonyl chlorides such as mesitylenesulfonyl chloride, 2,4,6-triisopropylbenzenesulfonyl chloride or 8-quinolinesulfonyl chloride in the presence of nucleophilic catalysts such as imidazole, triazole, tetrazole or their substituted derivatives such as N-methylimidazole, 3-nitrotriazole or 5-(p-nitrophenyl)-tetrazole. Particularly preferred condensing agents are 4-substituted derivatives of pyridine-N-oxide or quinoline-N-oxide (Efimov et al., Nucleic Acids Research 13 (1985) 3651). Compared with the H-phosphonate and phosphoramidite processes, the triester process has the advantage that no additional oxidation step is required.

If the oligonucleotide synthesis is carried out with a thio (x = 0) or sulfinyl (x = 1) support of the formula IV', these groups are then at the end oxidized to the sulfonyl radical in a manner known per se [Funakoshi et al., Proc. Natl. Acad. Sci. 88 (1991), 6982], in order to ensure ready cleavage with bases, preferably ammonia.

The nature of the amino-protective groups of the bases B' and the constitution of the linker arm A depend, in the individual case, on the nature of the substituent Z, since the latter must be removable without difficulty once synthesis has been completed. For example, in preparing an oligonucleotide 3'-phosphate isopropyl ester (Z = O-i-C_3H_7), Benzoyl (Bz) protective groups can be used for B = Ade and Cyt and isobutyryl (i-Bu) protective groups for B = Gua. On the other hand, to synthesize an oligonucleotide 3'-methylphosphonate ester (Z = CH_3) or ethyl ester (Z = O-C_2H_5), the more labile phenoxyacetyl (PAC) and isobutyryl protective groups are used for B = Ade and Gua, and for B = Cyt, respectively.

Many conjugates possess additional functional groups, which must be protected in a suitable manner before incorporation into the monomeric units of the formula V. For example, the carboxyl group of fluorescein must be protected as an alkyl ester. In psoralen, the amide group can be present as a N-Fmoc (fluorenylmethoxycarbonyl)-protected compound. Hydroxyl groups can be protected from side reactions by acylation or silylation (t-butyldimethylsilyl). Amino groups can also be present in the trifluoroacetyl-protected form. In exceptional cases, the conjugates may be so unstable that they would be decomposed under the conditions of protective-group removal during the oligonucleotide synthesis. In such cases it is convenient to incorporate only one linker arm with a functional group, for example Z = HN-$(CH_2)_x$-NH-Fmoc, where x is an integer from 2-12, preferably 4-6, in the monomer of the formula V. After incorporation into the oligonucleotide and removal of the protective groups, preferably with ammonia, the free amino group may be coupled to active esters. The base-labile acridinium ester, for example, was prepared in this way.

Characterization of the synthesized oligonucleotide derivatives takes place by electro-spray ionization mass

spectrometry (Stults and Masters, Rapid Commun. Mass. Spectr. 5 (1991) 350).

The oligonucleotide analogs of the formula I according to the invention were tested for their stability in serum and toward known exonucleases.

It was found, surprisingly, that, in comparison with the unmodified oligonucleotides, all oligonucleotide analogs of the formula I possess markedly increased stability toward the serum nucleases, while their hybridization behavior is only slightly affected.

While unmodified oligonucleotides have a half life of about two hours in fetal calf serum, all oligonucleotide analogs of the formula I are satisfactorily stable for about 16 hours. In addition, the oligonucleotide analogs of the formula I are stable toward snake venom phosphodiesterase, whereas only those where R^1 is not hydrogen are resistant to spleen phosphodiesterase. Unmodified oligonucleotides are degraded exonucleolytically from the 3'-end by snake venom phosphodiesterase and from the 5'-end by spleen phosphodiesterase.

With complementary single-stranded nucleotide sequences, the oligonucleotide analogs of the formula I form stable, double-stranded hybrids due to Watson-Crick base pairing, while they form triple helical structures with double-stranded nucleic acids due to Hoogsteen base pairing. In this way, the regulation or suppression of biological functions of nucleic acids is possible using the oligonucleotide analogs according to the invention, for example suppression of the expression of cellular genes as well as of oncogenes or of viral genome functions. Oligonucleotide analogs of the formula I may therefore be used as medicaments for the therapy or prophylaxis of viral infections or cancers.

The activity of the oligonucleotides according to the invention was determined on the basis of the inhibition of HSV-1 viral replication. By way of example, the following oligonucleotides of the formula 1 were found to be active against HSV-1:

Sequence	Points of attack in HSV-1
5′ GGG GCG GGG CTC CAT GGG GG	IE 110 (start)
5′ CCG GAA AAC ATC GCG GTT GT	UL 30 (middle)
5′ GGT GCT GGT GCT GGA CGA CA	UL 48 (middle)
5′ GGC CCT GCT GTT CCG TGG CG	UL 52 (middle)
5′ CGT CCA TGT CGG CAA ACA GCT	UL 48 (start)
5′ GAC GTT CCT CCT GCG GGA AG	IE4/5 (splice site)

In their natural form, i.e. without 3′-derivatization, the selected sequences are inactive toward HSV-1 in cell culture, probably since they are subject to rapid degradation in serum or have insufficient cell penetration. On the other hand, the 3′-derivatized oligonucleotides of the formula I inhibit HSV-1 replication to differing extents. The following served as control sequences with the appropriate chemical derivatization but with no antiviral activity:

5′ CCA GGG TAC AGG TGG CCG GC	control
5′ GAC TAA TCG GGA ATG TTA AG	control

An oligonucleotide of the formula I modified with psoralen at the 3′-end (Example 4s) recognizes the IE4/5 region of HSV-2 and inhibits the replication of HSV-2. The anti-viral activity of the psoralen conjugates may be significantly increased by irradiation with UV light. The HSV-1/2 genome, with its 160,000 bases, naturally offers innumerable alternative target sequences of diverse efficiency for inhibiting viral replication. By varying the nucleotide sequences, the therapeutic principle may be applied to any other viruses, bacteria or other pathogens. The sole prerequisite for transfer to other

pathogens is that the genes which are essential for the life cycle of these pathogens are known. The sequences of these genes are deposited in great variety in the so-called gene databases. This is also the case for oncogenes and other cellular genes whose function is to be suppressed. Examples of other cellular genes are those which encode enzymes, receptors, ion channels, immunomodulators, growth factors and other regulatory proteins. Examples of oncogenes are abl, neu, myc, myb, ras, fos, mos, erbB, ets, jun, p53, src and rel.

Antisense and triplex-forming oligonucleotide sequences are, for example, known as inhibitors of the cyclic AMP-dependent protein kinase (L. Sheffield, Exp. Cell Res. 192 (1991) 307), the strychnine-sensitive glycine receptor (Akagi et al., Proc. Natl. Acad. Sci. USA 86 (1989), 86, 8103), the chloride channel (Sorscher et al., Proc. Natl. Acad. Sci. USA 88 (1991), 7759), Interleukin-6 (Levy et al., J. Clin. Invest. 88 (1991), 696), the basic fibroblast growth factor (Becker et al., EMBO J. 8 (1989), 3685) and the c-myc oncogene (Postel et al., Proc. Natl. Acad. Sci. USA 88 (1991), 8227). The following further examples of sequences of other target molecules are intended to illustrate the broad applicability of the oligonucleotides according to the invention.

a) Antisense oligonucleotides against HIV-1:

5′ ACA CCC AAT TCT GAA AAT GG 3′	(splice site)
5′ AGG TCC CTG TTC GGG CGC CA 3′	(primer binding site)

b) EGF receptor (epidermal growth factor receptor)

5′ GGG ACT CCG GCG CAG CGC 3′	(5′ untranslated)
5′ GGC AAA CTT TCT TTT CCT CC 3′	(aminoterminal)

c) p53 tumor suppressor

5′ GGG AAG GAG GAG GAT GAG G 3′	(5′-noncoding)

5′ GGC AGT CAT CCA GCT TCG GAG 3′ (start of translation)

d) c-fos oncogene

5′ CCC GAG AAC ATC ATG GTC GAA G 3′ (start of translation)

5′ GGG GAA AGC CCG GCA AGG GG 3′ (5′-noncoding)

e) ELAM-1 (endothelial leucocyte adhesion molecule)

5′ ACT GCT GCC TCT TGT CTC AGG 3′ (5′-noncoding)

5′ CAA TCA ATG ACT TCA AGA GTT C 3′ (start of translation)

f) ICAM-1 (intracellular adhesion molecule)

5′ CTC CCC CAC CAC TTC CCC TC 3′ (3′-untranslated)

5′ GCT GGG AGC CAT AGC GAG G 3′ (start of translation)

g) BCR-ABL (Philadelphia chromosome translocation)

5′ GCT GAA GGG CTT CTT CCT TAT TG 3′ (BCR-ABL breakpoint)

Compared to the oligonucleotide derivatives with a 3′-hydroxyl group, known from the literature, DNA probes which comprise oligonucleotide analogs of the formula I on the one hand offer the advantage of increased nuclease stability and on the other permit the acceptance of identical or different marker molecules at both ends of the oligonucleotide. It is of advantage that different marker groupings can be selectively activated within one oligonucleotide (double labeling). The bifunctional derivatization can also be used to introduce a label at the one end and an additional function (for example an affinity label) at the other end. For this purpose, biotin, which recognizes avidin or streptavidin, can, for example, be incorporated at the 3′-end of the oligonucleotide, while an acridinium ester chemiluminescence label can be attached to the 5′-end via an

alkylamino linker.

