Antisense-Oligonucleotides as Inhibitors of TGF-R signaling

Specification

The present invention relates to antisense-oligonucleotides, their use as inhibitors of TGF-R signaling, pharmaceutical compositions containing such antisense-oligonucleotides and the use for prophylaxis and treatment of neurological, neurodegenerative and hyperproliferative including oncological diseases.

TGF-β exists in three known subtypes in humans, TGF-β1, TGF-β2, and TGF-β3. These are upregulated in neurodegenerative diseases, such as ALS, and some human cancers, and increased expression of this growth factor in pathological conditions of neurodegenerative diseases, acute trauma, and neuro-inflammation and ageing has been demonstrated. Isoforms of transforming growth factor-beta (TGF-β1) are also thought to be involved in the pathogenesis of pre-eclampsia.

Activated TGF-βs exert their effects on the target cell via three different receptor classes: type I (TGFRI), also termed activin-like kinases (ALK; 53 kDa), type II (TGFRII; 70-100 kDa), and type III (TGFRIII; 200–400 kDa. TGF-β receptors are single pass serine/threonine kinase receptors. Whereas type II receptor kinase is constitutively active, type I receptor needs to be activated. This process is initiated through binding of a ligand to TGFRII; this triggers the transient formation of a complex that includes the ligand and receptor types I and II. Taking into account the dimeric composition of the ligand, the receptor complex most likely consists of a tetrameric structure formed by two pairs of each receptor type.

TGF-β signal transduction takes place through its receptors and downstream through Smad proteins. Smad-dependent cellular signal transduction initiated by binding of the TGF-β isoform to a specific TGFRI/II receptor pair, leads to the phosphorylation of intracellular Smads and subsequently the translocation of an activated Smad complex into the nucleus in order to influence specific target gene expression. Signal divergence into other pathways and convergence from neighboring signaling pathways generate a highly complex network. Depending on the environmental and cellular context, TGF-beta signaling results in a variety of different cellular responses such as cellular proliferation, differentiation, motility, and apoptosis in tumor cells. In cancer, TGF-β can affect tumor growth directly (referred to as intrinsic effect of TGF-β signaling) or indirectly (referred to as extrinsic effect) by promoting tumor growth,

inducing epithelial-mesenchymal transition (EMT), blocking antitumor immune responses, increasing tumor-associated fibrosis, modulating extracellular matrix (ECN) and cell migration, and finally enhancing angiogenesis. The factors (e.g. concentration, timing, local exposure) determining whether TGF-β signaling has a tumor promoter or suppressor function are a matter of intense research and discussion. Currently, it is postulated that the tumor suppressor function of TGF-β signaling is lost in early stages of cancer similar to recessive loss-of-function mutations in other tumor suppressors. Therefore there are several pharmacological approaches for treatment of divers cancers by blocking TGF-beta signaling pathways, such as investigation of Galunisertib and TEW-7197, both are small molecule inhibitor of TGFRI and being in clinical investigation, and LY3022859, an antibody against TGFRII.

Signals provided by proteins of the transforming growth factor (TGF-β) family represent a system by which neural stem cells are controlled under physiological conditions but in analogy to other cell types are released from this control after transformation to cancer stem cells. TGF-β is a multifunctional cytokine involved in various physiological and patho-physiological processes of the brain. It is induced in the adult brain after injury or hypoxia and during neurodegeneration when it modulates and dampens inflammatory responses. After injury, although TGF-β is in general neuroprotective, it limits the self-repair of the brain by inhibiting neural stem cell proliferation and inducing fibrosis / gliosis for scar formation. Similar to its effect on neural stem cells, TGF-β reveals anti-proliferative control on most cell types; however, paradoxically, many tumors escape from TGF-β control. Moreover, these tumors develop mechanisms that change the anti-proliferative influence of TGF-β into oncogenic cues, mainly by orchestrating a multitude of TGF-β -mediated effects upon matrix, migration and invasion, angiogenesis, and, most importantly, immune escape mechanisms. Thus, TGF-β is involved in tumor progression (see Figure 3).

Consequently, the TGF Receptor II (transforming growth factor, beta receptor II; synonymously used symbols: TGF-beta type II receptor, TGFBR2 ; AAT3; FAA3; LDS1B; LDS2; LDS2B; MFS2; RIIC; TAAD2; TGFR-2; TGFbeta-RII, TGF-RII, TGF-R_{II}), and in particular its inhibition, was validated as target for the treatment of neurodegenerative diseases, such as ALS, and hyperproliferative diseases such as cancer and fibrotic dieases.

Thus objective of the present application is to provide pharmaceutically active compounds able inhibit expression of the TGF Receptor II (TGF-R_{II}) and therefore,

reduce the amount of TGF Receptor II (TGF-R_{II}) and decrease the activity of TGF-β downstream signaling.

The objective of the present invention is solved by the teaching of the independent claims. Further advantageous features, aspects and details of the invention are evident from the dependent claims, the description, the figures, and the examples of the present application.

Surprisingly under thousands of candidate substances, such as protein-nucleotide complexes, siRNA, microRNA (miRNA), ribozymes, aptamers, CpG-oligos, DNA-zymes, riboswitches, lipids, peptides, small molecules, modifyers of rafts or caveoli, modifyers of golgi apparatus, antibodies and their derivatives, especially chimeras, Fab-fragments, and Fc-fragments, antisense-oligonucleotides containing LNAs (LNA®: Locked Nucleic Acids) were found the most promising candidates for the uses disclosed herein.

Thus, the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **TGGTCCATTC** (Seq. ID No. 4) or the sequence **CCCTAAACAC** (Seq. ID No. 5) or the sequence **ACTACCAAAT** (Seq. ID No. 6) or the sequence **GGACGCGTAT** (Seq. ID No. 7) or the sequence **GTCTATGACG** (Seq. ID No. 8) or the sequence **TTATTAATGC** (Seq. ID No. 9), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **TGGTCCATTC** (Seq. ID No. 4) or sequence **CCCTAAACAC** (Seq. ID No. 5) or sequence **ACTACCAAAT** (Seq. ID No. 6) or sequence **GGACGCGTAT** (Seq. ID No. 7) or sequence **GTCTATGACG** (Seq. ID No. 8) or sequence **TTATTAATGC** (Seq. ID No. 9) respectively and salts and optical isomers of said antisense-oligonucleotide.

Slightly reworded the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of **the open reading frame of** the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of **the open reading frame of** the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **TGGTCCATTC** (Seq. ID No. 4) or the sequence **CCCTAAACAC** (Seq. ID No. 5) or the sequence **ACTACCAAAT** (Seq. ID No. 6) or the sequence **GGACGCGTAT** (Seq. ID No. 7) or the sequence **GTCTATGACG** (Seq. ID No. 8) or

the sequence **TTATTAATGC** (Seq. ID No. 9), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **TGGTCCATTC** (Seq. ID No. 4) or sequence **CCCTAAACAC** (Seq. ID No. 5) or sequence **ACTACCAAAT** (Seq. ID No. 6) or sequence **GGACGCGTAT** (Seq. ID No. 7) or sequence **GTCTATGACG** (Seq. ID No. 8) or sequence **TTATTAATGC** (Seq. ID No. 9) respectively and salts and optical isomers of said antisense-oligonucleotide.

Alternatively the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **TGGTCCATTC** (Seq. ID No. 4) or the sequence **CCCTAAACAC** (Seq. ID No. 5) or the sequence **ACTACCAAAT** (Seq. ID No. 6) or the sequence **GGACGCGTAT** (Seq. ID No. 7) or the sequence **GTCTATGACG** (Seq. ID No. 8) or the sequence **TTATTAATGC** (Seq. ID No. 9), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **TGGTCCATTC** (Seq. ID No. 4) or the sequence **CCCTAAACAC** (Seq. ID No. 5) or the sequence **ACTACCAAAT** (Seq. ID No. 6) or the sequence **GGACGCGTAT** (Seq. ID No. 7) or the sequence **GTCTATGACG** (Seq. ID No. 8) or the sequence **TTATTAATGC** (Seq. ID No. 9) respectively and salts and optical isomers of said antisense-oligonucleotide.

Slightly reworded the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of **the open reading frame of** the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of **the open reading frame of** the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **TGGTCCATTC** (Seq. ID No. 4) or the sequence **CCCTAAACAC** (Seq. ID No. 5) or the sequence **ACTACCAAAT** (Seq. ID No. 6) or the sequence **GGACGCGTAT** (Seq. ID No. 7) or the sequence **GTCTATGACG** (Seq. ID No. 8) or the sequence **TTATTAATGC** (Seq. ID No. 9), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **TGGTCCATTC** (Seq. ID No. 4) or the sequence **CCCTAAACAC** (Seq. ID No. 5) or the sequence **ACTACCAAAT** (Seq. ID No. 6) or the sequence **GGACGCGTAT** (Seq. ID No. 7) or the sequence **GTCTATGACG** (Seq. ID No. 8) or the sequence **TTATTAATGC** (Seq. ID No. 9) respectively and salts and optical isomers of said antisense-oligonucleotide.

Preferably the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **TGGTCCATTC** (Seq. ID No. 4), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **TGGTCCATTC** (Seq. ID No. 4) and salts and optical isomers of said antisense-oligonucleotide.

Slightly reworded the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of **the open reading frame of** the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of **the open reading frame of** the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **TGGTCCATTC** (Seq. ID No. 4), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **TGGTCCATTC** (Seq. ID No. 4) and salts and optical isomers of said antisense-oligonucleotide.

Alternatively the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **TGGTCCATTC** (Seq. ID No. 4), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **TGGTCCATTC** (Seq. ID No. 4) and salts and optical isomers of said antisense-oligonucleotide.

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Preferably the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **CCCTAAACAC** (Seq. ID No. 5), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **CCCTAAACAC** (Seq. ID No. 5) and salts and optical isomers of said antisense-oligonucleotide.

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Alternatively the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **CCCTAAACAC** (Seq. ID No. 5), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **CCCTAAACAC** (Seq. ID No. 5) and salts and optical isomers of said antisense-oligonucleotide.

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Preferably the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **ACTACCAAAT** (Seq. ID No. 6), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **ACTACCAAAT** (Seq. ID No. 6) and salts and optical isomers of said antisense-oligonucleotide.

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Preferably the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **GGACGCGTAT** (Seq. ID No. 7), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **GGACGCGTAT** (Seq. ID No. 7) and salts and optical isomers of said antisense-oligonucleotide.

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Slightly reworded consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of **the open reading frame of** the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of **the open reading frame of** the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **GGACGCGTAT** (Seq. ID No. 7), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **GGACGCGTAT** (Seq. ID No. 7) and salts and optical isomers of said antisense-oligonucleotide.

Preferably the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **GTCTATGACG** (Seq. ID No. 8), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **GTCTATGACG** (Seq. ID No. 8) and salts and optical isomers of said antisense-oligonucleotide.

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Preferably the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **TTATTAATGC** (Seq. ID No. 9), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **TTATTAATGC** (Seq. ID No. 9) and salts and optical isomers of said antisense-oligonucleotide.

Slightly reworded the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of **the open reading frame of** the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of **the open reading frame of** the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **TTATTAATGC** (Seq. ID No. 9), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **TTATTAATGC** (Seq. ID No. 9) and salts and optical isomers of said antisense-oligonucleotide.

Alternatively the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **TTATTAATGC** (Seq. ID No. 9), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **TTATTAATGC** (Seq. ID No. 9) and salts and optical isomers of said antisense-oligonucleotide.

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The antisense-oligonucleotides of the present invention preferably comprise 2 to 10 LNA units, more preferably 3 to 9 LNA units and still more preferably 4 to 8 LNA units and also preferably at least 6 non-LNA units, more preferably at least 7 non-LNA units and most preferably at least 8 non-LNA units. The non-LNA units are preferably DNA units. The LNA units are preferably positioned at the 3' terminal end (also named 3' terminus) and the 5' terminal end (also named 5' terminus). Preferably at least one and more preferably at least two LNA units are present at the 3' terminal end and/or at the 5' terminal end.

Thus, preferred are antisense-oligonucleotides of the present invention which contain 3 to 10 LNA units and which especially contain 1 to 5 LNA units at the 5' terminal end and 1 to 5 LNA units at the 3' terminal end of the antisense-oligonucleotide and between the LNA units at least 7 and more preferably at least 8 DNA units.

Moreover, the antisense-oligonucleotides may contain common nucleobases such as adenine, guanine, cytosine, thymine and uracil as well as common derivatives thereof. The antisense-oligonucleotides of the present invention may also contain modified internucleotide bridges such as phosphorothioate or phosphorodithioate instead of phosphate bridges. Such modifications may be present only in the LNA segments or only in the non-LNA segment of the antisense-oligonucleotide.

Thus, the present invention is also directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **CTGGTCCATTC** (Seq. ID No. 296), **TGGTCCATTCA** (Seq. ID No. 297), **CTGGTCCATTCA** (Seq. ID No. 298), **TCCCTAAACAC** (Seq. ID No. 299), **CCCTAAACACT** (Seq. ID No. 300), **TCCCTAAACACT** (Seq. ID No. 301), **CACTACCAAAT** (Seq. ID No. 302), **ACTACCAAATA** (Seq. ID No. 303), **CACTACCAAATA** (Seq. ID No. 304), **TGGACGCGTAT** (Seq. ID No. 305), **GGACGCGTATC** (Seq. ID No. 306), **TGGACGCGTATC** (Seq. ID No. 307), **GGTCTATGACG** (Seq. ID No. 308), **GTCTATGACGA** (Seq. ID No. 309), **GGTCTATGACGA** (Seq. ID No. 310), **TTTATTAATGC** (Seq. ID No. 311), **TTATTAATGCC** (Seq. ID No. 312), or **TTTATTAATGCC** (Seq. ID No. 313), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **CTGGTCCATTC** (Seq. ID No. 296), **TGGTCCATTCA** (Seq. ID No. 297), **CTGGTCCATTCA** (Seq. ID No. 298),

TCCCTAAACAC (Seq. ID No. 299), **CCCTAAACACT** (Seq. ID No. 300), **TCCCTAAACACT** (Seq. ID No. 301), **CACTACCAAAT** (Seq. ID No. 302), **ACTACCAAATA** (Seq. ID No. 303), **CACTACCAAATA** (Seq. ID No. 304), **TGGACGCGTAT** (Seq. ID No. 305), **GGACGCGTATC** (Seq. ID No. 306), **TGGACGCGTATC** (Seq. ID No. 307), **GGTCTATGACG** (Seq. ID No. 308), **GTCTATGACGA** (Seq. ID No. 309), **GGTCTATGACGA** (Seq. ID No. 310), **TTTATTAATGC** (Seq. ID No. 311), **TTATTAATGCC** (Seq. ID No. 312), or **TTTATTAATGCC** (Seq. ID No. 313) respectively and salts and optical isomers of said antisense-oligonucleotide.

Alternatively the present invention is directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **CTGGTCCATTC** (Seq. ID No. 296), **TGGTCCATTCA** (Seq. ID No. 297), **CTGGTCCATTCA** (Seq. ID No. 298), **TCCCTAAACAC** (Seq. ID No. 299), **CCCTAAACACT** (Seq. ID No. 300), **TCCCTAAACACT** (Seq. ID No. 301), **CACTACCAAAT** (Seq. ID No. 302), **ACTACCAAATA** (Seq. ID No. 303), **CACTACCAAATA** (Seq. ID No. 304), **TGGACGCGTAT** (Seq. ID No. 305), **GGACGCGTATC** (Seq. ID No. 306), **TGGACGCGTATC** (Seq. ID No. 307), **GGTCTATGACG** (Seq. ID No. 308), **GTCTATGACGA** (Seq. ID No. 309), **GGTCTATGACGA** (Seq. ID No. 310), **TTTATTAATGC** (Seq. ID No. 311), **TTATTAATGCC** (Seq. ID No. 312), or **TTTATTAATGCC** (Seq. ID No. 313), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **CTGGTCCATTC** (Seq. ID No. 296), **TGGTCCATTCA** (Seq. ID No. 297), **CTGGTCCATTCA** (Seq. ID No. 298), **TCCCTAAACAC** (Seq. ID No. 299), **CCCTAAACACT** (Seq. ID No. 300), **TCCCTAAACACT** (Seq. ID No. 301), **CACTACCAAAT** (Seq. ID No. 302), **ACTACCAAATA** (Seq. ID No. 303), **CACTACCAAATA** (Seq. ID No. 304), **TGGACGCGTAT** (Seq. ID No. 305), **GGACGCGTATC** (Seq. ID No. 306), **TGGACGCGTATC** (Seq. ID No. 307), **GGTCTATGACG** (Seq. ID No. 308), **GTCTATGACGA** (Seq. ID No. 309), **GGTCTATGACGA** (Seq. ID No. 310), **TTTATTAATGC** (Seq. ID No. 311), **TTATTAATGCC** (Seq. ID No. 312), or **TTTATTAATGCC** (Seq. ID No. 313) respectively and salts and optical isomers of said antisense-oligonucleotide.

Preferably, the present invention is also directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are

LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **CTGGTCCATTC** (Seq. ID No. 296), **TGGTCCATTCA** (Seq. ID No. 297), or **CTGGTCCATTCA** (Seq. ID No. 298), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **CTGGTCCATTC** (Seq. ID No. 296), **TGGTCCATTCA** (Seq. ID No. 297), or **CTGGTCCATTCA** (Seq. ID No. 298) respectively and salts and optical isomers of said antisense-oligonucleotide.

Slightly reworded, the present invention is also directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **CTGGTCCATTC** (Seq. ID No. 296), **TGGTCCATTCA** (Seq. ID No. 297), or **CTGGTCCATTCA** (Seq. ID No. 298), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **CTGGTCCATTC** (Seq. ID No. 296), **TGGTCCATTCA** (Seq. ID No. 297), or **CTGGTCCATTCA** (Seq. ID No. 298) respectively and salts and optical isomers of said antisense-oligonucleotide.

Preferably, the present invention is also directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **TCCCTAAACAC** (Seq. ID No. 299), **CCCTAAACACT** (Seq. ID No. 300), or **TCCCTAAACACT** (Seq. ID No. 301), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **TCCCTAAACAC** (Seq. ID No. 299), **CCCTAAACACT** (Seq. ID No. 300), or **TCCCTAAACACT** (Seq. ID No. 301) respectively and salts and optical isomers of said antisense-oligonucleotide.

Slightly reworded, the present invention is also directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the

mRNA encoding the TGF-R_{II} comprises the sequence **TCCCTAAACAC** (Seq. ID No. 299), **CCCTAAACACT** (Seq. ID No. 300), or **TCCCTAAACACT** (Seq. ID No. 301), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **TCCCTAAACAC** (Seq. ID No. 299), **CCCTAAACACT** (Seq. ID No. 300), or **TCCCTAAACACT** (Seq. ID No. 301) respectively and salts and optical isomers of said antisense-oligonucleotide.

Preferably, the present invention is also directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **CACTACCAAAT** (Seq. ID No. 302), **ACTACCAAATA** (Seq. ID No. 303), or **CACTACCAAATA** (Seq. ID No. 304), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **CACTACCAAAT** (Seq. ID No. 302), **ACTACCAAATA** (Seq. ID No. 303), or **CACTACCAAATA** (Seq. ID No. 304) respectively and salts and optical isomers of said antisense-oligonucleotide.

Slightly reworded, the present invention is also directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **CACTACCAAAT** (Seq. ID No. 302), **ACTACCAAATA** (Seq. ID No. 303), or **CACTACCAAATA** (Seq. ID No. 304), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **CACTACCAAAT** (Seq. ID No. 302), **ACTACCAAATA** (Seq. ID No. 303), or **CACTACCAAATA** (Seq. ID No. 304) respectively and salts and optical isomers of said antisense-oligonucleotide.

Preferably, the present invention is also directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **TGGACGCGTAT** (Seq. ID No. 305), **GGACGCGTATC** (Seq. ID No. 306), or **TGGACGCGTATC** (Seq. ID No. 307), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said

sequence **TGGACGCGTAT** (Seq. ID No. 305), **GGACGCGTATC** (Seq. ID No. 306), or **TGGACGCGTATC** (Seq. ID No. 307) respectively and salts and optical isomers of said antisense-oligonucleotide.

Slightly reworded, the present invention is also directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **TGGACGCGTAT** (Seq. ID No. 305), **GGACGCGTATC** (Seq. ID No. 306), or **TGGACGCGTATC** (Seq. ID No. 307), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **TGGACGCGTAT** (Seq. ID No. 305), **GGACGCGTATC** (Seq. ID No. 306), or **TGGACGCGTATC** (Seq. ID No. 307) respectively and salts and optical isomers of said antisense-oligonucleotide.

Preferably, the present invention is also directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **GGTCTATGACG** (Seq. ID No. 308), **GTCTATGACGA** (Seq. ID No. 309), or **GGTCTATGACGA** (Seq. ID No. 310), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **GGTCTATGACG** (Seq. ID No. 308), **GTCTATGACGA** (Seq. ID No. 309), or **GGTCTATGACGA** (Seq. ID No. 310) respectively and salts and optical isomers of said antisense-oligonucleotide.

Slightly reworded, the present invention is also directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **GGTCTATGACG** (Seq. ID No. 308), **GTCTATGACGA** (Seq. ID No. 309), or **GGTCTATGACGA** (Seq. ID No. 310), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **GGTCTATGACG** (Seq. ID No. 308), **GTCTATGACGA** (Seq. ID No. 309), or **GGTCTATGACGA** (Seq. ID No. 310) respectively and salts and optical isomers of said antisense-oligonucleotide.

Preferably, the present invention is also directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **TTTATTAATGC** (Seq. ID No. 311), **TTATTAATGCC** (Seq. ID No. 312), or **TTTATTAATGCC** (Seq. ID No. 313), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **TTTATTAATGC** (Seq. ID No. 311), **TTATTAATGCC** (Seq. ID No. 312), or **TTTATTAATGCC** (Seq. ID No. 313) respectively and salts and optical isomers of said antisense-oligonucleotide.

Slightly reworded, the present invention is also directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **TTTATTAATGC** (Seq. ID No. 311), **TTATTAATGCC** (Seq. ID No. 312), or **TTTATTAATGCC** (Seq. ID No. 313), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **TTTATTAATGC** (Seq. ID No. 311), **TTATTAATGCC** (Seq. ID No. 312), or **TTTATTAATGCC** (Seq. ID No. 313) respectively and salts and optical isomers of said antisense-oligonucleotide.

The antisense-oligonucleotides of the present invention preferably comprise 3 to 10 LNA units, more preferably 3 to 9 LNA units and still more preferably 4 to 8 LNA units and also preferably at least 6 non-LNA units, more preferably at least 7 non-LNA units and most preferably at least 8 non-LNA units. The non-LNA units are preferably DNA units. The LNA units are preferably positioned at the 3' terminal end (also named 3' terminus) and the 5' terminal end (also named 5' terminus). Preferably at least one and more preferably at least two LNA units are present at the 3' terminal end and/or at the 5' terminal end.

Thus, preferred are antisense-oligonucleotides of the present invention which contain 3 to 10 LNA units and which especially contain 1 to 5 LNA units at the 5' terminal end and 1 to 5 LNA units at the 3' terminal end of the antisense-oligonucleotide and between the LNA units at least 7 and more preferably at least 8 DNA units.

Moreover, the antisense-oligonucleotides may contain common nucleobases such as adenine, guanine, cytosine, thymine and uracil as well as common derivatives thereof. The antisense-oligonucleotides of the present invention may also contain

modified internucleotide bridges such as phosphorothioate or phosphorodithioate instead of phosphate bridges. Such modifications may be present only in the LNA segments or only in the non-LNA segment of the antisense-oligonucleotide.

Thus, the present invention is also directed to antisense-oligonucleotide(s) consisting of 14 to 20 more preferably 14 to 18 nucleotides and at least four of the 14 to 20 more preferably 14 to 18 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **ACTGGTCCATTC** (Seq. ID No. 314), **TGGTCCATTCAT** (Seq. ID No. 315), **CTGGTCCATTCAT** (Seq. ID No. 316), **ACTGGTCCATTCA** (Seq. ID No. 317), **ACTGGTCCATTCAT** (Seq. ID No. 318), **CTCCCTAAACAC** (Seq. ID No. 319), **CCCTAAACACTA** (Seq. ID No. 320), **TCCCTAAACACTA** (Seq. ID No. 321), **CTCCCTAAACACT** (Seq. ID No. 322), **CTCCCTAAACACTA** (Seq. ID No. 323), **ACACTACCAAAT** (Seq. ID No. 324), **ACTACCAAATAG** (Seq. ID No. 325), **CACTACCAAATAG** (Seq. ID No. 326), **ACACTACCAAATA** (Seq. ID No. 327), **ACACTACCAAATAG** (Seq. ID No. 328), **GTGGACGCGTAT** (Seq. ID No. 329), **GGACGCGTATCG** (Seq. ID No. 330), **TGGACGCGTATCG** (Seq. ID No. 331), **GTGGACGCGTATC** (Seq. ID No. 332), **GTGGACGCGTATCG** (Seq. ID No. 333), **CGGTCTATGACG** (Seq. ID No. 334), **GTCTATGACGAG** (Seq. ID No. 335), **GGTCTATGACGAG** (Seq. ID No. 336), **CGGTCTATGACGA** (Seq. ID No. 337), **CGGTCTATGACGAG** (Seq. ID No. 338), **CTTTATTAATGC** (Seq. ID No. 339), **TTATTAATGCCT** (Seq. ID No. 340), **TTTATTAATGCCT** (Seq. ID No. 341), **CTTTATTAATGCC** (Seq. ID No. 342), or **CTTTATTAATGCCT** (Seq. ID No. 343), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **ACTGGTCCATTC** (Seq. ID No. 314), **TGGTCCATTCAT** (Seq. ID No. 315), **CTGGTCCATTCAT** (Seq. ID No. 316), **ACTGGTCCATTCA** (Seq. ID No. 317), **ACTGGTCCATTCAT** (Seq. ID No. 318), **CTCCCTAAACAC** (Seq. ID No. 319), **CCCTAAACACTA** (Seq. ID No. 320), **TCCCTAAACACTA** (Seq. ID No. 321), **CTCCCTAAACACT** (Seq. ID No. 322), **CTCCCTAAACACTA** (Seq. ID No. 323), **ACACTACCAAAT** (Seq. ID No. 324), **ACTACCAAATAG** (Seq. ID No. 325), **CACTACCAAATAG** (Seq. ID No. 326), **ACACTACCAAATA** (Seq. ID No. 327), **ACACTACCAAATAG** (Seq. ID No. 328), **GTGGACGCGTAT** (Seq. ID No. 329), **GGACGCGTATCG** (Seq. ID No. 330), **TGGACGCGTATCG** (Seq. ID No. 331), **GTGGACGCGTATC** (Seq. ID No. 332), **GTGGACGCGTATCG** (Seq. ID No. 333), **CGGTCTATGACG** (Seq. ID No. 334), **GTCTATGACGAG** (Seq. ID No. 335), **GGTCTATGACGAG** (Seq. ID No. 336), **CGGTCTATGACGA** (Seq. ID No. 337), **CGGTCTATGACGAG** (Seq. ID No. 338), **CTTTATTAATGC** (Seq. ID No. 339),

TTATTAATGCCT (Seq. ID No. 340), **TTTATTAATGCCT** (Seq. ID No. 341), **CTTTATTAATGCC** (Seq. ID No. 342), or **CTTTATTAATGCCT** (Seq. ID No. 343), respectively and salts and optical isomers of said antisense-oligonucleotide.

Alternatively the present invention is directed to antisense-oligonucleotide(s) consisting of 14 to 20 more preferably 14 to 18 nucleotides and at least four of the 14 to 20 more preferably 14 to 18 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **ACTGGTCCATTC** (Seq. ID No. 314), **TGGTCCATTCAT** (Seq. ID No. 315), **CTGGTCCATTCAT** (Seq. ID No. 316), **ACTGGTCCATTCA** (Seq. ID No. 317), **ACTGGTCCATTCAT** (Seq. ID No. 318), **CTCCCTAAACAC** (Seq. ID No. 319), **CCCTAAACACTA** (Seq. ID No. 320), **TCCCTAAACACTA** (Seq. ID No. 321), **CTCCCTAAACACT** (Seq. ID No. 322), **CTCCCTAAACACTA** (Seq. ID No. 323), **ACACTACCAAAT** (Seq. ID No. 324), **ACTACCAAATAG** (Seq. ID No. 325), **CACTACCAAATAG** (Seq. ID No. 326), **ACACTACCAAATA** (Seq. ID No. 327), **ACACTACCAAATAG** (Seq. ID No. 328), **GTGGACGCGTAT** (Seq. ID No. 329), **GGACGCGTATCG** (Seq. ID No. 330), **TGGACGCGTATCG** (Seq. ID No. 331), **GTGGACGCGTATC** (Seq. ID No. 332), **GTGGACGCGTATCG** (Seq. ID No. 333), **CGGTCTATGACG** (Seq. ID No. 334), **GTCTATGACGAG** (Seq. ID No. 335), **GGTCTATGACGAG** (Seq. ID No. 336), **CGGTCTATGACGA** (Seq. ID No. 337), **CGGTCTATGACGAG** (Seq. ID No. 338), **CTTTATTAATGC** (Seq. ID No. 339), **TTATTAATGCCT** (Seq. ID No. 340), **TTTATTAATGCCT** (Seq. ID No. 341), **CTTTATTAATGCC** (Seq. ID No. 342), or **CTTTATTAATGCCT** (Seq. ID No. 343), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **ACTGGTCCATTC** (Seq. ID No. 314), **TGGTCCATTCAT** (Seq. ID No. 315), **CTGGTCCATTCAT** (Seq. ID No. 316), **ACTGGTCCATTCA** (Seq. ID No. 317), **ACTGGTCCATTCAT** (Seq. ID No. 318), **CTCCCTAAACAC** (Seq. ID No. 319), **CCCTAAACACTA** (Seq. ID No. 320), **TCCCTAAACACTA** (Seq. ID No. 321), **CTCCCTAAACACT** (Seq. ID No. 322), **CTCCCTAAACACTA** (Seq. ID No. 323), **ACACTACCAAAT** (Seq. ID No. 324), **ACTACCAAATAG** (Seq. ID No. 325), **CACTACCAAATAG** (Seq. ID No. 326), **ACACTACCAAATA** (Seq. ID No. 327), **ACACTACCAAATAG** (Seq. ID No. 328), **GTGGACGCGTAT** (Seq. ID No. 329), **GGACGCGTATCG** (Seq. ID No. 330), **TGGACGCGTATCG** (Seq. ID No. 331), **GTGGACGCGTATC** (Seq. ID No. 332), **GTGGACGCGTATCG** (Seq. ID No. 333), **CGGTCTATGACG** (Seq. ID No. 334), **GTCTATGACGAG** (Seq. ID No. 335), **GGTCTATGACGAG** (Seq. ID No. 336), **CGGTCTATGACGA** (Seq. ID No. 337), **CGGTCTATGACGAG** (Seq. ID No. 338), **CTTTATTAATGC** (Seq. ID No. 339),

TTATTAATGCCT (Seq. ID No. 340), **TTTATTAATGCCT** (Seq. ID No. 341), **CTTTATTAATGCC** (Seq. ID No. 342), or **CTTTATTAATGCCT** (Seq. ID No. 343), respectively and salts and optical isomers of said antisense-oligonucleotide.

Preferably the present invention is also directed to antisense-oligonucleotide(s) consisting of 14 to 20 more preferably 14 to 18 nucleotides and at least four of the 14 to 20 more preferably 14 to 18 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **ACTGGTCCATTC** (Seq. ID No. 314), **TGGTCCATTCAT** (Seq. ID No. 315), **CTGGTCCATTCAT** (Seq. ID No. 316), **ACTGGTCCATTCA** (Seq. ID No. 317), **ACTGGTCCATTCAT** (Seq. ID No. 318), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **ACTGGTCCATTC** (Seq. ID No. 314), **TGGTCCATTCAT** (Seq. ID No. 315), **CTGGTCCATTCAT** (Seq. ID No. 316), **ACTGGTCCATTCA** (Seq. ID No. 317), **ACTGGTCCATTCAT** (Seq. ID No. 318), respectively and salts and optical isomers of said antisense-oligonucleotide.

Slightly reworded, the present invention is directed to antisense-oligonucleotide(s) consisting of 14 to 20 more preferably 14 to 18 nucleotides and at least four of the 14 to 20 more preferably 14 to 18 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **ACTGGTCCATTC** (Seq. ID No. 314), **TGGTCCATTCAT** (Seq. ID No. 315), **CTGGTCCATTCAT** (Seq. ID No. 316), **ACTGGTCCATTCA** (Seq. ID No. 317), or **ACTGGTCCATTCAT** (Seq. ID No. 318), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **ACTGGTCCATTC** (Seq. ID No. 314), **TGGTCCATTCAT** (Seq. ID No. 315), **CTGGTCCATTCAT** (Seq. ID No. 316), **ACTGGTCCATTCA** (Seq. ID No. 317), or **ACTGGTCCATTCAT** (Seq. ID No. 318), respectively and salts and optical isomers of said antisense-oligonucleotide.

Preferably the present invention is also directed to antisense-oligonucleotide(s) consisting of 14 to 20 more preferably 14 to 18 nucleotides and at least four of the 14 to 20 more preferably 14 to 18 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the

gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **CTCCCTAAACAC** (Seq. ID No. 319), **CCCTAAACACTA** (Seq. ID No. 320), **TCCCTAAACACTA** (Seq. ID No. 321), **CTCCCTAAACACT** (Seq. ID No. 322), or **CTCCCTAAACACTA** (Seq. ID No. 323), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **CTCCCTAAACAC** (Seq. ID No. 319), **CCCTAAACACTA** (Seq. ID No. 320), **TCCCTAAACACTA** (Seq. ID No. 321), **CTCCCTAAACACT** (Seq. ID No. 322), or **CTCCCTAAACACTA** (Seq. ID No. 323), respectively and salts and optical isomers of said antisense-oligonucleotide.

Slightly reworded, the present invention is directed to antisense-oligonucleotide(s) consisting of 14 to 20 more preferably 14 to 18 nucleotides and at least four of the 14 to 20 more preferably 14 to 18 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **CTCCCTAAACAC** (Seq. ID No. 319), **CCCTAAACACTA** (Seq. ID No. 320), **TCCCTAAACACTA** (Seq. ID No. 321), **CTCCCTAAACACT** (Seq. ID No. 322), or **CTCCCTAAACACTA** (Seq. ID No. 323), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **CTCCCTAAACAC** (Seq. ID No. 319), **CCCTAAACACTA** (Seq. ID No. 320), **TCCCTAAACACTA** (Seq. ID No. 321), **CTCCCTAAACACT** (Seq. ID No. 322), or **CTCCCTAAACACTA** (Seq. ID No. 323), respectively and salts and optical isomers of said antisense-oligonucleotide.

Preferably the present invention is also directed to antisense-oligonucleotide(s) consisting of 14 to 20 more preferably 14 to 18 nucleotides and at least four of the 14 to 20 more preferably 14 to 18 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **ACACTACCAAAT** (Seq. ID No. 324), **ACTACCAAATAG** (Seq. ID No. 325), **CACTACCAAATAG** (Seq. ID No. 326), **ACACTACCAAATA** (Seq. ID No. 327), or **ACACTACCAAATAG** (Seq. ID No. 328), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **ACACTACCAAAT** (Seq. ID No. 324), **ACTACCAAATAG** (Seq. ID No. 325), **CACTACCAAATAG** (Seq. ID No. 326), **ACACTACCAAATA** (Seq. ID No. 327), or **ACACTACCAAATAG** (Seq. ID No. 328), respectively and salts and optical isomers of said antisense-oligonucleotide.

Slightly reworded, the present invention is directed to antisense-oligonucleotide(s) consisting of 14 to 20 more preferably 14 to 18 nucleotides and at least four of the 14 to 20 more preferably 14 to 18 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **ACACTACCAAAT** (Seq. ID No. 324), **ACTACCAAATAG** (Seq. ID No. 325), **CACTACCAAATAG** (Seq. ID No. 326), **ACACTACCAAATA** (Seq. ID No. 327), or **ACACTACCAAATAG** (Seq. ID No. 328), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **ACACTACCAAAT** (Seq. ID No. 324), **ACTACCAAATAG** (Seq. ID No. 325), **CACTACCAAATAG** (Seq. ID No. 326), **ACACTACCAAATA** (Seq. ID No. 327), or **ACACTACCAAATAG** (Seq. ID No. 328), respectively and salts and optical isomers of said antisense-oligonucleotide.

Preferably the present invention is also directed to antisense-oligonucleotide(s) consisting of 14 to 20 more preferably 14 to 18 nucleotides and at least four of the 14 to 20 more preferably 14 to 18 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **GTGGACGCGTAT** (Seq. ID No. 329), **GGACGCGTATCG** (Seq. ID No. 330), **TGGACGCGTATCG** (Seq. ID No. 331), **GTGGACGCGTATC** (Seq. ID No. 332), or **GTGGACGCGTATCG** (Seq. ID No. 333), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **GTGGACGCGTAT** (Seq. ID No. 329), **GGACGCGTATCG** (Seq. ID No. 330), **TGGACGCGTATCG** (Seq. ID No. 331), **GTGGACGCGTATC** (Seq. ID No. 332), or **GTGGACGCGTATCG** (Seq. ID No. 333), respectively and salts and optical isomers of said antisense-oligonucleotide.

Slightly reworded, the present invention is directed to antisense-oligonucleotide(s) consisting of 14 to 20 more preferably 14 to 18 nucleotides and at least four of the 14 to 20 more preferably 14 to 18 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **GTGGACGCGTAT** (Seq. ID No. 329), **GGACGCGTATCG** (Seq. ID No. 330), **TGGACGCGTATCG** (Seq. ID No. 331), **GTGGACGCGTATC** (Seq. ID No. 332), or **GTGGACGCGTATCG** (Seq. ID No. 333), and the antisense-

oligonucleotide comprises a sequence complementary to the sequence **GTGGACGCGTAT** (Seq. ID No. 329), **GGACGCGTATCG** (Seq. ID No. 330), **TGGACGCGTATCG** (Seq. ID No. 331), **GTGGACGCGTATC** (Seq. ID No. 332), or **GTGGACGCGTATCG** (Seq. ID No. 333), respectively and salts and optical isomers of said antisense-oligonucleotide.

Preferably the present invention is also directed to antisense-oligonucleotide(s) consisting of 14 to 20 more preferably 14 to 18 nucleotides and at least four of the 14 to 20 more preferably 14 to 18 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **CGGTCTATGACG** (Seq. ID No. 334), **GTCTATGACGAG** (Seq. ID No. 335), **GGTCTATGACGAG** (Seq. ID No. 336), **CGGTCTATGACGA** (Seq. ID No. 337), or **CGGTCTATGACGAG** (Seq. ID No. 338), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **CGGTCTATGACG** (Seq. ID No. 334), **GTCTATGACGAG** (Seq. ID No. 335), **GGTCTATGACGAG** (Seq. ID No. 336), **CGGTCTATGACGA** (Seq. ID No. 337), or **CGGTCTATGACGAG** (Seq. ID No. 338), respectively and salts and optical isomers of said antisense-oligonucleotide.

Slightly reworded, the present invention is directed to antisense-oligonucleotide(s) consisting of 14 to 20 more preferably 14 to 18 nucleotides and at least four of the 14 to 20 more preferably 14 to 18 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **CGGTCTATGACG** (Seq. ID No. 334), **GTCTATGACGAG** (Seq. ID No. 335), **GGTCTATGACGAG** (Seq. ID No. 336), **CGGTCTATGACGA** (Seq. ID No. 337), or **CGGTCTATGACGAG** (Seq. ID No. 338), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **CGGTCTATGACG** (Seq. ID No. 334), **GTCTATGACGAG** (Seq. ID No. 335), **GGTCTATGACGAG** (Seq. ID No. 336), **CGGTCTATGACGA** (Seq. ID No. 337), or **CGGTCTATGACGAG** (Seq. ID No. 338), respectively and salts and optical isomers of said antisense-oligonucleotide.

Preferably the present invention is also directed to antisense-oligonucleotide(s) consisting of 14 to 20 more preferably 14 to 18 nucleotides and at least four of the 14 to 20 more preferably 14 to 18 nucleotides are LNAs and the antisense-

oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **CTTTATTAATGC** (Seq. ID No. 339), **TTATTAATGCCT** (Seq. ID No. 340), **TTTATTAATGCCT** (Seq. ID No. 341), **CTTTATTAATGCC** (Seq. ID No. 342), or **CTTTATTAATGCCT** (Seq. ID No. 343), and the antisense-oligonucleotide comprises a sequence capable of hybridizing with said sequence **CTTTATTAATGC** (Seq. ID No. 339), **TTATTAATGCCT** (Seq. ID No. 340), **TTTATTAATGCCT** (Seq. ID No. 341), **CTTTATTAATGCC** (Seq. ID No. 342), or **CTTTATTAATGCCT** (Seq. ID No. 343), respectively and salts and optical isomers of said antisense-oligonucleotide.

Slightly reworded, the present invention is directed to antisense-oligonucleotide(s) consisting of 14 to 20 more preferably 14 to 18 nucleotides and at least four of the 14 to 20 more preferably 14 to 18 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **CTTTATTAATGC** (Seq. ID No. 339), **TTATTAATGCCT** (Seq. ID No. 340), **TTTATTAATGCCT** (Seq. ID No. 341), **CTTTATTAATGCC** (Seq. ID No. 342), or **CTTTATTAATGCCT** (Seq. ID No. 343), and the antisense-oligonucleotide comprises a sequence complementary to the sequence **CTTTATTAATGC** (Seq. ID No. 339), **TTATTAATGCCT** (Seq. ID No. 340), **TTTATTAATGCCT** (Seq. ID No. 341), **CTTTATTAATGCC** (Seq. ID No. 342), or **CTTTATTAATGCCT** (Seq. ID No. 343), respectively and salts and optical isomers of said antisense-oligonucleotide.

The antisense-oligonucleotides of the present invention preferably comprise 4 to 11 LNA units, more preferably 4 to 10 LNA units and still more preferably 4 to 8 LNA units and also preferably at least 6 non-LNA units, more preferably at least 7 non-LNA units and most preferably at least 8 non-LNA units. The non-LNA units are preferably DNA units. The LNA units are preferably positioned at the 3' terminal end (also named 3' terminus) and the 5' terminal end (also named 5' terminus). Preferably at least one and more preferably at least two LNA units are present at the 3' terminal end and/or at the 5' terminal end.

Thus, preferred are antisense-oligonucleotides of the present invention which contain 3 to 10 LNA units and which especially contain 1 to 5 LNA units at the 5' terminal end and 1 to 5 LNA units at the 3' terminal end of the antisense-oligonucleotide and between the LNA units at least 7 and more preferably at least 8 DNA units.

Moreover, the antisense-oligonucleotides may contain common nucleobases such as adenine, guanine, cytosine, thymine and uracil as well as common derivatives thereof. The antisense-oligonucleotides of the present invention may also contain modified internucleotide bridges such as phosphorothioate or phosphorodithioate instead of phosphate bridges. Such modifications may be present only in the LNA segments or only in the non-LNA segment of the antisense-oligonucleotide.

Thus, the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^1-**GTCATAGA**-N^2-3' (Seq. ID No. 12) or 5'-N^3-**ACGCGTCC**-N^4-3' (Seq. ID No. 98) or 5'-N^{11}-**TGTTTAGG**-N^{12}-3' (Seq. ID No. 10) or 5'-N^5-**TTTGGTAG**-N^6-3' (Seq. ID No. 11) or 5'-N^7-**AATGGACC**-N^8-3' (Seq. ID No. 100) or 5'-N^9-**ATTAATAA**-N^{10}-3' (Seq. ID No. 101), wherein

N^1 represents: CATGGCAGACCCCGCTGCTC-, ATGGCAGACCCCGCTGCTC-, TGGCAGACCCCGCTGCTC-, GGCAGACCCCGCTGCTC-, GCAGACCCCGCTGCTC-, CAGACCCCGCTGCTC-, AGACCCCGCTGCTC-, GACCCCGCTGCTC-, ACCCCGCTGCTC-, CCCCGCTGCTC-, CCCGCTGCTC-, CCGCTGCTC-, CGCTGCTC-, GCTGCTC-, CTGCTC-, TGCTC-, GCTC-, CTC-, TC-, or C-;

N^2 represents: -C, -CC, -CCG, -CCGA, -CCGAG, -CCGAGC, -CCGAGCC, -CCGAGCCC, -CCGAGCCCC, -CCGAGCCCCC, -CCGAGCCCCCA, -CCGAGCCCCCAG, -CCGAGCCCCCAGC, -CCGAGCCCCCAGCG, -CCGAGCCCCCAGCGC, -CCGAGCCCCCAGCGCA, -CCGAGCCCCCAGCGCAG, -CCGAGCCCCCAGCGCAGC, -CCGAGCCCCCAGCGCAGCG, or –CCGAGCCCCCAGCGCAGCGG;

N^3 represents: GGTGGGATCGTGCTGGCGAT-, GTGGGATCGTGCTGGCGAT-, TGGGATCGTGCTGGCGAT-, GGGATCGTGCTGGCGAT-, GGATCGTGCTGGCGAT-, GATCGTGCTGGCGAT-, ATCGTGCTGGCGAT-, TCGTGCTGGCGAT-, CGTGCTGGCGAT-, GTGCTGGCGAT-, TGCTGGCGAT-, GCTGGCGAT-, CTGGCGAT-, TGGCGAT-, GGCGAT-, GCGAT-, CGAT-, GAT-, AT-, or T-;

N^4 represents: -ACAGGACGATGTGCAGCGGC, -ACAGGACGATGTGCAGCGG, -ACAGGACGATGTGCAGCG, -ACAGGACGATGTGCAGC, -ACAGGACGATGTGCAG, -ACAGGACGATGTGCA, -ACAGGACGATGTGC, -ACAGGACGATGTG, -ACAGGACGATGT, -ACAGGACGATG, -ACAGGACGAT,

-ACAGGACGA, -ACAGGACG, -ACAGGAC, -ACAGGA, -ACAGG, -ACAG, -ACA, -AC, or -A;

N^5 represents: GCCCAGCCTGCCCCAGAAGAGCTA-, CCCAGCCTGCCCCAGAAGAGCTA-, CCAGCCTGCCCCAGAAGAGCTA-, CAGCCTGCCCCAGAAGAGCTA-, AGCCTGCCCCAGAAGAGCTA-, GCCTGCCCCAGAAGAGCTA-, CCTGCCCCAGAAGAGCTA-, CTGCCCCAGAAGAGCTA-, TGCCCCAGAAGAGCTA-, GCCCCAGAAGAGCTA-, CCCCAGAAGAGCTA-, CCCAGAAGAGCTA-, CCAGAAGAGCTA-, CAGAAGAGCTA-, AGAAGAGCTA-, GAAGAGCTA-, AAGAGCTA-, AGAGCTA-, GAGCTA-, AGCTA-, GCTA-, CTA-, TA-, or A-;

N^6 represents: -TGTTTAGGGAGCCGTCTTCAGGAA, -TGTTTAGGGAGCCGTCTTCAGGA, -TGTTTAGGGAGCCGTCTTCAGG, -TGTTTAGGGAGCCGTCTTCAG, -TGTTTAGGGAGCCGTCTTCA, -TGTTTAGGGAGCCGTCTTC, -TGTTTAGGGAGCCGTCTT, -TGTTTAGGGAGCCGTCT, -TGTTTAGGGAGCCGTC, -TGTTTAGGGAGCCGT, -TGTTTAGGGAGCCG, -TGTTTAGGGAGCC, -TGTTTAGGGAGC, -TGTTTAGGGAG, -TGTTTAGGGA, -TGTTTAGGG, -TGTTTAGG, -TGTTTAG, -TGTTTA, -TGTTT, -TGTT, -TGT, -TG, or –T;

N^7 represents: TGAATCTTGAATATCTCATG-, GAATCTTGAATATCTCATG-, AATCTTGAATATCTCATG-, ATCTTGAATATCTCATG-, TCTTGAATATCTCATG-, CTTGAATATCTCATG-, TTGAATATCTCATG-, TGAATATCTCATG-, GAATATCTCATG-, AATATCTCATG-, ATATCTCATG-, TATCTCATG-, ATCTCATG-, TCTCATG-, CTCATG-, TCATG-, CATG-, ATG-, TG-, or G-;

N^8 represents: -AGTATTCTAGAAACTCACCA, -AGTATTCTAGAAACTCACC, -AGTATTCTAGAAACTCAC, -AGTATTCTAGAAACTCA, -AGTATTCTAGAAACTC, -AGTATTCTAGAAACT, -AGTATTCTAGAAAC, -AGTATTCTAGAAA, -AGTATTCTAGAA, -AGTATTCTAGA, -AGTATTCTAG, -AGTATTCTA, -AGTATTCT, -AGTATTC, -AGTATT, -AGTAT, -AGTA, -AGT, -AG, or -A;

N^9 represents: ATTCATATTTATATACAGGC-, TTCATATTTATATACAGGC-, TCATATTTATATACAGGC-, CATATTTATATACAGGC-, ATATTTATATACAGGC-, TATTTATATACAGGC-, ATTTATATACAGGC-, TTTATATACAGGC-, TTATATACAGGC-, TATATACAGGC-, ATATACAGGC-, TATACAGGC-, ATACAGGC-, TACAGGC-, ACAGGC-, CAGGC-, AGGC-, GGC-, GC-, or C-;

N^{10} represents: -AGTGCAAATGTTATTGGCTA, -AGTGCAAATGTTATTGGCT, -AGTGCAAATGTTATTGGC, -AGTGCAAATGTTATTGG, -AGTGCAAATGTTATTG, -AGTGCAAATGTTATT, -AGTGCAAATGTTAT, -AGTGCAAATGTTA,

-AGTGCAAATGTT, -AGTGCAAATGT, -AGTGCAAATG, -AGTGCAAAT, -AGTGCAAA, -AGTGCAA, -AGTGCA, -AGTGC, -AGTG, -AGT, -AG, or -A;

N^{11} represents: TGCCCCAGAAGAGCTATTTGGTAG-, GCCCCAGAAGAGCTATTTGGTAG-, CCCCAGAAGAGCTATTTGGTAG-, CCCAGAAGAGCTATTTGGTAG-, CCAGAAGAGCTATTTGGTAG-, CAGAAGAGCTATTTGGTAG-, AGAAGAGCTATTTGGTAG-, GAAGAGCTATTTGGTAG-, AAGAGCTATTTGGTAG-, AGAGCTATTTGGTAG-, GAGCTATTTGGTAG-, AGCTATTTGGTAG-, GCTATTTGGTAG-, CTATTTGGTAG-, TATTTGGTAG-, ATTTGGTAG-, TTTGGTAG-, TTGGTAG-, TGGTAG-, GGTAG-, GTAG-, TAG-, AG- or G-,

N^{12} represents: -GAGCCGTCTTCAGGAATCTTCTCC, -GAGCCGTCTTCAGGAATCTTCTC, -GAGCCGTCTTCAGGAATCTTCT, -GAGCCGTCTTCAGGAATCTTC, -GAGCCGTCTTCAGGAATCTT, -GAGCCGTCTTCAGGAATCT, -GAGCCGTCTTCAGGAATC, -GAGCCGTCTTCAGGAAT, -GAGCCGTCTTCAGGAA, -GAGCCGTCTTCAGGA, -GAGCCGTCTTCAGG, -GAGCCGTCTTCAG, -GAGCCGTCTTCA, -GAGCCGTCTTC, -GAGCCGTCTT, -GAGCCGTCT, -GAGCCGTC, -GAGCCGT, -GAGCCG, -GAGCC, -GAGC, -GAG, -GA, or –G;

and salts and optical isomers of the antisense-oligonucleotide.

Thus, the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^1-**GTCATAGA**-N^2-3' (Seq. ID No. 12) or 5'-N^3-**ACGCGTCC**-N^4-3' (Seq. ID No. 98) or 5'-N^{11}-**TGTTTAGG**-N^{12}-3' (Seq. ID No. 10) or 5'-N^5-**TTTGGTAG**-N^6-3' (Seq. ID No. 11) or 5'-N^7-**AATGGACC**-N^8-3' (Seq. ID No. 100) or 5'-N^9-**ATTAATAA**-N^{10}-3' (Seq. ID No. 101), wherein the residues N^1 to N^{12} have the meanings especially the further limited meanings as disclosed herein and salts and optical isomers of said antisense-oligonucleotide.

Moreover, the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^1-**GTCATAGA**-N^2-3' (Seq. ID No. 12), wherein

N^1 represents: CATGGCAGACCCCGCTGCTC-, ATGGCAGACCCCGCTGCTC-, TGGCAGACCCCGCTGCTC-, GGCAGACCCCGCTGCTC-, GCAGACCCCGCTGCTC-, CAGACCCCGCTGCTC-, AGACCCCGCTGCTC-, GACCCCGCTGCTC-, ACCCCGCTGCTC-, CCCCGCTGCTC-, CCCGCTGCTC-, CCGCTGCTC-, CGCTGCTC-, GCTGCTC-, CTGCTC-, TGCTC-, GCTC-, CTC-, TC-, or C-;

N^2 represents: -C, -CC, -CCG, -CCGA, -CCGAG, -CCGAGC, -CCGAGCC, -CCGAGCCC, -CCGAGCCCC, -CCGAGCCCCC, -CCGAGCCCCCA, -CCGAGCCCCCAG, -CCGAGCCCCCAGC, -CCGAGCCCCCAGCG, -CCGAGCCCCCAGCGC, -CCGAGCCCCCAGCGCA, -CCGAGCCCCCAGCGCAG, -CCGAGCCCCCAGCGCAGC, -CCGAGCCCCCAGCGCAGCG, or –CCGAGCCCCCAGCGCAGCGG;

and salts and optical isomers of the antisense-oligonucleotide.

The antisense-oligonucleotides of formula S1 (Seq. ID No. 12) preferably comprise 2 to 10 LNA units, more preferably 3 to 9 LNA units and still more preferably 4 to 8 LNA units and also preferably at least 6 non-LNA units, more preferably at least 7 non-LNA units and most preferably at least 8 non-LNA units. The non-LNA units are preferably DNA units. The LNA units are preferably positioned at the 3' terminal end (also named 3' terminus) and the 5' terminal end (also named 5' terminus). Preferably at least one and more preferably at least two LNA units are present at the 3' terminal end and/or at the 5' terminal end.

Thus, preferred are antisense-oligonucleotides of the present invention designed as GAPmers which contain 2 to 10 LNA units and which especially contain 1 to 5 LNA units at the 5' terminal end and 1 to 5 LNA units at the 3' terminal end of the antisense-oligonucleotide and between the LNA units at least 7 and more preferably at least 8 DNA units. More preferably the antisense-oligonucleotides comprise 2 to 4 LNA units at the 5' terminal end and 2 to 4 LNA units at the 3' terminal end and still more preferred comprise 3 to 4 LNA units at the 5' terminal end and 3 to 4 LNA units at the 3' terminal end and contain preferably at least 7 non-LNA units and most preferably at least 8 non-LNA units such as DNA units in between both LNA segments.

Moreover, the antisense-oligonucleotides may contain common nucleobases such as adenine, guanine, cytosine, thymine and uracil as well as common derivatives thereof such as 5-methylcytosine or 2-aminoadenine. The antisense-oligonucleotides of the present invention may also contain modified internucleotide bridges such as phosphorothioate or phosphorodithioate instead of phosphate bridges. Such modifications may be present only in the LNA segments or only in the

non-LNA segment of the antisense-oligonucleotide. As LNA units especially the residues b^1 to b^9 as disclosed herein are preferred.

Thus, preferred are antisense-oligonucleotides of the formula (S1):

5'-N^1-**GTCATAGA**-N^2-3' (Seq. ID No. 12)

wherein

N^1 represents: CATGGCAGACCCCGCTGCTC-, ATGGCAGACCCCGCTGCTC-, TGGCAGACCCCGCTGCTC-, GGCAGACCCCGCTGCTC-, GCAGACCCCGCTGCTC-, CAGACCCCGCTGCTC-, AGACCCCGCTGCTC-, GACCCCGCTGCTC-, ACCCCGCTGCTC-, CCCCGCTGCTC-, CCCGCTGCTC-, CCGCTGCTC-, CGCTGCTC-, GCTGCTC-, CTGCTC-, TGCTC-, GCTC-, CTC-, TC-, or C-;

and

N^2 is selected from: -C, -CC, -CCG, -CCGA, -CCGAG, -CCGAGC, -CCGAGCC, -CCGAGCCC, -CCGAGCCCC, -CCGAGCCCCC, -CCGAGCCCCCA, -CCGAGCCCCCAG, -CCGAGCCCCCAGC, -CCGAGCCCCCAGCG, -CCGAGCCCCCAGCGC, -CCGAGCCCCCAGCGCA, -CCGAGCCCCCAGCGCAG, -CCGAGCCCCCAGCGCAGC, -CCGAGCCCCCAGCGCAGCG, or -CCGAGCCCCCAGCGCAGCGG.

Preferably the antisense-oligonucleotide of general formula (S1) has between 10 and 28 nucleotides and at least one LNA nucleotide at the 3' terminus and at least one LNA nucleotide at the 5' terminus. As LNA nucleotides (LNA units) especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably in the chapter "Preferred LNAs" are suitable and as internucleotides bridges especially these disclosed in the chapter "Internucleotide Linkages (IL)" are suitable.

More preferably the antisense-oligonucleotide of general formula (S1) has between 11 and 24 nucleotides and at least two LNA nucleotides at the 3' terminus and at least two LNA nucleotides at the 5' terminus.

Still more preferably the antisense-oligonucleotide of general formula (S1) has between 12 and 20, more preferably between 13 and 19 and still more preferable between 14 and 18 nucleotides and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 3' terminal end and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 5' terminal end. Preferably the antisense-oligonucleotides are GAPmers of the form LNA segment A – DNA segment – LNA segment B. Preferably the antisense-oligonucleotides contain at least 6, more preferably at least 7 and most preferably at least 8 non-LNA units such as DNA units in between the two LNA segments.

Suitable nucleobases for the non-LNA units and the LNA units are disclosed in the chapter “Nucleobases”.

Further preferred are antisense-oligonucleotides of the formula (S1):

5’-N^1-**GTCATAGA**-N^2-3’

wherein

N^1 represents: TGGCAGACCCCGCTGCTC-, GGCAGACCCCGCTGCTC-, GCAGACCCCGCTGCTC-, CAGACCCCGCTGCTC-, AGACCCCGCTGCTC-, GACCCCGCTGCTC-, ACCCCGCTGCTC-, CCCCGCTGCTC-, CCCGCTGCTC-, CCGCTGCTC-, CGCTGCTC-, GCTGCTC-, CTGCTC-, TGCTC-, GCTC-, CTC-, TC-, or C-;

and

N^2 is selected from: -C, -CC, -CCG, -CCGA, -CCGAG, -CCGAGC, -CCGAGCC, -CCGAGCCC, -CCGAGCCCC, -CCGAGCCCCC, -CCGAGCCCCCA, -CCGAGCCCCCAG, -CCGAGCCCCCAGC, -CCGAGCCCCCAGCG, -CCGAGCCCCCAGCGC, -CCGAGCCCCCAGCGCA, -CCGAGCCCCCAGCGCAG, or -CCGAGCCCCCAGCGCAGC.

Also preferred are antisense-oligonucleotides of the formula (S1):

5’-N^1-**GTCATAGA**-N^2-3’

wherein

N^1 represents: GACCCCGCTGCTC-, ACCCCGCTGCTC-, CCCCGCTGCTC-, CCCGCTGCTC-, CCGCTGCTC-, CGCTGCTC-, GCTGCTC-, CTGCTC-, TGCTC-, GCTC-, CTC-, TC-, or C-;

and

N^2 is selected from: -C, -CC, -CCG, -CCGA, -CCGAG, -CCGAGC, -CCGAGCC, -CCGAGCCC, -CCGAGCCCC, -CCGAGCCCCC, -CCGAGCCCCCA, -CCGAGCCCCCAG, or -CCGAGCCCCCAGC.

Also preferred are antisense-oligonucleotides of the formula (S1):

5’-N^1-**GTCATAGA**-N^2-3’

wherein

N^1 represents: CGCTGCTC-, GCTGCTC-, CTGCTC-, TGCTC-, GCTC-, CTC-, TC-, or C-;

and

N^2 is selected from: -C, -CC, -CCG, -CCGA, -CCGAG, -CCGAGC, -CCGAGCC, or -CCGAGCCC.

Preferably, the present invention is directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^{1A}-**CGTCATAGAC**-N^{2A}-3' (Seq. ID No. 69), wherein

N^{1A} represents: CATGGCAGACCCCGCTGCT-, ATGGCAGACCCCGCTGCT-, TGGCAGACCCCGCTGCT-, GGCAGACCCCGCTGCT-, GCAGACCCCGCTGCT-, CAGACCCCGCTGCT-, AGACCCCGCTGCT-, GACCCCGCTGCT-, ACCCCGCTGCT-, CCCCGCTGCT-, CCCGCTGCT-, CCGCTGCT-, CGCTGCT-, GCTGCT-, CTGCT-, TGCT-, GCT-, CT-, or T-;

N^{2A} represents: -C, -CG, -CGA, -CGAG, -CGAGC, -CGAGCC, -CGAGCCC, -CGAGCCCC, -CGAGCCCCC, -CGAGCCCCCA, -CGAGCCCCCAG, -CGAGCCCCCAGC, -CGAGCCCCCAGCG, -CGAGCCCCCAGCGC, -CGAGCCCCCAGCGCA, -CGAGCCCCCAGCGCAG, -CGAGCCCCCAGCGCAGC, -CGAGCCCCCAGCGCAGCG, or –CGAGCCCCCAGCGCAGCGG;

and salts and optical isomers of the antisense-oligonucleotide.

Preferably N^{1A} represents: TGGCAGACCCCGCTGCT-, GGCAGACCCCGCTGCT-, GCAGACCCCGCTGCT-, CAGACCCCGCTGCT-, AGACCCCGCTGCT-, GACCCCGCTGCT-, ACCCCGCTGCT-, CCCCGCTGCT-, CCCGCTGCT-, CCGCTGCT-, CGCTGCT-, GCTGCT-, CTGCT-, TGCT-, GCT-, CT-, or T-;

and

N^{2A} represents: -C, -CG, -CGA, -CGAG, -CGAGC, -CGAGCC, -CGAGCCC, -CGAGCCCC, -CGAGCCCCC, -CGAGCCCCCA, -CGAGCCCCCAG, -CGAGCCCCCAGC, -CGAGCCCCCAGCG, -CGAGCCCCCAGCGC, -CGAGCCCCCAGCGCA, -CGAGCCCCCAGCGCAG, or -CGAGCCCCCAGCGCAGC.

More preferably N^{1A} represents: GACCCCGCTGCT-, ACCCCGCTGCT-, CCCCGCTGCT-, CCCGCTGCT-, CCGCTGCT-, CGCTGCT-, GCTGCT-, CTGCT-, TGCT-, GCT-, CT-, or T-; and

N^{2A} represents: -C, -CG, -CGA, -CGAG, -CGAGC, -CGAGCC, -CGAGCCC, -CGAGCCCC, -CGAGCCCCC, -CGAGCCCCCA, -CGAGCCCCCAG, or -CGAGCCCCCAGC.

Still more preferably N^{1A} represents: CGCTGCT-, GCTGCT-, CTGCT-, TGCT-, GCT-, CT-, or T-; and

N^{2A} represents: -C, -CG, -CGA, -CGAG, -CGAGC, -CGAGCC, or -CGAGCCC.

Preferably the antisense-oligonucleotide of general formula (S1A / Seq. ID No. 69) has between 12 and 24 nucleotides and at least one LNA nucleotide at the 3' terminus and at least one LNA nucleotide at the 5' terminus. As LNA nucleotides (LNA units) especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably in the chapter "Preferred LNAs" are suitable and as internucleotides bridges especially these disclosed in the chapter "Internucleotide Linkages (IL)" are suitable.

More preferably the antisense-oligonucleotide of general formula (S1A) has between 12 and 22 nucleotides and at least two LNA nucleotides at the 3' terminus and at least two LNA nucleotides at the 5' terminus.
Still more preferably the antisense-oligonucleotide of general formula (S1A) has between 12 and 20, more preferably between 13 and 19 and still more preferable between 14 and 18 nucleotides and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 3' terminal end and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 5' terminal end. Preferably the antisense-oligonucleotides are GAPmers of the form LNA segment A – DNA segment – LNA segment B. Preferably the antisense-oligonucleotides contain at least 6, more preferably at least 7 and most preferably at least 8 non-LNA units such as DNA units in between the two LNA segments. Suitable nucleobases for the non-LNA units and the LNA units are disclosed in the chapter "Nucleobases".

Moreover, the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^3-**ACGCGTCC**-N^4-3' (Seq. ID No. 98), wherein
N^3 represents: GGTGGGATCGTGCTGGCGAT-, GTGGGATCGTGCTGGCGAT-, TGGGATCGTGCTGGCGAT-, GGGATCGTGCTGGCGAT-, GGATCGTGCTGGCGAT-, GATCGTGCTGGCGAT-, ATCGTGCTGGCGAT-, TCGTGCTGGCGAT-, CGTGCTGGCGAT-, GTGCTGGCGAT-, TGCTGGCGAT-, GCTGGCGAT-, CTGGCGAT-, TGGCGAT-, GGCGAT-, GCGAT-, CGAT-, GAT-, AT-, or T-;
N^4 represents: -ACAGGACGATGTGCAGCGGC, -ACAGGACGATGTGCAGCGG, -ACAGGACGATGTGCAGCG, -ACAGGACGATGTGCAGC, -ACAGGACGATGTGCAG, -ACAGGACGATGTGCA, -ACAGGACGATGTGC, -ACAGGACGATGTG, -ACAGGACGATGT, -ACAGGACGATG, -ACAGGACGAT,

-ACAGGACGA, -ACAGGACG, -ACAGGAC, -ACAGGA, -ACAGG, -ACAG, -ACA, -AC, or -A;
and salts and optical isomers of the antisense-oligonucleotide.

The antisense-oligonucleotides of formula S2 (Seq. ID No. 98) preferably comprise 2 to 10 LNA units, more preferably 3 to 9 LNA units and still more preferably 4 to 8 LNA units and also preferably at least 6 non-LNA units, more preferably at least 7 non-LNA units and most preferably at least 8 non-LNA units. The non-LNA units are preferably DNA units. The LNA units are preferably positioned at the 3' terminal end (also named 3' terminus) and the 5' terminal end (also named 5' terminus). Preferably at least one and more preferably at least two LNA units are present at the 3' terminal end and/or at the 5' terminal end.
Thus, preferred are antisense-oligonucleotides of the present invention designed as GAPmers which contain 2 to 10 LNA units and which especially contain 1 to 5 LNA units at the 5' terminal end and 1 to 5 LNA units at the 3' terminal end of the antisense-oligonucleotide and between the LNA units at least 7 and more preferably at least 8 DNA units. More preferably the antisense-oligonucleotides comprise 2 to 4 LNA units at the 5' terminal end and 2 to 4 LNA units at the 3' terminal end and still more preferred comprise 3 to 4 LNA units at the 5' terminal end and 3 to 4 LNA units at the 3' terminal end and contain preferably at least 7 non-LNA units and most preferably at least 8 non-LNA units such as DNA units in between both LNA segments.

Moreover the antisense-oligonucleotides may contain common nucleobases such as adenine, guanine, cytosine, thymine and uracil as well as common derivatives thereof such as 5-methylcytosine or 2-aminoadenine. The antisense-oligonucleotides of the present invention may also contain modified internucleotide bridges such as phosphorothioate or phosphorodithioate instead of phosphate bridges. Such modifications may be present only in the LNA segments or only in the non-LNA segment of the antisense-oligonucleotide. As LNA units especially the residues b^1 to b^9 as disclosed herein are preferred.

Thus, preferred are antisense-oligonucleotides of the formula (S2):

5'-N^3-**ACGCGTCC**-N^4-3' (Seq. ID No. 98)

wherein
N^3 represents: GGTGGGATCGTGCTGGCGAT-, GTGGGATCGTGCTGGCGAT-, TGGGATCGTGCTGGCGAT-, GGGATCGTGCTGGCGAT-, GGATCGTGCTGGCGAT-, GATCGTGCTGGCGAT-, ATCGTGCTGGCGAT-, TCGTGCTGGCGAT-, CGTGCTGGCGAT-, GTGCTGGCGAT-, TGCTGGCGAT-,

GCTGGCGAT-, CTGGCGAT-, TGGCGAT-, GGCGAT-, GCGAT-, CGAT-, GAT-, AT-, or T-;

and

N^4 represents: -ACAGGACGATGTGCAGCGGC, -ACAGGACGATGTGCAGCGG, -ACAGGACGATGTGCAGCG, -ACAGGACGATGTGCAGC, -ACAGGACGATGTGCAG, -ACAGGACGATGTGCA, -ACAGGACGATGTGC, -ACAGGACGATGTG, -ACAGGACGATGT, -ACAGGACGATG, -ACAGGACGAT, -ACAGGACGA, -ACAGGACG, -ACAGGAC, -ACAGGA, -ACAGG, -ACAG, -ACA, -AC, or -A.

Preferably the antisense-oligonucleotide of general formula (S2) has between 10 and 28 nucleotides and at least one LNA nucleotide at the 3' terminus and at least one LNA nucleotide at the 5' terminus. As LNA nucleotides (LNA units) especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably in the chapter "Preferred LNAs" are suitable and as internucleotides bridges especially these disclosed in the chapter "Internucleotide Linkages (IL)" are suitable.

More preferably the antisense-oligonucleotide of general formula (S2) has between 11 and 24 nucleotides and at least two LNA nucleotides at the 3' terminus and at least two LNA nucleotides at the 5' terminus.

Still more preferably the antisense-oligonucleotide of general formula (S2) has between 12 and 20, more preferably between 13 and 19 and still more preferable between 14 and 18 nucleotides and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 3' terminal end and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 5' terminal end. Preferably the antisense-oligonucleotides are GAPmers of the form LNA segment A – DNA segment – LNA segment B. Preferably the antisense-oligonucleotides contain at least 6, more preferably at least 7 and most preferably at least 8 non-LNA units such as DNA units in between the two LNA segments. Suitable nucleobases for the non-LNA units and the LNA units are disclosed in the chapter "Nucleobases".

Further preferred are antisense-oligonucleotides of the formula (S2):

5'-N^3-**ACGCGTCC**-N^4-3'

wherein

N^3 represents: TGGGATCGTGCTGGCGAT-, GGGATCGTGCTGGCGAT-, GGATCGTGCTGGCGAT-, GATCGTGCTGGCGAT-, ATCGTGCTGGCGAT-, TCGTGCTGGCGAT-, CGTGCTGGCGAT-, GTGCTGGCGAT-, TGCTGGCGAT-,

GCTGGCGAT-, CTGGCGAT-, TGGCGAT-, GGCGAT-, GCGAT-, CGAT-, GAT-, AT-, or T-;

and

N^4 represents: -ACAGGACGATGTGCAGCG, -ACAGGACGATGTGCAGC, -ACAGGACGATGTGCAG, -ACAGGACGATGTGCA, -ACAGGACGATGTGC, -ACAGGACGATGTG, -ACAGGACGATGT, -ACAGGACGATG, -ACAGGACGAT, -ACAGGACGA, -ACAGGACG, -ACAGGAC, -ACAGGA, -ACAGG, -ACAG, -ACA, -AC, or -A.

Also preferred are antisense-oligonucleotides of the formula (S2):

5'-N^3-**ACGCGTCC**-N^4-3'

wherein

N^3 represents: TCGTGCTGGCGAT-, CGTGCTGGCGAT-, GTGCTGGCGAT-, TGCTGGCGAT-, GCTGGCGAT-, CTGGCGAT-, TGGCGAT-, GGCGAT-, GCGAT-, CGAT-, GAT-, AT-, or T-;

and

N^4 represents: -ACAGGACGATGTG, -ACAGGACGATGT, -ACAGGACGATG, -ACAGGACGAT, -ACAGGACGA, -ACAGGACG, -ACAGGAC, -ACAGGA, -ACAGG, -ACAG, -ACA, -AC, or -A.

Also preferred are antisense-oligonucleotides of the formula (S2):

5'-N^3-**ACGCGTCC**-N^4-3'

wherein

N^3 represents: CTGGCGAT-, TGGCGAT-, GGCGAT-, GCGAT-, CGAT-, GAT-, AT-, or T-;

and

N^4 represents: -ACAGGACG, -ACAGGAC, -ACAGGA, -ACAGG, -ACAG, -ACA, -AC, or -A.

Preferably, the present invention is directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^{3A}-**TACGCGTCCA**-N^{4A}-3' (Seq. ID No. 70), wherein

N^{3A} represents: GGTGGGATCGTGCTGGCGA-, GTGGGATCGTGCTGGCGA-, TGGGATCGTGCTGGCGA-, GGGATCGTGCTGGCGA-, GGATCGTGCTGGCGA-, GATCGTGCTGGCGA-, ATCGTGCTGGCGA-, TCGTGCTGGCGA-,

CGTGCTGGCGA-, GTGCTGGCGA-, TGCTGGCGA-, GCTGGCGA-, CTGGCGA-, TGGCGA-, GGCGA-, GCGA-, CGA-, GA-, or A-;

N^{4A} represents: -CAGGACGATGTGCAGCGGC, -CAGGACGATGTGCAGCGG, -CAGGACGATGTGCAGCG, -CAGGACGATGTGCAGC, -CAGGACGATGTGCAG, -CAGGACGATGTGCA, -CAGGACGATGTGC, -CAGGACGATGTG, -CAGGACGATGT, -CAGGACGATG, -CAGGACGAT, -CAGGACGA, -CAGGACG, -CAGGAC, -CAGGA, -CAGG, -CAG, -CA, or -C;

and salts and optical isomers of the antisense-oligonucleotide.

Preferably N^{3A} represents: TGGGATCGTGCTGGCGA-, GGGATCGTGCTGGCGA-, GGATCGTGCTGGCGA-, GATCGTGCTGGCGA-, ATCGTGCTGGCGA-, TCGTGCTGGCGA-, CGTGCTGGCGA-, GTGCTGGCGA-, TGCTGGCGA-, GCTGGCGA-, CTGGCGA-, TGGCGA-, GGCGA-, GCGA-, CGA-, GA-, or A-;

and

N^{4A} represents: -CAGGACGATGTGCAGCG, -CAGGACGATGTGCAGC, -CAGGACGATGTGCAG, -CAGGACGATGTGCA, -CAGGACGATGTGC, -CAGGACGATGTG, -CAGGACGATGT, -CAGGACGATG, -CAGGACGAT, -CAGGACGA, -CAGGACG, -CAGGAC, -CAGGA, -CAGG, -CAG, -CA, or -C.

More preferably N^{3A} represents: TCGTGCTGGCGA-, CGTGCTGGCGA-, GTGCTGGCGA-, TGCTGGCGA-, GCTGGCGA-, CTGGCGA-, TGGCGA-, GGCGA-, GCGA-, CGA-, GA-, or A-; and

N^{4A} represents: -CAGGACGATGTG, -CAGGACGATGT, -CAGGACGATG, -CAGGACGAT, -CAGGACGA, -CAGGACG, -CAGGAC, -CAGGA, -CAGG, -CAG, -CA, or -C.

Still more preferably N^{3A} represents: CTGGCGA-, TGGCGA-, GGCGA-, GCGA-, CGA-, GA-, or A-; and

N^{4A} represents: -CAGGACG, -CAGGAC, -CAGGA, -CAGG, -CAG, -CA, or -C.

Preferably the antisense-oligonucleotide of general formula (S2A / Seq. ID No. 70) has between 12 and 24 nucleotides and at least one LNA nucleotide at the 3' terminus and at least one LNA nucleotide at the 5' terminus. As LNA nucleotides (LNA units) especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably in the chapter "Preferred LNAs" are suitable and as internucleotides bridges especially these disclosed in the chapter "Internucleotide Linkages (IL)" are suitable.

More preferably the antisense-oligonucleotide of general formula (S2A) has between 12 and 22 nucleotides and at least two LNA nucleotides at the 3' terminus and at least two LNA nucleotides at the 5' terminus.

Still more preferably the antisense-oligonucleotide of general formula (S2A) has between 12 and 20, more preferably between 13 and 19 and still more preferable between 14 and 18 nucleotides and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 3' terminal end and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 5' terminal end. Preferably the antisense-oligonucleotides are GAPmers of the form LNA segment A – DNA segment – LNA segment B. Preferably the antisense-oligonucleotides contain at least 6, more preferably at least 7 and most preferably at least 8 non-LNA units such as DNA units in between the two LNA segments. Suitable nucleobases for the non-LNA units and the LNA units are disclosed in the chapter "Nucleobases".

Moreover, the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^{11}-**TGTTTAGG**-N^{12}-3' (Seq. ID No. 10), wherein

N^{11} represents: TGCCCCAGAAGAGCTATTTGGTAG-, GCCCCAGAAGAGCTATTTGGTAG-, CCCCAGAAGAGCTATTTGGTAG-, CCCAGAAGAGCTATTTGGTAG-, CCAGAAGAGCTATTTGGTAG-, CAGAAGAGCTATTTGGTAG-, AGAAGAGCTATTTGGTAG-, GAAGAGCTATTTGGTAG-, AAGAGCTATTTGGTAG-, AGAGCTATTTGGTAG-, GAGCTATTTGGTAG-, AGCTATTTGGTAG-, GCTATTTGGTAG-, CTATTTGGTAG-, TATTTGGTAG-, ATTTGGTAG-, TTTGGTAG-, TTGGTAG-, TGGTAG-, GGTAG-, GTAG-, TAG-, AG- or G-,

N^{12} represents: -GAGCCGTCTTCAGGAATCTTCTCC, -GAGCCGTCTTCAGGAATCTTCTC, -GAGCCGTCTTCAGGAATCTTCT, -GAGCCGTCTTCAGGAATCTTC, -GAGCCGTCTTCAGGAATCTT, -GAGCCGTCTTCAGGAATCT, -GAGCCGTCTTCAGGAATC, -GAGCCGTCTTCAGGAAT, -GAGCCGTCTTCAGGAA, -GAGCCGTCTTCAGGA, -GAGCCGTCTTCAGG, -GAGCCGTCTTCAG, -GAGCCGTCTTCA, -GAGCCGTCTTC, -GAGCCGTCTT, -GAGCCGTCT, -GAGCCGTC, -GAGCCGT, -GAGCCG, -GAGCC, -GAGC, -GAG, -GA, or –G;

and salts and optical isomers of the antisense-oligonucleotide.

The antisense-oligonucleotides of formula S3 (Seq. ID No. 10) preferably comprise 2 to 10 LNA units, more preferably 3 to 9 LNA units and still more preferably 4 to 8 LNA units and also preferably at least 6 non-LNA units, more preferably at least 7 non-

LNA units and most preferably at least 8 non-LNA units. The non-LNA units are preferably DNA units. The LNA units are preferably positioned at the 3' terminal end (also named 3' terminus) and the 5' terminal end (also named 5' terminus). Preferably at least one and more preferably at least two LNA units are present at the 3' terminal end and/or at the 5' terminal end.

Thus, preferred are antisense-oligonucleotides of the present invention designed as GAPmers which contain 2 to 10 LNA units and which especially contain 1 to 5 LNA units at the 5' terminal end and 1 to 5 LNA units at the 3' terminal end of the antisense-oligonucleotide and between the LNA units at least 7 and more preferably at least 8 DNA units. More preferably the antisense-oligonucleotides comprise 2 to 4 LNA units at the 5' terminal end and 2 to 4 LNA units at the 3' terminal end and still more preferred comprise 3 to 4 LNA units at the 5' terminal end and 3 to 4 LNA units at the 3' terminal end and contain preferably at least 7 non-LNA units and most preferably at least 8 non-LNA units such as DNA units in between both LNA segments.

Moreover the antisense-oligonucleotides may contain common nucleobases such as adenine, guanine, cytosine, thymine and uracil as well as common derivatives thereof such as 5-methylcytosine or 2-aminoadenine. The antisense-oligonucleotides of the present invention may also contain modified internucleotide bridges such as phosphorothioate or phosphorodithioate instead of phosphate bridges. Such modifications may be present only in the LNA segments or only in the non-LNA segment of the antisense-oligonucleotide. As LNA units especially the residues b^1 to b^9 as disclosed herein are preferred.

Thus, preferred are antisense-oligonucleotides of the formula (S3):

5'-N^{11}-**TGTTTAGG**-N^{12}-3' (Seq. ID No. 10)

wherein

N^{11} represents: TGCCCCAGAAGAGCTATTTGGTAG-, GCCCCAGAAGAGCTATTTGGTAG-, CCCCAGAAGAGCTATTTGGTAG-, CCCAGAAGAGCTATTTGGTAG-, CCAGAAGAGCTATTTGGTAG-, CAGAAGAGCTATTTGGTAG-, AGAAGAGCTATTTGGTAG-, GAAGAGCTATTTGGTAG-, AAGAGCTATTTGGTAG-, AGAGCTATTTGGTAG-, GAGCTATTTGGTAG-, AGCTATTTGGTAG-, GCTATTTGGTAG-, CTATTTGGTAG-, TATTTGGTAG-, ATTTGGTAG-, TTTGGTAG-, TTGGTAG-, TGGTAG-, GGTAG-, GTAG-, TAG-, AG- or G-,

and

N^{12} represents: -GAGCCGTCTTCAGGAATCTTCTCC,

-GAGCCGTCTTCAGGAATCTTCTC, -GAGCCGTCTTCAGGAATCTTCT, -GAGCCGTCTTCAGGAATCTTC, -GAGCCGTCTTCAGGAATCTT, -GAGCCGTCTTCAGGAATCT, -GAGCCGTCTTCAGGAATC, -GAGCCGTCTTCAGGAAT, -GAGCCGTCTTCAGGAA, -GAGCCGTCTTCAGGA, -GAGCCGTCTTCAGG, -GAGCCGTCTTCAG, -GAGCCGTCTTCA, -GAGCCGTCTTC, -GAGCCGTCTT, -GAGCCGTCT, -GAGCCGTC, -GAGCCGT, -GAGCCG, -GAGCC, -GAGC, -GAG, -GA, or –G.

Preferably the antisense-oligonucleotide of general formula (S3) has between 10 and 28 nucleotides and at least one LNA nucleotide at the 3' terminus and at least one LNA nucleotide at the 5' terminus. As LNA nucleotides (LNA units) especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably in the chapter "Preferred LNAs" are suitable and as internucleotides bridges especially these disclosed in the chapter "Internucleotide Linkages (IL)" are suitable.

More preferably the antisense-oligonucleotide of general formula (S3) has between 11 and 24 nucleotides and at least two LNA nucleotides at the 3' terminus and at least two LNA nucleotides at the 5' terminus.

Still more preferably the antisense-oligonucleotide of general formula (S3) has between 12 and 20, more preferably between 13 and 19 and still more preferable between 14 and 18 nucleotides and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 3' terminal end and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 5' terminal end. Preferably the antisense-oligonucleotides are GAPmers of the form LNA segment A – DNA segment – LNA segment B. Preferably the antisense-oligonucleotides contain at least 6, more preferably at least 7 and most preferably at least 8 non-LNA units such as DNA units in between the two LNA segments. Suitable nucleobases for the non-LNA units and the LNA units are disclosed in the chapter "Nucleobases".

Further preferred are antisense-oligonucleotides of the formula (S3):

5'-N^{11}-**TGTTTAGG**-N^{12}-3'

wherein

N^{11} represents: AGAAGAGCTATTTGGTAG-, GAAGAGCTATTTGGTAG-, AAGAGCTATTTGGTAG-, AGAGCTATTTGGTAG-, GAGCTATTTGGTAG-, AGCTATTTGGTAG-, GCTATTTGGTAG-, CTATTTGGTAG-, TATTTGGTAG-, ATTTGGTAG-, TTTGGTAG-, TTGGTAG-, TGGTAG-, GGTAG-, GTAG-, TAG-, AG- or G-;

and

N^{12} represents: -GAGCCGTCTTCAGGAATC, -GAGCCGTCTTCAGGAAT, -GAGCCGTCTTCAGGAA, -GAGCCGTCTTCAGGA, -GAGCCGTCTTCAGG, -GAGCCGTCTTCAG, -GAGCCGTCTTCA, -GAGCCGTCTTC, -GAGCCGTCTT, -GAGCCGTCT, -GAGCCGTC, -GAGCCGT, -GAGCCG, -GAGCC, -GAGC, -GAG, -GA, or –G.

Also preferred are antisense-oligonucleotides of the formula (S3):

5'-N^{11}-**TGTTTAGG**-N^{12}-3'

wherein

N^{11} represents: AGCTATTTGGTAG-, GCTATTTGGTAG-, CTATTTGGTAG-, TATTTGGTAG-, ATTTGGTAG-, TTTGGTAG-, TTGGTAG-, TGGTAG-, GGTAG-, GTAG-, TAG-, AG- or G-;

and

N^{12} represents: -GAGCCGTCTTCAG, -GAGCCGTCTTCA, -GAGCCGTCTTC, -GAGCCGTCTT, -GAGCCGTCT, -GAGCCGTC, -GAGCCGT, -GAGCCG, -GAGCC, -GAGC, -GAG, -GA, or –G.

Also preferred are antisense-oligonucleotides of the formula (S3):

5'-N^{11}-**TGTTTAGG**-N^{12}-3'

wherein

N^{11} represents: TTTGGTAG-, TTGGTAG-, TGGTAG-, GGTAG-, GTAG-, TAG-, AG- or G-; and

N^{12} represents: -GAGCCGTC, -GAGCCGT, -GAGCCG, -GAGCC, -GAGC, -GAG, -GA, or –G.

Preferably, the present invention is directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^{11A}-**GTGTTTAGGG**-N^{12A}-3' (Seq. ID No. 71), wherein

N^{11A} represents: TGCCCCAGAAGAGCTATTTGGTA-, GCCCCAGAAGAGCTATTTGGTA-, CCCCAGAAGAGCTATTTGGTA-, CCCAGAAGAGCTATTTGGTA-, CCAGAAGAGCTATTTGGTA-, CAGAAGAGCTATTTGGTA-, AGAAGAGCTATTTGGTA-, GAAGAGCTATTTGGTA-, AAGAGCTATTTGGTA-, AGAGCTATTTGGTA-, GAGCTATTTGGTA-, AGCTATTTGGTA-, GCTATTTGGTA-, CTATTTGGTA-, TATTTGGTA-, ATTTGGTA-, TTTGGTA-, TTGGTA-, TGGTA-, GGTA-, GTA-, TA-, or A-,

N^{12A} represents: -AGCCGTCTTCAGGAATCTTCTCC, -AGCCGTCTTCAGGAATCTTCTC, -AGCCGTCTTCAGGAATCTTCT, -AGCCGTCTTCAGGAATCTTC, -AGCCGTCTTCAGGAATCTT, -AGCCGTCTTCAGGAATCT, -AGCCGTCTTCAGGAATC, -AGCCGTCTTCAGGAAT, -AGCCGTCTTCAGGAA, -AGCCGTCTTCAGGA, -AGCCGTCTTCAGG, -AGCCGTCTTCAG, -AGCCGTCTTCA, -AGCCGTCTTC, -AGCCGTCTT, -AGCCGTCT, -AGCCGTC, -AGCCGT, -AGCCG, -AGCC, -AGC, -AG, or -A;
and salts and optical isomers of the antisense-oligonucleotide.

Preferably N^{11A} represents: AGAAGAGCTATTTGGTA-, GAAGAGCTATTTGGTA-, AAGAGCTATTTGGTA-, AGAGCTATTTGGTA-, GAGCTATTTGGTA-, AGCTATTTGGTA-, GCTATTTGGTA-, CTATTTGGTA-, TATTTGGTA-, ATTTGGTA-, TTTGGTA-, TTGGTA-, TGGTA-, GGTA-, GTA-, TA-, or A-; and
N^{12A} represents: -AGCCGTCTTCAGGAATC, -AGCCGTCTTCAGGAAT, -AGCCGTCTTCAGGAA, -AGCCGTCTTCAGGA, -AGCCGTCTTCAGG, -AGCCGTCTTCAG, -AGCCGTCTTCA, -AGCCGTCTTC, -AGCCGTCTT, -AGCCGTCT, -AGCCGTC, -AGCCGT, -AGCCG, -AGCC, -AGC, -AG, or -A.

More preferably N^{11A} represents: AGCTATTTGGTA-, GCTATTTGGTA-, CTATTTGGTA-, TATTTGGTA-, ATTTGGTA-, TTTGGTA-, TTGGTA-, TGGTA-, GGTA-, GTA-, TA-, or A-; and
N^{12A} represents: -AGCCGTCTTCAG, -AGCCGTCTTCA, -AGCCGTCTTC, -AGCCGTCTT, -AGCCGTCT, -AGCCGTC, -AGCCGT, -AGCCG, -AGCC, -AGC, -AG, or -A.
Still more preferably N^{11A} represents: TTTGGTA-, TTGGTA-, TGGTA-, GGTA-, GTA-, TA-, or A-; and
N^{12A} represents: -AGCCGTC, -AGCCGT, -AGCCG, -AGCC, -AGC, -AG, or -A.

Preferably the antisense-oligonucleotide of general formula (S3A / Seq. ID No. 71) has between 12 and 24 nucleotides and at least one LNA nucleotide at the 3' terminus and at least one LNA nucleotide at the 5' terminus. As LNA nucleotides (LNA units) especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably in the chapter "Preferred LNAs" are suitable and as internucleotides bridges especially these disclosed in the chapter "Internucleotide Linkages (IL)" are suitable.

More preferably the antisense-oligonucleotide of general formula (S3A) has between 12 and 22 nucleotides and at least two LNA nucleotides at the 3' terminus and at least two LNA nucleotides at the 5' terminus.

Still more preferably the antisense-oligonucleotide of general formula (S3A) has between 12 and 20, more preferably between 13 and 19 and still more preferable between 14 and 18 nucleotides and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 3' terminal end and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 5' terminal end. Preferably the antisense-oligonucleotides are GAPmers of the form LNA segment A – DNA segment – LNA segment B. Preferably the antisense-oligonucleotides contain at least 6, more preferably at least 7 and most preferably at least 8 non-LNA units such as DNA units in between the two LNA segments. Suitable nucleobases for the non-LNA units and the LNA units are disclosed in the chapter "Nucleobases".

Moreover, the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^5-**TTTGGTAG**-N^6-3' (Seq. ID No. 11), wherein

N^5 represents: GCCCAGCCTGCCCCAGAAGAGCTA-, CCCAGCCTGCCCCAGAAGAGCTA-, CCAGCCTGCCCCAGAAGAGCTA-, CAGCCTGCCCCAGAAGAGCTA-, AGCCTGCCCCAGAAGAGCTA-, GCCTGCCCCAGAAGAGCTA-, CCTGCCCCAGAAGAGCTA-, CTGCCCCAGAAGAGCTA-, TGCCCCAGAAGAGCTA-, GCCCCAGAAGAGCTA-, CCCCAGAAGAGCTA-, CCCAGAAGAGCTA-, CCAGAAGAGCTA-, CAGAAGAGCTA-, AGAAGAGCTA-, GAAGAGCTA-, AAGAGCTA-, AGAGCTA-, GAGCTA-, AGCTA-, GCTA-, CTA-, TA-, or A-;

N^6 represents: -TGTTTAGGGAGCCGTCTTCAGGAA, -TGTTTAGGGAGCCGTCTTCAGGA, -TGTTTAGGGAGCCGTCTTCAGG, -TGTTTAGGGAGCCGTCTTCAG, -TGTTTAGGGAGCCGTCTTCA, -TGTTTAGGGAGCCGTCTTC, -TGTTTAGGGAGCCGTCTT, -TGTTTAGGGAGCCGTCT, -TGTTTAGGGAGCCGTC, -TGTTTAGGGAGCCGT, -TGTTTAGGGAGCCG, -TGTTTAGGGAGCC, -TGTTTAGGGAGC, -TGTTTAGGGAG, -TGTTTAGGGA, -TGTTTAGGG, -TGTTTAGG, -TGTTTAG, -TGTTTA, -TGTTT, -TGTT, -TGT, -TG, or –T;

and salts and optical isomers of the antisense-oligonucleotide.

The antisense-oligonucleotides of formula S4 (Seq. ID No. 11) preferably comprise 2 to 10 LNA units, more preferably 3 to 9 LNA units and still more preferably 4 to 8 LNA units and also preferably at least 6 non-LNA units, more preferably at least 7 non-LNA units and most preferably at least 8 non-LNA units. The non-LNA units are preferably DNA units. The LNA units are preferably positioned at the 3' terminal end (also named 3' terminus) and the 5' terminal end (also named 5' terminus). Preferably at least one and more preferably at least two LNA units are present at the 3' terminal end and/or at the 5' terminal end.

Thus, preferred are antisense-oligonucleotides of the present invention designed as GAPmers which contain 2 to 10 LNA units and which especially contain 1 to 5 LNA units at the 5' terminal end and 1 to 5 LNA units at the 3' terminal end of the antisense-oligonucleotide and between the LNA units at least 7 and more preferably at least 8 DNA units. More preferably the antisense-oligonucleotides comprise 2 to 4 LNA units at the 5' terminal end and 2 to 4 LNA units at the 3' terminal end and still more preferred comprise 3 to 4 LNA units at the 5' terminal end and 3 to 4 LNA units at the 3' terminal end and contain preferably at least 7 non-LNA units and most preferably at least 8 non-LNA units such as DNA units in between both LNA segments.

Moreover the antisense-oligonucleotides may contain common nucleobases such as adenine, guanine, cytosine, thymine and uracil as well as common derivatives thereof such as 5-methylcytosine or 2-aminoadenine. The antisense-oligonucleotides of the present invention may also contain modified internucleotide bridges such as phosphorothioate or phosphorodithioate instead of phosphate bridges. Such modifications may be present only in the LNA segments or only in the non-LNA segment of the antisense-oligonucleotide. As LNA units especially the residues b^1 to b^9 as disclosed herein are preferred.

Thus, preferred are antisense-oligonucleotides of the formula (S4):

5'-N^5-**TTTGGTAG**-N^6-3' (Seq. ID No. 11)

wherein

N^5 represents: GCCCAGCCTGCCCCAGAAGAGCTA-, CCCAGCCTGCCCCAGAAGAGCTA-, CCAGCCTGCCCCAGAAGAGCTA-, CAGCCTGCCCCAGAAGAGCTA-, AGCCTGCCCCAGAAGAGCTA-, GCCTGCCCCAGAAGAGCTA-, CCTGCCCCAGAAGAGCTA-, CTGCCCCAGAAGAGCTA-, TGCCCCAGAAGAGCTA-, GCCCCAGAAGAGCTA-, CCCCAGAAGAGCTA-, CCCAGAAGAGCTA-, CCAGAAGAGCTA-, CAGAAGAGCTA-, AGAAGAGCTA-, GAAGAGCTA-, AAGAGCTA-, AGAGCTA-, GAGCTA-, AGCTA-, GCTA-, CTA-, TA-, or A-;

and

N^6 is selected from: -TGTTTAGGGAGCCGTCTTCAGGAA, -TGTTTAGGGAGCCGTCTTCAGGA, -TGTTTAGGGAGCCGTCTTCAGG, -TGTTTAGGGAGCCGTCTTCAG, -TGTTTAGGGAGCCGTCTTCA, -TGTTTAGGGAGCCGTCTTC, -TGTTTAGGGAGCCGTCTT, -TGTTTAGGGAGCCGTCT, -TGTTTAGGGAGCCGTC, -TGTTTAGGGAGCCGT, -TGTTTAGGGAGCCG, -TGTTTAGGGAGCC, -TGTTTAGGGAGC, -TGTTTAGGGAG, -TGTTTAGGGA, -TGTTTAGGG, -TGTTTAGG, -TGTTTAG, -TGTTTA, -TGTTT, -TGTT, -TGT, -TG, or -T.

Preferably the antisense-oligonucleotide of general formula (S4) has between 10 and 28 nucleotides and at least one LNA nucleotide at the 3' terminus and at least one LNA nucleotide at the 5' terminus. As LNA nucleotides (LNA units) especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably in the chapter "Preferred LNAs" are suitable and as internucleotides bridges especially these disclosed in the chapter "Internucleotide Linkages (IL)" are suitable.

More preferably the antisense-oligonucleotide of general formula (S4) has between 11 and 24 nucleotides and at least two LNA nucleotides at the 3' terminus and at least two LNA nucleotides at the 5' terminus.
Still more preferably the antisense-oligonucleotide of general formula (S4) has between 12 and 20, more preferably between 13 and 19 and still more preferable between 14 and 18 nucleotides and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 3' terminal end and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 5' terminal end. Preferably the antisense-oligonucleotides are GAPmers of the form LNA segment A – DNA segment – LNA segment B. Preferably the antisense-oligonucleotides contain at least 6, more preferably at least 7 and most preferably at least 8 non-LNA units such as DNA units in between the two LNA segments. Suitable nucleobases for the non-LNA units and the LNA units are disclosed in the chapter "Nucleobases".

Further preferred are antisense-oligonucleotides of the formula (S4):

5'-N^5-**TTTGGTAG**-N^6-3'

wherein

N^5 represents: CCTGCCCCAGAAGAGCTA-, CTGCCCCAGAAGAGCTA-, TGCCCCAGAAGAGCTA-, GCCCCAGAAGAGCTA-, CCCCAGAAGAGCTA-, CCCAGAAGAGCTA-, CCAGAAGAGCTA-, CAGAAGAGCTA-, AGAAGAGCTA-,

GAAGAGCTA-, AAGAGCTA-, AGAGCTA-, GAGCTA-, AGCTA-, GCTA-, CTA-, TA-, or A-; and

N^6 is selected from: -TGTTTAGGGAGCCGTCTT, -TGTTTAGGGAGCCGTCT, -TGTTTAGGGAGCCGTC, -TGTTTAGGGAGCCGT, -TGTTTAGGGAGCCG, -TGTTTAGGGAGCC, -TGTTTAGGGAGC, -TGTTTAGGGAG, -TGTTTAGGGA, -TGTTTAGGG, -TGTTTAGG, -TGTTTAG, -TGTTTA, -TGTTT, -TGTT, -TGT, -TG, or -T.

Also preferred are antisense-oligonucleotides of the formula (S4):

5'-N^5-**TTTGGTAG**-N^6-3'

wherein

N^5 represents: CCCAGAAGAGCTA-, CCAGAAGAGCTA-, CAGAAGAGCTA-, AGAAGAGCTA-, GAAGAGCTA-, AAGAGCTA-, AGAGCTA-, GAGCTA-, AGCTA-, GCTA-, CTA-, TA-, or A-; and

N^6 is selected from: -TGTTTAGGGAGCC, -TGTTTAGGGAGC, -TGTTTAGGGAG, -TGTTTAGGGA, -TGTTTAGGG, -TGTTTAGG, -TGTTTAG, -TGTTTA, -TGTTT, -TGTT, -TGT, -TG, or -T.

Also preferred are antisense-oligonucleotides of the formula (S4):

5'-N^5-**TTTGGTAG**-N^6-3'

wherein

N^5 represents: AAGAGCTA-, AGAGCTA-, GAGCTA-, AGCTA-, GCTA-, CTA-, TA-, or A-; and

N^6 is selected from: -TGTTTAGG, -TGTTTAG, -TGTTTA, -TGTTT, -TGTT, -TGT, -TG, or -T.

Preferably, the present invention is directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^{5A}-**ATTTGGTAGT**-N^{6A}-3' (Seq. ID No. 72), wherein

N^{5A} represents: GCCCAGCCTGCCCCAGAAGAGCT-, CCCAGCCTGCCCCAGAAGAGCT-, CCAGCCTGCCCCAGAAGAGCT-, CAGCCTGCCCCAGAAGAGCT-, AGCCTGCCCCAGAAGAGCT-, GCCTGCCCCAGAAGAGCT-, CCTGCCCCAGAAGAGCT-, CTGCCCCAGAAGAGCT-, TGCCCCAGAAGAGCT-, GCCCCAGAAGAGCT-, CCCCAGAAGAGCT-, CCCAGAAGAGCT-, CCAGAAGAGCT-, CAGAAGAGCT-,

AGAAGAGCT-, GAAGAGCT-, AAGAGCT-, AGAGCT-, GAGCT-, AGCT-, GCT-, CT-, or T-; and

N^{6A} represents: -GTTTAGGGAGCCGTCTTCAGGAA, -GTTTAGGGAGCCGTCTTCAGGA, -GTTTAGGGAGCCGTCTTCAGG, -GTTTAGGGAGCCGTCTTCAG, -GTTTAGGGAGCCGTCTTCA, -GTTTAGGGAGCCGTCTTC, -GTTTAGGGAGCCGTCTT, -GTTTAGGGAGCCGTCT, -GTTTAGGGAGCCGTC, -GTTTAGGGAGCCGT, -GTTTAGGGAGCCG, -GTTTAGGGAGCC, -GTTTAGGGAGC, -GTTTAGGGAG, -GTTTAGGGA, -GTTTAGGG, -GTTTAGG, -GTTTAG, -GTTTA, -GTTT, -GTT, -GT, or -G;

and salts and optical isomers of the antisense-oligonucleotide.

Preferably N^{5A} represents: CCTGCCCCAGAAGAGCT-, CTGCCCCAGAAGAGCT-, TGCCCCAGAAGAGCT-, GCCCCAGAAGAGCT-, CCCCAGAAGAGCT-, CCCAGAAGAGCT-, CCAGAAGAGCT-, CAGAAGAGCT-, AGAAGAGCT-, GAAGAGCT-, AAGAGCT-, AGAGCT-, GAGCT-, AGCT-, GCT-, CT-, or T-; and

N^{6A} represents: -GTTTAGGGAGCCGTCTT, -GTTTAGGGAGCCGTCT, -GTTTAGGGAGCCGTC, -GTTTAGGGAGCCGT, -GTTTAGGGAGCCG, -GTTTAGGGAGCC, -GTTTAGGGAGC, -GTTTAGGGAG, -GTTTAGGGA, -GTTTAGGG, -GTTTAGG, -GTTTAG, -GTTTA, -GTTT, -GTT, -GT, or -G.

More preferably N^{5A} represents: CCCAGAAGAGCT-, CCAGAAGAGCT-, CAGAAGAGCT-, AGAAGAGCT-, GAAGAGCT-, AAGAGCT-, AGAGCT-, GAGCT-, AGCT-, GCT-, CT-, or T-; and

N^{6A} represents: -GTTTAGGGAGCC, -GTTTAGGGAGC, -GTTTAGGGAG, -GTTTAGGGA, -GTTTAGGG, -GTTTAGG, -GTTTAG, -GTTTA, -GTTT, -GTT, -GT, or -G.

Still more preferably N^{5A} represents: AAGAGCT-, AGAGCT-, GAGCT-, AGCT-, GCT-, CT-, or T-; and

N^{6A} represents: -GTTTAGG, -GTTTAG, -GTTTA, -GTTT, -GTT, -GT, or -G.

Preferably the antisense-oligonucleotide of general formula (S4A / Seq. ID No. 72) has between 12 and 24 nucleotides and at least one LNA nucleotide at the 3' terminus and at least one LNA nucleotide at the 5' terminus. As LNA nucleotides (LNA units) especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably in the chapter "Preferred LNAs" are suitable and as internucleotides bridges especially these disclosed in the chapter "Internucleotide Linkages (IL)" are suitable.

More preferably the antisense-oligonucleotide of general formula (S4A) has between 12 and 22 nucleotides and at least two LNA nucleotides at the 3' terminus and at least two LNA nucleotides at the 5' terminus.

Still more preferably the antisense-oligonucleotide of general formula (S4A) has between 12 and 20, more preferably between 13 and 19 and still more preferable between 14 and 18 nucleotides and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 3' terminal end and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 5' terminal end. Preferably the antisense-oligonucleotides are GAPmers of the form LNA segment A – DNA segment – LNA segment B. Preferably the antisense-oligonucleotides contain at least 6, more preferably at least 7 and most preferably at least 8 non-LNA units such as DNA units in between the two LNA segments. Suitable nucleobases for the non-LNA units and the LNA units are disclosed in the chapter "Nucleobases".

Moreover, the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^7-**AATGGACC**-N^8-3' (Seq. ID No. 100), wherein

N^7 represents: TGAATCTTGAATATCTCATG-, GAATCTTGAATATCTCATG-, AATCTTGAATATCTCATG-, ATCTTGAATATCTCATG-, TCTTGAATATCTCATG-, CTTGAATATCTCATG-, TTGAATATCTCATG-, TGAATATCTCATG-, GAATATCTCATG-, AATATCTCATG-, ATATCTCATG-, TATCTCATG-, ATCTCATG-, TCTCATG-, CTCATG-, TCATG-, CATG-, ATG-, TG-, or G-;

N^8 represents: -AGTATTCTAGAAACTCACCA, -AGTATTCTAGAAACTCACC, -AGTATTCTAGAAACTCAC, -AGTATTCTAGAAACTCA, -AGTATTCTAGAAACTC, -AGTATTCTAGAAACT, -AGTATTCTAGAAAC, -AGTATTCTAGAAA, -AGTATTCTAGAA, -AGTATTCTAGA, -AGTATTCTAG, -AGTATTCTA, -AGTATTCT, -AGTATTC, -AGTATT, -AGTAT, -AGTA, -AGT, -AG, or -A;

and salts and optical isomers of the antisense-oligonucleotide.

The antisense-oligonucleotides of formula S6 (Seq. ID No. 100) preferably comprise 2 to 10 LNA units, more preferably 3 to 9 LNA units and still more preferably 4 to 8 LNA units and also preferably at least 6 non-LNA units, more preferably at least 7 non-LNA units and most preferably at least 8 non-LNA units. The non-LNA units are preferably DNA units. The LNA units are preferably positioned at the 3' terminal end

(also named 3' terminus) and the 5' terminal end (also named 5' terminus). Preferably at least one and more preferably at least two LNA units are present at the 3' terminal end and/or at the 5' terminal end.

Thus, preferred are antisense-oligonucleotides of the present invention designed as GAPmers which contain 2 to 10 LNA units and which especially contain 1 to 5 LNA units at the 5' terminal end and 1 to 5 LNA units at the 3' terminal end of the antisense-oligonucleotide and between the LNA units at least 7 and more preferably at least 8 DNA units. More preferably the antisense-oligonucleotides comprise 2 to 4 LNA units at the 5' terminal end and 2 to 4 LNA units at the 3' terminal end and still more preferred comprise 3 to 4 LNA units at the 5' terminal end and 3 to 4 LNA units at the 3' terminal end and contain preferably at least 7 non-LNA units and most preferably at least 8 non-LNA units such as DNA units in between both LNA segments.

Moreover the antisense-oligonucleotides may contain common nucleobases such as adenine, guanine, cytosine, thymine and uracil as well as common derivatives thereof such as 5-methylcytosine or 2-aminoadenine. The antisense-oligonucleotides of the present invention may also contain modified internucleotide bridges such as phosphorothioate or phosphorodithioate instead of phosphate bridges. Such modifications may be present only in the LNA segments or only in the non-LNA segment of the antisense-oligonucleotide. As LNA units especially the residues b^1 to b^9 as disclosed herein are preferred.

Thus, preferred are antisense-oligonucleotides of the formula (S6):

5'-N^7-**AATGGACC**-N^8-3' (Seq. ID No. 100)

wherein

N^7 represents: TGAATCTTGAATATCTCATG-, GAATCTTGAATATCTCATG-, AATCTTGAATATCTCATG-, ATCTTGAATATCTCATG-, TCTTGAATATCTCATG-, CTTGAATATCTCATG-, TTGAATATCTCATG-, TGAATATCTCATG-, GAATATCTCATG-, AATATCTCATG-, ATATCTCATG-, TATCTCATG-, ATCTCATG-, TCTCATG-, CTCATG-, TCATG-, CATG-, ATG-, TG-, or G-;

and

N^8 is selected from: -AGTATTCTAGAAACTCACCA, -AGTATTCTAGAAACTCACC, -AGTATTCTAGAAACTCAC, -AGTATTCTAGAAACTCA, -AGTATTCTAGAAACTC, -AGTATTCTAGAAACT, -AGTATTCTAGAAAC, -AGTATTCTAGAAA, -AGTATTCTAGAA, -AGTATTCTAGA, -AGTATTCTAG, -AGTATTCTA, -AGTATTCT, -AGTATTC, -AGTATT, -AGTAT, -AGTA, -AGT, -AG, or -A.

Preferably the antisense-oligonucleotide of general formula (S6) has between 10 and 28 nucleotides and at least one LNA nucleotide at the 3' terminus and at least one LNA nucleotide at the 5' terminus. As LNA nucleotides (LNA units) especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably in the chapter "Preferred LNAs" are suitable and as internucleotides bridges especially these disclosed in the chapter "Internucleotide Linkages (IL)" are suitable.

More preferably the antisense-oligonucleotide of general formula (S6) has between 11 and 24 nucleotides and at least two LNA nucleotides at the 3' terminus and at least two LNA nucleotides at the 5' terminus.
Still more preferably the antisense-oligonucleotide of general formula (S6) has between 12 and 20, more preferably between 13 and 19 and still more preferable between 14 and 18 nucleotides and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 3' terminal end and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 5' terminal end. Preferably the antisense-oligonucleotides are GAPmers of the form LNA segment A – DNA segment – LNA segment B. Preferably the antisense-oligonucleotides contain at least 6, more preferably at least 7 and most preferably at least 8 non-LNA units such as DNA units in between the two LNA segments. Suitable nucleobases for the non-LNA units and the LNA units are disclosed in the chapter "Nucleobases".

Further preferred are antisense-oligonucleotides of the formula (S6):

5'-N^7-**AATGGACC**-N^8-3'

wherein
N^7 represents: AATCTTGAATATCTCATG-, ATCTTGAATATCTCATG-, TCTTGAATATCTCATG-, CTTGAATATCTCATG-, TTGAATATCTCATG-, TGAATATCTCATG-, GAATATCTCATG-, AATATCTCATG-, ATATCTCATG-, TATCTCATG-, ATCTCATG-, TCTCATG-, CTCATG-, TCATG-, CATG-, ATG-, TG-, or G-; and
N^8 is selected from: -AGTATTCTAGAAACTCAC, -AGTATTCTAGAAACTCA, -AGTATTCTAGAAACTC, -AGTATTCTAGAAACT, -AGTATTCTAGAAAC, -AGTATTCTAGAAA, -AGTATTCTAGAA, -AGTATTCTAGA, -AGTATTCTAG, -AGTATTCTA, -AGTATTCT, -AGTATTC, -AGTATT, -AGTAT, -AGTA, -AGT, -AG, or -A.

Also preferred are antisense-oligonucleotides of the formula (S6):

5'-N^7-**AATGGACC**-N^8-3'

wherein

N^7 represents: TGAATATCTCATG-, GAATATCTCATG-, AATATCTCATG-, ATATCTCATG-, TATCTCATG-, ATCTCATG-, TCTCATG-, CTCATG-, TCATG-, CATG-, ATG-, TG-, or G-; and

N^8 is selected from: -AGTATTCTAGAAA, -AGTATTCTAGAA, -AGTATTCTAGA, -AGTATTCTAG, -AGTATTCTA, -AGTATTCT, -AGTATTC, -AGTATT, -AGTAT, -AGTA, -AGT, -AG, or -A.

Also preferred are antisense-oligonucleotides of the formula (S6):

5'-N^7-**AATGGACC**-N^8-3'

wherein

N^7 represents: ATCTCATG-, TCTCATG-, CTCATG-, TCATG-, CATG-, ATG-, TG-, or G-; and

N^8 is selected from: -AGTATTCT, -AGTATTC, -AGTATT, -AGTAT, -AGTA, -AGT, -AG, or -A.

Preferably, the present invention is directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^{7A}-**GAATGGACCA**-N^{8A}-3' (Seq. ID No. 73), wherein

N^{7A} represents: TGAATCTTGAATATCTCAT-, GAATCTTGAATATCTCAT-, AATCTTGAATATCTCAT-, ATCTTGAATATCTCAT-, TCTTGAATATCTCAT-, CTTGAATATCTCAT-, TTGAATATCTCAT-, TGAATATCTCAT-, GAATATCTCAT-, AATATCTCAT-, ATATCTCAT-, TATCTCAT-, ATCTCAT-, TCTCAT-, CTCAT-, TCAT-, CAT-, AT-, or T-;

N^{8A} represents: -GTATTCTAGAAACTCACCA, -GTATTCTAGAAACTCACC, -GTATTCTAGAAACTCAC, -GTATTCTAGAAACTCA, -GTATTCTAGAAACTC, -GTATTCTAGAAACT, -GTATTCTAGAAAC, -GTATTCTAGAAA, -GTATTCTAGAA, -GTATTCTAGA, -GTATTCTAG, -GTATTCTA, -GTATTCT, -GTATTC, -GTATT, -GTAT, -GTA, -GT, or -G;

and salts and optical isomers of the antisense-oligonucleotide.

Preferably N^{7A} represents: AATCTTGAATATCTCAT-, ATCTTGAATATCTCAT-, TCTTGAATATCTCAT-, CTTGAATATCTCAT-, TTGAATATCTCAT-, TGAATATCTCAT-, GAATATCTCAT-, AATATCTCAT-, ATATCTCAT-, TATCTCAT-, ATCTCAT-, TCTCAT-, CTCAT-, TCAT-, CAT-, AT-, or T-;

and

N^{8A} represents: -GTATTCTAGAAACTCAC, -GTATTCTAGAAACTCA, -GTATTCTAGAAACTC, -GTATTCTAGAAACT, -GTATTCTAGAAAC, -GTATTCTAGAAA, -GTATTCTAGAA, -GTATTCTAGA, -GTATTCTAG, -GTATTCTA, -GTATTCT, -GTATTC, -GTATT, -GTAT, -GTA, -GT, or -G.

More preferably N^{7A} represents: TGAATATCTCAT-, GAATATCTCAT-, AATATCTCAT-, ATATCTCAT-, TATCTCAT-, ATCTCAT-, TCTCAT-, CTCAT-, TCAT-, CAT-, AT-, or T-; and
N^{8A} represents: -GTATTCTAGAAA, -GTATTCTAGAA, -GTATTCTAGA, -GTATTCTAG, -GTATTCTA, -GTATTCT, -GTATTC, -GTATT, -GTAT, -GTA, -GT, or -G.

Still more preferably N^{7A} represents: ATCTCAT-, TCTCAT-, CTCAT-, TCAT-, CAT-, AT-, or T-; and
N^{8A} represents: -GTATTCT, -GTATTC, -GTATT, -GTAT, -GTA, -GT, or -G.

Preferably the antisense-oligonucleotide of general formula (S6A / Seq. ID No. 73) has between 12 and 24 nucleotides and at least one LNA nucleotide at the 3' terminus and at least one LNA nucleotide at the 5' terminus. As LNA nucleotides (LNA units) especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably in the chapter "Preferred LNAs" are suitable and as internucleotides bridges especially these disclosed in the chapter "Internucleotide Linkages (IL)" are suitable.

More preferably the antisense-oligonucleotide of general formula (S6A) has between 12 and 22 nucleotides and at least two LNA nucleotides at the 3' terminus and at least two LNA nucleotides at the 5' terminus.
Still more preferably the antisense-oligonucleotide of general formula (S6A) has between 12 and 20, more preferably between 13 and 19 and still more preferable between 14 and 18 nucleotides and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 3' terminal end and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 5' terminal end. Preferably the antisense-oligonucleotides are GAPmers of the form LNA segment A – DNA segment – LNA segment B. Preferably the antisense-oligonucleotides contain at least 6, more preferably at least 7 and most preferably at least 8 non-LNA units such as DNA units in between the two LNA segments. Suitable nucleobases for the non-LNA units and the LNA units are disclosed in the chapter "Nucleobases".

Moreover, the present invention is directed to antisense-oligonucleotide(s) consisting of 10 to 28 nucleotides and at least two of the 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^9-**ATTAATAA**-N^{10}-3' (Seq. ID No. 101), wherein

N^9 represents: ATTCATATTTATATACAGGC-, TTCATATTTATATACAGGC-, TCATATTTATATACAGGC-, CATATTTATATACAGGC-, ATATTTATATACAGGC-, TATTTATATACAGGC-, ATTTATATACAGGC-, TTTATATACAGGC-, TTATATACAGGC-, TATATACAGGC-, ATATACAGGC-, TATACAGGC-, ATACAGGC-, TACAGGC-, ACAGGC-, CAGGC-, AGGC-, GGC-, GC-, or C-;

N^{10} represents: -AGTGCAAATGTTATTGGCTA, -AGTGCAAATGTTATTGGCT, -AGTGCAAATGTTATTGGC, -AGTGCAAATGTTATTGG, -AGTGCAAATGTTATTG, -AGTGCAAATGTTATT, -AGTGCAAATGTTAT, -AGTGCAAATGTTA, -AGTGCAAATGTT, -AGTGCAAATGT, -AGTGCAAATG, -AGTGCAAAT, -AGTGCAAA, -AGTGCAA, -AGTGCA, -AGTGC, -AGTG, -AGT, -AG, or -A;

and salts and optical isomers of the antisense-oligonucleotide.

The antisense-oligonucleotides of formula S7 (Seq. ID No. 101) preferably comprise 2 to 10 LNA units, more preferably 3 to 9 LNA units and still more preferably 4 to 8 LNA units and also preferably at least 6 non-LNA units, more preferably at least 7 non-LNA units and most preferably at least 8 non-LNA units. The non-LNA units are preferably DNA units. The LNA units are preferably positioned at the 3' terminal end (also named 3' terminus) and the 5' terminal end (also named 5' terminus). Preferably at least one and more preferably at least two LNA units are present at the 3' terminal end and/or at the 5' terminal end.

Thus, preferred are antisense-oligonucleotides of the present invention designed as GAPmers which contain 2 to 10 LNA units and which especially contain 1 to 5 LNA units at the 5' terminal end and 1 to 5 LNA units at the 3' terminal end of the antisense-oligonucleotide and between the LNA units at least 7 and more preferably at least 8 DNA units. More preferably the antisense-oligonucleotides comprise 2 to 4 LNA units at the 5' terminal end and 2 to 4 LNA units at the 3' terminal end and still more preferred comprise 3 to 4 LNA units at the 5' terminal end and 3 to 4 LNA units at the 3' terminal end and contain preferably at least 7 non-LNA units and most preferably at least 8 non-LNA units such as DNA units in between both LNA segments.

Moreover the antisense-oligonucleotides may contain common nucleobases such as adenine, guanine, cytosine, thymine and uracil as well as common derivatives

thereof such as 5-methylcytosine or 2-aminoadenine. The antisense-oligonucleotides of the present invention may also contain modified internucleotide bridges such as phosphorothioate or phosphorodithioate instead of phosphate bridges. Such modifications may be present only in the LNA segments or only in the non-LNA segment of the antisense-oligonucleotide. As LNA units especially the residues b^1 to b^9 as disclosed herein are preferred.

Thus, preferred are antisense-oligonucleotides of the formula (S7):

5'-N^9-**ATTAATAA**-N^{10}-3' (Seq. ID No. 101)

wherein

N^9 represents: ATTCATATTTATATACAGGC-, TTCATATTTATATACAGGC-, TCATATTTATATACAGGC-, CATATTTATATACAGGC-, ATATTTATATACAGGC-, TATTTATATACAGGC-, ATTTATATACAGGC-, TTTATATACAGGC-, TTATATACAGGC-, TATATACAGGC-, ATATACAGGC-, TATACAGGC-, ATACAGGC-, TACAGGC-, ACAGGC-, CAGGC-, AGGC-, GGC-, GC-, or C-;

and

N^{10} is selected from: -AGTGCAAATGTTATTGGCTA, -AGTGCAAATGTTATTGGCT, -AGTGCAAATGTTATTGGC, -AGTGCAAATGTTATTGG, -AGTGCAAATGTTATTG, -AGTGCAAATGTTATT, -AGTGCAAATGTTAT, -AGTGCAAATGTTA, -AGTGCAAATGTT, -AGTGCAAATGT, -AGTGCAAATG, -AGTGCAAAT, -AGTGCAAA, -AGTGCAA, -AGTGCA, -AGTGC, -AGTG, -AGT, -AG, or -A.

Preferably the antisense-oligonucleotide of general formula (S7) has between 10 and 28 nucleotides and at least one LNA nucleotide at the 3' terminus and at least one LNA nucleotide at the 5' terminus. As LNA nucleotides (LNA units) especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably in the chapter "Preferred LNAs" are suitable and as internucleotides bridges especially these disclosed in the chapter "Internucleotide Linkages (IL)" are suitable.

More preferably the antisense-oligonucleotide of general formula (S7) has between 11 and 24 nucleotides and at least two LNA nucleotides at the 3' terminus and at least two LNA nucleotides at the 5' terminus.

Still more preferably the antisense-oligonucleotide of general formula (S7) has between 12 and 20, more preferably between 13 and 19 and still more preferable between 14 and 18 nucleotides and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 3' terminal end and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 5' terminal

end. Preferably the antisense-oligonucleotides are GAPmers of the form LNA segment A – DNA segment – LNA segment B. Preferably the antisense-oligonucleotides contain at least 6, more preferably at least 7 and most preferably at least 8 non-LNA units such as DNA units in between the two LNA segments. Suitable nucleobases for the non-LNA units and the LNA units are disclosed in the chapter "Nucleobases".

Further preferred are antisense-oligonucleotides of the formula (S7):

5'-N^9-**ATTAATAA**-N^{10}-3'

wherein

N^9 represents: TCATATTTATATACAGGC-, CATATTTATATACAGGC-, ATATTTATATACAGGC-, TATTTATATACAGGC-, ATTTATATACAGGC-, TTTATATACAGGC-, TTATATACAGGC-, TATATACAGGC-, ATATACAGGC-, TATACAGGC-, ATACAGGC-, TACAGGC-, ACAGGC-, CAGGC-, AGGC-, GGC-, GC-, or C-; and

N^{10} is selected from: -AGTGCAAATGTTATTGGC, -AGTGCAAATGTTATTGG, -AGTGCAAATGTTATTG, -AGTGCAAATGTTATT, -AGTGCAAATGTTAT, -AGTGCAAATGTTA, -AGTGCAAATGTT, -AGTGCAAATGT, -AGTGCAAATG, -AGTGCAAAT, -AGTGCAAA, -AGTGCAA, -AGTGCA, -AGTGC, -AGTG, -AGT, -AG, or -A.

Also preferred are antisense-oligonucleotides of the formula (S7):

5'-N^9-**ATTAATAA**-N^{10}-3'

wherein

N^9 represents: TTTATATACAGGC-, TTATATACAGGC-, TATATACAGGC-, ATATACAGGC-, TATACAGGC-, ATACAGGC-, TACAGGC-, ACAGGC-, CAGGC-, AGGC-, GGC-, GC-, or C-; and

N^{10} is selected from: -AGTGCAAATGTTA, -AGTGCAAATGTT, -AGTGCAAATGT, -AGTGCAAATG, -AGTGCAAAT, -AGTGCAAA, -AGTGCAA, -AGTGCA, -AGTGC, -AGTG, -AGT, -AG, or -A.

Also preferred are antisense-oligonucleotides of the formula (S7):

5'-N^9-**ATTAATAA**-N^{10}-3'

wherein

N^9 represents: ATACAGGC-, TACAGGC-, ACAGGC-, CAGGC-, AGGC-, GGC-, GC-, or C-; and

N^{10} is selected from: -AGTGCAAA, -AGTGCAA, -AGTGCA, -AGTGC, -AGTG, -AGT, -AG, or -A.

Preferably, the present invention is directed to antisense-oligonucleotide(s) consisting of 12 to 24 nucleotides and at least three of the 12 to 24 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^{9A}-**CATTAATAAA**-N^{10A}-3' (Seq. ID No. 74), wherein

N^{9A} represents: ATTCATATTTATATACAGG-, TTCATATTTATATACAGG-, TCATATTTATATACAGG-, CATATTTATATACAGG-, ATATTTATATACAGG-, TATTTATATACAGG-, ATTTATATACAGG-, TTTATATACAGG-, TTATATACAGG-, TATATACAGG-, ATATACAGG-, TATACAGG-, ATACAGG-, TACAGG-, ACAGG-, CAGG-, AGG-, GG-, or G-;

N^{10A} represents: -GTGCAAATGTTATTGGCTA, -GTGCAAATGTTATTGGCT, -GTGCAAATGTTATTGGC, -GTGCAAATGTTATTGG, -GTGCAAATGTTATTG, -GTGCAAATGTTATT, -GTGCAAATGTTAT, -GTGCAAATGTTA, -GTGCAAATGTT, -GTGCAAATGT, -GTGCAAATG, -GTGCAAAT, -GTGCAAA, -GTGCAA, -GTGCA, -GTGC, -GTG, -GT, or -G;

and salts and optical isomers of the antisense-oligonucleotide.

Preferably N^{9A} represents: TCATATTTATATACAGG-, CATATTTATATACAGG-, ATATTTATATACAGG-, TATTTATATACAGG-, ATTTATATACAGG-, TTTATATACAGG-, TTATATACAGG-, TATATACAGG-, ATATACAGG-, TATACAGG-, ATACAGG-, TACAGG-, ACAGG-, CAGG-, AGG-, GG-, or G-;

and

N^{10A} represents: -GTGCAAATGTTATTGGC, -GTGCAAATGTTATTGG, -GTGCAAATGTTATTG, -GTGCAAATGTTATT, -GTGCAAATGTTAT, -GTGCAAATGTTA, -GTGCAAATGTT, -GTGCAAATGT, -GTGCAAATG, -GTGCAAAT, -GTGCAAA, -GTGCAA, -GTGCA, -GTGC, -GTG, -GT, or -G.

