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(57) Abstract

This invention relates to new oligonucleotide conjugates, which contain a coated chemically modified ferrite as signal-transmitting group, on which oligonucleotides are bound by connecting components. In this case, the oligonucleotides are modified in a way that prevents or at least significantly inhibits the degradation by naturally occurring nucleases. The oligonucleotide can bond specifically and with high bonding affinity on target structures and can thus have a specific diagnostic and/or therapeutic effect on the ferrite.

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Conjugates of Ferrites and Oligonucleotides, Which Bond Specifically to Certain Target Structures

Summary of the Invention

This invention relates to the object characterized in the claims, i.e., oligonucleotide conjugates, which contain a metal oxide sheathed with a coating agent. These conjugates are used in the field of diagnosis and treatment.

Imaging diagnosis has achieved great progress in past decades and continues to develop. It is now possible to make visible the blood vessel system, most organs and many tissues in the living body without major intervention. In many cases, diseases are diagnosed, since they lead to clear changes of shape, size and position of anatomical structures in the body. Such anatomical data from the inside of the body can be obtained by x-ray technology, ultrasound diagnosis and magnetic resonance tomography. Each of the mentioned technologies can be improved in efficiency by the use of pharmaceutical agents for enhancing the natural contrasts of the tissue and body fluids in the resulting image. The pharmaceutical agents in question are introduced into body cavities or injected into blood vessels with the purpose of changing in contrast the cavities or vessels. In addition, they are dispersed by the blood stream into the organism and can change organs and tissues in visibility. In exceptional cases, such substances are bound on certain structures in the body and/or actively transported and/or excreted from the latter. In this way, functions can also be made visible in individual cases and can be used for the diagnosis of diseases.

Contrast agents for NMR diagnosis influence the relaxation times of the protons of the body water and are not visible as such in the nuclear resonance image. Two classes of substances are distinguished in principle: paramagnetic and superparamagnetic compounds. In the case of the paramagnetic compounds, they are in most cases metal chelates or stable free radicals, superparamagnetic compounds are ferrite particles, which for stabilization in most cases are sheathed with a coating agent, for example a polysaccharide, a protein or a silane (see US 4,827,945, Groman et al.). Based on their pronounced relaxation effects, superparamagnetic contrast media, i.a., have the advantage that they can be dosed very low.

In contrast, nuclear diagnosis is based on substances which themselves can be made visible. In this case, radioactive isotopes, which emit far-reaching radiation, are introduced in the body. The dispersing of these substances in the organism can be tracked by means of suitable detectors. An advantage of the nuclear medicine process is the high effectiveness at low dosage of the signal-transmitting radioactive substances designated as radiopharmaceutical agents.

If isotopes are used which release α- or β-radiation or other toxic degradation products effective in the tissue, radiopharmaceutical agents can also be used for therapeutic purposes, e.g., for destruction of tumors. The same goal can also be achieved by the fact that harmless isotopes or substances are introduced into the body and are converted to a therapeutically effective form there by, e.g., neutron or x-ray radiation, ultrasound or radio waves.

A general problem is the diagnosis and localization of pathological changes at a time at which no marked changes of shape, structure and circulation of the organs and tissues in question are present. Such a diagnosis and follow-up are of decisive importance, e.g., in the case of tumor diseases including the search for metasta-

ses, the evaluation of an inferior supply of tissues with oxygen and in the case of certain infections as well as metabolic diseases.

The now available therapeutic and imaging diagnostic methods are dependent essentially on the availability of pharmaceutical preparations, which are concentrated at sites of otherwise undetectable pathological changes.

The contrast media in the trade are quite predominantly so-called unspecific preparations. They are spread passively in those spaces into which they are introduced, e.g., by injection.

In the past, a multiplicity of substances and classes of substances have been identified, which promise specificity with respect to their distribution in the living organism. Examples in this respect are, in addition to the antibodies, lectins, all types of receptor-bound substances, cells, membranes and membrane components, nucleic acids, natural metabolites and their derivatives, as well as countless pharmaceutical substances. Oligonucleotides were and are also being examined with special care.

US Patent No. 4,707,352 is concerned with a special process to label complexing molecules with radioactive isotopes, but no well-suited complexing agents for the bonding of metal ions are described.

EP-A-0 285 057 describes nucleotide-complexing agent conjugates, which are not suitable, i.a., because of the in vivo stability of the nucleotides used for the application as in vivo diagnostic agents or therapeutic agents and also meet hardly any of the other requirements of compatibility and pharmacokinetics.

A multiplicity of US patents, such as, for example, US Patent No. 4,707,440, is concerned with modified polymers, which contain a detectable chemical group. The polymers can be polynucleotides and oligonucleotides, but the latter are stabilized neither against degradation by naturally occurring nucleases, nor selected by a special process, so that they bond specifically with high bonding

affinity to target structures. Special embodiments of these detectable molecules are mentioned in US Patents No. 4,843,122 and 4,943,523. An individual nucleotide modified in this way is claimed in US Patent No. 4,952,685. The use of these agents in the imaging process is disclosed in US Patent No. 4,849,208.

The object of this invention is to make available specifically bonding agents for the detection of target structures, by which, for example, the visualization of organs, tissues and their pathological changes in vitro and in vivo is made possible.

It has now been found that conjugates consisting of oligonucleotides bonding specifically with high affinity to target structures, containing modifications significantly limiting the degradation by naturally occurring nucleases, reduced by one hydroxyl group in 3'-position or in 5'-position or by one hydroxymethyl group in 4'-position, and a chemically modified, optionally radioactively-labeled ferrite sheathed with a coating agent, achieve this object.

The conjugates according to the invention can be described by general formula I:

$$M\text{-}(B\text{-}L\text{-}A\text{-}PN)_m \qquad (I)$$

in which

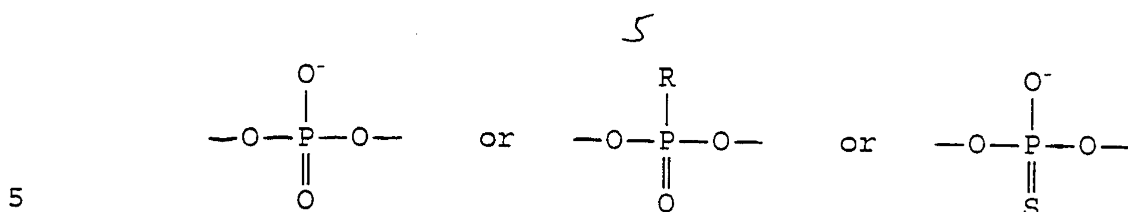
N means an oligonucleotide bonding specifically with high affinity to target structures, containing modifications significantly limiting the degradation by naturally occurring nucleases, reduced by one OH group in the terminal 3'-position or in the terminal 5'-position,

B means a bonding component X-Y-Z, in which X means a direct bond or a group -NH or -NR with R meaning a C1 to C4 alkyl chain,

Y means a direct bond or a spacer and Z means a direct bond or a sulfur atom,

L means a linker,

A means a group bound on the terminal 3'- or 5'-position



or a carbonyl group bound on 4'-position,

M means a chemically modified, optionally radioactively-labeled ferrite coated with a polysaccharide, and

m means a number between 1 and 100,

provided that at least one of groups X, Y and Z does not stand for a direct bond.

Oligonucleotide N consists of 5 to 200 nucleotides, preferably 15 to 100 nucleotides. Its structure is characterized in that

a) the 2'-position of the sugar units, independently of one another, is occupied with the following groups:

-- an optionally radioactively-labeled hydroxyl group, an optionally radioactively-labeled group OR^2, in which R^2 means an alkyl radical with 1-20 carbon atoms, which optionally contains up to 2 hydroxyl groups and which optionally is interrupted by 1-5 oxygen atoms,

a hydrogen atom,

a fluorine atom,

an amine radical,

an amino group

and hydroxyl groups present in 3'- and 5'-positions are etherified optionally with the radical R^2,

and/or

b) phosphodiesters, independently of one another, being used as internucleotide bond, are replaced by phosphorothioates, phosphorodithioates or methyl phosphonates,

and/or

c) it contains an internucleotide bond as described in b), which links the 3'-3'- or 5'-5'-positions

and/or

d) it contains a phosphodiester bond as described in b), which connects, ester-like, two thymidines via a C_2-C_{20} hydroxyalkyl radical present in 3-position or con-

nects, ester-like, an analogously-substituted thymidine radical with a hydroxyl group of another sugar in 2'- or 3'- or 5'-position,

and/or

5 e) the terminal radicals in 3'- and 5'-positions optionally contain modified internucleotide bonds as described in b), which connect up to 5 thymidines.

Oligonucleotide PN is characterized in that it bonds specifically with high bonding affinity to other target 10 structures, and in that it can be obtained in that a mixture of oligonucleotides, containing random sequences, is brought together with the target structure, and certain oligonucleotides exhibit an increased affinity to the target structure relative to the mixture of oligonucleo- 15 tides, the latter are separated from the remainder of the oligonucleotide mixture, then the oligonucleotides with increased affinity to the target structure are amplified to obtain a mixture of oligonucleotides, which exhibits an increased portion of oligonucleotides, which bond on 20 the target structures.

Preferably, oligonucleotide PN is characterized in that it bonds specifically with high bonding affinity to other target structures and in that it can be obtained in that

25 a) first a DNA strand is produced by chemical synthesis, so that this DNA strand exhibits a defined sequence on the 3'-end, which is complementary to a promoter for an RNA polymerase and at the same time complementary to a primer of the polymerase chain reaction 30 (PCR) and in that this DNA strand exhibits a defined DNA sequence on the 5'-end, which is complementary to a primer sequence for the polymerase chain reaction, and the sequence contains a random sequence between the defined sequences, and in that

35 b) this DNA strand is transferred with the help of an RNA polymerase in a complimentary RNA strand, and the nucleotides that are modified in 2'-position of the ribose unit are offered to the polymerase, and in that

c) the RNA oligonucleotides, produced in this way, with the target structure on which the oligonucleotide is to bond specifically, are brought together and in that

d) those oligonucleotides that have bonded on the target structure are separated first together with the target structure from the nonbonding oligonucleotides and then the bound oligonucleotides are again separated from the target structure and in that

e) these target-structure-specific RNA oligonucleotides are transferred with the help of reverse transcriptase to a complementary DNA strand and in that

f) these DNA strands are amplified by using the defined primer sequences with the polymerase chain reaction and in that

g) the DNA oligonucleotides amplified in this way are then transferred again with the help of the RNA polymerase and with modified nucleotides to RNA oligonucleotide and in that

h) above-mentioned selection steps c) to g) are optionally often repeated until the oligonucleotides, which are characterized by a high bonding affinity to the target structure, are sufficiently selected and then the sequences of the thus obtained oligonucleotides optionally can be determined. The target structure is selected from among macromolecules, tissue structures of higher organisms such as animals or humans, organs or parts of organs of an animal or human, cells, tumor cells or tumors.

The oligonucleotides that can be used according to the invention are stabilized against degradation by in vivo occurring nucleases.

Unmodified oligonucleotides or polynucleotides are cleaved in vivo by endonucleases and exonucleases. The degradation reaction in the RNA series begins with an activation of the 2'-hydroxy group. Other catabolic enzymes are, e.g., ribozymes, which cleave the phosphodiester bond of RNA (see Science 261, 709 (1993)). The in vivo stability of RNA derivatives can be increased by

partial or complete exchange of the 2'-hydroxyl group for other substituents. Such substituents are, e.g., alkoxy groups, especially the methoxy group (see, e.g., Chem. Pharm. Bull. 13, 1273 (1965), Biochemistry 10, 2581, (1971)), a hydrogen atom, a fluorine atom (see, e.g., Can. J. Chem. 46, 1131 (1968)) or an amino group (see, e.g., J. Org. Chem. 42, 714 (1977)). Several of these substituents, as well as others, can also be introduced at the 2'-position using the methods disclosed in U.S. application Ser. No. 08/264,029, filed June 22, 1994. Other possibilities for stabilizing the internucleotide bond are the replacement of one or two oxygen atoms in the phosphodiester bridge with the formation of phosphorothioates (Trends Biochem. Sci. 14, 97 (1989)) or phosphorodithioates (J. Chem. Soc., Chem. Commun. 591 (1983) and Nucleic Acids Res. 12, 9095 (1984) and the use of alkylphosphonates instead of phosphodiesters (Ann. rep. N. Y. Acad. Sci. 507, 220 (1988)).

The stabilization can be achieved in that the hydroxyl groups in 2'-position of the ribose units, independently of one another, are modified. Such a modification can be achieved by a replacement of this hydroxyl group by an OR^2 group, a halogen atom, especially a fluorine atom, a hydrogen atom or an amine radical, especially by an amino group. Radical R^2 of the alkoxy group stands, in this case, for a straight-chain or branched alkyl radical with 1 to 20 C atoms, such as methyl, ethyl, propyl, isopropyl, butyl, tert-butyl, pentyl or hexyl or a cyclic unsubstituted or substituted alkyl radical with 4 to 20 C atoms, such as cyclopentyl or cyclohexyl, which optionally contains 1-2 hydroxy groups and is optionally interrupted by 1-5 oxygen atoms. The stabilization is also increased in that existing hydroxyl groups in 3-' and 5'-positions are etherified with the radical R^2.