In addition, the penetration behavior of the oligonucleotide analogs according to the invention is in many cases more favorable than in the case of unmodified oligonucleotides, in particular if lipophilic radicals are introduced. The increased stability of the oligonucleotides and their improved cell penetration are expressed in the form of a higher biological activity as compared with the unmodified oligonucleotides.

The previously mentioned diagnostic, prophylactic and therapeutic applications of the oligonucleotide analogs according to the invention are only a selection of representative examples, and the use of the analogs is therefore not limited to them. In addition, the oligonucleotide analogs according to the invention may, for example, be employed as aids in biotechnology and molecular biology.

The invention relates furthermore to pharmaceutical preparations which contain an effective amount of one or more compounds of the formula I or their physiologically tolerated salts, where appropriate together with physiologically tolerated adjuvants and/or excipients, and/or other known active substances, as well as a process for preparing these preparations, wherein the active substance, together with the excipient and possibly further adjuvants, additives or active substances, is converted into a suitable presentation. Administration preferably takes place intravenously, topically or intranasally.

Example 1: Preparation of a support of the formula IV

a) Preparation of the support of the formula IVa by reacting aminopropyl-CPG with the succinate of bis-hydroxyethyl sulfone dimethoxytrityl ether

4.56 g of the dimethoxytrityl (DMTr) monoether of bis-(2-hydroxyethyl) sulfone (10 mmol) are dried by twice being taken up and concentrated in abs. pyridine, and are dissolved in 25 ml of abs. pyridine, then 1.78 g (14 mmol) of DMAP (dimethylaminopyridine) and 1.4 g of succinic anhydride (14 mmol) are added and this mixture is stirred at room temperature for 3 hours. After the reaction is complete, the mixture is concentrated, the residue is taken up and concentrated three times in toluene to remove the pyridine, and then taken up in 220 ml of methylene chloride. The organic phase is washed with 10% strength citric acid (110 ml) and 3 times with 110 ml of water, dried over sodium sulfate and concentrated. The resulting solid residue is dried in vacuo (5.64 g). 1.67 g (3 mmol) of this succinate are taken up and concentrated twice in abs. pyridine and dissolved in a mixture of 0.65 ml of abs. pyridine and 6 ml of tetrahydrofuran (THF). A solution of 420 mg (3 mmol) of p-nitrophenol and 687 mg of DCC (dicyclohexylcarbodiimide, 3.3 mmol) in 2.1 ml of abs. THF is then added and the mixture is stirred at room temperature for two hours. Once the reaction is complete, the precipitated dicyclohexylurea is removed by centrifugation. The sediment is suspended in 1 ml of abs. ether and centrifuged once again. 1.5 g of the aminopropyl-CPG support from Fluka (500 Å, 100 μmol/g of amino group) are suspended in a mixture of 1.8 ml of abs. DMF and 350 μl of triethylamine, and the combined solutions of the nitrophenyl succinate ester, which have been decanted from the sediment, are added, and the mixture shaken at room temperature for 16 hours. The solid support is separated off and shaken at room temperature for one hour with 3 ml of capping reagent (acetic anhydride/2,6-lutidine/DMAP; each 0.25 M in THF) to block reactive groups. The derivatized CPG support is then filtered off with suction, washed with methanol, THF, methylene chloride and ether and subsequently dried in vacuo at 40°C. The loading of the support of the

formula IVa with dimethoxytrityl-containing component is 38 μmol/g.

b) Preparation of the support of the formula IVb by reacting TentaGel® (® = registered trademark of the Rapp company, Tübingen) with the succinate of the bishydroxy ethyl sulfone dimethoxytrityl ether.

100 mg of the amino form of the TentaGel resin, a PS/POE copolymer with 250 μmol/g amino group, are suspended in a mixture of 360 μl of DMF and 70 μl of triethylamine, and 400 μmol of the p-nitrophenyl succinate ester (preparation see Ex. 1a) are added and the mixture is shaken at room temperature for 16 hours. The subsequent workup is as described in Ex. 1a). The loading of the TentaGel resin of the formula IVb with dimethoxytrityl-containing component is 98 μmol/g.

c) Preparation of the support IVc by reacting TentaGel (hydroxy form) with the phosphitylating reagent of the formula IX (Z''-DMTr-O-CH_2CH_2-S-CH_2CH_2-O-; R^9 = N(i-C_3H_7)$_2$; R^{10} = O-CH_2CH_2CN).

50 mg of the hydroxy form of the TentaGel resin with 500 μmol/g hydroxyl group are reacted in acetonitrile at 22°C with 10 equivalents of the phosphitylating reagent of the formula IX (Z''= DMTr-O-CH_2CH_2-S-CH_2CH_2-O-; R^9 = N(i-C_3H_7)$_2$; R^{10} = O-CH_2CH_2CN) in the presence of 25 equivalents of tetrazole. After oxidizing with iodine water (1.3 g of iodine in THF/water/pyridine; 70:20:5=v:v:v), working up is carried out as described in Example 1a. The loading of the support of the formula IVc with dimethoxytrityl-containing component is 247 μmol/g.

Example 2: Preparation of protected nucleoside 3'-phosphoramidites of the formula VIII

a) Preparation of VIIIa (B′ = Cyt^{iBu}, Z=O-CH_2CH_3, R^5=R^6=i-C_3H_7)

2 mmol of the nucleoside 3′-phosphorobisamidite of the formula VI (B′-Cyt^{iBu}, R^5=R^6=R^7=R^8=i-C_3H_7) are taken up and concentrated twice in 20 ml of abs. acetonitrile and then dissolved in 20 ml of abs. acetonitrile. A solution of 2.4 mmol of ethanol and 1.2 mmol of sublimed tetrazole in 5 ml of abs. acetonitrile is then added dropwise over a period of 15 minutes. After stirring has been continued for a further 2.5 hours, the mixture is diluted with 75 ml of methylene chloride, and the organic phase is extracted with 50 ml of 5% strength sodium bicarbonate solution. The aqueous solution is washed twice with 50 ml of methylene chloride, the combined organic phases are dried over sodium sulfate and concentrated in vacuo. The residue is purified by column chromatography on silica gel with methylene chloride/n-heptane/triethylamine (45:45:10;v:v:v). 0.7 g of the required diastereomeric substance is obtained as a compound which is pure by thin-layer chromatography. (^{31}P-NMR σ=146.7, 147.5 ppm). Traces of the corresponding bis-ethyl phosphite are isolated as byproduct (^{31}P-NMR σ=139.3 ppm).

b) Preparation of VIIIb (B′= Thy, Z = O-i-C_3H_7, R^5=R^6=i-C_3H_7)

The preparation takes place by phosphitylation of the 5′-O-dimethoxytritylthymidine of the formula X (B′ = Thy (β-position); R = DMTr, V = O, a=O, Y′′=O; 2mmol) with the bisamidite of the formula IX (Z′′ = O-i-C_3H_7, R^9=R^{10}=N(i-C_3H_7)$_2$; 4 mmol) in the presence of tetrazole (0.5 mmol) in 10 ml of abs. methylene chloride. The mixture is worked up as in Example 2a. (^{31}P-NMR σ=145.04 ppm, 145.66 ppm).

c) Preparation of VIIIc (B′= Cyt^{iBu}, Z = O-n-C_6H_{13}, R^5=R^6=i-C_3H_7)

In an analogous manner to Example 2a from the bisamidite of the formula VIa ($B'=Cyt^{iBu}$, $R^5=R^6=R^7=R^8=i\text{-}C_3H_7$) by reaction with one equivalent of n-hexanol with tetrazole catalysis. (^{31}P-NMR 148.1 ppm, 148.5 ppm).

d) Preparation of VIII d ($B'= Cyt^{iBu}$, $Z = O\text{-}n\text{-}C_{18}H_{37}$, $R^5=R^6=i\text{-}C_3H_7$)

In an analogous manner to Example 2a from the bisamidite of the formula VIa ($B'=Cyt^{iBu}$, $R^5=R^6=R^7=R^8=i\text{-}C_3H_7$) by reaction with one equivalent of n-octadecanol with tetrazole catalysis. (^{31}P-NMR 147.2 ppm, 147.9 ppm).

e) Preparation of VIIIe ($B'= Cyt^{Bz}$, Z = 3-pyridylpropan-3-oxy, $R^5=R^6=R^7=R^8=i\text{-}C_3H_7$)

In an analogous manner to Example 2a from the bisamidite of the formula VIa ($B'= Cyt^{Bz}$, $R^5=R^6=R^7=R^8=i\text{-}C_3H_7$, $R^{2'}$ = H) by reaction with one equivalent of 3-pyridine(propan-3-ol) with tetrazole catalysis. In this case it was possible to separate the two diastereomers by column chromatography. (^{31}P-NMR diastereomer 1: 147.7 ppm, diastereomer 2: 148.2 ppm)

f) Preparation of VIIIf ($B' = Cyt^{Bz}$, Z = p-nitrophenylethyl-2-oxy, $R^5=R^6=i\text{-}C_3H_7$)

In an analogous manner to Example 2a from the bisamidite of the formula VIa ($B'= Cyt^{Bz}$, $R^5=R^6=R^7=R^8=i\text{-}C_3H_7$) by reaction with one equivalent of p-nitrophenylethan-2-ol with tetrazole catalysis. (^{31}P-NMR 148.1 ppm, 148.6 ppm).

g) Preparation of VIIIg ($B' = Cyt^{Bz}$, $Z=-(OCH_2CH_2)_3OCH_3$, $R^5=R^6=i\text{-}C_3H_7$)

