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61/224,031 8 July 2009 (08.07.2009) US(71) Applicant (for all designated States except US): **RXI PHARMACEUTICALS CORPORATION** [US/US];
60 Prescott Street, Worcester, MA 01605 (US).

(72) Inventors; and

(71) Applicants : **KHVOROVA, Anastasia** [US/US]; 4550 Squires Circle, Boulder, CO 80305 (US). **SALOMON, William** [US/US]; 39 O Street, Apt. #1, Boston, MA 02127 (US). **KAMENS, Joanne** [US/US]; 21 Wessex Road, Newton, MA 02459 (US). **SAMARSKY, Dmitry** [US/US]; 15 Wayside Road, Westborough, MA 01581 (US). **WOOLF, Tod, M.** [US/US]; 14 Babe Ruth Drive, Sudbury, MA 01776-1942 (US). **CARDIA, James** [US/US]; 4 Peter Road, Weymouth, MA 02191 (US).(74) Agent: **LOCKHART, Helen, C.**; Wolf, Greenfield & Sacks, P.C., Federal Reserve Plaza, 600 Atlantic Avenue, Boston, MA 02210-2206 (US).

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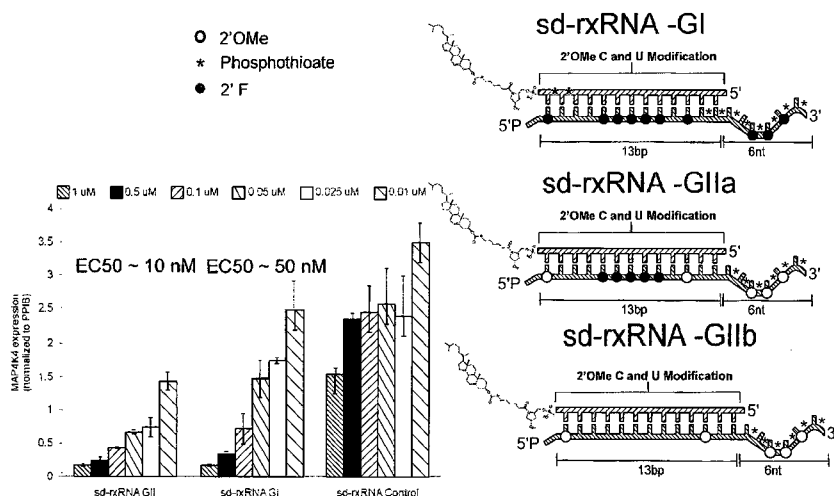
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Figure 61



(57) Abstract: The present invention relates to RNAi constructs with minimal double-stranded regions, and their use in gene silencing. RNAi constructs associated with the invention include a double stranded region of 8-14 nucleotides and a variety of chemical modifications, and are highly effective in gene silencing.

REDUCED SIZE SELF-DELIVERING RNAI COMPOUNDS

RELATED APPLICATIONS

5 This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional application serial number US 61/192,954, entitled "Chemically Modified Polyucleotides and Methods of Using the Same," filed on September 22, 2008, US 61/149,946, entitled "Minimum Length Triggers of RNA Interference," filed on February 4, 2009, and US 61/224,031, entitled "Minimum Length Triggers of RNA Interference," filed on July 8, 2009, the disclosure of each of which is incorporated by reference herein in its entirety.

10

FIELD OF INVENTION

The invention pertains to the field of RNA interference (RNAi). The invention more specifically relates to nucleic acid molecules with improved *in vivo* delivery properties without the use of a delivering agent and their use in efficient gene silencing.

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BACKGROUND OF INVENTION

Complementary oligonucleotide sequences are promising therapeutic agents and useful research tools in elucidating gene functions. However, prior art oligonucleotide molecules suffer from several problems that may impede their clinical development, and frequently make it difficult to achieve intended efficient inhibition of gene expression (including protein synthesis) using such compositions *in vivo*.

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A major problem has been the delivery of these compounds to cells and tissues. Conventional double-stranded RNAi compounds, 19-29 bases long, form a highly negatively-charged rigid helix of approximately 1.5 by 10-15 nm in size. This rod type molecule cannot get through the cell-membrane and as a result has very limited efficacy both *in vitro* and *in vivo*. As a result, all conventional RNAi compounds require some kind of a delivery vehicle to promote their tissue distribution and cellular uptake. This is considered to be a major limitation of the RNAi technology.

25

There have been previous attempts to apply chemical modifications to oligonucleotides to improve their cellular uptake properties. One such modification was the attachment of a cholesterol molecule to the oligonucleotide. A first report on this approach was by Letsinger *et al.*, in 1989. Subsequently, ISIS Pharmaceuticals, Inc.

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- 2 --

(Carlsbad, CA) reported on more advanced techniques in attaching the cholesterol molecule to the oligonucleotide (Manoharan, 1992).

With the discovery of siRNAs in the late nineties, similar types of modifications were attempted on these molecules to enhance their delivery profiles. Cholesterol molecules conjugated to slightly modified (Soutschek, 2004) and heavily modified (Wolfrum, 2007) siRNAs appeared in the literature. Yamada *et al.*, 2008 also reported on the use of advanced linker chemistries which further improved cholesterol mediated uptake of siRNAs. In spite of all this effort, the uptake of these types of compounds appears to be inhibited in the presence of biological fluids resulting in highly limited efficacy in gene silencing *in vivo*, limiting the applicability of these compounds in a clinical setting.

Therefore, it would be of great benefit to improve upon the prior art oligonucleotides by designing oligonucleotides that have improved delivery properties *in vivo* and are clinically meaningful.

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SUMMARY OF INVENTION

Described herein are asymmetric chemically modified nucleic acid molecules with minimal double stranded regions, and the use of such molecules in gene silencing. RNAi molecules associated with the invention contain single stranded regions and double stranded regions, and can contain a variety of chemical modifications within both the single stranded and double stranded regions of the molecule. Additionally, the RNAi molecules can be attached to a hydrophobic conjugate such as a conventional and advanced sterol-type molecule. This new class of RNAi molecules has superior efficacy both *in vitro* and *in vivo* than previously described RNAi molecules.

Aspects of the invention relate to asymmetric nucleic acid molecules including a guide strand, with a minimal length of 16 nucleotides, and a passenger strand forming a double stranded nucleic acid, having a double stranded region and a single stranded region, the double stranded region having 8-15 nucleotides in length, the single stranded region having 5-12 nucleotides in length, wherein the passenger strand is linked to a lipophilic group, wherein at least 40% of the nucleotides of the double stranded nucleic acid are modified, and wherein the single stranded region has at least 2 phosphorothioate modifications. In some embodiments position 1 of the guide strand is 5' phosphorylated.

- 3 --

In certain embodiments, position 1 of the guide strand is 2'-O-methyl modified and 5' phosphorylated.

Aspects of the invention relate to isolated double stranded nucleic acid molecules including a longer strand of 15-21 nucleotides in length that has complementarity to a miRNA sequence, a shorter strand of 8-15 nucleotides in length linked at the 3' end to a lipophilic group, wherein the longer strand and the passenger strand form the double stranded nucleic acid molecule having a double stranded region and a single stranded region, wherein the longer strand has a 3' single stranded region of 2-13 nucleotides in length, comprising at least two phosphorothioate modification, and at least 50% nucleotides are modified.

Further aspects of the invention relate to isolated double stranded nucleic acid molecules including a guide strand of 17-21 nucleotides in length that has complementarity to a target gene, a passenger strand of 8-16 nucleotides in length linked at the 3' end to a lipophilic group, wherein the guide strand and the passenger strand form the double stranded nucleic acid molecule having a double stranded region and a single stranded region, wherein the guide strand has a 3' single stranded region of 2-13 nucleotides in length, each nucleotide within the single stranded region having a phosphorothioate modification, wherein the guide strand has a 5' phosphate modification and wherein at least 50% of C and U nucleotides in the double stranded region include at least one 2' O-methyl modification or 2'-fluoro modification.

In another aspect, the invention is an isolated double stranded nucleic acid molecule having a guide strand of 17-21 nucleotides in length that has complementarity to a target gene, a passenger strand of 10-16 nucleotides in length linked at the 3' end to a lipophilic group, wherein the guide strand and the passenger strand form the double stranded nucleic acid molecule having a double stranded region and a single stranded region, wherein the guide strand has a 3' single stranded region of 5-11 nucleotides in length, at least two nucleotide within the single stranded region having a phosphorothioate modification, wherein the guide strand has a 5' phosphate modification and wherein at least 50% of C and U nucleotides in the double stranded region are 2' O-methyl modification or 2'-fluoro modified.

The invention in another aspect is an isolated double stranded nucleic acid molecule having a guide strand of 17-21 nucleotides in length that has complementarity to a target gene, a passenger strand of 8-16 nucleotides in length linked at the 3' end to a

- 4 --

lipophilic group, wherein the guide strand and the passenger strand form the double stranded nucleic acid molecule having a double stranded region and a single stranded region, wherein the guide strand has a 3' single stranded region of 6-8 nucleotides in length, each nucleotide within the single stranded region having a phosphorothioate modification, wherein the guide strand has a 5' phosphate modification, wherein the passenger strand includes at least two phosphorothioate modifications, wherein at least 50% of C and U nucleotides in the double stranded region include a 2' O-methyl modification or 2'-fluoro modification, and wherein the double stranded nucleic acid molecule has one end that is blunt or includes a one-two nucleotide overhang.

10 An isolated double stranded nucleic acid molecule having a guide strand of 17-21 nucleotides in length that has complementarity to a target gene, a passenger strand of 8-16 nucleotides in length linked at the 3' end to a lipophilic group, wherein the guide strand and the passenger strand form the double stranded nucleic acid molecule having a double stranded region and a single stranded region, wherein the guide strand has a 3' single stranded region, each nucleotide within the single stranded region having a phosphorothioate modification, wherein the guide strand has a 5' phosphate modification, wherein every C and U nucleotide in position 11-18 of the guide strand has a 2' O-methyl modification, wherein every nucleotide of the passenger strand is 2' O-methyl modified, and wherein the double stranded nucleic acid molecule has one end that is blunt or includes a one-two nucleotide overhang is provided in other aspects of the invention.

In another aspect the invention is an isolated double stranded nucleic acid molecule having a guide strand of 17-21 nucleotides in length that has complementarity to a target gene, a passenger strand of 8-15 nucleotides in length linked at the 3' end to a lipophilic group, wherein the lipophilic group is selected from the group consisting of cholesterol and a sterol type molecule with C17 polycarbon chain of 5-7 or 9-18 carbons in length, wherein the guide strand and the passenger strand form the double stranded nucleic acid molecule having a double stranded region and a single stranded region, wherein the guide strand has a 3' single stranded region, each nucleotide within the single stranded region having a phosphorothioate modification, wherein the guide strand has a 5' phosphate modification, wherein every C and U nucleotide in position 11-18 of the guide strand has a 2' O-methyl modification, wherein every C and U nucleotide in position 2-10 of the guide strand has a 2'F modification, wherein every nucleotide of the

- 5 -

passenger strand is 2' O-methyl modified, and wherein the double stranded nucleic acid molecule has one end that is blunt or includes a one-two nucleotide overhang.

In yet another aspect the invention is an isolated nucleic acid molecule having a guide sequence that has complementarity to a target gene, a passenger sequence linked at
5 the 3' end to a lipophilic group, wherein the guide sequence and the passenger sequence form a nucleic acid molecule having a double stranded region and a single stranded region, wherein the guide sequence has a 3' single stranded region of 2-13 nucleotides in length, each nucleotide within the single stranded region having a phosphorothioate modification, wherein the guide sequence has a 5' phosphate modification, wherein at
10 least 50% of C and U nucleotides in the double stranded region include at least one 2' O-methyl modification or 2'-fluoro modification, and wherein the double stranded nucleic acid molecule has one end that is blunt or includes a one-two nucleotide overhang.

An isolated double stranded nucleic acid molecule having a guide strand and a passenger strand, wherein the region of the molecule that is double stranded is from 8-14
15 nucleotides long, wherein the guide strand contains a single stranded region that is 4-12 nucleotides long, and wherein the single stranded region of the guide strand contains 2-12 phosphorothioate modifications is provided in other aspects of the invention.

In some embodiments the guide strand contains 6-8 phosphorothioate modifications. In other embodiments the single stranded region of the guide strand is 6
20 nucleotides long.

In yet other embodiments the double stranded region is 13 nucleotides long. Optionally the double stranded nucleic acid molecule has one end that is blunt or includes a one-two nucleotide overhang.

In another aspect the invention is an isolated double stranded nucleic acid
25 molecule having a guide strand, wherein the guide strand is 16-28 nucleotides long and has complementarity to a target gene, wherein the 3' terminal 10 nucleotides of the guide strand include at least two phosphate modifications, and wherein the guide strand has a 5' phosphate modification and includes at least one 2' O-methyl modification or 2'-fluoro modification, and a passenger strand, wherein the passenger strand is 8-14
30 nucleotides long and has complementarity to the guide strand, wherein the passenger strand is linked to a lipophilic group, wherein the guide strand and the passenger strand form the double stranded nucleic acid molecule.

- 6 --

In some embodiments the nucleotide in position one of the guide strand or sequence has a 2'-O-methyl modification. In other embodiments at least one C or U nucleotide in positions 2-10 of the guide strand or sequence has a 2'-fluoro modification. In yet other embodiments every C and U nucleotide in positions 2-10 of the guide strand or sequence has a 2'-fluoro modification. At least one C or U nucleotide in positions 11-18 of the guide strand or sequence may have a 2'-O-methyl modification. In some embodiments every C and U nucleotide in positions 11-18 of the guide strand or sequence has a 2'-O-methyl modification.

In yet other embodiments the 3' terminal 10 nucleotides of the guide strand include at least four phosphate modifications. Optionally the 3' terminal 10 nucleotides of the guide strand include at least eight phosphate modifications. In some embodiments the guide strand includes 4-14 phosphate modifications. In other embodiments the guide strand includes 4-10 phosphate modifications. In yet other embodiments the 3' terminal 6 nucleotides of the guide strand all include phosphate modifications. The phosphate modifications may be phosphorothioate modifications.

In some embodiments every C and U nucleotide on the passenger strand has a 2'-O-methyl modification. In other embodiments every nucleotide on the passenger strand has a 2'-O-methyl modification. In an embodiment at least one nucleotide on the passenger strand is phosphorothioate modified. At least two nucleotides on the passenger strand are phosphorothioate modified in other embodiments.

The lipophilic molecule may be a sterol, such as cholesterol.

In some embodiments the guide strand is 18-19 nucleotides long. In other embodiments the passenger strand is 11-13 nucleotides long.

The double stranded nucleic acid molecule has one end that is blunt or includes a one-two nucleotide overhang in other embodiments.

In other aspects the invention is an isolated double stranded nucleic acid molecule comprising a guide strand and a passenger strand, wherein the guide strand is from 16-29 nucleotides long and is substantially complementary to a target gene, wherein the passenger strand is from 8-14 nucleotides long and has complementarity to the guide strand, and wherein the guide strand has at least two chemical modifications. In some embodiments the at least two chemical modifications include at least two phosphorothioate modifications. In some embodiments the double stranded nucleic acid molecule has one end that is blunt or includes a one-two nucleotide overhang.

- 7 --

In some aspects the invention is an isolated double stranded nucleic acid molecule comprising a guide strand and a passenger strand, wherein the guide strand is from 16-29 nucleotides long and is substantially complementary to a target gene, wherein the passenger strand is from 8-14 nucleotides long and has complementarity to the guide strand, and wherein the guide strand has a single stranded 3' region that is 5 nucleotides or longer and a 5' region that is 1 nucleotide or less. The single stranded region may contain at least 2 phosphorothioate modifications.

An isolated double stranded nucleic acid molecule having a guide strand and a passenger strand, wherein the guide strand is from 16-29 nucleotides long and is substantially complementary to a target gene, wherein the passenger strand is from 8-16 nucleotides long and has complementarity to the guide strand, and wherein the guide strand has a single stranded 3' region that is 5 nucleotides or longer and a passenger strand has a sterol type molecule with C17 attached chain longer than 9 is provided in other aspects of the invention.

A duplex polynucleotide is provided in other aspects of the invention. The polynucleotide has a first polynucleotide wherein said first polynucleotide is complementary to a second polynucleotide and a target gene; and a second polynucleotide wherein said second polynucleotide is at least 6 nucleotides shorter than said first polynucleotide, wherein said first polynucleotide includes a single stranded region containing modifications selected from the group consisting of 40 – 90% hydrophobic base modifications, 40 – 90% phosphorothioates, and 40 – 90% modifications of the ribose moiety, or any combination thereof.

In other aspects the invention is a duplex polynucleotide having a first polynucleotide wherein said first polynucleotide is complementary to a second polynucleotide and a target gene; and a second polynucleotide wherein said second polynucleotide is at least 6 nucleotides shorter than said first polynucleotide, wherein the duplex polynucleotide includes a mismatch between nucleotides 9, 11, 12, 13 or 14 on the first polynucleotide and the opposite nucleotide on the second polynucleotide.

In other aspects the invention is a method for inhibiting the expression of a target gene in a mammalian cell, comprising contacting the mammalian cell with an isolated double stranded nucleic acid molecule of any one of claims 1-41 or a duplex polynucleotide of claim 43 or 44.

- 8 --

A method of inducing RNAi in a subject is provided in other aspects of the invention. The method involves administering to a subject an effective amount for inducing RNAi of an mRNA of a target gene, an isolated double stranded nucleic acid molecule of any one of claims 1-41 or a duplex polynucleotide of claim 43 or 44. In
5 other embodiment the subject is a human. In other embodiments the target gene is PPIB, MAP4K4, or SOD1.

In other aspects an isolated hydrophobic modified polynucleotide having a polynucleotide, wherein the polynucleotide is double stranded RNA, attached to a hydrophobic molecule, wherein the hydrophobic molecule is attached to a base, a ribose
10 or a backbone of a non-terminal nucleotide and wherein the isolated double stranded nucleic acid molecule comprises a guide strand and a passenger strand, wherein the guide strand is from 16-29 nucleotides long and is substantially complementary to a target gene, wherein the passenger strand is from 8-14 nucleotides long and has complementarity to the guide strand is provided.

15 In one embodiment the hydrophobic molecule is attached to the guide strand of the double stranded RNA. In another embodiment the 3' terminal 10 nucleotides of the guide strand include at least two phosphate modifications, and wherein the guide strand has a 5' phosphate modification and includes at least one 2' O-methyl modification or 2'-fluoro modification. In yet another embodiment the hydrophobic molecule is attached
20 to the passenger strand of the double stranded RNA.

The invention provides an isolated hydrophobic modified polynucleotide having a polynucleotide non-covalently complexed to a hydrophobic molecule, wherein the hydrophobic molecule is a polycationic molecule. In some embodiments the polycationic molecule is selected from the group consisting of protamine, arginine rich
25 peptides, and spermine.

In other aspects the invention an isolated hydrophobic modified polynucleotide having a polynucleotide, wherein the polynucleotide is double stranded RNA, directly complexed to a hydrophobic molecule without a linker, wherein the hydrophobic molecule is not cholesterol.

30 A composition having a hydrophobic modified polynucleotide, wherein the polynucleotide is double stranded RNA, attached to a hydrophobic molecule, wherein the double stranded nucleic acid molecule comprises a guide strand and a passenger strand, wherein the guide strand is from 16-29 nucleotides long and is substantially

- 9 --

complementary to a target gene, wherein the passenger strand is from 8-14 nucleotides long and has complementarity to the guide strand, wherein position 1 of the guide strand is 5' phosphorylated or has a 2' O-methyl modification, wherein at least 40% of the nucleotides of the double stranded nucleic acid are modified, and wherein the double
5 stranded nucleic acid molecule has one end that is blunt or includes a one-two nucleotide overhang; a neutral fatty mixture; and optionally a cargo molecule, wherein the hydrophobic modified polynucleotide and the neutral fatty mixture forms a micelle is provided in other aspects of the invention.

In some embodiments the 3' end of the passenger strand is linked to the
10 hydrophobic molecule. In other embodiments the composition is sterile. In yet other embodiments the neutral fatty mixture comprises a DOPC (dioleoylphosphatidylcholine). In further embodiments the neutral fatty mixture comprises a DSPC (distearoylphosphatidylcholine). The neutral fatty mixture further comprises a sterol such as cholesterol in other embodiments.

15 In yet other embodiments the composition includes at least 20% DOPC and at least 20% cholesterol. The hydrophobic portion of the hydrophobic modified polynucleotide is a sterol in other embodiments. The sterol may be a cholesterol, a cholesteryl or modified cholesteryl residue. In other embodiments the hydrophobic portion of the hydrophobic modified polynucleotide is selected from the group consisting
20 of bile acids, cholic acid or taurocholic acid, deoxycholate, oleyl lithocholic acid, oleoyl cholenic acid, glycolipids, phospholipids, sphingolipids, isoprenoids, vitamins, saturated fatty acids, unsaturated fatty acids, fatty acid esters, triglycerides, pyrenes, porphyrines, Texaphyrine, adamantane, acridines, biotin, coumarin, fluorescein, rhodamine, Texas-Red, digoxigenin, dimethoxytrityl, t-butyldimethylsilyl, t-butyldiphenylsilyl, cyanine
25 dyes (e.g. Cy3 or Cy5), Hoechst 33258 dye, psoralen, and ibuprofen.

In yet other embodiments the hydrophobic portion of the hydrophobic modified polynucleotide is a polycationic molecule, such as, for instance, protamine, arginine rich peptides, and/or spermine.

The composition optionally includes a cargo molecule such as a lipid, a peptide,
30 vitamin, and/or a small molecule. In some embodiments the cargo molecule is a commercially available fat emulsions available for a variety of purposes selected from the group consisting of parenteral feeding. In some embodiments the commercially available fat emulsion is an intralipid or a nutralipid. In other embodiments the cargo

- 10 -

molecule is a fatty acid mixture containing more than 74% of linoleic acid, a fatty acid mixture containing at least 6% of cardiolipin, or a fatty acid mixture containing at least 74% of linoleic acid and at least 6% of cardiolipin. In another embodiment the cargo molecule is a fusogenic lipid, such as for example, DOPE, and preferably is at least 10% fusogenic lipid

In some embodiments the polynucleotide includes chemical modifications. For instance it may be at least 40% modified.

A method of inducing RNAi in a subject is provided in another aspect of the invention. The method involves administering to a subject an effective amount for inducing RNAi of mRNA of a target gene, an isolated double stranded nucleic acid molecule or a duplex polynucleotide or a composition of the invention, wherein the polynucleotide has at least a region of sequence correspondence to the target gene, wherein the step of administering is systemic, intravenous, intraperitoneal, intradermal, topical, intranasal, inhalation, oral, intramucosal, local injection, subcutaneous, oral tracheal, or intraocular.

In other embodiment the subject is a human. In other embodiments the target gene is PPIB, MAP4K4, or SOD1.

In some aspects the invention is a single-stranded RNA of less than 35 nucleotides in length that forms a hairpin structure, said hairpin includes a double-stranded stem and a single-stranded loop, said double-stranded stem having a 5'-stem sequence having a 5'-end, and a 3'-stem sequence having a 3'-end; and said 5'-stem sequence and at least a portion of said loop form a guide sequence complementary to a transcript of a target gene, wherein said polynucleotide mediates sequence-dependent gene silencing of expression of said target gene, wherein each nucleotide within the single-stranded loop region has a phosphorothioate modification, and wherein at least 50% of C and U nucleotides in the double stranded region include a 2' O-methyl modification or 2'-fluoro modification. In one embodiment every C and U nucleotide in position 11-18 of the guide sequence has a 2' O-methyl modification.

A polynucleotide construct is provided in other aspects, the polynucleotide having two identical single-stranded polynucleotides, each of said single-stranded polynucleotide comprising a 5'-stem sequence having a 5'-end, a 3'-stem sequence having a 3'-end, and a linker sequence linking the 5'-stem sequence and the 3'-stem sequence, wherein: (1) the 5'-stem sequence of a first single-stranded polynucleotide

- 11 --

hybridizes with the 3'-stem sequence of a second single-stranded polynucleotide to form a first double-stranded stem region; (2) the 5'-stem sequence of the second single-stranded polynucleotide hybridize with the 3'-stem sequence of the first single-stranded polynucleotide to form a second double-stranded stem region; and, (3) the linker
 5 sequences of the first and the second single-stranded polynucleotides form a loop or bulge connecting said first and said second double-stranded stem regions, wherein the 5'-stem sequence and at least a portion of the linker sequence form a guide sequence complementary to a transcript of a target gene, wherein said polynucleotide construct mediates sequence-dependent gene silencing of expression of said target gene, wherein
 10 each nucleotide within the single-stranded loop region has a phosphorothioate modification, and wherein at least 50% of C and U nucleotides in the double stranded regions include a 2' O-methyl modification or 2'-fluoro modification.

In one embodiment every C and U nucleotide in position 11-18 of the guide sequence has a 2' O-methyl modification.

15 In some embodiments, the guide strand is 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 nucleotides long. In some embodiments, the passenger strand is 8, 9, 10, 11, 12, 13 or 14 nucleotides long. In some embodiments, the nucleic acid molecule has a thermodynamic stability (ΔG) of less than -20 kkal/mol.

Aspects of the invention relate to nucleic acid molecules that are chemically
 20 modified. In some embodiments, the chemical modification is selected from the group consisting of 5' Phosphate, 2'-O-methyl, 2'-O-ethyl, 2'-fluoro, ribothymidine, C-5 propynyl-dC (pdC), C-5 propynyl-dU (pdU), C-5 propynyl-C (pC), C-5 propynyl-U (pU), 5-methyl C, 5-methyl U, 5-methyl dC, 5-methyl dU methoxy, (2,6-diaminopurine), 5'-Dimethoxytrityl-N4-ethyl-2'-deoxyCytidine, C-5 propynyl-fC (pfC), C-5 propynyl-fU
 25 (pfU), 5-methyl fC, 5-methyl fU, C-5 propynyl-mC (pmC), C-5 propynyl-fU (pmU), 5-methyl mC, 5-methyl mU, LNA (locked nucleic acid), MGB (minor groove binder) and other base modifications which increase base hydrophobicity. More than one chemical modification may be present in the same molecule. In some embodiments, chemical modification increases stability and/or improves thermodynamic stability (ΔG). In some
 30 embodiments, at least 90 % of CU residues on a nucleic acid molecule are modified.

In some embodiments, the nucleotide in position one of the guide strand has a 2'-O-methyl modification and/or a 5' Phosphate modification. In some embodiments, at least one C or U nucleotide in positions 2-10 of the guide strand has a 2'-fluoro

- 12 -

modification. In certain embodiments, every C and U nucleotide in positions 2-10 of the guide strand has a 2'-fluoro modification. In some embodiments, at least one C or U nucleotide in positions 11-18 of the guide strand has a 2'-O-methyl modification. In certain embodiments, every C and U nucleotide in positions 11-18 of the guide strand
5 has a 2'-O-methyl modification. In some embodiments, every C and U nucleotide on the passenger strand has a 2'-O-methyl modification. In certain embodiments, every nucleotide on the passenger strand has a 2'-O-methyl modification.

In some embodiments, nucleic acid molecules associated with the invention contain a stretch of at least 4 nucleotides that are phosphorothioate modified. In certain
10 embodiments, the stretch of nucleotides that are phosphorothioate modified is at least 12 nucleotides long. In some embodiments, the stretch of nucleotides that are phosphorothioate modified is not fully single stranded.

Nucleic acid molecules associated with the invention may be attached to a conjugate. In some embodiments, the conjugate is attached to the guide strand, while in
15 other embodiments the conjugate is attached to the passenger strand. In some embodiments, the conjugate is hydrophobic. In some embodiments, the conjugate is a sterol such as cholesterol. In some embodiments, nucleic acid molecules associated with the invention are blunt-ended.

Aspects of the invention relate to double stranded nucleic acid molecule
20 including a guide strand and a passenger strand, wherein the region of the molecule that is double stranded is from 8-14 nucleotides long, and wherein the molecule has a thermodynamic stability (ΔG) of less than -13 kkal/mol.

In some embodiments, the region of the molecule that is double stranded is 8, 9, 10, 11, 12, 13, or 14 nucleotides long. In some embodiments, the molecule has a
25 thermodynamic stability (ΔG) of less than -20 kkal/mol. The nucleic acid molecules, in some embodiments are chemically modified. In certain embodiments, the chemical modification is selected from the group consisting of 5' Phosphate, 2'-O-methyl, 2'-O-ethyl, 2'-fluoro, ribothymidine, C-5 propynyl-dC (pdC), C-5 propynyl-dU (pdU), C-5 propynyl-C (pC), C-5 propynyl-U (pU), 5-methyl C, 5-methyl U, 5-methyl dC, 5-methyl
30 dU methoxy, (2,6-diaminopurine), 5'-Dimethoxytrityl-N4-ethyl-2'-deoxyCytidine, C-5 propynyl-fC (pfC), C-5 propynyl-fU (pfU), 5-methyl fC, 5-methyl fU, C-5 propynyl-mC (pmC), C-5 propynyl-fU (pmU), 5-methyl mC, 5-methyl mU, LNA (locked nucleic acid), MGB (minor groove binder) and other base modifications which increase base

- 13 --

hydrophobicity. More than one chemical modification may be present in the same molecule. In some embodiments, chemical modification increases stability and/or improves thermodynamic stability (ΔG). In some embodiments, at least 90 % of CU residues on a nucleic acid molecule are modified.

5 In some embodiments, the nucleotide in position one of the guide strand has a 2'-O-methyl modification and/or a 5' Phosphate modification. In some embodiments, at least one C or U nucleotide in positions 2-10 of the guide strand has a 2'-fluoro modification. In certain embodiments, every C and U nucleotide in positions 2-10 of the guide strand has a 2'-fluoro modification. In some embodiments, at least one C or U
10 nucleotide in positions 11-18 of the guide strand has a 2'-O-methyl modification. In certain embodiments, every C and U nucleotide in positions 11-18 of the guide strand has a 2'-O-methyl modification. In some embodiments, every C and U nucleotide on the passenger strand has a 2'-O-methyl modification. In certain embodiments, every nucleotide on the passenger strand has a 2'-O-methyl modification.

15 The nucleic acid molecules associated with the invention may contain a stretch of at least 4 nucleotides that are phosphorothioate modified. In certain embodiments, the stretch of nucleotides that are phosphorothioate modified is at least 12 nucleotides long. In some embodiments, the stretch of nucleotides that are phosphorothioate modified is not fully single stranded. In some embodiments, the nucleic acid molecules are attached
20 to a conjugate. In some embodiments, the conjugate is attached to the guide strand, while in other embodiments the conjugate is attached to the passenger strand. In some embodiments, the conjugate is hydrophobic. In some embodiments, the conjugate is a sterol such as cholesterol. In some embodiments, nucleic acid molecules associated with the invention are blunt-ended. In some embodiments, the nucleic acid molecules are
25 blunt ended at the 5' end. In certain embodiments, the nucleic acid molecules are blunt ended at the 5' end where the region of complementarity between the two strands of the molecule begins.

 Aspects of the invention relate to methods for inhibiting the expression of a target gene in a mammalian cell. Methods include contacting the mammalian cell with an
30 isolated double stranded nucleic acid molecule including a guide strand and a passenger strand, wherein the guide strand is from 16-29 nucleotides long and has complementarity to a target gene, wherein the passenger strand is from 8-14 nucleotides long and has

- 14 -

complementarity to the guide strand, and wherein the double stranded nucleic acid molecule has a thermodynamic stability (ΔG) of less than -13 kkal/mol.

The cell may be contacted in vivo or in vitro. In some embodiments, the guide strand is 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 nucleotides long. In some embodiments, the passenger strand is 8, 9, 10, 11, 12, 13 or 14 nucleotides long. In some embodiments, the nucleic acid molecule has a thermodynamic stability (ΔG) of less than -20 kkal/mol.

The nucleic acid molecules associated with methods described herein may be chemically modified. In some embodiments, the chemical modification is selected from the group consisting of 5' Phosphate, 2'-O-methyl, 2'-O-ethyl, 2'-fluoro, ribothymidine, C-5 propynyl-dC (pdC), C-5 propynyl-dU (pdU), C-5 propynyl-C (pC), C-5 propynyl-U (pU), 5-methyl C, 5-methyl U, 5-methyl dC, 5-methyl dU methoxy, (2,6-diaminopurine), 5'-Dimethoxytrityl-N4-ethyl-2'-deoxyCytidine, C-5 propynyl-fC (pfC), C-5 propynyl-fU (pfU), 5-methyl fC, 5-methyl fU, C-5 propynyl-mC (pmC), C-5 propynyl-fU (pmU), 5-methyl mC, 5-methyl mU, LNA (locked nucleic acid), MGB (minor groove binder) and other base modifications which increase base hydrophobicity. More than one chemical modification may be present in the same molecule. In some embodiments, chemical modification increases stability and/or improves thermodynamic stability (ΔG). In some embodiments, at least 90 % of CU residues on a nucleic acid molecule are modified.

In some embodiments, the nucleotide in position one of the guide strand has a 2'-O-methyl modification and/or a 5' Phosphate modification. In some embodiments, at least one C or U nucleotide in positions 2-10 of the guide strand has a 2'-fluoro modification. In certain embodiments, every C and U nucleotide in positions 2-10 of the guide strand has a 2'-fluoro modification. In some embodiments, at least one C or U nucleotide in positions 11-18 of the guide strand has a 2'-O-methyl modification. In certain embodiments, every C and U nucleotide in positions 11-18 of the guide strand has a 2'-O-methyl modification. In some embodiments, every C and U nucleotide on the passenger strand has a 2'-O-methyl modification. In certain embodiments, every nucleotide on the passenger strand has a 2'-O-methyl modification.

In some embodiments, nucleic acid molecules associated with the invention contain a stretch of at least 4 nucleotides that are phosphorothioate modified. In certain embodiments, the stretch of nucleotides that are phosphorothioate modified is at least 12

- 15 -

nucleotides long. In some embodiments, the stretch of nucleotides that are phosphorothioate modified is not fully single stranded.

Nucleic acid molecules associated with the invention may be attached to a conjugate. In some embodiments, the conjugate is attached to the guide strand, while in
5 other embodiments the conjugate is attached to the passenger strand. In some embodiments, the conjugate is hydrophobic. In some embodiments, the conjugate is a sterol such as cholesterol. In some embodiments, nucleic acid molecules associated with the invention are blunt-ended.

Methods for inhibiting the expression of a target gene in a mammalian cell
10 described herein include contacting the mammalian cell with an isolated double stranded nucleic acid molecule including a guide strand and a passenger strand, wherein the region of the molecule that is double stranded is from 8-14 nucleotides long, and wherein the molecule has a thermodynamic stability (ΔG) of less than -13 kkal/mol.

In some embodiments, the region of the molecule that is double stranded is 8, 9,
15 10, 11, 12, 13, or 14 nucleotides long. In some embodiments, the molecule has a thermodynamic stability (ΔG) of less than -20 kkal/mol. The nucleic acid molecules, in some embodiments are chemically modified. In certain embodiments, the chemical modification is selected from the group consisting of 5' Phosphate, 2'-O-methyl, 2'-O-ethyl, 2'-fluoro, ribothymidine, C-5 propynyl-dC (pdC), C-5 propynyl-dU (pdU), C-5
20 propynyl-C (pC), C-5 propynyl-U (pU), 5-methyl C, 5-methyl U, 5-methyl dC, 5-methyl dU methoxy, (2,6-diaminopurine), 5'-Dimethoxytrityl-N4-ethyl-2'-deoxyCytidine, C-5 propynyl-fC (pfC), C-5 propynyl-fU (pfU), 5-methyl fC, 5-methyl fU, , C-5 propynyl-mC (pmC), C-5 propynyl-fU (pmU), 5-methyl mC, 5-methyl mU, LNA (locked nucleic acid), MGB (minor groove binder) and other base modifications which increase base
25 hydrophobicity. More than one chemical modification may be present in the same molecule. In some embodiments, chemical modification increases stability and/or improves thermodynamic stability (ΔG). In some embodiments, at least 90 % of CU residues on a nucleic acid molecule are modified.

In some embodiments, the nucleotide in position one of the guide strand has a 2'-
30 O-methyl modification and/or a 5' Phosphate modification. In some embodiments, at least one C or U nucleotide in positions 2-10 of the guide strand has a 2'-fluoro modification. In certain embodiments, every C and U nucleotide in positions 2-10 of the guide strand has a 2'-fluoro modification. In some embodiments, at least one C or U

- 16 -

nucleotide in positions 11-18 of the guide strand has a 2'-O-methyl modification. In certain embodiments, every C and U nucleotide in positions 11-18 of the guide strand has a 2'-O-methyl modification. In some embodiments, every C and U nucleotide on the passenger strand has a 2'-O-methyl modification. In certain embodiments, every
5 nucleotide on the passenger strand has a 2'-O-methyl modification.

The nucleic acid molecules associated with the invention may contain a stretch of at least 4 nucleotides that are phosphorothioate modified. In certain embodiments, the stretch of nucleotides that are phosphorothioate modified is at least 12 nucleotides long. In some embodiments, the stretch of nucleotides that are phosphorothioate modified is
10 not fully single stranded. In some embodiments, the nucleic acid molecules are attached to a conjugate. In some embodiments, the conjugate is attached to the guide strand, while in other embodiments the conjugate is attached to the passenger strand. In some embodiments, the conjugate is hydrophobic. In some embodiments, the conjugate is a sterol such as cholesterol. In some embodiments, nucleic acid molecules associated with
15 the invention are blunt-ended.

In another embodiment, the invention provides a method for selecting an siRNA for gene silencing by (a) selecting a target gene, wherein the target gene comprises a target sequence; (b) selecting a candidate siRNA, wherein said candidate siRNA comprises a guide strand of 16-29 nucleotide base pairs and a passenger strand of 8-14
20 nucleotide base pairs that form a duplex comprised of an antisense region and a sense region and said antisense region of said candidate siRNA is at least 80% complementary to a region of said target sequence; (c) determining a thermodynamic stability (ΔG) of the candidate siRNA; and (e) selecting said candidate siRNA as an siRNA for gene silencing, if said thermodynamic stability is less than -13 kkal/mol.

25 Aspects of the invention relate to isolated double stranded nucleic acid molecules including a guide strand and a passenger strand, wherein the guide strand is 18-19 nucleotides long and has complementarity to a target gene, wherein the passenger strand is 11-13 nucleotides long and has complementarity to the guide strand, and wherein the double stranded nucleic acid molecule has a thermodynamic stability (ΔG) of less than -
30 13 kkal/mol.

In some embodiments, the nucleotide in position one of the guide strand has a 2'-O-methyl modification and/or a 5' Phosphate modification. In some embodiments, at least one C or U nucleotide in positions 2-10 of the guide strand has a 2'-fluoro

- 17 --

modification. In certain embodiments, every C and U nucleotide in positions 2-10 of the guide strand has a 2'-fluoro modification. In some embodiments, at least one C or U nucleotide in positions 11-18 of the guide strand has a 2'-O-methyl modification. In certain embodiments, every C and U nucleotide in positions 11-18 of the guide strand
5 has a 2'-O-methyl modification.

In some embodiments, the guide strand contains a stretch of at least 4 nucleotides that are phosphorothioate modified. In certain embodiments, the guide strand contains a stretch of at least 8 nucleotides that are phosphorothioate modified. In some
10 embodiments, every C and U nucleotide on the passenger strand has a 2'-O-methyl modification. In certain embodiments, every nucleotide on the passenger strand has a 2'-O-methyl modification. In some embodiments, at least one, or at least two nucleotides on the passenger strand is phosphorothioate modified. The nucleic acid molecule can be attached to a conjugate on either the guide or passenger strand. In some embodiments, the conjugate is a sterol such as cholesterol.

15 Aspects of the invention relate to isolated double stranded nucleic acid molecules including a guide strand, wherein the guide strand is 16-28 nucleotides long and has complementarity to a target gene, wherein the 3' terminal 10 nucleotides of the guide strand include at least two phosphate modifications, and wherein the guide strand includes at least one 2' O-methyl modification or 2'-fluoro modification, and a passenger
20 strand, wherein the passenger strand is 8-28 nucleotides long and has complementarity to the guide strand, wherein the passenger strand is linked to a lipophilic group, wherein the guide strand and the passenger strand form the double stranded nucleic acid molecule.

In some embodiments, the nucleotide in position one of the guide strand has a 2'-O-methyl modification and/or a 5' Phosphate modification. In some embodiments, at
25 least one C or U nucleotide in positions 2-10 of the guide strand has a 2'-fluoro modification. In certain embodiments, every C and U nucleotide in positions 2-10 of the guide strand has a 2'-fluoro modification. In some embodiments, at least one C or U nucleotide in positions 11-18 of the guide strand has a 2'-O-methyl modification. In certain embodiments, every C and U nucleotide in positions 11-18 of the guide strand
30 has a 2'-O-methyl modification.

In some embodiments, the 3' terminal 10 nucleotides of the guide strand include at least four, or at least eight phosphate modifications. In certain embodiments, the guide strand includes 2-14 or 4-10 phosphate modifications. In some embodiments, the 3'

- 18 -

terminal 6 nucleotides of the guide strand all include phosphate modifications. In certain embodiments, the phosphate modifications are phosphorothioate modifications.

In some embodiments, every C and U nucleotide on the passenger strand has a 2'-O-methyl modification. In certain embodiments, every nucleotide on the passenger strand has a 2'-O-methyl modification. In some embodiments, at least one, or at least two nucleotides on the passenger strand is phosphorothioate modified. In some embodiments, the lipophilic molecule is a sterol such as cholesterol. In some embodiments, the guide strand is 18-19 nucleotides long and the passenger strand is 11-13 nucleotides long.

Aspects of the invention relate to isolated double stranded nucleic acid molecules including a guide strand and a passenger strand, wherein the guide strand is from 16-29 nucleotides long and is substantially complementary to a target gene, wherein the passenger strand is from 8-14 nucleotides long and has complementarity to the guide strand, and wherein the guide strand has at least two chemical modifications. In some embodiments, the two chemical modifications are phosphorothioate modifications.

Further aspects of the invention relate to isolated double stranded nucleic acid molecule comprising a guide strand and a passenger strand, wherein the guide strand is from 16-29 nucleotides long and is substantially complementary to a target gene, wherein the passenger strand is from 8-14 nucleotides long and has complementarity to the guide strand, and wherein the guide strand has a single stranded 3' region that is 5 nucleotides or longer. In some embodiments, the single stranded region contains at least 2 phosphorothioate modifications.

Further aspects of the invention relate to isolated double stranded nucleic acid molecules including a guide strand and a passenger strand, wherein the guide strand is from 18-21 nucleotides long and is substantially complementary to a target gene, wherein the passenger strand is from 11-14 nucleotides long and has complementarity to the guide strand, and wherein position one of the guide strand has 2-OMe and 5' phosphate modifications, every C and U in positions 2 to 11 of the guide strand are 2-F modified, every C and U in positions 12-18 of the guide strand are 2'OMe modified, and 80% of Cs and Us on the passenger strand are 2'OMe modified

Another aspect of the invention relates to isolated double stranded nucleic acid molecules including a guide strand and a passenger strand, wherein the guide strand is from 18-21 nucleotides long and is substantially complementary to a target gene,

- 19 -

wherein the passenger strand is from 11-14 nucleotides long and has complementarity to the guide strand, and wherein the guide strand has 2-OMe and 5' phosphate modifications at position 1, every C and U in positions 2 to 11 of the guide strand are 2-F modified, every C and U in positions 12-18 of the guide strand are 2'OMe modified, 80% of Cs and Us on the passenger strand are 2'OMe and the 3' end of the passenger strand is attached to a conjugate. In some embodiments the conjugate is selected from sterols, sterol-type molecules, hydrophobic vitamins or fatty acids.

Each of the limitations of the invention can encompass various embodiments of the invention. It is, therefore, anticipated that each of the limitations of the invention involving any one element or combinations of elements can be included in each aspect of the invention. This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways.

BRIEF DESCRIPTION OF DRAWINGS

The accompanying drawings are not intended to be drawn to scale. In the drawings, each identical or nearly identical component that is illustrated in various figures is represented by a like numeral. For purposes of clarity, not every component may be labeled in every drawing. In the drawings:

FIG. 1 is a schematic depicting proposed structures of asymmetric double stranded RNA molecules (adsRNA). Bold lines represent sequences carrying modification patterns compatible with RISC loading. Striped lines represent polynucleotides carrying modifications compatible with passenger strands. Plain lines represent a single stranded polynucleotide with modification patterns optimized for cell interaction and uptake. FIG 1A depicts adsRNA with extended guide or passenger strands; FIG 1B depicts adsRNA with length variations of a cell penetrating polynucleotide; FIG 1C depicts adsRNA with 3' and 5' conjugates; FIG 1D depicts adsRNAs with mismatches.

FIG. 2 is a schematic depicting asymmetric dsRNA molecules with different chemical modification patterns. Several examples of chemical modifications that might be used to increase hydrophobicity are shown including 4-pyridyl, 2-pyridyl, isobutyl and indolyl based position 5 uridine modifications.

- 20 -

FIG. 3 is a schematic depicting the use of dsRNA binding domains, protamine (or other Arg rich peptides), spermidine or similar chemical structures to block duplex charge to facilitate cellular entry.

FIG. 4 is a schematic depicting positively charged chemicals that might be used
5 for polynucleotide charge blockage.

FIG. 5 is a schematic depicting examples of structural and chemical compositions of single stranded RISC entering polynucleotides. The combination of one or more modifications including 2'd, 2'Ome, 2'F, hydrophobic and phosphorothioate modifications can be used to optimize single strand entry into the RISC.

10 FIG. 6 is a schematic depicting examples of structural and chemical composition of RISC substrate inhibitors. Combinations of one or more chemical modifications can be used to mediate efficient uptake and efficient binding to preloaded RISC complex.

FIG. 7 is a schematic depicting structures of polynucleotides with sterol type molecules attached, where R represent a polycarbonic tail of 9 carbons or longer. FIG.
15 7A depicts an adsRNA molecule; FIG. 7B depicts an siRNA molecule of approximately 17-30 bp long; FIG. 7C depicts a RISC entering strand; FIG 7D depicts a substrate analog strand. Chemical modification patterns, as depicted in FIG. 7, can be optimized to promote desired function.

FIG. 8 is a schematic depicting examples of naturally occurring phytosterols with
20 a polycarbon chain that is longer than 8, attached at position 17. More than 250 different types of phytosterols are known.

FIG. 9 is a schematic depicting examples of sterol-like structures, with variations in the size of the polycarbon chains attached at position 17.

FIG. 10 presents schematics and graphs demonstrating that the percentage of
25 liver uptake and plasma clearance of lipid emulsions containing sterol type molecules is directly affected by the size of the polycarbon chain attached at position 17. This figure is adapted from Martins et al, Journal of Lipid Research (1998).

FIG. 11 is a schematic depicting micelle formation. FIG. 11A depicts a
polynucleotide with a hydrophobic conjugate; FIG. 11B depicts linoleic acid; FIG. 11C
30 depicts a micelle formed from a mixture of polynucleotides containing hydrophobic conjugates combined with fatty acids.

FIG. 12 is a schematic depicting how alteration in lipid composition can affect pharmacokinetic behavior and tissue distribution of hydrophobically modified and/or

- 21 --

hydrophobically conjugated polynucleotides. In particular, use of lipid mixtures enriched in linoleic acid and cardiolipin results in preferential uptake by cardiomyocytes.

FIG. 13 is a schematic showing examples of RNAi constructs and controls used to target MAP4K4 expression. RNAi construct 12083 corresponds to SEQ ID NOs:597
5 and 598. RNAi construct 12089 corresponds to SEQ ID NO:599.

FIG. 14 is a graph showing MAP4K4 expression following transfection with RNAi constructs associated with the invention. RNAi constructs tested were: 12083 (Nicked), 12085 (13nt Duplex), 12089 (No Stem Pairing) and 12134 (13nt miniRNA). Results of transfection were compared to an untransfected control sample. RNAi
10 construct 12083 corresponds to SEQ ID NOs:597 and 598. RNAi construct 12085 corresponds to SEQ ID NOs:600 and 601. RNAi construct 12089 corresponds to SEQ ID NO:599. RNAi construct 12134 corresponds to SEQ ID NOs:602 and 603.

FIG. 15 is a graph showing expression of MAP4K4 24 hours post-transfection with RNAi constructs associated with the invention. RNAi constructs tested were:
15 11546 (MAP4K4 rxRNA), 12083 (MAP4K4 Nicked Construct), 12134 (12bp soloRNA) and 12241 (14/3/14 soloRNA). Results of transfection were compared to a filler control sample. RNAi construct 11546 corresponds to SEQ ID NOs:604 and 605. RNAi construct 12083 corresponds to SEQ ID NOs:597 and 598. RNAi construct 12134 corresponds to SEQ ID NOs:602 and 603. RNAi construct 12241 corresponds to SEQ
20 ID NOs:606 and 607.

FIG. 16 presents a graph and several tables comparing parameters associated with silencing of MAP4K4 expression following transfection with RNAi constructs associated with the invention. The rxRNA construct corresponds to SEQ ID NOs:604 and 605. The 14-3-14 soloRNA construct corresponds to SEQ ID NOs:606 and 607. The 13/19
25 duplex (nicked construct) corresponds to SEQ ID NOs:597 and 598. The 12-bp soloRNA construct corresponds to SEQ ID NOs:602 and 603.

FIG. 17 is a schematic showing examples of RNAi constructs and controls used to target SOD1 expression. The 12084 RNAi construct corresponds to SEQ ID NOs:612 and 613.

30 FIG. 18 is a graph showing SOD1 expression following transfection with RNAi constructs associated with the invention. RNAi constructs tested were: 12084 (Nicked), 12086 (13nt Duplex), 12090 (No Stem Pairing) and 12035 (13nt MiniRNA). Results of transfection were compared to an untransfected control sample. The 12084 RNAi

- 22 --

construct corresponds to SEQ ID NOs:612 and 613. The 12086 RNAi construct corresponds to SEQ ID NOs:608 and 609. The 12035 RNAi construct corresponds to SEQ ID NOs:610 and 611.

FIG. 19 is a graph showing expression of SOD1 24 hours post-transfection with RNAi constructs associated with the invention. RNAi constructs tested were: 10015 (SOD1 rxRNA) and 12084 (SOD1 Nicked Construct). Results of transfection were compared to a filler control sample. The 10015 RNAi construct corresponds to SEQ ID NOs:614 and 615. The 12084 RNAi construct corresponds to SEQ ID NOs:612 and 613.

FIG. 20 is a schematic indicating that RNA molecules with double stranded regions that are less than 10 nucleotides are not cleaved by Dicer.

FIG. 21 is a schematic revealing a hypothetical RNAi model for RNA induced gene silencing.

FIG. 22 is a graph showing chemical optimization of asymmetric RNAi compounds. The presence of chemical modifications, in particular 2'F UC, phosphorothioate modifications on the guide strand, and complete CU 2'OMe modification of the passenger strands results in development of functional compounds. Silencing of MAP4K4 following lipid-mediated transfection is shown using RNAi molecules with specific modifications. RNAi molecules tested had sense strands that were 13 nucleotides long and contained the following modifications: unmodified; C and U 2'OMe; C and U 2'OMe and 3' Chl; rxRNA 2'OMe pattern; or full 2'OMe, except base 1. Additionally, the guide (anti-sense) strands of the RNAi molecules tested contained the following modifications: unmodified; unmodified with 5'P; C and U 2'F; C and U 2'F with 8 PS 3' end; and unmodified (17 nt length). Results for rxRNA 12/10 Duplex and negative controls are also shown.

FIG. 23 demonstrates that the chemical modifications described herein significantly increase in vitro efficacy in un-assisted delivery of RNAi molecules in HeLa cells. The structure and sequence of the compounds were not altered; only the chemical modification patterns of the molecules were modified. Compounds lacking 2'F, 2'O-me, phosphorothioate modification, or cholesterol conjugates were completely inactive in passive uptake. A combination of all 4 of these types of modifications produced the highest levels of activity (compound 12386).

- 23 -

FIG. 24 is a graph showing MAP4K4 expression in HeLa cells following passive uptake transfection of: NT Accell modified siRNA, MAP4K4 Accell siRNA, Non-Chl nanoRNA (12379) and sd-nanoRNA (12386).

FIG. 25 is a graph showing expression of MAP4K4 in HeLa cells following
5 passive uptake transfection of various concentrations of RNA molecules containing the following parameters: Nano Lead with no 3'Chl; Nano Lead; Accell MAP4K4; 21mer GS with 8 PS tail; 21mer GS with 12 PS tail; and 25mer GS with 12 PS tail.

FIG. 26 is a graph demonstrating that reduction in oligonucleotide content increases the efficacy of unassisted uptake. Similar chemical modifications were applied
10 to assymetric compounds, traditional siRNA compounds and 25 mer RNAi compounds. The assymetric small compounds demonstrated the most significant efficacy.

FIG. 27 is a graph demonstrating the importance of phosphorothioate content for un-assisted delivery. FIG. 27A demonstrates the results of a systematic screen that revealed that the presence of at least 2-12 phosphorothioates in the guide strand
15 significantly improves uptake; in some embodiments, 4-8 phosphorothioate modifications were found to be preferred. FIG. 27 B reveals that the presence or absence of phosphorothioate modifications in the sense strand did not alter efficacy.

FIG. 28 is a graph showing expression of MAP4K4 in primary mouse hepatocytes following passive uptake transfection of: Accell Media-Ctrl-UTC; MM
20 APOB Alnylam; Active APOB Alnylam; nanoRNA without chl; nanoRNA MAP4K4; Mouse MAP4K4 Accell Smartpool; DY547 Accell Control; Luc Ctrl rxRNA with Dy547; MAP4K4 rxRNA with DY547; and AS Strand Alone (nano).

FIG. 29 is a graph showing expression of ApoB in mouse primary hepatocytes following passive uptake transfection of: Accell Media-Ctrl-UTC; MM APOB Alnylam;
25 Active APOB Alnylam; nanoRNA without chl; nanoRNA MAP4K4; Mouse MAP4K4 Accell Smartpool; DY547 Accell Control; Luc Ctrl rxRNA with Dy547; MAP4K4 rxRNA with DY547; and AS Strand Alone (nano).

FIG. 30 is a graph showing expression of MAP4K4 in primary human hepatocytes following passive uptake transfection of: 11550 MAP4K4 rxRNA; 12544
30 MM MAP4K4 nanoRNA; 12539 Active MAP4K4 nanoRNA; Accell Media; and UTC.

FIG. 31 is a graph showing ApoB expression in primary human hepatocytes following passive uptake transfection of: 12505 Active ApoB chol-siRNA; 12506 MM ApoB chol-siRNA; Accell Media; and UTC.

- 24 -

FIG. 32 is an image depicting localization of sd-rxRNA^{nano} localization.

FIG. 33 is an image depicting localization of Chol-siRNA (Alnylam).

FIG. 34 is a schematic of 1st generation (G1) sd-rxRNA^{nano} molecules associated with the invention indicating regions that are targeted for modification, and functions
5 associated with different regions of the molecules.

FIG. 35 depicts modification patterns that were screened for optimization of sd-rxRNA^{nano} (G1). The modifications that were screened included, on the guide strand, lengths of 19, 21 and 25 nucleotides, phosphorothioate modifications of 0-18 nucleotides, and replacement of 2'F modifications with 2'OMe, 5 Methyl C and/or ribo
10 Thymidine modifications. Modifications on the sense strand that were screened included nucleotide lengths of 11, 13 and 19 nucleotides, phosphorothioate modifications of 0-4 nucleotides and 2'OMe modifications.

FIG. 36 is a schematic depicting modifications of sd- rxRNA^{nano} that were screened for optimization.

FIG. 37 is a graph showing percent MAP4K4 expression in Hek293 cells following transfection of: Risc Free siRNA; rxRNA; Nano (unmodified); GS alone; Nano Lead (no Chl); Nano (GS: (3) 2'OMe at positions 1, 18, and 19, 8 PS, 19 nt); Nano (GS: (3) 2'OMe at positions 1, 18, and 19, 8 PS, 21 nt); Nano (GS: (3) 2'OMe at positions 1, 18, and 19, 12 PS, 21 nt); and Nano (GS: (3) 2'OMe at positions 1, 18, and
20 19, 12 PS, 25 nt);

FIG. 38 is a graph showing percent MAP4K4 expression in HeLa cells following passive uptake transfection of: GS alone; Nano Lead; Nano (GS: (3) 2'OMe at positions 1, 18, and 19, 8 PS, 19 nt); Nano (GS: (3) 2'OMe at positions 1, 18, and 19, 8 PS, 21 nt); Nano (GS: (3) 2'OMe at positions 1, 18, and 19, 12 PS, 21 nt); Nano (GS: (3) 2'OMe at positions 1, 18, and 19, 12 PS, 25 nt).
25

FIG. 39 is a graph showing percent MAP4K4 expression in Hek293 cells following lipid mediated transfection of: Guide Strand alone (GS: 8PS, 19 nt); Guide Strand alone (GS: 18PS, 19 nt); Nano (GS: no PS, 19 nt); Nano (GS: 2 PS, 19 nt); Nano (GS: 4 PS, 19 nt); Nano (GS: 6 PS, 19 nt); Nano Lead (GS: 8 PS, 19 nt); Nano (GS: 10 PS, 19 nt); Nano (GS: 12 PS, 19 nt); and Nano (GS: 18 PS, 19 nt).
30

FIG. 40 is a graph showing percent MAP4K4 expression in Hek293 cells following lipid mediated transfection of: Guide Strand alone (GS: 8PS, 19 nt); Guide Strand alone (GS: 18PS, 19 nt); Nano (GS: no PS, 19 nt); Nano (GS: 2 PS, 19 nt); Nano

- 25 -

(GS: 4 PS, 19 nt); Nano (GS: 6 PS, 19 nt); Nano Lead (GS: 8 PS, 19 nt); Nano (GS: 10 PS, 19 nt); Nano (GS: 12 PS, 19 nt); and Nano (GS: 18 PS, 19 nt).

FIG. 41 is a graph showing percent MAP4K4 expression in HeLa cells following passive uptake transfection of: Nano Lead (no Chl); Guide Strand alone (18 PS); Nano
5 (GS: 0 PS, 19 nt); Nano (GS: 2 PS, 19 nt); Nano (GS: 4 PS, 19 nt); Nano (GS: 6 PS, 19 nt); Nano Lead (GS: 8 PS, 19 nt); Nano (GS: 10 PS, 19 nt); Nano (GS: 12 PS, 19 nt); and Nano (GS: 18 PS, 19 nt).

FIG. 42 is a graph showing percent MAP4K4 expression in HeLa cells following passive uptake transfection of: Nano Lead (no Chl); Guide Strand alone (18 PS); Nano
10 (GS: 0 PS, 19 nt); Nano (GS: 2 PS, 19 nt); Nano (GS: 4 PS, 19 nt); Nano (GS: 6 PS, 19 nt); Nano Lead (GS: 8 PS, 19 nt); Nano (GS: 10 PS, 19 nt); Nano (GS: 12 PS, 19 nt); and Nano (GS: 18 PS, 19 nt).

FIG. 43 is a schematic depicting guide strand chemical modifications that were screened for optimization.

15 FIG. 44 is a graph showing percent MAP4K4 expression in Hek293 cells following reverse transfection of: RISC free siRNA; GS only (2'F C and Us); GS only (2'OMe C and Us); Nano Lead (2'F C and Us); nano (GS: (3) 2'OMe, positions 16-18); nano (GS: (3) 2'OMe, positions 16, 17 and 19); nano (GS: (4) 2'OMe, positions 11, 16-18); nano (GS: (10) 2'OMe,C and Us); nano (GS: (6) 2'OMe, positions 1 and 5-9); nano
20 (GS: (3) 2'OMe, positions 1, 18 and 19); and nano (GS: (5) 2'OMe Cs).

FIG. 45 is a graph demonstrating efficacy of various chemical modification patterns. In particular, 2-OMe modification in positions 1 and 11-18 was well tolerated. 2'OMe modifications in the seed area resulted in a slight reduction of efficacy (but were still highly efficient). Ribo- modifications in the seed were well tolerated. This data
25 enabled the generation of self delivering compounds with reduced or no 2'F modifications. This is significant because 2'F modifications may be associated with toxicity *in vivo*.

FIG. 46 is a schematic depicting sense strand modifications.

FIG. 47 is a graph demonstrating sense strand length optimization. A sense
30 strand length between 10-15 bases was found to be optimal in this assay. Increasing sense strand length resulted in a reduction of passive uptake of these compounds but may be tolerated for other compounds. Sense strands containing LNA modification demonstrated similar efficacy to non-LNA containing compounds. In some embodiments,

- 26 --

the addition of LNA or other thermodynamically stabilizing compounds can be beneficial, resulting in converting non-functional sequences into functional sequences.

FIG. 48 is a graph showing percent MAP4K4 expression in HeLa cells following passive uptake transfection of: Guide Strand Alone (2'F C and U); Nano Lead; Nano
5 Lead (No Chl); Nano (SS: 11 nt 2'OMe C and Us, Chl); Nano (SS: 11nt, complete 2'OMe, Chl); Nano (SS: 19 nt, 2'OMe C and Us, Chl); Nano (SS: 19 nt, 2'OMe C and Us, no Chl).

FIG. 49 is a graph showing percent MAP4K4 expression in HeLa cells following passive uptake transfection of: Nano Lead (No Chl); Nano (SS no PS); Nano Lead (SS:2
10 PS); Nano (SS:4 PS).

FIG. 50 is a schematic depicting a *sd-rxRNA*^{nano} second generation (GII) lead molecule.

FIG. 51 presents a graph indicating EC₅₀ values for MAP4K4 silencing in the presence of *sd-rxRNA*, and images depicting localization of DY547-labeled *rxRNA*^{ori}
15 and DY547-labeled *sd-rxRNA*.

FIG. 52 is a graph showing percent MAP4K4 expression in HeLa cells in the presence of optimized *sd-rxRNA* molecules.

FIG. 53 is a graph depicting the relevance of chemistry content in optimization of *sd-rxRNA* efficacy.

FIG. 54 presents schematics of sterol-type molecules and a graph revealing that
20 *sd-rxRNA* compounds are fully functional with a variety of linker chemistries. GII asymmetric compounds were synthesized with steroltype molecules attached through TEG and amino caproic acid linkers. Both linkers showed identical potency. This functionality independent of linker chemistry indicates a significant difference between
25 the molecules described herein and previously described molecules, and offers significant advantages for the molecules described herein in terms of scale up and synthesis.

FIG. 55 demonstrates the stability of chemically modified *sd-rxRNA* compounds in human serum in comparison to non modified RNA. The oligonucleotides were
30 incubated in 75% serum at 37 °C for the number of hours indicated. The level of degradation was determined by running the samples on non-denaturing gels and staining with SYBGR.

- 27 -

FIG. 56 is a graph depicting optimization of cellular uptake of *sd*-rxRNA through minimizing oligonucleotide content.

FIG. 57 is a graph showing percent MAP4K4 expression after spontaneous cellular uptake of *sd*-rxRNA in mouse PEC-derived macrophages, and phase and
5 fluorescent images showing localization of *sd*-rxRNA.

FIG. 58 is a graph showing percent MAP4K4 expression after spontaneous cellular uptake of *sd*-rxRNA (targeting) and *sd*-rxRNA (mismatch) in mouse primary hepatocytes, and phase and fluorescent images showing localization of *sd*-rxRNA.

FIG. 59 presents images depicting localization of DY547-labeled *sd*-rxRNA
10 delivered to RPE cells with no formulation.

FIG. 60 is a graph showing silencing of MAP4K4 expression in RPE cells treated with *sd*-rxRNA^{nano} without formulation.

FIG. 61 presents a graph and schematics of RNAi compounds showing the chemical/ structural composition of highly effective *sd*-rxRNA compounds. Highly
15 effective compounds were found to have the following characteristics: antisense strands of 17-21 nucleotides, sense strands of 10-15 nucleotides, single-stranded regions that contained 2-12 phosphorothioate modifications, preferentially 6-8 phosphorothioate modifications, and sense strands in which the majority of nucleotides were 2'OMe modified, with or without phosphorothioate modification. Any linker chemistry can be
20 used to attach these molecules to hydrophobic moiety such as cholesterol at the 3' end of the sense strand. Version GIIa-b of these RNA compounds demonstrate that elimination of 2'F content has no impact on efficacy.

FIG. 62 presents a graph and schematics of RNAi compounds demonstrating the superior performance of *sd*-rxRNA compounds compared to compounds published by
25 Wolfrum et. al. Nature Biotech, 2007. Both generation I and II compounds (GI and GIIa) developed herein show great efficacy. By contrast, when the chemistry described in Wolfrum et al. (all oligos contain cholesterol conjugated to the 3' end of the sense strand) was applied to the same sequence in a context of conventional siRNA (19 bp duplex with two overhang) the compound was practically inactive. These data
30 emphasize the significance of the combination of chemical modifications and asymmetrical molecules described herein, producing highly effective RNA compounds.

FIG. 63 presents images showing that *sd*-rxRNA accumulates inside cells while other less effective conjugate RNAs accumulate on the surface of cells.

- 28 -

FIG. 64 presents images showing that *sd*-rxRNA molecules, but not other molecules, are internalized into cells within minutes.

FIG. 65 presents images demonstrating that *sd*-rxRNA compounds have drastically better cellular and tissue uptake characteristics when compared to conventional cholesterol conjugated siRNAs (such as those published by Soucheck et al). FIG. 65A,B compare uptake in RPE cells, FIG. 65C,D compare uptake upon local administration to skin and FIG. 65E,F compare uptake by the liver upon systemic administration. The level of uptake is at least an order of magnitude higher for the *sd*-rxRNA compounds relative to the regular siRNA-cholesterol compounds.

FIG. 66 presents images depicting localization of rxRNA^{ori} and *sd*-rxRNA following local delivery.

FIG. 67 presents images depicting localization of *sd*-rxRNA and other conjugate RNAs following local delivery.

FIG. 68 presents a graph revealing the results of a screen performed with *sd*-rxRNAGII chemistry to identify functional compounds targeting the SPP1 gene. Multiple effective compounds were identified, with 14131 being the most effective. The compounds were added to A-549 cells and the level of the ratio of SPP1/ PPIB was determined by B-DNA after 48 hours.

FIG. 69 presents a graph and several images demonstrating efficient cellular uptake of *sd*-rxRNA within minutes of exposure. This is a unique characteristics of the *sd*-rxRNA compounds described herein, not observed with any other RNAi compounds. The Soutschek et al. compound was used as a negative control.

FIG. 70 presents a graph and several images demonstrating efficient uptake and silencing of *sd*-rxRNA compounds in multiple cell types with multiple sequences. In each case silencing was confirmed by looking at target gene expression using a Branched DNA assay.

FIG. 71 presents a graph revealing that *sd*-rxRNA is active in the presence and absence of serum. A slight reduction in efficacy (2-5 fold) was observed in the presence of serum. This minimal reduction in efficacy in the presence of serum differentiates the *sd*-rxRNA compounds described herein from previously described RNAi compounds, which had a greater reduction in efficacy, and thus creates a foundation for *in vivo* efficacy of the *sd*-rxRNA molecules described herein.

- 29 -

FIG. 72 presents images demonstrating efficient tissue penetration and cellular uptake upon single intradermal injection of sd-rxRNA compounds described herein. This represents a model for local delivery of sd-rxRNA compounds as well as an effective demonstration of delivery of sd-rxRNA compounds and silencing of genes in dermatological applications.

FIG. 73 presents images and a graph demonstrating efficient cellular uptake and *in vivo* silencing with sd-rxRNA following intradermal injection.

FIG. 74 presents graphs demonstrating that sd-rxRNA compounds have improved blood clearance and induce effective gene silencing *in vivo* in the liver upon systemic administration.

FIG. 75 presents a graph demonstrating that the presence of 5-Methyl C in an RNAi compound resulted in an increase in potency of lipid mediated transfection, demonstrating that hydrophobic modification of Cs and Us in the content of RNAi compounds can be beneficial. In some embodiments, these types of modifications can be used in the context of 2' ribose modified bases to insure optimal stability and efficacy.

FIG. 76 presents a graph showing percent MAP4K4 expression in HeLa cells following passive uptake transfection of: Guide strand alone; Nano Lead; Nano Lead (No cholesterol); Guide Strand w/5MeC and 2'F Us Alone; Nano Lead w/GS 5MeC and 2'F Us; Nano Lead w/GS riboT and 5 Methyl Cs; and Nano Lead w/Guide dT and 5 Methyl Cs.

FIG. 77 presents images comparing localization of sd-rxRNA and other RNA conjugates following systemic delivery to the liver.

FIG. 78 presents schematics demonstrating 5-uridyl modifications with improved hydrophobicity characteristics. Incorporation of such modifications into sd-rxRNA compounds can increase cellular and tissue uptake properties. FIG. 78B presents a new type of RNAi compound modification which can be applied to compounds to improve cellular uptake and pharmacokinetic behavior. This type of modification, when applied to sd-rxRNA compounds, may contribute to making such compounds orally available.

FIG. 79 presents schematics revealing the structures of synthesized modified sterol type molecules, where the length and structure of the C17 attached tail is modified. Without wishing to be bound by any theory, the length of the C17 attached tail may contribute to improving *in vitro* and *in vivo* efficacy of sd-rxRNA compounds.

- 30 -

FIG. 80 presents a schematic demonstrating the lithocholic acid route to long side chain cholesterol.

FIG. 81 presents a schematic demonstrating a route to 5-uridyl phosphoramidite synthesis.

5 FIG. 82 presents a schematic demonstrating synthesis of tri-functional hydroxyprolinol linker for 3'-cholesterol attachment.

FIG. 83 presents a schematic demonstrating synthesis of solid support for the manufacture of a shorter asymmetric RNAi compound strand.

10 FIG. 84 demonstrates SPP1 sd-rxRNA compound selection. Sd-rxRNA compounds targeting SPP1 were added to A549 cells (using passive transfection) and the level of SPP1 expression was evaluated after 48 hours. Several novel compounds effective in SPP1 silencing were identified, the most potent of which was compound 14131.

15 FIG. 85 demonstrates independent validation of sd-rxRNA compounds 14116, 14121, 14131, 14134, 14139, 14149, and 14152 efficacy in SPP1 silencing.

FIG. 86 demonstrates results of sd-rxRNA compound screens to identify sd-rxRNA compounds functional in CTGF knockdown.

FIG. 87 demonstrates results of sd-rxRNA compound screens to identify sd-rxRNA functional in CTGF knockdown.

20 FIG. 88 demonstrates a systematic screen identifying the minimal length of the asymmetric compounds. The passenger strand of 10-19 bases was hybridized to a guide strand of 17-25 bases. In this assay, compounds with duplex regions as short as 10 bases were found to be effective in inducing.

25 FIG. 89 demonstrates that positioning of the sense strand relative to the guide strand is critical for RNAi Activity. In this assay, a blunt end was found to be optimal, a 3' overhang was tolerated, and a 5' overhang resulted in complete loss of functionality.

30 FIG. 90 demonstrates that the guide strand, which has homology to the target only at nucleotides 2-17, resulted in effective RNAi when hybridized with sense strands of different lengths. The compounds were introduced into HeLa cells via lipid mediated transfection.

FIG. 91 is a schematic depicting a panel of sterol-type molecules which can be used as a hydrophobic entity in place of cholesterol. In some instances, the use of sterol-

- 31 -

type molecules comprising longer chains results in generation of sd-rxRNA compounds with significantly better cellular uptake and tissue distribution properties.

FIG. 92 presents schematics depicting a panel of hydrophobic molecules which might be used as a hydrophobic entity in place of cholesterol. These list just provides
5 representative examples; any small molecule with substantial hydrophobicity can be used.

DETAILED DESCRIPTION

Aspects of the invention relate to methods and compositions involved in gene
10 silencing. The invention is based at least in part on the surprising discovery that asymmetric nucleic acid molecules with a double stranded region of a minimal length such as 8-14 nucleotides, are effective in silencing gene expression. Molecules with such a short double stranded region have not previously been demonstrated to be effective in mediating RNA interference. It had previously been assumed that that there
15 must be a double stranded region of 19 nucleotides or greater. The molecules described herein are optimized through chemical modification, and in some instances through attachment of hydrophobic conjugates.

The invention is based at least in part on another surprising discovery that asymmetric nucleic acid molecules with reduced double stranded regions are much more
20 effectively taken up by cells compared to conventional siRNAs. These molecules are highly efficient in silencing of target gene expression and offer significant advantages over previously described RNAi molecules including high activity in the presence of serum, efficient self delivery, compatibility with a wide variety of linkers, and reduced presence or complete absence of chemical modifications that are associated with toxicity.

25 In contrast to single-stranded polynucleotides, duplex polynucleotides have been difficult to deliver to a cell as they have rigid structures and a large number of negative charges which makes membrane transfer difficult. Unexpectedly, it was found that the polynucleotides of the present invention, although partially double-stranded, are recognized *in vivo* as single-stranded and, as such, are capable of efficiently being
30 delivered across cell membranes. As a result the polynucleotides of the invention are capable in many instances of self delivery. Thus, the polynucleotides of the invention may be formulated in a manner similar to conventional RNAi agents or they may be delivered to the cell or subject alone (or with non-delivery type carriers) and allowed to

- 32 -

self deliver. In one embodiment of the present invention, self delivering asymmetric double-stranded RNA molecules are provided in which one portion of the molecule resembles a conventional RNA duplex and a second portion of the molecule is single stranded.

5 The polynucleotides of the invention are referred to herein as isolated double stranded or duplex nucleic acids, oligonucleotides or polynucleotides, nano molecules, nano RNA, sd-rxRNA^{nano}, sd-rxRNA or RNA molecules of the invention.

 The oligonucleotides of the invention in some aspects have a combination of asymmetric structures including a double stranded region and a single stranded region of
10 5 nucleotides or longer, specific chemical modification patterns and are conjugated to lipophilic or hydrophobic molecules. This new class of RNAi like compounds have superior efficacy *in vitro* and *in vivo*. Based on the data described herein it is believed that the reduction in the size of the rigid duplex region in combination with phosphorothioate modifications applied to a single stranded region are new and
15 important for achieving the observed superior efficacy. Thus, the RNA molecules described herein are different in both structure and composition as well as *in vitro* and *in vivo* activity.

 In a preferred embodiment the RNAi compounds of the invention comprise an asymmetric compound comprising a duplex region (required for efficient RISC entry of
20 10-15 bases long) and single stranded region of 4-12 nucleotides long; with a 13 nucleotide duplex. A 6 nucleotide single stranded region is preferred in some embodiments. The single stranded region of the new RNAi compounds also comprises 2-12 phosphorothioate internucleotide linkages (referred to as phosphorothioate modifications). 6-8 phosphorothioate internucleotide linkages are preferred in some
25 embodiments. Additionally, the RNAi compounds of the invention also include a unique chemical modification pattern, which provides stability and is compatible with RISC entry. The combination of these elements has resulted in unexpected properties which are highly useful for delivery of RNAi reagents *in vitro* and *in vivo*.

 The chemical modification pattern, which provides stability and is compatible
30 with RISC entry includes modifications to the sense, or passenger, strand as well as the antisense, or guide, strand. For instance the passenger strand can be modified with any chemical entities which confer stability and do not interfere with activity. Such modifications include 2' ribo modifications (O-methyl, 2' F, 2 deoxy and others) and

- 33 -

backbone modification like phosphorothioate modifications. A preferred chemical modification pattern in the passenger strand includes Omethyl modification of C and U nucleotides within the passenger strand or alternatively the passenger strand may be completely Omethyl modified.

5 The guide strand, for example, may also be modified by any chemical modification which confirms stability without interfering with RISC entry. A preferred chemical modification pattern in the guide strand includes the majority of C and U nucleotides being 2' F modified and the 5' end being phosphorylated. Another preferred chemical modification pattern in the guide strand includes 2'Omethyl modification of position 1 and C/U in positions 11-18 and 5' end chemical phosphorylation. Yet another
10 preferred chemical modification pattern in the guide strand includes 2'Omethyl modification of position 1 and C/U in positions 11-18 and 5' end chemical phosphorylation and and 2'F modification of C/U in positions 2-10.

 It was surprisingly discovered according to the invention that the above-described
15 chemical modification patterns of the oligonucleotides of the invention are well tolerated and actually improved efficacy of asymmetric RNAi compounds. See, for instance, Figure 22.

 It was also demonstrated experimentally herein that the combination of modifications to RNAi when used together in a polynucleotide results in the achievement
20 of optimal efficacy in passive uptake of the RNAi. Elimination of any of the described components (Guide strand stabilization, phosphorothioate stretch, sense strand stabilization and hydrophobic conjugate) or increase in size results in sub-optimal efficacy and in some instances complete lost of efficacy. The combination of elements results in development of compound, which is fully active following passive delivery to
25 cells such as HeLa cells. (Figure 23). The degree to which the combination of elements results in efficient self delivery of RNAi molecules was completely unexpected.

 The data shown in Figures 26, 27 and 43 demonstrated the importance of the various modifications to the RNAi in achieving stabilization and activity. For instance, Figure 26 demonstrates that use off asymmetric configuration is important in getting
30 efficacy in passive uptake. When the same chemical composition is applied to compounds of traditional configurations (19-21 bases duplex and 25 mer duplex) the efficacy was drastically decreased in a length dependent manner. Figure 27 demonstrated a systematic screen of the impact of phosphorothioate chemical

- 34 -

modifications on activity. The sequence, structure, stabilization chemical modifications, hydrophobic conjugate were kept constant and compound phosphorothioate content was varied (from 0 to 18 PS bond). Both compounds having no phosphorothioate linkages and having 18 phosphorothioate linkages were completely inactive in passive uptake.

- 5 Compounds having 2-16 phosphorothioate linkages were active, with compounds having 4-10 phosphorothioate being the most active compounds.

The data in the Examples presented below demonstrates high efficacy of the oligonucleotides of the invention both *in vitro* in variety of cell types (supporting data) and *in vivo* upon local and systemic administration. For instance, the data compares the ability of several competitive RNAi molecules having different chemistries to silence a gene. Comparison of sd-rxRNA (oligonucleotides of the invention) with RNAs described in Soucheck et al. and Wolfrum et al., as applied to the same targeting region, demonstrated that only sd-rxRNA chemistry showed a significant functionality in passive uptake. The composition of the invention achieved EC₅₀ values of 10-50 pM.

10 This level of efficacy is un-attainable with conventional chemistries like those described in Soucheck et al and Accell. Similar comparisons were made in other systems, such as *in vitro* (RPE cell line), *in vivo* upon local administration (wounded skin) and systemic (50 mg/kg) as well as other genes (Figures 65 and 68). In each case the oligonucleotides of the invention achieved better results. Figure 64 includes data demonstrating efficient cellular uptake and resulting silencing by sd-rxRNA compounds only after 1 minute of exposure. Such an efficacy is unique to this composition and have not been seen with other types of molecules in this class. Figure 70 demonstrates efficient uptake and silencing of sd-rxRNA compounds in multiple cell types with multiple sequences. The sd-rxRNA compounds are also active in cells in presence and absence of serum and other biological liquids. Figure 71 demonstrates only a slight reduction in activity in the presence of serum. This ability to function in biologically aggressive environment effectively further differentiates sd-rxRNA compounds from other compounds described previously in this group, like Accell and Soucheck et al, in which uptake is drastically inhibited in a presence of serum.

30 Significant amounts of data also demonstrate the *in vivo* efficacy of the compounds of the invention. For instance Figures 72-74 involve multiple routes of *in vivo* delivery of the compounds of the invention resulting in significant activity. Figure 72, for example, demonstrates efficient tissue penetration and cellular uptake upon single

- 35 -

intradermal injection. This is a model for local delivery of sd-rxRNA compounds as well as an effective delivery mode for sd-rxRNA compounds and silencing genes in any dermatology applications. Figure 73 demonstrated efficient tissue penetration, cellular uptake and silencing upon local in vivo intradermal injection of sd-rxRNA compounds.

- 5 The data of Figure 74 demonstrate that sd-rxRNA compounds result in highly effective liver uptake upon IV administration. Comparison to Souicheck at al molecule showed that the level of liver uptake at identical dose level was quite surprisingly, at least 50 fold higher with the sd-rxRNA compound than the Souicheck at al molecule.

- The sd-rxRNA can be further improved in some instances by improving the
- 10 hydrophobicity of compounds using of novel types of chemistries. For example one chemistry is related to use of hydrophobic base modifications. Any base in any position might be modified, as long as modification results in an increase of the partition coefficient of the base. The preferred locations for modification chemistries are positions 4 and 5 of the pyrimidines. The major advantage of these positions is (a) ease of
- 15 synthesis and (b) lack of interference with base-pairing and A form helix formation, which are essential for RISC complex loading and target recognition. Examples of these chemistries is shown in Figures 75-83. A version of sd-rxRNA compounds where multiple deoxy Uridines are present without interfering with overall compound efficacy was used. In addition major improvement in tissue distribution and cellular uptake might
- 20 be obtained by optimizing the structure of the hydrophobic conjugate. In some of the preferred embodiment the structure of sterol is modified to alter (increase/ decrease) C17 attached chain. This type of modification results in significant increase in cellular uptake and improvement of tissue uptake prosperities in vivo.

- This invention is not limited in its application to the details of construction and
- 25 the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways. Also, the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of “including,” “comprising,” or “having,” “containing,” “involving,” and variations thereof
- 30 herein, is meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

Thus, aspects of the invention relate to isolated double stranded nucleic acid molecules comprising a guide (antisense) strand and a passenger (sense) strand. As used

- 36 -

herein, the term “double-stranded” refers to one or more nucleic acid molecules in which at least a portion of the nucleomonomers are complementary and hydrogen bond to form a double-stranded region. In some embodiments, the length of the guide strand ranges from 16-29 nucleotides long. In certain embodiments, the guide strand is 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 nucleotides long. The guide strand has complementarity to a target gene. Complementarity between the guide strand and the target gene may exist over any portion of the guide strand. Complementarity as used herein may be perfect complementarity or less than perfect complementarity as long as the guide strand is sufficiently complementary to the target that it mediates RNAi. In some embodiments complementarity refers to less than 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% mismatch between the guide strand and the target. Perfect complementarity refers to 100% complementarity. Thus the invention has the advantage of being able to tolerate sequence variations that might be expected due to genetic mutation, strain polymorphism, or evolutionary divergence. For example, siRNA sequences with insertions, deletions, and single point mutations relative to the target sequence have also been found to be effective for inhibition. Moreover, not all positions of a siRNA contribute equally to target recognition. Mismatches in the center of the siRNA are most critical and essentially abolish target RNA cleavage. Mismatches upstream of the center or upstream of the cleavage site referencing the antisense strand are tolerated but significantly reduce target RNA cleavage. Mismatches downstream of the center or cleavage site referencing the antisense strand, preferably located near the 3' end of the antisense strand, e.g. 1, 2, 3, 4, 5 or 6 nucleotides from the 3' end of the antisense strand, are tolerated and reduce target RNA cleavage only slightly.

While not wishing to be bound by any particular theory, in some embodiments, the guide strand is at least 16 nucleotides in length and anchors the Argonaute protein in RISC. In some embodiments, when the guide strand loads into RISC it has a defined seed region and target mRNA cleavage takes place across from position 10-11 of the guide strand. In some embodiments, the 5' end of the guide strand is or is able to be phosphorylated. The nucleic acid molecules described herein may be referred to as minimum trigger RNA.

In some embodiments, the length of the passenger strand ranges from 8-14 nucleotides long. In certain embodiments, the passenger strand is 8, 9, 10, 11, 12, 13 or 14 nucleotides long. The passenger strand has complementarity to the guide strand.

- 37 -

Complementarity between the passenger strand and the guide strand can exist over any portion of the passenger or guide strand. In some embodiments, there is 100% complementarity between the guide and passenger strands within the double stranded region of the molecule.

5 Aspects of the invention relate to double stranded nucleic acid molecules with minimal double stranded regions. In some embodiments the region of the molecule that is double stranded ranges from 8-14 nucleotides long. In certain embodiments, the region of the molecule that is double stranded is 8, 9, 10, 11, 12, 13 or 14 nucleotides long. In certain embodiments the double stranded region is 13 nucleotides long. There
10 can be 100% complementarity between the guide and passenger strands, or there may be one or more mismatches between the guide and passenger strands. In some embodiments, on one end of the double stranded molecule, the molecule is either blunt-ended or has a one-nucleotide overhang. The single stranded region of the molecule is in some embodiments between 4-12 nucleotides long. For example the single stranded
15 region can be 4, 5, 6, 7, 8, 9, 10, 11 or 12 nucleotides long. However, in certain embodiments, the single stranded region can also be less than 4 or greater than 12 nucleotides long. In certain embodiments, the single stranded region is 6 nucleotides long.

RNAi constructs associated with the invention can have a thermodynamic
20 stability (ΔG) of less than -13 kkal/mol. In some embodiments, the thermodynamic stability (ΔG) is less than -20 kkal/mol. In some embodiments there is a loss of efficacy when (ΔG) goes below -21 kkal/mol. In some embodiments a (ΔG) value higher than -13 kkal/mol is compatible with aspects of the invention. Without wishing to be bound by any theory, in some embodiments a molecule with a relatively higher (ΔG) value may
25 become active at a relatively higher concentration, while a molecule with a relatively lower (ΔG) value may become active at a relatively lower concentration. In some embodiments, the (ΔG) value may be higher than -9 kkal/mol. The gene silencing effects mediated by the RNAi constructs associated with the invention, containing minimal double stranded regions, are unexpected because molecules of almost identical
30 design but lower thermodynamic stability have been demonstrated to be inactive (Rana et al 2004).

Without wishing to be bound by any theory, results described herein suggest that a stretch of 8-10 bp of dsRNA or dsDNA will be structurally recognized by protein

- 38 -

components of RISC or co-factors of RISC. Additionally, there is a free energy requirement for the triggering compound that it may be either sensed by the protein components and/or stable enough to interact with such components so that it may be loaded into the Argonaute protein. If optimal thermodynamics are present and there is a double stranded portion that is preferably at least 8 nucleotides then the duplex will be recognized and loaded into the RNAi machinery.

In some embodiments, thermodynamic stability is increased through the use of LNA bases. In some embodiments, additional chemical modifications are introduced. Several non-limiting examples of chemical modifications include: 5' Phosphate, 2'-O-methyl, 2'-O-ethyl, 2'-fluoro, ribothymidine, C-5 propynyl-dC (pdC) and C-5 propynyl-dU (pdU); C-5 propynyl-C (pC) and C-5 propynyl-U (pU); 5-methyl C, 5-methyl U, 5-methyl dC, 5-methyl dU methoxy, (2,6-diaminopurine), 5'-Dimethoxytrityl-N4-ethyl-2'-deoxyCytidine and MGB (minor groove binder). It should be appreciated that more than one chemical modification can be combined within the same molecule.

Molecules associated with the invention are optimized for increased potency and/or reduced toxicity. For example, nucleotide length of the guide and/or passenger strand, and/or the number of phosphorothioate modifications in the guide and/or passenger strand, can in some aspects influence potency of the RNA molecule, while replacing 2'-fluoro (2'F) modifications with 2'-O-methyl (2'OMe) modifications can in some aspects influence toxicity of the molecule. Specifically, reduction in 2'F content of a molecule is predicted to reduce toxicity of the molecule. The Examples section presents molecules in which 2'F modifications have been eliminated, offering an advantage over previously described RNAi compounds due to a predicted reduction in toxicity. Furthermore, the number of phosphorothioate modifications in an RNA molecule can influence the uptake of the molecule into a cell, for example the efficiency of passive uptake of the molecule into a cell. Preferred embodiments of molecules described herein have no 2'F modification and yet are characterized by equal efficacy in cellular uptake and tissue penetration. Such molecules represent a significant improvement over prior art, such as molecules described by Accell and Wolfrum, which are heavily modified with extensive use of 2'F.

In some embodiments, a guide strand is approximately 18-19 nucleotides in length and has approximately 2-14 phosphate modifications. For example, a guide strand can contain 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or more than 14 nucleotides that

- 39 --

are phosphate-modified. The guide strand may contain one or more modifications that confer increased stability without interfering with RISC entry. The phosphate modified nucleotides, such as phosphorothioate modified nucleotides, can be at the 3' end, 5' end or spread throughout the guide strand. In some embodiments, the 3' terminal 10
5 nucleotides of the guide strand contains 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 phosphorothioate modified nucleotides. The guide strand can also contain 2'F and/or 2'OMe modifications, which can be located throughout the molecule. In some embodiments, the nucleotide in position one of the guide strand (the nucleotide in the most 5' position of the guide strand) is 2'OMe modified and/or phosphorylated. C and U nucleotides within
10 the guide strand can be 2'F modified. For example, C and U nucleotides in positions 2-10 of a 19 nt guide strand (or corresponding positions in a guide strand of a different length) can be 2'F modified. C and U nucleotides within the guide strand can also be 2'OMe modified. For example, C and U nucleotides in positions 11-18 of a 19 nt guide strand (or corresponding positions in a guide strand of a different length) can be 2'OMe
15 modified. In some embodiments, the nucleotide at the most 3' end of the guide strand is unmodified. In certain embodiments, the majority of Cs and Us within the guide strand are 2'F modified and the 5' end of the guide strand is phosphorylated. In other embodiments, position 1 and the Cs or Us in positions 11-18 are 2'OMe modified and the 5' end of the guide strand is phosphorylated. In other embodiments, position 1 and
20 the Cs or Us in positions 11-18 are 2'OMe modified, the 5' end of the guide strand is phosphorylated, and the Cs or Us in position 2-10 are 2'F modified.