More preferably N^{9A} represents: TTTATATACAGG-, TTATATACAGG-, TATATACAGG-, ATATACAGG-, TATACAGG-, ATACAGG-, TACAGG-, ACAGG-, CAGG-, AGG-, GG-, or G-; and

N^{10A} represents: -GTGCAAATGTTA, -GTGCAAATGTT, -GTGCAAATGT, -GTGCAAATG, -GTGCAAAT, -GTGCAAA, -GTGCAA, -GTGCA, -GTGC, -GTG, -GT, or -G.

Still more preferably N^{9A} represents: ATACAGG-, TACAGG-, ACAGG-, CAGG-, AGG-, GG-, or G-; and

N^{10A} represents: -GTGCAAA, -GTGCAA, -GTGCA, -GTGC, -GTG, -GT, or -G.

Preferably the antisense-oligonucleotide of general formula (S7A / Seq. ID No. 74) has between 12 and 24 nucleotides and at least one LNA nucleotide at the 3' terminus and at least one LNA nucleotide at the 5' terminus. As LNA nucleotides (LNA units) especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably in the chapter "Preferred LNAs" are suitable and as internucleotides bridges especially these disclosed in the chapter "Internucleotide Linkages (IL)" are suitable.

More preferably the antisense-oligonucleotide of general formula (S7A) has between 12 and 22 nucleotides and at least two LNA nucleotides at the 3' terminus and at least two LNA nucleotides at the 5' terminus.
Still more preferably the antisense-oligonucleotide of general formula (S7A) has between 12 and 20, more preferably between 13 and 19 and still more preferable between 14 and 18 nucleotides and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 3' terminal end and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 5' terminal end. Preferably the antisense-oligonucleotides are GAPmers of the form LNA segment A – DNA segment – LNA segment B. Preferably the antisense-oligonucleotides contain at least 6, more preferably at least 7 and most preferably at least 8 non-LNA units such as DNA units in between the two LNA segments. Suitable nucleobases for the non-LNA units and the LNA units are disclosed in the chapter "Nucleobases".

Moreover, the present invention is directed to antisense-oligonucleotide(s) consisting of 8 to 18, preferably 10 to 28 nucleotides and at least two of the 8 to 28, preferably 10 to 28 nucleotides are LNAs and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-$(N^{13})_m$-**GTAGTGTT**-$(N^{14})_n$-3' (Seq. ID No. 99), wherein
N^{13} represents: CCCAGCCTGCCCCAGAAGAGCTATTTG-, CCAGCCTGCCCCAGAAGAGCTATTTG-, CAGCCTGCCCCAGAAGAGCTATTTG-, AGCCTGCCCCAGAAGAGCTATTTG-, GCCTGCCCCAGAAGAGCTATTTG-, CCTGCCCCAGAAGAGCTATTTG-, CTGCCCCAGAAGAGCTATTTG-, TGCCCCAGAAGAGCTATTTG-, GCCCCAGAAGAGCTATTTG-, CCCCAGAAGAGCTATTTG-, CCCAGAAGAGCTATTTG-, CCAGAAGAGCTATTTG-, CAGAAGAGCTATTTG-, AGAAGAGCTATTTG-, GAAGAGCTATTTG-, AAGAGCTATTTG-, AGAGCTATTTG-, GAGCTATTTG-, AGCTATTTG-, GCTATTTG-, CTATTTG-, TATTTG-, ATTTG-, TTTG-, TTG-, TG-, or G-;
and

N^{14} is selected from: -TAGGGAGCCGTCTTCAGGAATCTTCTC, -TAGGGAGCCGTCTTCAGGAATCTTCT, -TAGGGAGCCGTCTTCAGGAATCTTC, -TAGGGAGCCGTCTTCAGGAATCTT, -TAGGGAGCCGTCTTCAGGAATCT, -TAGGGAGCCGTCTTCAGGAATC, -TAGGGAGCCGTCTTCAGGAAT, -TAGGGAGCCGTCTTCAGGAA, -TAGGGAGCCGTCTTCAGGA, -TAGGGAGCCGTCTTCAGG, -TAGGGAGCCGTCTTCAG, -TAGGGAGCCGTCTTCA, -TAGGGAGCCGTCTTC, -TAGGGAGCCGTCTT, -TAGGGAGCCGTCT, -TAGGGAGCCGTC, -TAGGGAGCCGT, -TAGGGAGCCG, -TAGGGAGCC, -TAGGGAGC, -TAGGGAG, -TAGGGA, -TAGGG, -TAGG, -TAG, -TA, or -T;

m represents 0 or 1;

n represents 0 or 1;

and n + m = 1 or 2;

and salts and optical isomers of the antisense-oligonucleotide.

The antisense-oligonucleotides of formula S5 (Seq. ID No. 99) preferably comprise 2 to 10 LNA units, more preferably 3 to 9 LNA units and still more preferably 4 to 8 LNA units and also preferably at least 6 non-LNA units, more preferably at least 7 non-LNA units and most preferably at least 8 non-LNA units. The non-LNA units are preferably DNA units. The LNA units are preferably positioned at the 3' terminal end (also named 3' terminus) and the 5' terminal end (also named 5' terminus). Preferably at least one and more preferably at least two LNA units are present at the 3' terminal end and/or at the 5' terminal end.

Thus, preferred are antisense-oligonucleotides of the present invention designed as GAPmers which contain 2 to 10 LNA units and which especially contain 1 to 5 LNA units at the 5' terminal end and 1 to 5 LNA units at the 3' terminal end of the antisense-oligonucleotide and between the LNA units at least 7 and more preferably at least 8 DNA units. More preferably the antisense-oligonucleotides comprise 2 to 4 LNA units at the 5' terminal end and 2 to 4 LNA units at the 3' terminal end and still more preferred comprise 3 to 4 LNA units at the 5' terminal end and 3 to 4 LNA units at the 3' terminal end and contain preferably at least 7 non-LNA units and most preferably at least 8 non-LNA units such as DNA units in between both LNA segments.

Moreover the antisense-oligonucleotides may contain common nucleobases such as adenine, guanine, cytosine, thymine and uracil as well as common derivatives thereof such as 5-methylcytosine or 2-aminoadenine. The antisense-oligonucleotides of the present invention may also contain modified internucleotide bridges such as phosphorothioate or phosphorodithioate instead of phosphate

bridges. Such modifications may be present only in the LNA segments or only in the non-LNA segment of the antisense-oligonucleotide. As LNA units especially the residues b^1 to b^9 as disclosed herein are preferred.

Thus, preferred are antisense-oligonucleotides of the formula (S5):

$$5'\text{-}(N^{13})_m\text{-}\mathbf{GTAGTGTT}\text{-}(N^{14})_n\text{-}3'$$

wherein

N^{13} represents: GCCTGCCCCAGAAGAGCTATTTG-, CCTGCCCCAGAAGAGCTATTTG-, CTGCCCCAGAAGAGCTATTTG-, TGCCCCAGAAGAGCTATTTG-, GCCCCAGAAGAGCTATTTG-, CCCCAGAAGAGCTATTTG-, CCCAGAAGAGCTATTTG-, CCAGAAGAGCTATTTG-, CAGAAGAGCTATTTG-, AGAAGAGCTATTTG-, GAAGAGCTATTTG-, AAGAGCTATTTG-, AGAGCTATTTG-, GAGCTATTTG-, AGCTATTTG-, GCTATTTG-, CTATTTG-, TATTTG-, ATTTG-, TTTG-, TTG-, TG-, or G-; and

N^{14} is selected from: -TAGGGAGCCGTCTTC, -TAGGGAGCCGTCTT, -TAGGGAGCCGTCT, -TAGGGAGCCGTC, -TAGGGAGCCGT, -TAGGGAGCCG, -TAGGGAGCC, -TAGGGAGC, -TAGGGAG, -TAGGGA, -TAGGG, -TAGG, -TAG, -TA, or -T; and

m represents 0 or 1; n represents 0 or 1; and n + m = 1 or 2.

Preferably the antisense-oligonucleotide of general formula (S5) has between 10 and 28 nucleotides and at least one LNA nucleotide at the 3' terminus and at least one LNA nucleotide at the 5' terminus. As LNA nucleotides (LNA units) especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably in the chapter "Preferred LNAs" are suitable and as internucleotides bridges especially these disclosed in the chapter "Internucleotide Linkages (IL)" are suitable.

More preferably the antisense-oligonucleotide of general formula (S5) has between 11 and 24 nucleotides and at least two LNA nucleotides at the 3' terminus and at least two LNA nucleotides at the 5' terminus.

Still more preferably the antisense-oligonucleotide of general formula (S5) has between 12 and 20, more preferably between 13 and 19 and still more preferable between 14 and 18 nucleotides and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 3' terminal end and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 5' terminal end. Preferably the antisense-oligonucleotides are GAPmers of the form LNA segment A – DNA segment – LNA segment B. Preferably the antisense-oligonucleotides contain at least 6, more preferably at least 7 and most preferably at

least 8 non-LNA units such as DNA units in between the two LNA segments. Suitable nucleobases for the non-LNA units and the LNA units are disclosed in the chapter “Nucleobases”.

Further preferred are antisense-oligonucleotides of the formula (S5):

$$5'\text{-}(N^{13})_m\text{-}\mathbf{GTAGTGTT}\text{-}(N^{14})_n\text{-}3'$$

wherein

N^{13} represents: CCCCAGAAGAGCTATTTG-, CCCAGAAGAGCTATTTG-, CCAGAAGAGCTATTTG-, CAGAAGAGCTATTTG-, AGAAGAGCTATTTG-, GAAGAGCTATTTG-, AAGAGCTATTTG-, AGAGCTATTTG-, GAGCTATTTG-, AGCTATTTG-, GCTATTTG-, CTATTTG-, TATTTG-, ATTTG-, TTTG-, TTG-, TG-, or G-; and

N^{14} is selected from: -TAGGGAGCCG, -TAGGGAGCC, -TAGGGAGC, -TAGGGAG, -TAGGGA, -TAGGG, -TAGG, -TAG, -TA, or -T; and

m represents 0 or 1; n represents 0 or 1; and n + m = 1 or 2.

Also preferred are antisense-oligonucleotides of the formula (S5):

$$5'\text{-}(N^{13})_m\text{-}\mathbf{GTAGTGTT}\text{-}(N^{14})_n\text{-}3'$$

wherein

N^{13} represents: GAAGAGCTATTTG-, AAGAGCTATTTG-, AGAGCTATTTG-, GAGCTATTTG-, AGCTATTTG-, GCTATTTG-, CTATTTG-, TATTTG-, ATTTG-, TTTG-, TTG-, TG-, or G-; and

N^{14} is selected from: -TAGGG, -TAGG, -TAG, -TA, or -T; and

m represents 0 or 1; n represents 0 or 1; and n + m = 1 or 2.

Also preferred are antisense-oligonucleotides of the formula (S5):

$$5'\text{-}(N^{13})_m\text{-}\mathbf{GTAGTGTT}\text{-}(N^{14})_n\text{-}3'$$

wherein

N^{13} represents: CAGAAGAGCTATTTG-, AGAAGAGCTATTTG-, GAAGAGCTATTTG-, AAGAGCTATTTG-, AGAGCTATTTG-, GAGCTATTTG-, AGCTATTTG-, GCTATTTG-, CTATTTG-, TATTTG-, ATTTG-, TTTG-, TTG-, TG-, or G-; and

N^{14} is selected from: -TAGGGAGCCGTCTTCAGGAATCT, -TAGGGAGCCGTCTTCAGGAATC, -TAGGGAGCCGTCTTCAGGAAT, -TAGGGAGCCGTCTTCAGGAA, -TAGGGAGCCGTCTTCAGGA, -TAGGGAGCCGTCTTCAGG, -TAGGGAGCCGTCTTCAG,

-TAGGGAGCCGTCTTCA, -TAGGGAGCCGTCTTC, -TAGGGAGCCGTCTT, -TAGGGAGCCGTCT, -TAGGGAGCCGTC, -TAGGGAGCCGT, -TAGGGAGCCG, -TAGGGAGCC, -TAGGGAGC, -TAGGGAG, -TAGGGA, -TAGGG, -TAGG, -TAG, -TA, or -T; and

m represents 0 or 1; n represents 0 or 1; and n + m = 1 or 2.

Also preferred are antisense-oligonucleotides of the formula (S5):

$$5'\text{-}(N^{13})_m\text{-}\mathbf{GTAGTGTT}\text{-}(N^{14})_n\text{-}3'$$

wherein

N^{13} represents: GAGCTATTTG-, AGCTATTTG-, GCTATTTG-, CTATTTG-, TATTTG-, ATTTG-, TTTG-, TTG-, TG-, or G-; and

N^{14} is selected from: -TAGGGAGCCGTCTTCAGG, -TAGGGAGCCGTCTTCAG, -TAGGGAGCCGTCTTCA, -TAGGGAGCCGTCTTC, -TAGGGAGCCGTCTT, -TAGGGAGCCGTCT, -TAGGGAGCCGTC, -TAGGGAGCCGT, -TAGGGAGCCG, -TAGGGAGCC, -TAGGGAGC, -TAGGGAG, -TAGGGA, -TAGGG, -TAGG, -TAG, -TA, or -T; and

m represents 0 or 1; n represents 0 or 1; and n + m = 1 or 2.

Also preferred are antisense-oligonucleotides of the formula (S5):

$$5'\text{-}(N^{13})_m\text{-}\mathbf{GTAGTGTT}\text{-}(N^{14})_n\text{-}3'$$

wherein

N^{13} represents: ATTTG-, TTTG-, TTG-, TG-, or G-; and

N^{14} is selected from: -TAGGGAGCCGTCT, -TAGGGAGCCGTC, -TAGGGAGCCGT, -TAGGGAGCCG, -TAGGGAGCC, -TAGGGAGC, -TAGGGAG, -TAGGGA, -TAGGG, -TAGG, -TAG, -TA, or -T; and

m represents 0 or 1; n represents 0 or 1; and n + m = 1 or 2.

Preferably the antisense-oligonucleotide of general formula (S5 / Seq. ID No. 99) has between 12 and 24 nucleotides and at least one LNA nucleotide at the 3' terminus and at least one LNA nucleotide at the 5' terminus. As LNA nucleotides (LNA units) especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably in the chapter "Preferred LNAs" are suitable and as internucleotides bridges especially these disclosed in the chapter "Internucleotide Linkages (IL)" are suitable.

More preferably the antisense-oligonucleotide of general formula (S5) has between 12 and 22 nucleotides and at least two LNA nucleotides at the 3' terminus and at least two LNA nucleotides at the 5' terminus.

Still more preferably the antisense-oligonucleotide of general formula (S5) has between 12 and 20, more preferably between 13 and 19 and still more preferable between 14 and 18 nucleotides and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 3' terminal end and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 5' terminal end. Preferably the antisense-oligonucleotides are GAPmers of the form LNA segment A – DNA segment – LNA segment B. Preferably the antisense-oligonucleotides contain at least 6, more preferably at least 7 and most preferably at least 8 non-LNA units such as DNA units in between the two LNA segments. Suitable nucleobases for the non-LNA units and the LNA units are disclosed in the chapter "Nucleobases".

Another aspect of the present invention relates to antisense-oligonucleotide(s) having a length of 10 to 28 nucleotides, preferably 10 to 24 nucleotides, more preferably 11 to 22 nucleotides or 12 to 20 nucleotides, still more preferably 13 to 19 nucleotides, and most preferably 14 to 18 nucleotides, wherein at least two of the nucleotides, preferably at least three of the nucleotides, and more preferably at least four of the nucleotides are LNAs and the sequence of the antisense-oligonucleotide of the 10 to 28 nucleotides, preferably 10 to 24 nucleotides, more preferably 11 to 22 nucleotides or 12 to 20 nucleotides, still more preferably 13 to 19 nucleotides, and most preferably 14 to 18 nucleotides is selected from the group of sequences of 10 to 28 nucleotides, preferably 10 to 24 nucleotides, more preferably 11 to 22 nucleotides or 12 to 20 nucleotides, still more preferably 13 to 19 nucleotides, and most preferably 14 to 18 nucleotides contained in a sequence selected from the following group:

GAATCTTGAATATCTCATGAATGGACCAGTATTCTAGAAAC

(Seq. ID No. 75: 383-423 of Seq. ID No. 1),

TTCATATTTATATACAGGCATTAATAAAGTGCAAATGTTAT

(Seq. ID No. 77: 2245-2285 of Seq. ID No. 1),

TGAGGAAGTGCTAACACAGCTTATCCTATGACAATGTCAAAG

(Seq. ID No. 78: 2315-2356 of Seq. ID No. 1),

GCCTGCCCCAGAAGAGCTATTTGGTAGTGTTTAGGGAGCCGTCTTCAGG

(Seq. ID No. 79: 2528-2576 of Seq. ID No. 1),

CGCAGGTCCTCCCAGCTGATGACATGCCGCGTCAGGTACTCCTGTAGGT

(Seq. ID No. 81: 3205-3253 of Seq. ID No. 1),

ATGTCGTTATTAACCGACTTCTGAACGTGCGGTGGGATCGTGCTGGCGATACGCGTCCACAGGA CGATGTGCAGCGGC

(Seq. ID No. 83: 4141-4218 of Seq. ID No. 1),

GGCCACAGGCCCCTGAGCAGCCCCCGACCCATGGCAGACCCCGCTGCTCGTCATAGACCGAGC CCCCAGCGCAG

(Seq. ID No. 84: 4216-4289 of Seq. ID No. 1),

ATGTCGTTATTAACCGACTTCTGAACGTGCGGTGGGATCGTGCTGGCGATACGCGTCCACAGGA CGATGTGCAGCGGCCACAGGCCCCTGAGCAGCCCCCGACCCATGGCAGACCCCGCTGCTCGTC ATAGACCGAGCCCCCAGCGCAG

(Seq. ID No. 86: 4141-4289 of Seq. ID No. 1),

TTGAATATCTCATGAATGGACCAGTATTCTA

(Seq. ID No. 87: 388-418 of Seq. ID No. 1),

CAAGTGGAATTTCTAGGCGCCTCTATGCTACTG

(Seq. ID No. 88: 483-515 of Seq. ID No. 1),

ATTTATATACAGGCATTAATAAAGTGCAAAT

(Seq. ID No. 89: 2250-2280 of Seq. ID No. 1),

AAGTGCTAACACAGCTTATCCTATGACAATGT

(Seq. ID No. 90: 2320-2351 of Seq. ID No. 1),

CCCCAGAAGAGCTATTTGGTAGTGTTTAGGGAGCCGTCT

(Seq. ID No. 91: 2533-2571 of Seq. ID No. 1),

CTGGTCGCCCTCGATCTCTCAACACGTTGTCCTTCATGCTTTCGACACAGGGGTGCTCCCGCAC CTTGGAACCAAATG

(Seq. ID No. 92: 2753-2830 of Seq. ID No. 1),

GTCCTCCCAGCTGATGACATGCCGCGTCAGGTACTCCTG

(Seq. ID No. 93: 3210-3248 of Seq. ID No. 1),

CTCAGCTTCTGCTGCCGGTTAACGCGGTAGCAGTAGAAGA

(Seq. ID No. 94: 3655-3694 of Seq. ID No. 1),

GTTATTAACCGACTTCTGAACGTGCGGTGGGATCGTGCTGGCGATACGCGTCCACAGGACGATG TGCA

(Seq. ID No. 95: 4146-4213 of Seq. ID No. 1),

CAGGCCCCTGAGCAGCCCCCGACCCATGGCAGACCCCGCTGCTCGTCATAGACCGAGCCCCCAG

(Seq. ID No. 96: 4221-4284 of Seq. ID No. 1),

CACGCGCGGGGGTGTCGTCGCTCCGTGCGCGCGAGTGACTCACTCAACTTCA

(Seq. ID No. 97: 4495-4546 of Seq. ID No. 1),

wherein the antisense-oligonucleotide is capable of selectively hybridizing in regard to the whole human transcriptome only with the gene encoding TGF-R_{II} or with the mRNA encoding TGF-R_{II} and salts and optical isomers of said antisense-oligonucleotide.

Said antisense-oligonucleotide having a sequence contained in the sequences No. 75, 77, 78, 79, 81, 83, 84, 86 – 97 have between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 3' terminal end and between 2 and 5, preferably 3 and 5 and more preferably between 3 and 4 LNA units at the 5' terminal end and have preferably the structure of GAPmers of the form LNA segment A – DNA segment – LNA segment B. As LNA nucleotides (LNA units) especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably in the chapter "Preferred LNAs" are suitable and as internucleotides bridges especially these disclosed in the chapter "Internucleotide Linkages (IL)" are suitable. Preferably said antisense-oligonucleotides contain at least 6, more preferably at least 7 and most preferably at least 8 non-LNA units such as DNA units in between the two LNA segments. Suitable nucleobases for the non-LNA units and the LNA units are disclosed in the chapter "Nucleobases". Suitable examples for said antisense-oligonucleotides are represented by the formulae (S1) to (S7), (S1A) to (S4A), (S6A) and (S7A).

The **Seq. ID No. 1** represents the antisense strand of the *c*DNA (cDNA) (5'-3' antisense-sequence) of the Homo sapiens transforming growth factor, beta receptor II (TGF-R_{II}), transcript variant 2.

The **Seq. ID No. 2** represents the sense strand of the cDNA (5'-3' sense-sequence) of the Homo sapiens transforming growth factor, beta receptor II (70/80kDa) (TGF-R_{II}), transcript variant 2. Alternatively, one can also regard the sequence of Seq. ID No. 2 to represent the sequence of the mRNA of the Homo sapiens transforming

growth factor, beta receptor II (TGF-R_{II}), transcript variant 2 (Seq. ID No. 3), but written in the DNA code, i.e. represented in G, C, A, T code, and not in the RNA code.

The **Seq. ID No. 3** represents the mRNA (5'-3' sense-sequence) of the Homo sapiens transforming growth factor, beta receptor II (TGF-R_{II}), transcript variant 2. It is evident that the mRNA displayed in Seq. ID No. 3 is written in the RNA code, i.e. represented in G, C, A, U code.

It shall be understood, that "coding DNA strand", as used herein, refers to the DNA strand that is identical to the mRNA (except that is written in the DNA code) and that encompasses the codons that used for protein translation. It is not used as template for the transcription into mRNA. Thus, the terms "coding DNA strand", "sense DNA strand" and "non-template DNA strand" can be used interchangeably. Furthermore, "non-coding DNA strand", as used herein, refers to the DNA strand that is complementary to the "coding DNA strand" and serves as a template for the transcription of mRNA. Thus, the terms "non-coding DNA strand", "antisense DNA strand" and "template DNA strand" can be used interchangeably

The term "**antisense-oligonucleotide**" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics or variants thereof such as antisense-oligonucleotides having a modified internucleotide linkage like a phosphorothioate linkage and/or one or more modified nucleobases such as 5-methylcytosine and/or one or more modified nucleotide units such as LNAs like β-D-oxy-LNA. The term "antisense-oligonucleotide" includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent internucleotide (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms, because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases. The antisense-oligonucleotides are short catalytic RNAs or catalytic oligonucleotides which hybridize to the target nucleic acid and inhibit its expression.

The term "**nucleoside**" is well known to a skilled person and refers to a pentose sugar moiety like ribose, desoxyribose or a modified or locked ribose or a modified or locked desoxyribose like the LNAs which are below disclosed in detail. A nucleobase is linked to the glycosidic carbon atom (position 1' of the pentose) and an internucleotide linkage is formed between the 3' oxygen or sulfur atom and preferably

the 3' oxygen atom of a nucleoside and the 5' oxygen or sulfur atom and preferably the 5' oxygen atom of the adjacent nucleoside, while the internucleotide linkage does not belong to the nucleoside (see Fig. 2).

The term "**nucleotide**" is well known to a skilled person and refers to a pentose sugar moiety like ribose, desoxyribose or a modified or locked ribose or a modified or locked desoxyribose like the LNAs which are below disclosed in detail. A nucleobase is linked to the glycosidic carbon atom (position 1' of the pentose) and an internucleotide linkage is formed between the 3' oxygen or sulfur atom and preferably the 3' oxygen atom of a nucleotide and the 5' oxygen or sulfur atom and preferably the 5' oxygen atom of the adjacent nucleotide, while the internucleotide linkage is a part of the nucleotide (see Fig. 2).

Nucleobases

The term "**nucleobase**" is herein abbreviated with "B" and refers to the five standard nucleotide bases adenine (A), thymine (T), guanine (G), cytosine (C), and uracil (U) as well as to modifications or analogues thereof or analogues with ability to form Watson-Crick base pair with bases in the complimentary strand. Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (C*), 5-hydroxymethyl cytosine, N^4-methylcytosine, xanthine, hypoxanthine, 7-deazaxanthine, 2-aminoadenine, 6-methyladenine, 6-methylguanine, 6-ethyladenine, 6-ethylguanine, 2-propyladenine, 2-propylguanine, 6-carboxyuracil, 5-halouracil, 5,6-dihydrouracil, 5-halocytosine, 5-propynyl uracil, 5-propynyl cytosine, 6-aza uracil, 6-aza cytosine, 6-aza thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-fluoroadenine, 8-chloroadenine, 8-bromoadenine, 8-iodoadenine, 8-aminoadenine, 8-thioladenine, 8-thioalkyladenine, 8-hydroxyladenine, 8-fluoroguanine, 8-chloroguanine, 8-bromoguanine, 8-iodoguanine, 8-aminoguanine, 8-thiolguanine, 8-thioalkylguanine, 8-hydroxylguanine, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, 5-trifluoromethyluracil, 5-fluorocytosine, 5-bromocytosine, 5-chlorocytosine, 5-iodocytosine, 5-trifluoromethylcytosine, 7-methylguanine, 7-methyladenine, 8-azaguanine, 8-azaadenine, 7-deazaguanine, 7-deazaadenine, 7-deaza-8-azaadenine, 3-deazaguanine, 3-deazaadenine, 2-thiouracil, 2-thiothymine and 2-thiocytosine etc., with 5-methylcytosine and/or 2-aminoadenine substitutions being preferred since these modifications have been shown to increase nucleic acid duplex stability.

Preferred antisense-oligonucleotides of the present invention can comprise analogues of nucleobases. The nucleobase of only one nucleotide unit of the

antisense-oligonucleotide could be replaced by an analogue of a nucleobase or two, three, four, five or even all nucleobases in an antisense-oligonucleotide could be replaced by analogues of nucleobases, such as 5-methylcytosine, or N^6-methyl-adenine or 2-aminoadenine. Preferably the LNA units might be connected to analogues of nucleobases such as 5-methylcytosine.

It will be recognized that when referring to a sequence of nucleotides or monomers, what is referred to, is the sequence of bases, such as A, T, G, C or U. However, except the specific examples disclosed in Tables 3 to 8 the representation of the antisense-oligonucleotides by the letter code A, T, G, C and U has to be understood that said antisense-oligonucleotide may contain any the nucleobases as disclosed herein, any of the 3' end groups as disclosed herein, any of the 5' end groups as disclosed herein, and any of the internucleotide linkages (also referred to as internucleotide bridges) as disclosed herein. The nucleotides A, T, G, C and U have also to be understood as being LNA nucleotides or non-LNA nucleotides such as preferably DNA nucleotides.
Only in regard to the specific examples as disclosed in Tables 4 to 9 the nucleobases, the LNA units, the non-LNA units, the internucleotide linkages and the end groups are further specified as outlined in the chapter "Legend" before Table 2.

The antisense-oligonucleotides as well as the salts of the antisense-oligonucleotides as disclosed herein have been proven to be complementary to the target which is the gene encoding for the TGF-R_{II} or the mRNA encoding the TGF-R_{II}, i.e., hybridize sufficiently well and with sufficient specificity and especially selectivity to give the desired inhibitory effect.

The term "**salt**" refers to physiologically and/or pharmaceutically acceptable salts of the antisense-oligonucleotides of the present invention. The antisense-oligonucleotides contain nucleobases like adenine, guanine, thymine, cytosine or derivatives thereof which are basic and which form a salt like a chloride or mesylate salt. The internucleotide linkage preferably contains a negatively charged oxygen or sulfur atom which form salts like the sodium, lithium or potassium salt. Thus, pharmaceutically acceptable base addition salts are formed with inorganic bases or organic bases. Examples for suitable organic and inorganic bases are bases derived from metal ions, e.g., aluminum, alkali metal ions, such as sodium or potassium, alkaline earth metal ions such as calcium or magnesium, or an amine salt ion or alkali- or alkaline-earth hydroxides, -carbonates or -bicarbonates. Examples include aqueous LiOH, NaOH, KOH, NH_4OH, potassium carbonate, ammonia and sodium bicarbonate, ammonium salts, primary, secondary and tertiary amines, such

as, e.g., tetraalkylammonium hydroxide, lower alkylamines such as methylamine, t-butylamine, procaine, ethanolamine, arylalkylamines such as dibenzylamine and N,N-dibenzylethylenediamine, lower alkylpiperidines such as N-ethylpiperidine, cycloalkylamines such as cyclohexylamine or dicyclohexylamine, morpholine, glucamine, N-methyl- and N,N-dimethylglucamine, 1-adamantylamine, benzathine, or salts derived from amino acids like arginine, lysine, ornithine or amides of originally neutral or acidic amino acids, chloroprocaine, choline, procaine or the like.

Since the antisense-oligonucleotides are basic, they form pharmaceutically acceptable salts with organic and inorganic acids. Examples of suitable acids for such acid addition salt formation are hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, acetic acid, citric acid, oxalic acid, malonic acid, salicylic acid, p-aminosalicylic acid, malic acid, fumaric acid, succinic acid, ascorbic acid, maleic acid, sulfonic acid, phosphonic acid, perchloric acid, nitric acid, formic acid, propionic acid, gluconic acid, lactic acid, tartaric acid, hydroxymaleic acid, pyruvic acid, phenylacetic acid, benzoic acid, p-aminobenzoic acid, p-hydroxybenzoic acid, methanesulfonic acid, ethanesulfonic acid, nitrous acid, hydroxyethanesulfonic acid, ethylenesulfonic acid, p-toluenesulfonic acid, naphthylsulfonic acid, sulfanilic acid, camphersulfonic acid, china acid, mandelic acid, o-methylmandelic acid, hydrogen-benzenesulfonic acid, picric acid, adipic acid, D-o-tolyltartaric acid, tartronic acid, □-toluic acid, (o, m, p)-toluic acid, naphthylamine sulfonic acid, and other mineral or carboxylic acids well known to those skilled in the art. The salts are prepared by contacting the free base form with a sufficient amount of the desired acid to produce a salt in the conventional manner.

In the context of this invention, "hybridization" means nucleic acid hybridization, wherein a single-stranded nucleic acid (DNA or RNA) interacts with another single-stranded nucleic acid having a very similar or even complementary sequence. Thereby the interaction takes place by hydrogen bonds between specific nucleobases (base pairing).

As used herein, the term "complementarity" (DNA and RNA base pair complementarity) refers to the capacity for precise pairing between two nucleic acids. The nucleotides in a base pair are complementary when their shape allows them to bond together by hydrogen bonds. Thereby forms the pair of adenine and thymidine (or uracil) two hydrogen bonds and the cytosine-guanine pair forms three hydrogen bonds. “Complementary sequences” as used herein means DNA or RNA sequences, being such that when they are aligned antiparallel to each other, the nucleotide bases at each position in the sequences will be complementary, much like looking in the mirror and seeing the reverse of things.

The term "specifically hybridizable" as used herein indicates a sufficient degree of complementarity or precise base pairing of the antisense-oligonucleotide to the target sequence such that stable and specific binding occurs between the antisense-oligonucleotide and the DNA or RNA target. The sequence of an –oligonucleotide according to the invention does not need to be 100% complementary to that of its target nucleic acid to be specifically hybridizable, although a 100% complementarity is preferred. Thereby “100% complementarity” means that the antisense-oligonucleotide hybridizes with the target over its complete or full length without mismatch. In other words, within the present invention it is defined that an antisense compound is specifically hybridizable when binding of the compound to the target DNA or RNA molecule takes place under physiological or pathological conditions but non-specific binding of the antisense-oligonucleotide to non-target sequences is highly unlikely or even impossible.

Therefore, the present invention refers preferably to antisense oligonucleotides, wherein the antisense oligonucleotides bind with 100% complementarity to the mRNA encoding TGF RII and do not bind to any other region in the complete human transcriptome. Further preferred the present invention refers to antisense oligonucleotides, wherein the antisense oligonucleotides have 100% complementarity over their complete length to the mRNA encoding TGF RII and have no off-target effects. Alternatively, the present invention refers preferably to antisense oligonucleotides having 100% complementarity to the mRNA encoding TGF RII but no complementarity to another mRNA of the human transcriptome. Thereby the term “human transcriptome” refers to the total set of transcripts in the human organism, which means transcripts of all cell types and environmental conditions (at any given time).

Specificity

The antisense-oligonucleotides of the present invention have in common that they are specific in regard to the region where they bind to the gene or to the mRNA encoding TGF-R_{II}. According to the present invention it is preferred that within the human transcriptome, the antisense-oligonucleotides have 100% complementarity over their full length only with the mRNA encoding TGF-RII. In addition, it was a goal of the present invention to find antisense-oligonucleotides without cross-reactivity within to the transcriptome of mammalian other than monkeys; in particular, the antisense-oligonucleotides have only cross-reactivity with the transcriptome of great apes. This should avoid off-effects. Thus the antisense-oligonucleotides of the present invention are highly specific concerning hybridization with the gene or with the mRNA encoding TGF-RII. The antisense-oligonucleotides of the invention bind

preferably over their complete length with 100% complementarity specific to the gene encoding TGF-RII or to the mRNA encoding TGF-RII and do not bind to any other region in the complete human transcriptome. This means, the antisense-oligonucleotides of the present invention hybridize with the target (TGF-RII mRNA) without mismatch.

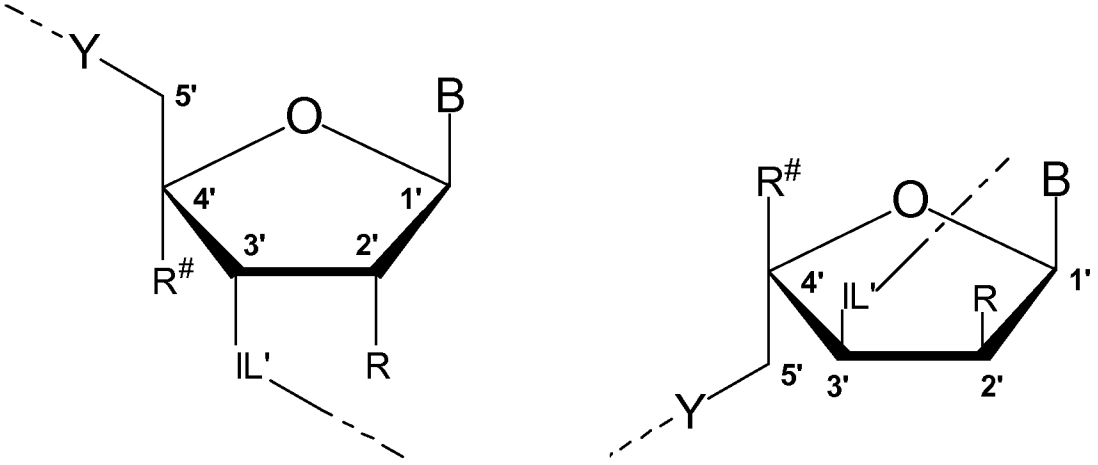
The term "**mRNA**", as used herein, may encompass both mRNA containing introns (also refered to as Pre-mRNA) as well as mRNA which does not contain any introns.

The antisense-oligonucleotides of the present invention are able to bind or hybridize with the Pre-mRNA and/or with the mRNA. That means the antisense-oligonucleotides can bind to or hybridize at an intron region or within an intron region of the Pre-mRNA or can bind to or hybridize at an overlapping intron – exon region of the Pre-mRNA or can bind to or hybridize at an exon region or within an exon region of the Pre-mRNA and the exon region of the mRNA (see Fig. 1). Preferred are antisense-oligonucleotides which are able to bind to or hybridize with Pre-mRNA and mRNA. Binding or hybridization of the antisense-oligonucleotides (ASO) to the Pre-mRNA inhibits the 5' cap formation, inhibits splicing of the Pre-mRNA in order to obtain the mRNA and activates RNase H which cleaves the Pre-mRNA. Binding or hybridization of the antisense-oligonucleotides (ASO) to the mRNA activates RNase H which cleaves the mRNA and inhibits binding of the ribosomal subunits.

The antisense-oligonucleotides of the present invention consist of at least 10 and no more than 28, preferably no more than 24 and more preferably no more than 20 nucleotides and consequently consist of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides, preferably of 11 to 20, or 11 to 19, or 12 to 19, or 13 to 19, or 13 to 18 nucleotides and more preferably of 14 to 18 nucleotides, wherein at least two, preferably three of these nucleotides are locked nucleic acis (LNA). Shorter antisense-oligonucleotides, i.e. antisense-oligonucleotides having less than 10 nucleotides, are also possible but the shorter the antisense-oligonucleotides the higher the risk that the hybridization is not sufficiently strong anymore and that selectivity will decrease or will get lost. Non-selective antisense-oligonucleotides bear the risk to bind to undesired regions in the human transcriptome and to undesired mRNAs coding for other proteins than TGF-R_{II} thereby causing undesired side effects. Longer antisense-oligonucleotides having more than 20 nucleotides are also possible but further increasing the length make the synthesis of such antisense-oligonucleotides even more complicated and expensive without any further benefit in increasing selectivity or strength of hybridization or better stability in regard to degradation.

Thus the present invention is directed to antisense-oligonucleotides consisting of 10 to 20 nucleotides, wherein at least two nucleotides and preferably the 3' and 5' terminal nucleotides are LNAs. Thus, it is preferred that at least the terminal 3' nucleotide is an LNA and also at least the 5' terminal nucleotide is an LNA. In case more than 2 LNAs are present, it is preferred that the further LNAs are linked to the 3' or 5' terminal LNA like it is the case in gapmers as disclosed herein.

One nucleotide building block present in an antisense-oligonucleotide of the present invention can be represented by the following general formula (B1) and (B2):



(**B1**) (**B2**)

wherein

B represents a nucleobase;

IL' represents –X''–P(=X')(X$^-$)–;

R represents –H, –F, –OH, –NH_2, –OCH_3, –$OCH_2CH_2OCH_3$ and $R^\#$ represents –H; or R and $R^\#$ form together the bridge –$R^\#$–R– which is selected from –CH_2–O–, –CH_2–S–, –CH_2–NH–, –CH_2–N(CH_3)–, –CH_2–N(C_2H_5)–, –CH_2–CH_2–O–, –CH_2–CH_2–S–, –CH_2–CH_2–NH–, –CH_2–CH_2–N(CH_3)–, or –CH_2–CH_2–N(C_2H_5)–;

X' represents =O or =S;

X$^-$ represents –O$^-$, –OH, –OR^H, –NHR^H, –$N(R^H)_2$, –$OCH_2CH_2OR^H$, –$OCH_2CH_2SR^H$, –BH_3^-, –R^H, –SH, –SR^H, or –S$^-$;

X'' represents –O–, –NH–, –NR^H–, –CH_2–, or –S–;

Y is –O–, –NH–, –NR^H–, –CH_2– or –S–;

R^H is selected from hydrogen and C_{1-4}-alkyl and preferably –CH_3 or –C_2H_5 and most preferably –CH_3.

Preferably X$^-$ represents –O$^-$, –OH, –OCH_3, –$NH(CH_3)$, –$N(CH_3)_2$, –$OCH_2CH_2OCH_3$, –$OCH_2CH_2SCH_3$, –BH_3^-, –CH_3, –SH, –SCH_3, or –S$^-$; and more preferably –O$^-$, –OH, –OCH_3, –$N(CH_3)_2$, –$OCH_2CH_2OCH_3$, –BH_3^-, –SH, –SCH_3, or –S$^-$.

IL' represents preferably –O–P(O)(O^-)–, –O–P(O)(S^-)–, –O–P(S)(S^-)–, –S–P(O)(O^-)–, –S–P(O)(S^-)–, –S–P(S)(S^-)–, –O–P(O)(O^-)–, –O–P(O)(S^-)–, –S–P(O)(O^-)–, –O–P(O)(R^H)–, –O–P(O)(OR^H)–, –O–P(O)(NHR^H)–, –O–P(O)[$N(R^H)_2$]–, –O–P(O)(BH_3^-)–, –O–P(O)($OCH_2CH_2OR^H$)-, –O–P(O)($OCH_2CH_2SR^H$)-, –O–P(O)(O^-)-, –NR^H–P(O)(O^-)–, wherein R^H is selected from hydrogen and C_{1-4}-alkyl.

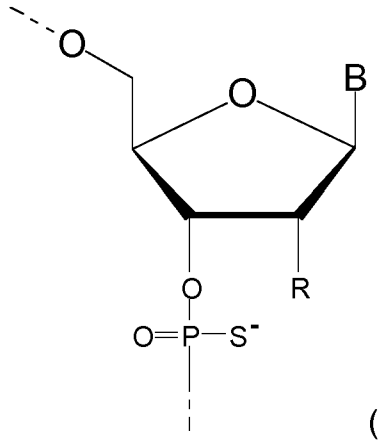
The group –O–P(O)(R^H)–O– is preferably –O–P(O)(CH_3)–O– or –O–P(O)(C_2H_5)–O– and most preferably –O–P(O)(CH_3)–O–.
The group –O–P(O)(OR^H)–O– is preferably –O–P(O)(OCH_3)–O– or –O–P(O)(OC_2H_5)–O– and most preferably –O–P(O)(OCH_3)–O–.
The group –O–P(O)(NHR^H)–O– is preferably –O–P(O)($NHCH_3$)–O– or –O–P(O)(NHC_2H_5)–O– and most preferably –O–P(O)($NHCH_3$)–O–.
The group –O–P(O)[$N(R^H)_2$]–O– is preferably –O–P(O)[$N(CH_3)_2$]–O– or –O–P(O)[$N(C_2H_5)_2$]–O– and most preferably –O–P(O)[$N(CH_3)_2$]–O–.
The group –O–P(O)($OCH_2CH_2OR^H$)–O– is preferably –O–P(O)($OCH_2CH_2OCH_3$)–O– or –O–P(O)($OCH_2CH_2OC_2H_5$)–O– and most preferably –O–P(O)($OCH_2CH_2OCH_3$)–O–.
The group –O–P(O)($OCH_2CH_2SR^H$)–O– is preferably –O–P(O)($OCH_2CH_2SCH_3$)–O– or –O–P(O)($OCH_2CH_2SC_2H_5$)–O– and most preferably –O–P(O)($OCH_2CH_2SCH_3$)–O–.
The group –O–P(O)(O^-)–NR^H– is preferably –O–P(O)(O^-)–NH– or –O–P(O)(O^-)–$N(CH_3)$– and most preferably –O–P(O)(O^-)–NH–.
The group –NR^H–P(O)(O^-)–O– is preferably –NH–P(O)(O^-)–O– or –$N(CH_3)$–P(O)(O^-)–O– and most preferably –NH–P(O)(O^-)–O–.

Even more preferably IL' represents –O–P(O)(O^-)–, –O–P(O)(S^-)–, –O–P(S)(S^-)–, –O–P(O)(NHR^H)–, or –O–P(O)[$N(R^H)_2$]–, and still more preferably IL' represents –O–P(O)(O^-)–, –O–P(O)(S^-)–, or –O–P(S)(S^-)–, and most preferably IL' represents –O–P(O)(S^-)–, or –O–P(S)(S^-)–.

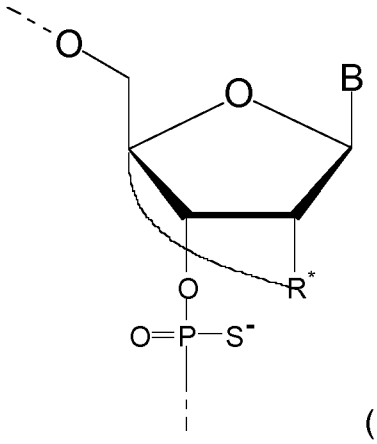
Preferably Y represents –O–.
Preferably B represents a standard nucleobase selected from A, T, G, C, U.
Preferably IL represents –O–P(=O)(S^-)– or –O–P(=S)(S^-)–.

The above definitions of B, Y and IL' apply also to the formula b^1 to b^9.

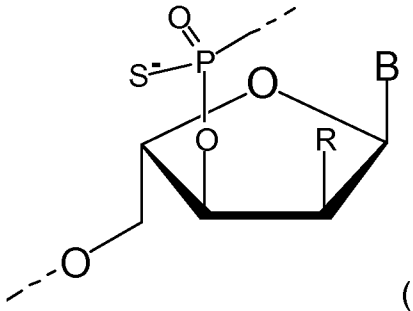
Thus the following general formula (B3) to (B6) are preferred:



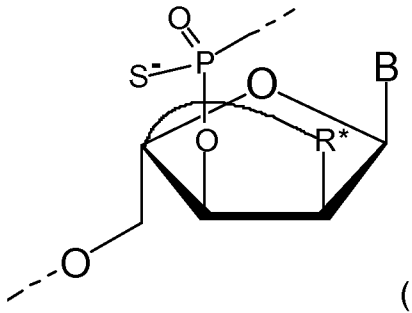
(**B3**)



(**B4**)



(**B5**)



(**B6**)

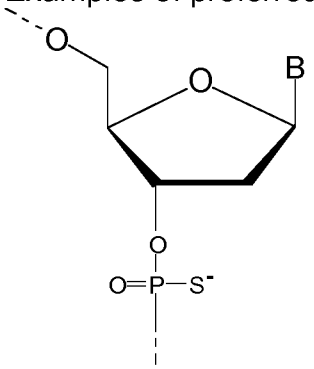
wherein

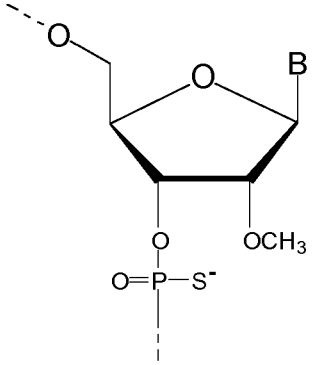
B represents a nucleobase and preferably A, T, G, C, U;

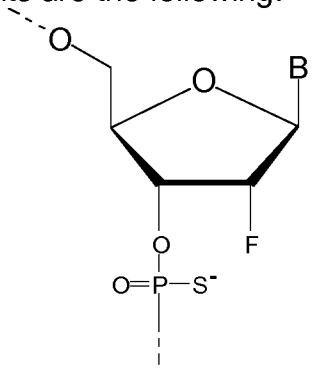
R represents –H, –F, –OH, $–NH_2$, $–N(CH_3)_2$, $–OCH_3$, $–OCH_2CH_2OCH_3$, $–OCH_2CH_2CH_2OH$, $–OCH_2CH_2CH_2NH_2$ and preferably –H;

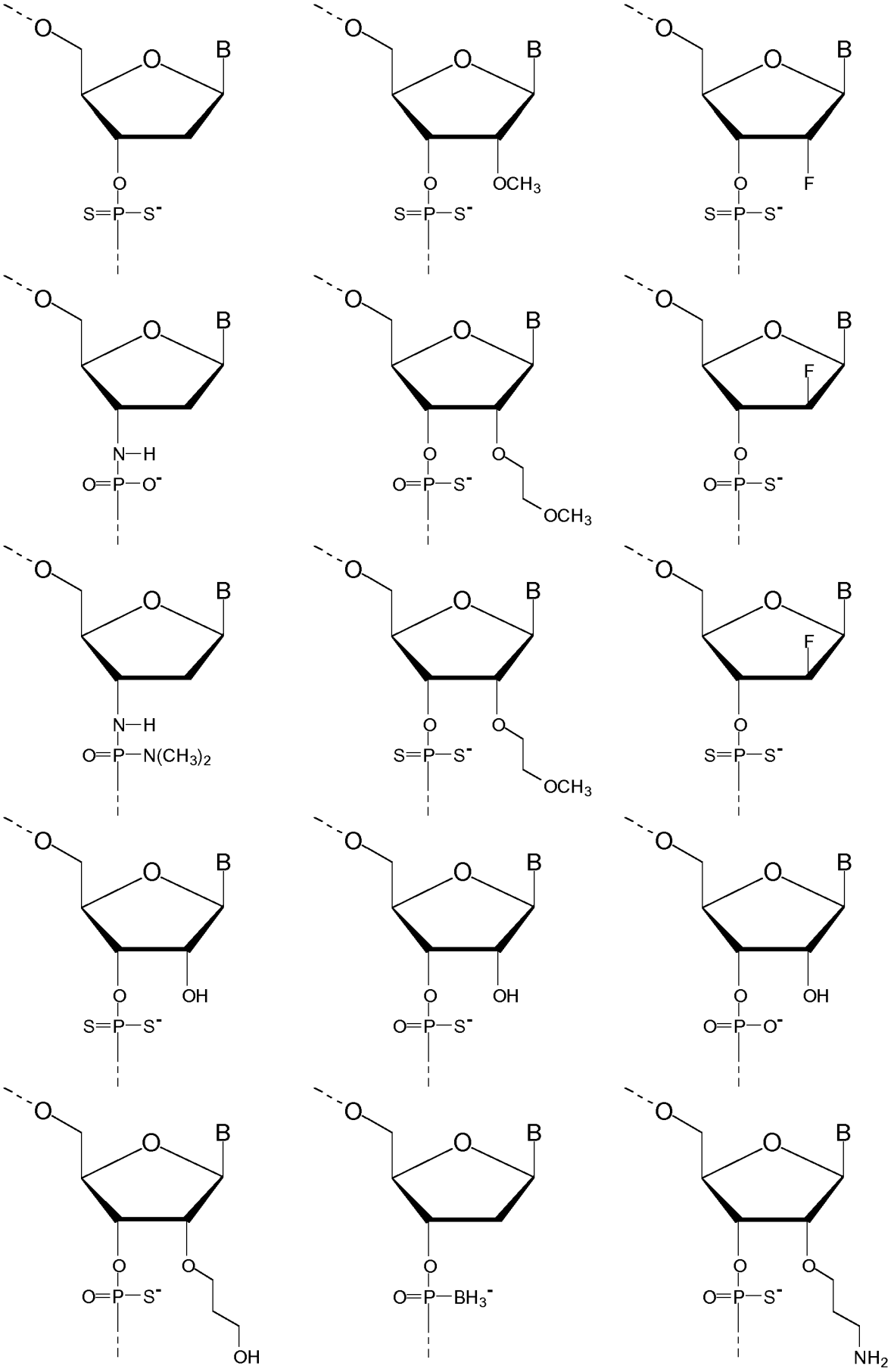
R^* represents the moiety $–R^{\#}–R–$ as defined below and is, for instance, preferably selected from $–C(R^aR^b)–O–$, $–C(R^aR^b)–NR^c–$, $–C(R^aR^b)–S–$, and $–C(R^aR^b)–C(R^aR^b)–O–$, wherein the substituents R^a, R^b and R^c have the meanings as defined herein. More preferably R^* is selected from $–CH_2–O–$, $–CH_2–S–$, $–CH_2–NH–$, $–CH_2–N(CH_3)–$, $–CH_2–CH_2–O–$, or $–CH_2–CH_2–S–$, and more preferably $–CH_2–O–$, $–CH_2–S–$, $–CH_2–CH_2–O–$, or $–CH_2–CH_2–S–$, and still more preferably $–CH_2–O–$, $–CH_2–S–$, or $–CH_2–CH_2–O–$, and still more preferably $–CH_2–O–$ or $–CH_2–S–$, and most preferably $–CH_2–O–$.

Examples of preferred nucleotides which are non-LNA units are the following:








B
O
S=P—S⁻
OCH3
F
N—H
O=P—O⁻
O=P—S⁻
O=P—N(CH3)2
OH
O=P—BH3⁻
NH2

Internucleotide Linkages (IL)

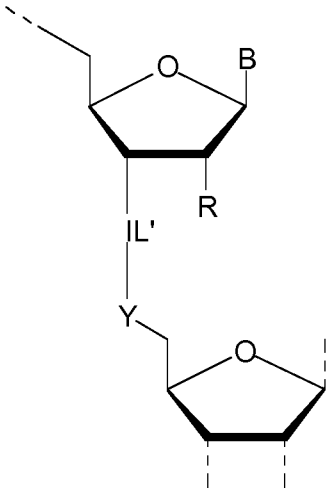
The monomers of the antisense-oligonucleotides described herein are coupled together via an internucleotide linkage. Suitably, each monomer is linked to the 3' adjacent monomer via an internucleotide linkage. The person having ordinary skill in the art would understand that, in the context of the present invention, the 5' monomer at the end of an oligomer does not comprise a 5' internucleotide linkage, although it may or may not comprise a 5' terminal group. The term "internucleotide linkage" is intended to mean a group capable of covalently coupling together two nucleotides, two nucleotide analogues like two LNAs, and a nucleotide and a nucleotide analogue like an LNA. Specific and preferred examples include phosphate groups and phosphorothioate groups.

The nucleotides of the antisense-oligonucleotides of the present invention or contiguous nucleotide sequences thereof are coupled together via internucleotide linkages. Suitably each nucleotide is linked through the 5' position to the 3' adjacent nucleotide via an internucleotide linkage.

The antisense-oligonucleotides can be modified by several different ways. Modifications within the backbone are possible and refer to antisense-oligonucleotides wherein the phosphate groups (also named phosphodiester groups) in their internucleotide backbone are partially or completely replaced by other groups. Preferred modified antisense-oligonucleotide backbones include, for instance, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriester, aminoalkylphosphotriesters, methyl, ethyl and C_3–C_{10}–alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogues of these, and those having inverted polarity wherein the adjacent pairs of nucleotide units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acids forms thereof are also included and disclosed herein in further detail.

Suitable internucleotide linkages include those listed within WO2007/031091, for example the internucleotide linkages listed on the first paragraph of page 34 of WO2007/031091 (hereby incorporated by reference). It is, in some embodiments, preferred to modify the internucleotide linkage from its normal phosphodiester to one that is more resistant to nuclease attack, such as phosphorothioate or boranophosphate - these two, accepted by RNase H mediated cleavage, also allow that route of antisense inhibition in reducing the expression of the target gene.

The internucleotide linkage consists of the group IL' which is the group bound to the 3' carbon atom of the ribose moiety and the group Y which is the group bound to the 5' carbon atom of the contiguous ribose moiety as shown in the formula (IL'Y) below



The internucleotide linkage IL is represented by –IL'–Y–. IL' represents –X''–P(=X')(X$^-$)– so that IL is represented by –X''–P(=X')(X$^-$)–Y–, wherein the substituents X$^-$, X', X'' and Y have the meanings as disclosed herein.

The internucleotide linkage IL = –X''–P(=X')(X$^-$)–Y– is preferably selected form the group consisting of:
–O–P(O)(O$^-$)–O–, –O–P(O)(S$^-$)–O–, –O–P(S)(S$^-$)–O–, –S–P(O)(O$^-$)–O–, –S–P(O)(S$^-$)–O–, –S–P(S)(S$^-$)–O–, –O–P(O)(O$^-$)–S–, –O–P(O)(S$^-$)–S–, –S–P(O)(O$^-$)–S–, –O–P(O)(R^H)–O–, –O–P(O)(OR^H)–O–, –O–P(O)(NHR^H)–O–, –O–P(O)[N(R^H)$_2$]–O–, –O–P(O)(BH$_3^-$)–O–, –O–P(O)(OCH$_2$CH$_2$OR^H)–O–, –O–P(O)(OCH$_2$CH$_2$SR^H)–O–, –O–P(O)(O$^-$)–NR^H–, –NR^H–P(O)(O$^-$)–O–, where R^H is selected from hydrogen and C1-4-alkyl.

The group –O–P(O)(R^H)–O– is preferably –O–P(O)(CH$_3$)–O– or –O–P(O)(C$_2$H$_5$)–O– and most preferably –O–P(O)(CH$_3$)–O–.
The group –O–P(O)(OR^H)–O– is preferably –O–P(O)(OCH$_3$)–O– or –O–P(O)(OC$_2$H$_5$)–O– and most preferably –O–P(O)(OCH$_3$)–O–.
The group –O–P(O)(NHR^H)–O– is preferably –O–P(O)(NHCH$_3$)–O– or –O–P(O)(NHC$_2$H$_5$)–O– and most preferably –O–P(O)(NHCH$_3$)–O–.
The group –O–P(O)[N(R^H)$_2$]–O– is preferably –O–P(O)[N(CH$_3$)$_2$]–O– or –O–P(O)[N(C$_2$H$_5$)$_2$]–O– and most preferably –O–P(O)[N(CH$_3$)$_2$]–O–.
The group –O–P(O)(OCH$_2$CH$_2$OR^H)–O– is preferably –O–P(O)(OCH$_2$CH$_2$OCH$_3$)–O– or –O–P(O)(OCH$_2$CH$_2$OC$_2$H$_5$)–O– and most preferably –O–P(O)(OCH$_2$CH$_2$OCH$_3$)–O–.

The group –O–P(O)($OCH_2CH_2SR^H$)–O– is preferably –O–P(O)($OCH_2CH_2SCH_3$)–O– or –O–P(O)($OCH_2CH_2SC_2H_5$)–O– and most preferably –O–P(O)($OCH_2CH_2SCH_3$)–O–.

The group –O–P(O)(O^-)–NR^H– is preferably –O–P(O)(O^-)–NH– or –O–P(O)(O^-)–$N(CH_3)$– and most preferably –O–P(O)(O^-)–NH–.

The group –NR^H–P(O)(O^-)–O– is preferably –NH–P(O)(O^-)–O– or –$N(CH_3)$–P(O)(O^-)–O– and most preferably –NH–P(O)(O^-)–O–.

Even more preferably IL represents –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –O–P(O)(NHR^H)–O–, or –O–P(O)[$N(R^H)_2$]–O–, and still more preferably IL represents –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, or –O–P(S)(S^-)–O–, and most preferably IL represents –O–P(O)(S^-)–O–, or –O–P(O)(O^-)–O–.

Thus IL is preferably a phosphate group (–O–P(O)(O^-)–O–), a phosphorothioate group (–O–P(O)(S^-)–O–) or a phosphorodithioate group (–O–P(S)(S^-)–O–).

The nucleotide units or the nucleosides of the antisense-oligonucleotides are connected to each other by internucleotide linkages so that within one antisense-oligonucleotide different internucleotide linkages can be present. The LNA units are preferably linked by internucleotide linkages which are not phosphate groups. The LNA units are linked to each other by a group IL which is preferably selected from –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –O–P(O)(NHR^H)–O–, and –O–P(O)[$N(R^H)_2$]–O– and more preferably from –O–P(O)(S^-)–O– and –O–P(S)(S^-)–O–.

The non-LNA units are linked to each other by a group IL which is preferably selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –O–P(O)(NHR^H)–O–, and –O–P(O)[$N(R^H)_2$]–O– and more preferably from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O– and –O–P(S)(S^-)–O–.

A non-LNA unit is linked to an LNA unit by a group IL which is preferably selected from –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –O–P(O)(NHR^H)–O–, and –O–P(O)[$N(R^H)_2$]–O– and more preferably from –O–P(O)(S^-)–O– and –O–P(S)(S^-)–O–.

The term "**LNA unit**" as used herein refers to a nucleotide which is locked, i.e. to a nucleotide which has a bicyclic structure and especially a bicyclic ribose structure and more especially a bicyclic ribose structure as shown in general formula (II). The bridge "locks" the ribose in the 3'-*endo* (North) conformation. The ribose moiety of an LNA nucleotide is modified with an extra bridge connecting the 2' oxygen and 4' carbon. Alternatively used terms for LNA are bicyclic nucleotides or bridged

nucleotides, thus, an alternative term for LNA unit is bicyclic nucleotide unit or bridged nucleotide unit.

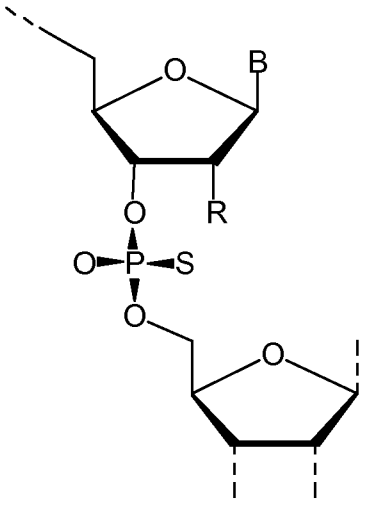
The term "**non-LNA unit**" as used herein refers to a nucleotide which is not locked, i.e. to a nucleotide which has no bicyclic sugar moiety and especially no bicyclic ribose structure and more especially no bicyclic ribose structure as shown in general formula (II). The non-LNA units are most preferably DNA units.

The term "**DNA unit**" as used herein refers to a nucleotide containing a 2-deoxyribose as sugar. Thus, the nucleotide is made of a nucleobase and a 2-deoxyribose.

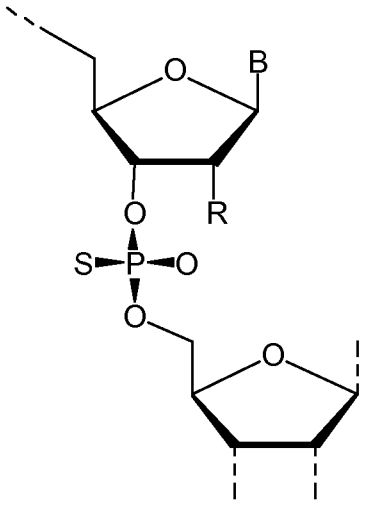
The term "**unit**" as used herein refers to a part or a fragment or a moiety of an antisense-oligonucleotide of the present invention. Thus a "unit" is not a complete molecule, it is a part or a fragment or a moiety of an antisense-oligonucleotide which has at least one position for a covalent linkage to another part or fragment or moiety of the antisense-oligonucleotide. For example, the general structures (**B1**) to (**B6**) are units, because they can be covalently linked through the group Y and IL' or –O– and –O–P(O)(S^-)– respectively. Preferably a unit is a moiety consisting of a pentose structure, a nucleobase connected to the pentose structure a 5' radical group and an IL' radical group.

The term "**building block**" or "**monomer**" as used herein refers to a molecule and especially to a nucleoside which is used in the synthesis of an antisense-oligonucleotide of the present invention. Examples are the LNA molecules of general formula (I), wherein Y represents a 5'-terminal group and IL' represents a 3'-terminal group.

Furthermore, pure diastereomeric anti-sense-oligonucleotides are preferred. Preferred are Sp- and Rp-diastereomers as shown at hand-right side:



Rp diastereomer



Sp diastereomer

Suitable sulphur (S) containing internucleotide linkages as provided herein are preferred.

Preferred are phosphorothioate moieties in the backbone where at least 50% of the internucleotide linkages are phosphorothioate groups. Also preferred is that the LNA units, if present, are linked through phosphorothioates as internucleotide linkages. Most preferred is a complete phosphorothioate backbone, i.e. most preferred is when all nucleotide units and also the LNA units (if present) are linked to each other through phosphorothioate groups which are defined as follows: –O–P(O)(S^-)–O– which is synonymous to –O–P(O,S)–O– or to –O–P(O^-)(S)–O–.

In case the antisense-oligonucleotide is a **gapmer**, it is preferred that the LNA regions have internucleotide linkages selected from –O–P(O)(S^-)–O– and –O–P(S)(S^-)–O– and that the non-LNA region, the middle part, has internucleotide linkages selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O– and –O–P(S)(S^-)–O– and that the LNA regions are connected to the non-LNA region through internucleotide linkages selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O– and –O–P(S)(S^-)–O–.

It is even more preferred if all internucleotide linkages which are 9 in a 10-mer and 19 in a 20-mer are selected from –O–P(O)(S^-)–O– and –O–P(S)(S^-)–O–. Still more preferred is that all internucleotide linkages are phosphorothioate groups (–O–P(O)(S^-)–O–) or are phosphorodithioate groups (–O–P(S)(S^-)–O–).

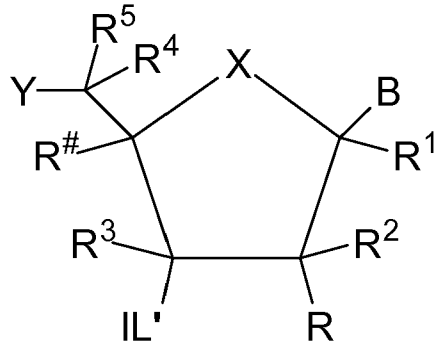
Locked Nucleic Acids (LNA®)

It is especially preferred that some of the nucleotides of the general formula (**B1**) or (**B2**) in the antisense-oligonucleotides are replaced by so-called LNAs (Locked Nucleic Acids). The abbreviation LNA is a registered trademark, but herein the term “LNA” is solely used in a descriptive manner.

Preferably the terminal nucleotides are replaced by LNAs and more preferred the last 1 to 4 nucleotides at the 3’ end and/or the last 1 to 4 nucleotides at the 5’ end are replaced by LNAs. It is also preferred to have at least the terminal nucleotide at the 3’ end and at the 5’ end replaced by an LNA each.

The term "**LNA**" as used herein, refers to a bicyclic nucleotide analogue, known as "Locked Nucleic Acid". It may refer to an LNA monomer, or, when used in the context of an "LNA antisense-oligonucleotide" or an "antisense-oligonucleotide containing LNAs", LNA refers to an oligonucleotide containing one or more such bicyclic nucleotide analogues. LNA nucleotides are characterized by the presence

of a linker group (such as a bridge) between C2' and C4' of the ribose sugar ring - for example as shown as the biradical $R^{\#}$ – R as described below. The LNA used in the antisense-oligonucleotides of the present invention preferably has the structure of the general formula (I)



(I)

wherein for all chiral centers, asymmetric groups may be found in either R or S orientation;

wherein X is selected from –O–, –S–, $–N(R^N)–$, $–C(R^6R^7)–$, and preferably X is –O–;

B is selected from hydrogen, optionally substituted C_{1-4}-alkoxy, optionally substituted C_{1-4}-alkyl, optionally substituted C_{1-4}-acyloxy, nucleobases and nucleobase analogues, and preferably B is a nucleobase or a nucleobase analogue and most preferred a standard nucleobase;

Y represents a part of an internucleotide linkage to an adjacent nucleotide in case the moiety of general formula (I) is an LNA unit of an antisense-oligonucleotide of the present invention, or a **5'-terminal group** in case the moiety of general formula (I) is a monomer or building block for synthesizing an antisense-oligonucleotide of the present invention. The 5' carbon atom optionally includes the substituent R^4 and R^5;

IL' represents a part of an internucleotide linkage to an adjacent nucleotide in case the moiety of general formula (I) is an LNA unit of an antisense-oligonucleotide of the present invention, or a **3'-terminal group** in case the moiety of general formula (I) is a monomer or building block for synthesizing an antisense-oligonucleotide of the present invention.

$R^{\#}$ and R together represent a bivalent linker group consisting of 1 – 4 groups or atoms selected from $–C(R^aR^b)–$, $–C(R^a)=C(R^b)–$, $–C(R^a)=N–$, –O–, $–Si(R^a)_2–$, –S–, $–SO_2–$, $–N(R^c)–$, and >C=Z, wherein Z is selected from –O–, –S–, and $–N(R^a)–$, and R^a, R^b and R^c are independently of each other selected from hydrogen, optionally substituted C_{1-12}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkynyl, hydroxy, optionally substituted C_{1-12}-alkoxy, C_{1-6}-alkoxy-C_{1-6}-alkyl, C_{2-6}-alkenyloxy, carboxy, C_{1-12}-alkoxycarbonyl, C_{1-12}-alkylcarbonyl, formyl, aryl,

aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkylenyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkylenyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, where aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together may represent optionally substituted methylene (=CH2), wherein for all chiral centers, asymmetric groups may be found in either *R* or *S* orientation, and;

each of the substituents R^1, R^2, R^3, R^4, R^5, R^6 and R^7, which are present is independently selected from hydrogen, optionally substituted C_{1-12}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkynyl, hydroxy, C_{1-12}-alkoxy, C_{1-6}-alkoxy-C_{1-6}-alkyl, C_{2-6}-alkenyloxy, carboxy, C_{1-12}-alkoxycarbonyl, C_{1-12}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, where aryl and heteroaryl may be optionally substituted, and where two geminal substituents together may designate oxo, thioxo, imino, or optionally substituted methylene;

wherein R^N is selected from hydrogen and C_{1-4}-alkyl, and where two adjacent (non-geminal) substituents may designate an additional bond resulting in a double bond; and R^N, when present and not involved in a biradical, is selected from hydrogen and C_{1-4}-alkyl; and basic salts and acid addition salts thereof. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

In preferred embodiments, $R^{\#}$ and R together represent a biradical consisting of a groups selected from the group consisting of –C(R^aR^b)–C(R^aR^b)–, –C(R^aR^b)–O–, –C(R^aR^b)–NR^c–, –C(R^aR^b)–S–, and –C(R^aR^b)–C(R^aR^b)–O–, wherein each R^a, R^b and R^c may optionally be independently selected.

In some embodiments, R^a and R^b may be, optionally independently selected from the group consisting of hydrogen and C_{1-6}-alkyl, such as methyl, and preferred is hydrogen.

In preferred embodiments, R^1, R^2, R^3, R^4, and R^5 are independently selected from the group consisting of hydrogen, halogen, C_{1-6}-alkyl, substituted C_{1-6}-alkyl, C_{2-6}-alkenyl, substituted C_{2-6}-alkenyl, C_{2-6}-alkynyl or substituted C_{2-6}-alkynyl, C_{1-6}-alkoxyl, substituted C_{1-6}-alkoxyl, acyl, substituted acyl, C_{1-6}-aminoalkyl or

substituted C_{1-6}-aminoalkyl. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

In preferred embodiments R^1, R^2, R^3, R^4, and R^5 are hydrogen.

In some embodiments, R^1, R^2, and R^3, are independently selected from the group consisting of hydrogen, halogen, C_{1-6}-alkyl, substituted C_{1-6}-alkyl, C_{2-6}-alkenyl, substituted C_{2-6}-alkenyl, C_{2-6}-alkynyl or substituted C_{2-6}-alkynyl, C_{1-6}-alkoxyl, substituted C_{1-6}-alkoxyl, acyl, substituted acyl, C_{1-6}-aminoalkyl or substituted C_{1-6}-aminoalkyl. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. In preferred embodiments R^1, R^2, and R^3 are hydrogen.

In preferred embodiments, R^4 and R^5 are each independently selected from the group consisting of –H, $–CH_3$, $–CH_2–CH_3$, $–CH_2–O–CH_3$, and $–CH=CH_2$. Suitably in some embodiments, either R^4 or R^5 are hydrogen, whereas the other group (R^4 or R^5 respectively) is selected from the group consisting of C_{1-6}-alkyl, C_{2-6}-alkenyl, C_{2-6}-alkynyl, substituted C_{1-6}-alkyl, substituted C_{2-6}-alkenyl, substituted C_{2-6}-alkynyl or substituted acyl (–C(=O)–); wherein each substituted group is mono or poly substituted with substituent groups independently selected from halogen, C_{1-6}-alkyl, substituted C_{1-6}-alkyl, C_{2-6}-alkenyl, substituted C_{2-6}-alkenyl, C_{2-6}-alkynyl, substituted C_{2-6}-alkynyl, $–OJ_1$, $–SJ_1$, $–NJ_1J_2$, $–N_3$, $–COOJ_1$, –CN, $–O–C(=O)NJ_1J_2$, $–N(H)C(=NH)NJ_1J_2$ or $–N(H)C(=X)N(H)J_2$, wherein X is O or S; and each J_1 and J_2 is, independently –H, C_{1-6}-alkyl, substituted C_{1-6}-alkyl, C_{2-6}-alkenyl, substituted C_{2-6}-alkenyl, C_{2-6}-alkynyl, substituted C_{2-6}-alkynyl, C_{1-6}-aminoalkyl, substituted C_{1-6}-aminoalkyl or a protecting group. In some embodiments either R^4 or R^5 is substituted C_{1-6}-alkyl. In some embodiments either R^4 or R^5 is substituted methylene, wherein preferred substituent groups include one or more groups independently selected from –F, $–NJ_1J_2$, $–N_3$, –CN, $–OJ_1$, $–SJ_1$, $–O–C(=O)NJ_1J_2$, $–N(H)C(=NH)NJ_1J_2$ or $–N(H)C(=O)N(H)J_2$. In some embodiments each J_1 and J_2 is, independently –H or C_{1-6}-alkyl. In some embodiments either R^4 or R^5 is methyl, ethyl or methoxymethyl. In some embodiments either R^4 or R^5 is methyl. In a further embodiment either R^4 or R^5 is ethylenyl. In some embodiments either R^4 or R^5 is substituted acyl. In some embodiments either R^4 or R^5 is $–O–C(=O)NJ_1J_2$. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. Such 5' modified bicyclic nucleotides are disclosed in WO 2007/134181 A, which is hereby incorporated by reference in its entirety.

In some embodiments B is a nucleobase, including nucleobase analogues and naturally occurring nucleobases, such as a purine or pyrimidine, or a substituted purine or substituted pyrimidine, such as a nucleobase referred to herein, such as a

nucleobase selected from the group consisting of adenine, cytosine, thymine, adenine, uracil, and/or a modified or substituted nucleobase, such as 5-thiazolo-uracil, 2-thio-uracil, 5-propynyl-uracil, 2'thio-thymine, 5-methyl cytosine, 5-thiozolo-cytosine, 5-propynyl-cytosine, and 2,6- diaminopurine.

In preferred embodiments, $R^{\#}$ and R together represent a biradical selected from $-C(R^aR^b)-O-$, $-C(R^aR^b)-C(R^cR^d)-O-$, $-C(R^aR^b)-C(R^cR^d)-C(R^eR^f)-O-$, $-C(R^aR^b)-O-C(R^dR^e)-$, $-C(R^aR^b)-O-C(R^dR^e)-O-$, $-C(R^aR^b)-C(R^dR^e)-$, $-C(R^aR^b)-C(R^cR^d)-C(R^eR^f)-$, $-C(R^a)=C(R^b)-C(R^dR^e)-$, $-C(R^aR^b)-N(R^c)-$, $-C(R^aR^b)-C(R^dR^e)-N(R^c)-$, $-C(R^aR^b)-N(R^c)-O-$, $-C(R^aR^b)-S-$, and $-C(R^aR^b)-C(R^dR^e)-S-$, wherein R^a, R^b, R^c, R^d, R^e, and R^f each is independently selected from hydrogen, optionally substituted C_{1-12}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkynyl, hydroxy, C_{1-12}-alkoxy, C_{1-6}-alkoxy-C_{1-6}-alkyl, C_{2-6}-alkenyloxy, carboxy, C_{1-12}-alkoxycarbonyl C_{1-12}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, where aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together may designate optionally substituted methylene ($=CH_2$). For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

In a further embodiment $R^{\#}$ and R together designate a biradical (bivalent group) selected from $-CH_2-O-$, $-CH_2-S-$, $-CH_2-NH-$, $-CH_2-N(CH_3)-$, $-CH_2-CH_2-O-$, $-CH_2-CH(CH_3)-$, $-CH_2-CH_2-S-$, $-CH_2-CH_2-NH-$, $-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-O-$, $-CH_2-CH_2-CH(CH_3)-$, $-CH=CH-CH_2-$, $-CH_2-O-CH_2-O-$, $-CH_2-NH-O-$, $-CH_2-N(CH_3)-O-$, $-CH_2-O-CH_2-$, $-CH(CH_3)-O-$, $-CH(CH_2-O-CH_3)-O-$, $-CH_2-CH_2-$, and $-CH=CH-$. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

In some embodiments, $R^{\#}$ and R together designate the biradical $-C(R^aR^b)-N(R^c)-O-$, wherein R^a and R^b are independently selected from the group consisting of hydrogen, halogen, C_{1-6}-alkyl, substituted C_{1-6}-alkyl, C_{2-6}-alkenyl, substituted C_{2-6}-alkenyl, C_{2-6}-alkynyl or substituted C_{2-6}-alkynyl, C_{1-6}-alkoxyl, substituted C_{1-6}-alkoxyl, acyl, substituted acyl, C_{1-6}-aminoalkyl or substituted C_{1-6}-aminoalkyl, such as hydrogen, and; wherein R^c is selected from the group consisting of hydrogen, halogen, C_{1-6}-alkyl, substituted C_{1-6}-alkyl, C_{2-6}-alkenyl,

substituted C_{2-6}-alkenyl, C_{2-6}-alkynyl or substituted C_{2-6}-alkynyl, C_{1-6}-alkoxyl, substituted C_{1-6}-alkoxyl, acyl, substituted acyl, C_{1-6}-aminoalkyl or substituted C_{1-6}-aminoalkyl, and preferably hydrogen.

In preferred embodiments, $R^{\#}$ and R together represent the biradical $–C(R^aR^b)–O–C(R^dR^e)–O–$, wherein R^a, R^b, R^d, and R^e are independently selected from the group consisting of hydrogen, halogen, C_{1-6}-alkyl, substituted C_{1-6}-alkyl, C_{2-6}-alkenyl, substituted C_{2-6}-alkenyl, C_{2-6}-alkynyl or substituted C_{2-6}-alkynyl, C_{1-6}-alkoxyl, substituted C_{1-6}-alkoxyl, acyl, substituted acyl, C_{1-6}-aminoalkyl or substituted C_{1-6}-aminoalkyl, and preferably hydrogen.

In preferred embodiments, $R^{\#}$ and R form the biradical $–CH(Z)–O–$, wherein Z is selected from the group consisting of C_{1-6}-alkyl, C_{2-6}-alkenyl, C_{2-6}-alkynyl, substituted C_{1-6}-alkyl, substituted C_{2-6}-alkenyl, substituted C_{2-6}-alkynyl, acyl, substituted acyl, substituted amide, thiol or substituted thio; and wherein each of the substituted groups, is, independently, mono or poly substituted with optionally protected substituent groups independently selected from halogen, oxo, hydroxyl, $–OJ_1$, $–NJ_1J_2$, $–SJ_1$, $–N_3$, $–OC(=X)J_1$, $–OC(=X)NJ_1J_2$, $–NJ^3C(=X)NJ_1J_2$ and –CN, wherein each J_1, J_2 and J_3 is, independently, –H or C_{1-6}-alkyl, and X is O, S or NJ_1. In preferred embodiments Z is C_{1-6}-alkyl or substituted C_{1-6}-alkyl. In further preferred embodiments Z is methyl. In preferred embodiments Z is substituted C_{1-6}-alkyl. In preferred embodiments said substituent group is C_{1-6}-alkoxy. In some embodiments Z is $CH_3OCH_2–$. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. Such bicyclic nucleotides are disclosed in US 7,399,845 which is hereby incorporated by reference in its entirety. In preferred embodiments, R^1, R^2, R^3, R^4, and R^5 are hydrogen. In preferred embodiments, R^1, R^2, and R^3 are hydrogen, and one or both of R^4, R^5 may be other than hydrogen as referred to above and in WO 2007/134181.

In preferred embodiments, $R^{\#}$ and R together represent a biradical which comprise a substituted amino group in the bridge such as the biradical $–CH_2–N(R^c)–$, wherein R^c is C_{1-12}-alkyloxy. In preferred embodiments $R^{\#}$ and R together represent a biradical $–Cq_3q_4–NOR–$, wherein q_3 and q_4 are independently selected from the group consisting of hydrogen, halogen, C_{1-6}-alkyl, substituted C_{1-6}-alkyl, C_{2-6}-alkenyl, substituted C_{2-6}-alkenyl, C_{2-6}-alkynyl or substituted C_{2-6}-alkynyl, C_{1-6}-alkoxyl, substituted C_{1-6}-alkoxyl, acyl, substituted acyl, C_{1-6}-aminoalkyl or substituted C_{1-6}-aminoalkyl; wherein each substituted group is, independently, mono or poly substituted with substituent groups independently selected from halogen, $–OJ_1$, $–SJ_1$, $–NJ_1J_2$, $–COOJ_1$, –CN, $–OC(=O)NJ_1J_2$, $–NH–C(=NH)NJ_1J_2$ or

–NH–C(=X)NHJ$_2$, wherein X is O or S; and each of J$_1$ and J$_2$ is, independently, –H, C$_{1-6}$-alkyl, C$_{2-6}$-alkenyl, C$_{2-6}$-alkynyl, C$_{1-6}$-aminoalkyl or a protecting group. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. Such bicyclic nucleotides are disclosed in WO2008/150729 which is hereby incorporated by reference in its entirety. In preferred embodiments, R^1, R^2, R^3, R^4, and R^5 are independently selected from the group consisting of hydrogen, halogen, C$_{1-6}$-alkyl, substituted C$_{1-6}$-alkyl, C$_{2-6}$-alkenyl, substituted C$_{2-6}$-alkenyl, C$_{2-6}$-alkynyl or substituted C$_{2-6}$-alkynyl, C$_{1-6}$-alkoxyl, substituted C$_{1-6}$-alkoxyl, acyl, substituted acyl, C$_{1-6}$-aminoalkyl or substituted C$_{1-6}$-aminoalkyl. In preferred embodiments, R^1, R^2, R^3, R^4, and R^5 are hydrogen. In preferred embodiments, R^1, R^2, and R^3 are hydrogen and one or both of R^4, R^5 may be other than hydrogen as referred to above and in WO 2007/134181.

In preferred embodiments R$^{\#}$ and R together represent a biradical (bivalent group) –C(R^{a}R^b)–O–, wherein R^a and R^b are each independently halogen, C$_{1-12}$-alkyl, substituted C$_{1-12}$-alkyl, C$_{2-6}$-alkenyl, substituted C$_{2-6}$-alkenyl, C$_{2-6}$-alkynyl, substituted C$_{2-6}$-alkynyl, C$_{1-12}$-alkoxy, substituted C$_{1-12}$-alkoxy, –OJ$_1$, –SJ$_1$, –S(O)J$_1$, –SO$_2$–J$_1$, –NJ$_1$J$_2$, –N$_3$, –CN, –C(=O)OJ$_1$, –C(=O)NJ$_1$J$_2$, –C(=O)J$_1$, –OC(=O)NJ$_1$J$_2$, –NH–C(=NH)NJ$_1$J$_2$, –NH–C(=O)NJ$_1$J$_2$, or , –NH–C(=S)NJ$_1$J$_2$; or R^a and R^b together are =C(q$_3$)(q$_4$); q$_3$ and q$_4$ are each, independently, – H, halogen, C$_{1-12}$-alkyl or substituted C$_{1-12}$-alkyl; each substituted group is, independently, mono or poly substituted with substituent groups independently selected from halogen, C$_{1-6}$-alkyl, substituted C$_{1-6}$-alkyl, C$_{2-6}$-alkenyl, substituted C$_{2-6}$-alkenyl, C$_{2-6}$-alkynyl, substituted C$_{2-6}$-alkynyl, –OJ$_1$, –SJ$_1$, –NJ$_1$J$_2$, –N$_3$, –CN, –C(=O)OJ$_1$, –C(=O)NJ$_1$J$_2$, –C(=O)J$_1$, –OC(=O)NJ$_1$J$_2$, –NH–C(=O)NJ$_1$J$_2$, or –NH–C(=S)NJ$_1$J$_2$ and; each J$_1$ and J$_2$ is independently, –H, C$_{1-6}$-alkyl, substituted C$_{1-6}$-alkyl, C$_{2-6}$-alkenyl, substituted C$_{2-6}$-alkenyl, C$_{2-6}$-alkynyl, substituted C$_{2-6}$-alkynyl, C$_{1-6}$-aminoalkyl, substituted C$_{1-6}$-aminoalkyl or a protecting group. Such compounds are disclosed in WO2009006478A, hereby incorporated in its entirety by reference.

In preferred embodiments, R$^{\#}$ and R form the biradical –Q–, wherein Q is –C(q$_1$)(q$_2$)C(q$_3$)(q$_4$)–, –C(q$_1$)=C(q$_3$)–, –C[=C(q$_1$)(q$_2$)]–C(q$_3$)(q$_4$)– or –C(q$_1$)(q$_2$)–C[=C(q$_3$)(q$_4$)]–;

q$_1$, q$_2$, q$_3$, q$_4$ are each independently of each other –H, halogen, C$_{1-12}$-alkyl, substituted C$_{1-12}$-alkyl, C$_{2-6}$-alkenyl, substituted C$_{1-12}$-alkoxy, –OJ$_1$, –SJ$_1$, –S(O)J$_1$, –SO$_2$–J$_1$, –NJ$_1$J$_2$, –N$_3$, –CN, –C(=O)OJ$_1$, –C(=O)NJ$_1$J$_2$, –C(=O)J$_1$, –OC(=O)NJ$_1$J$_2$, –NH–C(=NH)NJ$_1$J$_2$, –NH–C(=O)NJ$_1$J$_2$, or –NH–C(=S)NJ$_1$J$_2$; each J$_1$ and J$_2$ is independently of each other –H, C$_{1-6}$-alkyl, C$_{2-6}$-alkenyl, C$_{2-6}$-alkynyl, C$_{1-6}$-aminoalkyl or a protecting group; and optionally when Q is –C(q$_1$)(q$_2$)C(q$_3$)(q$_4$)– and one of q$_3$ or q$_4$ is –CH$_3$, then at least one of the other of q$_3$ or q$_4$ or one of q$_1$ and

q_2 is other than –H. In preferred embodiments R^1, R^2, R^3, R^4, and R^5 are hydrogen. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. Such bicyclic nucleotides are disclosed in WO2008/154401 which is hereby incorporated by reference in its entirety. In preferred embodiments R^1, R^2, R^3, R^4, and R^5 are independently of each other selected from the group consisting of hydrogen, halogen, C_{1-6}-alkyl, substituted C_{1-6}-alkyl, C_{2-6}-alkenyl, substituted C_{2-6}-alkenyl, C_{2-6}-alkynyl or substituted C_{2-6}-alkynyl, C_{1-6}-alkoxyl, substituted C_{1-6}-alkoxyl, acyl, substituted acyl, C_{1-6}-aminoalkyl or substituted C_{1-6}-aminoalkyl. In preferred embodiments R^1, R^2, R^3, R^4, and R^5 are hydrogen. In preferred embodiments R^1, R^2, and R^3 are hydrogen and one or both of R^4, R^5 may be other than hydrogen as referred to above and in WO 2007/134181 or WO2009/067647 (alpha-L-bicyclic nucleic acids analogues).

As used herein, the term "C_1-C_6-alkyl" refers to $–CH_3$, $–C_2H_5$, $–C_3H_7$, $–CH(CH_3)_2$, $–C_4H_9$, $–CH_2–CH(CH_3)_2$, $–CH(CH_3)–C_2H_5$, $–C(CH_3)_3$, $–C_5H_{11}$, $–CH(CH_3)–C_3H_7$, $–CH_2–CH(CH_3)–C_2H_5$, $–CH(CH_3)–CH(CH_3)_2$, $–C(CH_3)_2–C_2H_5$, $–CH_2–C(CH_3)_3$, $–CH(C_2H_5)_2$, $–C_2H_4–CH(CH_3)_2$, $–C_6H_{13}$, $–C_3H_6–CH(CH_3)_2$, $–C_2H_4–CH(CH_3)–C_2H_5$, $–CH(CH_3)–C_4H_9$, $–CH_2–CH(CH_3)–C_3H_7$, $–CH(CH_3)–CH_2–CH(CH_3)_2$, $–CH(CH_3)–CH(CH_3)–C_2H_5$, $–CH_2–CH(CH_3)–CH(CH_3)_2$, $–CH_2–C(CH_3)_2–C_2H_5$, $–C(CH_3)_2–C_3H_7$, $–C(CH_3)_2–CH(CH_3)_2$, $–C_2H_4–C(CH_3)_3$, $–CH_2–CH(C_2H_5)_2$, and $–CH(CH_3)–C(CH_3)_3$. The term "C_1-C_6-alkyl" shall also include "C_1-C_6-cycloalkyl" like cyclo-C_3H_5, cyclo-C_4H_7, cyclo-C_5H_9, and cyclo-C_6H_{11}.

Preferred are $–CH_3$, $–C_2H_5$, $–C_3H_7$, $–CH(CH_3)_2$, $–C_4H_9$, $–CH_2–CH(CH_3)_2$, $–CH(CH_3)–C_2H_5$, $–C(CH_3)_3$, and $–C_5H_{11}$. Especially preferred are $–CH_3$, $–C_2H_5$, $–C_3H_7$, and $–CH(CH_3)_2$.
The term "C_1-C_6-alkyl" shall also include "C_1-C_6-cycloalkyl" like cyclo-C_3H_5, cyclo-C_4H_7, cyclo-C_5H_9, and cyclo-C_6H_{11}.

As used herein, the term "C_1-C_{12}-alkyl" refers to C_1-C_6-alkyl, $–C_7H_{15}$, $–C_8H_{17}$, $–C_9H_{19}$, $–C_{10}H_{21}$, $–C_{11}H_{23}$, $–C_{12}H_{25}$.

As used herein, the term "C_1-C_6-alkylenyl" refers to $–CH_2–$, $–C_2H_4–$, $–CH(CH_3)–$, $–C_3H_6–$, $–CH_2–CH(CH_3)–$, $–CH(CH_3)–CH_2–$, $–C(CH_3)_2–$, $–C_4H_8–$, $–CH_2–C(CH_3)_2–$, $–C(CH_3)_2–CH_2–$, $–C_2H_4–CH(CH_3)–$, $–CH(CH_3)–C_2H_4–$, $–CH_2–CH(CH_3)–CH_2–$, $–CH(CH_3)–CH(CH_3)–$, $–C_5H_{10}–$, $–CH(CH_3)–C_3H_6–$, $–CH_2–CH(CH_3)–C_2H_4–$, $–C_2H_4–CH(CH_3)–CH_2–$, $–C_3H_6–CH(CH_3)–$, $–C_2H_4–C(CH_3)_2–$, $–C(CH_3)_2–C_2H_4–$, $–CH_2–C(CH_3)_2–CH_2–$,

–CH_2–CH(CH_3)–CH(CH_3)–, –CH(CH_3)–CH_2–CH(CH_3)–, –CH(CH_3)–CH(CH_3)–CH_2–, –CH(CH_3)–CH(CH_3)–CH(CH_3)–, –$C(CH_3)_2$–C_3H_6–, –CH_2–$C(CH_3)_2$–C_2H_4–, –C_2H_4–$C(CH_3)_2$–CH_2–, –C_3H_6–$C(CH_3)_2$–, –CH(CH_3)–C_4H_8–, –C_6H_{12}–, –CH_2–CH(CH_3)–C_3H_6–, –C_2H_4–CH(CH_3)–C_2H_4–, –C_3H_6–CH(CH_3)–CH_2–, –C_4H_8–CH(CH_3)–, –C_2H_4–CH(CH_3)–CH(CH_3)–, –CH_2–CH(CH_3)–CH(CH_3)–CH_2–, –CH_2–CH(CH_3)–CH_2–CH(CH_3)–, –CH(CH_3)–C_2H_4–CH(CH_3)–, –CH(CH_3)–CH_2–CH(CH_3)–CH_2–, and –CH(CH_3)–CH(CH_3)–C_2H_4–.

As used herein, the term "C_2-C_6-alkenyl" refers to –CH=CH_2, –CH_2–CH=CH_2, –$C(CH_3)$=CH_2, –CH=CH–CH_3, –C_2H_4–CH=CH_2, –CH_2–CH=CH–CH_3, –CH=CH–C_2H_5, –CH_2–$C(CH_3)$=CH_2, –CH(CH_3)–CH=CH, –CH=$C(CH_3)_2$, –$C(CH_3)$=CH–CH_3, –CH=CH–CH=CH_2, –C_3H_6–CH=CH_2, –C_2H_4–CH=CH–CH_3, –CH_2–CH=CH–C_2H_5, –CH=CH–C_3H_7, –CH_2–CH=CH–CH=CH_2, –CH=CH–CH=CH–CH_3, –CH=CH–CH_2–CH=CH_2, –$C(CH_3)$=CH–CH=CH_2, –CH=$C(CH_3)$–CH=CH_2, –CH=CH–$C(CH_3)$=CH_2, –C_4H_8–CH=CH_2, –C_3H_6–CH=CH–CH_3, –C_2H_4–CH=CH–C_2H_5, –CH_2–CH=CH–C_3H_7, –CH=CH–C_4H_9, –C_3H_6–$C(CH_3)$=CH_2, –C_2H_4–CH(CH_3)–CH=CH_2, –CH_2–CH(CH_3)–CH_2–CH=CH_2, –CH(CH_3)–C_2H_4–CH=CH_2, –C_2H_4–CH=$C(CH_3)_2$, –C_2H_4–$C(CH_3)$=CH–CH_3, –CH_2–CH(CH_3)–CH=CH–CH_3, –CH(CH_3)–CH_2–CH=CH–CH_3, –CH_2–CH=CH–$CH(CH_3)_2$, –CH_2–CH=$C(CH_3)$–C_2H_5, –CH_2–$C(CH_3)$=CH–C_2H_5, –CH(CH_3)–CH=CH–C_2H_5, –CH=CH–CH_2–$CH(CH_3)_2$, –CH=CH–CH(CH_3)–C_2H_5, –CH=$C(CH_3)$–C_3H_7, –$C(CH_3)$=CH–C_3H_7, –CH_2–CH(CH_3)–$C(CH_3)$=CH_2, –CH(CH_3)–CH_2–$C(CH_3)$=CH_2, –CH(CH_3)–CH(CH_3)–CH=CH_2, –CH_2–$C(CH_3)_2$–CH=CH_2, –$C(CH_3)_2$–CH_2–CH=CH_2, –CH_2–$C(CH_3)$=$C(CH_3)_2$, –CH(CH_3)–CH=$C(CH_3)_2$, –$C(CH_3)_2$–CH=CH–CH_3, –CH(CH_3)–$C(CH_3)$=CH–CH_3, –CH=$C(CH_3)$–CH(CH_3)$_2$, –$C(CH_3)$=CH–CH(CH_3)$_2$, –$C(CH_3)$=$C(CH_3)$–C_2H_5, –CH=CH–$C(CH_3)_3$, –$C(CH_3)_2$–$C(CH_3)$=CH_2, –CH(C_2H_5)–$C(CH_3)$=CH_2, –$C(CH_3)$(C_2H_5)–CH=CH_2, –CH(CH_3)–$C(C_2H_5)$=CH_2, –CH_2–$C(C_3H_7)$=CH_2, –CH_2–$C(C_2H_5)$=CH–CH_3, –CH(C_2H_5)–CH=CH–CH_3, –$C(C_4H_9)$=CH_2, –$C(C_3H_7)$=CH–CH_3, –$C(C_2H_5)$=CH–C_2H_5, –$C(C_2H_5)$=$C(CH_3)_2$, –C[$C(CH_3)_3$]=CH_2, –C[CH(CH_3)(C_2H_5)]=CH_2, –C[CH_2–$CH(CH_3)_2$]=CH_2, –C_2H_4–CH=CH–CH=CH_2, –CH_2–CH=CH–CH_2–CH=CH_2, –CH=CH–C_2H_4–CH=CH_2, –CH_2–CH=CH–CH=CH–CH_3, –CH=CH–CH_2–CH=CH–CH_3, –CH=CH–CH=CH–C_2H_5, –CH_2–CH=CH–$C(CH_3)$=CH_2,

$-CH_2-CH=C(CH_3)-CH=CH_2$, $-CH_2-C(CH_3)=CH-CH=CH_2$, $-CH(CH_3)-CH=CH-CH=CH_2$, $-CH=CH-CH_2-C(CH_3)=CH_2$, $-CH=CH-CH(CH_3)-CH=CH_2$, $-CH=C(CH_3)-CH_2-CH=CH_2$, $-C(CH_3)=CH-CH_2-CH=CH_2$, $-CH=CH-CH=C(CH_3)_2$, $-CH=CH-C(CH_3)=CH-CH_3$, $-CH=C(CH_3)-CH=CH-CH_3$, $-C(CH_3)=CH-CH=CH-CH_3$, $-CH=C(CH_3)-C(CH_3)=CH_2$, $-C(CH_3)=CH-C(CH_3)=CH_2$, $-C(CH_3)=C(CH_3)-CH=CH_2$, and $-CH=CH-CH=CH-CH=CH_2$.

Preferred are $-CH=CH_2$, $-CH_2-CH=CH_2$, $-C(CH_3)=CH_2$, $-CH=CH-CH_3$, $-C_2H_4-CH=CH_2$, $-CH_2-CH=CH-CH_3$. Especially preferred are $-CH=CH_2$, $-CH_2-CH=CH_2$, and $-CH=CH-CH_3$.

As used herein, the term "C_2-C_6-alkynyl" refers to $-C\equiv CH$, $-C\equiv C-CH_3$, $-CH_2-C\equiv CH$, $-C_2H_4-C\equiv CH$, $-CH_2-C\equiv C-CH_3$, $-C\equiv C-C_2H_5$, $-C_3H_6-C\equiv CH$, $-C_2H_4-C\equiv C-CH_3$, $-CH_2-C\equiv C-C_2H_5$, $-C\equiv C-C_3H_7$, $-CH(CH_3)-C\equiv CH$, $-CH_2-CH(CH_3)-C\equiv CH$, $-CH(CH_3)-CH_2-C\equiv CH$, $-CH(CH_3)-C\equiv C-CH_3$, $-C_4H_8-C\equiv CH$, $-C_3H_6-C\equiv C-CH_3$, $-C_2H_4-C\equiv C-C_2H_5$, $-CH_2-C\equiv C-C_3H_7$, $-C\equiv C-C_4H_9$, $-C_2H_4-CH(CH_3)-C\equiv CH$, $-CH_2-CH(CH_3)-CH_2-C\equiv CH$, $-CH(CH_3)-C_2H_4-C\equiv CH$, $-CH_2-CH(CH_3)-C\equiv C-CH_3$, $-CH(CH_3)-CH_2-C\equiv C-CH_3$, $-CH(CH_3)-C\equiv C-C_2H_5$, $-CH_2-C\equiv C-CH(CH_3)_2$, $-C\equiv C-CH(CH_3)-C_2H_5$, $-C\equiv C-CH_2-CH(CH_3)_2$, $-C\equiv C-C(CH_3)_3$, $-CH(C_2H_5)-C\equiv C-CH_3$, $-C(CH_3)_2-C\equiv C-CH_3$, $-CH(C_2H_5)-CH_2-C\equiv CH$, $-CH_2-CH(C_2H_5)-C\equiv CH$, $-C(CH_3)_2-CH_2-C\equiv CH$, $-CH_2-C(CH_3)_2-C\equiv CH$, $-CH(CH_3)-CH(CH_3)-C\equiv CH$, $-CH(C_3H_7)-C\equiv CH$, $-C(CH_3)(C_2H_5)-C\equiv CH$, $-C\equiv C-C\equiv CH$, $-CH_2-C\equiv C-C\equiv CH$, $-C\equiv C-C\equiv C-CH_3$, $-CH(C\equiv CH)_2$, $-C_2H_4-C\equiv C-C\equiv CH$, $-CH_2-C\equiv C-CH_2-C\equiv CH$, $-C\equiv C-C_2H_4-C\equiv CH$, $-CH_2-C\equiv C-C\equiv C-CH_3$, $-C\equiv C-CH_2-C\equiv C-CH_3$, $-C\equiv C-C\equiv C-C_2H_5$, $-C\equiv C-CH(CH_3)-C\equiv CH$, $-CH(CH_3)-C\equiv C-C\equiv CH$, $-CH(C\equiv CH)-CH_2-C\equiv CH$, $-C(C\equiv CH)_2-CH_3$, $-CH_2-CH(C\equiv CH)_2$, $-CH(C\equiv CH)-C\equiv C-CH_3$. Preferred are $-C\equiv CH$ and $-C\equiv C-CH_3$.

The term "$C_{1\text{-}6}$-alkoxyl" refers to "C_1-C_6-alkyl-O–".

The term "$C_{1\text{-}12}$-alkoxyl" refers to "C_1-C_{12}-alkyl-O–".

The term "$C_{1\text{-}6}$-aminoalkyl" refers to "H_2N–C_1-C_6-alkyl–".

The term "C_2-C_6-alkenyloxy" refers to "C_2-C_6-alkenyl–O–".

The term "$C_{1\text{-}6}$-alkylcarbonyl" refers to "C_1-C_6-alkyl-CO–". Also referred to as "acyl".

The term "C_{1-12}-alkylcarbonyl" refers to "C_1-C_{12}-alkyl-CO–". Also referred to as "acyl".

The term "C_{1-6}-alkoxycarbonyl" refers to "C_1-C_6-alkyl-O–CO–".

The term "C_{1-12}-alkoxycarbonyl" refers to "C_1-C_{12}-alkyl-O–CO–".

The term "C_1-C_6-alkanoyloxy" refers to "C_1-C_6-alkyl-CO–O–".

The term "C_{1-6}-alkylthio" refers to "C_1-C_6-alkyl–S–".

The term "C_{1-6}-alkylsulphonyloxy" refers to "C_1-C_6-alkyl–SO_2–O–".

The term "C_{1-6}-alkylcarbonylamino" refers to "C_1-C_6-alkyl–CO–NH–".

The term "C_{1-6}-alkylamino" refers to "C_1-C_6-alkyl–NH–".

The term "$(C_{1-6}\text{-})_2$alkylamino" refers to a dialkylamino group like "[C_1-C_6-alkyl][C_1-C_6-alkyl]N–".

The term "C_{1-6}-alkylaminocarbonyl" refers to "C_1-C_6-alkyl–NH–CO–"

The term "$(C_{1-6}\text{-})_2$alkylaminocarbonyl" refers to a dialkylaminocarbonyl group like "[C_1-C_6-alkyl][C_1-C_6-alkyl]N–CO–".

The term "amino-C_{1-6}-alkylaminocarbonyl" refers to "H_2N–[C_1-C_6-alkylenyl]–NH–CO–".

The term "C_{1-6}-alkyl-amino-C_{1-6}-alkylaminocarbonyl" refers to "C_{1-6}-alkyl–HN–[C_1-C_6-alkylenyl]–NH–CO–".

The term "$(C_{1-6}\text{-})_2$alkyl-amino-C_{1-6}-alkylaminocarbonyl" refers to "[C_1-C_6-alkyl][C_1-C_6-alkyl]N–[C_1-C_6-alkylenyl]–NH–CO–".

The term "aryl" refers to phenyl, toluyl, substituted phenyl and substituted toluyl.
The term "aryloxy" refers to "aryl–O–".
The term "arylcarbonyl" refers to "aryl–CO–".
The term "aryloxycarbonyl" refers to "aryl–O–CO–".

The term "heteroaryl" refers to substituted or not substituted heteroaromatic groups which have from 4 to 9 ring atoms, from 1 to 4 of which are selected from O, N and/or S. Preferred "heteroaryl" groups have 1 or 2 heteroatoms in a 5- or 6-membered aromatic ring. Mono and bicyclic ring systems are included. Typical "heteroaryl" groups are pyridyl, furyl, thienyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, pyridazinyl, pyrimidyl, pyrazinyl, 1,3,5-triazinyl, 1,2,3-triazolyl, 1,3,4-thiadiazolyl, indolizinyl, indolyl, isoindolyl, benzo[b]furyl, benzo[b]thienyl, indazolyl, benzimidazolyl, benzthiazolyl, purinyl, quinolizinyl, quinolyl, isoquinolyl, quinazolinyl, quinoxalinyl, 1,8-naphthyridinyl, tetrahydroquinolyl, benzooxazolyl, chrom-2-onyl, indazolyl, and the like.

The term "heteroaryloxy" refers to "heteroaryl–O–".
The term "heteroarylcarbonyl" refers to "heteroaryl–CO–".
The term "heteroaryloxycarbonyl" refers to "heteroaryl–O–CO–".

The term "substituted" refers to groups wherein one or more hydrogen atoms are replaced by one or more of the following substituents: –OH, $–OCH_3$, $–OC_2H_5$, $–OC_3H_7$, –O–cyclo-C_3H_5, $–OCH(CH_3)_2$, $–OCH_2Ph$, –F, –Cl, $–COCH_3$, $–COC_2H_5$, $–COC_3H_7$, –CO–cyclo-C_3H_5, $–COCH(CH_3)_2$, –COOH, $–CONH_2$, $–NH_2$, $–NHCH_3$, $–NHC_2H_5$, $–NHC_3H_7$, –NH–cyclo-C_3H_5, $–NHCH(CH_3)_2$, $–N(CH_3)_2$, $–N(C_2H_5)_2$, $–N(C_3H_7)_2$, –N(cyclo-$C_3H_5)_2$, $–N[CH(CH_3)_2]_2$, $–SO_3H$, $–OCF_3$, $–OC_2F_5$, cyclo-C_3H_5, $–CH_3$, $–C_2H_5$, $–C_3H_7$, $–CH(CH_3)_2$, $–CH=CH_2$, $–CH_2–CH=CH_2$, –C≡CH and/or $–C≡C–CH_3$.

In case the general structure (I) represents monomers or building blocks for synthesizing the antisense-oligonucleotides of the present invention, the terminal groups Y and IL' are selected independently of each other from hydrogen, azido, halogen, cyano, nitro, hydroxy, PG-O-, AG-O-, mercapto, PG-S-, AG-S-, C_{1-6}-alkylthio, amino, PG-N(R^H)-, AG-N(R^H)-, mono- or di(C_{1-6}-alkyl)amino, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkenyloxy, optionally substituted C_{2-6}-alkynyl, optionally substituted C_{2-6}-alkynyloxy, monophosphate, monothiophosphate, diphosphate, dithiophosphate triphosphate, trithiophosphate, carboxy, sulphono, hydroxymethyl, PG-O-CH_2-, AG-O-CH_2-, aminomethyl, PG-N(R^H)-CH_2-, AG-N(R^H)-CH_2-, carboxymethyl, sulphonomethyl, where PG is a protection group for -OH, -SH, and -NH(R^H), respectively, AG is an activation group for -OH, -SH, and -NH(R^H), respectively, and R^H is selected from hydrogen and C_{1-6}- alkyl.

The protection groups PG of hydroxy substituents comprise substituted trityl, such as 4,4'-dimethoxytrityl (DMT), 4-monomethoxytrityl (MMT), optionally substituted 9-(9-phenyl)xanthenyl (pixyl), optionally substituted methoxytetrahydropyranyl (mthp), silyl such as trimethylsilyl (TMS), triisopropylsilyl (TIPS), tert-butyldimethylsilyl (TBDMS), triethylsilyl, and phenyldimethylsilyl, tert-butylethers, acetals (including two hydroxy groups), acyl such as acetyl or halogen substituted acetyls, e.g. chloroacetyl or fluoroacetyl, isobutyryl, pivaloyl, benzoyl and substituted benzoyls, methoxymethyl (MOM), benzyl ethers or substituted benzyl ethers such as 2,6-dichlorobenzyl (2,6-Cl_2Bzl). Alternatively when Y or IL' is hydroxyl they may be protected by attachment to a solid support optionally through a linker.

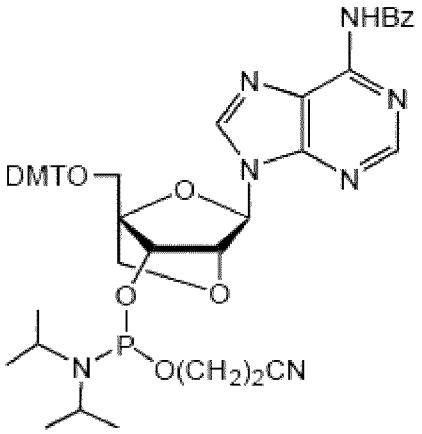
When Y or IL' is an amino group, illustrative examples of the amino protection groups are fluorenylmethoxycarbonyl (Fmoc), tert-butyloxycarbonyl (BOC), trifluoroacetyl, allyloxycarbonyl (alloc or AOC), benzyloxycarbonyl (Z or Cbz), substituted benzyloxycarbonyls such as 2-chloro benzyloxycarbonyl (2-ClZ), monomethoxytrityl (MMT), dimethoxytrityl (DMT), phthaloyl, and 9-(9-phenyl)xanthenyl (pixyl).

Act represents an activation group for -OH, -SH, and -NH(R^H), respectively. Such activation groups are, for instance, selected from optionally substituted O-phosphoramidite, optionally substituted O-phosphortriester, optionally substituted O-phosphordiester, optionally substituted H-phosphonate, and optionally substituted O-phosphonate.

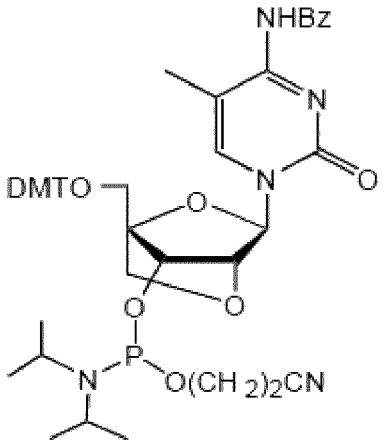
In the present context, the term "phosphoramidite" means a group of the formula -P(OR^x)-N(R^y)$_2$, wherein R^x designates an optionally substituted alkyl group, e.g. methyl, 2-cyanoethyl, or benzyl, and each of R^y designate optionally substituted alkyl groups, e.g. ethyl or isopropyl, or the group -N(R^y)$_2$ forms a morpholino group (-N(CH_2CH_2)$_2$O). R^x preferably designates 2-cyanoethyl and the two R^y are preferably identical and designate isopropyl. Thus, an especially relevant phosphoramidite is N,N-diisopropyl-O-(2-cyanoethyl)-phosphoramidite.

LNA monomers or LNA building blocks

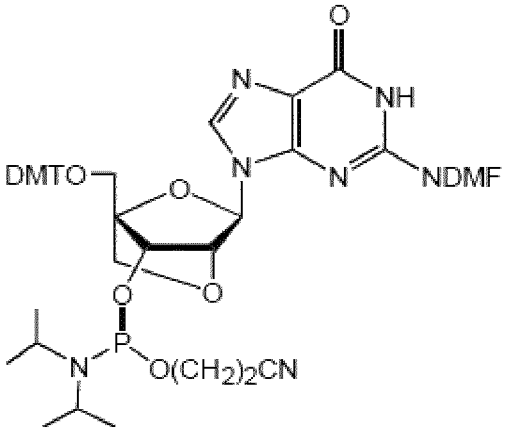
The LNA monomers or LNA building blocks used as starting materials in the synthesis of the antisense-oligonucleotides of the present invention are preferably LNA nucleosides of the following general formulae:



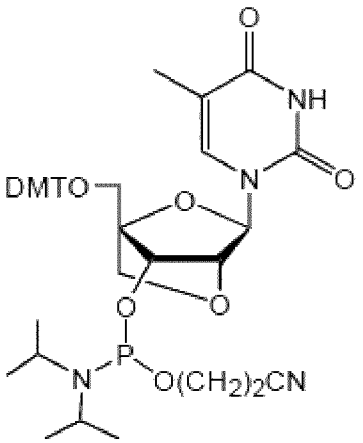
LNA-A^{Bz}



LNA-C*^{Bz}



LNA-G^{DMF}



LNA-T

The LNA building blocks are normally provided as LNA phosphoramidites with the four different nucleobases: adenine (A), guanine (G), 5-methyl-cytosine (C*) and thymine (T). The antisense-oligonucleotides of the present invention containing LNA units are synthesized by standard phosphoramidite chemistry. In the LNA building blocks the nucleobases are protected. A preferred protecting group for the amino group of the purin base is a benzoyl group (Bz), indicated as A^{Bz}. A preferred protecting group for the amino group of the 5-methylpyrimidinone base is a benzoyl group (Bz), indicated as C^{*Bz}. A preferred protecting group for the amino group of the purinone base is a dimethylformamidine (DMF) group, a diethylformamidine (DEF), a dipropylformamidine (DPF), a dibutylformamidine (DBF), or a iso-butyryl (-CO-CH(CH_3)$_2$) group, indicated as G^{DMF}, G^{DEF}, G^{DPF}, G^{DBF}, or G^{iBu}. Thus the group –NDMF refers to –N=CH–N(CH_3)$_2$. DMT refers to 4,4'-dimethoxytrityl.

Thus, **LNA-T** refers to 5'-O-(4,4'-dimethoxytrityl)-3'-O-(2-cyanoethyl-N,N-diisopropyl)-phosphoramidite-thymidine LNA. **LNA-C*^{Bz}** refers to 5'-O-(4,4'-dimethoxytrityl)-3'-O-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite-4-N-benzoyl-5-methyl-2'-cytidine LNA. **LNA-A^{Bz}** refers to 5'-O-(4,4'-dimethoxytrityl)-3'-O-(2-cyanoethyl-N,N-di-

isopropyl)phosphoramidite-6-N-benzoyl-2'-adenosine LNA. **LNA-G^{DMF}** refers to 5'-O-(4,4'-dimethoxytrityl)-3'-O-(2-cyanoethyl-N,N-diisopropyl)-phosphoramidite-2-N-dimethylformamidine-2'-guanosine LNA. **LNA-G^{iBu}** refers to 5'-O-(4,4'-dimethoxy-trityl)-3'-O-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite-2-N-butyryl-2'-guanosine LNA.

Terminal groups

In case Y represents the 5'-terminal group of an antisense-oligonucleotide of the present invention, the residue Y is also named $Y^{5'}$ and represents:

–OH, –O-C_{1-6}-alkyl, –S-C_{1-6}-alkyl, –O-C_{6-9}-phenyl, –O-C_{7-10}-benzyl, –NH-C_{1-6}-alkyl, –N(C_{1-6}-alkyl)$_2$, –O-C_{2-6}-alkenyl, –S-C_{2-6}-alkenyl, –NH-C_{2-6}-alkenyl, –N(C_{2-6}-alkenyl)$_2$, –O-C_{2-6}-alkynyl, –S-C_{2-6}-alkynyl, –NH-C_{2-6}-alkynyl, –N(C_{2-6}-alkynyl)$_2$, –O-C_{1-6}-alkylenyl–O-C_{1-6}-alkyl, –O-[C_{1-6}-alkylenyl–O]$_m$-C_{1-6}-alkyl, –O-CO-C_{1-6}-alkyl, –O-CO-C_{2-6}-alkenyl, –O-CO-C_{2-6}-alkynyl, –O-S(O)-C_{1-6}-alkyl, –O-SO_2-C_{1-6}-alkyl, –O-SO_2-O-C_{1-6}-alkyl, –O-P(O)(O^-)$_2$, –O-P(O)(O^-)(O-C_{1-6}-alkyl), –O-P(O)(O-C_{1-6}-alkyl)$_2$, –O-P(O)(S^-)$_2$, –O-P(O)(S-C_{1-6}-alkyl)$_2$, –O-P(O)(S^-)(O-C_{1-6}-alkyl), –O-P(O)(O^-)(NH-C_{1-6}-alkyl), –O-P(O)(O-C_{1-6}-alkyl)(NH-C_{1-6}-alkyl), –O-P(O)(O^-)[N(C_{1-6}-alkyl)$_2$], –O-P(O)(O-C_{1-6}-alkyl)[N(C_{1-6}-alkyl)$_2$], –O-P(O)(O^-)(BH_3^-), –O-P(O)(O-C_{1-6}-alkyl)(BH_3^-), –O-P(O)(O^-)(O-C_{1-6}-alkylenyl-O-C_{1-6}-alkyl), –O-P(O)(O-C_{1-6}-alkylenyl-O-C_{1-6}-alkyl)$_2$, –O-P(O)(O^-)(O-C_{1-6}-alkylenyl-S-C_{1-6}-alkyl), –O-P(O)(O-C_{1-6}-alkylenyl-S-C_{1-6}-alkyl)$_2$, –O-P(O)(O^-)(OCH_2CH_2O-C_{1-6}-alkyl), –O-P(O)(OCH_2CH_2O-C_{1-6}-alkyl)$_2$, –O-P(O)(O^-)(OCH_2CH_2S-C_{1-6}-alkyl), –O-P(O)(OCH_2CH_2S-C_{1-6}-alkyl)$_2$, –O-P(O)(O^-)OC_3H_6OH, –O-P(O)(S^-)OC_3H_6OH, –O-P(S)(S^-)OC_3H_6OH,

wherein the C_{1-6}-alkyl, C_{2-6}-alkenyl, C_{2-6}-alkynyl, –O-C_{6-9}-phenyl or –O-C_{7-10}-benzyl may be further substituted by –F, –OH, C_{1-4}-alkyl, C_{2-4}-alkenyl and/or C_{2-4}-alkynyl where m is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

More preferred are: $–OCH_3$, $–OC_2H_5$, $–OC_3H_7$, –O–cyclo-C_3H_5, $–OCH(CH_3)_2$, $–OC(CH_3)_3$, $–OC_4H_9$, –OPh, $–OCH_2$–Ph, –O-$COCH_3$, –O-COC_2H_5, –O-COC_3H_7, –O-CO–cyclo-C_3H_5, –O-$COCH(CH_3)_2$, $–OCF_3$, –O-S(O)CH_3, –O-S(O)C_2H_5, –O-S(O)C_3H_7, –O-S(O)–cyclo-C_3H_5, –O-SO_2CH_3, –O-$SO_2C_2H_5$, –O-$SO_2C_3H_7$, –O-SO_2–cyclo-C_3H_5, –O-SO_2-OCH_3, –O-SO_2-OC_2H_5, –O-SO_2-OC_3H_7, –O-SO_2-O-cyclo-C_3H_5, $–O(CH_2)_nN[(CH_2)_nOH]$, $–O(CH_2)_nN[(CH_2)_n$-H], –O-P(O)(O^-)OC_3H_6OH, –O-P(O)(S^-)OC_3H_6OH,

even more preferred are:

$-OCH_3$, $-OC_2H_5$, $-OCH_2CH_2OCH_3$ (also known as MOE), $-OCH_2CH_2-N(CH_3)_2$ (also known as DMAOE), $-O[(CH_2)_nO]_mCH_3$, $-O(CH_2)_nOCH_3$, $-O(CH_2)_nNH_2$, $-O(CH_2)_nN(CH_3)_2$, , $-O\text{-}P(O)(O^-)OC_3H_6OH$, $-O\text{-}P(O)(S^-)OC_3H_6OH$,

where n is selected from 1, 2, 3, 4, 5, or 6; and

where m is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

In case IL' represents the 3'-terminal group of an antisense-oligonucleotide of the present invention, the residue IL' is also named $IL'^{3'}$ and represents:

$-OH$, $-O\text{-}C_{1\text{-}6}\text{-alkyl}$, $-S\text{-}C_{1\text{-}6}\text{-alkyl}$, $-O\text{-}C_{6\text{-}9}\text{-phenyl}$, $-O\text{-}C_{7\text{-}10}\text{-benzyl}$, $-NH\text{-}C_{1\text{-}6}\text{-alkyl}$, $-N(C_{1\text{-}6}\text{-alkyl})_2$, $-O\text{-}C_{2\text{-}6}\text{-alkenyl}$, $-S\text{-}C_{2\text{-}6}\text{-alkenyl}$, $-NH\text{-}C_{2\text{-}6}\text{-alkenyl}$, $-N(C_{2\text{-}6}\text{-alkenyl})_2$, $-O\text{-}C_{2\text{-}6}\text{-alkynyl}$, $-S\text{-}C_{2\text{-}6}\text{-alkynyl}$, $-NH\text{-}C_{2\text{-}6}\text{-alkynyl}$, $-N(C_{2\text{-}6}\text{-alkynyl})_2$, $-O\text{-}C_{1\text{-}6}\text{-alkylenyl}\text{–}O\text{-}C_{1\text{-}6}\text{-alkyl}$, $-O\text{-}[C_{1\text{-}6}\text{-alkylenyl}\text{–}O]_m\text{-}C_{1\text{-}6}\text{-alkyl}$, $-O\text{-}CO\text{-}C_{1\text{-}6}\text{-alkyl}$, $-O\text{-}CO\text{-}C_{2\text{-}6}\text{-alkenyl}$, $-O\text{-}CO\text{-}C_{2\text{-}6}\text{-alkynyl}$, $-O\text{-}S(O)\text{-}C_{1\text{-}6}\text{-alkyl}$, $-O\text{-}SO_2\text{-}C_{1\text{-}6}\text{-alkyl}$, $-O\text{-}SO_2\text{-}O\text{-}C_{1\text{-}6}\text{-alkyl}$, $-O\text{-}P(O)(O^-)_2$, $-O\text{-}P(O)(O^-)(O\text{-}C_{1\text{-}6}\text{-alkyl})$, $-O\text{-}P(O)(O\text{-}C_{1\text{-}6}\text{-alkyl})_2$, $-O\text{-}P(O)(S^-)_2$, $-O\text{-}P(O)(S\text{-}C_{1\text{-}6}\text{-alkyl})_2$, $-O\text{-}P(O)(S^-)(O\text{-}C_{1\text{-}6}\text{-alkyl})$, $-O\text{-}P(O)(O^-)(NH\text{-}C_{1\text{-}6}\text{-alkyl})$, $-O\text{-}P(O)(O\text{-}C_{1\text{-}6}\text{-alkyl})(NH\text{-}C_{1\text{-}6}\text{-alkyl})$, $-O\text{-}P(O)(O^-)[N(C_{1\text{-}6}\text{-alkyl})_2]$, $-O\text{-}P(O)(O\text{-}C_{1\text{-}6}\text{-alkyl})[N(C_{1\text{-}6}\text{-alkyl})_2]$, $-O\text{-}P(O)(O^-)(BH_3^-)$, $-O\text{-}P(O)(O\text{-}C_{1\text{-}6}\text{-alkyl})(BH_3^-)$, $-O\text{-}P(O)(O^-)(O\text{-}C_{1\text{-}6}\text{-alkylenyl-}O\text{-}C_{1\text{-}6}\text{-alkyl})$, $-O\text{-}P(O)(O\text{-}C_{1\text{-}6}\text{-alkylenyl-}O\text{-}C_{1\text{-}6}\text{-alkyl})_2$, $-O\text{-}P(O)(O^-)(O\text{-}C_{1\text{-}6}\text{-alkylenyl-}S\text{-}C_{1\text{-}6}\text{-alkyl})$, $-O\text{-}P(O)(O\text{-}C_{1\text{-}6}\text{-alkylenyl-}S\text{-}C_{1\text{-}6}\text{-alkyl})_2$, $-O\text{-}P(O)(O^-)(OCH_2CH_2O\text{-}C_{1\text{-}6}\text{-alkyl})$, $-O\text{-}P(O)(OCH_2CH_2O\text{-}C_{1\text{-}6}\text{-alkyl})_2$, $-O\text{-}P(O)(O^-)(OCH_2CH_2S\text{-}C_{1\text{-}6}\text{-alkyl})$, $-O\text{-}P(O)(OCH_2CH_2S\text{-}C_{1\text{-}6}\text{-alkyl})_2$, $-O\text{-}P(O)(O^-)OC_3H_6OH$, $-O\text{-}P(O)(S^-)OC_3H_6OH$,

wherein the $C_{1\text{-}6}$-alkyl, $C_{2\text{-}6}$-alkenyl, $C_{2\text{-}6}$-alkynyl, $-O\text{-}C_{6\text{-}9}$-phenyl or $-O\text{-}C_{7\text{-}10}$-benzyl may be further substituted by –F, –OH, $C_{1\text{-}4}$-alkyl, $C_{2\text{-}4}$-alkenyl and/or $C_{2\text{-}4}$-alkynyl

where m is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

More preferred are: $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, $-O$–cyclo-C_3H_5, $-OCH(CH_3)_2$, $-OC(CH_3)_3$, $-OC_4H_9$, –OPh, $-OCH_2$–Ph, $-O\text{-}COCH_3$, $-O\text{-}COC_2H_5$, $-O\text{-}COC_3H_7$, $-O\text{-}CO$–cyclo-C_3H_5, $-O\text{-}COCH(CH_3)_2$, $-OCF_3$, $-O\text{-}S(O)CH_3$, $-O\text{-}S(O)C_2H_5$, $-O\text{-}S(O)C_3H_7$, $-O\text{-}S(O)$–cyclo-C_3H_5, $-O\text{-}SO_2CH_3$, $-O\text{-}SO_2C_2H_5$, $-O\text{-}SO_2C_3H_7$, $-O\text{-}SO_2$–cyclo-C_3H_5, $-O\text{-}SO_2\text{-}OCH_3$, $-O\text{-}SO_2\text{-}OC_2H_5$, $-O\text{-}SO_2\text{-}OC_3H_7$, $-O\text{-}SO_2\text{-}O\text{-cyclo-}C_3H_5$, $-O(CH_2)_nN[(CH_2)_nOH]$, $-O(CH_2)_nN[(CH_2)_n\text{-}H]$, , $-O\text{-}P(O)(O^-)OC_3H_6OH$, $-O\text{-}P(O)(S^-)OC_3H_6OH$,

even more preferred are:

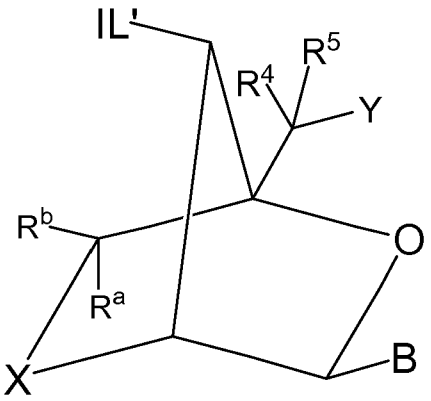
$-OCH_3$, $-OC_2H_5$, $-OCH_2CH_2OCH_3$ (also known as MOE), $-OCH_2CH_2-N(CH_3)_2$ (also known as DMAOE), $-O[(CH_2)_nO]_mCH_3$, $-O(CH_2)_nOCH_3$, $-O(CH_2)_nNH_2$, $-O(CH_2)_nN(CH_3)_2$, $-O\text{-}P(O)(O^-)OC_3H_6OH$, $-O\text{-}P(O)(S^-)OC_3H_6OH$,

where n is selected from 1, 2, 3, 4, 5, or 6; and

where m is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

Preferred LNAs

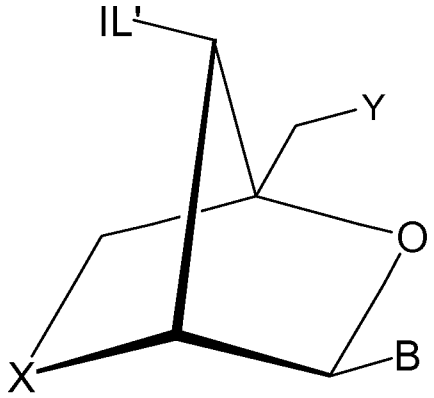
In preferred embodiments LNA units used in the antisense-oligonucleotides of the present invention preferably have the structure of general formula (II):



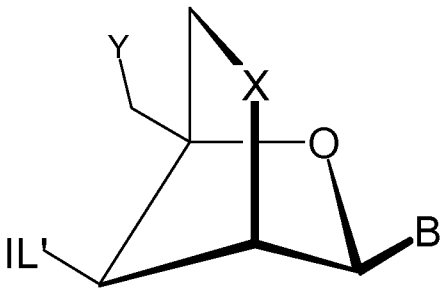
(II)

The moiety $-C(R^aR^b)-X-$ represents preferably $-C(R^aR^b)-O-$, $-C(R^aR^b)-NR^c-$, $-C(R^aR^b)-S-$, and $-C(R^aR^b)-C(R^aR^b)-O-$, wherein the substituents R^a, R^b and R^c have the meanings as defined herein and are preferably C_{1-6}-alkyl and more preferably C_{1-4}-alkyl. More preferably $-C(R^aR^b)-X-$ is selected from $-CH_2-O-$, $-CH_2-S-$, $-CH_2-NH-$, $-CH_2-N(CH_3)-$, $-CH_2-CH_2-O-$, or $-CH_2-CH_2-S-$, and more preferably $-CH_2-O-$, $-CH_2-S-$, $-CH_2-CH_2-O-$, or $-CH_2-CH_2-S-$, and still more preferably $-CH_2-O-$, $-CH_2-S-$, or $-CH_2-CH_2-O-$, and still more preferably $-CH_2-O-$ or $-CH_2-S-$, and most preferably $-CH_2-O-$.