In an analogous manner to Example 2a from the bisamidite of the formula VIa ($B'= Cyt^{Bz}$, $R^5=R^6=R^7=R^8=i\text{-}C_3H_7$) by

reaction with one equivalent of triethylene glycol monomethyl ether with tetrazole catalysis. (^{31}P-NMR 148.5 ppm, 148.9 ppm).

h) Preparation of VIII h (B'=Cyt^{Bz}, Z = $-(OCH_2CH_2)_4O(CH_2)_9CH_3$, R^5=R^6=i-C_3H_7)

In an analogous manner to Example 2a from the bisamidite of the formula VIa (B'= Cyt^{Bz}, R^5=R^6=R^7=R^8=i-C_3H_7) by reaction with one equivalent of tetraethylene glycol monodecyl ether with tetrazole catalysis. (^{31}P-NMR 148.4 ppm, 148.8 ppm).

i) Preparation of VIIIi (B'=Cyt^{Bz}, Z = $-(OCH_2CH_2)_5O(CH_2)_4CH_3$, R^5=R^6=i-C_3H_7)

In an analogous manner to Example 2a from the bisamidite of the formula VIa (B'= Cyt^{Bz}, R^5=R^6=R^7=R^8=i-C_3H_7) by reaction with one equivalent of pentaethylene glycol monopentyl ether with tetrazole catalysis.
(^{31}P-NMR 148.4 ppm, 148.9 ppm).

k) Preparation of VIII k (B'=Cyt^{Bz}, Z = $-(OCH_2CH_2)_8O(CH_2)_{13}CH_3$, R^5=R^6=i-C_3H_7)

In an analogous manner to Example 2a from the bisamidite of the formula VIa (B'= Cyt^{Bz}, R^5=R^6=R^7=R^8=i-C_3H_7) by reaction with one equivalent of octaethylene glycol monotetradecyl ether with tetrazole catalysis (^{31}P-NMR 148.4 ppm, 148.8 ppm).

l) Preparation of VIIIp (B' = Thy, Z = CH_3, R^5=R^6=i-C_3H_7)

In an analogous manner to Example 2b from 5'-O-dimethoxytritylthymidine by phosphitylation with the reagent of the formula IX (Z''= CH_3, R^9 = Cl, R^{10} = $N(i\text{-}C_3H_7)_2$, where, instead of tetrazole, catalysis is effected with two equivalents of diisopropylethylamine.

(^{31}P-NMR 120.6 ppm, 121.0 ppm).

m) Preparation of VIIIm (B′=Cyt^{Bz}, Z = acridine-9-(butyl-4-oxy)-, R^5=R^6=i-C_3H_7)

In an analogous manner to Example 2a from the bisamidite of the formula VIa (B′ = Cyt^{Bz}, R^5=R^6=R^7=R^8=i-C_3H_7) by reaction with one equivalent of 9-(4-hydroxybutyl)-acridine with tetrazole catalysis.
(^{31}P-NMR 146.7 ppm, 147.4 ppm).

Example 3: Preparation of the support-bound nucleotide of the formula VII

a) Method A: Preparation of a support of the formula VIIa-1 by coupling the nucleoside 3′-phosphoramidite of the formula VIIIb

7.5 mg of the support from Example 1a, to which is bound 0.2 μmol of the bishydroxyethyl sulfone dimethoxytrityl ether, are treated with 3% strength trichloroacetic acid, thereby removing the DMTr protective group, washed with acetonitrile, and subsequently reacted with 2 μmol of the nucleoside 3′-phosphoramidite of the formula VIIIb (B′ = Thy, Z=O-i-C_3H_7, R^5=R^6=i-C_3H_7) in the presence of tetrazole (10 μmol) in acetonitrile. The reaction time is 2.5 minutes. Oxidation with iodine (for W=O; 1.3 g of iodine in THF/water/pyridine; 70:20:5=v:v:v) then takes place.

b) Method B: Preparation of a support of the formula VIIa-2 by reaction via the phosphitylation reagent of the formula IX

The phosphitylation reagent of the formula IX (Z′′ = n-octyl, R^9=R^{10}=Cl; 1 equivalent) is reacted in the presence of 1.2 equivalents of diisopropylethylamine (DIPEA) in abs. acetonitrile or methylene chloride with a nucleoside of the formula X (1 equivalent of 5′-O-dimethoxytrityl-

thymidine, B′ = β-position, Y′′′ = O,) at -78°C to form the corresponding nucleoside-3′-O-n-octylphosphone monochloride. To remove the protective group D=DMTr, the support of the formula IVa is treated as described in Method A, and then washed with acetonitrile and reacted with an excess of the nucleoside-3′-O-n-octylphosphone monochloride, prepared in situ, in the presence of DIPEA. After oxidation with iodine water, a support-bound nucleotide of the formula VIIa-2 is obtained, which is available for the subsequent oligonucleotide synthesis.

Example 4: Preparation of oligonucleotides of the formula I (the monomer is in each case a β-D-deoxyribonucleoside)

a) Preparation of an oligonucleotide of the formula Ia (R^1=R^2=H, Z=O-i-C_3H_7, a=U=V=W=X=Y=Y′=O, B=Thy, n=9)

TpTpTpTpTpTpTpTpTpTp-(O-i-C_3H_7)

0.2 μmol of the support VIIa-1 (B′ = Thy, W=O, Z=O-i-C_3H_7) from Example 3a is treated with the following reagents in turn:

1. abs. acetonitrile
2. 3% trichloroacetic acid in dichloromethane
3. abs. acetonitrile
4. 4 μmol of β-cyanoethyl 5′-O-dimethoxytrityl-thymidine-3′-phosphite-diisopropylamidite and 25 μmol of tetrazole in 0.15 ml of abs. acetonitrile.
5. Acetonitrile
6. 20% acetic anhydride in THF with 40% lutidine and 10% dimethylaminopyridine
7. Acetonitrile
8. Iodine (1.3 g in THF/water/pyridine; 70:20:5=v:v:v)

The steps 1 to 8, hereinafter termed one reaction cycle, are repeated 8 times to construct the decathymidylate derivative. After the synthesis has been completed, removal of the dimethoxytrityl group takes place as described in steps 1 to 3. The oligonucleotide is cleaved from the support, and the β-cyanoethyl groups are simultaneously eliminated, by treatment for 1.5 hours with ammonia. Since the oligonucleotide does not contain any amino-protective groups, no further treatment with ammonia is necessary. The resultant crude product of isopropyl decathymidylate 3'-phosphate is purified by polyacrylamide gel electrophoresis or HPLC.

b) Preparation of an oligonucleotide of the formula Ib ($R^1 = R^2 = H$, $Z = O\text{-}i\text{-}C_3H_7$, a=U=V=W=X=Y=Y'=O)

d(CpGpTpCpCpApTpGpTpCpGpGpCpApApApCpApGpCpTp-O-i-C_3H_7)

The synthesis takes place in an analogous manner to Example 4a, but with different nucleotide bases in the monomer. In synthesis steps 1 to 8, the monomer is generally employed as a β-cyanoethyl 5'-O-dimethoxytrityl-nucleoside-3'-phosphite-dialkylamide, where the amino group of adenine (Ade), cytosine (Cyt) or guanine (Gua) is provided with suitable protective groups. In this example, N^6-benzoyl-Ade (Ade^{Bz}), N^4-benzoyl-Cyt (Cyt^{Bz}) and N^2-isobutyryl-Gua (Gua^{iBu}) are used. Chain construction takes place as described in Example 4a, starting with the support of the formula VIIa-1 (B' = Thy, W = O, Z = O-i-C_3H_7), and condensing on the corresponding monomers according to the above sequence. However, to remove the amino-protective groups, an additional treatment with ammonia (50°C for 16 hours) is carried out.

c) Preparation of an oligonucleotide of the formula Ic ($R^1 = R^2 = H$, $Z = O\text{-}CH_2CH_3$, a = U = V = W = Y = Y' = O)

d(CpGpTpCpCpApTpGpTpCpGpGpCpApApApCpApGpCp-O-CH_2CH_3

Starting with the support of the formula VIIa-3 (B′=Cyt^{iBu}, W=O, Z=O-C_2H_5), whose preparation takes place with the aid of the monomer of the formula VIIIa according to method A (Example 3a), the synthesis is carried out in an analogous manner to Example 4b. However, the more labile amino-protective groups N^6-phenoxyacetyl-Ade (Ade^{PAC}), N^4-isobutyryl-Cyt (Cyt^{iBu}) and N^2-phenoxyacetyl-Gua (Gua^{PAC}), which are easier to cleave at the end of the synthesis, are advantageously used to prepare base-labile substitutions (as here for Z = O-C_2H_5). Removal of the protective groups with ammonia then only takes 2 hours at 50°C. If the product is treated with ammonia for a further 6 hours at 50°C, about 5 to 10 percent of the oligonucleotide-3′-phosphate is obtained as a byproduct as a result of cleavage of the ethyl phosphate ester.

d) Preparation of an oligonucleotide of the formula Id (R^1 = R^2 = H, Z = O-$(CH_2)_{17}CH_3$, a = U = V = X = Y = Y′ = O; W = O, except for the last two 5′-terminal phosphorothioate internucleotide bonds, where W = S (indicated as p_s))

d($Cp_sGp_sTpCpCpApTpGpTpCpGpGpCpApApApCpApGpCp$-O-$(CH_2)_{17}CH_3$)