Further stabilization of the oligonucleotide takes place in that the phosphodiesters being used as internucleotide bond, independently of one another, are replaced by phosphorothioates, phosphorodithioates or alkylphos-

phonates, with a C1 to C6 alkyl -- especially a methyl group. These internucleotide bonds can also be linked to the terminal radicals in 3'- and 5'-positions or else also connect the 3'-3'- or 5'-5'-positions. The phospho-
5 diester bond further makes possible linkages with hydroxyalkyl radicals, which are present on nitrogen or carbon atoms of the nucleobases. Thus, for example, two thymidines can be linked with the hydroxyalkyl chains present in 3-position or two purine bases with the radi-
10 cal present in 8-position. The linkage can also take place in hydroxyl groups in 2'- or 3'- or 5'-position.

The modified internucleotide bonds can optionally occur preferably at the end of the polynucleotide, and they are especially preferably bound on the thymidine.

15 According to the invention, oligonucleotide radicals N used are not limited to certain oligonucleotide sequences. But those oligonucleotides are preferred that bond specifically with high bonding affinity to target structures.

20 A process for identifying suitable oligonucleotides, which are required as initial substances for the conjugates according to the invention, is described in US Patent 5,270,163. This process, termed SELEX, can be used to make a nucleic acid ligand to any desired target mole-
25 cule.

The SELEX method involves selection from a mixture of candidate oligonucleotides and step-wise iterations of binding, partitioning and amplification, using the same general selection scheme, to achieve virtually any
30 desired criterion of binding affinity and selectivity. Starting from a mixture of nucleic acids, preferably comprising a segment of randomized sequence, the SELEX method includes steps of contacting the mixture with the target under conditions favorable for binding, partition-
35 ing unbound nucleic acids from those nucleic acids which have bound specifically to target molecules, dissociating the nucleic acid-target complexes, amplifying the nucleic acids dissociated from the nucleic acid-target complexes

to yield a ligand-enriched mixture of nucleic acids, then reiterating the steps of binding, partitioning, dissociating and amplifying through as many cycles as desired to yield highly specific, high affinity nucleic acid ligands to the target molecule.

The basic SELEX method has been modified to achieve a number of specific objectives. For example, U.S. patent application Ser. No. 07/960,093, filed October 14, 1992, describes the use of SELEX in conjunction with gel electrophoresis to select nucleic acid molecules with specific structural characteristics, such as bent DNA. U.S. patent application Ser. No. 08/123,935, filed September 17, 1993, describes a SELEX-based method for selecting nucleic acid ligands containing photoreactive groups capable of binding and/or photocrosslinking to and/or photoinactivating a target molecule. U.S. patent application Ser. No. 08/134,028, filed October 7, 1993, describes a method for identifying highly specific nucleic acid ligands able to discriminate between closely related molecules, termed Counter-SELEX. U.S. patent application Ser. No. 08/143,564, filed October 25, 1993, describes a SELEX-based method which achieves highly efficient partitioning between oligonucleotides having high and low affinity for a target molecules. U.S. patent application Ser. No. 07/964,624, filed October 21, 1992, describes methods for obtaining improved nucleic acid ligands after SELEX has been performed. U.S. patent application Ser. No. 08/400,440, filed March 8, 1995, describes methods for covalently linking a ligand to its target.

The SELEX method encompasses the identification of high-affinity nucleic acid ligands containing modified nucleotides conferring improved characteristics on the ligand, such as improved in vivo stability or improved delivery characteristics. Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base substitutions. SELEX-identified nucleic acid ligands containing modified nucleotides are

described in U.S. patent application Ser. No. 08/117,991, filed September 8, 1993, that describes oligonucleotides containing nucleotide derivatives chemically modified at the 5- and 2'-positions of pyrimidines. U.S. patent application Ser. No. 08/134,028, supra, describes highly specific nucleic acid ligands containing one or more nucleotides modified with 2'-amino (2'-NH_2), 2'-fluoro (2'-F), and/or 2'-O-methyl (2'-OMe). U.S. patent application Ser. No. 08/264,029, filed June 22, 1994, describes oligonucleotides containing various 2'-modified pyrimidines.

The SELEX method encompasses combining selected oligonucleotides with other selected oligonucleotides and non-oligonucleotide functional units as described in U.S. patent applications Ser. No. 08/284,063, filed August 2, 1994, and Ser. No. 08/234,997, filed April 28, 1994, respectively. These applications allow the combination of the broad array of shapes and other properties, and the efficient amplification and replication properties, of oligonucleotides with the desirable properties of other molecules.

In its most basic form, the SELEX process may be defined by the following series of steps:

1) A candidate mixture of nucleic acids of differing sequence is prepared. The candidate mixture generally includes regions of fixed sequences (i.e., each of the members of the candidate mixture contains the same sequences in the same location) and regions of randomized sequences. The fixed sequence regions are selected either: (a) to assist in the amplification steps described below, (b) to mimic a sequence known to bind to the target, or (c) to enhance the concentration of a given structural arrangement of the nucleic acids in the candidate mixture. The randomized sequences can be totally randomized (i.e., the probability of finding a base at any position being one in four) or only partially randomized (e.g., the probability of finding a base at

any location can be selected at any level between 0 and 100 percent).

2) The candidate mixture is contacted with the selected target under conditions favorable for binding between the target and members of the candidate mixture. Under these circumstances, the interaction between the target and the nucleic acids of the candidate mixture can be considered as forming nucleic acid-target pairs between the target and those nucleic acids having the strongest affinity for the target.

3) The nucleic acids with the highest affinity for the target are partitioned from those nucleic acids with lesser affinity to the target. Because only an extremely small number of sequences (and possibly only one molecule of nucleic acid) corresponding to the highest affinity nucleic acids exist in the candidate mixture, it is generally desirable to set the partitioning criteria so that a significant amount of the nucleic acids in the candidate mixture (approximately 5-50%) are retained during partitioning.

4) Those nucleic acids selected during partitioning as having the relatively higher affinity to the target are then amplified to create a new candidate mixture that is enriched in nucleic acids having a relatively higher affinity for the target.

5) By repeating the partitioning and amplifying steps above, the newly formed candidate mixture contains fewer and fewer unique sequences, and the average degree of affinity of the nucleic acids to the target will generally increase. Taken to its extreme, the SELEX process will yield a candidate mixture containing one or a small number of unique nucleic acids representing those nucleic acids from the original candidate mixture having the highest affinity to the target molecule.

The SELEX patents and applications describe and elaborate on this process in great detail. Included are targets that can be used in the process; methods for partitioning nucleic acids within a candidate mixture;

and methods for amplifying partitioned nucleic acids to generate enriched candidate mixture. The SELEX patents and applications also describe ligands obtained to a number of target species, including both protein targets where the protein is and is not a nucleic acid binding protein. Therefore, the SELEX process can be used to provide high affinity ligands of a target molecule.

Target molecules are preferably proteins, but can also include among others carbohydrates, peptidoglycans and a variety of small molecules. As with conventional proteinaceous antibodies, nucleic acid antibodies (oligonucleotide ligands) can be employed to target biological structures, such as cell surfaces or viruses, through specific interaction with a molecule that is an integral part of that biological structure. Oligonucleotide ligands are advantageous in that they are not limited by self tolerance, as are conventional antibodies. Also nucleic acid antibodies do not require animals or cell cultures for synthesis or production, since SELEX is a wholly in vitro process. As is well-known, nucleic acids can bind to complementary nucleic acid sequences. This property of nucleic acids has been extensively utilized for the detection, quantitation and isolation of nucleic acid molecules. Thus, the methods of the present invention are not intended to encompass these well-known binding capabilities between nucleic acids. Specifically, the methods of the present invention related to the use of nucleic acid antibodies are not intended to encompass known binding affinities between nucleic acid molecules. A number of proteins are known to function via binding to nucleic sequences, such as regulatory proteins which bind to nucleic acid operator sequences. The known ability of certain nucleic acid binding proteins to bind to their natural sites, for example, has been employed in the detection, quantitation, isolation and purification of such proteins. The methods of the present invention related to the use of oligonucleotide ligands are not intended to encompass the known binding affinity between

nucleic acid binding proteins and nucleic acid sequences to which they are known to bind. However, novel, non-naturally-occurring sequences which bind to the same nucleic acid binding proteins can be developed using SELEX. In particular, the oligonucleotide ligands of the present invention bind to such target molecules which comprise a three dimensional chemical structure, other than a polynucleotide that binds to said oligonucleotide ligand through a mechanism which predominantly depends on Watson/Crick base pairing or triple helix binding, wherein said oligonucleotide ligand is not a nucleic acid having the known physiological function of being bound by the target molecule.

It should be noted that SELEX allows very rapid determination of nucleic acid sequences that will bind to a protein and, thus, can be readily employed to determine the structure of unknown operator and binding site sequences which sequences can then be employed for applications as described herein. SELEX is thus a general method for use of nucleic acid molecules for the detection, quantitation, isolation and purification of proteins which are not known to bind nucleic acids. In addition, certain nucleic acid antibodies isolatable by SELEX can also be employed to affect the function, for example inhibit, enhance or activate the function, of specific target molecules or structures. Specifically, nucleic acid antibodies can be employed to inhibit, enhance or activate the function of proteins.

The oligonucleotides used in the conjugates according to the invention are obtained in a preferred embodiment according to the process described below. Suitable oligonucleotides thus can be obtained in that a mixture of oligonucleotides, containing random sequences, is brought together with the target structure, certain oligonucleotides exhibiting an increased affinity to the target structure relative to the mixture of oligonucleotides, the latter being separated from the radical of the oligonucleotide mixture, then the oligonucleotides with

increased affinity to the target structure being amplified to obtain a mixture of oligonucleotides, which exhibits an increased portion of oligonucleotides, which bond on the target structures.

5 In the process, a DNA strand is first produced in a preferred way by chemical synthesis. This DNA strand has on the 3'-end a known sequence, which is used as a promoter for an RNA polymerase and at the same time is complementary to a primer sequence for the polymerase chain 10 reaction (PCR). In an especially preferred embodiment, in this case, the promoter for the T7 RNA polymerase is involved. Following this promoter, a random sequence is synthesized on the promoter. The random sequence can be obtained in that the four suitable bases are input in the 15 same ratio in the synthesis machine. Completely random DNA sequences thus result. The length of the random sequence is about 15 to 100 nucleotides in the preferred embodiment. On this DNA piece with the random sequence, another DNA sequence is synthesized, which can be used 20 for the polymerase chain reaction (PCR).

After synthesis of this DNA strand, the latter is transferred with the help of an RNA polymerase to a complementary RNA strand. In the preferred embodiment, in this case the T7 RNA polymerase is used. In the tran-25 scription, those nucleotides that are modified are supplied to the RNA polymerase. In an especially preferred embodiment, the ribose is modified in 2'-position. In this case, a substitution of the hydrogen atom or of the hydroxyl group by an alkoxy group, preferably a methoxy 30 group, an amino group or a fluorine atom can be involved. The RNA oligonucleotides produced in this way are then introduced into the selection process.

In the selection process, the RNA oligonucleotides with the target structure are introduced. Target struc-35 ture is defined as a structure on which the oligonucleotide is to bond specifically and with high affinity.

Such structures are, e.g., macromolecules, tissue structures of higher organisms such as animals or human,

organs or parts of organs, cells, especially tumor cells or tumors.

The target structure does not absolutely have to be present in pure form, it can also be present in a naturally occurring organ or on a cell surface. Stringency may applied to the selection process by the addition of polyamino (tRNA, heparin), plasma or whole blood to the SELEX reaction.

If an isolated protein is involved in this case, the latter can be bound on a solid phase, for example a filter. In the selection, an excess of target structure relative to the RNA mixture is used. In incubation, the specific oligonucleotide molecules bond on the target structures, while the nonbound oligonucleotides are separated from the mixture, for example by washing.

Then, the oligonucleotide molecules are separated from the target molecules or removed by washing with suitable buffers or solvents.

With the help of the reverse transcriptase, the RNA oligonucleotide found is then transferred to the complementary DNA strand.

Since the thus obtained DNA strand exhibits primer sequences (or promoter sequences) on both ends, an amplification of the DNA sequences found can be performed simply with the help of the polymerase chain reaction.

The DNA oligonucleotides amplified in this way are then transferred with the help of the RNA polymerase again to RNA oligonucleotides and the thus obtained RNA oligonucleotides can be used in another selection step (as described above).

After separating the bonding RNA oligonucleotides, obtained in the second selection step, from the target molecules, the latter are again transferred in DNA with the help of reverse transcriptase, the thus obtained complementary DNA oligonucleotides are amplified with the help of the polymerase chain reaction and then transcribed with the help of the RNA polymerase again to RNA

oligonucleotides, which are available for an additional selection step.

It has been found that the desired high specificities and high bonding affinities can be obtained if the selection steps are repeated several times. Rarely is the desired oligonucleotide sequence to be obtained already after one or two selection steps. As soon as the desired specificity and bonding affinity between target structure and oligonucleotide is obtained, the oligonucleotide(s) can be sequenced, by which the sequence of the specifically bonding oligonucleotides can be determined.

Especially advantageous in this process is that it can be used not only with suitable proteins, but also in vivo. But the above-mentioned selection process can also be performed on purified target structures. But it is essential especially for the in vivo diagnosis that specificity of the oligonucleotide is given for the target structure in the living environment. Therefore, the selection processes can be performed also on cells or cell cultures, on tissues or tissue sections, on perfused organs and even on living organisms.

In this case, it is advantageous that the modified oligonucleotides can withstand the degradation by the almost omnipresent RNAs. As a result, the desired oligonucleotide sequences are themselves concentrated in the selection processes on living organisms, since appropriate naturally occurring oligonucleotides are catabolized by the RNAs.

Ferrite M consists of a signal-transmitting metal oxide nucleus, which for stabilization is sheathed with a polysaccharide, preferably a dextran (see, e.g., EP 0 186 616, Gries et al.). For formation of the conjugates according to the invention, the polysaccharide is chemically modified, for example by glycol-splitting.

