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(54) Titre : COMPOSES D'ACIDES NUCLEIQUES DE TAILLE REDUITE A AUTO-ADMINISTRATION CIBLANT DES LONGS ARN NON CODANTS

(54) Title: REDUCED SIZE SELF-DELIVERING NUCLEIC ACID COMPOUNDS TARGETING LONG NON-CODING RNA

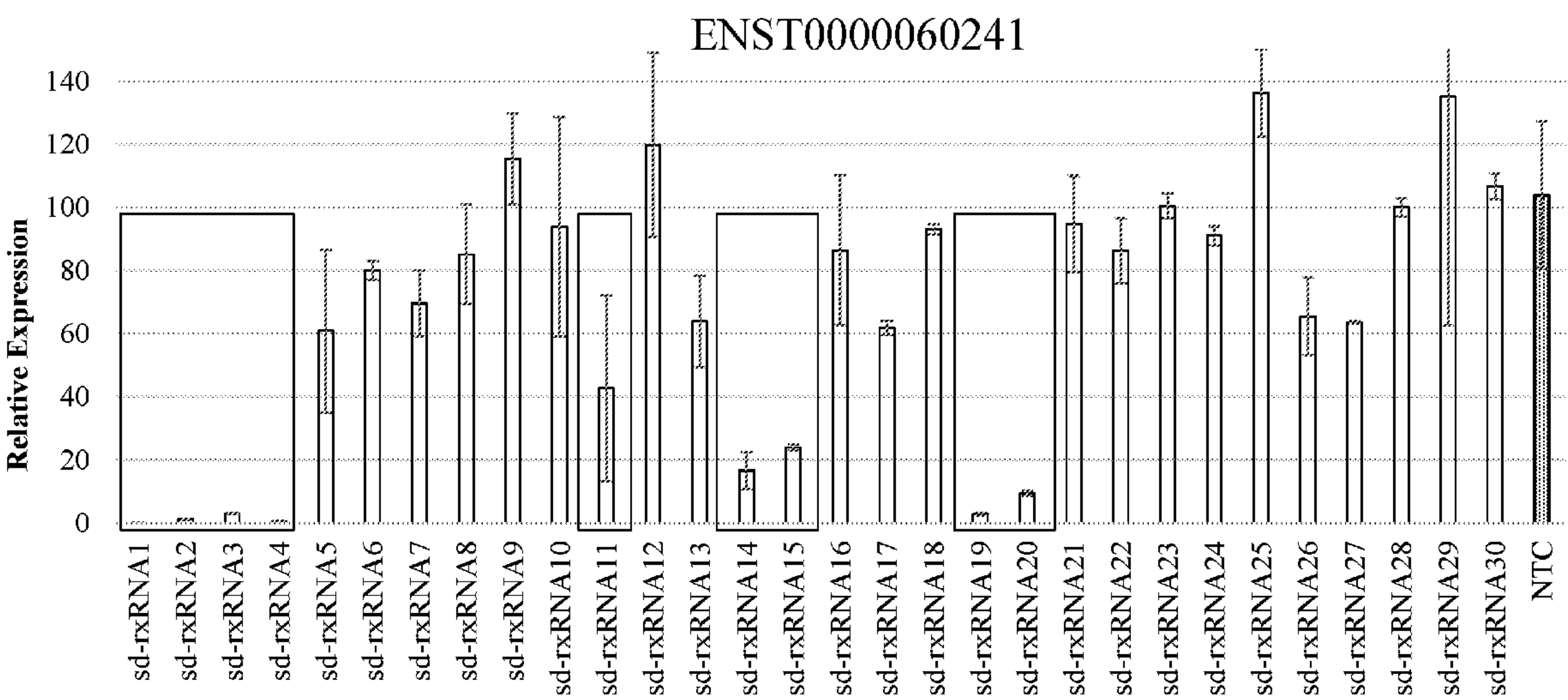

FIG. 1

(57) Abrégé/Abstract:
The present disclosure relates to RNAi constructs with improved cellular uptake characteristics and methods of use of these compounds for silencing expression of long coding RNAs (lncRNAs).

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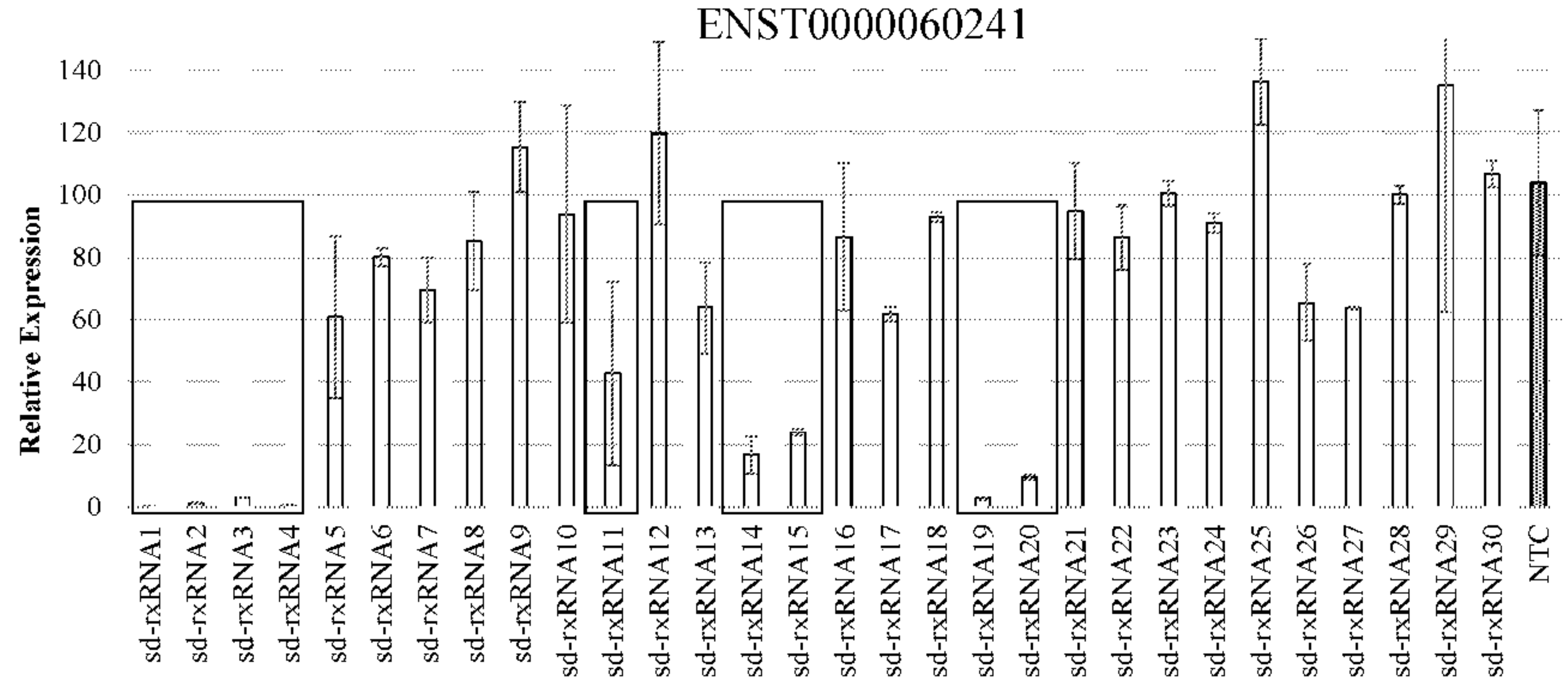


FIG. 1

(57) Abstract: The present disclosure relates to RNAi constructs with improved cellular uptake characteristics and methods of use of these compounds for silencing expression of long coding RNAs (lncRNAs).

WO 2017/070151 A1

REDUCED SIZE SELF-DELIVERING NUCLEIC ACID COMPOUNDS TARGETING LONG NON-CODING RNA

RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. 119(e) of U.S. Provisional Application Serial No. 62/243,565, filed on October 19, 2015, entitled "REDUCED SIZE SELF-DELIVERING NUCLEIC ACID COMPOUNDS TARGETING LONG NON-CODING RNA", the entire contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

The invention relates, at least in part, to the use of nucleic acid molecules with improved *in vivo* delivery properties and their use to reduce the expression of long non-coding RNAs (lncRNAs).

BACKGROUND OF THE 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 or increase of gene expression (including protein synthesis) using such compositions *in vivo*.

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 delivery vehicle to promote their tissue distribution and cellular uptake. This is considered to be a major limitation of the RNAi technology.

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. (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 impaired 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.

Following the sequencing of the mammalian genome, ~20,000 protein-coding genes were identified; however, 99% of the genome was thought to contain non-functional and repetitive sequences. More recently, researchers utilizing transcriptome profiling approaches have discovered that ~60,000 of these non-functional sequences of the genome are transcribed into long non-coding RNAs (lncRNAs), many of which are functional (Iyer et al. (2015)). Long non-coding RNAs (lncRNAs), containing >200 nucleotides, were found to function in the following biological processes: cell proliferation, differentiation, regulation of transcription, epigenetic regulation, post transcriptional regulation, organization of protein complexes, cell to cell communication and allosteric regulation of proteins (Chen, 2015; Geisler et al. 2013).

lncRNAs can be located throughout the cell; however, a majority of lncRNAs are localized in the nucleus (Cabili, 2015). Considering the machinery for RNAi is located in the cytoplasm and not the nucleus, it is believed that using RNAi compounds to reduce levels of lncRNAs (located in the nucleus) would not work. Indeed, researchers have shown that siRNAs can be used to target cytoplasmic-based lncRNAs; however, they have not been demonstrated to work to target nuclear lncRNAs.

SUMMARY

The present disclosure provides compositions and methods for the silencing of lncRNAs. The invention is based, at least in part, on the surprising discovery that self-delivering RNAi compounds are able to robustly and potently reduce levels of lncRNAs in cells, both in the cytoplasm and nucleus. Silencing of nuclear lncRNAs by the RNAi compounds described herein is particularly surprising since it had previously been demonstrated that siRNAs could be used to target cytoplasmic based lncRNAs, but not nuclear lncRNAs. Furthermore, self-delivering RNAi compounds described herein surprisingly mediate silencing of nuclear targets without the use of delivery vehicles (*e.g.*, lipid-mediated transfection agents).

Accordingly, in some aspects, the disclosure provides an isolated, double stranded nucleic acid molecule comprising a guide strand of 18-23 nucleotides in length that has complementarity to a lncRNA sequence, and a passenger strand of 8-16 nucleotides in length, wherein the molecule comprises a double stranded region and a single stranded region, wherein the single stranded region is the 3' end of the guide strand, is 2-13 nucleotides in length, and comprises at least two phosphorothioate modifications, and wherein at least 50% of the pyrimidines in the nucleic acid molecule are modified.

In some embodiments, the first nucleotide relative to the 5'end of the guide strand has a 2'-O-methyl modification, optionally wherein the 2'-O-methyl modification is a 5P-2'O-methyl U modification, or a 5' vinyl phosphonate 2'-O-methyl U modification.

In some embodiments, at least 60%, at least 80%, at least 90% or wherein 100% of the pyrimidines in the nucleic acid molecule are modified. In some embodiments, the modified pyrimidines are 2'-fluoro or 2'-O-methyl modified.

In some embodiments, at least one U or C includes a hydrophobic modification, optionally wherein a plurality of U's and/or C's include a hydrophobic modification. In some embodiments, the hydrophobic modification is a methyl or ethyl hydrophobic base modification.

In some embodiments, the guide strand comprises 6-8 phosphorothioate modifications. In some embodiments, the guide strand comprises at least eight phosphorothioate modifications located within the first 10 nucleotides relative to the 3'end of the guide strand. In some embodiments, the guide strand includes 4-14 phosphate modifications. In some embodiments, the single stranded region of the guide strand is 6 nucleotides long to 8 nucleotides long.

In some embodiments, the double stranded region is 13 nucleotides long. In some embodiments, the double stranded nucleic acid molecule has one end that is blunt or includes a one nucleotide overhang.

In some embodiments, the passenger strand is linked at the 3' end to a lipophilic group. In some embodiments, the lipophilic group is a sterol, optionally wherein the sterol is cholesterol.

In some embodiments, the isolated double stranded nucleic acid molecule is an sd-rxRNA and wherein the guide strand is complementary to a lncRNA, optionally wherein the lncRNA is selected from the group consisting of ENST00000585065, ENST00000602414,

ENST00000607352, ENST00000456581, ENST00000340510, ENST00000605920, ENST00000455699, ENST00000555578, ENST00000565493, ENST00000580048 and MALAT1.

In some embodiments, the isolated double stranded nucleic acid molecule is an sd-rxRNA and wherein the guide strand is complementary to MALAT1.

In some embodiments, the isolated double stranded nucleic acid molecule is a lncRNA inhibitor and wherein the lncRNA sequence to which the guide strand is complementary is an antisense strand of a mature lncRNA. In some embodiments, the guide strand of a double stranded nucleic acid molecule lncRNA inhibitor is at least 50% chemically modified.

In some embodiments, the nucleic acid molecule is directed against at least 12 contiguous nucleotides of a sequence within Table 1 or Table 2.

In some aspects, the disclosure provides a method for modulating lncRNA expression and/or activity in a cell, comprising contacting a cell with a double stranded nucleic acid molecule as described herein (*e.g.*, an sd-rxRNA) in an amount effective to modulate lncRNA expression and/or activity.

In some embodiments of the method, the lncRNA is localized in the nucleus of the cell. In some embodiments, of the method, the lncRNA is localized in the cytoplasm of the cell. In some embodiments of the method, the lncRNA is localized both in the nucleus and the cytoplasm of the cell. In some embodiments, the cell is a bacterial cell or a eukaryotic cell. In some embodiments, the eukaryotic cell is selected from the group consisting of plant cell, arthropod cell, and animal cell). In some embodiments, the eukaryotic cell is a mammalian cell, such as a human cell. In some embodiments, the cell is a stem cell, optionally a human stem cell.

In some embodiments of the method, the cell is contacted with the isolated nucleic acid molecule *in vivo* or *ex vivo*.

In some aspects, the disclosure relates to double stranded molecules configured to treat diseases associated with dysregulation of lncRNA expression. Dysregulation or alteration in lncRNAs levels has been shown to be associated with the progression of many diseases including: cancers (lung, breast, prostate, hepatocellular carcinoma, *etc.*), cardiovascular diseases, neurological disorders, diabetes, and HIV. Therefore in some embodiments, the disclosure provides a method of treating a subject having a disease associated with dysregulation of lncRNA expression, the method comprising administering to the subject a double stranded nucleic acid molecule as described herein in an amount effective to modulate the expression level or activity of a target lncRNA.

Without wishing to be bound by any particular theory, the sense strand of the double stranded molecules described herein (*e.g.*, sd-rxRNA sense strand) is not limited to delivery of the guide strand of the double stranded nucleic acid molecule. Rather, in some embodiments, a passenger strand described herein is joined (*e.g.*, covalently bound, non-covalently bound, conjugated, *etc.*) to certain molecules (*e.g.*, antisense oligonucleotides, ASO) for the purpose of targeting said other molecule to the nucleus of a cell. Accordingly, in some aspects, the disclosure provides a method of delivering a nucleic acid molecule to a cell, the method comprising administering an isolated nucleic acid molecule to a cell, wherein the isolated nucleic acid comprises a sense strand which is complementary to an anti-sense oligonucleotide (ASO), wherein the sense strand is between 8-15 nucleotides in length, comprises at least two phosphorothioate modifications, at least 50% of the pyrimidines in the sense strand are modified, and wherein the molecule comprises a hydrophobic conjugate.

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 THE FIGURES

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.

FIG. 1 shows the identification of potent sd-rxRNAs targeting lncRNA (ENST0000060241). sd-rxRNAs were screened against 11 lncRNA targets. Potent sd-rxRNAs (> 60% silencing) for 10 out of 11 lncRNAs, with an overall hit rate of 21% were identified. The lncRNA-targeting sd-rxRNAs described in this particular assay significantly reduced target gene lncRNA levels *in vitro* in a human hepatocarcinoma cell line.

FIG. 2 shows the identification of potent sd-rxRNAs targeting MALAT1 in a human colorectal carcinoma cell line. The MALAT1-targeting sd-rxRNAs described in this particular assay significantly reduced target gene lncRNA levels *in vitro* in a human hepatocarcinoma cell line.

FIG. 3 shows identification of potent sd-rxRNAs targeting lncRNAs. The lncRNA-targeting sd-rxRNAs described in this particular assay significantly reduced target gene lncRNA levels *in vitro* in a human hepatocarcinoma cell line or a human colorectal carcinoma cell line.

DETAILED DESCRIPTION

The present disclosure relates, in part, to compositions and methods for the silencing of long non-coding RNAs (lncRNAs) by double stranded nucleic acid molecules.

As used herein, a “long non-coding RNA” or “lncRNA” refers to a transcribed RNA molecule containing greater than 200 nucleotides that do not code for protein. LncRNAs are usually located within intergenic spaces of the genome. Generally, lncRNAs are a diverse class of molecules that play a variety of roles in modulation of gene function. For example lncRNAs are known to regulate gene transcription (for example, as described by Goodrichet al. *Nature Reviews Molecular Cell Biology*, 7 (8): 612–6, 2006), translation (for example, as described by Tiedge et al. *PNAS* 88:(6): 2093–7, 1991), and epigenetic regulation (for example, as described by Wutz et al. *Nature Genetics*, 30 (2): 167–74, 2002). Examples of lncRNAs include, but are not limited to Kcnq1ot1, Xlsirt, Xist, ANRIL and MALAT1. Further examples of lncRNAs are described, for example, in Amaralet al. *Nucleic Acids Research* 39((Database issue)): D146–D151, (2010).

The disclosure is based, at least in part, on the surprising discovery that the double stranded nucleic acid molecules described herein are able to robustly and potently reduce levels of long non-coding RNAs (lncRNAs) in cells, both in the cytoplasm and nucleus. Silencing of nuclear lncRNAs by the molecules described herein is particularly surprising in light of the fact that the prior art has demonstrated that siRNAs were not effective in targeting nuclear lncRNAs.

Accordingly, in some aspects, the disclosure provides an isolated, double stranded nucleic acid molecule comprising a guide strand of 18-23 nucleotides in length that has complementarity to a lncRNA sequence, and a passenger strand of 8-16 nucleotides in length, wherein the molecule comprises a double stranded region and a single stranded region, wherein the single stranded region is the 3' end of the guide strand, is 2-13 nucleotides in length, and comprises at least two phosphorothioate modifications, and wherein at least 50% of the pyrimidines in the nucleic acid molecule are modified.

As used herein, "nucleic acid molecule" includes but is not limited to: sd-rxRNA, rxRNAori, oligonucleotides, ASO, siRNA, shRNA, miRNA, ncRNA, cp-lasiRNA, aiRNA, BMT-101, RXI-109, EXC-001, single-stranded nucleic acid molecules, double-stranded nucleic acid molecules, RNA and DNA. In some embodiments, the nucleic acid molecule is a chemically modified nucleic acid molecule, such as a chemically modified oligonucleotide. Double stranded nucleic acid molecules of the invention are described in further detail below and in the Examples section.

Without wishing to be bound by any theory, dysregulation or alteration in lncRNAs levels has been shown to be associated with the progression of many diseases including: cancers (lung, breast, prostate, hepatocellular carcinoma, *etc.*), cardiovascular diseases, neurological disorders, diabetes, and HIV (Chen, 2015). Therefore in some embodiments, the disclosure provides a method of treating a subject having a disease associated with dysregulation of lncRNA expression, the method comprising administering to the subject a double stranded nucleic acid molecule as described herein in an amount effective to modulate the expression level or activity of a target lncRNA.

sd-rxRNA molecules

Aspects of the invention relate to sd-rxRNA molecules. As used herein, an "sd-rxRNA" or an "sd-rxRNA molecule" refers to a self-delivering RNA molecule such as those described in, and incorporated by reference from, US Patent No. 8,796,443, granted on August 5, 2014, entitled "REDUCED SIZE SELF-DELIVERING RNAI COMPOUNDS", US Patent No. 9,175,289, granted on November 3, 2015, entitled "REDUCED SIZE SELF-DELIVERING RNAI COMPOUNDS", and PCT Publication No. WO2010/033247 (Application No. PCT/US2009/005247), filed on September 22, 2009, and entitled "REDUCED SIZE SELF-DELIVERING RNAI COMPOUNDS." Briefly, an sd-rxRNA, (also referred to as an sd-rxRNA^{nano}) is an isolated asymmetric double stranded nucleic acid molecule comprising a guide strand, with a minimal length of 16 nucleotides, and a passenger strand of 8-18 nucleotides in length, wherein the double stranded nucleic acid molecule has a double stranded region and a single stranded region, the single stranded region having 4-12 nucleotides in length and having at least three nucleotide backbone modifications. In preferred embodiments, the double stranded nucleic acid molecule has one end that is blunt or includes a one or two nucleotide overhang. sd-rxRNA molecules can be optimized through chemical modification, and in some instances through attachment of hydrophobic conjugates.

In some embodiments, an sd-rxRNA comprises an isolated double stranded nucleic acid molecule comprising a guide strand and a passenger strand, wherein the region of the molecule that is double stranded is from 8-15 nucleotides long, wherein the guide strand contains a single stranded region that is 4-12 nucleotides long, wherein the single stranded region of the guide strand contains 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 phosphorothioate modifications, and wherein at least 40% of the nucleotides of the double stranded nucleic acid are modified.

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.

sd-rxRNAs are much more 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.

In contrast to single-stranded polynucleotides, duplex polynucleotides have traditionally been difficult to deliver to a cell as they have rigid structures and a large number of negative charges which makes membrane transfer difficult. sd-rxRNAs however, although partially double-stranded, are recognized *in vivo* as single-stranded and, as such, are capable of efficiently being 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 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.

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 5 nucleotides or longer, specific chemical modification patterns and are conjugated to lipophilic or hydrophobic molecules. In some embodiments, this class of RNAi like compounds have superior efficacy *in vitro* and *in vivo*. 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 contribute to the observed superior efficacy.

Methods of effectively administering sd-rxRNA to the skin and silencing gene expression have been demonstrated in US Patent No. 8,664,189, granted on March 4, 2014 and entitled "RNA INTERFERENCE IN SKIN INDICATIONS," US Patent Publication No. US2014/0113950, filed on April 4, 2013 and entitled "RNA INTERFERENCE IN DERMAL AND FIBROTIC INDICATIONS," PCT Publication No. WO 2010/033246, filed on September 22, 2009 and entitled "RNA INTERFERENCE IN SKIN INDICATIONS" and PCT Publication No. WO2011/119887, filed on March 24, 2011 and entitled "RNA INTERFERENCE IN DERMAL AND FIBROTIC INDICATIONS." Each of the above-referenced patents and publications are incorporated by reference herein in their entireties.

It should be appreciated that the sd-rxRNA molecules disclosed herein can be administered to the skin in the same manner as the sd-rxRNA molecules disclosed in US Patent Publication No. US2014/0113950, incorporated by reference in its entirety.

In a preferred embodiment the RNAi compounds of the invention comprise an asymmetric compound comprising a duplex region (required for efficient RISC entry of 8-15 bases long) and single stranded region of 4-12 nucleotides long. In some embodiments, the duplex region is 13 or 14 nucleotides long. A 6 or 7 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 embodiments. Additionally, the RNAi compounds of the invention also include a unique chemical modification pattern, which provides stability and is compatible with RISC entry. In some embodiments, 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 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 confirm stability and do not interfere with activity. Such modifications include 2' ribo modifications (O-methyl, 2' F, 2 deoxy and others) and backbone modification like phosphorothioate modifications. A preferred chemical modification pattern in the passenger strand includes O-methyl modification of C and U nucleotides within the passenger strand or alternatively the passenger strand may be completely O-methyl modified.

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'O-methyl modification of position 1 and C/U in positions 11-18 and 5' end chemical phosphorylation. Yet another preferred chemical modification pattern in the guide strand includes 2'O-methyl modification of position 1 and C/U in positions 11-18 and 5' end chemical phosphorylation and 2'F modification of C/U in positions 2-10. In some embodiments the passenger strand and/or the guide strand contains at least one 5-methyl C or U modifications.

In some embodiments, at least 30% of the nucleotides in the sd-rxRNA are modified. For example, at least 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% of the nucleotides in the sd-rxRNA are modified. In some embodiments, 100% of the nucleotides in the sd-rxRNA are modified.

The above-described chemical modification patterns of the oligonucleotides of the invention are well tolerated and actually improved efficacy of asymmetric RNAi compounds. In some embodiments, elimination of any of the described components (Guide strand stabilization, phosphorothioate stretch, sense strand stabilization and hydrophobic conjugate) or increase in size in some instances results in sub-optimal efficacy and in some instances complete loss of efficacy. The combination of elements results in development of a compound, which is fully active following passive delivery to cells such as HeLa cells.

The sd-rxRNA can be further improved in some instances by improving the 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 synthesis and (b) lack of interference with base-pairing and A form helix formation, which are essential for RISC complex loading and target recognition. 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 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*.

dsRNA formulated according to the invention also includes rxRNAori. rxRNAori refers to a class of RNA molecules described in and incorporated by reference from PCT Publication No. WO2009/102427 (Application No. PCT/US2009/000852), filed on February 11, 2009, and entitled, "MODIFIED RNAI POLYNUCLEOTIDES AND USES THEREOF," and US Patent Publication No. 2011/0039914, filed on November 1, 2010, and entitled "MODIFIED RNAI POLYNUCLEOTIDES AND USES THEREOF."

In some embodiments, an rxRNAori molecule comprises a double-stranded RNA (dsRNA) construct of 12-35 nucleotides in length, for inhibiting expression of a target gene, comprising: a sense strand having a 5'-end and a 3'-end, wherein the sense strand is highly modified with 2'-modified ribose sugars, and wherein 3-6 nucleotides in the central portion of the sense strand are not modified with 2'-modified ribose sugars and, an antisense strand having a 5'-end and a 3'-end, which hybridizes to the sense strand and to mRNA of the target gene, wherein the dsRNA inhibits expression of the target gene in a sequence-dependent manner.

rxRNAori can contain any of the modifications described herein. In some embodiments, at least 30% of the nucleotides in the rxRNAori are modified. For example, at least 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% of the nucleotides in the rxRNAori are modified. In some embodiments, 100% of the nucleotides in the sd-rxRNA are modified. In some embodiments, only the passenger strand of the rxRNAori contains modifications.

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. 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 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 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. In some embodiments, 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 8-15 nucleotides long. In certain embodiments, the passenger strand is 8, 9, 10, 11, 12, 13, 14 or 15 nucleotides long. The passenger strand has complementarity to the guide strand. 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.

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 8-15 nucleotides long. In certain embodiments, the region of the molecule that is double stranded is 8, 9, 10, 11, 12, 13, 14 or 15 nucleotides long. In certain embodiments the double stranded region is 13 or 14 nucleotides long. There 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 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 at least 6 or at least 7 nucleotides long.

RNAi constructs associated with the invention can have a thermodynamic 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 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 kkcal/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 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 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. 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 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 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 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 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 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 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 contain 2' ribo, 2'F and 2 deoxy modifications or any combination of the above. Chemical modification patterns on both the guide and passenger strand can be well tolerated and a combination of chemical modifications can lead to increased efficacy and self-delivery of RNA molecules.

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. In some embodiments, molecules that have a double stranded region of 8-15 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 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 (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.

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.

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.

In certain embodiments, the sd-rxRNA 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.

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 significantly decreasing RNAi activity (or no decrease in RNAi activity at all).

Certain combinations of modifications may result in further unexpected advantages, as partly manifested by enhanced ability to inhibit target gene expression, enhanced serum stability, and/or 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 sd-rxRNA structures of the present invention mediates 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" ("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.

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.

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 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 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 O-methyl modified with the phosphorothioate content described previously. For these types of compounds the 5' phosphorylation is not necessary in some embodiments. The presence of double stranded region is preferred as it is 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 ~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 complementarity.

Some aspects of biogenesis, protein complexes, and function are shared between the siRNA pathway and the miRNA pathway. 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 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, 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.

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.

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.

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 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, 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 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 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 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 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 Cytosine (4-pyridyl, 2-pyridyl, indolyl, phenyl (C_6H_5OH); 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, 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 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' 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 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% 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 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 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 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 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.*, the first molecule comprising an antisense sequence, can be longer than the second molecule hybridizing thereto (leaving a portion of the molecule single-stranded). 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.

Modifications

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

In some embodiments, the base moiety of a nucleoside may be modified. For example, a pyrimidine base may be modified at the 2, 3, 4, 5, and/or 6 position of the pyrimidine ring. In some embodiments, the exocyclic amine of cytosine may be modified. A purine base may also be modified. For example, a purine base may be modified at the 1, 2, 3, 6, 7, or 8 position. In some embodiments, the exocyclic amine of adenine may be modified. In some cases, a nitrogen atom in a ring of a base moiety may be substituted with another atom, such as carbon. A modification to a base moiety may be any suitable modification. Examples of modifications are known to those of ordinary skill in the art. In some embodiments, the base modifications include alkylated purines or pyrimidines, acylated purines or pyrimidines, or other heterocycles.

In some embodiments, a pyrimidine may be modified at the 5 position. For example, the 5 position of a pyrimidine may be modified with an alkyl group, an alkynyl group, an alkenyl group, an acyl group, or substituted derivatives thereof. In other examples, the 5 position of a pyrimidine may be modified with a hydroxyl group or an alkoxyl group or substituted derivative thereof. Also, the N^4 position of a pyrimidine may be alkylated. In still further examples, the pyrimidine 5-6 bond may be saturated, a nitrogen atom within the pyrimidine ring may be substituted with a carbon atom, and/or the O^2 and O^4 atoms may be substituted with sulfur atoms. It should be understood that other modifications are possible as well.

In other examples, the N^7 position and/or N^2 and/or N^3 position of a purine may be modified with an alkyl group or substituted derivative thereof. In further examples, a third ring may be fused to the purine bicyclic ring system and/or a nitrogen atom within the purine ring system may be substituted with a carbon atom. It should be understood that other modifications are possible as well.

Non-limiting examples of pyrimidines modified at the 5 position are disclosed in U.S. Patent 5591843, U.S. Patent 7,205,297, U.S. Patent 6,432,963, and U.S. Patent 6,020,483; non-limiting examples of pyrimidines modified at the N^4 position are disclosed in U.S. Patent 5,580,731; non-limiting examples of purines modified at the 8 position are disclosed in U.S.

Patent 6,355,787 and U.S. Patent 5,580,972; non-limiting examples of purines modified at the N^6 position are disclosed in U.S. Patent 4,853,386, U.S. Patent 5,789,416, and U.S. Patent 7,041,824; and non-limiting examples of purines modified at the 2 position are disclosed in U.S. Patent 4,201,860 and U.S. Patent 5,587,469, all of which are incorporated herein by reference.

Non-limiting examples of modified bases include N^4,N^4-ethanocytosine, 7-deazaxanthosine, 7-deazaguanosine, 8-oxo-N^6-methyladenine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyl uracil, dihydrouracil, inosine, N^6-isopentenyl-adenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N^6 -methyladenine, 7-methylguanine, 5-methylaminomethyl uracil, 5-methoxy aminomethyl-2-thiouracil, 5-methoxyuracil, 2-methylthio-N^6-isopentenyladenine, pseudouracil, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, 2-thiocytosine, and 2,6-diaminopurine. In some embodiments, the base moiety may be a heterocyclic base other than a purine or pyrimidine. The heterocyclic base may be optionally modified and/or substituted.

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, alxoxy (or OR), R or alkyl, halogen, SH, SR, amino (such as NH_2, NHR, NR_2,), 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.

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 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 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.

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 ($-OCH_2CH=CH_2$), 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.*, *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.

Definitions of specific functional groups and chemical terms are described in more detail below. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, *Handbook of Chemistry and Physics, 75th Ed.*, inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in *Organic Chemistry*, Thomas Sorrell, University Science Books, Sausalito: 1999, the entire contents of which are incorporated herein by reference.

Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including *cis*- and *trans*-isomers, *R*- and *S*-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

Isomeric mixtures containing any of a variety of isomer ratios may be utilized in accordance with the present invention. For example, where only two isomers are combined, mixtures containing 50:50, 60:40, 70:30, 80:20, 90:10, 95:5, 96:4, 97:3, 98:2, 99:1, or 100:0 isomer ratios are all contemplated by the present invention. Those of ordinary skill in the art will readily appreciate that analogous ratios are contemplated for more complex isomer mixtures.

If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

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 "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 (CH_2-CH_2-CH_3), glycol (-O-CH_2-CH_2-O-) phosphate (PO_3^{2-}), 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.

One of ordinary skill in the art will appreciate that the synthetic methods, as described herein, utilize a variety of protecting groups. By the term "protecting group," as used herein, it is meant that a particular functional moiety, *e.g.*, O, S, or N, is temporarily blocked so that a reaction can be carried out selectively at another reactive site in a multifunctional compound. In certain embodiments, a protecting group reacts selectively in good yield to give a protected substrate that is stable to the projected reactions; the protecting group should be selectively removable in good yield by readily available, preferably non-toxic reagents that do not attack the other functional groups; the protecting group forms an easily separable derivative (more preferably without the generation of new stereogenic centers); and the protecting group has a minimum of additional functionality to avoid further sites of reaction. As detailed herein, oxygen, sulfur, nitrogen, and carbon protecting groups may be utilized. Hydroxyl protecting groups include methyl, methoxylmethyl (MOM), methylthiomethyl (MTM), *t*-butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), *p*-methoxybenzyloxymethyl (PMBM), (4-methoxyphenoxy)methyl (*p*-AOM), guaiacolmethyl (GUM), *t*-butoxymethyl, 4-pentenyloxymethyl (POM), siloxymethyl, 2-methoxyethoxymethyl (MEM), 2,2,2-trichloroethoxymethyl, bis(2-chloroethoxy)methyl, 2-(trimethylsilyl)ethoxymethyl (SEMOR), tetrahydropyranyl (THP), 3-

bromotetrahydropyranyl, tetrahydrothiopyranyl, 1-methoxycyclohexyl, 4-methoxytetrahydropyranyl (MTHP), 4-methoxytetrahydrothiopyranyl, 4-methoxytetrahydrothiopyranyl S,S-dioxide, 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl (CTMP), 1,4-dioxan-2-yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a-octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 1-methyl-1-methoxyethyl, 1-methyl-1-benzyloxyethyl, 1-methyl-1-benzyloxy-2-fluoroethyl, 2,2,2-trichloroethyl, 2-trimethylsilylethyl, 2-(phenylselenyl)ethyl, *t*-butyl, allyl, *p*-chlorophenyl, *p*-methoxyphenyl, 2,4-dinitrophenyl, benzyl, *p*-methoxybenzyl, 3,4-dimethoxybenzyl, *o*-nitrobenzyl, *p*-nitrobenzyl, *p*-halobenzyl, 2,6-dichlorobenzyl, *p*-cyanobenzyl, *p*-phenylbenzyl, 2-picolyl, 4-picolyl, 3-methyl-2-picolyl *N*-oxido, diphenylmethyl, *p,p'*-dinitrobenzhydryl, 5-dibenzosuberyl, triphenylmethyl, α-naphthyldiphenylmethyl, *p*-methoxyphenyldiphenylmethyl, di(*p*-methoxyphenyl)phenylmethyl, tri(*p*-methoxyphenyl)methyl, 4-(4′-bromophenacyloxyphenyl)diphenylmethyl, 4,4′,4″-tris(4,5-dichlorophthalimidophenyl)methyl, 4,4′,4″-tris(levulinoyloxyphenyl)methyl, 4,4′,4″-tris(benzoyloxyphenyl)methyl, 3-(imidazol-1-yl)bis(4′,4″-dimethoxyphenyl)methyl, 1,1-bis(4-methoxyphenyl)-1′-pyrenylmethyl, 9-anthryl, 9-(9-phenyl)xanthenyl, 9-(9-phenyl-10-oxo)anthryl, 1,3-benzodithiolan-2-yl, benzisothiazolyl S,S-dioxido, trimethylsilyl (TMS), triethylsilyl (TES), triisopropylsilyl (TIPS), dimethylisopropylsilyl (IPDMS), diethylisopropylsilyl (DEIPS), dimethylthexylsilyl, *t*-butyldimethylsilyl (TBDMS), *t*-butyldiphenylsilyl (TBDPS), tribenzylsilyl, tri-*p*-xylylsilyl, triphenylsilyl, diphenylmethylsilyl (DPMS), *t*-butylmethoxyphenylsilyl (TBMPS), formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, *p*-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate (levulinate), 4,4-(ethylenedithio)pentanoate (levulinoyldithioacetal), pivaloate, adamantoate, crotonate, 4-methoxycrotonate, benzoate, *p*-phenylbenzoate, 2,4,6-trimethylbenzoate (mesitoate), alkyl methyl carbonate, 9-fluorenylmethyl carbonate (Fmoc), alkyl ethyl carbonate, alkyl 2,2,2-trichloroethyl carbonate (Troc), 2-(trimethylsilyl)ethyl carbonate (TMSEC), 2-(phenylsulfonyl) ethyl carbonate (Psec), 2-(triphenylphosphonio) ethyl carbonate (Peoc), alkyl isobutyl carbonate, alkyl vinyl carbonate alkyl allyl carbonate, alkyl *p*-nitrophenyl carbonate, alkyl benzyl carbonate, alkyl *p*-methoxybenzyl carbonate, alkyl 3,4-dimethoxybenzyl carbonate, alkyl *o*-nitrobenzyl carbonate, alkyl *p*-nitrobenzyl carbonate, alkyl *S*-benzyl thiocarbonate, 4-ethoxy-1-napththyl carbonate, methyl dithiocarbonate, 2-iodobenzoate, 4-azidobutyrate, 4-nitro-4-methylpentanoate, *o*-

(dibromomethyl)benzoate, 2-formylbenzenesulfonate, 2-(methylthiomethoxy)ethyl, 4-(methylthiomethoxy)butyrate, 2-(methylthiomethoxymethyl)benzoate, 2,6-dichloro-4-methylphenoxyacetate, 2,6-dichloro-4-(1,1,3,3-tetramethylbutyl)phenoxyacetate, 2,4-bis(1,1-dimethylpropyl)phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccinoate, (*E*)-2-methyl-2-butenoate, *o*-(methoxycarbonyl)benzoate, α-naphthoate, nitrate, alkyl *N,N,N',N'*-tetramethylphosphorodiamidate, alkyl *N*-phenylcarbamate, borate, dimethylphosphinothioyl, alkyl 2,4-dinitrophenylsulfenate, sulfate, methanesulfonate (mesylate), benzylsulfonate, and tosylate (Ts). For protecting 1,2- or 1,3-diols, the protecting groups include methylene acetal, ethylidene acetal, 1-*t*-butylethylidene ketal, 1-phenylethylidene ketal, (4-methoxyphenyl)ethylidene acetal, 2,2,2-trichloroethylidene acetal, acetonide, cyclopentylidene ketal, cyclohexylidene ketal, cycloheptylidene ketal, benzylidene acetal, *p*-methoxybenzylidene acetal, 2,4-dimethoxybenzylidene ketal, 3,4-dimethoxybenzylidene acetal, 2-nitrobenzylidene acetal, methoxymethylene acetal, ethoxymethylene acetal, dimethoxymethylene ortho ester, 1-methoxyethylidene ortho ester, 1-ethoxyethylidine ortho ester, 1,2-dimethoxyethylidene ortho ester, α-methoxybenzylidene ortho ester, 1-(*N,N*-dimethylamino)ethylidene derivative, α-(*N,N'*-dimethylamino)benzylidene derivative, 2-oxacyclopentylidene ortho ester, di-*t*-butylsilylene group (DTBS), 1,3-(1,1,3,3-tetraisopropyldisiloxanylidene) derivative (TIPDS), tetra-*t*-butoxydisiloxane-1,3-diylidene derivative (TBDS), cyclic carbonates, cyclic boronates, ethyl boronate, and phenyl boronate. Amino-protecting groups include methyl carbamate, ethyl carbamante, 9-fluorenylmethyl carbamate (Fmoc), 9-(2-sulfo)fluorenylmethyl carbamate, 9-(2,7-dibromo)fluoroenylmethyl carbamate, 2,7-di-*t*-butyl-[9-(10,10-dioxo-10,10,10,10-tetrahydrothioxanthyl)]methyl carbamate (DBD-Tmoc), 4-methoxyphenacyl carbamate (Phenoc), 2,2,2-trichloroethyl carbamate (Troc), 2-trimethylsilylethyl carbamate (Teoc), 2-phenylethyl carbamate (hZ), 1-(1-adamantyl)-1-methylethyl carbamate (Adpoc), 1,1-dimethyl-2-haloethyl carbamate, 1,1-dimethyl-2,2-dibromoethyl carbamate (DB-*t*-BOC), 1,1-dimethyl-2,2,2-trichloroethyl carbamate (TCBOC), 1-methyl-1-(4-biphenylyl)ethyl carbamate (Bpoc), 1-(3,5-di-*t*-butylphenyl)-1-methylethyl carbamate (*t*-Bumeoc), 2-(2′- and 4′-pyridyl)ethyl carbamate (Pyoc), 2-(*N,N*-dicyclohexylcarboxamido)ethyl carbamate, *t*-butyl carbamate (BOC), 1-adamantyl carbamate (Adoc), vinyl carbamate (Voc), allyl carbamate (Alloc), 1-isopropylallyl carbamate (Ipaoc), cinnamyl carbamate (Coc), 4-nitrocinnamyl carbamate (Noc), 8-quinolyl carbamate, *N*-hydroxypiperidinyl carbamate, alkyldithio carbamate, benzyl carbamate (Cbz), *p*-methoxybenzyl carbamate (Moz), *p*-nitobenzyl carbamate, *p*-bromobenzyl carbamate, *p*-chlorobenzyl carbamate, 2,4-dichlorobenzyl carbamate, 4-

methylsulfinylbenzyl carbamate (Msz), 9-anthrylmethyl carbamate, diphenylmethyl carbamate, 2-methylthioethyl carbamate, 2-methylsulfonylethyl carbamate, 2-(*p*-toluenesulfonyl)ethyl carbamate, [2-(1,3-dithianyl)]methyl carbamate (Dmoc), 4-methylthiophenyl carbamate (Mtpc), 2,4-dimethylthiophenyl carbamate (Bmpc), 2-phosphonioethyl carbamate (Peoc), 2-triphenylphosphonioisopropyl carbamate (Ppoc), 1,1-dimethyl-2-cyanoethyl carbamate, *m*-chloro-*p*-acyloxybenzyl carbamate, *p*-(dihydroxyboryl)benzyl carbamate, 5-benzisoxazolylmethyl carbamate, 2-(trifluoromethyl)-6-chromonylmethyl carbamate (Tcroc), *m*-nitrophenyl carbamate, 3,5-dimethoxybenzyl carbamate, *o*-nitrobenzyl carbamate, 3,4-dimethoxy-6-nitrobenzyl carbamate, phenyl(*o*-nitrophenyl)methyl carbamate, phenothiazinyl-(10)-carbonyl derivative, *N*′-*p*-toluenesulfonylaminocarbonyl derivative, *N*′-phenylaminothiocarbonyl derivative, *t*-amyl carbamate, *S*-benzyl thiocarbamate, *p*-cyanobenzyl carbamate, cyclobutyl carbamate, cyclohexyl carbamate, cyclopentyl carbamate, cyclopropylmethyl carbamate, *p*-decyloxybenzyl carbamate, 2,2-dimethoxycarbonylvinyl carbamate, *o*-(*N,N*-dimethylcarboxamido)benzyl carbamate, 1,1-dimethyl-3-(*N,N*-dimethylcarboxamido)propyl carbamate, 1,1-dimethylpropynyl carbamate, di(2-pyridyl)methyl carbamate, 2-furanylmethyl carbamate, 2-iodoethyl carbamate, isoborynl carbamate, isobutyl carbamate, isonicotinyl carbamate, *p*-(*p*′-methoxyphenylazo)benzyl carbamate, 1-methylcyclobutyl carbamate, 1-methylcyclohexyl carbamate, 1-methyl-1-cyclopropylmethyl carbamate, 1-methyl-1-(3,5-dimethoxyphenyl)ethyl carbamate, 1-methyl-1-(*p*-phenylazophenyl)ethyl carbamate, 1-methyl-1-phenylethyl carbamate, 1-methyl-1-(4-pyridyl)ethyl carbamate, phenyl carbamate, *p*-(phenylazo)benzyl carbamate, 2,4,6-tri-*t*-butylphenyl carbamate, 4-(trimethylammonium)benzyl carbamate, 2,4,6-trimethylbenzyl carbamate, formamide, acetamide, chloroacetamide, trichloroacetamide, trifluoroacetamide, phenylacetamide, 3-phenylpropanamide, picolinamide, 3-pyridylcarboxamide, *N*-benzoylphenylalanyl derivative, benzamide, *p*-phenylbenzamide, *o*-nitophenylacetamide, *o*-nitrophenoxyacetamide, acetoacetamide, (*N*’-dithiobenzyloxycarbonylamino)acetamide, 3-(*p*-hydroxyphenyl)propanamide, 3-(*o*-nitrophenyl)propanamide, 2-methyl-2-(*o*-nitrophenoxy)propanamide, 2-methyl-2-(*o*-phenylazophenoxy)propanamide, 4-chlorobutanamide, 3-methyl-3-nitrobutanamide, *o*-nitrocinnamide, *N*-acetylmethionine derivative, *o*-nitrobenzamide, *o*-(benzoyloxymethyl)benzamide, 4,5-diphenyl-3-oxazolin-2-one, *N*-phthalimide, *N*-dithiasuccinimide (Dts), *N*-2,3-diphenylmaleimide, *N*-2,5-dimethylpyrrole, *N*-1,1,4,4-tetramethyldisilylazacyclopentane adduct (STABASE), 5-substituted 1,3-dimethyl-1,3,5-triazacyclohexan-2-one, 5-substituted 1,3-dibenzyl-1,3,5-

triazacyclohexan-2-one, 1-substituted 3,5-dinitro-4-pyridone, *N*-methylamine, *N*-allylamine, *N*-[2-(trimethylsilyl)ethoxy]methylamine (SEM), *N*-3-acetoxypropylamine, *N*-(1-isopropyl-4-nitro-2-oxo-3-pyroolin-3-yl)amine, quaternary ammonium salts, *N*-benzylamine, *N*-di(4-methoxyphenyl)methylamine, *N*-5-dibenzosuberylamine, *N*-triphenylmethylamine (Tr), *N*-[(4-methoxyphenyl)diphenylmethyl]amine (MMTr), *N*-9-phenylfluorenylamine (PhF), *N*-2,7-dichloro-9-fluorenylmethyleneamine, *N*-ferrocenylmethylamino (Fcm), *N*-2-picolylamino *N'*-oxide, *N*-1,1-dimethylthiomethyleneamine, *N*-benzylideneamine, *N*-*p*-methoxybenzylideneamine, *N*-diphenylmethyleneamine, *N*-[(2-pyridyl)mesityl]methyleneamine, *N*-(*N'*,*N'*-dimethylaminomethylene)amine, *N,N'*-isopropylidenediamine, *N*-*p*-nitrobenzylideneamine, *N*-salicylideneamine, *N*-5-chlorosalicylideneamine, *N*-(5-chloro-2-hydroxyphenyl)phenylmethyleneamine, *N*-cyclohexylideneamine, *N*-(5,5-dimethyl-3-oxo-1-cyclohexenyl)amine, *N*-borane derivative, *N*-diphenylborinic acid derivative, *N*-[phenyl(pentacarbonylchromium- or tungsten)carbonyl]amine, *N*-copper chelate, *N*-zinc chelate, *N*-nitroamine, *N*-nitrosoamine, amine *N*-oxide, diphenylphosphinamide (Dpp), dimethylthiophosphinamide (Mpt), diphenylthiophosphinamide (Ppt), dialkyl phosphoramidates, dibenzyl phosphoramidate, diphenyl phosphoramidate, benzenesulfenamide, *o*-nitrobenzenesulfenamide (Nps), 2,4-dinitrobenzenesulfenamide, pentachlorobenzenesulfenamide, 2-nitro-4-methoxybenzenesulfenamide, triphenylmethylsulfenamide, 3-nitropyridinesulfenamide (Npys), *p*-toluenesulfonamide (Ts), benzenesulfonamide, 2,3,6,-trimethyl-4-methoxybenzenesulfonamide (Mtr), 2,4,6-trimethoxybenzenesulfonamide (Mtb), 2,6-dimethyl-4-methoxybenzenesulfonamide (Pme), 2,3,5,6-tetramethyl-4-methoxybenzenesulfonamide (Mte), 4-methoxybenzenesulfonamide (Mbs), 2,4,6-trimethylbenzenesulfonamide (Mts), 2,6-dimethoxy-4-methylbenzenesulfonamide (iMds), 2,2,5,7,8-pentamethylchroman-6-sulfonamide (Pmc), methanesulfonamide (Ms), β-trimethylsilylethanesulfonamide (SES), 9-anthracenesulfonamide, 4-(4′,8′-dimethoxynaphthylmethyl)benzenesulfonamide (DNMBS), benzylsulfonamide, trifluoromethylsulfonamide, and phenacylsulfonamide. Exemplary protecting groups are detailed herein. However, it will be appreciated that the present invention is not intended to be limited to these protecting groups; rather, a variety of additional equivalent protecting groups can be readily identified using the above criteria and utilized in the method of the present invention. Additionally, a variety of protecting groups are described in *Protective Groups in Organic Synthesis,* Third Ed. Greene, T.W. and Wuts, P.G., Eds., John Wiley & Sons, New York: 1999, the entire contents of which are hereby incorporated by reference.