Starting with the support of the formula VIIa-4 (B′ = Cyt^{iBu}, W = O, Z = O-$(CH_2)_{17}CH_3$), whose preparation takes place with the aid of the monomer of the formula VIIId according to method A (Example 3a), the synthesis is carried out with the more labile protective groups in an analogous manner to that described in Example 4c. After coupling the penultimate nucleotide (G) and the last nucleotide (C), a TETD solution (0.4 M tetraethylthiuram disulfide in acetonitrile) is employed for the sulfur oxidation instead of iodine water. The protective groups are removed by treatment with ammonia for 2 hours. An oligonucleotide of the formula Id is obtained with two 5′-terminal phosphorothioate internucleotide bonds and a 3′-O-n-octadecyl phosphate ester residue.

e) Preparation of an oligonucleotide of the formula Ie (R^1 = R^2 = H, Z = CH_3, a = V = W = X = Y = Y′ = O; U = O, except for the two 5′-terminal methylphosphonate internucleotide bonds, where U = CH_3 (indicated as p_{Me}))

d($Cp_{Me}Gp_{Me}TpCpCpApTpGpTpCpGpGpCpApApApCpApGpCpTp_{Me}$)

Starting with the support of the formula VIIa-5 (B′ = Thy, W = O, Z = CH_3), whose preparation takes place with the aid of the monomer of the formula VIIIp according to method A (Example 3a), the synthesis is carried out in an analogous manner to Example 4c. Instead of the normal cyanoethyl-protected monomers (formula VIII, Z = OCH_2CH_2CN), the corresponding methylphosphonamidites (formula VIII, Z = CH_3) are employed for coupling the last two nucleotide units (G and C). Cleavage from the support with conc. ammonia (1.5 hours at room temperature) is followed by a 6-hour treatment with ethylenediamine/ethanol/water (5:4:1; v:v:v) to liberate the amino groups of the bases. The result is an oligonucleotide-3′-methylphosphonate with two 5′-terminal methylphosphonate internucleotide bonds of the formula Ie.

f) Preparation of an oligonucleotide of the formula If (R^1 = R^2 = H, Z = CH_3, X = S, a = U = V = W = Y = Y′ = O)

d(CpGpTpCpCpApTpGpTpCpGpGpCpApApApCpApGpCp$_{(S)Me}$)

Starting with the support of the formula IVa from Example 1a, the methylphosphonamidite of the formula VIII (Z = CH_3, B′ = Cyt^{iBu}, R_5 = R_6 = i-C_3H_7) is coupled in the first reaction cycle as described in Example 3a. Oxidation is carried out with TETD. Further synthesis is as described in Example 3c. After removal of the protective groups in an analogous manner to Example 3e, an oligonucleotide-3′-methylphosphonothioate of the formula If is obtained.

g) Preparation of an oligonucleotide of the formula Ig (R^1 = R^2 = H, Z = X = S, a = U = V = W = Y = Y′ = O)

d(CpGpTpCpCpApTpGpTpCpGpGpCpApApApCpApGpCp$_{(s)s}$)

In analogy with the synthesis described in Example 4b, starting with the support of the formula IVa from Example 1a, with the difference, however, that in the first condensation step a nucleoside-3′-phosphoramidite of the formula VIII (Z = 2,4-dichlorothiobenzyl; R^5 = R^6 = ethyl) is employed instead of the methylphosphonamidite. Once again the introduction of the second S atom takes place by oxidation with TETD (0.4 M in acetonitrile). Cleavage of the dichlorobenzylthio group takes place in a known manner with thiophenol/triethylamine. After removal of the protective groups with conc. ammonia, an oligonucleotide-3′-phosphorodithioate of the formula Ig is obtained.

h) Preparation of an oligonucleotide of the formula Ih (R^1 = R^2 = H, Z = p-nitrophenylethyl-2-oxy, a = U = V = W = X = Y = Y′ = O)

d(CpGpTpCpCpApTpGpTpCpGpGpCpApApApCpApGpCp-O-CH_2CH_2--NO_2)

Starting with the support of the formula VIIa-6 (B′ = Cyt^{Bz}, W = O, Z = p-nitrophenylethyl-2-oxy), whose preparation takes place with the aid of the monomer of the formula VIIIf according to method A (Example 3a), the synthesis is carried out in an analogous manner to Example 4b. After removal of the protective groups by 10-hour treatment with ammonia at 55°C, an oligonucleotide-3′-O-(p-nitrophenylethyl) phosphate of the formula Ih is obtained.

i) Preparation of an oligonucleotide of the formula Ii (R^1 = R^2 = H, Z = 3-pyridylpropan-3-oxy, a = U = V = W = X = Y = Y′ = O)

d(CpGpTpCpCpApTpGpTpCpGpGpCpApApApCpApGpCp-O-$(CH_2)_3$-)

Starting with the support of the formula VIIa-7 (B′ = Cyt^{iBu}, W = O, Z = -O-$(CH_2)_3$-), which was prepared with the aid of the amidite of the formula VIIIe as described in Example 3a, the oligonucleotide synthesis takes place in analogy with Example 4c.

k) Preparation of an oligonucleotide of the formula Ik (R^1 = R^2 = H, Z = -O-$(CH_2CH_2O)_3CH_3$, a = U = V = W = X = Y = Y′ = O)

d(GpApGpGpApCpGpTpTpCpCpTpCpCpTpGpCpGpGpGpApApGpGpCp-O-$(CH_2CH_2O)_3CH_3$)

Starting with the support of formula VIIa-8 (B′ = Cyt^{Bz}, W = O, Z = -O-$(CH_2CH_2O)_3CH_3$, which was prepared with the aid of the amidite of the formula VIIIg as described in Example 3a, the oligonucleotide synthesis corresponding to the above sequence takes place in analogy with Example 4b.

l) Preparation of an oligonucleotide of the formula Il (R^1 = R^2 = H, Z = -O-$(CH_2CH_2O)_5(CH_2)_4CH_3$, a = U = V = W = X = Y = Y′ = O)

d(CpGpTpCpCpApTpGpTpCpGpGpCpApApApCpApGpCp-O-$(CH_2CH_2O)_5$-$(CH_2)_4CH_3$)

Starting with the support of the formula VIIa-9 (B′ = Cyt^{Bz}, W = O, Z = -O-$(CH_2CH_2O)_5(CH_2)_4CH_3$, which was prepared with the aid of the amidite of the formula VIIIi as described in Example 3a, the oligonucleotide synthesis takes place in analogy with Example 4b.

m) Preparation of an oligonucleotide of the formula Im (R^1 = R^2 = H, Z = -O-$(CH_2CH_2O)_8(CH_2)_{13}CH_3$, a = U = V = W = X =

Y = Y′ = O)

d(CpGpTpCpCpApTpGpTpCpGpGpCpApApApCpApGpCp-O-$(CH_2CH_2O)_8$-$(CH_2)_{13}CH_3$)

Starting with the support of the formula VIIa-10 (B′ = Cyt^{Bz}, W = O, Z = -O-$(CH_2CH_2O)_8(CH_2)_{13}CH_3$), which was prepared with the aid of the amidite of the formula VIIIk as described in Example 3a, the oligonucleotide synthesis takes place in analogy with Example 4b.

n) Preparation of an oligonucleotide of the formula In (R^1 = R^2 = H, Z = -$(CH_3)N(CH_2)_2N(CH_3)_2$, a = U = V = W = X = Y = Y′ = O)

d(CpGpTpCpCpApTpGpTpCpGpGpCpApApApCpApGpCp-N$(CH_3)(CH_2)_2$N-$(CH_3)_2$

Starting with the support of the formula IVc from Example 1c, the oligonucleotide synthesis is carried out as described in Example 4a with the exception that a methoxyphosphoramidite of the formula VIII (B′ = $Cyt^{i\text{-}Bu}$, Z = OCH_3, R^5 = R^6 = $N(i\text{-}C_3H_7)_2$ is employed for the first condensation reaction and the oxidative amidation takes place for two times 15 minutes with a 0.1 M iodine solution in THF/ N,N′,N′-trimethylethylenediamine (2:1; v:v). After construction of the oligonucleotide sequence, the base-stable sulfide support is oxidized with $NaIO_4$ in a manner known per se to the base-labile sulfone support. Cleavage from the support and removal of the protective groups (PAC for Ade and Gua; i-Bu for Cyt) is effected with t-butylamine/methanol (1:1, v:v) at 50°C for 16 hours. An oligonucleotide-3′-trimethylethylenediamine-phosphoramidate of the formula In is obtained.

o) Preparation of an oligonucleotide of the formula Io (R^1 = R^2 = H, Z = -$HN(CH_2)_2O$-CH_3, a = U = V = W = X = Y = Y′ = O)

d(CpGpTpCpCpApTpGpTpCpGpGpCpApApApCpApGpCp-HN$(CH_2)_2$O-CH_3)

In analogy with Example 4n, the oxidative amidation with a 0.1 M iodine solution in THF/2-methoxy-ethylamine (2:1; v:v) takes place for two times 15 minutes. After removal of the protective groups, an oligonucleotide-3'-(2-methoxyethyl)-phosphoramidate of the formula Io is obtained.

p) Preparation of an oligonucleotide of the formula Ip (R^1 = formula II, R^2 = H, Z = S, a = U = V = W = X = Y = Y' = Z' = O)

d(p_sCpGpTpCpCpApTpGpTpCpGpGpCpApApApCpApGpCp$_s$)

The synthesis is carried out as described in Example 4b, starting with the support of the formula IVa. However, after coupling the first unit (formula VIII; B' = Cyt^{Bz}; Z = O-CH_2CH_2CN; R^5 = R^6 = N(i-C_3H_7)$_2$) oxidation is carried out with TETD. After removal of the DMTr protective group of the last base added, the free 5'-hydroxyl group is phosphitylated with the bis-cyanoethyloxy-phosphoramidite of the formula IX (R^9 = N(i-C_3H_7)$_2$, Z" = R^{10} = OCH_2CH_2CN, and subsequently oxidized to the thiophosphate with TETD. The cyanoethyl-protective groups are eliminated during ammonia treatment. The result is an oligonucleotide-3'5'-bis-thiophosphate of the formula Ip.