As ferrites, in principle all microcrystalline metal oxides stabilized by coating agents, proposed for NMR diagnosis, are suitable, especially superparamagnetic materials, such as, for example, a magnetite AMI-25

designated as Endorem(R) (Guerbet Company, Paris), a magnetite designated as AMI-227 (Advanced Magnetics Company, Cambridge, Mass.), dextran-magnetites produced according to US Patent 4,101,435 (M. Hasegawa et al.),
5 monocrystalline iron oxide nanoparticles designated as USPIO or MION [R. Weissleder et al., Radiology (1992); 182: 381-385] or superparamagnetic particles with the designation MSM [A. K. Fahlvik et al., Invest. Radiol. (1990), 25: 113-120]. Preferred are particles that are
10 sheathed with an alkali-treated polysaccharide [see EP 0 186 616, Gries et al.; EP 0 525 199, Kito et al.; WO 92/22586, Hasegawa et al.]. Ferrite particles, which are stabilized with glycosaminoglycans as coating agent (see, for example, EP 0 516 252, M. Kresse et al.), are also
15 suitable. Dextran-magnetites are especially preferred.

The ferrites or the ferrite conjugates according to the invention have the value of nanoparticles, in which the metal oxide nucleus exhibits a diameter less than 30 nm, preferably less than 15 nm.

20 The ferrites optionally can also be radioactive. Thus, for example, numerous mixed oxides of iron with rare earths are known (V. K. Sankaranayanan et al., J. Mater. Sci. 1994, 29(3): 762-767), which can be radioactively labeled by the incorporation of an isotope suit-
25 able for radiodiagnosis and/or radiotherapy, such as, for example, Yb-169 or Y-90. In this case, the co-precipitation process described by R. Weissleder et al., Radiology 1994, 191:225 is advantageously used.

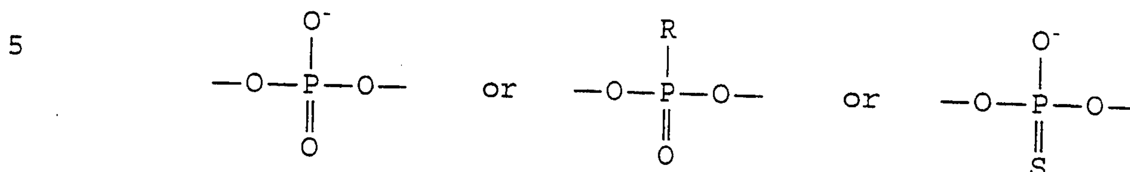
As suitable isotopes, there can be mentioned, for
30 example: Fe-57, Ga-67, Tc-99m, In-111, I-123, Ti-201 and Yb-169 for radiodiagnosis and Y-90 for radiotherapy.

The bonding of oligonucleotide N to chemically modified ferrite M takes place by bonding elements B-L-A, in which

35 B means a bonding component X-Y-Z, in which X is a direct bond or a group -NH or -NR with R meaning a C1 to C4 alkyl chain, Y is a direct bond or a spacer and Z is a direct bond or a sulfur atom,

L means a linker and

A means a carbonyl group bound on the terminal 3-' or 5'-position

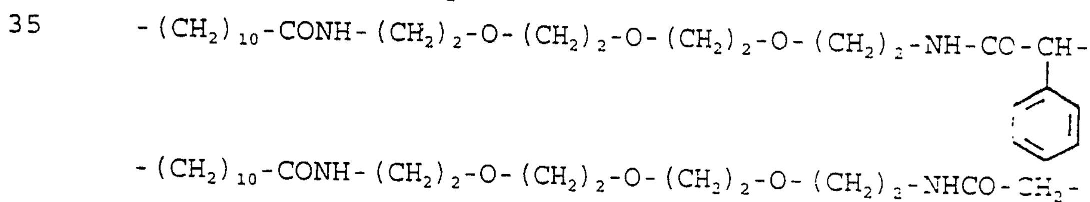


or a carbonyl group bound on the terminal 4'-position.

The linkage of group A with oligonucleotide N takes place on an oligonucleotide radical reduced on the terminal 3'-end or 5'-end by a hydroxyl group or on an oligonucleotide radical reduced in 4'-position by a hydroxymethyl group, the linkage of group X with ferrite M takes place on a ferrite derivative carrying functional groups. As functional groups, the formyl group, the carboxyl group, the glycidyl group and the amino group can be mentioned as examples.

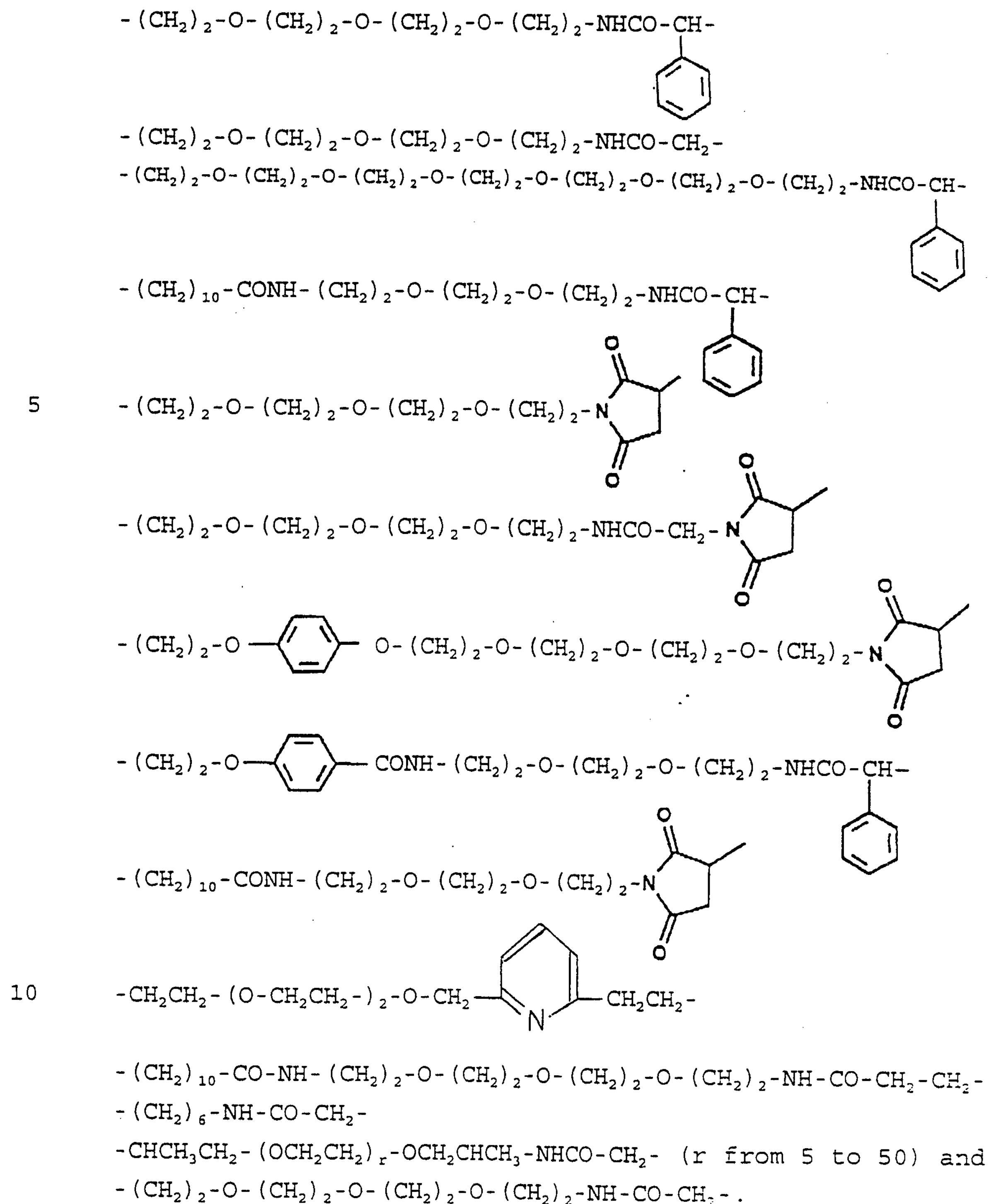
For the case that Y has the meaning of a spacer, it stands for a straight-chain or branched, saturated or unsaturated C1 to C200, preferably C1 to C50 alkylene chain, which optionally contains 1 to 10 imino, preferably 1 to 5 imino, 1 to 3 phenylene, 1 to 3 phenylenoxy, 1 to 10 amido, 1 to 2 hydrazido, 1 to 10 carbonyl, 1 succinimido, 1 6-ethylpyridin-2-yl groups, and which optionally is interrupted by 1 to 60 oxygen atoms and/or 1 to 5 sulfur atoms and which optionally is substituted by 1 to 5 hydroxy, 1 to 10 oxo, 1 to 3 carboxy, 1 to 5 carboxy-C1 to C4 alkyl, 1 to 3 hydroxy-C1 to C4 alkyl, 1 to 3 C1 to C7 alkoxy and/or 1 to 2 phenyl groups, which optionally are substituted by a carboxy, a cyano, a nitro, a sulfonyl and/or an acetyl group.

As spacers Y, the following structures can be mentioned as examples:



$-(CH_2)_{10}-CONH-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-NH-CO-CH-$ (with phenyl on CH)

$-(CH_2)_{10}-CONH-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-NHCO-CH_2-$



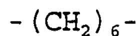
5

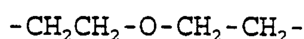
10

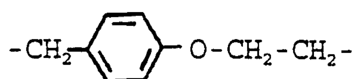
$-(CH_2)_{10}-CO-NH-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-NH-CO-CH_2-CH_2-$
$-(CH_2)_6-NH-CO-CH_2-$
$-CHCH_3CH_2-(OCH_2CH_2)_r-OCH_2CHCH_3-NHCO-CH_2-$ (r from 5 to 50) and
$-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-NH-CO-CH_2-$.

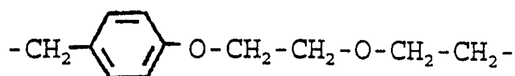
15 Linker L stands for a straight-chain or branched, saturated or unsaturated C1 to C20 alkylene chain, which optionally contains 1 to 3 imino, 1 to 3 oxo, a phenylene or a phenylenoxy group and optionally is interrupted by 1 to 6 oxygen atoms and/or 1 to 3 sulfur atoms.

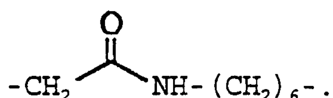
20 The following structures can be mentioned as examples:











In addition, the invention relates to a process for the production of the conjugates according to the invention.

The production of the compounds of general formula I according to the invention

in which

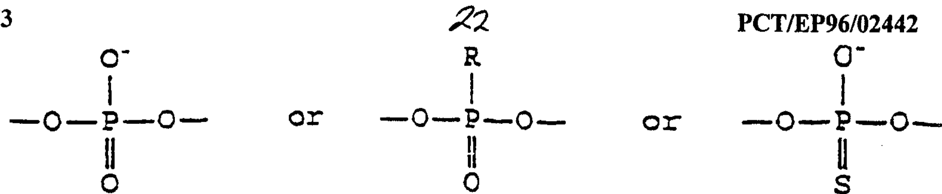
PN means an oligonucleotide bonding specifically with high affinity to target structures, containing modifications significantly limiting the degradation by naturally occurring nucleases, reduced by one hydroxyl group in the terminal 3'-position or 5'-position, or by one hydroxymethyl group in the terminal 4'-position,

B means a bonding component X-Y-Z, in which X means a direct bond or a group -NH or -NR with R meaning a C1 to C4 alkylene chain,

Y means a direct bond or a spacer and Z is a direct bond or a sulfur atom,

L means a linker,

A means a group bound on the terminal 3'- or 5'-position



or a carbonyl group bound on the terminal 4'-position,

M means an optionally radioactively-labeled ferrite coated with a polysaccharide and chemically modified, and

m means a number between 1 and 100,

provided that at least one of groups X, Y and Z does not stand for a direct bond,

takes place, in that in a way known in the art, compounds of general formula II

$M-(X-Y'-E)_m$ (II),

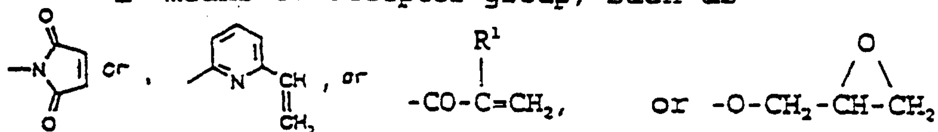
in which

a) X and m have the above-mentioned meaning and E stands for a reactive group

E^1 means a leaving group such as F, Br, Cl, OTs or OMes and

Y' has the same meaning as Y or

E^2 means an acceptor group, such as



and

Y' means spacer Y reduced by E^2-H, and

R^1 stands for hydrogen, C1 to C10 alkyl or for phenyl optionally substituted with $-OCH_3$, -CN, $-SO_2R$, -COCH, $-NO_2$, -Cl, $-CO_2H$, $-OCH_3$, $-CF_3$,

are reacted with m equivalents of a compound of general formula III

H, S-L-A-PN (III),

in which L, A and PN have the above-mentioned meaning

or in which

b) X and Y each respectively stand for a direct bond and

E stands for a functional group

E^3 means a formyl, carboxyl, carboxylalkyl or glycidyl group,

with m equivalents of a compound of general formula X

5 HX-L-A-PN (XI),

in which X stands for an -NH or -NR group with R in the above-mentioned meaning and L, A and PN have the above-mentioned meaning,

and in the case of reaction of formyl groups with alde-
10 hyde groups, a reduction step is necessary.