In some aspects, an optimal passenger strand is approximately 11-14 nucleotides in length. The passenger strand may contain modifications that confer increased stability. One or more nucleotides in the passenger strand can be 2'OMe modified. In
25 some embodiments, one or more of the C and/or U nucleotides in the passenger strand is 2'OMe modified, or all of the C and U nucleotides in the passenger strand are 2'OMe modified. In certain embodiments, all of the nucleotides in the passenger strand are 2'OMe modified. One or more of the nucleotides on the passenger strand can also be phosphate-modified such as phosphorothioate modified. The passenger strand can also
30 contain 2' ribo, 2'F and 2 deoxy modifications or any combination of the above. As demonstrated in the Examples, chemical modification patterns on both the guide and passenger strand are well tolerated and a combination of chemical modifications is shown herein to lead to increased efficacy and self-delivery of RNA molecules.

- 40 --

Aspects of the invention relate to RNAi constructs that have extended single-stranded regions relative to double stranded regions, as compared to molecules that have been used previously for RNAi. The single stranded region of the molecules may be modified to promote cellular uptake or gene silencing. In some embodiments, phosphorothioate modification of the single stranded region influences cellular uptake and/or gene silencing. The region of the guide strand that is phosphorothioate modified can include nucleotides within both the single stranded and double stranded regions of the molecule. In some embodiments, the single stranded region includes 2-12 phosphorothioate modifications. For example, the single stranded region can include 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 phosphorothioate modifications. In some instances, the single stranded region contains 6-8 phosphorothioate modifications.

Molecules associated with the invention are also optimized for cellular uptake. In RNA molecules described herein, the guide and/or passenger strands can be attached to a conjugate. In certain embodiments the conjugate is hydrophobic. The hydrophobic conjugate can be a small molecule with a partition coefficient that is higher than 10. The conjugate can be a sterol-type molecule such as cholesterol, or a molecule with an increased length polycarbon chain attached to C17, and the presence of a conjugate can influence the ability of an RNA molecule to be taken into a cell with or without a lipid transfection reagent. The conjugate can be attached to the passenger or guide strand through a hydrophobic linker. In some embodiments, a hydrophobic linker is 5-12C in length, and/or is hydroxypyrrolidine-based. In some embodiments, a hydrophobic conjugate is attached to the passenger strand and the CU residues of either the passenger and/or guide strand are modified. In some embodiments, at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of the CU residues on the passenger strand and/or the guide strand are modified. In some aspects, molecules associated with the invention are self-delivering (sd). As used herein, "self-delivery" refers to the ability of a molecule to be delivered into a cell without the need for an additional delivery vehicle such as a transfection reagent.

Aspects of the invention relate to selecting molecules for use in RNAi. Based on the data described herein, molecules that have a double stranded region of 8-14 nucleotides can be selected for use in RNAi. In some embodiments, molecules are selected based on their thermodynamic stability (ΔG). In some embodiments, molecules will be selected that have a (ΔG) of less than -13 kkal/mol. For example, the (ΔG) value

- 41 -

may be -13, -14, -15, -16, -17, -18, -19, -21, -22 or less than -22 kkal/mol. In other embodiments, the (ΔG) value may be higher than -13 kkal/mol. For example, the (ΔG) value may be -12, -11, -10, -9, -8, -7 or more than -7 kkal/mol. It should be appreciated that ΔG can be calculated using any method known in the art. In some embodiments ΔG is calculated using Mfold, available through the Mfold internet site (<http://mfold.bioinfo.rpi.edu/cgi-bin/rna-form1.cgi>). Methods for calculating ΔG are described in, and are incorporated by reference from, the following references: Zuker, M. (2003) *Nucleic Acids Res.*, 31(13):3406-15; Mathews, D. H., Sabina, J., Zuker, M. and Turner, D. H. (1999) *J. Mol. Biol.* 288:911-940; Mathews, D. H., Disney, M. D., Childs, J. L., Schroeder, S. J., Zuker, M., and Turner, D. H. (2004) *Proc. Natl. Acad. Sci.* 101:7287-7292; Duan, S., Mathews, D. H., and Turner, D. H. (2006) *Biochemistry* 45:9819-9832; Wuchty, S., Fontana, W., Hofacker, I. L., and Schuster, P. (1999) *Biopolymers* 49:145-165.

Aspects of the invention relate to using nucleic acid molecules described herein, with minimal double stranded regions and/or with a (ΔG) of less than -13 kkal/mol, for gene silencing. RNAi molecules can be administered in vivo or in vitro, and gene silencing effects can be achieved in vivo or in vitro.

In certain embodiments, the polynucleotide contains 5'- and/or 3'-end overhangs. The number and/or sequence of nucleotides overhang on one end of the polynucleotide may be the same or different from the other end of the polynucleotide. In certain embodiments, one or more of the overhang nucleotides may contain chemical modification(s), such as phosphorothioate or 2'-OMe modification.

In certain embodiments, the polynucleotide is unmodified. In other embodiments, at least one nucleotide is modified. In further embodiments, the modification includes a 2'-H or 2'-modified ribose sugar at the 2nd nucleotide from the 5'-end of the guide sequence. The "2nd nucleotide" is defined as the second nucleotide from the 5'-end of the polynucleotide.

As used herein, "2'-modified ribose sugar" includes those ribose sugars that do not have a 2'-OH group. "2'-modified ribose sugar" does not include 2'-deoxyribose (found in unmodified canonical DNA nucleotides). For example, the 2'-modified ribose sugar may be 2'-O-alkyl nucleotides, 2'-deoxy-2'-fluoro nucleotides, 2'-deoxy nucleotides, or combination thereof.

- 42 -

In certain embodiments, the 2'-modified nucleotides are pyrimidine nucleotides (*e.g.*, C /U). Examples of 2'-O-alkyl nucleotides include 2'-O-methyl nucleotides, or 2'-O-allyl nucleotides.

5 In certain embodiments, the miniRNA polynucleotide of the invention with the above-referenced 5'-end modification exhibits significantly (*e.g.*, at least about 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or more) less "off-target" gene silencing when compared to similar constructs without the specified 5'-end modification, thus greatly improving the overall specificity of the RNAi reagent or therapeutics.

10 As used herein, "off-target" gene silencing refers to unintended gene silencing due to, for example, spurious sequence homology between the antisense (guide) sequence and the unintended target mRNA sequence.

According to this aspect of the invention, certain guide strand modifications further increase nuclease stability, and/or lower interferon induction, without
15 significantly decreasing RNAi activity (or no decrease in RNAi activity at all).

In some embodiments, the 5'-stem sequence may comprise a 2'-modified ribose sugar, such as 2'-O-methyl modified nucleotide, at the 2nd nucleotide on the 5'-end of the polynucleotide and, in some embodiments, no other modified nucleotides. The hairpin structure having such modification may have enhanced target specificity or reduced off-
20 target silencing compared to a similar construct without the 2'-O-methyl modification at said position.

Certain combinations of specific 5'-stem sequence and 3'-stem sequence modifications may result in further unexpected advantages, as partly manifested by enhanced ability to inhibit target gene expression, enhanced serum stability, and/or
25 increased target specificity, *etc.*

In certain embodiments, the guide strand comprises a 2'-O-methyl modified nucleotide at the 2nd nucleotide on the 5'-end of the guide strand and no other modified nucleotides.

In other aspects, the miniRNA structures of the present invention mediates
30 sequence-dependent gene silencing by a microRNA mechanism. As used herein, the term "microRNA" ("miRNA"), also referred to in the art as "small temporal RNAs"

- 43 --

("stRNAs"), refers to a small (10-50 nucleotide) RNA which are genetically encoded (e.g., by viral, mammalian, or plant genomes) and are capable of directing or mediating RNA silencing. An "miRNA disorder" shall refer to a disease or disorder characterized by an aberrant expression or activity of an miRNA.

5 microRNAs are involved in down-regulating target genes in critical pathways, such as development and cancer, in mice, worms and mammals. Gene silencing through a microRNA mechanism is achieved by specific yet imperfect base-pairing of the miRNA and its target messenger RNA (mRNA). Various mechanisms may be used in microRNA-mediated down-regulation of target mRNA expression.

10 miRNAs are noncoding RNAs of approximately 22 nucleotides which can regulate gene expression at the post transcriptional or translational level during plant and animal development. One common feature of miRNAs is that they are all excised from an approximately 70 nucleotide precursor RNA stem-loop termed pre-miRNA, probably by Dicer, an RNase III-type enzyme, or a homolog thereof. Naturally-occurring
15 miRNAs are expressed by endogenous genes in vivo and are processed from a hairpin or stem-loop precursor (pre-miRNA or pri-miRNAs) by Dicer or other RNases. miRNAs can exist transiently in vivo as a double-stranded duplex but only one strand is taken up by the RISC complex to direct gene silencing.

 In some embodiments a version of sd-rxRNA compounds, which are effective in
20 cellular uptake and inhibiting of miRNA activity are described. Essentially the compounds are similar to RISC entering version but large strand chemical modification patterns are optimized in the way to block cleavage and act as an effective inhibitor of the RISC action. For example, the compound might be completely or mostly Omethyl modified with the PS content described previously. For these types of compounds the 5'
25 phosphorilation is not necessary. The presence of double stranded region is preferred as it promotes cellular uptake and efficient RISC loading.

 Another pathway that uses small RNAs as sequence-specific regulators is the RNA interference (RNAi) pathway, which is an evolutionarily conserved response to the presence of double-stranded RNA (dsRNA) in the cell. The dsRNAs are cleaved into
30 ~20-base pair (bp) duplexes of small-interfering RNAs (siRNAs) by Dicer. These small RNAs get assembled into multiprotein effector complexes called RNA-induced silencing complexes (RISCs). The siRNAs then guide the cleavage of target mRNAs with perfect

- 44 -

complementarity.

Some aspects of biogenesis, protein complexes, and function are shared between the siRNA pathway and the miRNA pathway. The subject single-stranded polynucleotides may mimic the dsRNA in the siRNA mechanism, or the microRNA in the miRNA mechanism.

In certain embodiments, the modified RNAi constructs may have improved stability in serum and/or cerebral spinal fluid compared to an unmodified RNAi constructs having the same sequence.

In certain embodiments, the structure of the RNAi construct does not induce interferon response in primary cells, such as mammalian primary cells, including primary cells from human, mouse and other rodents, and other non-human mammals. In certain embodiments, the RNAi construct may also be used to inhibit expression of a target gene in an invertebrate organism.

To further increase the stability of the subject constructs *in vivo*, the 3'-end of the hairpin structure may be blocked by protective group(s). For example, protective groups such as inverted nucleotides, inverted abasic moieties, or amino-end modified nucleotides may be used. Inverted nucleotides may comprise an inverted deoxynucleotide. Inverted abasic moieties may comprise an inverted deoxyabasic moiety, such as a 3',3'-linked or 5',5'-linked deoxyabasic moiety.

The RNAi constructs of the invention are capable of inhibiting the synthesis of any target protein encoded by target gene(s). The invention includes methods to inhibit expression of a target gene either in a cell *in vitro*, or *in vivo*. As such, the RNAi constructs of the invention are useful for treating a patient with a disease characterized by the overexpression of a target gene.

The target gene can be endogenous or exogenous (*e.g.*, introduced into a cell by a virus or using recombinant DNA technology) to a cell. Such methods may include introduction of RNA into a cell in an amount sufficient to inhibit expression of the target gene. By way of example, such an RNA molecule may have a guide strand that is complementary to the nucleotide sequence of the target gene, such that the composition inhibits expression of the target gene.

The invention also relates to vectors expressing the nucleic acids of the invention,

- 45 -

and cells comprising such vectors or the nucleic acids. The cell may be a mammalian cell *in vivo* or in culture, such as a human cell.

The invention further relates to compositions comprising the subject RNAi constructs, and a pharmaceutically acceptable carrier or diluent.

5 Another aspect of the invention provides a method for inhibiting the expression of a target gene in a mammalian cell, comprising contacting the mammalian cell with any of the subject RNAi constructs.

The method may be carried out *in vitro*, *ex vivo*, or *in vivo*, in, for example, mammalian cells in culture, such as a human cell in culture.

10 The target cells (*e.g.*, mammalian cell) may be contacted in the presence of a delivery reagent, such as a lipid (*e.g.*, a cationic lipid) or a liposome.

Another aspect of the invention provides a method for inhibiting the expression of a target gene in a mammalian cell, comprising contacting the mammalian cell with a vector expressing the subject RNAi constructs.

15 In one aspect of the invention, a longer duplex polynucleotide is provided, including a first polynucleotide that ranges in size from about 16 to about 30 nucleotides; a second polynucleotide that ranges in size from about 26 to about 46 nucleotides, wherein the first polynucleotide (the antisense strand) is complementary to both the second polynucleotide (the sense strand) and a target gene, and wherein both
20 polynucleotides form a duplex and wherein the first polynucleotide contains a single stranded region longer than 6 bases in length and is modified with alternative chemical modification pattern, and/or includes a conjugate moiety that facilitates cellular delivery. In this embodiment, between about 40% to about 90% of the nucleotides of the passenger strand between about 40% to about 90% of the nucleotides of the guide strand,
25 and between about 40% to about 90% of the nucleotides of the single stranded region of the first polynucleotide are chemically modified nucleotides.

In an embodiment, the chemically modified nucleotide in the polynucleotide duplex may be any chemically modified nucleotide known in the art, such as those discussed in detail above. In a particular embodiment, the chemically modified
30 nucleotide is selected from the group consisting of 2' F modified nucleotides, 2'-O-methyl modified and 2'-deoxy nucleotides. In another particular embodiment, the

- 46 --

chemically modified nucleotides results from "hydrophobic modifications" of the nucleotide base. In another particular embodiment, the chemically modified nucleotides are phosphorothioates. In an additional particular embodiment, chemically modified nucleotides are combination of phosphorothioates, 2'-O-methyl, 2' deoxy, hydrophobic
5 modifications and phosphorothioates. As these groups of modifications refer to modification of the ribose ring, back bone and nucleotide, it is feasible that some modified nucleotides will carry a combination of all three modification types.

In another embodiment, the chemical modification is not the same across the various regions of the duplex. In a particular embodiment, the first polynucleotide (the
10 passenger strand), has a large number of diverse chemical modifications in various positions. For this polynucleotide up to 90% of nucleotides might be chemically modified and/or have mismatches introduced.

In another embodiment, chemical modifications of the first or second polynucleotide include, but not limited to, 5' position modification of Uridine and
15 Cytosine (4-pyridyl, 2-pyridyl, indolyl, phenyl (C₆H₅OH); tryptophanyl (C₈H₆N)CH₂CH(NH₂)CO), isobutyl, butyl, aminobenzyl; phenyl; naphthyl, etc), where the chemical modification might alter base pairing capabilities of a nucleotide. For the guide strand an important feature of this aspect of the invention is the position of the chemical modification relative to the 5' end of the antisense and sequence. For example,
20 chemical phosphorylation of the 5' end of the guide strand is usually beneficial for efficacy. O-methyl modifications in the seed region of the sense strand (position 2-7 relative to the 5' end) are not generally well tolerated, whereas 2'F and deoxy are well tolerated. The mid part of the guide strand and the 3' end of the guide strand are more permissive in a type of chemical modifications applied. Deoxy modifications are not
25 tolerated at the 3' end of the guide strand.

A unique feature of this aspect of the invention involves the use of hydrophobic modification on the bases. In one embodiment, the hydrophobic modifications are preferably positioned near the 5' end of the guide strand, in other embodiments, they localized in the middle of the guides strand, in other embodiment they localized at the 3'
30 end of the guide strand and yet in another embodiment they are distributed thought the whole length of the polynucleotide. The same type of patterns is applicable to the passenger strand of the duplex.

The other part of the molecule is a single stranded region. The single stranded

- 47 --

region is expected to range from 7 to 40 nucleotides.

In one embodiment, the single stranded region of the first polynucleotide contains modifications selected from the group consisting of between 40% and 90% hydrophobic base modifications, between 40%-90% phosphorothioates, between 40% -90%

5 modification of the ribose moiety, and any combination of the preceding.

Efficiency of guide strand (first polynucleotide) loading into the RISC complex might be altered for heavily modified polynucleotides, so in one embodiment, the duplex polynucleotide includes a mismatch between nucleotide 9, 11, 12, 13, or 14 on the guide strand (first polynucleotide) and the opposite nucleotide on the sense strand (second
10 polynucleotide) to promote efficient guide strand loading.

More detailed aspects of the invention are described in the sections below.

Duplex Characteristics

Double-stranded oligonucleotides of the invention may be formed by two separate complementary nucleic acid strands. Duplex formation can occur either inside
15 or outside the cell containing the target gene.

As used herein, the term “duplex” includes the region of the double-stranded nucleic acid molecule(s) that is (are) hydrogen bonded to a complementary sequence. Double-stranded oligonucleotides of the invention may comprise a nucleotide sequence that is sense to a target gene and a complementary sequence that is antisense to the target
20 gene. The sense and antisense nucleotide sequences correspond to the target gene sequence, *e.g.*, are identical or are sufficiently identical to effect target gene inhibition (*e.g.*, are about at least about 98% identical, 96% identical, 94%, 90% identical, 85% identical, or 80% identical) to the target gene sequence.

In certain embodiments, the double-stranded oligonucleotide of the invention is
25 double-stranded over its entire length, *i.e.*, with no overhanging single-stranded sequence at either end of the molecule, *i.e.*, is blunt-ended. In other embodiments, the individual nucleic acid molecules can be of different lengths. In other words, a double-stranded oligonucleotide of the invention is not double-stranded over its entire length. For instance, when two separate nucleic acid molecules are used, one of the molecules, *e.g.*,
30 the first molecule comprising an antisense sequence, can be longer than the second molecule hybridizing thereto (leaving a portion of the molecule single-stranded).

- 48 -

Likewise, when a single nucleic acid molecule is used a portion of the molecule at either end can remain single-stranded.

In one embodiment, a double-stranded oligonucleotide of the invention contains mismatches and/or loops or bulges, but is double-stranded over at least about 70% of the length of the oligonucleotide. In another embodiment, a double-stranded oligonucleotide of the invention is double-stranded over at least about 80% of the length of the oligonucleotide. In another embodiment, a double-stranded oligonucleotide of the invention is double-stranded over at least about 90%-95% of the length of the oligonucleotide. In another embodiment, a double-stranded oligonucleotide of the invention is double-stranded over at least about 96%-98% of the length of the oligonucleotide. In certain embodiments, the double-stranded oligonucleotide of the invention contains at least or up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 mismatches.

15 ***Modifications***

The nucleotides of the invention may be modified at various locations, including the sugar moiety, the phosphodiester linkage, and/or the base.

Sugar moieties include natural, unmodified sugars, *e.g.*, monosaccharide (such as pentose, *e.g.*, ribose, deoxyribose), modified sugars and sugar analogs. In general, possible modifications of nucleomonomers, particularly of a sugar moiety, include, for example, replacement of one or more of the hydroxyl groups with a halogen, a heteroatom, an aliphatic group, or the functionalization of the hydroxyl group as an ether, an amine, a thiol, or the like.

One particularly useful group of modified nucleomonomers are 2'-O-methyl nucleotides. Such 2'-O-methyl nucleotides may be referred to as "methylated," and the corresponding nucleotides may be made from unmethylated nucleotides followed by alkylation or directly from methylated nucleotide reagents. Modified nucleomonomers may be used in combination with unmodified nucleomonomers. For example, an oligonucleotide of the invention may contain both methylated and unmethylated nucleomonomers.

Some exemplary modified nucleomonomers include sugar- or backbone-modified ribonucleotides. Modified ribonucleotides may contain a non-naturally occurring base (instead of a naturally occurring base), such as uridines or cytidines modified at the 5'-

position, *e.g.*, 5'-(2-amino)propyl uridine and 5'-bromo uridine; adenosines and guanosines modified at the 8-position, *e.g.*, 8-bromo guanosine; deaza nucleotides, *e.g.*, 7-deaza-adenosine; and N-alkylated nucleotides, *e.g.*, N6-methyl adenosine. Also, sugar-modified ribonucleotides may have the 2'-OH group replaced by a H, alkoxy (or
5 OR), R or alkyl, halogen, SH, SR, amino (such as NH₂, NHR, NR₂), or CN group, wherein R is lower alkyl, alkenyl, or alkynyl.

Modified ribonucleotides may also have the phosphodiester group connecting to adjacent ribonucleotides replaced by a modified group, *e.g.*, of phosphorothioate group. More generally, the various nucleotide modifications may be combined.

10 Although the antisense (guide) strand may be substantially identical to at least a portion of the target gene (or genes), at least with respect to the base pairing properties, the sequence need not be perfectly identical to be useful, *e.g.*, to inhibit expression of a target gene's phenotype. Generally, higher homology can be used to compensate for the use of a shorter antisense gene. In some cases, the antisense strand generally will be
15 substantially identical (although in antisense orientation) to the target gene.

The use of 2'-O-methyl modified RNA may also be beneficial in circumstances in which it is desirable to minimize cellular stress responses. RNA having 2'-O-methyl nucleomonomers may not be recognized by cellular machinery that is thought to recognize unmodified RNA. The use of 2'-O-methylated or partially 2'-O-methylated
20 RNA may avoid the interferon response to double-stranded nucleic acids, while maintaining target RNA inhibition. This may be useful, for example, for avoiding the interferon or other cellular stress responses, both in short RNAi (*e.g.*, siRNA) sequences that induce the interferon response, and in longer RNAi sequences that may induce the interferon response.

25 Overall, modified sugars may include D-ribose, 2'-O-alkyl (including 2'-O-methyl and 2'-O-ethyl), *i.e.*, 2'-alkoxy, 2'-amino, 2'-S-alkyl, 2'-halo (including 2'-fluoro), 2'-methoxyethoxy, 2'-allyloxy (-OCH₂CH=CH₂), 2'-propargyl, 2'-propyl, ethynyl, ethenyl, propenyl, and cyano and the like. In one embodiment, the sugar moiety can be a hexose and incorporated into an oligonucleotide as described (Augustyns, K., *et al.*,
30 *Nucl. Acids. Res.* 18:4711 (1992)). Exemplary nucleomonomers can be found, *e.g.*, in U.S. Pat. No. 5,849,902, incorporated by reference herein.

The term "alkyl" includes saturated aliphatic groups, including straight-chain alkyl groups (*e.g.*, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl,

- 50 -

etc.), branched-chain alkyl groups (isopropyl, tert-butyl, isobutyl, *etc.*), cycloalkyl (alicyclic) groups (cyclopropyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl), alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In certain embodiments, a straight chain or branched chain alkyl has 6 or fewer carbon atoms in its backbone (*e.g.*, C₁-C₆ for straight chain, C₃-C₆ for branched chain), and more preferably 4 or fewer. Likewise, preferred cycloalkyls have from 3-8 carbon atoms in their ring structure, and more preferably have 5 or 6 carbons in the ring structure. The term C₁-C₆ includes alkyl groups containing 1 to 6 carbon atoms.

Moreover, unless otherwise specified, the term alkyl includes both “unsubstituted alkyls” and “substituted alkyls,” the latter of which refers to alkyl moieties having independently selected substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, alkenyl, alkynyl, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxy carbonyloxy, aryloxy carbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxy carbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxy, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. Cycloalkyls can be further substituted, *e.g.*, with the substituents described above. An “alkylaryl” or an “arylalkyl” moiety is an alkyl substituted with an aryl (*e.g.*, phenylmethyl (benzyl)). The term “alkyl” also includes the side chains of natural and unnatural amino acids. The term “n-alkyl” means a straight chain (*i.e.*, unbranched) unsubstituted alkyl group.

The term “alkenyl” includes unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double bond. For example, the term “alkenyl” includes straight-chain alkenyl groups (*e.g.*, ethylenyl, propenyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, nonenyl, decenyl, *etc.*), branched-chain alkenyl groups, cycloalkenyl (alicyclic) groups (cyclopropenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl), alkyl or alkenyl substituted cycloalkenyl groups, and cycloalkyl or cycloalkenyl substituted alkenyl groups. In certain embodiments, a straight chain or branched chain alkenyl group has 6 or fewer

- 51 -

carbon atoms in its backbone (*e.g.*, C₂-C₆ for straight chain, C₃-C₆ for branched chain). Likewise, cycloalkenyl groups may have from 3-8 carbon atoms in their ring structure, and more preferably have 5 or 6 carbons in the ring structure. The term C₂-C₆ includes alkenyl groups containing 2 to 6 carbon atoms.

5 Moreover, unless otherwise specified, the term alkenyl includes both “unsubstituted alkenyls” and “substituted alkenyls,” the latter of which refers to alkenyl moieties having independently selected substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, alkyl groups, alkynyl groups, halogens, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, 10 alkoxycarbonyloxy, aryloxy carbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), 15 amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfanyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety.

 The term “alkynyl” includes unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but which contain at least one triple 20 bond. For example, the term “alkynyl” includes straight-chain alkynyl groups (*e.g.*, ethynyl, propynyl, butynyl, pentynyl, hexynyl, heptynyl, octynyl, nonynyl, decynyl, *etc.*), branched-chain alkynyl groups, and cycloalkyl or cycloalkenyl substituted alkynyl groups. In certain embodiments, a straight chain or branched chain alkynyl group has 6 or fewer carbon atoms in its backbone (*e.g.*, C₂-C₆ for straight chain, C₃-C₆ for branched 25 chain). The term C₂-C₆ includes alkynyl groups containing 2 to 6 carbon atoms.

 Moreover, unless otherwise specified, the term alkynyl includes both “unsubstituted alkynyls” and “substituted alkynyls,” the latter of which refers to alkynyl moieties having independently selected substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, alkyl 30 groups, alkynyl groups, halogens, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxy carbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino

(including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety.

Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, but having from one to five carbon atoms in its backbone structure. "Lower alkenyl" and "lower alkynyl" have chain lengths of, for example, 2-5 carbon atoms.

The term "alkoxy" includes substituted and unsubstituted alkyl, alkenyl, and alkynyl groups covalently linked to an oxygen atom. Examples of alkoxy groups include methoxy, ethoxy, isopropoxy, propoxy, butoxy, and pentoxy groups. Examples of substituted alkoxy groups include halogenated alkoxy groups. The alkoxy groups can be substituted with independently selected groups such as alkenyl, alkynyl, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxy carbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moieties. Examples of halogen substituted alkoxy groups include, but are not limited to, fluoromethoxy, difluoromethoxy, trifluoromethoxy, chloromethoxy, dichloromethoxy, trichloromethoxy, *etc.*

The term "heteroatom" includes atoms of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, sulfur and phosphorus.

The term "hydroxy" or "hydroxyl" includes groups with an -OH or -O⁻ (with an appropriate counterion).

The term "halogen" includes fluorine, bromine, chlorine, iodine, *etc.* The term "perhalogenated" generally refers to a moiety wherein all hydrogens are replaced by halogen atoms.

The term “substituted” includes independently selected substituents which can be placed on the moiety and which allow the molecule to perform its intended function.

Examples of substituents include alkyl, alkenyl, alkynyl, aryl, (CR'R'')₀₋₃NR'R'', (CR'R'')₀₋₃CN, NO₂, halogen, (CR'R'')₀₋₃C(halogen)₃, (CR'R'')₀₋₃CH(halogen)₂, (CR'R'')₀₋₃CH₂(halogen), (CR'R'')₀₋₃CONR'R'', (CR'R'')₀₋₃S(O)₁₋₂NR'R'', (CR'R'')₀₋₃CHO, (CR'R'')₀₋₃O(CR'R'')₀₋₃H, (CR'R'')₀₋₃S(O)₀₋₂R', (CR'R'')₀₋₃O(CR'R'')₀₋₃H, (CR'R'')₀₋₃COR', (CR'R'')₀₋₃CO₂R', or (CR'R'')₀₋₃OR' groups; wherein each R' and R'' are each independently hydrogen, a C₁-C₅ alkyl, C₂-C₅ alkenyl, C₂-C₅ alkynyl, or aryl group, or R' and R'' taken together are a benzylidene group or a —(CH₂)₂O(CH₂)₂— group.

The term “amine” or “amino” includes compounds or moieties in which a nitrogen atom is covalently bonded to at least one carbon or heteroatom. The term “alkyl amino” includes groups and compounds wherein the nitrogen is bound to at least one additional alkyl group. The term “dialkyl amino” includes groups wherein the nitrogen atom is bound to at least two additional alkyl groups.

The term “ether” includes compounds or moieties which contain an oxygen bonded to two different carbon atoms or heteroatoms. For example, the term includes “alkoxyalkyl,” which refers to an alkyl, alkenyl, or alkynyl group covalently bonded to an oxygen atom which is covalently bonded to another alkyl group.

The term “base” includes the known purine and pyrimidine heterocyclic bases, deazapurines, and analogs (including heterocyclic substituted analogs, *e.g.*, aminoethoxy phenoxazine), derivatives (*e.g.*, 1-alkyl-, 1-alkenyl-, heteroaromatic- and 1-alkynyl derivatives) and tautomers thereof. Examples of purines include adenine, guanine, inosine, diaminopurine, and xanthine and analogs (*e.g.*, 8-oxo-N⁶-methyladenine or 7-diazaxanthine) and derivatives thereof. Pyrimidines include, for example, thymine, uracil, and cytosine, and their analogs (*e.g.*, 5-methylcytosine, 5-methyluracil, 5-(1-propynyl)uracil, 5-(1-propynyl)cytosine and 4,4-ethanocytosine). Other examples of suitable bases include non-purinyl and non-pyrimidinyl bases such as 2-aminopyridine and triazines.

In a preferred embodiment, the nucleomonomers of an oligonucleotide of the invention are RNA nucleotides. In another preferred embodiment, the nucleomonomers of an oligonucleotide of the invention are modified RNA nucleotides. Thus, the oligonucleotides contain modified RNA nucleotides.

- 54 -

The term “nucleoside” includes bases which are covalently attached to a sugar moiety, preferably ribose or deoxyribose. Examples of preferred nucleosides include ribonucleosides and deoxyribonucleosides. Nucleosides also include bases linked to amino acids or amino acid analogs which may comprise free carboxyl groups, free amino groups, or protecting groups. Suitable protecting groups are well known in the art (see P. G. M. Wuts and T. W. Greene, “Protective Groups in Organic Synthesis”, 2nd Ed., Wiley-Interscience, New York, 1999).

The term “nucleotide” includes nucleosides which further comprise a phosphate group or a phosphate analog.

As used herein, the term “linkage” includes a naturally occurring, unmodified phosphodiester moiety ($-O-(PO^2-)-O-$) that covalently couples adjacent nucleomonomers. As used herein, the term “substitute linkage” includes any analog or derivative of the native phosphodiester group that covalently couples adjacent nucleomonomers. Substitute linkages include phosphodiester analogs, *e.g.*, phosphorothioate, phosphorodithioate, and P-ethoxyphosphodiester, P-ethoxyphosphodiester, P-alkyloxyphosphotriester, methylphosphonate, and nonphosphorus containing linkages, *e.g.*, acetals and amides. Such substitute linkages are known in the art (*e.g.*, Bjergarde *et al.* 1991. Nucleic Acids Res. 19:5843; Caruthers *et al.* 1991. Nucleosides Nucleotides. 10:47). In certain embodiments, non-hydrolyzable linkages are preferred, such as phosphorothiate linkages.

In certain embodiments, oligonucleotides of the invention comprise hydrophobically modified nucleotides or “hydrophobic modifications.” As used herein “hydrophobic modifications” refers to bases that are modified such that (1) overall hydrophobicity of the base is significantly increased, and/or (2) the base is still capable of forming close to regular Watson –Crick interaction. Several non-limiting examples of base modifications include 5-position uridine and cytidine modifications such as phenyl, 4-pyridyl, 2-pyridyl, indolyl, and isobutyl, phenyl (C₆H₅OH); tryptophanyl (C₈H₆N)CH₂CH(NH₂)CO), Isobutyl, butyl, aminobenzyl; phenyl; and naphthyl.

In certain embodiments, oligonucleotides of the invention comprise 3' and 5' termini (except for circular oligonucleotides). In one embodiment, the 3' and 5' termini of an oligonucleotide can be substantially protected from nucleases *e.g.*, by modifying the 3' or 5' linkages (*e.g.*, U.S. Pat. No. 5,849,902 and WO 98/13526). For example, oligonucleotides can be made resistant by the inclusion of a “blocking group.” The term

- 55 -

“blocking group” as used herein refers to substituents (*e.g.*, other than OH groups) that can be attached to oligonucleotides or nucleomonomers, either as protecting groups or coupling groups for synthesis (*e.g.*, FITC, propyl (CH₂-CH₂-CH₃), glycol (-O-CH₂-CH₂-O-) phosphate (PO₃²⁻), hydrogen phosphonate, or phosphoramidite). “Blocking groups” also include “end blocking groups” or “exonuclease blocking groups” which protect the 5' and 3' termini of the oligonucleotide, including modified nucleotides and non-nucleotide exonuclease resistant structures.

Exemplary end-blocking groups include cap structures (*e.g.*, a 7-methylguanosine cap), inverted nucleomonomers, *e.g.*, with 3'-3' or 5'-5' end inversions (see, *e.g.*, Ortiagao *et al.* 1992. *Antisense Res. Dev.* 2:129), methylphosphonate, phosphoramidite, non-nucleotide groups (*e.g.*, non-nucleotide linkers, amino linkers, conjugates) and the like. The 3' terminal nucleomonomer can comprise a modified sugar moiety. The 3' terminal nucleomonomer comprises a 3'-O that can optionally be substituted by a blocking group that prevents 3'-exonuclease degradation of the oligonucleotide. For example, the 3'-hydroxyl can be esterified to a nucleotide through a 3'→3' internucleotide linkage. For example, the alkyloxy radical can be methoxy, ethoxy, or isopropoxy, and preferably, ethoxy. Optionally, the 3'→3'linked nucleotide at the 3' terminus can be linked by a substitute linkage. To reduce nuclease degradation, the 5' most 3'→5' linkage can be a modified linkage, *e.g.*, a phosphorothioate or a P-alkyloxyphosphotriester linkage. Preferably, the two 5' most 3'→5' linkages are modified linkages. Optionally, the 5' terminal hydroxy moiety can be esterified with a phosphorus containing moiety, *e.g.*, phosphate, phosphorothioate, or P-ethoxyphosphate.

Another type of conjugates that can be attached to the end (3' or 5' end), the loop region, or any other parts of the miniRNA might include a sterol, sterol type molecule, peptide, small molecule, protein, etc. In some embodiments, a miniRNA may contain more than one conjugates (same or different chemical nature). In some embodiments, the conjugate is cholesterol.

Another way to increase target gene specificity, or to reduce off-target silencing effect, is to introduce a 2'-modification (such as the 2'-O methyl modification) at a position corresponding to the second 5'-end nucleotide of the guide sequence. This allows the positioning of this 2'-modification in the Dicer-resistant hairpin structure, thus enabling one to design better RNAi constructs with less or no off-target silencing.

- 56 -

In one embodiment, a hairpin polynucleotide of the invention can comprise one nucleic acid portion which is DNA and one nucleic acid portion which is RNA.

Antisense (guide) sequences of the invention can be "chimeric oligonucleotides" which comprise an RNA-like and a DNA-like region.

5 The language "RNase H activating region" includes a region of an oligonucleotide, *e.g.*, a chimeric oligonucleotide, that is capable of recruiting RNase H to cleave the target RNA strand to which the oligonucleotide binds. Typically, the RNase activating region contains a minimal core (of at least about 3-5, typically between about 3-12, more typically, between about 5-12, and more preferably between about 5-10
10 contiguous nucleomonomers) of DNA or DNA-like nucleomonomers. (See, *e.g.*, U.S. Pat. No. 5,849,902). Preferably, the RNase H activating region comprises about nine contiguous deoxyribose containing nucleomonomers.

 The language "non-activating region" includes a region of an antisense sequence, *e.g.*, a chimeric oligonucleotide, that does not recruit or activate RNase H. Preferably, a
15 non-activating region does not comprise phosphorothioate DNA. The oligonucleotides of the invention comprise at least one non-activating region. In one embodiment, the non-activating region can be stabilized against nucleases or can provide specificity for the target by being complementary to the target and forming hydrogen bonds with the target nucleic acid molecule, which is to be bound by the oligonucleotide.

20 In one embodiment, at least a portion of the contiguous polynucleotides are linked by a substitute linkage, *e.g.*, a phosphorothioate linkage.

 In certain embodiments, most or all of the nucleotides beyond the guide sequence (2'-modified or not) are linked by phosphorothioate linkages. Such constructs tend to have improved pharmacokinetics due to their higher affinity for serum proteins. The
25 phosphorothioate linkages in the non-guide sequence portion of the polynucleotide generally do not interfere with guide strand activity, once the latter is loaded into RISC.

 Antisense (guide) sequences of the present invention may include "morpholino oligonucleotides." Morpholino oligonucleotides are non-ionic and function by an RNase H-independent mechanism. Each of the 4 genetic bases (Adenine, Cytosine, Guanine,
30 and Thymine/Uracil) of the morpholino oligonucleotides is linked to a 6-membered morpholine ring. Morpholino oligonucleotides are made by joining the 4 different subunit types by, *e.g.*, non-ionic phosphorodiamidate inter-subunit linkages. Morpholino oligonucleotides have many advantages including: complete resistance to nucleases

- 57 -

(Antisense & Nucl. Acid Drug Dev. 1996. 6:267); predictable targeting (Biochemica Biophysica Acta. 1999. 1489:141); reliable activity in cells (Antisense & Nucl. Acid Drug Dev. 1997. 7:63); excellent sequence specificity (Antisense & Nucl. Acid Drug Dev. 1997. 7:151); minimal non-antisense activity (Biochemica Biophysica Acta. 1999. 1489:141); and simple osmotic or scrape delivery (Antisense & Nucl. Acid Drug Dev. 1997. 7:291). Morpholino oligonucleotides are also preferred because of their non-toxicity at high doses. A discussion of the preparation of morpholino oligonucleotides can be found in Antisense & Nucl. Acid Drug Dev. 1997. 7:187.

The chemical modifications described herein are believed, based on the data described herein, to promote single stranded polynucleotide loading into the RISC. Single stranded polynucleotides have been shown to be active in loading into RISC and inducing gene silencing. However, the level of activity for single stranded polynucleotides appears to be 2 to 4 orders of magnitude lower when compared to a duplex polynucleotide.

The present invention provides a description of the chemical modification patterns, which may (a) significantly increase stability of the single stranded polynucleotide (b) promote efficient loading of the polynucleotide into the RISC complex and (c) improve uptake of the single stranded nucleotide by the cell. Figure 5 provides some non-limiting examples of the chemical modification patterns which may be beneficial for achieving single stranded polynucleotide efficacy inside the cell. The chemical modification patterns may include combination of ribose, backbone, hydrophobic nucleoside and conjugate type of modifications. In addition, in some of the embodiments, the 5' end of the single polynucleotide may be chemically phosphorylated.

In yet another embodiment, the present invention provides a description of the chemical modifications patterns, which improve functionality of RISC inhibiting polynucleotides. Single stranded polynucleotides have been shown to inhibit activity of a preloaded RISC complex through the substrate competition mechanism. For these types of molecules, conventionally called antagonomers, the activity usually requires high concentration and in vivo delivery is not very effective. The present invention provides a description of the chemical modification patterns, which may (a) significantly increase stability of the single stranded polynucleotide (b) promote efficient recognition of the polynucleotide by the RISC as a substrate and/or (c) improve uptake of the single stranded nucleotide by the cell. Figure 6 provides some non-limiting examples of the

- 58 -

chemical modification patterns that may be beneficial for achieving single stranded polynucleotide efficacy inside the cell. The chemical modification patterns may include combination of ribose, backbone, hydrophobic nucleoside and conjugate type of modifications.

5 The modifications provided by the present invention are applicable to all polynucleotides. This includes single stranded RISC entering polynucleotides, single stranded RISC inhibiting polynucleotides, conventional duplexed polynucleotides of variable length (15- 40 bp), asymmetric duplexed polynucleotides, and the like. Polynucleotides may be modified with wide variety of chemical modification patterns,
10 including 5' end, ribose, backbone and hydrophobic nucleoside modifications.

Synthesis

 Oligonucleotides of the invention can be synthesized by any method known in the art, *e.g.*, using enzymatic synthesis and/or chemical synthesis. The oligonucleotides can be synthesized *in vitro* (*e.g.*, using enzymatic synthesis and chemical synthesis) or *in*
15 *vivo* (using recombinant DNA technology well known in the art).

 In a preferred embodiment, chemical synthesis is used for modified polynucleotides. Chemical synthesis of linear oligonucleotides is well known in the art and can be achieved by solution or solid phase techniques. Preferably, synthesis is by solid phase methods. Oligonucleotides can be made by any of several different synthetic
20 procedures including the phosphoramidite, phosphite triester, H-phosphonate, and phosphotriester methods, typically by automated synthesis methods.

 Oligonucleotide synthesis protocols are well known in the art and can be found, *e.g.*, in U.S. Pat. No. 5,830,653; WO 98/13526; Stec *et al.* 1984. *J. Am. Chem. Soc.* 106:6077; Stec *et al.* 1985. *J. Org. Chem.* 50:3908; Stec *et al.* *J. Chromatog.* 1985.
25 326:263; LaPlanche *et al.* 1986. *Nucl. Acid. Res.* 1986. 14:9081; Fasman G. D., 1989. *Practical Handbook of Biochemistry and Molecular Biology.* 1989. CRC Press, Boca Raton, Fla.; Lamone. 1993. *Biochem. Soc. Trans.* 21:1; U.S. Pat. No. 5,013,830; U.S. Pat. No. 5,214,135; U.S. Pat. No. 5,525,719; Kawasaki *et al.* 1993. *J. Med. Chem.* 36:831; WO 92/03568; U.S. Pat. No. 5,276,019; and U.S. Pat. No. 5,264,423.

30 The synthesis method selected can depend on the length of the desired oligonucleotide and such choice is within the skill of the ordinary artisan. For example, the phosphoramidite and phosphite triester method can produce oligonucleotides having

- 59 -

175 or more nucleotides, while the H-phosphonate method works well for oligonucleotides of less than 100 nucleotides. If modified bases are incorporated into the oligonucleotide, and particularly if modified phosphodiester linkages are used, then the synthetic procedures are altered as needed according to known procedures. In this regard, Uhlmann *et al.* (1990, *Chemical Reviews* 90:543-584) provide references and outline procedures for making oligonucleotides with modified bases and modified phosphodiester linkages. Other exemplary methods for making oligonucleotides are taught in Sonveaux. 1994. "Protecting Groups in Oligonucleotide Synthesis"; Agrawal. *Methods in Molecular Biology* 26:1. Exemplary synthesis methods are also taught in "Oligonucleotide Synthesis - A Practical Approach" (Gait, M. J. IRL Press at Oxford University Press. 1984). Moreover, linear oligonucleotides of defined sequence, including some sequences with modified nucleotides, are readily available from several commercial sources.

The oligonucleotides may be purified by polyacrylamide gel electrophoresis, or by any of a number of chromatographic methods, including gel chromatography and high pressure liquid chromatography. To confirm a nucleotide sequence, especially unmodified nucleotide sequences, oligonucleotides may be subjected to DNA sequencing by any of the known procedures, including Maxam and Gilbert sequencing, Sanger sequencing, capillary electrophoresis sequencing, the wandering spot sequencing procedure or by using selective chemical degradation of oligonucleotides bound to Hybond paper. Sequences of short oligonucleotides can also be analyzed by laser desorption mass spectroscopy or by fast atom bombardment (McNeal, *et al.*, 1982, *J. Am. Chem. Soc.* 104:976; Viari, *et al.*, 1987, *Biomed. Environ. Mass Spectrom.* 14:83; Grotjahn *et al.*, 1982, *Nuc. Acid Res.* 10:4671). Sequencing methods are also available for RNA oligonucleotides.

The quality of oligonucleotides synthesized can be verified by testing the oligonucleotide by capillary electrophoresis and denaturing strong anion HPLC (SAX-HPLC) using, *e.g.*, the method of Bergot and Egan. 1992. *J. Chrom.* 599:35.

Other exemplary synthesis techniques are well known in the art (see, *e.g.*, Sambrook *et al.*, *Molecular Cloning: a Laboratory Manual*, Second Edition (1989); *DNA Cloning*, Volumes I and II (DN Glover Ed. 1985); *Oligonucleotide Synthesis* (M J Gait Ed, 1984; *Nucleic Acid Hybridisation* (B D Hames and S J Higgins eds. 1984); A

- 60 --

Practical Guide to Molecular Cloning (1984); or the series, Methods in Enzymology (Academic Press, Inc.)).

In certain embodiments, the subject RNAi constructs or at least portions thereof are transcribed from expression vectors encoding the subject constructs. Any art
5 recognized vectors may be use for this purpose. The transcribed RNAi constructs may be isolated and purified, before desired modifications (such as replacing an unmodified sense strand with a modified one, *etc.*) are carried out.

Delivery/Carrier

Uptake of Oligonucleotides by Cells

10 Oligonucleotides and oligonucleotide compositions are contacted with (*i.e.*, brought into contact with, also referred to herein as administered or delivered to) and taken up by one or more cells or a cell lysate. The term "cells" includes prokaryotic and eukaryotic cells, preferably vertebrate cells, and, more preferably, mammalian cells. In a preferred embodiment, the oligonucleotide compositions of the invention are contacted
15 with human cells.

Oligonucleotide compositions of the invention can be contacted with cells *in vitro*, *e.g.*, in a test tube or culture dish, (and may or may not be introduced into a subject) or *in vivo*, *e.g.*, in a subject such as a mammalian subject. Oligonucleotides are taken up by cells at a slow rate by endocytosis, but endocytosed oligonucleotides are
20 generally sequestered and not available, *e.g.*, for hybridization to a target nucleic acid molecule. In one embodiment, cellular uptake can be facilitated by electroporation or calcium phosphate precipitation. However, these procedures are only useful for *in vitro* or ex vivo embodiments, are not convenient and, in some cases, are associated with cell toxicity.

25 In another embodiment, delivery of oligonucleotides into cells can be enhanced by suitable art recognized methods including calcium phosphate, DMSO, glycerol or dextran, electroporation, or by transfection, *e.g.*, using cationic, anionic, or neutral lipid compositions or liposomes using methods known in the art (see *e.g.*, WO 90/14074; WO 91/16024; WO 91/17424; U.S. Pat. No. 4,897,355; Bergan *et al.* 1993. *Nucleic Acids*
30 *Research*. 21:3567). Enhanced delivery of oligonucleotides can also be mediated by the use of vectors (See *e.g.*, Shi, Y. 2003. Trends Genet 2003 Jan. 19:9; Reichhart J M *et al.*

- 61 -

Genesis. 2002. 34(1-2):1604, Yu *et al.* 2002. Proc. Natl. Acad Sci. USA 99:6047; Sui *et al.* 2002. Proc. Natl. Acad Sci. USA 99:5515) viruses, polyamine or polycation conjugates using compounds such as polylysine, protamine, or Ni, N12-bis (ethyl) spermine (see, *e.g.*, Bartzatt, R. *et al.* 1989. *Biotechnol. Appl. Biochem.* 11:133; Wagner
5 E. *et al.* 1992. *Proc. Natl. Acad. Sci.* 88:4255).

In certain embodiments, the miniRNA of the invention may be delivered by using various beta-glucan containing particles, such as those described in US 2005/0281781 A1, WO 2006/007372, and WO 2007/050643 (all incorporated herein by reference). In certain embodiments, the beta-glucan particle is derived from yeast. In
10 certain embodiments, the payload trapping molecule is a polymer, such as those with a molecular weight of at least about 1000 Da, 10,000 Da, 50,000 Da, 100 kDa, 500 kDa, etc. Preferred polymers include (without limitation) cationic polymers, chitosans, or PEI (polyethylenimine), etc.

Such beta-glucan based delivery system may be formulated for oral delivery,
15 where the orally delivered beta-glucan / miniRNA constructs may be engulfed by macrophages or other related phagocytic cells, which may in turn release the miniRNA constructs in selected *in vivo* sites. Alternatively or in addition, the miniRNA may changes the expression of certain macrophage target genes.

The optimal protocol for uptake of oligonucleotides will depend upon a number
20 of factors, the most crucial being the type of cells that are being used. Other factors that are important in uptake include, but are not limited to, the nature and concentration of the oligonucleotide, the confluence of the cells, the type of culture the cells are in (*e.g.*, a suspension culture or plated) and the type of media in which the cells are grown.

25 ***Encapsulating Agents***

Encapsulating agents entrap oligonucleotides within vesicles. In another embodiment of the invention, an oligonucleotide may be associated with a carrier or vehicle, *e.g.*, liposomes or micelles, although other carriers could be used, as would be appreciated by one skilled in the art. Liposomes are vesicles made of a lipid bilayer
30 having a structure similar to biological membranes. Such carriers are used to facilitate the cellular uptake or targeting of the oligonucleotide, or improve the oligonucleotide's pharmacokinetic or toxicologic properties.

- 62 --

For example, the oligonucleotides of the present invention may also be administered encapsulated in liposomes, pharmaceutical compositions wherein the active ingredient is contained either dispersed or variously present in corpuscles consisting of aqueous concentric layers adherent to lipidic layers. The oligonucleotides, depending upon solubility, may be present both in the aqueous layer and in the lipidic layer, or in what is generally termed a liposomic suspension. The hydrophobic layer, generally but not exclusively, comprises phospholipids such as lecithin and sphingomyelin, steroids such as cholesterol, more or less ionic surfactants such as diacetylphosphate, stearylamine, or phosphatidic acid, or other materials of a hydrophobic nature. The diameters of the liposomes generally range from about 15 nm to about 5 microns.

The use of liposomes as drug delivery vehicles offers several advantages. Liposomes increase intracellular stability, increase uptake efficiency and improve biological activity. Liposomes are hollow spherical vesicles composed of lipids arranged in a similar fashion as those lipids which make up the cell membrane. They have an internal aqueous space for entrapping water soluble compounds and range in size from 0.05 to several microns in diameter. Several studies have shown that liposomes can deliver nucleic acids to cells and that the nucleic acids remain biologically active. For example, a lipid delivery vehicle originally designed as a research tool, such as Lipofectin or LIPOFECTAMINE™ 2000, can deliver intact nucleic acid molecules to cells.

Specific advantages of using liposomes include the following: they are non-toxic and biodegradable in composition; they display long circulation half-lives; and recognition molecules can be readily attached to their surface for targeting to tissues. Finally, cost-effective manufacture of liposome-based pharmaceuticals, either in a liquid suspension or lyophilized product, has demonstrated the viability of this technology as an acceptable drug delivery system.

In some aspects, formulations associated with the invention might be selected for a class of naturally occurring or chemically synthesized or modified saturated and unsaturated fatty acid residues. Fatty acids might exist in a form of triglycerides, diglycerides or individual fatty acids. In another embodiment, the use of well-validated mixtures of fatty acids and/or fat emulsions currently used in pharmacology for parenteral nutrition may be utilized.

- 63 --

Liposome based formulations are widely used for oligonucleotide delivery. However, most of commercially available lipid or liposome formulations contain at least one positively charged lipid (cationic lipids). The presence of this positively charged lipid is believed to be essential for obtaining a high degree of oligonucleotide loading and for enhancing liposome fusogenic properties. Several methods have been performed and published to identify optimal positively charged lipid chemistries. However, the commercially available liposome formulations containing cationic lipids are characterized by a high level of toxicity. In vivo limited therapeutic indexes have revealed that liposome formulations containing positive charged lipids are associated with toxicity (i.e. elevation in liver enzymes) at concentrations only slightly higher than concentration required to achieve RNA silencing.

New liposome formulations, lacking the toxicity of the prior art liposomes have been developed according to the invention. These new liposome formulations are neutral fat-based formulations for the efficient delivery of oligonucleotides, and in particular for the delivery of the RNA molecules of the invention. The compositions are referred to as neutral nanotransporters because they enable quantitative oligonucleotide incorporation into non-charged lipids mixtures. The lack of toxic levels of cationic lipids in the neutral nanotransporter compositions of the invention is an important feature.

The neutral nanotransporters compositions enable efficient loading of oligonucleotide into neutral fat formulation. The composition includes an oligonucleotide that is modified in a manner such that the hydrophobicity of the molecule is increased (for example a hydrophobic molecule is attached (covalently or non-covalently) to a hydrophobic molecule on the oligonucleotide terminus or a non-terminal nucleotide, base, sugar, or backbone), the modified oligonucleotide being mixed with a neutral fat formulation (for example containing at least 25 % of cholesterol and 25% of DOPC or analogs thereof). A cargo molecule, such as another lipid can also be included in the composition. This composition, where part of the formulation is build into the oligonucleotide itself, enables efficient encapsulation of oligonucleotide in neutral lipid particles.

One of several unexpected observations associated with the invention was that the oligonucleotides of the invention could effectively be incorporated in a lipid mixture that was free of cationic lipids and that such a composition could effectively deliver the therapeutic oligonucleotide to a cell in a manner that it is functional. Another

- 64 -

unexpected observation was the high level of activity observed when the fatty mixture is composed of a phosphatidylcholine base fatty acid and a sterol such as a cholesterol. For instance, one preferred formulation of neutral fatty mixture is composed of at least 20% of DOPC or DSPC and at least 20% of sterol such as cholesterol. Even as low as 1:5 lipid to oligonucleotide ratio was shown to be sufficient to get complete encapsulation of the oligonucleotide in a non charged formulation. The prior art demonstrated only a 1-5% oligonucleotide encapsulation with non-charged formulations, which is not sufficient to get to a desired amount of in vivo efficacy. Compared to the prior art using neutral lipids the level of oligonucleotide delivery to a cell was quite unexpected.

Stable particles ranging in size from 50 to 140 nm were formed upon complexing of hydrophobic oligonucleotides with preferred formulations. It is interesting to mention that the formulation by itself typically does not form small particles, but rather, forms agglomerates, which are transformed into stable 50-120 nm particles upon addition of the hydrophobic modified oligonucleotide.

The neutral nanotransporter compositions of the invention include a hydrophobic modified polynucleotide, a neutral fatty mixture, and optionally a cargo molecule. A "hydrophobic modified polynucleotide" as used herein is a polynucleotide of the invention (i.e. sd-rxRNA) that has at least one modification that renders the polynucleotide more hydrophobic than the polynucleotide was prior to modification.

The modification may be achieved by attaching (covalently or non-covalently) a hydrophobic molecule to the polynucleotide. In some instances the hydrophobic molecule is or includes a lipophilic group.

The term "lipophilic group" means a group that has a higher affinity for lipids than its affinity for water. Examples of lipophilic groups include, but are not limited to, cholesterol, a cholesteryl or modified cholesteryl residue, adamantane, dihydrotestosterone, long chain alkyl, long chain alkenyl, long chain alkynyl, olelyl-lithocholic, cholenic, oleoyl-cholenic, palmityl, heptadecyl, myristyl, bile acids, cholic acid or taurocholic acid, deoxycholate, oleyl lithocholic acid, oleoyl cholenic acid, glycolipids, phospholipids, sphingolipids, isoprenoids, such as steroids, vitamins, such as vitamin E, fatty acids either saturated or unsaturated, fatty acid esters, such as triglycerides, pyrenes, porphyrines, Texaphyrine, adamantane, acridines, biotin, coumarin, fluorescein, rhodamine, Texas-Red, digoxigenin, dimethoxytrityl, t-butyldimethylsilyl, t-butyldiphenylsilyl, cyanine dyes (e.g. Cy3 or Cy5), Hoechst 33258 dye, psoralen, or

- 65 --

ibuprofen. The cholesterol moiety may be reduced (e.g. as in cholestan) or may be substituted (e.g. by halogen). A combination of different lipophilic groups in one molecule is also possible.

The hydrophobic molecule may be attached at various positions of the polynucleotide. As described above, the hydrophobic molecule may be linked to the terminal residue of the polynucleotide such as the 3' or 5'-end of the polynucleotide. Alternatively, it may be linked to an internal nucleotide or a nucleotide on a branch of the polynucleotide. The hydrophobic molecule may be attached, for instance to a 2'-position of the nucleotide. The hydrophobic molecule may also be linked to the heterocyclic base, the sugar or the backbone of a nucleotide of the polynucleotide.

The hydrophobic molecule may be connected to the polynucleotide by a linker moiety. Optionally the linker moiety is a non-nucleotidic linker moiety. Non-nucleotidic linkers are e.g. abasic residues (dSpacer), oligoethyleneglycol, such as triethyleneglycol (spacer 9) or hexaethyleneglycol (spacer 18), or alkane-diol, such as butanediol. The spacer units are preferably linked by phosphodiester or phosphorothioate bonds. The linker units may appear just once in the molecule or may be incorporated several times, e.g. via phosphodiester, phosphorothioate, methylphosphonate, or amide linkages.

Typical conjugation protocols involve the synthesis of polynucleotides bearing an aminolinker at one or more positions of the sequence, however, a linker is not required. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction may be performed either with the polynucleotide still bound to a solid support or following cleavage of the polynucleotide in solution phase. Purification of the modified polynucleotide by HPLC typically results in a pure material.

In some embodiments the hydrophobic molecule is a sterol type conjugate, a PhytoSterol conjugate, cholesterol conjugate, sterol type conjugate with altered side chain length, fatty acid conjugate, any other hydrophobic group conjugate, and/or hydrophobic modifications of the internal nucleoside, which provide sufficient hydrophobicity to be incorporated into micelles.

For purposes of the present invention, the term "sterols", refers or steroid alcohols are a subgroup of steroids with a hydroxyl group at the 3-position of the A-ring. They are amphipathic lipids synthesized from acetyl-coenzyme A via the HMG-CoA reductase pathway. The overall molecule is quite flat. The hydroxyl group on the A ring

- 66 -

is polar. The rest of the aliphatic chain is non-polar. Usually sterols are considered to have an 8 carbon chain at position 17.

For purposes of the present invention, the term “sterol type molecules”, refers to steroid alcohols, which are similar in structure to sterols. The main difference is the
5 structure of the ring and number of carbons in a position 21 attached side chain.

For purposes of the present invention, the term “PhytoSterols” (also called plant sterols) are a group of steroid alcohols, phytochemicals naturally occurring in plants. There are more than 200 different known PhytoSterols.

For purposes of the present invention, the term “Sterol side chain” refers to a
10 chemical composition of a side chain attached at the position 17 of sterol-type molecule. In a standard definition sterols are limited to a 4 ring structure carrying a 8 carbon chain at position 17. In this invention, the sterol type molecules with side chain longer and shorter than conventional are described. The side chain may branched or contain double back bones.

15 Thus, sterols useful in the invention, for example, include cholesterol, as well as unique sterols in which position 17 has attached side chain of 2-7 or longer than 9 carbons. In a particular embodiment, the length of the polycarbon tail is varied between 5 and 9 carbons. Figure 9 demonstrates that there is a correlation between plasma clearance, liver uptake and the length of the polycarbon chain. Such conjugates may
20 have significantly better in vivo efficacy, in particular delivery to liver. These types of molecules are expected to work at concentrations 5 to 9 fold lower than oligonucleotides conjugated to conventional cholesterol.

Alternatively the polynucleotide may be bound to a protein, peptide or positively charged chemical that functions as the hydrophobic molecule. The proteins may be
25 selected from the group consisting of protamine, dsRNA binding domain, and arginine rich peptides. Exemplary positively charged chemicals include spermine, spermidine, cadaverine, and putrescine.

In another embodiment hydrophobic molecule conjugates may demonstrate even higher efficacy when it is combined with optimal chemical modification patterns of the
30 polynucleotide (as described herein in detail), containing but not limited to hydrophobic modifications, phosphorothioate modifications, and 2' ribo modifications.

In another embodiment the sterol type molecule may be a naturally occurring PhytoSterols such as those shown in Figure 8. The polycarbon chain may be longer than

- 67 --

9 and may be linear, branched and/or contain double bonds. Some PhytoSterol containing polynucleotide conjugates may be significantly more potent and active in delivery of polynucleotides to various tissues. Some PhytoSterols may demonstrate tissue preference and thus be used as a way to delivery RNAi specifically to particular
5 tissues.

The hydrophobic modified polynucleotide is mixed with a neutral fatty mixture to form a micelle. The neutral fatty acid mixture is a mixture of fats that has a net neutral or slightly net negative charge at or around physiological pH that can form a micelle with the hydrophobic modified polynucleotide. For purposes of the present invention, the
10 term "micelle" refers to a small nanoparticle formed by a mixture of non charged fatty acids and phospholipids. The neutral fatty mixture may include cationic lipids as long as they are present in an amount that does not cause toxicity. In preferred embodiments the neutral fatty mixture is free of cationic lipids. A mixture that is free of cationic lipids is one that has less than 1% and preferably 0% of the total lipid being cationic lipid. The
15 term "cationic lipid" includes lipids and synthetic lipids having a net positive charge at or around physiological pH. The term "anionic lipid" includes lipids and synthetic lipids having a net negative charge at or around physiological pH.

The neutral fats bind to the oligonucleotides of the invention by a strong but non-covalent attraction (*e.g.*, an electrostatic, van der Waals, pi-stacking, *etc.* interaction).

20 The neutral fat mixture may include formulations selected from a class of naturally occurring or chemically synthesized or modified saturated and unsaturated fatty acid residues. Fatty acids might exist in a form of triglycerides, diglycerides or individual fatty acids. In another embodiment the use of well-validated mixtures of fatty acids and/or fat emulsions currently used in pharmacology for parenteral nutrition may be
25 utilized.

The neutral fatty mixture is preferably a mixture of a choline based fatty acid and a sterol. Choline based fatty acids include for instance, synthetic phosphocholine derivatives such as DDPC, DLPC, DMPC, DPPC, DSPC, DOPC, POPC, and DEPC. DOPC (chemical registry number 4235-95-4) is dioleoylphosphatidylcholine (also
30 known as dielaidoylphosphatidylcholine, dioleoyl-PC, dioleoylphosphocholine, dioleoyl-sn-glycero-3-phosphocholine, dioleoylphosphatidylcholine). DSPC (chemical registry number 816-94-4) is distearoylphosphatidylcholine (also known as 1,2-Distearoyl-sn-Glycero-3-phosphocholine).

- 68 -

The sterol in the neutral fatty mixture may be for instance cholesterol. The neutral fatty mixture may be made up completely of a choline based fatty acid and a sterol or it may optionally include a cargo molecule. For instance, the neutral fatty mixture may have at least 20% or 25% fatty acid and 20% or 25% sterol.

5 For purposes of the present invention, the term "Fatty acids" relates to conventional description of fatty acid. They may exist as individual entities or in a form of two-and triglycerides. For purposes of the present invention, the term "fat emulsions" refers to safe fat formulations given intravenously to subjects who are unable to get enough fat in their diet. It is an emulsion of soy bean oil (or other naturally occurring
10 oils) and egg phospholipids. Fat emulsions are being used for formulation of some insoluble anesthetics. In this disclosure, fat emulsions might be part of commercially available preparations like Intralipid, Liposyn, Nutrilipid, modified commercial preparations, where they are enriched with particular fatty acids or fully de novo-formulated combinations of fatty acids and phospholipids.

15 In one embodiment, the cells to be contacted with an oligonucleotide composition of the invention are contacted with a mixture comprising the oligonucleotide and a mixture comprising a lipid, *e.g.*, one of the lipids or lipid compositions described supra for between about 12 hours to about 24 hours. In another embodiment, the cells to be contacted with an oligonucleotide composition are contacted with a mixture comprising
20 the oligonucleotide and a mixture comprising a lipid, *e.g.*, one of the lipids or lipid compositions described supra for between about 1 and about five days. In one embodiment, the cells are contacted with a mixture comprising a lipid and the oligonucleotide for between about three days to as long as about 30 days. In another embodiment, a mixture comprising a lipid is left in contact with the cells for at least
25 about five to about 20 days. In another embodiment, a mixture comprising a lipid is left in contact with the cells for at least about seven to about 15 days.

50%-60% of the formulation can optionally be any other lipid or molecule. Such a lipid or molecule is referred to herein as a cargo lipid or cargo molecule. Cargo molecules include but are not limited to intralipid, small molecules, fusogenic peptides
30 or lipids or other small molecules might be added to alter cellular uptake, endosomal release or tissue distribution properties. The ability to tolerate cargo molecules is important for modulation of properties of these particles, if such properties are desirable. For instance the presence of some tissue specific metabolites might drastically alter

- 69 -

tissue distribution profiles. For example use of Intralipid type formulation enriched in shorter or longer fatty chains with various degrees of saturation affects tissue distribution profiles of these type of formulations (and their loads).

An example of a cargo lipid useful according to the invention is a fusogenic lipid. For instance, the zwitterionic lipid DOPE (chemical registry number 4004-5-1, 1,2-Dioleoyl-sn-Glycero-3-phosphoethanolamine) is a preferred cargo lipid.

Intralipid may be comprised of the following composition: 1 000 mL contain: purified soybean oil 90 g, purified egg phospholipids 12 g, glycerol anhydrous 22 g, water for injection q.s. ad 1 000 mL. pH is adjusted with sodium hydroxide to pH approximately 8. Energy content/L: 4.6 MJ (190 kcal). Osmolality (approx.): 300 mOsm/kg water. In another embodiment fat emulsion is Liposyn that contains 5% safflower oil, 5% soybean oil, up to 1.2% egg phosphatides added as an emulsifier and 2.5% glycerin in water for injection. It may also contain sodium hydroxide for pH adjustment. pH 8.0 (6.0 - 9.0). Liposyn has an osmolality of 276 m Osmol/liter (actual).

Variation in the identity, amounts and ratios of cargo lipids affects the cellular uptake and tissue distribution characteristics of these compounds. For example, the length of lipid tails and level of saturability will affect differential uptake to liver, lung, fat and cardiomyocytes. Addition of special hydrophobic molecules like vitamins or different forms of sterols can favor distribution to special tissues which are involved in the metabolism of particular compounds. Complexes are formed at different oligonucleotide concentrations, with higher concentrations favoring more efficient complex formation (Figs. 21-22).

In another embodiment, the fat emulsion is based on a mixture of lipids. Such lipids may include natural compounds, chemically synthesized compounds, purified fatty acids or any other lipids. In yet another embodiment the composition of fat emulsion is entirely artificial. In a particular embodiment, the fat emulsion is more than 70% linoleic acid. In yet another particular embodiment the fat emulsion is at least 1% of cardiolipin. Linoleic acid (LA) is an unsaturated omega-6 fatty acid. It is a colorless liquid made of a carboxylic acid with an 18-carbon chain and two cis double bonds.

In yet another embodiment of the present invention, the alteration of the composition of the fat emulsion is used as a way to alter tissue distribution of hydrophobically modified polynucleotides. This methodology provides for the specific delivery of the polynucleotides to particular tissues (Figure 12).

- 70 -

In another embodiment the fat emulsions of the cargo molecule contain more than 70% of Linoleic acid (C₁₈H₃₂O₂) and/or cardiolipin are used for specifically delivering RNAi to heart muscle.

Fat emulsions, like intralipid have been used before as a delivery formulation for some non-water soluble drugs (such as Propofol, re-formulated as Diprivan). Unique features of the present invention include (a) the concept of combining modified polynucleotides with the hydrophobic compound(s), so it can be incorporated in the fat micelles and (b) mixing it with the fat emulsions to provide a reversible carrier. After injection into a blood stream, micelles usually bind to serum proteins, including albumin, HDL, LDL and other. This binding is reversible and eventually the fat is absorbed by cells. The polynucleotide, incorporated as a part of the micelle will then be delivered closely to the surface of the cells. After that cellular uptake might be happening through variable mechanisms, including but not limited to sterol type delivery.

15 ***Complexing Agents***

Complexing agents bind to the oligonucleotides of the invention by a strong but non-covalent attraction (*e.g.*, an electrostatic, van der Waals, pi-stacking, *etc.* interaction). In one embodiment, oligonucleotides of the invention can be complexed with a complexing agent to increase cellular uptake of oligonucleotides. An example of a complexing agent includes cationic lipids. Cationic lipids can be used to deliver oligonucleotides to cells. However, as discussed above, formulations free in cationic lipids are preferred in some embodiments.

The term “cationic lipid” includes lipids and synthetic lipids having both polar and non-polar domains and which are capable of being positively charged at or around physiological pH and which bind to polyanions, such as nucleic acids, and facilitate the delivery of nucleic acids into cells. In general cationic lipids include saturated and unsaturated alkyl and alicyclic ethers and esters of amines, amides, or derivatives thereof. Straight-chain and branched alkyl and alkenyl groups of cationic lipids can contain, *e.g.*, from 1 to about 25 carbon atoms. Preferred straight chain or branched alkyl or alkene groups have six or more carbon atoms. Alicyclic groups include cholesterol and other steroid groups. Cationic lipids can be prepared with a variety of counterions (anions) including, *e.g.*, Cl⁻, Br⁻, I⁻, F⁻, acetate, trifluoroacetate, sulfate, nitrite, and nitrate.

- 71 -

Examples of cationic lipids include polyethylenimine, polyamidoamine (PAMAM) starburst dendrimers, Lipofectin (a combination of DOTMA and DOPE), Lipofectase, LIPOFECTAMINE™ (*e.g.*, LIPOFECTAMINE™ 2000), DOPE, Cytofectin (Gilead Sciences, Foster City, Calif.), and Eufectins (JBL, San Luis Obispo, Calif.). Exemplary cationic liposomes can be made from N-[1-(2,3-dioleloxy)-propyl]-N,N,N-trimethylammonium chloride (DOTMA), N-[1-(2,3-dioleloxy)-propyl]-N,N,N-trimethylammonium methylsulfate (DOTAP), 3β-[N-(N',N'-dimethylaminoethane)carbamoyl]cholesterol (DC-Chol), 2,3,-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA), 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide; and dimethyldioctadecylammonium bromide (DDAB). The cationic lipid N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), for example, was found to increase 1000-fold the antisense effect of a phosphorothioate oligonucleotide. (Vlassov *et al.*, 1994, *Biochimica et Biophysica Acta* 1197:95-108). Oligonucleotides can also be complexed with, *e.g.*, poly (L-lysine) or avidin and lipids may, or may not, be included in this mixture, *e.g.*, steryl-poly (L-lysine).

Cationic lipids have been used in the art to deliver oligonucleotides to cells (see, *e.g.*, U.S. Pat. Nos. 5,855,910; 5,851,548; 5,830,430; 5,780,053; 5,767,099; Lewis *et al.* 1996. *Proc. Natl. Acad. Sci. USA* 93:3176; Hope *et al.* 1998. *Molecular Membrane Biology* 15:1). Other lipid compositions which can be used to facilitate uptake of the instant oligonucleotides can be used in connection with the claimed methods. In addition to those listed supra, other lipid compositions are also known in the art and include, *e.g.*, those taught in U.S. Pat. No. 4,235,871; U.S. Pat. Nos. 4,501,728; 4,837,028; 4,737,323.

In one embodiment lipid compositions can further comprise agents, *e.g.*, viral proteins to enhance lipid-mediated transfections of oligonucleotides (Kamata, *et al.*, 1994. *Nucl. Acids. Res.* 22:536). In another embodiment, oligonucleotides are contacted with cells as part of a composition comprising an oligonucleotide, a peptide, and a lipid as taught, *e.g.*, in U.S. patent 5,736,392. Improved lipids have also been described which are serum resistant (Lewis, *et al.*, 1996. *Proc. Natl. Acad. Sci.* 93:3176). Cationic lipids and other complexing agents act to increase the number of oligonucleotides carried into the cell through endocytosis.

In another embodiment N-substituted glycine oligonucleotides (peptoids) can be

- 72 -

used to optimize uptake of oligonucleotides. Peptoids have been used to create cationic lipid-like compounds for transfection (Murphy, *et al.*, 1998. *Proc. Natl. Acad. Sci.* 95:1517). Peptoids can be synthesized using standard methods (*e.g.*, Zuckermann, R. N., *et al.* 1992. *J. Am. Chem. Soc.* 114:10646; Zuckermann, R. N., *et al.* 1992. *Int. J. Peptide Protein Res.* 40:497). Combinations of cationic lipids and peptoids, liptoids, can also be used to optimize uptake of the subject oligonucleotides (Hunag, *et al.*, 1998. *Chemistry and Biology.* 5:345). Liptoids can be synthesized by elaborating peptoid oligonucleotides and coupling the amino terminal submonomer to a lipid via its amino group (Hunag, *et al.*, 1998. *Chemistry and Biology.* 5:345).

10 It is known in the art that positively charged amino acids can be used for creating highly active cationic lipids (Lewis *et al.* 1996. *Proc. Natl. Acad. Sci. U.S.A.* 93:3176). In one embodiment, a composition for delivering oligonucleotides of the invention comprises a number of arginine, lysine, histidine or ornithine residues linked to a lipophilic moiety (see *e.g.*, U.S. Pat. No. 5,777,153).

15 In another embodiment, a composition for delivering oligonucleotides of the invention comprises a peptide having from between about one to about four basic residues. These basic residues can be located, *e.g.*, on the amino terminal, C-terminal, or internal region of the peptide. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine (can also be considered non-polar), asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Apart from the basic amino acids, a majority or all of the other residues of the peptide can be selected from the non-basic amino acids, *e.g.*, amino acids other than lysine, arginine, or histidine. Preferably a preponderance of neutral amino acids with long neutral side chains are used.

30 In one embodiment, a composition for delivering oligonucleotides of the invention comprises a natural or synthetic polypeptide having one or more gamma carboxyglutamic acid residues, or γ -Gla residues. These gamma carboxyglutamic acid residues may enable the polypeptide to bind to each other and to membrane surfaces. In

- 73 --

other words, a polypeptide having a series of γ -Gla may be used as a general delivery modality that helps an RNAi construct to stick to whatever membrane to which it comes in contact. This may at least slow RNAi constructs from being cleared from the blood stream and enhance their chance of homing to the target.

5 The gamma carboxyglutamic acid residues may exist in natural proteins (for example, prothrombin has 10 γ -Gla residues). Alternatively, they can be introduced into the purified, recombinantly produced, or chemically synthesized polypeptides by carboxylation using, for example, a vitamin K-dependent carboxylase. The gamma carboxyglutamic acid residues may be consecutive or non-consecutive, and the total
10 number and location of such gamma carboxyglutamic acid residues in the polypeptide can be regulated / fine tuned to achieve different levels of "stickiness" of the polypeptide.

 In one embodiment, the cells to be contacted with an oligonucleotide composition of the invention are contacted with a mixture comprising the oligonucleotide and a mixture comprising a lipid, *e.g.*, one of the lipids or lipid compositions described supra
15 for between about 12 hours to about 24 hours. In another embodiment, the cells to be contacted with an oligonucleotide composition are contacted with a mixture comprising the oligonucleotide and a mixture comprising a lipid, *e.g.*, one of the lipids or lipid compositions described supra for between about 1 and about five days. In one
20 embodiment, the cells are contacted with a mixture comprising a lipid and the oligonucleotide for between about three days to as long as about 30 days. In another embodiment, a mixture comprising a lipid is left in contact with the cells for at least about five to about 20 days. In another embodiment, a mixture comprising a lipid is left in contact with the cells for at least about seven to about 15 days.

 For example, in one embodiment, an oligonucleotide composition can be
25 contacted with cells in the presence of a lipid such as cytofectin CS or GSV (available from Glen Research; Sterling, Va.), GS3815, GS2888 for prolonged incubation periods as described herein.

 In one embodiment, the incubation of the cells with the mixture comprising a lipid and an oligonucleotide composition does not reduce the viability of the cells.
30 Preferably, after the transfection period the cells are substantially viable. In one embodiment, after transfection, the cells are between at least about 70% and at least about 100% viable. In another embodiment, the cells are between at least about 80% and

- 74 -

at least about 95% viable. In yet another embodiment, the cells are between at least about 85% and at least about 90% viable.

In one embodiment, oligonucleotides are modified by attaching a peptide sequence that transports the oligonucleotide into a cell, referred to herein as a
5 “transporting peptide.” In one embodiment, the composition includes an oligonucleotide which is complementary to a target nucleic acid molecule encoding the protein, and a covalently attached transporting peptide.

The language “transporting peptide” includes an amino acid sequence that facilitates the transport of an oligonucleotide into a cell. Exemplary peptides which
10 facilitate the transport of the moieties to which they are linked into cells are known in the art, and include, *e.g.*, HIV TAT transcription factor, lactoferrin, Herpes VP22 protein, and fibroblast growth factor 2 (Pooga *et al.* 1998. *Nature Biotechnology*. 16:857; and Derossi *et al.* 1998. *Trends in Cell Biology*. 8:84; Elliott and O'Hare. 1997. *Cell* 88:223).

Oligonucleotides can be attached to the transporting peptide using known
15 techniques, *e.g.*, (Prochiantz, A. 1996. *Curr. Opin. Neurobiol.* 6:629; Derossi *et al.* 1998. *Trends Cell Biol.* 8:84; Troy *et al.* 1996. *J. Neurosci.* 16:253), Vives *et al.* 1997. *J. Biol. Chem.* 272:16010). For example, in one embodiment, oligonucleotides bearing an activated thiol group are linked via that thiol group to a cysteine present in a transport peptide (*e.g.*, to the cysteine present in the β turn between the second and the third helix
20 of the antennapedia homeodomain as taught, *e.g.*, in Derossi *et al.* 1998. *Trends Cell Biol.* 8:84; Prochiantz. 1996. *Current Opinion in Neurobiol.* 6:629; Allinquant *et al.* 1995. *J Cell Biol.* 128:919). In another embodiment, a Boc-Cys-(Npys)OH group can be coupled to the transport peptide as the last (N-terminal) amino acid and an oligonucleotide bearing an SH group can be coupled to the peptide (Troy *et al.* 1996. *J.*
25 *Neurosci.* 16:253).

In one embodiment, a linking group can be attached to a nucleomonomer and the transporting peptide can be covalently attached to the linker. In one embodiment, a linker can function as both an attachment site for a transporting peptide and can provide stability against nucleases. Examples of suitable linkers include substituted or
30 unsubstituted C₁-C₂₀ alkyl chains, C₂-C₂₀ alkenyl chains, C₂-C₂₀ alkynyl chains, peptides, and heteroatoms (*e.g.*, S, O, NH, *etc.*). Other exemplary linkers include bifunctional crosslinking agents such as sulfosuccinimidyl-4-(maleimidophenyl)-butyrate (SMPB)

- 75 -

(see, e.g., Smith *et al.* Biochem J 1991.276: 417-2).

In one embodiment, oligonucleotides of the invention are synthesized as molecular conjugates which utilize receptor-mediated endocytotic mechanisms for delivering genes into cells (see, e.g., Bunnell *et al.* 1992. *Somatic Cell and Molecular*
5 *Genetics*. 18:559, and the references cited therein).

Targeting Agents

The delivery of oligonucleotides can also be improved by targeting the oligonucleotides to a cellular receptor. The targeting moieties can be conjugated to the
10 oligonucleotides or attached to a carrier group (*i.e.*, poly(L-lysine) or liposomes) linked to the oligonucleotides. This method is well suited to cells that display specific receptor-mediated endocytosis.

For instance, oligonucleotide conjugates to 6-phosphomannosylated proteins are internalized 20-fold more efficiently by cells expressing mannose 6-phosphate specific
15 receptors than free oligonucleotides. The oligonucleotides may also be coupled to a ligand for a cellular receptor using a biodegradable linker. In another example, the delivery construct is mannosylated streptavidin which forms a tight complex with biotinylated oligonucleotides. Mannosylated streptavidin was found to increase 20-fold the internalization of biotinylated oligonucleotides. (Vlassov *et al.* 1994. *Biochimica et*
20 *Biophysica Acta* 1197:95-108).

In addition specific ligands can be conjugated to the polylysine component of polylysine-based delivery systems. For example, transferrin-polylysine, adenovirus-polylysine, and influenza virus hemagglutinin HA-2 N-terminal fusogenic peptides-polylysine conjugates greatly enhance receptor-mediated DNA delivery in eucaryotic
25 cells. Mannosylated glycoprotein conjugated to poly(L-lysine) in aveolar macrophages has been employed to enhance the cellular uptake of oligonucleotides. Liang *et al.* 1999. *Pharmazie* 54:559-566.

Because malignant cells have an increased need for essential nutrients such as folic acid and transferrin, these nutrients can be used to target oligonucleotides to
30 cancerous cells. For example, when folic acid is linked to poly(L-lysine) enhanced oligonucleotide uptake is seen in promyelocytic leukaemia (HL-60) cells and human melanoma (M-14) cells. Ginobbi *et al.* 1997. *Anticancer Res.* 17:29. In another example,

- 76 --

liposomes coated with maleylated bovine serum albumin, folic acid, or ferric protoporphyrin IX, show enhanced cellular uptake of oligonucleotides in murine macrophages, KB cells, and 2.2.15 human hepatoma cells. Liang *et al.* 1999. *Pharmazie* 54:559-566.

5 Liposomes naturally accumulate in the liver, spleen, and reticuloendothelial system (so-called, passive targeting). By coupling liposomes to various ligands such as antibodies or protein A, they can be actively targeted to specific cell populations. For example, protein A-bearing liposomes may be pretreated with H-2K specific antibodies which are targeted to the mouse major histocompatibility complex-encoded H-2K
10 protein expressed on L cells. (Vlassov *et al.* 1994. *Biochimica et Biophysica Acta* 1197:95-108).

Other *in vitro* and/or *in vivo* delivery of RNAi reagents are known in the art, and can be used to deliver the subject RNAi constructs. See, for example, U.S. patent application publications 20080152661, 20080112916, 20080107694, 20080038296,
15 20070231392, 20060240093, 20060178327, 20060008910, 20050265957, 20050064595, 20050042227, 20050037496, 20050026286, 20040162235, 20040072785, 20040063654, 20030157030, WO 2008/036825, WO04/065601, and AU2004206255B2, just to name a few (all incorporated by reference).

Administration

20 The optimal course of administration or delivery of the oligonucleotides may vary depending upon the desired result and/or on the subject to be treated. As used herein "administration" refers to contacting cells with oligonucleotides and can be performed *in vitro* or *in vivo*. The dosage of oligonucleotides may be adjusted to optimally reduce expression of a protein translated from a target nucleic acid molecule,
25 *e.g.*, as measured by a readout of RNA stability or by a therapeutic response, without undue experimentation.

For example, expression of the protein encoded by the nucleic acid target can be measured to determine whether or not the dosage regimen needs to be adjusted accordingly. In addition, an increase or decrease in RNA or protein levels in a cell or
30 produced by a cell can be measured using any art recognized technique. By determining whether transcription has been decreased, the effectiveness of the oligonucleotide in

- 77 -

inducing the cleavage of a target RNA can be determined.

Any of the above-described oligonucleotide compositions can be used alone or in conjunction with a pharmaceutically acceptable carrier. As used herein, “pharmaceutically acceptable carrier” includes appropriate solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, it can be used in the therapeutic compositions. Supplementary active ingredients can also be incorporated into the compositions.

Oligonucleotides may be incorporated into liposomes or liposomes modified with polyethylene glycol or admixed with cationic lipids for parenteral administration. Incorporation of additional substances into the liposome, for example, antibodies reactive against membrane proteins found on specific target cells, can help target the oligonucleotides to specific cell types.

Moreover, the present invention provides for administering the subject oligonucleotides with an osmotic pump providing continuous infusion of such oligonucleotides, for example, as described in Rataiczak *et al.* (1992 *Proc. Natl. Acad. Sci. USA* 89:11823-11827). Such osmotic pumps are commercially available, *e.g.*, from Alzet Inc. (Palo Alto, Calif.). Topical administration and parenteral administration in a cationic lipid carrier are preferred.

With respect to *in vivo* applications, the formulations of the present invention can be administered to a patient in a variety of forms adapted to the chosen route of administration, *e.g.*, parenterally, orally, or intraperitoneally. Parenteral administration, which is preferred, includes administration by the following routes: intravenous; intramuscular; interstitially; intraarterially; subcutaneous; intra ocular; intrasynovial; trans epithelial, including transdermal; pulmonary via inhalation; ophthalmic; sublingual and buccal; topically, including ophthalmic; dermal; ocular; rectal; and nasal inhalation via insufflation.

Pharmaceutical preparations for parenteral administration include aqueous solutions of the active compounds in water-soluble or water-dispersible form. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for

- 78 -

example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, or dextran, optionally, the suspension may also contain stabilizers. The

5 oligonucleotides of the invention can be formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the oligonucleotides may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included in the invention.

10 Pharmaceutical preparations for topical administration include transdermal patches, ointments, lotions, creams, gels, drops, sprays, suppositories, liquids and powders. In addition, conventional pharmaceutical carriers, aqueous, powder or oily bases, or thickeners may be used in pharmaceutical preparations for topical administration.

15 Pharmaceutical preparations for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets or tablets. In addition, thickeners, flavoring agents, diluents, emulsifiers, dispersing aids, or binders may be used in pharmaceutical preparations for oral administration.

 For transmucosal or transdermal administration, penetrants appropriate to the

20 barrier to be permeated are used in the formulation. Such penetrants are known in the art, and include, for example, for transmucosal administration bile salts and fusidic acid derivatives, and detergents. Transmucosal administration may be through nasal sprays or using suppositories. For oral administration, the oligonucleotides are formulated into conventional oral administration forms such as capsules, tablets, and tonics. For topical

25 administration, the oligonucleotides of the invention are formulated into ointments, salves, gels, or creams as known in the art.

 Drug delivery vehicles can be chosen *e.g.*, for *in vitro*, for systemic, or for topical administration. These vehicles can be designed to serve as a slow release reservoir or to deliver their contents directly to the target cell. An advantage of using some direct

30 delivery drug vehicles is that multiple molecules are delivered per uptake. Such vehicles have been shown to increase the circulation half-life of drugs that would otherwise be rapidly cleared from the blood stream. Some examples of such specialized drug delivery

- 79 -

vehicles which fall into this category are liposomes, hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres.

The described oligonucleotides may be administered systemically to a subject. Systemic absorption refers to the entry of drugs into the blood stream followed by
5 distribution throughout the entire body. Administration routes which lead to systemic absorption include: intravenous, subcutaneous, intraperitoneal, and intranasal. Each of these administration routes delivers the oligonucleotide to accessible diseased cells. Following subcutaneous administration, the therapeutic agent drains into local lymph nodes and proceeds through the lymphatic network into the circulation. The rate of entry
10 into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier localizes the oligonucleotide at the lymph node. The oligonucleotide can be modified to diffuse into the cell, or the liposome can directly participate in the delivery of either the unmodified or modified oligonucleotide into the cell.

15 The chosen method of delivery will result in entry into cells. Preferred delivery methods include liposomes (10-400 nm), hydrogels, controlled-release polymers, and other pharmaceutically applicable vehicles, and microinjection or electroporation (for ex vivo treatments).

The pharmaceutical preparations of the present invention may be prepared and
20 formulated as emulsions. Emulsions are usually heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter. The emulsions of the present invention may contain excipients such as emulsifiers, stabilizers, dyes, fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives, and anti-oxidants may also be present in emulsions as
25 needed. These excipients may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase.

Examples of naturally occurring emulsifiers that may be used in emulsion formulations of the present invention include lanolin, beeswax, phosphatides, lecithin and acacia. Finely divided solids have also been used as good emulsifiers especially in
30 combination with surfactants and in viscous preparations. Examples of finely divided solids that may be used as emulsifiers include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin,

- 80 -

montrorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

Examples of preservatives that may be included in the emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Examples of antioxidants that may be included in the emulsion formulations include free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

10 In one embodiment, the compositions of oligonucleotides are formulated as microemulsions. A microemulsion is a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution. Typically microemulsions are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a 4th component, generally an intermediate chain-
15 length alcohol to form a transparent system.

Surfactants that may be used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate
20 (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (S0750), decaglycerol decaoleate (DA0750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because
25 of the void space generated among surfactant molecules.

Microemulsions may, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase may typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of
30 ethylene glycol. The oil phase may include, but is not limited to, materials such as Captex 300, Captex 355, Capmul MCM, fatty acid esters, medium chain (C₈-C₁₂) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols,

- 81 -

polyglycolized glycerides, saturated polyglycolized C₈-C₁₀ glycerides, vegetable oils and silicone oil.

Microemulsions are particularly of interest from the standpoint of drug solubilization and the enhanced absorption of drugs. Lipid based microemulsions (both
5 oil/water and water/oil) have been proposed to enhance the oral bioavailability of drugs.

Microemulsions offer improved drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, ease of oral administration over solid dosage forms, improved clinical potency, and decreased
10 toxicity (Constantinides *et al.*, Pharmaceutical Research, 1994, 11:1385; Ho *et al.*, J. Pharm. Sci., 1996, 85:138-143). Microemulsions have also been effective in the transdermal delivery of active components in both cosmetic and pharmaceutical applications. It is expected that the microemulsion compositions and formulations of the present invention will facilitate the increased systemic absorption of oligonucleotides
15 from the gastrointestinal tract, as well as improve the local cellular uptake of oligonucleotides within the gastrointestinal tract, vagina, buccal cavity and other areas of administration.

In an embodiment, the present invention employs various penetration enhancers to affect the efficient delivery of nucleic acids, particularly oligonucleotides, to the skin
20 of animals. Even non-lipophilic drugs may cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to increasing the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also act to enhance the permeability of lipophilic drugs.

Five categories of penetration enhancers that may be used in the present
25 invention include: surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants. Other agents may be utilized to enhance the penetration of the administered oligonucleotides include: glycols such as ethylene glycol and propylene glycol, pyrrols such as 2-15 pyrrol, azones, and terpenes such as limonene, and menthone.

30 The oligonucleotides, especially in lipid formulations, can also be administered by coating a medical device, for example, a catheter, such as an angioplasty balloon catheter, with a cationic lipid formulation. Coating may be achieved, for example, by

- 82 -

dipping the medical device into a lipid formulation or a mixture of a lipid formulation and a suitable solvent, for example, an aqueous-based buffer, an aqueous solvent, ethanol, methylene chloride, chloroform and the like. An amount of the formulation will naturally adhere to the surface of the device which is subsequently administered to a patient, as appropriate. Alternatively, a lyophilized mixture of a lipid formulation may be specifically bound to the surface of the device. Such binding techniques are described, for example, in K. Ishihara *et al.*, Journal of Biomedical Materials Research, Vol. 27, pp. 1309-1314 (1993), the disclosures of which are incorporated herein by reference in their entirety.