It will be appreciated that the compounds, as described herein, may be substituted with any number of substituents or functional moieties. In general, the term "substituted" whether preceded by the term "optionally" or not, and substituents contained in formulas of this invention, refer to the replacement of hydrogen radicals in a given structure with the radical of a specified substituent. When more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. Heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valencies of the heteroatoms. Furthermore, this invention is not intended to be limited in any manner by the permissible substituents of organic compounds. Combinations of substituents and variables envisioned by this invention are preferably those that result in the formation of stable compounds useful in the treatment, for example, of infectious diseases or proliferative disorders. The term "stable", as used herein, preferably refers to compounds which possess stability sufficient to allow manufacture and which maintain the integrity of the compound for a sufficient period of time to be detected and preferably for a sufficient period of time to be useful for the purposes detailed herein.

The term "aliphatic," as used herein, includes both saturated and unsaturated, straight chain (*i.e.*, unbranched), branched, acyclic, cyclic, or polycyclic aliphatic hydrocarbons, which are optionally substituted with one or more functional groups. As will be appreciated by one of ordinary skill in the art, "aliphatic" is intended herein to include, but is not limited to, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, and cycloalkynyl moieties. Thus, as used herein, the term "alkyl" includes straight, branched and cyclic alkyl groups. An analogous convention applies to other generic terms such as "alkenyl," "alkynyl," and the like. Furthermore, as used herein, the terms "alkyl," "alkenyl," "alkynyl," and the like encompass both substituted and unsubstituted groups. In certain embodiments, as used herein, "lower alkyl" is used to indicate those alkyl groups (cyclic, acyclic, substituted, unsubstituted, branched, or unbranched) having 1-6 carbon atoms.

In certain embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-4 carbon atoms. Illustrative aliphatic groups thus include, but are not limited to, for example, methyl, ethyl, *n*-propyl, isopropyl, cyclopropyl, $-CH_2$-cyclopropyl, vinyl, allyl, *n*-butyl, *sec*-butyl, isobutyl, *tert*-butyl, cyclobutyl, $-CH_2$-cyclobutyl, *n*-pentyl, sec-pentyl, isopentyl, *tert*-pentyl, cyclopentyl, $-CH_2$-cyclopentyl, *n*-hexyl, *sec*-hexyl, cyclohexyl, $-CH_2$-cyclohexyl moieties and the like, which again, may bear one or more substituents. Alkenyl groups include, but are not limited to, for example, ethenyl, propenyl, butenyl, 1-methyl-2-buten-1-yl, and the like. Representative alkynyl groups include, but are not limited to, ethynyl, 2-propynyl (propargyl), 1-propynyl, and the like.

Some examples of substituents of the above-described aliphatic (and other) moieties of compounds of the invention include, but are not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; arylalkyl; heteroarylalkyl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; -F; -Cl; -Br; -I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein any of the aliphatic, heteroaliphatic, arylalkyl, or heteroarylalkyl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments described herein.

The term "heteroaliphatic," as used herein, refers to aliphatic moieties that contain one or more oxygen, sulfur, nitrogen, phosphorus, or silicon atoms, *e.g.*, in place of carbon atoms. Heteroaliphatic moieties may be branched, unbranched, cyclic or acyclic and include saturated and unsaturated heterocycles such as morpholino, pyrrolidinyl, *etc.* In certain embodiments, heteroaliphatic moieties are substituted by independent replacement of one or more of the hydrogen atoms thereon with one or more moieties including, but not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; arylalkyl; heteroarylalkyl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; -F; -Cl; -Br;

-I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$, wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein any of the aliphatic, heteroaliphatic, arylalkyl, or heteroarylalkyl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments described herein.

The terms “halo” and “halogen” as used herein refer to an atom selected from fluorine, chlorine, bromine, and iodine.

The term “alkyl” includes saturated aliphatic groups, including straight-chain alkyl groups (*e.g.*, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, *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.*, C_1-C_6 for straight chain, C_3-C_6 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 C_1-C_6 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, alkoxycarbonyloxy, aryloxycarbonyloxy, 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. 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 carbon atoms in its backbone (*e.g.*, C_2-C_6 for straight chain, C_3-C_6 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 C_2-C_6 includes alkenyl groups containing 2 to 6 carbon atoms.

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, alkoxycarbonyloxy, aryloxycarbonyloxy, 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.

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 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.*, C_2-C_6 for straight chain, C_3-C_6 for branched chain). The term C_2-C_6 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 groups, alkynyl groups, halogens, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, 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, isopropyloxy, 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, aryloxycarbonyloxy, 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, sulffiydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfmyl, 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, $(CR'R'')_{0-3}NR'R''$, $(CR'R'')_{0-3}CN$, NO_2, halogen, $(CR'R'')_{0-3}C(halogen)_3$, $(CR'R'')_{0-3}CH(halogen)_2$, $(CR'R'')_{0-3}CH_2(halogen)$, $(CR'R'')_{0-3}CONR'R''$, $(CR'R'')_{0-3}S(O)_{1-2}NR'R''$, $(CR'R'')_{0-3}CHO$, $(CR'R'')_{0-3}O(CR'R'')_{0-3}H$, $(CR'R'')_{0-3}S(O)_{0-2}R'$, $(CR'R'')_{0-3}O(CR'R'')_{0-3}H$, $(CR'R'')_{0-3}COR'$, $(CR'R'')_{0-3}CO_2R'$, or $(CR'R'')_{0-3}OR'$ groups; wherein each R′ and R″ are each independently hydrogen, a C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, or aryl group, or R′ and R″ taken together are a benzylidene group or a —$(CH_2)_2O(CH_2)_2$- 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 terms "polynucleotide," "nucleotide sequence," "nucleic acid," "nucleic acid molecule," "nucleic acid sequence," and "oligonucleotide" refer to a polymer of two or more nucleotides. The polynucleotides can be DNA, RNA, or derivatives or modified versions thereof. The polynucleotide may be single-stranded or double-stranded. The polynucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, its hybridization parameters, *etc*. The polynucleotide may comprise a modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-

thiouridine, 5- carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2- dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5- methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5- methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'- methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil- 5-oxyacetic acid methylester, uracil-5-oxyacetic acid, 5-methyl-2- thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. The olynucleotide may compirse a modified sugar moiety (*e.g.*, 2′-fluororibose, ribose, 2′-deoxyribose, 2′-O-methylcytidine, arabinose, and hexose), and/or a modified phosphate moiety (*e.g.*, phosphorothioates and 5′ -N-phosphoramidite linkages). A nucleotide sequence typically carries genetic information, including the information used by cellular machinery to make proteins and enzymes. These terms include double- or single-stranded genomic and cDNA, RNA, any synthetic and genetically manipulated polynucleotide, and both sense and antisense polynucleotides. This includes single- and double-stranded molecules, *i.e.*, DNA-DNA, DNA-RNA, and RNA-RNA hybrids, as well as "protein nucleic acids" (PNA) formed by conjugating bases to an amino acid backbone.

The term "base" includes the known purine and pyrimidine heterocyclic bases, deazapurines, and analogs (including heterocyclic substituted analogs, *e.g.*, aminoethyoxy 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-N^6-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.

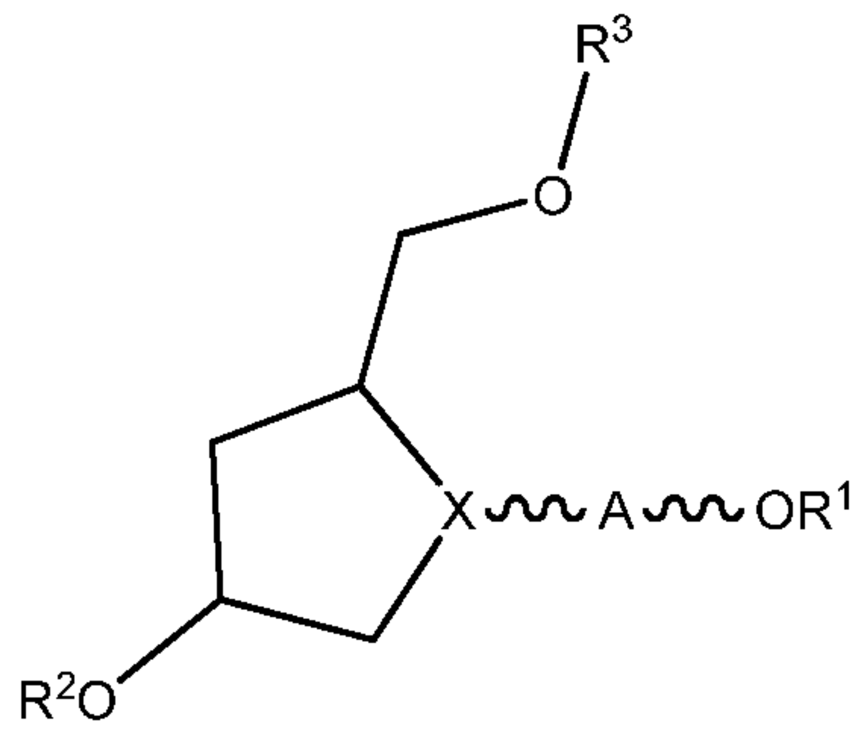
In a preferred embodiment, the nucleomonomers of an oligonucleotide of the invention are RNA nucleotides. In another preferred embodiment, the nucleomonomers of an oligonucleotide of the invention are modified RNA nucleotides. Thus, the oligonucleotides contain modified RNA nucleotides.

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.

The nucleic acid molecules may be associated with a hydrophobic moiety for targeting and/or delivery of the molecule to a cell. In certain embodiments, the hydrophobic moiety is associated with the nucleic acid molecule through a linker. In certain embodiments, the association is through non-covalent interactions. In other embodiments, the association is through a covalent bond. Any linker known in the art may be used to associate the nucleic acid with the hydrophobic moiety. Linkers known in the art are described in published international PCT applications, WO 92/03464, WO 95/23162, WO 2008/021157, WO 2009/021157, WO 2009/134487, WO 2009/126933, U.S. Patent Application Publication 2005/0107325, U.S. Patent 5,414,077, U.S. Patent 5,419,966, U.S. Patent 5,512,667, U.S. Patent 5,646,126, and U.S. Patent 5,652,359, which are incorporated herein by reference. The linker may be as simple as a covalent bond to a multi-atom linker. The linker may be cyclic or acyclic. The linker may be optionally substituted. In certain embodiments, the linker is capable of being cleaved from the nucleic acid. In certain embodiments, the linker is capable of being hydrolyzed under physiological conditions. In certain embodiments, the linker is capable of being cleaved by an enzyme (*e.g.*, an esterase or phosphodiesterase). In certain embodiments, the linker comprises a spacer element to separate the nucleic acid from the hydrophobic moiety. The spacer element may include one to thirty carbon or heteroatoms. In certain embodiments, the linker and/or spacer element comprises protonatable functional groups. Such protonatable functional groups may promote the endosomal escape of the nucleic acid molecule. The protonatable functional groups may also aid in the delivery of the nucleic acid to a cell, for example, neutralizing the overall charge of the molecule. In other embodiments, the linker and/or spacer element is biologically inert (that is, it does not impart biological activity or function to the resulting nucleic acid molecule).

In certain embodiments, the nucleic acid molecule with a linker and hydrophobic moiety is of the formulae described herein. In certain embodiments, the nucleic acid molecule is of the formula:



wherein

X is N or CH;

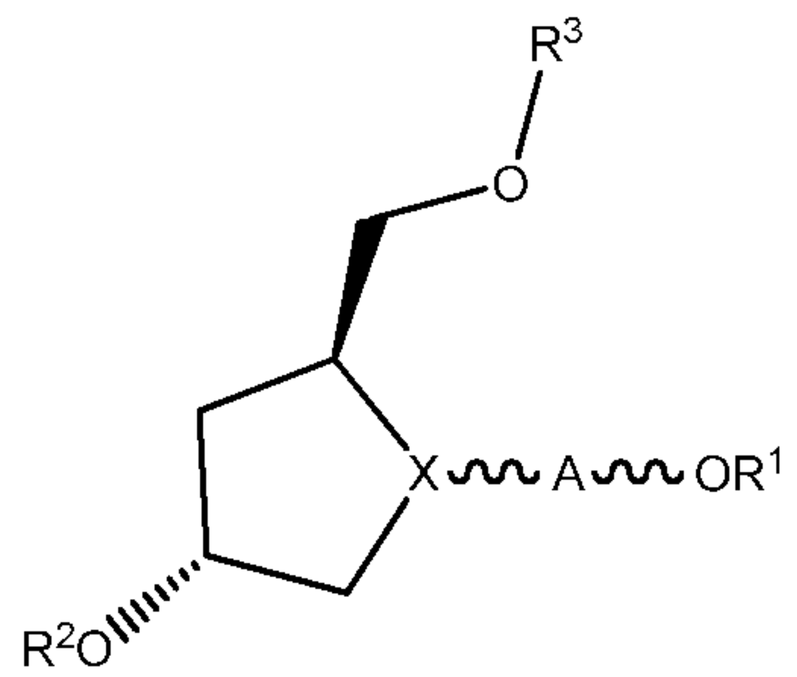
A is a bond; substituted or unsubstituted, cyclic or acyclic, branched or unbranched aliphatic; or substituted or unsubstituted, cyclic or acyclic, branched or unbranched heteroaliphatic;

R^1 is a hydrophobic moiety;

R^2 is hydrogen; an oxygen-protecting group; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; and

R^3 is a nucleic acid.

In certain embodiments, the molecule is of the formula:



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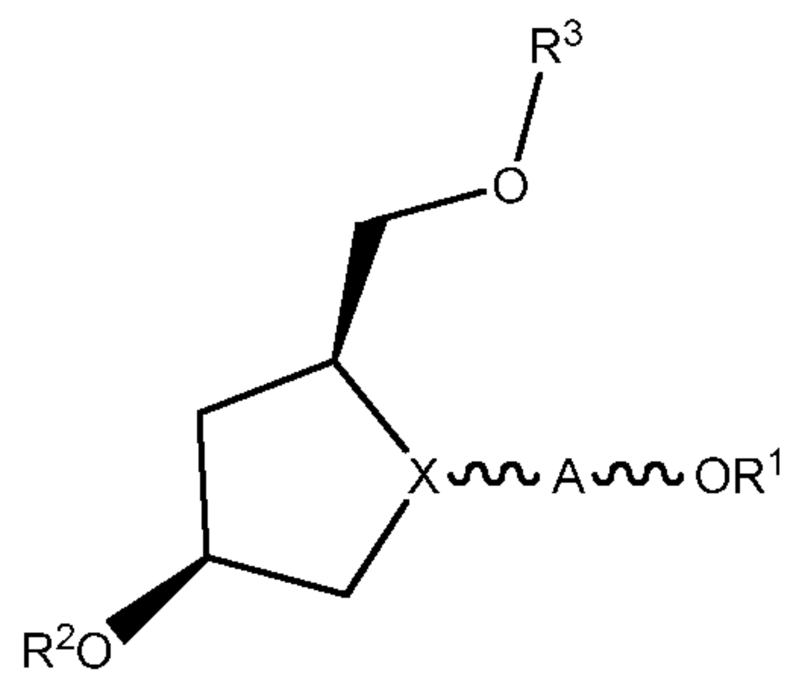
In certain embodiments, the molecule is of the formula:



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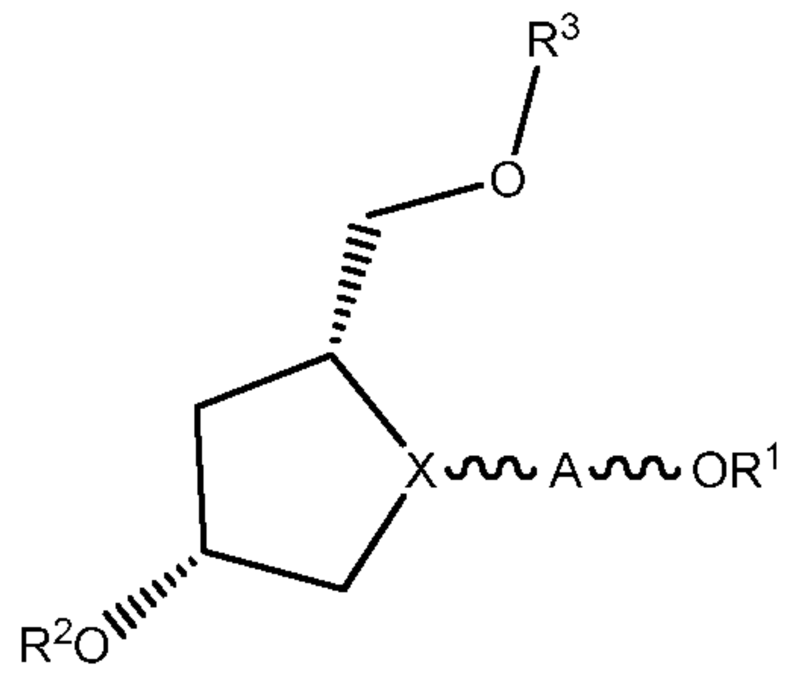
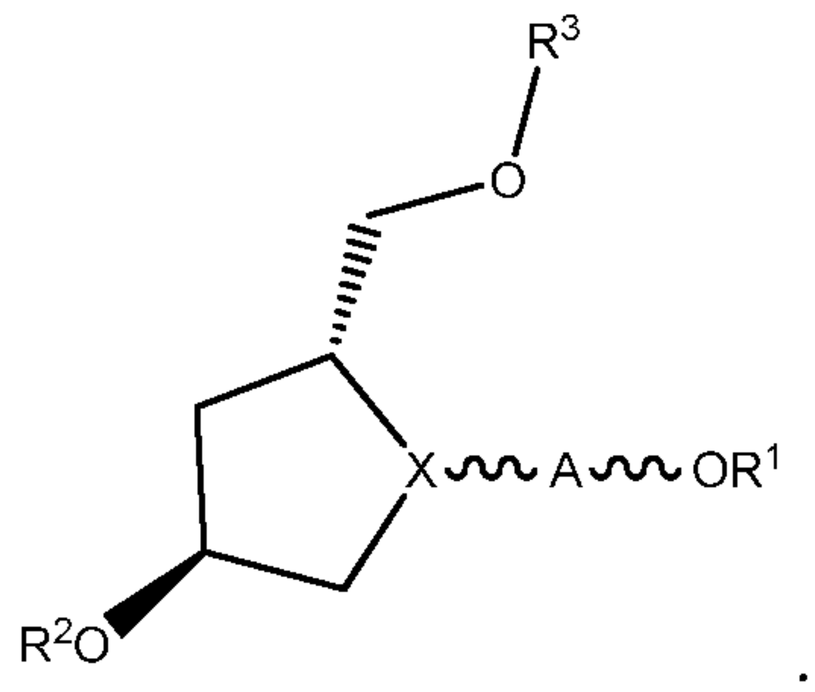
In certain embodiments, the molecule is of the formula:



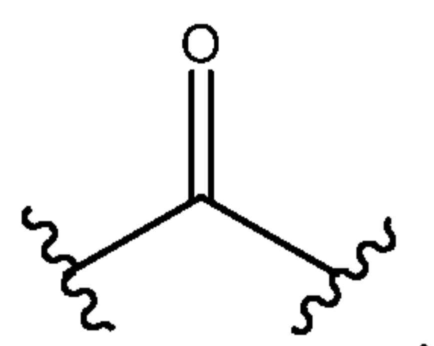
In certain embodiments, the molecule is of the formula:



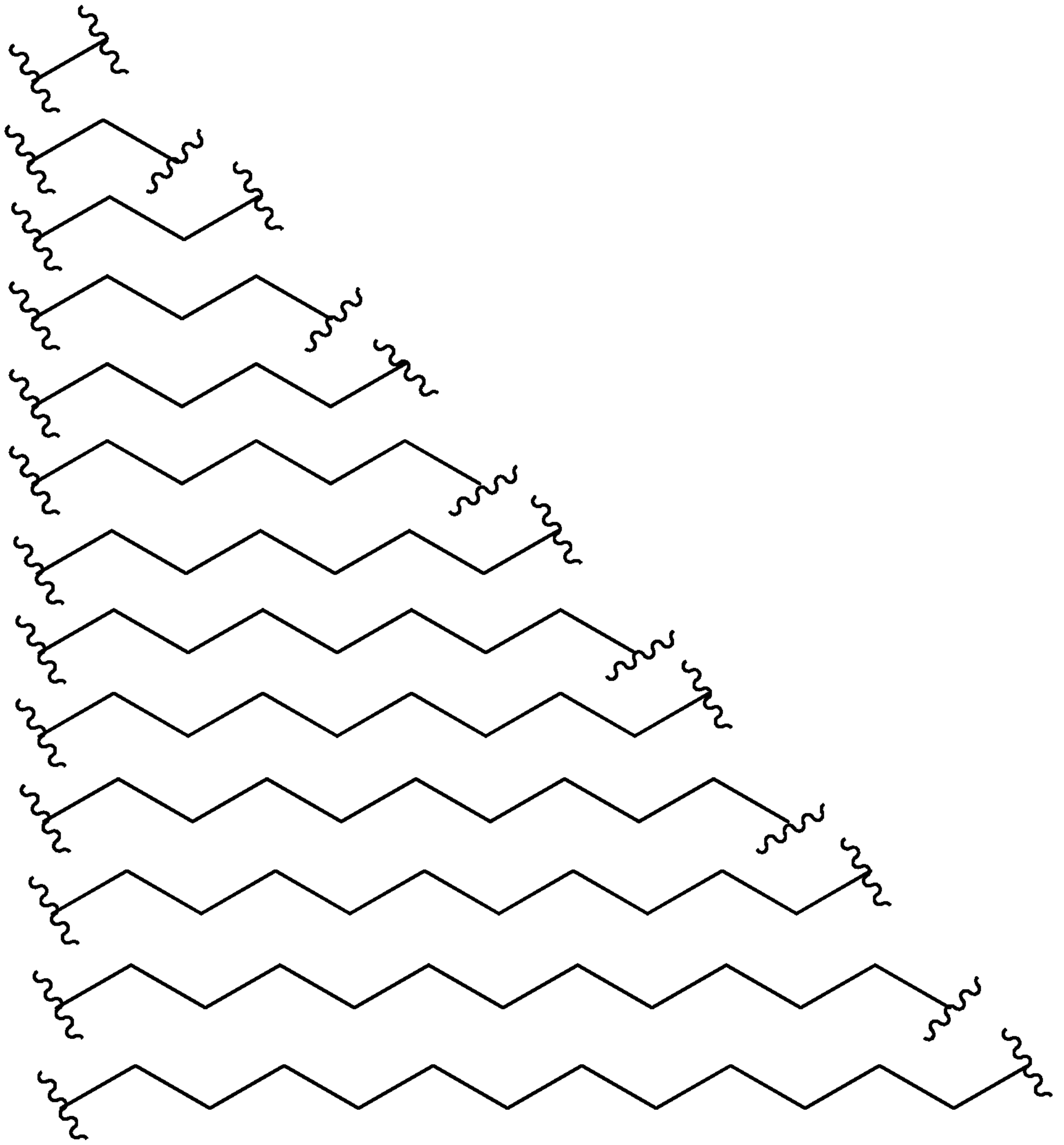
In certain embodiments, X is N. In certain embodiments, X is CH.

In certain embodiments, A is a bond. In certain embodiments, A is substituted or unsubstituted, cyclic or acyclic, branched or unbranched aliphatic. In certain embodiments, A is acyclic, substituted or unsubstituted, branched or unbranched aliphatic. In certain embodiments, A is acyclic, substituted, branched or unbranched aliphatic. In certain embodiments, A is acyclic, substituted, unbranched aliphatic. In certain embodiments, A is acyclic, substituted, unbranched alkyl. In certain embodiments, A is acyclic, substituted, unbranched C_{1-20} alkyl. In certain embodiments, A is acyclic, substituted, unbranched C_{1-12} alkyl. In certain embodiments, A is acyclic, substituted, unbranched C_{1-10} alkyl. In certain embodiments, A is acyclic, substituted, unbranched C_{1-8} alkyl. In certain embodiments, A is acyclic, substituted, unbranched C_{1-6} alkyl. In certain embodiments, A is substituted or unsubstituted, cyclic or acyclic, branched or unbranched heteroaliphatic. In certain embodiments, A is acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic. In certain embodiments, A is acyclic, substituted, branched or unbranched heteroaliphatic. In certain embodiments, A is acyclic, substituted, unbranched heteroaliphatic.

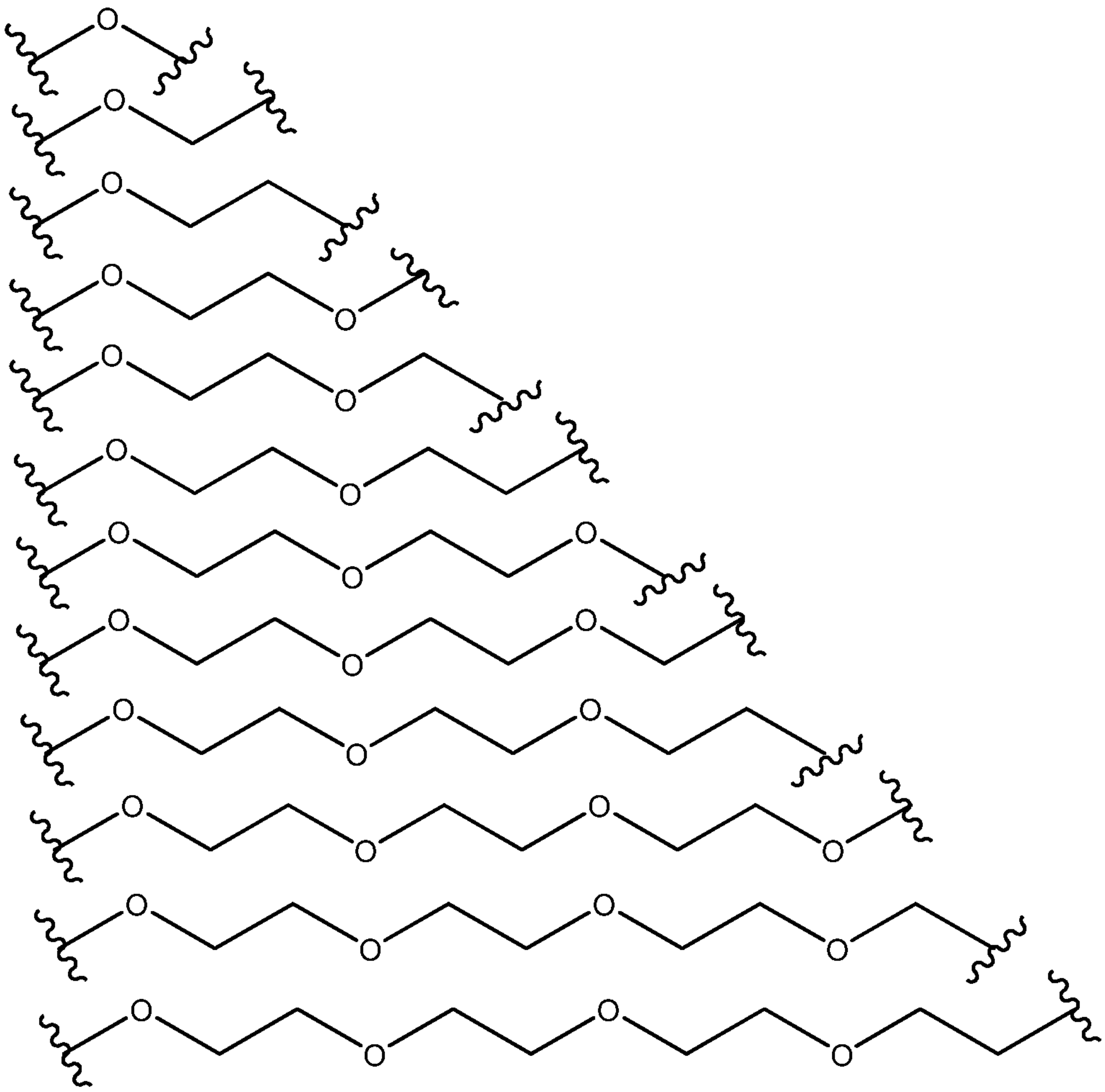
In certain embodiments, A is of the formula:



In certain embodiments, A is of one of the formulae:



In certain embodiments, A is of one of the formulae:



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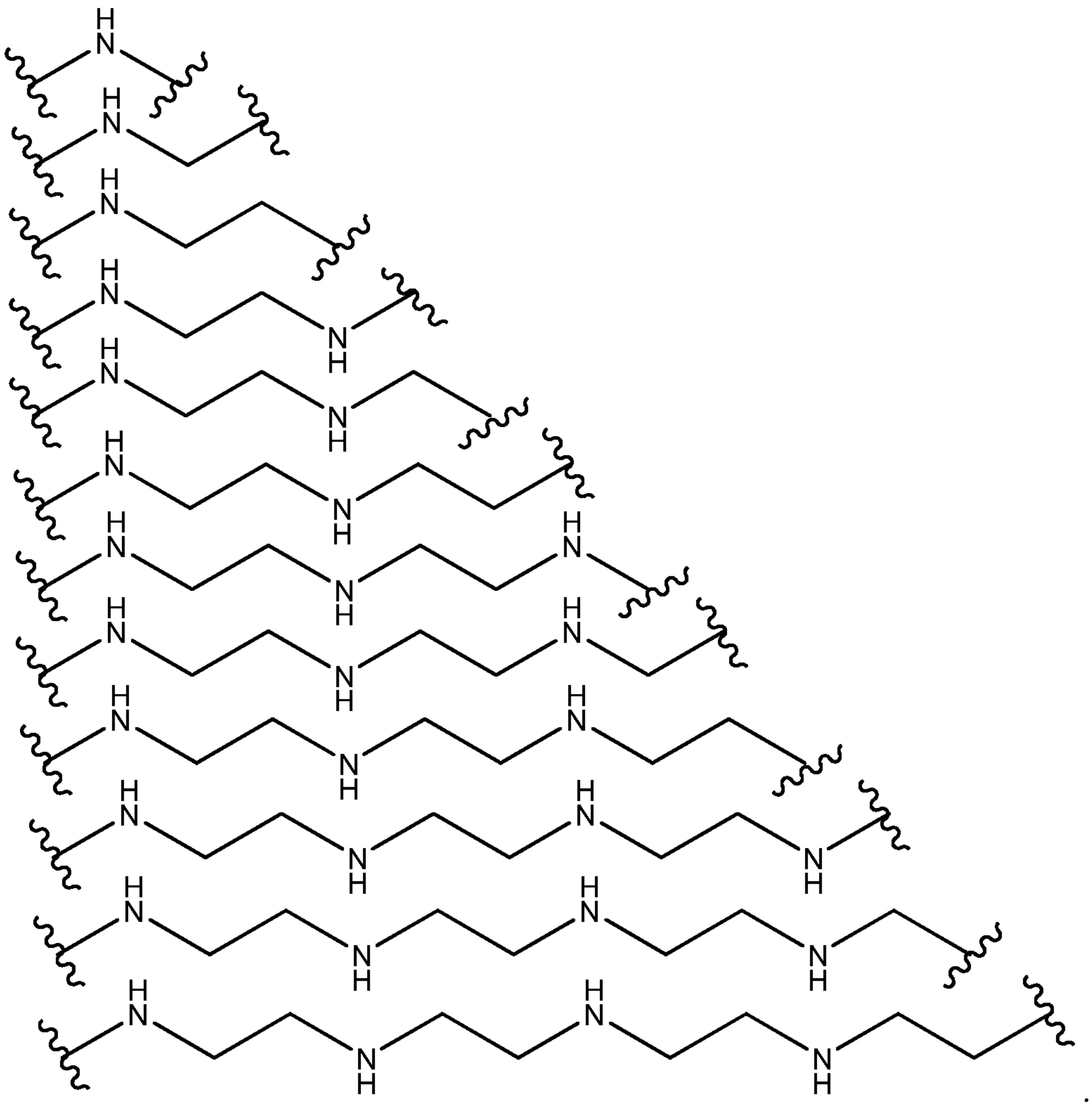
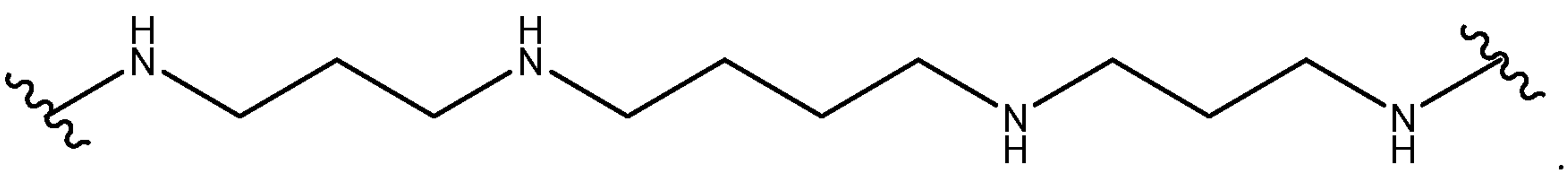
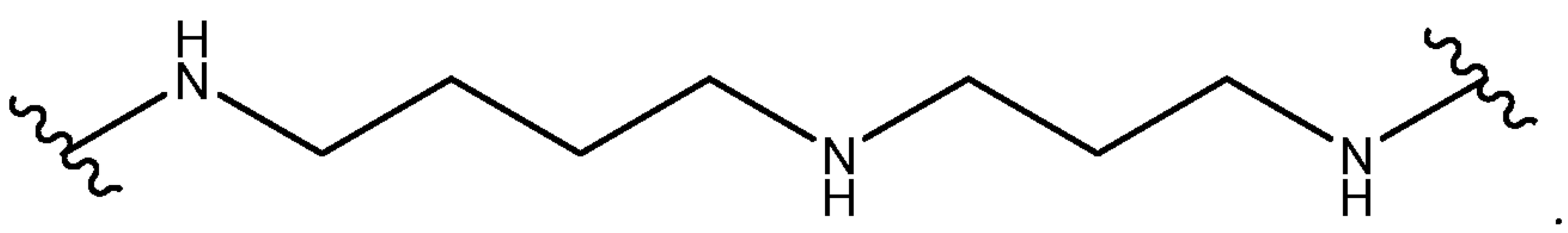
In certain embodiments, A is of one of the formulae:



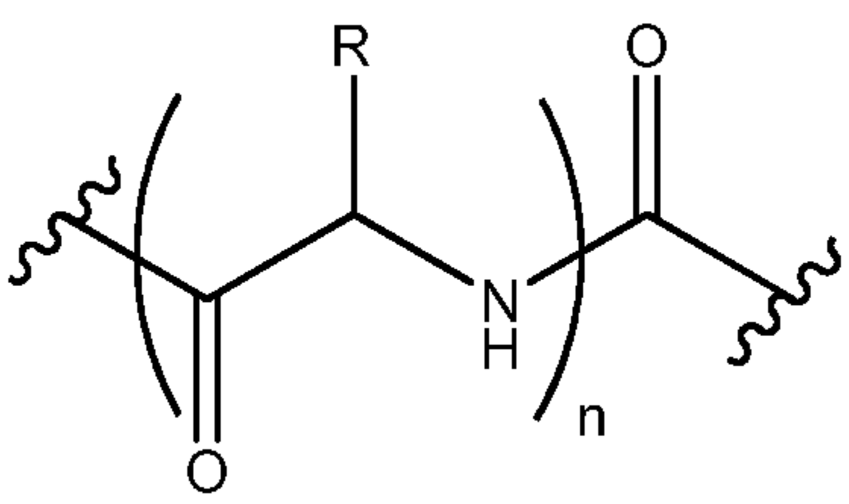
In certain embodiments, A is of the formula:



In certain embodiments, A is of the formula:



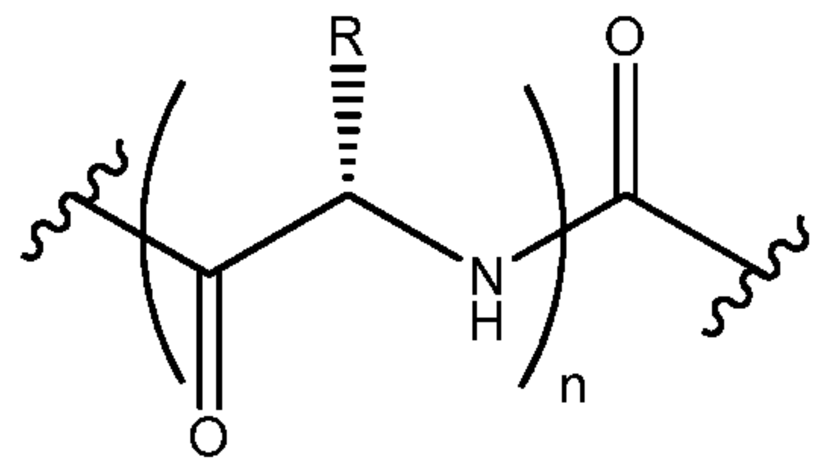
In certain embodiments, A is of the formula:



wherein

each occurrence of R is independently the side chain of a natural or unnatural amino acid; and

n is an integer between 1 and 20, inclusive. In certain embodiments, A is of the formula:



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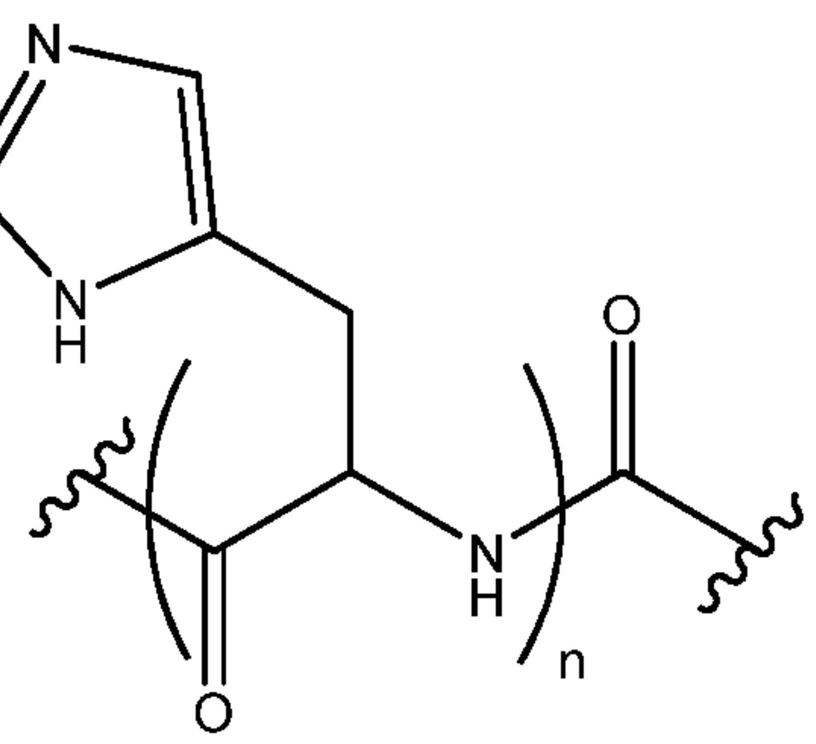
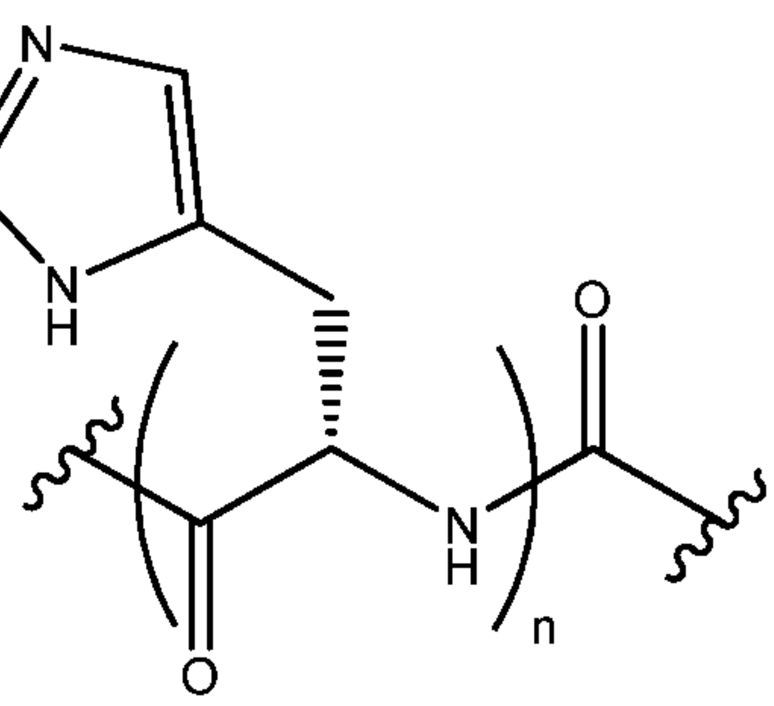
In certain embodiments, each occurrence of R is independently the side chain of a natural amino acid. In certain embodiments, n is an integer between 1 and 15, inclusive. In certain embodiments, n is an integer between 1 and 10, inclusive. In certain embodiments, n is an integer between 1 and 5, inclusive.