The reaction cited under a) takes place in aqueous medium at an alkaline pH, preferably at 8 to 9, at room temperature under protective gas, preferably under argon.

For the production of compounds of general formula
15 II, a start is made from a chemically modified, optionally radioactively-labeled ferrite coated with a polysaccharide, which contains, for example, the following functional groups:

a) formyl groups obtained by glycol-splitting (G.
20 Jayme et al., B. 77, 383 (1944) and B. 85, 1840 (1952)), or

b) carboxyl groups obtained by alkali treatment (see US Pat. 4,101,435), or

c) carboxyalkyl groups -O-$(CH_2)_n$-COOH with n meaning
25 1 to 10, obtained by carboxyalkylation of OH groups (see EP 0 525 199), or

d) glycidyl groups obtained by reaction of OH groups with halohydrins according to standard methods, or

30 e) amino groups introduced according to the process indicated on page 22 of EP 0 125 995.

The reaction to the compounds of general formula II, in which Y stands for a spacer, can take place, for example, in the following ways:

35 α) by reductive amination of formyl groups with m amino compounds of general formula IV,

X-Y-E (IV)

in which

X stands for -NH and Y and E have the above-mentioned meanings, in aqueous medium at room temperature with sodium cyanoborohydride as reducing agent,

5 β) by reaction, for example, with carbodiimide of activated carboxyl groups with amino compounds of general formula IV
in which

X stands for -NH or -NR with R in the
10 above-mentioned meaning and Y and E have the above-mentioned meanings,

in aqueous medium at room temperature under neutral conditions, preferably with the addition of sulfo-N-hydroxysuccinimide,

15 γ) by opening the epoxide ring of glycidyl groups with amino compounds of general formula IV in the meaning mentioned under β) in aqueous medium at room temperature under neutral conditions.

Compounds of general formula IV are obtained by
20 protective group G being cleaved in compounds of general formula V

G-X-Y-E (V)

in which

X, Y and E have the above-mentioned meaning,
25 and

G represents a protective group familiar to one skilled in the art, such as, for example, a tert-butyl-oxycarbonyl, an Fmoc or a trifluoroacetyl group

30 (see T. W. Greene et al., John Wiley & Sons, New York 1991: Protective Groups in Organic Syntheses).

Compounds of general formula V are obtained by reaction of a compound of general formula VI

$G\text{-}X\text{-}Y^1\text{-}NRH$ (VI)

35 in which

G and X have the above-mentioned meaning,

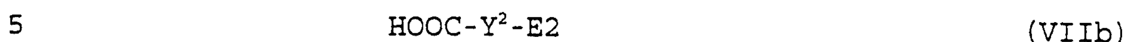
a) with an acid of general formula VIIa

$HOOC\text{-}CH(R^1)\text{-}E1$ (VIIa),

in which

E^1 and R^1 have the above-mentioned meaning and Y^1-NR-CO-CHR^1 stands for Y, or

b) by reaction with an acid of general formula VIIb



HOOC-Y^2-E2 (VIIb)

in which

E^2 has the above-mentioned meaning and Y^1-NR-CO-Y^2 stands for Y.

The reactions take place in organic solvents, such as, for example, dichloromethane, dimethylformamide, tetrahydrofuran, dioxane or dichloromethane at temperatures between -5°C and 50°C, preferably at room temperature. In this case, the acid group is activated in a way known to one skilled in the art, for example, by boiling with thionyl chloride as acid chloride; also, mixed anhydrides are suitable [Krejcarek and Tucker, Biochem. Biophys. Res. Commun. 77, 581 (1977)]. The use of N-hydroxysuccinimide esters, acylimidazoles is also known in the literature [Houben-Weyl, Methoden der organischen Chemie [Methods of Organic Chemistry], Georg Thieme Verlag, Stuttgart, Volume E, 5: 633 (1985); Org. React. 12: 157 (1962)]. As acid-binding agents, organic amines, such as triethylamine, pyridine, N-ethylmorpholine, are used.

The acids of general formula VIIa are commercially available or can be obtained easily from the also commercially available compounds of general formula VIII

$HO_2C-CH_2R^1$ (VIII)

with R^1 in the above-mentioned meaning. Thus, α-halogen acids of formula VIIa are obtained by reacting the acids with elementary bromine according to the process of C. Hell, Vol. 14: 891 (1881), J. Volhard, A. 242: 141 (1887) or N. Zelinsky, Vol. 20: 2026, (1887). By suitable performance of the reaction [C. K. Ingold, Soc. 119: 316 (1921)], it is even possible to pass in a reaction step from the acid to the activated halogen acid. ϖ-halogen acids can be obtained by opening the corresponding lactones with hydrogen halides.

The production of acids of general formula VIIb takes place according to methods known to one skilled in the art, e.g., by reaction of maleic anhydride with an amine of general formula IX

$$HOOC\text{-}Y^2\text{-}NH_2 \qquad (IX)$$

or by treatment of an aminocarboxylic acid of general formula VIII with maleic anhydride.

The partially protected amines of general formula VI can be obtained from the commercially available compounds of general formula X

$$HX\text{-}Y^1\text{-}XH \qquad (X)$$

in which

X and Y^1 have the above-mentioned meaning,

e.g., by reaction with di-t-butyl pyrocarbonate [Hoppe-Seyler's Z. Physiol. Chem., 357, 1651 (1976)].

The compounds of general formula III are obtained according to standard methods of nucleotide chemistry (Oligonucleotides and Analogues, A Practical Approach, Ed. F. Eckstein, Oxford University Press, Oxford, New York, Tokyo, 1991) from the partially protected polynucleotide, which has a free 3'- or 5'-position, by reaction with suitable linker precursors. Thus, e.g., the 5'-[6-mercaptohexylphosphoric acid ester] of the oligonucleotide is obtained by a condensation with β-cyanoethyl-N,N-diisopropylamino-S-trityl-6-mercapto-phosphoramidite, subsequent oxidation of the formed phosphite with iodine to the phosphotriester and downstream hydrolysis with ammonia solution to the S-trityl derivative of the target compound. The free SH compound is obtained by hydrolysis of the trityl compound with silver nitrate solution.

Further, the 2-mercaptopyridyl group and the mercaptomethyl group are suitable as sulfur protective groups, which can be cleaved reductively (see T. W. Greene, loc. cit.).

The compounds of general formula III can be produced easily with the help of an automatic synthesizer of the Pharmacia Company (see F. Eckstein, loc. cit.).

Compounds of general formula I with Y and Z meaning a direct bond are obtained by reaction of a chemically modified ferrite, as described under a) to d), with amines of general formula XI

5 HX-L-A-PN (XI)

in which

X stands for a group -NH or -NR with R in the above-mentioned meaning, and L, A and N have the above-mentioned meaning,

10 analogously to the reactions described above under α to γ, and these amines can be obtained according to standard methods of nucleotide chemistry (see F. Eckstein et al., loc. cit.), for example, by reaction with cyanoethyl-N,N-diisopropylamino-(3,6,9-trioxa-11-phthalimido-1-undecyl)-
15 phosphoramidite (Proc. Natl. Acad. Sci., USA, 86: 6230-6234 (1988)). 4'-Carboxyamides are also obtained according to standard methods.

Compounds of general formula I with Y and Z meaning a direct bond are obtained by reductive amination of
20 amino groups, introduced according to the method in M, with formyl groups of the compounds of general formula XII, produced, for example, according to J. Haralambidis et al., Bioorg. Med. Chem. Letters, 4: 1005-1010 (1994)

OHC-L'-A-PN (XII)

25 in which

A and PN have the above-mentioned meaning, and L' means radical L reduced by a methylene group, and the reaction preferably is performed in aqueous medium at neutral pH, at room temperature and under
30 protective gas.

The production of the pharmaceutical agents according to the invention takes place in a way known in the art, by the oligonucleotide-conjugates according to the invention -- optionally by the addition of the additives
35 usual in galenicals -- being suspended or dissolved in aqueous medium and then the suspension or solution optionally being sterilized or sterilized by filtration. Suitable additives are, for example, physiologically

harmless buffers (such as, e.g., sodium citrate) or electrolytes, such as, e.g., sodium chloride or antioxidants, such as, e.g., ascorbic acid or mannitol (or other osmotically active substances) or stabilizers, such as, e.g., sodium lactate.

5

The pharmaceutical agents according to the invention contain preferably 0.1 μmol/l to 0.1 mmol/l of the oligonucleotide conjugates according to the invention and are generally administered in amounts of 0.01 nmol/kg to 60 μmol/kg, preferably 5 to 20 μmol of metal/kg. They are intended for enteral and parenteral administration.

10

In the preparations of nanoparticles, low-viscosity aqueous colloidal solutions or suspensions of metal oxides containing stabilized particles in the nanometer range are involved. The solutions of nanoparticles do not contain any substantial aggregates, so that requirements of international pharmacopoeia on parenteralia with respect to the particle size are met.

15

The solutions or suspensions are colored reddish-brown to black, which is attributable to the intensive colors of crystals containing iron. The pronounced inherent colors can be used for visual detection, e.g., as a marker substance in surgical medicine. For detection with the MR technique, the nanoparticles are superparamagnetic or contain superparamagnetic portions. The particles physically show very high saturated magnetizations, which are achieved even in the case of low applied field strengths and, after turning off an external magnet, exhibit no more residual magnetization, they show no remanence.

20

25

30

The nanoparticles are formulated as solutions (suspensions) and can be administered without further preparation. Since the solutions of the nanoparticles are compatible with usual medicinal solvents, such as physiological sodium chloride solution, electrolyte solutions or sugar solutions, the particles can be diluted at will and are also infused, e.g., for special applications.

35

This invention further relates to a process for detecting target structures. In this case, one or more of the above-described compounds are brought together with the samples to be examined in vivo or in vitro. In this case, the oligonucleotide bonds specifically and with high bonding affinity to the target structure to be detected.

If the target structure is present in the sample, it can be detected there based on the signal. The process is especially suitable for a noninvasive diagnosis of diseases. In this case, one or more of the above-described compounds, preferably labeled with radioisotopes, are administered in vivo. Based on the signal, it can be detected whether the target structure, on which the oligonucleotide bonds specifically and with high affinity, is present in the organism to be examined.

The conjugates and agents according to the invention meet the varied requirements, which are to be imposed on a diagnostic agent. They are distinguished especially by a high specificity or affinity relative to the target structures in question. Relative to known oligonucleotide conjugates, the conjugates according to the invention exhibit an especially high in vivo stability. This was achieved by a substitution of the 2'-hydroxy group. Surprisingly, the specificity of the oligonucleotide is significantly impaired neither by this modification, nor by the coupling with the ferrite. Other advantages are the controllable pharmacokinetics as well as the low dosage that is advantageous with respect to the compatibility.

Depending on the properties of the oligonucleotide components, many areas of use arise for special indications such as the MR-lymphography after intravenous or local interstitial administration, the tumor visualization, the visualization of functions or disturbed functions, the plaque visualization (atherosclerotic imaging), the visualization of clots and vessel occlusions, MR angiography, perfusion tests, the visualization of

infarctions, the visualization of endothelial impairments, receptor imaging, the visualization of the integrity of the blood-brain barrier, etc. and for the differential diagnosis, especially for distinguishing tumors/metastases and hyperplastic tissue.

The following examples are used for a more detailed explanation of the object of the invention.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative and not limitative of the remainder of the disclosure in any way whatsoever.

In the foregoing and in the following examples, all temperatures are set forth uncorrected in degrees Celsius; and, unless otherwise indicated, all parts and percentages are by weight.

The entire disclosures of all applications, patents and publications, cited above and below, if any, are hereby incorporated by reference.

EXAMPLES

Example A

1-Amino-3,6,9-trioxa-11-(2-bromo-2-phenyl-acetylamino)-undecane

5 a) 1-Amino-3,6,9-trioxa-11-(t-butyloxycarbonylamino)-undecane

43.65 g (200 mmol) of di-t-butyloxypyrocarbonate is instilled with stirring in a solution of 38.45 g (200 mmol) of 1,11-diamino-3,6,9-trioxa-undecane in 200 ml of 10 dry tetrahydrofuran. It is stirred overnight, then evaporated to dryness in a vacuum and purified by chromatography on silica gel. As eluant, a mixture of ethyl acetate and ethanol is used. The title compound is obtained as oil.

15 Yield: 40.17 g (68.7% of theory)

Elementary analysis:

Cld:	C 53.41	H 9.65	N 9.58
Fnd:	C 53.49	H 9.71	N 9.51

b) 1-(2-Bromo-2-phenyl-acetylamino)-3,6,9-trioxa-11-(t-20 butyloxycarbonylamino)-undecane

29.24 g (100 mmol) of the amine produced under 1a) is dissolved in 300 ml of dichloromethane. It is mixed with 10.12 g (100 mmol) of triethylamine and then, 23.35 g (100 mmol) of 2-bromo-2-phenyl-acetyl chloride, dis-25 solved in 30 ml of dichloromethane, is instilled in it with stirring and cooling with ice water. After stirring overnight, it is poured into ice water, the organic solution is separated, it is washed quickly with cold 2 N hydrochloric acid, then with saturated sodium bicarbonate 30 solution, it is dried on sodium sulfate and evaporated to dryness in a vacuum. The title compound is obtained as oil.

