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(54) RNA INTERFERENCE MEDIATED INHIBITION OF STEROL REGULATORY ELEMENT BINDING PROTEIN 1 (SREBP1) GENE EXPRESSION USING SHORT INTERFERING NUCLEIC ACID (SINA)

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Related U.S. Application Data

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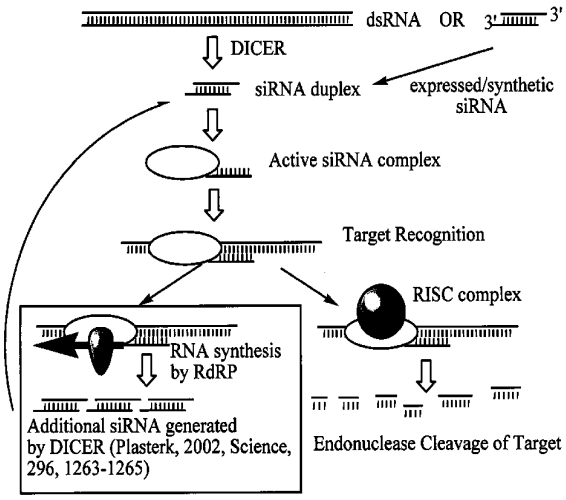
(52) **U.S. Cl.** **514/44**; 536/23.1

(57) **ABSTRACT**

The present invention relates to compounds, compositions, and methods for the study, diagnosis, and treatment of traits, diseases and conditions that respond to the modulation of Sterol Regulatory Element-Binding Protein 1 (SREBP1) gene expression and/or activity. The present invention is also directed to compounds, compositions, and methods relating to traits, diseases and conditions that respond to the modulation of expression and/or activity of genes involved in Sterol Regulatory Element-Binding Protein 1 (SREBP1) gene expression pathways or other cellular processes that mediate the maintenance or development of such traits, diseases and conditions. Specifically, the invention relates to double stranded nucleic acid molecules including small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules capable of mediating RNA interference (RNAi) against Sterol Regulatory Element-Binding Protein 1 (SREBP1) gene expression, including cocktails of such small nucleic acid molecules and lipid nanoparticle (LNP) formulations of such small nucleic acid molecules. The present invention also relates to small nucleic acid molecules, such as siNA, siRNA, and others that can inhibit the function of endogenous RNA molecules, such as endogenous micro-RNA (miRNA) (e.g, miRNA inhibitors) or endogenous short interfering RNA (siRNA), (e.g., siRNA inhibitors) or that can inhibit the function of RISC (e.g., RISC inhibitors), to modulate SREBP1 gene expression by interfering with the regulatory function of such endogenous RNAs or proteins associated with such endogenous RNAs (e.g., RISC), including cocktails of such small nucleic acid molecules and lipid nanoparticle (LNP) formulations of such small nucleic acid molecules. Such small nucleic acid molecules and are useful, for example, in providing compositions to prevent, inhibit, or reduce metabolic diseases traits and conditions, including but not limited to hyperlipidemia, hypercholesterolemia, cardiovascular disease, atherosclerosis, hypertension, diabetis (e.g., type I and/or type II diabetis), insulin resistance, obesity and/or other disease states, conditions, or traits associated with SREBP1 gene expression or activity in a subject or organism.

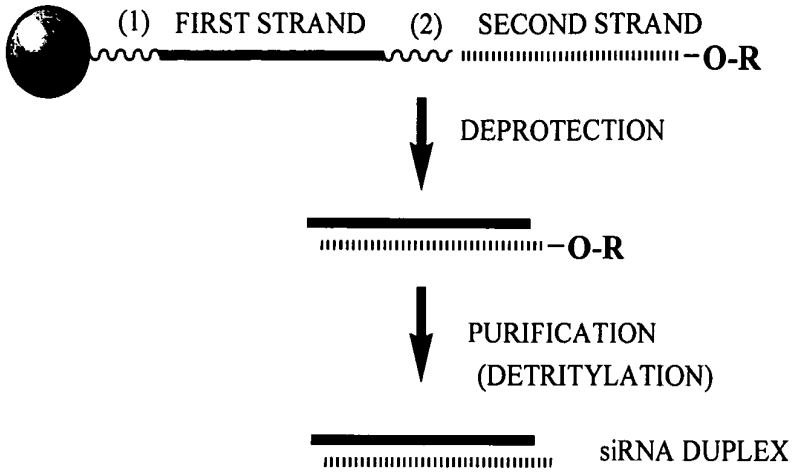


Related U.S. Application Data

(63) which is a continuation-in-part of application No. 11/098,303, filed on Apr. 4, 2005, now abandoned, which is a continuation-in-part of application No. 10/923,536, filed on Aug. 20, 2004, which is a continuation-in-part of application No. PCT/US04/16390, filed on May 24, 2004, which is a continuation-in-part of application No. 10/826,966, filed on Apr. 16, 2004, now abandoned, which is a continuation-in-part of application No. 10/757,803, filed on Jan. 14, 2004, which is a continuation-in-part of application No. 10/720,448, filed on Nov. 24, 2003, which is a continuation-in-part of application No. 10/693,059, filed on Oct. 23, 2003, which is a continuation-in-part of application No. 10/444,853, filed on May 23, 2003, which is a continuation-in-part of application No. PCT/US03/05346, filed on Feb. 20, 2003, which is a continuation-in-part of application No. PCT/US03/05028, filed on Feb. 20, 2003, Continuation-in-part of application No. PCT/US04/13456, filed on Apr. 30, 2004, which is a continuation-in-part of application No. 10/780,447, filed on Feb. 13, 2004, which is a continuation-in-part of application No. 10/427,160, filed on Apr. 30, 2003, which is a continuation-in-part of application No. PCT/US02/15876, filed on May 17, 2002, Continuation-in-part of application No. 10/727,780, filed on Dec. 3, 2003, now abandoned, Continuation-in-part of application No. PCT/US05/04270, filed on Feb. 9, 2005, Continuation-in-part of application No. 11/353,630, filed on Feb. 14, 2006.

(60) Provisional application No. 60/358,580, filed on Feb. 20, 2002, provisional application No. 60/358,580, filed on Feb. 20, 2002, provisional application No. 60/363,124, filed on Mar. 11, 2002, provisional application No. 60/363,124, filed on Mar. 11, 2002, provisional application No. 60/386,782, filed on Jun. 6, 2002, provisional application No. 60/386,782, filed on Jun. 6, 2002, provisional application No. 60/406,784, filed on Aug. 29, 2002, provisional application No. 60/406,784, filed on Aug. 29, 2002, provisional application No. 60/408,378, filed on Sep. 5, 2002, provisional application No. 60/408,378, filed on Sep. 5, 2002, provisional application No. 60/409,293, filed on Sep. 9, 2002, provisional application No. 60/409,293, filed on Sep. 9, 2002, provisional application No. 60/440,129, filed on Jan. 15, 2003, provisional application No. 60/440,129, filed on Jan. 15, 2003, provisional application No. 60/292,217, filed on May 18, 2001, provisional application No. 60/362,016, filed on Mar. 6, 2002, provisional application No. 60/306,883, filed on Jul. 20, 2001, provisional application No. 60/311,865, filed on Aug. 13, 2001, provisional application No. 60/543,480, filed on Feb. 10, 2004, provisional application No. 60/652,787, filed on Feb. 14, 2005, provisional application No. 60/678,531, filed on May 6, 2005, provisional application No. 60/703,946, filed on Jul. 29, 2005, provisional application No. 60/737,024, filed on Nov. 15, 2005.

Figure 1

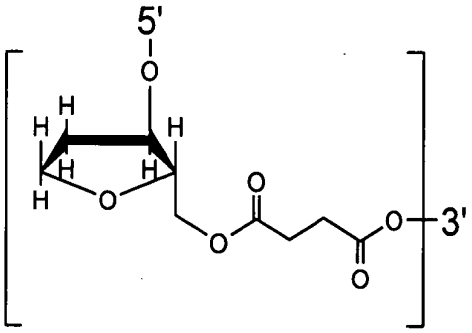


= SOLID SUPPORT

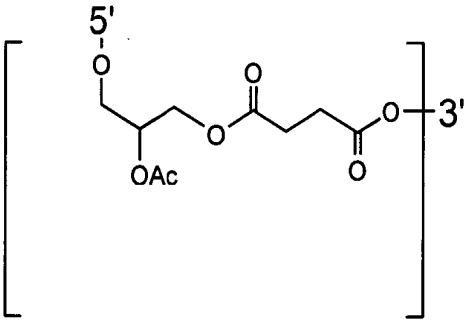
R = TERMINAL PROTECTING GROUP
FOR EXAMPLE:
DIMETHOXYTRITYL (DMT)

(1)
= CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)

(2)
= CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)



INVERTED DEOXYABASIC SUCCINATE LINKAGE



GLYCERYL SUCCINATE LINKAGE

Figure 2

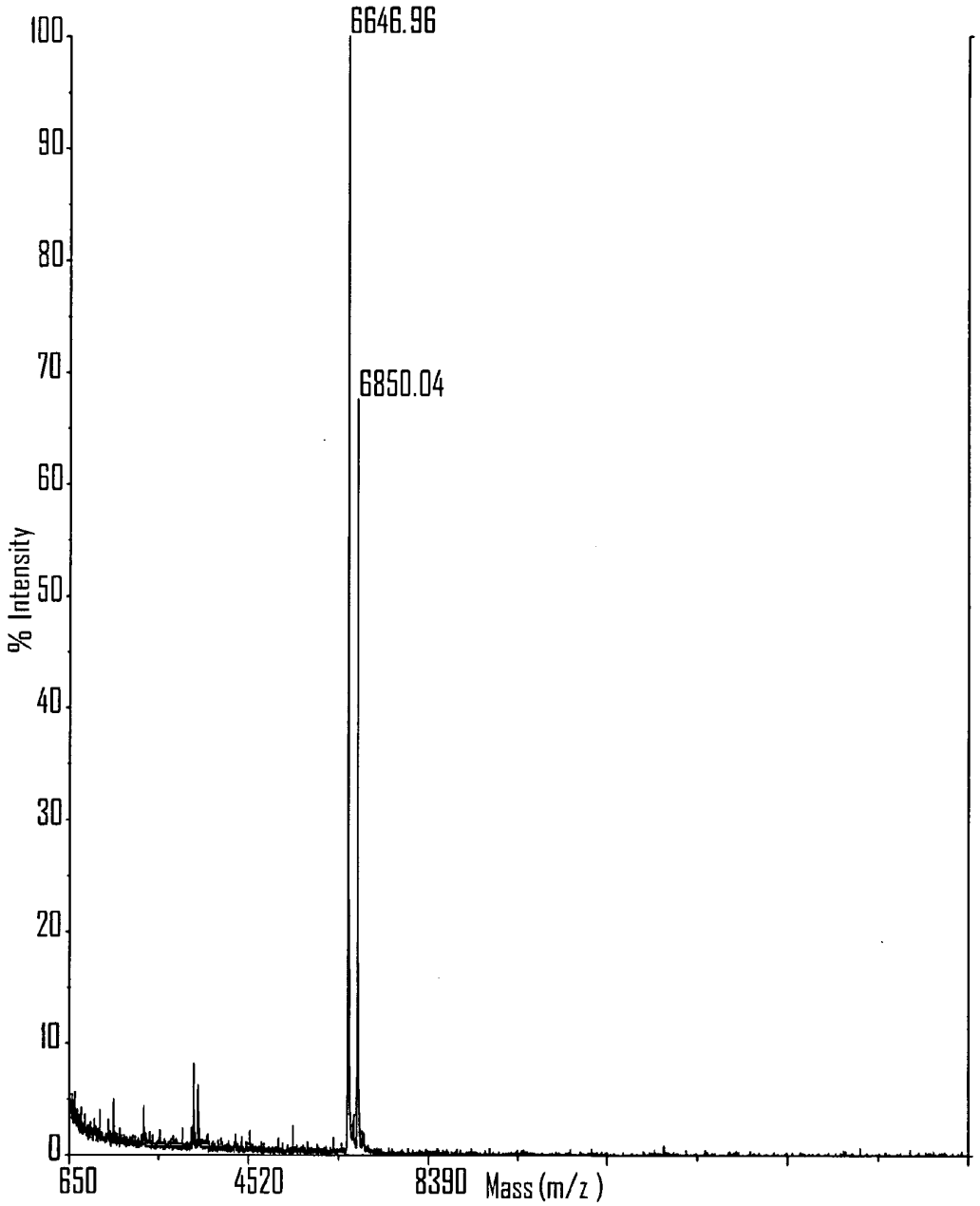

6646.96
6850.04
100
90
80
70
60
50
40
30
20
10
0
% Intensity
650
4520
8390
Mass (m/z)

Figure 3

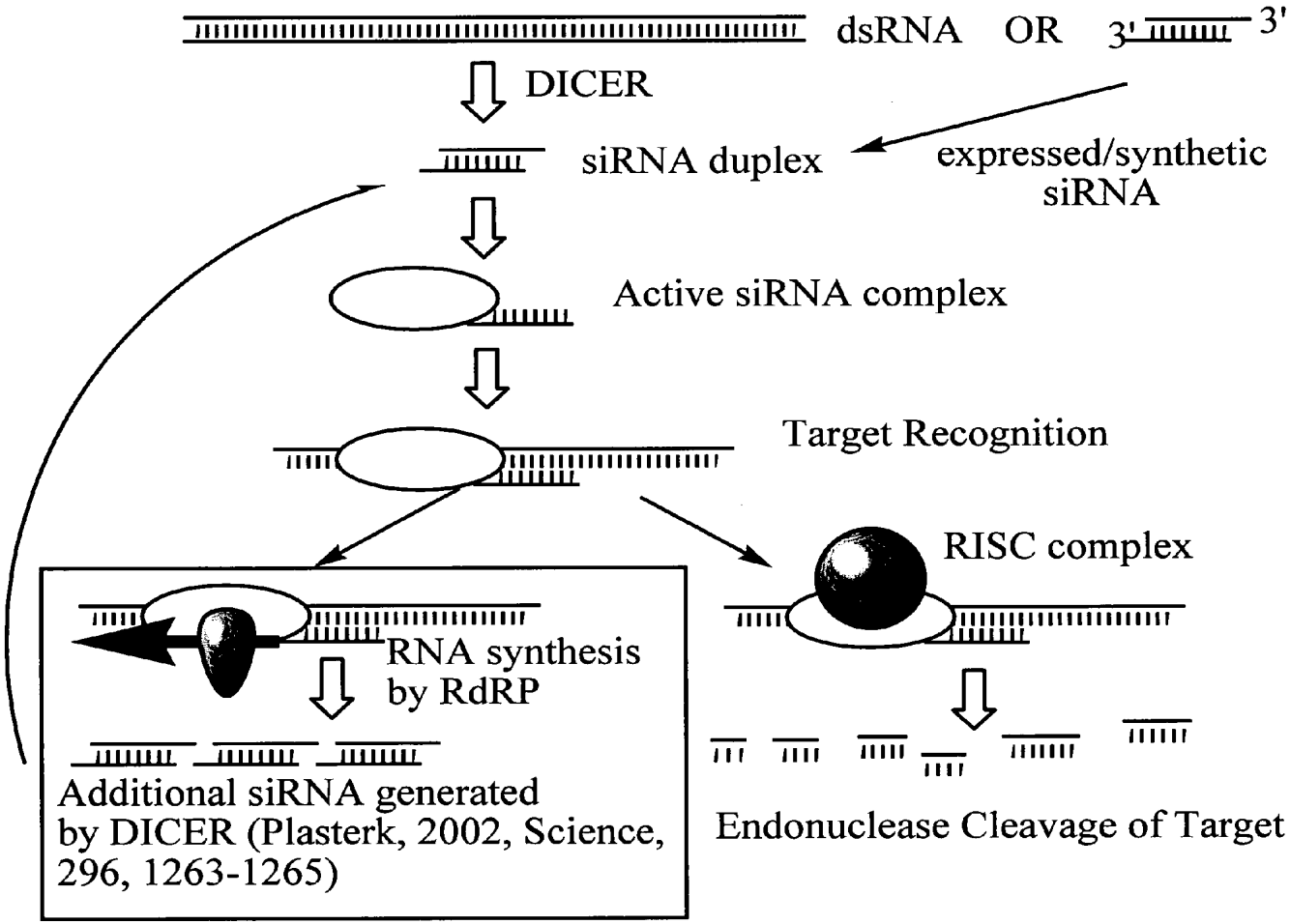

dsRNA OR 3' 3'
DICER
siRNA duplex
expressed/synthetic siRNA
Active siRNA complex
Target Recognition
RISC complex
RNA synthesis by RdRP
Additional siRNA generated by DICER (Plasterk, 2002, Science, 296, 1263-1265)
Endonuclease Cleavage of Target

Figure 4

A

SENSE STRAND (SEQ ID NO 767)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 768)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

B

SENSE STRAND (SEQ ID NO 769)
ALL PYRIMIDINES = 2'-F or OCF3 AND ALL PURINES = 2'-OMe EXCEPT POSITIONS (N N)

5'- N N N N N N N N N N N N N N N N N N N N (N_sN) -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 770)
ALL PYRIMIDINES = 2'-F or OCF3 AND ALL PURINES = 2'-O-Me EXCEPT POSITIONS (N N)

C

SENSE STRAND (SEQ ID NO 771)
ALL PYRIMIDINES = 2'-F or OCF3 EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 772)
ALL PYRIMIDINES = 2'-F or OCF3 EXCEPT POSITIONS (N N)

D

SENSE STRAND (SEQ ID NO 773)
ALL PYRIMIDINES = 2'-F or OCF3 EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 770)
ALL PYRIMIDINES = 2'-F or OCF3 AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

E

SENSE STRAND (SEQ ID NO 771)
ALL PYRIMIDINES = 2'-F or OCF3 EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 770)
ALL PYRIMIDINES = 2'-F or OCF3 AND ALL PURINES = 2'-O-Me EXCEPT POSITIONS (N N)

F

SENSE STRAND (SEQ ID NO 773)
ALL PYRIMIDINES = 2'-F or OCF3 EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 774)
ALL PYRIMIDINES = 2'-F or OCF3 EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE), 2'-O-METHYL, 2'-DEOXY-2'-FLUORO, OR UNIVERSAL BASES

B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT

L = GLYCERYL or B THAT IS OPTIONALLY PRESENT

S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE that is optionally absent

Figure 5

A

SENSE STRAND (SEQ ID NO 775)

5'- B- G A U C G C G G A G C C A U G G A U U *N N*-B -3'

3'- L-N_S*N* C U A G C G C C U C G G U A C C U A A -5'

ANTISENSE STRAND (SEQ ID NO 776)

B

SENSE STRAND (SEQ ID NO 777)

5'- g a *u* c g c g g a g *c c* a *u* g g a *u u* N_S*N* -3'

3'- L-N_S*N c u* a g *c* g *c c u c* g g *u* a *c c u* a a -5'

ANTISENSE STRAND (SEQ ID NO 778)

C

SENSE STRAND (SEQ ID NO 779)

5'- B-G A *u c* G *c* G G A G *c c* A *u* G G A *u u N N*-B -3'

3'- L-N_S*N c u* A G *c* G *c c u c* G G *u* A *c c u* A A -5'

ANTISENSE STRAND (SEQ ID NO 780)

D

SENSE STRAND (SEQ ID NO 781)

5'- B-*G A u c G c G G A G c c A u G G A u u N N*-B -3'

3'- L-N_S*N c u* a g *c* g *c c u c* g g *u* a *c c u* a a -5'

ANTISENSE STRAND (SEQ ID NO 778)

E

SENSE STRAND (SEQ ID NO 779)

5'- B-G A *u c* G *c* G G A G *c c* A *u* G G A *u u N N*-B -3'

3'- L-N_S*N c u* a g *c* g *c c u c* g g *u* a *c c u* a a -5'

ANTISENSE STRAND (SEQ ID NO 778)

F

SENSE STRAND (SEQ ID NO 781)

5'- B-*G A u c G c G G A G c c A u G G A u u N N*-B -3'

3'- L-N_S*N c u A G c G c c u c G G u A c c u A A* -5'

ANTISENSE STRAND (SEQ ID NO 782)

italic lower case = 2'-deoxy-2'-fluoro or 2'-OCF$_3$
underline = 2'-O-methyl
ITALIC UPPER CASE = DEOXY
N = Deoxy, 2'-OMe, 2'-deoxy-2'-fluoro, LNA etc.

B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT
L = GLYCERYL MOIETY or B OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE OPTIONALLY PRESENT

Figure 6A

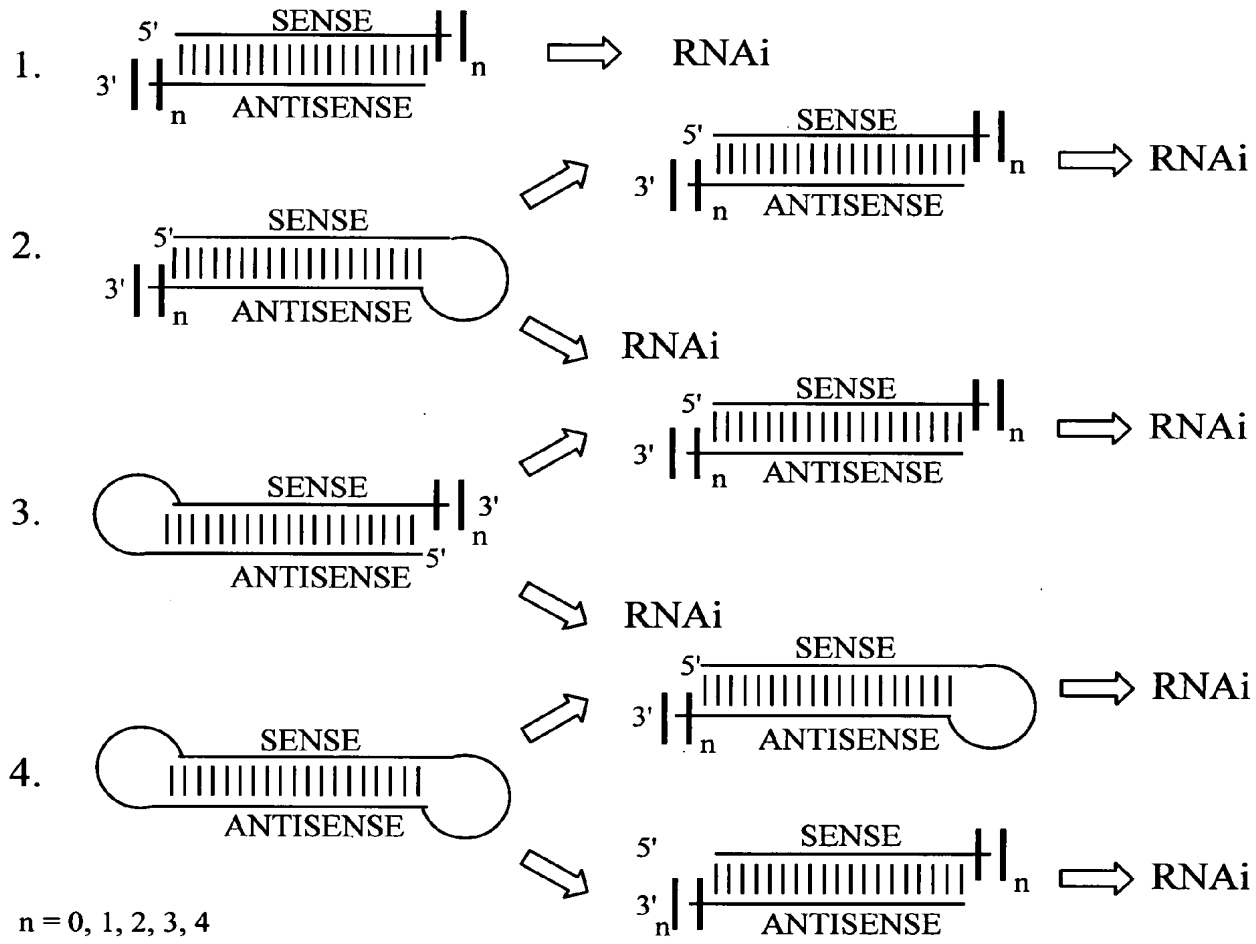
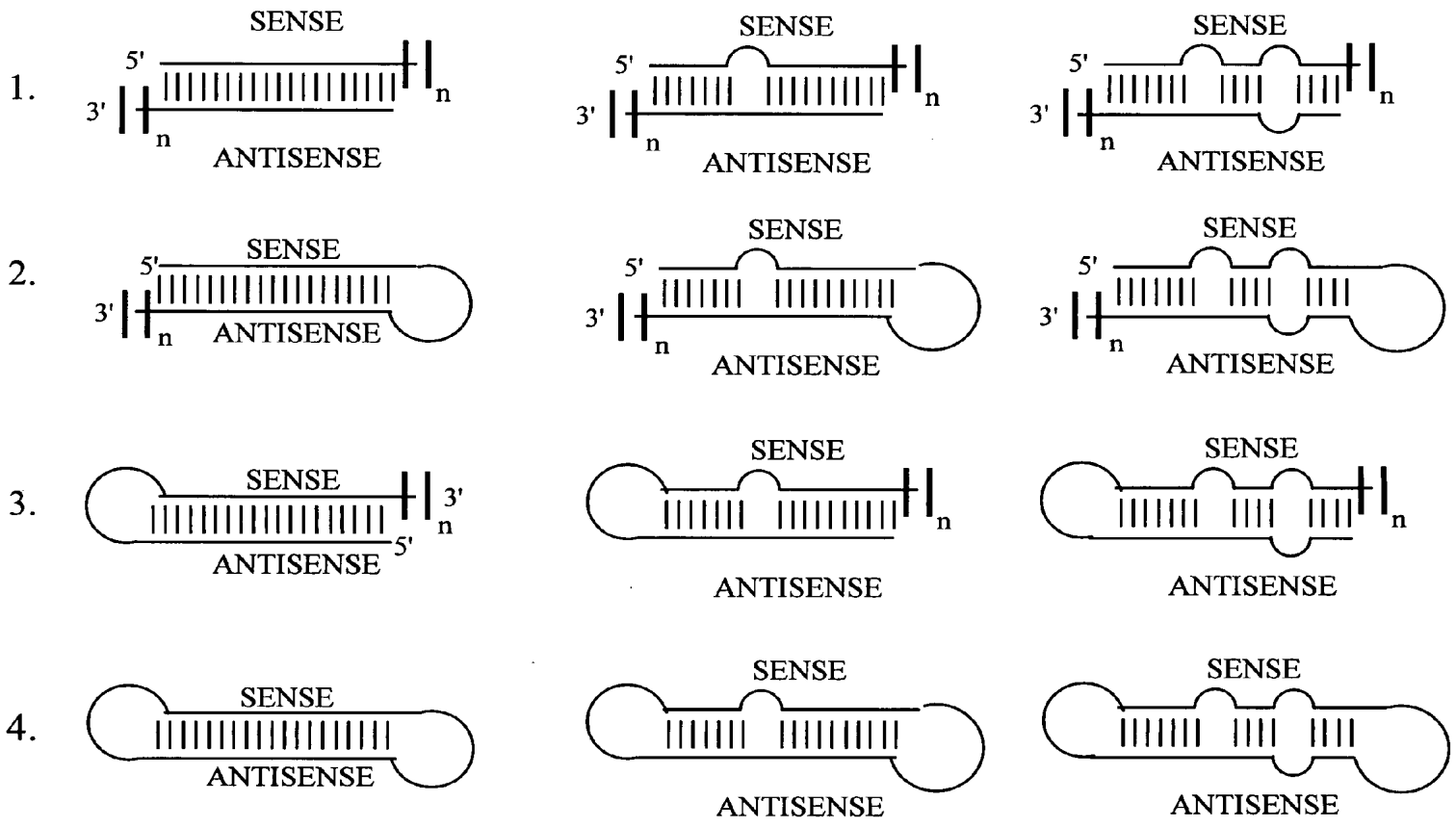

1.
5'
SENSE
3'
n
ANTISENSE
n
RNAi
2.
5'
SENSE
3'
n
ANTISENSE
5'
SENSE
3'
n
ANTISENSE
RNAi
RNAi
3.
SENSE
3'
n
ANTISENSE
5'
5'
SENSE
3'
n
ANTISENSE
RNAi
RNAi
4.
SENSE
ANTISENSE
5'
SENSE
3'
n
ANTISENSE
RNAi
5'
SENSE
3'
n
ANTISENSE
RNAi
n = 0, 1, 2, 3, 4

Figure 6B



n = 0, 1, 2, 3, 4

Figure 6C

11th nucleotide position based on
5'-end of guide strand

1. 5'- B-N N N N N N N N N *N* N N N N N N N N N N (N N)-B -3'
2. 3'- B-(N N) N N N N N N N N N N N N N N N N N N N N N N -5'
3. 5'-------[N N] N N N N N N N N N N N N N N N N N N N N N N ---------- -3'

1. = sense strand (passenger strand)
2. = antisense strand (guide strand)
3. = target polynucleotide sequence

The guide strand is complementary to the target sequence and the passenger strand is complementary to the guide strand.
Overhang nucleotides (NN) in the guide strand can be complementary to nucleotides [NN] in target sequence.
Overhang nucleotides (NN) in the passenger strand can comprise nucleotides [NN] in target sequence.
Position *N* of the passenger strand can comprise a ribonucleotide. For the representative 19 base pair 21 mer duplex shown, position *N* is 9 nucleotides in from the 3' end of the passenger strand. However, in duplexes of differing length, the position *N* is determined based on the 5'-end of the guide strand by counting 11 nucleotide positions in from the 5'-terminus of the guide strand and picking the corresponding base paired nucleotide in the passenger strand. Cleavage by Ago2 takes place between positions 10 and 11 as indicated by the arrow.
Representative 2 nucleotide overhangs are shown, but can vary for example from 0 to about 4 nucleotides.
B = terminal cap which can be present or absent
This generalized motif can be applied to all Stab 00-34 chemistries herein.

Figure 7

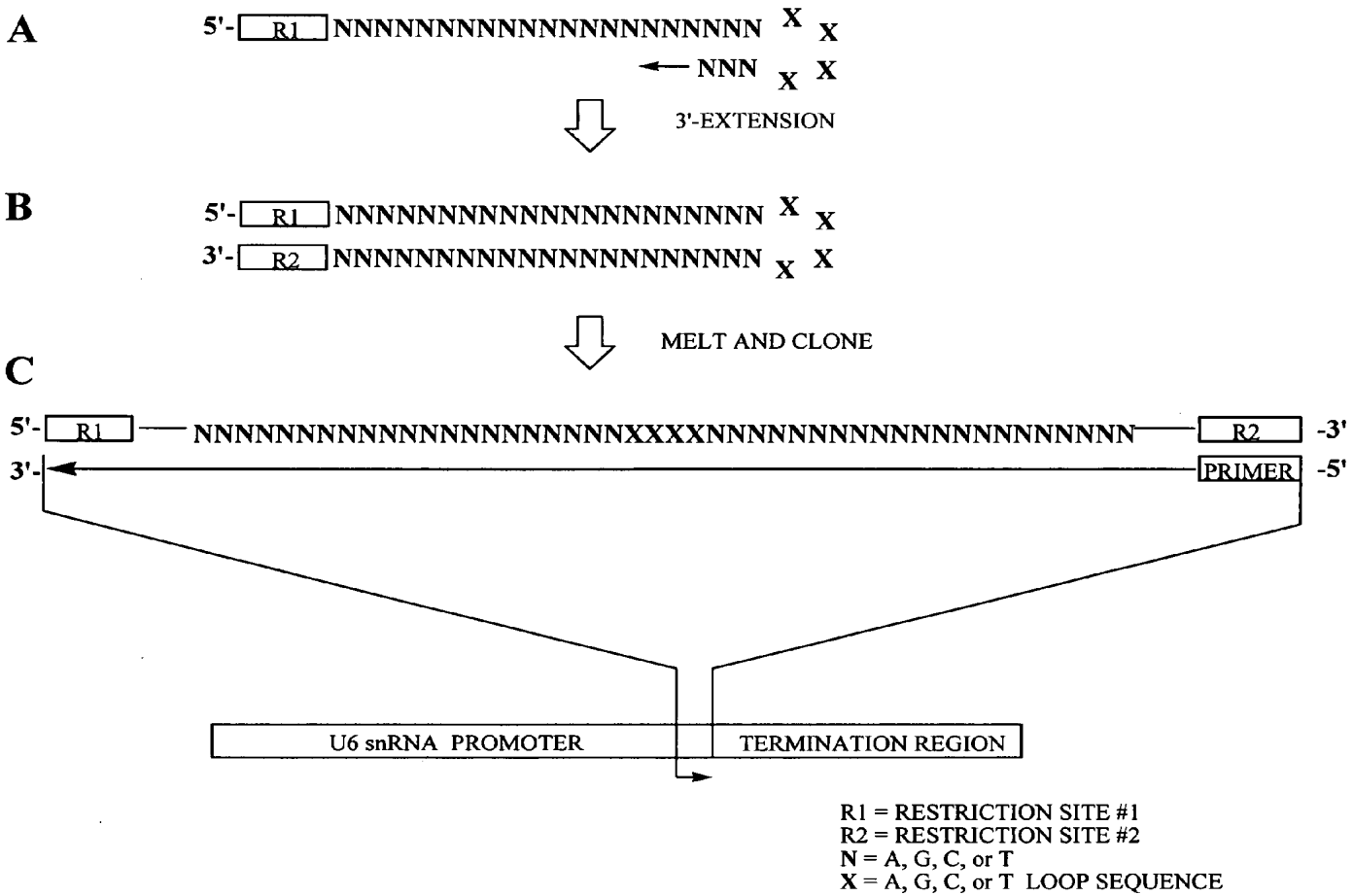

A
5'- R1 NNNNNNNNNNNNNNNNNNNNNN X X
NNN X X
3'-EXTENSION
B
5'- R1 NNNNNNNNNNNNNNNNNNNNNN X X
3'- R2 NNNNNNNNNNNNNNNNNNNNNN X X
MELT AND CLONE
C
5'- R1 NNNNNNNNNNNNNNNNNNNNNNXXXXNNNNNNNNNNNNNNNNNNNNNNNN R2 -3'
3'- PRIMER -5'
U6 snRNA PROMOTER
TERMINATION REGION
R1 = RESTRICTION SITE #1
R2 = RESTRICTION SITE #2
N = A, G, C, or T
X = A, G, C, or T LOOP SEQUENCE

Figure 8

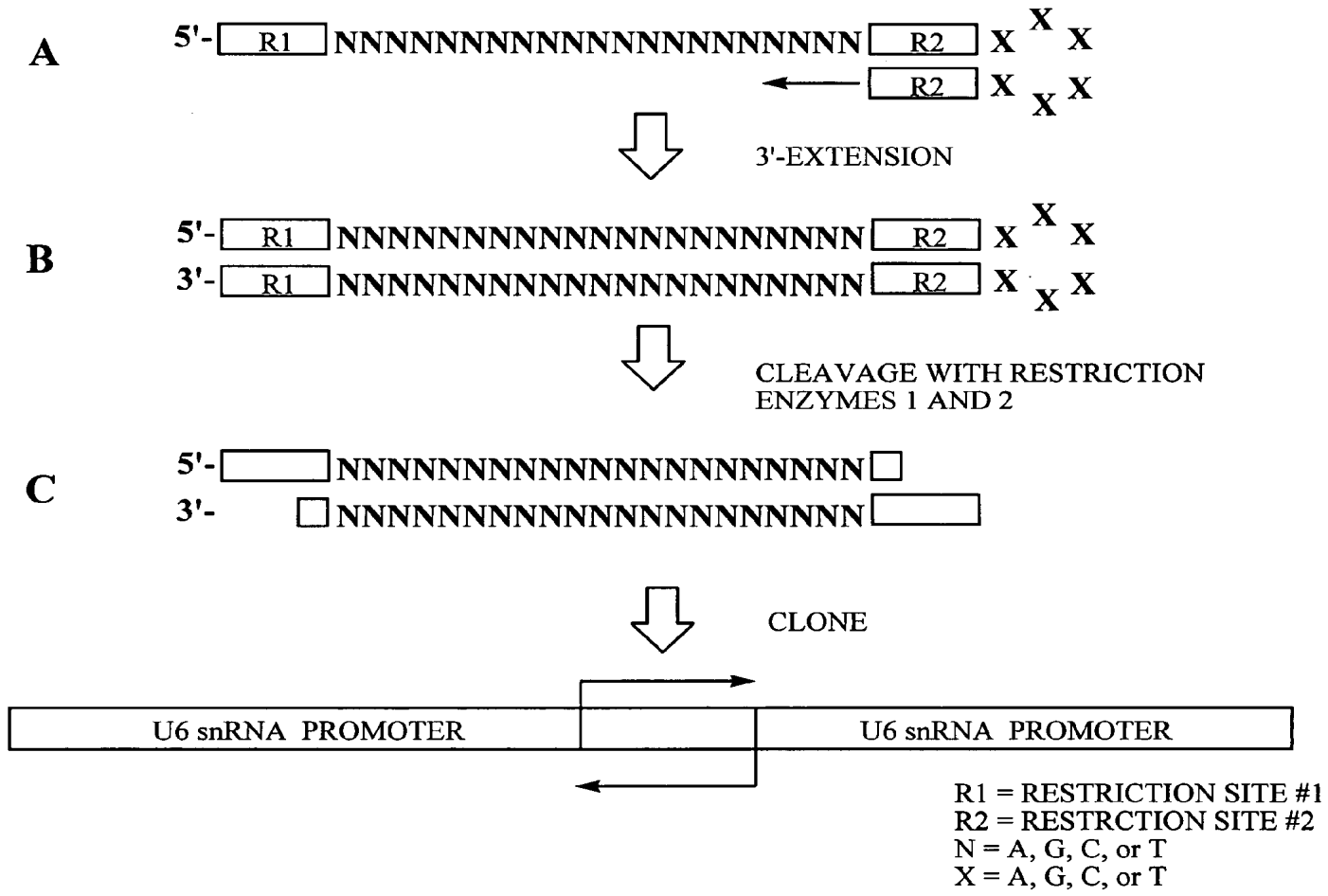

A
5'- R1 NNNNNNNNNNNNNNNNNNNNNN R2 X X X
R2 X X X
3'-EXTENSION
B
5'- R1 NNNNNNNNNNNNNNNNNNNNNN R2 X X X
3'- R1 NNNNNNNNNNNNNNNNNNNNNN R2 X X X
CLEAVAGE WITH RESTRICTION ENZYMES 1 AND 2
C
5'- NNNNNNNNNNNNNNNNNNNNNN
3'- NNNNNNNNNNNNNNNNNNNNNN
CLONE
U6 snRNA PROMOTER
U6 snRNA PROMOTER
R1 = RESTRICTION SITE #1
R2 = RESTRCTION SITE #2
N = A, G, C, or T
X = A, G, C, or T

Figure 9: Target site Selection using siRNA

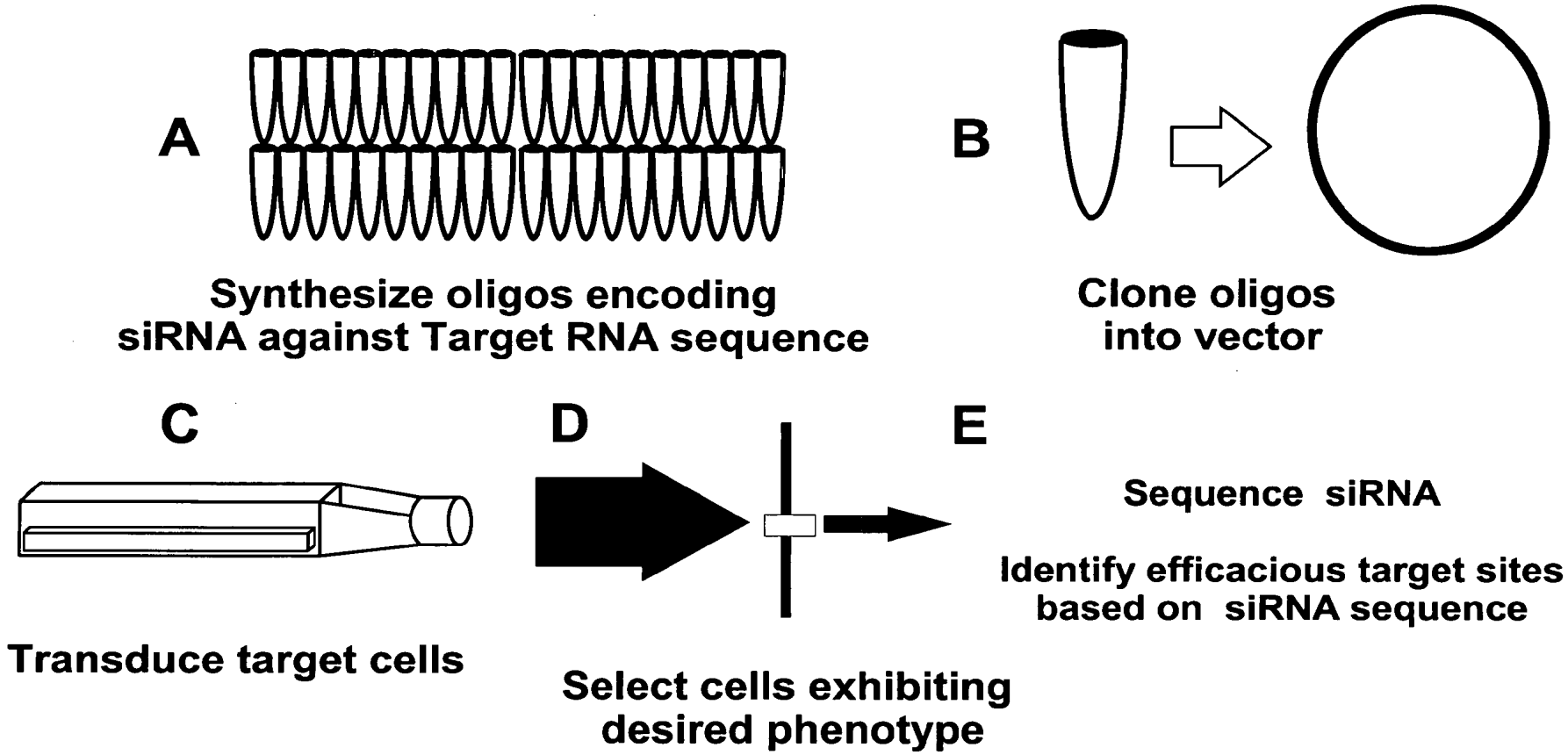
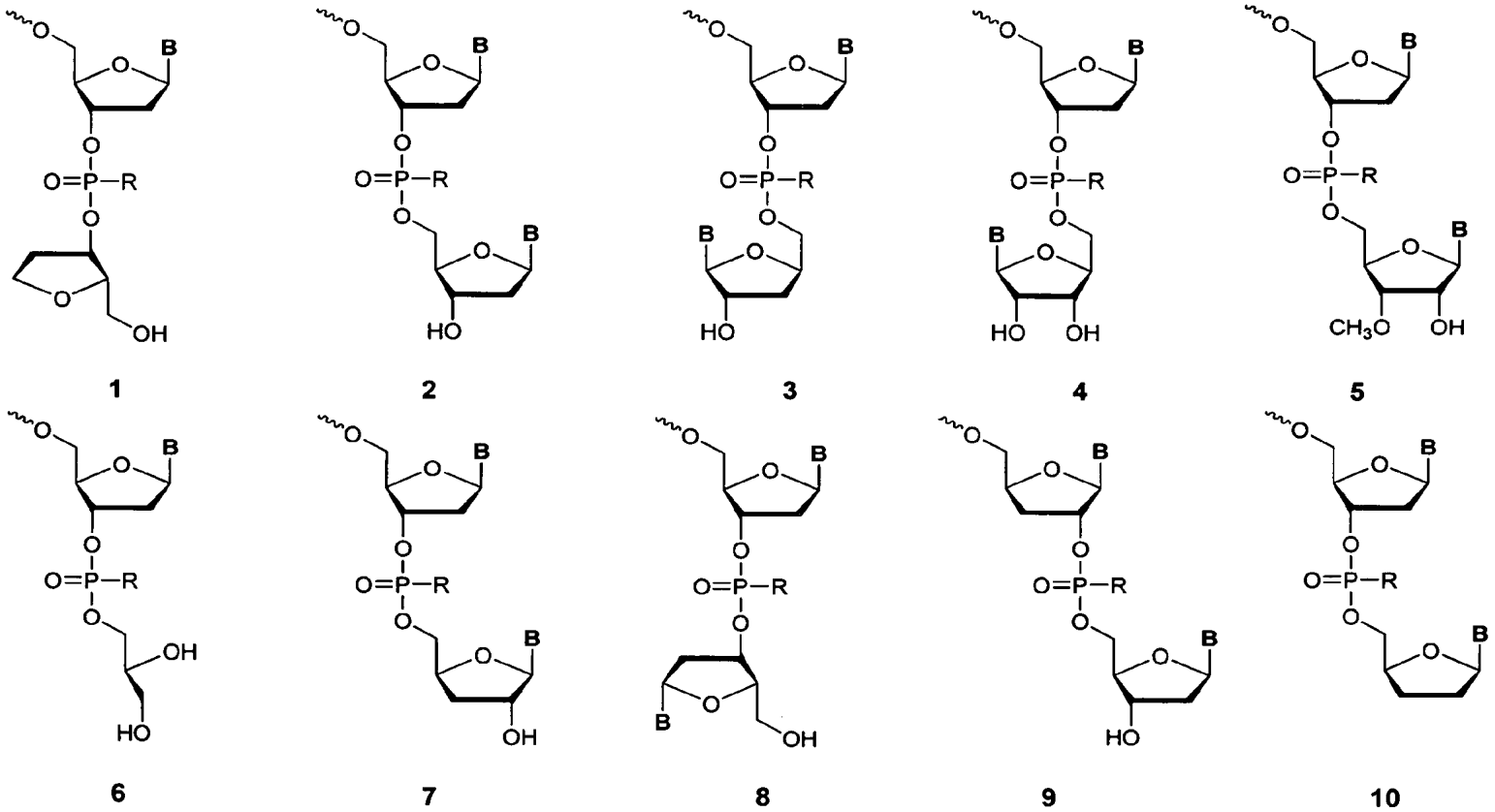


Figure 10



R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl
B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

Figure 11: Modification Strategy

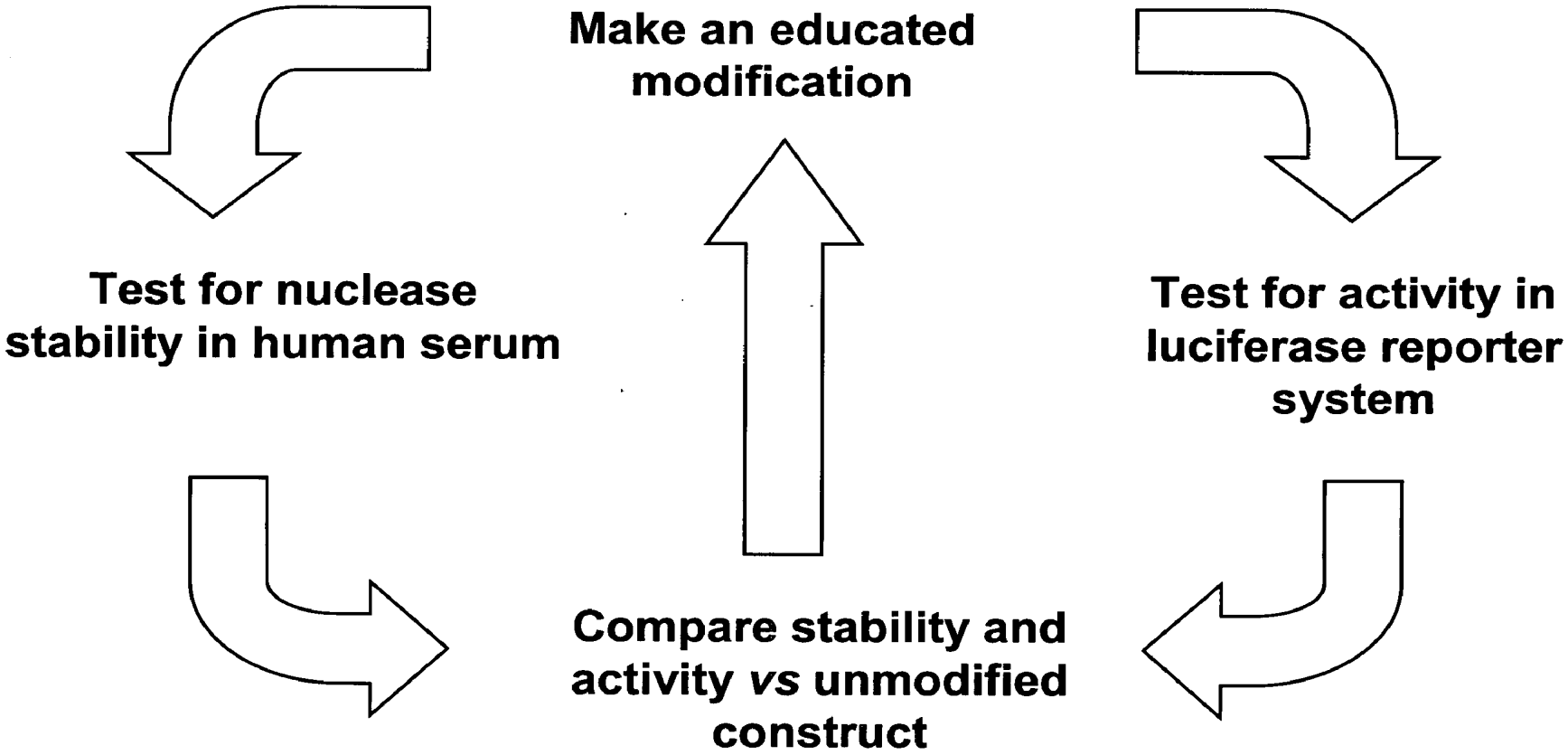


Figure 12: Phosphorylated siNA constructs

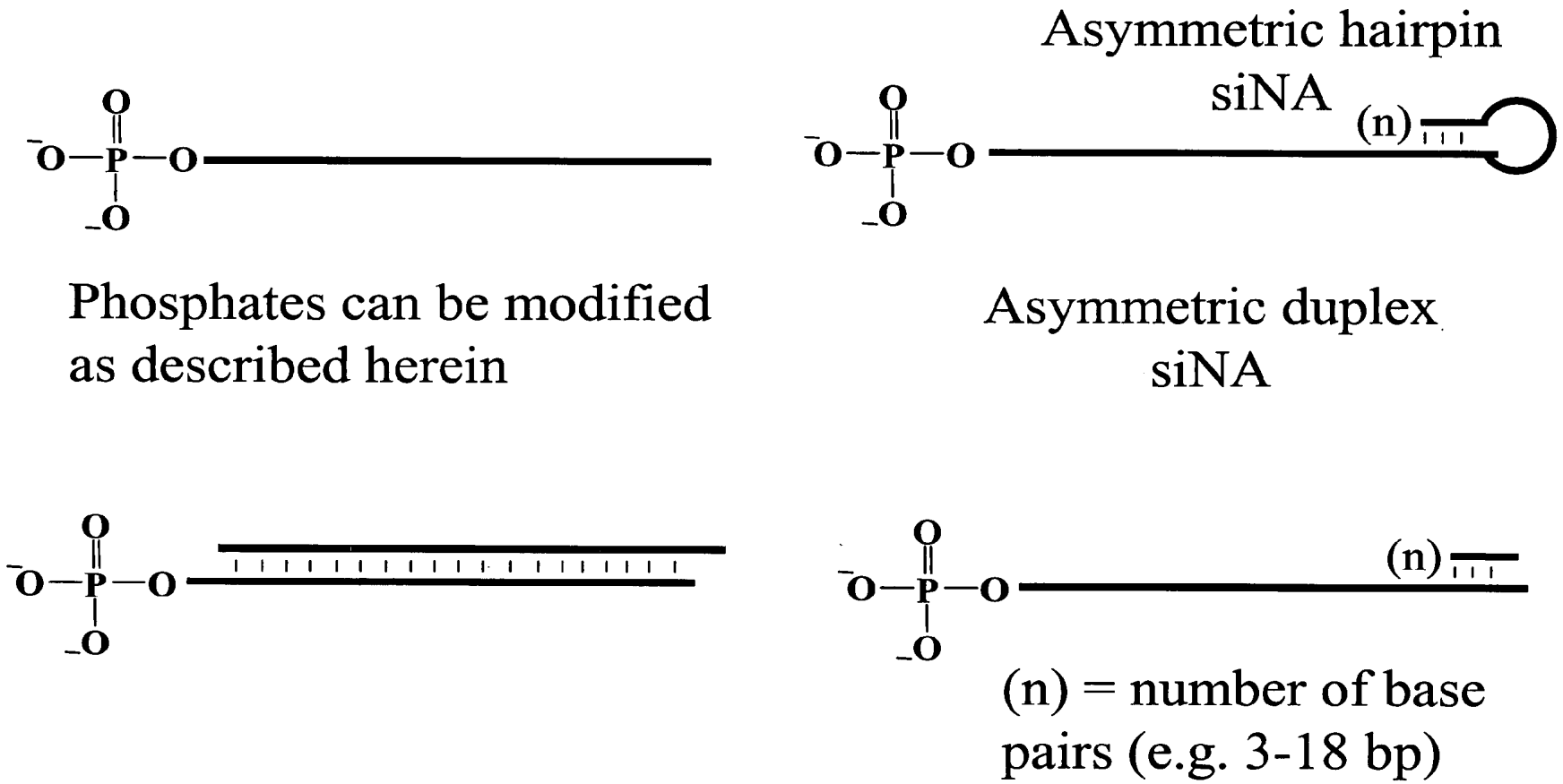


Figure 13: 5'-phosphate modifications

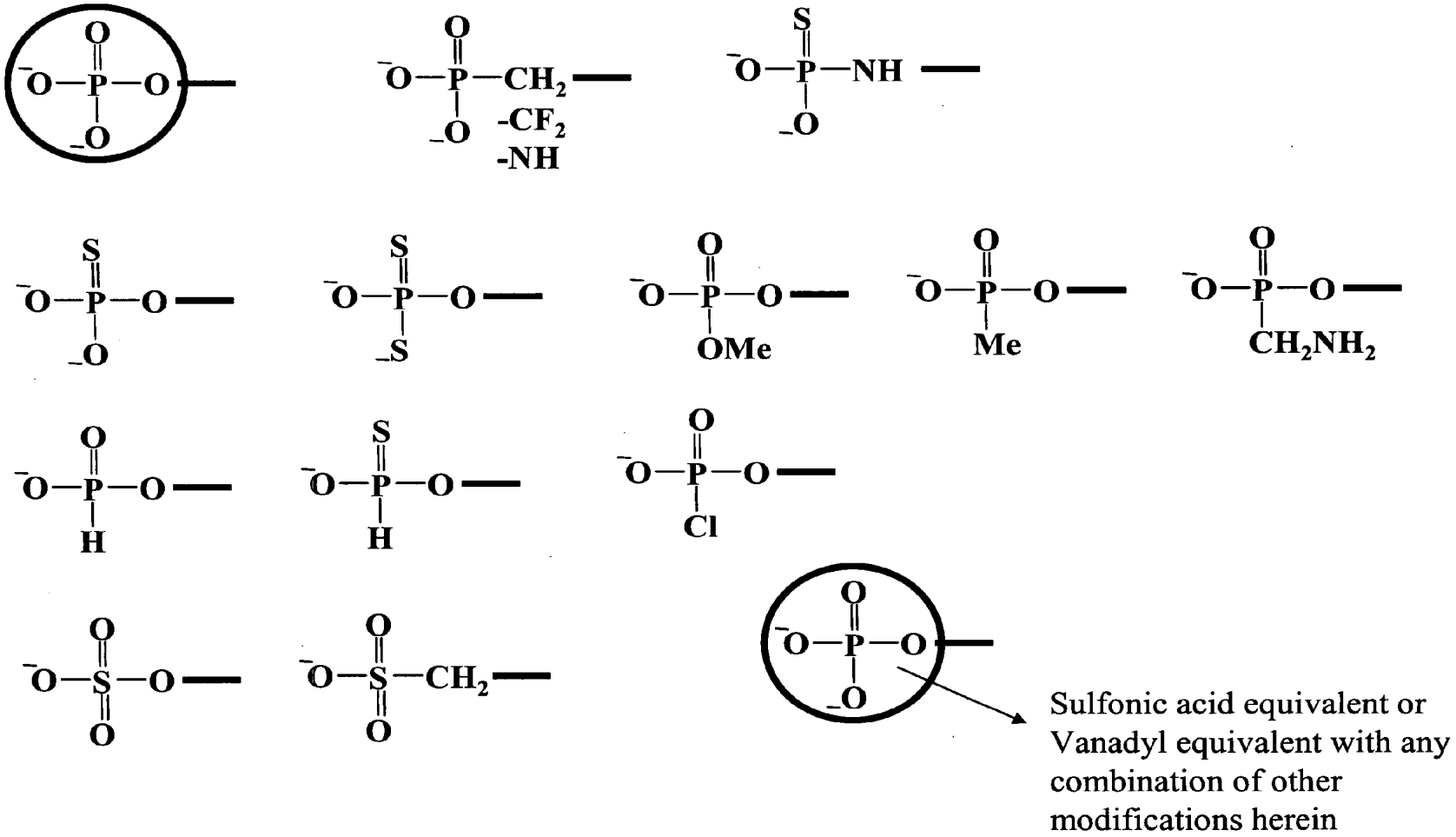


Figure 14A: Duplex forming oligonucleotide constructs that utilize Palindrome or repeat sequences

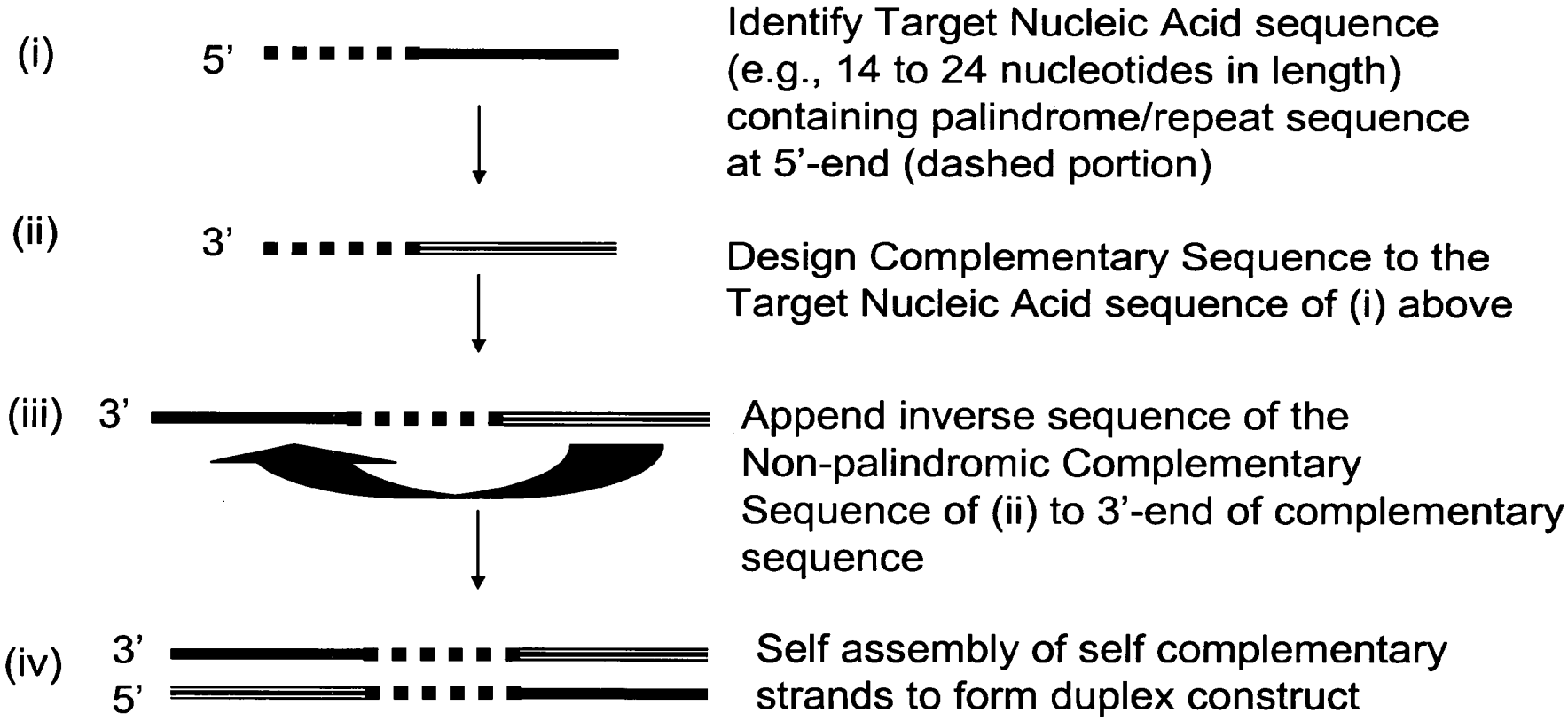


Figure 14B: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence

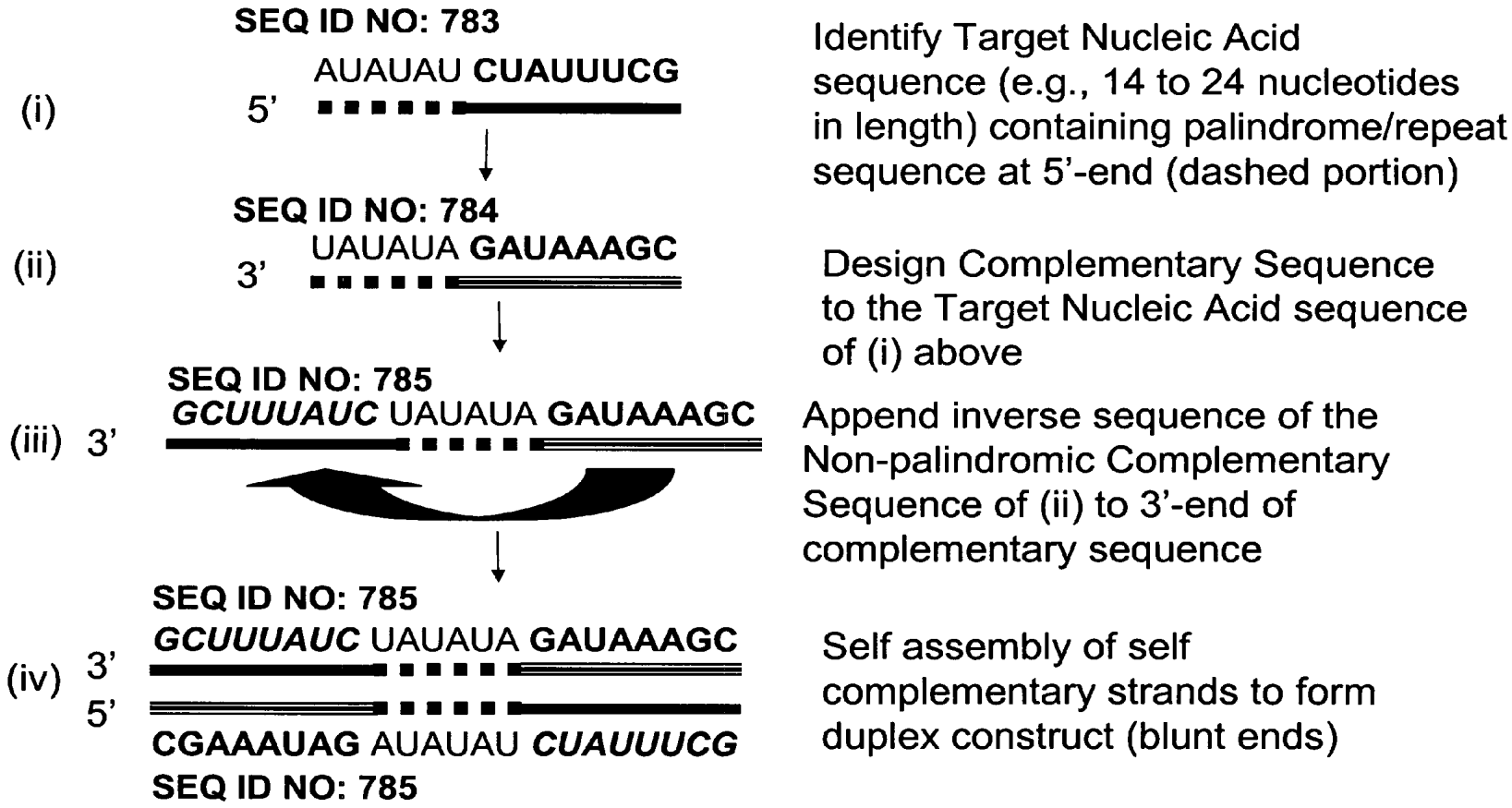


Figure 14C: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly

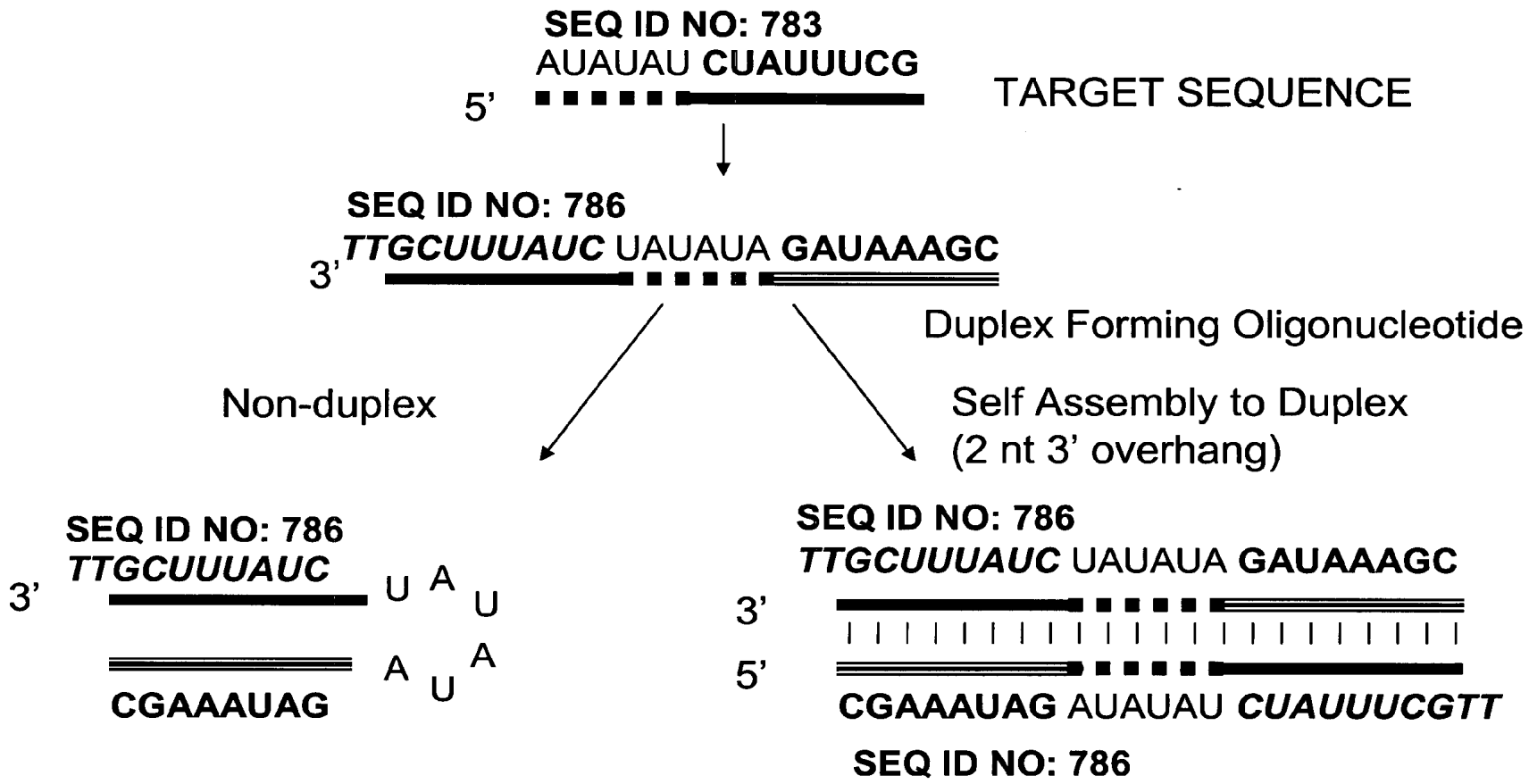


Figure 14D: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly and inhibition of Target Sequence Expression

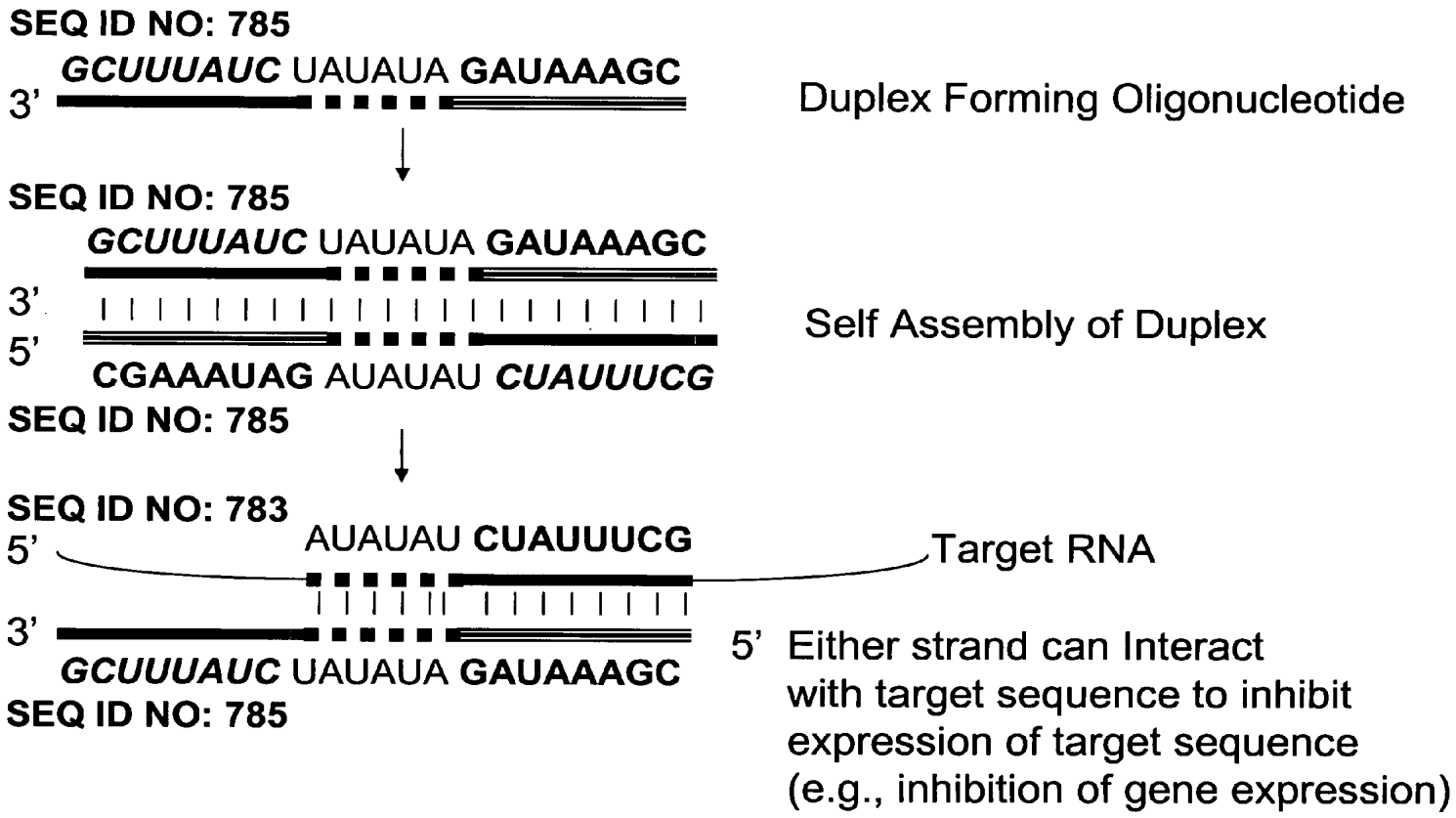
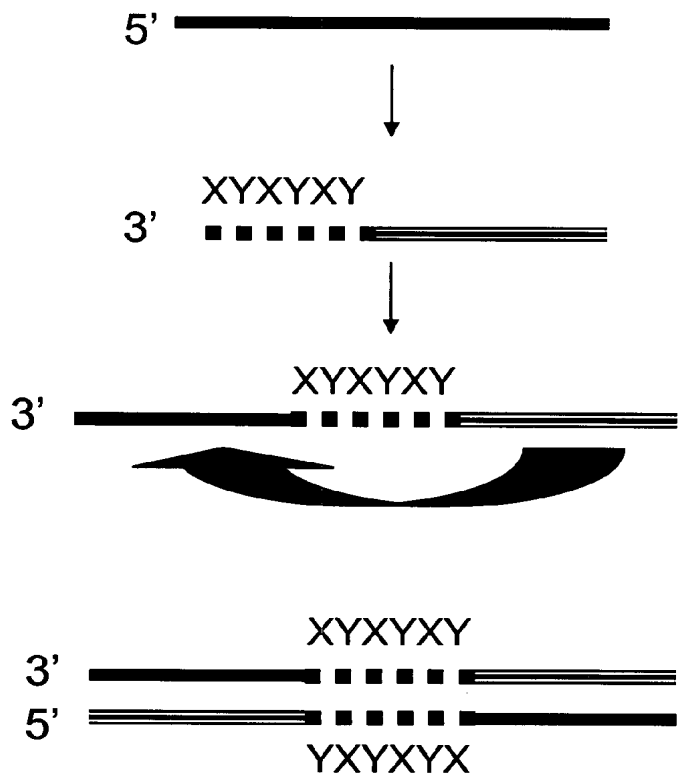