In certain embodiments, A is of the formula:



wherein n is an integer between 1 and 20, inclusive. In certain embodiments, A is of the formula:



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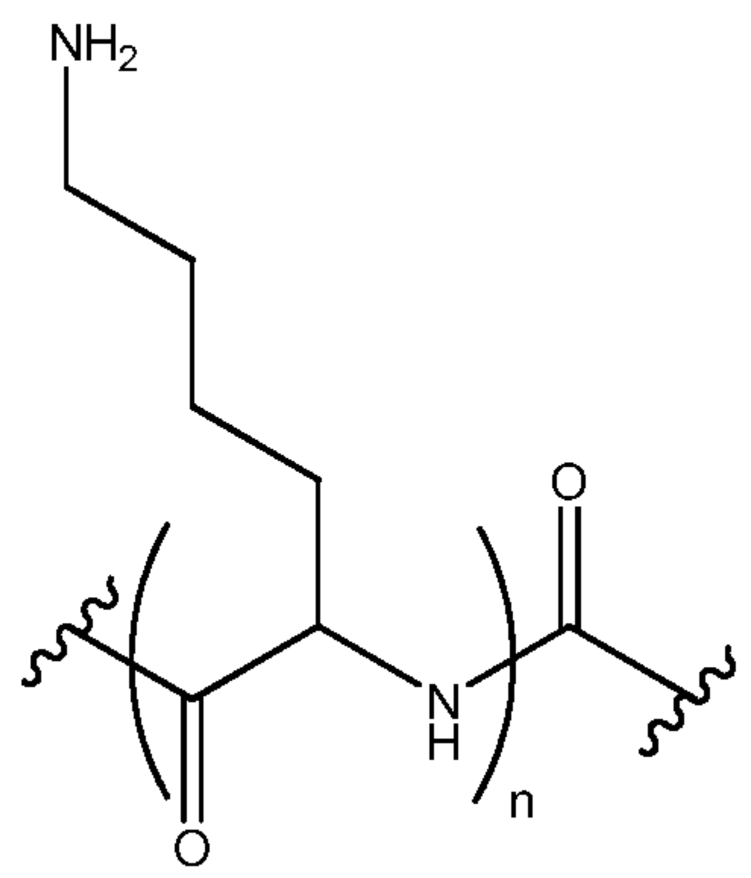
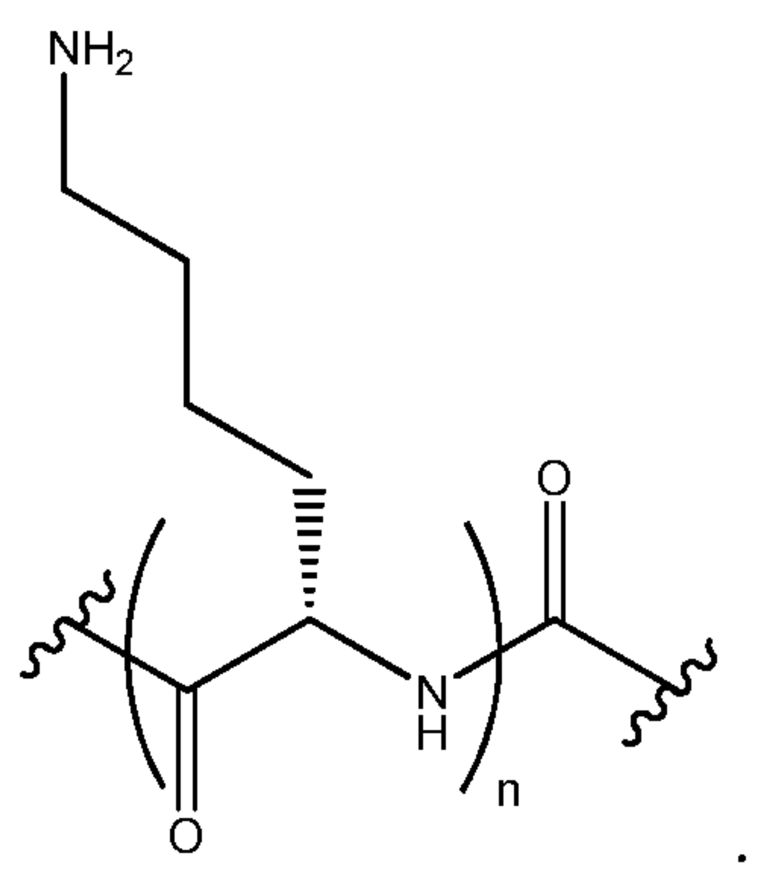
In certain embodiments, n is an integer between 1 and 15, inclusive. In certain embodiments, n is an integer between 1 and 10, inclusive. In certain embodiments, n is an integer between 1 and 5, inclusive.

In certain embodiments, A is of the formula:

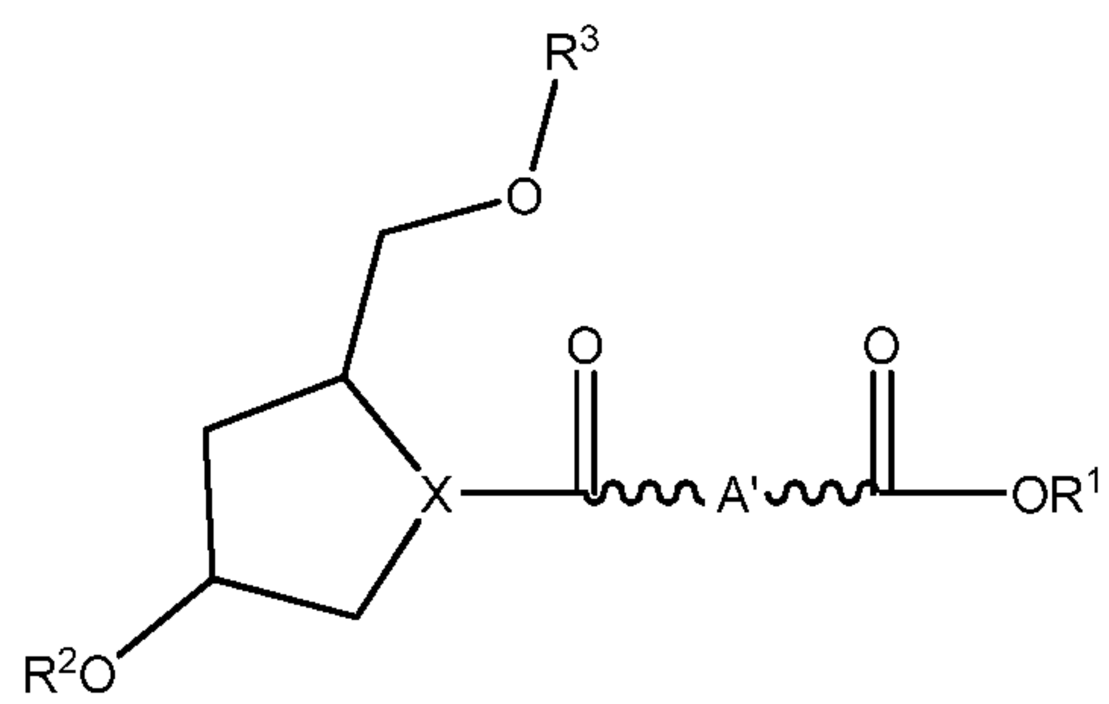


wherein n is an integer between 1 and 20, inclusive. In certain embodiments, A is of the formula:



In certain embodiments, n is an integer between 1 and 15, inclusive. In certain embodiments, n is an integer between 1 and 10, inclusive. In certain embodiments, n is an integer between 1 and 5, inclusive.

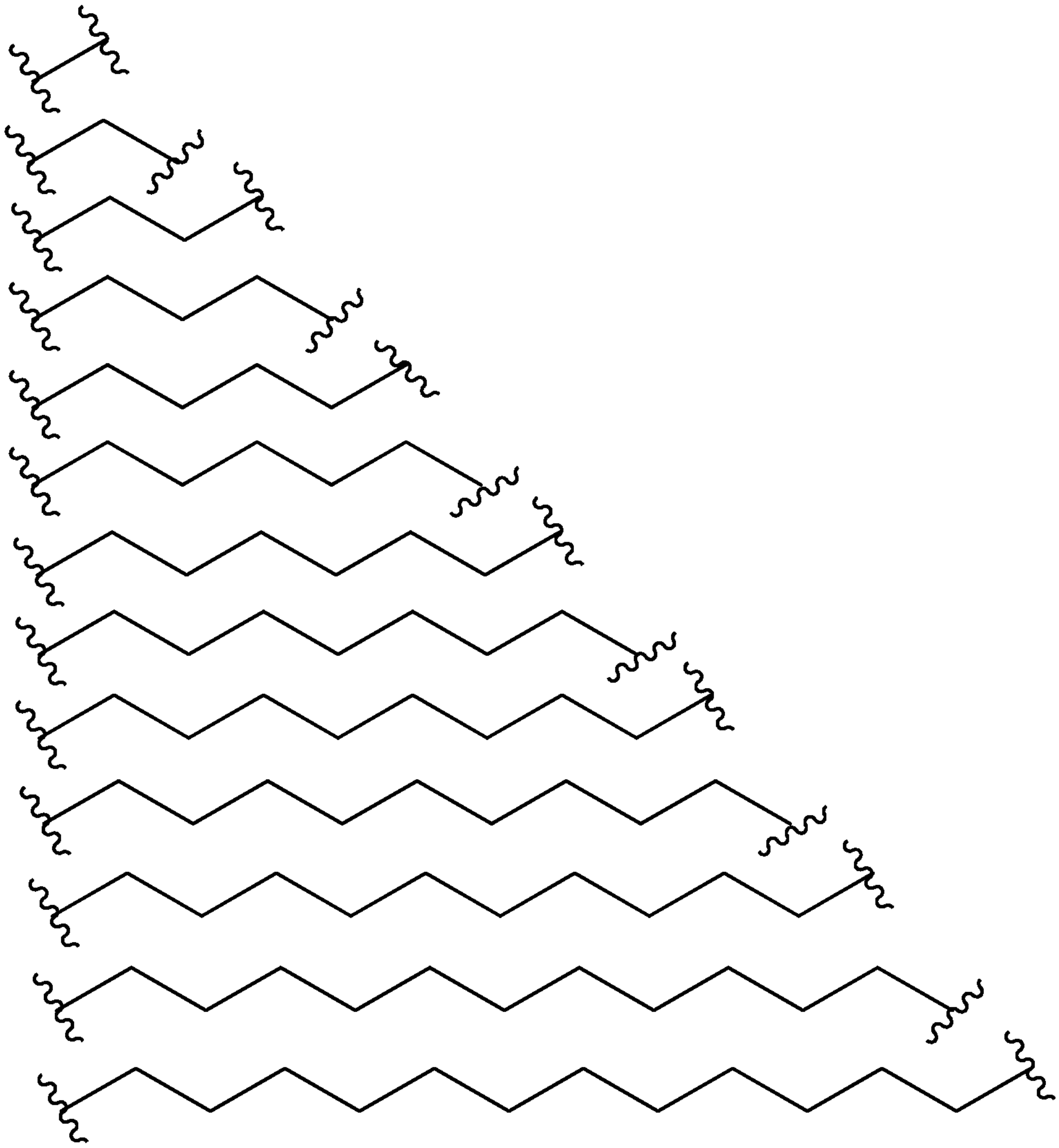
In certain embodiments, the molecule is of the formula:



wherein X, R^1, R^2, and R^3 are as defined herein; and

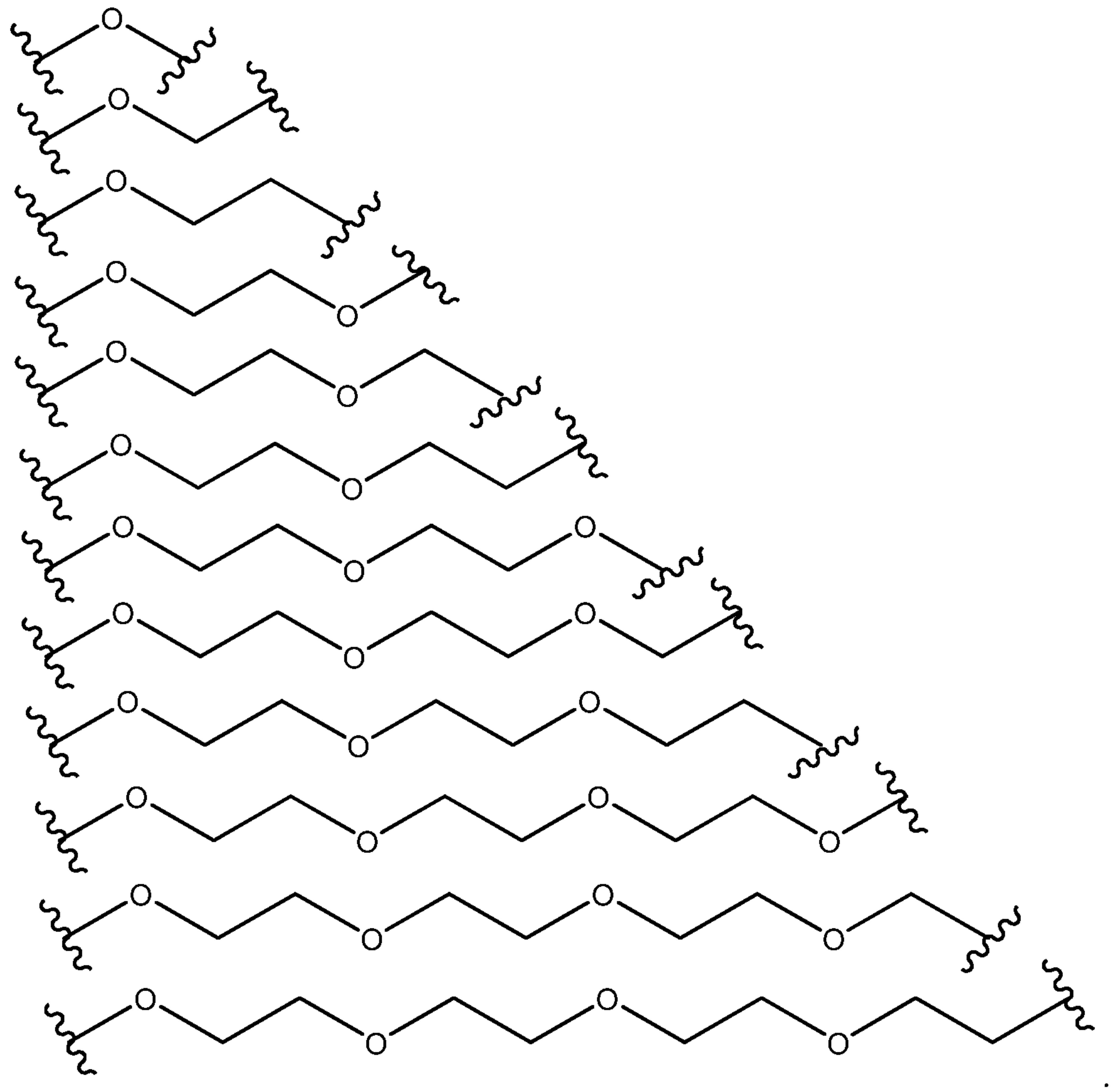
A′ is substituted or unsubstituted, cyclic or acyclic, branched or unbranched aliphatic; or substituted or unsubstituted, cyclic or acyclic, branched or unbranched heteroaliphatic.

In certain embodiments, A′ is of one of the formulae:

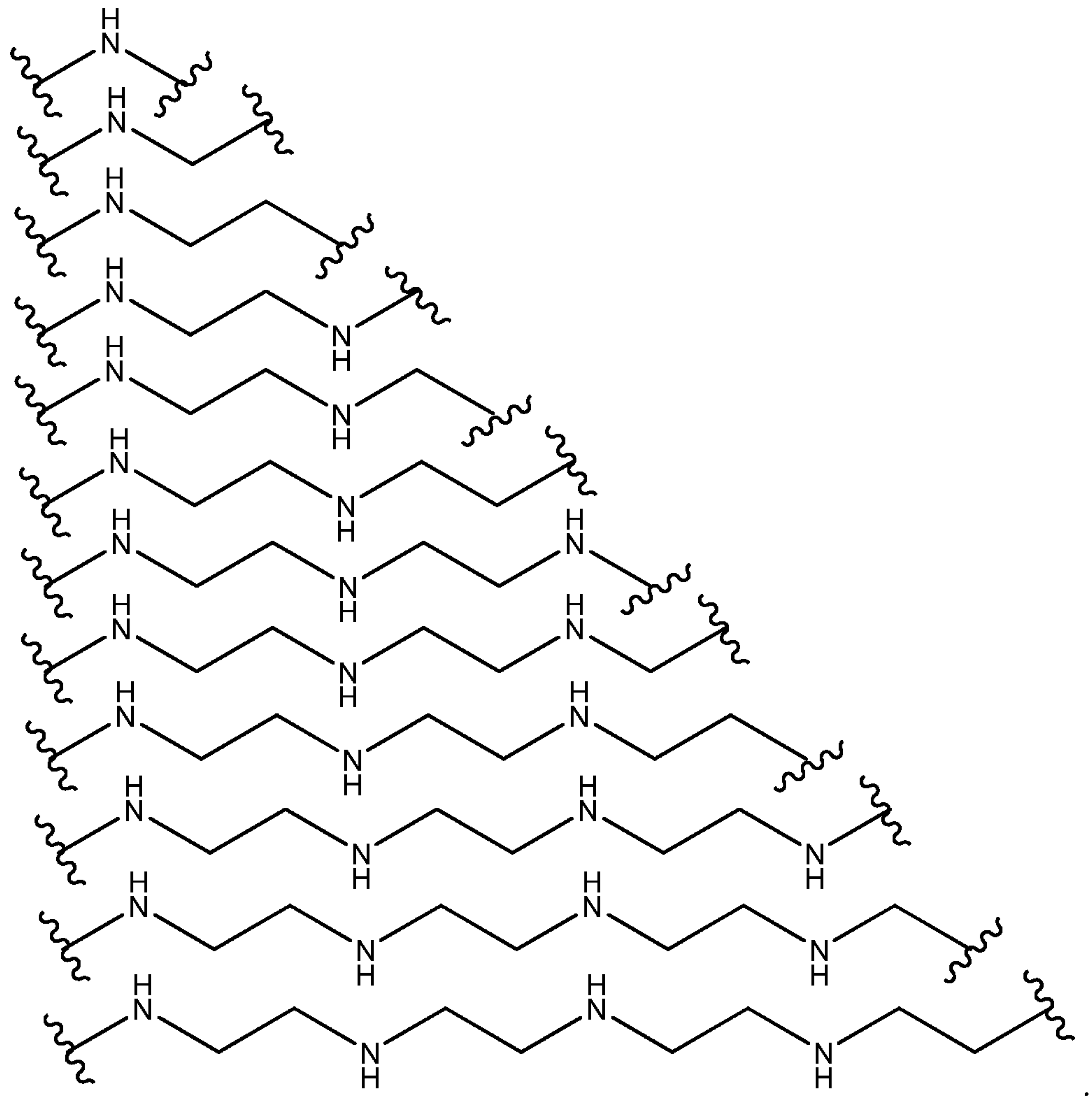


.

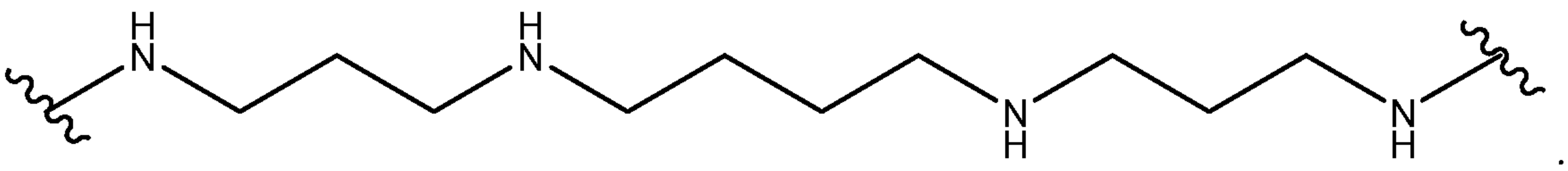
In certain embodiments, A is of one of the formulae:



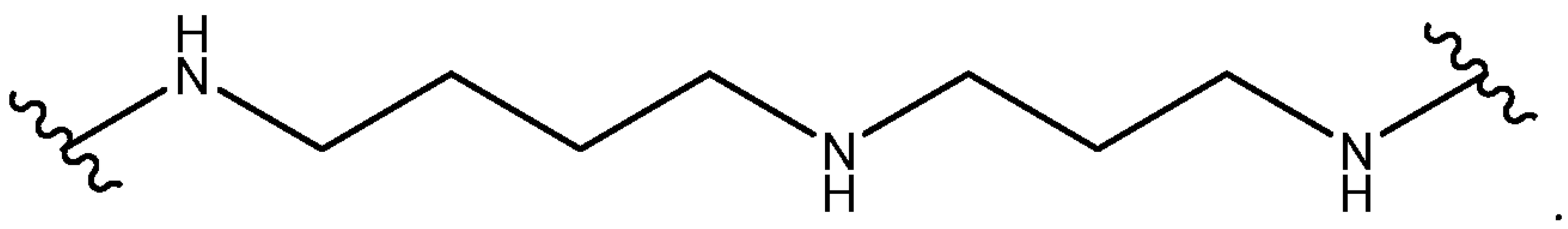
In certain embodiments, A is of one of the formulae:



In certain embodiments, A is of the formula:

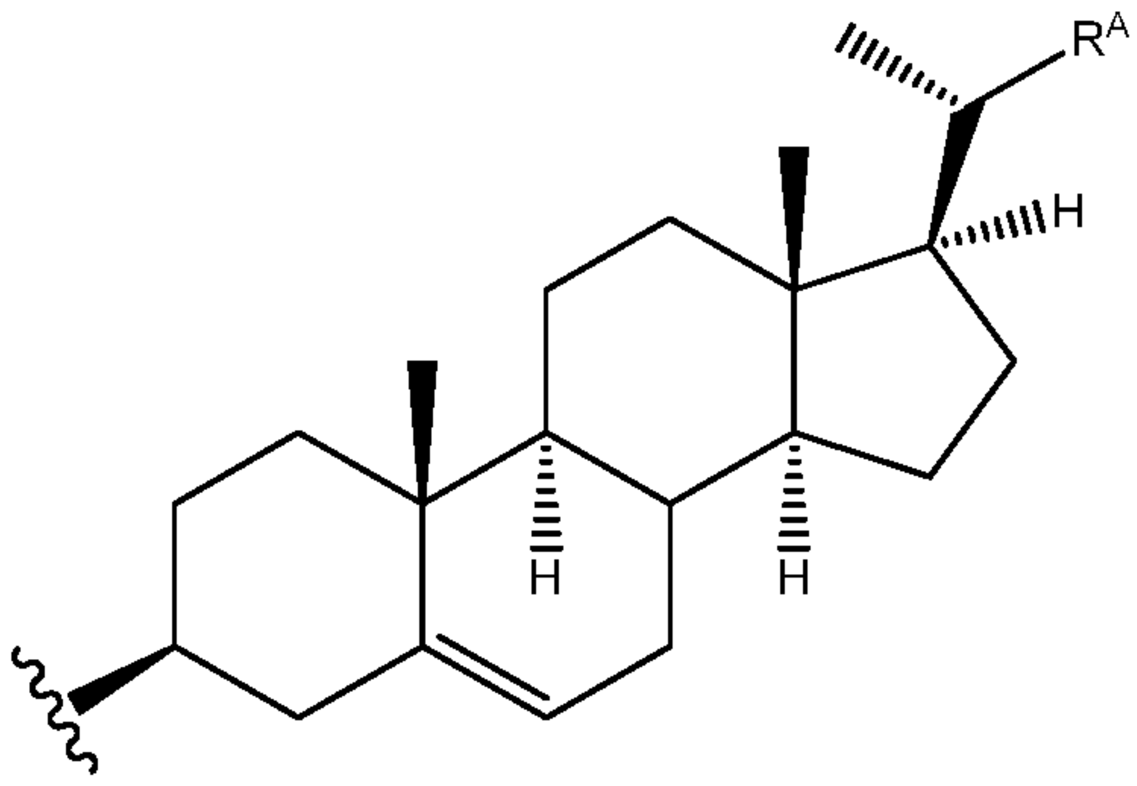


In certain embodiments, A is of the formula:



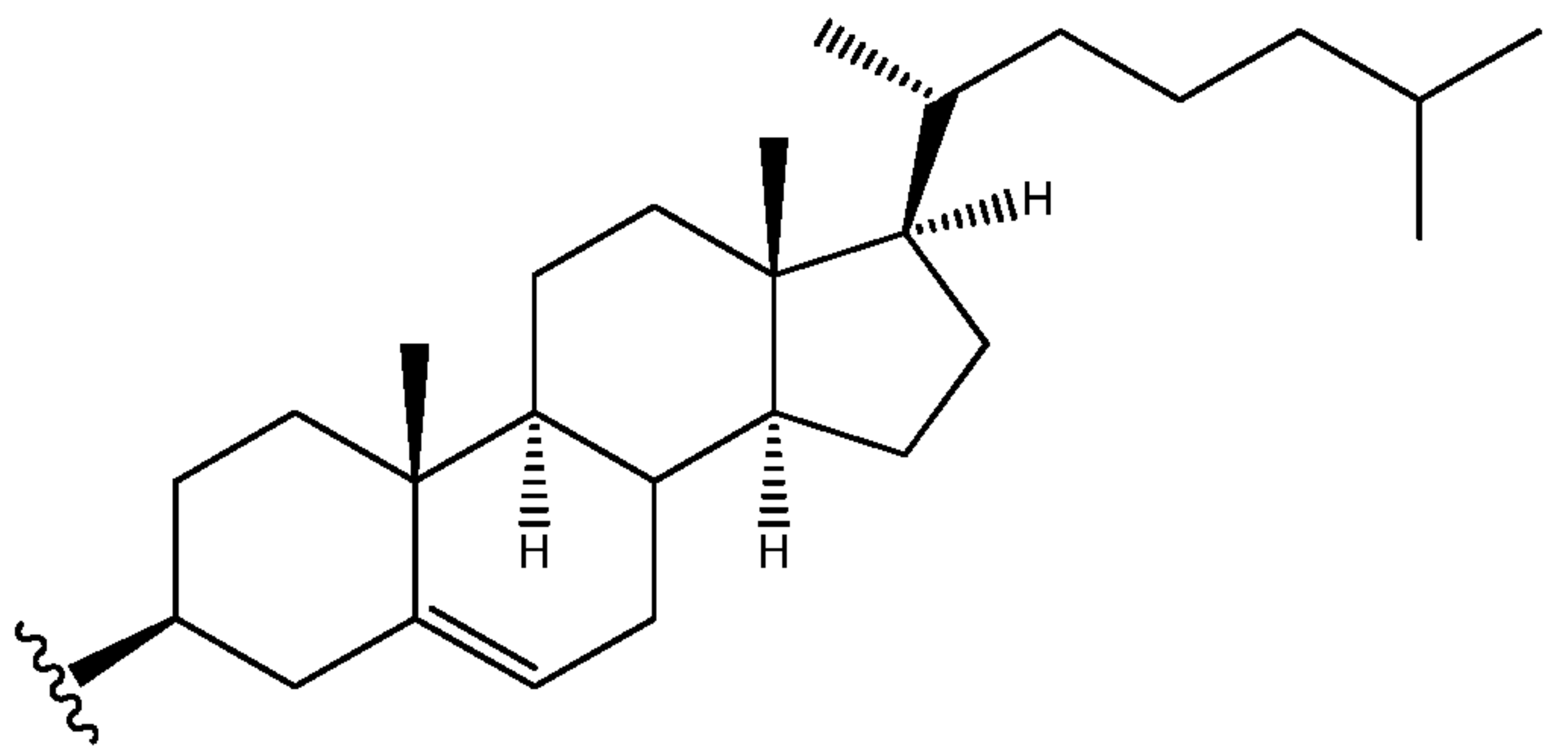
In certain embodiments, R^1 is a steroid. In certain embodiments, R^1 is a cholesterol. In certain embodiments, R^1 is a lipophilic vitamin. In certain embodiments, R^1 is a vitamin A. In certain embodiments, R^1 is a vitamin E.

In certain embodiments, R^1 is of the formula:



wherein R^A is substituted or unsubstituted, cyclic or acyclic, branched or unbranched aliphatic; or substituted or unsubstituted, cyclic or acyclic, branched or unbranched heteroaliphatic.

In certain embodiments, R^1 is of the formula:



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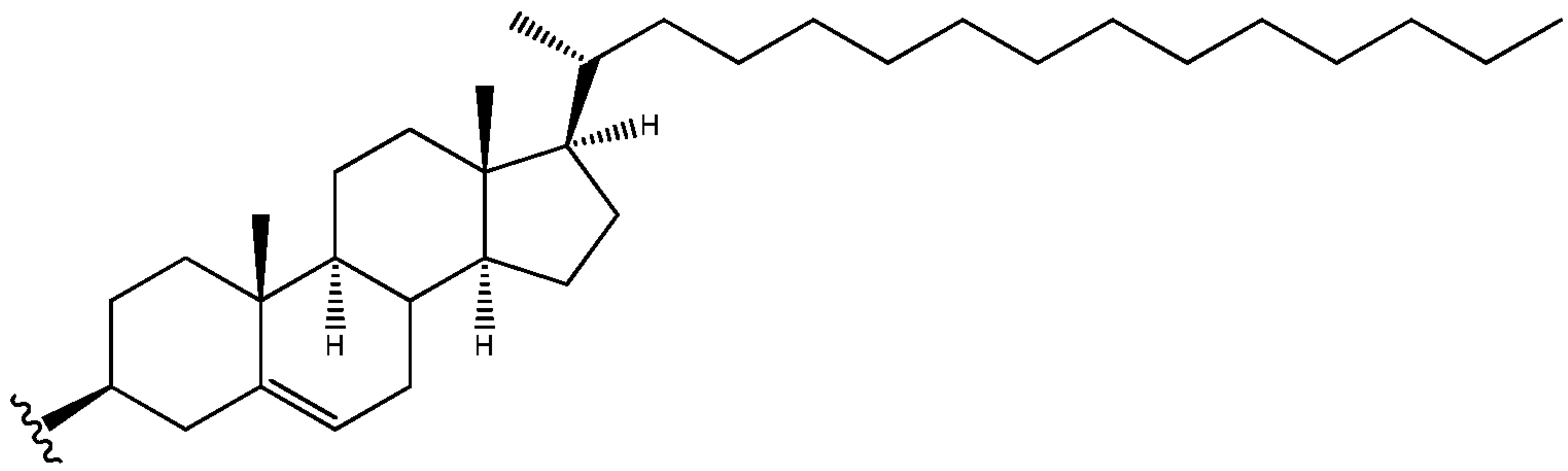
In certain embodiments, R^1 is of the formula:



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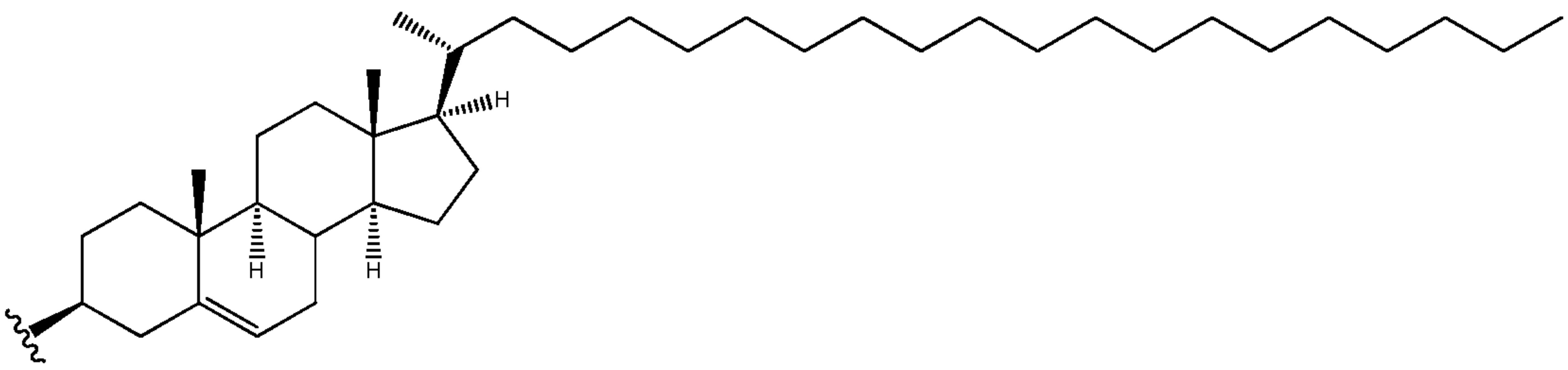
In certain embodiments, R^1 is of the formula:



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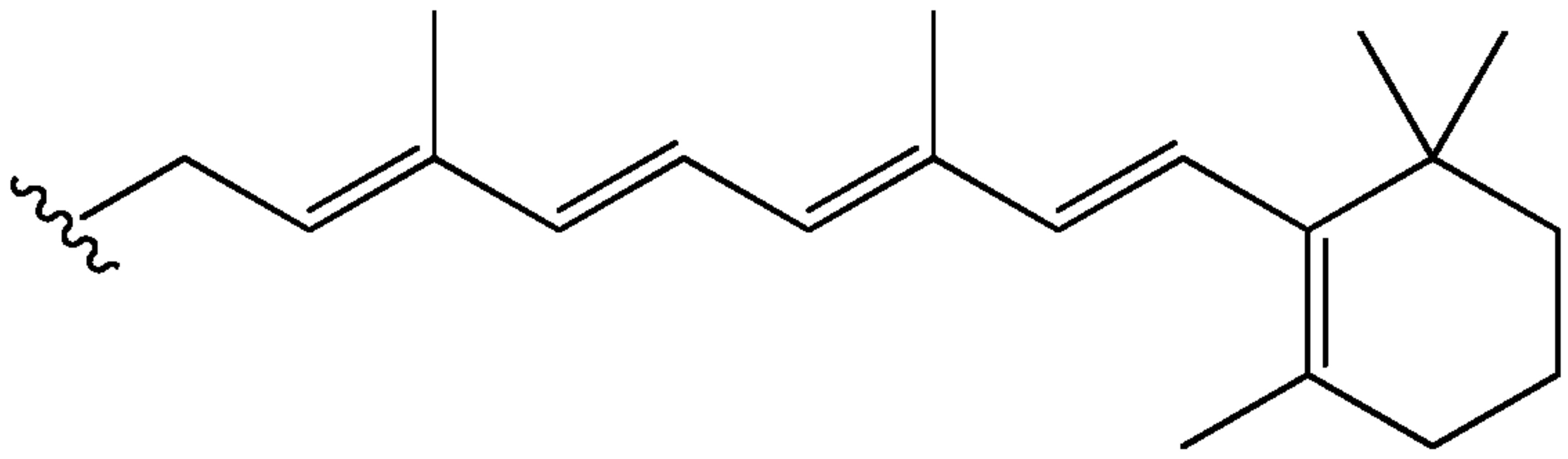
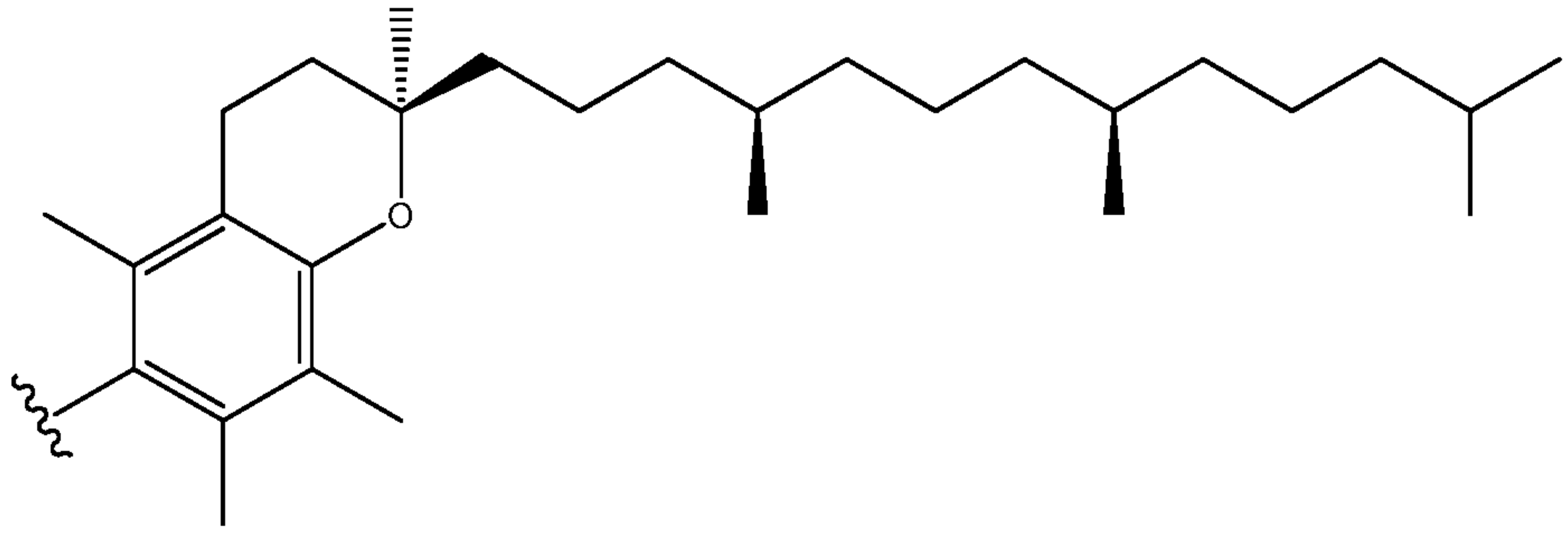
In certain embodiments, R^1 is of the formula:



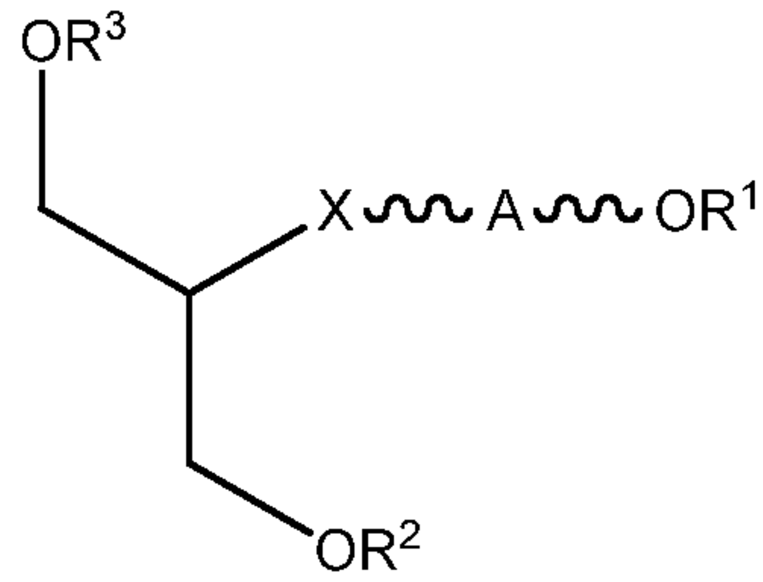
.

In certain embodiments, R^1 is of the formula:



.

In certain embodiments, the nucleic acid molecule is of the formula:



wherein

X is N or CH;

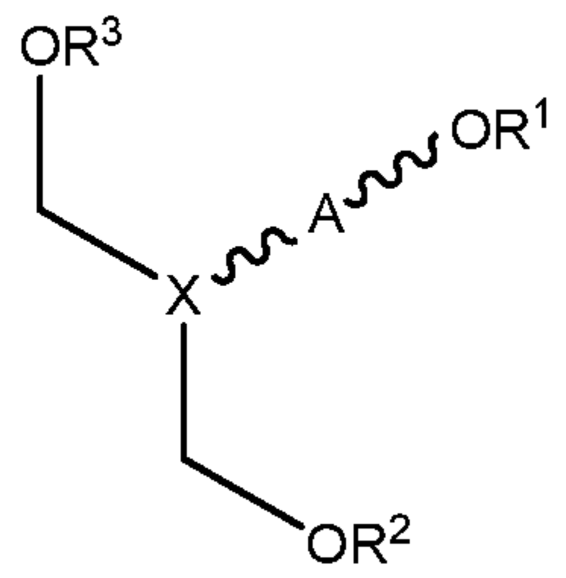
A is a bond; substituted or unsubstituted, cyclic or acyclic, branched or unbranched aliphatic; or substituted or unsubstituted, cyclic or acyclic, branched or unbranched heteroaliphatic;

R^1 is a hydrophobic moiety;

R^2 is hydrogen; an oxygen-protecting group; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; and

R^3 is a nucleic acid.

In certain embodiments, the nucleic acid molecule is of the formula:



wherein

X is N or CH;

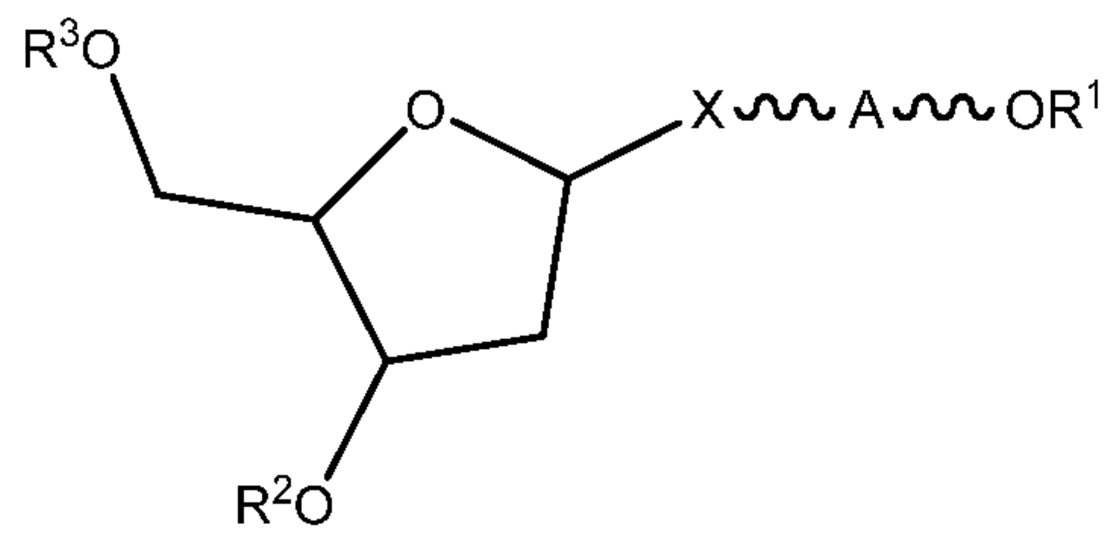
A is a bond; substituted or unsubstituted, cyclic or acyclic, branched or unbranched aliphatic; or substituted or unsubstituted, cyclic or acyclic, branched or unbranched heteroaliphatic;

R^1 is a hydrophobic moiety;

R^2 is hydrogen; an oxygen-protecting group; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; and

R^3 is a nucleic acid.

In certain embodiments, the nucleic acid molecule is of the formula:



wherein

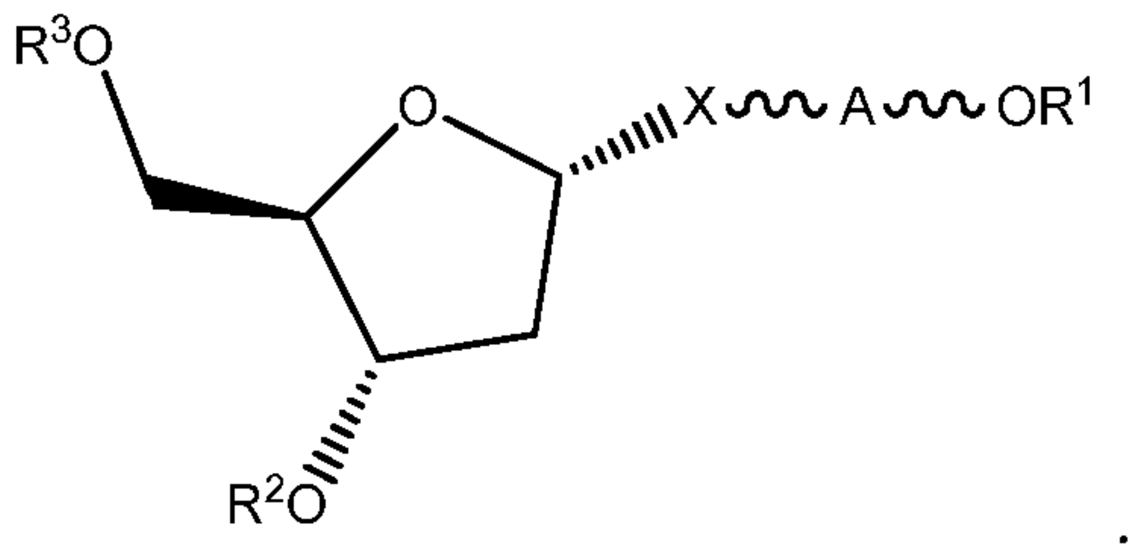
X is N or CH;

A is a bond; substituted or unsubstituted, cyclic or acyclic, branched or unbranched aliphatic; or substituted or unsubstituted, cyclic or acyclic, branched or unbranched heteroaliphatic;

R^1 is a hydrophobic moiety;

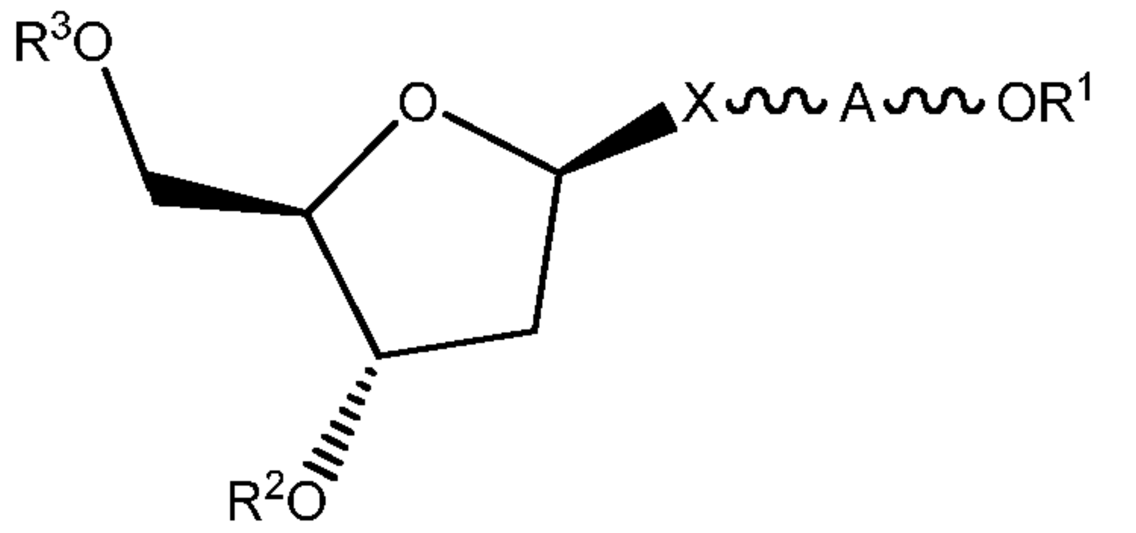
R^2 is hydrogen; an oxygen-protecting group; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; and

R^3 is a nucleic acid. In certain embodiments, the nucleic acid molecule is of the formula:



.