The useful dosage to be administered and the particular mode of administration will vary depending upon such factors as the cell type, or for *in vivo* use, the age, weight and the particular animal and region thereof to be treated, the particular oligonucleotide and delivery method used, the therapeutic or diagnostic use contemplated, and the form of the formulation, for example, suspension, emulsion, micelle or liposome, as will be readily apparent to those skilled in the art. Typically, dosage is administered at lower levels and increased until the desired effect is achieved. When lipids are used to deliver the oligonucleotides, the amount of lipid compound that is administered can vary and generally depends upon the amount of oligonucleotide agent being administered. For example, the weight ratio of lipid compound to oligonucleotide agent is preferably from about 1:1 to about 15:1, with a weight ratio of about 5:1 to about 10:1 being more preferred. Generally, the amount of cationic lipid compound which is administered will vary from between about 0.1 milligram (mg) to about 1 gram (g). By way of general guidance, typically between about 0.1 mg and about 10 mg of the particular oligonucleotide agent, and about 1 mg to about 100 mg of the lipid compositions, each per kilogram of patient body weight, is administered, although higher and lower amounts can be used.

The agents of the invention are administered to subjects or contacted with cells in a biologically compatible form suitable for pharmaceutical administration. By “biologically compatible form suitable for administration” is meant that the oligonucleotide is administered in a form in which any toxic effects are outweighed by the therapeutic effects of the oligonucleotide. In one embodiment, oligonucleotides can be administered to subjects. Examples of subjects include mammals, *e.g.*, humans and other primates; cows, pigs, horses, and farming (agricultural) animals; dogs, cats, and

- 83 -

other domesticated pets; mice, rats, and transgenic non-human animals.

Administration of an active amount of an oligonucleotide of the present invention is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, an active amount of an oligonucleotide may vary
5 according to factors such as the type of cell, the oligonucleotide used, and for *in vivo* uses the disease state, age, sex, and weight of the individual, and the ability of the oligonucleotide to elicit a desired response in the individual. Establishment of therapeutic levels of oligonucleotides within the cell is dependent upon the rates of uptake and efflux or degradation. Decreasing the degree of degradation prolongs the
10 intracellular half-life of the oligonucleotide. Thus, chemically-modified oligonucleotides, *e.g.*, with modification of the phosphate backbone, may require different dosing.

The exact dosage of an oligonucleotide and number of doses administered will depend upon the data generated experimentally and in clinical trials. Several factors
15 such as the desired effect, the delivery vehicle, disease indication, and the route of administration, will affect the dosage. Dosages can be readily determined by one of ordinary skill in the art and formulated into the subject pharmaceutical compositions. Preferably, the duration of treatment will extend at least through the course of the disease symptoms.

20 Dosage regimen may be adjusted to provide the optimum therapeutic response. For example, the oligonucleotide may be repeatedly administered, *e.g.*, several doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. One of ordinary skill in the art will readily be able to determine appropriate doses and schedules of administration of the subject
25 oligonucleotides, whether the oligonucleotides are to be administered to cells or to subjects.

Physical methods of introducing nucleic acids include injection of a solution containing the nucleic acid, bombardment by particles covered by the nucleic acid, soaking the cell or organism in a solution of the nucleic acid, or electroporation of cell
30 membranes in the presence of the nucleic acid. A viral construct packaged into a viral particle would accomplish both efficient introduction of an expression construct into the cell and transcription of nucleic acid encoded by the expression construct. Other methods

- 84 -

known in the art for introducing nucleic acids to cells may be used, such as lipid-mediated carrier transport, chemical-mediated transport, such as calcium phosphate, and the like. Thus the nucleic acid may be introduced along with components that perform one or more of the following activities: enhance nucleic acid uptake by the cell, inhibit
5 annealing of single strands, stabilize the single strands, or other-wise increase inhibition of the target gene.

Nucleic acid may be directly introduced into the cell (i.e., intracellularly); or introduced extracellularly into a cavity, interstitial space, into the circulation of an organism, introduced orally or by inhalation, or may be introduced by bathing a cell or
10 organism in a solution containing the nucleic acid. Vascular or extravascular circulation, the blood or lymph system, and the cerebrospinal fluid are sites where the nucleic acid may be introduced.

The cell with the target gene may be derived from or contained in any organism. The organism may a plant, animal, protozoan, bacterium, virus, or fungus. The plant may
15 be a monocot, dicot or gymnosperm; the animal may be a vertebrate or invertebrate. Preferred microbes are those used in agriculture or by industry, and those that are pathogenic for plants or animals.

Alternatively, vectors, e.g., transgenes encoding a siRNA of the invention can be engineered into a host cell or transgenic animal using art recognized techniques.

20 Another use for the nucleic acids of the present invention (or vectors or transgenes encoding same) is a functional analysis to be carried out in eukaryotic cells, or eukaryotic non-human organisms, preferably mammalian cells or organisms and most preferably human cells, e.g. cell lines such as HeLa or 293 or rodents, e.g. rats and mice. By administering a suitable nucleic acid of the invention which is sufficiently
25 complementary to a target mRNA sequence to direct target-specific RNA interference, a specific knockout or knockdown phenotype can be obtained in a target cell, e.g. in cell culture or in a target organism.

Thus, a further subject matter of the invention is a eukaryotic cell or a eukaryotic non-human organism exhibiting a target gene-specific knockout or knockdown
30 phenotype comprising a fully or at least partially deficient expression of at least one endogenous target gene wherein said cell or organism is transfected with at least one vector comprising DNA encoding an RNAi agent capable of inhibiting the expression of

- 85 -

the target gene. It should be noted that the present invention allows a target-specific knockout or knockdown of several different endogenous genes due to the specificity of the RNAi agent.

Gene-specific knockout or knockdown phenotypes of cells or non-human organisms, particularly of human cells or non-human mammals may be used in analytic to procedures, e.g. in the functional and/or phenotypical analysis of complex physiological processes such as analysis of gene expression profiles and/or proteomes. Preferably the analysis is carried out by high throughput methods using oligonucleotide based chips.

10 *Assays of Oligonucleotide Stability*

In some embodiments, the oligonucleotides of the invention are stabilized, *i.e.*, substantially resistant to endonuclease and exonuclease degradation. An oligonucleotide is defined as being substantially resistant to nucleases when it is at least about 3-fold more resistant to attack by an endogenous cellular nuclease, and is highly nuclease resistant when it is at least about 6-fold more resistant than a corresponding oligonucleotide. This can be demonstrated by showing that the oligonucleotides of the invention are substantially resistant to nucleases using techniques which are known in the art.

One way in which substantial stability can be demonstrated is by showing that the oligonucleotides of the invention function when delivered to a cell, *e.g.*, that they reduce transcription or translation of target nucleic acid molecules, *e.g.*, by measuring protein levels or by measuring cleavage of mRNA. Assays which measure the stability of target RNA can be performed at about 24 hours post-transfection (*e.g.*, using Northern blot techniques, RNase Protection Assays, or QC-PCR assays as known in the art). Alternatively, levels of the target protein can be measured. Preferably, in addition to testing the RNA or protein levels of interest, the RNA or protein levels of a control, non-targeted gene will be measured (*e.g.*, actin, or preferably a control with sequence similarity to the target) as a specificity control. RNA or protein measurements can be made using any art-recognized technique. Preferably, measurements will be made beginning at about 16-24 hours post transfection. (M. Y. Chiang, *et al.* 1991. J Biol Chem. 266:18162-71; T. Fisher, *et al.* 1993. Nucleic Acids Research. 21 3857).

- 86 -

The ability of an oligonucleotide composition of the invention to inhibit protein synthesis can be measured using techniques which are known in the art, for example, by detecting an inhibition in gene transcription or protein synthesis. For example, Nuclease S1 mapping can be performed. In another example, Northern blot analysis can be used to measure the presence of RNA encoding a particular protein. For example, total RNA
5 can be prepared over a cesium chloride cushion (see, *e.g.*, Ausebel *et al.*, 1987. Current Protocols in Molecular Biology (Greene & Wiley, New York)). Northern blots can then be made using the RNA and probed (see, *e.g.*, *Id.*). In another example, the level of the specific mRNA produced by the target protein can be measured, *e.g.*, using PCR. In yet
10 another example, Western blots can be used to measure the amount of target protein present. In still another embodiment, a phenotype influenced by the amount of the protein can be detected. Techniques for performing Western blots are well known in the art, see, *e.g.*, Chen *et al.* J. Biol. Chem. 271:28259.

In another example, the promoter sequence of a target gene can be linked to a
15 reporter gene and reporter gene transcription (*e.g.*, as described in more detail below) can be monitored. Alternatively, oligonucleotide compositions that do not target a promoter can be identified by fusing a portion of the target nucleic acid molecule with a reporter gene so that the reporter gene is transcribed. By monitoring a change in the expression of the reporter gene in the presence of the oligonucleotide composition, it is possible to
20 determine the effectiveness of the oligonucleotide composition in inhibiting the expression of the reporter gene. For example, in one embodiment, an effective oligonucleotide composition will reduce the expression of the reporter gene.

A "reporter gene" is a nucleic acid that expresses a detectable gene product, which may be RNA or protein. Detection of mRNA expression may be accomplished by
25 Northern blotting and detection of protein may be accomplished by staining with antibodies specific to the protein. Preferred reporter genes produce a readily detectable product. A reporter gene may be operably linked with a regulatory DNA sequence such that detection of the reporter gene product provides a measure of the transcriptional activity of the regulatory sequence. In preferred embodiments, the gene product of the
30 reporter gene is detected by an intrinsic activity associated with that product. For instance, the reporter gene may encode a gene product that, by enzymatic activity, gives rise to a detectable signal based on color, fluorescence, or luminescence. Examples of reporter genes include, but are not limited to, those coding for chloramphenicol acetyl

- 87 -

transferase (CAT), luciferase, beta-galactosidase, and alkaline phosphatase.

One skilled in the art would readily recognize numerous reporter genes suitable for use in the present invention. These include, but are not limited to, chloramphenicol acetyltransferase (CAT), luciferase, human growth hormone (hGH), and beta-galactosidase. Examples of such reporter genes can be found in F. A. Ausubel *et al.*,
5 Eds., Current Protocols in Molecular Biology, John Wiley & Sons, New York, (1989). Any gene that encodes a detectable product, *e.g.*, any product having detectable enzymatic activity or against which a specific antibody can be raised, can be used as a reporter gene in the present methods.

10 One reporter gene system is the firefly luciferase reporter system. (Gould, S. J., and Subramani, S. 1988. Anal. Biochem., 7:404-408 incorporated herein by reference). The luciferase assay is fast and sensitive. In this assay, a lysate of the test cell is prepared and combined with ATP and the substrate luciferin. The encoded enzyme luciferase catalyzes a rapid, ATP dependent oxidation of the substrate to generate a light-
15 emitting product. The total light output is measured and is proportional to the amount of luciferase present over a wide range of enzyme concentrations.

CAT is another frequently used reporter gene system; a major advantage of this system is that it has been extensively validated and is widely accepted as a measure of promoter activity. (Gorman C. M., Moffat, L. F., and Howard, B. H. 1982. Mol. Cell.
20 Biol., 2:1044-1051). In this system, test cells are transfected with CAT expression vectors and incubated with the candidate substance within 2-3 days of the initial transfection. Thereafter, cell extracts are prepared. The extracts are incubated with acetyl CoA and radioactive chloramphenicol. Following the incubation, acetylated chloramphenicol is separated from nonacetylated form by thin layer chromatography. In
25 this assay, the degree of acetylation reflects the CAT gene activity with the particular promoter.

Another suitable reporter gene system is based on immunologic detection of hGH. This system is also quick and easy to use. (Selden, R., Burke-Howie, K. Rowe, M. E., Goodman, H. M., and Moore, D. D. (1986), Mol. Cell, Biol., 6:3173-3179
30 incorporated herein by reference). The hGH system is advantageous in that the expressed hGH polypeptide is assayed in the media, rather than in a cell extract. Thus, this system does not require the destruction of the test cells. It will be appreciated that

- 88 -

the principle of this reporter gene system is not limited to hGH but rather adapted for use with any polypeptide for which an antibody of acceptable specificity is available or can be prepared.

In one embodiment, nuclease stability of a double-stranded oligonucleotide of the invention is measured and compared to a control, *e.g.*, an RNAi molecule typically used in the art (*e.g.*, a duplex oligonucleotide of less than 25 nucleotides in length and comprising 2 nucleotide base overhangs) or an unmodified RNA duplex with blunt ends.

The target RNA cleavage reaction achieved using the siRNAs of the invention is highly sequence specific. Sequence identity may be determined by sequence comparison and alignment algorithms known in the art. To determine the percent identity of two nucleic acid sequences (or of two amino acid sequences), the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the first sequence or second sequence for optimal alignment). A preferred, non-limiting example of a local alignment algorithm utilized for the comparison of sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-68, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-77. Such an algorithm is incorporated into the BLAST programs (version 2.0) of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-10. Additionally, numerous commercial entities, such as Dharmacon, and Invitrogen provide access to algorithms on their website. The Whitehead Institute also offers a free siRNA Selection Program. Greater than 90% sequence identity, *e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or even 100% sequence identity, between the siRNA and the portion of the target gene is preferred. Alternatively, the siRNA may be defined functionally as a nucleotide sequence (or oligonucleotide sequence) that is capable of hybridizing with a portion of the target gene transcript. Examples of stringency conditions for polynucleotide hybridization are provided in Sambrook, J., E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., chapters 9 and 11, and *Current Protocols in Molecular Biology*, 1995, F. M. Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4, incorporated herein by reference.

30 *Therapeutic use*

By inhibiting the expression of a gene, the oligonucleotide compositions of the present invention can be used to treat any disease involving the expression of a protein.

- 89 -

Examples of diseases that can be treated by oligonucleotide compositions, just to illustrate, include: cancer, retinopathies, autoimmune diseases, inflammatory diseases (*i.e.*, ICAM-1 related disorders, Psoriasis, Ulcerative Colitis, Crohn's disease), viral diseases (*i.e.*, HIV, Hepatitis C), miRNA disorders, and cardiovascular diseases.

5 In one embodiment, *in vitro* treatment of cells with oligonucleotides can be used for ex vivo therapy of cells removed from a subject (*e.g.*, for treatment of leukemia or viral infection) or for treatment of cells which did not originate in the subject, but are to be administered to the subject (*e.g.*, to eliminate transplantation antigen expression on cells to be transplanted into a subject). In addition, *in vitro* treatment of cells can be used
10 in non-therapeutic settings, *e.g.*, to evaluate gene function, to study gene regulation and protein synthesis or to evaluate improvements made to oligonucleotides designed to modulate gene expression or protein synthesis. *In vivo* treatment of cells can be useful in certain clinical settings where it is desirable to inhibit the expression of a protein. There are numerous medical conditions for which antisense therapy is reported to be suitable
15 (see, *e.g.*, U.S. Pat. No. 5,830,653) as well as respiratory syncytial virus infection (WO 95/22,553) influenza virus (WO 94/23,028), and malignancies (WO 94/08,003). Other examples of clinical uses of antisense sequences are reviewed, *e.g.*, in Glaser. 1996. *Genetic Engineering News* 16:1. Exemplary targets for cleavage by oligonucleotides include, *e.g.*, protein kinase Ca, ICAM-1, c-raf kinase, p53, c-myb, and the bcr/abl fusion
20 gene found in chronic myelogenous leukemia.

 The subject nucleic acids can be used in RNAi-based therapy in any animal having RNAi pathway, such as human, non-human primate, non-human mammal, non-human vertebrates, rodents (mice, rats, hamsters, rabbits, etc.), domestic livestock animals, pets (cats, dogs, etc.), *Xenopus*, fish, insects (*Drosophila*, etc.), and worms (*C. elegans*), etc.
25

 The invention provides methods for inhibiting or preventing in a subject, a disease or condition associated with an aberrant or unwanted target gene expression or activity, by administering to the subject a nucleic acid of the invention. If appropriate, subjects are first treated with a priming agent so as to be more responsive to the
30 subsequent RNAi therapy. Subjects at risk for a disease which is caused or contributed to by aberrant or unwanted target gene expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays known in the art.

- 90 -

Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the target gene aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of target gene aberrancy, for example, a target gene, target gene agonist or target gene antagonist agent can be
5 used for treating the subject.

In another aspect, the invention pertains to methods of modulating target gene expression, protein expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the methods of the invention involve contacting a cell capable of expressing target gene with a nucleic acid of the invention that is specific for the target
10 gene or protein (e.g., is specific for the mRNA encoded by said gene or specifying the amino acid sequence of said protein) such that expression or one or more of the activities of target protein is modulated. These methods can be performed in vitro (e.g., by culturing the cell with the agent), in vivo (e.g., by administering the agent to a subject), or ex vivo. The subjects may be first treated with a priming agent so as to be more
15 responsive to the subsequent RNAi therapy if desired. As such, the present invention provides methods of treating a subject afflicted with a disease or disorder characterized by aberrant or unwanted expression or activity of a target gene polypeptide or nucleic acid molecule. Inhibition of target gene activity is desirable in situations in which target gene is abnormally unregulated and/or in which decreased target gene activity is likely to
20 have a beneficial effect.

Thus the therapeutic agents of the invention can be administered to subjects to treat (prophylactically or therapeutically) disorders associated with aberrant or unwanted target gene activity. In conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response
25 to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a therapeutic agent as
30 well as tailoring the dosage and/or therapeutic regimen of treatment with a therapeutic agent. Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons.

- 91 -

For the purposes of the invention, ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

Moreover, for the purposes of the present invention, the term "a" or "an" entity refers to one or more of that entity; for example, "a protein" or "a nucleic acid molecule" refers to one or more of those compounds or at least one compound. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising", "including", and "having" can be used interchangeably. Furthermore, a compound "selected from the group consisting of" refers to one or more of the compounds in the list that follows, including mixtures (i.e., combinations) of two or more of the compounds. According to the present invention, an isolated, or biologically pure, protein or nucleic acid molecule is a compound that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the compound has been purified. An isolated compound of the present invention can be obtained from its natural source, can be produced using molecular biology techniques or can be produced by chemical synthesis.

The present invention is further illustrated by the following Examples, which in no way should be construed as further limiting. The entire contents of all of the references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference.

EXAMPLES

Example 1: Inhibition of Gene Expression using Minimum Length Trigger RNAs

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Transfection of Minimum Length Trigger (mlt) RNA

mltRNA constructs were chemically synthesized (Integrated DNA Technologies, Coralville, IA) and transfected into HEK293 cells (ATCC, Manassas, VA) using the

- 92 --

Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA) reagent according to manufacturer's instructions. In brief, RNA was diluted to a 12X concentration and then combined with a 12X concentration of Lipofectamine RNAiMAX to complex. The RNA and transfection reagent were allowed to complex at room temperature for 20 minutes and make a 6X concentration. While complexing, HEK293 cells were washed, trypsinized and counted. The cells were diluted to a concentration recommended by the manufacturer and previously described conditions which was at 1×10^5 cells/ml. When RNA had completed complexing with the RNAiMAX transfection reagent, 20ul of the complexes were added to the appropriate well of the 96-well plate in triplicate. Cells were added to each well (100ul volume) to make the final cell count per well at 1×10^4 cells/well. The volume of cells diluted the 6X concentration of complex to 1X which was equal to a concentration noted (between 10-0.05 nM). Cells were incubated for 24 or 48 hours under normal growth conditions.

After 24 or 48 hour incubation cells were lysed and gene silencing activity was measured using the QuantiGene assay (Panomics, Freemont, CA) which employs bDNA hybridization technology. The assay was carried out according to manufacturer's instructions.

ΔG calculation

ΔG was calculated using Mfold, available through the Mfold internet site (<http://mfold.bioinfo.rpi.edu/cgi-bin/rna-form1.cgi>). Methods for calculating ΔG are described in, and are incorporated by reference from, the following references: Zuker, M. (2003) Nucleic Acids Res., 31(13):3406-15; Mathews, D. H., Sabina, J., Zuker, M. and Turner, D. H. (1999) J. Mol. Biol. 288:911-940; Mathews, D. H., Disney, M. D., Childs, J. L., Schroeder, S. J., Zuker, M., and Turner, D. H. (2004) Proc. Natl. Acad. Sci. 101:7287-7292; Duan, S., Mathews, D. H., and Turner, D. H. (2006) Biochemistry 45:9819-9832; Wuchty, S., Fontana, W., Hofacker, I. L., and Schuster, P. (1999) Biopolymers 49:145-165.

Example 2: Optimization of *sd-rxRNA*^{nano} Molecules for Gene Silencing

Asymmetric double stranded RNAi molecules, with minimal double stranded regions, were developed herein and are highly effective at gene silencing. These molecules can contain a variety of chemical modifications on the sense and/or anti-sense strands, and can be conjugated to sterol-like compounds such as cholesterol.

- 93 --

Figs. 1-3 present schematics of RNAi molecules associated with the invention. In the asymmetric molecules, which contain a sense and anti-sense strand, either of the strands can be the longer strand. Either strand can also contain a single-stranded region. There can also be mismatches between the sense and anti-sense strand, as indicated in Fig. 1D. Preferably, one end of the double-stranded molecule is either blunt-ended or contains a short overhang such as an overhang of one nucleotide. Fig. 2 indicates types of chemical modifications applied to the sense and anti-sense strands including 2'F, 2'OMe, hydrophobic modifications and phosphorothioate modifications. Preferably, the single stranded region of the molecule contains multiple phosphorothioate modifications. Hydrophobicity of molecules can be increased using such compounds as 4-pyridyl at 5-U, 2-pyridyl at 5-U, isobutyl at 5-U and indolyl at 5-U (Fig. 2). Proteins or peptides such as protamine (or other Arg rich peptides), spermidine or other similar chemical structures can also be used to block duplex charge and facilitate cellular entry (Fig. 3). Increased hydrophobicity can be achieved through either covalent or non-covalent modifications. Several positively charged chemicals, which might be used for polynucleotide charge blockage are depicted in Fig. 4.

Chemical modifications of polynucleotides, such as the guide strand in a duplex molecule, can facilitate RISC entry. Fig. 5 depicts single stranded polynucleotides, representing a guide strand in a duplex molecule, with a variety of chemical modifications including 2'd, 2'OMe, 2'F, hydrophobic modifications, phosphorothioate modifications, and attachment of conjugates such as "X" in Fig. 5, where X can be a small molecule with high affinity to a PAZ domain, or sterol-type entity. Similarly, Fig. 6 depicts single stranded polynucleotides, representing a passenger strand in a duplex molecule, with proposed structural and chemical compositions of RISC substrate inhibitors. Combinations of chemical modifications can ensure efficient uptake and efficient binding to preloaded RISC complexes.

Fig. 7 depicts structures of polynucleotides with sterol-type molecules attached, where R represents a polycarbonic tail of 9 carbons or longer. Fig. 8 presents examples of naturally occurring phytosterols with a polycarbon chain longer than 8 attached at position 17. More than 250 different types of phytosterols are known. Fig. 9 presents examples of sterol-like structures with variations in the sizes of the polycarbon chains attached at position 17. Fig. 91 presents further examples of sterol-type molecules that can be used as a hydrophobic entity in place of cholesterol. Fig. 92 presents further

- 94 -

examples of hydrophobic molecules that might be used as hydrophobic entities in place of cholestesterol. Optimization of such characteristics can improve uptake properties of the RNAi molecules. Fig. 10 presents data adapted from Martins et al. (J Lipid Research), showing that the percentage of liver uptake and plasma clearance of lipid emulsions containing sterol-type molecules is directly affected by the size of the attached polycarbon chain at position 17. Fig. 11 depicts a micelle formed from a mixture of polynucleotides attached to hydrophobic conjugates and fatty acids. Fig. 12 describes how alteration in lipid composition can affect pharmacokinetic behavior and tissue distribution of hydrophobically modified and/or hydrophobically conjugated polynucleotides. In particular, the use of lipid mixtures that are enriched in linoleic acid and cardiolipin results in preferential uptake by cardiomyocytes.

Fig. 13 depicts examples of RNAi constructs and controls designed to target MAP4K4 expression. Figs. 14 and 15 reveal that RNAi constructs with minimal duplex regions (such as duplex regions of approximately 13 nucleotides) are effective in mediating RNA silencing in cell culture. Parameters associated with these RNA molecules are shown in Fig. 16. Fig. 17 depicts examples of RNAi constructs and controls designed to target SOD1 expression. Figs. 18 and 19 reveal the results of gene silencing experiments using these RNAi molecules to target SOD1 in cells. Fig. 20 presents a schematic indicating that RNA molecules with double stranded regions that are less than 10 nucleotides are not cleaved by Dicer, and Fig. 21 presents a schematic of a hypothetical RNAi model for RNA induced gene silencing.

The RNA molecules described herein were subject to a variety of chemical modifications on the sense and antisense strands, and the effects of such modifications were observed. RNAi molecules were synthesized and optimized through testing of a variety of modifications. In first generation optimization, the sense (passenger) and anti-sense (guide) strands of the *sd-rxRNA*^{nano} molecules were modified for example through incorporation of C and U 2'OMe modifications, 2'F modifications, phosphorothioate modifications, phosphorylation, and conjugation of cholesterol. Molecules were tested for inhibition of MAP4K4 expression in cells including HeLa, primary mouse hepatocytes and primary human hepatocytes through both lipid-mediated and passive uptake transfection.

Fig. 22 reveals that chemical modifications can enhance gene silencing. In particular, modifying the guide strand with 2'F UC modifications, and with a stretch of

- 95 -

phosphorothioate modifications, combined with complete CU O'Me modification of the passenger strands, resulted in molecules that were highly effective in gene silencing.

The effect of chemical modification on *in vitro* efficacy in un-assisted delivery in HeLa cells was also examined. Fig. 23 reveals that compounds lacking any of 2'F, 2'OMe, a stretch of phosphorothioate modifications, or cholesterol conjugates, were completely inactive in passive uptake. A combination of all 4 types of chemical modifications, for example in compound 12386, was found to be highly effective in gene silencing. Fig. 24 also shows the effectiveness of compound 12386 in gene silencing.

Optimization of the length of the oligonucleotide was also investigated. Figs. 25 and 26 reveal that oligonucleotides with a length of 21 nucleotides were more effective than oligonucleotides with a length of 25 nucleotides, indicating that reduction in the size of an RNA molecule can improve efficiency, potentially by assisting in its uptake.

Screening was also conducted to optimize the size of the duplex region of double stranded RNA molecules. Fig. 88 reveals that compounds with duplexes of 10 nucleotides were effective in inducing gene silencing. Positioning of the sense strand relative to the guide strand can also be critical for silencing gene expression (Fig. 89). In this assay, a blunt end was found to be most effective. 3' overhangs were tolerated, but 5' overhangs resulted in a complete loss of functionality. The guide strand can be effective in gene silencing when hybridized to a sense strand of varying lengths (Fig. 90). In this assay presented in Fig. 90, the compounds were introduced into HeLa cells via lipid mediated transfection.

The importance of phosphorothioate content of the RNA molecule for unassisted delivery was also investigated. Fig. 27 presents the results of a systematic screen that identified that the presence of at least 2-12 phosphorothioates in the guide strand as being highly advantageous for achieving uptake, with 4-8 being the preferred number. Fig. 27 also shows that presence or absence of phosphorothioate modifications in the sense strand did not alter efficacy.

Figs. 28-29 reveal the effects of passive uptake of RNA compounds on gene silencing in primary mouse hepatocytes. nanoRNA molecules were found to be highly effective, especially at a concentration of 1 μ M (Fig. 28). Figs. 30 and 31 reveal that the RNA compounds associated with the invention were also effective in gene silencing following passive uptake in primary human hepatocytes. The cellular localization of the

- 96 --

RNA molecules associated with the invention was examined and compared to the localization of Chol-siRNA (Alnylam) molecules, as shown in Figs. 32 and 33.

A summary of 1st generation sd-rxRNA molecules is presented in Fig. 21. Chemical modifications were introduced into the RNA molecules, at least in part, to increase potency, such as through optimization of nucleotide length and phosphorothioate content, to reduce toxicity, such as through replacing 2'F modifications on the guide strand with other modifications, to improve delivery such as by adding or conjugating the RNA molecules to linker and sterol modalities, and to improve the ease of manufacturing the RNA molecules. Fig. 35 presents schematic depictions of some of the chemical modifications that were screened in 1st generation molecules. Parameters that were optimized for the guide strand included nucleotide length (e.g., 19, 21 and 25 nucleotides), phosphorothioate content (e.g., 0-18 phosphorothioate linkages) and replacement of 2'F groups with 2'OMe and 5 Me C or riboThymidine. Parameters that were optimized for the sense strand included nucleotide length (e.g., 11, 13 and 19 nucleotides), phosphorothioate content (e.g., 0-4 phosphorothioate linkages), and 2'OMe modifications. Fig. 36 summarizes parameters that were screened. For example, the nucleotide length and the phosphorothioate tail length were modified and screened for optimization, as were the additions of 2'OMe C and U modifications. Guide strand length and the length of the phosphorothioate modified stretch of nucleotides were found to influence efficacy (Figs. 37-38). Phosphorothioate modifications were tolerated in the guide strand and were found to influence passive uptake (Figs. 39-42).

Fig. 43 presents a schematic revealing guide strand chemical modifications that were screened. Figs. 44 and 45 reveal that 2'OMe modifications were tolerated in the 3' end of the guide strand. In particular, 2'OMe modifications in positions 1 and 11-18 were well tolerated. The 2'OMe modifications in the seed area were tolerated but resulted in slight reduction of efficacy. Ribo- modifications in the seed were also well tolerated. These data indicate that the molecules associated with the invention offer the significant advantage of having reduced or no 2'F modification content. This is advantageous because 2'F modifications are thought to generate toxicity *in vivo*. In some instances, a complete substitution of 2'F modifications with 2'OMe was found to lead to some reduction in potency. However, the 2' OMe substituted molecules were still very active. A molecule with 50% reduction in 2'F content (including at positions 11, 16-18 which were changed to 2'OMe modifications), was found to have comparable

- 97 -

efficacy to a compound with complete 2'F C and U modification. 2'OMe modification in position was found in some instances to reduce efficacy, although this can be at least partially compensated by 2'OMe modification in position 1 (with chemical phosphate). In some instances, 5 Me C and/or ribothymidine substitution for 2'F modifications led to a reduction in passive uptake efficacy, but increased potency in lipid mediated transfections compared to 2'F modifications. Optimization results for lipid mediated transfection were not necessarily the same as for passive uptake.

Modifications to the sense strand were also developed and tested, as depicted in Fig. 46. Fig. 47 reveals that in some instances, a sense strand length between 10-15 bases was found to be optimal. For the molecules tested in Fig. 47, an increase in the sense strand length resulted in reduction of passive uptake, however an increase in sense strand length may be tolerated for some compounds. Fig. 47 also reveals that LNA modification of the sense strand demonstrated similar efficacy to non-LNA containing compounds. In general, the addition of LNA or other thermodynamically stabilizing compounds has been found to be beneficial, in some instances resulting in converting non-functional sequences to functional sequences. Fig. 48 also presents data on sense strand length optimization, while Fig. 49 shows that phosphorothioate modification of the sense strand is not required for passive uptake.

Based on the above-described optimization experiments, 2nd generation RNA molecules were developed. As shown in Fig. 50, these molecules contained reduced phosphorothioate modification content and reduced 2'F modification content, relative to 1st generation RNA molecules. Significantly, these RNA molecules exhibit spontaneous cellular uptake and efficacy without a delivery vehicle (Fig. 51). These molecules can achieve self-delivery (i.e., with no transfection reagent) and following self-delivery can exhibit nanomolar activity in cell culture. These molecules can also be delivered using lipid-mediated transfection, and exhibit picomolar activity levels following transfection. Significantly, these molecules exhibit highly efficient uptake, 95% by most cells in cell culture, and are stable for more than three days in the presence of 100% human serum. These molecules are also highly specific and exhibit little or no immune induction. Figs. 52 and 53 reveal the significance of chemical modifications and the configurations of such modifications in influencing the properties of the RNA molecules associated with the invention.

- 98 -

Linker chemistry was also tested in conjunction with the RNA molecules associated with the invention. As depicted in Fig. 54, 2nd generation RNA molecules were synthesized with sterol-type molecules attached through TEG and amino caproic acid linkers. Both linkers showed identical potency. This functionality of the RNA molecules, independent of linker chemistry offers additional advantages in terms of scale up and synthesis and demonstrates that the mechanism of function of these RNA molecules is very different from other previously described RNA molecules.

Stability of the chemically modified sd-rxRNA molecules described herein in human serum is shown in Fig. 55 in comparison to unmodified RNA. The duplex molecules were incubated in 75% serum at 37 °C for the indicated periods of time. The level of degradation was determined by running the samples on non-denaturing gels and staining with SYBGR.

Figs. 56 and 57 present data on cellular uptake of the sd-rxRNA molecules. Fig. 56 shows that minimizing the length of the RNA molecule is importance for cellular uptake, while Fig. 57 presents data showing target gene silencing after spontaneous cellular uptake in mouse PEC-derived macrophages. Fig. 58 demonstrates spontaneous uptake and target gene silencing in primary cells. Fig. 59 shows the results of delivery of sd-rxRNA molecules associated with the invention to RPE cells with no formulation. Imaging with Hoechst and DY547 reveals the clear presence of a signal representing the RNA molecule in the sd-rxRNA sample, while no signal is detectable in the other samples including the samples competing a competing conjugate, an rxRNA, and an untransfected control. Fig. 60 reveals silencing of target gene expression in RPE cells treated with sd-rxRNA molecules associated with the invention following 24-48 hours without any transfection formulation.

Fig. 61 shows further optimization of the chemical/structural composition of sd-rxRNA compounds. In some instances, preferred properties included an antisense strand that was 17-21 nucleotides long, a sense strand that was 10-15 nucleotides long, phosphorothioate modification of 2-12 nucleotides within the single stranded region of the molecule, preferentially phosphorothioate modification of 6-8 nucleotides within the single stranded region, and 2'OMe modification at the majority of positions within the sense strand, with or without phosphorothioate modification. Any linker chemistry can be used to attach the hydrophobic moiety, such as cholesterol, to the 3' end of the sense

- 99 -

strand. Version GIIb molecules, as shown in Fig. 61, have no 2'F modifications. Significantly, there is was no impact on efficacy in these molecules.

FIG. 62 demonstrates the superior performance of sd-rxRNA compounds compared to compounds published by Wolfrum et. al. Nature Biotech, 2007. Both
5 generation I and II compounds (GI and GIIa) developed herein show great efficacy in reducing target gene expression. By contrast, when the chemistry described in Wolfrum et al. (all oligos contain cholesterol conjugated to the 3' end of the sense strand) was applied to the same sequence in a context of conventional siRNA (19 bp duplex with two overhang) the compound was practically inactive. These data emphasize the significance
10 of the combination of chemical modifications and assymetrical molecules described herein, producing highly effective RNA compounds.

Fig. 63 shows localization of sd-rxRNA molecules developed herein compared to localization of other RNA molecules such as those described in Soutschek et al. (2004) Nature, 432:173. sd-rxRNA molecules accumulate inside the cells whereas competing
15 conjugate RNAs accumulate on the surface of cells. Significantly, Fig. 64 shows that sd-rxRNA molecules, but not competitor molecules such as those described in Soutschek et al. are internalized within minutes. Fig. 65 compares localization of sd-rxRNA molecules compared to regular siRNA-cholesterol, as described in Soutschek et al. A signal representing the RNA molecule is clearly detected for the sd-rxRNA molecule in
20 tissue culture RPE cells, following local delivery to compromised skin, and following systemic delivery where uptake to the liver is seen. In each case, no signal is detected for the regular siRNA-cholesterol molecule. The sd-rxRNA molecule thus has drastically better cellular and tissue uptake characteristics when compared to
conventional cholesterol conjugated siRNAs such as those described in Soutschek et al.
25 The level of uptake is at least order of magnitude higher and is due at least in part to the unique combination of chemistries and conjugated structure. Superior delivery of sd-rxRNA relative to previously described RNA molecules is also demonstrated in Figs. 66 and 67.

Based on the analysis of 2nd generation RNA molecules associated with the
30 invention, a screen was performed to identify functional molecules for targeting the SPP1/PPIB gene. As revealed in Fig. 68, several effective molecules were identified, with 14131 being the most effective. The compounds were added to A-549 cells and then the level of SPP1/ PPIB ratio was determined by B-DNA after 48 hours.

- 100 --

Fig. 69 reveals efficient cellular uptake of sd-rxRNA within minutes of exposure. This is a unique characteristics of these molecules, not observed with any other RNAi compounds. Compounds described in Soutschek et al. were used as negative controls. Fig. 70 reveals that the uptake and gene silencing of the sd-rxRNA is effective in
5 multiple different cell types including SH-SY5Y neuroblastoma derived cells, ARPE-19 (retinal pigment epithelium) cells, primary hepatocytes, and primary macrophages. In each case silencing was confirmed by looking at target gene expression by a Branched DNA assay.

Fig. 70 reveals that sd-rxRNA is active in the presence or absence of serum.
10 While a slight reduction in efficacy (2-5 fold) was observed in the presence of serum, this small reduction in efficacy in the presence of serum differentiate the sd-rxRNA molecules from previously described molecules which exhibited a larger reduction in efficacy in the presence of serum. This demonstrated level of efficacy in the presence of serum creates a foundation for in vivo efficacy.

Fig. 72 reveals efficient tissue penetration and cellular uptake upon single
15 intradermal injection. This data indicates the potential of the sd-rxRNA compounds described herein for silencing genes in any dermatology applications, and also represents a model for local delivery of sd-rxRNA compounds. Fig. 73 also demonstrates efficient cellular uptake and *in vivo* silencing with sd-rxRNA following intradermal injection.
20 Silencing is determined as the level of MAP4K4 knockdown in several individual biopsies taken from the site of injection as compared to biopsies taken from a site injected with a negative control. Fig. 74 reveals that sd-rxRNA compounds has improved blood clearance and induced effective gene silencing *in vivo* in the liver upon systemic administration. In comparison to the RNA molecules described by Soutschek
25 et al., the level of liver uptake at identical dose level is at least 50 fold higher with the sd-rxRNA molecules. The uptake results in productive silencing. sd-rxRNA compounds are also characterized by improved blood clearance kinetics.

The effect of 5-Methyl C modifications was also examined. Fig. 75 demonstrates that the presence of 5-Methyl C in an RNAi molecule resulted in increased potency in
30 lipid mediated transfection. This suggests that hydrophobic modification of Cs and Us in an RNAi molecule can be beneficial. These types of modifications can also be used in the context 2' ribose modified bases to ensure optimal stability and efficacy. Fig. 76

- 101 -

presents data showing that incorporation of 5-Methyl C and/or ribothymidine in the guide strand can in some instances reduce efficacy.

Fig. 77 reveals that sd-rxRNA molecules are more effective than competitor molecules such as molecules described in Soutschek et al., in systemic delivery to the liver. A signal representing the RNA molecule is clearly visible in the sample containing
5 sd-rxRNA, while no signal representing the RNA molecule is visible in the sample containing the competitor RNA molecule.

The addition of hydrophobic conjugates to the sd-rxRNA molecules was also explored (Figs 78-83). FIG. 78 presents schematics demonstrating 5-uridyl
10 modifications with improved hydrophobicity characteristics. Incorporation of such modifications into sd-rxRNA compounds can increase cellular and tissue uptake properties. FIG. 78B presents a new type of RNAi compound modification which can be applied to compounds to improve cellular uptake and pharmacokinetic behavior. Significantly, this type of modification, when applied to sd-rxRNA compounds, may
15 contribute to making such compounds orally available. FIG. 79 presents schematics revealing the structures of synthesized modified sterol-type molecules, where the length and structure of the C17 attached tail is modified. Without wishing to be bound by any theory, the length of the C17 attached tail may contribute to improving *in vitro* and *in vivo* efficacy of sd-rxRNA compounds.

FIG. 80 presents a schematic demonstrating the lithocholic acid route to long side
20 chain cholesterol. FIG. 81 presents a schematic demonstrating a route to 5-uridyl phosphoramidite synthesis. FIG. 82 presents a schematic demonstrating synthesis of tri-functional hydroxyprolinol linker for 3'-cholesterol attachment. FIG. 83 presents a schematic demonstrating synthesis of solid support for the manufacture of a shorter
25 asymmetric RNAi compound strand.

A screen was conducted to identify compounds that could effectively silence expression of SPP1 (Osteopontin). Compounds targeting SPP1 were added to A549 cells (using passive transfection), and the level of SPP1 expression was evaluated at 48 hours. Several novel compounds effective in SPP1 silencing were identified. Compounds that
30 were effective in silencing of SPP1 included 14116, 14121, 14131, 14134, 14139, 14149, and 14152 (Figs. 84-86). The most potent compound in this assay was 14131 (Fig. 84). The efficacy of these sd-rxRNA compounds in silencing SPP1 expression was independently validated (Fig. 85).

- 102 --

A similar screen was conducted to identify compounds that could effectively silence expression of CTGF (Figs. 86-87). Compounds that were effective in silencing of CTGF included 14017, 14013, 14016, 14022, 14025, 14027.

5

Methods

Transfection of sd-rxRNA^{nano}

Lipid mediated transfection

sd-rxRNA^{nano} constructs were chemically synthesized (Dharmacon, Lafayette, CO) and transfected into HEK293 cells (ATCC, Manassas, VA) using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. In brief, RNA was diluted to a 12X concentration in Opti-MEM®1 Reduced Serum Media (Invitrogen, Carlsbad, California) and then combined with a 12X concentration of Lipofectamine RNAiMAX. The RNA and transfection reagent were allowed to complex at room temperature for 20 minutes and make a 6X concentration. While complexing, HEK293 cells were washed, trypsinized and counted. The cells were diluted to a concentration recommended by the manufacturer and previously described of 1×10^5 cells/ml. When RNA had completed complexing with the RNAiMAX transfection reagent, 20 ul of the complexes were added to the appropriate well of the 96-well plate in triplicate. Cells were added to each well (100 ul volume) to make the final cell count per well 1×10^4 cells/well. The volume of cells diluted the 6X concentration of complex to 1X (between 10-0.05 nM). Cells were incubated for 24 or 48 hours under normal growth conditions. After 24 or 48 hour incubation, cells were lysed and gene silencing activity was measured using the QuantiGene assay (Panomics, Fremont, CA) which employs bDNA hybridization technology. The assay was carried out according to manufacturer's instructions.

Passive uptake transfection

sd-rxRNA^{nano} constructs were chemically synthesized (Dharmacon, Lafayette, CO). 24 hours prior to transfection, HeLa cells (ATCC, Manassas, VA) were plated at 1×10^4 cells/well in a 96 well plate under normal growth conditions (DMEM, 10 % FBS and 1% Penicillin and Streptomycin). Prior to transfection of HeLa cells, sd-rxRNA^{nano} were diluted to a final concentration of 0.01 uM to 1 uM in Accell siRNA Delivery

30

- 103 -

Media (Dharmacon, Lafayette, CO). Normal growth media was aspirated off cells and 100 uL of Accell Delivery media containing the appropriate concentration of sd-rxRNA_{nano} was applied to the cells. 48 hours post transfection, delivery media was aspirated off the cells and normal growth media was applied to cells for an additional 24
5 hours.

After 48 or 72 hour incubation, cells were lysed and gene silencing activity was measured using the QuantiGene assay (Panomics, Fremont, CA) according to manufacturer's instructions.

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
APOB-10167-20-12138	12138	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB-10167-20-12139	12139	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
MAP4K4-2931-13-12266	12266	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4-2931-16-12293	12293	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4-2931-16-12383	12383	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4-2931-16-12384	12384	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4-2931-16-12385	12385	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4-2931-16-12386	12386	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4-2931-16-12387	12387	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4-2931-15-12388	12388	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4-2931-13-12432	12432	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4-2931-13-12266.2	12266.2	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
APOB--21-12434	12434	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB--21-12435	12435	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
MAP4K4-2931-16-12451	12451	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4-2931-16-12452	12452	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4-2931-16-12453	12453	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4-2931-17-12454	12454	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4

- 105 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
MAP4K4-2931-17-12455	12455	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4-2931-19-12456	12456	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
--27-12480	12480			
--27-12481	12481			
APOB-10167-21-12505	12505	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB-10167-21-12506	12506	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
MAP4K4-2931-16-12539	12539	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
APOB-10167-21-12505.2	12505.2	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB-10167-21-12506.2	12506.2	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
MAP4K4--13-12565	12565			MAP4K4
MAP4K4-2931-16-12386.2	12386.2	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4-2931-13-12815	12815	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
APOB--13-12957	12957	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
MAP4K4--16-12983	12983		Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4--16-12984	12984		Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4--16-12985	12985		Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4--16-12986	12986		Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4--16-12987	12987		Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4--16-12988	12988		Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4),	MAP4K4

- 106 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
			transcript variant 1	
MAP4K4--16-12989	12989		Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4--16-12990	12990		Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4--16-12991	12991		Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4--16-12992	12992		Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4--16-12993	12993		Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4--16-12994	12994		Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4--16-12995	12995		Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4-2931-19-13012	13012	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
MAP4K4-2931-19-13016	13016	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
PPIB--13-13021	13021	NM_000942	Peptidylprolyl Isomerase B (cyclophilin B)	PPIB
pGL3-1172-13-13038	13038	U47296	Cloning vector pGL3-Control	pGL3
pGL3-1172-13-13040	13040	U47296	Cloning vector pGL3-Control	pGL3
--16-13047	13047			
SOD1-530-13-13090	13090	NM_000454	Superoxide Dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	SOD1
SOD1-523-13-13091	13091	NM_000454	Superoxide Dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	SOD1
SOD1-535-13-13092	13092	NM_000454	Superoxide Dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	SOD1
SOD1-536-13-13093	13093	NM_000454	Superoxide Dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	SOD1

- 107 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
SOD1-396-13-13094	13094	NM_000454	Superoxide Dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	SOD1
SOD1-385-13-13095	13095	NM_000454	Superoxide Dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	SOD1
SOD1-195-13-13096	13096	NM_000454	Superoxide Dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	SOD1
APOB-4314-13-13115	13115	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB-3384-13-13116	13116	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB-3547-13-13117	13117	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB-4318-13-13118	13118	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB-3741-13-13119	13119	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
PPIB--16-13136	13136	NM_000942	Peptidylprolyl Isomerase B (cyclophilin B)	PPIB
APOB-4314-15-13154	13154	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB-3547-15-13155	13155	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB-4318-15-13157	13157	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB-3741-15-13158	13158	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB--13-13159	13159	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB--15-13160	13160	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
SOD1-530-16-13163	13163	NM_000454	Superoxide Dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	SOD1
SOD1-523-16-13164	13164	NM_000454	Superoxide Dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	SOD1
SOD1-535-16-13165	13165	NM_000454	Superoxide Dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	SOD1
SOD1-536-16-13166	13166	NM_000454	Superoxide Dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	SOD1

- 108 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
SOD1-396-16-13167	13167	NM_000454	Superoxide Dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	SOD1
SOD1-385-16-13168	13168	NM_000454	Superoxide Dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	SOD1
SOD1-195-16-13169	13169	NM_000454	Superoxide Dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	SOD1
pGL3-1172-16-13170	13170	U47296	Cloning vector pGL3-Control	pGL3
pGL3-1172-16-13171	13171	U47296	Cloning vector pGL3-Control	pGL3
MAP4k4-2931-19-13189	13189	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4k4
CTGF-1222-13-13190	13190	NM_001901.2	connective tissue growth factor	CTGF
CTGF-813-13-13192	13192	NM_001901.2	connective tissue growth factor	CTGF
CTGF-747-13-13194	13194	NM_001901.2	connective tissue growth factor	CTGF
CTGF-817-13-13196	13196	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1174-13-13198	13198	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1005-13-13200	13200	NM_001901.2	connective tissue growth factor	CTGF
CTGF-814-13-13202	13202	NM_001901.2	connective tissue growth factor	CTGF
CTGF-816-13-13204	13204	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1001-13-13206	13206	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1173-13-13208	13208	NM_001901.2	connective tissue growth factor	CTGF
CTGF-749-13-13210	13210	NM_001901.2	connective tissue growth factor	CTGF
CTGF-792-13-13212	13212	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1162-13-13214	13214	NM_001901.2	connective tissue growth factor	CTGF
CTGF-811-13-13216	13216	NM_001901.2	connective tissue growth factor	CTGF

- 109 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
CTGF-797-13-13218	13218	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1175-13-13220	13220	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1172-13-13222	13222	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1177-13-13224	13224	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1176-13-13226	13226	NM_001901.2	connective tissue growth factor	CTGF
CTGF-812-13-13228	13228	NM_001901.2	connective tissue growth factor	CTGF
CTGF-745-13-13230	13230	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1230-13-13232	13232	NM_001901.2	connective tissue growth factor	CTGF
CTGF-920-13-13234	13234	NM_001901.2	connective tissue growth factor	CTGF
CTGF-679-13-13236	13236	NM_001901.2	connective tissue growth factor	CTGF
CTGF-992-13-13238	13238	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1045-13-13240	13240	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1231-13-13242	13242	NM_001901.2	connective tissue growth factor	CTGF
CTGF-991-13-13244	13244	NM_001901.2	connective tissue growth factor	CTGF
CTGF-998-13-13246	13246	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1049-13-13248	13248	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1044-13-13250	13250	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1327-13-13252	13252	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1196-13-13254	13254	NM_001901.2	connective tissue growth factor	CTGF
CTGF-562-13-13256	13256	NM_001901.2	connective tissue growth factor	CTGF

- 110 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
CTGF-752-13-13258	13258	NM_001901.2	connective tissue growth factor	CTGF
CTGF-994-13-13260	13260	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1040-13-13262	13262	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1984-13-13264	13264	NM_001901.2	connective tissue growth factor	CTGF
CTGF-2195-13-13266	13266	NM_001901.2	connective tissue growth factor	CTGF
CTGF-2043-13-13268	13268	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1892-13-13270	13270	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1567-13-13272	13272	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1780-13-13274	13274	NM_001901.2	connective tissue growth factor	CTGF
CTGF-2162-13-13276	13276	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1034-13-13278	13278	NM_001901.2	connective tissue growth factor	CTGF
CTGF-2264-13-13280	13280	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1032-13-13282	13282	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1535-13-13284	13284	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1694-13-13286	13286	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1588-13-13288	13288	NM_001901.2	connective tissue growth factor	CTGF
CTGF-928-13-13290	13290	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1133-13-13292	13292	NM_001901.2	connective tissue growth factor	CTGF
CTGF-912-13-13294	13294	NM_001901.2	connective tissue growth factor	CTGF
CTGF-753-13-13296	13296	NM_001901.2	connective tissue growth factor	CTGF

- 111 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
CTGF-918-13-13298	13298	NM_001901.2	connective tissue growth factor	CTGF
CTGF-744-13-13300	13300	NM_001901.2	connective tissue growth factor	CTGF
CTGF-466-13-13302	13302	NM_001901.2	connective tissue growth factor	CTGF
CTGF-917-13-13304	13304	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1038-13-13306	13306	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1048-13-13308	13308	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1235-13-13310	13310	NM_001901.2	connective tissue growth factor	CTGF
CTGF-868-13-13312	13312	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1131-13-13314	13314	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1043-13-13316	13316	NM_001901.2	connective tissue growth factor	CTGF
CTGF-751-13-13318	13318	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1227-13-13320	13320	NM_001901.2	connective tissue growth factor	CTGF
CTGF-867-13-13322	13322	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1128-13-13324	13324	NM_001901.2	connective tissue growth factor	CTGF
CTGF-756-13-13326	13326	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1234-13-13328	13328	NM_001901.2	connective tissue growth factor	CTGF
CTGF-916-13-13330	13330	NM_001901.2	connective tissue growth factor	CTGF
CTGF-925-13-13332	13332	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1225-13-13334	13334	NM_001901.2	connective tissue growth factor	CTGF
CTGF-445-13-13336	13336	NM_001901.2	connective tissue growth factor	CTGF

- 112 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
CTGF-446-13-13338	13338	NM_001901.2	connective tissue growth factor	CTGF
CTGF-913-13-13340	13340	NM_001901.2	connective tissue growth factor	CTGF
CTGF-997-13-13342	13342	NM_001901.2	connective tissue growth factor	CTGF
CTGF-277-13-13344	13344	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1052-13-13346	13346	NM_001901.2	connective tissue growth factor	CTGF
CTGF-887-13-13348	13348	NM_001901.2	connective tissue growth factor	CTGF
CTGF-914-13-13350	13350	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1039-13-13352	13352	NM_001901.2	connective tissue growth factor	CTGF
CTGF-754-13-13354	13354	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1130-13-13356	13356	NM_001901.2	connective tissue growth factor	CTGF
CTGF-919-13-13358	13358	NM_001901.2	connective tissue growth factor	CTGF
CTGF-922-13-13360	13360	NM_001901.2	connective tissue growth factor	CTGF
CTGF-746-13-13362	13362	NM_001901.2	connective tissue growth factor	CTGF
CTGF-993-13-13364	13364	NM_001901.2	connective tissue growth factor	CTGF
CTGF-825-13-13366	13366	NM_001901.2	connective tissue growth factor	CTGF
CTGF-926-13-13368	13368	NM_001901.2	connective tissue growth factor	CTGF
CTGF-923-13-13370	13370	NM_001901.2	connective tissue growth factor	CTGF
CTGF-866-13-13372	13372	NM_001901.2	connective tissue growth factor	CTGF
CTGF-563-13-13374	13374	NM_001901.2	connective tissue growth factor	CTGF
CTGF-823-13-13376	13376	NM_001901.2	connective tissue growth factor	CTGF

- 113 --

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
CTGF-1233-13-13378	13378	NM_001901.2	connective tissue growth factor	CTGF
CTGF-924-13-13380	13380	NM_001901.2	connective tissue growth factor	CTGF
CTGF-921-13-13382	13382	NM_001901.2	connective tissue growth factor	CTGF
CTGF-443-13-13384	13384	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1041-13-13386	13386	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1042-13-13388	13388	NM_001901.2	connective tissue growth factor	CTGF
CTGF-755-13-13390	13390	NM_001901.2	connective tissue growth factor	CTGF
CTGF-467-13-13392	13392	NM_001901.2	connective tissue growth factor	CTGF
CTGF-995-13-13394	13394	NM_001901.2	connective tissue growth factor	CTGF
CTGF-927-13-13396	13396	NM_001901.2	connective tissue growth factor	CTGF
SPP1-1025-13-13398	13398	NM_000582.2	Osteopontin	SPP1
SPP1-1049-13-13400	13400	NM_000582.2	Osteopontin	SPP1
SPP1-1051-13-13402	13402	NM_000582.2	Osteopontin	SPP1
SPP1-1048-13-13404	13404	NM_000582.2	Osteopontin	SPP1
SPP1-1050-13-13406	13406	NM_000582.2	Osteopontin	SPP1
SPP1-1047-13-13408	13408	NM_000582.2	Osteopontin	SPP1
SPP1-800-13-13410	13410	NM_000582.2	Osteopontin	SPP1
SPP1-492-13-13412	13412	NM_000582.2	Osteopontin	SPP1
SPP1-612-13-13414	13414	NM_000582.2	Osteopontin	SPP1
SPP1-481-13-13416	13416	NM_000582.2	Osteopontin	SPP1

- 114 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
SPP1-614-13-13418	13418	NM_000582.2	Osteopontin	SPP1
SPP1-951-13-13420	13420	NM_000582.2	Osteopontin	SPP1
SPP1-482-13-13422	13422	NM_000582.2	Osteopontin	SPP1
SPP1-856-13-13424	13424	NM_000582.2	Osteopontin	SPP1
SPP1-857-13-13426	13426	NM_000582.2	Osteopontin	SPP1
SPP1-365-13-13428	13428	NM_000582.2	Osteopontin	SPP1
SPP1-359-13-13430	13430	NM_000582.2	Osteopontin	SPP1
SPP1-357-13-13432	13432	NM_000582.2	Osteopontin	SPP1
SPP1-858-13-13434	13434	NM_000582.2	Osteopontin	SPP1
SPP1-1012-13-13436	13436	NM_000582.2	Osteopontin	SPP1
SPP1-1014-13-13438	13438	NM_000582.2	Osteopontin	SPP1
SPP1-356-13-13440	13440	NM_000582.2	Osteopontin	SPP1
SPP1-368-13-13442	13442	NM_000582.2	Osteopontin	SPP1
SPP1-1011-13-13444	13444	NM_000582.2	Osteopontin	SPP1
SPP1-754-13-13446	13446	NM_000582.2	Osteopontin	SPP1
SPP1-1021-13-13448	13448	NM_000582.2	Osteopontin	SPP1
SPP1-1330-13-13450	13450	NM_000582.2	Osteopontin	SPP1
SPP1-346-13-13452	13452	NM_000582.2	Osteopontin	SPP1
SPP1-869-13-13454	13454	NM_000582.2	Osteopontin	SPP1
SPP1-701-13-13456	13456	NM_000582.2	Osteopontin	SPP1

- 115 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
SPP1-896-13-13458	13458	NM_000582.2	Osteopontin	SPP1
SPP1-1035-13-13460	13460	NM_000582.2	Osteopontin	SPP1
SPP1-1170-13-13462	13462	NM_000582.2	Osteopontin	SPP1
SPP1-1282-13-13464	13464	NM_000582.2	Osteopontin	SPP1
SPP1-1537-13-13466	13466	NM_000582.2	Osteopontin	SPP1
SPP1-692-13-13468	13468	NM_000582.2	Osteopontin	SPP1
SPP1-840-13-13470	13470	NM_000582.2	Osteopontin	SPP1
SPP1-1163-13-13472	13472	NM_000582.2	Osteopontin	SPP1
SPP1-789-13-13474	13474	NM_000582.2	Osteopontin	SPP1
SPP1-841-13-13476	13476	NM_000582.2	Osteopontin	SPP1
SPP1-852-13-13478	13478	NM_000582.2	Osteopontin	SPP1
SPP1-209-13-13480	13480	NM_000582.2	Osteopontin	SPP1
SPP1-1276-13-13482	13482	NM_000582.2	Osteopontin	SPP1
SPP1-137-13-13484	13484	NM_000582.2	Osteopontin	SPP1
SPP1-711-13-13486	13486	NM_000582.2	Osteopontin	SPP1
SPP1-582-13-13488	13488	NM_000582.2	Osteopontin	SPP1
SPP1-839-13-13490	13490	NM_000582.2	Osteopontin	SPP1
SPP1-1091-13-13492	13492	NM_000582.2	Osteopontin	SPP1
SPP1-884-13-13494	13494	NM_000582.2	Osteopontin	SPP1
SPP1-903-13-13496	13496	NM_000582.2	Osteopontin	SPP1

- 116 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
SPP1-1090-13-13498	13498	NM_000582.2	Osteopontin	SPP1
SPP1-474-13-13500	13500	NM_000582.2	Osteopontin	SPP1
SPP1-575-13-13502	13502	NM_000582.2	Osteopontin	SPP1
SPP1-671-13-13504	13504	NM_000582.2	Osteopontin	SPP1
SPP1-924-13-13506	13506	NM_000582.2	Osteopontin	SPP1
SPP1-1185-13-13508	13508	NM_000582.2	Osteopontin	SPP1
SPP1-1221-13-13510	13510	NM_000582.2	Osteopontin	SPP1
SPP1-347-13-13512	13512	NM_000582.2	Osteopontin	SPP1
SPP1-634-13-13514	13514	NM_000582.2	Osteopontin	SPP1
SPP1-877-13-13516	13516	NM_000582.2	Osteopontin	SPP1
SPP1-1033-13-13518	13518	NM_000582.2	Osteopontin	SPP1
SPP1-714-13-13520	13520	NM_000582.2	Osteopontin	SPP1
SPP1-791-13-13522	13522	NM_000582.2	Osteopontin	SPP1
SPP1-813-13-13524	13524	NM_000582.2	Osteopontin	SPP1
SPP1-939-13-13526	13526	NM_000582.2	Osteopontin	SPP1
SPP1-1161-13-13528	13528	NM_000582.2	Osteopontin	SPP1
SPP1-1164-13-13530	13530	NM_000582.2	Osteopontin	SPP1
SPP1-1190-13-13532	13532	NM_000582.2	Osteopontin	SPP1
SPP1-1333-13-13534	13534	NM_000582.2	Osteopontin	SPP1
SPP1-537-13-13536	13536	NM_000582.2	Osteopontin	SPP1

- 117 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
SPP1-684-13-13538	13538	NM_000582.2	Osteopontin	SPP1
SPP1-707-13-13540	13540	NM_000582.2	Osteopontin	SPP1
SPP1-799-13-13542	13542	NM_000582.2	Osteopontin	SPP1
SPP1-853-13-13544	13544	NM_000582.2	Osteopontin	SPP1
SPP1-888-13-13546	13546	NM_000582.2	Osteopontin	SPP1
SPP1-1194-13-13548	13548	NM_000582.2	Osteopontin	SPP1
SPP1-1279-13-13550	13550	NM_000582.2	Osteopontin	SPP1
SPP1-1300-13-13552	13552	NM_000582.2	Osteopontin	SPP1
SPP1-1510-13-13554	13554	NM_000582.2	Osteopontin	SPP1
SPP1-1543-13-13556	13556	NM_000582.2	Osteopontin	SPP1
SPP1-434-13-13558	13558	NM_000582.2	Osteopontin	SPP1
SPP1-600-13-13560	13560	NM_000582.2	Osteopontin	SPP1
SPP1-863-13-13562	13562	NM_000582.2	Osteopontin	SPP1
SPP1-902-13-13564	13564	NM_000582.2	Osteopontin	SPP1
SPP1-921-13-13566	13566	NM_000582.2	Osteopontin	SPP1
SPP1-154-13-13568	13568	NM_000582.2	Osteopontin	SPP1
SPP1-217-13-13570	13570	NM_000582.2	Osteopontin	SPP1
SPP1-816-13-13572	13572	NM_000582.2	Osteopontin	SPP1
SPP1-882-13-13574	13574	NM_000582.2	Osteopontin	SPP1
SPP1-932-13-13576	13576	NM_000582.2	Osteopontin	SPP1

- 118 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
SPP1-1509-13-13578	13578	NM_000582.2	Osteopontin	SPP1
SPP1-157-13-13580	13580	NM_000582.2	Osteopontin	SPP1
SPP1-350-13-13582	13582	NM_000582.2	Osteopontin	SPP1
SPP1-511-13-13584	13584	NM_000582.2	Osteopontin	SPP1
SPP1-605-13-13586	13586	NM_000582.2	Osteopontin	SPP1
SPP1-811-13-13588	13588	NM_000582.2	Osteopontin	SPP1
SPP1-892-13-13590	13590	NM_000582.2	Osteopontin	SPP1
SPP1-922-13-13592	13592	NM_000582.2	Osteopontin	SPP1
SPP1-1169-13-13594	13594	NM_000582.2	Osteopontin	SPP1
SPP1-1182-13-13596	13596	NM_000582.2	Osteopontin	SPP1
SPP1-1539-13-13598	13598	NM_000582.2	Osteopontin	SPP1
SPP1-1541-13-13600	13600	NM_000582.2	Osteopontin	SPP1
SPP1-427-13-13602	13602	NM_000582.2	Osteopontin	SPP1
SPP1-533-13-13604	13604	NM_000582.2	Osteopontin	SPP1
APOB--13-13763	13763	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB--13-13764	13764	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
MAP4K4--16-13766	13766			MAP4K4
PPIB--13-13767	13767	NM_000942	peptidylprolyl isomerase B (cyclophilin B)	PPIB
PPIB--15-13768	13768	NM_000942	peptidylprolyl isomerase B (cyclophilin B)	PPIB
PPIB--17-13769	13769	NM_000942	peptidylprolyl isomerase B (cyclophilin B)	PPIB

- 119 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
MAP4K4--16-13939	13939			MAP4K4
APOB-4314-16-13940	13940	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB-4314-17-13941	13941	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB--16-13942	13942	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB--18-13943	13943	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB--17-13944	13944	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB--19-13945	13945	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB-4314-16-13946	13946	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB-4314-17-13947	13947	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB--16-13948	13948	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB--17-13949	13949	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB--16-13950	13950	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB--18-13951	13951	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB--17-13952	13952	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
APOB--19-13953	13953	NM_000384	Apolipoprotein B (including Ag(x) antigen)	APOB
MAP4K4--16-13766.2	13766.2			MAP4K4
CTGF-1222-16-13980	13980	NM_001901.2	connective tissue growth factor	CTGF
CTGF-813-16-13981	13981	NM_001901.2	connective tissue growth factor	CTGF
CTGF-747-16-13982	13982	NM_001901.2	connective tissue growth factor	CTGF
CTGF-817-16-13983	13983	NM_001901.2	connective tissue growth factor	CTGF

- 120 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
CTGF-1174-16-13984	13984	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1005-16-13985	13985	NM_001901.2	connective tissue growth factor	CTGF
CTGF-814-16-13986	13986	NM_001901.2	connective tissue growth factor	CTGF
CTGF-816-16-13987	13987	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1001-16-13988	13988	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1173-16-13989	13989	NM_001901.2	connective tissue growth factor	CTGF
CTGF-749-16-13990	13990	NM_001901.2	connective tissue growth factor	CTGF
CTGF-792-16-13991	13991	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1162-16-13992	13992	NM_001901.2	connective tissue growth factor	CTGF
CTGF-811-16-13993	13993	NM_001901.2	connective tissue growth factor	CTGF
CTGF-797-16-13994	13994	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1175-16-13995	13995	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1172-16-13996	13996	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1177-16-13997	13997	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1176-16-13998	13998	NM_001901.2	connective tissue growth factor	CTGF
CTGF-812-16-13999	13999	NM_001901.2	connective tissue growth factor	CTGF
CTGF-745-16-14000	14000	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1230-16-14001	14001	NM_001901.2	connective tissue growth factor	CTGF
CTGF-920-16-14002	14002	NM_001901.2	connective tissue growth factor	CTGF
CTGF-679-16-14003	14003	NM_001901.2	connective tissue growth factor	CTGF

- 121 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
CTGF-992-16-14004	14004	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1045-16-14005	14005	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1231-16-14006	14006	NM_001901.2	connective tissue growth factor	CTGF
CTGF-991-16-14007	14007	NM_001901.2	connective tissue growth factor	CTGF
CTGF-998-16-14008	14008	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1049-16-14009	14009	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1044-16-14010	14010	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1327-16-14011	14011	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1196-16-14012	14012	NM_001901.2	connective tissue growth factor	CTGF
CTGF-562-16-14013	14013	NM_001901.2	connective tissue growth factor	CTGF
CTGF-752-16-14014	14014	NM_001901.2	connective tissue growth factor	CTGF
CTGF-994-16-14015	14015	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1040-16-14016	14016	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1984-16-14017	14017	NM_001901.2	connective tissue growth factor	CTGF
CTGF-2195-16-14018	14018	NM_001901.2	connective tissue growth factor	CTGF
CTGF-2043-16-14019	14019	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1892-16-14020	14020	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1567-16-14021	14021	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1780-16-14022	14022	NM_001901.2	connective tissue growth factor	CTGF
CTGF-2162-16-14023	14023	NM_001901.2	connective tissue growth factor	CTGF

- 122 --

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
CTGF-1034-16-14024	14024	NM_001901.2	connective tissue growth factor	CTGF
CTGF-2264-16-14025	14025	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1032-16-14026	14026	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1535-16-14027	14027	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1694-16-14028	14028	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1588-16-14029	14029	NM_001901.2	connective tissue growth factor	CTGF
CTGF-928-16-14030	14030	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1133-16-14031	14031	NM_001901.2	connective tissue growth factor	CTGF
CTGF-912-16-14032	14032	NM_001901.2	connective tissue growth factor	CTGF
CTGF-753-16-14033	14033	NM_001901.2	connective tissue growth factor	CTGF
CTGF-918-16-14034	14034	NM_001901.2	connective tissue growth factor	CTGF
CTGF-744-16-14035	14035	NM_001901.2	connective tissue growth factor	CTGF
CTGF-466-16-14036	14036	NM_001901.2	connective tissue growth factor	CTGF
CTGF-917-16-14037	14037	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1038-16-14038	14038	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1048-16-14039	14039	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1235-16-14040	14040	NM_001901.2	connective tissue growth factor	CTGF
CTGF-868-16-14041	14041	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1131-16-14042	14042	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1043-16-14043	14043	NM_001901.2	connective tissue growth factor	CTGF

- 123 --

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
CTGF-751-16-14044	14044	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1227-16-14045	14045	NM_001901.2	connective tissue growth factor	CTGF
CTGF-867-16-14046	14046	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1128-16-14047	14047	NM_001901.2	connective tissue growth factor	CTGF
CTGF-756-16-14048	14048	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1234-16-14049	14049	NM_001901.2	connective tissue growth factor	CTGF
CTGF-916-16-14050	14050	NM_001901.2	connective tissue growth factor	CTGF
CTGF-925-16-14051	14051	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1225-16-14052	14052	NM_001901.2	connective tissue growth factor	CTGF
CTGF-445-16-14053	14053	NM_001901.2	connective tissue growth factor	CTGF
CTGF-446-16-14054	14054	NM_001901.2	connective tissue growth factor	CTGF
CTGF-913-16-14055	14055	NM_001901.2	connective tissue growth factor	CTGF
CTGF-997-16-14056	14056	NM_001901.2	connective tissue growth factor	CTGF
CTGF-277-16-14057	14057	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1052-16-14058	14058	NM_001901.2	connective tissue growth factor	CTGF
CTGF-887-16-14059	14059	NM_001901.2	connective tissue growth factor	CTGF
CTGF-914-16-14060	14060	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1039-16-14061	14061	NM_001901.2	connective tissue growth factor	CTGF
CTGF-754-16-14062	14062	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1130-16-14063	14063	NM_001901.2	connective tissue growth factor	CTGF

- 124 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
CTGF-919-16-14064	14064	NM_001901.2	connective tissue growth factor	CTGF
CTGF-922-16-14065	14065	NM_001901.2	connective tissue growth factor	CTGF
CTGF-746-16-14066	14066	NM_001901.2	connective tissue growth factor	CTGF
CTGF-993-16-14067	14067	NM_001901.2	connective tissue growth factor	CTGF
CTGF-825-16-14068	14068	NM_001901.2	connective tissue growth factor	CTGF
CTGF-926-16-14069	14069	NM_001901.2	connective tissue growth factor	CTGF
CTGF-923-16-14070	14070	NM_001901.2	connective tissue growth factor	CTGF
CTGF-866-16-14071	14071	NM_001901.2	connective tissue growth factor	CTGF
CTGF-563-16-14072	14072	NM_001901.2	connective tissue growth factor	CTGF
CTGF-823-16-14073	14073	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1233-16-14074	14074	NM_001901.2	connective tissue growth factor	CTGF
CTGF-924-16-14075	14075	NM_001901.2	connective tissue growth factor	CTGF
CTGF-921-16-14076	14076	NM_001901.2	connective tissue growth factor	CTGF
CTGF-443-16-14077	14077	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1041-16-14078	14078	NM_001901.2	connective tissue growth factor	CTGF
CTGF-1042-16-14079	14079	NM_001901.2	connective tissue growth factor	CTGF
CTGF-755-16-14080	14080	NM_001901.2	connective tissue growth factor	CTGF
CTGF-467-16-14081	14081	NM_001901.2	connective tissue growth factor	CTGF
CTGF-995-16-14082	14082	NM_001901.2	connective tissue growth factor	CTGF
CTGF-927-16-14083	14083	NM_001901.2	connective tissue growth factor	CTGF

- 125 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
SPP1-1091-16-14131	14131	NM_000582.2	Osteopontin	SPP1
PPIB--16-14188	14188	NM_000942	peptidylprolyl isomerase B (cyclophilin B)	PPIB
PPIB--17-14189	14189	NM_000942	peptidylprolyl isomerase B (cyclophilin B)	PPIB
PPIB--18-14190	14190	NM_000942	peptidylprolyl isomerase B (cyclophilin B)	PPIB
pGL3-1172-16-14386	14386	U47296	Cloning vector pGL3-Control	pGL3
pGL3-1172-16-14387	14387	U47296	Cloning vector pGL3-Control	pGL3
MAP4K4-2931-25-14390	14390	NM_004834	Mitogen-Activated Protein Kinase Kinase Kinase 4 (MAP4K4), transcript variant 1	MAP4K4
miR-122--23-14391	14391			miR-122
	14084	NM_000582.2	Osteopontin	SPP1
	14085	NM_000582.2	Osteopontin	SPP1
	14086	NM_000582.2	Osteopontin	SPP1
	14087	NM_000582.2	Osteopontin	SPP1
	14088	NM_000582.2	Osteopontin	SPP1
	14089	NM_000582.2	Osteopontin	SPP1
	14090	NM_000582.2	Osteopontin	SPP1
	14091	NM_000582.2	Osteopontin	SPP1
	14092	NM_000582.2	Osteopontin	SPP1
	14093	NM_000582.2	Osteopontin	SPP1
	14094	NM_000582.2	Osteopontin	SPP1
	14095	NM_000582.2	Osteopontin	SPP1

- 126 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
	14096	NM_000582.2	Osteopontin	SPP1
	14097	NM_000582.2	Osteopontin	SPP1
	14098	NM_000582.2	Osteopontin	SPP1
	14099	NM_000582.2	Osteopontin	SPP1
	14100	NM_000582.2	Osteopontin	SPP1
	14101	NM_000582.2	Osteopontin	SPP1
	14102	NM_000582.2	Osteopontin	SPP1
	14103	NM_000582.2	Osteopontin	SPP1
	14104	NM_000582.2	Osteopontin	SPP1
	14105	NM_000582.2	Osteopontin	SPP1
	14106	NM_000582.2	Osteopontin	SPP1
	14107	NM_000582.2	Osteopontin	SPP1
	14108	NM_000582.2	Osteopontin	SPP1
	14109	NM_000582.2	Osteopontin	SPP1
	14110	NM_000582.2	Osteopontin	SPP1
	14111	NM_000582.2	Osteopontin	SPP1
	14112	NM_000582.2	Osteopontin	SPP1
	14113	NM_000582.2	Osteopontin	SPP1
	14114	NM_000582.2	Osteopontin	SPP1
	14115	NM_000582.2	Osteopontin	SPP1

- 127 --

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
	14116	NM_000582.2	Osteopontin	SPP1
	14117	NM_000582.2	Osteopontin	SPP1
	14118	NM_000582.2	Osteopontin	SPP1
	14119	NM_000582.2	Osteopontin	SPP1
	14120	NM_000582.2	Osteopontin	SPP1
	14121	NM_000582.2	Osteopontin	SPP1
	14122	NM_000582.2	Osteopontin	SPP1
	14123	NM_000582.2	Osteopontin	SPP1
	14124	NM_000582.2	Osteopontin	SPP1
	14125	NM_000582.2	Osteopontin	SPP1
	14126	NM_000582.2	Osteopontin	SPP1
	14127	NM_000582.2	Osteopontin	SPP1
	14128	NM_000582.2	Osteopontin	SPP1
	14129	NM_000582.2	Osteopontin	SPP1
	14130	NM_000582.2	Osteopontin	SPP1
	14132	NM_000582.2	Osteopontin	SPP1
	14133	NM_000582.2	Osteopontin	SPP1
	14134	NM_000582.2	Osteopontin	SPP1
	14135	NM_000582.2	Osteopontin	SPP1
	14136	NM_000582.2	Osteopontin	SPP1

- 128 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
	14137	NM_000582.2	Osteopontin	SPP1
	14138	NM_000582.2	Osteopontin	SPP1
	14139	NM_000582.2	Osteopontin	SPP1
	14140	NM_000582.2	Osteopontin	SPP1
	14141	NM_000582.2	Osteopontin	SPP1
	14142	NM_000582.2	Osteopontin	SPP1
	14143	NM_000582.2	Osteopontin	SPP1
	14144	NM_000582.2	Osteopontin	SPP1
	14145	NM_000582.2	Osteopontin	SPP1
	14146	NM_000582.2	Osteopontin	SPP1
	14147	NM_000582.2	Osteopontin	SPP1
	14148	NM_000582.2	Osteopontin	SPP1
	14149	NM_000582.2	Osteopontin	SPP1
	14150	NM_000582.2	Osteopontin	SPP1
	14151	NM_000582.2	Osteopontin	SPP1
	14152	NM_000582.2	Osteopontin	SPP1
	14153	NM_000582.2	Osteopontin	SPP1
	14154	NM_000582.2	Osteopontin	SPP1
	14155	NM_000582.2	Osteopontin	SPP1
	14156	NM_000582.2	Osteopontin	SPP1

- 129 -

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
	14157	NM_000582.2	Osteopontin	SPP1
	14158	NM_000582.2	Osteopontin	SPP1
	14159	NM_000582.2	Osteopontin	SPP1
	14160	NM_000582.2	Osteopontin	SPP1
	14161	NM_000582.2	Osteopontin	SPP1
	14162	NM_000582.2	Osteopontin	SPP1
	14163	NM_000582.2	Osteopontin	SPP1
	14164	NM_000582.2	Osteopontin	SPP1
	14165	NM_000582.2	Osteopontin	SPP1
	14166	NM_000582.2	Osteopontin	SPP1
	14167	NM_000582.2	Osteopontin	SPP1
	14168	NM_000582.2	Osteopontin	SPP1
	14169	NM_000582.2	Osteopontin	SPP1
	14170	NM_000582.2	Osteopontin	SPP1
	14171	NM_000582.2	Osteopontin	SPP1
	14172	NM_000582.2	Osteopontin	SPP1
	14173	NM_000582.2	Osteopontin	SPP1
	14174	NM_000582.2	Osteopontin	SPP1
	14175	NM_000582.2	Osteopontin	SPP1
	14176	NM_000582.2	Osteopontin	SPP1

- 130 --

ID Number	Oligo Number	Accession number	Gene Name	Gene Symbol
	14177	NM_000582.2	Osteopontin	SPP1
	14178	NM_000582.2	Osteopontin	SPP1
	14179	NM_000582.2	Osteopontin	SPP1
	14180	NM_000582.2	Osteopontin	SPP1
	14181	NM_000582.2	Osteopontin	SPP1
	14182	NM_000582.2	Osteopontin	SPP1
	14183	NM_000582.2	Osteopontin	SPP1
	14184	NM_000582.2	Osteopontin	SPP1
	14185	NM_000582.2	Osteopontin	SPP1
	14186	NM_000582.2	Osteopontin	SPP1
	14187	NM_000582.2	Osteopontin	SPP1

TABLE 1

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
APOB-10167-20-12138	12138	oooooooooooooooooooo ooo	0000000000000000 000000m	AUUGGUAUUCAGUGUGA UG	1
APOB-10167-20-12139	12139	oooooooooooooooooooo ooo	0000000000000000 000000m	AUUCGUAUUGAGUCUGA UC	2
MAP4K4-2931-13-12266	12266				
MAP4K4-2931-16-12293	12293	oooooooooooooooooooo ooo	Pf000fffff0f00 00fff0	UAGACUUCCACAGAACU CU	3
MAP4K4-2931-16-12383	12383	oooooooooooooooooooo ooo	0000000000000000 000000	UAGACUUCCACAGAACU CU	4
MAP4K4-2931-16-12384	12384	oooooooooooooooooooo ooo	P000000000000000 000000	UAGACUUCCACAGAACU CU	5
MAP4K4-2931-16-12385	12385	oooooooooooooooooooo ooo	Pf000fffff0f00 00fff0	UAGACUUCCACAGAACU CU	6
MAP4K4-2931-16-12386	12386	ooooooooooooossssss sso	Pf000fffff0f00 00fff0	UAGACUUCCACAGAACU CU	7
MAP4K4-2931-16-12387	12387	ooooooooooooossssss sso	P000000000000000 000000	UAGACUUCCACAGAACU CU	8
MAP4K4-2931-15-12388	12388	oooooooooooooooooooo o	0000000000000000 000	UAGACUUCCACAGAACU	9
MAP4K4-2931-13-12432	12432				
MAP4K4-2931-13-12266.2	12266.2				
APOB--21-12434	12434	oooooooooooooooooooo ooooo	0000000000000000 000000m	AUUGGUAUUCAGUGUGA UGAC	10
APOB--21-12435	12435	oooooooooooooooooooo ooooo	0000000000000000 000000m	AUUCGUAUUGAGUCUGA UCAC	11

- 132 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
MAP4K4-2931-16-12451	12451	oooooooooooooSSSSSSSSO	Pf000fffff0f0000ffmm	UAGACUCCACAGAACUCU	12
MAP4K4-2931-16-12452	12452	oooooooooooooSSSSSSSSO	Pm000fffff0f0000ffmm	UAGACUCCACAGAACUCU	13
MAP4K4-2931-16-12453	12453	ooooooooSSSSSSSSSSSSSSO	Pm000fffff0f0000ffmm	UAGACUCCACAGAACUCU	14
MAP4K4-2931-17-12454	12454	oooooooooooooSSSSSSSSSO	Pm000fffff0f0000ffffmm	UAGACUCCACAGAACUCUUC	15
MAP4K4-2931-17-12455	12455	oooooooooSSSSSSSSSSSSSO	Pm000fffff0f0000ffffmm	UAGACUCCACAGAACUCUUC	16
MAP4K4-2931-19-12456	12456	oooooooooooooSSSSSSSSSSSO	Pm000fffff0f0000fffff00mm	UAGACUCCACAGAACUCUCAAAG	17
--27-12480	12480				
--27-12481	12481				
APOB-10167-21-12505	12505	ooooooooooooooooooooo ooooo	000000000000000 000000m	AUUGGUAUUCAGUGUGAUGAC	18
APOB-10167-21-12506	12506	ooooooooooooooooooooo ooooo	000000000000000 000000m	AUUCGUAUUGAGUCUGAUCAC	19
MAP4K4-2931-16-12539	12539	oooooooooooooSSSSSSSSS	Pf000fffff0f0000fff0	UAGACUCCACAGAACUCU	20
APOB-10167-21-12505.2	12505.2	ooooooooooooooooooooo ooooo	000000000000000 000000m	AUUGGUAUUCAGUGUGAUGAC	21
APOB-10167-21-12506.2	12506.2	ooooooooooooooooooooo ooooo	000000000000000 000000m	AUUCGUAUUGAGUCUGAUCAC	22
MAP4K4--13-12565	12565				

- 133 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
MAP4K4-2931-16-12386.2	12386.2	ooooooooooooSSSSSS	Pf000fffff0f0000fff0	UAGACUCCACAGAACU CU	23
MAP4K4-2931-13-12815	12815				
APOB--13-12957	12957				
MAP4K4--16-12983	12983	ooooooooooooSSSSSS	Pm000fffff0m0000mmm0	uagacuuccacagaacu cu	24
MAP4K4--16-12984	12984	ooooooooooooSSSSSS	Pm000fffff0m0000mmm0	uagacuuccacagaacu cu	25
MAP4K4--16-12985	12985	ooooooooooooSSSSSS	Pm000fffff0m0000mmm0	uagacuuccacagaacu cu	26
MAP4K4--16-12986	12986	ooooooooooooSSSSSS	Pf000fffff0f0000fff0	UAGACUCCACAGAACU CU	27
MAP4K4--16-12987	12987	ooooooooooooSSSSSS	P0000f00ff0m0000m0m0	UagacUuccacagaacU cU	28
MAP4K4--16-12988	12988	ooooooooooooSSSSSS	P0000f00ff0m0000m0m0	UagacUuccacagaacU cu	29
MAP4K4--16-12989	12989	ooooooooooooSSSSSS	P0000ff0ff0m0000m0m0	UagacuUccacagaacU cu	30
MAP4K4--16-12990	12990	ooooooooooooSSSSSS	Pf0000ff0000000000m00	uagaCuuCCaCagaaCu Cu	31
MAP4K4--16-12991	12991	ooooooooooooSSSSSS	Pf0000fff00m00000mm0	uagaCuucCacagaaCu cu	32
MAP4K4--16-12992	12992	ooooooooooooSSSSSS	Pf000fffff0000000m00	uagacuuccaCagaaCu Cu	33
MAP4K4--16-12993	12993	ooooooooooooSSSSSS	P00000000000000000000	UagaCUUCCaCagaaCU CU	34

- 134 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
MAP4K4-- 16-12994	12994	ooooooooooooooooosss sss	P0000f0f0f0000 000m00	UagacUuCcaCagaaCu Cu	35
MAP4K4-- 16-12995	12995	ooooooooooooooooossss sso	Pf000fffff0000 000000	uagacuuccaCagaaCU CU	36
MAP4K4- 2931-19- 13012	13012				
MAP4K4- 2931-19- 13016	13016				
PPIB-- 13-13021	13021				
pGL3- 1172-13- 13038	13038				
pGL3- 1172-13- 13040	13040				
--16- 13047	13047	ooooooooooooooooossss ss	Pm000000000m00 00mmm0	UAGACUUCCACAGAACU CU	37
SOD1- 530-13- 13090	13090				
SOD1- 523-13- 13091	13091				
SOD1- 535-13- 13092	13092				
SOD1- 536-13- 13093	13093				
SOD1- 396-13- 13094	13094				
SOD1- 385-13- 13095	13095				

- 135 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
SOD1-195-13-13096	13096				
APOB-4314-13-13115	13115				
APOB-3384-13-13116	13116				
APOB-3547-13-13117	13117				
APOB-4318-13-13118	13118				
APOB-3741-13-13119	13119				
PPIB--16-13136	13136	oooooooooooooooossss	Pm0fffff0f00mm000mm0	UGUUUUUGUAGCCAAUCC	38
APOB-4314-15-13154	13154				
APOB-3547-15-13155	13155				
APOB-4318-15-13157	13157				
APOB-3741-15-13158	13158				
APOB--13-13159	13159				
APOB--15-13160	13160				
SOD1-530-16-13163	13163	oooooooooooooooossso	Pm0fffffffff0mmmmm0m0	UACUUUCUUCAUUCCACC	39

- 136 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
SOD1-523-16-13164	13164	ooooooooooooSSSSSSO	Pmfff0fffff0fmmmm0mm0	UUCAUUUCCACCUUUGCC	40
SOD1-535-16-13165	13165	ooooooooooooSSSSSSO	Pmfff0f0fffffmmmm0mm0	CUUUGUACUUUCUUCAUU	41
SOD1-536-16-13166	13166	ooooooooooooSSSSSSO	Pmfff0f0fffmmmmmm0m0	UCUUUGUACUUUCUUCAUU	42
SOD1-396-16-13167	13167	ooooooooooooSSSSSSO	Pmf00f00ff0f0mm0mmm0	UCAGCAGUCACAUUGCCCA	43
SOD1-385-16-13168	13168	ooooooooooooSSSSSSO	Pmfff0fff000fmmmm00m0	AUUGCCCAAGUCUCCACA	44
SOD1-195-16-13169	13169	ooooooooooooSSSSSSO	Pmfff0fff0000mm00m00	UUCUGCUCGAAAUUGAUGA	45
pGL3-1172-16-13170	13170	ooooooooooooSSSSSSO	Pm00ff0f0ffm0ff00mm0	AAAUCGUAAUUGUCAAUCA	46
pGL3-1172-16-13171	13171	ooooooooooooSSSSSSS	Pm00ff0f0ffm0ff00mm0	AAAUCGUAAUUGUCAAUCA	47
MAP4k4-2931-19-13189	13189	ooooooooooooooooooooooo	00000000000000000000	UAGACUCCACAGAACUCU	48
CTGF-1222-13-13190	13190				
CTGF-813-13-13192	13192				
CTGF-747-13-13194	13194				
CTGF-817-13-13196	13196				
CTGF-1174-13-13198	13198				

- 137 --

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
CTGF-1005-13-13200	13200				
CTGF-814-13-13202	13202				
CTGF-816-13-13204	13204				
CTGF-1001-13-13206	13206				
CTGF-1173-13-13208	13208				
CTGF-749-13-13210	13210				
CTGF-792-13-13212	13212				
CTGF-1162-13-13214	13214				
CTGF-811-13-13216	13216				
CTGF-797-13-13218	13218				
CTGF-1175-13-13220	13220				
CTGF-1172-13-13222	13222				
CTGF-1177-13-13224	13224				
CTGF-1176-13-13226	13226				

- 138 --

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
CTGF-812-13-13228	13228				
CTGF-745-13-13230	13230				
CTGF-1230-13-13232	13232				
CTGF-920-13-13234	13234				
CTGF-679-13-13236	13236				
CTGF-992-13-13238	13238				
CTGF-1045-13-13240	13240				
CTGF-1231-13-13242	13242				
CTGF-991-13-13244	13244				
CTGF-998-13-13246	13246				
CTGF-1049-13-13248	13248				
CTGF-1044-13-13250	13250				
CTGF-1327-13-13252	13252				
CTGF-1196-13-13254	13254				

- 139 --

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
CTGF-562-13-13256	13256				
CTGF-752-13-13258	13258				
CTGF-994-13-13260	13260				
CTGF-1040-13-13262	13262				
CTGF-1984-13-13264	13264				
CTGF-2195-13-13266	13266				
CTGF-2043-13-13268	13268				
CTGF-1892-13-13270	13270				
CTGF-1567-13-13272	13272				
CTGF-1780-13-13274	13274				
CTGF-2162-13-13276	13276				
CTGF-1034-13-13278	13278				
CTGF-2264-13-13280	13280				
CTGF-1032-13-13282	13282				

- 140 --

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
CTGF-1535-13-13284	13284				
CTGF-1694-13-13286	13286				
CTGF-1588-13-13288	13288				
CTGF-928-13-13290	13290				
CTGF-1133-13-13292	13292				
CTGF-912-13-13294	13294				
CTGF-753-13-13296	13296				
CTGF-918-13-13298	13298				
CTGF-744-13-13300	13300				
CTGF-466-13-13302	13302				
CTGF-917-13-13304	13304				
CTGF-1038-13-13306	13306				
CTGF-1048-13-13308	13308				
CTGF-1235-13-13310	13310				