Figure 15: Duplex forming oligonucleotide constructs that utilize artificial palindrome or repeat sequences



Identify Target Nucleic Acid sequence (e.g., 14 to 24 nucleotides in length)

Design Complementary Sequence and utilize modified nucleotides (shown as X, Y) that interact with a portion of the target sequence and result in the formation of a palindrome/repeat sequence (e.g., 2 to 12 nucleotides) at 3'-end (dashed portion)

Append inverse sequence of Complementary region to 3'-end of palindrome/repeat sequence

Hybridize self complementary strands to form duplex siNA construct

Figure 16: Examples of double stranded multifunctional siNA constructs with distinct complementary regions

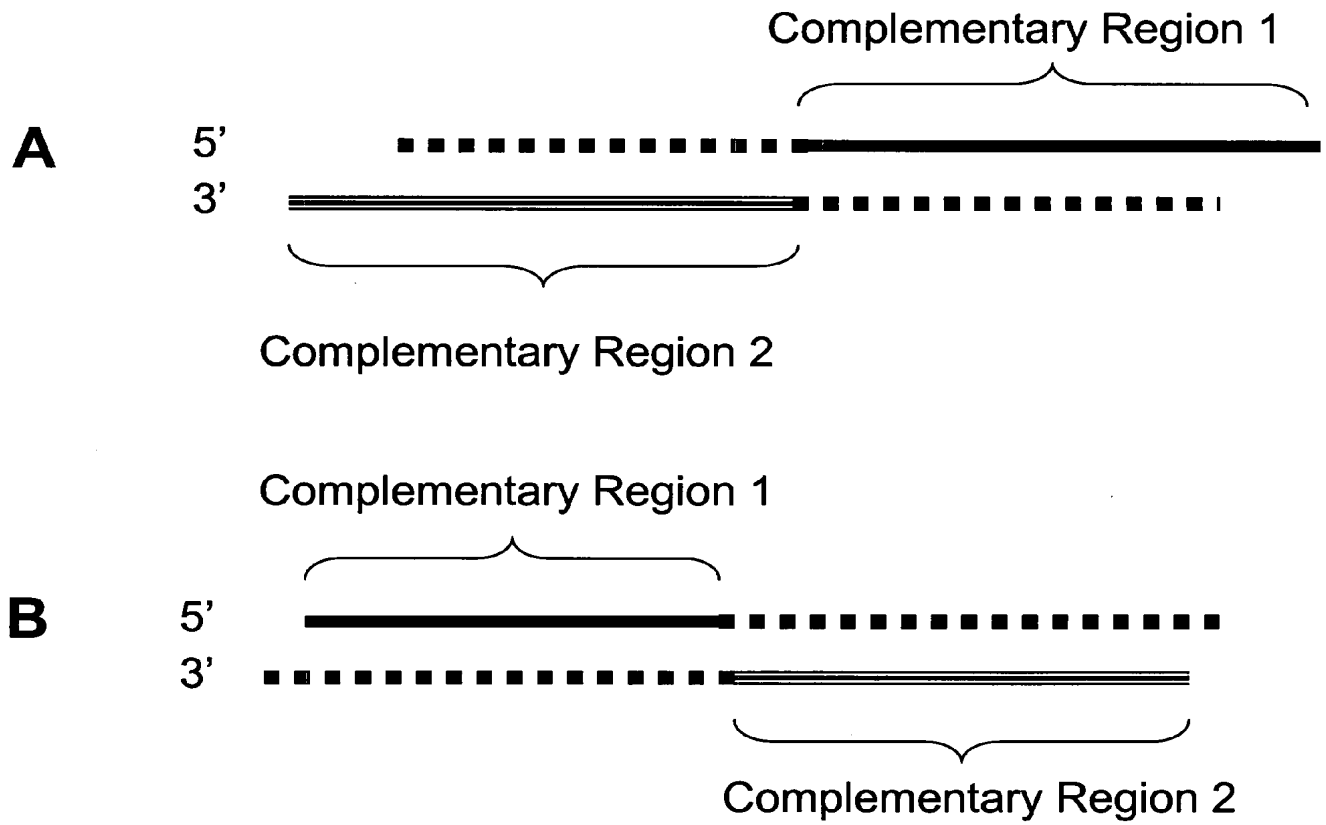


Figure 17: Examples of hairpin multifunctional siNA constructs with distinct complementary regions

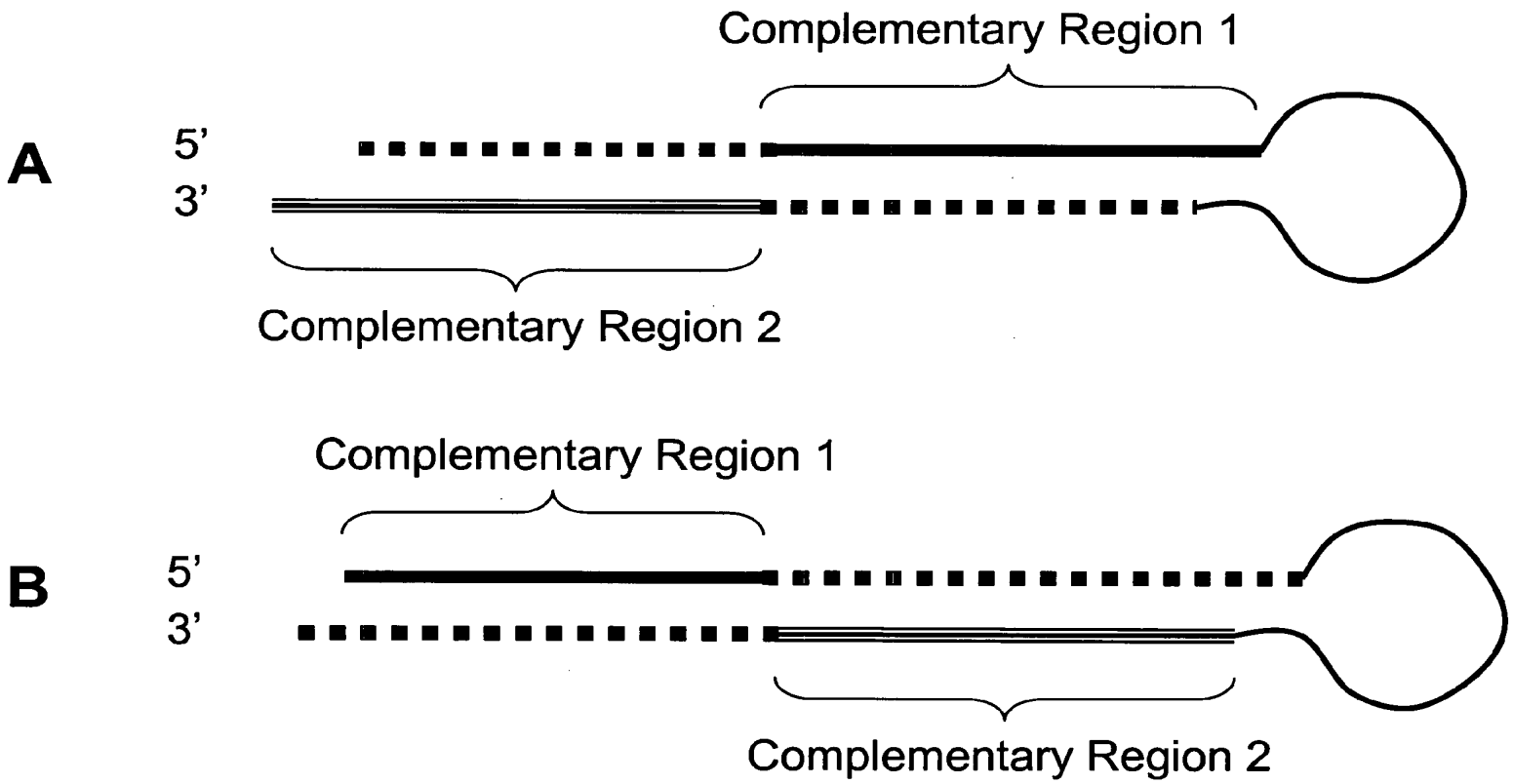


Figure 18: Examples of double stranded multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region

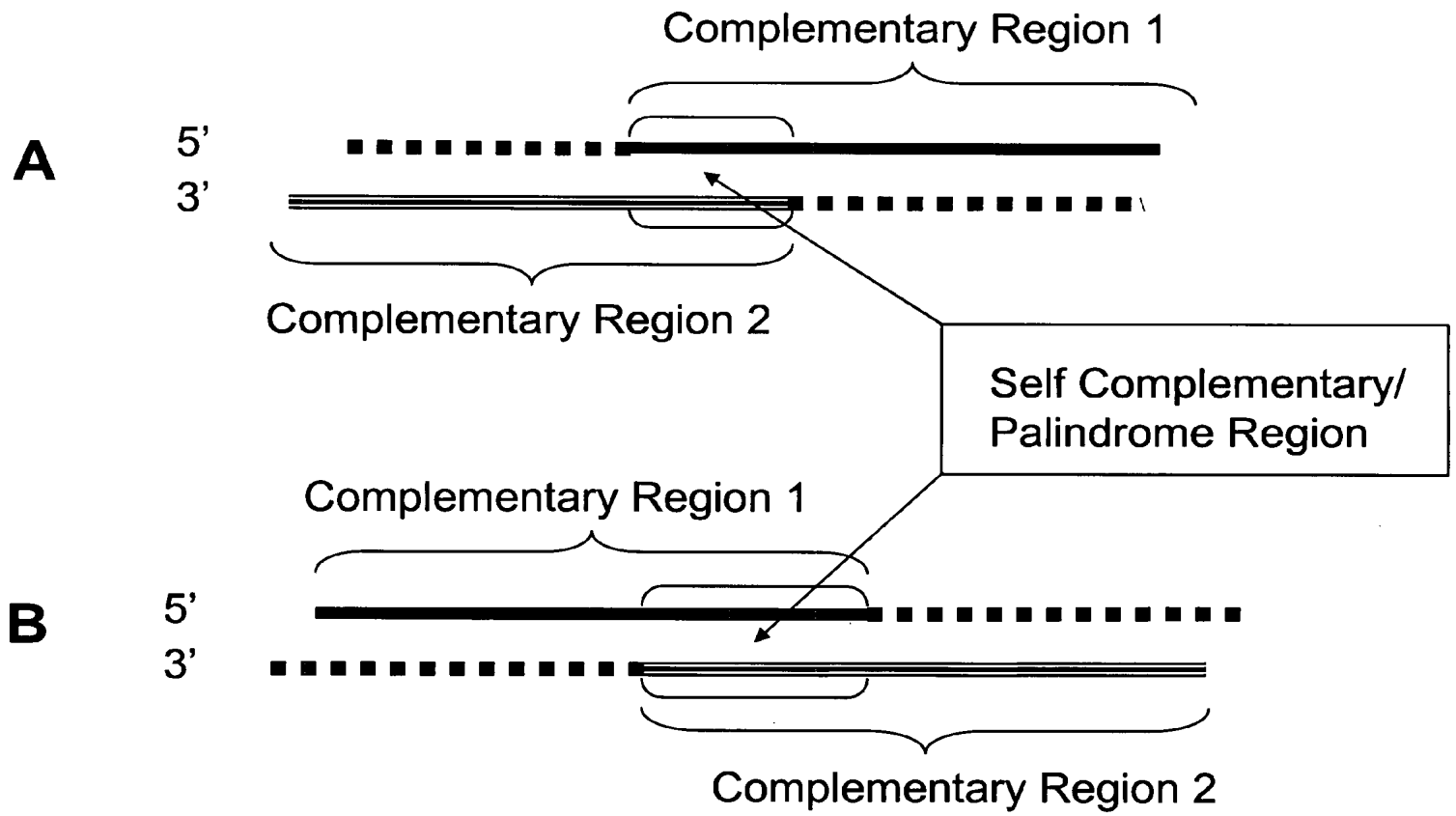


Figure 19: Examples of hairpin multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region

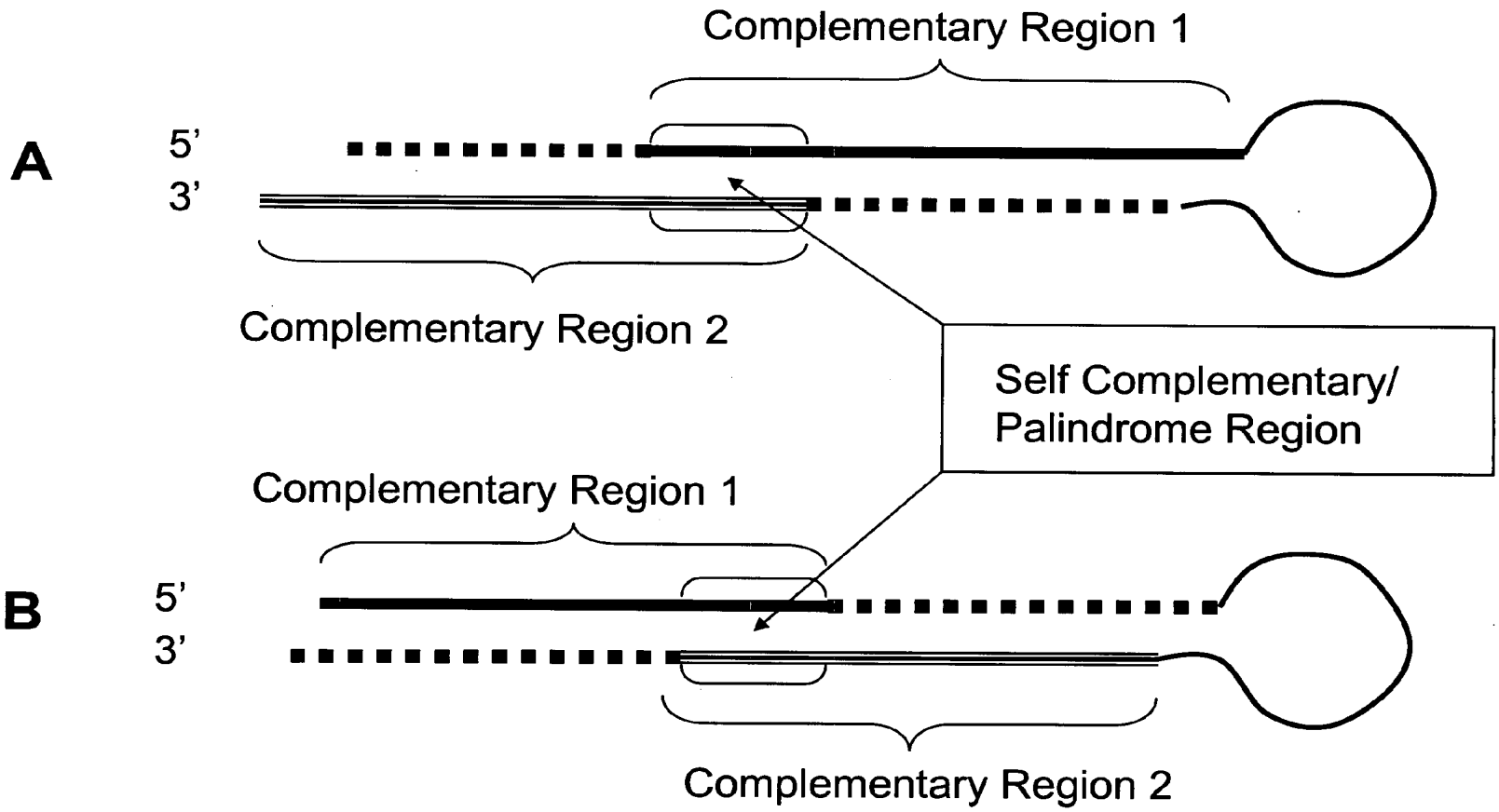


Figure 20: Example of multifunctional siNA targeting two Separate Target nucleic acid sequences

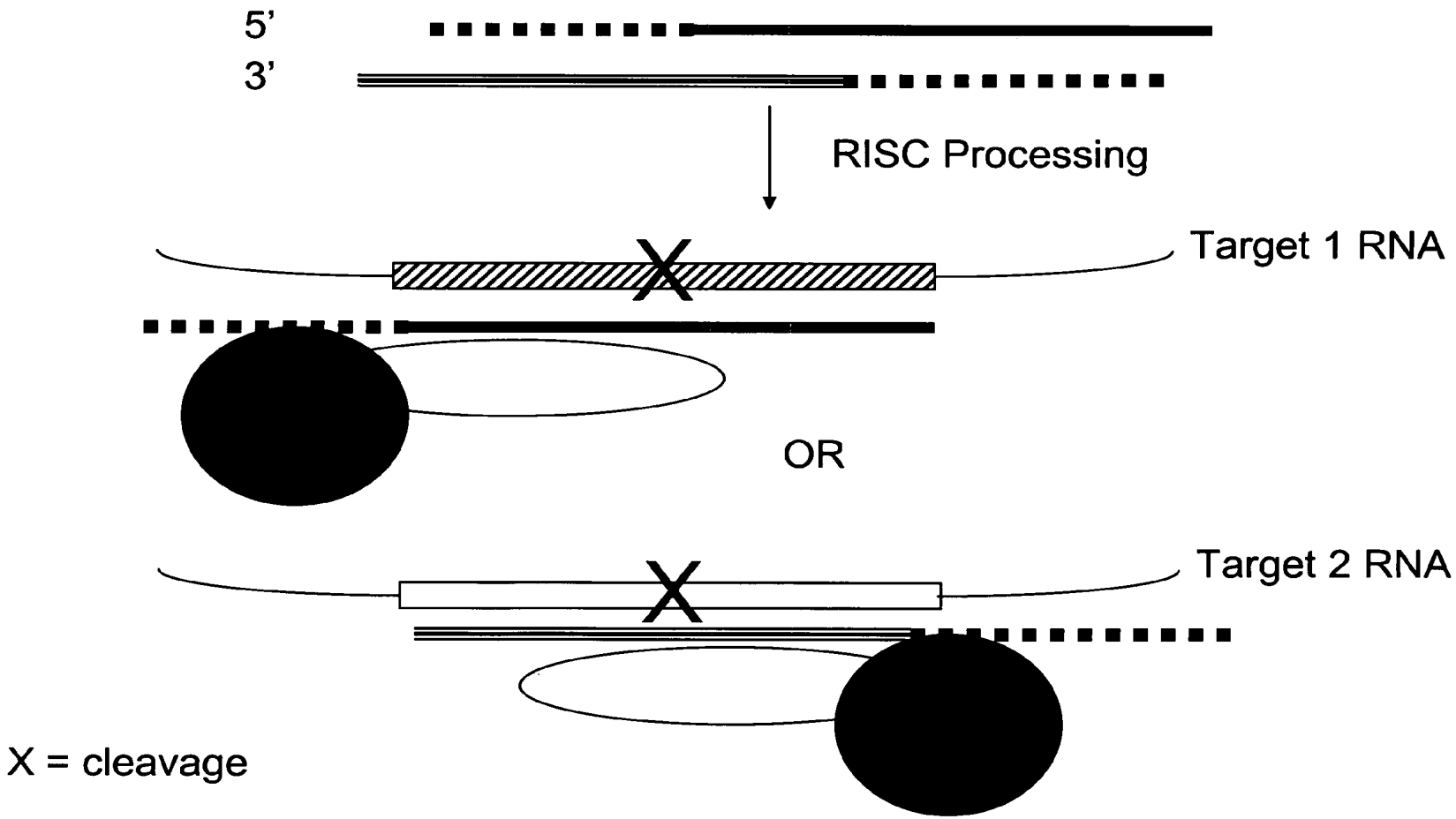
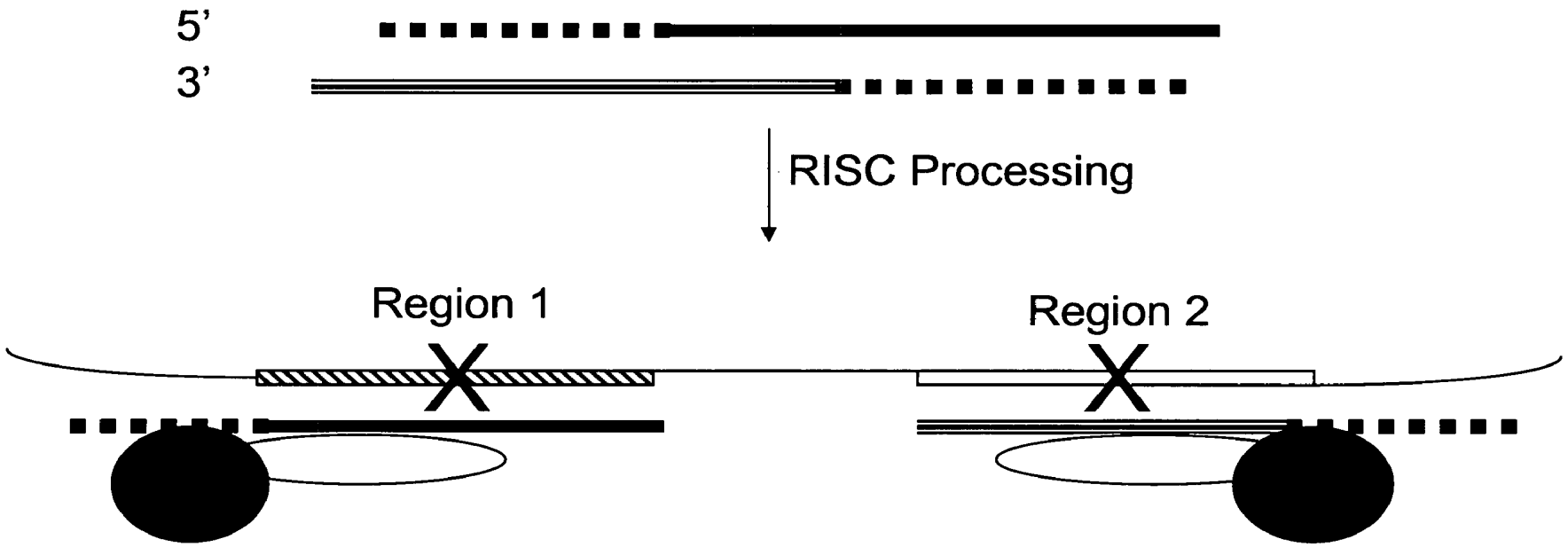
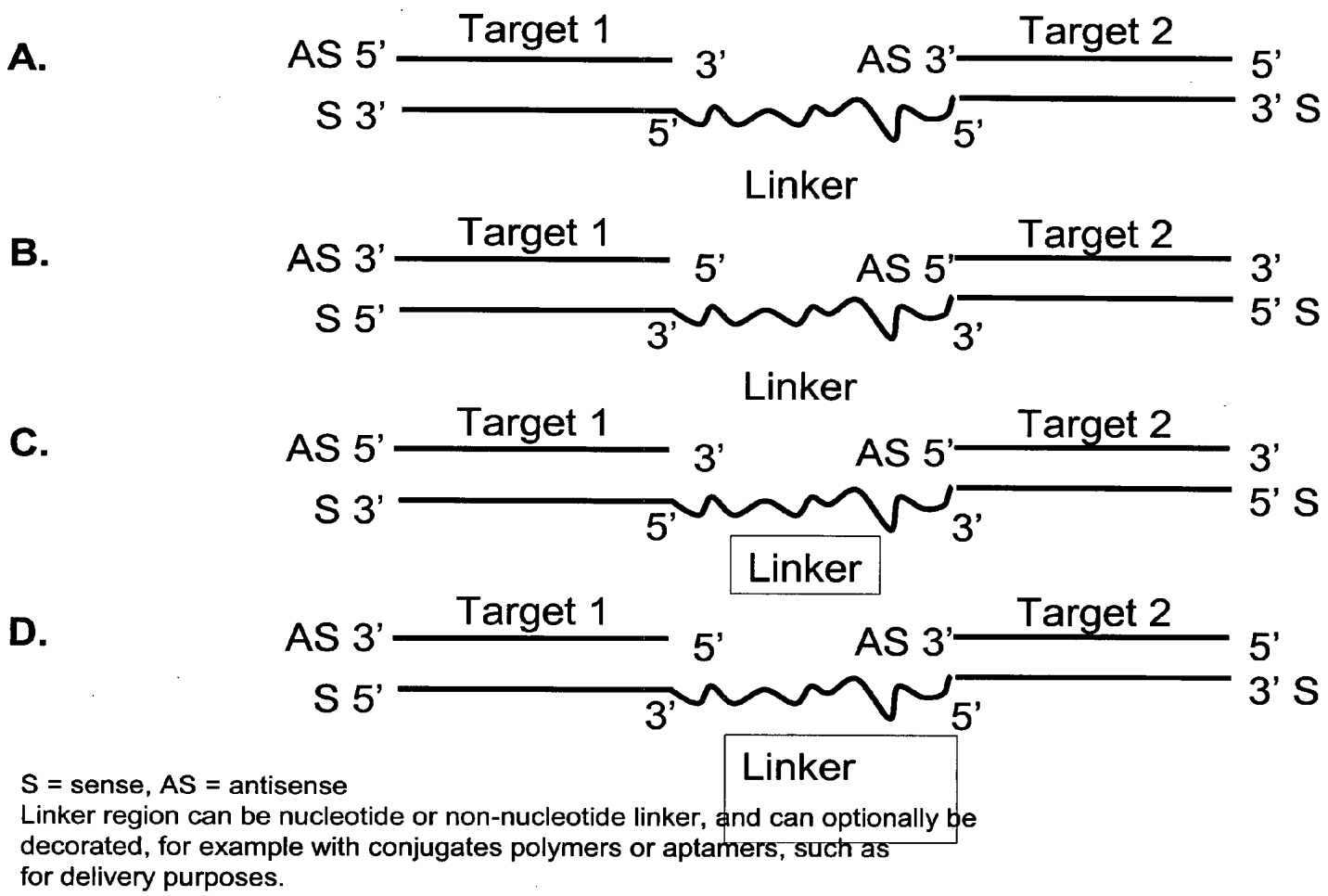


Figure 21: Example of multifunctional siNA targeting two regions within the same target nucleic acid sequence



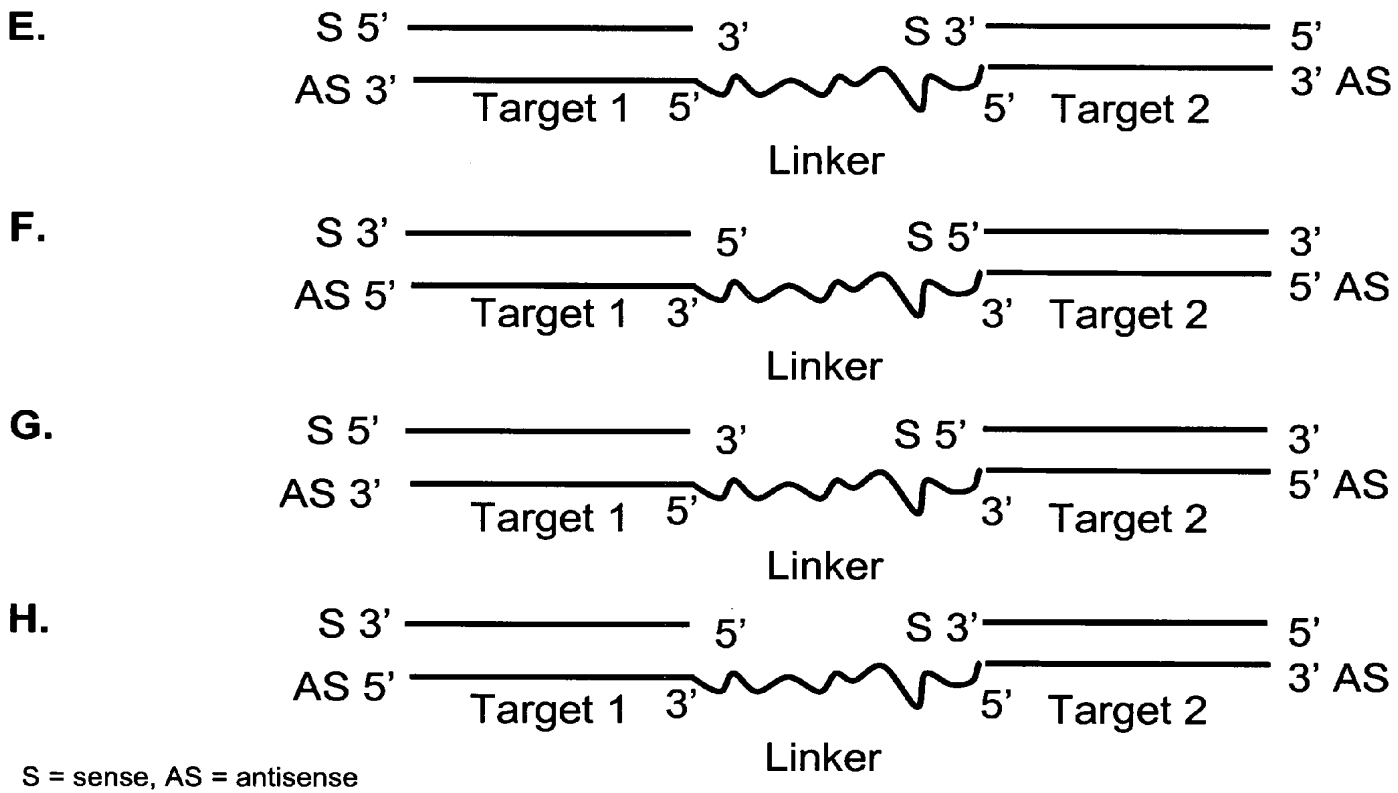
X = cleavage

Figure 22: Tethered Multifunctional siNA design



S = sense, AS = antisense
Linker region can be nucleotide or non-nucleotide linker, and can optionally be decorated, for example with conjugates polymers or aptamers, such as for delivery purposes.

Figure 22: Tethered Multifunctional siNA design



S = sense, AS = antisense
Linker region can be nucleotide or non-nucleotide linker, and can optionally be decorated, for example with conjugates polymers or aptamers, such as for delivery purposes.

Figure 23: Dendrimer Multifunctional siNA designs

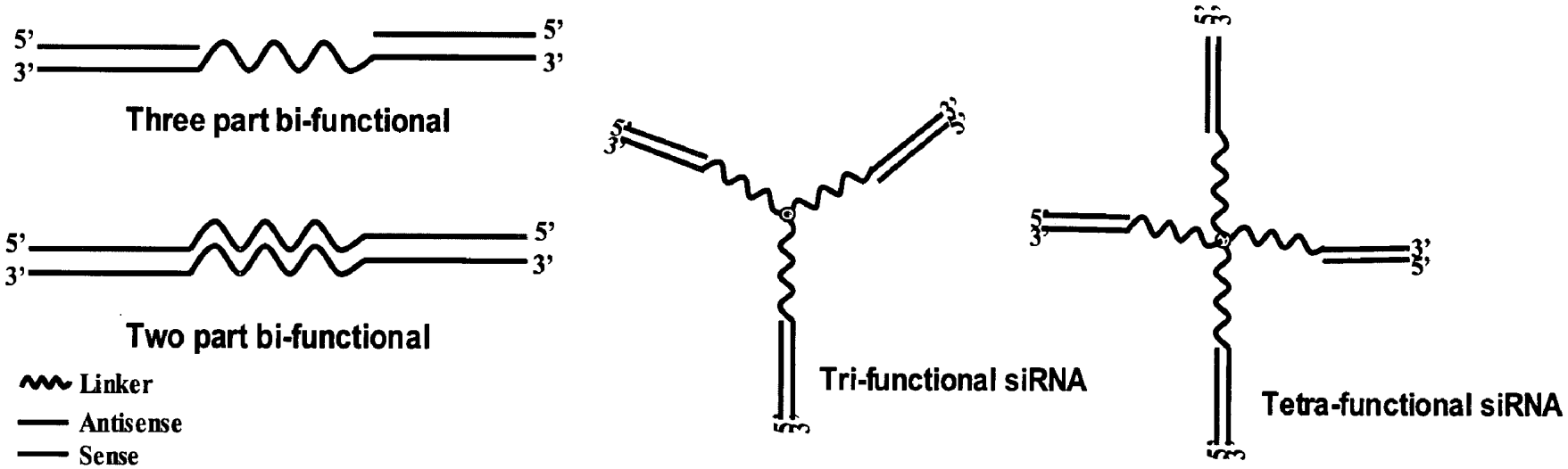


Figure 24: Supramolecular Multifunctional siNA designs

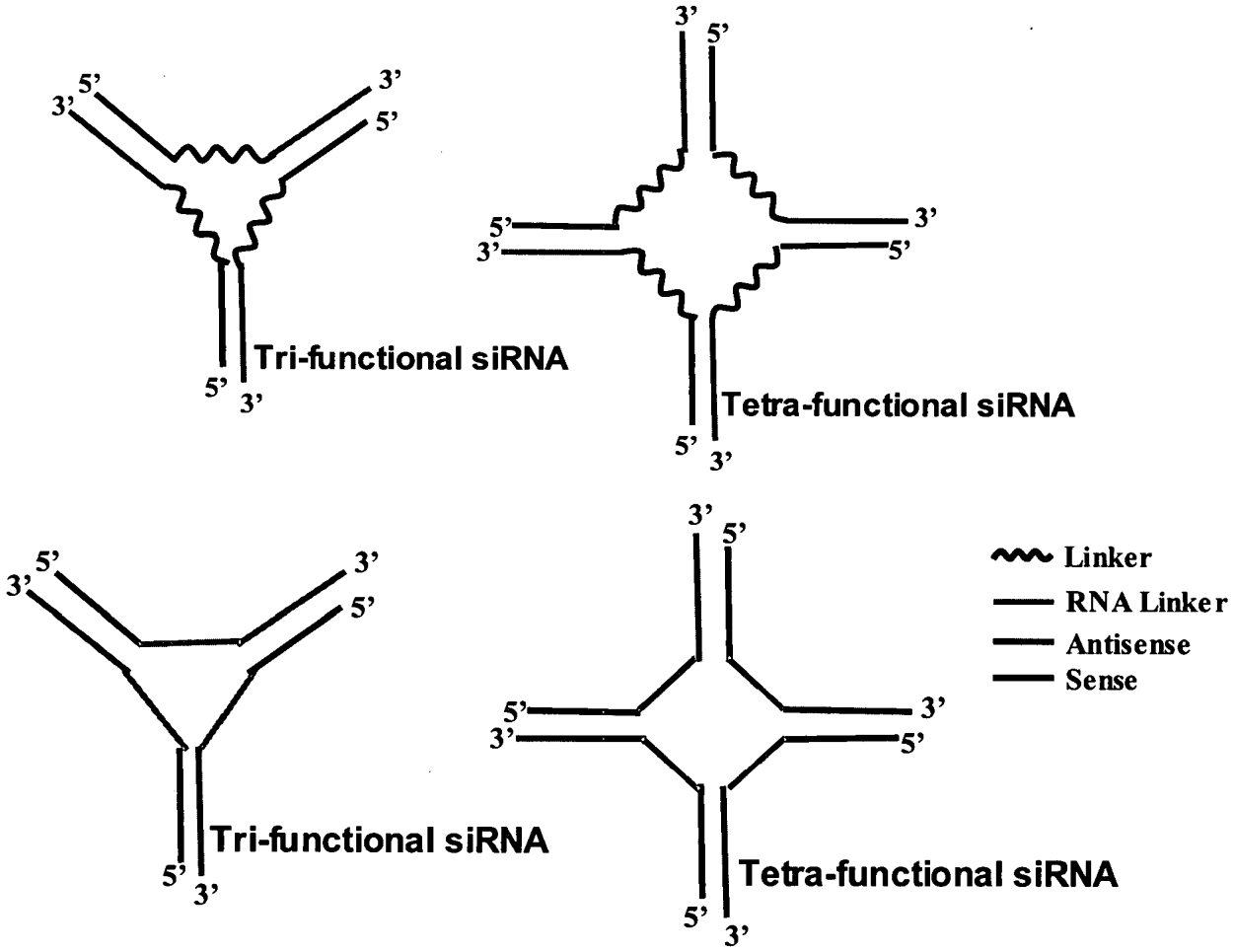


Figure 25: Dicer enabled multifunctional siNA design

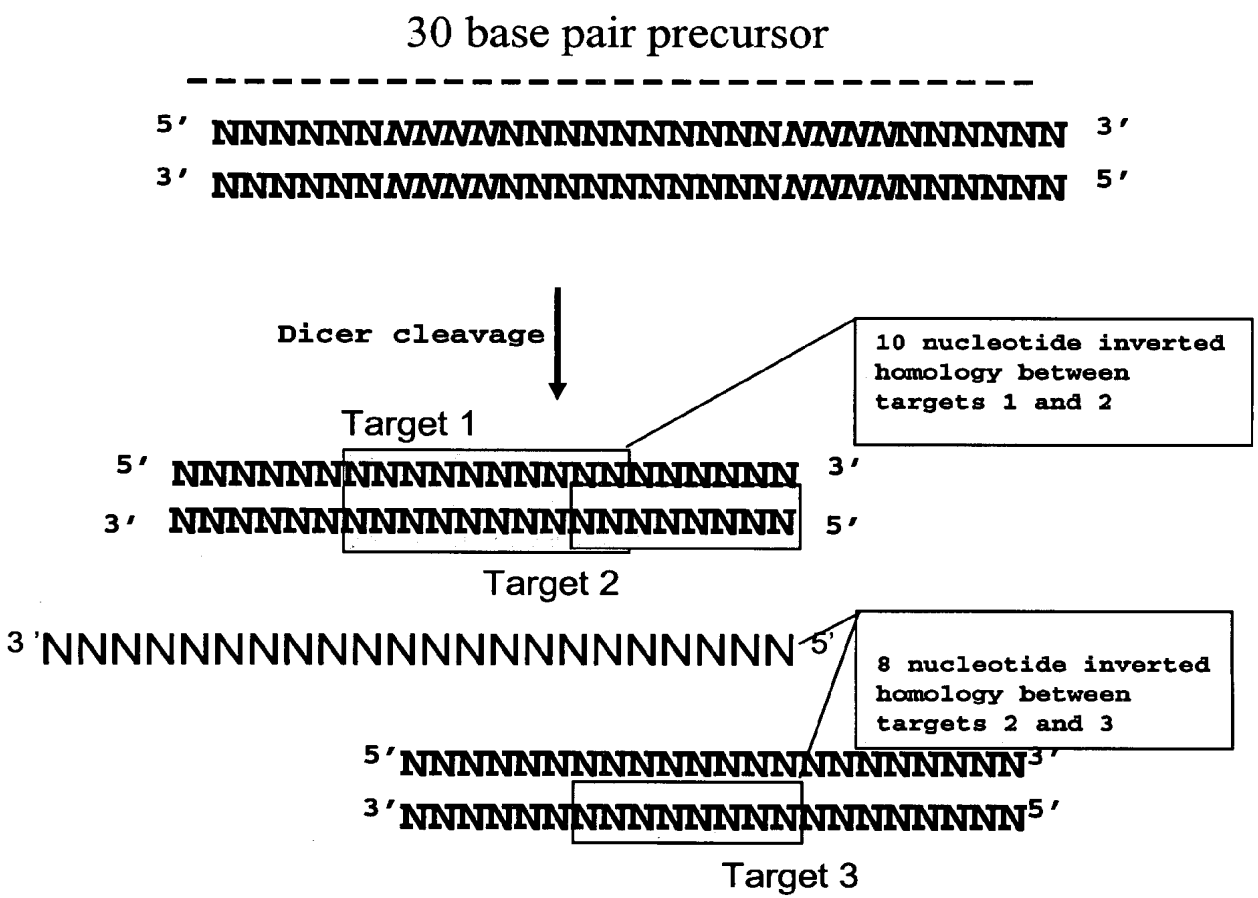


Figure 26: Dicer enabled multifunctional siNA design

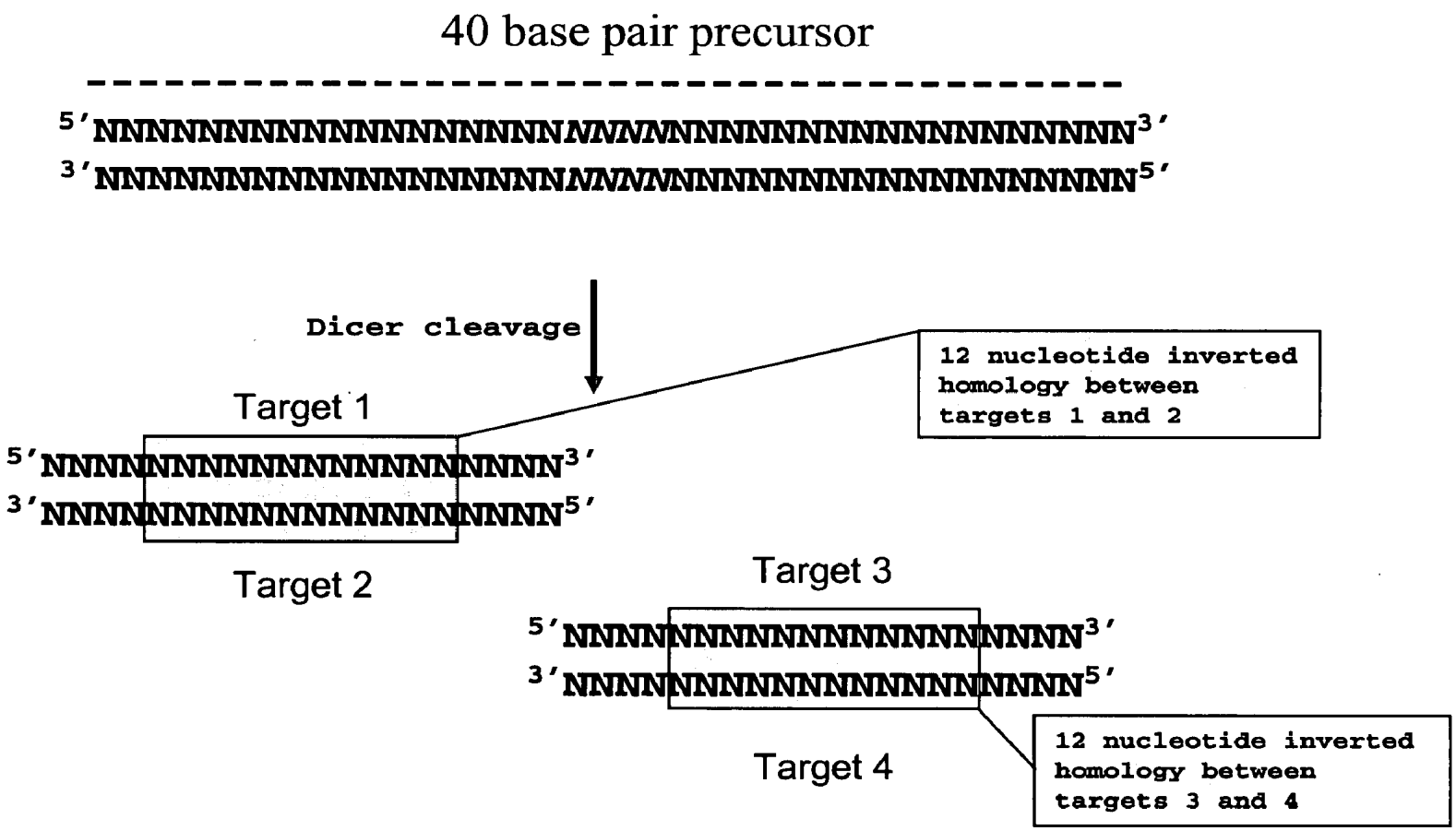

40 base pair precursor
5'NNNN

Figure 27: Additional Multifunctional siNA designs

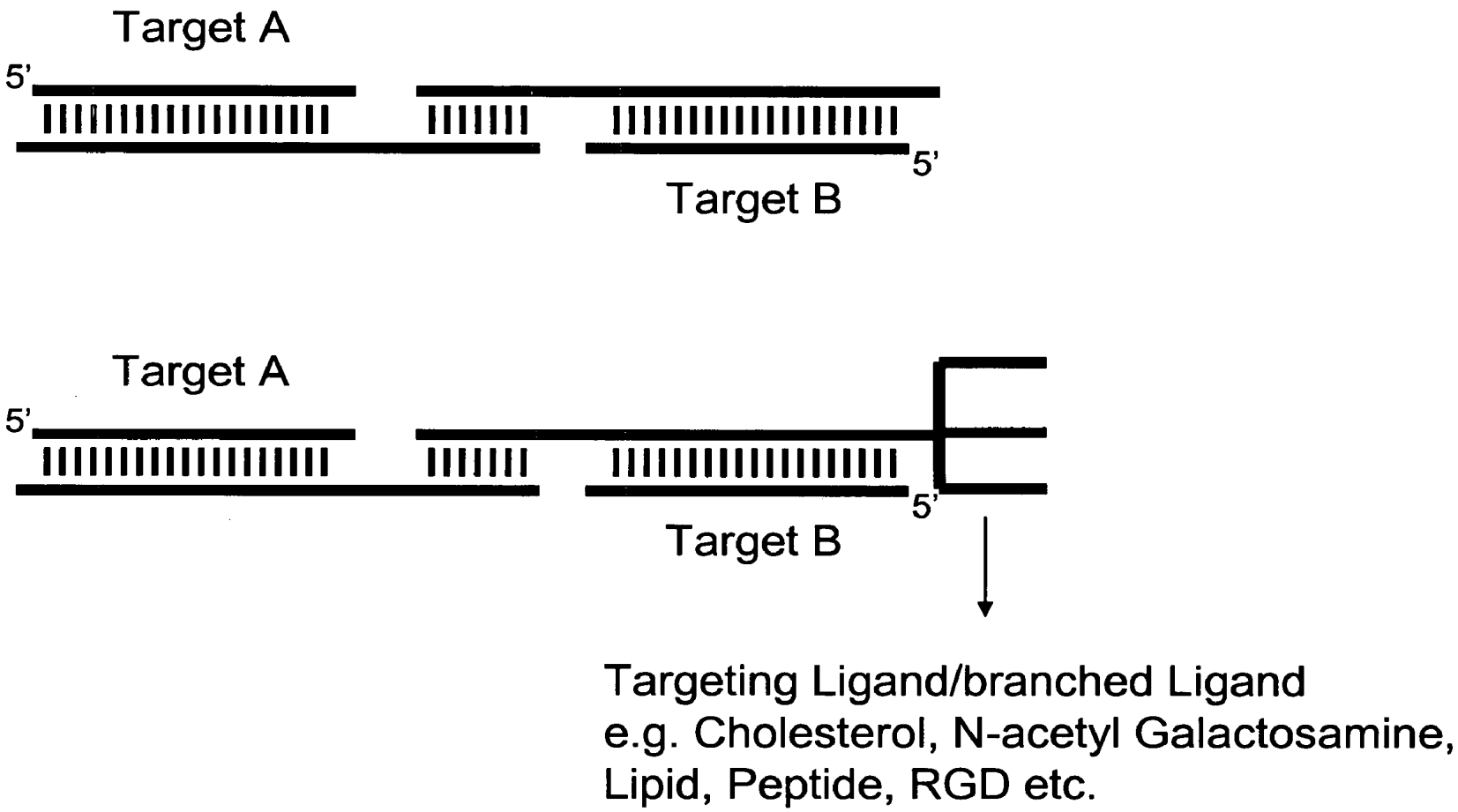


Figure 28: Additional Multifunctional siNA designs

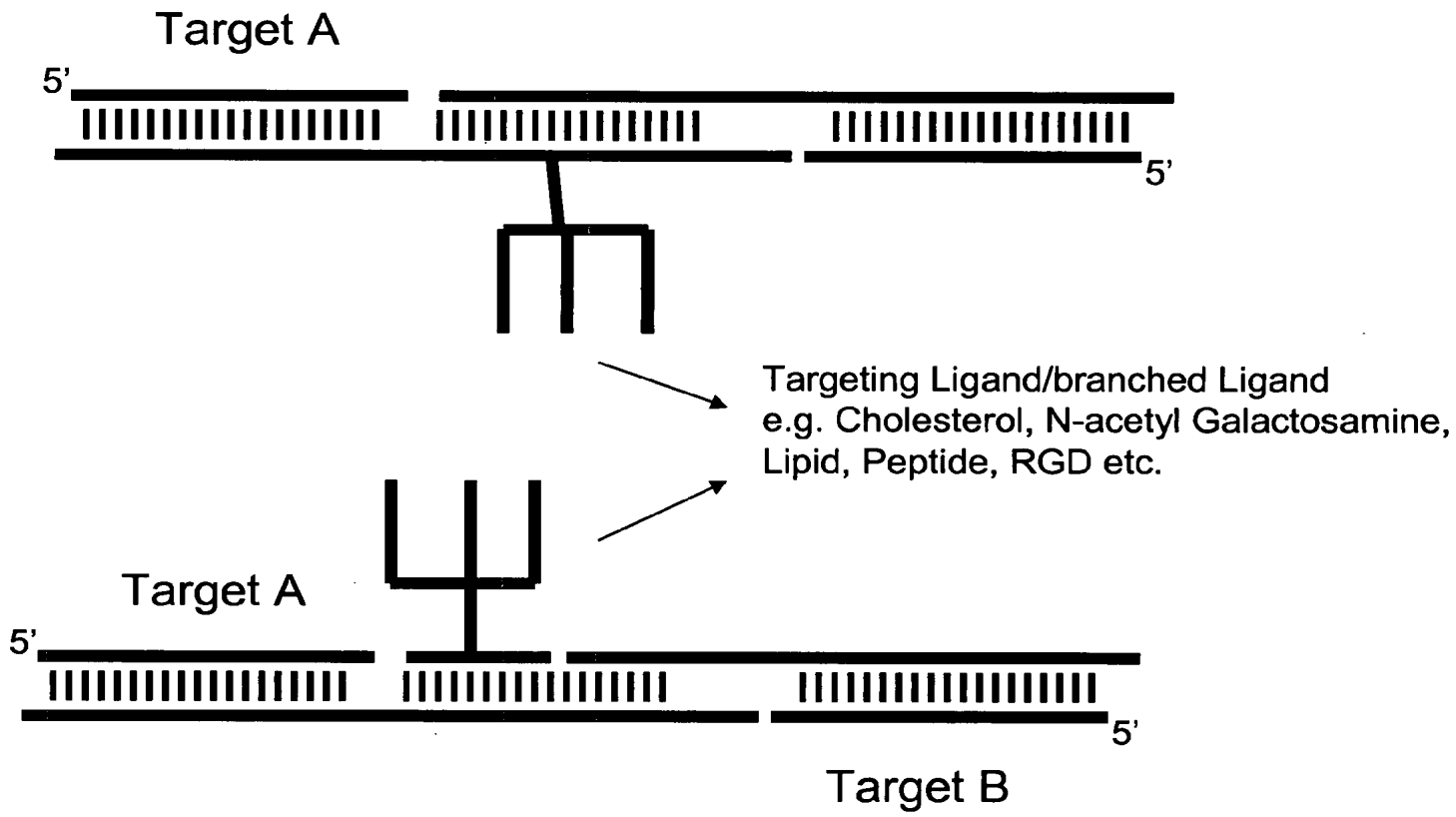
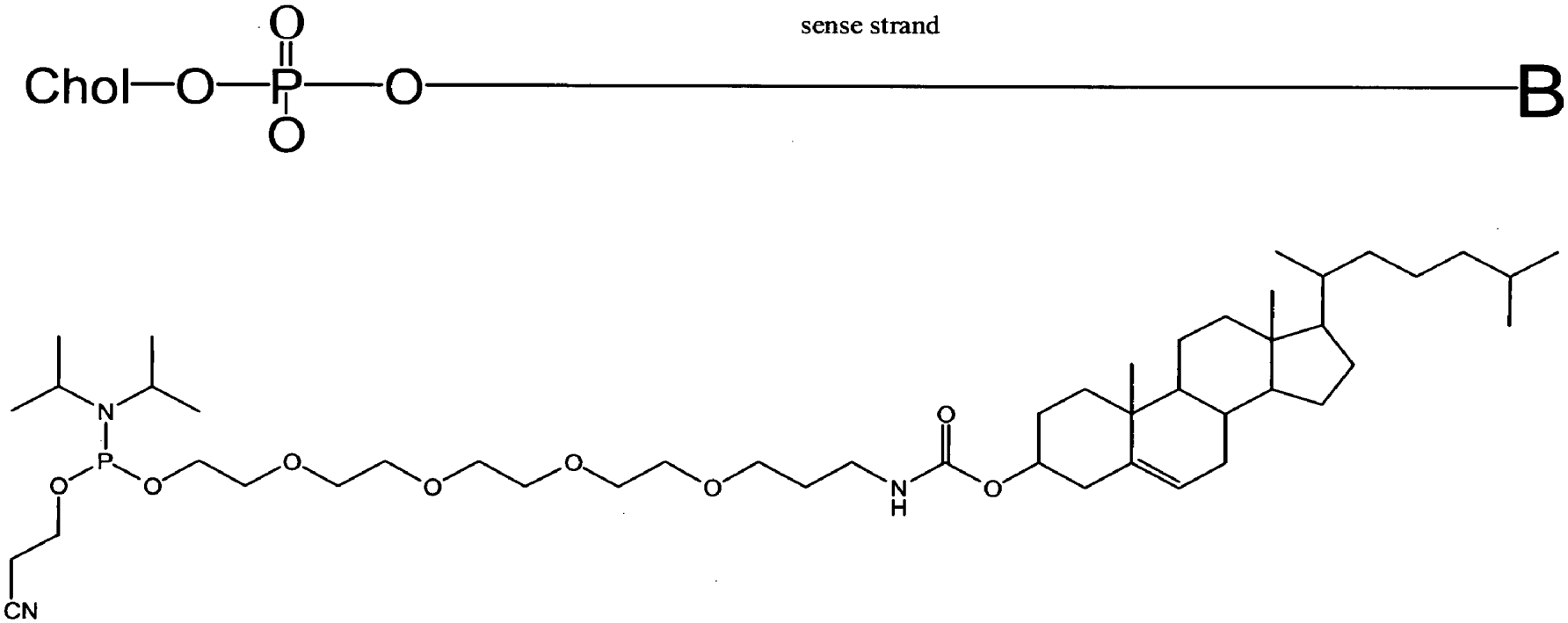


Figure 29: Cholesterol Conjugate Approach



$C_{48}H_{86}N_3O_8P$
Exact Mass: 863.62
Mol. Wt.: 864.19
C, 66.71; H, 10.03; N, 4.86; O, 14.81; P, 3.58

RNA INTERFERENCE MEDIATED INHIBITION OF STEROL REGULATORY ELEMENT BINDING PROTEIN 1 (SREBP1) GENE EXPRESSION USING SHORT INTERFERING NUCLEIC ACID (SINA)

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 11/369,108 filed Mar. 6, 2006. This application is also a continuation-in-part of U.S. patent application Ser. No. 11/299,254, filed Dec. 8, 2005, which is a continuation-in-part of U.S. patent application Ser. No. 11/234,730, filed Sep. 23, 2005, which is a continuation-in-part of U.S. patent application Ser. No. 11/205,646, filed Aug. 17, 2005, which is a continuation-in-part of U.S. patent application Ser. No. 11/098,303, filed Apr. 4, 2005, which is a continuation-in-part of U.S. patent application Ser. No. 10/923,536, filed Aug. 20, 2004, which is a continuation-in-part of International Patent Application No. PCT/US04/16390, filed May 24, 2004, which is a continuation-in-part of U.S. patent application Ser. No. 10/826,966, filed Apr. 16, 2004, which is continuation-in-part of U.S. patent application Ser. No. 10/757,803, filed Jan. 14, 2004, which is a continuation-in-part of U.S. patent application Ser. No. 10/720,448, filed Nov. 24, 2003, which is a continuation-in-part of U.S. patent application Ser. No. 10/693,059, filed Oct. 23, 2003, which is a continuation-in-part of U.S. patent application Ser. No. 10/444,853, filed May 23, 2003, which is a continuation-in-part of International Patent Application No. PCT/US03/05346, filed Feb. 20, 2003, and a continuation-in-part of International Patent Application No. PCT/US03/05028, filed Feb. 20, 2003, both of which claim the benefit of U.S. Provisional Application No. 60/358,580 filed Feb. 20, 2002, U.S. Provisional Application No. 60/363,124 filed Mar. 11, 2002, U.S. Provisional Application No. 60/386,782 filed Jun. 6, 2002, U.S. Provisional Application No. 60/406,784 filed Aug. 29, 2002, U.S. Provisional Application No. 60/408,378 filed Sep. 5, 2002, U.S. Provisional Application No. 60/409,293 filed Sep. 9, 2002, and U.S. Provisional Application No. 60/440,129 filed Jan. 15, 2003. This application is also a continuation-in-part of International Patent Application No. PCT/US04/13456, filed Apr. 30, 2004, which is a continuation-in-part of U.S. patent application Ser. No. 10/780,447, filed Feb. 13, 2004, which is a continuation-in-part of U.S. patent application Ser. No. 10/427,160, filed Apr. 30, 2003, which is a continuation-in-part of International Patent Application No. PCT/US02/15876 filed May 17, 2002, which claims the benefit of U.S. Provisional Application No. 60/292,217, filed May 18, 2001, U.S. Provisional Application No. 60/362,016, filed Mar. 6, 2002, U.S. Provisional Application No. 60/306,883, filed Jul. 20, 2001, and U.S. Provisional Application No. 60/311,865, filed Aug. 13, 2001. This application is also a continuation-in-part of U.S. patent application Ser. No. 10/727,780 filed Dec. 3, 2003. This application is also a continuation-in-part of International Patent Application No. PCT/US05/04270, filed Feb. 9, 2005 which claims the benefit of U.S. Provisional Application No. 60/543,480, filed Feb. 10, 2004. This application is also a a continuation-in-part of U.S. patent application Ser. No. 11/353,630, filed Feb. 14, 2006, which claims the benefit of U.S. Provisional Patent Application No. 60/652,787 filed Feb. 14, 2005, U.S. Provisional Patent Application No. 60/678,531 filed May 6, 2005, U.S. Provisional Patent Application No. 60/703,946, filed Jul. 29, 2005, and U.S. Provisional Patent Application No. 60/737,024, filed Nov. 15, 2005. The instant application claims the benefit of all the listed applications, which are hereby incorporated by reference herein in their entireties, including the drawings.

FIELD OF THE INVENTION

[0002] The present invention relates to compounds, compositions, and methods for the study, diagnosis, and treatment of traits, diseases and conditions that respond to the modulation of Sterol Regulatory Element-Binding Protein 1 (SREBP1) gene expression and/or activity. The present invention is also directed to compounds, compositions, and methods relating to traits, diseases and conditions that respond to the modulation of expression and/or activity of genes involved in Sterol Regulatory Element-Binding Protein 1 (SREBP1) gene expression pathways or other cellular processes that mediate the maintenance or development of such traits, diseases and conditions. Specifically, the invention relates to double stranded nucleic acid molecules including small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules capable of mediating RNA interference (RNAi) against Sterol Regulatory Element-Binding Protein 1 (SREBP1) gene expression, including cocktails of such small nucleic acid molecules and lipid nanoparticle (LNP) formulations of such small nucleic acid molecules. The present invention also relates to small nucleic acid molecules, such as siNA, siRNA, and others that can inhibit the function of endogenous RNA molecules, such as endogenous micro-RNA (miRNA) (e.g, miRNA inhibitors) or endogenous short interfering RNA (siRNA), (e.g., siRNA inhibitors) or that can inhibit the function of RISC (e.g., RISC inhibitors), to modulate SREBP1 gene expression by interfering with the regulatory function of such endogenous RNAs or proteins associated with such endogenous RNAs (e.g., RISC), including cocktails of such small nucleic acid molecules and lipid nanoparticle (LNP) formulations of such small nucleic acid molecules. Such small nucleic acid molecules and are useful, for example, in providing compositions to prevent, inhibit, or reduce metabolic diseases traits and conditions, including but not limited to hyperlipidemia, hypercholesterolemia, cardiovascular disease, atherosclerosis, hypertension, diabetis (e.g., type I and/or type II diabetis), insulin resistance, obesity and/or other disease states, conditions, or traits associated with SREBP1 gene expression or activity in a subject or organism.

BACKGROUND OF THE INVENTION

[0003] The following is a discussion of relevant art pertaining to RNAi. The discussion is provided only for understanding of the invention that follows. The summary is not an admission that any of the work described below is prior art to the claimed invention.

[0004] RNA interference refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Zamore et al., 2000, Cell, 101, 25-33; Fire et al., 1998, *Nature,* 391, 806; Hamilton et al., 1999, *Science,* 286, 950-951; Lin et al., 1999, *Nature,* 402, 128-129; Sharp, 1999, Genes & Dev., 13:139-141; and Strauss, 1999, *Science,* 286, 886). The corresponding process in plants (Heifetz et al., International PCT Publication No. WO 99/61631) is commonly referred to as post-

transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes and is commonly shared by diverse flora and phyla (Fire et al., 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or from the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response through a mechanism that has yet to be fully characterized. This mechanism appears to be different from other known mechanisms involving double stranded RNA-specific ribonucleases, such as the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2',5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L (see for example U.S. Pat. Nos. 6,107,094; 5,898,031; Clemens et al., 1997, *J. Interferon & Cytokine Res.*, 17, 503-524; Adah et al., 2001, *Curr. Med. Chem.*, 8, 1189).

[0005] The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer (Bass, 2000, Cell, 101, 235; Zamore et al., 2000, Cell, 101, 25-33; Hammond et al., 2000, Nature, 404, 293). Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Zamore et al., 2000, Cell, 101, 25-33; Bass, 2000, Cell, 101, 235; Berstein et al., 2001, *Nature*, 409, 363). Short interfering RNAs derived from dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes (Zamore et al., 2000, Cell, 101, 25-33; Elbashir et al., 2001, *Genes Dev.*, 15, 188). Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner et al., 2001, *Science*, 293, 834). The RNAi response also features an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence complementary to the antisense strand of the siRNA duplex. Cleavage of the target RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex (Elbashir et al., 2001, *Genes Dev.*, 15, 188).

[0006] RNAi has been studied in a variety of systems. Fire et al., 1998, *Nature*, 391, 806, were the first to observe RNAi in *C. elegans*. Bahramian and Zarbl, 1999, *Molecular and Cellular Biology*, 19, 274-283 and Wianny and Goetz, 1999, *Nature Cell Biol.*, 2, 70, describe RNAi mediated by dsRNA in mammalian systems. Hammond et al., 2000, *Nature*, 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir et al., 2001, *Nature*, 411, 494 and Tuschl et al., International PCT Publication No. WO 01/75164, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in *Drosophila* embryonic lysates (Elbashir et al., 2001, *EMBO J.*, 20, 6877 and Tuschl et al., International PCT Publication No. WO 01/75164) has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21-nucleotide siRNA duplexes are most active when containing 3'-terminal dinucleotide overhangs. Furthermore, complete substitution of one or both siRNA strands with 2'-deoxy (2'-H) or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of the 3'-terminal siRNA overhang nucleotides with 2'-deoxy nucleotides (2'-H) was shown to be tolerated. Single mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end of the guide sequence (Elbashir et al., 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen et al., 2001, *Cell*, 107, 309).

[0007] Studies have shown that replacing the 3'-terminal nucleotide overhanging segments of a 21-mer siRNA duplex having two-nucleotide 3'-overhangs with deoxyribonucleotides does not have an adverse effect on RNAi activity. Replacing up to four nucleotides on each end of the siRNA with deoxyribonucleotides has been reported to be well tolerated, whereas complete substitution with deoxyribonucleotides results in no RNAi activity (Elbashir et al., 2001, *EMBO J.*, 20, 6877 and Tuschl et al., International PCT Publication No. WO 01/75164). In addition, Elbashir et al., supra, also report that substitution of siRNA with 2'-O-methyl nucleotides completely abolishes RNAi activity. Li et al., International PCT Publication No. WO 00/44914, and Beach et al., International PCT Publication No. WO 01/68836 preliminarily suggest that siRNA may include modifications to either the phosphate-sugar backbone or the nucleoside to include at least one of a nitrogen or sulfur heteroatom, however, neither application postulates to what extent such modifications would be tolerated in siRNA molecules, nor provides any further guidance or examples of such modified siRNA. Kreutzer et al., Canadian Patent Application No. 2,359,180, also describe certain chemical modifications for use in dsRNA constructs in order to counteract activation of double-stranded RNA-dependent protein kinase PKR, specifically 2'-amino or 2'-O-methyl nucleotides, and nucleotides containing a 2'-O or 4'-C methylene bridge. However, Kreutzer et al. similarly fails to provide examples or guidance as to what extent these modifications would be tolerated in dsRNA molecules.

[0008] Parrish et al., 2000, *Molecular Cell*, 6, 1077-1087, tested certain chemical modifications targeting the unc-22 gene in *C. elegans* using long (>25 nt) siRNA transcripts. The authors describe the introduction of thiophosphate residues into these siRNA transcripts by incorporating thiophosphate nucleotide analogs with T7 and T3 RNA polymerase and observed that RNAs with two phosphorothioate modified bases also had substantial decreases in effectiveness as RNAi. Further, Parrish et al. reported that phosphorothioate modification of more than two residues greatly destabilized the RNAs in vitro such that interference activities could not be assayed. Id. at 1081. The authors also tested certain modifications at the 2'-position of the nucleotide sugar in the long siRNA transcripts and found that substituting deoxynucleotides for ribonucleotides produced a substantial decrease in interference activity, especially in the case of Uridine to Thymidine and/or Cytidine to deoxy-Cytidine substitutions. Id. In addition, the authors tested certain base modifications, including substituting, in sense and antisense strands of the

siRNA, 4-thiouracil, 5-bromouracil, 5-iodouracil, and 3-(aminoallyl)uracil for uracil, and inosine for guanosine. Whereas 4-thiouracil and 5-bromouracil substitution appeared to be tolerated, Parrish reported that inosine produced a substantial decrease in interference activity when incorporated in either strand. Parrish also reported that incorporation of 5-iodouracil and 3-(aminoallyl)uracil in the antisense strand resulted in a substantial decrease in RNAi activity as well.

[0009] The use of longer dsRNA has been described. For example, Beach et al., International PCT Publication No. WO 01/68836, describes specific methods for attenuating gene expression using endogenously-derived dsRNA. Tuschl et al., International PCT Publication No. WO 01/75164, describe a *Drosophila* in vitro RNAi system and the use of specific siRNA molecules for certain functional genomic and certain therapeutic applications; although Tuschl, 2001, *Chem. Biochem.*, 2, 239-245, doubts that RNAi can be used to cure genetic diseases or viral infection due to the danger of activating interferon response. Li et al., International PCT Publication No. WO 00/44914, describe the use of specific long (141 bp-488 bp) enzymatically synthesized or vector expressed dsRNAs for attenuating the expression of certain target genes. Zernicka-Goetz et al., International PCT Publication No. WO 01/36646, describe certain methods for inhibiting the expression of particular genes in mammalian cells using certain long (550 bp-714 bp), enzymatically synthesized or vector expressed dsRNA molecules. Fire et al., International PCT Publication No. WO 99/32619, describe particular methods for introducing certain long dsRNA molecules into cells for use in inhibiting gene expression in nematodes. Plaetinck et al., International PCT Publication No. WO 00/01846, describe certain methods for identifying specific genes responsible for conferring a particular phenotype in a cell using specific long dsRNA molecules. Mello et al., International PCT Publication No. WO 01/29058, describe the identification of specific genes involved in dsRNA-mediated RNAi. Pachuck et al., International PCT Publication No. WO 00/63364, describe certain long (at least 200 nucleotide) dsRNA constructs. Deschamps Depaillette et al., International PCT Publication No. WO 99/07409, describe specific compositions consisting of particular dsRNA molecules combined with certain anti-viral agents. Waterhouse et al., International PCT Publication No. 99/53050 and 1998, *PNAS*, 95, 13959-13964, describe certain methods for decreasing the phenotypic expression of a nucleic acid in plant cells using certain dsRNAs. Driscoll et al., International PCT Publication No. WO 01/49844, describe specific DNA expression constructs for use in facilitating gene silencing in targeted organisms.

[0010] Others have reported on various RNAi and gene-silencing systems. For example, Parrish et al., 2000, *Molecular Cell*, 6, 1077-1087, describe specific chemically-modified dsRNA constructs targeting the unc-22 gene of *C. elegans*. Grossniklaus, International PCT Publication No. WO 01/38551, describes certain methods for regulating polycomb gene expression in plants using certain dsRNAs. Churikov et al., International PCT Publication No. WO 01/42443, describe certain methods for modifying genetic characteristics of an organism using certain dsRNAs. Cogoni et al, International PCT Publication No. WO 01/53475, describe certain methods for isolating a *Neurospora* silencing gene and uses thereof. Reed et al., International PCT Publication No. WO 01/68836, describe certain methods for gene silencing in plants. Honer et al., International PCT Publication No. WO 01/70944, describe certain methods of drug screening using transgenic nematodes as Parkinson's Disease models using certain dsRNAs. Deak et al., International PCT Publication No. WO 01/72774, describe certain *Drosophila*-derived gene products that may be related to RNAi in *Drosophila*. Arndt et al., International PCT Publication No. WO 01/92513 describe certain methods for mediating gene suppression by using factors that enhance RNAi. Tuschl et al., International PCT Publication No. WO 02/44321, describe certain synthetic siRNA constructs. Pachuk et al., International PCT Publication No. WO 00/63364, and Satishchandran et al., International PCT Publication No. WO 01/04313, describe certain methods and compositions for inhibiting the function of certain polynucleotide sequences using certain long (over 250 bp), vector expressed dsRNAs. Echeverri et al., International PCT Publication No. WO 02/38805, describe certain *C. elegans* genes identified via RNAi. Kreutzer et al., International PCT Publications Nos. WO 02/055692, WO 02/055693, and EP 1144623 B1 describes certain methods for inhibiting gene expression using dsRNA. Graham et al., International PCT Publications Nos. WO 99/49029 and WO 01/70949, and AU 4037501 describe certain vector expressed siRNA molecules. Fire et al., U.S. Pat. No. 6,506,559, describe certain methods for inhibiting gene expression in vitro using certain long dsRNA (299 bp-1033 bp) constructs that mediate RNAi. Martinez et al., 2002, *Cell*, 110, 563-574, describe certain single stranded siRNA constructs, including certain 5'-phosphorylated single stranded siRNAs that mediate RNA interference in Hela cells. Harborth et al., 2003, Antisense & Nucleic Acid Drug Development, 13, 83-105, describe certain chemically and structurally modified siRNA molecules. Chiu and Rana, 2003, RNA, 9, 1034-1048, describe certain chemically and structurally modified siRNA molecules. Woolf et al., International PCT Publication Nos. WO 03/064626 and WO 03/064625 describe certain chemically modified dsRNA constructs. Hornung et al., 2005, *Nature Medicine*, 11, 263-270, describe the sequence-specific potent induction of IFN-alpha by short interfering RNA in plasmacytoid dendritic cells through TLR7. Judge et al., 2005, Nature Biotechnology, Published online: 20 Mar. 2005, describe the sequence-dependent stimulation of the mammalian innate immune response by synthetic siRNA. Yuki et al., International PCT Publication Nos. WO 05/049821 and WO 04/048566, describe certain methods for designing short interfering RNA sequences and certain short interfering RNA sequences with optimized activity. Saigo et al., US Patent Application Publication No. US20040539332, describe certain methods of designing oligo- or polynucleotide sequences, including short interfering RNA sequences, for achieving RNA interference. Tei et al., International PCT Publication No. WO 03/044188, describe certain methods for inhibiting expression of a target gene, which comprises transfecting a cell, tissue, or individual organism with a double-stranded polynucleotide comprising DNA and RNA having a substantially identical nucleotide sequence with at least a partial nucleotide sequence of the target gene.

[0011] Mattick, 2005, *Science*, 309, 1527-1528; Clayerie, 2005, *Science*, 309, 1529-1530; Sethupathy et al., 2006, *RNA*, 12, 192-197; and Czech, 2006 *NEJM*, 354, 11: 1194-1195; Hutvagner et al., US 20050227256, and Tuschl et al., US 20050182005, all describe antisense molecules that can

inhibit miRNA function via steric blocking and are all incorporated by reference herein in their entirety.

SUMMARY OF THE INVENTION

[0012] This invention relates to compounds, compositions, and methods useful for modulating the expression of Sterol Regulatory Element-Binding Protein 1 (SREBP1) genes, such as those SREBP1 genes associated with the development or maintenance of metabolic diseases traits and conditions, including but not limited to hyperlipidemia, hypercholesterolemia, cardiovascular disease, atherosclerosis, hypertension, diabetis (e.g., type I and/or type II diabetis), insulin resistance, obesity and/or any other diseases, traits, and conditions that are related to SREBP1 gene expression or activity, by RNA interference (RNAi) using short interfering nucleic acid (siNA) molecules. This invention also relates to compounds, compositions, and methods useful for modulating the expression and activity of other genes involved in pathways of SREBP1 gene expression and/or activity by RNA interference (RNAi) using small nucleic acid molecules. In particular, the instant invention features small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules and methods used to modulate the expression of SREBP1 genes and/or other genes involved in pathways of SREBP1 gene expression and/or activity.