In certain embodiments, the nucleic acid molecule is of the formula:



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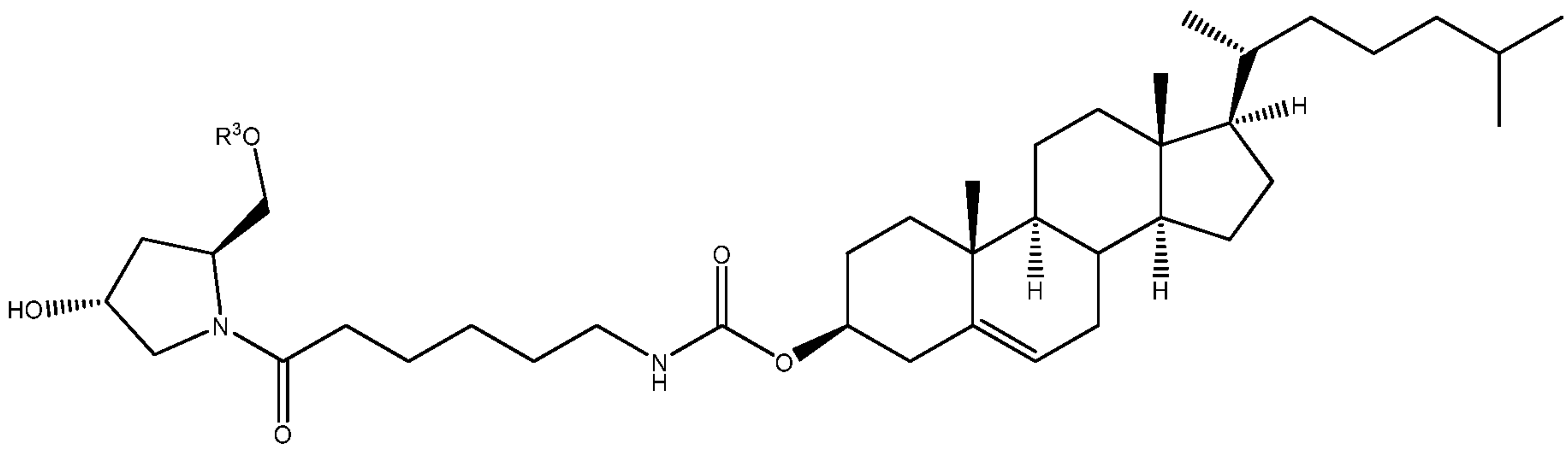
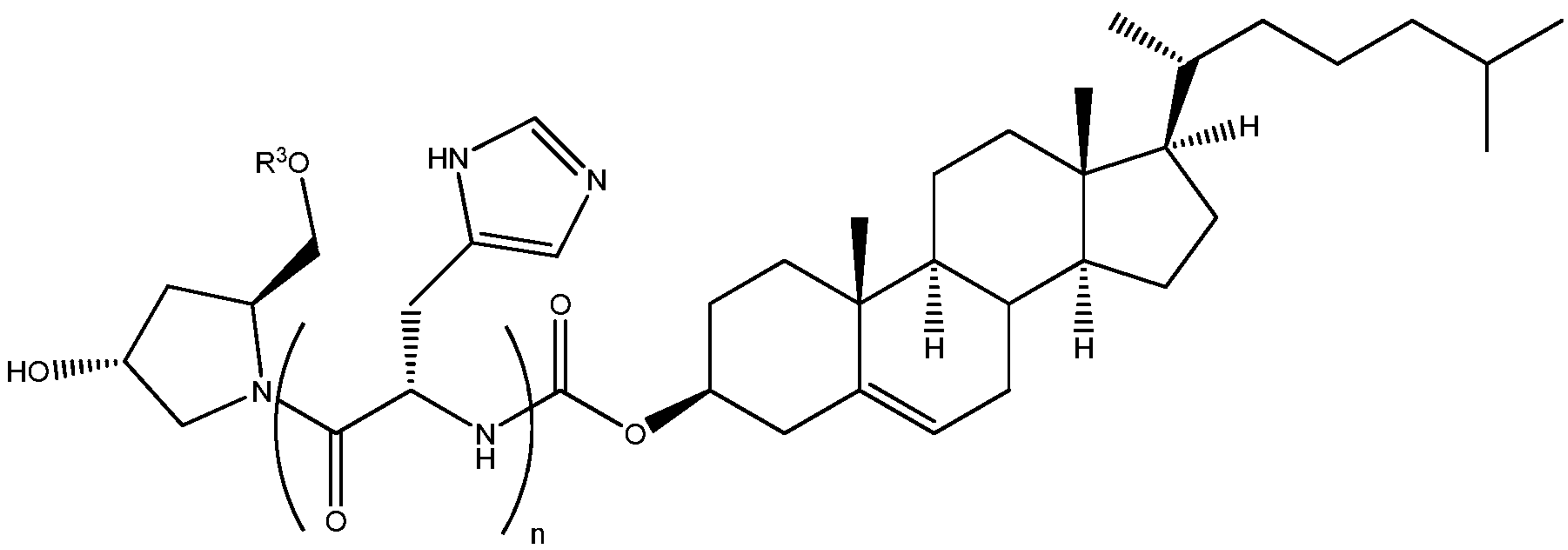
In certain embodiments, the nucleic acid molecule is of the formula:



wherein R^3 is a nucleic acid.

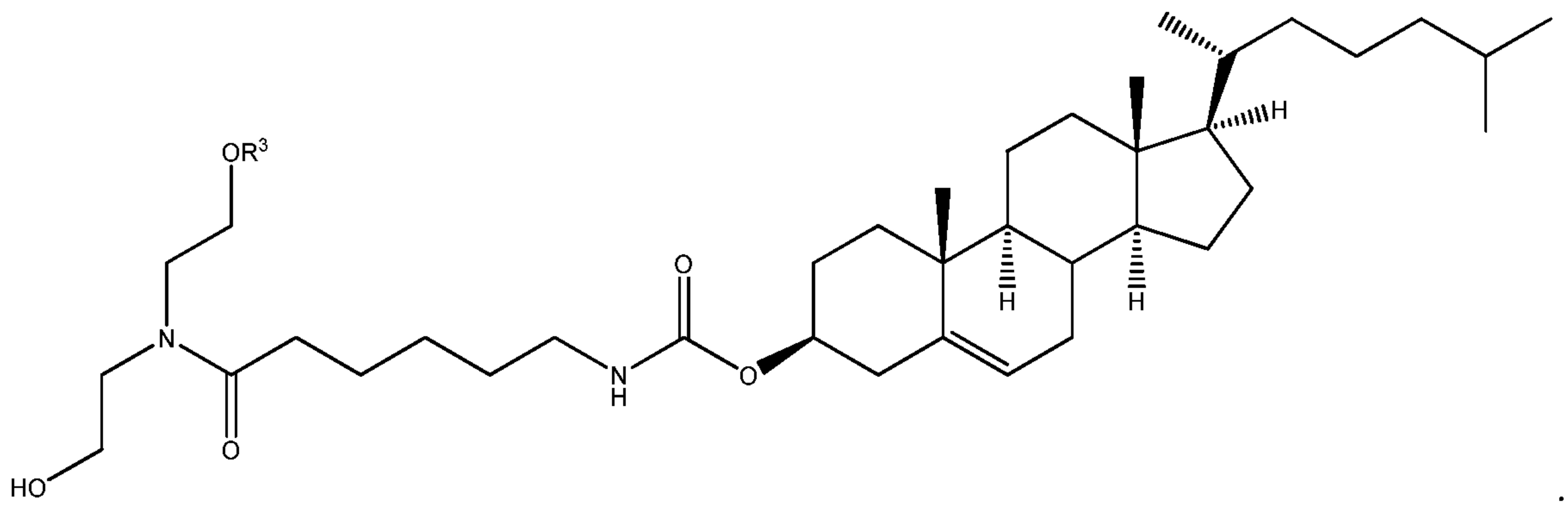
In certain embodiments, the nucleic acid molecule is of the formula:



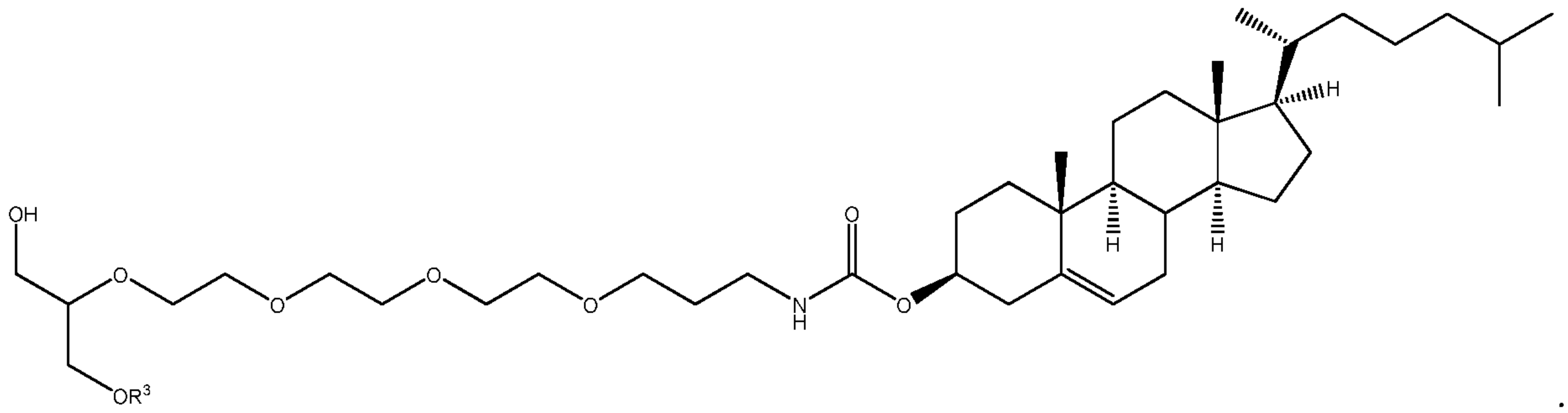
wherein R^3 is a nucleic acid; and

n is an integer between 1 and 20, inclusive.

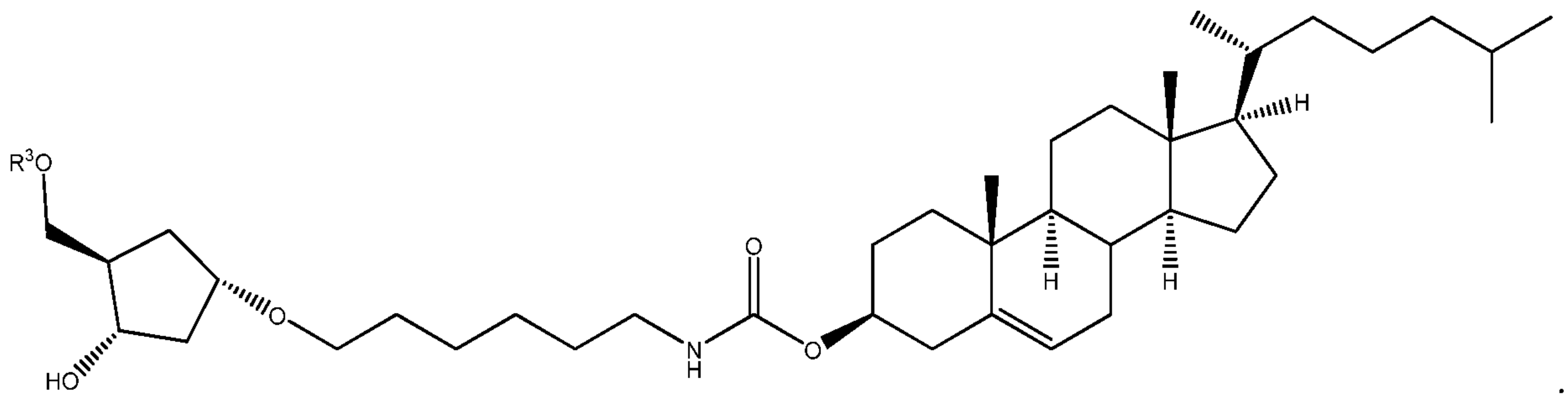
In certain embodiments, the nucleic acid molecule is of the formula:



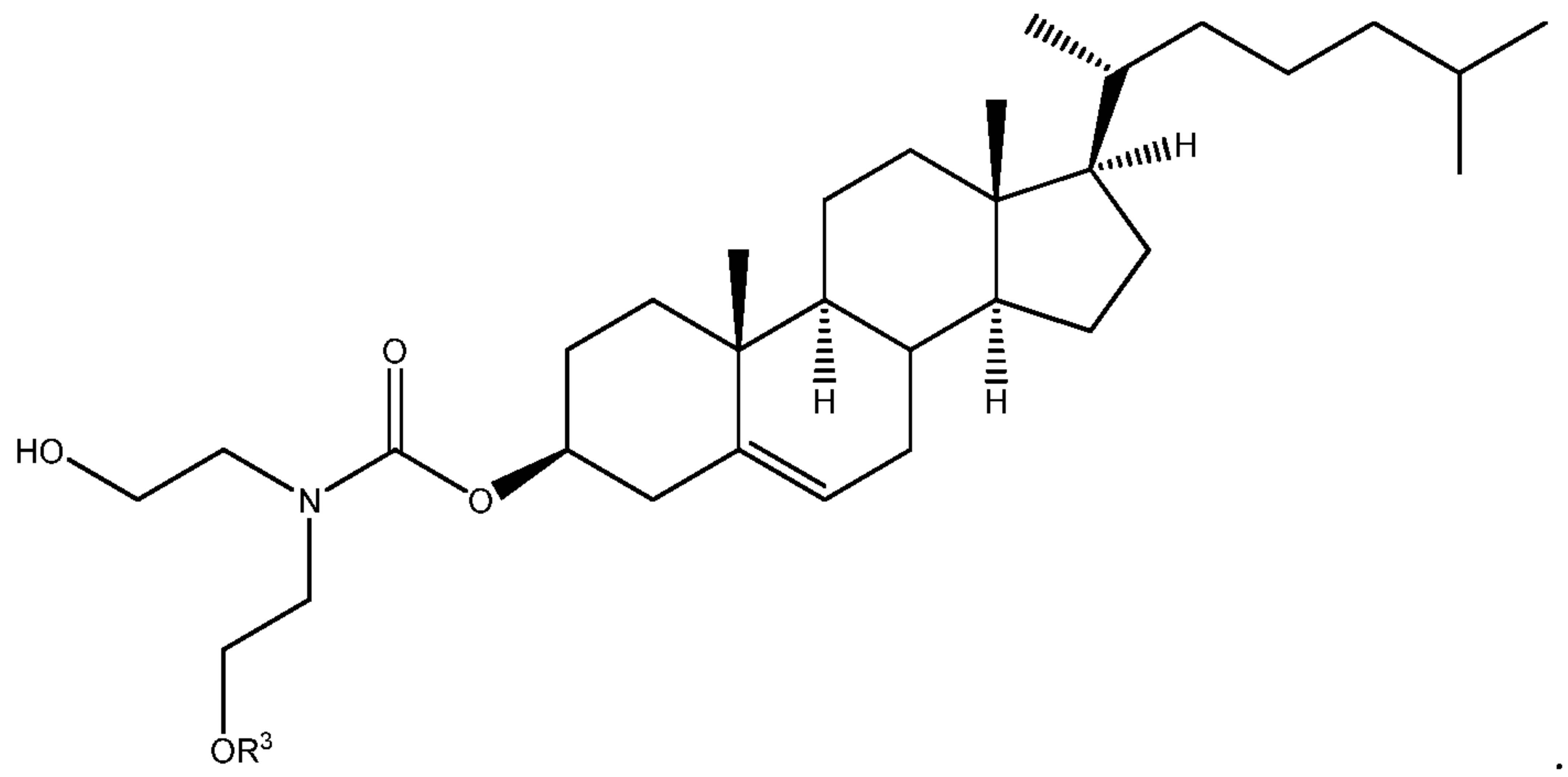
In certain embodiments, the nucleic acid molecule is of the formula:



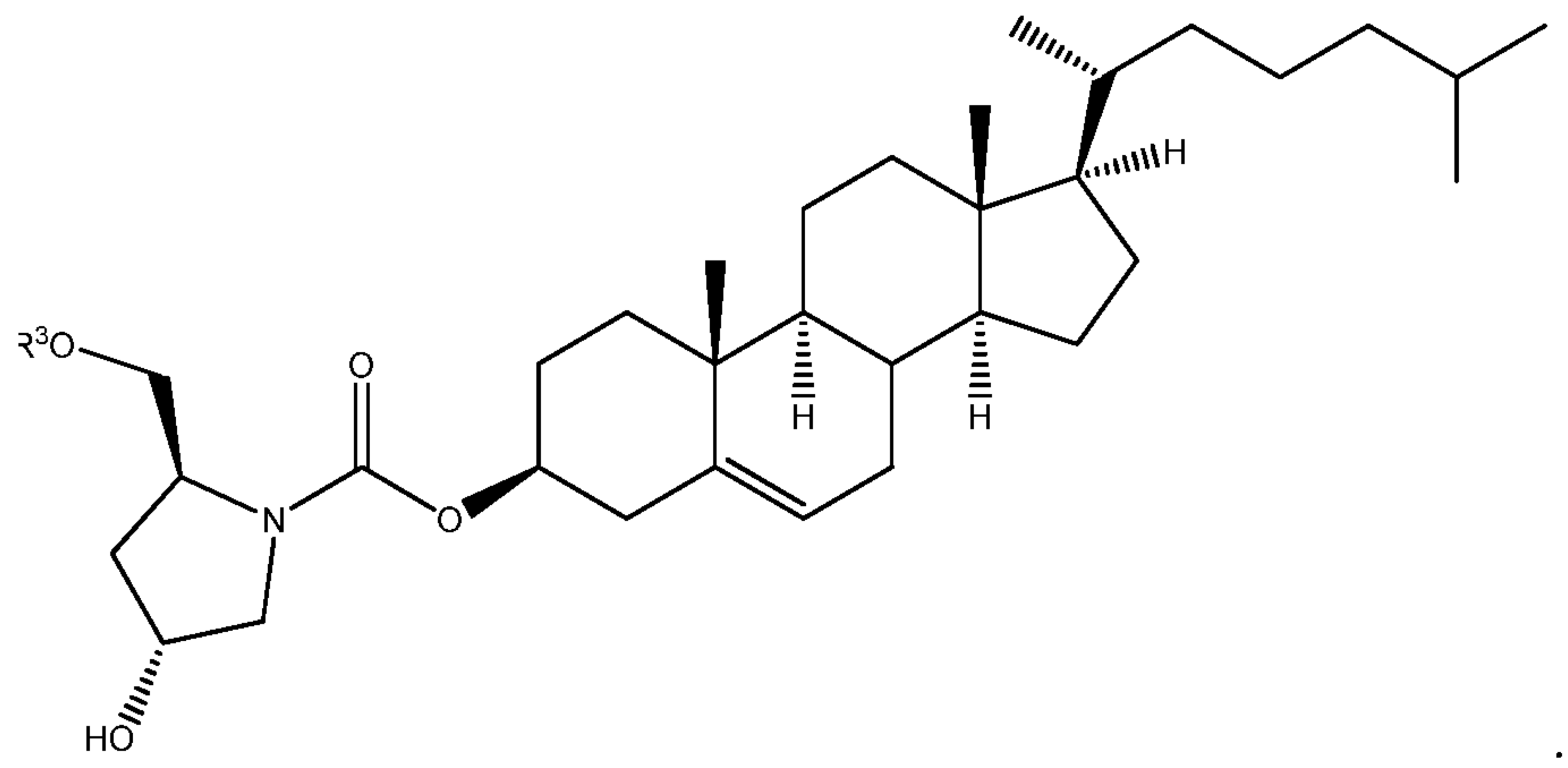
In certain embodiments, the nucleic acid molecule is of the formula:



In certain embodiments, the nucleic acid molecule is of the formula:



In certain embodiments, the nucleic acid molecule is of the formula:



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-ethyoxyphosphodiester, 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-hydrolizable 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.

Another type of conjugates that can be attached to the end (3’ or 5’ end), the loop region, or any other parts of the sd-rxRNA might include a sterol, sterol type molecule, peptide, small molecule, protein, etc. In some embodiments, a sd-rxRNA 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. Antisense (guide) sequences of the invention can be "chimeric oligonucleotides" which comprise an RNA-like and a DNA-like region.

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 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 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.

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 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. In some embodiments, high levels of phosphorothioate modification can lead to improved delivery. In some embodiments, the guide and/or passenger strand is completely phosphorothioated.

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, 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 (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. 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 antagomers, 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. The chemical modification patterns may include combination of ribose, backbone, hydrophobic nucleoside and conjugate type of modifications.

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, 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 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 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. 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.

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 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 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 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

The invention is based, in part, on the surprising discovery that the double stranded nucleic acid molecules described herein are able to robustly and potently reduce levels of long non-coding RNAs (lncRNAs) in cells, both in the cytoplasm and nucleus. Without wishing to be bound by any particular theory, the inventors believe that the particular patterns of modifications on the passenger strand and guide strand of the double stranded nucleic acid molecules described herein (*e.g.*, sd-rxRNAs) facilitate entry of the guide strand into the nucleus, where the guide strand mediates gene silencing (*e.g.*, silencing of lncRNAs).

Without wishing to be bound by any theory, several potential mechanisms of action could account for this activity. For example, in some embodiments, the guide strand (*e.g.*, antisense strand) of the nucleic acid molecule (*e.g.*, sd-rxRNA) may dissociate from the passenger strand and enter into the nucleus as a single strand. Once in the nucleus the single stranded guide strand may associate with RNAse H or another ribonuclease and cleave the target (*e.g.*, lncRNA) ("Antisense mechanism of action"). In some embodiments, the guide strand (*e.g.*, antisense strand) of the nucleic acid molecule (*e.g.*, sd-rxRNA) may associate with an Argonaute (Ago) protein in the cytoplasm or outside the nucleus, forming a loaded Ago complex. This loaded Ago complex may translocate into the nucleus and then cleave the target (*e.g.*, lncRNA). In some embodiments, both strands (*e.g.* a duplex) of the nucleic acid molecule (*e.g.*, sd-rxRNA) may enter the nucleus and the guide strand may associate with RNAse H, an Ago protein or another ribonuclease and cleaves the target (*e.g.*, lncRNA).

The skilled artisan appreciates that the sense strand of the double stranded molecules described herein (*e.g.*, sd-rxRNA sense strand) is not limited to delivery of a guide strand of the double stranded nucleic acid molecule described herein. Rather, in some embodiments, a passenger strand described herein is joined (*e.g.*, covalently bound, non-covalently bound, conjugated, hybridized via a region of complementarity, *etc.*) to certain molecules (*e.g.*, antisense oligonucleotides, ASO) for the purpose of targeting said other molecule to the nucleus of a cell. In some embodiments, the molecule joined to a sense strand described herein is a synthetic antisense oligonucleotide (ASO). In some embodiments, the sense strand joined to an anti-sense oligonucleotide is between 8-15 nucleotides long, chemically modified, and comprises a hydrophobic conjugate.

Without wishing to be bound by any particular theory, an ASO can be joined to a complementary passenger strand by hydrogen bonding. Accordingly, in some aspects, the disclosure provides a method of delivering a nucleic acid molecule to a cell, the method comprising administering an isolated nucleic acid molecule to a cell, wherein the isolated nucleic acid comprises a sense strand which is complementary to an anti-sense oligonucleotide (ASO), wherein the sense strand is between 8-15 nucleotides in length, comprises at least two phosphorothioate modifications, at least 50% of the pyrimidines in the sense strand are modified, and wherein the molecule comprises a hydrophobic conjugate.

Uptake of Oligonucleotides by Cells

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 some embodiments, the oligonucleotide compositions of the invention are contacted with bacterial cells. In some embodiments, the oligonucleotide compositions of the invention are contacted with eukaryotic cells (*e.g.*, plant cell, mammalian cell, arthropod cell, such as insect cell). In some embodiments, the oligonucleotide compositions of the invention are contacted with stem cells. In a preferred embodiment, the oligonucleotide compositions of the invention are contacted 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. In some embodiments, Oligonucleotides are administered topically or through electroporation. Oligonucleotides are taken up by cells at a slow rate by endocytosis, but endocytosed oligonucleotides are 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.

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 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.* 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 E. *et al.* 1992. *Proc. Natl. Acad. Sci.* 88:4255).

In certain embodiments, the sd-rxRNA of the invention may be delivered by using various beta-glucan containing particles, referred to as GeRPs (glucan encapsulated RNA loaded particle), described in, and incorporated by reference from, US Provisional Application No. 61/310,611, filed on March 4, 2010 and entitled "Formulations and Methods for Targeted Delivery to Phagocyte Cells." Such particles are also described in, and incorporated by reference from US Patent Publications US 2005/0281781 A1, and US 2010/0040656, and in PCT publications WO 2006/007372, and WO 2007/050643. The sd-rxRNA molecule may be hydrophobically modified and optionally may be associated with a lipid and/or amphiphilic peptide. In certain embodiments, the beta-glucan particle is derived from yeast. In 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.

Glucan particles can be derived from insoluble components of fungal cell walls such as yeast cell walls. In some embodiments, the yeast is Baker's yeast. Yeast-derived glucan molecules can include one or more of ß-(1,3)-Glucan, ß-(1,6)-Glucan, mannan and chitin. In some embodiments, a glucan particle comprises a hollow yeast cell wall whereby the particle maintains a three dimensional structure resembling a cell, within which it can complex with or encapsulate a molecule such as an RNA molecule. Some of the advantages associated with the use of yeast cell wall particles are availability of the components, their biodegradable nature, and their ability to be targeted to phagocytic cells.

In some embodiments, glucan particles can be prepared by extraction of insoluble components from cell walls, for example by extracting Baker's yeast (Fleischmann's) with 1M NaOH/pH 4.0 H2O, followed by washing and drying. Methods of preparing yeast cell wall particles are discussed in, and incorporated by reference from U.S. Patents 4,810,646, 4,992,540, 5,082,936, 5,028,703, 5,032,401, 5,322,841, 5,401,727, 5,504,079, 5,607,677, 5,968,811, 6,242,594, 6,444,448, 6,476,003, US Patent Publications 2003/0216346, 2004/0014715 and 2010/0040656, and PCT published application WO02/12348.

Protocols for preparing glucan particles are also described in, and incorporated by reference from, the following references: Soto and Ostroff (2008), "Characterization of multilayered nanoparticles encapsulated in yeast cell wall particles for DNA delivery."

Bioconjug Chem 19(4):840-8; Soto and Ostroff (2007), "Oral Macrophage Mediated Gene Delivery System," *Nanotech*, Volume 2, Chapter 5 ("Drug Delivery"), pages 378-381; and Li et al. (2007), "Yeast glucan particles activate murine resident macrophages to secrete proinflammatory cytokines via MyD88-and Syk kinase-dependent pathways." *Clinical Immunology* 124(2):170-181.

Glucan containing particles such as yeast cell wall particles can also be obtained commercially. Several non-limiting examples include: Nutricell MOS 55 from Biorigin (Sao Paolo, Brazil), SAF-Mannan (SAF Agri, Minneapolis, Minn.), Nutrex (Sensient Technologies, Milwaukee, Wis.), alkali-extracted particles such as those produced by Nutricepts (Nutricepts Inc., Burnsville, Minn.) and ASA Biotech, acid-extracted WGP particles from Biopolymer Engineering, and organic solvent-extracted particles such as Adjuvax™ from Alpha-beta Technology, Inc. (Worcester, Mass.) and microparticulate glucan from Novogen (Stamford, Conn.).

Glucan particles such as yeast cell wall particles can have varying levels of purity depending on the method of production and/or extraction. In some instances, particles are alkali-extracted, acid-extracted or organic solvent-extracted to remove intracellular components and/or the outer mannoprotein layer of the cell wall. Such protocols can produce particles that have a glucan (w/w) content in the range of 50% - 90%. In some instances, a particle of lower purity, meaning lower glucan w/w content may be preferred, while in other embodiments, a particle of higher purity, meaning higher glucan w/w content may be preferred.

Glucan particles, such as yeast cell wall particles, can have a natural lipid content. For example, the particles can contain 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20% or more than 20% w/w lipid. In the Examples section, the effectiveness of two glucan particle batches are tested: YGP SAF and YGP SAF + L (containing natural lipids). In some instances, the presence of natural lipids may assist in complexation or capture of RNA molecules.

Glucan containing particles typically have a diameter of approximately 2-4 microns, although particles with a diameter of less than 2 microns or greater than 4 microns are also compatible with aspects of the invention.

The RNA molecule(s) to be delivered are complexed or "trapped" within the shell of the glucan particle. The shell or RNA component of the particle can be labeled for visualization, as described in, and incorporated by reference from, Soto and Ostroff (2008) *Bioconjug Chem* 19:840. Methods of loading GeRPs are discussed further below.

The optimal protocol for uptake of oligonucleotides will depend upon a number 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.

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 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.

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 phopholipids 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.

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.

Nucleic acids associated with the invention can be hydrophobically modified and can be encompassed within neutral nanotransporters. Further description of neutral nanotransporters is incorporated by reference from PCT Application PCT/US2009/005251, filed on September 22, 2009, and entitled “Neutral Nanotransporters.” Such particles enable quantitative oligonucleotide incorporation into non-charged lipid mixtures. The lack of toxic levels of cationic lipids in such neutral nanotransporter compositions is an important feature.

As demonstrated in PCT/US2009/005251, oligonucleotides can effectively be incorporated into a lipid mixture that is free of cationic lipids and such a composition can effectively deliver a therapeutic oligonucleotide to a cell in a manner that it is functional. For example, a high level of activity was observed when the fatty mixture was 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 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 no-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 built into the oligonucleotide itself, enables efficient encapsulation of oligonucleotide in neutral lipid particles.

In some aspects, stable particles ranging in size from 50 to 140 nm can be 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, adamantine, dihydrotesterone, long chain alkyl, long chain alkenyl, long chain alkynyl, olely-lithocholic, cholenic, oleoyl-cholenic, palmityl, heptadecyl, myrisityl, bile acids, cholic acid or taurocholic acid, deoxycholate, oleyl litocholic 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, digoxygenin, dimethoxytrityl, t-butyldimethylsilyl, t-butyldiphenylsilyl, cyanine dyes (e.g. Cy3 or Cy5), Hoechst 33258 dye, psoralen, or 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' of 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 hexaethylenegylcol (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 amino linker 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 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 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 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.

Thus, sterols useful in the invention, for example, include cholesterols, 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. Such conjugates may 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 then oligonucleotides conjugated to conventional cholesterols.

Alternatively the polynucleotide may be bound to a protein, peptide or positively charged chemical that functions as the hydrophobic molecule. The proteins may be 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 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. The polycarbon chain may be longer than 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 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 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 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).

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 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 known as dielaidoylphosphatidylcholine, dioleoyl-PC, dioleoylphosphocholine, dioleoyl-sn-glycero-3-phosphocholine, dioleylphosphatidylcholine). DSPC (chemical registry number 816-94-4) is distearoylphosphatidylcholine (also known as 1,2-Distearoyl-sn-Glycero-3-phosphocholine).

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.

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 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.

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 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 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 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 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 zwiterionic 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 osmolarity 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. In some embodiments, vitamin A or E is used. Complexes are formed at different oligonucleotide concentrations, with higher concentrations favoring more efficient complex formation.

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 hydrophobicly modified polynucleotides. This methodology provides for the specific delivery of the polynucleotides to particular tissues.

In another embodiment the fat emulsions of the cargo molecule contain more than 70% of Linoleic acid ($C_{18}H_{32}O_2$) and/or cardiolipin.

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 though variable mechanisms, including but not limited to sterol type delivery.

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.

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-dioleoloxy)-propyl]-N,N,N-trimethylammonium chloride (DOTMA), N-[1 -(2,3-dioleoloxy)-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 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).

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. US.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).

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.

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 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.

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 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 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 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 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. 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 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 "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 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 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 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. 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 unsubstituted C_1-C_{20} alkyl chains, C_2-C_{20} alkenyl chains, C_2-C_{20} alkynyl chains, peptides, and heteroatoms (*e.g.*, S, O, NH, *etc.*). Other exemplary linkers include bifinctional crosslinking agents such as sulfosuccinimidyl-4-(maleimidophenyl)-butyrate (SMPB) (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 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 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 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 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 eukaryotic cells. Mannosylated glycoprotein conjugated to poly(L-lysine) in alveolar 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 cancerous cells. For example, when folic acid is linked to poly(L-lysine) enhanced oligonucleotide uptake is seen in promyelocytic leukemia (HL-60) cells and human melanoma (M-14) cells. Ginobbi *et al.* 1997. *Anticancer Res.* 17:29. In another example, 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.

Liposomes naturally accumulate in the liver, spleen, and reticuloendothelial system (so-called, passive targeting). By coupling liposomes to various ligands such as antibodies are 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 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, 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).

Treatment Indications

In some aspects, the instant disclosure relates to the use of sd-rxRNA to target a lncRNA associated with disease. In some embodiments, the lncRNA associated with disease is associated with a neoplasm (*e.g.*, cancer). Examples of cancers include lung, hepatocellular carcinoma, uterine endometrial stromal sarcoma, cervical cancer, breast cancer, osteosarcoma and colorectal cancer. In some embodiments, the lncRNA associated with disease is associated with alcoholism (see, for example, Eiβmann et al. 2012). In some embodiments, the lncRNA associated with disease is associated with viral infections (see, for example, Eiβmann et al. 2012). In some embodiments, the lncRNA associated with disease is associated with diabetes (see, for example, Liu et al. Cell Death and Disease 2014, 5).

In some instances, an sd-rxRNA is targeted to a neoplasm or a neoplastic tissue and is used to ameliorate at least one symptom of a condition or disorder associated with neoplasia. Neoplasia refers to the abnormal proliferation of cells, often resulting in an abnormal mass of tissue (*i.e.*, a neoplasm). Neoplasm may be benign, pre-malignant (*e.g.*, a carcinoma in situ), or malignant (cancerous). Benign neoplasms include uterine fibroids and melanocytic nevi (*i.e.*, skin moles) that do not transform into cancer. Potentially malignant, or pre-cancerous, neoplasms include carcinoma in situ, which is an early form of carcinoma that does not invade surrounding tissue, but rather proliferate in their normal environment. Malignant neoplasms are commonly referred to as cancer, and they invade and destroy surrounding tissue, may form metastases, and eventually may be fatal to the host.

In some instances, the sd-rxRNA is targeted to a neoplasm or neoplastic cells of epithelial origin. Epithelial cells reside in one or more layers which cover the entire surface of the body and which line most of the hollow structures of the body, excluding the blood vessels, lymph vessels, and the heart interior, which are lined with endothelium, and the chest and abdominal cavities which are lined with mesothelium.

Epithelial neoplasms include, but are not limited to, benign and premalignant epithelial tumors, such as breast fibroadenoma and colon adenoma, and malignant epithelial tumors. Malignant epithelial tumors include primary tumors, also referred to as carcinomas, and secondary tumors, also referred to as metastases of epithelial origin. Carcinomas include, but are not limited to, acinar carcinoma, acinous carcinoma, alveolar adenocarcinoma (also called adenocystic carcinoma, adenomyoepithelioma, cribriform carcinoma and cylindroma), carcinoma adenomatosum, adenocarcinoma, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma (also called bronchiolar carcinoma, alveolar cell tumor and pulmonary adenomatosis), basal cell carcinoma, carcinoma basocellulare (also called basaloma, or basiloma, and hair matrix carcinoma), basaloid carcinoma, basosquamous cell carcinoma, breast carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma (also called cholangioma and cholangiocarcinoma), chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epibulbar carcinoma, epidermoid carcinoma, carcinoma epitheliale adenoides, carcinoma exulcere, carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant cell carcinoma, gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma (also called hepatoma, malignant hepatoma and hepatocarcinoma), Hurthle cell carcinoma, hyaline carcinoma, hypernephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma mastitoides, carcinoma medullare, medullary carcinoma, carcinoma melanodes, melanotic carcinoma, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, carcinoma nigrum, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, ovarian carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prostate carcinoma, renal cell carcinoma of kidney (also called adenocarcinoma of kidney and hypernephoroid carcinoma), reserve cell carcinoma, carcinoma sarcomatodes, scheinderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, carcinoma vilosum.

In other instances, the sd-rxRNA is targeted to a neoplasm or neoplastic cells of mesenchymal origin, for example, neoplastic cells forming a sarcoma. Sarcomas are rare mesenchymal neoplasms that arise in bone and soft tissues. Different types of sarcomas are recognized, including liposarcomas (including myxoid liposarcomas and pleiomorphic liposarcomas), leiomyosarcomas, rhabdomyosarcomas, malignant peripheral nerve sheath tumors (also called malignant schwannomas, neurofibrosarcomas, or neurogenic sarcomas), Ewing's tumors (including Ewing's sarcoma of bone, extra skeletal [not bone] Ewing's sarcoma, and primitive neuroectodermal tumor [PNET]), synovial sarcoma, angiosarcomas, hemangiosarcomas, lymphangiosarcomas, Kaposi's sarcoma, hemangioendothelioma, fibrosarcoma, desmoid tumor (also called aggressive fibromatosis), dermatofibrosarcoma protuberans (DFSP), malignant fibrous histiocytoma (MFH), hemangiopericytoma, malignant mesenchymoma, alveolar soft-part sarcoma, epithelioid sarcoma, clear cell sarcoma, desmoplastic small cell tumor, gastrointestinal stromal tumor (GIST) (also known as GI stromal sarcoma), osteosarcoma (also known as osteogenic sarcoma)-skeletal and extra skeletal, and chondrosarcoma.

In yet other instances, the sd-rxRNA targets neoplasms or neoplastic cells of melanocytic origin. Melanomas are tumors arising from the melanocytic system of the skin and other organs. Examples of melanoma include lentigo maligna melanoma, superficial spreading melanoma, nodular melanoma, and acral lentiginous melanoma.
In still other instances, the sd-rxRNA targets malignant neoplasms or neoplastic cells including, but not limited to, those found in biliary tract cancer, endometrial cancer, esophageal cancer, gastric cancer, intraepithelial neoplasms, including Bowen's disease and Paget's disease, liver cancer, oral cancer, including squamous cell carcinoma, sarcomas, including fibrosarcoma and osteosarcoma, skin cancer, including melanoma, Kaposi's sarcoma, testicular cancer, including germinal tumors (seminoma, non-seminoma (teratomas, choriocarcinomas)), stromal tumors and germ cell tumors, thyroid cancer, including thyroid adenocarcinoma and medullar carcinoma, and renal cancer including adenocarcinoma and Wilms tumor.

In other instances, the sd-rxRNA targets neoplasms or neoplastic cells originating in bone, muscle or connective tissue. The neoplastic cells may be found in primary tumors (*e.g.*, sarcomas) of bone and connective tissue.

In some instances, the sd-rxRNA is delivered directly to a neoplasm, for example, by injection using a needle and syringe. Injection into the neoplasm permits large quantities of the sd-rxRNA to be delivered directly to the target cells while minimizing delivery to systemic sites. By direct injection into the neoplasm, an effective amount to promote RNA interference by the sd-rxRNA is distributed throughout at least a substantial volume of the neoplasm. In some instances, delivery of the sd-rxRNA requires a single injection into the neoplasm. In other instances, delivery of the sd-rxRNA requires multiple injections into separate regions of the neoplasm such that the entire mass of the neoplasm is invested with an effective amount to promote RNA interference by the sd-rxRNA. See U.S. Patent Nos.

5,162,115 and 5,051,257, and Livraghi et al, *Tumori* 72 (1986), pp. 81-87, each of which is incorporated herein by reference.

The total dose, concentration, volume of the sd-rxRNA delivered, and rate of delivery can be optimized for a given neoplasm type, size and architecture. The zone of RNA interference can be controlled by optimizing these parameters. The volume and concentration of the sd-rxRNA delivered into the neoplasm must be sufficient to promote RNA interference throughout the tumor. Depending on the number of injections, and their placement with respect to neoplasm architecture, it can be useful to administer total sd-rxRNA volumes less than the neoplasm volume, greater than the neoplasm volume, or approximately equal to the neoplasm volume.

In some instances, the sd-rxRNA is delivered directly to the neoplasm using an implantable device.

In some instances sd-rxRNA injection into a neoplasm can be accompanied by ultrasound guidance.

In other instances, the sd-rxRNA is administered systemically, for example, intravenously, intraarterially, intramuscularly, or subcutaneously.

The sd-rxRNA that is targeted to a neoplasm, in some instances target a lncRNA that regulates or modulates a proliferative gene or a gene that is expressed at higher levels in a neoplastic tissue than in other tissues. In some embodiments, the sd-rxRNA is targeted to a lncRNA associated with a neoplasm. As used herein, a lncRNA "associated with a neoplasm" is a lncRNA that is dysregulated in a subject having a neoplasm (*e.g.*, overexpressed or under expressed in the subject relative to the expression level in a subject not having a neoplasm).

lncRNAs have been shown to be involved in several different cancer types including: neuroblastoma , acute lymphocytic leukemia , melanoma , prostate cancer, hepatocellular carcinoma , colorectal cancer , breast cancer, ovarian cancer and non-small-cell lung cancer.

For example, the lncRNA MALAT1 is known to be dysregulated in several cancers, such as lung, hepatocellular carcinoma, uterine endometrial stromal sarcoma, cervical cancer, breast cancer, osteosarcoma and colorectal cancer (see, for example, Eißmann et al. RNA Biology, 2012 Aug 1 ; 9(8): 1076-1087).

MALAT1 has also been found to be upregulated in diabetes-induced microvascular dysfunction (Liu et al. 2014). In some embodiments, Malat1 is a target for anti-angiogenic therapy for diabetes-related microvascular complications such as diabetic retinopathy.

MALAT1 has also been linked to viral infection and alcoholism. In some embodiments, MALAT1 is a target for treatment of viral infection or alcoholism.

In some aspects, the disorder to be treated according to methods described herein is selected from the group consisting of: cardiovascular diseases, including hypertension, stroke, hypertrophy and heart failure; neurological and psychiatric disorders, including Alzheimer's Disease, schizophrenia, schizoaffective disorder, dipolar disorder, major depression and autistic disorders; metabolic diseases; and diseases associated with immune dysfunction or inflammation.

Administration

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, *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 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 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.

In some embodiments, the disclosure relates to a composition (*e.g.*, pharmaceutical composition) comprising an oligonucleotide (*e.g.*, an isolated double stranded nucleic acid molecule). In some embodiments, the composition comprises an additional therapeutic agent.

Non-limiting examples of additional therapeutic agents include but are not limited to nucleic acids (*e.g.*, sd-rxRNA, *etc.*), small molecules (*e.g.*, small molecules useful for treating cancer, neurodegenerative diseases, infectious diseases, autoimmune diseases, *etc.*), peptides (*e.g.*, peptides useful for treating cancer, neurodegenerative diseases, infectious diseases, autoimmune diseases, *etc.*), and polypeptides (*e.g.*, antibodies useful for treating cancer, neurodegenerative diseases, infectious diseases, autoimmune diseases, *etc.*). Compositions of the disclosure can have, in some embodiments, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more additional therapeutic agents. In some embodiments, a composition comprises more than 10 additional therapeutic agents.

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.

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. In preferred embodiments, the sd-rxRNA molecules are administered by intradermal injection or subcutaneously.

With respect to *in vivo* applications, in some embodiments, the formulations of the present invention can be administered to a patient in a variety of forms adapted to deliver the construct to the eye. In some embodiments, parenteral administration is ocular. Ocular administration can be intravitreal, intracameral, subretinal, subconjunctival, or subtenon.

The sd-rxRNA molecules, when it is desirable to deliver them systemically, may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

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 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 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.

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.

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

For administration by inhalation, such as by insufflation, the sd-rxRNA molecules for use according to the present invention may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

Also contemplated herein is pulmonary delivery of the sd-rxRNA molecules. The sd-rxRNA molecule is delivered to the lungs of a mammal while inhaling and traverses across the lung epithelial lining to the blood stream. Other reports of inhaled molecules include Adjei et al., 1990, Pharmaceutical Research, 7:565 569; Adjei et al., 1990, International Journal of Pharmaceutics, 63:135 144 (leuprolide acetate); Braquet et al., 1989, Journal of Cardiovascular Pharmacology, 13(suppl. 5):143 146 (endothelin-1); Hubbard et al., 1989, Annals of Internal Medicine, Vol. III, pp. 206 212 (a1 antitrypsin); Smith et al., 1989, J. Clin. Invest. 84:1145-1146 (a 1-proteinase); Oswein et al., 1990, "Aerosolization of Proteins", Proceedings of Symposium on Respiratory Drug Delivery II, Keystone, Colorado, March, (recombinant human growth hormone); Debs et al., 1988, J. Immunol. 140:3482 3488 (interferon g and tumor necrosis factor alpha) and Platz et al., U.S. Patent No. 5,284,656 (granulocyte colony stimulating factor). A method and composition for pulmonary delivery of drugs for systemic effect is described in, and incorporated by reference from, U.S. Patent No. 5,451,569, issued September 19, 1995 to Wong et al.

Contemplated for use in the practice of this invention are a wide range of mechanical devices designed for pulmonary delivery of therapeutic products, including but not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art.

Some specific examples of commercially available devices suitable for the practice of this invention are the Ultravent nebulizer, manufactured by Mallinckrodt, Inc., St. Louis, Missouri; the Acorn II nebulizer, manufactured by Marquest Medical Products, Englewood, Colorado; the Ventolin metered dose inhaler, manufactured by Glaxo Inc., Research Triangle Park, North Carolina; and the Spinhaler powder inhaler, manufactured by Fisons Corp., Bedford, Massachusetts.

All such devices require the use of formulations suitable for the dispensing of oligonucleotide (or derivative). Typically, each formulation is specific to the type of device employed and may involve the use of an appropriate propellant material, in addition to the usual diluents, adjuvants and/or carriers useful in therapy. Also, the use of liposomes, microcapsules or microspheres, inclusion complexes, or other types of carriers is contemplated. Chemically modified oligonucleotide may also be prepared in different formulations depending on the type of chemical modification or the type of device employed.

Formulations suitable for use with a nebulizer, either jet or ultrasonic, will typically comprise oligonucleotide (or derivative) dissolved in water at a concentration of about 0.1 to 25 mg of biologically active oligonucleotide per mL of solution. The formulation may also include a buffer and a simple sugar (e.g., for oligonucleotide stabilization and regulation of osmotic pressure). The nebulizer formulation may also contain a surfactant, to reduce or prevent surface induced aggregation of the oligonucleotide caused by atomization of the solution in forming the aerosol.

Formulations for use with a metered dose inhaler device will generally comprise a finely divided powder, such as a dry powder formulation, containing the sd-rxRNA molecule suspended in a propellant with the aid of a surfactant. The propellant may be any conventional material employed for this purpose, such as a chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol, and 1,1,1,2 tetrafluoroethane, or combinations thereof. Suitable surfactants include sorbitan trioleate and soya lecithin. Oleic acid may also be useful as a surfactant.

Formulations for dispensing from a powder inhaler device will comprise a finely divided dry powder containing oligonucleotide (or derivative) and may also include a bulking agent, such as lactose, sorbitol, sucrose, or mannitol in amounts which facilitate dispersal of the powder from the device, e.g., 50 to 90% by weight of the formulation. The sd-rxRNA molecule can be prepared in particulate form with an average particle size of less than 10 mm (or microns), most preferably 0.5 to 5 mm, for most effective delivery to the distal lung.

Nasal delivery of a pharmaceutical composition of the present invention is also contemplated. Nasal delivery allows the passage of a pharmaceutical composition of the present invention to the blood stream directly after administering the therapeutic product to the nose, without the necessity for deposition of the product in the lung. Formulations for nasal delivery include those with dextran or cyclodextran.