q) Preparation of an oligonucleotide of the formula Iq (R^1 = formula II, R^2 = H, Z = O-i-C_3H_7, a = U = V = W = X = Y = Y' = Z' = O)

d(i-C_3H_7-O-pCpGpTpCpCpApTpGpTpCpGpGpCpApApApCpApGpCpTp-O-i-C_3H_7)

The synthesis is carried out as described in Example 4b. After removal of the DMTr protective group of the last base added, the free 5'-hydroxyl group is phosphitylated

with the cyanoethyloxy-i-propyloxy-phosphoramidite of the formula IX (R^9 = $N(i\text{-}C_3H_7)_2$, R^{10} = OCH_2CH_2CN, Z" = O-i-C_3H_7, and subsequently oxidized with iodine water. The result is an oligonucleotide-3'5'-bis-isopropyl phosphate ester of the formula Iq.

r) Preparation of an oligonucleotide of the formula Ir (R^1 = formula II, R^2 = H, Z = n-C_8H_{17}, a = U = V = W = X = Y = Y' = Z' = O)

d($CH_3(CH_2)_7$-pCpGpTpCpCpApTpGpTpCpGpGpCpApApApCpApGpCpTp-$(CH_2)_7CH_3$)

Starting with the support of the formula VIIa-2 (B' = Thy, W = O, Z = $(CH_2)_7CH_3$), whose preparation is described in Example 3b, the synthesis is carried out in analogy with Example 4c. After removal of the DMTr protective groupof the last base added, the free 5'-hydroxyl group is phosphitylated with n-octyldichlorophosphane of the formula IX (Z" = $(CH_2)_7CH_3$, R^9 = R^{10} = Cl) using DIPEA (diisopropylethylamine). After oxidation and hydrolysis, the oligonucleotide is cleaved from the support as in Example 4e. An oligonucleotide-3'5'-bis-(n-octylphosphonate) of the formula Ir is obtained.

s) Preparation of an oligonucleotide of the formula Is (R^1 = R^2 = H, Z" = "psoralen", a = U = V = W = X = Y = Y' = O)

d(GpGpCpGpCpCpCpGpGpCpCpTpGpCpGpApGpApApApGpCpGpCpGp-"psoralen")

The synthesis takes place in analogy with Example 4c starting with the support of the formula VIIa-11 (B' = Gua^{PAC}, Z = "psoralen", W = O), which was prepared in analogy with Example 3a from the monomer of the formula VIII (B' = Gua^{PAC}, Z = "psoralen", R^5 = R^6 = i-C_3H_7), which had previously been obtained from the bisamidite VIa (B' = Gua^{PAC}, R^5-R^8 = i-C_3H_7) by reaction with "psoralen"-H

(U. Pieles and U. Englisch, Nucleic Acids Research (1989) 17, 285.) in analogy with Example 2a. After removal of the protective groups with ammonia, an oligonucleotide of the formula Is is obtained, to which a "psoralen" phosphate ester is bound at the 3'-end.

t) Preparation of an oligonucleotide of the formula It (R^1 = R^2 = H, Z = "biotin", a = U = V = W = X = Y = Y' = O)

d(GpGpCpGpCpCpCpGpGpCpCpTpGpCpGpApGpApApApGpCpGpCpGp-"biotin")

The synthesis takes place in analogy with Example 4c starting with the support of the formula VIIa-12 (B'= Gua^{PAC}, Z = "biotin", W = O), which was prepared in analogy with Example 3a from the monomer of the formula VIII (B' = Gua^{PAC}, Z = "biotin", R^5 = R^6 = i-C_3H_7), which had previously been obtained from the bisamidite VIa (B' = Gua^{PAC}, R^5-R^8 = i-C_3H_7) by reaction with "biotin"-H (R. Pon, Tetrahedron Lett. (1991) 32, 1715) in analogy with Example 2a. After removal of the protective groups with ammonia, an oligonucleotide of the formula It is obtained, to which a "biotin" phosphate ester is bound at the 3'-end.

u) Preparation of an oligonucleotide of the formula Iu (R^1 = R^2 = H, Z = "fluorescein", a = U = V = W = X = Y = Y' = O)

d(GpGpCpGpCpCpCpGpGpCpCpTpGpCpGpApGpApApApGpCpGpCpGp-"fluorescein")

The synthesis takes place in analogy with Example 4c, starting with the support of the formula VIIa-13 (B' = Gua^{PAC}, Z = "fluorescein", W = O), which was prepared in analogy with Example 3a from the monomer of the formula VIII (B' = Gua^{PAC}, Z = "fluorescein", R^5 = R^6 = i-C_3H_7), which had previously been obtained from the bisamidite

VIa (B′ = Gua^{PAC}, R^5-R^8 = i-C_3H_7) by reaction with "fluorescein"-H (Schubert et al., Nucleic Acids Research (1991) 18, 3427) in analogy with Example 2a. After removal of the protective groups with ammonia, an oligonucleotide of the formula Iu is obtained, to which a "fluorescein" phosphate ester is bound at the 3′-end.

v) Preparation of an oligonucleotide of the formula Iv (R^1 = R^2 = H, Z = acridin-9-yl-but-4-oxy, a = U = V = W = X = Y = Y′ = O)

d(CpGpTpCpCpApTpGpTpCpGpGpCpApApApCpApGpCp-(acridin-9-yl-but-4-oxy))

Starting with the support of the formula VIIa-14 (B′ = Cyt^{Bz}, W = O, Z = acridin-9-yl-but-4-oxy), whose preparation takes place using the monomer of the formula VIIIm in analogy with Example 3a, the oligonucleotide synthesis is carried out as described in Example 4b. After deprotection, an oligonucleotide of the formula Iv is obtained, which contains an acridin-9-yl-but-4-yl phosphate ester at the 3′-end.

w) Preparation of an oligonucleotide of the formula Iw (R^1 = R^2 = H, Z = $HN(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$, a = U = V = W = X = Y = Y′ = O)

d(CpGpTpCpCpApTpGpTpCpGpGpCpApApApCpApGpCp-$HN(CH_2)_3NH$-$(CH_2)_4NH(CH_2)_3NH_2$)

The synthesis takes place in analogy with Example 4n, with the oxidative amidation being carried out with spermine. A capping reaction is then carried out with trifluoroacetic anhydride instead of acetic anhydride. After removing the protective groups, an oligonucleotide of the formula Iw is obtained, which contains a spermine-phosphoramidate residue at the 3′-end.

x) Preparation of an oligonucleotide of the formula Ix (R^1 = R^2 = H, Z = aziridyl-N-ethyl-2-oxy, a = U = V = W = X = Y = Y′ = O)

d(CpGpTpCpCpApTpGpTpCpGpGpCpApApApCpApGpCp-O$(CH_2)_2$N$\begin{matrix}CH_2\\CH_2\end{matrix}$

The synthesis takes place in analogy with Example 4c, starting with the support of the formula VIIa-15 (B′ = Cyt^{Bz}, Z = aziridyl-N-ethyl-2-oxy, W = O), which was prepared in analogy with Example 3a from the monomer of the formula VIII (B′ = Cyt^{Bz}, Z = aziridyl-N-ethyl-2-oxy, R^5 = R^6 = i-C_3H_7), which had previously been obtained from the bisamidite of the formula VIa (B′ = Cyt^{Bz}, R^5-R^8 = i-C_3H_7) by reaction with N-(2-hydroxyethyl)aziridine in analogy with Example 2a. After removal of the protective groups with ammonia, an oligonucleotide of the formula Ix is obtained, to which an aziridine-N-eth-2-yl phosphate ester is bound at the 3′-end.

y-1) Preparation of an oligonucleotide of the formula Iy-1 (R1 = R2 = H, Z = -O-farnesyl, for ps is W = S)

5′ CpSCpSGpSGpSApSApSApSApSCpSApSTpSCpSGpSCpSGpSGpSTp-STpSGpSTpS-O-farnesyl

The synthesis takes place in analogy with Example 4d, starting with the support of the formula VIIa-16 (B′ = Thy, Z = O-farnesyl), which, in analogy with Example 3a, was prepared from the monomer of the formula VIII (B′ = Thy, Z = O-farnesyl, R^5 = R^6 = i-C_3H_7), which had previously been prepared from the bisamidite VIa (B′ = Thy, R^5-R^8 = i-C_3H_7) by reaction with farnesol in analogy with Example 2b. In this case the oxidation is carried out on each occasion with TETD solution as described in Example 4d. After removal of the protective groups with ammonia, an allophosphorothioate oligonucleotide of the formula

Iy-1 is obtained, to which a farnesyl thiophosphate ester is bound at the 3'-end.

y-2) Preparation of an oligonucleotide of the formula Iy-2 (R^1 = R^2 = H, Z = -O-phytyl for ps(s) is W = U = S)

5' CpS(S)CpS(S)GpGpApApApApCpApTpCpGpCpGGpTpTpS(S)Gp-S(S)Tp-O-phytyl3'