All chiral centers and asymmetric substituents (if any) can be either in *R* or in *S* orientation. For example, two exemplary stereochemical isomers are the beta-D and alpha-L isoforms as shown below:

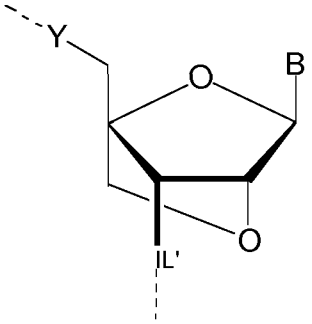


Formula (IIA)

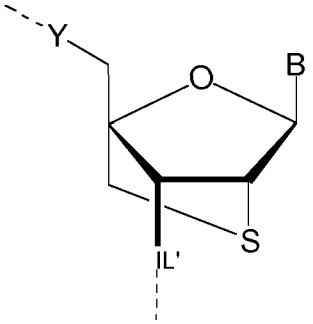


Formula (IIB)

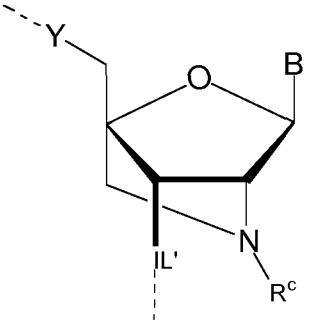
Preferred LNA units are selected from general formula (b^1) to (b^9):



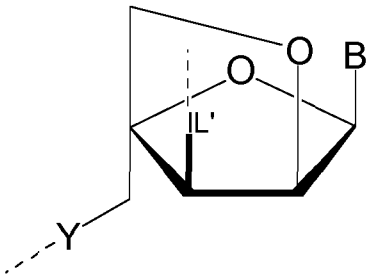
β-D-oxy-LNA (b^1)



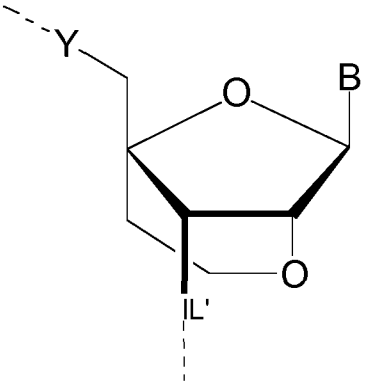
β-D-thio-LNA (b^2)



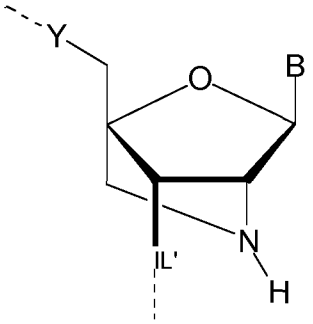
β-D-amino-LNA (b^3)



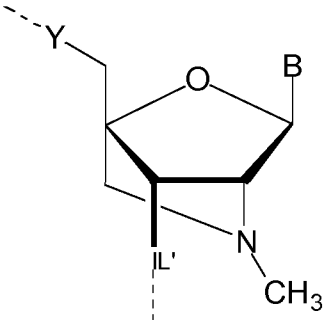
α-L-oxy-LNA (b^4)



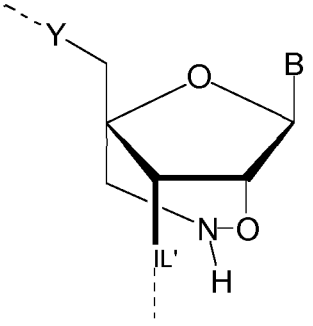
β-D-ENA (b^5)



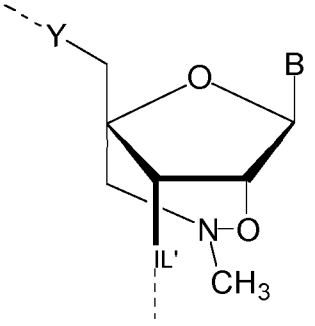
β-D-(NH)-LNA (b^6)



β-D-(NCH_3)-LNA (b^7)



β-D-(ONH)-LNA (b^8)



β-D-($ONCH_3$)-LNA (b^9)

The term "thio-LNA" comprises a locked nucleotide in which X in the general formula (II) is selected from –S– or –CH_2–S–. Thio-LNA can be in both beta-D and alpha-L-configuration.

The term "amino-LNA" comprises a locked nucleotide in which X in the general formula (II) is selected from –NH–, –N(R)–, $–CH_2–NH–$, and $–CH_2–N(R)–$, where R is selected from hydrogen and C_{1-4}-alkyl. Amino-LNA can be in both beta-D and alpha-L-configuration.

The term "oxy-LNA" comprises a locked nucleotide in which X in the general formula (II) is –O–. Oxy-LNA can be in both beta-D and alpha-L-configuration.

The term "ENA" comprises a locked nucleotide in which X in the general formula (II) is $–CH_2–O–$ (where the oxygen atom of $–CH_2–O–$ is attached to the 2'-position relative to the base B). R^a and R^b are independently of each other hydrogen or methyl.

In preferred exemplary embodiments LNA is selected from beta-D-oxy-LNA, alpha-L-oxy-LNA, beta-D-amino-LNA and beta-D-thio-LNA, in particular beta-D-oxy-LNA.

Still more preferred are the following antisense-oligonucleotides (Table 1):

SP	L	Seq ID No.	Sequence, 5'-3'
89	17	102a	GCGAGTGACTCACTCAA
90	15	103a	CGAGTGACTCACTCA
90	16	104a	GCGAGTGACTCACTCA
90	17	105a	CGCGAGTGACTCACTCA
91	14	106a	CGAGTGACTCACTC
91	16	107a	CGCGAGTGACTCACTC
91	17	108a	GCGCGAGTGACTCACTC
92	14	109a	GCGAGTGACTCACT
92	16	110a	GCGCGAGTGACTCACT
92	17	111a	CGCGCGAGTGACTCACT
93	12	112a	CGAGTGACTCAC
93	13	113a	GCGAGTGACTCAC
93	14	114a	CGCGAGTGACTCAC
93	16	115a	CGCGCGAGTGACTCAC
93	17	116a	GCGCGCGAGTGACTCAC
94	13	117a	CGCGAGTGACTCA
94	14	118a	GCGCGAGTGACTCA
94	15	119a	CGCGCGAGTGACTCA
94	16	120a	GCGCGCGAGTGACTCA

94	17	121a	TGCGCGCGAGTGACTCA
95	14	122a	CGCGCGAGTGACTC
95	16	123a	TGCGCGCGAGTGACTC
95	17	124a	GTGCGCGCGAGTGACTC
96	13	125a	CGCGCGAGTGACT
97	14	126a	TGCGCGCGAGTGAC
97	16	127a	CGTGCGCGCGAGTGAC
98	13	128a	TGCGCGCGAGTGA
107	16	129a	GTCGTCGCTCCGTGCG
108	15	130a	GTCGTCGCTCCGTGC
108	17	131a	GTGTCGTCGCTCCGTGC
109	13	132a	TCGTCGCTCCGTG
109	15	133a	TGTCGTCGCTCCGTG
110	12	134a	TCGTCGCTCCGT
110	13	135a	GTCGTCGCTCCGT
110	14	136a	TGTCGTCGCTCCGT
110	15	137a	GTGTCGTCGCTCCGT
110	16	138a	GGTGTCGTCGCTCCGT
351	16	139a	CGTCATAGACCGAGCC
351	12	140a	ATAGACCGAGCC
354	16	141a	GCTCGTCATAGACCGA
354	13	142a	CGTCATAGACCGA
355	14	143a	CTCGTCATAGACCG
355	15	144a	GCTCGTCATAGACCG
356	14	145a	GCTCGTCATAGACC
381	17	146a	CAGCCCCCGACCCATGG
382	16	147a	CAGCCCCCGACCCATG
383	14	148a	AGCCCCCGACCCAT
384	14	149a	CAGCCCCCGACCCA
422	17	150a	CGCGTCCACAGGACGAT
425	14	151a	CGCGTCCACAGGAC
429	15	152a	CGATACGCGTCCACA
431	13	153a	CGATACGCGTCCA
431	16	154a	TGGCGATACGCGTCCA
432	12	155a	CGATACGCGTCC
432	13	156a	GCGATACGCGTCC
432	17	157a	GCTGGCGATACGCGTCC
433	15	158a	CTGGCGATACGCGTC

433	12	159a	GCGATACGCGTC
433	16	160a	GCTGGCGATACGCGTC
433	14	161a	TGGCGATACGCGTC
434	13	162a	TGGCGATACGCGT
434	14	163a	CTGGCGATACGCGT
434	12	164a	GGCGATACGCGT
435	13	165a	CTGGCGATACGCG
435	12	166a	TGGCGATACGCG
437	17	167a	ATCGTGCTGGCGATACG
449	16	168a	CGTGCGGTGGGATCGT
449	17	169a	ACGTGCGGTGGGATCGT
450	17	170a	AACGTGCGGTGGGATCG
452	15	171a	AACGTGCGGTGGGAT
452	17	172a	TGAACGTGCGGTGGGAT
459	17	173a	CGACTTCTGAACGTGCG
941	17	174a	TTAACGCGGTAGCAGTA
941	16	175a	TAACGCGGTAGCAGTA
942	17	176a	GTTAACGCGGTAGCAGT
943	15	177a	TTAACGCGGTAGCAG
944	13	178a	TAACGCGGTAGCA
945	12	179a	TAACGCGGTAGC
945	13	180a	TTAACGCGGTAGC
946	12	181a	TTAACGCGGTAG
946	13	182a	GTTAACGCGGTAG
946	15	183a	CGGTTAACGCGGTAG
946	16	184a	CCGGTTAACGCGGTAG
947	14	185a	CGGTTAACGCGGTA
947	13	186a	GGTTAACGCGGTA
947	15	187a	CCGGTTAACGCGGTA
947	16	188a	GCCGGTTAACGCGGTA
947	17	189a	TGCCGGTTAACGCGGTA
948	13	190a	CGGTTAACGCGGT
949	13	191a	CCGGTTAACGCGG
949	14	192a	GCCGGTTAACGCGG
949	15	193a	TGCCGGTTAACGCGG
950	13	194a	GCCGGTTAACGCG
950	15	195a	CTGCCGGTTAACGCG
950	16	196a	GCTGCCGGTTAACGCG

1387	16	197a	ATGCCGCGTCAGGTAC
1392	13	198a	ACATGCCGCGTCA
1393	16	199a	GATGACATGCCGCGTC
1394	12	200a	GACATGCCGCGT
1394	15	201a	GATGACATGCCGCGT
1395	13	202a	ATGACATGCCGCG
1805	17	203a	TCCCGCACCTTGGAACC
1851	16	204a	CGATCTCTCAACACGT
1851	17	205a	TCGATCTCTCAACACGT
1852	15	206a	CGATCTCTCAACACG
1852	16	207a	TCGATCTCTCAACACG
1852	17	208a	CTCGATCTCTCAACACG
2064	16	209a	GTAGTGTTTAGGGAGC
2072	16	210a	GCTATTTGGTAGTGTT
2284	15	211a	AGCTTATCCTATGAC
2285	14	212a	AGCTTATCCTATGA
2355	17	213a	CAGGCATTAATAAAGTG
4120	16	214a	CTAGGCGCCTCTATGC
4121	14	215a	TAGGCGCCTCTATG
4121	15	216a	CTAGGCGCCTCTATG
4122	13	217a	TAGGCGCCTCTAT
4217	16	218a	CATGAATGGACCAGTA

SP: start position or start nucleotide on **Seq. ID No. 2**

L: length of the sequence

The antisense-oligonucleotides as disclosed herein such as the antisense-oligonucleotides of **Tables 1 to 3** and especially the antisense-oligonucleotides of **Tables 4 to 9** consist of nucleotides, preferably DNA nucleotides, which are non-LNA units (also named herein non-LNA nucleotides) as well as LNA units (also named herein LNA nucleotides).

Although not explicitly indicated, the antisense-oligonucleotides of the sequences Seq. ID No.s 102a-218a of Table 1 comprise 2 to 4 LNA nucleotides (LNA units) at the 3' terminus and 2 to 4 LNA nucleotides (LNA units) at the 5' terminus. Although not explicitly indicated, the "C" in Table 2 which refer to LNA units preferably contain 5-methylcytosine (C*) as nucleobase.

That means, as long as not explicitly indicated, the antisense-oligonucleotides of the present invention or as disclosed herein by the letter code A, C, G, T and U may contain any internucleotide linkage, any end group and any nucleobase as disclosed

herein. Moreover the antisense-oligonucleotides of the present invention or as disclosed herein are gapmers of any gapmer structure as disclosed herein with at least one LNA unit at the 3' terminus and at least one LNA unit at the 5' terminus. Moreover any LNA unit as disclosed herein can be used within the antisense-oligonucleotides of the present invention or as disclosed herein. Thus, for instance, the antisense-oligonucleotide GCTC**GTCATAGA**CCGA (Seq. ID No. 13) or CGAT**ACGCGTCC**ACAG (Seq. ID No. 14) or GTAG**TGTTTAGG**GAGC (Seq. ID No. 15) or GCTA**TTTGGTAG**TGTT (Seq. ID No. 16) or CATG**AATGGACC**AGTA (Seq. ID No. 17) or AGGC**ATTAATAA**AGTG (Seq. ID No. 18) contains at least one LNA unit at the 5' terminus and at least one LNA unit at the 3' terminus, any nucleobase, any 3' end group, any 5' end group, any gapmer structure, and any internucleotide linkage as disclosed herein and covers also salts and optical isomers of that antisense-oligonucleotide.

The use of LNA units is preferred especially at the 3' terminal and the 5' terminal end. Thus it is preferred if the last 1 – 5 nucleotides at the 3' terminal end and also the last 1 – 5 nucleotides at the 5' terminal end especially of the sequences disclosed herein and particularly of Seq. ID No.s 102a – 218a of Table 1 are LNA units (also named LNA nucleotides) while in between the 1 – 5 LNA units at the 3' and 5' end 2 – 14, preferably 3 – 12, more preferably 4 – 10, more preferably 5 – 9, still more preferably 6 – 8, non-LNA units (also named non-LNA nucleotides) are present. Such kind of antisense-oligonucleotides are called gapmers and are disclosed in more detail below. More preferred are 2 – 5 LNA nucleotides at the 3' end and 2 – 5 LNA nucleotides at the 5' end or 1 – 4 LNA nucleotides at the 3' end and 1 – 4 LNA nucleotides at the 5' end and still more preferred are 2 – 4 LNA nucleotides at the 3' end and 2 – 4 LNA nucleotides at the 5' end of the antisense-oligonucleotides with a number of preferably 4 – 10, more preferably 5 – 9, still more preferably 6 – 8 non-LNA units in between the LNA units at the 3' and the 5' end.

Moreover as internucleotide linkages between the LNA units and between the LNA units and the non-LNA units, the use of phosphorothioates or phosphorodithioates and preferably phosphorothioates is preferred.

Thus further preferred are antisense-oligonucleotides wherein more than 50%, preferably more than 60%, more preferably more than 70%, still more preferably more than 80%, and most preferably more than 90% of the internucleotide linkages are phosphorothioates or phosphates and more preferably phosphorothioate linkages and wherein the last 1 – 4 or 2 – 5 nucleotides at the 3' end are LNA units and the last 1 – 4 or 2 – 5 nucleotides at the 5' end are LNA units and between the LNA units

at the ends a sequence of 6 – 14 nucleotides, preferably 7 – 12, preferably 8 – 11, more preferably 8 – 10 are present which are non-LNA units, preferably DNA units. Moreover it is preferred that these antisense-oligonucleotides in form of gapmers consist in total of 12 to 20, preferably 12 to 18 nucleotides.

Gapmers

The antisense-oligonucleotides of the invention may consist of nucleotide sequences which comprise both DNA nucleotides which are non-LNA units as well as LNA nucleotides, and may be arranged in the form of a gapmer.

Thus, the antisense-oligonucleotides of the present invention are preferably gapmers. A gapmer consists of a middle part of DNA nucleotide units which are not locked, thus which are non-LNA units. The DNA nucleotides of this middle part could be linked to each other by the internucleotide linkages (IL) as disclosed herein which preferably may be phosphate groups, phosphorothioate groups or phosphorodithioate groups and which may contain nucleobase analogues such as 5-propynyl cytosine, 7-methylguanine, 7-methyladenine, 2-aminoadenine, 2-thiothymine, 2-thiocytosine, or 5-methylcytosine. That DNA units or DNA nucleotides are not bicyclic pentose structures. The middle part of non-LNA units is flanked at the 3' end and the 5' end by sequences consisting of LNA units. Thus gapmers have the general formula:

LNA sequence 1 – non-LNA sequence – LNA sequence 2

or

region A – region B – region C

The middle part of the antisense-oligonucleotide which consists of DNA nucleotide units which are non-LNA units is, when formed in a duplex with the complementary target RNA, capable of recruiting RNase. The 3' and 5' terminal nucleotide units are LNA units which are preferably in alpha-L configuration, particularly preferred being beta-D-oxy-LNA and alpha-L-oxy LNAs.

Thus, a gapmer is an antisense-oligonucleotide which comprises a contiguous stretch of DNA nucleotides which is capable of recruiting an RNase, such as RNaseH, such as a region of at least 6 or 7 DNA nucleotides which are non-LNA units, referred to herein as middle part or region B, wherein region B is flanked both 5' and 3' by regions of affinity enhancing nucleotide analogues which are LNA units, such as between 1 – 6 LNA units 5' and 3' to the contiguous stretch of DNA nucleotides which is capable of recruiting RNase – these flanking regions are referred to as regions A and C respectively.

Preferably the gapmer comprises a (poly)nucleotide sequence of formula (5' to 3'), A-B-C, or optionally A-B-C-D or D-A-B-C, wherein; region A (5' region) consists of at least one nucleotide analogue, such as at least one LNA unit, such as between 1-6 LNA units, and region B consists of at least five consecutive DNA nucleotides which are non-LNA units and which are capable of recruiting RNase (when formed in a duplex with a complementary RNA molecule, such as the mRNA target), and region C (3'region) consists of at least one nucleotide analogue, such as at least one LNA unit, such as between 1-6 LNA units, and region D, when present consists of 1, 2 or 3 DNA nucleotide units which are non-LNA units.

In some embodiments, region A consists of 1, 2, 3, 4, 5 or 6 LNA units, such as between 2-5 LNA units, such as 3 or 4 LNA units; and/or region C consists of 1, 2, 3, 4, 5 or 6 LNA units, such as between 2-5 LNA units, such as 3 or 4 LNA units.

In some embodiments B consists of 5, 6, 7, 8, 9, 10, 11 or 12 consecutive DNA nucleotides which are capable of recruiting RNase, or between 6-10, or between 7-9, such as 8 consecutive nucleotides which are capable of recruiting RNase. In some embodiments region B consists of at least one DNA nucleotide unit, such as 1-12 DNA nucleotide units, preferably between 4-12 DNA nucleotide units, more preferably between 6-10 DNA nucleotide units, still more preferred such as between 7-10 DNA nucleotide units, and most preferably 8, 9 or 10 DNA nucleotide units which are non-LNA units.

In some embodiments region A consist of 3 or 4 LNA, region B consists of 7, 8, 9 or 10 DNA nucleotide units, and region C consists of 3 or 4 LNA units. Such designs include (A-B-C): 1-7-2, 2-7-1, 2-7-2, 3-7-1, 3-7-2, 1-7-3, 2-7-3, 3-7-3, 2-7-4, 3-7-4, 4-7-2, 4-7-3, 4-7-4, 1-8-1, 1-8-2, 2-8-1, 2-8-2, 1-8-3, 3-8-1, 3-8-3, 2-8-3, 3-8-2, 4-8-1, 4-8-2, 1-8-4, 2-8-4, 3-8-4, 4-8-3, 4-8-4, 1-9-1, 1-9-2, 2-9-1, 2-9-2, 2-9-3, 3-9-2, 3-9-3, 1-9-3, 3-9-1, 4-9-1, 1-9-4, 4-9-2, 2-9-4, 4-9-3, 3-9-4, 4-9-4, 1-10-1, 1-10-2, 2-10-1, 2-10-2, 1-10-3, 3-10-1, 2-10-2, 2-10-3, 3-10-2, 3-10-3, 2-10-4, 4-10-2, 3-10-4, 4-10-3, 4-10-4, 1-11-1, 1-11-2, 2-11-1, 2-11-2, 1-11-3, 3-11-1, 2-11-2, 2-11-3, 3-11-2, 3-11-3, 2-11-4, 4-11-2, 3-11-4, 4-11-3, 4-11-4, and may further include region D, which may have one or 2 DNA nucleotide units, which are non-LNA units.

Further gapmer designs are disclosed in WO2004/046160A and are hereby incorporated by reference. US provisional application, 60/977409, hereby incorporated by reference, refers to 'shortmer' gapmer antisense-oligonucleotide, which are also suitable for the present invention.

In some embodiments the antisense-oligonucleotide consists of a contiguous nucleotide sequence of a total of 10, 11, 12, 13 or 14 nucleotide units (LNA units and non-LNA units together), wherein the contiguous nucleotide sequence is of formula (5' – 3'), A-B-C, or optionally A-B-C-D or D-A-B-C, wherein A consists of 1, 2 or 3 LNA units, and B consists of 7, 8 or 9 contiguous DNA nucleotide units which are non-LNA units and which are capable of recruiting RNase when formed in a duplex with a complementary RNA molecule (such as a mRNA target), and C consists of 1, 2 or 3 LNA units. When present, D consists of a single DNA nucleotide unit which is a non-LNA unit.

In some embodiments A consists of 1 LNA unit. In some embodiments A consists of 2 LNA units. In some embodiments A consists of 3 LNA units. In some embodiments C consists of 1 LNA unit. In some embodiments C consists of 2 LNA units. In some embodiments C consists of 3 LNA units. In some embodiments B consists of 7 DNA nucleotide units which are non-LNA units. In some embodiments B consists of 8 DNA nucleotide units. In some embodiments B consists of 9 DNA nucleotide units. In some embodiments B consists of 1 – 9 DNA nucleotide units, such as 2, 3, 4, 5, 6, 7 or 8 DNA nucleotide units. The DNA nucleotide units are always non-LNA units. In some embodiments B comprises 1, 2 or 3 LNA units which are preferably in the alpha-L configuration and which are more preferably alpha-L-oxy LNA units. In some embodiments the number of nucleotides present in A-B-C are selected from the group consisting of (LNA units - region B - LNA units and more preferably alpha-L-oxy LNA units (region A) – region B – (region C) alpha-L-oxy LNA units): 1-8-1, 1-8-2, 2-8-1, 2-8-2, 1-8-3, 3-8-1, 3-8-3, 2-8-3, 3-8-2, 4-8-1, 4-8-2, 1-8-4, 2-8-4, 3-8-4, 4-8-3, 4-8-4, 1-9-1, 1-9-2, 2-9-1, 2-9-2, 2-9-3, 3-9-2, 3-9-3, 1-9-3, 3-9-1, 4-9-1, 1-9-4, 4-9-2, 2-9-4, 4-9-3, 3-9-4, 4-9-4, 1-10-1, 1-10-2, 2-10-1, 2-10-2, 1-10-3, 3-10-1, 2-10-2, 2-10-3, 3-10-2, 3-10-3, 2-10-4, 4-10-2, 3-10-4, 4-10-3, 4-10-4, 1-11-1, 1-11-2, 2-11-1, 2-11-2, 1-11-3, 3-11-1, 2-11-2, 2-11-3, 3-11-2, 3-11-3, 2-11-4, 4-11-2, 3-11-4, 4-11-3, 4-11-4. In further preferred embodiments the number of nucleotides in A-B-C are selected from the group consisting of: 3-8-3, 4-8-2, 2-8-4, 3-8-4, 4-8-3, 4-8-4, 3-9-3, 4-9-2, 2-9-4, 4-9-3, 3-9-4, 4-9-4, 3-10-3, 2-10-4, 4-10-2, 3-10-4, 4-10-3, 4-10-4, 2-11-4, 4-11-2, 3-11-4, 4-11-3 and still more preferred are: 3-8-3, 3-8-4, 4-8-3, 4-8-4, 3-9-3, 4-9-3, 3-9-4, 4-9-4, 3-10-3, 3-10-4, 4-10-3, 4-10-4, 3-11-4, and 4-11-3.

Phosphorothioate, phosphate or phosphorodithioate and especially phosphorothioate internucleotide linkages are also preferred, particularly for the gapmer region B. Phosphorothioate, phosphate or phosphorodithioate linkages and especially phosphorothioate internucleotide linkages may also be used for the flanking regions (A and C, and for linking A or C to D, and within region D, if present).

Regions A, B and C, may however comprise internucleotide linkages other than phosphorothioate or phosphorodithioate, such as phosphodiester linkages, particularly, for instance when the use of nucleotide analogues protects the internucleotide linkages within regions A and C from endo-nuclease degradation - such as when regions A and C consist of LNA units.

The internucleotide linkages in the antisense-oligonucleotide may be phosphodiester, phosphorothioate, phosphorodithioate or boranophosphate so as to allow RNase H cleavage of targeted RNA. Phosphorothioate or phosphorodithioate is preferred, for improved nuclease resistance and other reasons, such as ease of manufacture. In one aspect of the oligomer of the invention, the LNA units and/or the non-LNA units are linked together by means of phosphorothioate groups.

It is recognized that the inclusion of phosphodiester linkages, such as one or two linkages, into an otherwise phosphorothioate antisense-oligonucleotide, particularly between or adjacent to LNA units (typically in region A and or C) can modify the bioavailability and/or bio-distribution of an antisense-oligonucleotide (see WO2008/053314A which is hereby incorporated by reference).

In some embodiments, such as in the sequences of the antisense-oligonucleotides disclosed herein and where suitable and not specifically indicated, all remaining internucleotide linkage groups are either phosphodiester groups or phosphorothioate groups, or a mixture thereof.

In some embodiments all the internucleotide linkage groups are phosphorothioate groups. When referring to specific gapmer antisense-oligonucleotide sequences, such as those provided herein, it will be understood that, in various embodiments, when the linkages are phosphorothioate linkages, alternative linkages, such as those disclosed herein may be used, for example phosphate (also named phosphodiester) linkages may be used, particularly for linkages between nucleotide analogues, such as LNA units. Likewise, when referring to specific gapmer antisense-oligonucleotide sequences, such as those provided herein, when the C residues are annotated as 5'-methyl modified cytosine, in various embodiments, one or more of the Cs present in the oligomer may be unmodified C residues.

Legend

As used herein the abbreviations b, d, s, ss have the following meaning:

b LNA unit or LNA nucleotide (any one selected from b^1 – b^7)

b^1 β-D-oxy-LNA

b^2 β-D-thio-LNA
b^3 β-D-amino-LNA
b^4 α-L-oxy-LNA
b^5 β-D-ENA
b^6 β-D-(NH)-LNA
b^7 β-D-(NCH_3)-LNA
d 2-deoxy, that means 2-deoxyribose units (e.g. formula **B3** or **B5** with R = –H)
C* methyl-C (5-methylcytosine); [consequently dC* is 5-methyl-2'-deoxycytidine]
A* 2-aminoadenine [consequently dA* is 2-amino-2'-deoxyadenosine]
s the internucleotide linkage is a phosphorothioate group (-O-P(O)(S^-)-O-)
ss the internucleotide linkage is a phosphorodithioate group (-O-P(S)(S^-)-O-)
/5SpC3/ –O-P(O)(O^-)OC_3H_6OH at 5'-terminal group of an antisense-oligonucleotide
/3SpC3/ –O-P(O)(O^-)OC_3H_6OH at 3'-terminal group of an antisense-oligonucleotide
/5SpC3s/ –O-P(O)(S^-)OC_3H_6OH at 5'-terminal group of an antisense-oligonucleotide
/3SpC3s/ –O-P(O)(S^-)OC_3H_6OH at 3'-terminal group of an antisense-oligonucleotide

nucleotides in **bold** are LNA nucleotides
nucleotides not in bold are non-LNA nucleotides

Gapmer Sequences

The following antisense-oligonucleotides in form of gapmers as listed in Table 2 to Table 9 and more preferably in Table 4 to 9 are especially preferred.

Table 2

SP	L	Seq ID No.	Sequence, 5'-3'
89	17	102b	**GbsCbsGbsAb**sdGsdTsdGsdAsdCsdTsdCsdAsdCs**TbsCbsAbsAb**
90	15	103b	**CbsGbsAb**sdGsdTsdGsdAsdCsdTsdCsdAsdCs**TbsCbsAb**
90	16	104b	**GbsCbsGb**sdAsdGsdTsdGsdAsdCsdTsdCsdAsdCs**TbsCbsAb**
90	17	105b	**CbsGbsCbsGb**sdAsdGsdTsdGsdAsdCsdTsdCsdAs**CbsTbsCbsAb**
91	14	106b	**CbsGbsAb**sdGsdTsdGsdAsdCsdTsdCsdAs**CbsTbsCb**
91	16	107b	**CbsGbsCb**sdGsdAsdGsdTsdGsdAsdCsdTsdCsdAs**CbsTbsCb**
91	17	108b	**GbsCbsGbsCb**sdGsdAsdGsdTsdGsdAsdCsdTsdCs**AbsCbsTbsCb**
92	14	109b	**GbsCbsGb**sdAsdGsdTsdGsdAsdCsdTsdCs**AbsCbsTb**
92	16	110b	**GbsCbsGb**sdCsdGsdAsdGsdTsdGsdAsdCsdTsdCs**AbsCbsTb**
92	17	111b	**CbsGbsCbsGb**sdCsdGsdAsdGsdTsdGsdAsdCsdTs**CbsAbsCbsTb**

93	12	112b	**CbsGb**sdAsdGsdTsdGsdAsdCsdTsdCs**AbsCb**
93	13	113b	**GbsCbsGb**sdAsdGsdTsdGsdAsdCsdTsdCs**AbsCb**
93	14	114b	**CbsGbsCb**sdGsdAsdGsdTsdGsdAsdCsdTs**CbsAbsCb**
93	16	115b	**CbsGbsCb**sdGsdCsdGsdAsdGsdTsdGsdAsdCsdTs**CbsAbsCb**
93	17	116b	**GbsCbsGbsCb**sdGsdCsdGsdAsdGsdTsdGsdAsdCs**TbsCbsAbsCb**
94	13	117b	**CbsGbsCb**sdGsdAsdGsdTsdGsdAsdCsdTs**CbsAb**
94	14	118b	**GbsCbsGb**sdCsdGsdAsdGsdTsdGsdAsdCs**TbsCbsAb**
94	15	119b	**CbsGbsCb**sdGsdCsdGsdAsdGsdTsdGsdAsdCs**TbsCbsAb**
94	16	120b	**GbsCbsGb**sdCsdGsdCsdGsdAsdGsdTsdGsdAsdCs**TbsCbsAb**
94	17	121b	**TbsGbsCbsGb**sdCsdGsdCsdGsdAsdGsdTsdGsdAs**CbsTbsCbsAb**
95	14	122b	**CbsGbsCb**sdGsdCsdGsdAsdGsdTsdGsdAs**CbsTbsCb**
95	16	123b	**TbsGbsCb**sdGsdCsdGsdCsdGsdAsdGsdTsdGsdAs**CbsTbsCb**
95	17	124b	**GbsTbsGbsCb**sdGsdCsdGsdCsdGsdAsdGsdTsdGs**AbsCbsTbsCb**
96	13	125b	**CbsGbsCb**sdGsdCsdGsdAsdGsdTsdGsdAs**CbsTb**
97	14	126b	**TbsGbsCb**sdGsdCsdGsdCsdGsdAsdGsdTs**GbsAbsCb**
97	16	127b	**CbsGbsTb**sdGsdCsdGsdCsdGsdCsdGsdAsdGsdTs**GbsAbsCb**
98	13	128b	**TbsGbsCb**sdGsdCsdGsdCsdGsdAsdGsdTs**GbsAb**
107	16	129b	**GbsTbsCb**sdGsdTsdCsdGsdCsdTsdCsdCsdGsdTs**GbsCbsGb**
108	15	130b	**GbsTbsCb**sdGsdTsdCsdGsdCsdTsdCsdCsdGs**TbsGbsCb**
108	17	131b	**GbsTbsGbsTb**sdCsdGsdTsdCsdGsdCsdTsdCsdCs**GbsTbsGbsCb**
109	13	132b	**TbsCbsGb**sdTsdCsdGsdCsdTsdCsdCsdGs**TbsGb**
109	15	133b	**TbsGbsTb**sdCsdGsdTsdCsdGsdCsdTsdCsdCs**GbsTbsGb**
110	12	134b	**TbsCb**sdGsdTsdCsdGsdCsdTsdCsdCs**GbsTb**
110	13	135b	**GbsTbsCb**sdGsdTsdCsdGsdCsdTsdCsdCs**GbsTb**
110	14	136b	**TbsGbsTb**sdCsdGsdTsdCsdGsdCsdTsdCs**CbsGbsTb**
110	15	137b	**GbsTbsGb**sdTsdCsdGsdTsdCsdGsdCsdTsdCs**CbsGbsTb**
110	16	138b	**GbsGbsTb**sdGsdTsdCsdGsdTsdCsdGsdCsdTsdCs**CbsGbsTb**
351	16	139b	**CbsGbsTb**sdCsdAsdTsdAsdGsdAsdCsdCsdGsdAs**GbsCbsCb**
351	12	140b	**AbsTb**sdAsdGsdAsdCsdCsdGsdAsdGs**CbsCb**
354	16	141b	**GbsCbsTb**sdCsdGsdTsdCsdAsdTsdAsdGsdAsdCs**CbsGbsAb**
354	13	142b	**CbsGbsTb**sdCsdAsdTsdAsdGsdAsdCsdCs**GbsAb**
355	14	143b	**CbsTb**sdCsdGsdTsdCsdAsdTsdAsdGsdAs**CbsCbsGb**
355	14	143c	**CbsTbsCb**sdGsdTsdCsdAsdTsdAsdGsdAsdCs**CbsGb**
355	14	143d	**CbsTbsCb**sdGsdTsdCsdAsdTsdAsdGsdAs**CbsCbsGb**
355	15	144b	**GbsCbsTb**sdCsdGsdTsdCsdAsdTsdAsdGsdAs**CbsCbsGb**
356	14	145b	**GbsCbsTb**sdCsdGsdTsdCsdAsdTsdAsdGs**AbsCbsCb**
381	17	146b	**CbsAbsGbsCb**sdCsdCsdCsdCsdGsdAsdCsdCsdCs**AbsTbsGbsGb**
382	16	147b	**CbsAbsGb**sdCsdCsdCsdCsdCsdGsdAsdCsdCsdCs**AbsTbsGb**

382	16	147c	**CbsAbsGb**sdCsdCsdCsdCsdCsdGsdAsdCsdCsdCs**AsTsG**
382	16	147d	**CbsAbsGb**sdCsdCsdCsdCsdCsdGsdAsdCsdCs**CbsAbsTbsGb**
382	16	147e	**CbsAbsGbsCb**sdCsdCsdCsdCsdGsdAsdCsdCsdCs**AbsTbsGb**
382	16	147f	**CbsAbsGbsCb**sdCsdCsdCsdCsdGsdAsdCsdCs**CbsAbsTbsGb**
383	14	148b	**AbsGb**sdCsdCsdCsdCsdCsdGsdAsdCsdCs**CbsAbsTb**
383	14	148c	**AbsGbsCb**sdCsdCsdCsdCsdGsdAsdCsdCsdCs**AbsTb**
383	14	148d	**AbsGbsCb**sdCsdCsdCsdCsdGsdAsdCsdCs**CbsAbsTb**
384	14	149	**CbsAbsGb**sdCsdCsdCsdCsdCsdGsdAsdCs**CbsCbsAb**
422	17	150b	**CbsGbsCbsGb**sdTsdCsdCsdAsdCsdAsdGsdGsdAs**CbsGbsAbsTb**
425	14	151b	**CbsGbsCb**sdGsdTsdCsdCsdAsdCsdAsdGs**GbsAbsCb**
429	15	152b	**CbsGbsAb**sdTsdAsdCsdGsdCsdGsdTsdCsdCs**AbsCbsAb**
429	15	152c	**CbsGbsAb**sdTsdAsdCsdGsdCsdGsdTsdCs**CbsAbsCbsAb**
429	15	152d	**CbsGbsAbsTb**sdAsdCsdGsdCsdGsdTsdCsdCs**AbsCbsAb**
432	12	155b	**CbsGb**sdAsdTsdAsdCsdGsdCsdGsdTs**CbsCb**
431	13	153b	**CbsGbsAb**sdTsdAsdCsdGsdCsdGsdTsdCs**CbsAb**
431	13	153c	**CbsGb**sdAsdTsdAsdCsdGsdCsdGsdTs**CbsCbsAb**
431	16	154b	**TbsGbsGb**sdCsdGsdAsdTsdAsdCsdGsdCsdGsdTs**CbsCbsAb**
432	12	155c	**CbsGb**sdAsdTsdAsdCsdGsdCsdGsdTsdCs**Cb**
432	12	155d	**Cb**sdGsdAsdTsdAsdCsdGsdCsdGsdTs**CbsCb**
432	13	156b	**GbsCbsGb**sdAsdTsdAsdCsdGsdCsdGsdTs**CbsCb**
432	17	157b	**GbsCbsTbsGb**sdGsdCsdGsdAsdTsdAsdCsdGsdCs**GbsTbsCbsCb**
433	15	158b	**CbsTbsGb**sdGsdCsdGsdAsdTsdAsdCsdGsdCs**GbsTbsCb**
433	12	159b	**GbsCb**sdGsdAsdTsdAsdCsdGsdCsdGs**TbsCb**
433	16	160b	**GbsCbsTb**sdGsdGsdCsdGsdAsdTsdAsdCsdGsdCs**GbsTbsCb**
433	14	161b	**TbsGbsGb**sdCsdGsdAsdTsdAsdCsdGsdCs**GbsTbsCb**
434	12	164b	**GbsGb**sdCsdGsdAsdTsdAsdCsdGsdCs**GbsTb**
434	13	162b	**TbsGbsGb**sdCsdGsdAsdTsdAsdCsdGsdCs**GbsTb**
434	13	162c	**TbsGb**sdGsdCsdGsdAsdTsdAsdCsdGs**CbsGbsTb**
434	14	163b	**CbsTbsGb**sdGsdCsdGsdAsdTsdAsdCsdGs**CbsGbsTb**
435	13	165b	**CbsTbsGb**sdGsdCsdGsdAsdTsdAsdCsdGs**CbsGb**
435	12	166b	**TbsGb**sdGsdCsdGsdAsdTsdAsdCsdGs**CbsGb**
437	17	167b	**AbsTbsCbsGb**sdTsdGsdCsdTsdGsdGsdCsdGsdAs**TbsAbsCbsGb**
449	16	168b	**CbsGbsTb**sdGsdCsdGsdGsdTsdGsdGsdGsdAsdTs**CbsGbsTb**
449	17	169b	**AbsCbsGbsTb**sdGsdCsdGsdGsdTsdGsdGsdGsdAs**TbsCbsGbsTb**
450	17	170b	**AbsAbsCbsGb**sdTsdGsdCsdGsdGsdTsdGsdGsdGs**AbsTbsCbsGb**
452	15	171b	**AbsAbsCb**sdGsdTsdGsdCsdGsdGsdTsdGsdGs**GbsAbsTb**
452	17	172b	**TbsGbsAbsAb**sdCsdGsdTsdGsdCsdGsdGsdTsdGs**GbsGbsAbsTb**
459	17	173b	**CbsGbsAbsCb**sdTsdTsdCsdTsdGsdAsdAsdCsdGs**TbsGbsCbsGb**

941	17	174b	**TbsTbsAbsAb**sdCsdGsdCsdGsdGsdTsdAsdGsdCs**AbsGbsTbsAb**
941	16	175b	**TbsAbsAb**sdCsdGsdCsdGsdGsdTsdAsdGsdCsdAs**GbsTbsAb**
942	17	176b	**GbsTbsTbsAb**sdAsdCsdGsdCsdGsdGsdTsdAsdGs**CbsAbsGbsTb**
943	15	177b	**TbsTbsAb**sdAsdCsdGsdCsdGsdGsdTsdAsdGs**CbsAbsGb**
944	13	178b	**TbsAbsAb**sdCsdGsdCsdGsdGsdTsdAsdGs**CbsAb**
945	12	179b	**TbsAb**sdAsdCsdGsdCsdGsdGsdTsdAs**GbsCb**
945	13	180b	**TbsTbsAb**sdAsdCsdGsdCsdGsdGsdTsdAs**GbsCb**
946	12	181b	**TbsTb**sdAsdAsdCsdGsdCsdGsdGsdTs**AbsGb**
946	13	182b	**GbsTbsTb**sdAsdAsdCsdGsdCsdGsdGsdTs**AbsGb**
946	15	183b	**CbsGbsGb**sdTsdTsdAsdAsdCsdGsdCsdGsdGs**TbsAbsGb**
946	16	184b	**CbsCbsGb**sdGsdTsdTsdAsdAsdCsdGsdCsdGsdGs**TbsAbsGb**
947	14	185b	**CbsGbsGb**sdTsdTsdAsdAsdCsdGsdCsdGs**GbsTbsAb**
947	13	186b	**GbsGbsTb**sdTsdAsdAsdCsdGsdCsdGsdGs**TbsAb**
947	15	187b	**CbsCbsGb**sdGsdTsdTsdAsdAsdCsdGsdCsdGs**GbsTbsAb**
947	16	188b	**GbsCbsCb**sdGsdGsdTsdTsdAsdAsdCsdGsdCsdGs**GbsTbsAb**
947	17	189b	**TbsGbsCbsCb**sdGsdGsdTsdTsdAsdAsdCsdGsdCs**GbsGbsTbsAb**
948	13	190b	**CbsGbsGb**sdTsdTsdAsdAsdCsdGsdCsdGs**GbsTb**
949	13	191b	**CbsCbsGb**sdGsdTsdTsdAsdAsdCsdGsdCs**GbsGb**
949	14	192b	**GbsCbsCb**sdGsdGsdTsdTsdAsdAsdCsdGs**CbsGbsGb**
949	15	193b	**TbsGbsCb**sdCsdGsdGsdTsdTsdAsdAsdCsdGs**CbsGbsGb**
950	13	194b	**GbsCbsCb**sdGsdGsdTsdTsdAsdAsdCsdGs**CbsGb**
950	15	195b	**CbsTbsGb**sdCsdCsdGsdGsdTsdTsdAsdAsdCs**GbsCbsGb**
950	16	196b	**GbsCbsTb**sdGsdCsdCsdGsdGsdTsdTsdAsdAsdCs**GbsCbsGb**
1387	16	197b	**AbsTbsGb**sdCsdCsdGsdCsdGsdTsdCsdAsdGsdGs**TbsAbsCb**
1392	13	198b	**AbsCbsAb**sdTsdGsdCsdCsdGsdCsdGsdTs**CbsAb**
1393	16	199b	**GbsAbsTb**sdGsdAsdCsdAsdTsdGsdCsdCsdGsdCs**GbsTbsCb**
1393	16	199c	**GbsAbsTb**sdGsdAsdCsdAsdTsdGsdCsdCsdGs**CbsGbsTbsCb**
1393	16	199d	**GbsAbsTbsGb**sdAsdCsdAsdTsdGsdCsdCsdGsdCs**GbsTbsCb**
1393	16	199e	**GbsAbsTbsGb**sdAsdCsdAsdTsdGsdCsdCsdGs**CbsGbsTbsCb**
1394	12	200b	**GbsAb**sdCsdAsdTsdGsdCsdCsdGsdCs**GbsTb**
1394	15	201b	**GbsAbsTb**sdGsdAsdCsdAsdTsdGsdCsdCsdGs**CbsGbsTb**
1395	13	202b	**AbsTbsGb**sdAsdCsdAsdTsdGsdCsdCsdGs**CbsGb**
1805	17	203b	**TbsCbsCbsCb**sdGsdCsdAsdCsdCsdTsdTsdGsdGs**AbsAbsCbsCb**
1851	16	204b	**CbsGbsAb**sdTsdCsdTsdCsdTsdCsdAsdAsdCsdAs**CbsGbsTb**
1851	17	205b	**TbsCbsGbsAb**sdTsdCsdTsdCsdTsdCsdAsdAsdCs**AbsCbsGbsTb**
1852	15	206b	**CbsGbsAb**sdTsdCsdTsdCsdTsdCsdAsdAsdCs**AbsCbsGb**
1852	16	207b	**TbsCbsGb**sdAsdTsdCsdTsdCsdTsdCsdAsdAsdCs**AbsCbsGb**
1852	17	208b	**CbsTbsCbsGb**sdAsdTsdCsdTsdCsdTsdCsdAsdAs**CbsAbsCbsGb**

2064	16	209b	**GbsTb**sdAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsAbs**GbsCb**
2064	16	209c	**GbsTbsAbsGb**sdTsdGsdTsdTsdTsdAsdGsdGsdGs**AbsGbsCb**
2064	16	209d	**GbsTbsAb**sdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**AbsGbsCb**
2064	16	209e	**GbsTbsAb**sdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsdAs**GbsCb**
2064	16	209f	**GbsTbsAb**sdGsdTsdGsdTsdTsdTsdAsdGsdGs**GbsAbsGbsCb**
2064	16	209g	**GbsTbsAbsGb**sdTsdGsdTsdTsdTsdAsdGsdGs**GbsAbsGbsCb**
2064	16	209h	**GbsTb**sdAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**AbsGbsCb**
2064	16	209i	**GbsTbsAbsGbsTb**sdGsdTsdTsdTsdAsdGsdGs**GbsAbsGbsCb**
2064	16	209j	**GbsTbsAbsGb**sdTsdGsdTsdTsdTsdAsdGs**GbsGbsAbsGbsCb**
2064	16	209k	**GbsTbsAbsGbsTb**sdGsdTsdTsdTsdAsdGs**GbsGbsAbsGbsCb**
2072	16	210b	**GbsCb**sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGsdTs**GbsTbsTb**
2072	16	210c	**GbsCbsTb**sdAsdTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**TbsTb**
2072	16	210d	**GbsCbsTb**sdAsdTsdTsdTsdGsdGsdTsdAsdGsdTs**GbsTbsTb**
2072	16	210e	**GbsCbsTbsAb**sdTsdTsdTsdGsdGsdTsdAsdGsdTs**GbsTbsTb**
2072	16	210f	**GbsCbsTb**sdAsdTsdTsdTsdGsdGsdTsdAsdGs**TbsGbsTbsTb**
2072	16	210g	**GbsCbsTbsAb**sdTsdTsdTsdGsdGsdTsdAsdGs**TbsGbsTbsTb**
2284	15	211b	**AbsGbsCb**sdTsdTsdAsdTsdCsdCsdTsdAsdTs**GbsAbsCb**
2284	15	211c	**AbsGbsCb**sdTsdTsdAsdTsdCsdCsdTsdAs**TbsGbsAbsCb**
2284	15	211d	**AbsGbsCbsTb**sdTsdAsdTsdCsdCsdTsdAsdTs**GbsAbsCb**
2285	14	212b	**AbsGb**sdCsdTsdTsdAsdTsdCsdCsdTsdAs**TbsGbsAb**
2285	14	212c	**AbsGbsCb**sdTsdTsdAsdTsdCsdCsdTsdAsdTs**GbsAb**
2285	14	212d	**AbsGbsCb**sdTsdTsdAsdTsdCsdCsdTsdAs**TbsGbsAb**
2355	17	213b	**CbsAbsGb**sdGsdCsdAsdTsdTsdAsdAsdTsdAsdAsdAs**GbsTbsGb**
2355	17	213c	**CbsAbsGbsGb**sdCsdAsdTsdTsdAsdAsdTsdAsdAsdAs**GbsTbsGb**
2355	17	213d	**CbsAbsGb**sdGsdCsdAsdTsdTsdAsdAsdTsdAsdAs**AbsGbsTbsGb**
2355	17	213e	**CbsAbsGbsGb**sdCsdAsdTsdTsdAsdAsdTsdAsdAs**AbsGbsTbsGb**
4217	16	218d	**CbsAbsTb**sdGsdAsdAsdTsdGsdGsdAsdCsdCsdAs**GbsTbsAb**
4217	16	218e	**CbsAbsTb**sdGsdAsdAsdTsdGsdGsdAsdCsdCs**AbsGbsTbsAb**
4217	16	218f	**CbsAbsTbsGb**sdAsdAsdTsdGsdGsdAsdCsdCsdAs**GbsTbsAb**
4217	16	218g	**CbsAbsTbsGb**sdAsdAsdTsdGsdGsdAsdCsdCs**AbsGbsTbsAb**
4120	16	214	**CbsTbsAb**sdGsdGsdCsdGsdCsdCsdTsdCsdTsdAs**TbsGbsCb**
4121	14	215b	**TbsAbsGb**sdGsdCsdGsdCsdCsdTsdCsdTs**AbsTbsGb**
4121	15	216b	**CbsTbsAb**sdGsdGsdCsdGsdCsdCsdTsdCsdTs**AbsTbsGb**
4122	13	217b	**TbsAbsGb**sdGsdCsdGsdCsdCsdTsdCsdTs**AbsTb**

Table 3

SP	L	Seq ID No.	Sequence, 5'-3'
2064	16	209m	**GbsTbsAbsGb**sdTsdGsdTsdTsdTsdAsdGsdGsdGs**AbsGbsC*b**

2064	16	209n	**GbsTbsAb**sdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**AbsGbsC*b**
2064	16	209o	**GbsTbsAb**sdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsdAs**GbsC*b**
2064	16	209p	**GbsTbsAb**sdGsdTsdGsdTsdTsdTsdAsdGsdGs**GbsAbsGbsC*b**
2064	16	209q	**GbsTbsAbsGb**sdTsdGsdTsdTsdTsdAsdGsdGs**GbsAbsGbsC*b**
2064	16	209r	**GbsTb**sdAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**AbsGbsC*b**
429	15	152e	**C*bsGbsAbsTb**sdAsdC*sdGsdC*sdGsdTsdC*sdC*s**AbsC*bsAb**
4217	16	-218j	**C*bsAbsTb**sdGsdAsdAsdTsdGsdGsdAsdC*sdC*s**AbsGbsTbsAb**
2355	17	213f	**C*bsAbsGb**sdGsdC*sdAsdTsdTsdAsdAsdTsdAsdAs**AbsGbsTbsGb**
2355	17	213g	**C*bsAbsGb**sdGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**GbsTbsGb**
432	12	155e	**C*bsGb**sdAsdTsdAsdC*sdGsdC*sdGsdTs**C*bsC*b**
4217	16	218h	**C*bsAbsTbsGb**sdAsdAsdTsdGsdGsdAsdC*sdC*s**AbsGbsTbsAb**
2072	16	210h	**GbsC*bsTbsAb**sdTsdTsdTsdGsdGsdTsdAsdGsdTs**GbsTbsTb**
2072	16	210i	**GbsC*b**sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGsdTs**GbsTbsTb**
432	12	155f	**C*bsGb**sdAsdTsdAsdC*sdGsdC*sdGsdTsdC*s**C*b**
2072	16	210j	**GbsC*bsTb**sdAsdTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**TbsTb**
432	12	155g	**C*b**sdGsdAsdTsdAsdC*sdGsdC*sdGsdTs**C*bsC*b**
431	13	153d	**C*bsGbsAb**sdTsdAsdC*sdGsdC*sdGsdTsdC*s**C*bsAb**
429	15	152f	**C*bsGbsAb**sdTsdAsdC*sdGsdC*sdGsdTsdC*sdC*s**AbsC*bsAb**
4217	16	218i	**C*bsAbsTbsGb**sdAsdAsdTsdGsdGsdAsdC*sdC*sdAs**GbsTbsAb**
1393	16	199f	**GbsAbsTb**sdGsdAsdC*sdAsdTsdGsdC*sdC*sdGsdC*s**GbsTbsC*b**
2285	14	212e	**AbsGbsC*b**sdTsdTsdAsdTsdC*sdC*sdTsdAsdTs**GbsAb**
355	14	143e	**C*bsTb**sdC*sdGsdTsdC*sdAsdTsdAsdGsdAs**C*bsC*bsGb**
2072	16	210k	**GbsC*bsTb**sdAsdTsdTsdTsdGsdGsdTsdAsdGsdTs**GbsTbsTb**
1393	16	199g	**GbsAbsTb**sdGsdAsdC*sdAsdTsdGsdC*sdC*sdGs**C*bsGbsTbsC*b**
2355	17	213h	**C*bsAbsGbsGb**sdC*sdAsdTsdTsdAsdAsdTsdAsdAs**AbsGbsTbsGb**
429	15	152g	**C*bsGbsAb**sdTsdAsdC*sdGsdC*sdGsdTsdC*s**C*bsAbsC*bsAb**
2285	14	212f	**AbsGbsC*b**sdTsdTsdAsdTsdC*sdC*sdTsdAs**TbsGbsAb**
355	14	143f	**C*bsTbsC*b**sdGsdTsdC*sdAsdTsdAsdGsdAs**C*bsC*bsGb**
1393	16	199h	**GbsAbsTbsGb**sdAsdC*sdAsdTsdGsdC*sdC*sdGs**C*bsGbsTbsC*b**
1393	16	199i	**GbsAbsTbsGb**sdAsdC*sdAsdTsdGsdC*sdC*sdGsdC*s**GbsTbsC*b**
4217	16	218k	**C*bsAbsTb**sdGsdAsdAsdTsdGsdGsdAsdC*sdC*sdAs**GbsTbsAb**
2285	14	212g	**AbsGb**sdC*sdTsdTsdAsdTsdC*sdC*sdTsdAs**TbsGbsAb**
434	13	162d	**TbsGbsGb**sdC*sdGsdAsdTsdAsdC*sdGsdC*s**GbsTb**
383	14	148e	**AbsGbsC*b**sdC*sdC*sdC*sdC*sdGsdAsdC*sdC*sdC*s**AbsTb**
431	13	153e	**C*bsGb**sdAsdTsdAsdC*sdGsdC*sdGsdTs**C*bsC*bsAb**
2284	15	211e	**AbsGbsC*b**sdTsdTsdAsdTsdC*sdC*sdTsdAsdTs**GbsAbsC*b**
355	14	143g	**C*bsTbsC*b**sdGsdTsdC*sdAsdTsdAsdGsdAsdC*s**C*bsGb**
2284	15	211f	**AbsGbsC*b**sdTsdTsdAsdTsdC*sdC*sdTsdAs**TbsGbsAbsC*b**

383	14	148f	**AbsGbsC*b**sdC*sdC*sdC*sdC*sdGsdAsdC*sdC*s**C*bsAbsTb**
383	14	148g	**AbsGb**sdC*sdC*sdC*sdC*sdC*sdGsdAsdC*sdC*s**C*bsAbsTb**
382	16	147g	**C*bsAbsGb**sdC*sdC*sdC*sdC*sdC*sdGsdAsdC*sdC*sdC*s**AbsTbsGb**
2072	16	210m	**GbsC*bsTb**sdAsdTsdTsdTsdGsdGsdTsdAsdGs**TbsGbsTbsTb**
2072	16	210n	**GbsC*bsTbsAb**sdTsdTsdTsdGsdGsdTsdAsdGs**TbsGbsTbsTb**
434	13	162e	**TbsGb**sdGsdC*sdGsdAsdTsdAsdC*sdGs**C*bsGbsTb**
2284	15	211g	**AbsGbsC*bsTb**sdTsdAsdTsdC*sdC*sdTsdAsdTs**GbsAbsC*b**
382	16	147h	**C*bsAbsGb**sdC*sdC*sdC*sdC*sdC*sdC*sdGsdAsdC*sdC*s**C*bsAbsTbsGb**
382	16	147i	**C*bsAbsGbsC*b**sdC*sdC*sdC*sdC*sdC*sdGsdAsdC*sdC*sdC*s**AbsTbsGb**
382	16	147j	**C*bsAbsGb**sdC*sdC*sdC*sdC*sdC*sdC*sdGsdAsdC*sdC*sdC*s**AbsTbsGb**
2355	17	213i	**C*bsAbsGbsGb**sdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**GbsTbsGb**
382	16	147k	**C*bsAbsGbsC*b**sdC*sdC*sdC*sdC*sdC*sdGsdAsdC*sdC*s**C*bsAbsTbsGb**

Preferred antisense-oligonucleotides

In the following preferred antisense-oligonucleotides of the present invention are disclosed.

Thus, the present invention is preferably directed to an antisense-oligonucleotide in form of a gapmer consisting of 10 to 28 nucleotides, preferably 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides and 1 to 5 of these nucleotides at the 5' terminal end and 1 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 6, preferably 7 and more preferably 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^1-**GTCATAGA**-N^2-3' (Seq. ID No. 12) or 5'-N^3-**ACGCGTCC**-N^4-3' (Seq. ID No. 98) or 5'-N^{11}-**TGTTTAGG**-N^{12}-3' (Seq. ID No. 10) or 5'-N^5-**TTTGGTAG**-N^6-3' (Seq. ID No. 11) or 5'-N^7-**AATGGACC**-N^8-3' (Seq. ID No. 100) or 5'-N^9-**ATTAATAA**-N^{10}-3' (Seq. ID No. 101), wherein

N^1 represents: CATGGCAGACCCCGCTGCTC-, ATGGCAGACCCCGCTGCTC-, TGGCAGACCCCGCTGCTC-, GGCAGACCCCGCTGCTC-, GCAGACCCCGCTGCTC-, CAGACCCCGCTGCTC-, AGACCCCGCTGCTC-, GACCCCGCTGCTC-, ACCCCGCTGCTC-, CCCCGCTGCTC-, CCCGCTGCTC-, CCGCTGCTC-, CGCTGCTC-, GCTGCTC-, CTGCTC-, TGCTC-, GCTC-, CTC-, TC-, or C-;

N^2 represents: -C, -CC, -CCG, -CCGA, -CCGAG, -CCGAGC, -CCGAGCC, -CCGAGCCC, -CCGAGCCCC, -CCGAGCCCCC, -CCGAGCCCCCA, -CCGAGCCCCCAG, -CCGAGCCCCCAGC, -CCGAGCCCCCAGCG, -CCGAGCCCCCAGCGC, -CCGAGCCCCCAGCGCA, -CCGAGCCCCCAGCGCAG, -CCGAGCCCCCAGCGCAGC, -CCGAGCCCCCAGCGCAGCG, or –CCGAGCCCCCAGCGCAGCGG;

N^3 represents: GGTGGGATCGTGCTGGCGAT-, GTGGGATCGTGCTGGCGAT-, TGGGATCGTGCTGGCGAT-, GGGATCGTGCTGGCGAT-, GGATCGTGCTGGCGAT-, GATCGTGCTGGCGAT-, ATCGTGCTGGCGAT-, TCGTGCTGGCGAT-, CGTGCTGGCGAT-, GTGCTGGCGAT-, TGCTGGCGAT-, GCTGGCGAT-, CTGGCGAT-, TGGCGAT-, GGCGAT-, GCGAT-, CGAT-, GAT-, AT-, or T-;

N^4 represents: -ACAGGACGATGTGCAGCGGC, -ACAGGACGATGTGCAGCGG, -ACAGGACGATGTGCAGCG, -ACAGGACGATGTGCAGC, -ACAGGACGATGTGCAG, -ACAGGACGATGTGCA, -ACAGGACGATGTGC, -ACAGGACGATGTG, -ACAGGACGATGT, -ACAGGACGATG, -ACAGGACGAT, -ACAGGACGA, -ACAGGACG, -ACAGGAC, -ACAGGA, -ACAGG, -ACAG, -ACA, -AC, or -A;

N^5 represents: GCCCAGCCTGCCCCAGAAGAGCTA-, CCCAGCCTGCCCCAGAAGAGCTA-, CCAGCCTGCCCCAGAAGAGCTA-, CAGCCTGCCCCAGAAGAGCTA-, AGCCTGCCCCAGAAGAGCTA-, GCCTGCCCCAGAAGAGCTA-, CCTGCCCCAGAAGAGCTA-, CTGCCCCAGAAGAGCTA-, TGCCCCAGAAGAGCTA-, GCCCCAGAAGAGCTA-, CCCCAGAAGAGCTA-, CCCAGAAGAGCTA-, CCAGAAGAGCTA-, CAGAAGAGCTA-, AGAAGAGCTA-, GAAGAGCTA-, AAGAGCTA-, AGAGCTA-, GAGCTA-, AGCTA-, GCTA-, CTA-, TA-, or A-;

N^6 represents: -TGTTTAGGGAGCCGTCTTCAGGAA, -TGTTTAGGGAGCCGTCTTCAGGA, -TGTTTAGGGAGCCGTCTTCAGG, -TGTTTAGGGAGCCGTCTTCAG, -TGTTTAGGGAGCCGTCTTCA, -TGTTTAGGGAGCCGTCTTC, -TGTTTAGGGAGCCGTCTT, -TGTTTAGGGAGCCGTCT, -TGTTTAGGGAGCCGTC, -TGTTTAGGGAGCCGT, -TGTTTAGGGAGCCG, -TGTTTAGGGAGCC, -TGTTTAGGGAGC, -TGTTTAGGGAG, -TGTTTAGGGA, -TGTTTAGGG, -TGTTTAGG, -TGTTTAG, -TGTTTA, -TGTTT, -TGTT, -TGT, -TG, or –T;

N^7 represents: TGAATCTTGAATATCTCATG-, GAATCTTGAATATCTCATG-, AATCTTGAATATCTCATG-, ATCTTGAATATCTCATG-, TCTTGAATATCTCATG-, CTTGAATATCTCATG-, TTGAATATCTCATG-, TGAATATCTCATG-, GAATATCTCATG-, AATATCTCATG-, ATATCTCATG-, TATCTCATG-, ATCTCATG-, TCTCATG-, CTCATG-, TCATG-, CATG-, ATG-, TG-, or G-;

N^8 represents: -AGTATTCTAGAAACTCACCA, -AGTATTCTAGAAACTCACC, -AGTATTCTAGAAACTCAC, -AGTATTCTAGAAACTCA, -AGTATTCTAGAAACTC, -AGTATTCTAGAAACT, -AGTATTCTAGAAAC, -AGTATTCTAGAAA, -AGTATTCTAGAA, -AGTATTCTAGA, -AGTATTCTAG, -AGTATTCTA, -AGTATTCT, -AGTATTC, -AGTATT, -AGTAT, -AGTA, -AGT, -AG, or -A;

N^9 represents: ATTCATATTTATATACAGGC-, TTCATATTTATATACAGGC-, TCATATTTATATACAGGC-, CATATTTATATACAGGC-, ATATTTATATACAGGC-, TATTTATATACAGGC-, ATTTATATACAGGC-, TTTATATACAGGC-, TTATATACAGGC-, TATATACAGGC-, ATATACAGGC-, TATACAGGC-, ATACAGGC-, TACAGGC-, ACAGGC-, CAGGC-, AGGC-, GGC-, GC-, or C-;

N^{10} represents: -AGTGCAAATGTTATTGGCTA, -AGTGCAAATGTTATTGGCT, -AGTGCAAATGTTATTGGC, -AGTGCAAATGTTATTGG, -AGTGCAAATGTTATTG, -AGTGCAAATGTTATT, -AGTGCAAATGTTAT, -AGTGCAAATGTTA, -AGTGCAAATGTT, -AGTGCAAATGT, -AGTGCAAATG, -AGTGCAAAT, -AGTGCAAA, -AGTGCAA, -AGTGCA, -AGTGC, -AGTG, -AGT, -AG, or -A;

N^{11} represents: TGCCCCAGAAGAGCTATTTGGTAG-, GCCCCAGAAGAGCTATTTGGTAG-, CCCCAGAAGAGCTATTTGGTAG-, CCCAGAAGAGCTATTTGGTAG-, CCAGAAGAGCTATTTGGTAG-, CAGAAGAGCTATTTGGTAG-, AGAAGAGCTATTTGGTAG-, GAAGAGCTATTTGGTAG-, AAGAGCTATTTGGTAG-, AGAGCTATTTGGTAG-, GAGCTATTTGGTAG-, AGCTATTTGGTAG-, GCTATTTGGTAG-, CTATTTGGTAG-, TATTTGGTAG-, ATTTGGTAG-, TTTGGTAG-, TTGGTAG-, TGGTAG-, GGTAG-, GTAG-, TAG-, AG- or G-,

N^{12} represents: -GAGCCGTCTTCAGGAATCTTCTCC, -GAGCCGTCTTCAGGAATCTTCTC, -GAGCCGTCTTCAGGAATCTTCT, -GAGCCGTCTTCAGGAATCTTC, -GAGCCGTCTTCAGGAATCTT, -GAGCCGTCTTCAGGAATCT, -GAGCCGTCTTCAGGAATC, -GAGCCGTCTTCAGGAAT, -GAGCCGTCTTCAGGAA, -GAGCCGTCTTCAGGA, -GAGCCGTCTTCAGG, -GAGCCGTCTTCAG, -GAGCCGTCTTCA, -GAGCCGTCTTC, -GAGCCGTCTT, -GAGCCGTCT, -GAGCCGTC, -GAGCCGT, -GAGCCG, -GAGCC, -GAGC, -GAG, -GA, or –G;

or wherein N^1 to N^{12} represent any of the limited lists of residues as disclosed herein, and salts and optical isomers of the antisense-oligonucleotide.

Moreover, the present invention is preferably directed to an antisense-oligonucleotide in form of a gapmer consisting of 10 to 28 nucleotides, preferably 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18

nucleotides and 1 to 5 of these nucleotides at the 5' terminal end and 1 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 6, preferably 7 and more preferably 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^1-**GTCATAGA**-N^2-3' (Seq. ID No. 12), wherein

N^1 represents: GGCAGACCCCGCTGCTC-, GCAGACCCCGCTGCTC-, CAGACCCCGCTGCTC-, AGACCCCGCTGCTC-, GACCCCGCTGCTC-, ACCCCGCTGCTC-, CCCCGCTGCTC-, CCCGCTGCTC-, CCGCTGCTC-, CGCTGCTC-, GCTGCTC-, CTGCTC-, TGCTC-, GCTC-, CTC-, TC-, or C-;

N^2 represents: -C, -CC, -CCG, -CCGA, -CCGAG, -CCGAGC, -CCGAGCC, -CCGAGCCC, -CCGAGCCCC, -CCGAGCCCCC, -CCGAGCCCCCA, -CCGAGCCCCCAG, -CCGAGCCCCCAGC, -CCGAGCCCCCAGCG, -CCGAGCCCCCAGCGC, -CCGAGCCCCCAGCGCA, or -CCGAGCCCCCAGCGCAG;

and salts and optical isomers of the antisense-oligonucleotide.

N^1 and/or N^2 may also represent any of the further limited lists of 3' and 5' residues as disclosed herein.

Especially preferred gapmer antisense-oligonucleotides falling under general formula S1:

5'-N^1-**GTCATAGA**-N^2-3' (Seq. ID No. 12) **S1**

are the following:

CCGCTGCT**CGTCATAGAC**	(Seq. ID No. 19)
CGCTGCT**CGTCATAGACC**	(Seq. ID No. 20)
GCTGCT**CGTCATAGAC**CG	(Seq. ID No. 21)
CTGCT**CGTCATAGAC**CGA	(Seq. ID No. 22)
TGCT**CGTCATAGAC**CGAG	(Seq. ID No. 23)
GCT**CGTCATAGAC**CGAGC	(Seq. ID No. 24)
CT**CGTCATAGAC**CGAGCC	(Seq. ID No. 25)
T**CGTCATAGAC**CGAGCCC	(Seq. ID No. 26)
CGTCATAGACCGAGCCCC	(Seq. ID No. 27)
CGCTGCT**CGTCATAGAC**	(Seq. ID No. 28)
GCTGCT**CGTCATAGACC**	(Seq. ID No. 29)
CTGCT**CGTCATAGAC**CG	(Seq. ID No. 30)

TGCT**CGTCATAGAC**CGA	(Seq. ID No. 31)
GCT**CGTCATAGAC**CGAG	(Seq. ID No. 32)
CT**CGTCATAGAC**CGAGC	(Seq. ID No. 33)
T**CGTCATAGAC**CGAGCC	(Seq. ID No. 34)
CGTCATAGACCGAGCCC	(Seq. ID No. 35)
GCTGCT**CGTCATAGAC**	(Seq. ID No. 36)
CTGCT**CGTCATAGACC**	(Seq. ID No. 37)
TGCT**CGTCATAGAC**CG	(Seq. ID No. 38)
GCT**CGTCATAGAC**CGA	(Seq. ID No. 39)
CT**CGTCATAGAC**CGAG	(Seq. ID No. 40)
T**CGTCATAGAC**CGAGC	(Seq. ID No. 41)
CGTCATAGACCGAGCC	(Seq. ID No. 42)
CTGCT**CGTCATAGAC**	(Seq. ID No. 43)
TGCT**CGTCATAGACC**	(Seq. ID No. 44)
GCT**CGTCATAGAC**CG	(Seq. ID No. 45)
CT**CGTCATAGAC**CGA	(Seq. ID No. 46)
T**CGTCATAGAC**CGAG	(Seq. ID No. 47)
CGTCATAGACCGAGC	(Seq. ID No. 48)
TGCT**CGTCATAGAC**	(Seq. ID No. 49)
GCT**CGTCATAGACC**	(Seq. ID No. 50)
CT**CGTCATAGAC**CG	(Seq. ID No. 51)
T**CGTCATAGAC**CGA	(Seq. ID No. 52)
CGTCATAGACCGAG	(Seq. ID No. 53)

The antisense-oligonucleotides of formula **S1** in form of gapmers (LNA segment 1 – DNA segment – LNA segment 2) contain an LNA segment at the 5' terminal end consisting of 2 to 5, preferably 2 to 4 LNA units and contain an LNA segment at the 3' terminal end consisting of 2 to 5, preferably 2 to 4 LNA units and between the two LNA segments one DNA segment consisting of 6 to 14, preferably 7 to 12 and more preferably 8 to 11 DNA units.

The antisense-oligonucleotides of formula **S1** contain the LNA nucleotides (LNA units) as disclosed herein, especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably these disclosed in the chapter "Preferred LNAs". The LNA units and the DNA units may comprise standard nucleobases such as adenine (A), cytosine (C), guanine (G), thymine (T) and uracil (U), but may also contain modified nucleobases as disclosed in the chapter "Nucleobases". The antisense-oligonucleotides of formula **S1** or the LNA segments and the DNA segment of the antisense-oligonucleotide may contain any internucleotide linkage as disclosed

herein and especially these disclosed in the chapter "Internucleotide Linkages (IL)". The antisense-oligonucleotides of formula **S1** may optionally also contain endgroups at the 3' terminal end and/or the 5' terminal end and especially these disclosed in the chapter "Terminal groups".

Experiments have shown that modified nucleobases do not considerably increase or change the activity of the inventive antisense-oligonucleotides in regard to tested neurological and oncological indications. The modified nucleobases 5-methylcytosine or 2-aminoadenine have been demonstrated to further increase the activity of the antisense-oligonucleotides of formula **S1** especially if 5-methylcytosine is used in the LNA nucleotides only or in the LNA nucleotides and in the DNA nucleotides and/or if 2-aminoadenine is used in the DNA nucleotides and not in the LNA nucleotides.

The preferred gapmer structure of the antisense-oligonucleotides of formula **S1** is as follows: 3-8-3, 4-8-2, 2-8-4, 3-8-4, 4-8-3, 4-8-4, 3-9-3, 4-9-2, 2-9-4, 4-9-3, 3-9-4, 4-9-4, 3-10-3, 2-10-4, 4-10-2, 3-10-4, 4-10-3, 4-10-4, 2-11-4, 4-11-2, 3-11-4, 4-11-3 and still more preferred: 3-8-3, 3-8-4, 4-8-3, 4-8-4, 3-9-3, 4-9-3, 3-9-4, 4-9-4, 3-10-3, 3-10-4, 4-10-3, 4-10-4, 3-11-4, and 4-11-3.

As LNA units for the antisense-oligonucleotides of formula **S1** especially β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-ENA (**b^5**), β-D-(NH)-LNA (**b^6**), β-D-(NCH_3)-LNA (**b^7**), β-D-(ONH)-LNA (**b^8**) and β-D-($ONCH_3$)-LNA (**b^9**) are preferred. Experiments have been shown that all of these LNA units b^1, b^2, b^4, b^5, b^6, b^7, b^8, and b^9 can be synthesized with the required effort and lead to antisense-oligonucleotides of comparable stability and activity. However based on the expermients the LNA units b^1, b^2, b^4, b^5, b^6, and b^7 are further preferred. Still further preferred are the LNA units b^1, b^2, b^4, b^6, and b^7, and even more preferred are the LNA units b^1 and b^4 and most preferred also in regard to the complexity of the chemical synthesis is the β-D-oxy-LNA (**b^1**).

So far no special 3' terminal group or 5' terminal group could be found which remarkably had changed or increased the stability or activity for oncological or neurological indications, so that 3' and 5' end groups are possible but not explicitly preferred.

Various internucleotide bridges or internucleotide linkages are possible. In the formulae disclosed herein the internucleotide linkage IL is represented by –IL'–Y–.

Thus, IL = –IL'–Y– = –X''–P(=X')(X^-)–Y–, wherein IL is preferably selected form the group consisting of:
–O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, –O–P(O)(CH_3)–O–, –O–P(O)(OCH_3)–O–, –O–P(O)($NH(CH_3)$)–O–, –O–P(O)[$N(CH_3)_2$]–O–, –O–P(O)(BH_3^-)–O–, –O–P(O)($OCH_2CH_2OCH_3$)–O–, –O–P(O)($OCH_2CH_2SCH_3$)–O–, –O–P(O)(O^-)–$N(CH_3)$–, –$N(CH_3)$–P(O)(O^-)–O–.
Preferred are the internucleotide linkages IL selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, –O–P(O)(OCH_3)–O–, –O–P(O)($NH(CH_3)$)–O–, –O–P(O)[$N(CH_3)_2$]–O–, –O–P(O)($OCH_2CH_2OCH_3$)–O–, and more preferred selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, and still more preferred selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, and most preferably selected from –O–P(O)(O^-)–O– and –O–P(O)(S^-)–O–.

Thus, the present invention is preferably directed to an antisense-oligonucleotide in form of a gapmer consisting of 10 to 28 nucleotides, preferably 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides and 1 to 5 of these nucleotides at the 5' terminal end and 1 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 6, preferably 7 and more preferably 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^1-**GTCATAGA**-N^2-3' (Seq. ID No. 12), wherein
N^1 represents: GGCAGACCCCGCTGCTC-, GCAGACCCCGCTGCTC-, CAGACCCCGCTGCTC-, AGACCCCGCTGCTC-, GACCCCGCTGCTC-, ACCCCGCTGCTC-, CCCCGCTGCTC-, CCCGCTGCTC-, CCGCTGCTC-, CGCTGCTC-, GCTGCTC-, CTGCTC-, TGCTC-, GCTC-, CTC-, TC-, or C-;
N^2 represents: -C, -CC, -CCG, -CCGA, -CCGAG, -CCGAGC, -CCGAGCC, -CCGAGCCC, -CCGAGCCCC, -CCGAGCCCCC, -CCGAGCCCCCA, -CCGAGCCCCCAG, -CCGAGCCCCCAGC, -CCGAGCCCCCAGCG, -CCGAGCCCCCAGCGC, -CCGAGCCCCCAGCGCA, or -CCGAGCCCCCAGCGCAG; and
the LNA nucleotides are selected from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-ENA (**b^5**), β-D-(NH)-LNA (**b^6**), β-D-(NCH_3)-LNA (**b^7**),

β-D-(ONH)-LNA (**b**[8]) and β-D-(ONCH$_3$)-LNA (**b**[9]); and preferably from β-D-oxy-LNA (**b**[1]), β-D-thio-LNA (**b**[2]), α-L-oxy-LNA (**b**[4]), β-D-(NH)-LNA (**b**[6]), and β-D-(NCH$_3$)-LNA (**b**[7]); and

the internucleotide linkages are selected from

–O–P(O)(O$^-$)–O–, –O–P(O)(S$^-$)–O–, –O–P(S)(S$^-$)–O–, –S–P(O)(O$^-$)–O–, –S–P(O)(S$^-$)–O–, –O–P(O)(O$^-$)–S–, –O–P(O)(S$^-$)–S–, –S–P(O)(O$^-$)–S–, –O–P(O)(CH$_3$)–O–, –O–P(O)(OCH$_3$)–O–, –O–P(O)(NH(CH$_3$))–O–, –O–P(O)[N(CH$_3$)$_2$]–O–, –O–P(O)(BH$_3^-$)–O–, –O–P(O)(OCH$_2$CH$_2$OCH$_3$)–O–, –O–P(O)(OCH$_2$CH$_2$SCH$_3$)–O–, –O–P(O)(O$^-$)–N(CH$_3$)–, –N(CH$_3$)–P(O)(O$^-$)–O–;

and preferably from –O–P(O)(O$^-$)–O–, –O–P(O)(S$^-$)–O–, –O–P(S)(S$^-$)–O–, –S–P(O)(O$^-$)–O–, –S–P(O)(S$^-$)–O–, –O–P(O)(O$^-$)–S–, –O–P(O)(S$^-$)–S–, –S–P(O)(O$^-$)–S–;

and salts and optical isomers of the antisense-oligonucleotide. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine and/or 2-aminoadenine.

More preferably N^1 represents: CAGACCCCGCTGCTC-, AGACCCCGCTGCTC-, GACCCCGCTGCTC-, ACCCCGCTGCTC-, CCCCGCTGCTC-, CCCGCTGCTC-, CCGCTGCTC-, CGCTGCTC-, GCTGCTC-, CTGCTC-, TGCTC-, GCTC-, CTC-, TC-, or C-; and

N^2 represents: -C, -CC, -CCG, -CCGA, -CCGAG, -CCGAGC, -CCGAGCC, -CCGAGCCC, -CCGAGCCCC, -CCGAGCCCCC, -CCGAGCCCCCA, -CCGAGCCCCCAG, -CCGAGCCCCCAGC, -CCGAGCCCCCAGCG, or -CCGAGCCCCCAGCGC.

Still further preferred, the present invention is directed to an antisense-oligonucleotide in form of a gapmer consisting of 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides and 2 to 5 of these nucleotides at the 5' terminal end and 2 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 7, preferably at least 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R$_{II}$ or with a region of the mRNA encoding the TGF-R$_{II}$, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^1-**GTCATAGA**-N^2-3' (Seq. ID No. 12), wherein

N^1 represents: ACCCCGCTGCTC-, CCCCGCTGCTC-, CCCGCTGCTC-, CCGCTGCTC-, CGCTGCTC-, GCTGCTC-, CTGCTC-, TGCTC-, GCTC-, CTC-, TC-,

or C-; preferably N^1 represents: CCCGCTGCTC-, CCGCTGCTC-, CGCTGCTC-, GCTGCTC-, CTGCTC-, TGCTC-, GCTC-, CTC-, TC-, or C-; and
N^2 represents: -C, -CC, -CCG, -CCGA, -CCGAG, -CCGAGC, -CCGAGCC, -CCGAGCCC, -CCGAGCCCC, or -CCGAGCCCCC, -CCGAGCCCCCA, or -CCGAGCCCCCAG; preferably N^2 represents: -C, -CC, -CCG, -CCGA, -CCGAG, -CCGAGC, -CCGAGCC, -CCGAGCCC, -CCGAGCCCC, or -CCGAGCCCCC;
and the LNA nucleotides are selected from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-(NH)-LNA (**b^6**), and β-D-(NCH_3)-LNA (**b^7**); and
the internucleotide linkages are selected from
–O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–; and preferably selected from phosphate, phosphorothioate and phosphorodithioate;
and salts and optical isomers of the antisense-oligonucleotide. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine and/or 2-aminoadenine.

Especially preferred are the gapmer antisense-oligonucleotides of Seq. ID No. 19 to Seq. ID No. 53 containing a segment of 2 to 5, preferably 2 to 4 and more preferably 3 to 4 LNA units at the 3' terminus and a segment of 2 to 5, preferably 2 to 4 and more preferably 3 to 4 LNA units at the 5' terminus and a segment of at least 6, preferably 7 and more preferably 8 DNA units between the two segments of LNA units, wherein the LNA units are selected from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-(NH)-LNA (**b^6**), and β-D-(NCH_3)-LNA (**b^7**) and the internucleotide linkages are selected from phosphate, phosphorothioate and phosphorodithioate. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine in the LNA units, preferably all the LNA units and/or 2-aminoadenine in some or all DNA units and/or 5-methylcytosine in some or all DNA units.

Also especially preferred are the gapmer antisense-oligonucleotides of Table 4 (Seq. ID No. 232a to 244b).

Moreover, the present invention is preferably directed to an antisense-oligonucleotide in form of a gapmer consisting of 10 to 28 nucleotides, preferably 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides and 1 to 5 of these nucleotides at the 5' terminal end and 1 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA

nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 6, preferably 7 and more preferably 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^3-**ACGCGTCC**-N^4-3' (Seq. ID No. 98), wherein

N^3 represents: GGGATCGTGCTGGCGAT-, GGATCGTGCTGGCGAT-, GATCGTGCTGGCGAT-, ATCGTGCTGGCGAT-, TCGTGCTGGCGAT-, CGTGCTGGCGAT-, GTGCTGGCGAT-, TGCTGGCGAT-, GCTGGCGAT-, CTGGCGAT-, TGGCGAT-, GGCGAT-, GCGAT-, CGAT-, GAT-, AT-, or T-;

N^4 represents: -ACAGGACGATGTGCAGC, -ACAGGACGATGTGCAG, -ACAGGACGATGTGCA, -ACAGGACGATGTGC, -ACAGGACGATGTG, -ACAGGACGATGT, -ACAGGACGATG, -ACAGGACGAT, -ACAGGACGA, -ACAGGACG, -ACAGGAC, -ACAGGA, -ACAGG, -ACAG, -ACA, -AC, or -A;

and salts and optical isomers of the antisense-oligonucleotide.

N^3 and/or N^4 may also represent any of the further limited lists of 3' and 5' residues as disclosed herein.

Especially preferred gapmer antisense-oligonucleotides falling under general formula S2:

5'-N^3-**ACGCGTCC**-N^4-3' (Seq. ID No. 98) **S2**

are the following:

GCTGGCGA**TACGCGTCCA**	(Seq. ID No. 54)
CTGGCGA**TACGCGTCCA**C	(Seq. ID No. 55)
TGGCGA**TACGCGTCCA**CA	(Seq. ID No. 56)
GGCGA**TACGCGTCCA**CAG	(Seq. ID No. 57)
GCGA**TACGCGTCCA**CAGG	(Seq. ID No. 58)
CGA**TACGCGTCCA**CAGGA	(Seq. ID No. 59)
GA**TACGCGTCCA**CAGGAC	(Seq. ID No. 60)
A**TACGCGTCCA**CAGGACG	(Seq. ID No. 61)
TACGCGTCCACAGGACGA	(Seq. ID No. 62)
CTGGCGA**TACGCGTCCA**	(Seq. ID No. 63)
TGGCGA**TACGCGTCCA**C	(Seq. ID No. 64)
GGCGA**TACGCGTCCA**CA	(Seq. ID No. 65)
GCGA**TACGCGTCCA**CAG	(Seq. ID No. 66)
CGA**TACGCGTCCA**CAGG	(Seq. ID No. 67)
GA**TACGCGTCCA**CAGGA	(Seq. ID No. 68)

A**TACGCGTCCA**CAGGAC	(Seq. ID No. 349)
TACGCGTCCACAGGACG	(Seq. ID No. 350)
TGGCGA**TACGCGTCCA**	(Seq. ID No. 351)
GGCGA**TACGCGTCCA**C	(Seq. ID No. 352)
GCGA**TACGCGTCCA**CA	(Seq. ID No. 353)
CGA**TACGCGTCCA**CAG	(Seq. ID No. 354)
GA**TACGCGTCCA**CAGG	(Seq. ID No. 355)
A**TACGCGTCCA**CAGGA	(Seq. ID No. 356)
TACGCGTCCACAGGAC	(Seq. ID No. 357)
GGCGA**TACGCGTCCA**	(Seq. ID No. 358)
GCGA**TACGCGTCCA**C	(Seq. ID No. 359)
CGA**TACGCGTCCA**CA	(Seq. ID No. 360)
GA**TACGCGTCCA**CAG	(Seq. ID No. 361)
A**TACGCGTCCA**CAGG	(Seq. ID No. 362)
TACGCGTCCACAGGA	(Seq. ID No. 363)
GCGA**TACGCGTCCA**	(Seq. ID No. 364)
CGA**TACGCGTCCA**C	(Seq. ID No. 365)
GA**TACGCGTCCA**CA	(Seq. ID No. 366)
A**TACGCGTCCA**CAG	(Seq. ID No. 367)
TACGCGTCCACAGG	(Seq. ID No. 368)

The antisense-oligonucleotides of formula **S2** in form of gapmers (LNA segment 1 – DNA segment – LNA segment 2) contain an LNA segment at the 5' terminal end consisting of 2 to 5, preferably 2 to 4 LNA units and contain an LNA segment at the 3' terminal end consisting of 2 to 5, preferably 2 to 4 LNA units and between the two LNA segments one DNA segment consisting of 6 to 14, preferably 7 to 12 and more preferably 8 to 11 DNA units.

The antisense-oligonucleotides of formula **S2** contain the LNA nucleotides (LNA units) as disclosed herein, especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably these disclosed in the chapter "Preferred LNAs". The LNA units and the DNA units may comprise standard nucleobases such as adenine (A), cytosine (C), guanine (G), thymine (T) and uracil (U), but may also contain modified nucleobases as disclosed in the chapter "Nucleobases". The antisense-oligonucleotides of formula **S2** or the LNA segments and the DNA segment of the antisense-oligonucleotide may contain any internucleotide linkage as disclosed herein and especially these disclosed in the chapter "Internucleotide Linkages (IL)". The antisense-oligonucleotides of formula **S2** may optionally also contain endgroups

at the 3' terminal end and/or the 5' terminal end and especially these disclosed in the chapter "Terminal groups".

Experiments have shown that modified nucleobases do not considerably increase or change the activity of the inventive antisense-oligonucleotides in regard to tested neurological and oncological indications. The modified nucleobases 5-methylcytosine or 2-aminoadenine have been demonstrated to further increase the activity of the antisense-oligonucleotides of formula **S2** especially if 5-methylcytosine is used in the LNA nucleotides only or in the LNA nucleotides and in the DNA nucleotides and/or if 2-aminoadenine is used in the DNA nucleotides and not in the LNA nucleotides.

The preferred gapmer structure of the antisense-oligonucleotides of formula **S2** is as follows: 3-8-3, 4-8-2, 2-8-4, 3-8-4, 4-8-3, 4-8-4, 3-9-3, 4-9-2, 2-9-4, 4-9-3, 3-9-4, 4-9-4, 3-10-3, 2-10-4, 4-10-2, 3-10-4, 4-10-3, 4-10-4, 2-11-4, 4-11-2, 3-11-4, 4-11-3 and still more preferred: 3-8-3, 3-8-4, 4-8-3, 4-8-4, 3-9-3, 4-9-3, 3-9-4, 4-9-4, 3-10-3, 3-10-4, 4-10-3, 4-10-4, 3-11-4, and 4-11-3.

As LNA units for the antisense-oligonucleotides of formula **S2** especially β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-ENA (**b^5**), β-D-(NH)-LNA (**b^6**), β-D-(NCH_3)-LNA (**b^7**), β-D-(ONH)-LNA (**b^8**) and β-D-($ONCH_3$)-LNA (**b^9**) are preferred. Experiments have been shown that all of these LNA units b^1, b^2, b^4, b^5, b^6, b^7, b^8, and b^9 can be synthesized with the required effort and lead to antisense-oligonucleotides of comparable stability and activity. However based on the expermients the LNA units b^1, b^2, b^4, b^5, b^6, and b^7 are further preferred. Still further preferred are the LNA units b^1, b^2, b^4, b^6, and b^7, and even more preferred are the LNA units b^1 and b^4 and most preferred also in regard to the complexity of the chemical synthesis is the β-D-oxy-LNA (**b^1**).

So far no special 3' terminal group or 5' terminal group could be found which remarkably had changed or increased the stability or activity for oncological or neurological indications, so that 3' and 5' end groups are possible but not explicitly preferred.

Various internucleotide bridges or internucleotide linkages are possible. In the formulae disclosed herein the internucleotide linkage IL is represented by –IL'–Y–. Thus, IL = –IL'–Y– = –X''–P(=X')(X^-)–Y–, wherein IL is preferably selected form the group consisting of:

–O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, –O–P(O)(CH_3)–O–, –O–P(O)(OCH_3)–O–, –O–P(O)($NH(CH_3)$)–O–, –O–P(O)[$N(CH_3)_2$]–O–, –O–P(O)(BH_3^-)–O–, –O–P(O)($OCH_2CH_2OCH_3$)–O–, –O–P(O)($OCH_2CH_2SCH_3$)–O–, –O–P(O)(O^-)–$N(CH_3)$–, –$N(CH_3)$–P(O)(O^-)–O–. Preferred are the internucleotide linkages IL selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, –O–P(O)(OCH_3)–O–, –O–P(O)($NH(CH_3)$)–O–, –O–P(O)[$N(CH_3)_2$]–O–, –O–P(O)($OCH_2CH_2OCH_3$)–O–, and more preferred selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, and still more preferred selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, and most preferably selected from –O–P(O)(O^-)–O– and –O–P(O)(S^-)–O–.

Thus, the present invention is preferably directed to an antisense-oligonucleotide in form of a gapmer consisting of 10 to 28 nucleotides, preferably 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides and 1 to 5 of these nucleotides at the 5' terminal end and 1 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 6, preferably 7 and more preferably 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^3-**ACGCGTCC**-N^4-3' (Seq. ID No. 98), wherein

N^3 represents: GGGATCGTGCTGGCGAT-, GGATCGTGCTGGCGAT-, GATCGTGCTGGCGAT-, ATCGTGCTGGCGAT-, TCGTGCTGGCGAT-, CGTGCTGGCGAT-, GTGCTGGCGAT-, TGCTGGCGAT-, GCTGGCGAT-, CTGGCGAT-, TGGCGAT-, GGCGAT-, GCGAT-, CGAT-, GAT-, AT-, or T-; and

N^4 represents: -ACAGGACGATGTGCAGC, -ACAGGACGATGTGCAG, -ACAGGACGATGTGCA, -ACAGGACGATGTGC, -ACAGGACGATGTG, -ACAGGACGATGT, -ACAGGACGATG, -ACAGGACGAT, -ACAGGACGA, -ACAGGACG, -ACAGGAC, -ACAGGA, -ACAGG, -ACAG, -ACA, -AC, or -A, and

the LNA nucleotides are selected from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-ENA (**b^5**), β-D-(NH)-LNA (**b^6**), β-D-(NCH_3)-LNA (**b^7**), β-D-(ONH)-LNA (**b^8**) and β-D-($ONCH_3$)-LNA (**b^9**); and preferably from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-(NH)-LNA (**b^6**), and β-D-(NCH_3)-LNA (**b^7**); and

the internucleotide linkages are selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, –O–P(O)(CH_3)–O–, –O–P(O)(OCH_3)–O–, –O–P(O)($NH(CH_3)$)–O–, –O–P(O)[$N(CH_3)_2$]–O–, –O–P(O)(BH_3^-)–O–, –O–P(O)($OCH_2CH_2OCH_3$)–O–, –O–P(O)($OCH_2CH_2SCH_3$)–O–, –O–P(O)(O^-)–$N(CH_3)$–, –$N(CH_3)$–P(O)(O^-)–O–;
and preferably from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–;
and salts and optical isomers of the antisense-oligonucleotide. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine and/or 2-aminoadenine.

More preferably N^3 represents: GATCGTGCTGGCGAT-, ATCGTGCTGGCGAT-, TCGTGCTGGCGAT-, CGTGCTGGCGAT-, GTGCTGGCGAT-, TGCTGGCGAT-, GCTGGCGAT-, CTGGCGAT-, TGGCGAT-, GGCGAT-, GCGAT-, CGAT-, GAT-, AT-, or T-; and
N^4 represents: -ACAGGACGATGTGCA, -ACAGGACGATGTGC, -ACAGGACGATGTG, -ACAGGACGATGT, -ACAGGACGATG, -ACAGGACGAT, -ACAGGACGA, -ACAGGACG, -ACAGGAC, -ACAGGA, -ACAGG, -ACAG, -ACA, -AC, or -A.

Still further preferred, the present invention is directed to an antisense-oligonucleotide in form of a gapmer consisting of 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides and 2 to 5 of these nucleotides at the 5' terminal end and 2 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 7, preferably at least 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^3-**ACGCGTCC**-N^4-3' (Seq. ID No. 98), wherein
N^3 represents: CGTGCTGGCGAT-, GTGCTGGCGAT-, TGCTGGCGAT-, GCTGGCGAT-, CTGGCGAT-, TGGCGAT-, GGCGAT-, GCGAT-, CGAT-, GAT-, AT-, or T-; preferably N^3 represents: TGCTGGCGAT-, GCTGGCGAT-, CTGGCGAT-, TGGCGAT-, GGCGAT-, GCGAT-, CGAT-, GAT-, AT-, or T-;
and

N^4 represents: -ACAGGACGATGT, -ACAGGACGATG, -ACAGGACGAT, -ACAGGACGA, -ACAGGACG, -ACAGGAC, -ACAGGA, -ACAGG, -ACAG, -ACA, -AC, or -A; preferably N^4 represents: -ACAGGACGAT, -ACAGGACGA, -ACAGGACG, -ACAGGAC, -ACAGGA, -ACAGG, -ACAG, -ACA, -AC, or -A; and the LNA nucleotides are selected from β-D-oxy-LNA (**b**^{1}), β-D-thio-LNA (**b**^{2}), α-L-oxy-LNA (**b**^{4}), β-D-(NH)-LNA (**b**^{6}), and β-D-(NCH_3)-LNA (**b**^{7}); and the internucleotide linkages are selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–; and preferably selected from phosphate, phosphorothioate and phosphorodithioate; and salts and optical isomers of the antisense-oligonucleotide. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine and/or 2-aminoadenine.

Especially preferred are the gapmer antisense-oligonucleotides of Seq. ID No. 54 to Seq. ID No. 68 and Seq. ID No. 349 to Seq. ID No. 368 containing a segment of 2 to 5, preferably 2 to 4 and more preferably 3 to 4 LNA units at the 3' terminus and a segment of 2 to 5, preferably 2 to 4 and more preferably 3 to 4 LNA units at the 5' terminus and a segment of at least 6, preferably 7 and more preferably 8 DNA units between the two segments of LNA units, wherein the LNA units are selected from β-D-oxy-LNA (**b**^{1}), β-D-thio-LNA (**b**^{2}), α-L-oxy-LNA (**b**^{4}), β-D-(NH)-LNA (**b**^{6}), and β-D-(NCH_3)-LNA (**b**^{7}) and the internucleotide linkages are selected from phosphate, phosphorothioate and phosphorodithioate. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine in the LNA units, preferably all the LNA units and/or 2-aminoadenine in some or all DNA units and/or 5-methylcytosine in some or all DNA units.

Also especially preferred are the gapmer antisense-oligonucleotides of Table 5 (Seq. ID No. 245a to 257b).

Moreover, the present invention is preferably directed to an antisense-oligonucleotide in form of a gapmer consisting of 10 to 28 nucleotides, preferably 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides and 1 to 5 of these nucleotides at the 5' terminal end and 1 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 6, preferably 7 and more preferably 8 DNA

nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^{11}-**TGTTTAGG**-N^{12}-3' (Seq. ID No. 10), wherein

N^{11} represents: GAAGAGCTATTTGGTAG-, AAGAGCTATTTGGTAG-, AGAGCTATTTGGTAG-, GAGCTATTTGGTAG-, AGCTATTTGGTAG-, GCTATTTGGTAG-, CTATTTGGTAG-, TATTTGGTAG-, ATTTGGTAG-, TTTGGTAG-, TTGGTAG-, TGGTAG-, GGTAG-, GTAG-, TAG-, AG- or G-,

N^{12} represents: -GAGCCGTCTTCAGGAAT, -GAGCCGTCTTCAGGAA, -GAGCCGTCTTCAGGA, -GAGCCGTCTTCAGG, -GAGCCGTCTTCAG, -GAGCCGTCTTCA, -GAGCCGTCTTC, -GAGCCGTCTT, -GAGCCGTCT, -GAGCCGTC, -GAGCCGT, -GAGCCG, -GAGCC, -GAGC, -GAG, -GA, or –G;

and salts and optical isomers of the antisense-oligonucleotide.

N^{11} and/or N^{12} may also represent any of the further limited lists of 3' and 5' residues as disclosed herein.

Especially preferred gapmer antisense-oligonucleotides falling under general formula S3:

5'-N^{11}-**TGTTTAGG**-N^{12}-3' (Seq. ID No. 10) **S3**

are the following:

ATTTGGTA**GTGTTTAGGG**	(Seq. ID No. 369)
TTTGGTA**GTGTTTAGGG**A	(Seq. ID No. 370)
TTGGTA**GTGTTTAGGG**AG	(Seq. ID No. 371)
TGGTA**GTGTTTAGGG**AGC	(Seq. ID No. 372)
GGTA**GTGTTTAGGG**AGCC	(Seq. ID No. 373)
GTA**GTGTTTAGGG**AGCCG	(Seq. ID No. 374)
TA**GTGTTTAGGG**AGCCGT	(Seq. ID No. 375)
A**GTGTTTAGGG**AGCCGTC	(Seq. ID No. 376)
GTGTTTAGGGAGCCGTCT	(Seq. ID No. 377)
TTTGGTA**GTGTTTAGGG**	(Seq. ID No. 378)
TTGGTA**GTGTTTAGGG**A	(Seq. ID No. 379)
TGGTA**GTGTTTAGGG**AG	(Seq. ID No. 380)
GGTA**GTGTTTAGGG**AGC	(Seq. ID No. 381)
GTA**GTGTTTAGGG**AGCC	(Seq. ID No. 382)
TA**GTGTTTAGGG**AGCCG	(Seq. ID No. 383)
A**GTGTTTAGGG**AGCCGT	(Seq. ID No. 384)
GTGTTTAGGGAGCCGTC	(Seq. ID No. 385)

TTGGTA**GTGTTTAGGG**	(Seq. ID No. 386)
TGGTA**GTGTTTAGGG**A	(Seq. ID No. 387)
GGTA**GTGTTTAGGG**AG	(Seq. ID No. 388)
GTA**GTGTTTAGGG**AGC	(Seq. ID No. 389)
TA**GTGTTTAGGG**AGCC	(Seq. ID No. 390)
A**GTGTTTAGGG**AGCCG	(Seq. ID No. 391)
GTGTTTAGGGAGCCGT	(Seq. ID No. 392)
TGGTA**GTGTTTAGGG**	(Seq. ID No. 393)
GGTA**GTGTTTAGGG**A	(Seq. ID No. 394)
GTA**GTGTTTAGGG**AG	(Seq. ID No. 395)
TA**GTGTTTAGGG**AGC	(Seq. ID No. 396)
A**GTGTTTAGGG**AGCC	(Seq. ID No. 397)
GTGTTTAGGGAGCCG	(Seq. ID No. 398)
GGTA**GTGTTTAGGG**	(Seq. ID No. 399)
GTA**GTGTTTAGGG**A	(Seq. ID No. 400)
TA**GTGTTTAGGG**AG	(Seq. ID No. 401)
A**GTGTTTAGGG**AGC	(Seq. ID No. 402)
GTGTTTAGGGAGCC	(Seq. ID No. 403)

The antisense-oligonucleotides of formula **S3** in form of gapmers (LNA segment 1 – DNA segment – LNA segment 2) contain an LNA segment at the 5' terminal end consisting of 2 to 5, preferably 2 to 4 LNA units and contain an LNA segment at the 3' terminal end consisting of 2 to 5, preferably 2 to 4 LNA units and between the two LNA segments one DNA segment consisting of 6 to 14, preferably 7 to 12 and more preferably 8 to 11 DNA units.

The antisense-oligonucleotides of formula **S3** contain the LNA nucleotides (LNA units) as disclosed herein, especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably these disclosed in the chapter "Preferred LNAs". The LNA units and the DNA units may comprise standard nucleobases such as adenine (A), cytosine (C), guanine (G), thymine (T) and uracil (U), but may also contain modified nucleobases as disclosed in the chapter "Nucleobases". The antisense-oligonucleotides of formula **S3** or the LNA segments and the DNA segment of the antisense-oligonucleotide may contain any internucleotide linkage as disclosed herein and especially these disclosed in the chapter "Internucleotide Linkages (IL)". The antisense-oligonucleotides of formula **S3** may optionally also contain endgroups at the 3' terminal end and/or the 5' terminal end and especially these disclosed in the chapter "Terminal groups".

Experiments have shown that modified nucleobases do not considerably increase or change the activity of the inventive antisense-oligonucleotides in regard to tested neurological and oncological indications. The modified nucleobases 5-methylcytosine or 2-aminoadenine have been demonstrated to further increase the activity of the antisense-oligonucleotides of formula **S3** especially if 5-methylcytosine is used in the LNA nucleotides only or in the LNA nucleotides and in the DNA nucleotides and/or if 2-aminoadenine is used in the DNA nucleotides and not in the LNA nucleotides.

The preferred gapmer structure of the antisense-oligonucleotides of formula **S3** is as follows: 3-8-3, 4-8-2, 2-8-4, 3-8-4, 4-8-3, 4-8-4, 3-9-3, 4-9-2, 2-9-4, 4-9-3, 3-9-4, 4-9-4, 3-10-3, 2-10-4, 4-10-2, 3-10-4, 4-10-3, 4-10-4, 2-11-4, 4-11-2, 3-11-4, 4-11-3 and still more preferred: 3-8-3, 3-8-4, 4-8-3, 4-8-4, 3-9-3, 4-9-3, 3-9-4, 4-9-4, 3-10-3, 3-10-4, 4-10-3, 4-10-4, 3-11-4, and 4-11-3.

As LNA units for the antisense-oligonucleotides of formula **S3** especially β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-ENA (**b^5**), β-D-(NH)-LNA (**b^6**), β-D-(NCH_3)-LNA (**b^7**), β-D-(ONH)-LNA (**b^8**) and β-D-($ONCH_3$)-LNA (**b^9**) are preferred. Experiments have been shown that all of these LNA units b^1, b^2, b^4, b^5, b^6, b^7, b^8, and b^9 can be synthesized with the required effort and lead to antisense-oligonucleotides of comparable stability and activity. However based on the expermients the LNA units b^1, b^2, b^4, b^5, b^6, and b^7 are further preferred. Still further preferred are the LNA units b^1, b^2, b^4, b^6, and b^7, and even more preferred are the LNA units b^1 and b^4 and most preferred also in regard to the complexity of the chemical synthesis is the β-D-oxy-LNA (**b^1**).

So far no special 3' terminal group or 5' terminal group could be found which remarkably had changed or increased the stability or activity for oncological or neurological indications, so that 3' and 5' end groups are possible but not explicitly preferred.

Various internucleotide bridges or internucleotide linkages are possible. In the formulae disclosed herein the internucleotide linkage IL is represented by –IL'–Y–. Thus, IL = –IL'–Y– = –X''–P(=X')(X^-)–Y–, wherein IL is preferably selected form the group consisting of:
–O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, –O–P(O)(CH_3)–O–, –O–P(O)(OCH_3)–O–, –O–P(O)($NH(CH_3)$)–O–, –O–P(O)[$N(CH_3)_2$]–O–, –O–P(O)(BH_3^-)–O–, –O–P(O)($OCH_2CH_2OCH_3$)–O–,

–O–P(O)($OCH_2CH_2SCH_3$)–O–, –O–P(O)(O^-)–N(CH_3)–, –N(CH_3)–P(O)(O^-)–O–. Preferred are the internucleotide linkages IL selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, –O–P(O)(OCH_3)–O–, –O–P(O)(NH(CH_3))–O–, –O–P(O)[N(CH_3)$_2$]–O–, –O–P(O)($OCH_2CH_2OCH_3$)–O–, and more preferred selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, and still more preferred selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, and most preferably selected from –O–P(O)(O^-)–O– and –O–P(O)(S^-)–O–.

Thus, the present invention is preferably directed to an antisense-oligonucleotide in form of a gapmer consisting of 10 to 28 nucleotides, preferably 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides and 1 to 5 of these nucleotides at the 5' terminal end and 1 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 6, preferably 7 and more preferably 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^{11}-**TGTTTAGG**-N^{12}-3' (Seq. ID No. 10), wherein

N^{11} represents: GAAGAGCTATTTGGTAG-, AAGAGCTATTTGGTAG-, AGAGCTATTTGGTAG-, GAGCTATTTGGTAG-, AGCTATTTGGTAG-, GCTATTTGGTAG-, CTATTTGGTAG-, TATTTGGTAG-, ATTTGGTAG-, TTTGGTAG-, TTGGTAG-, TGGTAG-, GGTAG-, GTAG-, TAG-, AG- or G-,

N^{12} represents: -GAGCCGTCTTCAGGAAT, -GAGCCGTCTTCAGGAA, -GAGCCGTCTTCAGGA, -GAGCCGTCTTCAGG, -GAGCCGTCTTCAG, -GAGCCGTCTTCA, -GAGCCGTCTTC, -GAGCCGTCTT, -GAGCCGTCT, -GAGCCGTC, -GAGCCGT, -GAGCCG, -GAGCC, -GAGC, -GAG, -GA, or –G;

the LNA nucleotides are selected from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-ENA (**b^5**), β-D-(NH)-LNA (**b^6**), β-D-(NCH_3)-LNA (**b^7**), β-D-(ONH)-LNA (**b^8**) and β-D-($ONCH_3$)-LNA (**b^9**); and preferably from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-(NH)-LNA (**b^6**), and β-D-(NCH_3)-LNA (**b^7**); and

the internucleotide linkages are selected from

–O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, –O–P(O)(CH_3)–O–, –O–P(O)(OCH_3)–O–, –O–P(O)(NH(CH_3))–O–,

–O–P(O)[N(CH_3)$_2$]–O–, –O–P(O)(BH_3^-)–O–, –O–P(O)($OCH_2CH_2OCH_3$)–O–, –O–P(O)($OCH_2CH_2SCH_3$)–O–, –O–P(O)(O^-)–N(CH_3)–, –N(CH_3)–P(O)(O^-)–O–;

and preferably from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–;

and salts and optical isomers of the antisense-oligonucleotide. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine and/or 2-aminoadenine.

More preferably N^{11} represents: AGAGCTATTTGGTAG-, GAGCTATTTGGTAG-, AGCTATTTGGTAG-, GCTATTTGGTAG-, CTATTTGGTAG-, TATTTGGTAG-, ATTTGGTAG-, TTTGGTAG-, TTGGTAG-, TGGTAG-, GGTAG-, GTAG-, TAG-, AG- or G-; and

N^{12} represents: -GAGCCGTCTTCAGGA, -GAGCCGTCTTCAGG, -GAGCCGTCTTCAG, -GAGCCGTCTTCA, -GAGCCGTCTTC, -GAGCCGTCTT, -GAGCCGTCT, -GAGCCGTC, -GAGCCGT, -GAGCCG, -GAGCC, -GAGC, -GAG, -GA, or –G.

Still further preferred, the present invention is directed to an antisense-oligonucleotide in form of a gapmer consisting of 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides and 2 to 5 of these nucleotides at the 5' terminal end and 2 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 7, preferably at least 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^{11}-**TGTTTAGG**-N^{12}-3' (Seq. ID No. 10), wherein

N^{11} represents: GCTATTTGGTAG-, CTATTTGGTAG-, TATTTGGTAG-, ATTTGGTAG-, TTTGGTAG-, TTGGTAG-, TGGTAG-, GGTAG-, GTAG-, TAG-, AG- or G-; preferably N^{11} represents: TATTTGGTAG-, ATTTGGTAG-, TTTGGTAG-, TTGGTAG-, TGGTAG-, GGTAG-, GTAG-, TAG-, AG- or G-; and

N^{12} represents: -GAGCCGTCTTCA, -GAGCCGTCTTC, -GAGCCGTCTT, -GAGCCGTCT, -GAGCCGTC, -GAGCCGT, -GAGCCG, -GAGCC, -GAGC, -GAG, -GA, or –G; preferably N^{12} represents: -GAGCCGTCTT, -GAGCCGTCT, -GAGCCGTC, -GAGCCGT, -GAGCCG, -GAGCC, -GAGC, -GAG, -GA, or –G; and

the LNA nucleotides are selected from β-D-oxy-LNA (**b**^{1}), β-D-thio-LNA (**b**^{2}), α-L-oxy-LNA (**b**^{4}), β-D-(NH)-LNA (**b**^{6}), and β-D-(NCH_3)-LNA (**b**^{7}); and
the internucleotide linkages are selected from
–O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–; and preferably selected from phosphate, phosphorothioate and phosphorodithioate;
and salts and optical isomers of the antisense-oligonucleotide. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine and/or 2-aminoadenine.

Especially preferred are the gapmer antisense-oligonucleotides of Seq. ID No. 369 to Seq. ID No. 403 containing a segment of 2 to 5, preferably 2 to 4 and more preferably 3 to 4 LNA units at the 3' terminus and a segment of 2 to 5, preferably 2 to 4 and more preferably 3 to 4 LNA units at the 5' terminus and a segment of at least 6, preferably 7 and more preferably 8 DNA units between the two segments of LNA units, wherein the LNA units are selected from β-D-oxy-LNA (**b**^{1}), β-D-thio-LNA (**b**^{2}), α-L-oxy-LNA (**b**^{4}), β-D-(NH)-LNA (**b**^{6}), and β-D-(NCH_3)-LNA (**b**^{7}) and the internucleotide linkages are selected from phosphate, phosphorothioate and phosphorodithioate. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine in the LNA units, preferably all the LNA units and/or 2-aminoadenine in some or all DNA units and/or 5-methylcytosine in some or all DNA units.

Also especially preferred are the gapmer antisense-oligonucleotides of Table 6 (Seq. ID No. 258a to 270b).

Moreover, the present invention is preferably directed to an antisense-oligonucleotide in form of a gapmer consisting of 10 to 28 nucleotides, preferably 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides and 1 to 5 of these nucleotides at the 5' terminal end and 1 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 6, preferably 7 and more preferably 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^5-**TTTGGTAG**-N^6-3' (Seq. ID No. 11), wherein

N^5 represents: CTGCCCCAGAAGAGCTA-, TGCCCCAGAAGAGCTA-, GCCCCAGAAGAGCTA-, CCCCAGAAGAGCTA-, CCCAGAAGAGCTA-, CCAGAAGAGCTA-, CAGAAGAGCTA-, AGAAGAGCTA-, GAAGAGCTA-, AAGAGCTA-, AGAGCTA-, GAGCTA-, AGCTA-, GCTA-, CTA-, TA-, or A-;

N^6 represents: -TGTTTAGGGAGCCGTCT, -TGTTTAGGGAGCCGTC, -TGTTTAGGGAGCCGT, -TGTTTAGGGAGCCG, -TGTTTAGGGAGCC, -TGTTTAGGGAGC, -TGTTTAGGGAG, -TGTTTAGGGA, -TGTTTAGGG, -TGTTTAGG, -TGTTTAG, -TGTTTA, -TGTTT, -TGTT, -TGT, -TG, or –T;

and salts and optical isomers of the antisense-oligonucleotide.

N^5 and/or N^6 may also represent any of the further limited lists of 3' and 5' residues as disclosed herein.

Especially preferred gapmer antisense-oligonucleotides falling under general formula S4:

5'-N^5-**TTTGGTAG**-N^6-3' (Seq. ID No. 11) **S4**

are the following:

GAAGAGCT**ATTTGGTAGT**	(Seq. ID No. 404)
AAGAGCT**ATTTGGTAGT**G	(Seq. ID No. 405)
AGAGCT**ATTTGGTAGT**GT	(Seq. ID No. 406)
GAGCT**ATTTGGTAGT**GTT	(Seq. ID No. 407)
AGCT**ATTTGGTAGT**GTTT	(Seq. ID No. 408)
GCT**ATTTGGTAGT**GTTTA	(Seq. ID No. 409)
CT**ATTTGGTAGT**GTTTAG	(Seq. ID No. 410)
T**ATTTGGTAGT**GTTTAGG	(Seq. ID No. 411)
ATTTGGTAGTGTTTAGGG	(Seq. ID No. 412)
AAGAGCT**ATTTGGTAGT**	(Seq. ID No. 413)
AGAGCT**ATTTGGTAGT**G	(Seq. ID No. 414)
GAGCT**ATTTGGTAGT**GT	(Seq. ID No. 415)
AGCT**ATTTGGTAGT**GTT	(Seq. ID No. 416)
GCT**ATTTGGTAGT**GTTT	(Seq. ID No. 417)
CT**ATTTGGTAGT**GTTTA	(Seq. ID No. 418)
T**ATTTGGTAGT**GTTTAG	(Seq. ID No. 419)
ATTTGGTAGTGTTTAGG	(Seq. ID No. 420)
AGAGCT**ATTTGGTAGT**	(Seq. ID No. 421)
GAGCT**ATTTGGTAGT**G	(Seq. ID No. 422)
AGCT**ATTTGGTAGT**GT	(Seq. ID No. 423)
GCT**ATTTGGTAGT**GTT	(Seq. ID No. 424)

CT**ATTTGGTAGT**GTTT	(Seq. ID No. 425)
T**ATTTGGTAGT**GTTTA	(Seq. ID No. 426)
ATTTGGTAGTGTTTAG	(Seq. ID No. 427)
GAGCT**ATTTGGTAGT**	(Seq. ID No. 428)
AGCT**ATTTGGTAGT**G	(Seq. ID No. 429)
GCT**ATTTGGTAGT**GT	(Seq. ID No. 430)
CT**ATTTGGTAGT**GTT	(Seq. ID No. 431)
T**ATTTGGTAGT**GTTT	(Seq. ID No. 432)
ATTTGGTAGTGTTTA	(Seq. ID No. 433)
AGCT**ATTTGGTAGT**	(Seq. ID No. 434)
GCT**ATTTGGTAGT**G	(Seq. ID No. 435)
CT**ATTTGGTAGT**GT	(Seq. ID No. 436)
T**ATTTGGTAGT**GTT	(Seq. ID No. 437)
ATTTGGTAGTGTTT	(Seq. ID No. 438)

The antisense-oligonucleotides of formula **S4** in form of gapmers (LNA segment 1 – DNA segment – LNA segment 2) contain an LNA segment at the 5' terminal end consisting of 2 to 5, preferably 2 to 4 LNA units and contain an LNA segment at the 3' terminal end consisting of 2 to 5, preferably 2 to 4 LNA units and between the two LNA segments one DNA segment consisting of 6 to 14, preferably 7 to 12 and more preferably 8 to 11 DNA units.

The antisense-oligonucleotides of formula **S4** contain the LNA nucleotides (LNA units) as disclosed herein, especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably these disclosed in the chapter "Preferred LNAs". The LNA units and the DNA units may comprise standard nucleobases such as adenine (A), cytosine (C), guanine (G), thymine (T) and uracil (U), but may also contain modified nucleobases as disclosed in the chapter "Nucleobases". The antisense-oligonucleotides of formula **S4** or the LNA segments and the DNA segment of the antisense-oligonucleotide may contain any internucleotide linkage as disclosed herein and especially these disclosed in the chapter "Internucleotide Linkages (IL)". The antisense-oligonucleotides of formula **S4** may optionally also contain endgroups at the 3' terminal end and/or the 5' terminal end and especially these disclosed in the chapter "Terminal groups".

Experiments have shown that modified nucleobases do not considerably increase or change the activity of the inventive antisense-oligonucleotides in regard to tested neurological and oncological indications. The modified nucleobases 5-methylcytosine or 2-aminoadenine have been demonstrated to further increase the

activity of the antisense-oligonucleotides of formula **S4** especially if 5-methylcytosine is used in the LNA nucleotides only or in the LNA nucleotides and in the DNA nucleotides and/or if 2-aminoadenine is used in the DNA nucleotides and not in the LNA nucleotides.

The preferred gapmer structure of the antisense-oligonucleotides of formula **S4** is as follows: 3-8-3, 4-8-2, 2-8-4, 3-8-4, 4-8-3, 4-8-4, 3-9-3, 4-9-2, 2-9-4, 4-9-3, 3-9-4, 4-9-4, 3-10-3, 2-10-4, 4-10-2, 3-10-4, 4-10-3, 4-10-4, 2-11-4, 4-11-2, 3-11-4, 4-11-3 and still more preferred: 3-8-3, 3-8-4, 4-8-3, 4-8-4, 3-9-3, 4-9-3, 3-9-4, 4-9-4, 3-10-3, 3-10-4, 4-10-3, 4-10-4, 3-11-4, and 4-11-3.

As LNA units for the antisense-oligonucleotides of formula **S4** especially β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-ENA (**b^5**), β-D-(NH)-LNA (**b^6**), β-D-(NCH_3)-LNA (**b^7**), β-D-(ONH)-LNA (**b^8**) and β-D-($ONCH_3$)-LNA (**b^9**) are preferred. Experiments have been shown that all of these LNA units b^1, b^2, b^4, b^5, b^6, b^7, b^8, and b^9 can be synthesized with the required effort and lead to antisense-oligonucleotides of comparable stability and activity. However based on the expermients the LNA units b^1, b^2, b^4, b^5, b^6, and b^7 are further preferred. Still further preferred are the LNA units b^1, b^2, b^4, b^6, and b^7, and even more preferred are the LNA units b^1 and b^4 and most preferred also in regard to the complexity of the chemical synthesis is the β-D-oxy-LNA (**b^1**).

So far no special 3' terminal group or 5' terminal group could be found which remarkably had changed or increased the stability or activity for oncological or neurological indications, so that 3' and 5' end groups are possible but not explicitly preferred.

Various internucleotide bridges or internucleotide linkages are possible. In the formulae disclosed herein the internucleotide linkage IL is represented by –IL'–Y–. Thus, IL = –IL'–Y– = –X''–P(=X')(X^-)–Y–, wherein IL is preferably selected form the group consisting of:
–O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, –O–P(O)(CH_3)–O–, –O–P(O)(OCH_3)–O–, –O–P(O)($NH(CH_3)$)–O–, –O–P(O)[$N(CH_3)_2$]–O–, –O–P(O)(BH_3^-)–O–, –O–P(O)($OCH_2CH_2OCH_3$)–O–, –O–P(O)($OCH_2CH_2SCH_3$)–O–, –O–P(O)(O^-)–N(CH_3)–, –N(CH_3)–P(O)(O^-)–O–. Preferred are the internucleotide linkages IL selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, –O–P(O)(OCH_3)–O–,

–O–P(O)(NH(CH_3))–O–, –O–P(O)[N(CH_3)$_2$]–O–, –O–P(O)($OCH_2CH_2OCH_3$)–O–, and more preferred selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, and still more preferred selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, and most preferably selected from –O–P(O)(O^-)–O– and –O–P(O)(S^-)–O–.

Thus, the present invention is preferably directed to an antisense-oligonucleotide in form of a gapmer consisting of 10 to 28 nucleotides, preferably 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides and 1 to 5 of these nucleotides at the 5' terminal end and 1 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 6, preferably 7 and more preferably 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^5-**TTTGGTAG**-N^6-3' (Seq. ID No. 11), wherein

N^5 represents: CTGCCCCAGAAGAGCTA-, TGCCCCAGAAGAGCTA-, GCCCCAGAAGAGCTA-, CCCCAGAAGAGCTA-, CCCAGAAGAGCTA-, CCAGAAGAGCTA-, CAGAAGAGCTA-, AGAAGAGCTA-, GAAGAGCTA-, AAGAGCTA-, AGAGCTA-, GAGCTA-, AGCTA-, GCTA-, CTA-, TA-, or A-; and

N^6 represents: -TGTTTAGGGAGCCGTCT, -TGTTTAGGGAGCCGTC, -TGTTTAGGGAGCCGT, -TGTTTAGGGAGCCG, -TGTTTAGGGAGCC, -TGTTTAGGGAGC, -TGTTTAGGGAG, -TGTTTAGGGA, -TGTTTAGGG, -TGTTTAGG, -TGTTTAG, -TGTTTA, -TGTTT, -TGTT, -TGT, -TG, or –T; and

the LNA nucleotides are selected from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-ENA (**b^5**), β-D-(NH)-LNA (**b^6**), β-D-(NCH_3)-LNA (**b^7**), β-D-(ONH)-LNA (**b^8**) and β-D-($ONCH_3$)-LNA (**b^9**); and preferably from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-(NH)-LNA (**b^6**), and β-D-(NCH_3)-LNA (**b^7**); and

the internucleotide linkages are selected from

–O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, –O–P(O)(CH_3)–O–, –O–P(O)(OCH_3)–O–, –O–P(O)(NH(CH_3))–O–, –O–P(O)[N(CH_3)$_2$]–O–, –O–P(O)(BH_3^-)–O–, –O–P(O)($OCH_2CH_2OCH_3$)–O–, –O–P(O)($OCH_2CH_2SCH_3$)–O–, –O–P(O)(O^-)–N(CH_3)–, –N(CH_3)–P(O)(O^-)–O–; and preferably from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–,

–S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–;

and salts and optical isomers of the antisense-oligonucleotide. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine and/or 2-aminoadenine.

More preferably N^5 represents: GCCCCAGAAGAGCTA-, CCCCAGAAGAGCTA-, CCCAGAAGAGCTA-, CCAGAAGAGCTA-, CAGAAGAGCTA-, AGAAGAGCTA-, GAAGAGCTA-, AAGAGCTA-, AGAGCTA-, GAGCTA-, AGCTA-, GCTA-, CTA-, TA-, or A-; and

N^6 represents: -TGTTTAGGGAGCCGT, -TGTTTAGGGAGCCG, -TGTTTAGGGAGCC, -TGTTTAGGGAGC, -TGTTTAGGGAG, -TGTTTAGGGA, -TGTTTAGGG, -TGTTTAGG, -TGTTTAG, -TGTTTA, -TGTTT, -TGTT, -TGT, -TG, or –T.

Still further preferred, the present invention is directed to an antisense-oligonucleotide in form of a gapmer consisting of 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides and 2 to 5 of these nucleotides at the 5' terminal end and 2 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 7, preferably at least 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^5-**TTTGGTAG**-N^6-3' (Seq. ID No. 11)), wherein

N^5 represents: CCAGAAGAGCTA-, CAGAAGAGCTA-, AGAAGAGCTA-, GAAGAGCTA-, AAGAGCTA-, AGAGCTA-, GAGCTA-, AGCTA-, GCTA-, CTA-, TA-, or A-; preferably N^5 represents: AGAAGAGCTA-, GAAGAGCTA-, AAGAGCTA-, AGAGCTA-, GAGCTA-, AGCTA-, GCTA-, CTA-, TA-, or A-; and

N^6 represents: -TGTTTAGGGAGC, -TGTTTAGGGAG, -TGTTTAGGGA, -TGTTTAGGG, -TGTTTAGG, -TGTTTAG, -TGTTTA, -TGTTT, -TGTT, -TGT, -TG, or –T; preferably N^6 represents: -TGTTTAGGGA, -TGTTTAGGG, -TGTTTAGG, -TGTTTAG, -TGTTTA, -TGTTT, -TGTT, -TGT, -TG, or –T; and

the LNA nucleotides are selected from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-(NH)-LNA (**b^6**), and β-D-(NCH_3)-LNA (**b^7**); and

the internucleotide linkages are selected from

–O–P(O)(O$^-$)–O–, –O–P(O)(S$^-$)–O–, –O–P(S)(S$^-$)–O–, –S–P(O)(O$^-$)–O–, –S–P(O)(S$^-$)–O–, –O–P(O)(O$^-$)–S–, –O–P(O)(S$^-$)–S–, –S–P(O)(O$^-$)–S–; and preferably selected from phosphate, phosphorothioate and phosphorodithioate;
and salts and optical isomers of the antisense-oligonucleotide. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine and/or 2-aminoadenine.