For nasal administration, a useful device is a small, hard bottle to which a metered dose sprayer is attached. In one embodiment, the metered dose is delivered by drawing the pharmaceutical composition of the present invention solution into a chamber of defined volume, which chamber has an aperture dimensioned to aerosolize and aerosol formulation by forming a spray when a liquid in the chamber is compressed. The chamber is compressed to administer the pharmaceutical composition of the present invention. In a specific embodiment, the chamber is a piston arrangement. Such devices are commercially available.

Alternatively, a plastic squeeze bottle with an aperture or opening dimensioned to aerosolize an aerosol formulation by forming a spray when squeezed is used. The opening is usually found in the top of the bottle, and the top is generally tapered to partially fit in the nasal passages for efficient administration of the aerosol formulation. Preferably, the nasal inhaler will provide a metered amount of the aerosol formulation, for administration of a measured dose of the drug.

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 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 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 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 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.

The chosen method of delivery will result in entry into cells. In some embodiments, 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 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 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 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, montrnorillonite, 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.

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-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 (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 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 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 (C_8-C_{12}) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C_8-C_{10} 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 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 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 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 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 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.

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 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 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 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 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 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.

Dosage regimens 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 oligonucleotides, whether the oligonucleotides are to be administered to cells or to subjects.

Ocular administration of sd-rxRNAs, including intravitreal, intracameral, subretinal, subconjunctival, and subtenon administration, can be optimized through testing of dosing regimens. In some embodiments, a single administration is sufficient. To further prolong the effect of the administered sd-rxRNA, the sd-rxRNA can be administered in a slow-release formulation or device, as would be familiar to one of ordinary skill in the art. The hydrophobic nature of sd-rxRNA compounds can enable use of a wide variety of polymers, some of which are not compatible with conventional oligonucleotide delivery.

Intravenous administration of sd-rxRNAs can be optimized through testing of dosing regimens. In some instances, intravenous administration is achieved through infusion, for example through the use of an infusion pump to infuse molecules into the circulatory system of a subject. The infusion can be continuous or intermittent. In some instances, it is preferred if the dosing regimen involves repetitive administration of a short-term continuous infusion. For example, the continuous infusion can last for approximately 5 min, 10 min, 20 min, 30 min, 40 min, 50 min, 1.0 hour, 1.1 hours, 1.2 hours, 1.3 hours, 1.4 hours, 1.5 hours, 1.6 hours, 1.7 hours, 1.8 hours, 1.9 hours, 2.0 hours, 2.1 hours, 2.2 hours, 2.3 hours, 2.4 hours, 2.5 hours, 2.6 hours, 2.7 hours, 2.8 hours, 2.9 hours, 3.0 hours, 3.1 hours, 3.2 hours, 3.3 hours, 3.4 hours. 3.5 hours, 3.6 hours, 3.7 hours, 3.8 hours, 3.9 hours, 4.0 hours, 4.1 hours, 4.2 hours, 4.3 hours, 4.4 hours, 4.5 hours, 4.6 hours, 4.7 hours, 4.8 hours, 4.9 hours, 5.0 hours, 5.1 hours, 5.2 hours, 5.3 hours, 5.4 hours, 5.5 hours, 5.6 hours, 5.7 hours, 5.8 hours, 5.9 hours, 6.0 hours, or more than 6.0 hours, including any intermediate values.

The infusion can be repetitive. In some instances it is administered daily, bi-weekly, weekly, every two weeks, every three weeks, monthly, every two months, every three months, every four months, every five months, every six months or less frequently than every six months. In some instances, it is administered multiple times per day, week, month and/or year. For example, it can be administered approximately every hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours 10 hours, 12 hours or more than twelve hours. It can be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 times per day.

Administration of sd-rxRNAs, such as through intradermal injection or subcutaneous delivery, can be optimized through testing of dosing regimens. In some embodiments, a single administration is sufficient. To further prolong the effect of the administered sd-rxRNA, the sd-rxRNA can be administered in a slow-release formulation or device, as would be familiar to one of ordinary skill in the art. The hydrophobic nature of sd-rxRNA compounds can enable use of a wide variety of polymers, some of which are not compatible with conventional oligonucleotide delivery.

In other embodiments, the sd-rxRNA is administered multiple times. In some instances it is administered daily, bi-weekly, weekly, every two weeks, every three weeks, monthly, every two months, every three months, every four months, every five months, every six months or less frequently than every six months. In some instances, it is administered multiple times per day, week, month and/or year. For example, it can be administered approximately every hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours 10 hours, 12 hours or more than twelve hours. It can be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 times per day.

Aspects of the invention relate to administering sd-rxRNA molecules to a subject. In some instances the subject is a patient and administering the sd-rxRNA molecule involves administering the sd-rxRNA molecule in a doctor's office. Without wishing to be bound by any theory, a continuous infusion may saturate the normal clearance mechanism and maintain relatively high compound levels in the blood to ensure tissue distribution. sd-rxRNA are well suited to such an approach due to their low levels of toxicity.

In some instances, the effective amount of sd-rxRNA that is delivered through ocular administration is at least approximately 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or more than 100 μg including any intermediate values.

sd-rxRNA molecules administered through methods described herein are effectively targeted to all the cell types in the eye.

In some embodiments, more than one sd-rxRNA molecule is administered simultaneously. For example a composition may be administered that contains 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 different sd-rxRNA molecules. In certain embodiments, a composition comprises 2 or 3 different sd-rxRNA molecules. When a composition comprises more than one sd-rxRNA, the sd-rxRNA molecules within the composition can be directed to the same gene or to different genes.

In some instances, the effective amount of sd-rxRNA that is delivered by subcutaneous administration is at least approximately 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or more than 100 mg/kg including any intermediate values.

Subcutaneous administration can also be repetitive. In some instances it is administered daily, bi-weekly, weekly, every two weeks, every three weeks, monthly, every two months, every three months, every four months, every five months, every six months or less frequently than every six months. In some instances, it is administered multiple times per day, week, month and/or year. For example, it can be administered approximately every hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours 10 hours, 12 hours or more than twelve hours. It can be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 times per day.

In some instances, sd-rxRNA is administered through insufflation. In some instances, the effective amount of sd-rxRNA that is delivered by insufflation is at least approximately 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or more than 100 mg/kg including any intermediate values.

Administration by insufflation can also be repetitive. In some instances it is administered daily, bi-weekly, weekly, every two weeks, every three weeks, monthly, every two months, every three months, every four months, every five months, every six months or less frequently than every six months. In some instances, it is administered multiple times per day, week, month and/or year. For example, it can be administered approximately every hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours 10 hours, 12 hours or more than twelve hours. It can be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 times per day.

sd-rxRNA molecules administered by methods described herein including intravenous, subcutaneous and insufflation, can be targeted to a variety of remote tissues in the body including liver, heart, lung, kidney, spleen and skin.

In some instances, the effective amount of sd-rxRNA that is delivered through intradermal injection is at least approximately 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or more than 950 μg including any intermediate values.

sd-rxRNA molecules administered through methods described herein are effectively targeted to all the cell types in the skin.

Various modalities of introducing nucleic acids into a subject (*e.g.*, a cell of a subject) are contemplated by the disclosure. For example, nucleic acids (*e.g.,* a solution containing the nucleic acids) can be injected into a subject (*e.g.*, injected into a cell) or a subject (*e.g.*, a cell) can be bombarded by particles covered by the nucleic acids. In some embodiments, the cell or organism is soaked in a solution of the nucleic acid. In some embodiments, a nucleic acid is introduced into an organism or cell by electroporation of cell membranes in the presence of the nucleic acid. In some embodiments, a viral construct comprising the nucleic acid is packaged into a viral particle and accomplishes introduction of the nucleic acid into the cell and transcription of nucleic acid. Further examples of modalities for introducing nucleic acids into a subject (*e.g.*, a cell of a subject) include but are not limited to lipid-mediated carrier transport, chemical-mediated transport (*e.g.*, calcium phosphate), etc.

Nucleic acids can be introduced with additional components. For example, in some embodiments, the nucleic acid is introduced with a component that enhances nucleic acid uptake by the cell. In some embodiments, the nucleic acid is introduced with a component that inhibits annealing of single strands. In some embodiments, the nucleic acid is introduced with a component that stabilizes the nucleic acid molecule, or other-wise increases 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 may be introduced by bathing a cell or 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.

In some embodiments, the cell with the target gene may be derived from any organism. In some embodiments, the cell with the target gene may be contained in (*e.g.*, housed by, or present within) any organism. For example, the organism may a plant, animal, protozoan, bacterium, arthropod, virus, or fungus. The plant may 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.

A further preferred use for the agents 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 priming agent/RNAi agent which is sufficiently 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 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 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.

Therapeutic use

By inhibiting the expression of a gene (*e.g.*, a lncRNA), the oligonucleotide compositions of the present invention can be used to treat any disease involving the expression of a lncRNA. 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 Colitus, Crohn's disease), viral diseases (*i.e.*, HIV, Hepatitis C), miRNA disorders, and cardiovascular diseases.

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 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 (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 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.

The invention provides methods for 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 therapeutic agent (e.g., a RNAi agent or vector or transgene encoding same). If appropriate, subjects are first treated with a priming agent so as to be more responsive to the 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 as described herein. 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 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 modulatory method of the invention involves contacting a cell capable of expressing target gene with a therapeutic agent of the invention that is specific for the target 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 modulatory 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*. Typically, subjects are first treated with a priming agent so as to be more responsive to the subsequent RNAi therapy. As such, the present invention provides methods of treating an individual 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 have a beneficial effect.

The therapeutic agents of the invention can be administered to individuals to treat (prophylactically or therapeutically) disorders associated with aberrant or unwanted target gene (*e.g.*, lncRNA) activity. In conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response 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 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. See, for example, Eichelbaum, M. et al. (1996) Clin. Exp. Pharmacol. Physiol. 23(10-11): 983-985 and Linder, M. W. et al. (1997) Clin. Chem. 43(2):254-266

RNAi in skin indications

Nucleic acid molecules, or compositions comprising nucleic acid molecules, described herein may in some embodiments be administered to pre-treat, treat or prevent compromised skin. As used herein “compromised skin” refers to skin which exhibits characteristics distinct from normal skin. Compromised skin may occur in association with a dermatological condition. Several non-limiting examples of dermatological conditions include rosacea, common acne, seborrheic dermatitis, perioral dermatitis, acneform rashes, transient acantholytic dermatosis, and acne necrotica miliaris. In some instances, compromised skin may comprise a wound and/or scar tissue. In some instances, methods and compositions associated with the invention may be used to promote wound healing, prevention, reduction or inhibition of scarring, and/or promotion of re-epithelialisation of wounds.

A subject can be pre-treated or treated prophylactically with a molecule associated with the invention, prior to the skin of the subject becoming compromised. As used herein “pre-treatment” or “prophylactic treatment” refers to administering a nucleic acid to the skin prior to the skin becoming compromised. For example, a subject could be pre-treated 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 24 hours, 48 hours, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days or more than 8 days prior to the skin becoming compromised. In other embodiments, a subject can be treated with a molecule associated with the invention immediately before the skin becomes compromised and/or simultaneous to the skin becoming compromised and/or after the skin has been compromised. In some embodiments, the skin is compromised through a medical procedure such as surgery, including elective surgery. In certain embodiments methods and compositions may be applied to areas of the skin that are believed to be at risk of becoming compromised. It should be appreciated that one of ordinary skill in the art would be able to optimize timing of administration using no more than routine experimentation.

In some aspects, methods associated with the invention can be applied to promote healing of compromised skin. Administration can occur at any time up until the compromised skin has healed, even if the compromised skin has already partially healed. The timing of administration can depend on several factors including the nature of the compromised skin, the degree of damage within the compromised skin, and the size of the compromised area. In some embodiments administration may occur immediately after the skin is compromised, or 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, 24 hours, 48 hours, or more than 48 hours after the skin has been compromised. Methods and compositions of the invention may be administered one or more times as necessary. For example, in some embodiments, compositions may be administered daily or twice daily. In some instances, compositions may be administered both before and after formation of compromised skin.

Compositions associated with the invention may be administered by any suitable route. In some embodiments, administration occurs locally at an area of compromised skin. For example, compositions may be administered by intradermal injection. Compositions for intradermal injection may include injectable solutions. Intradermal injection may in some embodiments occur around the area of compromised skin or at a site where the skin is likely to become compromised. In some embodiments, compositions may also be administered in a topical form, such as in a cream or ointment. In some embodiments, administration of compositions described herein comprises part of an initial treatment or pre-treatment of compromised skin, while in other embodiments, administration of such compositions comprises follow-up care for an area of compromised skin.

The appropriate amount of a composition or medicament to be applied can depend on many different factors and can be determined by one of ordinary skill in the art through routine experimentation. Several non-limiting factors that might be considered include biological activity and bioavailability of the agent, nature of the agent, mode of administration, half-life, and characteristics of the subject to be treated.

In some aspects, nucleic acid molecules associated with the invention may also be used in treatment and/or prevention of fibrotic disorders, including pulmonary fibrosis, liver cirrhosis, scleroderma and glomerulonephritis, lung fibrosis, liver fibrosis, skin fibrosis, muscle fibrosis, radiation fibrosis, kidney fibrosis, proliferative vitreoretinopathy and uterine fibrosis.

A therapeutically effective amount of a nucleic acid molecule described herein may in some embodiments be an amount sufficient to prevent the formation of compromised skin and/or improve the condition of compromised skin. In some embodiments, improvement of the condition of compromised skin may correspond to promotion of wound healing and/or inhibition of scarring and/or promotion of epithelial regeneration. The extent of prevention of formation of compromised skin and/or improvement to the condition of compromised skin may in some instances be determined by, for example, a doctor or clinician.

The ability of nucleic acid molecules associated with the invention to prevent the formation of compromised skin and/or improve the condition of compromised skin may in some instances be measured with reference to properties exhibited by the skin. In some instances, these properties may include rate of epithelialisation and/or decreased size of an area of compromised skin compared to control skin at comparable time points.

As used herein, prevention of formation of compromised skin, for example prior to a surgical procedure, and/or improvement of the condition of compromised skin, for example after a surgical procedure, can encompass any increase in the rate of healing in the compromised skin as compared with the rate of healing occurring in a control sample. In some instances, the condition of compromised skin may be assessed with respect to either comparison of the rate of re-epithelialisation achieved in treated and control skin, or comparison of the relative areas of treated and control areas of compromised skin at comparable time points. In some aspects, a molecule that prevents formation of compromised skin or promotes healing of compromised skin may be a molecule that, upon administration, causes the area of compromised skin to exhibit an increased rate of re-epithelialisation and/or a reduction of the size of compromised skin compared to a control at comparable time points. In some embodiments, the healing of compromised skin may give rise to a rate of healing that is 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% greater than the rate occurring in controls.

In some aspects, subjects to be treated by methods and compositions associated with the invention may be subjects who will undergo, are undergoing or have undergone a medical procedure such as a surgery. In some embodiments, the subject may be prone to defective, delayed or otherwise impaired re-epithelialisation, such as dermal wounds in the aged. Other non-limiting examples of conditions or disorders in which wound healing is associated with delayed or otherwise impaired re-epithelialisation include patients suffering from diabetes, patients with polypharmacy, post-menopausal women, patients susceptible to pressure injuries, patients with venous disease, clinically obese patients, patients receiving chemotherapy, patients receiving radiotherapy, patients receiving steroid treatment, and immuno-compromised patients. In some instances, defective re-epithelialisation response can contributes to infections at the wound site, and to the formation of chronic wounds such as ulcers.

In some embodiments, methods associated with the invention may promote the re-epithelialisation of compromised skin in chronic wounds, such as ulcers, and may also inhibit scarring associated with wound healing. In other embodiments, methods associated with the invention are applied to prevention or treatment of compromised skin in acute wounds in patients predisposed to impaired wound healing developing into chronic wounds. In other aspects, methods associated with the invention are applied to promote accelerated healing of compromised skin while preventing, reducing or inhibiting scarring for use in general clinical contexts. In some aspects, this can involve the treatment of surgical incisions and application of such methods may result in the prevention, reduction or inhibition of scarring that may otherwise occur on such healing. Such treatment may result in the scars being less noticeable and exhibiting regeneration of a more normal skin structure. In other embodiments, the compromised skin that is treated is not compromised skin that is caused by a surgical incision. The compromised skin may be subject to continued care and continued application of medicaments to encourage re-epithelialisation and healing.

In some aspects, methods associated with the invention may also be used in the treatment of compromised skin associated with grafting procedures. This can involve treatment at a graft donor site and/or at a graft recipient site. Grafts can in some embodiments involve skin, artificial skin, or skin substitutes. Methods associated with the invention can also be used for promoting epithelial regeneration. As used herein, promotion of epithelial regeneration encompasses any increase in the rate of epithelial regeneration as compared to the regeneration occurring in a control-treated or untreated epithelium. The rate of epithelial regeneration attained can in some instances be compared with that taking place in control-treated or untreated epithelia using any suitable model of epithelial regeneration known in the art. Promotion of epithelial regeneration may be of use to induce effective re-epithelialisation in contexts in which the re-epithelialisation response is impaired, inhibited, retarded or otherwise defective. Promotion of epithelial regeneration may be also effected to accelerate the rate of defective or normal epithelial regeneration responses in patients suffering from epithelial damage.

Some instances where re-epithelialisation response may be defective include conditions such as pemphigus, Hailey-Hailey disease (familial benign pemphigus), toxic epidermal necrolysis (TEN)/Lyell's syndrome, epidermolysis bullosa, cutaneous leishmaniasis and actinic keratosis. Defective re-epithelialisation of the lungs may be associated with idiopathic pulmonary fibrosis (IPF) or interstitial lung disease. Defective re-epithelialisation of the eye may be associated with conditions such as partial limbal stem cell deficiency or corneal erosions. Defective re-epithelialisation of the gastrointestinal tract or colon may be associated with conditions such as chronic anal fissures (fissure in ano), ulcerative colitis or Crohn's disease, and other inflammatory bowel disorders.

In some aspects, methods associated with the invention are used to prevent, reduce or otherwise inhibit compromised skin associated with scarring. This can be applied to any site within the body and any tissue or organ, including the skin, eye, nerves, tendons, ligaments, muscle, and oral cavity (including the lips and palate), as well as internal organs (such as the liver, heart, brain, abdominal cavity, pelvic cavity, thoracic cavity, guts and reproductive tissue). In the skin, treatment may change the morphology and organization of collagen fibers and may result in making the scars less visible and blend in with the surrounding skin. As used herein, prevention, reduction or inhibition of scarring encompasses any degree of prevention, reduction or inhibition in scarring as compared to the level of scarring occurring in a control-treated or untreated wound.

Prevention, reduction or inhibition of compromised skin, such as compromised skin associated with dermal scarring, can be assessed and/or measured with reference to microscopic and/or macroscopic characteristics. Macroscopic characteristics may include color, height, surface texture and stiffness of the skin. In some instances, prevention, reduction or inhibition of compromised skin may be demonstrated when the color, height, surface texture and stiffness of the skin resembles that of normal skin more closely after treatment than does a control that is untreated. Microscopic assessment of compromised skin may involve examining characteristics such as thickness and/or orientation and/or composition of the extracellular matrix (ECM) fibers, and cellularity of the compromised skin. In some instances, prevention, reduction or inhibition of compromised skin may be demonstrated when the thickness and/or orientation and/or composition of the extracellular matrix (ECM) fibers, and/or cellularity of the compromised skin resembles that of normal skin more closely after treatment than does a control that is untreated.

In some aspects, methods associated with the invention are used for cosmetic purposes, at least in part to contribute to improving the cosmetic appearance of compromised skin. In some embodiments, methods associated with the invention may be used to prevent, reduce or inhibit compromised skin such as scarring of wounds covering joints of the body. In other embodiments, methods associated with the invention may be used to promote accelerated wound healing and/or prevent, reduce or inhibit scarring of wounds at increased risk of forming a contractile scar, and/or of wounds located at sites of high skin tension.

In some embodiments, methods associated with the invention can be applied to promoting healing of compromised skin in instances where there is an increased risk of pathological scar formation, such as hypertrophic scars and keloids, which may have more pronounced deleterious effects than normal scarring. In some embodiments, methods described herein for promoting accelerated healing of compromised skin and/or preventing, reducing or inhibiting scarring are applied to compromised skin produced by surgical revision of pathological scars.

Aspects of the invention can be applied to compromised skin caused by burn injuries. Healing in response to burn injuries can lead to adverse scarring, including the formation of hypertrophic scars. Methods associated with the invention can be applied to treatment of all injuries involving damage to an epithelial layer, such as injuries to the skin in which the epidermis is damaged. Other non-limiting examples of injuries to epithelial tissue include injuries involving the respiratory epithelia, digestive epithelia or epithelia surrounding internal tissues or organs.

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: Identification of potent sd-rxRNAs targeting lncRNA ENST00000602414

sd-rxRNAs targeting lncRNA ENST00000602414 were designed, synthesized and screened *in vitro* to determine the ability of the sd-rxRNAs to reduce target lncRNA levels. The sd-rxRNAs were tested for activity in a human hepatocellular carcinoma cell line (40,000 cells/well, 96 well plate). The cells were treated with a panel of ENST00000602414 lncRNA-targeting sd-rxRNAs or non-targeting control (#26247) in media containing 10% FCS. The concentration of sd-rxRNA tested was 5 µM. The non-targeting control sd-rxRNA (#26247) is of similar structure to the lncRNA-targeting sd-rxRNAs and contains similar stabilizing modifications throughout both strands. Forty eight hours post-administration, cells were lysed and lncRNA levels determined with lncRNA-specific SYBR Green I qPCR assays and SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) according to the manufacturer's protocol. FIG.1 demonstrates the lncRNA-targeting sd-rxRNAs, comprising sense strands and antisense strands found in Tables 1 and 2, respectively, significantly reduce target gene lncRNA levels *in vitro* in a human hepatocarcinoma cell line. All sense sequences in Table 1 have the following modification: TEG-Chl, wherein Chl stands for cholesterol and TEG is a linker. Data were normalized, using geometric average to a panel of 4 house-keeping genes and graphed with respect to the mock (non-transfected) control. Samples were run in biological duplicates.

The human lncRNA sequence is represented by Ensembl transcript ID: ENST00000602414 (SEQ ID NO: 1), as shown below.

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GGAATAGCGTCATCAGTTCTATAAGAGAGCGTGTGCCGAAGGCCTCGGCCTTTCACATTCGGGAAGCGTCGGGAT
TAGGTGAAAGTACGTAGTTGTCTTTCGTAAGTTAAAATGATAATTGGGCCGAAACTTACTGCCTTACCTAAAAGG
CAGCGCAGTCAGGATATTGGTAGGTCGGGGGCGGCTTTGGAAACCCTTAAGTTTACAAGCATGCGCGGACTTGAG
TGCTCATTAGGTCGCCGGGCGTCCACGTGCAGCCCTGGACCCTGAACCCCGGCGTGCGTGGGCCGTGGGCCCTCG
GGGAAAGGTTCCGTGCACTCGGGGACTCCGGTGAAGCCTGTTCAGCCGTCTGTGTCATGTGGCCATCTTGAGTCT
ACTCTGTCGCTCTTGTGCCCTAGCACCCCGAGAACCGTCAGTTTGAGCCAGATGGAAGCTGAGCTGAACACATTA
CGATGGATGATGGAAACATAAGACTATCAAGAAATCCAAGTGGTAATGGGCGAAGTTTATTCAGCATCCGGCAAT
GGACTTATCGTAGTTGGGGAAACGGGTGTTCCGAATAATATCCTGGAAGTTATCAGGACACCTATTTTAAATATA
GGCCTGAATTTTGTAAAGTAATATTTAAGGTGGTCCGTGATAATTAAATAAAATGCTTAATTCATGTGGCTA
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Example 2: Identification of potent sd-rxRNAs targeting lncRNA MALAT1

sd-rxRNAs targeting lncRNA MALAT1 were designed, synthesized and screened *in vitro* to determine the ability of the sd-rxRNAs to reduce target lncRNA levels. The sd-rxRNAs were tested for activity in a human hepatocellular carcinoma cell line (40,000 cells/well, 96 well plate) and a human colorectal carcinoma cell line (40,000 cells/well). Cells were treated with a panel of MALAT1-targeting sd-rxRNAs or non-targeting control

(#26247) in media containing 10% FCS. The concentration of sd-rxRNA tested was 5 μM. The non-targeting control sd-rxRNA (#26247) is of similar structure to the MALAT1-targeting sd-rxRNAs and contains similar stabilizing modifications throughout both strands. Forty eight hours post-administration, cells were lysed and MALAT1 levels determined with MALAT1-specific SYBR Green I qPCR assays and SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) according to manufacturer's protocols. FIG. 2 demonstrates the MALAT1-targeting sd-rxRNAs, comprising sense and antisense sequences found in Tables 1 and 2, respectively, significantly reduce target gene lncRNA levels *in vitro* in a human hepatocellular carcinoma cell line. All sense sequences in Table 1 have the following modification: TEG-Chl, wherein Chl stands for cholesterol and TEG is a linker. Data were normalized, using geometric average, to a panel of 4 house-keeping genes and graphed with respect to the mock (non-transfected) control. Samples were run in biological duplicates.

The human MALAT1 sequence is represented by GenBank accession number EF177381 (SEQ ID NO: 2), as shown below.

GTAAAGGACTGGGGCCCCGCAACTGGCCTCTCCTGCCCTCTTAAGCGCAGCGCCATTTTAGCAACGCAGAAGCCC
GGCGCCGGGAAGCCTCAGCTCGCCTGAAGGCAGGTCCCCTCTGACGCCTCCGGGAGCCCAGGTTTCCCAGAGTCC
TTGGGACGCAGCGACGAGTTGTGCTGCTATCTTAGCTGTCCTTATAGGCTGGCCATTCCAGGTGGTGGTATTTAG
ATAAAACCACTCAAACTCTGCAGTTTGGTCTTGGGGTTTGGAGGAAAGCTTTTATTTTTCTTCCTGCTCCGGTTC
AGAAGGTCTGAAGCTCATACCTAACCAGGCATAACACAGAATCTGCAAAACAAAAACCCCTAAAAAAGCAGACCC
AGAGCAGTGTAAACACTTCTGGGTGTGTCCCTGACTGGCTGCCCAAGGTCTCTGTGTCTTCGGAGACAAAGCCAT
TCGCTTAGTTGGTCTACTTTAAAAGGCCACTTGAACTCGCTTTCCATGGCGATTTGCCTTGTGAGCACTTTCAGG
AGAGCCTGGAAGCTGAAAAACGGTAGAAAAATTTCCGTGCGGGCCGTGGGGGGCTGGCGGCAACTGGGGGGCCGC
AGATCAGAGTGGGCCACTGGCAGCCAACGGCCCCCGGGGCTCAGGCGGGGAGCAGCTCTGTGGTGTGGGATTGAG
GCGTTTTCCAAGAGTGGGTTTTCACGTTTCTAAGATTTCCCAAGCAGACAGCCCGTGCTGCTCCGATTTCTCGAA
CAAAAAAGCAAAACGTGTGGCTGTCTTGGGAGCAAGTCGCAGGACTGCAAGCAGTTGGGGGAGAAAGTCCGCCAT
TTTGCCACTTCTCAACCGTCCCTGCAAGGCTGGGGCTCAGTTGCGTAATGGAAAGTAAAGCCCTGAACTATCACA
CTTTAATCTTCCTTCAAAAGGTGGTAAACTATACCTACTGTCCCTCAAGAGAACACAAGAAGTGCTTTAAGAGGT
ATTTTAAAAGTTCCGGGGGTTTTGTGAGGTGTTTGATGACCCGTTTAAAATATGATTTCCATGTTTCTTTTGTCT
AAAGTTTGCAGCTCAAATCTTTCCACACGCTAGTAATTTAAGTATTTCTGCATGTGTAGTTTGCATTCAAGTTCC
ATAAGCTGTTAAGAAAAATCTAGAAAAGTAAAACTAGAACCTATTTTTAACCGAAGAACTACTTTTTGCCTCCCT
CACAAAGGCGGCGGAAGGTGATCGAATTCCGGTGATGCGAGTTGTTCTCCGTCTATAAATACGCCTCGCCCGAGC
TGTGCGGTAGGCATTGAGGCAGCCAGCGCAGGGGCTTCTGCTGAGGGGGCAGGCGGAGCTTGAGGAAACCGCAGA
TAAGTTTTTTTCTCTTTGAAAGATAGAGATTAATACAACTACTTAAAAAATATAGTCAATAGGTTACTAAGATAT
TGCTTAGCGTTAAGTTTTTAACGTAATTTTAATAGCTTAAGATTTTAAGAGAAAATATGAAGACTTAGAAGAGTA
GCATGAGGAAGGAAAAGATAAAAGGTTTCTAAAACATGACGGAGGTTGAGATGAAGCTTCTTCATGGAGTAAAAA
ATGTATTTAAAAGAAAATTGAGAGAAAGGACTACAGAGCCCCGAATTAATACCAATAGAAGGGCAATGCTTTTAG
ATTAAAATGAAGGTGACTTAAACAGCTTAAAGTTTAGTTTAAAAGTTGTAGGTGATTAAAATAATTTGAAGGCGA

TCTTTTAAAAAGAGATTAAACCGAAGGTGATTAAAAGACCTTGAAATCCATGACGCAGGGAGAATTGCGTCATTT
AAAGCCTAGTTAACGCATTTACTAAACGCAGACGAAAATGGAAAGATTAATTGGGAGTGGTAGGATGAAACAATT
TGGAGAAGATAGAAGTTTGAAGTGGAAAACTGGAAGACAGAAGTACGGGAAGGCGAAGAAAAGAATAGAGAAGAT
AGGGAAATTAGAAGATAAAAACATACTTTTAGAAGAAAAAAGATAAATTTAAACCTGAAAAGTAGGAAGCAGAAG
AAAAAAGACAAGCTAGGAAACAAAAAGCTAAGGGCAAAATGTACAAACTTAGAAGAAAATTGGAAGATAGAAACA
AGATAGAAAATGAAAATATTGTCAAGAGTTTCAGATAGAAAATGAAAAACAAGCTAAGACAAGTATTGGAGAAGT
ATAGAAGATAGAAAAATATAAAGCCAAAAATTGGATAAAATAGCACTGAAAAAATGAGGAAATTATTGGTAACCA
ATTTATTTTAAAAGCCCATCAATTTAATTTCTGGTGGTGCAGAAGTTAGAAGGTAAAGCTTGAGAAGATGAGGGT
GTTTACGTAGACCAGAACCAATTTAGAAGAATACTTGAAGCTAGAAGGGGAAGTTGGTTAAAAATCACATCAAAA
AGCTACTAAAAGGACTGGTGTAATTTAAAAAAAACTAAGGCAGAAGGCTTTTGGAAGAGTTAGAAGAATTTGGAA
GGCCTTAAATATAGTAGCTTAGTTTGAAAAATGTGAAGGACTTTCGTAACGGAAGTAATTCAAGATCAAGAGTAA
TTACCAACTTAATGTTTTTGCATTGGACTTTGAGTTAAGATTATTTTTTAAATCCTGAGGACTAGCATTAATTGA
CAGCTGACCCAGGTGCTACACAGAAGTGGATTCAGTGAATCTAGGAAGACAGCAGCAGACAGGATTCCAGGAACC
AGTGTTTGATGAAGCTAGGACTGAGGAGCAAGCGAGCAAGCAGCAGTTCGTGGTGAAGATAGGAAAAGAGTCCAG
GAGCCAGTGCGATTTGGTGAAGGAAGCTAGGAAGAAGGAAGGAGCGCTAACGATTTGGTGGTGAAGCTAGGAAAA
AGGATTCCAGGAAGGAGCGAGTGCAATTTGGTGATGAAGGTAGCAGGCGGCTTGGCTTGGCAACCACACGGAGGA
GGCGAGCAGGCGTTGTGCGTAGAGGATCCTAGACCAGCATGCCAGTGTGCCAAGGCCACAGGGAAAGCGAGTGGT
TGGTAAAAATCCGTGAGGTCGGCAATATGTTGTTTTTCTGGAACTTACTTATGGTAACCTTTTATTTATTTTCTA
ATATAATGGGGGAGTTTCGTACTGAGGTGTAAAGGGATTTATATGGGGACGTAGGCCGATTTCCGGGTGTTGTAG
GTTTCTCTTTTTCAGGCTTATACTCATGAATCTTGTCTGAAGCTTTTGAGGGCAGACTGCCAAGTCCTGGAGAAA
TAGTAGATGGCAAGTTTGTGGGTTTTTTTTTTTTACACGAATTTGAGGAAAACCAAATGAATTTGATAGCCAAAT
TGAGACAATTTCAGCAAATCTGTAAGCAGTTTGTATGTTTAGTTGGGGTAATGAAGTATTTCAGTTTTGTGAATA
GATGACCTGTTTTTACTTCCTCACCCTGAATTCGTTTTGTAAATGTAGAGTTTGGATGTGTAACTGAGGCGGGGG
GGAGTTTTCAGTATTTTTTTTTGTGGGGGTGGGGGCAAAATATGTTTTCAGTTCTTTTTCCCTTAGGTCTGTCTA
GAATCCTAAAGGCAAATGACTCAAGGTGTAACAGAAAACAAGAAAATCCAATATCAGGATAATCAGACCACCACA
GGTTTACAGTTTATAGAAACTAGAGCAGTTCTCACGTTGAGGTCTGTGGAAGAGATGTCCATTGGAGAAATGGCT
GGTAGTTACTCTTTTTTCCCCCCACCCCCTTAATCAGACTTTAAAAGTGCTTAACCCCTTAAACTTGTTATTTTT
TACTTGAAGCATTTTGGGATGGTCTTAACAGGGAAGAGAGAGGGTGGGGGAGAAAATGTTTTTTTCTAAGATTTT
CCACAGATGCTATAGTACTATTGACAAACTGGGTTAGAGAAGGAGTGTACCGCTGTGCTGTTGGCACGAACACCT
TCAGGGACTGGAGCTGCTTTTATCCTTGGAAGAGTATTCCCAGTTGAAGCTGAAAAGTACAGCACAGTGCAGCTT
TGGTTCATATTCAGTCATCTCAGGAGAACTTCAGAAGAGCTTGAGTAGGCCAAATGTTGAAGTTAAGTTTTCCAA
TAATGTGACTTCTTAAAAGTTTATTAAAGGGGAGGGGCAAATATTGGCAATTAGTTGGCAGTGGCCTGTTACGG
TTGGGATTGGTGGGGTGGGTTTAGGTAATTGTTTAGTTTATGATTGCAGATAAACTCATGCCAGAGAACTTAAAG
TCTTAGAATGGAAAAAGTAAAGAAATATCAACTTCCAAGTTGGCAAGTAACTCCCAATGATTTAGTTTTTTTCCC
CCCAGTTTGAATTGGGAAGCTGGGGGAAGTTAAATATGAGCCACTGGGTGTACCAGTGCATTAATTTGGGCAAGG
AAAGTGTCATAATTTGATACTGTATCTGTTTTCCTTCAAAGTATAGAGCTTTTGGGGAAGGAAAGTATTGAACTG
GGGGTTGGTCTGGCCTACTGGGCTGACATTAACTACAATTATGGGAAATGCAAAAGTTGTTTGGATATGGTAGTG
TGTGGTTCTCTTTTGGAATTTTTTTCAGGTGATTTAATAATAATTTAAAACTACTATAGAAACTGCAGAGCAAAG
GAAGTGGCTTAATGATCCTGAAGGGATTTCTTCTGATGGTAGCTTTTGTATTATCAAGTAAGATTCTATTTTCAG
TTGTGTGTAAGCAAGTTTTTTTTTAGTGTAGGAGAAATACTTTTCCATTGTTTAACTGCAAAACAAGATGTTAAG
GTATGCTTCAAAAATTTTGTAAATTGTTTATTTTAAACTTATCTGTTTGTAAATTGTAACTGATTAAGAATTGTG

ATAGTTCAGCTTGAATGTCTCTTAGAGGGTGGGCTTTTGTTGATGAGGGAGGGGAAACTTTTTTTTTTTCTATAG
ACTTTTTTCAGATAACATCTTCTGAGTCATAACCAGCCTGGCAGTATGATGGCCTAGATGCAGAGAAAACAGCTC
CTTGGTGAATTGATAAGTAAAGGCAGAAAAGATTATATGTCATACCTCCATTGGGGAATAAGCATAACCCTGAGA
TTCTTACTACTGATGAGAACATTATCTGCATATGCCAAAAAATTTTAAGCAAATGAAAGCTACCAATTTAAAGTT
ACGGAATCTACCATTTTAAAGTTAATTGCTTGTCAAGCTATAACCACAAAAATAATGAATTGATGAGAAATACAA
TGAAGAGGCAATGTCCATCTCAAAATACTGCTTTTACAAAAGCAGAATAAAAGCGAAAAGAAATGAAAATGTTAC
ACTACATTAATCCTGGAATAAAAGAAGCCGAAATAAATGAGAGATGAGTTGGGATCAAGTGGATTGAGGAGGCTG
TGCTGTGTGCCAATGTTTCGTTTGCCTCAGACAGGTATCTCTTCGTTATCAGAAGAGTTGCTTCATTTCATCTGG
GAGCAGAAAACAGCAGGCAGCTGTTAACAGATAAGTTTAACTTGCATCTGCAGTATTGCATGTTAGGGATAAGTG
CTTATTTTTAAGAGCTGTGGAGTTCTTAAATATCAACCATGGCACTTTCTCCTGACCCCTTCCCTAGGGGATTTC
AGGATTGAGAAATTTTTCCATCGAGCCTTTTTAAAATTGTAGGACTTGTTCCTGTGGGCTTCAGTGATGGGATAG
TACACTTCACTCAGAGGCATTTGCATCTTTAAATAATTTCTTAAAAGCCTCTAAAGTGATCAGTGCCTTGATGCC
AACTAAGGAAATTTGTTTAGCATTGAATCTCTGAAGGCTCTATGAAAGGAATAGCATGATGTGCTGTTAGAATCA
GATGTTACTGCTAAAATTTACATGTTGTGATGTAAATTGTGTAGAAAACCATTAAATCATTCAAAATAATAAACT
ATTTTTATTAGAGAATGTATACTTTTAGAAAGCTGTCTCCTTATTTAAATAAAATAGTGTTTGTCTGTAGTTCAG
TGTTGGGGCAATCTTGGGGGGGATTCTTCTCTAATCTTTCAGAAACTTTGTCTGCGAACACTCTTTAATGGACCA
GATCAGGATTTGAGCGGAAGAACGAATGTAACTTTAAGGCAGGAAAGACAAATTTTATTCTTCATAAAGTGATGA
GCATATAATAATTCCAGGCACATGGCAATAGAGGCCCTCTAAATAAGGAATAAATAACCTCTTAGACAGGTGGGA
GATTATGATCAGAGTAAAAGGTAATTACACATTTTATTTCCAGAAAGTCAGGGGTCTATAAATTGACAGTGATTA
GAGTAATACTTTTTCACATTTCCAAAGTTTGCATGTTAACTTTAAATGCTTACAATCTTAGAGTGGTAGGCAATG
TTTTACACTATTGACCTTATATAGGGAAGGGAGGGGGTGCCTGTGGGGTTTTAAAGAATTTTCCTTTGCAGAGGC
ATTTCATCCTTCATGAAGCCATTCAGGATTTTGAATTGCATATGAGTGCTTGGCTCTTCCTTCTGTTCTAGTGAG
TGTATGAGACCTTGCAGTGAGTTTATCAGCATACTCAAAATTTTTTTCCTGGAATTTGGAGGGATGGGAGGAGGG
GGTGGGGCTTACTTGTTGTAGCTTTTTTTTTTTTTACAGACTTCACAGAGAATGCAGTTGTCTTGACTTCAGGTC
TGTCTGTTCTGTTGGCAAGTAAATGCAGTACTGTTCTGATCCCGCTGCTATTAGAATGCATTGTGAAACGACTGG
AGTATGATTAAAAGTTGTGTTCCCCAATGCTTGGAGTAGTGATTGTTGAAGGAAAAAAATCCAGCTGAGTGATAAA
GGCTGAGTGTTGAGGAAATTTCTGCAGTTTTAAGCAGTCGTATTTGTGATTGAAGCTGAGTACATTTTGCTGGTG
TATTTTTAGGTAAAATGCTTTTTGTTCATTTCTGGTGGTGGGAGGGGACTGAAGCCTTTAGTCTTTTCCAGATGC
AACCTTAAAATCAGTGACAAGAAACATTCCAAACAAGCAACAGTCTTCAAGAAATTAAACTGGCAAGTGGAAATG
TTTAAACAGTTCAGTGATCTTTAGTGCATTGTTTATGTGTGGGTTTCTCTCTCCCCTCCCTTGGTCTTAATTCTT
ACATGCAGGAACACTCAGCAGACACACGTATGCGAAGGGCCAGAGAAGCCAGACCCAGTAAGAAAAAATAGCCTA
TTTACTTTAAATAAACCAAACATTCCATTTTAAATGTGGGGATTGGGAACCACTAGTTCTTTCAGATGGTATTCT
TCAGACTATAGAAGGAGCTTCCAGTTGAATTCACCAGTGGACAAAATGAGGAAAACAGGTGAACAAGCTTTTTCT
GTATTTACATACAAAGTCAGATCAGTTATGGGACAATAGTATTGAATAGATTTCAGCTTTATGCTGGAGTAACTG
GCATGTGAGCAAACTGTGTTGGCGTGGGGGTGGAGGGGTGAGGTGGGCGCTAAGCCTTTTTTTAAGATTTTTCAG
GTACCCCTCACTAAAGGCACCGAAGGCTTAAAGTAGGACAACCATGGAGCCTTCCTGTGGCAGGAGAGACAACAA
AGCGCTATTATCCTAAGGTCAAGAGAAGTGTCAGCCTCACCTGATTTTTATTAGTAATGAGGACTTGCCTCAACT
CCCTCTTTCTGGAGTGAAGCATCCGAAGGAATGCTTGAAGTACCCCTGGGCTTCTCTTAACATTTAAGCAAGCTG
TTTTTATAGCAGCTCTTAATAATAAAGCCCAAATCTCAAGCGGTGCTTGAAGGGGAGGGAAAGGGGGAAAGCGGG
CAACCACTTTTCCCTAGCTTTTCCAGAAGCCTGTTAAAAGCAAGGTCTCCCCACAAGCAACTTCTCTGCCACATC
GCCACCCCGTGCCTTTTGATCTAGCACAGACCCTTCACCCCTCACCTCGATGCAGCCAGTAGCTTGGATCCTTGT

GGGCATGATCCATAATCGGTTTCAAGGTAACGATGGTGTCGAGGTCTTTGGTGGGTTGAACTATGTTAGAAAAGG
CCATTAATTTGCCTGCAAATTGTTAACAGAAGGGTATTAAAACCACAGCTAAGTAGCTCTATTATAATACTTATC
CAGTGACTAAAACCAACTTAAACCAGTAAGTGGAGAAATAACATGTTCAAGAACTGTAATGCTGGGTGGGAACAT
GTAACTTGTAGACTGGAGAAGATAGGCATTTGAGTGGCTGAGAGGGCTTTTGGGTGGGAATGCAAAAATTCTCTG
CTAAGACTTTTTCAGGTGAACATAACAGACTTGGCCAAGCTAGCATCTTAGCGGAAGCTGATCTCCAATGCTCTT
CAGTAGGGTCATGAAGGTTTTTCTTTTCCTGAGAAAACAACACGTATTGTTTTCTCAGGTTTTGCTTTTTGGCCT
TTTTCTAGCTTAAAAAAAAAAAAAGCAAAAGATGCTGGTGGTTGGCACTCCTGGTTTCCAGGACGGGGTTCAAAT
CCCTGCGGCGTCTTTGCTTTGACTACTAATCTGTCTTCAGGACTCTTTCTGTATTTCTCCTTTTCTCTGCAGGTG
CTAGTTCTTGGAGTTTTGGGGAGGTGGGAGGTAACAGCACAATATCTTTGAACTATATACATCCTTGATGTATAA
TTTGTCAGGAGCTTGACTTGATTGTATATTCATATTTACACGAGAACCTAATATAACTGCCTTGTCTTTTTCAGG
TAATAGCCTGCAGCTGGTGTTTTGAGAAGCCCTACTGCTGAAAACTTAACAATTTTGTGTAATAAAAATGGAGAA
GCTCTAAA

<u>Example 3: Identification of sd-rxRNAs Targeting lncRNAs</u>

sd-rxRNAs targeting the following lncRNAs; ENST00000585065, ENST00000607352, ENST00000456581, ENST00000340510, ENST00000605920, ENST00000455699, ENST00000555578, ENST00000565493, 580048 were designed, synthesized and screened *in vitro* to determine the ability of the sd-rxRNAs to reduce target lncRNA levels. The sd-rxRNAs were tested for activity in a human hepatocellular carcinoma cell line (40,000 cells/well, 96 well plate) or a human colorectal carcinoma cell line (40,000 cells/well, 96 well plate). Cells were treated with a panel of lncRNA-targeting sd-rxRNAs or non-targeting control (#26247) in media containing 10% FCS. The concentration of sd-rxRNA tested was 5 µM. The non-targeting control sd-rxRNA (#26247) is of similar structure to the lncRNA-targeting sd-rxRNAs and contains similar stabilizing modifications throughout both strands. Forty eight hours post-administration, cells were lysed and lncRNA levels determined with lncRNA-specific SYBR Green I qPCR assays and SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) according to manufacturer's protocol. FIG. 3 demonstrates the lncRNA-targeting sd-rxRNAs, comprising sense and antisense sequences found in Tables 1 and 2, respectively, significantly reduce target gene lncRNA levels *in vitro* in a human hepatocellular carcinoma cell line or a human colorectal carcinoma cell line. All sense sequences in Table 1 have the following modification: TEG-Chl, wherein Chl stands for cholesterol and TEG is a linker. Data were normalized, using geometric average, to a panel of 4 house-keeping genes and graphed with respect to the mock (non-transfected) control. Samples were run in biological duplicates.