- 141 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
CTGF-868-13-13312	13312				
CTGF-1131-13-13314	13314				
CTGF-1043-13-13316	13316				
CTGF-751-13-13318	13318				
CTGF-1227-13-13320	13320				
CTGF-867-13-13322	13322				
CTGF-1128-13-13324	13324				
CTGF-756-13-13326	13326				
CTGF-1234-13-13328	13328				
CTGF-916-13-13330	13330				
CTGF-925-13-13332	13332				
CTGF-1225-13-13334	13334				
CTGF-445-13-13336	13336				
CTGF-446-13-13338	13338				

- 142 --

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
CTGF-913-13-13340	13340				
CTGF-997-13-13342	13342				
CTGF-277-13-13344	13344				
CTGF-1052-13-13346	13346				
CTGF-887-13-13348	13348				
CTGF-914-13-13350	13350				
CTGF-1039-13-13352	13352				
CTGF-754-13-13354	13354				
CTGF-1130-13-13356	13356				
CTGF-919-13-13358	13358				
CTGF-922-13-13360	13360				
CTGF-746-13-13362	13362				
CTGF-993-13-13364	13364				
CTGF-825-13-13366	13366				

- 143 --

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
CTGF-926-13-13368	13368				
CTGF-923-13-13370	13370				
CTGF-866-13-13372	13372				
CTGF-563-13-13374	13374				
CTGF-823-13-13376	13376				
CTGF-1233-13-13378	13378				
CTGF-924-13-13380	13380				
CTGF-921-13-13382	13382				
CTGF-443-13-13384	13384				
CTGF-1041-13-13386	13386				
CTGF-1042-13-13388	13388				
CTGF-755-13-13390	13390				
CTGF-467-13-13392	13392				
CTGF-995-13-13394	13394				

- 144 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
CTGF-927-13-13396	13396				
SPP1-1025-13-13398	13398				
SPP1-1049-13-13400	13400				
SPP1-1051-13-13402	13402				
SPP1-1048-13-13404	13404				
SPP1-1050-13-13406	13406				
SPP1-1047-13-13408	13408				
SPP1-800-13-13410	13410				
SPP1-492-13-13412	13412				
SPP1-612-13-13414	13414				
SPP1-481-13-13416	13416				
SPP1-614-13-13418	13418				
SPP1-951-13-13420	13420				
SPP1-482-13-13422	13422				

- 145 --

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
SPP1-856-13-13424	13424				
SPP1-857-13-13426	13426				
SPP1-365-13-13428	13428				
SPP1-359-13-13430	13430				
SPP1-357-13-13432	13432				
SPP1-858-13-13434	13434				
SPP1-1012-13-13436	13436				
SPP1-1014-13-13438	13438				
SPP1-356-13-13440	13440				
SPP1-368-13-13442	13442				
SPP1-1011-13-13444	13444				
SPP1-754-13-13446	13446				
SPP1-1021-13-13448	13448				
SPP1-1330-13-13450	13450				

- 146 --

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
SPP1-346-13-13452	13452				
SPP1-869-13-13454	13454				
SPP1-701-13-13456	13456				
SPP1-896-13-13458	13458				
SPP1-1035-13-13460	13460				
SPP1-1170-13-13462	13462				
SPP1-1282-13-13464	13464				
SPP1-1537-13-13466	13466				
SPP1-692-13-13468	13468				
SPP1-840-13-13470	13470				
SPP1-1163-13-13472	13472				
SPP1-789-13-13474	13474				
SPP1-841-13-13476	13476				
SPP1-852-13-13478	13478				

- 147 --

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
SPP1- 209-13- 13480	13480				
SPP1- 1276-13- 13482	13482				
SPP1- 137-13- 13484	13484				
SPP1- 711-13- 13486	13486				
SPP1- 582-13- 13488	13488				
SPP1- 839-13- 13490	13490				
SPP1- 1091-13- 13492	13492				
SPP1- 884-13- 13494	13494				
SPP1- 903-13- 13496	13496				
SPP1- 1090-13- 13498	13498				
SPP1- 474-13- 13500	13500				
SPP1- 575-13- 13502	13502				
SPP1- 671-13- 13504	13504				
SPP1- 924-13- 13506	13506				

- 148 --

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
SPP1-1185-13-13508	13508				
SPP1-1221-13-13510	13510				
SPP1-347-13-13512	13512				
SPP1-634-13-13514	13514				
SPP1-877-13-13516	13516				
SPP1-1033-13-13518	13518				
SPP1-714-13-13520	13520				
SPP1-791-13-13522	13522				
SPP1-813-13-13524	13524				
SPP1-939-13-13526	13526				
SPP1-1161-13-13528	13528				
SPP1-1164-13-13530	13530				
SPP1-1190-13-13532	13532				
SPP1-1333-13-13534	13534				

- 149 --

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
SPP1- 537-13- 13536	13536				
SPP1- 684-13- 13538	13538				
SPP1- 707-13- 13540	13540				
SPP1- 799-13- 13542	13542				
SPP1- 853-13- 13544	13544				
SPP1- 888-13- 13546	13546				
SPP1- 1194-13- 13548	13548				
SPP1- 1279-13- 13550	13550				
SPP1- 1300-13- 13552	13552				
SPP1- 1510-13- 13554	13554				
SPP1- 1543-13- 13556	13556				
SPP1- 434-13- 13558	13558				
SPP1- 600-13- 13560	13560				
SPP1- 863-13- 13562	13562				

- 150 --

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
SPP1-902-13-13564	13564				
SPP1-921-13-13566	13566				
SPP1-154-13-13568	13568				
SPP1-217-13-13570	13570				
SPP1-816-13-13572	13572				
SPP1-882-13-13574	13574				
SPP1-932-13-13576	13576				
SPP1-1509-13-13578	13578				
SPP1-157-13-13580	13580				
SPP1-350-13-13582	13582				
SPP1-511-13-13584	13584				
SPP1-605-13-13586	13586				
SPP1-811-13-13588	13588				
SPP1-892-13-13590	13590				

- 151 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
SPP1-922-13-13592	13592				
SPP1-1169-13-13594	13594				
SPP1-1182-13-13596	13596				
SPP1-1539-13-13598	13598				
SPP1-1541-13-13600	13600				
SPP1-427-13-13602	13602				
SPP1-533-13-13604	13604				
APOB--13-13763	13763				
APOB--13-13764	13764				
MAP4K4--16-13766	13766	ooooooooooooossss sso	Pm000fffff0m00 00mmm0	UAGACUCCACAGAACU CU	49
PPIB--13-13767	13767				
PPIB--15-13768	13768				
PPIB--17-13769	13769				
MAP4K4--16-13939	13939	ooooooooooooooooossss sso	m000f0fffff0m0m 00m0m	UAGACAUCCUACACAGC AC	50

- 152 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
APOB-4314-16-13940	13940	ooooooooooooSSSSSSO	Pm0ffffff000mmmm00	UGUUUCUCCAGAUCCUUGC	51
APOB-4314-17-13941	13941	ooooooooooooSSSSSSO	Pm0ffffff000mmmm00	UGUUUCUCCAGAUCCUUGC	52
APOB--16-13942	13942	ooooooooooooSSSSSSO	Pm00f000f000mm0mmm0	UAGCAGAUAGAUCCAUG	53
APOB--18-13943	13943	ooooooooooooooooSSSSSSO	Pm00f000f000mm0mmm00000	UAGCAGAUAGAUCCAUGGAGA	54
APOB--17-13944	13944	ooooooooooooSSSSSSO	Pm00f000f000mm0mmm0	UAGCAGAUAGAUCCAUG	55
APOB--19-13945	13945	ooooooooooooooooSSSSSSO	Pm00f000f000mm0mmm00000	UAGCAGAUAGAUCCAUGGAGA	56
APOB-4314-16-13946	13946	ooooooooooooSSSSSSO	Pmf0ff0ffffmmm000mm0	AUGUUGUUUCUCCAGAUCC	57
APOB-4314-17-13947	13947	ooooooooooooSSSSSSO	Pmf0ff0ffffmmm000mm0	AUGUUGUUUCUCCAGAUCC	58
APOB--16-13948	13948	ooooooooooooSSSSSSO	Pm0fff000000mmmm0m00	UGUUUGAGGGACUCUGUGA	59
APOB--17-13949	13949	ooooooooooooSSSSSSO	Pm0fff000000mmmm0m00	UGUUUGAGGGACUCUGUGA	60
APOB--16-13950	13950	ooooooooooooSSSSSSO	Pmff00f0fff00m0m00m0	AUUGGUAUUCAGUGUGAUG	61
APOB--18-13951	13951	ooooooooooooooooSSSSSSO	Pmff00f0fff00m0m0m0m00	AUUGGUAUUCAGUGUGAUGACAC	62
APOB--17-13952	13952	ooooooooooooSSSSSSO	Pmff00f0fff00m0m00m0	AUUGGUAUUCAGUGUGAUG	63
APOB--19-13953	13953	ooooooooooooooooSSSSSSO	Pmff00f0fff00m0m0m0m00	AUUGGUAUUCAGUGUGAUGACAC	64

- 153 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
MAP4K4--16-13766.2	13766.2	ooooooooooooSSSSSSO	Pm000fffff0m0000mm0	UAGACUCCACAGAACUCU	65
CTGF-1222-16-13980	13980	ooooooooooooSSSSSSO	Pm0f0fffff0m000m0	UACAUCUCCUGUAGUACA	66
CTGF-813-16-13981	13981	ooooooooooooSSSSSSO	Pm0f0ffff0mmm0m000	AGGCGCUCCACUCUGUGU	67
CTGF-747-16-13982	13982	ooooooooooooSSSSSSO	Pm0fffff00mm0m0000	UGUCUCCAGUCGGUAA GC	68
CTGF-817-16-13983	13983	ooooooooooooSSSSSSO	Pm00f000f0fmm00mmm0	GAACAGGCGCUCCACUCUG	69
CTGF-1174-16-13984	13984	ooooooooooooSSSSSSO	Pm00ff0f00f00m000m0	CAGUUGUAAUGGCAGGCAC	70
CTGF-1005-16-13985	13985	ooooooooooooSSSSSSO	Pmff000000mmm000mm0	AGCCAGAAAGCUCAAACUU	71
CTGF-814-16-13986	13986	ooooooooooooSSSSSSO	Pm000f0ffff0mmmm0m0	CAGGCGCUCCACUCUGUGG	72
CTGF-816-16-13987	13987	ooooooooooooSSSSSSO	Pm0f000f0ffmm0mmmm0	AACAGGCGCUCCACUCUGU	73
CTGF-1001-16-13988	13988	ooooooooooooSSSSSSO	Pm0000fff000mm00m0	AGAAAGCUCAAACUUGAU	74
CTGF-1173-16-13989	13989	ooooooooooooSSSSSSO	Pmff0f00f00m000m0m0	AGUUGUAAUGGCAGGCA CA	75
CTGF-749-16-13990	13990	ooooooooooooSSSSSSO	Pmf0fffff00mm00m0	CGUGUCUCCAGUCGGUAA	76
CTGF-792-16-13991	13991	ooooooooooooSSSSSSO	Pm00ff000f00mm00mmm0	GGACCAGGCAGUUGGCU CU	77
CTGF-1162-16-13992	13992	ooooooooooooSSSSSSO	Pm000f0f000mmm00m0	CAGGCACAGGUCUUGAU GA	78

- 154 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
CTGF-811-16-13993	13993	ooooooooooooSSSS SSO	Pmf0ffff0ffmm0 m00mm0	GCGCUCCACUCUGUGGU CU	79
CTGF-797-16-13994	13994	ooooooooooooSSSS SSO	Pm0fff000ff000 m00mm0	GGUCUGGACCAGGCAGU UG	80
CTGF-1175-16-13995	13995	ooooooooooooSSSS SSO	Pmf00ff0f00m00 m000m0	ACAGUUGUAAUGGCAGG CA	81
CTGF-1172-16-13996	13996	ooooooooooooSSSS SSO	Pmff0f00f00m00 0m0m00	GUUGUAAUGGCAGGCAC AG	82
CTGF-1177-16-13997	13997	ooooooooooooSSSS SSO	Pm00f00ff0f00m 00m000	GGACAGUUGUAAUGGCA GG	83
CTGF-1176-16-13998	13998	ooooooooooooSSSS SSO	Pm0f00ff0f00m0 0m0000	GACAGUUGUAAUGGCAG GC	84
CTGF-812-16-13999	13999	ooooooooooooSSSS SSO	Pm0f0ffff0fmmm 0m00m0	GGCGCUCCACUCUGUGG UC	85
CTGF-745-16-14000	14000	ooooooooooooSSSS SSO	Pmffffff00ff00m 000mm0	UCUCCAGUCGGUAAGC CG	86
CTGF-1230-16-14001	14001	ooooooooooooSSSS SSO	Pm0ffffff0f0m0m mmmmm0	UGUCUCCGUACAUCUUC CU	87
CTGF-920-16-14002	14002	ooooooooooooSSSS SSO	Pmffff0f0000mm m00m0	AGCUUCGCAAGGCCUGA CC	88
CTGF-679-16-14003	14003	ooooooooooooSSSS SSO	Pm0ffffff0f00m 0mmmm0	CACUCCUCGCAGCAUUU CC	89
CTGF-992-16-14004	14004	ooooooooooooSSSS SSO	Pm00fff00f000m mm0000	AAACUUGAUAGGCUUGG AG	90
CTGF-1045-16-14005	14005	ooooooooooooSSSS SSO	Pmffff0f0000mm m00mm0	ACUCCACAGAAUUUAGC UC	91
CTGF-1231-16-14006	14006	ooooooooooooSSSS SSO	Pmf0ffffff0f0m0 mmmmm0	AUGUCUCCGUACAUCUU CC	92

- 155 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
CTGF-991-16-14007	14007	ooooooooooooooooSSSSSSO	Pm0ffff00f000mm000000	AACUUGAUAGGCUUGGAGA	93
CTGF-998-16-14008	14008	ooooooooooooooooSSSSSSO	Pm00ffff000fmm00m00000	AAGCUCAAACUUGAUAGGC	94
CTGF-1049-16-14009	14009	ooooooooooooooooSSSSSSO	Pmf0f0ffff0m0000mm00	ACAUACUCCACAGAAUUA	95
CTGF-1044-16-14010	14010	ooooooooooooooooSSSSSSO	Pmfff0f0000mmm00mm00	CUCCACAGAAUUUAGCUCG	96
CTGF-1327-16-14011	14011	ooooooooooooooooSSSSSSO	Pm0f0ff0ff0000mm0mm0	UGUGCUACUGAAAUCAUUU	97
CTGF-1196-16-14012	14012	ooooooooooooooooSSSSSSO	Pm0000f0ff0mm0mmmm00	AAAGAUGUCAUUGUCUCG	98
CTGF-562-16-14013	14013	ooooooooooooooooSSSSSSO	Pmf0f0ff00f0mm0m0m000	GUGCACUGGUACUUGCAGC	99
CTGF-752-16-14014	14014	ooooooooooooooooSSSSSSO	Pm00f0f0fffmmm00mm00	AAACGUGUCUCCAGUCGG	100
CTGF-994-16-14015	14015	ooooooooooooooooSSSSSSO	Pmf000fff00m000mmmm00	UCAAACUUGAUAGGCUUGG	101
CTGF-1040-16-14016	14016	ooooooooooooooooSSSSSSO	Pmf0000fff00mm00m0m00	ACAGAAUUUAGCUCGGUAU	102
CTGF-1984-16-14017	14017	ooooooooooooooooSSSSSSO	Pmf0f0ffff0mmm0m00m0	UUACAUUCUACCUAUGGUG	103
CTGF-2195-16-14018	14018	ooooooooooooooooSSSSSSO	Pm00ff00ff00mm0m0m00	AAACUGAUCAGCUAUUAUAG	104
CTGF-2043-16-14019	14019	ooooooooooooooooSSSSSSO	Pm0fff000f0000mmmm00	UAUCUGAGCAGAAUUUCAC	105
CTGF-1892-16-14020	14020	ooooooooooooooooSSSSSSO	Pmf00fff000m00mm0m00	UUAACUUAGUAACUGUAC	106

- 156 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
CTGF-1567-16-14021	14021	ooooooooooooSSSS	PmOff0fff0f0m000m00	UAUUACUCGUUAAGAUGC	107
CTGF-1780-16-14022	14022	ooooooooooooSSSS	Pm00ff0fff00mm00mm0	AAGCUGUCCAGUCUAAUCG	108
CTGF-2162-16-14023	14023	ooooooooooooSSSS	Pm00f00000fm0mm0mm0	UAAUAAAGGCCAUUUGUC	109
CTGF-1034-16-14024	14024	ooooooooooooSSSS	Pmff00fff00m0m0mmmm0	UUUAGCUCGGUAUGUCUCU	110
CTGF-2264-16-14025	14025	ooooooooooooSSSS	Pmf0fffff00m000m0000	ACACUCUCAACAAAUAAAC	111
CTGF-1032-16-14026	14026	ooooooooooooSSSS	Pm00fff00f0m0mmmm00	UAGCUCGGUAUGUCUUCAU	112
CTGF-1535-16-14027	14027	ooooooooooooSSSS	Pm00fffffff0mm00m0m0	UAACCUUUCUGCUGGUACC	113
CTGF-1694-16-14028	14028	ooooooooooooSSSS	Pmf000000f00mm00mm0	UUAAGGAACAACUUGACUC	114
CTGF-1588-16-14029	14029	ooooooooooooSSSS	Pmf0f0ffff000m00m000	UUACACUCAAUAGCAGG	115
CTGF-928-16-14030	14030	ooooooooooooSSSS	Pmff000ff00mmm0m0000	UCCAGGUCAGCUUCGCAAG	116
CTGF-1133-16-14031	14031	ooooooooooooSSSS	Pmfffff0f00mmmm0mm0	CUUCUUAUGACCUCGCGC	117
CTGF-912-16-14032	14032	ooooooooooooSSSS	Pm000fff00fm0m0m0m00	AAGGCCUGACCAUGCACAG	118
CTGF-753-16-14033	14033	ooooooooooooSSSS	Pm000f0f0ffmmmm00mm0	CAAACGUGUCUCCAGUCG	119
CTGF-918-16-14034	14034	ooooooooooooSSSS	Pmfff0f0000mmmm00mm00	CUUCGCAAGGCCUGACCAU	120

- 157 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
CTGF-744-16-14035	14035	ooooooooooooSSSS SSO	Pmffff00ff00m0 00mm00	CUUCCAGUCGGUAAGCC GC	121
CTGF-466-16-14036	14036	ooooooooooooSSSS SSO	Pmf00ffff0f00m m00mm0	CCGAUCUUGCGGUUGGC CG	122
CTGF-917-16-14037	14037	ooooooooooooSSSS SSO	Pmff0f0000fmm0 0mm0m0	UUCGCAAGGCCUGACCA UG	123
CTGF-1038-16-14038	14038	ooooooooooooSSSS SSO	Pm00fff00fmm0m 0m00	AGAAUUUAGCUCGGUAU GU	124
CTGF-1048-16-14039	14039	ooooooooooooSSSS SSO	Pm0f0ffff0f000 0mmm00	CAUACUCCACAGAAUUU AG	125
CTGF-1235-16-14040	14040	ooooooooooooSSSS SSO	Pm0ff0f0fffmmm 0m0m0	UGCCAUGUCUCCGUACA UC	126
CTGF-868-16-14041	14041	ooooooooooooSSSS SSO	Pm000f0ff0fm0m m00m00	GAGGCGUUGUCAUUGGU AA	127
CTGF-1131-16-14042	14042	ooooooooooooSSSS SSO	Pmffff0f00fmmm 0mm0m0	UCUUCAUGACCUCGCCG UC	128
CTGF-1043-16-14043	14043	ooooooooooooSSSS SSO	Pmff0f0000fmm0 0mmm00	UCCACAGAAUUUAGCUC GG	129
CTGF-751-16-14044	14044	ooooooooooooSSSS SSO	Pm0f0f0ffffmm0 0mm000	AACGUGUCUCCAGUCG GU	130
CTGF-1227-16-14045	14045	ooooooooooooSSSS SSO	Pmfff0f0f0fmmm mmm0m0	CUCCGUACAUCUCCUG UA	131
CTGF-867-16-14046	14046	ooooooooooooSSSS SSO	Pm0f0ff0ff0mm0 0m000	AGGCGUUGUCAUUGGUA AC	132
CTGF-1128-16-14047	14047	ooooooooooooSSSS SSO	Pmf0f00ffff0mm 0mm000	UCAUGACCUCGCCGUCA GG	133
CTGF-756-16-14048	14048	ooooooooooooSSSS SSO	Pm0ff000f0f0mm mmmm00	GGCCAAACGUGUCUCC AG	134

- 158 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
CTGF-1234-16-14049	14049	ooooooooooooooooSSSSSSO	Pmf0f0f0ffffmm0m0mm0	GCCAUGUCUCCGUACAU CU	135
CTGF-916-16-14050	14050	ooooooooooooooooSSSSSSO	Pmf0f0000ffm00mm0m00	UCGCAAGGCCUGACCAU GC	136
CTGF-925-16-14051	14051	ooooooooooooooooSSSSSSO	Pm0ff00ffffmm000m0	AGGUCAGCUUCGCAAGG CC	137
CTGF-1225-16-14052	14052	ooooooooooooooooSSSSSSO	Pmf0f0f0ffffmmm0m000	CCGUACAUCUCCUGUA GU	138
CTGF-445-16-14053	14053	ooooooooooooooooSSSSSSO	Pm00ff0000fm0m000000	GAGCCGAAGUCACAGAA GA	139
CTGF-446-16-14054	14054	ooooooooooooooooSSSSSSO	Pm000ff0000mm0m00000	GGAGCCGAAGUCACAGA AG	140
CTGF-913-16-14055	14055	ooooooooooooooooSSSSSSO	Pm0000fff00mm0m0m0m0	CAAGGCCUGACCAUGCA CA	141
CTGF-997-16-14056	14056	ooooooooooooooooSSSSSSO	Pmfff000ffm00m000m0	AGCUCAAACUUGAUAGG CU	142
CTGF-277-16-14057	14057	ooooooooooooooooSSSSSSO	Pmf0f00ffff00m000m00	CUGCAGUUCUGGCCGAC GG	143
CTGF-1052-16-14058	14058	ooooooooooooooooSSSSSSO	Pm0f0f0f0ffmm0m00000	GGUACAUAUCCACAGA AU	144
CTGF-887-16-14059	14059	ooooooooooooooooSSSSSSO	Pmf0ffffff00mm0m00	CUGCUCUCUAGCCUGC AG	145
CTGF-914-16-14060	14060	ooooooooooooooooSSSSSSO	Pmf0000fff00mm0m0m00	GCAAGGCCUGACCAUGC AC	146
CTGF-1039-16-14061	14061	ooooooooooooooooSSSSSSO	Pm0000fff00mmm00m0m0	CAGAAUUUAGCUCGGUA UG	147
CTGF-754-16-14062	14062	ooooooooooooooooSSSSSSO	Pmf000f0f0fmmm0m00m0	CCAAACGUGUCUCCAG UC	148

- 159 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
CTGF-1130-16-14063	14063	ooooooooooooSSSS SSO	Pmfff0f00ffmmm m0mm0	CUUCAUGACCUCGCCGU CA	149
CTGF-919-16-14064	14064	ooooooooooooSSSS SSO	Pmffff0f0000mm m00mm0	GCUUCGCAAGGCCUGAC CA	150
CTGF-922-16-14065	14065	ooooooooooooSSSS SSO	Pmf00ffff0f000 Ommm00	UCAGCUUCGCAAGGCCU GA	151
CTGF-746-16-14066	14066	ooooooooooooSSSS SSO	Pmffffff00fm0m 000m0	GUCUCCAGUCGGUAAG CC	152
CTGF-993-16-14067	14067	ooooooooooooSSSS SSO	Pm000fff00f000 mmm000	CAAACUUGAUAGGCUUG GA	153
CTGF-825-16-14068	14068	ooooooooooooSSSS SSO	Pm0ffff0000m00 0m0m0	AGGUCUUGGAACAGGCG CU	154
CTGF-926-16-14069	14069	ooooooooooooSSSS SSO	Pm000ff00ffmmm 00000	CAGGUCAGCUUCGCAAG GC	155
CTGF-923-16-14070	14070	ooooooooooooSSSS SSO	Pmff00ffff0m00 00mmm0	GUCAGCUUCGCAAGGCC UG	156
CTGF-866-16-14071	14071	ooooooooooooSSSS SSO	Pm0f0ff0ff0mm0 0m00m0	GGCGUUGUCAUUGGUAA CC	157
CTGF-563-16-14072	14072	ooooooooooooSSSS SSO	Pmf0f0ff00m0mm m0m00	CGUGCACUGGUACUUGC AG	158
CTGF-823-16-14073	14073	ooooooooooooSSSS SSO	Pmffff0000f000 m0mmm0	GUCUUGGAACAGGCGCU CC	159
CTGF-1233-16-14074	14074	ooooooooooooSSSS SSO	Pmf0f0ffff0m0 m0mmm0	CCAUGUCUCCGUACAUC UU	160
CTGF-924-16-14075	14075	ooooooooooooSSSS SSO	Pm0ff00ffff0m0 000mm0	GGUCAGCUUCGCAAGGC CU	161
CTGF-921-16-14076	14076	ooooooooooooSSSS SSO	Pm00ffff0f0000 mmm000	CAGCUUCGCAAGGCCUG AC	162

- 160 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
CTGF-443-16-14077	14077	ooooooooooooSSSS	Pmff0000ff0m0000000	GCCGAAGUCACAGAAGAGG	163
CTGF-1041-16-14078	14078	ooooooooooooSSSS	Pm0f0000fff00mmm00m0	CACAGAAUUUAGCUCGGUA	164
CTGF-1042-16-14079	14079	ooooooooooooSSSS	Pmf0f0000ffm00mmm000	CCACAGAAUUUAGCUCGGU	165
CTGF-755-16-14080	14080	ooooooooooooSSSS	Pmff000f0f0mmm000	GCCAAACGUGUCUCCAUGU	166
CTGF-467-16-14081	14081	ooooooooooooSSSS	Pmf0f00ffff0m0mm00m0	GCCGAUCUUGCGGUUGGCC	167
CTGF-995-16-14082	14082	ooooooooooooSSSS	Pmff000fff00m000mmm0	CUCAAACUUGAUAGGCUUG	168
CTGF-927-16-14083	14083	ooooooooooooSSSS	Pmf000ff00fmmm0m0000	CCAGGUCAGCUUCGCAAGG	169
SPP1-1091-16-14131	14131	ooooooooooooSSSS	Pmff00ff000m0m0000m0	UUUGACUAAAUGCAAAGUG	170
PPIB--16-14188	14188	ooooooooooooSSSS	Pm0fffff0f00mm000mm0	UGUUUUUGUAGCCAAAUCC	171
PPIB--17-14189	14189	ooooooooooooSSSS	Pm0fffff0f00mm000mm0	UGUUUUUGUAGCCAAAUCC	172
PPIB--18-14190	14190	ooooooooooooSSSS	Pm0fffff0f00mm000mm0	UGUUUUUGUAGCCAAAUCC	173
pGL3-1172-16-14386	14386	ooooooooooooSSSS	Pm00ff0f0ffm0mm00mm0	AAAUCGUUUUGUCAAUCA	174
pGL3-1172-16-14387	14387	ooooooooooooSSSS	Pm00ff0f0ffm0mm00mm0	AAAUCGUUUUGUCAAUCA	175
MAP4K4-2931-25-14390	14390				

- 161 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
miR-122-23-14391	14391				
	14084	ooooooooooooooooSSSSSSO	Pmff00fff0f000000m00	UCUAAUUAUGAGAAUAC	616
	14085	ooooooooooooooooSSSSSSO	Pm00ff00fffm00000m0	UAAUUGACCUCAGAAGAU	617
	14086	ooooooooooooooooSSSSSSO	Pmff00ff00fmm000000	UUUAAUUGACCUCAGAA	618
	14087	ooooooooooooooooSSSSSSO	Pm0ff00ffff000000m00	AAUUGACCUCAGAAGAU	619
	14088	ooooooooooooooooSSSSSSO	Pmf00ff00ffmm000000	UUAAUUGACCUCAGAAG	620
	14089	ooooooooooooooooSSSSSSO	Pmff00ffff000000m0m0	AUUGACCUCAGAAGAUG	621
	14090	ooooooooooooooooSSSSSSO	Pmf0fff00ff00mm0mm0	UCAUCCAGCUGACUCGU	622
	14091	ooooooooooooooooSSSSSSO	Pm0fff0ff00000m00m00	AGAUUCAUCAGAAUGGU	623
	14092	ooooooooooooooooSSSSSSO	Pm00ffff00fmm0m000m0	UGACCUCAGUCCAUA	624
	14093	ooooooooooooooooSSSSSSO	Pm0f00f0000mmm0mm000	AAUGGUGAGACUCAU	625
	14094	ooooooooooooooooSSSSSSO	Pmff00ffff00mm0m0m000	UUUGACCUCAGUCCAUA	626
	14095	ooooooooooooooooSSSSSSO	Pmff0f00ff0m0000mmm0	UUCAUGGCUGUGAAAU	627
	14096	ooooooooooooooooSSSSSSO	Pm00f00f0000mm0m0mm00	GAAUGGUGAGACUCAUC	628

- 162 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
	14097	ooooooooooooSSSS SSO	Pm00ffffff0mmm 0m0m00	UGGCUUCCGCUUAUAU AA	629
	14098	ooooooooooooSSSS SSO	Pmf00ffffff0mm m0m0m0	UUGGCUUCCGCUUAUA UA	630
	14099	ooooooooooooSSSS SSO	Pmf0fff0f0f00m m0m000	UCAUCCAUGUGGUCAUG GC	631
	14100	ooooooooooooSSSS SSO	Pmf0f00ff0f00m mmmm00	AUGUGGUCAUGGCUUUC GU	632
	14101	ooooooooooooSSSS SSO	Pmf00ff0f00mmm mm0mm0	GUGGUCAUGGCUUUCGU UG	633
	14102	ooooooooooooSSSS SSO	Pmff00fffffmmm m0m00	AUUGGCUUCCGCUUAU AU	634
	14103	ooooooooooooSSSS SSO	Pm00f0f0000mmm m000m0	AAAUACGAAAUUUCAGG UG	635
	14104	ooooooooooooSSSS SSO	Pm000f0f0000mm mm000	AGAAAUACGAAAUUCA GG	636
	14105	ooooooooooooSSSS SSO	Pm00ff0f00fmmm m0mm00	UGGUCAUGGCUUUCGUU GG	637
	14106	ooooooooooooSSSS SSO	Pmf0ff0fff0m0m 00mm00	AUAUCAUCCAUGUGGUC AU	638
	14107	ooooooooooooSSSS SSO	Pm0f0f0000fmmm 000m00	AAUACGAAAUUUCAGGU GU	639
	14108	ooooooooooooSSSS SSO	Pm0ff000000mm0 mmm00	AAUCAGAAGGCGCGUUC AG	640
	14109	ooooooooooooSSSS SSO	Pmfff0f000000m 0m0000	AUUCAUGAGAAAUACGA AA	641
	14110	ooooooooooooSSSS SSO	Pmf0fff0f00000 00m000	CUAUUCAUGAGAGAAUA AC	642

- 163 --

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
	14111	ooooooooooooSSSS SSO	Pmfff0ff000mmm 0mmm00	UUUCGUUGGACUUACUU GG	643
	14112	ooooooooooooSSSS SSO	Pmf0fffff0fm0m m00mm0	UUGCUCUCAUCAUUGGC UU	644
	14113	ooooooooooooSSSS SSO	Pmff00fffffmmm mmmm0	UUCAACUCCUCGCUUUC CA	645
	14114	ooooooooooooSSSS SSO	Pm00ff0ff00mm0 m0mm00	UGACUAUCAAUACAUC GG	646
	14115	ooooooooooooSSSS SSO	Pm0f0f0ff0mmm0 0mmm0	AGAUGCACUAUCUAAUU CA	647
	14116	ooooooooooooSSSS SSO	Pm0f000f0f0m0m mm00m0	AAUAGAUACACAUUCA CC	648
	14117	ooooooooooooSSSS SSO	Pmffffff0f0000 m000m0	UUCUUCUAUAGAAUGAA CA	649
	14118	ooooooooooooSSSS SSO	Pm0ff0ff000m00 mm0m00	AAUUGCUGGACAACCGU GG	650
	14119	ooooooooooooSSSS SSO	Pmf0fffff0m0m 0m0000	UCGCUUCCAUGUGUGA GG	651
	14120	ooooooooooooSSSS SSO	Pm00fff000fm0m mm0m00	UAAUCUGGACUGCUUGU GG	652
	14121	ooooooooooooSSSS SSO	Pmf0f0fff00mm0 0m0000	ACACAUUCAACCAUAA AC	653
	14122	ooooooooooooSSSS SSO	Pmfff0ffff0m00 mm0mm0	ACUCGUUUAUAACUGU CC	654
	14123	ooooooooooooSSSS SSO	Pmf00fff000mm0 mmm0m0	AUAAUCUGGACUGCUUG UG	655
	14124	ooooooooooooSSSS SSO	Pmffff0fff0m0m 00mmm0	UUUCCGCUUAUAUAUC UG	656

- 164 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
	14125	ooooooooooooooooSSSS SSO	Pm0fff00ff00m0 m00m00	UGUUUAACUGGUAUGGC AC	657
	14126	ooooooooooooooooSSSS SSO	Pm0f0000f000m0 m000m0	UAUAGAAUGAACAUAGA CA	658
	14127	ooooooooooooooooSSSS SSO	Pmffffff00fm0m 0mmm0	UUCCUUGGUCGGCGUU UG	659
	14128	ooooooooooooooooSSSS SSO	Pmf0f0f0ff0mmm 00mmm0	GUAUGCACCAUUAACU CC	660
	14129	ooooooooooooooooSSSS SSO	Pmf00ff0ff0m0m 0m0mm0	UCGGCCAUCAUAUGUGU CU	661
	14130	ooooooooooooooooSSSS SSO	Pm0fff000ff0mm m0m000	AAUCUGGACUGCUUGUG GC	662
	14132	ooooooooooooooooSSSS SSO	Pmf0ff0000f0mm m0mm00	ACAUCGGAAUGCUCAUU GC	663
	14133	ooooooooooooooooSSSS SSO	Pm00fffff00mm0 mm00m0	AAGUCCUGACUAUCAA UC	664
	14134	ooooooooooooooooSSSS SSO	Pmf00ff000f0m0 000m00	UUGACUAAAUGCAAAGU GA	665
	14135	ooooooooooooooooSSSS SSO	Pm0fff0ff000mm 00m00	AGACUCAUCAGACUGGU GA	666
	14136	ooooooooooooooooSSSS SSO	Pmf0f0f0f0fmm0 mm0m00	UCAUAUGUGUCUACUGU GG	667
	14137	ooooooooooooooooSSSS SSO	Pmf0fffff0fmm0 m00m00	AUGUCCUCGUCUGUAGC AU	668
	14138	ooooooooooooooooSSSS SSO	Pm0fff0f00mm0 0mmmm0	GAAUUCACGGCUGACUU UG	669
	14139	ooooooooooooooooSSSS SSO	Pmf0fffff000mm m000m0	UUUUUCCAGACUAAA UA	670

- 165 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
	14140	ooooooooooooSSSS SSO	Pm000ff0f000mm 000mm0	GAAGCCACAAACUAAAC UA	671
	14141	ooooooooooooSSSS SSO	Pmffff0ff000mm m0mmm0	CUUUCGUUGGACUUACU UG	672
	14142	ooooooooooooSSSS SSO	Pmfff0f0000mmm mmm000	GUCUGCGAAACUUCUUA GA	673
	14143	ooooooooooooSSSS SSO	Pm0f0fff0ff0mm mmm0m0	AAUGCUCAUUGCUCUCA UC	674
	14144	ooooooooooooSSSS SSO	Pmf0f0ff0ffm00 mmm0m0	AUGCACUAUCUAAUUA UG	675
	14145	ooooooooooooSSSS SSO	Pmff0f0f0f0mm0 mmm000	CUUGUAUGCACCAUUA AC	676
	14146	ooooooooooooSSSS SSO	Pm00fff0fffm0m 00mm00	UGACUCGUUUAUAACU GU	677
	14147	ooooooooooooSSSS SSO	Pmff00f0fffm00 mm0mm0	UUCAGCACUCUGGUCAU CC	678
	14148	ooooooooooooSSSS SSO	Pm00fff0f00mm0 m00000	AAAUUCAUGGCUGUGGA AU	679
	14149	ooooooooooooSSSS SSO	Pmf0fff00ff00m 000mm0	ACAUUCAACCAUAAAC UG	680
	14150	ooooooooooooSSSS SSO	Pm0f0f0fff00mm 00m000	UACACAUUCAACCAUA AA	681
	14151	ooooooooooooSSSS SSO	Pmff00ff0ffmmm 000mm0	AUUAGUUUUUCCAGAC UC	682
	14152	ooooooooooooSSSS SSO	Pmffff0fff0m00 000000	UUUCUAUUAUGAGAGA AU	683
	14153	ooooooooooooSSSS SSO	Pmff00ff0ff00m 000mm0	UUCGGUUGCUGGCAGGU CC	684

- 166 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
	14154	ooooooooooooooooSSSS SSO	Pm0f0f0f0000m0 0m0mm0	CAUGUGUGAGGUGAUGU CC	685
	14155	ooooooooooooooooSSSS SSO	Pmf0ff0fff00mm mmmm00	GCACCAUUCACUCCUC GC	686
	14156	ooooooooooooooooSSSS SSO	Pm0fff00ff00mm m0mmm0	CAUCCAGCUGACUCGUU UC	687
	14157	ooooooooooooooooSSSS SSO	Pmffffff0fff0m0 m00mm0	CUUCCGCUUAUAUAAU CU	688
	14158	ooooooooooooooooSSSS SSO	Pm0ff0f0ff0000 m0mmm0	AAUCACAUCGGAAUGCU CA	689
	14159	ooooooooooooooooSSSS SSO	Pmf0f0ff00fm0m mmmm00	ACACAUUAGUUAUUUCC AG	690
	14160	ooooooooooooooooSSSS SSO	Pmfff0f0000m00 0m0m00	UUCUAUAGAAUGAACAU AG	691
	14161	ooooooooooooooooSSSS SSO	Pm0f00f00f00mm m0m0m0	UACAGUGAUAGUUUGCA UU	692
	14162	ooooooooooooooooSSSS SSO	Pmf000f00ff00m 0mm0m0	AUAAGCAAUUGACACCA CC	693
	14163	ooooooooooooooooSSSS SSO	Pmff0ff00ff0mm 000m00	UUUAUUAAUUGCUGGAC AA	694
	14164	ooooooooooooooooSSSS SSO	Pmf0ff0000fmmm m0000	UCAUCAGAGUCGUUCGA GU	695
	14165	ooooooooooooooooSSSS SSO	Pmf000ff0f0mm0 mm0mm0	AUAAACCACACUAUCAC CU	696
	14166	ooooooooooooooooSSSS SSO	Pmf0ff0ff00mmm mmm0m0	UCAUCAUUGGCUUCCG CU	697
	14167	ooooooooooooooooSSSS SSO	Pmffffff00fm0mm 00mm0	AGUCCUGACUAUCAAU CA	698

- 167 -

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
	14168	ooooooooooooSSSS SSO	Pmff0f00ff00mm mm0000	UUCACGGCUGACUUUGG AA	699
	14169	ooooooooooooSSSS SSO	Pmffff0f00f00m 000mm0	UUCUCAUGGUAGUGAGU UU	700
	14170	ooooooooooooSSSS SSO	Pm0ff00fff0mmm 00mm00	AAUCAGCCUGUUUAACU GG	701
	14171	ooooooooooooSSSS SSO	Pm0ffff00f0mmm m00mm0	GGUUUCAGCACUCUGGU CA	702
	14172	ooooooooooooSSSS SSO	Pmff0000f0fmm0 mm0mm0	AUCGGAAUGCUCAUUGC UC	703
	14173	ooooooooooooSSSS SSO	Pm00ff0f0000mm m0m000	UGGCUGUGAAUUCACG GC	704
	14174	ooooooooooooSSSS SSO	Pm000f00ff00m0 mm0mm0	UAAGCAAUUGACACCAC CA	705
	14175	ooooooooooooSSSS SSO	Pm00fffff0f00m 00m000	CAAUUCUCAUGGUAGUG AG	706
	14176	ooooooooooooSSSS SSO	Pm00fffff0fm00 0mmm00	UGGCUUUCGUUGGACUU AC	707
	14177	ooooooooooooSSSS SSO	Pm0ff00f00fm00 mmm0m0	AAUCAGUGACCAGUUCA UC	708
	14178	ooooooooooooSSSS SSO	Pmfff0f000mm0m 0mm00	AGUCCAUAACCACACU AU	709
	14179	ooooooooooooSSSS SSO	Pm00f0ffff00mm 0mmm00	CAGCACUCUGGUCAUCC AG	710
	14180	ooooooooooooSSSS SSO	Pm0ff00ff0f0mm 0000m0	UAUCAAUCAUCGGAA UG	711
	14181	ooooooooooooSSSS SSO	Pmfff0f00ff00m mmm000	AUUCACGGCUGACUUUG GA	712

- 168 --

ID Number	Oligo Number	AntiSense Backbone	AntiSense Chemistry	AntiSense Sequence	SEQ ID NO:
	14182	ooooooooooooooooSSSS SSO	Pmf000f0f0f0mm m00mm0	AUAGAUACACAUUCAAC CA	713
	14183	ooooooooooooooooSSSS SSO	Pmffff000ffm00 0m0000	UUUCCAGACUCAAUAG AU	714
	14184	ooooooooooooooooSSSS SSO	Pmf00ff0ff000m 00mm00	UUAUUGCUGGACAACC GU	715
	14185	ooooooooooooooooSSSS SSO	Pm0ff00ff0fm00 0m00m0	UAUUAUUGCUGGACAA CC	716
	14186	ooooooooooooooooSSSS SSO	Pmff0fff000mm0 0m000	AGUCGUUCGAGUCAUG GA	717
	14187	ooooooooooooooooSSSS SSO	Pmff0ff00f000m mm0m00	GUUGCUGGCAGGUCCGU GG	718

TABLE 2: Antisense backbone, chemistry, and sequence information. o: phosphodiester; s: phosphorothioate; P: 5' phosphorylation; 0: 2'-OH; F: 2'-fluoro; m: 2' O-methyl; +: LNA modification. Capital letters in the sequence signify ribonucleotides, lower case letters

5

signify deoxyribonucleotides.

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
APOB-10167-20-12138	12138	chl	oooooooooooo oooooooooso	0000000000000 0000000	GUCAUCACACUGA AUACCAAU	176
APOB-10167-20-12139	12139	chl	oooooooooooo oooooooooso	0000000000000 0000000	GUGAUCAGACUCA AUACGAAU	177
MAP4K4-2931-13-12266	12266	chl	oooooooooooo ss o	mm0m00000mmm0	CUGUGGAAGUCUA	178
MAP4K4-2931-16-12293	12293	chl	oooooooooooo ss o	mm0m00000mmm0	CUGUGGAAGUCUA	179
MAP4K4-2931-16-12383	12383	chl	oooooooooooo o	mm0m00000mmm0	CUGUGGAAGUCUA	180
MAP4K4-2931-16-12384	12384	chl	oooooooooooo o	mm0m00000mmm0	CUGUGGAAGUCUA	181
MAP4K4-2931-16-12385	12385	chl	oooooooooooo o	mm0m00000mmm0	CUGUGGAAGUCUA	182
MAP4K4-2931-16-12386	12386	chl	oooooooooooo ss o	0mm0m00000mmm 0	CUGUGGAAGUCUA	183
MAP4K4-2931-16-12387	12387	chl	oooooooooooo o	mm0m00000mmm0	CUGUGGAAGUCUA	184
MAP4K4-2931-15-12388	12388	chl	oooooooooooo o	mm0m00000mmm0	CUGUGGAAGUCUA	185
MAP4K4-2931-13-12432	12432	chl	oooooooooooo o	DY547mm0m0000 0mmm0	CUGUGGAAGUCUA	186
MAP4K4-2931-13-12266.2	12266.2	chl	oooooooooooo ss	mm0m00000mmm0	CUGUGGAAGUCUA	187
APOB--21-12434	12434	chl	oooooooooooo oooooooooso	0000000000000 0000000	GUCAUCACACUGA AUACCAAU	188
APOB--21-12435	12435	chl	oooooooooooo oooooooooso	DY54700000000 000000000000	GUGAUCAGACUCA AUACGAAU	189
MAP4K4-2931-16-12451	12451	chl	oooooooooooo ss	0mm0m00000mmm 0	CUGUGGAAGUCUA	190
MAP4K4-2931-16-12452	12452	chl	oooooooooooo ss	mm0m00000mmm0	CUGUGGAAGUCUA	191
MAP4K4-2931-16-12453	12453	chl	oooooooooooo ss	mm0m00000mmm0	CUGUGGAAGUCUA	192
MAP4K4-2931-17-12454	12454	chl	oooooooooooo ss	0mm0m00000mmm 0	CUGUGGAAGUCUA	193
MAP4K4-2931-17-12455	12455	chl	oooooooooooo ss	mm0m00000mmm0	CUGUGGAAGUCUA	194
MAP4K4-2931-19-	12456	chl	oooooooooooo ss	mm0m00000mmm0	CUGUGGAAGUCUA	195

- 170 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
12456						
--27-12480	12480	chl	oooooooooooo oooooooooooo sso	DY547mm0f000f 0055f5f00mm00 000m000	UCAUAGGUAACCU CUGGUUGAAAGUG A	196
--27-12481	12481	chl	oooooooooooo oooooooooooo sso	DY547mm05f050 00f05ff0m0000 0000m00	CGGCUACAGGUGC UUAUGAAGAAAGU A	197
APOB-10167-21-12505	12505	chl	oooooooooooo oooooooooos	0000000000000 00000000	GUCAUCACACUGA AUACCAAU	198
APOB-10167-21-12506	12506	chl	oooooooooooo oooooooooos	0000000000000 00000000	GUGAUCAGACUCA AUACGAAU	199
MAP4K4-2931-16-12539	12539	chl	oooooooooooo s	DY547mm0m0000 0mmm0	CUGUGGAAGUCUA	200
APOB-10167-21-12505.2	12505.2	chl	oooooooooooo oooooooooso	0000000000000 0000000	GUCAUCACACUGA AUACCAAU	201
APOB-10167-21-12506.2	12506.2	chl	oooooooooooo oooooooooso	0000000000000 0000000	GUGAUCAGACUCA AUACGAAU	202
MAP4K4--13-12565	12565	Chl	oooooooooooo o	m0m0000m0mmm0	UGUAGGAUGUCUA	203
MAP4K4-2931-16-12386.2	12386.2	chl	oooooooooooo o	0mm0m00000mmm 0	CUGUGGAAGUCUA	204
MAP4K4-2931-13-12815	12815	chl	oooooooooooo o	m0m0m0m0m0m0m 0m0m0m0m0m0m0	CUGUGGAAGUCUA	205
APOB--13-12957	12957	Chl TEG	oooooooooooo s	0mmmmmmmmmmmm m	ACUGAAUACCAAU	206
MAP4K4--16-12983	12983	chl	oooooooooooo s	mm0m00000mmm0	CUGUGGAAGUCUA	207
MAP4K4--16-12984	12984	Chl	oooooooooooo oo	mm0m00000mmm0	CUGUGGAAGUCUA	208
MAP4K4--16-12985	12985	chl	oooooooooooo o	mmmmmmmmmmmmmm	CUGUGGAAGUCUA	209
MAP4K4--16-12986	12986	chl	oooooooooooo o	mmmmmmmmmmmmmm	CUGUGGAAGUCUA	210
MAP4K4--16-12987	12987	chl	oooooooooooo o	mm0m00000mmm0	CUGUGGAAGUCUA	211
MAP4K4--16-12988	12988	chl	oooooooooooo o	mm0m00000mmm0	CUGUGGAAGUCUA	212
MAP4K4--16-12989	12989	chl	oooooooooooo o	mm0m00000mmm0	CUGUGGAAGUCUA	213
MAP4K4--16-12990	12990	chl	oooooooooooo o	mm0m00000mmm0	CUGUGGAAGUCUA	214
MAP4K4--16-12991	12991	chl	oooooooooooo o	mm0m00000mmm0	CUGUGGAAGUCUA	215
MAP4K4--16-12992	12992	chl	oooooooooooo o	mm0m00000mmm0	CUGUGGAAGUCUA	216
MAP4K4--16-12993	12993	chl	oooooooooooo o	mm0m00000mmm0	CUGUGGAAGUCUA	217
MAP4K4--16-12994	12994	chl	oooooooooooo o	mm0m00000mmm0	CUGUGGAAGUCUA	218
MAP4K4--16-12995	12995	chl	oooooooooooo o	mm0m00000mmm0	CUGUGGAAGUCUA	219

- 171 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
MAP4K4-2931-19-13012	13012	chl	oooooooooooo oooooooo	0000000000000 00000000	AGAGUUCUGUGGA AGUCUA	220
MAP4K4-2931-19-13016	13016	chl	oooooooooooo oooooooo	DY54700000000 0000000000000	AGAGUUCUGUGGA AGUCUA	221
PPIB--13-13021	13021	Chl	oooooooooooo o	0mmm00mm0m000	AUUUGGCUACAAA	222
pGL3-1172-13-13038	13038	chl	oooooooooooo o	00m000m0m00mm m	ACAAAUACGAUUU	223
pGL3-1172-13-13040	13040	chl	oooooooooooo o	DY5470m000m0m 00mmm	ACAAAUACGAUUU	224
--16-13047	13047	Chl	oooooooooooo oo	mm0m00000mmm0	CUGUGGAAGUCUA	225
SOD1-530-13-13090	13090	chl	oooooooooooo o	00m00000000m0	AAUGAAGAAAGUA	226
SOD1-523-13-13091	13091	chl	oooooooooooo o	000m00000m000	AGGUGGAAAUGAA	227
SOD1-535-13-13092	13092	chl	oooooooooooo o	000000m0m0000	AGAAAGUACAAAG	228
SOD1-536-13-13093	13093	chl	oooooooooooo o	00000m0m00000	GAAAGUACAAAGA	229
SOD1-396-13-13094	13094	chl	oooooooooooo o	0m0m00mm0mm00	AUGUGACUGCUGA	230
SOD1-385-13-13095	13095	chl	oooooooooooo o	000mmm000m00m	AGACUUGGGCAAU	231
SOD1-195-13-13096	13096	chl	oooooooooooo o	0mmmm000m0000	AUUUCGAGCAGAA	232
APOB-4314-13-13115	13115	Chl	oooooooooooo o	0mmmm0000000m0	AUCUGGAGAAACA	233
APOB-3384-13-13116	13116	Chl	oooooooooooo o	mm0000m000000	UCAGAACAAGAAA	234
APOB-3547-13-13117	13117	Chl	oooooooooooo o	00mmm0mmm0mm0	GACUCAUCUGCUA	235
APOB-4318-13-13118	13118	Chl	oooooooooooo o	0000000m00m0m	GGAGAAACAACAU	236
APOB-3741-13-13119	13119	Chl	oooooooooooo o	00mmmmmm000m0	AGUCCCUCAAACA	237
PPIB--16-13136	13136	Chl	oooooooooooo oo	00mm0m00000m0	GGCUACAAAAACA	238
APOB-4314-15-13154	13154	chl	oooooooooooo oo	000mmm0000000 m0	AGAUCUGGAGAAA CA	239
APOB-3547-15-13155	13155	chl	oooooooooooo oo	m000mmm0mmm0m m0	UGGACUCAUCUGC UA	240
APOB-4318-15-13157	13157	chl	oooooooooooo oo	mm0000000m00m 0m	CUGGAGAAACAAC AU	241
APOB-3741-15-13158	13158	chl	oooooooooooo oo	0000mmmmmm000 m0	AGAGUCCCUCAAA CA	242
APOB--13-13159	13159	chl	oooooooooooo	0mm000m0mm00m	ACUGAAUACCAAU	243
APOB--15-13160	13160	chl	oooooooooooo oo	0m0mm000m0mm0 0m	ACACUGAAUACCA AU	244
SOD1-530-16-13163	13163	chl	oooooooooooo o	00m00000000m0	AAUGAAGAAAGUA	245

- 172 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
SOD1-523-16-13164	13164	chl	oooooooooooo o	000m00000m000	AGGUGGAAAUGAA	246
SOD1-535-16-13165	13165	chl	oooooooooooo o	000000m0m0000	AGAAAGUACAAAG	247
SOD1-536-16-13166	13166	chl	oooooooooooo o	00000m0m00000	GAAAGUACAAAGA	248
SOD1-396-16-13167	13167	chl	oooooooooooo o	0m0m00mm0mm00	AUGUGACUGCUGA	249
SOD1-385-16-13168	13168	chl	oooooooooooo o	000mmm000m00m	AGACUUGGGCAAU	250
SOD1-195-16-13169	13169	chl	oooooooooooo o	0mmmm000m0000	AUUUCGAGCAGAA	251
pGL3-1172-16-13170	13170	chl	oooooooooooo o	0m000m0m00mmm	ACAAAUACGAUUU	252
pGL3-1172-16-13171	13171	chl	oooooooooooo o	DY5470m000m0m00mmm	ACAAAUACGAUUU	253
MAP4k4-2931-19-13189	13189	chl	oooooooooooo oooooooo	00000000000000 00000000	AGAGUUCUGUGGA AGUCUA	254
CTGF-1222-13-13190	13190	Chl	oooooooooooo o	0m0000000m0m0	ACAGGAAGAUGUA	255
CTGF-813-13-13192	13192	Chl	oooooooooooo o	000m0000m0mmm	GAGUGGAGCGCCU	256
CTGF-747-13-13194	13194	Chl	oooooooooooo o	m00mm000000m0	CGACUGGAAGACA	257
CTGF-817-13-13196	13196	Chl	oooooooooooo o	0000m0mmm0mmm	GGAGCGCCUGUUC	258
CTGF-1174-13-13198	13198	Chl	oooooooooooo o	0mm0mm0m00mm0	GCCAUUACAACUG	259
CTGF-1005-13-13200	13200	Chl	oooooooooooo o	000mmmmmm00mm	GAGCUUUCUGGCU	260
CTGF-814-13-13202	13202	Chl	oooooooooooo o	00m0000m0mmm0	AGUGGAGCGCCUG	261
CTGF-816-13-13204	13204	Chl	oooooooooooo o	m0000m0mmm0mm	UGGAGCGCCUGUU	262
CTGF-1001-13-13206	13206	Chl	oooooooooooo o	0mmm000mmmmmm	GUUUGAGCUUUCU	263
CTGF-1173-13-13208	13208	Chl	oooooooooooo o	m0mm0mm0m00mm	UGCCAUUACAACU	264
CTGF-749-13-13210	13210	Chl	oooooooooooo o	0mm000000m0m0	ACUGGAAGACACG	265
CTGF-792-13-13212	13212	Chl	oooooooooooo o	00mm0mmm00mmm	AACUGCCUGGUCC	266
CTGF-1162-13-13214	13214	Chl	oooooooooooo o	000mmm0m0mmm0	AGACCUGUGCCUG	267
CTGF-811-13-13216	13216	Chl	oooooooooooo o	m0000m0000m0m	CAGAGUGGAGCGC	268
CTGF-797-13-13218	13218	Chl	oooooooooooo o	mmm00mmm000mm	CCUGGUCCAGACC	269
CTGF-1175-13-13220	13220	Chl	oooooooooooo o	mm0mm0m00mm0m	CCAUUACAACUGU	270
CTGF-1172-13-13222	13222	Chl	oooooooooooo o	mm0mm0mm0m00m	CUGCCAUUACAAC	271
CTGF-1177-13-13224	13224	Chl	oooooooooooo o	0mm0m00mm0mmm	AUUACAACUGUCC	272

- 173 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
CTGF-1176-13-13226	13226	Ch1	oooooooooooo o	m0mm0m00mm0mm	CAUUACAACUGUC	273
CTGF-812-13-13228	13228	Ch1	oooooooooooo o	0000m0000m0mm	AGAGUGGAGCGCC	274
CTGF-745-13-13230	13230	Ch1	oooooooooooo o	0mm00mm000000	ACCGACUGGAAGA	275
CTGF-1230-13-13232	13232	Ch1	oooooooooooo o	0m0m0m00000m0	AUGUACGGAGACA	276
CTGF-920-13-13234	13234	Ch1	oooooooooooo o	0mmmm0m0000mm	GCCUUGCGAAGCU	277
CTGF-679-13-13236	13236	Ch1	oooooooooooo o	0mm0m000000m0	GCUGCGAGGAGUG	278
CTGF-992-13-13238	13238	Ch1	oooooooooooo o	0mmm0mm000mmm	GCCUAUCAAGUUU	279
CTGF-1045-13-13240	13240	Ch1	oooooooooooo o	00mmmm0m0000m	AAUUCUGUGGAGU	280
CTGF-1231-13-13242	13242	Ch1	oooooooooooo o	m0m0m00000m0m	UGUACGGAGACAU	281
CTGF-991-13-13244	13244	Ch1	oooooooooooo o	00mmm0mm000mm	AGCCUAUCAAGUU	282
CTGF-998-13-13246	13246	Ch1	oooooooooooo o	m000mmm000mmm	CAAGUUUGAGCUU	283
CTGF-1049-13-13248	13248	Ch1	oooooooooooo o	mm0m0000m0m0m	CUGUGGAGUAUGU	284
CTGF-1044-13-13250	13250	Ch1	oooooooooooo o	000mmmm0m0000	AAAUUCUGUGGAG	285
CTGF-1327-13-13252	13252	Ch1	oooooooooooo o	mmmm00m00m0m0	UUUCAGUAGCACA	286
CTGF-1196-13-13254	13254	Ch1	oooooooooooo o	m00m00m0mmmmmm	CAAUGACAUCUUU	287
CTGF-562-13-13256	13256	Ch1	oooooooooooo o	00m0mm00m0m0m	AGUACCAGUGCAC	288
CTGF-752-13-13258	13258	Ch1	oooooooooooo o	000000m0m0mmm	GGAAGACACGUUU	289
CTGF-994-13-13260	13260	Ch1	oooooooooooo o	mm0mm000mmm00	CUAUAAGUUUGA	290
CTGF-1040-13-13262	13262	Ch1	oooooooooooo o	00mm000mmmm0m	AGCUAAAUUCUGU	291
CTGF-1984-13-13264	13264	Ch1	oooooooooooo o	000m0000m0m00	AGGUAGAAUGUAA	292
CTGF-2195-13-13266	13266	Ch1	oooooooooooo o	00mm00mm00mmm	AGCUGAUCAGUUU	293
CTGF-2043-13-13268	13268	Ch1	oooooooooooo o	mmmm0mmm000m0	UUCUGCUCAGAU	294
CTGF-1892-13-13270	13270	Ch1	oooooooooooo o	mm0mmm000mm00	UUAUCUAAGUUAA	295
CTGF-1567-13-13272	13272	Ch1	oooooooooooo o	m0m0m000m0m0m	UAUACGAGUAAUA	296
CTGF-1780-13-13274	13274	Ch1	oooooooooooo o	00mm000m00mmm	GACUGGACAGCUU	297
CTGF-2162-13-13276	13276	Ch1	oooooooooooo o	0m00mmmmmm0mm0	AUGGCCUUUAUUA	298
CTGF-1034-13-13278	13278	Ch1	oooooooooooo o	0m0mm000mm000	AUACCGAGCUAAA	299

- 174 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
CTGF-2264-13-13280	13280	Ch1	oooooooooooo o	mm0mm00000m0m	UUGUUGAGAGUGU	300
CTGF-1032-13-13282	13282	Ch1	oooooooooooo o	0m0m0mm000mm0	ACAUACCGAGCUA	301
CTGF-1535-13-13284	13284	Ch1	oooooooooooo o	00m0000000mm0	AGCAGAAAGGUUA	302
CTGF-1694-13-13286	13286	Ch1	oooooooooooo o	00mm0mmmmmm00	AGUUGUCCUUA	303
CTGF-1588-13-13288	13288	Ch1	oooooooooooo o	0mmm0000m0m00	AUUUGAAGUGUAA	304
CTGF-928-13-13290	13290	Ch1	oooooooooooo o	000mm00mmm000	AAGCUGACCUGGA	305
CTGF-1133-13-13292	13292	Ch1	oooooooooooo o	00mm0m0000000	GGUCAUGAAGAAG	306
CTGF-912-13-13294	13294	Ch1	oooooooooooo o	0m00mm000mmm	AUGGUCAGGCCUU	307
CTGF-753-13-13296	13296	Ch1	oooooooooooo o	00000m0m0mmm0	GAAGACACGUUUG	308
CTGF-918-13-13298	13298	Ch1	oooooooooooo o	000mmmm0m0000	AGGCCUUGCGAAG	309
CTGF-744-13-13300	13300	Ch1	oooooooooooo o	m0mm0mm00000	UACCGACUGGAAG	310
CTGF-466-13-13302	13302	Ch1	oooooooooooo o	0mm0m0000mm0	ACCGCAAGAUCCG	311
CTGF-917-13-13304	13304	Ch1	oooooooooooo o	m000mmmm0m000	CAGGCCUUGCGAA	312
CTGF-1038-13-13306	13306	Ch1	oooooooooooo o	m000mm000mmm	CGAGCUAAAUUCU	313
CTGF-1048-13-13308	13308	Ch1	oooooooooooo o	mmm0m0000m0m0	UCUGUGGAGUAUG	314
CTGF-1235-13-13310	13310	Ch1	oooooooooooo o	m00000m0m00m0	CGGAGACAUGGCA	315
CTGF-868-13-13312	13312	Ch1	oooooooooooo o	0m00m00m0mmm	AUGACAACGCCUC	316
CTGF-1131-13-13314	13314	Ch1	oooooooooooo o	0000mm0m00000	GAGGUCAUGAAGA	317
CTGF-1043-13-13316	13316	Ch1	oooooooooooo o	m000mmmm0m000	UAAAUUCUGUGGA	318
CTGF-751-13-13318	13318	Ch1	oooooooooooo o	m000000m0m0mm	UGGAAGACACGUU	319
CTGF-1227-13-13320	13320	Ch1	oooooooooooo o	0000m0m0m0000	AAGAUGUACGGAG	320
CTGF-867-13-13322	13322	Ch1	oooooooooooo o	00m00m00m0mmm	AAUGACAACGCCU	321
CTGF-1128-13-13324	13324	Ch1	oooooooooooo o	00m0000mm0m00	GGCGAGGUCAUGA	322
CTGF-756-13-13326	13326	Ch1	oooooooooooo o	00m0m0mmm00mm	GACACGUUUGGCC	323
CTGF-1234-13-13328	13328	Ch1	oooooooooooo o	0m00000m0m00m	ACGGAGACAUGGC	324
CTGF-916-13-13330	13330	Ch1	oooooooooooo o	mm000mmmm0m00	UCAGGCCUUGCGA	325
CTGF-925-13-13332	13332	Ch1	oooooooooooo o	0m0000mm00mmm	GCGAAGCUGACCU	326

- 175 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
CTGF-1225-13-13334	13334	Ch1	oooooooooooo o	000000m0m0m00	GGAAGAUGUACGG	327
CTGF-445-13-13336	13336	Ch1	oooooooooooo o	0m00mmmm00mmm	GUGACUUCGGCUC	328
CTGF-446-13-13338	13338	Ch1	oooooooooooo o	m00mmmm00mmmm	UGACUUCGGCUCC	329
CTGF-913-13-13340	13340	Ch1	oooooooooooo o	m00mm000mmmm0	UGGUCAGGCCUUG	330
CTGF-997-13-13342	13342	Ch1	oooooooooooo o	mm000mmmm000mm	UCAAGUUUGAGCU	331
CTGF-277-13-13344	13344	Ch1	oooooooooooo o	0mm0000mm0m00	GCCAGAACUGCAG	332
CTGF-1052-13-13346	13346	Ch1	oooooooooooo o	m0000m0m0m0mm	UGGAGUAUGUACC	333
CTGF-887-13-13348	13348	Ch1	oooooooooooo o	0mm0000000m00	GCUAGAGAAGCAG	334
CTGF-914-13-13350	13350	Ch1	oooooooooooo o	00mm000mmmm0m	GGUCAGGCCUUGC	335
CTGF-1039-13-13352	13352	Ch1	oooooooooooo o	000mm000mmmm0	GAGCUAAAUUCUG	336
CTGF-754-13-13354	13354	Ch1	oooooooooooo o	0000m0m0mmmm00	AAGACACGUUUGG	337
CTGF-1130-13-13356	13356	Ch1	oooooooooooo o	m0000mm0m0000	CGAGGUCAUGAAG	338
CTGF-919-13-13358	13358	Ch1	oooooooooooo o	00mmmm0m0000m	GGCCUUGCGAAGC	339
CTGF-922-13-13360	13360	Ch1	oooooooooooo o	mmmm0m0000mm00	CUUGCGAAGCUGA	340
CTGF-746-13-13362	13362	Ch1	oooooooooooo o	mm00mm000000m	CCGACUGGAAGAC	341
CTGF-993-13-13364	13364	Ch1	oooooooooooo o	mmmm0mm000mmmm0	CCUAUCAAGUUUG	342
CTGF-825-13-13366	13366	Ch1	oooooooooooo o	m0mmmm0000mmmm	UGUUGCAAGACCU	343
CTGF-926-13-13368	13368	Ch1	oooooooooooo o	m0000mm00mmmm0	CGAAGCUGACCUG	344
CTGF-923-13-13370	13370	Ch1	oooooooooooo o	mm0m0000mm00m	UUGCGAAGCUGAC	345
CTGF-866-13-13372	13372	Ch1	oooooooooooo o	m00m00m00m0mm	CAAUGACAACGCC	346
CTGF-563-13-13374	13374	Ch1	oooooooooooo o	0m0mm00m0m0m0	GUACCAGUGCACG	347
CTGF-823-13-13376	13376	Ch1	oooooooooooo o	mmmm0mmmm0000m	CCUGUUGCAAGAC	348
CTGF-1233-13-13378	13378	Ch1	oooooooooooo o	m0m00000m0m00	UACGGAGACAUGG	349
CTGF-924-13-13380	13380	Ch1	oooooooooooo o	m0m0000mm00mm	UGCGAAGCUGACC	350
CTGF-921-13-13382	13382	Ch1	oooooooooooo o	mmmm0m0000mm0	CCUUGCGAAGCUG	351
CTGF-443-13-13384	13384	Ch1	oooooooooooo o	mm0m00mmmm00m	CUGUGACUUCGGC	352
CTGF-1041-13-13386	13386	Ch1	oooooooooooo o	0mm000mmmm0m0	GCUAAAUUCUGUG	353

- 176 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
CTGF-1042-13-13388	13388	Ch1	oooooooooooo o	mm000mmmm0m00	CUAAAUUCUGUGG	354
CTGF-755-13-13390	13390	Ch1	oooooooooooo o	000m0m0mmm00m	AGACACGUUUGGC	355
CTGF-467-13-13392	13392	Ch1	oooooooooooo o	mm0m0000mm00m	CCGCAAGAUCCGC	356
CTGF-995-13-13394	13394	Ch1	oooooooooooo o	m0mm000mmm000	UAUCAAGUUUGAG	357
CTGF-927-13-13396	13396	Ch1	oooooooooooo o	0000mm00mmm00	GAAGCUGACCUGG	358
SPP1-1025-13-13398	13398	Ch1	oooooooooooo o	mmm0m000mm000	CUCAUGAAUUAGA	359
SPP1-1049-13-13400	13400	Ch1	oooooooooooo o	mm0000mm00mm0	CUGAGGUCAAUUA	360
SPP1-1051-13-13402	13402	Ch1	oooooooooooo o	0000mm00mm000	GAGGUCAAUAAA	361
SPP1-1048-13-13404	13404	Ch1	oooooooooooo o	mmm0000mm00mm	UCUGAGGUCAAUU	362
SPP1-1050-13-13406	13406	Ch1	oooooooooooo o	m0000mm00mm00	UGAGGUCAAUUA	363
SPP1-1047-13-13408	13408	Ch1	oooooooooooo o	mmmm0000mm00m	UUCUGAGGUCAAU	364
SPP1-800-13-13410	13410	Ch1	oooooooooooo o	0mm00mm000m00	GUCAGCUGGAUGA	365
SPP1-492-13-13412	13412	Ch1	oooooooooooo o	mmmm00m000mmm	UUCUGAUGAAUCU	366
SPP1-612-13-13414	13414	Ch1	oooooooooooo o	m000mm0000mm0	UGGACUGAGGUCA	367
SPP1-481-13-13416	13416	Ch1	oooooooooooo o	000mmmm0mm0mm	GAGUCUCACCAUU	368
SPP1-614-13-13418	13418	Ch1	oooooooooooo o	00mm0000mm000	GACUGAGGUCAAA	369
SPP1-951-13-13420	13420	Ch1	oooooooooooo o	mm0m00mm0m000	UCACAGCCAUGAA	370
SPP1-482-13-13422	13422	Ch1	oooooooooooo o	00mmmm0mm0mmm	AGUCUCACCAUUC	371
SPP1-856-13-13424	13424	Ch1	oooooooooooo o	000m000000mm0	AAGCGGAAAGCCA	372
SPP1-857-13-13426	13426	Ch1	oooooooooooo o	00m000000mm00	AGCGGAAAGCCAA	373
SPP1-365-13-13428	13428	Ch1	oooooooooooo o	0mm0m0m000m00	ACCACAUGGAUGA	374
SPP1-359-13-13430	13430	Ch1	oooooooooooo o	0mm0m00mm0m0m	GCCAUGACCACAU	375
SPP1-357-13-13432	13432	Ch1	oooooooooooo o	000mm0m00mm0m	AAGCCAUGACCAC	376
SPP1-858-13-13434	13434	Ch1	oooooooooooo o	0m000000mm00m	GCGGAAAGCCAAU	377
SPP1-1012-13-13436	13436	Ch1	oooooooooooo o	000mmmm0m0mmm	AAAUUUCGUUUUU	378
SPP1-1014-13-13438	13438	Ch1	oooooooooooo o	0mmmm0m0mmmmm	AUUUCGUUUUCU	379
SPP1-356-13-13440	13440	Ch1	oooooooooooo o	0000mm0m00mm0	AAAGCCAUGACCA	380

- 177 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
SPP1-368-13-13442	13442	Ch1	oooooooooooo o	0m0m000m00m0m	ACAUGGAUGAUAU	381
SPP1-1011-13-13444	13444	Ch1	oooooooooooo o	0000mmmm0m0mm	GAAAUUUCGUUU	382
SPP1-754-13-13446	13446	Ch1	oooooooooooo o	0m0mmmmmm00mm	GCGCCUUCUGAUU	383
SPP1-1021-13-13448	13448	Ch1	oooooooooooo o	0mmmmmm0m000m	AUUUCUCAUGAAU	384
SPP1-1330-13-13450	13450	Ch1	oooooooooooo o	mmmm0m000m00	CUCUCAUGAAUAG	385
SPP1-346-13-13452	13452	Ch1	oooooooooooo o	000mm00m0000	AAGUCCAACGAAA	386
SPP1-869-13-13454	13454	Ch1	oooooooooooo o	0m00m00000m00	AUGAUGAGAGCAA	387
SPP1-701-13-13456	13456	Ch1	oooooooooooo o	0m000000mm000	GCGAGGAGUUGAA	388
SPP1-896-13-13458	13458	Ch1	oooooooooooo o	m00mm00m00mm0	UGAUUGAUAGUCA	389
SPP1-1035-13-13460	13460	Ch1	oooooooooooo o	000m00m0m0mm	AGAUAGUGCAUCU	390
SPP1-1170-13-13462	13462	Ch1	oooooooooooo o	0m0m0m0mm0mm	AUGUGUAUCUAUU	391
SPP1-1282-13-13464	13464	Ch1	oooooooooooo o	mmmm0m0000000	UUCUAUAGAAGAA	392
SPP1-1537-13-13466	13466	Ch1	oooooooooooo o	mm0mm00m00mm	UUGUCCAGCAAUU	393
SPP1-692-13-13468	13468	Ch1	oooooooooooo o	0m0m000000m00	ACAUGGAAAGCGA	394
SPP1-840-13-13470	13470	Ch1	oooooooooooo o	0m00mm000mm0	GCAGUCCAGAUUA	395
SPP1-1163-13-13472	13472	Ch1	oooooooooooo o	m00mm000m0m0m	UGGUUGAAUGUGU	396
SPP1-789-13-13474	13474	Ch1	oooooooooooo o	mm0m0000m000m	UUAUGAAACGAGU	397
SPP1-841-13-13476	13476	Ch1	oooooooooooo o	m00mm000mm0m	CAGUCCAGAUUUA	398
SPP1-852-13-13478	13478	Ch1	oooooooooooo o	0m0m000m00000	AUAUAAGCGGAAA	399
SPP1-209-13-13480	13480	Ch1	oooooooooooo o	m0mm00mm000m0	UACCAGUUAACA	400
SPP1-1276-13-13482	13482	Ch1	oooooooooooo o	m0mm0mmmm0m0	UGUUCAUUCUAUA	401
SPP1-137-13-13484	13484	Ch1	oooooooooooo o	mm00mm0000000	CCGACCAAGGAAA	402
SPP1-711-13-13486	13486	Ch1	oooooooooooo o	000m00m0m0m	GAAUGGUGCAUAC	403
SPP1-582-13-13488	13488	Ch1	oooooooooooo o	0m0m00m00mm00	AUAUGAUGGCCGA	404
SPP1-839-13-13490	13490	Ch1	oooooooooooo o	00m00mm000mm	AGCAGUCCAGAUU	405
SPP1-1091-13-13492	13492	Ch1	oooooooooooo o	0m0mm00mm000	GCAUUUAGUCAAA	406
SPP1-884-13-13494	13494	Ch1	oooooooooooo o	00m0mmmm00m0m	AGCAUCCGAUGU	407

- 178 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
SPP1-903-13-13496	13496	Ch1	oooooooooooo o	m00mm00000mmm	UAGUCAGGAACUU	408
SPP1-1090-13-13498	13498	Ch1	oooooooooooo o	m0m0mmm00mm00	UGCAUUUAGUCAA	409
SPP1-474-13-13500	13500	Ch1	oooooooooooo o	0mmm00m000mmm	GUCUGAUGAGUCU	410
SPP1-575-13-13502	13502	Ch1	oooooooooooo o	m000m0m0m0m00	UAGACACAU AUGA	411
SPP1-671-13-13504	13504	Ch1	oooooooooooo o	m000m00000m0m	CAGACGAGGACAU	412
SPP1-924-13-13506	13506	Ch1	oooooooooooo o	m00mm0m000mmm	CAGCCGUGAAUUC	413
SPP1-1185-13-13508	13508	Ch1	oooooooooooo o	00mmm00000m00	AGUCUGGAAAUA	414
SPP1-1221-13-13510	13510	Ch1	oooooooooooo o	00mmm0m00mmm	AGUUUGUGGCUUC	415
SPP1-347-13-13512	13512	Ch1	oooooooooooo o	00mmm00m00000	AGUCCAACGAAAG	416
SPP1-634-13-13514	13514	Ch1	oooooooooooo o	000mmmm0m000m	AAGUUUCGCAGAC	417
SPP1-877-13-13516	13516	Ch1	oooooooooooo o	00m00m000m0mm	AGCAAUGAGCAUU	418
SPP1-1033-13-13518	13518	Ch1	oooooooooooo o	mm000m00m0m0m	UUAGAUAGUGCAU	419
SPP1-714-13-13520	13520	Ch1	oooooooooooo o	m00m0m0m0m000	UGGUGCAUACAAG	420
SPP1-791-13-13522	13522	Ch1	oooooooooooo o	0m0000m000mm0	AUGAAACGAGUCA	421
SPP1-813-13-13524	13524	Ch1	oooooooooooo o	mm0000m0mm000	CCAGAGUGCUGAA	422
SPP1-939-13-13526	13526	Ch1	oooooooooooo o	m00mm0m000mmm	CAGCCAUGAAUUU	423
SPP1-1161-13-13528	13528	Ch1	oooooooooooo o	0mm00mm000m0m	AUUGGUUGAAUGU	424
SPP1-1164-13-13530	13530	Ch1	oooooooooooo o	00mm000m0m0m0	GGUUGAAUGUGUA	425
SPP1-1190-13-13532	13532	Ch1	oooooooooooo o	00000m00mm00m	GGAAAUAACUAAU	426
SPP1-1333-13-13534	13534	Ch1	oooooooooooo o	mm0m000m00000	UCAUGAAUAGAAA	427
SPP1-537-13-13536	13536	Ch1	oooooooooooo o	0mm00m00mm000	GCCAGCAACCGAA	428
SPP1-684-13-13538	13538	Ch1	oooooooooooo o	m0mmmm0m0m0m0	CACCUCACACAUG	429
SPP1-707-13-13540	13540	Ch1	oooooooooooo o	00mm000m00m0m	AGUUGAAUGGUGC	430
SPP1-799-13-13542	13542	Ch1	oooooooooooo o	00mm00mm000m0	AGUCAGCUGGAUG	431
SPP1-853-13-13544	13544	Ch1	oooooooooooo o	m0m000m000000	UAUAAGCGGAAAG	432
SPP1-888-13-13546	13546	Ch1	oooooooooooo o	mmmm00m0m00mm	UUCCGAUGUGAUU	433
SPP1-1194-13-13548	13548	Ch1	oooooooooooo o	0m00mm00m0m0m	AUAACUAAUGUGU	434

- 179 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
SPP1-1279-13-13550	13550	Ch1	oooooooooooo o	mm0mmmm0m0000	UCAUUCUAUAGAA	435
SPP1-1300-13-13552	13552	Ch1	oooooooooooo o	00mm0mm0mm0m0	AACUAUCACUGUA	436
SPP1-1510-13-13554	13554	Ch1	oooooooooooo o	0mm00mm0mmm0m	GUCAAUUGCUUUAU	437
SPP1-1543-13-13556	13556	Ch1	oooooooooooo o	00m00mm00m000	AGCAAUUAUAAA	438
SPP1-434-13-13558	13558	Ch1	oooooooooooo o	0m00mmmm00m00	ACGACUCUGAUGA	439
SPP1-600-13-13560	13560	Ch1	oooooooooooo o	m00m0m00mmm0m	UAGUGUGGUUUUAU	440
SPP1-863-13-13562	13562	Ch1	oooooooooooo o	000mm00m00m00	AAGCCAAUGAUGA	441
SPP1-902-13-13564	13564	Ch1	oooooooooooo o	0m00mm00000mm	AUAGUCAGGAACU	442
SPP1-921-13-13566	13566	Ch1	oooooooooooo o	00mm00mm0m000	AGUCAGCCGUGAA	443
SPP1-154-13-13568	13568	Ch1	oooooooooooo o	0mm0mm0m00000	ACUACCAUGAGAA	444
SPP1-217-13-13570	13570	Ch1	oooooooooooo o	000m000mm00mm	AAACAGGCUGAUU	445
SPP1-816-13-13572	13572	Ch1	oooooooooooo o	000m0mm0000mm	GAGUGCUGAAACC	446
SPP1-882-13-13574	13574	Ch1	oooooooooooo o	m000m0mmmm00m	UGAGCAUUCGGAU	447
SPP1-932-13-13576	13576	Ch1	oooooooooooo o	00mmmm0m00mm0	AAUUCCACAGCCA	448
SPP1-1509-13-13578	13578	Ch1	oooooooooooo o	m0mm00mm0mmm0	UGUCAAUUGCUIA	449
SPP1-157-13-13580	13580	Ch1	oooooooooooo o	0mm0m00000mm0	ACCAUGAGAAUUG	450
SPP1-350-13-13582	13582	Ch1	oooooooooooo o	mm00m00000mm0	CCAACGAAAGCCA	451
SPP1-511-13-13584	13584	Ch1	oooooooooooo o	mm00mm0mm00mm	CUGGUCACUGAUU	452
SPP1-605-13-13586	13586	Ch1	oooooooooooo o	m00mmm0m000mm	UGGUUUUUGGACU	453
SPP1-811-13-13588	13588	Ch1	oooooooooooo o	00mm0000m0mm0	GACCAGAGUGCUG	454
SPP1-892-13-13590	13590	Ch1	oooooooooooo o	00m0m00mm00m0	GAUGUGAUUGAUA	455
SPP1-922-13-13592	13592	Ch1	oooooooooooo o	0mm00mm0m000m	GUCAGCCGUGAAU	456
SPP1-1169-13-13594	13594	Ch1	oooooooooooo o	00m0m0m0mmm0m	AAUGUGUAUCUAU	457
SPP1-1182-13-13596	13596	Ch1	oooooooooooo o	mm000mmm00000	UUGAGUCUGGAAA	458
SPP1-1539-13-13598	13598	Ch1	oooooooooooo o	0mmm00m00mm00	GUCCAGCAAUUA	459
SPP1-1541-13-13600	13600	Ch1	oooooooooooo o	mm00m00mm00m0	CCAGCAAUUAUA	460
SPP1-427-13-13602	13602	Ch1	oooooooooooo o	00mmmm000m00mm	GACUCGAACGACU	461

- 180 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
SPP1-533-13-13604	13604	Ch1	oooooooooooo o	0mmm0mm00m00m	ACCUGCCAGCAAC	462
APOB--13-13763	13763	Ch1 TEG	oooooooooooo o	0m+00+m0+m0+m	ACtGAaUAcCAaU	463
APOB--13-13764	13764	Ch1 TEG	oooooooooooo o	0mm000m0mm00m	ACUGAAUACCAAU	464
MAP4K4--16-13766	13766	Ch1	oooooooooooo o	DY547mm0m0000 0mmm0	CUGUGGAAGUCUA	465
PPIB--13-13767	13767	Ch1	oooooooooooo o	mmmmmmmmmmmm	GGCUACAAAACA	466
PPIB--15-13768	13768	Ch1	oooooooooooo ooo	mm00mm0m00000 m0	UUGGCUACAAAA CA	467
PPIB--17-13769	13769	Ch1	oooooooooooo ooooo	0mmm00mm0m000 00m0	AUUUGGCUACAAA AACA	468
MAP4K4--16-13939	13939	Ch1	oooooooooooo o	m0m0000m0mmm0	UGUAGGAUGUCUA	469
APOB-4314-16-13940	13940	Ch1	oooooooooooo o	0mmm0000000m0	AUCUGGAGAAACA	470
APOB-4314-17-13941	13941	Ch1	oooooooooooo ooo	000mmm0000000 m0	AGAUCUGGAGAAA CA	471
APOB--16-13942	13942	Ch1	oooooooooooo o	00mmm0mmm0mm0	GACUCAUCUGCUA	472
APOB--18-13943	13943	Ch1	oooooooooooo o	00mmm0mmm0mm0	GACUCAUCUGCUA	473
APOB--17-13944	13944	Ch1	oooooooooooo ooo	m000mmm0mmm0m m0	UGGACUCAUCUGC UA	474
APOB--19-13945	13945	Ch1	oooooooooooo ooo	m000mmm0mmm0m m0	UGGACUCAUCUGC UA	475
APOB-4314-16-13946	13946	Ch1	oooooooooooo o	0000000m00m0m	GGAGAAACAACAU	476
APOB-4314-17-13947	13947	Ch1	oooooooooooo ooo	mm0000000m00m 0m	CUGGAGAAACAAC AU	477
APOB--16-13948	13948	Ch1	oooooooooooo o	00mmmmmm000m0	AGUCCCUCAAACA	478
APOB--17-13949	13949	Ch1	oooooooooooo ooo	0000mmmmmm000 m0	AGAGUCCCUCAAA CA	479
APOB--16-13950	13950	Ch1	oooooooooooo o	0mm000m0mm00m	ACUGAAUACCAAU	480
APOB--18-13951	13951	Ch1	oooooooooooo o	0mm000m0mm00m	ACUGAAUACCAAU	481
APOB--17-13952	13952	Ch1	oooooooooooo ooo	0m0mm000m0mm0 0m	ACACUGAAUACCA AU	482
APOB--19-13953	13953	Ch1	oooooooooooo ooo	0m0mm000m0mm0 0m	ACACUGAAUACCA AU	483
MAP4K4--16-13766.2	13766.2	Ch1	oooooooooooo o	DY547mm0m0000 0mmm0	CUGUGGAAGUCUA	484
CTGF-1222-16-13980	13980	Ch1	oooooooooooo o	0m0000000m0m0	ACAGGAAGAUGUA	485
CTGF-813-16-13981	13981	Ch1	oooooooooooo o	000m0000mmmm	GAGUGGAGCGCCU	486
CTGF-747-16-13982	13982	Ch1	oooooooooooo o	m0mm000000m0	CGACUGGAAGACA	487
CTGF-817-16-13983	13983	Ch1	oooooooooooo o	0000mmmmUmmm	GGAGCGCCUGUUC	488

- 181 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
CTGF-1174-16-13984	13984	Ch1	oooooooooooo o	0mm0mm0m00mm0	GCCAUUACAACUG	489
CTGF-1005-16-13985	13985	Ch1	oooooooooooo o	000mmmmmm00mm	GAGCUUUCUGGCU	490
CTGF-814-16-13986	13986	Ch1	oooooooooooo o	00m0000mmmm0	AGUGGAGCGCCUG	491
CTGF-816-16-13987	13987	Ch1	oooooooooooo o	m0000mmmm0mm	UGGAGCGCCUGUU	492
CTGF-1001-16-13988	13988	Ch1	oooooooooooo o	0mmm000mmmmmm	GUUUGAGCUUUCU	493
CTGF-1173-16-13989	13989	Ch1	oooooooooooo o	m0mm0mm0m00mm	UGCCAUUACAACU	494
CTGF-749-16-13990	13990	Ch1	oooooooooooo o	0mm000000m0m	ACUGGAAGACACG	495
CTGF-792-16-13991	13991	Ch1	oooooooooooo o	00mm0mmm00mmm	AACUGCCUGGUCC	496
CTGF-1162-16-13992	13992	Ch1	oooooooooooo o	000mmmm0m0mmm0	AGACCUGUGCCUG	497
CTGF-811-16-13993	13993	Ch1	oooooooooooo o	m0000m0000mm	CAGAGUGGAGCGC	498
CTGF-797-16-13994	13994	Ch1	oooooooooooo o	mmm00mmm000mm	CCUGGUCCAGACC	499
CTGF-1175-16-13995	13995	Ch1	oooooooooooo o	mm0mm0m00mm0m	CCAUUACAACUGU	500
CTGF-1172-16-13996	13996	Ch1	oooooooooooo o	mm0mm0mm0m00m	CUGCCAUUACAAC	501
CTGF-1177-16-13997	13997	Ch1	oooooooooooo o	0mm0m00mm0mmm	AUUACAACUGUCC	502
CTGF-1176-16-13998	13998	Ch1	oooooooooooo o	m0mm0m00mm0mm	CAUUACAACUGUC	503
CTGF-812-16-13999	13999	Ch1	oooooooooooo o	0000m0000mmm	AGAGUGGAGCGCC	504
CTGF-745-16-14000	14000	Ch1	oooooooooooo o	0mm0mm000000	ACCGACUGGAAGA	505
CTGF-1230-16-14001	14001	Ch1	oooooooooooo o	0m0m0m0000m0	AUGUACGGAGACA	506
CTGF-920-16-14002	14002	Ch1	oooooooooooo o	0mmmm0m000mm	GCCUUGCGAAGCU	507
CTGF-679-16-14003	14003	Ch1	oooooooooooo o	0mm0m00000m0	GCUGCGAGGAGUG	508
CTGF-992-16-14004	14004	Ch1	oooooooooooo o	0mmm0mm000mmm	GCCUAUCAAGUUU	509
CTGF-1045-16-14005	14005	Ch1	oooooooooooo o	00mmmm0m0000m	AAUUCUGUGGAGU	510
CTGF-1231-16-14006	14006	Ch1	oooooooooooo o	m0m0m0000m0m	UGUACGGAGACAU	511
CTGF-991-16-14007	14007	Ch1	oooooooooooo o	00mmmm0mm000mm	AGCCUAUCAAGUU	512
CTGF-998-16-14008	14008	Ch1	oooooooooooo o	m000mmmm000mmm	CAAGUUUGAGCUU	513
CTGF-1049-16-14009	14009	Ch1	oooooooooooo o	mm0m0000m0m0m	CUGUGGAGUAUGU	514
CTGF-1044-16-14010	14010	Ch1	oooooooooooo o	000mmmm0m0000	AAAUUCUGUGGAG	515