Especially preferred are the gapmer antisense-oligonucleotides of Seq. ID No. 404 to Seq. ID No. 438 containing a segment of 2 to 5, preferably 2 to 4 and more preferably 3 to 4 LNA units at the 3' terminus and a segment of 2 to 5, preferably 2 to 4 and more preferably 3 to 4 LNA units at the 5' terminus and a segment of at least 6, preferably 7 and more preferably 8 DNA units between the two segments of LNA units, wherein the LNA units are selected from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-(NH)-LNA (**b^6**), and β-D-(NCH_3)-LNA (**b^7**) and the internucleotide linkages are selected from phosphate, phosphorothioate and phosphorodithioate. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine in the LNA units, preferably all the LNA units and/or 2-aminoadenine in some or all DNA units and/or 5-methylcytosine in some or all DNA units.

Also especially preferred are the gapmer antisense-oligonucleotides of Table 7 (Seq. ID No. 271a to 283b).

Moreover, the present invention is preferably directed to an antisense-oligonucleotide in form of a gapmer consisting of 10 to 28 nucleotides, preferably 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides and 1 to 5 of these nucleotides at the 5' terminal end and 1 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 6, preferably 7 and more preferably 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^7-**AATGGACC**-N^8-3' (Seq. ID No. 100), wherein
N^7 represents: ATCTTGAATATCTCATG-, TCTTGAATATCTCATG-, CTTGAATATCTCATG-, TTGAATATCTCATG-, TGAATATCTCATG-,

GAATATCTCATG-, AATATCTCATG-, ATATCTCATG-, TATCTCATG-, ATCTCATG-, TCTCATG-, CTCATG-, TCATG-, CATG-, ATG-, TG-, or G-;

N^8 represents: -AGTATTCTAGAAACTCA, -AGTATTCTAGAAACTC, -AGTATTCTAGAAACT, -AGTATTCTAGAAAC, -AGTATTCTAGAAA, -AGTATTCTAGAA, -AGTATTCTAGA, -AGTATTCTAG, -AGTATTCTA, -AGTATTCT, -AGTATTC, -AGTATT, -AGTAT, -AGTA, -AGT, -AG, or -A;

and salts and optical isomers of the antisense-oligonucleotide.

N^7 and/or N^8 may also represent any of the further limited lists of 3' and 5' residues as disclosed herein.

Especially preferred gapmer antisense-oligonucleotides falling under general formula S6:

5'-N^7-**AATGGACC**-N^8-3' (Seq. ID No. 100) **S6**

are the following:

TATCTCAT**GAATGGACCA**	(Seq. ID No. 439)
ATCTCAT**GAATGGACCA**G	(Seq. ID No. 440)
TCTCAT**GAATGGACCA**GT	(Seq. ID No. 441)
CTCAT**GAATGGACCA**GTA	(Seq. ID No. 442)
TCAT**GAATGGACCA**GTAT	(Seq. ID No. 443)
CAT**GAATGGACCA**GTATT	(Seq. ID No. 444)
AT**GAATGGACCA**GTATTC	(Seq. ID No. 445)
T**GAATGGACCA**GTATTCT	(Seq. ID No. 446)
GAATGGACCAGTATTCTA	(Seq. ID No. 447)
ATCTCAT**GAATGGACCA**	(Seq. ID No. 448)
TCTCAT**GAATGGACCA**G	(Seq. ID No. 449)
CTCAT**GAATGGACCA**GT	(Seq. ID No. 450)
TCAT**GAATGGACCA**GTA	(Seq. ID No. 451)
CAT**GAATGGACCA**GTAT	(Seq. ID No. 452)
AT**GAATGGACCA**GTATT	(Seq. ID No. 453)
T**GAATGGACCA**GTATTC	(Seq. ID No. 454)
GAATGGACCAGTATTCT	(Seq. ID No. 455)
TCTCAT**GAATGGACCA**	(Seq. ID No. 456)
CTCAT**GAATGGACCA**G	(Seq. ID No. 457)
TCAT**GAATGGACCA**GT	(Seq. ID No. 458)
CAT**GAATGGACCA**GTA	(Seq. ID No. 459)
AT**GAATGGACCA**GTAT	(Seq. ID No. 460)
T**GAATGGACCA**GTATT	(Seq. ID No. 461)
GAATGGACCAGTATTC	(Seq. ID No. 462)
CTCAT**GAATGGACCA**	(Seq. ID No. 463)

TCAT**GAATGGACCA**G	(Seq. ID No. 464)
CAT**GAATGGACCA**GT	(Seq. ID No. 465)
AT**GAATGGACCA**GTA	(Seq. ID No. 466)
T**GAATGGACCA**GTAT	(Seq. ID No. 467)
GAATGGACCAGTATT	(Seq. ID No. 468)
TCAT**GAATGGACCA**	(Seq. ID No. 469)
CAT**GAATGGACCA**G	(Seq. ID No. 470)
AT**GAATGGACCA**GT	(Seq. ID No. 471)
T**GAATGGACCA**GTA	(Seq. ID No. 472)
GAATGGACCAGTAT	(Seq. ID No. 473)

The antisense-oligonucleotides of formula **S6** in form of gapmers (LNA segment 1 – DNA segment – LNA segment 2) contain an LNA segment at the 5' terminal end consisting of 2 to 5, preferably 2 to 4 LNA units and contain an LNA segment at the 3' terminal end consisting of 2 to 5, preferably 2 to 4 LNA units and between the two LNA segments one DNA segment consisting of 6 to 14, preferably 7 to 12 and more preferably 8 to 11 DNA units.

The antisense-oligonucleotides of formula **S6** contain the LNA nucleotides (LNA units) as disclosed herein, especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably these disclosed in the chapter "Preferred LNAs". The LNA units and the DNA units may comprise standard nucleobases such as adenine (A), cytosine (C), guanine (G), thymine (T) and uracil (U), but may also contain modified nucleobases as disclosed in the chapter "Nucleobases". The antisense-oligonucleotides of formula **S6** or the LNA segments and the DNA segment of the antisense-oligonucleotide may contain any internucleotide linkage as disclosed herein and especially these disclosed in the chapter "Internucleotide Linkages (IL)". The antisense-oligonucleotides of formula **S6** may optionally also contain endgroups at the 3' terminal end and/or the 5' terminal end and especially these disclosed in the chapter "Terminal groups".

Experiments have shown that modified nucleobases do not considerably increase or change the activity of the inventive antisense-oligonucleotides in regard to tested neurological and oncological indications. The modified nucleobases 5-methylcytosine or 2-aminoadenine have been demonstrated to further increase the activity of the antisense-oligonucleotides of formula **S6** especially if 5-methylcytosine is used in the LNA nucleotides only or in the LNA nucleotides and in the DNA nucleotides and/or if 2-aminoadenine is used in the DNA nucleotides and not in the LNA nucleotides.

The preferred gapmer structure of the antisense-oligonucleotides of formula **S6** is as follows: 3-8-3, 4-8-2, 2-8-4, 3-8-4, 4-8-3, 4-8-4, 3-9-3, 4-9-2, 2-9-4, 4-9-3, 3-9-4, 4-9-4, 3-10-3, 2-10-4, 4-10-2, 3-10-4, 4-10-3, 4-10-4, 2-11-4, 4-11-2, 3-11-4, 4-11-3 and still more preferred: 3-8-3, 3-8-4, 4-8-3, 4-8-4, 3-9-3, 4-9-3, 3-9-4, 4-9-4, 3-10-3, 3-10-4, 4-10-3, 4-10-4, 3-11-4, and 4-11-3.

As LNA units for the antisense-oligonucleotides of formula **S6** especially β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-ENA (**b^5**), β-D-(NH)-LNA (**b^6**), β-D-(NCH_3)-LNA (**b^7**), β-D-(ONH)-LNA (**b^8**) and β-D-($ONCH_3$)-LNA (**b^9**) are preferred. Experiments have been shown that all of these LNA units b^1, b^2, b^4, b^5, b^6, b^7, b^8, and b^9 can be synthesized with the required effort and lead to antisense-oligonucleotides of comparable stability and activity. However based on the expermients the LNA units b^1, b^2, b^4, b^5, b^6, and b^7 are further preferred. Still further preferred are the LNA units b^1, b^2, b^4, b^6, and b^7, and even more preferred are the LNA units b^1 and b^4 and most preferred also in regard to the complexity of the chemical synthesis is the β-D-oxy-LNA (**b^1**).

So far no special 3' terminal group or 5' terminal group could be found which remarkably had changed or increased the stability or activity for oncological or neurological indications, so that 3' and 5' end groups are possible but not explicitly preferred.

Various internucleotide bridges or internucleotide linkages are possible. In the formulae disclosed herein the internucleotide linkage IL is represented by –IL'–Y–. Thus, IL = –IL'–Y– = –X''–P(=X')(X^-)–Y–, wherein IL is preferably selected form the group consisting of:
–O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, –O–P(O)(CH_3)–O–, –O–P(O)(OCH_3)–O–, –O–P(O)($NH(CH_3)$)–O–, –O–P(O)[$N(CH_3)_2$]–O–, –O–P(O)(BH_3^-)–O–, –O–P(O)($OCH_2CH_2OCH_3$)–O–, –O–P(O)($OCH_2CH_2SCH_3$)–O–, –O–P(O)(O^-)–$N(CH_3)$–, –$N(CH_3)$–P(O)(O^-)–O–. Preferred are the internucleotide linkages IL selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, –O–P(O)(OCH_3)–O–, –O–P(O)($NH(CH_3)$)–O–, –O–P(O)[$N(CH_3)_2$]–O–, –O–P(O)($OCH_2CH_2OCH_3$)–O–, and more preferred selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, and still more preferred selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, and most preferably selected from –O–P(O)(O^-)–O– and –O–P(O)(S^-)–O–.

Thus, the present invention is preferably directed to an antisense-oligonucleotide in form of a gapmer consisting of 10 to 28 nucleotides, preferably 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides and 1 to 5 of these nucleotides at the 5' terminal end and 1 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 6, preferably 7 and more preferably 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^7-**AATGGACC**-N^8-3' (Seq. ID No. 100), wherein

N^7 represents: ATCTTGAATATCTCATG-, TCTTGAATATCTCATG-, CTTGAATATCTCATG-, TTGAATATCTCATG-, TGAATATCTCATG-, GAATATCTCATG-, AATATCTCATG-, ATATCTCATG-, TATCTCATG-, ATCTCATG-, TCTCATG-, CTCATG-, TCATG-, CATG-, ATG-, TG-, or G-; and

N^8 represents: -AGTATTCTAGAAACTCA, -AGTATTCTAGAAACTC, -AGTATTCTAGAAACT, -AGTATTCTAGAAAC, -AGTATTCTAGAAA, -AGTATTCTAGAA, -AGTATTCTAGA, -AGTATTCTAG, -AGTATTCTA, -AGTATTCT, -AGTATTC, -AGTATT, -AGTAT, -AGTA, -AGT, -AG, or -A; and

the LNA nucleotides are selected from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-ENA (**b^5**), β-D-(NH)-LNA (**b^6**), β-D-(NCH_3)-LNA (**b^7**), β-D-(ONH)-LNA (**b^8**) and β-D-($ONCH_3$)-LNA (**b^9**); and preferably from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-(NH)-LNA (**b^6**), and β-D-(NCH_3)-LNA (**b^7**); and

the internucleotide linkages are selected from

–O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, –O–P(O)(CH_3)–O–, –O–P(O)(OCH_3)–O–, –O–P(O)($NH(CH_3)$)–O–, –O–P(O)[$N(CH_3)_2$]–O–, –O–P(O)(BH_3^-)–O–, –O–P(O)($OCH_2CH_2OCH_3$)–O–, –O–P(O)($OCH_2CH_2SCH_3$)–O–, –O–P(O)(O^-)–$N(CH_3)$–, –$N(CH_3)$–P(O)(O^-)–O–;

and preferably from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–;

and salts and optical isomers of the antisense-oligonucleotide. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine and/or 2-aminoadenine.

More preferably N^7 represents: CTTGAATATCTCATG-, TTGAATATCTCATG-, TGAATATCTCATG-, GAATATCTCATG-, AATATCTCATG-, ATATCTCATG-, TATCTCATG-, ATCTCATG-, TCTCATG-, CTCATG-, TCATG-, CATG-, ATG-, TG-, or G-; and

N^8 represents: -AGTATTCTAGAAACT, -AGTATTCTAGAAAC, -AGTATTCTAGAAA, -AGTATTCTAGAA, -AGTATTCTAGA, -AGTATTCTAG, -AGTATTCTA, -AGTATTCT, -AGTATTC, -AGTATT, -AGTAT, -AGTA, -AGT, -AG, or -A.

Still further preferred, the present invention is directed to an antisense-oligonucleotide in form of a gapmer consisting of 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides and 2 to 5 of these nucleotides at the 5' terminal end and 2 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 7, preferably at least 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^7-**AATGGACC**-N^8-3' (Seq. ID No. 100), wherein

N^7 represents: GAATATCTCATG-, AATATCTCATG-, ATATCTCATG-, TATCTCATG-, ATCTCATG-, TCTCATG-, CTCATG-, TCATG-, CATG-, ATG-, TG-, or G-; preferably N^7 represents: ATATCTCATG-, TATCTCATG-, ATCTCATG-, TCTCATG-, CTCATG-, TCATG-, CATG-, ATG-, TG-, or G-; and

N^8 represents: -AGTATTCTAGAA, -AGTATTCTAGA, -AGTATTCTAG, -AGTATTCTA, -AGTATTCT, -AGTATTC, -AGTATT, -AGTAT, -AGTA, -AGT, -AG, or -A; preferably N^8 represents: -AGTATTCTAG, -AGTATTCTA, -AGTATTCT, -AGTATTC, -AGTATT, -AGTAT, -AGTA, -AGT, -AG, or -A; and

the LNA nucleotides are selected from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-(NH)-LNA (**b^6**), and β-D-(NCH_3)-LNA (**b^7**); and

the internucleotide linkages are selected from

–O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–; and preferably selected from phosphate, phosphorothioate and phosphorodithioate;

and salts and optical isomers of the antisense-oligonucleotide. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine and/or 2-aminoadenine.

Especially preferred are the gapmer antisense-oligonucleotides of Seq. ID No. 439 to Seq. ID No. 473 containing a segment of 2 to 5, preferably 2 to 4 and more preferably 3 to 4 LNA units at the 3' terminus and a segment of 2 to 5, preferably 2 to 4 and more preferably 3 to 4 LNA units at the 5' terminus and a segment of at least 6, preferably 7 and more preferably 8 DNA units between the two segments of LNA units, wherein the LNA units are selected from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-(NH)-LNA (**b^6**), and β-D-(NCH_3)-LNA (**b^7**) and the internucleotide linkages are selected from phosphate, phosphorothioate and phosphorodithioate. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine in the LNA units, preferably all the LNA units and/or 2-aminoadenine in some or all DNA units and/or 5-methylcytosine in some or all DNA units.

Also especially preferred are the gapmer antisense-oligonucleotides of Table 8 (Seq. ID No. 219a to 231b).

Moreover, the present invention is preferably directed to an antisense-oligonucleotide in form of a gapmer consisting of 10 to 28 nucleotides, preferably 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides and 1 to 5 of these nucleotides at the 5' terminal end and 1 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 6, preferably 7 and more preferably 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^9-**ATTAATAA**-N^{10}-3' (Seq. ID No. 101), wherein

N^9 represents: CATATTTATATACAGGC-, ATATTTATATACAGGC-, TATTTATATACAGGC-, ATTTATATACAGGC-, TTTATATACAGGC-, TTATATACAGGC-, TATATACAGGC-, ATATACAGGC-, TATACAGGC-, ATACAGGC-, TACAGGC-, ACAGGC-, CAGGC-, AGGC-, GGC-, GC-, or C-;

N^{10} represents: -AGTGCAAATGTTATTGG, -AGTGCAAATGTTATTG, -AGTGCAAATGTTATT, -AGTGCAAATGTTAT, -AGTGCAAATGTTA, -AGTGCAAATGTT, -AGTGCAAATGT, -AGTGCAAATG, -AGTGCAAAT, -AGTGCAAA, -AGTGCAA, -AGTGCA, -AGTGC, -AGTG, -AGT, -AG, or -A;

and salts and optical isomers of the antisense-oligonucleotide.

N^9 and/or N^{10} may also represent any of the further limited lists of 3' and 5' residues as disclosed herein.

Especially preferred gapmer antisense-oligonucleotides falling under general formula S7:

5'-N^9-**ATTAATAA**-N^{10}-3' (Seq. ID No. 101) **S7**

are the following:

TATACAGG**CATTAATAAA**	(Seq. ID No. 474)
ATACAGG**CATTAATAAA**G	(Seq. ID No. 475)
TACAGG**CATTAATAAA**GT	(Seq. ID No. 476)
ACAGG**CATTAATAAA**GTG	(Seq. ID No. 477)
CAGG**CATTAATAAA**GTGC	(Seq. ID No. 478)
AGG**CATTAATAAA**GTGCA	(Seq. ID No. 479)
GG**CATTAATAAA**GTGCAA	(Seq. ID No. 480)
G**CATTAATAAA**GTGCAAA	(Seq. ID No. 481)
CATTAATAAAGTGCAAAT	(Seq. ID No. 482)
ATACAGG**CATTAATAA**	(Seq. ID No. 483)
TACAGG**CATTAATAAA**G	(Seq. ID No. 484)
ACAGG**CATTAATAAA**GT	(Seq. ID No. 485)
CAGG**CATTAATAAA**GTG	(Seq. ID No. 486)
AGG**CATTAATAAA**GTGC	(Seq. ID No. 487)
GG**CATTAATAAA**GTGCA	(Seq. ID No. 488)
G**CATTAATAAA**GTGCAA	(Seq. ID No. 489)
CATTAATAAAGTGCAAA	(Seq. ID No. 490)
TACAGG**CATTAATAA**	(Seq. ID No. 491)
ACAGG**CATTAATAAA**G	(Seq. ID No. 492)
CAGG**CATTAATAAA**GT	(Seq. ID No. 493)
AGG**CATTAATAAA**GTG	(Seq. ID No. 494)
GG**CATTAATAAA**GTGC	(Seq. ID No. 495)
G**CATTAATAAA**GTGCA	(Seq. ID No. 496)
CATTAATAAAGTGCAA	(Seq. ID No. 497)
ACAGG**CATTAATAA**	(Seq. ID No. 498)
CAGG**CATTAATAAA**G	(Seq. ID No. 499)
AGG**CATTAATAAA**GT	(Seq. ID No. 500)
GG**CATTAATAAA**GTG	(Seq. ID No. 501)
G**CATTAATAAA**GTGC	(Seq. ID No. 502)
CATTAATAAAGTGCA	(Seq. ID No. 503)
CAGG**CATTAATAA**	(Seq. ID No. 504)

AGG**CATTAATAAA**G	(Seq. ID No. 505)
GG**CATTAATAAA**GT	(Seq. ID No. 506)
G**CATTAATAAA**GTG	(Seq. ID No. 507)
CATTAATAAAGTGC	(Seq. ID No. 508)

The antisense-oligonucleotides of formula **S7** in form of gapmers (LNA segment 1 – DNA segment – LNA segment 2) contain an LNA segment at the 5' terminal end consisting of 2 to 5, preferably 2 to 4 LNA units and contain an LNA segment at the 3' terminal end consisting of 2 to 5, preferably 2 to 4 LNA units and between the two LNA segments one DNA segment consisting of 6 to 14, preferably 7 to 12 and more preferably 8 to 11 DNA units.

The antisense-oligonucleotides of formula **S7** contain the LNA nucleotides (LNA units) as disclosed herein, especially these disclosed in the chapter "Locked Nucleic Acids (LNA®)" and preferably these disclosed in the chapter "Preferred LNAs". The LNA units and the DNA units may comprise standard nucleobases such as adenine (A), cytosine (C), guanine (G), thymine (T) and uracil (U), but may also contain modified nucleobases as disclosed in the chapter "Nucleobases". The antisense-oligonucleotides of formula **S7** or the LNA segments and the DNA segment of the antisense-oligonucleotide may contain any internucleotide linkage as disclosed herein and especially these disclosed in the chapter "Internucleotide Linkages (IL)". The antisense-oligonucleotides of formula **S7** may optionally also contain endgroups at the 3' terminal end and/or the 5' terminal end and especially these disclosed in the chapter "Terminal groups".

Experiments have shown that modified nucleobases do not considerably increase or change the activity of the inventive antisense-oligonucleotides in regard to tested neurological and oncological indications. The modified nucleobases 5-methylcytosine or 2-aminoadenine have been demonstrated to further increase the activity of the antisense-oligonucleotides of formula **S7** especially if 5-methylcytosine is used in the LNA nucleotides only or in the LNA nucleotides and in the DNA nucleotides and/or if 2-aminoadenine is used in the DNA nucleotides and not in the LNA nucleotides.

The preferred gapmer structure of the antisense-oligonucleotides of formula **S7** is as follows: 3-8-3, 4-8-2, 2-8-4, 3-8-4, 4-8-3, 4-8-4, 3-9-3, 4-9-2, 2-9-4, 4-9-3, 3-9-4, 4-9-4, 3-10-3, 2-10-4, 4-10-2, 3-10-4, 4-10-3, 4-10-4, 2-11-4, 4-11-2, 3-11-4, 4-11-3 and still more preferred: 3-8-3, 3-8-4, 4-8-3, 4-8-4, 3-9-3, 4-9-3, 3-9-4, 4-9-4, 3-10-3, 3-10-4, 4-10-3, 4-10-4, 3-11-4, and 4-11-3.

As LNA units for the antisense-oligonucleotides of formula **S7** especially β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-ENA (**b^5**), β-D-(NH)-LNA (**b^6**), β-D-(NCH_3)-LNA (**b^7**), β-D-(ONH)-LNA (**b^8**) and β-D-($ONCH_3$)-LNA (**b^9**) are preferred. Experiments have been shown that all of these LNA units b^1, b^2, b^4, b^5, b^6, b^7, b^8, and b^9 can be synthesized with the required effort and lead to antisense-oligonucleotides of comparable stability and activity. However based on the expermients the LNA units b^1, b^2, b^4, b^5, b^6, and b^7 are further preferred. Still further preferred are the LNA units b^1, b^2, b^4, b^6, and b^7, and even more preferred are the LNA units b^1 and b^4 and most preferred also in regard to the complexity of the chemical synthesis is the β-D-oxy-LNA (**b^1**).

So far no special 3' terminal group or 5' terminal group could be found which remarkably had changed or increased the stability or activity for oncological or neurological indications, so that 3' and 5' end groups are possible but not explicitly preferred.

Various internucleotide bridges or internucleotide linkages are possible. In the formulae disclosed herein the internucleotide linkage IL is represented by –IL'–Y–. Thus, IL = –IL'–Y– = –X''–P(=X')(X^-)–Y–, wherein IL is preferably selected form the group consisting of:
–O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, –O–P(O)(CH_3)–O–, –O–P(O)(OCH_3)–O–, –O–P(O)($NH(CH_3)$)–O–, –O–P(O)[$N(CH_3)_2$]–O–, –O–P(O)(BH_3^-)–O–, –O–P(O)($OCH_2CH_2OCH_3$)–O–, –O–P(O)($OCH_2CH_2SCH_3$)–O–, –O–P(O)(O^-)–$N(CH_3)$–, –$N(CH_3)$–P(O)(O^-)–O–.
Preferred are the internucleotide linkages IL selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, –O–P(O)(OCH_3)–O–, –O–P(O)($NH(CH_3)$)–O–, –O–P(O)[$N(CH_3)_2$]–O–, –O–P(O)($OCH_2CH_2OCH_3$)–O–, and more preferred selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, and still more preferred selected from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, and most preferably selected from –O–P(O)(O^-)–O– and –O–P(O)(S^-)–O–.

Thus, the present invention is preferably directed to an antisense-oligonucleotide in form of a gapmer consisting of 10 to 28 nucleotides, preferably 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides

and 1 to 5 of these nucleotides at the 5' terminal end and 1 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 6, preferably 7 and more preferably 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^9-**ATTAATAA**-N^{10}-3' (Seq. ID No. 101), wherein

N^9 represents: CATATTTATATACAGGC-, ATATTTATATACAGGC-, TATTTATATACAGGC-, ATTTATATACAGGC-, TTTATATACAGGC-, TTATATACAGGC-, TATATACAGGC-, ATATACAGGC-, TATACAGGC-, ATACAGGC-, TACAGGC-, ACAGGC-, CAGGC-, AGGC-, GGC-, GC-, or C-;

N^{10} represents: -AGTGCAAATGTTATTGG, -AGTGCAAATGTTATTG, -AGTGCAAATGTTATT, -AGTGCAAATGTTAT, -AGTGCAAATGTTA, -AGTGCAAATGTT, -AGTGCAAATGT, -AGTGCAAATG, -AGTGCAAAT, -AGTGCAAA, -AGTGCAA, -AGTGCA, -AGTGC, -AGTG, -AGT, -AG, or -A; and

the LNA nucleotides are selected from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-ENA (**b^5**), β-D-(NH)-LNA (**b^6**), β-D-(NCH_3)-LNA (**b^7**), β-D-(ONH)-LNA (**b^8**) and β-D-($ONCH_3$)-LNA (**b^9**); and preferably from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-(NH)-LNA (**b^6**), and β-D-(NCH_3)-LNA (**b^7**); and

the internucleotide linkages are selected from

–O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–, –O–P(O)(CH_3)–O–, –O–P(O)(OCH_3)–O–, –O–P(O)($NH(CH_3)$)–O–, –O–P(O)[$N(CH_3)_2$]–O–, –O–P(O)(BH_3^-)–O–, –O–P(O)($OCH_2CH_2OCH_3$)–O–, –O–P(O)($OCH_2CH_2SCH_3$)–O–, –O–P(O)(O^-)–N(CH_3)–, –N(CH_3)–P(O)(O^-)–O–;

and preferably from –O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–;

and salts and optical isomers of the antisense-oligonucleotide. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine and/or 2-aminoadenine.

More preferably N^9 represents: TATTTATATACAGGC-, ATTTATATACAGGC-, TTTATATACAGGC-, TTATATACAGGC-, TATATACAGGC-, ATATACAGGC-, TATACAGGC-, ATACAGGC-, TACAGGC-, ACAGGC-, CAGGC-, AGGC-, GGC-, GC-, or C-; and

N^{10} represents: -AGTGCAAATGTTATT, -AGTGCAAATGTTAT, -AGTGCAAATGTTA, -AGTGCAAATGTT, -AGTGCAAATGT, -AGTGCAAATG, -AGTGCAAAT, -AGTGCAAA, -AGTGCAA, -AGTGCA, -AGTGC, -AGTG, -AGT, -AG, or -A.

Still further preferred, the present invention is directed to an antisense-oligonucleotide in form of a gapmer consisting of 11 to 24 nucleotides, more preferably 12 to 20, and still more preferably 13 to 19 or 14 to 18 nucleotides and 2 to 5 of these nucleotides at the 5' terminal end and 2 to 5 nucleotides at the 3' terminal end of the antisense-oligonucleotide are LNA nucleotides and between the LNA nucleotides at the 5' terminal end and the 3' terminal end a sequence of at least 7, preferably at least 8 DNA nucleotides is present, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the antisense-oligonucleotide is represented by the following sequence 5'-N^9-**ATTAATAA**-N^{10}-3' (Seq. ID No. 101), wherein

N^9 represents: TTATATACAGGC-, TATATACAGGC-, ATATACAGGC-, TATACAGGC-, ATACAGGC-, TACAGGC-, ACAGGC-, CAGGC-, AGGC-, GGC-, GC-, or C-; preferably N^9 represents: ATATACAGGC-, TATACAGGC-, ATACAGGC-, TACAGGC-, ACAGGC-, CAGGC-, AGGC-, GGC-, GC-, or C-; and

N^{10} represents: -AGTGCAAATGTT, -AGTGCAAATGT, -AGTGCAAATG, -AGTGCAAAT, -AGTGCAAA, -AGTGCAA, -AGTGCA, -AGTGC, -AGTG, -AGT, -AG, or -A; preferably N^{10} represents: -AGTGCAAATG, -AGTGCAAAT, -AGTGCAAA, -AGTGCAA, -AGTGCA, -AGTGC, -AGTG, -AGT, -AG, or -A; and

the LNA nucleotides are selected from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-(NH)-LNA (**b^6**), and β-D-(NCH_3)-LNA (**b^7**); and

the internucleotide linkages are selected from

–O–P(O)(O^-)–O–, –O–P(O)(S^-)–O–, –O–P(S)(S^-)–O–, –S–P(O)(O^-)–O–, –S–P(O)(S^-)–O–, –O–P(O)(O^-)–S–, –O–P(O)(S^-)–S–, –S–P(O)(O^-)–S–; and

preferably selected from phosphate, phosphorothioate and phosphorodithioate;

and salts and optical isomers of the antisense-oligonucleotide. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine and/or 2-aminoadenine.

Especially preferred are the gapmer antisense-oligonucleotides of Seq. ID No. 474 to Seq. ID No. 508 containing a segment of 2 to 5, preferably 2 to 4 and more preferably 3 to 4 LNA units at the 3' terminus and a segment of 2 to 5, preferably 2 to 4 and more preferably 3 to 4 LNA units at the 5' terminus and a segment of at least 6,

preferably 7 and more preferably 8 DNA units between the two segments of LNA units, wherein the LNA units are selected from β-D-oxy-LNA (**b^1**), β-D-thio-LNA (**b^2**), α-L-oxy-LNA (**b^4**), β-D-(NH)-LNA (**b^6**), and β-D-(NCH_3)-LNA (**b^7**) and the internucleotide linkages are selected from phosphate, phosphorothioate and phosphorodithioate. Such preferred antisense-oligonucleotides may not contain any modified 3' and 5' terminal end or may not contain any 3' and 5' terminal group and may as modified nucleobase contain 5-methylcytosine in the LNA units, preferably all the LNA units and/or 2-aminoadenine in some or all DNA units and/or 5-methylcytosine in some or all DNA units.

Also especially preferred are the gapmer antisense-oligonucleotides of Table 9 (Seq. ID No. 284a to 236b).

Table 4

SP	L	Seq ID No.	Sequence, 5'-3'
357	10	232a	**C*b^{1}sGb^{1}s**dTsdC*sdAsdTsdAsdGs**Ab^{1}sC*b^1**
357	10	232b	**C*b^{1}Gb^1**dTdC*dAdTdAdG**Ab^{1}C*b^1**
356	12	233a	**Tb^{1}sC*b^{1}sGb^{1}s**dTsdC*sdAsdTsdAsdGs**Ab^{1}sC*b^{1}sC*b^1**
356	12	233b	**Tb^{1}C*b^{1}Gb^1**dTdC*dAdTdAdG**Ab^{1}C*b^{1}C*b^1**
356	12	233c	**Tb^{1}sC*b^{1}sGb^{1}s**dTsdC*sdAsdTsdAsdGsdAs**C*b^{1}sC*b^1**
356	12	233d	**Tb^{1}s**dC*sdGsdTsdC*sdAsdTsdAsdGsdAs**C*b^{1}sC*b^1**
356	12	233e	**Tb^{1}sC*b^{1}s**dGsdTsdCsdAsdTsdAsdGsdAsdC*s**C*b^1**
355	13	234a	**Tb^{1}sC*b^{1}sGb^{1}sTb^{1}s**dCsdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^1**
355	13	234b	**Tb^{1}C*b^{1}Gb^{1}Tb^1**dCdAdUdAdGdA**C*b^{1}C*b^{1}Gb^1**
355	13	234c	**Tb^{1}sC*b^{1}sGb^{1}sTb^{1}s**dC*sdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^1**
355	13	234d	**Tb^{1}sC*b^{1}sGb^{1}s**dTsdC*sdA*sdTsdA*sdGsdA*sdC*s**C*b^{1}sGb^1**
355	13	234e	**Tb^{1}sC*b^{1}s**dGsdTsdC*sdAsdTsdA*sdGsdA*sdC*sdCs**Gb^1**
355	13	234f	**Tb^{1}s**dCsdGsdTsdC*sdA*sdTsdAsdGs**Ab^{1}sC*b^{1}sC*b^{1}sGb^1**
354	13	142c	**C*b^{1}sGb^{1}sTb^1**sdCsdAsdTsdAsdGsdAsdCsdCs**Gb^{1}sAb^1**
355	14	143i	**C*b^{1}sTb^{1}sC*b^{1}sGb^{1}s**dTsdCsdAsdTsdAsdGs**Ab^{1}sC*b^{1}sC*b^{1}sGb^1**
355	14	143j	**C*b^{4}ssTb^{4}ssC*b^{4}ss**dGssdTssdCssdAssdTssdAssdGssdA*ss**C*b^{4}ssC*b^{4}ss Gb^4**
355	14	143h	**C*b^{1}sTb^{1}s**dCsdGsdTsdCsdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^1**
355	14	143k	**C*b^{2}ssTb^{2}ssC*b^{2}ss**dGssdTssdCssdAssdTssdAssdGssdAss**C*b^{2}ssC*b^{2}ssG b^2**
355	14	143m	**C*b^{1}Tb^{1}C*b^{1}Gb^1**dUsdCsdAsdTsdAsdGs**Ab^{1}C*b^{1}C*b^{1}Gb^1**
355	14	143n	**C*b^{1}sTb^{1}sC*b^{1}sGb^{1}sTb^{1}s**dCsdA*sdTsdA*sdGsdA*s**C*b^{1}sC*b^{1}sGb^1**
355	14	143o	**C*b^{1}sTb^{1}s**dCsdGsdUsdCsdAsdUsdAs**Gb^{1}sAb^{1}sC*b^{1}sC*b^{1}sGb^1**

355	14	143p	**C*b^{6}sTb^{6}sC*b^{6}sGb^{6}s**dTsdCsdAsdTsdAsdGsdAs**C*b^{6}sC*b^{6}sGb^6**
355	14	143q	**C*b^{7}sTb^{7}sC*b^{7}s**dGsdUsdCsdA*sdUsdA*sdGsdA*s**C*b^{7}sC*b^{7}sGb^7**
355	14	143r	**C*b^{4}sTb^{4}sC*b^{4}sGb^{4}s**dTsdC*sdA*sdTsdAsdGsdAsdC*s**C*b^{4}sGb^4**
355	14	143s	**C*b^{4}Tb^{4}C*b^{4}Gb^4**dTdCdAdTdAdGdAdC**C*b^{4}Gb^4**
355	14	143t	**C*b^{1}ssTb^{1}ssC*b^{1}ss**dGssdTssdC*ssdAssdTssdAssdGssdAss**C*b^{1}ssC*b^{1}ss Gb^1**
355	14	143u	**C*b^{1}Tb^{1}s**dCsdGsdUsdC*sdAsdUsdAsdGsdAs**C*b^{1}C*b^{1}Gb^1**
355	14	143v	**C*b^{1}Tb^{1}s**dC*sdGsdTsdC*sdA*sdTsdAsdGsdAs**C*b^{1}C*b^{1}Gb^1**
355	14	143w	**C*b^{6}sTb^{6}s**dC*dGdTdC*dAdTdAdGdAs**C*b^{6}sC*b^{6}sGb^6**
355	14	143x	**C*b^{7}sTb^{7}sC*b^{7}sGb^{7}s**dTsdC*sdAsdTsdAsdGsdAs**C*b^{7}sC*b^{7}sGb^7**
355	14	143y	**C*b^{7}sTb^{7}s**dC*sdGsdTsdCsdAsdUsdAsdGs**Ab^{7}sC*b^{7}sC*b^{7}sGb^7**
355	14	143z	**C*b^{1}sTb^{1}s**dC*sdGsdTsdC*sdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^1**
355	14	143aa	**C*b^{1}Tb^{1}s**dC*sdGsdTsdC*sdAsdTsdAsdGsdAs**C*b^{1}C*b^{1}Gb^1**
355	14	143ab	**C*b^{1}sTb^{1}s**dC*sdGsdTsdC*sdA*sdTsdAsdGsdA*s**C*b^{1}sC*b^{1}sGb^1**
355	14	143ac	**C*b^{1}sTb^{1}s**dC*sdGsdTsdCsdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^1**
355	14	143ad	**C*b^{1}Tb^1**dC*dGdTdCdAdTdAdGdA**C*b^{1}C*b^{1}Gb^1**
355	14	143ae	**C*b^{1}sTb^{1}s**dC*dGdTdC*dAdTdAdGdAs**C*b^{1}sC*b^{1}sGb^1**
355	14	143af	**/5SpC3s/C*b^{1}sTb^{1}s**dC*dGdTdC*dA*dTdAdGdA*s**C*b^{1}sC*b^{1}sGb^1**
355	14	143ag	**C*b^{1}sTb^{1}s**dC*dGdTdC*dA*dTdAdGdA*s**C*b^{1}sC*b^{1}sGb^1/3SpC3s/**
355	14	143ah	**/5SpC3s/C*b^{1}sTb^{1}s**dC*dGdTdC*dA*dTdAdGdA*s**C*b^{1}sC*b^{1}sGb^1/3SpC3s/**
355	14	143ai	**C*b^{1}sTb^{1}s**dC*sdGsdUsdC*sdA*sdUsdA*sdGsdA*s**C*b^{1}sC*b^{1}sGb^1**
355	14	143aj	**C*b^{1}sTb^{1}sC*b^1**sdGsdTsdCsdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^1**
356	14	145c	**Gb^{1}sC*b^{1}sTb^1**sdCsdGsdTsdCsdAsdTsdAsdGs**Ab^{1}sC*b^{1}sC*b^1**
354	15	235i	**C*b^{1}sTb^{1}sC*b^{1}sGb^{1}s**dTdC*dAdTdAdGdAs**C*b^{1}sC*b^{1}sGb^{1}sAb^1**
354	15	235a	**C*b^{1}ssTb^{1}ss**dCssdGssdTssdCssdAssdTssdAssdGssdAssdCssdCssdGss**Ab^1**
354	15	235b	**C*b^{1}Tb^1**dCdGdTdCdAdTdAdGdAdCdCdG**Ab^1**
354	15	235c	**C*b^{1}sTb^{1}s**dCsdGsdTsdCsdA*sdUsdAsdGsdAsdCs**C*b^{1}sGb^{1}sAb^1**
354	15	235d	**C*b^{1}Tb^{1}s**dCsdGsdTsdCsdAsdTsdAsdGsdAs**C*b^{1}C*b^{1}Gb^{1}Ab^1**
354	15	235e	**C*b^{4}sTb^{4}sC*b^{4}s**dGsdTsdCsdAsdTsdAsdGsdAsdCsdCs**Gb^{4}sAb^4**
354	15	235f	**C*b^{6}sTb^{6}sC*b^{6}s**dGdTdCdA*dTdAdGdAdC*s**C*b^{6}sGb^{6}sAb^6**
354	15	235g	**C*b^{1}sTb^{1}sC*b^{1}sGb^{1}s**dTsdC*sdAsdTsdAsdGsdAsdC*sdC*sdGs**Ab^1**
354	15	235h	**C*b^{1}ssTb^{1}ss**dCssdGssdUssdCssdAssdUssdAssdGssdAssdCssdCss**Gb^1 ssAb^1**
355	15	144c	**Gb^{1}sC*b^{1}sTb^1**sdCsdGsdTsdCsdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^1**
354	16	141c	**Gb^{1}sC*b^{1}sTb^{1}sC*b^{1}s**dGsdTsdC*sdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^{1}sAb^1**
354	16	141d	**Gb^{1}C*b^{1}Tb^{1}C*b^{1}s**dGsdTsdC*sdAsdTsdAsdGsdAsdCs**C*b^{1}Gb^{1}Ab^1**
354	16	141e	**Gb^{4}sC*b^{4}sTb^{4}sC*b^{4}s**dGsdTsdC*sdAsdTsdAsdGsdA*sdC*sdC*s**Gb^{4}sAb^4**

354	16	141f	**Gb^{1}s**dC*sdTsdCsdGsdTsdC*sdA*sdTsdAsdGsdA*sdC*sdC*sdGs**Ab^1**
354	16	141g	**Gb^{2}sC*b^{2}sTb^{2}s**dCsdGsdUsdCsdAsdTsdA*sdGsdAsdCs**C*b^{2}sGb^{2}sAb^2**
354	16	141h	**Gb^{4}ssC*b^{4}ssTb^{4}ss**dCssdGssdTssdCssdAssdTssdAssdGssdAss**C*b^{4}ssC*b^{4}ssGb^{4}ssAb^4**
354	16	141i	**Gb^{1}C*b^1**dTdCdGdTdCdA*dTdA*dGdA*dC**C*b^{1}Gb^{1}Ab^1**
354	16	141j	**Gb^{1}sC*b^{1}sTb^1**sdCsdGsdTsdCsdAsdTsdAsdGsdAsdCs**C*b^{1}sGb^{1}sAb^1**
351	16	139c	**C*b^{1}sGb^{1}sTb^1**sdCsdAsdTsdAsdGsdAsdCsdCsdGsdAs**Gb^{1}sC*b^{1}sC*b^1**
354	17	237a	**Tb^{1}sGb^{1}sC*b^{1}sTb^{1}sC*b^{1}s**dGsdTsdC*sdAsdTsdAsdGs**Ab^{1}sC*b^{1}sC*b^{1}sGb^{1}sAb^1**
354	17	237b	**Tb^{2}sGb^{2}sC*b^{2}s**dTsdGsdTsdC*sdAsdTsdAsdGs**Ab^{2}sC*b^{2}sC*b^{2}sGb^{2}sAb^2**
354	17	237c	**Tb^{1}sGb^{1}sC*b^{1}sTb^{1}s**dC*sdGsdTsdCsdAsdTsdAsdGsdAsdC*s**C*b^{1}sGb^{1}sAb^1**
354	17	237d	**Tb^{1}s**dGsdCsdUsdC*sdGsdTsdC*sdAsdUsdAsdGs**Ab^{1}sC*b^{1}sC*b^{1}sGb^{1}sAb^1**
354	17	237e	**Tb^{1}sGb^{1}sC*b^{1}s**dTsdGsdTsdC*sdA*sdTsdA*sdGs**Ab^{1}sC*b^{1}sC*b^{1}sGb^{1}sAb^1**
354	17	237f	**Tb^{1}Gb^1**dC*dTdGdTdC*dAdTdAdGdA**C*b^{1}C*b^{1}Gb^{1}Ab^1**
354	17	237g	**Tb^{1}s**dGsdC*sdTsdGsdTsdC*sdAsdTsdAsdGsdAsdC*s**C*b^{1}sGb^{1}sAb^1**
354	17	237h	**Tb^{1}Gb^{1}C*b^{1}Tb^{1}C*b^1**dGdTdC*dA*dTdA*dGdA*dC*dC***Gb^{1}Ab^1**
354	17	237i	**Tb^{1}ssGb^{1}ssC*b^{1}ssTb^{1}ssC*b^{1}ss**dGssdTssdCssdAssdTssdAssdGssdAssdCss**C*b^{1}ssGb^{1}ssAb^1**
354	17	237j	**Tb^{4}sGb^{4}sC*b^{4}s**dTdGdTdCdA*dTdA*dGdA*s**C*b^{4}sC*b^{4}sGb^{4}sAb^4**
354	17	237k	**Tb^{6}sGb^{6}sC*b^{6}s**dUsdGsdUsdC*sdA*sdUsdA*sdGsdA*sdC*s**C*b^{6}sGb^{6}sAb^6**
354	17	237m	**Tb^{7}sGb^{7}sC*b^{7}sTb^{7}s**dC*dGdTdC*dAdTdAdGdAs**C*b^{7}sC*b^{7}sGb^{7}sAb^7**
353	18	238a	**Tb^{1}sGb^{1}sC*b^{1}sTb^{1}sC*b^{1}s**dGsdTsdC*sdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^{1}sAb^{1}sGb^1**
353	18	238b	**Tb^{7}sGb^{7}sC*b^{7}sTb^{7}sC*b^{7}s**dGsdTsdC*sdAsdTsdAsdGsdAsdC*sdC*sdGsdAs**Gb^7**
353	18	238c	**Tb^{1}sGb^{1}sC*b^{1}sTb^{1}s**dC*sdGsdTsdCsdAsdTsdAsdGsdAsdC*s**C*b^{1}sGb^{1}sAb^{1}sGb^1**
353	18	238d	**Tb^{1}sGb^{1}s**dC*sdTsdC*sdGsdTsdCsdAsdTsdAsdGsdAsdC*sdC*s**Gb^{1}sAb^{1}sGb^1**
353	18	238e	**Tb^{1}sGb^{1}sC*b^{1}sTb^{1}s**dC*sdGsdTsdCsdAsdTsdAsdGsdAsdC*sdC*s**Gb^{1}sAb^{1}sGb^1**
353	18	238f	**Tb^{1}Gb^1**dC*dUdC*dGdTdC*dAdTdAdGdA***C*b^{1}C*b^{1}Gb^{1}Ab^{1}Gb^1**
353	18	238g	**Tb^{4}Gb^{4}C*b^{4}Tb^{4}s**dCsdGsdTsdCsdAsdTsdAsdGsdAs**C*b^{4}C*b^{4}Gb^{4}Ab^{4}Gb^4**
353	18	238h	**Tb^{1}ssGb^{1}ssC*b^{1}ss**dTssdC*ssdGssdTssdC*ssdAssdTssdA*ssdGssdAssdC*ssdC*ss**Gb^{1}ssAb^{1}ssGb^1**
353	18	238i	**Tb^{2}Gb^{2}C*b^2**dTdCdGdTdC*dAdTdAdGdA**C*b^{2}C*b^{2}Gb^{2}Ab^{2}Gb^2**
352	19	239a	**Tb^{1}sGb^{1}sC*b^{1}sTb^{1}sC*b^{1}s**dGsdTsdCsdAsdTsdAsdGsdAsdC*s**C*b^{1}sGb^{1}sAb^{1}sGb^{1}sC*b^1**

352	19	239b	**Tb^{6}Gb^{6}C*b^{6}Tb^{6}C*b^6**dGdTdC*dAdTdAdGdAdC***C*b^{6}Gb^{6}Ab^{6}Gb^{6}C*b^6**
352	19	239c	**Tb^{1}sGb^{1}sC*b^{1}sTb^{1}s**dC*sdGsdTsdCsdAsdTsdAsdGsdAsdCsdCsdGs**Ab^1 sGb^{1}sC*b^1**
352	19	239d	**Tb^{1}s**dGsdCsdTsdCsdGsdTsdCsdAsdTsdAsdGsdA*sdC*s**C*b^{1}sGb^{1}sAb^1 sGb^{1}sC*b^1**
352	19	239e	**Tb^{4}sGb^{4}s**dCsdUsdCsdGsdUsdCsdAsdTsdAsdGsdA*sdC*sdC*s**Gb^{4}sAb^4 sGb^{4}sC*b^4**
352	19	239f	**Tb^{2}ssGb^{2}ssC*b^{2}ssTb^{2}ssC*b^{2}ss**dGssdTssdCssdAssdTssdAssdGssdAssdC ssdCssdGssdAss**Gb^{2}ssC*b^2**
352	20	240a	**C*b^{1}sTb^{1}sGb^{1}sC*b^{1}sTb^{1}s**dC*sdGsdTsdCsdAsdTsdAsdGsdAsdC*s**C*b^1 sGb^{1}sAb^{1}sGb^{1}sC*b^1**
352	20	240b	**C*b^{2}sTb^{2}sGb^{2}s**dC*sdTsdC*sdGsdTsdCsdAsdTsdAsdGsdAsdC*s**C*b^{2}sGb^2 sAb^{2}sGb^{2}sC*b^2**
352	20	240c	**C*b^{1}Tb^{1}Gb^1**dC*dTdC*dGdTdCdAdTdAdGdAdC*dC***Gb^{1}Ab^{1}Gb^{1}C*b^1**
352	20	240d	**C*b^{1}s**dUsdGsdCsdUsdC*sdGsdTsdCsdAsdTsdAsdGsdAsdC*s**C*b^{1}sGb^1 sAb^{1}sGb^{1}sC*b^1**
352	20	240e	**C*b^{4}sTb^{4}sGb^{4}sC*b^{4}s**dTsdCsdGsdTsdCsdAsdTsdAsdGsdAsdCsdCs**Gb^4 sAb^{4}sGb^{4}sC*b^4**
351	22	241a	**Gb^{1}sC*b^{1}sTb^{1}sGb^{1}sC*b^{1}s**dTsdC*sdGsdTsdCsdAsdTsdAsdGsdAsdCsdC* s**Gb^{1}sAb^{1}sGb^{1}sC*b^{1}sC*b^1**
351	22	241b	**Gb^{1}C*b^{1}Tb^{1}Gb^{1}C*b^1**dTdC*dGdTdC*dAdTdAdGdAdC*dC***Gb^{1}Ab^{1}Gb^{1}C*b^1 C*b^1**
351	22	241c	**Gb^{1}sC*b^{1}sTb^{1}sGb^{1}sC*b^{1}s**dTsdCsdGsdTsdCsdAsdTsdAsdGsdAsdCsdCs**Gb^1 sAb^{1}sGb^{1}sC*b^{1}sC*b^1**
350	24	242a	**C*b^{1}sGb^{1}sC*b^{1}sTb^{1}sGb^{1}s**dCsdTsdCsdGsdTsdCsdAsdTsdAsdGsdAsdCsdC* sdGs**Ab^{1}sGb^{1}sC*b^{1}sC*b^{1}sC*b^1**
350	24	242b	**C*b^{1}Gb^{1}C*b^{1}Tb^{1}Gb^1**dC*dTdCdGdTdCdAdTdAdGdAdCdC*dG**Ab^{1}Gb^{1}C*b^1 C*b^{1}C*b^1**
349	26	243a	**C*b^{1}sC*b^{1}sGb^{1}sC*b^{1}sTb^{1}s**dGsdC*sdTsdCsdGsdTsdC*sdAsdTsdAsdGsdAs dCsdC*sdGsdAs**Gb^{1}sC*b^{1}sC*b^{1}sC*b^{1}sC*b^1**
349	26	243b	**C*b^{1}C*b^{1}Gb^{1}C*b^{1}Tb^1**dGdC*dTdCdGdTdC*dAdTdAdGdAdCdC*dGdA**Gb^{1}C*b^1 C*b^{1}C*b^{1}C*b^1**
348	28	244a	**C*b^{1}sC*b^{1}sC*b^{1}sGb^{1}sC*b^{1}s**dTsdGsdCsdTsdCsdGsdTsdC*sdAsdTsdAsdGs dAsdC*sdCsdGsdAsdGs**C*b^{1}sC*b^{1}sC*b^{1}sC*b^{1}sC*b^1**
348	28	244b	**C*b^{1}C*b^{1}C*b^{1}Gb^{1}C*b^1**dTdGdC*dTdCdGdTdC*dAdTdAdGdAdC*dCdGdAdG **C*b^{1}C*b^{1}C*b^{1}C*b^{1}C*b^1**

Table 5

SP	L	Seq ID No.	Sequence, 5'-3'
431	10	245a	**Tb^{1}sAb^{1}s**dC*sdGsdCsdGsdTsdC*s**C*b^{1}sAb^1**
431	10	245b	**Tb^{1}Ab^1**dCdGdC*dGdTdC**C*b^{1}Ab^1**
430	12	246a	**Ab^{1}sTb^{1}sAb^{1}s**dC*sdGsdCsdGsdTsdCs**C*b^{1}sAb^{1}sC*b^1**
430	12	246b	**Ab^{1}Tb^{1}Ab^1**dCdGdCdGdTdC***C*b^{1}Ab^{1}C*b^1**
430	12	246c	**Ab^{1}sTb^{1}sAb^{1}s**dCsdGsdCsdGsdTsdC*sdC*s**Ab^{1}sC*b^1**
430	12	246d	**Ab^{1}sTb^{1}s**dA*sdC*sdGsdCsdGsdTsdC*sdC*sdA*s**C*b^1**

430	12	246e	**Ab^{1}s**dTsdA*sdC*sdGsdC*sdGsdTsdC*sdC*s**Ab^{1}sC*b^1**
430	13	247a	**Gb^{1}sAb^{1}sTb^{1}sAb^{1}s**dCsdGsdCsdGsdTsdCs**C*b^{1}sAb^{1}sC*b^1**
430	13	247b	**Gb^{1}Ab^{1}Tb^{1}Ab^1**dCdGdCdGdUdC**C*b^{1}Ab^{1}C*b^1**
430	13	247c	**Gb^{1}sAb^{1}sTb^{1}sAb^{1}s**dC*sdGsdCsdGsdTsdC*s**C*b^{1}sAb^{1}sC*b^1**
430	13	247d	**Gb^{1}sAb^{1}sTb^{1}s**dA*sdCsdGsdCsdGsdTsdCsdC*s**Ab^{1}sC*b^1**
430	13	247e	**Gb^{1}sAb^{1}s**dTsdA*sdCsdGsdC*sdGsdTsdCsdC*sdA*s**C*b^1**
430	13	247f	**Gb^{1}s**dA*sdTsdA*sdC*sdGsdCsdGsdTs**C*b^{1}sC*b^{1}sAb^{1}sC*b^1**
431	13	153f	**C*b^{1}sGb^{1}sAb^1**sdTsdAsdCsdGsdCsdGsdTsdCs**C*b^{1}sAb^1**
429	14	248a	**Gb^{1}sAb^{1}sTb^{1}sAb^{1}s**dC*sdGsdC*sdGsdTsdC*s**C*b^{1}sAb^{1}sC*b^{1}sAb^1**
429	14	248b	**Gb^{1}Ab^{1}Tb^{1}Ab^{1}s**dC*sdGsdCsdGsdTsdC*sdC*s**Ab^{1}C*b^{1}Ab^1**
429	14	248c	**Gb^{4}sAb^{4}sTb^{4}sAb^{4}s**dC*sdGsdCsdGsdTsdC*sdC*sdA*s**C*b^{4}sAb^4**
429	14	248d	**Gb^{1}s**dA*sdTsdAsdCsdGsdCsdGsdTsdCsdC*sdA*sdCs**Ab^1**
429	14	248e	**Gb^{2}sAb^{2}sTb^{2}s**dA*sdCsdGsdCsdGsdUsdCsdCs**Ab^{2}sC*b^{2}sAb^2**
429	14	248f	**Gb^{4}ssAb^{4}ss**dTssdAssdCssdGssdCssdGssdTssdCssdCss**Ab^{4}ssC*b^{4}ssAb^4**
429	14	248g	**Gb^{1}Ab^1**dTdA*dCdGdCdGdTdC**C*b^{1}Ab^{1}C*b^{1}Ab^1**
429	15	152h	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdCsdGsdCsdGsdTsdCsdCs**Ab^{1}sC*b^{1}sAb^1**
429	15	152i	**C*b^{1}Gb^{1}Ab^{1}Tb^{1}s**dAsdCsdGsdCsdGsdUsdCsdC*s**Ab^{1}C*b^{1}Ab^1**
429	15	152j	**C*b^{1}Gb^{1}Ab^{1}Tb^{1}s**dA*sdCsdGsdCsdGsdUsdCsdCs**Ab^{1}C*b^{1}Ab^1**
429	15	152k	**C*b^{6}sGb^{6}sAb^{6}sTb^{6}s**dAdC*dGdCdGdTdCdC*s**Ab^{6}sC*b^{6}sAb^6**
429	15	152m	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdCsdGsdCsdGsdTsdC*sdC*s**Ab^{1}sC*b^{1}sAb^1**
429	15	152n	**C*b^{1}Gb^{1}Ab^{1}Tb^{1}s**dAsdC*sdGsdC*sdGsdTsdC*sdC*s**Ab^{1}C*b^{1}Ab^1**
429	15	152o	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dA*sdCsdGsdCsdGsdTsdCsdC*s**Ab^{1}sC*b^{1}sAb^1**
429	15	152p	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdCsdGsdCsdGsdTsdCsdC*s**Ab^{1}sC*b^{1}sAb^1**
429	15	152q	**C*b^{1}Gb^{1}Ab^{1}Tb^1**dAdCdGdC*dGdTdCdC***Ab^{1}C*b^{1}Ab^1**
429	15	152r	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAdC*dGdC*dGdTdC*dC*s**Ab^{1}sC*b^{1}sAb^1**
429	15	152s	**/5SpC3s/C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdC*sdGsdC*sdGsdTsdCsdCs**Ab^{1}sC*b^1 sAb^1**
429	15	152t	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdC*sdGsdCsdGsdTsdCsdC*s**Ab^{1}sC*b^{1}sAb^1 /3SpC3s/**
429	15	152u	**/5SpC3s/C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdC*sdGsdC*sdGsdTsdCsdCs**Ab^{1}sC*b^1 sAb^1/3SpC3s/**
429	15	152v	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dA*sdC*sdGsdC*sdGsdUsdC*sdC*s**Ab^{1}sC*b^{1}sAb^1**
429	15	152w	**C*b^{7}sGb^{7}sAb^{7}s**dTsdAsdCsdGsdC*sdGsdTsdCs**C*b^{7}sAb^{7}sC*b^{7}sAb^7**
429	15	152z	**C*b^{7}sGb^{7}s**dAsdUsdAsdCsdGsdC*sdGsdUsdCs**C*b^{7}sAb^{7}sC*b^{7}sAb^7**
429	15	152aa	**C*b^{1}ssGb^{1}ssAb^{1}ss**dTssdAssdC*ssdGssdCssdGssdTssdCssdC*ss**Ab^1 ssC*b^{1}ssAb^1**
429	15	152ab	**C*b^{4}ssGb^{4}ssAb^{4}ss**dTssdA*ssdCssdGssdCssdGssdTssdCssdCssdA*ss **C*b^{4}ssAb^4**
429	15	152ac	**C*b^{2}ssGb^{2}ssAb^{2}ssTb^{2}ss**dAssdCssdGssdCssdGssdTssdCssdCssdAssdCss

			Ab^2
429	15	152ad	**C*b^{1}Gb^{1}Ab^{1}Tb^1**dAdCdGdCdGdUdC**C*b^{1}Ab^{1}C*b^{1}Ab^1**
429	15	152ae	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}sAb^{1}s**dCsdGsdCsdGsdUsdCsdCs**Ab^{1}sC*b^{1}sAb^1**
429	15	152af	**C*b^{1}sGb^{1}s**dA*sdTsdA*sdCsdGsdCsdGsdTs**C*b^{1}sC*b^{1}sAb^{1}sC*b^{1}sAb^1**
429	15	152ag	**C*b^{6}sGb^{6}sAb^{6}s**dTsdAsdCsdGsdCsdGsdTsdCs**C*b^{6}sAb^{6}sC*b^{6}sAb^6**
429	15	152ah	**C*b^{7}sGb^{7}sAb^{7}s**dUsdA*sdCsdGsdCsdGsdUsdCsdCs**Ab^{7}sC*b^{7}sAb^7**
429	15	152ai	**C*b^{4}sGb^{4}sAb^{4}sTb^{4}s**dA*sdCsdGsdCsdGsdTsdC*sdC*sdA*s**C*b^{4}sAb^4**
429	15	152aj	**C*b^{4}Gb^{4}Ab^{4}Tb^4**dAdCdGdCdGdTdCdCdA**C*b^{4}Ab^4**
429	15	152ak	**C*b^{1}sGb^{1}sAb^1**sdTsdAsdCsdGsdCsdGsdTsdCsdCs**Ab^{1}sC*b^{1}sAb^1**
428	16	249a	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAdCdGdCdGdTdCdC*s**Ab^{1}sC*b^{1}sAb^{1}sGb^1**
428	16	249b	**C*b^{1}ssGb^{1}ss**dAssdTssdAssdCssdGssdCssdGssdTssdCssdCssdAssdCssdAss**Gb^1**
428	16	249c	**C*b^{1}Gb^1**dAdTdAdCdGdCdGdTdCdCdAdCdA**Gb^1**
428	16	249d	**C*b^{1}sGb^{1}s**dAsdUsdAsdC*sdGsdCsdGsdUsdCsdC*sdAs**C*b^{1}sAb^{1}sGb^1**
428	16	249e	**C*b^{1}Gb^{1}s**dAsdTsdAsdC*sdGsdC*sdGsdTsdCsdC*s**Ab^{1}C*b^{1}Ab^{1}Gb^1**
428	16	249f	**C*b^{4}sGb^{4}sAb^{4}s**dTsdAsdCsdGsdCsdGsdTsdCsdCsdAsdCs**Ab^{4}sGb^4**
428	16	249g	**C*b^{6}Gb^{6}Ab^6**dTdA*dCdGdCdGdTdC*dCdA***C*b^{6}Ab^{6}Gb^6**
428	16	249h	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdC*sdGsdCsdGsdTsdCsdC*sdAsdC*sdAs**Gb^1**
428	16	249i	**C*b^{1}ssGb^{1}ss**dAssdUssdAssdCssdGssdCssdGssdUssdCssdCssdAssdCss**Ab^{1}ssGb^1**
428	17	250a	**Gb^{1}sC*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdCsdGsdC*sdGsdTsdCs**C*b^{1}sAb^{1}sC*b^{1}sAb^{1}sGb^1**
428	17	250b	**Gb^{1}sC*b^{1}sGb^{1}sAb^{1}s**dTsdAsdC*sdGsdC*sdGsdTsdC*sdC*sdAs**C*b^{1}sAb^{1}sGb^1**
428	17	250c	**Gb^{1}s**dC*sdGsdAsdUsdAsdCsdGsdC*sdGsdUsdCs**C*b^{1}sAb^{1}sC*b^{1}sAb^{1}sGb^1**
428	17	250d	**Gb^{1}sC*b^{1}sGb^{1}s**dA*sdTsdA*sdC*sdGsdC*sdGsdTsdC*s**C*b^{1}sAb^{1}sC*b^{1}sAb^{1}sGb^1**
428	17	250e	**Gb^{1}C*b^1**dGdAdTdAdCdGdC*dGdTdCdC***Ab^{1}C*b^{1}Ab^{1}Gb^1**
428	17	250f	**Gb^{1}s**dC*sdGsdAsdTsdAsdCsdGsdC*sdGsdTsdCsdCsdAs**C*b^{1}sAb^{1}sGb^1**
428	17	250g	**Gb^{2}sC*b^{2}sGb^{2}s**dAsdTsdAsdCsdGsdC*sdGsdTsdC*s**C*b^{2}sAb^{2}sC*b^{2}sAb^{2}sGb^2**
428	17	250h	**Gb^{1}C*b^{1}Gb^{1}Ab^{1}Tb^1**dA*dCdGdC*dGdTdC*dCdA*dC***Ab^{1}Gb^1**
428	17	250i	**Gb^{1}ssC*b^{1}ssGb^{1}ssAb^{1}ssTb^{1}ss**dAssdCssdGssdCssdGssdTssdCssdCssdAss**C*b^{1}ssAb^{1}ssGb^1**
428	17	250j	**Gb^{4}sC*b^{4}sGb^{4}s**dA*sdTsdA*sdCsdGsdCsdGsdTsdCsdCs**Ab^{4}sC*b^{4}sAb^{4}sGb^4**
428	17	250k	**Gb^{6}sC*b^{6}sGb^{6}s**dA*sdUsdAsdCsdGsdCsdGsdUsdC*sdCsdA*s**C*b^{6}sAb^{6}sGb^6**
428	17	250m	**Gb^{7}sC*b^{7}sGb^{7}sAb^{7}s**dTdAdCdGdCdGdTdC*dCs**Ab^{7}sC*b^{7}sAb^{7}sGb^7**
427	18	251a	**Gb^{1}sC*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdCsdGsdCsdGsdTsdCsdC*s**Ab^{1}sC*b^{1}sAb^{1}sGb^{1}sGb^1**

427	18	251b	**Gb^{7}sC*b^{7}sGb^{7}sAb^{7}sTb^{7}s**dAsdC*sdGsdCsdGsdTsdCsdCsdAsdCsdAsdGs **Gb^{7}**
427	18	251c	**Gb^{1}sC*b^{1}sGb^{1}sAb^{1}s**dTsdAsdC*sdGsdCsdGsdTsdCsdC*sdAs**C*b^{1}sAb^{1}sGb^{1} sGb^{1}**
427	18	251d	**Gb^{1}sC*b^{1}sGb^{1}s**dAsdTsdAsdC*sdGsdC*sdGsdTsdCsdC*sdAs**C*b^{1}sAb^{1}sGb^{1} sGb^{1}**
427	18	251e	**Gb^{1}sC*b^{1}sGb^{1}sAb^{1}s**dTsdAsdC*sdGsdCsdGsdTsdCsdC*sdAsdC*s**Ab^{1}sGb^{1} sGb^{1}**
427	18	251f	**Gb^{1}C*b^{1}**dGdAdUdA*dCdGdCdGdTdC*dC***Ab^{1}C*b^{1}Ab^{1}Gb^{1}Gb^{1}**
427	18	251g	**Gb^{4}C*b^{4}Gb^{4}Ab^{4}**sdTsdAsdCsdGsdCsdGsdTsdCsdCs**Ab^{4}C*b^{4}Ab^{4}Gb^{4}Gb^{4}**
427	18	251h	**Gb^{1}ssC*b^{1}ssGb^{1}ss**dA*ssdTssdA*ssdCssdGssdCssdGssdTssdCssdCssdA* ssdC*ss**Ab^{1}ssGb^{1}ssGb^{1}**
427	18	251i	**Gb^{2}C*b^{2}Gb^{2}**dAdTdAdCdGdC*dGdTdCdC***Ab^{2}C*b^{2}Ab^{2}Gb^{2}Gb^{2}**
426	19	252a	**Gb^{1}sC*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdC*sdGsdCsdGsdTsdCsdCsdAs**C*b^{1}sAb^{1}sGb^{1} sGb^{1}sAb^{1}**
426	19	252b	**Gb^{6}C*b^{6}Gb^{6}Ab^{6}Tb^{6}**dAdC*dGdCdGdTdCdC*dA**C*b^{6}Ab^{6}Gb^{6}Gb^{6}Ab^{6}**
426	19	252c	**Gb^{1}sC*b^{1}sGb^{1}s**dAsdTsdAsdCsdGsdCsdGsdTsdCsdCsdAsdC*s**Ab^{1}sGb^{1} sGb^{1}sAb^{1}**
426	19	252d	**Gb^{1}s**dC*sdGsdA*sdTsdA*sdC*sdGsdCsdGsdTsdCsdCsdA*s**C*b^{1}sAb^{1}sGb^{1} sGb^{1}sAb^{1}**
426	19	252e	**Gb^{4}sC*b^{4}s**dGsdAsdUsdAsdCsdGsdCsdGsdUsdCsdCsdAsdC*s**Ab^{4}sGb^{4}sGb^{4} sAb^{4}**
426	19	252f	**Gb^{2}ssC*b^{2}ssGb^{2}ssAb^{2}ssTb^{2}ss**dAssdCssdGssdCssdGssdTssdCssdCssdAss dCssdAssdGss**Gb^{2}ssAb^{2}**
426	20	253a	**Gb^{1}sGb^{1}sC*b^{1}sGb^{1}sAb^{1}s**dTsdAsdCsdGsdCsdGsdTsdC*sdC*sdAs**C*b^{1}sAb^{1} sGb^{1}sGb^{1}sAb^{1}**
426	20	253b	**Gb^{2}sGb^{2}sC*b^{2}s**dGsdAsdTsdAsdC*sdGsdCsdGsdTsdC*sdC*sdAs**C*b^{2}sAb^{2} sGb^{2}sGb^{2}sAb^{2}**
426	20	253c	**Gb^{1}Gb^{1}C*b^{1}**dGdAdTdAdCdGdCdGdTdCdCdAdC***Ab^{1}Gb^{1}Gb^{1}Ab^{1}**
426	20	253d	**Gb^{1}s**dGsdCsdGsdAsdTsdAsdCsdGsdC*sdGsdUsdCsdCsdAs**C*b^{1}sAb^{1}sGb^{1} sGb^{1}sAb^{1}**
426	20	253e	**Gb^{4}sGb^{4}sC*b^{4}sGb^{4}s**dAsdTsdAsdCsdGsdCsdGsdTsdCsdCsdAsdCs**Ab^{4}sGb^{4} sGb^{4}sAb^{4}**
425	22	254a	**Tb^{1}sGb^{1}sGb^{1}sC*Gb^{1}s**dAsdTsdAsdCsdGsdCsdGsdTsdCsdCsdAsdC*s**Ab^{1} sGb^{1}sGb^{1}sAb^{1}sC*b^{1}**
425	22	254b	**Tb^{1}Gb^{1}Gb^{1}C*b^{1}Gb^{1}**dAdTdAdC*dGdCdGdTdCdC*dAdC**Ab^{1}Gb^{1}Gb^{1}Ab^{1}C*b^{1}**
425	22	254c	**Tb^{6}sGb^{6}sGb^{6}sC*b^{6}s**dGsdAsdTsdAsdCsdGsdCsdGsdTsdCsdCsdAsdCsdA sdGs**Gb^{6}sAb^{6}sC*b^{6}**
424	24	255a	**C*b^{1}sTb^{1}sGb^{1}sGb^{1}sC*b^{1}s**dGsdAsdTsdAsdCsdGsdC*sdGsdTsdCsdC*sdA sdCsdAs**Gb^{1}sGb^{1}sAb^{1}sC*b^{1}sGb^{1}**
424	24	255b	**C*b^{1}Tb^{1}Gb^{1}Gb^{1}C*b^{1}**dGdAdTdAdCdGdC*dGdTdCdC*dAdC*dA**Gb^{1}Gb^{1}Ab^{1} C*b^{1}Gb^{1}**
423	26	256a	**Gb^{1}sC*b^{1}sTb^{1}sGb^{1}sGb^{1}s**dC*sdGsdAsdTsdAsdCsdGsdCsdGsdTsdCsdCsdA sdCsdAsdGs**Gb^{1}sAb^{1}sC*b^{1}sGb^{1}sAb^{1}**

423	26	256b	**Gb^{1}C*b^{1}Tb^{1}Gb^{1}Gb^1**dC*dGdAdTdAdCdGdCdGdTdCdCdAdC*dAdG**Gb^{1}Ab^{1}C*b^{1}Gb^{1}Ab^1**
422	28	257a	**Tb^{1}sGb^{1}sC*b^{1}sTb^{1}sGb^{1}s**dGsdCsdGsdAsdTsdAsdC*sdGsdC*sdGsdTsdCsdCsdAsdCsdAsdGs**Gb^{1}sAb^{1}sC*b^{1}sGb^{1}sAb^1**
422	28	257b	**Tb^{1}Gb^{1}C*b^{1}Tb^{1}Gb^1**dGdCdGdAdTdAdCdGdCdGdTdCdC*dAdC*dAdG**Gb^{1}Ab^{1}C*b^{1}Gb^{1}Ab^1**

Table 6

SP	L	Seq ID No.	Sequence, 5'-3'
2067	10	258a	**Gb^{1}sTb^{1}s**dGsdTsdTsdTsdA*sdGs**Gb^{1}sGb^1**
2067	10	258b	**Gb^{1}sTb^{1}s**dGsdUsdTsdTsdA*sdGs**Gb^{1}sGb^1**
2066	12	259a	**Ab^{1}sGb^{1}sTb^{1}s**dGsdTsdTsdTsdA*sdGs**Gb^{1}sGb^{1}sAb^1**
2066	12	259b	**Ab^{1}Gb^{1}Tb^1**dGdUdUdUdA*dG**Gb^{1}Gb^{1}Ab^1**
2066	12	259c	**Ab^{1}sGb^{1}sTb^{1}s**dGsdTsdTsdTsdA*sdGsdGs**Gb^{1}sAb^1**
2066	12	259d	**Ab^{1}sGb^{1}s**dTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^1**
2066	12	259e	**Ab^{1}s**dGsdTsdGsdTsdTsdTsdA*sdGsdGs**Gb^{1}sAb^1**
2066	13	260a	**Tb^{1}sAb^{1}sGb^{1}sTb^{1}s**dGsdTsdTsdTsdAsdGs**Gb^{1}sGb^{1}sAb^1**
2066	13	260b	**Tb^{1}Ab^{1}Gb^{1}Tb^1**dGdUdUdUdAdG**Gb^{1}Gb^{1}Ab^1**
2066	13	260c	**Tb^{1}sAb^{1}sGb^{1}sTb^{1}s**dGsdTsdTsdTsdA*sdGs**Gb^{1}sGb^{1}sAb^1**
2066	13	260d	**Tb^{1}sAb^{1}sGb^{1}s**dUsdGsdTsdTsdTsdA*sdGsdGs**Gb^{1}sAb^1**
2066	13	260e	**Tb^{1}sAb^{1}s**dGsdUsdGsdUsdUsdUsdA*sdGsdGsdGs**Ab^1**
2066	13	260f	**Tb^{1}s**dA*sdGsdTsdGsdTsdTsdUsdA*s**Gb^{1}sGb^{1}sGb^{1}sAb^1**
2065	14	261a	**Tb^{1}sAb^{1}sGb^{1}sTb^{1}s**dGsdTsdTsdTsdA*sdGs**Gb^{1}sGb^{1}sAb^{1}sGb^1**
2065	14	261b	**Tb^{1}Ab^{1}Gb^{1}Tb^{1}s**dGsdTsdTsdTsdA*sdGsdG**Gb^{1}Ab^{1}Gb^1**
2065	14	261c	**Tb^{4}sAb^{4}sGb^{4}sTb^{4}s**dGsdUsdTsdUsdA*sdGsdGsdGs**Ab^{4}sGb^4**
2065	14	261d	**Tb^{1}s**dA*sdGsdUsdGsdTsdTsdUsdA*sdGsdGsdGsdA*s**Gb^1**
2065	14	261e	**Tb^{2}sAb^{2}sGb^{2}s**dUsdGsdUsdUsdTsdAsdGsdGs**Gb^{2}sAb^{2}sGb^2**
2065	14	261f	**Tb^{4}sAb^{4}s**dGsdTsdGsdTsdTsdTsdAsdGsdGs**Gb^{4}sAb^{4}sGb^4**
2065	14	261g	**Tb^{1}Ab^1**dGdTdGdTdTdTdA*dG**Gb^{1}Gb^{1}Ab^{1}Gb^1**
2064	15	262a	**Tb^{1}sAb^{1}sGb^{1}sTb^{1}s**dGdTdTdTdA*dGdGs**Gb^{1}sAb^{1}sGb^{1}sC*b^1**
2064	15	262b	**Tb^{1}ssAb^{1}ss**dGssdTssdGssdTssdTssdTssdAssdGssdGssdGssdAssdGss**C*b^1**
2064	15	262c	**Tb^{1}sAb^{1}s**dGsdUsdGsdUsdUsdUsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1**
2064	15	262d	**Tb^1**dAdGdTdGdTdTdTdAdGdGdGdAdG**C*b^1**
2064	15	262e	**Tb^{1}Ab^1**sdGsdUsdGsdUsdTsdUsdAsdGsdGs**Gb^{1}Ab^{1}Gb^{1}C*b^1**
2064	15	262f	**Tb^{4}sAb^{4}sGb^{4}s**dTsdGsdTsdTsdTsdAsdGsdGsdsdGsdAs**Gb^{4}sC*b^4**
2064	15	262g	**Tb^{6}Ab^{6}Gb^6**dUdGdTdTdUdAdGdGdG**Ab^{6}Gb^{6}C*b^6**
2064	15	262h	**Tb^{1}sAb^{1}sGb^{1}sTb^{1}s**dGsdTsdTsdTsdAsdGsdGsdGsdAsdGs**C*b^1**
2064	15	262i	**Tb^{1}ssAb^{1}ss**dGssdTssdGssdUssdUssdUssdAssdGssdGssdGssdAss**Gb^1**

			ssC*b^{1}
2064	16	209s	**Gb**^{1}**Tb**^{1}dAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab**^{1}**Gb**^{1}**C*b**^{1}
2064	16	209t	**Gb**^{1}**sTb**^{1}**s**dA*sdGsdTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab**^{1}**sGb**^{1}**sC*b**^{1}
2064	16	209u	**Gb**^{1}**Tb**^{1}dAdGdTdGdTdTdTdAdGdGdG**Ab**^{1}**Gb**^{1}**C*b**^{1}
2064	16	209v	/5SpC3s/**Gb**^{1}**sTb**^{1}**s**dAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab**^{1}**sGb**^{1}**sC*b**^{1}
2064	16	209w	**Gb**^{1}**sTb**^{1}**s**dAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab**^{1}**sGb**^{1}**sC*b**^{1}/s3SpC3/
2064	16	209x	/5SpC3s/**Gb**^{1}**sTb**^{1}**s**dAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab**^{1}**sGb**^{1}**sC*b**^{1} /3SpC3s/
2064	16	209y	**Gb**^{1}**sTb**^{1}**s**dAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab**^{1}**sGb**^{1}**sC*b**^{1}
2064	16	209aa	**Gb**^{1}**Tb**^{1}dA*sdGsdUsdGsdUsdUsdUsdAsdGsdGsdGs**Ab**^{1}**Gb**^{1}**C*b**^{1}
2064	16	209ab	**Gb**^{1}**Tb**^{1}dA*sdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab**^{1}**Gb**^{1}**C*b**^{1}
2064	16	209ac	**Gb**^{6}**sTb**^{6}**s**dA*dGdTdGdTdTdTdA*dGdGdG**Ab**^{6}**sGb**^{6}**sC*b**^{6}
2064	16	209ad	**Gb**^{1}**sTb**^{1}**s**dA*sdGsdUsdGsdUsdUsdUsdA*sdGsdGsdGs**Ab**^{1}**sGb**^{1}**sC*b**^{1}
2064	16	209ae	**Gb**^{1}**sTb**^{1}**s**dA*sdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab**^{1}**sGb**^{1}**sC*b**^{1}
2064	16	209af	**Gb**^{1}**sTb**^{1}**s**dAsdGsdTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab**^{1}**sGb**^{1}**sC*b**^{1}
2064	16	209ag	**Gb**^{1}**Tb**^{1}dA*dGdTdGdTdTdTdA*dGdGdG**Ab**^{1}**Gb**^{1}**C*b**^{1}
2064	16	209ah	**Gb**^{1}**Tb**^{1}dAdGdTdGdTdTdTdA*dGdGdG**Ab**^{1}**Gb**^{1}**C*b**^{1}
2064	16	209ai	**Gb**^{6}**sTb**^{6}**s**dA*dGdTdGdTdTdTdAdGdGdG**Ab**^{6}**sGb**^{6}**sC*b**^{6}
2064	16	209aj	**Gb**^{1}**sTb**^{1}**s**dA*sdGsdUsdGsdTsdTsdUsdA*sdGsdGsdGs**Ab**^{1}**sGb**^{1}**sC*b**^{1}
2064	16	209ak	**Gb**^{7}**sTb**^{7}**sAb**^{7}**sGb**^{7}**s**dTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab**^{7}**sGb**^{7}**sC*b**^{7}
2064	16	209am	**Gb**^{7}**sTb**^{7}**s**dAsdGsdTsdGsdTsdTsdUsdA*sdGsdGs**Gb**^{7}**sAb**^{7}**sGb**^{7}**sC*b**^{7}
2064	16	209an	**Gb**^{1}**ssTb**^{1}**ssAb**^{1}**ss**dGssdTssdGssdTssdTssdTssdA*ssdGssdGssdGss**Ab**^{1} **ssGb**^{1}**ssC*b**^{1}
2064	16	209ao	**Gb**^{4}**ssTb**^{4}**ssAb**^{4}**ss**dGssdTssdGssdTssdTssdTssdAssdGssdGssdGssdA*ss **Gb**^{4}**ssC*b**^{4}
2064	16	209ap	**Gb**^{2}**ssTb**^{2}**ssAb**^{2}**ssGb**^{2}**ss**dTssdGssdTssdTssdTssdAssdGssdGssdGssdAssd Gss**C*b**^{2}
2064	16	209aq	**Gb**^{1}**Tb**^{1}**Ab**^{1}**Gb**^{1}dUdGdUdUdUdAdGdG**Gb**^{1}**Ab**^{1}**Gb**^{1}**C*b**^{1}
2064	16	209ar	**Gb**^{1}**sTb**^{1}**sAb**^{1}**sGb**^{1}**sTb**^{1}**s**dGsdTsdTsdTsdA*sdGsdGsdGs**Ab**^{1}**sGb**^{1}**sC*b**^{1}
2064	16	209as	**Gb**^{1}**sTb**^{1}**s**dAsdGsdTsdGsdTsdTsdUsdAsdGs**Gb**^{1}**sGb**^{1}**sAb**^{1}**sGb**^{1}**sC*b**^{1}
2064	16	209at	**Gb**^{6}**sTb**^{6}**sAb**^{6}**sGb**^{6}**s**dTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab**^{6}**sGb**^{6}**sC*b**^{6}
2064	16	209au	**Gb**^{7}**sTb**^{7}**sAb**^{7}**s**dGsdUsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab**^{7}**sGb**^{7}**sC*b**^{7}
2064	16	209av	**Gb**^{4}**sTb**^{4}**sAb**^{4}**sGb**^{4}**s**dUsdGsdTsdUsdTsdA*sdGsdGsdGsdA*s**Gb**^{4}**sC*b**^{4}
2064	16	209aw	**Gb**^{4}**Tb**^{4}**Ab**^{4}**Gb**^{4}dTdGdTdTdTdAdGdGdGdA**Gb**^{4}**C*b**^{4}
2064	16	209ax	**Gb**^{1}**sTb**^{1}**sAb**^{1}sdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab**^{1}**sGb**^{1}**sC*b**^{1}
2064	16	209az	**Gb**^{1}**sTb**^{1}**sAb**^{1}**s**dGsdTsdGsdTsdTsdTsdAsdGsdGs**Gb**^{1}**sAb**^{1}**sGb**^{1}**sC*b**^{1}
2064	16	209ba	**Gb**^{1}**sTb**^{1}**sAb**^{1}**sGb**^{1}**s**dTsdGsdTsdTsdTsdAsdGsdGs**Gb**^{1}**sAb**^{1}**sGb**^{1}**sC*b**^{1}
2064	16	209bb	**Gb**^{1}**sTb**^{1}**sAb**^{1}**s**dGsdTsdGsdTsdTsdTsdAsdGsdGsdGsdAs**Gb**^{1}**sC*b**^{1}
2063	17	263a	**Gb**^{1}**sTb**^{1}**sAb**^{1}**sGb**^{1}**sTb**^{1}**s**dGsdTsdTsdTsdA*sdGsdGs**Gb**^{1}**sAb**^{1}**sGb**^{1}**sC*b**^{1} **sC*b**^{1}

2063	17	263b	**Gb^{2}sTb^{2}sAb^{2}s**dGsdTsdGsdTsdTsdTsdA*sdGsdGs**Gb^{2}sAb^{2}sGb^{2}sC*b^{2}sC*b^2**
2063	17	263c	**Gb^{1}sTb^{1}sAb^{1}sGb^{1}s**dTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^{1}sC*b^1**
2063	17	263d	**Gb^{1}s**dUsdA*sdGsdUsdGsdUsdTsdTsdA*sdGsdGs**Gb^{1}sAb^{1}sGb^{1}sC*b^{1}sC*b^1**
2063	17	263e	**Gb^{1}sTb^{1}sAb^{1}s**dGsdTsdGsdUsdTsdTsdA*sdGsdGs**Gb^{1}sAb^{1}sGb^{1}sC*b^{1}sC*b^1**
2063	17	263f	**Gb^{1}Tb^1**dA*dGdTdGdTdTdTdA*dGdGdG**Ab^{1}Gb^{1}C*b^{1}C*b^1**
2063	17	263g	**Gb^{1}s**dTsdA*sdGsdTsdGsdTsdTsdTsdA*sdGsdGsdGsdA*s**Gb^{1}sC*b^{1}sC*b^1**
2063	17	263h	**Gb^{1}Tb^{1}Ab^{1}Gb^{1}Tb^1**dGdTdUdTdAdGdGdGdA*dG**C*b^{1}C*b^1**
2063	17	263i	**Gb^{1}ssTb^{1}ssAb^{1}ssGb^{1}ssTb^{1}ss**dGssdTssdTssdTssdAssdGssdGssdGssdAss **Gb^{1}ssC*b^{1}ssC*b^1**
2063	17	263j	**Gb^{4}Tb^4**dA*dGdTdGdTdTdTdAdGdGdGdA***Gb^{4}C*b^{4}C*b^4**
2063	17	263k	**Gb^{6}sTb^{6}sAb^{6}s**dGsdTsdGsdUsdUsdTsdAsdGsdGsdGsdA*s**Gb^{6}sC*b^{6}sC*b^6**
2063	17	263m	**Gb^{7}sTb^{7}sAb^{7}sGb^{7}s**dTdGdTdTdTdA*dGdGdGs**Ab^{7}sGb^{7}sC*b^{7}sC*b^7**
2063	18	264a	**Gb^{1}sGb^{1}sTb^{1}sAb^{1}sGb^{1}s**dTsdGsdTsdTsdTsdA*sdGsdGs**Gb^{1}sAb^{1}sGb^{1}sC*b^1 sC*b^1**
2063	18	264b	**Gb^{7}sGb^{7}sTb^{7}sAb^{7}sGb^{7}s**dTsdGsdTsdTsdTsdAsdGsdGsdGsdAsdGsdC*s**C*b^7**
2063	18	264c	**Gb^{1}sGb^{1}sTb^{1}sAb^{1}sGb^{1}s**dTsdGsdTsdTsdTsdAsdGsdGsdGsdA*sdGsdC*s **C*b^1**
2063	18	264d	**Gb^{1}sGb^{1}sTb^{1}sAb^{1}sGb^{1}s**dUsdGsdTsdTsdTsdAsdGsdGsdGsdA*sdGsdC*s **C*b^1**
2063	18	264e	**Gb^{1}sGb^{1}sTb^{1}sAb^{1}s**dGsdTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1 sC*b^1**
2063	18	264f	**Gb^{1}sGb^{1}sTb^{1}s**dA*sdGsdTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1 sC*b^1**
2063	18	264g	**Gb^{1}sGb^{1}sTb^{1}sAb^{1}s**dGsdTsdGsdTsdTsdTsdA*sdGsdGsdGsdA*s**Gb^{1}sC*b^1 sC*b^1**
2063	18	264h	**Gb^{1}Gb^1**dUdA*dGdTdGdTdTdTdAdGdG**Gb^{1}Ab^{1}Gb^{1}C*b^{1}C*b^1**
2063	18	264i	**Gb^{4}Gb^{4}Tb^{4}Ab^4**dGsdTsdGsdTsdTsdTsdAsdGsdGs**Gb^{4}Ab^{4}Gb^{4}C*b^{4}C*b^4**
2063	18	264j	**Gb^{1}ssGb^{1}ssTb^{1}ss**dA*ssdGssdTssdGssdUssdTssdTssdA*ssdGssdGssdGss dA*ss**Gb^{1}ssC*b^{1}ssC*b^1**
2063	18	264k	**Gb^{2}Gb^{2}Tb^2**dA*dGdTdGdTdTdTdAdGdG**Gb^{2}Ab^{2}Gb^{2}C*b^{2}C*b^2**
2062	19	265a	**Gb^{1}sGb^{1}sTb^{1}sAb^{1}sGb^{1}s**dTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1 sC*b^{1}sGb^1**
2062	19	265b	**Gb^{6}Gb^{6}Tb^{6}Ab^{6}Gb^6**dTdGdTdTdTdA*dGdGdG**Ab^{6}Gb^{6}C*b^{6}C*b^{6}Gb^6**
2062	19	265c	**Gb^{1}sGb^{1}sTb^{1}sAb^{1}s**dGsdTsdGsdTsdTsdTsdA*sdGsdGsdGsdA*sdGs**C*b^1 sC*b^{1}sGb^1**
2062	19	265d	**Gb^{1}s**dGsdTsdA*sdGsdUsdGsdTsdUsdTsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1 sC*b^{1}sGb^1**
2062	19	265e	**Gb^{4}sGb^{4}s**dUsdAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsdA*s**Gb^{4}sC*b^{4}sC*b^4 sGb^4**
2062	19	265f	**Gb^{2}ssGb^{2}ssTb^{2}ssAb^{2}ssGb^{2}ss**dTssdGssdTssdTssdTssdAssdGssdGssdGss dAssdGssdCss**C*b^{2}ssGb^2**
2062	20	266a	**Tb^{1}sGb^{1}sGb^{1}sTb^{1}sAb^{1}s**dGsdTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^{1}sGb^1 sC*b^{1}sC*b^{1}sGb^1**
2062	20	266b	**Tb^{2}sGb^{2}sGb^{2}s**dTsdA*sdGsdTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^{2}sGb^2 sC*b^{2}sC*b^{2}sGb^2**
2062	20	266c	**Gb^{1}Gb^{1}Tb^1**dA*dGdTdGdTdTdTdA*dGdGdGdA***Gb^{1}C*b^{1}C*b^{1}Gb^1**

2062	20	266d	**Tb^{1}s**dGsdGsdUsdA*sdGsdTsdGsdTsdUsdTsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1 sC*b^{1}sGb^1**
2062	20	266e	**Tb^{4}sGb^{4}sGb^{4}sTb^{4}s**dAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsdAs**Gb^{4}sC*b^4 sC*b^{4}sGb^4**
2061	22	267a	**Tb^{1}sTb^{1}sGb^{1}sGb^{1}sTb^{1}s**dAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsdA*s**Gb^1 sC*b^{1}sC*b^{1}sGb^{1}sTb^1**
2061	22	267b	**Tb^{1}Tb^{1}Gb^{1}Gb^{1}Tb^1**dA*dGdTdGdTdTdTdAdGdGdGdA***Gb^{1}C*b^{1}C*b^{1}Gb^{1}Tb^1**
2061	22	267c	**Tb^{6}sTb^{6}sGb^{6}s**dGsdTsdAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsdAs**Gb^6 sC*b^{6}sC*b^{6}sGb^{6}sTb^6**
2060	24	268a	**Tb^{1}sTb^{1}sTb^{1}sGb^{1}sGb^{1}s**dTsdA*sdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsdA sdG**C*b^{1}sC*b^{1}sGb^{1}sTb^{1}sC*b^1**
2060	24	268b	**Tb^{1}Tb^{1}Tb^{1}Gb^{1}Gb^1**dTdA*dGdTdGdTdTdTdAdGdGdGdA*dG**C*b^{1}C*b^{1}Gb^{1}Tb^1 C*b^1**
2059	26	269a	**Ab^{1}sTb^{1}sTb^{1}sTb^{1}sGb^{1}s**dGsdTsdAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdG sdAsdGsdC*s**C*b^{1}sGb^{1}sTb^{1}sC*b^{1}sTb^1**
2059	26	269b	**Ab^{1}Tb^{1}Tb^{1}Tb^{1}Gb^1**dGdTdAdGdTdGdTdTdTdAdGdGdGdAdGdC***C*b^{1}Gb^{1}Tb^1 C*b^{1}Tb^1**
2058	28	270a	T**b^{1}s**A**b^{1}s**T**b^{1}s**T**b^{1}s**T**b^{1}s**dGsdGsdTsdAsdGsdTsdGsdTsdTsdTsdAsdGsdG sdGsdAsdGsdC*sdCs**Gb^{1}sTb^{1}sC*b^{1}sTb^{1}sTb^1**
2058	28	270b	T**b^1**A**b^1**T**b^1**T**b^1**T**b^1**dGdGdTdAdGdTdGdTdTdTdAdGdGdGdAdGdC*dC***Gb^{1}Tb^1 C*b^{1}Tb^{1}Tb^1**

Table 7

SP	L	Seq ID No.	Sequence, 5'-3'
2075	10	271a	**Ab^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*s**Gb^{1}sTb^1**
2075	10	271b	**Ab^{1}Tb^1**dTdTdGdGdTdA***Gb^{1}Tb^1**
2074	12	272a	**Tb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*s**Gb^{1}sTb^{1}sGb^1**
2074	12	272b	**Tb^{1}Ab^{1}Tb^1**dTdTdGdGdTdA***Gb^{1}Tb^{1}Gb^1**
2074	12	272c	**Tb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*sdGs**Tb^{1}sGb^1**
2074	12	272d	**Tb^{1}sAb^{1}s**dTsdTsdTsdGsdGsdTsdA*sdGsdUs**Gb^1**
2074	12	272e	**Tb^{1}s**dAsdTsdUsdTsdGsdGsdUsdA*sdGs**Tb^{1}sGb^1**
2073	13	273a	**Tb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdAs**Gb^{1}sTb^{1}sGb^{1}sTb^1**
2073	13	273b	**Tb^{1}Ab^{1}Tb^1**dUdUdGdGdUdA**Gb^{1}Tb^{1}Gb^{1}Tb^1**
2073	13	273c	**Tb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*s**Gb^{1}sTb^{1}sGb^{1}sTb^1**
2073	13	273d	**Tb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*sdGsdUs**Gb^{1}sTb^1**
2073	13	273e	**Tb^{1}sAb^{1}s**dUsdUsdUsdGsdGsdUsdA*sdGsdUsdGs**Tb^1**
2073	13	273f	**Tb^{1}s**dA*sdTsdTsdUsdGsdGsdTsdA*s**Gb^{1}sTb^{1}sGb^{1}sTb^1**
2073	14	274a	**C*b^{1}Tb^{1}Ab^{1}s**dUsdTsdTsdGsdGsdTsdA*s**Gb^{1}Tb^{1}Gb^{1}Tb^1**
2073	14	274b	**C*b^{4}sTb^{4}sAb^{4}sTb^{4}s**dTsdTsdGsdGsdTsdA*sdGsdUs**Gb^{4}sTb^4**
2073	14	274c	**C*b^{1}s**dUsdA*sdTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**Tb^1**
2073	14	274d	**C*b^{2}sTb^{2}sAb^{2}s**dTsdTsdUsdGsdGsdTsdA*sdGs**Tb^{2}sGb^{2}sTb^2**
2073	14	274e	**C*b^{4}ssTb^{4}ss**dAssdTssdTssdTssdGssdGssdTssdAssdGss**Tb^{4}ssGb^{4}ssTb^4**
2073	14	274f	**C*b^{1}Tb^{1}Ab^1**dTdTdTdGdGdTdA***Gb^{1}Tb^{1}Gb^{1}Tb^1**

2073	14	274g	**C*b^{1}sTb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*s**Gb^{1}sTb^{1}sGb^{1}sTb^1**
2072	15	275a	**C*b^{1}sTb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*sdGsdTsdGsdTs**Tb^1**
2072	15	275b	**C*b^{1}sTb^{1}s**dA*sdUsdTsdUsdGsdGsdTsdAsdGsdUs**Gb^{1}sTb^{1}sTb^1**
2072	15	275c	**C*b^{4}sTb^{4}sAb^{4}s**dTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**Tb^{4}sTb^4**
2072	15	275d	**C*b^{1}ssTb^{1}ss**dAssdTssdTssdTssdGssdGssdTssdAssdGssdTssdGssdTss**Tb^1**
2072	15	275e	**C*b^{1}ssTb^{1}ss**dAssdUssdTssdTssdGssdGssdTssdAssdGssdUssdGss**Tb^1 ssTb^1**
2072	15	275f	**C*b^{1}sTb^{1}sAb^{1}sTb^{1}s**dTdTdGdGdTdA*dGs**Tb^{1}sGb^{1}sTb^{1}sTb^1**
2072	15	275g	**C*b^{1}Tb^{1}s**dAsdTsdTsdTsdGsdGsdTsdA*sdGs**Tb^{1}Gb^{1}Tb^{1}Tb^1**
2072	15	275h	**C*b^{6}Tb^{6}Ab^6**dUdTdTdGdGdTdA*dGdU**Gb^{6}Tb^{6}Tb^6**
2072	15	275i	**C*b^1**dTdAdTdTdTdGdGdTdAdGdTdGdT**Tb^1**
2072	16	210o	**Gb^{1}C*b^{1}Tb^{1}Ab^1**dTsdTsdTsdGsdGsdTsdAsdGsdTs**Gb^{1}Tb^{1}Tb^1**
2072	16	210p	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^1**
2072	16	210q	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dTsdTsdTsdGsdGsdTsdAsdGsdTs**Gb^{1}sTb^{1}sTb^1**
2072	16	210r	**Gb^{1}C*b^{1}Tb^{1}Ab^1**dTdTdTdGdGdTdA*dGdT**Gb^{1}Tb^{1}Tb^1**
2072	16	210s	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dUsdUsdTdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^1**
2072	16	210t	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dUsdTsdTsdGsdGsdTsdAsdGsdUs**Gb^{1}sTb^{1}sTb^1**
2072	16	210u	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dUsdUsdUsdGsdGsdUsdA*sdGsdUs**Gb^{1}sTb^{1}sTb^1**
2072	16	210v	**/5SpC3s/Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dTsdTsdTsdGsdGsdTsdAsdGsdTs**Gb^{1}sTb^1 sTb^1**
2072	16	210w	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dTsdTsdTsdGsdGsdTsdAsdGsdTs**Gb^{1}sTb^{1}sTb^1 /3SpC3s/**
2072	16	210x	**/5SpC3s/Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dTsdTsdTsdGsdGsdTsdAsdGsdTs**Gb^{1}sTb^1 sTb^1/3SpC3s/**
2072	16	210y	**Gb^{1}C*b^{1}Tb^{1}Ab^{1}s**dTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}Tb^{1}Tb^1**
2072	16	210z	**Gb^{1}C*b^{1}Tb^{1}Ab^{1}s**dUsdTsdTsdGsdGsdUsdA*sdGsdTs**Gb^{1}Tb^{1}Tb^1**
2072	16	210aa	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dTdTdTdGdGdTdA*dGdTs**Gb^{1}sTb^{1}sTb^1**
2072	16	210ab	**Gb^{6}sC*b^{6}sTb^{6}sAb^{6}s**dTdTdTdGdGdTdA*dGdTs**Gb^{6}sTb^{6}sTb^6**
2072	16	210ac	**Gb^{6}sC*b^{6}sTb^{6}s**dAsdTsdTsdTsdGsdGsdTsdAsdGs**Tb^{6}sGb^{6}sTb^{6}sTb^6**
2072	16	210ad	**Gb^{7}sC*b^{7}sTb^{7}s**dA*sdTsdTsdTsdGsdGsdTsdA*sdGs**Tb^{7}sGb^{7}sTb^{7}sTb^7**
2072	16	210ae	**Gb^{7}sC*b^{7}s**dUsdAsdTsdTsdUsdGsdGsdUsdA*sdGs**Tb^{7}sGb^{7}sTb^{7}sTb^7**
2072	16	210af	**Gb^{1}ssC*b^{1}ssTb^{1}ss**dAssdTssdTssdTssdGssdGssdTssdA*ssdGssdTss**Gb^1 ssTb^{1}ssTb^1**
2072	16	210ag	**Gb^{4}ssC*b^{4}ssTb^{4}ss**dA*ssdTssdTssdTssdGssdGssdTssdAssdGssdTssdGss **Tb^{4}ssTb^4**
2072	16	210ah	**Gb^{2}ssC*b^{2}ssTb^{2}ssAb^{2}ss**dTssdTssdTssdGssdGssdTssdAssdGssdTssdGss dTss**Tb^2**
2072	16	210ai	**Gb^{1}C*b^{1}Tb^{1}Ab^1**dUsdTsdTsdGsdGsdTsdAsdGs**Tb^{1}Gb^{1}Tb^{1}Tb^1**

2072	16	210aj	**Gb^{4}C*b^{4}Tb^{4}Ab^4**dTsdTsdTsdGsdGsdTsdAsdGsdTdG**Tb^{4}Tb^4**
2072	16	210ak	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^1**
2072	16	210am	**Gb^{4}sC*b^{4}sTb^{4}sAb^{4}s**dTsdTsdUsdGsdGsdTsdA*sdGsdTsdGs**Tb^{4}sTb^4**
2072	16	210an	**Gb^{7}sC*b^{7}sTb^{7}s**dA*sdTsdTsdUsdGsdGsdTsdA*sdGsdTs**Gb^{7}sTb^{7}sTb^7**
2072	16	210ao	**Gb^{1}sC*b^{1}s**dUsdAsdUsdUsdTsdGsdGsdUsdAs**Gb^{1}sTb^{1}sGb^{1}sTb^{1}sTb^1**
2072	16	210ap	**Gb^{1}sC*b^{1}sTb^1**sdAsdTsdTsdTsdGsdGsdTsdAsdGsdTs**Gb^{1}sTb^{1}sTb^1**
2072	16	210aq	**Gb^{1}sC*b^{1}sTb^{1}s**dAsdTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**Tb^{1}sTb^1**
2071	17	276a	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*sdGs**Tb^{1}sGb^{1}sTb^{1}sTb^{1}sTb^1**
2071	17	276b	**Gb^{2}sC*b^{2}sTb^{2}s**dAsdTsdTsdTsdGsdGsdTsdA*sdGs**Tb^{2}sGb^{2}sTb^{2}sTb^{2}sTb^2**
2071	17	276c	**Gb^{1}sC*b^{1}sTb^{1}s**dAsdTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^{1}sTb^1**
2071	17	276d	**Gb^{2}s**dC*sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGs**Tb^{2}sGb^{2}sTb^{2}sTb^{2}sTb^2**
2071	17	276e	**Gb^{6}sC*b^{6}sTb^{6}s**dA*sdUsdUsdUsdGsdGsdUsdA*sdGsdUsdGs**Tb^{6}sTb^{6}sTb^6**
2071	17	276f	**Gb^{1}s**dC*sdTsdA*sdUsdUsdUsdGsdGsdUsdAsdGsdUsdGs**Tb^{1}sTb^{1}sTb^1**
2071	17	276g	**Gb^{1}C*b^1**dTdA*dTdTdTdGdGdTdA*dGdT**Gb^{1}Tb^{1}Tb^{1}Tb^1**
2071	17	276h	**Gb^{4}C*b^{4}Tb^{4}Ab^4**dTdTdTdGdGdTdA*dGdTdG**Tb^{4}Tb^{4}Tb^4**
2071	17	276i	**Gb^{1}C*b^{1}Tb^{1}Ab^{1}Tb^1**dUdTdGdGdTdA*dGdTdGdU**Tb^{1}Tb^1**
2071	17	276j	**Gb^{1}ssC*b^{1}ssTb^{1}ssAb^{1}ssTb^{1}ss**dTssdTssdGssdGssdTssdAssdGssdTssdGss **Tb^{1}ssTb^{1}ssTb^1**
2071	17	276k	**Gb^{7}sC*b^{7}sTb^{7}sAb^{7}s**dTdTdTdGdGdTdA*dGdTs**Gb^{7}sTb^{7}sTb^{7}sTb^7**
2071	18	277a	**Ab^{1}sGb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dTsdTsdTsdGsdGsdTsdA*sdGs**Tb^{1}sGb^{1}sTb^{1}sTb^1 sTb^1**
2071	18	277b	**Ab^{7}sGb^{7}sC*b^{7}sTb^{7}sAb^{7}s**dTsdTsdTsdGsdGsdTsdA*sdGsdTsdGsdTsdTs**Tb^7**
2071	18	277c	**Ab^{1}sGb^{1}s**dC*sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGs**Tb^{1}sGb^{1}sTb^{1}sTb^{1}sTb^1**
2071	18	277d	**Ab^{1}sGb^{1}s**dC*sdTsdAsdTsdTsdTsdGsdGsdTsdA*sdGs**Tb^{1}sGb^{1}sTb^{1}sTb^{1}sTb^1**
2071	18	277e	**Ab^{1}Gb^1**dC*dTdAdUdTdTdGdGdTdA*dG**Tb^{1}Gb^{1}Tb^{1}Tb^{1}Tb^1**
2071	18	277f	**Ab^{2}Gb^{2}C*b^2**dTdAdTdTdTdGdGdTdA*dG**Tb^{2}Gb^{2}Tb^{2}Tb^{2}Tb^2**
2071	18	277g	**Ab^{1}sGb^{1}sC*b^{1}sTb^{1}s**dA*sdTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^1 sTb^1**
2071	18	277h	**Ab^{1}sGb^{1}sC*b^{1}sTb^{1}s**dA*sdTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**Tb^{1}sTb^{1}sTb^1**
2071	18	277i	**Ab^{1}sGb^{1}sC*b^{1}s**dTsdA*sdTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^1 sTb^1**
2071	18	277j	**Ab^{4}Gb^{4}C*b^{4}Tb^{4}s**dAsdTsdTsdTsdGsdGsdTsdAsdGs**Tb^{4}Gb^{4}Tb^{4}Tb^{4}Tb^4**
2071	18	277k	**Ab^{1}ssGb^{1}ssC*b^{1}ss**dTssdA*ssdTssdTssdTssdGssdGssdTssdA*ssdGssdUssd Gss**Tb^{1}ssTb^{1}ssTb^1**
2070	19	278a	**Ab^{1}sGb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^1 sTb^{1}sAb^1**
2070	19	278b	**Ab^{2}ssGb^{2}ssC*b^{2}ssTb^{2}ssAb^{2}ss**dTssdTssdTssdGssdGssdTssdAssdGssdTss dGssdTssdTss**Tb^{2}ssAb^2**
2070	19	278c	**Ab^{1}s**dGsdC*sdTsdAsdTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^{1}sTb^1 sAb^1**
2070	19	278d	**Ab^{1}s**dGsdC*sdTsdAsdTsdTsdTsdGsdGsdUsdA*sdGsdUs**Gb^{1}sTb^{1}sTb^{1}sTb^1**

			sAb^{1}
2070	19	278e	**Ab**^{1}**sGb**^{1}**sC*b**^{1}**s**dTsdAsdTsdTsdTsdGsdGsdTsdA*sdGsdTsdGs**Tb**^{1}**sTb**^{1}**sTb**^{1} **sAb**^{1}
2070	19	278f	**Ab**^{4}**sGb**^{4}**s**dC*sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**Tb**^{4}**sTb**^{4}**sTb**^{4} **sAb**^{4}
2070	19	278g	**Ab**^{6}**Gb**^{6}**C*b**^{6}**Tb**^{6}**Ab**^{6}dTdTdTdGdGdTdA*dGdT**Gb**^{6}**Tb**^{6}**Tb**^{6}**Tb**^{6}**Ab**^{6}
2070	20	279a	**Gb**^{1}**sAb**^{1}**sGb**^{1}**sC*b**^{1}**sTb**^{1}**s**dAsdTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb**^{1}**sTb**^{1} **sTb**^{1}**sTb**^{1}**sAb**^{1}
2070	20	279b	**Gb**^{2}**sAb**^{2}**sGb**^{2}**s**dC*sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGsdTs**Gb**^{2}**sTb**^{2}**sTb**^{2} **sTb**^{2}**sAb**^{2}
2070	20	279c	**Gb**^{1}**s**dAsdGsdC*sdUsdAsdTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb**^{1}**sTb**^{1}**sTb**^{1} **sTb**^{1}**sAb**^{1}
2070	20	279d	**Gb**^{4}**sAb**^{4}**sGb**^{4}**sC*b**^{4}**s**dTsdAsdTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**Tb**^{4}**sTb**^{4} **sTb**^{4}**sAb**^{4}
2070	20	279e	**Gb**^{1}**Ab**^{1}**Gb**^{1}dC*dTdAdTdTdTdGdGdTdAdGdTdG**Tb**^{1}**Tb**^{1}**Tb**^{1}**Ab**^{1}
2069	22	280a	**Ab**^{1}**sGb**^{1}**sAb**^{1}**sGb**^{1}**sC*b**^{1}**s**dTsdAsdTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**Tb**^{1} **sTb**^{1}**sTb**^{1}**sAb**^{1}**sGb**^{1}
2069	22	280b	**Ab**^{1}**Gb**^{1}**Ab**^{1}**Gb**^{1}**C*b**^{1}dTdAdTdTdTdGdGdTdAdGdTdG**Tb**^{1}**Tb**^{1}**Tb**^{1}**Ab**^{1}**Gb**^{1}
2069	22	280c	**Ab**^{1}**sGb**^{1}**sAb**^{1}**sGb**^{1}**s**dC*sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**Tb**^{1} **sTb**^{1}**sTb**^{1}**sAb**^{1}**sGb**^{1}
2069	22	280d	**Ab**^{6}**sGb**^{6}**sAb**^{6}**sGb**^{6}**s**dC*sdTsdAsdTsdTsdTsdGsdGsdTsdA*sdGsdTsdGsdTsd TsTb^{6}**sAb**^{6}**sGb**^{6}
2068	24	281a	**Ab**^{1}**sAb**^{1}**sGb**^{1}**sAb**^{1}**sGb**^{1}**s**dC*sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGsdTsdGs dTs**Tb**^{1}**sTb**^{1}**sAb**^{1}**sGb**^{1}**sGb**^{1}
2068	24	281b	**Ab**^{1}**Ab**^{1}**Gb**^{1}**Ab**^{1}**Gb**^{1}dC*dTdAdTdTdTdGdGdTdAdGdTdGdT**Tb**^{1}**Tb**^{1}**Ab**^{1}**Gb**^{1}**Gb**^{1}
2067	26	282a	**Gb**^{1}**sAb**^{1}**sAb**^{1}**sGb**^{1}**sAb**^{1}**s**dGsdC*sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGsdTs dGsdTsdTs**Tb**^{1}**sAb**^{1}**sGb**^{1}**sGb**^{1}**sGb**^{1}
2067	26	282b	**Gb**^{1}**Ab**^{1}**Ab**^{1}**Gb**^{1}**Ab**^{1}dGdC*dTdAdTdTdTdGdGdTdAdGdTdGdTdT**Tb**^{1}**Ab**^{1}**Gb**^{1} **Gb**^{1}**Gb**^{1}
2066	28	283a	**Ab**^{1}**sGb**^{1}**sAb**^{1}**sAb**^{1}**sGb**^{1}**s**dAsdGsdC*sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGs dTsdGsdTsdTsTs**Ab**^{1}**sGb**^{1}**sGb**^{1}**sGb**^{1}**sAb**^{1}
2066	28	283b	**Ab**^{1}**Gb**^{1}**Ab**^{1}**Ab**^{1}**Gb**^{1}dAdGdC*dTdAdTdTdTdGdGdTdAdGdTdGdTdTdT**Ab**^{1}**Gb**^{1} **Gb**^{1}**Gb**^{1}**Ab**^{1}

Table 8

SP	L	Seq ID No.	Sequence, 5'-3'
4220	10	219a	**Gb**^{1}**sAb**^{1}**s**dAsdTsdGsdGsdAsdCs**C*b**^{1}**sAb**^{1}
4220	10	219b	**Gb**^{1}**Ab**^{1}dAdTdGdGdAdC**C*b**^{1}**Ab**^{1}
4219	12	220a	**Tb**^{1}**sGb**^{1}**sAb**^{1}**s**dAsdTsdGsdGsdAsdCs**C*b**^{1}**sAb**^{1}**sGb**^{1}
4219	12	220b	**Tb**^{1}**Gb**^{1}**Ab**^{1}dAdTdGdGdAdC**C*b**^{1}**Ab**^{1}**Gb**^{1}
4219	12	220c	**Tb**^{1}**sGb**^{1}**sAb**^{1}**s**dAsdTsdGsdGsdAsdCsdC*s**Ab**^{1}**sGb**^{1}
4219	12	220d	**Tb**^{1}**s**dGsdA*sdAsdTsdGsdGsdAsdC*sdCs**Ab**^{1}**sGb**^{1}
4219	12	220e	**Tb**^{1}**sGb**^{1}**s**dA*sdA*sdTsdGsdGsdA*sdC*sdC*sdAs**Gb**^{1}

4218	13	221a	**Tb^{1}sGb^{1}sAb^{1}sAb^{1}s**dTsdGsdGsdAsdCsdCs**Ab^{1}sGb^{1}sTb^1**
4218	13	221b	**Tb^{1}Gb^{1}Ab^{1}Ab^1**dUdGdGdAdCdC**Ab^{1}Gb^{1}Tb^1**
4218	13	221c	**Tb^{1}sGb^{1}sAb^{1}sAb^{1}s**dTsdGsdGsdAsdCsdC*s**Ab^{1}sGb^{1}sTb^1**
4218	13	221d	**Tb^{1}sGb^{1}sAb^{1}s**dAsdTsdGsdGsdA*sdCsdC*sdAs**Gb^{1}sTb^1**
4218	13	221e	**Tb^{1}sGb^{1}s**dA*sdAsdTsdGsdGsdAsdC*sdCsdAsdGs**Tb^1**
4218	13	221f	**Tb^{1}s**dGsdAsdA*sdTsdGsdGsdAsdCs**C*b^{1}sAb^{1}sGb^{1}sTb^1**
4218	14	222a	**Ab^{1}sTb^{1}sGb^{1}sAb^{1}s**dAsdTsdGsdGsdAsdCs**C*b^{1}sAb^{1}sGb^{1}sTb^1**
4218	14	222b	**Ab^{1}Tb^{1}Gb^{1}Ab^1**dAsdTsdGsdGsdAsdCsdC*s**Ab^{1}Gb^{1}Tb^1**
4218	14	222c	**Ab^{1}Tb^1**dGdA*dAdTdGdGdA*dC**C*b^{1}Ab^{1}Gb^{1}Tb^1**
4218	14	222d	**Ab^{4}sTb^{4}sGb^{4}s**dA*sdAsdTsdGsdGsdAsdCsdC*sAbs**Gb^{4}sTb^4**
4218	14	222e	**Ab^{1}s**dTsdGsdA*sdA*sdTsdGsdGsdA*sdC*sdC*sdA*sdGs**Tb^1**
4218	14	222f	**Ab^{2}sTb^{2}sGb^{2}s**dA*sdAsdUsdGsdGsdAsdCsdCs**Ab^{2}sGb^{2}sTb^2**
4218	14	222g	**Ab^{4}ssTb^{4}ss**dGssdAssdAssdTssdGssdGssdAssdCssdCss**Ab^{4}ssGb^{4}ssTb^4**
4217	15	223a	**Ab^{1}sTb^{1}sGb^{1}sAb^{1}s**dAdTdGdGdAdCdC*s**Ab^{1}sGb^{1}sTb^{1}sAb^1**
4217	15	223b	**Ab^{1}ssTb^{1}ss**dGssdAssdAssdTssdGssdGssdAssdCssdCssdAssdGssdTss**Ab^1**
4217	15	223c	**Ab^1**dTdGdAdAdTdGdGdAdCdCdAdGdT**Ab^1**
4217	15	223d	**Ab^{1}sTb^{1}s**dGsdAsdAsdUsdGsdGsdA*sdCsdCsdAs**Gb^{1}sTb^{1}sAb^1**
4217	15	223e	**Ab^{6}Tb^{6}Gb^6**dA*dAdTdGdGdAdCdC*dA**Gb^{6}Tb^{6}Ab^6**
4217	15	223f	**Ab^{1}Tb^1**dGsdAsdAsdTsdGsdGsdAsdC*sdC*s**Ab^{1}Gb^{1}Tb^{1}Ab^1**
4217	15	223g	**Ab^{4}sTb^{4}sGb^{4}s**dAsdAsdTsdGsdGsdAsdCsdCsdAsdGs**Tb^{4}sAb^4**
4217	15	223h	**Ab^{1}sTb^{1}sGb^{1}sAb^{1}s**dAsdTsdGsdGsdAsdC*sdC*sdAsdGsdTs**Ab^1**
4217	15	223i	**Ab^{1}ssTb^{1}ss**dGssdAssdAssdUssdGssdGssdA*ssdCssdCssdAssdGss**Tb^{1}ssAb^1**
4217	16	218y	**C*b^{2}sAb^{2}sTb^{2}s**dGsdAsdAsdTsdGsdGsdAsdCsdCs**Ab^{2}sGb^{2}sTb^{2}sAb^2**
4217	16	218z	**C*b^{1}sAb^{1}sTb^{1}s**dGsdAsdAsdTsdGsdGsdAsdC*sdC*s**Ab^{1}sGb^{1}sTb^{1}sAb^1**
4217	16	218aa	**C*b^{1}ssAb^{1}ssTb^{1}ss**dGssdAssdAssdTssdGssdGssdAssdCssdCss**Ab^{1}ssGb^{1}ssTb^{1}ssAb^1**
4217	16	218ab	**C*b^{1}Ab^{1}Tb^1**dGsdAsdAsdUsdGsdGsdAsdC*sdC*s**Ab^{1}Gb^{1}Tb^{1}Ab^1**
4217	16	218ac	**C*b^{1}Ab^{1}Tb^1**dGsdA*sdA*sdTsdGsdGsdA*sdCsdCs**Ab^{1}Gb^{1}Tb^{1}Ab^1**
4217	16	218ad	**C*b^{6}sAb^{6}sTb^{6}s**dGdAdAdTdGdGdAdCdC**Ab^{6}sGb^{6}sTb^{6}sAb^6**
4217	16	218ae	**C*b^{7}sAb^{7}sTb^{7}sGb^{7}s**dAsdAsdTsdGsdGsdAsdCsdCsdAs**Gb^{7}sTb^{7}sAb^7**
4217	16	218af	**C*bs^{1}Ab^{1}s**dUsdGsdAsdAsdUsdGsdGsdUsdCsdCs**Ab^{1}sGb^{1}sTb^{1}sAb^1**
4217	16	218b	**C*b^{1}sAb^{1}sTb^1**sdGsdAsdAsdTsdGsdGsdAsdCsdCs**Ab^{1}sGb^{1}sTb^{1}sAb^1**
4217	16	218m	**C*b^{1}sAb^{1}sTb^{1}s**dGsdAsdAsdTsdGsdGsdAsdC*sdC*s**Ab^{1}sGb^{1}sTb^{1}sAb^1**
4217	16	218n	**C*b^{1}Ab^{1}Tb^1**dGsdAsdAsdTsdGsdGsdAsdC*sdC*s**Ab^{1}Gb^{1}Tb^{1}Ab^1**
4217	16	218o	**C*b^{1}sAb^{1}sTb^{1}s**dGsdA*sdA*sdTsdGsdGsdA*sdCsdCs**Ab^{1}sGb^{1}sTb^{1}sAb^1**
4217	16	218p	**C*b^{1}sAb^{1}sTb^{1}s**dGsdA*sdA*sdTsdGsdGsdA*sdC*sdC*s**Ab^{1}sGb^{1}sTb^{1}sAb^1**
4217	16	218q	**C*b^{1}sAb^{1}sTb^{1}s**dGsdAsdAsdTsdGsdGsdAsdC*sdCs**Ab^{1}sGb^{1}sTb^{1}sAb^1**

4217	16	218c	**C*b^{1}sAb^{1}sTb^{1}s**dGsdAsdAsdTsdGsdGsdAsdCsdC*s**Ab^{1}sGb^{1}sTb^{1}sAb^1**
4217	16	218r	**C*b^{1}Ab^{1}Tb^1**dGdAdAdTdGdGdAdCdC**Ab^{1}Gb^{1}Tb^{1}Ab^1**
4217	16	218s	**C*b^{1}sAb^{1}sTb^{1}s**dGdAdAdTdGdGdAdC*sdC*s**Ab^{1}sGb^{1}sTb^{1}sAb^1**
4217	16	218t	**/5SpC3s/C*b^{1}sAb^{1}sTb^{1}s**dGsdAsdAsdTsdGsdGsdAsdCsdCs**Ab^{1}sGb^{1}sTb^1 sAb^1**
4217	16	218u	**C*b^{1}sAb^{1}sTb^{1}s**dGsdAsdAsdTsdGsdGsdAsdCsdCs**Ab^{1}sGb^{1}sTb^{1}sAb^1 /3SpC3s/**
4217	16	218v	**/5SpC3s/C*b^{1}sAb^{1}sTb^{1}s**dGsdAsdAsdTsdGsdGsdAsdCsdCs**Ab^{1}sGb^{1}sTb^1 sAb^1/3SpC3s/**
4217	16	218ag	**C*b^{1}sAb^{1}sTb^{1}s**dGsdA*sdA*sdUsdGsdGsdA*sdCsdCs**Ab^{1}sGb^{1}sTb^{1}sAb^1**
4217	16	218ah	**C*b^{4}ssAb^{4}ssTb^{4}ss**dGssdA*ssdA*ssdTssdGssdGssdA*ssdCssdCssdAssdGss **Tb^{4}ssAb^4**
4217	16	218ai	**C*b^{2}ssAb^{2}ssTb^{2}ssGb^{2}ss**dAssdAssdTssdGssdGssdAssdCssdCssdAssdGss dTss**Ab^2**
4217	16	218aj	**C*b^{1}Ab^{1}Tb^{1}Gb^1**dAdAdUdGdGdAdCdC**Ab^{1}Gb^{1}Tb^{1}Ab^1**
4217	16	218ak	**C*b^{1}sAb^{1}sTb^{1}sGb^{1}sAb^{1}s**dA*sdUsdGsdGsdAsdCsdCsdA*s**Gb^{1}sTb^{1}sAb^1**
4217	16	218am	**C*b^{1}sAb^{1}s**dUsdGsdAsdAsdUsdGsdGsdAsdCs**C*b^{1}sAb^{1}sGb^{1}sTb^{1}sAb^1**
4217	16	218an	**C*b^{6}sAb^{6}sTb^{6}sGb^{6}s**dAsdAsdTsdGsdGsdAsdCsdCsdAs**Gb^{6}sTb^{6}sAb^6**
4217	16	218ao	**C*b^{7}sAb^{7}sTb^{7}s**dGsdA*sdA*sdUsdGsdGsdAsdCsdCsdA*s**Gb^{7}sTb^{7}sAb^7**
4217	16	218ap	**C*b^{4}sAb^{4}sTb^{4}sGb^{4}s**dA*sdAsdTsdGsdGsdAsdCsdC*sdAsdGs**Tb^{4}sAb^4**
4217	16	218aq	**C*b^{4}Ab^{4}Tb^{4}Gb^4**dAdAdTdGdGdAdCdCdAdG**Tb^{4}Ab^4**
4217	16	218ar	**C*b^{1}sAb^{1}sTb^1**sdGsdAsdAsdTsdGsdGsdAsdCsdCsdAs**Gb^{1}sTb^{1}sAb^1**
4216	17	224a	**C*b^{1}sAb^{1}sTb^{1}sGb^{1}sAb^{1}s**dAsdTsdGsdGsdAsdCsdCs**Ab^{1}sGb^{1}sTb^{1}sAb^{1}sTb^1**
4216	17	224b	**C*b^{2}sAb^{2}sTb^{2}s**dGsdAsdAsdTsdGsdGsdAsdCsdCs**Ab^{2}sGb^{2}sTb^{2}sAb^{2}sTb^2**
4216	17	224c	**C*b^{1}sAb^{1}sTb^{1}sGb^{1}s**dAsdAsdTsdGsdGsdAsdCsdCsdAsdGs**Tb^{1}sAb^{1}sTb^1**
4216	17	224d	**C*b^{1}s**dAsdUsdGsdAsdAsdUsdGsdGsdAsdC*sdC*s**Ab^{1}sGb^{1}sTb^{1}sAb^{1}sTb^1**
4216	17	224e	**C*b^{1}sAb^{1}sTb^{1}s**dGsdA*sdA*sdTsdGsdGsdA*sdC*sdC*s**Ab^{1}sGb^{1}sTb^{1}sAb^1 sTb^1**
4216	17	224f	**C*b^{1}Ab^1**dTdGdAdAdTdGdGdAdCdCdA**Gb^{1}Tb^{1}Ab^{1}Tb^1**
4216	17	224g	**C*b^{1}s**dAsdTsdGsdAsdAsdTsdGsdGsdAsdCsdCsdAsdGs**Tb^{1}sAb^{1}sTb^1**
4216	17	224h	**C*b^{1}Ab^{1}Tb^{1}Gb^{1}Ab^1**dA*dTdGdGdA*dC*dC*dAdGdT**Ab^{1}Tb^1**
4216	17	224i	**C*b^{1}ssAb^{1}ssTb^{1}ssGb^{1}ssAb^{1}ss**dAssdTssdGssdGssdAssdCssdCssdAssdGss **Tb^{1}ssAb^{1}ssTb^1**
4216	17	224j	**C*b^{4}Ab^{4}Tb^4**dGdA*dA*dTdGdGdA*dCdCdA**Gb^{4}Tb^{4}Ab^{4}Tb^4**
4216	17	224k	**C*b^{6}sAb^{6}sTb^{6}s**dGsdA*sdA*sdUsdGsdGsdA*sdC*sdC*sdAsdGs**Tb^{6}sAb^{6}sTb^6**
4216	17	224m	**C*b^{7}sAb^{7}sTb^{7}sGb^{7}s**dAdAdTdGdGdAdC*dC*dAs**Gb^{7}sTb^{7}sAb^{7}sTb^7**
4216	18	225a	**Tb^{1}sC*b^{1}sAb^{1}sTb^{1}sGb^{1}s**dAsdAsdTsdGsdGsdAsdCsdCs**Ab^{1}sGb^{1}sTb^{1}sAb^1 sTb^1**
4216	18	225b	**Tb^{7}sC*b^{7}sAb^{7}sTb^{7}sGb^{7}s**dAsdAsdTsdGsdGsdAsdCsdCsdAsdGsdTsdAs**Tb^7**
4216	18	225c	**Tb^{1}sC*b^{1}sAb^{1}sTb^{1}s**dGsdAsdAsdTsdGsdGsdAsdC*sdC*sdAs**Gb^{1}sTb^{1}sAb^1**

			sTb^{1}
4216	18	225d	**Tb**^{1}**sC*b**^{1}**sAb**^{1}**s**dTsdGsdAsdAsdTsdGsdGsdAsdCsdCsdAs**Gb**^{1}**sTb**^{1}**sAb**^{1}**sTb**^{1}
4216	18	225e	**Tb**^{1}**sC*b**^{1}**sAb**^{1}**sTb**^{1}**s**dGsdAsdAsdTsdGsdGsdAsdCsdCsdAsdGs**Tb**^{1}**sAb**^{1}**sTb**^{1}
4216	18	225f	**Tb**^{1}**C*b**^{1}dA*dTdGdAdAdUdGdGdAdCdC***Ab**^{1}**Gb**^{1}**Tb**^{1}**Ab**^{1}**Tb**^{1}
4216	18	225g	**Tb**^{4}**C*b**^{4}**Ab**^{4}**Tb**^{4}**s**dGsdAsdAsdTsdGsdGsdAsdCsdCs**Ab**^{4}**Gb**^{4}**Tb**^{4}**Ab**^{4}**Tb**^{4}
4216	18	225h	**Tb**^{1}**ssC*b**^{1}**ssAb**^{1}**ss**dTssdGssdA*ssdA*ssdTssdGssdGssdAssdCssdC*ssdA*ssdGss**Tb**^{1}**ssAb**^{1}**ssTb**^{1}
4216	18	225i	**Tb**^{2}**C*b**^{2}**Ab**^{2}dTdGdAdAdTdGdGdAdC*dC***Ab**^{2}**Gb**^{2}**Tb**^{2}**Ab**^{2}**Tb**^{2}
4215	19	226a	**Tb**^{1}**sC*b**^{1}**sAb**^{1}**sTb**^{1}**sGb**^{1}**s**dAsdAsdTsdGsdGsdAsdCsdCsdAs**Gb**^{1}**sTb**^{1}**sAb**^{1}**sTb**^{1}**sTb**^{1}
4215	19	226b	**Tb**^{6}**C*b**^{6}**Ab**^{6}**Tb**^{6}**Gb**^{6}dAdAdTdGdGdAdCdCdA**Gb**^{6}**Tb**^{6}**Ab**^{6}**Tb**^{6}**Tb**^{6}
4215	19	226c	**Tb**^{1}**sC*b**^{1}**sAb**^{1}**sTb**^{1}**s**dGsdAsdAsdTsdGsdGsdAsdCsdCsdAsdGsdTs**Ab**^{1}**sTb**^{1}**sTb**^{1}
4215	19	226d	**Tb**^{1}**s**dCsdAsdTsdGsdAsdA*sdUsdGsdGsdAsdCsdCsdAs**Gb**^{1}**sTb**^{1}**sAb**^{1}**sTb**^{1}**sTb**^{1}
4215	19	226e	**Tb**^{4}**sC*b**^{4}**s**dAsdUsdGsdAsdAsdUsdGsdGsdAsdCsdC*sdAsdGs**Tb**^{4}**sAb**^{4}**sTb**^{4}**sTb**^{4}
4215	19	226f	**Tb**^{2}**ssC*b**^{2}**ssAb**^{2}**ssTb**^{2}**ssGb**^{2}**ss**dAssdAssdTssdGssdGssdAssdCssdCssdAssdGssdTssdAss**Tb**^{2}**ssTb**^{2}
4215	20	227a	**C*b**^{1}**sTb**^{1}**sC*b**^{1}**sAb**^{1}**sTb**^{1}**s**dGsdAsdAsdTsdGsdGsdAsdCsdCsdAs**Gb**^{1}**sTb**^{1}**sAb**^{1}**sTb**^{1}**sTb**^{1}
4215	20	227b	**C*b**^{2}**sTb**^{2}**sC*b**^{2}**s**dAsdTsdGsdAsdAsdTsdGsdGsdAsdCsdCsdAs**Gb**^{2}**sTb**^{2}**sAb**^{2}**sTb**^{2}**sTb**^{2}
4215	20	227c	**C*b**^{1}**Tb**^{1}**C*b**^{1}dAdTdGdAdAdTdGdGdAdCdC*dAdG**Tb**^{1}**Ab**^{1}**Tb**^{1}**Tb**^{1}
4215	20	227d	**C*b**^{1}**s**dUsdCsdAsdTsdGsdAsdAsdTsdGsdGsdAsdCsdCsdAs**Gb**^{1}**sTb**^{1}**sAb**^{1}**sTb**^{1}**sTb**^{1}
4215	20	227e	**C*b**^{4}**sTb**^{4}**sC*b**^{4}**sAb**^{4}**s**dTsdGsdAsdAsdTsdGsdGsdAsdCsdCsdAsdGs**Tb**^{4}**sAb**^{4}**sTb**^{4}**sTb**^{4}
4214	22	228a	**Tb**^{1}**sC*b**^{1}**sTb**^{1}**sC*b**^{1}**sAb**^{1}**s**dTsdGsdAsdAsdTsdGsdGsdAsdCsdCsdAsdGs**Tb**^{1}**sAb**^{1}**sTb**^{1}**sTb**^{1}**sC*b**^{1}
4214	22	228b	**Tb**^{1}**C*b**^{1}**Tb**^{1}**C*b**^{1}**Ab**^{1}dTdGdAdAdTdGdGdAdC*dC*dAdG**Tb**^{1}**Ab**^{1}**Tb**^{1}**Tb**^{1}**C*b**^{1}
4214	22	228c	**Tb**^{6}**sC*b**^{6}**sTb**^{6}**s**dCsdAsdTsdGsdAsdAsdTsdGsdGsdAsdCsdCsdAsdGsdTs**Ab**^{6}**sTb**^{6}**sTb**^{6}**sC*b**^{6}
4213	24	229a	**Ab**^{1}**sTb**^{1}**sC*b**^{1}**sTb**^{1}**sC*b**^{1}**s**dAsdTsdGsdAsdAsdTsdGsdGsdAsdC*sdCsdAsdGsdTs**Ab**^{1}**sTb**^{1}**sTb**^{1}**sC*b**^{1}**sTb**^{1}
4213	24	229b	**Ab**^{1}**Tb**^{1}**C*b**^{1}**Tb**^{1}**C*b**^{1}AdTdGdAdAdTdGdGdAdCdCdAdGdT**Ab**^{1}**Tb**^{1}**Tb**^{1}**C*b**^{1}**Tb**^{1}
4212	26	230a	**Tb**^{1}**sAb**^{1}**sTb**^{1}**sC*b**^{1}**sTb**^{1}**s**dCsdAsdTsdGsdAsdAsdTsdGsdGsdAsdCsdCsdAsdGsdTsdAs**Tb**^{1}**sTb**^{1}**sC*b**^{1}**sTb**^{1}**sAb**^{1}
4212	26	230a	**Tb**^{1}**sAb**^{1}**sTb**^{1}**sC*b**^{1}**sTb**^{1}**s**dCsdAsdTsdGsdAsdAsdTsdGsdGsdAsdCsdCsdAsdGsdTsdAs**Tb**^{1}**sTb**^{1}**sC*b**^{1}**sTb**^{1}**sAb**^{1}
4212	26	230b	**Tb**^{1}**Ab**^{1}**Tb**^{1}**C*b**^{1}**Tb**^{1}dCdAdTdGdAdAdTdGdGdAdCdCdAdGdTdA**Tb**^{1}**Tb**^{1}**C*b**^{1}**Tb**^{1}**Ab**^{1}
4211	28	231a	**Ab**^{1}**sTb**^{1}**sAb**^{1}**sTb**^{1}**sC*b**^{1}**s**dTsdCsdAsdTsdGsdAsdAsdTsdGsdGsdAsdCsdCsdAsdGsdTsdAsdTs**Tb**^{1}**sC*b**^{1}**sTb**^{1}**sAb**^{1}**sGb**^{1}