Table 1. Sense Strand Oligonucleotides

Oligo ID	Gene Name	Accession number	Start Site	SEQ ID NO:	Sense sequence	Sense Chemistry	Sense Backbone
lncRala1 1	LNC Rala1	ENST00000340510	140	3	CCGCUUCAGAAUCA	mm0mmmm00m0mmm	ooooooooooosso
lncRala1 2	LNC Rala1	ENST00000340510	296	4	UGAUCCCGAGCCUA	mm0mmmm000mmmm	ooooooooooosso
lncRala1 3	LNC Rala1	ENST00000340510	366	5	UUUUUCCGCUGUAA	mmmmmmm0mm0mmm	ooooooooooosso
lncRala1 4	LNC Rala1	ENST00000340510	367	6	UUUUCCGCUGUAAA	mmmmmm0mm0m0mm	ooooooooooosso
lncRala1 5	LNC Rala1	ENST00000340510	368	7	UUUCCGCUGUAAAA	mmmmm0mm0m00mm	ooooooooooosso
lncRala1 6	LNC Rala1	ENST00000340510	369	8	UUCCGCUGUAAAUA	mmmm0mm0m000mm	ooooooooooosso
lncRala1 7	LNC Rala1	ENST00000340510	370	9	UCCGCUGUAAAUAA	mmm0mm0m000mmm	ooooooooooosso
lncRala1 8	LNC Rala1	ENST00000340510	487	10	GCCAAGCGGAAUUA	mmm000m00m0mmm	ooooooooooosso
lncRala1 9	LNC Rala1	ENST00000340510	488	11	CCAAGCGGAAUUUA	mm000m0000mmmm	ooooooooooosso
lncRala1 10	LNC Rala1	ENST00000340510	489	12	CAAGCGGAAUUUAA	mm00m00m0mmmmm	ooooooooooosso
lncRala1 11	LNC Rala1	ENST00000340510	490	13	AAGCGGAAUUUAAA	mm0m00m0mmm0mm	ooooooooooosso
lncRala1 12	LNC Rala1	ENST00000340510	491	14	AGCGGAAUUUAAAA	mmm00m0mmm00mm	ooooooooooosso
lncRala1 13	LNC Rala1	ENST00000340510	492	15	GCGGAAUUUAAAUA	mm00m0mmm000mm	ooooooooooosso
lncRala1 14	LNC Rala1	ENST00000340510	620	16	UGAGCCGCAGAGAA	mm00mm0m00m0mm	ooooooooooosso
lncRala1 15	LNC Rala1	ENST00000340510	622	17	AGCCGCAGAGAUCA	mmmm0m00m00mmm	ooooooooooosso
lncRala1 16	LNC Rala1	ENST00000340510	852	18	UACCACGUCAGUCA	mmmm0m0mm00mmm	ooooooooooosso
lncRala1 17	LNC Rala1	ENST00000340510	853	19	ACCACGUCAGUCUA	mmm0m0mm00mmmm	ooooooooooosso

lncRala1 18	LNC Rala1	ENST00000 340510	1662	20	ACGAGCUUAA CACA	mm000mmm00m 0mm	ooooooooo oosso
lncRala1 19	LNC Rala1	ENST00000 340510	1663	21	CGAGCUUAAC ACGA	mmm0mmm00m 0mmm	ooooooooo oosso
lncRala1 20	LNC Rala1	ENST00000 340510	1664	22	GAGCUUAACA CGCA	mm0mmm00m0 m0mm	ooooooooo oosso
lncRala1 21	LNC Rala1	ENST00000 340510	1205	23	CCUUUCGAAU GCAA	mmmmmm000m 0mmm	ooooooooo oosso
lncRala1 22	LNC Rala1	ENST00000 340510	1208	24	UUCGAAUGCA CUUA	mmm000m0m0m mmm	ooooooooo oosso
lncRala1 23	LNC Rala1	ENST00000 340510	1926	25	UCAAGUCGAC GUCA	mm000mm00m0 mmm	ooooooooo oosso
lncRala1 24	LNC Rala1	ENST00000 340510	2933	26	AGGCCCCGAA CUUA	mm0mmmm000 mmmm	ooooooooo oosso
lncRala1 25	LNC Rala1	ENST00000 340510	1857	27	CCAUCGUUAC AAUA	mm0mm0mm0m 00mm	ooooooooo oosso
lncRala1 26	LNC Rala1	ENST00000 340510	1203	28	AUCCUUUCGA AUGA	mmmmmmmm00 0mmm	ooooooooo oosso
lncRala1 27	LNC Rala1	ENST00000 340510	1784	29	GGCCCAUACC CUAA	mmmmm0m0mm mmmm	ooooooooo oosso
lncRala1 28	LNC Rala1	ENST00000 340510	99	30	UAUAGACCCU GAAA	mmm000mmmm 00mm	ooooooooo oosso
lncRala1 29	LNC Rala1	ENST00000 340510	1480	31	UAGUGCUAUC ACAA	mm0m0mm0mm 0mmm	ooooooooo oosso
lncRala1 30	LNC Rala1	ENST00000 340510	1154	32	GUUGACCACU GCAA	mmm00mm0mm 0mmm	ooooooooo oosso
lncZBTB42 1	LNC ZBTB42	ENST00000 555578	588	33	UCUGCCCGAA UCUA	mmm0mmm000 mmmm	ooooooooo oosso
lncZBTB42 2	LNC ZBTB42	ENST00000 555578	590	34	UGCCCGAAUC UUCA	mmmmm000mm mmmm	ooooooooo oosso
lncZBTB42 3	LNC ZBTB42	ENST00000 555578	593	35	CCGAAUCUUC ACAA	mm000mmmmm 0mmm	ooooooooo oosso
lncZBTB42 4	LNC ZBTB42	ENST00000 555578	801	36	AAUUCGACCC GUAA	mmmmm00mmm 0mmm	ooooooooo oosso
lncZBTB42 5	LNC ZBTB42	ENST00000 555578	804	37	UCGACCCGUA ACAA	mm00mmm0m00 mmm	ooooooooo oosso
lncZBTB42 6	LNC ZBTB42	ENST00000 555578	807	38	ACCCGUAACA GCUA	mmmm0m00m00 mmm	ooooooooo oosso

lncZBTB42 7	LNC ZBTB42	ENST00000 555578	836	39	UCCGAUGUGC UUCA	mmm00m0m0m mmmm	ooooooooo oosso
lncZBTB42 8	LNC ZBTB42	ENST00000 555578	960	40	ACGGACCUUU AUUA	mm000mmmmm 0mmm	ooooooooo oosso
lncZBTB42 9	LNC ZBTB42	ENST00000 555578	1073	41	UCUCCGAAGA GAUA	mmmmm000m00 0mm	ooooooooo oosso
lncZBTB42 10	LNC ZBTB42	ENST00000 555578	1075	42	UCCGAAGAGA UUCA	mmm000m000m mmm	ooooooooo oosso
lncZBTB42 11	LNC ZBTB42	ENST00000 555578	1076	43	CCGAAGAGAU UCCA	mm000m000mm mmm	ooooooooo oosso
lncZBTB42 12	LNC ZBTB42	ENST00000 555578	1281	44	AGCCGAUUAG CUGA	mmmm00mm00 mmmm	ooooooooo oosso
lncZBTB42 13	LNC ZBTB42	ENST00000 555578	1581	45	CUUAUCGCCA CACA	mmm0mm0mm0 m0mm	ooooooooo oosso
lncZBTB42 14	LNC ZBTB42	ENST00000 555578	2212	46	UGGACGUUUG AAAA	mm00m0mmm00 0mm	ooooooooo oosso
lncZBTB42 15	LNC ZBTB42	ENST00000 555578	2213	47	GGACGUUUGA AAAA	mm0m0mmm00 m0mm	ooooooooo oosso
lncZBTB42 16	LNC ZBTB42	ENST00000 555578	2137	48	UAGGCCUAAU CAAA	mm00mmm00m m0mm	ooooooooo oosso
lncZBTB42 17	LNC ZBTB42	ENST00000 555578	2141	49	CCUAAUCAAC GUAA	mmm00mm00m0 mmm	ooooooooo oosso
lncZBTB42 18	LNC ZBTB42	ENST00000 555578	636	50	UUCCCGUCUU UAUA	mmmmm0mmm mm0mm	ooooooooo oosso
lncZBTB42 19	LNC ZBTB42	ENST00000 555578	1574	51	ACACAAGCUU AUCA	mm0m000mmm0 mmm	ooooooooo oosso
lncZBTB42 20	LNC ZBTB42	ENST00000 555578	1575	52	CACAAGCUUA UCGA	mmm000mmm0 mmmm	ooooooooo oosso
lncZBTB42 21	LNC ZBTB42	ENST00000 555578	694	53	CUCACCCUAA CUUA	mmm0mmmm00 mmmm	ooooooooo oosso
lncZBTB42 22	LNC ZBTB42	ENST00000 555578	699	54	CCUAACUUGA UGGA	mmm00mmm00 m0mm	ooooooooo oosso
lncZBTB42 23	LNC ZBTB42	ENST00000 555578	2145	55	AUCAACGUAA AUCA	mmm00m0m000 mmm	ooooooooo oosso
lncZBTB42 24	LNC ZBTB42	ENST00000 555578	2149	56	ACGUAAAUCU GUCA	mm0m000mmm0 mmm	ooooooooo oosso
lncZBTB42 25	LNC ZBTB42	ENST00000 555578	700	57	CUAACUUGAU GGAA	mm00mmm00m0 0mm	ooooooooo oosso

lncZBTB42 26	LNC ZBTB42	ENST00000 555578	2134	58	AGUUAGGCCU AAUA	mmmm000mmm 00mm	ooooooooo oosso
lncZBTB42 27	LNC ZBTB42	ENST00000 555578	1307	59	GUGUAAGGAC UGCA	mm0m00m00mm 0mm	ooooooooo oosso
lncZBTB42 28	LNC ZBTB42	ENST00000 555578	640	60	CGUCUUUAUA AGGA	mmmmmmm0m0 00mm	ooooooooo oosso
lncZBTB42 29	LNC ZBTB42	ENST00000 555578	1616	61	CCUGGAUUAC AAGA	mmm000mm0m0 0mm	ooooooooo oosso
lncZBTB42 30	LNC ZBTB42	ENST00000 555578	2133	62	GAGUUAGGCC UAAA	mm0mm000mm m0mm	ooooooooo oosso
lncPANK1 1	LNC PANK1	ENST00000 455699	174	63	AUUGGAGCUC AACA	mmm00m0mmm 00mm	ooooooooo oosso
lncPANK1 2	LNC PANK1	ENST00000 455699	176	64	UGGAGCUCAA CUAA	mm000mmm00m mmm	ooooooooo oosso
lncPANK1 3	LNC PANK1	ENST00000 455699	179	65	AGCUCAACUA CCGA	mmmmm00mm0 mmmm	ooooooooo oosso
lncPANK1 4	LNC PANK1	ENST00000 455699	188	66	ACCGACUGUG UCAA	mmm00mm0m0 mmmm	ooooooooo oosso
lncPANK1 5	LNC PANK1	ENST00000 455699	191	67	GACUGUGUCA AUCA	mmmm0m0mm0 0mmm	ooooooooo oosso
lncPANK1 6	LNC PANK1	ENST00000 455699	211	68	AGUAUCAGGU UCCA	mmm0mm000m mmmm	ooooooooo oosso
lncPANK1 7	LNC PANK1	ENST00000 455699	419	69	GGUCUAUAGU CUUA	mmmmm0m00m mmmm	ooooooooo oosso
lncPANK1 8	LNC PANK1	ENST00000 455699	565	70	CUUGUAUCCG UAAA	mmm0m0mmm0 m0mm	ooooooooo oosso
lncPANK1 9	LNC PANK1	ENST00000 455699	568	71	GUAUCCGUAA GUCA	mm0mmm0m000 mmm	ooooooooo oosso
lncPANK1 10	LNC PANK1	ENST00000 455699	571	72	UCCGUAAGUC ACAA	mmm0m000mm0 mmm	ooooooooo oosso
lncPANK1 11	LNC PANK1	ENST00000 455699	573	73	CGUAAGUCAC ACAA	mmm000mm0m0 mmm	ooooooooo oosso
lncPANK1 12	LNC PANK1	ENST00000 455699	636	74	AAAUGUCGAA AAGA	mm0m0mm000m 0mm	ooooooooo oosso
lncPANK1 13	LNC PANK1	ENST00000 455699	415	75	UGCAGGUCUA UAGA	mmm000mmm0 m0mm	ooooooooo oosso
lncPANK1 14	LNC PANK1	ENST00000 455699	418	76	AGGUCUAUAG UCUA	mm0mmm0m00 mmmm	ooooooooo oosso

lncPANK1 15	LNC PANK1	ENST00000 455699	505	77	AGGAUUAUA UGCCA	mm00mm0m0m0 mmm	ooooooooo oosso
lncPANK1 16	LNC PANK1	ENST00000 455699	259	78	AGACAAUACC AGAA	mm0m00m0mm0 0mm	ooooooooo oosso
lncPANK1 17	LNC PANK1	ENST00000 455699	421	79	UCUAUAGUCU UUAA	mmm0m00mmm mmmm	ooooooooo oosso
lncPANK1 18	LNC PANK1	ENST00000 455699	502	80	ACCAGGAUUA UAUA	mmm00m0mm0 m0mm	ooooooooo oosso
lncPANK1 19	LNC PANK1	ENST00000 455699	341	81	AGUAAUAGCU GCAA	mmm00m00mm0 mmm	ooooooooo oosso
lncPANK1 20	LNC PANK1	ENST00000 455699	351	82	GCAUAACCUU GAGA	mm0m00mmmm 00mm	ooooooooo oosso
lncPANK1 21	LNC PANK1	ENST00000 455699	257	83	GCAGACAAUA CCAA	mm000m00m0m mmm	ooooooooo oosso
lncPANK1 22	LNC PANK1	ENST00000 455699	367	84	GAUACUGACU GAGA	mmm0mm00mm 00mm	ooooooooo oosso
lncPANK1 23	LNC PANK1	ENST00000 455699	55	85	UGAGUCUUAU GUCA	mm00mmmm0m 0mmm	ooooooooo oosso
lncPANK1 24	LNC PANK1	ENST00000 455699	424	86	AUAGUCUUUA CUCA	mm00mmmmm0 mmmm	ooooooooo oosso
lncPANK1 25	LNC PANK1	ENST00000 455699	253	87	CUUGGCAGAC AAUA	mmm00m000m0 0mm	ooooooooo oosso
lncPANK1 26	LNC PANK1	ENST00000 455699	217	88	AGGUUCCUGU GCUA	mm0mmmmm0m 0mmm	ooooooooo oosso
lncPANK1 27	LNC PANK1	ENST00000 455699	545	89	AAGCCUCUAU UGUA	mm0mmmmm0m m0mm	ooooooooo oosso
lncPANK1 28	LNC PANK1	ENST00000 455699	304	90	CCAAAUGUUA GGAA	mm000m0mm00 0mm	ooooooooo oosso
lncPANK1 29	LNC PANK1	ENST00000 455699	115	91	AGGAUGUAG AAGUA	mm00m0m00m0 0mm	ooooooooo oosso
lncPANK1 30	LNC PANK1	ENST00000 455699	150	92	CAAAGCAUCU CCAA	mm000m0mmm mmmm	ooooooooo oosso
lncEBF3 1	LNC EBF3	ENST00000 456581	744	93	UGGCGACUUU UGUA	mm0m00mmmm m0mm	ooooooooo oosso
lncEBF3 2	LNC EBF3	ENST00000 456581	746	94	GCGACUUUUG UAUA	mm00mmmmm0 m0mm	ooooooooo oosso
lncEBF3 3	LNC EBF3	ENST00000 456581	1506	95	UAAAGACGGA UGAA	mm0m00m000m 0mm	ooooooooo oosso

lncEBF3 4	LNC EBF3	ENST00000 456581	1593	96	UAAAGACGAA UAUA	mm0000m000m0 mm	ooooooooo oosso
lncEBF3 5	LNC EBF3	ENST00000 456581	1596	97	AGACGAAUAU GCUA	mm0m000m0m0 mmm	ooooooooo oosso
lncEBF3 6	LNC EBF3	ENST00000 456581	1652	98	AGGAAUCGUC AACA	mm000mm0mm0 0mm	ooooooooo oosso
lncEBF3 7	LNC EBF3	ENST00000 456581	1655	99	AAUCGUCAAC AUCA	mmmm0mm00m 0mmm	ooooooooo oosso
lncEBF3 8	LNC EBF3	ENST00000 456581	1656	100	AUCGUCAACA UCUA	mmm0mm00m0 mmmm	ooooooooo oosso
lncEBF3 9	LNC EBF3	ENST00000 456581	1657	101	UCGUCAACAU CUUA	mm0mm00m0m mmmm	ooooooooo oosso
lncEBF3 10	LNC EBF3	ENST00000 456581	2032	102	GAAGCCGUUG CAGA	mm00mm0mm0 m0mm	ooooooooo oosso
lncEBF3 11	LNC EBF3	ENST00000 456581	2209	103	CCGUGGAAUU GUGA	mm0m00m0mm0 mmm	ooooooooo oosso
lncEBF3 12	LNC EBF3	ENST00000 456581	2593	104	CAAUUUCGAA AGGA	mm0mmmm000 m0mm	ooooooooo oosso
lncEBF3 13	LNC EBF3	ENST00000 456581	2595	105	AUUUCGAAAG GUUA	mmmmm000m00 mmm	ooooooooo oosso
lncEBF3 14	LNC EBF3	ENST00000 456581	2597	106	UUCGAAAGGU UCCA	mmm000m00mm mmm	ooooooooo oosso
lncEBF3 15	LNC EBF3	ENST00000 456581	240	107	UGCUCGGCUU UUUA	mmmmm00mmm mmmm	ooooooooo oosso
lncEBF3 16	LNC EBF3	ENST00000 456581	2193	108	ACAUCGUUCU CUUA	mm0mm0mmmm mmmm	ooooooooo oosso
lncEBF3 17	LNC EBF3	ENST00000 456581	1878	109	CGUAAUGGUC CCAA	mmm00m00mm mmmm	ooooooooo oosso
lncEBF3 18	LNC EBF3	ENST00000 456581	2205	110	UGCUCCGUGG AAUA	mmmmmm0m00 m0mm	ooooooooo oosso
lncEBF3 19	LNC EBF3	ENST00000 456581	1511	111	ACGGAUGAUU GUCA	mm000m00mm0 mmm	ooooooooo oosso
lncEBF3 20	LNC EBF3	ENST00000 456581	1843	112	GUACCAGAGG UGAA	mm0mm00m0m0 mm	ooooooooo oosso
lncEBF3 21	LNC EBF3	ENST00000 456581	1879	113	GUAAUGGUCC CAGA	mm00m00mmm m0mm	ooooooooo oosso
lncEBF3 22	LNC EBF3	ENST00000 456581	1354	114	UGACUGGUAC AGAA	mm0mm00m0m0 0mm	ooooooooo oosso

lncEBF3 23	LNC EBF3	ENST00000456581	2317	115	AGUAAGACUCACAA	mmm00m0mmm0mmm	ooooooooooosso
lncEBF3 24	LNC EBF3	ENST00000456581	1527	116	GAGGUCCAAGCUUA	mm00mmm000mmmm	ooooooooooosso
lncEBF3 25	LNC EBF3	ENST00000456581	1544	117	UGUAGGCCUUUGUA	mmm000mmmmm0mm	ooooooooooosso
lncEBF3 26	LNC EBF3	ENST00000456581	1325	118	GCCCAUGUAUCUGA	mmmm0m0m0mmmmm	ooooooooooosso
lncEBF3 27	LNC EBF3	ENST00000456581	2409	119	CUGAUGACUUGAGA	mm00m00mmm00mm	ooooooooooosso
lncEBF3 28	LNC EBF3	ENST00000456581	933	120	UCUGGUAAGUUCAA	mmm00m000mmmmm	ooooooooooosso
lncEBF3 29	LNC EBF3	ENST00000456581	1296	121	UAAUAACCCCUUUA	mm0m00mmmmmmmm	ooooooooooosso
lncEBF3 30	LNC EBF3	ENST00000456581	1297	122	AAUAACCCCUUUGA	mmm00mmmmmmmmm	ooooooooooosso
lncScand1 1	LNC Scand 1	ENST00000565493	849	123	GCCGACGUAUGAUA	mmm00m0m0m00mm	ooooooooooosso
lncScand1 2	LNC Scand 1	ENST00000565493	851	124	CGACGUAUGAUAAA	mm0m0m0m00m0mm	ooooooooooosso
lncScand1 3	LNC Scand 1	ENST00000565493	985	125	AUACGUCCACGUUA	mm0m0mmm0m0mmm	ooooooooooosso
lncScand1 4	LNC Scand 1	ENST00000565493	2663	126	UAGUCCCGAUUUUA	mm0mmmm00mmmmm	ooooooooooosso
lncScand1 5	LNC Scand 1	ENST00000565493	2971	127	UAUAGCGGACAAAA	m0m00m000m00mm	ooooooooooosso
lncScand1 6	LNC Scand 1	ENST00000565493	2973	128	UAGCGGACAAACUA	mm0m000m000mmm	ooooooooooosso
lncScand1 7	LNC Scand 1	ENST00000565493	3283	129	UAUAAGCGGACAUA	mmm000m000m0mm	ooooooooooosso
lncScand1 8	LNC Scand 1	ENST00000565493	3285	130	UAAGCGGACAUAGA	mm00m000m0m0mm	ooooooooooosso
lncScand1 9	LNC Scand 1	ENST00000565493	3288	131	GCGGACAUAGGAGA	mm000m0m00m0mm	ooooooooooosso
lncScand1 10	LNC Scand 1	ENST00000565493	3312	132	GUCUAGUCGAUGUA	mmmm00mm00m0mm	ooooooooooosso
lncScand1 11	LNC Scand 1	ENST00000565493	3313	133	UCUAGUCGAUGUUA	mmm00mm00m0mmm	ooooooooooosso

lncScand1 12	LNC Scand 1	ENST00000 565493	3314	134	CUAGUCGAUG UUAA	mm00mm00m0m mmm	ooooooooo oosso
lncScand1 13	LNC Scand 1	ENST00000 565493	4972	135	UAGAGGCGUG UUGA	mm00m0m0m0m mmm	ooooooooo oosso
lncScand1 14	LNC Scand 1	ENST00000 565493	654	136	GCUGUCGGAA GAGA	mmm0mm000m0 0mm	ooooooooo oosso
lncScand1 15	LNC Scand 1	ENST00000 565493	656	137	UGUCGGAAGA GAGA	mmmm000m0m0 0mm	ooooooooo oosso
lncScand1 16	LNC Scand 1	ENST00000 565493	733	138	ACUGGCCGUU UAUA	mmm00mm0mm m0mm	ooooooooo oosso
lncScand1 17	LNC Scand 1	ENST00000 565493	736	139	GGCCGUUUAU GGAA	mmmm0mmm0m 00mm	ooooooooo oosso
lncScand1 18	LNC Scand 1	ENST00000 565493	991	140	CCACGUUUGU UAAA	mm0m0mmm0m m0mm	ooooooooo oosso
lncScand1 19	LNC Scand 1	ENST00000 565493	1057	141	UAUGCUAGAC UGGA	mmm0mm000m m0mm	ooooooooo oosso
lncScand1 20	LNC Scand 1	ENST00000 565493	1386	142	CAGCGAGGCA AGAA	mm0m00m0m00 0mm	ooooooooo oosso
lncScand1 21	LNC Scand 1	ENST00000 565493	1459	143	CAGACGAGUC CUAA	mm00m000mmm mmm	ooooooooo oosso
lncScand1 22	LNC Scand 1	ENST00000 565493	1778	144	UGCCCGAUGU AUGA	mmmmm00m0m 0mmm	ooooooooo oosso
lncScand1 23	LNC Scand 1	ENST00000 565493	2158	145	AAUUCGUAGG AAAA	mmmmm0m00m 00mm	ooooooooo oosso
lncScand1 24	LNC Scand 1	ENST00000 565493	3981	146	AACACCCCUC UAAA	mmm0mmmmm mm00m	ooooooooo oosso
lncScand1 25	LNC Scand 1	ENST00000 565493	4064	147	AGCGAAUGCA GACA	mmm000m0m00 0mm	ooooooooo oosso
lncScand1 26	LNC Scand 1	ENST00000 565493	4168	148	GGUCUAACCA UUGA	mmmmm00mm0 mmmm	ooooooooo oosso
lncScand1 27	LNC Scand 1	ENST00000 565493	4435	149	UCUAGACGAU GGUA	mmm000m00m0 0mm	ooooooooo oosso
lncScand1 28	LNC Scand 1	ENST00000 565493	4440	150	ACGAUGGUUU UAGA	mm00m00mmm m0mm	ooooooooo oosso
lncScand1 29	LNC Scand 1	ENST00000 565493	4474	151	GAGCGUUUUU AGUA	mm0m0mmmmm 00mm	ooooooooo oosso
lncScand1 30	LNC Scand 1	ENST00000 565493	4535	152	AGCUUUACGA AUGA	mmmmmm0m00 0mmm	ooooooooo oosso

lncFAM69 C2 1	LNC FAM69C2	ENST00000 580048	166	153	CCGCUAAGAG AUAA	mm0mm000m00 mmm	ooooooooo oosso
lncFAM69 C2 2	LNC FAM69C2	ENST00000 580048	240	154	AAUUCGAUGA GCGA	mmmmm00m000 mmm	ooooooooo oosso
lncFAM69 C2 3	LNC FAM69C2	ENST00000 580048	241	155	AUUCGAUGAG CGCA	mmmm00m000m 0mm	ooooooooo oosso
lncFAM69 C2 4	LNC FAM69C2	ENST00000 580048	242	156	UUCGAUGAGC GCGA	mmm00m000m0 mmm	ooooooooo oosso
lncFAM69 C2 5	LNC FAM69C2	ENST00000 580048	764	157	AACGUUCGAC AAGA	mmm0mmm00m 00mm	ooooooooo oosso
lncFAM69 C2 6	LNC FAM69C2	ENST00000 580048	766	158	CGUUCGACAA GGAA	mmmmm00m00 m0mm	ooooooooo oosso
lncFAM69 C2 7	LNC FAM69C2	ENST00000 580048	768	159	UUCGACAAGG ACUA	mmm00m00m00 mmm	ooooooooo oosso
lncFAM69 C2 8	LNC FAM69C2	ENST00000 580048	790	160	ACGUUAACGG CACA	mm0mm00m00m 0mm	ooooooooo oosso
lncFAM69 C2 9	LNC FAM69C2	ENST00000 580048	795	161	AACGGCACAG CAUA	mmm00m0m00m 0mm	ooooooooo oosso
lncFAM69 C2 10	LNC FAM69C2	ENST00000 580048	932	162	UGUAGACGAA UAAA	mmm000m000m 0mm	ooooooooo oosso
lncFAM69 C2 11	LNC FAM69C2	ENST00000 580048	1391	163	UUCCAACGAG UGGA	mmmm00m000m 0mm	ooooooooo oosso
lncFAM69 C2 12	LNC FAM69C2	ENST00000 580048	1999	164	UUAUAACGAC AUUA	mm0m00m00m0 mmm	ooooooooo oosso
lncFAM69 C2 13	LNC FAM69C2	ENST00000 580048	2001	165	AUAACGACAU UGCA	mm00m00m0mm 0mm	ooooooooo oosso
lncFAM69 C2 14	LNC FAM69C2	ENST00000 580048	531	166	CGAUUUCGAG AAAA	mm0mmmm000 m0mm	ooooooooo oosso
lncFAM69 C2 15	LNC FAM69C2	ENST00000 580048	535	167	UUCGAGAAAU GACA	mmm000m00m0 0mm	ooooooooo oosso
lncFAM69 C2 16	LNC FAM69C2	ENST00000 580048	597	168	UCUCGAAUGG CUCA	mmmm000m00m mmm	ooooooooo oosso
lncFAM69 C2 17	LNC FAM69C2	ENST00000 580048	876	169	GAACCUCGAG UUAA	mm0mmmm000 mmmm	ooooooooo oosso
lncFAM69 C2 18	LNC FAM69C2	ENST00000 580048	879	170	CCUCGAGUUA GAGA	mmmm000mm00 0mm	ooooooooo oosso
lncFAM69 C2 19	LNC FAM69C2	ENST00000 580048	1573	171	CUGCGAAGAU GCAA	mm0m000m0m0 mmm	ooooooooo oosso

lncFAM69 C2 20	LNC FAM69C2	ENST00000 580048	1575	172	GCGAAGAUGC AAAA	mm000m0m0m0 0mm	ooooooooo oosso
lncFAM69 C2 21	LNC FAM69C2	ENST00000 580048	1927	173	UUAUGCUUAG UGGA	mm0m0mmm00 m0mm	ooooooooo oosso
lncFAM69 C2 22	LNC FAM69C2	ENST00000 580048	2019	174	GCUACACUCC AUGA	mmm0m0mmmm 0mmm	ooooooooo oosso
lncFAM69 C2 23	LNC FAM69C2	ENST00000 580048	2674	175	GUAUCAAGGA CCUA	mm0mm00m00m mmm	ooooooooo oosso
lncFAM69 C2 24	LNC FAM69C2	ENST00000 580048	2721	176	AUGCCCUAUU GAAA	mm0mmmm0mm 00mm	ooooooooo oosso
lncFAM69 C2 25	LNC FAM69C2	ENST00000 580048	3316	177	AUCCCAACUU GUAA	mmmmm00mmm 0mmm	ooooooooo oosso
lncFAM69 C2 26	LNC FAM69C2	ENST00000 580048	1749	178	ACUAUCGAAA UAAA	mmm0mm00m0 m0mm	ooooooooo oosso
lncFAM69 C2 27	LNC FAM69C2	ENST00000 580048	2532	179	CUUAUACCAG GAGA	mmm0m0mm00 m0mm	ooooooooo oosso
lncFAM69 C2 28	LNC FAM69C2	ENST00000 580048	2724	180	CCCUAUUGAA CAUA	mmmm0mm000 m0mm	ooooooooo oosso
lncFAM69 C2 29	LNC FAM69C2	ENST00000 580048	2744	181	UAGUAAGAU GGCUA	mm0m00m0m00 mmm	ooooooooo oosso
lncFAM69 C2 30	LNC FAM69C2	ENST00000 580048	3321	182	AACUUGUAGC UGCA	mmmmm0m00m m0mm	ooooooooo oosso
lncVEZF1 1	LNC VEZF1	ENST00000 585065	239	183	AUAUCGAGUA CUGA	mm0mm000m0m m0m	ooooooooo oosso
lncVEZF1 2	LNC VEZF1	ENST00000 585065	2307	184	UGUACUCGAG AAAA	mmm0mmm00m 00mm	ooooooooo oosso
lncVEZF1 3	LNC VEZF1	ENST00000 585065	2637	185	UGCGAUUUGU UGGA	mmm00mmm0m m0mm	ooooooooo oosso
lncVEZF1 4	LNC VEZF1	ENST00000 585065	2638	186	GCGAUUUGUU GGAA	mm00mmm0mm 00mm	ooooooooo oosso
lncVEZF1 5	LNC VEZF1	ENST00000 585065	2863	187	GCCCUCGACU ACCA	mmmmmm00mm 0mmm	ooooooooo oosso
lncVEZF1 6	LNC VEZF1	ENST00000 585065	3477	188	UGACAACGGC AGAA	mm0m00m00m0 0mm	ooooooooo oosso
lncVEZF1 7	LNC VEZF1	ENST00000 585065	3478	189	GACAACGGCA GAGA	mmm00m00m00 0mm	ooooooooo oosso
lncVEZF1 8	LNC VEZF1	ENST00000 585065	3675	190	CGUUUACCUU AGA	mmmmm0mmm m0mm	ooooooooo oosso

lncVEZF1 9	LNC VEZF1	ENST00000 585065	3804	191	CCACUCGAUA ACAA	mm0mmm00m00 mmm	ooooooooo oosso
lncVEZF1 10	LNC VEZF1	ENST00000 585065	3805	192	CACUCGAUAA CACA	mmmmm00m00 m0mm	ooooooooo oosso
lncVEZF1 11	LNC VEZF1	ENST00000 585065	3806	193	ACUCGAUAAC ACCA	mmmm00m00m0 mmm	ooooooooo oosso
lncVEZF1 12	LNC VEZF1	ENST00000 585065	3808	194	UCGAUAACAC CAAA	mm00m00m0mm 0mm	ooooooooo oosso
lncVEZF1 13	LNC VEZF1	ENST00000 585065	4348	195	AAUGCGUCCA UCUA	mmm0m0mmm0 mmmm	ooooooooo oosso
lncVEZF1 14	LNC VEZF1	ENST00000 585065	4349	196	AUGCGUCCAU CUGA	mm0m0mmm0m mmmm	ooooooooo oosso
lncVEZF1 15	LNC VEZF1	ENST00000 585065	4350	197	UGCGUCCAUC UGAA	m0m0mmm0mm m0mm	ooooooooo oosso
lncVEZF1 16	LNC VEZF1	ENST00000 585065	4351	198	GCGUCCAUCU GAAA	mm0mmm0mmm 00mm	ooooooooo oosso
lncVEZF1 17	LNC VEZF1	ENST00000 585065	2309	199	UACUCGAGAA ACUA	mmmmm000m00 mmm	ooooooooo oosso
lncVEZF1 18	LNC VEZF1	ENST00000 585065	2312	200	UCGAGAAACU UUGA	mm000m00mmm mmm	ooooooooo oosso
lncVEZF1 19	LNC VEZF1	ENST00000 585065	2449	201	ACCCAUUACC UACA	mmmm0mm0mm m0mm	ooooooooo oosso
lncVEZF1 20	LNC VEZF1	ENST00000 585065	2539	202	GGUGCCUAUG AGUA	mmm0mmm0m0 00mm	ooooooooo oosso
lncVEZF1 21	LNC VEZF1	ENST00000 585065	2541	203	UGCCUAUGAG UAUA	mmmmm0m000 m0mm	ooooooooo oosso
lncVEZF1 22	LNC VEZF1	ENST00000 585065	3674	204	CCCGUUUACC UUAA	mmm0mmm0mm mmmm	ooooooooo oosso
lncVEZF1 23	LNC VEZF1	ENST00000 585065	3727	205	CUUGGCGAAA GUAA	mmm00m00m00 mmm	ooooooooo oosso
lncVEZF1 24	LNC VEZF1	ENST00000 585065	3730	206	GGCGAAAGUA AAAA	mmm00000m000 mm	ooooooooo oosso
lncVEZF1 25	LNC VEZF1	ENST00000 585065	4441	207	UCUUGGACUA GAGA	mmmm000mm00 0mm	ooooooooo oosso
lncVEZF1 26	LNC VEZF1	ENST00000 585065	4444	208	UGGACUAGAG ACAA	mm00mm00m00 mmm	ooooooooo oosso
lncVEZF1 27	LNC VEZF1	ENST00000 585065	4650	209	AAGUUCGAUU UUUA	mm0mmm00mm mmmm	ooooooooo oosso

lncVEZF1 28	LNC VEZF1	ENST00000 585065	2723	210	UGAUAGGUU UAGCA	mm0m000mmm0 0mm	ooooooooo oosso
lncVEZF1 29	LNC VEZF1	ENST00000 585065	3116	211	CCUUAGUGUG CUUA	mmmm00m0m0 mmmm	ooooooooo oosso
lncVEZF1 30	LNC VEZF1	ENST00000 585065	3369	212	AGUUGGUCCA UUAA	mmmm00mmm0 mmmm	ooooooooo oosso
lncFBXO 1	LNC FBXO 256	ENST00000 607352	198	213	UUUAUAUGUC GUCA	mmm0m0m0mm 0mmm	ooooooooo oosso
lncFBXO 2	LNC FBXO 256	ENST00000 607352	199	214	UUAUAUGUCG UCUA	mm0m0m0mm0 mmmm	ooooooooo oosso
lncFBXO 3	LNC FBXO 256	ENST00000 607352	886	215	CUUUGUCGUA AGUA	mmmm0mm0m0 00mm	ooooooooo oosso
lncFBXO 4	LNC FBXO 256	ENST00000 607352	887	216	UUUGUCGUAA GUUA	mmm0mm0m000 mmm	ooooooooo oosso
lncFBXO 5	LNC FBXO 256	ENST00000 607352	888	217	UUGUCGUAAG UUAA	mm0mm0m000m mmm	ooooooooo oosso
lncFBXO 6	LNC FBXO 256	ENST00000 607352	889	218	UGUCGUAAGU UAUA	mmmm0m000m m0mm	ooooooooo oosso
lncFBXO 7	LNC FBXO 256	ENST00000 607352	890	219	GUCGUAAGUU AUGA	mmm0m000mm0 mmm	ooooooooo oosso
lncFBXO 8	LNC FBXO 256	ENST00000 607352	2596	220	UGAGAGCGUU GUUA	mm00m0m0mm0 mmm	ooooooooo oosso
lncFBXO 9	LNC FBXO 256	ENST00000 607352	2598	221	AGAGCGUUGU UUAA	mm00m0mm0m mmmm	ooooooooo oosso
lncFBXO 10	LNC FBXO 256	ENST00000 607352	2842	222	GUCUUGCGAC UGAA	mmmmm0m00m m0mm	ooooooooo oosso
lncFBXO 11	LNC FBXO 256	ENST00000 607352	2844	223	CUUGCGACUG AUCA	mmm0m00mm00 mmm	ooooooooo oosso
lncFBXO 12	LNC FBXO 256	ENST00000 607352	2846	224	UGCGACUGAU CUUA	mmm00mm00m mmmm	ooooooooo oosso
lncFBXO 13	LNC FBXO 256	ENST00000 607352	2845	225	UUGCGACUGA UCUA	mm0m00mm00m mmm	ooooooooo oosso
lncFBXO 14	LNC FBXO 256	ENST00000 607352	2847	226	GCGACUGAUC UUCA	mm00mm00mm mmmm	ooooooooo oosso
lncFBXO 15	LNC FBXO 256	ENST00000 607352	2871	227	CCUAUCCGUU ACUA	mmm0mmm0mm 0mmm	ooooooooo oosso
lncFBXO 16	LNC FBXO 256	ENST00000 607352	2873	228	UAUCCGUUAC UGAA	mmmmm0mm0m m0mm	ooooooooo oosso

lncFBXO 17	LNC FBXO 256	ENST00000 607352	3806	229	ACUCGAUAAC ACCA	mmmm00m00m0 mmm	ooooooooo oosso
lncFBXO 18	LNC FBXO 256	ENST00000 607352	685	230	GGUAGAUCUA GCUA	mmm000mmm00 mmm	ooooooooo oosso
lncFBXO 19	LNC FBXO 256	ENST00000 607352	687	231	UAGAUCUAGC UUCA	mm00mmm00m mmmm	ooooooooo oosso
lncFBXO 20	LNC FBXO 256	ENST00000 607352	689	232	GAUCUAGCUU CAUA	mmmmm00mmm m0mm	ooooooooo oosso
lncFBXO 21	LNC FBXO 256	ENST00000 607352	1073	233	AGGUAUCCAA UCCA	mm0m0mmm00 mmmm	ooooooooo oosso
lncFBXO 22	LNC FBXO 256	ENST00000 607352	1071	234	UAAGGUAUCC AAUA	mm000m0mmm0 0mm	ooooooooo oosso
lncFBXO 23	LNC FBXO 256	ENST00000 607352	2071	235	GACUAGCAUA GGUA	mmmm00m0m00 0mm	ooooooooo oosso
lncFBXO 24	LNC FBXO 256	ENST00000 607352	2074	236	UAGCAUAGGU CUGA	mm0m0m000mm mmm	ooooooooo oosso
lncFBXO 25	LNC FBXO 256	ENST00000 607352	2076	237	GCAUAGGUCU GUUA	mm0m000mmm0 mmm	ooooooooo oosso
lncFBXO 26	LNC FBXO 256	ENST00000 607352	2600	238	AGCGUUGUUU AAUA	mmm0mm0mmm 00mm	ooooooooo oosso
lncFBXO 27	LNC FBXO 256	ENST00000 607352	2870	239	UCCUAUCCGU UACA	mmmm0mmm0m m0mm	ooooooooo oosso
lncFBXO 28	LNC FBXO 256	ENST00000 607352	2874	240	AUCCGUUACU GAAA	mmmm0mm0mm 00mm	ooooooooo oosso
lncFBXO 29	LNC FBXO 256	ENST00000 607352	2876	241	CCGUUACUGA AAGA	mm0mm0mm000 0mm	ooooooooo oosso
lncFBXO 30	LNC FBXO 256	ENST00000 607352	200	242	UAUAUGUCGU CUUA	mmm0m0mm0m mmmm	ooooooooo oosso
lncNDST3 1	LNC NDST3	ENST00000 602414	77	243	AAAGUACGUA GUUA	mm00m0m0m00 mmm	ooooooooo osso
lncNDST3 2	LNC NDST3	ENST00000 602414	78	244	AAGUACGUAG UUGA	mm0m0m0m00m mmm	ooooooooo osso
lncNDST3 3	LNC NDST3	ENST00000 602414	79	245	AGUACGUAGU UGUA	mmm0m0m00m m0mm	ooooooooo osso
lncNDST3 4	LNC NDST3	ENST00000 602414	81	246	UACGUAGUUG UCUA	mmm0m00mm0 mmmm	ooooooooo osso
lncNDST3 5	LNC NDST3	ENST00000 602414	440	247	ACAUUACGAU GGAA	mm0mm0m00m0 0mm	ooooooooo osso

lncNDST36	LNCNDST3	ENST00000602414	441	248	CAUUACGAUGGAUA	mmmm0m00m000mm	ooooooooosso
lncNDST37	LNCNDST3	ENST00000602414	442	249	AUUACGAUGGAUGA	mmm0m00m000mmm	ooooooooosso
lncNDST38	LNCNDST3	ENST00000602414	443	250	UUACGAUGGAUGAA	mm0m00m000m0mm	ooooooooosso
lncNDST39	LNCNDST3	ENST00000602414	444	251	UACGAUGGAUGAUA	mmm00m000m00mm	ooooooooosso
lncNDST310	LNCNDST3	ENST00000602414	445	252	ACGAUGGAUGAUGA	mm00m000m00mmm	ooooooooosso
lncNDST311	LNCNDST3	ENST00000602414	508	253	AGCAUCCGGCAAUA	mmm0mmm00m00mm	ooooooooosso
lncNDST312	LNCNDST3	ENST00000602414	523	254	ACUUAUCGUAGUUA	mmmm0mm0m00mmm	ooooooooosso
lncNDST313	LNCNDST3	ENST00000602414	524	255	CUUAUCGUAGUUGA	mmm0mm0m00mmmm	ooooooooosso
lncNDST314	LNCNDST3	ENST00000602414	625	256	GUGGUCCGUGAUAA	mm00mmm0m00mmm	ooooooooosso
lncNDST315	LNCNDST3	ENST00000602414	626	257	UGGUCCGUGAUAAA	mm0mmm0m00m0mm	ooooooooosso
lncNDST316	LNCNDST3	ENST00000602414	627	258	GGUCCGUGAUAAUA	mmmmm0m00m00mm	ooooooooosso
lncNDST317	LNCNDST3	ENST00000602414	628	259	GUCCGUGAUAAUUA	mmmm0m00m00mmm	ooooooooosso
lncNDST318	LNCNDST3	ENST00000602414	629	260	UCCGUGAUAAUUAA	mmm0m00m00mmmm	ooooooooosso
lncNDST319	LNCNDST3	ENST00000602414	91	261	UCUUUCGUAAGUUA	mmmmmm0m000mmm	ooooooooosso
lncNDST320	LNCNDST3	ENST00000602414	92	262	CUUUCGUAAGUUAA	mmmm00m000mmmm	ooooooooosso
lncNDST321	LNCNDST3	ENST00000602414	515	263	GGCAAUGGACUUAA	mmm00m000mmmmm	ooooooooosso
lncNDST322	LNCNDST3	ENST00000602414	550	264	UCCGAAUAAUAUCA	mmm000m00m0mmm	ooooooooosso
lncNDST323	LNCNDST3	ENST00000602414	551	265	CCGAAUAAUAUCCA	mm000m00m0mmmm	ooooooooosso
lncNDST324	LNCNDST3	ENST00000602414	623	266	AGGUGGUCCGUGAA	mm0m00mmm0m0mm	ooooooooosso

lncNDST3 25	LNC NDST3	ENST00000 602414	624	267	GGUGGUCCGU GAUA	mmm00mmm0m 00mm	ooooooooo osso
lncNDST3 26	LNC NDST3	ENST00000 602414	630	268	CCGUGAUAAU UAAA	mm0m00m00mm 0mm	ooooooooo osso
lncNDST3 27	LNC NDST3	ENST00000 602414	130	269	UGCCUUACCU AAAA	mmmmmm0mm m00mm	ooooooooo osso
lncNDST3 28	LNC NDST3	ENST00000 602414	131	270	GCCUUACCUA AAAA	mmmmm0mmm0 00mm	ooooooooo osso
lncNDST3 29	LNC NDST3	ENST00000 602414	516	271	GCAAUGGACU UAUA	mm00m000mmm 0mm	ooooooooo osso
lncNDST3 30	LNC NDST3	ENST00000 602414	519	272	AUGGACUUAU CGUA	mm000mmm0m m0mm	ooooooooo osso
lncMALAT 1 1	LNC Malat1	MALAT1	445	273	UUCGCUUAGU UGGA	mmm0mmm00m m0mm	ooooooooo oosso
lncMALAT 1 2	LNC Malat1	MALAT1	860	274	GUUGCGUAAU GGAA	mmm0m0m00m0 0mm	ooooooooo oosso
lncMALAT 1 3	LNC Malat1	MALAT1	1006	275	AUGACCCGUU UAAA	mm00mmm0mm m0mm	ooooooooo oosso
lncMALAT 1 4	LNC Malat1	MALAT1	1007	276	UGACCCGUUU AAAA	mm0mmm0mmm 00mm	ooooooooo oosso
lncMALAT 1 5	LNC Malat1	MALAT1	1818	277	UAAACGCAGA CGAA	mm00m0m000m 0mm	ooooooooo oosso
lncMALAT 1 6	LNC Malat1	MALAT1	1821	278	ACGCAGACGA AAAA	mm0m000m00m 0mm	ooooooooo oosso
lncMALAT 1 7	LNC Malat1	MALAT1	2513	279	UUCGUAACGG AAGA	mmm0m00m00m 0mm	ooooooooo oosso
lncMALAT 1 8	LNC Malat1	MALAT1	2813	280	AGCGCUAACG AUUA	mmm0mm00m00 mmm	ooooooooo oosso
lncMALAT 1 9	LNC Malat1	MALAT1	3087	281	UCGUACUGAG GUGA	mm0m0mm00m0 mmm	ooooooooo oosso
lncMALAT 1 10	LNC Malat1	MALAT1	7883	282	UAAUCGGUUU CAAA	mm0mm00mmm m0mm	ooooooooo oosso
lncMALAT 1 11	LNC Malat1	MALAT1	8585	283	ACGAGAACCU AAUA	mm000m0mmm0 0mm	ooooooooo oosso
lncMALAT 1 12	LNC Malat1	MALAT1	1218	284	CGAAUUCCGG UGAA	mm00mmmm00 m0mm	ooooooooo oosso
lncMALAT 1 13	LNC Malat1	MALAT1	1251	285	UAAAUACGCC UCGA	mm00m0m0mm mmmm	ooooooooo oosso