Yield: 41.38 g (87.4% of theory)

Elementary analysis:

35 Cld:	C 53.28	H 7.03	Br 16.88	N 5.92
Fnd:	C 53.38	H 7.15	Br 16.94	N 5.86

c) 1-Amino-3,6,9-trioxa-11-(2-bromo-2-phenyl-acetylamino)-undecane

28.40 g (60 mmol) of the amino compound produced under 1b) is dissolved with stirring and cooling with ice water in 100 ml of trifluoroacetic acid and 10.81 g (100 mmol) of anisole. It is stirred for one more hour at room temperature and the progress of the reaction is examined by thin-layer chromatography. It is shown that initial material is no longer present. It is largely concentrated by evaporation in a vacuum, stirred with addition of dry hexane and the hexane phase is separated. The residue is dried in an oil pump vacuum. The trifluoroacetate of the title compound is obtained as viscous oil.

Yield: 49.68 g (98.7% of theory)

Elementary analysis:

Cld:	C 42.96	H 5.21	Br 15.88	F 11.32	N 5.57
Fnd:	C 43.07	H 5.27	Br 15.94	F 11.41	N 5.50

Example B

1-Amino-11-oxo-12-aza-15,18,21-trioxa-23-(2-bromo-2-phenyl-acetylamino)-tricosane

a) 1-Amino-3,6,9-trioxa-12-aza-13-oxo-23-(t-butyloxycarbonylamino)-tricosane

30.14 g (100 mmol) of 11-t-butyloxycarbonylamino-undecanoic acid is dissolved in 250 ml of dry tetrahydrofuran. It is cooled to 0°C, then 16.22 g (100 mmol) of carbonyldiimidazole is added with stirring and allowed to stir for one more hour at 0°C. Then, this solution is instilled in the solution of 28.84 g (150 mmol) of 1,11-diamino-3,6,9-trioxa-undecane in 150 ml of dry tetrahydrofuran, and it is stirred intensively. It is allowed to stir overnight, then concentrated by evaporation in a vacuum, and the product is purified by chromatography on silica gel. As eluant, a mixture of ethyl acetate and ethanol is used. The title compound is obtained as syrup.

Yield: 37.20 (78.2% of theory)

Elementary analysis:
Cld: C 60.60 H 10.38 N 8.83
Fnd: C 60.69 H 10.45 N 8.89

5 b) 1-(2-Bromo-2-phenyl-acetylamino)-3,6,9-trioxa-12-aza-13-oxo-23-(t-butyloxycarbonylamino)-tricosane

47.57 g (100 mmol) of the amino compound produced under a) is dissolved in 400 ml of dichloromethane. It is mixed with 10.12 g (100 mmol) of triethylamine, cooled
10 to 0°C and then 23.35 g (100 mmol) of 2-bromo-2-phenyl-acetyl chloride, dissolved in 30 ml of dichloromethane, is instilled in it with stirring and cooling. After stirring overnight, it is poured into ice water, the organic solution is separated, it is washed quickly with
15 2N hydrochloric acid and then with saturated sodium bicarbonate solution, it is dried on sodium sulfate and concentrated by evaporation in a vacuum. The title compound is obtained as viscous oil.

Yield: 58.46 g (86.9% of theory)

20 Elementary analysis:
Cld: C 57.14 H 8.09 Br 11.88 N 6.25
Fnd: C 57.01 H 8.17 Br 11.96 N 6.37

c) 1-Amino-11-oxo-12-aza-15,18,21-trioxa-23-(2-bromo-2-phenyl-acetylamino-tricosane

25 5.41 g (50 mmol) of anisole is added to 100 ml of trifluoroacetic acid. With cooling, 14.27 mg (30 mmol) of the compound produced under b) is now stirred in. It is allowed to stir for one more hour at room temperature and then the progress of the reaction is examined by
30 thin-layer chromatography. It is shown that the reaction is complete. It is concentrated by evaporation in a vacuum, mixed with dry hexane and stirred with exclusion of moisture. The hexane solution is separated and the

residue is dried in an oil pump vacuum. The title compound is obtained as trifluoroacetate.

Yield: 5.91 g (86.1% of theory)

Elementary analysis:

	C	H	Br	F	N
Cld:	C 50.73	H 6.90	Br 11.64	F 8.30	N 6.12
Fnd:	C 50.62	H 6.99	Br 11.72	F 8.40	N 6.19

Example C

5'-(6-Mercapto-1-hexyl-phosphoric acid ester) of the 35-mer oligonucleotide
5'-T*T*T*T*TAGGAGGAGGAGGGAGAGCGCAAAUGAGAUU-3' (modified ligand for serine protease)

The 35-mer oligonucleotide 5'-T*T*T*T*TAGGAGGAGGAGGGAGAGCGCAAAUGAGAUU-3' (seq. no. 13 from US Patent No. 5,270,163) identified according to the SELEX process, modified in the sugar units and by a 5'-linked sequence, is produced in the usual way in an automatic synthesizer of the Pharmacia Company (see Oligonucleotides and Analogues, A Practical Approach, Ed. F. Eckstein, Oxford University Press, Oxford, New York, Tokyo, 1991), in which the oligonucleotide is also present on the column of the solid support. By reaction with trichloroacetic acid solution in dichloromethane, the 5'-hydroxy group is opened. The loading of the column is about 10 mg of the 35-mer oligonucleotide. To link the linker, the column is reacted with a solution of 50 µmol of β-cyanoethyl-N,N-diisopropylamino-S-trityl-6-mercapto)-phosphoramite in acetonitrile in the presence of tetrazole. The oxidation of the formed phosphite to the completely protected phosphotriester takes place with iodine in tetrahydrofuran. Then, the column is washed in succession with methanol and water. To remove the modified oligonucleotide from the solid support, the content of the column is transferred to a multivial, mixed with 5 ml of 30% ammonia solution, the vessel is sealed and shaken overnight at 55°C. It is then cooled to 0°C, centrifuged, the support is washed with 5 ml of water and

the combined aqueous phases are subjected to a freeze-drying. For purification, the solid material is taken up in 2 ml of water, mixed with 2 ml of 0.5 M ammonium acetate solution, then with 10 ml of ethanol. It is allowed to stand overnight at -20°C, centrifuged, the residue is washed with 1 ml of ethanol (-20°C) and finally dried in a vacuum at room temperature. 9 mg of the S-tritylated title compound is obtained. For cleavage of the trityl protective group, the product is dissolved in 0.5 ml of water, mixed with 0.1 ml of 1 M silver nitrate solution and stirred for 1 hour at room temperature. Then, it is mixed with 0.1 ml of 1 M dithiothreitol solution. After 15 minutes, it is centrifuged and the supernatant solution is extracted several times with ethyl acetate. After the freeze-drying, 8 mg of the desired title compound is obtained from the aqueous solution.

Note: *: The internucleotide bond is a methyl phosphonate group.

Example D

5'-(6-Amino-hexyl-phosphoric acid ester) of the 35-mer oligonucleotide

5'-CUCAUGGAGCGCAAGACGAAUAGCUACAUAT*T*T*T*T-3'

The 30-mer oligonucleotide 5'-CUCAUGGAGCGCAAGACGAAUAGCUACAUA-3' identified according to the SELEX process, with the modification of upstream sequence T*T*T*T*T-3' and modified sugar units, is produced in the usual way in an automatic synthesizer of the Pharmacia Company (see Oligonucleotides and Analogues, A Practical Approach, Ed. F. Eckstein, Oxford University Press, Oxford, New York, Tokyo, 1991), the oligonucleotide also being present on the column of the solid support. By reaction with trichloroacetic acid solution in dichloromethane, the 5'-hydroxy group is opened. The loading of the column is about 10 mg of the 35-mer oligonucleotide. To link the linker, the column is reacted with an acetonitrile solution of 50 μmol of β-cyanoethyl-

N,N-diisopropylamino-6-(trifluoroacetamido)-1-hexyl-phosphoramidite (produced according to Nucl. Acids. Res. 16, 2659-2669 (1988)) in the presence of tetrazole. The oxidation of the formed phosphite to the completely protected phosphotriester takes place with iodine in tetrahydrofuran. Then, the column is washed in succession with methanol and water. To remove the modified oligonucleotide from the solid support, the content of the column is transferred to a multivial, mixed with 5 ml of 30% ammonia solution, the vessel is sealed and shaken overnight at 55°C. It is then cooled to 0°C, centrifuged, the support is washed with 5 ml of water and the combined aqueous phases are subjected to a freeze-drying. For purification, the solid material is taken up in 2 ml of water, mixed with 2 ml of 0.5 M ammonium acetate solution and mixed with 10 ml of ethanol, it is allowed to stand overnight at -20°C, centrifuged, the residue is washed with 1 ml of ethanol (-20°C) and finally dried in a vacuum at room temperature. 8 mg of the title compound is obtained as colorless powder.

Example 1

Production of a conjugate from oligonucleotide and carboxydextran-ferrite (hydrodynamic diameter ≤ 20 nm; loading factor m = 26)

2.0 ml of the ferrite solution according to Hasegawa et al., Example 11, WO 94/03501 (108 mg of iron; 108 mg of carboxydextran) is added to an Amicon Diaflo stirring cell with a membrane filter (cutoff 30 kDa) and ultrafiltered. The retentate is again filled up with bidistilled water and the ultrafiltration is continued. The retentate is obtained and diluted to 20.0 ml with bidistilled water. The iron determination with ICP-AES (inductively coupled plasma atomic emission spectroscopy) produces an iron content of 5.4 mg/ml and the carboxydextran content was determined as 1.6 mg/ml (photometric determination with anthrone, Dische Z, in Whistler and Wolfrom, Methods in Carbohydrate Chemistry I, Academic Press, New York,

London, 490-491, 1962). For activation of the ferrite solution, 10.0 ml (54 mg of iron; 16 mg of dextran) is cooled in the ice bath and then mixed with 7.6 mg of sodium periodate (Merck, Germany). After completion of the reaction, it is again ultrafiltered in an Amicon Diaflo stirring cell until iodate can no longer be determined in the filtrate (detection with aqueous silver nitrate solution, Jander, G. and Blasius, E., Lehrbuch der analytischen und präparativen anorganischen Chemie [Textbook of Analytical and Preparative Inorganic Chemistry], S. Hirzel Verlag Stuttgart, 161, 1979). The generated aldehydes were determined with hydroxylamine (Makromol. Chemie [Macromol. Chemistry]; 182; 1641-1648; 1981) to 0.036 mmol.

The activated ferrite is mixed with a 5-fold excess (relative to the aldehyde groups) of spacer according to Example A (69.2 mg) and stirred for 30 minutes. The purification of nonbound spacer takes place by an Amicon Diaflo stirring cell with a cutoff 30 kDa membrane. The retentate is again filled up right through the middle with water and the purification is continued as long as the conductivity in the filtrate is less than 10 μS/cm. The filtrates are combined and the nonbound spacers are determined by the determination of the bromine content (elementary analysis). The portion of bound spacer is produced by the difference in the amount used and was determined as 13.8 mg.

The ferrite solution is adjusted to pH 8.5 with 0.1 N NaOH and under protective gas argon mixed with a 2-fold excess (relative to the spacer content) of the oligonucleotide according to Example C (711 mg). The reaction solution is stirred for 3 days and nonbound oligonucleotide is then separated by filtration on Amicon Diaflo stirring cells with a cutoff 30 kDa filter and recovered. The purification is completed when the extinction in the filtrate (260 nm; reference bidistilled water) can no longer be measured (OD < 0.002 AU). The determination of the bound oligonucleotide takes place destructively after

conversion of the phosphoric acid ester to inorganic phosphate (heating in sulfuric acid) and yields a bound portion of a total of 355.6 mg of oligonucleotide. The oligonucleotide-ferrite solution is then filtered by a membrane filter with 0.22 μm pore size and freeze-dried.

Yield: 460 mg of brownish-black powder with an uncharacteristic decomposition point

The analysis of the lyophilizate provides the following characteristic values:

Determination	Result	Method
Iron	11.6%	ICP-AES
carboxydextran	2.9%	anthrone method
spacer	3.0%	elementary analysis bromine
oligonucleotide	77.8%	elementary analysis phosphorus
water content	0.7%	Karl-Fischer titration
nuclear size	3.2 nm	electron microscopy in transmission arrangement
hydrodynamic value	18 nm	dynamic laser light scattering (volume weighting); Nicomp A370 Autodilute Nanosizer; USA)
loading factor m	26 oligonucleotides per ferrite particle	
relaxivity	r1 = 18; r2 = 59	minispec pc120; Bruker Deutschland [Germany]; 0.47 T; water; 39 ± 1°C
susceptibility (10 mmol of Fe/l of solution)	68.3 emu/g of iron	magnetic scales MSB auto; Johnson Matthey; Germany; 4.5 kGauss; 26°C

Example 2

Production of a conjugate made of oligonucleotide and carboxydextran-ferrite (hydrodynamic diameter > 20 nm; loading factor m = 45)

5 2.0 ml of the ferrite solution according to Hasegawa et al., Comparative Example 1, WO 94/03501 (104 mg of iron; 120 mg of carboxydextran) is purified by nonbound carboxydextran as in Example 1. The iron determination with ICP-AES (inductively coupled plasma atomic emission 10 spectroscopy) provides an iron content of 5.2 mg/ml and the carboxydextran content was determined as 1.7 mg/ml (photometric determination with anthrone). For activation of the ferrite solution, 10.0 ml (50.5 mg of iron; 17 mg of carboxydextran) is cooled in an ice bath and 15 then mixed with 3.03 mg of sodium periodate (Merck, Germany). After the reaction and purification as in Example 1), a CHO content of 0.014 mmol is produced. The activated ferrite is mixed with a 5-fold excess (relative to the aldehyde groups) of spacer according to Example B 20 (40.5 mg) and treated as in Example 1. The portion of bound spacer arises by the difference in the amount used and was determined as 8.1. The ferrite solution is adjusted to pH 8.5 with 0.1 N NaOH and under argon as protective gas mixed with a 2-fold excess (relative to 25 the spacer content) of the oligonucleotide according to Example C) (283 mg) and reacted with the oligonucleotide analogously to Example 1). The bound portion of the oligonucleotide was determined as 141.7 mg. The oligonucleotide-ferrite solution is then filtered by membrane 30 filter with 0.22 μm pore size and freeze-dried.