4211	28	231b	**Ab^{1}Tb^{1}Ab^{1}Tb^{1}C*b^1**dTdCdAdTdGdAdAdTdGdGdAdCdCdAdGdTdAdT**Tb^{1}C*b^1 Tb^{1}Ab^{1}Gb^1**

Table 9

SP	L	Seq ID No.	Sequence, 5'-3'
2358	10	284a	**C*b^{1}sAb^{1}s**dTsdTsdAsdAsdTsdA*s**Ab^{1}sAb^1**
2358	10	284b	**C*b^{1}Ab^1**dTdTdA*dAdTdA***Ab^{1}Ab^1**
2357	12	285a	**Gb^{1}sC*b^{1}sAb^{1}s**dTsdTsdA*sdA*sdTsdAs**Ab^{1}sAb^{1}sGb^1**
2357	12	285b	**Gb^{1}sC*b^{1}sAb^{1}s**dTsdTsdA*sdA*sdTsdAsdA*s**Ab^{1}sGb^1**
2357	12	285c	**Gb^{1}sC*b^{1}s**dAsdTsdTsdA*sdA*sdUsdAsdA*sdA*s**Gb^1**
2357	12	285d	**Gb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAs**Ab^{1}sGb^1**
2357	12	285e	**Gb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdA*s**Ab^{1}sGb^1**
2357	12	285f	**Gb^{1}C*b^{1}Ab^1**dTdTdA*dA*dTdA**Ab^{1}Ab^{1}Gb^1**
2356	13	286a	**Gb^{1}sC*b^{1}sAb^{1}sTb^{1}s**dTsdAsdAsdTsdAsdAs**Ab^{1}sGb^{1}sTb^1**
2356	13	286b	**Gb^{1}sC*b^{1}sAb^{1}sTb^{1}s**dTsdA*sdA*sdTsdAsdAs**Ab^{1}sGb^{1}sTb^1**
2356	13	286c	**Gb^{1}sC*b^{1}sAb^{1}s**dUsdTsdAsdAsdTsdAsdAsdA*s**Gb^{1}sTb^1**
2356	13	286d	**Gb^{1}sC*b^{1}s**dAsdTsdTsdAsdA*sdUsdAsdAsdAsdGs**Tb^1**
2356	13	286e	**Gb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAs**Ab^{1}sAb^{1}sGb^{1}sTb^1**
2356	13	286f	**Gb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdA*s**Ab^{1}sAb^{1}sGb^{1}sTb^1**
2356	13	286g	**Gb^{1}C*b^{1}Ab^{1}Tb^1**dUdAdAdUdAdA**Ab^{1}Gb^{1}Tb^1**
2356	14	287a	**Gb^{1}sGb^{1}sC*b^{1}sAb^{1}s**dTsdTsdA*sdAsdTsdAs**Ab^{1}sAb^{1}sGb^{1}sTb^1**
2356	14	287b	**Gb^{4}sGb^{4}sC*b^{4}sAb^{4}s**dTsdTsdA*sdAsdUsdAsdAsdA*s**Gb^{4}sTb^4**
2356	14	287c	**Gb^{1}s**dGsdCsdAsdUsdUsdAsdAsdTsdA*sdA*sdA*sdGs**Tb^1**
2356	14	287d	**Gb^{2}sGb^{2}sC*b^{2}s**dA*sdUsdTsdA*sdAsdTsdAsdA*s**Ab^{2}sGb^{2}sTb^2**
2356	14	287e	**Gb^{1}Gb^{1}C*b^{1}Ab^{1}s**dTsdTsdAsdAsdTsdA*sdA*s**Ab^{1}Gb^{1}Tb^1**
2356	14	287f	**Gb^{1}sGb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAs**Ab^{1}sAb^{1}sGb^{1}sTb^1**
2356	14	287g	**Gb^{1}sGb^{1}s**dC*sdA*sdTsdTsdA*sdA*sdTsdA*s**Ab^{1}sAb^{1}sGb^{1}sTb^1**
2356	14	287h	**Gb^{1}Gb^1**dC*dAdTdTdAdAdTdA**Ab^{1}Ab^{1}Gb^{1}Tb^1**
2356	14	287i	**Gb^{4}ssGb^{4}ss**dCssdAssdTssdTssdAssdAssdTssdAss**Ab^{4}ssAb^{4}ssGb^{4}ssTb^4**
2356	14	287j	**Gb^{4}ssGb^{4}ss**dC*ssdAssdTssdTssdAssdAssdTssdAss**Ab^{4}ssAb^{4}ssGb^{4}ssTb^4**
2355	15	288a	Gb^{1}sGb^{1}s**d**C*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}s**Tb^{1}sGb^1
2355	15	288b	Gb^{1}sGb^{1}sC*b^{1}sAb^{1}s**d**TsdTsdA*sdA*sdTsdAsdAsdAsdGsdTsG**b^1**
2355	15	288c	Gb^{4}sGb^{4}sC*b^{4}s**d**AsdTsdTsdAsdAsdTsdAsdAsdAsdGsT**b^{4}s**Gb^4
2355	15	288d	**Gb^{1}sGb^{1}sC*b^{1}sAb^{1}s**dTdTdAdAdTdAdAs**Ab^{1}sGb^{1}sTb^{1}sGb^1**
2355	15	288e	**Gb^{1}Gb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAs**Ab^{1}Gb^{1}Tb^{1}Gb^1**
2355	15	288f	Gb^1**ss**Gb^1**ss**dCssdAssdUssdUssdAssdAssdUssdAssdAssdAssdGss**Tb^1 ssGb^1**
2355	15	288g	Gb^1**ss**Gb^1**ss**dCssdAssdTssdTssdAssdAssdTssdAssdAssdAssdGssdTss**Gb^1**

2355	15	288h	**Gb^{6}Gb^{6}C*b^6**dA*dTdTdAdAdUdA*dA*dA**Gb^{6}Tb^{6}Gb^6**
2355	15	288i	**Gb^{1}Gb^{1}C*b^1**dAdTdTdAdAdUdAdAdA**Gb^{1}Tb^{1}Gb^1**
2355	16	289a	**Ab^{1}sGb^{1}sGb^{1}sC*b^{1}sAb^{1}s**dTsdTsdA*sdA*sdTsdA*s**Ab^{1}sAb^{1}sGb^{1}sTb^{1}sGb^1**
2355	16	289b	**Ab^{1}sGb^{1}sGb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAs**Ab^{1}sAb^{1}sGb^{1}sTb^{1}sGb^1**
2355	16	289c	**Ab^{1}sGb^{1}sGb^{1}s**dC*sdA*sdTsdTsdA*sdA*sdTsdA*s**Ab^{1}sAb^{1}sGb^{1}sTb^{1}sGb^1**
2355	16	289d	**Ab^{2}sGb^{2}sGb^{2}s**dC*sdAsdTsdTsdAsdAsdTsdAs**Ab^{2}sAb^{2}sGb^{2}sTb^{2}sGb^2**
2355	16	289e	**Ab^{1}s**dGsdGsdC*sdAsdTsdTsdAsdAsdTsdAs**Ab^{1}sAb^{1}sGb^{1}sTb^{1}sGb^1**
2355	16	289f	**Ab^{1}s**dGsdGsdC*sdAsdTsdUsdAsdAsdUsdAs**Ab^{1}sAb^{1}sGb^{1}sTb^{1}sGb^1**
2355	16	289g	**Ab^{1}sGb^{1}sGb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAs**Ab^{1}sGb^{1}sTb^{1}sGb^1**
2355	16	289h	**Ab^{1}sGb^{1}sGb^{1}sC*b^{1}s**dA*sdTsdTsdA*sdA*sdTsdA*sdAsdAs**Gb^{1}sTb^{1}sGb^1**
2355	16	289i	**Ab^{6}sGb^{6}sGb^{6}s**dC*sdA*sdUsdTsdAsdAsdTsdAsdAsdAs**Gb^{6}sTb^{6}sGb^6**
2355	16	289j	**Ab^{1}sGb^{1}sGb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^1**
2355	16	289k	**Ab^{1}s**dGsdGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^1**
2355	16	289m	**Ab^{1}Gb^{1}Gb^{1}C*b^{1}Ab^1**dUdTdA*dA*dTdAdAdAdG**Tb^{1}Gb^1**
2355	16	289n	**Ab^{1}Gb^1**dGdC*dAdTdTdAdAdTdAdA**Ab^{1}Gb^{1}Tb^{1}Gb^1**
2355	16	289o	**Ab^{4}Gb^{4}Gb^4**dCdA*dTdTdAdAdTdAdA***Ab^{4}Gb^{4}Tb^{4}Gb^4**
2355	16	289p	**Ab^{1}ssGb^{1}ssGb^{1}ssC*b^{1}ssAb^{1}ss**dTssdTssdAssdAssdTssdAssdAssdAss **Gb^{1}ssTb^{1}ssGb^1**
2355	16	289q	**Ab^{7}sGb^{7}sGb^{7}sC*b^{7}s**dA*dTdTdAdAdTdAdA*s**Ab^{7}sGb^{7}sTb^{7}sGb^7**
2355	17	213j	**C*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^1**
2355	17	213k	**C*b^{1}sAb^{1}sGb^{1}s**dGsdCsdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^1**
2355	17	213m	**C*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdA*sdA*s**Gb^{1}sTb^{1}sGb^1**
2355	17	213n	**C*b^{1}Ab^{1}Gb^1**dGdC*dAdTdTdAdAdTdAdAdA**Gb^{1}Tb^{1}Gb^1**
2355	17	213o	**/5SpC3s/C*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^1 sGb^1**
2355	17	213p	**C*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^1 /3SpC3s/**
2355	17	213q	**/5SpC3s/C*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^1 sGb^1/3SpC3s/**
2355	17	213r	**C*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdUsdAsdAsdA*s**Gb^{1}sTb^{1}sGb^1**
2355	17	213s	**C*b^{6}sAb^{6}sGb^{6}s**dGdC*dAdTdTdAdAdTdAdAdAs**Gb^{6}sTb^{6}sGb^6**
2355	17	213t	**C*b^{1}sAb^{1}sGb^{1}s**dGdC*dAdTdTdAdAdTdAdAdAs**Gb^{1}sTb^{1}sGb^1**
2355	17	213u	**C*b^{1}Ab^{1}Gb^{1}s**dGsdC*sdAsdUsdUsdAsdAsdUsdAsdAsdAs**Gb^{1}Tb^{1}Gb^1**
2355	17	213v	**C*b^{1}Ab^{1}Gb^{1}s**dGsdC*sdAsdTsdTsdAsdA*sdTsdAsdAsdA*s**Gb^{1}Tb^{1}Gb^1**
2355	17	213w	**C*b^{1}Ab^{1}Gb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}Tb^{1}Gb^1**
2355	17	213x	**C*b^{7}sAb^{7}sGb^{7}sGb^{7}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{7}sTb^{7}sGb^7**
2355	17	213y	**C*b^{6}sAb^{6}sGb^{6}sGb^{6}s**dCsdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{6}sTb^{6}sGb^6**
2355	17	213z	**C*b^{7}sAb^{7}sGb^{7}s**dGsdCsdA*sdUsdTsdAsdAsdTsdAsdAs**Ab^{7}sGb^{7}sTb^{7}sGb^7**

2355	17	213aa	**C*b^{4}sAb^{4}sGb^{4}sGb^{4}s**dC*sdA*sdTsdTsdAsdAsdTsdAsdA*sdAsdGs**Tb^{4}sGb^4**
2355	17	213ab	**C*b^{1}sAb^{1}sGb^{1}sGb^{1}sC*b^{1}s**dA*sdTsdTsdA*sdA*sdTsdAsdA*sdA*s**Gb^{1}sTb^1 sGb^1**
2355	17	213ac	**C*b^{1}sAb^{1}sGb^{1}s**dGsdCsdAsdTsdTsdA*sdA*sdTsdAs**Ab^{1}sAb^{1}sGb^{1}sTb^{1}sGb^1**
2355	17	213ad	**C*b^{1}sAb^{1}s**dGsdGsdC*sdAsdTsdTsdAsdAsdUsdAsdAs**Ab^{1}sGb^{1}sTb^{1}sGb^1**
2355	17	213ae	**C*b^{1}ssAb^{1}ssGb^{1}ss**dGssdC*ssdAssdTssdTssdAssdAssdTssdAssdAss**Ab^1 ssGb^{1}ssTb^{1}ssGb^1**
2355	17	213af	**C*b^{4}ssAb^{4}ssGb^{4}ss**dGssdCssdAssdTssdTssdA*ssdAssdTssdAssdAssdAss dGss**Tb^{4}ssGb^4**
2355	17	213ag	**C*b^{2}ssAb^{2}ssGb^{2}ssGb^{2}ss**dCssdAssdTssdTssdAssdAssdTssdAssdAssdAss dGssdTss**Gb^2**
2355	17	213ah	**C*b^{1}Ab^{1}Gb^{1}Gb^1**dCdAdTdTdAdAdUdAdA**Ab^{1}Gb^{1}Tb^{1}Gb^1**
2355	17	213ai	**C*b^{4}Ab^{4}Gb^{4}Gb^4**dCdAdTdTdAdAdTdAdAdAdG**Tb^{4}Gb^4**
2355	17	213aj	**C*b^{1}Ab^{1}Gb^1**dGdCdAdTdTdAdAdUdAdAdA**Gb^{1}Tb^{1}Gb^1**
2355	17	213ak	**C*b^{1}sAb^{1}sGb^{1}sGb^1**sdCsdAsdTsdTsdAsdAsdTsdAsdAs**Ab^{1}sGb^{1}sTb^{1}sGb^1**
2354	18	290a	**C*b^{1}sAb^{1}sGb^{1}sGb^{1}sC*b^{1}s**dAsdTsdTsdAsdAsdTsdA*sdAs**Ab^{1}sGb^{1}sTb^{1}sGb^1 sC*b^1**
2354	18	290b	**C*b^{1}sAb^{1}sGb^{1}sGb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^1 sC*b^1**
2354	18	290c	**C*b^{1}sAb^{1}sGb^{1}sGb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAsdAsdGs**Tb^{1}sGb^1 sC*b^1**
2354	18	290d	**C*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^1 sC*b^1**
2354	18	290e	**C*b^{7}sAb^{7}sGb^{7}sGb^{7}sC*b^{7}s**dA*sdTsdTsdAsdAsdTsdAsdAsdAsdGsdTsdG **sC*b^7**
2354	18	290f	**C*b^{4}Ab^{4}Gb^{4}Gb^{4}s**dCsdAsdTsdTsdAsdAsdTsdA*sdAs**Ab^{4}Gb^{4}Tb^{4}Gb^{4}C*b^4**
2354	18	290g	**C*b^{1}ssAb^{1}ssGb^{1}ss**dGssdC*ssdAssdTssdTssdA*ssdAssdTssdA*ssdAssdA* ssdGss**Tb^{1}ssGb^{1}ssC*b^1**
2354	18	290h	**C*b^{2}Ab^{2}Gb^2**dGdC*dAdTdTdAdAdTdAdA**Ab^{2}Gb^{2}Tb^{2}Gb^{2}C*b^2**
2354	18	290i	**C*b^{1}Ab^1**dGdGdC*dA*dUdUdAdAdUdA*dA***Ab^{1}Gb^{1}Tb^{1}Gb^{1}C*b^1**
2354	19	291a	**Ab^{1}sC*b^{1}sAb^{1}sGb^{1}sGb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAs**Ab^{1}sGb^{1}sTb^1 sGb^{1}sC*b^1**
2354	19	291b	**Ab^{1}sC*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAsdGs**Tb^{1}sGb^1 sC*b^1**
2354	19	291c	**Ab^{4}sC*b^{4}s**dAsdGsdGsdC*sdAsdTsdTsdAsdAsdUsdAsdAsdAs**Gb^{4}sTb^{4}sGb^4 sC*b^4**
2354	19	291d	**Ab^{1}s**dC*sdAsdGsdGsdC*sdA*sdTsdTsdAsdAsdTsdAsdAs**Ab^{1}sGb^{1}sTb^{1}sGb^1 sC*b^1**
2354	19	291e	**Ab^{2}ssC*b^{2}ssAb^{2}ssGb^{2}ssGb^{2}ss**dCssdAssdTssdTssdAssdAssdTssdAssdAss dAssdGssdTss**Gb^{2}ssC*b^2**
2354	19	291f	**Ab^{6}C*b^{6}Ab^{6}Gb^{6}Gb^6**dC*dAdTdTdAdAdTdAdA**Ab^{6}Gb^{6}Tb^{6}Gb^{6}C*b^6**
2353	20	292a	**Ab^{1}sC*b^{1}sAb^{1}sGb^{1}sGb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^1 sGb^{1}sC*b^{1}sAb^1**

2353	20	292b	**Ab^{2}sC*b^{2}sAb^{2}s**dGsdGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{2}sTb^{2}sGb^2 sC*b^{2}sAb^2**
2353	20	292c	**Ab^{1}s**dC*sdAsdGsdGsdC*sdAsdUsdUsdAsdAsdUsdAsdAsdAs**Gb^{1}sTb^{1}sGb^1 sC*b^{1}sAb^1**
2353	20	292d	**Ab^{4}sC*b^{4}sAb^{4}sGb^{4}s**dGsdCsdAsdTsdTsdAsdAsdTsdAsdAsdAsdGs**Tb^{4}sGb^4 sC*b^{4}sAb^4**
2353	20	292e	**Ab^{1}C*b^{1}Ab^1**dGdGdC*dAdTdTdAdAdTdAdAdAdG**Tb^{1}Gb^{1}C*b^{1}Ab^1**
2352	22	293a	**Tb^{1}sAb^{1}sC*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAsdGs**Tb^1 sGb^{1}sC*b^{1}sAb^{1}sAb^1**
2352	22	293b	**Tb^{1}Ab^{1}C*b^{1}Ab^{1}Gb^1**dGdC*dAdTdTdAdAdTdAdAdAdG**Tb^{1}Gb^{1}C*b^{1}Ab^{1}Ab^1**
2352	22	293c	**Tb^{6}sAb^{6}sC*b^{6}s**dAsdGsdGsdCsdAsdTsdTsdAsdAsdTsdAsdAsdAsdGs**Tb^6 sGb^{6}sC*b^{6}sAb^{6}sAb^6**
2351	24	294a	**Ab^{1}sTb^{1}sAb^{1}sC*b^{1}sAb^{1}s**dGsdGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAsdG sdTs**Gb^{1}sC*b^{1}sAb^{1}sAb^{1}sAb^1**
2351	24	294b	**Ab^{1}Tb^{1}Ab^{1}C*b^{1}Ab^1**dGdGdC*dAdTdTdAdAdTdAdAdAdGdT**Gb^{1}C*b^{1}Ab^{1}Ab^1 Ab^1**
2350	26	295a	**Tb^{1}sAb^{1}sTb^{1}sAb^{1}sC*b^{1}s**dAsdGsdGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs dGsdTsdGs**C*b^{1}sAb^{1}sAb^{1}sAb^{1}sTb^1**
2350	26	295b	**Tb^{1}Ab^{1}Tb^{1}Ab^{1}C*b^1**dAdGdGdC*dAdTdTdAdAdTdAdAdAdGdTdG**C*b^{1}Ab^{1}Ab^1 Ab^{1}Tb^1**
2349	28	236a	**Ab^{1}sTb^{1}sAb^{1}sTb^{1}sAb^{1}s**dC*sdAsdGsdGsdC*sdAsdTsdTsdAsdAsdTsdAsdAs dAsdGsdTsdGsdC*s**Ab^{1}sAb^{1}sAb^{1}sTb^{1}sGb^1**
2349	28	236b	**Ab^{1}Tb^{1}Ab^{1}Tb^{1}Ab^1**dC*dAdGdGdCdAdTdTdAdAdTdAdAdAdGdTdGdC***Ab^{1}Ab^1 Ab^{1}Tb^{1}Gb^1**

Pharmaceutical Compositions

The antisense-oligonucleotides of the present invention are preferably administered in form of their pharmaceutically active salts optionally using substantially nontoxic pharmaceutically acceptable carriers, excipients, adjuvants, solvents or diluents. The medications of the present invention are prepared in a conventional solid or liquid carrier or diluents and a conventional pharmaceutically-made adjuvant at suitable dosage level in a known way. The preferred preparations and formulations are in administrable form which is suitable for infusion or injection (intrathecal, intracerebroventricular, intracranial, intravenous, intraparenchymal, intratumoral, intra- or extraocular, intraperitoneal, intramuscular, subcutaneous), local administration into the brain, inhalation, local administration into a solid tumor or oral application. However also other application forms are possible such as absorption through epithelial or mucocutaneous linings (oral mucosa, rectal and vaginal epithelial linings, nasopharyngial mucosa, intestinal mucosa), rectally, transdermally,

topically, intradermally, intragastrically, intracutaneously, intravaginally, intravasally, intranasally, intrabuccally, percutaneously, sublingually application, or any other means available within the pharmaceutical arts.

The administrable formulations, for example, include injectable liquid formulations, retard formulations, powders especially for inhalation, pills, tablets, film tablets, coated tablets, dispersible granules, dragees, gels, syrups, slurries, suspensions, emulsions, capsules and deposits. Other administratable galenical formulations are also possible like a continuous injection through an implantable pump or a catheter into the brain.

As used herein the term "pharmaceutically acceptable" refers to any carrier which does not interfere with the effectiveness of the biological activity of the antisense-oligonucleotides as active ingredient in the formulation and that is not toxic to the host to which it is administered. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc.. Such carriers can be formulated by conventional methods and the active compound can be administered to the subject at an effective dose.

An "effective dose" refers to an amount of the antisense-oligonucleotide as active ingredient that is sufficient to affect the course and the severity of the disease, leading to the reduction or remission of such pathology. An "effective dose" useful for treating and/or preventing these diseases or disorders may be determined using methods known to one skilled in the art. Furthermore, the antisense-oligonucleotides of the present invention may be mixed and administered together with liposomes, complex forming agents, receptor targeted molecules, solvents, preservatives and/or diluents.

Preferred are pharmaceutical preparations in form of **infusion** solutions or solid matrices for continuous release of the active ingredient, especially for continuous infusion for **intrathecal** administration, intracerebroventricular administration or intracranial administration of at least one antisense-oligonucleotide of the present invention. Also preferred are pharmaceutical preparations in form of solutions or solid matrices suitable for local administration into the brain. For fibrotic diseases of the lung, inhalation formulations are especially preferred.

A ready-to-use sterile solution comprises for example at least one antisense-oligonucleotide at a concentration ranging from 1 to 10 mg/ml, preferably from 5 to 10 mg/ml and an isotonic agent selected, for example, amongst sugars such as sucrose, lactose, mannitol or sorbitol. A suitable buffering agent, to control

the solution pH to 6 to 8 (preferably 7 - 8), may be also included. Another optional ingredient of the formulation can be a non-ionic surfactant, such as Tween 20 or Tween 80.

A sterile lyophilized **powder** to be reconstituted for use comprises at least one antisense-oligonucleotide, and optionally a bulking agent (e.g. mannitol, trehalose, sorbitol, glycine) and/or a cryoprotectent (e.g. trehalose, mannitol). The solvent for reconstitution can be water for injectable compounds, with or without a buffering salt to control the pH to 6 to 8.

Aerosol preparations suitable for **inhalation** may include solutions and solids in powder form, which may be in combination with a pharmaceutically acceptable carrier such as inert compressed gas, e.g. nitrogen.

A particularly preferred pharmaceutical composition is a lyophilized (freeze-dried) preparation (lyophilisate) suitable for administration by inhalation or for intravenous administration. To prepare the preferred lyophilized preparation at least one antisense-oligonucleotide of the invention is solubilized in a 4 to 5% (w/v) mannitol solution and the solution is then lyophilized. The mannitol solution can also be prepared in a suitable buffer solution as described above.

Further examples of suitable cryo- / lyoprotectants (otherwise referred to as bulking agents or stabilizers) include thiol-free albumin, immunoglobulins, polyalkyleneoxides (e.g. PEG, polypropylene glycols), trehalose, glucose, sucrose, sorbitol, dextran, maltose, raffinose, stachyose and other saccharides (cf. for instance WO 97/29782), while mannitol is used preferably. These can be used in conventional amounts in conventional lyophilization techniques. Methods of lyophilization are well known in the art of preparing pharmaceutical formulations.

For administration by inhalation the particle diameter of the lyophilized preparation is preferably between 2 to 5 µm, more preferably between 3 to 4 µm. The lyophilized preparation is particularly suitable for administration using an inhalator, for example the OPTINEB® or VENTA-NEB® inhalator (NEBU-TEC, Elsenfeld, Germany). The lyophilized product can be rehydrated in sterile distilled water or any other suitable liquid for inhalation administration. Alternatively, for intravenous administration the lyophilized product can be rehydrated in sterile distilled water or any other suitable liquid for intravenous administration.

After rehydration for administration in sterile distilled water or another suitable liquid the lyophilized preparation should have the approximate physiological osmolality of the target tissue for the rehydrated peptide preparation i.e. blood for intravenous administration or lung tissue for inhalation administration. Thus it is preferred that the rehydrated formulation is substantially isotonic.

The preferred dosage concentration for either intravenous, oral, or inhalation administration is between 10 to 2000 µmol/ml, and more preferably is between 200 to 800 µmol / ml.

For **oral** administration in the form of tablets or capsules, the at least one antisense-oligonucleotide may be combined with any oral nontoxic pharmaceutically acceptable inert carrier, such as lactose, starch, sucrose, cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, talc, mannitol, ethyl alcohol (liquid forms) and the like. Moreover, when desired or needed, suitable binders, lubricants, disintegrating agents and coloring agents may also be incorporated in the mixture. Powders and tablets may be comprised of from about 5 to about 95 percent inventive composition.

Suitable binders include starch, gelatin, natural sugars, corn sweeteners, natural and synthetic gums such as acacia, sodium alginate, carboxymethyl-cellulose, polyethylene glycol and waxes. Among the lubricants that may be mentioned for use in these dosage forms, boric acid, sodium benzoate, sodium acetate, sodium chloride, and the like. Disintegrants include starch, methylcellulose, guar gum and the like.

Additionally, the compositions of the present invention may be formulated in sustained release form to provide the rate controlled release of the at least one antisense-oligonucleotide to optimize the therapeutic effects. Suitable dosage forms for sustained release include implantable biodegradable matrices for sustained release containing the at least one antisense-oligonucleotide, layered tablets containing layers of varying disintegration rates or controlled release polymeric matrices impregnated with the at least one antisense-oligonucleotide.

Liquid form preparations include solutions, suspensions and emulsions. As an example may be mentioned water or water-propylene glycol solutions for parenteral injections or addition of sweeteners and opacifiers for oral solutions, suspensions and emulsions.

Suitable diluents are substances that usually make up the major portion of the composition or dosage form. Suitable diluents include sugars such as lactose, sucrose, mannitol and sorbitol, starches derived from wheat, corn rice and potato, and celluloses such as microcrystalline cellulose. The amount of diluents in the composition can range from about 5% to about 95% by weight of the total composition, preferably from about 25% to about 75% by weight.

The term disintegrants refers to materials added to the composition to help it break apart (disintegrate) and release the medicaments. Suitable disintegrants include starches, "cold water soluble" modified starches such as sodium carboxymethyl starch, natural and synthetic gums such as locust bean, karaya, guar, tragacanth and agar, cellulose derivatives such as methylcellulose and sodium carboxymethylcellulose, microcrystalline celluloses and cross-linked microcrystalline celluloses such as sodium croscarmellose, alginates such as alginic acid and sodium alginate, clays such as bentonites, and effervescent mixtures. The amount of disintegrant in the composition can range from about 1 to about 40% by weight of the composition, preferably 2 to about 30% by weight of the composition, more preferably from about 3 to 20% by weight of the composition, and most preferably from about 5 to about 10% by weight.

Binders characterize substances that bind or "glue" powders together and make them cohesive by forming granules, thus serving as the "adhesive" in the formulation. Binders add cohesive strength already available in the diluents or bulking agent. Suitable binders include sugars such as sucrose, starches derived from wheat, corn rice and potato; natural gums such as acacia, gelatin and tragacanth; derivatives of seaweed such as alginic acid, sodium alginate and ammonium calcium alginate; cellulosic materials such as methylcellulose and sodium carboxymethylcellulose and hydroxypropyl-methylcellulose; polyvinylpyrrolidone; and inorganics such as magnesium aluminum silicate. The amount of binder in the composition can range from about 1 to 30% by weight of the composition, preferably from about 2 to about 20% by weight of the composition, more preferably from about 3 to about 10% by weight, even more preferably from about 3 to about 6% by weight.

Lubricant refers to a substance added to the dosage form to enable the tablet, granules, etc. after it has been compressed, to release from the mold or die by reducing friction or wear. Suitable lubricants include metallic stearates, such as magnesium stearate, calcium stearate or potassium stearate, stearic acid; high melting point waxes; and water soluble lubricants, such as sodium chloride, sodium benzoate, sodium acetate, sodium oleate, polyethylene glycols and D,L-leucine.

Lubricants are usually added at the very last step before compression, since they must be present on the surfaces of the granules and in between them and the parts of the tablet press. The amount of lubricant in the composition can range from about 0.05 to about 15% by weight of the composition, preferably 0.2 to about 5% by weight of the composition, more preferably from about 0.3 to about 3%, and most preferably from about 0.3 to about 1.5% by weight of the composition.

Glidents are materials that prevent caking and improve the flow characteristics of granulations, so that flow is smooth and uniform. Suitable glidents include silicon dioxide and talc. The amount of glident in the composition can range from about 0.01 to 10% by weight of the composition, preferably 0.1% to about 7% by weight of the total composition, more preferably from about 0.2 to 5% by weight, and most preferably from about 0.5 to about 2% by weight.

In the pharmaceutical compositions disclosed herein the antisense-oligonucleotides are incorporated preferably in the form of their salts and optionally together with other components which increase stability of the antisense-oligonucleotides, increase recruitment of RNase H, increase target finding properties, enhance cellular uptake and the like. In order to achieve these goals, the antisense-oligonucleotides may be chemically modified instead of or in addition to the use of the further components useful for achieving these purposes. Thus the antisense-oligonucleotides of the invention may be chemically linked to moieties or components which enhance the activity, cellular distribution or cellular uptake etc. of the antisense-oligonucleotides. Such moieties include lipid moieties such as a cholesterol moiety, cholic acid, a thioether, hexyl-S-tritylthiol, a thiocholesterol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipid such as dihexadecyl-rac-glycerol or triethylammonium-1,2-di-O-hexadecyl-rac-glycero-3H-phosphonate, a polyamine or a polyethylene glycol chain, or adamantine acetic acid, a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety. The present invention also includes antisense-oligonucleotides which are chimeric compounds. "Chimeric" antisense-oligonucleotides in the context of this invention, are antisense-oligonucleotides, which contain two or more chemically distinct regions, one is the oligonucleotide sequence as disclosed herein which is connected to a moiety or component for increasing cellular uptake, increasing resistance to nuclease degradation, increasing binding affinity for the target nucleic acid, increasing recruitment of RNase H and so on. For instance, the additional region or moiety or component of the antisense-oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA hybrids or RNA:RNA molecules. By way of example, RNase H is a cellular endoribonuclease which cleaves the RNA strand of an

RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target which is the mRNA coding for the TGF-R_{II}, thereby greatly enhancing the efficiency of antisense-oligonucleotide inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used.

Indications

The present invention relates to the use of the antisense-oligonucleotides disclosed herein for prophylaxis and treatment of neurodegenerative diseases, neurotrauma, neurovascular and neuroinflammatory diseases, including postinfectious and inflammatory disorders of the central nervous system (CNS).

The antisense-oligonucleotides of the present invention are especially useful for promoting regeneration and functional reconnection of damaged nerve pathways and/or for the treatment and compensation of age induced decreases in neuronal stem cell renewal.

Thus, another aspect of the present invention relates to the use of an antisense-oligonucleotide as disclosed herein for promoting regeneration neuronal tissue by reactivating neurogenesis, allowing neuronal differentiation and migration, and inducing integration of new neurons into anatomic and functional neuronal circuits.

A further aspect of the present invention relates to the use of an antisense-oligonucleotide as disclosed herein for promoting regeneration and clinical (structural) repair in patients with damage to the nervous system or damage to other organ systems induced by fibrosis or loss of stem cell turnover.

Moreover, the antisense-oligonucleotides are useful for compensation and treatment of decreases in neuronal stem cell renewal induced by age, inflammation or a gene defect.

The antisense-oligonucleotides of the present invention inhibit the TGF-R_{II} expression and are consequently used for the treatment of diseases associated with up-regulated or enhanced TGF-R_{II} and/or TGF-R_{II} levels.

Thus, another aspect of the present invention relates to the use of the antisense-oligonucleotides in the prophylaxis and treatment of neurodegenerative

diseases, neuroinflammatory disorders, traumatic or posttraumatic disorders, vascular or more precisely neurovascular disorders, hypoxic disorders, postinfectious central nervous system disorders, fibrotic diseases, hyperproliferative diseases, cancer, tumors, presbyakusis and presbyopie.

The term "neurodegenerative disease" or "neurological disease" or "neuroinflammatory disorder" refers to any disease, disorder, or condition affecting the central or peripheral nervous system, including ADHD, AIDS-neurological complications, absence of the Septum Pellucidum, acquired epileptiform aphasia, acute disseminated encephalomyelitis, adrenoleukodystrophy, agenesis of the Corpus Callosum, agnosia, Aicardi Syndrome, Alexander Disease, Alpers' Disease, alternating hemiplegia, Alzheimer's Disease, amyotrophic lateral sclerosis (ALS), anencephaly, aneurysm, Angelman Syndrome, angiomatosis, anoxia, aphasia, apraxia, arachnoid cysts, arachnoiditis, Arnold-Chiari Malformation, arteriovenous malformation, aspartame, Asperger Syndrome, ataxia telangiectasia, ataxia, attention deficit-hyperactivity disorder, autism, autonomic dysfunction, back pain, Barth Syndrome, Batten Disease, Behcet's Disease, Bell's Palsy, benign essential blepharospasm, benign focal amyotrophy, benign intracranial hypertension, Bernhardt-Roth Syndrome, Binswanger's Disease, blepharospasm, Bloch-Sulzberger Syndrome, brachial plexus birth injuries, brachial plexus injuries, Bradbury-Eggleston Syndrome, brain aneurysm, brain injury, brain and spinal tumors, Brown-Sequard Syndrome, bulbospinal muscular atrophy, Canavan Disease, Carpal Tunnel Syndrome, causalgia, cavernomas, cavernous angioma, cavernous malformation, central cervical cord syndrome, central cord syndrome, central pain syndrome, cephalic disorders, cerebellar degeneration, cerebellar hypoplasia, cerebral aneurysm, cerebral arteriosclerosis, cerebral atrophy, cerebral beriberi, cerebral gigantism, cerebral hypoxia, cerebral palsy, cerebro-oculo-facio-skeletal syndrome, Charcot-Marie-Tooth Disorder, Chiari Malformation, chorea, choreoacanthocytosis, chronic inflammatory demyelinating polyneuropathy (CIDP), chronic orthostatic intolerance, chronic pain, Cockayne Syndrome Type II, Coffin Lowry Syndrome, coma, including persistent vegetative state, complex regional pain syndrome, congenital facial diplegia, congenital myasthenia, congenital myopathy, congenital vascular cavernous malformations, corticobasal degeneration, cranial arteritis, craniosynostosis, Creutzfeldt-Jakob Disease, cumulative trauma disorders, Cushing's Syndrome, cytomegalic inclusion body disease (CIBD), cytomegalovirus infection, dancing eyes-dancing feet syndrome, Dandy-Walker Syndrome, Dawson Disease, De Morsier's Syndrome, Dejerine-Klumpke Palsy, dementia-multi-infarct, dementia-subcortical, dementia with Lewy Bodies, dermatomyositis, developmental dyspraxia, Devic's Syndrome, diabetic neuropathy, diffuse sclerosis, Dravet's

Syndrome, dysautonomia, dysgraphia, dyslexia, dysphagia, dyspraxia, dystonias, early infantile epileptic encephalopathy, Empty Sella Syndrome, encephalitis lethargica, encephalitis and meningitis, encephaloceles, encephalopathy, encephalotrigeminal angiomatosis, epilepsy, Erb's Palsy, Erb-Duchenne and Dejerine-Klumpke Palsies, Fabry's Disease, Fahr's Syndrome, fainting, familial dysautonomia, familial hemangioma, familial idiopathic basal ganglia calcification, familial spastic paralysis, febrile seizures (e.g., GEFS and GEFS plus), Fisher Syndrome, Floppy Infant Syndrome, Friedreich's Ataxia, Gaucher's Disease, Gerstmann's Syndrome, Gerstmann-Straussler-Scheinker Disease, giant cell arteritis, giant cell inclusion disease, globoid cell leukodystrophy, glossopharyngeal neuralgia, Guillain-Barre Syndrome, HTLV-1 associated myelopathy, Hallervorden-Spatz Disease, head injury, headache, hemicrania continua, hemifacial spasm, hemiplegia alterans, hereditary neuropathies, hereditary spastic paraplegia, heredopathia atactica polyneuritiformis, Herpes Zoster Oticus, Herpes Zoster, Hirayama Syndrome, holoprosencephaly, Huntington's Disease, hydranencephaly, hydrocephalus-normal pressure, hydrocephalus (in particular TGFβ-induced hydrocephalus), hydromyelia, hypercortisolism, hypersomnia, hypertonia, hypotonia, hypoxia, immune-mediated encephalomyelitis, inclusion body myositis, incontinentia pigmenti, infantile hypotonia, infantile phytanic acid storage disease, infantile refsum disease, infantile spasms, inflammatory myopathy, intestinal lipodystrophy, intracranial cysts, intracranial hypertension, Isaac's Syndrome, Joubert Syndrome, Kearns-Sayre Syndrome, Kennedy's Disease, Kinsbourne syndrome, Kleine-Levin syndrome, Klippel Feil Syndrome, Klippel-Trenaunay Syndrome (KTS), Klüver-Bucy Syndrome, Korsakoff's Amnesic Syndrome, Krabbe Disease, Kugelberg-Welander Disease, kuru, Lambert-Eaton Myasthenic Syndrome, Landau-Kleffner Syndrome, lateral femoral cutaneous nerve entrapment, lateral medullary syndrome, learning disabilities, Leigh's Disease, Lennox-Gastaut Syndrome, Lesch-Nyhan Syndrome, leukodystrophy, Levine-Critchley Syndrome, Lewy Body Dementia, lissencephaly, locked-in syndrome, Lou Gehrig's Disease, lupus-neurological sequelae, Lyme Disease-Neurological Complications, Machado-Joseph Disease, macrencephaly, megalencephaly, Melkersson-Rosenthal Syndrome, meningitis, Menkes Disease, meralgia paresthetica, metachromatic leukodystrophy, microcephaly, migraine, Miller Fisher Syndrome, mini-strokes, mitochondrial myopathies, Mobius Syndrome, monomelic amyotrophy, motor neuron diseases, Moyamoya Disease, mucolipidoses, mucopolysaccharidoses, multi-infarct dementia, multifocal motor neuropathy, multiple sclerosis (MS), multiple systems atrophy (MSA-C and MSA-P), multiple system atrophy with orthostatic hypotension, muscular dystrophy, myasthenia-congenital, myasthenia gravis, myelinoclastic diffuse sclerosis, myoclonic encephalopathy of infants, myoclonus, myopathy-congenital, myopathy-thyrotoxic, myopathy, myotonia

congenita, myotonia, narcolepsy, neuroacanthocytosis, neurodegeneration with brain iron accumulation, neurofibromatosis, neuroleptic malignant syndrome, neurological complications of AIDS, neurological manifestations of Pompe Disease, neuromyelitis optica, neuromyotonia, neuronal ceroid lipofuscinosis, neuronal migration disorders, neuropathy-hereditary, neurosarcoidosis, neurotoxicity, nevus cavernosus, Niemann-Pick Disease, O'Sullivan-McLeod Syndrome, occipital neuralgia, occult spinal dysraphism sequence, Ohtahara Syndrome, olivopontocerebellar atrophy, opsoclonus myoclonus, orthostatic hypotension, Overuse Syndrome, pain-chronic, paraneoplastic syndromes, paresthesia, Parkinson's Disease, parmyotonia congenita, paroxysmal choreoathetosis, paroxysmal hemicrania, Parry-Romberg, Pelizaeus-Merzbacher Disease, Pena Shokeir II Syndrome, perineural cysts, periodic paralyses, peripheral neuropathy, periventricular leukomalacia, persistent vegetative state, pervasive developmental disorders, phytanic acid storage disease, Pick's Disease, Piriformis Syndrome, pituitary tumors, polymyositis, Pompe Disease, porencephaly, Post-Polio Syndrome, postherpetic neuralgia, postinfectious encephalomyelitis, postural hypotension, postural orthostatic tachycardia syndrome, postural tachycardia syndrome, primary lateral sclerosis, prion diseases, progressive hemifacial atrophy, progressive locomotor ataxia, progressive multifocal leukoencephalopathy, progressive sclerosing poliodystrophy, progressive supranuclear palsy, pseudotumor cerebri, pyridoxine dependent and pyridoxine responsive siezure disorders, Ramsay Hunt Syndrome Type I, Ramsay Hunt Syndrome Type II, Rasmussen's Encephalitis and other autoimmune epilepsies, reflex sympathetic dystrophy syndrome, refsum disease-infantile, refsum disease, repetitive motion disorders, repetitive stress injuries, restless legs syndrome, retrovirus-associated myelopathy, Rett Syndrome, Reye's Syndrome, Riley-Day Syndrome, SUNCT headache, sacral nerve root cysts, Saint Vitus Dance, Salivary Gland Disease, Sandhoff Disease, Schilder's Disease, schizencephaly, seizure disorders, septo-optic dysplasia, severe myoclonic epilepsy of infancy (SMEI), shaken baby syndrome, shingles, Shy-Drager Syndrome, Sjogren's Syndrome, sleep apnea, sleeping sickness, Soto's Syndrome, spasticity, spina bifida, spinal cord infarction, spinal cord injury, spinal cord tumors, spinal muscular atrophy, spinocerebellar atrophy, Steele-Richardson-Olszewski Syndrome, Stiff-Person Syndrome, striatonigral degeneration, stroke, Sturge-Weber Syndrome, subacute sclerosing panencephalitis, subcortical arteriosclerotic encephalopathy, Swallowing Disorders, Sydenham Chorea, syncope, syphilitic spinal sclerosis, syringohydromyelia, syringomyelia, systemic lupus erythematosus, Tabes Dorsalis, Tardive Dyskinesia, Tarlov Cysts, Tay-Sachs Disease, temporal arteritis, tethered spinal cord syndrome, Thomsen Disease, thoracic outlet syndrome, thyrotoxic myopathy, Tic Douloureux, Todd's Paralysis, Tourette Syndrome, transient ischemic

attack, transmissible spongiform encephalopathies, transverse myelitis, traumatic brain injury, tremor, trigeminal neuralgia, tropical spastic paraparesis, tuberous sclerosis, vascular erectile tumor, vasculitis including temporal arteritis, Von Economo's Disease, Von Hippel-Lindau disease (VHL), Von Recklinghausen's Disease, Wallenberg's Syndrome, Werdnig-Hoffinan Disease, Wernicke-Korsakoff Syndrome, West Syndrome, Whipple's Disease, Williams Syndrome, Wilson's Disease, X-Linked Spinal and Bulbar Muscular Atrophy, and Zellweger Syndrome.

Preferred examples of neurodegenerative diseases and neuroinflammatory disorders are selected from the group comprising or consisting of:
Alzheimer's disease, Parkinson's disease, Creutzfeldt Jakob disease (CJD), new variant of Creutzfeldt Jakobs disease (nvCJD), Hallervorden Spatz disease, Huntington's disease, multisystem atrophy, dementia, frontotemporal dementia, motor neuron disorders of multiple spontaneous or genetic background, amyotrophic lateral sclerosis (ALS), spinal muscular atrophy, spinocerebellar atrophies (SCAs), schizophrenia, affective disorders, major depression, meningoencephalitis, bacterial meningoencephalitis, viral meningoencephalitis, CNS autoimmune disorders, multiple sclerosis (MS), acute ischemic / hypoxic lesions, stroke, CNS and spinal cord trauma, head and spinal trauma, brain traumatic injuries, arteriosclerosis, atherosclerosis, microangiopathic dementia, Binswanger' disease (Leukoaraiosis), retinal degeneration, cochlear degeneration, macular degeneration, cochlear deafness, AIDS-related dementia, retinitis pigmentosa, fragile X-associated tremor/ataxia syndrome (FXTAS), progressive supranuclear palsy (PSP), striatonigral degeneration (SND), olivopontocerebellear degeneration (OPCD), Shy Drager syndrome (SDS), age dependant memory deficits, neurodevelopmental disorders associated with dementia, Down`s Syndrome, synucleinopathies, superoxide dismutase mutations, trinucleotide repeat disorders as Huntington`s Disease, trauma, hypoxia, vascular diseases, vascular inflammations, CNS-ageing. Also age dependant decrease of stem cell renewal may be addressed.

Particularly referred examples of neurodegenerative diseases and neuroinflammatory disorders are selected from the group comprising or consisting of:
Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), hydrocephalus (in particular TGFβ-induced hydrocephalus), CNS and spinal cord trauma such as spinal cord injury, head and spinal trauma, brain traumatic injuries, retinal degeneration, macular degeneration, cochlear deafness, AIDS-related dementia, trinucleotide repeat disorders as Huntington`s Disease, and CNS-ageing.

The antisense-oligonucleotides are also useful for prophylaxis and treatment of **fibrotic diseases**. Fibrosis or fibrotic disease is the formation of excess fibrous connective tissue in an organ or tissue in a reparative or reactive process. This can be a reactive, benign, or pathological state. In response to injury this is called scarring and if fibrosis arises from a single cell line this is called a fibroma. Physiologically this acts to deposit extracellular matrix, which can obliterate the architecture and function of the underlying organ or tissue. Fibrosis can be used to describe the pathological state of excess deposition of fibrous tissue, as well as the process of connective tissue deposition in healing. Fibrosis is a process involving stimulated cells to form connective tissue, including collagen and glycosaminoglycans. Subsequently macrophages and damaged tissue between the interstitium release TGF-β. TGF-β stimulates the proliferation and activation of fibroblasts which deposit connective tissue. Reducing the TGF-β levels prevents and decreases the formation of connective tissue and thus prevents and treats fibrosis.

Examples for fibrotic diseases are

Lungs:
- pulmonary fibrosis
- idiopathic pulmonary fibrosis (idiopathic means cause is unknown)
- cystic fibrosis

Liver:
- hepatic cirrhosis of multiple origin

Heart:
- endomyocardial fibrosis
- old myocardial infarction
- atrial fibrosis

Other:
- mediastinal fibrosis (soft tissue of the mediastinum)
- glaucoma (eye, ocular)
- myelofibrosis (bone marrow)
- retroperitoneal fibrosis (soft tissue of the retroperitoneum)
- progressive massive fibrosis (lungs); a complication of coal workers' pneumoconiosis
- nephrogenic systemic fibrosis (skin)
- Crohn's Disease (intestine)
- keloid (skin)
- scleroderma/systemic sclerosis (skin, lungs)
- arthrofibrosis (knee, shoulder, other joints)
- Peyronie's disease (penis)
- Dupuytren's contracture (hands, fingers)
- some forms of adhesive capsulitis (shoulder)
- residuums after Lupus erythematodes

Thus another aspect of the present invention relates to the use of an antisense-oligonucleotide for prophylaxis and/or treatment of or to the use of an antisense-oligonucleotide for the preparation of a pharmaceutical composition for prophylaxis and/or treatment of pulmonary fibrosis, cystic fibrosis, hepatic cirrhosis, endomyocardial fibrosis, old myocardial infarction, atrial fibrosis, mediastinal fibrosis, myelofibrosis, retroperitoneal fibrosis, progressive massive fibrosis, nephrogenic systemic fibrosis, glaucoma, such as primary open angle glaucoma, Crohn's Disease, keloid, systemic sclerosis, arthrofibrosis, Peyronie's disease, Dupuytren's contracture, and residuums after Lupus erythematodes.

Still another aspect of the present invention relates to the use of an antisense-oligonucleotide for prophylaxis and/or treatment of hyperproliferative diseases, cancer, tumors and their metastases or to the use of an antisense-oligonucleotide for the preparation of a pharmaceutical composition for prophylaxis and/or treatment of hyperproliferative diseases, cancer, tumors and their metastases.

Examples for hyperproliferative diseases, cancer, tumors are selected from the group comprising or consisting of: adenocarcinoma, melanoma, acute leukemia, acoustic neurinoma, ampullary carcinoma, anal carcinoma, astrocytoma, basal cell carcinoma, pancreatic cancer, desmoid tumor, bladder cancer, bronchial carcinoma, non-small cell lung cancer (NSCLC), breast cancer, Burkitt's lymphoma, corpus cancer, CUP-syndrome (carcinoma of unknown primary), colorectal cancer, small intestine cancer, small intestinal tumors, ovarian cancer, endometrial carcinoma, ependymoma, epithelial cancer types, Ewing's tumors, gastrointestinal tumors, gastric cancer, gallbladder cancer, gall bladder carcinomas, uterine cancer, cervical cancer, cervix, glioblastomas, gynecologic tumors, ear, nose and throat tumors, hematologic neoplasias, hairy cell leukemia, urethral cancer, skin cancer, skin testis cancer, brain tumors (gliomas, e.g. astrocytomas, oligodendrogliomas, medulloblastomas, PNET`s, mixed gliomas), brain metastases, testicle cancer, hypophysis tumor, carcinoids, Kaposi's sarcoma, laryngeal cancer, germ cell tumor, bone cancer, colorectal carcinoma, head and neck tumors (tumors of the ear, nose and throat area), colon carcinoma, craniopharyngiomas, oral cancer (cancer in the mouth area and on lips), cancer of the central nervous system, liver cancer, liver metastases, leukemia, eyelid tumor, lung cancer, lymph node cancer (Hodgkin's/Non-Hodgkin's), lymphomas, stomach cancer, malignant melanoma, malignant neoplasia, malignant tumors gastrointestinal tract, breast carcinoma, rectal cancer, medulloblastomas, melanoma, meningiomas, Hodgkin's disease, mycosis

fungoides, nasal cancer, neurinoma, neuroblastoma, kidney cancer, renal cell carcinomas, non-Hodgkin's lymphomas, oligodendroglioma, esophageal carcinoma, osteolytic carcinomas and osteoplastic carcinomas, osteosarcomas, ovarial carcinoma, pancreatic carcinoma, penile cancer, plasmocytoma, squamous cell carcinoma of the head and neck (SCCHN), prostate cancer, pharyngeal cancer, rectal carcinoma, retinoblastoma, vaginal cancer, thyroid carcinoma, Schneeberger disease, esophageal cancer, spinalioms, T-cell lymphoma (mycosis fungoides), thymoma, tube carcinoma, eye/ocular tumors, urethral cancer, urologic tumors, urothelial carcinoma, vulva cancer, wart appearance, soft tissue tumors, soft tissue sarcoma, Wilm's tumor, cervical carcinoma and tongue cancer.

The term "cancer" refers preferably to a cancer selected from the group consisting of or comprising Lung cancer, such as Lung carcinoma, liver cancer such as hepatocellular carcinoma, melanoma or malignant melanoma, pancreatic cancer, such as pancreatic epithelioid carcinoma or pancreatic adenocarcinoma, colon cancer, such as colorectal adenocarcinoma, gastric cancer or gastric carcinoma, mamma carcinoma, malignant astrocytoma, prostatic cancer, such as gastric carcinoma, leukemia, such as acute myelogenous leukemia, chronic myelogenous leukemia, monocytic leukemia, promyelocytic leukemia, lymphocytic leukemia, acute lymphoblastic leukemia, lymphocytic leukemia, and acute lymphoblastic leukemia, and lymphoma, such as histiocytic lymphoma.

For the treatment of hyperproliferative diseases, cancer, tumors and their metastases the antisense-oligonucleotides may be administered at regular intervals (dose intervals, DI) of between 3 days and two weeks, such as 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 days, such as about 1 week, such as 6, 7 or 8 days. Suitably at least two doses are provide with a DI period between the two dosages, such as 3, 4, 5, 6, 7, 8, 9 or 10 dosages, each with a dose interval (DI) between each dose of the antisense-oligonucleotide. The DI period between each dosage may the same, such as between 3 days and two weeks, such as 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 days, such as about 1 week, such as 6, 7 or 8 days.
Preferably, each dose of the antisense-oligonucleotide may be between about 0.25mg/kg - about 10mg/kg, such as about 0.5mg/kg, about 1 mg/kg, about 2mg/kg, about 3mg/kg, about 4mg/kg, about 5mg/kg, about 6mg/kg, about 7mg/kg, about 8mg/kg, about 9mg/kg. In some embodiments, each does of the antisense-oligonucleotide may be between about 2 mg/kg - about 8mg/kg, or about 4 to about 6 mg/kg or about 4mg/kg to about 5mg/kg. In some embodiments, each does of the antisense-oligonucleotide is at least 2mg/kg, such as 2, 3, 4, 5, 6, 7 or 8 mg/kg, such as 6 mg/kg. In some embodiments the dosage regime for the

antisense-oligonucleotide may be repeated after an initial dosage regime, for example after a rest period where no antisense-oligonucleotide is administered. Such as rest period may be more than 2 weeks in duration, such as about 3 weeks or about 4 weeks, or about 5 weeks or about 6 weeks. In some embodiments the dosage regimen for the antisense-oligonucleotide is one weekly dosage, repeated three, four or five times. This dosage regimen may then be repeated after a rest period of, for example, about 3 - 5 weeks, such as about 4 weeks. In some embodiments, the antisense-oligonucleotide is administered during a first dosage regimen at regular dosage intervals (DI) of between 4 and 13 days for between 2 - 10 administrations.

Administration of the antisense-oligonucleotide is typically performed by parenteral administration, such as subcutaneous, intramuscular, intravenous or intraperitoneal administration.

Description of Figures

Fig. 1 shows the inhibitory effect of the antisense-oligonucleotides (ASO). The DNA is transcribed to the Pre-mRNA to which in the nucleus of the cell, the antisense-oligonucleotides (ASO) can bind or hybridize to the complementary sequence within an exon (as represented by the first ASO from the right side and the first ASO from the left side) or within an intron (as represented by the second ASO from the right side) or at allocation consisting of an area of an exon and an area of an adjacent intron (as represented by the second ASO from the left side). By post-transcriptional modification, i.e. the splicing, the mRNA is formed to which the ASO can bind or hybridize in the cytoplasma of the cell in order to inhibit translation of the mRNA into the protein sequence. Thus, the ASO knock down the target gene and the protein expression selectively.

Fig. 2 shows a nucleoside unit (without internucleotide linkage) or nucleotide unit (with internucleotide linkage) which are non-LNA units and which may be contained in the antisense-oligonucleotides of the present invention especially in the region B in case the antisense-oligonucleotide of the present invention is a gapmer.

Fig. 3 shows TGF-beta and its effects on neural stem cells, cancer stem cells, and tumors. TGFbeta inhibits neural stem cell proliferation. It may affect the transition to a cancer stem cell, which might escape from TGF-beta growth control. Later in tumor progression, TGF-beta acts as an oncogene; it further promotes tumor growth by

promoting angiogenesis and suppressing the immune system. In addition, it promotes cellular migration, thereby driving cells into metastasis.

Fig. 4 shows the antisense-oligonucleotide of **Seq ID No 218b** in form of a gapmer consisting of 16 nucleotides with 3 LNA units (**C*b^1 and Ab^1 and Tb^1**) at the 5' terminal end and 4 LNA units (**Ab^1 and Gb^1 and Tb^1 and Ab^1**) at the 3' terminal end and 9 DNA nucleotides (dG, dA, dA, dT, dG, dG, dA, dC, and dC) in between the LNA segments, with phosphorothioate internucleotiodes linkages (s) and the nucleobase 5-methylcytosine (C*) in the first LNA unit from the 5' terminal end.

SP	L	Seq ID No	Sequence, 5'-3'
4217	16	218b	**C*b^{1}sAb^{1}sTb^1**sdGsdAsdAsdTsdGsdGsdAsdCsdCs**Ab^{1}sGb^{1}sTb^{1}sAb^1**

Fig. 5: ASO (Seq. ID No. 218b) treatment leads to intracellular pSmad2 protein reduction. Labeling with an antibody against pSmad2 (left coulmn, red) in A549 (Fig. 5A) and ReNcell CX® (Fig. 5B) cells after gymnotic transfer with ASO Seq. ID No. 218b for 72 h or 96 h respectively. Nuclear DNA was stained with DAPI (central column, blue). Examination of cells was performed by fluorescence microscopy (Zeiss Axio® Observer.Z1). Images were analyzed with Image J Software and CorelDRAW® X7 Software. A = untreated control, B = Ref.1, C = Seq. ID No. 218b.

Fig. 6: ASO (Seq. ID No. 218c) treatment leads to intracellular pSmad2 protein reduction. Labeling with an antibody against pSmad2 (left column, red) in A549 (Fig. 6A) and ReNcell CX® (Fig. 6B) cells after gymnotic transfer with ASO Seq. ID No. 218c for 72 h or 96 h respectively. Nuclear DNA was stained with DAPI (central column, blue). Examination of cells was performed by fluorescence microscopy (Zeiss Axio® Observer.Z1). Images were analyzed with Image J Software and Corel DRAW®X7 Software. A = untreated control, B = Ref.1, D= Seq. ID No. 218c.

Fig. 7: In presence of TGF-β1, ASO (Seq. ID No. 218b) treatment leads to downregulation of TGF-R$_{II}$ mRNA. Potent downregulation of TGF-R$_{II}$ mRNA after gymnotic transfer of TGF-R$_{II}$ specific ASO in TGF-β1 pre-incubated (48 h) A549 (Fig. 7A) and ReNcell CX® (Fig. 7B) cells. ASOs were incubated for 72 h or 96 h in presence of TGF-β1, respectively. mRNA expression levels were quantified relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and normalized to untreated controls. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E= TGF-β1, ± = SEM, *p < 0.05, **p < 0.01 in reference to A, $^{++}$p < 0.01 in reference to E+B. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey's" *post hoc* comparisons.

Fig. 8: In presence of TGF-β1, ASO (Seq. ID No. 218c) treatment leads to downregulation of TGF-R_{II} mRNA. Potent downregulation of TGF-R_{II} mRNA after gymnotic transfer of TGF-R_{II} specific ASO in TGF-β1 pre-incubated (48 h) A549 (Fig. 8A) and ReNcell CX® (Fig. 8B) cells. ASOs were incubated for 72 h or 96 h in presence of TGF-β1, respectively. mRNA expression levels were quantified relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and normalized to untreated controls. A = untreated control, B = Ref.1, D = Seq. ID No. 218c, E = TGF-β1, ± = SEM, *p < 0.05, **p < 0.01 in reference to A, $^{++}$p < 0.01 in reference to E+B. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey's" *post hoc* comparisons.

Fig. 9 shows the antisense-oligonucleotide of **Seq ID No 209y** in form of a gapmer consisting of 16 nucleotides with 2 LNA units (**Gb^1 and Tb^1**) at the 5' terminal end and 3 LNA units (**Ab^1 and Gb^1 and C^*b^1**) at the 3' terminal end and 11 DNA nucleotides (dA, dG, dT, dG, dT, dT, dT, dA, dG, dG, and dG) in between the LNA segments, with phosphorothioate internucleotiodes linkages (s) and the nucleobase 5-methylcytosine (C*) in the last LNA unit from the 5' terminal end.

SP	L	Seq ID No	Sequence, 5'-3'
2064	16	209y	**Gb^1sTb^1s**dAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab^1sGb^1sC^*b^1**

Fig. 10 shows the antisense-oligonucleotide of **Seq ID No 210q** in form of a gapmer consisting of 16 nucleotides with 4 LNA units (**Gb^1 and C^*b^1 and Tb^1 and Ab^1**) at the 5' terminal end and 3 LNA units (**Gb^1 and Tb^1 and Tb^1**) at the 3' terminal end and 9 DNA nucleotides (dT, dT, dT, dG, dG, dT, dA, dG, and dTs) in between the LNA segments, with phosphorothioate internucleotiodes linkages (s) and the nucleobase 5-methylcytosine (C*) in the second LNA unit from the 5' terminal end.

SP	L	Seq ID No	Sequence, 5'-3'
2072	16	210q	**Gb^1sC^*b^1sTb^1sAb^1**sdTsdTsdTsdGsdGsdTsdAsdGsdTs**Gb^1sTb^1sTb^1**

Fig. 11: In presence of TGF-β1, ASO (Seq. ID No. 218b) treatment leads to downregulation of CTGF mRNA. Potent downregulation of CTGF mRNA after gymnotic transfer of TGF-R_{II} specific ASO in TGF-β1 pre-incubated (48 h) A549 (Fig. 11A) and ReNcell CX® (Fig. 11B) cells. ASOs were incubated for 72 h or 96 h in presence of TGF-β1, respectively. mRNA expression levels were quantified relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and normalized to untreated controls. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E =

TGF-β1, ± = SEM, *p < 0.05, **p < 0.01 in reference to A, $^{++}$p < 0.01 in reference to E+B. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey's" *post hoc* comparisons.

Fig. 12: In presence of TGF-β1, ASO (Seq. ID No. 218b) treatment leads to reduction of CTGF cellular protein. CTGF protein expression was reduced after gymnotic transfer of TGF-R_{II} specific ASO in TGF-β1 pre-incubated (48 h) A549 (Fig. 12A) and ReNcell CX® (Fig. 12B) cells. ASOs were incubated for 72 h or 96 h in presence of TGF-β1, respectively. Cells were labeled with an antibody against CTGF (left column, red). Nuclear DNA was stained with DAPI (central column, blue). Examination of cells was performed by fluorescence microscopy (Zeiss Axio® Observer.Z1). Images were analyzed with Image J Software and CorelDRAW® X7 Software. A = untreated control, B = Ref.1, C = Seq. ID. 218b, E = TGF-β1.

Fig. 13: In presence of TGF-β1, ASO (Seq. ID No. 218b) treatment leads to intracellular pSmad2 protein reduction. pSmad2 protein expression was reduced after gymnotic transfer of TGF-R_{II} specific ASO in TGF-β1 pre-incubated (48 h) A549 (Fig. 13A) and ReNcell CX® (Fig. 13B) cells. ASOs were incubated for 72 h or 96 h in presence of TGF-β1, respectively. Cells were labeled with an antibody against pSmad2 (left column, red). Nuclear DNA was stained with DAPI (central column, blue). Examination of cells was performed by fluorescence microscopy (Zeiss Axio® Observer.Z1). Images were analyzed with Image J Software and CorelDRAW® X7 Software. A = untreated control, B = Ref.1, C = Seq. ID. 218b, E = TGF-β1.

Fig. 14: In presence of TGF-β1, ASO (Seq. ID No. 218c) treatment leads to downregulation of CTGF mRNA. Potent downregulation of CTGF mRNA after gymnotic transfer of TGF-R_{II} specific ASO in TGF-β1 pre-incubated (48 h) A549 (Fig. 14A) and ReNcell CX® (Fig. 14B) cells. ASOs were incubated for 72 h or 96 h in presence of TGF-β1, respectively. mRNA expression levels were quantified relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and normalized to untreated controls. A = untreated control, B = Ref.1, D = Seq. ID No. 218c, E = TGF-β1, ± = SEM, *p < 0.05, **p < 0.01 in reference to A, Statistics were calculated using the Ordinary-one-way-ANOVA followed by "Dunnett's" *post hoc* comparisons. Note different scales.

Fig. 15: In presence of TGF-β1, ASO (Seq. ID No. 218c) treatment leads to reduction of CTGF cellular protein. CTGF protein expression was reduced after gymnotic transfer of TGF-R_{II} specific ASO in TGF-β1 pre-incubated (48 h) A549 cells. ASOs were incubated for 72 h in presence of TGF-β1. Cells were labeled with an

antibody against CTGF (left column, red). Nuclear DNA was stained with DAPI (central column, blue). Examination of cells was performed by fluorescence microscopy (Zeiss Axio® Observer.Z1). Images were analyzed with Image J Software and CorelDRAW® X7 Software. A = untreated control, B = Ref.1, D = Seq. ID. 218c, E = TGF-β1.

Fig. 16: In presence of TGF-β1, ASO (Seq. ID No. 218c) treatment leads to intracellular pSmad2 protein reduction. pSmad2 protein expression was reduced after gymnotic transfer of TGF-R_{II} specific ASO in TGF-β1 pre-incubated (48 h) A549 (Fig. 16A) and ReNcell CX® (Fig. 16B) cells. ASOs were incubated for 72 h or 96 h in presence of TGF-β1, respectively. Cells were labeled with an antibody against pSmad2 (left column, red). Nuclear DNA was stained with DAPI (central column, blue). Examination of cells was performed by fluorescence microscopy (Zeiss Axio® Observer.Z1). Images were analyzed with Image J Software and CorelDRAW® X7 Software. A = untreated control, B = Ref.1, D = Seq. ID. 218c, E = TGF-β1.

Fig. 17: ASO (Seq. ID No. 218b) pretreatment and subsequent TGF-β1 co-exposure leads to reduction of TGF-R_{II} membrane protein. TGF-R_{II} protein was reduced after gymnotic transfer of TGF-R_{II} specific ASO followed by co-exposure of TGF-β1 (48 h) A549 (Fig. 17A) and ReNcell CX® (Fig. 17B) cells. ASOs were incubated for 72 h or 96 h, respectively, in advance to 48 h TGF-β1 co-exposure. Cells were labeled with an antibody against TGF-R_{II} (left column, red). Nuclear DNA was stained with DAPI (central column, blue). Examination of cells was performed by fluorescence microscopy (Zeiss Axio® Observer.Z1). Images were analyzed with Image J Software and CorelDRAW® X7 Software. A = untreated control, B = Ref.1, C = Seq. ID. 218b, E = TGF-β1.

Fig. 18: ASO (Seq. ID No. 218b) pretreatment and subsequent TGF-β1 co-exposure leads to intracellular pSmad3 protein reduction. pSmad3 protein expression was reduced after gymnotic transfer of TGF-R_{II} specific ASO followed by co-exposure of TGF-β1 (48 h) A549 (Fig. 18A) and ReNcell CX® (Fig. 18B) cells. ASOs were incubated for 72 h or 96 h, respectively, in advance to 48 h TGF-β1 co-exposure. Cells were labeled with an antibody against pSmad3 (left column, red). Nuclear DNA was stained with DAPI (central column, blue). Examination of cells was performed by fluorescence microscopy (Zeiss Axio® Observer.Z1). Images were analyzed with Image J Software and CorelDRAW® X7 Software. A = untreated control, B = Ref.1, C = Seq. ID. 218b, E = TGF β1.

Fig. 19: ASO (Seq. ID No. 218b) enhances and TGF-β1 reduces neurogenesis in human neural precursor ReNcell CX® cells. Neurogenesis marker DCX mRNA is upregulated in ReNcell CX® cells after repeated gymnotic transfer (2 x 96 h) of inventive ASOs. A strong reduction of DCX mRNA expression was recognized after an 8-day TGF-β1 exposure. mRNA levels were quantified relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and normalized to untreated controls. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post-hoc* comparison. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E= TGF-β1, ± = SEM, +p < 0.05 in reference to C 2.5 µM, #p < 0.05 in reference to C 10 µM.

Fig. 20: ASO (Seq. ID No. 218b) enhances and TGF-β1 reduces proliferation in human neural precursor ReNcell CX® cells. Proliferation marker Ki67 protein expression is increased in ReNcell CX® cells after repeated gymnotic transfer (2 x 96 h) of inventive ASOs. Reduced Ki67 protein expression was recognized after an 8-day TGF-β1 exposure. Cells were labeled with an antibody against Ki67 (left column, green). Nuclear DNA was stained with DAPI (central column, blue). Examination of cells was performed by fluorescence microscopy (Zeiss Axio® Observer.Z1). Images were analyzed with Image J Software and CorelDRAW® X7 Software. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E = TGF-β1.

Fig. 21: Despite proliferative conditions ASO (Seq. ID No. 218b) enhances differentiation in human neural precursor ReNcell CX® cells. Neural markers NeuN (Fig. 23 A, left column, red) and βIII-Tubulin (Fig. 23 B, left column, red) in ReNcell CX® were observed. ASO treatment was applied for initial 4 days under proliferative conditions followed by further 4 days under either proliferative (+ EGF/FGF) or differentiating conditions (- EGF/FGF). Nuclear DNA was stained with DAPI (central column, blue). Examination of cells was performed by fluorescence microscopy (Zeiss Axio® Observer.Z1). Images were analyzed with Image J Software and CorelDRAW® X7 Software. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E = TGF-β1, + EGF/FGF = proliferation, - EGF/FGF = differentiation.

Fig. 22: ASO-mediated (Seq. ID No. 218b) rescue from TGF-β-induced neural stem cell proliferation arrest. Human neural precursor ReNcell CX® cells proliferation was observed with or without TGF-β1 exposure for 7 days followed by ASO treatment for 8 days. Upregulation of GFAP (Fig. 24A), Ki67 (Fig. 24B) and DCX (Fig. 24C) mRNA 7 days after TGF-β1 pre-incubation indicates recovery of stem cell proliferation. mRNA expression levels were quantified relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and normalized to

untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E= TGF-β1, ± = SEM, *p < 0.05 in reference to A, Statistics were calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" *post hoc* multiple comparisons.

Fig. 23: ASO reduces proliferation of human lung-cancer cells (A549). Proliferation marker Ki67 protein expression is decreased in A549 cells after gymnotic transfer (72 h) of inventive ASOs. Reduced Ki67 protein expression was recognized (left column, green). Nuclear DNA was stained with DAPI (central column, blue). Examination of cells was performed by fluorescence microscopy (Zeiss Axio® Observer.Z1). Images were analyzed with Image J Software and CorelDRAW® X7 Software. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E = TGF-β1.

Fig. 24: ASO reduces proliferation of several human tumor cell-lines. HPAFII, K562, MCF-7, Panc-1, and HTZ-19 cells were exposed 4x 72 h to inventive ASOs and proliferation was analyzed by light microscopy (Nikon, TS-100® F LED). A = untreated control, B = Ref.1, C = Seq. ID No. 218b.

Fig. 25: ASO treatment mediates neural anti-fibrotic effects and ameliorates cellular stress. ReNcell CX® cells were observed after TGF-β1-preincubation (48 h) followed by gymnotic transfer of inventive ASO and co-exposure with TGF-β1 treatment for 96 h. Cells were labeled with an antibody against CTGF (Fig. 29A, left column, red), FN (Fig. 29B, left column, green) and of Phalloidin (actin-cytoskeleton, Fig. 29C, left column, red). Nuclear DNA was stained with DAPI (central column, blue). Examination of cells was performed by fluorescence microscopy (Zeiss Axio® Observer.Z1). Images were analyzed with Image J Software and CorelDRAW® X7 Software. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E = TGF-β1.

Fig. 26: ASO treatment mediates tumor anti-fibrotic effects and ameliorates cellular stress. A549 cells were observed after treatment with either TGF-β1 or gymnotic transfer of inventive ASO (72 h). Cells were labeled with an antibody against FN (Fig. 30A, left column, green), Phalloidin (actin-cytoskeleton, Fig. 30B, left column, red). Nuclear DNA was stained with DAPI (central column, blue). Examination of cells was performed by fluorescence microscopy (Zeiss Axio® Observer.Z1). Images were analyzed with Image J Software and CorelDRAW® X7 Software. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E = TGF-β1.

Fig. 27: ASO treatment mediates tumor anti-fibrotic effects. A549 human lung cancer cells were observed after TGF-β1-preincubation (48 h) followed by gymnotic

transfer of inventive ASO and co-exposure with TGF-β1 treatment for 72 h. Cells were labeled with an antibody against CTGF (Fig. 31A, left column, red) and FN (Fig. 31B, left column, green). Nuclear DNA was stained with DAPI (central column, blue). Examination of cells was performed by fluorescence microscopy (Zeiss Axio® Observer.Z1). Images were analyzed with Image J Software and CorelDRAW® X7 Software. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E = TGF-β1.

Fig. 28: ASO treatment mediates tumor anti-fibrotic effects. A549 human lung cancer cells were observed after TGF-β1-preincubation (48 h) followed by gymnotic transfer of inventive ASO and co-exposure with TGF-β1 treatment for 72 h. Cells were labeled with an antibody against CTGF (Fig. 32A, left column, red) and FN (Fig. 32B, left column, green). Nuclear DNA was stained with DAPI (central column, blue). Examination of cells was performed by fluorescence microscopy (Zeiss Axio® Observer.Z1). Images were analyzed with Image J Software and CorelDRAW® X7 Software. A = untreated control, B = Ref.1, D = Seq. ID No. 218c, E = TGF-β1.

Fig. 29 shows the antisense-oligonucleotide of **Seq ID No 209x** in form of a gapmer consisting of 16 nucleotides with 2 LNA units (**Gb[1] and Tb[1]**) at the 5' terminal end and 3 LNA units (**Ab[1] and Gb[1] and C*b[1]**) at the 3' terminal end and 11 DNA nucleotides (dA, dG, dT, dG, dT, dT, dT, dA, dG, dG, and dG) in between the LNA segments, with phosphorothioate internucleotiodes linkages (s), the nucleobase 5-methylcytosine (C*) in the last LNA unit from the 5' terminal end, and with –O-P(O)(S^-)OC_3H_6OH as terminal end groups at the 5' terminal end and at the 3' terminal end.

SP	L	Seq ID No	Sequence, 5'-3'
2064	16	209x	/5SpC3s/**Gb[1]sTb[1]s**dAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs **Ab[1]sGb[1]sC*b[1]**/3SpC3s/

Fig. 30 shows the antisense-oligonucleotide of **Seq ID No 152h** in form of a gapmer consisting of 15 nucleotides with 4 LNA units (**C*b[1] and Gb[1] and Ab[1] and Tb[1]**) at the 5' terminal end and 3 LNA units (**Ab[1] and C*b[1] and Ab[1]**) at the 3' terminal end and 8 DNA nucleotides (dA, dC, dG, dC, dG, dT, dC, and dC) in between the LNA segments, with phosphorothioate internucleotiodes linkages (s) and the nucleobase 5-methylcytosine (C*) in the first and second last LNA unit from the 5' terminal end.

SP	L	Seq ID No	Sequence, 5'-3'
429	15	152h	**C*b[1]sGb[1]sAb[1]sTb[1]s**dAsdCsdGsdCsdGsdTsdCsdCs**Ab[1]sC*b[1]sAb[1]**

Fig. 31 shows the antisense-oligonucleotide of **Seq ID No 143h** in form of a gapmer consisting of 14 nucleotides with 2 LNA units (**C*b^1 and Tb^{1}s**) at the 5' terminal end and 3 LNA units (**C*b^1 and C*b^1 and Gb^1**) at the 3' terminal end and 9 DNA nucleotides (dC, dG, dT, dC, dA, dT, dA, dG, and dA) in between the LNA segments, with phosphorothioate internucleotiodes linkages (s) and the nucleobase 5-methylcytosine (C*) in the first, third from last and second LNA unit from the 5' terminal end.

SP	L	Seq ID No	Sequence, 5'-3'
355	14	143h	**C*b^{1}sTb^{1}s**dCsdGsdTsdCsdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^1**

Fig. 32 shows the antisense-oligonucleotide of **Seq ID No 213k** in form of a gapmer consisting of 17 nucleotides with 3 LNA units (**C*b^1 and Ab^1 and Gb^1**) at the 5' terminal end and 3 LNA units (**Gb^1 and Tb^1 and Gb^1**) at the 3' terminal end and 11 DNA nucleotides (dG, dC, dA, dT, dT, dA, dA, dT, dA, dA, and dA) in between the LNA segments, with phosphorothioate internucleotiodes linkages (s) and the nucleobase 5-methylcytosine (C*) in the first LNA unit from the 5' terminal end.

SP	L	Seq ID No	Sequence, 5'-3'
2355	17	213k	**C*b^{1}sAb^{1}sGb^{1}s**dGsdCsdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^1**

Examples

Material and Methods

Most Antisense-Oligonucleotides as well as control or reference oligonucleotides used herein were synthesized by EXIQON as custom oligonucleotides according to the needs of the inventors/applicant. Oligonucleotides having the following sequences were used as references:

Ref.0 = dCsdAsdGsdCsdCsdCsdCsdCsdGsdAsdCsdCsdCsdAsdTsdG (Seq. ID No. 147c);

Ref. 1= Ab1sAb1sC*b1sdAsdCsdGsdTsdCsdTsdAsdTsdAsC*b1sGb1sC*b1 (Seq. ID No. 76);

Ref. 2= C*b1sAb1sGb1sdCsdCsdCsdCsdCsdGsdAsdCsdCsdCsAb1sTb1sGb1 (Seq. ID No. 147m);

Ref. 3= TTGAATATCTCATGAATGGA; having 2'-MOE-wings (5 units 5' and 3') and phosphorothioate linkages (Seq. ID No. 80);

Ref. 4=; CAGAAGAGCTATTTGGTAGT, having 2'-MOE-wings (5 units 5' and 3') and phosphorothioate linkages (Seq. ID No. 82);
Ref. 5 = TGGTAGTGTTTAGGGAGCCG (Seq. ID No. 85),
Ref. 6 = GTGCAGGGGAAAGATGAAAA (Seq. ID No. 344),
Ref. 7 = GAGCTCTTGAGGTCCCTGTG (Seq. ID No. 345),
Ref. 8 = AGCCTCTTTCCTCATGCAAA (Seq. ID No. 346),
Ref. 9 = CCTTCTCTGCTTGGTTCTGG (Seq. ID No. 347), and
Ref. 10 = GCCATGGAGTAGACATCGGT (Seq. ID No. 348).

Standard procedures protocols

Cell culture:

Table 10: The following human cell lines were used for antisense-oligonucleotide experiments:

Description	Cell line	CO_2-Content	Medium
Melanoma	HTZ-19	5%	DMEM F12 (Gibco 31331-018) + 1 % dM-Mix (Transferrin (30 mg/ml in water 835 µl, non-essential AS (100x) 10 ml, Sodium-selenite (0.2 mg/ml in water) 70 µl, 10 ml PBS), 1% P/S
Lung carcinoma	A549	5%	Kaighn´s F12 K + 10% FCS + 1% P/S
hepatocellular carcinoma	HepG2	5%	DMEM (Sigma D6429) + 10% FCS + 1% P/S
hepatocellular carcinoma	Hep3B	5%	DMEM (Sigma D6429) + 10% FCS + 1% P/S
pancreatic epithelioid carcinoma	Panc-1	5%	DMEM (Sigma D6429) + 10% FCS + 1% P/S
pancreatic adenocarcinoma	HPAFII	5%	DMEM (Sigma D5796) + 15 % FCS, 1 % P/S, 1% Antibiotic/Antimycotic, 1% MEM Vitamin Solution, 1% non-essential AS (100x)
pancreatic adenocarcinoma	BxPC-3	5%	RPMI (Gibco A10491-01) + 10% FCS + 1% P/S + 1% Antibiotic/Antimycotic, 1% MEM Vitamin Solution
pancreatic cancer liver metastasis	L3.6pl	5%	DMEM (Sigma D5796) + 15 % FCS, 1% P/S, 1% Antibiotic/Antimycotic, 1% Vitamin, 1% non-essential AS (100x)
colorectal adenocarcinoma	HT-29	5%	DMEM (Sigma D5796) + 15 % FCS, 1% P/S, 1% Antibiotic/Antimycotic, 1% MEM Vitamin Solution, 1% non-essential AS (100x)

epithelial colorectal adenocarcinoma	CaCo2	5%	DMEM (Sigma D5796) + 20 % FCS + 1% P/S
gastric carcinoma	TMK-1	5%	DMEM (Sigma D5796) + 10% FCS + 1% P/S, 1% Antibiotic/Antimycotic, 1% MEM Vitamin Solution
malignant astrocytoma	HTZ-243	5%	DMEM (Sigma D6046) + 10% FCS + 1% P/S + 1% non-essential AS + 1% MEM Vitamin Solution
Mamma-Carcinoma	MCF-7	5%	DMEM (Sigma D6046) + 10 % FCS + 1% P/S
prostatic adenocarcinoma	PC-3M	5%	RPMI (Gibco #61870-010), 10 % FCS, 1% Sodium pyruvate, 1% Sodium bicarbonate, 1 % P/S
acute myelogenous leukemia	KG-1	5%	RPMI (Gibco #61870-010) + 10 %FCS + 1% P/S
chronic myelogenous leukemia	K562	5%	RPMI (Gibco #61870-010) + 10 % FCS + 1% P/S
monocytic leukemia	THP-1	5%	RPMI (Gibco #61870-010) + 10 % FCS + 0.5 % P/S
promyelocytic leukemia	HL60	5%	RPMI (Gibco #61870-010) + 10 % FCS + 0.5 % P/S
lymphocytic leukemia	CEM-C7H2	5%	RPMI (Gibco #61870-010) + 10 % FCS + 0.5 % P/S
acute lymphoblastic leukemia	Pre-B697	5%	RPMI (Gibco #61870-010) + 10 % FCS + 0.5 % P/S
histiocytic lymphoma	U937	5%	RPMI (Gibco #61870-010) + 10 % FCS + 0.5 % P/S
Neuronal precursor cells of cortical brain region	ReNcell CX	5%	ReNcell Neural Stem Cell Maintenance Medium (Millipore #SCM005) + human FGF Basic human + human EGF + N2-Supplement

Material:

FCS (ATCC #30-2020)

Sodium pyruvate (Sigma #S8636)

Sodium bicarbonate (Sigma #S8761-100ML)

Transferrin (Sigma #T8158-100MG)

Natrium Selenite (Sigma #S5261-10G)

Penicillin / Streptomycin (P/S) (Sigma-Aldrich #P4458)
Non-essential Amino Acids (AS) 100x (Sigma #M7145)
Antibiotic/Antimycotic (Sigma #A5955)
MEM Vitamin Solution (Sigma #M6895)
PBS (Sigma #D8537)
FGF Basic human (Millipore #GF003)
EGF human (Millipore #GF144)
N-2 Supplement (Life Technologies #17502048)
ReNcell Neural Stem Cell Maintenance Medium (Millipore #SCM005)

Culturing and disseminating cells:

After removing the medium, cells were washed with PBS and incubated with accutase (Sigma-Aldrich #P4458) (5 min, RT). Following incubation, cells were peened and full medium (3 ml, company: see Tab.10 for respective cell lines) was added. Afterwards, cells were transferred into a 5 ml Eppendorf Cup and centrifuged (5 min, 1000 rpm, RT). Pellet from 1 T75-bottle (Sarstedt #833.910.302) was resuspended in 2.5 ml fresh medium. Cell number of cell suspension was determined with Luna-FL™ automated cell counter (Biozym #872040) by staining with acridine orange/propidium iodide assay viability kit (Biozym #872045). Laminin-coating (Millipore #CC095) of dishes was necessary for adhesion of ReNcell CX® cells before seeding the cells for experiments in a concentration of 2 µg/cm^2. Laminin-PBS solution was given in the respective amount directly to wells and flasks and was incubated for 1.5 h at 37 °C. For experiments cells were seeded and harvested as mentioned in method part of respective experimental chapter. After overnight incubation of cells at 37 °C and 5 % CO_2, cells were treated as explained in respective experimental description. 500 µl of remaining cell suspension was given into a new T75-bottle filled with 10 ml fresh full medium for culturing cells.

RNA-Analysis

Total RNA for cDNA synthesis was isolated using innuPREP® RNA Mini Kit (Analytik Jena #845-KS-2040250) according to manufacturer´s instructions. In order to synthesize cDNA, total RNA content was determined using a photometer (Eppendorf, BioPhotometer D30 #6133000907), diluted with nuclease-free water. Afterwards first-strand cDNA was prepared with iScript™ cDNA Synthesis Kit (BioRad #170-8891) according to manufacturer´s recommendations. For mRNA analysis real-time RT-PCR was performed using a CFX96 Touch™ Real Time PCR Detection System (BioRad #185-5196).

All primer pairs were ready-to-use standardized and were mixed with the respective ready-to-use Mastermix solution (SsoAdvanced™ Universial SYBR® Green

Supermix (BioRad #172-5271) according to manufacturer´s instructions (BioRad Prime PCR Quick Guide). Primer-pairs for *in vivo* experiments were adapted according to individual species.

Table 11: Primer pairs used for mRNA Analysis

Primer pair	Company	Unique Assay ID
Human CDKN1A	BioRad	qHsaCID0014498
Human CDNK1B	BioRad	qHsaCID0012509
Human CFLAR	BioRad	qHsaCID0038905
Human Col4A1	BioRad	qHsaCID0010223
Human CTGF	BioRad	qHsaCED0002044
Human DCX	BioRad	qHsaCID0010869
Human FN1	BioRad	qHsaCID0012349
Human GFAP	BioRad	qHsaCID0022307
Human GNB2L1	BioRad	qHsaCEP0057912
Human ID-2	BioRad	qHsaCED0043637
Human MKi67	BioRad	qHsaCID0011882
Human Nestin	BioRad	qHsaCED0044457
Human SERPINE1	BioRad	qHsaCED0043144
Human SOX2	BioRad	qHsaCED0036871
Human TGFβ-RII	BioRad	qHsaCID0016240
Human TP53	BioRad	qHsaCID0013658

As template, 1 µl of respective cDNA was used. RNA that was not reverse transcribed served as negative control for real-time RT-PCR. For relative quantification housekeeping gene Guanine nucleotide-binding protein subunit beta-2-like 1 (GNB2L1) was used. Real-time RT-PCR was performed with the following protocol:

Table 12: Protocol for real-time RT-PCR.

Initiation period	2 min	95 °C	1x
Denaturation	5 s	95 °C	40x
Annealing, Extension	30 s	60 °C	40x
Melting curve		65 °C – 95 °C (0.5 °C gradient)	1x

Afterwards, BioRad CFX Manager 3.1 was used for quantification of respective mRNA-level relative to GNB2L1 mRNA and then normalized to untreated control.

Western Blot:

For protein analysis, cells/tissues were lysed using M-PER® Mammalian Protein Extraction Reagent/ T-PER® Tissue Protein Extraction Reagent (Thermo Scientific, #78501/ #78510) according to manufacturer instructions, respectively. SDS-acrylamide-gels (10%) were produced using TGX Stain Free™ Fast Cast™ Acrylamide Kit (BioRad #161-0183) according to manufacturer instructions. Protein samples (20 µl) were diluted 1:5 with Lämmli-buffer (6.5 µl, Roti®-Load1, Roth #K929.1), incubated at 60 °C for 30 min and loaded on the gel with the entire volume of the protein solution. Separation of proteins was performed by electrophoresis using PowerPac™ Basic Power Supply (Biorad #164-5050SP) and Mini-PROTEAN® Tetra cell electrophoresis chamber (BioRad #165-8001-SP) (200 V, 45 min). Following electrophoresis, the proteins were blotted using Trans-Blot® Turbo Transfer System (BioRad #170-4155SP). All materials for western blotting were included in Trans-Blot® Turbo RTA PVDF-Midi Kit (BioRad #170-4273).

The PVDF-membrane for blotting procedure was activated in methanol (Merck #1.06009.2511) and equilibrated in 1x transfer buffer. Following blotting (25 V, 1 A, 30 min), membranes were washed (3x, 10 min, RT) with 1x TBS (Roth #10.60.1) containing 0.5 ml Tween-20 (Roth #9127.1). Afterwards, the membranes were blocked with 5% BSA (Albumin-IgG-free, Roth #3737.3) diluted with TBS-T for 1 h at RT, the primary antibodies (diluted in 0.5% BSA in TBS-T, Table 13) were added and incubated at 4 °C for 2 days. Antibodies for *in vivo* experiments were chosen for species specificity accordingly.

Table 13: Antibodies used for Western Blot analysis.

Primary Antibody	**Dilution**	**Company**	**Order Number**
Alpha-Tubulin HRP-linked (rabbit)	1:2000	Cell Signaling	cs12351s
ColIV (rabbit)	1:1000	Abcam	ab6586
CTGF (rabbit)	1:1000	Genetex	GTX-26992
FN (rabbit)	1:250	Proteintech	15613-1-AP
GAPDH XP HRP-linked (rabbit)	1:1000	Cell Signaling	cs8884s
Ki67 (rabbit)	1:500	Abcam	ab15580
pAkt (rabbit)	1:1000	Cell signaling	cs4060s
pErk1/2 (rabbit)	1:1000	Cell signaling	cs4370s
pSmad2 (rabbit)	1:500	Cell Signaling	cs3104
TGF-βRII (rabbit)	1:400	Aviva	ARP44743-T100
Secondary Antibody	**Dilution**	**Company**	**Order Number**
Anti-rabbit IgG, HRP-linked	1:10000	Cell signaling	cs#12351S

In the next step, membranes were washed in TBS-T (3x 10 min, RT) and incubated with the secondary antibody (1h, RT, Table 13). Following incubation, blots were washed with TBS-T, emerged using Luminata™Forte Western HRP Substrate (Millipore #WBLUF0500) and bands were detected with a luminescent image analyzer (ImageQuant™ LAS 4000, GE Healthcare). Afterwards, the blots were washed in TBS-T (3x 10min, RT) and blocked with 5% BSA diluted in TBS-T (1h, RT). For housekeeper comparison, the membranes were incubated with HRP-conjugated anti alpha-tubulin (1:2000 in 0.5 % BSA, 4 °C, overnight). The next day blots were emerged using Luminata™Forte Western HRP Substrate (Millipore #WBLUF0500) and bands were detected with the luminescent image analyzer. Finally, the blots were washed with TBS-T (3x, 5 min) and stained using 1x Roti®-Blue solution (Roth #A152.2) and dried at RT.

Immunocytochemistry

Cells were treated and harvested as described before. Following fixation of cells with Roti®-Histofix 4 % (Roth #P087.4) on 8-well, cell culture slide dishes (6 min, RT) were washed three times with PBS. After blocking cells for 1 h at RT with Blocking Solution (Zytomed #ZUC007-100) cells were incubated with respective primary antibodies listed in Table 14 and incubated at 4 °C overnight.

Afterwards, cell culture slides were washed three times with PBS following incubation with secondary antibody (1 h, RT). All antibody-dilutions were prepared with Antibody-Diluent (Zytomed #ZUC025-100).

Table 14: Antibodies used for immunocytochemistry.

Primary Antibody	**Dilution**	**Company**	**Order Number**
ColIV (rabbit)	1:50	Abcam	ab6586
CTGF (rabbit)	1:50	Genetex	GTX26992
βIII-Tubulin (rabbit)	1:100	cell signaling	cs5568
FN (rabbit)	1:50	Proteintech	15613-1-AP
Ki67 (rabbit)	1:100	Abcam	ab15580
NeuN (rabbit)	1:250	Abcam	Ab104225
Phalloidin Alexa Fluor 555	1:20	Cell signaling	cs8953
pSmad2 (rabbit)	1:50	Cell signaling	cs3104s
pSmad3 (rabbit)	1:50	Cell signaling	cs9520s
TGF-R_{II} (rabbit)	1:50	Millipore	06-227
Secondary Antibody	**Dilution**	**Company**	**Order Number**
Alexa Fluor 488	1:750	Life Technologies	A21441
Cy3 goat-anti-rabbit	1:1000	Life Technologies	A10520

Following incubation with secondary antibody, cells were washed three times with PBS, coverslips were separated from cell culture dish and mounted with VECTASHIELD® HardSet™ with DAPI (Biozol #VEC-H-1500). Slides were dried overnight at 4 °C before fluorescence microscopy (Zeiss, Axio® Observer.Z1). Images were analyzed with Image J Software and CorelDRAW® X7 Software.

***In vivo* experiments**

Peripheral blood mononuclear cell (PBMC) assay

PBMCs were isolated from buffy coats corresponding to 500 ml full blood transfusion units. Each unit was obtained from healthy volunteers and glucose-citrate was used as an anti-agglutinant. The buffy coat blood was prepared and delivered by the Blood Bank Suhl of the Institute for Transfusion Medicine, Germany. Each blood donation was monitored for HIV antibody, HCV antibody, HBs antigen, TPHA, HIV RNA, and SPGT (ALAT). Only blood samples tested negative for infectious agents and with a normal SPGT value were used for leukocyte and erythrocyte separation by low-speed centrifugation. The isolation of PBMCs was performed about 40 h following blood donation by gradient centrifugation using Ficoll-Histopague® 1077 (Heraeus™ Multifuge™ 3 SR). For IFNα assay, PBMCs were seeded at 100,000 cells/ 96-well in 100 µl complete medium plus additives (RPMI1640, + L-Glu, + 10% FCS, + PHA-P (5 µg/ ml), + IL-3 (10 µg/ ml)) and test compounds (5 µl) were added for direct incubation (24 h, 37 °C, 5% CO_2). For TNFα assay, PBMCs were seeded at 100,000 cells/ 96-well in 100 µl complete medium w/o additives (RPMI1640, + L-Glu, + 10% FCS) and test compounds (5 µl) were added for direct incubation (24 h, 37 °C, 5% CO_2). ELISA (duplicate measurement out of pooled supernatants, 20 µl) for huIFNα (eBioscience, #BMS216INSTCE) was performed according to the manufacturer's protocol. ELISA (duplicate measurement out of pooled supernatants, 20 µl) for huTNFα (eBioscience, #BMS223INSTCE) was performed according to the manufacturer's protocol.

bDNA assay

TGF-R_{II} mRNA levels were determined in liver, kidney, and lung lysate by bDNA assay according to manufacturer´s instructions (QuantiGene® kit, Panomics/ Affimetrix).

Immunofluorescence

Paraffin-embedded spinal cord and brain tissue was cut into 5 µm sections (3-4 slides per object plate). Paraffin sections were deparaffinized and demasked by

heating in citrate buffer (10 mM, 40 min) in a microwave oven. Afterwards, deparaffinized sections were incubated with 0.3 % H_2O_2 (30 min, RT), washed with PBS (10 min, RT) and blocked with Blocking Solution (Zytomed #ZUC007-100) for 30 min. After blocking for 1 h at RT with Blocking Solution (Zytomed) slides were incubated with 150 µl of the respective primary antibodies and incubated at 4 °C overnight. After washing with PBS (three times, 5 min RT) the slices were incubated with the secondary antibody for 1 h at RT. All antibody dilutions were prepared with Antibody Diluent (Zytomed #ZUC025-100). Afterwards the slices were washed again with PBS (three times, 5 min, RT) and mounted using VECTASHIELD® Mounting Medium with DAPI (Vector). Antibodies for immunofluorescence were comparable to cell culture experiments and adapted for each species.

Electrochemiluminescence

For immunological and hematological alterations, electrochemiluminescence technique (MesoScale Discovery®, Maryland, United States) was used. For each assay, 25 µl of the protein, blood, and liquor samples were used and the procedure was performed according to manufacturer's instructions.

BrdU assay

Labeling of dividing cells was performed by intraperitoneal injection of the thymidine analogue BrdU (Sigma, Steinheim, Germany) at 50 mg/kg of body weight using a sterile solution of 10 mg/ml of BrdU dissolved in a 0.9% (w/v) NaCl solution. The BrdU injections were performed daily within the last experimental week.

Surgery

For chronic central infusion, animals underwent surgery for an icv cannula attached to an Alzet® osmotic minipump (mice, rats, infusion rate: 0.25 µl/h, Alzet®, Model 2004, Cupertino, USA) or a gas pressure pump (Cynomolgus monkeys, infusion rate 0.25 ml/24 h, Tricumed®, Model IP 2000V, Germany). The cannula and the pump were stereotaxically implanted under ketamine/xylacin anesthesia (Baxter, GmbH, Germany) and semi-sterile conditions. Each osmotic minipump/ gas pressure pump was implanted subcutaneously in the abdominal region *via* a skin incision at the neck of the animals and connected with the icv cannula by silicone tubing. Animals were placed into a stereotaxic frame, and the icv cannula was lowered into the right lateral ventricle. The cannula was fixed with two stainless steel screws using dental cement (Kallocryl, Speiko®-Dr. Speier GmbH, Münster, Germany). The skin of the neck was closed with sutures. During surgery, the body temperature was maintained by a heating pad. To avoid post-surgical infections, animals were locally treated with betaisodona® (Mundipharma GmbH, Limburg, Germany) and received antibiotics

(sc, Baytril® 2.5% Bayer Vital GmbH, Leverkusen, Germany). The tubing was filled with the respective solution. Blood, liquor, and tissues were collected for analysis. Histological verification of the icv implantation sites was performed at 40 µm coronal, cresyl violet-stained brain sections.

Outcome parameters and functional analysis

Onset of symptomatic disease, onset of first paresis and survival were used as *in vivo* endpoints. Onset of symptomatic disease was defined as a lack of leg stretching in reaction to tail suspending. Time point at which gait impairments were first detected (e.g., hobbling or waddling) was classified as onset of first paresis. These parameters were determined daily starting at age 40 days.

To monitor disease progression, running wheel testing (LMTB, Berlin, Germany) was performed. Animals were caged separately with access to a running wheel starting at 33 days of age. Motor activity was directly correlated with the rotations per minute, generated by each animal in the running wheel. Each full turn of the wheel triggered two electromagnetic signals, directly fed into a computer attached to a maximum of 120 wheels. Running wheel data were recorded and analyzed with "Maus Vital" software (Laser- und Medizin-Technologie, Berlin, Germany). Assessment time lasted for 12 hours from 6:00 pm to 6:00 am.

Spatial learning test (Morris-Water-Maze)

Behavioral testing was performed between 8:00 and 13:00.

Rats were trained in a black circular pool (1.4 m in diameter, 50 cm high, filled with 20°C warm water to a height of 30 cm) to find a visible white target (10 cm in diameter, raised above the water's surface of approximately 1 cm) that was located throughout the study in the center of the same imaginary quadrant (proximally cued). Each animal was trained to navigate to the platform in 3 consecutive sessions with 12 trials/sessions, one session per day and an inter-trial interval of 10–20 s.

Microbiological analysis

Antisense-oligonucleotide samples were microbiologically analyzed according to Ph. Eur. 2.6.12, USP 30 <61> regarding the Total Aerobic Microbial Count (TAMC) and the Total Combined Yeast and Mould Count (TYMC).

Anion-exchange high-performance liquid chromatography (AEX-HPLC)

Integrity and stability of antisense-oligonucleotide (ASO) samples was determined by AEX-HPLC using ÄKTAexplorer™ System (GE healthcare, Freiburg, Germany). The purified ASO samples were desalinated by ethanol precipitation. The identity of the ASO was confirmed by electrospray-ionization-mass-spectrometry (ESI-MS) and the

purity was determined by AEX-HPLC with a Dionex DNAPac™ 200 (4 x 250 mm) column.

Example 1: Determination of inhibitory activity of inventive antisense-oligonucleotides on mRNA level

1.1 Transfection of Antisense-Oligonucleotides

The inhibitory activity of several antisense-oligonucleotides directed to TGF-R_{II} was tested in human epithelial lung cancer cells (A549). TGF-R_{II} mRNA was quantified by branched DNA assay in total mRNA isolated from cells incubated with TGF-R_{II} specific oligonucleotides.

Description of Method:

Cells were obtained and cultured as described above. Transfection of antisense-oligonucleotides was performed directly after seeding 10,000 A549 cells / well on a 96-well plate, and was carried out with Lipofectamine® 2000 (Invitrogen GmbH, Karlsruhe, Germany, cat. No. 11668-019) as described by the manufacturer. In two independent single dose experiments performed in quadruplicates, oligonucleotides were transfected at a concentration of 20 nM. After transfection, cells were incubated for 24 h at 37 °C and 5 % CO_2 in a humidified incubator (Heraeus GmbH, Hanau, Germany). For measurement of TGF-R_{II} mRNA, cells were harvested and lysed at 53 °C following procedures recommended by the manufacturer of the QuantiGene® Explore Kit (Panomics, Fremont, Calif., USA, cat. No. QG0004) for isolation of branched DNA (bDNA). For quantitation of housekeeping gene Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA the QuantiGene® Explore Kit was used, whereas quantitation of TGF-R_{II} mRNA was conducted with QuantiGene® 2.0 (custom manufacturing for Axolabs GmbH, Kulmbach, Germany). After incubation and lysis, 10 µl of the lysates were incubated with probe sets specific to human TGF-R_{II} and human GAPDH. Both reaction types were processed according to the manufacturer's protocol for the respective QuantiGene® kit. Chemoluminescence was measured in a Victor^2™ multilabel counter (Perkin Elmer, Wiesbaden, Germany) as RLUs (relative light units) and values obtained with the TGF-R_{II} probe sets were normalized to the respective GAPDH values for each well and then normalized to the corresponding mRNA readout from mock-treated cells.

Results

Results show the efficient downregulation of TGF-R_{II} by several ASOs after transfection of A549 cells. Downregulation after transfection of reference

oligonucleotides Ref. 6 – Ref. 10 was not as efficient and resulted in downregulation of > 60%.

Table 15: Downregulation of TGF-R_{II} mRNA. Transfection with TGF-R_{II} specific antisense-oligonucleotides (ASOs) in human epithelial lung carcinoma cells (A549). Quantitation of mRNA expression levels was performed relative to housekeeping gene GAPDH using QuantiGene® Kit. Probes were then normalized to the corresponding mRNA readout from mock-treated cells.

	A549 (c= 20 nM)			
	GAPDH		TGF-R_{II}	
	mean	SD	mean	SD
ASO				
Seq. ID No. 141j	1.41	0.05	0.02	0.01
Seq. ID No. 143aj	0.76	0.03	0.02	0.01
Seq. ID No. 139c	0.9	0.03	0.02	0.01
Seq. ID No. 145c	0.91	0.05	0.03	0.01
Seq. ID No. 209ax	1.52	0.58	0.03	0.01
Seq. ID No. 152ak	0.88	0.03	0.04	0
Seq. ID No. 218ar	1.08	0.03	0.04	0
Seq. ID No. 144c	0.5	0.07	0.05	0.03
Seq. ID No. 210ap	0.92	0.05	0.05	0.01
Seq. ID No. 142c	1.33	0.05	0.06	0.03
Seq. ID No. 213ak	1.2	0.03	0.07	0.01
Seq. ID No. 153f	1.09	0.07	0.08	0.03

Conclusion

TGF-R_{II} mRNA was efficiently targeted by the inventive ASOs. The named ASOs achieved an effective target mRNA downregulation after transfection of A549 cells.

1.2 Gymnotic uptake of Antisense-Oligonucleotides

1.2.1a Comparison of Target-Knockdown between inventive ASOs and prior-Art sequences by gymnotic transfer in A549 and Panc-1 cells

The downregulatory activity of several antisense-oligonucleotides directed to TGF-R_{II} was tested in human epithelial lung tumor cells (A549) by direct uptake without transfection reagents ("gymnotic uptake"). TGF-R_{II} mRNA was quantified by branched DNA assay in total mRNA isolated from cells incubated with TGF-R_{II} specific oligonucleotides.

Description of Method:

Cells were obtained and cultured as described in general methods. Gymnotic transfer of antisense-oligonucleotides was performed by preparing a 96-well plate with the respective antisense-oligonucleotides and subsequently seeding of 10,000 cells (Panc-1) or 8,000 cells (A549) /well. Experiments were performed in quadruplicates, oligonucleotides were used at final concentrations of 5 µM (Panc-1) and 7.5 µM (A549). Cells were incubated for 72 h at 37 °C and 5 % CO_2 in a humidified incubator (Heraeus GmbH, Hanau, Germany). For measurement of TGF-R_{II} mRNA, cells were harvested and lysed at 53 °C following procedures recommended by the manufacturer of the QuantiGene® Explore Kit (Panomics, Fremont, Calif., USA, cat. No. QG0004) for branched DNA (bDNA). For quantitation of housekeeping gene GAPDH mRNA the QuantiGene® Explore Kit was used, whereas quantitation of TGF-R_{II} mRNA was conducted with QuantiGene® 2.0 (custom manufacturing for Axolabs GmbH, Kulmbach, Germany). After incubation and lysis, 10 µl of the lysates were incubated with probe sets specific to human TGF-R_{II} and human GAPDH. Both reaction types were processed according to the manufacturer's protocol for the respective QuantiGene® kit. Chemoluminescence was measured in a Victor$^{2}{}_{TM}$ multilabel counter (Perkin Elmer, Wiesbaden, Germany) as RLUs (relative light units) and values obtained with the TGF-R_{II} probe sets were normalized to the respective GAPDH values for each well and then normalized to the corresponding mRNA readout from PBS treated cells.

Selected results are shown in Table 16a. Further modifications of Seq. ID No. 209ay, Seq. ID No. 209ax and Seq. ID No. 209y, namely ASOs listed in Table 6 of the description, showed comparable values to these three antisense-oligonucleotides. In addition, modifications of Seq. ID No. 152h, namely ASOs listed in Table 5 of the description, showed comparable values to this antisense-oligonucleotide. Modifications of Seq. ID No. 218b, namely ASOs listed in Table 8 of the description, showed comparable values to the antisense-oligonucleotide Seq. ID No. 218b. Modifications of Seq. ID No. 213k, namely ASOs listed in Table 9 of the description, showed comparable values to the antisense-oligonucleotide Seq. ID No. 213k. Also modifications of Seq. ID No. 210q, namely ASOs listed in Table 7 of the description, showed comparable values to the antisense-oligonucleotide Seq. ID No. 210q. Finally, modifications of Seq. ID No. 143h, namely ASOs listed in Table 4 of the description, showed comparable values to the antisense-oligonucleotide Seq. ID No. 143h. Transfer of antisense-oligonucleotides listed in Tables 4 – 9 resulted in a more potent downregulation of the target TGF-RII mRNA compared to the transfer of tested reference sequences (A549: downregulation < 0.5; Panc1 cells: downregulation < 0.4).

Table 16a Efficacy of target mRNA downregulation by gymnotic transfer. Remaining TGF-R_{II} mRNA after gymnotic uptake of selected TGF-R_{II} specific ASOs in A549 and Panc-1 cells. mRNA expression levels were determined relative to housekeeping gene Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and compared to PBS treated cells as reference control (=1) using QuantiGene® Kit.

ASO	Remaining mRNA of TGF-R_{II} (PBS treated cells = 1)			
	A549 cells		Panc1 cells	
	mean	SD	mean	SD
Seq. ID No. 209ay	0.11	0.01	0.07	0.02
Seq. ID No. 209ax	0.14	0.02	0.08	0.01
Seq. ID No. 209bb	0.19	0.01	0.11	0.01
Seq. ID No. 209az	0.19	0.03	0.13	0.02
Seq. ID No. 209ba	0.23	0.02	0.18	0.03
Seq. ID No. 209y	0.27	0.04	0.17	0.01
Seq. ID No. 152h	0.29	0.04	0.12	0.02
Seq. ID No. 218b	0.30	0.02	0.07	0.01
Seq. ID No. 213k	0.34	0.04	0.17	0.04
Seq. ID No. 210q	0.37	0.05	0.18	0.02
Seq. ID No. 210aq	0.39	0.03	0.18	0.02
Seq. ID No. 143h	0.43	0.04	0.35	0.05
Ref. 2	0.59	0.05	0.40	0.04
Ref. 0	0.89	0.06	1.10	0.07
Ref. 3	0.68	0.03	0.62	0.03
Ref. 4	0.74	0.04	0.71	0.01

Conclusion

Gymnotic transfer of inventive ASOs results in a continuously stronger downregulation of the target TGF-R_{II} mRNA than the transfer of tested reference sequences. The claimed antisense-oligonucleotides outperformed all tested sequences known from prior-art, independently of the chosen human cell line. Nevertheless, in general antisense-oligonucleotides having a length of 12 – 20 nucleotides result in a more effective downregulation of the target TGF-R_{II} mRNA than shorter or longer antisense-oligonucleotides. This effect was even more noticeable for antisense-oligonucleotides having a length of 14 – 18 nucleotides, which in general show the most potent effects.

1.2.1b Analysis of gymnotic transfer in A549 cells by branched DNA assay

Most effective antisense-oligonucleotides against TGF-R_{II} from the transfection screens were further characterized by gymnotic uptake in A549 cells. TGF-R_{II} mRNA

was quantified by branched DNA in total mRNA isolated from cells incubated with TGF-R_{II} specific antisense-oligonucleotides.

Description of Method:

A549 cells were cultured as described before under standard conditions. For single-dose and dose-response experiments 80,000 A549 cells / well were seeded in a 6-well culture dish and incubated directly with oligonucleotides at a concentration of 7.5 µM. For measurement of TGF-R_{II} mRNA, cells were harvested, lysed at 53 °C and analyzed by branched DNA Assay following procedures recommended by the manufacturer of the QuantiGene® Explore Kit (Panomics, Fremont, Calif., USA, cat. No. QG0004) as described above (see 1.1).

Results

Listed ASOs in Table 16b showed reduced target mRNA level of TGF-R_{II} relative to the housekeeping gene GAPDH in A549 cells. The ten most efficient ASOs were also tested for inhibitory concentration 50 (IC_{50}). All together Seq. ID No. 209t, Seq. ID No. 218b, Seq. ID No. 218c and Seq. ID No. 209y lead to most proper knockdown of TGF-R_{II} at low concentration levels.

Table 16b: Downregulation of TGF-R_{II} mRNA after gymnotic uptake of TGF-R_{II} specific ASOs in A549 cells. mRNA levels were determined relative to housekeeping gene GAPDH using QuantiGene® Kit. IC_{50} = inhibitory concentration for 50 % of downregulation, Pos. Ctrl: aha-1= activator of heat shock 90kDa protein ATPase homolog 1 (Aha1) directed LNA as positive control, Ref.1 = Scrambled control.

ASO	TGF-R_{II} n=4	SD	GAPDH n=4	SD	IC_{50} n=4
Seq. ID No. 209t	0.19	0.05	1.13	0.11	1.63
Seq. ID No. 218c	0.25	0.04	0.94	0.18	1.17
Seq. ID No. 218b	0.26	0.08	1.08	0.28	2.54
Seq. ID No. 218q	0.27	0.07	1.11	0.08	2.39
Seq. ID No. 209y	0.34	0.06	0.96	0.06	1.57
Seq. ID No. 218t	0.36	0.12	0.76	0.04	2.57
Seq. ID No. 218m	0.41	0.06	1.16	0.29	1.66
Seq. ID No. 209w	0.44	0.07	1.00	0.11	5.76
Seq. ID No. 218p	0.46	0.12	0.88	0.07	
Seq. ID No. 209v	0.48	0.25	0.96	0.07	3.10
Seq. ID No. 209x	0.52	0.02	0.87	0.06	5.60
Seq. ID No. 218u	0.53	0.20	0.79	0.05	

Seq. ID No.218v	0.54	0.13	0.77	0.04	
Seq. ID No. 210q	0.60	0.23	1.11	0.11	
Seq. ID No. 218o	0.61	0.15	0.96	0.06	
Seq. ID No. 210p	0.65	0.24	1.01	0.23	
Seq. ID No.218n	0.89	0.36	1.07	0.22	
Seq. ID No. 210o	0.95	0.08	0.97	0.14	
Seq. ID No. 209s	0.96	0.31	1.14	0.24	
pos. Ctrl aha-1	0.22	0.04	0.77	0.02	
Ref.1	1.43	0.40	1.27	0.18	

Conclusion

The target downregulation by the most efficient inventive ASOs was again excellent without transfection reagents. Thus, gymnotic transfer is feasible and the preferred method for further drug development.

1.2.2 Analysis of gymnotic uptake in A549 and ReNcell CX® cells

Inhibitory activity on the target mRNA by antisense-oligonucleotides (ASOs) was determined in human neuronal progenitor cells from cortical brain region (ReNcell CX® cells, Millipore #SCM007). Questions regarding adult neurogenesis as therapeutic target were assessed by gymnotic transfer studies with most effective ASOs. A549 cells were used as reference cell line.

Description of Method:

A549 and ReNcell CX® cells were cultured as described above. For treatment studies cells were seeded in a 24-well culture dish (Sarstedt #83.1836.300) (50,000 cells / well) and were incubated overnight at 37 °C and 5 % CO_2. For treatment of A549 and ReNcell CX® cells, medium was removed and replaced by fresh full medium (0.5 ml for 24-well). Ref.1, ASO with Seq. ID No. 218b , and ASO with Seq. ID No. 218c were then added in medium at concentrations of 2.5 and 10 µM for analysis of target downregulation at different time points (A549 cells: 18 h, 72 h, 6 d, ReNcell CX® cells: 18 h, 96 h, 8 d) at 37 °C and 5 % CO_2. For harvesting, cells were washed twice with PBS and frozen at -20 °C. For analysis of mRNA by real-time RT-PCR, cells were processed as described above. Ready-to-use and standardized primer pairs for real-time RT-PCR (see Table 11) were used and mixed with the respective ready-to-use Mastermix solution (SsoAdvanced™ Universial SYBR® Green Supermix (BioRad #172-5271) according to manufacturer´s instructions (BioRad Prime PCR Quick Guide). Probes were analyzed as triplicates and data was quantified relative to GNB2L1 mRNA using BioRad CFX Manager™ 3.1 and then

normalized to untreated control. Statistics were calculated using the Ordinary-one-way-ANOVA followed by "Dunnett's" *post hoc* comparisons.

Results:

Results showed that gymnotic transfer with Seq. ID No. 218b and 218c result in a proper downregulation of TGF-R_{II} mRNA in A549 and ReNcell CX® cells in a dose- and time dependent manner (Table 17). Target mRNA in A549 cells was significantly reduced after 18 h, and was even more efficient reduced after 72 h and 6 d. After 18h in ReNcell CX® only a depression of TGF-R_{II} mRNA after gymnotic uptake of 10µM could be observed, but target downregulation was significant after 72 h for both tested concentrations and was stable until day 8.

Table 17: Dose- and time-dependent downregulation of TGF-R_{II} mRNA after gymnotic transfer with TGF-R_{II} specific ASO in A549 and ReNcell CX® cells. mRNA expression levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, D = Seq. ID No. 218c, ± = SEM, *p < 0.05, **p < 0.01 in reference to A, +p < 0.05, ++p < 0.01 in reference to B. Statistics were calculated using the Ordinary-one-way-ANOVA followed by "Dunnett's" *post hoc* comparisons.

Cell line	**A549**		
Target Time point	TGF-R_{II} 18 h, n=3	TGF-R_{II} 72 h, n=3	TGF-R_{II} 6 d, n=3
A	1.00 ± 0.03	1.00 ± 0.20	1.00 ± 0.38
B 2.5 µM	1.17 ± 0.06	0.87 ± 0.21	0.88 ± 0.14
B 10 µM	0.98 ± 0.10	0.77 ± 0.06	1.03 ± 0.10
C 2.5 µM	**0.60*++ ± 0.09**	**0.41* ± 0.07**	0.13 ± 0.03
C 10 µM	**0.49**++ ± 0.02**	**0.15** ± 0.02**	**0.02*+ ± 0.00**
D 2.5 µM		**0.46** ± 0.09**	
D 10 µM		**0.21* ± 0.04**	

Cell line	**ReNcell CX**		
Target Time point	TGF-R_{II} 18 h, n=3	TGF-R_{II} 96 h, n=3	TGF-R_{II} 8 d, n=3
A	1.00 ± 0.41	1.00 ± 0.04	1.00 ± 0.18
B 2.5 µM	1.38 ± 0.58	0.89 ± 0.09	0.80 ± 0.33
B 10 µM	1.70 ± 0.68	0.81 ± 0.10	1.16 ± 0.43

C 2.5 µM	1.04 ± 0.36	**0.32** ± 0.06**	0.42 ± 0.16
C 10 µM	0.64 ± 0.24	**0.16** ± 0.02**	0.21 ± 0.09
D 2.5 µM		0.53 ± 0.07	
D 10 µM		**0.23** ± 0.03**	

Conclusion:

Efficient and stable downregulation of target mRNA by gymnotic uptake of ASOs is achieved even in long-term applications. ReNcell CX® cells could therefore be used e.g. for experiments addressing recovery of adult neurogenesis as a therapeutic option in patients. The same applies for other indications as shown by A549 experiments.

Taken together, efficient downregulation of TGF-R_{II} is suitable independently from method of transfer and cell type. Gymnotic uptake of ASOs is the preferred transfer method as in clinical applications the absence of additional transfection agents suggests high safety for patients.

Example 2: Determination of inhibitory activity of the antisense-oligonucleotides directed to TGF-R_{II} on protein level

Western Blot Analysis and Immunocytochemistry was performed to determine whether reduced TGF-R_{II} mRNA level, mediated by inventive antisense-oligonucleotides (ASOs) in human lung cancer cells (A549) and human neuronal precursor cells (ReNcell CX®) results in a reduction of target protein.

Description of Method:

Cells were cultured as described above. For treatment, cells were seeded in a 6-well culture dish (Sarstedt #83.3920.300, 80,000 cells / well) and 8-well cell culture slide dishes (Sarstedt #94.6140.802, 10,000 cells / well) and were incubated overnight at 37 °C and 5 % CO_2. For gymnotic transfer of A549 and ReNcell CX® cell medium was removed and replaced by fresh full medium (1 ml for 6-well and 0.5 ml for 8-well). Ref. 1 (scrambled control), the respective inventive ASO was then added in medium at concentrations of 2.5 and 10 µM for protein analysis of target downregulation after 72 h in A549 cells and 96 h in ReNcell CX® cells. The cells were lysed and examined by Western Blot as described in general method part. The primary antibody anti-TGF-R_{II} was diluted in 0.5% BSA in TBS-T and incubated at 4 °C for 2 days. Afterward membranes were incubated with the second antibody anti-rabbit IgG HRP-linked diluted in 0.5 % BSA in TBS-T (1h, RT). Following incubation, blots were washed with TBS-T, emerged using Luminata™Forte Western HRP Substrate (Millipore #WBLUF0500) and bands were detected with a luminescent

image analyzer (ImageQuant™ LAS 4000, GE Healthcare). For housekeeper comparison, the membranes were incubated with HRP-conjugated anti-GAPDH (1:1000 in 0.5 % Blotto, 4 °C, overnight). Densitometric quantification was calculated relative to GAPDH and then normalized to untreated control with Image Studio™ Lite Software.

Procedure for immunocytochemistry was performed as described in standard protocol. For verification of target-downregulation anti-TGF-R_{II} was diluted and incubated overnight at 4 °C. Cy3 goat-anti-rabbit was used as secondary antibody. All antibody-dilutions were prepared with Antibody-Diluent (Zytomed® #ZUC025-100). Examination of cells was performed by fluorescence microscopy (Zeiss Axio® Observer.Z1). Images were analyzed with Image J Software and CorelDRAW® X7 Software.