The synthesis takes place in analogy with Example 4y-1, starting with the support of the formula VIIa-17 (B' = Thy, Z = O-phytyl), which, in analogy with Example 3a, was prepared from the monomer of the formula VIII (B' = Thy, Z = O-phytyl, R^5 = R^6 = i-C_3H_7), which had previously been obtained from the bisamidite VIa (B' = Thy, R^5-R^8 = i-C_3H_7) by reaction with phytol in analogy with Example 2b. The nucleotides 2, 3, 19 and 20 (counting of the nucleotides corresponds to the direction of synthesis from 3' to 5') are [?] via the units of the formula VIII (Z = 2,4-dichlorothiobenzyl, R^5, R^6 = C_2H_5). In the case of these nucleotides oxidation is carried out with TETD solution. In the other reaction cycles oxidation is with iodine water. After removal of the protective groups with ammonia, an oligonucleotide of the formula Iy-2 is obtained, which in each case has two phosphorodithioate internucleoside bonds 3'- and 5'-terminally, and to which is bound a farnesyl phosphate ester at the 3'-end.

y-3) Preparation of an oligonucleotide of the formula Iy-3 (R^1 = R^2 = H, Z = "-O-cholesterol", for pMe is U = CH_3)

5' CpMeCpMeGpGpApApApApCpApTpCpGpCpGpGpTpTpMeGpMeTp-"O-cholesterol"

The synthesis takes place in analogy with Example 4y-1, starting with the support of the formula VIIa-18 (B' = Thy, Z = O-"cholesterol"), which, in analogy with

Example 3a, was prepared from the monomer of the formula VIII (B′ = Thy, Z = O-"cholesterol", R^5 = R^6 = i-C_3H_7), which had previously been obtained from the bisamidite VIa (B′ = Thy, R^5-R^8 = i-C_3H_7) by reaction with "cholesterol" in analogy with Example 2b. The nucleotides 2, 3, 19 and 20 are introduced, as described in Example 4e, via the methylphosphonamidites of the formula VIII (Z = CH_3). In each case oxidation is with iodine water. After removal of the protective groups (cf. Example 4d), an oligonucleotide of the formula Iy-3 is obtained, which in each case has two methylphosphonate internucleoside bonds 3′- and 5′-terminally, and to which a "cholesterol" phosphate ester is bound at the 3′-end.

y-4) Preparation of an oligonucleotide of the formula Iy-4 (R^1 = R^2 = H, Z = -O-testosterone, for pMe is U = CH_3)

5′ CpMeCpMeGpMeGpMeApMeApMeApApCpApTpCpGpCpMeGpMeGpMe-TpMeTpMeGpMeTp-"testosterone"

The synthesis takes place in analogy with Example 4y-3, starting with the support of the formula VIIa-19 (B′ = Thy, Z = O-"testosterone"), which, in analogy with Example 3a, was prepared from the monomer of the formula VIII (B′ = Thy, Z = O-"testosterone", R^5 = R^6 = i-C_3H_7), which had previously been obtained from the bisamidite VIa (B′ = Thy, R^5-R^8 = i-C_3H_7) by reaction with "testosterone" in analogy with Example 2b. The nucleotides 2 to 7 and 15 to 20 are, as described in Example 4e, introduced via the methylphosphonamidites of the formula VIII (Z = CH_3). In each case oxidation is with iodine water. After removal of the protective groups, an oligonucleotide of the formula Ly-4 is obtained, which in each case has six methylphosphonate internucleoside bonds 3′- and 5′-terminally, and to which a "testosterone" phosphate ester is bound at the 3′-end.

y-5) Preparation of an oligonucleotide of the formula Iy-5 ($R^1 = R^2 = H$, Z = -O-vitamin-A, for pMe(S) is U = CH_3 and W = S, for pS is U = S and W = O)

5' CpMe(S)CpMe(S)GpMe(S)GpMe(S)ApMe(S)ApMe(S)ApSApSCpS ApSTpSCpSGpSCpMe(S)GpMe(S)GpMe(S)TpMe(S)TpMe(S)GpMe (S)Tp-O-"Vitamin A"

The synthesis takes place in analogy with Example 4y-4 starting with the support of the formula VIIa-20 (B'=Thy, Z=O-"Vitamin A"), which, in analogy with Example 3a, was prepared from the monomer of the formula VIII (B'=Thy, Z=O-"Vitamin A", $R^5=R^6=IC_3H_7$), which had previously obtained from the bisamidite VIa (B'=Thy, R^5-R^8=i-C_3H_7) by reaction with "Vitamin A-alcohol" in analogy with Example 2b. The nucleotides 2 to 7 and 15 to 20 are introduced via the methylphosphoramidites of the formula VIII (Z=CH_3) as described in Example 4e. Oxidation is with TETD as described in Example 4d. After removing the protective groups, an oligonucleotide of the formula Iy-5 is obtained, which contains methylphosphonothioate and internally seven phosphorothioate internucleoside bonds. A "vitamin A" phosphate ester is additionally located at the 3'-end of this oligonucleotide.

y-6) Preparation of an oligonucleotide of the formula Iy-6 ($R^1 = H$, R^2 = O-CH_3; $R^2 = H$ for T, Z = -O-vitamin E)

5' 2'-O-CH_3(CpCpGpGpApApApApCpApUpCpGpCpGpGpUpUpGp)Tp-O-"vitamin E"

The synthesis takes place in analogy with Example 4y-4, starting with the support of the formula VIIa-21 (B' = Thy, Z = O-"vitamin E"), which, in analogy with Example 3a, was prepared from the monomer of the formula VIII (B' = Thy, Z = O-"vitamin E", $R^5 = R^6$ = i-C_3H_7), which had previously been obtained from the bisamidite VIa (B' = Thy, R^5-R^8 = i-C_3H_7) by reaction with tocopherol in analogy with Example 2b. The nucleotides 2 to 20 are introduced via the 2'-O-methylribonucleoside-phosphoramidites of the formula V (R = DMTr, R^2 = O-CH_3H_7). Oxidation is with iodine water, as described in Example 4a. After removing the labile phenoxyacetyl protective groups, a 2'-O-methyloligoribonucleotide of the formula Iy-6 is obtained, which contains a "viatamin E" phosphate ester at the 3'-end.

Example 5: Testing for nuclease stability

10 nmol of the oligonucleotide under investigation are dissolved in 450 µl of 20% strength fetal calf serum in RPMI medium and 50 ml of double-distilled water and incubated at 37°C. 10 µl samples, for gel electrophoresis, and 20 µl samples, for HPLC, are then removed immediately and after 1, 2, 4, 7 and 24 hours and in each case mixed with 5 or 10 µl of formamide, respectively, to stop the reaction, and then heated at 95°C for 5 minutes. For the gel electrophoresis, the samples are loaded onto a 15% polyacrylamide gel (2% bis), which is then run for about 3,000 volt hours. The bands are visualized by silver staining. For the HPLC analysis, the samples are injected onto a Gen-Pak Fax™ HPLC column (from Waters/Millipore) and chromatographed at 1 ml/min with 5 to 50% Buffer A in B (Buffer A: 10 mM sodium dihydrogen phosphate, 0.1 M NaCl in acetonitrile/water 1:4 (v:v) pH 6.8; Buffer B: as A, but 1.5 M NaCl).

Example 6: Anti-viral activity

The anti-viral activity of the compounds according to the invention is examined in in vitro experiments. For this purpose, the compounds according to the invention are added in various dilutions to cell cultures of HeLa and Vero cells in microtiter plates. After 3 h, the cultures are infected with various viruses which are pathogenic for man (e.g.: herpes viruses HSV-1, HSV-2, orthomyxoviruses influenza A2, picornaviruses rhinovirus 2). 48 to 72 h after the infection, therapeutic success is determined on the basis of the cytopathic effect, as measured microscopically, and photometrically following uptake of neutral red (Finter's color test) (Finter, N.B. in "Interferons", N.B. Finter et al., North Holland Publishing Co., Amsterdam, 1966). The minimum concentration at which half the infected cells show no cytopathic

effect is considered to be the minimum inhibitory concentration (MIC).

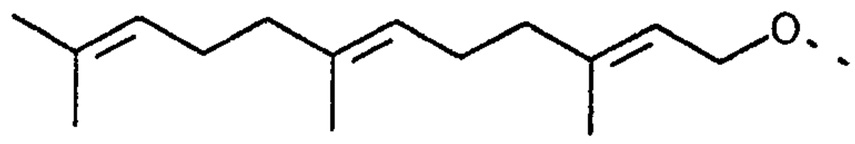
Example 7: Preparation of the phosphitylating reagent DMTr-O-CH_2CH_2-S-CH_2CH_2O-P-{OCH_2CH_2CN}{$N(i\text{-}C_3H_7)_2$} [?] (claim 9; X′ = O, x′ = zero, R^9 = OCH_2CH_2CN, R^{10} = $N(i\text{-}C_3H_7)_2$

The bis-hydroxyethyl sulfide (3.05 g) is dissolved in 75 ml of absolute pyridine and this solution is cooled to 0°C. The dimethoxytrityl chloride (8.04 g) dissolved in 60 ml of abs. pyridine is then added dropwise with stirring over a period one hour. After warming the reaction mixture at room temperature, this is stirred for a further 1.5 hours. 5 ml of water are added to the solution which is then concentrated in vacuo. The residue is dissolved in 250 ml of methylene chloride. This solution is extracted three times with 125 ml of 0.1 M phosphate buffer, pH7, on each occasion, and the organic phase is dried over sodium sulfate and concentrated in vacuo. The crude product is chromatographed on a silica gel column using ethyl acetate/n-heptane/triethylamine (gradient 6:14:1 to 2:2:1, v:v:v). 5.3 g of the bis-hydroxyethyl sulfide-mono-(dimethoxytrityl) ether DMTr-O-CH_2CH_2-S-CH_2CH_2OH (52%) are obtained.