- 182 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
CTGF-1327-16-14011	14011	Ch1	oooooooooooo o	mmmm00m00m0m0	UUUCAGUAGCACA	516
CTGF-1196-16-14012	14012	Ch1	oooooooooooo o	m00m00m0mmmm	CAAUGACAUCUUU	517
CTGF-562-16-14013	14013	Ch1	oooooooooooo o	00m0mm00m0m0m	AGUACCAGUGCAC	518
CTGF-752-16-14014	14014	Ch1	oooooooooooo o	000000m0mmmm	GGAAGACACGUUU	519
CTGF-994-16-14015	14015	Ch1	oooooooooooo o	mm0mm000mm00	CUAUCAGUUUGA	520
CTGF-1040-16-14016	14016	Ch1	oooooooooooo o	00mm000mmmm0m	AGCUAAAUUCUGU	521
CTGF-1984-16-14017	14017	Ch1	oooooooooooo o	000m0000m0m00	AGGUAGAAUGUAA	522
CTGF-2195-16-14018	14018	Ch1	oooooooooooo o	00mm00mm00mmm	AGCUGAUCAGUUU	523
CTGF-2043-16-14019	14019	Ch1	oooooooooooo o	mmmm0mmmm000m0	UUCUGCUCAGAU	524
CTGF-1892-16-14020	14020	Ch1	oooooooooooo o	mm0mm000mm00	UUAUCUAAGUUAA	525
CTGF-1567-16-14021	14021	Ch1	oooooooooooo o	m0m0m00m00m0	UAUACGAGUAAUA	526
CTGF-1780-16-14022	14022	Ch1	oooooooooooo o	00mm000m00mmm	GACUGGACAGCUU	527
CTGF-2162-16-14023	14023	Ch1	oooooooooooo o	0m00mmmm0mm0	AUGGCCUUUAUUA	528
CTGF-1034-16-14024	14024	Ch1	oooooooooooo o	0m0mm00mm000	AUACCGAGCUAAA	529
CTGF-2264-16-14025	14025	Ch1	oooooooooooo o	mm0mm00000m0m	UUGUUGAGAGUGU	530
CTGF-1032-16-14026	14026	Ch1	oooooooooooo o	0m0m0mm00mm0	ACAUACCGAGCUA	531
CTGF-1535-16-14027	14027	Ch1	oooooooooooo o	00m0000000mm0	AGCAGAAAGGUUA	532
CTGF-1694-16-14028	14028	Ch1	oooooooooooo o	00mm0mmmmmm00	AGUUGUUCUUAA	533
CTGF-1588-16-14029	14029	Ch1	oooooooooooo o	0mmm0000m0m00	AUUUGAAGUGUAA	534
CTGF-928-16-14030	14030	Ch1	oooooooooooo o	000mm00mm000	AAGCUGACCUGGA	535
CTGF-1133-16-14031	14031	Ch1	oooooooooooo o	00mm0m0000000	GGUCAUGAAGAAG	536
CTGF-912-16-14032	14032	Ch1	oooooooooooo o	0m00mm000mmmm	AUGGUCAGGCCUU	537
CTGF-753-16-14033	14033	Ch1	oooooooooooo o	00000m0mmmm0	GAAGACACGUUUG	538
CTGF-918-16-14034	14034	Ch1	oooooooooooo o	000mmmm0m000	AGGCCUUGCGAAG	539
CTGF-744-16-14035	14035	Ch1	oooooooooooo o	m0mm0mm00000	UACCGACUGGAAG	540
CTGF-466-16-14036	14036	Ch1	oooooooooooo o	0mmm0000mm0	ACCGCAAGAUCGG	541
CTGF-917-16-14037	14037	Ch1	oooooooooooo o	m000mmmm0m00	CAGGCCUUGCGAA	542

- 183 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
CTGF-1038-16-14038	14038	Ch1	oooooooooooo o	m00mm000mmmm	CGAGCUAAAUUCU	543
CTGF-1048-16-14039	14039	Ch1	oooooooooooo o	mmm0m0000m0m0	UCUGUGGAGUAUG	544
CTGF-1235-16-14040	14040	Ch1	oooooooooooo o	m0000m0m00m0	CGGAGACAUGGCA	545
CTGF-868-16-14041	14041	Ch1	oooooooooooo o	0m00m00mmmm	AUGACAACGCCUC	546
CTGF-1131-16-14042	14042	Ch1	oooooooooooo o	0000mm0m00000	GAGGUCAUGAAGA	547
CTGF-1043-16-14043	14043	Ch1	oooooooooooo o	m000mmmm0m000	UAAAUUCUGUGGA	548
CTGF-751-16-14044	14044	Ch1	oooooooooooo o	m000000m0mmm	UGGAAGACACGUU	549
CTGF-1227-16-14045	14045	Ch1	oooooooooooo o	0000m0m0m000	AAGAUGUACGGAG	550
CTGF-867-16-14046	14046	Ch1	oooooooooooo o	00m00m00mmmm	AAUGACAACGCCU	551
CTGF-1128-16-14047	14047	Ch1	oooooooooooo o	00m000mm0m00	GGCGAGGUCAUGA	552
CTGF-756-16-14048	14048	Ch1	oooooooooooo o	00m0m0mmm00mm	GACACGUUUGGCC	553
CTGF-1234-16-14049	14049	Ch1	oooooooooooo o	0m00000m0m00m	ACGGAGACAUGGC	554
CTGF-916-16-14050	14050	Ch1	oooooooooooo o	mm000mmmm0m00	UCAGGCCUUGCGA	555
CTGF-925-16-14051	14051	Ch1	oooooooooooo o	0m0000mm00mmm	GCGAAGCUGACCU	556
CTGF-1225-16-14052	14052	Ch1	oooooooooooo o	000000m0m0m00	GGAAGAUGUACGG	557
CTGF-445-16-14053	14053	Ch1	oooooooooooo o	0m00mmmm00mmm	GUGACUUCGGCUC	558
CTGF-446-16-14054	14054	Ch1	oooooooooooo o	m00mmmm00mmmm	UGACUUCGGCUCC	559
CTGF-913-16-14055	14055	Ch1	oooooooooooo o	m00mm000mmmm0	UGGUCAGGCCUUG	560
CTGF-997-16-14056	14056	Ch1	oooooooooooo o	mm000mmmm000mm	UCAAGUUUGAGCU	561
CTGF-277-16-14057	14057	Ch1	oooooooooooo o	0mm0000mm0m00	GCCAGAACUGCAG	562
CTGF-1052-16-14058	14058	Ch1	oooooooooooo o	m0000m0m0m0mm	UGGAGUAUGUACC	563
CTGF-887-16-14059	14059	Ch1	oooooooooooo o	0mm000000m00	GCUAGAGAAGCAG	564
CTGF-914-16-14060	14060	Ch1	oooooooooooo o	00mm000mmmm0m	GGUCAGGCCUUGC	565
CTGF-1039-16-14061	14061	Ch1	oooooooooooo o	000mm000mmmm0	GAGCUAAAUUCUG	566
CTGF-754-16-14062	14062	Ch1	oooooooooooo o	0000m0m0mmm00	AAGACACGUUUGG	567
CTGF-1130-16-14063	14063	Ch1	oooooooooooo o	m0000mm0m0000	CGAGGUCAUGAAG	568
CTGF-919-16-14064	14064	Ch1	oooooooooooo o	00mmmm0m0000m	GGCCUUGCGAAGC	569

- 184 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
CTGF-922-16-14065	14065	Ch1	oooooooooooo o	mmm0m0000mm00	CUUGCGAAGCUGA	570
CTGF-746-16-14066	14066	Ch1	oooooooooooo o	mm00mm000000m	CCGACUGGAAGAC	571
CTGF-993-16-14067	14067	Ch1	oooooooooooo o	mmm0mm000mmm0	CCUAUCAAGUUUG	572
CTGF-825-16-14068	14068	Ch1	oooooooooooo o	m0mmmm0000mmm	UGUUGCAAGACCU	573
CTGF-926-16-14069	14069	Ch1	oooooooooooo o	m0000mm00mmm0	CGAAGCUGACCUG	574
CTGF-923-16-14070	14070	Ch1	oooooooooooo o	mm0m0000mm00m	UUGCGAAGCUGAC	575
CTGF-866-16-14071	14071	Ch1	oooooooooooo o	m00m00m00m0mm	CAAUGACAACGCC	576
CTGF-563-16-14072	14072	Ch1	oooooooooooo o	0m0mm00m0m0m0	GUACCAGUGCACG	577
CTGF-823-16-14073	14073	Ch1	oooooooooooo o	mmm0mmmm0000m	CCUGUUGCAAGAC	578
CTGF-1233-16-14074	14074	Ch1	oooooooooooo o	m0m00000m0m00	UACGGAGACAUGG	579
CTGF-924-16-14075	14075	Ch1	oooooooooooo o	m0m0000mm00mm	UGCGAAGCUGACC	580
CTGF-921-16-14076	14076	Ch1	oooooooooooo o	mmmm0m0000mm0	CCUUGCGAAGCUG	581
CTGF-443-16-14077	14077	Ch1	oooooooooooo o	mm0m00mmmm00m	CUGUGACUUCGGC	582
CTGF-1041-16-14078	14078	Ch1	oooooooooooo o	0mm000mmmm0m0	GCUAAAUUCUGUG	583
CTGF-1042-16-14079	14079	Ch1	oooooooooooo o	mm000mmmm0m00	CUAAAUUCUGUGG	584
CTGF-755-16-14080	14080	Ch1	oooooooooooo o	000m0m0mm00m	AGACACGUUUGGC	585
CTGF-467-16-14081	14081	Ch1	oooooooooooo o	mm0m0000mm00m	CCGCAAGAUCGGC	586
CTGF-995-16-14082	14082	Ch1	oooooooooooo o	m0mm000mmm000	UAUCAAGUUUGAG	587
CTGF-927-16-14083	14083	Ch1	oooooooooooo o	0000mm00mmm00	GAAGCUGACCUGG	588
SPP1-1091-16-14131	14131	Ch1	oooooooooooo o	0m0mm00mm000	GCAUUUAGUCAAA	589
PPIB--16-14188	14188	Ch1	oooooooooooo o	mmmmmmmmmmmm	GGCUACAAAACA	590
PPIB--17-14189	14189	Ch1	oooooooooooo ooo	mm00mm0m00000 m0	UUGGCUACAAAA CA	591
PPIB--18-14190	14190	Ch1	oooooooooooo ooooo	0mm00mm0m000 00m0	AUUUGGCUACAAA AACA	592
pGL3-1172-16-14386	14386	chl	oooooooooooo o	0m000m0m00mmm	ACAAUACGAUUU	593
pGL3-1172-16-14387	14387	chl	oooooooooooo o	DY5470m000m0m 00mmm	ACAAUACGAUUU	594
MAP4K4-2931-25-14390	14390	Ch1	oooooooooooo oooooooooooo o	Pmmmmmmmmmmmm 000mmmmmmmmmm	CUUUGAAGAGUUC UGUGGAAGUCUA	595
miR-122--23-14391	14391	Ch1	ssoooooooooooo ooooooooossss	mmmmmmmmmmmm mmmmmmmmmm	ACAAACACCAUUG UCACACUCCA	596

- 185 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
	14084	Ch1	oooooooooooo o	mmm0m000mm000	CUCAUGAAUUAGA	719
	14085	Ch1	oooooooooooo o	mm0000mm00mm0	CUGAGGUCAAUUA	720
	14086	Ch1	oooooooooooo o	0000mm00mm000	GAGGUCAAUUAAA	721
	14087	Ch1	oooooooooooo o	mmm0000mm00mm	UCUGAGGUCAAUU	722
	14088	Ch1	oooooooooooo o	m0000mm00mm00	UGAGGUCAAUUAA	723
	14089	Ch1	oooooooooooo o	mmmm0000mm00m	UUCUGAGGUCAAU	724
	14090	Ch1	oooooooooooo o	0mm00mm000m00	GUCAGCUGGAUGA	725
	14091	Ch1	oooooooooooo o	mmmm00m000mmm	UUCUGAUGAAUCU	726
	14092	Ch1	oooooooooooo o	m000mm0000mm0	UGGACUGAGGUCA	727
	14093	Ch1	oooooooooooo o	000mmmm0mm0mm	GAGUCUCACCAUU	728
	14094	Ch1	oooooooooooo o	00mm0000mm000	GACUGAGGUCAAA	729
	14095	Ch1	oooooooooooo o	mm0m00mm0m000	UCACAGCCAUGAA	730
	14096	Ch1	oooooooooooo o	00mmmm0mm0mmm	AGUCUCACCAUUC	731
	14097	Ch1	oooooooooooo o	000m00000mm0	AAGCGGAAAGCCA	732
	14098	Ch1	oooooooooooo o	00m00000mm00	AGCGGAAAGCCAA	733
	14099	Ch1	oooooooooooo o	0mm0m0m000m00	ACCACAUGGAUGA	734
	14100	Ch1	oooooooooooo o	0mm0m00mm0m0m	GCCAUGACCACAU	735
	14101	Ch1	oooooooooooo o	000mm0m00mm0m	AAGCCAUGACCAC	736
	14102	Ch1	oooooooooooo o	0m00000mm00m	GCGGAAAGCCAAU	737
	14103	Ch1	oooooooooooo o	000mmmm0mmm	AAAUUCGUUUUU	738
	14104	Ch1	oooooooooooo o	0mmmm0mmmmmm	AUUUCGUUUUCU	739
	14105	Ch1	oooooooooooo o	0000mm0m00mm0	AAAGCCAUGACCA	740
	14106	Ch1	oooooooooooo o	0m0m000m00m0m	ACAUGGAUGAUAU	741
	14107	Ch1	oooooooooooo o	0000mmmmmm0mm	GAAAUUCGUUUU	742
	14108	Ch1	oooooooooooo o	0mmmmmm00mm	GCGCCUUCUGAUU	743
	14109	Ch1	oooooooooooo o	0mmmmmm0m000m	AUUUCUCAUGAAU	744
	14110	Ch1	oooooooooooo o	mmmmmm0m000m00	CUCUCAUGAAUAG	745

- 186 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
	14111	Ch1	oooooooooooo o	000mmm00m000	AAGUCCAACGAAA	746
	14112	Ch1	oooooooooooo o	0m00m00000m00	AUGAUGAGAGCAA	747
	14113	Ch1	oooooooooooo o	0m00000mm000	GCGAGGAGUUGAA	748
	14114	Ch1	oooooooooooo o	m00mm00m00mm0	UGAUUGAUAGUCA	749
	14115	Ch1	oooooooooooo o	000m00m0m0mmm	AGAUAGUGCAUCU	750
	14116	Ch1	oooooooooooo o	0m0m0m0mmm0mm	AUGUGUAUCUAUU	751
	14117	Ch1	oooooooooooo o	mmmm0m0000000	UUCUAUAGAAGAA	752
	14118	Ch1	oooooooooooo o	mm0mmm00m00mm	UUGUCCAGCAAUU	753
	14119	Ch1	oooooooooooo o	0m0m000000m0	ACAUGGAAAGCGA	754
	14120	Ch1	oooooooooooo o	0m00mmm000mm0	GCAGUCCAGAUUA	755
	14121	Ch1	oooooooooooo o	m00mm000m0m0m	UGGUUGAAUGUGU	756
	14122	Ch1	oooooooooooo o	mm0m0000m00m	UUAUGAAACGAGU	757
	14123	Ch1	oooooooooooo o	m00mmm000mm0m	CAGUCCAGAUUAU	758
	14124	Ch1	oooooooooooo o	0m0m000m0000	AUAUAAGCGGAAA	759
	14125	Ch1	oooooooooooo o	m0mm00mm000m0	UACCAGUUAACA	760
	14126	Ch1	oooooooooooo o	m0mmm0mmm0m0	UGUUCAUUCUAUA	761
	14127	Ch1	oooooooooooo o	mm0mm0000000	CCGACCAAGGAAA	762
	14128	Ch1	oooooooooooo o	000m00m0m0m0m	GAAUGGUGCAUAC	763
	14129	Ch1	oooooooooooo o	0m0m00m00mm0	AUAUGAUGGCCGA	764
	14130	Ch1	oooooooooooo o	00m00mmm000mm	AGCAGUCCAGAUU	765
	14132	Ch1	oooooooooooo o	00m0mmm0m0m	AGCAUUCGAGUGU	766
	14133	Ch1	oooooooooooo o	m00mm00000mmm	UAGUCAGGAACUU	767
	14134	Ch1	oooooooooooo o	m0m0mmm00mm00	UGCAUUUAGUCAA	768
	14135	Ch1	oooooooooooo o	0mmm00m000mmm	GUCUGAUGAGUCU	769
	14136	Ch1	oooooooooooo o	m000m0m0m000	UAGACACAUUGA	770
	14137	Ch1	oooooooooooo o	m000m0000m0m	CAGACGAGGACAU	771
	14138	Ch1	oooooooooooo o	m00mmm000mmm	CAGCCGUGAAUUC	772

- 187 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
	14139	Ch1	oooooooooooo o	00mmm00000m00	AGUCUGGAAAUA	773
	14140	Ch1	oooooooooooo o	00mmm0m00mmm	AGUUUGUGGCUUC	774
	14141	Ch1	oooooooooooo o	00mmm00m0000	AGUCCAACGAAAG	775
	14142	Ch1	oooooooooooo o	000mmmm000m	AAGUUUCGCAGAC	776
	14143	Ch1	oooooooooooo o	00m00m000m0mm	AGCAAUGAGCAUU	777
	14144	Ch1	oooooooooooo o	mm000m00m0m0m	UUAGAUAGUGCAU	778
	14145	Ch1	oooooooooooo o	m00m0m0m0m000	UGGUGCAUACAAG	779
	14146	Ch1	oooooooooooo o	0m0000m00mm0	AUGAAACGAGUCA	780
	14147	Ch1	oooooooooooo o	mm0000m0mm000	CCAGAGUGCUGAA	781
	14148	Ch1	oooooooooooo o	m00mm0m000mmm	CAGCCAUGAAUUU	782
	14149	Ch1	oooooooooooo o	0mm00mm000m0m	AUUGGUUGAAUGU	783
	14150	Ch1	oooooooooooo o	00mm000m0m0m0	GGUUGAAUGUGUA	784
	14151	Ch1	oooooooooooo o	00000m00mm00m	GGAAUAACUAAU	785
	14152	Ch1	oooooooooooo o	mm0m000m00000	UCAUGAAUAGAAA	786
	14153	Ch1	oooooooooooo o	0mm00m00mm00	GCCAGCAACCGAA	787
	14154	Ch1	oooooooooooo o	m0mmmm0m0m0m0	CACCUCACACAUG	788
	14155	Ch1	oooooooooooo o	00mm000m00m0m	AGUUGAAUGGUGC	789
	14156	Ch1	oooooooooooo o	00mm00mm000m0	AGUCAGCUGGAUG	790
	14157	Ch1	oooooooooooo o	m0m000m00000	UAUAAGCGGAAAG	791
	14158	Ch1	oooooooooooo o	mmmm0m0m00mm	UUCCGAUGUGAUU	792
	14159	Ch1	oooooooooooo o	0m00mm00m0m0m	AUAACUAAUGUGU	793
	14160	Ch1	oooooooooooo o	mm0mmmm0m0000	UCAUUCUAUAGAA	794
	14161	Ch1	oooooooooooo o	00mm0mm0mm0m0	AACUAUCACUGUA	795
	14162	Ch1	oooooooooooo o	0mm00mm0mmm0m	GUCAAUUGCUUUAU	796
	14163	Ch1	oooooooooooo o	00m00mm00m000	AGCAAUUAUAAA	797
	14164	Ch1	oooooooooooo o	0m0mmmm00m00	ACGACUCUGAUGA	798
	14165	Ch1	oooooooooooo o	m00m0m00mmm0m	UAGUGUGGUUUAU	799

- 188 -

ID Number	Oligo Number	OHang Sense Chem.	Sense Backbone	Sense Chemistry	Sense Sequence	SEQ ID NO:
	14166	Ch1	oooooooooooo o	000mm00m00m00	AAGCCAAUGAUGA	800
	14167	Ch1	oooooooooooo o	0m00mm00000mm	AUAGUCAGGAACU	801
	14168	Ch1	oooooooooooo o	00mm00mmm000	AGUCAGCCGUGAA	802
	14169	Ch1	oooooooooooo o	0mm0mm0m00000	ACUACCAUGAGAA	803
	14170	Ch1	oooooooooooo o	000m000mm00mm	AAACAGGCUGAUU	804
	14171	Ch1	oooooooooooo o	000m0mm0000mm	GAGUGCUGAAACC	805
	14172	Ch1	oooooooooooo o	m000m0mmmm0m	UGAGCAUCCGAU	806
	14173	Ch1	oooooooooooo o	00mmmm0m00mm0	AAUCCACAGCCA	807
	14174	Ch1	oooooooooooo o	m0mm00mm0mm0	UGUCAAUUGCUGA	808
	14175	Ch1	oooooooooooo o	0mm0m00000mm0	ACCAUGAGAAUUG	809
	14176	Ch1	oooooooooooo o	mm00m0000mm0	CCAACGAAAGCCA	810
	14177	Ch1	oooooooooooo o	mm00mm0mm00mm	CUGGUCACUGAUU	811
	14178	Ch1	oooooooooooo o	m00mmm0m000mm	UGGUUUAUGGACU	812
	14179	Ch1	oooooooooooo o	00mm0000m0mm0	GACCAGAGUGCUG	813
	14180	Ch1	oooooooooooo o	00m0m00mm00m0	GAUGUGAUUGAUA	814
	14181	Ch1	oooooooooooo o	0mm00mmm000m	GUCAGCCGUGAAU	815
	14182	Ch1	oooooooooooo o	00m0m0m0mm0m	AAUGUGUAUCUAU	816
	14183	Ch1	oooooooooooo o	mm000mmm00000	UUGAGUCUGGAAA	817
	14184	Ch1	oooooooooooo o	0mmm00m00mm00	GUCCAGCAAUUA	818
	14185	Ch1	oooooooooooo o	mm00m00mm00m0	CCAGCAAUUAUA	819
	14186	Ch1	oooooooooooo o	00mmm00m0mm	GACUCGAACGACU	820
	14187	Ch1	oooooooooooo o	0mmm0mm00m00m	ACCUGCCAGCAAC	821

TABLE 3: Sense backbone, chemistry, and sequence information. o: phosphodiester; s: phosphorothioate; P: 5' phosphorylation; 0: 2'-OH; F: 2'-fluoro; m: 2' O-methyl; +: LNA modification. Capital letters in the sequence signify ribonucleotides, lower case letters signify deoxyribonucleotides.

- 189 -

Having thus described several aspects of at least one embodiment of this invention, it is to be appreciated various alterations, modifications, and improvements will readily occur to those skilled in the art. Such alterations, modifications, and improvements are intended to be part of this disclosure, and are intended to be within the spirit and scope of the invention. Accordingly, the foregoing description and drawings are by way of example only.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

All references, including patent documents, disclosed herein are incorporated by reference in their entirety. This application incorporates by reference the entire contents, including all the drawings and all parts of the specification (including sequence listing or amino acid / polynucleotide sequences) of the co-pending U.S. Provisional Application No. 61/135,855, filed on July 24, 2008, entitled "SHORT HAIRPIN RNAI CONSTRUCTS AND USES THEROF," and U.S. Provisional Application No. 61/197,768, filed on October 30, 2008, entitled "MINIRNA CONSTRUCTS AND USES THEREOF."

What is claimed is:

- 190 --

CLAIMS

1. An isolated asymmetric nucleic acid molecule comprising
a guide strand, with a minimal length of 16 nucleotides, and a passenger strand
forming a double stranded nucleic acid, having a double stranded region and a single
5 stranded region, the double stranded region having 8-15 nucleotides in length, the single
stranded region having 5-12 nucleotides in length, wherein the passenger strand is linked to
a lipophilic group, wherein at least 40% of the nucleotides of the double stranded nucleic
acid are modified, and wherein the single stranded region has at least 2 phosphorothioate
modifications.

10 2. The isolated asymmetric nucleic acid molecule of claim 1 wherein position 1
of the guide strand is 5' phosphorylated

3. The isolated asymmetric nucleic acid molecule of claim 1 wherein position 1
of the guide strand is 2'O-methyl modified and 5' phosphorylated.

4. An isolated double stranded nucleic acid molecule comprising
15 a longer strand of 15-21 nucleotides in length that has complementarity to a miRNA
sequence,
a shorter strand of 8-16 nucleotides in length linked at the 3' end to a lipophilic
group,

wherein the longer strand and the passenger strand form the double stranded nucleic
20 acid molecule having a double stranded region and a single stranded region, wherein the
longer strand has a 3' single stranded region of 2-13 nucleotides in length, comprising at
least two phosphorothioate modification, and at least 50% nucleotides are modified.

5. An isolated double stranded nucleic acid molecule comprising
a guide strand of 17-21 nucleotides in length that has complementarity to a target
25 gene,

a passenger strand of 8-16 nucleotides in length linked at the 3' end to a lipophilic
group,

wherein the guide strand and the passenger strand form the double stranded nucleic
acid molecule having a double stranded region and a single stranded region, wherein the
30 guide strand has a 3' single stranded region of 2-13 nucleotides in length, each nucleotide
within the single stranded region having a phosphorothioate modification, wherein the guide

- 191 --

strand has a 5' phosphate modification and wherein at least 50% of C and U nucleotides in the double stranded region include at least one 2' O-methyl modification or 2'-fluoro modification.

6. An isolated double stranded nucleic acid molecule comprising
5 a guide strand of 17-21 nucleotides in length that has complementarity to a target gene,
a passenger strand of 10-16 nucleotides in length linked at the 3' end to a lipophilic group,

wherein the guide strand and the passenger strand form the double stranded nucleic
10 acid molecule having a double stranded region and a single stranded region, wherein the guide strand has a 3' single stranded region of 5-11 nucleotides in length, at least two nucleotide within the single stranded region having a phosphorothioate modification, wherein the guide strand has a 5' phosphate modification and wherein at least 50% of C and U nucleotides in the double stranded region are 2' O-methyl modification or 2'-fluoro
15 modified.

7. An isolated double stranded nucleic acid molecule comprising
a guide strand of 17-21 nucleotides in length that has complementarity to a target gene,

a passenger strand of 8-16 nucleotides in length linked at the 3' end to a lipophilic
20 group,

wherein the guide strand and the passenger strand form the double stranded nucleic acid molecule having a double stranded region and a single stranded region, wherein the guide strand has a 3' single stranded region of 6-8 nucleotides in length, each nucleotide within the single stranded region having a phosphorothioate modification, wherein the guide
25 strand has a 5' phosphate modification, wherein the passenger strand includes at least two phosphorothioate modifications, wherein at least 50% of C and U nucleotides in the double stranded region include a 2' O-methyl modification or 2'-fluoro modification, and wherein the double stranded nucleic acid molecule has one end that is blunt or includes a one-two nucleotide overhang.

8. An isolated double stranded nucleic acid molecule comprising

- 192 -

a guide strand of 17-21 nucleotides in length that has complementarity to a target gene,

a passenger strand of 8-16 nucleotides in length linked at the 3' end to a lipophilic group,

5 wherein the guide strand and the passenger strand form the double stranded nucleic acid molecule having a double stranded region and a single stranded region, wherein the guide strand has a 3' single stranded region, each nucleotide within the single stranded region having a phosphorothioate modification, wherein the guide strand has a 5' phosphate modification, wherein every C and U nucleotide in position 11-18 of the guide strand has a
10 2' O-methyl modification, wherein every nucleotide of the passenger strand is 2' O-methyl modified, and wherein the double stranded nucleic acid molecule has one end that is blunt or includes a one-two nucleotide overhang.

9. An isolated double stranded nucleic acid molecule comprising
a guide strand of 17-21 nucleotides in length that has complementarity to a target
15 gene,

a passenger strand of 8-15 nucleotides in length linked at the 3' end to a lipophilic group, wherein the lipophilic group is selected from the group consisting of cholesterol and a sterol type molecule with C17 polycarbon chain of 5-7 or 9-18 carbons in length,

wherein the guide strand and the passenger strand form the double stranded nucleic
20 acid molecule having a double stranded region and a single stranded region, wherein the guide strand has a 3' single stranded region, each nucleotide within the single stranded region having a phosphorothioate modification, wherein the guide strand has a 5' phosphate modification, wherein every C and U nucleotide in position 11-18 of the guide strand has a
25 2' O-methyl modification, wherein every C and U nucleotide in position 2-10 of the guide strand has a 2'F modification, wherein every nucleotide of the passenger strand is 2' O-methyl modified, and wherein the double stranded nucleic acid molecule has one end that is blunt or includes a one-two nucleotide overhang.

10. An isolated nucleic acid molecule comprising
a guide sequence that has complementarity to a target gene,
30 a passenger sequence linked at the 3' end to a lipophilic group,

- 193 --

wherein the guide sequence and the passenger sequence form a nucleic acid molecule having a double stranded region and a single stranded region, wherein the guide sequence has a 3' single stranded region of 2-13 nucleotides in length, each nucleotide within the single stranded region having a phosphorothioate modification, wherein the guide
5 sequence has a 5' phosphate modification, wherein at least 50% of C and U nucleotides in the double stranded region include at least one 2' O-methyl modification or 2'-fluoro modification, and wherein the double stranded nucleic acid molecule has one end that is blunt or includes a one-two nucleotide overhang.

11. An isolated double stranded nucleic acid molecule comprising a guide strand
10 and a passenger strand, wherein the region of the molecule that is double stranded is from 8-14 nucleotides long, wherein the guide strand contains a single stranded region that is 4-12 nucleotides long, and wherein the single stranded region of the guide strand contains 2-12 phosphorothioate modifications.

12. The nucleic acid molecule of claim 11, wherein the guide strand contains 6-8
15 phosphorothioate modifications.

13. The nucleic acid molecule of claim 11 or 12, wherein the single stranded region of the guide strand is 6 nucleotides long.

14. The nucleic acid molecule of any one of claims 11-13, wherein the double stranded region is 13 nucleotides long.

15. The nucleic acid molecule of any one of claims 11-14, wherein the double stranded nucleic acid molecule has one end that is blunt or includes a one-two nucleotide
20 overhang.

16. An isolated double stranded nucleic acid molecule comprising
a guide strand, wherein the guide strand is 16-28 nucleotides long and has
25 complementarity to a target gene, wherein the 3' terminal 10 nucleotides of the guide strand include at least two phosphate modifications, and wherein the guide strand has a 5' phosphate modification and includes at least one 2' O-methyl modification or 2'-fluoro modification, and

a passenger strand, wherein the passenger strand is 8-14 nucleotides long and has
30 complementarity to the guide strand, wherein the passenger strand is linked to a lipophilic group,

- 194 -

wherein the guide strand and the passenger strand form the double stranded nucleic acid molecule.

17. The nucleic acid molecule of any one of claims 1, 7-10 or 16 wherein the nucleotide in position one of the guide strand or sequence has a 2'-O-methyl modification.

18. The nucleic acid molecule of any one of claims 1-4, 6-10. or 16-17 wherein at least one C or U nucleotide in positions 2-10 of the guide strand or sequence has a 2'-fluoro modification.

19. The nucleic acid molecule of claim 18 wherein every C and U nucleotide in positions 2-10 of the guide strand or sequence has a 2'-fluoro modification.

20. The nucleic acid molecule of any one of claims 1-3, 5, 7, 9 or 16-19 wherein at least one C or U nucleotide in positions 11-18 of the guide strand or sequence has a 2'-O-methyl modification.

21. The nucleic acid molecule of claim 20 wherein every C and U nucleotide in positions 11-18 of the guide strand or sequence has a 2'-O-methyl modification.

22. The nucleic acid molecule of any one of claims 1, 2, 4, 5 or 16-21 wherein the 3' terminal 10 nucleotides of the guide strand include at least four phosphate modifications.

23. The nucleic acid molecule of claim 22 wherein the 3' terminal 10 nucleotides of the guide strand include at least eight phosphate modifications.

24. The nucleic acid molecule of claim 16, wherein the guide strand includes 4-14 phosphate modifications.

25. The nucleic acid molecule of claim 16, wherein the guide strand includes 4-10 phosphate modifications.

26. The nucleic acid molecule of any one of claims 1-5 or 16, wherein the 3' terminal 6 nucleotides of the guide strand all include phosphate modifications.

27. The nucleic acid molecule of any one of claims 16-26, wherein the phosphate modifications are phosphorothioate modifications.

28. The nucleic acid molecule of any one of claims 16-27 wherein every C and U nucleotide on the passenger strand has a 2'-O-methyl modification.

29. The nucleic acid molecule of claim 28 wherein every nucleotide on the passenger strand has a 2'-O-methyl modification.

- 195 --

30. The nucleic acid molecule of any one of claims 16-30 wherein at least one nucleotide on the passenger strand is phosphorothioate modified.

31. The nucleic acid molecule of claim 30 wherein at least two nucleotides on the passenger strand are phosphorothioate modified.

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32. The nucleic acid molecule of any one of claims 1-5 or 16-31 wherein the lipophilic molecule is a sterol.

33. The nucleic acid molecule of claim 32 wherein the sterol is cholesterol.

34. The nucleic acid molecule of any one of claims 16-33, wherein the guide strand
10 is 18-19 nucleotides long.

35. The nucleic acid molecule of any one of claims 16-34, wherein the passenger strand is 11-13 nucleotides long.

36. The nucleic acid molecule of any one of claims 16-35, wherein the double stranded nucleic acid molecule has one end that is blunt or includes a one-two nucleotide
15 overhang.

37. An isolated double stranded nucleic acid molecule comprising a guide strand and a passenger strand, wherein the guide strand is from 16-29 nucleotides long and is substantially complementary to a target gene, wherein the passenger strand is from 8-14 nucleotides long and has complementarity to the guide strand, and wherein the guide strand
20 has at least two chemical modifications.

38. The isolated double stranded nucleic acid molecule of claim 37, wherein the at least two chemical modifications include at least two phosphorothioate modifications.

39. The nucleic acid molecule of any one of claims 37-38, wherein the double stranded nucleic acid molecule has one end that is blunt or includes a one-two nucleotide
25 overhang.

40. An isolated double stranded nucleic acid molecule comprising a guide strand and a passenger strand, wherein the guide strand is from 16-29 nucleotides long and is substantially complementary to a target gene, wherein the passenger strand is from 8-14 nucleotides long and has complementarity to the guide strand, and wherein the guide strand
30 has a single stranded 3' region that is 5 nucleotides or longer and a 5' region that is 1 nucleotide or less.

- 196 -

41. The nucleic acid molecule of claim 40, wherein the single stranded region contains at least 2 phosphorothioate modifications.

42. An isolated double stranded nucleic acid molecule comprising a guide strand and a passenger strand, wherein the guide strand is from 16-29 nucleotides long and is substantially complementary to a target gene, wherein the passenger strand is from 8-16 nucleotides long and has complementarity to the guide strand, and wherein the guide strand has a single stranded 3' region that is 5 nucleotides or longer and a passenger strand has a sterol type molecule with C17 attached chain longer than 9.

43. A duplex polynucleotide comprising:
a first polynucleotide wherein said first polynucleotide is complementary to a second polynucleotide and a target gene; and
a second polynucleotide wherein said second polynucleotide is at least 6 nucleotides shorter than said first polynucleotide,
wherein said first polynucleotide includes a single stranded region containing modifications selected from the group consisting of 40 – 90% hydrophobic base modifications, 40 – 90% phosphorothioates, and 40 – 90% modifications of the ribose moiety, or any combination thereof.

44. A duplex polynucleotide comprising:
a first polynucleotide wherein said first polynucleotide is complementary to a second polynucleotide and a target gene; and
a second polynucleotide wherein said second polynucleotide is at least 6 nucleotides shorter than said first polynucleotide,
wherein the duplex polynucleotide includes a mismatch between nucleotides 9, 11, 12, 13 or 14 on the first polynucleotide and the opposite nucleotide on the second polynucleotide.

45. A method for inhibiting the expression of a target gene in a mammalian cell, comprising contacting the mammalian cell with an isolated double stranded nucleic acid molecule of any one of claims 1-41 or a duplex polynucleotide of claim 43 or 44.

46. A method of inducing RNAi in a subject comprising:

- 197 -

administering to a subject an effective amount for inducing RNAi of an mRNA of a target gene, an isolated double stranded nucleic acid molecule of any one of claims 1-41 or a duplex polynucleotide of claim 43 or 44.

47. The method of claim 46, wherein the subject is a human.

5 48. The method of claim 45, wherein the target gene is PPIB.

49. The method of claim 45, wherein the target gene is MAP4K4.

50. The method of claim 45, wherein the target gene is SOD1.

51. An isolated hydrophobic modified polynucleotide comprising:

10 a polynucleotide, wherein the polynucleotide is double stranded RNA, attached to a hydrophobic molecule, wherein the hydrophobic molecule is attached to a base, a ribose or a backbone of a non-terminal nucleotide and wherein the isolated double stranded nucleic acid molecule comprises a guide strand and a passenger strand, wherein the guide strand is from 16-29 nucleotides long and is substantially complementary to a target gene, wherein the passenger strand is from 8-14 nucleotides long and has complementarity to the guide strand.

15 52. The isolated hydrophobic modified polynucleotide of claim 51, wherein the hydrophobic molecule is attached to the guide strand of the double stranded RNA.

53. The isolated hydrophobic modified polynucleotide of claim 51, wherein the 3' terminal 10 nucleotides of the guide strand include at least two phosphate modifications, and wherein the guide strand has a 5' phosphate modification and includes at least one 2' O-methyl modification or 2'-fluoro modification.

20 54. The isolated hydrophobic modified polynucleotide of claim 51, wherein the hydrophobic molecule is attached to the passenger strand of the double stranded RNA.

55. An isolated hydrophobic modified polynucleotide comprising:

25 a polynucleotide non-covalently complexed to a hydrophobic molecule, wherein the hydrophobic molecule is a polycationic molecule.

56. The isolated hydrophobic modified polynucleotide of claim 55, wherein the polycationic molecule is selected from the group consisting of protamine, arginine rich peptides, and spermine.

57. An isolated hydrophobic modified polynucleotide comprising:

- 198 -

a polynucleotide, wherein the polynucleotide is double stranded RNA, directly complexed to a hydrophobic molecule without a linker, wherein the hydrophobic molecule is not cholesterol.

58. A composition comprising:

5 a hydrophobic modified polynucleotide, wherein the polynucleotide is double stranded RNA, attached to a hydrophobic molecule, wherein the double stranded nucleic acid molecule comprises a guide strand and a passenger strand, wherein the guide strand is from 16-29 nucleotides long and is substantially complementary to a target gene, wherein the passenger strand is from 8-14 nucleotides long and has complementarity to the guide
10 strand, wherein position 1 of the guide strand is 5' phosphorylated or has a 2' O-methyl modification, wherein at least 40% of the nucleotides of the double stranded nucleic acid are modified, and wherein the double stranded nucleic acid molecule has one end that is blunt or includes a one-two nucleotide overhang;

a neutral fatty mixture; and

15 optionally a cargo molecule,

wherein the hydrophobic modified polynucleotide and the neutral fatty mixture forms a micelle.

59. The composition of claim 58, wherein the 3' end of the passenger strand is linked to the hydrophobic molecule.

20 60. The composition of any one of claims 58-60, wherein the composition is sterile.

61. The composition of any one of claims 58-60, wherein the neutral fatty mixture comprises a DOPC (dioleoylphosphatidylcholine).

62. The composition of any one of claims 58-60, wherein the neutral fatty mixture comprises a DSPC (distearoylphosphatidylcholine).

25 63. The composition of any one of claims 58-62, wherein the neutral fatty mixture further comprises a sterol.

64. The composition of claim 62, wherein the sterol is cholesterol.

65. The composition of any one of claims 57-55, wherein the composition includes at least 20% DOPC and at least 20% cholesterol.

30 66. The composition of any one of claims 57-64, wherein the hydrophobic portion of the hydrophobic modified polynucleotide is a sterol.

- 199 -

67. The composition of claim 65, wherein the sterol is a cholesterol, a cholesteryl or modified cholesteryl residue.

68. The composition of any one of claims 57-64, wherein the hydrophobic portion of the hydrophobic modified polynucleotide is selected from the group consisting of bile acids, cholic acid or taurocholic acid, deoxycholate, oleyl lithocholic acid, oleoyl cholenic acid, glycolipids, phospholipids, sphingolipids, isoprenoids, vitamins, saturated fatty acids, unsaturated fatty acids, fatty acid esters, triglycerides, pyrenes, porphyrines, Texaphyrine, adamantane, acridines, biotin, coumarin, fluorescein, rhodamine, Texas-Red, digoxigenin, dimethoxytrityl, t-butyldimethylsilyl, t-butyldiphenylsilyl, cyanine dyes (e.g. Cy3 or Cy5), Hoechst 33258 dye, psoralen, and ibuprofen.

69. The composition of any one of claims 57-64 or 67, wherein the hydrophobic portion of the hydrophobic modified polynucleotide is a polycationic molecule.

70. The composition of claim 18, wherein the polycationic molecule is selected from the group consisting of protamine, arginine rich peptides, and spermine.

71. The composition of any one of claims 57-69, wherein the cargo molecule is a lipid, a peptide, vitamin, or a small molecule.

72. The composition of any one of claims 57-69, wherein the cargo molecule is a commercially available fat emulsions available for a variety of purposes selected from the group consisting of parenteral feeding.

73. The composition of claim 71, wherein the commercially available fat emulsion is an intralipid or a nutralipid.

74. The composition of any one of claims 57-69, wherein the cargo molecule is a fatty acid mixture containing more than 74% of linoleic acid.

75. The composition of any one of claims 57-69, wherein the cargo molecule is a fatty acid mixture containing at least 6% of cardiolipin.

76. The composition of any one of claims 57-69, wherein the cargo molecule is a fatty acid mixture containing at least 74% of linoleic acid and at least 6% of cardiolipin.

77. The composition of any one of claims 57-75, wherein at least 40% of the nucleotides of the the polynucleotide are modified.

78. The composition of any one of claims 57-69, wherein the cargo molecule is a fusogenic lipid and preferably is at least 10% fusogenic lipid.

- 200 -

79. The composition of claims 77, wherein the fusogenic lipid is DOPE.

80. A method of inducing RNAi in a subject comprising:

administering to a subject an effective amount for inducing RNAi of mRNA of a target gene, an isolated double stranded nucleic acid molecule of any one of claims 1-41 or a duplex polynucleotide of claim 43 or 44 or a composition of any one of claims 57-78, wherein the polynucleotide has at least a region of sequence correspondence to the target gene, wherein the step of administering is systemic, intravenous, intraperitoneal, intradermal, topical, intranasal, inhalation, oral, intramucosal, local injection, subcutaneous, oral tracheal, or intraocular.

81. The method of claim 79, wherein the administration is systemic.

82. The method of claim 79, wherein the administration is intravenous.

83. The method of claim 79, wherein the administration is intraperitoneal.

84. The method of claim 79, wherein the administration is intradermal.

85. The method of claim 79, wherein the administration is topical.

86. The method of claim 79, wherein the administration is intraocular.

87. The method of claim 79, wherein the subject is a human.

88. The method of claim 79, wherein the target gene is PPIB.

89. The method of claim 79, wherein the target gene is MAP4K4.

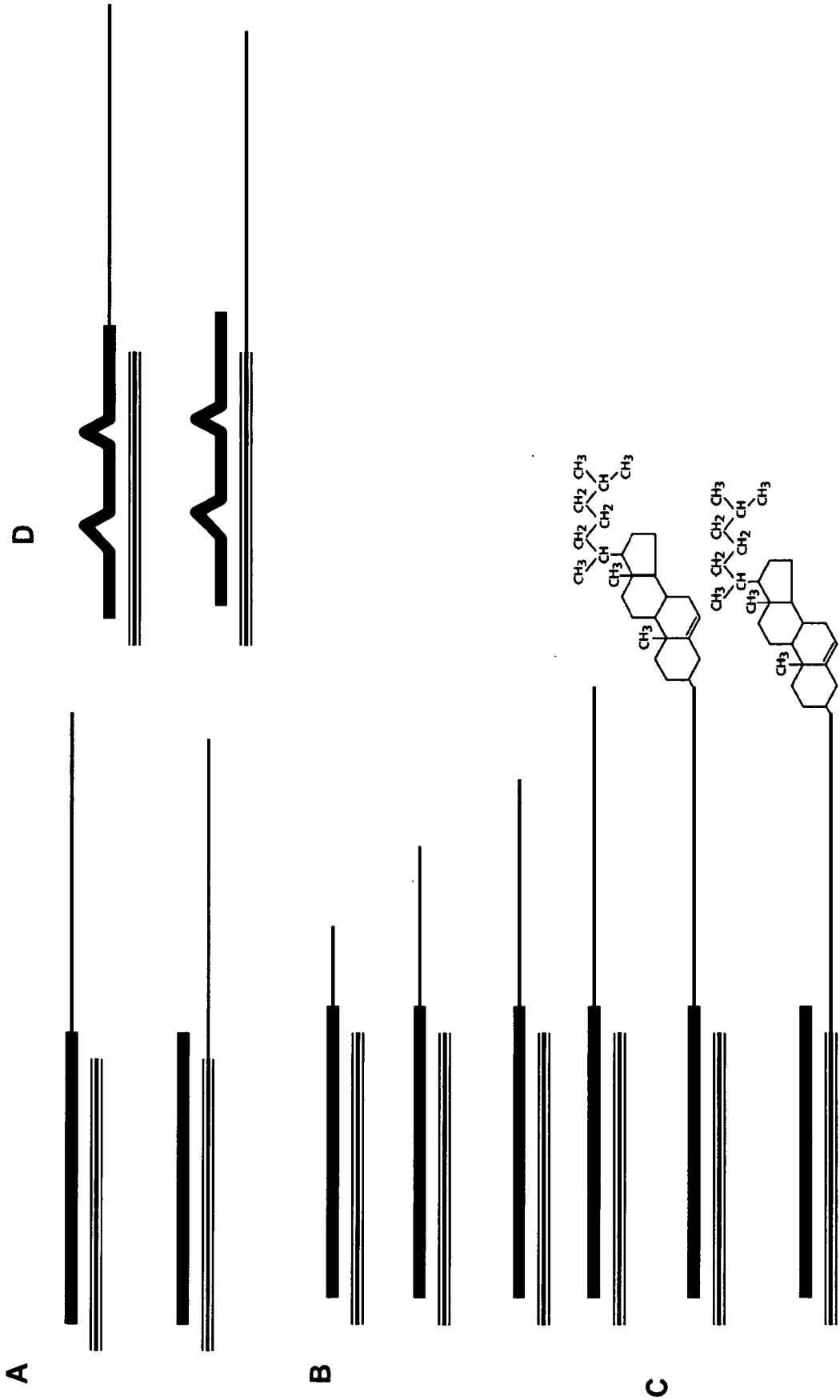
90. The method of claim 79, wherein the target gene is SOD1.

91. An isolated double stranded nucleic acid molecule comprising a guide strand of 17-21 nucleotides in length that has complementarity to a target gene,

a passenger strand of 10-17 nucleotides in length linked at the 3' end to a lipophilic group,

wherein the guide strand and the passenger strand form the double stranded nucleic acid molecule having a double stranded region and a single stranded region, wherein the guide strand has a 3' single stranded region of 5-11 nucleotides in length, at least two nucleotide within the single stranded region having a phosphorothioate modification, wherein the guide strand has a 5' phosphate modification and wherein at least 50% of C and U nucleotides in the double stranded region are 2' O-methyl modification or 2'-fluoro modified.

Figure 1



2/92

Figure 2

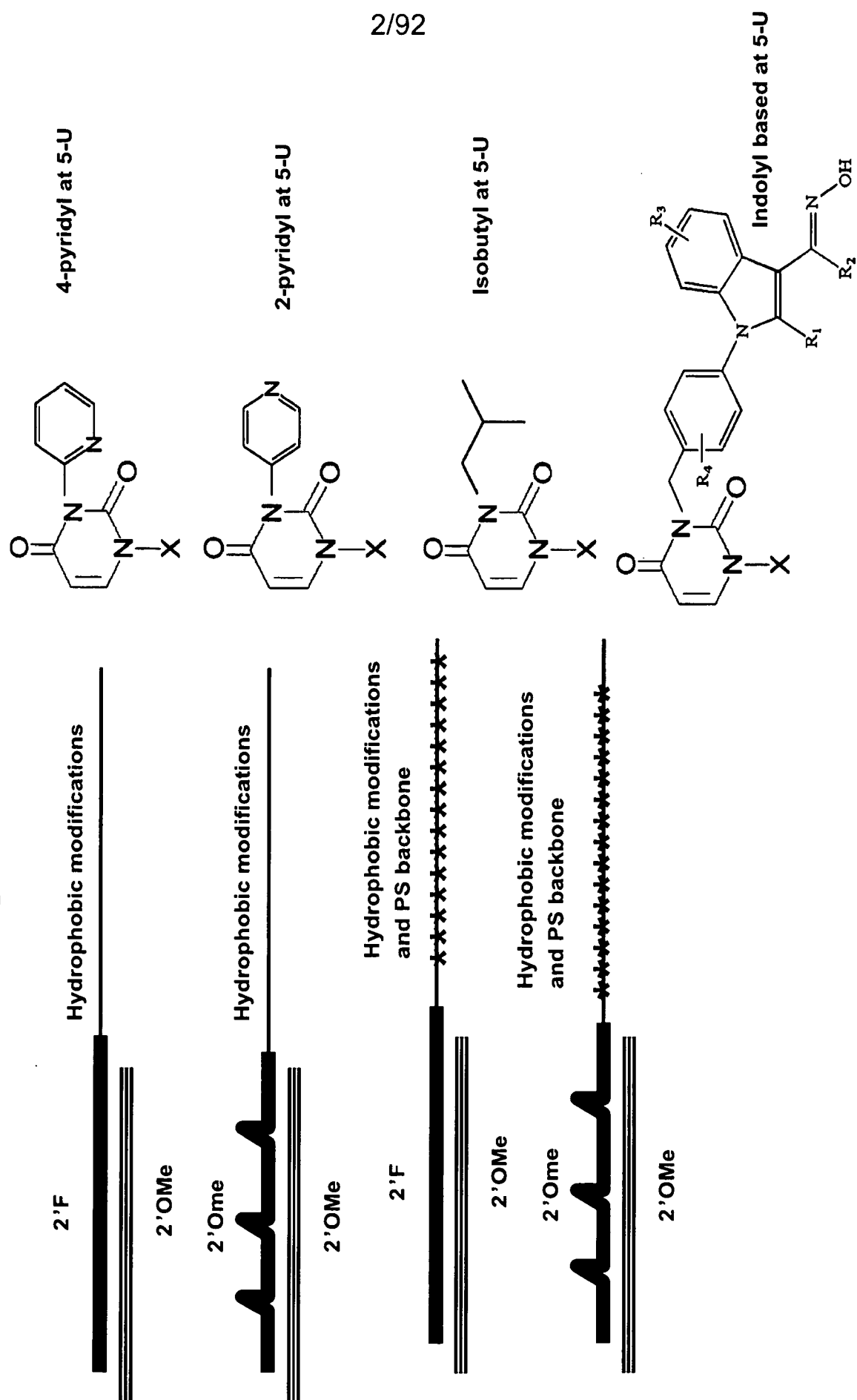
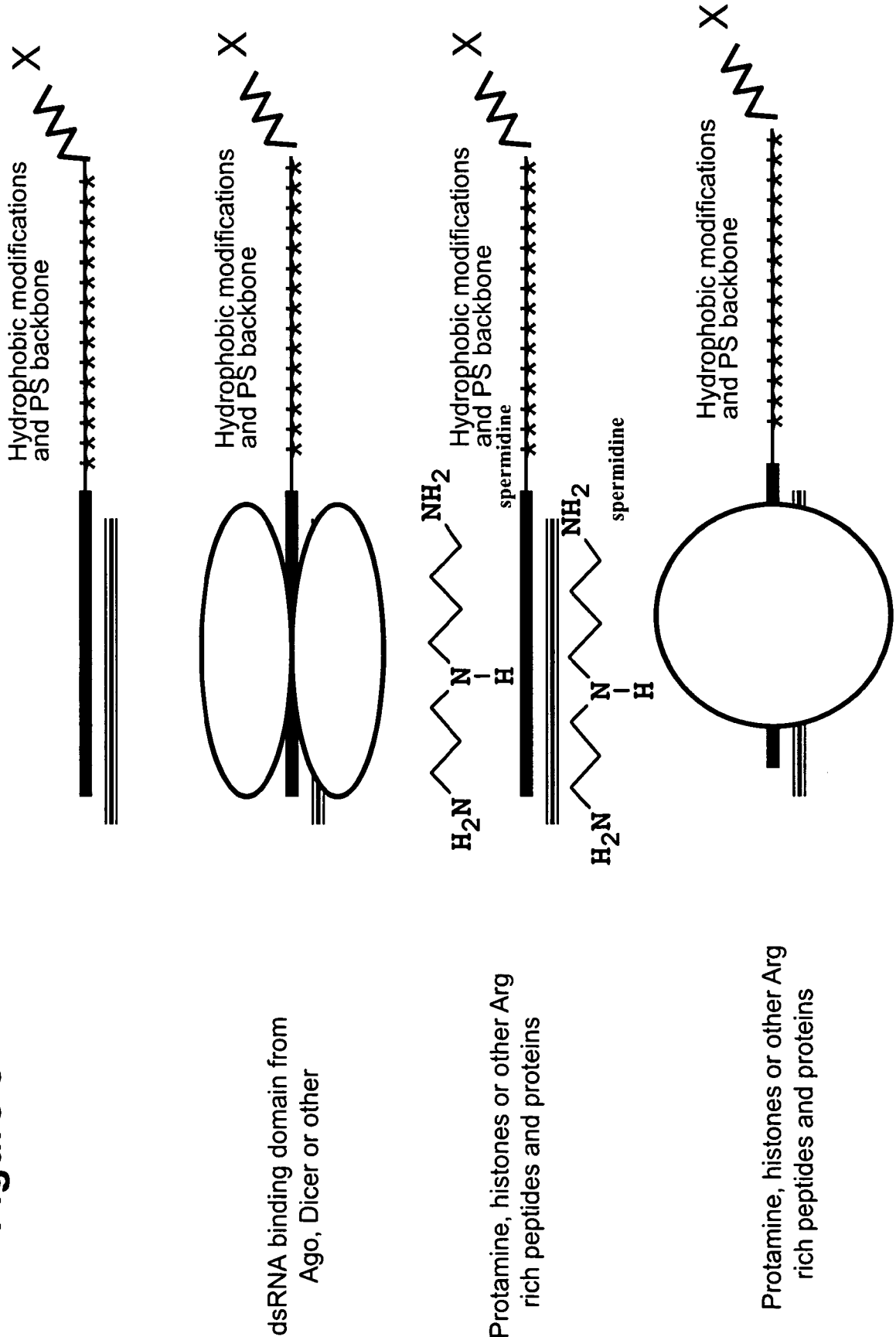
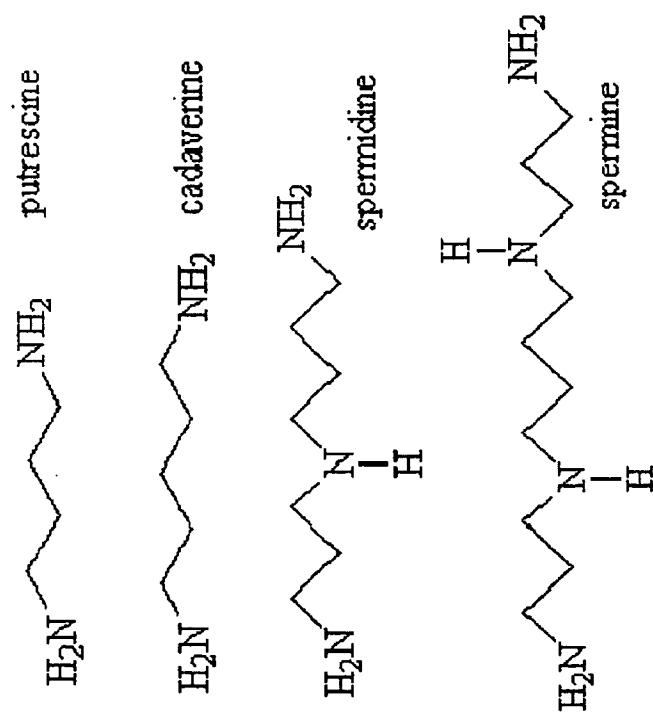


Figure 3



4/92

Figure 4

5/92

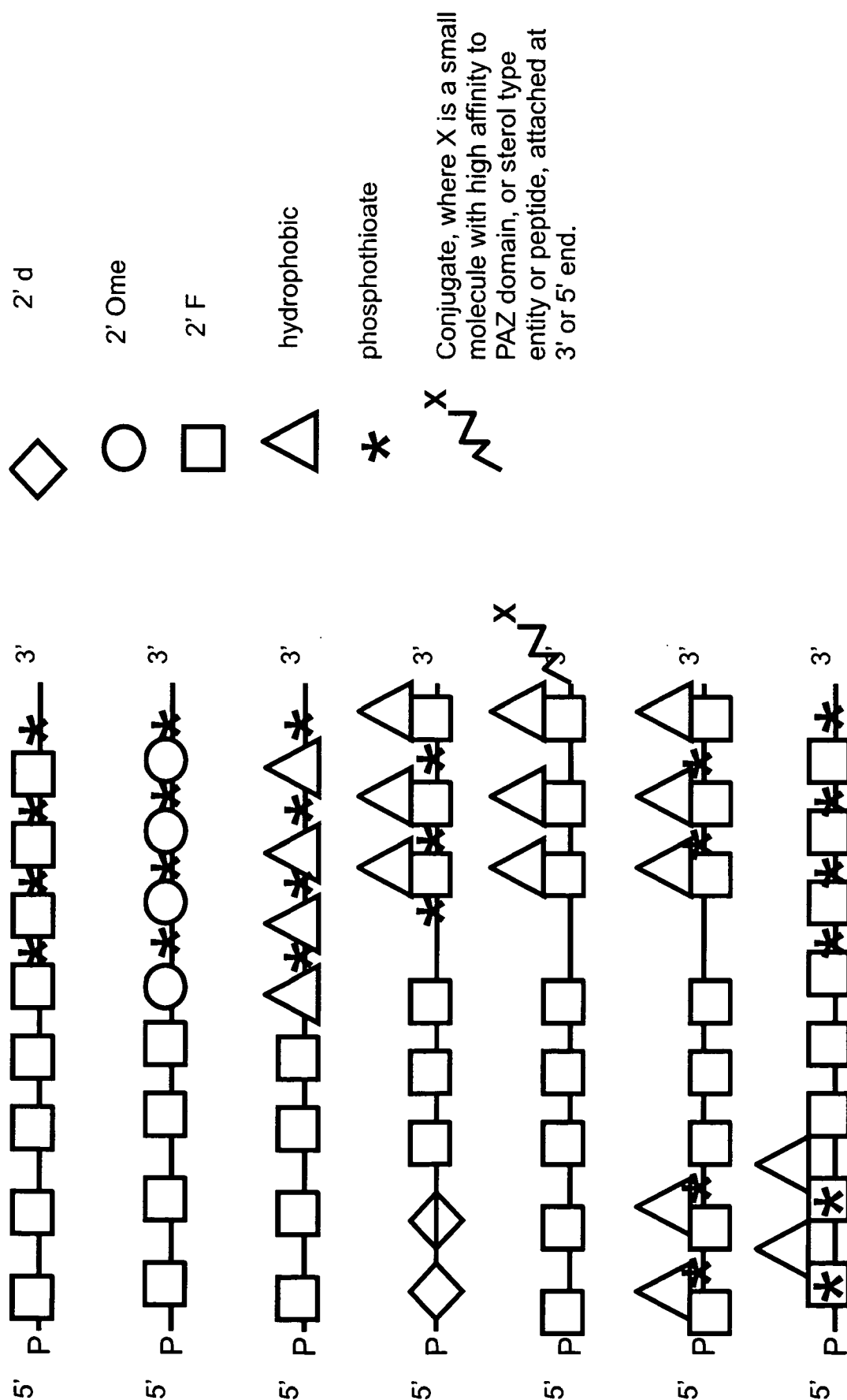


Figure 5

6/92

Figure 6

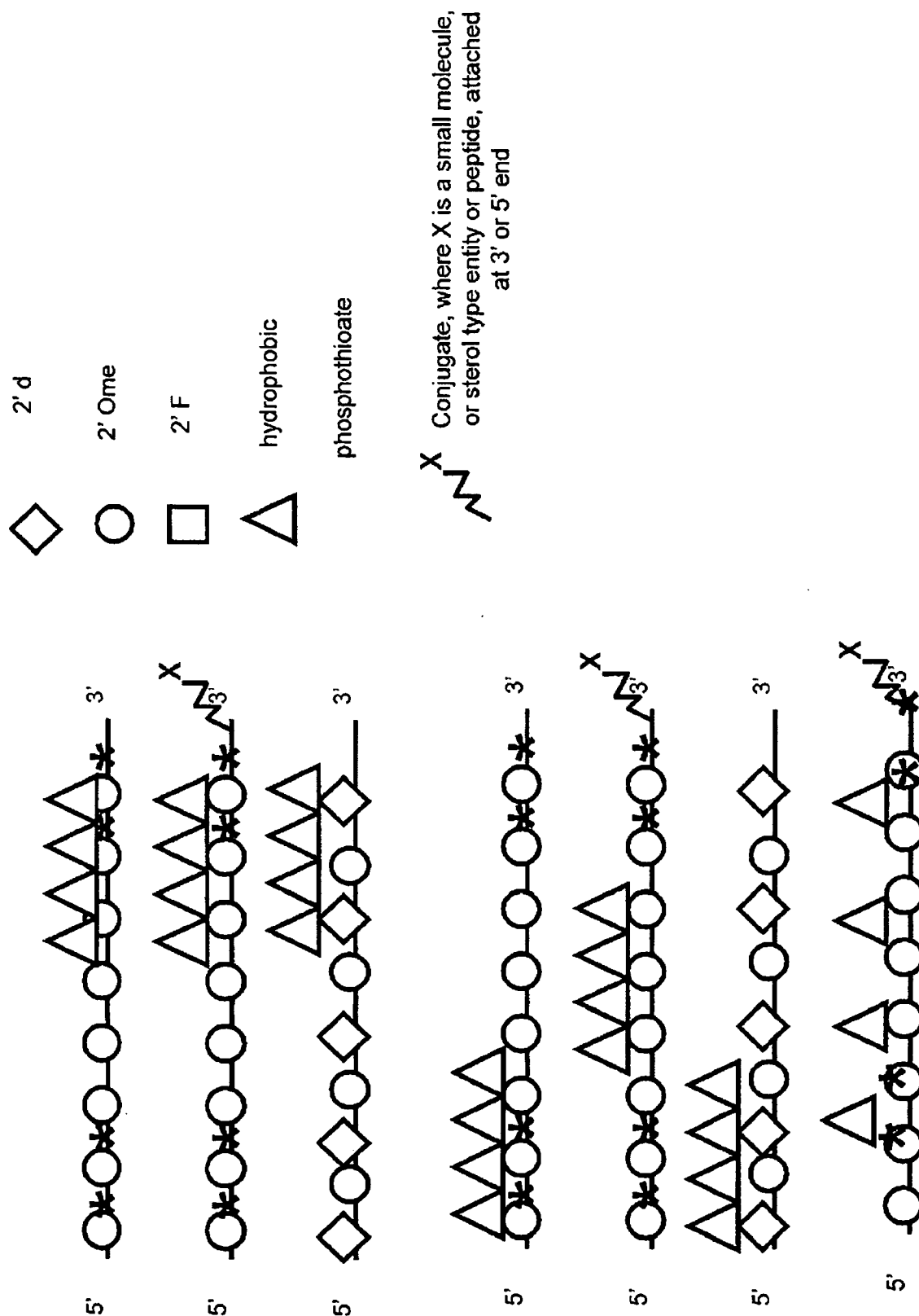
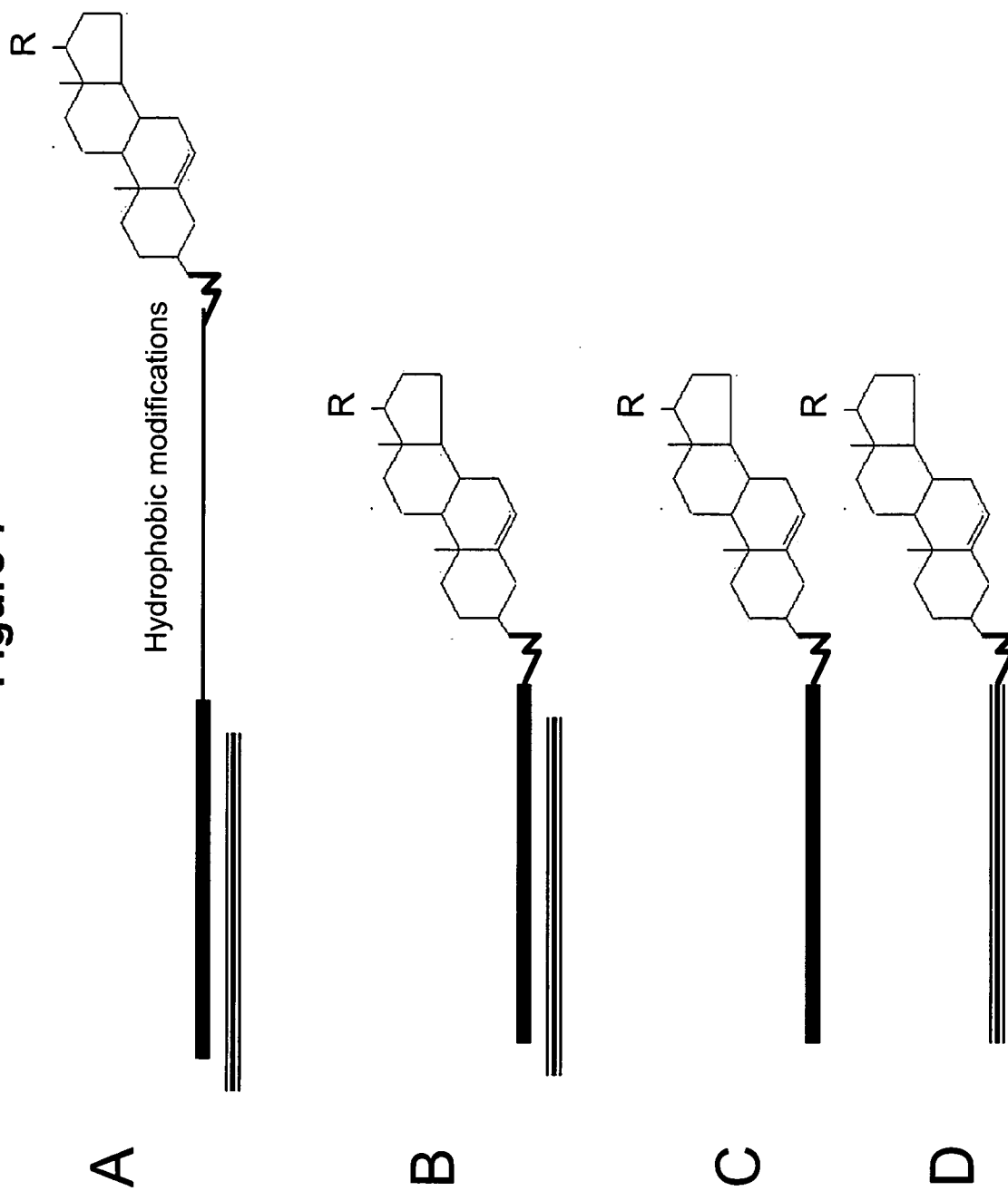


Figure 7

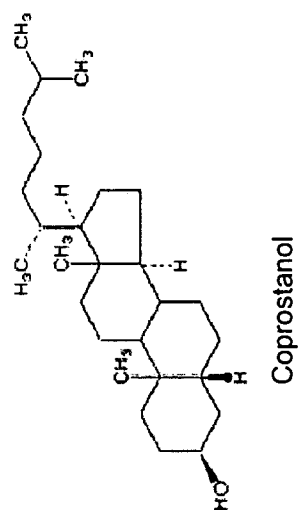
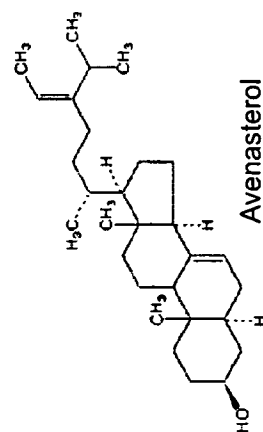
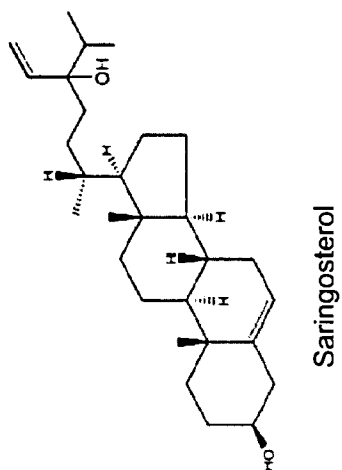
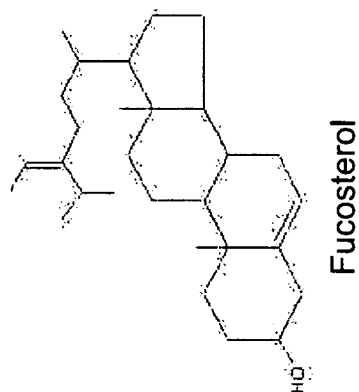
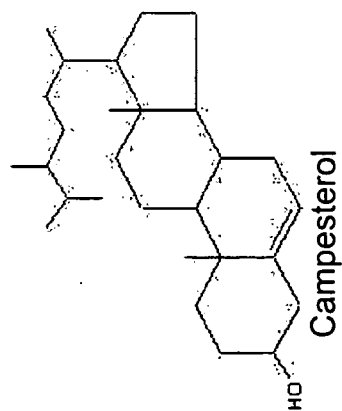
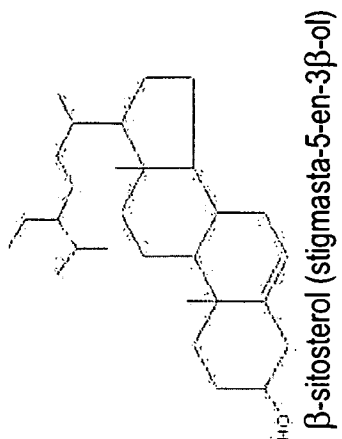
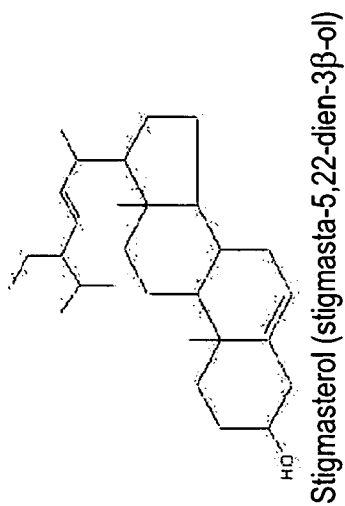
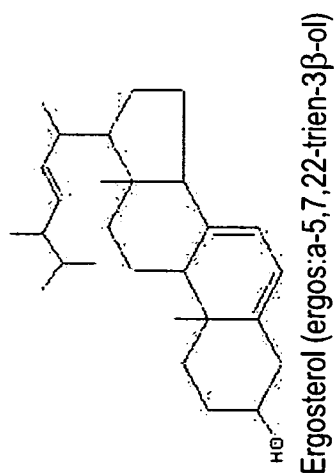
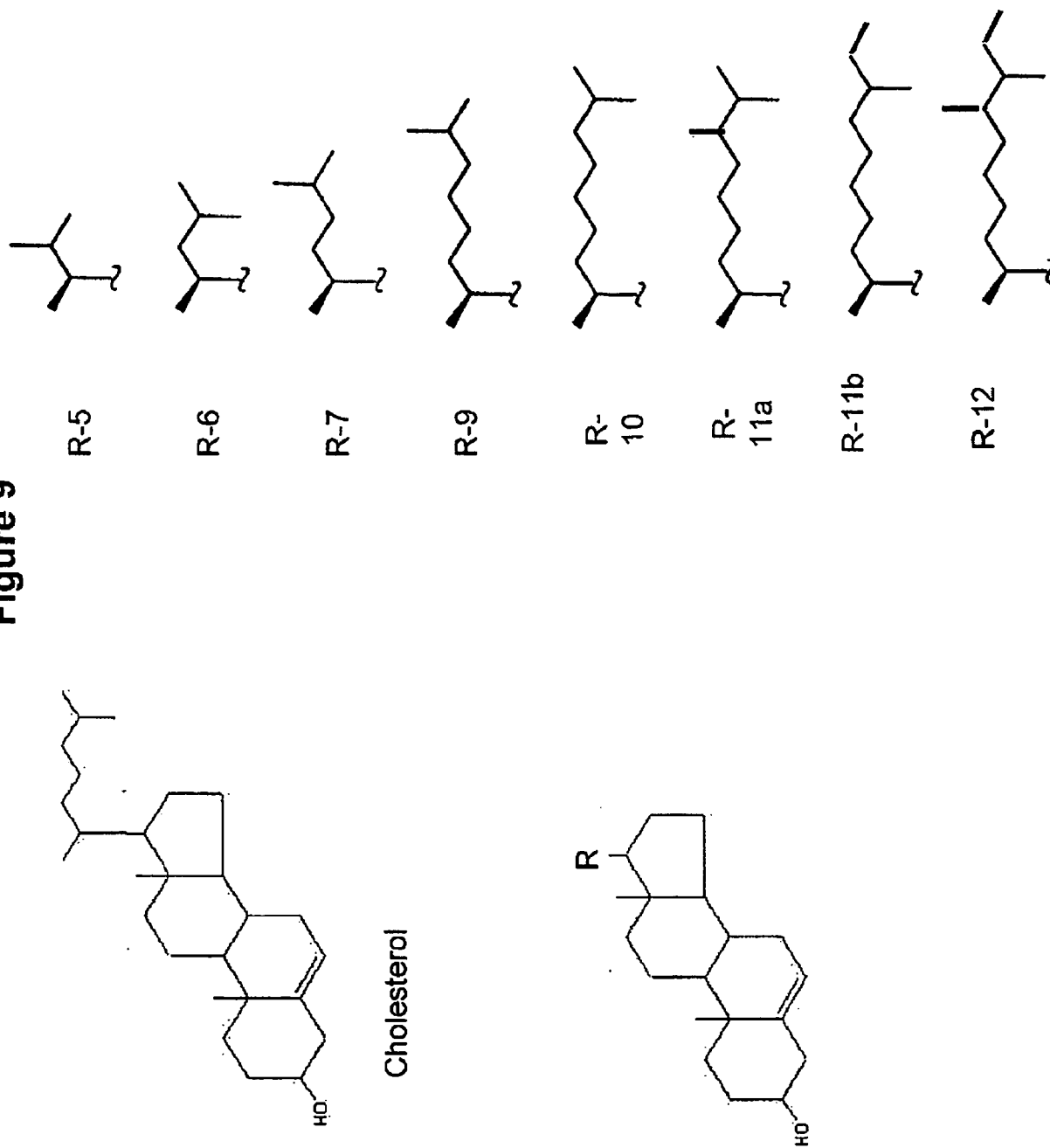
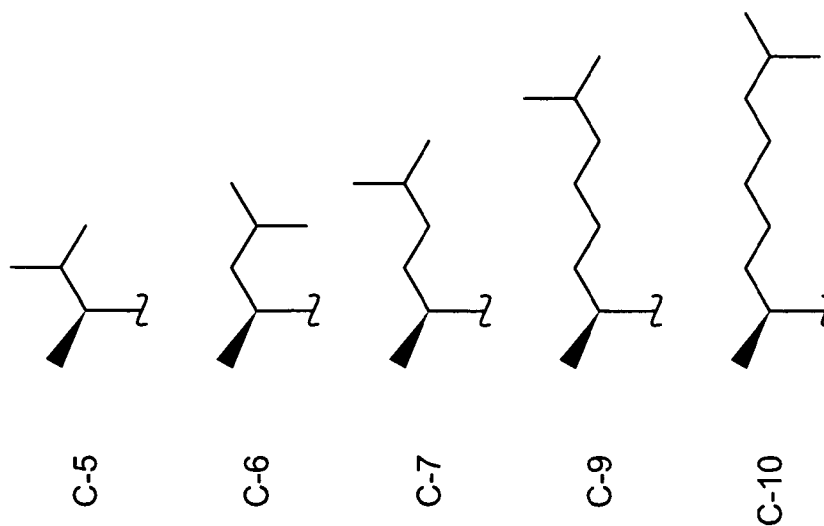
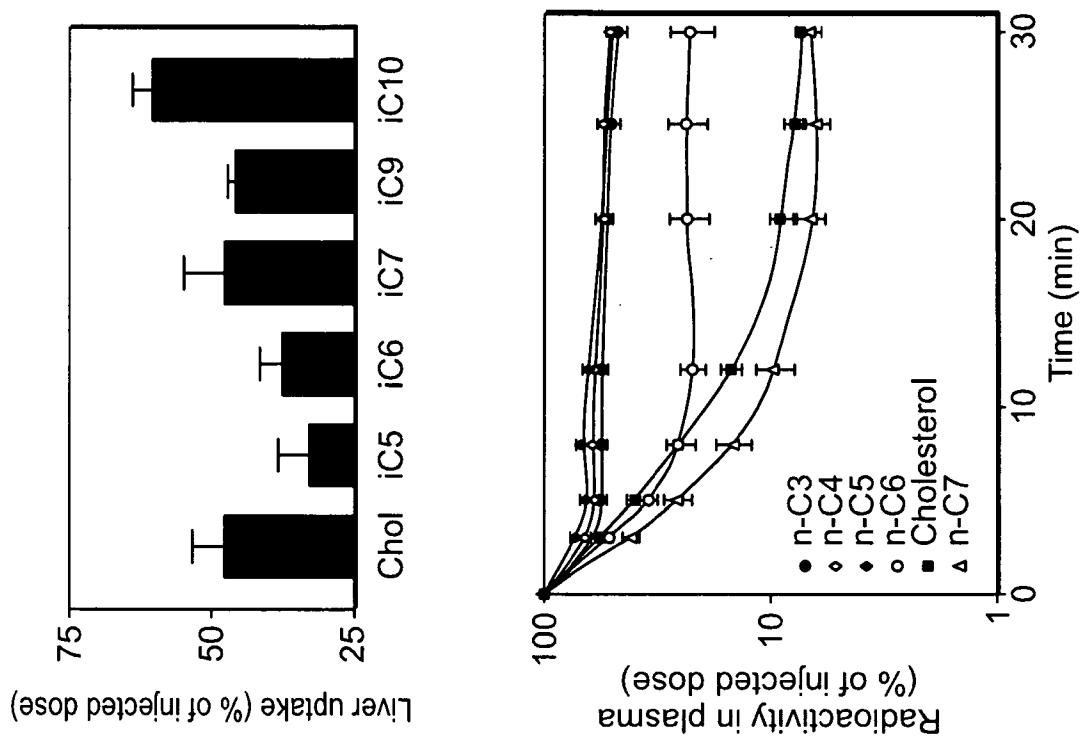


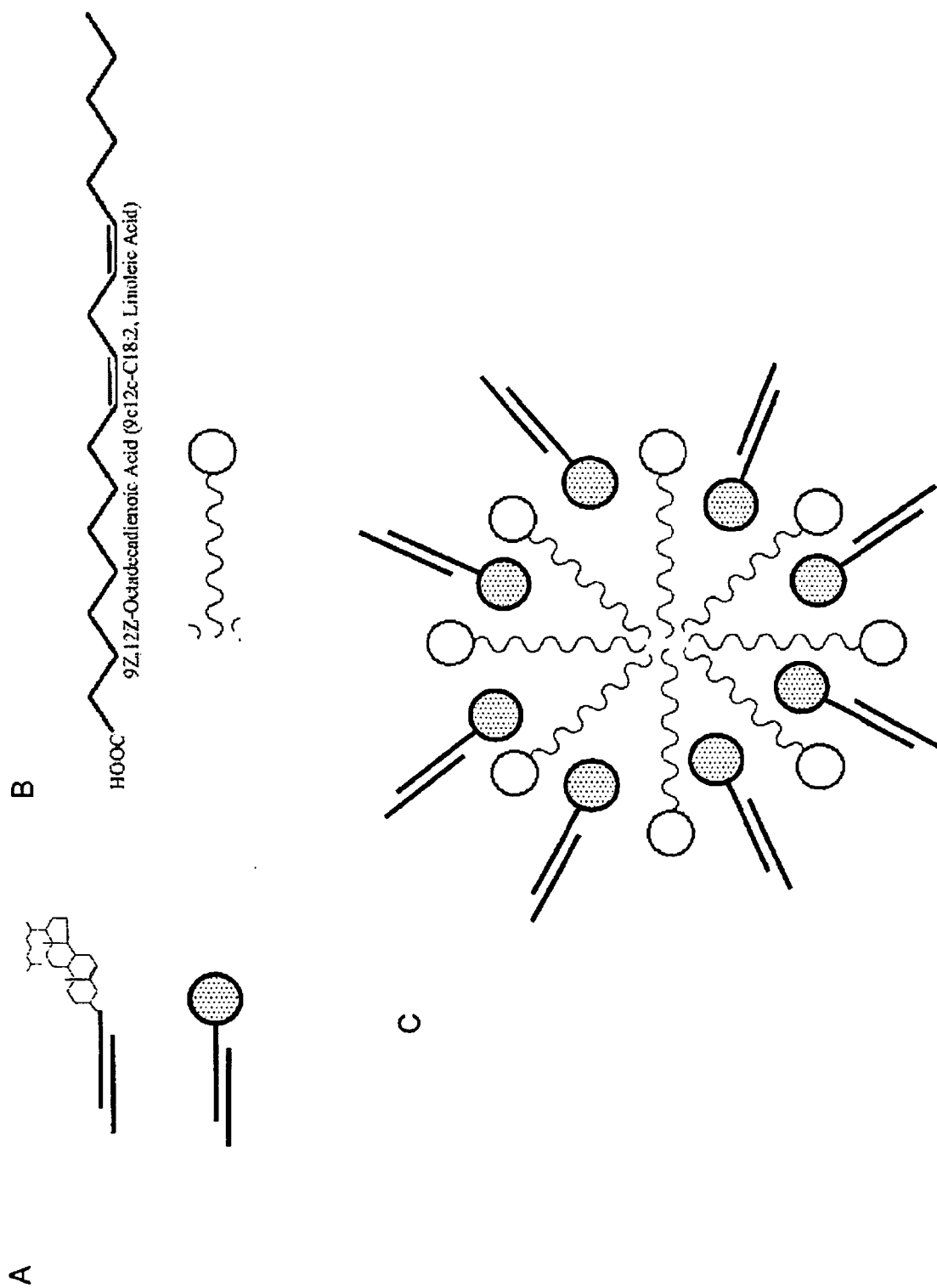
Figure 9



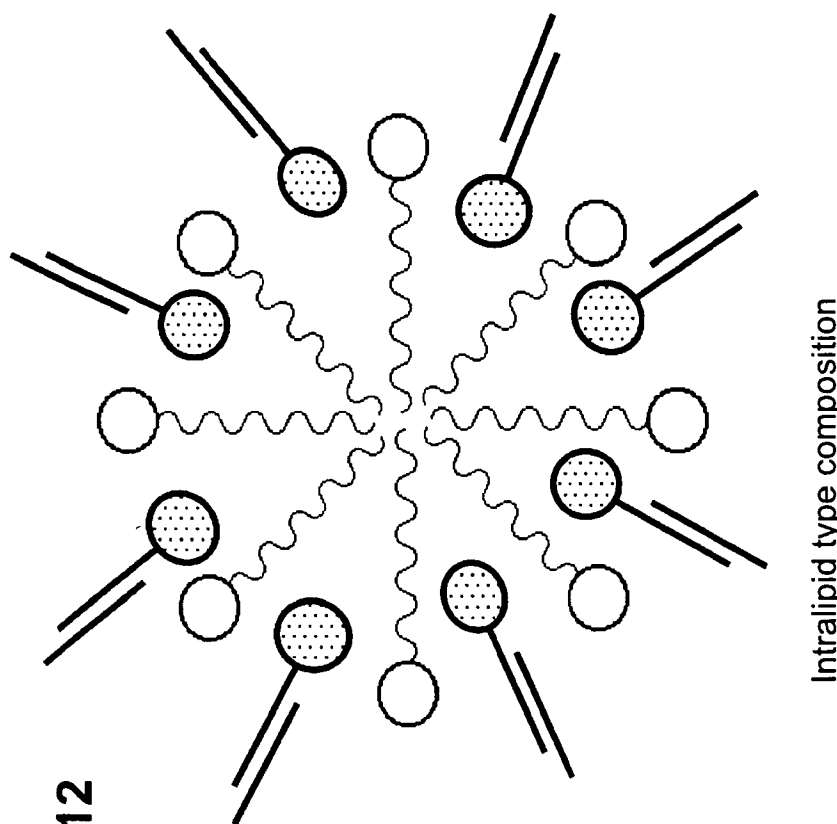
10/92

Figure 10

11/92

Figure 11

12/92

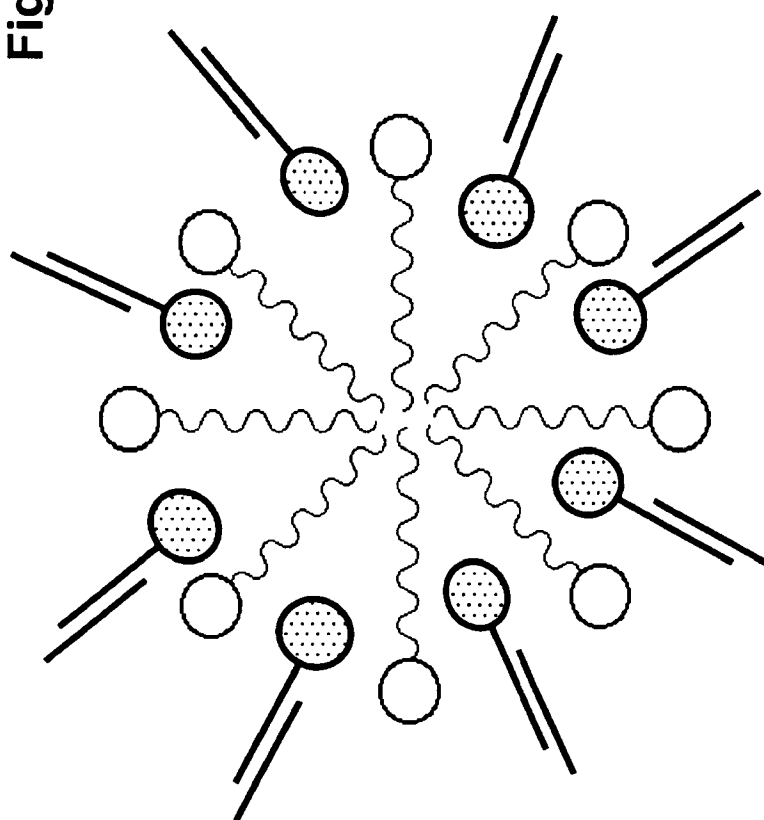


Intralipid type composition

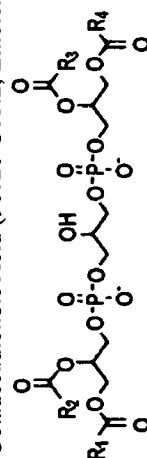


Preferential delivery to liver

Figure 12



HOOC-9Z,12Z-Octadecadienoic Acid (9c12c-C18:2, Linoleic Acid)



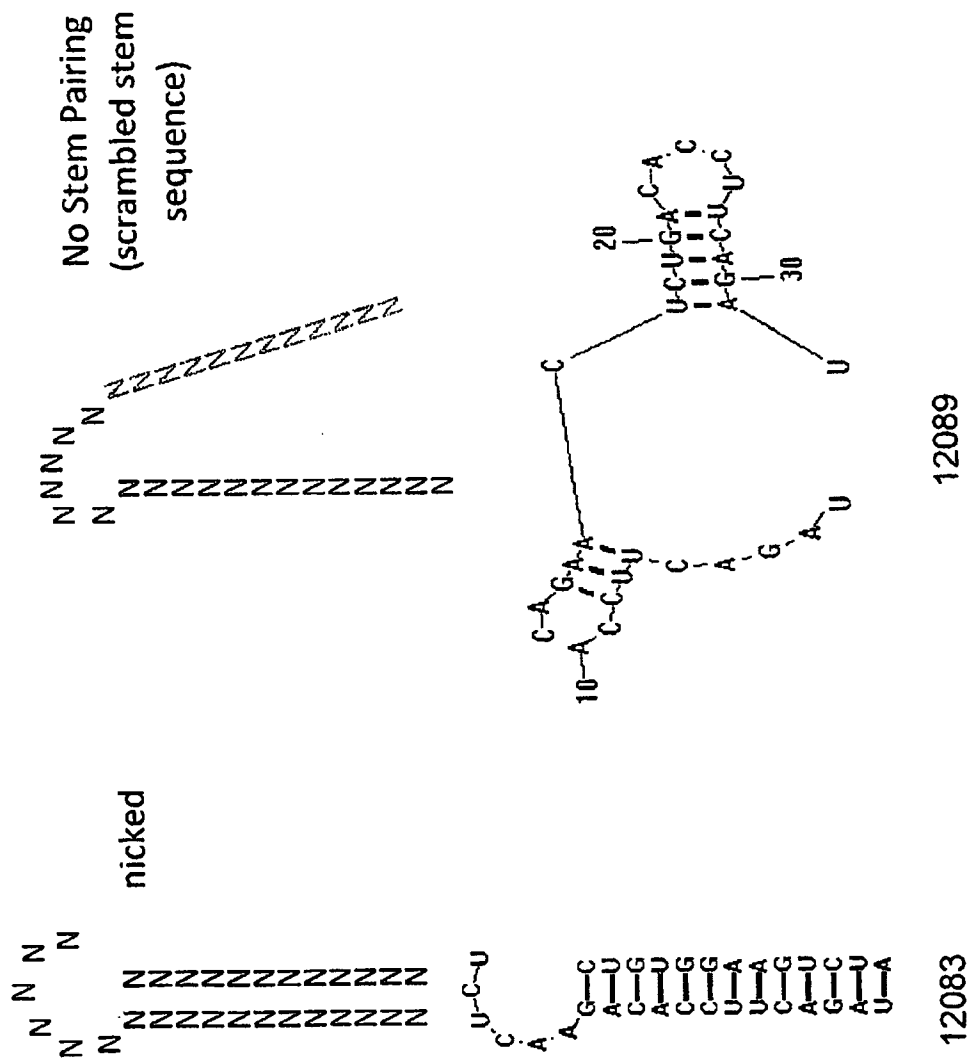
Linoleic acid and cardiolipin enriched micelles