Results after gymnotic transfer:

Western Blot Analysis and immunocytochemistry were used to verify the reduction of TGF-R_{II} protein level. 72 h after gymnotic transfer, TGF-R_{II} protein was significantly reduced using high concentration of different ASOs according to the invention in comparison to untreated control in A549 cells (Table 18). Reduced TGF-R_{II} levels were also observed in ReNcell CX® cells (Table 18). For both cell lines, reduction of TGF-R_{II} protein level was shown by Western Blot Analysis. Immunocytochemistry revealed a strong dose-dependent reduction of TGF-R_{II} protein in both cell lines in comparison to untreated cells and scrambled control treated cells.

Table 18: Densitometric analysis after TGF-R_{II} Western Blot. Reduction of TGF-R_{II} protein after gymnotic transfer with TGF-R_{II} specific ASOs in A549 and ReNcell CX® cells could be observed after 72 h or 96 h, respectively. Protein levels were determined relative to housekeeping gene GAPDH using Image Studio™ Lite Software and were normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, D = Seq. ID No. 218c, F = Seq. ID No. 210q, G = Seq. ID No. 213k, H = Seq. ID No. 143h, I = Seq. ID No. 152h, J = Seq. ID No. 209az, K = Seq. ID No. 209y, ± = SEM, *p < 0.05 in reference to A. Statistics were calculated using the Ordinary-one-way-ANOVA followed by “Dunnett’s” *post hoc* comparisons.

Cell line	**A549**	**ReNcell CX**
Target Time point	TGF-R_{II} 72 h, n=3	TGF-R_{II} 96 h, n=2
A	1.00 ± 0.00	1.00 ± 0.00
B 2.5 µM	0.85 ± 0.13	0.91 ± 0.12
B 10 µM	1.06 ± 0.47	1.23 ± 0.16
C 2.5 µM	0.34 ± 0.11	0.59 ± 0.05

C 10 µM	**0.39* ± 0.11**	0.63 ± 0.17
D 2.5 µM	0.68 ± 0.14	1.21 ± 0.28
D 10 µM	**0.39* ± 0.07**	0.77 ± 0.10
F 2.5 µM	0.51 ± 0.08	0.71 ± 0.16
F 10 µM	0.45 ± 0.09	0.57 ± 0.12
G 2.5 µM	0.41 ± 0.13	0.61 ± 0.10
G 10 µM	**0.40* ± 0.06**	0.58 ± 0.08
H 2.5 µM	0.75 ± 0.12	0.83 ± 0.13
H 10 µM	0.51 ± 0.07	0.77 ± 0.06
I 2.5 µM	0.58 ± 0.14	0.91 ± 0.21
I 10 µM	**0.38* ± 0.14**	0.67 ± 0.09
J 2.5 µM	0.42 ± 0.15	0.75 ± 0.23
J 10 µM	**0.34* ± 0.05**	0.59 ± 0.08
K 2.5 µM	0.45 ± 0.17	0.69 ± 0.16
K 10 µM	**0.36* ± 0.09**	0.49 ± 0.09

Conclusion:

In addition to target mRNA downregulation, gymnotic transfer of Seq. ID No. 218b, Seq. ID No. 218c, Seq. ID No. 210q, Seq. ID No. 213k, Seq. ID No. 143h, Seq. ID No. 152h, Seq. ID No. 209az, and Seq. ID No. 209y resulted in excellent reduction of protein level in A549 and ReNcell CX® cells. Staining of TGF-R_{II} revealed a dose-dependent reduction of TGF-R_{II} protein after treatment with these ASOs in both cell lines.

Results after gymnotic transfer with further ASOs:

Protein analysis showed a reduced amount of TGF-R_{II} in A549 cells and ReNcell CX® cells gymnotic transfer of tested ASOs (10 µM, Table 19). This was also verified by immunocytochemistry. For both cell lines, reduction of TGF-R_{II} protein level by gymnotic transfer of the tested ASOs could be detected in comparison to untreated cells and scrambled control treated cells.

Table 19: Densitometric analysis after TGF-R_{II} Western Blot. Reduction of TGF-R_{II} protein after gymnotic transfer with further TGF-R_{II}-specific antisense-oligonucleotides (ASO) in A549 and ReNcell CX® cells could be observed after 72 h or 96 h, respectively. Protein levels were determined relative to housekeeping-gene GAPDH using Studio™ Lite Software and were then normalized to untreated control. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Dunnett's" *post hoc* comparisons. Reduction of protein level for ASO 1 - 25 is indicated with the following key: A = less than 10% inferior to SEQ ID No. 143h; B = more than 10 % but less than 20% inferior to SEQ ID No. 143h; C = more than 20 % but less than 30% inferior to SEQ ID No. 143h. Reduction of protein level for

ASO 26 - 48 is indicated with the following key: A = less than 10% inferior to SEQ ID No. 152h; B = more than 10 % but less than 20% inferior to SEQ ID No. 152h; C = more than 20 % but less than 30% inferior to SEQ ID No. 152h. Reduction of protein level for ASO 49 - 74 is indicated with the following key: A = less than 10% inferior to the mean value derived from Seq. ID No. 209az and Seq. ID No. 209y; B = more than 10 % but less than 20% inferior to the mean value derived from Seq. ID No. 209az and Seq. ID No. 209y; C = more than 20 % but less than 30% inferior to the mean value derived from Seq. ID No. 209az and Seq. ID No. 209y. Reduction of protein level for ASO 75 - 100 is indicated with the following key: A = less than 10% inferior to SEQ ID No. 210q; B = more than 10 % but less than 20% inferior to SEQ ID No. 210q; C = more than 20 % but less than 30% inferior to SEQ ID No. 210q. Reduction of protein level for ASO 101 - 124 is indicated with the following key: A = less than 10% inferior to the mean value derived from Seq. ID No. 218b and Seq. ID No. 218c; B = more than 10 % but less than 20% inferior to the mean value derived from Seq. ID No. 218b and Seq. ID No. 218c; C = more than 20 % but less than 30% inferior to the mean value derived from Seq. ID No. 218b and Seq. ID No. 218c. Reduction of protein level for ASO 125 - 152 is indicated with the following key: A = less than 10% inferior to SEQ ID No. 213k; B = more than 10 % but less than 20% inferior to SEQ ID No. 213k; C = more than 20 % but less than 30% inferior to SEQ ID No. 213k.

ASO No. in Test	Seq ID No.	Result
1	233d	**B**
2	234d	**A**
3	143j	**C**
4	143p	**A**
5	143q	**A**
6	143r	**A**
7	143w	**A**
8	143af	**C**
9	143ag	**C**
10	143ah	**C**
11	235b	**B**
12	235d	**A**
13	141d	**A**
14	141g	**A**
15	141i	**A**
16	237b	**A**
17	237c	**A**
18	237i	**C**
19	237m	**A**
20	238c	**A**
21	238f	**A**

22	239e	**B**
23	240c	**B**
24	241b	**C**
25	242a	**C**
26	246e	**C**
27	247d	**A**
28	248b	**A**
29	248e	**B**
30	248g	**A**
31	152k	**B**
32	152s	**B**
33	152t	**B**
34	152u	**B**
35	152ab	**C**
36	152ag	**B**
37	152ah	**C**
38	152ai	**C**
39	249c	**A**
40	249e	**A**
41	250b	**A**
42	250g	**B**
43	251c	**A**
44	251f	**A**
45	252e	**B**
46	253c	**A**
47	254b	**C**
48	255a	**C**
49	259e	**C**
50	260d	**B**
51	261b	**A**
52	261e	**B**
53	261g	**A**
54	262d	**B**
55	262e	**A**
56	209s	**A**
57	209v	**B**
58	209w	**B**
59	209x	**C**
60	209ai	**B**
61	209an	**C**
62	209at	**A**
63	209au	**B**
64	209av	**B**
65	263b	**B**
66	263c	**A**
67	263i	**B**
68	263m	**A**
69	264e	**A**
70	264h	**A**

71	265e	**B**
72	266c	**A**
73	267b	**B**
74	268a	**B**
75	272e	**B**
76	273d	**A**
77	274a	**A**
78	274d	**B**
79	274f	**A**
80	275g	**A**
81	275i	**B**
82	210o	**A**
83	210v	**B**
84	210w	**B**
85	210x	**C**
86	210ab	**B**
87	210ac	**A**
88	210ad	**B**
89	210af	**A**
90	210am	**B**
91	276b	**B**
92	276c	**A**
93	276j	**B**
94	276k	**B**
95	277d	**A**
96	277e	**A**
97	278f	**B**
98	279c	**B**
99	280b	**C**
100	281a	**C**
101	220d	**C**
102	221d	**B**
103	222b	**A**
104	222c	**A**
105	222f	**B**
106	223c	**B**
107	223f	**A**
108	218ad	**B**
109	218n	**A**
110	218t	**B**
111	218u	**B**
112	218v	**C**
113	218ah	**C**
114	218an	**A**
115	218ao	**B**
116	218ap	**B**
117	224i	**B**
118	224m	**B**
119	225c	**A**

120	225f	**A**
121	226e	**B**
122	227c	**A**
123	228b	**C**
124	229a	**C**
125	285d	**C**
126	286d	**A**
127	287d	**B**
128	287e	**A**
129	287f	**A**
130	288e	**A**
131	288i	**A**
132	289d	**B**
134	289h	**A**
135	289o	**B**
136	289p	**B**
137	289q	**B**
138	213o	**B**
139	213p	**B**
140	213q	**B**
141	213s	**B**
142	213y	**B**
143	213z	**B**
144	213aa	**B**
145	213af	**B**
146	290c	**A**
147	290f	**B**
148	290i	**A**
149	291c	**B**
150	292c	**C**
151	293b	**C**
152	294a	**C**

Conclusion:

Taken together, dose-dependent downregulation of TGF-R_{II} mRNA by gymnotic transfer in A549 and ReNcell CX® cells resulted in a dose-dependent reduction of protein levels. Inventive ASOs are potent in protein target downregulation as demonstrated in A549 and ReNcell CX® cells.

Example 3: Analysis of the effects of the antisense-oligonucleotides to the downstream signaling pathway of TGF-R_{II}.

Functional analyses were performed in human lung cancer cells (A549) and human neuronal precursor cells (ReNcell CX®). TGF-β downstream signaling pathway was analyzed, following to an effective downregulation of TGF-R_{II} mRNA and reduction of protein levels by gymnotic transfer of the inventive ASOs. Therefore, mRNA and

protein levels of Connective Tissue Growth Factor (CTGF), known as downstream-mediator of TGF-β, were evaluated. In addition, phosphorylation of Smad2 (mothers against decapentaphlegic homolog 2) was examined. The phosphorylation of Smad2 is a marker for an active TGF-β pathway followed by the upregulation of the downstream target gene CTGF.

Description of Method:

Cells were cultured as described before. For treatment, cells were seeded in a 6-well culture dish (Sarstedt #83.3920.300) (80,000 cells / well) and 8-well cell culture slide dishes (Sarstedt #94.6140.802) (10,000 cells / well) and were incubated overnight at 37 °C and 5 % CO_2. For gymnotic transfer, A549 and ReNcell CX® cell medium was removed and replaced by fresh full medium (1 ml for 6-well and 0.5 ml for 8-well). Ref. 1 (Scrambled control), ASO with sequence identification number 218b (Seq. ID No. 218b), No. 218c (Seq.ID No. 218c) was then added in medium at concentrations of 2.5 and 10 μM and respective analysis was performed after 72 h in A549 cells and 96 h in ReNcell CX® cells. To evaluate effects on CTGF mRNA level, real-time RT-PCR was performed as described before. The primer pair for analysis of CTGF was ready-to-use and standardized. To check for CTGF and pSmad2 protein levels, Western Blot and immunocytochemistry were used as described before. Type and used dilutions of antibodies for respective method are listed in Table 13 and 14.

3.1. Results for Seq.ID No.218b

3.1.1 Effects on CTGF mRNA and protein level

CTGF mRNA was significantly and dose-dependently reduced after gymnotic transfer with ASO Seq. ID No. 218b in A549 (72 h) and ReNcell CX® (96 h) cells. Downstream-mediator of TGF-β was reduced to 52 % ± 0.02 in ReNcell CX® cells and to 39 % ± 0.03 in A549 cells after gymnotic transfer with 10 µM Seq.ID No.218b (Table 20). According to these downregulated CTGF mRNA levels, a strong reduction of CTGF protein expression was observed in A549 cells (Table 21).

Table 20: Dose-dependent and significant downregulation of CTGF mRNA after gymnotic transfer with Seq. ID No. 218b in A549 and ReNcell CX® cells.

mRNA expression levels were quantified relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b. ± = SEM, *p < 0.05, **p < 0.01 in reference to A. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Dunnett's" *post hoc* comparisons.

Cell line	A549	ReNcell CX
Target	CTGF	CTGF

Time point	72 h, n=3	96 h, n=3
A	1.00 ± 0.08	1.00 ± 0.04
B 2.5 µM	0.87 ± 0.06	0.97 ± 0.06
B 10 µM	0.80 ± 0.03	0.86 ± 0.17
C 2.5 µM	**0.60** ± 0.04**	**0.66** ± 0.02**
C 10 µM	**0.39** ± 0.03**	**0.52** ± 0.02**

Table 21: Densitometric analysis of CTGF Western Blot. Downregulation of CTGF protein 72 h after gymnotic transfer with ASO Seq. ID No. 218b in A549 was recognized. Protein levels were determined relative to housekeeping gene alpha-Tubulin using Studio™ Lite Software and were normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b.

Cell line	**A549**
Target Time point	CTGF 72 h, n=1
A	1.00
B 2.5 µM	0.91
B 10 µM	1.31
C 2.5 µM	0.05
C 10 µM	0.086

Conclusion:

Functional inhibition of TGF-β signaling was achieved with gymnotic transfer of Seq. ID No. 218b as shown by downregulation of target CTGF mRNA and reduced CTGF protein levels in A549 and ReNcell CX® cells.

3.1.2 Effects on pSmad2 protein level

pSmad2 protein levels were analyzed to proof the CTGF downregulation as a specific result of the ASO-mediated TGF-β signaling inhibition.

Staining against pSmad2 after gymnotic transfer of ASO Seq. ID No. 218b after 72 h in A549 and 96 h in ReNcell CX® cells showed a dose-dependent inhibition of Smad2 phosphorylation (Figure 5). In addition, reduction of pSmad2 expression levels by ASO Seq. ID No. 218b was verified by Western Blot Analysis in A549 cells (Table 22).

Table 22: Densitometric analysis of pSmad2 Western Blot. Downregulation of pSmad2 protein 72 h after gymnotic transfer with ASO Seq. ID No. 218b in A549 was recognized. Protein levels were determined relative to housekeeping gene GAPDH

using Studio™ Lite Software and normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b.

Cell line	A549
Target Time point	pSmad2 72 h, n=1
A	1.00
B 2.5 µM	1.81
B 10 µM	1.79
C 2.5 µM	0.66
C 10 µM	0.72

Conclusion:

Gymnotic transfer of Seq. ID No. 218b in A549 and ReNcell CX® cells resulted in a dose-dependent inhibition of downstream mediators of TGF-β signaling. CTGF and phosphorylation of Smad2 was reduced by ASO Seq. ID No. 218b, both indicating an inhibited TGF-β pathway.

3.2 Results for Seq.ID No. 218c

3.2.1 Effects on CTGF mRNA and pSmad2 protein level

Gymnotic transfer of ASO Seq. ID No. 218c downregulates CTGF mRNA in A549 and ReNcell CX® cells (Table 23). Immunocytochemistry against pSmad2 confirmed an inhibition of TGF-β signaling (Figure 6). Therefore, downregulation of CTGF mRNA is an direct effect of reduced TGF-β signaling.

Table 23: Significant downregulation of CTGF mRNA was observed in A549 and ReNcell CX® cells. mRNA expression levels were quantified relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and normalized to untreated controls. A = untreated control, B = Ref.1, D = Seq. ID No. 218c. ± = SEM, **p < 0.01 in reference to A. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Dunnett's" *post hoc* comparisons.

Cell line	A549	ReNcell CX
Target Time point	CTGF 72 h, n=4	CTGF 96 h, n=3
A	1.00 ± 0.08	1.00 ± 0.10
B 2.5 µM	0.97 ± 0.07	0.88 ± 0.08
B 10 µM	0.85 ± 0.06	0.89 ± 0.07
D 2.5 µM	**0.49** ± 0.05**	1.10 ± 0.08
D 10 µM	**0.31** ± 0.03**	0.82 ± 0.02

Conclusion:

ASO Seq. ID No. 218c was efficient in inhibiting TGF-β signaling after downregulation of target TGF-R_{II} mRNA. This was examined by determination of downregulated CTGF mRNA and reduced pSmad2 protein levels as a marker for TGF-β signaling.

Taken together, inventive ASOs are efficient in mediating a functional inhibition of TGF-β signaling by downregulation of TGF-R_{II}. Thus, inventive ASOs will be beneficial for medical indications in which elevated TGF-β levels are involved, e.g. neurological disorders, fibrosis and tumor progression.

Example 4: Inhibitory activity of the inventive ASOs on target mRNA levels in TGF-β1 treated cells.

4.1 Gymnotic uptake of ASOs in A549 and ReNcell CX® cells after TGF-β1 pre-treatment

To analyze inhibitory activity of antisense oligonucleotides (ASOs) in human neuronal progenitor cells from cortical brain region (ReNcell CX®) under pathological conditions, cells were pre-treated with Transforming Growth Factor-β 1 (TGF-β1). From previous studies it is known that TGF-β1 is found in high concentrations in Cerebrospinal Fluid (CSF) of all neural disorders e.g. ALS. Therefore, inhibitory efficacy of ASOs on TGFβ-signaling was examined after pre-treatment and in presence with TGF-β1. A549 cells were used as reference cell line.

Description of Method:

A549 and ReNcell CX® were cultured as described above. For treatment studies cells were seeded in a 24-well culture dish (Sarstedt #83.1836.300) (50,000 cells / well) and were incubated overnight at 37 °C and 5 % CO_2. For treatment of A549 and ReNcell CX® cells, medium was removed and replaced by fresh full medium (0.5 ml for 24-well). Following TGF-β1 (10 ng/ml, PromoCell #C-63499) exposition for 48 h, medium was changed, TGF-β1 re-treatment was performed in combination with Ref.1 (Scrambled control, 10 µM), ASO Seq. ID No. 218b (10 µM), or ASO Seq. ID No. 218c (10 µM) in medium. A549 cells were incubated for further 72 h, whereas ReNcell CX® cells were harvested after 96 h. Therefore, cells were washed twice with PBS and subsequently used for RNA isolation (24-well dishes) as described before. Used primer pairs for real-time RT-PCR are listed in Table 11.

4.1.1 Results for Seq. ID No. 218b

Efficacy in mRNA downregulation of TGF-R_{II} by ASO Seq. ID No. 218b was not influenced by TGF-β1 pre-incubation in A549 and ReNcell CX® cells (Table 24,

Figure 7). Target mRNA in A549 cells was significantly downregulated after single treatment (remaining mRNA: 15 % ± 0.05) with ASO, but also after treatment in presence of TGF-β1, following pre-treatment (remaining mRNA: 7 % ± 0.01). In ReNcell CX® cells ASO Seq. ID No. 218b showed similar potency in inhibiting TGF-R_{II} mRNA in absence of TGF-β1 (25 % ± 0.01) or in presence of TGF-β1, following pre-treatment of TGF-β1 (17 % ± 0.02).

Table 24: In presence of TGF-β1, ASO Seq. ID No. 218b leads to a potent downregulation of TGF-R_{II} mRNA after gymnotic transfer in A549 and ReNcell CX® cells. mRNA expression levels were quantified relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and normalized to untreated controls. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E = TGF-β1, ± = SEM, *p < 0.05, **p < 0.01 in reference to A. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Dunnett's" *post hoc* comparisons.

Target **Time point**	**TGF-R_{II}** 48 h TGF-β1 -> 72 h / 96 h TGF-β1 + ASOs / single treatment	
Cell line	**A549** n= 4	**ReNcell CX** n= 3
A	1.00 ± 0.07	1.00 ± 0.11
B 10 µM	0.90 ± 0.17	0.89 ± 0.26
C 10 µM	**0.15** ± 0.05**	0.25 ± 0.01
E 10 ng/ml	0.71 ± 0.05	0.79 ± 0.34
E 10 ng/ml + B 10 µM	0.74 ± 0.05	0.89 ± 0.25
E 10 ng/ml + C 10 µM	**0.07** ± 0.01**	0.27 ± 0.02

Conclusion:

Target mRNA was efficiently downregulated to approx. 20 % by gymnotic uptake of inventive ASOs in presence of TGF-β1, following pre-incubation in both tested cell lines.

4.1.2 Results for Seq. ID No. 218c

Downregulation of TGF-R_{II} mRNA by ASO Seq. ID No. 218c was effective in presence of TGF-β1 in A549 and ReNcell CX® cells (Table 25, Figure 8). Target mRNA in both tested cell lines was significantly downregulated, regardless of a single treatment with ASO Seq. ID No. 218c or in presence with TGF-β1.

Table 25: In presence of TGF-β1, ASO Seq. ID No. 218c leads to a potent downregulation of TGF-R_{II} mRNA after gymnotic transfer in A549 and ReNcell

CX® cells. mRNA expression levels were quantified relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and normalized to untreated control. A = untreated control, B = Ref.1, D = Seq. ID No. 218c, E = TGF-β1, ± = SEM, *p < 0.05, **p < 0.01 in reference to A. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Dunnett's" *post hoc* comparisons.

Target **Time point**	**TGF-R_{II}** 48 h TGF-β1 -> 72 h / 96 h TGF-β1 + ASOs / single treatment	
Cell line	**A549** n= 2	**ReNcell CX** n= 2
A	1.00 ± 0.12	1.00 ± 0.18
B 10 µM	0.92 ± 0.06	0.51 ± 0.14
D 10 µM	**0.31** ± 0.04**	**0.05** ± 0.01**
E 10 ng/ml	0.68 ± 0.05	0.88 ± 0.73
E 10 ng/ml + B 10 µM	0.86 ± 0.04	0.45 ± 0.09
E 10 ng/ml + D 10 µM	**0.16** ± 0.05**	**0.03** ± 0.01**

Conclusion:

Taken together, the inventive ASOs were effective in downregulating TGF-R_{II} mRNA in presence of TGF-β1, indicating that ASOs are functional under pathological conditions.

Example 5: Inhibitory activity of the inventive ASOs on target protein levels in TGF-β1 treated cells.

To analyze inhibitory activity of antisense oligonucleotides (ASOs) in human neuronal progenitor cells from cortical brain region (ReNcell CX®) under pathological conditions, cells were pre-treated with Transforming Growth Factor-β 1 (TGF-β1). From previous studies it is known that TGF-β1 is found in high concentrations in Cerebrospinal Fluid (CSF) of all neural disorders e.g. ALS. Therefore, inhibitory efficacy of ASOs on TGFβ-signaling was examined after pre-treatment and in presence with TGF-β1. A549 cells were used as reference cell line.

Description of Method:

Cells were cultured as described before in standard protocol. For treatment, cells were seeded in a 6-well culture dish (Sarstedt #83.3920.300) (80,000 cells / well) and 8-well cell culture slide dishes (Sarstedt #94.6140.802) (10,000 cells / well) and were

incubated overnight at 37 °C and 5 % CO_2. For investigation of gymnotic transfer effects (A549 and ReNcell CX), after pre-incubation with TGF-β1 (Promocell # C-63499), medium was removed and replaced by fresh full medium (1 ml for 6-well dishes and 8-well cell culture slide dishes). Following exposition of TGF-β1 (10 ng/ml, 48 h) medium was changed, TGF-β1 (10 ng/ml), Ref.1 (Scrambled control, 10 μM), and inventive ASOs (10 μM) was added, in combination and in single treatment, to the cells. A549 cells were incubated for further 72 h, whereas ReNcell CX® cells were harvested after 96 h. Therefore, cells were washed twice with PBS and subsequently used for protein isolation (6-well dishes) following Western Blot analysis or immunocytochemical examination of cells (in 8-well cell culture slide dishes). Procedures for used techniques were performed as described before. Used antibodies and dilutions for respective methods are listed in Table 13 and 14.

Results after gymnotic transfer

Western Blot and immunocytochemical analysis for A549 cells showed that the ASOs having Seq. ID No. 218b, Seq. ID No. 218c, Seq. ID No. 210q, Seq. ID No. 213k, Seq. ID No. 143h, Seq. ID No. 152h, Seq. ID No. 209az, Seq. ID No. 209y generate a potent target downregulation in presence of TGF-β1 (Table 26). Staining of TGF-R_{II} on fixed ReNcell CX® cells confirmed the results observed in A549 cells. Tested ASOs revealed a strong target downregulation after single treatment but also in presence with TGF-β1.

Table 26: Densitometric analysis of TGF-R_{II} Western Blot. Reduction of TGF-R_{II} protein after TGF-β1 pre-incubation followed by gymnotic transfer with different ASOs in A549 was observed. Protein levels were determined relative to housekeeping gene GAPDH using Studio™ Lite Software and were then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq.ID No. 218b, C = Seq. ID No. 218b, D = Seq. ID No. 218c, F = Seq. ID No. 210q, G = Seq. ID No. 213k, H = Seq. ID No. 143h, I = Seq. ID No. 152h, J = Seq. ID No. 209az, K = Seq. ID No. 209y, E = TGF-β1.

Target Time point	**TGF-R_{II}** 48 h TGF-β1 -> 72 h TGF-β1 + ASOs / single treatment
Cell line	**A549** n= 1
A	1.00
B 10 µM	1.20
C 10 µM	0.31
D 10 µM	0.42

F 10 µM	0.45
G 10 µM	0.35
H 10 µM	0.47
I 10 µM	0.33
J 10 µM	0.27
K 10 µM	0.30
E 10 ng/ml	2.03
E 10 ng/ml + B 10 µM	1.50
E 10 ng/ml + C 10 µM	0.78
E 10 ng/ml + D 10 µM	1.16
E 10 ng/ml + F 10 µM	1.21
E 10 ng/ml + G 10 µM	0.83
E 10 ng/ml + H 10 µM	1.02
E 10 ng/ml + I 10 µM	0.76
E 10 ng/ml + J 10 µM	0.69
E 10 ng/ml + K 10 µM	0.77

Conclusion:

TGF-β1 pre-incubation followed by gymnotic transfer of Seq. ID No. 218b, Seq. ID No. 218c, Seq. ID No. 210q, Seq. ID No. 213k, Seq. ID No. 143h, Seq. ID No. 152h, Seq. ID No. 209az, and Seq. ID No. 209y resulted, in addition to target mRNA downregulation, in a reduction of protein level in A549 and ReNcell CX® cells.

Results after gymnotic transfer with further ASOs:

Western Blot analysis showed a reduced amount of TGF-R_{II} protein in A549 cells (Table 27) after gymnotic transfer for 72 h in comparison to untreated cells and cells treated with scrambled control. Pre-incubation of TGF-β1 followed by gymnotic transfer of tested ASOs evoked a reduction in comparison to cells which were pre-treated with TGF-β1 followed by gymnotic transfer with scrambled control. Immunocytochemical examination of A549 and ReNcell CX® after staining against TGF-R_{II} showed that tested ASOs mediated a strong reduction of target protein after gymnotic transfer with or without pre-treatment of TGF-β1.

Table 27 Densitometric analysis of TGF-R_{II} Western Blot. Reduction of TGF-R_{II} protein after TGF-β1 pre-incubation followed by gymnotic transfer with further TGF-RII-specific antisense oligonucleotides (ASOs) in A549 could be detected. Protein levels were determined relative to housekeeping gene GAPDH using Studio™ Lite Software and were then normalized to untreated control. Reduction of protein level for ASO 1 - 25 is indicated with the following key: A = less than 10% inferior to SEQ

ID No. 143h; B = more than 10 % but less than 20% inferior to SEQ ID No. 143h; C = more than 20 % but less than 30% inferior to SEQ ID No. 143h. Reduction of protein level for ASO 26 - 48 is indicated with the following key: A = less than 10% inferior to SEQ ID No. 152h; B = more than 10 % but less than 20% inferior to SEQ ID No. 152h; C = more than 20 % but less than 30% inferior to SEQ ID No. 152h. Reduction of protein level for ASO 49 - 74 is indicated with the following key: A = less than 10% inferior to the mean value derived from Seq. ID No. 209az and Seq. ID No. 209y; B = more than 10 % but less than 20% inferior to the mean value derived from Seq. ID No. 209az and Seq. ID No. 209y; C = more than 20 % but less than 30% inferior to the mean value derived from Seq. ID No. 209az and Seq. ID No. 209y. Reduction of protein level for ASO 75 - 100 is indicated with the following key: A = less than 10% inferior to SEQ ID No. 210q; B = more than 10 % but less than 20% inferior to SEQ ID No. 210q; C = more than 20 % but less than 30% inferior to SEQ ID No. 210q. Reduction of protein level for ASO 101 - 124 is indicated with the following key: A = less than 10% inferior to the mean value derived from Seq. ID No. 218b and Seq. ID No. 218c; B = more than 10 % but less than 20% inferior to the mean value derived from Seq. ID No. 218b and Seq. ID No. 218c; C = more than 20 % but less than 30% inferior to the mean value derived from Seq. ID No. 218b and Seq. ID No. 218c. Reduction of protein level for ASO 125 - 152 is indicated with the following key: A = less than 10% inferior to SEQ ID No. 213k; B = more than 10 % but less than 20% inferior to SEQ ID No. 213k; C = more than 20 % but less than 30% inferior to SEQ ID No. 213k.

ASO No. in Test	Seq ID No.	Result
1	233d	**B**
2	234d	**A**
3	143j	**C**
4	143p	**A**
5	143q	**A**
6	143r	**A**
7	143w	**A**
8	143af	**B**
9	143ag	**C**
10	143ah	**C**
11	235b	**B**
12	235d	**A**
13	141d	**A**
14	141g	**A**
15	141i	**B**
16	237b	**A**
17	237c	**A**
18	237i	**C**

19	237m	**A**
20	238c	**A**
21	238f	**A**
22	239e	**B**
23	240c	**B**
24	241b	**C**
25	242a	**C**
26	246e	**C**
27	247d	**A**
28	248b	**A**
29	248e	**B**
30	248g	**A**
31	152k	**B**
32	152s	**B**
33	152t	**B**
34	152u	**B**
35	152ab	**C**
36	152ag	**B**
37	152ah	**A**
38	152ai	**A**
39	249c	**A**
40	249e	**A**
41	250b	**A**
42	250g	**B**
43	251c	**A**
44	251f	**A**
45	252e	**B**
46	253c	**A**
47	254b	**C**
48	255a	**C**
49	259e	**C**
50	260d	**B**
51	261b	**A**
52	261e	**B**
53	261g	**A**
54	262d	**B**
55	262e	**A**
56	209s	**A**
57	209v	**B**
58	209w	**A**
59	209x	**C**
60	209ai	**B**
61	209an	**C**
62	209at	**B**
63	209au	**B**
64	209av	**B**
65	263b	**B**
66	263c	**A**
67	263i	**B**

68	263m	**A**
69	264e	**A**
70	264h	**A**
71	265e	**B**
72	266c	**A**
73	267b	**B**
74	268a	**B**
75	272e	**B**
76	273d	**B**
77	274a	**A**
78	274d	**B**
79	274f	**A**
80	275g	**A**
81	275i	**B**
82	210o	**A**
83	210v	**B**
84	210w	**B**
85	210x	**C**
86	210ab	**B**
87	210ac	**A**
88	210ad	**B**
89	210af	**A**
90	210am	**B**
91	276b	**B**
92	276c	**A**
93	276j	**B**
94	276k	**B**
95	277d	**A**
96	277e	**A**
97	278f	**B**
98	279c	**B**
99	280b	**C**
100	281a	**C**
101	220d	**C**
102	221d	**B**
103	222b	**A**
104	222c	**A**
105	222f	**B**
106	223c	**B**
107	223f	**A**
108	218ad	**B**
109	218n	**A**
110	218t	**B**
111	218u	**B**
112	218v	**C**
113	218ah	**C**
114	218an	**A**
115	218ao	**B**
116	218ap	**B**

117	224i	**B**
118	224m	**B**
119	225c	**A**
120	225f	**A**
121	226e	**B**
122	227c	**B**
123	228b	**C**
124	229a	**C**
125	285d	**C**
126	286d	**A**
127	287d	**B**
128	287e	**A**
129	287f	**A**
130	288e	**A**
131	288i	**A**
132	289d	**B**
134	289h	**A**
135	289o	**B**
136	289p	**A**
137	289q	**B**
138	213o	**B**
139	213p	**B**
140	213q	**B**
141	213s	**B**
142	213y	**B**
143	213z	**B**
144	213aa	**B**
145	213af	**C**
146	290c	**A**
147	290f	**B**
148	290i	**A**
149	291c	**B**
150	292c	**C**
151	293b	**C**
152	294a	**C**
125	285d	**C**

Conclusion:

Even after TGF-β1 pre-incubation, gymnotic transfer of inventive ASOs results in reduction of TGF-R_{II} protein in A549 and ReNcell CX® cells.

Example 6: Analysis of the effects of the inventive ASOs to the downstream signaling pathway of TGF-R_{II} after TGF-β1-preincubation

Functional analyses were performed in human lung cancer cells (A549) and human neuronal precursor cells (ReNcell CX®). TGF-β1 downstream signaling pathway was analyzed, following to an effective downregulation of TGF-R_{II} mRNA and reduction of protein levels by gymnotic transfer of the inventive ASOs in presence of TGF-β1.

Therefore, mRNA and protein levels of Connective Tissue Growth Factor (CTGF), known as downstream-mediator of TGF-β, were evaluated. In addition, phosphorylation of Smad2 (mothers against decapentaphlegic homolog 2) was examined. The phosphorylation of Smad2 is a marker for an active TGF-β pathway followed by the upregulation of the downstream target gene CTGF.

Description of Method:

Cells were cultured as described before in standard protocol. For treatment, cells were seeded in 24-well culture dishes (Sarstedt #83.1836.300) (50,000 cells / well), 6-well culture dishes (Sarstedt #83.3920.300) (80,000 cells / well) and 8-well cell culture slide dishes (Sarstedt #94.6140.802) (10,000 cells / well) and were incubated overnight at 37 °C and 5 % CO_2. For investigation of gymnotic transfer effects (A549 and ReNcell CX® cells), after pre-incubation with TGF-β1, medium was removed and replaced by fresh full medium (1 ml for 6-well dishes and 8-well cell culture slide dishes). Following exposition of TGF-β1 (10 ng/ml, 48 h) medium was changed, TGF-β1 (10 ng/ml), Ref.1 (Scrambled control, 10 μM), ASO with Seq. ID No. 218b (10 μM), and ASO with Seq. ID No. 218c (10 μM) was added in combination and in single treatment to cells. A549 cells were incubated for further 72h, whereas ReNcell CX® cells were harvested after 96 h. Therefore, cells were washed twice with PBS and subsequently used for RNA (24-well dishes) and protein isolation (6-well dishes) or immunocytochemical examination of cells (in 8-well cell culture slide dishes). To evaluate effects on CTGF mRNA level, real-time RT-PCR was performed as described before. The primer pair for analysis of CTGF was ready-to-use and standardized. To check for CTGF and pSmad2 protein levels, Western Blot and immunocytochemistry were used as described before. Type and used dilutions of antibodies for respective method are listed in Table 13 and 14.

6.1. Results for Seq. ID No. 218b

6.1.1 Effects on CTGF mRNA and protein levels

CTGF mRNA was downregulated after gymnotic transfer with ASO Seq. ID No. 218b in A549 (72 h, 0.52 ± 0.05) and ReNcell CX® (96 h, 0.70 ± 0.25) cells, whereas TGF-β1 incubation for 5 days (A549: 48 h + 72 h, 6.92 ± 2.32) or 6 days (ReNcell CX: 48 h + 96 h, 1.60 ± 015) respectively, caused significant upregulation of CTGF mRNA. ASO Seq. ID No. 218b was potent enough to evoke a CTGF mRNA downregulation by blocking TGF-β1 effects in presence of TGF-β1 (Table 28, Figure 11). According to observations for mRNA levels, immunochemical staining against CTGF also confirmed these observations for protein levels (Figure 12).

Table 28: Downregulation of CTGF mRNA in presence of TGF-β1 followed by gymnotic transfer with Seq. ID No. 218b in A549 and ReNcell CX® cells. mRNA expression levels were quantified relative to housekeeping GNB2L1 using quantitative real-time RT-PCR normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E = TGF-β1, ± = SEM, *p < 0.05, **p < 0.01 in reference to A, $^{++}$p < 0.01 in reference to E+B. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey's" *post hoc* comparisons.

Target Time point	**CTGF** 48 h TGF-β1 -> 72 h / 96 h TGF-β1 + ASOs / single treatment	
Cell line	**A549** n= 5	**ReNcell CX** n= 3
A	1.00 ± 0.22	1.00 ± 0.04
B 10 µM	0.89 ± 0.19	0.85 ± 0.01
C 10 µM	0.52 ± 0.05	**0.70* ± 0.25**
E 10 ng/ml	**6.92* ± 2.32**	**1.60** ± 0.15**
E 10 ng/ml + B 10 µM	**8.79** ± 2.72**	**1.71** ± 0.03**
E 10 ng/ml + C 10 µM	**2.53^{++} ± 0.59**	**1.19^{++} ± 0.04**

Conclusion:

In presence of TGF-β1 and following treatment of ASO Seq. ID No. 218b resulted firstly in downregulation of TGF-R_{II} mRNA and secondary in reduced CTGF mRNA and protein levels in A549 and ReNcell CX® cells. That indicates that ASO Seq. ID No. 218b is potent enough to be active under high TGF-β1 pathological conditions and is able to rescue from TGF-β1 mediated effects.

6.1.2 Effects on pSmad2 protein level

To verify if CTGF downregulation is a consequence of specific TGF-β signaling inhibition, mediated by ASO Seq. ID No. 218b in presence of TGF-β1, pSmad2 protein levels were analyzed.

Staining pSmad2 after TGF-β1 pre-incubation followed by gymnotic transfer of ASO Seq. ID No. 218b with parallel TGF-β1 exposition leads to an inhibition of Smad2 phosphorylation in both tested cell lines (Figure 13). In addition, reduced pSmad2 protein levels were verified by Western Blot Analysis in A549 and ReNcell CX® cells (Table 29).

Table 29: Densitometric analysis of pSmad2 Western Blot. Downregulation of pSmad2 protein after gymnotic transfer with ASO Seq. ID No. 218b was recognized. Also reversion of TGF-β1 mediated effects by inventive ASOs was found, when

combination treatments were compared. Protein levels were determined relative to housekeeping gene GAPDH using Studio™ Lite Software and were then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E = TGF-β1. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Dunnett's" *post hoc* comparisons.

Target Time point	**pSmad2** 48 h TGF-β1-> 72 h / 96 h TGF-β1 + ASOs / single treatment	
Cell line	**A549** n= 2	**ReNcell CX** n= 2
A	1.00 ± 0.00	1.00 ± 0.00
B 10 µM	1.23 ± 0.47	0.89 ± 0.22
C 10 µM	0.58 ± 0.08	0.66 ± 0.14
E 10 ng/ml	1.40 ± 0.31	1.19 ± 0.61
E 10 ng/ml + B 10 µM	1.27 ± 0.46	2.19 ± 0.76
E 10 ng/ml + C 10 µM	0.81 ± 0.31	1.55 ± 0.42

Conclusion:

ASO Seq. ID No. 218b results in a functional inhibition of TGF-β signaling in A549 and ReNcell CX® cells in presence of TGF-β1, confirmed by reduced phosphorylation of Smad2.

6.2 Results for Seq. ID No. 218c

6.2.1 Effects on CTGF mRNA and protein level

Data show CTGF mRNA downregulation after combination treatment with ASO Seq. ID No. 218c and TGF-β1 (A549: 0.86, ReNcell CX®: 0.23) compared to combination treatment with scrambled control and TGF-β1 (A549: 5.89, ReNcell CX®: 1.25) (Table 30 and Figure 14). In addition to these observations, immunochemical staining of CTGF confirmed prevention of TGF-β1 mediated effects on protein level by ASO Seq. ID No. 218c (Figure 15).

Table 30: CTGF mRNA levels after TGF-β1 pre-incubation followed by gymnotic transfer of Seq. ID No. 218c and parallel TGF-β1 treatment in A549 and ReNcell CX® cells. Data confirmed effective prevention of TGF-β1 effects on CTGF mRNA levels by ASO Seq. ID No. 218c. mRNA expression levels were quantified relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR normalized to untreated controls. A = untreated control, B = Ref.1, D = Seq. ID No. 218c, E = TGF-β1, ± = SEM, **p < 0.01 in reference to A, $^{++}$p < 0.01 in reference to E+B. Statistics

was calculated using the Ordinary-one-way-ANOVA followed by "Tukey's" *post hoc* comparisons.

Target Time point	**CTGF** 48 h TGF-β1 -> 72 h / 96 h TGF-β1 + ASOs / single treatment	
Cell line	**A549** n= 3	**ReNcell CX** n= 2
A	1.00 ± 0.05	1.00 ± 0.03
B 10 µM	0.86 ± 0.11	0.85 ± 0.01
D 10 µM	0.53 ± 0.10	**0.17* ± 0.02**
E 10 ng/ml	4.71 ± 1.76	1.39 ± 0.08
E 10 ng/ml + B 10 µM	**5.89* ± 2.16**	1.25 ± 0.44
E 10 ng/ml + D 10 µM	**0.86^{++} ± 0.06**	**0.23^{*++} ± 0.02**

Conclusion:
Data confirmed an effective prevention of TGF-β1 induced effects on CTGF mRNA and protein levels by ASO Seq. ID No. 218c.

6.2.2 Effects on pSmad2 protein level

To verify if CTGF downregulation (6.2.1) is a consequence of TGF-β1 signaling-inhibition mediated by ASO Seq. ID No. 218c, even in presence of TGF-β1-preincubation, pSmad2 protein levels were analyzed.
Phosphorylation of Smad2 was induced by TGF-β1 incubation (1.52 ± 0.19), whereas ASO gymnotic transfer mediated a reduction of pSmad2 in A549 cells (0.89 ± 0.05). TGF-β1 pre-incubation with following combination treatment results in suppression of TGF-β1 effects on phosphorylation of Smad2 (Western Blot Analysis, Table 31). Immunocytochemistry supported the data observed by Western Blot Analysis (Figure 16).

Table 31: Densitometric analysis of pSmad2 Western Blot. Downregulation of pSmad2 protein after gymnotic transfer with ASO Seq. ID No. 218c was measured. Suppression of TGF-β1 mediated effects by inventive ASOs was shown, when combination treatments were compared. Protein levels were determined relative to housekeeping gene GAPDH using Studio™ Lite Software and normalized to untreated controls. A = untreated control, B = Ref.1, D = Seq. ID No. 218c, E = TGF-β1. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Dunnett's" *post hoc* comparisons.

Target Time point	**pSmad2** 48 h TGF-β1 -> 72 h TGF-β1 + ASOs / single treatment
Cell line	**A549** n= 2
A	1.00 ± 0.00
B 10 µM	1.23 ± 0.27
D 10 µM	0.89 ± 0.05
E 10 ng/ml	1.52 ± 0.19
E 10 ng/ml + B 10 µM	1.27 ± 0.29
E 10 ng/ml + D 10 µM	**0.93 ± 0.35**

Conclusion:

ASO Seq. ID No. 218c is efficiently inhibiting TGF-β signaling after TGF-β1 pre-incubation followed by ASO gymnotic transfer. This was shown by examination of downstream pSmad2 protein levels.

Taken together, inventive ASOs are extraordinary capable in mediating a functional inhibition of TGF-β signaling **in presence of pathological, high TGF-β1 levels** by efficiently downregulating TGF-R_{II} mRNA. Thus, inventive ASOs will be beneficial in medical indications in which elevated TGF-β levels are involved, e.g. neurological disorders, fibrosis, tumor progression and others.

Example 7: Determination of prophylactic activity of the antisense-oligonucleotides on mRNA level (TGF-β1 post-treatment)

To analyze prophylactic activity of antisense-oligonucleotides (ASOs) in human neuronal progenitor cells from cortical brain region (ReNcell CX®), ASOs were transferred to cells by gymnotic uptake following Transforming Growth Factor-β1 (TGF-β1) treatment.

Description of Method:

A549 and ReNcell CX® cells were cultured as described above. For prophylactic treatment studies, cells were seeded in a 24-well culture dish (Sarstedt #83.1836.300) (50,000 cells / well) and were incubated overnight at 37 °C and 5 % CO_2. Afterwards, Ref.1 (Scrambled control, 10 µM) or ASO with Seq. ID No. 218b (10 µM) were added to media for 72 h (A549) or 96 h (ReNcell CX®). Following incubation time after gymnotic transfer, TGF-β1 (10 ng/ml, Promocell #C-63499) was added, without medium replacement, to the cells for further 48 h. For harvesting, cells were washed twice with PBS and subsequently used for RNA isolation (24-well dishes) following mRNA analysis by real-time RT-PCR. Ready-to-use and

standardized primer pairs for real-time RT-PCR were used and mixed with the respective ready-to-use Mastermix solution (SsoAdvanced™ Universial SYBR® Green Supermix (BioRad #172-5271) according to manufacturer´s instructions (BioRad Prime PCR Quick Guide). Methods were performed as described above.

7.1 Results for Seq. ID No. 218b

Efficacy in TGF-R_{II} mRNA downregulation by ASO Seq. ID No. 218b was not influenced by TGF-β1 post-incubation in A549 and ReNcell CX® cells (Table 32). Significant decrease of target mRNA in ReNcell CX® cells was shown after single treatment (0.33* ± 0.11) with ASO Seq. ID No. 218b. ASO gymnotic transfer with post-treatment of TGF-β1, strongly reduced the target TGF-R_{II} mRNA. In A549 cells, Seq. ID No. 218b showed similar potency in inhibiting TGF-R_{II} mRNA in single (0.25 ± 0.07) or combination treatment with post-incubation of TGF-β1 (0.24 ± 0.06).

Table 32: Downregulation of TGF-R_{II} mRNA after gymnotic transfer following TGF-β1 treatment of inventive ASO in A549 and ReNcell CX® cells. mRNA expression levels were quantified relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR normalized to untreated control. A = untreated control, B = Ref.1, C = Seq.ID No. 218b, E = TGF-β1. ± = SEM, *p < 0.05 in reference to A, $^{++}$p < 0.01 in reference to E+B. Statistics was calculated using the Ordinary-one-way-ANOVA followed by “Tukey’s” *post hoc* comparisons.

Target Time point	**TGF-R_{II}** 72 h / 96 h ASOs -> 48 h TGF-β1	
Cell line	**A549** n= 3	**ReNcell CX** n= 3
A	1.00 ± 0.44	1.00 ± 0.19
B 10 µM	0.95 ± 0.22	1.42 ± 0.14
C 10 µM	0.25 ± 0.07	**0.33* ± 0.11**
E 10 ng/ml	1.96 ± 0.16	1.42 ± 0.08
E 10 ng/ml + B 10 µM	1.14 ± 0.39	1.25 ± 0.14
E 10 ng/ml + C 10 µM	**0.24^{++} ± 0.06**	**0.56^{++} ± 0.10**

Conclusion:

Gymnotic uptake of ASO Seq. ID No. 218b followed by TGF-β1 post-incubation was effective in target TGF-R_{II} mRNA downregulation, indicating that ASO Seq. ID No. 218b is feasible for prophylactic treatment in medical indications.

Example 8: Determination of inhibitory activity of the inventive ASOs on protein level following TGF-β1 treatment

To analyze prophylactic activity of inventive ASOs in human neuronal progenitor cells from cortical brain region (ReNcell CX®), ASOs were transferred to cells by gymnotic uptake following TGF-β1 treatment.

Description of Method:

Cells were cultured as described before in standard protocol. For treatment cells were seeded in 8-well cell culture slide dishes (Sarstedt #94.6140.802) (10,000 cells / well) and were incubated overnight at 37 °C and 5 % CO_2. Afterwards, Ref.1 (Scrambled control, 10 µM) or ASO sequence identification number 218b (Seq. ID No. 218b, 10 µM) were added to media for 72 h (A549) or 96 h (ReNcell CX®). Following gymnotic transfer TGF-β1 (10 ng/ml, Promocell #C-63499) was added, without medium replacement, to the cells for further 48 h. For harvesting, cells were washed twice with PBS and subsequently used for immunocytochemical analysis. Procedure was performed as described before. Used antibodies and dilutions for respective methods are listed in Table 13 and 14.

8.1 Results of TGF-R_{II} protein reduction after gymnotic transfer with Seq. ID No. 218b following TGF-β1 treatment

Immunocytochemical analysis against TGF-R_{II} for A549 and ReNcell CX® cells showed that ASO Seq. ID No. 218b generates potent TGF-R_{II} mRNA target downregulation after following TGF-β1 treatment (Figure 17).

Conclusion:

Gymnotic transfer of ASO Seq. ID No. 218b following TGF-β1 treatment resulted in target mRNA downregulation, as well as a strong reduction of TGF-R_{II} protein level in A549 and ReNcell CX® cells.

Taken together, efficacy of downregulating TGF-R_{II} protein mediated by ASO Seq. ID No. 218b in combination with post-treatment of TGF-β1 was still given, concluding that the inventive ASOs are effective for prophylactic applications.

Example 9: ASO treatment effects on downstream signaling pathway of TGF-R_{II} following TGF-β1 treatment.

Efficacy of inventive ASOs in mediating an inhibition of TGF-β signaling was evaluated for TGF-β1 treatment followed gymnotic transfer in human lung cancer cells (A549) and human neuronal precursor cells (ReNcell CX®). Therefore, downstream molecules of TGF-β signaling, Smad3 (mothers against

decapentaphlegic homolog 3) and Connective Tissue Growth factor (CTGF), were analyzed.

Description of Method:

Cells were cultured as described before in standard protocol. For treatment, cells were seeded in 24-well culture dishes (Sarstedt #83.1836.300) (50,000 cells / well) and 8-well cell culture slide dishes (Sarstedt #94.6140.802) (10,000 cells / well) and were incubated overnight at 37 °C and 5 % CO_2. Afterwards, Ref.1 (Scrambled control, 10 µM) or ASO Seq. ID No. 218b (10 µM) were added to media for 72 h (A549) or 96 h (ReNcell CX®). Following gymnotic transfer, TGF-β1 (10 ng/ml, Promocell #C-63499) was added without medium replacement for further 48 h. For harvesting, cells were washed twice with PBS and subsequently used for RNA isolation (24-well dishes) or immunocytochemical examination of cells (in 8-well cell culture slide dishes). To evaluate effects on CTGF mRNA level, real-time RT-PCR was performed as described before. The primer pair for analysis of CTGF was ready-to-use and standardized. To determine pSmad3 protein levels, immunocytochemistry was used as described before. Type and used dilutions of antibodies for respective method are listed in Table 13 and 14.

9.1. Results for Seq. ID No. 218b

9.1.1 Effects on CTGF mRNA and pSmad3 protein level

CTGF mRNA was reduced after gymnotic transfer with ASO Seq. ID No. 218b in A549 (5 days: 0.67 ± 0.02) and ReNcell CX® (6 days: 0.70 ± 0.02) cells. Adding TGF-β1 after 72 h or 96 h respectively, cells react with an increase of CTGF mRNA, but in comparison to gymnotic transfer of scrambled control following TGF-β1 treatment, induction of CTGF mRNA was strongly reduced (Table 33). To verify if CTGF mRNA downregulation was a consequence of TGF-β signaling inhibition, mediated by ASO Seq. ID No. 218b, also after followed TGF-β1 treatment, pSmad3 protein levels were examined. Figure 18 demonstrates that TGF-β signaling was in fact blocked by gymnotic transfer of ASO Seq. ID No. 218b in A549 (Figure 18 A) and ReNcell CX® cells (Figure 18 B). This effect was also present after gymnotic transfer of tested ASO following TGF-β1 treatment.

Table 33: Downregulation of CTGF mRNA after gymnotic transfer of ASO Seq. ID No. 218b followed by TGF-β1 treatment in A549 and ReNcell CX® cells. Quantification of mRNA expression levels were performed relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E = TGF-β1. ± = SEM, *p < 0.05, **p < 0.01 in reference to A, $^{+}$p < 0.05, $^{++}$p < 0.01 in reference to

E+B. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey's" *post hoc* comparisons.

Target **Time point**	**CTGF** 72 h /96 h ASOs -> +/- 48 h TGF-β1	
Cell line	**A549** n= 3	**ReNcell CX** n= 3
A	1.00 ± 0.13	1.00 ± 0.09
B 10 µM	0.80 ± 0.03	1.07 ± 0.07
C 10 µM	0.67 ± 0.02	0.70 ± 0.02
E 10 ng/ml	**4.54** ± 0.68**	**1.56* ± 0.08**
E 10 ng/ml + B 10 µM	**4.07** ± 0.38**	**1.62* ± 0.09**
E 10 ng/ml + C 10 µM	**1.90^{+} ± 0.03**	**0.97^{++} ± 0.10**

Conclusion:

Gymnotic transfer of ASO Seq. ID No. 218b resulted in downregulation of TGF-R_{II} mRNA and protein, as well as in reduced CTGF mRNA and pSmad3 protein levels in A549 and ReNcell CX® cells, independently of TGF-β1 treatment.

That indicates that ASO Seq. ID No. 218b is potent enough to be also active under prophylactic conditions to resume or reduce ongoing TGF-β1 mediated effects.

Example 10: Analysis of potential proinflammatory and toxicological effects of antisense-oligonucleotides

10.1 Peripheral blood mononuclear cell (PBMC) assay

To analyze antisense-oligonucleotide (ASO) for immunostimulatory properties, peripheral blood mononuclear cells (PBMCs) were incubated with control ASOs and test compounds followed by ELISAs for IFNα and TGFα.

Description of Method:

PBMCs were isolated from buffy coats corresponding to 500 ml full blood transfusion units. Each unit was obtained from healthy volunteers and glucose-citrate was used as an anti-agglutinant. The buffy coat was prepared and delivered by the Blood Bank Suhl on the Institute for Transfusion Medicine, Germany. Each blood donation was monitored for HIV antibody, HCV antibody, HBs antigen, TPHA, HIV RNA, and SPGT (ALAT). Only blood samples tested negative for infectious agents and with a normal SPGT value were used for leukocyte and erythrocyte separation by low-speed centrifugation. The isolation of PBMCs was performed about 40 h following blood donation by gradient centrifugation using Ficoll-Histopague® 1077 (Heraeus™ Multifuge™ 3 SR). For IFNα assay, PBMCs were seeded at 100,000 cells/ 96-well in

100 µl complete medium plus additives (RPMI1640, + L-Glu, + 10% FCS, + PHA-P (5 µg/ ml), + IL-3 (10 µg/ ml)) and test compounds (5 µl) were added for direct incubation (24 h, 37 °C, 5% CO_2). For TNFα assay, PBMCs were seeded at 100,000 cells/ 96-well in 100 µl complete medium w/o additives (RPMI1640, + L-Glu, + 10% FCS) and test compounds (5 µl) were added for direct incubation (24 h, 37 °C, 5% CO_2). ELISA (duplicate measurement out of pooled supernatants, 20 µl) for huIFNα (eBioscience, #BMS216INSTCE) was performed according to the manufacturer's protocol. ELISA (duplicate measurement out of pooled supernatants, 20 µl) for huTNFα (eBioscience, #BMS223INSTCE) was performed according to the manufacturer's protocol.

Results:

There was no immunostimulatory effect of ASO treatment on PBMCs indicated by no detectable IFNα (Table 34) and TNFα (Table 35) secretion upon ASO incubation. Assay functionality is proven by the immunostimulatory effect of immunostimulatory, cholesterol-conjugated siRNA (XD-01024; IFNα) and polyinosinic:polycytidylic acid (poly I:C; TNFα; InvivoGen # tlrl-pic) which is a synthetic analog of double-stranded RNA, binds to TLR3 and stimulates the immune system..

Table 34 IFNα response to inventive ASO exposure: shows the IFNα response of PBMCs upon ASO incubation. Quantification of expression levels were determined to positive controls (ODN2216 [class A CpG oligonucleotide; recognized by TLR9 and leading to strong immunostimulatory effects; InvivoGen tlrl-2216], poly I:C, XD-01024) using ELISA assay.

Test candidate	Mean of duplicates [pg/ml]	
	Donor 1	Donor 2
mock	- 0.084	0.720
Seq. ID No. 209y	- 0.061	- 0.039
Seq. ID No. 209t	- 0.308	- 0.520
Seq. ID No. 209v	- 0.191	- 1.252
Seq. ID No. 218b	- 0.001	- 0.093
Seq. ID No. 218m	- 0.140	- 0.163
Seq. ID No. 218q	- 0.755	0.005
Seq. ID No. 218c	- 0.852	- 0.805
Seq. ID No. 218t	- 0.469	0.450
ODN2216	0.300	1.311
poly I:C	- 1.378	2.053
XD-01024	13.961	26.821
All values except positive control (XD-01024) below limit of quantification		

Table 35 TNFα response to inventive ASO exposure: Quantification of expression levels were determined to control candidates (ODN2216, poly I:C, XD-01024) using ELISA assay.

Test candidate	Mean of duplicates [pg/ ml]	
	Donor 1	Donor 2
mock	0.647	-0.137
Seq. ID No. 209y	2.397	-0.117
Seq. ID No. 209t	0.734	0.193
Seq. ID No. 209v	0.360	0.063
Seq. ID No. 218b	0.670	0.183
Seq. ID No. 218m	0.594	0.519
Seq. ID No. 218q	0.049	0.194
Seq. ID No. 218c	-0.212	0.029
Seq. ID No. 218t	0.593	0.758
ODN2216	0.085	0.894
poly I:C	115.026	102.042
XD-01024	1.188	1.418
All values except positive control (poly I:C) below limit of quantification		

10. 2 *In vivo* toxicology of inventive antisense-oligonucleotides

To analyze antisense-oligonucleotides (ASOs) for toxicological properties, C57/Bl6N mice received three intravenous ASO injections, and following sacrification, transaminase levels within serum, liver and kidney were examined.

Description of Method:

Female C57/Bl6N mice at the age of 6 weeks were treated with test compounds (Seq. ID No. 218b, Seq. ID No. 218c) for seven days. ASOs (200 µl, 15 mg/ kg/ BW) were injected intravenously on day one, two, and three of the treatment period. Body weight development (Seq. ID No. 218c) was monitored on every consecutive day and on day four serum was collected from the vena fascicularis. On day eight the animals were sacrificed (CO_2) and serum from the vena cava, the liver (pieces of ≈ 50 mg), the kidneys, and the lung were collected for mRNA and transaminase quantification. TGF-R_{II} mRNA levels were determined in liver, kidney, and lung lysate by bDNA assay (QuantiGene® kit, Panomics/ Affimetrix). Aspartate transaminase (ASP) and alanine transaminase (ALT) were measured on Cobas Integra® 400 from 1:10 diluted serum.

Table 36: Serum expression levels of alanine transaminase and aspartate transaminase of C57/Bl6N mice following repeated ASO iv injection. Quantification of expression levels was achieved by comparing to the expression levels of saline-treated animals. ± = SEM.

Test compound	Serum transaminases [U/L]			
	3 days post injection		7 days post injection	
	ALT	AST	ALT	AST
Seq. ID No. 209ax	13.87 ± 1.44	47.33 ± 15.88	64.91 ± 21.01	108.99 ± 13.56
Seq. ID No. 143h	13.68 ± 3.33	53.50 ± 6.99	12.47 ± 1.64	33.35 ± 8.17
Seq. ID No. 152h	16.66 ± 6.29	67.23 ± 29.91	17.49 ± 2.81	45.75 ± 17.14
Seq. ID No. 209ay	18.29 ± 6.37	69.96 ± 35.44	287.29 ± 65.39	273.45 ± 101.33
Seq. ID No. 210q	11.70 ± 3.80	36.44 ± 5.36	11.11 ± 6.31	40.81 ± 13.32
Seq. ID No. 218b	19.60 ± 8.62	67.61 ± 42.75	18.38 ± 4.60	48.91 ± 17.86
Seq. ID No. 213k	13.59 ± 3.28	54.47 ± 36.15	96.00 ± 46.74	89.12 ± 21.82
Saline	**9.52 ± 9.21**	**67.18 ± 28.60**	**9.99 ± 2.29**	**28.29 ± 2.23**

Table 37: Expression levels of TGF-R_{II} within liver, kidney, and lung tissue of C57/Bl6N mice following repeated ASO iv injection. Quantification of expression levels was achieved by comparing to the expression levels of saline-treated animals. ± = SEM.

Test compound	TGF-RII mRNA/GAPDH mRNA expression		
	Liver	Kidney	Lung
Seq. ID No. 209ax	0.64 ± 0.03	1.31 ± 0.11	13.25 ± 0.67
Seq. ID No. 143h	0.26 ± 0.02	0.65 ± 0.22	11.10 ± 0.11
Seq. ID No. 152h	0.58 ± 0.10	0.87 ± 0.17	13.42 ± 0.69
Seq. ID No. 209ay	0.62 ± 0.06	1.30 ± 0.10	13.93 ± 0.57
Seq. ID No.210q	0.39 ± 0.06	0.83 ± 0.15	13.53 ± 1.23
Seq. ID No. 218b	0.72 ± 0.08	0.97 ± 0.06	15.63 ± 1.45
Seq. ID No. 213k	0.42 ± 0.01	1.20 ± 0.04	14.44 ± 1.03
Saline	**0.66 ± 0.04**	**1.10 ± 0.08**	**15.14 ± 0.65**

Table 38: Serum expression levels of alanine transaminase and aspartate transaminase of C57/Bl6N mice following repeated ASO iv injection. Quantification of expression levels was achieved by comparing to the expression levels of saline-treated animals. ± = SEM.

Test compound	Serum transaminases [U/L]			
	3 days post injection		7 days post injection	
	ALT	AST	ALT	AST
Seq. ID No. 218c	24.63 ± 2.10	51.87 ± 5.99	18.10 ± 4.01	39.99 ± 2.09
Saline	28.68 ± 3.23	79.95 ± 30.24	14.52 ± 4.89	36.08 ± 3.32

Table 39: Expression levels of TGF-R_{II} within liver and kidney tissue of C57/Bl6N mice following repeated ASO iv injection. Quantification of expression levels was achieved by comparing to the expression levels of saline-treated animals. ± = SEM.

Test compound	TGF-RII mRNA/GAPDH mRNA expression	
	Liver	Kidney
Seq. ID No. 218c	0.21 ± 0.03	0.16 ± 0.02
Saline	**0.35 ± 0.05**	**0.24 ± 0.03**

Table 40: Body weight development during the 7-day ASO treatment paradigm. Body weight gain was quantified compared to body weight on day 0, which was set to 100%.

Test compound	Body weight development [%]					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 7
Seq. ID No. 218c	100%	99%	99%	99%	102%	104%
Saline	**100%**	**99%**	**100%**	**100%**	**101%**	**103%**

Conclusion: There were no proinflammatory or toxic effects of relevant inventive ASOs on PBMCs or C57/Bl6N mice. Therefore, ASO treatment targeting TGF-R_{II} reflects a safe method to treat a variety of TGF-ß associated disorders.

Example 11: Determination of intracerebroventricular infusion of inventive ASOs on TGF-β induced neural stem inhibition and neural progenitor cell proliferation *in vivo*

The goal of the present study was to evaluate the potential of inventive ASOs against TGF-R_{II} i) to prevent and ii) to treat the TGF-β1 induced effects on neural stem and progenitor cell proliferation *in vivo*.

Description of Method:

11.1 Prevention of TGF-β1 associated downregulation of neurogenesis

Two-month-old female Fischer-344 rats (n = 32) received intracerebroventricular infusions via osmotic minipumps (Model 2002, Alzet) connected to stainless steel cannulas. The surgical implantation of the minipumps was performed under deep anesthesia using intramuscular injections. Animals were infused with inventive ASOs according to the invention (1.64 mM concentration present in the pump), scrambled ASO (1.64 mM concentration present in the pump) or aCSF (artificial cerebrospinal fluid) for 7 days. At day 8, pumps were changed and the animals were infused with either i) aCSF, ii) TGF-β1 (500 ng/ml present in the pump), iii) TGF-β1 (500 ng/ml present in the pump) plus scrambled ASO (1,64 mM concentration present in the pump), or iv) TGF-β1 (500 ng/ml present in the pump) plus inventive ASO (1,64 mM concentration present in the pump) for 14 days. At the end of the infusion-period all animals were transcardially perfused with 4% paraformaldehyde. The brains were analyzed for cannula tract localization and animals with incorrect cannula placement were excluded from the analysis. During the last 24 hours of the pump period, the animals received an intraperitoneal injection of 200 mg/kg bromo-deoxyuridine (BrdU).

The tissue was processed for chromogenic immunodetection of BrdU-positive cells in 40 µm sagital sections. BrdU positive cells were counted within three 50 µm x 50 µm counting frames per section located at the lowest, middle and upper part of the subventricular zone. Positive profiles that intersected the uppermost focal plane (exclusion plane) or the lateral exclusion boundaries of the counting frame were not counted. For hippocampal analysis, the volume of the hippocampus was determined and all positive cells within and adjacent to the boundaries were counted. The total counts of positive profiles were multiplied by the ratio of reference volume to sampling volume in order to obtain the estimated number of BrdU-positive cells for each structure. All extrapolations were calculated for one cerebral hemisphere and should be doubled to represent the total brain values. Data are presented as mean values ± standard deviations (SD). Statistical analysis was performed using the unpaired, two-sided t-test comparison –Student's t-test between the TGF-β1 treated and control groups (GraphPad Prism 4 software, USA). The significance level was assumed at $p < 0.05$.

11.2 Treatment of TGF-β1 associated down-regulation of neurogenesis

Animals received either aCSF or recombinant human TGF-ß1 (500 ng/ml present in pump) at a flow rate of 0.5 µl per hour for 14 days. After 14 days, pumps were

changed and the animals were infused with either i) aCSF, ii) recombinant human TGF-ß1 (500 ng/ml present in pump) or co-infused with iii) inventive ASO (1.64 mM concentration present in the pump) plus recombinant human TGF-ß1 (500 ng/ml present in pump) or iv) scrambled ASO (1.64 mM concentration present in the pump) plus recombinant human TGF-ß1 (500 ng/ml present in pump). At the end of the infusion-period all animals were transcardially perfused with 4% paraformaldehyde. The brains were analyzed for cannula tract localization and animals with incorrect cannula placement were excluded from the analysis. During the last 24 hours of the pump period, the animals received an intraperitoneal injection of 200 mg/kg bromo-deoxyuridine (BrdU).
Histological analysis was done as described above (11.1).

Results:
The treatment with ASO of Seq. ID No. 143aj, Seq. ID No. 143h and Seq. ID No. 210q specifically and partially reduced the effect of TGF-β1 on cell proliferation in the hippocampus and in the ventricle wall. Treatment with an inventive ASO specifically and partially rescues from the inhibitory effect of TGF-β1 on neurogenesis.

Conclusion: The ASOs of the present invention demonstrating cross-reactivity with rodents induce neurogenesis in this *in vivo* experiment. The ASOs of the present invention demonstrating no cross-reactivity, exert mostly even more potential effects in *in vitro* experiments. As a result, it is assumed that these inventive ASOs are also more effective in *in vivo* set ups for non-human primates and humans and therefore act as a highly potent medication for preventing or treating TGF-ß1 induced inhibition of neural stem and progenitor proliferation.

Example 12: Analysis of the effect of the inventive antisense-oligonucleotides on proliferation and specific markers of human neural progenitor cells

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative lethal disorder with no effective treatment so far. The current molecular genetic campaign is increasingly elucidating the molecular pathogenesis of this fatal disease, from previous studies it is known that TGF-β is found in high concentrations in Cerebrospinal Fluid (CSF) of ALS patients. These high levels of circulating TGF-β are known to promote stem cell quiescence and therefore cause inhibition of adult neurogenesis within the subventricular zone (SVZ) of the brain. Thus, regeneration of degenerating neurons seems to be prevented by an enhanced TGF-β signaling.
To figure out if selective inhibition of TGF-β signaling mediated by the inventive antisense-oligonucleotides might allow reactivation of adult neurogenesis, evidence of TGF-β mediated cell cycle arrest has to be proofed.

Description of Methods:

Cell cycle arrest studies: Cells were cultured as described before in standard protocol. For experiments, cells were seeded in 24-well culture dishes (Sarstedt #83.1836.300) (30,000 cells / well) and were incubated overnight at 37 °C and 5 % CO_2. For determination of TGF-β1 mediated effects on cell cycle under proliferative (+EGF/FGF) (Millipore: EGF #GF144, bFGF #GF003) or differentiating (-EGF/FGF) conditions, cells were treated for 4 d with TGF-β1 (PromoCell #C-63499, 10 or 50 ng/ml) after removing and replacement of respective medium. At day 4 medium was refreshed and TGF-β1 treatment was repeated until day 7. On day 7, cells were harvested by washing twice with PBS and subsequently used for RNA (24-well dishes) isolation as described above. For evaluating TGF-β1 -mediated effects on cell cycle by real-time RT-PCR, mRNA of proliferation marker Ki67, tumor suppressor gene p53, cyclin-dependent kinase inhibitor 1 (p21) and of neurogenesis marker Doublecortin (DCX) were analyzed. Respective primer pairs are listed in Table 11.

mRNA Analysis for effects of ASO Seq. ID No. 218b on human neural progenitor cells: Cells were cultured as described before in standard protocol. For experiments, cells were seeded in 24-well culture dishes (Sarstedt #83.1836.300) (30,000 cells / well) and were incubated overnight at 37 °C and 5 % CO_2. For present experiments, cell medium was changed and Ref.1 (Scrambled control, 2.5 and 10 μM), ASO with Seq. ID No. 218b (2.5 and 10 μM) or TGF-β1 (10 ng/ml, Promocell #C-63499) were added to cells for 96 h. After incubation time, medium was changed once more and further treatment was performed for further 96 h. After 8 days of treatment cells were harvested. Cells were washed twice with PBS and subsequently used for RNA (24-well dishes) isolation. To evaluate effects on progenitor cells, Nestin (early neuronal marker), Sox2 (early neuronal marker), DCX (indicator of neurogenesis) and Ki67 (proliferation marker) mRNA levels were determined by real-time RT-PCR as described before. Respective primer pairs are listed in Table 11.

Proliferative and differentiating effects of $TGFR_{II}$ specific ASOs by gymnotic transfer on ReNcell CX® cells: The next goal was to investigate, whether TGF-R_{II} specific ASO influence the proliferation of ReNcell CX® cells. Therefore, cells were cultured as described before and seeded in 24-well culture dishes (Sarstedt #83.1836.300) (30,000 cells / well) or 8-well cell culture slide dishes (Sarstedt #94.6140.802) (10,000 cells / well) and were incubated overnight at 37 °C and 5 % CO_2. For obtaining a proliferation curve, cells were treated after medium change for 72 h with Ref.1 (Scrambled control, 2.5 and 10 μM,) and with ASO Seq. ID No. 218b (2.5 and 10 μM). After incubation time, medium change and treatment was repeated two

times. After collecting supernatant, remaining cells were harvested from 24-well dishes for determination of cell number. For this purpose, remaining cells were washed with PBS (2x), treated with accutase (500 µl/ well) and incubated for 5 min at 37 °C. Afterwards 500 µl medium were added and cell number was determined using Luna FL™ Automated Cell Counter Fluorescence and Bright Field (Biozym, #872040) according to manufacturer´s instructions. Briefly, 18 µl of the cell suspension were added to 2 µl of acridine orange/propidium iodide assay viability kit (Biozym #872045). After 1 min of settling, 10 µl were added onto Cell Counting Slide (Biozym # 872011), cells were counted and calculated in total cells/ml and percentage of alive cells compared to dead cells. After gymnotic transfer of Ref.1 (10 µM), Seq. ID No. 218b (10 µM) and corresponding treatment of TGF-β1 (10 ng/ml) for 8 days, cells of 8-well cell culture slide dishes were fixed and stained with an antibody against Ki67. For investigating differentiation ability of ReNcell CX® cells after gymnotic transfer, other 8-well cell culture slide dishes were treated with Ref.1 (10 µM), Seq. ID No. 218b (10 µM) and corresponding treatment of TGF-β1 (10 ng/ml) for 96 h under proliferative conditions (+ EGF/FGF). Afterwards, one part of the cells was treated for further 96 h under proliferative conditions whereas the other part of cells was treated and hold under differentiating conditions (- EGF/FGF). Following staining of cells, Neurofilament N (NeuN) and βIII-Tubulin expression levels were determined by fluorescence microscopy. Protocol for harvesting, fixing and staining cells was described above and respective antibody dilutions are listed in Table 14.

mRNA Analysis of markers for proliferation and neurogenesis after gymnotic transfer following TGF-β1 pre-incubation: Cells were cultured as described before in standard protocol. For experiments cells were seeded in 24-well culture dishes (Sarstedt #83.1836.300) (30,000 cells / well) and incubated overnight at 37 °C and 5 % CO_2. For inducing cell cycle arrest, ReNcell CX® cells were treated with TGF-β1 for 4 days. Afterwards medium was changed and TGF-β1 (10 ng/ml) was added freshly. One day 8 medium was changed on more time, and gymnotic transfer was performed for 96 h by adding Ref.1 (10 µM), Seq. ID No. 218b (10 µM) in combination with TGF-β1 (10 ng/ml). Cells were harvested after incubation by washing twice with PBS. Following RNA isolation and mRNA analysis by real-time RT-PCR were performed as described.

12.1.1 Mediation of cell cycle arrest by TGF-β1 in human neural progenitor cells

Detection of stem cell quiescence markers showed that TGF-β1 mediates cell cycle arrest 7 days after exposure of cells. Proliferation marker Ki67 mRNA expression was dose-dependently reduced. Also mRNA expression of tumor suppressor gene p53 was downregulated correlating to TGF-β1 concentration. In contrast, cyclin-

dependent kinase inhibitor 1 (p21) was significantly upregulated by TGF-β1. In summary these results indicate stem cell quiescence induced by TGF-β1. Interestingly, DCX, a marker for neurogenesis, was strongly reduced by TGF-β1 (Table 41).

Table 41: mRNA expression of Ki67, p27, p21, and DCX 7 days after TGF-β1 treatment in ReNcell CX® cells. mRNA expression levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, E = TGF-β1. ± = SEM, *p < 0.05 in reference to A. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparison.

Cell line	**ReNcell CX** mRNA levels 7 days after TGF-β1 exposure			
Target	Ki67 n = 3	p53 n = 3	p21 n = 3	DCX n = 3
A + EGF/FGF	1.00 ± 0.38	1.00 ± 0.38	1.00 ± 0.25	1.00 ± 0.49
E 10 ng/ml + EGF/FGF	0.67 ± 0.20	0.66 ± 0.18	**1.90* ± 0.22**	0.37 ± 0.06
E 50 ng/ml + EGF/FGF	0.43 ± 0.09	0.42 ± 0.06	1.45 ± 0.16	0.16 ± 0.01
A - EGF/FGF	1.00 ± 0.15	1.00 ± 0.13	1.00 ± 0.14	1.00 ± 0.31
E 10 ng/ml - EGF/FGF	0.87 ± 0.08	0.97 ± 0.10	1.00 ± 0.04	0.72 ± 0.14
E 50 ng/ml - EGF/FGF	0.93 ± 0.11	0.93 ± 0.09	0.90 ± 0.09	0.71 ± 0.24

Conclusion

Proliferation of ReNcell CX® cells was blocked by TGF-β1.

12.1.2 Results of antisense-oligonucleotide effects on markers of human neuronal stem cells

To figure out the effect of ASO Seq. ID No. 218b on stem cell markers, 8 days after repeated gymnotic transfer (2 x 96 h) in ReNcell CX® cells, different markers of early neural progenitor cells were tested (Table 42). Gene expression levels of Nestin and Sox2 were not influenced by ASO Seq. ID No. 218b. GFAP mRNA was slightly upregulated after gymnotic transfer with 10 µM ASO Seq. ID No. 218b and in contrast, DCX was clearly induced after gymnotic uptake of ASO Seq. ID No. 218b. Expression of all tested markers was strongly reduced after TGF-β1 treatment (8d) (Table 42, Figure 19).

Table 42: mRNA expression of Nestin, Sox2, GFAP and DCX 8 days after gymnotic transfer of Seq. ID No. 218b in ReNcell CX® cells. mRNA expression levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E= TGF-β1, ± = SEM, +p < 0.05 in reference to C 2.5 µM, #p < 0.05 in reference to C 10 µM. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparison.

Cell line	**ReNcell CX** mRNA levels 8 days after gymnotic transfer or TGF-β1 exposure			
Target	Nestin n = 4	Sox2 n = 4	GFAP n = 4	DCX n = 4
A	1.00 ± 0.18	1.00 ± 0.25	1.00 ± 0.22	1.00 ± 0.32
B 2.5 µM	0.97 ± 0.32	0.88 ± 0.33	0.78 ± 0.13	1.31 ± 0.42
B 10 µM	0.89 ± 0.16	0.79 ± 0.13	1.02 ± 0.20	1.44 ± 0.48
C 2.5 µM	1.09 ± 0.21	0.93 ± 0.09	0.99 ± 0.14	1.67 ± 0.46
C 10 µM	0.90 ± 0.09	0.89 ± 0.11	1.21 ± 0.11	1.95 ± 0.37
E 10 ng/ml	0.48 ± 0.12	0.32 ± 0.06	**0.41# ± 0.13**	**0.05+# ± 0.01**

Conclusion:

Results for mRNA analysis indicate that ASO Seq. ID No. 218b guides ReNcell CX® cells into the direction of an even more stem cell like state (GFAP upregulation). In addition, induction of DCX indicates an elevated neurogenesis. TGF-β1 treatment results in an opposite direction.

12.1.3 Results of antisense-oligonucleotide effects on proliferation of human neuronal stem cells

Further analysis was performed to investigate whether gymnotic transfer of ASO Seq. ID No. 218b has really effects on proliferation rate by counting cells 9 days after repeated gymnotic transfer (3 x 72 h) and determination of Ki67 protein levels 8 days after gymnotic uptake (2 x 96 h).

Results

Cell number was increased after gymnotic uptake of ASO Seq. ID No. 218b in accordance to an increased protein expression of proliferation marker Ki67 observed in immunochemical staining of cells (Table 43, Figure 20). Fluorescence analysis of immunocytochemical staining also revealed a proliferation stop mediated by TGF-β1.

Table 43: Increased cell number 9 days after repeated gymnotic transfer (3 x 72 h) of ReNcell CX® cells. Cell number was determined using Luna FL™ Automated Cell Counter Fluorescence and Bright Field (Biozym, #872040) according to manufacturer´s instructions. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, ± = SEM.

Cell line	**ReNcell CX**	
Cell number	alive cells x 10^5, n = 2	dead cells x 10^5, n = 2
A	3.34 ± 0.09	0.51 ± 0.05
B 2.5 µM	4.34 ± 0.56	0.60 ± 0.09
B 10 µM	4.36 ± 0.96	0.58 ± 0.09
C 2.5 µM	4.63 ± 1.28	0.47 ± 0.02
C 10 µM	5.24 ± 0.42	0.37 ± 0.02

Conclusion

Gymnotic transfer of ASO Seq. ID No. 218b in ReNcell CX® cells results in an increased cell number, paralleled by an enhanced Ki67 protein expression, altogether indicating increased neuronal precursor proliferation.

12.1.3 Results of antisense-oligonucleotide effects on differentiation ability of human neuronal stem cells

To exclude an influence of ASO Seq. ID No. 218b on cell ability to differentiate, ASO Seq. ID No. 218b was transferred to cells by gymnotic uptake for 96 h under proliferative conditions (+ EGF/FGF). After incubation time, medium was changed and to one part of cells proliferative medium was added whereas to the other part of cells differentiating medium (- EGF/FGF) was added. Afterwards, another gymnotic transfer for 96 h was performed. Cells were analyzed by expression levels of neuronal markers Neurofilament N (NeuN) and βIII-Tubulin.

Results

Immunochemical staining against NeuN (Figure 23A) and βIII-Tubulin (Figure 23B) demonstrates no effects on the ability to differentiate after gymnotic ASO transfer under proliferative conditions followed by gymnotic transfer under differentiating conditions. Signal for βIII-Tubulin, a human neuron specific protein, was not influenced by ASO Seq. ID No. 218b under differentiating conditions and was comparable to untreated control. Also NeuN expression was not influenced after gymnotic transfer under differentiating conditions. Thus, cells are still capable to differentiate into neural cells. Strikingly, ReNcell CX® cells expressed neuronal marker NeuN and βIII-Tubulin after gymnotic transfer of ASO under proliferative

conditions (2 x 96 h) for both periods, indicating that gymnotic transfer of ASO could promote a specific shift into differentiation of neurons even under proliferative conditions. In addition, elevated proliferation rates of neural precursor cells were observed (Table 43, Figure 20). Further, staining against NeuN revealed that cells treated with ASO Seq. ID 218b look more viable compared to all other treatments (Figure 21A). Obviously, cells which were treated with TGF-β1 were significantly less proliferative.

Conclusion

The ability to differentiate was not influenced by inventive ASO Seq. ID No. 218b. Interestingly, ReNcell CX® cells showed differentiation to neurons after gymnotic transfer under proliferative and differentiating conditions. This indicates in context to the observation of an increased proliferation rate, that inventive ASO Seq. ID No. 218b promotes neurogenesis with a tendency towards elevated neuronal differentiation.

12.1.4 Results of inventive antisense-oligonucleotides on proliferation of human neuronal stem cells after TGF-β1 pre-incubation

To analyze whether gymnotic transfer of ASO Seq. ID No. 218b is efficient in reversing TGF-β1 mediated effects on ReNcell CX® cells, further studies were performed with TGF-β1 pre-incubation for 7 days followed by gymnotic transfer for 8 days (2 x 96 h).

Results

Gene expression of GFAP (Table 44, Figure 22A) as an early neuronal marker, Ki67 (Table 44, Figure 22B), as a marker for proliferation, and DCX (Table 44, Figure 22C) as marker for neurogenesis were elevated after single ASO treatment, whereas TGF-β1 resulted in the opposite. In addition, 7 days after TGF-β1 pre-incubation, inventive ASO treatment reversed TGF-β1-induced effects. Thus the analysis demonstrates that ASO Seq. ID No. 218b is potent in recovering TGF-β1 mediated effects upon stem cell and proliferation markers

Table 44: mRNA expression of GFAP, Ki67 and DCX 7 days after TGF-β1 **pre-incubation followed by 2 x 96 h gymnotic transfer of Seq. ID No. 218b in ReNcell CX® cells**. mRNA expression levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E= TGF-β1, ± = SEM, Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparisons.

Cell line	ReNcell CX mRNA levels 7 d after TGF-β1 pre-incubation followed by 2 x 96 h gymnotic transfer		
Target	GFAP n = 2	Ki67 n = 1	DCX n = 2
A	1.00 ± 0.20	1.00	1.00 ± 0.16
B 10 µM	1.62 ± 0.15	0.91	1.52 ± 0.24
C 10 µM	2.23 ± 0.52	1.52	4.82 ± 1.15
E 10 ng/ml	0.76 ± 0.01	0.48	0.68 ± 0.03
E 10 ng/ml + B 10 µM	0.58 ± 0.07	0.61	0.83 ± 0.10
E 10 ng/ml + C 10 µM	2.04 ± 1.04	7.40	1.55 ± 0.24

Conclusion

Results indicate that adult neurogenesis could be reactivated by inventive TGF-R_{II} specific ASO-mediated blocking of TGF-β signaling.

Taken together, TGF-R_{II} specific ASO Seq. ID No. 218b rescued cells from TGF-β mediated stem cell quiescence and promotes adult neurogenesis without having an impact on differentiation. This makes it an ideal treatment drug for brain repair.

Example 13: Determination of therapeutic activity of inventive antisense-oligonucleotides disease progression of ALS in SOD1 mice

To analyze the therapeutic potential of ASOs as a medication for amyotrophic lateral sclerosis (ALS) male and female transgenic, SOD1 G93A mice were treated with different doses of inventive ASOs by icv administration into the lateral ventricle via osmotic ALZET® minipumps. In addition, riluzole was used as a reference. Riluzole is a drug used to treat amyotrophic lateral sclerosis and is marketed by Sanofi Pharmaceuticals. It delays the onset of ventilator-dependence or tracheostomy in selected patients and may increase survival by approximately two to three months

Description of Method:

For long-lasting central infusion an icv cannula attached to an Alzet® osmotic minipump (infusion rate: 0.25 µl/h, Alzet®, Model 2004, Cupertino, USA), was stereotaxically implanted under isoflurane anesthesia (Baxter, GmbH, Germany) and semi-sterile conditions. Each osmotic minipump was implanted subcutaneously in the abdominal region *via* a 1 cm long skin incision at the neck of the mouse and connected with the icv cannula by silicone tubing. Animals were placed into a stereotaxic frame, and the icv cannula (23G, 3 mm length) was lowered into the right

lateral ventricle (posterior 0.3 mm, lateral 1mm, depth 3 mm relative to bregma). The cannula was fixed with two stainless steel screws using dental cement (Kallocryl, Speiko® Dr. Speier GmbH, Münster, Germany). The skin of the neck was closed with sutures. During surgery, the body temperature was maintained by a heating pad. To avoid post-surgical infections, mice were locally treated with betaisodona® (Mundipharma GmbH, Limburg, Germany) and received 0.1 ml antibiotics (sc, Baytril® 2.5% Bayer Vital GmbH, Leverkusen, Germany). The tubing was filled with the respective solution. To determine the effects of ASOs on the development and the progression of ALS, the onset of symptoms, paresis, and survival were used as *in vivo* endpoints. At the age of nine weeks, mice were sacrificed and brains were removed for neuropathology analysis. Histological verification of the icv implantation sites was performed at 40-µm coronal, cresyl violet-stained brain sections.

The inventive ASOs exert potential effects in *in vitro* experiments. Quite in line, the rodent cross-reactive inventive ASOs with Seq. ID No. 143aj, Seq. ID No. 143h and Seq. ID No. 210q were also effective in the above experiments proving an effect in the treatment of ALS model animals. The ASOs of the present invention demonstrating no cross-reactivity exert more potential effects in *in vitro* experiments. As a result, it is assumed that these inventive ASOs are also more effective in *in vivo* set ups for non-human primates and humans and therefore act as a highly potent medication for preventing or treating TGF-β1 induced inhibition of neural stem and progenitor proliferation, and thereby treating ALS and other neurodegenerative disorders.

<u>Examples 14:</u> Determination of the therapeutic activity of antisense-inventive ASOs directed to TGF-R_{II} on disease development and progression of Huntington´s disease in R6/2 mice

To analyze the therapeutic potential of ASOs as a medication for Huntington´s disease (HD), male and female transgenic R6/2 mice were treated with different doses of inventive TGF-R_{II} specific ASO by icv administration into the lateral ventricle via osmotic minipumps.

<u>Description of Method:</u> For chronic central infusion, mice underwent surgery for an icv cannula attached to an Alzet® osmotic minipump (infusion rate: 0.25 µl/h, Alzet®, Model 2004, Cupertino, USA) at the age of five weeks. The cannula and the pump were stereotaxically implanted under ketamine/xylacin anesthesia (Baxter, GmbH, Germany) and semi-sterile conditions. Each osmotic minipump was implanted subcutaneously in the abdominal region *via* a 1cm long skin incision at the neck of the mouse and connected with the icv cannula by a silicone tubing. Animals were

placed into a stereotaxic frame, and the icv cannula (23G, 3 mm length) was lowered into the right lateral ventricle (posterior 0.3 mm, lateral 1mm, depth 3 mm relative to bregma). The cannula was fixed with two stainless steel screws using dental cement (Kallocryl, Speiko®-Dr. Speier GmbH, Münster, Germany). The skin of the neck was closed with sutures. During surgery, the body temperature was maintained by a heating pad. To avoid post-surgical infections, mice were locally treated with betaisodona® (Mundipharma GmbH, Limburg, Germany) and received 0.1 ml antibiotics (sc, Baytril® 2.5% Bayer Vital GmbH, Leverkusen, Germany). The tubing was filled with the respective solution. To determine the effects of ASOs on the development and the progression of HD the onset of symptoms, grip strength, general motoric, and survival were used as *in vivo* endpoints. At the age of nine weeks, mice were sacrificed and brains were removed for histological analyzation. Histological verification of the icv implantation sites was performed at 40-µm coronal, cresyl violet-stained brain sections.

The inventive ASOs exert potential effects in *in vitro* experiments. Quite in line, the rodent cross-reactive inventive ASOs with Seq. ID No. 143aj, Seq. ID No. 143h and Seq. ID No. 210q were also effective in the above experiments proving an effect in the treatment of Huntington model animals. The ASOs of the present invention demonstrating no cross-reactivity exert more potential effects in *in vitro* experiments. As a result, it is assumed that these inventive ASOs are also more effective in *in vivo* set ups for non-human primates and humans and therefore act as a highly potent medication for preventing or treating TGF-β1 induced inhibition of neural stem and progenitor proliferation, and thereby treating HD and other neurodegenerative disorders.

Example 15: Determination of therapeutic activity of the inventive ASOs on disease progression of TGFβ-induced hydrocephalus and associated cognitive deficits in Fischer-344 rats

The goal of the present study is to treat animals suffering from the TGFβ induced effects on i) neural stem cell proliferation and neurogenesis, ii) formation of hydrocephalus, and iii) spatial learning deficits by intraventricular infusion of inventive ASO in a dose-dependent manner.

Description of Method: Osmotic minipumps for intracerebroventricular infusion were implanted into female Fischer-344 rats of 180 to 200 g body weight (n_{total}=70, n_{group}=10). Infused were a) artificial cerebrospinal fluid (aCSF: 148.0 mM NaCl, 3.0 mM KCl, 1.4 mM $CaCl_2$, 0.8 mM $MgCl_2$, 1.5 mM Na_2HPO_4, 0.2 mM NaH_2PO_4, 100

µg/ml rat serum albumin, 50 µg/ml Gentamycin, pH 7.4) as control, or b) TGF-β1 1 µg/mL in aCSF using an Alzet® osmotic pump 2004 with flow rate of 0.25 µl/h for 14 days. After 14 days the pumps are changed and Alzet® osmotic pumps 2004 (flow rate 0.25 µl/h) were used for the following infusions: aCSF or TGF-β1 (1 µg/ml) in combination with varying concentrations of TGF-R_{II} ASO (1.1 mmol/L, 3.28 mmol/l, 9.84 mmol/l) or scrambled ASO (3.28 mmol/l) were infused (2 x 4 weeks). During the last four days of the infusion period, animals received a daily intraperitoneal injection of BrdU (50 mg/kg of body weight) to label proliferating cells. Pumps are removed, and two weeks later animals are functionally analyzed in a spatial learning test (Morris-Water-Maze) for 14 days. One day later, animals are perfused with 0.9% NaCl, brains are removed, the ipsilateral hemisphere is postfixed in 4% paraformaldehyde for quantitative histological analysis of PCNA, BrdU, DCX, BrdU/NeuN, and BrdU/GFAP, and for stereological analysis of the volume of the lateral ventricles as a measure for the hydrocephalus. The contralateral hemisphere is further dissected and different areas (ventricle wall, hippocampus, cortex) are processed for quantitative RT-PCR to analyze TGF-R_{II} expression levels. MR images were taken of 4 animals of group 1, group 3, and group 6 at day four before pump implantation, one week after pump implantation, at the day of the first pump change and from then on every 2 weeks until the end of the infusion period. Histological verification of the icv implantation sites was performed at 40-µm coronal, cresyl violet-stained brain sections.

Table 46: Treatment scheme and the group classification of the Hydrocephalus experiment.

Group	1. aCSF	2. aCSF + ASO	3. TGF-β1	4. TGF-β1 + scramb-ASO	5.-7. TGF-β1 + ASO
treatment	aCSF-infusion	aCSF plus ASO infusion	TGF-β1-infusion	TGF-β1 plus ASO infusion	TGF-β1 plus ASO infusion
treatment scheme	**week 1 to 10**	**week 1 and 2:** aCSF **week 3 to 10**: ASO: 3.28 mmol/l	**week 1 and 2:** 1 µg/ml **week 3 to 10:** 1 µg/ml	**week 1 and 2:** TGF-β1: 1 µg/ml **week 3 to 10:** TGF-β1: 1 µg/ml scramb.-ASO: 3.28 mmol/l	**week 1 and 2:** TGF-β1: 1 µg/ml **week 3 to 10:** TGF-β1: 1 µg/ml ASO: 1.1 mmol/l 3.28 mmol/l 9.84 mmol/l
n	10	10	10	10	10 per dose
n-total	10	10	10	10	30

The inventive ASOs exert potential effects in *in vitro* experiments. Quite in line, the rodent cross-reactive inventive ASOs with Seq. ID No. 143aj, Seq. ID No. 143h and Seq. ID No. 210q were also effective in the above experiments proving an effect in the treatment of Hydrocephalus model animals. The ASOs of the present invention demonstrating no cross-reactivity exert more potential effects in *in vitro* experiments. As a result, it is assumed that these inventive ASOs are also more effective in *in vivo* set ups for non-human primates and humans and therefore act as a highly potent medication for preventing or treating TGF-β1 induced inhibition of neural stem and progenitor proliferation, and thereby treating Hydrocephalus and other neurodegenerative disorders.

Example 16: Determination of therapeutic activity of the antisense-oligonucleotides directed to TGF-R_{II} on rehabilitation of spinal cord injury in Fischer 344 rats

To analyze the therapeutic potential of ASOs as a medication for spinal cord injury (SCI), male and female Fischer-344 rats were treated with different doses of inventive ASOs by icv administration into the lateral ventricle via osmotic minipumps.

Description of Method: SCI was simulated by cervical tungsten wire knife dorsal column transection at the C3 level. In the next step, for chronic central infusion rats, (180 – 200 g body weight) underwent surgery for an icv cannula attached to an Alzet® osmotic minipump (infusion rate: 0.25 µl/h, Alzet®, Model 2004, Cupertino, USA). The cannula and the pump were stereotaxically implanted under ketamine/xylacin anesthesia (Baxter, GmbH, Germany) and semi-sterile conditions. Each osmotic minipump was implanted subcutaneously in the abdominal region *via* a 1 cm long skin incision at the neck of the rat and connected with the icv cannula by a silicone tubing. Animals were placed into a stereotaxic frame, and the icv cannula (23G, 3 mm length) was lowered into the right lateral ventricle (posterior 1.0 mm, lateral 1.0 mm, depth 1.8 mm relative to bregma). The cannula was fixed with two stainless steel screws using dental cement (Kallocryl, Speiko®-Dr. Speier GmbH, Münster, Germany). The skin of the neck was closed with sutures. During surgery, the body temperature was maintained by a heating pad. To avoid post-surgical infections, rats were locally treated with betaisodona® (Mundipharma GmbH, Limburg, Germany) and received 0.5 ml antibiotics (sc, Baytril® 2.5% Bayer Vital GmbH, Leverkusen, Germany). The tubing was filled with the respective solution. To determine the effects of ASOs on the rehabilitation process following SCI, 4 weeks post-surgery an *in vivo* MRI structural analysis was performed (3T MRI, Allegra

Siemens, phased array - small animal coil). 6 weeks after surgery, animals were sacrificed and the spinal cord was removed for histological and immunohistochemical analysis. Histological verification of the icv implantation sites was performed at 40-µm coronal, cresyl violet-stained brain sections.

The inventive ASOs exert potential effects in *in vitro* experiments. Quite in line, the rodent cross-reactive inventive ASOs with Seq. ID No. 143aj, Seq. ID No. 143h and Seq. ID No. 210q were also effective in the above experiments proving an effect in the treatment of a Fischer-344 – rat spinal cord paraplegia model. In MRI images and neuropathological analysis, the inventive ASOs showed high treatment efficacy. The ASOs of the present invention demonstrating no cross-reactivity exert more potential effects in *in vitro* experiments. As a result, it is assumed that these inventive ASOs are also more effective in *in vivo* set ups for non-human primates and humans and therefore act as a highly potent medication for preventing or treating TGF-β1 induced inhibition of neural stem and progenitor proliferation, and thereby treating spinal cord injury and other neurodegenerative disorders.

Example 17: ASO-mediated effects on proliferation of human lung cancer cell line A549.

mRNA of Ki67, p53, Caspase 8 (Casp8) and of DNA-binding protein inhibitor 2 (ID2) were analyzed as representative markers on proliferation in several tumor cells. It is known from previous studies, that expression of tumor suppressor gene p53 and ID2 is often dramatically elevated in tumor tissues. Ki67 is a proliferation marker and Casp8 is an indicator for apoptosis. In addition, cell numbers were determined after gymnotic transfer.

Description of Method:

A549 were cultured as described above. For treating cells, medium was removed and replaced by fresh full medium in 24-well culture dishes (Sarstedt #83.1836.300) (30,000 cells / well), 6-well culture dishes (Sarstedt #83.3920.300) (50,000 cells / well) or 8-x-well cell culture slide dishes (Sarstedt #94.6140.802) (20,000 cells / well) (0.5 ml for 24-well and 8-well cell culture slide dishes and 1 ml for 6-well dishes) and were incubated overnight at 37 °C and 5 % CO_2. To analyze mRNA expression and influence on proliferation, cells were treated with Ref.1 (Scrambled control) and ASO Seq. ID No. 218b at concentrations of 2.5 µM and 10 µM and were incubated for 72 h at 37 °C and 5 % CO_2. Treatment including medium replacement was repeated for 3 times every 72 h (12 days in total). For immunocytochemical analysis of proliferation (Ki67), gymnotic transfer of ASO Seq. ID No. 218b was limited to 72 h. Afterwards,

cells were washed twice with PBS and subsequently used for protein isolation (6-well dishes), immunocytochemistry (in 8-well cell culture slide dishes), proliferation curve and RNA isolation (24-well dishes). Protocols for RNA, protein and immunocytochemistry were performed as described above. For proliferation curve, remaining cells were harvested from 24-well dishes for determination of cell number. For this purpose, remaining cells were washed with PBS (2x), treated with accutase (500 µl/ well) and incubated for 7 min at 37 °C. Afterwards 500 µl medium was added and cell number was determined using Luna FL™ Automated Cell Counter Fluorescence and Bright Field (Biozym, #872040) according to manufacturer´s instructions. Briefly, 18 µl of the cell suspension was added to 2 µl of acridine orange/propidium iodide assay viability kit (Biozym #872045). After 1 min of settling, 10 µl were added onto Cell Counting Slide (Biozym # 872011). Cells were counted and calculated in distinction of alive and dead cells.

17.1 Results for ASO Seq. ID No. 218b

mRNA analysis showed reduced Ki67, p53 and ID2 expression levels 12 days after gymnotic transfer of ASO Seq. ID No. 218b. In contrast, Casp8 was elevated at low levels of ASO Seq. ID No. 218b (Table 46). These observations indicate that a reduced tumor growth is associated with a slight increase in apoptotic cells. Furthermore, Western Blot analysis showed reduction in protein level of Ki67 and pAkt 12 days after gymnotic transfer of inventive ASOs (Table 47). Immunochemical examination of A549 cells after gymnotic transfer of ASO Seq. ID No. 218b showed a reduced level of Ki67 signals in comparison to scrambled control for both concentrations applied (Figure 23). Finally, cell number of A549 cells was reduced about nearly 50 % 12 days after gymnotic transfer of ASO Seq. ID No. 218b (Table 48).

Table 46: mRNA expression of Ki67, p53, Casp8 and ID2, 12 days after gymnotic transfer of ASO Seq. ID No. 218b in A549 cells. Regulation of examined genes demonstrates diminished proliferation rates after gymnotic transfer of inventive ASOs. Reduced ID2 mRNA levels are beneficial in dampening expansion of tumor cells. mRNA expression levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, ± = SEM, Statistics was calculated using the Ordinary-one-way-ANOVA followed by “Tukey´s” multiple *post hoc* comparisons.

Cell line	A549 mRNA levels 12 days after repeated gymnotic transfer (4x 72 h)			
Target	Ki67 n = 2	p53 n = 2	Casp8 n = 2	ID2 n = 2
A	1.00 ± 0.37	1.00 ± 0.31	1.00 ± 0.05	1.00 ± 0.03
B 2.5 µM	0.92 ± 0.05	1.06 ± 0.02	1.36 ± 0.37	0.73 ± 0.01
B 10 µM	0.96 ± 0.03	1.11 ± 0.92	1.52 ± 0.15	0.82 ± 0.15
C 2.5 µM	0.55 ± 0.33	0.27 ± 0.04	1.59 ± 0.48	0.59 ± 0.01
C 10 µM	0.57 ± 0.20	0.53 ± 0.07	0.98 ± 0.17	0.35 ± 0.02

Table 47: Densitometric analysis of Ki67 and pAkt Western Blot. Downregulation of Ki67 and pAkt protein 12 days after gymnotic transfer with TGF-R_{II} specific ASO Seq. ID No. 218b was observed in A549 cells. Protein levels were determined relative to housekeeping gene GAPDH using Image Studio™ Lite Software and were then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, ± = SEM.

Cell line	A549 protein levels 12 days after repeated gymnotic transfer (4x 72 h)	
Target	Ki67 n = 1	pAKT n = 1
A	1.00	1.00
B 10 µM	1.18	0.80
C 10 µM	0.57	0.39

Table 48: Cell numbers 12 days after repeated gymnotic transfer. Cell numbers were determined 12 days after repeated gymnotic transfers (4 x 72 h) of A549 cells using Luna FL™ Automated Cell Counter Fluorescence and Bright Field (Biozym, #872040) according to manufacturer´s instructions. A = untreated control, C = Seq. ID No. 218b, ± = SEM.

Cell line	A549 cell number 12 days after repeated gymnotic transfer (4x 72 h)	
Cell number	alive cells x 10^5 n = 3	dead cells x 10^5 n = 3
A	4.25 ± 0.50	0.47 ± 0.09
B 10 µM	3.88 ± 0.95	0.31 ± 0.11
C 10 µM	2.35 ± 0.07	0.35 ± 0.16

Conclusion

These observations indicate that reduced tumorous growth is associated with an increase in apoptotic cells. Notably, ID2, which is a possible therapeutic target gene in tumors, is reduced after gymnotic transfer of TGF-R_{II} specific ASO Seq. ID No. 218b.

Taken together, ASO Seq. ID No. 218b is efficient in minimizing proliferation rates and reduces tumor promoting gene expression.

Example 18: Effect of ASO gymnotic transfer on proliferation of several tumor cell lines

TGF-β signaling is a critical pathway in cancer development. On the one hand TGF-β promotes factors, which act tumor suppressive but on the other hand, this growth factor leads to stimulation of cell migration, cell invasion, cell proliferation, immune regulation, and promotes an environmental reorganization in advantage to progression and metastasis of tumor cells. Thus, TGF-β is a key target in cancer treatment. mRNA and protein levels of proliferation marker (Ki67) and cell numbers were determined after gymnotic uptake of inventive ASOs as markers of proliferation rate in tumor cells. Furthermore, mRNA levels of tumor suppressor gene p53 and of DNA-binding protein inhibitor 2 (ID2) were examined.

Description of methods

Several tumor cell lines were cultured as described above (Table 10). For treating cells, medium was removed and replaced by fresh full medium in 24-well culture dishes (Sarstedt #83.1836.300) (30,000 cells / well), 6-well culture dishes (Sarstedt #83.3920.300) (50,000 cells / well) (0.5 ml for 24-well and 1 ml for 6-well dishes) and were incubated overnight at 37 °C and 5 % CO_2. To analyze mRNA expression and influence on proliferation, cells were treated with Ref.1 (Scrambled control) and ASO Seq. ID No. 218b at concentrations of 2.5 µM and 10 µM and were incubated for 72 h at 37 °C and 5 % CO_2. Treatment including medium replacement was repeated 3 times every 72 h (12 days in total). For harvesting, cells were washed twice with PBS and subsequently used for RNA isolation (24-well dishes), protein isolation (6-well dishes), or proliferation curve. Protocols for RNA and protein isolation were performed as described above. Before counting cells for proliferation curve, cells were analyzed by using light microscopy (Nikon, TS-100 F LED #MFA33500). Remaining cells were then harvested from 24-well dishes for determination of cell number. For this purpose, remaining cells were washed with PBS (2x), treated with accutase (500 µl/ well) and incubated for 5 - 7 min at 37 °C. Afterwards 500 µl

medium was added and cell number was determined using Luna FL™ Automated Cell Counter Fluorescence and Bright Field (Biozym, #872040) according to manufacturer´s instructions. Briefly, 18 µl of the cell suspension were added to 2 µl of acridine orange/propidium iodide assay viability kit (Biozym #872045). After 1 min of settling, 10 µl were added onto Cell Counting Slide (Biozym # 872011). Cells were counted and calculated in distinction of alive and dead cells.

18.1 Results for Seq. ID No. 218b

Ki67 mRNA levels were efficiently decreased independently (A549, L3.6pl, Panc-1) or dependently (HT-29, Panc-1, CaCo2) of used ASO concentrations, 12 days after gymnotic transfer (Table 40). Gene expression level of p53 was also affected in A549, HT-29, K562, KG-1, CaCo2 and TMK-1 by tested ASO (Table 50). Verification of reduced Ki67 protein expression was shown for A549, L3.6pl, TMK-1, HT-29 and K562 (Table 51). Notably, ID2 mRNA expression showed a consistent efficiently and dose-dependently downregulation in A549, HT-29, K562 and TMK-1 cells mediated by ASO Seq. ID No. 218b (Table 51). In addition, ASO Seq. ID No. 218b resulted in a reduced proliferation rate of several tumor cell lines (Table 53). A dose-dependent decrease of cell number was recognized for HPAFII, MCF-7, KG1, K562, U937 and HTZ-19 cells. Lung cancer cells (A549) showed approx. 50 % reduction of cell numbers elicited by ASO Seq. ID No. 218b. Reduced cell numbers were additionally confirmed by light microscopy for HPAFII, K562, MCF-7, Panc-1 and HTZ-1, 12 days after gymnotic transfer of ASO Seq. ID No. 218b (Figure 24).

Comparable results are obtainable for the antisense-oligonucleotides of the Seq. ID No.s 141d, 141g, 141i, 143r, 143w, 143af, 143ag, 143ah, 143j, 143p, 143q, 233d, 234d, 235b, 235d, 237b, 237c, 237i, 237m, 238c, 238f, 239e, 240c, 241b, 242a, 246e, 247d, 248b, 248e, 248g, 152k, 152s, 152t, 152u, 152ab, 152ag, 152ah, 152ai, 249c, 249e, 250b, 250g, 251c, 251f, 252e, 253c, 254b, 255a, 259e, 260d, 261b, 261e, 261g, 262d, 262e, 209s, 209v, 209w, 209x, 209ai, 209an, 209at, 209au, 209av, 210o, 210v, 210w, 210x, 210ab, 210ac, 210ad, 210af, 210am, 263b, 263c, 263i, 263m, 264e, 264h, 265e, 266c, 267b, 268a, 272e, 273d, 274a, 274d, 274f, 275g, 275i, 276b, 276c, 276j, 276k, 277d, 277e, 278f, 279c, 280b, 281a, 218ad, 218n, 218t, 218u, 218v, 218ah, 218an, 218ao, 218ap, 220d, 221d, 222b, 222c,222f, 223c, 223f, 224i, 224m, 225c, 225f, 226e, 227c, 213o, 213p, 213q, 213s, 213y, 213z, 213aa, 213af, 228b, 229a, 285d, 286d, 287d, 287e, 287f, 288e, 288i, 289d, 289h, 289o, 289p, 289q, 290c, 290f, 290i, 291c, 292c, 293b, and 294a. Most of the afore-mentioned antisense-oligonucleotides could not beat Seq. ID Nos. 218b and 218c, but are still far more advantageous than the state of the art antisense-oligonucleotides. Thus the antisense-oligonucleotides of the present invention are

highly useful for the treatment of hyperproliferative diseases such as cancer and tumors.

Table 49: mRNA expression of proliferation marker Ki67. 12 days after gymnotic transfer of ASO Seq. ID No. 218b in A549, HT-29, L3.6pl, KG1, Panc-1 and CaCo2 cells, Ki67 mRNA was decreased in all cell lines, respectively. mRNA levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, ± = SEM, Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparisons.

Target	**Ki67** mRNA levels 12 days after repeated gymnotic transfer (4x 72 h)					
Cell line	**A549** n = 2	**HT-29** n = 2	**L3.6pl** n = 2	**KG1** n = 1	**Panc-1** n = 1	**CaCo2** n = 1
A	1.00 ± 0.37	1.00 ± 0.00	1.00 ± 0.25	1.00	1.00	1.00
B 2.5 µM	0.92 ± 0.05	0.89 ± 0.46	0.93 ± 0.03	0.72	0.76	1.21
B 10 µM	0.96 ± 0.03	0.60 ± 0.11	0.96 ± 0.16	0.76	0.79	1.07
C 2.5 µM	0.55 ± 0.33	0.34 ± 0.11	0.42 ± 0.03	0.16	0.68	0.99
C 10 µM	0.57 ± 0.20	0.17 ± 0.02	0.64 ± 0.05	0.33	0.37	0.37

Table 50: mRNA expression of tumor suppressor p53. 12 days after gymnotic transfer of ASO Seq. ID No. 218b in A549, HT-29, K562, KG1, CaCo2 and TMK-1 cells, p53 mRNA was decreased in all cell lines, respectively. mRNA expression levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, ± = SEM, *p < 0.05 in reference to A, Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparisons.

Target	**p53** mRNA levels 12 days after repeated gymnotic transfer (4x 72 h)					
Cell line	**A549** n = 2	**HT-29** n = 1	**K562** n = 1	**KG1** n = 1	**TMK-1** n = 1	**CaCo2** n = 1
A	1.00 ± 0.31	1.00	1.00	1.00	1.00 ± 0.04	1.00
B 2.5 µM	1.06 ± 0.02	0.72	0.90	1.37	0.74 ± 0.11	0.82
B 10 µM	1.11 ± 0.92	0.68	1.35	0.87	0.71 ± 0.15	1.25
C 2.5 µM	0.27 ± 0.04	0.51	0.27	0.65	**0.14*± 0.14**	0.99
C 10 µM	0.53 ± 0.07	0.32	0.46	0.67	**0.21* ± 0.05**	0.30

Table 51: mRNA expression of ID2. 12 days after gymnotic transfer of ASO Seq. ID No. 218b in A549, HT-29, K562 and TMK-1 cells, ID2 mRNA was dose-dependently downregulated in all cell lines, respectively. mRNA expression levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, ± = SEM, *p < 0.05 in reference to A, Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" *post hoc* multiple comparisons.

Target	**ID2** mRNA levels 12 days after repeated gymnotic transfer (4x 72 h)			
Cell line	**A549** n = 2	**HT-29** n = 1	**K562** n = 1	**TMK-1** n = 1
A	1.00 ± 0.03	1.00	1.00 ± 0.23	1.00 ± 0.23
B 2.5 µM	0.73 ± 0.01	0.93	0.97 ± 0.15	0.88 ± 0.15
B 10 µM	0.82 ± 0.15	1.00	0.82 ± 0.05	0.82 ± 0.05
C 2.5 µM	0.59 ± 0.01	0.31	0.70 ± 0.10	0.70 ± 0.10
C 10 µM	0.35 ± 0.02	0.25	**0.29* ± 0.09**	**0.30* ± 0.09**

Table 52: Densitometric analysis of Ki67 Western Blot. Downregulation of Ki67 protein after gymnotic transfer with ASO Seq. ID No. 218b was recognized. Protein level was quantified relative to housekeeping gene alpha-tubulin using Image Studio™ Lite Software and normalized to untreated controls. A = untreated control, B = Ref.1, C = Seq. ID No. 218b. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" *post hoc* comparisons.

Target	**Ki67** protein level 12 days after repeated gymnotic transfer (4x 72 h)				
Cell line	**A549** n = 1	**L3.6pl** n = 2	**TMK-1** n = 2	**HT29** n = 2	**K562** n = 1
A	1.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00
B 10 µM	1.18	0.59 ± 0.00	0.75 ± 0.00	1.19 ± 0.68	1.05
C 10 µM	0.57	0.19 ± 0.17	0.53 ± 0.26	0.69 ± 0.05	0.35

Table 53: Cell numbers in several cancer cell lines 12 days after repeated gymnotic transfer (4 x 72 h). ASO Seq. ID No. 218b was transferred to several cancer cell lines. Cell numbers were determined using Luna FL™ Automated Cell Counter Fluorescence and Bright Field (Biozym, #872040) according to manufacturer´s instructions. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, a = alive cells, d = dead cells. ± = SEM. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" *post hoc* comparisons test.

ll e	Treatment											
	A		B 2.5 µM		B 10 µM		C 2.5 µM		C 10 µM			
	cell number x 10^5											
	a	d	a	d	a	d	a	d	a	d	n	p=
l9	4.25 ± 0.50	0.47 ± 0,09			3.88 ± 0,95	0.31 ± 0,11			2.35 ± 0.07	0.35 ± 0.16	3	
\Fl	2.80 ± 0.33	0.35 ± 0.11	2.88 ± 2.04	0.36 ± 0.06	2.56 ± 0.45	0.39 ± 0.06	0.66 ± 0.47	0.25 ± 0.07	0.20 ± 0.09	0.06 ± 0.02	2	
1	17.40 ± 3.00	0.43 ± 0.16	16.5 ± 0.85	0.58 ± 0.24	13.80 ± 0.80	0.26 ± 0.17	10.90 ± 0.20	0.59 ± 0.18	7.63 ± 3.08	**0.48*+ ± 0.14**	3	A vs. C 10 µM *p < 0.01 B 2.5 µM vs. C 10 µM +p < 0.01 C 10 µM vs. D 10 µM #p < 0.01
52	10.93 ± 1.58	1.37 ± 0.40	7.44 ± 1.05	2.40 ± 0.62	6.40 ± 0.38	2.36 ± 0.30	5.60 ± 0.08	2.66 ± 0.41	3.33 ± 0.54	**0.62* ± 0.07**	3	A vs. C 10 µM *p < 0.01
-7	6.73	2.37	6.51	1.57	6.51	3.35	5.21	1.64	2.47	0.73	1	
37	26.43 ± 2.05	7.04 ± 0.28	14.5 ± 2.73	2.88 ± 0.37	17.67 ± 0.50	2.36 ± 0.30	**11.34* ± 2.85**	3.07 ± 0.97	**7.56* ± 1.49**	2.25 ± 0.44	3	A vs. C 2.5 µM *p < 0.01 A vs. C 10 µM *p < 0.01
c-	2.16 ± 0.08	0.11 ± 0.02	1.82 ± 0.36	0.15 ± 0.04	2.98 ± 0.27	0.16 ± 0.02	**1.15* ± 0.51**	0.07 ± 0.02	**1.20*+ ± 0.23**	0.36 ± 0.02	3	A vs. C 2.5 µM *p < 0.05 A vs. C 10 µM *p < 0.05 B 10µM vs. C 10 µM +p < 0.01
Z-)	2.06 ± 0.02	3.05 ± 0.36	2.57 ± 0.16	1.78 ± 0.15	2.55 ± 0.22	1.22 ± 0.15	1.78 ± 0.25	0.88 ± 0.09	**1.17+ ± 0.14**	0.49 ± 0.05	3	B 10µM vs. C 10 µM +p < 0.05

Conclusion

Modulation of Ki67, p53 and ID2 mRNA by ASO Seq. ID No. 218b indicates a beneficial effect in dampening tumor expansion in several organs and with different origin. Ki67, ID2 and p53 are known to be upregulated and promote cell proliferation in different cancer types. Proliferation marker Ki67, p53 and ID2 were efficiently downregulated. Cell counting and light microscopy of several tumor cells 12 days after gymnotic transfer revealed ASO Seq. ID No. 218b as a potent agent to reduce cell proliferation.

Taken together, TGF-R_{II} specific ASO Seq. ID No. 218b was efficiently reducing proliferation rates parallel to recognized mRNA modulations of Ki67, p53 and ID2. These data suggest that the inventive ASOs are promising drug candidates for dampening tumor cell progression and metastasis of tumor cells.

Example 19: Analysis of the effect of the antisense-oligonucleotides to Angiogenesis in several tumor cell lines

Modulation of angiogenesis is essential for organ growth and repair. An imbalance in blood vessel growth contributes to different diseases like e.g. tumor growth, ischemia, inflammatory and immune disorders. TGF-β is known to be a pro-angiogenic factor. This may be most relevant in inflammatory and neoplastic processes, when overshooting angiogenesis is responsible for disease progression. These effects may go hand in hand with TGF-β1 induced fibrosis. Therefore Inhibition of TGF-β signaling by $TGFR_{II}$ specific ASO may represent an adequate therapeutic approach.

To test this assumption, these ASOs were transferred to several tumor cell lines by gymnotic uptake. 12 days after repeated gymnotic transfers, cell supernatant was analyzed for protein levels of pro-angiogenic factors by multiplex analysis. This technology allowed investigation of multiple pro-angiogenic proteins (VEGF, Tie-2, FLt-1, PIGF and bFGF) by electro-chemiluminescence. Vascular endothelial growth factor (VEGF) is a potent tumor secreted cytokine that promotes angiogenesis and therewith contributes to e.g. tumor proliferation. Tie-2 is a protein which is expressed from actively growing blood vessels. Fms-like tyrosine kinase 1 (Flt-1), also known as vascular endothelial growth factor receptor 1 (VEGFR1), is a transmembrane tyrosine receptor kinase that is highly expressed in vascular endothelial cells and Placental Growth Factor (PlGF) acts together with VEGF and is upregulated under pathological conditions e.g. in tumor formation. Besides, basic Fibroblast Growth Factor (bFGF) is a growth factor that also induces angiogenesis. PAI-1 is a target gene of TGF-β and mediates scar formation and angiogenic effects of TGF-β. Therefore, PAI-1 demonstrates also a key factor for tumor invasion and metastasis. Patients showing a high PAI-1 concentration level are considered to a poor

prognostic factor e.g. in breast cancer, lung, colorectal and gastric cancer. High PAI-1 concentrations also are a risk factor for diseases where thrombosis plays a role (e.g. myocardial infarction, stroke). Thus, PAI-1 mRNA regulation by TGF-β specific antisense oligonucleotides was also tested.

Description of methods:

Tumor cell lines were cultured as described above (Table 10). For treating cells, medium was removed and replaced by fresh full medium in 24-well culture dishes (Sarstedt #83.1836.300) (30,000 cells / well) incubated overnight at 37 °C and 5 % CO_2. The next day, Ref.1 (Scrambled control,) and ASO Seq. ID No. 218b (were added to refreshed medium at concentrations of 2.5 and 10 μM and were incubated for 72 h at 37 °C and 5 % CO_2. Treatment including medium replacement was repeated 3 times every 72 h (12 days in total). Afterwards cell supernatant was collected and analyzed by a MesoScale Discovery® Assay (MSD Discovery). This technology allowed investigation of multiple pro-angiogenic proteins (VEGF, Tie-2, FLt-1, PIGF and bFGF) by electro-chemiluminescence. Experiment performance and information about the individual growth factors were extracted by manufacturer instructions (MSD MesoScale Discovery®, #K15198G). The results were evaluated by GraphPad Prism® 6.0 Software.

Afterwards, cells were washed twice with PBS and subsequently used for RNA isolation (24-well dishes) to analyze, whether gymnotic transfer of ASO may regulate mRNA levels of Plasminogen Activator inhibitor-1 (PAI-1) by real-time RT-PCR. Protocols and primers were used and listed as described before.

19.1 Results for Seq. ID 218b

Table 54 demonstrates that PAI-1 mRNA was downregulated in a dose-dependent manner in several tested cancer cells (A549: lung cancer, HPAFII: pancreatic adenocarcinoma, HT-29: colorectal adenocarcinoma, HTZ-19: melanoma, TMK-1: gastric carcinoma, THP-1: monocytic leukemia) after repeated gymnotic transfer of ASO Seq. ID No. 218b. In addition, VEGF protein levels in stimulated cell supernatants showed also a dose-dependent decrease in A549, HTZ-19, HPAFII and PC3M (prostatic adenocarcinoma). For HPAFII and PC3M cells downregulation was significant (Table 55). Influence of ASO Seq. ID No. 218b to bFGF confirmed observations for VEGF, meaning that ASO Seq. ID No. 218b is potent to suppress angiogenesis (Table 56) In A549 and PC3M results showed also a significant reduction of bFGF. Protein amount of PIGF in cell supernatants was only slightly but dose-dependently depressed in A549 and HTZ-19 cells. In PC3M cells basic endogenous PIGF level was higher than in all other tested cells and ASO effect was also stronger (Table 57). Finally, downregulation of Flt-1 protein in HT-29 cells (Table

58) and Tie-2 depression in HTZ-19 (ASO Seq. ID No. 218b 2.5 µM) and MCF-7 (mamma-carcinoma, 10 µM) could be detected (Table 59).

Comparable results are obtainable for the antisense-oligonucleotides of the Seq. ID No.s 141d, 141g, 141i, 143r, 143w, 143af, 143ag, 143ah, 143j, 143p, 143q, 152k, 152s, 152t, 152u, 152ab, 152ag, 152ah, 152ai, 209s, 209v, 209w, 209x, 209ai, 209an, 209at, 209au, 209av, 210o, 210v, 210w, 210x, 210ab, 210ac, 210ad, 210af, 210am, 213o, 213p, 213q, 213s, 213y, 213z, 213aa, 213af, 218ad, 218n, 218t, 218u, 218v, 218ah, 218an, 218ao, 218ap, 220d, 221d, 222b, 222c,222f, 223c, 223f, 224i, 224m, 225c, 225f, 226e, 227c, 228b, 229a, 233d, 234d, 235b, 235d, 237b, 237c, 237i, 237m, 238c, 238f, 239e, 240c, 241b, 242a, 246e, 247d, 248b, 248e, 248g, 249c, 249e, 250b, 250g, 251c, 251f, 252e, 253c, 254b, 255a, 259e, 260d, 261b, 261e, 261g, 262d, 262e, 263b, 263c, 263i, 263m, 264e, 264h, 265e, 266c, 267b, 268a, 272e, 273d, 274a, 274d, 274f, 275g, 275i, 276b, 276c, 276j, 276k, 277d, 277e, 278f, 279c, 280b, 281a, 285d, 286d, 287d, 287e, 287f, 288e, 288i, 289d, 289h, 289o, 289p, 289q, 290c, 290f, 290i, 291c, 292c, 293b, and 294a. Most of the afore-mentioned antisense-oligonucleotides could not beat Seq. ID Nos. 218b and 218c, but are still far more advantageous than the state of the art antisense-oligonucleotides. Thus the antisense-oligonucleotides of the present invention are highly useful for the treatment of hyperproliferative diseases such as cancer and tumors.

Table 54: mRNA expression of PAI-1 12 days after gymnotic transfer of Seq. ID No. 218b in A549, HPAFII, HT-29, HTZ-19, TMK-1 and THP-1 cells. Regulation of PAI-1 gene expression is dose-dependently affected by ASO Seq. ID No. 218b in a manner for an improved disease prognosis. mRNA levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, ± = SEM, Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparisons.

Target	**PAI-1** mRNA levels 12 days after repeated gymnotic transfer (4x 72 h)					
Cell line	**A549** n = 3	**HPAFII** n = 1	**HT-29** n = 2	**HTZ-19** n = 2	**TMK-1** n = 2	**THP-1** n = 2
A	1.00 ± 0.10	1.00	1.00 ± 0.11	1.00 ± 0.21	1.00 ± 0.06	1.00 ± 0.11
B 2.5 µM	1.28 ± 0.03	1.48	0.88 ± 0.27	0.99 ± 0.34	0.89 ± 0.04	1.14 ± 0.79
B 10 µM	1.03 ± 0.27	1.05	0.81 ± 0.08	1.30 ± 0.00	1.16 ± 0.00	1.21 ± 0.37
C 2.5 µM	0.91 ± 0.28	0.62	0.60 ± 0.13	1.13 ± 0.10	0.56 ± 0.04	0.83 ± 0.20
C 10 µM	0.56 ± 0.13	0.32	0.50 ± 0.18	0.77 ± 0.10	0.45 ± 0.23	0.09 ± 0.02

Table 55: VEGF protein levels in cell supernatant 12 days after gymnotic transfer of Seq. ID No. 218b in A549, HPAFII, HTZ-19, PC3M cells by MesoScale Discovery® Assay (MSD Mesoscale Discovery, #K15198G). Protein levels were determined by measuring electro-chemiluminescence. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, ± = SEM, *p < 0.05 and **p < 0.01 in reference to A, +p < 0.05 and ++p < 0.01 in reference to B 2.5 µM. Statistics were calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparisons.

Target	**VEGF** protein (pg / ml) 12 days after repeated gymnotic transfer (4x 72 h)			
Cell line	**A549** n = 1	**HPAFII** n = 2	**HTZ-19** n = 2	**PC3M** n = 2
A	8186	23266 ± 876	4411 ± 66	2657 ± 103
B 2.5 µM	8387	22278 ± 5711	3385 ± 57	1993 ± 5.4
B 10 µM	8623	20776 ± 497	4044 ± 21	813 ± 0.8
C 2.5 µM	8846	**15479**++ ± 512**	3444 ± 197	**1266*+ ± 20.5**
C 10 µM	6842	**11214** ± 898**	2882 ± 90	**442** ± 14.3**

Table 56: bFGF protein levels in cell supernatant 12 days after gymnotic transfer of Seq. ID No. 218b in A549 and PC3M cells by MesoScale Discovery® Assay (MSD Mesoscale Discovery, #K15198G). Protein levels were determined by measuring electro-chemiluminescence. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, ± = SEM, *p < 0.05 and **p < 0.01 in reference to A, +p < 0.05 and ++p < 0.01 in reference to B 2.5 µM, #p < 0.05 and ##p < 0.01 in reference to B 10 µM. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparisons.