Yield: 237 mg of brownish-black powder with an uncharacteristic decomposition point.

The analysis of the lyophilizate provides the following characteristic values:

Determination	Result	Method
Iron	21%	ICP-AES
carboxydextran	6.2%	anthrone method
spacer	3.5	elementary analysis bromine
oligonucleotide	60.9%	elementary analysis phosphorus
water content	1.98%	Karl-Fischer titration
nuclear size	5.1 nm	electron microscopy in transmission arrangement
hydrodynamic value	34 nm	dynamic laser light scattering (volume weighting); Nicomp A370 Autodilute Nanosizer; USA)
loading factor m	45 oligonucleotides per ferrite particle	
relaxivity	r1 = 34; r2 = 162	minispec pc120; Bruker Deutschland [Germany]; 0.47 T; water; 39 ± 1°C
susceptibility (10 mmol of Fe/l of solution)	117.3 emu/g of iron	magnetic scales MSB auto; Johnson Matthey; Germany; 4.5 kGauss; 26°C

Example 3

Production of a conjugate made of oligonucleotide and carboxydextran-ferrite with chromatographic preliminary purification (hydrodynamic diameter < 20 nm; loading factor m = 20)

2.0 ml of the ferrite solution according to Hasegawa et al., Example 1, WO 94/03501 (118 mg of iron; 150 mg of carboxydextran) is applied on a PD 10 column equilibrated with bidistilled water (Pharmacia, Germany), filled with Sephadex G-25. After the infiltration of the sample into gel, elution takes place with bidistilled water in 1 ml steps. The first 4 fractions at 1 ml each are collected and pooled. The iron determination with ICP-AES (inductively coupled plasma atomic emission spectroscopy) provides an iron content of 27 mg/ml and the carboxydextran content was determined as 23.1 mg/ml (photometric determination with anthrone).

For activation of the ferrite solution, 3.0 ml (81 mg of iron; 69.4 mg of carboxydextran) is cooled in an ice bath and then mixed with 3.3 mg of sodium periodate (Merck, Germany). After the reaction and purification as in example 1, an aldehyde content of 0.015 mmol is produced.

The activated ferrite is mixed with a 5-fold excess (relative to the aldehyde groups) of spacer according to Example A (30 mg) and treated as in Example 1. The portion of bound spacer is produced by the difference in the amount used and was determined as 6 mg.

The ferrite solution is adjusted to pH 8.5 with 0.1 N NaOH and under argon as protective gas mixed with a 2-fold excess (relative to the spacer content) of the oligonucleotide according to Example C (308.5 mg) and reacted with the oligonucleotide analogously to Example 1. The bound portion of oligonucleotide was determined as 154 mg.

The oligonucleotide ferrite solution is then filtered via membrane filter with 0.22 μm pore size and is freeze-dried.

Yield: 331 mg of brownish-black powder with an uncharacteristic decomposition point

The analysis of the lyophilizate provides the following characteristic values:

Determination	**Result**	**Method**
Iron	23.9%	ICP-AES
carboxydextran	17.9%	anthrone method
spacer	1.8%	elementary analysis bromine
oligonucleotide	46.8%	elementary analysis phosphorus
water content	0.57%	Karl-Fischer titration
nuclear size	4.4 nm	electron microscopy in trans mission arrangement
hydrodynamic value	18.2 nm	dynamic laser light scattering (volume weighting); Nicomp A370 Autodilute Nanosizer; USA)
loading factor m	20 oligonucleotides per ferrite particle	
relaxivity	r1 = 23; r2 = 54	minispec pc120; Bruker Deutschland [Germany]; 0.47 T; water; 39 ± 1°C
susceptibility (10 mmol of Fe/l of solution)	77 emu/g of iron	magnetic scales MSB auto; Johnson Matthey; Germany; 4.5 kGauss; 26°C

Example 4

Production of a conjugate made of oligonucleotide and dextran-ferrite (hydrodynamic diameter ≤ 20 nm; loading factor m = 55)

25.0 ml of the ferrite solution according to Shen, T. et al.; MRM 29; 599-604; 1993 (67.5 mg of iron; 60 mg of dextran) is purified in an Amicon Diaflo stirring cell as in Example 1. The iron determination in the retentate with ICP-AES (inductively coupled plasma atomic emission spectroscopy) provides an iron content of 3.24 mg/ml and the dextran content was determined as 0.88 mg/ml (photometric determination with anthrone).

For activation of the ferrite solution, 10.0 ml (3.24 mg of iron; 8.8 mg of dextran) is cooled in an ice bath and then mixed with 3.14 mg of sodium periodate

(Merck, Germany). After the reaction and purification as in Example 1), an aldehyde content of 0.015 mmol arises.

The activated ferrite is mixed with a 5-fold excess (relative to the aldehyde groups) of spacer according to
5 Example A (28.5 mg) and treated as in Example 1. The portion of bound spacer arises by the difference in the amount used and was determined as 5.7 mg.

The ferrite solution is adjusted to pH 8.5 with 0.1 N NaOH and under argon as protective gas mixed with a
10 2-fold excess (relative to the spacer content) of the oligonucleotide according to Example C (293.3 mg) and reacted with the oligonucleotide analogously to Example 1. The bound portion of the oligonucleotide was determined as 146.7 mg.

15 The oligonucleotide-ferrite solution is then filtered via membrane filter with 0.22 μm pore size and freeze-dried.

Yield: 208 mg of brownish-black powder with an uncharacteristic decomposition point

20 The analysis of the lyophilizate provides the following characteristic values:

Determination	Result	Method
Iron	15.4%	ICP-AES
dextran	3.7%	anthrone method
spacer	2.8%	elementary analysis bromine
oligonucleotide	72%	elementary analysis phosphorus
water content	2.1%	Karl-Fischer titration
nuclear size	4.6 nm	electron microscopy in transmission arrangement
hydrodynamic value	20 nm	dynamic laser light scattering (volume weighting); Nicomp A370 Autodilute Nanosizer; USA)
loading factor m	55 oligonucleotides per ferrite particle	
relaxivity	r1 = 17; r2 = 35	minispec pc120; Bruker Deutschland [Germany]; 0.47 T; water; 39 ± 1°C
susceptibility (10 mmol of Fe/l of solution)	68 emu/g of iron	magnetic scales MSB auto; Johnson Matthey; Germany; 4.5 kGauss; 26°C

Example 5

Production of a conjugate made of oligonucleotide and dextran-ferrite (hydrodynamic diameter > 20 nm; loading factor m = 56)

10.0 ml of the ferrite solution Endorem(R); Guerbet GmbH, Germany (112 mg of iron; 132 mg of dextran) is purified in an Amicon Diaflo stirring cell as in Example 1. The iron determination in the retentate with ICP-AES (inductively coupled plasma atomic emission spectroscopy) provides an iron content of 5.15 mg/ml and the dextran content was determined as 1.8 mg/ml (photometric determination with anthrone).

For activation of the ferrite solution, 10.0 ml (5.51 mg of iron; 18 mg of carboxydextran) is cooled in an ice bath and then mixed with 0.5 mg of sodium periodate (Merck, Germany). After the reaction and purification as in Example 1, an aldehyde content of 0.002 mmol is produced.

The activated ferrite is mixed with a 5-fold excess (relative to the aldehyde groups) of spacer according to Example B (6.7 mg) and treated as in Example 1. The portion of bound spacer arises by the difference in the amount used and was determined as 1.34 mg.

The ferrite solution is adjusted to pH 8.5 with 0.1 N NaOH and under argon as protective gas mixed with a 2-fold excess (relative to the spacer content) of the oligonucleotide according to Example C (46.8 mg) and reacted with the oligonucleotide analogously to Example 1. The bound portion of the oligonucleotide was determined as 23.4 mg.

The oligonucleotide-ferrite solution is then filtered by membrane filter with 0.22 μm pore size and freeze-dried.

Yield: 112 mg of brownish-black powder with an uncharacteristic decomposition point

The analysis of the lyophilizate provides the following characteristic values:

Determination	**Result**	**Method**
Iron	45.5%	ICP-AES
dextran	13.9%	anthrone method
spacer	1.2%	elementary analysis bromine
oligonucleotide	21.3%	elementary analysis phosphorus
water content	2.4%	Karl-Fischer titration
nuclear size	10 nm	electron microscopy in transmission arrangement
hydrodynamic value	85 nm	dynamic laser light scattering (volume weighting); Nicomp A370 Autodilute Nanosizer; USA)
loading factor m	56 oligonucleotides per ferrite particle	
relaxivity	r1 = 26; r2 = 104	minispec pc120; Bruker Deutschland [Germany]; 0.47 T; water; 39 ± 1°C
susceptibility (10 mmol of Fe/l of solution)	88 emu/g of iron	magnetic scales MSB auto; Johnson Matthey; Germany; 4.5 kGauss; 26°C

Example 6

Production of a conjugate made of oligonucleotide and dextran-ferrite (hydrodynamic diameter > 20 nm; loading factor m = 32)

5 200 mg of lyophilizate of ferrite AMI-227 (Advanced Magnetics Inc.; USA) (44 mg of iron; 137 mg of dextran) is dissolved in 25 ml of bidistilled water and then purified in an Amicon Diaflo stirring cell as in Example 1. The iron determination in the retentate with ICP-AES
10 (inductively coupled plasma atomic emission spectroscopy) provides an iron content of 4.3 mg/ml and the dextran content was determined as 1.9 mg/ml (photometric determination with anthrone).

For activation of the ferrite solution, 5.0 ml (21.6
15 mg of iron; 9.5 mg of dextran) is cooled in an ice bath and then mixed with 1.13 mg of sodium periodate (Merck, Germany). After the reaction and purification as in Example 1, an aldehyde content of 0.005 mmol is produced.

The activated ferrite is mixed with a 5-fold excess
20 (relative to the aldehyde groups) of spacer according to Example A (10.3 mg) and treated as in Example 1. The portion of bound spacer arises by the difference in the amount used and was determined as 2.0 mg.

The ferrite solution is adjusted to pH 8.5 with 0.1
25 N NaOH and under argon as protective gas mixed with a 2-fold excess (relative to the spacer content) of the oligonucleotide according to Example C (105.5 mg) and reacted with the oligonucleotide analogously to Example 1. The bound portion of the oligonucleotide was determined
30 as 53 mg.

The oligonucleotide-ferrite solution is then filtered via membrane filter with 0.22 μm pore size and freeze-dried.

Yield: 94 mg of brownish-black powder with an
35 uncharacteristic decomposition point

The analysis of the lyophilizate provides the following characteristic values:

Determination	Result	Method
Iron	22.7%	ICP-AES
dextran	8.8%	anthrone method
spacer	2.2%	elementary analysis bromine
oligonucleotide	57.3%	elementary analysis phosphorus
water content	1.8%	Karl-Fischer titration
nuclear size	4.75 nm	electron microscopy in transmission arrangement
hydrodynamic value	27 nm	dynamic laser light scattering (volume weighting); Nicomp A370 Autodilute Nanosizer; USA)
loading factor m	32 oligonucleotides per ferrite particle	
relaxivity	r1 = 22; r2 = 44	minispec pc120; Bruker Deutschland [Germany]; 0.47 T; water; 39 ± 1°C
susceptibility (10 mmol of Fe/l of solution)	59.8 emu/g of iron	magnetic scales MSB auto; Johnson Matthey; Germany; 4.5 kGauss; 26°C

Example 7

Production of a conjugate made of oligonucleotide and Chondroitin-4-sulfate-ferrite (hydrodynamic diameter > 20 nm; loading factor m = 86)

1.0 ml of chondroitin-4-sulfate-ferrite according to EP 0,516,252, Example 1 (56 mg of iron, 24 mg of chondroitin-4-sulfate) is diluted with bidistilled water 1:10 and adjusted to pH 4.75 with 0.1 N HCl. The ferrite solution is mixed with 1.0 ml of a freshly-prepared solution of 9.2 mg of EDC [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl] in 10 ml of water and the pH is kept constant at 4.75 for two hours (titration system TPC 2000; Schott, Germany). The reaction of the EDC can be tracked by the acid consumption in the pH-STAT titration and is calculated as 2.4 µmol of activated acid. The separation of low-molecular reactants takes place by an Amicon Diaflo ultrafiltration with a cutoff of 30 kDa. The iron determination in the retentate with ICP-AES (inductively

coupled plasma atomic emission spectroscopy) provides an iron content of 5 mg/ml and the chondroitin content was determined as 2.1 mg/ml (photometric determination with the hexosamine method according to Morgan-Elson, Morgan,
5 W. T., and Elson, L. A., J. Biochem. 51, 1824, 1933).

The activated ferrite is mixed with a 5-fold excess (relative to the activated acid groups) of spacer according to Example B (13.7 mg) and treated as in Example 1. The portion of bound spacer arises by the difference in
10 the amount used and was determined as 2.7 mg.

The ferrite solution is adjusted to pH 8.5 with 0.1 N NaOH and under argon as protective gas mixed with a 2-fold excess (relative to the spacer content) of the oligonucleotide according to Example C (96 mg) and reac-
15 ted with the oligonucleotide analogously to Example 1. The bound portion of the oligonucleotide was determined as 46 mg.