lncMALAT 1 14	LNC Malat1	MALAT1	3014	286	UCGGCAAUAU GUUA	mm00m00m0m0 mmm	ooooooooo oosso
lncMALAT 1 15	LNC Malat1	MALAT1	5094	287	UUACGGAAUC UACA	mm0m00m0mm m0mm	ooooooooo oosso
lncMALAT 1 16	LNC Malat1	MALAT1	5338	288	UCGUUUGCCU CAGA	mm0mmm0mmm m0mm	ooooooooo oosso
lncMALAT 1 17	LNC Malat1	MALAT1	5970	289	GUCUGCGAAC ACUA	mmmm0m000m0 mmm	ooooooooo oosso
lncMALAT 1 18	LNC Malat1	MALAT1	6008	290	AGCGGAAGAA CGAA	mmm000m00mm 0mm	ooooooooo oosso
lncMALAT 1 19	LNC Malat1	MALAT1	6634	291	AUCCCGCUGC UAUA	mmmmm0mm0m m0mm	ooooooooo oosso
lncMALAT 1 20	LNC Malat1	MALAT1	6662	292	AACGACUGGA GUAA	mmm00mm00m0 mmm	ooooooooo oosso
lncMALAT 1 21	LNC Malat1	MALAT1	6782	293	GUCGUAUUUG UGAA	mmm0m0mmm0 m0mm	ooooooooo oosso
lncMALAT 1 22	LNC Malat1	MALAT1	7439	294	ACCGAAGGCU UAAA	mmm000m0mm m0mm	ooooooooo oosso
lncMALAT 1 23	LNC Malat1	MALAT1	7681	295	UCAAGCGGUG CUUA	mm000m00m0m mmm	ooooooooo oosso
lncMALAT 1 24	LNC Malat1	MALAT1	8219	296	UAGCGGAAGC UGAA	mm0m00m00mm 0mm	ooooooooo oosso
lncMALAT 1 25	LNC Malat1	MALAT1	4012	297	UGAGUAGGCC AAAA	mm00m000mm0 0mm	ooooooooo oosso
lncMALAT 1 26	LNC Malat1	MALAT1	2325	298	ACGUAGACCA GAAA	mm0m000mm00 0mm	ooooooooo oosso
lncMALAT 1 27	LNC Malat1	MALAT1	2742	299	UUCGUGGUGA AGAA	mmm0m00m000 0mm	ooooooooo oosso
lncMALAT 1 28	LNC Malat1	MALAT1	1423	300	CUUAGCGUUA AGUA	mmm00m0mm00 0mm	ooooooooo oosso
lncMALAT 1 29	LNC Malat1	MALAT1	1610	301	CCCGAAUUAA UACA	mmm000mm00m 0mm	ooooooooo oosso
lncMALAT 1 30	LNC Malat1	MALAT1	810	302	AAGUCCGCCA UUUA	mm0mmm0mm0 mmmm	ooooooooo oosso
lncFAM22 E1 1	LNC FAM22E1	ENST00000 605920	509	303	UAGAGGUAU UCCCA	mm00m0m0mm mmmm	ooooooooo osso
lncFAM22 E1 2	LNC FAM22E1	ENST00000 605920	716	304	CCGUGCGCUU UAUA	mm0m0m0mmm m0mm	ooooooooo oosso

lncFAM22 E1 3	LNC FAM22E1	ENST00000 605920	1139	305	CCAGCCUUAA AUCA	mm00mmmm000 mmm	ooooooooo oosso
lncFAM22 E1 4	LNC FAM22E1	ENST00000 605920	1148	306	AAUCGAGCCG ACUA	mmmm000mm00 mmm	ooooooooo oosso
lncFAM22 E1 5	LNC FAM22E1	ENST00000 605920	1149	307	AUCGAGCCGA CUAA	mmm000mm00m mmm	ooooooooo oosso
lncFAM22 E1 6	LNC FAM22E1	ENST00000 605920	1150	308	UCGAGCCGAC UACA	mm000mm00mm 0mm	ooooooooo oosso
lncFAM22 E1 7	LNC FAM22E1	ENST00000 605920	1328	309	GCUUCAGCGG AAUA	mmmmm00m00 m0mm	ooooooooo oosso
lncFAM22 E1 8	LNC FAM22E1	ENST00000 605920	1334	310	GCGGAAUACC UACA	mm00m0m0mm m0mm	ooooooooo oosso
lncFAM22 E1 9	LNC FAM22E1	ENST00000 605920	1335	311	CGGAAUACCU ACUA	mm000m0mmm0 mmm	ooooooooo oosso
lncFAM22 E1 10	LNC FAM22E1	ENST00000 605920	1362	312	AACAAGCCGA UUGA	mmm000mm00m mmm	ooooooooo oosso
lncFAM22 E1 11	LNC FAM22E1	ENST00000 605920	1363	313	ACAAGCCGAU UGAA	mm000mm00mm 0mm	ooooooooo oosso
lncFAM22 E1 12	LNC FAM22E1	ENST00000 605920	1364	314	CAAGCCGAUU GAUA	mm00mm00mm0 0mm	ooooooooo oosso
lncFAM22 E1 13	LNC FAM22E1	ENST00000 605920	1365	315	AAGCCGAUUG AUCA	mm0mm00mm00 mmm	ooooooooo oosso
lncFAM22 E1 14	LNC FAM22E1	ENST00000 605920	1366	316	AGCCGAUUGA UCAA	mmmm00mm00 mmmm	ooooooooo oosso
lncFAM22 E1 15	LNC FAM22E1	ENST00000 605920	1367	317	GCCGAUUGAU CACA	mmm00mm00m m0mm	ooooooooo oosso
lncFAM22 E1 16	LNC FAM22E1	ENST00000 605920	1368	318	CCGAUUGAUC ACAA	mm00mm00mm0 mmm	ooooooooo oosso
lncFAM22 E1 17	LNC FAM22E1	ENST00000 605920	1369	319	CGAUUGAUCA CAUA	mm0mm00mm0 m0mm	ooooooooo oosso
lncFAM22 E1 18	LNC FAM22E1	ENST00000 605920	1562	320	UACCCUUAUG GCUA	mmmmmmm0m0 0mmm	ooooooooo oosso
lncFAM22 E1 19	LNC FAM22E1	ENST00000 605920	1563	321	ACCCUUAUGG CUAA	mmmmmm0m00 mmmm	ooooooooo oosso
lncFAM22 E1 20	LNC FAM22E1	ENST00000 605920	1564	322	CCCUUAUGGC UAAA	mmmmm0m00m m0mm	ooooooooo oosso
lncFAM22 E1 21	LNC FAM22E1	ENST00000 605920	1140	323	CAGCCUUAAA UCGA	mm0mmmm000 mmmm	ooooooooo oosso

lncFAM22E1 22	LNC FAM22E1	ENST00000605920	1565	324	CCUUAUGGCUAAAA	mmmm0m00mm00mm	ooooooooooosso
lncFAM22E1 23	LNC FAM22E1	ENST00000605920	507	325	ACUAGAGGUAUUCA	mmm000m0m0mmmm	ooooooooooosso
lncFAM22E1 24	LNC FAM22E1	ENST00000605920	508	326	CUAGAGGUAUUCCA	mm00m00m0mmmmm	ooooooooooosso
lncFAM22E1 25	LNC FAM22E1	ENST00000605920	1141	327	AGCCUUAAAUCGAA	mmmmmm000mm0mm	ooooooooooosso
lncFAM22E1 26	LNC FAM22E1	ENST00000605920	1142	328	GCCUUAAAUCGAGA	mmmmm000mm00mm	ooooooooooosso
lncFAM22E1 27	LNC FAM22E1	ENST00000605920	1370	329	GAUUGAUCACAUUA	mmmm00mm0m0mmm	ooooooooooosso
lncFAM22E1 28	LNC FAM22E1	ENST00000605920	1389	330	CUCUAGCAGUGCAA	mmmm00m00m0mmm	ooooooooooosso
lncFAM22E1 29	LNC FAM22E1	ENST00000605920	1390	331	UCUAGCAGUGCAAA	mmm00m00m0m0mm	ooooooooooosso
lncFAM22E1 30	LNC FAM22E1	ENST00000605920	1492	332	UCUUAUGACAGCAA	mmmm0m00m00mmm	ooooooooooosso

Figure 1 Legend:

o: phosphodiester s: phosphorothioate

P: 5' phosphorylation 0: 2'-OH

f: 2'-fluoro m: 2' O-methyl

Table 2: Antisense Strand Oligonucleotides

Oligo ID	Gene Name	Accession number	Start Site	SEQ ID NO:	Antisense sequence	AntiSense Chemistry	AntiSense Backbone
lncRala1 1	LNC Rala1	ENST00000340510	140	333	UGAUUCUGAAGCGGAACCU	Pm00ffff00m0f00m0ff0	oooooooooooosssssso
lncRala1 2	LNC Rala1	ENST00000340510	296	334	UAGGCUCGGGAUCAUGUAA	Pm000fff0m00ff0f0f00	oooooooooooosssssso
lncRala1 3	LNC Rala1	ENST00000340510	366	335	UUACAGCGGAAAAAGGCAG	Pmf0f00f00m00m000f00	oooooooooooosssssso
lncRala1 4	LNC Rala1	ENST00000340510	367	336	UUUACAGCGGAAAAAGGCA	Pmff0f00f000m000m0f0	oooooooooooosssssso
lncRala1 5	LNC Rala1	ENST00000340510	368	337	UUUUACAGCGGAAAAAGGC	Pmfff0f00f000m00m000	oooooooooooosssssso
lncRala1 6	LNC Rala1	ENST00000340510	369	338	UAUUUACAGCGGAAAAAGG	Pm0fff0f00f00m00m0m0	oooooooooooosssssso

lncRala1 7	LNC Rala1	ENST00000 340510	370	339	UUAUUUACAGC GGAAAAAG	Pmf0fff0f00f00 0m0m00	ooooooooo ooossssso
lncRala1 8	LNC Rala1	ENST00000 340510	487	340	UAAUUCCGCUU GGCAAGAA	Pm00ffff0fff00 f00m00	ooooooooo ooossssso
lncRala1 9	LNC Rala1	ENST00000 340510	488	341	UAAAUUCCGCU UGGCAAGA	Pm000ffff0fff0 0f0000	ooooooooo ooossssso
lncRala1 10	LNC Rala1	ENST00000 340510	489	342	UUAAAUUCCGC UUGGCAAG	Pmf000ffff0fff 00f000	ooooooooo ooossssso
lncRala1 11	LNC Rala1	ENST00000 340510	490	343	UUUAAAUUCCG CUUGGCAA	Pmff000ffff0fff 00f00	ooooooooo ooossssso
lncRala1 12	LNC Rala1	ENST00000 340510	491	344	UUUUAAAUUCC GCUUGGCA	Pmfff000ffff0ff f00f0	ooooooooo ooossssso
lncRala1 13	LNC Rala1	ENST00000 340510	492	345	UAUUUAAAUUC CGCUUGGC	Pm0fff000ffff0 fff000	ooooooooo ooossssso
lncRala1 14	LNC Rala1	ENST00000 340510	620	346	UUCUCUGCGGC UCAAAUGU	Pmfffff0f00fff0 00f00	ooooooooo ooossssso
lncRala1 15	LNC Rala1	ENST00000 340510	622	347	UGAUCUCUGCG GCUCAAAU	Pm00fffff0f00f ff00m0	ooooooooo ooossssso
lncRala1 16	LNC Rala1	ENST00000 340510	852	348	UGACUGACGUG GUAGGAUU	Pm00ff00f0f00 f00m0f0	ooooooooo ooossssso
lncRala1 17	LNC Rala1	ENST00000 340510	853	349	UAGACUGACGU GGUAGGAU	Pm000ff00f0f0 0f00m00	ooooooooo ooossssso
lncRala1 18	LNC Rala1	ENST00000 340510	1662	350	UGUGUUAAGCU CGUUUCC	Pm0f0ff000fff0 fffff0	ooooooooo ooossssso
lncRala1 19	LNC Rala1	ENST00000 340510	1663	351	UCGUGUUAAGC UCGUUUC	Pmf0f0ff000fff 0ffff0	ooooooooo ooossssso
lncRala1 20	LNC Rala1	ENST00000 340510	1664	352	UGCGUGUUAAG CUCGUUU	Pm0f0f0ff000ff f0fff0	ooooooooo ooossssso
lncRala1 21	LNC Rala1	ENST00000 340510	1205	353	UUGCAUUCGAA AGGAUCCA	Pmf0f0fff0m00 m00fff0	ooooooooo ooossssso
lncRala1 22	LNC Rala1	ENST00000 340510	1208	354	UAAGUGCAUUC GAAAGGAU	Pm000f0f0fff0 00m00m0	ooooooooo ooossssso
lncRala1 23	LNC Rala1	ENST00000 340510	1926	355	UGACGUCGACU UGAGAAAG	Pm00f0ff00fff0 00m0m0	ooooooooo ooossssso
lncRala1 24	LNC Rala1	ENST00000 340510	2933	356	UAAGUUCGGGG CCUACAAA	Pm000fff0000f ff0f000	ooooooooo ooossssso
lncRala1 25	LNC Rala1	ENST00000 340510	1857	357	UAUUGUAACGA UGGAGCUG	Pm0ff0f00f00f 0000ff0	ooooooooo ooossssso

lncRala1 26	LNC Rala1	ENST00000 340510	1203	358	UCAUUCGAAAG GAUCCAUC	Pmf0fff000m0 00fff0f0	ooooooooo ooossssso
lncRala1 27	LNC Rala1	ENST00000 340510	1784	359	UUAGGGUAUGG GCCUAAAU	Pmf00m0f0f00 0fff0000	ooooooooo ooossssso
lncRala1 28	LNC Rala1	ENST00000 340510	99	360	UUUCAGGGUCU AUAUAAGA	Pmfff0000fff0f 0f00m0	ooooooooo ooossssso
lncRala1 29	LNC Rala1	ENST00000 340510	1480	361	UUGUGAUAGCA CUACUACA	Pmf0f00f00f0ff 0ff0f0	ooooooooo ooossssso
lncRala1 30	LNC Rala1	ENST00000 340510	1154	362	UUGCAGUGGUC AACUUGUA	Pmf0f00f00ff0 0fff0f0	ooooooooo ooossssso
lncZBTB 42 1	LNC ZBTB42	ENST00000 555578	588	363	UAGAUUCGGGC AGAGAUUG	Pm000fff000f0 m000ff0	ooooooooo ooossssso
lncZBTB 42 2	LNC ZBTB42	ENST00000 555578	590	364	UGAAGAUUCGG GCAGAGAU	Pm00m00fff00 0f000m00	ooooooooo ooossssso
lncZBTB 42 3	LNC ZBTB42	ENST00000 555578	593	365	UUGUGAAGAUU CGGGCAGA	Pmf0f0m000fff 000f000	ooooooooo ooossssso
lncZBTB 42 4	LNC ZBTB42	ENST00000 555578	801	366	UUACGGGUCGA AUUGUGUC	Pmf0f000ff000 ff0f0f0	ooooooooo ooossssso
lncZBTB 42 5	LNC ZBTB42	ENST00000 555578	804	367	UUGUUACGGGU CGAAUUGU	Pmf0ff0f000ff0 00ff00	ooooooooo ooossssso
lncZBTB 42 6	LNC ZBTB42	ENST00000 555578	807	368	UAGCUGUUACG GGUCGAAU	Pm00ff0ff0f00 0ff0000	ooooooooo ooossssso
lncZBTB 42 7	LNC ZBTB42	ENST00000 555578	836	369	UGAAGCACAUC GGAUGUGU	Pmm000f0f0ff 000f0f00	ooooooooo ooossssso
lncZBTB 42 8	LNC ZBTB42	ENST00000 555578	960	370	UAAUAAAGGUC CGUGGAAA	Pm00f000m0ff f0f000m0	ooooooooo ooossssso
lncZBTB 42 9	LNC ZBTB42	ENST00000 555578	1073	371	UAUCUCUUCGG AGAGAUCC	Pm0ffffff000 m000ff0	ooooooooo ooossssso
lncZBTB 42 10	LNC ZBTB42	ENST00000 555578	1075	372	UGAAUCUCUUC GGAGAGAU	Pm000ffffff00 000m00	ooooooooo ooossssso
lncZBTB 42 11	LNC ZBTB42	ENST00000 555578	1076	373	UGGAAUCUCUU CGGAGAGA	Pmm000ffffff 0000m00	ooooooooo ooossssso
lncZBTB 42 12	LNC ZBTB42	ENST00000 555578	1281	374	UCAGCUAAUCG GCUAUGGA	Pmf00ff00ff00f f0f000	ooooooooo ooossssso
lncZBTB 42 13	LNC ZBTB42	ENST00000 555578	1581	375	UGUGUGGCGAU AAGCUUGU	Pm0f0f00f00f0 00fff00	ooooooooo ooossssso
lncZBTB 42 14	LNC ZBTB42	ENST00000 555578	2212	376	UUUUCAAACGU CCAGCAGC	Pmffff000f0fff 00f000	ooooooooo ooossssso

lncZBTB 42 15	LNC ZBTB42	ENST00000 555578	2213	377	UUUUUCAAACG UCCAGCAG	Pmfffff000f0fff 00f00	ooooooooo ooosssssso
lncZBTB 42 16	LNC ZBTB42	ENST00000 555578	2137	378	UUUGAUUAGGC CUAACUCA	Pmff00ff000fff 00fff0	ooooooooo ooosssssso
lncZBTB 42 17	LNC ZBTB42	ENST00000 555578	2141	379	UUACGUUGAUU AGGCCUAA	Pmf0f0ff00ff00 0fff00	ooooooooo ooosssssso
lncZBTB 42 18	LNC ZBTB42	ENST00000 555578	636	380	UAUAAAGACGG GAAAUUUG	Pm0f00m00f00 0m00fff0	ooooooooo ooosssssso
lncZBTB 42 19	LNC ZBTB42	ENST00000 555578	1574	381	UGAUAAGCUUG UGUCCAUC	Pm00f000fff0f 0fff0f0	ooooooooo ooosssssso
lncZBTB 42 20	LNC ZBTB42	ENST00000 555578	1575	382	UCGAUAAGCUU GUGUCCAU	Pmf00f000fff0f 0fff00	ooooooooo ooosssssso
lncZBTB 42 21	LNC ZBTB42	ENST00000 555578	694	383	UAAGUUAGGGU GAGUCAUC	Pm000ff00m0f 000ff0f0	ooooooooo ooosssssso
lncZBTB 42 22	LNC ZBTB42	ENST00000 555578	699	384	UCCAUCAAGUU AGGGUGAG	Pmff0ff000ff0 m00f000	ooooooooo ooosssssso
lncZBTB 42 23	LNC ZBTB42	ENST00000 555578	2145	385	UGAUUUACGUU GAUUAGGC	Pm00fff0f0ff00 ff0000	ooooooooo ooosssssso
lncZBTB 42 24	LNC ZBTB42	ENST00000 555578	2149	386	UGACAGAUUUA CGUUGAUU	Pm00f000fff0f 0ff00f0	ooooooooo ooosssssso
lncZBTB 42 25	LNC ZBTB42	ENST00000 555578	700	387	UUCCAUCAAGU UAGGGUGA	Pmfff0ff000ff0 00mf00	ooooooooo ooosssssso
lncZBTB 42 26	LNC ZBTB42	ENST00000 555578	2134	388	UAUUAGGCCUA ACUCACAG	Pm0ff000fff00f ff0f00	ooooooooo ooosssssso
lncZBTB 42 27	LNC ZBTB42	ENST00000 555578	1307	389	UGCAGUCCUUA CACAGAGU	Pm0f00fffff0f0 f000m0	ooooooooo ooosssssso
lncZBTB 42 28	LNC ZBTB42	ENST00000 555578	640	390	UCCUUAUAAAG ACGGGAAA	Pmffff0f0m000 f000m00	ooooooooo ooosssssso
lncZBTB 42 29	LNC ZBTB42	ENST00000 555578	1616	391	UCUUGUAAUCC AGGGCCUU	Pmfff0f00fff0 m00fff0	ooooooooo ooosssssso
lncZBTB 42 30	LNC ZBTB42	ENST00000 555578	2133	392	UUUAGGCCUAA CUCACAGG	Pmff000fff00ff f0f000	ooooooooo ooosssssso
lncPAN K1 1	LNC PANK1	ENST00000 455699	174	393	UGUUGAGCUCC AAUGCUGA	Pm0ff000ffff00 f0ff00	ooooooooo ooosssssso
lncPAN K1 2	LNC PANK1	ENST00000 455699	176	394	UUAGUUGAGCU CCAAUGCU	Pmf00ff000ffff 00f0f0	ooooooooo ooosssssso
lncPAN K1 3	LNC PANK1	ENST00000 455699	179	395	UCGGUAGUUGA GCUCCAAU	Pmf00f00ff000 ffff000	ooooooooo ooosssssso

lncPANK1 4	LNC PANK1	ENST00000455699	188	396	UUGACACAGUCGGUAGUUG	Pmf00f0f00ff00f00ff0	oooooooooooosssssso
lncPANK1 5	LNC PANK1	ENST00000455699	191	397	UGAUUGACACAGUCGGUAG	Pm00ff00f0f00ff00f00	oooooooooooosssssso
lncPANK1 6	LNC PANK1	ENST00000455699	211	398	UGGAACCUGAUACUCUUAU	Pmm000fff00f0fffff00	oooooooooooosssssso
lncPANK1 7	LNC PANK1	ENST00000455699	419	399	UAAGACUAUAGACCUGCAU	Pmm000ff0f000fff0f00	oooooooooooosssssso
lncPANK1 8	LNC PANK1	ENST00000455699	565	400	UUUACGGAUACAAGUGCUG	Pmff0f000f0f000f0ff0	oooooooooooosssssso
lncPANK1 9	LNC PANK1	ENST00000455699	568	401	UGACUUACGGAUACAAGUG	Pm00fff0f000f0f000f0	oooooooooooosssssso
lncPANK1 10	LNC PANK1	ENST00000455699	571	402	UUGUGACUUACGGAUACAA	Pmf0f00fff0f000f0f00	oooooooooooosssssso
lncPANK1 11	LNC PANK1	ENST00000455699	573	403	UUGUGUGACUUACGGAUAC	Pmf0f0f00fff0f000f00	oooooooooooosssssso
lncPANK1 12	LNC PANK1	ENST00000455699	636	404	UCUUUUCGACAUUUUCCAU	Pmffffff00f0ffffff00	oooooooooooosssssso
lncPANK1 13	LNC PANK1	ENST00000455699	415	405	UCUAUAGACCUGCAUUAAA	Pmff0f000fff0f0ff000	oooooooooooosssssso
lncPANK1 14	LNC PANK1	ENST00000455699	418	406	UAGACUAUAGACCUGCAUU	Pm000ff0f000fff0f0f0	oooooooooooosssssso
lncPANK1 15	LNC PANK1	ENST00000455699	505	407	UGGCAUAUAAUCCUGGUGC	Pm00f0f0f00ffff00f00	oooooooooooosssssso
lncPANK1 16	LNC PANK1	ENST00000455699	259	408	UUCUGGUAUUGUCUGCCAA	Pmfff00f0ff0fff0ff00	oooooooooooosssssso
lncPANK1 17	LNC PANK1	ENST00000455699	421	409	UUAAAGACUAUAGACCUGC	Pmf00m00ff0f000fff00	oooooooooooosssssso
lncPANK1 18	LNC PANK1	ENST00000455699	502	410	UAUAUAAUCCUGGUGCCAA	Pm0f0f00ffff00f0ff00	oooooooooooosssssso
lncPANK1 19	LNC PANK1	ENST00000455699	341	411	UUGCAGCUAUUACUUGUCU	Pmf0f00ff0ff0fff0ff0	oooooooooooosssssso
lncPANK1 20	LNC PANK1	ENST00000455699	351	412	UCUCAAGGUUAUGCAGCUA	Pmfff00m0ff0f0f00ff0	oooooooooooosssssso
lncPANK1 21	LNC PANK1	ENST00000455699	257	413	UUGGUAUUGUCUGCCAAGA	Pmf00f0ff0fff0ff0000	oooooooooooosssssso
lncPANK1 22	LNC PANK1	ENST00000455699	367	414	UCUCAGUCAGUAUCUUGCU	Pmfff00ff00f0ffff0f0	oooooooooooosssssso

lncPANK1 23	LNC PANK1	ENST00000455699	55	415	UGACAUAAGACUCAAUCCU	Pm00f0f0m00fff00fff0	ooooooooooooSSSSSSo
lncPANK1 24	LNC PANK1	ENST00000455699	424	416	UGAGUAAAGACUAUAGACC	Pm000f00m00ff0f000f0	ooooooooooooSSSSSSo
lncPANK1 25	LNC PANK1	ENST00000455699	253	417	UAUUGUCUGCCAAGAUGAU	Pm0ff0fff0ff0000f000	ooooooooooooSSSSSSo
lncPANK1 26	LNC PANK1	ENST00000455699	217	418	UAGCACAGGAACCUGAUAC	Pm00f0f00m00fff00f00	ooooooooooooSSSSSSo
lncPANK1 27	LNC PANK1	ENST00000455699	545	419	UACAAUAGAGGCUUCAUAU	Pm0f00f00m00ffff0f00	ooooooooooooSSSSSSo
lncPANK1 28	LNC PANK1	ENST00000455699	304	420	UUCCUAACAUUUGGUCACU	Pmffff00f0fff00ff0f0	ooooooooooooSSSSSSo
lncPANK1 29	LNC PANK1	ENST00000455699	115	421	UACUUCUACAUCCUGUUGU	Pm0fffff0f0ffff0ff00	ooooooooooooSSSSSSo
lncPANK1 30	LNC PANK1	ENST00000455699	150	422	UUGGAGAUGCUUUGCACAC	Pmf00m00f0ffff0f0f00	ooooooooooooSSSSSSo
lncEBF3 1	LNC EBF3	ENST00000456581	744	423	UACAAAAGUCGCCAGGCAU	Pm0f00m00ff0ff000f00	ooooooooooooSSSSSSo
lncEBF3 2	LNC EBF3	ENST00000456581	746	424	UAUACAAAAGUCGCCAGGC	Pm0f0f00m00ff0ff0m00	ooooooooooooSSSSSSo
lncEBF3 3	LNC EBF3	ENST00000456581	1506	425	UUCAUCCGUCUUUACCAGC	Pmff0fff0fffff0ff000	ooooooooooooSSSSSSo
lncEBF3 4	LNC EBF3	ENST00000456581	1593	426	UAUAUUCGUCUUUACUACC	Pm0f0fff0fffff0ff0f0	ooooooooooooSSSSSSo
lncEBF3 5	LNC EBF3	ENST00000456581	1596	427	UAGCAUAUUCGUCUUUACU	Pm00f0f0fff0fffff0f0	ooooooooooooSSSSSSo
lncEBF3 6	LNC EBF3	ENST00000456581	1652	428	UGUUGACGAUUCCUGCCAU	Pm0ff00f00fffff0ff00	ooooooooooooSSSSSSo
lncEBF3 7	LNC EBF3	ENST00000456581	1655	429	UGAUGUUGACGAUUCCUGC	Pm00f0ff00f00fffff00	ooooooooooooSSSSSSo
lncEBF3 8	LNC EBF3	ENST00000456581	1656	430	UAGAUGUUGACGAUUCCUG	Pm000f0ff00f00fffff0	ooooooooooooSSSSSSo
lncEBF3 9	LNC EBF3	ENST00000456581	1657	431	UAAGAUGUUGACGAUUCCU	Pmm000f0ff00f00ffff0	ooooooooooooSSSSSSo
lncEBF3 10	LNC EBF3	ENST00000456581	2032	432	UCUGCAACGGCUUCUUUGU	Pmff0f00f00fffffff00	ooooooooooooSSSSSSo
lncEBF3 11	LNC EBF3	ENST00000456581	2209	433	UCACAAUUCCACGGAGCAA	Pmf0f00ffff0f0000f00	ooooooooooooSSSSSSo

lncEBF3 12	LNC EBF3	ENST00000 456581	2593	434	UCCUUUCGAAA UUGCUCAU	Pmffffff0m00ff 0fff00	ooooooooo ooosssssso
lncEBF3 13	LNC EBF3	ENST00000 456581	2595	435	UAACCUUUCGA AAUUGCUC	Pm00ffffff00m 0ff0ff0	ooooooooo ooosssssso
lncEBF3 14	LNC EBF3	ENST00000 456581	2597	436	UGGAACCUUUC GAAAUUGC	Pmm000ffffff0 00mff00	ooooooooo ooosssssso
lncEBF3 15	LNC EBF3	ENST00000 456581	240	437	UAAAAAGCCGA GCACUGGA	Pm000m00ff00 0f0ff000	ooooooooo ooosssssso
lncEBF3 16	LNC EBF3	ENST00000 456581	2193	438	UAAGAGAACGA UGUUUGUG	Pm000m000f0 0f0fff0f0	ooooooooo ooosssssso
lncEBF3 17	LNC EBF3	ENST00000 456581	1878	439	UUGGGACCAUU ACGUGAAA	Pmf0m00ff0ff0 f0f00m0	ooooooooo ooosssssso
lncEBF3 18	LNC EBF3	ENST00000 456581	2205	440	UAUUCCACGGA GCAAGAGA	Pm0ffff0fm000 f00m000	ooooooooo ooosssssso
lncEBF3 19	LNC EBF3	ENST00000 456581	1511	441	UGACAAUCAUC CGUCUUUA	Pm00f00ff0fff0 fffff0	ooooooooo ooosssssso
lncEBF3 20	LNC EBF3	ENST00000 456581	1843	442	UUCACCUCUGG UACAUCUA	Pmff0fffff00f0f 0fff0	ooooooooo ooosssssso
lncEBF3 21	LNC EBF3	ENST00000 456581	1879	443	UCUGGGACCAU UACGUGAA	Pmff00m0ff0ff 0f0f000	ooooooooo ooosssssso
lncEBF3 22	LNC EBF3	ENST00000 456581	1354	444	UUCUGUACCAG UCAUAGCC	Pmfff0f0ff00ff 0f00f0	ooooooooo ooosssssso
lncEBF3 23	LNC EBF3	ENST00000 456581	2317	445	UUGUGAGUCUU ACUGCAGA	Pmf0f000ffff0f f0f000	ooooooooo ooosssssso
lncEBF3 24	LNC EBF3	ENST00000 456581	1527	446	UAAGCUUGGAC CUCUAAGA	Pm000fff000fff ff00m0	ooooooooo ooosssssso
lncEBF3 25	LNC EBF3	ENST00000 456581	1544	447	UACAAAGGCCU ACAGUAAA	Pm0f00m00fff 0f00f000	ooooooooo ooosssssso
lncEBF3 26	LNC EBF3	ENST00000 456581	1325	448	UCAGAUACAUG GGCGAACA	Pmf000f0f0f00 0f000f0	ooooooooo ooosssssso
lncEBF3 27	LNC EBF3	ENST00000 456581	2409	449	UCUCAAGUCAU CAGACUCU	Pmfff000ff0ff0 00fff0	ooooooooo ooosssssso
lncEBF3 28	LNC EBF3	ENST00000 456581	933	450	UUGAACUUACC AGAGACUU	Pmf000fff0ff00 0m0ff0	ooooooooo ooosssssso
lncEBF3 29	LNC EBF3	ENST00000 456581	1296	451	UAAAGGGGUUA UUACAAAA	Pm000m000ff0 ff0f00m0	ooooooooo ooosssssso
lncEBF3 30	LNC EBF3	ENST00000 456581	1297	452	UCAAAGGGGUU AUUACAAA	Pmf000m000ff 0ff0f000	ooooooooo ooosssssso

lncScand 1 1	LNC Scand 1	ENST00000 565493	849	453	UAUCAUACGUC GGCAACCU	Pm0ff0f0f0ff00 f00ff0	ooooooooo ooosssssso
lncScand 1 2	LNC Scand 1	ENST00000 565493	851	454	UUUAUCAUACG UCGGCAAC	Pmff0ff0f0f0ff 00f000	ooooooooo ooosssssso
lncScand 1 3	LNC Scand 1	ENST00000 565493	985	455	UAACGUGGACG UAUCGCUU	Pm00f0f000f0f 0ff0ff0	ooooooooo ooosssssso
lncScand 1 4	LNC Scand 1	ENST00000 565493	2663	456	UAAAAUCGGGA CUAAUUUG	Pmm000ff0m0 0ff00fff0	ooooooooo ooosssssso
lncScand 1 5	LNC Scand 1	ENST00000 565493	2971	457	UUUUGUCCGCU AUAUACAC	Pmfff0fff0ff0f0 f0f00	ooooooooo ooosssssso
lncScand 1 6	LNC Scand 1	ENST00000 565493	2973	458	UAGUUUGUCCG CUAUAUAC	Pm00fff0fff0ff 0f0f00	ooooooooo ooosssssso
lncScand 1 7	LNC Scand 1	ENST00000 565493	3283	459	UAUGUCCGCUU AUAUACAC	Pm0f0fff0fff0f 0f0f00	ooooooooo ooosssssso
lncScand 1 8	LNC Scand 1	ENST00000 565493	3285	460	UCUAUGUCCGC UUAUAUAC	Pmff0f0fff0fff0 f0f00	ooooooooo ooosssssso
lncScand 1 9	LNC Scand 1	ENST00000 565493	3288	461	UCUCCUAUGUC CGCUUAUA	Pmffff0f0fff0f ff0f0	ooooooooo ooosssssso
lncScand 1 10	LNC Scand 1	ENST00000 565493	3312	462	UACAUCGACUA GACGUAAA	Pm0f0ff00ff00 0f0f000	ooooooooo ooosssssso
lncScand 1 11	LNC Scand 1	ENST00000 565493	3313	463	UAACAUCGACU AGACGUAA	Pm00f0ff00ff0 00f0f00	ooooooooo ooosssssso
lncScand 1 12	LNC Scand 1	ENST00000 565493	3314	464	UUAACAUCGAC UAGACGUA	Pmf00f0ff00ff0 00f0f0	ooooooooo ooosssssso
lncScand 1 13	LNC Scand 1	ENST00000 565493	4972	465	UCAACACGCCU CUAGAUAA	Pmf00f0f0ffff 000f00	ooooooooo ooosssssso
lncScand 1 14	LNC Scand 1	ENST00000 565493	654	466	UCUCUUCCGAC AGCAAAGU	Pmffffff00f00f 00m00	ooooooooo ooosssssso
lncScand 1 15	LNC Scand 1	ENST00000 565493	656	467	UCUCUCUUCCG ACAGCAAA	Pmffffffff00f0 0f000	ooooooooo ooosssssso
lncScand 1 16	LNC Scand 1	ENST00000 565493	733	468	UAUAAACGGCC AGUAAAUC	Pm0f000f00ff0 0f000f0	ooooooooo ooosssssso
lncScand 1 17	LNC Scand 1	ENST00000 565493	736	469	UUCCAUAAACG GCCAGUAA	Pmfff0f000f00f f00f00	ooooooooo ooosssssso
lncScand 1 18	LNC Scand 1	ENST00000 565493	991	470	UUUAACAAACG UGGACGUA	Pmff00f000f0f 000f0f0	ooooooooo ooosssssso
lncScand 1 19	LNC Scand 1	ENST00000 565493	1057	471	UCCAGUCUAGC AUAGAACC	Pmff00fff00f0f 00m0f0	ooooooooo ooosssssso

lncScand 1 20	LNC Scand 1	ENST00000 565493	1386	472	UUCUUGCCUCG CUGUAAAC	Pmffff0ffff0ff0 f0000	ooooooooo ooossssso
lncScand 1 21	LNC Scand 1	ENST00000 565493	1459	473	UUAGGACUCGU CUGUCCUU	Pmf00m0fff0ff f0ffff0	ooooooooo ooossssso
lncScand 1 22	LNC Scand 1	ENST00000 565493	1778	474	UCAUACAUCGG GCACUUCU	Pmf0f0f0ff000f 0ffff0	ooooooooo ooossssso
lncScand 1 23	LNC Scand 1	ENST00000 565493	2158	475	UUUUCCUACGA AUUUCAAC	Pmfffff0f000ff ff000	ooooooooo ooossssso
lncScand 1 24	LNC Scand 1	ENST00000 565493	3981	476	UUUAGAGGGGU GUUACUUA	Pmff000m000f 0ff00ff0	ooooooooo ooossssso
lncScand 1 25	LNC Scand 1	ENST00000 565493	4064	477	UGUCUGCAUUC GCUCCUAA	Pm0fff0f0fff0ff fff00	ooooooooo ooossssso
lncScand 1 26	LNC Scand 1	ENST00000 565493	4168	478	UCAAUGGUUAG ACCAUCUG	Pmf00f00ff000 ff0fff0	ooooooooo ooossssso
lncScand 1 27	LNC Scand 1	ENST00000 565493	4435	479	UACCAUCGUCU AGAUAUGG	Pm0ff0ff0fff00 0f0f00	ooooooooo ooossssso
lncScand 1 28	LNC Scand 1	ENST00000 565493	4440	480	UCUAAAACCAU CGUCUAGA	Pmff00m0ff0ff 0fff000	ooooooooo ooossssso
lncScand 1 29	LNC Scand 1	ENST00000 565493	4474	481	UACUAAAAACG CUCUUGUA	Pm0ff00m00f0 fffff0f0	ooooooooo ooossssso
lncScand 1 30	LNC Scand 1	ENST00000 565493	4535	482	UCAUUCGUAAA GCUUAGAU	Pmf0fff0f000m fff00m0	ooooooooo ooossssso
lncFAM6 9C2 1	LNC FAM69C2	ENST00000 580048	166	483	UUAUCUCUUAG CGGCUUCC	Pmf0fffff00f0 0ffff0	ooooooooo ooossssso
lncFAM6 9C2 2	LNC FAM69C2	ENST00000 580048	240	484	UCGCUCAUCGA AUUUAGAU	Pmf0fff0ff000f ff0000	ooooooooo ooossssso
lncFAM6 9C2 3	LNC FAM69C2	ENST00000 580048	241	485	UGCGCUCAUCG AAUUUAGA	Pm0f0fff0ff000 fff000	ooooooooo ooossssso
lncFAM6 9C2 4	LNC FAM69C2	ENST00000 580048	242	486	UCGCGCUCAUC GAAUUUAG	Pmf0f0fff0ff00 0fff00	ooooooooo ooossssso
lncFAM6 9C2 5	LNC FAM69C2	ENST00000 580048	764	487	UCUUGUCGAAC GUUUUAAA	Pmfff0ff000f0f fff000	ooooooooo ooossssso
lncFAM6 9C2 6	LNC FAM69C2	ENST00000 580048	766	488	UUCCUUGUCGA ACGUUUUA	Pmffff0ff000f 0ffff0	ooooooooo ooossssso
lncFAM6 9C2 7	LNC FAM69C2	ENST00000 580048	768	489	UAGUCCUUGUC GAACGUUU	Pm00ffff0ff00 0f0ff0	ooooooooo ooossssso
lncFAM6 9C2 8	LNC FAM69C2	ENST00000 580048	790	490	UGUGCCGUUAA CGUUCAUA	Pm0f0ff0ff00f0 fff0f0	ooooooooo ooossssso

lncFAM69C2 9	LNC FAM69C2	ENST00000580048	795	491	UAUGCUGUGCCGUUAACGU	Pm0f0ff0f0ff0ff00f00	ooooooooooosssssso
lncFAM69C2 10	LNC FAM69C2	ENST00000580048	932	492	UUUAUUCGUCUACACAGGU	Pmff0fff0fff0f0f0000	ooooooooooosssssso
lncFAM69C2 11	LNC FAM69C2	ENST00000580048	1391	493	UCCACUCGUUGGAAUGAUU	Pmff0fff0ff0m00f00f0	ooooooooooosssssso
lncFAM69C2 12	LNC FAM69C2	ENST00000580048	1999	494	UAAUGUCGUUAUAAACUUG	Pm00f0ff0ff0f000fff0	ooooooooooosssssso
lncFAM69C2 13	LNC FAM69C2	ENST00000580048	2001	495	UGCAAUGUCGUUAUAAACU	Pm0f00f0ff0fff0f000f0	ooooooooooosssssso
lncFAM69C2 14	LNC FAM69C2	ENST00000580048	531	496	UUUUCUCGAAAUCGGAGCG	Pmffffff0000ff0m00f0	ooooooooooosssssso
lncFAM69C2 15	LNC FAM69C2	ENST00000580048	535	497	UGUCAUUUCUCGAAAUCGG	Pm0ff0ffffff000mff00	ooooooooooosssssso
lncFAM69C2 16	LNC FAM69C2	ENST00000580048	597	498	UGAGCCAUUCGAGAGAUUU	Pm000ff0fff00000mff0	ooooooooooosssssso
lncFAM69C2 17	LNC FAM69C2	ENST00000580048	876	499	UUAACUCGAGGUUCAUGAA	Pmf00fff0000fff0f000	ooooooooooosssssso
lncFAM69C2 18	LNC FAM69C2	ENST00000580048	879	500	UCUCUAACUCGAGGUUCAU	Pmffff00fff0000fff00	ooooooooooosssssso
lncFAM69C2 19	LNC FAM69C2	ENST00000580048	1573	501	UUGCAUCUUCGCAGCUUAG	Pmf0f0fffff0f00fff00	ooooooooooosssssso
lncFAM69C2 20	LNC FAM69C2	ENST00000580048	1575	502	UUUUGCAUCUUCGCAGCUU	Pmfff0f0fffff0f00ff0	ooooooooooosssssso
lncFAM69C2 21	LNC FAM69C2	ENST00000580048	1927	503	UCCACUAAGCAUAACCUAG	Pmff0ff000f0f00fff00	ooooooooooosssssso
lncFAM69C2 22	LNC FAM69C2	ENST00000580048	2019	504	UCAUGGAGUGUAGCAUCCA	Pmf0f0000f0f00f0fff0	ooooooooooosssssso
lncFAM69C2 23	LNC FAM69C2	ENST00000580048	2674	505	UAGGUCCUUGAUACCAACA	Pm000fffff00f0ff00f0	ooooooooooosssssso
lncFAM69C2 24	LNC FAM69C2	ENST00000580048	2721	506	UUUCAAUAGGGCAUUGAGA	Pmfff00f0m00f0ff0m00	ooooooooooosssssso
lncFAM69C2 25	LNC FAM69C2	ENST00000580048	3316	507	UUACAAGUUGGGAUCCUCU	Pmf0f000ff0000fffff0	ooooooooooosssssso
lncFAM69C2 26	LNC FAM69C2	ENST00000580048	1749	508	UUUAUUUCGAUAGUUUCUG	Pmff0ffff00f00fffff0	ooooooooooosssssso
lncFAM69C2 27	LNC FAM69C2	ENST00000580048	2532	509	UCUCCUGGUAUAAGUGCUU	Pmfffff00f0f000f0ff0	ooooooooooosssssso

lncFAM69C2 28	LNC FAM69C2	ENST00000580048	2724	510	UAUGUUCAAUAGGGCAUUG	Pm0f0fff00f00m0f0ff0	ooooooooooosssssso
lncFAM69C2 29	LNC FAM69C2	ENST00000580048	2744	511	UAGCCAUCUUACUACAGCC	Pm00ff0ffff0ff0f00f0	ooooooooooosssssso
lncFAM69C2 30	LNC FAM69C2	ENST00000580048	3321	512	UGCAGCUACAAGUUGGGAU	Pm0f00ff0f000ff000m0	ooooooooooosssssso
lncVEZF1 1	LNC VEZF1	ENST00000585065	239	513	UCAGUACUCGAUAUAUCAA	Pmf00f0fff00f0f0ff00	ooooooooooosssssso
lncVEZF1 2	LNC VEZF1	ENST00000585065	2307	514	UUUUCUCGAGUACAGAGGU	Pmfffff000f0f00m000	ooooooooooosssssso
lncVEZF1 3	LNC VEZF1	ENST00000585065	2637	515	UCCAACAAAUCGCAAGUAA	Pmff00f000ff0f000f00	ooooooooooosssssso
lncVEZF1 4	LNC VEZF1	ENST00000585065	2638	516	UUCCAACAAAUCGCAAGUA	Pmfff00f000ff0f000f0	ooooooooooosssssso
lncVEZF1 5	LNC VEZF1	ENST00000585065	2863	517	UGGUAGUCGAGGGCUUUUA	Pm00f00ff000m0ffff0	ooooooooooosssssso
lncVEZF1 6	LNC VEZF1	ENST00000585065	3477	518	UUCUGCCGUUGUCAAUUAC	Pmfff0ff0ff0ff00ff00	ooooooooooosssssso
lncVEZF1 7	LNC VEZF1	ENST00000585065	3478	519	UCUCUGCCGUUGUCAAUUA	Pmffff0ff0ff0ff00ff0	ooooooooooosssssso
lncVEZF1 8	LNC VEZF1	ENST00000585065	3675	520	UCUAAGGUAAACGGGCAAA	Pmff00m0f000f000f000	ooooooooooosssssso
lncVEZF1 9	LNC VEZF1	ENST00000585065	3804	521	UUGUUAUCGAGUGGUUCUA	Pmf0ff0ff000f00ffff0	ooooooooooosssssso
lncVEZF1 10	LNC VEZF1	ENST00000585065	3805	522	UGUGUUAUCGAGUGGUUCU	Pm0f0ff0ff000f00fff0	ooooooooooosssssso
lncVEZF1 11	LNC VEZF1	ENST00000585065	3806	523	UGGUGUUAUCGAGUGGUUC	Pm00f0ff0ff000f00ff0	ooooooooooosssssso
lncVEZF1 12	LNC VEZF1	ENST00000585065	3808	524	UUUGGUGUUAUCGAGUGGU	Pmff00f0ff0ff000f000	ooooooooooosssssso
lncVEZF1 13	LNC VEZF1	ENST00000585065	4348	525	UAGAUGGACGCAUUAUUUU	Pm000f000f0f0ff0fff0	ooooooooooosssssso
lncVEZF1 14	LNC VEZF1	ENST00000585065	4349	526	UCAGAUGGACGCAUUAUUU	Pmf000f000f0f0ff0ff0	ooooooooooosssssso
lncVEZF1 15	LNC VEZF1	ENST00000585065	4350	527	UUCAGAUGGACGCAUUAUU	Pmff000f000f0f0ff0f0	ooooooooooosssssso
lncVEZF1 16	LNC VEZF1	ENST00000585065	4351	528	UUUCAGAUGGACGCAUUAU	Pmfff000f000f0f0ff00	ooooooooooosssssso