Target	**bFGF** protein (pg / ml) 12 days after repeated gymnotic transfer (4x 72 h)	
Cell line	**A549** n = 2	**PC3M** n = 2
A	50.7 ± 2.9	21.2 ± 0.2
B 2.5 µM	54.4 ± 3.1	16.8 ± 0.1
B 10 µM	51.8 ± 2.7	14.7 ± 0.2
C 2.5 µM	**26.7**++ ± 2.1**	**11.3**+ ± 0.0**
C 10 µM	24.2 ± 3.4	**7.6**++ ± 0.0**

Table 57: PlGF protein levels in cell supernatant 12 days after gymnotic transfer of Seq. ID No. 218b in A549, HTZ-19 and PC3M cells by MesoScale Discovery® Assay (MSD MesoScale Discovery®, #K15198G). Protein levels were

determined by measuring electro-chemiluminescence. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, ± = SEM, **p < 0.01 in reference to A, Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparisons

Target	**PlGF** protein (pg / ml) 12 days after repeated gymnotic transfer (4x 72 h)		
Cell line	**A549** n = 2	**HTZ-19** n = 1	**PC3M** n = 2
A	9.9 ± 0.4	11.6	61.7 ± 2.1
B 2.5 µM	9.6 ± 0.2	8.1	54.1 ± 1.9
B 10 µM	8.6 ± 0.1	8.4	59.5 ± 3.2
C 2.5 µM	8.2 ± 0.8	8.2	69.4 ± 2.4
C 10 µM	**6.3** ± 0.9**	6.5	45.0 ± 3.5

Table 58: Flt-1 protein levels in cell supernatant 12 days after gymnotic transfer of Seq. ID No. 218b in HTZ-19 cells by MesoScale Discovery® assay (MSD Mesoscale Discovery, #K15198G). Protein levels were determined by measuring electro-chemiluminescence. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, ± = SEM, **p < 0.01 in reference to A, Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparisons.

Target	**Flt-1** protein (pg / ml) 12 days after repeated gymnotic transfer (4x 72 h)
Cell line	**HT-29** n = 1
A	33.9
B 2.5 µM	27.7
B 10 µM	27.7
C 2.5 µM	18.2
C 10 µM	18.7

Table 59: shows Tie-2 protein levels in cell supernatant 12 days after gymnotic transfer of Seq. ID No. 218b in HTZ-19 and MCF-7 cells by MesoScale Discovery® Assay (MSD Mesoscale Discovery, #K15198G). Protein levels were determined by measuring electro-chemiluminescence. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, ± = SEM, **p < 0.01 in reference to A, Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparisons.

Target	Tie-2 protein (pg / ml) 12 days after repeated gymnotic transfer (4x 72 h)	
Cell line	**HTZ-19** n = 1	**MCF-7** n = 1
A	13.5	98.1
B 2.5 µM	6.2	
B 10 µM		149.2
C 2.5 µM	3.2	
C 10 µM		76.9

Conclusion

All analyzed pro-angiogenic factors (VEGF, bFGF, PIGF, Flt-1 and Tie-2) could be regulated by ASO Seq. ID No. 218b in a manner that would have a favorable impact on suppressing tumor progression and other pathological mechanisms dependent on enhanced angiogenesis. Furthermore, PAI-1 mRNA was dose-dependently reduced by ASO Seq. ID No. 218b. This factor, a TGF-β target gene and e.g. an approved prognostic marker in breast cancer, was also dose-dependently downregulated.

Taken together, all tested inventive ASOs were efficient in reducing angiogenic processes that favors tumor progression, metastasis, inflammation, and thrombosis. Thus, the inventive ASOs directed against TGF-R_{II} are potent therapeutic candidate in different types of cancer and thrombosis related diseases.

Example 20: Analysis of the effect of inventive ASOs upon fibrosis

TGF-β is involved in a lot of processes such as cell proliferation, migration, wound healing, angiogenesis and cell-cell interactions. It´s known from several studies, that this factor is often elevated during pathogenesis in several diseases including primary open angle glaucoma, Alzheimer disease, pulmonal fibrosis and diabetic nephropathy. These diseases are related to pathologic modifications in extracellular matrix (ECM) and the aktin-cytoskeleton. Often, these observed alterations correlate with severity disease progression and resistance to treatment (Epithelial Mesenchymal transition - EMT - in tumors). Connective tissue growth factor (CTGF) is a downstream-mediator of TGF-β and mediates fibrotic effects of TGF-β. Thus, it is shown that CTGF mediates deposition of ECM and modulates reorganization of aktin-cytoskeleton. To investigate whether the inventive ASOs contribute to a resolution of fibrotic processes by inhibiting TGF-β signaling, CTGF levels were evaluated in addition to fibronectin (FN) and Collagen IV (ColIV), which represent two main components of ECM in several different cancer cells. Furthermore, effects of ASOs on CTGF, FN and on aktin-cytoskeleton were examined in neural precursor (ReNcell CX) and human lung cancer (A549) cells.

20.1 Fibrosis in neurodegeneration

Description of methods

Cells were cultured as described before in standard protocol. For treatment, cells were seeded in 24-well culture dishes (Sarstedt #83.1836.300) (50,000 cells / well), 6-well culture dishes (Sarstedt #83.3920.300) (80,000 cells / well) and 8-well cell culture slide dishes (Sarstedt #94.6140.802) (10,000 cells / well) and were incubated overnight at 37 °C and 5 % CO_2. To investigate a response of ReNcell CX® cells to TGF-β1 cells were treated after refreshing of medium with TGF-β1 (2 and 10 ng/ml, PromoCell #C63499) for 48 h, followed by mRNA analysis for CTGF. To figure out the ASO effect on CTGF and FN, ReNcell CX® cells, medium was removed and replaced by fresh full medium (1 ml for 6-well and 0.5 ml for 8-well). Ref. 1 (Scrambled control), ASO Seq. ID No. 218b and Seq.ID No. 218b were then added in medium at concentrations of 2.5 and 10 μM and respective analysis (real-time RT-PCR, Western Blot analysis and Immunocytochemistry) was performed after 96 h. To examine the ASO impact after investigation of pre-incubation with TGF-β1, medium was removed and replaced by fresh full medium (1 ml for 6-well dishes and 8-well cell culture slide dishes). Following exposition of TGF-β1 (10 ng/ml, 48 h) medium was changed, TGF-β1 (10 ng/ml), Ref.1 (10 μM), ASO with Seq. ID No. 218b (10 μM) and ASO with Seq. ID No. 218c (10 μM) were added in combination and in single treatment to cells. ReNcell CX® cells were then harvested 96 h after gymnotic transfer. Therefore, cells were washed twice with PBS and subsequently used for RNA (24-well dishes) and protein isolation (6-well dishes) or immunocytochemical examination of cells (in 8-well cell culture slide dishes). Protocols, antibodies, dilutions and primers were used as described before.

20.1.1 Results of TGF-β1 effects on neural precursor cells (ReNcell CX)

Nothing was known about reaction of ReNcell CX® to TGF-β1 exposure. Thus ReNcell CX® cells were treated for 48 h with TGF-β1 in two different concentrations (Table 60). Evaluation of real-time RT-PCR revealed a dose-dependent induction of CTGF- and TGF-β1 gene expression.

Table 60: CTGF and TGF-β1 mRNA expression 48 h after stimulation with TGF-β1. mRNA levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, E = TGF-β1. ± = SEM, Statistics was calculated using the Ordinary-one-way-ANOVA followed by “Tukey´s” multiple *post hoc* comparison.

Cell line	ReNcell CX mRNA levels after 48 h TGF-β1 treatment	
Target Time point	**CTGF** 48 h n=3	TGF-β1 48 h n=3
A	1.00 ± 0.43	1.00 ± 0.10
E 2 ng/ml	1.73 ± 0.92	1.34 ± 0.45
E 10 ng/ml	2.15 ± 1.14	1.85 ± 0.65

Conclusion

ReNcell CX® cells showed a response to TGF-β1 exposure presenting self-induction of TGF-β1 and elevation of TGF-β1 target gene CTGF. Taken together, ReNcell CX® cells are ideal to examine questions addressing TGF-β effects.

20.1.2 Results for Seq. ID No. 218b

20.1.2.1 Effects of gymnotic transfer

Gymnotic transfer of ASO Seq. ID No. 218b results in a dose-dependent and significant reduction of CTGF and FN (Table 61). This impact of ASO Seq. ID No. 218b was verified for FN protein level. FN protein level was depressed by about 70 % 96 h after gymnotic transfer of tested ASO, whereas TGF-β1 treatment of ReNcell CX® cells resulted in a 3.4-fold induction of FN (Table 62).

Table 61: Dose-dependent and significant downregulation of CTGF mRNA after gymnotic transfer with Seq. ID No. 218b in ReNcell CX® cells. mRNA levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b. ± = SEM, *p < 0.05, **p < 0.01 in reference to A. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Dunnett's" post *hoc* comparisons.

Cell line	ReNcell CX mRNA levels after gymnotic transfer	
Target Time point	**CTGF** 96 h, n=3	**FN** 96 h, n=3
A	1.00 ± 0.04	1.00 ± 0.00
B 2.5 µM	0.97 ± 0.06	0.81 ± 0.14
B 10 µM	0.86 ± 0.17	0.67 ± 0.07
C 2.5 µM	**0.66** ± 0.02**	**0.59 ± 0.02**
C 10 µM	**0.52** ± 0.02**	**0.39* ± 0.03**

Table 62: Densitometric analysis after Western Blotting for Fibronectin. Downregulation of FN protein 96 h after gymnotic transfer of ASO Seq. ID No. 218b in ReNcell CX® cells could be recognized. Protein level was determined relative to housekeeping gene alpha-Tubulin using Image Studio™ Lite Software and was then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b.

Cell line	**ReNcell CX** protein levels after gymnotic transfer
Target Time point	FN 96 h, n=1
A	1.00
B 2.5 µM	1.06
B 10 µM	0.60
C 2.5 µM	0.46
C 10 µM	0.30
E 10 ng/ml	3.43

Conclusion

ASO Seq. ID No. 218b was potent in downregulating mRNA levels of CTGF and FN in human neuronal precursor cells. ASO Seq. ID No. 218b treatment reduced FN protein, 96 h after gymnotic transfer. Thus, TGF-R_{II} specific ASO mediates blocking of TGF-β induced fibrotic effects ReNcell CX® cells.

20.1.2.2 Effects of gymnotic transfer after TGF-β pre-incubation

To analyze whether ASO Seq. ID No. 218b is also potent in inhibiting fibrotic effects mediated by TGF-β under pathological conditions, ReNcell CX® cells were pre-incubated with TGF-β pre-incubation followed by gymnotic transfer for 96 h. Afterwards, determined mRNA levels of CTGF and FN indicate a strong anti-fibrotic effect of ASO Seq. ID No. 218b also after TGF-β induction of CTGF and FN gene expression (Table 63). Immunocytochemical staining for CTGF (Figure 25A) and FN (Figure 25B) confirmed data from mRNA analysis. In addition, staining with phalloidin for analysis of actin-cytoskeleton showed an induction of stress-fibers after TGF-β treatment, whereas ASO Seq. ID No. 218b was efficient in blocking TGF-β-mediated stress fiber induction (Figure 25C).

Table 63: Downregulation of CTGF and FN mRNA after TGF-β1-pre-incubation followed by gymnotic transfer with Seq. ID No. 218b in ReNcell CX® cells (compared to scrambled control). mRNA levels were determined relative to

housekeeping gene GNB2L1 using quantitative real-time RT-PCR and was then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E = TGF-β, ± = SEM, *p < 0.05, **p < 0.01 in reference to A. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Dunnett's" *post hoc* comparisons.

Cell line	**ReNcell CX** mRNA levels after 48 h TGF-β1 -> 96 h TGF-β1 + ASOs / single treatment	
Target Time point	**CTGF** 96 h, n=3	**FN** 96 h, n=3
A	1.00 ± 0.04	1.00 ± 0.10
B 10 µM	0.85 ± 0.01	0.78 ± 0.20
C 10 µM	**0.70* ± 0.25**	0.44 ± 0.04
E 10 ng/ml	**1.60** ± 0.15**	2.25 ± 0.31
E 10 ng/ml + B 10 µM	**1.71** ± 0.03**	**4.08*++ ± 0.90**
E 10 ng/ml + C 10 µM	**1.19++ ± 0.04**	**1.74++ ± 0.61**

Conclusion

ASO Seq. ID No. 218b showed strong anti-fibrotic effects under simulated pathological conditions (TGF-β1 pre-incubation). Aside from downregulation of FN as one main component of ECM, actin-cytoskeleton was also affected by inventive ASO in a manner that may be beneficial for a better outcome in fibrotic diseases.

20.1.3 Results for Seq. ID No. 218c

20.1.3.1 Effects of gymnotic transfer

Gymnotic transfer of ASO Seq. ID No. 218c results in a strong and significant reduction of CTGF mRNA after gymnotic transfer of 10 µM ASO Seq. ID No. 218c (Table 64).

Table 64: Downregulation of CTGF mRNA after gymnotic transfer of Seq. ID No. 218c in ReNcell CX® cells. mRNA levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, B = Ref.1, D = Seq. ID No. 218c. ± = SEM, *p < 0.05 in reference to A. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Dunnett's" *post hoc* comparisons.

Cell line	**ReNcell CX** mRNA levels after gymnotic transfer
Target Time point	**CTGF** 96 h, n=3

A	1.00 ± 0.10
B 2.5 µM	0.88 ± 0.08
B 10 µM	0.89 ± 0.07
D 2.5 µM	0.48 ± 0.08
D 10 µM	**0.17* ± 0.02**

Conclusion

ASO Seq. ID No. 218c was efficient in dose-dependent reduction of CTGF mRNA.

20.1.3.2 Effects of gymnotic transfer after TGF-β pre-incubation

Results for gymnotic transfer for ASO Seq. ID 218c followed by TGF-β1 pre-incubation verified an effective blockage of TGF-β1 induced effects on CTGF mRNA levels (Table 65). ASO was such potent in blocking TGF-β1 effect on CTGF that combination treatment is comparable to ASO Seq. ID No. 218c single treatment.

Table 65: CTGF mRNA level after TGF-β1 pre-incubation following gymnotic transfer of Seq. ID No. 218c and parallel TGF-β1 treatment in ReNcell CX® cells. Data confirmed an effective blocking of TGF-β1 induced effects on CTGF mRNA levels by ASO Seq. ID No. 218c in comparison to combination treatments. mRNA levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, B = Ref.1, D = Seq. ID No. 218c, E = TGF-β1. ± = SEM, *p < 0.05 in reference to A. Statistics was calculated using the Ordinary-one-way-ANOVA followed by “Dunnett’s” post *hoc* comparisons.

Target Time point	**ReNcell CX** mRNA levels 48 h TGF-β1 -> 96 h TGF-β1 + ASOs / single treatment
Cell line	**CTGF** n= 3
A	1.00 ± 0.03
B 10 µM	0.85 ± 0.01
D 10 µM	**0.17* ± 0.02**
E 10 ng/ml	1.39 ± 0.08
E 10 ng/ml + B 10 µM	1.25 ± 0.44
E 10 ng/ml + D 10 µM	**0.23* ± 0.02**

Conclusion

ASO Seq. ID No. 218c showed a strong downregulation of CTGF mRNA and protein even under artificial pathological conditions (TGF-β1 pre-incubation).

Taken together, aside from strong anti-fibrotic effects, TGF-R_{II} specific ASOs showed a modulation of actin-cytoskeleton. Induction of stress fibers may cause an elevation of cell rigidity and stiffness that may play a role e.g. in Alzheimer disease and other Neurodegenerative Disorders. ECM deposition may also mediate fast pathogenic modifications e.g. in primary open angle glaucoma. Thus, reduction of ECM deposition and suppression of stress fiber formation may be profitable for a better prognosis in fibrotic related neurological disorders. Thereby, TGF-R_{II} specific ASOs are potent therapeutic agents for the treatment e.g. Alzheimer disease and primary open angle glaucoma.

20.2. Pulmonary fibrosis

Description of methods

For investigation of ASO effects to ECM and actin-cytoskeleton in lung, human lung cancer (A549) cells were examined and cultured as described before. For treatment, cells were seeded in 24-well culture dishes (Sarstedt #83.1836.300) (50,000 cells / well), 6-well culture dishes (Sarstedt #83.3920.300) (80,000 cells / well) and 8-well cell culture slide dishes (Sarstedt #94.6140.802) (10,000 cells / well) and were incubated overnight at 37 °C and 5 % CO_2. To investigate a response of A549 cells to TGF-β1 cells were treated after refreshing of medium with TGF-β1 (2 and 10 ng/ml, PromoCell #C63499) for 48 h following mRNA analysis for CTGF. To investigate the ASO effect on CTGF and FN A549 cells, medium was removed and replaced by fresh full medium (1 ml for 6-well and 0.5 ml for 8-x-well). Ref. 1 (scrambled control), ASO Seq. ID No. 218b and Seq.ID No. 218b were then added in medium at concentrations of 2.5 and 10 μM and respective analysis (real-time RT-PCR, Western Blot analysis and Immunocytochemistry) was performed after 72 h in ReNcell CX® cells. To show possible ASO impact after pre-incubation with TGF-β1, medium was removed and replaced by fresh full medium (1 ml for 6-well dishes and 8-well cell culture slide dishes). Following exposition of TGF-β1 (10 ng/ml, 48 h) medium was changed, TGF-β1 (10 ng/ml), Ref.1 (10 μM), ASO with Seq. ID No. 218b (10 μM) and ASO with Seq. ID No. 218c (10 μM) was added in combination and in single treatment to cells. A549 cells were then harvested 72 h after gymnotic transfer. Therefore, cells were washed twice with PBS and subsequently used for RNA (24-well dishes) and protein isolation (6-well dishes) or immunocytochemical examination of cells (in 8-well cell culture slide dishes). Protocols, used antibodies, dilutions and primers were as described before.

20.2.1 Results of TGF-β1 effects on lung cancer cells (A549)

To investigate the ability of A549 cells to react to TGF-β1 exposure, cells were treated for 48 h with TGF-β1 in two different concentrations (Table 66). Evaluation of

real-time RT-PCR revealed for CTGF and TGF-β1 itself a dose-dependent induction of gene expression.

Table 66: Induced CTGF and TGF-β1 mRNA expression 48 h after stimulation with TGF-β1 in A549 cells. mRNA expression levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, E = TGF-β1. ± = SEM, *p < 0.05 and **p < 0.01 in reference to A, ++p < 0.05 in reference to E 2 ng/ml. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparison.

Cell line	**A549** mRNA levels after 48 h TGF-β1 treatment	
Target Time point	**CTGF** 48 h, n=3	TGF-β**1** 48 h, n=3
A	1.00 ± 0.23	1.00 ± 0.31
E 2 ng/ml	**2.44* ± 0.18**	1.60 ± 0.34
E 10 ng/ml	**11.35**++ ± 0.52**	2.37 ± 0.36

Conclusion

A549 cells showed a dose-dependent and significant mRNA upregulation of CTGF upon TGF-β1 exposure. In addition, self-induction of TGF-β1 was observed. Taken together, A549 cells are a good model to examine questions addressing TGF-β effects in lung and lung cancer.

20.2.2 Results for Seq. ID No. 218b

20.2.2.1 Results for effects of gymnotic transfer

Gymnotic transfer of ASO Seq. ID No. 218b causes a dose-dependent and highly significant reduction of CTGF gene expression (Table 67). FN mRNA level was also affected by tested ASO but not dose-dependently. In contrast, staining against FN revealed a dose-dependent reduction of FN in comparison to scrambled control (Figure 260A). Furthermore, ASO and TGF-β impact on actin-cytoskeleton was examined. Figure 26B showed an induction of actin-fibers including stress-fiber formation after TGF-β1 treatment in A549 cells in doss-dependent manner, whereas signal after gymnotic transfer of ASO Seq. ID No. 218b in A549 cells was significantly downregulated parallel to recognized reversion of TGF-β1-mediated effects. For protein analysis a proper downregulation of CTGF parallel to an inhibition of pErk1/2 by which CTGF mediates its fibrotic effects could have been shown (Table 68).

Furthermore, 72 h after gymnotic transfer of ASO Seq. ID No. 218b a decrease of both ECM main components FN and ColIV was remarkable (Table 68).

Table 67: Dose-dependent and significant downregulation of CTGF mRNA after gymnotic transfer with Seq. ID No. 218b in A549 cells. mRNA levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq.ID No.218b. ± = SEM, **p < 0.01 in reference to A. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Dunnett's" *post hoc* comparisons.

Cell line	**A549** mRNA levels after gymnotic transfer	
Target Time point	**CTGF** 72 h, n=3	**FN** 72 h, n=3
A	1.00 ± 0.08	1.00 ± 0.07
B 2.5 µM	0.87 ± 0.06	1.08 ± 0.02
B 10 µM	0.80 ± 0.03	0.87 ± 0.08
C 2.5 µM	**0.60** ± 0.04**	0.77 ± 0.17
C 10 µM	**0.39** ± 0.03**	0.74 ± 0.16

Table 68: **Densitometric analysis after CTGF, FN, ColIV and pErk11/2 Western Blot :** 72 h after gymnotic transfer with ASO Seq. ID No 218b in A549. Protein level was determined relative to housekeeping gene alpha-Tubulin using Image Studio™ Lite Software and was then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b.

Cell line	**A549** protein levels after gymnotic transfer			
Target Time point	**CTGF** 72 h n = 1	**FN** 72 h n = 1	**ColIV** 72 h n = 1	**pErk1/2** 72 h n=2
A	1.00	1.00	1.00	1.00 ± 0.00
B 2.5 µM	0.91	0.89	1.19	1.00 ± 0.14
B 10 µM	1.31	0.76	0.87	0.98 ± 0.02
C 2.5 µM	0.05	0.81	1.16	0.67 ± 0.26
C 10 µM	0.09	0.46	0.65	0.61 ± 0.13

Conclusion

Gymnotic transfer of Seq. ID No. 218b was efficient in modulating factors which are involved in ECM deposition and actin-cytoskeleton reorganization in human lung cells.

20.2.2.2 Results for effects of gymnotic transfer after TGF-β1 pre-incubation

Results for gymnotic transfer of ASO Seq. ID 218b following TGF-β1 pre-incubation verified an effective blockage of strong TGF-β1 induced effects on CTGF and FN mRNA levels (Table 69). Immunocytochemical staining against CTGF (Figure 27A) and FN (Figure 27B) confirmed mRNA detection on protein level.

Table 69: CTGF and FN mRNA level after TGF-β1-pre-incubation following gymnotic transfer of Seq. ID No. 218b and parallel TGF-β1 treatment in A549 cells. Data confirmed an effective blocking of TGF-β1 induced effects on CTGF and FN mRNA levels by ASO Seq. ID No. 218b in comparison to combination treatments. mRNA levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, E = TGF-β1. ± = SEM, *p < 0.05, **p < 0.01 in reference to A. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Dunnett's" *post hoc* comparisons.

Target Time point	**A549** mRNA levels 48 h TGF-β1 -> 72 h TGF-β1 + ASOs / single treatment	
Cell line	**CTGF** n= 5	**FN** n= 3
A	1.00 ± 0.22	1.00 ± 0.45
B 10 µM	0.89 ± 0.19	1.02 ± 0.37
C 10 µM	0.52 ± 0.05	0.35 ± 0.06
E 10 ng/ml	**6.92* ± 2.32**	2.92 ± 1.02
E 10 ng/ml + B 10 µM	**8.79** ± 2.72**	2.90 ± 0.56
E 10 ng/ml + C 10 µM	2.53 ± 0.59	1.18 ± 0.28

Conclusion

ASO Seq. ID No. 218b was efficient in mediating anti-fibrotic effects in A549 cells under artificial pathological conditions mimicked excessive concentrations of TGF-β1.

20.2.3 Results for Seq. ID No. 218c

20.2.3.1 Results for effects of gymnotic transfer

Gymnotic transfer of ASO Seq. ID No. 218c mediates a strong dose-dependent and significant reduction of CTGF mRNA 72 h after gymnotic transfer in A549 cells (Table 70).

Table 70: Downregulation of CTGF mRNA 72 h after gymnotic transfer of Seq. ID No. 218c in A549 cells. mRNA levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, B = Ref.1, D = Seq. ID No. 218c. ± = SEM, **p < 0.01 in reference to A. Statistics were calculated using the Ordinary-one-way-ANOVA followed by "Dunnett's" *post hoc* comparisons.

Cell line	**A549** mRNA level after gymnotic transfer
Target Time point	CTGF 72 h n=4
A	1.00 ± 0.08
B 2.5 µM	0.97 ± 0.07
B 10 µM	0.85 ± 0.06
D 2.5 µM	**0.49** ± 0.05**
D 10 µM	**0.31** ± 0.03**

Conclusion

Gymnotic transfer of ASO Seq. ID No. 218c was efficient in reducing mRNA of TGF-β downstream-mediator CTGF.

20.2.2.2 Results for effects of gymnotic transfer after TGF-β pre-incubation

Results for gymnotic transfer for ASO Seq. ID No. 218c following TGF-β1 pre-incubation verified an effective blockage of strong TGF-β1 induced effects on CTGF mRNA levels (Table 71). Immunocytochemical staining against CTGF confirmed these findings on protein level (Figure 28).

Table 71: CTGF mRNA levels after TGF-β1 pre-incubation followed by gymnotic transfer of Seq. ID No. 218c and parallel TGF-β1 treatment in A549. Data verified an effective blockage of TGF-β1 induced effects on CTGF mRNA levels by ASO Seq. ID No. 218c in comparison to combination treatments. mRNA levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, B = Ref.1, D = Seq. ID No. 218c, E = TGF-β1. ± = SEM, **p < 0.01 in reference to A, ++p < 0.01 in reference to E+B. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey's" *post hoc* comparisons.

Target Time point	**A549** 48 h TGF-β1 -> 72 h TGF-β1 + ASOs / single treatment
Cell line	**CTGF** n= 3
A	1.00 ± 0.05
B 10 µM	0.86 ± 0.11
D 10 µM	0.53 ± 0.10
E 10 ng/ml	4.71 ± 1.76
E 10 ng/ml + B 10 µM	**5.89* ± 2.16**
E 10 ng/ml + D 10 µM	**0.86++ ± 0.06**

Conclusion

ASO Seq. ID 218c was potent in mediating anti-fibrotic effects in A549 cells under artificial pathological conditions mimicked by excessive TGF-β1 concentrations.

Taken together, ASO Seq. ID 218c is an effective therapeutic agent, because pathology of lung fibrosis could be slowed down by reducing CTGF, FN and ColIV. In addition, stress fiber formation can be reduced effectively by TGF-R_{II} specific ASO, making inventive ASOs ideal therapeutic agents.

20.3 Effects on several cancer cells

Description of methods

For investigation of ASO effects addressing ECM (CTGF, FN, ColIV) cells were used and cultured as described before in standard protocol (Table 10). For treatment, cells were seeded in 24-well culture dishes (Sarstedt #83.1836.300) (30,000 cells / well), 6-well culture dishes (Sarstedt #83.3920.300) (50,000 cells / well) and were incubated overnight at 37 °C and 5 % CO_2. To analyze mRNA expression and influence on CTGF, FN and ColIV mRNA and protein levels cells were treated with Ref.1 (Scrambled control) or ASO Seq. ID No. 218b at concentrations of 2.5 and 10 µM and were incubated for 72 h at 37 °C and 5 % CO_2. Treatment including medium replacement was repeated 3 times every 72 h (12 days in total). For harvesting, cells were washed twice with PBS and subsequently used for RNA isolation (24-well dishes) or protein isolation (6-well dishes). Protocols for RNA and protein isolation as well as used antibodies and dilutions were performed as described above.

20.3.1 Results for Seq. ID No. 218b

Anti-fibrotic effects were detected by analysis of CTGF, FN, ColIV mRNA and protein levels. CTGF mRNA (Table 72) was dose-dependently reduced by Seq. ID No. 218b in HT-29, HTZ-19, MCF-7 and THP-1 cells. For KG-1 cells downregulation of TGF-β

downstream-mediator was recognized for 2.5 µM ASO Seq. ID No. 218b. For A549, Panc-1 and CaCo2 cells a decrease of FN was demonstrated (Table 73) in accordance to a dose-dependently decline of ColIV mRNA (Table 74) in THP-1, HTZ-19 and L3.6pl cells (Table 65). Western Blot analysis revealed a strong reduction of CTGF protein in HT-29, MCF-7, TMK-1 and L3.6pl cells. Result for MCF-7 was significant (Table 75). In addition, phosphorylation of Erk1/2 in A549 and TMK-1 cells was inhibited by ASO Seq. ID No. 218b. pErk1/2 is normally activated by CTGF to induce TGF-β mediated fibrotic effects (Table 76). For FN (A549, MCF-7, HT-29, HTZ-19, HPAFII) and Col IV (A549, HTZ-19, HPAFII, PC3M) (Table 77 and 78), the two main components of ECM, protein levels were minimized by about 50 %.

Table 72: mRNA expression of CTGF 12 days after gymnotic transfer of Seq. ID No. 218b in HT-29, HTZ-19, KG1, MCF-7 and THP-1 cells. CTGF mRNA was decreased after gymnotic transfer of Seq. ID No. 218b for all tested cell lines. mRNA levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, ± = SEM, Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparisons.

Target	**CTGF** mRNA levels 12 days after repeated gymnotic transfer (4x 72 h)				
Cell line	**HT-29** n = 2	**HTZ-19** n = 1	**KG-1** n = 1	**MCF-7** n = 1	**THP-1** n = 2
A	1.00 ± 0.28	1.00	1.00	1.00	1.00 ± 0.28
B 2.5 µM	0.68± 0.11	1.30		0.93	0.99 ± 0.68
B 10 µM	0.65 ± 0.03	1.20	0.88	0.91	1.15 ± 0.34
C 2.5 µM	0.40 ± 0.20	0.64		0.24	0.98 ± 0.11
C 10 µM	0.33 ± 0.19	0.55	0.26	0.22	0.09 ± 0.03

Table 73: mRNA expression of FN 12 days after gymnotic transfer of Seq. ID No. 218b in A549, Panc-1 and CaCo2 cells. FN mRNA was decreased after gymnotic transfer of Seq. ID No. 218b for all tested cell lines. mRNA levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, ± = SEM, **p < 0.01 in reference to A. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparisons.

Target	**FN** mRNA levels 12 days after repeated gymnotic transfer (4x 72 h)

Cell line	**A549** n = 2	**Panc-1** n = 1	**CaCo2** n = 2
A	1.00 ± 0.39	1.00	1.00 ± 0.30
B 2.5 µM	0.83 ± 0.08	1.29	0.55 ± 0.13
B 10 µM			1.00 ± 0.76
C 2.5 µM	0.35 ± 0.20	0.15	0.73 ± 0.54
C 10 µM			0.18 ± 0.17

Table 74: mRNA expression of ColIV 12 days after gymnotic transfer of Seq. ID No. 218b in A549, HTZ-19, THP-1, L3.6pl, Panc-1 and CaCo2 cells. ColIV mRNA was decreased after gymnotic transfer of Seq. ID No. 218b for all tested cell lines. mRNA levels were determined relative to housekeeping gene GNB2L1 using quantitative real-time RT-PCR and then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b, ± = SEM, Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparisons.

Target	**Col IV** mRNA levels 12 days after repeated gymnotic transfer (4x 72 h)					
Cell line	**A549** n = 2	**THP-1** n = 2	**HTZ-19** n = 1	**L3.6pl** n = 2	**Panc-1** n = 1	**CaCo2** n = 2
A	1.00 ± 0.00	1.00 ± 0.22	1.00	1.00 ± 0.20	1.00	1.00 ± 0.71
B 2.5 µM	1.18 ± 0.31	0.71 ± 0.25	0.94	0.83 ± 0.09	0.98	1.37 ± 0.19
B 10 µM	1.11 ± 0.60	0.61 ± 0.03		0.91 ± 0.29	0.57	2.61 ± 0.01
C 2.5 µM	0.84± 0.02	0.65 ± 0.19	0.51	1.14 ± 0.13	0.59	1.30± 0.03
C 10 µM	0.75± 0.02	0.30 ± 0.13		0.69 ± 0.05	0.30	0.57± 0.14

Table 75: Densitometric analysis after Western Blotting in HT-29, MCF-7, L3.6pl and TMK-1 cells 12 days after gymnotic transfer of Seq. ID No. 218b. Downregulation of CTGF protein by ASO Seq. ID No. 218b could be recognized. Protein levels were determined relative to housekeeping gene alpha-Tubulin using Image Studio™ Lite Software and was then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparisons.

Target	CTGF protein levels 12 days after repeated gymnotic transfer (4x 72 h)			
Cell line	**HT-29** n = 1	**MCF-7** n = 2	**TMK-1** n =1	**L3.6pl** n = 1
A	1.00	1.00 ± 0.0	1.00	1.00
B 10 µM	1.19	1.12 ± 0.11	0.85	0.93
C 10 µM	0.50	**0.22**++ ± 0.03**	0.38	0.22

Table 76: Densitometric analysis after Western Blotting in A549 and TMK-1 cells 12 days after gymnotic transfer of Seq. ID No. 218b. Downregulation of pErk1/2 protein by ASO Seq. ID No. 218b was determined. Quantification of protein level was done relative to housekeeping gene alpha-Tubulin using Image Studio™ Lite Software and was then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparisons.

Target	pErk1/2 protein levels 12 days after repeated gymnotic transfer (4x 72 h)	
Cell line	**A549** n = 1	**TMK-1** n =1
A	1.00	1.00
B 10 µM	1.21	1.14
C 10 µM	0.58	0.76

Table 77: Densitometric analysis after Western Blotting in A549, MCF-7, HT-29, HTZ-19 and HPAFII cells 12 days after gymnotic transfer of Seq. ID No. 218b. Downregulation of FN protein by ASO Seq. ID No. 218b was determined. Quantification of protein level was done relative to housekeeping gene alpha-Tubulin using Image Studio™ Lite Software and was then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparisons.

Target	FN protein levels 12 days after repeated gymnotic transfer (4x 72 h)				
Cell line	**A549** n = 1	**MCF-7** n = 2	**HT-29** n =1	**HTZ-19** n =1	**HPAFII** n = 1
A	1.00	1.00 ± 0.22	1.00	1.00	1.00
B 10 µM	1.10	1.08 ± 0.25	0.81	1.20	1.12
C 10 µM	0.56	0.69 ± 0.18	0.40	0.83	0.56

Table 78: Densitometric analysis after Western Blotting in A549, MCF-7, HT-29, HTZ-19 and HPAFII cells 12 days after gymnotic transfer of Seq. ID No. 218b. Downregulation of FN protein by ASO Seq. ID No. 218b was determined. Protein levels were analyzed relative to housekeeping gene alpha-Tubulin using Image Studio™ Lite Software and was then normalized to untreated control. A = untreated control, B = Ref.1, C = Seq. ID No. 218b. Statistics was calculated using the Ordinary-one-way-ANOVA followed by "Tukey´s" multiple *post hoc* comparisons.

Target	**Col IV** protein levels 12 days after repeated gymnotic transfer (4x 72 h)			
Cell line	**A549** n = 1	**HTZ-19** n =1	**HPAFII** n =1	**PC3M** n = 1
A	1.00	1.00	1.00	1.00
B 10 µM	1.31	1.01	1.05	1.07
C 10 µM	0.61	0.36	0.76	0.43

Conclusion

Increased deposition of ECM mediated by TGF-β1, through its downstream-mediator CTGF, could be efficiently reversed by TGF-R_{II} specific inventive ASOs in different tumor cell lines. A reduced level of ECM components could contribute to a less aggressive in tumor progression. Taken together, tested ASOs may demonstrate a new therapeutic strategy in different fibrosis-associated diseases.

Example 21: Threshold for toxicity of inventive ASOs by chronic intracerebroventricular administration using a dose-escalation paradigm in Cynomolgus.

To evaluate the ideal dose range for the GLP-toxicity study, a pre-experiment using chronic intracerebroventricular (icv) antisense-oligonucleotide (ASO) administration with escalating doses was performed in Cynomolgus monkeys. During the administration paradigm animals were monitored for immunological, hematological and physiological alterations.

Description of Method:

For chronic central ASO infusion in male and female Cynomolgus monkeys, a gas-pressure pump (0.25 ml/ 24 h, Tricumed-IP 2000V®) connected to a silicone catheter, targeting the right lateral ventricle was implanted subcutaneously under ketamine/xylacin anesthesia and semi-sterile conditions. A single male and a single

female monkey were used for each treatment condition (Seq. ID No. 218b, Seq. ID No. 218c, concentrations given in Table 79). Each pump was implanted subcutaneously in the abdominal region *via* a 10 cm long skin incision at the neck of the monkey and was connected with the icv cannula by a silicone catheter. Animals were placed into a stereotaxic frame, and the icv cannula was lowered into the right lateral ventricle. The cannula was fixed with two stainless steel screws using dental cement (Kallocryl, Speiko®-Dr. Speier GmbH, Münster, Germany). The skin of the neck was closed with sutures. During surgery, the body temperature was maintained by a heating pad. To avoid post-surgical infections, monkeys were locally treated with betaisodona® (Mundipharma GmbH, Limburg, Germany) and received 1 ml antibiotics (sc, Baytril® 2.5% Bayer Vital GmbH, Leverkusen, Germany). The tubing and the resp. pump was filled with the respective treatment solution. ASO infusion periods (1 week for each dose) were interrupted by a one-week wash out period with 0.9% NaCl being administered exclusively. During the entire administration paradigm body weight development and food consumption were monitored. Further, blood and CSF samples were taken once a week to determine hematological as well as immunological alterations but also systemic ASO concentrations. On the last day, animals were sacrificed, and organs (liver, kidneys, brain) were removed, and analyzed for proliferation, apoptosis, mRNA knock down, and tumor formation.

Table 79: Experimental design and the doses of ASOs given during the 7-week administration paradigm.

Treatment condition	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Seq. ID No.218b	0.048 mM	0.9% NaCl	0.24 mM	0.9% NaCl	1.2 mM	0.9% NaCl	6 mM
Seq. ID No 218c	0.048 mM	0.9% NaCl	0.24 mM	0.9% NaCl	1.2 mM	0.9% NaCl	6 mM

Conclusion:

All tested, inventive ASOs were at least non-toxic in weeks 1 – 6 and were therefore used for further research and toxicological examination. However, infusion of antisense-oligonucleotides Seq. ID No. 214, Seq. ID No. 138b, Seq. ID No. 172b as well as Ref. 0 and Ref. 5. resulted in toxic effects early in the above scheme. Therefore, these antisense-oligonucleotides are not suitable as therapeutic agent and were not used for further studies.

Example 22: Determination of behavioral and physiological abnormalities following central antisense-oligonucleotide administration

The goal of this study was to investigate the effects of a single intracerebroventricular (icv) antisense-oligonucleotide administration on neurological and resulting behavioral parameters in rats.

Description of Method:

Stereotaxic procedures were performed under ketamine/xylacin anesthesia and semi-sterile conditions. Following surgery, rats had two days for recovery.

Implantation of icv guide cannula

Animals were placed into a stereotaxic frame, and the guide cannula (12 mm) was implanted 2 mm above the left lateral ventricle (coordinates relative to bregma: 1.0 mm posterior, -1.6 mm lateral to midline, 1.8 mm beneath the surface of the skull.
The guide cannula was anchored to two stainless steel screws using dental acrylic cement (Kallocryl, Speiko®-Dr. Speier GmbH, Münster, Germany) and was closed with a dummy cannula. During surgery, the body temperature was maintained by a heating pad. To avoid post-surgical infections, mice were locally treated with betaisodona® (Mundipharma GmbH, Limburg, Germany) and received 0.1 ml antibiotics (sc, Baytril® 2.5% Bayer Vital GmbH, Leverkusen, Germany).

ICV infusion

Slightly restrained rats received an icv infusion of either ASO (2 µM/5 µl, 10 µM/5 µl, 50 µM/5 µl, 250 µM/5 µl) or vehicle (5 µl, 0.9% NaCl, pH 7.4, Braun) using a 27-gauge cannula, which extended 2 mm beyond the guide cannula and remained in place for 30 s to allow diffusion. Rats were monitored 15, 30, 60 and 120 minutes following icv administration for behavioral reactions, motor activity, CNS excitation, posture, motor coordination, muscle tone, reflexes, and body temperature.

Verification of cannula and microdialysis probe placement

After scarification, brains were removed, snap frozen and stored at -80 °C until analyzation. Histological verification of the icv implantation sites was performed at 40-µm coronal, cresyl violet-stained brain sections.

The present results demonstrate a single ASO (for both sequences Seq. ID No. 218b, Seq. ID No. 218c) icv administration, for different doses, to be a safe and secure technique in rats due to no effects on neurological parameters.

Example 23: Determination of the ideal dose range for the Cynomolgus GLP-toxicity study (pre-toxicity experiment in rats)

To investigate any general toxicological effects of a daily intravenous (iv) antisense-oligonucleotide (ASO) administration, and to localize the perfect dose-range for the GLP-pre-toxicity study in rats, a pre-toxicity experiment in rats was performed.

Description of Method:

For repeated intravenous ASO injection 20 male and 20 female rats were divided into four treatment groups, a vehicle group, an ASO_{low}, an $ASO_{intermediate}$, and an ASO_{high} group. This paradigm was performed for Seq. ID No. 218b and Seq. ID No. 218c. Rats received a daily iv bolus ASO injection for 15 consecutive days. Rats were monitored for mortality (twice daily), clinical symptoms (once daily, bod weight development (weekly), food consumption (weekly). On day 15 of the experimental paradigm, animals were sacrificed, organs (liver, kidney, brain) were removed and trunk blood was collected. Afterwards tissues and blood was analyzed for immunological and hematological alterations.

The results of the present study demonstrate the two ASOs Seq. ID No. 218b and Seq. ID No. 218c to be a safe medication for a variety of different disorders with no toxic effects when administered at low and intermediate doses.

Example 24: Determination of any general toxicological effects by repeated intravenous antisense-oligonucleotide injection

The goal of this study was to investigate at which dose a daily intravenous (iv) antisense-oligonucleotide (ASO) administration exerts any general toxicological effects in rats.

Description of Method:

For repeated intravenous ASO injection 80 male and 80 female rats were divided into four treatment groups, a vehicle group, an ASO_{low}, an $ASO_{intermediate}$, and an ASO_{high} group. Rats received a daily iv bolus ASO injection for 29 consecutive days. Rats were monitored for mortality (twice daily), clinical symptoms (once daily, bod weight development (weekly), food consumption (weekly). On day 29 of the experimental paradigm, animals were sacrificed, organs (liver, kidney, brain) were removed and trunk blood was collected. In addition, bone marrow smears were collected. Afterwards tissues and blood was analyzed for immunological and hematological, and histopathological alterations.

The results of the present study demonstrate the two ASOs Seq. ID No. 218b and Seq. ID No. 218c to be a safe medication for a variety of different disorders with no toxic effects when administered at low and intermediate doses.

Example 25: Determination of the toxicological properties of a chronic central antisense-oligonucleotide administration in Cynomolgus Monkeys

To determine the effective, and to identify the toxic dose, male and female Cynomolgus monkeys received different doses of an inventive antisense-oligonucleotide (ASO) by chronic intracerebroventricular administration. During the administration paradigm, animals were monitored for immunological, hematological and physiological alterations.

Description of Method:

For chronic central ASO infusion in male and female Cynomolgus monkeys, a gas-pressure pump (0.25 ml/ 24 h, Tricumed IP-2000V®) connected to a silicone catheter, targeting the right lateral ventricle, was implanted subcutaneously under ketamine/xylacin anesthesia and semi-sterile conditions. Three male and three female monkeys were used for each treatment condition (vehicle, ASO_{low}, ASO_{high}, concentrations given in Table 79). Further, for investigating the timeframe for recovery, two male and two female monkeys (vehicle, and ASO_{high}) were sacrificed four weeks after ASO administration was terminated. Each pump was implanted subcutaneously in the abdominal region *via* a 10cm long skin incision at the neck of the monkey and connected with the icv cannula by a silicone catheter. Animals were placed into a stereotaxic frame, and the icv cannula was lowered into the right lateral ventricle. The cannula was fixed with two stainless steel screws using dental cement (Kallocryl, Speiko®-Dr. Speier GmbH, Münster, Germany). The skin of the neck was closed with sutures. During surgery, the body temperature was maintained by a heating pad. To avoid post-surgical infections, monkeys were locally treated with betaisodona® (Mundipharma GmbH, Limburg, Germany) and received 1 ml antibiotics (sc, Baytril® 2.5% Bayer Vital GmbH, Leverkusen, Germany). The tubing was filled with the respective treatment solution. During the entire administration paradigm body weight development and food consumption was monitored. Further, blood and aCSF samples were taken once a week to determine hematological as well as immunological alterations but also systemic ASO concentrations. On the last day, animals of the main study were sacrificed, and organs (liver, kidneys, brain) were removed, and analyzed for proliferation, apoptosis, mRNA knock down, and tumor formation. After week 57, the additional animals used for investigating recovery periods were also sacrificed and the same read out parameters were determined.

Table 80. Treatment conditions and the animals per group for the 4-week GLP-toxicity study and for the additional 4-week recovery period.

Treatment condition	Main study		4-week recovery period	
	Males [n]	Females [n]	Males [n]	Females [n]
Vehicle	3	3	2	2
ASO_{low}	3	3	/	/
ASO_{high}	3	3	2	2

The results of the present study demonstrate a chronic intracerebroventricular ASO administration to be a non-toxic and safe medication for the treatment of a variety of different diseases.

Example 26: Determination of the stability and the biological activity of an antisense-oligonucleotide in different vehicle solutions

To investigate, whether there are any interaction effects of the antisense-oligonucleotides (Seq. ID No. 218b, Seq. ID No. 218c) and the infusion solution, a 29-day pre-experiment was performed. Therefore, the two ASOs were reconstituted in different endotoxin-free vehicle solutions (PBS, water for injection [WFI], 0.9 % NaCl) and stored at different conditions, respectively. Samples were collected every single week and were analyzed for pH-value, ASO stability, content, and integrity by AEX-HPLC. Any change in efficacy conditions were tested by proving the potency of TGF-RII mRNA knockdown in cell-culture assay with each sample, respectively.

Description of Method:

The lyophilized ASOs were diluted with the respective vehicle solution (Water for injection, 0.9%NaCl, PBS) under sterile conditions (laminar flow, BIOWIZARD Golden GL-170 Ergoscience®, S1 conditions). The 1.5 ml Eppendorf Cups were labeled and filled with 100 µl (AEX-HPLC) or 250 µl (target knock down) of the respective ASO solution (all steps under laminar flow, BIOWIZARD Golden GL-170 Ergoscience®, S1 conditions, see pipetting/labeling scheme table 81). In the next step, all samples were stored at their respective storing conditions and samples were collected every single week (see sampling scheme table 82) and stored at -80 °C until analyzation.

Table 81: Labeling scheme for the ASO-vehicle-stability study. The labeling scheme was performed for Seq. ID No. 218b and Seq. ID No. 218c (each 10 µM and 0.24 mM) and for all three vehicles WFI, 0.9% NaCl, and PBS (=> 12 different schemes).

	Day							
Label	**Vehicle (WFI, 0.9%NaCL or PBS)**	**Condition**	**0**	**6**	**12**	**18**	**24**	**29**
ASO [10 µM]	X	Baseline	ASO [10 µM] X_Baseline					
ASO [10 µM]	X	-20°C		ASO [10 µM] X_-20°C_Day 6	ASO [10 µM] X_-20°C_Day 12	ASO [10 µM] X_-20°C_Day 18	ASO [10 µM] X_-20°C_Day 24	ASO [10 µM] X_-20°C_Day 29
ASO [10 µM]	X	+4°C		ASO [10 µM] X_+4°C_Day 6	ASO [10 µM] X_+4°C _Day 12	ASO [10 µM] X_+4°C _Day 18	ASO [10 µM] X_+4°C _Day 24	ASO [10 µM] X_+4°C _Day 29
ASO [10 µM]	X	+20°C		ASO [10 µM] X_+20°C _Day 6	ASO [10 µM] X_+20°C _Day 12	ASO [10 µM] X_+20°C _Day 18	ASO [10 µM] X_+20°C _Day 24	ASO [10 µM] X_+20°C _Day 29
ASO [10 µM]	X	+37°C		ASO [10 µM] X_+37°C_Day 6	ASO [10 µM] X_+37°C _Day 12	ASO [10 µM] X_+37°_Day 18	ASO [10 µM] X_+37°_Day 24	ASO [10 µM] X_+37°C _Day 29
ASO [10 µM]	X	+40°C		ASO [10 µM] X_40°C_Day 6	ASO [10 µM] X_40°C _Day 12	ASO [10 µM] X_40°C _Day 18	ASO [10 µM] X_40°C _Day 24	ASO [10 µM] X_40°C _Day 29
ASO [10 µM]	X	pH value	ASO [10 µM] X_pH value_Day 0					ASO [10 µM] X_pH value_ Day 29
ASO [0.24 mM]	X	Baseline	ASO [0.24 mM] X_Baseline					
ASO [0.24 mM]	X	-20°C		ASO [0.24 mM] X_-20°C_Day 6	ASO [0.24 mM] X_-20°C_Day 12	ASO [0.24 mM] X_-20°C_Day 18	ASO [0.24 mM] X_-20°C_Day 24	ASO [0.24 mM] X_-20°C_Day 29
ASO [0.24 mM]	X	+4°C		ASO [0.24 mM] X_+4°C_Day 6	ASO [0.24 mM] X_+4°C _Day 12	ASO [0.24 mM] X_+4°C _Day 18	ASO [0.24 mM] X_+4°C _Day 24	ASO [0.24 mM] X_+4°C _Day 29

ASO [0.24 mM]	X	+20°C		ASO [0.24 mM] X_+20°C _Day 6	ASO [0.24 mM] X_+20°C _Day 12	ASO [0.24 mM] X_+20°C _Day 18	ASO [0.24 mM] X_+20°C _Day 24	ASO [0.24 mM] X_+20°C _Day 29
ASO [0.24 mM]	X	+37°C		ASO [0.24 mM] X_+37°C_Day 6	ASO [0.24 mM] X_+37°C _Day 12	ASO [0.24 mM] X_+37°_Day 18	ASO [0.24 mM] X_+37°_Day 24	ASO [0.24 mM] X_+37°C _Day 29
ASO [0.24 mM]	X	+40°C		ASO [0.24 mM] X_40°C_Day 6	ASO [0.24 mM] X_40°C _Day 12	ASO [0.24 mM] X_40°C _Day 18	ASO [0.24 mM] X_40°C _Day 24	ASO [0.24 mM] X_40°C _Day 29
ASO [0.24 mM]	X	pH value	ASO [0.24 mM] X_pH value_Day 0					ASO [0.24 mM] X_pH_value_ Day 29

Table 82: Collection scheme for the ASO-vehicle-stability study. The collection scheme was performed for Seq. ID No. 218b and Seq. ID No. 218c (each 10 µM and 0.24 mM) and for all three vehicles WFI, 0.9% NaCl, and PBS (=> 12 different schemes).

Sample	**Day**					
Condition	**0**	**6**	**12**	**18**	**24**	**29**
Baseline	X					
-20°C		X	X	X	X	X
+4°C		X	X	X	X	X
+20°C		X	X	X	X	X
+37°C		X	X	X	X	X
+40°C		X	X	X	X	X
pH value	X					X

Since there were no effects of any of the vehicle solutions on stability, content, and integrity of Seq. ID No. 218b and Seq. ID No. 218c, 0.9% NaCl was used for the ASO-*in use*-stability experiment.

Example 27: Determination of the *in-use* stability and the biological activity of inventive antisense-oligonucleotides (ASOs) in vehicle solution

To investigate, whether there are any interaction effects of the antisense oligonucleotides (ASO) (Seq. ID No. 218b, Seq. ID No. 218c) and a gas pressure pump or a catheter, a 29-day pre-experiment was performed. Therefore, the two ASOs were reconstituted in 0.9% NaCl and the pump and the catheter were filled according to manufacturer´s description. Samples were collected every single week and were analyzed for pH-value, microbiology, and oligo stability, content, and integrity by AEX-HPLC. Any change in efficacy conditions were also tested by proofing the potency to knockdown TGF-R_{II} mRNA in cell-culture assay with every sample, respectively.

Description of Method:

The lyophilized ASOs were diluted with 0.9% NaCl under sterile conditions (laminar flow, BIOWIZARD Golden GL-170 Ergoscience®, S1 conditions). The 5 ml Eppendorf Cups were labeled according to the labeling scheme (see table 83) under sterile conditions (laminar flow, BIOWIZARD Golden GL-170 Ergoscience, S1 conditions). The two gas pressure pumps (Tricumed Model IP-2000 V®) and the catheter (spinal catheter set 4000) were filled according to manufacturer´s description with the respective ASO solution (all steps under laminar flow, BIOWIZARD Golden GL-170 Ergoscience®, S1 conditions, see pipetting/labeling scheme table 83). In the next step, the pump connected to the catheter which was connected to the lid of a 5 ml Eppendorf Cup and the remaining Cups were stored in a storage box with all openings being closed with Parafilm®, to avoid any contamination. Every single week the samples were collected, stored at -80 °C until analysis and the catheter connected to the lid of a 5 ml Eppendorf Cup was transferred to the following Cup to continue the sampling procedure. In addition, one sample was taken directly from the pump via the bolus port and stored at -80 °C. On the last day, an additional sample for microbiological analysis was collected.

Table 83: Labeling scheme for the ASO *in-use*-stability study. The labeling scheme was performed for Seq. ID No. 218b and Seq. ID No. 218c (each 0.24 mM). PS: (PumpSample: sample directly from the catheter), AS: (AdditionalSample: sample directly from the reservoir inside the pump via bolus port, MB: (MicroBiology: 500 µM from PS and AS)

Sample			Day					
Oligo	**Cup**	**Condition**	**0**	**6**	**12**	**18**	**24**	**29**
Seq. ID No. 218b [0.24 mM]	5ml	PS	Seq. ID No. 218b [10 µM] Baseline	Seq. ID No. 218b _+37°C PS_Day 6	Seq. ID No. 218b _+37°C PS_Day 12	Seq. ID No. 218b _+37°C PS_Day 18	Seq. ID No. 218b _+37°C PS_Day 24	Seq. ID No. 218b _+37°C PS_Day 29
Seq. ID No. 218b [0.24 mM]	5 ml	AS		Seq. ID No. 218b _+37°C AS_Day 6	Seq. ID No. 218b _+37°C AS_Day 12	Seq. ID No. 218b _+37°C AS_Day 18	Seq. ID No. 218b _+37°C AS_Day 24	Seq. ID No. 218b _+37°C AS_Day 29
Seq. ID No. 218b [0.24 mM]	5 ml	MB						Seq. ID No. 218b _+37°C MB_Day 29
Seq. ID No. 218b [0.24 mM]	5 ml	pH value	Seq. ID No. 218b pH value Day 0					Seq. ID No. 218b pH value Day 29
Seq. ID No. 218c [0.24 mM]	5 ml	PS	Seq. ID No. 218c Baseline	Seq. ID No. 218c _+37°C PS_Day 6	Seq. ID No. 218c _+37°C PS_Day 12	Seq. ID No. 218c _+37°C PS_Day 18	Seq. ID No. 218c _+37°C PS_Day 24	Seq. ID No. 218c _+37°C PS_Day 29
Seq. ID No. 218c [0.24 mM]	5 ml	AS		Seq. ID No. 218c _+37°C AS_Day 6	Seq. ID No. 218c _+37°C AS_Day 12	Seq. ID No. 218c _+37°C AS_Day 18	Seq. ID No. 218c _+37°C AS_Day 24	Seq. ID No. 218c _+37°C AS_Day 29
Seq. ID No. 218c [0.24 mM]	5 ml	MB						Seq. ID No. 218c _+37°C MB_Day 29
Seq. ID No. 218c [0.24 mM]	5 ml	pH value	Seq. ID No. 218c pH value Day 0					Seq. ID No. 218c pH value Day 29

Table 84: Collection scheme for the ASO *in-use*-stability study. The collection scheme was performed for Seq. ID No. 218b and Seq. ID No. 218c (0.24 mM). PS: (PumpSample: sample directly from the catheter), AS: (AdditionalSample: sample directly from the reservoir inside the pump via bolus port, MB: (MicroBiology: 500 µM from PS and AS)

Sample		Day					
Cup	**Condition**	**0**	**6**	**12**	**18**	**24**	**29**
5 ml	Baseline	X					
5 ml	PS		X	X	X	X	X
5 ml	AS		X	X	X	X	X
5 ml	MB						X
5 ml	pH value	X					X

Since there were no effects of the pump and the catheter on the stability, content, and integrity of Seq. ID No. 218b and Seq. ID No. 218c, and there were also no noticeable microbiological problems, this application paradigm represents the optimal technique for the intrathecal and intracerebroventricular administration in Cynomolgus monkeys and humans.

Chemical Synthesis

Abbreviations

Pybop: (Benzotriazol-1-yl-oxy)tripyrrolidinophosphonium-hexafluorophosphat
DCM: Dichloromethane
DMF: Dimethylformamide
DMAP: 4-Dimethylaminopyridine
DMT: 4,4'-dimethoxytrityl
LCAA: Long Chain Alkyl Amino
TRIS: Tris(hydroxymethyl)-aminomethan
TRIS-HCl: Tris(hydroxymethyl)-aminomethan hydrochloride
DEPC: Diethyl dicarbonate

Gapmer antisense-oligonucleotide synthesis and purification

The antisense-oligonucleotides in form of gapmers were assembled on an ABI 3900 or on an ABI 394 synthesizer, or on an Expedite™ (Applied Biosystems) according to the phosphoramidite oligomerization chemistry. On the ABI3900, the solid support was polystyrene loaded with UnySupport (purchased from Glen Research, Sterling,

Virginia, USA) to give a synthesis scale of 0.2 µmol. On the ABI 394 the solid support was 500 A controlled pore glass (CPG) loaded with Unylinker™ purchased from Chemgenes (Wilmington, MA, USA) to give a 3 µmol synthesis scale.

Ancillary synthesis reagents such as "Deblock", "Oxidizer", "CapA" and "CapB" as well as DNA phosphoramidites were obtained from SAFC Proligo (Hamburg, Germany).
Specifically, 5'-O-(4,4'-dimethoxytrityl)-2'-O,3'-O-(2-cyanoethyl-N,N-diisopropyl) phosphoramidite monomers of deoxy thymidine (dT), 4-N-benzoyl-2'-deoxy-cytidine (dC^{Bz}), 6-N-benzoyl-2'-deoxy-adenosine (dA^{Bz}) and 2-N-isobutyryl-2'-deoxy-guanosine (dG^{iBu}) were used as DNA building-units. 5'-O-DMT-2'-O,4'-C-methylene-N^2- dimethylformamidine-guanosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (LNA-G^{DMF}), 5'-O-DMT-2'-O,4'-C-methylene-thymidine 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]phosphoramidite (LNA-Tb), 5'-O-DMT-2'-O,4'-C-methylene-N^6-benzoyladenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (LNA-A^{Bz}), 5'-O-DMT-2'-O-,4'-C-methylene-5-methyl-N^4-benzoylcytidine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (LNA-C^{*Bz}) were used as LNA-building-units. The LNA phosphoramidites were purchased from Exiqon (Vebaek, Denmark).

As shown by the examples of the LNAs in table 85, the phosphoramidites were dissolved in dry acetonitrile to give 0.07 M-oligonucleotide except LNA-C^{*Bz} which was dissolved in a mixture of THF/acetonitrile (25/75 (v/v)).

Table 85:

	Molecular weight g/mole	CAS No.	Solvent	To obtain a 0.07 M solution 100 mg
LNA-A^{Bz}	885.9	[206055-79-0]	Anhydrous Acetonitrile	1.6 ml
LNA-C^{*Bz}	875.9	[206055-82-5]	THF/Acetonitrile 25/75 (v/v)	1.6 ml
LNA-G^{DMF}	852.9	[709641-79-2]	Anhydrous Acetonitrile	1.7 ml
LNA-T	772.8	[206055-75-6]	Anhydrous Acetonitrile	1.8 ml

The β-D-thio-LNAs 5'-O-DMT-2'-deoxy-2'-mercapto-2'-S,4'-C-methylene-N^6-benzoyladenosine-3'-[(2-cyanoethyl-N,N-diisopropyl)]-

phosphoramidite, 5‘-O-DMT-2‘-deoxy-2’-mercapto-2’-S,4‘-C-methylene-5-methyl-N^4-benzoylcytidine-3‘-(2-cyanoethyl-N,N-diisopropyl)phosphoramidites, 5'-O-DMT-2‘-deoxy-2’-mercapto-2’-S,4’-C-methylene-N^2-dimethyformamidineguanosine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, 5’-O-DMT-2‘-deoxy-2’-mercapto-2’-S,4’-C-methylene-thymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, and 5‘-O-DMT-2‘-deoxy-2’-amino-2’-N,4‘-C-methylene-N^6-benzoyladenosine-3‘-(2-cyanoethyl-N,N-diisopropyl)phosphoramidites were synthesized as described in J. Org. Chem. 1998, 63, 6078 – 6079.

The synthesis of the β-D-amino-LNA 5‘-O-DMT-2‘-deoxy-2’-amino-2’-N,4‘-C-methylene-5-methyl-N^4-benzoylcytidine-3‘-[(2-cyanoethyl-N,N-diisopropyl)]-phosphoramidites, 5'-O-DMT-2‘-deoxy-2’-amino-2’-N,4’-C-methylene-N^2-dimethyformamidineguanosine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, 5’-O-DMT-2‘-deoxy-2’-amino-2’-N,4’-C-methylene-thymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, 5‘-O-DMT-2‘-deoxy-2’-methylamino-2’-N,4‘-C-methylene-N^6-benzoyladenosine-3‘-[(2-cyanoethyl-N,N-diisopropyl)]-phosphoramidite, and 5‘-O-DMT-2‘-deoxy-2’-methylamino-2’-N,4‘-C-methylene-5-methyl-N^4-benzoylcytidine-3‘-(2-cyanoethyl-N,N-diisopropyl)phosphoramidites, 5'-O-DMT-2‘-deoxy-2’-methylamino-2’-N,4’-C-methylene-N^2-dimethyformamidineguanosine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite 5’-O-DMT-2‘-deoxy-2’-methylamino-2’-N,4’-C-methylene-thymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite were carry out according to the literature procedure (J. Org Chem. 1998, 63, 6078 – 6079).

The α-L-oxy-LNAs__α-L-5‘-O-DMT-2‘-O,4‘-C-methylene-N^6-benzoyladenosine-3‘-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite, α-L-5‘-O-DMT-2‘-O-,4‘-C-methylene-5-methyl-N^4-benzoylcytidine-3‘-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite, α-L-5'-O-DMT-2'-O,4’-C-methylene-N^2-dimethyformamidineguanosine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, and α-L-5’-O-DMT-2'-O,4’-C-methylene-thymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite were performed similar to the procedures described in the literature (J. Am. Chem. Soc. 2002, 124, 2164-2176; Angew. Chem. Int. Ed. 200, 39, 1656 – 1659).

The (β-benzoylmercapto)ethyl)pyrrolidinolthiophosphoramidites for the synthesis of the oligonucleotide with phosphorothioate backbone were prepared in analogy to the protocol reported by Caruthers (J. Org. Chem. 1996, 61, 4272-4281).

The “phosphoramidites-C3” (3-(4,4'-Dimethoxytrityloxy)propyl-1-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite and the „3‘-Spacer C3 CPG“ (1-Dimethoxytrityloxy-

propanediol-3-succinoyl)-long chain alkylamino-CPG were purchased from Glen Research.

General procedure

Preparation of the LNA-solid support:

1) Preparation of the LNA succinyl hemiester (WO2007/112754)
5'-O-DMT-3'-hydroxy-nucleoside monomer, succinic anhydride (1.2 eq.) and DMAP (1.2 eq.) were dissolved in 35 ml dichloromethane (DCM). The reaction was stirred at room temperature overnight. After extractions with NaH_2PO_4 0.1 M pH 5.5 (2x) and brine (1x), the organic layer was further dried with anhydrous $NaSO_4$ filtered and evaporated. The hemiester derivative was obtained in 95% yield and was used without any further purification.

2) Preparation of the LNA-support (WO2007/112754)
The above prepared hemiester derivative (90 µmol) was dissolved in a minimum amount of DMF, DIEA and pyBOP (90 µmol) were added and mixed together for 1 min. This pre-activated mixture was combined with LCAA-CPG (500 Å, 80-120 mesh size, 300 mg) in a manual synthesizer and stirred. After 1.5 hours at room temperature, the support was filtered off and washed with DMF, DCM and MeOH. After drying, the loading was determined to be 57 µmol/g (see Tom Brown, Dorcas J. S. Brown. Modern machine-aided methods of oligodeoxyribonucleotide synthesis. In: F.Eckstein, editor. Oligonucleotides and 35 Analogues A Practical Approach. Oxford: IRL Press, 1991: 13-14).

Elongation of the oligonucleotide (Coupling)

5-ethylthio-1H-tetrazole (ETT) as activator (0.5 M in acetonitrile) was employed for the coupling of the phosphoramidites. Instead of ETT other reagents such as 4,5-dicyanoimidazole (DCI) as described in WO2007/112754, 5-benzylthio-1H-tetrazole or saccharin-1-methylimidazol can be used as activator. 0.25 M DCI in acetonitrile was used for the coupling with LNA.

Capping

10% acetic anhydride (Ac_2O) in THF (HPLC grade) and 10% N-methylimidazol (NMI) in THF/pyridine (8:1) (HPLC grade) were added and allowed to react.

Oxidation

Phosphorous(III) to Phosphorous(V) is normally done with e.g. iodine/THF/pyridine/H_2O using 0.02 M iodine in THF/Pyridine/H_2O purchased from Glen Research or 0.5 M 1S)-(+)-(10-camphorsulfonyl)-oxaziridine (CSO) in anhydrous acetonitrile from Glen Research.

In the case that a phosphorthioate internucleoside linkage is prepared, a thiolation step is performed using a 0.05 M solution of 3-((dimethylamino-methylidene)amino)-3H-1,2,4-dithiazole-3-thione (DDTT, obtained from Chemgenes (Wilmington, MA, USA)) in anhydrous acetonitrile/pyridine (1:1 v/v). In case LNAs are used, the thiolation was carried out usind 0.2 M 3,H-1,2-benzothiole-3-one 1,1-dioxide (Beaucage reagent) in anhydrous acetonitrile.
In general, the thiolation can also be carried out by using xanthane chloride (0.01 M in acetonitrile/pyridine 10%) as described in WO2007/112754.
Alternative, other reagents for the thiolation step such as xanthane hydride (5-imino-(1,2,4)dithiazolidine-3-thione), phenylacetyl disulfide (PADS) can be applied.

In the case that a phosphordithioate was synthesized, the resulting thiophosphite triester was oxidized to the phosphorothiotriester by addition of 0.05 M DDTT (3-((Dimethylamino-methylidene)amino)-3H-1,2,4-dithiazole-3-thione) in pyridine/acteonitrile (4:1 v/v).

Cleavage from the solid support and deprotection

At the end of the solid phase synthesis, the antisense-oligonucleotide can either be cleaved "DMT-on" or "DMT-off". "DMT off" means that the final 5'-O-(4,4'-dimethoxytrityl) group was removed on the synthesizer using the "Deblock" reagent and DMT-on means that the group is present while the oligonucleotide is cleaved from the solid support. The DMT groups were removed with trichloroacetic acid.

"DMT-off"

Upon completion of the solid phase synthesis antisense-oligonucleotides were treated with a 20% diethylamine solution in acetonitrile (Biosolve BV, Valkenswaard, The Netherlands) for 20 min. to remove the cyanoethyl protecting groups on the phosphate backbone. Subsequently, the antisense-oligonucleotides were cleaved from the solid support and deprotected using 1 to 5 mL concentrated aqueous ammonia (obtained from Sigma Aldrich) for 16 hours at 55 °C. The solid support was separated from the antisense-oligonucleotides by filtration or centrifugation.

If the oligonucleotides contain phosphorodithioate triester, the thiol-groups were deprotected with thiophenol:triethylamine:dioxane, 1:1:2, v/v/v for 24 h, then the oligonucleotides were cleaved from the solid support using aqueous ammonia for 1-2 hours at room temperature, and further deprotected for 4 hours at 65 °C.

"DMT-on"
The oligonucleotides were cleaved from the solid support using aqueous ammonia for 1-2 hours at room temperature, and further deprotected for 4 hours at 65 °C. The oligonucleotides were purified by reverse phase HPLC (RP-HPLC), and then the DMT-group is removed wih trichloroacetic acid.

If the oligonucleotides contain phosphorodithioate triester, the cleavage from the solid-support and the deprotection of the thiol-group were performed by the addition of 850 µl ammonia in concentrated ethanol (ammonia/ethanol 3:1 v/v) at 55 °C for 15 - 16h.

Terminal groups
Terminal groups at the 5'-end of the antisense oligonucleotide
The solid supported oligonucleotide was treated with 3% trichloroacetic acid in dichloromethane (w/v) to completely remove the 5'-DMT protection group. Further, the compound was converted with an appropriate terminal group with cyanoethyl-N,N-diisopropyl)phosphoramidite-moiety. After the oxidation of the phosphorus(III) to phosphorus(V), the deprotection, detachment from the solid support and deprotection sequence were performed as described above.

Purification
Next, the crude antisense-oligonucleotides were purified by anion-exchange high-performance liquid chromatography (HPLC) on an AKTA Explorer System (GE Healthcare, Freiburg, Germany) and a column packed with Source Q15 (GE Helthcare). Buffer A was 10 mM sodium perchlorate, 20 mM Tris, 1 mM EDTA, pH 7.4 and contained 20% acetonitrile and buffer B was the same as buffer A with the exception of 500 mM sodium perchlorate. A gradient of 15%B to 55%B within 32 column volumes (CV) was employed. UV traces at 280 nm were recorded. Appropriate fractions were pooled and precipitated with 3 M NaOAc, pH= 5.2 and 70% ethanol. Finally, the pellet was washed with 70% ethanol.Analytics
Identity of the antisense-oligonucleotides was confirmed by electrospray ionization mass spectrometry (ESI-MS) and purity was by analytical OligoPro Capillary Electrophoresis (CE).

The purification oft he dithioate was performed on an Amersham Biosciences P920 FPLC instrument fitted with a Mono Q 10/100 GL column. The buffers were prepared with DEPC-treated water, and their compositions were as follows: Buffer A: 25 mM Tris-HCl, 1 mM EDTA, pH 8.0; Buffer B: 25 mM Tris-HCl, 1 mM EDTA, 1 M NaCl, pH 8.0.

Example 28

$Gb^1sTb^1sdAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsAb^1sGb^1sC*b^1$ (Seq. ID No. 209y)

5'-O-DMT-2'-O,4'-C-methylene-5-methyl-N^4-benzoxylcytidine-3'-O-succinoyl- linked LCAA CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. After several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene-N^2-dimethyformamidineguanosine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene-N^6-benzoyladenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidites (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^2-isobutyryl-2'-deoxyguanosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidites (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^2-isobutyryl-2'-deoxyguanosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidites (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^2-isobutyryl-2'-deoxyguanosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidites (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^6-benzoyl-2'-deoxyadenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9

v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-deoxythymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-deoxythymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-deoxythymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^2-isobutyryl-2'-deoxyguanosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidites (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-deoxythymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^2-isobutyryl-2'-deoxyguanosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^6-benzoyl-2'-deoxyadenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidites (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9

v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene thymidine 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene-N^2-dimethyformamidineguanosine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

Upon completion of the solid phase synthesis, the antisense-oligonucleotides were treated with a 20% diethylamine solution in acetonitrile (Biosolve BV, Valkenswaard, The Netherlands) for 20 min. to remove the cyanoethyl protecting groups on the phosphate backbone.
Subsequently, the antisense-oligonucleotides were cleaved from the solid support and further deprotected using 5 mL concentrated aqueous ammonia for 16 hours at 55°C. The solid support was separated from the antisense-oligonucleotides by filtration or centrifugation.

Next, the crude antisense-oligonucleotides were purified by anion-exchange high-performance liquid chromatography (HPLC) on an AKTA Explorer System (GE

Healthcare, Freiburg, Germany) and a column packed with Source Q15 (GE Helthcare). Buffer A was 10 mM sodium perchlorate, 20 mM Tris, 1 mM EDTA, pH 7.4 and contained 20% acetonitrile and buffer B was the same as buffer A with the exception of 500 mM sodium perchlorate. A gradient of 15%B to 55%B within 32 column volumes (CV) was employed. UV traces at 280 nm were recorded. Appropriate fractions were pooled and precipitated with 3 M NaOAc, pH= 5.2 and 70% ethanol. Finally, the pellet was washed with 70% ethanol. The antisense oligonucleotide was received with a purity of 93.7%. ESI-MS: experimental: 5387.3 Da; calculated: 5387.80 Da.

Example 29

$Gb^1Tb^1dAdGdTdGdTdTdTdAdGdGdGAb^1Gb^1C*b^1$ (Seq. ID No. 209u)

The LNA was bound to CPG according to the general procedure. The coupling reaction and capping step were also carried out as described in example 28. After the capping step, the system was flushed out with 800 µl acetonitrile, and 400 µl of 0.02 M Iodine in THF/pyridine/H_2O were inserted to the column for 45 s. The system was flushed after the oxidation step with 24 µl acetonitrile. After purification, the antisense oligonucleotide was received with a purity of 95.3%. ESI-Ms: experimental: 5146.80 Da; calculated: 5146.4 Da.

Example 30

/5SpC3s/$Gb^1sTb^1sdAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsAb^1sGb^1sC*b^1$ (Seq. ID No. 209v)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 28. But with the exception that after the last nucleotide has been coupled to the oligonucleotide and the subseuqent oxidation and capping steps were carried out, 80 µl of phosphoramidite-C3 (0.07 M) and 236 µl DCI in acetonitrile (0.25 M) were added. The coupling was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile. The subsequent steps were performed as described in example 28. After purification, the antisense oligonucleotide was received with a purity of 97.4%. HRMS (ESI): experimental: 5540.70 Da; calculated: 5541.4 Da.

Example 31

$Gb^1sTb^1sdAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsAb^1sGb^1sC*b^1$/3SpC3s/ (Seq. ID No. 209w)

3'-Spacer C3 CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. After

several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 80 µl 5'-O-DMT-2'-O-,4'-C-methylene-5-methyl-N^4-benzoylcytidine-3'-[(2-cyanoethyl-N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. The subsequent reactions were performed as described in example 28. After purification, the antisense oligonucleotide was received with a purity of 92.7%. ESI-sMS: experimental: 5541.70 Da; calculated: 5541.4 Da.

Example 32

$Gb^1ssTb^1ssAb^1ssdGssdTssdGssdTssdTssdTssdA*ssdGssdGssdGssAb^1ssGb^1ssC*b^1$ (Seq. ID No. 209an)

5'-O-DMT-2'-O,4'-C-methylene-5-methyl-N^4-benzoxylcytidine-3'-O-succinoyl- linked LCAA CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. After several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 38 µl 5'-O-DMT-2'-O,4'-C-methylene-N^2-dimethyformamidineguanosine-3'-[(β-benzoylmercapto)ethyl]pyrrolidinolthiophosphoramidite (0.15 M) in 10% dichloromethane (v/V) in acetonitrile and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 900 µl of DDTT (0.05 M in pyridine/acetonitrile 4:1 v/v) were inserted to the column for 240 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 38 µl 5'-O-DMT-2'-O,4'-C-methylene-N^6-benzoyladenosine-3'-[(β-benzoylmercapto)ethyl]pyrrolidinolthiophosphoramidite (0.15 M) in 10%

dichloromethane (v/v) in acetonitrile and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 900 µl of DDTT (0.05 M in pyridine/acetonitrile 4:1 v/v) were inserted to the column for 240 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The further elongation of the oligonucleotide was performed in the same way.

Upon completion of the solid phase synthesis, the antisense-oligonucleotides were treated 850 µl ammonia in concentrated ethanol (ammonia/ethanol 3:1 v/v) at 55 °C for 15 - 16h in order to cleave antisense-oligonucleotide from the solid-support and to deprotect the thiol-group.

Next, the crude antisense-oligonucleotide was purified by anion-exchange chromatogtraophy using a Mono Q 10/100 GL column. The buffers were prepared with DEPC-treated water, and their compositions were as follows: Buffer A: 25 mM Tris-HCl, 1 mM EDTA, pH 8.0; Buffer B: 25 mM Tris-HCl, 1 mM EDTA, 1 M NaCl, pH 8.0.

Example 33

$Gb^1sTb^1sAb^1sdGsdTsdGsdTsdTsdTsdAsdGsdGsGbsAb^1sGb^1sC*b^1$ (Seq. ID No. 209az)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 28. After purification, the antisense oligonucleotide was received with a purity of 90.5%. ESI-MS: experimental: 5442.9 Da; calculated: 5443.3 Da.

Example 34

$Gb^1sTb^1sAb^1sGb^1sdTsdGsdTsdTsdTsdAsdGsdGsGb^1sAb^1sGb^1sC*b^1$ (Seq. ID No. 209ba)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 28. After purification, the antisense oligonucleotide was received with a purity of 89.4%. ESI-MS: experimental: 5469.9 Da; calculated: 5471.3 Da.

Example 35

$Gb^1sTb^1sAb^1sdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsdAsGb^1sC*b^1$ (Seq. ID No. 209bb)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 28. After purification, the antisense oligonucleotide was received with a purity of 88.4%. ESI-MS: experimental: 5386.5 Da; calculated: 5387.3 Da.

Example 36

$Gb^1Tb^1dAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsAb^1Gb^1C*b^1$ (Seq. ID No. 209s)

The compound was synthesized according to the pocedure as described in example 28 and example 29 with the appropriate DNA, DNA-derivatives and the LNA building units. After purification, the antisense oligonucleotide was received with a purity of 96.8%. ESI-MS: experimental: 5323.30 Da; calculated: 5323.0 Da.

Example 37

$Gb^1sTb^1sdA*sdGsdTsdGsdTsdTsdTsdA*sdGsdGsdGsAb^1sGb^1sC*b^1$ (Seq. ID No. 209t)

The compound was synthesized according to the general procedure and as described in example 28 with the appropriate DNA and LNA building units. After purification, the antisense oligonucleotide was received with a purity of 91.4%. ESI-MS: experimental: 5416.30 Da; calculated: 5417.3 Da.

Example 38

/5SpC3s/$Gb^1sTb^1sdAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsAb^1sGb^1sC*b^1$/3SpC3s/ (Seq. ID No. 209x)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 28, example 30 and example 31. After purification, the antisense oligonucleotide was received with a purity of 95.1%. ESI-MS: experimental: 5696.30 Da; calculated: 5695.5 Da.

Examples 39 - 132

The other oligonucleotides of Table 6 were synthesized according to the general procedure and as shown in the examples. The preparation of the antisense-oligonucleotide including β-D-thio-LNA, α-L-oxy-LNA, β-D-(NH)-LNA, or β-D-(NCH_3)-LNA units were performed in the same way as the antisense-oligonucleotides containing β-D-oxy-LNA units.

Example 133

$Gb^1sC*b^1sTb^1sAb^1sdTsdTsdTsdGsdGsdTsdAsdGsdTsGb^1sTb^1sTb^1$ (Seq. ID No. 210q)

5'-O-DMT-2'-O,4'-C-methylene thymidine-3'-O-succinoyl- linked LCAA CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. After several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene thymidine 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene-N^2-dimethyformamidineguanosine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-deoxythymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl

acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^2-isobutyryl-2'-deoxyguanosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidites (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^6-benzoyl-2'-deoxyadenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-deoxythymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^2-isobutyryl-2'-deoxyguanosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec.,

and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^2-isobutyryl-2'-deoxyguanosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-deoxythymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-deoxythymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl

acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5‘-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2’-deoxythymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5‘-DMT protection group.

The coupling was carried out with 80 µl 5‘-O-DMT-2‘-O,4‘-C-methylene-N^6-benzoyladenosine-3‘-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5‘-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O,4’-C-methylene thymidine 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5‘-DMT protection group.

The coupling was carried out with 80 µl 5‘-O-DMT-2‘-O-,4‘-C-methylene-5-methyl-N^4-benzoylcytidine-3‘-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and

236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene-N^2-dimethyformamidineguanosine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

Upon completion of the solid phase synthesis antisense-oligonucleotides were treated with a 20% diethylamine solution in acetonitrile (Biosolve BV, Valkenswaard, The Netherlands) for 20 min. to remove the cyanoethyl protecting groups on the phosphate backbone.
Subsequently, the antisense-oligonucleotides were cleaved from the solid support and further deprotected using 5 ml concentrated aqueous ammonia for 16 hours at 55 °C. The solid support was separated from the antisense-oligonucleotides by filtration or centrifugation.

Next, the crude antisense-oligonucleotides were purified by anion-exchange high-performance liquid chromatography (HPLC) on an AKTA Explorer System (GE Healthcare, Freiburg, Germany) and a column packed with Source Q15 (GE Helthcare). Buffer A was 10 mM sodium perchlorate, 20 mM Tris, 1 mM EDTA, pH 7.4 and contained 20% acetonitrile and buffer B was the same as buffer A with the exception of 500 mM sodium perchlorate. A gradient of 15%B to 55%B within 32 column volumes (CV) was employed. UV traces at 280 nm were recorded. Appropriate fractions were pooled and precipitated with 3 M NaOAc, pH= 5.2 and 70% ethanol. Finally, the pellet was washed with 70% ethanol.

The antisense oligonucleotide was received with a purity of 87.1%. ESI-MS: experimental: 5384.30 Da; calculated: 5384.3 Da.

Example 134

Gb^{1}C*b^{1}Tb^{1}Ab^{1}dTdTdTdGdGdTdA*dGdT**Gb^{1}Tb^{1}Tb^1** (Seq. ID No. 210r)

The LNA was bound to CPG according to the general procedure. The coupling reaction and capping step were also carried out as described in example 133. After the capping step, the system was flushed out with 800 µl acetonitrile, and 400 µl of 0.02 M Iodine in THF/pyridine/H_2O were inserted to the column for 45 s. The system was flushed after the oxidation step with 24 µl acetonitrile. After purification, the antisense oligonucleotide was received with a purity of 95.3%.

Example 135

/5SpC3s/**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dTsdTsdTsdGsdGsdTsdAsdGsdTs**Gb^{1}sTb^{1}sTb^1** (Seq. ID No. 210v)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 133. But with the exception that after the last nucleotide has been coupled to the oligonucleotide and the subseuqent oxidation and capping steps were carried out, 80 µl of phosphoramidite-C3 (0.07 M) and 236 µl DCI in acetonitrile (0.25 M) were added. The coupling was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile. The subsequent steps were performed as described in example 133. After purification, the antisense oligonucleotide was received with a purity of 93.9%.

Example 136

Gb^{1}sC*b^{1}sTb^{1}sAb^{1}sdTsdTsdTsdGsdGsdTsdAsdGsdTs**Gb^{1}sTb^{1}sTb^1**/3SpC3s/ (Seq. ID No. 210w)

3'-Spacer C3 CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. After several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene-thymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45

sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. The subsequent reactions were performed as described in example 133. After purification, the antisense oligonucleotide was received with a purity of 89.7%.

Example 137

$Gb^1C*b^1Tb^1Ab^1dTsdTsdTsdGsdGsdTsdAsdGsdTsGb^1Tb^1Tb^1$ (Seq. ID No. 210o)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 133 and example 134. After purification, the antisense oligonucleotide was received with a purity of 83.8%. ESI-MS: experimental: 5288.10 Da; calculated: 5287.9 Da.

Example 138

$Gb^1sC*b^1sTb^1sAb^1sdTsdTsdTdGsdGsdTsdA*sdGsdTsGb^1sTb^1sTb^1$ (Seq. ID No. 210p)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 133. After purification, the antisense oligonucleotide was received with a purity of 80.7%. ESI-MS: experimental: 5398.40 Da; calculated: 5399.3 Da.

Example 139

$Gb^1ssC*b^1ssTb^1ss$dAssdTssdTssdTssdGssdGssdTssdA*ssdGssdTss**$Gb^1ssTb^1ssTb^1$** (Seq. ID No. 210af)

5'-O-DMT-2'-O,4'-C-methylene-thymidine-3'-O-succinoyl- linked LCAA CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. After several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 38 µl 5'-O-DMT-2'-O,4'-C-methylene-thymidine-3'-[(β-benzoylmercapto)ethyl]pyrrolidinolthiophosphoramidite (0.15 M) in 10% dichloromethane (v/V) in acetonitrile and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 900 µl of DDTT (0.05 M in pyridine/acetonitrile 4:1 v/v) were inserted to the column for 240 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 38 µl 5'-O-DMT-2'-O,4'-C-methylene-N^2-dimethyformamidineguanosine-3'-[(β-benzoylmercapto)ethyl]pyrrolidinolthiophosphoramidite (0.15 M) in 10% dichloromethane (v/v) in acetonitrile and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 900 µl of DDTT (0.05 M in pyridine/acetonitrile 4:1 v/v) were inserted to the column for 240 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The further elongation of the oligonucleotide was performed in the same way.

Upon completion of the solid phase synthesis, the antisense-oligonucleotides were treated 850 µl ammonia in concentrated ethanol (ammonia/ethanol 3:1 v/v) at 55 °C for 15 - 16h in order to cleave antisense-oligonucleotide from the solid-support and to deprotect the thiol-group.

Next, the crude antisense-oligonucleotide was purified by anion-exchange chromatogtraophy using a Mono Q 10/100 GL column. The buffers were prepared with DEPC-treated water, and their compositions were as follows: Buffer A: 25 mM Tris-HCl, 1 mM EDTA, pH 8.0; Buffer B: 25 mM Tris-HCl, 1 mM EDTA, 1 M NaCl, pH 8.0.

Example 140- 233

The other oligonucleotides of Table 7 were synthesized according to the general procedure and as shown in the examples. The preparation of the antisense-oligonucleotide including β-D-thio-LNA, α-L-oxy-LNA, β-D-(NH)-LNA, or β-D-(NCH_3)-LNA units were performed in the same way as the antisense-oligonucleotides containing β-D-oxy-LNA units.

Example 234

C*b^1sAb^1sTb^1sdGsdAsdAsdTsdGsdGsdAsdCsdCsAb^1sGb^1sTb^1sAb^1 (Seq. ID No. 218b)

<u>5'-O-DMT-2'-O,4'-C-methylene-N^6-benzoyladenosine-3'-O-succinate</u>

5'-O-DMT-2'-O,4'-C-methylene-N^6-benzoyladenosine (500 mg, 0,73 mmol), 95 mg succinic anhydride (0.95 mmol, 1.2 eq.) and 116 mg DMAP (0.95 mmol, 1.2 eq.) were dissolved in 35 ml dichloromethane. The reaction was stirred at room temperature overnight. The reaction solution was washed 2 times with 10 ml NaH_2PO_4 (0.1 M, pH 5.5) and one time with 10 ml brine. The organic phase was dried under anhydrous $NaSO_4$, filtered and concentrated to dryness in vacuo. The hemiester derivative was obtained in 95% yield and was used without further purification for the next step.

5'-O-DMT-2'-O,4'-C-methylene-N^6-benzoyladenosine-3'-O-succinoyl- linked LCAA CPG

70 mg hemiester derivative (90 µmol) was dissolved in 0.3 ml DMF, 11.6 µl DIEA (90 µmol) and pyBOP (90 µmol) were added and mixed together for 1 min. This mixture was combined with LCAA-CPG (500 Å, 80-120 mesh size, 300 mg) in a manual synthesizer and stirred for 1.5 hours at room temperature. The support was filtered off and washed with DMF, DCM and MeOH. After drying, the loading was determined to be 57 µmol/g.

Elongation

5'-O-DMT-2'-O,4'-C-methylene-N^6-benzoxyladenosine-3'-O-succinoyl- linked LCAA CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. After several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene-thymidine 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene-N^2-dimethyformamidineguanosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl

acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene-N^6-benzoyladenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^4-benzoyl-2'-deoxycytidine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^4-benzoyl-2'-deoxycytidine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile.

The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5‘-DMT protection group.

The coupling was carried out with 80 µl 5‘-O-DMT-N^6-benzoyl-2‘-deoxyadenosine-3‘-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5‘-DMT protection group.

The coupling was carried out with 80 µl 5’-O-DMT-N^2-isobutyryl-2’-deoxyguanosine-3’-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5‘-DMT protection group.

The coupling was carried out with 80 µl 5’-O-DMT-N^2-isobutyryl-2’-deoxyguanosine-3’-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5‘-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2’-deoxythymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec.,

and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^6-benzoyl-2'-deoxyadenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^6-benzoyl-2'-deoxyadenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^2-isobutyryl-2'-deoxyguanosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl

acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5‘-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O,4’-C-methylene thymidine 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5‘-DMT protection group.

The coupling was carried out with 80 µl 5‘-O-DMT-2‘-O,4‘-C-methylene-N^6-benzoyladenosine-3‘-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5‘-DMT protection group.

The coupling was carried out with 80 µl 5‘-O-DMT-2‘-O-,4‘-C-methylene-5-methyl-N^4-benzoylcytidine-3‘-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5‘-DMT protection group.

Upon completion of the solid phase synthesis antisense-oligonucleotides were treated with a 20% diethylamine solution in acetonitrile (Biosolve BV, Valkenswaard,

The Netherlands) for 20 min. to remove the cyanoethyl protecting groups on the phosphate backbone.
Subsequently, the antisense-oligonucleotides were cleaved from the solid support and further deprotected using 5 mL concentrated aqueous ammonia for 16 hours at 55°C. The solid support was separated from the antisense-oligonucleotides by filtration or centrifugation.

Next, the crude antisense-oligonucleotides were purified by anion-exchange high-performance liquid chromatography (HPLC) on an AKTA Explorer System (GE Healthcare, Freiburg, Germany) and a column packed with Source Q15 (GE Helthcare). Buffer A was 10 mM sodium perchlorate, 20 mM Tris, 1 mM EDTA, pH 7.4 and contained 20% acetonitrile and buffer B was the same as buffer A with the exception of 500 mM sodium perchlorate. A gradient of 15%B to 55%B within 32 column volumes (CV) was employed. UV traces at 280 nm were recorded. Appropriate fractions were pooled and precipitated with 3 M NaOAc, pH= 5.2 and 70% ethanol. Finally, the pellet was washed with 70% ethanol. The antisense oligonucleotide was received with a purity of 94.8%. ESI-MS: experimental: 5365.80 Da; calculated: 5365.30 Da.

Example 235

C*b^{1}Ab^{1}Tb^{1}dGdAdAdTdGdGdAdCdCAb^{1}Gb^{1}Tb^{1}Ab^1 (Seq. ID No. 218r)

The LNA was bound to CPG according the general procedure. The coupling reaction and capping step were also carried out as described in example 234. After the capping step, the system was flushed out with 800 µl acetonitrile, and 400 µl of 0.02 M Iodine in THF/pyridine/H_2O were inserted to the column for 45 s. The system was flushed after the oxidation step with 24 µl acetonitrile. After purification, the antisense oligonucleotide was received with a purity of 97.8%. ESI-MS: experimental: 5125.10 Da.; calculated: 5124.4 Da.

Example 236

/5SpC3s/**C*b^{1}sAb^{1}sTb^{1}s**dGsdAsdAsdTsdGsdGsdAsdCsdCs**Ab^{1}sGb^{1}sTb^{1}sAb^1**

(Seq. ID No. 218t)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 234. But with the exception that after the last nucleotide has been coupled to the oligonucleotide and the subsequent oxidation and capping steps were carried out, 80 µl of phosphoramidite-C3 (0.07 M) and 236 µl DCI in acetonitrile (0.25 M) were added. The coupling was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile. The subsequent steps were performed as

described in example 234. After purification, the antisense oligonucleotide was received with a purity of 94.2%. ESI-MS: experimental: 5519.60 Da; calculated: 5519.4 Da.

Example 237

C*b[1]sAb[1]sTb[1]sdGsdAsdAsdTsdGsdGsdAsdCsdCsAb[1]sGb[1]sTb[1]sAb[1]s/3SpC3/ (Seq. ID No. 218u)

3'-Spacer C3 CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. After several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene-N^6-benzoyladenosine-3'-[(2-cyanoethyl-N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. The subsequent reactions were performed as described in example 234. After purification, the antisense oligonucleotide was received with a purity of 94.3%. ESI-MS: experimental: 5519.10 Da; calculated: 5519.4 Da.

Example 238

C*b[1]ssAb[1]ssTb[1]ssdGssdAssdAssdTssdGssdGssdAssdCssdCss**Ab[1]ssGb[1]ssTb[1]ssAb[1]** (Seq. ID No. 218aa)

5'-O-DMT-2'-O,4'-C-methylene-N^6-benzoyladenosine-3'-O-succinoyl- linked LCAA CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. After several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 38 µl 5'-O-DMT-2'-O,4'-C-methylene-thymidine-3'-[(β-benzoylmercapto)ethyl]pyrrolidinolthiophosphoramidite (0.15 M) in 10% dichloromethane (v/V) in acetonitrile and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 900 µl of DDTT (0.05 M in pyridine/acetonitrile 4:1 v/v) were inserted to the column for 240 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in

THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 38 µl 5'-O-DMT-2'-O,4'-C-methylene-N^2-dimethyformamidineguanosine-3'-[(β-benzoylmercapto)ethyl]pyrrolidinolthiophosphoramidite (0.15 M) in 10% dichloromethane (v/v) in acetonitrile and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 900 µl of DDTT (0.05 M in pyridine/acetonitrile 4:1 v/v) were inserted to the column for 240 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The further elongation of the oligonucleotide was performed in the same way.

Upon completion of the solid phase synthesis, the antisense-oligonucleotides were treated 850 µl ammonia in concentrated ethanol (ammonia/ethanol 3:1 v/v) at 55 °C for 15 - 16h in order to cleave antisense-oligonucleotide from the solid-support and to deprotect the thiol-group.

Next, the crude antisense-oligonucleotide was purified by anion-exchange chromatogtraophy using a Mono Q 10/100 GL column. The buffers were prepared with DEPC-treated water, and their compositions were as follows: Buffer A: 25 mM Tris-HCl, 1 mM EDTA, pH 8.0; Buffer B: 25 mM Tris-HCl, 1 mM EDTA, 1 M NaCl, pH 8.0.

Example 239

C*b^{1}sAb^{1}sTb^{1}sdGsdAsdAsdTsdGsdGsdAsdC*sdC*sAb^{1}sGb^{1}sTb^{1}sAb^1

(Seq. ID No. 218m)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 234. After purification, the antisense oligonucleotide was received with a purity of 93.8%. ESI-MS: experimental: 5394.00 Da; calculated: 5393.3 Da.

Example 240

C*b[1]Ab[1]Tb[1]dGsdAsdAsdTsdGsdGsdAsdC*sdC*sAb[1]Gb[1]Tb[1]Ab[1] (Seq. ID No. 218n)

The compound was synthesized according to the general procedure with the appropriate DNA building units and LNA building units as exemplified in example 234 and example 235. After purification, the antisense oligonucleotide was received with a purity of 94.7%. ESI-MS: experimental: 5297.30 Da; calculated: 5297.0 Da.

Example 241

C*b[1]sAb[1]sTb[1]sdGsdA*sdA*sdTsdGsdGsdA*sdCsdCsAb[1]sGb[1]sTb[1]sAb[1] (Seq. ID No. 218o)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 234. After purification, the antisense oligonucleotide was received with a purity of 92.8%. ESI-MS: experimental: 5410.40 Da; calculated: 5410.3 Da.

Example 242

C*b[1]sAb[1]sTb[1]sdGsdA*sdA*sdTsdGsdGsdA*sdC*sdC*sAb[1]sGb[1]sTb[1]sAb[1]
(Seq. ID. No. 218p)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 234. After purification, the antisense oligonucleotide was received with a purity of 95.3%. ESI-MS: experimental: 5437.40 Da; calculated: 5438.4 Da.

Example 243

C*b[1]sAb[1]sTb[1]sdGsdAsdAsdTsdGsdGsdAsdC*sdCsAbsGb[1]sTb[1]sAb[1] (Seq. ID No. 218q)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 234. After purification, the antisense oligonucleotide was received with a purity of 93.9%. ESI-MS: experimental: 5378.80 Da; calculated: 5379.3 Da.

Example 244

C*b[1]sAb[1]sTb[1]sdGsdAsdAsdTsdGsdGsdAsdCsdC*sAb[1]sGb[1]sTb[1]sAb[1] (Seq. ID No. 218c)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 234. After purification, the antisense oligonucleotide was received with a purity of 92.9%. ESI-MS: experimental: 5379.10 Da ; calculated: 5379.3 Da.

Example 245

C*b^{1}sAb^{1}sTb^{1}sdGdAdAdTdGdGdAdC*sdC*sAb^{1}sGb^{1}sTb^{1}sAb^1 (Seq. ID No. 218s)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 234. After purification, the antisense oligonucleotide was received with a purity of 94.5%. ESI-MS: experimental: 5152.70 Da; calculated: 5152.4 Da.

Example 246

/5SpC3/sC*b^{1}sAb^{1}sTb^{1}sdGsdAsdAsdTsdGsdGsdAsdCsdCsAb^{1}sGb^{1}sTb^{1}sAb^{1}s/3SpC3/ (Seq. ID No. 218v)

The compound was synthesized according to the general procedure with the appropriate DNA building units and LNA building units as exemplified in example 234, example 236 and example 237. After purification, the antisense oligonucleotide was received with a purity of 94.4%. ESI-MS: experimental: 5673.50 Da; calculated: 5673.5 Da

Example 247 - 335

The other oligonucleotides of Table 8 were synthesized according to the general procedure and as shown in the examples. The preparation of the antisense-oligonucleotide including β-D-thio-LNA, α-L-oxy-LNA, β-D-(NH)-LNA, or β-D-(NCH_3)-LNA units were performed in the same way as the antisense-oligonucleotides containing β-D-oxy-LNA units.

Example 336

C*b^{1}sGb^{1}sAb^{1}sTb^{1}sdAsdCsdGsdCsdGsdTsdCsdCsAb^{1}sC*b^{1}sAb^1 (Seq. ID No. 152h)

5'-O-DMT-2'-O,4'-C-methylene-N^6-benzoyladenosine-3'-O-succinoyl- linked LCAA CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. After several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 80 µl 5'-O-DMT-2'-O-,4'-C-methylene-5-methyl-N^4-benzoylcytidine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl

N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene-N^6-benzoyladenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^4-benzoyl-2'-deoxycytidine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^4-benzoyl-2'-deoxycytidine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s. The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-deoxythymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^2-isobutyryl-2'-deoxyguanosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^4-benzoyl-2'-deoxycytidine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^2-isobutyryl-2'-deoxyguanosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9

v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^4-benzoyl-2'-deoxycytidine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^6-benzoyl-2'-deoxyadenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene thymidine 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5‘-O-DMT-2‘-O,4‘-C-methylene-N^6-benzoyladenosine-3‘-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5‘-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O,4’-C-methylene-N^2-dimethyformamidineguanosine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5‘-DMT protection group.

The coupling was carried out with 80 µl 5‘-O-DMT-2‘-O-,4‘-C-methylene-5-methyl-N^4-benzoylcytidine-3‘-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5‘-DMT protection group.

Upon completion of the solid phase synthesis antisense-oligonucleotides were treated with a 20% diethylamine solution in acetonitrile (Biosolve BV, Valkenswaard, The Netherlands) for 20 min. to remove the cyanoethyl protecting groups on the phosphate backbone.

Subsequently, the antisense-oligonucleotides were cleaved from the solid support and further deprotected using 5 mL concentrated aqueous ammonia for 16 hours at 55°C. The solid support was separated from the antisense-oligonucleotides by filtration or centrifugation.

Next, the crude antisense-oligonucleotides were purified by anion-exchange high-performance liquid chromatography (HPLC) on an AKTA Explorer System (GE Healthcare, Freiburg, Germany) and a column packed with Source Q15 (GE Helthcare). Buffer A was 10 mM sodium perchlorate, 20 mM Tris, 1 mM EDTA, pH 7.4 and contained 20% acetonitrile and buffer B was the same as buffer A with the exception of 500 mM sodium perchlorate. A gradient of 15%B to 55%B within 32 column volumes (CV) was employed. UV traces at 280 nm were recorded. Appropriate fractions were pooled and precipitated with 3 M NaOAc, pH= 5.2 and 70% ethanol. Finally, the pellet was washed with 70% ethanol.

Example 337

C*b^{1}Gb^{1}Ab^{1}Tb^{1}dAdCdGdC*dGdTdCdC***Ab^{1}C*b^{1}Ab^1** (Seq. ID No. 152q)

The LNA was bound to CPG according to the general procedure. The coupling reaction and capping step were also carried out as described in example 336. After the coupling step, the system was flushed out with 800 µl acetonitrile, and 400 µl of 0.02 M Iodine in THF/pyridine/H_2O were inserted to the column for 45 s. After the oxidation step, the system was flushed with 24 µl acetonitrile. After purification, the antisense oligonucleotide was received with a purity of 93.1%.

Example 338

/5SpC3s/C*b^{1}sGb^{1}sAb^{1}sTb^{1}sdAsdC*sdGsdC*sdGsdTsdCsdCs**Ab^{1}sC*b^{1}sAb^1**
(Seq. ID. No. 152s)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 336. But with the exception that after the last nucleotide has been coupled to the oligonucleotide and the subseuqent oxidation and capping steps were carried out, 80 µl of phosphoramidite-C3 (0.07 M) and 236 µl DCI in acetonitrile (0.25 M) were added. The coupling was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile. The subsequent steps were performed as described in example 336. After purification, the antisense oligonucleotide was received with a purity of 96.5%.

Example 339

C*b^{1}sGb^{1}sAb^{1}sTb^{1}sdAsdC*sdGsdCsdGsdTsdCsdC*s**Ab^{1}sC*b^{1}sAb^1/3SpC3s/**
(Seq. ID No. 152t)

3'-Spacer C3 CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. After several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene-N^6-benzoyladenosine-3'-[(2-cyanoethyl-N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. The subsequent reactions were performed as described in example 336. After purification, the antisense oligonucleotide was received with a purity of 92.1%.

Example 340

C*b^{1}ssGb^{1}ssAb^{1}ssdTssdAssdC*ssdGssdCssdGssdTssdCssdC*ss**Ab^{1}ssC*b^{1}ssAb^1**
(Seq. ID No. 152aa)

5'-O-DMT-2'-O,4'-C-methylene-N^6-benzoyladenosine-3'-O-succinoyl- linked LCAA CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. After several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 38 µl 5'-O-DMT-2'-O-,4'-C-methylene-5-methyl-N^4-benzoylcytidine-3'-[(β-benzoylmercapto)ethyl]pyrrolidinolthiophosphoramidite (0.15 M) in 10% dichloromethane (v/V) in acetonitrile and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 900 µl of DDTT (0.05 M in pyridine/acetonitrile 4:1 v/v) were inserted to the column for 240 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 38 µl 5'-O-DMT-2'-O,4'-C-methylene-N^6-benzoyladenosine-3'-[(β-benzoylmercapto)ethyl]pyrrolidinolthiophosphoramidite (0.15 M) in 10% dichloromethane (v/v) in acetonitrile and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 900 µl of DDTT (0.05 M in pyridine/acetonitrile 4:1 v/v) were inserted to the column for 240 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The further elongation of the oligonucleotide was performed in the same way.

Upon completion of the solid phase synthesis, the antisense-oligonucleotides were treated 850 µl ammonia in concentrated ethanol (ammonia/ethanol 3:1 v/v) at 55 °C for 15 - 16h in order to cleave antisense-oligonucleotide from the solid-support and to deprotect the thiol-group.

Next, the crude antisense-oligonucleotide was purified by anion-exchange chromatogtraophy using a Mono Q 10/100 GL column. The buffers were prepared with DEPC-treated water, and their compositions were as follows: Buffer A: 25 mM Tris-HCl, 1 mM EDTA, pH 8.0; Buffer B: 25 mM Tris-HCl, 1 mM EDTA, 1 M NaCl, pH 8.0.

Example 341 - 433

The other oligonucleotides of Table 5 were synthesized according to the general procedure and as shown in the examples. The preparation of the antisense-oligonucleotide including β-D-thio-LNA, α-L-oxy-LNA, β-D-(NH)-LNA, or β-D-(NCH_3)-LNA units were performed in the same way as the antisense-oligonucleotides containing β-D-oxy-LNA units.

Example 434

C*b^1sTb^1sdCsdGsdTsdCsdAsdTsdAsdGsdAsC*b^1sC*b^1sGb^1 (Seq. ID No. 143h)

5'-O-DMT-2'-O,4'-C-methylene-N^2-dimethyformamidineguanosine -3'-O-succinoyl-linked LCAA CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in

dichloromethane for 60 s to completely remove the 5'-DMT protection group. After several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 80 µl 5'-O-DMT-2'-O-,4'-C-methylene-5-methyl-N^4-benzoylcytidine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O-,4'-C-methylene-5-methyl-N^4-benzoylcytidine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^6-benzoyl-2'-deoxyadenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^2-isobutyryl-2'-deoxyguanosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of

Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^6-benzoyl-2'-deoxyadenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-deoxythymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^6-benzoyl-2'-deoxyadenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^4-benzoyl-2'-deoxycytidine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-deoxythymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^2-isobutyryl-2'-deoxyguanosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^4-benzoyl-2'-deoxycytidine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl

acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene thymidine 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O-,4'-C-methylene-5-methyl-N^4-benzoylcytidine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

Upon completion of the solid phase synthesis antisense-oligonucleotides were treated with a 20% diethylamine solution in acetonitrile (Biosolve BV, Valkenswaard, The Netherlands) for 20 min. to remove the cyanoethyl protecting groups on the phosphate backbone.
Subsequently, the antisense-oligonucleotides were cleaved from the solid support and further deprotected using 5 mL concentrated aqueous ammonia for 16 hours at 55°C. The solid support was separated from the antisense-oligonucleotides by filtration or centrifugation.

Next, the crude antisense-oligonucleotides were purified by anion-exchange high-performance liquid chromatography (HPLC) on an AKTA Explorer System (GE

Healthcare, Freiburg, Germany) and a column packed with Source Q15 (GE Helthcare). Buffer A was 10 mM sodium perchlorate, 20 mM Tris, 1 mM EDTA, pH 7.4 and contained 20% acetonitrile and buffer B was the same as buffer A with the exception of 500 mM sodium perchlorate. A gradient of 15%B to 55%B within 32 column volumes (CV) was employed. UV traces at 280 nm were recorded. Appropriate fractions were pooled and precipitated with 3 M NaOAc, pH= 5.2 and 70% ethanol. Finally, the pellet was washed with 70% ethanol.

Example 435

C*b^{1}Tb^{1}dC*dGdTdCdAdTdAdGdA**C*b^{1}C*b^{1}Gb^1** (Seq. ID No. 143ad)

The LNA was bound to CPG according to the general procedure. The coupling reaction and capping step were also carried out as described in example 434. After the capping step, the system was flushed out with 800 µl acetonitrile, and 400 µl of 0.02 M Iodine in THF/pyridine/H_2O were inserted to the column for 45 s. The system was flushed after the oxidation step with 24 µl acetonitrile. After purification, the antisense oligonucleotide was received with a purity of 88.7%.

Example 436

/5SpC3s/C*b^{1}sTb^{1}sdC*dGdTdC*dA*dTdAdGdA*s**C*b^{1}sC*b^{1}sGb^1** (Seq. ID No. 143af)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 434 and example 435. But with the exception that after the last nucleotide has been coupled to the oligonucleotide and the subsequent oxidation and capping steps were carried out, 80 µl of phosphoramidite-C3 (0.07 M) and 236 µl DCI in acetonitrile (0.25 M) were added. The coupling was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile. The subsequent steps were performed as described in example 434 and example 435. After purification, the antisense oligonucleotide was received with a purity of 94.4%.

Example 437

C*b^{1}sTb^{1}sdC*dGdTdC*dA*dTdAdGdA*s**C*b^{1}sC*b^{1}sGb^1/3SpC3s/** (Seq. ID No. 143ag)

3'-Spacer C3 CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. After several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene-N^2-diemthyformamidineguanosine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed

to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. The subsequent reactions were performed as described in example 434 and example 435. After purification, the antisense oligonucleotide was received with a purity of 91.6%.

Example 438

C*b[1]ssTb[1]ssC*b[1]ssdGssdTssdC*ssdAssdTssdAssdGssdAss**C*b[1]ssC*b[1]ssGb[1]**

(Seq. ID No. 143t)

5'-O-DMT-2'-O,4'-C-methylene-N^2-dimethyformamidineguanosine-3'-O-succinoyl-linked LCAA CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. After several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 38 µl 5'-O-DMT-2'-O-,4'-C-methylene-5-methyl-N^4-benzoylcytidine-3'-[(β-benzoylmercapto)ethyl]pyrrolidinolthiophosphoramidite (0.15 M) in 10% dichloromethane (v/V) in acetonitrile and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 900 µl of DDTT (0.05 M in pyridine/acetonitrile 4:1 v/v) were inserted to the column for 240 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 38 µl 5'-O-DMT-2'-O-,4'-C-methylene-5-methyl-N^4-benzoylcytidine-3'-[(β-benzoylmercapto)ethyl]pyrrolidinolthiophosphoramidite (0.15 M) in 10% dichloromethane (v/v) in acetonitrile and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 900 µl of DDTT (0.05 M in pyridine/acetonitrile 4:1 v/v) were inserted to the column for 240 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed

with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The further elongation of the oligonucleotide was performed in the same way.

Upon completion of the solid phase synthesis, the antisense-oligonucleotides were treated 850 µl ammonia in concentrated ethanol (ammonia/ethanol 3:1 v/v) at 55 °C for 15 - 16h in order to cleave antisense-oligonucleotide from the solid-support and to deprotect the thiol-group.

Next, the crude antisense-oligonucleotide was purified by anion-exchange chromatogtraophy using a Mono Q 10/100 GL column. The buffers were prepared with DEPC-treated water, and their compositions were as follows: Buffer A: 25 mM Tris-HCl, 1 mM EDTA, pH 8.0; Buffer B: 25 mM Tris-HCl, 1 mM EDTA, 1 M NaCl, pH 8.0.

Example 439 - 534

The other oligonucleotides of Table 4 were synthesized according to the general procedure and as shown in the examples. The preparation of the antisense-oligonucleotide including β-D-thio-LNA, α-L-oxy-LNA, β-D-(NH)-LNA, or β-D-(NCH_3)-LNA units were performed in the same way as the antisense-oligonucleotides containing β-D-oxy-LNA units.

Example 535

C*b^{1}sAb^{1}sGb^{1}sdGsdCsdAsdTsdTsdAsdAsdTsdAsdAsdAsGb^{1}sTb^{1}sGb^1 (Seq. ID No. 213k)

5'-O-DMT-2'-O,4'-C-methylene-N^2-dimethyformamidineguanosine-3'-O-succinoyl-linked LCAA CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. After several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene thymidine 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The

compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene-N^2-dimethyformamidineguanosine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^6-benzoyl-2'-deoxyadenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^6-benzoyl-2'-deoxyadenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^6-benzoyl-2'-deoxyadenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in

acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-deoxythymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^6-benzoyl-2'-deoxyadenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^6-benzoyl-2'-deoxyadenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl

acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-deoxythymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-deoxythymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^6-benzoyl-2'-deoxyadenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^4-benzoyl-2'-deoxycytidine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess

reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-N^2-isobutyryl-2'-deoxyguanosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene-N^2-dimethyformamidineguanosine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene-N^6-benzoyladenosine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s. The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end

of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 80 µl 5'-O-DMT-2'-O-,4'-C-methylene-5-methyl-N^4-benzoylcytidine-3'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

Upon completion of the solid phase synthesis antisense-oligonucleotides were treated with a 20% diethylamine solution in acetonitrile (Biosolve BV, Valkenswaard, The Netherlands) for 20 min. to remove the cyanoethyl protecting groups on the phosphate backbone.
Subsequently, the antisense-oligonucleotides were cleaved from the solid support and further deprotected using 5 mL concentrated aqueous ammonia for 16 hours at 55°C. The solid support was separated from the antisense-oligonucleotides by filtration or centrifugation.

Next, the crude antisense-oligonucleotides were purified by anion-exchange high-performance liquid chromatography (HPLC) on an AKTA Explorer System (GE Healthcare, Freiburg, Germany) and a column packed with Source Q15 (GE Helthcare). Buffer A was 10 mM sodium perchlorate, 20 mM Tris, 1 mM EDTA, pH 7.4 and contained 20% acetonitrile and buffer B was the same as buffer A with the exception of 500 mM sodium perchlorate. A gradient of 15%B to 55%B within 32 column volumes (CV) was employed. UV traces at 280 nm were recorded. Appropriate fractions were pooled and precipitated with 3 M NaOAc, pH= 5.2 and 70% ethanol. Finally, the pellet was washed with 70% ethanol.

Example 536

C*b^{1}Ab^{1}Gb^{1}dGdC*dAdTdTdAdAdTdAdAdA**Gb^{1}Tb^{1}Gb^1** (Seq. ID No. 213n)

The LNA was bound to CPG according to general procedure. The coupling reaction and capping step were also carried out as described in example 535. After the

ccapping step, the system was flushed out with 800 µl acetonitrile, and 400 µl of 0.02 M Iodine in THF/pyridine/H_2O were inserted to the column for 45 s. The system was flushed after the oxidation step with 24 µl acetonitrile. After purification, the antisense oligonucleotide was received with a purity of 91.4%.

Example 537

/5SpC3s/C*b^{1}sAb^{1}sGb^{1}sdGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^1**
(Seq. ID No. 213o)

The compound was synthesized according to the general procedure with the appropriate DNA and LNA building units as exemplified in example 535. But with the exception that after the last nucleotide has been coupled to the oligonucleotide and the subseuqent oxidation and capping steps were carried out, 80 µl of phosphoramidite-C3 (0.07 M) and 236 µl DCI in acetonitrile (0.25 M) were added. The coupling was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile. The subsequent steps were performed as described in example 535. After purification, the antisense oligonucleotide was received with a purity of 87.1%.

Example 538

C*b^{1}sAb^{1}sGb^{1}sdGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^1/3SpC3s/**
(Seq. ID No. 213p)

3'-Spacer C3 CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. After several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 80 µl 5'-O-DMT-2'-O,4'-C-methylene-N^2-diemthyformamidineguanosine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (0.07 M) and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 640 µl of Beaucage (0.2 M) were inserted to the column for 180 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. The subsequent reactions were performed as described in example 535. After purification, the antisense oligonucleotide was received with a purity of 95.7%.

Example 539
C*b[1]ssAb[1]ssGb[1]ssdGssdC*ssdAssdTssdTssdAssdAssdTssdAssdAss**Ab[1]ssGb[1]ssTb[1]ssGb[1]** (Seq. ID No. 213ae)

5'-O-DMT-2'-O,4'-C-methylene-N^2-dimethyformamidineguanosine-3'-O-succinoyl-linked LCAA CPG (0.2 µmol) was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group. After several washes with a total amount of 800 µl acetonitrile, the coupling reaction was carried out with 38 µl 5'-O-DMT-2'-O,4'-C-methylene-thymidine-3'-[(β-benzoylmercapto)ethyl]pyrrolidinolthiophosphoramidite (0.15 M) in 10% dichloromethane (v/V) in acetonitrile and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 900 µl of DDTT (0.05 M in pyridine/acetonitrile 4:1 v/v) were inserted to the column for 240 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The coupling was carried out with 38 µl 5'-O-DMT-2'-O,4'-C-methylene-N^2-dimethyformamidineguanosine-3'-[(β-benzoylmercapto)ethyl]pyrrolidinolthiophosphoramidite (0.15 M) in 10% dichloromethane (v/v) in acetonitrile and 236 µl DCI in acetonitrile (0.25 M). The coupling reaction was allowed to take place for 250 sec., and excess reagents were flashed out with 800 µl acetonitrile, and 900 µl of DDTT (0.05 M in pyridine/acetonitrile 4:1 v/v) were inserted to the column for 240 s The system was flushed with 320 µl acetonitrile. For the capping step, 448 µl of acetic anhydride in THF (1:9 v/v) and 448 µl N-methylimidazol (NMI)/THF/pyridine (1:8:1) were added and allowed to react for 45 sec. At the end of this cycle, the system was washed with 480 µl acetonitrile. The compound was treated with 1400 µl 3% trichloroacetic acid in dichloromethane for 60 s to completely remove the 5'-DMT protection group.

The further elongation of the oligonucleotide was performed in the same way.

Upon completion of the solid phase synthesis, the antisense-oligonucleotides were treated 850 µl ammonia in concentrated ethanol (ammonia/ethanol 3:1 v/v) at 55 °C for 15 - 16h in order to cleave antisense-oligonucleotide from the solid-support and to deprotect the thiol-group.

Next, the crude antisense-oligonucleotide was purified by anion-exchange chromatogtraophy using a Mono Q 10/100 GL column. The buffers were prepared with DEPC-treated water, and their compositions were as follows: Buffer A: 25 mM Tris-HCl, 1 mM EDTA, pH 8.0; Buffer B: 25 mM Tris-HCl, 1 mM EDTA, 1 M NaCl, pH 8.0.

Examples 540 - 640

The other oligonucleotides of Table 9 were synthesized according to the general procedure and as shown in the examples. The preparation of the antisense-oligonucleotide including β-D-thio-LNA, α-L-oxy-LNA, β-D-(NH)-LNA, or β-D-(NCH_3)-LNA units were performed in the same way as the antisense-oligonucleotides containing β-D-oxy-LNA units.