Preferential delivery to heart

Figure 13

Structural Elucidation-MAP4K4



14/92

Figure 14
MAP4K4 Targeting Structure Testing

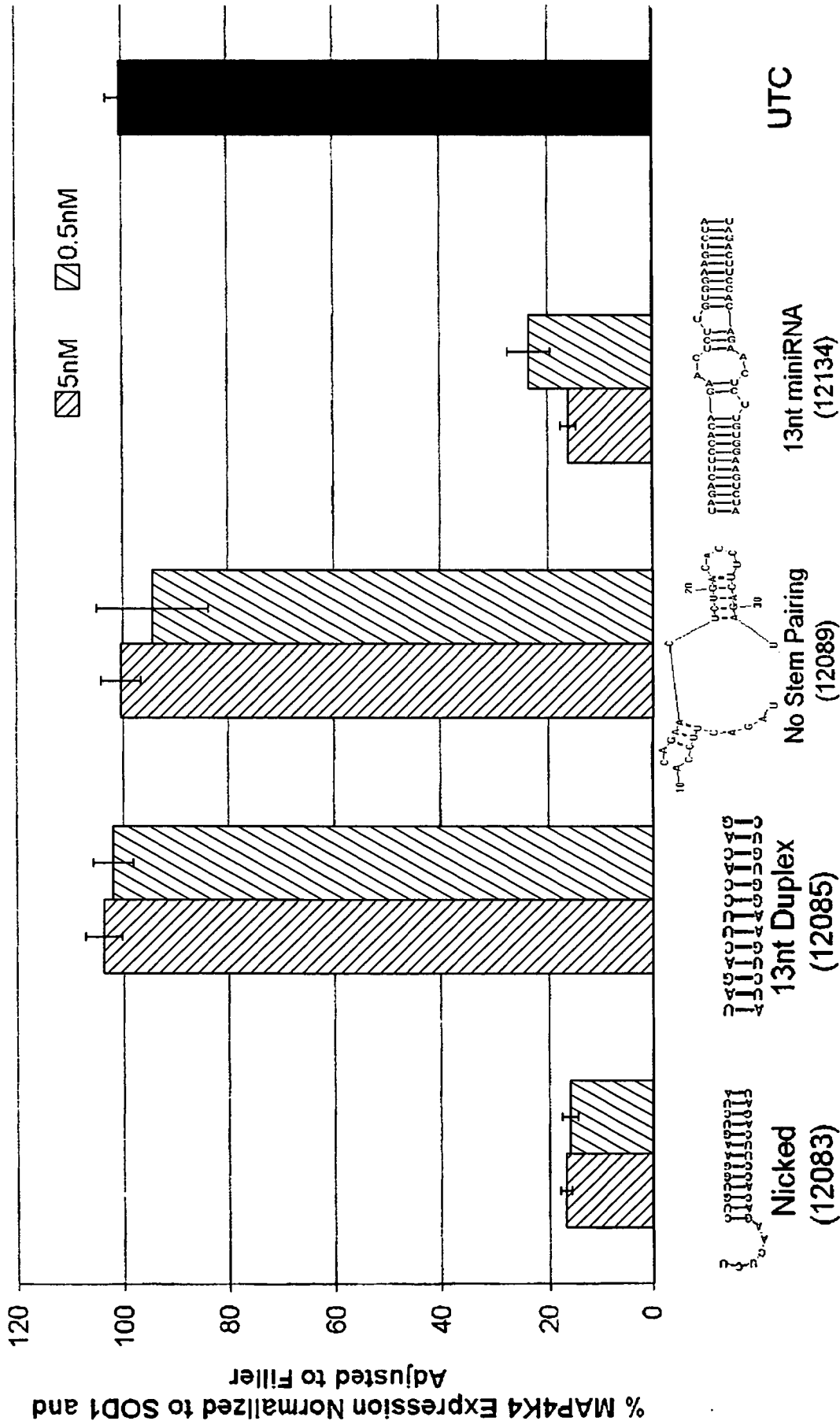


Figure 15

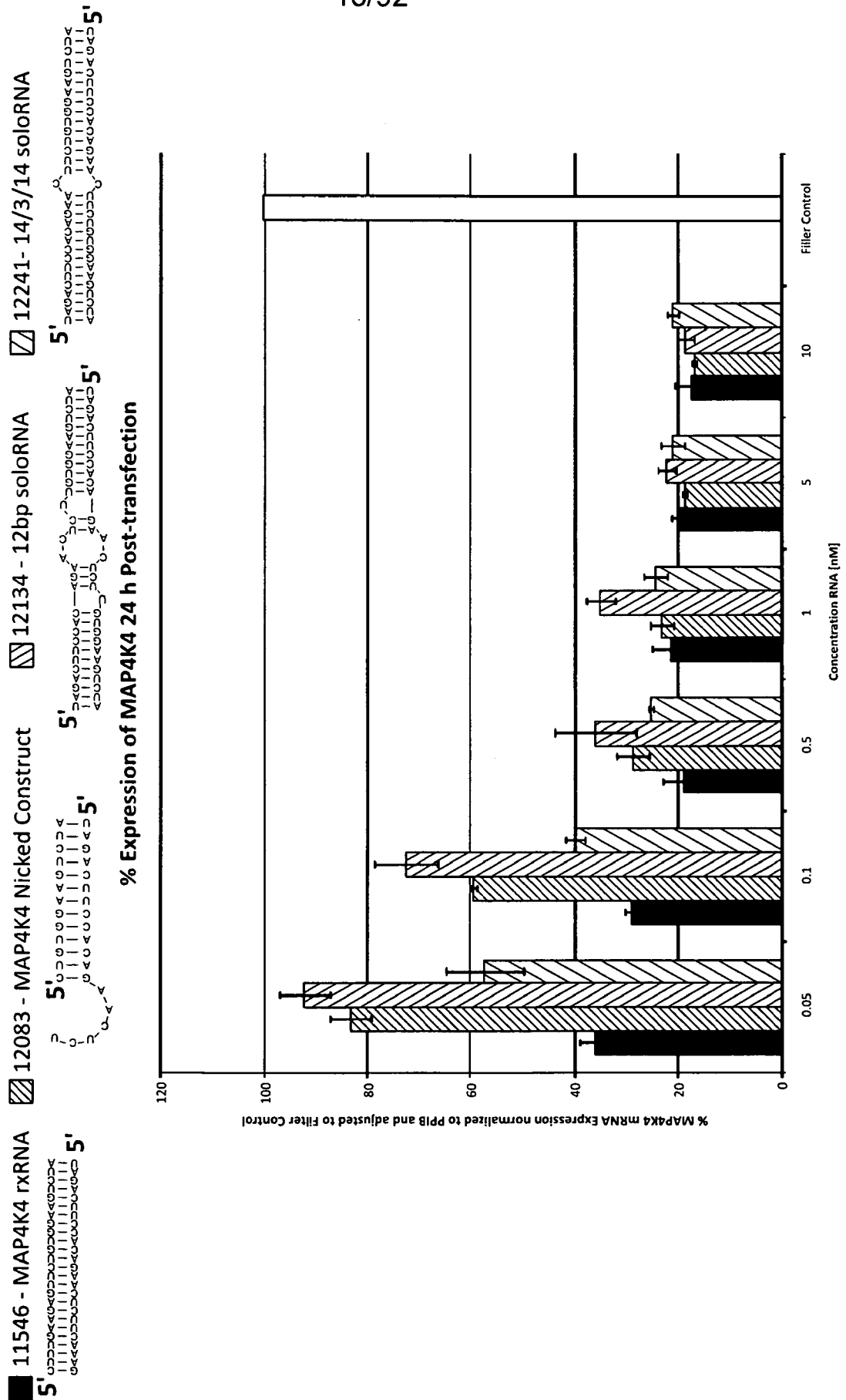
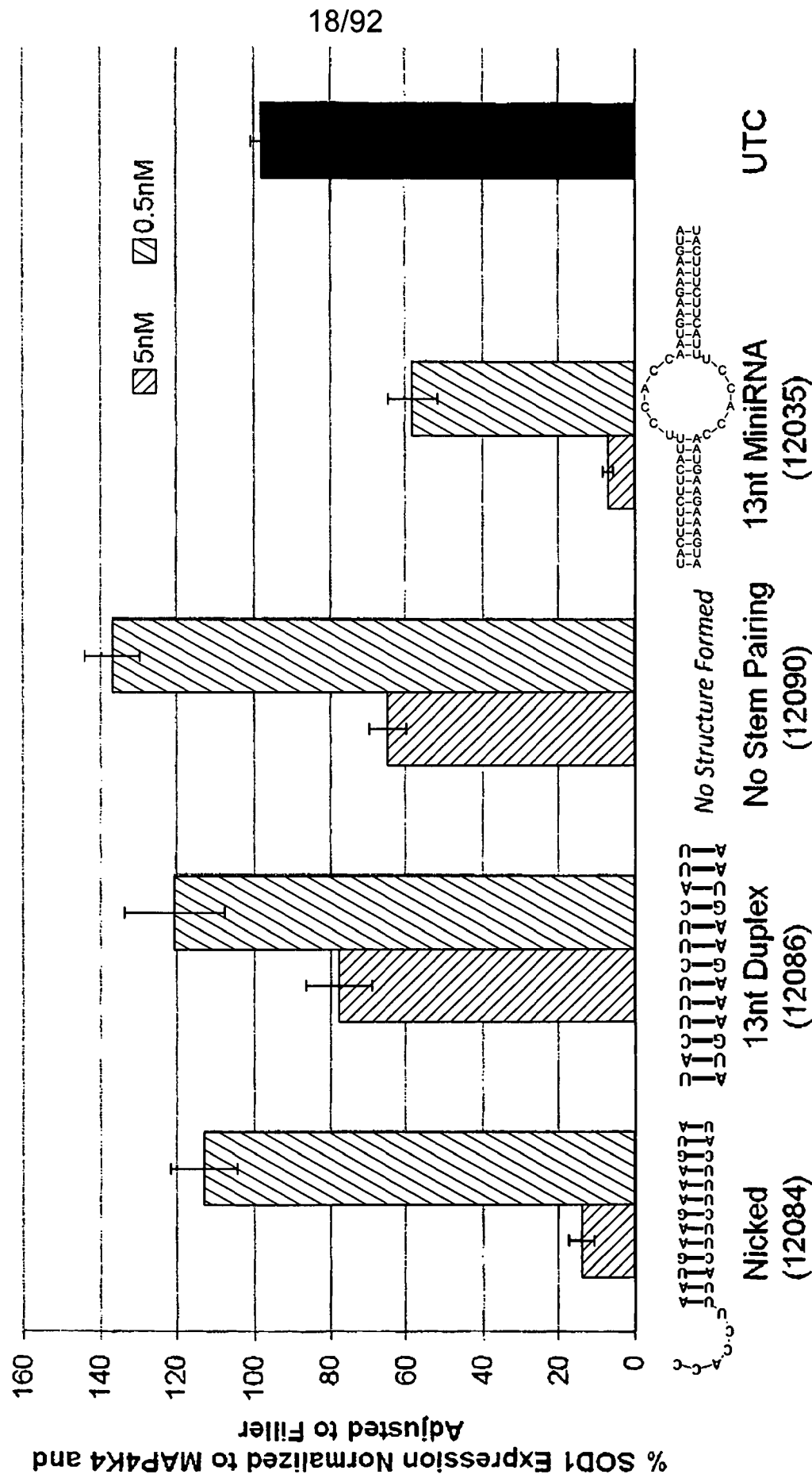


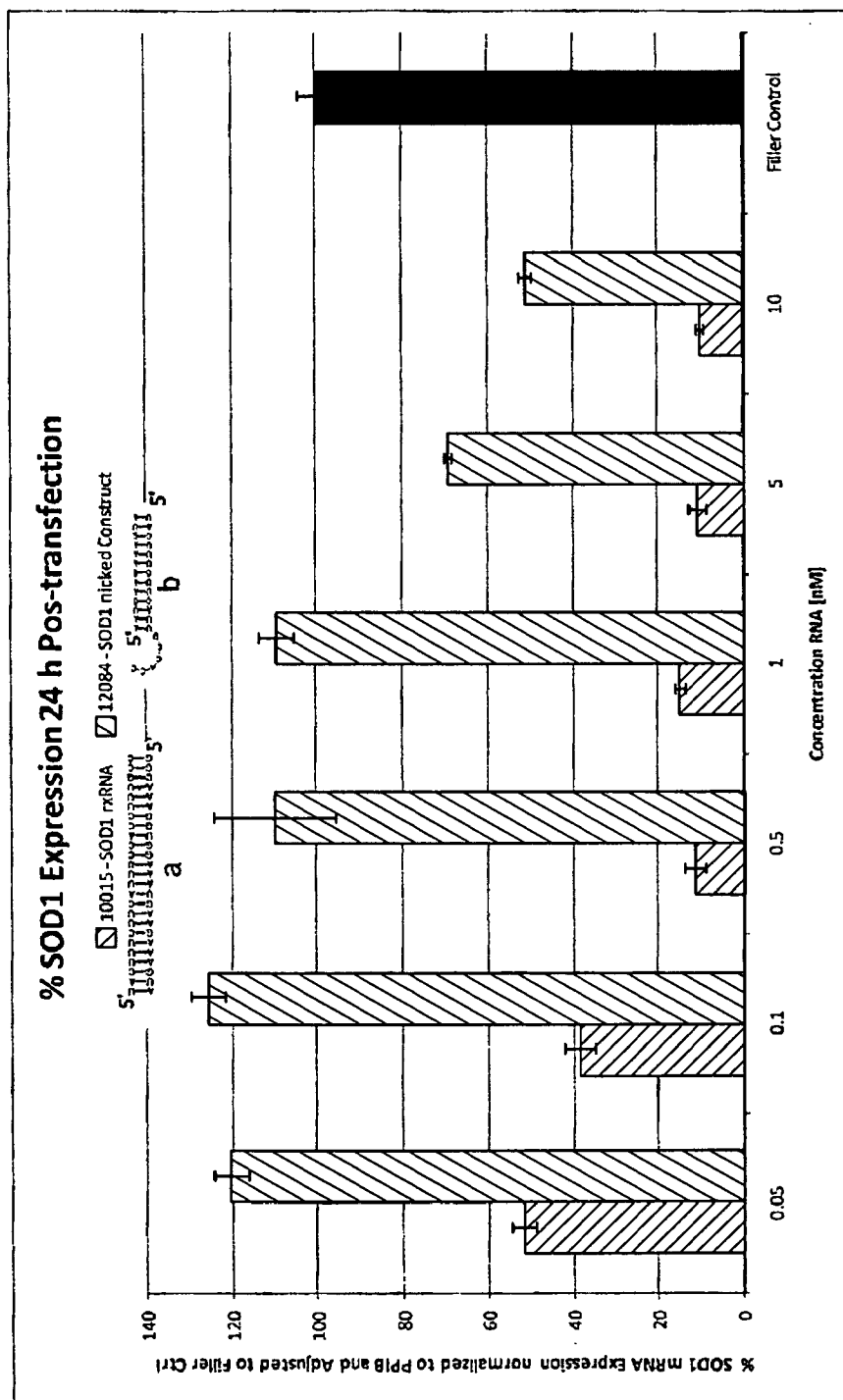
Figure 18
SOD1 Targeting Structure Testing



19/92

Figure 19

SOD1 Minimum Length RNAi Trigger



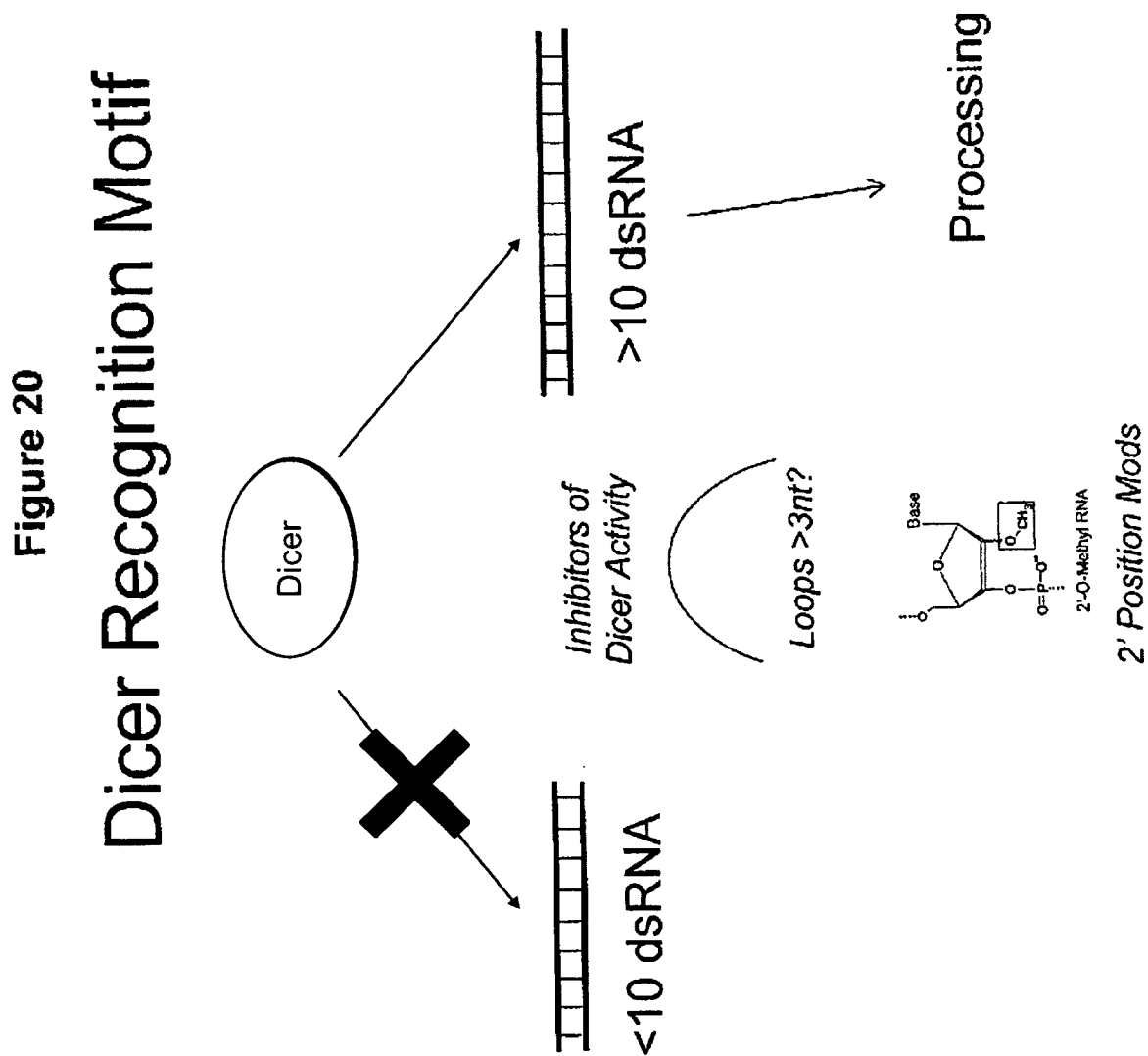
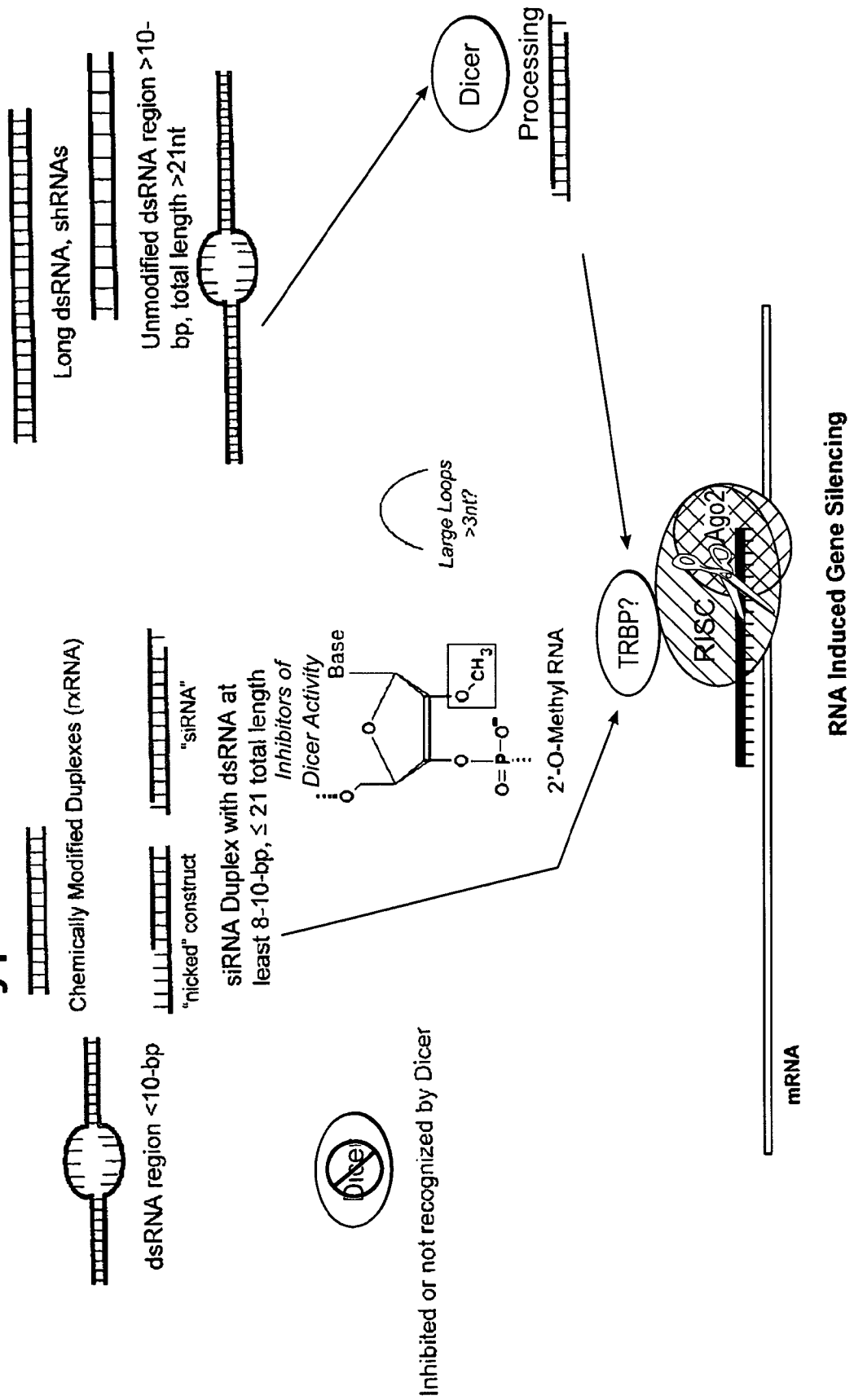


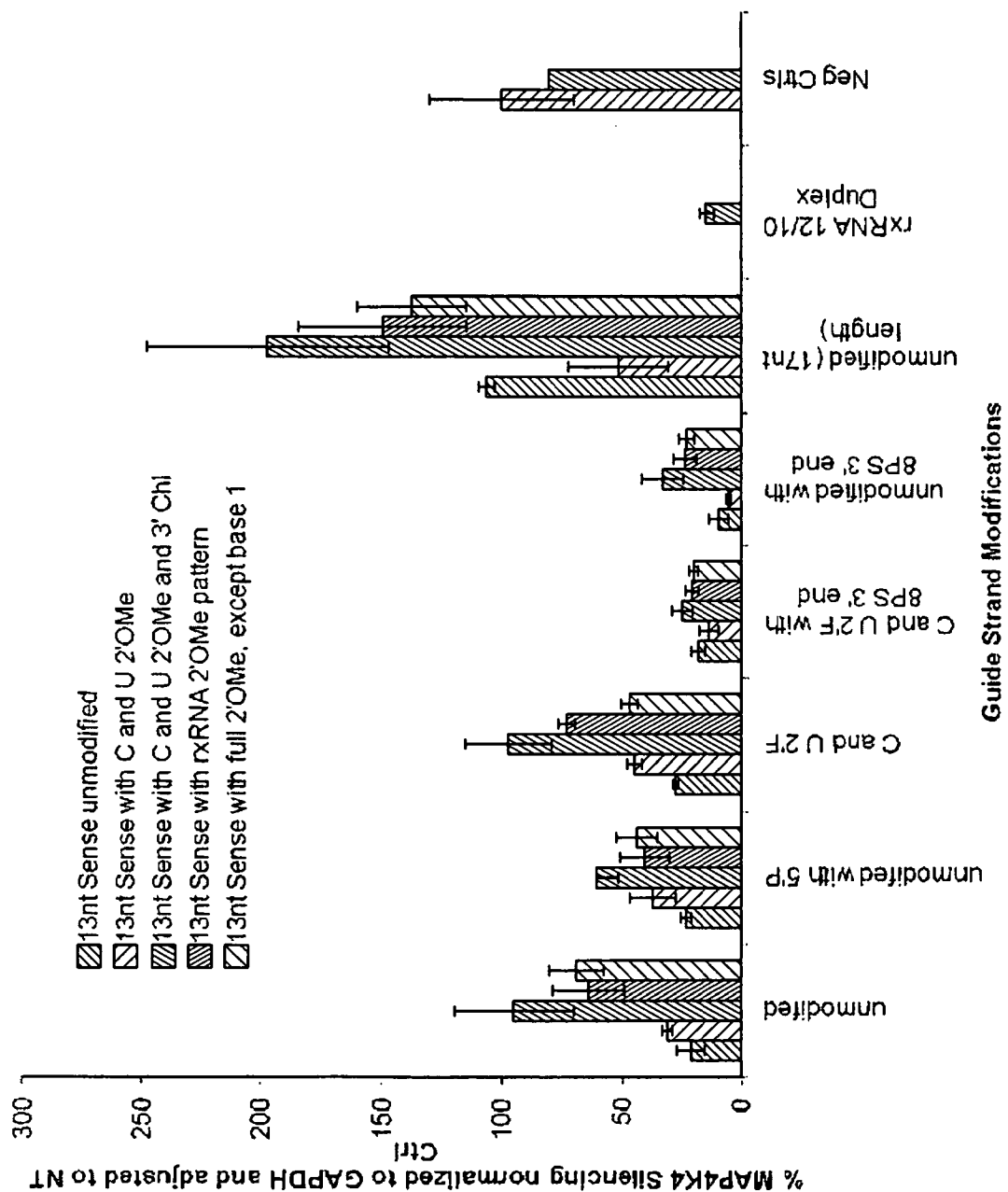
Figure 21

Hypothetical RNAi Model



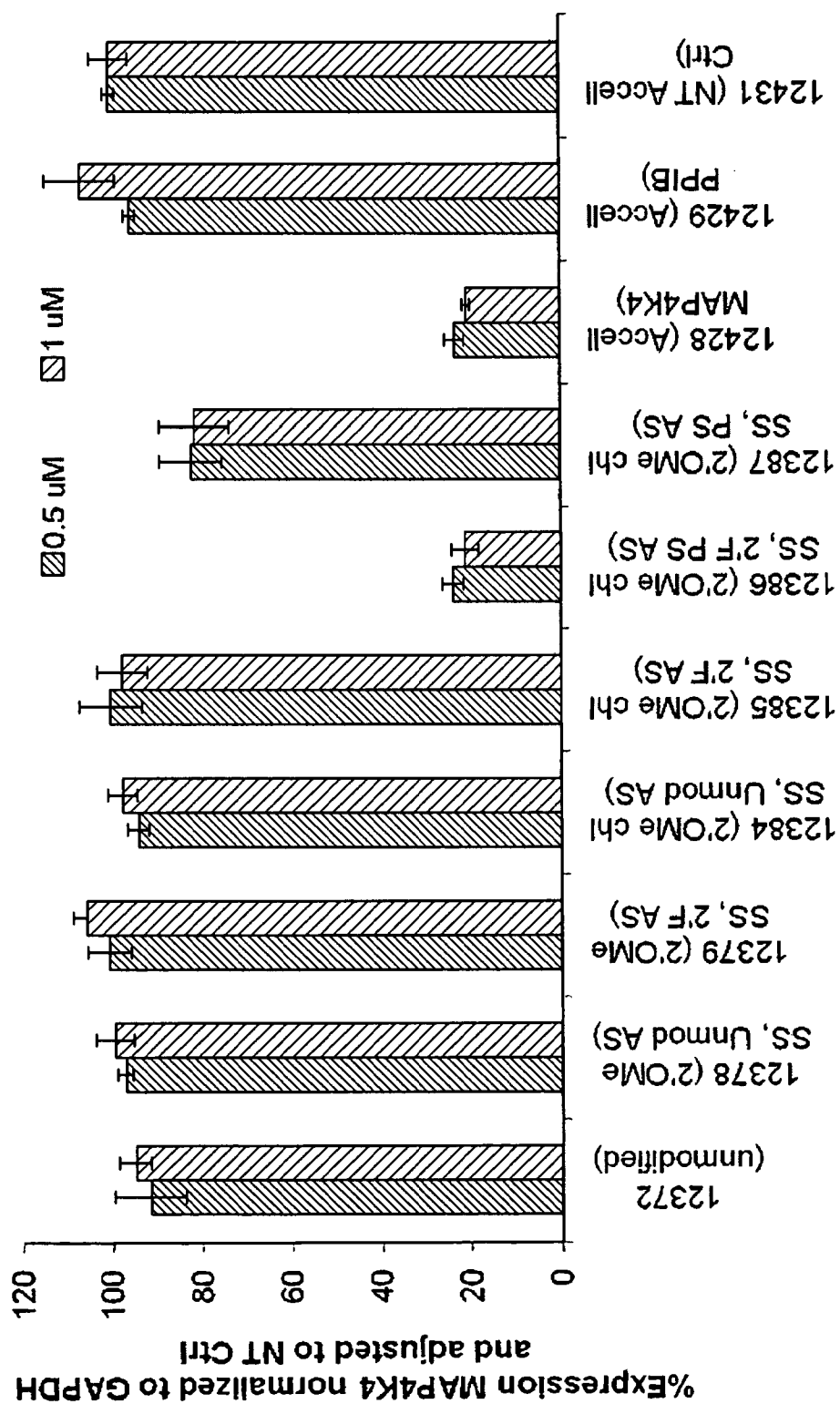
22/92

Figure 22



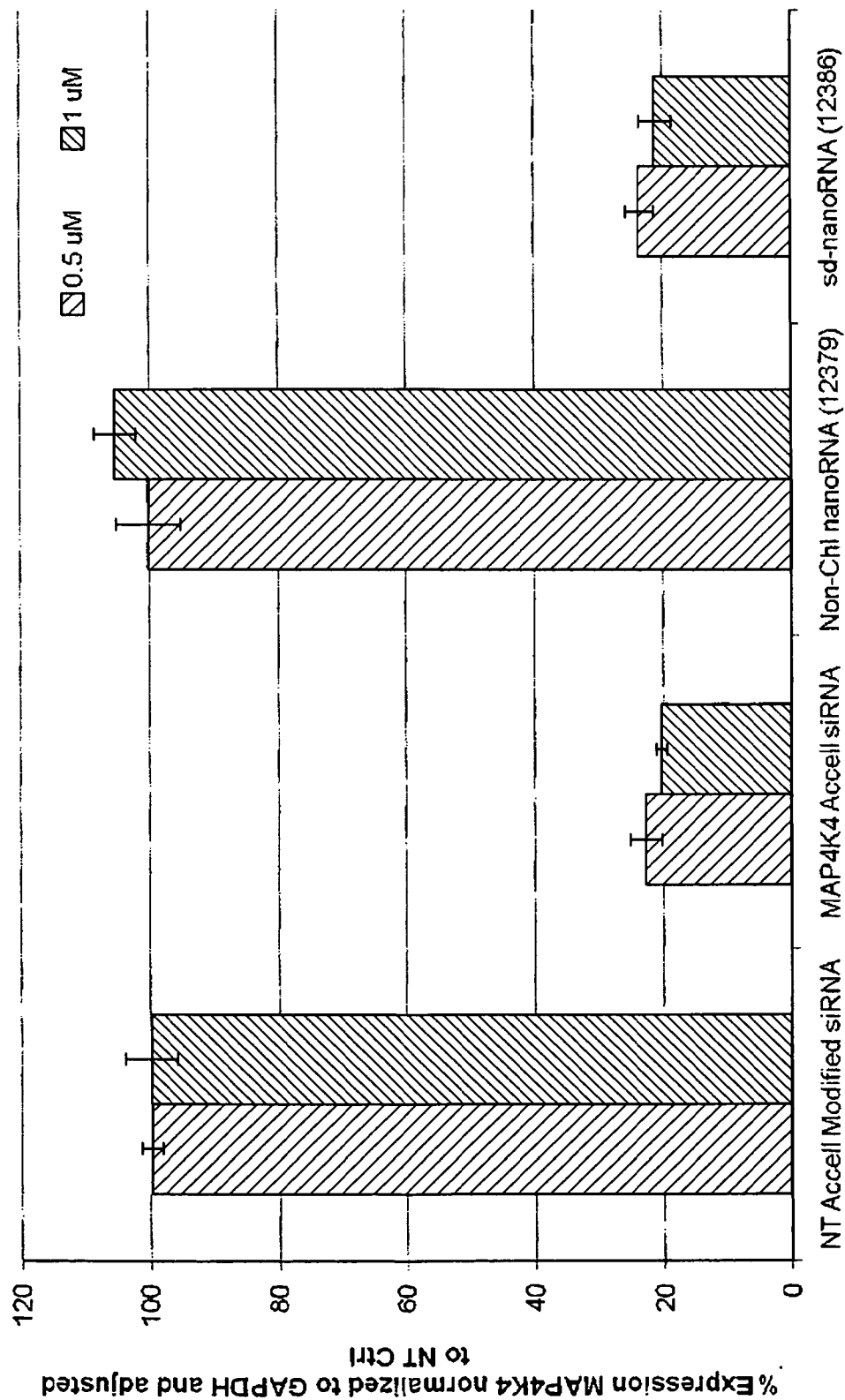
23/92

Figure 23



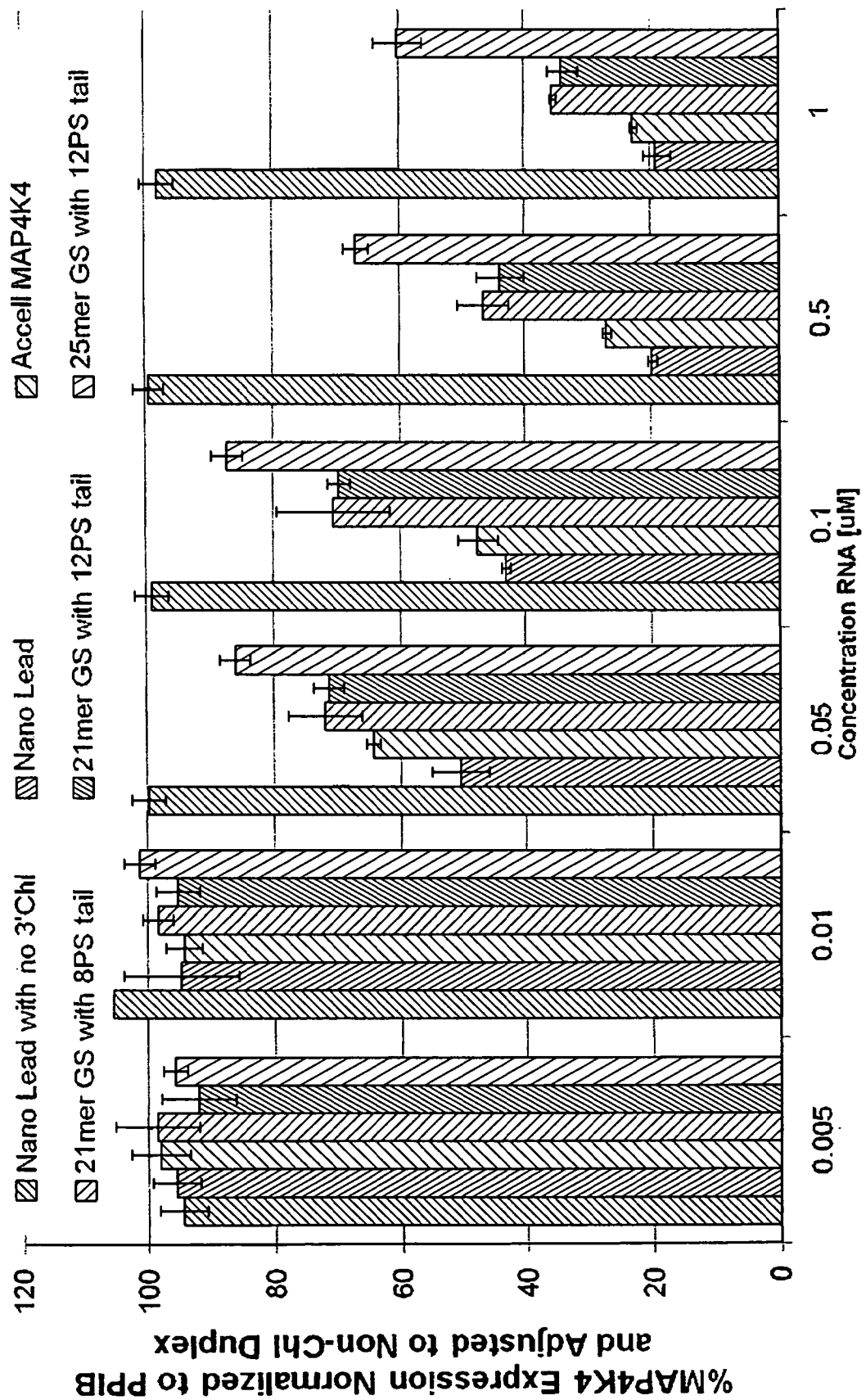
24/92

Figure 24
Self-Delivering nano-rxRNA



25/92

Figure 25
Dose Response Passive Uptake



26/92

Figure 26

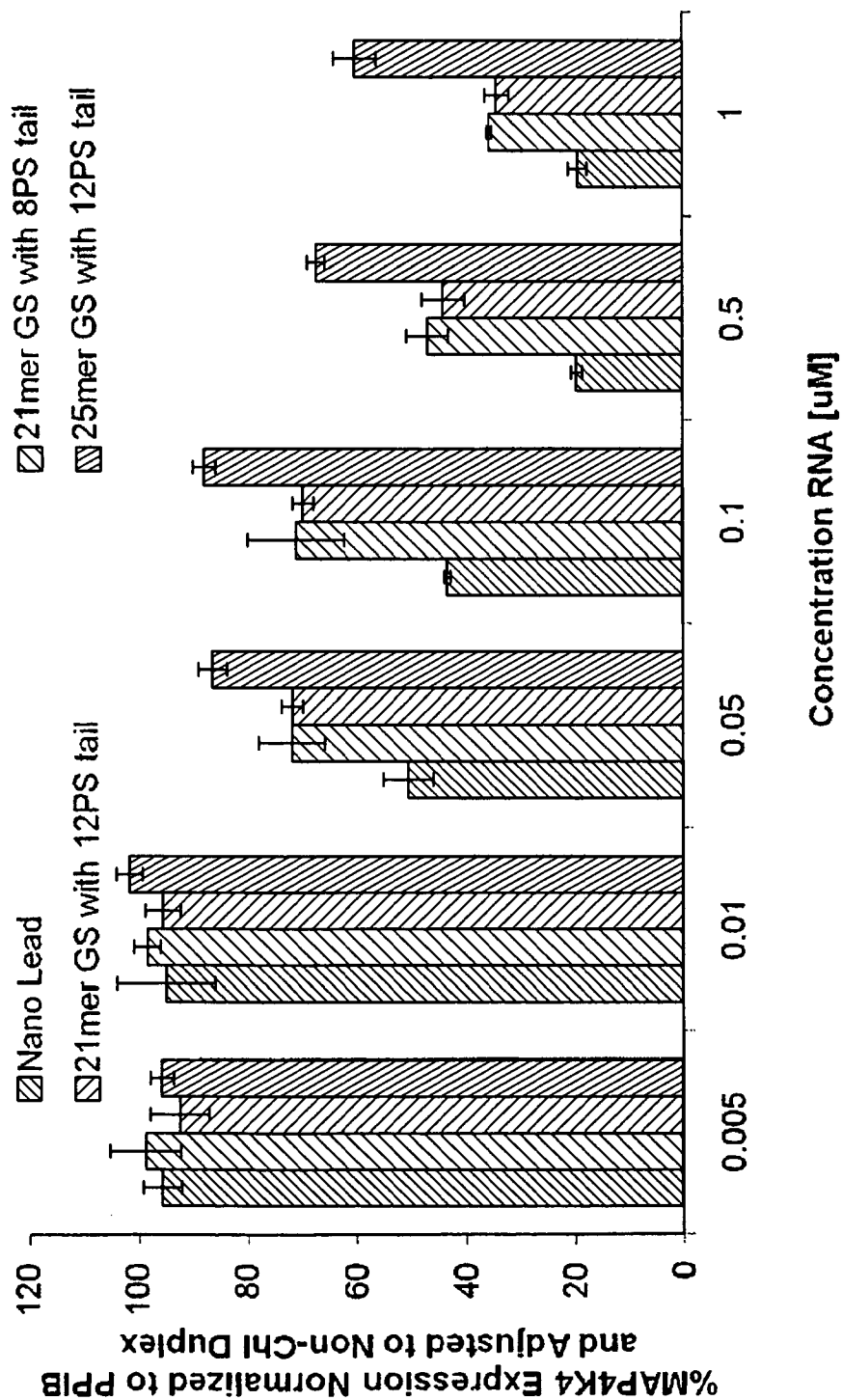


Figure 27

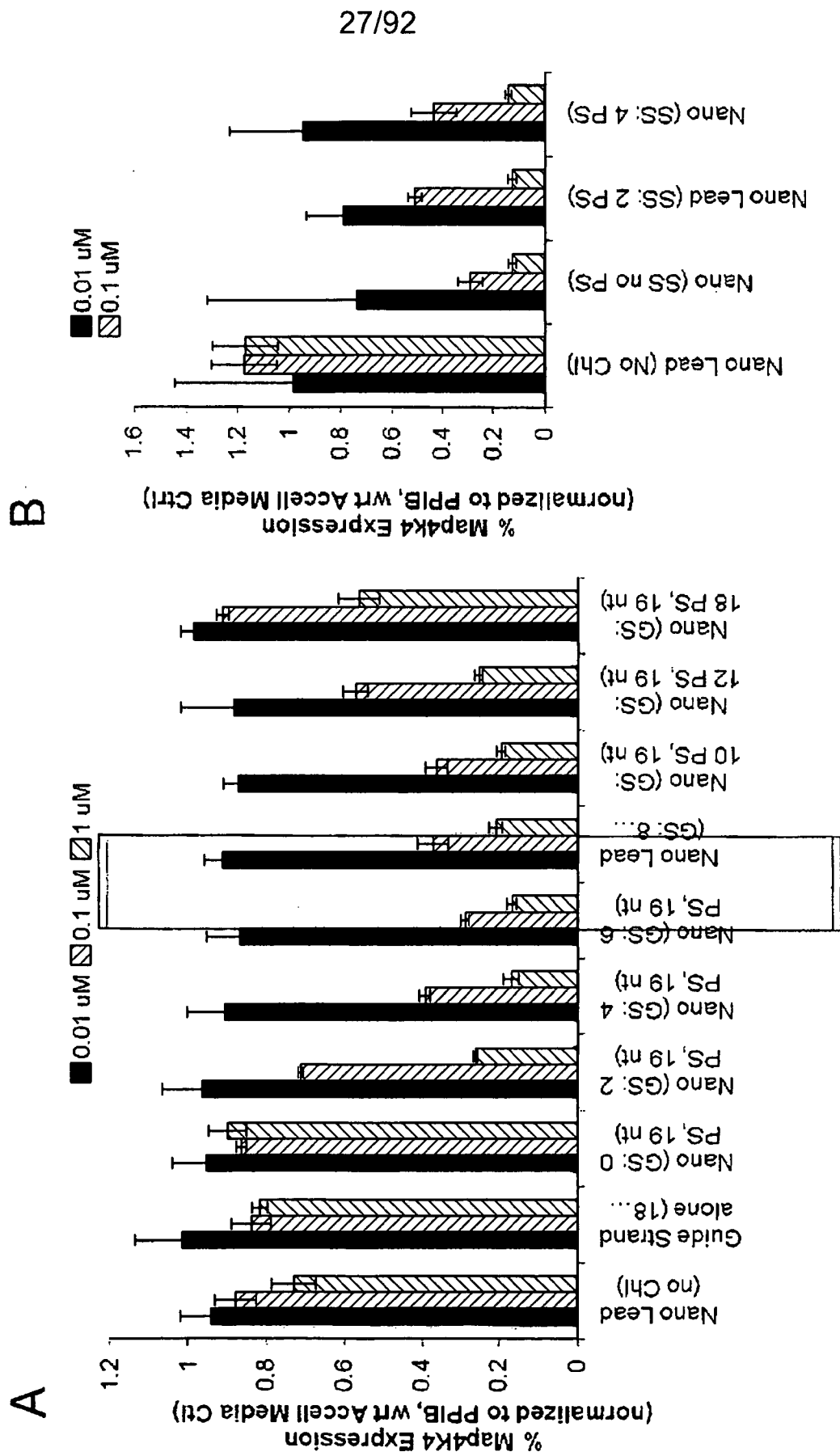
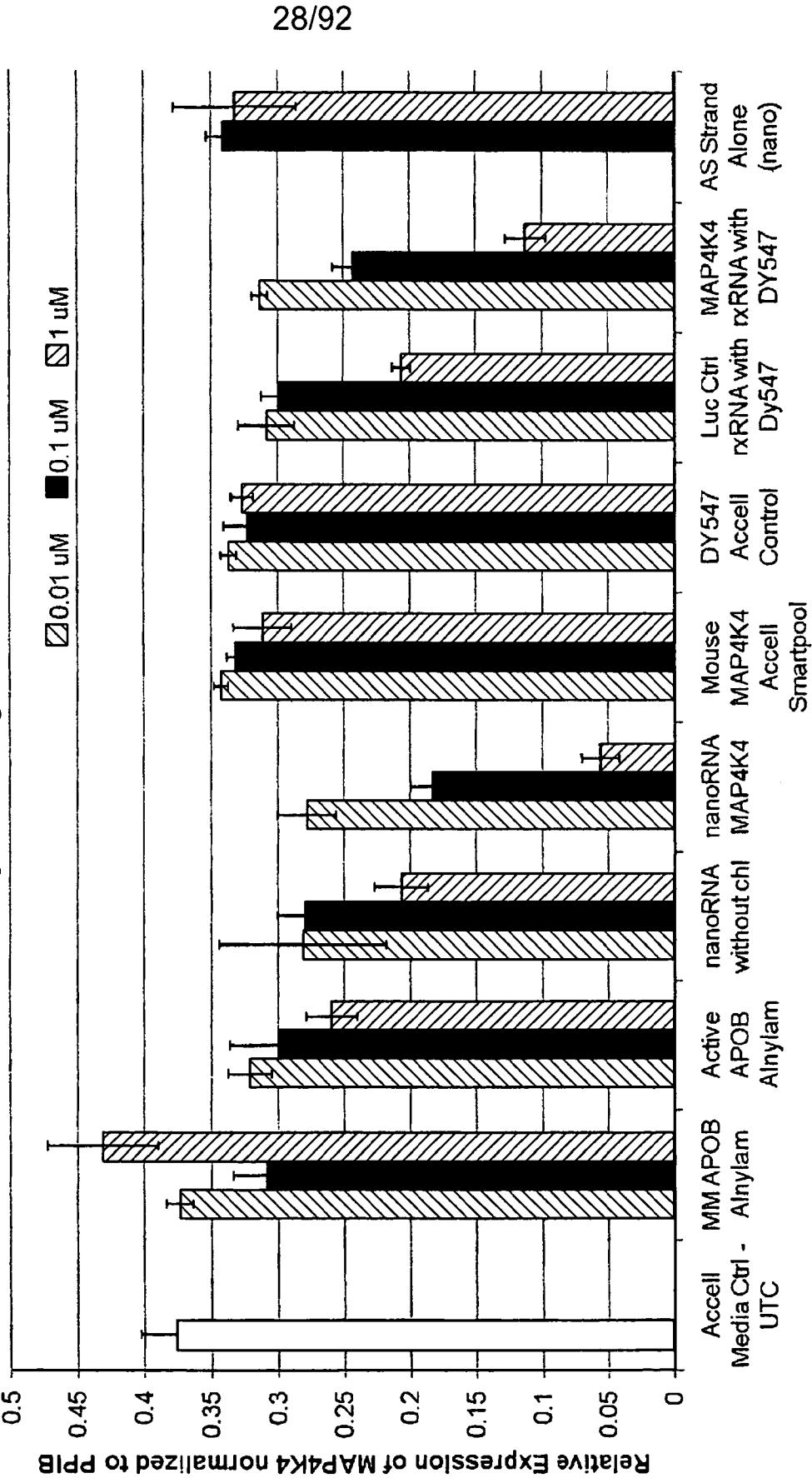


Figure 28
sd-nanoRNA silencing in Primary Mouse
Hepatocytes



29/92

Figure 29
Passive Uptake with Alnylam siRNA in
Primary Mouse Hepatocytes

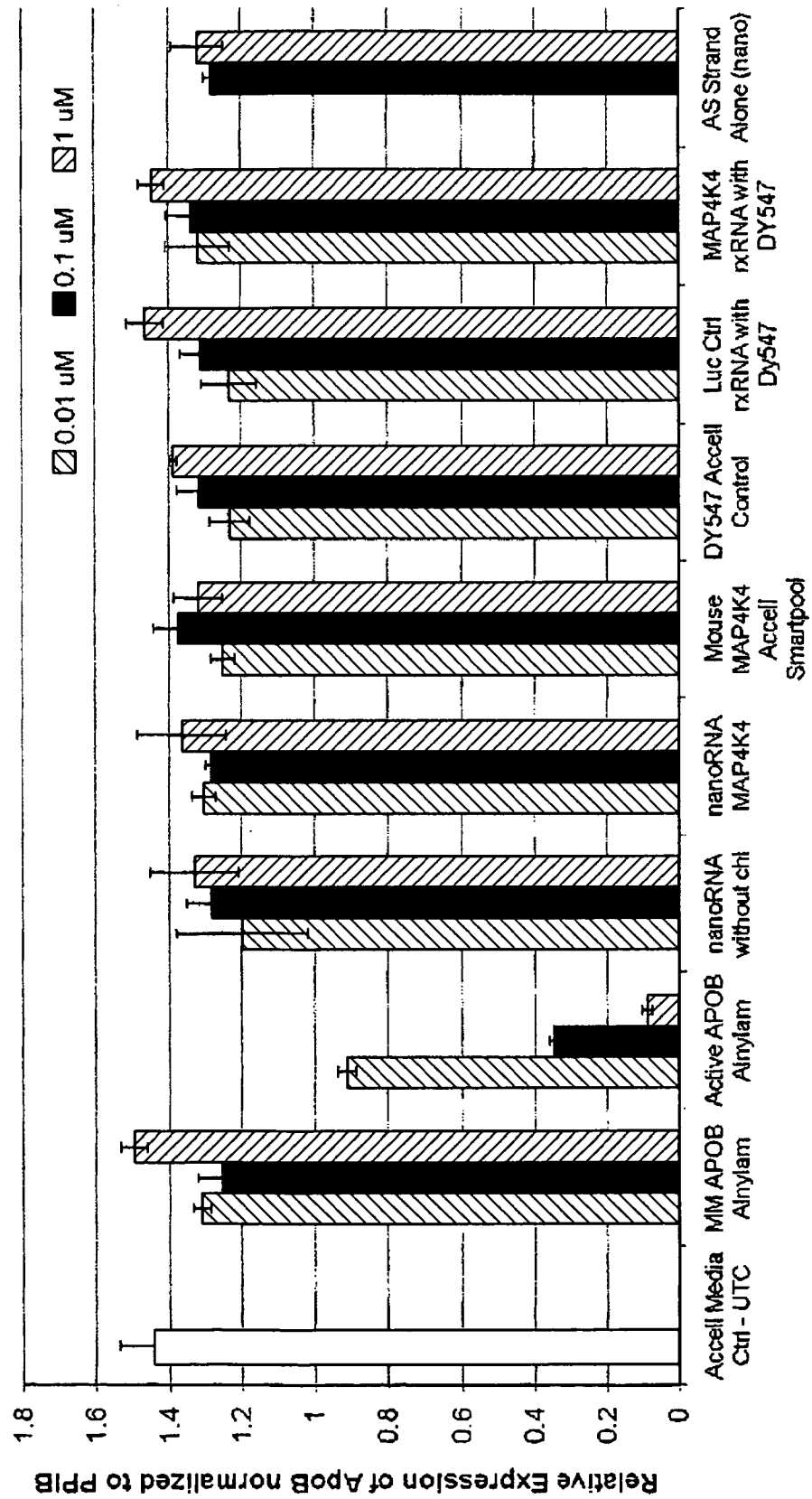
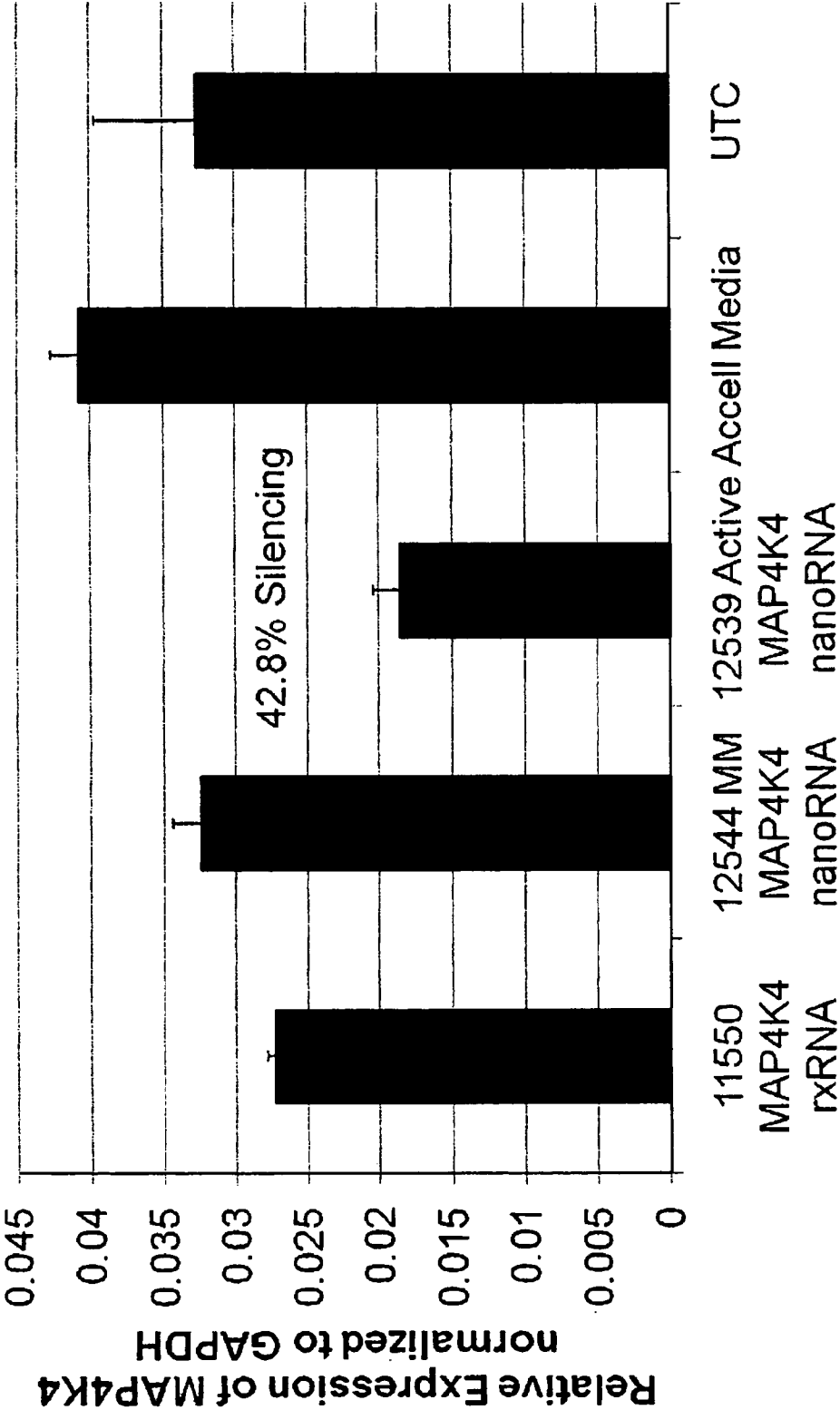


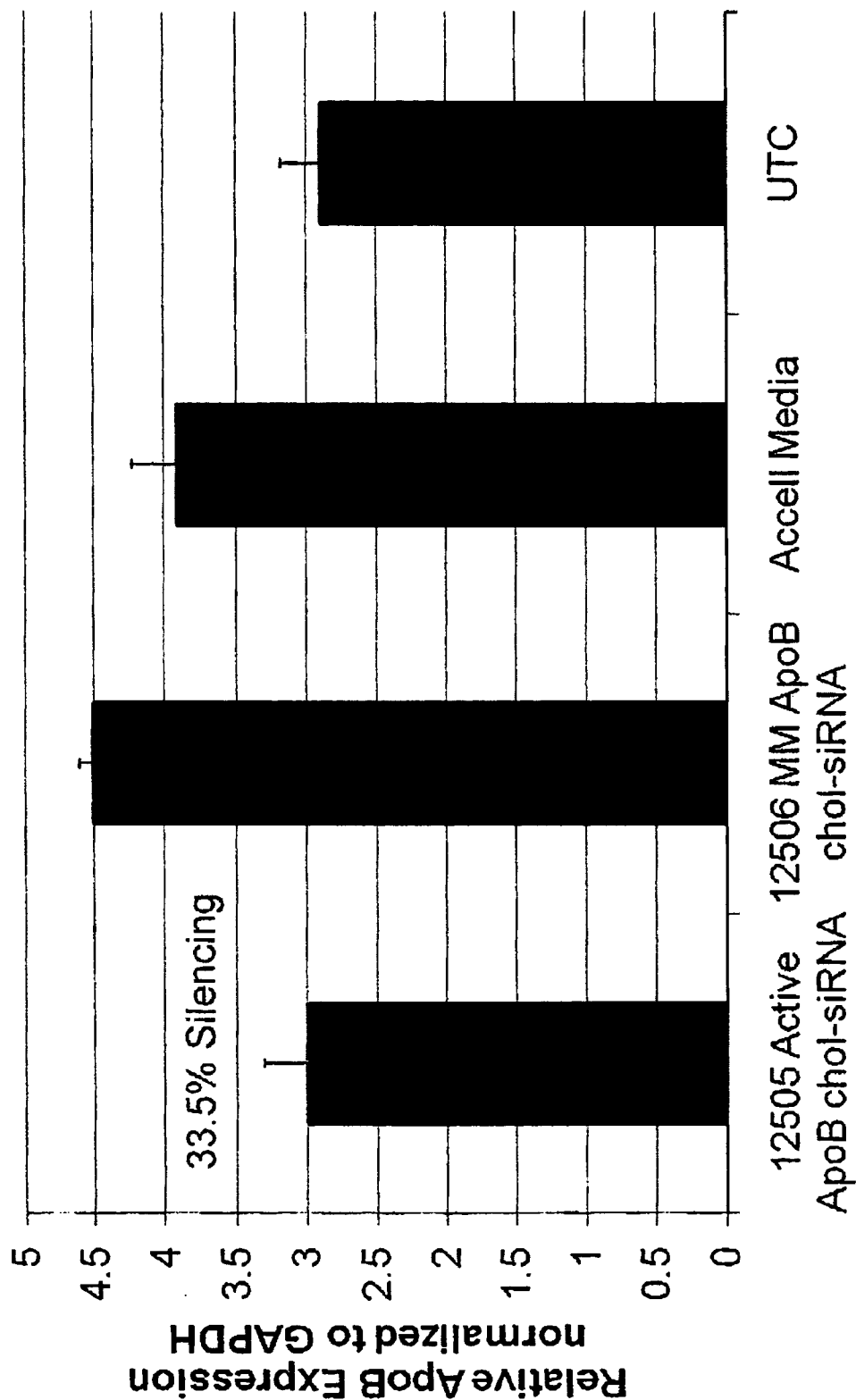
Figure 30

Sd-nanoRNA can silence genes in Primary Human Hepatocytes



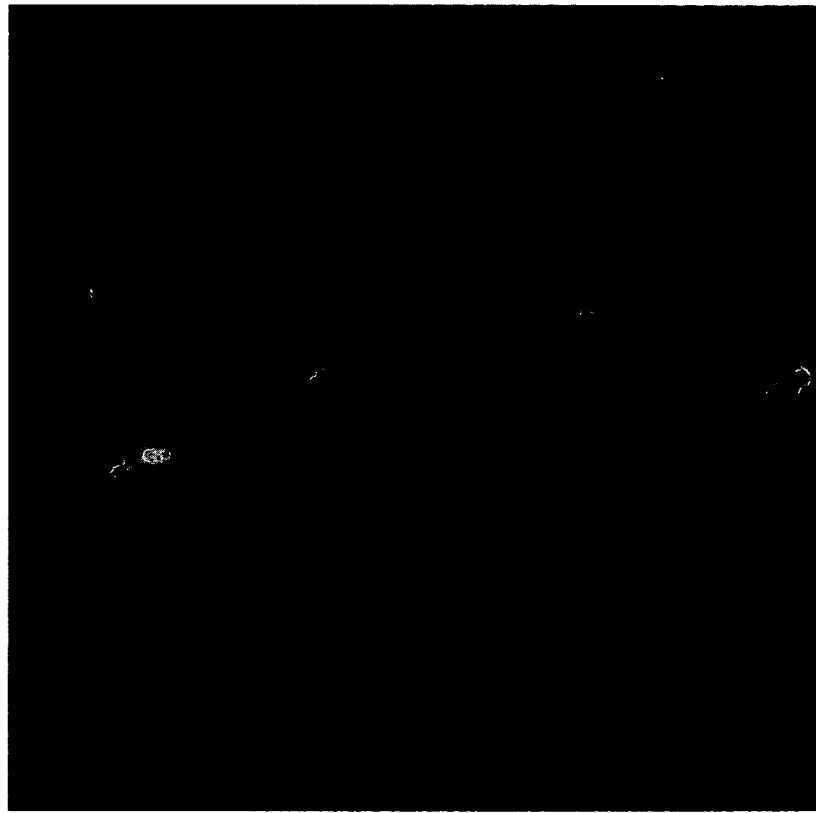
31/92

Figure 31
chol-siRNA silencing in Primary Human
Hepatocytes

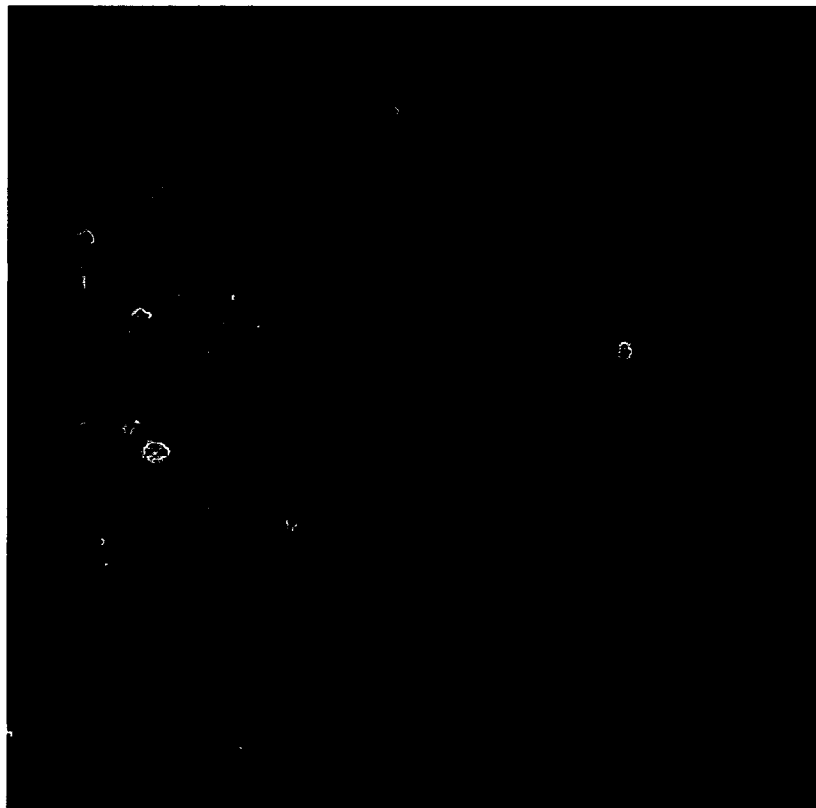


32/92

Figure 32
sd-rxRNA_{nano} Localization



Inside



Outside

Figure 33
Chol-siRNA (Alnylam) Localization

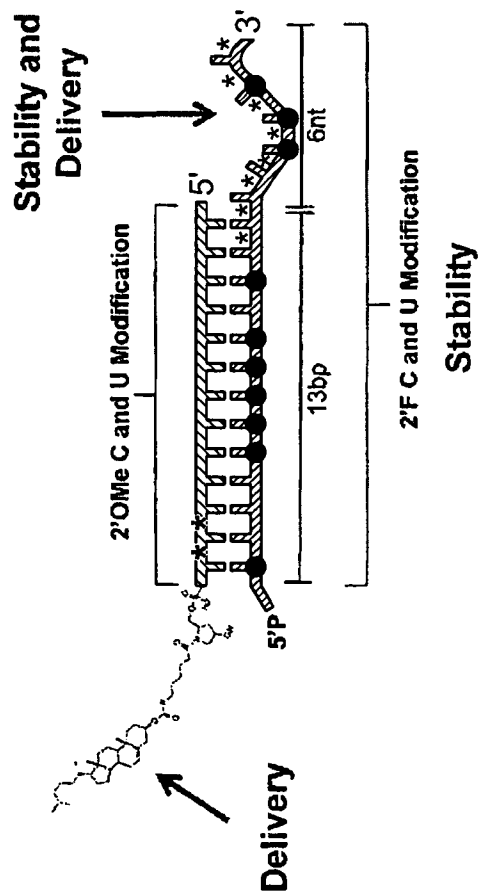


Inside

Outside

34/92

1st Generation sd-rxRNA^{nano}



• Why do we need to optimize chemistry?

- Increased potency
 - Nucleotide Length
 - PS Content
- Reduced toxicity
 - Replacing 2'F on GS
- Delivery
 - Linker and Sterol modalities
- Ease of manufacturing
 - Replacing OH-PS

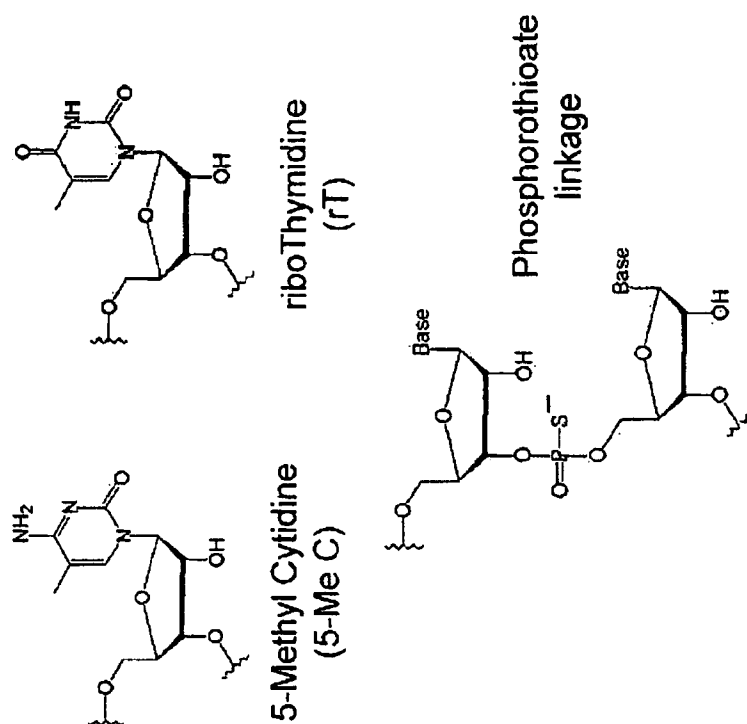
Key

* Phosphorothioate

● 2' F

Figure 34

Chemical Modifications Screened for Optimization of sd-rxRNA ^{nano} (G1)



Above pictures from
www.dharmacon.com

Figure 35

Optimization of GS Length and PS Content

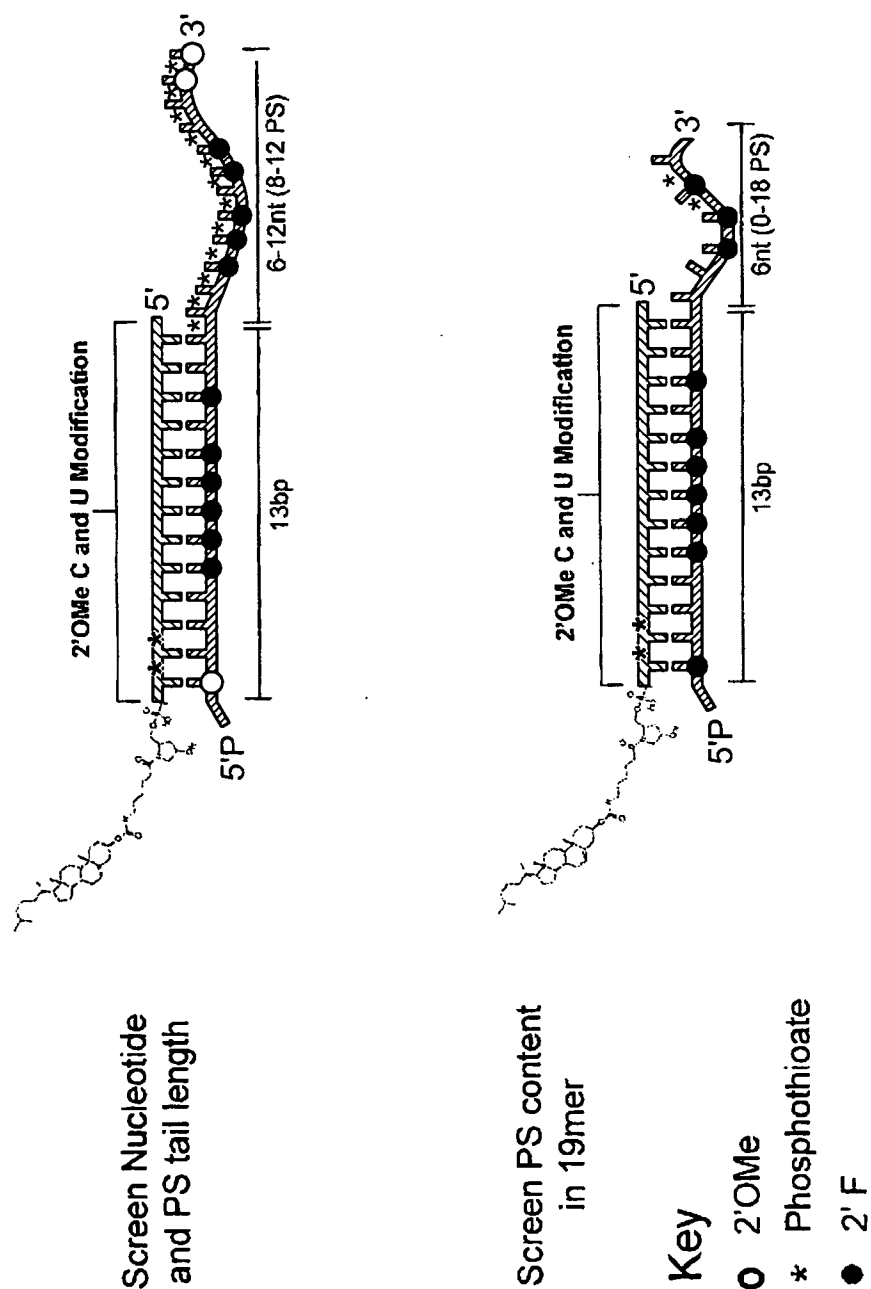


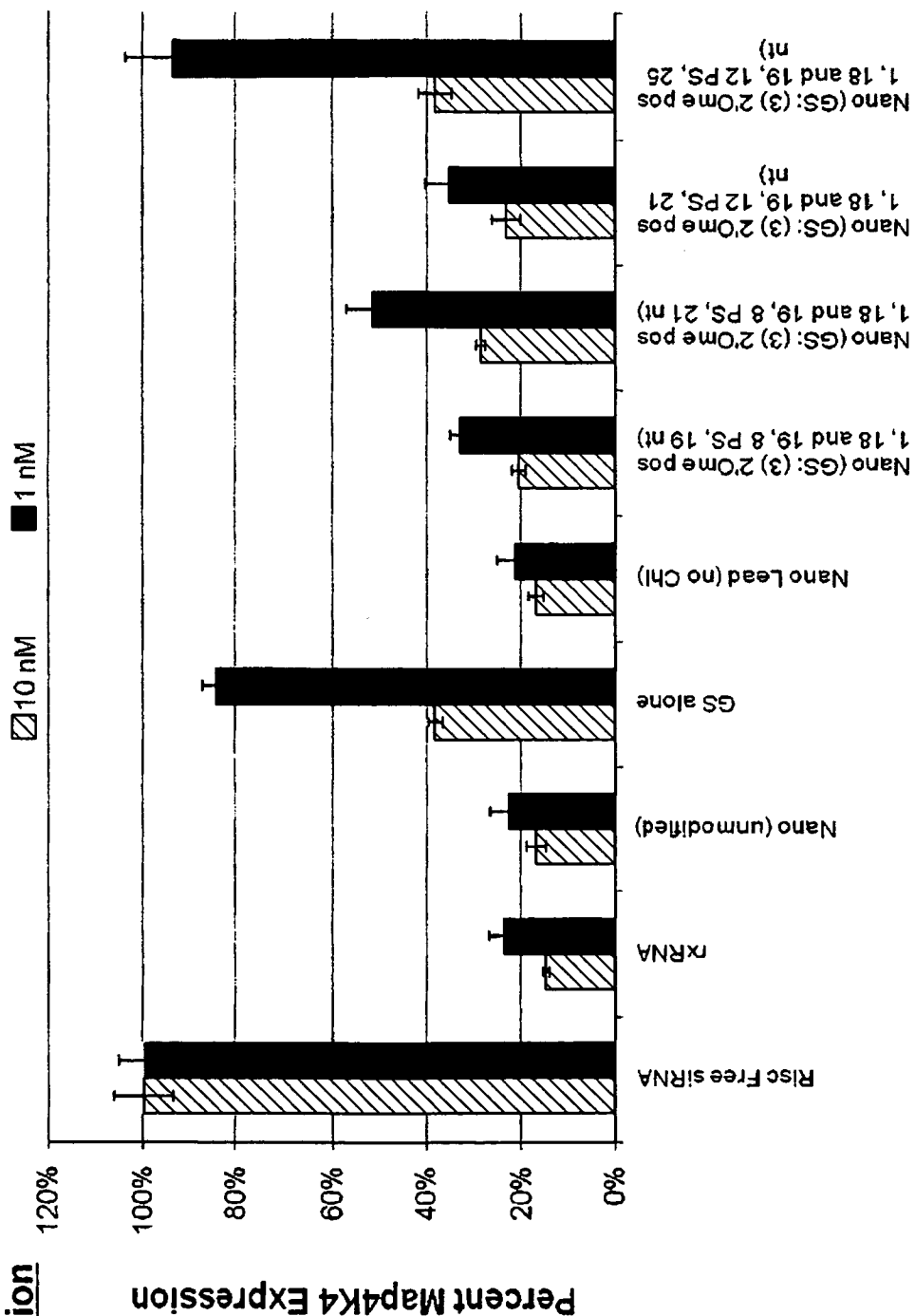
Figure 36

37/92

Figure 37

Increasing Nucleotide Length Reduces Efficacy

Lipid mediated
transfection



Increasing Nucleotide and/or PS Tail Length Reduces Efficacy

Passive Uptake

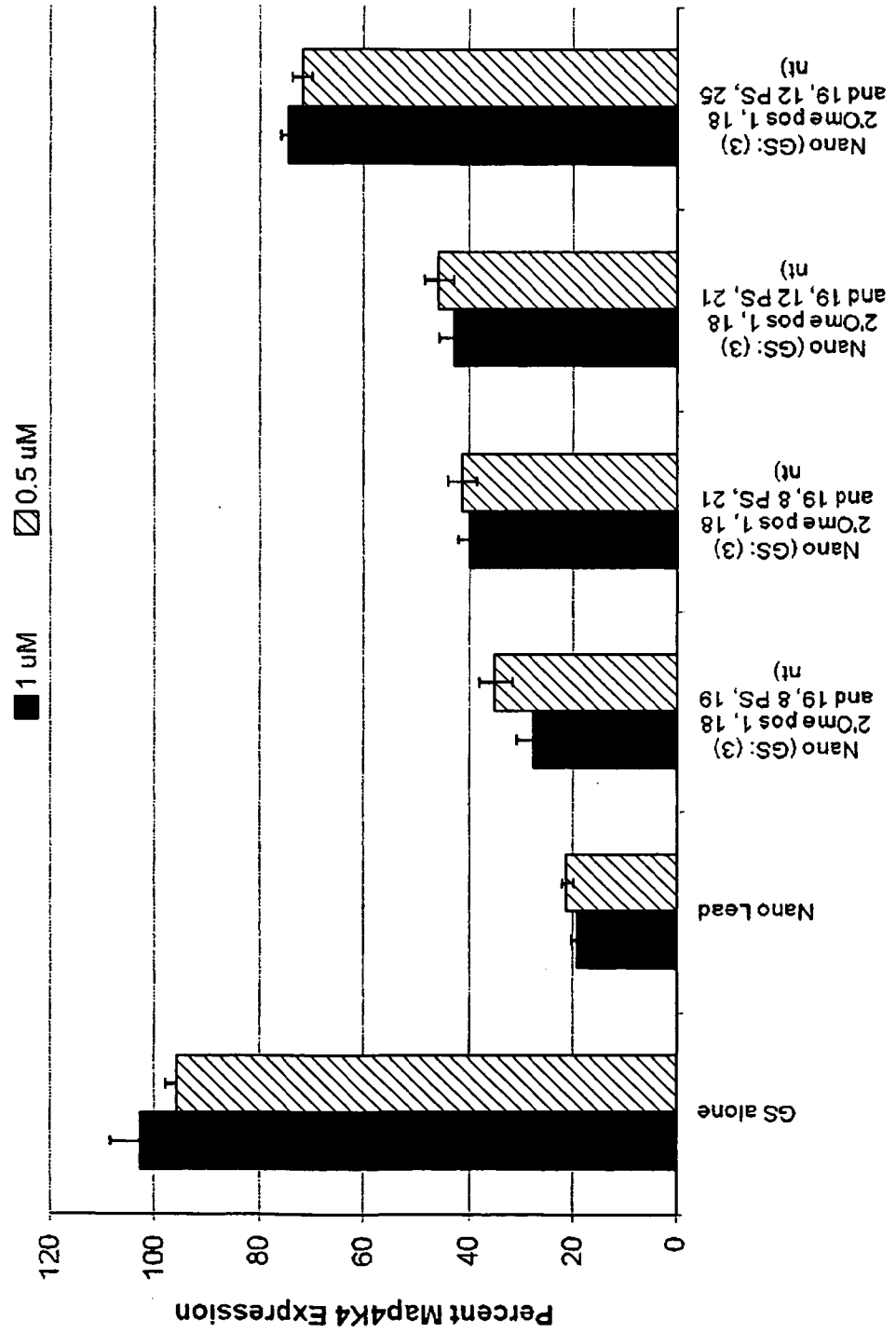
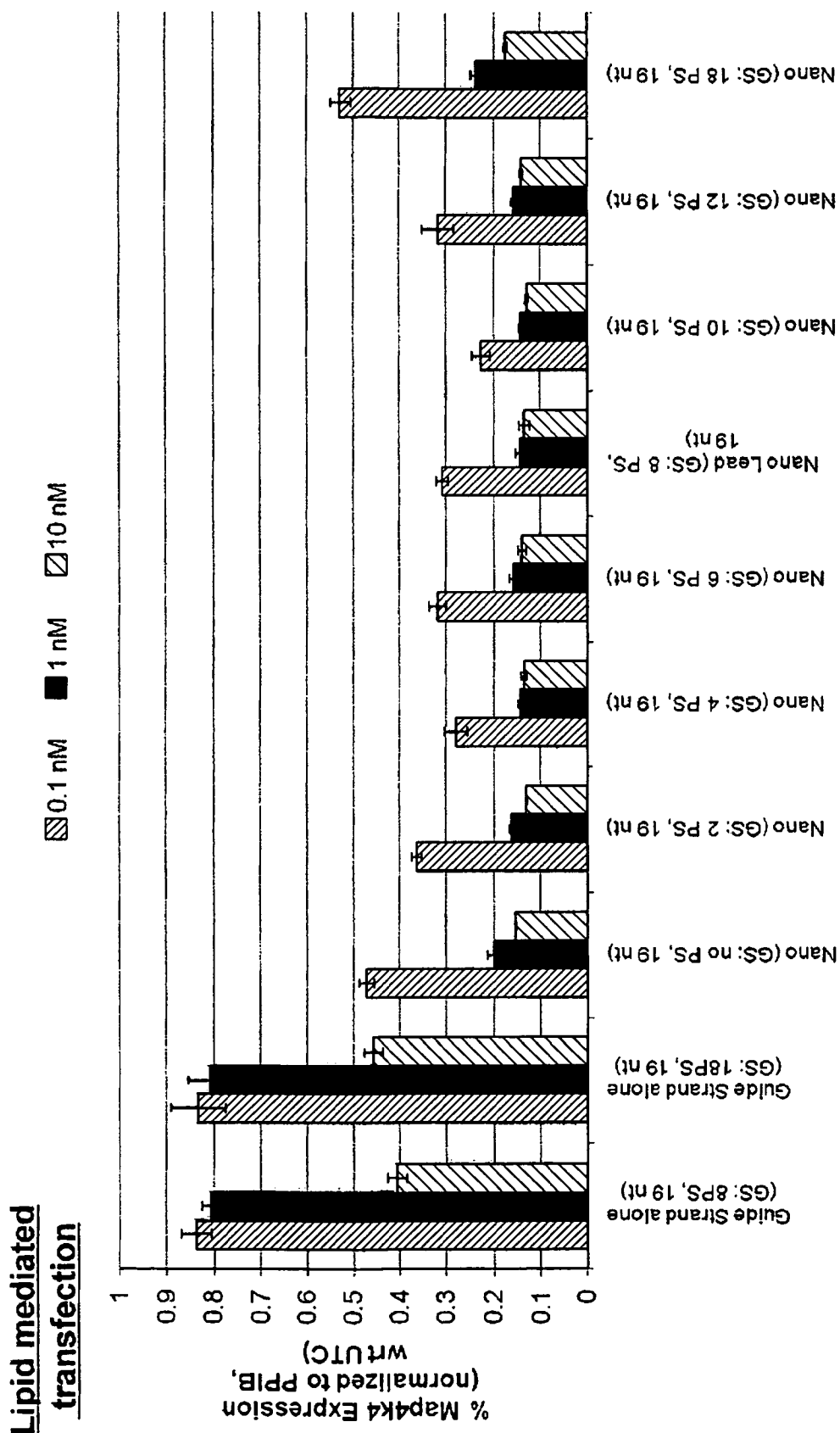


Figure 38

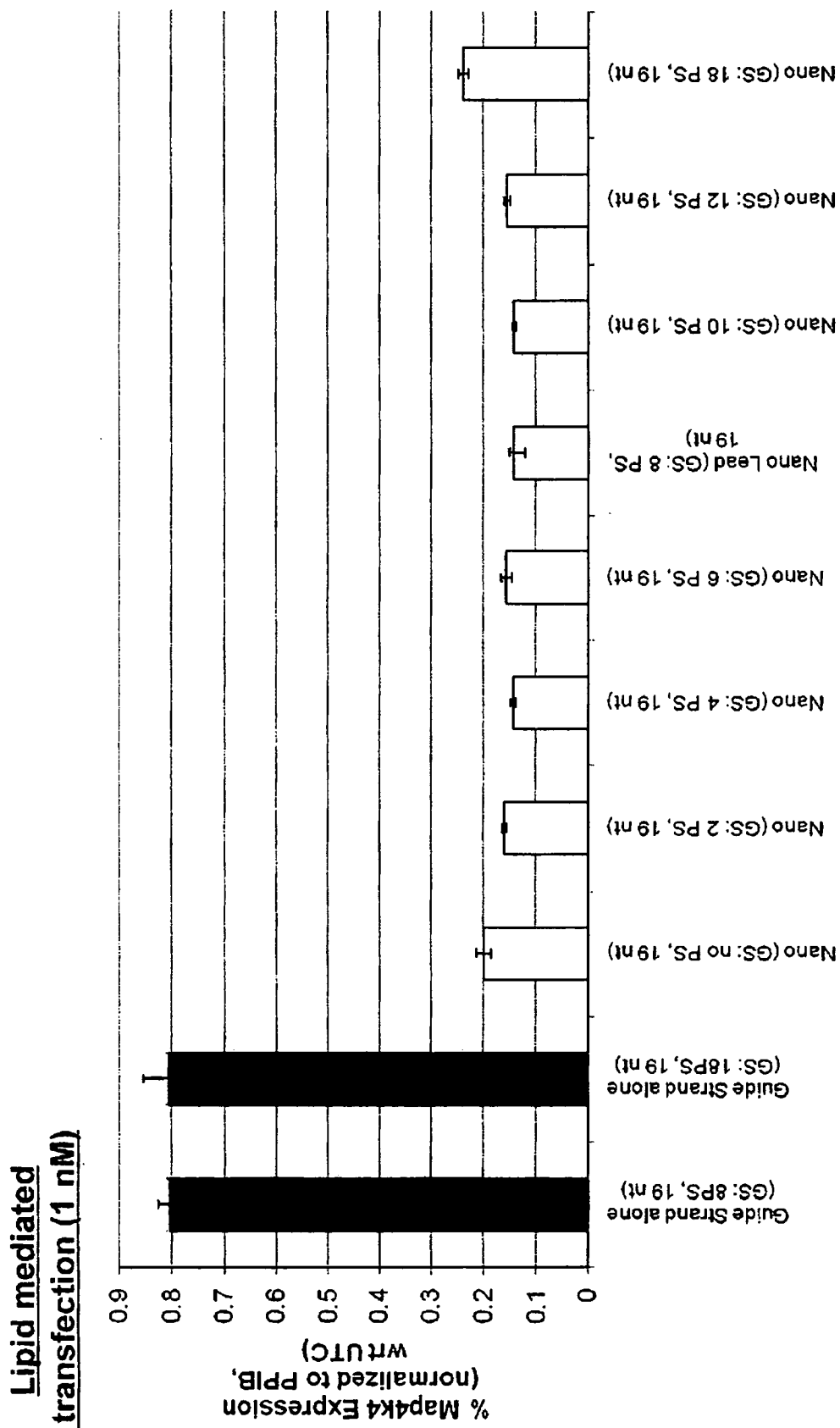
39/92

Figure 39
4 to 10 Phosphorothioates Tolerated in GS (19 mer)



40/92

Figure 40
4 to 10 Phosphorothioates Tolerated in GS (19 mer)



Phosphorothioate Content Vital for Passive Uptake

Passive Uptake (0.1 μ M)