[0013] The instant invention also relates to small nucleic acid molecules, such as siNA, siRNA, and others that can inhibit the function of endogenous RNA molecules, such as endogenous micro-RNA (miRNA) (e.g, miRNA inhibitors) or endogenous short interfering RNA (siRNA), (e.g., siRNA inhibitors) or that can inhibit the function of RISC (e.g., RISC inhibitors), to modulate SREBP1 gene expression by interfering with the regulatory function of such endogenous RNAs or proteins associated with such endogenous RNAs (e.g., RISC). Such molecules are collectively referred to herein as RNAi inhibitors.

[0014] A siNA or RNAi inhibitor of the invention can be unmodified or chemically-modified. A siNA or RNAi inhibitor of the instant invention can be chemically synthesized, expressed from a vector or enzymatically synthesized. The instant invention also features various chemically-modified synthetic short interfering nucleic acid (siNA) molecules capable of modulating target gene expression or activity in cells by RNA interference (RNAi). The instant invention also features various chemically-modified synthetic short nucleic acid (siNA) molecules capable of modulating RNAi activity in cells by interacting with miRNA, siRNA, or RISC, and hence down regulating or inhibiting RNA interference (RNAi), translational inhibition, or transcriptional silencing in a cell or organism. The use of chemically-modified siNA and/or RNAi inhibitors improves various properties of native siNA molecules and/or RNAi inhibitors through increased resistance to nuclease degradation in vivo and/or through improved cellular uptake. Further, contrary to earlier published studies, siNA molecules of the invention having multiple chemical modifications, including fully modified siNA, retains its RNAi activity. Therefore, Applicant teaches herein chemically modified siRNA that retains or improves upon the activity of native siRNA. The siNA molecules of the instant invention provide useful reagents and methods for a variety of therapeutic, prophylactic, veterinary, diagnostic, target validation, genomic discovery, genetic engineering, and pharmacogenomic applications.

[0015] In one embodiment, the invention features one or more siNA molecules and/or RNAi inhibitors and methods that independently or in combination modulate the expression of gene(s) encoding Sterol Regulatory Element-Binding Protein 1 (SREBP1) and/or SREBP1 pathway genes, such as genes encoding sequences comprising those sequences referred to by GenBank Accession Nos. shown in Table I, referred to herein generally as "SREBP1". The description below of the various aspects and embodiments of the invention is provided with reference to exemplary Sterol Regulatory Element-Binding Protein 1 (SREBP1) gene referred to herein generally as SREBP1, but also known as Sterol Regulatory Element-Binding Transcription Factor 1 or SREBF1. The present invention is also directed to compounds, compositions, and methods relating to traits, diseases and conditions that respond to the modulation of expression and/or activity of genes involved in Sterol Regulatory Element-Binding Protein 1 (SREBP1) gene expression pathways or other cellular processes that mediate the maintenance or development of such traits, diseases and conditions, including Sterol Regulatory Element-Binding Protein 2 (SREBP2), also referred to as Sterol Regulatory Element-Binding Transcription Factor 2 or SREBF2. However, such reference is meant to be exemplary only and the various aspects and embodiments of the invention are also directed to other genes that express alternate SREBP1 and SREBP2 genes, such as mutant SREBP1 and SREBP2 genes, splice variants of SREBP1 and SREBP2 genes, SREBP1 and SREBP2 variants from species to species or subject to subject, and other SREBP1 and SREBP2 pathway genes including certain genes described in PCT/US03/05028 and U.S. Ser. No. 10/923,536, both incorporated by reference herein. Such additional genes can be analyzed for target sites using the methods described herein for exemplary SREBP1 and SREBP2 genes and sequences herein. Thus, the modulation and the effects of such modulation of the other genes can be performed as described herein. In other words, the term "SREBP1" as it is defined herein below and recited in the described embodiments, is meant to encompass genes associated with the development and/or maintenance of diseases, traits and conditions herein, such as genes which encode SREBP1 and SREBP2 polypeptides, SREBP1 and SREBP2 regulatory polynucleotides (e.g., SREBP1 and SREBP2 miRNAs and siRNAs), mutant SREBP1 and SREBP2 genes, and splice variants of SREBP1 and SREBP2 genes, as well as other genes involved in SREBP1 and SREBP2 pathways of gene expression and/or activity. Thus, each of the embodiments described herein with reference to the term "SREBP1" are applicable to all of the protein, peptide, polypeptide, and/or polynucleotide molecules covered by the term "SREBP1", as that term is defined herein. Comprehensively, such gene targets are also referred to herein generally as "target" sequences.

[0016] In one embodiment, the invention features a composition comprising two or more different siNA molecules and/or RNAi inhibitors of the invention (e.g., siNA, duplex forming siNA, or multifunctional siNA or any combination thereof) targeting different polynucleotide targets, such as different regions of SREBP1 and SREBP2 RNA or DNA (e.g., two different target sites such as provided herein or any combination of SREBP1 and SREBP2 targets or SREBP1 and SREBP2 pathway targets, e.g. PCSK9) or both coding

and non-coding targets. Such pools of siNA molecules can provide increased therapeutic effect.

[0017] In one embodiment, the invention features a pool of two or more different siNA molecules of the invention (e.g., siNA, duplex forming siNA, or multifunctional siNA or any combination thereof) that have specificity for different polynucleotide targets, such as different regions of SREBP1 and SREBP2 RNA or DNA (e.g., two different target sites herein or any combination of SREBP1 and SREBP2 targets or SREBP1 and SREBP2 pathway targets, e.g. PCSK9) or both coding and non-coding targets, wherein the pool comprises siNA molecules targeting about 2, 3, 4, 5, 6, 7, 8, 9, 10 or more different SREBP1 and SREBP2 targets.

[0018] Due to the potential for sequence variability of the SREBP1 genome across different organisms or different subjects, selection of siNA molecules for broad therapeutic applications likely involve the conserved regions of the SREBP1 gene. In one embodiment, the present invention relates to siNA molecules and/or RNAi inhibitors that target conserved regions of the SREBP1 genome or regions that are conserved across different SREBP1 targets. siNA molecules and/or RNAi inhibitors designed to target conserved regions of various SREBP1 targets enable efficient inhibition of SREBP1 target gene expression in diverse patient populations.

[0019] In one embodiment, the invention features a double stranded nucleic acid molecule, such as an siNA molecule, where one of the strands comprises nucleotide sequence having complementarity to a predetermined nucleotide sequence in a SREBP1 target nucleic acid molecule, or a portion thereof. The predetermined nucleotide sequence can be a nucleotide SREBP1 target sequence, such as a sequence described herein or known in the art. In another embodiment, the predetermined nucleotide sequence is a SREBP1 target sequence or SREBP1 pathway target sequence as is known in the art.

[0020] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a SREBP1 target gene or that directs cleavage of a SREBP1 target RNA, wherein said siNA molecule comprises about 15 to about 28 base pairs.

[0021] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a SREBP1 target RNA, wherein said siNA molecule comprises about 15 to about 28 base pairs.

[0022] In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a SREBP1 target RNA via RNA interference (RNAi), wherein the double stranded siNA molecule comprises a first strand and a second strand, each strand of the siNA molecule is about 18 to about 28 (e.g., about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28) nucleotides in length, the first strand of the siNA molecule comprises nucleotide sequence having sufficient complementarity to the SREBP1 target RNA for the siNA molecule to direct cleavage of the SREBP1 target RNA via RNA interference, and the second strand of said siNA molecule comprises nucleotide sequence that is complementary to the first strand. In one specific embodiment, for example, each strand of the siNA molecule is about 18 to about 27 nucleotides in length.

[0023] In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a SREBP1 target RNA via RNA interference (RNAi), wherein the double stranded siNA molecule comprises a first strand and a second strand, each strand of the siNA molecule is about 18 to about 23 (e.g., about 18, 19, 20, 21, 22, or 23) nucleotides in length, the first strand of the siNA molecule comprises nucleotide sequence having sufficient complementarity to the SREBP1 target RNA for the siNA molecule to direct cleavage of the SREBP1 target RNA via RNA interference, and the second strand of said siNA molecule comprises nucleotide sequence that is complementary to the first strand.

[0024] In one embodiment, the invention features a chemically synthesized double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a SREBP1 target RNA via RNA interference (RNAi), wherein each strand of the siNA molecule is about 18 to about 28 nucleotides in length; and one strand of the siNA molecule comprises nucleotide sequence having sufficient complementarity to the SREBP1 target RNA for the siNA molecule to direct cleavage of the SREBP1 target RNA via RNA interference.

[0025] In one embodiment, the invention features a chemically synthesized double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a SREBP1 target RNA via RNA interference (RNAi), wherein each strand of the siNA molecule is about 18 to about 23 nucleotides in length; and one strand of the siNA molecule comprises nucleotide sequence having sufficient complementarity to the SREBP1 target RNA for the siNA molecule to direct cleavage of the SREBP1 target RNA via RNA interference.

[0026] In one embodiment, the invention features a siNA molecule that down-regulates expression of a SREBP1 target gene or that directs cleavage of a SREBP1 target RNA, for example, wherein the SREBP1 target gene or RNA comprises protein encoding sequence. In one embodiment, the invention features a siNA molecule that down-regulates expression of a SREBP1 target gene or that directs cleavage of a SREBP1 target RNA, for example, wherein the SREBP1 target gene or RNA comprises non-coding sequence or regulatory elements involved in SREBP1 target gene expression (e.g., non-coding RNA, miRNA, stRNA etc.).

[0027] In one embodiment, a siNA of the invention is used to inhibit the expression of SREBP1 target genes or a SREBP1 target gene family (e.g., SREBP1 and SREBP2), wherein the genes or gene family sequences share sequence homology. Such homologous sequences can be identified as is known in the art, for example using sequence alignments. siNA molecules can be designed to target such homologous sequences, for example using perfectly complementary sequences or by incorporating non-canonical base pairs, for example mismatches and/or wobble base pairs, that can provide additional SREBP1 target sequences. In instances where mismatches are identified, non-canonical base pairs (for example, mismatches and/or wobble bases) can be used to generate siNA molecules that SREBP1 target more than one gene sequence. In a non-limiting example, non-canonical base pairs such as UU and CC base pairs are used to generate siNA molecules that are capable of SREBP1 targeting sequences for differing polynucleotide SREBP1 targets that share sequence homology. As such, one advantage of using siNAs of the invention is that a single siNA can be designed to include nucleic acid sequence that is complementary to the nucleotide sequence that is conserved between the homologous genes. In this approach, a single siNA can be used to inhibit expression of more than one gene instead of using more than one siNA molecule to target the different genes.

[0028] In one embodiment, the invention features a siNA molecule having RNAi activity against SREBP1 target RNA (e.g., coding or non-coding RNA), wherein the siNA molecule comprises a sequence complementary to any RNA sequence, such as those sequences having GenBank Accession Nos. shown in Table I herein, or in PCT/US03/05028 and U.S. Ser. No. 10/923,536, both incorporated by reference herein. In another embodiment, the invention features a siNA molecule having RNAi activity against SREBP1 target RNA, wherein the siNA molecule comprises a sequence complementary to an RNA having variant encoding sequence, for example other mutant genes known in the art to be associated with the maintenance and/or development of diseases, traits, disorders, and/or conditions described herein or otherwise known in the art. Chemical modifications as shown in Table IV or otherwise described herein can be applied to any siNA construct of the invention. In another embodiment, a siNA molecule of the invention includes a nucleotide sequence that can interact with nucleotide sequence of a SREBP1 target gene and thereby mediate silencing of SREBP1 target gene expression, for example, wherein the siNA mediates regulation of SREBP1 target gene expression by cellular processes that modulate the chromatin structure or methylation patterns of the SREBP1 target gene and prevent transcription of the SREBP1 target gene.

[0029] In one embodiment, siNA molecules of the invention are used to down regulate or inhibit the expression of proteins arising from haplotype polymorphisms that are associated with a trait, disease or condition in a subject or organism. Analysis of genes, or protein or RNA levels can be used to identify subjects with such polymorphisms or those subjects who are at risk of developing traits, conditions, or diseases described herein. These subjects are amenable to treatment, for example, treatment with siNA molecules of the invention and any other composition useful in treating diseases related to target gene expression. As such, analysis of protein or RNA levels can be used to determine treatment type and the course of therapy in treating a subject. Monitoring of protein or RNA levels can be used to predict treatment outcome and to determine the efficacy of compounds and compositions that modulate the level and/or activity of certain proteins associated with a trait, disorder, condition, or disease.

[0030] In one embodiment of the invention a siNA molecule comprises an antisense strand comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof encoding a SREBP1 target protein. The siNA further comprises a sense strand, wherein said sense strand comprises a nucleotide sequence of a SREBP1 target gene or a portion thereof.

[0031] In another embodiment, a siNA molecule comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence encoding a SREBP1 target protein or a portion thereof. The siNA molecule further comprises a sense region, wherein said sense region comprises a nucleotide sequence of a SREBP1 target gene or a portion thereof.

[0032] In another embodiment, the invention features a siNA molecule comprising nucleotide sequence, for example, nucleotide sequence in the antisense region of the siNA molecule that is complementary to a nucleotide sequence or portion of sequence of a SREBP1 target gene. In another embodiment, the invention features a siNA molecule comprising a region, for example, the antisense region of the siNA construct, complementary to a sequence comprising a SREBP1 target gene sequence or a portion thereof.

[0033] In one embodiment, the sense region or sense strand of a siNA molecule of the invention is complementary to that portion of the antisense region or antisense strand of the siNA molecule that is complementary to a SREBP1 target polynucleotide sequence.

[0034] In yet another embodiment, the invention features a siNA molecule comprising a sequence, for example, the antisense sequence of the siNA construct, complementary to a sequence or portion of sequence comprising sequence represented by GenBank Accession Nos. shown in Table I and in PCT/US03/05028 and U.S. Ser. No. 10/923,536, both incorporated by reference herein. Chemical modifications in Tables III and IV and described herein can be applied to any siNA construct of the invention. LNP formulations described in Table VI can be applied to any siNA molecule or combination of siNA molecules herein.

[0035] In one embodiment of the invention a siNA molecule comprises an antisense strand having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein the antisense strand is complementary to a SREBP1 target RNA sequence or a portion thereof, and wherein said siNA further comprises a sense strand having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, and wherein said sense strand and said antisense strand are distinct nucleotide sequences where at least about 15 nucleotides in each strand are complementary to the other strand.

[0036] In one embodiment, a siNA molecule of the invention (e.g., a double stranded nucleic acid molecule) comprises an antisense (guide) strand having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to a RNA sequence of SREBP1 or a portion thereof. In one embodiment, at least 15 nucleotides (e.g., 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides) of a SREBP1 RNA sequence are complementary to the antisense (guide) strand of a siNA molecule of the invention.

[0037] In one embodiment, a siNA molecule of the invention (e.g., a double stranded nucleic acid molecule) comprises a sense (passenger) strand having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that comprise sequence of a SREBP1 RNA or a portion thereof. In one embodiment, at least 15 nucleotides (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides of a SREBP1 RNA sequence comprise the sense (passenger) strand of a siNA molecule of the invention.

[0038] In another embodiment of the invention a siNA molecule of the invention comprises an antisense region having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein the antisense region is complementary to a SREBP1 target DNA sequence, and wherein said siNA further comprises a sense region having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein said sense region and said antisense region are comprised in a linear molecule where the sense region comprises at least about 15 nucleotides that are complementary to the antisense region.

[0039] In one embodiment, a siNA molecule of the invention has RNAi activity that modulates expression of RNA encoded by a SREBP1 gene. Because SREBP1 genes can

share some degree of sequence homology with each other, siNA molecules can be designed to target a class of SREBP1 genes by selecting sequences that are either shared amongst different SREBP1 targets or alternatively that are unique for a specific SREBP1 target. Therefore, in one embodiment, the siNA molecule can be designed to target conserved regions of SREBP1 polynucleotide sequences having homology among several SREBP1 gene variants so as to target a class of SREBP1 genes with one siNA molecule. Accordingly, in one embodiment, the siNA molecule of the invention modulates the expression of one or more SREBP1 isoforms or variants in a subject or organism. In another embodiment, the siNA molecule can be designed to target a sequence that is unique to a specific SREBP1 polynucleotide sequence (e.g., a single SREBP1 isoform or SREBP1 single nucleotide polymorphism (SNP)) due to the high degree of specificity that the siNA molecule requires to mediate RNAi activity.

[0040] In one embodiment, nucleic acid molecules of the invention that act as mediators of the RNA interference gene silencing response are double-stranded nucleic acid molecules. In another embodiment, the siNA molecules of the invention consist of duplex nucleic acid molecules containing about 15 to about 30 base pairs between oligonucleotides comprising about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides. In yet another embodiment, siNA molecules of the invention comprise duplex nucleic acid molecules with overhanging ends of about 1 to about 3 (e.g., about 1, 2, or 3) nucleotides, for example, about 21-nucleotide duplexes with about 19 base pairs and 3'-terminal mononucleotide, dinucleotide, or trinucleotide overhangs. In yet another embodiment, siNA molecules of the invention comprise duplex nucleic acid molecules with blunt ends, where both ends are blunt, or alternatively, where one of the ends is blunt.

[0041] In one embodiment, a double stranded nucleic acid (e.g., siNA) molecule comprises nucleotide or non-nucleotide overhangs. By "overhang" is meant a terminal portion of the nucleotide sequence that is not base paired between the two strands of a double stranded nucleic acid molecule (see for example FIG. **6**). In one embodiment, a double stranded nucleic acid molecule of the invention can comprise nucleotide or non-nucleotide overhangs at the 3'-end of one or both strands of the double stranded nucleic acid molecule. For example, a double stranded nucleic acid molecule of the invention can comprise a nucleotide or non-nucleotide overhang at the 3'-end of the guide strand or antisense strand/region, the 3'-end of the passenger strand or sense strand/region, or both the guide strand or antisense strand/region and the passenger strand or sense strand/region of the double stranded nucleic acid molecule. In another embodiment, the nucleotide overhang portion of a double stranded nucleic acid (siNA) molecule of the invention comprises 2'-O-methyl, 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-deoxy-2'-fluoroarabino (FANA), 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, 2'-O-difluoromethoxy-ethoxy, universal base, acyclic, or 5-C-methyl nucleotides. In another embodiment, the non-nucleotide overhang portion of a double stranded nucleic acid (siNA) molecule of the invention comprises glyceryl, abasic, or inverted deoxy abasic non-nucleotides.

[0042] In one embodiment, the nucleotides comprising the overhang portions of a double stranded nucleic acid (e.g., siNA) molecule of the invention correspond to the nucleotides comprising the SREBP1 target polynucleotide sequence of the siNA molecule. Accordingly, in such embodiments, the nucleotides comprising the overhang portion of a siNA molecule of the invention comprise sequence based on the SREBP1 target polynucleotide sequence in which nucleotides comprising the overhang portion of the guide strand or antisense strand/region of a siNA molecule of the invention can be complementary to nucleotides in the SREBP1 target polynucleotide sequence and nucleotides comprising the overhang portion of the passenger strand or sense strand/region of a siNA molecule of the invention can comprise the nucleotides in the SREBP1 target polynucleotide sequence. Such nucleotide overhangs comprise sequence that would result from Dicer processing of a native dsRNA into siRNA.

[0043] In one embodiment, the nucleotides comprising the overhang portion of a double stranded nucleic acid (e.g., siNA) molecule of the invention are complementary to the SREBP1 target polynucleotide sequence and are optionally chemically modified as described herein. As such, in one embodiment, the nucleotides comprising the overhang portion of the guide strand or antisense strand/region of a siNA molecule of the invention can be complementary to nucleotides in the SREBP1 target polynucleotide sequence, i.e. those nucleotide positions in the SREBP1 target polynucleotide sequence that are complementary to the nucleotide positions of the overhang nucleotides in the guide strand or antisense strand/region of a siNA molecule. In another embodiment, the nucleotides comprising the overhang portion of the passenger strand or sense strand/region of a siNA molecule of the invention can comprise the nucleotides in the SREBP1 target polynucleotide sequence, i.e. those nucleotide positions in the SREBP1 target polynucleotide sequence that correspond to same the nucleotide positions of the overhang nucleotides in the passenger strand or sense strand/region of a siNA molecule. In one embodiment, the overhang comprises a two nucleotide (e.g., 3'-GA; 3'-GU; 3'-GG; 3'GC; 3'-CA; 3'-CU; 3'-CG; 3'CC; 3'-UA; 3'-UU; 3'-UG; 3'UC; 3'-AA; 3'-AU; 3'-AG; 3'-AC; 3'-TA; 3'-TU; 3'-TG; 3'-TC; 3'-AT; 3'-UT; 3'-GT; 3'-CT) overhang that is complementary to a portion of the SREBP1 target polynucleotide sequence. In one embodiment, the overhang comprises a two nucleotide (e.g., 3'-GA; 3'-GU; 3'-GG; 3'GC; 3'-CA; 3'-CU; 3'-CG; 3'CC; 3'-UA; 3'-UU; 3'-UG; 3'UC; 3'-AA; 3'-AU; 3'-AG; 3'-AC; 3'-TA; 3'-TU; 3'-TG; 3'-TC; 3'-AT; 3'-UT; 3'-GT; 3'-CT) overhang that is not complementary to a portion of the SREBP1 target polynucleotide sequence. In another embodiment, the overhang nucleotides of a siNA molecule of the invention are 2'-O-methyl nucleotides, 2'-deoxy-2'-fluoroarabino, and/or 2'-deoxy-2'-fluoro nucleotides. In another embodiment, the overhang nucleotides of a siNA molecule of the invention are 2'-O-methyl nucleotides in the event the overhang nucleotides are purine nucleotides and/or 2'-deoxy-2'-fluoro nucleotides or 2'-deoxy-2'-fluoroarabino nucleotides in the event the overhang nucleotides are pyrimidines nucleotides. In another embodiment, the purine nucleotide (when present) in an overhang of siNA molecule of the invention is 2'-O-methyl nucleotides. In another embodiment, the pyrimidine nucleotide (when present) in an overhang of siNA molecule of the invention are 2'-deoxy-2'-fluoro or 2'-deoxy-2'-fluoroarabino nucleotides.

[0044] In one embodiment, the nucleotides comprising the overhang portion of a double stranded nucleic acid (e.g., siNA) molecule of the invention are not complementary to the SREBP1 target polynucleotide sequence and are optionally chemically modified as described herein. In one embodiment, the overhang comprises a 3'-UU overhang that is not comple-

mentary to a portion of the SREBP1 target polynucleotide sequence. In another embodiment, the nucleotides comprising the overhanging portion of a siNA molecule of the invention are 2'-O-methyl nucleotides, 2'-deoxy-2'-fluoroarabino and/or 2'-deoxy-2'-fluoro nucleotides.

[0045] In one embodiment, the double stranded nucleic molecule (e.g. siNA) of the invention comprises a two or three nucleotide overhang, wherein the nucleotides in the overhang are the same or different. In one embodiment, the double stranded nucleic molecule (e.g. siNA) of the invention comprises a two or three nucleotide overhang, wherein the nucleotides in the overhang are the same or different and wherein one or more nucleotides in the overhang are chemically modified at the base, sugar and/or phosphate backbone.

[0046] In one embodiment, the invention features one or more chemically-modified siNA constructs having specificity for SREBP1 target nucleic acid molecules, such as DNA, or RNA encoding a protein or non-coding RNA associated with the expression of SREBP1 target genes. In one embodiment, the invention features a RNA based siNA molecule (e.g., a siNA comprising 2'-OH nucleotides) having specificity for nucleic acid molecules that includes one or more chemical modifications described herein. Non-limiting examples of such chemical modifications include without limitation phosphorothioate internucleotide linkages, 2'-deoxyribonucleotides, 2'-O-methyl ribonucleotides, 2'-deoxy-2'-fluoro ribonucleotides, 4'-thio ribonucleotides, 2'-O-trifluoromethyl nucleotides, 2'-O-ethyl-trifluoromethoxy nucleotides, 2'-O-difluoromethoxy-ethoxy nucleotides (see for example U.S. Ser. No. 10/981,966 filed Nov. 5, 2004, incorporated by reference herein), "universal base" nucleotides, "acyclic" nucleotides, 5-C-methyl nucleotides, 2'-deoxy-2'-fluoroarabino (FANA, see for example Dowler et al., 2006, Nucleic Acids Research, 34, 1669-1675) and terminal glyceryl and/or inverted deoxy abasic residue incorporation. These chemical modifications, when used in various siNA constructs, (e.g., RNA based siNA constructs), are shown to preserve RNAi activity in cells while at the same time, dramatically increasing the serum stability of these compounds.

[0047] In one embodiment, a siNA molecule of the invention comprises chemical modifications described herein (e.g., 2'-O-methyl ribonucleotides, 2'-deoxy-2'-fluoro ribonucleotides, 4'-thio ribonucleotides, 2'-O-trifluoromethyl nucleotides, 2'-O-ethyl-trifluoromethoxy nucleotides, 2'-O-difluoromethoxy-ethoxy nucleotides, LNA) at the internal positions of the siNA molecule. By "internal position" is meant the base paired positions of a siNA duplex.

[0048] In one embodiment, a siNA molecule of the invention comprises modified nucleotides while maintaining the ability to mediate RNAi. The modified nucleotides can be used to improve in vitro or in vivo characteristics such as stability, activity, toxicity, immune response, and/or bioavailability. For example, a siNA molecule of the invention can comprise modified nucleotides as a percentage of the total number of nucleotides present in the siNA molecule. As such, a siNA molecule of the invention can generally comprise about 5% to about 100% modified nucleotides (e.g., about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% modified nucleotides). For example, in one embodiment, between about 5% to about 100% (e.g., about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% modified nucleotides) of the nucleotide positions in a siNA molecule of the invention comprise a nucleic acid sugar modification, such as a 2'-sugar modification, e.g., 2'-O-methyl nucleotides, 2'-deoxy-2'-fluoro nucleotides, 2'-deoxy-2'-fluoroarabino, 2'-O-methoxyethyl nucleotides, 2'-O-trifluoromethyl nucleotides, 2'-O-ethyl-trifluoromethoxy nucleotides, 2'-O-difluoromethoxy-ethoxy nucleotides, or 2'-deoxy nucleotides. In another embodiment, between about 5% to about 100% (e.g., about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% modified nucleotides) of the nucleotide positions in a siNA molecule of the invention comprise a nucleic acid base modification, such as inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2,4,6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (e.g., 5-methylcytidine), 5-alkyluridines (e.g., ribothymidine), 5-halouridine (e.g., 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (e.g. 6-methyluridine), or propyne modifications. In another embodiment, between about 5% to about 100% (e.g., about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% modified nucleotides) of the nucleotide positions in a siNA molecule of the invention comprise a nucleic acid backbone modification, such as a backbone modification having Formula I herein. In another embodiment, between about 5% to about 100% (e.g., about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% modified nucleotides) of the nucleotide positions in a siNA molecule of the invention comprise a nucleic acid sugar, base, or backbone modification or any combination thereof (e.g., any combination of nucleic acid sugar, base, backbone or non-nucleotide modifications herein). In one embodiment, a siNA molecule of the invention comprises at least about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% modified nucleotides. The actual percentage of modified nucleotides present in a given siNA molecule will depend on the total number of nucleotides present in the siNA. If the siNA molecule is single stranded, the percent modification can be based upon the total number of nucleotides present in the single stranded siNA molecules. Likewise, if the siNA molecule is double stranded, the percent modification can be based upon the total number of nucleotides present in the sense strand, antisense strand, or both the sense and antisense strands.

[0049] A siNA molecule of the invention can comprise modified nucleotides at various locations within the siNA molecule. In one embodiment, a double stranded siNA molecule of the invention comprises modified nucleotides at internal base paired positions within the siNA duplex. For example, internal positions can comprise positions from about 3 to about 19 nucleotides from the 5'-end of either sense or antisense strand or region of a 21 nucleotide siNA duplex having 19 base pairs and two nucleotide 3'-overhangs. In another embodiment, a double stranded siNA molecule of the invention comprises modified nucleotides at non-base paired or overhang regions of the siNA molecule. By "non-base paired" is meant, the nucleotides are not base paired between the sense strand or sense region and the antisense strand or antisense region or the siNA molecule. The overhang nucleotides can be complementary or base paired to a corresponding SREBP1 target polynucleotide sequence (see for example FIG. **6**C). For example, overhang positions can comprise positions from about 20 to about 21 nucleotides from the

5'-end of either sense or antisense strand or region of a 21 nucleotide siNA duplex having 19 base pairs and two nucleotide 3'-overhangs. In another embodiment, a double stranded siNA molecule of the invention comprises modified nucleotides at terminal positions of the siNA molecule. For example, such terminal regions include the 3'-position, 5'-position, for both 3' and 5'-positions of the sense and/or antisense strand or region of the siNA molecule. In another embodiment, a double stranded siNA molecule of the invention comprises modified nucleotides at base-paired or internal positions, non-base paired or overhang regions, and/or terminal regions, or any combination thereof.

[0050] One aspect of the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a SREBP1 target gene or that directs cleavage of a SREBP1 target RNA. In one embodiment, the double stranded siNA molecule comprises one or more chemical modifications and each strand of the double-stranded siNA is about 21 nucleotides long. In one embodiment, the double-stranded siNA molecule does not contain any ribonucleotides. In another embodiment, the double-stranded siNA molecule comprises one or more ribonucleotides. In one embodiment, each strand of the double-stranded siNA molecule independently comprises about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein each strand comprises about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to the nucleotides of the other strand. In one embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of the SREBP1 target gene, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence of the SREBP1 target gene or a portion thereof.

[0051] In another embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a SREBP1 target gene or that directs cleavage of a SREBP1 target RNA, comprising an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of the SREBP1 target gene or a portion thereof, and a sense region, wherein the sense region comprises a nucleotide sequence substantially similar to the nucleotide sequence of the SREBP1 target gene or a portion thereof. In one embodiment, the antisense region and the sense region independently comprise about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein the antisense region comprises about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to nucleotides of the sense region.

[0052] In another embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a SREBP1 target gene or that directs cleavage of a SREBP1 target RNA, comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the SREBP1 target gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region.

[0053] In one embodiment, a siNA molecule of the invention comprises blunt ends, i.e., ends that do not include any overhanging nucleotides. For example, a siNA molecule comprising modifications described herein (e.g., comprising nucleotides having Formulae I-VII or siNA constructs comprising "Stab 00"-"Stab 36" or "Stab 3F"-"Stab 36F" (Table IV) or any combination thereof (see Table IV)) and/or any length described herein can comprise blunt ends or ends with no overhanging nucleotides.

[0054] In one embodiment, any siNA molecule of the invention can comprise one or more blunt ends, i.e. where a blunt end does not have any overhanging nucleotides. In one embodiment, the blunt ended siNA molecule has a number of base pairs equal to the number of nucleotides present in each strand of the siNA molecule. In another embodiment, the siNA molecule comprises one blunt end, for example wherein the 5'-end of the antisense strand and the 3'-end of the sense strand do not have any overhanging nucleotides. In another example, the siNA molecule comprises one blunt end, for example, wherein the 3'-end of the antisense strand and the 5'-end of the sense strand do not have any overhanging nucleotides. In another example, a siNA molecule comprises two blunt ends, for example, wherein the 3'-end of the antisense strand and the 5'-end of the sense strand as well as the 5'-end of the antisense strand and 3'-end of the sense strand do not have any overhanging nucleotides. A blunt ended siNA molecule can comprise, for example, from about 15 to about 30 nucleotides (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides). Other nucleotides present in a blunt ended siNA molecule can comprise, for example, mismatches, bulges, loops, or wobble base pairs to modulate the activity of the siNA molecule to mediate RNA interference.

[0055] By "blunt ends" is meant symmetric termini or termini of a double stranded siNA molecule having no overhanging nucleotides. The two strands of a double stranded siNA molecule align with each other without over-hanging nucleotides at the termini. For example, a blunt ended siNA construct comprises terminal nucleotides that are complementary between the sense and antisense regions of the siNA molecule.

[0056] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a SREBP1 target gene or that directs cleavage of a SREBP1 target RNA, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. The sense region can be connected to the antisense region via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker.

[0057] In one embodiment, a double stranded nucleic acid molecule (e.g., siNA) molecule of the invention comprises ribonucleotides at positions that maintain or enhance RNAi activity. In one embodiment, ribonucleotides are present in the sense strand or sense region of the siNA molecule, which can provide for RNAi activity by allowing cleavage of the sense strand or sense region by an enzyme within the RISC (e.g., ribonucleotides present at the position of passenger strand, sense strand, or sense region cleavage, such as position 9 of the passenger strand of a 19 base-pair duplex, which is cleaved in the RISC by AGO2 enzyme, see, for example, Matranga et al., 2005, *Cell,* 123:1-114 and Rand et al., 2005, *Cell,* 123:621-629). In another embodiment, one or more (for

example 1, 2, 3, 4 or 5) nucleotides at the 5'-end of the guide strand or guide region (also known as antisense strand or antisense region) of the siNA molecule are ribonucleotides.

[0058] In one embodiment, a double stranded nucleic acid molecule (e.g., siNA) molecule of the invention comprises one or more ribonucleotides at positions within the passenger strand or passenger region (also known as the sense strand or sense region) that allows cleavage of the passenger strand or passenger region by an enzyme in the RISC complex, (e.g., ribonucleotides present at the position of passenger strand, such as position 9 of the passenger strand of a 19 base-pair duplex that is cleaved in the RISC, see, for example, Matranga et al., 2005, *Cell,* 123:1-114 and Rand et al., 2005, *Cell,* 123:621-629).

[0059] In one embodiment, a siNA molecule of the invention contains at least 2, 3, 4, 5, or more chemical modifications that can be the same of different. In one embodiment, a siNA molecule of the invention contains at least 2, 3, 4, 5, or more different chemical modifications.

[0060] In one embodiment, a siNA molecule of the invention is a double-stranded short interfering nucleic acid (siNA), wherein the double stranded nucleic acid molecule comprises about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) base pairs, and wherein one or more (e.g., at least 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, or 30) of the nucleotide positions in each strand of the siNA molecule comprises a chemical modification. In another embodiment, the siNA contains at least 2, 3, 4, 5, or more different chemical modifications.

[0061] In one embodiment, the invention features double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a SREBP1 target gene or that directs cleavage of a SREBP1 target RNA, wherein the siNA molecule comprises about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) base pairs, and wherein each strand of the siNA molecule comprises one or more chemical modifications. In one embodiment, each strand of the double stranded siNA molecule comprises at least two (e.g., 2, 3, 4, 5, or more) different chemical modifications, e.g., different nucleotide sugar, base, or backbone modifications. In another embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of a SREBP1 target gene or a portion thereof, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of the SREBP1 target gene. In another embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of a SREBP1 target gene or portion thereof, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or portion thereof of the SREBP1 target gene. In another embodiment, each strand of the siNA molecule comprises about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, and each strand comprises at least about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to the nucleotides of the other strand. The SREBP1 target gene can comprise, for example, sequences referred to herein or incorporated herein by reference. The SREBP1 gene can comprise, for example, sequences referred to in Table I.

[0062] In one embodiment, each strand of a double stranded siNA molecule of the invention comprises a different pattern of chemical modifications, such as any "Stab 00"-"Stab 36" or "Stab 3F"-"Stab 36F" (Table IV) modification patterns herein or any combination thereof (see Table IV). Non-limiting examples of sense and antisense strands of such siNA molecules having various modification patterns are shown in Table III and FIGS. **4** and **5**.

[0063] In one embodiment, a siNA molecule of the invention comprises no ribonucleotides. In another embodiment, a siNA molecule of the invention comprises one or more ribonucleotides (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more ribonucleotides).

[0064] In one embodiment, a siNA molecule of the invention comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence of a SREBP1 target gene or a portion thereof, and the siNA further comprises a sense region comprising a nucleotide sequence substantially similar to the nucleotide sequence of the SREBP1 target gene or a portion thereof. In another embodiment, the antisense region and the sense region each comprise about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides and the antisense region comprises at least about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to nucleotides of the sense region. In one embodiment, each strand of the double stranded siNA molecule comprises at least two (e.g., 2, 3, 4, 5, or more) different chemical modifications, e.g., different nucleotide sugar, base, or backbone modifications. The SREBP1 target gene can comprise, for example, sequences referred to herein or incorporated by reference herein. In another embodiment, the siNA is a double stranded nucleic acid molecule, where each of the two strands of the siNA molecule independently comprise about 15 to about 40 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 23, 33, 34, 35, 36, 37, 38, 39, or 40) nucleotides, and where one of the strands of the siNA molecule comprises at least about 15 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 or more) nucleotides that are complementary to the nucleic acid sequence of the SREBP1 target gene or a portion thereof.

[0065] In one embodiment, a siNA molecule of the invention comprises a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by a SREBP1 target gene, or a portion thereof, and the sense region comprises a nucleotide sequence that is complementary to the antisense region. In one embodiment, the siNA molecule is assembled from two separate oligonucleotide fragments, wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. In another embodiment, the sense region is connected to the antisense region via a linker molecule. In another embodiment, the sense region is connected to the antisense region via a linker molecule, such as a nucleotide or non-nucleotide linker. In one embodiment, each strand of the double stranded siNA molecule comprises at least two (e.g., 2, 3, 4, 5, or more) different chemical modifications, e.g., different nucleotide sugar, base, or backbone

modifications. The SREBP1 target gene can comprise, for example, sequences referred herein or incorporated by reference herein.

[0066] In one embodiment, a siNA molecule of the invention comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) 2'-deoxy-2'-fluoro pyrimidine modifications (e.g., where one or more or all pyrimidine (e.g., U or C) positions of the siNA are modified with 2'-deoxy-2'-fluoro nucleotides). In one embodiment, the 2'-deoxy-2'-fluoro pyrimidine modifications are present in the sense strand. In one embodiment, the 2'-deoxy-2'-fluoro pyrimidine modifications are present in the antisense strand. In one embodiment, the 2'-deoxy-2'-fluoro pyrimidine modifications are present in both the sense strand and the antisense strand of the siNA molecule.

[0067] In one embodiment, a siNA molecule of the invention comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) 2'-O-methyl purine modifications (e.g., where one or more or all purine (e.g., A or G) positions of the siNA are modified with 2'-O-methyl nucleotides). In one embodiment, the 2'-O-methyl purine modifications are present in the sense strand. In one embodiment, the 2'-O-methyl purine modifications are present in the antisense strand. In one embodiment, the 2'-O-methyl purine modifications are present in both the sense strand and the antisense strand of the siNA molecule.

[0068] In one embodiment, a siNA molecule of the invention comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) 2'-deoxy purine modifications (e.g., where one or more or all purine (e.g., A or G) positions of the siNA are modified with 2'-deoxy nucleotides). In one embodiment, the 2'-deoxy purine modifications are present in the sense strand. In one embodiment, the 2'-deoxy purine modifications are present in the antisense strand. In one embodiment, the 2'-deoxy purine modifications are present in both the sense strand and the antisense strand of the siNA molecule.

[0069] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a SREBP1 target gene or that directs cleavage of a SREBP1 target RNA, comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the SREBP1 target gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region, and wherein the siNA molecule has one or more modified pyrimidine and/or purine nucleotides. In one embodiment, each strand of the double stranded siNA molecule comprises at least two (e.g., 2, 3, 4, 5, or more) different chemical modifications, e.g., different nucleotide sugar, base, or backbone modifications. In one embodiment, the pyrimidine nucleotides in the sense region are 2'-O-methylpyrimidine nucleotides or 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In another embodiment, the pyrimidine nucleotides in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides. In another embodiment, the pyrimidine nucleotides in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In one embodiment, the pyrimidine nucleotides in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the antisense region are 2'-O-methyl or 2'-deoxy purine nucleotides. In another embodiment of any of the above-described siNA molecules, any nucleotides present in a non-complementary region of the sense strand (e.g. overhang region) are 2'-deoxy nucleotides.

[0070] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a SREBP1 target gene or that directs cleavage of a SREBP1 target RNA, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule, and wherein the fragment comprising the sense region includes a terminal cap moiety at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the fragment. In one embodiment, the terminal cap moiety is an inverted deoxy abasic moiety or glyceryl moiety. In one embodiment, each of the two fragments of the siNA molecule independently comprise about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides. In another embodiment, each of the two fragments of the siNA molecule independently comprise about 15 to about 40 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 23, 33, 34, 35, 36, 37, 38, 39, or 40) nucleotides. In a non-limiting example, each of the two fragments of the siNA molecule comprise about 21 nucleotides.

[0071] In one embodiment, the invention features a siNA molecule comprising at least one modified nucleotide, wherein the modified nucleotide is a 2'-deoxy-2'-fluoro nucleotide, 2'-deoxy-2'-fluoroarabino, 2'-O-trifluoromethyl nucleotide, 2'-O-ethyl-trifluoromethoxy nucleotide, or 2'-O-difluoromethoxy-ethoxy nucleotide or any other modified nucleoside/nucleotide described herein and in U.S. Ser. No. 10/981,966, filed Nov. 5, 2004, incorporated by reference herein. In one embodiment, the invention features a siNA molecule comprising at least two (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) modified nucleotides, wherein the modified nucleotide is selected from the group consisting of 2'-deoxy-2'-fluoro nucleotide, 2'-deoxy-2'-fluoroarabino, 2'-O-trifluoromethyl nucleotide, 2'-O-ethyl-trifluoromethoxy nucleotide, or 2'-O-difluoromethoxy-ethoxy nucleotide or any other modified nucleoside/nucleotide described herein and in U.S. Ser. No. 10/981,966, filed Nov. 5, 2004, incorporated by reference herein. The modified nucleotide/nucleoside can be the same or different. The siNA can be, for example, about 15 to about 40 nucleotides in length. In one embodiment, all pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro, 2'-deoxy-2'-fluoroarabino, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy, 4'-thio pyrimidine nucleotides. In one embodiment, the modified nucleotides in the siNA include at least one 2'-deoxy-2'-fluoro cytidine or 2'-deoxy-2'-fluoro uridine nucleotide. In another embodiment, the modified nucleotides in the siNA include at least one 2'-deoxy-2'-fluoro cytidine and at least one 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all uridine nucleotides present in the siNA are 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all cytidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro cytidine nucleotides. In one embodiment, all adenosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro adenosine nucleotides. In one embodiment, all guanosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro guanosine nucleotides. The siNA can further comprise at least

one modified internucleotidic linkage, such as phosphorothioate linkage. In one embodiment, the 2'-deoxy-2'-fluoronucleotides are present at specifically selected locations in the siNA that are sensitive to cleavage by ribonucleases, such as locations having pyrimidine nucleotides.

[0072] In one embodiment, the invention features a method of increasing the stability of a siNA molecule against cleavage by ribonucleases comprising introducing at least one modified nucleotide into the siNA molecule, wherein the modified nucleotide is a 2'-deoxy-2'-fluoro nucleotide. In one embodiment, all pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In one embodiment, the modified nucleotides in the siNA include at least one 2'-deoxy-2'-fluoro cytidine or 2'-deoxy-2'-fluoro uridine nucleotide. In another embodiment, the modified nucleotides in the siNA include at least one 2'-fluoro cytidine and at least one 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all uridine nucleotides present in the siNA are 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all cytidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro cytidine nucleotides. In one embodiment, all adenosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro adenosine nucleotides. In one embodiment, all guanosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro guanosine nucleotides. The siNA can further comprise at least one modified internucleotidic linkage, such as a phosphorothioate linkage. In one embodiment, the 2'-deoxy-2'-fluoronucleotides are present at specifically selected locations in the siNA that are sensitive to cleavage by ribonucleases, such as locations having pyrimidine nucleotides.

[0073] In one embodiment, the invention features a method of increasing the stability of a siNA molecule against cleavage by ribonucleases comprising introducing at least one modified nucleotide into the siNA molecule, wherein the modified nucleotide is a 2'-deoxy-2'-fluoroarabino nucleotide. In one embodiment, all pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoroarabino pyrimidine nucleotides. In one embodiment, the modified nucleotides in the siNA include at least one 2'-deoxy-2'-fluoroarabino cytidine or 2'-deoxy-2'-fluoroarabino uridine nucleotide. In another embodiment, the modified nucleotides in the siNA include at least one 2'-fluoro cytidine and at least one 2'-deoxy-2'-fluoroarabino uridine nucleotides. In one embodiment, all uridine nucleotides present in the siNA are 2'-deoxy-2'-fluoroarabino uridine nucleotides. In one embodiment, all cytidine nucleotides present in the siNA are 2'-deoxy-2'-fluoroarabino cytidine nucleotides. In one embodiment, all adenosine nucleotides present in the siNA are 2'-deoxy-2'-fluoroarabino adenosine nucleotides. In one embodiment, all guanosine nucleotides present in the siNA are 2'-deoxy-2'-fluoroarabino guanosine nucleotides. The siNA can further comprise at least one modified internucleotidic linkage, such as a phosphorothioate linkage. In one embodiment, the 2'-deoxy-2'-fluoroarabinonucleotides are present at specifically selected locations in the siNA that are sensitive to cleavage by ribonucleases, such as locations having pyrimidine nucleotides.

[0074] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a SREBP1 target gene or that directs cleavage of a SREBP1 target RNA, comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the SREBP1 target gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region, and wherein the purine nucleotides present in the antisense region comprise 2'-deoxy-purine nucleotides. In an alternative embodiment, the purine nucleotides present in the antisense region comprise 2'-O-methyl purine nucleotides. In either of the above embodiments, the antisense region can comprise a phosphorothioate internucleotide linkage at the 3' end of the antisense region. Alternatively, in either of the above embodiments, the antisense region can comprise a glyceryl modification at the 3' end of the antisense region. In another embodiment of any of the above-described siNA molecules, any nucleotides present in a non-complementary region of the antisense strand (e.g. overhang region) are 2'-deoxy nucleotides.

[0075] In one embodiment, the antisense region of a siNA molecule of the invention comprises sequence complementary to a portion of an endogenous transcript having sequence unique to a particular disease or trait related allele in a subject or organism, such as sequence comprising a single nucleotide polymorphism (SNP) associated with the disease or trait specific allele. As such, the antisense region of a siNA molecule of the invention can comprise sequence complementary to sequences that are unique to a particular allele to provide specificity in mediating selective RNAi against the disease, condition, or trait related allele.

[0076] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a SREBP1 target gene or that directs cleavage of a SREBP1 target RNA, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. In one embodiment, each strand of the double stranded siNA molecule is about 21 nucleotides long where about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule, wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule, where each strand is about 19 nucleotide long and where the nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule to form at least about 15 (e.g., 15, 16, 17, 18, or 19) base pairs, wherein one or both ends of the siNA molecule are blunt ends. In one embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-deoxy-pyrimidine nucleotide, such as a 2'-deoxy-thymidine. In one embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-O-methylpyrimidine nucleotide, such as a 2'-O-methyl uridine, cytidine, or thymidine. In another embodiment, all nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule of about 19 to about 25 base pairs having a sense region and an antisense region, where about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the SREBP1 target gene. In another embodiment, about 21 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of

the RNA encoded by the SREBP1 target gene. In any of the above embodiments, the 5'-end of the fragment comprising said antisense region can optionally include a phosphate group.

[0077] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a SREBP1 target RNA sequence, wherein the siNA molecule does not contain any ribonucleotides and wherein each strand of the double-stranded siNA molecule is about 15 to about 30 nucleotides. In one embodiment, the siNA molecule is 21 nucleotides in length. Examples of non-ribonucleotide containing siNA constructs are combinations of stabilization chemistries shown in Table IV in any combination of Sense/Antisense chemistries, such as Stab 7/8, Stab 7/11, Stab 8/8, Stab 18/8, Stab 18/11, Stab 12/13, Stab 7/13, Stab 18/13, Stab 7/19, Stab 8/19, Stab 18/19, Stab 7/20, Stab 8/20, Stab 18/20, Stab 7/32, Stab 8/32, or Stab 18/32 (e.g., any siNA having Stab 7, 8, 11, 12, 13, 14, 15, 17, 18, 19, 20, or 32 sense or antisense strands or any combination thereof). Herein, numeric Stab chemistries can include both 2'-fluoro and 2'-OCF3 versions of the chemistries shown in Table IV. For example, "Stab 7/8" refers to both Stab 7/8 and Stab 7F/8F etc. In one embodiment, the invention features a chemically synthesized double stranded RNA molecule that directs cleavage of a SREBP1 target RNA via RNA interference, wherein each strand of said RNA molecule is about 15 to about 30 nucleotides in length; one strand of the RNA molecule comprises nucleotide sequence having sufficient complementarity to the SREBP1 target RNA for the RNA molecule to direct cleavage of the SREBP1 target RNA via RNA interference; and wherein at least one strand of the RNA molecule optionally comprises one or more chemically modified nucleotides described herein, such as without limitation deoxynucleotides, 2'-O-methyl nucleotides, 2'-deoxy-2'-fluoro nucleotides, 2'-deoxy-2'-fluoroarabino, 2'-O-methoxyethyl nucleotides, 4'-thio nucleotides, 2'-O-trifluoromethyl nucleotides, 2'-O-ethyl-trifluoromethoxy nucleotides, 2'-O-difluoromethoxy-ethoxy nucleotides, etc. or any combination thereof.

[0078] In one embodiment, a SREBP1 target RNA of the invention comprises sequence encoding a protein.

[0079] In one embodiment, SREBP1 target RNA of the invention comprises non-coding RNA sequence (e.g., miRNA, snRNA, siRNA etc.), see for example Mattick, 2005, *Science,* 309, 1527-1528; Clayerie, 2005, *Science,* 309, 1529-1530; Sethupathy et al., 2006, RNA, 12, 192-197; and Czech, 2006 NEJM, 354, 11: 1194-1195.

[0080] In one embodiment, the invention features a medicament comprising a siNA molecule of the invention.

[0081] In one embodiment, the invention features an active ingredient comprising a siNA molecule of the invention.

[0082] In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule to inhibit, down-regulate, or reduce expression of a SREBP1 target gene, wherein the siNA molecule comprises one or more chemical modifications and each strand of the double-stranded siNA is independently about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 or more) nucleotides long. In one embodiment, the siNA molecule of the invention is a double stranded nucleic acid molecule comprising one or more chemical modifications, where each of the two fragments of the siNA molecule independently comprise about 15 to about 40 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 23, 33, 34, 35, 36, 37, 38, 39, or 40) nucleotides and where one of the strands comprises at least 15 nucleotides that are complementary to nucleotide sequence of SREBP1 target encoding RNA or a portion thereof. In a non-limiting example, each of the two fragments of the siNA molecule comprise about 21 nucleotides. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule comprising one or more chemical modifications, where each strand is about 21 nucleotide long and where about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule, wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule comprising one or more chemical modifications, where each strand is about 19 nucleotide long and where the nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule to form at least about 15 (e.g., 15, 16, 17, 18, or 19) base pairs, wherein one or both ends of the siNA molecule are blunt ends. In one embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-deoxy-pyrimidine nucleotide, such as a 2'-deoxy-thymidine. In one embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-O-methylpyrimidine nucleotide, such as a 2'-O-methyl uridine, cytidine, or thymidine. In another embodiment, all nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule of about 19 to about 25 base pairs having a sense region and an antisense region and comprising one or more chemical modifications, where about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the SREBP1 target gene. In another embodiment, about 21 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the SREBP1 target gene. In any of the above embodiments, the 5'-end of the fragment comprising said antisense region can optionally include a phosphate group.

[0083] In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule that inhibits, down-regulates, or reduces expression of a SREBP1 target gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of SREBP1 target RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand. In one embodiment, each strand has at least two (e.g., 2, 3, 4, 5, or more) chemical modifications, which can be the same or different, such as nucleotide, sugar, base, or backbone modifications. In one embodiment, a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification. In one embodiment, a majority of the purine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

[0084] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits, down-regulates, or reduces expression of a SREBP1

target gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of SREBP1 target RNA or a portion thereof, wherein the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand. In one embodiment, each strand has at least two (e.g., 2, 3, 4, 5, or more) chemical modifications, which can be the same or different, such as nucleotide, sugar, base, or backbone modifications. In one embodiment, a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification. In one embodiment, a majority of the purine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

[0085] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits, down-regulates, or reduces expression of a SREBP1 target gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of SREBP1 target RNA that encodes a protein or portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification. In one embodiment, each strand of the siNA molecule comprises about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides, wherein each strand comprises at least about 15 nucleotides that are complementary to the nucleotides of the other strand. In one embodiment, the siNA molecule is assembled from two oligonucleotide fragments, wherein one fragment comprises the nucleotide sequence of the antisense strand of the siNA molecule and a second fragment comprises nucleotide sequence of the sense region of the siNA molecule. In one embodiment, the sense strand is connected to the antisense strand via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker. In a further embodiment, the pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In another embodiment, the pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides. In still another embodiment, the pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and any purine nucleotides present in the antisense strand are 2'-deoxy purine nucleotides. In another embodiment, the antisense strand comprises one or more 2'-deoxy-2'-fluoro pyrimidine nucleotides and one or more 2'-O-methyl purine nucleotides. In another embodiment, the pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and any purine nucleotides present in the antisense strand are 2'-O-methyl purine nucleotides. In a further embodiment the sense strand comprises a 3'-end and a 5'-end, wherein a terminal cap moiety (e.g., an inverted deoxy abasic moiety or inverted deoxy nucleotide moiety such as inverted thymidine) is present at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the sense strand. In another embodiment, the antisense strand comprises a phosphorothioate internucleotide linkage at the 3' end of the antisense strand. In another embodiment, the antisense strand comprises a glyceryl modification at the 3' end. In another embodiment, the 5'-end of the antisense strand optionally includes a phosphate group.

[0086] In any of the above-described embodiments of a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a SREBP1 target gene, wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, each of the two strands of the siNA molecule can comprise about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides. In one embodiment, about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides of each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule. In another embodiment, about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides of each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule, wherein at least two 3' terminal nucleotides of each strand of the siNA molecule are not base-paired to the nucleotides of the other strand of the siNA molecule. In another embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-deoxy-pyrimidine, such as 2'-deoxy-thymidine. In one embodiment, each strand of the siNA molecule is base-paired to the complementary nucleotides of the other strand of the siNA molecule. In one embodiment, about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides of the antisense strand are base-paired to the nucleotide sequence of the SREBP1 target RNA or a portion thereof. In one embodiment, about 18 to about 25 (e.g., about 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides of the antisense strand are base-paired to the nucleotide sequence of the SREBP1 target RNA or a portion thereof.

[0087] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a SREBP1 target gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of SREBP1 target RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand. In one embodiment, each strand has at least two (e.g., 2, 3, 4, 5, or more) different chemical modifications, such as nucleotide sugar, base, or backbone modifications. In one embodiment, a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification. In one embodiment, a majority of the purine nucleotides present in the double-stranded siNA molecule comprises a sugar modification. In one embodiment, the 5'-end of the antisense strand optionally includes a phosphate group.

[0088] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a SREBP1 target gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of SREBP1 target RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the

double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence or a portion thereof of the antisense strand is complementary to a nucleotide sequence of the untranslated region or a portion thereof of the SREBP1 target RNA.

[0089] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a SREBP1 target gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of SREBP1 target RNA or a portion thereof, wherein the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand, wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence of the antisense strand is complementary to a nucleotide sequence of the SREBP1 target RNA or a portion thereof that is present in the SREBP1 target RNA.

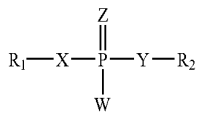
[0090] In one embodiment, the invention features a composition comprising a siNA molecule of the invention and a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features two or more differing siNA molecules of the invention (e.g. siNA molecules that target different regions of SREBP1 target RNA or siNA molecules that target SREBP1 pathway RNA) and a pharmaceutically acceptable carrier or diluent.

[0091] In a non-limiting example, the introduction of chemically-modified nucleotides into nucleic acid molecules provides a powerful tool in overcoming potential limitations of in vivo stability and bioavailability inherent to native RNA molecules that are delivered exogenously. For example, the use of chemically-modified nucleic acid molecules can enable a lower dose of a particular nucleic acid molecule for a given therapeutic effect since chemically-modified nucleic acid molecules tend to have a longer half-life in serum. Furthermore, certain chemical modifications can improve the bioavailability of nucleic acid molecules by SREBP1 targeting particular cells or tissues and/or improving cellular uptake of the nucleic acid molecule. Therefore, even if the activity of a chemically-modified nucleic acid molecule is reduced as compared to a native nucleic acid molecule, for example, when compared to an all-RNA nucleic acid molecule, the overall activity of the modified nucleic acid molecule can be greater than that of the native molecule due to improved stability and/or delivery of the molecule. Unlike native unmodified siNA, chemically-modified siNA can also minimize the possibility of activating interferon activity or immunostimulation in humans. These properties therefore improve upon native siRNA or minimally modified siRNA's ability to mediate RNAi in various in vitro and in vivo settings, including use in both research and therapeutic applications. Applicant describes herein chemically modified siNA molecules with improved RNAi activity compared to corresponding unmodified or minimally modified siRNA molecules. The chemically modified siNA motifs disclosed herein provide the capacity to maintain RNAi activity that is substantially similar to unmodified or minimally modified active siRNA (see for example Elbashir et al., 2001, EMBO J., 20:6877-6888) while at the same time providing nuclease resistance and pharmacoketic properties suitable for use in therapeutic applications.

[0092] In any of the embodiments of siNA molecules described herein, the antisense region of a siNA molecule of the invention can comprise a phosphorothioate internucleotide linkage at the 3'-end of said antisense region. In any of the embodiments of siNA molecules described herein, the antisense region can comprise about one to about five phosphorothioate internucleotide linkages at the 5'-end of said antisense region. In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs of a siNA molecule of the invention can comprise ribonucleotides or deoxyribonucleotides that are chemically-modified at a nucleic acid sugar, base, or backbone. In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs can comprise one or more universal base ribonucleotides. In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs can comprise one or more acyclic nucleotides.

[0093] One embodiment of the invention provides an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention in a manner that allows expression of the nucleic acid molecule. Another embodiment of the invention provides a mammalian cell comprising such an expression vector. The mammalian cell can be a human cell. The siNA molecule of the expression vector can comprise a sense region and an antisense region. The antisense region can comprise sequence complementary to a RNA or DNA sequence encoding a SREBP1 target and the sense region can comprise sequence complementary to the antisense region. The siNA molecule can comprise two distinct strands having complementary sense and antisense regions. The siNA molecule can comprise a single strand having complementary sense and antisense regions.

[0094] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) inside a cell or reconstituted in vitro system, wherein the chemical modification comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides comprising a backbone modified internucleotide linkage having Formula I:

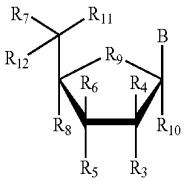


[0095] wherein each R1 and R2 is independently any nucleotide, non-nucleotide, or polynucleotide which can be naturally-occurring or chemically-modified and which can be included in the structure of the siNA molecule or serve as a point of attachment to the siNA molecule, each X and Y is independently O, S, N, alkyl, or substituted alkyl, each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, or acetyl and wherein W, X, Y, and Z are optionally not all O. In another embodiment, a backbone modification of the invention comprises a phosphonoacetate and/or thiophosphonoacetate internucleotide linkage (see for example Sheehan et al., 2003, Nucleic Acids Research, 31, 4109-4118).

[0096] The chemically-modified internucleotide linkages having Formula I, for example, wherein any Z, W, X, and/or Y independently comprises a sulphur atom, can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both

strands. The siNA molecules of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) chemically-modified internucleotide linkages having Formula I at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified internucleotide linkages having Formula I at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In another embodiment, a siNA molecule of the invention having internucleotide linkage(s) of Formula I also comprises a chemically-modified nucleotide or non-nucleotide having any of Formulae I-VII.

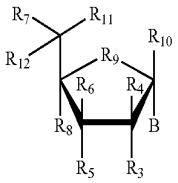
[0097] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) inside a cell or reconstituted in vitro system, wherein the chemical modification comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula II:



wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCH3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or a group having any of Formula I, II, III, IV, V, VI and/or VII, any of which can be included in the structure of the siNA molecule or serve as a point of attachment to the siNA molecule; R9 is O, S, CH2, S═O, CHF, or CF2, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA. In one embodiment, R3 and/or R7 comprises a conjugate moiety and a linker (e.g., a nucleotide or non-nucleotide linker as described herein or otherwise known in the art). Non-limiting examples of conjugate moieties include ligands for cellular receptors, such as peptides derived from naturally occurring protein ligands; protein localization sequences, including cellular ZIP code sequences; antibodies; nucleic acid aptamers; vitamins and other co-factors, such as folate and N-acetylgalactosamine; polymers, such as polyethyleneglycol (PEG); phospholipids; cholesterol; steroids, and polyamines, such as PEI, spermine or spermidine. In one embodiment, a nucleotide of the invention having Formula II is a 2'-deoxy-2'-fluoro nucleotide. In one embodiment, a nucleotide of the invention having Formula II is a 2'-O-methyl nucleotide. In one embodiment, a nucleotide of the invention having Formula II is a 2'-deoxy nucleotide.

[0098] The chemically-modified nucleotide or non-nucleotide of Formula II can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotides or non-nucleotides of Formula II at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 3'-end of the sense strand, the antisense strand, or both strands.

[0099] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) inside a cell or reconstituted in vitro system, wherein the chemical modification comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula III:



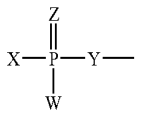
wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCH3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-5-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or a group having any of Formula I, II, III, IV, V, VI and/or VII, any of which can be included in the structure of the siNA molecule or serve as a point of attachment to the siNA molecule; R9 is O, S, CH2, S═O, CHF, or CF2, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can

be employed to be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA. In one embodiment, R3 and/or R7 comprises a conjugate moiety and a linker (e.g., a nucleotide or non-nucleotide linker as described herein or otherwise known in the art). Non-limiting examples of conjugate moieties include ligands for cellular receptors, such as peptides derived from naturally occurring protein ligands; protein localization sequences, including cellular ZIP code sequences; antibodies; nucleic acid aptamers; vitamins and other co-factors, such as folate and N-acetylgalactosamine; polymers, such as polyethyleneglycol (PEG); phospholipids; cholesterol; steroids, and polyamines, such as PEI, spermine or spermidine.

[0100] The chemically-modified nucleotide or non-nucleotide of Formula III can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotides or non-nucleotides of Formula III at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide(s) or non-nucleotide(s) of Formula III at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide or non-nucleotide of Formula III at the 3'-end of the sense strand, the antisense strand, or both strands.

[0101] In another embodiment, a siNA molecule of the invention comprises a nucleotide having Formula II or III, wherein the nucleotide having Formula II or III is in an inverted configuration. For example, the nucleotide having Formula II or III is connected to the siNA construct in a 3'-3',3'-2',2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

[0102] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) inside a cell or reconstituted in vitro system, wherein the chemical modification comprises a 5'-terminal phosphate group having Formula IV:



wherein each X and Y is independently O, S, N, alkyl, substituted alkyl, or alkylhalo; wherein each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, alkylhalo, or acetyl; and wherein W, X, Y and Z are optionally not all O and Y serves as a point of attachment to the siNA molecule.

[0103] In one embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the SREBP1 target-complementary strand, for example, a strand complementary to a SREBP1 target RNA, wherein the siNA molecule comprises an all RNA siNA molecule. In another embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the SREBP1 target-complementary strand wherein the siNA molecule also comprises about 1 to about 3 (e.g., about 1, 2, or 3) nucleotide 3'-terminal nucleotide overhangs having about 1 to about 4 (e.g., about 1, 2, 3, or 4) deoxyribonucleotides on the 3'-end of one or both strands. In another embodiment, a 5'-terminal phosphate group having Formula IV is present on the SREBP1 target-complementary strand of a siNA molecule of the invention, for example a siNA molecule having chemical modifications having any of Formulae I-VII.

[0104] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) inside a cell or reconstituted in vitro system, wherein the chemical modification comprises one or more phosphorothioate internucleotide linkages. For example, in a non-limiting example, the invention features a chemically-modified short interfering nucleic acid (siNA) having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in one siNA strand. In yet another embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) individually having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in both siNA strands. The phosphorothioate internucleotide linkages can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more phosphorothioate internucleotide linkages at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) consecutive phosphorothioate internucleotide linkages at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands.

[0105] Each strand of the double stranded siNA molecule can have one or more chemical modifications such that each strand comprises a different pattern of chemical modifications. Several non-limiting examples of modification schemes that could give rise to different patterns of modifications are provided herein.

[0106] In one embodiment, the invention features a siNA molecule, wherein the sense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, 2'-O-difluoromethoxy-ethoxy and/or about one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense

strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, 2'-O-difluoromethoxy-ethoxy, 4'-thio and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, 2'-O-difluoromethoxy-ethoxy, 4'-thio and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

[0107] In another embodiment, the invention features a siNA molecule, wherein the sense strand comprises about 1 to about 5, specifically about 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, 2'-O-difluoromethoxy-ethoxy, 4'-thio and/or one or more (e.g., about 1, 2, 3, 4, 5, or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, 2'-O-difluoromethoxy-ethoxy, 4'-thio and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, 2'-O-difluoromethoxy-ethoxy, 4'-thio and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5 or more, for example about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

[0108] In one embodiment, the invention features a siNA molecule, wherein the antisense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or about one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, 2'-O-difluoromethoxy-ethoxy, 4'-thio and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, 2'-O-difluoromethoxy-ethoxy, 4'-thio and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, 2'-O-difluoromethoxy-ethoxy, 4'-thio and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3' and 5'-ends, being present in the same or different strand.

[0109] In another embodiment, the invention features a siNA molecule, wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, 2'-O-difluoromethoxy-ethoxy, 4'-thio and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, 2'-O-difluoromethoxy-ethoxy, 4'-thio and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, 2'-O-difluoromethoxy-ethoxy, 4'-thio and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5, for example about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

[0110] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule having about 1 to about 5 or more (specifically about 1, 2, 3, 4, 5 or more) phosphorothioate internucleotide linkages in each strand of the siNA molecule.

[0111] In another embodiment, the invention features a siNA molecule comprising 2'-5' internucleotide linkages. The 2'-5' internucleotide linkage(s) can be at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of one or both siNA sequence strands. In addition, the 2'-5' internucleotide linkage(s) can be present at various other positions within one or both siNA sequence strands, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a pyrimidine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide

linkage of a purine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage.

[0112] In another embodiment, a chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified, wherein each strand is independently about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in length, wherein the duplex has about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) base pairs, and wherein the chemical modification comprises a structure having any of Formulae I-VII. For example, an exemplary chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein each strand consists of about 21 nucleotides, each having a 2-nucleotide 3'-terminal nucleotide overhang, and wherein the duplex has about 19 base pairs. In another embodiment, a siNA molecule of the invention comprises a single stranded hairpin structure, wherein the siNA is about 36 to about 70 (e.g., about 36, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) base pairs, and wherein the siNA can include a chemical modification comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 42 to about 50 (e.g., about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms a hairpin structure having about 19 to about 21 (e.g., 19, 20, or 21) base pairs and a 2-nucleotide 3'-terminal nucleotide overhang. In another embodiment, a linear hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. For example, a linear hairpin siNA molecule of the invention is designed such that degradation of the loop portion of the siNA molecule in vivo can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

[0113] In another embodiment, a siNA molecule of the invention comprises a hairpin structure, wherein the siNA is about 25 to about 50 (e.g., about 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, or 50) nucleotides in length having about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 25 to about 35 (e.g., about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35) nucleotides that is chemically-modified with one or more chemical modifications having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms a hairpin structure having about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs and a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV). In another embodiment, a linear hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. In one embodiment, a linear hairpin siNA molecule of the invention comprises a loop portion comprising a non-nucleotide linker.

[0114] In another embodiment, a siNA molecule of the invention comprises an asymmetric hairpin structure, wherein the siNA is about 25 to about 50 (e.g., about 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, or 50) nucleotides in length having about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 25 to about 35 (e.g., about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35) nucleotides that is chemically-modified with one or more chemical modifications having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms an asymmetric hairpin structure having about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs and a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV). In one embodiment, an asymmetric hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. In another embodiment, an asymmetric hairpin siNA molecule of the invention comprises a loop portion comprising a non-nucleotide linker.

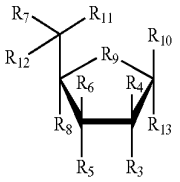
[0115] In another embodiment, a siNA molecule of the invention comprises an asymmetric double stranded structure having separate polynucleotide strands comprising sense and antisense regions, wherein the antisense region is about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in length, wherein the sense region is about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides in length, wherein the sense region and the antisense region have at least 3 complementary nucleotides, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises an asymmetric double stranded structure having separate polynucleotide strands comprising sense and antisense regions, wherein the antisense region is about 18 to about 23 (e.g., about 18, 19, 20, 21, 22, or 23) nucleotides in length and wherein the sense region is about 3 to about 15 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) nucleotides in length, wherein the sense region the antisense region have at least 3 complementary nucleotides, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. In another embodiment, the asymmetric double stranded siNA molecule can also have a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV).

[0116] In another embodiment, a siNA molecule of the invention comprises a circular nucleic acid molecule, wherein the siNA is about 38 to about 70 (e.g., about 38, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 15

to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) base pairs, and wherein the siNA can include a chemical modification, which comprises a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a circular oligonucleotide having about 42 to about 50 (e.g., about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the circular oligonucleotide forms a dumbbell shaped structure having about 19 base pairs and 2 loops.

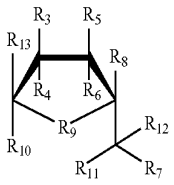
[0117] In another embodiment, a circular siNA molecule of the invention contains two loop motifs, wherein one or both loop portions of the siNA molecule is biodegradable. For example, a circular siNA molecule of the invention is designed such that degradation of the loop portions of the siNA molecule in vivo can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

[0118] In one embodiment, a siNA molecule of the invention comprises at least one (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) abasic moiety, for example a compound having Formula V:



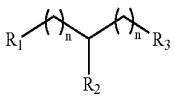
wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCH3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or a group having any of Formula I, II, III, IV, V, VI and/or VII, any of which can be included in the structure of the siNA molecule or serve as a point of attachment to the siNA molecule; R9 is O, S, CH2, S═O, CHF, or CF2. In one embodiment, R3 and/or R7 comprises a conjugate moiety and a linker (e.g., a nucleotide or non-nucleotide linker as described herein or otherwise known in the art). Non-limiting examples of conjugate moieties include ligands for cellular receptors, such as peptides derived from naturally occurring protein ligands; protein localization sequences, including cellular ZIP code sequences; antibodies; nucleic acid aptamers; vitamins and other co-factors, such as folate and N-acetylgalactosamine; polymers, such as polyethyleneglycol (PEG); phospholipids; cholesterol; steroids, and polyamines, such as PEI, spermine or spermidine.

[0119] In one embodiment, a siNA molecule of the invention comprises at least one (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) inverted abasic moiety, for example a compound having Formula VI:



wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCH3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-5-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or a group having any of Formula I, II, III, IV, V, VI and/or VII, any of which can be included in the structure of the siNA molecule or serve as a point of attachment to the siNA molecule; R9 is O, S, CH2, S═O, CHF, or CF2, and either R2, R3, R8 or R13 serve as points of attachment to the siNA molecule of the invention. In one embodiment, R3 and/or R7 comprises a conjugate moiety and a linker (e.g., a nucleotide or non-nucleotide linker as described herein or otherwise known in the art). Non-limiting examples of conjugate moieties include ligands for cellular receptors, such as peptides derived from naturally occurring protein ligands; protein localization sequences, including cellular ZIP code sequences; antibodies; nucleic acid aptamers; vitamins and other co-factors, such as folate and N-acetylgalactosamine; polymers, such as polyethyleneglycol (PEG); phospholipids; cholesterol; steroids, and polyamines, such as PEI, spermine or spermidine.

[0120] In another embodiment, a siNA molecule of the invention comprises at least one (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) substituted polyalkyl moieties, for example a compound having Formula VII:



wherein each n is independently an integer from 1 to 12, each R1, R2 and R3 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCH3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or a group having any of Formula I, II, III, IV, V, VI and/or VII, any of which can be included in the structure of the siNA molecule or serve as a point of attachment to the siNA molecule. In one embodiment, R3 and/or R1 comprises a conjugate moiety and a linker (e.g., a nucleotide or non-nucleotide linker as described herein or otherwise known in the art). Non-limiting examples of conjugate moieties include ligands for cellular receptors, such as peptides

derived from naturally occurring protein ligands; protein localization sequences, including cellular ZIP code sequences; antibodies; nucleic acid aptamers; vitamins and other co-factors, such as folate and N-acetylgalactosamine; polymers, such as polyethyleneglycol (PEG); phospholipids; cholesterol; steroids, and polyamines, such as PEI, spermine or spermidine.

[0121] By "ZIP code" sequences is meant, any peptide or protein sequence that is involved in cellular topogenic signaling mediated transport (see for example Ray et al., 2004, *Science,* 306(1501): 1505).