Sequence Listing

Seq. ID No. 1: Homo sapiens transforming growth factor, beta receptor II (TGFBR2), transcript variant 2, (antisense; DNA code)

TTTAGCTACT AGGAATGGGA ACAGGAGGCA GGATGCTCAC CTGAGTATTT TGCTTTATTC 60
AATCTAATAA ACATTTTATT TATGTAAAAG ACAAACAATG CATAGAATAA AAATAAGTGC 120
TTGAGACTTT TGATATAAAA AGAGTATATA GCATTCACAT TCCTATTTTA ATACATGAGT 180
ACAGCTGAAG TGTTCCATAA AAGAATAAAA CTTTCCCTTT ATGTATAGTA GTGAAAAAAG 240
TCAGTATTTT TAGGAACTAC AGAATGTTAT TCCTTGGTCT TTTTTCTTGA ATAAGAAAAA 300
AAAACATAAA CAAAACAAGC CACAGTATCC TCTGACACTA CATTCCAGTT TATGCTGATA 360
ACCCAGAAGT GAGAATACTC TTGAATCTTG AATATCTCAT GAATGGACCA GTATTCTAGA 420
AACTCACCAC TAGAGGTCAA TGGGCAACAG CTATTGGGAT GGTATCAGCA TGCCCTACGG 480
TGCAAGTGGA ATTTCTAGGC GCCTCTATGC TACTGCAGCC ACACTGTCTT TAACTCTCAG 540
CCCACCCACA CTGAGGAGGG TGCCTAGAGG TTCTATTTCC AAACCTTTGC ATGTATCTTA 600
AAAATCTCAA TAAAATGAGA CCTTCCACCA TCCAAACAGA GCTGATATTC TCACTACCAG 660
TCCCTCTCTA ATATTCCTAT TTGGCTGAAA ATAAGTAGCT TCAAAAAGTT TTAAAAAAGA 720
GATTACTTGC AGCATTAACA CTTCTTTGTT GATTAACAAG TTTCCTATGG AGTTTTAAAG 780
CTCATACTTT GTTCTTGTCC TTGTGGACAC AAATTTTCTA ACTGCAAATG GGACCTTTGT 840
GTCCCACATT CAAATCCTCT CTAGTAATTT CTGCAAAGGT TGAGAAGGCT GGCATGATGG 900
AGAGAACGGT AACCATGAGG AAAGCTTCTT GGAGTAAAGC ACTCCTCTCT CCAATGCAGA 960
GGGTAAAACT ATTAACATAT AAGCAAAAGA AACTTGGGCT AACTGAGACC CTTAAAGGAG 1020
TTCCCCTTTA GTCCAATAAA AGGCCAACTT CAAATCTTAA CACCAGATAA GGTAGTCAAA 1080
ATCATATTAT ATACCCAGAG AATGACTGCT TGAATGGACA TTTCTTACAA GGGACCTTGG 1140
TTAGGTGCAG ATTTAATTCC TAGACTGGGG TCCAGGTAGG CAGTGGAAAG AGCTAATGTT 1200
TACAGTGAGA AGTGAGGCAG CTTTGTAAGT GTCTCCACAC CTTCACATTT TGTGAACGTG 1260
GACTGGAGAT AACTGAAAAC CATCTGCTAT CCTTACCTGG GGATCCAGAT TTTCCTGCAA 1320
AATCTCCAAA TATTTATAAA GTGGCTTCAC TTTTTGAAAC GCTGTGCTGA CCAAACAAAA 1380
CATATGTTTA GAGTGCCTGA GGTCATAGTC CTGACAATGA TAGTATTGTG TAGTTGAAAT 1440
CCTCTTCATC AGGCCAAACT GTGCTTGAGC AATCAGGAGC CCAGAAAGAT GGAACCCATT 1500
GGTGTTTGTA TAGAAAACTA GAAAATCAAG TCAAGTGTAA TGAAAAAGTA AACACGATAA 1560
AGCCTAGAGT GAGAATTTGC TCCTTTTTAG AAAAGGATGA AGGCTGGGAG CAGAGAATAG 1620
TAACATAAGT GCAGGGGAAA GATGAAAAAA AGAACAATTT TTCATTAGTA GATGGTGGGG 1680
CAATCGCATG GATGGGGACA TCTGTTCTGA TTTTTCTGCA ACCCATGAAG GTAAAAAGTG 1740
GGGTTCAAAA CATTCAAGGT ATTAAAGATG GGGTAGAGTT TCTAAACTAG GTTGAGGGAG 1800
AGTTTCTAAA CTAGCCCCCC AGATTTGGGG CTTGGAGCTT AAATGAAAAG TCCAGGAGAA 1860
ATAAGGGCAC ACAGGAACCC CGGGAACACT GGTCCTCAAA CAGTGCCACT GTACTTAGTT 1920
CCATGGCCAG AAGAGAAGTG CTAGGCAGGG AATGATTATT TTGCAAAAGC AAGTGCAATG 1980
TGGTCATAGC TGGCTGTGAG ACATGGAGCC TCTTTCCTCA TGCAAAGTTC ACTGTTTTAC 2040
AGTCAGAGAA CCACTGCATG TGTGATTGTC AAATGCTAAT GCTGTCATGG GTCCCTTCCT 2100
TCTCTGCTTG GTTCTGGAGT TCTCCAATAA AACCAATTTC CTGGGAATAT TTGATGTTTT 2160

TCCTTGTCTC TTTTCAAGGT ATGGCTATAT ATATAGAGCT ATAGACATAT ATAGATATAT 2220
ATATATATAT ATAAAACATA GCTATTCATA TTTATATACA GGCATTAATA AAGTGCAAAT 2280
GTTATTGGCT ATTGTAAAAA TCAATCTCAT TTCCTGAGGA AGTGCTAACA CAGCTTATCC 2340
TATGACAATG TCAAAGGCAT AGAATGCTCT ATGTCACCCA CTCCCTGCTG CTGTTGTTTC 2400
TGCTTATCCC CACAGCTTAC AGGGAGGGGA GTGACCCCCT TGGTTTTCCA GGAAGCATCA 2460
GTTCAGGGGC AGCTTCCTGC TGCCTCTGTT CTTTGGTGAG AGGGGCAGCC TCTTTGGACA 2520
TGGCCCAGCC TGCCCCAGAA GAGCTATTTG GTAGTGTTTA GGGAGCCGTC TTCAGGAATC 2580
TTCTCCTCCG AGCAGCTCCT CCCCGAGAGC CTGTCCAGAT GCTCCAGCTC ACTGAAGCGT 2640
TCTGCCACAC ACTGGGCTGT GAGACGGGCC TCTGGGTCGT GGTCCCAGCA CTCAGTCAAC 2700
GTCTCACACA CCATCTGGAT GCCCTGGTGG TTGAGCCAGA AGCTGGGAAT TTCTGGTCGC 2760
CCTCGATCTC TCAACACGTT GTCCTTCATG CTTTCGACAC AGGGGTGCTC CCGCACCTTG 2820
GAACCAAATG GAGGCTCATA ATCTTTTACT TCTCCCACTG CATTACAGCG AGATGTCATT 2880
TCCCAGAGCA CCAGAGCCAT GGAGTAGACA TCGGTCTGCT TGAAGGACTC AACATTCTCC 2940
AAATTCATCC TGGATTCTAG GACTTCTGGA GCCATGTATC TTGCAGTTCC CACCTGCCCA 3000
CTGTTAGCCA GGTCATCCAC AGACAGAGTA GGGTCCAGAC GCAGGGAAAG CCCAAAGTCA 3060
CACAGGCAGC AGGTTAGGTC GTTCTTCACG AGGATATTGG AGCTCTTGAG GTCCCTGTGC 3120
ACGATGGGCA TCTTGGGCCT CCCACATGGA GTGTGATCAC TGTGGAGGTG AGCAATCCCC 3180
CGGGCGAGGG AGCTGCCCAG CTTGCGCAGG TCCTCCCAGC TGATGACATG CCGCGTCAGG 3240
TACTCCTGTA GGTTGCCCTT GGCGTGGAAG GCGGTGATCA GCCAGTATTG TTTCCCCAAC 3300
TCCGTCTTCC GCTCCTCAGC CGTCAGGAAC TGGAGTATGT TCTCATGCTT CAGATTGATG 3360
TCTGAGAAGA TGTCCTTCTC TGTCTTCCAA GAGGCATACT CCTCATAGGG AAAGATCTTG 3420
ACTGCCACTG TCTCAAACTG CTCTGAAGTG TTCTGCTTCA GCTTGGCCTT ATAGACCTCA 3480
GCAAAGCGAC CTTTCCCCAC CAGGGTGTCC AGCTCAATGG GCAGCAGCTC TGTGTTGTGG 3540
TTGATGTTGT TGGCACACGT GGAGCTGATG TCAGAGCGGT CATCTTCCAG GATGATGGCA 3600
CAGTGCTCGC TGAACTCCAT GAGCTTCCGC GTCTTGCCGG TTTCCCAGGT TGAACTCAGC 3660
TTCTGCTGCC GGTTAACGCG GTAGCAGTAG AAGATGATGA TGACAGATAT GGCAACTCCC 3720
AGTGGTGGCA GGAGGCTGAT GCCTGTCACT TGAAATATGA CTAGCAACAA GTCAGGATTG 3780
CTGGTGTTAT ATTCTTCTGA GAAGATGATG TTGTCATTGC ACTCATCAGA GCTACAGGAA 3840
CACATGAAGA AAGTCTCACC AGGCTTTTTT TTTTCCTTCA TAATGCACTT TGGAGAAGCA 3900
GCATCTTCCA GAATAAAGTC ATGGTAGGGG AGCTTGGGGT CATGGCAAAC TGTCTCTAGT 3960
GTTATGTTCT CGTCATTCTT TCTCCATACA GCCACACAGA CTTCCTGTGG CTTCTCACAG 4020
ATGGAGGTGA TGCTGCAGTT GCTCATGCAG GATTTCTGGT TGTCACAGGT GGAAAATCTC 4080
ACATCACAAA ATTTACACAG TTGTGGAAAC TTGACTGCAC CGTTGTTGTC AGTGACTATC 4140
ATGTCGTTAT TAACCGACTT CTGAACGTGC GGTGGGATCG TGCTGGCGAT ACGCGTCCAC 4200
AGGACGATGT GCAGCGGCCA CAGGCCCCTG AGCAGCCCCC GACCCATGGC AGACCCCGCT 4260
GCTCGTCATA GACCGAGCCC CCAGCGCAGC GGACGGCGCC TTCCCGGACC CCTGGCTGCG 4320
CCTCCGCGCC GCGCCCTCTC CGGACCCCGC GCCGGGCCGG CAGCGCAGAT GTGCGGGCCA 4380
GATGTGGCGC CCGCTCGCCA GCCAGGAGGG GGCCTGGAGG CCGGCGAGGC GCGGGGAGGC 4440
CCCCGGCGGC CGAGGGAAGC TGCACAGGAG TCCGGCTCCT GTCCCGAGCG GGTGCACGCG 4500
CGGGGGTGTC GTCGCTCCGT GCGCGCGAGT GACTCACTCA ACTTCAACTC AGCGCTGCGG 4560
GGGAAACAGG AAACTCCTCG CCAACAGCTG GGCAGGACCT CTCTCCGCCC GAGAGCCTTC 4620
TCCCTCTCC 4629

Seq. ID No. 2: Homo sapiens transforming growth factor, beta receptor II (TGFBR2), transcript variant 2, mRNA (sense; written in DNA code)

GGAGAGGGAG AAGGCTCTCG GGCGGAGAGA GGTCCTGCCC AGCTGTTGGC GAGGAGTTTC 60
CTGTTTCCCC CGCAGCGCTG AGTTGAAGTT GAGTGAGTCA CTCGCGCGCA CGGAGCGACG 120
ACACCCCCGC GCGTGCACCC GCTCGGGACA GGAGCCGGAC TCCTGTGCAG CTTCCCTCGG 180
CCGCCGGGGG CCTCCCCGCG CCTCGCCGGC CTCCAGGCCC CCTCCTGGCT GGCGAGCGGG 240
CGCCACATCT GGCCCGCACA TCTGCGCTGC CGGCCCGGCG CGGGGTCCGG AGAGGGCGCG 300
GCGCGGAGGC GCAGCCAGGG GTCCGGGAAG GCGCCGTCCG CTGCGCTGGG GGCTCGGTCT 360
ATGACGAGCA GCGGGGTCTG CCATGGGTCG GGGGCTGCTC AGGGGCCTGT GGCCGCTGCA 420
CATCGTCCTG TGGACGCGTA TCGCCAGCAC GATCCCACCG CACGTTCAGA AGTCGGTTAA 480
TAACGACATG ATAGTCACTG ACAACAACGG TGCAGTCAAG TTTCCACAAC TGTGTAAATT 540
TTGTGATGTG AGATTTTCCA CCTGTGACAA CCAGAAATCC TGCATGAGCA ACTGCAGCAT 600
CACCTCCATC TGTGAGAAGC CACAGGAAGT CTGTGTGGCT GTATGGAGAA AGAATGACGA 660
GAACATAACA CTAGAGACAG TTTGCCATGA CCCCAAGCTC CCCTACCATG ACTTTATTCT 720
GGAAGATGCT GCTTCTCCAA AGTGCATTAT GAAGGAAAAA AAAAAGCCTG GTGAGACTTT 780
CTTCATGTGT TCCTGTAGCT CTGATGAGTG CAATGACAAC ATCATCTTCT CAGAAGAATA 840
TAACACCAGC AATCCTGACT TGTTGCTAGT CATATTTCAA GTGACAGGCA TCAGCCTCCT 900
GCCACCACTG GGAGTTGCCA TATCTGTCAT CATCATCTTC TACTGCTACC GCGTTAACCG 960
GCAGCAGAAG CTGAGTTCAA CCTGGGAAAC CGGCAAGACG CGGAAGCTCA TGGAGTTCAG 1020
CGAGCACTGT GCCATCATCC TGGAAGATGA CCGCTCTGAC ATCAGCTCCA CGTGTGCCAA 1080
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ACAGGAGTAC CTGACGCGGC ATGTCATCAG CTGGGAGGAC CTGCGCAAGC TGGGCAGCTC 1440
CCTCGCCCGG GGGATTGCTC ACCTCCACAG TGATCACACT CCATGTGGGA GGCCCAAGAT 1500
GCCCATCGTG CACAGGGACC TCAAGAGCTC CAATATCCTC GTGAAGAACG ACCTAACCTG 1560
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GTGTGAGACG TTGACTGAGT GCTGGGACCA CGACCCAGAG GCCCGTCTCA CAGCCCAGTG 1980
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ATGCGATTGC CCCACCATCT ACTAATGAAA AATTGTTCTT TTTTTCATCT TTCCCCTGCA 3000
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ATGAAGAGGA TTTCAACTAC ACAATACTAT CATTGTCAGG ACTATGACCT CAGGCACTCT 3240
AAACATATGT TTTGTTTGGT CAGCACAGCG TTTCAAAAAG TGAAGCCACT TTATAAATAT 3300
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TCTCCAGTCC ACGTTCACAA AATGTGAAGG TGTGGAGACA CTTACAAAGC TGCCTCACTT 3420
CTCACTGTAA ACATTAGCTC TTTCCACTGC CTACCTGGAC CCCAGTCTAG GAATTAAATC 3480
TGCACCTAAC CAAGGTCCCT TGTAAGAAAT GTCCATTCAA GCAGTCATTC TCTGGGTATA 3540
TAATATGATT TTGACTACCT TATCTGGTGT TAAGATTTGA AGTTGGCCTT TTATTGGACT 3600
AAAGGGGAAC TCCTTTAAGG GTCTCAGTTA GCCCAAGTTT CTTTTGCTTA TATGTTAATA 3660
GTTTTACCCT CTGCATTGGA GAGAGGAGTG CTTTACTCCA AGAAGCTTTC CTCATGGTTA 3720
CCGTTCTCTC CATCATGCCA GCCTTCTCAA CCTTTGCAGA AATTACTAGA GAGGATTTGA 3780
ATGTGGGACA CAAAGGTCCC ATTTGCAGTT AGAAAATTTG TGTCCACAAG GACAAGAACA 3840
AAGTATGAGC TTTAAAACTC CATAGGAAAC TTGTTAATCA ACAAAGAAGT GTTAATGCTG 3900
CAAGTAATCT CTTTTTTAAA ACTTTTTGAA GCTACTTATT TTCAGCCAAA TAGGAATATT 3960
AGAGAGGGAC TGGTAGTGAG AATATCAGCT CTGTTTGGAT GGTGGAAGGT CTCATTTTAT 4020
TGAGATTTTT AAGATACATG CAAAGGTTTG GAAATAGAAC CTCTAGGCAC CCTCCTCAGT 4080
GTGGGTGGGC TGAGAGTTAA AGACAGTGTG GCTGCAGTAG CATAGAGGCG CCTAGAAATT 4140
CCACTTGCAC CGTAGGGCAT GCTGATACCA TCCCAATAGC TGTTGCCCAT TGACCTCTAG 4200
TGGTGAGTTT CTAGAATACT GGTCCATTCA TGAGATATTC AAGATTCAAG AGTATTCTCA 4260
CTTCTGGGTT ATCAGCATAA ACTGGAATGT AGTGTCAGAG GATACTGTGG CTTGTTTTGT 4320
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AAGTCTCAAG CACTTATTTT TATTCTATGC ATTGTTTGTC TTTTACATAA ATAAAATGTT 4560
TATTAGATTG AATAAAGCAA AATACTCAGG TGAGCATCCT GCCTCCTGTT CCCATTCCTA 4620
GTAGCTAAA 4629

Seq. ID No. 3: Homo sapiens transforming growth factor, beta receptor II (TGFBR2), transcript variant 2, mRNA (sense; written in RNA code)

GGAGAGGGAG AAGGCUCUCG GGCGGAGAGA GGUCCUGCCC AGCUGUUGGC GAGGAGUUUC 60
CUGUUUCCCC CGCAGCGCUG AGUUGAAGUU GAGUGAGUCA CUCGCGCGCA CGGAGCGACG 120
ACACCCCCGC GCGUGCACCC GCUCGGGACA GGAGCCGGAC UCCUGUGCAG CUUCCCUCGG 180
CCGCCGGGGG CCUCCCCGCG CCUCGCCGGC CUCCAGGCCC CCUCCUGGCU GGCGAGCGGG 240
CGCCACAUCU GGCCCGCACA UCUGCGCUGC CGGCCCGGCG CGGGGUCCGG AGAGGGCGCG 300
GCGCGGAGGC GCAGCCAGGG GUCCGGGAAG GCGCCGUCCG CUGCGCUGGG GGCUCGGUCU 360
AUGACGAGCA GCGGGGUCUG CCAUGGGUCG GGGGCUGCUC AGGGGCCUGU GGCCGCUGCA 420
CAUCGUCCUG UGGACGCGUA UCGCCAGCAC GAUCCCACCG CACGUUCAGA AGUCGGUUAA 480
UAACGACAUG AUAGUCACUG ACAACAACGG UGCAGUCAAG UUUCCACAAC UGUGUAAAUU 540
UUGUGAUGUG AGAUUUUCCA CCUGUGACAA CCAGAAAUCC UGCAUGAGCA ACUGCAGCAU 600
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GAACAUAACA CUAGAGACAG UUUGCCAUGA CCCCAAGCUC CCCUACCAUG ACUUUAUUCU 720
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GCCACCACUG GGAGUUGCCA UAUCUGUCAU CAUCAUCUUC UACUGCUACC GCGUUAACCG 960
GCAGCAGAAG CUGAGUUCAA CCUGGGAAAC CGGCAAGACG CGGAAGCUCA UGGAGUUCAG 1020
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UCUCUGACUG UAAAACAGUG AACUUUGCAU GAGGAAAGAG GCUCCAUGUC UCACAGCCAG 2640
CUAUGACCAC AUUGCACUUG CUUUUGCAAA AUAAUCAUUC CCUGCCUAGC ACUUCUCUUC 2700
UGGCCAUGGA ACUAAGUACA GUGGCACUGU UUGAGGACCA GUGUUCCCGG GGUUCCUGUG 2760
UGCCCUUAUU UCUCCUGGAC UUUUCAUUUA AGCUCCAAGC CCCAAAUCUG GGGGGCUAGU 2820
UUAGAAACUC UCCCUCAACC UAGUUUAGAA ACUCUACCCC AUCUUUAAUA CCUUGAAUGU 2880
UUUGAACCCC ACUUUUUACC UUCAUGGGUU GCAGAAAAAU CAGAACAGAU GUCCCCAUCC 2940
AUGCGAUUGC CCCACCAUCU ACUAAUGAAA AAUUGUUCUU UUUUUCAUCU UUCCCCUGCA 3000
CUUAUGUUAC UAUUCUCUGC UCCCAGCCUU CAUCCUUUUC UAAAAAGGAG CAAAUUCUCA 3060
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AUGAAGAGGA UUUCAACUAC ACAAUACUAU CAUUGUCAGG ACUAUGACCU CAGGCACUCU 3240
AAACAUAUGU UUUGUUUGGU CAGCACAGCG UUUCAAAAAG UGAAGCCACU UUAUAAAUAU 3300
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CAAGUAAUCU CUUUUUUAAA ACUUUUUGAA GCUACUUAUU UUCAGCCAAA UAGGAAUAUU 3960
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UAUUAGAUUG AAUAAAGCAA AAUACUCAGG UGAGCAUCCU GCCUCCUGUU CCCAUUCCUA 4620
GUAGCUAAA 4629

1. Antisense-oligonucleotide consisting of 10 to 28 nucleotides and being LNA gapmers which contain at least one LNA at the 3’ terminal end and at least one LNA at the 5’ terminal end, and the antisense-oligonucleotide is capable of hybridizing with a region of the gene encoding the TGF-R_{II} or with a region of the mRNA encoding the TGF-R_{II}, wherein the region of the gene encoding the TGF-R_{II} or the region of the mRNA encoding the TGF-R_{II} comprises the sequence **CCCTAAACAC** (Seq. ID No. 5) or the sequence **ACTACCAAAT** (Seq. ID No. 6) or the sequence **GGACGCGTAT** (Seq. ID No. 7) or the sequence **GTCTATGACG** (Seq. ID No. 8) or the sequence **TTATTAATGC** (Seq. ID No. 9), and the antisense-oligonucleotide comprises a sequence complementary to said sequence **CCCTAAACAC** (Seq. ID No. 5) or sequence **ACTACCAAAT** (Seq. ID No. 6) or sequence **GGACGCGTAT** (Seq. ID No. 7) or sequence **GTCTATGACG** (Seq. ID No. 8) or sequence **TTATTAATGC** (Seq. ID No. 9) respectively and salts and optical isomers of said antisense-oligonucleotide.

2. Antisense-oligonucleotide according to claim 1, wherein the antisense-oligonucleotide hybridizes selectively only with the sequence **CCCTAAACAC** (Seq. ID No. 5) or the sequence **ACTACCAAAT** (Seq. ID No. 6) or the sequence **GGACGCGTAT** (Seq. ID No. 7) or the sequence **GTCTATGACG** (Seq. ID No. 8) or the sequence **TTATTAATGC** (Seq. ID No. 9) of the region of the gene encoding the TGF-R_{II} or of the region of the mRNA encoding the TGF-R_{II}.

3. Antisense-oligonucleotide according to claim 1 or 2, wherein the antisense-oligonucleotide has a length of 12 to 20 nucleotides and/or wherein the antisense-oligonucleotide has a gapmer structure with 1 to 5 LNA units at the 3’ terminal end and 1 to 5 LNA units at the 5’ terminal end and/or wherein the antisense-oligonucleotide has phosphate, phosphorothioate and/or phosphorodithioate as internucleotide linkages.

4. Antisense-oligonucleotide according to any one of the claims 1 – 3, wherein the antisense-oligonucleotide is represented by the following sequence:

5’-N^1-**GTCATAGA**-N^2-3’ (Seq. ID No. 12) or

5’-N^3-**ACGCGTCC**-N^4-3’ (Seq. ID No. 98) or

5’-N^5-**TTTGGTAG**-N^6-3’ (Seq. ID No. 11) or

5’-N^9-**ATTAATAA**-N^{10}-3’ (Seq. ID No. 101)

5’-N^{11}-**TGTTTAGG**-N^{12}-3’ (Seq. ID No. 10) or

wherein

N^1 represents: CATGGCAGACCCCGCTGCTC-, ATGGCAGACCCCGCTGCTC-, TGGCAGACCCCGCTGCTC-, GGCAGACCCCGCTGCTC-, GCAGACCCCGCTGCTC-, CAGACCCCGCTGCTC-, AGACCCCGCTGCTC-, GACCCCGCTGCTC-, ACCCCGCTGCTC-, CCCCGCTGCTC-, CCCGCTGCTC-, CCGCTGCTC-, CGCTGCTC-, GCTGCTC-, CTGCTC-, TGCTC-, GCTC-, CTC-, TC-, or C-;

N^2 represents: -C, -CC, -CCG, -CCGA, -CCGAG, -CCGAGC, -CCGAGCC, -CCGAGCCC, -CCGAGCCCC, -CCGAGCCCCC, -CCGAGCCCCCA, -CCGAGCCCCCAG, -CCGAGCCCCCAGC, -CCGAGCCCCCAGCG, -CCGAGCCCCCAGCGC, -CCGAGCCCCCAGCGCA, -CCGAGCCCCCAGCGCAG, -CCGAGCCCCCAGCGCAGC, -CCGAGCCCCCAGCGCAGCG, or –CCGAGCCCCCAGCGCAGCGG;

N^3 represents: GGTGGGATCGTGCTGGCGAT-, GTGGGATCGTGCTGGCGAT-, TGGGATCGTGCTGGCGAT-, GGGATCGTGCTGGCGAT-, GGATCGTGCTGGCGAT-, GATCGTGCTGGCGAT-, ATCGTGCTGGCGAT-, TCGTGCTGGCGAT-, CGTGCTGGCGAT-, GTGCTGGCGAT-, TGCTGGCGAT-, GCTGGCGAT-, CTGGCGAT-, TGGCGAT-, GGCGAT-, GCGAT-, CGAT-, GAT-, AT-, or T-;

N^4 represents: -ACAGGACGATGTGCAGCGGC, -ACAGGACGATGTGCAGCGG, -ACAGGACGATGTGCAGCG, -ACAGGACGATGTGCAGC, -ACAGGACGATGTGCAG, -ACAGGACGATGTGCA, -ACAGGACGATGTGC, -ACAGGACGATGTG, -ACAGGACGATGT, -ACAGGACGATG, -ACAGGACGAT, -ACAGGACGA, -ACAGGACG, -ACAGGAC, -ACAGGA, -ACAGG, -ACAG, -ACA, -AC, or -A;

N^5 represents: GCCCAGCCTGCCCCAGAAGAGCTA-, CCCAGCCTGCCCCAGAAGAGCTA-, CCAGCCTGCCCCAGAAGAGCTA-, CAGCCTGCCCCAGAAGAGCTA-, AGCCTGCCCCAGAAGAGCTA-, GCCTGCCCCAGAAGAGCTA-, CCTGCCCCAGAAGAGCTA-, CTGCCCCAGAAGAGCTA-, TGCCCCAGAAGAGCTA-, GCCCCAGAAGAGCTA-, CCCCAGAAGAGCTA-, CCCAGAAGAGCTA-, CCAGAAGAGCTA-, CAGAAGAGCTA-, AGAAGAGCTA-, GAAGAGCTA-, AAGAGCTA-, AGAGCTA-, GAGCTA-, AGCTA-, GCTA-, CTA-, TA-, or A-;

N^6 represents: -TGTTTAGGGAGCCGTCTTCAGGAA, -TGTTTAGGGAGCCGTCTTCAGGA, -TGTTTAGGGAGCCGTCTTCAGG,

-TGTTTAGGGAGCCGTCTTCAG, -TGTTTAGGGAGCCGTCTTCA, -TGTTTAGGGAGCCGTCTTC, -TGTTTAGGGAGCCGTCTT, -TGTTTAGGGAGCCGTCT, -TGTTTAGGGAGCCGTC, -TGTTTAGGGAGCCGT, -TGTTTAGGGAGCCG, -TGTTTAGGGAGCC, -TGTTTAGGGAGC, -TGTTTAGGGAG, -TGTTTAGGGA, -TGTTTAGGG, -TGTTTAGG, -TGTTTAG, -TGTTTA, -TGTTT, -TGTT, -TGT, -TG, or -T;

N^9 represents: ATTCATATTTATATACAGGC-, TTCATATTTATATACAGGC-, TCATATTTATATACAGGC-, CATATTTATATACAGGC-, ATATTTATATACAGGC-, TATTTATATACAGGC-, ATTTATATACAGGC-, TTTATATACAGGC-, TTATATACAGGC-, TATATACAGGC-, ATATACAGGC-, TATACAGGC-, ATACAGGC-, TACAGGC-, ACAGGC-, CAGGC-, AGGC-, GGC-, GC-, or C-;

N^{10} represents: -AGTGCAAATGTTATTGGCTA, -AGTGCAAATGTTATTGGCT, -AGTGCAAATGTTATTGGC, -AGTGCAAATGTTATTGG, -AGTGCAAATGTTATTG, -AGTGCAAATGTTATT, -AGTGCAAATGTTAT, -AGTGCAAATGTTA, -AGTGCAAATGTT, -AGTGCAAATGT, -AGTGCAAATG, -AGTGCAAAT, -AGTGCAAA, -AGTGCAA, -AGTGCA, -AGTGC, -AGTG, -AGT, -AG, or -A;

N^{11} represents: TGCCCCAGAAGAGCTATTTGGTAG-, GCCCCAGAAGAGCTATTTGGTAG-, CCCCAGAAGAGCTATTTGGTAG-, CCCAGAAGAGCTATTTGGTAG-, CCAGAAGAGCTATTTGGTAG-, CAGAAGAGCTATTTGGTAG-, AGAAGAGCTATTTGGTAG-, GAAGAGCTATTTGGTAG-, AAGAGCTATTTGGTAG-, AGAGCTATTTGGTAG-, GAGCTATTTGGTAG-, AGCTATTTGGTAG-, GCTATTTGGTAG-, CTATTTGGTAG-, TATTTGGTAG-, ATTTGGTAG-, TTTGGTAG-, TTGGTAG-, TGGTAG-, GGTAG-, GTAG-, TAG-, AG- or G-,

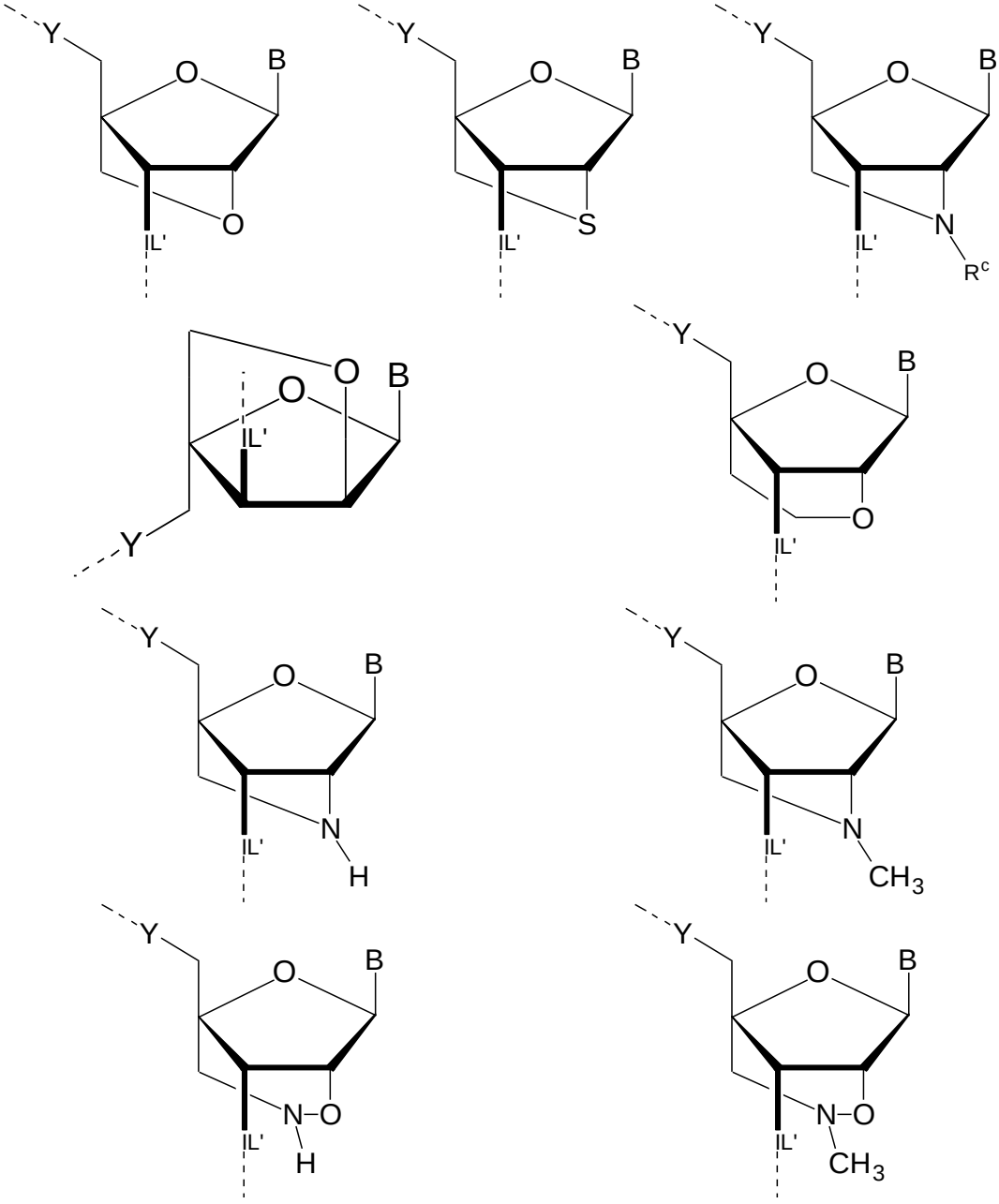
N^{12} represents: -GAGCCGTCTTCAGGAATCTTCTCC, -GAGCCGTCTTCAGGAATCTTCTC, -GAGCCGTCTTCAGGAATCTTCT, -GAGCCGTCTTCAGGAATCTTC, -GAGCCGTCTTCAGGAATCTT, -GAGCCGTCTTCAGGAATCT, -GAGCCGTCTTCAGGAATC, -GAGCCGTCTTCAGGAAT, -GAGCCGTCTTCAGGAA, -GAGCCGTCTTCAGGA, -GAGCCGTCTTCAGG, -GAGCCGTCTTCAG, -GAGCCGTCTTCA, -GAGCCGTCTTC, -GAGCCGTCTT, -GAGCCGTCT, -GAGCCGTC, -GAGCCGT, -GAGCCG, -GAGCC, -GAGC, -GAG, -GA, or -G.

5. Antisense-oligonucleotide according to any one of the claims 1 – 4, wherein the last 2 to 4 nucleotides at the 5’ terminal end are LNA nucleotides and the last 2 to 4 nucleotides at the 3’ terminal end are LNA nucleotides and between the LNA nucleotides at the 5’ terminal end and the LNA nucleotides at the 3’

terminal end at least 6 consecutive nucleotides are present which are non-LNA nucleotides or which are DNA nucleotides.

6. Antisense-oligonucleotide according to any one of the claims 1 – 5, wherein the LNA nucleotides are linked to each other through a phosphorothioate group or a phosphorodithioate group or wherein all nucleotides are linked to each other through a phosphate group or a phosphorothioate group or a phosphorodithioate group.

7. Antisense-oligonucleotide according to any one of the claims 1 – 6, wherein the LNA nucleotides are selected from the following group:



wherein

IL' represents –X''–P(=X')(X^-)–;

X' represents =O or =S;

X^- represents $–O^-$, –OH, $–OR^H$, $–NHR^H$, $–N(R^H)_2$, $–OCH_2CH_2OR^H$, $–OCH_2CH_2SR^H$, $–BH_3^-$, $–R^H$, –SH, $–SR^H$, or $–S^-$;

X'' represents –O–, –NH–, $–NR^H–$, $–CH_2–$, or –S–;

Y is –O–, –NH–, $–NR^H–$, $–CH_2–$ or –S–;

R^C and R^H are independently of each other selected from hydrogen and C_{1-4}-alkyl;

B represents a nucleobase selected from the following group:
adenine, thymine, guanine, cytosine, uracil, 5-methylcytosine, 5-hydroxymethyl cytosine, N^4-methylcytosine, xanthine, hypoxanthine, 7-deazaxanthine, 2-aminoadenine, 6-methyladenine, 6-methylguanine, 6-ethyladenine, 6-ethylguanine, 2-propyladenine, 2-propylguanine, 6-carboxyuracil, 5,6-dihydrouracil, 5-propynyl uracil, 5-propynyl cytosine, 6-aza uracil, 6-aza cytosine, 6-aza thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-fluoroadenine, 8-chloroadenine, 8-bromoadenine, 8-iodoadenine, 8-aminoadenine, 8-thioladenine, 8-thioalkyladenine, 8-hydroxyladenine, 8-fluoroguanine, 8-chloroguanine, 8-bromoguanine, 8-iodoguanine, 8-aminoguanine, 8-thiolguanine, 8-thioalkylguanine, 8-hydroxylguanine, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, 5-trifluoromethyluracil, 5-fluorocytosine, 5-bromocytosine, 5-chlorocytosine, 5-iodocytosine, 5-trifluoromethylcytosine, 7-methylguanine, 7-methyladenine, 8-azaguanine, 8-azaadenine, 7-deazaguanine, 7-deazaadenine, 7-deaza-8-azaadenine, 3-deazaguanine, 3-deazaadenine, 2-thiouracil, 2-thiothymine and 2-thiocytosine.

8. Antisense-oligonucleotide according to any one of the claims 1 – 7 having one of the following gapmer structures: 3-8-3, 4-8-2, 2-8-4, 3-8-4, 4-8-3, 4-8-4, 3-9-3, 4-9-2, 2-9-4, 4-9-3, 3-9-4, 4-9-4, 3-10-3, 2-10-4, 4-10-2, 3-10-4, 4-10-3, 4-10-4, 2-11-4, 4-11-2, 3-11-4, 4-11-3.

9. Antisense oligonucleotide according to any one of the claims 1 – 8, wherein the antisense oligonucleotides bind with 100% complementarity to the mRNA encoding TGF-RII and do not bind to any other region in the human transcriptome.

10. Antisense-oligonucleotide selected from the following group:

Seq ID No.	Sequence, 5'-3'
232a	**C*b^{1}sGb^{1}s**dTsdC*sdAsdTsdAsdGs**Ab^{1}sC*b^1**
232b	**C*b^{1}Gb^1**dTdC*dAdTdAdG**Ab^{1}C*b^1**
233a	**Tb^{1}sC*b^{1}sGb^{1}s**dTsdC*sdAsdTsdAsdGs**Ab^{1}sC*b^{1}sC*b^1**
233b	**Tb^{1}C*b^{1}Gb^1**dTdC*dAdTdAdG**Ab^{1}C*b^{1}C*b^1**
233c	**Tb^{1}sC*b^{1}sGb^{1}s**dTsdC*sdAsdTsdAsdGsdAs**C*b^{1}sC*b^1**
233d	**Tb^{1}s**dC*sdGsdTsdC*sdAsdTsdAsdGsdAs**C*b^{1}sC*b^1**
233e	**Tb^{1}sC*b^{1}s**dGsdTsdCsdAsdTsdAsdGsdAsdC*s**C*b^1**
234a	**Tb^{1}sC*b^{1}sGb^{1}sTb^{1}s**dCsdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^1**
234b	**Tb^{1}C*b^{1}Gb^{1}Tb^1**dCdAdUdAdGdA**C*b^{1}C*b^{1}Gb^1**
234c	**Tb^{1}sC*b^{1}sGb^{1}sTb^{1}s**dC*sdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^1**
234d	**Tb^{1}sC*b^{1}sGb^{1}s**dTsdC*sdA*sdTsdA*sdGsdA*sdC*s**C*b^{1}sGb^1**
234e	**Tb^{1}sC*b^{1}s**dGsdTsdC*sdAsdTsdA*sdGsdA*sdC*sdCs**Gb^1**
234f	**Tb^{1}s**dCsdGsdTsdC*sdA*sdTsdAsdGs**Ab^{1}sC*b^{1}sC*b^{1}sGb^1**
142c	**C*b^{1}sGb^{1}sTb^1**sdCsdAsdTsdAsdGsdAsdCsdCs**Gb^{1}sAb^1**
143i	**C*b^{1}sTb^{1}sC*b^{1}sGb^{1}s**dTsdCsdAsdTsdAsdGs**Ab^{1}sC*b^{1}sC*b^{1}sGb^1**
143j	**C*b^{4}ssTb^{4}ssC*b^{4}ss**dGssdTssdCssdAssdTssdAssdGssdA*ss**C*b^{4}ssC*b^{4}ssGb^4**
143h	**C*b^{1}sTb^{1}s**dCsdGsdTsdCsdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^1**
143k	**C*b^{2}ssTb^{2}ssC*b^{2}ss**dGssdTssdCssdAssdTssdAssdGssdAss**C*b^{2}ssC*b^{2}ssGb^2**
143m	**C*b^{1}Tb^{1}C*b^{1}Gb^1**dUsdCsdAsdTsdAsdGs**Ab^{1}C*b^{1}C*b^{1}Gb^1**
143n	**C*b^{1}sTb^{1}sC*b^{1}sGb^{1}sTb^{1}s**dCsdA*sdTsdA*sdGsdA*s**C*b^{1}sC*b^{1}sGb^1**
143o	**C*b^{1}sTb^{1}s**dCsdGsdUsdCsdAsdUsdAs**Gb^{1}sAb^{1}sC*b^{1}sC*b^{1}sGb^1**
143p	**C*b^{6}sTb^{6}sC*b^{6}sGb^{6}s**dTsdCsdAsdTsdAsdGsdAs**C*b^{6}sC*b^{6}sGb^6**
143q	**C*b^{7}sTb^{7}sC*b^{7}s**dGsdUsdCsdA*sdUsdA*sdGsdA*s**C*b^{7}sC*b^{7}sGb^7**
143r	**C*b^{4}sTb^{4}sC*b^{4}sGb^{4}s**dTsdC*sdA*sdTsdAsdGsdAsdC*s**C*b^{4}sGb^4**
143s	**C*b^{4}Tb^{4}C*b^{4}Gb^4**dTdCdAdTdAdGdAdC**C*b^{4}Gb^4**
143t	**C*b^{1}ssTb^{1}ssC*b^{1}ss**dGssdTssdC*ssdAssdTssdAssdGssdAss**C*b^{1}ssC*b^{1}ssGb^1**
143u	**C*b^{1}Tb^{1}s**dCsdGsdUsdC*sdAsdUsdAsdGsdAs**C*b^{1}C*b^{1}Gb^1**
143v	**C*b^{1}Tb^{1}s**dC*sdGsdTsdC*sdA*sdTsdAsdGsdAs**C*b^{1}C*b^{1}Gb^1**
143w	**C*b^{6}sTb^{6}s**dC*dGdTdC*dAdTdAdGdAs**C*b^{6}sC*b^{6}sGb^6**
143x	**C*b^{7}sTb^{7}sC*b^{7}sGb^{7}s**dTsdC*sdAsdTsdAsdGsdAs**C*b^{7}sC*b^{7}sGb^7**
143y	**C*b^{7}sTb^{7}s**dC*sdGsdTsdCsdAsdUsdAsdGs**Ab^{7}sC*b^{7}sC*b^{7}sGb^7**
143z	**C*b^{1}sTb^{1}s**dC*sdGsdTsdC*sdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^1**
143aa	**C*b^{1}Tb^{1}s**dC*sdGsdTsdC*sdAsdTsdAsdGsdAs**C*b^{1}C*b^{1}Gb^1**
143ab	**C*b^{1}sTb^{1}s**dC*sdGsdTsdC*sdA*sdTsdAsdGsdA*s**C*b^{1}sC*b^{1}sGb^1**

143ac	**C*b^{1}sTb^{1}s**dC*sdGsdTsdCsdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^1**
143ad	**C*b^{1}Tb^1**dC*dGdTdCdAdTdAdGdA**C*b^{1}C*b^{1}Gb^1**
143ae	**C*b^{1}sTb^{1}s**dC*dGdTdC*dAdTdAdGdAs**C*b^{1}sC*b^{1}sGb^1**
143af	**/5SpC3s/C*b^{1}sTb^{1}s**dC*dGdTdC*dA*dTdAdGdA*s**C*b^{1}sC*b^{1}sGb^1**
143ag	**C*b^{1}sTb^{1}s**dC*dGdTdC*dA*dTdAdGdA*s**C*b^{1}sC*b^{1}sGb^1/3SpC3s/**
143ah	**/5SpC3s/C*b^{1}sTb^{1}s**dC*dGdTdC*dA*dTdAdGdA*s**C*b^{1}sC*b^{1}sGb^1/3SpC3s/**
143ai	**C*b^{1}sTb^{1}s**dC*sdGsdUsdC*sdA*sdUsdA*sdGsdA*s**C*b^{1}sC*b^{1}sGb^1**
143aj	**C*b^{1}sTb^{1}sC*b^1**sdGsdTsdCsdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^1**
145c	**Gb^{1}sC*b^{1}sTb^1**sdCsdGsdTsdCsdAsdTsdAsdGs**Ab^{1}sC*b^{1}sC*b^1**
235i	**C*b^{1}sTb^{1}sC*b^{1}sGb^{1}s**dTdC*dAdTdAdGdAs**C*b^{1}sC*b^{1}sGb^{1}sAb^1**
235a	**C*b^{1}ssTb^{1}ss**dCssdGssdTssdCssdAssdTssdAssdGssdAssdCssdCssdGss**Ab^1**
235b	**C*b^{1}Tb^1**dCdGdTdCdAdTdAdGdAdCdCdG**Ab^1**
235c	**C*b^{1}sTb^{1}s**dCsdGsdTsdCsdA*sdUsdAsdGsdAsdCs**C*b^{1}sGb^{1}sAb^1**
235d	**C*b^{1}Tb^{1}s**dCsdGsdTsdCsdAsdTsdAsdGsdAs**C*b^{1}C*b^{1}Gb^{1}Ab^1**
235e	**C*b^{4}sTb^{4}sC*b^{4}s**dGsdTsdCsdAsdTsdAsdGsdAsdCsdCs**Gb^{4}sAb^4**
235f	**C*b^{6}sTb^{6}sC*b^{6}s**dGdTdCdA*dTdAdGdAdC*s**C*b^{6}sGb^{6}sAb^6**
235g	**C*b^{1}sTb^{1}sC*b^{1}sGb^{1}s**dTsdC*sdAsdTsdAsdGsdAsdC*sdC*sdGs**Ab^1**
235h	**C*b^{1}ssTb^{1}ss**dCssdGssdUssdCssdAssdUssdAssdGssdAssdCssdCss**Gb^1 ssAb^1**
144c	**Gb^{1}sC*b^{1}sTb^1**sdCsdGsdTsdCsdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^1**
141c	**Gb^{1}sC*b^{1}sTb^{1}sC*b^{1}s**dGsdTsdC*sdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^{1}sAb^1**
141d	**Gb^{1}C*b^{1}Tb^{1}C*b^{1}s**dGsdTsdC*sdAsdTsdAsdGsdAsdCs**C*b^{1}Gb^{1}Ab^1**
141e	**Gb^{4}sC*b^{4}sTb^{4}sC*b^{4}s**dGsdTsdC*sdAsdTsdAsdGsdA*sdC*sdC*s**Gb^{4}sAb^4**
141f	**Gb^{1}s**dC*sdTsdCsdGsdTsdC*sdA*sdTsdAsdGsdA*sdC*sdC*sdGs**Ab^1**
141g	**Gb^{2}sC*b^{2}sTb^{2}s**dCsdGsdUsdCsdAsdTsdA*sdGsdAsdCs**C*b^{2}sGb^{2}sAb^2**
141h	**Gb^{4}ssC*b^{4}ssTb^{4}ss**dCssdGssdTssdCssdAssdTssdAssdGssdAss**C*b^{4}ssC*b^4 ssGb^{4}ssAb^4**
141i	**Gb^{1}C*b^1**dTdCdGdTdCdA*dTdA*dGdA*dC**C*b^{1}Gb^{1}Ab^1**
141j	**Gb^{1}sC*b^{1}sTb^1**sdCsdGsdTsdCsdAsdTsdAsdGsdAsdCs**C*b^{1}sGb^{1}sAb^1**
139c	**C*b^{1}sGb^{1}sTb^1**sdCsdAsdTsdAsdGsdAsdCsdCsdGsdAs**Gb^{1}sC*b^{1}sC*b^1**
237a	**Tb^{1}sGb^{1}sC*b^{1}sTb^{1}sC*b^{1}s**dGsdTsdC*sdAsdTsdAsdGs**Ab^{1}sC*b^{1}sC*b^{1}sGb^1 sAb^1**
237b	**Tb^{2}sGb^{2}sC*b^{2}s**dTsdGsdTsdC*sdAsdTsdAsdGs**Ab^{2}sC*b^{2}sC*b^{2}sGb^{2}sAb^2**
237c	**Tb^{1}sGb^{1}sC*b^{1}sTb^{1}s**dC*sdGsdTsdCsdAsdTsdAsdGsdAsdC*s**C*b^{1}sGb^{1}sAb^1**
237d	**Tb^{1}s**dGsdCsdUsdC*sdGsdTsdC*sdAsdUsdAsdGs**Ab^{1}sC*b^{1}sC*b^{1}sGb^{1}sAb^1**
237e	**Tb^{1}sGb^{1}sC*b^{1}s**dTsdGsdTsdC*sdA*sdTsdA*sdGs**Ab^{1}sC*b^{1}sC*b^{1}sGb^{1}sAb^1**
237f	**Tb^{1}Gb^1**dC*dTdGdTdC*dAdTdAdGdA**C*b^{1}C*b^{1}Gb^{1}Ab^1**
237g	**Tb^{1}s**dGsdC*sdTsdGsdTsdC*sdAsdTsdAsdGsdAsdC*s**C*b^{1}sGb^{1}sAb^1**

237h	**Tb^{1}Gb^{1}C*b^{1}Tb^{1}C*b^1**dGdTdC*dA*dTdA*dGdA*dC*dC***Gb^{1}Ab^1**
237i	**Tb^{1}ssGb^{1}ssC*b^{1}ssTb^{1}ssC*b^{1}ss**dGssdTssdCssdAssdTssdAssdGssdAssdCss**C*b^{1}ssGb^{1}ssAb^1**
237j	**Tb^{4}sGb^{4}sC*b^{4}s**dTdGdTdCdA*dTdA*dGdA*s**C*b^{4}sC*b^{4}sGb^{4}sAb^4**
237k	**Tb^{6}sGb^{6}sC*b^{6}s**dUsdGsdUsdC*sdA*sdUsdA*sdGsdA*sdC*s**C*b^{6}sGb^{6}sAb^6**
237m	**Tb^{7}sGb^{7}sC*b^{7}sTb^{7}s**dC*dGdTdC*dAdTdAdGdAs**C*b^{7}sC*b^{7}sGb^{7}sAb^7**
238a	**Tb^{1}sGb^{1}sC*b^{1}sTb^{1}sC*b^{1}s**dGsdTsdC*sdAsdTsdAsdGsdAs**C*b^{1}sC*b^{1}sGb^1 sAb^{1}sGb^1**
238b	**Tb^{7}sGb^{7}sC*b^{7}sTb^{7}sC*b^{7}s**dGsdTsdC*sdAsdTsdAsdGsdAsdC*sdC*sdGsdAs**Gb^7**
238c	**Tb^{1}sGb^{1}sC*b^{1}sTb^{1}s**dC*sdGsdTsdCsdAsdTsdAsdGsdAsdC*s**C*b^{1}sGb^{1}sAb^1 sGb^1**
238d	**Tb^{1}sGb^{1}s**dC*sdTsdC*sdGsdTsdCsdAsdTsdAsdGsdAsdC*sdC*s**Gb^{1}sAb^1 sGb^1**
238e	**Tb^{1}sGb^{1}sC*b^{1}sTb^{1}s**dC*sdGsdTsdCsdAsdTsdAsdGsdAsdC*sdC*s**Gb^{1}sAb^1 sGb^1**
238f	**Tb^{1}Gb^1**dC*dUdC*dGdTdC*dAdTdAdGdA***C*b^{1}C*b^{1}Gb^{1}Ab^{1}Gb^1**
238g	**Tb^{4}Gb^{4}C*b^{4}Tb^{4}s**dCsdGsdTsdCsdAsdTsdAsdGsdAs**C*b^{4}C*b^{4}Gb^{4}Ab^{4}Gb^4**
238h	**Tb^{1}ssGb^{1}ssC*b^{1}ss**dTssdC*ssdGssdTssdC*ssdAssdTssdA*ssdGssdAssdC*ssdC*ss**Gb^{1}ssAb^{1}ssGb^1**
238i	**Tb^{2}Gb^{2}C*b^2**dTdCdGdTdC*dAdTdAdGdA**C*b^{2}C*b^{2}Gb^{2}Ab^{2}Gb^2**
239a	**Tb^{1}sGb^{1}sC*b^{1}sTb^{1}sC*b^{1}s**dGsdTsdCsdAsdTsdAsdGsdAsdC*s**C*b^{1}sGb^1 sAb^{1}sGb^{1}sC*b^1**
239b	**Tb^{6}Gb^{6}C*b^{6}Tb^{6}C*b^6**dGdTdC*dAdTdAdGdAdC***C*b^{6}Gb^{6}Ab^{6}Gb^{6}C*b^6**
239c	**Tb^{1}sGb^{1}sC*b^{1}sTb^{1}s**dC*sdGsdTsdCsdAsdTsdAsdGsdAsdCsdCsdGs**Ab^1 sGb^{1}sC*b^1**
239d	**Tb^{1}s**dGsdCsdTsdCsdGsdTsdCsdAsdTsdAsdGsdA*sdC*s**C*b^{1}sGb^{1}sAb^1 sGb^{1}sC*b^1**
239e	**Tb^{4}sGb^{4}s**dCsdUsdCsdGsdUsdCsdAsdTsdAsdGsdA*sdC*sdC*s**Gb^{4}sAb^4 sGb^{4}sC*b^4**
239f	**Tb^{2}ssGb^{2}ssC*b^{2}ssTb^{2}ssC*b^{2}ss**dGssdTssdCssdAssdTssdAssdGssdAssdCssdCssdGssdAss**Gb^{2}ssC*b^2**
240a	**C*b^{1}sTb^{1}sGb^{1}sC*b^{1}sTb^{1}s**dC*sdGsdTsdCsdAsdTsdAsdGsdAsdC*s**C*b^1 sGb^{1}sAb^{1}sGb^{1}sC*b^1**
240b	**C*b^{2}sTb^{2}sGb^{2}s**dC*sdTsdC*sdGsdTsdCsdAsdTsdAsdGsdAsdC*s**C*b^{2}sGb^2 sAb^{2}sGb^{2}sC*b^2**
240c	**C*b^{1}Tb^{1}Gb^1**dC*dTdC*dGdTdCdAdTdAdGdAdC*dC***Gb^{1}Ab^{1}Gb^{1}C*b^1**
240d	**C*b^{1}s**dUsdGsdCsdUsdC*sdGsdTsdCsdAsdTsdAsdGsdAsdC*s**C*b^{1}sGb^1 sAb^{1}sGb^{1}sC*b^1**

240e	**C*b^{4}sTb^{4}sGb^{4}sC*b^{4}s**dTsdCsdGsdTsdCsdAsdTsdAsdGsdAsdCsdCs**Gb^4 sAb^{4}sGb^{4}sC*b^4**
241a	**Gb^{1}sC*b^{1}sTb^{1}sGb^{1}sC*b^{1}s**dTsdC*sdGsdTsdCsdAsdTsdAsdGsdAsdCsdC* s**Gb^{1}sAb^{1}sGb^{1}sC*b^{1}sC*b^1**
241b	**Gb^{1}C*b^{1}Tb^{1}Gb^{1}C*b^1**dTdC*dGdTdC*dAdTdAdGdAdC*dC***Gb^{1}Ab^{1}Gb^{1}C*b^1 C*b^1**
241c	**Gb^{1}sC*b^{1}sTb^{1}sGb^{1}sC*b^{1}s**dTsdCsdGsdTsdCsdAsdTsdAsdGsdAsdCsdCs**Gb^1 sAb^{1}sGb^{1}sC*b^{1}sC*b^1**
242a	**C*b^{1}sGb^{1}sC*b^{1}sTb^{1}sGb^{1}s**dCsdTsdCsdGsdTsdCsdAsdTsdAsdGsdAsdCsdC* sdGs**Ab^{1}sGb^{1}sC*b^{1}sC*b^{1}sC*b^1**
242b	**C*b^{1}Gb^{1}C*b^{1}Tb^{1}Gb^1**dC*dTdCdGdTdCdAdTdAdGdAdCdC*dG**Ab^{1}Gb^{1}C*b^1 C*b^{1}C*b^1**
243a	**C*b^{1}sC*b^{1}sGb^{1}sC*b^{1}sTb^{1}s**dGsdC*sdTsdCsdGsdTsdC*sdAsdTsdAsdGsdAs dCsdC*sdGsdAs**Gb^{1}sC*b^{1}sC*b^{1}sC*b^{1}sC*b^1**
243b	**C*b^{1}C*b^{1}Gb^{1}C*b^{1}Tb^1**dGdC*dTdCdGdTdC*dAdTdAdGdAdCdC*dGdA**Gb^{1}C*b^1 C*b^{1}C*b^{1}C*b^1**
244a	**C*b^{1}sC*b^{1}sC*b^{1}sGb^{1}sC*b^{1}s**dTsdGsdCsdTsdCsdGsdTsdC*sdAsdTsdAsdGs dAsdC*sdCsdGsdAsdGs**C*b^{1}sC*b^{1}sC*b^{1}sC*b^{1}sC*b^1**
244b	**C*b^{1}C*b^{1}C*b^{1}Gb^{1}C*b^1**dTdGdC*dTdCdGdTdC*dAdTdAdGdAdC*dCdGdAdG **C*b^{1}C*b^{1}C*b^{1}C*b^{1}C*b^1**
245a	**Tb^{1}sAb^{1}s**dC*sdGsdCsdGsdTsdC*s**C*b^{1}sAb^1**
245b	**Tb^{1}Ab^1**dCdGdC*dGdTdC**C*b^{1}Ab^1**
246a	**Ab^{1}sTb^{1}sAb^{1}s**dC*sdGsdCsdGsdTsdCs**C*b^{1}sAb^{1}sC*b^1**
246b	**Ab^{1}Tb^{1}Ab^1**dCdGdCdGdTdC***C*b^{1}Ab^{1}C*b^1**
246c	**Ab^{1}sTb^{1}sAb^{1}s**dCsdGsdCsdGsdTsdC*sdC*s**Ab^{1}sC*b^1**
246d	**Ab^{1}sTb^{1}s**dA*sdC*sdGsdCsdGsdTsdC*sdC*sdA*s**C*b^1**
246e	**Ab^{1}s**dTsdA*sdC*sdGsdC*sdGsdTsdC*sdC*s**Ab^{1}sC*b^1**
247a	**Gb^{1}sAb^{1}sTb^{1}sAb^{1}s**dCsdGsdCsdGsdTsdCs**C*b^{1}sAb^{1}sC*b^1**
247b	**Gb^{1}Ab^{1}Tb^{1}Ab^1**dCdGdCdGdUdC**C*b^{1}Ab^{1}C*b^1**
247c	**Gb^{1}sAb^{1}sTb^{1}sAb^{1}s**dC*sdGsdCsdGsdTsdC*s**C*b^{1}sAb^{1}sC*b^1**
247d	**Gb^{1}sAb^{1}sTb^{1}s**dA*sdCsdGsdCsdGsdTsdCsdC*s**Ab^{1}sC*b^1**
247e	**Gb^{1}sAb^{1}s**dTsdA*sdCsdGsdC*sdGsdTsdCsdC*sdA*s**C*b^1**
247f	**Gb^{1}s**dA*sdTsdA*sdC*sdGsdCsdGsdTs**C*b^{1}sC*b^{1}sAb^{1}sC*b^1**
153f	**C*b^{1}sGb^{1}sAb^1**sdTsdAsdCsdGsdCsdGsdTsdCs**C*b^{1}sAb^1**
248a	**Gb^{1}sAb^{1}sTb^{1}sAb^{1}s**dC*sdGsdC*sdGsdTsdC*s**C*b^{1}sAb^{1}sC*b^{1}sAb^1**
248b	**Gb^{1}Ab^{1}Tb^{1}Ab^{1}s**dC*sdGsdCsdGsdTsdC*sdC*s**Ab^{1}C*b^{1}Ab^1**
248c	**Gb^{4}sAb^{4}sTb^{4}sAb^{4}s**dC*sdGsdCsdGsdTsdC*sdC*sdA*s**C*b^{4}sAb^4**
248d	**Gb^{1}s**dA*sdTsdAsdCsdGsdCsdGsdTsdCsdC*sdA*sdCs**Ab^1**
248e	**Gb^{2}sAb^{2}sTb^{2}s**dA*sdCsdGsdCsdGsdUsdCsdCs**Ab^{2}sC*b^{2}sAb^2**
248f	**Gb^{4}ssAb^{4}ss**dTssdAssdCssdGssdCssdGssdTssdCssdCss**Ab^{4}ssC*b^{4}ssAb^4**
248g	**Gb^{1}Ab^1**dTdA*dCdGdCdGdTdC**C*b^{1}Ab^{1}C*b^{1}Ab^1**
152h	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdCsdGsdCsdGsdTsdCsdCs**Ab^{1}sC*b^{1}sAb^1**
152i	**C*b^{1}Gb^{1}Ab^{1}Tb^{1}s**dAsdCsdGsdCsdGsdUsdCsdC*s**Ab^{1}C*b^{1}Ab^1**

152j	**C*b^{1}Gb^{1}Ab^{1}Tb^{1}s**dA*sdCsdGsdCsdGsdUsdCsdCs**Ab^{1}C*b^{1}Ab^1**
152k	**C*b^{6}sGb^{6}sAb^{6}sTb^{6}s**dAdC*dGdCdGdTdCdC*s**Ab^{6}sC*b^{6}sAb^6**
152m	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdCsdGsdCsdGsdTsdC*sdC*s**Ab^{1}sC*b^{1}sAb^1**
152n	**C*b^{1}Gb^{1}Ab^{1}Tb^{1}s**dAsdC*sdGsdC*sdGsdTsdC*sdC*s**Ab^{1}C*b^{1}Ab^1**
152o	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dA*sdCsdGsdCsdGsdTsdCsdC*s**Ab^{1}sC*b^{1}sAb^1**
152p	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdCsdGsdCsdGsdTsdCsdC*s**Ab^{1}sC*b^{1}sAb^1**
152q	**C*b^{1}Gb^{1}Ab^{1}Tb^1**dAdCdGdC*dGdTdCdC***Ab^{1}C*b^{1}Ab^1**
152r	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAdC*dGdC*dGdTdC*dC*s**Ab^{1}sC*b^{1}sAb^1**
152s	**/5SpC3s/C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdC*sdGsdC*sdGsdTsdCsdCs**Ab^{1}sC*b^1 sAb^1**
152t	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdC*sdGsdCsdGsdTsdCsdC*s**Ab^{1}sC*b^{1}sAb^1 /3SpC3s/**
152u	**/5SpC3s/C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdC*sdGsdC*sdGsdTsdCsdCs**Ab^{1}sC*b^1 sAb^1/3SpC3s/**
152v	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dA*sdC*sdGsdC*sdGsdUsdC*sdC*s**Ab^{1}sC*b^{1}sAb^1**
152w	**C*b^{7}sGb^{7}sAb^{7}s**dTsdAsdCsdGsdC*sdGsdTsdCs**C*b^{7}sAb^{7}sC*b^{7}sAb^7**
152z	**C*b^{7}sGb^{7}s**dAsdUsdAsdCsdGsdC*sdGsdUsdCs**C*b^{7}sAb^{7}sC*b^{7}sAb^7**
152aa	**C*b^{1}ssGb^{1}ssAb^{1}ss**dTssdAssdC*ssdGssdCssdGssdTssdCssdC*ss**Ab^1 ssC*b^{1}ssAb^1**
152ab	**C*b^{4}ssGb^{4}ssAb^{4}ss**dTssdA*ssdCssdGssdCssdGssdTssdCssdCssdA*ss **C*b^{4}ssAb^4**
152ac	**C*b^{2}ssGb^{2}ssAb^{2}ssTb^{2}ss**dAssdCssdGssdCssdGssdTssdCssdCssdAssdCss **Ab^2**
152ad	**C*b^{1}Gb^{1}Ab^{1}Tb^1**dAdCdGdCdGdUdC**C*b^{1}Ab^{1}C*b^{1}Ab^1**
152ae	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}sAb^{1}s**dCsdGsdCsdGsdUsdCsdCs**Ab^{1}sC*b^{1}sAb^1**
152af	**C*b^{1}sGb^{1}s**dA*sdTsdA*sdCsdGsdCsdGsdTs**C*b^{1}sC*b^{1}sAb^{1}sC*b^{1}sAb^1**
152ag	**C*b^{6}sGb^{6}sAb^{6}s**dTsdAsdCsdGsdCsdGsdTsdCs**C*b^{6}sAb^{6}sC*b^{6}sAb^6**
152ah	**C*b^{7}sGb^{7}sAb^{7}s**dUsdA*sdCsdGsdCsdGsdUsdCsdCs**Ab^{7}sC*b^{7}sAb^7**
152ai	**C*b^{4}sGb^{4}sAb^{4}sTb^{4}s**dA*sdCsdGsdCsdGsdTsdC*sdC*sdA*s**C*b^{4}sAb^4**
152aj	**C*b^{4}Gb^{4}Ab^{4}Tb^4**dAdCdGdCdGdTdCdCdA**C*b^{4}Ab^4**
152ak	**C*b^{1}sGb^{1}sAb^1**sdTsdAsdCsdGsdCsdGsdTsdCsdCs**Ab^{1}sC*b^{1}sAb^1**
249a	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAdCdGdCdGdTdCdC*s**Ab^{1}sC*b^{1}sAb^{1}sGb^1**
249b	**C*b^{1}ssGb^{1}ss**dAssdTssdAssdCssdGssdCssdGssdTssdCssdCssdAssdCssdA ss**Gb^1**
249c	**C*b^{1}Gb^1**dAdTdAdCdGdCdGdTdCdCdAdCdA**Gb^1**
249d	**C*b^{1}sGb^{1}s**dAsdUsdAsdC*sdGsdCsdGsdUsdCsdC*sdAs**C*b^{1}sAb^{1}sGb^1**
249e	**C*b^{1}Gb^{1}s**dAsdTsdAsdC*sdGsdC*sdGsdTsdCsdC*s**Ab^{1}C*b^{1}Ab^{1}Gb^1**
249f	**C*b^{4}sGb^{4}sAb^{4}s**dTsdAsdCsdGsdCsdGsdTsdCsdCsdAsdCs**Ab^{4}sGb^4**
249g	**C*b^{6}Gb^{6}Ab^6**dTdA*dCdGdCdGdTdC*dCdA***C*b^{6}Ab^{6}Gb^6**
249h	**C*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdC*sdGsdCsdGsdTsdCsdC*sdAsdC*sdAs**Gb^1**

249i	**C*b^{1}ssGb^{1}ss**dAssdUssdAssdCssdGssdCssdGssdUssdCssdCssdAssdCss **Ab^{1}ssGb^1**
250a	**Gb^{1}sC*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdCsdGsdC*sdGsdTsdCs**C*b^{1}sAb^{1}sC*b^{1}sAb^1 sGb^1**
250b	**Gb^{1}sC*b^{1}sGb^{1}sAb^{1}s**dTsdAsdC*sdGsdC*sdGsdTsdC*sdC*sdAs**C*b^{1}sAb^1 sGb^1**
250c	**Gb^{1}s**dC*sdGsdAsdUsdAsdCsdGsdC*sdGsdUsdCs**C*b^{1}sAb^{1}sC*b^{1}sAb^{1}sGb^1**
250d	**Gb^{1}sC*b^{1}sGb^{1}s**dA*sdTsdA*sdC*sdGsdC*sdGsdTsdC*s**C*b^{1}sAb^{1}sC*b^{1}sAb^1 sGb^1**
250e	**Gb^{1}C*b^1**dGdAdTdAdCdGdC*dGdTdCdC***Ab^{1}C*b^{1}Ab^{1}Gb^1**
250f	**Gb^{1}s**dC*sdGsdAsdTsdAsdCsdGsdC*sdGsdTsdCsdCsdAs**C*b^{1}sAb^{1}sGb^1**
250g	**Gb^{2}sC*b^{2}sGb^{2}s**dAsdTsdAsdCsdGsdC*sdGsdTsdC*s**C*b^{2}sAb^{2}sC*b^{2}sAb^2 sGb^2**
250h	**Gb^{1}C*b^{1}Gb^{1}Ab^{1}Tb^1**dA*dCdGdC*dGdTdC*dCdA*dC***Ab^{1}Gb^1**
250i	**Gb^{1}ssC*b^{1}ssGb^{1}ssAb^{1}ssTb^{1}ss**dAssdCssdGssdCssdGssdTssdCssdCssdAss **C*b^{1}ssAb^{1}ssGb^1**
250j	**Gb^{4}sC*b^{4}sGb^{4}s**dA*sdTsdA*sdCsdGsdCsdGsdTsdCsdCs**Ab^{4}sC*b^{4}sAb^{4}sGb^4**
250k	**Gb^{6}sC*b^{6}sGb^{6}s**dA*sdUsdAsdCsdGsdCsdGsdUsdC*sdCsdA*s**C*b^{6}sAb^{6}sGb^6**
250m	**Gb^{7}sC*b^{7}sGb^{7}sAb^{7}s**dTdAdCdGdCdGdTdC*dCs**Ab^{7}sC*b^{7}sAb^{7}sGb^7**
251a	**Gb^{1}sC*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdCsdGsdCsdGsdTsdCsdC*s**Ab^{1}sC*b^{1}sAb^1 sGb^{1}sGb^1**
251b	**Gb^{7}sC*b^{7}sGb^{7}sAb^{7}sTb^{7}s**dAsdC*sdGsdCsdGsdTsdCsdCsdAsdCsdAsdGs **Gb^7**
251c	**Gb^{1}sC*b^{1}sGb^{1}sAb^{1}s**dTsdAsdC*sdGsdCsdGsdTsdCsdC*sdAs**C*b^{1}sAb^{1}sGb^1 sGb^1**
251d	**Gb^{1}sC*b^{1}sGb^{1}s**dAsdTsdAsdC*sdGsdC*sdGsdTsdCsdC*sdAs**C*b^{1}sAb^{1}sGb^1 sGb^1**
251e	**Gb^{1}sC*b^{1}sGb^{1}sAb^{1}s**dTsdAsdC*sdGsdCsdGsdTsdCsdC*sdAsdC*s**Ab^{1}sGb^1 sGb^1**
251f	**Gb^{1}C*b^1**dGdAdUdA*dCdGdCdGdTdC*dC***Ab^{1}C*b^{1}Ab^{1}Gb^{1}Gb^1**
251g	**Gb^{4}C*b^{4}Gb^{4}Ab^4**sdTsdAsdCsdGsdCsdGsdTsdCsdCs**Ab^{4}C*b^{4}Ab^{4}Gb^{4}Gb^4**
251h	**Gb^{1}ssC*b^{1}ssGb^{1}ss**dA*ssdTssdA*ssdCssdGssdCssdGssdTssdCssdCssdA* ssdC*ss**Ab^{1}ssGb^{1}ssGb^1**
251i	**Gb^{2}C*b^{2}Gb^2**dAdTdAdCdGdC*dGdTdCdC***Ab^{2}C*b^{2}Ab^{2}Gb^{2}Gb^2**
252a	**Gb^{1}sC*b^{1}sGb^{1}sAb^{1}sTb^{1}s**dAsdC*sdGsdCsdGsdTsdCsdCsdAs**C*b^{1}sAb^{1}sGb^1 sGb^{1}sAb^1**
252b	**Gb^{6}C*b^{6}Gb^{6}Ab^{6}Tb^6**dAdC*dGdCdGdTdCdC*dA**C*b^{6}Ab^{6}Gb^{6}Gb^{6}Ab^6**
252c	**Gb^{1}sC*b^{1}sGb^{1}s**dAsdTsdAsdCsdGsdCsdGsdTsdCsdCsdAsdC*s**Ab^{1}sGb^1 sGb^{1}sAb^1**
252d	**Gb^{1}s**dC*sdGsdA*sdTsdA*sdC*sdGsdCsdGsdTsdCsdCsdA*s**C*b^{1}sAb^{1}sGb^1 sGb^{1}sAb^1**
252e	**Gb^{4}sC*b^{4}s**dGsdAsdUsdAsdCsdGsdCsdGsdUsdCsdCsdAsdC*s**Ab^{4}sGb^{4}sGb^4**

	sAb^4
252f	**Gb^{2}ssC*b^{2}ssGb^{2}ssAb^{2}ssTb^{2}ss**dAssdCssdGssdCssdGssdTssdCssdCssdAssdCssdAssdGss**Gb^{2}ssAb^2**
253a	**Gb^{1}sGb^{1}sC*b^{1}sGb^{1}sAb^{1}s**dTsdAsdCsdGsdCsdGsdTsdC*sdC*sdAs**C*b^{1}sAb^{1}sGb^{1}sGb^{1}sAb^1**
253b	**Gb^{2}sGb^{2}sC*b^{2}s**dGsdAsdTsdAsdC*sdGsdCsdGsdTsdC*sdC*sdAs**C*b^{2}sAb^{2}sGb^{2}sGb^{2}sAb^2**
253c	**Gb^{1}Gb^{1}C*b^1**dGdAdTdAdCdGdCdGdTdCdCdAdC***Ab^{1}Gb^{1}Gb^{1}Ab^1**
253d	**Gb^{1}s**dGsdCsdGsdAsdTsdAsdCsdGsdC*sdGsdUsdCsdCsdAs**C*b^{1}sAb^{1}sGb^{1}sGb^{1}sAb^1**
253e	**Gb^{4}sGb^{4}sC*b^{4}sGb^{4}s**dAsdTsdAsdCsdGsdCsdGsdTsdCsdCsdAsdCs**Ab^{4}sGb^{4}sGb^{4}sAb^4**
254a	**Tb^{1}sGb^{1}sGb^{1}sC*Gb^{1}s**dAsdTsdAsdCsdGsdCsdGsdTsdCsdCsdAsdC*s**Ab^{1}sGb^{1}sGb^{1}sAb^{1}sC*b^1**
254b	**Tb^{1}Gb^{1}Gb^{1}C*b^{1}Gb^1**dAdTdAdC*dGdCdGdTdCdC*dAdC**Ab^{1}Gb^{1}Gb^{1}Ab^{1}C*b^1**
254c	**Tb^{6}sGb^{6}sGb^{6}sC*b^{6}s**dGsdAsdTsdAsdCsdGsdCsdGsdTsdCsdCsdAsdCsdAsdGs**Gb^{6}sAb^{6}sC*b^6**
255a	**C*b^{1}sTb^{1}sGb^{1}sGb^{1}sC*b^{1}s**dGsdAsdTsdAsdCsdGsdC*sdGsdTsdCsdC*sdAsdCsdAs**Gb^{1}sGb^{1}sAb^{1}sC*b^{1}sGb^1**
255b	**C*b^{1}Tb^{1}Gb^{1}Gb^{1}C*b^1**dGdAdTdAdCdGdC*dGdTdCdC*dAdC*dA**Gb^{1}Gb^{1}Ab^{1}C*b^{1}Gb^1**
256a	**Gb^{1}sC*b^{1}sTb^{1}sGb^{1}sGb^{1}s**dC*sdGsdAsdTsdAsdCsdGsdCsdGsdTsdCsdCsdAsdCsdAsdGs**Gb^{1}sAb^{1}sC*b^{1}sGb^{1}sAb^1**
256b	**Gb^{1}C*b^{1}Tb^{1}Gb^{1}Gb^1**dC*dGdAdTdAdCdGdCdGdTdCdCdAdC*dAdG**Gb^{1}Ab^{1}C*b^{1}Gb^{1}Ab^1**
257a	**Tb^{1}sGb^{1}sC*b^{1}sTb^{1}sGb^{1}s**dGsdCsdGsdAsdTsdAsdC*sdGsdC*sdGsdTsdCsdCsdAsdCsdAsdGs**Gb^{1}sAb^{1}sC*b^{1}sGb^{1}sAb^1**
257b	**Tb^{1}Gb^{1}C*b^{1}Tb^{1}Gb^1**dGdCdGdAdTdAdCdGdCdGdTdCdC*dAdC*dAdG**Gb^{1}Ab^{1}C*b^{1}Gb^{1}Ab^1**
258a	**Gb^{1}sTb^{1}s**dGsdTsdTsdTsdA*sdGs**Gb^{1}sGb^1**
258b	**Gb^{1}sTb^{1}s**dGsdUsdTsdTsdA*sdGs**Gb^{1}sGb^1**
259a	**Ab^{1}sGb^{1}sTb^{1}s**dGsdTsdTsdTsdA*sdGs**Gb^{1}sGb^{1}sAb^1**
259b	**Ab^{1}Gb^{1}Tb^1**dGdUdUdUdA*dG**Gb^{1}Gb^{1}Ab^1**
259c	**Ab^{1}sGb^{1}sTb^{1}s**dGsdTsdTsdTsdA*sdGsdGs**Gb^{1}sAb^1**
259d	**Ab^{1}sGb^{1}s**dTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^1**
259e	**Ab^{1}s**dGsdTsdGsdTsdTsdTsdA*sdGsdGs**Gb^{1}sAb^1**
260a	**Tb^{1}sAb^{1}sGb^{1}sTb^{1}s**dGsdTsdTsdTsdAsdGs**Gb^{1}sGb^{1}sAb^1**
260b	**Tb^{1}Ab^{1}Gb^{1}Tb^1**dGdUdUdUdAdG**Gb^{1}Gb^{1}Ab^1**
260c	**Tb^{1}sAb^{1}sGb^{1}sTb^{1}s**dGsdTsdTsdTsdA*sdGs**Gb^{1}sGb^{1}sAb^1**
260d	**Tb^{1}sAb^{1}sGb^{1}s**dUsdGsdTsdTsdTsdA*sdGsdGs**Gb^{1}sAb^1**
260e	**Tb^{1}sAb^{1}s**dGsdUsdGsdUsdUsdUsdA*sdGsdGsdGs**Ab^1**
260f	**Tb^{1}s**dA*sdGsdTsdGsdTsdTsdUsdA*s**Gb^{1}sGb^{1}sGb^{1}sAb^1**

261a	**Tb^{1}sAb^{1}sGb^{1}sTb^{1}s**dGsdTsdTsdTsdA*sdGs**Gb^{1}sGb^{1}sAb^{1}sGb^1**
261b	**Tb^{1}Ab^{1}Gb^{1}Tb^{1}s**dGsdTsdTsdTsdA*sdGsdG**Gb^{1}Ab^{1}Gb^1**
261c	**Tb^{4}sAb^{4}sGb^{4}sTb^{4}s**dGsdUsdTsdUsdA*sdGsdGsdGs**Ab^{4}sGb^4**
261d	**Tb^{1}s**dA*sdGsdUsdGsdTsdTsdUsdA*sdGsdGsdGsdA*s**Gb^1**
261e	**Tb^{2}sAb^{2}sGb^{2}s**dUsdGsdUsdUsdTsdAsdGsdGs**Gb^{2}sAb^{2}sGb^2**
261f	**Tb^{4}sAb^{4}s**dGsdTsdGsdTsdTsdTsdAsdGsdGs**Gb^{4}sAb^{4}sGb^4**
261g	**Tb^{1}Ab^1**dGdTdGdTdTdTdA*dG**Gb^{1}Gb^{1}Ab^{1}Gb^1**
262a	**Tb^{1}sAb^{1}sGb^{1}sTb^{1}s**dGdTdTdTdA*dGdGs**Gb^{1}sAb^{1}sGb^{1}sC*b^1**
262b	**Tb^{1}ssAb^{1}ss**dGssdTssdGssdTssdTssdTssdAssdGssdGssdGssdGssdAssdGss**C*b^1**
262c	**Tb^{1}sAb^{1}s**dGsdUsdGsdUsdUsdUsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1**
262d	**Tb^1**dAdGdTdGdTdTdTdAdGdGdGdAdG**C*b^1**
262e	**Tb^{1}Ab^1**sdGsdUsdGsdUsdTsdUsdAsdGsdGs**Gb^{1}Ab^{1}Gb^{1}C*b^1**
262f	**Tb^{4}sAb^{4}sGb^{4}s**dTsdGsdTsdTsdTsdAsdGsdGsdsdGsdAs**Gb^{4}sC*b^4**
262g	**Tb^{6}Ab^{6}Gb^6**dUdGdTdTdUdAdGdGdG**Ab^{6}Gb^{6}C*b^6**
262h	**Tb^{1}sAb^{1}sGb^{1}sTb^{1}s**dGsdTsdTsdTsdAsdGsdGsdGsdAsdGs**C*b^1**
262i	**Tb^{1}ssAb^{1}ss**dGssdTssdGssdUssdUssdUssdAssdGssdGssdGssdAss**Gb^1 ssC*b^1**
209s	**Gb^{1}Tb^1**dAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab^{1}Gb^{1}C*b^1**
209t	**Gb^{1}sTb^{1}s**dA*sdGsdTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1**
209u	**Gb^{1}Tb^1**dAdGdTdGdTdTdTdAdGdGdG**Ab^{1}Gb^{1}C*b^1**
209v	/5SpC3s/**Gb^{1}sTb^{1}s**dAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1**
209w	**Gb^{1}sTb^{1}s**dAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1**/s3SpC3/
209x	/5SpC3s/**Gb^{1}sTb^{1}s**dAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1** /3SpC3s/
209y	**Gb^{1}sTb^{1}s**dAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1**
209aa	**Gb^{1}Tb^1**dA*sdGsdUsdGsdUsdUsdUsdAsdGsdGsdGs**Ab^{1}Gb^{1}C*b^1**
209ab	**Gb^{1}Tb^1**dA*sdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab^{1}Gb^{1}C*b^1**
209ac	**Gb^{6}sTb^{6}s**dA*dGdTdGdTdTdTdA*dGdGdG**Ab^{6}sGb^{6}sC*b^6**
209ad	**Gb^{1}sTb^{1}s**dA*sdGsdUsdGsdUsdUsdUsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1**
209ae	**Gb^{1}sTb^{1}s**dA*sdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1**
209af	**Gb^{1}sTb^{1}s**dAsdGsdTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1**
209ag	**Gb^{1}Tb^1**dA*dGdTdGdTdTdTdA*dGdGdG**Ab^{1}Gb^{1}C*b^1**
209ah	**Gb^{1}Tb^1**dAdGdTdGdTdTdTdA*dGdGdG**Ab^{1}Gb^{1}C*b^1**
209ai	**Gb^{6}sTb^{6}s**dA*dGdTdGdTdTdTdAdGdGdG**Ab^{6}sGb^{6}sC*b^6**
209aj	**Gb^{1}sTb^{1}s**dA*sdGsdUsdGsdTsdTsdUsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1**
209ak	**Gb^{7}sTb^{7}sAb^{7}sGb^{7}s**dTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^{7}sGb^{7}sC*b^7**
209am	**Gb^{7}sTb^{7}s**dAsdGsdTsdGsdTsdTsdUsdA*sdGsdGs**Gb^{7}sAb^{7}sGb^{7}sC*b^7**
209an	**Gb^{1}ssTb^{1}ssAb^{1}ss**dGssdTssdGssdTssdTssdTssdA*ssdGssdGssdGss**Ab^1**

	ssGb^{1}ssC*b^1
209ao	**Gb^{4}ssTb^{4}ssAb^{4}ss**dGssdTssdGssdTssdTssdTssdAssdGssdGssdGssdA*ss **Gb^{4}ssC*b^4**
209ap	**Gb^{2}ssTb^{2}ssAb^{2}ssGb^{2}ss**dTssdGssdTssdTssdTssdAssdGssdGssdGssdAssd Gss**C*b^2**
209aq	**Gb^{1}Tb^{1}Ab^{1}Gb^1**dUdGdUdUdUdAdGdG**Gb^{1}Ab^{1}Gb^{1}C*b^1**
209ar	**Gb^{1}sTb^{1}sAb^{1}sGb^{1}sTb^{1}s**dGsdTsdTsdTsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1**
209as	**Gb^{1}sTb^{1}s**dAsdGsdTsdGsdTsdTsdUsdAsdGs**Gb^{1}sGb^{1}sAb^{1}sGb^{1}sC*b^1**
209at	**Gb^{6}sTb^{6}sAb^{6}sGb^{6}s**dTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab^{6}sGb^{6}sC*b^6**
209au	**Gb^{7}sTb^{7}sAb^{7}s**dGsdUsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^{7}sGb^{7}sC*b^7**
209av	**Gb^{4}sTb^{4}sAb^{4}sGb^{4}s**dUsdGsdTsdUsdTsdA*sdGsdGsdGsdA*s**Gb^{4}sC*b^4**
209aw	**Gb^{4}Tb^{4}Ab^{4}Gb^4**dTdGdTdTdTdAdGdGdGdA**Gb^{4}C*b^4**
209ax	**Gb^{1}sTb^{1}sAb^1**sdGsdTsdGsdTsdTsdTsdAsdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1**
209az	**Gb^{1}sTb^{1}sAb^{1}s**dGsdTsdGsdTsdTsdTsdAsdGsdGs**Gb^{1}sAb^{1}sGb^{1}sC*b^1**
209ba	**Gb^{1}sTb^{1}sAb^{1}sGb^{1}s**dTsdGsdTsdTsdTsdAsdGsdGs**Gb^{1}sAb^{1}sGb^{1}sC*b^1**
209bb	**Gb^{1}sTb^{1}sAb^{1}s**dGsdTsdGsdTsdTsdTsdAsdGsdGsdGsdAs**Gb^{1}sC*b^1**
263a	**Gb^{1}sTb^{1}sAb^{1}sGb^{1}sTb^{1}s**dGsdTsdTsdTsdA*sdGsdGs**Gb^{1}sAb^{1}sGb^{1}sC*b^1 sC*b^1**
263b	**Gb^{2}sTb^{2}sAb^{2}s**dGsdTsdGsdTsdTsdTsdA*sdGsdGs**Gb^{2}sAb^{2}sGb^{2}sC*b^{2}sC*b^2**
263c	**Gb^{1}sTb^{1}sAb^{1}sGb^{1}s**dTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^{1}sC*b^1**
263d	**Gb^{1}s**dUsdA*sdGsdUsdGsdUsdTsdTsdA*sdGsdGs**Gb^{1}sAb^{1}sGb^{1}sC*b^{1}sC*b^1**
263e	**Gb^{1}sTb^{1}sAb^{1}s**dGsdTsdGsdUsdTsdTsdA*sdGsdGs**Gb^{1}sAb^{1}sGb^{1}sC*b^{1}sC*b^1**
263f	**Gb^{1}Tb^1**dA*dGdTdGdTdTdTdA*dGdGdG**Ab^{1}Gb^{1}C*b^{1}C*b^1**
263g	**Gb^{1}s**dTsdA*sdGsdTsdGsdTsdTsdTsdA*sdGsdGsdGsdA*s**Gb^{1}sC*b^{1}sC*b^1**
263h	**Gb^{1}Tb^{1}Ab^{1}Gb^{1}Tb^1**dGdTdUdTdAdGdGdGdA*dG**C*b^{1}C*b^1**
263i	**Gb^{1}ssTb^{1}ssAb^{1}ssGb^{1}ssTb^{1}ss**dGssdTssdTssdTssdAssdGssdGssdGssdAss **Gb^{1}ssC*b^{1}ssC*b^1**
263j	**Gb^{4}Tb^4**dA*dGdTdGdTdTdTdAdGdGdGdA***Gb^{4}C*b^{4}C*b^4**
263k	**Gb^{6}sTb^{6}sAb^{6}s**dGsdTsdGsdUsdUsdTsdAsdGsdGsdGsdA*s**Gb^{6}sC*b^{6}sC*b^6**
263m	**Gb^{7}sTb^{7}sAb^{7}sGb^{7}s**dTdGdTdTdTdA*dGdGdGs**Ab^{7}sGb^{7}sC*b^{7}sC*b^7**
264a	**Gb^{1}sGb^{1}sTb^{1}sAb^{1}sGb^{1}s**dTsdGsdTsdTsdTsdA*sdGsdGs**Gb^{1}sAb^{1}sGb^{1}sC*b^1 sC*b^1**
264b	**Gb^{7}sGb^{7}sTb^{7}sAb^{7}sGb^{7}s**dTsdGsdTsdTsdTsdAsdGsdGsdGsdAsdGsdC*s**C*b^7**
264c	**Gb^{1}sGb^{1}sTb^{1}sAb^{1}sGb^{1}s**dTsdGsdTsdTsdTsdAsdGsdGsdGsdA*sdGsdC*s **C*b^1**
264d	**Gb^{1}sGb^{1}sTb^{1}sAb^{1}sGb^{1}s**dUsdGsdTsdTsdTsdAsdGsdGsdGsdA*sdGsdC*s **C*b^1**
264e	**Gb^{1}sGb^{1}sTb^{1}sAb^{1}s**dGsdTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1 sC*b^1**
264f	**Gb^{1}sGb^{1}sTb^{1}s**dA*sdGsdTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1 sC*b^1**
264g	**Gb^{1}sGb^{1}sTb^{1}sAb^{1}s**dGsdTsdGsdTsdTsdTsdA*sdGsdGsdGsdA*s**Gb^{1}sC*b^1 sC*b^1**
264h	**Gb^{1}Gb^1**dUdA*dGdTdGdTdTdTdAdGdG**Gb^{1}Ab^{1}Gb^{1}C*b^{1}C*b^1**

264i	**Gb^{4}Gb^{4}Tb^{4}Ab^4**dGsdTsdGsdTsdTsdTsdAsdGsdGs**Gb^{4}Ab^{4}Gb^{4}C*b^{4}C*b^4**
264j	**Gb^{1}ssGb^{1}ssTb^{1}ss**dA*ssdGssdTssdGssdUssdTssdTssdA*ssdGssdGssdGssdA*ss**Gb^{1}ssC*b^{1}ssC*b^1**
264k	**Gb^{2}Gb^{2}Tb^2**dA*dGdTdGdTdTdTdAdGdG**Gb^{2}Ab^{2}Gb^{2}C*b^{2}C*b^2**
265a	**Gb^{1}sGb^{1}sTb^{1}sAb^{1}sGb^{1}s**dTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1 sC*b^{1}sGb^1**
265b	**Gb^{6}Gb^{6}Tb^{6}Ab^{6}Gb^6**dTdGdTdTdTdA*dGdGdG**Ab^{6}Gb^{6}C*b^{6}C*b^{6}Gb^6**
265c	**Gb^{1}sGb^{1}sTb^{1}sAb^{1}s**dGsdTsdGsdTsdTsdTsdA*sdGsdGsdGsdA*sdGs**C*b^1 sC*b^{1}sGb^1**
265d	**Gb^{1}s**dGsdTsdA*sdGsdUsdGsdTsdUsdTsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1 sC*b^{1}sGb^1**
265e	**Gb^{4}sGb^{4}s**dUsdAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsdA*s**Gb^{4}sC*b^{4}sC*b^4 sGb^4**
265f	**Gb^{2}ssGb^{2}ssTb^{2}ssAb^{2}ssGb^{2}ss**dTssdGssdTssdTssdTssdAssdGssdGssdGssdAssdGssdCss**C*b^{2}ssGb^2**
266a	**Tb^{1}sGb^{1}sGb^{1}sTb^{1}sAb^{1}s**dGsdTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^{1}sGb^1 sC*b^{1}sC*b^{1}sGb^1**
266b	**Tb^{2}sGb^{2}sGb^{2}s**dTsdA*sdGsdTsdGsdTsdTsdTsdA*sdGsdGsdGs**Ab^{2}sGb^2 sC*b^{2}sC*b^{2}sGb^2**
266c	**Gb^{1}Gb^{1}Tb^1**dA*dGdTdGdTdTdTdA*dGdGdGdA***Gb^{1}C*b^{1}C*b^{1}Gb^1**
266d	**Tb^{1}s**dGsdGsdUsdA*sdGsdTsdGsdTsdUsdTsdA*sdGsdGsdGs**Ab^{1}sGb^{1}sC*b^1 sC*b^{1}sGb^1**
266e	**Tb^{4}sGb^{4}sGb^{4}sTb^{4}s**dAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsdAs**Gb^{4}sC*b^4 sC*b^{4}sGb^4**
267a	**Tb^{1}sTb^{1}sGb^{1}sGb^{1}sTb^{1}s**dAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsdA*s**Gb^1 sC*b^{1}sC*b^{1}sGb^{1}sTb^1**
267b	**Tb^{1}Tb^{1}Gb^{1}Gb^{1}Tb^1**dA*dGdTdGdTdTdTdAdGdGdGdA***Gb^{1}C*b^{1}C*b^{1}Gb^{1}Tb^1**
267c	**Tb^{6}sTb^{6}sGb^{6}s**dGsdTsdAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsdAs**Gb^6 sC*b^{6}sC*b^{6}sGb^{6}sTb^6**
268a	**Tb^{1}sTb^{1}sTb^{1}sGb^{1}sGb^{1}s**dTsdA*sdGsdTsdGsdTsdTsdTsdAsdGsdGsdGsdA sdG**C*b^{1}sC*b^{1}sGb^{1}sTb^{1}sC*b^1**
268b	**Tb^{1}Tb^{1}Tb^{1}Gb^{1}Gb^1**dTdA*dGdTdGdTdTdTdAdGdGdGdA*dG**C*b^{1}C*b^{1}Gb^{1}Tb^1 C*b^1**
269a	**Ab^{1}sTb^{1}sTb^{1}sTb^{1}sGb^{1}s**dGsdTsdAsdGsdTsdGsdTsdTsdTsdAsdGsdGsdG sdAsdGsdC*s**C*b^{1}sGb^{1}sTb^{1}sC*b^{1}sTb^1**
269b	**Ab^{1}Tb^{1}Tb^{1}Tb^{1}Gb^1**dGdTdAdGdTdGdTdTdTdAdGdGdGdAdGdC***C*b^{1}Gb^{1}Tb^1 C*b^{1}Tb^1**
270a	T**b^{1}s**A**b^{1}s**T**b^{1}s**T**b^{1}s**T**b^{1}s**dGsdGsdTsdAsdGsdTsdGsdTsdTsdTsdAsdGsdG sdGsdAsdGsdC*sdCs**Gb^{1}sTb^{1}sC*b^{1}sTb^{1}sTb^1**
270b	T**b^1**A**b^1**T**b^1**T**b^1**T**b^1**dGdGdTdAdGdTdGdTdTdTdAdGdGdGdAdGdC*dC***Gb^{1}Tb^1 C*b^{1}Tb^{1}Tb^1**
271a	**Ab^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*s**Gb^{1}sTb^1**
271b	**Ab^{1}Tb^1**dTdTdGdGdTdA***Gb^{1}Tb^1**
272a	**Tb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*s**Gb^{1}sTb^{1}sGb^1**
272b	**Tb^{1}Ab^{1}Tb^1**dTdTdGdGdTdA***Gb^{1}Tb^{1}Gb^1**
272c	**Tb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*sdGs**Tb^{1}sGb^1**
272d	**Tb^{1}sAb^{1}s**dTsdTsdTsdGsdGsdTsdA*sdGsdUs**Gb^1**
272e	**Tb^{1}s**dAsdTsdUsdTsdGsdGsdUsdA*sdGs**Tb^{1}sGb^1**

273a	**Tb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdAs**Gb^{1}sTb^{1}sGb^{1}sTb^1**
273b	**Tb^{1}Ab^{1}Tb^1**dUdUdGdGdUdA**Gb^{1}Tb^{1}Gb^{1}Tb^1**
273c	**Tb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*s**Gb^{1}sTb^{1}sGb^{1}sTb^1**
273d	**Tb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*sdGsdUs**Gb^{1}sTb^1**
273e	**Tb^{1}sAb^{1}s**dUsdUsdUsdGsdGsdUsdA*sdGsdUsdGs**Tb^1**
273f	**Tb^{1}s**dA*sdTsdTsdUsdGsdGsdTsdA*s**Gb^{1}sTb^{1}sGb^{1}sTb^1**
274a	**C*b^{1}Tb^{1}Ab^{1}s**dUsdTsdTsdGsdGsdTsdA*s**Gb^{1}Tb^{1}Gb^{1}Tb^1**
274b	**C*b^{4}sTb^{4}sAb^{4}sTb^{4}s**dTsdTsdGsdGsdTsdA*sdGsdUs**Gb^{4}sTb^4**
274c	**C*b^{1}s**dUsdA*sdTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**Tb^1**
274d	**C*b^{2}sTb^{2}sAb^{2}s**dTsdTsdUsdGsdGsdTsdA*sdGs**Tb^{2}sGb^{2}sTb^2**
274e	**C*b^{4}ssTb^{4}ss**dAssdTssdTssdTssdGssdGssdTssdAssdGss**Tb^{4}ssGb^{4}ssTb^4**
274f	**C*b^{1}Tb^{1}Ab^1**dTdTdTdGdGdTdA***Gb^{1}Tb^{1}Gb^{1}Tb^1**
274g	**C*b^{1}sTb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*s**Gb^{1}sTb^{1}sGb^{1}sTb^1**
275a	**C*b^{1}sTb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*sdGsdTsdGsdTs**Tb^1**
275b	**C*b^{1}sTb^{1}s**dA*sdUsdTsdUsdGsdGsdTsdAsdGsdUs**Gb^{1}sTb^{1}sTb^1**
275c	**C*b^{4}sTb^{4}sAb^{4}s**dTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**Tb^{4}sTb^4**
275d	**C*b^{1}ssTb^{1}ss**dAssdTssdTssdTssdGssdGssdTssdAssdGssdTssdGssdTss**Tb^1**
275e	**C*b^{1}ssTb^{1}ss**dAssdUssdTssdTssdGssdGssdTssdAssdGssdUssdGss**Tb^1 ssTb^1**
275f	**C*b^{1}sTb^{1}sAb^{1}sTb^{1}s**dTdTdGdGdTdA*dGs**Tb^{1}sGb^{1}sTb^{1}sTb^1**
275g	**C*b^{1}Tb^{1}s**dAsdTsdTsdTsdGsdGsdTsdA*sdGs**Tb^{1}Gb^{1}Tb^{1}Tb^1**
275h	**C*b^{6}Tb^{6}Ab^6**dUdTdTdGdGdTdA*dGdU**Gb^{6}Tb^{6}Tb^6**
275i	**C*b^1**dTdAdTdTdTdGdGdTdAdGdTdGdT**Tb^1**
210o	**Gb^{1}C*b^{1}Tb^{1}Ab^1**dTsdTsdTsdGsdGsdTsdAsdGsdTs**Gb^{1}Tb^{1}Tb^1**
210p	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^1**
210q	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dTsdTsdTsdGsdGsdTsdAsdGsdTs**Gb^{1}sTb^{1}sTb^1**
210r	**Gb^{1}C*b^{1}Tb^{1}Ab^1**dTdTdTdGdGdTdA*dGdT**Gb^{1}Tb^{1}Tb^1**
210s	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dUsdUsdTdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^1**
210t	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dUsdTsdTsdGsdGsdTsdAsdGsdUs**Gb^{1}sTb^{1}sTb^1**
210u	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dUsdUsdUsdGsdGsdUsdA*sdGsdUs**Gb^{1}sTb^{1}sTb^1**
210v	**/5SpC3s/Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dTsdTsdTsdGsdGsdTsdAsdGsdTs**Gb^{1}sTb^1 sTb^1**
210w	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dTsdTsdTsdGsdGsdTsdAsdGsdTs**Gb^{1}sTb^{1}sTb^1 /3SpC3s/**
210x	**/5SpC3s/Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dTsdTsdTsdGsdGsdTsdAsdGsdTs**Gb^{1}sTb^1 sTb^1/3SpC3s/**
210y	**Gb^{1}C*b^{1}Tb^{1}Ab^{1}s**dTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}Tb^{1}Tb^1**
210z	**Gb^{1}C*b^{1}Tb^{1}Ab^{1}s**dUsdTsdTsdGsdGsdUsdA*sdGsdTs**Gb^{1}Tb^{1}Tb^1**

210aa	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dTdTdTdGdGdTdA*dGdTs**Gb^{1}sTb^{1}sTb^1**
210ab	**Gb^{6}sC*b^{6}sTb^{6}sAb^{6}s**dTdTdTdGdGdTdA*dGdTs**Gb^{6}sTb^{6}sTb^6**
210ac	**Gb^{6}sC*b^{6}sTb^{6}s**dAsdTsdTsdTsdGsdGsdTsdAsdGs**Tb^{6}sGb^{6}sTb^{6}sTb^6**
210ad	**Gb^{7}sC*b^{7}sTb^{7}s**dA*sdTsdTsdTsdGsdGsdTsdA*sdGs**Tb^{7}sGb^{7}sTb^{7}sTb^7**
210ae	**Gb^{7}sC*b^{7}s**dUsdAsdTsdTsdUsdGsdGsdUsdA*sdGs**Tb^{7}sGb^{7}sTb^{7}sTb^7**
210af	**Gb^{1}ssC*b^{1}ssTb^{1}ss**dAssdTssdTssdTssdGssdGssdTssdA*ssdGssdTss**Gb^{1}ssTb^{1}ssTb^1**
210ag	**Gb^{4}ssC*b^{4}ssTb^{4}ss**dA*ssdTssdTssdTssdGssdGssdTssdAssdGssdTssdGss**Tb^{4}ssTb^4**
210ah	**Gb^{2}ssC*b^{2}ssTb^{2}ssAb^{2}ss**dTssdTssdTssdGssdGssdTssdAssdGssdTssdGssdTss**Tb^2**
210ai	**Gb^{1}C*b^{1}Tb^{1}Ab^1**dUsdTsdTsdGsdGsdTsdAsdGs**Tb^{1}Gb^{1}Tb^{1}Tb^1**
210aj	**Gb^{4}C*b^{4}Tb^{4}Ab^4**dTsdTsdTsdGsdGsdTsdAsdGsdTdG**Tb^{4}Tb^4**
210ak	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^1**
210am	**Gb^{4}sC*b^{4}sTb^{4}sAb^{4}s**dTsdTsdUsdGsdGsdTsdA*sdGsdTsdGs**Tb^{4}sTb^4**
210an	**Gb^{7}sC*b^{7}sTb^{7}s**dA*sdTsdTsdUsdGsdGsdTsdA*sdGsdTs**Gb^{7}sTb^{7}sTb^7**
210ao	**Gb^{1}sC*b^{1}s**dUsdAsdUsdUsdTsdGsdGsdUsdAs**Gb^{1}sTb^{1}sGb^{1}sTb^{1}sTb^1**
210ap	**Gb^{1}sC*b^{1}sTb^1**sdAsdTsdTsdTsdGsdGsdTsdAsdGsdTs**Gb^{1}sTb^{1}sTb^1**
210aq	**Gb^{1}sC*b^{1}sTb^{1}s**dAsdTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**Tb^{1}sTb^1**
276a	**Gb^{1}sC*b^{1}sTb^{1}sAb^{1}sTb^{1}s**dTsdTsdGsdGsdTsdA*sdGs**Tb^{1}sGb^{1}sTb^{1}sTb^{1}sTb^1**
276b	**Gb^{2}sC*b^{2}sTb^{2}s**dAsdTsdTsdTsdGsdGsdTsdA*sdGs**Tb^{2}sGb^{2}sTb^{2}sTb^{2}sTb^2**
276c	**Gb^{1}sC*b^{1}sTb^{1}s**dAsdTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^{1}sTb^1**
276d	**Gb^{2}s**dC*sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGs**Tb^{2}sGb^{2}sTb^{2}sTb^{2}sTb^2**
276e	**Gb^{6}sC*b^{6}sTb^{6}s**dA*sdUsdUsdUsdGsdGsdUsdA*sdGsdUsdGs**Tb^{6}sTb^{6}sTb^6**
276f	**Gb^{1}s**dC*sdTsdA*sdUsdUsdUsdGsdGsdUsdAsdGsdUsdGs**Tb^{1}sTb^{1}sTb^1**
276g	**Gb^{1}C*b^1**dTdA*dTdTdTdGdGdTdA*dGdT**Gb^{1}Tb^{1}Tb^{1}Tb^1**
276h	**Gb^{4}C*b^{4}Tb^{4}Ab^4**dTdTdTdGdGdTdA*dGdTdG**Tb^{4}Tb^{4}Tb^4**
276i	**Gb^{1}C*b^{1}Tb^{1}Ab^{1}Tb^1**dUdTdGdGdTdA*dGdTdGdU**Tb^{1}Tb^1**
276j	**Gb^{1}ssC*b^{1}ssTb^{1}ssAb^{1}ssTb^{1}ss**dTssdTssdGssdGssdTssdAssdGssdTssdGss**Tb^{1}ssTb^{1}ssTb^1**
276k	**Gb^{7}sC*b^{7}sTb^{7}sAb^{7}s**dTdTdTdGdGdTdA*dGdTs**Gb^{7}sTb^{7}sTb^{7}sTb^7**
277a	**Ab^{1}sGb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dTsdTsdTsdGsdGsdTsdA*sdGs**Tb^{1}sGb^{1}sTb^{1}sTb^{1}sTb^1**
277b	**Ab^{7}sGb^{7}sC*b^{7}sTb^{7}sAb^{7}s**dTsdTsdTsdGsdGsdTsdA*sdGsdTsdGsdTsdTs**Tb^7**
277c	**Ab^{1}sGb^{1}s**dC*sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGs**Tb^{1}sGb^{1}sTb^{1}sTb^{1}sTb^1**
277d	**Ab^{1}sGb^{1}s**dC*sdTsdAsdTsdTsdTsdGsdGsdTsdA*sdGs**Tb^{1}sGb^{1}sTb^{1}sTb^{1}sTb^1**
277e	**Ab^{1}Gb^1**dC*dTdAdUdTdTdGdGdTdA*dG**Tb^{1}Gb^{1}Tb^{1}Tb^{1}Tb^1**
277f	**Ab^{2}Gb^{2}C*b^2**dTdAdTdTdTdGdGdTdA*dG**Tb^{2}Gb^{2}Tb^{2}Tb^{2}Tb^2**

277g	**Ab^{1}sGb^{1}sC*b^{1}sTb^{1}s**dA*sdTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^{1}sTb^1**
277h	**Ab^{1}sGb^{1}sC*b^{1}sTb^{1}s**dA*sdTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**Tb^{1}sTb^{1}sTb^1**
277i	**Ab^{1}sGb^{1}sC*b^{1}s**dTsdA*sdTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^{1}sTb^1**
277j	**Ab^{4}Gb^{4}C*b^{4}Tb^{4}s**dAsdTsdTsdTsdGsdGsdTsdAsdGs**Tb^{4}Gb^{4}Tb^{4}Tb^{4}Tb^4**
277k	**Ab^{1}ssGb^{1}ssC*b^{1}ss**dTssdA*ssdTssdTssdTssdGssdGssdTssdA*ssdGssdUssdGss**Tb^{1}ssTb^{1}ssTb^1**
278a	**Ab^{1}sGb^{1}sC*b^{1}sTb^{1}sAb^{1}s**dTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^{1}sTb^{1}sAb^1**
278b	**Ab^{2}ssGb^{2}ssC*b^{2}ssTb^{2}ssAb^{2}ss**dTssdTssdTssdGssdGssdTssdAssdGssdTssdGssdTssdTss**Tb^{2}ssAb^2**
278c	**Ab^{1}s**dGsdC*sdTsdAsdTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^{1}sTb^{1}sTb^{1}sAb^1**
278d	**Ab^{1}s**dGsdC*sdTsdAsdTsdTsdTsdGsdGsdUsdA*sdGsdUs**Gb^{1}sTb^{1}sTb^{1}sTb^{1}sTb^{1}sAb^1**
278e	**Ab^{1}sGb^{1}sC*b^{1}s**dTsdAsdTsdTsdTsdGsdGsdTsdA*sdGsdTsdGs**Tb^{1}sTb^{1}sTb^{1}sTb^{1}sAb^1**
278f	**Ab^{4}sGb^{4}s**dC*sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**Tb^{4}sTb^{4}sTb^{4}sAb^4**
278g	**Ab^{6}Gb^{6}C*b^{6}Tb^{6}Ab^6**dTdTdTdGdGdTdA*dGdT**Gb^{6}Tb^{6}Tb^{6}Tb^{6}Ab^6**
279a	**Gb^{1}sAb^{1}sGb^{1}sC*b^{1}sTb^{1}s**dAsdTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^{1}sTb^{1}sAb^1**
279b	**Gb^{2}sAb^{2}sGb^{2}s**dC*sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGsdTs**Gb^{2}sTb^{2}sTb^{2}sTb^{2}sAb^2**
279c	**Gb^{1}s**dAsdGsdC*sdUsdAsdTsdTsdTsdGsdGsdTsdA*sdGsdTs**Gb^{1}sTb^{1}sTb^{1}sTb^{1}sAb^1**
279d	**Gb^{4}sAb^{4}sGb^{4}sC*b^{4}s**dTsdAsdTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**Tb^{4}sTb^{4}sTb^{4}sAb^4**
279e	**Gb^{1}Ab^{1}Gb^1**dC*dTdAdTdTdTdGdGdTdAdGdTdG**Tb^{1}Tb^{1}Tb^{1}Ab^1**
280a	**Ab^{1}sGb^{1}sAb^{1}sGb^{1}sC*b^{1}s**dTsdAsdTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**Tb^{1}sTb^{1}sTb^{1}sAb^{1}sGb^1**
280b	**Ab^{1}Gb^{1}Ab^{1}Gb^{1}C*b^1**dTdAdTdTdTdGdGdTdAdGdTdG**Tb^{1}Tb^{1}Tb^{1}Ab^{1}Gb^1**
280c	**Ab^{1}sGb^{1}sAb^{1}sGb^{1}s**dC*sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGsdTsdGs**Tb^{1}sTb^{1}sTb^{1}sAb^{1}sGb^1**
280d	**Ab^{6}sGb^{6}sAb^{6}sGb^{6}s**dC*sdTsdAsdTsdTsdTsdGsdGsdTsdA*sdGsdTsdGsdTsdTs**Tb^{6}sAb^{6}sGb^6**
281a	**Ab^{1}sAb^{1}sGb^{1}sAb^{1}sGb^{1}s**dC*sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGsdTsdGsdTs**Tb^{1}sTb^{1}sAb^{1}sGb^{1}sGb^1**
281b	**Ab^{1}Ab^{1}Gb^{1}Ab^{1}Gb^1**dC*dTdAdTdTdTdGdGdTdAdGdTdGdT**Tb^{1}Tb^{1}Ab^{1}Gb^{1}Gb^1**
282a	**Gb^{1}sAb^{1}sAb^{1}sGb^{1}sAb^{1}s**dGsdC*sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGsdTsdGsdTsdTs**Tb^{1}sAb^{1}sGb^{1}sGb^{1}sGb^1**
282b	**Gb^{1}Ab^{1}Ab^{1}Gb^{1}Ab^1**dGdC*dTdAdTdTdTdGdGdTdAdGdTdGdTdT**Tb^{1}Ab^{1}Gb^{1}Gb^{1}Gb^1**
283a	**Ab^{1}sGb^{1}sAb^{1}sAb^{1}sGb^{1}s**dAsdGsdC*sdTsdAsdTsdTsdTsdGsdGsdTsdAsdGs

	dTsdGsdTsdTsdTs**Ab^{1}sGb^{1}sGb^{1}sGb^{1}sAb^1**
283b	**Ab^{1}Gb^{1}Ab^{1}Ab^{1}Gb^1**dAdGdC*dTdAdTdTdTdGdGdTdAdGdTdGdTdTdT**Ab^{1}Gb^{1}Gb^{1}Gb^{1}Ab^1**
284a	**C*b^{1}sAb^{1}s**dTsdTsdAsdAsdTsdA*s**Ab^{1}sAb^1**
284b	**C*b^{1}Ab^1**dTdTdA*dAdTdA***Ab^{1}Ab^1**
285a	**Gb^{1}sC*b^{1}sAb^{1}s**dTsdTsdA*sdA*sdTsdAs**Ab^{1}sAb^{1}sGb^1**
285b	**Gb^{1}sC*b^{1}sAb^{1}s**dTsdTsdA*sdA*sdTsdAsdA*s**Ab^{1}sGb^1**
285c	**Gb^{1}sC*b^{1}s**dAsdTsdTsdA*sdA*sdUsdAsdA*sdA*s**Gb^1**
285d	**Gb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAs**Ab^{1}sGb^1**
285e	**Gb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdA*s**Ab^{1}sGb^1**
285f	**Gb^{1}C*b^{1}Ab^1**dTdTdA*dA*dTdA**Ab^{1}Ab^{1}Gb^1**
286a	**Gb^{1}sC*b^{1}sAb^{1}sTb^{1}s**dTsdAsdAsdTsdAsdAs**Ab^{1}sGb^{1}sTb^1**
286b	**Gb^{1}sC*b^{1}sAb^{1}sTb^{1}s**dTsdA*sdA*sdTsdAsdAs**Ab^{1}sGb^{1}sTb^1**
286c	**Gb^{1}sC*b^{1}sAb^{1}s**dUsdTsdAsdAsdTsdAsdAsdA*s**Gb^{1}sTb^1**
286d	**Gb^{1}sC*b^{1}s**dAsdTsdTsdAsdA*sdUsdAsdAsdAsdGs**Tb^1**
286e	**Gb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAs**Ab^{1}sAb^{1}sGb^{1}sTb^1**
286f	**Gb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdA*s**Ab^{1}sAb^{1}sGb^{1}sTb^1**
286g	**Gb^{1}C*b^{1}Ab^{1}Tb^1**dUdAdAdUdAdA**Ab^{1}Gb^{1}Tb^1**
287a	**Gb^{1}sGb^{1}sC*b^{1}sAb^{1}s**dTsdTsdA*sdAsdTsdAs**Ab^{1}sAb^{1}sGb^{1}sTb^1**
287b	**Gb^{4}sGb^{4}sC*b^{4}sAb^{4}s**dTsdTsdA*sdAsdUsdAsdAsdA*s**Gb^{4}sTb^4**
287c	**Gb^{1}s**dGsdCsdAsdUsdUsdAsdAsdTsdA*sdA*sdA*sdGs**Tb^1**
287d	**Gb^{2}sGb^{2}sC*b^{2}s**dA*sdUsdTsdA*sdAsdTsdAsdA*s**Ab^{2}sGb^{2}sTb^2**
287e	**Gb^{1}Gb^{1}C*b^{1}Ab^{1}s**dTsdTsdAsdAsdTsdA*sdA*s**Ab^{1}Gb^{1}Tb^1**
287f	**Gb^{1}sGb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAs**Ab^{1}sAb^{1}sGb^{1}sTb^1**
287g	**Gb^{1}sGb^{1}s**dC*sdA*sdTsdTsdA*sdA*sdTsdA*s**Ab^{1}sAb^{1}sGb^{1}sTb^1**
287h	**Gb^{1}Gb^1**dC*dAdTdTdAdAdTdA**Ab^{1}Ab^{1}Gb^{1}Tb^1**
287i	**Gb^{4}ssGb^{4}ss**dCssdAssdTssdTssdAssdAssdTssdAss**Ab^{4}ssAb^{4}ssGb^{4}ssTb^4**
287j	**Gb^{4}ssGb^{4}ss**dC*ssdAssdTssdTssdAssdAssdTssdAss**Ab^{4}ssAb^{4}ssGb^{4}ssTb^4**
288a	G**b^{1}s**G**b^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}s**T**b^{1}s**G**b^1**
288b	G**b^{1}s**G**b^{1}s**C***b^{1}s**A**b^{1}s**dTsdTsdA*sdA*sdTsdAsdAsdAsdGsdTsG**b^1**
288c	G**b^{4}s**G**b^{4}sC*b^{4}s**dAsdTsdTsdAsdAsdTsdAsdAsdAsdGsT**b^{4}s**G**b^4**
288d	**Gb^{1}sGb^{1}sC*b^{1}sAb^{1}s**dTdTdAdAdTdAdAs**Ab^{1}sGb^{1}sTb^{1}sGb^1**
288e	**Gb^{1}Gb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAs**Ab^{1}Gb^{1}Tb^{1}Gb^1**
288f	G**b^{1}ss**G**b^{1}ss**dCssdAssdUssdUssdAssdAssdUssdAssdAssdAssdGss**Tb^1 ssGb^1**
288g	G**b^{1}ss**G**b^{1}ss**dCssdAssdTssdTssdAssdAssdTssdAssdAssdAssdGssdTss**Gb^1**
288h	**Gb^{6}Gb^{6}C*b^6**dA*dTdTdAdAdUdA*dA*dA**Gb^{6}Tb^{6}Gb^6**
288i	**Gb^{1}Gb^{1}C*b^1**dAdTdTdAdAdUdAdAdA**Gb^{1}Tb^{1}Gb^1**

289a	**Ab^{1}sGb^{1}sGb^{1}sC*b^{1}sAb^{1}s**dTsdTsdA*sdA*sdTsdA*s**Ab^{1}sAb^{1}sGb^{1}sTb^{1}sGb^1**
289b	**Ab^{1}sGb^{1}sGb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAs**Ab^{1}sAb^{1}sGb^{1}sTb^{1}sGb^1**
289c	**Ab^{1}sGb^{1}sGb^{1}s**dC*sdA*sdTsdTsdA*sdA*sdTsdA*s**Ab^{1}sAb^{1}sGb^{1}sTb^{1}sGb^1**
289d	**Ab^{2}sGb^{2}sGb^{2}s**dC*sdAsdTsdTsdAsdAsdTsdAs**Ab^{2}sAb^{2}sGb^{2}sTb^{2}sGb^2**
289e	**Ab^{1}s**dGsdGsdC*sdAsdTsdTsdAsdAsdTsdAs**Ab^{1}sAb^{1}sGb^{1}sTb^{1}sGb^1**
289f	**Ab^{1}s**dGsdGsdC*sdAsdTsdUsdAsdAsdUsdAs**Ab^{1}sAb^{1}sGb^{1}sTb^{1}sGb^1**
289g	**Ab^{1}sGb^{1}sGb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAs**Ab^{1}sGb^{1}sTb^{1}sGb^1**
289h	**Ab^{1}sGb^{1}sGb^{1}sC*b^{1}s**dA*sdTsdTsdA*sdA*sdTsdA*sdAsdAs**Gb^{1}sTb^{1}sGb^1**
289i	**Ab^{6}sGb^{6}sGb^{6}s**dC*sdA*sdUsdTsdAsdAsdTsdAsdAsdAs**Gb^{6}sTb^{6}sGb^6**
289j	**Ab^{1}sGb^{1}sGb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^1**
289k	**Ab^{1}s**dGsdGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^1**
289m	**Ab^{1}Gb^{1}Gb^{1}C*b^{1}Ab^1**dUdTdA*dA*dTdAdAdAdG**Tb^{1}Gb^1**
289n	**Ab^{1}Gb^1**dGdC*dAdTdTdAdAdTdAdA**Ab^{1}Gb^{1}Tb^{1}Gb^1**
289o	**Ab^{4}Gb^{4}Gb^4**dCdA*dTdTdAdAdTdAdA***Ab^{4}Gb^{4}Tb^{4}Gb^4**
289p	**Ab^{1}ssGb^{1}ssGb^{1}ssC*b^{1}ssAb^{1}ss**dTssdTssdAssdAssdTssdAssdAssdAss **Gb^{1}ssTb^{1}ssGb^1**
289q	**Ab^{7}sGb^{7}sGb^{7}sC*b^{7}s**dA*dTdTdAdAdTdAdA*s**Ab^{7}sGb^{7}sTb^{7}sGb^7**
213j	**C*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^1**
213k	**C*b^{1}sAb^{1}sGb^{1}s**dGsdCsdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^1**
213m	**C*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdA*sdA*s**Gb^{1}sTb^{1}sGb^1**
213n	**C*b^{1}Ab^{1}Gb^1**dGdC*dAdTdTdAdAdTdAdAdA**Gb^{1}Tb^{1}Gb^1**
213o	**/5SpC3s/C*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^1 sGb^1**
213p	**C*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^1 /3SpC3s/**
213q	**/5SpC3s/C*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^1 sGb^1/3SpC3s/**
213r	**C*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdUsdAsdAsdA*s**Gb^{1}sTb^{1}sGb^1**
213s	**C*b^{6}sAb^{6}sGb^{6}s**dGdC*dAdTdTdAdAdTdAdAdAs**Gb^{6}sTb^{6}sGb^6**
213t	**C*b^{1}sAb^{1}sGb^{1}s**dGdC*dAdTdTdAdAdTdAdAdAs**Gb^{1}sTb^{1}sGb^1**
213u	**C*b^{1}Ab^{1}Gb^{1}s**dGsdC*sdAsdUsdUsdAsdAsdUsdAsdAsdAs**Gb^{1}Tb^{1}Gb^1**
213v	**C*b^{1}Ab^{1}Gb^{1}s**dGsdC*sdAsdTsdTsdAsdA*sdTsdAsdAsdA*s**Gb^{1}Tb^{1}Gb^1**
213w	**C*b^{1}Ab^{1}Gb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}Tb^{1}Gb^1**
213x	**C*b^{7}sAb^{7}sGb^{7}sGb^{7}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{7}sTb^{7}sGb^7**
213y	**C*b^{6}sAb^{6}sGb^{6}sGb^{6}s**dCsdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{6}sTb^{6}sGb^6**
213z	**C*b^{7}sAb^{7}sGb^{7}s**dGsdCsdA*sdUsdTsdAsdAsdTsdAsdAs**Ab^{7}sGb^{7}sTb^{7}sGb^7**
213aa	**C*b^{4}sAb^{4}sGb^{4}sGb^{4}s**dC*sdA*sdTsdTsdAsdAsdTsdAsdA*sdAsdGs**Tb^{4}sGb^4**
213ab	**C*b^{1}sAb^{1}sGb^{1}sGb^{1}sC*b^{1}s**dA*sdTsdTsdA*sdA*sdTsdAsdA*sdA*s**Gb^{1}sTb^1**

	sGb^1
213ac	**C*b^{1}sAb^{1}sGb^{1}s**dGsdCsdAsdTsdTsdA*sdA*sdTsdAs**Ab^{1}sAb^{1}sGb^{1}sTb^{1}sGb^1**
213ad	**C*b^{1}sAb^{1}s**dGsdGsdC*sdAsdTsdTsdAsdAsdUsdAsdAs**Ab^{1}sGb^{1}sTb^{1}sGb^1**
213ae	**C*b^{1}ssAb^{1}ssGb^{1}ss**dGssdC*ssdAssdTssdTssdAssdAssdTssdAssdAss**Ab^{1}ssGb^{1}ssTb^{1}ssGb^1**
213af	**C*b^{4}ssAb^{4}ssGb^{4}ss**dGssdCssdAssdTssdTssdA*ssdAssdTssdAssdAssdAssdGss**Tb^{4}ssGb^4**
213ag	**C*b^{2}ssAb^{2}ssGb^{2}ssGb^{2}ss**dCssdAssdTssdTssdAssdAssdTssdAssdAssdAssdGssdTss**Gb^2**
213ah	**C*b^{1}Ab^{1}Gb^{1}Gb^1**dCdAdTdTdAdAdUdAdA**Ab^{1}Gb^{1}Tb^{1}Gb^1**
213ai	**C*b^{4}Ab^{4}Gb^{4}Gb^4**dCdAdTdTdAdAdTdAdAdAdG**Tb^{4}Gb^4**
213aj	**C*b^{1}Ab^{1}Gb^1**dGdCdAdTdTdAdAdUdAdAdA**Gb^{1}Tb^{1}Gb^1**
213ak	**C*b^{1}sAb^{1}sGb^{1}sGb^1**sdCsdAsdTsdTsdAsdAsdTsdAsdAs**Ab^{1}sGb^{1}sTb^{1}sGb^1**
290a	**C*b^{1}sAb^{1}sGb^{1}sGb^{1}sC*b^{1}s**dAsdTsdTsdAsdAsdTsdA*sdAs**Ab^{1}sGb^{1}sTb^{1}sGb^{1}sC*b^1**
290b	**C*b^{1}sAb^{1}sGb^{1}sGb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^{1}sC*b^1**
290c	**C*b^{1}sAb^{1}sGb^{1}sGb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAsdAsdGs**Tb^{1}sGb^{1}sC*b^1**
290d	**C*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^{1}sC*b^1**
290e	**C*b^{7}sAb^{7}sGb^{7}sGb^{7}sC*b^{7}s**dA*sdTsdTsdAsdAsdTsdAsdAsdAsdGsdTsdG**sC*b^7**
290f	**C*b^{4}Ab^{4}Gb^{4}Gb^{4}s**dCsdAsdTsdTsdAsdAsdTsdA*sdAs**Ab^{4}Gb^{4}Tb^{4}Gb^{4}C*b^4**
290g	**C*b^{1}ssAb^{1}ssGb^{1}ss**dGssdC*ssdAssdTssdTssdA*ssdAssdTssdA*ssdAssdA*ssdGss**Tb^{1}ssGb^{1}ssC*b^1**
290h	**C*b^{2}Ab^{2}Gb^2**dGdC*dAdTdTdAdAdTdAdA**Ab^{2}Gb^{2}Tb^{2}Gb^{2}C*b^2**
290i	**C*b^{1}Ab^1**dGdGdC*dA*dUdUdAdAdUdA*dA***Ab^{1}Gb^{1}Tb^{1}Gb^{1}C*b^1**
291a	**Ab^{1}sC*b^{1}sAb^{1}sGb^{1}sGb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAs**Ab^{1}sGb^{1}sTb^{1}sGb^{1}sC*b^1**
291b	**Ab^{1}sC*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAsdGs**Tb^{1}sGb^{1}sC*b^1**
291c	**Ab^{4}sC*b^{4}s**dAsdGsdGsdC*sdAsdTsdTsdAsdAsdUsdAsdAsdAs**Gb^{4}sTb^{4}sGb^{4}sC*b^4**
291d	**Ab^{1}s**dC*sdAsdGsdGsdC*sdA*sdTsdTsdAsdAsdTsdAsdAs**Ab^{1}sGb^{1}sTb^{1}sGb^{1}sC*b^1**
291e	**Ab^{2}ssC*b^{2}ssAb^{2}ssGb^{2}ssGb^{2}ss**dCssdAssdTssdTssdAssdAssdTssdAssdAssdAssdGssdTss**Gb^{2}ssC*b^2**
291f	**Ab^{6}C*b^{6}Ab^{6}Gb^{6}Gb^6**dC*dAdTdTdAdAdTdAdA**Ab^{6}Gb^{6}Tb^{6}Gb^{6}C*b^6**
292a	**Ab^{1}sC*b^{1}sAb^{1}sGb^{1}sGb^{1}s**dC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{1}sTb^{1}sGb^{1}sC*b^{1}sAb^1**
292b	**Ab^{2}sC*b^{2}sAb^{2}s**dGsdGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs**Gb^{2}sTb^{2}sGb^{2}sC*b^{2}sAb^2**

292c	**Ab^{1}s**dC*sdAsdGsdGsdC*sdAsdUsdUsdAsdAsdUsdAsdAsdAs**Gb^{1}sTb^{1}sGb^1 sC*b^{1}sAb^1**
292d	**Ab^{4}sC*b^{4}sAb^{4}sGb^{4}s**dGsdCsdAsdTsdTsdAsdAsdTsdAsdAsdAsdGs**Tb^{4}sGb^4 sC*b^{4}sAb^4**
292e	**Ab^{1}C*b^{1}Ab^1**dGdGdC*dAdTdTdAdAdTdAdAdAdG**Tb^{1}Gb^{1}C*b^{1}Ab^1**
293a	**Tb^{1}sAb^{1}sC*b^{1}sAb^{1}sGb^{1}s**dGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAsdGs**Tb^1 sGb^{1}sC*b^{1}sAb^{1}sAb^1**
293b	**Tb^{1}Ab^{1}C*b^{1}Ab^{1}Gb^1**dGdC*dAdTdTdAdAdTdAdAdAdG**Tb^{1}Gb^{1}C*b^{1}Ab^{1}Ab^1**
293c	**Tb^{6}sAb^{6}sC*b^{6}s**dAsdGsdGsdCsdAsdTsdTsdAsdAsdTsdAsdAsdAsdGs**Tb^6 sGb^{6}sC*b^{6}sAb^{6}sAb^6**
294a	**Ab^{1}sTb^{1}sAb^{1}sC*b^{1}sAb^{1}s**dGsdGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAsdG sdTs**Gb^{1}sC*b^{1}sAb^{1}sAb^{1}sAb^1**
294b	**Ab^{1}Tb^{1}Ab^{1}C*b^{1}Ab^1**dGdGdC*dAdTdTdAdAdTdAdAdAdGdT**Gb^{1}C*b^{1}Ab^{1}Ab^1 Ab^1**
295a	**Tb^{1}sAb^{1}sTb^{1}sAb^{1}sC*b^{1}s**dAsdGsdGsdC*sdAsdTsdTsdAsdAsdTsdAsdAsdAs dGsdTsdGs**C*b^{1}sAb^{1}sAb^{1}sAb^{1}sTb^1**
295b	**Tb^{1}Ab^{1}Tb^{1}Ab^{1}C*b^1**dAdGdGdC*dAdTdTdAdAdTdAdAdAdGdTdG**C*b^{1}Ab^{1}Ab^1 Ab^{1}Tb^1**
236a	**Ab^{1}sTb^{1}sAb^{1}sTb^{1}sAb^{1}s**dC*sdAsdGsdGsdC*sdAsdTsdTsdAsdAsdTsdAsdAs dAsdGsdTsdGsdC*s**Ab^{1}sAb^{1}sAb^{1}sTb^{1}sGb^1**
236b	**Ab^{1}Tb^{1}Ab^{1}Tb^{1}Ab^1**dC*dAdGdGdCdAdTdTdAdAdTdAdAdAdGdTdGdC***Ab^{1}Ab^1 Ab^{1}Tb^{1}Gb^1**

wherein the abbreviations b, d, C*, A*, s, ss have the following meaning:

b^1 β-D-oxy-LNA,

b^2 β-D-thio-LNA,

b^3 β-D-amino-LNA,

b^4 α-L-oxy-LNA,

b^5 β-D-ENA,

b^6 β-D-(NH)-LNA,

b^7 β-D-(NCH_3)-LNA,

d 2-deoxy,

C* 5-methylcytosine,

A* 2-aminoadenine,

s the internucleotide linkage is a phosphorothioate group (-O-P(O)(S^-)-O-),

ss the internucleotide linkage is a phosphorodithioate group (-O-P(S)(S^-)-O-),

/5SpC3s/ (-O-P(O)(S^-)-OC_3H_6OH at 5'-terminal group of an antisense-oligonucleotide,

/3SpC3s/ (-O-P(O)(S^-)-OC_3H_6OH at 3'-terminal group of an antisense-oligonucleotide,

nucleotides in **bold** are LNA nucleotides,

nucleotides not in bold are non-LNA nucleotides.

11. Antisense-oligonucleotide according to any one of the claims 1 – 10 for promoting regeneration and functional reconnection of damaged nerve pathways and/or for treatment and compensation of age induced decreases in neuronal stem cell renewal.

12. Antisense-oligonucleotide according to any one of the claims 1 – 10 for use in the prophylaxis and treatment of neurodegenerative diseases, neuroinflammatory disorders, traumatic or posttraumatic disorders, neurovascular disorders, hypoxic disorders, postinfectious central nervous system disorders, fibrotic diseases, hyperproliferative diseases, cancer, tumors, presbycusis and presbyopia.

13. Antisense-oligonucleotide for use according to claim 12, wherein the neurodegenerative diseases and neuroinflammatory disorders are selected from the group consisting of: Alzheimer's disease, Parkinson's disease, Creutzfeldt Jakob disease, new variant of Creutzfeldt Jakobs disease, Hallervorden Spatz disease, Huntington's disease, multisystem atrophy, dementia, frontotemporal dementia, motor neuron disorders, amyotrophic lateral sclerosis, spinal muscular atrophy, spinocerebellar atrophies, schizophrenia, affective disorders, major depression, meningoencephalitis, bacterial meningoencephalitis, viral meningoencephalitis, CNS autoimmune disorders, multiple sclerosis, acute ischemic / hypoxic lesions, stroke, CNS and spinal cord trauma, head and spinal trauma, brain traumatic injuries, arteriosclerosis, atherosclerosis, microangiopathic dementia, Binswanger' disease, retinal degeneration, cochlear degeneration, macular degeneration, cochlear deafness, AIDS-related dementia, retinitis pigmentosa, fragile X-associated tremor/ataxia syndrome, progressive supranuclear palsy, striatonigral degeneration, olivopontocerebellear degeneration, Shy Drager syndrome, age dependant memory deficits, neurodevelopmental disorders associated with dementia, Down`s Syndrome, synucleinopathies, trinucleotide repeat disorders, trauma, , vascular diseases, vascular inflammations, and CNS-ageing and wherein the fibrotic diseases are selected from the group consisting of: pulmonary fibrosis, cystic fibrosis, hepatic cirrhosis, endomyocardial fibrosis, old myocardial infarction, atrial fibrosis, mediastinal fibrosis, myelofibrosis, retroperitoneal fibrosis, progressive massive fibrosis, nephrogenic systemic fibrosis, Crohn's Disease, keloid, systemic sclerosis, arthrofibrosis, Peyronie's disease, Dupuytren's contracture, and residuums after Lupus erythematodes.

14. Pharmaceutical composition containing at least one antisense-oligonucleotide according to any one of claims 1 – 10 together with at least one pharmaceutically acceptable carrier, excipient, adjuvant, solvent or diluent.