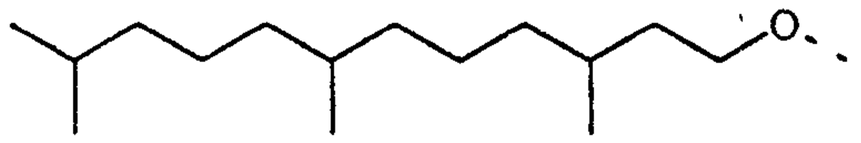
A solution of this dimethoxytrityl compound (1.06 g) and of tetrazole (88 mg) in 12.5 ml of absolute acetonitrile is slowly (20 min) added dropwise to a solution of cyanoethoxy-di-isopropylamino-phosphane (0.75 g) in abs. acetonitrile (20 ml). After a further 3 hours of reaction, the reaction solution is diluted with 95 ml of methylene chloride and washed with 5% strength sodium carbonate solution (65 ml). The organic phase is dried over sodium sulfate and concentrated in vacuo. The residue is purified by chromatography on a silica gel column with ethyl acetate/n-hexane/triethylamine (11:8:1, v:v:v). 1.25 g (80%) of the required phosphitylating reagent (^{31}P-NMR: δ 148 ppm [d], 99% of the total

phosphorus content) are obtained.

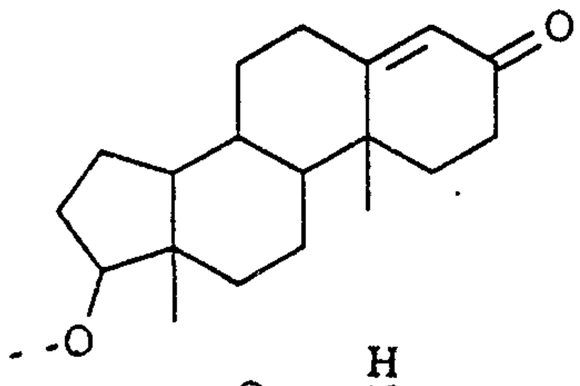
The compounds y-1 to y-6 described in Example 4 possess residues of the following definition:



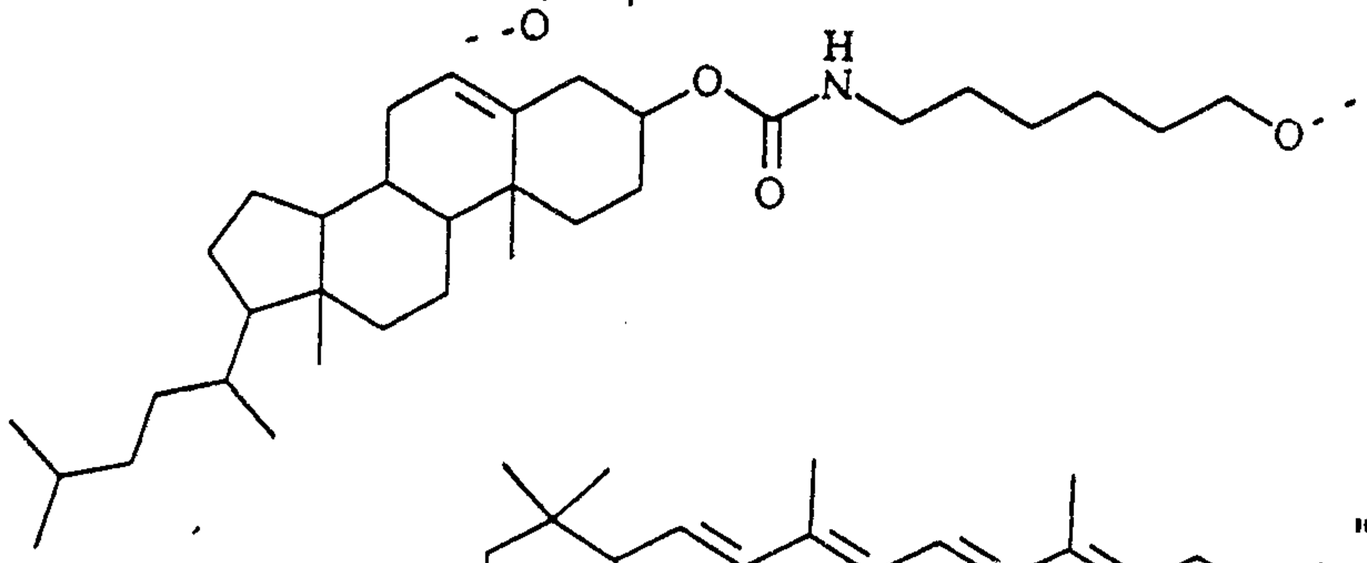
farnesyl



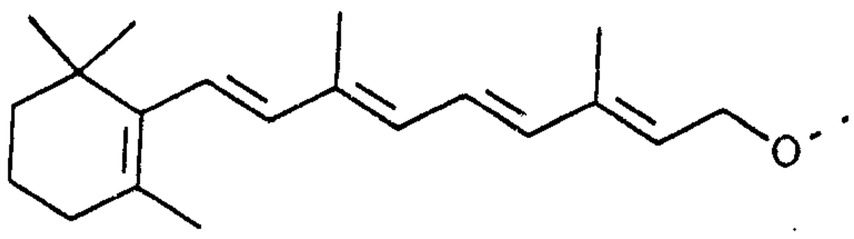
phytyl



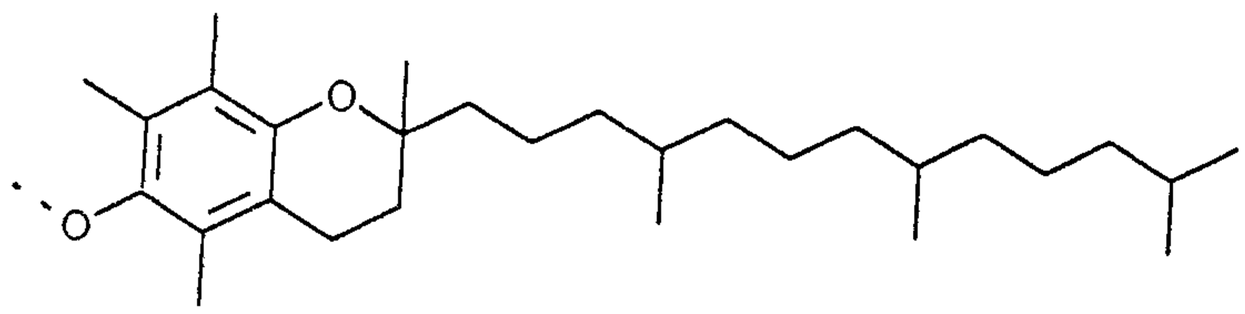
"vitamin A"



"vitamin E"



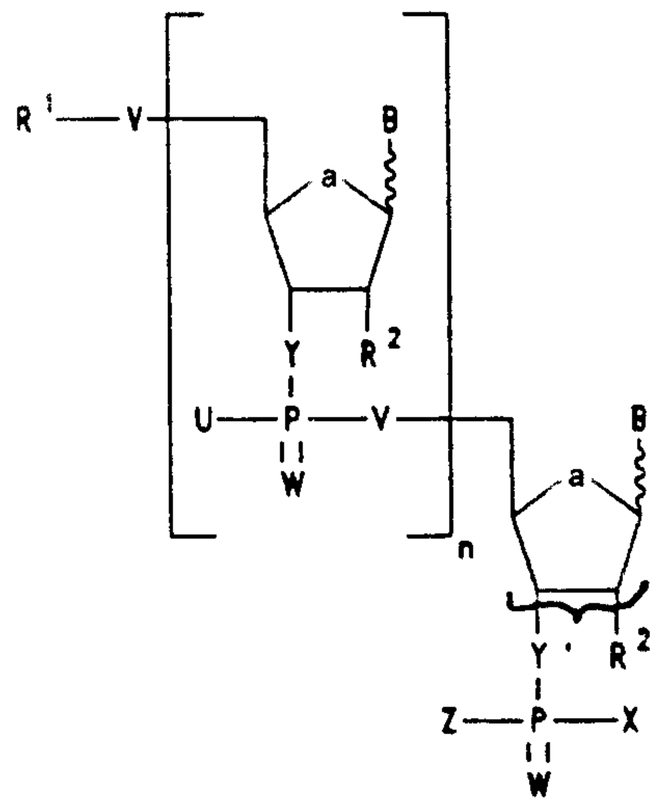
"cholesterol"



testosterone

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

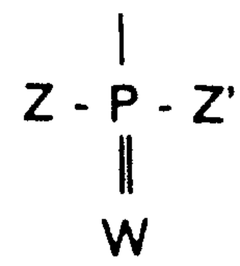
1. An oligonucleotide analog of the formula I



(I)

and its physiologically tolerated salts, wherein

R^1 is hydrogen, C_1-C_{18}-alkyl, C_2-C_{18}-alkenyl, C_2-C_{18}-alkynyl, C_2-C_{18}-alkylcarbonyl, C_3-C_{19}-alkenylcarbonyl, C_3-C_{19}-alkynylcarbonyl, C_6-C_{20}-aryl, (C_6-C_{14})-aryl-(C_1-C_8)-alkyl, or a radical of the formula II



(II);

R^2 is hydrogen, C_1-C_{18}-alkoxy, halogen, azido or NH_2;

B is a conventional base in nucleotide chemistry;

a is oxy or methylene;

n is an integer from 1 to 100;

W is oxo, thioxo or selenoxo;

V is oxy, thio, or imino;

Y is oxy, thio, imino or methylene;

Y' is oxy, thio, imino, $(CH_2)_m$ or $V(CH_2)_m$, where

m is an integer from 1 to 18;

X is hydroxyl or mercapto;