[0122] Each nucleotide within the double stranded siNA molecule can independently have a chemical modification comprising the structure of any of Formulae I-VIII. Thus, in one embodiment, one or more nucleotide positions of a siNA molecule of the invention comprises a chemical modification having structure of any of Formulae I-VII or any other modification herein. In one embodiment, each nucleotide position of a siNA molecule of the invention comprises a chemical modification having structure of any of Formulae I-VII or any other modification herein.

[0123] In one embodiment, one or more nucleotide positions of one or both strands of a double stranded siNA molecule of the invention comprises a chemical modification having structure of any of Formulae 1-VII or any other modification herein. In one embodiment, each nucleotide position of one or both strands of a double stranded siNA molecule of the invention comprises a chemical modification having structure of any of Formulae I-VII or any other modification herein.

[0124] In another embodiment, the invention features a compound having Formula VII, wherein R1 and R2 are hydroxyl (OH) groups, n=1, and R3 comprises O and is the point of attachment to the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both strands of a double-stranded siNA molecule of the invention or to a single-stranded siNA molecule of the invention. This modification is referred to herein as "glyceryl" (for example modification 6 in FIG. **10**).

[0125] In another embodiment, a chemically modified nucleoside or non-nucleoside (e.g. a moiety having any of Formula V, VI or VII) of the invention is at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of a siNA molecule of the invention. For example, chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) can be present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense strand, the sense strand, or both antisense and sense strands of the siNA molecule. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the terminal position of the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the two terminal positions of the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the penultimate position of the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In addition, a moiety having Formula VII can be present at the 3'-end or the 5'-end of a hairpin siNA molecule as described herein.

[0126] In another embodiment, a siNA molecule of the invention comprises an abasic residue having Formula V or VI, wherein the abasic residue having Formula VI or VI is connected to the siNA construct in a 3'-3',3'-2',2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

[0127] In one embodiment, a siNA molecule of the invention comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) locked nucleic acid (LNA) nucleotides, for example, at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

[0128] In one embodiment, a siNA molecule of the invention comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) 4'-thio nucleotides, for example, at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

[0129] In another embodiment, a siNA molecule of the invention comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) acyclic nucleotides, for example, at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

[0130] In one embodiment, a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprises a sense strand or sense region having one or more (e.g., 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 or more) 2'-O-alkyl (e.g. 2'-O-methyl), 2'-deoxy-2'-fluoro, 2'-deoxy, FANA, or abasic chemical modifications or any combination thereof.

[0131] In one embodiment, a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprises an antisense strand or antisense region having one or more (e.g., 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 or more) 2'-O-alkyl (e.g. 2'-O-methyl), 2'-deoxy-2'-fluoro, 2'-deoxy, FANA, or abasic chemical modifications or any combination thereof.

[0132] In one embodiment, a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprises a sense strand or sense region and an antisense strand or antisense region, each having one or more (e.g., 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 or more) 2'-O-alkyl (e.g. 2'-O-methyl), 2'-deoxy-2'-fluoro, 2'-deoxy, FANA, or abasic chemical modifications or any combination thereof.

[0133] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality (ie. more than one) of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides).

[0134] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the sense region are FANA pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are FANA pyrimidine nucleotides or

alternately a plurality (ie. more than one) of pyrimidine nucleotides are FANA pyrimidine nucleotides).

[0135] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality (ie. more than one) of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides).

[0136] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region and an antisense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the sense region and the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality (ie. more than one) of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides).

[0137] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (e.g., one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality (ie. more than one) of purine nucleotides are 2'-deoxy purine nucleotides).

[0138] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (e.g., one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality (ie. more than one) of pyrimidine nucleotides are 2'-O-methyl purine nucleotides).

[0139] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality (ie. more than one) of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality (ie. more than one) of purine nucleotides are 2'-deoxy purine nucleotides).

[0140] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides or alternately a plurality (ie. more than one) of pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality (ie. more than one) of purine nucleotides are 2'-deoxy purine nucleotides), wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said sense region are 2'-deoxy nucleotides.

[0141] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides or alternately a plurality (ie. more than one) of pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides or alternately a plurality (ie. more than one) of purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides).

[0142] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides or alternately a plurality (ie. more than one) of pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides), wherein any (e.g., one or more or all) purine nucleotides present in the sense region are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides or alternately a plurality (ie. more than one) of purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides), and wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said sense region are 2'-deoxy nucleotides.

[0143] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-

trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides or alternately a plurality (ie. more than one) of pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides or alternately a plurality (ie. more than one) of purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides).

[0144] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides or alternately a plurality (ie. more than one) of pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides), wherein any (e.g., one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides or alternately a plurality (ie. more than one) of purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides), and wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said antisense region are 2'-deoxy nucleotides.

[0145] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides or alternately a plurality (ie. more than one) of pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the antisense region are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality (ie. more than one) of purine nucleotides are 2'-deoxy purine nucleotides).

[0146] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides or alternately a plurality (ie. more than one) of pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides or alternately a plurality (ie. more than one) of purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides).

[0147] In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention capable of mediating RNA interference (RNAi) inside a cell or reconstituted in vitro system comprising a sense region, wherein one or more pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides or alternately a plurality (ie. more than one) of pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides), and one or more purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality (ie. more than one) of purine nucleotides are 2'-deoxy purine nucleotides), and an antisense region, wherein one or more pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides or alternately a plurality (ie. more than one) of pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides or alternately a plurality (ie. more than one) of purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides). The sense region and/or the antisense region can have a terminal cap modification, such as any modification described herein or shown in FIG. **10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends

of the sense and/or antisense sequence. The sense and/or antisense region can optionally further comprise a 3'-terminal nucleotide overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxynucleotides. The overhang nucleotides can further comprise one or more (e.g., about 1, 2, 3, 4 or more) phosphorothioate, phosphonoacetate, and/or thiophosphonoacetate internucleotide linkages. Non-limiting examples of these chemically-modified siNAs are shown in FIGS. **4** and **5** and Table III herein. In any of these described embodiments, the purine nucleotides present in the sense region are alternatively 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides) and one or more purine nucleotides present in the antisense region are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides or alternately a plurality (ie. more than one) of purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides). Also, in any of these embodiments, one or more purine nucleotides present in the sense region are alternatively purine ribonucleotides (e.g., wherein all purine nucleotides are purine ribonucleotides or alternately a plurality (ie. more than one) of purine nucleotides are purine ribonucleotides) and any purine nucleotides present in the antisense region are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides or alternately a plurality (ie. more than one) of purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides). Additionally, in any of these embodiments, one or more purine nucleotides present in the sense region and/or present in the antisense region are alternatively selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, 2'-O-trifluoromethyl nucleotides, 2'-O-ethyl-trifluoromethoxy nucleotides, 2'-O-difluoromethoxy-ethoxy nucleotides and 2'-O-methyl nucleotides (e.g., wherein all purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, 2'-O-trifluoromethyl nucleotides, 2'-O-ethyl-trifluoromethoxy nucleotides, 2'-O-difluoromethoxy-ethoxy nucleotides and 2'-O-methyl nucleotides or alternately a plurality (ie. more than one) of purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, 2'-O-trifluoromethyl nucleotides, 2'-O-ethyl-trifluoromethoxy nucleotides, 2'-O-difluoromethoxy-ethoxy nucleotides and 2'-O-methyl nucleotides).

[0148] In another embodiment, any modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984) otherwise known as a "ribo-like" or "A-form helix" configuration. As such, chemically modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, are resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi. Non-limiting examples of nucleotides having a northern configuration include locked nucleic acid (LNA) nucleotides (e.g., 2'-O, 4'-C-methylene-(D-ribofuranosyl) nucleotides); 2'-methoxyethoxy (MOE) nucleotides; 2'-methyl-thio-ethyl, 2'-deoxy-2'-fluoro nucleotides, 2'-deoxy-2'-chloro nucleotides, 2'-azido nucleotides, 2'-O-trifluoromethyl nucleotides, 2'-O-ethyl-trifluoromethoxy nucleotides, 2'-O-difluoromethoxy-ethoxy nucleotides, 4'-thio nucleotides and 2'-O-methyl nucleotides.

[0149] In one embodiment, the sense strand of a double stranded siNA molecule of the invention comprises a terminal cap moiety, (see for example FIG. **10**) such as an inverted deoxyabaisc moiety, at the 3'-end, 5'-end, or both 3' and 5'-ends of the sense strand.

[0150] In one embodiment, the invention features a chemically-modified short interfering nucleic acid molecule (siNA) capable of mediating RNA interference (RNAi) inside a cell or reconstituted in vitro system, wherein the chemical modification comprises a conjugate covalently attached to the chemically-modified siNA molecule. Non-limiting examples of conjugates contemplated by the invention include conjugates and ligands described in Vargeese et al., U.S. Ser. No. 10/427,160, filed Apr. 30, 2003, incorporated by reference herein in its entirety, including the drawings. In another embodiment, the conjugate is covalently attached to the chemically-modified siNA molecule via a biodegradable linker. In one embodiment, the conjugate molecule is attached at the 3'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In another embodiment, the conjugate molecule is attached at the 5'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In yet another embodiment, the conjugate molecule is attached both the 3'-end and 5'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule, or any combination thereof. In one embodiment, a conjugate molecule of the invention comprises a molecule that facilitates delivery of a chemically-modified siNA molecule into a biological system, such as a cell. In another embodiment, the conjugate molecule attached to the chemically-modified siNA molecule is a ligand for a cellular receptor, such as peptides derived from naturally occurring protein ligands; protein localization sequences, including cellular ZIP code sequences; antibodies; nucleic acid aptamers; vitamins and other co-factors, such as folate and N-acetylgalactosamine; polymers, such as polyethyleneglycol (PEG); phospholipids; cholesterol; steroids, and polyamines, such as PEI, spermine or spermidine. Examples of specific conjugate molecules contemplated by the instant

invention that can be attached to chemically-modified siNA molecules are described in Vargeese et al., U.S. Ser. No. 10/201,394, filed Jul. 22, 2002 incorporated by reference herein. The type of conjugates used and the extent of conjugation of siNA molecules of the invention can be evaluated for improved pharmacokinetic profiles, bioavailability, and/or stability of siNA constructs while at the same time maintaining the ability of the siNA to mediate RNAi activity. As such, one skilled in the art can screen siNA constructs that are modified with various conjugates to determine whether the siNA conjugate complex possesses improved properties while maintaining the ability to mediate RNAi, for example in animal models as are generally known in the art.

[0151] In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule of the invention, wherein the siNA further comprises a nucleotide, non-nucleotide, or mixed nucleotide/non-nucleotide linker that joins the sense region of the siNA to the antisense region of the siNA. In one embodiment, a nucleotide, non-nucleotide, or mixed nucleotide/non-nucleotide linker is used, for example, to attach a conjugate moiety to the siNA. In one embodiment, a nucleotide linker of the invention can be a linker of >2 nucleotides in length, for example about 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length. In another embodiment, the nucleotide linker can be a nucleic acid aptamer. By "aptamer" or "nucleic acid aptamer" as used herein is meant a nucleic acid molecule that binds specifically to a SREBP1 target molecule wherein the nucleic acid molecule has sequence that comprises a sequence recognized by the SREBP1 target molecule in its natural setting. Alternately, an aptamer can be a nucleic acid molecule that binds to a SREBP1 target molecule where the SREBP1 target molecule does not naturally bind to a nucleic acid. The SREBP1 target molecule can be any molecule of interest. For example, the aptamer can be used to bind to a ligand-binding domain of a protein, thereby preventing interaction of the naturally occurring ligand with the protein. This is a non-limiting example and those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art. (See, for example, Gold et al., 1995, *Annu. Rev. Biochem.,* 64, 763; Brody and Gold, 2000, *J. Biotechnol.,* 74, 5; Sun, 2000, *Curr. Opin. Mol. Ther.,* 2, 100; Kusser, 2000, *J. Biotechnol.,* 74, 27; Hermann and Patel, 2000, *Science,* 287, 820; and Jayasena, 1999, *Clinical Chemistry,* 45, 1628.)

[0152] In yet another embodiment, a non-nucleotide linker of the invention comprises abasic nucleotide, polyether, polyamine, polyamide, peptide, carbohydrate, lipid, polyhydrocarbon, or other polymeric compounds (e.g. polyethylene glycols such as those having between 2 and 100 ethylene glycol units). Specific examples include those described by Seela and Kaiser, *Nucleic Acids Res.* 1990, 18:6353 and *Nucleic Acids Res.* 1987, 15:3113; Cload and Schepartz, *J. Am. Chem. Soc.* 1991, 113:6324; Richardson and Schepartz, *J. Am. Chem. Soc.* 1991, 113:5109; Ma et al., *Nucleic Acids Res.* 1993, 21:2585 and *Biochemistry* 1993, 32:1751; Durand et al., *Nucleic Acids Res.* 1990, 18:6353; McCurdy et al., *Nucleosides & Nucleotides* 1991, 10:287; Jschke et al., *Tetrahedron Lett.* 1993, 34:301; Ono et al., *Biochemistry* 1991, 30:9914; Arnold et al., International Publication No. WO 89/02439; Usman et al., International Publication No. WO 95/06731; Dudycz et al., International Publication No. WO 95/11910 and Ferentz and Verdine, *J. Am. Chem. Soc.* 1991, 113:4000, all hereby incorporated by reference herein. A "non-nucleotide" further means any group or compound that can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound can be abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine, for example at the C1 position of the sugar.

[0153] In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) inside a cell or reconstituted in vitro system, wherein one or both strands of the siNA molecule that are assembled from two separate oligonucleotides do not comprise any ribonucleotides. For example, a siNA molecule can be assembled from a single oligonucleotide where the sense and antisense regions of the siNA comprise separate oligonucleotides that do not have any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotides. In another example, a siNA molecule can be assembled from a single oligonucleotide where the sense and antisense regions of the siNA are linked or circularized by a nucleotide or non-nucleotide linker as described herein, wherein the oligonucleotide does not have any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotide. Applicant has surprisingly found that the presence of ribonucleotides (e.g., nucleotides having a 2'-hydroxyl group) within the siNA molecule is not required or essential to support RNAi activity. As such, in one embodiment, all positions within the siNA can include chemically modified nucleotides and/or non-nucleotides such as nucleotides and or non-nucleotides having Formula I, II, III, IV, V, VI, or VII or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

[0154] In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system comprising a single stranded polynucleotide having complementarity to a SREBP1 target nucleic acid sequence. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group and a 3'-terminal phosphate group (e.g., a 2',3'-cyclic phosphate). In another embodiment, the single stranded siNA molecule of the invention comprises about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides. In yet another embodiment, the single stranded siNA molecule of the invention comprises one or more chemically modified nucleotides or non-nucleotides described herein. For example, all the positions within the siNA molecule can include chemically-modified nucleotides such as nucleotides having any of Formulae I-VII, or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

[0155] In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity or that alternately modulates RNAi activity in a cell or reconstituted in vitro system comprising a single stranded polynucleotide having complementarity to a SREBP1 target nucleic acid sequence, wherein one or more pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro,

4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, or 2'-O-difluoromethoxy-ethoxy purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in FIG. **10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence. The siNA optionally further comprises about 1 to about 4 or more (e.g., about 1, 2, 3, 4 or more) terminal 2'-deoxynucleotides at the 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, 4 or more) phosphorothioate, phosphonoacetate, and/or thiophosphonoacetate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group. In any of these embodiments, any purine nucleotides present in the antisense region are alternatively 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides). Also, in any of these embodiments, any purine nucleotides present in the siNA (i.e., purine nucleotides present in the sense and/or antisense region) can alternatively be locked nucleic acid (LNA) nucleotides (e.g., wherein all purine nucleotides are LNA nucleotides or alternately a plurality of purine nucleotides are LNA nucleotides). Also, in any of these embodiments, any purine nucleotides present in the siNA are alternatively 2'-methoxyethyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-methoxyethyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-methoxyethyl purine nucleotides). In another embodiment, any modified nucleotides present in the single stranded siNA molecules of the invention comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As such, chemically modified nucleotides present in the single stranded siNA molecules of the invention are preferably resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi.

[0156] In one embodiment, a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprises a sense strand or sense region having two or more (e.g., 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 or more) 2'-O-alkyl (e.g. 2'-O-methyl) modifications or any combination thereof. In another embodiment, the 2'-O-alkyl modification is at alternating position in the sense strand or sense region of the siNA, such as position 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 etc. or position 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 etc.

[0157] In one embodiment, a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprises an antisense strand or antisense region having two or more (e.g., 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 or more) 2'-O-alkyl (e.g. 2'-O-methyl) modifications or any combination thereof. In another embodiment, the 2'-O-alkyl modification is at alternating position in the antisense strand or antisense region of the siNA, such as position 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 etc. or position 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 etc.

[0158] In one embodiment, a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprises a sense strand or sense region and an antisense strand or antisense region, each having two or more (e.g., 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 or more) 2'-O-alkyl (e.g. 2'-O-methyl), 2'-deoxy-2'-fluoro, 2'-deoxy, or abasic chemical modifications or any combination thereof. In another embodiment, the 2'-O-alkyl modification is at alternating position in the sense strand or sense region of the siNA, such as position 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 etc. or position 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 etc. In another embodiment, the 2'-O-alkyl modification is at alternating position in the antisense strand or antisense region of the siNA, such as position 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 etc. or position 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 etc.

[0159] In one embodiment, a siNA molecule of the invention comprises chemically modified nucleotides or non-nucleotides (e.g., having any of Formulae I-VII, such as 2'-deoxy, 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, 2'-O-difluoromethoxy-ethoxy or 2'-O-methyl nucleotides) at alternating positions within one or more strands or regions of the siNA molecule. For example, such chemical modifications can be introduced at every other position of a RNA based siNA molecule, starting at either the first or second nucleotide from the 3'-end or 5'-end of the siNA. In a non-limiting example, a double stranded siNA molecule of the invention in which each strand of the siNA is 21 nucleotides in length is featured wherein positions 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21 of each strand are chemically modified (e.g., with compounds having any of Formulae I-VII, such as such as 2'-deoxy, 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, 2'-O-difluoromethoxy-ethoxy or 2'-O-methyl nucleotides). In another non-limiting example, a double stranded siNA molecule of the invention in which each strand of the siNA is 21 nucleotides in length is featured wherein positions 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 of each strand are chemically modified (e.g., with compounds having any of Formulae I-VII, such as such as 2'-deoxy, 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, 2'-O-difluoromethoxy-ethoxy or 2'-O-methyl nucleotides). In one embodiment, one strand of the double stranded siNA molecule comprises chemical modifications at positions 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 and chemical modifications at positions 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21. Such siNA molecules can further comprise terminal cap moieties and/or backbone modifications as described herein.

[0160] In one embodiment, a siNA molecule of the invention comprises the following features: if purine nucleotides are present at the 5'-end (e.g., at any of terminal nucleotide positions 1, 2, 3, 4, 5, or 6 from the 5'-end) of the antisense strand or antisense region (otherwise referred to as the guide

sequence or guide strand) of the siNA molecule then such purine nucleosides are ribonucleotides. In another embodiment, the purine ribonucleotides, when present, are base paired to nucleotides of the sense strand or sense region (otherwise referred to as the passenger strand) of the siNA molecule. Such purine ribonucleotides can be present in a siNA stabilization motif that otherwise comprises modified nucleotides.

[0161] In one embodiment, a siNA molecule of the invention comprises the following features: if pyrimidine nucleotides are present at the 5'-end (e.g., at any of terminal nucleotide positions 1, 2, 3, 4, 5, or 6 from the 5'-end) of the antisense strand or antisense region (otherwise referred to as the guide sequence or guide strand) of the siNA molecule then such pyrimidine nucleosides are ribonucleotides. In another embodiment, the pyrimidine ribonucleotides, when present, are base paired to nucleotides of the sense strand or sense region (otherwise referred to as the passenger strand) of the siNA molecule. Such pyrimidine ribonucleotides can be present in a siNA stabilization motif that otherwise comprises modified nucleotides.

[0162] In one embodiment, a siNA molecule of the invention comprises the following features: if pyrimidine nucleotides are present at the 5'-end (e.g., at any of terminal nucleotide positions 1, 2, 3, 4, 5, or 6 from the 5'-end) of the antisense strand or antisense region (otherwise referred to as the guide sequence or guide strand) of the siNA molecule then such pyrimidine nucleosides are modified nucleotides. In another embodiment, the modified pyrimidine nucleotides, when present, are base paired to nucleotides of the sense strand or sense region (otherwise referred to as the passenger strand) of the siNA molecule. Non-limiting examples of modified pyrimidine nucleotides include those having any of Formulae I-VII, such as such as 2'-deoxy, 2'-deoxy-2'-fluoro, 4'-thio, 2'-O-trifluoromethyl, 2'-O-ethyl-trifluoromethoxy, 2'-O-difluoromethoxy-ethoxy or 2'-O-methyl nucleotides.

[0163] In one embodiment, the invention features a double stranded nucleic acid molecule having structure SI:

$$B\text{—}N_{X3}\text{—}(N)_{X2}\ B\text{-}3' \quad B(N)_{X1}\text{—}N_{X4}\text{—}[N]_{X5}\text{-}5' \qquad \text{SI}$$

[0164] wherein each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides which can be unmodified or chemically modified; [N] represents nucleotide positions wherein any purine nucleotides when present are ribonucleotides; X1 and X2 are independently integers from about 0 to about 4; X3 is an integer from about 9 to about 30; X4 is an integer from about 11 to about 30, provided that the sum of X4 and X5 is between 17-36; X5 is an integer from about 1 to about 6; NX3 is complementary to NX4 and NX5, and

[0165] (a) any pyrimidine nucleotides present in the antisense strand (lower strand) are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the antisense strand (lower strand) other than the purines nucleotides in the [N] nucleotide positions, are independently 2'-O-methyl nucleotides, 2'-deoxyribonucleotides or a combination of 2'-deoxyribonucleotides and 2'-O-methyl nucleotides;

[0166] (b) any pyrimidine nucleotides present in the sense strand (upper strand) are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the sense strand (upper strand) are independently 2'-deoxyribonucleotides, 2'-O-methyl nucleotides or a combination of 2'-deoxyribonucleotides and 2'-O-methyl nucleotides; and

[0167] (c) any (N) nucleotides are optionally 2'-O-methyl, 2'-deoxy-2'-fluoro, or deoxyribonucleotides.

[0168] In one embodiment, the invention features a double stranded nucleic acid molecule having structure SII:

$$\begin{array}{l} \qquad\qquad B\text{-}N_{X3}\text{—}(N)_{X2}B\text{-}3' \\ B(N)_{X1}\text{—}N_{X4}\text{–}[N]_{X5}\text{-}5' \end{array} \qquad \text{SII}$$

[0169] wherein each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides which can be unmodified or chemically modified; [N] represents nucleotide positions wherein any purine nucleotides when present are ribonucleotides; X1 and X2 are independently integers from about 0 to about 4; X3 is an integer from about 9 to about 30; X4 is an integer from about 11 to about 30, provided that the sum of X4 and X5 is between 17-36; X5 is an integer from about 1 to about 6; NX3 is complementary to NX4 and NX5, and

[0170] (a) any pyrimidine nucleotides present in the antisense strand (lower strand) are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the antisense strand (lower strand) other than the purines nucleotides in the [N] nucleotide positions, are 2'-O-methyl nucleotides;

[0171] (b) any pyrimidine nucleotides present in the sense strand (upper strand) are ribonucleotides; any purine nucleotides present in the sense strand (upper strand) are ribonucleotides; and

[0172] (c) any (N) nucleotides are optionally 2'-O-methyl, 2'-deoxy-2'-fluoro, or deoxyribonucleotides.

[0173] In one embodiment, the invention features a double stranded nucleic acid molecule having structure SIII:

$$\begin{array}{l} \qquad\qquad B\text{-}N_{X3}\text{—}(N)_{X2}B\text{-}3' \\ B(N)_{X1}\text{—}N_{X4}\text{–}[N]_{X5}\text{-}5' \end{array} \qquad \text{SIII}$$

[0174] wherein each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides which can be unmodified or chemically modified; [N] represents nucleotide positions wherein any purine nucleotides when present are ribonucleotides; X1 and X2 are independently integers from about 0 to about 4; X3 is an integer from about 9 to about 30; X4 is an integer from about 11 to about 30, provided that the sum of X4 and X5 is between 17-36; X5 is an integer from about 1 to about 6; NX3 is complementary to NX4 and NX5, and

[0175] (a) any pyrimidine nucleotides present in the antisense strand (lower strand) are 2'-deoxy-2'-fluoro nucle-

otides; any purine nucleotides present in the antisense strand (lower strand) other than the purines nucleotides in the [N] nucleotide positions, are 2'-O-methyl nucleotides;

[0176] (b) any pyrimidine nucleotides present in the sense strand (upper strand) are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the sense strand (upper strand) are ribonucleotides; and

[0177] (c) any (N) nucleotides are optionally 2'-O-methyl, 2'-deoxy-2'-fluoro, or deoxyribonucleotides.

[0178] In one embodiment, the invention features a double stranded nucleic acid molecule having structure SIV:

SIV

$$B—N_{X3}—(N)_{X2}\ B\text{-}3' \quad B(N)_{X1}—N_{X4}—[N]_{X5}\text{-}5'$$

[0179] wherein each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides which can be unmodified or chemically modified; [N] represents nucleotide positions wherein any purine nucleotides when present are ribonucleotides; X1 and X2 are independently integers from about 0 to about 4; X3 is an integer from about 9 to about 30; X4 is an integer from about 11 to about 30, provided that the sum of X4 and X5 is between 17-36; X5 is an integer from about 1 to about 6; NX3 is complementary to NX4 and NX5, and

[0180] (a) any pyrimidine nucleotides present in the antisense strand (lower strand) are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the antisense strand (lower strand) other than the purines nucleotides in the [N] nucleotide positions, are 2'-O-methyl nucleotides;

[0181] (b) any pyrimidine nucleotides present in the sense strand (upper strand) are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the sense strand (upper strand) are deoxyribonucleotides; and

[0182] (c) any (N) nucleotides are optionally 2'-O-methyl, 2'-deoxy-2'-fluoro, or deoxyribonucleotides.

[0183] In one embodiment, the invention features a double stranded nucleic acid molecule having structure SV:

SV

$$B—N_{X3}—(N)_{X2}\ B\text{-}3'$$
$$B\ (N)_{X1}—N_{X4}—[N]_{X5}\text{-}5'$$

[0184] wherein each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides which can be unmodified or chemically modified; [N] represents nucleotide positions wherein any purine nucleotides when present are ribonucleotides; X1 and X2 are independently integers from about 0 to about 4; X3 is an integer from about 9 to about 30; X4 is an integer from about 11 to about 30, provided that the sum of X4 and X5 is between 17-36; X5 is an integer from about 1 to about 6; NX3 is complementary to NX4 and NX5, and

[0185] (a) any pyrimidine nucleotides present in the antisense strand (lower strand) are nucleotides having a ribo-like configuration (e.g., Northern or A-form helix configuration); any purine nucleotides present in the antisense strand (lower strand) other than the purines nucleotides in the [N] nucleotide positions, are 2'-O-methyl nucleotides;

[0186] (b) any pyrimidine nucleotides present in the sense strand (upper strand) are nucleotides having a ribo-like configuration (e.g., Northern or A-form helix configuration); any purine nucleotides present in the sense strand (upper strand) are 2'-O-methyl nucleotides; and

[0187] (c) any (N) nucleotides are optionally 2'-O-methyl, 2'-deoxy-2'-fluoro, or deoxyribonucleotides.

[0188] In one embodiment, the invention features a double stranded nucleic acid molecule having structure SVI:

SVI

$$B—N_{X3}—(N)_{X2}\ B\text{-}3'$$
$$B\ (N)_{X1}—N_{X4}—[N]_{X5}\text{-}5'$$

[0189] wherein each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides which can be unmodified or chemically modified; [N] represents nucleotide positions comprising sequence that renders the 5'-end of the antisense strand (lower strand) less thermally stable than the 5'-end of the sense strand (upper strand); X1 and X2 are independently integers from about 0 to about 4; X3 is an integer from about 9 to about 30; X4 is an integer from about 11 to about 30, provided that the sum of X4 and X5 is between 17-36; X5 is an integer from about 1 to about 6; NX3 is complementary to NX4 and NX5, and

[0190] (a) any pyrimidine nucleotides present in the antisense strand (lower strand) are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the antisense strand (lower strand) other than the purines nucleotides in the [N] nucleotide positions, are independently 2'-O-methyl nucleotides, 2'-deoxyribonucleotides or a combination of 2'-deoxyribonucleotides and 2'-O-methyl nucleotides;

[0191] (b) any pyrimidine nucleotides present in the sense strand (upper strand) are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the sense strand (upper strand) are independently 2'-deoxyribonucleotides, 2'-O-methyl nucleotides or a combination of 2'-deoxyribonucleotides and 2'-O-methyl nucleotides; and

[0192] (c) any (N) nucleotides are optionally 2'-O-methyl, 2'-deoxy-2'-fluoro, or deoxyribonucleotides.

[0193] In one embodiment, the invention features a double stranded nucleic acid molecule having structure SVII:

SVII

$$B\text{-}N_{X3}—(N)_{X2}\ B\text{-}3'$$
$$B\ (N)_{X1}—N_{X4}\text{-}5'$$

[0194] wherein each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides; X1 and X2 are independently integers from

about 0 to about 4; X3 is an integer from about 9 to about 30; X4 is an integer from about 11 to about 30; NX3 is complementary to NX4, and any (N) nucleotides are 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides.

[0195] In one embodiment, the invention features a double stranded nucleic acid molecule having structure SVIII:

SVIII

$$\begin{array}{l} B\text{—}N_{X7}\text{—}[N]_{X6}\text{—}N_{X3}\text{—}(N)_{X2}\ B\text{-}3' \\ B\ (N)_{X1}\text{—}N_{X4}\text{—}[N]_{X5}\text{-}5' \end{array}$$

[0196] wherein each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides which can be unmodified or chemically modified; [N] represents nucleotide positions comprising sequence that renders the 5'-end of the antisense strand (lower strand) less thermally stable than the 5'-end of the sense strand (upper strand); [N] represents nucleotide positions that are ribonucleotides; X1 and X2 are independently integers from about 0 to about 4; X3 is an integer from about 9 to about 15; X4 is an integer from about 11 to about 30, provided that the sum of X4 and X5 is between 17-36; X5 is an integer from about 1 to about 6; X6 is an integer from about 1 to about 4; X7 is an integer from about 9 to about 15; NX7, NX6, and NX3 are complementary to NX4 and NX5, and

[0197] (a) any pyrimidine nucleotides present in the antisense strand (lower strand) are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the antisense strand (lower strand) other than the purines nucleotides in the [N] nucleotide positions, are independently 2'-O-methyl nucleotides, 2'-deoxyribonucleotides or a combination of 2'-deoxyribonucleotides and 2'-O-methyl nucleotides;

[0198] (b) any pyrimidine nucleotides present in the sense strand (upper strand) are 2'-deoxy-2'-fluoro nucleotides other than [N] nucleotides; any purine nucleotides present in the sense strand (upper strand) are independently 2'-deoxyribonucleotides, 2'-O-methyl nucleotides or a combination of 2'-deoxyribonucleotides and 2'-O-methyl nucleotides other than [N] nucleotides; and

[0199] (c) any (N) nucleotides are optionally 2'-O-methyl, 2'-deoxy-2'-fluoro, or deoxyribonucleotides.

[0200] In one embodiment, the invention features a double stranded nucleic acid molecule having structure SIX:

SIX

$$\begin{array}{l} B\text{—}N_{X3}\text{—}(N)_{X2}\ B\text{-}3' \\ B\ (N)_{X1}\text{—}N_{X4}\text{—}[N]_{X5}\text{-}5' \end{array}$$

[0201] wherein each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides which can be unmodified or chemically modified; [N] represents nucleotide positions that are ribonucleotides; X1 and X2 are independently integers from about 0 to about 4; X3 is an integer from about 9 to about 30; X4 is an integer from about 11 to about 30, provided that the sum of X4 and X5 is between 17-36; X5 is an integer from about 1 to about 6; NX3 is complementary to NX4 and NX5, and

[0202] (a) any pyrimidine nucleotides present in the antisense strand (lower strand) are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the antisense strand (lower strand) other than the purines nucleotides in the [N] nucleotide positions, are independently 2'-O-methyl nucleotides, 2'-deoxyribonucleotides or a combination of 2'-deoxyribonucleotides and 2'-O-methyl nucleotides;

[0203] (b) any pyrimidine nucleotides present in the sense strand (upper strand) are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the sense strand (upper strand) are independently 2'-deoxyribonucleotides, 2'-O-methyl nucleotides or a combination of 2'-deoxyribonucleotides and 2'-O-methyl nucleotides; and

[0204] (c) any (N) nucleotides are optionally 2'-O-methyl, 2'-deoxy-2'-fluoro, or deoxyribonucleotides.

[0205] In one embodiment, the invention features a double stranded nucleic acid molecule having structure SX:

SX

$$\begin{array}{l} B\text{—}N_{X3}\text{—}(N)_{X2}\ B\text{-}3' \\ B\ (N)_{X1}\text{—}N_{X4}\text{—}[N]_{X5}\text{-}5' \end{array}$$

[0206] wherein each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides which can be unmodified or chemically modified; [N] represents nucleotide positions that are ribonucleotides; X1 and X2 are independently integers from about 0 to about 4; X3 is an integer from about 9 to about 30; X4 is an integer from about 11 to about 30, provided that the sum of X4 and X5 is between 17-36; X5 is an integer from about 1 to about 6; NX3 is complementary to NX4 and NX5, and

[0207] (a) any pyrimidine nucleotides present in the antisense strand (lower strand) are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the antisense strand (lower strand) other than the purines nucleotides in the [N] nucleotide positions, are 2'-O-methyl nucleotides;

[0208] (b) any pyrimidine nucleotides present in the sense strand (upper strand) are ribonucleotides; any purine nucleotides present in the sense strand (upper strand) are ribonucleotides; and

[0209] (c) any (N) nucleotides are optionally 2'-O-methyl, 2'-deoxy-2'-fluoro, or deoxyribonucleotides.

[0210] In one embodiment, the invention features a double stranded nucleic acid molecule having structure SXI:

SXI

$$\begin{array}{l} B\text{—}N_{X3}\text{—}(N)_{X2}\ B\text{-}3' \\ B\ (N)_{X1}\text{—}N_{X4}\text{—}[N]_{X5}\text{-}5' \end{array}$$

[0211] wherein each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides which can be unmodified or chemically modified; [N] represents nucleotide positions that are

ribonucleotides; X1 and X2 are independently integers from about 0 to about 4; X3 is an integer from about 9 to about 30; X4 is an integer from about 11 to about 30, provided that the sum of X4 and X5 is between 17-36; X5 is an integer from about 1 to about 6; NX3 is complementary to NX4 and NX5, and

[0212] (a) any pyrimidine nucleotides present in the antisense strand (lower strand) are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the antisense strand (lower strand) other than the purines nucleotides in the [N] nucleotide positions, are 2'-O-methyl nucleotides;

[0213] (b) any pyrimidine nucleotides present in the sense strand (upper strand) are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the sense strand (upper strand) are ribonucleotides; and

[0214] (c) any (N) nucleotides are optionally 2'-O-methyl, 2'-deoxy-2'-fluoro, or deoxyribonucleotides.

[0215] In one embodiment, the invention features a double stranded nucleic acid molecule having structure SXII:

SXII

$$\begin{array}{l} B\text{—}N_{X3}\text{—}(N)_{X2}\ B\text{-}3' \\ B\ (N)_{X1}\text{—}N_{X4}\text{—}[N]_{X5}\text{-}5' \end{array}$$

[0216] wherein each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides which can be unmodified or chemically modified; [N] represents nucleotide positions that are ribonucleotides; X1 and X2 are independently integers from about 0 to about 4; X3 is an integer from about 9 to about 30; X4 is an integer from about 11 to about 30, provided that the sum of X4 and X5 is between 17-36; X5 is an integer from about 1 to about 6; NX3 is complementary to NX4 and NX5, and

[0217] (a) any pyrimidine nucleotides present in the antisense strand (lower strand) are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the antisense strand (lower strand) other than the purines nucleotides in the [N] nucleotide positions, are 2'-O-methyl nucleotides;

[0218] (b) any pyrimidine nucleotides present in the sense strand (upper strand) are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the sense strand (upper strand) are deoxyribonucleotides; and

[0219] (c) any (N) nucleotides are optionally 2'-O-methyl, 2'-deoxy-2'-fluoro, or deoxyribonucleotides.

[0220] In one embodiment, the invention features a double stranded nucleic acid molecule having structure SXIII:

SXIII

$$\begin{array}{l} B\text{—}N_{X3}\text{—}(N)_{X2}\ B\text{-}3' \\ B\ (N)_{X1}\text{—}N_{X4}\text{—}[N]_{X5}\text{-}5' \end{array}$$

[0221] wherein each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides which can be unmodified or chemically modified; [N] represents nucleotide positions that are ribonucleotides; X1 and X2 are independently integers from about 0 to about 4; X3 is an integer from about 9 to about 30; X4 is an integer from about 11 to about 30, provided that the sum of X4 and X5 is between 17-36; X5 is an integer from about 1 to about 6; NX3 is complementary to NX4 and NX5, and

[0222] (a) any pyrimidine nucleotides present in the antisense strand (lower strand) are nucleotides having a ribo-like configuration (e.g., Northern or A-form helix configuration); any purine nucleotides present in the antisense strand (lower strand) other than the purines nucleotides in the [N] nucleotide positions, are 2'-O-methyl nucleotides;

[0223] (b) any pyrimidine nucleotides present in the sense strand (upper strand) are nucleotides having a ribo-like configuration (e.g., Northern or A-form helix configuration); any purine nucleotides present in the sense strand (upper strand) are 2'-O-methyl nucleotides; and

[0224] (c) any (N) nucleotides are optionally 2'-O-methyl, 2'-deoxy-2'-fluoro, or deoxyribonucleotides.

[0225] In one embodiment, the invention features a double stranded nucleic acid molecule having structure SXIV:

SXIV

$$\begin{array}{l} B\text{—}N_{X7}\text{—}[N]_{X6}\text{—}N_{X3}\text{—}(N)_{X2}\ B\text{-}3' \\ \qquad\qquad\ \ |\ \ \ \

[0231] In one embodiment, a double stranded nucleic acid molecule having any of structure SI, SII, SIII, SIV, SV, SVI, SVII, SVIII, SIX, SX, SXII, SXIII, or SXIV comprises X5=1, 2, or 3; each X1 and X2=1 or 2; X3=12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, and X4=15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30.

[0232] In one embodiment, a double stranded nucleic acid molecule having any of structure SI, SII, SIII, SIV, SV, SVI, SVII, SVIII, SIX, SX, SXII, SXIII, or SXIV comprises X5=1; each X1 and X2=2; X3=19, and X4=18.

[0233] In one embodiment, a double stranded nucleic acid molecule having any of structure SI, SII, SIII, SIV, SV, SVI, SVII, SVIII, SIX, SX, SXII, SXIII, or SXIV comprises X5=2; each X1 and X2=2; X3=19, and X4=17

[0234] In one embodiment, a double stranded nucleic acid molecule having any of structure SI, SII, SIII, SIV, SV, SVI, SVII, SVIII, SIX, SX, SXII, SXIII, or SXIV comprises X5=3; each X1 and X2=2; X3=19, and X4=16.

[0235] In one embodiment, a double stranded nucleic acid molecule having any of structure SI, SII, SIII, SIV, SV, SVI, SVII, SVIII, SIX, SX, SXII, SXIII, or SXIV comprises B at the 3' and 5' ends of the sense strand or sense region.

[0236] In one embodiment, a double stranded nucleic acid molecule having any of structure SI, SII, SIII, SIV, SV, SVI, SVII, SVIII, SIX, SX, SXII, SXIII, or SXIV comprises B at the 3'-end of the antisense strand or antisense region.

[0237] In one embodiment, a double stranded nucleic acid molecule having any of structure SI, SII, SIII, SIV, SV, SVI, SVII, SVIII, SIX, SX, SXII, SXIII, or SXIV comprises B at the 3' and 5' ends of the sense strand or sense region and B at the 3'-end of the antisense strand or antisense region.

[0238] In one embodiment, a double stranded nucleic acid molecule having any of structure SI, SII, SIII, SIV, SV, SVI, SVII, SVIII, SIX, SX, SXII, SXIII, or SXIV further comprises one or more phosphorothioate internucleotide linkages at the first terminal (N) on the 3' end of the sense strand, antisense strand, or both sense strand and antisense strands of the nucleic acid molecule. For example, a double stranded nucleic acid molecule can comprise X1 and/or X2=2 having overhanging nucleotide positions with a phosphorothioate internucleotide linkage, e.g., (NsN) where "s" indicates phosphorothioate.

[0239] In one embodiment, a double stranded nucleic acid molecule having any of structure SI, SII, SIII, SIV, SV, SVI, SVII, SVIII, SIX, SX, SXII, SXIII, or SXIV comprises (N) nucleotides that are 2'-O-methyl nucleotides.

[0240] In one embodiment, a double stranded nucleic acid molecule having any of structure SI, SII, SIII, SIV, SV, SVI, SVII, SVIII, SIX, SX, SXII, SXIII, or SXIV comprises (N) nucleotides that are 2'-O-methyl nucleotides.

[0241] In one embodiment, a double stranded nucleic acid molecule having any of structure SI, SII, SIII, SIV, SV, SVI, SVII, SVIII, SIX, SX, SXII, SXIII, or SXIV comprises (N) nucleotides in the antisense strand (lower strand) that are complementary to nucleotides in a SREBP1 target polynucleotide sequence having complementary to the N and [N] nucleotides of the antisense (lower) strand.

[0242] In one embodiment, a double stranded nucleic acid molecule having any of structure SI, SII, SIII, SIV, SV, SVI, SVII, SVIII, SIX, SX, SXII, SXIII, or SXIV comprises (N) nucleotides in the sense strand (upper strand) that comprise a contiguous nucleotide sequence of about 15 to about 30 nucleotides of a SREBP1 target polynucleotide sequence.

[0243] In one embodiment, a double stranded nucleic acid molecule having any of structure SI, SII, SIII, SIV, SV, SVI, SVII, SVIII, SIX, SX, SXII, SXIII, or SXIV comprises (N) nucleotides in the sense strand (upper strand) that comprise nucleotide sequence corresponding a SREBP1 target polynucleotide sequence having complementary to the antisense (lower) strand such that the contiguous (N) and N nucleotide sequence of the sense strand comprises nucleotide sequence of the SREBP1 target nucleic acid sequence.

[0244] In one embodiment, a double stranded nucleic acid molecule having any of structure SVIII or SXIV comprises B only at the 5'-end of the sense (upper) strand of the double stranded nucleic acid molecule.

[0245] In one embodiment, a double stranded nucleic acid molecule having any of structure SI, SII, SIII, SIV, SV, SVI, SVII, SVIII, SIX, SX, SXII, SXIII, or SXIV further comprises an unpaired terminal nucleotide at the 5'-end of the antisense (lower) strand. The unpaired nucleotide is not complementary to the sense (upper) strand. In one embodiment, the unpaired terminal nucleotide is complementary to a SREBP1 target polynucleotide sequence having complementary to the N and [N] nucleotides of the antisense (lower) strand. In another embodiment, the unpaired terminal nucleotide is not complementary to a SREBP1 target polynucleotide sequence having complementary to the N and [N] nucleotides of the antisense (lower) strand.

[0246] In one embodiment, a double stranded nucleic acid molecule having any of structure SVIII or SXIV comprises X6=1 and X3=10.

[0247] In one embodiment, a double stranded nucleic acid molecule having any of structure SVIII or SXIV comprises X6=2 and X3=9.

[0248] In one embodiment, the invention features a composition comprising a siNA molecule or double stranded nucleic acid molecule or RNAi inhibitor formulated as any of formulation LNP-051; LNP-053; LNP-054; LNP-069; LNP-073; LNP-077; LNP-080; LNP-082; LNP-083; LNP-060; LNP-061; LNP-086; LNP-097; LNP-098; LNP-099; LNP-100; LNP-101; LNP-102; LNP-103; or LNP-104 (see Table VI).

[0249] In one embodiment, the invention features a composition comprising a first double stranded nucleic and a second double stranded nucleic acid molecule each having a first strand and a second strand that are complementary to each other, wherein the second strand of the first double stranded nucleic acid molecule comprises sequence complementary to a first SREBP1 target sequence and the second strand of the second double stranded nucleic acid molecule comprises sequence complementary to a second SREBP1 target or SREBP1 pathway target sequence (e.g., PCSK9). In one embodiment, the composition further comprises a cationic lipid, a neutral lipid, and a polyethyleneglycol-conjugate. In one embodiment, the composition further comprises a cationic lipid, a neutral lipid, a polyethyleneglycol-conjugate, and a cholesterol. In one embodiment, the composition further comprises a polyethyleneglycol-conjugate, a cholesterol, and a surfactant. In one embodiment, the cationic lipid is selected from the group consisting of CLinDMA, pCLinDMA, eCLinDMA, DMOBA, and DMLBA. In one embodiment, the neutral lipid is selected from the group consisting of DSPC, DOBA, and cholesterol. In one embodiment, the polyethyleneglycol-conjugate is selected from the

group consisting of a PEG-dimyristoyl glycerol and PEG-cholesterol. In one embodiment, the PEG is 2 KPEG. In one embodiment, the surfactant is selected from the group consisting of palmityl alcohol, stearyl alcohol, oleyl alcohol and linoleyl alcohol. In one embodiment, the cationic lipid is CLinDMA, the neutral lipid is DSPC, the polyethylene glycol conjugate is 2 KPEG-DMG, the cholesterol is cholesterol, and the surfactant is linoleyl alcohol. In one embodiment, the CLinDMA, the DSPC, the 2 KPEG-DMG, the cholesterol, and the linoleyl alcohol are present in molar ratio of 43:38:10:2:7 respectively.

[0250] In any of the embodiments herein, the siNA molecule of the invention modulates expression of one or more SREBP1 targets via RNA interference or the inhibition of RNA interference. In one embodiment, the RNA interference is RISC mediated cleavage of the SREBP1 target (e.g., siRNA mediated RNA interference). In one embodiment, the RNA interference is translational inhibition of the SREBP1 target (e.g., miRNA mediated RNA interference). In one embodiment, the RNA interference is transcriptional inhibition of the SREBP1 target (e.g., siRNA mediated transcriptional silencing). In one embodiment, the RNA interference takes place in the cytoplasm. In one embodiment, the RNA interference takes place in the nucleus.

[0251] In any of the embodiments herein, the siNA molecule of the invention modulates expression of one or more SREBP1 targets via inhibition of an endogenous SREBP1 RNA, such as an endogenous SREBP1 mRNA, SREBP1 siRNA, SREBP1 miRNA, or alternately though inhibition of RISC.

[0252] In one embodiment, the invention features one or more RNAi inhibitors that modulate the expression of one or more SREBP1 gene targets by miRNA inhibition, siRNA inhibition, or RISC inhibition.

[0253] In one embodiment, a RNAi inhibitor of the invention is a siNA molecule as described herein that has one or more strands that are complementary to one or more target miRNA or siRNA molecules.

[0254] In one embodiment, the RNAi inhibitor of the invention is an antisense molecule that is complementary to a target miRNA or siRNA molecule or a portion thereof. An antisense RNAi inhibitor of the invention can be of length of about 10 to about 40 nucleotides in length (e.g., 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, or 40 nucleotides in length). An antisense RNAi inhibitor of the invention can comprise one or more modified nucleotides or non-nucleotides as described herein (see for example molecules having any of Formulae I-VII herein or any combination thereof). In one embodiment, an antisense RNAi inhibitor of the invention can comprise one or more or all 2'-O-methyl nucleotides. In one embodiment, an antisense RNAi inhibitor of the invention can comprise one or more or all 2'-deoxy-2'-fluoro nucleotides. In one embodiment, an antisense RNAi inhibitor of the invention can comprise one or more or all 2'-O-methoxy-ethyl (also known as 2'-methoxyethoxy or MOE) nucleotides. In one embodiment, an antisense RNAi inhibitor of the invention can comprise one or more or all phosphorothioate internucleotide linkages. In one embodiment, an antisense RNA inhibitor or the invention can comprise a terminal cap moiety at the 3'-end, the 5;'-end, or both the 5' and 3' ends of the antisense RNA inhibitor.

[0255] In one embodiment, a RNAi inhibitor of the invention is a nucleic acid aptamer having binding affinity for RISC, such as a regulatable aptamer (see for example An et al., 2006, *RNA,* 12:710-716). An aptamer RNAi inhibitor of the invention can be of length of about 10 to about 50 nucleotides in length (e.g., 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, or 50 nucleotides in length). An aptamer RNAi inhibitor of the invention can comprise one or more modified nucleotides or non-nucleotides as described herein (see for example molecules having any of Formulae I-VII herein or any combination thereof). In one embodiment, an aptamer RNAi inhibitor of the invention can comprise one or more or all 2'-O-methyl nucleotides. In one embodiment, an aptamer RNAi inhibitor of the invention can comprise one or more or all 2'-deoxy-2'-fluoro nucleotides. In one embodiment, an aptamer RNAi inhibitor of the invention can comprise one or more or all 2'-O-methoxy-ethyl (also known as 2'-methoxyethoxy or MOE) nucleotides. In one embodiment, an aptamer RNAi inhibitor of the invention can comprise one or more or all phosphorothioate internucleotide linkages. In one embodiment, an aptamer RNA inhibitor or the invention can comprise a terminal cap moiety at the 3'-end, the 5;'-end, or both the 5' and 3' ends of the aptamer RNA inhibitor.

[0256] In one embodiment, the invention features a method for modulating the expression of a SREBP1 target gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified or unmodified, wherein one of the siNA strands comprises a sequence complementary to RNA of the SREBP1 target gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target gene in the cell.

[0257] In one embodiment, the invention features a method for modulating the expression of a SREBP1 target gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified or unmodified, wherein one of the siNA strands comprises a sequence complementary to RNA of the SREBP1 target gene and wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the sequence of the SREBP1 target RNA; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target gene in the cell.

[0258] In another embodiment, the invention features a method for modulating the expression of more than one SREBP1 target gene within a cell comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified or unmodified, wherein one of the siNA strands comprises a sequence complementary to RNA of the SREBP1 target genes; and (b) introducing the siNA molecules into a cell under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target genes in the cell.

[0259] In another embodiment, the invention features a method for modulating the expression of two or more SREBP1 target genes within a cell comprising: (a) synthesizing one or more siNA molecules of the invention, which can be chemically-modified or unmodified, wherein the siNA strands comprise sequences complementary to RNA of the SREBP1 target genes and wherein the sense strand sequences of the siNAs comprise sequences identical or substantially similar to the sequences of the SREBP1 target RNAs; and (b) introducing the siNA molecules into a cell under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target genes in the cell.

[0260] In another embodiment, the invention features a method for modulating the expression of more than one SREBP1 target gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified or unmodified, wherein one of the siNA strands comprises a sequence complementary to RNA of the SREBP1 target gene and wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the sequences of the SREBP1 target RNAs; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target genes in the cell.

[0261] In another embodiment, the invention features a method for modulating the expression of a SREBP1 target gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified or unmodified, wherein one of the siNA strands comprises a sequence complementary to RNA of the SREBP1 target gene, wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the sequences of the SREBP1 target RNA; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target gene in the cell.

[0262] In one embodiment, siNA molecules of the invention are used as reagents in ex vivo applications. For example, siNA reagents are introduced into tissue or cells that are transplanted into a subject for therapeutic effect. The cells and/or tissue can be derived from an organism or subject that later receives the explant, or can be derived from another organism or subject prior to transplantation. The siNA molecules can be used to modulate the expression of one or more genes in the cells or tissue, such that the cells or tissue obtain a desired phenotype or are able to perform a function when transplanted in vivo. In one embodiment, certain SREBP1 target cells from a patient are extracted. These extracted cells are contacted with siNAs SREBP1 targeting a specific nucleotide sequence within the cells under conditions suitable for uptake of the siNAs by these cells (e.g. using delivery reagents such as cationic lipids, liposomes and the like or using techniques such as electroporation to facilitate the delivery of siNAs into cells). The cells are then reintroduced back into the same patient or other patients.

[0263] In one embodiment, the invention features a method of modulating the expression of a SREBP1 target gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the SREBP1 target gene; and (b) introducing the siNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target gene in that organism.

[0264] In one embodiment, the invention features a method of modulating the expression of a SREBP1 target gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the SREBP1 target gene and wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the sequence of the SREBP1 target RNA; and (b) introducing the siNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target gene in that organism.

[0265] In another embodiment, the invention features a method of modulating the expression of more than one SREBP1 target gene in a tissue explant comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the SREBP1 target genes; and (b) introducing the siNA molecules into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target genes in that organism.

[0266] In one embodiment, the invention features a method of modulating the expression of a SREBP1 target gene in a subject or organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the SREBP1 target gene; and (b) introducing the siNA molecule into the subject or organism under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target gene in the subject or organism. The level of SREBP1 target protein or RNA can be determined using various methods well-known in the art.

[0267] In another embodiment, the invention features a method of modulating the expression of more than one SREBP1 target gene in a subject or organism comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the SREBP1 target genes; and (b) introducing the siNA molecules into the subject or organism under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target genes in the subject or organism. The level of SREBP1 target protein or RNA can be determined as is known in the art.

[0268] In one embodiment, the invention features a method for modulating the expression of a SREBP1 target gene within a cell (e.g., a liver or adipose tissue cell) comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the SREBP1 target gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target gene in the cell.

[0269] In another embodiment, the invention features a method for modulating the expression of more than one SREBP1 target gene within a cell (e.g., a liver or adipose tissue cell) comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the SREBP1 target gene; and (b) contacting the cell in vitro or in vivo with the siNA molecule

under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target genes in the cell.

[0270] In one embodiment, the invention features a method of modulating the expression of a SREBP1 target gene in a tissue explant ((e.g., liver or any other organ, tissue or cell as can be transplanted from one organism to another or back to the same organism from which the organ, tissue or cell is derived) comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the SREBP1 target gene; and (b) contacting a cell of the tissue explant derived from a particular subject or organism with the siNA molecule under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the subject or organism the tissue was derived from or into another subject or organism under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target gene in that subject or organism.

[0271] In another embodiment, the invention features a method of modulating the expression of more than one SREBP1 target gene in a tissue explant (e.g., liver or any other organ, tissue or cell as can be transplanted from one organism to another or back to the same organism from which the organ, tissue or cell is derived) comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the SREBP1 target gene; and (b) introducing the siNA molecules into a cell of the tissue explant derived from a particular subject or organism under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the subject or organism the tissue was derived from or into another subject or organism under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target genes in that subject or organism.

[0272] In one embodiment, the invention features a method of modulating the expression of a SREBP1 target gene in a subject or organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the SREBP1 target gene; and (b) introducing the siNA molecule into the subject or organism under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target gene in the subject or organism.

[0273] In another embodiment, the invention features a method of modulating the expression of more than one SREBP1 target gene in a subject or organism comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the SREBP1 target gene; and (b) introducing the siNA molecules into the subject or organism under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target genes in the subject or organism.

[0274] In one embodiment, the invention features a method of modulating the expression of a SREBP1 target gene in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target gene in the subject or organism.

[0275] In one embodiment, the invention features a method for treating or preventing a disease, disorder, trait or condition related to SREBP1 gene expression or SREBP1 activity in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the SREBP1 target gene in the subject or organism. The reduction of gene expression and thus reduction in the level of the respective protein/RNA relieves, to some extent, the symptoms of the disease, disorder, trait or condition.

[0276] In one embodiment, the invention features a method for treating or preventing one or more metabolic diseases, traits, or conditions in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the SREBP1 target gene in the subject or organism whereby the treatment or prevention of the metabolic disease (s), trait(s), or condition(s) can be achieved. In one embodiment, the invention features contacting the subject or organism with a siNA molecule of the invention via local administration to relevant tissues or cells. In one embodiment, the invention features contacting the subject or organism with a siNA molecule of the invention via systemic administration (such as via intravenous, intramuscular, subcutaneous, or GI administration of siNA) to relevant tissues or cells, such as tissues or cells involved in the maintenance or development of the metabolic disease, trait, or condition in a subject or organism (e.g., liver, pancreas, small intestine, adipose tissue or cells). The siNA molecule of the invention can be formulated or conjugated as described herein or otherwise known in the art to target appropriate tissues or cells in the subject or organism (e.g., liver, pancreas, small intestine, adipose tissue or cells). The siNA molecule can be combined with other therapeutic treatments and modalities as are known in the art for the treatment of or prevention of metabolic diseases, traits, or conditions in a subject or organism. In one embodiment, the metabolic disease is selected from the group consisting of hypercholesterolemia, hyperlipidemia, dyslipidemia, diabetis (e.g., type I and/or type II diabetis), insulin resistance, obesity, or related conditions, including but not limited to sleep apnea, hiatal hernia, reflux esophagisitis, osteoarthritis, gout, cancers associated with weight gain, gallstones, kidney stones, pulmonary hypertension, infertility, cardiovascular disease, above normal weight, and above normal lipid levels, uric acid levels, or oxalate levels.

[0277] In one embodiment, the invention features a method for treating or preventing one or more metabolic diseases, traits, or conditions in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate (e.g., inhibit) the expression of an inhibitor of SREBP1 gene expression in the subject or organism. In one embodiment, the inhibitor of SREBP1 gene expression is a miRNA.

[0278] In one embodiment, the invention features a method for treating or preventing one or more cardiovascular diseases, traits, or conditions in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the SREBP1 target gene in the subject or organism whereby the treatment or prevention of the cardiovascular disease(s), trait(s), or condition(s) can be achieved. In one embodiment, the invention features contacting the subject or

organism with a siNA molecule of the invention via local administration to relevant tissues or cells, e.g., liver, pancreas, small intestine, adipose tissue or cells tissues or cells. In one embodiment, the invention features contacting the subject or organism with a siNA molecule of the invention via systemic administration (such as via intravenous, intramuscular, subcutaneous, or GI administration of siNA) to relevant tissues or cells, such as tissues or cells involved in the maintenance or development of the cardiovascular disease, trait, or condition in a subject or organism. The siNA molecule of the invention can be formulated or conjugated as described herein or otherwise known in the art to target appropriate tissues or cells in the subject or organism. The siNA molecule can be combined with other therapeutic treatments and modalities as are known in the art for the treatment of or prevention of cardiovascular diseases, traits, or conditions in a subject or organism. In one embodiment the cardiovascular disease is selected from the group consisting of hypertension, coronary thrombosis, stroke, lipid syndromes, hyperglycemia, hypertriglyceridemia, hyperlipidemia, ischemia, congestive heart failure, and myocardial infarction.

[0279] In one embodiment, the invention features a method for treating or preventing one or more cardiovascular diseases, traits, or conditions in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate (e.g., inhibit) the expression of an inhibitor of SREBP1 gene expression in the subject or organism. In one embodiment, the inhibitor of SREBP1 gene expression is a miRNA.

[0280] In one embodiment, the invention features a method for weight loss in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the SREBP1 target gene in the subject or organism whereby the weight loss can be achieved. In one embodiment, the invention features contacting the subject or organism with a siNA molecule of the invention via local administration to relevant tissues or cells, e.g., liver, pancreas, small intestine, adipose tissue or cells. In one embodiment, the invention features contacting the subject or organism with a siNA molecule of the invention via systemic administration (such as via intravenous, intramuscular, subcutaneous, or GI administration of siNA) to relevant tissues or cells. The siNA molecule of the invention can be formulated or conjugated as described herein or otherwise known in the art to target appropriate tissues or cells in the subject or organism. The siNA molecule can be combined with other therapeutic treatments and modalities as are known in the art for weight loss in a subject or organism.

[0281] In one embodiment, the siNA molecule or double stranded nucleic acid molecule of the invention is formulated as a composition described in U.S. Provisional patent application No. 60/678,531 and in related U.S. Provisional patent application No. 60/703,946, filed Jul. 29, 2005, U.S. Provisional patent application No. 60/737,024, filed Nov. 15, 2005, and U.S. Ser. No. 11/353,630, filed Feb. 14, 2006 (Vargeese et al.).

[0282] In any of the methods herein for modulating the expression of one or more SREBP1 targets or for treating or preventing SREBP1 related diseases, traits, conditions, or phenotypes in a cell, subject, or organism, the siNA molecule of the invention modulates expression of one or more SREBP1 targets via RNA interference. In one embodiment, the RNA interference is RISC mediated cleavage of the SREBP1 target (e.g., siRNA mediated RNA interference). In one embodiment, the RNA interference is translational inhibition of the SREBP1 target (e.g., miRNA mediated RNA interference). In one embodiment, the RNA interference is transcriptional inhibition of the SREBP1 target (e.g., siRNA mediated transcriptional silencing). In one embodiment, the RNA interference takes place in the cytoplasm. In one embodiment, the RNA interference takes place in the nucleus.

[0283] In any of the methods of treatment of the invention, the siNA can be administered to the subject as a course of treatment, for example administration at various time intervals, such as once per day over the course of treatment, once every two days over the course of treatment, once every three days over the course of treatment, once every four days over the course of treatment, once every five days over the course of treatment, once every six days over the course of treatment, once per week over the course of treatment, once every other week over the course of treatment, once per month over the course of treatment, etc. In one embodiment, the course of treatment is once every 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks. In one embodiment, the course of treatment is from about one to about 52 weeks or longer (e.g., indefinitely). In one embodiment, the course of treatment is from about one to about 48 months or longer (e.g., indefinitely).

[0284] In one embodiment, a course of treatment involves an initial course of treatment, such as once every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more weeks for a fixed interval (e.g., 1×, 2×, 3×, 4×, 5×, 6×, 7×, 8×, 9×, 10× or more) followed by a maintenance course of treatment, such as once every 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, or more weeks for an additional fixed interval (e.g., 1×, 2×, 3×, 4×, 5×, 6×, 7×, 8×, 9×, 10× or more).

[0285] In any of the methods of treatment of the invention, the siNA can be administered to the subject systemically as described herein or otherwise known in the art, either alone as a monotherapy or in combination with additional therapies described herein or as are known in the art. Systemic administration can include, for example, pulmonary (inhalation, nebulization etc.) intravenous, subcutaneous, intramuscular, catheterization, nasopharangeal, transdermal, or oral/gastrointestinal administration as is generally known in the art.

[0286] In one embodiment, in any of the methods of treatment or prevention of the invention, the siNA can be administered to the subject locally or to local tissues as described herein or otherwise known in the art, either alone as a monotherapy or in combination with additional therapies as are known in the art. Local administration can include, for example, inhalation, nebulization, catheterization, implantation, direct injection, dermal/transdermal application, stenting, ear/eye drops, or portal vein administration to relevant tissues, or any other local administration technique, method or procedure, as is generally known in the art.

[0287] In another embodiment, the invention features a method of modulating the expression of more than one SREBP1 target gene in a subject or organism comprising contacting the subject or organism with one or more siNA molecules of the invention under conditions suitable to modulate (e.g., inhibit) the expression of the SREBP1 target genes in the subject or organism.

[0288] The siNA molecules of the invention can be designed to down regulate or inhibit target gene expression through RNAi targeting of a variety of nucleic acid molecules. In one embodiment, the siNA molecules of the invention are used to target various DNA corresponding to a target

gene, for example via heterochromatic silencing or transcriptional inhibition. In one embodiment, the siNA molecules of the invention are used to target various RNAs corresponding to a target gene, for example via RNA target cleavage or translational inhibition. Non-limiting examples of such RNAs include messenger RNA (mRNA), non-coding RNA (ncRNA) or regulatory elements (see for example Mattick, 2005, *Science,* 309, 1527-1528 and Clayerie, 2005, *Science,* 309, 1529-1530) which includes miRNA and other small RNAs, alternate RNA splice variants of target gene(s), post-transcriptionally modified RNA of target gene(s), pre-mRNA of target gene(s), and/or RNA templates. If alternate splicing produces a family of transcripts that are distinguished by usage of appropriate exons, the instant invention can be used to inhibit gene expression through the appropriate exons to specifically inhibit or to distinguish among the functions of gene family members. For example, a protein that contains an alternatively spliced transmembrane domain can be expressed in both membrane bound and secreted forms. Use of the invention to target the exon containing the transmembrane domain can be used to determine the functional consequences of pharmaceutical targeting of the membrane bound as opposed to the secreted form of the protein. Non-limiting examples of applications of the invention relating to targeting these RNA molecules include therapeutic pharmaceutical applications, cosmetic applications, veterinary applications, pharmaceutical discovery applications, molecular diagnostic and gene function applications, and gene mapping, for example using single nucleotide polymorphism mapping with siNA molecules of the invention. Such applications can be implemented using known gene sequences or from partial sequences available from an expressed sequence tag (EST).

[0289] In another embodiment, the siNA molecules of the invention are used to target conserved sequences corresponding to a gene family or gene families such as SREBP1 family genes (e.g., all known SREBP1 isotypes, or select groupings of SREBP1 isotypes, or both SREBP1 and SREBP2). As such, siNA molecules targeting multiple SREBP1 targets can provide increased therapeutic effect. In addition, by avoiding other SREBP1 isotypes, toxicity may be avoided.

[0290] In one embodiment, siNA molecules can be used to characterize pathways of gene function in a variety of applications. For example, the present invention can be used to inhibit the activity of target gene(s) in a pathway to determine the function of uncharacterized gene(s) in gene function analysis, mRNA function analysis, or translational analysis. The invention can be used to determine potential target gene pathways involved in various diseases and conditions toward pharmaceutical development. The invention can be used to understand pathways of gene expression involved in, for example metabolic and/or cardiovascular diseases, disorders, traits and conditions.

[0291] In one embodiment, siNA molecule(s) and/or methods of the invention are used to down regulate the expression of gene(s) that encode RNA referred to by Genbank Accession, for example, target genes encoding RNA sequence(s) referred to herein by Genbank Accession number, for example, Genbank Accession Nos. shown herein (e.g. in Table I) and in U.S. Ser. No. 10/923,536 and PCT/US03/05028, both incorporated by reference herein.

[0292] In one embodiment, the invention features a method comprising: (a) generating a library of siNA constructs having a predetermined complexity; and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within the target RNA sequence. In one embodiment, the siNA molecules of (a) have strands of a fixed length, for example, about 23 nucleotides in length. In another embodiment, the siNA molecules of (a) are of differing length, for example having strands of about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in length. In one embodiment, the assay can comprise a reconstituted in vitro siNA assay as described herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNAse protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for in vitro systems, and by cellular expression in in vivo systems.

[0293] In one embodiment, the invention features a method comprising: (a) generating a randomized library of siNA constructs having a predetermined complexity, such as of 4^N, where N represents the number of base paired nucleotides in each of the siNA construct strands (eg. for a siNA construct having 21 nucleotide sense and antisense strands with 19 base pairs, the complexity would be 4^{19}); and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within the target RNA sequence. In another embodiment, the siNA molecules of (a) have strands of a fixed length, for example about 23 nucleotides in length. In yet another embodiment, the siNA molecules of (a) are of differing length, for example having strands of about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in length. In one embodiment, the assay can comprise a reconstituted in vitro siNA assay as described in Example 6 herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments of target RNA are analyzed for detectable levels of cleavage, for example, by gel electrophoresis, northern blot analysis, or RNAse protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for in vitro systems, and by cellular expression in in vivo systems.

[0294] In another embodiment, the invention features a method comprising: (a) analyzing the sequence of a RNA target encoded by a target gene; (b) synthesizing one or more sets of siNA molecules having sequence complementary to one or more regions of the RNA of (a); and (c) assaying the siNA molecules of (b) under conditions suitable to determine RNAi targets within the target RNA sequence. In one embodiment, the siNA molecules of (b) have strands of a fixed length, for example about 23 nucleotides in length. In another embodiment, the siNA molecules of (b) are of differing length, for example having strands of about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in length. In one embodiment, the assay can comprise a reconstituted in vitro siNA assay as described herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. Fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNAse protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA

sequence can be obtained as is known in the art, for example, by cloning and/or transcription for in vitro systems, and by expression in in vivo systems.

[0295] By "target site" is meant a sequence within a target RNA that is "targeted" for cleavage mediated by a siNA construct which contains sequences within its antisense region that are complementary to the target sequence.

[0296] By "detectable level of cleavage" is meant cleavage of target RNA (and formation of cleaved product RNAs) to an extent sufficient to discern cleavage products above the background of RNAs produced by random degradation of the target RNA. Production of cleavage products from 1-5% of the target RNA is sufficient to detect above the background for most methods of detection.

[0297] In one embodiment, the invention features a composition comprising a siNA molecule of the invention, which can be chemically-modified, in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a pharmaceutical composition comprising siNA molecules of the invention, which can be chemically-modified, targeting one or more genes in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a method for diagnosing a disease, trait, or condition in a subject comprising administering to the subject a composition of the invention under conditions suitable for the diagnosis of the disease, trait, or condition in the subject. In another embodiment, the invention features a method for treating or preventing a disease, trait, or condition, such as metabolic and/or cardiovascular diseases, traits, conditions, or disorders in a subject, comprising administering to the subject a composition of the invention under conditions suitable for the treatment or prevention of the disease, trait, or condition in the subject, alone or in conjunction with one or more other therapeutic compounds.

[0298] In another embodiment, the invention features a method for validating a target gene target, comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands includes a sequence complementary to RNA of a target gene; (b) introducing the siNA molecule into a cell, tissue, subject, or organism under conditions suitable for modulating expression of the target gene in the cell, tissue, subject, or organism; and (c) determining the function of the gene by assaying for any phenotypic change in the cell, tissue, subject, or organism.

[0299] In another embodiment, the invention features a method for validating a target comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands includes a sequence complementary to RNA of a target gene; (b) introducing the siNA molecule into a biological system under conditions suitable for modulating expression of the target gene in the biological system; and (c) determining the function of the gene by assaying for any phenotypic change in the biological system.

[0300] By "biological system" is meant, material, in a purified or unpurified form, from biological sources, including but not limited to human or animal, wherein the system comprises the components required for RNAi activity. The term "biological system" includes, for example, a cell, tissue, subject, or organism, or extract thereof. The term biological system also includes reconstituted RNAi systems that can be used in an in vitro setting.

[0301] By "phenotypic change" is meant any detectable change to a cell that occurs in response to contact or treatment with a nucleic acid molecule of the invention (e.g., siNA). Such detectable changes include, but are not limited to, changes in shape, size, proliferation, motility, protein expression or RNA expression or other physical or chemical changes as can be assayed by methods known in the art. The detectable change can also include expression of reporter genes/molecules such as Green Florescent Protein (GFP) or various tags that are used to identify an expressed protein or any other cellular component that can be assayed.

[0302] In one embodiment, the invention features a kit containing a siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of a target gene in a biological system, including, for example, in a cell, tissue, subject, or organism. In another embodiment, the invention features a kit containing more than one siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of more than one target gene in a biological system, including, for example, in a cell, tissue, subject, or organism.

[0303] In one embodiment, the invention features a cell containing one or more siNA molecules of the invention, which can be chemically-modified. In another embodiment, the cell containing a siNA molecule of the invention is a mammalian cell. In yet another embodiment, the cell containing a siNA molecule of the invention is a human cell.

[0304] In one embodiment, the synthesis of a siNA molecule of the invention, which can be chemically-modified, comprises: (a) synthesis of two complementary strands of the siNA molecule; (b) annealing the two complementary strands together under conditions suitable to obtain a double-stranded siNA molecule. In another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase oligonucleotide synthesis. In yet another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase tandem oligonucleotide synthesis.

[0305] In one embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing a first oligonucleotide sequence strand of the siNA molecule, wherein the first oligonucleotide sequence strand comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of the second oligonucleotide sequence strand of the siNA; (b) synthesizing the second oligonucleotide sequence strand of siNA on the scaffold of the first oligonucleotide sequence strand, wherein the second oligonucleotide sequence strand further comprises a chemical moiety than can be used to purify the siNA duplex; (c) cleaving the linker molecule of (a) under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex; and (d) purifying the siNA duplex utilizing the chemical moiety of the second oligonucleotide sequence strand. In one embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for example, under hydrolysis conditions using an alkylamine base such as methylamine. In one embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise similar reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized

linker and the cleavable linker of (a) takes place concomitantly. In another embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group, which can be employed in a trityl-on synthesis strategy as described herein. In yet another embodiment, the chemical moiety, such as a dimethoxytrityl group, is removed during purification, for example, using acidic conditions.

[0306] In a further embodiment, the method for siNA synthesis is a solution phase synthesis or hybrid phase synthesis wherein both strands of the siNA duplex are synthesized in tandem using a cleavable linker attached to the first sequence which acts a scaffold for synthesis of the second sequence. Cleavage of the linker under conditions suitable for hybridization of the separate siNA sequence strands results in formation of the double-stranded siNA molecule.

[0307] In another embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing one oligonucleotide sequence strand of the siNA molecule, wherein the sequence comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of another oligonucleotide sequence; (b) synthesizing a second oligonucleotide sequence having complementarity to the first sequence strand on the scaffold of (a), wherein the second sequence comprises the other strand of the double-stranded siNA molecule and wherein the second sequence further comprises a chemical moiety than can be used to isolate the attached oligonucleotide sequence; (c) purifying the product of (b) utilizing the chemical moiety of the second oligonucleotide sequence strand under conditions suitable for isolating the full-length sequence comprising both siNA oligonucleotide strands connected by the cleavable linker and under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex. In one embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for example, under hydrolysis conditions. In another embodiment, cleavage of the linker molecule in (c) above takes place after deprotection of the oligonucleotide. In another embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise similar reactivity or differing reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place either concomitantly or sequentially. In one embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group.