The oligonucleotide-ferrite solution is then filtered via membrane filter with 0.22 μm pore size and
20 freeze-dried.

Yield: 148 mg of brownish-black powder with an uncharacteristic decomposition point

The analysis of the lyophilizate provides the following characteristic values:

Determination	Result	Method
Iron	37.3%	ICP-AES
chondroitin-4-sulfate	13.4%	Morgan-Elson assay
spacer	1.9%	elementary analysis bromine
oligonucleotide	32.6%	elementary analysis phosphorus
water content	0.6%	Karl-Fischer titration
nuclear size	9.4 nm	electron microscopy in transmission arrangement
hydrodynamic value	106 nm	dynamic laser light scattering (volume weighting); Nicomp A370 Autodilute Nanosizer; USA)
loading factor m	86 oligonucleotides per ferrite particle	
relaxivity	r1 = 43; r2 = 255	minispec pc120; Bruker Deutschland [Germany]; 0.47 T; water; 39 ± 1°C
susceptibility (10 mmol of Fe/l of solution)	125 emu/g of iron	magnetic scales MSB auto; Johnson Matthey; Germany; 4.5 kGauss; 26°C

Example 8

Production of a conjugate made of oligonucleotide and chondroitin-4-sulfate-ferrite (hydrodynamic diameter < 20 nm; loading factor m = 22)

1.0 ml of chondroitin-4-sulfate-ferrite according to EP 0,516,252, Example 13 (22.4 mg of iron, 19.6 mg of chondroitin-4-sulfate) is diluted with bidistilled water 1:200 and diafiltered from bidistilled water in an Amicon ultrafiltration unit with an RS2000 tank and a hollow fiber membrane with a cutoff of 100 kDA to separate free coating polymer. Then, the solution is concentrated up to about 8 ml and then filled up to 10.0 ml with bidistilled water. For activation, the ferrite solution is adjusted to pH 4.75 with 0.1 N HCl. The ferrite solution is then mixed with 1.0 ml of a freshly-prepared solution of 4.2 mg of EDC [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl] in 10 ml of water and the pH is kept constant at 4.75 for two hours (titration system TPC 2000;

Schott, Germany). The reaction of the EDC can be tracked by the acid consumption in the pH-STAT titration and is calculated at 2.2 μmol of activated acid. The separation of low-molecular reactants takes place by an Amicon Diaflo ultrafiltration with a cutoff of 30 kDa. The iron determination in the retentate with ICP-AES (inductively coupled plasma atomic emission spectroscopy) provides an iron content of 1.8 mg/ml and the chondroitin content was determined as 0.8 mg/ml (photometric determination of the hexosamine method according to Morgan-Elson).

The activated ferrite is mixed with a 5-fold excess (relative to the aldehyde groups) of spacer according to Example A (4.3 mg) and treated as in Example 1. The portion of bound spacer arises by the difference in the amount used and was determined as 0.86 mg.

The ferrite solution is adjusted to pH 8.5 with 0.1 N NaOH and under argon as protective gas mixed with a 2-fold excess (relative to the spacer content) of the oligonucleotide according to Example C (44 mg) and reacted with the oligonucleotide analogously to Example 1. The bound portion of the oligonucleotide was determined as 22 mg.

The oligonucleotide-ferrite solution is then filtered by membrane filter with 0.22 μm pore size and freeze-dried.

Yield: 61.5 mg of brownish-black powder with an uncharacteristic decomposition point

The analysis of the lyophilizate provides the following characteristic values:

Determination	Result	Method
Iron	33.9%	ICP-AES
chondroitin-4-sulfate	14.9%	Morgan-Elson assay
spacer	1.4%	elementary analysis bromine
oligonucleotide	36.3%	elementary analysis phosphorus
water content	1.5%	Karl-Fischer titration
nuclear size	5.6 nm	electron microscopy in transmission arrangement
hydrodynamic value	18.7 nm	dynamic laser light scattering (volume weighting); Nicomp A370 Autodilute Nanosizer; USA)
loading factor m	22 oligonucleotides per ferrite particle	
relaxivity	r1 = 34; r2 = 94	minispec pc120; Bruker Deutschland [Germany]; 0.47 T; water; 39 ± 1°C
susceptibility (10 mmol of Fe/l of solution)	122 emu/g of iron	magnetic scales MSB auto; Johnson Matthey; Germany; 4.5 kGauss; 26°C

Example 9

Production of a conjugate made of oligonucleotide and polycarboxyhexyldextran-ferrite (hydrodynamic diameter < 20 nm; loading factor m = 39)

5.0 ml of ferrite solution (according to Example 11; WO 94/03501; Hasegawa et al.) with a concentration of 56 mg of iron/ml (carboxydextran 60 mg/ml) is filled up with 2 N NaOH at 20 ml and heated under reflux conditions to 80°C (magnetic stirrer). The slow addition of 1 g of 6-bromohexanoic acid (Aldrich, Germany) takes place with stirring. After 3 hours of reaction time, it is cooled to room temperature and then neutralized under a hood with about 6 N HCl. For purification, the acid-substituted ferrite is mixed with the double volume of ethanol and the precipitate is centrifuged off (10 minutes, 1000 g). Then, the precipitate is resuspended again in 5 ml of bidistilled water, and pressed through a 0.22 μm cellulose acetate filter and then filled up with bidis-

tilled water at 5.0 ml. For determining the degree of substitution of the carboxyldextran with the hexanoic acid, the reaction was performed under the same conditions on pure stabilizer polymer (carboxydextran) and the content of acid groups was determined by potentiometric titration as 19.9 ± 1.2%. 1.0 ml of ferrite solution (54 mg of iron, 48 mg of carboxydextran) is diluted to 5 ml with bidistilled water and mixed with 103.8 mg of spacer according to Example A, and the mixture is then adjusted to pH 4.75 with 0.1 N HCl. The solution is mixed with 10.2 mg of EDC-HCl/1 ml of bidistilled water (freshly prepared), and the pH is kept constant at pH 4.75 for 2 hours by an automatic titration system (TPC 2000, Schott, Germany). The purification of low-molecular reactants and free, nonbound or adsorbed carboxydextran stabilizers takes place by dialysis (Visking dialyzer tube, Serva, Germany). The portion of bound spacer is produced by the difference in the amount used and was determined as 20.5 mg.

The ferrite solution is adjusted to pH 8.5 with 0.1 N NaOH and under argon as protective gas mixed with a 2-fold excess (relative to the spacer content) of the oligonucleotide according to Example C (1067 mg) and reacted with the oligonucleotide analogously to Example 1. The bound portion of the oligonucleotide was determined as 533 mg.

The oligonucleotide-ferrite solution is then filtered via membrane filter with 0.22 μm pore size and freeze-dried.

Yield: 674 mg of brownish-black powder with an uncharacteristic decomposition point

The analysis of the lyophilizate provides the following characteristic values:

Determination	Result	Method
Iron	7.9%	ICP-AES
polycarboxyhexyl-dextran	5.9%	anthrone assay
spacer	3.1%	elementary analysis bromine
oligonucleotide	79.9%	elementary analysis phosphorus
water content	0.9%	Karl-Fischer titration
nuclear size	3.2 nm	electron microscopy in transmission arrangement
hydrodynamic value	18 nm	dynamic laser light scattering (volume weighting); Nicomp A370 Autodilute Nanosizer; USA)
loading factor m	39 oligonucleotides per ferrite particle	
relaxivity	r1 = 18; r2 = 60	minispec pc120; Bruker Deutschland [Germany]; 0.47 T; water; 39 ± 1°C
susceptibility (10 mmol of Fe/l of solution)	71 emu/g of iron	magnetic scales MSB auto; Johnson Matthey; Germany; 4.5 kGauss; 26°C

Example 10

Production of a conjugate made of oligonucleotide and polycarboxymethyldextran-ferrite (hydrodynamic diameter < 20 nm; loading factor m = 56)

5.0 ml of ferrite solution (according to Example 11; WO 94/03501; Hasegawa et al.) with a concentration of 56 mg of iron/ml (carboxydextran about 60 mg/ml) is filled up with 2N NaOH at 20 ml. In a separate vessel, 600 mg of monochloroacetic acid is dissolved in 5 ml of water and slowly mixed with 340 mg of anhydrous sodium carbonate. The two separate solutions are then mixed and heated for 1 hour to 65°C. Then, purification takes place by pouring the solution into double the volume of ethanol. The precipitate is again dissolved in water and precipitated again with ethanol. Then, the precipitate is redispersed in 5 ml of water and pressed through a 0.22 µm cellulose acetate filter and then filled up to 5.0 ml with bidistilled water.

For determining the degree of substitution with the carboxymethyl group of the monochloroacetic acid, the reaction was performed under the same conditions on pure stabilizer polymer (carboxydextran) and the content of acid groups was determined by potentiometric titration. One carboxymethyl group per glucose unit is produced on the average.

1.0 ml of the ferrite solution (53 mg of iron, 49 mg of carboxydextran) is diluted to 5 ml with bidistilled water and mixed with 105.9 mg of spacer according to Example A, and the mixture is then adjusted to pH 4.75 with 0.1 N HCl. The solution is mixed with 10.2 mg of EDC-HCl/1 ml of bidistilled water (freshly prepared), and the pH is kept constant at pH 4.75 for 2 hours by an automatic titration system (TPC 2000, Schott, Germany). The purification of low-molecular reactants and free, not tightly bound or adsorbed carboxydextran stabilizers takes place by dialysis (Visking dialyzer tube, Serva, Germany). The portion of bound spacer is produced by the difference in the amount used and was determined as 21.2 mg.

The ferrite solution is adjusted to pH 8.5 with 0.1 N NaOH and under argon as protective gas mixed with a 2-fold excess (relative to the spacer content) of the oligonucleotide according to Example C (1088.9 mg) and reacted with the oligonucleotide analogously to Example 1. The bound portion of the oligonucleotide was determined as 544 mg.

The oligonucleotide-ferrite solution is then filtered by membrane filter with 0.22 μm pore size and freeze-dried.

Yield: 687 mg of brownish-black powder with an uncharacteristic decomposition point

The analysis of the lyophilizate provides the following characteristic values:

Determination	Result	Method
Iron	7.65%	ICP-AES
polycarboxymethyl-dextran	5.9%	anthrone assay
spacer	3.1%	elementary analysis bromine
oligonucleotide	80%	elementary analysis phosphorus
water content	1.2%	Karl-Fischer titration
nuclear size	3.6 nm	electron microscopy in transmission arrangement
hydrodynamic value	18.2 nm	dynamic laser light scattering (volume weighting); Nicomp A370 Autodilute Nanosizer; USA)
loading factor m	58 oligonucleotides per ferrite particle	
relaxivity	r1 = 19; r2 = 59	minispec pc120; Bruker Germany; 0.47 T; water; 39 ± 1°C
susceptibility (10 mmol of Fe/1 of solution)	69 emu/g of iron	magnetic scales MSB auto; Johnson Matthey; Germany; 4.5 kGauss; 26°C

Example 11

Production of a conjugate made of oligonucleotide and dextran-ferrite (hydrodynamic diameter > 20 nm; loading factor m = 65)

200 mg of lyophilizate of ferrite AMI-227 (Advanced Magnetics Inc.; USA) (44 mg of iron; 137 mg of dextran) is dissolved in 25 ml of bidistilled water and then purified in an Amicon Diaflo stirring cell as in Example 1. The iron determination in the retentate with ICP-AES (inductively coupled plasma atomic emission spectroscopy) provides an iron content of 4.3 mg/ml and the dextran content was determined as 1.9 mg/ml (photometric determination with anthrone). For activation of the ferrite solution, 5.0 ml (21.6 mg of iron; 9.5 mg of dextran) is cooled in an ice bath and then mixed with 2.26 mg of sodium periodate (Merck, Germany). After the reaction and purification as in Example 1, a CHO content of 0.011 mmol is produced. The activated ferrite is mixed with a 5-

fold excess (relative to the aldehyde groups) of spacer according to Example A (20.5 mg) and treated as in Example 1. The portion of bound spacer is produced by the difference in the amount used and was determined as 4.1 mg.

The ferrite solution is adjusted to pH 8.5 with 0.1 N NaOH and under argon as protective gas mixed with a 2-fold excess (relative to the spacer content) of the oligonucleotide according to Example D (211.1 mg) and reacted with the oligonucleotide analogously to Example 1. The bound portion of the oligonucleotide was determined as 105.6 mg. The oligonucleotide-ferrite solution is then filtered by membrane filter with 0.22 μm pore size and freeze-dried.

The yield is 94 mg of brownish-black powder with an uncharacteristic decomposition point.