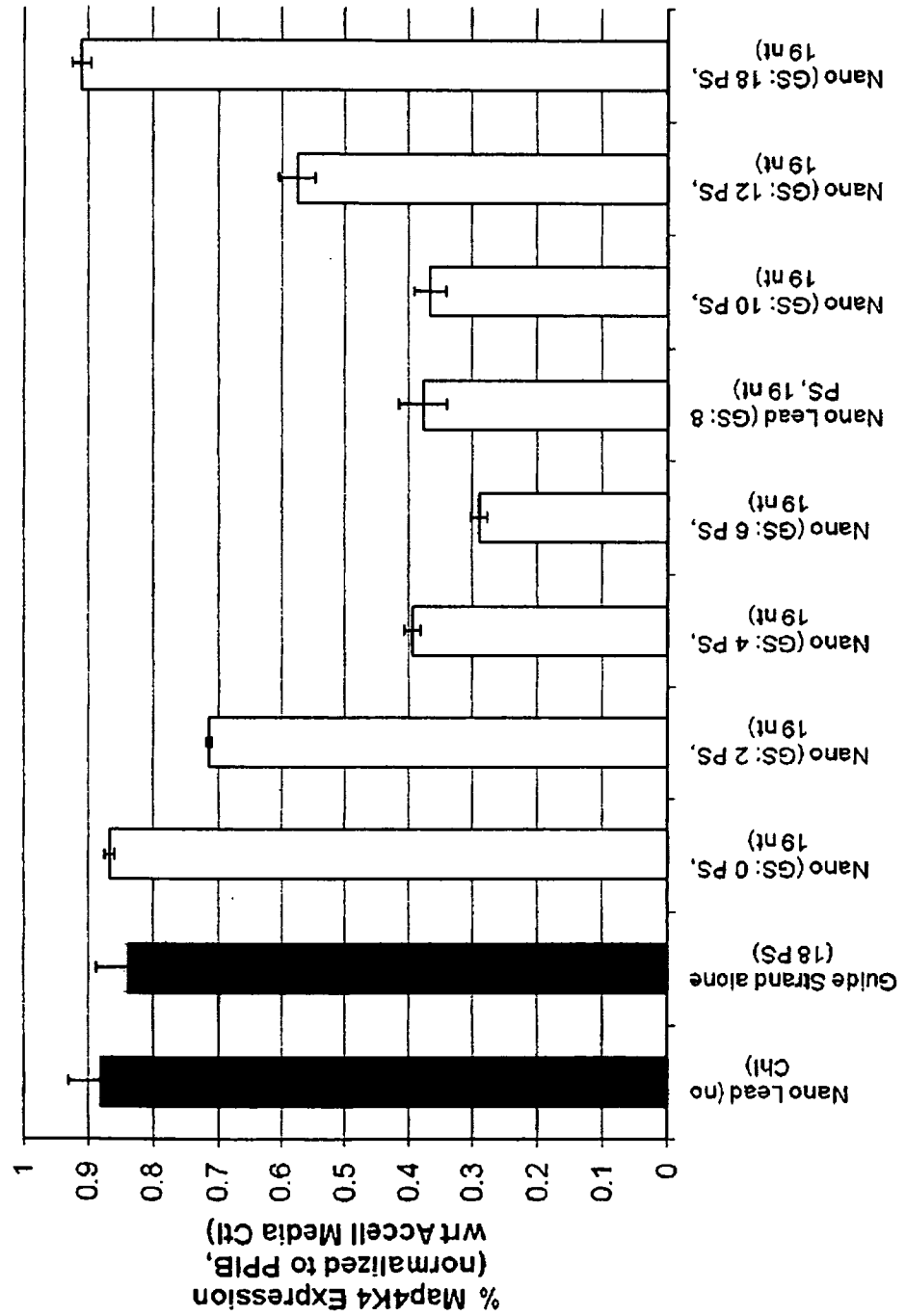


Figure 41

Phosphorothioate Content Vital for Passive Uptake

Passive Uptake

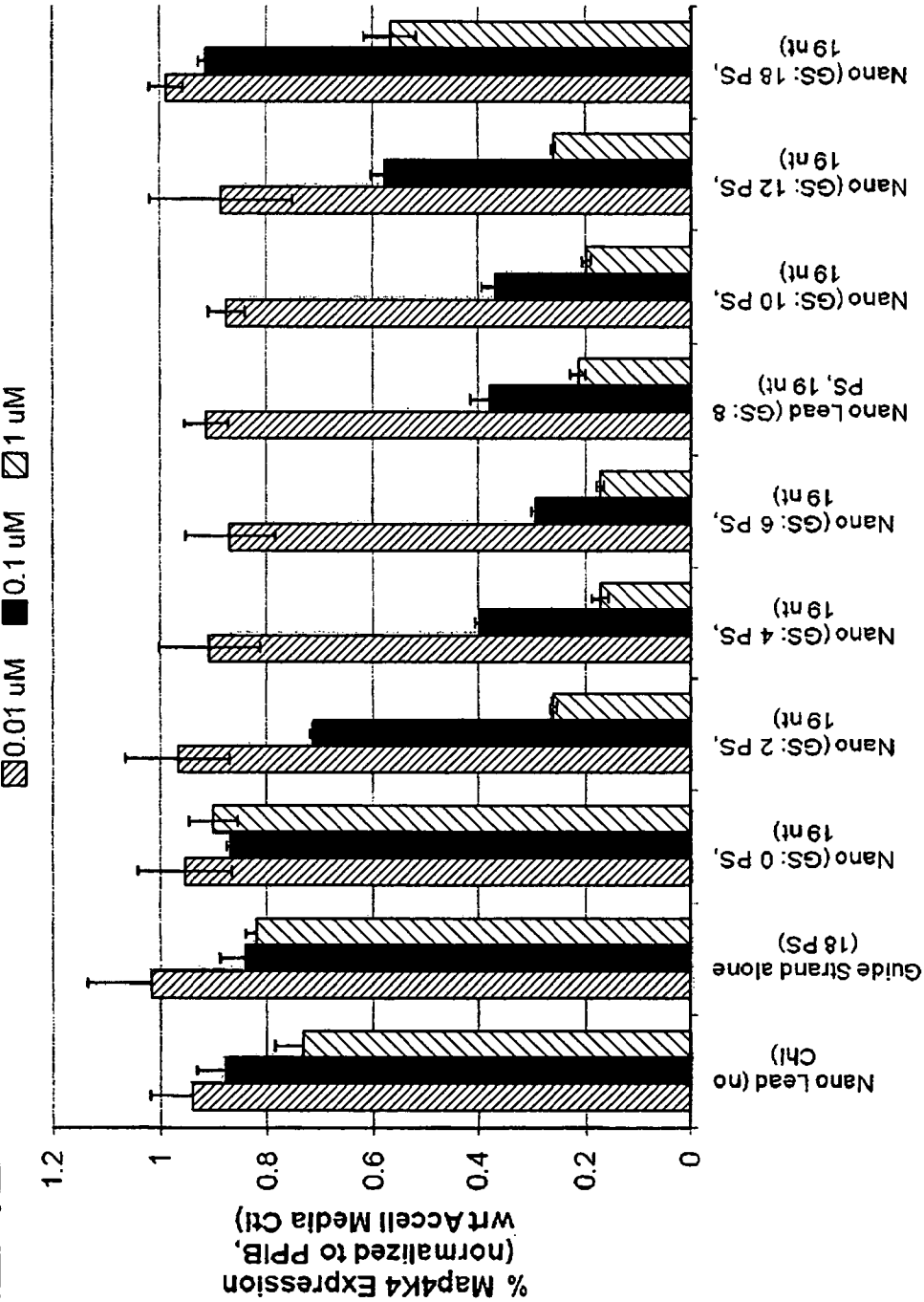
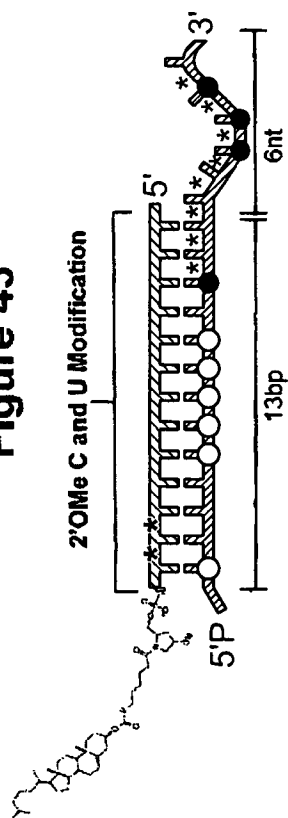
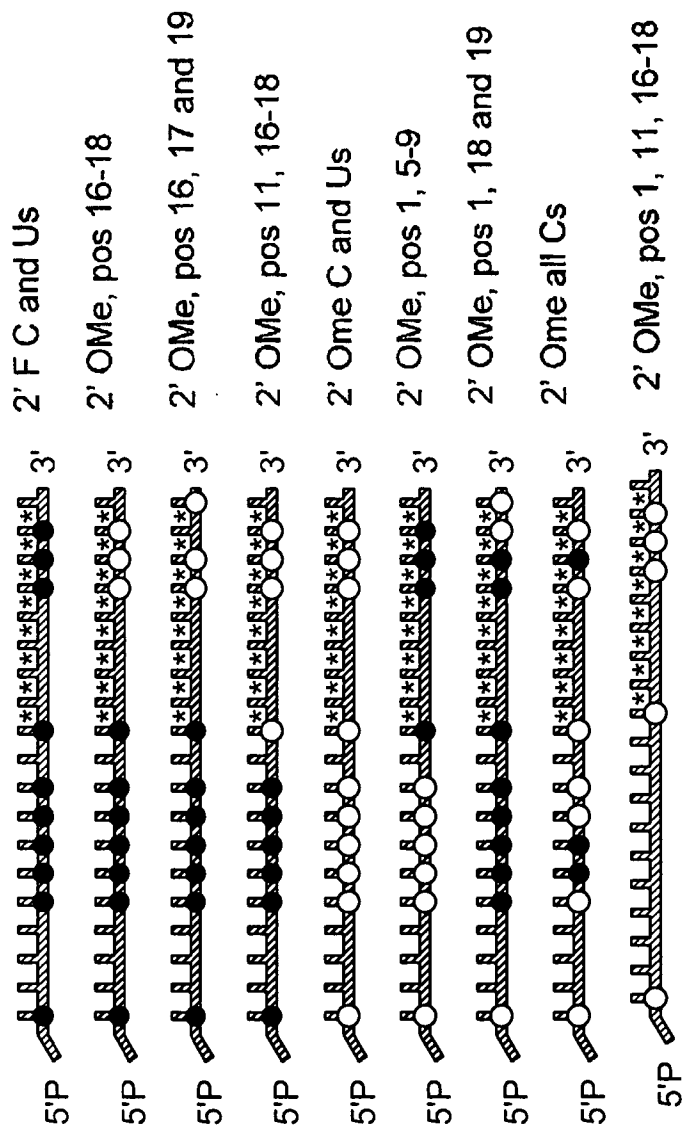


Figure 42

Figure 43



Guide strands screened



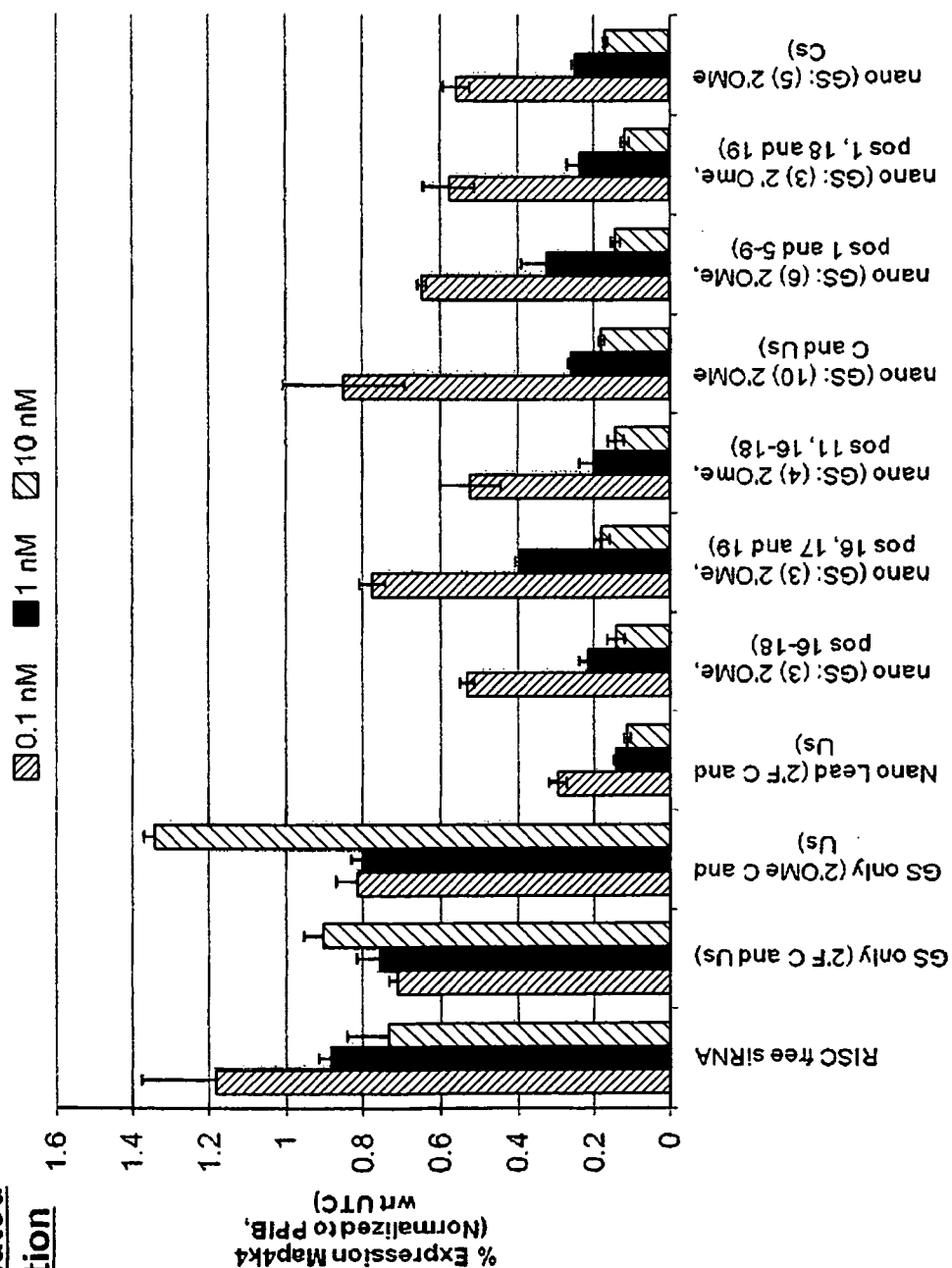
Key

- 2'OMe
- * Phosphothioate
- 2' F

Figure 44

2'OMe Tolerated in 3'end of Guide Strand

Lipid mediated
transfection



45/92

Figure 45

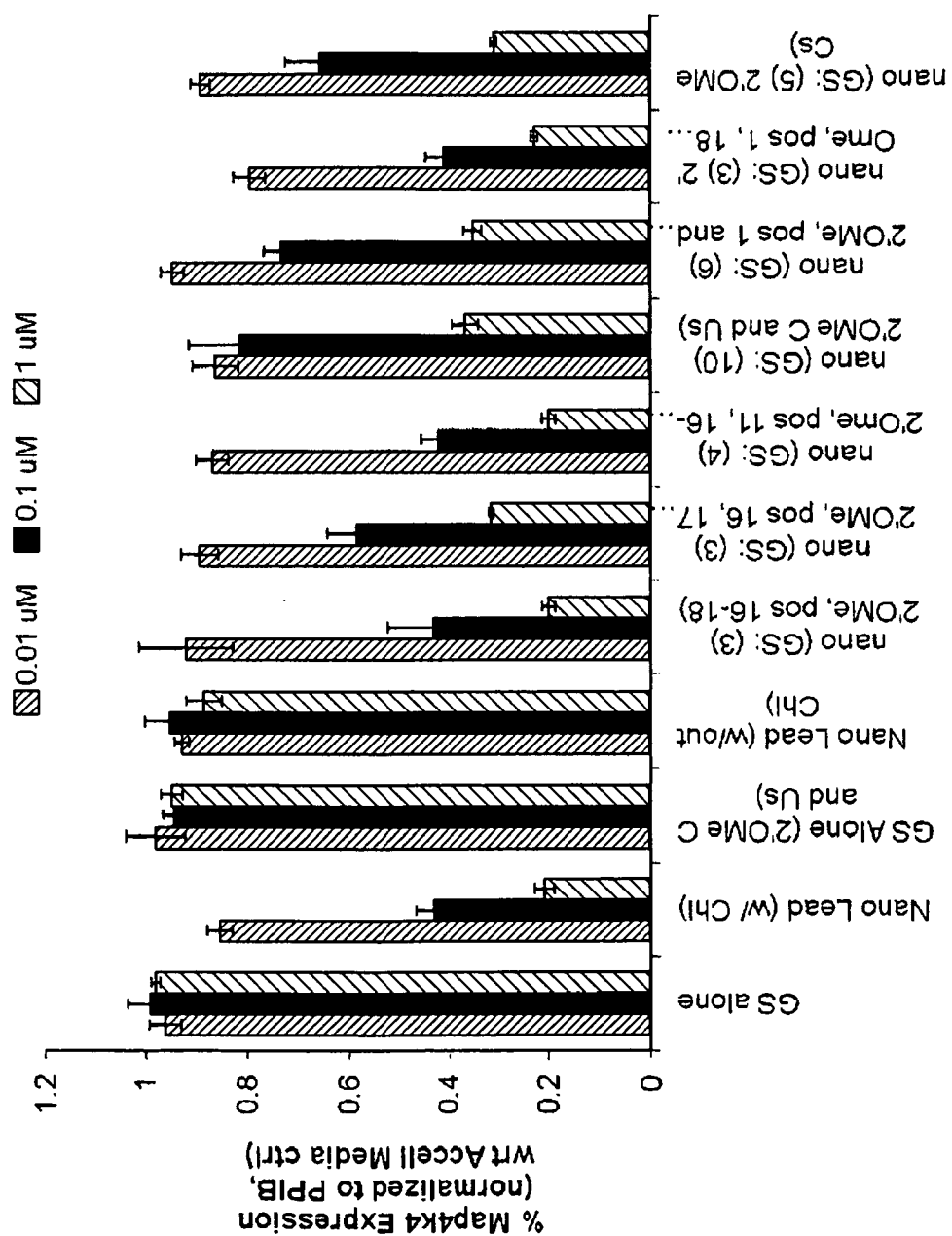
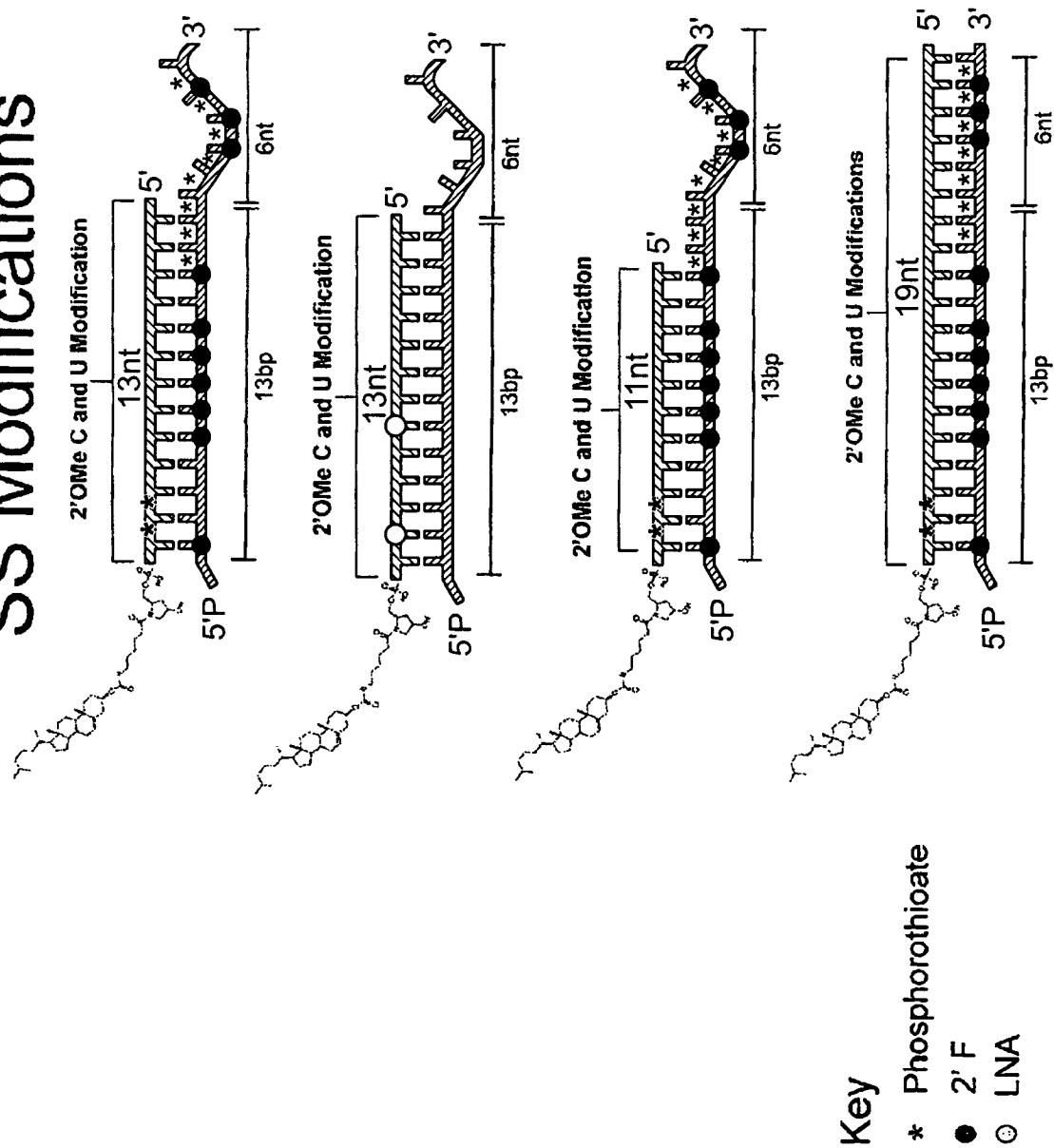


Figure 46
SS Modifications



47/92

Figure 47

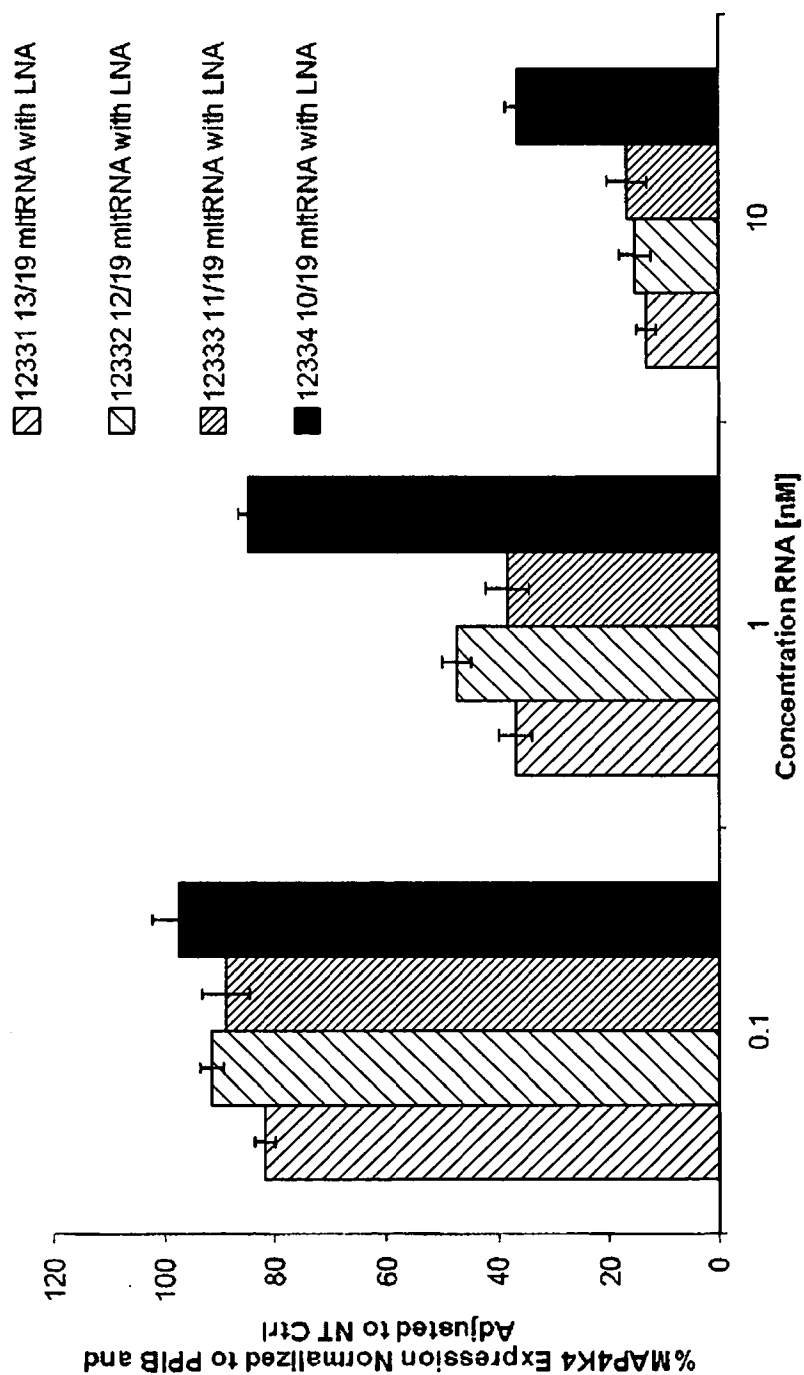
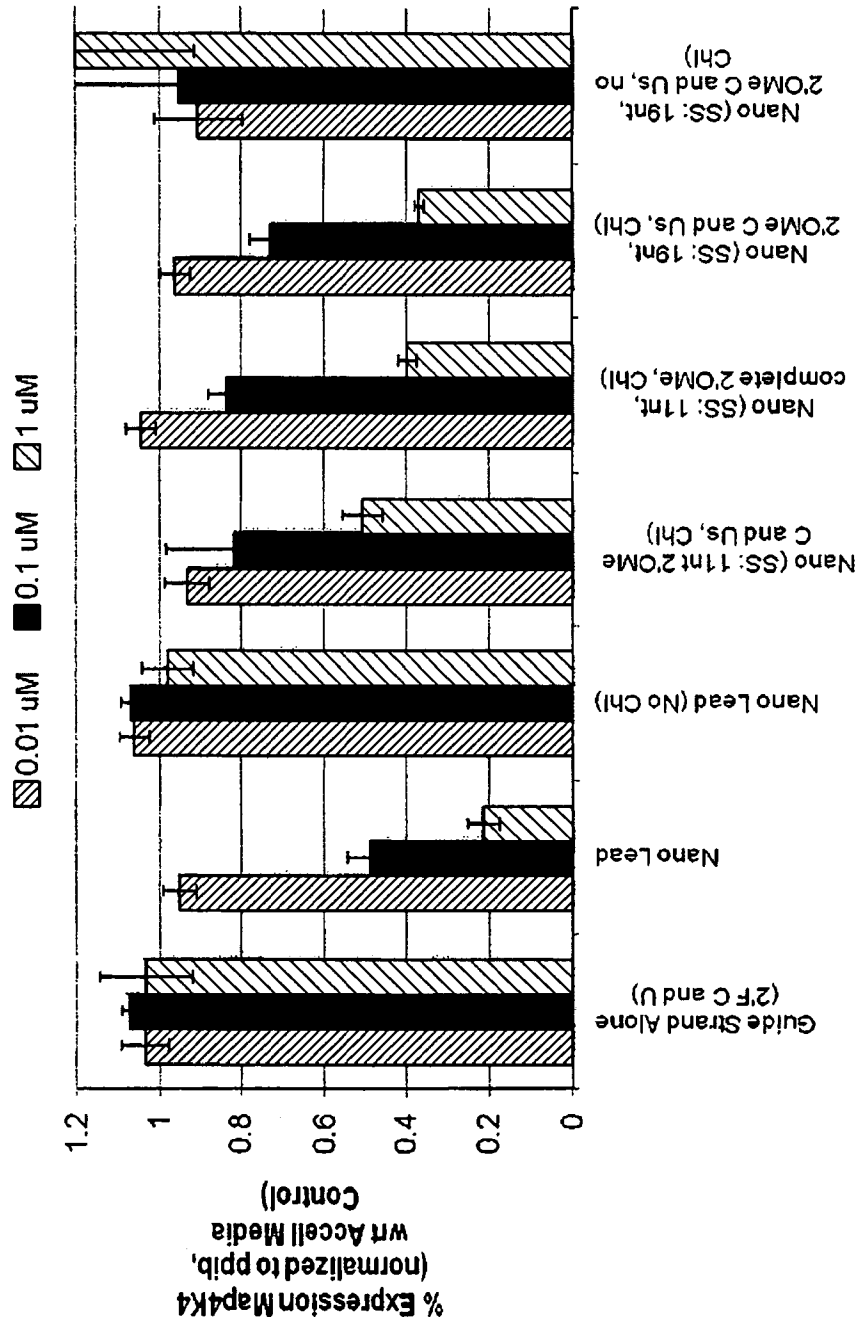


Figure 48

13 Bases Optimal Length in Sense Strand

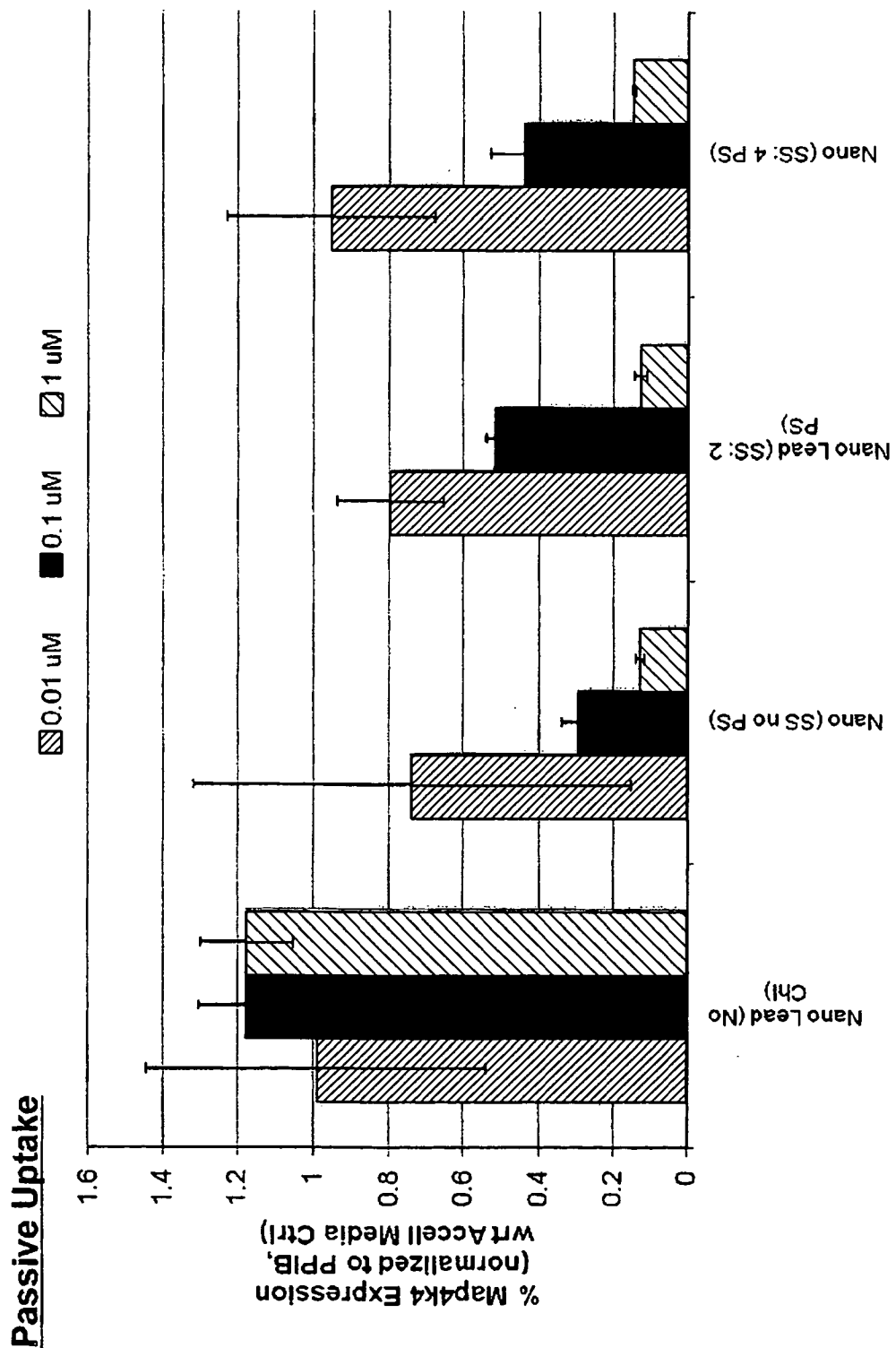
Passive Uptake



49/92

Figure 49

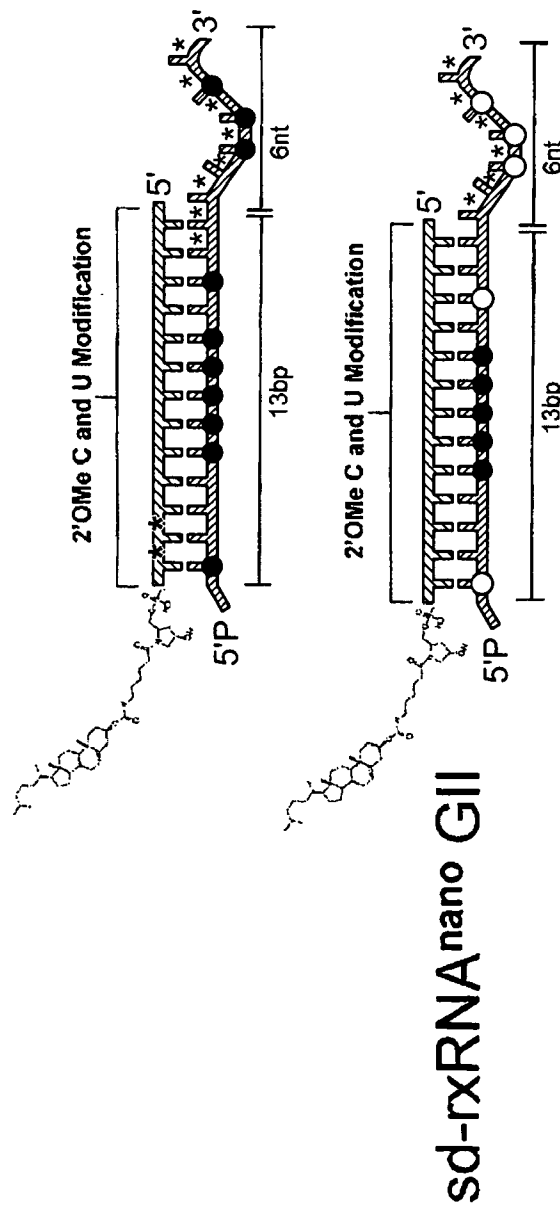
Phosphorothioates Not Required for Passive Uptake in Sense Strand



50/92

Figure 50

2nd Generation sd-rxRNA ^{nano} Lead



- 40 % reduction in PS content

- 40-50% reduction in 2'F

Key

○ 2'OMe

* Phosphothioate

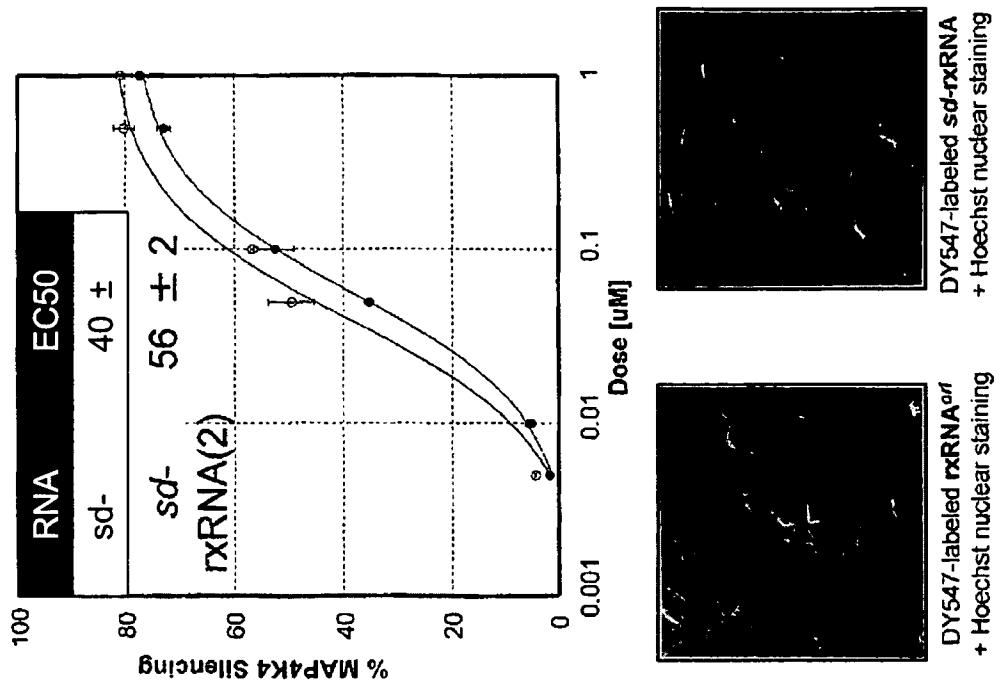
● 2' F

51/92

Figure 51

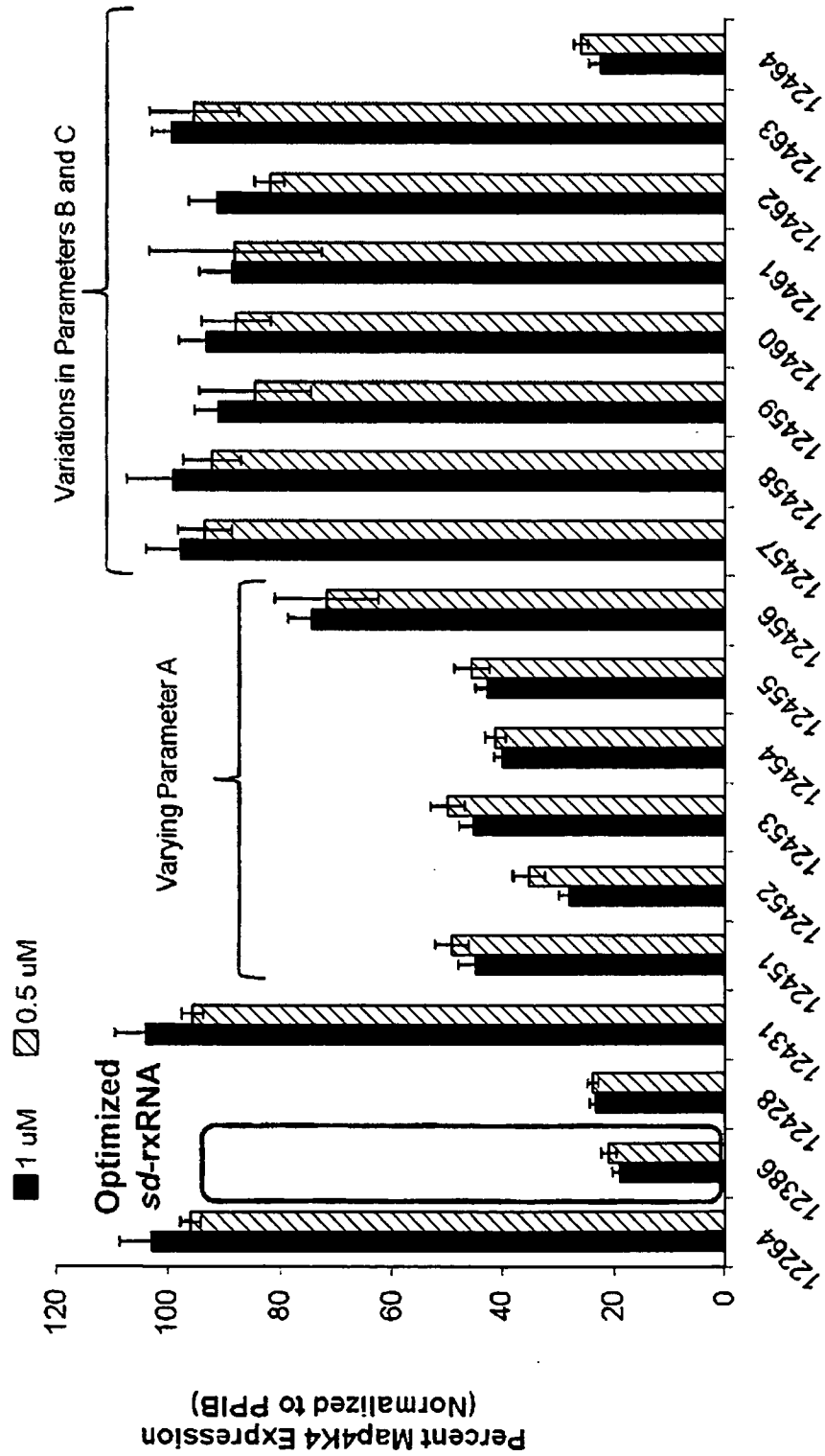
sd-rxRNA™: Spontaneous Cellular Uptake and Efficacy Without Delivery Vehicle

- Chemically modified bipartite RNAi molecules with self-delivering moiety(s)
- *Picomolar* activity after facilitated delivery(lipid-mediated transfection)
- *Nanomolar* activity in cell culture with NO transfection reagent (**self-delivery**)
- Efficient uptake (>95%) by most cell types in cell culture
- Stable (more then 3 days in 100% human serum)
- Results in distribution to tissues; reduced kidney clearance
- Compatible with SC administration
- Highly specific (little or no immune induction)



52/92

Interplay Between Chemistry and Configuration Yields Potent sd-rxRNA™

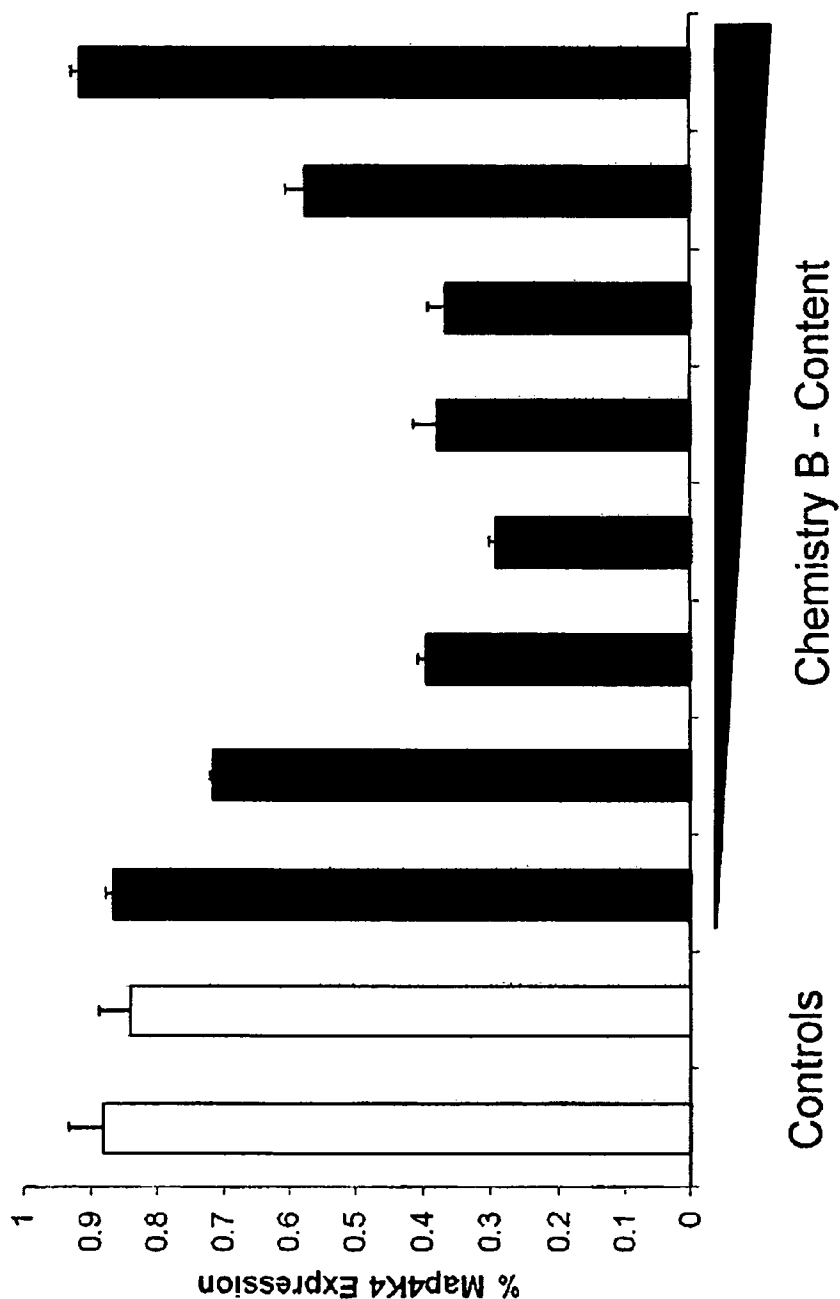


- HeLa cells; 72 hours
- Varying three different parameters

Figure 52

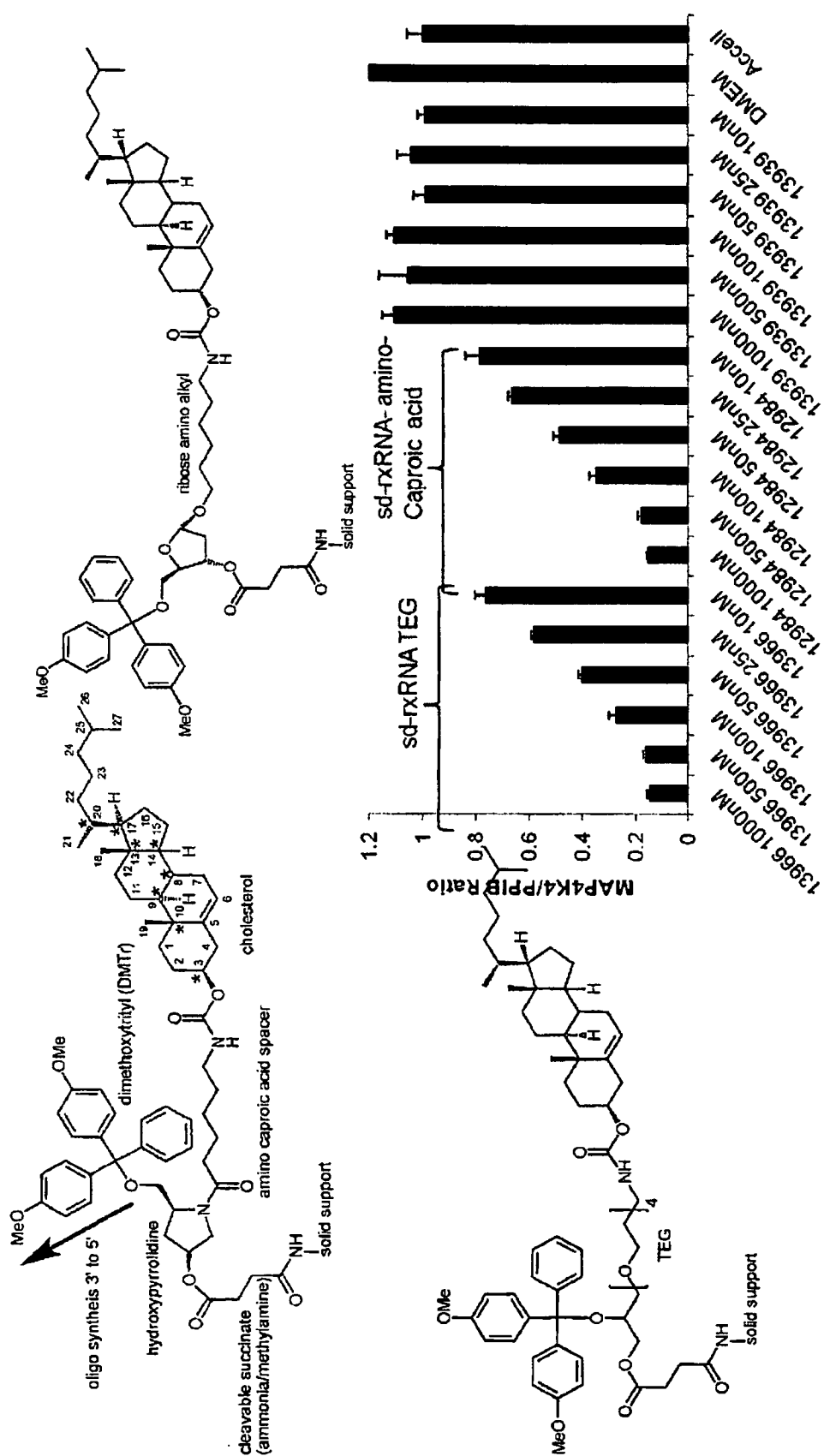
53/92

Figure 53
Chemistry Type and Content is Essential for
***sd*-rxRNA™ Efficacy**
Passive Uptake (0.1 uM)



54/92

Figure 54



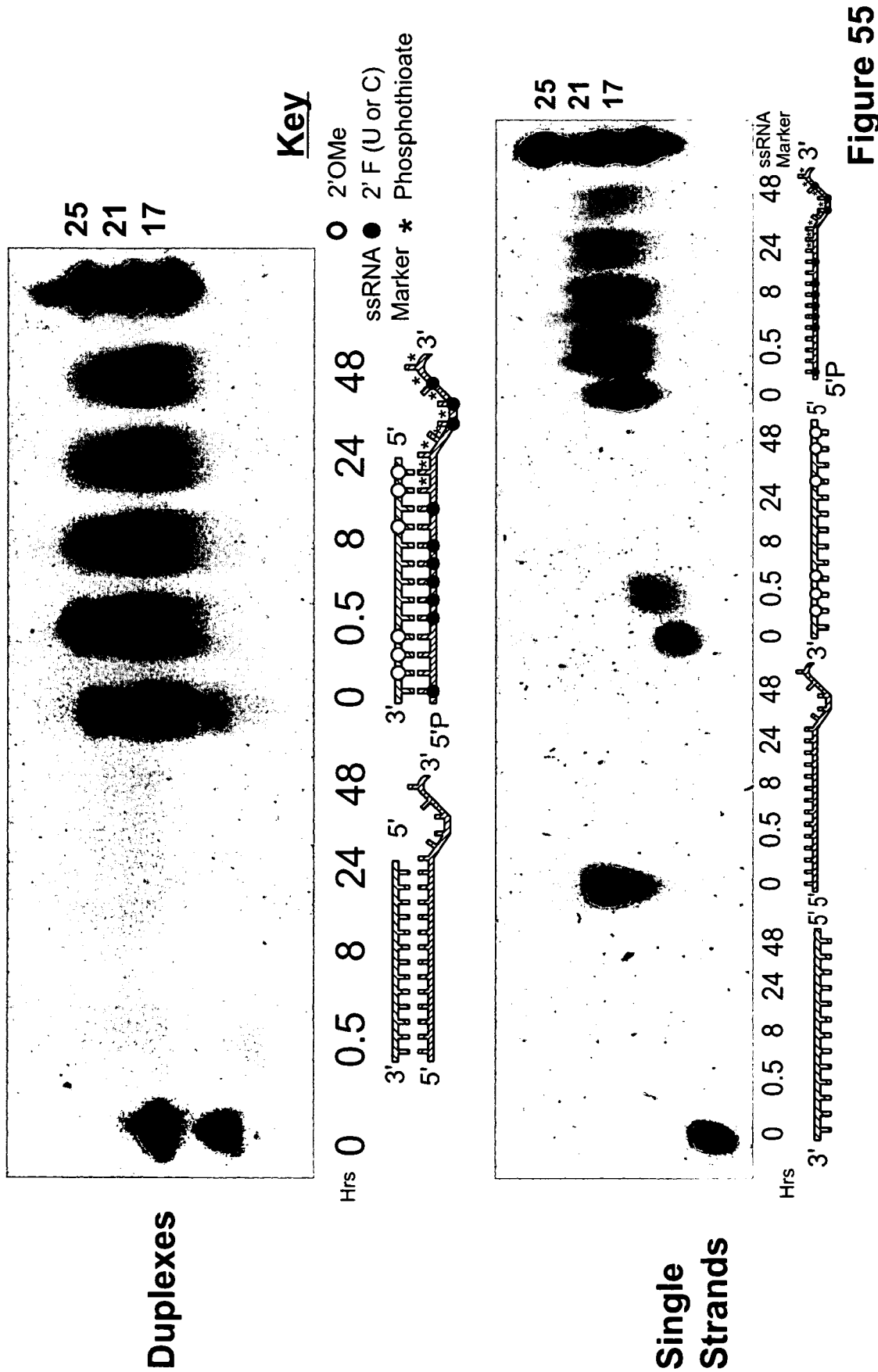
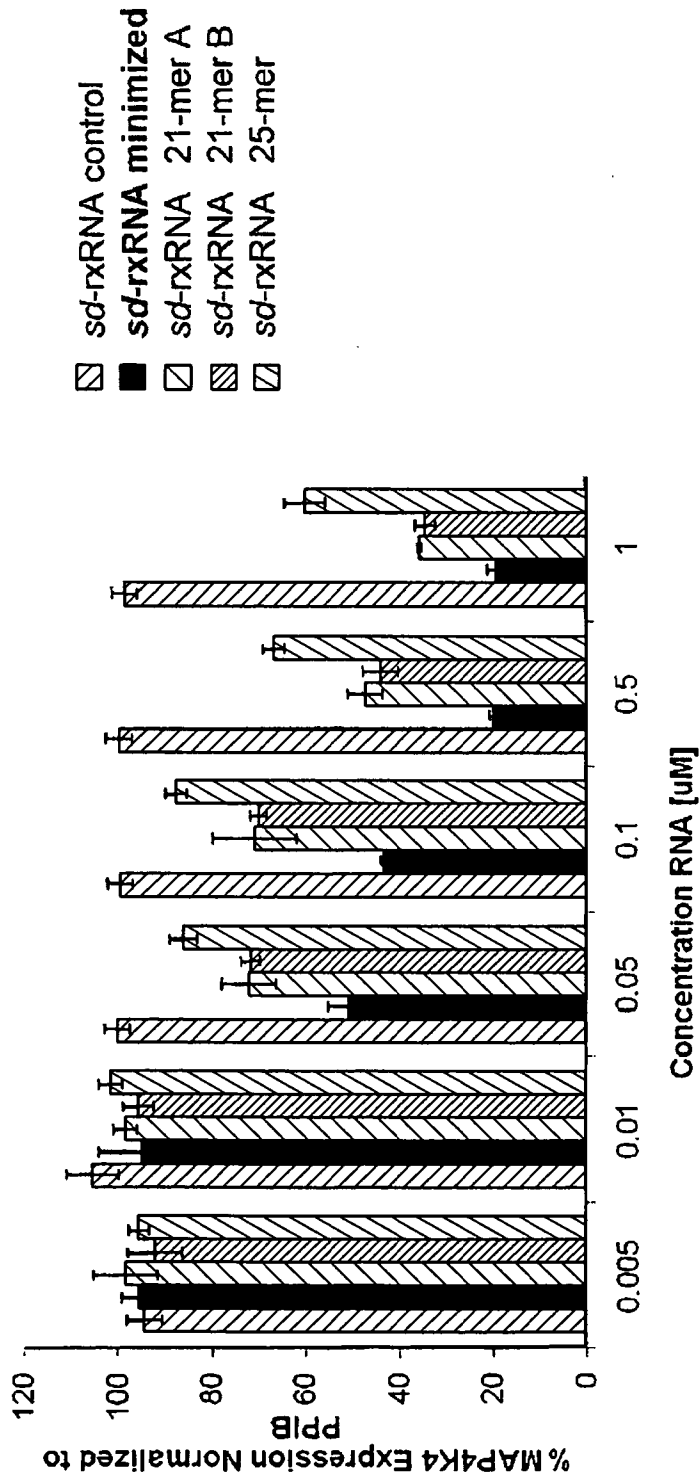


Figure 56

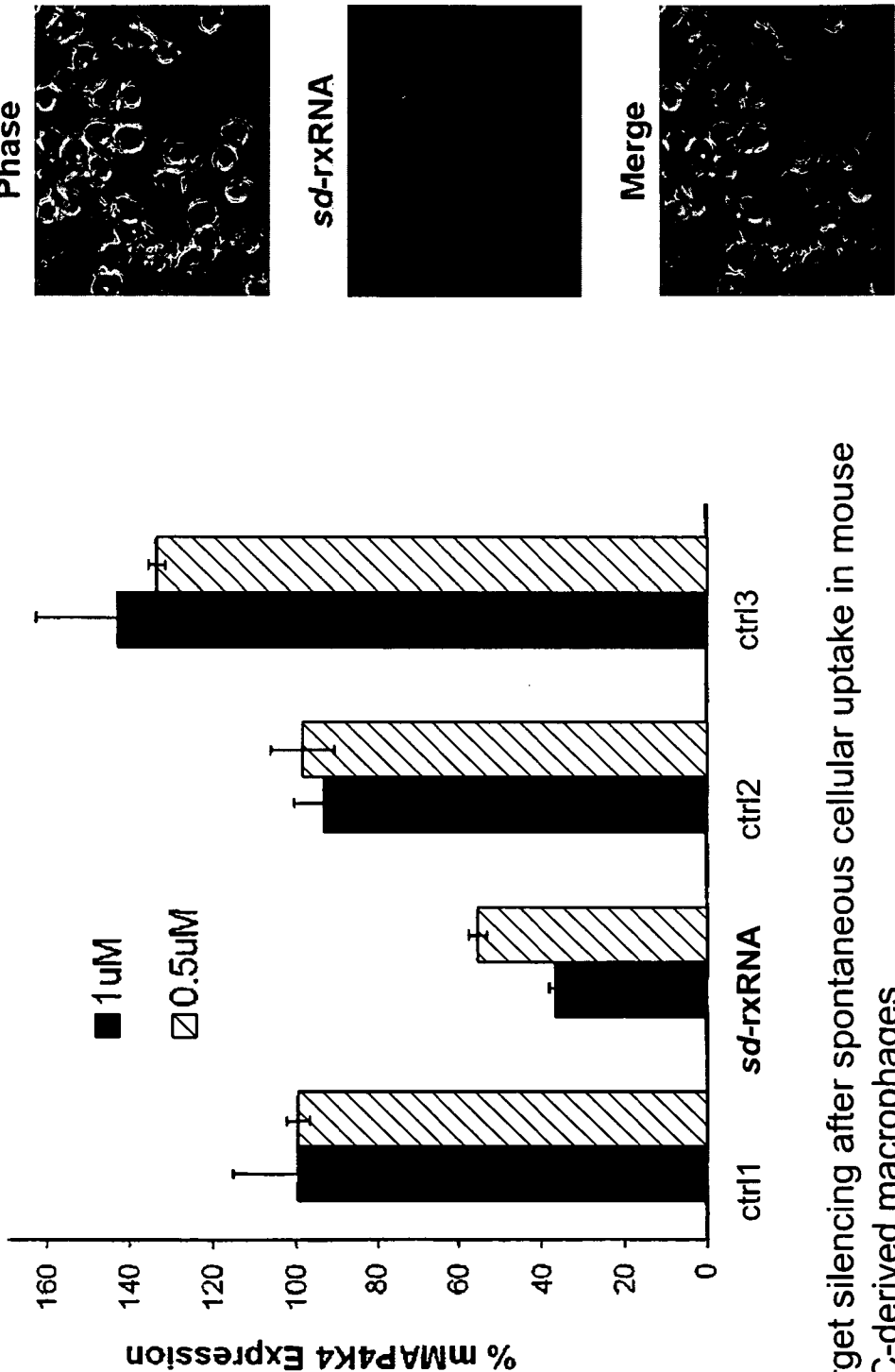
sd-rxRNA™: Minimizing Oligonucleotide Content is Important for Cellular Uptake



- Spontaneous cellular uptake (HeLa)
- Lead **sd-rxRNA** compound is based on rxRNA^{nano}
- Minimizing oligonucleotide content is critical for efficient uptake

Figure 57

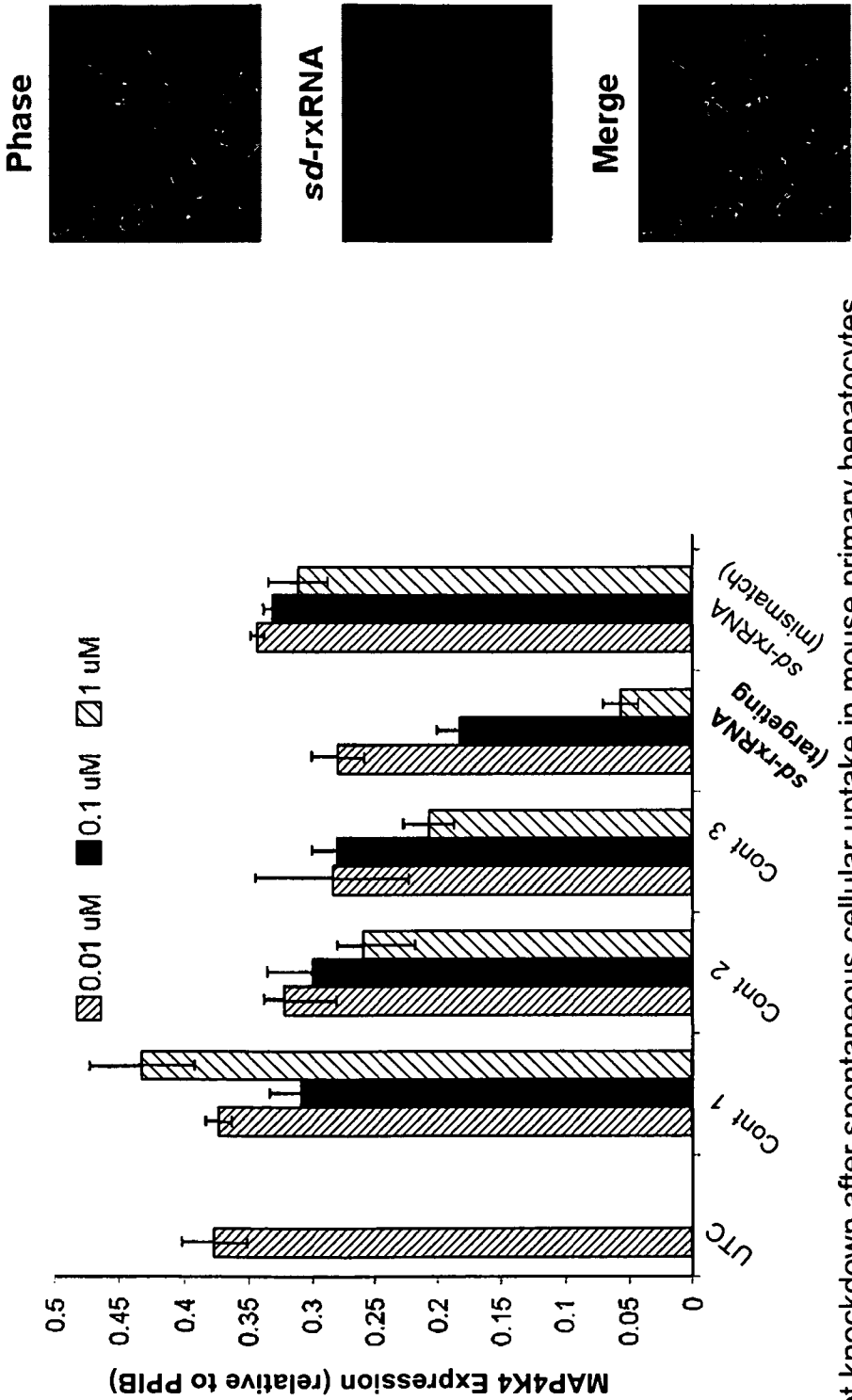
*sd-rxRNA*TM: Spontaneous Uptake and Target Gene Silencing in Primary Cells



• Target silencing after spontaneous cellular uptake in mouse PEC-derived macrophages

Figure 58

*sd-rxRNA*TM: Spontaneous Uptake and Target Gene Silencing in Primary Cells



• Target knockdown after spontaneous cellular uptake in mouse primary hepatocytes

Figure 59

sd-rxRNA Delivery to RPE Cells With No Formulation

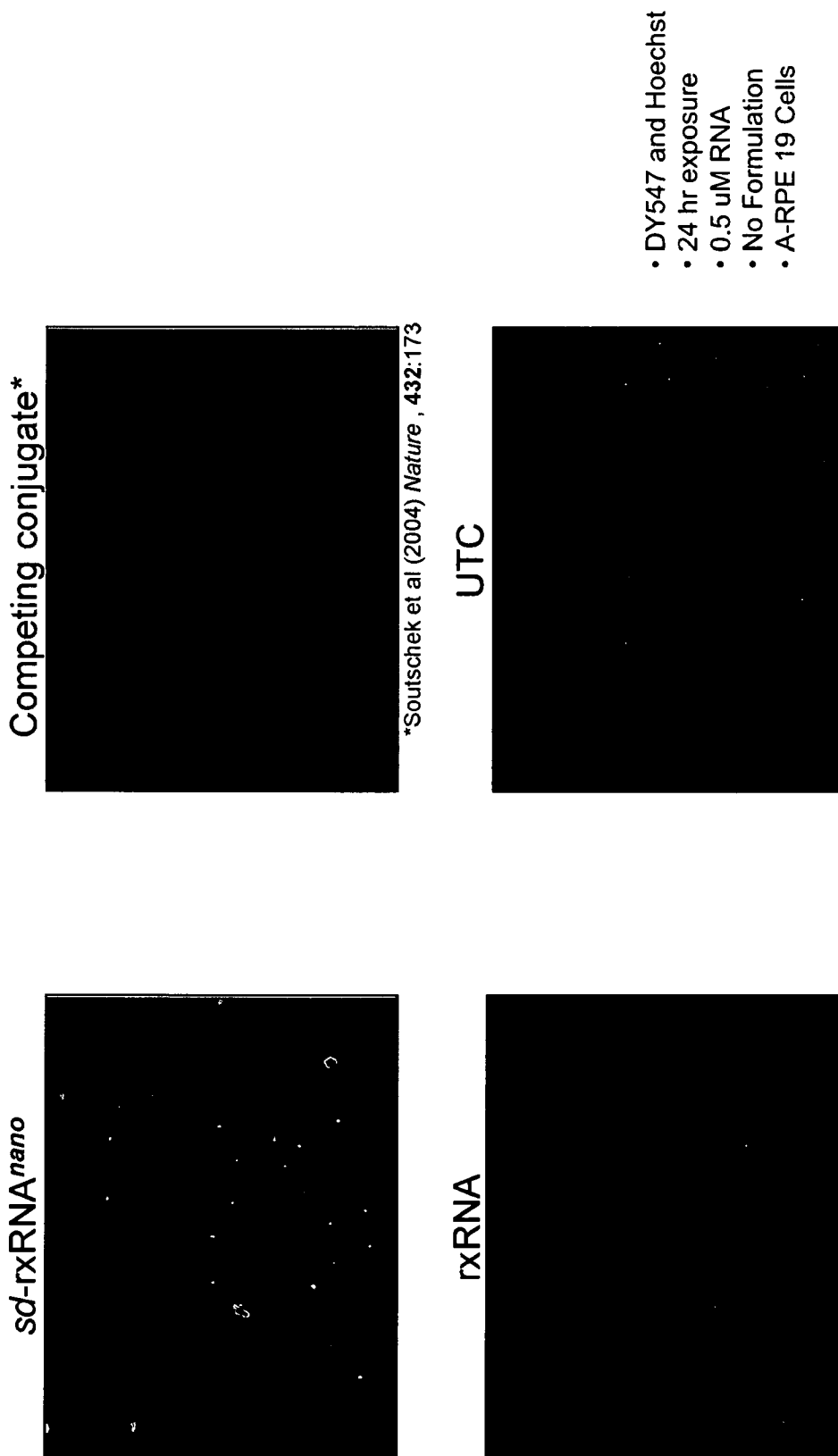
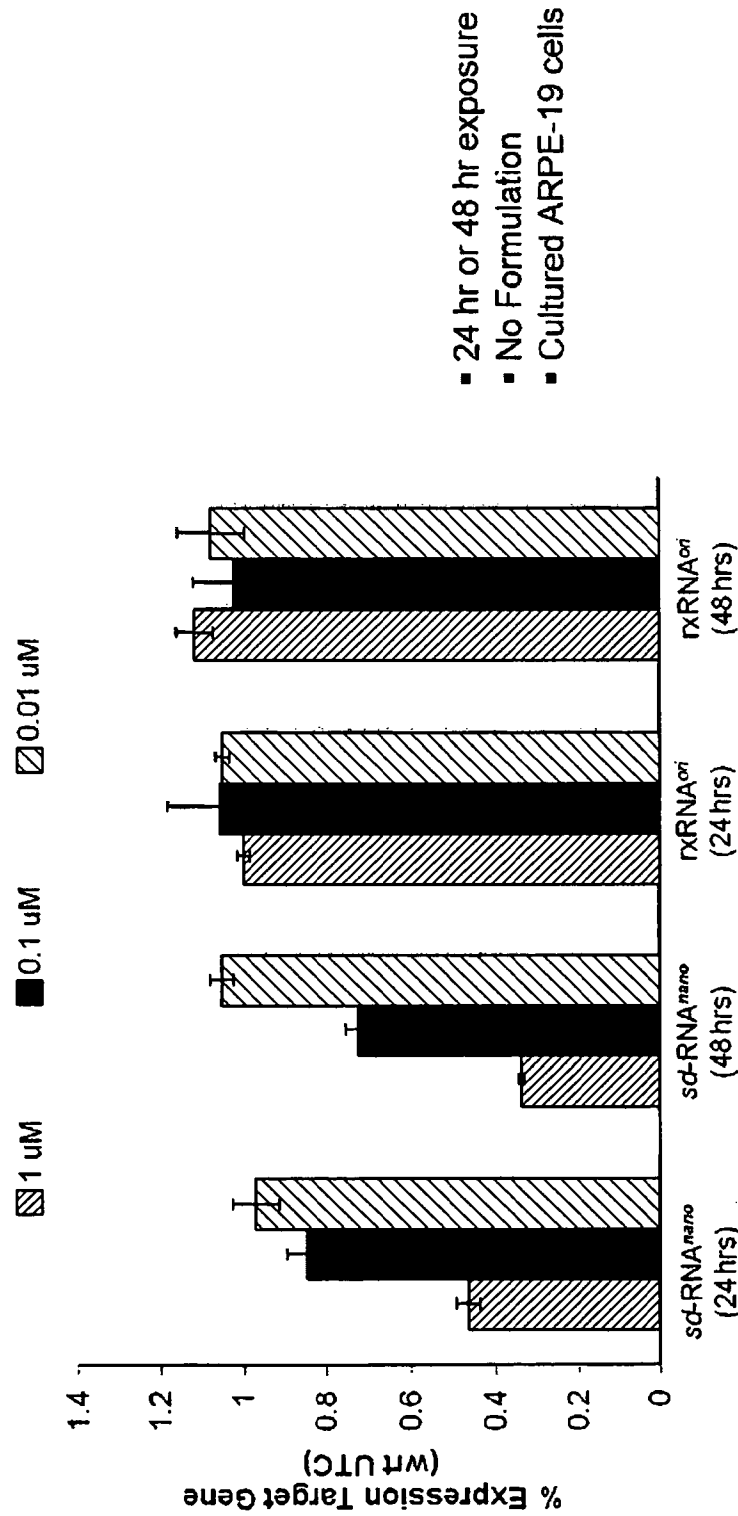


Figure 60

Silencing of MAP4K4 in RPE Cells Treated with *sd-rxRNA^{nano}*



61/92

Figure 61

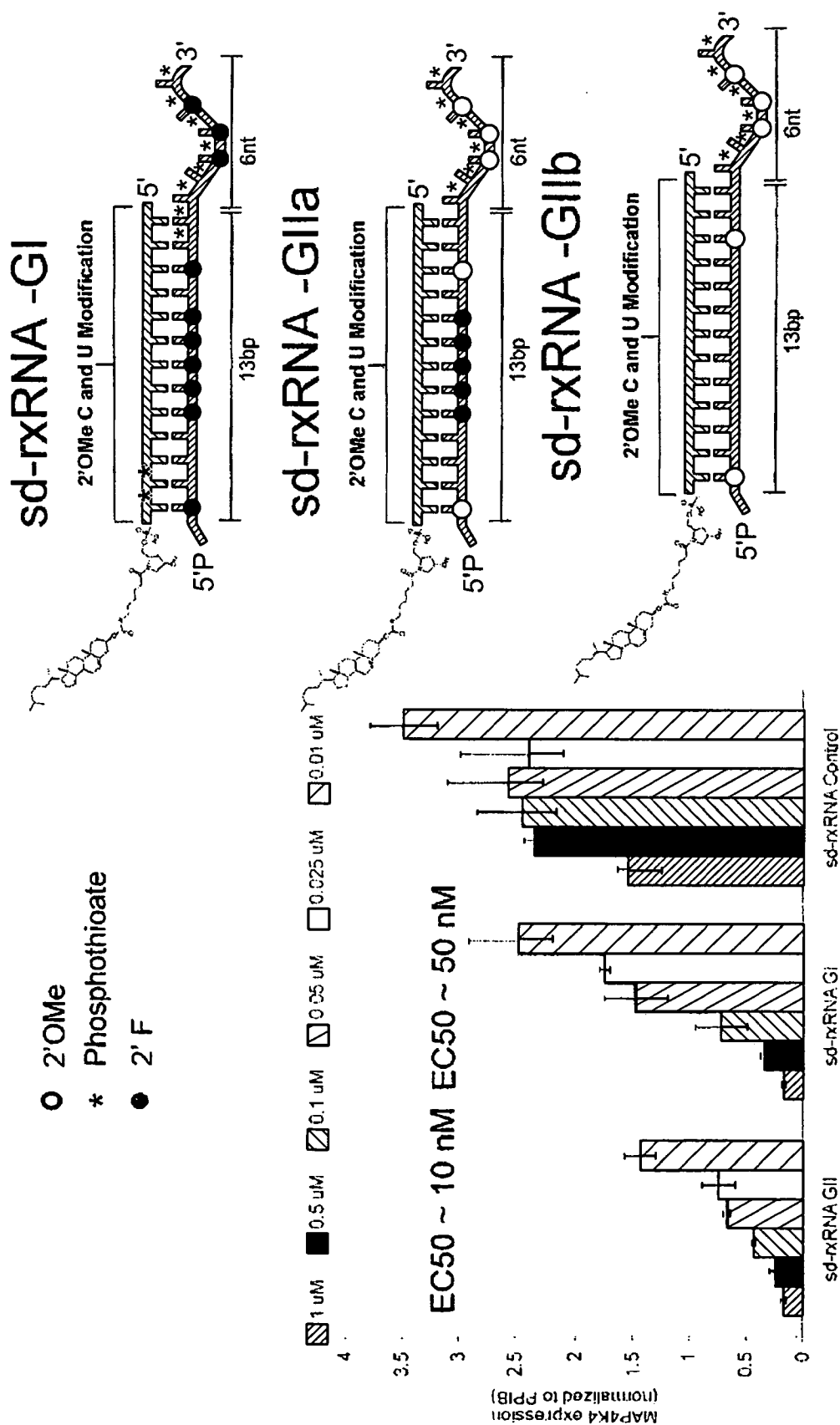


Figure 62

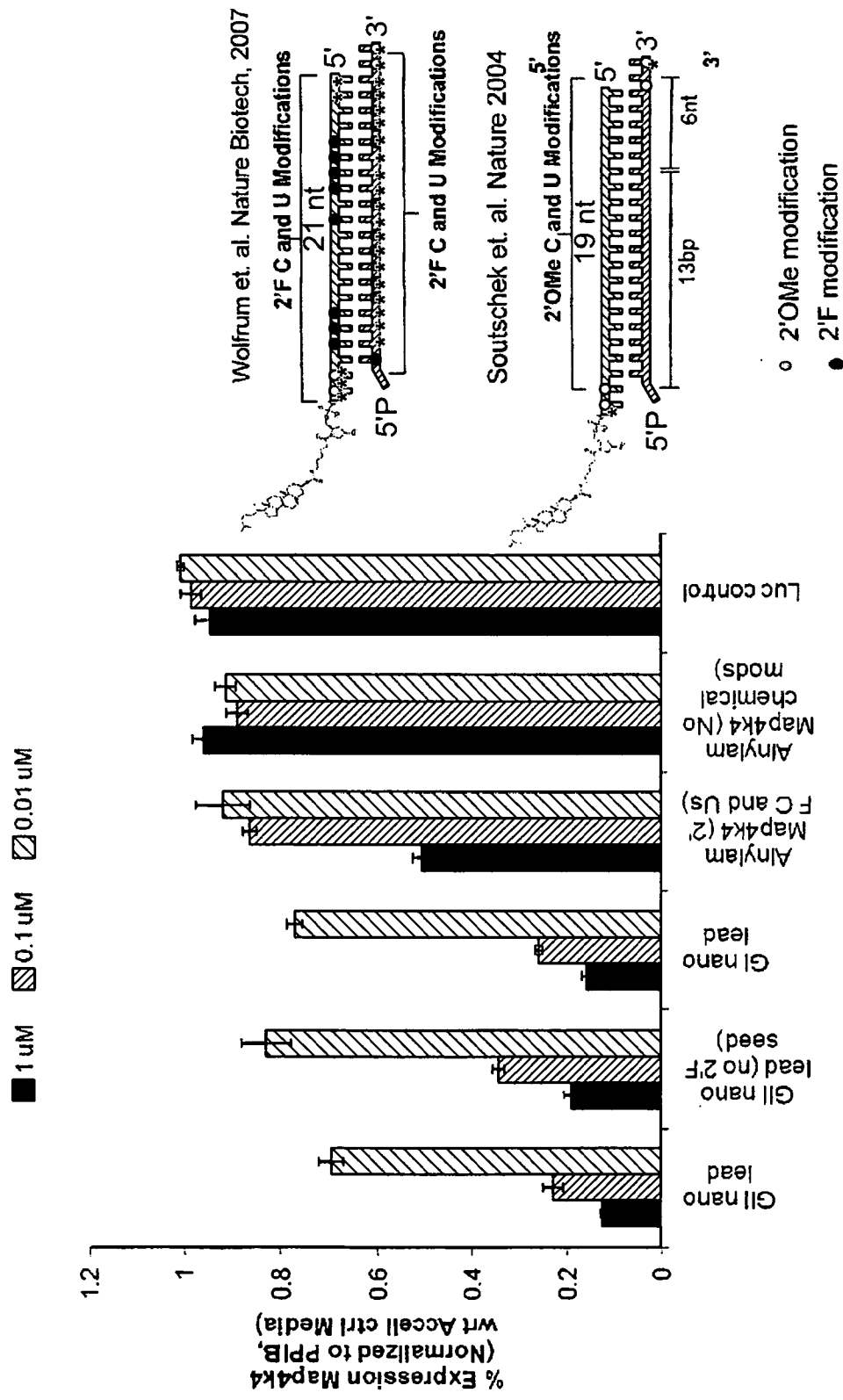
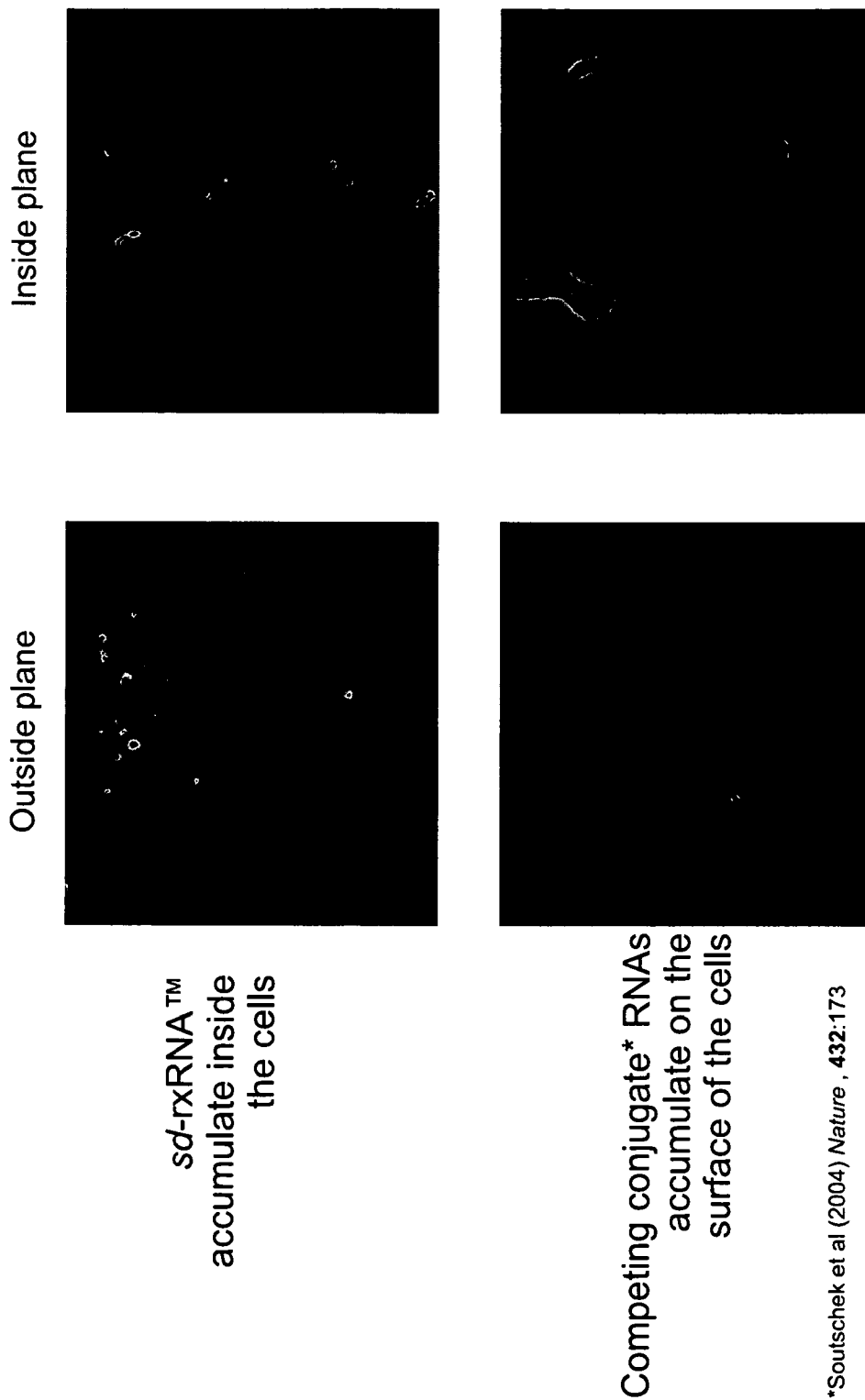


Figure 63

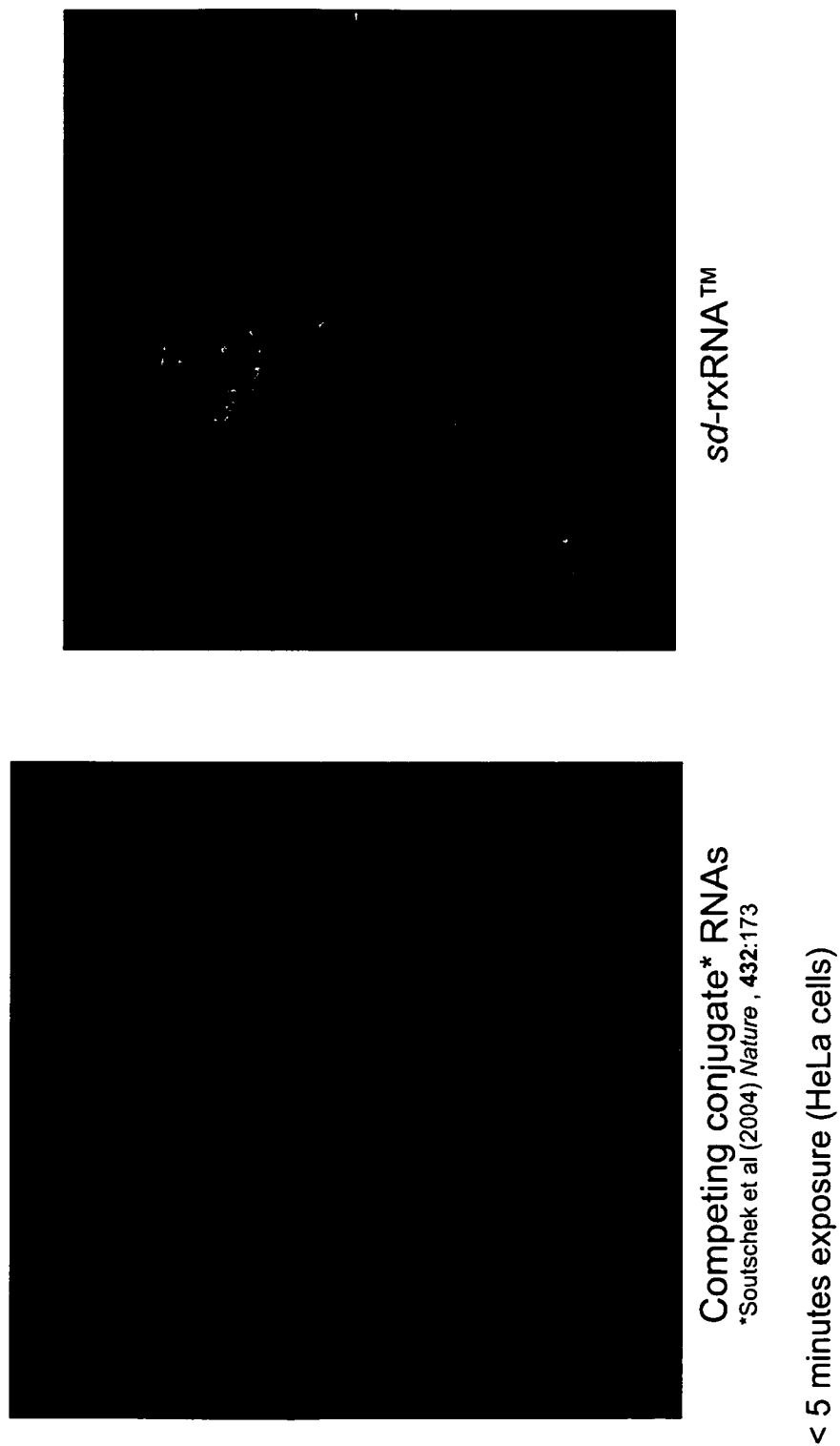
sd-rxRNA™ Is Efficiently Delivered to Cytoplasm



64/92

Figure 64

***sd*-rxRNA™ but not Competitor Molecules Are
Internalized within Minutes**



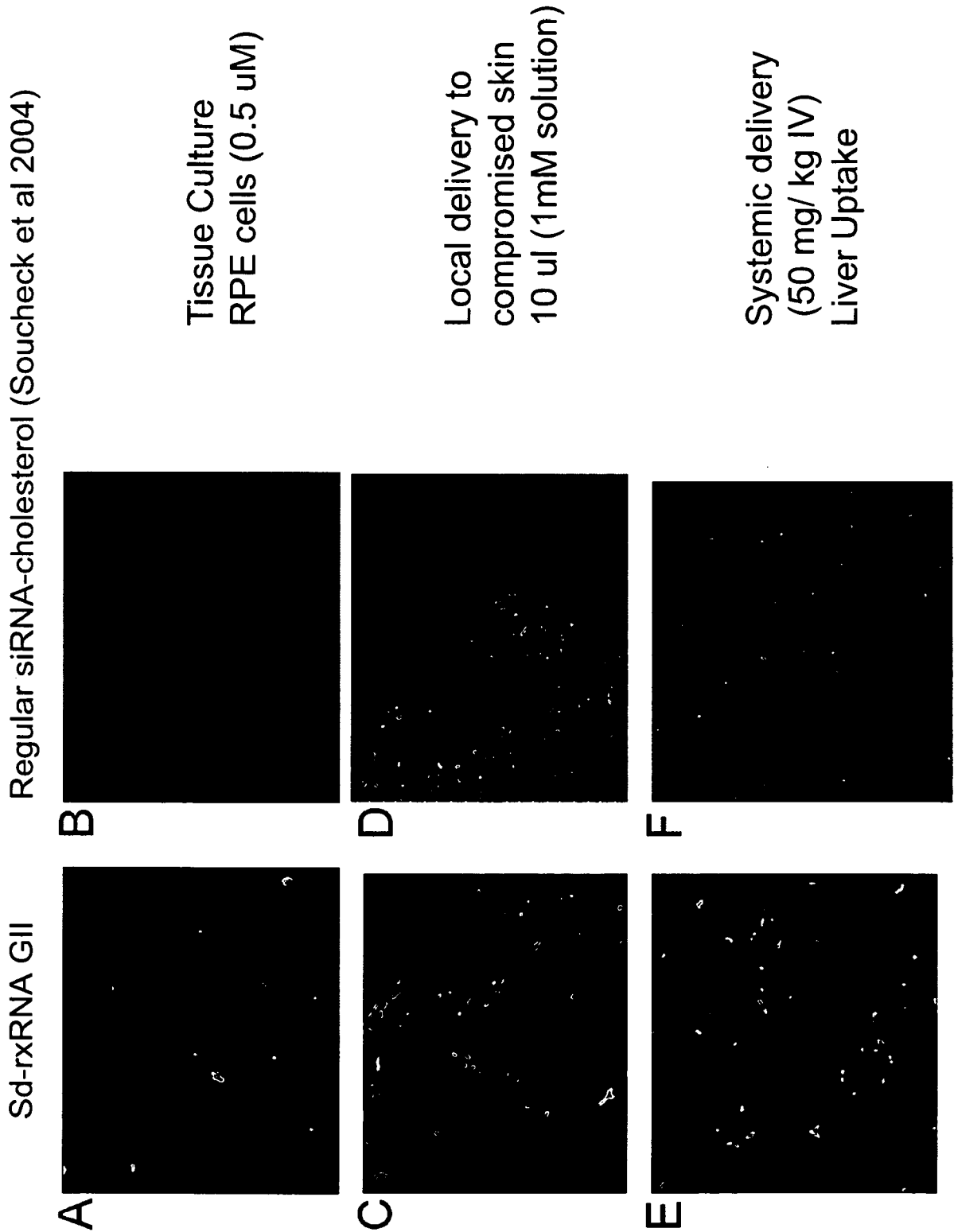
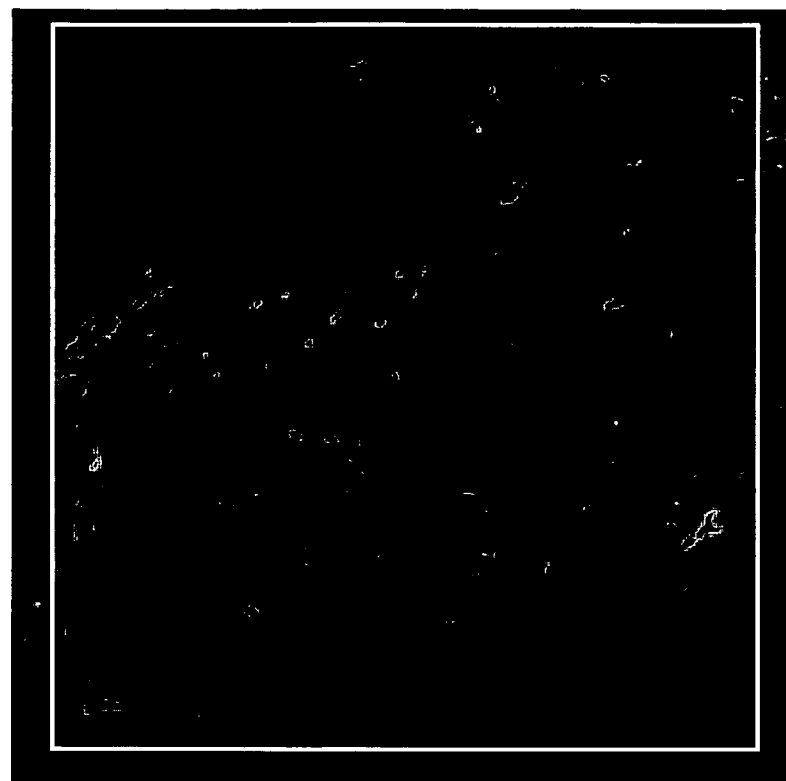