[0308] In another embodiment, the invention features a method for making a double-stranded siNA molecule in a single synthetic process comprising: (a) synthesizing an oligonucleotide having a first and a second sequence, wherein the first sequence is complementary to the second sequence, and the first oligonucleotide sequence is linked to the second sequence via a cleavable linker, and wherein a terminal 5'-protecting group, for example, a 5'-O-dimethoxytrityl group (5'-O-DMT) remains on the oligonucleotide having the second sequence; (b) deprotecting the oligonucleotide whereby the deprotection results in the cleavage of the linker joining the two oligonucleotide sequences; and (c) purifying the product of (b) under conditions suitable for isolating the double-stranded siNA molecule, for example using a trityl-on synthesis strategy as described herein.

[0309] In another embodiment, the method of synthesis of siNA molecules of the invention comprises the teachings of Scaringe et al., U.S. Pat. Nos. 5,889,136; 6,008,400; and 6,111,086, incorporated by reference herein in their entirety.

[0310] In one embodiment, the invention features siNA constructs that mediate RNAi against a target polynucleotide (e.g., RNA or DNA target), wherein the siNA construct comprises one or more chemical modifications, for example, one or more chemical modifications having any of Formulae I-VII or any combination thereof that increases the nuclease resistance of the siNA construct.

[0311] In another embodiment, the invention features a method for generating siNA molecules with increased nuclease resistance comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased nuclease resistance.

[0312] In another embodiment, the invention features a method for generating siNA molecules with improved toxicologic profiles (e.g., having attenuated or no immunostimulatory properties) comprising (a) introducing nucleotides having any of Formula I-VII (e.g., siNA motifs referred to in Table IV) or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved toxicologic profiles.

[0313] In another embodiment, the invention features a method for generating siNA formulations with improved toxicologic profiles (e.g., having attenuated or no immunostimulatory properties) comprising (a) generating a siNA formulation comprising a siNA molecule of the invention and a delivery vehicle or delivery particle as described herein or as otherwise known in the art, and (b) assaying the siNA formulation of step (a) under conditions suitable for isolating siNA formulations having improved toxicologic profiles.

[0314] In another embodiment, the invention features a method for generating siNA molecules that do not stimulate an interferon response (e.g., no interferon response or attenuated interferon response) in a cell, subject, or organism, comprising (a) introducing nucleotides having any of Formula I-VII (e.g., siNA motifs referred to in Table IV) or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules that do not stimulate an interferon response.

[0315] In another embodiment, the invention features a method for generating siNA formulations that do not stimulate an interferon response (e.g., no interferon response or attenuated interferon response) in a cell, subject, or organism, comprising (a) generating a siNA formulation comprising a siNA molecule of the invention and a delivery vehicle or delivery particle as described herein or as otherwise known in the art, and (b) assaying the siNA formulation of step (a) under conditions suitable for isolating siNA formulations that do not stimulate an interferon response. In one embodiment, the interferon comprises interferon alpha.

[0316] In another embodiment, the invention features a method for generating siNA molecules that do not stimulate an inflammatory or proinflammatory cytokine response (e.g., no cytokine response or attenuated cytokine response) in a cell, subject, or organism, comprising (a) introducing nucle-

otides having any of Formula I-VII (e.g., siNA motifs referred to in Table IV) or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules that do not stimulate a cytokine response. In one embodiment, the cytokine comprises an interleukin such as interleukin-6 (IL-6) and/or tumor necrosis alpha (TNF-α).

[0317] In another embodiment, the invention features a method for generating siNA formulations that do not stimulate an inflammatory or proinflammatory cytokine response (e.g., no cytokine response or attenuated cytokine response) in a cell, subject, or organism, comprising (a) generating a siNA formulation comprising a siNA molecule of the invention and a delivery vehicle or delivery particle as described herein or as otherwise known in the art, and (b) assaying the siNA formulation of step (a) under conditions suitable for isolating siNA formulations that do not stimulate a cytokine response. In one embodiment, the cytokine comprises an interleukin such as interleukin-6 (IL-6) and/or tumor necrosis alpha (TNF-α).

[0318] In another embodiment, the invention features a method for generating siNA molecules that do not stimulate Toll-like Receptor (TLR) response (e.g., no TLR response or attenuated TLR response) in a cell, subject, or organism, comprising (a) introducing nucleotides having any of Formula I-VII (e.g., siNA motifs referred to in Table IV) or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules that do not stimulate a TLR response. In one embodiment, the TLR comprises TLR3, TLR7, TLR8 and/or TLR9.

[0319] In another embodiment, the invention features a method for generating siNA formulations that do not stimulate a Toll-like Receptor (TLR) response (e.g., no TLR response or attenuated TLR response) in a cell, subject, or organism, comprising (a) generating a siNA formulation comprising a siNA molecule of the invention and a delivery vehicle or delivery particle as described herein or as otherwise known in the art, and (b) assaying the siNA formulation of step (a) under conditions suitable for isolating siNA formulations that do not stimulate a TLR response. In one embodiment, the TLR comprises TLR3, TLR7, TLR8 and/or TLR9.

[0320] In one embodiment, the invention features a chemically synthesized double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a target RNA via RNA interference (RNAi), wherein: (a) each strand of said siNA molecule is about 18 to about 38 nucleotides in length; (b) one strand of said siNA molecule comprises nucleotide sequence having sufficient complementarity to said target RNA for the siNA molecule to direct cleavage of the target RNA via RNA interference; and (c) wherein the nucleotide positions within said siNA molecule are chemically modified to reduce the immunostimulatory properties of the siNA molecule to a level below that of a corresponding unmodified siRNA molecule. Such siNA molecules are said to have an improved toxicologic profile compared to an unmodified or minimally modified siNA.

[0321] By "improved toxicologic profile", is meant that the chemically modified or formulated siNA construct exhibits decreased toxicity in a cell, subject, or organism compared to an unmodified or unformulated siNA, or siNA molecule having fewer modifications or modifications that are less effective in imparting improved toxicology. Such siNA molecules are also considered to have "improved RNAi activity". In a non-limiting example, siNA molecules and formulations with improved toxicologic profiles are associated with reduced immunostimulatory properties, such as a reduced, decreased or attenuated immunostimulatory response in a cell, subject, or organism compared to an unmodified or unformulated siNA, or siNA molecule having fewer modifications or modifications that are less effective in imparting improved toxicology. Such an improved toxicologic profile is characterized by abrogated or reduced immunostimulation, such as reduction or abrogation of induction of interferons (e.g., interferon alpha), inflammatory cytokines (e.g., interleukins such as IL-6, and/or TNF-alpha), and/or toll like receptors (e.g., TLR-3, TLR-7, TLR-8, and/or TLR-9). In one embodiment, a siNA molecule or formulation with an improved toxicological profile comprises no ribonucleotides. In one embodiment, a siNA molecule or formulation with an improved toxicological profile comprises less than 5 ribonucleotides (e.g., 1, 2, 3, or 4 ribonucleotides). In one embodiment, a siNA molecule or formulation with an improved toxicological profile comprises Stab 7, Stab 8, Stab 11, Stab 12, Stab 13, Stab 16, Stab 17, Stab 18, Stab 19, Stab 20, Stab 23, Stab 24, Stab 25, Stab 26, Stab 27, Stab 28, Stab 29, Stab 30, Stab 31, Stab 32, Stab 33, Stab 34, Stab 35, Stab 36 or any combination thereof (see Table IV). Herein, numeric Stab chemistries include both 2'-fluoro and 2'-OCF3 versions of the chemistries shown in Table IV. For example, "Stab 7/8" refers to both Stab 7/8 and Stab 7F/8F etc. In one embodiment, a siNA molecule or formulation with an improved toxicological profile comprises a siNA molecule of the invention and a formulation as described in United States Patent Application Publication No. 20030077829, incorporated by reference herein in its entirety including the drawings.

[0322] In one embodiment, the level of immunostimulatory response associated with a given siNA molecule can be measured as is described herein or as is otherwise known in the art, for example by determining the level of PKR/interferon response, proliferation, B-cell activation, and/or cytokine production in assays to quantitate the immunostimulatory response of particular siNA molecules (see, for example, Leifer et al., 2003, *J Immunother.* 26, 313-9; and U.S. Pat. No. 5,968,909, incorporated in its entirety by reference). In one embodiment, the reduced immunostimulatory response is between about 10% and about 100% compared to an unmodified or minimally modified siRNA molecule, e.g., about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% reduced immunostimulatory response. In one embodiment, the immunostimulatory response associated with a siNA molecule can be modulated by the degree of chemical modification. For example, a siNA molecule having between about 10% and about 100%, e.g., about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% or at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% of the nucleotide positions in the siNA molecule modified can be selected to have a corresponding degree of immunostimulatory properties as described herein.

[0323] In one embodiment, the degree of reduced immunostimulatory response is selected for optimized RNAi activity. For example, retaining a certain degree of immunostimulation can be preferred to treat viral infection, where less than 100% reduction in immunostimulation may be preferred for maximal antiviral activity (e.g., about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% reduction in immunostimulation) whereas the inhibition of expression of an endogenous

gene target may be preferred with siNA molecules that possess minimal immunostimulatory properties to prevent non-specific toxicity or off target effects (e.g., about 90% to about 100% reduction in immunostimulation).

[0324] In one embodiment, the invention features a chemically synthesized double stranded siNA molecule that directs cleavage of a target RNA via RNA interference (RNAi), wherein (a) each strand of said siNA molecule is about 18 to about 38 nucleotides in length; (b) one strand of said siNA molecule comprises nucleotide sequence having sufficient complementarity to said target RNA for the siNA molecule to direct cleavage of the target RNA via RNA interference; and (c) wherein one or more nucleotides of said siNA molecule are chemically modified to reduce the immunostimulatory properties of the siNA molecule to a level below that of a corresponding unmodified siNA molecule. In one embodiment, each starnd comprises at least about 18 nucleotides that are complementary to the nucleotides of the other strand.

[0325] In another embodiment, the siNA molecule comprising modified nucleotides to reduce the immunostimulatory properties of the siNA molecule comprises an antisense region having nucleotide sequence that is complementary to a nucleotide sequence of a target gene or a portion thereof and further comprises a sense region, wherein said sense region comprises a nucleotide sequence substantially similar to the nucleotide sequence of said target gene or portion thereof. In one embodiment thereof, the antisense region and the sense region comprise about 18 to about 38 nucleotides, wherein said antisense region comprises at least about 18 nucleotides that are complementary to nucleotides of the sense region. In one embodiment thereof, the pyrimidine nucleotides in the sense region are 2'-O-methyl pyrimidine nucleotides. In another embodiment thereof, the purine nucleotides in the sense region are 2'-deoxy purine nucleotides. In yet another embodiment thereof, the pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In another embodiment thereof, the pyrimidine nucleotides of said antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In yet another embodiment thereof, the purine nucleotides of said antisense region are 2'-O-methyl purine nucleotides. In still another embodiment thereof, the purine nucleotides present in said antisense region comprise 2'-deoxypurine nucleotides. In another embodiment, the antisense region comprises a phosphorothioate internucleotide linkage at the 3' end of said antisense region. In another embodiment, the antisense region comprises a glyceryl modification at a 3' end of said antisense region.

[0326] In other embodiments, the siNA molecule comprising modified nucleotides to reduce the immunostimulatory properties of the siNA molecule can comprise any of the structural features of siNA molecules described herein. In other embodiments, the siNA molecule comprising modified nucleotides to reduce the immunostimulatory properties of the siNA molecule can comprise any of the chemical modifications of siNA molecules described herein.

[0327] In one embodiment, the invention features a method for generating a chemically synthesized double stranded siNA molecule having chemically modified nucleotides to reduce the immunostimulatory properties of the siNA molecule, comprising (a) introducing one or more modified nucleotides in the siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating an siNA molecule having reduced immunostimulatory properties compared to a corresponding siNA molecule having unmodified nucleotides. Each strand of the siNA molecule is about 18 to about 38 nucleotides in length. One strand of the siNA molecule comprises nucleotide sequence having sufficient complementarity to the target RNA for the siNA molecule to direct cleavage of the target RNA via RNA interference. In one embodiment, the reduced immunostimulatory properties comprise an abrogated or reduced induction of inflammatory or proinflammatory cytokines, such as interleukin-6 (IL-6) or tumor necrosis alpha (TNF-α), in response to the siNA being introduced in a cell, tissue, or organism. In another embodiment, the reduced immunostimulatory properties comprise an abrogated or reduced induction of Toll Like Receptors (TLRs), such as TLR3, TLR7, TLR8 or TLR9, in response to the siNA being introduced in a cell, tissue, or organism. In another embodiment, the reduced immunostimulatory properties comprise an abrogated or reduced induction of interferons, such as interferon alpha, in response to the siNA being introduced in a cell, tissue, or organism.

[0328] In one embodiment, the invention features siNA constructs that mediate RNAi against a target polynucleotide, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the sense and antisense strands of the siNA construct.

[0329] In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the sense and antisense strands of the siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the sense and antisense strands of the siNA molecule.

[0330] In one embodiment, the invention features siNA constructs that mediate RNAi against a target polynucleotide, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target RNA sequence within a cell.

[0331] In one embodiment, the invention features siNA constructs that mediate RNAi against a target polynucleotide, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target DNA sequence within a cell.

[0332] In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence.

[0333] In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity

between the antisense strand of the siNA molecule and a complementary target DNA sequence.

[0334] In one embodiment, the invention features siNA constructs that mediate RNAi against a target polynucleotide, wherein the siNA construct comprises one or more chemical modifications described herein that modulate the polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA construct.

[0335] In another embodiment, the invention features a method for generating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to a chemically-modified siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA molecule.

[0336] In one embodiment, the invention features chemically-modified siNA constructs that mediate RNAi against a target polynucleotide in a cell, wherein the chemical modifications do not significantly effect the interaction of siNA with a target RNA molecule, DNA molecule and/or proteins or other factors that are essential for RNAi in a manner that would decrease the efficacy of RNAi mediated by such siNA constructs.

[0337] In another embodiment, the invention features a method for generating siNA molecules with improved RNAi specificity against polynucleotide targets comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi specificity. In one embodiment, improved specificity comprises having reduced off target effects compared to an unmodified siNA molecule. For example, introduction of terminal cap moieties at the 3'-end, 5'-end, or both 3' and 5'-ends of the sense strand or region of a siNA molecule of the invention can direct the siNA to have improved specificity by preventing the sense strand or sense region from acting as a template for RNAi activity against a corresponding target having complementarity to the sense strand or sense region.

[0338] In another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against a target polynucleotide comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity.

[0339] In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against a target RNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target RNA.

[0340] In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against a target DNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target DNA.

[0341] In one embodiment, the invention features siNA constructs that mediate RNAi against a target polynucleotide, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the cellular uptake of the siNA construct, such as cholesterol conjugation of the siNA.

[0342] In another embodiment, the invention features a method for generating siNA molecules against a target polynucleotide with improved cellular uptake comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved cellular uptake.

[0343] In one embodiment, the invention features siNA constructs that mediate RNAi against a target polynucleotide, wherein the siNA construct comprises one or more chemical modifications described herein that increases the bioavailability of the siNA construct, for example, by attaching polymeric conjugates such as polyethyleneglycol or equivalent conjugates that improve the pharmacokinetics of the siNA construct, or by attaching conjugates that target specific tissue types or cell types in vivo. Non-limiting examples of such conjugates are described in Vargeese et al., U.S. Ser. No. 10/201,394 incorporated by reference herein.

[0344] In one embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing a conjugate into the structure of a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such conjugates can include ligands for cellular receptors, such as peptides derived from naturally occurring protein ligands; protein localization sequences, including cellular ZIP code sequences; antibodies; nucleic acid aptamers; vitamins and other co-factors, such as folate and N-acetylgalactosamine; polymers, such as polyethyleneglycol (PEG); phospholipids; cholesterol; cholesterol derivatives, polyamines, such as spermine or spermidine; and others.

[0345] In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence is chemically modified in a manner that it can no longer act as a guide sequence for efficiently mediating RNA interference and/or be recognized by cellular proteins that facilitate RNAi. In one embodiment, the first nucleotide sequence of the siNA is chemically modified as described herein. In one embodiment, the first nucleotide sequence of the siNA is not modified (e.g., is all RNA).

[0346] In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein the second sequence is designed or modified in a manner that prevents its entry into the RNAi pathway as a guide sequence or as a sequence that is complementary to a target nucleic acid (e.g., RNA) sequence. In one embodiment,

the first nucleotide sequence of the siNA is chemically modified as described herein. In one embodiment, the first nucleotide sequence of the siNA is not modified (e.g., is all RNA). Such design or modifications are expected to enhance the activity of siNA and/or improve the specificity of siNA molecules of the invention. These modifications are also expected to minimize any off-target effects and/or associated toxicity.

[0347] In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence is incapable of acting as a guide sequence for mediating RNA interference. In one embodiment, the first nucleotide sequence of the siNA is chemically modified as described herein. In one embodiment, the first nucleotide sequence of the siNA is not modified (e.g., is all RNA).

[0348] In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence does not have a terminal 5'-hydroxyl (5'-OH) or 5'-phosphate group.

[0349] In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence comprises a terminal cap moiety at the 5'-end of said second sequence. In one embodiment, the terminal cap moiety comprises an inverted abasic, inverted deoxy abasic, inverted nucleotide moiety, a group shown in FIG. **10**, an alkyl or cycloalkyl group, a heterocycle, or any other group that prevents RNAi activity in which the second sequence serves as a guide sequence or template for RNAi.

[0350] In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence comprises a terminal cap moiety at the 5'-end and 3'-end of said second sequence. In one embodiment, each terminal cap moiety individually comprises an inverted abasic, inverted deoxy abasic, inverted nucleotide moiety, a group shown in FIG. **10**, an alkyl or cycloalkyl group, a heterocycle, or any other group that prevents RNAi activity in which the second sequence serves as a guide sequence or template for RNAi.

[0351] In one embodiment, the invention features a method for generating siNA molecules of the invention with improved specificity for down regulating or inhibiting the expression of a target nucleic acid (e.g., a DNA or RNA such as a gene or its corresponding RNA), comprising (a) introducing one or more chemical modifications into the structure of a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved specificity. In another embodiment, the chemical modification used to improve specificity comprises terminal cap modifications at the 5'-end, 3'-end, or both 5' and 3'-ends of the siNA molecule. The terminal cap modifications can comprise, for example, structures shown in FIG. **10** (e.g. inverted deoxyabasic moieties) or any other chemical modification that renders a portion of the siNA molecule (e.g. the sense strand) incapable of mediating RNA interference against an off target nucleic acid sequence. In a non-limiting example, a siNA molecule is designed such that only the antisense sequence of the siNA molecule can serve as a guide sequence for RISC mediated degradation of a corresponding target RNA sequence. This can be accomplished by rendering the sense sequence of the siNA inactive by introducing chemical modifications to the sense strand that preclude recognition of the sense strand as a guide sequence by RNAi machinery. In one embodiment, such chemical modifications comprise any chemical group at the 5'-end of the sense strand of the siNA, or any other group that serves to render the sense strand inactive as a guide sequence for mediating RNA interference. These modifications, for example, can result in a molecule where the 5'-end of the sense strand no longer has a free 5'-hydroxyl (5'-OH) or a free 5'-phosphate group (e.g., phosphate, diphosphate, triphosphate, cyclic phosphate etc.). Non-limiting examples of such siNA constructs are described herein, such as "Stab 9/10", "Stab 7/8", "Stab 7/19", "Stab 17/22", "Stab 23/24", "Stab 24/25", and "Stab 24/26" (e.g., any siNA having Stab 7, 9, 17, 23, or 24 sense strands) chemistries and variants thereof (see Table IV) wherein the 5'-end and 3'-end of the sense strand of the siNA do not comprise a hydroxyl group or phosphate group. Herein, numeric Stab chemistries include both 2'-fluoro and 2'-OCF3 versions of the chemistries shown in Table IV. For example, "Stab 7/8" refers to both Stab 7/8 and Stab 7F/8F etc.

[0352] In one embodiment, the invention features a method for generating siNA molecules of the invention with improved specificity for down regulating or inhibiting the expression of a SREBP1 target nucleic acid (e.g., a DNA or RNA such as a gene or its corresponding RNA), comprising introducing one or more chemical modifications into the structure of a siNA molecule that prevent a strand or portion of the siNA molecule from acting as a template or guide sequence for RNAi activity. In one embodiment, the inactive strand or sense region of the siNA molecule is the sense strand or sense region of the siNA molecule, i.e. the strand or region of the siNA that does not have complementarity to the target nucleic acid sequence. In one embodiment, such chemical modifications comprise any chemical group at the 5'-end of the sense strand or region of the siNA that does not comprise a 5'-hydroxyl (5'-OH) or 5'-phosphate group, or any other group that serves to render the sense strand or sense region inactive as a guide sequence for mediating RNA interference. Non-limiting examples of such siNA constructs are described herein, such as "Stab 9/10", "Stab 7/8", "Stab 7/19", "Stab 17/22", "Stab 23/24", "Stab 24/25", and "Stab 24/26" (e.g., any siNA having Stab 7, 9, 17, 23, or 24 sense strands) chemistries and variants thereof (see Table IV) wherein the 5'-end and 3'-end of the sense strand of the siNA do not comprise a hydroxyl group or phosphate group. Herein, numeric Stab chemistries include both 2'-fluoro and 2'-OCF3 versions of the chemistries shown in Table IV. For example, "Stab 7/8" refers to both Stab 7/8 and Stab 7F/8F etc.

[0353] In one embodiment, the invention features a method for screening siNA molecules that are active in mediating RNA interference against a target nucleic acid sequence comprising (a) generating a plurality of unmodified siNA molecules, (b) screening the siNA molecules of step (a) under conditions suitable for isolating siNA molecules that are active in mediating RNA interference against the target

nucleic acid sequence, and (c) introducing chemical modifications (e.g. chemical modifications as described herein or as otherwise known in the art) into the active siNA molecules of (b). In one embodiment, the method further comprises re-screening the chemically modified siNA molecules of step (c) under conditions suitable for isolating chemically modified siNA molecules that are active in mediating RNA interference against the target nucleic acid sequence.

[0354] In one embodiment, the invention features a method for screening chemically modified siNA molecules that are active in mediating RNA interference against a target nucleic acid sequence comprising (a) generating a plurality of chemically modified siNA molecules (e.g. siNA molecules as described herein or as otherwise known in the art), and (b) screening the siNA molecules of step (a) under conditions suitable for isolating chemically modified siNA molecules that are active in mediating RNA interference against the target nucleic acid sequence.

[0355] The term "ligand" refers to any compound or molecule, such as a drug, peptide, hormone, or neurotransmitter, that is capable of interacting with another compound, such as a receptor, either directly or indirectly. The receptor that interacts with a ligand can be present on the surface of a cell or can alternately be an intercellular receptor. Interaction of the ligand with the receptor can result in a biochemical reaction, or can simply be a physical interaction or association.

[0356] In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing an excipient formulation to a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such excipients include polymers such as cyclodextrins, lipids, cationic lipids, polyamines, phospholipids, nanoparticles, receptors, ligands, and others.

[0357] In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing nucleotides having any of Formulae I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability.

[0358] In another embodiment, polyethylene glycol (PEG) can be covalently attached to siNA compounds of the present invention. The attached PEG can be any molecular weight, preferably from about 100 to about 50,000 daltons (Da).

[0359] The present invention can be used alone or as a component of a kit having at least one of the reagents necessary to carry out the in vitro or in vivo introduction of RNA to test samples and/or subjects. For example, preferred components of the kit include a siNA molecule of the invention and a vehicle that promotes introduction of the siNA into cells of interest as described herein (e.g., using lipids and other methods of transfection known in the art, see for example Beigelman et al, U.S. Pat. No. 6,395,713). The kit can be used for target validation, such as in determining gene function and/or activity, or in drug optimization, and in drug discovery (see for example Usman et al., U.S. Ser. No. 60/402,996). Such a kit can also include instructions to allow a user of the kit to practice the invention.

[0360] The term "short interfering nucleic acid", "siNA", "short interfering RNA", "siRNA", "short interfering nucleic acid molecule", "short interfering oligonucleotide molecule", or "chemically-modified short interfering nucleic acid molecule" as used herein refers to any nucleic acid molecule capable of inhibiting or down regulating gene expression or viral replication by mediating RNA interference "RNAi" or gene silencing in a sequence-specific manner. These terms can refer to both individual nucleic acid molecules, a plurality of such nucleic acid molecules, or pools of such nucleic acid molecules. The siNA can be a double-stranded nucleic acid molecule comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be assembled from two separate oligonucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (i.e., each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double stranded structure, for example wherein the double stranded region is about 15 to about 30, e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 base pairs; the antisense strand comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof (e.g., about 15 to about 25 or more nucleotides of the siNA molecule are complementary to the target nucleic acid or a portion thereof). Alternatively, the siNA is assembled from a single oligonucleotide, where the self-complementary sense and antisense regions of the siNA are linked by means of a nucleic acid based or non-nucleic acid-based linker(s). The siNA can be a polynucleotide with a duplex, asymmetric duplex, hairpin or asymmetric hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide can be processed either in vivo or in vitro to generate an active siNA molecule capable of mediating RNAi. The siNA can also comprise a single stranded polynucleotide having nucleotide sequence complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof (for example, where such siNA molecule does not require the presence within the siNA molecule of nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof), wherein the single stranded polynucleotide can further comprise a terminal phosphate group, such as a 5'-phosphate (see for example Martinez et al., 2002, *Cell.*, 110, 563-574 and Schwarz et al., 2002, *Molecular Cell*, 10, 537-568), or 5',3'-diphosphate. In certain embodiments, the siNA molecule of the invention comprises separate sense and anti-

sense sequences or regions, wherein the sense and antisense regions are covalently linked by nucleotide or non-nucleotide linkers molecules as is known in the art, or are alternately non-covalently linked by ionic interactions, hydrogen bonding, van der waals interactions, hydrophobic interactions, and/or stacking interactions. In certain embodiments, the siNA molecules of the invention comprise nucleotide sequence that is complementary to nucleotide sequence of a target gene. In another embodiment, the siNA molecule of the invention interacts with nucleotide sequence of a target gene in a manner that causes inhibition of expression of the target gene. As used herein, siNA molecules need not be limited to those molecules containing only RNA, but further encompasses chemically-modified nucleotides and non-nucleotides. In certain embodiments, the short interfering nucleic acid molecules of the invention lack 2'-hydroxy (2'-OH) containing nucleotides. Applicant describes in certain embodiments short interfering nucleic acids that do not require the presence of nucleotides having a 2'-hydroxy group for mediating RNAi and as such, short interfering nucleic acid molecules of the invention optionally do not include any ribonucleotides (e.g., nucleotides having a 2'-OH group). Such siNA molecules that do not require the presence of ribonucleotides within the siNA molecule to support RNAi can however have an attached linker or linkers or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. Optionally, siNA molecules can comprise ribonucleotides at about 5, 10, 20, 30, 40, or 50% of the nucleotide positions. The modified short interfering nucleic acid molecules of the invention can also be referred to as short interfering modified oligonucleotides "siMON." As used herein, the term siNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically-modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. Non limiting examples of siNA molecules of the invention are shown in FIGS. **4-6**, and Tables II and III herein. Such siNA molecules are distinct from other nucleic acid technologies known in the art that mediate inhibition of gene expression, such as ribozymes, antisense, triplex forming, aptamer, 2,5-A chimera, or decoy oligonucleotides.

[0361] By "RNA interference" or "RNAi" is meant a biological process of inhibiting or down regulating gene expression in a cell as is generally known in the art and which is mediated by short interfering nucleic acid molecules, see for example Zamore and Haley, 2005, *Science,* 309, 1519-1524; Vaughn and Martienssen, 2005, *Science,* 309, 1525-1526; Zamore et al., 2000, *Cell,* 101, 25-33; Bass, 2001, *Nature,* 411, 428-429; Elbashir et al., 2001, *Nature,* 411, 494-498; and Kreutzer et al., International PCT Publication No. WO 00/44895; Zernicka-Goetz et al., International PCT Publication No. WO 01/36646; Fire, International PCT Publication No. WO 99/32619; Plaetinck et al., International PCT Publication No. WO 00/01846; Mello and Fire, International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; and Li et al., International PCT Publication No. WO 00/44914; Allshire, 2002, *Science,* 297, 1818-1819; Volpe et al., 2002, *Science,* 297, 1833-1837; Jenuwein, 2002, *Science,* 297, 2215-2218; and Hall et al., 2002, *Science,* 297, 2232-2237; Hutvagner and Zamore, 2002, *Science,* 297, 2056-60; McManus et al., 2002, *RNA,* 8, 842-850; Reinhart et al., 2002, *Gene & Dev.,* 16, 1616-1626; and Reinhart & Bartel, 2002, *Science,* 297, 1831). In addition, as used herein, the term RNAi is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, translational inhibition, transcriptional inhibition, or epigenetics. For example, siNA molecules of the invention can be used to epigenetically silence genes at both the post-transcriptional level or the pre-transcriptional level. In a non-limiting example, epigenetic modulation of gene expression by siNA molecules of the invention can result from siNA mediated modification of chromatin structure or methylation patterns to alter gene expression (see, for example, Verdel et al., 2004, *Science,* 303, 672-676; Pal-Bhadra et al., 2004, *Science,* 303, 669-672; Allshire, 2002, *Science,* 297, 1818-1819; Volpe et al., 2002, *Science,* 297, 1833-1837; Jenuwein, 2002, *Science,* 297, 2215-2218; and Hall et al., 2002, *Science,* 297, 2232-2237). In another non-limiting example, modulation of gene expression by siNA molecules of the invention can result from siNA mediated cleavage of RNA (either coding or non-coding RNA) via RISC, or alternately, translational inhibition as is known in the art. In another embodiment, modulation of gene expression by siNA molecules of the invention can result from transcriptional inhibition (see for example Janowski et al., 2005, *Nature Chemical Biology,* 1, 216-222).

[0362] In one embodiment, a siNA molecule of the invention is a duplex forming oligonucleotide "DFO", (see for example FIGS. **14-15** and Vaish et al., U.S. Ser. No. 10/727,780 filed Dec. 3, 2003 and International PCT Application No. US04/16390, filed May 24, 2004).

[0363] In one embodiment, a siNA molecule of the invention is a multifunctional siNA, (see for example FIGS. **16-28** and Jadhav et al., U.S. Ser. No. 60/543,480 filed Feb. 10, 2004 and International PCT Application No. US04/16390, filed May 24, 2004). In one embodiment, the multifunctional siNA of the invention can comprise sequence targeting, for example, two or more regions of SREBP1 RNA (see for example target sequences in Tables II and III). In one embodiment, the multifunctional siNA of the invention can comprise sequence targeting one or more different SREBP1 targets, including coding regions and non-coding regions of SREBP1.

[0364] By "asymmetric hairpin" as used herein is meant a linear siNA molecule comprising an antisense region, a loop portion that can comprise nucleotides or non-nucleotides, and a sense region that comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complementary nucleotides to base pair with the antisense region and form a duplex with loop. For example, an asymmetric hairpin siNA molecule of the invention can comprise an antisense region having length sufficient to mediate RNAi in a cell or in vitro system (e.g. about 15 to about 30, or about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides) and a loop region comprising about 4 to about 12 (e.g., about 4, 5, 6, 7, 8, 9, 10, 11, or 12) nucleotides, and a sense region having about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides that are complementary to the antisense region. The asymmetric hairpin siNA molecule can also comprise a 5'-terminal phosphate group that can be chemically modified. The loop portion of the asymmetric hairpin siNA

molecule can comprise nucleotides, non-nucleotides, linker molecules, or conjugate molecules as described herein.

[0365] By "asymmetric duplex" as used herein is meant a siNA molecule having two separate strands comprising a sense region and an antisense region, wherein the sense region comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complementary nucleotides to base pair with the antisense region and form a duplex. For example, an asymmetric duplex siNA molecule of the invention can comprise an antisense region having length sufficient to mediate RNAi in a cell or in vitro system (e.g., about 15 to about 30, or about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides) and a sense region having about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides that are complementary to the antisense region.

[0366] By "RNAi inhibitor" is meant any molecule that can down regulate, reduce or inhibit RNA interference function or activity in a cell or organism. An RNAi inhibitor can down regulate, reduce or inhibit RNAi (e.g., RNAi mediated cleavage of a target polynucleotide, translational inhibition, or transcriptional silencing) by interaction with or interfering the function of any component of the RNAi pathway, including protein components such as RISC, or nucleic acid components such as miRNAs or siRNAs. A RNAi inhibitor can be a siNA molecule, an antisense molecule, an aptamer, or a small molecule that interacts with or interferes with the function of RISC, a miRNA, or a siRNA or any other component of the RNAi pathway in a cell or organism. By inhibiting RNAi (e.g., RNAi mediated cleavage of a target polynucleotide, translational inhibition, or transcriptional silencing), a RNAi inhibitor of the invention can be used to modulate (e.g, up-regulate or down regulate) the expression of a target gene. In one embodiment, a RNA inhibitor of the invention is used to up-regulate gene expression by interfering with (e.g., reducing or preventing) endogenous down-regulation or inhibition of gene expression through translational inhibition, transcriptional silencing, or RISC mediated cleavage of a polynucleotide (e.g., mRNA). By interfering with mechanisms of endogenous repression, silencing, or inhibition of gene expression, RNAi inhibitors of the invention can therefore be used to up-regulate gene expression for the treatment of diseases, traits, or conditions resulting from a loss of function.

[0367] By "aptamer" or "nucleic acid aptamer" as used herein is meant a polynucleotide that binds specifically to a target molecule wherein the nucleic acid molecule has sequence that is distinct from sequence recognized by the target molecule in its natural setting. Alternately, an aptamer can be a nucleic acid molecule that binds to a target molecule where the target molecule does not naturally bind to a nucleic acid. The target molecule can be any molecule of interest. For example, the aptamer can be used to bind to a ligand-binding domain of a protein, thereby preventing interaction of the naturally occurring ligand with the protein. This is a non-limiting example and those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art, see for example Gold et al., 1995, *Annu. Rev. Biochem.*, 64, 763; Brody and Gold, 2000, *J. Biotechnol.*, 74, 5; Sun, 2000, *Curr. Opin. Mol. Ther.*, 2, 100; Kusser, 2000, *J. Biotechnol.*, 74, 27; Hermann and Patel, 2000, *Science*, 287, 820; and Jayasena, 1999, *Clinical Chemistry*, 45, 1628. Aptamer molecules of the invention can be chemically modified as is generally known in the art or as described herein.

[0368] The term "antisense nucleic acid", as used herein, refers to a nucleic acid molecule that binds to target RNA by means of RNA-RNA or RNA-DNA or RNA-PNA (protein nucleic acid; Egholm et al., 1993 Nature 365, 566) interactions and alters the activity of the target RNA (for a review, see Stein and Cheng, 1993 Science 261, 1004 and Woolf et al., U.S. Pat. No. 5,849,902) by steric interaction or by RNase H mediated target recognition. Typically, antisense molecules are complementary to a target sequence along a single contiguous sequence of the antisense molecule. However, in certain embodiments, an antisense molecule can bind to substrate such that the substrate molecule forms a loop, and/or an antisense molecule can bind such that the antisense molecule forms a loop. Thus, the antisense molecule can be complementary to two (or even more) non-contiguous substrate sequences or two (or even more) non-contiguous sequence portions of an antisense molecule can be complementary to a target sequence or both. For a review of current antisense strategies, see Schmajuk et al., 1999, J. Biol. Chem., 274, 21783-21789, Delihas et al., 1997, Nature, 15, 751-753, Stein et al., 1997, Antisense N. A. Drug Dev., 7, 151, Crooke, 2000, Methods Enzymol., 313, 345; Crooke, 1998, Biotech. Genet. Eng. Rev., 15, 121-157, Crooke, 1997, Ad. Pharmacol., 40, 149. In addition, antisense DNA or antisense modified with 2'-MOE and other modifications as are known in the art can be used to target RNA by means of DNA-RNA interactions, thereby activating RNase H, which digests the target RNA in the duplex. The antisense oligonucleotides can comprise one or more RNAse H activating region, which is capable of activating RNAse H cleavage of a target RNA. Antisense DNA can be synthesized chemically or expressed via the use of a single stranded DNA expression vector or equivalent thereof. Antisense molecules of the invention can be chemically modified as is generally known in the art or as described herein.

[0369] By "modulate" is meant that the expression of the gene, or level of a RNA molecule or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits is up regulated or down regulated, such that expression, level, or activity is greater than or less than that observed in the absence of the modulator. For example, the term "modulate" can mean "inhibit," but the use of the word "modulate" is not limited to this definition.

[0370] By "inhibit", "down-regulate", or "reduce", it is meant that the expression of the gene, or level of RNA molecules or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits, is reduced below that observed in the absence of the nucleic acid molecules (e.g., siNA) of the invention. In one embodiment, inhibition, down-regulation or reduction with an siNA molecule is below that level observed in the presence of an inactive or attenuated molecule. In another embodiment, inhibition, down-regulation, or reduction with siNA molecules is below that level observed in the presence of, for example, an siNA molecule with scrambled sequence or with mismatches. In another embodiment, inhibition, down-regulation, or reduction of gene expression with a nucleic acid molecule of the instant invention is greater in the presence of the nucleic acid molecule than in its absence. In one embodiment, inhibition, down regulation, or reduction

of gene expression is associated with post transcriptional silencing, such as RNAi mediated cleavage of a target nucleic acid molecule (e.g. RNA) or inhibition of translation. In one embodiment, inhibition, down regulation, or reduction of gene expression is associated with pretranscriptional silencing, such as by alterations in DNA methylation patterns and DNA chromatin structure.

[0371] By "up-regulate", or "promote", it is meant that the expression of the gene, or level of RNA molecules or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits, is increased above that observed in the absence of the nucleic acid molecules (e.g., siNA) of the invention. In one embodiment, up-regulation or promotion of gene expression with an siNA molecule is above that level observed in the presence of an inactive or attenuated molecule. In another embodiment, up-regulation or promotion of gene expression with siNA molecules is above that level observed in the presence of, for example, an siNA molecule with scrambled sequence or with mismatches. In another embodiment, up-regulation or promotion of gene expression with a nucleic acid molecule of the instant invention is greater in the presence of the nucleic acid molecule than in its absence. In one embodiment, up-regulation or promotion of gene expression is associated with inhibition of RNA mediated gene silencing, such as RNAi mediated cleavage or silencing of a coding or non-coding RNA target that down regulates, inhibits, or silences the expression of the gene of interest to be up-regulated. The down regulation of gene expression can, for example, be induced by a coding RNA or its encoded protein, such as through negative feedback or antagonistic effects. The down regulation of gene expression can, for example, be induced by a non-coding RNA having regulatory control over a gene of interest, for example by silencing expression of the gene via translational inhibition, chromatin structure, methylation, RISC mediated RNA cleavage, or translational inhibition. As such, inhibition or down regulation of targets that down regulate, suppress, or silence a gene of interest can be used to up-regulate or promote expression of the gene of interest toward therapeutic use.

[0372] By "gene", or "target gene" or "target DNA", is meant a nucleic acid that encodes an RNA, for example, nucleic acid sequences including, but not limited to, structural genes encoding a polypeptide. A gene or target gene can also encode a functional RNA (fRNA) or non-coding RNA (ncRNA), such as small temporal RNA (stRNA), micro RNA (miRNA), small nuclear RNA (snRNA), short interfering RNA (siRNA), small nucleolar RNA (snRNA), ribosomal RNA (rRNA), transfer RNA (tRNA) and precursor RNAs thereof. Such non-coding RNAs can serve as target nucleic acid molecules for siNA mediated RNA interference in modulating the activity of fRNA or ncRNA involved in functional or regulatory cellular processes. Abberant fRNA or ncRNA activity leading to disease can therefore be modulated by siNA molecules of the invention. siNA molecules targeting fRNA and ncRNA can also be used to manipulate or alter the genotype or phenotype of a subject, organism or cell, by intervening in cellular processes such as genetic imprinting, transcription, translation, or nucleic acid processing (e.g., transamination, methylation etc.). The target gene can be a gene derived from a cell, an endogenous gene, a transgene, or exogenous genes such as genes of a pathogen, for example a virus, which is present in the cell after infection thereof. The cell containing the target gene can be derived from or contained in any organism, for example a plant, animal, protozoan, virus, bacterium, or fungus. Non-limiting examples of plants include monocots, dicots, or gymnosperms. Non-limiting examples of animals include vertebrates or invertebrates. Non-limiting examples of fungi include molds or yeasts. For a review, see for example Snyder and Gerstein, 2003, *Science,* 300, 258-260.

[0373] By "non-canonical base pair" is meant any non-Watson Crick base pair, such as mismatches and/or wobble base pairs, including flipped mismatches, single hydrogen bond mismatches, trans-type mismatches, triple base interactions, and quadruple base interactions. Non-limiting examples of such non-canonical base pairs include, but are not limited to, AC reverse Hoogsteen, AC wobble, AU reverse Hoogsteen, GU wobble, AA N7 amino, CC 2-carbonyl-amino(H1)-N-3-amino(H2), GA sheared, UC 4-carbonyl-amino, UU imino-carbonyl, AC reverse wobble, AU Hoogsteen, AU reverse Watson Crick, CG reverse Watson Crick, GC N3-amino-amino N3, AA N1-amino symmetric, AA N7-amino symmetric, GA N7-N1 amino-carbonyl, GA+carbonyl-amino N7-NI, GG N1-carbonyl symmetric, GG N3-amino symmetric, CC carbonyl-amino symmetric, CC N3-amino symmetric, UU 2-carbonyl-imino symmetric, UU 4-carbonyl-imino symmetric, AA amino-N3, AA N1-amino, AC amino 2-carbonyl, AC N3-amino, AC N7-amino, AU amino-4-carbonyl, AU N1-imino, AU N3-imino, AU N7-imino, CC carbonyl-amino, GA amino-N1, GA amino-N7, GA carbonyl-amino, GA N3-amino, GC amino-N3, GC carbonyl-amino, GC N3-amino, GC N7-amino, GG amino-N7, GG carbonyl-imino, GG N7-amino, GU amino-2-carbonyl, GU carbonyl-imino, GU imino-2-carbonyl, GU N7-imino, psiU imino-2-carbonyl, UC 4-carbonyl-amino, UC imino-carbonyl, UU imino-4-carbonyl, AC C2-H-N3, GA carbonyl-C2-H, UU imino-4-carbonyl 2 carbonyl-C5-H, AC amino(A) N3(C)-carbonyl, GC imino amino-carbonyl, Gpsi imino-2-carbonyl amino-2-carbonyl, and GU imino amino-2-carbonyl base pairs.

[0374] By "SREBP1" as used herein is meant, any Sterol Regulatory Element-Binding Protein 1 or SREBP1 protein, peptide, or polypeptide having SREBP1 activity, such as encoded by SREBP1 Genbank Accession Nos. shown in Table I. The term SREBP1 also refers to nucleic acid sequences encoding or regulating the expression of any SREBP1 protein, peptide, or polypeptide having SREBP1 activity. The term "SREBP1" is also meant to include other SREBP1 encoding sequence, such as SREBP1 sequences derived from various subjects or organisms, including other SRFBP isoforms (e.g., SREBP2), mutant SREBP1 genes, splice variants of SREBP1 genes, and SREBP1 gene polymorphisms.

[0375] By "SREBP1 pathway target" is meant any target involved in SREBP1 pathways of gene expression or activity, including such targets as ApoB, PCSK9, calcium/calmodulin-dependent protein kinase 1D, fatty acid binding protein 5, proprotein convertase subtilisin/kexin 9), LXR target genes, metalloprotease domain 11, apoptosis-inhibitory 6, and F-box-only protein 3.

[0376] By "target" as used herein is meant, any target protein, peptide, or polypeptide, such as encoded by Genbank Accession Nos. herein (see Table I) and in U.S. Ser. No. 10/923,536 and PCT/US03/05028, both incorporated by reference herein. The term "target" also refers to nucleic acid sequences or target polynucleotide sequence encoding any target protein, peptide, or polypeptide, such as proteins, pep-

tides, or polypeptides encoded by sequences having Genbank Accession Nos. shown herein or in U.S. Ser. No. 10/923,536 and USSN PCT/US03/05028. The target of interest can include target polynucleotide sequences, such as target DNA or target RNA. The term "target" is also meant to include other sequences, such as differing isoforms, mutant target genes, splice variants of target polynucleotides, target polymorphisms, and non-coding (e.g., ncRNA, miRNA, stRNA) or other regulatory polynucleotide sequences as described herein. Therefore, in various embodiments of the invention, a double stranded nucleic acid molecule of the invention (e.g. siNA) having complementarity to a target RNA can be used to inhibit or down regulate miRNA or other ncRNA activity. In one embodiment, inhibition of miRNA or ncRNA activity can be used to down regulate or inhibit gene expression (e.g., gene targets described herein or otherwise known in the art) that is dependent on miRNA or ncRNA activity. In another embodiment, inhibition of miRNA or ncRNA activity by double stranded nucleic acid molecules of the invention (e.g. siNA) having complementarity to the miRNA or ncRNA can be used to up regulate or promote target gene expression (e.g., gene targets described herein or otherwise known in the art) where the expression of such genes is down regulated, suppressed, or silenced by the miRNA or ncRNA. Such up-regulation of gene expression can be used to treat diseases and conditions associated with a loss of function or haploinsufficiency as are generally known in the art.

[0377] In one embodiment, the target is any of SREBP1 RNA or a portion thereof.

[0378] In one embodiment, the target is any of SREBP1 DNA or a portion thereof.

[0379] In one embodiment, the target is any of SREBP1 mRNA or a portion thereof.

[0380] In one embodiment, the target is any of SREBP1 miRNA or a portion thereof.

[0381] In one embodiment, the target is any of SREBP1 siRNA or a portion thereof.

[0382] In one embodiment, the target is any of SREBP1 stRNA or a portion thereof.

[0383] In one embodiment, the target is a SREBP1 target and or SREBP1 pathway target or a portion thereof.

[0384] In one embodiment, the target is any (e.g., one or more) of target sequences shown in Table I or a portion thereof. In one embodiment, the target is any (e.g., one or more) of target sequences shown in Table II and III or a portion thereof. In another embodiment, the target is a siRNA, miRNA, or stRNA corresponding to any (e.g., one or more) target, upper strand, or lower strand sequence shown in Table II or III or a portion thereof. In another embodiment, the target is any siRNA, miRNA, or stRNA corresponding any (e.g., one or more) sequence shown in Table I or a portion thereof.

[0385] By "homologous sequence" is meant, a nucleotide sequence that is shared by one or more polynucleotide sequences, such as genes, gene transcripts and/or non-coding polynucleotides. For example, a homologous sequence can be a nucleotide sequence that is shared by two or more genes encoding related but different proteins, such as different members of a gene family, different protein epitopes, different protein isoforms or completely divergent genes, such as a cytokine and its corresponding receptors. A homologous sequence can be a nucleotide sequence that is shared by two or more non-coding polynucleotides, such as noncoding DNA or RNA, regulatory sequences, introns, and sites of transcriptional control or regulation. Homologous sequences can also include conserved sequence regions shared by more than one polynucleotide sequence. Homology does not need to be perfect homology (e.g., 100%), as partially homologous sequences are also contemplated by the instant invention (e.g., 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80% etc.).

[0386] By "conserved sequence region" is meant, a nucleotide sequence of one or more regions in a polynucleotide does not vary significantly between generations or from one biological system, subject, or organism to another biological system, subject, or organism. The polynucleotide can include both coding and non-coding DNA and RNA.

[0387] By "sense region" is meant a nucleotide sequence of a siNA molecule having complementarity to an antisense region of the siNA molecule. In addition, the sense region of a siNA molecule can comprise a nucleic acid sequence having homology with a target nucleic acid sequence. In one embodiment, the sense region of the siNA molecule is referred to as the sense strand or passenger strand.

[0388] By "antisense region" is meant a nucleotide sequence of a siNA molecule having complementarity to a target nucleic acid sequence. In addition, the antisense region of a siNA molecule can optionally comprise a nucleic acid sequence having complementarity to a sense region of the siNA molecule. In one embodiment, the antisense region of the siNA molecule is referred to as the antisense strand or guide strand.

[0389] By "target nucleic acid" or "target polynucleotide" is meant any nucleic acid sequence (e.g, any SREBP1 and/or SREBP1 pathway sequence) whose expression or activity is to be modulated. The target nucleic acid can be DNA or RNA. In one embodiment, a target nucleic acid of the invention is target RNA or DNA.

[0390] By "complementarity" is meant that a nucleic acid can form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types as described herein. In one embodiment, a double stranded nucleic acid molecule of the invention, such as an siNA molecule, wherein each strand is between 15 and 30 nucleotides in length, comprises between about 10% and about 100% (e.g., about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) complementarity between the two strands of the double stranded nucleic acid molecule. In another embodiment, a double stranded nucleic acid molecule of the invention, such as an siNA molecule, where one strand is the sense strand and the other stand is the antisense strand, wherein each strand is between 15 and 30 nucleotides in length, comprises between at least about 10% and about 100% (e.g., at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) complementarity between the nucleotide sequence in the antisense strand of the double stranded nucleic acid molecule and the nucleotide sequence of its corresponding target nucleic acid molecule, such as a target RNA or target mRNA or viral RNA. In one embodiment, a double stranded nucleic acid molecule of the invention, such as an siNA molecule, where one strand comprises nucleotide sequence that is referred to as the sense region and the other strand comprises a nucleotide sequence that is referred to as the antisense region, wherein each strand is between 15 and 30 nucleotides in length, comprises between about 10% and about 100% (e.g., about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) complementarity between the sense region and the antisense region of the

double stranded nucleic acid molecule. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., RNAi activity. Determination of binding free energies for nucleic acid molecules is well known in the art (see, e.g., Turner et al., 1987, *CSH Symp. Quant. Biol.* LII pp. 123-133; Frier et al., 1986, *Proc. Nat. Acad. Sci.* USA 83:9373-9377; Turner et al., 1987, *J. Am. Chem. Soc.* 109:3783-3785). A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, or 10 nucleotides out of a total of 10 nucleotides in the first oligonucleotide being based paired to a second nucleic acid sequence having 10 nucleotides represents 50%, 60%, 70%, 80%, 90%, and 100% complementary respectively). In one embodiment, a siNA molecule of the invention has perfect complementarity between the sense strand or sense region and the antisense strand or antisense region of the siNA molecule. In one embodiment, a siNA molecule of the invention is perfectly complementary to a corresponding target nucleic acid molecule. "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence. In one embodiment, a siNA molecule of the invention comprises about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides that are complementary to one or more target nucleic acid molecules or a portion thereof. In one embodiment, a siNA molecule of the invention has partial complementarity (i.e., less than 100% complementarity) between the sense strand or sense region and the antisense strand or antisense region of the siNA molecule or between the antisense strand or antisense region of the siNA molecule and a corresponding target nucleic acid molecule. For example, partial complementarity can include various mismatches or non-based paired nucleotides (e.g., 1, 2, 3, 4, 5 or more mismatches or non-based paired nucleotides) within the siNA structure which can result in bulges, loops, or overhangs that result between the between the sense strand or sense region and the antisense strand or antisense region of the siNA molecule or between the antisense strand or antisense region of the siNA molecule and a corresponding target nucleic acid molecule.

[0391] In one embodiment, a double stranded nucleic acid molecule of the invention, such as siNA molecule, has perfect complementarity between the sense strand or sense region and the antisense strand or antisense region of the nucleic acid molecule. In one embodiment, double stranded nucleic acid molecule of the invention, such as siNA molecule, is perfectly complementary to a corresponding target nucleic acid molecule.

[0392] In one embodiment, double stranded nucleic acid molecule of the invention, such as siNA molecule, has partial complementarity (i.e., less than 100% complementarity) between the sense strand or sense region and the antisense strand or antisense region of the double stranded nucleic acid molecule or between the antisense strand or antisense region of the nucleic acid molecule and a corresponding target nucleic acid molecule. For example, partial complementarity can include various mismatches or non-base paired nucleotides (e.g., 1, 2, 3, 4, 5 or more mismatches or non-based paired nucleotides, such as nucleotide bulges) within the double stranded nucleic acid molecule, structure which can result in bulges, loops, or overhangs that result between the sense strand or sense region and the antisense strand or antisense region of the double stranded nucleic acid molecule or between the antisense strand or antisense region of the double stranded nucleic acid molecule and a corresponding target nucleic acid molecule.

[0393] In one embodiment, double stranded nucleic acid molecule of the invention is a microRNA (miRNA). By "microRNA" or "miRNA" is meant, a small double stranded RNA that regulates the expression of target messenger RNAs either by mRNA cleavage, translational repression/inhibition or heterochromatic silencing (see for example Ambros, 2004, Nature, 431, 350-355; Bartel, 2004, Cell, 116, 281-297; Cullen, 2004, Virus Research., 102, 3-9; He et al., 2004, Nat. Rev. Genet., 5, 522-531; Ying et al., 2004, Gene, 342, 25-28; and Sethupathy et al., 2006, RNA, 12:192-197). In one embodiment, the microRNA of the invention, has partial complementarity (i.e., less than 100% complementarity) between the sense strand or sense region and the antisense strand or antisense region of the miRNA molecule or between the antisense strand or antisense region of the miRNA and a corresponding target nucleic acid molecule. For example, partial complementarity can include various mismatches or non-base paired nucleotides (e.g., 1, 2, 3, 4, 5 or more mismatches or non-based paired nucleotides, such as nucleotide bulges) within the double stranded nucleic acid molecule, structure which can result in bulges, loops, or overhangs that result between the sense strand or sense region and the antisense strand or antisense region of the miRNA or between the antisense strand or antisense region of the miRNA and a corresponding target nucleic acid molecule.

[0394] In one embodiment, siNA molecules of the invention that down regulate or reduce SREBP1 gene expression are used for preventing or treating metabolic and/or cardiovascular and related diseases, disorders, conditions, or traits in a subject or organism as described herein or otherwise known in the art.

[0395] By "metabolic disease" is meant any disease or condition affecting metabolic pathways as in known in the art. Metabolic disease can result in an abnormal metabolic process, either congenital due to inherited enzyme abnormality (inborn errors of metabolism) or acquired due to disease of an endocrine organ or failure of a metabolically important organ such as the liver. In one embodiment, metabolic disease includes hyperlipidemia, hypercholesterolemia, cardiovascular disease, atherosclerosis, hypertension, diabetis (e.g., type I and/or type II diabetis), insulin resistance, and/or obesity.

[0396] By "cardiovascular disease" is meant and disease or condition affecting the heart and vasculature, including but not limited to, coronary heart disease (CHD), cerebrovascular disease (CVD), aortic stenosis, peripheral vascular disease, atherosclerosis, arteriosclerosis, myocardial infarction (heart attack), cerebrovascular diseases (stroke), transient ischaemic attacks (TIA), angina (stable and unstable), atrial fibrillation, arrhythmia, valvular disease, congestive heart failure, hypercholesterolemia, type I hyperlipoproteinemia, type II hyperlipoproteinemia, type III hyperlipoproteinemia, type IV hyperlipoproteinemia, type V hyperlipoproteinemia, secondary hypertriglyceridemia, and familial lecithin cholesterol acyltransferase deficiency.

[0397] In one embodiment of the present invention, each sequence of a siNA molecule of the invention is independently about 15 to about 30 nucleotides in length, in specific

embodiments about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. In another embodiment, the siNA duplexes of the invention independently comprise about 15 to about 30 base pairs (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30). In another embodiment, one or more strands of the siNA molecule of the invention independently comprises about 15 to about 30 nucleotides (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) that are complementary to a target nucleic acid molecule. In yet another embodiment, siNA molecules of the invention comprising hairpin or circular structures are about 35 to about 55 (e.g., about 35, 40, 45, 50 or 55) nucleotides in length, or about 38 to about 44 (e.g., about 38, 39, 40, 41, 42, 43, or 44) nucleotides in length and comprising about 15 to about 25 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs. Exemplary siNA molecules of the invention are shown in Tables II and III and/or FIGS. **4-5**.

[0398] As used herein "cell" is used in its usual biological sense, and does not refer to an entire multicellular organism, e.g., specifically does not refer to a human. The cell can be present in an organism, e.g., birds, plants and mammals such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell can be prokaryotic (e.g., bacterial cell) or eukaryotic (e.g., mammalian or plant cell). The cell can be of somatic or germ line origin, totipotent or pluripotent, dividing or non-dividing. The cell can also be derived from or can comprise a gamete or embryo, a stem cell, or a fully differentiated cell.

[0399] The siNA molecules of the invention are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells or tissues. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues ex vivo, or in vivo through local delivery to the lung, with or without their incorporation in biopolymers. In particular embodiments, the nucleic acid molecules of the invention comprise sequences shown in Tables II-II and/or FIGS. **4-5**. Examples of such nucleic acid molecules consist essentially of sequences defined in these tables and figures. Furthermore, the chemically modified constructs described in Table IV and the lipid nanoparticle (LNP) formulations shown in Table VI can be applied to any siNA sequence or group of siNA sequences of the invention.

[0400] In another aspect, the invention provides mammalian cells containing one or more siNA molecules of this invention. The one or more siNA molecules can independently be targeted to the same or different sites within a target polynucleotide of the invention.

[0401] By "RNA" is meant a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" is meant a nucleotide with a hydroxyl group at the 2' position of a β-D-ribofuranose moiety. The terms include double-stranded RNA, single-stranded RNA, isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the siNA or internally, for example at one or more nucleotides of the RNA. Nucleotides in the RNA molecules of the instant invention can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs or analogs of naturally-occurring RNA.

[0402] By "subject" is meant an organism, which is a donor or recipient of explanted cells or the cells themselves. "Subject" also refers to an organism to which the nucleic acid molecules of the invention can be administered. A subject can be a mammal or mammalian cells, including a human or human cells. In one embodiment, the subject is an infant (e.g., subjects that are less than 1 month old, or 1, 2, 3, 4, 5, 6, 7, 8, 9 10, 11, or 12 months old). In one embodiment, the subject is a toddler (e.g., 1, 2, 3, 4, 5 or 6 years old). In one embodiment, the subject is a senior (e.g., anyone over the age of about 65 years of age).

[0403] By "chemical modification" as used herein is meant any modification of chemical structure of the nucleotides that differs from nucleotides of native siRNA or RNA. The term "chemical modification" encompasses the addition, substitution, or modification of native siRNA or RNA nucleosides and nucleotides with modified nucleosides and modified nucleotides as described herein or as is otherwise known in the art. Non-limiting examples of such chemical modifications include without limitation compositions having any of Formulae I, II, III, IV, V, VI, or VII herein, phosphorothioate internucleotide linkages, 2'-deoxyribonucleotides, 2'-O-methyl ribonucleotides, 2'-deoxy-2'-fluoro ribonucleotides, 4'-thio ribonucleotides, 2'-O-trifluoromethyl nucleotides, 2'-O-ethyl-trifluoromethoxy nucleotides, 2'-O-difluoromethoxy-ethoxy nucleotides (see for example U.S. Ser. No. 10/981,966 filed Nov. 5, 2004, incorporated by reference herein), FANA, "universal base" nucleotides, "acyclic" nucleotides, 5-C-methyl nucleotides, terminal glyceryl and/or inverted deoxy abasic residue incorporation, or a modification having any of Formulae I-VII herein. In one embodiment, the nucleic acid molecules of the invention (e.g, dsRNA, siNA etc.) are partially modified (e.g., about 5%, 10,%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% modified) with chemical modifications. In another embodiment, the nucleic acid molecules of the invention (e.g, dsRNA, siNA etc.) are completely modified (e.g., about 100% modified) with chemical modifications.

[0404] The term "phosphorothioate" as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise a sulfur atom. Hence, the term phosphorothioate refers to both phosphorothioate and phosphorodithioate internucleotide linkages.

[0405] The term "phosphonoacetate" as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise an acetyl or protected acetyl group.

[0406] The term "thiophosphonoacetate" as used herein refers to an internucleotide linkage having Formula I, wherein Z comprises an acetyl or protected acetyl group and W comprises a sulfur atom or alternately W comprises an acetyl or protected acetyl group and Z comprises a sulfur atom.

[0407] The term "universal base" as used herein refers to nucleotide base analogs that form base pairs with each of the natural DNA/RNA bases with little discrimination between them. Non-limiting examples of universal bases include C-phenyl, C-naphthyl and other aromatic derivatives, inosine, azole carboxamides, and nitroazole derivatives such as 3-nitropyrrole, 4-nitroindole, 5-nitroindole, and 6-nitroindole as known in the art (see for example Loakes, 2001, *Nucleic Acids Research,* 29, 2437-2447).

[0408] The term "acyclic nucleotide" as used herein refers to any nucleotide having an acyclic ribose sugar, for example where any of the ribose carbons (C1, C2, C3, C4, or C5), are independently or in combination absent from the nucleotide.

[0409] The nucleic acid molecules of the instant invention, individually, or in combination or in conjunction with other drugs, can be used to for preventing or treating diseases, disorders, conditions, and traits described herein or otherwise known in the art, in a subject or organism.

[0410] In one embodiment, the siNA molecules of the invention can be administered to a subject or can be administered to other appropriate cells evident to those skilled in the art, individually or in combination with one or more drugs under conditions suitable for the treatment.

[0411] In a further embodiment, the siNA molecules can be used in combination with other known treatments to prevent or treat respiratory diseases, disorders, or conditions in a subject or organism. For example, the described molecules could be used in combination with one or more known compounds, treatments, or procedures to prevent or treat diseases, disorders, conditions, and traits described herein in a subject or organism as are known in the art.

[0412] In one embodiment, the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention, in a manner which allows expression of the siNA molecule. For example, the vector can contain sequence(s) encoding both strands of a siNA molecule comprising a duplex. The vector can also contain sequence(s) encoding a single nucleic acid molecule that is self-complementary and thus forms a siNA molecule. Non-limiting examples of such expression vectors are described in Paul et al., 2002, *Nature Biotechnology,* 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology,* 19, 497; Lee et al., 2002, *Nature Biotechnology,* 19, 500; and Novina et al., 2002, *Nature Medicine*, advance online publication doi:10.1038/nm725.

[0413] In another embodiment, the invention features a mammalian cell, for example, a human cell, including an expression vector of the invention.

[0414] In yet another embodiment, the expression vector of the invention comprises a sequence for a siNA molecule having complementarity to a RNA molecule referred to by a Genbank Accession numbers, for example Genbank Accession Nos. shown herein (Table I) or in PCT/US03/05028 and U.S. Ser. No. 10/923,536, both incorporated by reference herein.

[0415] In one embodiment, an expression vector of the invention comprises a nucleic acid sequence encoding two or more siNA molecules, which can be the same or different.

[0416] In another aspect of the invention, siNA molecules that interact with target RNA molecules and down-regulate gene encoding target RNA molecules (for example target RNA molecules referred to by Genbank Accession numbers herein) are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siNA molecules bind and down-regulate gene function or expression via RNA interference (RNAi). Delivery of siNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired target cell.

[0417] By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

[0418] Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0419] FIG. **1** shows a non-limiting example of a scheme for the synthesis of siNA molecules. The complementary siNA sequence strands, strand 1 and strand 2, are synthesized in tandem and are connected by a cleavable linkage, such as a nucleotide succinate or abasic succinate, which can be the same or different from the cleavable linker used for solid phase synthesis on a solid support. The synthesis can be either solid phase or solution phase, in the example shown, the synthesis is a solid phase synthesis. The synthesis is performed such that a protecting group, such as a dimethoxytrityl group, remains intact on the terminal nucleotide of the tandem oligonucleotide. Upon cleavage and deprotection of the oligonucleotide, the two siNA strands spontaneously hybridize to form a siNA duplex, which allows the purification of the duplex by utilizing the properties of the terminal protecting group, for example by applying a trityl on purification method wherein only duplexes/oligonucleotides with the terminal protecting group are isolated.

[0420] FIG. **2** shows a MALDI-TOF mass spectrum of a purified siNA duplex synthesized by a method of the invention. The two peaks shown correspond to the predicted mass of the separate siNA sequence strands. This result demonstrates that the siNA duplex generated from tandem synthesis can be purified as a single entity using a simple trityl-on purification methodology.

[0421] FIG. **3** shows a non-limiting proposed mechanistic representation of target RNA degradation involved in RNAi. Double-stranded RNA (dsRNA), which is generated by RNA-dependent RNA polymerase (RdRP) from foreign single-stranded RNA, for example viral, transposon, or other exogenous RNA, activates the DICER enzyme that in turn generates siNA duplexes. Alternately, synthetic or expressed siNA can be introduced directly into a cell by appropriate means. An active siNA complex forms which recognizes a target RNA, resulting in degradation of the target RNA by the RISC endonuclease complex or in the synthesis of additional RNA by RNA-dependent RNA polymerase (RdRP), which can activate DICER and result in additional siNA molecules, thereby amplifying the RNAi response.

[0422] FIG. **4**A-F shows non-limiting examples of chemically-modified siNA constructs of the present invention. In the figure, N stands for any nucleotide (adenosine, guanosine, cytosine, uridine, or optionally thymidine, for example thymidine can be substituted in the overhanging regions designated by parenthesis (N N). Various modifications are shown for the sense and antisense strands of the siNA constructs. The (N N) nucleotide positions can be chemically modified as described herein (e.g., 2'-O-methyl, 2'-deoxy-2'-fluoro etc.) and can be either derived from a corresponding target nucleic acid sequence or not (see for example FIG. **6**C). Furthermore, the sequences shown in FIG. **4** can optionally include a ribo-

nucleotide at the 9th position from the 5'-end of the sense strand or the 11th position based on the 5'-end of the guide strand by counting 11 nucleotide positions in from the 5'-terminus of the guide strand (see FIG. 6C).

[0423] FIG. 4A: The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all nucleotides present are ribonucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all nucleotides present are ribonucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

[0424] FIG. 4B: The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the sense and antisense strand.

[0425] FIG. 4C: The sense strand comprises 21 nucleotides having 5'- and 3'-terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl or 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

[0426] FIG. 4D: The sense strand comprises 21 nucleotides having 5'- and 3'-terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein and wherein and all purine nucleotides that may be present are 2'-deoxy nucleotides. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

[0427] FIG. 4E: The sense strand comprises 21 nucleotides having 5'- and 3'-terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

[0428] FIG. 4F: The sense strand comprises 21 nucleotides having 5'- and 3'-terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein and wherein and all purine nucleotides that may be present are 2'-deoxy nucleotides. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-deoxy nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand. The antisense strand of constructs A-F comprise sequence complementary to any

target nucleic acid sequence of the invention. Furthermore, when a glyceryl moiety (L) is present at the 3'-end of the antisense strand for any construct shown in FIG. **4** A-F, the modified internucleotide linkage is optional.

[0429] FIG. **5**A-F shows non-limiting examples of specific chemically-modified siNA sequences of the invention. A-F applies the chemical modifications described in FIG. **4**A-F to an exemplary SREBP1 siNA sequence. Such chemical modifications can be applied to any SREBP1 sequence. Furthermore, the sequences shown in FIG. **5** can optionally include a ribonucleotide at the 9th position from the 5'-end of the sense strand or the 11th position based on the 5'-end of the guide strand by counting 11 nucleotide positions in from the 5'-terminus of the guide strand (see FIG. **6**C). In addition, the sequences shown in FIG. **5** can optionally include terminal ribonucleotides at up to about 4 positions at the 5'-end of the antisense strand (e.g., about 1, 2, 3, or 4 terminal ribonucleotides at the 5'-end of the antisense strand).

[0430] FIG. **6**A-C shows non-limiting examples of different siNA constructs of the invention.

[0431] The examples shown in FIG. **6**A (constructs 1, 2, and 3) have 19 representative base pairs; however, different embodiments of the invention include any number of base pairs described herein. Bracketed regions represent nucleotide overhangs, for example, comprising about 1, 2, 3, or 4 nucleotides in length, preferably about 2 nucleotides. Constructs 1 and 2 can be used independently for RNAi activity. Construct 2 can comprise a polynucleotide or non-nucleotide linker, which can optionally be designed as a biodegradable linker. In one embodiment, the loop structure shown in construct 2 can comprise a biodegradable linker that results in the formation of construct 1 in vivo and/or in vitro. In another example, construct 3 can be used to generate construct 2 under the same principle wherein a linker is used to generate the active siNA construct 2 in vivo and/or in vitro, which can optionally utilize another biodegradable linker to generate the active siNA construct 1 in vivo and/or in vitro. As such, the stability and/or activity of the siNA constructs can be modulated based on the design of the siNA construct for use in vivo or in vitro and/or in vitro.

[0432] The examples shown in FIG. **6**B represent different variations of double stranded nucleic acid molecule of the invention, such as microRNA, that can include overhangs, bulges, loops, and stem-loops resulting from partial complementarity. Such motifs having bulges, loops, and stem-loops are generally characteristics of miRNA. The bulges, loops, and stem-loops can result from any degree of partial complementarity, such as mismatches or bulges of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleotides in one or both strands of the double stranded nucleic acid molecule of the invention.

[0433] The example shown in FIG. **6**C represents a model double stranded nucleic acid molecule of the invention comprising a 19 base pair duplex of two 21 nucleotide sequences having dinucleotide 3'-overhangs. The top strand (1) represents the sense strand (passenger strand), the middle strand (2) represents the antisense (guide strand), and the lower strand (3) represents a target polynucleotide sequence. The dinucleotide overhangs (NN) can comprise sequence derived from the target polynucleotide. For example, the 3'-(NN) sequence in the guide strand can be complementary to the 5'-[NN] sequence of the target polynucleotide. In addition, the 5'-(NN) sequence of the passenger strand can comprise the same sequence as the 5'-[NN] sequence of the target polynucleotide sequence. In other embodiments, the overhangs (NN) are not derived from the target polynucleotide sequence, for example where the 3'-(NN) sequence in the guide strand are not complementary to the 5'-[NN] sequence of the target polynucleotide and the 5'-(NN) sequence of the passenger strand can comprise different sequence from the 5'-[NN] sequence of the target polynucleotide sequence. In additional embodiments, any (NN) nucleotides are chemically modified, e.g., as 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or other modifications herein. Furthermore, the passenger strand can comprise a ribonucleotide position N of the passenger strand. For the representative 19 base pair 21 mer duplex shown, position N can be 9 nucleotides in from the 3' end of the passenger strand. However, in duplexes of differing length, the position N is determined based on the 5'-end of the guide strand by counting 11 nucleotide positions in from the 5'-terminus of the guide strand and picking the corresponding base paired nucleotide in the passenger strand. Cleavage by Ago2 takes place between positions 10 and 11 as indicated by the arrow. In additional embodiments, there are two ribonucleotides, NN, at positions 10 and 11 based on the 5'-end of the guide strand by counting 10 and 11 nucleotide positions in from the 5'-terminus of the guide strand and picking the corresponding base paired nucleotides in the passenger strand.

[0434] FIG. **7**A-C is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate siNA hairpin constructs.

[0435] FIG. **7**A: A DNA oligomer is synthesized with a 5'-restriction site (R1) sequence followed by a region having sequence identical (sense region of siNA) to a predetermined target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, which is followed by a loop sequence of defined sequence (X), comprising, for example, about 3 to about 10 nucleotides.

[0436] FIG. **7**B: The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence that will result in a siNA transcript having specificity for a target sequence and having self-complementary sense and antisense regions.

[0437] FIG. **7**C: The construct is heated (for example to about 95° C.) to linearize the sequence, thus allowing extension of a complementary second DNA strand using a primer to the 3'-restriction sequence of the first strand. The double-stranded DNA is then inserted into an appropriate vector for expression in cells. The construct can be designed such that a 3'-terminal nucleotide overhang results from the transcription, for example, by engineering restriction sites and/or utilizing a poly-U termination region as described in Paul et al., 2002, *Nature Biotechnology*, 29, 505-508.

[0438] FIG. **8**A-C is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate double-stranded siNA constructs.

[0439] FIG. **8**A: A DNA oligomer is synthesized with a 5'-restriction (R1) site sequence followed by a region having sequence identical (sense region of siNA) to a predetermined target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, and which is followed by a 3'-restriction site (R2) which is adjacent to a loop sequence of defined sequence (X).

[0440] FIG. **8**B: The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence.

[0441] FIG. **8**C: The construct is processed by restriction enzymes specific to R1 and R2 to generate a double-stranded DNA which is then inserted into an appropriate vector for expression in cells. The transcription cassette is designed such that a U6 promoter region flanks each side of the dsDNA which generates the separate sense and antisense strands of the siNA. Poly T termination sequences can be added to the constructs to generate U overhangs in the resulting transcript.

[0442] FIG. **9**A-E is a diagrammatic representation of a method used to determine target sites for siNA mediated RNAi within a particular target nucleic acid sequence, such as messenger RNA.

[0443] FIG. **9**A: A pool of siNA oligonucleotides are synthesized wherein the antisense region of the siNA constructs has complementarity to target sites across the target nucleic acid sequence, and wherein the sense region comprises sequence complementary to the antisense region of the siNA.