The analysis of the lyophilizate provides the following characteristic values:

Determination	Result	Method
Iron	22.7%	ICP-AES
dextran	8.8%	anthrone method
spacer	2.2%	elementary analysis bromine
oligonucleotide	57.3%	elementary analysis phosphorus
water content	1.8%	Karl-Fischer titration
nuclear size	4.75 nm	electron microscopy in transmission arrangement
hydrodynamic value	27 nm	dynamic laser light scattering (volume weighting); Nicomp A370 Autodilute Nanosizer; USA)
loading factor m	32 oligonucleotides per ferrite particle	
relaxivity	r1 = 22; r2 = 44	minispec pc120; Bruker Deutschland [Germany]; 0.47 T; water; 39 ± 1°C
susceptibility (10 mmol of Fe/l of solution)	59.8 emu/g of iron	magnetic scales MSB auto; Johnson Matthey; Germany; 4.5 kGauss; 26°C

Example 12

Production of a radioactively-labelled solution of an oligonucleotide-magnetite conjugate

200 mg of lyophilizate of a MION 46-magnetite labeled with indium-111, produced according to R. Weissleder et al., Radiology 1994; 191: 225-230, is dissolved with bidistilled water to 10 ml. The solution is mixed with 4.1 mg of sodium periodate with stirring and cooling in an ice bath. After 20 minutes, 5 ml of ethylene glycol: water 1:1 (v/v) is added and purified by a YM-300-membrane (Amicon) by ultrafiltration. The solution is reacted with 1.8 mg of the spacer obtained according to Example A with stirring at room temperature. After 30 minutes, it is mixed with 62 mg of sodium cyanoborohydride and the solution is purified after another 30 minutes by ultrafiltration. It is then adjusted to pH 8.5 by adding 0.1 N sodium hydroxide solution and reacted for 2 hours at room temperature under argon protective gassing with 3.5 mg of the 35-mer oligonucleotide obtained according to Example C. After another ultrafiltration, the solution is adjusted to a concentration of 20 mmol/l of iron and then sterilized by filtration.

The analysis of the lyophilizate provides the following characteristic values:

Determination	Result	Method
Iron	20 mmol/l	ICP-AES
glucose equivalent	0.48 mg/ml	anthrone assay
oligonucleotide	0.33 mg/ml	P-determination
nuclear size	4.0-5.5 nm	electron microscope (TIM)
total value	20 nm	laser light scattering
susceptibility	54.5 emu/g Fe	magnetic scales MSB auto; Johnson Matthey; Germany; 4.5 kGauss; 26°C
loading factor m	30 oligonucleotides per ferrite particle	
relaxivity	T1 = 20 l/m Molxs T2 = 48	Bruker Minispec pc 120; 0.47 T; water: 39 ± 1°C
radioactivity	23 µCi/µmol of FE	gamma counter

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

5 From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

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What is claimed is:

1. A conjugate, consisting of an oligonucleotide bonding specifically with high affinity to a target structure, containing a modification significantly limiting the degradation by naturally occurring nucleases, reduced by one hydroxyl group in 3′-position or in 5′-position or by one hydroxymethyl group in 4′-position, and a chemically modified, optionally radioactively-labeled ferrite sheathed with a coating agent.

2. A conjugate according to claim 1 of formula I:

$$M\text{-}(B\text{-}L\text{-}A\text{-}PN)_m \qquad (I)$$

wherein

PN is an oligonucleotide bonding specifically with high affinity to target structures, containing modifications significantly limiting the degradation by naturally occurring nucleases, reduced by one hydroxyl group in 3′-position or in 5′-position or by one hydroxymethyl group in 4′-position,

B is a bonding component X-Y-Z,
wherein

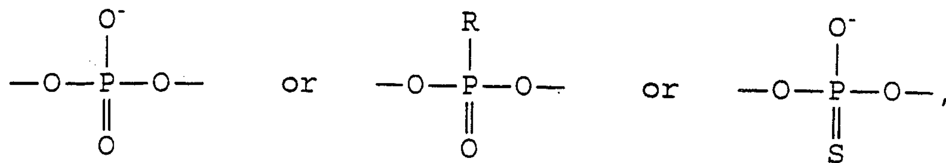
X is a direct bond or a group -NH or -NR,
wherein

R is a C1 to C4 alkyl chain,

Y a direct bond or a spacer and Z means a direct bond or a sulfur atom,

L means a linker,

A is a group bound on the terminal 3′- or 5′-position,



or a carbonyl group bound on the terminal 4′-position,

M is a chemically modified, optionally radioactively-labeled ferrite coated with a polysaccharide, and

m is a number between 1 and 100,

provided that at least one of groups X, Y and Z does not stand for a direct bond.

3. A compound according to claim 1, wherein PN is an oligonucleotide with 5 to 200 nucleotides, and wherein

a) the 2′-position of the sugar units, independently of one another, is occupied with the following groups:

-- an optional hydroxyl group, an optional group OR^2, in which R^2 means an alkyl radical with 1-20 carbon atoms, which optionally contains up to 2 hydroxyl groups and which optionally is interrupted by 1-5 oxygen atoms,

a hydrogen atom,

a fluorine atom,

an amine radical,

an amino group

and hydroxyl groups present in 3′- and 5′-positions optionally are etherified with radical R^2,

and/or

b) the phosphodiesters, independently of one another, being used as internucleotide bond, are replaced by phosphorothioates, phosphorodithioates or methyl phosphonates,

and/or

c) it contains an internucleotide bond as described in b), which links the 3′-3′- or 5′-5′-positions

and/or

d) it contains a phosphodiester bond as described in b), which connects, ester-like, two thymidines via a C_2-C_{20} hydroxyalkyl radical present in 3-position or connects, ester-like, an analogously-substituted thymidine radical with a hydroxyl group of another sugar in 2'- or 3'- or 5'-position,

and/or

e) the terminal radicals in 3'- and 5'-positions optionally contain modified internucleotide bonds as described in b), which connect up to 5 thymidines.

4. A compound according to claim 3, wherein oligonucleotide PN comprises 15 to 100 nucleotides.

5. A compound according to claim 1, wherein PN is an oligonucleotide, which bonds specifically with high bonding affinity to other target structures and which can be obtained in that a mixture of oligonucleotides, containing random sequences, is brought together with the target structure, certain oligonucleotides exhibiting an increased affinity to the target structure relative to the mixture of oligonucleotides, the latter being separated from the remainder of the oligonucleotide mixture, then the oligonucleotides with increased affinity to the target structure being amplified to obtain a mixture of oligonucleotides, which exhibits an increased portion of oligonucleotides, which bond on the target structures.

6. A compound according to claim 1, wherein PN is an oligonucleotide, which bonds specifically with high bonding affinity to other target structures and which can be obtained by a process comprising

a) producing a DNA strand by chemical synthesis, wherein said DNA strand exhibits a defined sequence on the 3'-end, which is complementary to a promoter for an RNA polymerase and at the same time complementary to a primer of the polymerase chain reaction

(PCR) and wherein said DNA strand exhibits a defined DNA sequence on the 5'-end, which is complementary to a primer sequence for the polymerase chain reaction, the sequence containing a random sequence between the defined sequences,

b) transferring said DNA strand with the help of an RNA polymerase into a complimentary RNA strand, wherein nucleotides that are modified in 2'-position of the ribose unit are supplied to the polymerase,

c) bring together the RNA oligonucleotides produced in this way with the target structure on which the oligonucleotide is to bond specifically,

d) separating those oligonucleotides that have bonded on the target structure, together with the target structure from the nonbonding oligonucleotides, and again separating the bound oligonucleotides from the target structure,

e) transferring said target-structure-specific RNA oligonucleotides with the help of reverse transcriptase to a complementary DNA strand,

f) amplifying said DNA strands by using the defined primer sequences with the polymerase chain reaction,

g) again transferring the DNA oligonucleotides amplified in this way, with the help of the RNA polymerase and with modified nucleotides into RNA oligonucleotide,

h) optionally repeating above-mentioned selection steps c) to g) frequently until the oligonucleotides, which are characterized by a high bonding affinity to the target structure, are sufficiently selected, and then optionally determining the sequences of the thus obtained oligonucleotides.

7. A compound according to claim 6, wherein the target structure is selected from among macromolecules, tissue structures of higher organisms such as animals or humans, organs or parts of organs of an animal or human, cells, tumor cells or tumors.

8. A conjugate according to claim 2, containing a chemically modified dextran-magnetite as ferrite M.

9. A conjugate according to claim 2, wherein Y in bonding component B means a spacer, which contains a straight-chain or branched, saturated or unsaturated C1 to C200 alkylene chain, preferably C1 to C50 alkylene chain, which optionally contains 1 to 10 imino, preferably 1 to 5 imino, 1 to 3 phenylene, 1 to 3 phenylenoxy, 1 to 10 amido, 1 to 2 hydrazido, 1 to 10 carbonyl, 1 succinimido, 1 6-ethylpyridin-2-yl groups, and which optionally is interrupted by 1 to 60 oxygen atoms and/or 1 to 5 sulfur atoms and which optionally is substituted by 1 to 5 hydroxy, 1 to 10 oxo, 1 to 3 carboxy, 1 to 5 carboxy-C1 to C4 alkyl, 1 to 3 hydroxy-C1 to C4 alkyl, 1 to 3 C1 to C7 alkoxy and/or 1 to 2 phenyl groups, which optionally are substituted by a carboxy, a cyano, a nitro, a sulfonyl and/or an acetyl group.

10. A conjugate according to claim 2, wherein Y and Z each stand for a direct bond, and X stands for NH or NR.

11. A conjugate according to claim 2, wherein X stands for -NH or NR, Y stands for a spacer and Z stands for a sulfur atom.

12. A conjugate according to claim 2, wherein X stands for a direct bond, Y stands for a spacer and Z stands for a sulfur atom.

13. A conjugate according to claim 2, wherein linker L is a straight-chain or branched, saturated or unsaturated C1 to C20 alkylene chain, which optionally contains 1 to 3 imino, 1 to 3 oxo, one phenylene or one phenylenoxy group and which optionally is interrupted by 1 to 6 oxygen atoms and/or 1 to 3 sulfur atoms.

14. A conjugate according to claim 2, containing Fe-57, Ga-67, Y-90, Tc-99m, In-111, I-123, Tl-201, Yb-169 as a radioisotope.

15. A process for the production of a compound of formula I

$$M\text{-}(B\text{-}L\text{-}A\text{-}PN)_m \qquad (I)$$

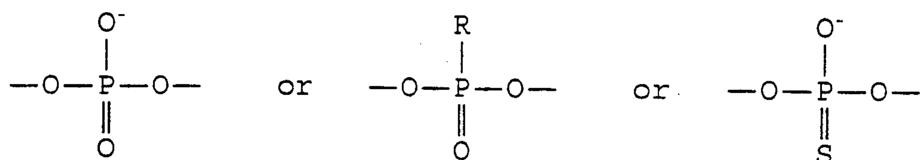
wherein

PN is an oligonucleotide bonding specifically with high affinity to target structures, containing modifications significantly limiting the degradation by naturally occurring nucleases, reduced by one hydroxyl group in the terminal 3'- or 5'-position, or by one hydroxymethyl group in the terminal 4'-position,

B is a bonding component X-Y-Z,
wherein
X is a direct bond or a group -NH or -NR,
wherein
R is a C1 to C4 alkyl chain,
Y is a direct bond or a spacer and Z is a direct bond or a sulfur atom,

L is a linker,

A is a group bound on 3'- or 5'-position



or a carbonyl group bound on 4'-position,

M is a chemically modified, optionally radio-actively-labeled ferrite coated with a poly-saccharide, and

m is a number between 1 and 100,

provided that at least one of groups X, Y and Z does not stand for a direct bond,

wherein in a way known in the art, compounds of general formula II

$$M\text{-}(X\text{-}Y'\text{-}E)_m \qquad (II),$$

wherein

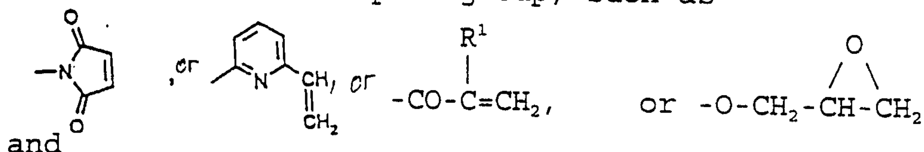
a) X and m have the above-mentioned meaning and

E is a reactive group

E^2 is a leaving group such as F, Br, Cl, OTs or OMes and

Y′ has the same meaning as Y or

E^2 is an acceptor group, such as



and

Y′ is spacer Y reduced by E2-H, and

R^1 is for hydrogen, C1 to C10 alkyl or for phenyl optionally substituted with $-OCH_3$, -CN, $-SO_2R$, -COCH, $-NO_2$, -Cl, $-CO_2H$, $-OCH_3$, $-CF_3$,

are reacted with m equivalents of a compound of general formula III

H,S-L-A-PN (III),

wherein L, A and PN have the above-mentioned meaning or wherein

b) X and Y each respectively are a direct bond and

E is a functional group

E^3 is a formyl, carboxyl, carboxylalkyl or glycidyl group,

with m equivalents of a compound of general formula X

HX-L-A-PN (XI),

wherein

X is an -NH or -NR group with R in the above-mentioned meaning and L, A and PN have the above-mentioned meaning,

and in the case of reaction of formyl groups with aldehyde groups, a reduction step is necessary.

16. An agent containing at least one conjugate according to claim 1, optionally with the additives usual in galenicals.

17. A diagnostic agent, containing at least one conjugate according to claim 1, optionally with the additives usual in galenicals for NMR imaging.

18. An agent, containing at least one conjugate according to claim 14, optionally with the additives usual in galenicals, for radiodiagnosis.

19. An agent, containing at least one conjugate according to claim 14, optionally with the additives usual in galenicals, for radiotherapy.

20. A method of NMR diagnosis, comprising administering at least one physiologically compatible conjugate according to claim 1.

21. A method of radiodiagnosis, comprising administering at least one physiologically compatible conjugate according to claim 14.

22. A method of radiotherapy, comprising administering at least one physiologically compatible conjugate according to claim 14.

23. A conjugate according to claim 1, wherein N is a non-naturally occuring oligonucleotide ligand having a specific binding affinity for a target molecule, such target molecule being a three dimensional chemical structure other than a polynucleotide that binds to said oligonucleotide ligand through a mechanism which predominantly depends on Watson/Crick base pairing or triple helix binding, wherein said oligonucleotide ligand is not a nucleic acid having the known physiological function of being bound by the target molecule.