U is hydroxyl, mercapto, SeH, C_1-C_{18}-alkoxy, C_1-C_{18}-alkyl, C_6-C_{20}-aryl, (C_6-C_{14})-aryl-(C_1-C_8)-alkyl, NHR^3, NR^3R^4 or a radical of the formula III $(OCH_2CH_2)_pO(CH_2)_qCH_2R^{11}$ (III), where

R^3 is C_1-C_{18}-alkyl, C_6-C_{20}-aryl, (C_6-C_{14})-aryl-(C_1-C_8)-alkyl, 2-$(CH_2)_c$-$[NH(CH_2)_c]_d$-$NR^{12}R^{12}$, where c is an integer from 2 to 6 and d is an integer from 0 to 6, and each R^{12} independently of the other is hydrogen or C_1-C_6-alkyl or C_1-C_4-alkoxy-C_1-C_6-alkyl;

R^4 is C_1-C_{18}-alkyl, C_6-C_{20}-aryl, or (C_6-C_{10})-aryl-(C_1-C_8)-alkyl, or, in the case of NR^3R^4, is, together with R^3 and the nitrogen atom carrying them, a 5-6-membered heterocyclic ring, which can additionally contain a further hereto atom selected from the group comprising O, S, N,

p is an integer from 1 to 100,

q is an integer form 0 to 22,

R^{11} is hydrogen or a functional group;

Z = Z' are hydroxyl, mercapto, SeH, C_1-C_{22}-alkoxy, -O-$(CH_2)_b$-$NR^{12}R^{13}$, where b is an integer from 1 to 6, and R^{13} is C_1-C_6-alkyl or R^{12} and R^{13} together with the nitrogen atom carrying them form a 3-6-membered ring, C_1-C_{18}-alkyl, C_6-C_{20}-aryl, (C_6-C_{14})-aryl-(C_1-C_8)-alkyl, (C_6-C_{14})-aryl-(C_1-C_8)-alkoxy, where aryl includes heteroaryl, and aryl is optionally substituted by 1, 2 or 3 identical or different radicals selected from the group comprising carboxyl, amino, nitro, C_1-C_4-alkylamino, C_1-C_6-alkoxy, hydroxyl, halogen and cyano, C_1-C_{18}-alkylmercapto, NHR^3, NR^3R^4, a radical of the formula III or a group which favors intracellular uptake or serves as the label for a DNA probe, or, during hybridization of the oligonucleotide analog to the target

nucleic acid, attacks the latter with binding, crosslinking or cleavage;

the curved bracket indicates that R^2 and the neighboring phosphoryl residue can be located in the 2'- and 3' position or else the opposite way round in the 3'- and 2'-position,

where each nucleotide can be present in its D- or L-configuration and the base B can be located in the α- or β-position, with the proviso that, if Z = hydroxyl, mercapto, methyl or ethoxy, at least one of the groups X, Y, Y', V and W is not hydroxyl, oxy or oxo, or R^1 is not hydrogen.

2. An oligonucleotide analog as claimed in claim 1, wherein the base B is located in the β-position, the nucleotides are present in the D-configuration, R^2 is located in the 2'-position and a is oxy.

3. An oligonucleotide analog as claimed in claim 1 or 2, wherein

R^1 is hydrogen, C_1-C_6-alkyl or a radical of the formula II;

R^2 is hydrogen;

n is an integer from 10 to 40;

m is an integer from 1 to 6;

U is hydroxyl, mercapto, C_1-C_6-alkoxy, C_1-C_6-alkyl, NR^3R^4 or NHR^3, where

R^3 is C_1-C_8-alkyl or methoxyethyl, and B, W, V, Y, Y', X and Z are defined as in claim 1.

4. An oligonucleotide analog as claimed in claim 3, wherein R^1 is methyl.

5. An oligonucleotide analog as claimed in claim 3, wherein R^2 is hydrogen.

6. An oligonucleotide analog as claimed in claim 3, wherein n is an integer from 12 to 30.

7. An oligonucleotide analog as claimed in claim 3, wherein m is the integer 1.

8. An oligonucleotide analog as claimed in claim 3, wherein U is hydroxyl or C_1-C_6-alkyl.

9. An oligonucleotide analog as claimed in claim 3, wherein R^3 is C_1-C_4-alkyl.

10. An oligonucleotide analog as claimed in any one of claims 1 to 9, wherein V, Y, and Y' have the meaning of oxy.

11. An oligonucleotide analog as claimed in any one of claims 1 to 10, wherein W has the meaning of oxo.

12. An oligonucleotide analog as claimed in any one of claims 1 to 11, wherein U has the meaning of hydroxyl.

13. An oligonucleotide analog as claimed in any one of claims 1 to 11, wherein R^1 is hydrogen.

14. A process for preparing an oligonucleotide analog of the formula I as claimed in claim 1, wherein

a) a nucleotide unit with a 3'(2')-terminal phosphorus(V) grouping and a free 5'-hydroxyl or mercapto group is reacted with a further nucleotide unit with in the 3' position a phosphorus(III) or phosphorus(V) grouping or an activated derivative thereof, or

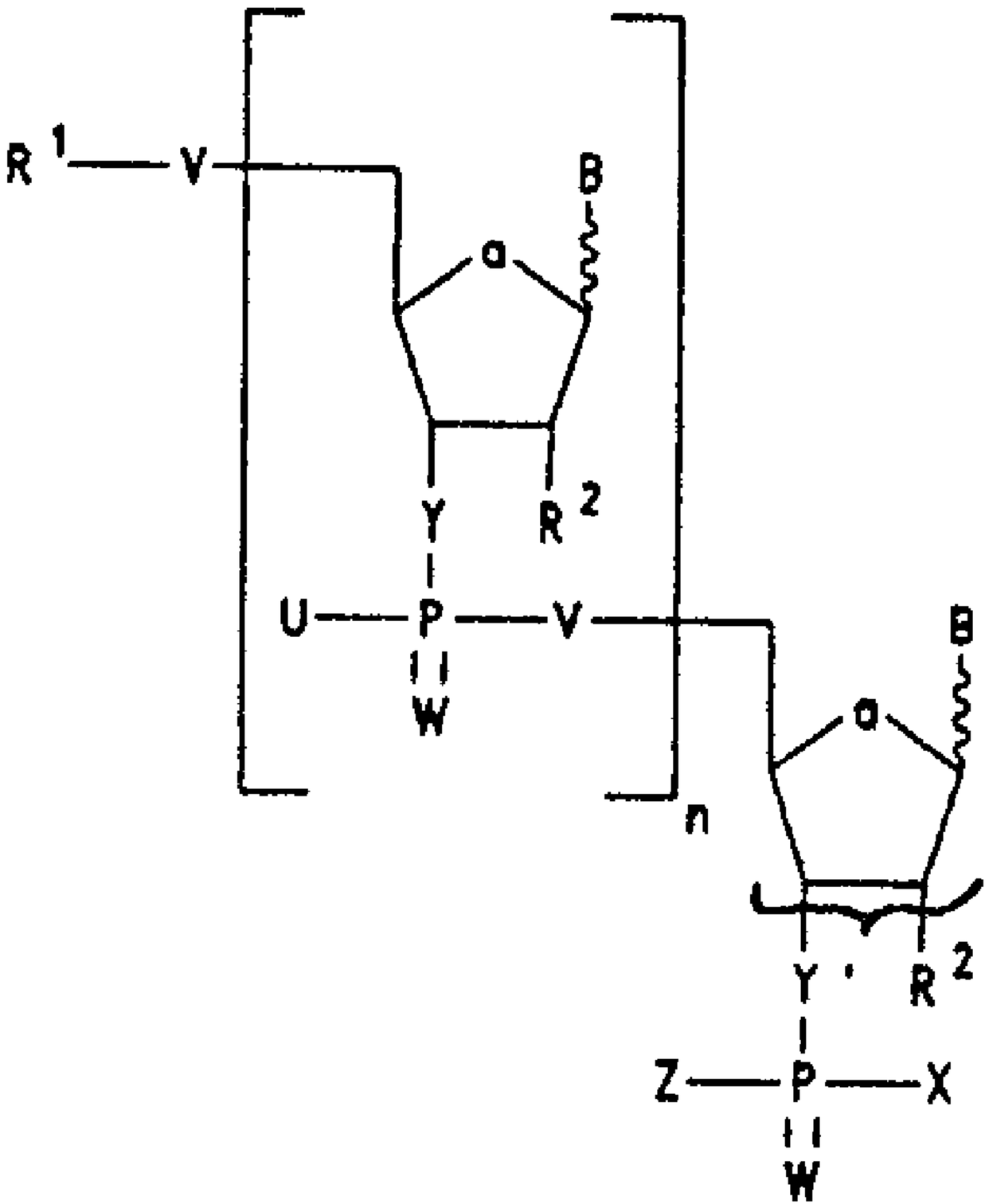
b) the oligonucleotide analog is constructed with fragments in a similar manner,

and protective groups, which have been temporarily introduced in the oligonucleotides obtained according to (a) or (b) in order to protect other functions, are removed and the oligonucleotide analog of the formula I thus obtained is, where appropriate, converted into its physiologically tolerated salt.

15. The use of an oligonucleotide analog as claimed in any one of claims 1 to 13 as an inhibitor of gene expression.

16. The use of an oligonucleotide analog as claimed in any one of claims 1 to 13 as a probe for detecting nucleic acids or as an aid in molecular biology.

17. A pharmaceutical preparation containing one or more oligonucleotide analogs of the formula I as claimed in any one of claims 1 to 13 together with physiologically tolerated adjuvants and/or excipients and/or together with other known active substances.



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