[0444] FIGS. **9**B&C: (FIG. **9**B) The sequences are pooled and are inserted into vectors such that (FIG. **9**C) transfection of a vector into cells results in the expression of the siNA.

[0445] FIG. **9**D: Cells are sorted based on phenotypic change that is associated with modulation of the target nucleic acid sequence.

[0446] FIG. **9**E: The siNA is isolated from the sorted cells and is sequenced to identify efficacious target sites within the target nucleic acid sequence.

[0447] FIG. **10** shows non-limiting examples of different stabilization chemistries (1-10) that can be used, for example, to stabilize the 3'-end of siNA sequences of the invention, including (1) [3-3']-inverted deoxyribose; (2) deoxyribonucleotide; (3) [5'-3']-3'-deoxyribonucleotide; (4) [5'-3']-ribonucleotide; (5) [5'-3']-3'-O-methyl ribonucleotide; (6) 3'-glyceryl; (7) [3'-5']-3'-deoxyribonucleotide; (8) [3'-3']-deoxyribonucleotide; (9) [5'-2']-deoxyribonucleotide; and (10) [5-3']-dideoxyribonucleotide. In addition to modified and unmodified backbone chemistries indicated in the figure, these chemistries can be combined with different backbone modifications as described herein, for example, backbone modifications having Formula I. In addition, the 2'-deoxy nucleotide shown 5' to the terminal modifications shown can be another modified or unmodified nucleotide or non-nucleotide described herein, for example modifications having any of Formulae I-VII or any combination thereof.

[0448] FIG. **11** shows a non-limiting example of a strategy used to identify chemically modified siNA constructs of the invention that are nuclease resistant while preserving the ability to mediate RNAi activity. Chemical modifications are introduced into the siNA construct based on educated design parameters (e.g. introducing 2'-modifications, base modifications, backbone modifications, terminal cap modifications etc). The modified construct in tested in an appropriate system (e.g. human serum for nuclease resistance, shown, or an animal model for PK/delivery parameters). In parallel, the siNA construct is tested for RNAi activity, for example in a cell culture system such as a luciferase reporter assay). Lead siNA constructs are then identified which possess a particular characteristic while maintaining RNAi activity, and can be further modified and assayed once again. This same approach can be used to identify siNA-conjugate molecules with improved pharmacokinetic profiles, delivery, and RNAi activity.

[0449] FIG. **12** shows non-limiting examples of phosphorylated siNA molecules of the invention, including linear and duplex constructs and asymmetric derivatives thereof.

[0450] FIG. **13** shows non-limiting examples of chemically modified terminal phosphate groups of the invention.

[0451] FIG. **14**A shows a non-limiting example of methodology used to design self complementary DFO constructs utilizing palindrome and/or repeat nucleic acid sequences that are identified in a target nucleic acid sequence. (i) A palindrome or repeat sequence is identified in a nucleic acid target sequence. (ii) A sequence is designed that is complementary to the target nucleic acid sequence and the palindrome sequence. (iii) An inverse repeat sequence of the non-palindrome/repeat portion of the complementary sequence is appended to the 3'-end of the complementary sequence to generate a self complementary DFO molecule comprising sequence complementary to the nucleic acid target. (iv) The DFO molecule can self-assemble to form a double stranded oligonucleotide. FIG. **14**B shows a non-limiting representative example of a duplex forming oligonucleotide sequence. FIG. **14**C shows a non-limiting example of the self assembly schematic of a representative duplex forming oligonucleotide sequence. FIG. **14**D shows a non-limiting example of the self assembly schematic of a representative duplex forming oligonucleotide sequence followed by interaction with a target nucleic acid sequence resulting in modulation of gene expression.

[0452] FIG. **15** shows a non-limiting example of the design of self complementary DFO constructs utilizing palindrome and/or repeat nucleic acid sequences that are incorporated into the DFO constructs that have sequence complementary to any target nucleic acid sequence of interest. Incorporation of these palindrome/repeat sequences allow the design of DFO constructs that form duplexes in which each strand is capable of mediating modulation of target gene expression, for example by RNAi. First, the target sequence is identified. A complementary sequence is then generated in which nucleotide or non-nucleotide modifications (shown as X or Y) are introduced into the complementary sequence that generate an artificial palindrome (shown as XYXYXY in the Figure). An inverse repeat of the non-palindrome/repeat complementary sequence is appended to the 3'-end of the complementary sequence to generate a self complementary DFO comprising sequence complementary to the nucleic acid target. The DFO can self-assemble to form a double stranded oligonucleotide.

[0453] FIG. **16** shows non-limiting examples of multifunctional siNA molecules of the invention comprising two separate polynucleotide sequences that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences. FIG. **16**A shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 3'-ends of each polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. FIG. **16**B shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 5'-ends

of each polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences.

[0454] FIG. **17** shows non-limiting examples of multifunctional siNA molecules of the invention comprising a single polynucleotide sequence comprising distinct regions that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences. FIG. **17**A shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the second complementary region is situated at the 3'-end of the polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. FIG. **17**B shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first complementary region is situated at the 5'-end of the polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. In one embodiment, these multifunctional siNA constructs are processed in vivo or in vitro to generate multifunctional siNA constructs as shown in FIG. **16**.

[0455] FIG. **18** shows non-limiting examples of multifunctional siNA molecules of the invention comprising two separate polynucleotide sequences that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences and wherein the multifunctional siNA construct further comprises a self complementary, palindrome, or repeat region, thus enabling shorter bifunctional siNA constructs that can mediate RNA interference against differing target nucleic acid sequences. FIG. **18**A shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 3'-ends of each polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. FIG. **18**B shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 5'-ends of each polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences.

[0456] FIG. **19** shows non-limiting examples of multifunctional siNA molecules of the invention comprising a single polynucleotide sequence comprising distinct regions that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences and wherein the multifunctional siNA construct further comprises a self complementary, palindrome, or repeat region, thus enabling shorter bifunctional siNA constructs that can mediate RNA interference against differing target nucleic acid sequences. FIG. **19**A shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the second complementary region is situated at the 3'-end of the polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. FIG. **19**B shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first complementary region is situated at the 5'-end of the polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. In one embodiment, these multifunctional siNA constructs are processed in vivo or in vitro to generate multifunctional siNA constructs as shown in FIG. **18**.

[0457] FIG. **20** shows a non-limiting example of how multifunctional siNA molecules of the invention can target two separate target nucleic acid molecules, such as separate RNA molecules encoding differing proteins (e.g., any of SREBP1 targets herein), for example, a cytokine and its corresponding receptor, differing viral strains, a virus and a cellular protein involved in viral infection or replication, or differing proteins involved in a common or divergent biologic pathway that is implicated in the maintenance of progression of disease. Each strand of the multifunctional siNA construct comprises a region having complementarity to separate target nucleic acid molecules. The multifunctional siNA molecule is designed such that each strand of the siNA can be utilized by the RISC complex to initiate RNA interference mediated cleavage of its corresponding target. These design parameters can include destabilization of each end of the siNA construct (see for example Schwarz et al., 2003, *Cell,* 115, 199-208). Such

destabilization can be accomplished for example by using guanosine-cytidine base pairs, alternate base pairs (e.g., wobbles), or destabilizing chemically modified nucleotides at terminal nucleotide positions as is known in the art.

[0458] FIG. **21** shows a non-limiting example of how multifunctional siNA molecules of the invention can target two separate target nucleic acid sequences within the same target nucleic acid molecule, such as alternate coding regions of a RNA, coding and non-coding regions of a RNA, or alternate splice variant regions of a RNA. Each strand of the multifunctional siNA construct comprises a region having complementarity to the separate regions of the target nucleic acid molecule. The multifunctional siNA molecule is designed such that each strand of the siNA can be utilized by the RISC complex to initiate RNA interference mediated cleavage of its corresponding target region. These design parameters can include destabilization of each end of the siNA construct (see for example Schwarz et al., 2003, *Cell,* 115, 199-208). Such destabilization can be accomplished for example by using guanosine-cytidine base pairs, alternate base pairs (e.g., wobbles), or destabilizing chemically modified nucleotides at terminal nucleotide positions as is known in the art.

[0459] FIG. **22**(A-H) shows non-limiting examples of tethered multifunctional siNA constructs of the invention. In the examples shown, a linker (e.g., nucleotide or non-nucleotide linker) connects two siNA regions (e.g., two sense, two antisense, or alternately a sense and an antisense region together. Separate sense (or sense and antisense) sequences corresponding to a first target sequence and second target sequence are hybridized to their corresponding sense and/or antisense sequences in the multifunctional siNA. In addition, various conjugates, ligands, aptamers, polymers or reporter molecules can be attached to the linker region for selective or improved delivery and/or pharmacokinetic properties.

[0460] FIG. **23** shows a non-limiting example of various dendrimer based multifunctional siNA designs.

[0461] FIG. **24** shows a non-limiting example of various supramolecular multifunctional siNA designs.

[0462] FIG. **25** shows a non-limiting example of a dicer enabled multifunctional siNA design using a 30 nucleotide precursor siNA construct. A 30 base pair duplex is cleaved by Dicer into 22 and 8 base pair products from either end (8 b.p. fragments not shown). For ease of presentation the overhangs generated by dicer are not shown—but can be compensated for. Three targeting sequences are shown. The required sequence identity overlapped is indicated by grey boxes. The N's of the parent 30 b.p. siNA are suggested sites of 2'-OH positions to enable Dicer cleavage if this is tested in stabilized chemistries. Note that processing of a 30mer duplex by Dicer RNase III does not give a precise 22+8 cleavage, but rather produces a series of closely related products (with 22+8 being the primary site). Therefore, processing by Dicer will yield a series of active siNAs.

[0463] FIG. **26** shows a non-limiting example of a dicer enabled multifunctional siNA design using a 40 nucleotide precursor siNA construct. A 40 base pair duplex is cleaved by Dicer into 20 base pair products from either end. For ease of presentation the overhangs generated by dicer are not shown—but can be compensated for. Four targeting sequences are shown. The target sequences having homology are enclosed by boxes. This design format can be extended to larger RNAs. If chemically stabilized siNAs are bound by Dicer, then strategically located ribonucleotide linkages can enable designer cleavage products that permit our more extensive repertoire of multifunctional designs. For example cleavage products not limited to the Dicer standard of approximately 22-nucleotides can allow multifunctional siNA constructs with a target sequence identity overlap ranging from, for example, about 3 to about 15 nucleotides.

[0464] FIG. **27** shows a non-limiting example of additional multifunctional siNA construct designs of the invention. In one example, a conjugate, ligand, aptamer, label, or other moiety is attached to a region of the multifunctional siNA to enable improved delivery or pharmacokinetic profiling.

[0465] FIG. **28** shows a non-limiting example of additional multifunctional siNA construct designs of the invention. In one example, a conjugate, ligand, aptamer, label, or other moiety is attached to a region of the multifunctional siNA to enable improved delivery or pharmacokinetic profiling.

[0466] FIG. **29** shows a non-limiting example of a cholesterol linked phosphoramidite that can be used to synthesize cholesterol conjugated siNA molecules of the invention. An example is shown with the cholesterol moiety linked to the 5'-end of the sense strand of a siNA molecule.

DETAILED DESCRIPTION OF THE INVENTION

Mechanism of Action of Nucleic Acid Molecules of the Invention

[0467] The discussion that follows discusses the proposed mechanism of RNA interference mediated by short interfering RNA as is presently known, and is not meant to be limiting and is not an admission of prior art. Applicant demonstrates herein that chemically-modified short interfering nucleic acids possess similar or improved capacity to mediate RNAi as do siRNA molecules and are expected to possess improved stability and activity in vivo; therefore, this discussion is not meant to be limiting only to siRNA and can be applied to siNA as a whole. By "improved capacity to mediate RNAi" or "improved RNAi activity" is meant to include RNAi activity measured in vitro and/or in vivo where the RNAi activity is a reflection of both the ability of the siNA to mediate RNAi and the stability of the siNAs of the invention. In this invention, the product of these activities can be increased in vitro and/or in vivo compared to an all RNA siRNA or a siNA containing a plurality of ribonucleotides. In some cases, the activity or stability of the siNA molecule can be decreased (i.e., less than ten-fold), but the overall activity of the siNA molecule is enhanced in vitro and/or in vivo.

[0468] RNA interference refers to the process of sequence specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Fire et al., 1998, Nature, 391, 806). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes which is commonly shared by diverse flora and phyla (Fire et al., 1999, *Trends Genet.,* 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response though a mechanism that has yet to be fully characterized. This mechanism appears to be different from the interferon

response that results from dsRNA-mediated activation of protein kinase PKR and 2',5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

[0469] The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as Dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Berstein et al., 2001, Nature, 409, 363). Short interfering RNAs derived from Dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes. Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner et al., 2001, *Science,* 293, 834). The RNAi response also features an endonuclease complex containing a siRNA, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence homologous to the siRNA. Cleavage of the target RNA takes place in the middle of the region complementary to the guide sequence of the siRNA duplex (Elbashir et al., 2001, *Genes Dev.,* 15, 188). In addition, RNA interference can also involve small RNA (e.g., micro-RNA or miRNA) mediated gene silencing, presumably though cellular mechanisms that regulate chromatin structure and thereby prevent transcription of target gene sequences (see for example Allshire, 2002, *Science,* 297, 1818-1819; Volpe et al., 2002, *Science,* 297, 1833-1837; Jenuwein, 2002, *Science,* 297, 2215-2218; and Hall et al., 2002, *Science,* 297, 2232-2237). As such, siNA molecules of the invention can be used to mediate gene silencing via interaction with RNA transcripts or alternately by interaction with particular gene sequences, wherein such interaction results in gene silencing either at the transcriptional level or post-transcriptional level.

[0470] RNAi has been studied in a variety of systems. Fire et al., 1998, *Nature,* 391, 806, were the first to observe RNAi in *C. elegans*. Wianny and Goetz, 1999, *Nature Cell Biol.,* 2, 70, describe RNAi mediated by dsRNA in mouse embryos. Hammond et al., 2000, *Nature,* 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir et al., 2001, *Nature,* 411, 494, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in *Drosophila* embryonic lysates has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21 nucleotide siRNA duplexes are most active when containing two 2-nucleotide 3'-terminal nucleotide overhangs. Furthermore, substitution of one or both siRNA strands with 2'-deoxy or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of 3'-terminal siRNA nucleotides with deoxy nucleotides was shown to be tolerated. Mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end (Elbashir et al., 2001, *EMBO J.,* 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen et al., 2001, *Cell,* 107, 309); however, siRNA molecules lacking a 5'-phosphate are active when introduced exogenously, suggesting that 5'-phosphorylation of siRNA constructs may occur in vivo.

Duplex Forming Oligonucleotides (DFO) of the Invention

[0471] In one embodiment, the invention features siNA molecules comprising duplex forming oligonucleotides (DFO) that can self-assemble into double stranded oligonucleotides. The duplex forming oligonucleotides of the invention can be chemically synthesized or expressed from transcription units and/or vectors. The DFO molecules of the instant invention provide useful reagents and methods for a variety of therapeutic, diagnostic, agricultural, veterinary, target validation, genomic discovery, genetic engineering and pharmacogenomic applications.

[0472] Applicant demonstrates herein that certain oligonucleotides, referred to herein for convenience but not limitation as duplex forming oligonucleotides or DFO molecules, are potent mediators of sequence specific regulation of gene expression. The oligonucleotides of the invention are distinct from other nucleic acid sequences known in the art (e.g., siRNA, miRNA, stRNA, shRNA, antisense oligonucleotides etc.) in that they represent a class of linear polynucleotide sequences that are designed to self-assemble into double stranded oligonucleotides, where each strand in the double stranded oligonucleotides comprises a nucleotide sequence that is complementary to a SREBP1 target nucleic acid molecule. Nucleic acid molecules of the invention can thus self assemble into functional duplexes in which each strand of the duplex comprises the same polynucleotide sequence and each strand comprises a nucleotide sequence that is complementary to a SREBP1 target nucleic acid molecule.

[0473] Generally, double stranded oligonucleotides are formed by the assembly of two distinct oligonucleotide sequences where the oligonucleotide sequence of one strand is complementary to the oligonucleotide sequence of the second strand; such double stranded oligonucleotides are assembled from two separate oligonucleotides, or from a single molecule that folds on itself to form a double stranded structure, often referred to in the field as hairpin stem-loop structure (e.g., shRNA or short hairpin RNA). These double stranded oligonucleotides known in the art all have a common feature in that each strand of the duplex has a distinct nucleotide sequence.

[0474] Distinct from the double stranded nucleic acid molecules known in the art, the applicants have developed a novel, potentially cost effective and simplified method of forming a double stranded nucleic acid molecule starting from a single stranded or linear oligonucleotide. The two strands of the double stranded oligonucleotide formed according to the instant invention have the same nucleotide sequence and are not covalently linked to each other. Such double-stranded oligonucleotides molecules can be readily linked post-synthetically by methods and reagents known in the art and are within the scope of the invention. In one embodiment, the single stranded oligonucleotide of the invention (the duplex forming oligonucleotide) that forms a double stranded oligonucleotide comprises a first region and a second region, where the second region includes a nucleotide sequence that is an inverted repeat of the nucleotide sequence in the first region, or a portion thereof, such that the single stranded oligonucleotide self assembles to form a duplex oligonucleotide in which the nucleotide sequence of one strand of the duplex is the same as the nucleotide

sequence of the second strand. Non-limiting examples of such duplex forming oligonucleotides are illustrated in FIGS. **14** and **15**. These duplex forming oligonucleotides (DFOs) can optionally include certain palindrome or repeat sequences where such palindrome or repeat sequences are present in between the first region and the second region of the DFO.

[0475] In one embodiment, the invention features a duplex forming oligonucleotide (DFO) molecule, wherein the DFO comprises a duplex forming self complementary nucleic acid sequence that has nucleotide sequence complementary to a SREBP1 target nucleic acid sequence. The DFO molecule can comprise a single self complementary sequence or a duplex resulting from assembly of such self complementary sequences.

[0476] In one embodiment, a duplex forming oligonucleotide (DFO) of the invention comprises a first region and a second region, wherein the second region comprises a nucleotide sequence comprising an inverted repeat of nucleotide sequence of the first region such that the DFO molecule can assemble into a double stranded oligonucleotide. Such double stranded oligonucleotides can act as a short interfering nucleic acid (siNA) to modulate gene expression. Each strand of the double stranded oligonucleotide duplex formed by DFO molecules of the invention can comprise a nucleotide sequence region that is complementary to the same nucleotide sequence in a SREBP1 target nucleic acid molecule (e.g., SREBP1 target RNA).

[0477] In one embodiment, the invention features a single stranded DFO that can assemble into a double stranded oligonucleotide. The applicant has surprisingly found that a single stranded oligonucleotide with nucleotide regions of self complementarity can readily assemble into duplex oligonucleotide constructs. Such DFOs can assemble into duplexes that can inhibit gene expression in a sequence specific manner. The DFO molecules of the invention comprise a first region with nucleotide sequence that is complementary to the nucleotide sequence of a second region and where the sequence of the first region is complementary to a SREBP1 target nucleic acid (e.g., RNA). The DFO can form a double stranded oligonucleotide wherein a portion of each strand of the double stranded oligonucleotide comprises a sequence complementary to a SREBP1 target nucleic acid sequence.

[0478] In one embodiment, the invention features a double stranded oligonucleotide, wherein the two strands of the double stranded oligonucleotide are not covalently linked to each other, and wherein each strand of the double stranded oligonucleotide comprises a nucleotide sequence that is complementary to the same nucleotide sequence in a SREBP1 target nucleic acid molecule or a portion thereof (e.g., SREBP1 RNA target). In another embodiment, the two strands of the double stranded oligonucleotide share an identical nucleotide sequence of at least about 15, preferably at least about 16, 17, 18, 19, 20, or 21 nucleotides.

[0479] In one embodiment, a DFO molecule of the invention comprises a structure having Formula DFO-I:

5'-*p*-XZX'-3'

wherein Z comprises a palindromic or repeat nucleic acid sequence optionally with one or more modified nucleotides (e.g., nucleotide with a modified base, such as 2-amino purine, 2-amino-1,6-dihydro purine or a universal base), for example of length about 2 to about 24 nucleotides in even numbers (e.g., about 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, or 22 or 24 nucleotides), X represents a nucleic acid sequence, for example of length of about 1 to about 21 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 nucleotides), X' comprises a nucleic acid sequence, for example of length about 1 and about 21 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 21 nucleotides) having nucleotide sequence complementarity to sequence X or a portion thereof, p comprises a terminal phosphate group that can be present or absent, and wherein sequence X and Z, either independently or together, comprise nucleotide sequence that is complementary to a SREBP1 target nucleic acid sequence or a portion thereof and is of length sufficient to interact (e.g., base pair) with the SREBP1 target nucleic acid sequence or a portion thereof (e.g., SREBP1 RNA target). For example, X independently can comprise a sequence from about 12 to about 21 or more (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more) nucleotides in length that is complementary to nucleotide sequence in a SREBP1 target RNA or a portion thereof. In another non-limiting example, the length of the nucleotide sequence of X and Z together, when X is present, that is complementary to the SREBP1 target or a portion thereof (e.g., SREBP1 RNA target) is from about 12 to about 21 or more nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more). In yet another non-limiting example, when X is absent, the length of the nucleotide sequence of Z that is complementary to the SREBP1 target or a portion thereof is from about 12 to about 24 or more nucleotides (e.g., about 12, 14, 16, 18, 20, 22, 24, or more). In one embodiment X, Z and X' are independently oligonucleotides, where X and/or Z comprises a nucleotide sequence of length sufficient to interact (e.g., base pair) with a nucleotide sequence in the SREBP1 target or a portion thereof (e.g., SREBP1 RNA target). In one embodiment, the lengths of oligonucleotides X and X' are identical. In another embodiment, the lengths of oligonucleotides X and X' are not identical. In another embodiment, the lengths of oligonucleotides X and Z, or Z and X', or X, Z and X' are either identical or different.

[0480] When a sequence is described in this specification as being of "sufficient" length to interact (i.e., base pair) with another sequence, it is meant that the length is such that the number of bonds (e.g., hydrogen bonds) formed between the two sequences is enough to enable the two sequence to form a duplex under the conditions of interest. Such conditions can be in vitro (e.g., for diagnostic or assay purposes) or in vivo (e.g., for therapeutic purposes). It is a simple and routine matter to determine such lengths.

[0481] In one embodiment, the invention features a double stranded oligonucleotide construct having Formula DFO-I (a):

5'-*p*-XZX'-3'

3'-X'ZX-*p*-5'

wherein Z comprises a palindromic or repeat nucleic acid sequence or palindromic or repeat-like nucleic acid sequence with one or more modified nucleotides (e.g., nucleotides with a modified base, such as 2-amino purine, 2-amino-1,6-dihydro purine or a universal base), for example of length about 2 to about 24 nucleotides in even numbers (e.g., about 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 24 nucleotides), X represents a nucleic acid sequence, for example of length about 1 to about 21 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 nucleotides), X' comprises a nucleic acid sequence, for example of length about 1 to

about 21 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleotides) having nucleotide sequence complementarity to sequence X or a portion thereof, p comprises a terminal phosphate group that can be present or absent, and wherein each X and Z independently comprises a nucleotide sequence that is complementary to a SREBP1 target nucleic acid sequence or a portion thereof (e.g., SREBP1 RNA target) and is of length sufficient to interact with the SREBP1 target nucleic acid sequence of a portion thereof (e.g., SREBP1 RNA target). For example, sequence X independently can comprise a sequence from about 12 to about 21 or more nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more) in length that is complementary to a nucleotide sequence in a SREBP1 target or a portion thereof (e.g., SREBP1 RNA target). In another non-limiting example, the length of the nucleotide sequence of X and Z together (when X is present) that is complementary to the SREBP1 target or a portion thereof is from about 12 to about 21 or more nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more). In yet another non-limiting example, when X is absent, the length of the nucleotide sequence of Z that is complementary to the SREBP1 target or a portion thereof is from about 12 to about 24 or more nucleotides (e.g., about 12, 14, 16, 18, 20, 22, 24 or more). In one embodiment X, Z and X' are independently oligonucleotides, where X and/or Z comprises a nucleotide sequence of length sufficient to interact (e.g., base pair) with nucleotide sequence in the SREBP1 target or a portion thereof (e.g., SREBP1 RNA target). In one embodiment, the lengths of oligonucleotides X and X' are identical. In another embodiment, the lengths of oligonucleotides X and X' are not identical. In another embodiment, the lengths of oligonucleotides X and Z or Z and X' or X, Z and X' are either identical or different. In one embodiment, the double stranded oligonucleotide construct of Formula I(a) includes one or more, specifically 1, 2, 3 or 4, mismatches, to the extent such mismatches do not significantly diminish the ability of the double stranded oligonucleotide to inhibit SREBP1 target gene expression.

[0482] In one embodiment, a DFO molecule of the invention comprises structure having Formula DFO-II:

5'-*p*-XX'-3'

wherein each X and X' are independently oligonucleotides of length about 12 nucleotides to about 21 nucleotides, wherein X comprises, for example, a nucleic acid sequence of length about 12 to about 21 nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleotides), X' comprises a nucleic acid sequence, for example of length about 12 to about 21 nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 nucleotides) having nucleotide sequence complementarity to sequence X or a portion thereof, p comprises a terminal phosphate group that can be present or absent, and wherein X comprises a nucleotide sequence that is complementary to a SREBP1 target nucleic acid sequence (e.g., SREBP1 target RNA) or a portion thereof and is of length sufficient to interact (e.g., base pair) with the SREBP1 target nucleic acid sequence of a portion thereof. In one embodiment, the length of oligonucleotides X and X' are identical. In another embodiment the length of oligonucleotides X and X' are not identical. In one embodiment, length of the oligonucleotides X and X' are sufficient to form a relatively stable double stranded oligonucleotide.

[0483] In one embodiment, the invention features a double stranded oligonucleotide construct having Formula DFO-II (a):

5'-*p*-XX'-3'

3'-X'X-*p*-5'

wherein each X and X' are independently oligonucleotides of length about 12 nucleotides to about 21 nucleotides, wherein X comprises a nucleic acid sequence, for example of length about 12 to about 21 nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleotides), X' comprises a nucleic acid sequence, for example of length about 12 to about 21 nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleotides) having nucleotide sequence complementarity to sequence X or a portion thereof, p comprises a terminal phosphate group that can be present or absent, and wherein X comprises nucleotide sequence that is complementary to a SREBP1 target nucleic acid sequence or a portion thereof (e.g., SREBP1 RNA target) and is of length sufficient to interact (e.g., base pair) with the SREBP1 target nucleic acid sequence (e.g., SREBP1 target RNA) or a portion thereof. In one embodiment, the lengths of oligonucleotides X and X' are identical. In another embodiment, the lengths of oligonucleotides X and X' are not identical. In one embodiment, the lengths of the oligonucleotides X and X' are sufficient to form a relatively stable double stranded oligonucleotide. In one embodiment, the double stranded oligonucleotide construct of Formula II(a) includes one or more, specifically 1, 2, 3 or 4, mismatches, to the extent such mismatches do not significantly diminish the ability of the double stranded oligonucleotide to inhibit SREBP1 target gene expression.

[0484] In one embodiment, the invention features a DFO molecule having Formula DFO-I(b):

5'-*p*-Z-3'

where Z comprises a palindromic or repeat nucleic acid sequence optionally including one or more non-standard or modified nucleotides (e.g., nucleotide with a modified base, such as 2-amino purine or a universal base) that can facilitate base-pairing with other nucleotides. Z can be, for example, of length sufficient to interact (e.g., base pair) with nucleotide sequence of a SREBP1 target nucleic acid (e.g., SREBP1 target RNA) molecule, preferably of length of at least 12 nucleotides, specifically about 12 to about 24 nucleotides (e.g., about 12, 14, 16, 18, 20, 22 or 24 nucleotides). p represents a terminal phosphate group that can be present or absent.

[0485] In one embodiment, a DFO molecule having any of Formula DFO-I, DFO-I(a), DFO-I(b), DFO-II(a) or DFO-II can comprise chemical modifications as described herein without limitation, such as, for example, nucleotides having any of Formulae I-VII, stabilization chemistries as described in Table IV, or any other combination of modified nucleotides and non-nucleotides as described in the various embodiments herein.

[0486] In one embodiment, the palindrome or repeat sequence or modified nucleotide (e.g., nucleotide with a modified base, such as 2-amino purine or a universal base) in Z of DFO constructs having Formula DFO-I, DFO-I(a) and DFO-I(b), comprises chemically modified nucleotides that are able to interact with a portion of the SREBP1 target nucleic acid sequence (e.g., modified base analogs that can form Watson Crick base pairs or non-Watson Crick base pairs).

[0487] In one embodiment, a DFO molecule of the invention, for example a DFO having Formula DFO-I or DFO-II, comprises about 15 to about 40 nucleotides (e.g., about 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, or 40 nucleotides). In one embodiment, a DFO molecule of the invention comprises one or more chemical modifications. In a non-limiting example, the introduction of chemically modified nucleotides and/or non-nucleotides into nucleic acid molecules of the invention provides a powerful tool in overcoming potential limitations of in vivo stability and bioavailability inherent to unmodified RNA molecules that are delivered exogenously. For example, the use of chemically modified nucleic acid molecules can enable a lower dose of a particular nucleic acid molecule for a given therapeutic effect since chemically modified nucleic acid molecules tend to have a longer half-life in serum or in cells or tissues. Furthermore, certain chemical modifications can improve the bioavailability and/or potency of nucleic acid molecules by not only enhancing half-life but also facilitating the targeting of nucleic acid molecules to particular organs, cells or tissues and/or improving cellular uptake of the nucleic acid molecules. Therefore, even if the activity of a chemically modified nucleic acid molecule is reduced in vitro as compared to a native/unmodified nucleic acid molecule, for example when compared to an unmodified RNA molecule, the overall activity of the modified nucleic acid molecule can be greater than the native or unmodified nucleic acid molecule due to improved stability, potency, duration of effect, bioavailability and/or delivery of the molecule.

Multifunctional or Multi-Targeted siNA Molecules of the Invention

[0488] In one embodiment, the invention features siNA molecules comprising multifunctional short interfering nucleic acid (multifunctional siNA) molecules that modulate the expression of one or more SREBP1 target genes in a biologic system, such as a cell, tissue, or organism. The multifunctional short interfering nucleic acid (multifunctional siNA) molecules of the invention can target more than one region of the SREBP1 target nucleic acid sequence or can target sequences of more than one distinct target nucleic acid molecules (e.g., SREBP1 or SREBP1 pathway RNA and/or DNA targets). The multifunctional siNA molecules of the invention can be chemically synthesized or expressed from transcription units and/or vectors. The multifunctional siNA molecules of the instant invention provide useful reagents and methods for a variety of human applications, therapeutic, diagnostic, agricultural, veterinary, target validation, genomic discovery, genetic engineering and pharmacogenomic applications.

[0489] Applicant demonstrates herein that certain oligonucleotides, referred to herein for convenience but not limitation as multifunctional short interfering nucleic acid or multifunctional siNA molecules, are potent mediators of sequence specific regulation of gene expression. The multifunctional siNA molecules of the invention are distinct from other nucleic acid sequences known in the art (e.g., siRNA, miRNA, stRNA, shRNA, antisense oligonucleotides, etc.) in that they represent a class of polynucleotide molecules that are designed such that each strand in the multifunctional siNA construct comprises a nucleotide sequence that is complementary to a distinct nucleic acid sequence in one or more target nucleic acid molecules. A single multifunctional siNA molecule (generally a double-stranded molecule) of the invention can thus target more than one (e.g., 2, 3, 4, 5, or more) differing target nucleic acid target molecules. Nucleic acid molecules of the invention can also target more than one (e.g., 2, 3, 4, 5, or more) region of the same target nucleic acid sequence. As such multifunctional siNA molecules of the invention are useful in down regulating or inhibiting the expression of one or more target nucleic acid molecules. For example, a multifunctional siNA molecule of the invention can target (e.g., have complementarity to) nucleic acid molecules selected from the group consisting of SREBP1, H6PD, PTP-1B, apoAI/CIII/AIV, CETP, ApoB, PCSK9, calcium/calmodulin-dependent protein kinase ID, fatty acid binding protein 5, proprotein convertase subtilisin/kexin 9, LXR target genes, metalloprotease domain 11, apoptosis-inhibitory 6, F-box-only protein 3 or any combination thereof. By reducing or inhibiting expression of more than one target nucleic acid molecule with one multifunctional siNA construct, multifunctional siNA molecules of the invention represent a class of potent therapeutic agents that can provide simultaneous inhibition of multiple targets within a disease (e.g., angiogenic) related pathway. Such simultaneous inhibition can provide synergistic therapeutic treatment strategies without the need for separate preclinical and clinical development efforts or complex regulatory approval process.

[0490] Use of multifunctional siNA molecules that target more then one region of a target nucleic acid molecule (e.g., SREBP1 target RNA or DNA) is expected to provide potent inhibition of gene expression. For example, a single multifunctional siNA construct of the invention can target both conserved and variable regions of a target nucleic acid molecule (e.g., SREBP1 RNA or DNA), thereby allowing down regulation or inhibition of different SREBP1 isoforms or variants to optimize therapeutic efficacy and minimize toxicity, or allowing for targeting of both coding and non-coding regions of the SREBP1 target nucleic acid molecule.

[0491] Generally, double stranded oligonucleotides are formed by the assembly of two distinct oligonucleotides where the oligonucleotide sequence of one strand is complementary to the oligonucleotide sequence of the second strand; such double stranded oligonucleotides are generally assembled from two separate oligonucleotides (e.g., siRNA). Alternately, a duplex can be formed from a single molecule that folds on itself (e.g., shRNA or short hairpin RNA). These double stranded oligonucleotides are known in the art to mediate RNA interference and all have a common feature wherein only one nucleotide sequence region (guide sequence or the antisense sequence) has complementarity to a target nucleic acid sequence, and the other strand (sense sequence) comprises nucleotide sequence that is homologous to the target nucleic acid sequence. Generally, the antisense sequence is retained in the active RISC complex and guides the RISC to the target nucleotide sequence by means of complementary base-pairing of the antisense sequence with the target sequence for mediating sequence-specific RNA interference. It is known in the art that in some cell culture systems, certain types of unmodified siRNAs can exhibit "off target" effects. It is hypothesized that this off-target effect involves the participation of the sense sequence instead of the antisense sequence of the siRNA in the RISC complex (see for example Schwarz et al., 2003, Cell, 115, 199-208). In this instance the sense sequence is believed to direct the RISC complex to a sequence (off-target sequence) that is distinct from the intended target sequence, resulting in the inhibition of the off-target sequence. In these double stranded nucleic acid molecules, each strand is complementary to a distinct

target nucleic acid sequence. However, the off-targets that are affected by these dsRNAs are not entirely predictable and are non-specific.

[0492] Distinct from the double stranded nucleic acid molecules known in the art, the applicants have developed a novel, potentially cost effective and simplified method of down regulating or inhibiting the expression of more than one target nucleic acid sequence using a single multifunctional siNA construct. The multifunctional siNA molecules of the invention are designed to be double-stranded or partially double stranded, such that a portion of each strand or region of the multifunctional siNA is complementary to a target nucleic acid sequence of choice. As such, the multifunctional siNA molecules of the invention are not limited to targeting sequences that are complementary to each other, but rather to any two differing target nucleic acid sequences. Multifunctional siNA molecules of the invention are designed such that each strand or region of the multifunctional siNA molecule, that is complementary to a given target nucleic acid sequence, is of suitable length (e.g., from about 16 to about 28 nucleotides in length, preferably from about 18 to about 28 nucleotides in length) for mediating RNA interference against the target nucleic acid sequence. The complementarity between the target nucleic acid sequence and a strand or region of the multifunctional siNA must be sufficient (at least about 8 base pairs) for cleavage of the target nucleic acid sequence by RNA interference. Multifunctional siNA of the invention is expected to minimize off-target effects seen with certain siRNA sequences, such as those described in Schwarz et al., supra.

[0493] It has been reported that dsRNAs of length between 29 base pairs and 36 base pairs (Tuschl et al., International PCT Publication No. WO 02/44321) do not mediate RNAi. One reason these dsRNAs are inactive may be the lack of turnover or dissociation of the strand that interacts with the target RNA sequence, such that the RISC complex is not able to efficiently interact with multiple copies of the target RNA resulting in a significant decrease in the potency and efficiency of the RNAi process. Applicant has surprisingly found that the multifunctional siNAs of the invention can overcome this hurdle and are capable of enhancing the efficiency and potency of RNAi process. As such, in certain embodiments of the invention, multifunctional siNAs of length of about 29 to about 36 base pairs can be designed such that, a portion of each strand of the multifunctional siNA molecule comprises a nucleotide sequence region that is complementary to a target nucleic acid of length sufficient to mediate RNAi efficiently (e.g., about 15 to about 23 base pairs) and a nucleotide sequence region that is not complementary to the target nucleic acid. By having both complementary and non-complementary portions in each strand of the multifunctional siNA, the multifunctional siNA can mediate RNA interference against a target nucleic acid sequence without being prohibitive to turnover or dissociation (e.g., where the length of each strand is too long to mediate RNAi against the respective target nucleic acid sequence). Furthermore, design of multifunctional siNA molecules of the invention with internal overlapping regions allows the multifunctional siNA molecules to be of favorable (decreased) size for mediating RNA interference and of size that is well suited for use as a therapeutic agent (e.g., wherein each strand is independently from about 18 to about 28 nucleotides in length). Non-limiting examples are illustrated in FIGS. **16-28** and Table III.

[0494] In one embodiment, a multifunctional siNA molecule of the invention comprises a first region and a second region, where the first region of the multifunctional siNA comprises a nucleotide sequence complementary to a nucleic acid sequence of a first target nucleic acid molecule, and the second region of the multifunctional siNA comprises nucleic acid sequence complementary to a nucleic acid sequence of a second target nucleic acid molecule. In one embodiment, a multifunctional siNA molecule of the invention comprises a first region and a second region, where the first region of the multifunctional siNA comprises nucleotide sequence complementary to a nucleic acid sequence of the first region of a target nucleic acid molecule, and the second region of the multifunctional siNA comprises nucleotide sequence complementary to a nucleic acid sequence of a second region of a the target nucleic acid molecule. In another embodiment, the first region and second region of the multifunctional siNA can comprise separate nucleic acid sequences that share some degree of complementarity (e.g., from about 1 to about 10 complementary nucleotides). In certain embodiments, multifunctional siNA constructs comprising separate nucleic acid sequences can be readily linked post-synthetically by methods and reagents known in the art and such linked constructs are within the scope of the invention. Alternately, the first region and second region of the multifunctional siNA can comprise a single nucleic acid sequence having some degree of self complementarity, such as in a hairpin or stem-loop structure. Non-limiting examples of such double stranded and hairpin multifunctional short interfering nucleic acids are illustrated in FIGS. **16** and **17** respectively. These multifunctional short interfering nucleic acids (multifunctional siNAs) can optionally include certain overlapping nucleotide sequence where such overlapping nucleotide sequence is present in between the first region and the second region of the multifunctional siNA (see for example FIGS. **18** and **19**).

[0495] In one embodiment, the invention features a multifunctional short interfering nucleic acid (multifunctional siNA) molecule, wherein each strand of the multifunctional siNA independently comprises a first region of nucleic acid sequence that is complementary to a distinct target nucleic acid sequence and the second region of nucleotide sequence that is not complementary to the target sequence. The target nucleic acid sequence of each strand is in the same target nucleic acid molecule or different target nucleic acid molecules.

[0496] In another embodiment, the multifunctional siNA comprises two strands, where: (a) the first strand comprises a region having sequence complementarity to a target nucleic acid sequence (complementary region 1) and a region having no sequence complementarity to the target nucleotide sequence (non-complementary region 1); (b) the second strand of the multifunction siNA comprises a region having sequence complementarity to a target nucleic acid sequence that is distinct from the target nucleotide sequence complementary to the first strand nucleotide sequence (complementary region 2), and a region having no sequence complementarity to the target nucleotide sequence of complementary region 2 (non-complementary region 2); (c) the complementary region 1 of the first strand comprises a nucleotide sequence that is complementary to a nucleotide sequence in the non-complementary region 2 of the second strand and the complementary region 2 of the second strand comprises a nucleotide sequence that is complementary to a nucleotide sequence in the non-complementary region 1 of the first

strand. The target nucleic acid sequence of complementary region 1 and complementary region 2 is in the same target nucleic acid molecule or different target nucleic acid molecules.

[0497] In another embodiment, the multifunctional siNA comprises two strands, where: (a) the first strand comprises a region having sequence complementarity to a target nucleic acid sequence derived from a gene (e.g., a first SREBP1 gene) (complementary region 1) and a region having no sequence complementarity to the target nucleotide sequence of complementary region 1 (non-complementary region 1); (b) the second strand of the multifunction siNA comprises a region having sequence complementarity to a target nucleic acid sequence derived from a gene (e.g., a second SREBP1 gene) that is distinct from the gene of complementary region 1 (complementary region 2), and a region having no sequence complementarity to the target nucleotide sequence of complementary region 2 (non-complementary region 2); (c) the complementary region 1 of the first strand comprises a nucleotide sequence that is complementary to a nucleotide sequence in the non-complementary region 2 of the second strand and the complementary region 2 of the second strand comprises a nucleotide sequence that is complementary to a nucleotide sequence in the non-complementary region 1 of the first strand.

[0498] In another embodiment, the multifunctional siNA comprises two strands, where: (a) the first strand comprises a region having sequence complementarity to a target nucleic acid sequence derived from a gene (e.g., SREBP1 gene) (complementary region 1) and a region having no sequence complementarity to the target nucleotide sequence of complementary region 1 (non-complementary region 1); (b) the second strand of the multifunction siNA comprises a region having sequence complementarity to a target nucleic acid sequence distinct from the target nucleic acid sequence of complementary region 1 (complementary region 2), provided, however, that the target nucleic acid sequence for complementary region 1 and target nucleic acid sequence for complementary region 2 are both derived from the same gene, and a region having no sequence complementarity to the target nucleotide sequence of complementary region 2 (non-complementary region 2); (c) the complementary region 1 of the first strand comprises a nucleotide sequence that is complementary to a nucleotide sequence in the non-complementary region 2 of the second strand and the complementary region 2 of the second strand comprises a nucleotide sequence that is complementary to nucleotide sequence in the non-complementary region 1 of the first strand.

[0499] In one embodiment, the invention features a multifunctional short interfering nucleic acid (multifunctional siNA) molecule, wherein the multifunctional siNA comprises two complementary nucleic acid sequences in which the first sequence comprises a first region having nucleotide sequence complementary to nucleotide sequence within a first target nucleic acid molecule, and in which the second sequence comprises a first region having nucleotide sequence complementary to a distinct nucleotide sequence within the same target nucleic acid molecule. Preferably, the first region of the first sequence is also complementary to the nucleotide sequence of the second region of the second sequence, and where the first region of the second sequence is complementary to the nucleotide sequence of the second region of the first sequence.

[0500] In one embodiment, the invention features a multifunctional short interfering nucleic acid (multifunctional siNA) molecule, wherein the multifunctional siNA comprises two complementary nucleic acid sequences in which the first sequence comprises a first region having a nucleotide sequence complementary to a nucleotide sequence within a first target nucleic acid molecule, and in which the second sequence comprises a first region having a nucleotide sequence complementary to a distinct nucleotide sequence within a second target nucleic acid molecule. Preferably, the first region of the first sequence is also complementary to the nucleotide sequence of the second region of the second sequence, and where the first region of the second sequence is complementary to the nucleotide sequence of the second region of the first sequence.

[0501] In one embodiment, the invention features a multifunctional siNA molecule comprising a first region and a second region, where the first region comprises a nucleic acid sequence having about 18 to about 28 nucleotides complementary to a nucleic acid sequence within a first target nucleic acid molecule, and the second region comprises nucleotide sequence having about 18 to about 28 nucleotides complementary to a distinct nucleic acid sequence within a second target nucleic acid molecule.

[0502] In one embodiment, the invention features a multifunctional siNA molecule comprising a first region and a second region, where the first region comprises nucleic acid sequence having about 18 to about 28 nucleotides complementary to a nucleic acid sequence within a target nucleic acid molecule, and the second region comprises nucleotide sequence having about 18 to about 28 nucleotides complementary to a distinct nucleic acid sequence within the same target nucleic acid molecule.

[0503] In one embodiment, the invention features a double stranded multifunctional short interfering nucleic acid (multifunctional siNA) molecule, wherein one strand of the multifunctional siNA comprises a first region having nucleotide sequence complementary to a first target nucleic acid sequence, and the second strand comprises a first region having a nucleotide sequence complementary to a second target nucleic acid sequence. The first and second target nucleic acid sequences can be present in separate target nucleic acid molecules or can be different regions within the same target nucleic acid molecule. As such, multifunctional siNA molecules of the invention can be used to target the expression of different genes, splice variants of the same gene, both mutant and conserved regions of one or more gene transcripts, or both coding and non-coding sequences of the same or differing genes or gene transcripts.

[0504] In one embodiment, a target nucleic acid molecule of the invention encodes a single protein. In another embodiment, a target nucleic acid molecule encodes more than one protein (e.g., 1, 2, 3, 4, 5 or more proteins). As such, a multifunctional siNA construct of the invention can be used to down regulate or inhibit the expression of several proteins. For example, a multifunctional siNA molecule comprising a region in one strand having nucleotide sequence complementarity to a first target nucleic acid sequence derived from a SREBP1 target, and the second strand comprising a region with nucleotide sequence complementarity to a second target nucleic acid sequence present in target nucleic acid molecules from genes encoding two proteins (e.g., two differing proteins) selected from the group consisting of SREBP1, H6PD, PTP-1B, apoAI/CIII/AIV, CETP, ApoB, PCSK9, calcium/

calmodulin-dependent protein kinase 1D, fatty acid binding protein 5, proprotein convertase subtilisin/kexin 9, LXR target genes, metalloprotease domain 11, apoptosis-inhibitory 6, F-box-only protein 3 or any combination thereof, which can be used to down regulate, inhibit, or shut down a particular biologic pathway by targeting multiple SREBP1 or SREBP1 pathway genes.

[0505] In one embodiment the invention takes advantage of conserved nucleotide sequences present in different SREBP1 variants. By designing multifunctional siNAs in a manner where one strand includes a sequence that is complementary to one or more target nucleic acid sequences that are conserved among various SREBP1 family members and the other strand optionally includes sequence that is complementary to SREBP1 pathway target nucleic acid sequence, such as any of SREBP1, H6PD, PTP-1B, apoAI/CIII/AIV, CETP, ApoB, PCSK9, calcium/calmodulin-dependent protein kinase ID, fatty acid binding protein 5, proprotein convertase subtilisin/kexin 9, LXR target genes, metalloprotease domain 11, apoptosis-inhibitory 6, F-box-only protein 3, it is possible to selectively and effectively inhibit a SREBP1 disease related biological pathway (e.g., hyperlipidemia and/or insulin resistance) using a single multifunctional siNA.

[0506] In one embodiment, a multifunctional short interfering nucleic acid (multifunctional siNA) of the invention comprises a first region and a second region, wherein the first region comprises nucleotide sequence complementary to a first SREBP1 RNA of a first SREBP1 target and the second region comprises nucleotide sequence complementary to a second SREBP1 RNA of a second SREBP1 target. In one embodiment, the first and second regions can comprise nucleotide sequence complementary to shared or conserved RNA sequences of differing SREBP1 target sites within the same SREBP1 target sequence or shared amongst different SREBP1 target sequences.

[0507] In one embodiment, a double stranded multifunctional siNA molecule of the invention comprises a structure having Formula MF-I:

5'-*p*-XZX'-3'

3'-Y'ZY-*p*-5'

wherein each 5'-p-XZX'-3' and 5'-p-YZY'-3' are independently an oligonucleotide of length about 20 nucleotides to about 300 nucleotides, preferably about 20 to about 200 nucleotides, about 20 to about 100 nucleotides, about 20 to about 40 nucleotides, about 20 to about 40 nucleotides, about 24 to about 38 nucleotides, or about 26 to about 38 nucleotides; XZ comprises a nucleic acid sequence that is complementary to a first SREBP1 target nucleic acid sequence; YZ is an oligonucleotide comprising nucleic acid sequence that is complementary to a second SREBP1 target nucleic acid sequence; Z comprises nucleotide sequence of length about 1 to about 24 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 nucleotides) that is self complementary; X comprises nucleotide sequence of length about 1 to about 100 nucleotides, preferably about 1 to about 21 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 nucleotides) that is complementary to nucleotide sequence present in region Y'; Y comprises nucleotide sequence of length about 1 to about 100 nucleotides, preferably about 1 to about 21 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleotides) that is complementary to nucleotide sequence present in region X'; each p comprises a terminal phosphate group that is independently present or absent; each XZ and YZ is independently of length sufficient to stably interact (i.e., base pair) with the first and second target nucleic acid sequence, respectively, or a portion thereof. For example, each sequence X and Y can independently comprise sequence from about 12 to about 21 or more nucleotides in length (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more) that is complementary to a target nucleotide sequence in different target nucleic acid molecules, such as target RNAs or a portion thereof. In another non-limiting example, the length of the nucleotide sequence of X and Z together that is complementary to the first SREBP1 target nucleic acid sequence or a portion thereof is from about 12 to about 21 or more nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more). In another non-limiting example, the length of the nucleotide sequence of Y and Z together, that is complementary to the second SREBP1 target nucleic acid sequence or a portion thereof is from about 12 to about 21 or more nucleotides (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more). In one embodiment, the first SREBP1 target nucleic acid sequence and the second SREBP1 target nucleic acid sequence are present in the same target nucleic acid molecule (e.g., SREBP1 target RNA or SREBP1 pathway target RNA such as SREBP1, H6PD, PTP-1B, apoAI/CIII/AIV, CETP, ApoB, PCSK9, calcium/calmodulin-dependent protein kinase 1D, fatty acid binding protein 5, proprotein convertase subtilisin/kexin 9, LXR target genes, metalloprotease domain 11, apoptosis-inhibitory 6, and/or F-box-only protein 3 RNA). In another embodiment, the first SREBP1 target nucleic acid sequence and the second SREBP1 target nucleic acid sequence are present in different target nucleic acid molecules (e.g., SREBP1 target RNA and SREBP1 pathway target RNA). In one embodiment, Z comprises a palindrome or a repeat sequence. In one embodiment, the lengths of oligonucleotides X and X' are identical. In another embodiment, the lengths of oligonucleotides X and X' are not identical. In one embodiment, the lengths of oligonucleotides Y and Y' are identical. In another embodiment, the lengths of oligonucleotides Y and Y' are not identical. In one embodiment, the double stranded oligonucleotide construct of Formula I(a) includes one or more, specifically 1, 2, 3 or 4, mismatches, to the extent such mismatches do not significantly diminish the ability of the double stranded oligonucleotide to inhibit target gene expression.

[0508] In one embodiment, a multifunctional siNA molecule of the invention comprises a structure having Formula MF-II:

5'-*p*-XX'-3'

3'-Y'Y-*p*-5'

wherein each 5'-p-XX'-3' and 5'-p-YY'-3' are independently an oligonucleotide of length about 20 nucleotides to about 300 nucleotides, preferably about 20 to about 200 nucleotides, about 20 to about 100 nucleotides, about 20 to about 40 nucleotides, about 20 to about 40 nucleotides, about 24 to about 38 nucleotides, or about 26 to about 38 nucleotides; X comprises a nucleic acid sequence that is complementary to a first target nucleic acid sequence; Y is an oligonucleotide comprising nucleic acid sequence that is complementary to a second target nucleic acid sequence; X comprises a nucleotide sequence of length about 1 to about 100 nucleotides, preferably about 1 to about 21 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 nucleotides) that is complementary to nucleotide sequence

present in region Y'; Y comprises nucleotide sequence of length about 1 to about 100 nucleotides, preferably about 1 to about 21 nucleotides (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleotides) that is complementary to nucleotide sequence present in region X'; each p comprises a terminal phosphate group that is independently present or absent; each X and Y independently is of length sufficient to stably interact (i.e., base pair) with the first and second target nucleic acid sequence, respectively, or a portion thereof. For example, each sequence X and Y can independently comprise sequence from about 12 to about 21 or more nucleotides in length (e.g., about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or more) that is complementary to a target nucleotide sequence in different target nucleic acid molecules, such as SREBP1 target RNAs or a portion thereof. In one embodiment, the first SREBP1 target nucleic acid sequence and the second SREBP1 target nucleic acid sequence are present in the same target nucleic acid molecule (e.g., SREBP1 target RNA or SREBP1 pathway target RNA such as H6PD, PTP-1B, apoAI/CIII/AIV, and/or CETP RNA). In another embodiment, the first SREBP1 target nucleic acid sequence and the second SREBP1 target nucleic acid sequence are present in different target nucleic acid molecules (e.g., SREBP1 target RNA and SREBP1 pathway target RNA, such as H6PD, PTP-1B, apoAI/CIII/AIV, and/or CETP RNA). In one embodiment, Z comprises a palindrome or a repeat sequence. In one embodiment, the lengths of oligonucleotides X and X' are identical. In another embodiment, the lengths of oligonucleotides X and X' are not identical. In one embodiment, the lengths of oligonucleotides Y and Y' are identical. In another embodiment, the lengths of oligonucleotides Y and Y' are not identical. In one embodiment, the double stranded oligonucleotide construct of Formula I(a) includes one or more, specifically 1, 2, 3 or 4, mismatches, to the extent such mismatches do not significantly diminish the ability of the double stranded oligonucleotide to inhibit target gene expression.

[0509] In one embodiment, a multifunctional siNA molecule of the invention comprises a structure having Formula MF-III:

XX'

Y'—W—Y

wherein each X, X', Y, and Y' is independently an oligonucleotide of length about 15 nucleotides to about 50 nucleotides, preferably about 18 to about 40 nucleotides, or about 19 to about 23 nucleotides; X comprises nucleotide sequence that is complementary to nucleotide sequence present in region Y'; X' comprises nucleotide sequence that is complementary to nucleotide sequence present in region Y; each X and X' is independently of length sufficient to stably interact (i.e., base pair) with a first and a second SREBP1 target nucleic acid sequence, respectively, or a portion thereof; W represents a nucleotide or non-nucleotide linker that connects sequences Y' and Y; and the multifunctional siNA directs cleavage of the first and second SREBP1 target sequence via RNA interference. In one embodiment, the first SREBP1 target nucleic acid sequence and the second SREBP1 target nucleic acid sequence are present in the same target nucleic acid molecule (e.g., SREBP1 target RNA or SREBP1 pathway target RNA such as SREBP1, H6PD, PTP-1B, apoAI/CIII/AIV, CETP, ApoB, PCSK9, calcium/calmodulin-dependent protein kinase ID, fatty acid binding protein 5, proprotein convertase subtilisin/kexin 9, LXR target genes, metalloprotease domain 11, apoptosis-inhibitory 6, and/or F-box-only protein 3 RNA). In another embodiment, the first SREBP1 target nucleic acid sequence and the second SREBP1 target nucleic acid sequence are present in different target nucleic acid molecules (e.g., SREBP1 target RNA and SREBP1 pathway target RNA such as SREBP1, H6PD, PTP-1B, apoAI/CIII/AIV, CETP, ApoB, PCSK9, calcium/calmodulin-dependent protein kinase 1D, fatty acid binding protein 5, proprotein convertase subtilisin/kexin 9, LXR target genes, metalloprotease domain 11, apoptosis-inhibitory 6, and/or F-box-only protein 3 RNA). In one embodiment, region W connects the 3'-end of sequence Y' with the 3'-end of sequence Y. In one embodiment, region W connects the 3'-end of sequence Y' with the 5'-end of sequence Y. In one embodiment, region W connects the 5'-end of sequence Y' with the 5'-end of sequence Y. In one embodiment, region W connects the 5'-end of sequence Y' with the 3'-end of sequence Y. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence X. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence X'. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence Y. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence Y'. In one embodiment, W connects sequences Y and Y' via a biodegradable linker. In one embodiment, W further comprises a conjugate, label, aptamer, ligand, lipid, or polymer.

[0510] In one embodiment, a multifunctional siNA molecule of the invention comprises a structure having Formula MF-IV:

XX'

Y'—W—Y

wherein each X, X', Y, and Y' is independently an oligonucleotide of length about 15 nucleotides to about 50 nucleotides, preferably about 18 to about 40 nucleotides, or about 19 to about 23 nucleotides; X comprises nucleotide sequence that is complementary to nucleotide sequence present in region Y'; X' comprises nucleotide sequence that is complementary to nucleotide sequence present in region Y; each Y and Y' is independently of length sufficient to stably interact (i.e., base pair) with a first and a second SREBP1 target nucleic acid sequence, respectively, or a portion thereof; W represents a nucleotide or non-nucleotide linker that connects sequences Y' and Y; and the multifunctional siNA directs cleavage of the first and second SREBP1 target sequence via RNA interference. In one embodiment, the first SREBP1 target nucleic acid sequence and the second SREBP1 target nucleic acid sequence are present in the same target nucleic acid molecule (e.g., SREBP1, H6PD, PTP-1B, apoAI/CIII/AIV, CETP, ApoB, PCSK9, calcium/calmodulin-dependent protein kinase 1D, fatty acid binding protein 5, proprotein convertase subtilisin/kexin 9, LXR target genes, metalloprotease domain 11, apoptosis-inhibitory 6, and/or F-box-only protein 3 RNA). In another embodiment, the first SREBP1 target nucleic acid sequence and the second SREBP1 target nucleic acid sequence are present in different target nucleic acid molecules (e.g., SREBP1 target RNA and SREBP1 pathway target RNA such as SREBP1, H6PD, PTP-1B, apoAI/CIII/AIV, CETP, ApoB, PCSK9, calcium/calmodulin-dependent protein kinase 1D, fatty acid binding protein 5, proprotein convertase subtilisin/kexin 9, LXR target genes, metalloprotease domain 11, apoptosis-inhibitory 6, and/or F-box-only protein 3 RNA). In one embodiment, region W connects the 3'-end of sequence Y' with the 3'-end of sequence Y. In one

embodiment, region W connects the 3'-end of sequence Y' with the 5'-end of sequence Y. In one embodiment, region W connects the 5'-end of sequence Y' with the 5'-end of sequence Y. In one embodiment, region W connects the 5'-end of sequence Y' with the 3'-end of sequence Y. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence X. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence X'. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence Y. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence Y'. In one embodiment, W connects sequences Y and Y' via a biodegradable linker. In one embodiment, W further comprises a conjugate, lable, aptamer, ligand, lipid, or polymer.

[0511] In one embodiment, a multifunctional siNA molecule of the invention comprises a structure having Formula MF-V:

XX'

Y'—W—Y

wherein each X, X', Y, and Y' is independently an oligonucleotide of length about 15 nucleotides to about 50 nucleotides, preferably about 18 to about 40 nucleotides, or about 19 to about 23 nucleotides; X comprises nucleotide sequence that is complementary to nucleotide sequence present in region Y'; X' comprises nucleotide sequence that is complementary to nucleotide sequence present in region Y; each X, X', Y, or Y' is independently of length sufficient to stably interact (i.e., base pair) with a first, second, third, or fourth SREBP1 target nucleic acid sequence, respectively, or a portion thereof; W represents a nucleotide or non-nucleotide linker that connects sequences Y' and Y; and the multifunctional siNA directs cleavage of the first, second, third, and/or fourth target sequence via RNA interference. In one embodiment, the first, second, third and fourth SREBP1 target nucleic acid sequence are all present in the same target nucleic acid molecule (e.g., SREBP1 target RNA or SREBP1 pathway target RNA such as SREBP1, H6PD, PTP-1B, apoAI/CIII/AIV, CETP, ApoB, PCSK9, calcium/calmodulin-dependent protein kinase 1D, fatty acid binding protein 5, proprotein convertase subtilisin/kexin 9, LXR target genes, metalloprotease domain 11, apoptosis-inhibitory 6, and/or F-box-only protein 3 RNA). In another embodiment, the first, second, third and fourth SREBP1 target nucleic acid sequence are independently present in different target nucleic acid molecules (e.g., SREBP1 target RNA and SREBP1 pathway target RNA such as SREBP1, H6PD, PTP-1B, apoAI/CIII/AIV, CETP, ApoB, PCSK9, calcium/calmodulin-dependent protein kinase 1D, fatty acid binding protein 5, proprotein convertase subtilisin/kexin 9, LXR target genes, metalloprotease domain 11, apoptosis-inhibitory 6, and/or F-box-only protein 3 RNA). In one embodiment, region W connects the 3'-end of sequence Y' with the 3'-end of sequence Y. In one embodiment, region W connects the 3'-end of sequence Y' with the 5'-end of sequence Y. In one embodiment, region W connects the 5'-end of sequence Y' with the 5'-end of sequence Y. In one embodiment, region W connects the 5'-end of sequence Y' with the 3'-end of sequence Y. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence X. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence X'. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence Y. In one embodiment, a terminal phosphate group is present at the 5'-end of sequence Y'. In one embodiment, W connects sequences Y and Y' via a biodegradable linker. In one embodiment, W further comprises a conjugate, lable, aptamer, ligand, lipid, or polymer.

[0512] In one embodiment, regions X and Y of multifunctional siNA molecule of the invention (e.g., having any of Formula MF-I-MF-V), are complementary to different target nucleic acid sequences that are portions of the same target nucleic acid molecule. In one embodiment, such target nucleic acid sequences are at different locations within the coding region of a RNA transcript. In one embodiment, such target nucleic acid sequences comprise coding and non-coding regions of the same RNA transcript. In one embodiment, such target nucleic acid sequences comprise regions of alternately spliced transcripts or precursors of such alternately spliced transcripts.

[0513] In one embodiment, a multifunctional siNA molecule having any of Formula MF-I-MF-V can comprise chemical modifications as described herein without limitation, such as, for example, nucleotides having any of Formulae I-VII described herein, stabilization chemistries as described in Table IV, or any other combination of modified nucleotides and non-nucleotides as described in the various embodiments herein.

[0514] In one embodiment, the palindrome or repeat sequence or modified nucleotide (e.g., nucleotide with a modified base, such as 2-amino purine or a universal base) in Z of multifunctional siNA constructs having Formula MF-I or MF-II comprises chemically modified nucleotides that are able to interact with a portion of the target nucleic acid sequence (e.g., modified base analogs that can form Watson Crick base pairs or non-Watson Crick base pairs).

[0515] In one embodiment, a multifunctional siNA molecule of the invention, for example each strand of a multifunctional siNA having MF-I-MF-V, independently comprises about 15 to about 40 nucleotides (e.g., about 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, or 40 nucleotides). In one embodiment, a multifunctional siNA molecule of the invention comprises one or more chemical modifications. In a non-limiting example, the introduction of chemically modified nucleotides and/or non-nucleotides into nucleic acid molecules of the invention provides a powerful tool in overcoming potential limitations of in vivo stability and bioavailability inherent to unmodified RNA molecules that are delivered exogenously. For example, the use of chemically modified nucleic acid molecules can enable a lower dose of a particular nucleic acid molecule for a given therapeutic effect since chemically modified nucleic acid molecules tend to have a longer half-life in serum or in cells or tissues. Furthermore, certain chemical modifications can improve the bioavailability and/or potency of nucleic acid molecules by not only enhancing half-life but also facilitating the targeting of nucleic acid molecules to particular organs, cells or tissues and/or improving cellular uptake of the nucleic acid molecules. Therefore, even if the activity of a chemically modified nucleic acid molecule is reduced in vitro as compared to a native/unmodified nucleic acid molecule, for example when compared to an unmodified RNA molecule, the overall activity of the modified nucleic acid molecule can be greater than the native or unmodified nucleic acid molecule due to improved stability, potency, duration of effect, bioavailability and/or delivery of the molecule.

[0516] In another embodiment, the invention features multifunctional siNAs, wherein the multifunctional siNAs are

assembled from two separate double-stranded siNAs, with one of the ends of each sense strand is tethered to the end of the sense strand of the other siNA molecule, such that the two antisense siNA strands are annealed to their corresponding sense strand that are tethered to each other at one end (see FIG. **22**). The tethers or linkers can be nucleotide-based linkers or non-nucleotide based linkers as generally known in the art and as described herein.

[0517] In one embodiment, the invention features a multifunctional siNA, wherein the multifunctional siNA is assembled from two separate double-stranded siNAs, with the 5'-end of one sense strand of the siNA is tethered to the 5'-end of the sense strand of the other siNA molecule, such that the 5'-ends of the two antisense siNA strands, annealed to their corresponding sense strand that are tethered to each other at one end, point away (in the opposite direction) from each other (see FIG. **22** (A)). The tethers or linkers can be nucleotide-based linkers or non-nucleotide based linkers as generally known in the art and as described herein.

[0518] In one embodiment, the invention features a multifunctional siNA, wherein the multifunctional siNA is assembled from two separate double-stranded siNAs, with the 3'-end of one sense strand of the siNA is tethered to the 3'-end of the sense strand of the other siNA molecule, such that the 5'-ends of the two antisense siNA strands, annealed to their corresponding sense strand that are tethered to each other at one end, face each other (see FIG. **22** (B)). The tethers or linkers can be nucleotide-based linkers or non-nucleotide based linkers as generally known in the art and as described herein.

[0519] In one embodiment, the invention features a multifunctional siNA, wherein the multifunctional siNA is assembled from two separate double-stranded siNAs, with the 5'-end of one sense strand of the siNA is tethered to the 3'-end of the sense strand of the other siNA molecule, such that the 5'-end of the one of the antisense siNA strands annealed to their corresponding sense strand that are tethered to each other at one end, faces the 3'-end of the other antisense strand (see FIG. **22** (C-D)). The tethers or linkers can be nucleotide-based linkers or non-nucleotide based linkers as generally known in the art and as described herein.

[0520] In one embodiment, the invention features a multifunctional siNA, wherein the multifunctional siNA is assembled from two separate double-stranded siNAs, with the 5'-end of one antisense strand of the siNA is tethered to the 3'-end of the antisense strand of the other siNA molecule, such that the 5'-end of the one of the sense siNA strands annealed to their corresponding antisense sense strand that are tethered to each other at one end, faces the 3'-end of the other sense strand (see FIG. **22** (G-H)). In one embodiment, the linkage between the 5'-end of the first antisense strand and the 3'-end of the second antisense strand is designed in such a way as to be readily cleavable (e.g., biodegradable linker) such that the 5' end of each antisense strand of the multifunctional siNA has a free 5'-end suitable to mediate RNA interference-based cleavage of the target RNA. The tethers or linkers can be nucleotide-based linkers or non-nucleotide based linkers as generally known in the art and as described herein.

[0521] In one embodiment, the invention features a multifunctional siNA, wherein the multifunctional siNA is assembled from two separate double-stranded siNAs, with the 5'-end of one antisense strand of the siNA is tethered to the 5'-end of the antisense strand of the other siNA molecule, such that the 3'-end of the one of the sense siNA strands annealed to their corresponding antisense sense strand that are tethered to each other at one end, faces the 3'-end of the other sense strand (see FIG. **22** (E)). In one embodiment, the linkage between the 5'-end of the first antisense strand and the 5'-end of the second antisense strand is designed in such a way as to be readily cleavable (e.g., biodegradable linker) such that the 5' end of each antisense strand of the multifunctional siNA has a free 5'-end suitable to mediate RNA interference-based cleavage of the target RNA. The tethers or linkers can be nucleotide-based linkers or non-nucleotide based linkers as generally known in the art and as described herein.

[0522] In one embodiment, the invention features a multifunctional siNA, wherein the multifunctional siNA is assembled from two separate double-stranded siNAs, with the 3'-end of one antisense strand of the siNA is tethered to the 3'-end of the antisense strand of the other siNA molecule, such that the 5'-end of the one of the sense siNA strands annealed to their corresponding antisense sense strand that are tethered to each other at one end, faces the 3'-end of the other sense strand (see FIG. **22** (F)). In one embodiment, the linkage between the 5'-end of the first antisense strand and the 5'-end of the second antisense strand is designed in such a way as to be readily cleavable (e.g., biodegradable linker) such that the 5' end of each antisense strand of the multifunctional siNA has a free 5'-end suitable to mediate RNA interference-based cleavage of the target RNA. The tethers or linkers can be nucleotide-based linkers or non-nucleotide based linkers as generally known in the art and as described herein.

[0523] In any of the above embodiments, a first target nucleic acid sequence or second target nucleic acid sequence can independently comprise SREBP1, H6PD, PTP-1B, apoAI/CIII/AIV, CETP, ApoB, PCSK9, calcium/calmodulin-dependent protein kinase 1D, fatty acid binding protein 5, proprotein convertase subtilisin/kexin 9, LXR target genes, metalloprotease domain 11, apoptosis-inhibitory 6, and/or F-box-only protein 3 RNA. In one embodiment, the first SREBP1 target nucleic acid sequence is a SREBP1 target RNA or a portion thereof and the second SREBP1 target nucleic acid sequence is a SREBP1 pathway target RNA, such as SREBP1, H6PD, PTP-1B, apoAI/CIII/AIV, CETP, ApoB, PCSK9, calcium/calmodulin-dependent protein kinase ID, fatty acid binding protein 5, proprotein convertase subtilisin/kexin 9, LXR target genes, metalloprotease domain 11, apoptosis-inhibitory 6, and/or F-box-only protein 3 RNA of a portion thereof.

Synthesis of Nucleic Acid Molecules

[0524] Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small nucleic acid motifs ("small" refers to nucleic acid motifs no more than 100 nucleotides in length, preferably no more than 80 nucleotides in length, and most preferably no more than 50 nucleotides in length; e.g., individual siNA oligonucleotide sequences or siNA sequences synthesized in tandem) are preferably used for exogenous delivery. The simple structure of these molecules increases the ability of the nucleic acid to invade targeted regions of protein and/or RNA structure. Exemplary molecules of the instant invention are chemically synthesized, and others can similarly be synthesized.

[0525] Oligonucleotides (e.g., certain modified oligonucleotides or portions of oligonucleotides lacking ribonucle-

otides) are synthesized using protocols known in the art, for example as described in Caruthers et al., 1992, *Methods in Enzymology* 211, 3-19, Thompson et al., International PCT Publication No. WO 99/54459, Wincott et al., 1995, *Nucleic Acids Res.* 23, 2677-2684, Wincott et al., 1997, *Methods Mol. Bio.*, 74, 59, Brennan et al., 1998, *Biotechnol Bioeng.*, 61, 33-45, and Brennan, U.S. Pat. No. 6,001,311. All of these references are incorporated herein by reference. The synthesis of oligonucleotides makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μmol scale protocol with a 2.5 min coupling step for 2'-O-methylated nucleotides and a 45 second coupling step for 2'-deoxy nucleotides or 2'-deoxy-2'-fluoro nucleotides. Table V outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μmol scale can be performed on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, Calif.) with minimal modification to the cycle. A 33-fold excess (60 μL of 0.11 M=6.6 μmol) of 2'-O-methyl phosphoramidite and a 105-fold excess of S-ethyl tetrazole (60 μL of 0.25 M=15 μmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 22-fold excess (40 μL of 0.11 M=4.4 μmol) of deoxy phosphoramidite and a 70-fold excess of S-ethyl tetrazole (40 μL of 0.25 M=10 μmol) can be used in each coupling cycle of deoxy residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by calorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% N-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); and oxidation solution is 16.9 mM 12, 49 mM pyridine, 9% water in THF (PerSeptive Biosystems, Inc.). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide, 0.05 M in acetonitrile) is used.

[0526] Deprotection of the DNA-based oligonucleotides is performed as follows: the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aqueous methylamine (1 mL) at 65° C. for 10 minutes. After cooling to −20° C., the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder. In one embodiment, the nucleic acid molecules of the invention are synthesized, deprotected, and analyzed according to methods described in U.S. Pat. No. 6,995,259, U.S. Pat. No. 6,686,463, U.S. Pat. No. 6,673,918, U.S. Pat. No. 6,649,751, U.S. Pat. No. 6,989,442, and U.S. Ser. No. 10/190,359, all incorporated by reference herein in their entirety.

[0527] The method of synthesis used for RNA including certain siNA molecules of the invention follows the procedure as described in Usman et al., 1987, *J. Am. Chem. Soc.*, 109, 7845; Scaringe et al., 1990, *Nucleic Acids Res.*, 18, 5433; and Wincott et al., 1995, *Nucleic Acids Res.* 23, 2677-2684 Wincott et al., 1997, *Methods Mol. Bio.*, 74, 59, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μmol scale protocol with a 7.5 min coupling step for alkylsilyl protected nucleotides and a 2.5 min coupling step for 2'-O-methylated nucleotides. Table V outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μmol scale can be done on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, Calif.) with minimal modification to the cycle. A 33-fold excess (60 μL of 0.11 M=6.6 μmol) of 2'-O-methyl phosphoramidite and a 75-fold excess of S-ethyl tetrazole (60 μL of 0.25 M=15 μmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 66-fold excess (120 μL of 0.11 M=13.2 μmol) of alkylsilyl (ribo) protected phosphoramidite and a 150-fold excess of S-ethyl tetrazole (120 μL of 0.25 M=30 μmol) can be used in each coupling cycle of ribo residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% N-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution is 16.9 mM 12, 49 mM pyridine, 9% water in THF (PerSeptive Biosystems, Inc.). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide 0.05 M in acetonitrile) is used.