lncVEZF 1 17	LNC VEZF1	ENST00000 585065	2309	529	UAGUUUCUCGA GUACAGAG	Pm00ffffff000f 0f0m00	ooooooooo ooossssso
lncVEZF 1 18	LNC VEZF1	ENST00000 585065	2312	530	UCAAAGUUUCU CGAGUACA	Pmf00m0ffffff 000f0f0	ooooooooo ooossssso
lncVEZF 1 19	LNC VEZF1	ENST00000 585065	2449	531	UGUAGGUAAUG GGUCACAC	Pm0f000f00f00 0ff0f00	ooooooooo ooossssso
lncVEZF 1 20	LNC VEZF1	ENST00000 585065	2539	532	UACUCAUAGGC ACCAACAU	Pm0fff0f000f0f f00f00	ooooooooo ooossssso
lncVEZF 1 21	LNC VEZF1	ENST00000 585065	2541	533	UAUACUCAUAG GCACCAAC	Pm0f0fff0f000f 0ff000	ooooooooo ooossssso
lncVEZF 1 22	LNC VEZF1	ENST00000 585065	3674	534	UUAAGGUAAAC GGGCAAAG	Pmf00m0f000f 000f0m00	ooooooooo ooossssso
lncVEZF 1 23	LNC VEZF1	ENST00000 585065	3727	535	UUACUUUCGCC AAGUGACA	Pmf0fffff0ff00 0f00f0	ooooooooo ooossssso
lncVEZF 1 24	LNC VEZF1	ENST00000 585065	3730	536	UUUUUACUUUC GCCAAGUG	Pmffff0fffff0ff 000f0	ooooooooo ooossssso
lncVEZF 1 25	LNC VEZF1	ENST00000 585065	4441	537	UCUCUAGUCCA AGACAUCU	Pmffff00fff0m 00f0ff0	ooooooooo ooossssso
lncVEZF 1 26	LNC VEZF1	ENST00000 585065	4444	538	UUGUCUCUAGU CCAAGACA	Pmf0fffff00fff0 00mf0	ooooooooo ooossssso
lncVEZF 1 27	LNC VEZF1	ENST00000 585065	4650	539	UAAAAAUCGAA CUUCUGGU	Pm00m00ff000 fffff000	ooooooooo ooossssso
lncVEZF 1 28	LNC VEZF1	ENST00000 585065	2723	540	UGCUAAACCUA UCAGCUUC	Pm0ff000fff0ff 00fff0	ooooooooo ooossssso
lncVEZF 1 29	LNC VEZF1	ENST00000 585065	3116	541	UAAGCACACUA AGGGCUUU	Pm000f0f0ff0 m000fff0	ooooooooo ooossssso
lncVEZF 1 30	LNC VEZF1	ENST00000 585065	3369	542	UUAAUGGACCA ACUCUUUA	Pmf00f000ff00 ffffff0	ooooooooo ooossssso
lncFBXO 1	LNC FBXO 256	ENST00000 607352	198	543	UGACGACAUAU AAACGGCC	Pm00f00f0f0f0 00f00f0	ooooooooo ooossssso
lncFBXO 2	LNC FBXO 256	ENST00000 607352	199	544	UAGACGACAUA UAAACGGC	Pm000f00f0f0f 000f000	ooooooooo ooossssso
lncFBXO 3	LNC FBXO 256	ENST00000 607352	886	545	UACUUACGACA AAGCUACA	Pm0fff0f00f00 m0ff0f0	ooooooooo ooossssso
lncFBXO 4	LNC FBXO 256	ENST00000 607352	887	546	UAACUUACGAC AAAGCUAC	Pm00fff0f00f0 m00ff00	ooooooooo ooossssso
lncFBXO 5	LNC FBXO 256	ENST00000 607352	888	547	UUAACUUACGA CAAAGCUA	Pmf00fff0f00f0 000ff0	ooooooooo ooossssso

lncFBXO 6	LNC FBXO 256	ENST00000 607352	889	548	UAUAACUUACG ACAAAGCU	Pm0f00fff0f00f 00m0f0	ooooooooo ooosssssso
lncFBXO 7	LNC FBXO 256	ENST00000 607352	890	549	UCAUAACUUAC GACAAAGC	Pmf0f00fff0f00 f00m00	ooooooooo ooosssssso
lncFBXO 8	LNC FBXO 256	ENST00000 607352	2596	550	UAACAACGCUC UCAACCAG	Pm00f00f0ffff 00ff00	ooooooooo ooosssssso
lncFBXO 9	LNC FBXO 256	ENST00000 607352	2598	551	UUAAACAACGC UCUCAACC	Pmf000f00f0fff ff00f0	ooooooooo ooosssssso
lncFBXO 10	LNC FBXO 256	ENST00000 607352	2842	552	UUCAGUCGCAA GACAGAAC	Pmff00ff0f000 mf00m00	ooooooooo ooosssssso
lncFBXO 11	LNC FBXO 256	ENST00000 607352	2844	553	UGAUCAGUCGC AAGACAGA	Pm00ff00ff0f0 0m0f000	ooooooooo ooosssssso
lncFBXO 12	LNC FBXO 256	ENST00000 607352	2846	554	UAAGAUCAGUC GCAAGACA	Pm0000ff00ff0 f0m00f0	ooooooooo ooosssssso
lncFBXO 13	LNC FBXO 256	ENST00000 607352	2845	555	UAGAUCAGUCG CAAGACAG	Pm000ff00ff0f 0000f00	ooooooooo ooosssssso
lncFBXO 14	LNC FBXO 256	ENST00000 607352	2847	556	UGAAGAUCAGU CGCAAGAC	Pm00m00ff00f f0f00m00	ooooooooo ooosssssso
lncFBXO 15	LNC FBXO 256	ENST00000 607352	2871	557	UAGUAACGGAU AGGACAAC	Pm00f00f000f0 000f000	ooooooooo ooosssssso
lncFBXO 16	LNC FBXO 256	ENST00000 607352	2873	558	UUCAGUAACGG AUAGGACA	Pmff00f00f000 f00m0f0	ooooooooo ooosssssso
lncFBXO 17	LNC FBXO 256	ENST00000 607352	3806	559	UGGUGUUAUCG AGUGGUUC	Pm00f0ff0ff00 0f00ff0	ooooooooo ooosssssso
lncFBXO 18	LNC FBXO 256	ENST00000 607352	685	560	UAGCUAGAUCU ACCUCACA	Pm00ff000fff0f fff0f0	ooooooooo ooosssssso
lncFBXO 19	LNC FBXO 256	ENST00000 607352	687	561	UGAAGCUAGAU CUACCUCA	Pmm000ff000f ff0ffff0	ooooooooo ooosssssso
lncFBXO 20	LNC FBXO 256	ENST00000 607352	689	562	UAUGAAGCUAG AUCUACCU	Pm0f00m0ff00 0fff0ff0	ooooooooo ooosssssso
lncFBXO 21	LNC FBXO 256	ENST00000 607352	1073	563	UGGAUUGGAUA CCUUAAGA	Pm000ff000f0f fff00m0	ooooooooo ooosssssso
lncFBXO 22	LNC FBXO 256	ENST00000 607352	1071	564	UAUUGGAUACC UUAAGAUG	Pm0ff000f0fff 0000f0	ooooooooo ooosssssso
lncFBXO 23	LNC FBXO 256	ENST00000 607352	2071	565	UACCUAUGCUA GUCAAGAG	Pm0fff0f0ff00f f000m0	ooooooooo ooosssssso
lncFBXO 24	LNC FBXO 256	ENST00000 607352	2074	566	UCAGACCUAUG CUAGUCAA	Pmf000fff0f0ff 00ff00	ooooooooo ooosssssso

lncFBXO 25	LNC FBXO 256	ENST00000 607352	2076	567	UAACAGACCUA UGCUAGUC	Pm00f000fff0f 0ff00f0	ooooooooo ooossssso
lncFBXO 26	LNC FBXO 256	ENST00000 607352	2600	568	UAUUAAACAAC GCUCUCAA	Pm0ff000f00f0 fffff00	ooooooooo ooossssso
lncFBXO 27	LNC FBXO 256	ENST00000 607352	2870	569	UGUAACGGAUA GGACAACC	Pm0f00f000f00 m0f00f0	ooooooooo ooossssso
lncFBXO 28	LNC FBXO 256	ENST00000 607352	2874	570	UUUCAGUAACG GAUAGGAC	Pmfff00f00f00 0f000m0	ooooooooo ooossssso
lncFBXO 29	LNC FBXO 256	ENST00000 607352	2876	571	UCUUUCAGUAA CGGAUAGG	Pmffff00f00f0 00f000	ooooooooo ooossssso
lncFBXO 30	LNC FBXO 256	ENST00000 607352	200	572	UAAGACGACAU AUAAACGG	Pmm000f00f0f 0f000f00	ooooooooo ooossssso
lncNDST 3 1	LNC NDST3	ENST00000 602414	77	573	UAACUACGUAC UUUCACCU	Pm00ff0f0fff ff0ff0	ooooooooo ooossssso
lncNDST 3 2	LNC NDST3	ENST00000 602414	78	574	UCAACUACGUA CUUUCACC	Pmf00ff0f0ff fff0f0	ooooooooo ooossssso
lncNDST 3 3	LNC NDST3	ENST00000 602414	79	575	UACAACUACGU ACUUUCAC	Pm0f00ff0f0f ffff00	ooooooooo ooossssso
lncNDST 3 4	LNC NDST3	ENST00000 602414	81	576	UAGACAACUAC GUACUUUC	Pm000f00ff0f0 f0ffff0	ooooooooo ooossssso
lncNDST 3 5	LNC NDST3	ENST00000 602414	440	577	UUCCAUCGUAA UGUGUUCA	Pmfff0ff0f00f0 f0fff0	ooooooooo ooossssso
lncNDST 3 6	LNC NDST3	ENST00000 602414	441	578	UAUCCAUCGUA AUGUGUUC	Pm0fff0ff0f00f 0f0ff0	ooooooooo ooossssso
lncNDST 3 7	LNC NDST3	ENST00000 602414	442	579	UCAUCCAUCGU AAUGUGUU	Pmf0fff0ff0f00 f0f0f0	ooooooooo ooossssso
lncNDST 3 8	LNC NDST3	ENST00000 602414	443	580	UUCAUCCAUCG UAAUGUGU	Pmff0fff0ff0f0 0f0f00	ooooooooo ooossssso
lncNDST 3 9	LNC NDST3	ENST00000 602414	444	581	UAUCAUCCAUC GUAAUGUG	Pm0ff0fff0ff0f 00f0f0	ooooooooo ooossssso
lncNDST 3 10	LNC NDST3	ENST00000 602414	445	582	UCAUCAUCCAU CGUAAUGU	Pmf0ff0fff0ff0f 00f00	ooooooooo ooossssso
lncNDST 3 11	LNC NDST3	ENST00000 602414	508	583	UAUUGCCGGAU GCUGAAUA	Pm0ff0ff000f0f f000f0	ooooooooo ooossssso
lncNDST 3 12	LNC NDST3	ENST00000 602414	523	584	UAACUACGAUA AGUCCAUU	Pm00ff0f00f00 0fff0f0	ooooooooo ooossssso
lncNDST 3 13	LNC NDST3	ENST00000 602414	524	585	UCAACUACGAU AAGUCCAU	Pmf00ff0f00f0 00fff00	ooooooooo ooossssso

lncNDST 3 14	LNC NDST3	ENST00000 602414	625	586	UUAUCACGGAC CACCUUAA	Pmf0ff0f000ff0 ffff00	ooooooooo ooosssssso
lncNDST 3 15	LNC NDST3	ENST00000 602414	626	587	UUUAUCACGGA CCACCUUA	Pmff0ff0f000ff 0ffff0	ooooooooo ooosssssso
lncNDST 3 16	LNC NDST3	ENST00000 602414	627	588	UAUUAUCACGG ACCACCUU	Pm0ff0ff0f000f f0fff0	ooooooooo ooosssssso
lncNDST 3 17	LNC NDST3	ENST00000 602414	628	589	UAAUUAUCACG GACCACCU	Pm00ff0ff0f00 0ff0ff0	ooooooooo ooosssssso
lncNDST 3 18	LNC NDST3	ENST00000 602414	629	590	UUAAUUAUCAC GGACCACC	Pmf00ff0ff0f00 0ff0f0	ooooooooo ooosssssso
lncNDST 3 19	LNC NDST3	ENST00000 602414	91	591	UAACUUACGAA AGACAACU	Pm00fff0f000 m00f00f0	ooooooooo ooosssssso
lncNDST 3 20	LNC NDST3	ENST00000 602414	92	592	UUAACUUACGA AAGACAAC	Pmf00fff0f00m 000f000	ooooooooo ooosssssso
lncNDST 3 21	LNC NDST3	ENST00000 602414	515	593	UUAAGUCCAUU GCCGGAUG	Pmf000fff0ff0f f000f0	ooooooooo ooosssssso
lncNDST 3 22	LNC NDST3	ENST00000 602414	550	594	UGAUAUUAUUC GGAACACC	Pm00f0ff0fff0 m00f0f0	ooooooooo ooosssssso
lncNDST 3 23	LNC NDST3	ENST00000 602414	551	595	UGGAUAUUAUU CGGAACAC	Pm000f0ff0fff0 0m0f00	ooooooooo ooosssssso
lncNDST 3 24	LNC NDST3	ENST00000 602414	623	596	UUCACGGACCA CCUUAAAU	Pmff0f000ff0ff ff00m0	ooooooooo ooosssssso
lncNDST 3 25	LNC NDST3	ENST00000 602414	624	597	UAUCACGGACC ACCUUAAA	Pm0ff0f000ff0f fff000	ooooooooo ooosssssso
lncNDST 3 26	LNC NDST3	ENST00000 602414	630	598	UUUAAUUAUCA CGGACCAC	Pmff00ff0ff0f0 00ff00	ooooooooo ooosssssso
lncNDST 3 27	LNC NDST3	ENST00000 602414	130	599	UUUUAGGUAAG GCAGUAAG	Pmfff000f0m0 0f00f000	ooooooooo ooosssssso
lncNDST 3 28	LNC NDST3	ENST00000 602414	131	600	UUUUUAGGUAA GGCAGUAA	Pmffff000f000 mf00f00	ooooooooo ooosssssso
lncNDST 3 29	LNC NDST3	ENST00000 602414	516	601	UAUAAGUCCAU UGCCGGAU	Pm0f000fff0ff0 ff00m0	ooooooooo ooosssssso
lncNDST 3 30	LNC NDST3	ENST00000 602414	519	602	UACGAUAAGUC CAUUGCCG	Pm0f00f000fff 0ff0ff0	ooooooooo ooosssssso
lncMAL AT1 1	LNC Malat1	MALAT1	445	603	UCCAACUAAGC GAAUGGCU	Pmff00ff000f0 00f00f0	ooooooooo ooosssssso
lncMAL AT1 2	LNC Malat1	MALAT1	860	604	UUCCAUUACGC AACUGAGC	Pmfff0ff0f0f00 ff00m0	ooooooooo ooosssssso

lncMAL AT1 3	LNC Malat1	MALAT1	1006	605	UUUAAACGGGU CAUCAAAC	Pmff000f000f0 0ff00m0	ooooooooo ooossssso
lncMAL AT1 4	LNC Malat1	MALAT1	1007	606	UUUUAAACGGG UCAUCAAA	Pmfff000f000ff 0ff000	ooooooooo ooossssso
lncMAL AT1 5	LNC Malat1	MALAT1	1818	607	UUCGUCUGCGU UUAGUAAA	Pmff0fff0f0fff0 0f000	ooooooooo ooossssso
lncMAL AT1 6	LNC Malat1	MALAT1	1821	608	UUUUUCGUCUG CGUUUAGU	Pmfffff0fff0f0f ff000	ooooooooo ooossssso
lncMAL AT1 7	LNC Malat1	MALAT1	2513	609	UCUUCCGUUAC GAAAGUCC	Pmfffff0ff0f00 0m0ff0	ooooooooo ooossssso
lncMAL AT1 8	LNC Malat1	MALAT1	2813	610	UAAUCGUUAGC GCUCCUUC	Pm00ff0ff00f0f fffff0	ooooooooo ooossssso
lncMAL AT1 9	LNC Malat1	MALAT1	3087	611	UCACCUCAGUA CGAAACUC	Pmf0ffff00f0f0 0m0fff	ooooooooo ooossssso
lncMAL AT1 10	LNC Malat1	MALAT1	7883	612	UUUGAAACCGA UUAUGGAU	Pmff0m00ff00f f0f00m0	ooooooooo ooossssso
lncMAL AT1 11	LNC Malat1	MALAT1	8585	613	UAUUAGGUUCU CGUGUAAA	Pm0ff000fffff0 f0f000	ooooooooo ooossssso
lncMAL AT1 12	LNC Malat1	MALAT1	1218	614	UUCACCGGAAU UCGAUCAC	Pmff0ff0m00ff f00ff00	ooooooooo ooossssso
lncMAL AT1 13	LNC Malat1	MALAT1	1251	615	UCGAGGCGUAU UUAUAGAC	Pmf00m0f0f0ff f0f00m0	ooooooooo ooossssso
lncMAL AT1 14	LNC Malat1	MALAT1	3014	616	UAACAUAUUGC CGACCUCA	Pm00f0f0ff0ff0 0ffff0	ooooooooo ooossssso
lncMAL AT1 15	LNC Malat1	MALAT1	5094	617	UGUAGAUUCCG UAACUUUA	Pm0f000ffff0f0 0ffff0	ooooooooo ooossssso
lncMAL AT1 16	LNC Malat1	MALAT1	5338	618	UCUGAGGCAAA CGAAACAU	Pmff0000f000f 00m0f00	ooooooooo ooossssso
lncMAL AT1 17	LNC Malat1	MALAT1	5970	619	UAGUGUUCGCA GACAAAGU	Pm00f0fff0f00 0f00m00	ooooooooo ooossssso
lncMAL AT1 18	LNC Malat1	MALAT1	6008	620	UUCGUUCUUCC GCUCAAAU	Pmff0ffffff0fff 00m0	ooooooooo ooossssso
lncMAL AT1 19	LNC Malat1	MALAT1	6634	621	UAUAGCAGCGG GAUCAGAA	Pm0f00f00f00 m0ff00m0	ooooooooo ooossssso
lncMAL AT1 20	LNC Malat1	MALAT1	6662	622	UUACUCCAGUC GUUUCACA	Pmf0ffff00ff0ff ff0f0	ooooooooo ooossssso
lncMAL AT1 21	LNC Malat1	MALAT1	6782	623	UUCACAAAUAC GACUGCUU	Pmff0f000f0f0 0ff0ff0	ooooooooo ooossssso

lncMALAT1 22	LNC Malat1	MALAT1	7439	624	UUUAAGCCUUCGGUGCCUU	Pmff000fffff00f0fff0	ooooooooooooosssssso
lncMALAT1 23	LNC Malat1	MALAT1	7681	625	UAAGCACCGCUUGAGAUUU	Pm000f0ff0fff0000ff0	ooooooooooooosssssso
lncMALAT1 24	LNC Malat1	MALAT1	8219	626	UUCAGCUUCCGCUAAGAUG	Pmff00fffff0ff000mf0	ooooooooooooosssssso
lncMALAT1 25	LNC Malat1	MALAT1	4012	627	UUUUGGCCUACUCAAGCUC	Pmfff00fff0fff000ff0	ooooooooooooosssssso
lncMALAT1 26	LNC Malat1	MALAT1	2325	628	UUUCUGGUCUACGUAAACA	Pmffff00fff0f0f000f0	ooooooooooooosssssso
lncMALAT1 27	LNC Malat1	MALAT1	2742	629	UUCUUCACCACGAACUGCU	Pmfffff0ff0f000ff0f0	ooooooooooooosssssso
lncMALAT1 28	LNC Malat1	MALAT1	1423	630	UACUUAACGCUAAGCAAUA	Pm0fff00f0ff000f00f0	ooooooooooooosssssso
lncMALAT1 29	LNC Malat1	MALAT1	1610	631	UGUAUUAAUUCGGGGCUCU	Pm0f0ff00fff0m00fff0	ooooooooooooosssssso
lncMALAT1 30	LNC Malat1	MALAT1	810	632	UAAAUGGCGGACUUUCUCC	Pm000f00f000fffffff0	ooooooooooooosssssso
lncFAM22E1 1	LNC FAM22E1	ENST00000605920	509	633	UGGGAAUACCUCUAGUUCU	Pm00m00f0fffff00fff0	ooooooooooooosssssso
lncFAM22E1 2	LNC FAM22E1	ENST00000605920	716	634	UAUAAAGCGCACGGAUGGA	Pm0f00m0f0f0f000f000	ooooooooooooosssssso
lncFAM22E1 3	LNC FAM22E1	ENST00000605920	1139	635	UGAUUUAAGGCUGGUAUCC	Pm00fff0m00ff00f0ff0	ooooooooooooosssssso
lncFAM22E1 4	LNC FAM22E1	ENST00000605920	1148	636	UAGUCGGCUCGAUUUAAGG	Pm00ff00fff00fff00m0	ooooooooooooosssssso
lncFAM22E1 5	LNC FAM22E1	ENST00000605920	1149	637	UUAGUCGGCUCGAUUUAAG	Pmf00ff00fff00fff000	ooooooooooooosssssso
lncFAM22E1 6	LNC FAM22E1	ENST00000605920	1150	638	UGUAGUCGGCUCGAUUUAA	Pm0f00ff00fff00fff00	ooooooooooooosssssso
lncFAM22E1 7	LNC FAM22E1	ENST00000605920	1328	639	UAUUCCGCUGAAGCCAACU	Pm0ffff0ff00m0ff00f0	ooooooooooooosssssso
lncFAM22E1 8	LNC FAM22E1	ENST00000605920	1334	640	UGUAGGUAUUCCGCUGAAG	Pm0f000f0ffff00f00m0	ooooooooooooosssssso
lncFAM22E1 9	LNC FAM22E1	ENST00000605920	1335	641	UAGUAGGUAUUCCGCUGAA	Pm00f000f0ffff0ff000	ooooooooooooosssssso
lncFAM22E1 10	LNC FAM22E1	ENST00000605920	1362	642	UCAAUCGGCUUGUUGAAUA	Pmf00ff00fff0ff000f0	ooooooooooooosssssso

lncFAM22E1 11	LNC FAM22E1	ENST00000605920	1363	643	UUCAAUCGGCUUGUUGAAU	Pmff00ff00fff0ff00m0	ooooooooooosssssso
lncFAM22E1 12	LNC FAM22E1	ENST00000605920	1364	644	UAUCAAUCGGCUUGUUGAA	Pm0ff00ff00fff0ff000	ooooooooooosssssso
lncFAM22E1 13	LNC FAM22E1	ENST00000605920	1365	645	UGAUCAAUCGGCUUGUUGA	Pm00ff00ff00fff0ff00	ooooooooooosssssso
lncFAM22E1 14	LNC FAM22E1	ENST00000605920	1366	646	UUGAUCAAUCGGCUUGUUG	Pmf00ff00ff00fff0ff0	ooooooooooosssssso
lncFAM22E1 15	LNC FAM22E1	ENST00000605920	1367	647	UGUGAUCAAUCGGCUUGUU	Pm0f00ff00ff00fff0f0	ooooooooooosssssso
lncFAM22E1 16	LNC FAM22E1	ENST00000605920	1368	648	UUGUGAUCAAUCGGCUUGU	Pmf0f00ff00ff00fff00	ooooooooooosssssso
lncFAM22E1 17	LNC FAM22E1	ENST00000605920	1369	649	UAUGUGAUCAAUCGGCUUG	Pm0f0f00ff00ff00fff0	ooooooooooosssssso
lncFAM22E1 18	LNC FAM22E1	ENST00000605920	1562	650	UAGCCAUAAGGGUAAGGGA	Pm00ff0f0m000f000m00	ooooooooooosssssso
lncFAM22E1 19	LNC FAM22E1	ENST00000605920	1563	651	UUAGCCAUAAGGGUAAGGG	Pmf00ff0f000m0f000m0	ooooooooooosssssso
lncFAM22E1 20	LNC FAM22E1	ENST00000605920	1564	652	UUUAGCCAUAAGGGUAAGG	Pmff00ff0f00m00f00m0	ooooooooooosssssso
lncFAM22E1 21	LNC FAM22E1	ENST00000605920	1140	653	UCGAUUUAAGGCUGGUAUC	Pmf00fff0m00ff00f0f0	ooooooooooosssssso
lncFAM22E1 22	LNC FAM22E1	ENST00000605920	1565	654	UUUUAGCCAUAAGGGUAAG	Pmfff00ff0f0m000f000	ooooooooooosssssso
lncFAM22E1 23	LNC FAM22E1	ENST00000605920	507	655	UGAAUACCUCUAGUUCUUC	Pm000f0fffff00fffff0	ooooooooooosssssso
lncFAM22E1 24	LNC FAM22E1	ENST00000605920	508	656	UGGAAUACCUCUAGUUCUU	Pm00m0f0fffff00ffff0	ooooooooooosssssso
lncFAM22E1 25	LNC FAM22E1	ENST00000605920	1141	657	UUCGAUUUAAGGCUGGUAU	Pmff00fff0m00ff00f00	ooooooooooosssssso
lncFAM22E1 26	LNC FAM22E1	ENST00000605920	1142	658	UCUCGAUUUAAGGCUGGUA	Pmfff00fff00m0ff00f0	ooooooooooosssssso
lncFAM22E1 27	LNC FAM22E1	ENST00000605920	1370	659	UAAUGUGAUCAAUCGGCUU	Pm00f0f00ff00ff00ff0	ooooooooooosssssso
lncFAM22E1 28	LNC FAM22E1	ENST00000605920	1389	660	UUGCACUGCUAGAGCUGAA	Pmf0f0ff0ff0m00ff000	ooooooooooosssssso
lncFAM22E1 29	LNC FAM22E1	ENST00000605920	1390	661	UUUGCACUGCUAGAGCUGA	Pmff0f0ff0ffm000ff00	ooooooooooosssssso

lncFAM22E1 30	LNC FAM22E1	ENST00000605920	1492	662	UUGCUGUCAUAAGAUCAAA	Pmf0ff0ff0f0m00ff000	ooooooooooooossssso

Table 2 Legend:

o: phosphodiester	s: phosphorothioate
P: 5' phosphorylation	0: 2'-OH
f: 2'-fluoro	m: 2' O-methyl

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 PCT Publication No. WO2010/033247 (Application No. PCT/US2009/005247), filed on September 22, 2009, and entitled "REDUCED SIZE SELF-DELIVERING RNAI COMPOUNDS," US Patent No. 8,796,443, issued on August 5, 2014, published as US 2012/0040459 on February 16, 2012, entitled "REDUCED SIZE SELF-DELIVERING RNAI COMPOUNDS," PCT Publication No. WO2009/102427 (Application No. PCT/US2009/000852), filed on February 11, 2009, and entitled, "MODIFIED RNAI POLYNUCLEOTIDES AND USES THEREOF," and US Patent Publication No. 2011/0039914, published on February 17, 2011 and entitled "MODIFIED RNAI POLYNUCLEOTIDES AND USES THEREOF," PCT Publication No. WO 2011/119887 (Application No. PCT/US2011/029867), filed on March 24, 2011, and entitled RNA INTERFERENCE IN DERMAL AND FIBROTIC INDICATIONS, and US Patent No. 8,664,189, issued on March 4, 2014, published as US 2011/0237648 on September 29, 2011, entitled "RNA INTERFERENCE IN DERMAL AND FIBROTIC INDICATIONS."

CLAIMS

1. An isolated, double stranded nucleic acid molecule comprising a guide strand of 18-23 nucleotides in length that has complementarity to a lncRNA sequence, and a passenger strand of 8-16 nucleotides in length, wherein the molecule comprises a double stranded region and a single stranded region, wherein the single stranded region is the 3' end of the guide strand, is 2-13 nucleotides in length, and comprises at least two phosphorothioate modifications, and wherein at least 50% of the pyrimidines in the nucleic acid molecule are modified.

2. The nucleic acid molecule of claim 1, wherein the first nucleotide relative to the 5'end of the guide strand has a 2'-O-methyl modification, optionally wherein the 2'-O-methyl modification is a 5P-2'-O-methyl U modification, or a 5' vinyl phosphonate 2'-O-methyl U modification.

3. The nucleic acid molecule of claim 1 or claim 2, wherein at least 60%, at least 80%, at least 90% or wherein 100% of the pyrimidines in the nucleic acid molecule are modified.

4. The nucleic acid molecule of any one of claims 1 to 3, wherein the modified pyrimidines are 2'-fluoro or 2'-O-methyl modified.

5. The nucleic acid molecule of any one of claims 1 to 4, wherein at least one U or C includes a hydrophobic modification, optionally wherein a plurality of U's and/or C's include a hydrophobic modification.

6. The nucleic acid molecule of claim 5, wherein the hydrophobic modification is a methyl or ethyl hydrophobic base modification.

7. The nucleic acid molecule of any one of claims 1 to 6, wherein the guide strand comprises 6-8 phosphorothioate modifications.

8. The nucleic acid molecule of claim 7, wherein the guide strand comprises at least eight phosphorothioate modifications located within the first 10 nucleotides relative to the 3'end of the guide strand.

9. The nucleic acid molecule of any one of claims 1 to 8, wherein the guide strand includes 4-14 phosphate modifications.

10. The nucleic acid molecule of any one of claims 1 to 9, wherein the single stranded region of the guide strand is 6 nucleotides long to 8 nucleotides long.

11. The nucleic acid molecule of any one of claims 1 to 10, wherein the double stranded region is 13 nucleotides long.

12. The nucleic acid molecule of any one of claims 1 to 11, wherein the double stranded nucleic acid molecule has one end that is blunt or includes a one nucleotide overhang.

13. The nucleic acid molecule of any one of claims 1 to 12, wherein the passenger strand is linked at the 3' end to a lipophilic group.

14. The nucleic acid molecule of claim 13, wherein the lipophilic group is a sterol, optionally wherein the sterol is cholesterol.

15. The nucleic acid molecule of any one of claims 1 to 14, wherein the nucleic acid molecule is an sd-rxRNA, and wherein the guide strand of the sd-rxRNA is complementary to a lncRNA, optionally wherein the lncRNA is selected from the group consisting of ENST00000585065, ENST00000602414, ENST00000607352, ENST00000456581, ENST00000340510, ENST00000605920, ENST00000455699, ENST00000555578, ENST00000565493, ENST00000580048 and MALAT1.

16. The nucleic acid molecule of any one of claims 1 to 15, wherein the isolated double stranded nucleic acid molecule is an sd-rxRNA and wherein the guide strand of the sd-rxRNA is complementary to MALAT1.

17. The nucleic acid molecule of any one of claims 1 to 16, wherein the isolated double stranded nucleic acid molecule is a lncRNA inhibitor and wherein the lncRNA sequence to which the guide strand is complementary is an antisense strand of a mature lncRNA.

18. The nucleic acid molecule of claim 17, wherein the guide strand is at least 50% chemically modified.

19. The nucleic acid molecule of claim 17 or 18, wherein the nucleic acid molecule is directed against at least 12 contiguous nucleotides of a sequence within Table 1 or Table 2.

20. A method for modulating lncRNA expression and/or activity in a cell, comprising contacting a cell with the nucleic acid molecule of any one of claims 1 to 19 in an amount effective to modulate lncRNA expression and/or activity.

21. The method of claim 20, wherein the lncRNA is localized in the nucleus of the cell.

22. The method of claim 20, wherein the lncRNA is localized in the cytoplasm of the cell.

23. The method of claim 20, wherein the lncRNA is localized both in the nucleus and the cytoplasm of the cell.

24. The method of any one of claims 20 to 23, wherein the cell is a bacterial cell or a eukaryotic cell.

25. The method of claim 24, wherein the cell is a mammalian cell.

26. The method of claim 25, wherein the mammalian cell is a mammalian stem cell.

27. The method of any one of claims 20 to 26, wherein the cell is contacted with the isolated nucleic acid molecule *in vivo* or *ex vivo*.

28. A method of delivering a nucleic acid molecule to a cell, the method comprising administering an isolated nucleic acid molecule to a cell, wherein the isolated nucleic acid comprises a sense strand which is complementary to an anti-sense oligonucleotide (ASO), wherein the sense strand is between 8-15 nucleotides in length, comprises at least two phosphorothioate modifications, at least 50% of the pyrimidines in the sense strand are modified, and wherein the molecule comprises a hydrophobic conjugate.

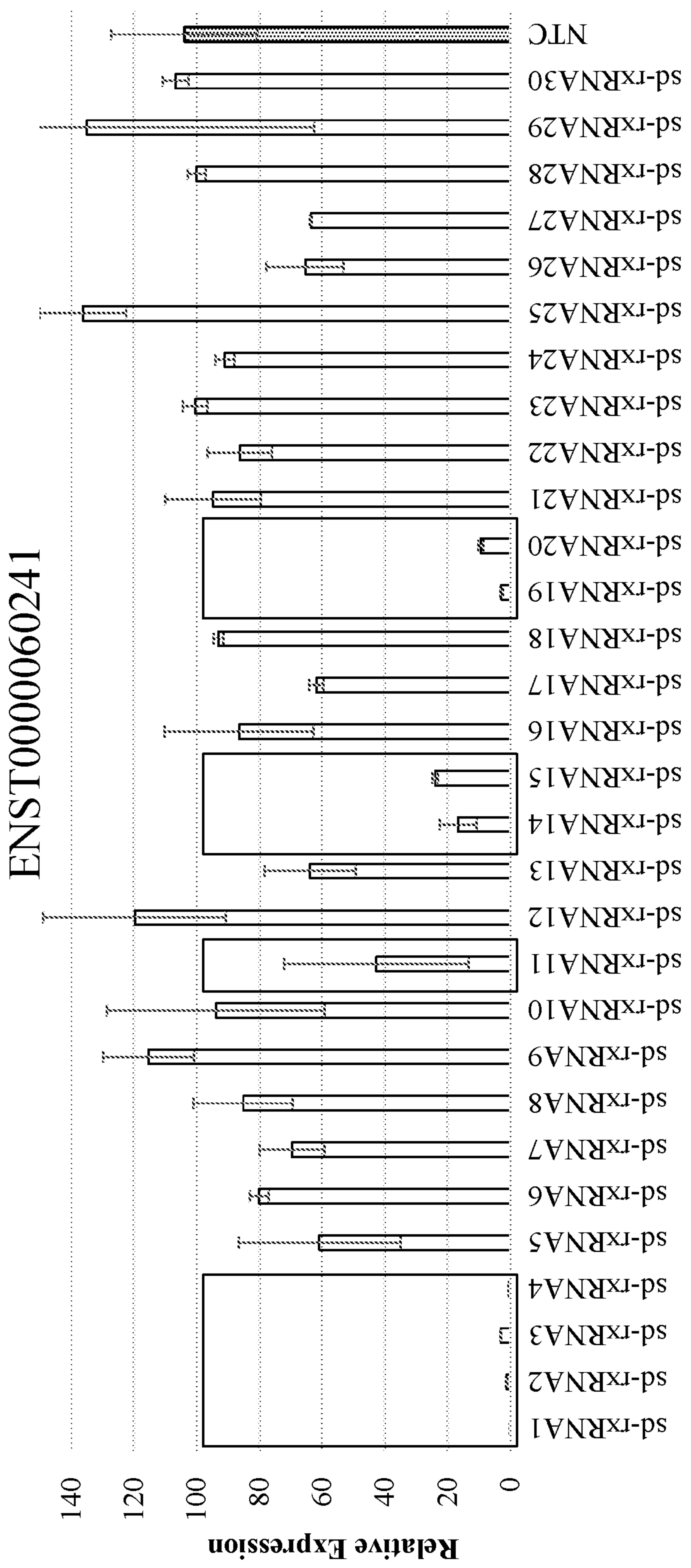
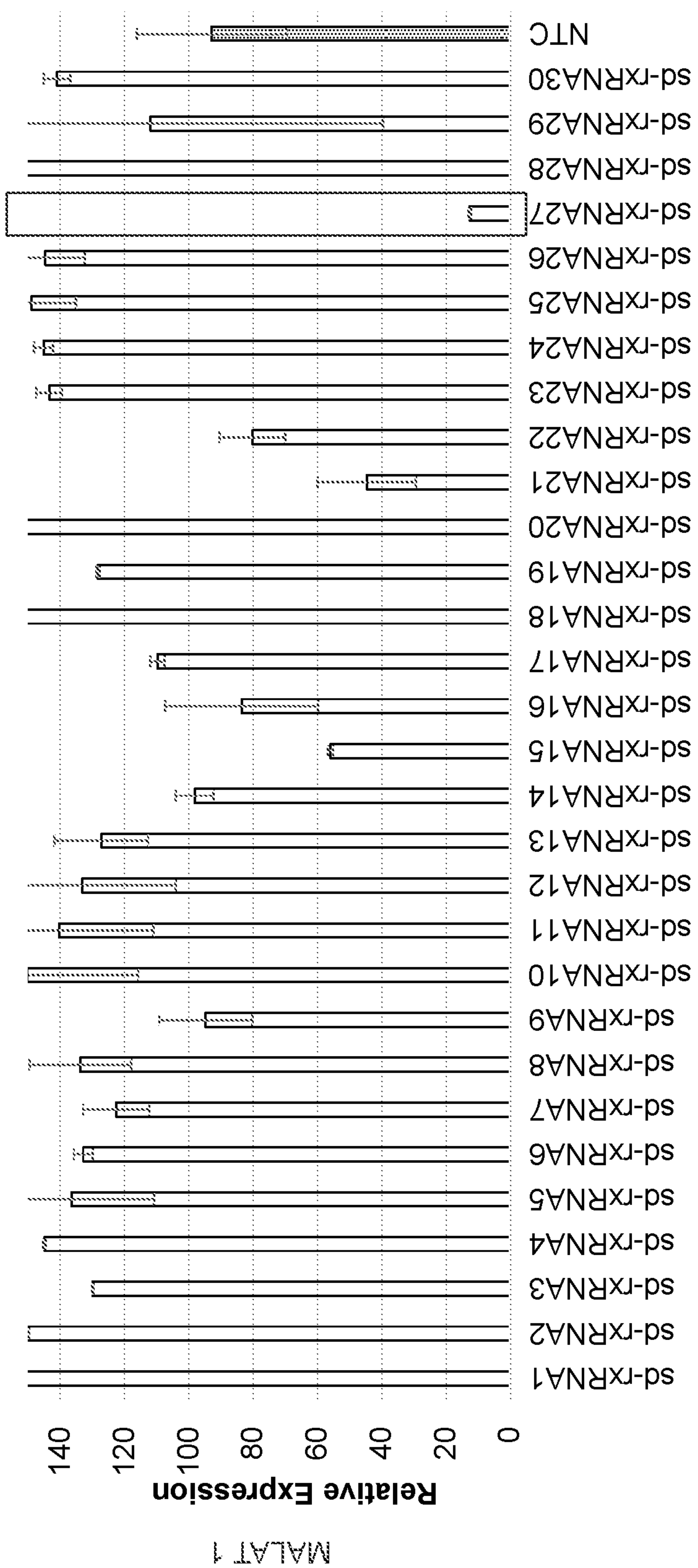

ENST00000060241
Relative Expression
0
20
40
60
80
100
120
140
sd-rxRNA1
sd-rxRNA2
sd-rxRNA3
sd-rxRNA4
sd-rxRNA5
sd-rxRNA6
sd-rxRNA7
sd-rxRNA8
sd-rxRNA9
sd-rxRNA10
sd-rxRNA11
sd-rxRNA12
sd-rxRNA13
sd-rxRNA14
sd-rxRNA15
sd-rxRNA16
sd-rxRNA17
sd-rxRNA18
sd-rxRNA19
sd-rxRNA20
sd-rxRNA21
sd-rxRNA22
sd-rxRNA23
sd-rxRNA24
sd-rxRNA25
sd-rxRNA26
sd-rxRNA27
sd-rxRNA28
sd-rxRNA29
sd-rxRNA30
NTC

FIG. 1

2/3


MALAT 1
Relative Expression
0
20
40
60
80
100
120
140
sd-rxRNA1
sd-rxRNA2
sd-rxRNA3
sd-rxRNA4
sd-rxRNA5
sd-rxRNA6
sd-rxRNA7
sd-rxRNA8
sd-rxRNA9
sd-rxRNA10
sd-rxRNA11
sd-rxRNA12
sd-rxRNA13
sd-rxRNA14
sd-rxRNA15
sd-rxRNA16
sd-rxRNA17
sd-rxRNA18
sd-rxRNA19
sd-rxRNA20
sd-rxRNA21
sd-rxRNA22
sd-rxRNA23
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sd-rxRNA29
sd-rxRNA30
NTC

FIG. 2

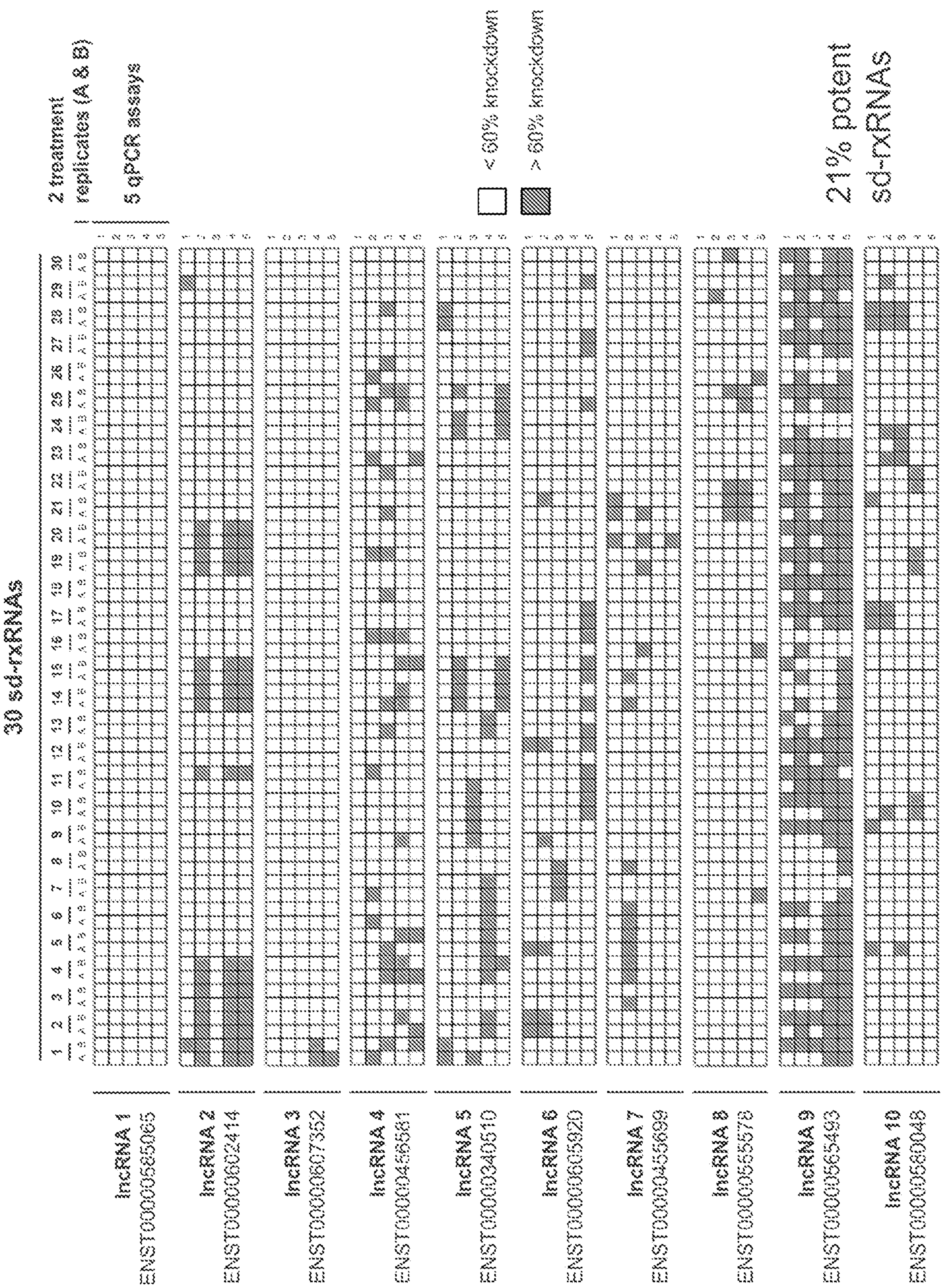
30 sd-rxRNAs
2 treatment replicates (A & B)
5 qPCR assays
< 60% knockdown
> 60% knockdown
21% potent sd-rxRNAs
lncRNA 1 ENST00000585065
lncRNA 2 ENST00000602414
lncRNA 3 ENST00000607352
lncRNA 4 ENST00000456581
lncRNA 5 ENST00000340510
lncRNA 6 ENST00000605920
lncRNA 7 ENST00000455699
lncRNA 8 ENST00000555578
lncRNA 9 ENST00000565493
lncRNA 10 ENST00000580048

FIG. 3


ENST0000060241
Relative Expression
0
20
40
60
80
100
120
140
sd-rxRNA1
sd-rxRNA2
sd-rxRNA3
sd-rxRNA4
sd-rxRNA5
sd-rxRNA6
sd-rxRNA7
sd-rxRNA8
sd-rxRNA9
sd-rxRNA10
sd-rxRNA11
sd-rxRNA12
sd-rxRNA13
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sd-rxRNA18
sd-rxRNA19
sd-rxRNA20
sd-rxRNA21
sd-rxRNA22
sd-rxRNA23
sd-rxRNA24
sd-rxRNA25
sd-rxRNA26
sd-rxRNA27
sd-rxRNA28
sd-rxRNA29
sd-rxRNA30
NTC

FIG. 1