Figure 65

66/92

Figure 66

Local Delivery of *sd-rxRNA*TM: Pilot Study

rxRNA^{ori}*sd-rxRNA*TM

- 24 hours post delivery
- Hoechst and DY547

67/92

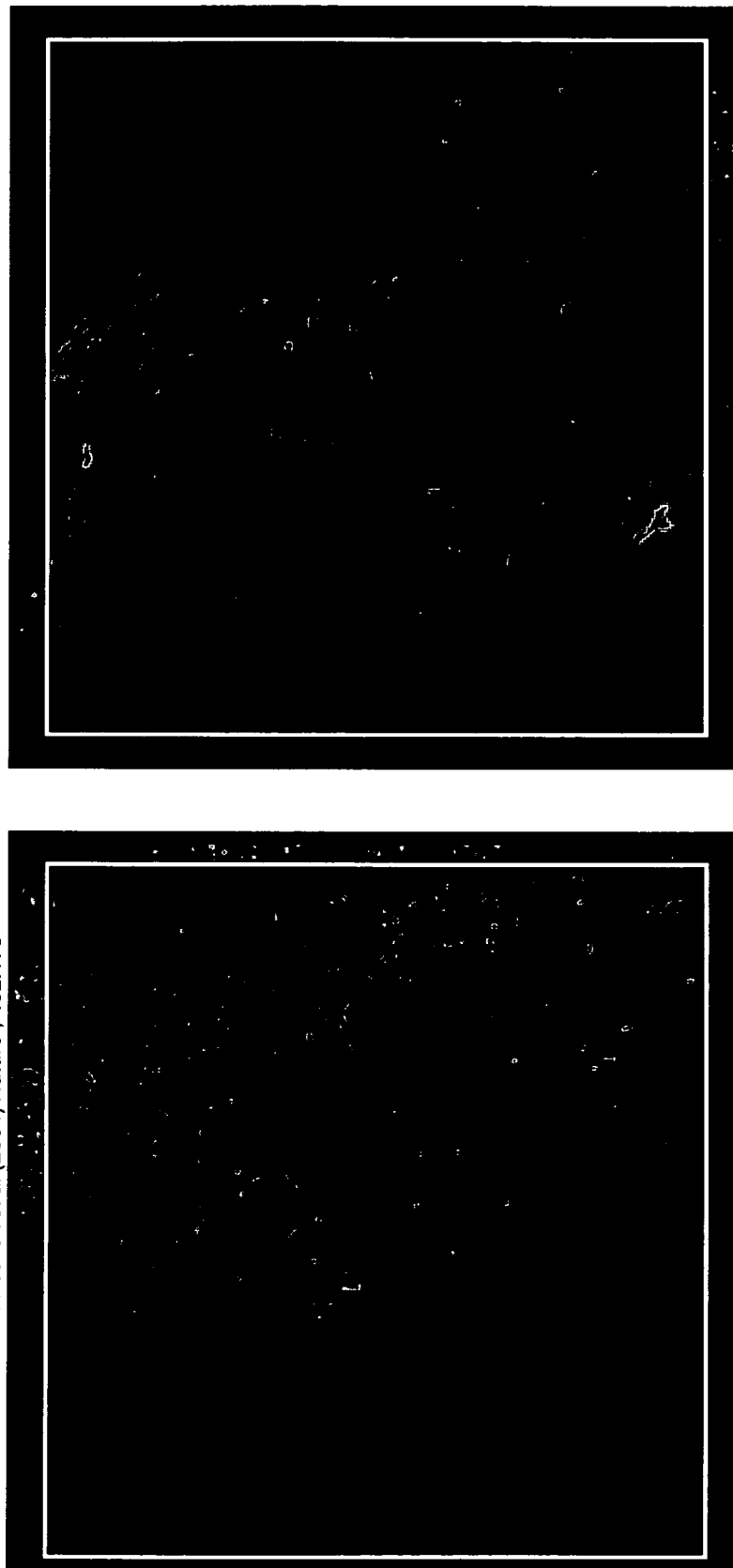
Figure 67

Local Delivery of sd-rxRNA™: Pilot Study

Competing conjugate* RNAs

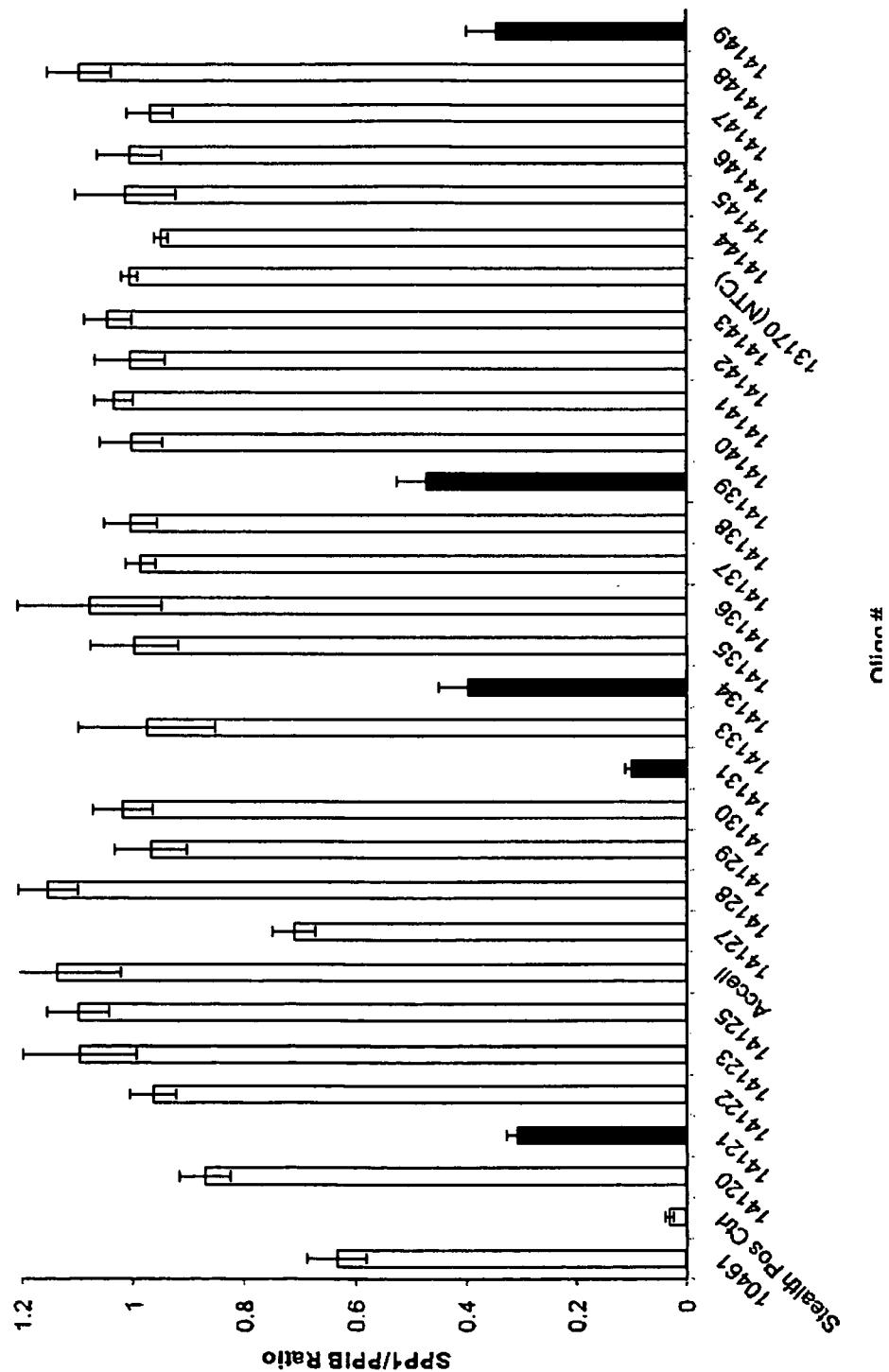
*Soutschek et al (2004) *Nature*, 432:173

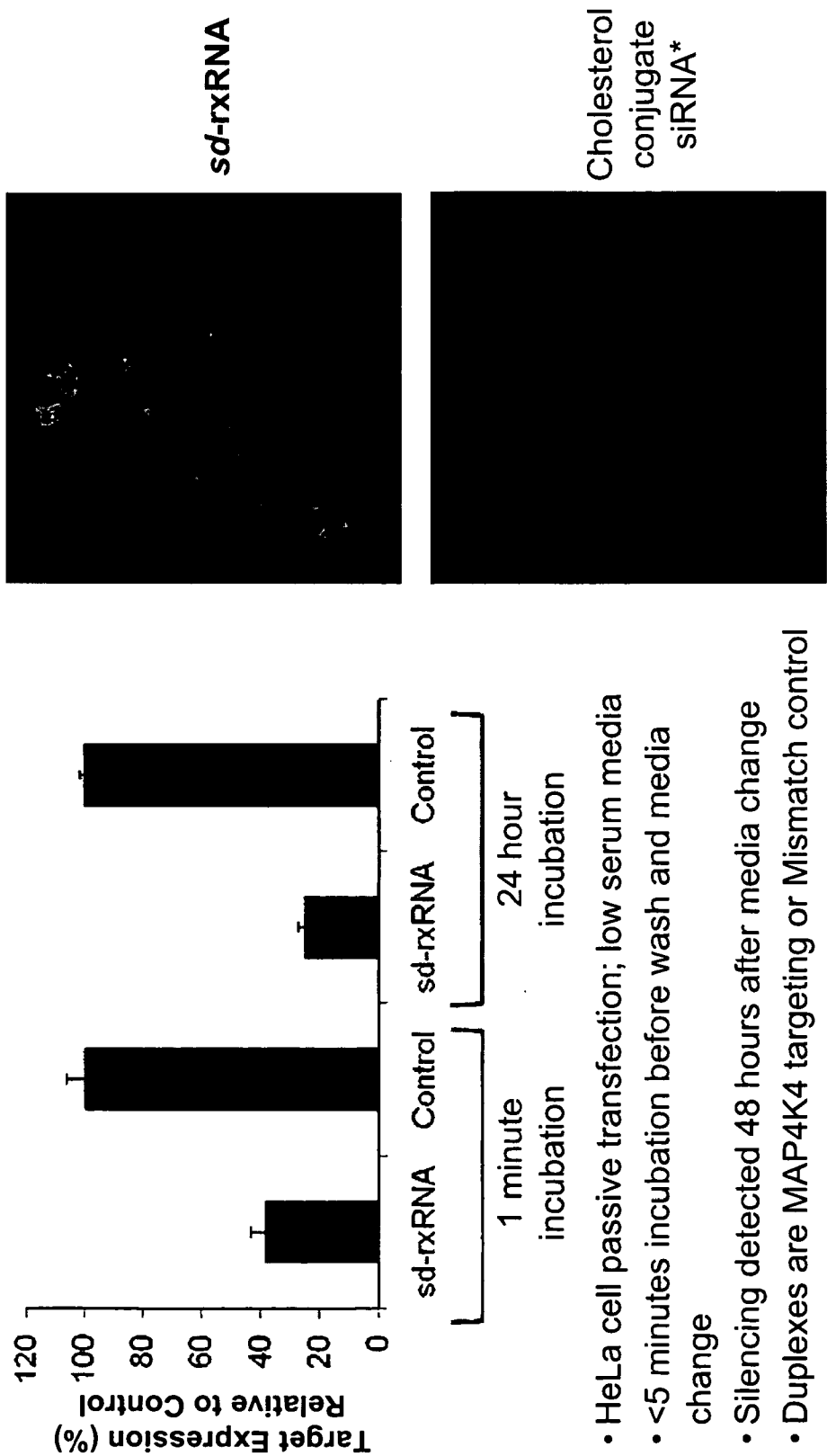
sd-rxRNA™



- 24 hours post delivery
- Hoechst and DY547

Figure 68
TARGET Screen Normalized T751 SPP1/PP1B Ratio
/bDNA/A-549 JL.032.1967 Cells rxRNA





*Soutschek et al (2004) Nature

Figure 69

- HeLa cell passive transfection; low serum media
- <5 minutes incubation before wash and media change
- Silencing detected 48 hours after media change
- Duplexes are MAP4K4 targeting or Mismatch control

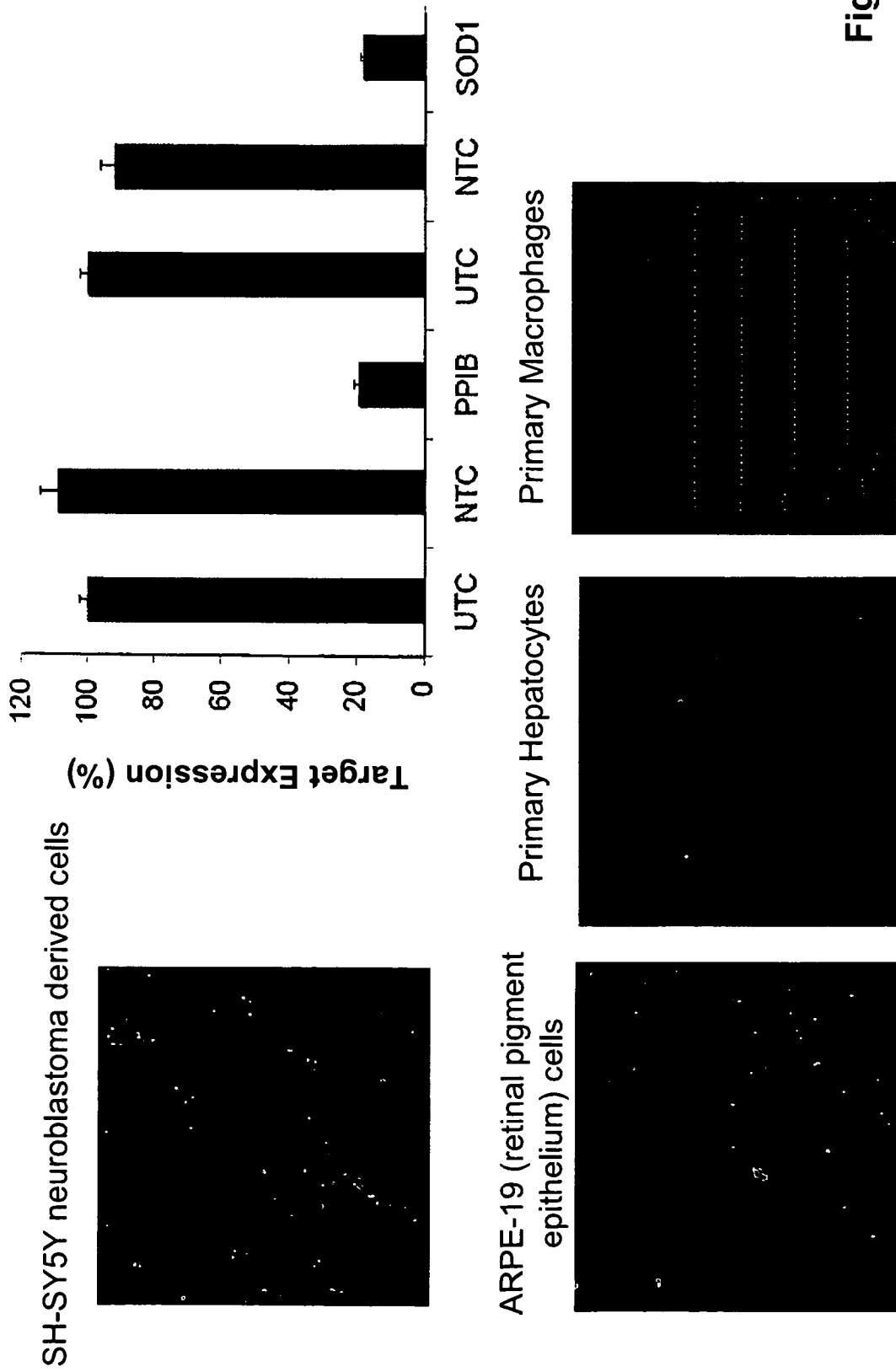


Figure 70

71/92

Figure 71

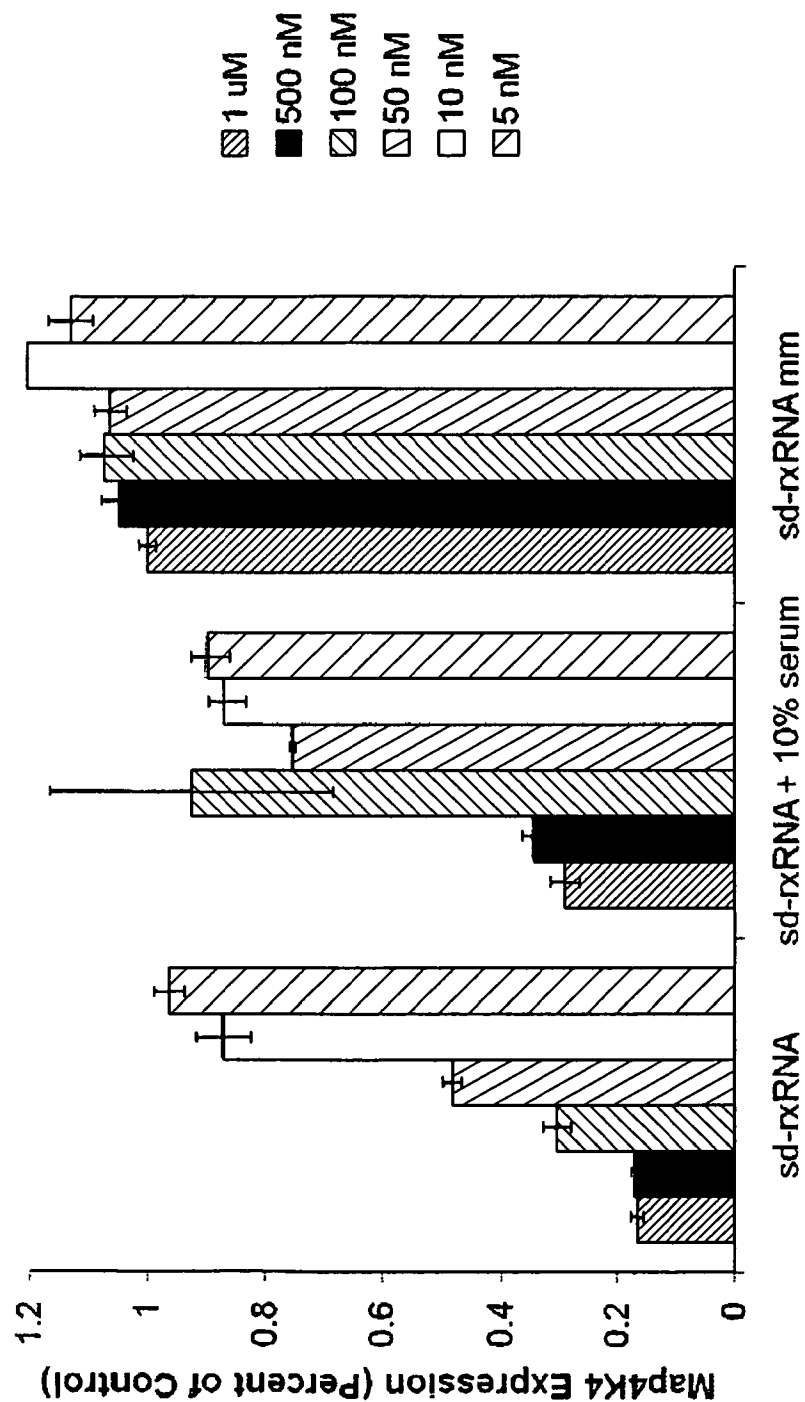


Figure 72

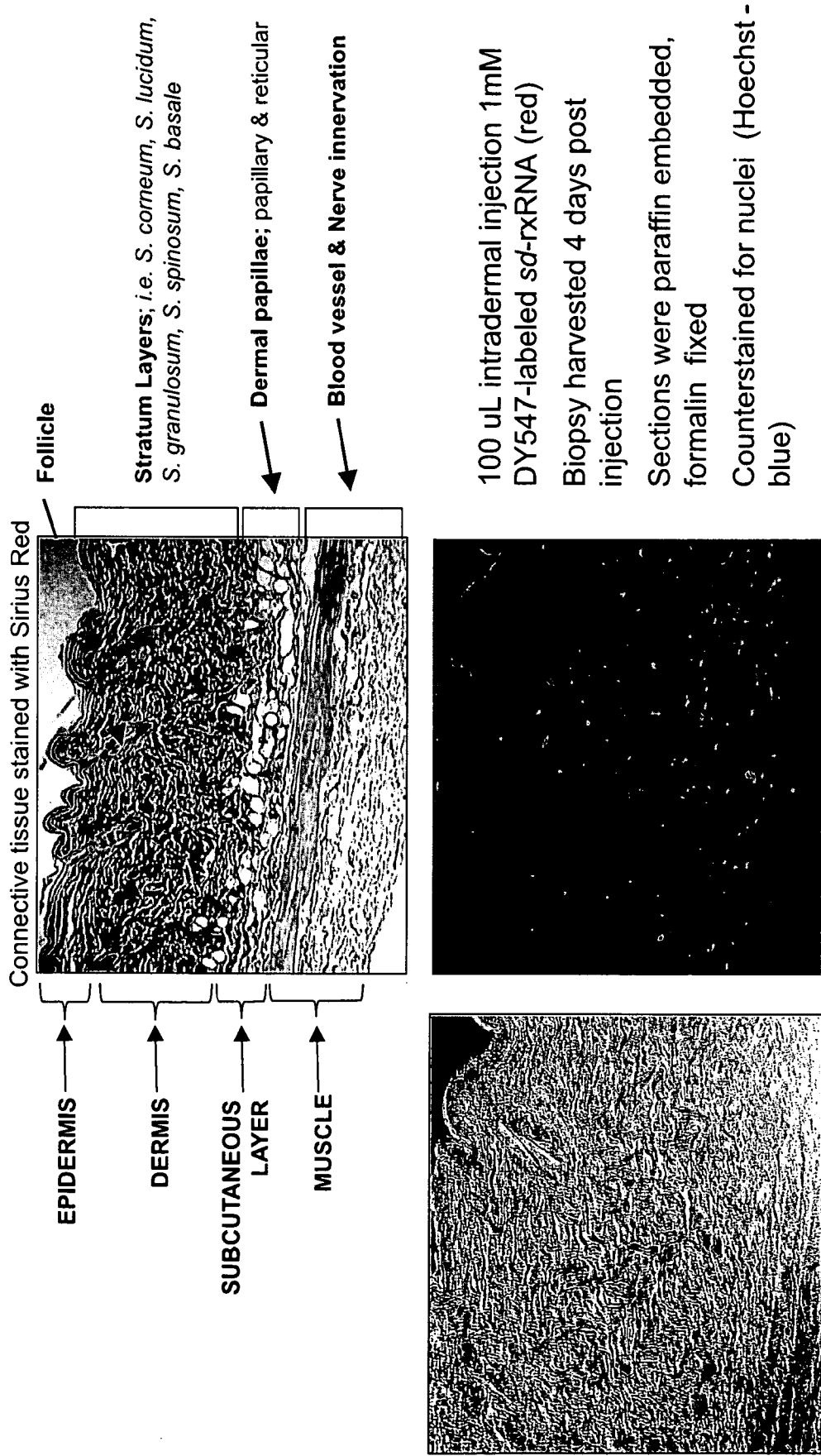


Figure 73

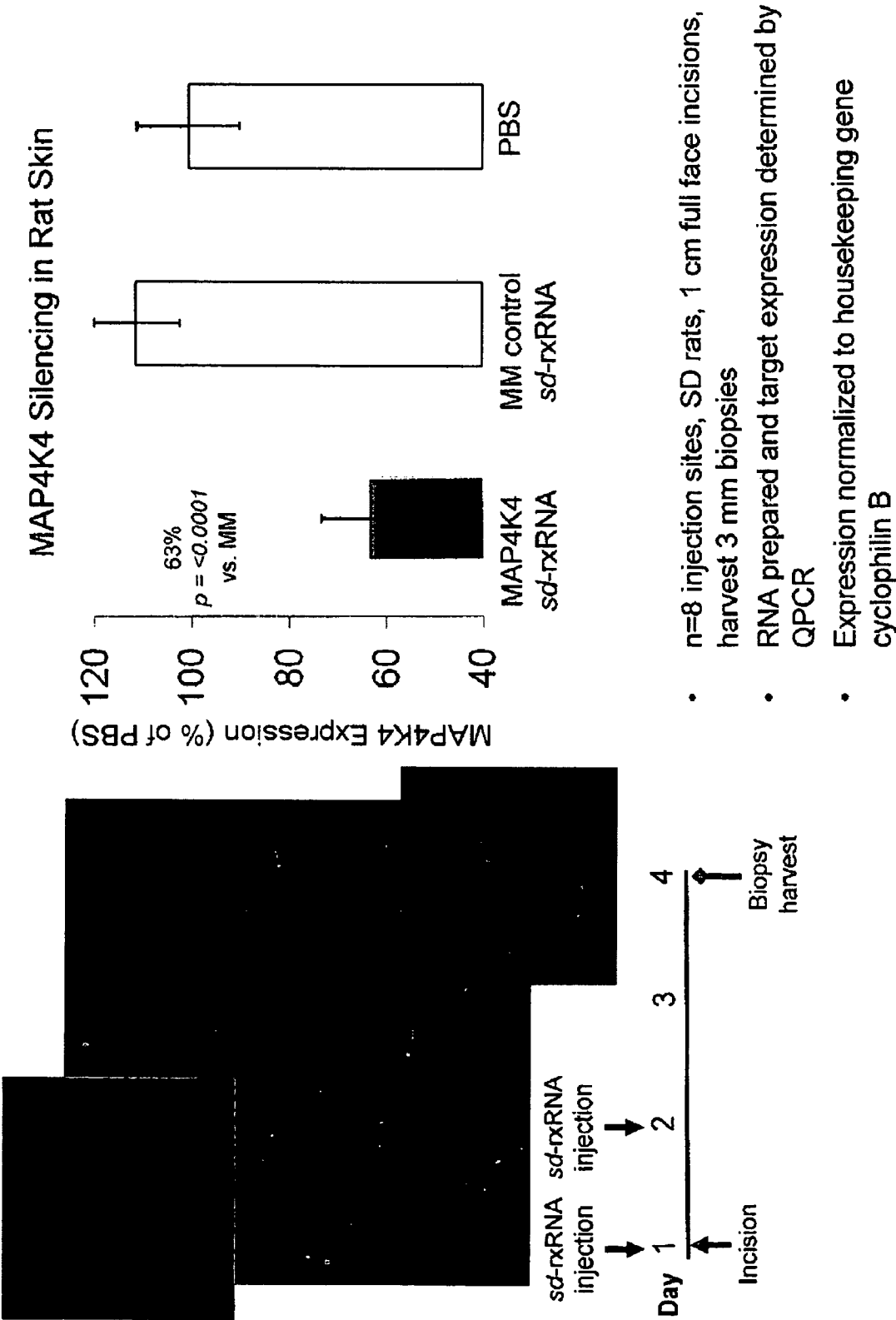
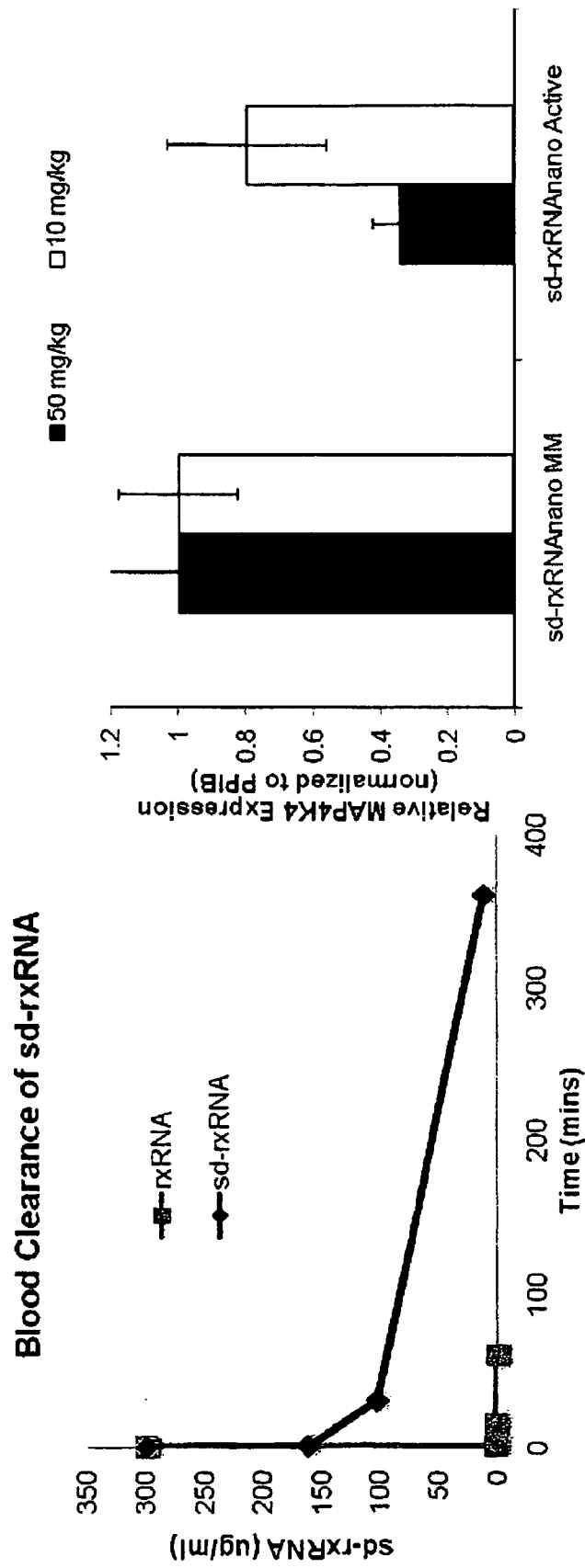


Figure 74

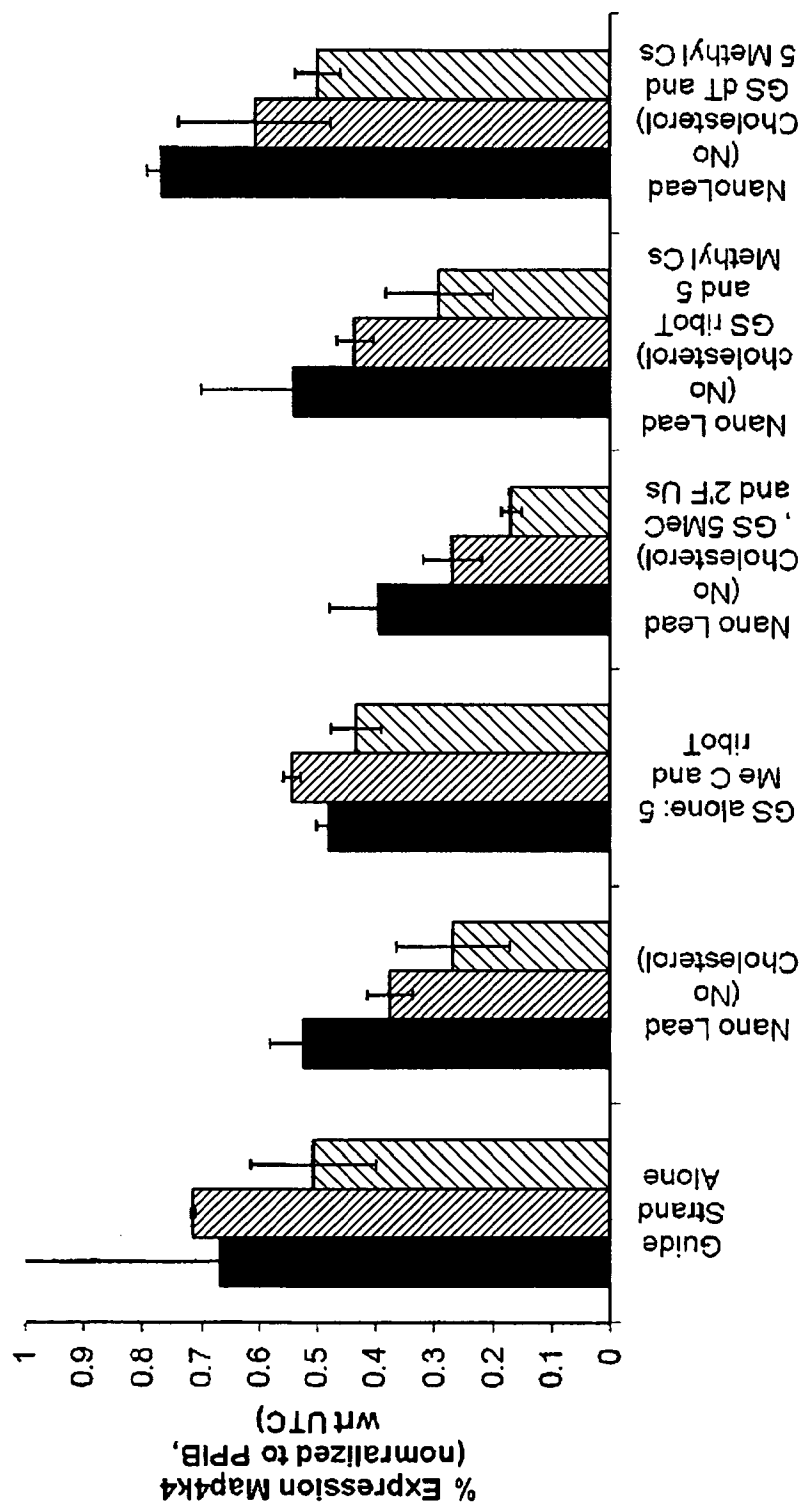


- Pilot Study: sd-rxRNA remaining in mouse blood (n=1)
- Detection of DY547-labeled RNA by fluorescence

- Visual fluorescence detection in tissue lysates (50 mg/kg dose)
- Confocal imaging confirms intracellular delivery
- Efficient silencing of targeted gene in liver as detected by RT-PCR

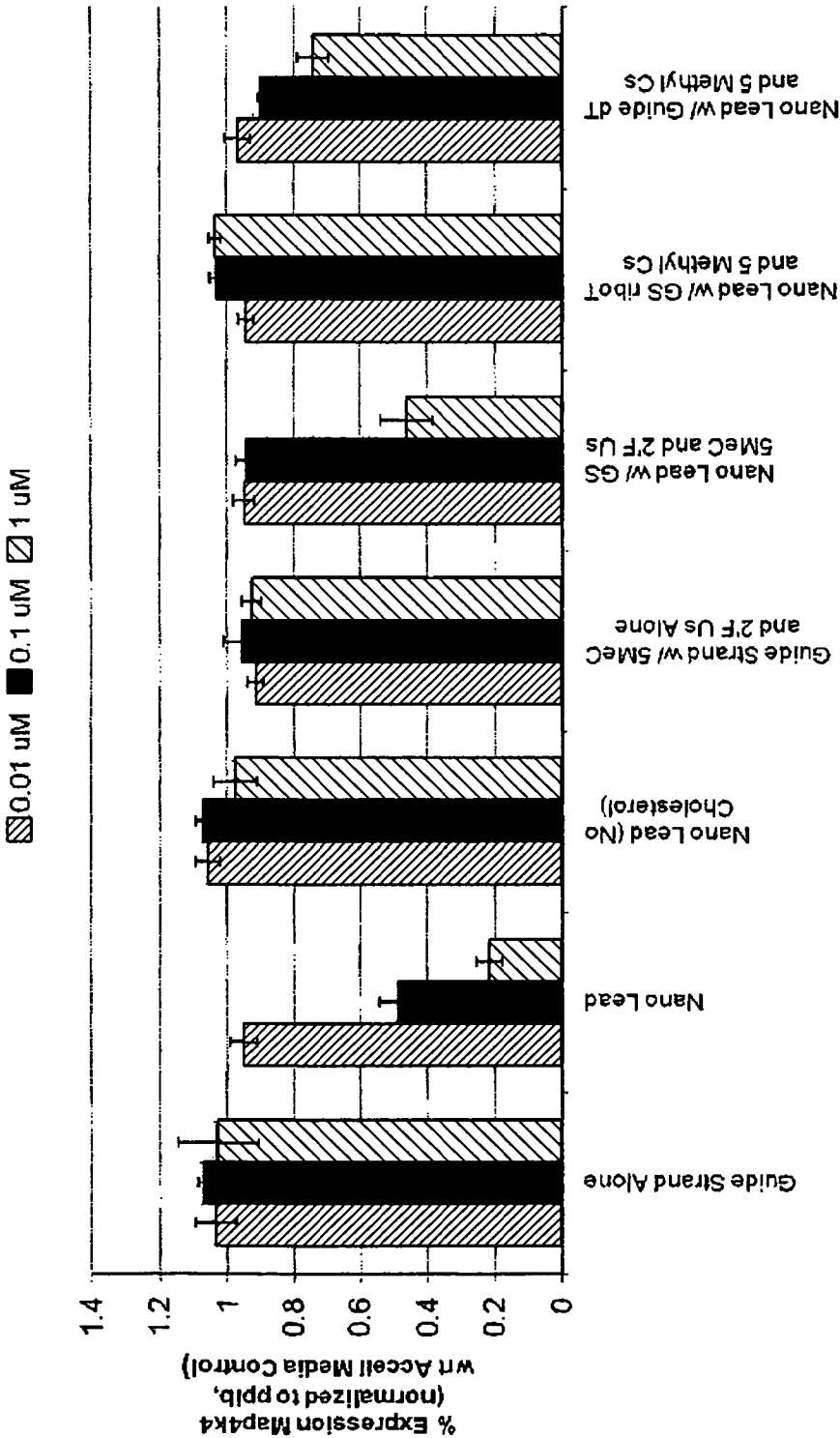
75/92

Figure 75



Incorporation of 5 Me C and/or ribothymidine in Guide Strand Reduces Efficacy

Passive Uptake



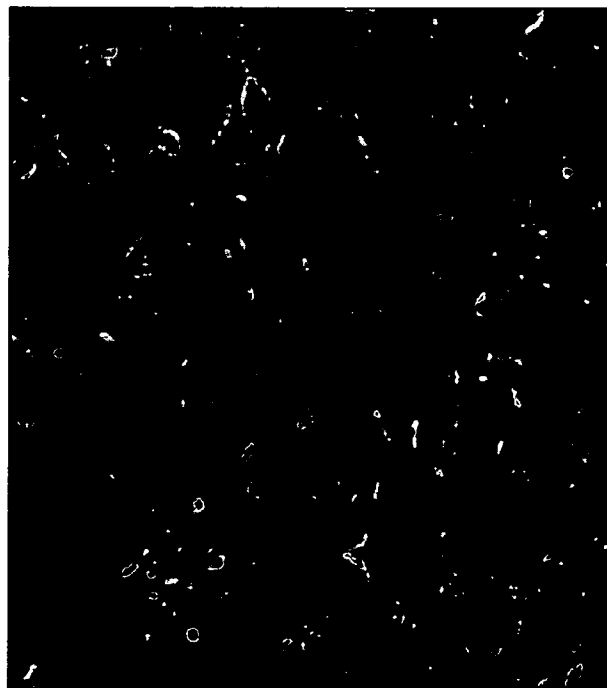
76/92

Figure 76

Figure 77

sd-rxRNA^{nano} vs. Competitor: Systemic Delivery to the Liver

sd-rxRNA^{nano}



gain = 380; 50 mg/kg

Competitor conjugate*

*Soutschek et al (2004) *Nature* , 432:173

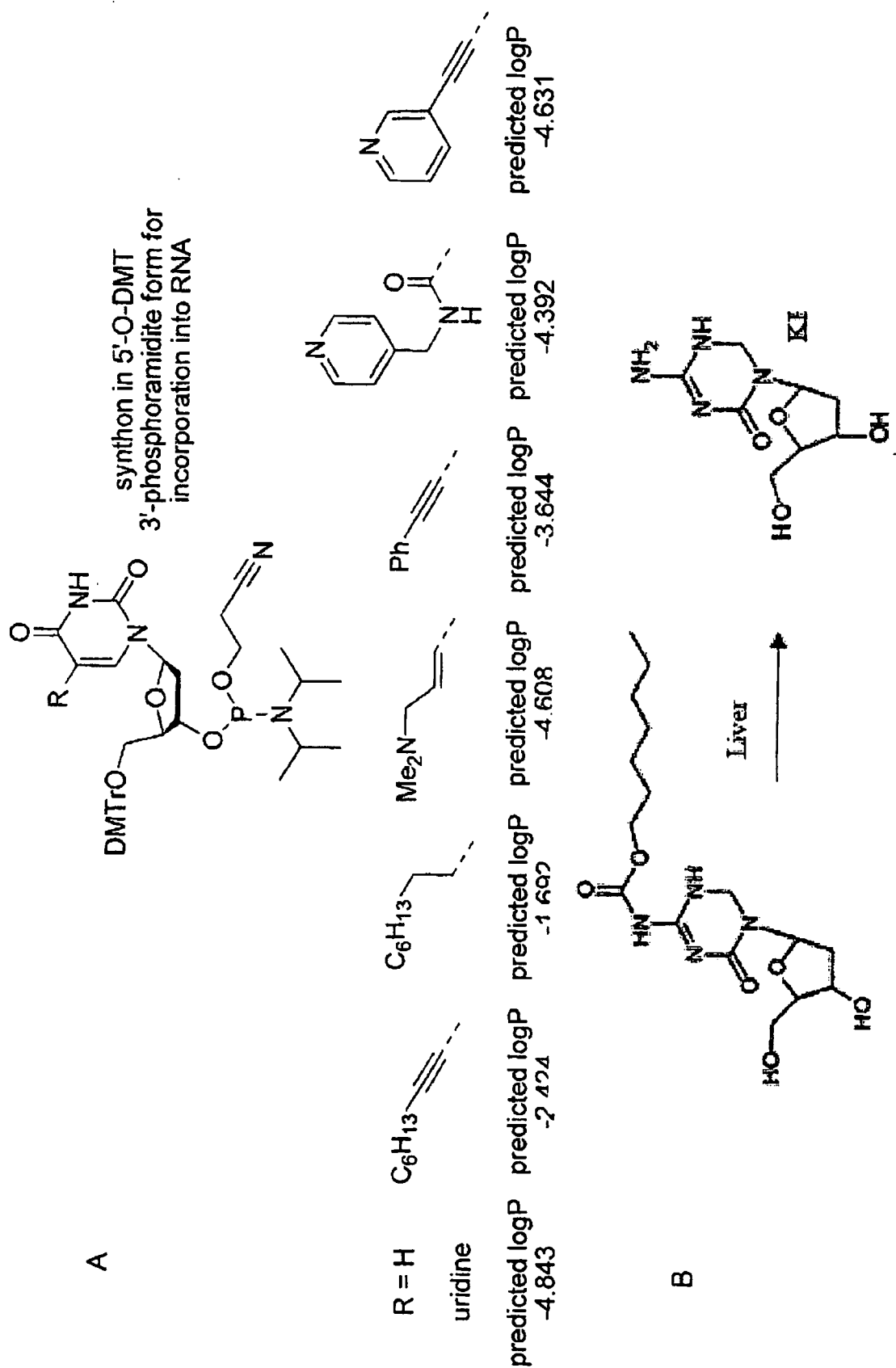


gain =400; 50 mg/kg

77/92

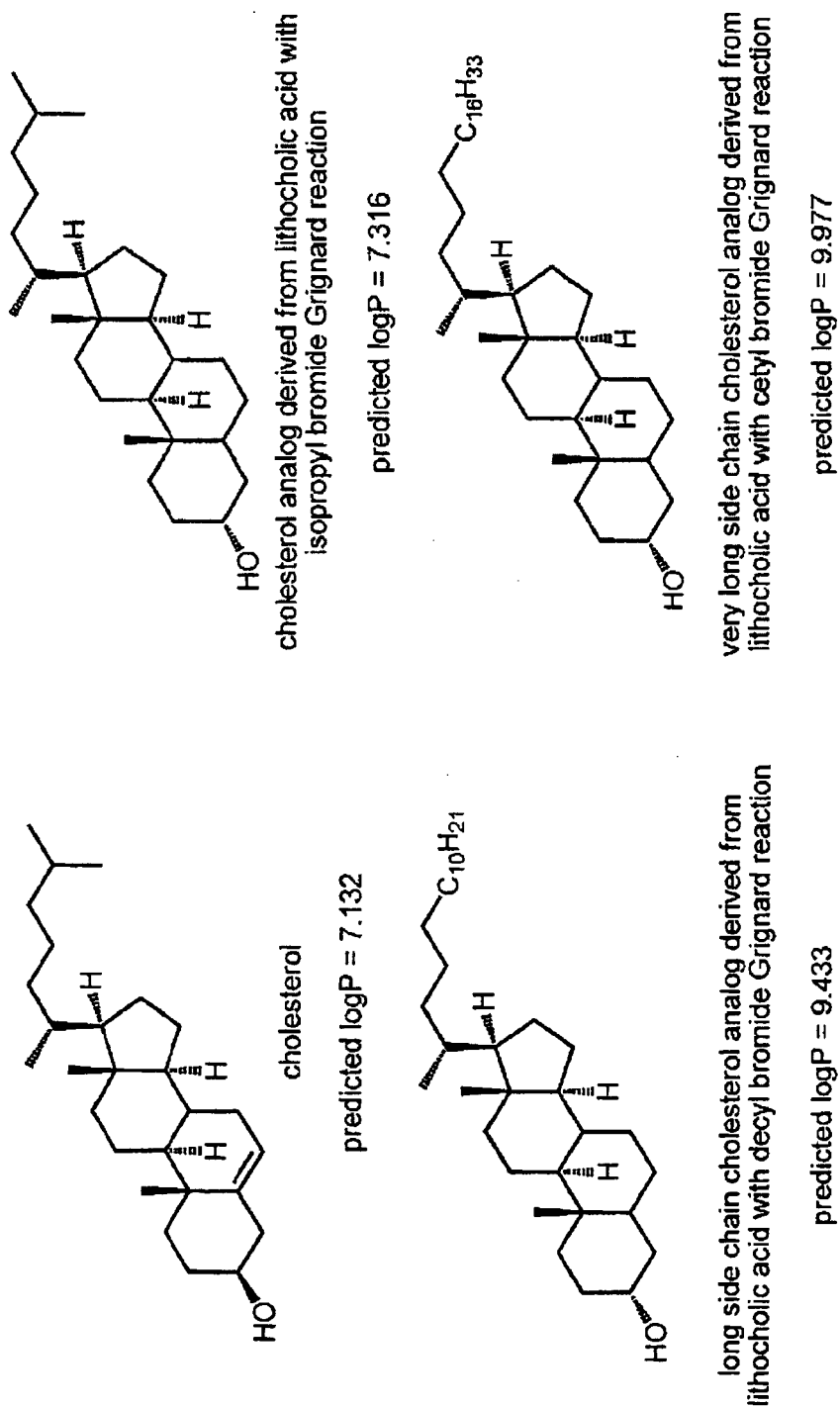
78/92

Figure 78



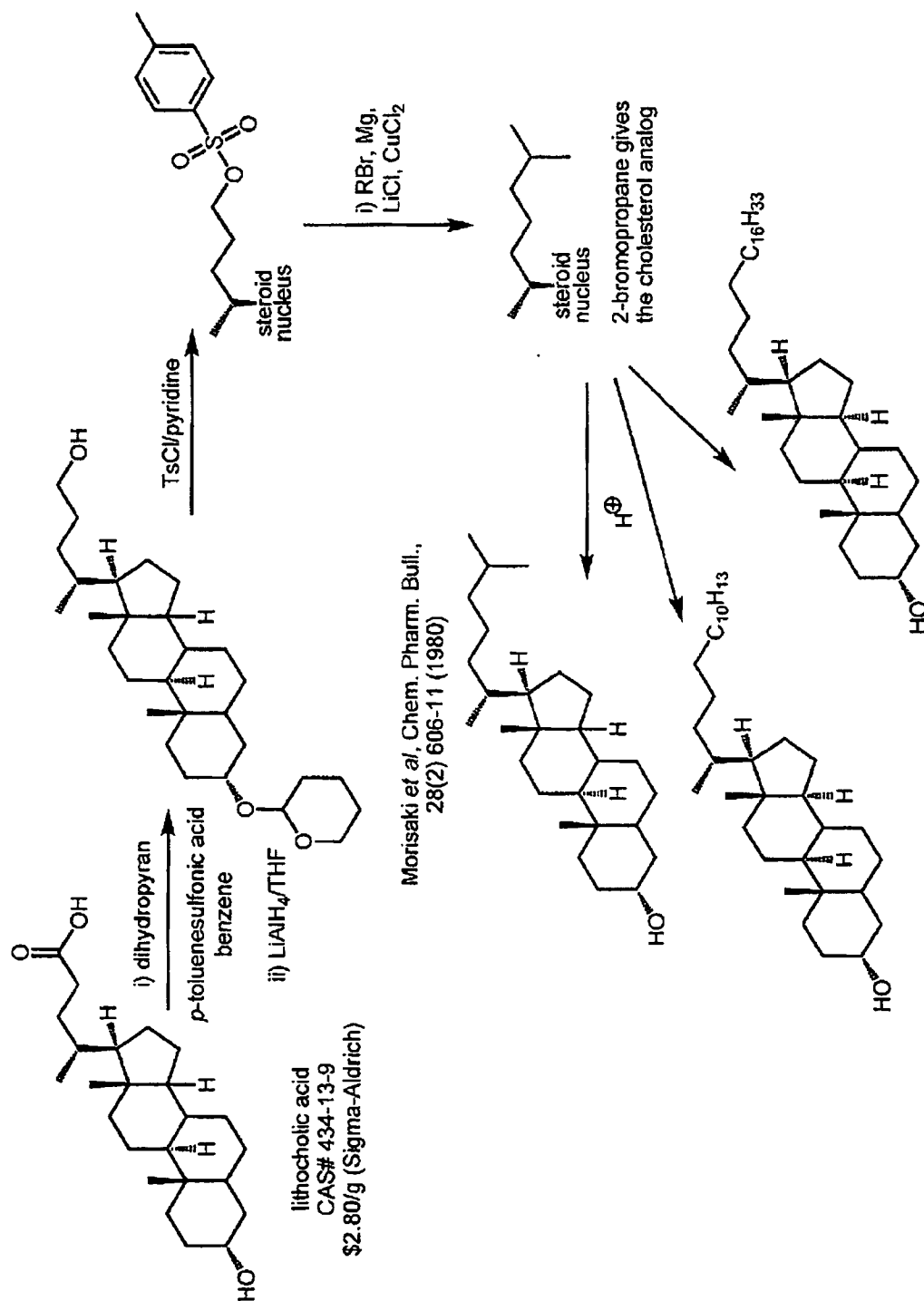
79/92

Figure 79



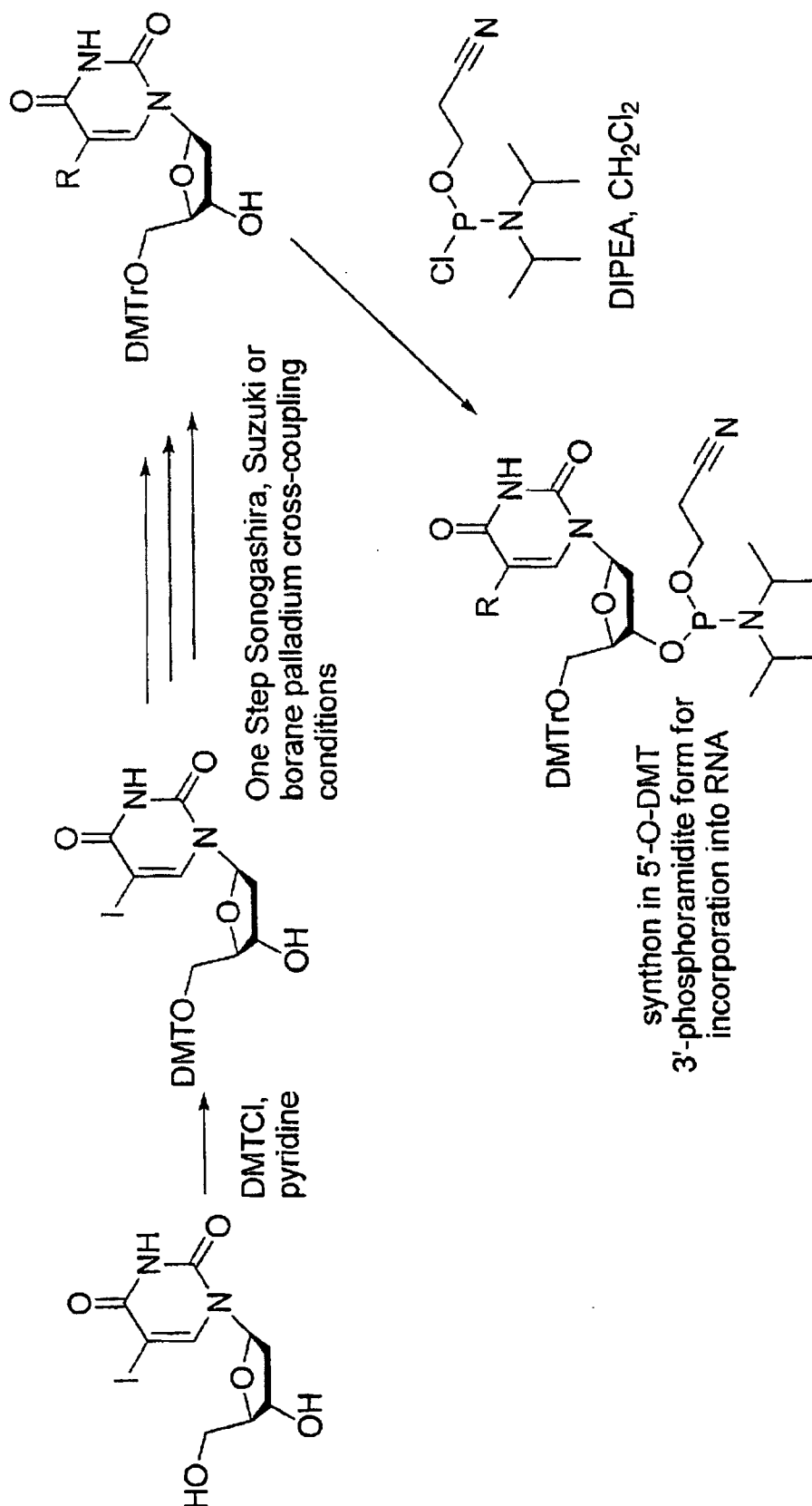
80/92

Figure 80



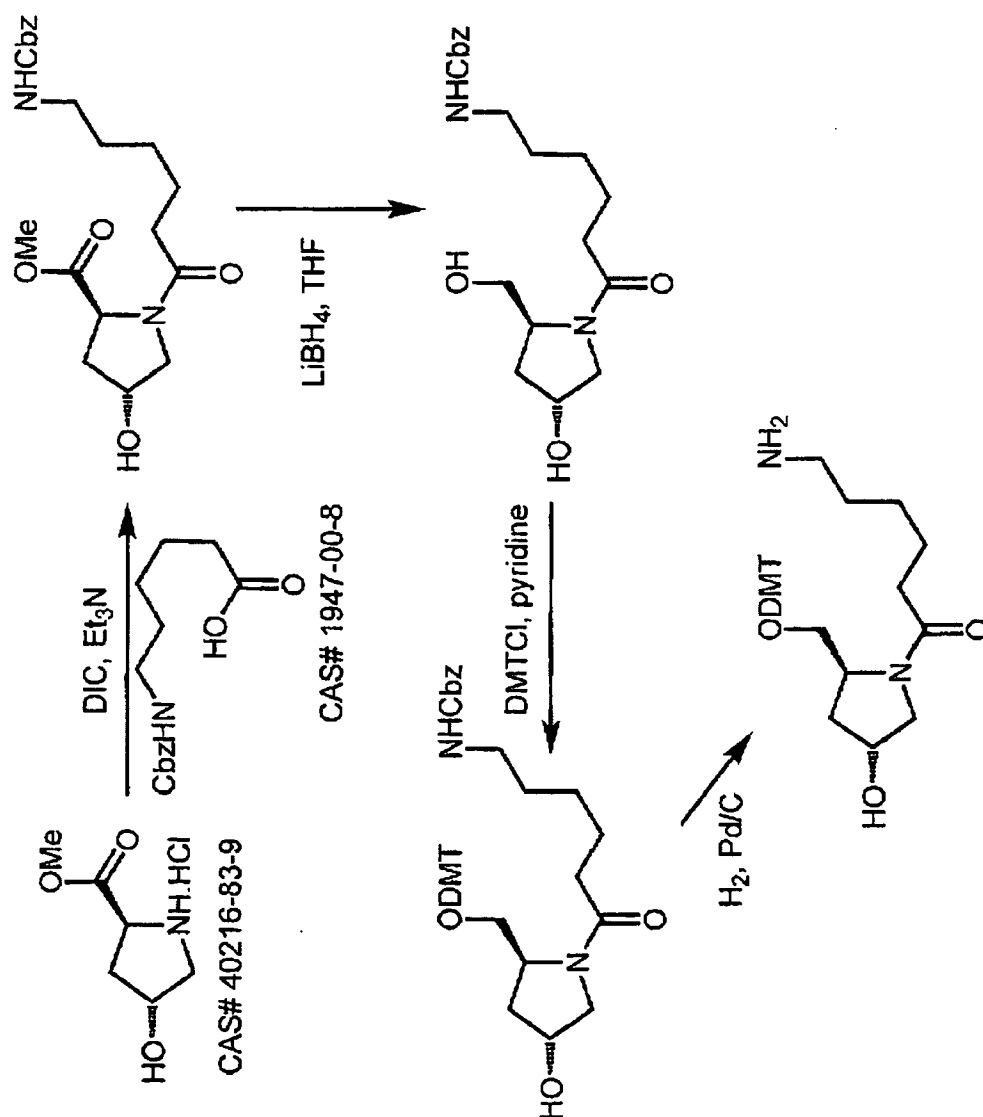
81/92

Figure 81



82/92

Figure 82



83/92

Figure 83

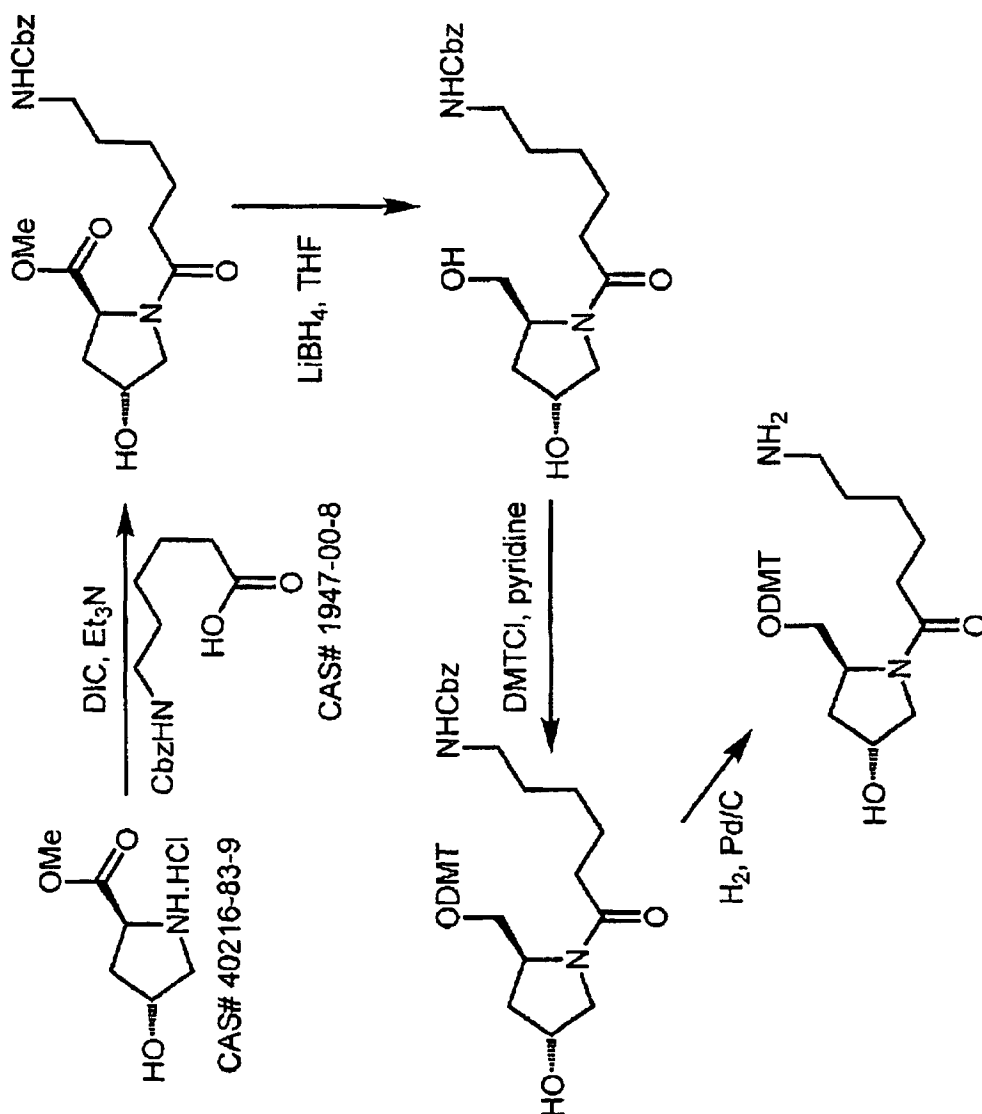
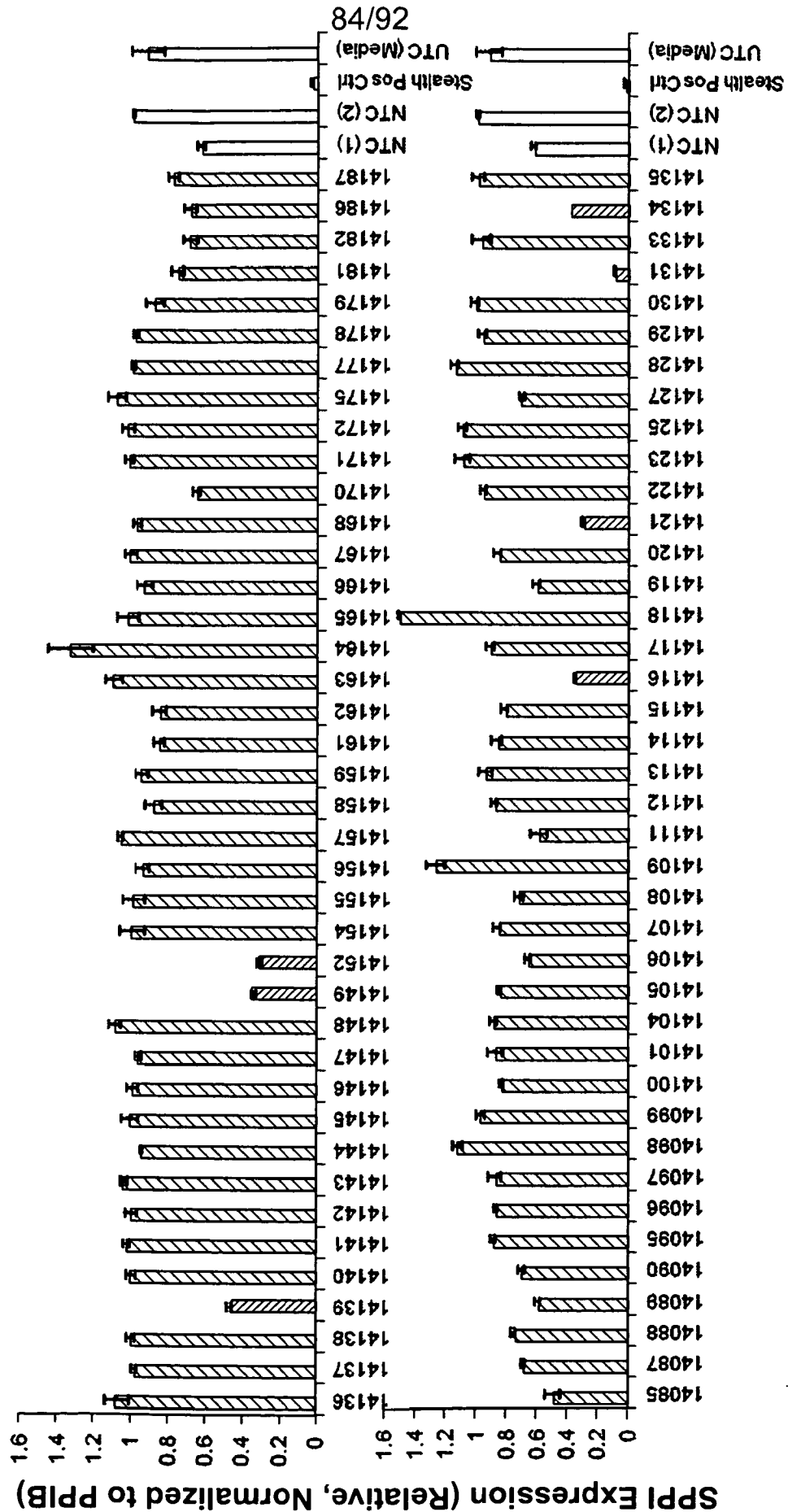


Figure 84



85/92

Figure 85

TARGET Screen Normalized T755 SPP1/PPIB Ratio /bDNA/A-549 1 and 0.1 uM Cells rxRNA

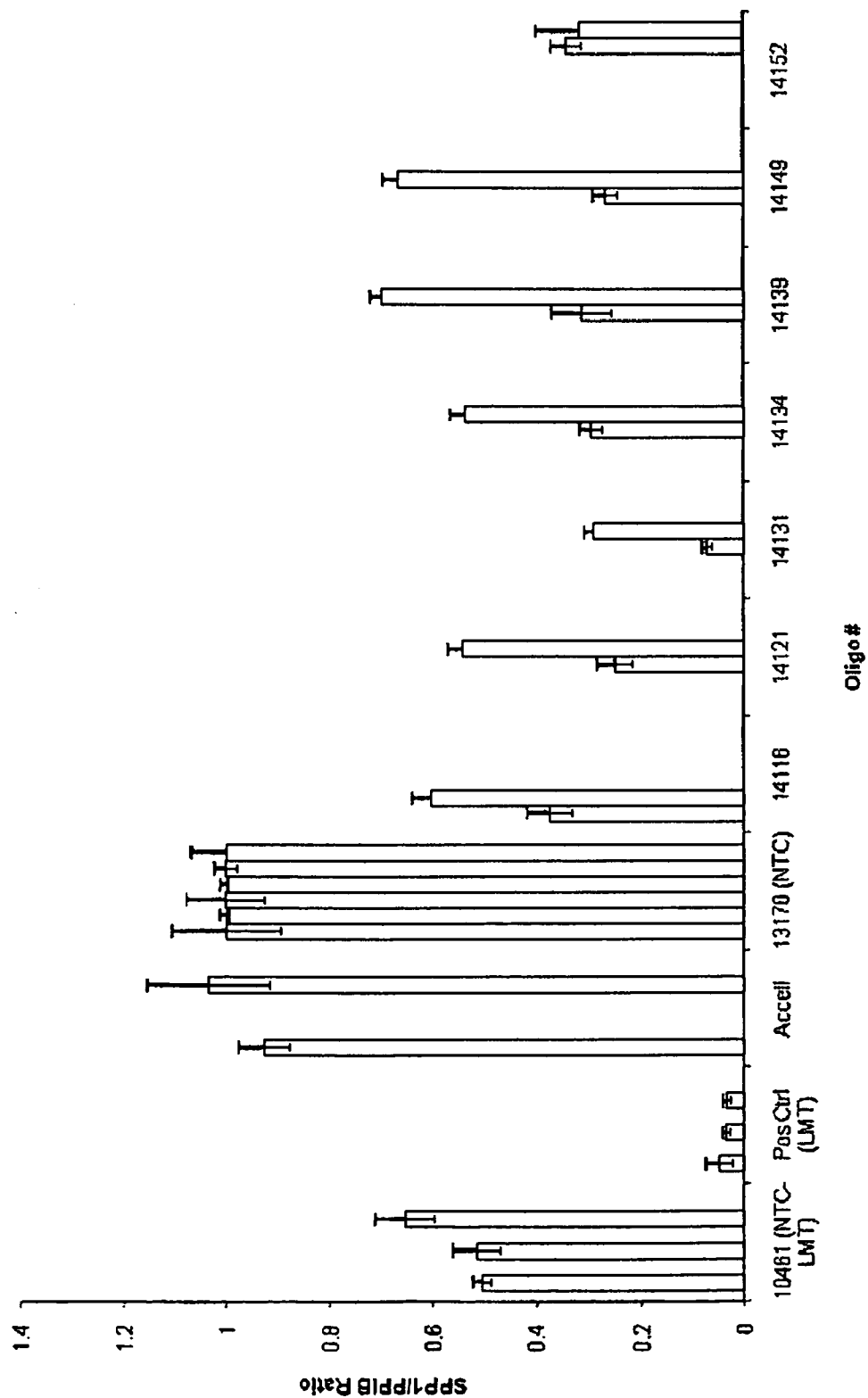
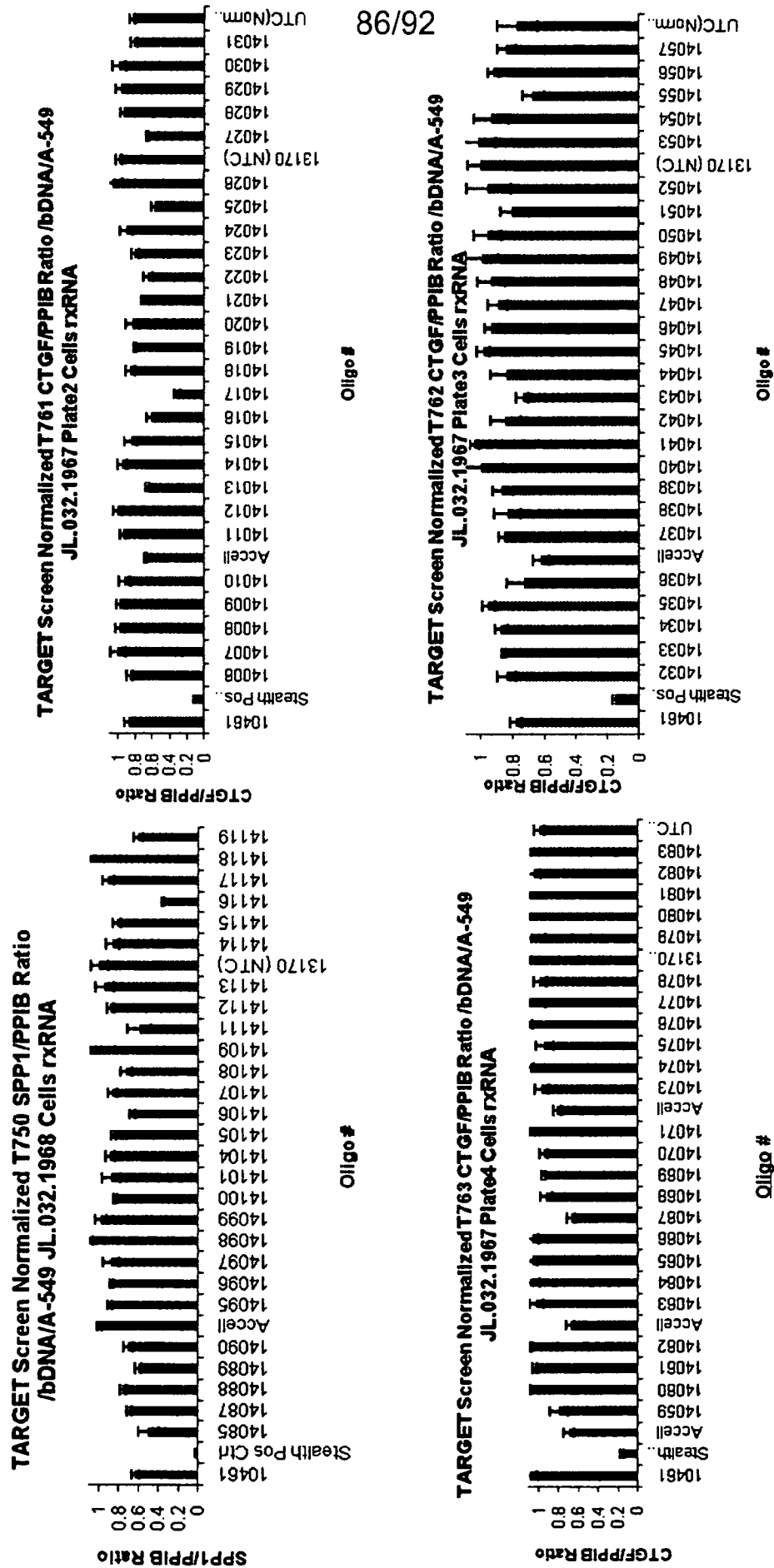
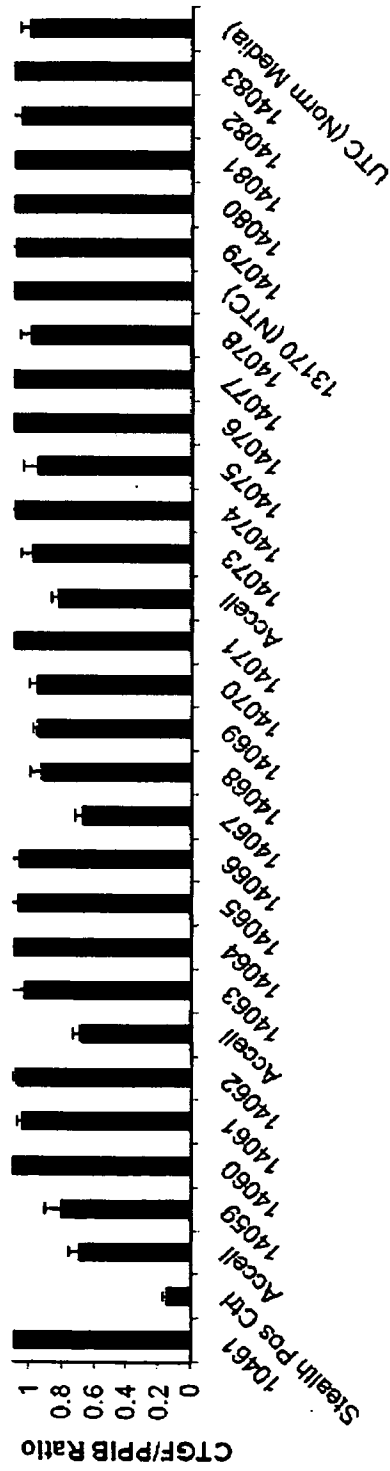


Figure 86



TARGET Screen Normalized T763 CTGF/PPiB Ratio /bDNA/A-549 JL.032.1967

Plate4 Cells rxRNA



TARGET Screen Normalized T762 CTGF/PPiB Ratio /bDNA/A-549 JL.032.1967 Plate3 Cells

rxRNA

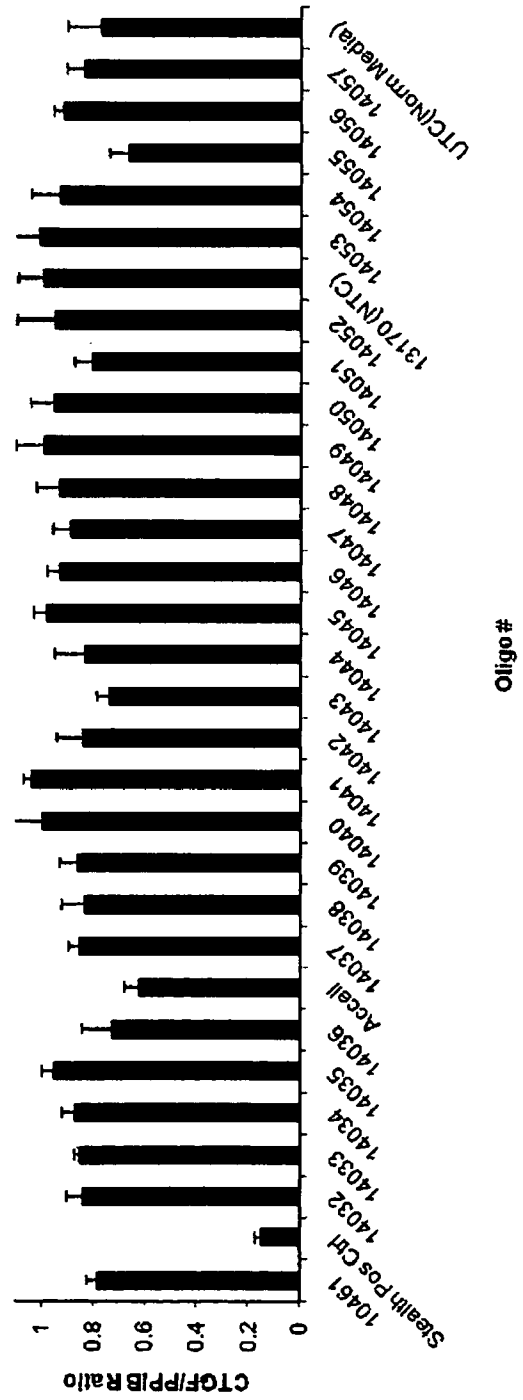


Figure 87

Figure 88

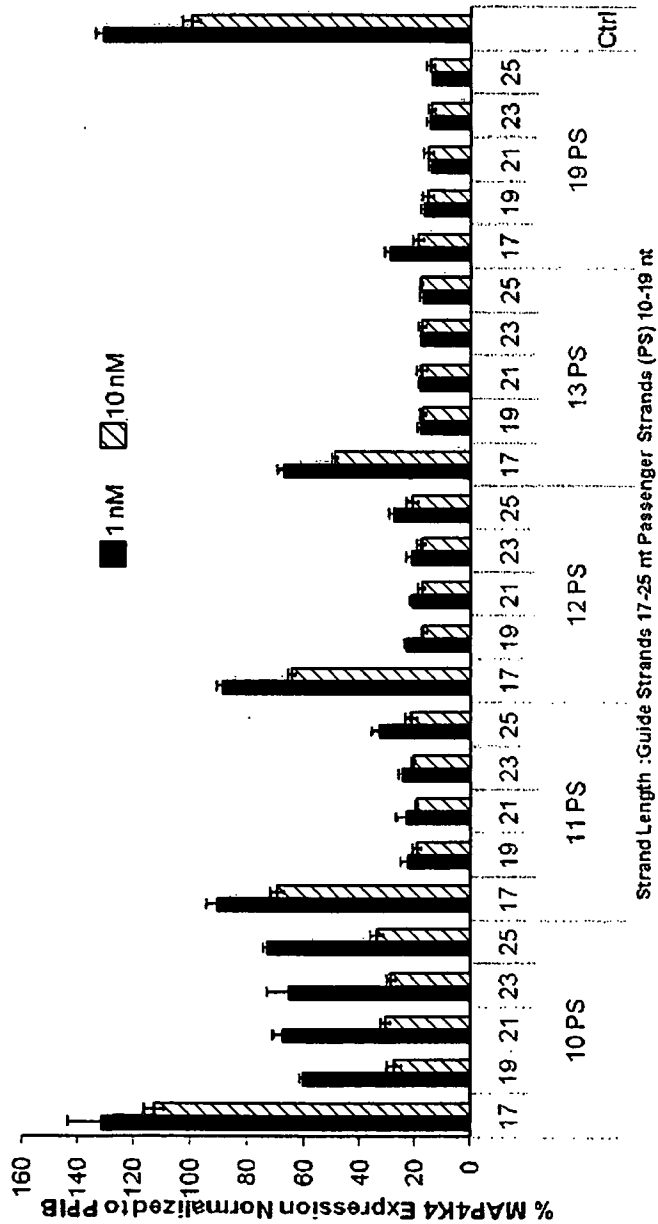
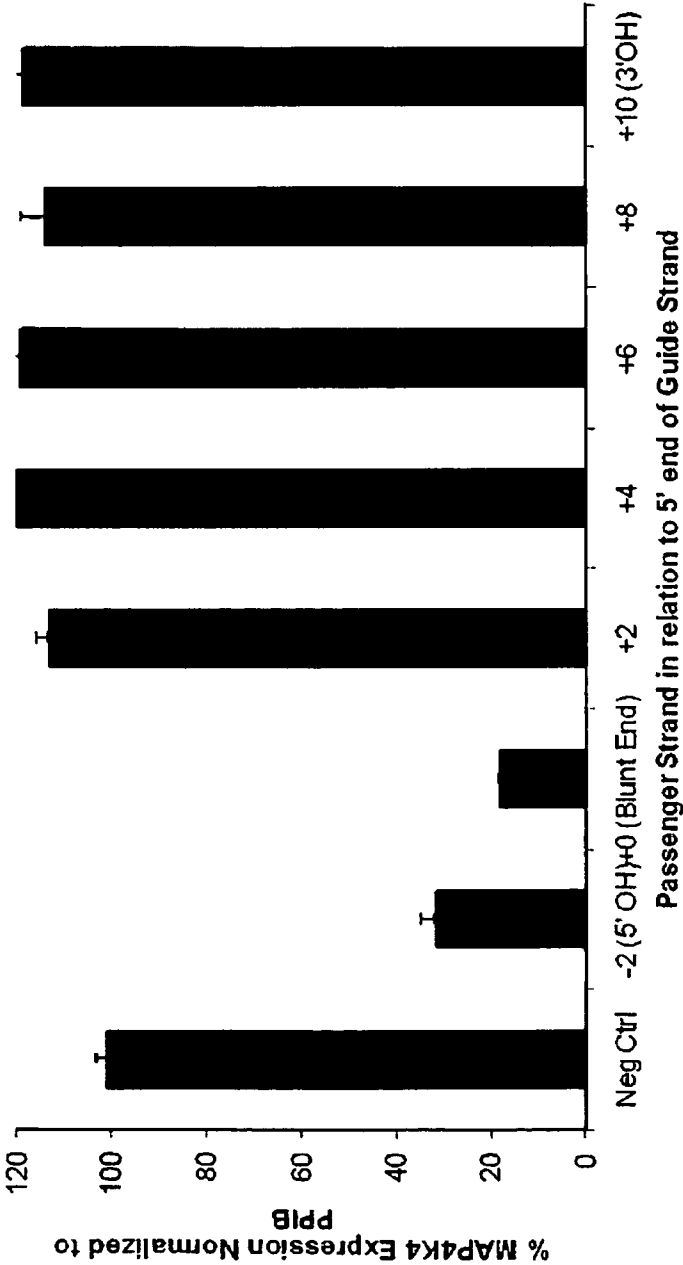
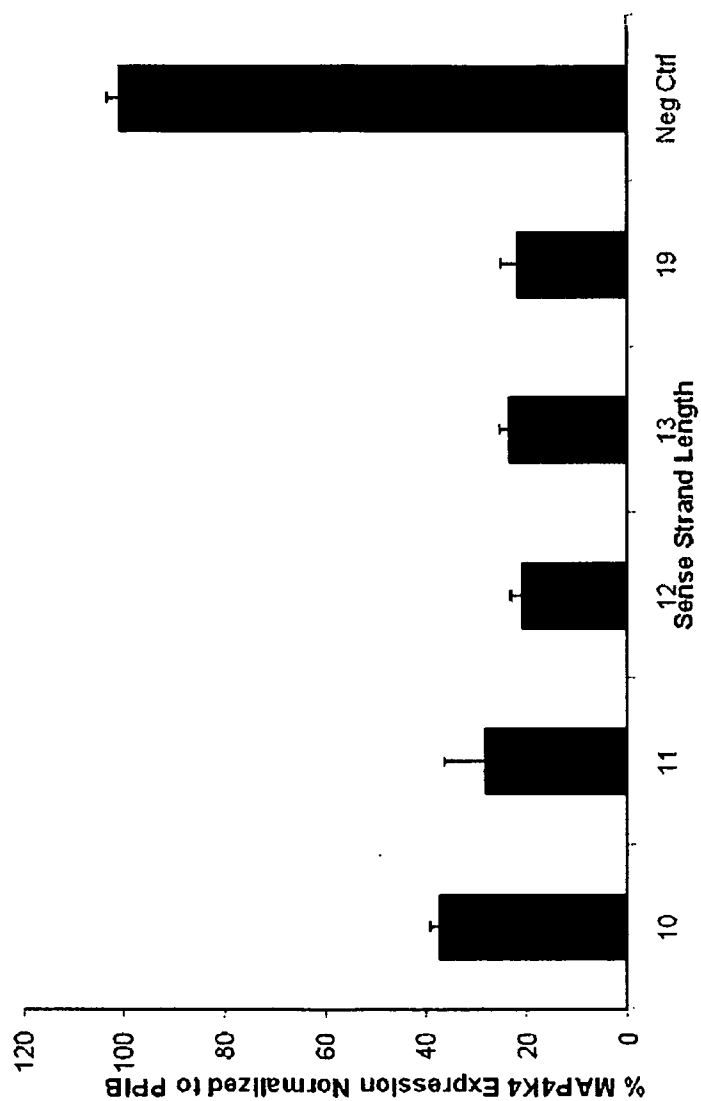


Figure 89



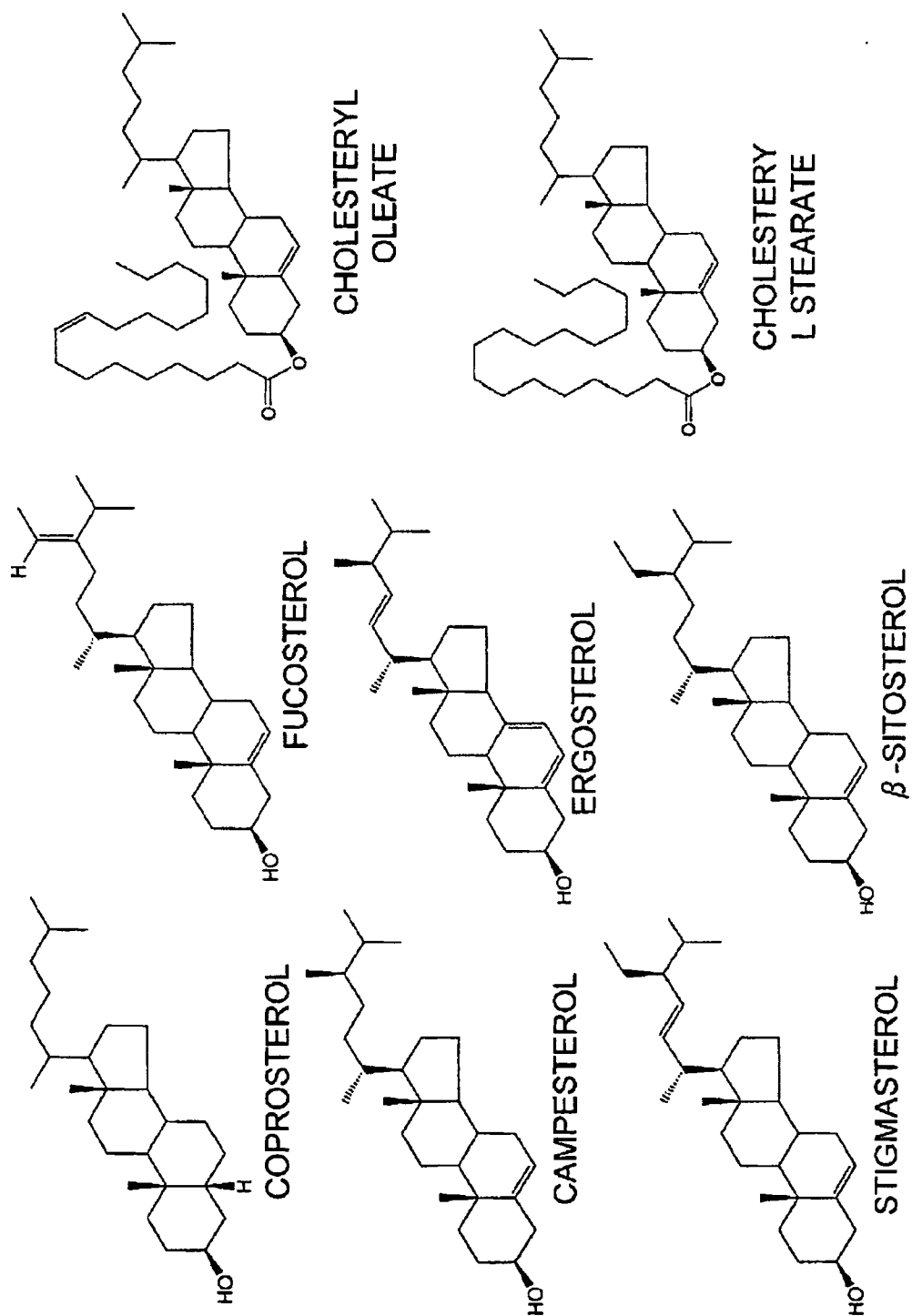
90/92

Figure 90



91/92

Figure 91



92/92

Figure 92