[0528] Deprotection of the RNA is performed using either a two-pot or one-pot protocol. For the two-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65° C. for 10 min. After cooling to −20° C., the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder. The base deprotected oligoribonucleotide is resuspended in anhydrous TEA/HF/NMP solution (300 μL of a solution of 1.5 mL N-methylpyrrolidinone, 750 μL TEA and 1 mL TEA-3HF to provide a 1.4 M HF concentration) and heated to 65° C. After 1.5 h, the oligomer is quenched with 1.5 M NH_4HCO_3. In one embodiment, the nucleic acid molecules of the invention are synthesized, deprotected, and analyzed according to methods described in U.S. Pat. No. 6,995,259, U.S. Pat. No. 6,686,463, U.S. Pat. No. 6,673,918, U.S. Pat. No. 6,649,751, U.S. Pat. No. 6,989,442, and U.S. Ser. No. 10/190,359, all incorporated by reference herein in their entirety.

[0529] Alternatively, for the one-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 33%

ethanolic methylamine/DMSO: 1/1 (0.8 mL) at 65° C. for 15 minutes. The vial is brought to room temperature TEA-3HF (0.1 mL) is added and the vial is heated at 65° C. for 15 minutes. The sample is cooled at −20° C. and then quenched with 1.5 M NH_4HCO_3.

[0530] For purification of the trityl-on oligomers, the quenched NH_4HCO_3 solution is loaded onto a C-18 containing cartridge that had been prewashed with acetonitrile followed by 50 mM TEAA. After washing the loaded cartridge with water, the RNA is detritylated with 0.5% TFA for 13 minutes. The cartridge is then washed again with water, salt exchanged with 1 M NaCl and washed with water again. The oligonucleotide is then eluted with 30% acetonitrile.

[0531] The average stepwise coupling yields are typically >98% (Wincott et al., 1995 *Nucleic Acids Res.* 23, 2677-2684). Those of ordinary skill in the art will recognize that the scale of synthesis can be adapted to be larger or smaller than the example described above including but not limited to 96-well format.

[0532] Alternatively, the nucleic acid molecules of the present invention can be synthesized separately and joined together post-synthetically, for example, by ligation (Moore et al., 1992, *Science* 256, 9923; Draper et al., International PCT publication No. WO 93/23569; Shabarova et al., 1991, *Nucleic Acids Research* 19, 4247; Bellon et al., 1997, *Nucleosides & Nucleotides,* 16, 951; Bellon et al., 1997, *Bioconjugate Chem.* 8, 204), or by hybridization following synthesis and/or deprotection.

[0533] The siNA molecules of the invention can also be synthesized via a tandem synthesis methodology as described in Example 1 herein, wherein both siNA strands are synthesized as a single contiguous oligonucleotide fragment or strand separated by a cleavable linker which is subsequently cleaved to provide separate siNA fragments or strands that hybridize and permit purification of the siNA duplex. The linker can be a polynucleotide linker or a non-nucleotide linker. The tandem synthesis of siNA as described herein can be readily adapted to both multiwell/multiplate synthesis platforms such as 96 well or similarly larger multi-well platforms. The tandem synthesis of siNA as described herein can also be readily adapted to large scale synthesis platforms employing batch reactors, synthesis columns and the like.

[0534] A siNA molecule can also be assembled from two distinct nucleic acid strands or fragments wherein one fragment includes the sense region and the second fragment includes the antisense region of the RNA molecule.

[0535] The nucleic acid molecules of the present invention can be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992, *TIBS* 17, 34; Usman et al., 1994, *Nucleic Acids Symp. Ser.* 31, 163). siNA constructs can be purified by gel electrophoresis using general methods or can be purified by high pressure liquid chromatography (HPLC; see Wincott et al., supra, the totality of which is hereby incorporated herein by reference) and re-suspended in water.

[0536] In another aspect of the invention, siNA molecules of the invention are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules.

Optimizing Activity of the Nucleic Acid Molecule of the Invention.

[0537] Chemically synthesizing nucleic acid molecules with modifications (base, sugar and/or phosphate) can prevent their degradation by serum ribonucleases, which can increase their potency (see e.g., Eckstein et al., International Publication No. WO 92/07065; Perrault et al., 1990 *Nature* 344, 565; Pieken et al., 1991, *Science* 253, 314; Usman and Cedergren, 1992, *Trends in Biochem. Sci.* 17, 334; Usman et al., International Publication No. WO 93/15187; and Rossi et al., International Publication No. WO 91/03162; Sproat, U.S. Pat. No. 5,334,711; Gold et al., U.S. Pat. No. 6,300,074; and Burgin et al., supra; all of which are incorporated by reference herein). All of the above references describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of the nucleic acid molecules described herein. Modifications that enhance their efficacy in cells, and removal of bases from nucleic acid molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired.

[0538] There are several examples in the art describing sugar, base and phosphate modifications that can be introduced into nucleic acid molecules with significant enhancement in their nuclease stability and efficacy. For example, oligonucleotides are modified to enhance stability and/or enhance biological activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-O-allyl, 2'-H, nucleotide base modifications (for a review see Usman and Cedergren, 1992, *TIBS.* 17, 34; Usman et al., 1994, *Nucleic Acids Symp. Ser.* 31, 163; Burgin et al., 1996, *Biochemistry,* 35, 14090). Sugar modification of nucleic acid molecules have been extensively described in the art (see Eckstein et al., International Publication PCT No. WO 92/07065; Perrault et al. *Nature,* 1990, 344, 565-568; Pieken et al. *Science,* 1991, 253, 314-317; Usman and Cedergren, *Trends in Biochem. Sci.,* 1992, 17, 334-339; Usman et al. International Publication PCT No. WO 93/15187; Sproat, U.S. Pat. No. 5,334,711 and Beigelman et al., 1995, *J. Biol. Chem.,* 270, 25702; Beigelman et al., International PCT publication No. WO 97/26270; Beigelman et al., U.S. Pat. No. 5,716,824; Usman et al., U.S. Pat. No. 5,627,053; Woolf et al., International PCT Publication No. WO 98/13526; Thompson et al., U.S. Ser. No. 60/082,404 which was filed on Apr. 20, 1998; Karpeisky et al., 1998, *Tetrahedron Lett.,* 39, 1131; Earnshaw and Gait, 1998, *Biopolymers* (*Nucleic Acid Sciences*), 48, 39-55; Verma and Eckstein, 1998, *Annu. Rev. Biochem.,* 67, 99-134; and Burlina et al., 1997, *Bioorg. Med. Chem.,* 5, 1999-2010; all of the references are hereby incorporated in their totality by reference herein). Such publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into nucleic acid molecules without modulating catalysis, and are incorporated by reference herein. In view of such teachings, similar modifications can be used as described herein to modify the siNA nucleic acid molecules of the instant invention so long as the ability of siNA to promote RNAi is cells is not significantly inhibited.

[0539] In one embodiment, a nucleic acid molecule of the invention is chemically modified as described in US 20050020521, incorporated by reference herein in its entirety.

[0540] While chemical modification of oligonucleotide internucleotide linkages with phosphorothioate, phosphorodithioate, and/or 5'-methylphosphonate linkages improves stability, excessive modifications can cause some toxicity or decreased activity. Therefore, when designing nucleic acid molecules, the amount of these internucleotide linkages should be minimized. The reduction in the concentration of these linkages should lower toxicity, resulting in increased efficacy and higher specificity of these molecules.

[0541] Short interfering nucleic acid (siNA) molecules having chemical modifications that maintain or enhance activity are provided. Such a nucleic acid is also generally more resistant to nucleases than an unmodified nucleic acid. Accordingly, the in vitro and/or in vivo activity should not be significantly lowered. In cases in which modulation is the goal, therapeutic nucleic acid molecules delivered exogenously should optimally be stable within cells until translation of the target RNA has been modulated long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Improvements in the chemical synthesis of RNA and DNA (Wincott et al., 1995, *Nucleic Acids Res.* 23, 2677; Caruthers et al., 1992, *Methods in Enzymology* 211, 3-19 (incorporated by reference herein)) have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability, as described above.

[0542] In one embodiment, nucleic acid molecules of the invention include one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) G-clamp nucleotides. A G-clamp nucleotide is a modified cytosine analog wherein the modifications confer the ability to hydrogen bond both Watson-Crick and Hoogsteen faces of a complementary guanine within a duplex, see for example Lin and Matteucci, 1998, *J. Am. Chem. Soc.*, 120, 8531-8532. A single G-clamp analog substitution within an oligonucleotide can result in substantially enhanced helical thermal stability and mismatch discrimination when hybridized to complementary oligonucleotides. The inclusion of such nucleotides in nucleic acid molecules of the invention results in both enhanced affinity and specificity to nucleic acid targets, complementary sequences, or template strands. In another embodiment, nucleic acid molecules of the invention include one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) LNA "locked nucleic acid" nucleotides such as a 2',4'-C methylene bicyclo nucleotide (see for example Wengel et al., International PCT Publication No. WO 00/66604 and WO 99/14226).

[0543] In another embodiment, the invention features conjugates and/or complexes of siNA molecules of the invention. Such conjugates and/or complexes can be used to facilitate delivery of siNA molecules into a biological system, such as a cell. The conjugates and complexes provided by the instant invention can impart therapeutic activity by transferring therapeutic compounds across cellular membranes, altering the pharmacokinetics, and/or modulating the localization of nucleic acid molecules of the invention. The present invention encompasses the design and synthesis of novel conjugates and complexes for the delivery of molecules, including, but not limited to, small molecules, lipids, cholesterol, phospholipids, nucleosides, nucleotides, nucleic acids, antibodies, toxins, negatively charged polymers and other polymers, for example proteins, peptides, hormones, carbohydrates, polyethylene glycols, or polyamines, across cellular membranes. In general, the transporters described are designed to be used either individually or as part of a multi-component system, with or without degradable linkers. These compounds are expected to improve delivery and/or localization of nucleic acid molecules of the invention into a number of cell types originating from different tissues, in the presence or absence of serum (see Sullenger and Cech, U.S. Pat. No. 5,854,038). Conjugates of the molecules described herein can be attached to biologically active molecules via linkers that are biodegradable, such as biodegradable nucleic acid linker molecules.

[0544] The term "biodegradable linker" as used herein, refers to a nucleic acid or non-nucleic acid linker molecule that is designed as a biodegradable linker to connect one molecule to another molecule, for example, a biologically active molecule to a siNA molecule of the invention or the sense and antisense strands of a siNA molecule of the invention. The biodegradable linker is designed such that its stability can be modulated for a particular purpose, such as delivery to a particular tissue or cell type. The stability of a nucleic acid-based biodegradable linker molecule can be modulated by using various chemistries, for example combinations of ribonucleotides, deoxyribonucleotides, and chemically-modified nucleotides, such as 2'-O-methyl, 2'-fluoro, 2'-amino, 2'-O-amino, 2'-C-allyl, 2'-O-allyl, and other 2'-modified or base modified nucleotides. The biodegradable nucleic acid linker molecule can be a dimer, trimer, tetramer or longer nucleic acid molecule, for example, an oligonucleotide of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length, or can comprise a single nucleotide with a phosphorus-based linkage, for example, a phosphoramidate or phosphodiester linkage. The biodegradable nucleic acid linker molecule can also comprise nucleic acid backbone, nucleic acid sugar, or nucleic acid base modifications.

[0545] The term "biodegradable" as used herein, refers to degradation in a biological system, for example, enzymatic degradation or chemical degradation.

[0546] The term "biologically active molecule" as used herein refers to compounds or molecules that are capable of eliciting or modifying a biological response in a system. Non-limiting examples of biologically active siNA molecules either alone or in combination with other molecules contemplated by the instant invention include therapeutically active molecules such as antibodies, cholesterol, hormones, antivirals, peptides, proteins, chemotherapeutics, small molecules, vitamins, co-factors, nucleosides, nucleotides, oligonucleotides, enzymatic nucleic acids, antisense nucleic acids, triplex forming oligonucleotides, 2,5-A chimeras, siNA, dsRNA, allozymes, aptamers, decoys and analogs thereof. Biologically active molecules of the invention also include molecules capable of modulating the pharmacokinetics and/or pharmacodynamics of other biologically active molecules, for example, lipids and polymers such as polyamines, polyamides, polyethylene glycol and other polyethers.

[0547] The term "phospholipid" as used herein, refers to a hydrophobic molecule comprising at least one phosphorus group. For example, a phospholipid can comprise a phosphorus-containing group and saturated or unsaturated alkyl group, optionally substituted with OH, COOH, oxo, amine, or substituted or unsubstituted aryl groups.

[0548] Therapeutic nucleic acid molecules (e.g., siNA molecules) delivered exogenously optimally are stable within cells until reverse transcription of the RNA has been modulated long enough to reduce the levels of the RNA transcript.

The nucleic acid molecules are resistant to nucleases in order to function as effective intracellular therapeutic agents. Improvements in the chemical synthesis of nucleic acid molecules described in the instant invention and in the art have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

[0549] In yet another embodiment, siNA molecules having chemical modifications that maintain or enhance enzymatic activity of proteins involved in RNAi are provided. Such nucleic acids are also generally more resistant to nucleases than unmodified nucleic acids. Thus, in vitro and/or in vivo the activity should not be significantly lowered.

[0550] Use of the nucleic acid-based molecules of the invention will lead to better treatments by affording the possibility of combination therapies (e.g., multiple siNA molecules targeted to different genes; nucleic acid molecules coupled with known small molecule modulators; or intermittent treatment with combinations of molecules, including different motifs and/or other chemical or biological molecules). The treatment of subjects with siNA molecules can also include combinations of different types of nucleic acid molecules, such as enzymatic nucleic acid molecules (ribozymes), allozymes, antisense, 2,5-A oligoadenylate, decoys, and aptamers.

[0551] In another aspect a siNA molecule of the invention comprises one or more 5' and/or a 3'-cap structure, for example, on only the sense siNA strand, the antisense siNA strand, or both siNA strands.

[0552] By "cap structure" is meant chemical modifications, which have been incorporated at either terminus of the oligonucleotide (see, for example, Adamic et al., U.S. Pat. No. 5,998,203, incorporated by reference herein). These terminal modifications protect the nucleic acid molecule from exonuclease degradation, and may help in delivery and/or localization within a cell. The cap may be present at the 5'-terminus (5'-cap) or at the 3'-terminal (3'-cap) or may be present on both termini. In non-limiting examples, the 5'-cap includes, but is not limited to, glyceryl, inverted deoxy abasic residue (moiety); 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide, 4'-thio nucleotide; carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; threo-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety. Non-limiting examples of cap moieties are shown in FIG. **10**.

[0553] Non-limiting examples of the 3'-cap include, but are not limited to, glyceryl, inverted deoxy abasic residue (moiety), 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate; 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; threo-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details see Beaucage and Iyer, 1993, *Tetrahedron* 49, 1925; incorporated by reference herein).

[0554] By the term "non-nucleotide" is meant any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine and therefore lacks a base at the 1'-position.

[0555] An "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably, it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group can be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino, or SH. The term also includes alkenyl groups that are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably, it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2, halogen, $N(CH_3)_2$, amino, or SH. The term "alkyl" also includes alkynyl groups that have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably, it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino or SH.

[0556] Such alkyl groups can also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group that has at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an —C(O)—NH—R, where R is either

alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an —C(O)—OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

[0557] By "nucleotide" as used herein is as recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see, for example, Usman and McSwiggen, supra; Eckstein et al., International PCT Publication No. WO 92/07065; Usman et al., International PCT Publication No. WO 93/15187; Uhlman & Peyman, supra, all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach et al., 1994, Nucleic Acids Res. 22, 2183. Some of the non-limiting examples of base modifications that can be introduced into nucleic acid molecules include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2,4,6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (e.g., 5-methylcytidine), 5-alkyluridines (e.g., ribothymidine), 5-halouridine (e.g., 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (e.g. 6-methyluridine), propyne, and others (Burgin et al., 1996, *Biochemistry,* 35, 14090; Uhlman & Peyman, supra). By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents.

[0558] In one embodiment, the invention features modified siNA molecules, with phosphate backbone modifications comprising one or more phosphorothioate, phosphorodithioate, methylphosphonate, phosphotriester, morpholino, amidate carbamate, carboxymethyl, acetamidate, polyamide, sulfonate, sulfonamide, sulfamate, formacetal, thioformacetal, and/or alkylsilyl, substitutions. For a review of oligonucleotide backbone modifications, see Hunziker and Leumann, 1995, *Nucleic Acid Analogues: Synthesis and Properties*, in *Modern Synthetic Methods*, VCH, 331417, and Mesmaeker et al., 1994, *Novel Backbone Replacements for Oligonucleotides*, in *Carbohydrate Modifications in Antisense Research*, ACS, 24-39.

[0559] By "abasic" is meant sugar moieties lacking a nucleobase or having a hydrogen atom (H) or other non-nucleobase chemical groups in place of a nucleobase at the 1' position of the sugar moiety, see for example Adamic et al., U.S. Pat. No. 5,998,203. In one embodiment, an abasic moiety of the invention is a ribose, deoxyribose, or dideoxyribose sugar.

[0560] By "unmodified nucleoside" is meant one of the bases adenine, cytosine, guanine, thymine, or uracil joined to the 1' carbon of β-D-ribo-furanose.

[0561] By "modified nucleoside" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base, sugar and/or phosphate. Non-limiting examples of modified nucleotides are shown by Formulae I-VII and/or other modifications described herein.

[0562] In connection with 2'-modified nucleotides as described for the present invention, by "amino" is meant 2'-NH_2 or 2'-O—NH_2, which can be modified or unmodified. Such modified groups are described, for example, in Eckstein et al., U.S. Pat. No. 5,672,695 and Matulic-Adamic et al., U.S. Pat. No. 6,248,878, which are both incorporated by reference in their entireties.

[0563] Various modifications to nucleic acid siNA structure can be made to enhance the utility of these molecules. Such modifications will enhance shelf-life, half-life in vitro, stability, and ease of introduction of such oligonucleotides to the target site, e.g., to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

Administration of Nucleic Acid Molecules

[0564] A siNA molecule of the invention can be adapted for use to prevent or treat diseases, traits, disorders, and/or conditions described herein or otherwise known in the art to be related to SREBP1 gene or SREBP1 pathway gene expression, and/or any other trait, disease, disorder or condition that is related to or will respond to the levels of SREBP1 polynucleotides or proteins expressed therefrom in a cell or tissue, alone or in combination with other therapies. In one embodiment, the siNA molecules of the invention and formulations or compositions thereof are administered to a cell, subject, or organism as is described herein and as is generally known in the art.

[0565] In one embodiment, a siNA composition of the invention can comprise a delivery vehicle, including liposomes, for administration to a subject, carriers and diluents and their salts, and/or can be present in pharmaceutically acceptable formulations. Methods for the delivery of nucleic acid molecules are described in Akhtar et al., 1992, *Trends Cell Bio.,* 2, 139; *Delivery Strategies for Antisense Oligonucleotide Therapeutics*, ed. Akhtar, 1995, Maurer et al., 1999, *Mol. Membr. Biol.,* 16, 129-140; Hofland and Huang, 1999, *Handb. Exp. Pharmacol.,* 137, 165-192; and Lee et al., 2000, *ACS Symp. Ser.,* 752, 184-192, all of which are incorporated herein by reference. Beigelman et al., U.S. Pat. No. 6,395,713 and Sullivan et al., PCT WO 94/02595 further describe the general methods for delivery of nucleic acid molecules. These protocols can be utilized for the delivery of virtually any nucleic acid molecule. Nucleic acid molecules can be administered to cells by a variety of methods known to those of skill in the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as biodegradable polymers, hydrogels, cyclodextrins (see for example Gonzalez et al., 1999, *Bioconjugate Chem.,* 10, 1068-1074; Wang et al., International PCT publication Nos. WO 03/47518 and WO 03/46185), poly(lactic-co-glycolic)acid (PLGA) and PLCA microspheres (see for example U.S. Pat. No. 6,447,796 and US Patent Application Publication No. US 2002130430), biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors (O'Hare and Normand, International PCT Publication No. WO 00/53722). In another embodiment, the nucleic acid molecules of the invention can also be formulated or complexed with polyethyleneimine and derivatives thereof, such as polyethyleneimine-polyethyleneglycol-N-acetylgalactosamine (PEI-PEG-GAL) or polyethyleneimine-polyethyleneglycol-tri-N-acetylgalactosamine (PEI-PEG-triGAL) derivatives. In one embodiment, the nucleic acid molecules of the invention are formulated as described in United States Patent Application Publication No. 20030077829, incorporated by reference herein in its entirety.

[0566] In one embodiment, a siNA molecule of the invention is formulated as a composition described in U.S. Provisional patent application No. 60/678,531 and in related U.S. Provisional patent application No. 60/703,946, filed Jul. 29, 2005, U.S. Provisional patent application No. 60/737,024, filed Nov. 15, 2005, and U.S. Ser. No. 11/353,630, filed Feb. 14, 2006 (Vargeese et al.), all of which are incorporated by reference herein in their entirety. Such siNA formulations are generally referred to as "lipid nucleic acid particles" (LNP). In one embodiment, a siNA molecule of the invention is formulated with one or more LNP compositions described herein in Table IV (see U.S. Ser. No. 11/353,630 supra).

[0567] In one embodiment, the siNA molecules of the invention and formulations or compositions thereof are administered to lung tissues and cells as is described in US 2006/0062758; US 2006/0014289; and US 2004/0077540.

[0568] In one embodiment, a siNA molecule of the invention is complexed with membrane disruptive agents such as those described in U.S. Patent Application Publication No. 20010007666, incorporated by reference herein in its entirety including the drawings. In another embodiment, the membrane disruptive agent or agents and the siNA molecule are also complexed with a cationic lipid or helper lipid molecule, such as those lipids described in U.S. Pat. No. 6,235,310, incorporated by reference herein in its entirety including the drawings.

[0569] In one embodiment, a siNA molecule of the invention is complexed with delivery systems as described in U.S. Patent Application Publication No. 2003077829 and International PCT Publication Nos. WO 00/03683 and WO 02/087541, all incorporated by reference herein in their entirety including the drawings.

[0570] In one embodiment, a siNA molecule of the invention is complexed with delivery systems as is generally described in U.S. Patent Application Publication Nos. US-20050287551; US-20050164220; US-20050191627; US-20050118594; US-20050153919; US-20050085486; and US-20030158133; all incorporated by reference herein in their entirety including the drawings.

[0571] In one embodiment, the nucleic acid molecules of the invention and formulations thereof (e.g., LNP formulations of double stranded nucleic acid molecules of the invention) are administered via pulmonary delivery, such as by inhalation of an aerosol or spray dried formulation administered by an inhalation device or nebulizer, providing rapid local uptake of the nucleic acid molecules into relevant pulmonary tissues. Solid particulate compositions containing respirable dry particles of micronized nucleic acid compositions can be prepared by grinding dried or lyophilized nucleic acid compositions, and then passing the micronized composition through, for example, a 400 mesh screen to break up or separate out large agglomerates. A solid particulate composition comprising the nucleic acid compositions of the invention can optionally contain a dispersant which serves to facilitate the formation of an aerosol as well as other therapeutic compounds. A suitable dispersant is lactose, which can be blended with the nucleic acid compound in any suitable ratio, such as a 1 to 1 ratio by weight.

[0572] Aerosols of liquid particles comprising a nucleic acid composition of the invention can be produced by any suitable means, such as with a nebulizer (see for example U.S. Pat. No. 4,501,729). Nebulizers are commercially available devices which transform solutions or suspensions of an active ingredient into a therapeutic aerosol mist either by means of acceleration of a compressed gas, typically air or oxygen, through a narrow venturi orifice or by means of ultrasonic agitation. Suitable formulations for use in nebulizers comprise the active ingredient in a liquid carrier in an amount of up to 40% w/w preferably less than 20% w/w of the formulation. The carrier is typically water or a dilute aqueous alcoholic solution, preferably made isotonic with body fluids by the addition of, for example, sodium chloride or other suitable salts. Optional additives include preservatives if the formulation is not prepared sterile, for example, methyl hydroxybenzoate, anti-oxidants, flavorings, volatile oils, buffering agents and emulsifiers and other formulation surfactants. The aerosols of solid particles comprising the active composition and surfactant can likewise be produced with any solid particulate aerosol generator. Aerosol generators for administering solid particulate therapeutics to a subject produce particles which are respirable, as explained above, and generate a volume of aerosol containing a predetermined metered dose of a therapeutic composition at a rate suitable for human administration.

[0573] In one embodiment, a solid particulate aerosol generator of the invention is an insufflator. Suitable formulations for administration by insufflation include finely comminuted powders which can be delivered by means of an insufflator. In the insufflator, the powder, e.g., a metered dose thereof effective to carry out the treatments described herein, is contained in capsules or cartridges, typically made of gelatin or plastic, which are either pierced or opened in situ and the powder delivered by air drawn through the device upon inhalation or by means of a manually-operated pump. The powder employed in the insufflator consists either solely of the active ingredient or of a powder blend comprising the active ingredient, a suitable powder diluent, such as lactose, and an optional surfactant. The active ingredient typically comprises from 0.1 to 100 w/w of the formulation. A second type of illustrative aerosol generator comprises a metered dose inhaler. Metered dose inhalers are pressurized aerosol dispensers, typically containing a suspension or solution formulation of the active ingredient in a liquified propellant. During use these devices discharge the formulation through a valve adapted to deliver a metered volume to produce a fine particle spray containing the active ingredient. Suitable propellants include certain chlorofluorocarbon compounds, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane and mixtures thereof. The formulation can additionally contain one or more co-solvents, for example, ethanol, emulsifiers and other formulation surfactants, such as oleic acid or sorbitan trioleate, anti-oxidants and suitable flavoring agents. Other methods for pulmonary delivery are described in, for example US Patent Application No. 20040037780, and U.S. Pat. Nos. 6,592,904; 6,582,728; 6,565,885, all incorporated by reference herein.

[0574] In one embodiment, the siNA and LNP compositions and formulations provided herein for use in pulmonary delivery further comprise one or more surfactants. Suitable surfactants or surfactant components for enhancing the uptake of the compositions of the invention include synthetic and natural as well as full and truncated forms of surfactant protein A, surfactant protein B, surfactant protein C, surfactant protein D and surfactant Protein E, di-saturated phosphatidylcholine (other than dipalmitoyl), dipalmitoylphosphatidylcholine, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine; phosphatidic acid, ubiquinones, lysophosphati-

dylethanolamine, lysophosphatidylcholine, palmitoyl-lysophosphatidylcholine, dehydroepiandrosterone, dolichols, sulfatidic acid, glycerol-3-phosphate, dihydroxyacetone phosphate, glycerol, glycero-3-phosphocholine, dihydroxyacetone, palmitate, cytidine diphosphate (CDP) diacylglycerol, CDP choline, choline, choline phosphate; as well as natural and artificial lamelar bodies which are the natural carrier vehicles for the components of surfactant, omega-3 fatty acids, polyenic acid, polyenoic acid, lecithin, palmitinic acid, non-ionic block copolymers of ethylene or propylene oxides, polyoxypropylene, monomeric and polymeric, polyoxyethylene, monomeric and polymeric, poly (vinyl amine) with dextran and/or alkanoyl side chains, Brij 35, Triton X-100 and synthetic surfactants ALEC, Exosurf, Survan and Atovaquone, among others. These surfactants may be used either as single or part of a multiple component surfactant in a formulation, or as covalently bound additions to the 5' and/or 3' ends of the nucleic acid component of a pharmaceutical composition herein.

[0575] The composition of the present invention may be administered into the respiratory system as a formulation including particles of respirable size, e.g. particles of a size sufficiently small to pass through the nose, mouth and larynx upon inhalation and through the bronchi and alveoli of the lungs. In general, respirable particles range from about 0.5 to 10 microns in size. Particles of non-respirable size which are included in the aerosol tend to deposit in the throat and be swallowed, and the quantity of non-respirable particles in the aerosol is thus minimized. For nasal administration, a particle size in the range of 10-500 um is preferred to ensure retention in the nasal cavity.

[0576] In one embodiment, the siNA molecules of the invention and formulations or compositions thereof are administered to the liver as is generally known in the art (see for example Wen et al., 2004, *World J Gastroenterol.*, 10, 244-9; Murao et al., 2002, *Pharm Res.*, 19, 1808-14; Liu et al., 2003, *Gene Ther.*, 10, 180-7; Hong et al., 2003, *J Pharm Pharmacol.*, 54, 51-8; Herrmann et al., 2004, *Arch Virol.*, 149, 1611-7; and Matsuno et al., 2003, *Gene Ther.*, 10, 1559-66).

[0577] In one embodiment, a siNA molecule of the invention is administered iontophoretically, for example to a particular organ or compartment (e.g., the eye, back of the eye, heart, liver, kidney, bladder, prostate, tumor, CNS etc.). Non-limiting examples of iontophoretic delivery are described in, for example, WO 03/043689 and WO 03/030989, which are incorporated by reference in their entireties herein.

[0578] In one embodiment, siNA compounds and compositions of the invention are administered either systemically or locally about every 1-50 weeks (e.g., about every 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, or 50 weeks), alone or in combination with other compounds and/or therapies herein. In one embodiment, siNA compounds and compositions of the invention are administered systemically (e.g., via intravenous, subcutaneous, intramuscular, infusion, pump, implant etc.) about every 1-50 weeks (e.g., about every 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, or 50 weeks), alone or in combination with other compounds and/or therapies described herein and/or otherwise known in the art.

[0579] In one embodiment, delivery systems of the invention include, for example, aqueous and nonaqueous gels, creams, multiple emulsions, microemulsions, liposomes, ointments, aqueous and nonaqueous solutions, lotions, aerosols, hydrocarbon bases and powders, and can contain excipients such as solubilizers, permeation enhancers (e.g., fatty acids, fatty acid esters, fatty alcohols and amino acids), and hydrophilic polymers (e.g., polycarbophil and polyvinylpyrolidone). In one embodiment, the pharmaceutically acceptable carrier is a liposome or a transdermal enhancer. Examples of liposomes which can be used in this invention include the following: (1) CellFectin, 1:1.5 (M/M) liposome formulation of the cationic lipid N,NI,NII,NIII-tetramethyl-N,NI,NII,NIII-tetrapalmit-y-spermine and dioleoyl phosphatidylethanolamine (DOPE) (GIBCO BRL); (2) Cytofectin GSV, 2:1 (M/M) liposome formulation of a cationic lipid and DOPE (Glen Research); (3) DOTAP (N-[1-(2,3-dioleoyloxy)-N,N,N-tri-methyl-ammoniummethylsulfate) (Boehringer Manheim); and (4) Lipofectamine, 3:1 (M/M) liposome formulation of the polycationic lipid DOSPA and the neutral lipid DOPE (GIBCO BRL).

[0580] In one embodiment, delivery systems of the invention include patches, tablets, suppositories, pessaries, gels and creams, and can contain excipients such as solubilizers and enhancers (e.g., propylene glycol, bile salts and amino acids), and other vehicles (e.g., polyethylene glycol, fatty acid esters and derivatives, and hydrophilic polymers such as hydroxypropylmethylcellulose and hyaluronic acid).

[0581] In one embodiment, siNA molecules of the invention are formulated or complexed with polyethylenimine (e.g:, linear or branched PEI) and/or polyethylenimine derivatives, including for example grafted PEIs such as galactose PEI, cholesterol PEI, antibody derivatized PEI, and polyethylene glycol PEI (PEG-PEI) derivatives thereof (see for example Ogris et al., 2001, *AAPA Pharm Sci*, 3, 1-11; Furgeson et al., 2003, Bioconjugate Chem., 14, 840-847; Kunath et al., 2002, Pharmaceutical Research, 19, 810-817; Choi et al., 2001, Bull. Korean Chem. Soc., 22, 46-52; Bettinger et al., 1999, Bioconjugate Chem., 10, 558-561; Peterson et al., 2002, Bioconjugate Chem., 13, 845-854; Erbacher et al., 1999, Journal of Gene Medicine Preprint, 1, 1-18; Godbey et al., 1999, PNAS USA, 96, 5177-5181; Godbey et al., 1999, Journal of Controlled Release, 60, 149-160; Diebold et al., 1999, Journal of Biological Chemistry, 274, 19087-19094; Thomas and Klibanov, 2002, PNAS USA, 99, 14640-14645; and Sagara, U.S. Pat. No. 6,586,524, incorporated by reference herein.

[0582] In one embodiment, a siNA molecule of the invention comprises a bioconjugate, for example a nucleic acid conjugate as described in Vargeese et al., U.S. Ser. No. 10/427,160, filed Apr. 30, 2003; U.S. Pat. No. 6,528,631; U.S. Pat. No. 6,335,434; U.S. Pat. No. 6,235,886; U.S. Pat. No. 6,153,737; U.S. Pat. No. 5,214,136; U.S. Pat. No. 5,138,045, all incorporated by reference herein.

[0583] Thus, the invention features a pharmaceutical composition comprising one or more nucleic acid(s) of the invention in an acceptable carrier, such as a stabilizer, buffer, and the like. The polynucleotides of the invention can be administered (e.g., RNA, DNA or protein) and introduced to a subject by any standard means, with or without stabilizers, buffers, and the like, to form a pharmaceutical composition. When it is desired to use a liposome delivery mechanism, standard protocols for formation of liposomes can be followed. The compositions of the present invention can also be formulated and used as creams, gels, sprays, oils and other

suitable compositions for topical, dermal, or transdermal administration as is known in the art.

[0584] The present invention also includes pharmaceutically acceptable formulations of the compounds described. These formulations include salts of the above compounds, e.g., acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

[0585] A pharmacological composition or formulation refers to a composition or formulation in a form suitable for administration, e.g., systemic or local administration, into a cell or subject, including for example a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should not prevent the composition or formulation from reaching a target cell (i.e., a cell to which the negatively charged nucleic acid is desirable for delivery). For example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms that prevent the composition or formulation from exerting its effect.

[0586] In one embodiment, siNA molecules of the invention are administered to a subject by systemic administration in a pharmaceutically acceptable composition or formulation. By "systemic administration" is meant in vivo systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes that lead to systemic absorption include, without limitation: intravenous, subcutaneous, portal vein, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes exposes the siNA molecules of the invention to an accessible diseased tissue (e.g., lung). The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES). A liposome formulation that can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful. This approach can provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells.

[0587] By "pharmaceutically acceptable formulation" or "pharmaceutically acceptable composition" is meant, a composition or formulation that allows for the effective distribution of the nucleic acid molecules of the instant invention in the physical location most suitable for their desired activity. Non-limiting examples of agents suitable for formulation with the nucleic acid molecules of the instant invention include: P-glycoprotein inhibitors (such as Pluronic P85); biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery (Emerich, D F et al, 1999, *Cell Transplant,* 8, 47-58); and loaded nanoparticles, such as those made of polybutylcyanoacrylate. Other non-limiting examples of delivery strategies for the nucleic acid molecules of the instant invention include material described in Boado et al., 1998, *J. Pharm. Sci.,* 87, 1308-1315; Tyler et al., 1999, *FEBS Lett.,* 421, 280-284; Pardridge et al., 1995, *PNAS USA.,* 92, 5592-5596; Boado, 1995, *Adv. Drug Delivery Rev.,* 15, 73-107; Aldrian-Herrada et al., 1998, *Nucleic Acids Res.,* 26, 49104916; and Tyler et al., 1999, *PNAS USA.,* 96, 7053-7058.

[0588] The invention also features the use of a composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes) and nucleic acid molecules of the invention. These formulations offer a method for increasing the accumulation of drugs (e.g., siNA) in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic et al. *Chem. Rev.* 1995, 95, 2601-2627; Ishiwata et al., *Chem. Pharm. Bull.* 1995, 43, 1005-1011). Such liposomes have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic et al., *Science* 1995, 267, 1275-1276; Oku et al., 1995, *Biochim. Biophys. Acta,* 1238, 86-90). The long-circulating liposomes enhance the pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu et al., *J. Biol. Chem.* 1995, 42, 24864-24870; Choi et al., International PCT Publication No. WO 96/10391; Ansell et al., International PCT Publication No. WO 96/10390; Holland et al., International PCT Publication No. WO 96/10392). Long-circulating liposomes are also likely to protect drugs from nuclease degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen.

[0589] In one embodiment, a liposomal formulation of the invention comprises a double stranded nucleic acid molecule of the invention (e.g, siNA) formulated or complexed with compounds and compositions described in U.S. Pat. Nos. 6,858,224; 6,534,484; 6,287,591; 6,835,395; 6,586,410; 6,858,225; 6,815,432; U.S. Pat. Nos. 6,586,001; 6,120,798; U.S. Pat. No. 6,977,223; U.S. Pat. Nos. 6,998,115; 5,981,501; 5,976,567; 5,705,385; US 2006/0019912; US 2006/0019258; US 2006/0008909; US 2005/0255153; US 2005/0079212; US 2005/0008689; US 2003/0077829, US 2005/0064595, US 2005/0175682, US 2005/0118253; US 2004/0071654; US 2005/0244504; US 2005/0265961 and US 2003/0077829, all of which are incorporated by reference herein in their entirety.

[0590] The present invention also includes compositions prepared for storage or administration that include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A. R. Gennaro edit. 1985), hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents can be provided. These include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

[0591] A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors that those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day

of active ingredients is administered dependent upon potency of the negatively charged polymer.

[0592] The nucleic acid molecules of the invention and formulations thereof can be administered orally, topically, parenterally, by inhalation or spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and/or vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (e.g., intravenous), intramuscular, or intrathecal injection or infusion techniques and the like. In addition, there is provided a pharmaceutical formulation comprising a nucleic acid molecule of the invention and a pharmaceutically acceptable carrier. One or more nucleic acid molecules of the invention can be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants, and if desired other active ingredients. The pharmaceutical compositions containing nucleic acid molecules of the invention can be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

[0593] Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more such sweetening agents, flavoring agents, coloring agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients can be, for example, inert diluents; such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques. In some cases such coatings can be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate can be employed.

[0594] Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

[0595] Aqueous suspensions contain the active materials in a mixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropyl-methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

[0596] Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents can be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid

[0597] Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, can also be present.

[0598] Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

[0599] Syrups and elixirs can be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

[0600] The nucleic acid molecules of the invention can also be administered in the form of suppositories, e.g., for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

[0601] Nucleic acid molecules of the invention can be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either

be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

[0602] Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 0.5 mg to about 7 g per subject per day). The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending upon the host treated and the particular mode of administration. Dosage unit forms generally contain between from about 1 mg to about 500 mg of an active ingredient.

[0603] It is understood that the specific dose level for any particular subject depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

[0604] For administration to non-human animals, the composition can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and drinking water compositions so that the animal takes in a therapeutically appropriate quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

[0605] The nucleic acid molecules of the present invention can also be administered to a subject in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

[0606] In one embodiment, the invention comprises compositions suitable for administering nucleic acid molecules of the invention to specific cell types. For example, the asialoglycoprotein receptor (ASGPr) (Wu and Wu, 1987, *J. Biol. Chem.* 262, 4429-4432) is unique to hepatocytes and binds branched galactose-terminal glycoproteins, such as asialoorosomucoid (ASOR). In another example, the folate receptor is overexpressed in many cancer cells. Binding of such glycoproteins, synthetic glycoconjugates, or folates to the receptor takes place with an affinity that strongly depends on the degree of branching of the oligosaccharide chain, for example, triatennary structures are bound with greater affinity than biatenarry or monoatennary chains (Baenziger and Fiete, 1980, *Cell,* 22, 611-620; Connolly et al., 1982, *J. Biol. Chem.,* 257, 939-945). Lee and Lee, 1987, *Glycoconjugate J.,* 4, 317-328, obtained this high specificity through the use of N-acetyl-D-galactosamine as the carbohydrate moiety, which has higher affinity for the receptor, compared to galactose. This "clustering effect" has also been described for the binding and uptake of mannosyl-terminating glycoproteins or glycoconjugates (Ponpipom et al., 1981, *J. Med. Chem.,* 24, 1388-1395). The use of galactose, galactosamine, or folate based conjugates to transport exogenous compounds across cell membranes can provide a targeted delivery approach to, for example, the treatment of liver disease, cancers of the liver, or other cancers. The use of bioconjugates can also provide a reduction in the required dose of therapeutic compounds required for treatment. Furthermore, therapeutic bioavailability, pharmacodynamics, and pharmacokinetic parameters can be modulated through the use of nucleic acid bioconjugates of the invention. Non-limiting examples of such bioconjugates are described in Vargeese et al., U.S. Ser. No. 10/201,394, filed Aug. 13, 2001; and Matulic-Adamic et al., U.S. Ser. No. 60/362,016, filed Mar. 6, 2002.

[0607] Alternatively, certain siNA molecules of the instant invention can be expressed within cells from eukaryotic promoters (e.g., Izant and Weintraub, 1985, *Science,* 229, 345; McGarry and Lindquist, 1986, *Proc. Natl. Acad. Sci., USA* 83, 399; Scanlon et al., 1991, *Proc. Natl. Acad. Sci. USA,* 88, 10591-5; Kashani-Sabet et al., 1992, *Antisense Res. Dev.,* 2, 3-15; Dropulic et al., 1992, *J. Virol.,* 66, 1432-41; Weerasinghe et al., 1991, *J. Virol.,* 65, 5531-4; Ojwang et al., 1992, *Proc. Natl. Acad. Sci. USA,* 89, 10802-6; Chen et al., 1992, *Nucleic Acids Res.,* 20, 4581-9; Sarver et al., 1990 *Science,* 247, 1222-1225; Thompson et al., 1995, *Nucleic Acids Res.,* 23, 2259; Good et al., 1997, *Gene Therapy,* 4, 45. Those skilled in the art realize that any nucleic acid can be expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of such nucleic acids can be augmented by their release from the primary transcript by a enzymatic nucleic acid (Draper et al., PCT WO 93/23569, and Sullivan et al., PCT WO 94/02595; Ohkawa et al., 1992, *Nucleic Acids Symp. Ser.,* 27, 15-6; Taira et al., 1991, *Nucleic Acids Res.,* 19, 5125-30; Ventura et al., 1993, *Nucleic Acids Res.,* 21, 3249-55; Chowrira et al., 1994, *J. Biol. Chem.,* 269, 25856.

[0608] In another aspect of the invention, RNA molecules of the present invention can be expressed from transcription units (see for example Couture et al., 1996, *TIG.,* 12, 510) inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. In another embodiment, pol III based constructs are used to express nucleic acid molecules of the invention (see for example Thompson, U.S. Pats. Nos. 5,902,880 and 6,146,886). The recombinant vectors capable of expressing the siNA molecules can be delivered as described above, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of nucleic acid molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siNA molecule interacts with the target mRNA and generates an RNAi response. Delivery of siNA molecule expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired target cell (for a review see Couture et al., 1996, *TIG.,* 12, 510).

[0609] In one aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the instant invention. The expression vector can encode one or both strands of a siNA duplex, or a single self-complementary strand that self hybridizes into a siNA duplex. The nucleic acid sequences encoding the siNA molecules of the instant invention can be operably linked in a manner that allows expression of the siNA molecule (see for example Paul et al., 2002, *Nature Biotechnology,* 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology,* 19, 497; Lee et al., 2002, *Nature Biotechnology,* 19, 500; and Novina et al., 2002, *Nature Medicine*, advance online publication doi:10.1038/nm725).

[0610] In another aspect, the invention features an expression vector comprising: a) a transcription initiation region (e.g., eukaryotic pol I, II or III initiation region); b) a transcription termination region (e.g., eukaryotic pol I, II or III termination region); and c) a nucleic acid sequence encoding

at least one of the siNA molecules of the instant invention, wherein said sequence is operably linked to said initiation region and said termination region in a manner that allows expression and/or delivery of the siNA molecule. The vector can optionally include an open reading frame (ORF) for a protein operably linked on the 5' side or the 3'-side of the sequence encoding the siNA of the invention; and/or an intron (intervening sequences).

[0611] Transcription of the siNA molecule sequences can be driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters are expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type depends on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990, *Proc. Natl. Acad. Sci. USA,* 87, 6743-7; Gao and Huang 1993, *Nucleic Acids Res.,* 21, 2867-72; Lieber et al., 1993, *Methods Enzymol.,* 217, 47-66; Zhou et al., 1990, *Mol. Cell. Biol.,* 10, 4529-37). Several investigators have demonstrated that nucleic acid molecules expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992, *Antisense Res. Dev.,* 2, 3-15; Ojwang et al., 1992, *Proc. Natl. Acad. Sci. USA,* 89, 10802-6; Chen et al., 1992, *Nucleic Acids Res.,* 20, 4581-9; Yu et al., 1993, *Proc. Natl. Acad. Sci. USA,* 90, 6340-4; L'Huillier et al., 1992, *EMBO J.,* 11, 4411-8; Lisziewicz et al., 1993, *Proc. Natl. Acad. Sci. U.S.A.,* 90, 8000-4; Thompson et al., 1995, *Nucleic Acids Res.,* 23, 2259; Sullenger & Cech, 1993, *Science,* 262, 1566). More specifically, transcription units such as the ones derived from genes encoding U6 small nuclear (snRNA), transfer RNA (tRNA) and adenovirus VA RNA are useful in generating high concentrations of desired RNA molecules such as siNA in cells (Thompson et al., supra; Couture and Stinchcomb, 1996, supra; Noonberg et al., 1994, *Nucleic Acid Res.,* 22, 2830; Noonberg et al., U.S. Pat. No. 5,624,803; Good et al., 1997, *Gene Ther.,* 4, 45; Beigelman et al., International PCT Publication No. WO 96/18736. The above siNA transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review see Couture and Stinchcomb, 1996, supra).

[0612] In another aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one of the siNA molecules of the invention in a manner that allows expression of that siNA molecule. The expression vector comprises in one embodiment; a) a transcription initiation region; b) a transcription termination region; and c) a nucleic acid sequence encoding at least one strand of the siNA molecule, wherein the sequence is operably linked to the initiation region and the termination region in a manner that allows expression and/or delivery of the siNA molecule.

[0613] In another embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an open reading frame; and d) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and wherein the sequence is operably linked to the initiation region, the open reading frame and the termination region in a manner that allows expression and/or delivery of the siNA molecule. In yet another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; and d) a nucleic acid sequence encoding at least one siNA molecule, wherein the sequence is operably linked to the initiation region, the intron and the termination region in a manner which allows expression and/or delivery of the nucleic acid molecule.

[0614] In another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) an open reading frame; and e) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and wherein the sequence is operably linked to the initiation region, the intron, the open reading frame and the termination region in a manner which allows expression and/or delivery of the siNA molecule.

SREBP1 Biology and Biochemistry

[0615] Cardiovascular disease is the number one killer in the United States, and atherosclerosis is the major cause of heart disease and stroke ("*Heart and Stroke Statistical Update*", American Heart Association, Dallas, Tex., USA, 1997). It is known that cholesterol plays an important role in atherogenesis (Kannel et al., 1971, *Ann. Intern. Med.* 74:1-12). In mammals, most cholesterol serves as a structural element in the walls of cells, whereas the rest either serves as the starting material for the synthesis of bile acids, steroid hormones and vitamin D or is in transit in the blood to a given location.

[0616] The transport of cholesterol and other lipids through the circulatory system is mediated by their packaging into lipoprotein carriers. These spherical particles comprise protein and phospholipid shells surrounding a core of neutral lipid, including unesterified or esterified cholesterol and triglycerides.

[0617] It is known that the risk of atherosclerosis increases with increasing concentrations of low-density lipoprotein (LDL) cholesterol. In contrast, risk of cardiovascular disease is inversely related to concentrations of high-density lipoprotein (HDL) cholesterol (Gordon et al., 1977, *Am. J. Med.* 62:707-14).

[0618] Although cholesterol plays a crucial role in the etiology of atherosclerosis, numerous other genetic and environmental factors are known to contribute to the disease. For example, non-modifiable factors that influence disease predisposition include age (risk increases with age), gender (men are more prone than are pre-menopausal women) and family history (i.e., genetics). Modifiable factors that increase risk of disease include obesity, blood pressure, diet, exercise, poor control of diabetes, alcohol intake and smoking. Thus, the risk of atherosclerosis is determined by a complex interplay of genetic and environmental risk factors (Pearson, 2002, *Circulation,* 105:886-892). While there is considerable knowledge of the environmental risk factors, present understanding of the genetics of atherosclerosis is poor.

[0619] Because LDL and HDL have opposing and significant effects on the risk of atherosclerosis, understanding the genetic contribution to levels of these lipoproteins in the blood not only will enhance the understanding of atherosclerosis but can also facilitate identification of at-risk individuals, even before abnormalities in their plasma HDL and LDL levels become apparent. Indeed, mutations in the LDL recep-

tor gene result in familial hypercholesterolemia, a disease characterized by elevated plasma LDL levels and premature atherosclerosis (Goldstein & Brown, in *The Metabolic Basis of Inherited Disease,* 6th ed., (Scriver, Beaudet, Sly and Valle, eds.), McGraw-Hill, New York, N.Y., USA, (1989) pp. 1215-1250). Similarly, mutations in the ATP-binding cassette 1 (ABC1) gene cause Tangier disease, a disorder that is characterized by the near-absence of HDL in plasma, peripheral neuropathy and premature heart disease (Bodzioch et al., 1999, *Nature Genet.* 22:347-351). Thus, genes that influence plasma HDL and LDL levels can serve as targets for drugs that modulate lipid levels and therefore the risk of atherosclerosis. In fact, drugs known as "statins" inhibit the enzyme, 3-hydroxy-3-methylglutaryl-CoA-reductase (HMG CoA reductase), and thereby reduce both levels of plasma LDL levels and risk of atherosclerosis (Sacks et al., 1996, *N. Engl. J. Med.* 335:1001-1009). Analogous drugs that significantly increase plasma HDL levels are not presently known.

[0620] A family of transcription factors called Sterol Response Element Binding Proteins (SREBPs) regulates lipid levels in mammals. It is known that genes called Sterol Response Element Binding Protein 1 (SREBP1) and called Sterol Response Element Binding Protein 2 (SREBP2) directly activate the expression of over thirty different genes implicated in the synthesis and uptake of cholesterol, fatty acids, triglycerides, phospholipids and the NADPH co-factor required to synthesize these molecules (Horton et al., 2001, *J. Clin. Invest.* 109:1125-1131). Further, it is known that the expression of both the LDL receptor and HDL receptor genes is regulated by SREBPs (Smith et al., 1990, *J. Biol. Chem.* 265: 2306-2310; Lopez & McLean, 1999, *Endocrinol.* 140: 5669-5681). Thus, the inter-individual variation in plasma HDL and LDL levels (and therefore variation in risk of atherosclerosis) seen in the general population can be attributed, at least in part, to genetic variation in SREBPs.

[0621] Based upon the current understanding of SREBP1 function, the modulation of SREBP1 and other related genes is instrumental in the development of new therapeutics for metabolic disease. As such, modulation of SREBP1 using small interfering nucleic acid (siNA) mediated RNAi represents a novel approach to the treatment and study of diseases and conditions related to SREBP1 activity and/or gene expression including hyperlipidemia, hypercholesterolemia, cardiovascular disease, atherosclerosis, hypertension, diabetis (e.g., type I and/or type II diabetis), insulin resistance, obesity and/or other disease states, conditions, or traits associated with SREBP1 gene expression or activity in a subject or organism.

EXAMPLES

[0622] The following are non-limiting examples showing the selection, isolation, synthesis and activity of nucleic acids of the instant invention.

Example 1

Tandem Synthesis of siNA Constructs

[0623] Exemplary siNA molecules of the invention are synthesized in tandem using a cleavable linker, for example, a succinyl-based linker. Tandem synthesis as described herein is followed by a one-step purification process that provides RNAi molecules in high yield. This approach is highly amenable to siNA synthesis in support of high throughput RNAi screening, and can be readily adapted to multi-column or multi-well synthesis platforms.

[0624] After completing a tandem synthesis of a siNA oligo and its complement in which the 5'-terminal dimethoxytrityl (5'-O-DMT) group remains intact (trityl on synthesis), the oligonucleotides are deprotected as described above. Following deprotection, the siNA sequence strands are allowed to spontaneously hybridize. This hybridization yields a duplex in which one strand has retained the 5'-O-DMT group while the complementary strand comprises a terminal 5'-hydroxyl. The newly formed duplex behaves as a single molecule during routine solid-phase extraction purification (Trityl-On purification) even though only one molecule has a dimethoxytrityl group. Because the strands form a stable duplex, this dimethoxytrityl group (or an equivalent group, such as other trityl groups or other hydrophobic moieties) is all that is required to purify the pair of oligos, for example, by using a C18 cartridge.

[0625] Standard phosphoramidite synthesis chemistry is used up to the point of introducing a tandem linker, such as an inverted deoxy abasic succinate or glyceryl succinate linker (see FIG. **1**) or an equivalent cleavable linker. A non-limiting example of linker coupling conditions that can be used includes a hindered base such as diisopropylethylamine (DIPA) and/or DMAP in the presence of an activator reagent such as Bromotripyrrolidinophosphoniumhexafluororophosphate (PyBrOP). After the linker is coupled, standard synthesis chemistry is utilized to complete synthesis of the second sequence leaving the terminal the 5'-O-DMT intact. Following synthesis, the resulting oligonucleotide is deprotected according to the procedures described herein and quenched with a suitable buffer, for example with 50 mM NaOAc or 1.5M $NH_4H_2CO_3$.

[0626] Purification of the siNA duplex can be readily accomplished using solid phase extraction, for example, using a Waters C18 SepPak 1 g cartridge conditioned with 1 column volume (CV) of acetonitrile, 2 CV H2O, and 2 CV 50 mM NaOAc. The sample is loaded and then washed with 1 CV H2O or 50 mM NaOAc. Failure sequences are eluted with 1 CV 14% ACN (Aqueous with 50 mM NaOAc and 50 mM NaCl). The column is then washed, for example with 1 CV H2O followed by on-column detritylation, for example by passing 1 CV of 1% aqueous trifluoroacetic acid (TFA) over the column, then adding a second CV of 1% aqueous TFA to the column and allowing to stand for approximately 10 minutes. The remaining TFA solution is removed and the column washed with H20 followed by 1 CV 1M NaCl and additional H2O. The siNA duplex product is then eluted, for example, using 1 CV 20% aqueous CAN.

[0627] FIG. **2** provides an example of MALDI-TOF mass spectrometry analysis of a purified siNA construct in which each peak corresponds to the calculated mass of an individual siNA strand of the siNA duplex. The same purified siNA provides three peaks when analyzed by capillary gel electrophoresis (CGE), one peak presumably corresponding to the duplex siNA, and two peaks presumably corresponding to the separate siNA sequence strands. Ion exchange HPLC analysis of the same siNA contract only shows a single peak. Testing of the purified siNA construct using a luciferase reporter assay described below demonstrated the same RNAi activity compared to siNA constructs generated from separately synthesized oligonucleotide sequence strands.

Example 2

Identification of Potential siNA Target Sites in any RNA Sequence

[0628] The sequence of an RNA target of interest, such as a human SREBP1 mRNA transcript, is screened for target sites, for example by using a computer folding algorithm. In a non-limiting example, the sequence of a gene or RNA gene transcript derived from a database, such as Genbank, is used to generate siNA targets having complementarity to the target. Such sequences can be obtained from a database, or can be determined experimentally as known in the art. Target sites that are known, for example, those target sites determined to be effective target sites based on studies with other nucleic acid molecules, for example ribozymes or antisense, or those targets known to be associated with a disease, trait, or condition such as those sites containing mutations or deletions, can be used to design siNA molecules targeting those sites. Various parameters can be used to determine which sites are the most suitable target sites within the target RNA sequence. These parameters include but are not limited to secondary or tertiary RNA structure, the nucleotide base composition of the target sequence, the degree of homology between various regions of the target sequence, or the relative position of the target sequence within the RNA transcript. Based on these determinations, any number of target sites within the RNA transcript can be chosen to screen siNA molecules for efficacy, for example by using in vitro RNA cleavage assays, cell culture, or animal models. In a non-limiting example, anywhere from 1 to 1000 target sites are chosen within the transcript based on the size of the siNA construct to be used. High throughput screening assays can be developed for screening siNA molecules using methods known in the art, such as with multi-well or multi-plate assays to determine efficient reduction in target gene expression.

Example 3

Selection of siNA Molecule Target Sites in a RNA

[0629] The following non-limiting steps can be used to carry out the selection of siNAs targeting a given gene sequence or transcript.

[0630] 1. The target sequence is parsed in silico into a list of all fragments or subsequences of a particular length, for example 23 nucleotide fragments, contained within the target sequence. This step is typically carried out using a custom Perl script, but commercial sequence analysis programs such as Oligo, MacVector, or the GCG Wisconsin Package can be employed as well.

[0631] 2. In some instances the siNAs correspond to more than one target sequence; such would be the case for example in targeting different transcripts of the same gene, targeting different transcripts of more than one gene, or for targeting both the human gene and an animal homolog. In this case, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find matching sequences in each list. The subsequences are then ranked according to the number of target sequences that contain the given subsequence; the goal is to find subsequences that are present in most or all of the target sequences. Alternately, the ranking can identify subsequences that are unique to a target sequence, such as a mutant target sequence. Such an approach would enable the use of siNA to target specifically the mutant sequence and not effect the expression of the normal sequence.

[0632] 3. In some instances the siNA subsequences are absent in one or more sequences while present in the desired target sequence; such would be the case if the siNA targets a gene with a paralogous family member that is to remain untargeted. As in case 2 above, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find sequences that are present in the target gene but are absent in the untargeted paralog.

[0633] 4. The ranked siNA subsequences can be further analyzed and ranked according to GC content. A preference can be given to sites containing 30-70% GC, with a further preference to sites containing 40-60% GC.

[0634] 5. The ranked siNA subsequences can be further analyzed and ranked according to self-folding and internal hairpins. Weaker internal folds are preferred; strong hairpin structures are to be avoided.

[0635] 6. The ranked siNA subsequences can be further analyzed and ranked according to whether they have runs of GGG or CCC in the sequence. GGG (or even more Gs) in either strand can make oligonucleotide synthesis problematic and can potentially interfere with RNAi activity, so it is avoided whenever better sequences are available. CCC is searched in the target strand because that will place GGG in the antisense strand.

[0636] 7. The ranked siNA subsequences can be further analyzed and ranked according to whether they have the dinucleotide UU (uridine dinucleotide) on the 3'-end of the sequence, and/or AA on the 5'-end of the sequence (to yield 3' UU on the antisense sequence). These sequences allow one to design siNA molecules with terminal TT thymidine dinucleotides.

[0637] 8. Four or five target sites are chosen from the ranked list of subsequences as described above. For example, in subsequences having 23 nucleotides, the right 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the upper (sense) strand of the siNA duplex, while the reverse complement of the left 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the lower (antisense) strand of the siNA duplex (see Table II). If terminal TT residues are desired for the sequence (as described in paragraph 7), then the two 3' terminal nucleotides of both the sense and antisense strands are replaced by TT prior to synthesizing the oligos.

[0638] 9. The siNA molecules are screened in an in vitro, cell culture or animal model system to identify the most active siNA molecule or the most preferred target site within the target RNA sequence.

[0639] 10. Other design considerations can be used when selecting target nucleic acid sequences, see, for example, Reynolds et al., 2004, *Nature Biotechnology Advanced Online Publication,* 1 Feb. 2004, doi:10.1038/nbt936 and Ui-Tei et al., 2004, Nucleic Acids Research, 32, doi:10.1093/nar/gkh247.

[0640] In an alternate approach, a pool of siNA constructs specific to a target sequence is used to screen for target sites in cells expressing target RNA, such as cultured Jurkat, HeLa, A549 or 293T cells. The general strategy used in this approach is shown in FIG. **9**. Cells expressing the target RNA are transfected with the pool of siNA constructs and cells that demonstrate a phenotype associated with target inhibition are sorted. The pool of siNA constructs can be expressed from

transcription cassettes inserted into appropriate vectors (see for example FIG. **7** and FIG. **8**). The siNA from cells demonstrating a positive phenotypic change (e.g., decreased proliferation, decreased target mRNA levels or decreased target protein expression), are sequenced to determine the most suitable target site(s) within the target RNA sequence.

[0641] In one embodiment, siNA molecules of the invention are selected using the following methodology. The following guidelines were compiled to predict hyper-active siNAs that contain chemical modifications described herein. These rules emerged from a comparative analysis of hyper-active (>75% knockdown of target mRNA levels) and inactive (<75% knockdown of target mRNA levels) siNAs against several different targets. A total of 242 siNA sequences were analyzed. Thirty-five siNAs out of 242 siRNAs were grouped into hyper-active and the remaining siNAs were grouped into inactive groups. The hyper-active siNAs clearly showed a preference for certain bases at particular nucleotide positions within the siNA sequence. For example, A or U nucleobase was overwhelmingly present at position 19 of the sense strand in hyper-active siNAs and opposite was true for inactive siNAs. There was also a pattern of a A/U rich (3 out of 5 bases as A or U) region between positions 15-19 and G/C rich region between positions 1-5 (3 out of 5 bases as G or C) of the sense strand in hyperactive siNAs. As shown in Table VII, 12 such patterns were identified that were characteristics of hyper-active siNAs. It is to be noted that not every pattern was present in each hyper-active siNA. Thus, to design an algorithm for predicting hyper-active siNAs, a different score was assigned for each pattern. Depending on how frequently such patterns occur in hyper-active siRNAs versus inactive siRNAs, the design parameters were assigned a score with the highest being 10. If a certain nucleobase is not preferred at a position, then a negative score was assigned. For example, at positions 9 and 13 of the sense strand, a G nucleotide was not preferred in hyper-active siNAs and therefore they were given score of −3 (minus 3). The differential score for each pattern is given in Table VII. The pattern # 4 was given a maximum score of −100. This is mainly to weed out any sequence that contains string of 4Gs or 4Cs as they can be highly incompatible for synthesis and can allow sequences to self-aggregate, thus rendering the siNA inactive. Using this algorithm, the highest score possible for any siNA is 66. As there are numerous siNA sequences possible against any given target of reasonable size (~1000 nucleotides), this algorithm is useful to generate hyper-active siNAs.

[0642] In one embodiment, rules 1-11 shown in Table VII are used to generate active siNA molecules of the invention. In another embodiment, rules 1-12 shown in Table VII are used to generate active siNA molecules of the invention.

Example 4

SREBP1 siNA Design

[0643] siNA target sites were chosen by analyzing sequences of the SREBP1 target and optionally prioritizing the target sites on the basis of the rules presented in Example 3 above, and alternately on the basis of folding (structure of any given sequence analyzed to determine siNA accessibility to the target), or by using a library of siNA molecules as described in Example 3, or alternately by using an in vitro siNA system as described in Example 6 herein. siNA molecules were designed that could bind each target and are selected using the algorithm above and are optionally individually analyzed by computer folding to assess whether the siNA molecule can interact with the target sequence. Varying the length of the siNA molecules can be chosen to optimize activity. Generally, a sufficient number of complementary nucleotide bases are chosen to bind to, or otherwise interact with, the target RNA, but the degree of complementarity can be modulated to accommodate siNA duplexes or varying length or base composition. By using such methodologies, siNA molecules can be designed to target sites within any known RNA sequence, for example those RNA sequences corresponding to the any gene transcript.

[0644] SREBP1 sequences (Table I) were analysed to generate SREBP1 targets from which double stranded siNA were designed (Table II). To generate synthetic siNA constructs (Table III), the algorithm described in Example 3 was utilized to pick active double stranded constructs and chemically modified versions thereof. In each of Tables II and III, the target sequence is shown, along with the upper (sense strand) and lower (antisense strand) of the siNA duplex. Multifunctional siNAs are designed by searching for homologous sites between different target sequences (e.g., 11 nucleotide regions of shared homology) and allowing for up to 3 wobble bp (G:U) or up to 2 mismatches.

[0645] Chemically modified siNA constructs were designed as described herein (see for example Table V) to provide nuclease stability for systemic administration in vivo and/or improved pharmacokinetic, localization, and delivery properties while preserving the ability to mediate RNAi activity. Chemical modifications as described herein are introduced synthetically using synthetic methods described herein and those generally known in the art. The synthetic siNA constructs are then assayed for nuclease stability in serum and/or cellular/tissue extracts (e.g. liver extracts). The synthetic siNA constructs are also tested in parallel for RNAi activity using an appropriate assay, such as a luciferase reporter assay as described herein or another suitable assay that can quantity RNAi activity. Synthetic siNA constructs that possess both nuclease stability and RNAi activity can be further modified and re-evaluated in stability and activity assays. The chemical modifications of the stabilized active siNA constructs can then be applied to any siNA sequence targeting any chosen RNA and used, for example, in target screening assays to pick lead siNA compounds for therapeutic development (see for example FIG. **11**).

Example 5

Chemical Synthesis and Purification of siNA

[0646] siNA molecules can be designed to interact with various sites in the RNA message, for example, target sequences within the RNA sequences described herein. The sequence of one strand of the siNA molecule(s) is complementary to the target site sequences described above. The siNA molecules can be chemically synthesized using methods described herein. Inactive siNA molecules that are used as control sequences can be synthesized by scrambling the sequence of the siNA molecules such that it is not complementary to the target sequence. Generally, siNA constructs can by synthesized using solid phase oligonucleotide synthesis methods as described herein (see for example Usman et al., U.S. Pat. Nos. 5,804,683; 5,831,071; 5,998,203; 6,117,657; 6,353,098; 6,362,323; 6,437,117; 6,469,158; Scaringe et al., U.S. Pat. Nos. 6,111,086; 6,008,400; 6,111,086 all incorporated by reference herein in their entirety).

[0647] In a non-limiting example, RNA oligonucleotides are synthesized in a stepwise fashion using the phosphoramidite chemistry as is known in the art. Standard phosphoramidite chemistry involves the use of nucleosides comprising any of 5'-O-dimethoxytrityl, 2'-O-tert-butyldimethylsilyl, 3'-O-2-Cyanoethyl N,N-diisopropylphosphoroamidite groups, and exocyclic amine protecting groups (e.g. N6-benzoyl adenosine, N4 acetyl cytidine, and N2-isobutyryl guanosine). Alternately, 2'-O-Silyl Ethers can be used in conjunction with acid-labile 2'-O-orthoester protecting groups in the synthesis of RNA as described by Scaringe supra. Differing 2' chemistries can require different protecting groups, for example 2'-deoxy-2'-amino nucleosides can utilize N-phthaloyl protection as described by Usman et al., U.S. Pat. No. 5,631,360, incorporated by reference herein in its entirety).

[0648] During solid phase synthesis, each nucleotide is added sequentially (3'- to 5'-direction) to the solid support-bound oligonucleotide. The first nucleoside at the 3'-end of the chain is covalently attached to a solid support (e.g., controlled pore glass or polystyrene) using various linkers. The nucleotide precursor, a ribonucleoside phosphoramidite, and activator are combined resulting in the coupling of the second nucleoside phosphoramidite onto the 5'-end of the first nucleoside. The support is then washed and any unreacted 5'-hydroxyl groups are capped with a capping reagent such as acetic anhydride to yield inactive 5'-acetyl moieties. The trivalent phosphorus linkage is then oxidized to a more stable phosphate linkage. At the end of the nucleotide addition cycle, the 5'-O-protecting group is cleaved under suitable conditions (e.g., acidic conditions for trityl-based groups and fluoride for silyl-based groups). The cycle is repeated for each subsequent nucleotide.

[0649] Modification of synthesis conditions can be used to optimize coupling efficiency, for example by using differing coupling times, differing reagent/phosphoramidite concentrations, differing contact times, differing solid supports and solid support linker chemistries depending on the particular chemical composition of the siNA to be synthesized. Deprotection and purification of the siNA can be performed as is generally described in Usman et al., U.S. Pat. No. 5,831,071, U.S. Pat. No. 6,353,098, U.S. Pat. No. 6,437,117, and Bellon et al., U.S. Pat. No. 6,054,576, U.S. Pat. No. 6,162,909, U.S. Pat. No. 6,303,773, or Scaringe supra, incorporated by reference herein in their entireties. Additionally, deprotection conditions can be modified to provide the best possible yield and purity of siNA constructs. For example, applicant has observed that oligonucleotides comprising 2'-deoxy-2'-fluoro nucleotides can degrade under inappropriate deprotection conditions. Such oligonucleotides are deprotected using aqueous methylamine at about 35° C. for 30 minutes. If the 2'-deoxy-2'-fluoro containing oligonucleotide also comprises ribonucleotides, after deprotection with aqueous methylamine at about 35° C. for 30 minutes, TEA-HF is added and the reaction maintained at about 65° C. for an additional 15 minutes.

Example 6

RNAi In Vitro Assay to Assess siNA Activity

[0650] An in vitro assay that recapitulates RNAi in a cell-free system is used to evaluate siNA constructs targeting target RNA targets. The assay comprises the system described by Tuschl et al., 1999, *Genes and Development,* 13, 3191-3197 and Zamore et al., 2000, *Cell,* 101, 25-33 adapted for use with a target RNA. A *Drosophila* extract derived from syncytial blastoderm is used to reconstitute RNAi activity in vitro. Target RNA is generated via in vitro transcription from an appropriate target expressing plasmid using T7 RNA polymerase or via chemical synthesis as described herein. Sense and antisense siNA strands (for example 20 uM each) are annealed by incubation in buffer (such as 100 mM potassium acetate, 30 mM HEPES-KOH, pH 7.4, 2 mM magnesium acetate) for 1 minute at 90° C. followed by 1 hour at 37° C., then diluted in lysis buffer (for example 100 mM potassium acetate, 30 mM HEPES-KOH at pH 7.4, 2 mM magnesium acetate). Annealing can be monitored by gel electrophoresis on an agarose gel in TBE buffer and stained with ethidium bromide. The *Drosophila* lysate is prepared using zero to two-hour-old embryos from Oregon R flies collected on yeasted molasses agar that are dechorionated and lysed. The lysate is centrifuged and the supernatant isolated. The assay comprises a reaction mixture containing 50% lysate [vol/vol], RNA (10-50 pM final concentration), and 10% [vol/vol] lysis buffer containing siNA (10 nM final concentration). The reaction mixture also contains 10 mM creatine phosphate, 10 ug/ml creatine phosphokinase, 100 um GTP, 100 uM UTP, 100 uM CTP, 500 uM ATP, 5 mM DTT, 0.1 U/uL RNasin (Promega), and 100 uM of each amino acid. The final concentration of potassium acetate is adjusted to 100 mM. The reactions are pre-assembled on ice and preincubated at 25° C. for 10 minutes before adding RNA, then incubated at 25° C. for an additional 60 minutes. Reactions are quenched with 4 volumes of 1.25× Passive Lysis Buffer (Promega). Target RNA cleavage is assayed by RT-PCR analysis or other methods known in the art and are compared to control reactions in which siNA is omitted from the reaction.

[0651] Alternately, internally-labeled target RNA for the assay is prepared by in vitro transcription in the presence of [alpha-^{32}P] CTP, passed over a G50 Sephadex column by spin chromatography and used as target RNA without further purification. Optionally, target RNA is 5'-^{32}P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed as described above and target RNA and the specific RNA cleavage products generated by RNAi are visualized on an autoradiograph of a gel. The percentage of cleavage is determined by PHOSPHOR IMAGER® (autoradiography) quantitation of bands representing intact control RNA or RNA from control reactions without siNA and the cleavage products generated by the assay.

[0652] In one embodiment, this assay is used to determine target sites in the target RNA target for siNA mediated RNAi cleavage, wherein a plurality of siNA constructs are screened for RNAi mediated cleavage of the target RNA target, for example, by analyzing the assay reaction by electrophoresis of labeled target RNA, or by northern blotting, as well as by other methodology well known in the art.

Example 7

Nucleic Acid Inhibition of SREBP1 Target RNA

[0653] siNA molecules targeted to SREBP1 RNA are designed and synthesized as described above. These nucleic acid molecules can be tested for cleavage activity in vivo, for example, using the following procedure. The target sequences and the nucleotide location within the SREBP1 RNA are given in Table II and III.

[0654] Two formats are used to test the efficacy of siNAs targeting SREBP1. First, the reagents are tested in cell culture

using HepG2, Jurkat, HeLa, A549 or 293T cells, to determine the extent of RNA and protein inhibition. siNA reagents (e.g.; see Tables II and III) are selected against the SREBP1 target as described herein. RNA inhibition is measured after delivery of these reagents by a suitable transfection agent to, for example, HepG2, Jurkat, HeLa, A549 or 293T cells. Relative amounts of target RNA are measured versus actin using real-time PCR monitoring of amplification (eg., ABI 7700 TAQMAN®). A comparison is made to a mixture of oligonucleotide sequences made to unrelated targets or to a randomized siNA control with the same overall length and chemistry, but randomly substituted at each position. Primary and secondary lead reagents are chosen for the target and optimization performed. After an optimal transfection agent concentration is chosen, a RNA time-course of inhibition is performed with the lead siNA molecule. In addition, a cell-plating format can be used to determine RNA inhibition.

Delivery of siNA to Cells

[0655] Cells (e.g., HepG2, Jurkat, HeLa, A549 or 293T cells) are seeded, for example, at 1×10^5 cells per well of a six-well dish in EGM-2 (BioWhittaker) the day before transfection. siNA (final concentration, for example 20 nM) and cationic lipid (e.g., LNP formulations herein, or another suitable lipid such as Lipofectamine, final concentration 2 μg/ml) are complexed in EGM basal media (Biowhittaker) at 37° C. for 30 minutes in polystyrene tubes. Following vortexing, the complexed siNA is added to each well and incubated for the times indicated. For initial optimization experiments, cells are seeded, for example, at 1×10^3 in 96 well plates and siNA complex added as described. Efficiency of delivery of siNA to cells is determined using a fluorescent siNA complexed with lipid. Cells in 6-well dishes are incubated with siNA for 24 hours, rinsed with PBS and fixed in 2% paraformaldehyde for 15 minutes at room temperature. Uptake of siNA is visualized using a fluorescent microscope.

TAQMAN® (Real-Time PCR Monitoring of Amplification) and Lightcycler Quantification of mRNA

[0656] Total RNA is prepared from cells following siNA delivery, for example, using Qiagen RNA purification kits for 6-well or Rneasy extraction kits for 96-well assays. For TAQMAN® analysis (real-time PCR monitoring of amplification), dual-labeled probes are synthesized with the reporter dye, FAM or JOE, covalently linked at the 5'-end and the quencher dye TAMRA conjugated to the 3'-end. One-step RT-PCR amplifications are performed on, for example, an ABI PRISM 7700 Sequence Detector using 50 μl reactions consisting of 10 μl total RNA, 100 nM forward primer, 900 mM reverse primer, 100 nM probe, 1× TaqMan PCR reaction buffer (PE-Applied Biosystems), 5.5 mM $MgCl_2$, 300 μM each dATP, dCTP, dGTP, and dTTP, 10 U RNase Inhibitor (Promega), 1.25 U AMPLITAQ GOLDS (DNA polymerase) (PE-Applied Biosystems) and 10 U M-MLV Reverse Transcriptase (Promega). The thermal cycling conditions can consist of 30 minutes at 48° C., 10 minutes at 95° C., followed by 40 cycles of 15 seconds at 95° C. and 1 minute at 60° C. Quantitation of mRNA levels is determined relative to standards generated from serially diluted total cellular RNA (300, 100, 33, 11 ng/reaction) and normalizing to β-actin or GAPDH mRNA in parallel TAQMAN® reactions (real-time PCR monitoring of amplification). For each gene of interest an upper and lower primer and a fluorescently labeled probe are designed. Real time incorporation of SYBR Green I dye into a specific PCR product can be measured in glass capillary tubes using a lightcyler. A standard curve is generated for each primer pair using control cRNA. Values are represented as relative expression to GAPDH in each sample.

Western Blotting

[0657] Nuclear extracts can be prepared using a standard micro preparation technique (see for example Andrews and Faller, 1991, *Nucleic Acids Research,* 19, 2499). Protein extracts from supernatants are prepared, for example using TCA precipitation. An equal volume of 20% TCA is added to the cell supernatant, incubated on ice for 1 hour and pelleted by centrifugation for 5 minutes. Pellets are washed in acetone, dried and resuspended in water. Cellular protein extracts are run on a 10% Bis-Tris NuPage (nuclear extracts) or 4-12% Tris-Glycine (supernatant extracts) polyacrylamide gel and transferred onto nitro-cellulose membranes. Non-specific binding can be blocked by incubation, for example, with 5% non-fat milk for 1 hour followed by primary antibody for 16 hour at 4° C. Following washes, the secondary antibody is applied, for example (1:10,000 dilution) for 1 hour at room temperature and the signal detected with SuperSignal reagent (Pierce).

Example 8

Models Useful to Evaluate the Down-Regulation of SREBP1 Gene Expression

[0658] Evaluating the efficacy of siNA molecules of the invention in animal models is an important prerequisite to human clinical trials. Various animal models of metabolic and cardiovascular diseases, conditions, or disorders as are known in the art can be adapted for use for pre-clinical evaluation of the efficacy of nucleic acid compositions of the invention in modulating target (e.g., SREBP1) gene expression toward therapeutic or research use. Goldberg, 2004, *J Clin Invest.* 114(5): 613-615, provides a review of animal models useful for evaluating therapeutic agents for metabolic disease, including hyperlipidemia and diabetes. These models and others can be utilized to evaluate the efficacy of siNA molecules and compositions of the invention in treating metabolic and related diseases and conditions.

Example 9

RNAi Mediated Inhibition of SREBP1 Gene Expression

[0659] In Vitro siNA Mediated Inhibition of SREBP1 RNA

[0660] siNA constructs are tested for efficacy in reducing SREBP1 RNA expression in cells, (e.g., HepG2 cells). Cells are plated approximately 24 hours before transfection in 96-well plates at 5,000-7,500 cells/well, 100 μl/well, such that at the time of transfection cells are 70-90% confluent. For transfection, annealed siNAs are mixed with the transfection reagent (Lipofectamine 2000, Invitrogen) in a volume of 50 μl/well and incubated for 20 minutes at room temperature. The siNA transfection mixtures are added to cells to give a final siNA concentration of 25 nM in a volume of 150 μl. Each siNA transfection mixture is added to 3 wells for triplicate siNA treatments. Cells are incubated at 37° for 24 hours in the continued presence of the siNA transfection mixture. At 24 hours, RNA is prepared from each well of treated cells. The supernatants with the transfection mixtures are first removed and discarded, then the cells are lysed and RNA prepared from each well. Target gene expression following treatment is evaluated by RT-PCR for the SREBP1 gene and for a control

gene (36B4, an RNA polymerase subunit) for normalization. The triplicate data is averaged and the standard deviations determined for each treatment. Normalized data are graphed and the percent reduction of target mRNA by active siNAs in comparison to their respective inverted control siNAs is determined.

Example 10

Indications

[0661] Particular conditions and disease states that can be associated with gene expression modulation include, but are not limited to metabolic and cardiovascular diseases, conditions, or disorders as described herein or otherwise known in the art, and any other diseases, conditions or disorders that are related to or will respond to the levels of a target (e.g., target SREBP1 protein or target SREBP1 polynucleotide) in a cell or tissue, alone or in combination with other therapies. Non-limiting examples of such diseases and conditions include hyperlipidemia, hypercholesterolemia, dyslipidemia, cardiovascular disease, atherosclerosis, hypertension, diabetis (e.g., type I and/or type II diabetis), insulin resistance, obesity, and/or metabolic syndrome.

Example 11

Multifunctional siNA Inhibition of Target RNA Expression

[0662] Multifunctional siNA Design

[0663] Once target sites have been identified for multifunctional siNA constructs, each strand of the siNA is designed with a complementary region of length, for example, of about 18 to about 28 nucleotides, that is complementary to a different target nucleic acid sequence. Each complementary region is designed with an adjacent flanking region of about 4 to about 22 nucleotides that is not complementary to the target sequence, but which comprises complementarity to the complementary region of the other sequence (see for example FIG. **16**). Hairpin constructs can likewise be designed (see for example FIG. **17**). Identification of complementary, palindrome or repeat sequences that are shared between the different target nucleic acid sequences can be used to shorten the overall length of the multifunctional siNA constructs (see for example FIGS. **18** and **19**).

[0664] In a non-limiting example, three additional categories of additional multifunctional siNA designs are presented that allow a single siNA molecule to silence multiple targets. The first method utilizes linkers to join siNAs (or multifunctional siNAs) in a direct manner. This can allow the most potent siNAs to be joined without creating a long, continuous stretch of RNA that has potential to trigger an interferon response. The second method is a dendrimeric extension of the overlapping or the linked multifunctional design; or alternatively the organization of siNA in a supramolecular format. The third method uses helix lengths greater than 30 base pairs. Processing of these siNAs by Dicer will reveal new, active 5' antisense ends. Therefore, the long siNAs can target the sites defined by the original 5' ends and those defined by the new ends that are created by Dicer processing. When used in combination with traditional multifunctional siNAs (where the sense and antisense strands each define a target) the approach can be used for example to target 4 or more sites.

I. Tethered Bifunctional siNAs

[0665] The basic idea is a novel approach to the design of multifunctional siNAs in which two antisense siNA strands are annealed to a single sense strand. The sense strand oligonucleotide contains a linker (e.g., non-nucleotide linker as described herein) and two segments that anneal to the antisense siNA strands (see FIG. **22**). The linkers can also optionally comprise nucleotide-based linkers. Several potential advantages and variations to this approach include, but are not limited to:

[0666] 1. The two antisense siNAs are independent. Therefore, the choice of target sites is not constrained by a requirement for sequence conservation between two sites. Any two highly active siNAs can be combined to form a multifunctional siNA.

[0667] 2. When used in combination with target sites having homology, siNAs that target a sequence present in two genes (e.g., different isoforms), the design can be used to target more than two sites. A single multifunctional siNA can be for example, used to target RNA of two different target RNAs.

[0668] 3. Multifunctional siNAs that use both the sense and antisense strands to target a gene can also be incorporated into a tethered multifuctional design. This leaves open the possibility of targeting 6 or more sites with a single complex.

[0669] 4. It can be possible to anneal more than two antisense strand siNAs to a single tethered sense strand.

[0670] 5. The design avoids long continuous stretches of dsRNA. Therefore, it is less likely to initiate an interferon response.

[0671] 6. The linker (or modifications attached to it, such as conjugates described herein) can improve the pharmacokinetic properties of the complex or improve its incorporation into liposomes. Modifications introduced to the linker should not impact siNA activity to the same extent that they would if directly attached to the siNA (see for example FIGS. **27** and **28**).

[0672] 7. The sense strand can extend beyond the annealed antisense strands to provide additional sites for the attachment of conjugates.

[0673] 8. The polarity of the complex can be switched such that both of the antisense 3' ends are adjacent to the linker and the 5' ends are distal to the linker or combination thereof.

Dendrimer and Supramolecular siNAs

[0674] In the dendrimer siNA approach, the synthesis of siNA is initiated by first synthesizing the dendrimer template followed by attaching various functional siNAs. Various constructs are depicted in FIG. **23**. The number of functional siNAs that can be attached is only limited by the dimensions of the dendrimer used.

Supramolecular Approach to Multifunctional siNA

[0675] The supramolecular format simplifies the challenges of dendrimer synthesis. In this format, the siNA strands are synthesized by standard RNA chemistry, followed by annealing of various complementary strands. The individual strand synthesis contains an antisense sense sequence of one siNA at the 5'-end followed by a nucleic acid or synthetic linker, such as hexaethyleneglycol, which in turn is followed by sense strand of another siNA in 5' to 3' direction. Thus, the synthesis of siNA strands can be carried out in a standard 3' to 5' direction. Representative examples of trifunctional and tetrafunctional siNAs are depicted in FIG. **24**.

Based on a similar principle, higher functionality siNA constructs can be designed as long as efficient annealing of various strands is achieved.

Dicer Enabled Multifunctional siNA

[0676] Using bioinformatic analysis of multiple targets, stretches of identical sequences shared between differing target sequences can be identified ranging from about two to about fourteen nucleotides in length. These identical regions can be designed into extended siNA helixes (e.g., >30 base pairs) such that the processing by Dicer reveals a secondary functional 5'-antisense site (see for example FIG. **25**). For example, when the first 17 nucleotides of a siNA antisense strand (e.g., 21 nucleotide strands in a duplex with 3'-TT overhangs) are complementary to a target RNA, robust silencing was observed at 25 nM. 80% silencing was observed with only 16 nucleotide complementarity in the same format.

[0677] Incorporation of this property into the designs of siNAs of about 30 to 40 or more base pairs results in additional multifunctional siNA constructs. The example in FIG. **25** illustrates how a 30 base-pair duplex can target three distinct sequences after processing by Dicer-RNaseIII; these sequences can be on the same mRNA or separate RNAs, such as viral and host factor messages, or multiple points along a given pathway (e.g., inflammatory cascades). Furthermore, a 40 base-pair duplex can combine a bifunctional design in tandem, to provide a single duplex targeting four target sequences. An even more extensive approach can include use of homologous sequences to enable five or six targets silenced for one multifunctional duplex. The example in FIG. **25** demonstrates how this can be achieved. A 30 base pair duplex is cleaved by Dicer into 22 and 8 base pair products from either end (8 b.p. fragments not shown). For ease of presentation the overhangs generated by dicer are not shown—but can be compensated for. Three targeting sequences are shown. The required sequence identity overlapped is indicated by grey boxes. The N's of the parent 30 b.p. siNA are suggested sites of 2'-OH positions to enable Dicer cleavage if this is tested in stabilized chemistries. Note that processing of a 30mer duplex by Dicer RNase III does not give a precise 22+8 cleavage, but rather produces a series of closely related products (with 22+8 being the primary site). Therefore, processing by Dicer will yield a series of active siNAs. Another non-limiting example is shown in FIG. **26**. A 40 base pair duplex is cleaved by Dicer into 20 base pair products from either end. For ease of presentation the overhangs generated by dicer are not shown, but can be compensated for. Four targeting sequences are shown. The required sequence identity overlapped is indicated by shaded boxes. This design format can be extended to larger RNAs. If chemically stabilized siNAs are bound by Dicer, then strategically located ribonucleotide linkages can enable designer cleavage products that permit our more extensive repertoire of multifunctional designs. For example, cleavage products not limited to the Dicer standard of approximately 22-nucleotides can allow multifunctional siNA constructs with a target sequence identity overlap ranging from, for example, about 3 to about 15 nucleotides.

Example 12

Diagnostic Uses

[0678] The siNA molecules of the invention can be used in a variety of diagnostic applications, such as in the identification of molecular targets (e.g., RNA) in a variety of applications, for example, in clinical, industrial, environmental, agricultural and/or research settings. Such diagnostic use of siNA molecules involves utilizing reconstituted RNAi systems, for example, using cellular lysates or partially purified cellular lysates. siNA molecules of this invention can be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of endogenous or exogenous, for example viral, RNA in a cell. The close relationship between siNA activity and the structure of the target RNA allows the detection of mutations in any region of the molecule, which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple siNA molecules described in this invention, one can map nucleotide changes, which are important to RNA structure and function in vitro, as well as in cells and tissues. Cleavage of target RNAs with siNA molecules can be used to inhibit gene expression and define the role of specified gene products in the progression of disease or infection. In this manner, other genetic targets can be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple siNA molecules targeted to different genes, siNA molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations siNA molecules and/or other chemical or biological molecules). Other in vitro uses of siNA molecules of this invention are well known in the art, and include detection of the presence of mRNAs associated with a disease, infection, or related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a siNA using standard methodologies, for example, fluorescence resonance emission transfer (FRET).

[0679] In a specific example, siNA molecules that cleave only wild-type or mutant forms of the target RNA are used for the assay. The first siNA molecules (i.e., those that cleave only wild-type forms of target RNA) are used to identify wild-type RNA present in the sample and the second siNA molecules (i.e., those that cleave only mutant forms of target RNA) are used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA are cleaved by both siNA molecules to demonstrate the relative siNA efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus, each analysis requires two siNA molecules, two substrates and one unknown sample, which is combined into six reactions. The presence of cleavage products is determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (i.e., disease related or infection related) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels is adequate and decreases the cost of the initial diagnosis. Higher mutant form to wild-type ratios are correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

[0680] All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in

the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

[0681] One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

[0682] It will be readily apparent to one skilled in the art that varying substitutions and modifications can be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims. The present invention teaches one skilled in the art to test various combinations and/or substitutions of chemical modifications described herein toward generating nucleic acid constructs with improved activity for mediating RNAi activity. Such improved activity can comprise improved stability, improved bioavailability, and/or improved activation of cellular responses mediating RNAi. Therefore, the specific embodiments described herein are not limiting and one skilled in the art can readily appreciate that specific combinations of the modifications described herein can be tested without undue experimentation toward identifying siNA molecules with improved RNAi activity.

[0683] The invention illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

[0684] In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

TABLE I

SREBP1 Accession Numbers
NM_001005291 *Homo sapiens* sterol regulatory element binding transcription factor 1 (SREBF1), transcript variant 1, mRNA gi|52630418|ref|NM_001005291.1|[52630418] (SEQ ID NO: 787) agcagagcug cggccggggg aacccaguuu ccgaggaacu uuucgccggc gccgggccgc cucugaggcc agggcaggac acgaacgcgc ggagcggcgg cggcgacuga gagccggggc cgcggcggcg cucccuagga agggccguac gaggcggcgg gcccggcggg ccucccggag gaggcggcug cgccauggac gagccacccu ucagcgaggc ggcuuuggag caggcgcugg gcgagccgug cgaucuggac gcggcgcugc ugaccgacau cgaaggugaa gucggcgcgg ggagggguag ggccaacggc cuggacgccc caagggcggg cgcagaucgc ggagccaugg auugcacuuu cgaagacaug cuucagcuua ucaacaacca agacagugac uucccuggcc uauuugaccc acccuaugcu gggagugggg caggggcac agacccugcc agccccgaua ccagcucccc aggcagcuug ucuccaccuc cugccacauu gagcuccucu cuugaagccu uccugagcgg gccgcaggca gcgcccucac cccugucccc uccccagccu gcacccacuc cauugaagau guacccgucc augcccgcuu ucucccccugg gccugguauc aaggaagagu cagugccacu gagcauccug cagacccccca ccccacagcc ccugccaggg gcccuccugc cacagagcuu cccagcccca gccccaccgc aguucagcuc cacccccugug uuaggcuacc ccagcccucc gggaggcuuc ucuacaggaa gcccucccgg gaacacccag cagccgcugc cuggccugcc acuggcuucc ccgccagggg ucccgcccgu cuccuugcac acccaggucc agaguguggu cccccagcag cuacugacag ucacagcugc ccccacggca gccccuguaa cgaccacugu gaccucgcag auccagcagg ucccgguccu gcugcagccc cacuucauca aggcagacuc gcugcuucug acagccauga agacagacgg agccacugug aaggcggcag gucucagucc ccuggucucu ggcaccacug ugcagacagg gccuuugccg acccugguga guggcggaac caucuuggca acaguccccac uggucguaga ugcggagaag cugccuauca accggcucgc agcuggcagc aaggccccgg ccucugccca gagccgugga gagaagcgca cagcccacaa cgccauugag aagcgcuacc gcuccuccau caaugacaaa aucauugagc ucaaggaucu gguggugggc acugaggcaa agcugaauaa aucugcuguc uugcgcaagg ccaucgacua cauucgcuuu cugcaacaca gcaaccagaa acucaagcag gagaaccuaa gucugcgcac ugcuguccac aaaagcaaau cucugaagga ucuggugucg gccuguggca guggagggaa cacagacgug cucauggagg gcgugaagac ugagguggag gacacacuga ccccaccccc cucggaugcu ggcucaccuu uccagagcag ccccuugucc cuuggcagca ggggcagugg cagcgguggc aguggcagug acucggagcc ugacagccca gucuuugagg acagcaaggc aaagccagag cagcggccgu cucugcacag ccggggcaug cuggaccgcu cccgccuggc ccugugcacg cucgucuucc ucugccuguc cugcaacccc uuggccuccu ugcugggggc ccgggggcuu cccagccccu cagauaccac cagcgucuac cauagcccug ggcgcaacgu gcugggcacc gagagcagag auggcccugg cugggcccag uggcugcugc ccccaguggu cuggcugcuc aaugggcugu uggugcucgu cuccuuggug cuucucuuug

TABLE I-continued

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NM_004176
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gi|52630417|ref|NM_004176.3|[52630417]
(SEQ ID NO: 788)

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ggccaucgac uacauucgcu uucugcaaca cagcaaccag aaacucaagc aggagaaccu
aagucugcgc acugcugucc acaaaagcaa aucucugaag gaucuggugu cggccugugg
caguggaggg aacacagacg ugcucaugga gggcgugaag acugaggugg aggacacacu
gaccccaccc cccucggaug cuggcucacc uuuccagagc agccccuugu cccuuggcag
caggggcagu ggcagcggug gcaguggcag ugacucggag ccugacagcc cagucuuuga
ggacagcaag gcaaagccag agcagcggcc gucucugcac agccggggca ugcuggaccg
cucccgccug gcccugugca cgcucgucuu ccucugccug uccugcaacc ccuuggccuc
cuugcugggg gcccgggggc uucccagccc cucagauacc accagcgucu accauagccc

TABLE I-continued

SREBP1 Accession Numbers
ugggcgcaac gugcugggca ccgagagcag agauggcccu ggcugggccc aguggcugcu gcccccagug gucuggcugc ucaaugggcu guuggugcuc gucuccuugg ugcuucucuu ugucuacggu gagccaguca cacggcccca cucaggcccc gccguguacu ucuggaggca ucgcaagcag gcugaccugg accuggcccg gggagacuuu gcccaggcug cccagcagcu guggcuggcc cugcgggcac ugggccggcc ccugcccacc ucccaccugg accuggcuug uagccucccuc uggaaccuca uccgucaccu gcugcagcgu cucugggugg gccgcuggcu ggcaggccgg gcagggggcc ugcagcagga cugugcucug cgaguggaug cuagcgccag cgcccgagac gcagcccugg ucuaccauaa gcugcaccag cugcacacca ugggggaagca cacaggcggg caccucacug ccaccaaccu ggcgcugagu gcccugaacc uggcagagug ugcaggggau gccgugucug uggcgacgcu ggccgagauc uauguggcgg cugcauugag agugaagacc agucucccac gggccuugca uuuucugaca cgcuucuucc ugagcagugc ccgccaggcc ugccuggcac agaguggcuc agugccuccu gccaugcagu ggcucugcca ccccgugggc caccguuucu ucguggaugg ggacuggucc gugcucagua ccccauggga gagccuguac agcuuggccg ggaacccagu ggacccccug gcccagguga cucagcuauu ccgggaacau cucuuagagc gagcacugaa cugugugacc cagcccaacc ccagcccugg gucagcugau ggggacaagg aauucucgga ugcccucggg uaccugcagc ugcugaacag cuguucugau gcugcggggg cuccugccua cagcuucucc aucaguucca gcauggccac caccaccggc guagacccgg uggccaagug gugggccucu cugacagcug uggugaucca cuggcugcgg cgggaugagg aggcggcuga gcggcugugc ccgcuggugg agcaccugcc ccgggugcug caggagucug agagaccccu gcccagggca gcucugcacu ccuucaaggc ugcccgggcc cugcugggcu gugccaaggc agagucuggu ccagccagcc ugaccaucug ugagaaggcc aguggguacc ugcaggacag ccuggcuacc acaccagcca gcagcuccau ugacaaggcc gugcagcugu uccuguguga ccugcuucuu guggugcgca ccagccugug gcggcagcag cagcccccgg ccccggcccc agcagcccag ggcgccagca gcaggcccca ggcuuccgcc cuugagcugc guggcuucca acgggaccug agcagccuga ggcggcuggc acagagcuuc cggcccgcca ugcggagggu guuccuacau gaggccacgg cccggcugau ggcgggggcc agccccacac ggacacacca gcuccucgac cgcagucuga ggcggcgggc aggccccggu ggcaaaggag gcgcgguggc ggagcuggag ccgcggccca cgcggcggga gcacgcggag gccuugcugc uggccuccug cuaccugccc cccggcuucc ugucggcgcc cgggcagcgc gugggcaugc uggcugaggc ggcgcgcaca cucgagaagc uuggcgaucg ccggcugcug cacgacuguc agcagaugcu caugcgccug ggcgguggga ccacugucac uuccagcuag accccguguc cccggccuca gcaccccugu cucuagccac uuugguccccg ugcagcuucu guccugcguc gaagcuuuga aggccgaagg cagugcaaga gacucuggcc uccacaguuc gaccugcggc ugcugugugc cuucgcggug gaaggcccga ggggcgcgau cuugacccua agaccggcgg ccaugauggu gcugaccucu gguggccgau cggggcacug caggggccga gccauuuugg ggggcccccc uccuugcucu gcaggcaccu uaguggcuuu uuuccuccug uguacaggga agagaggggu acauuucccu gugcugacgg aagccaacuu ggcuuucccg gacugcaagc agggcucugc cccagaggcc ucucucuccg ucgugggaga gagacgugua cauaguguag gucagcgugc uuagccuccu gaccugaggc uccugugcua cuuugccuuu ugcaaacuuu auuuucauag auugagaagu uuuguacaga gaauuaaaaa ugaaauuauu uaua

NM_005896
Homo sapiens isocitrate dehydrogenase 1 (NADP+), soluble (IDH1), mRNA
gi|28178824|ref|NM_005896.2|[28178824]

NM_004599
Homo sapiens sterol regulatory element binding transcription factor 2 (SREBF2), mRNA
gi|27477112|ref|NM_004599.2|[27477112]

1AM9H
Chain H, Human Srebp-1a Bound To Ldl Receptor Promoter
gi|3318717|pdb|1AM9|H[3318717]

1AM9G
Chain G, Human Srebp-1a Bound To Ldl Receptor Promoter
gi|3318716|pdb|1AM9|G[3318716]

1AM9F
Chain F, Human Srebp-1a Bound To Ldl Receptor Promoter
gi|3318715|pdb|1AM9|F[3318715]

1AM9E
Chain E, Human Srebp-1a Bound To Ldl Receptor Promoter
gi|3318714|pdb|1AM9|E[3318714]

NM_000389
Homo sapiens cyclin-dependent kinase inhibitor 1A (p21, Cip1) (CDKN1A), transcript variant 1, mRNA
gi|17978496|ref|NM_000389.2|[17978496]

TABLE I-continued

SREBP1 Accession Numbers
NM_078467 *Homo sapiens* cyclin-dependent kinase inhibitor 1A (p21, Cip1) (CDKN1A), transcript variant 2, mRNA gi|17978494|ref|NM_078467.1|[17978494]
NM_000384 *Homo sapiens* apolipoprotein B (including Ag(x) antigen) (APOB), mRNA gi|105990531|refNM_000384.2|[105990531]
NM_002746 *Homo sapiens* mitogen-activated protein kinase 3 (MAPK3), transcript variant 1, mRNA gi|91718898|ref|NM_002746.2|[91718898]
NM_001040056 *Homo sapiens* mitogen-activated protein kinase 3 (MAPK3), transcript variant 2, mRNA gi|91718896|ref|NM_001040056.1|[91718896]
NM_138957 *Homo sapiens* mitogen-activated protein kinase 1 (MAPK1), transcript variant 2, mRNA gi|75709179|ref|NM_138957.2|[75709179]
NM_002745 *Homo sapiens* mitogen-activated protein kinase 1 (MAPK1), transcript variant 1, mRNA gi|75709178|ref|NM_002745.4|[75709178]
NM_174936 *Homo sapiens* proprotein convertase subtilisin/kexin type 9 (PCSK9), mRNA gi|31317306|ref|NM_174936.2|[31317306]
AK075365 *Homo sapiens* cDNA PSEC0052 fis, clone NT2RP2000279, weakly similar to AQUALYSIN I PRECURSOR (EC 3.4.21.-) gi|22761404|dbj|AK075365.1|[22761404]
AK122717 *Homo sapiens* cDNA FLJ16214 fis, clone CTONG2024614, weakly similar to AQUALYSIN I PRECURSOR (EC 3.4.21.-) gi|34527909|dbj|AK122717.1|[34527909]
BC042095 *Homo sapiens* proprotein convertase subtilisin/kexin type 9, mRNA (cDNA clone IMAGE:5767792), partial cds gi|27694359|gb|BC042095.1|[27694359]
AK124635 *Homo sapiens* cDNA FLJ42644 fis, clone BRACE3025630 gi|34530476|dbj|AK124635.1|[34530476]
AC091609 *Homo sapiens* chromosome 1 clone RP11-109I2, complete sequence gi|15042908|gb|AC091609.2|[15042908]
AY829011 *Homo sapiens* proprotein convertase subtilisin/kexin type 9 (PCSK9) gene, complete cds gi|55925153|gb|AY829011.1|[55925153]
AL589790 Human DNA sequence from clone GS1-118I5 on chromosome 1 Contains the 3' end of the gene for barttin (BSND) and the 5' end of the PCSK9 gene for proprotein convertase subtilisin/kexin type 9, complete sequence gi|17973948|emb|AL589790.22|[17973948]

TABLE II

SREBP1 siNA and Target Sequences

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
hmrSREBP1 (NM_001005291.1)								
3	CAGAGCUGCGGCCGGGGGA	1	3	CAGAGCUGCGGCCGGGGGA	1	21	UCCCCCGGCCGCAGCUCUG	328
21	AACCCAGUUUCCGAGGAAC	2	21	AACCCAGUUUCCGAGGAAC	2	39	GUUCCUCGGAAACUGGGUU	329
39	CUUUUCGCCGGCGCCGGGC	3	39	CUUUUCGCCGGCGCCGGGC	3	57	GCCCGGCGCCGGCGAAAAG	330
57	CCGCCUCUGAGGCCAGGGC	4	57	CCGCCUCUGAGGCCAGGGC	4	75	GCCCUGGCCUCAGAGGCGG	331
75	CAGGACACGAACGCGCGGA	5	75	CAGGACACGAACGCGCGGA	5	93	UCCGCGCGUUCGUGUCCUG	332
93	AGCGGCGGCGGCGACUGAG	6	93	AGCGGCGGCGGCGACUGAG	6	111	CUCAGUCGCCGCCGCCGCU	333
111	GAGCCGGGGCCGCGGCGGC	7	111	GAGCCGGGGCCGCGGCGGC	7	129	GCCGCCGCGGCCCCGGCUC	334
129	CGCUCCCUAGGAAGGGCCG	8	129	CGCUCCCUAGGAAGGGCCG	8	147	CGGCCCUUCCUAGGGAGCG	335
147	GUACGAGGCGGCGGGCCCG	9	147	GUACGAGGCGGCGGGCCCG	9	165	CGGGCCCGCCGCCUCGUAC	336
165	GGCGGGCCUCCCGGAGGAG	10	165	GGCGGGCCUCCCGGAGGAG	10	183	CUCCUCCGGGAGGCCCGCC	337
183	GGCGGCUGCGCCAUGGACG	11	183	GGCGGCUGCGCCAUGGACG	11	201	CGUCCAUGGCGCAGCCGCC	338
201	GAGCCACCCUUCAGCGAGG	12	201	GAGCCACCCUUCAGCGAGG	12	219	CCUCGCUGAAGGGUGGCUC	339
219	GCGGCUUUGGAGCAGGCGC	13	219	GCGGCUUUGGAGCAGGCGC	13	237	GCGCCUGCUCCAAAGCCGC	340
237	CUGGGCGAGCCGUGCGAUC	14	237	CUGGGCGAGCCGUGCGAUC	14	255	GAUCGCACGGCUCGCCCAG	341
255	CUGGACGCGGCGCUGCUGA	15	255	CUGGACGCGGCGCUGCUGA	15	273	UCAGCAGCGCCGCGUCCAG	342
273	ACCGACAUCGAAGGUGAAG	16	273	ACCGACAUCGAAGGUGAAG	16	291	CUUCACCUUCGAUGUCGGU	343
291	GUCGGCGCGGGGAGGGGUA	17	291	GUCGGCGCGGGGAGGGGUA	17	309	UACCCCUCCCCGCGCCGAC	344
309	AGGGCCAACGGCCUGGACG	18	309	AGGGCCAACGGCCUGGACG	18	327	CGUCCAGGCCGUUGGCCCU	345
327	GCCCCAAGGGCGGGCGCAG	19	327	GCCCCAAGGGCGGGCGCAG	19	345	CUGCGCCCGCCCUUGGGGC	346
345	GAUCGCGGAGCCAUGGAUU	20	345	GAUCGCGGAGCCAUGGAUU	20	363	AAUCCAUGGCUCCGCGAUC	347
363	UGCACUUUCGAAGACAUGC	21	363	UGCACUUUCGAAGACAUGC	21	381	GCAUGUCUUCGAAAGUGCA	348
381	CUUCAGCUUAUCAACAACC	22	381	CUUCAGCUUAUCAACAACC	22	399	GGUUGUUGAUAAGCUGAAG	349
399	CAAGACAGUGACUUCCCUG	23	399	CAAGACAGUGACUUCCCUG	23	417	CAGGGAAGUCACUGUCUUG	350
417	GGCCUAUUUGACCCACCCU	24	417	GGCCUAUUUGACCCACCCU	24	435	AGGGUGGGUCAAAUAGGCC	351
435	UAUGCUGGGAGUGGGGCAG	25	435	UAUGCUGGGAGUGGGGCAG	25	453	CUGCCCCACUCCCAGCAUA	352
453	GGGGGCACAGACCCUGCCA	26	453	GGGGGCACAGACCCUGCCA	26	471	UGGCAGGGUCUGUGCCCCC	353
471	AGCCCCGAUACCAGCUCCC	27	471	AGCCCCGAUACCAGCUCCC	27	489	GGGAGCUGGUAUCGGGGCU	354
489	CCAGGCAGCUUGUCUCCAC	28	489	CCAGGCAGCUUGUCUCCAC	28	507	GUGGAGACAAGCUGCCUGG	355
507	CCUCCUGCCACAUUGAGCU	29	507	CCUCCUGCCACAUUGAGCU	29	525	AGCUCAAUGUGGCAGGAGG	356
525	UCCUCUCUUGAAGCCUUCC	30	525	UCCUCUCUUGAAGCCUUCC	30	543	GGAAGGCUUCAAGAGAGGA	357
543	CUGAGCGGGCCGCAGGCAG	31	543	CUGAGCGGGCCGCAGGCAG	31	561	CUGCCUGCGGCCCGCUCAG	358
561	GCGCCCUCACCCCUGUCCC	32	561	GCGCCCUCACCCCUGUCCC	32	579	GGGACAGGGGUGAGGGCGC	359
579	CCUCCCCAGCCUGCACCCA	33	579	CCUCCCCAGCCUGCACCCA	33	597	UGGGUGCAGGCUGGGGAGG	360
597	ACUCCAUUGAAGAUGUACC	34	597	ACUCCAUUGAAGAUGUACC	34	615	GGUACAUCUUCAAUGGAGU	361
615	CCGUCCAUGCCCGCUUUCU	35	615	CCGUCCAUGCCCGCUUUCU	35	633	AGAAAGCGGGCAUGGACGG	362

TABLE II-continued

SREBP1 siNA and Target Sequences

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
633	UCCCCUGGGCCUGGUAUCA	36	633	UCCCCUGGGCCUGGUAUCA	36	651	UGAUACCAGGCCCAGGGGA	363
651	AAGGAAGAGUCAGUGCCAC	37	651	AAGGAAGAGUCAGUGCCAC	37	669	GUGGCACUGACUCUUCCUU	364
669	CUGAGCAUCCUGCAGACCC	38	669	CUGAGCAUCCUGCAGACCC	38	687	GGGUCUGCAGGAUGCUCAG	365
687	CCCACCCCACAGCCCCUGC	39	687	CCCACCCCACAGCCCCUGC	39	705	GCAGGGGCUGUGGGGUGGG	366
705	CCAGGGGCCCUCCUGCCAC	40	705	CCAGGGGCCCUCCUGCCAC	40	723	GUGGCAGGAGGGCCCCUGG	367
723	CAGAGCUUCCCAGCCCCAG	41	723	CAGAGCUUCCCAGCCCCAG	41	741	CUGGGGCUGGGAAGCUCUG	368
741	GCCCCACCGCAGUUCAGCU	42	741	GCCCCACCGCAGUUCAGCU	42	759	AGCUGAACUGCGGUGGGGC	369
759	UCCACCCCUGUGUUAGGCU	43	759	UCCACCCCUGUGUUAGGCU	43	777	AGCCUAACACAGGGGUGGA	370
777	UACCCCAGCCCUCCGGGAG	44	777	UACCCCAGCCCUCCGGGAG	44	795	CUCCCGGAGGGCUGGGGUA	371
795	GGCUUCUCUACAGGAAGCC	45	795	GGCUUCUCUACAGGAAGCC	45	813	GGCUUCCUGUAGAGAAGCC	372
813	CCUCCCGGGAACACCCAGC	46	813	CCUCCCGGGAACACCCAGC	46	831	GCUGGGUGUUCCCGGGAGG	373
831	CAGCCGCUGCCUGGCCUGC	47	831	CAGCCGCUGCCUGGCCUGC	47	849	GCAGGCCAGGCAGCGGCUG	374
849	CCACUGGCUUCCCCGCCAG	48	849	CCACUGGCUUCCCCGCCAG	48	867	CUGGCGGGGAAGCCAGUGG	375
867	GGGGUCCCGCCCGUCUCCU	49	867	GGGGUCCCGCCCGUCUCCU	49	885	AGGAGACGGGCGGGACCCC	376
885	UUGCACACCCAGGUCCAGA	50	885	UUGCACACCCAGGUCCAGA	50	903	UCUGGACCUGGGUGUGCAA	377
903	AGUGUGGUCCCCCAGCAGC	51	903	AGUGUGGUCCCCCAGCAGC	51	921	GCUGCUGGGGGACCACACU	378
921	CUACUGACAGUCACAGCUG	52	921	CUACUGACAGUCACAGCUG	52	939	CAGCUGUGACUGUCAGUAG	379
939	GCCCCCACGGCAGCCCCUG	53	939	GCCCCCACGGCAGCCCCUG	53	957	CAGGGGCUGCCGUGGGGGC	380
957	GUAACGACCACUGUGACCU	54	957	GUAACGACCACUGUGACCU	54	975	AGGUCACAGUGGUCGUUAC	381
975	UCGCAGAUCCAGCAGGUCC	55	975	UCGCAGAUCCAGCAGGUCC	55	993	GGACCUGCUGGAUCUGCGA	382
993	CCGGUCCUGCUGCAGCCCC	56	993	CCGGUCCUGCUGCAGCCCC	56	1011	GGGGCUGCAGCAGGACCGG	383
1011	CACUUCAUCAAGGCAGACU	57	1011	CACUUCAUCAAGGCAGACU	57	1029	AGUCUGCCUUGAUGAAGUG	384
1029	UCGCUGCUUCUGACAGCCA	58	1029	UCGCUGCUUCUGACAGCCA	58	1047	UGGCUGUCAGAAGCAGCGA	385
1047	AUGAAGACAGACGGAGCCA	59	1047	AUGAAGACAGACGGAGCCA	59	1065	UGGCUCCGUCUGUCUUCAU	386
1065	ACUGUGAAGGCGGCAGGUC	60	1065	ACUGUGAAGGCGGCAGGUC	60	1083	GACCUGCCGCCUUCACAGU	387
1083	CUCAGUCCCCUGGUCUCUG	61	1083	CUCAGUCCCCUGGUCUCUG	61	1101	CAGAGACCAGGGGACUGAG	388
1101	GGCACCACUGUGCAGACAG	62	1101	GGCACCACUGUGCAGACAG	62	1119	CUGUCUGCACAGUGGUGCC	389
1119	GGGCCUUUGCCGACCCUGG	63	1119	GGGCCUUUGCCGACCCUGG	63	1137	CCAGGGUCGGCAAAGGCCC	390
1137	GUGAGUGGCGGAACCAUCU	64	1137	GUGAGUGGCGGAACCAUCU	64	1155	AGAUGGUUCCGCCACUCAC	391
1155	UUGGCAACAGUCCCACUGG	65	1155	UUGGCAACAGUCCCACUGG	65	1173	CCAGUGGGACUGUUGCCAA	392
1173	GUCGUAGAUGCGGAGAAGC	66	1173	GUCGUAGAUGCGGAGAAGC	66	1191	GCUUCUCCGCAUCUACGAC	393
1191	CUGCCUAUCAACCGGCUCG	67	1191	CUGCCUAUCAACCGGCUCG	67	1209	CGAGCCGGUUGAUAGGCAG	394
1209	GCAGCUGGCAGCAAGGCCC	68	1209	GCAGCUGGCAGCAAGGCCC	68	1227	GGGCCUUGCUGCCAGCUGC	395
1227	CCGGCCUCUGCCCAGAGCC	69	1227	CCGGCCUCUGCCCAGAGCC	69	1245	GGCUCUGGGCAGAGGCCGG	396
1245	CGUGGAGAGAAGCGCACAG	70	1245	CGUGGAGAGAAGCGCACAG	70	1263	CUGUGCGCUUCUCUCCACG	397
1263	GCCCACAACGCCAUUGAGA	71	1263	GCCCACAACGCCAUUGAGA	71	1281	UCUCAAUGGCGUUGUGGGC	398

TABLE II-continued

SREBP1 siNA and Target Sequences

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
1281	AAGCGCUACCGCUCCUCCA	72	1281	AAGCGCUACCGCUCCUCCA	72	1299	UGGAGGAGCGGUAGCGCUU	399
1299	AUCAAUGACAAAAUCAUUG	73	1299	AUCAAUGACAAAAUCAUUG	73	1317	CAAUGAUUUUGUCAUUGAU	400
1317	GAGCUCAAGGAUCUGGUGG	74	1317	GAGCUCAAGGAUCUGGUGG	74	1335	CCACCAGAUCCUUGAGCUC	401
1335	GUGGGCACUGAGGCAAAGC	75	1335	GUGGGCACUGAGGCAAAGC	75	1353	GCUUUGCCUCAGUGCCCAC	402
1353	CUGAAUAAAUCUGCUGUCU	76	1353	CUGAAUAAAUCUGCUGUCU	76	1371	AGACAGCAGAUUUAUUCAG	403
1371	UUGCGCAAGGCCAUCGACU	77	1371	UUGCGCAAGGCCAUCGACU	77	1389	AGUCGAUGGCCUUGCGCAA	404
1389	UACAUUCGCUUUCUGCAAC	78	1389	UACAUUCGCUUUCUGCAAC	78	1407	GUUGCAGAAAGCGAAUGUA	405
1407	CACAGCAACCAGAAACUCA	79	1407	CACAGCAACCAGAAACUCA	79	1425	UGAGUUUCUGGUUGCUGUG	406
1425	AAGCAGGAGAACCUAAGUC	80	1425	AAGCAGGAGAACCUAAGUC	80	1443	GACUUAGGUUCUCCUGCUU	407
1443	CUGCGCACUGCUGUCCACA	81	1443	CUGCGCACUGCUGUCCACA	81	1461	UGUGGACAGCAGUGCGCAG	408
1461	AAAAGCAAAUCUCUGAAGG	82	1461	AAAAGCAAAUCUCUGAAGG	82	1479	CCUUCAGAGAUUUGCUUUU	409
1479	GAUCUGGUGUCGGCCUGUG	83	1479	GAUCUGGUGUCGGCCUGUG	83	1497	CACAGGCCGACACCAGAUC	410
1497	GGCAGUGGAGGGAACACAG	84	1497	GGCAGUGGAGGGAACACAG	84	1515	CUGUGUUCCCUCCACUGCC	411
1515	GACGUGCUCAUGGAGGGCG	85	1515	GACGUGCUCAUGGAGGGCG	85	1533	CGCCCUCCAUGAGCACGUC	412
1533	GUGAAGACUGAGGUGGAGG	86	1533	GUGAAGACUGAGGUGGAGG	86	1551	CCUCCACCUCAGUCUUCAC	413
1551	GACACACUGACCCCACCCC	87	1551	GACACACUGACCCCACCCC	87	1569	GGGGUGGGGUCAGUGUGUC	414
1569	CCCUCGGAUGCUGGCUCAC	88	1569	CCCUCGGAUGCUGGCUCAC	88	1587	GUGAGCCAGCAUCCGAGGG	415
1587	CCUUUCCAGAGCAGCCCCU	89	1587	CCUUUCCAGAGCAGCCCCU	89	1605	AGGGGCUGCUCUGGAAAGG	416
1605	UUGUCCCUUGGCAGCAGGG	90	1605	UUGUCCCUUGGCAGCAGGG	90	1623	CCCUGCUGCCAAGGGACAA	417
1623	GGCAGUGGCAGCGGUGGCA	91	1623	GGCAGUGGCAGCGGUGGCA	91	1641	UGCCACCGCUGCCACUGCC	418
1641	AGUGGCAGUGACUCGGAGC	92	1641	AGUGGCAGUGACUCGGAGC	92	1659	GCUCCGAGUCACUGCCACU	419
1659	CCUGACAGCCCAGUCUUUG	93	1659	CCUGACAGCCCAGUCUUUG	93	1677	CAAAGACUGGGCUGUCAGG	420
1677	GAGGACAGCAAGGCAAAGC	94	1677	GAGGACAGCAAGGCAAAGC	94	1695	GCUUUGCCUUGCUGUCCUC	421
1695	CCAGAGCAGCGGCCGUCUC	95	1695	CCAGAGCAGCGGCCGUCUC	95	1713	GAGACGGCCGCUGCUCUGG	422
1713	CUGCACAGCCGGGGCAUGC	96	1713	CUGCACAGCCGGGGCAUGC	96	1731	GCAUGCCCCGGCUGUGCAG	423
1731	CUGGACCGCUCCCGCCUGG	97	1731	CUGGACCGCUCCCGCCUGG	97	1749	CCAGGCGGGAGCGGUCCAG	424
1749	GCCCUGUGCACGCUCGUCU	98	1749	GCCCUGUGCACGCUCGUCU	98	1767	AGACGAGCGUGCACAGGGC	425
1767	UUCCUCUGCCUGUCCUGCA	99	1767	UUCCUCUGCCUGUCCUGCA	99	1785	UGCAGGACAGGCAGAGGAA	426
1785	AACCCCUUGGCCUCCUUGC	100	1785	AACCCCUUGGCCUCCUUGC	100	1803	GCAAGGAGGCCAAGGGGUU	427
1803	CUGGGGGCCCGGGGGCUUC	101	1803	CUGGGGGCCCGGGGGCUUC	101	1821	GAAGCCCCCGGGCCCCCAG	428
1821	CCCAGCCCCUCAGAUACCA	102	1821	CCCAGCCCCUCAGAUACCA	102	1839	UGGUAUCUGAGGGGCUGGG	429
1839	ACCAGCGUCUACCAUAGCC	103	1839	ACCAGCGUCUACCAUAGCC	103	1857	GGCUAUGGUAGACGCUGGU	430
1857	CCUGGGCGCAACGUGCUGG	104	1857	CCUGGGCGCAACGUGCUGG	104	1875	CCAGCACGUUGCGCCCAGG	431
1875	GGCACCGAGAGCAGAGAUG	105	1875	GGCACCGAGAGCAGAGAUG	105	1893	CAUCUCUGCUCUCGGUGCC	432
1893	GGCCCUGGCUGGGCCCAGU	106	1893	GGCCCUGGCUGGGCCCAGU	106	1911	ACUGGGCCCAGCCAGGGCC	433

TABLE II-continued

SREBP1 siNA and Target Sequences

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
1911	UGGCUGCUGCCCCCAGUGG	107	1911	UGGCUGCUGCCCCCAGUGG	107	1929	CCACUGGGGGCAGCAGCCA	434
1929	GUCUGGCUGCUCAAUGGGC	108	1929	GUCUGGCUGCUCAAUGGGC	108	1947	GCCCAUUGAGCAGCCAGAC	435
1947	CUGUUGGUGCUCGUCUCCU	109	1947	CUGUUGGUGCUCGUCUCCU	109	1965	AGGAGACGAGCACCAACAG	436
1965	UUGGUGCUUCUCUUUGUCU	110	1965	UUGGUGCUUCUCUUUGUCU	110	1983	AGACAAAGAGAAGCACCAA	437
1983	UACGGUGAGCCAGUCACAC	111	1983	UACGGUGAGCCAGUCACAC	111	2001	GUGUGACUGGCUCACCGUA	438
2001	CGGCCCCACUCAGGCCCCG	112	2001	CGGCCCCACUCAGGCCCCG	112	2019	CGGGGCCUGAGUGGGGCCG	439
2019	GCCGUGUACUUCUGGAGGC	113	2019	GCCGUGUACUUCUGGAGGC	113	2037	GCCUCCAGAAGUACACGGC	440
2037	CAUCGCAAGCAGGCUGACC	114	2037	CAUCGCAAGCAGGCUGACC	114	2055	GGUCAGCCUGCUUGCGAUG	441
2055	CUGGACCUGGCCCGGGGAG	115	2055	CUGGACCUGGCCCGGGGAG	115	2073	CUCCCCGGGCCAGGUCCAG	442
2073	GACUUUGCCCAGGCUGCCC	116	2073	GACUUUGCCCAGGCUGCCC	116	2091	GGGCAGCCUGGGCAAAGUC	443
2091	CAGCAGCUGUGGCUGGCCC	117	2091	CAGCAGCUGUGGCUGGCCC	117	2109	GGGCCAGCCACAGCUGCUG	444
2109	CUGCGGGCACUGGGCCGGC	118	2109	CUGCGGGCACUGGGCCGGC	118	2127	GCCGGCCCAGUGCCCGCAG	445
2127	CCCCUGCCCACCUCCCACC	119	2127	CCCCUGCCCACCUCCCACC	119	2145	GGUGGGAGGUGGGCAGGGG	446
2145	CUGGACCUGGCUUGUAGCC	120	2145	CUGGACCUGGCUUGUAGCC	120	2163	GGCUACAAGCCAGGUCCAG	447
2163	CUCCUCUGGAACCUCAUCC	121	2163	CUCCUCUGGAACCUCAUCC	121	2181	GGAUGAGGUUCCAGAGGAG	448
2181	CGUCACCUGCUGCAGCGUC	122	2181	CGUCACCUGCUGCAGCGUC	122	2199	GACGCUGCAGCAGGUGACG	449
2199	CUCUGGGUGGGCCGCUGGC	123	2199	CUCUGGGUGGGCCGCUGGC	123	2217	GCCAGCGGCCCACCCAGAG	450
2217	CUGGCAGGCCGGGCAGGGG	124	2217	CUGGCAGGCCGGGCAGGGG	124	2235	CCCCUGCCCGGCCUGCCAG	451
2235	GGCCUGCAGCAGGACUGUG	125	2235	GGCCUGCAGCAGGACUGUG	125	2253	CACAGUCCUGCUGCAGGCC	452
2253	GCUCUGCGAGUGGAUGCUA	126	2253	GCUCUGCGAGUGGAUGCUA	126	2271	UAGCAUCCACUCGCAGAGC	453
2271	AGCGCCAGCGCCCGAGACG	127	2271	AGCGCCAGCGCCCGAGACG	127	2289	CGUCUCGGGCGCUGGCGCU	454
2289	GCAGCCCUGGUCUACCAUA	128	2289	GCAGCCCUGGUCUACCAUA	128	2307	UAUGGUAGACCAGGGCUGC	455
2307	AAGCUGCACCAGCUGCACA	129	2307	AAGCUGCACCAGCUGCACA	129	2325	UGUGCAGCUGGUGCAGCUU	456
2325	ACCAUGGGGAAGCACACAG	130	2325	ACCAUGGGGAAGCACACAG	130	2343	CUGUGUGCUUCCCCAUGGU	457
2343	GGCGGGCACCUCACUGCCA	131	2343	GGCGGGCACCUCACUGCCA	131	2361	UGGCAGUGAGGUGCCCGCC	458
2361	ACCAACCUGGCGCUGAGUG	132	2361	ACCAACCUGGCGCUGAGUG	132	2379	CACUCAGCGCCAGGUUGGU	459
2379	GCCCUGAACCUGGCAGAGU	133	2379	GCCCUGAACCUGGCAGAGU	133	2397	ACUCUGCCAGGUUCAGGGC	460
2397	UGUGCAGGGGAUGCCGUGU	134	2397	UGUGCAGGGGAUGCCGUGU	134	2415	ACACGGCAUCCCCUGCACA	461
2415	UCUGUGGCGACGCUGGCCG	135	2415	UCUGUGGCGACGCUGGCCG	135	2433	CGGCCAGCGUCGCCACAGA	462
2433	GAGAUCUAUGUGGCGGCUG	136	2433	GAGAUCUAUGUGGCGGCUG	136	2451	CAGCCGCCACAUAGAUCUC	463
2451	GCAUUGAGAGUGAAGACCA	137	2451	GCAUUGAGAGUGAAGACCA	137	2469	UGGUCUUCACUCUCAAUGC	464
2469	AGUCUCCCACGGGCCUUGC	138	2469	AGUCUCCCACGGGCCUUGC	138	2487	GCAAGGCCCGUGGGAGACU	465
2487	CAUUUUCUGACACGCUUCU	139	2487	CAUUUUCUGACACGCUUCU	139	2505	AGAAGCGUGUCAGAAAAUG	466
2505	UUCCUGAGCAGUGCCCGCC	140	2505	UUCCUGAGCAGUGCCCGCC	140	2523	GGCGGGCACUGCUCAGGAA	467
2523	CAGGCCUGCCUGGCACAGA	141	2523	CAGGCCUGCCUGGCACAGA	141	2541	UCUGUGCCAGGCAGGCCUG	468
2541	AGUGGCUCAGUGCCUCCUG	142	2541	AGUGGCUCAGUGCCUCCUG	142	2559	CAGGAGGCACUGAGCCACU	469

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SREBP1 siNA and Target Sequences

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
2559	GCCAUGCAGUGGCUCUGCC	143	2559	GCCAUGCAGUGGCUCUGCC	143	2577	GGCAGAGCCACUGCAUGGC	470
2577	CACCCCGUGGGCCACCGUU	144	2577	CACCCCGUGGGCCACCGUU	144	2595	AACGGUGGCCCACGGGGUG	471
2595	UUCUUCGUGGAUGGGGACU	145	2595	UUCUUCGUGGAUGGGGACU	145	2613	AGUCCCCAUCCACGAAGAA	472
2613	UGGUCCGUGCUCAGUACCC	146	2613	UGGUCCGUGCUCAGUACCC	146	2631	GGGUACUGAGCACGGACCA	473
2631	CCAUGGGAGAGCCUGUACA	147	2631	CCAUGGGAGAGCCUGUACA	147	2649	UGUACAGGCUCUCCCAUGG	474
2649	AGCUUGGCCGGGAACCCAG	148	2649	AGCUUGGCCGGGAACCCAG	148	2667	CUGGGUUCCCGGCCAAGCU	475
2667	GUGGACCCCCUGGCCCAGG	149	2667	GUGGACCCCCUGGCCCAGG	149	2685	CCUGGGCCAGGGGGUCCAC	476
2685	GUGACUCAGCUAUUCCGGG	150	2685	GUGACUCAGCUAUUCCGGG	150	2703	CCCGGAAUAGCUGAGUCAC	477
2703	GAACAUCUCUUAGAGCGAG	151	2703	GAACAUCUCUUAGAGCGAG	151	2721	CUCGCUCUAAGAGAUGUUC	478
2721	GCACUGAACUGUGUGACCC	152	2721	GCACUGAACUGUGUGACCC	152	2739	GGGUCACACAGUUCAGUGC	479
2739	CAGCCCAACCCCAGCCCUG	153	2739	CAGCCCAACCCCAGCCCUG	153	2757	CAGGGCUGGGGUUGGGCUG	480
2757	GGGUCAGCUGAUGGGGACA	154	2757	GGGUCAGCUGAUGGGGACA	154	2775	UGUCCCCAUCAGCUGACCC	481
2775	AAGGAAUUCUCGGAUGCCC	155	2775	AAGGAAUUCUCGGAUGCCC	155	2793	GGGCAUCCGAGAAUUCCUU	482
2793	CUCGGGUACCUGCAGCUGC	156	2793	CUCGGGUACCUGCAGCUGC	156	2811	GCAGCUGCAGGUACCCGAG	483
2811	CUGAACAGCUGUUCUGAUG	157	2811	CUGAACAGCUGUUCUGAUG	157	2829	CAUCAGAACAGCUGUUCAG	484
2829	GCUGCGGGGGCUCCUGCCU	158	2829	GCUGCGGGGGCUCCUGCCU	158	2847	AGGCAGGAGCCCCCGCAGC	485
2847	UACAGCUUCUCCAUCAGUU	159	2847	UACAGCUUCUCCAUCAGUU	159	2865	AACUGAUGGAGAAGCUGUA	486
2865	UCCAGCAUGGCCACCACCA	160	2865	UCCAGCAUGGCCACCACCA	160	2883	UGGUGGUGGCCAUGCUGGA	487
2883	ACCGGCGUAGACCCGGUGG	161	2883	ACCGGCGUAGACCCGGUGG	161	2901	CCACCGGGUCUACGCCGGU	488
2901	GCCAAGUGGUGGGCCUCUC	162	2901	GCCAAGUGGUGGGCCUCUC	162	2919	GAGAGGCCCACCACUUGGC	489
2919	CUGACAGCUGUGGUGAUCC	163	2919	CUGACAGCUGUGGUGAUCC	163	2937	GGAUCACCACAGCUGUCAG	490
2937	CACUGGCUGCGGCGGGAUG	164	2937	CACUGGCUGCGGCGGGAUG	164	2955	CAUCCCGCCGCAGCCAGUG	491
2955	GAGGAGGCGGCUGAGCGGC	165	2955	GAGGAGGCGGCUGAGCGGC	165	2973	GCCGCUCAGCCGCCUCCUC	492
2973	CUGUGCCCGCUGGUGGAGC	166	2973	CUGUGCCCGCUGGUGGAGC	166	2991	GCUCCACCAGCGGGCACAG	493
2991	CACCUGCCCCGGGUGCUGC	167	2991	CACCUGCCCCGGGUGCUGC	167	3009	GCAGCACCCGGGGCAGGUG	494
3009	CAGGAGUCUGAGAGACCCC	168	3009	CAGGAGUCUGAGAGACCCC	168	3027	GGGGUCUCUCAGACUCCUG	495
3027	CUGCCCAGGGCAGCUCUGC	169	3027	CUGCCCAGGGCAGCUCUGC	169	3045	GCAGAGCUGCCCUGGGCAG	496
3045	CACUCCUUCAAGGCUGCCC	170	3045	CACUCCUUCAAGGCUGCCC	170	3063	GGGCAGCCUUGAAGGAGUG	497
3063	CGGGCCCUGCUGGGCUGUG	171	3063	CGGGCCCUGCUGGGCUGUG	171	3081	CACAGCCCAGCAGGGCCCG	498
3081	GCCAAGGCAGAGUCUGGUC	172	3081	GCCAAGGCAGAGUCUGGUC	172	3099	GACCAGACUCUGCCUUGGC	499
3099	CCAGCCAGCCUGACCAUCU	173	3099	CCAGCCAGCCUGACCAUCU	173	3117	AGAUGGUCAGGCUGGCUGG	500
3117	UGUGAGAAGGCCAGUGGGU	174	3117	UGUGAGAAGGCCAGUGGGU	174	3135	ACCCACUGGCCUUCUCACA	501
3135	UACCUGCAGGACAGCCUGG	175	3135	UACCUGCAGGACAGCCUGG	175	3153	CCAGGCUGUCCUGCAGGUA	502
3153	GCUACCACACCAGCCAGCA	176	3153	GCUACCACACCAGCCAGCA	176	3171	UGCUGGCUGGUGUGGUAGC	503
3171	AGCUCCAUUGACAAGGCCG	177	3171	AGCUCCAUUGACAAGGCCG	177	3189	CGGCCUUGUCAAUGGAGCU	504

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SREBP1 siNA and Target Sequences

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
3189	GUGCAGCUGUUCCUGUGUG	178	3189	GUGCAGCUGUUCCUGUGUG	178	3207	CACACAGGAACAGCUGCAC	505
3207	GACCUGCUUCUUGUGGUGC	179	3207	GACCUGCUUCUUGUGGUGC	179	3225	GCACCACAAGAAGCAGGUC	506
3225	CGCACCAGCCUGUGGCGGC	180	3225	CGCACCAGCCUGUGGCGGC	180	3243	GCCGCCACAGGCUGGUGCG	507
3243	CAGCAGCAGCCCCCGGCCC	181	3243	CAGCAGCAGCCCCCGGCCC	181	3261	GGGCCGGGGGCUGCUGCUG	508
3261	CCGGCCCCAGCAGCCCAGG	182	3261	CCGGCCCCAGCAGCCCAGG	182	3279	CCUGGGCUGCUGGGGCCGG	509
3279	GGCACCAGCAGCAGGCCCC	183	3279	GGCACCAGCAGCAGGCCCC	183	3297	GGGGCCUGCUGCUGGUGCC	510
3297	CAGGCUUCCGCCCUUGAGC	184	3297	CAGGCUUCCGCCCUUGAGC	184	3315	GCUCAAGGGCGGAAGCCUG	511
3315	CUGCGUGGCUUCCAACGGG	185	3315	CUGCGUGGCUUCCAACGGG	185	3333	CCCGUUGGAAGCCACGCAG	512
3333	GACCUGAGCAGCCUGAGGC	186	3333	GACCUGAGCAGCCUGAGGC	186	3351	GCCUCAGGCUGCUCAGGUC	513
3351	CGGCUGGCACAGAGCUUCC	187	3351	CGGCUGGCACAGAGCUUCC	187	3369	GGAAGCUCUGUGCCAGCCG	514
3369	CGGCCCGCCAUGCGGAGGG	188	3369	CGGCCCGCCAUGCGGAGGG	188	3387	CCCUCCGCAUGGCGGGCCG	515
3387	GUGUUCCUACAUGAGGCCA	189	3387	GUGUUCCUACAUGAGGCCA	189	3405	UGGCCUCAUGUAGGAACAC	516
3405	ACGGCCCGGCUGAUGGCGG	190	3405	ACGGCCCGGCUGAUGGCGG	190	3423	CCGCCAUCAGCCGGGCCGU	517
3423	GGGGCCAGCCCCACACGGA	191	3423	GGGGCCAGCCCCACACGGA	191	3441	UCCGUGUGGGGCUGGCCCC	518
3441	ACACACCAGCUCCUCGACC	192	3441	ACACACCAGCUCCUCGACC	192	3459	GGUCGAGGAGCUGGUGUGU	519
3459	CGCAGUCUGAGGCGGCGGG	193	3459	CGCAGUCUGAGGCGGCGGG	193	3477	CCCGCCGCCUCAGACUGCG	520
3477	GCAGGCCCCGGUGGCAAAG	194	3477	GCAGGCCCCGGUGGCAAAG	194	3495	CUUUGCCACCGGGGCCUGC	521
3495	GGAGGCGCGGUGGCGGAGC	195	3495	GGAGGCGCGGUGGCGGAGC	195	3513	GCUCCGCCACCGCGCCUCC	522
3513	CUGGAGCCGCGGCCCACGC	196	3513	CUGGAGCCGCGGCCCACGC	196	3531	GCGUGGGCCGCGGCUCCAG	523
3531	CGGCGGGAGCACGCGGAGG	197	3531	CGGCGGGAGCACGCGGAGG	197	3549	CCUCCGCGUGCUCCCGCCG	524
3549	GCCUUGCUGCUGGCCUCCU	198	3549	GCCUUGCUGCUGGCCUCCU	198	3567	AGGAGGCCAGCAGCAAGGC	525
3567	UGCUACCUGCCCCCCGGCU	199	3567	UGCUACCUGCCCCCCGGCU	199	3585	AGCCGGGGGGCAGGUAGCA	526
3585	UUCCUGUCGGCGCCCGGGC	200	3585	UUCCUGUCGGCGCCCGGGC	200	3603	GCCCGGGCGCCGACAGGAA	527
3603	CAGCGCGUGGGCAUGCUGG	201	3603	CAGCGCGUGGGCAUGCUGG	201	3621	CCAGCAUGCCCACGCGCUG	528
3621	GCUGAGGCGGCGCGCACAC	202	3621	GCUGAGGCGGCGCGCACAC	202	3639	GUGUGCGCGCCGCCUCAGC	529
3639	CUCGAGAAGCUUGGCGAUC	203	3639	CUCGAGAAGCUUGGCGAUC	203	3657	GAUCGCCAAGCUUCUCGAG	530
3657	CGCCGGCUGCUGCACGACU	204	3657	CGCCGGCUGCUGCACGACU	204	3675	AGUCGUGCAGCAGCCGGCG	531
3675	UGUCAGCAGAUGCUCAUGC	205	3675	UGUCAGCAGAUGCUCAUGC	205	3693	GCAUGAGCAUCUGCUGACA	532
3693	CGCCUGGGCGGUGGGACCA	206	3693	CGCCUGGGCGGUGGGACCA	206	3711	UGGUCCCACCGCCCAGGCG	533
3711	ACUGUCACUUCCAGCUAGA	207	3711	ACUGUCACUUCCAGCUAGA	207	3729	UCUAGCUGGAAGUGACAGU	534
3729	ACCCCGUGUCCCCGGCCUC	208	3729	ACCCCGUGUCCCCGGCCUC	208	3747	GAGGCCGGGGACACGGGGU	535
3747	CAGCACCCCUGUCUCUAGC	209	3747	CAGCACCCCUGUCUCUAGC	209	3765	GCUAGAGACAGGGGUGCUG	536
3765	CCACUUUGGUCCCGUGCAG	210	3765	CCACUUUGGUCCCGUGCAG	210	3783	CUGCACGGGACCAAAGUGG	537
3783	GCUUCUGUCCUGCGUCGAA	211	3783	GCUUCUGUCCUGCGUCGAA	211	3801	UUCGACGCAGGACAGAAGC	538
3801	AGCUUUGAAGGCCGAAGGC	212	3801	AGCUUUGAAGGCCGAAGGC	212	3819	GCCUUCGGCCUUCAAAGCU	539
3819	CAGUGCAAGAGACUCUGGC	213	3819	CAGUGCAAGAGACUCUGGC	213	3837	GCCAGAGUCUCUUGCACUG	540

TABLE II-continued

SREBP1 siNA and Target Sequences

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
3837	CCUCCACAGUUCGACCUGC	214	3837	CCUCCACAGUUCGACCUGC	214	3855	GCAGGUCGAACUGUGGAGG	541
3855	CGGCUGCUGUGUGCCUUCG	215	3855	CGGCUGCUGUGUGCCUUCG	215	3873	CGAAGGCACACAGCAGCCG	542
3873	GCGGUGGAAGGCCCGAGGG	216	3873	GCGGUGGAAGGCCCGAGGG	216	3891	CCCUCGGGCCUUCCACCGC	543
3891	GGCGCGAUCUUGACCCUAA	217	3891	GGCGCGAUCUUGACCCUAA	217	3909	UUAGGGUCAAGAUCGCGCC	544
3909	AGACCGGCGGCCAUGAUGG	218	3909	AGACCGGCGGCCAUGAUGG	218	3927	CCAUCAUGGCCGCCGGUCU	545
3927	GUGCUGACCUCUGGUGGCC	219	3927	GUGCUGACCUCUGGUGGCC	219	3945	GGCCACCAGAGGUCAGCAC	546
3945	CGAUCGGGGCACUGCAGGG	220	3945	CGAUCGGGGCACUGCAGGG	220	3963	CCCUGCAGUGCCCCGAUCG	547
3963	GGCCGAGCCAUUUUGGGGG	221	3963	GGCCGAGCCAUUUUGGGGG	221	3981	CCCCCAAAAUGGCUCGGCC	548
3981	GGCCCCCCUCCUUGCUCUG	222	3981	GGCCCCCCUCCUUGCUCUG	222	3999	CAGAGCAAGGAGGGGGGCC	549
3999	GCAGGCACCUUAGUGGCUU	223	3999	GCAGGCACCUUAGUGGCUU	223	4017	AAGCCACUAAGGUGCCUGC	550
4017	UUUUUCCUCCUGUGUACAG	224	4017	UUUUUCCUCCUGUGUACAG	224	4035	CUGUACACAGGAGGAAAAA	551
4035	GGGAAGAGAGGGGUACAUU	225	4035	GGGAAGAGAGGGGUACAUU	225	4053	AAUGUACCCCUCUCUUCCC	552
4053	UUCCCUGUGCUGACGGAAG	226	4053	UUCCCUGUGCUGACGGAAG	226	4071	CUUCCGUCAGCACAGGGAA	553
4071	GCCAACUUGGCUUUCCCGG	227	4071	GCCAACUUGGCUUUCCCGG	227	4089	CCGGGAAAGCCAAGUUGGC	554
4089	GACUGCAAGCAGGGCUCUG	228	4089	GACUGCAAGCAGGGCUCUG	228	4107	CAGAGCCCUGCUUGCAGUC	555
4107	GCCCCAGAGGCCUCUCUCU	229	4107	GCCCCAGAGGCCUCUCUCU	229	4125	AGAGAGAGGCCUCUGGGGC	556
4125	UCCGUCGUGGGAGAGAGAC	230	4125	UCCGUCGUGGGAGAGAGAC	230	4143	GUCUCUCUCCCACGACGGA	557
4143	CGUGUACAUAGUGUAGGUC	231	4143	CGUGUACAUAGUGUAGGUC	231	4161	GACCUACACUAUGUACACG	558
4161	CAGCGUGCUUAGCCUCCUG	232	4161	CAGCGUGCUUAGCCUCCUG	232	4179	CAGGAGGCUAAGCACGCUG	559
4179	GACCUGAGGCUCCUGUGCU	233	4179	GACCUGAGGCUCCUGUGCU	233	4197	AGCACAGGAGCCUCAGGUC	560
4197	UACUUUGCCUUUUGCAAAC	234	4197	UACUUUGCCUUUUGCAAAC	234	4215	GUUUGCAAAAGGCAAAGUA	561
4215	CUUUAUUUUCAUAGAUUGA	235	4215	CUUUAUUUUCAUAGAUUGA	235	4233	UCAAUCUAUGAAAAUAAAG	562
4233	AGAAGUUUUGUACAGAGAA	236	4233	AGAAGUUUUGUACAGAGAA	236	4251	UUCUCUGUACAAAACUUCU	563
4251	AUUAAAAAUGAAAUUAUUU	237	4251	AUUAAAAAUGAAAUUAUUU	237	4269	AAAUAAUUUCAUUUUUAAU	564
1372	UGCGCAAGGCCAUCGACUA	238	1372	UGCGCAAGGCCAUCGACUA	238	1390	UAGUCGAUGGCCUUGCGCA	565
2898	GUGGCCAAGUGGUGGGCCU	239	2898	GUGGCCAAGUGGUGGGCCU	239	2916	AGGCCCACCACUUGGCCAC	566
1347	GCAAAGCUGAAUAAAUCUG	240	1347	GCAAAGCUGAAUAAAUCUG	240	1365	CAGAUUUAUUCAGCUUUGC	567
1355	GAAUAAAUCUGCUGUCUUG	241	1355	GAAUAAAUCUGCUGUCUUG	241	1373	CAAGACAGCAGAUUUAUUC	568
1373	GCGCAAGGCCAUCGACUAC	242	1373	GCGCAAGGCCAUCGACUAC	242	1391	GUAGUCGAUGGCCUUGCGC	569
1738	GCUCCCGCCUGGCCCUGUG	243	1738	GCUCCCGCCUGGCCCUGUG	243	1756	CACAGGGCCAGGCGGGAGC	570
2205	GUGGGCCGCUGGCUGGCAG	244	2205	GUGGGCCGCUGGCUGGCAG	244	2223	CUGCCAGCCAGCGGCCCAC	571
3112	CCAUCUGUGAGAAGGCCAG	245	3112	CCAUCUGUGAGAAGGCCAG	245	3130	CUGGCCUUCUCACAGAUGG	572
394	ACAACCAAGACAGUGACUU	246	394	ACAACCAAGACAGUGACUU	246	412	AAGUCACUGUCUUGGUUGU	573
1252	AGAAGCGCACAGCCCACAA	247	1252	AGAAGCGCACAGCCCACAA	247	1270	UUGUGGGCUGUGCGCUUCU	574
393	AACAACCAAGACAGUGACU	248	393	AACAACCAAGACAGUGACU	248	411	AGUCACUGUCUUGGUUGUU	575

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SREBP1 siNA and Target Sequences

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
1349	AAAGCUGAAUAAAUCUGCU	249	1349	AAAGCUGAAUAAAUCUGCU	249	1367	AGCAGAUUUAUUCAGCUUU	576
1354	UGAAUAAAUCUGCUGUCUU	250	1354	UGAAUAAAUCUGCUGUCUU	250	1372	AAGACAGCAGAUUUAUUCA	577
1365	GCUGUCUUGCGCAAGGCCA	251	1365	GCUGUCUUGCGCAAGGCCA	251	1383	UGGCCUUGCGCAAGACAGC	578
2560	CCAUGCAGUGGCUCUGCCA	252	2560	CCAUGCAGUGGCUCUGCCA	252	2578	UGGCAGAGCCACUGCAUGG	579
3714	GUCACUUCCAGCUAGACCC	253	3714	GUCACUUCCAGCUAGACCC	253	3732	GGGUCUAGCUGGAAGUGAC	580
3118	GUGAGAAGGCCAGUGGGUA	254	3118	GUGAGAAGGCCAGUGGGUA	254	3136	UACCCACUGGCCUUCUCAC	581
3122	GAAGGCCAGUGGGUACCUG	255	3122	GAAGGCCAGUGGGUACCUG	255	3140	CAGGUACCCACUGGCCUUC	582
392	CAACAACCAAGACAGUGAC	256	392	CAACAACCAAGACAGUGAC	256	410	GUCACUGUCUUGGUUGUUG	583
1370	CUUGCGCAAGGCCAUCGAC	257	1370	CUUGCGCAAGGCCAUCGAC	257	1388	GUCGAUGGCCUUGCGCAAG	584
2299	UCUACCAUAAGCUGCACCA	258	2299	UCUACCAUAAGCUGCACCA	258	2317	UGGUGCAGCUUAUGGUAGA	585
2302	ACCAUAAGCUGCACCAGCU	259	2302	ACCAUAAGCUGCACCAGCU	259	2320	AGCUGGUGCAGCUUAUGGU	586
2305	AUAAGCUGCACCAGCUGCA	260	2305	AUAAGCUGCACCAGCUGCA	260	2323	UGCAGCUGGUGCAGCUUAU	587
3046	ACUCCUUCAAGGCUGCCCG	261	3046	ACUCCUUCAAGGCUGCCCG	261	3064	CGGGCAGCCUUGAAGGAGU	588
1251	GAGAAGCGCACAGCCCACA	262	1251	GAGAAGCGCACAGCCCACA	262	1269	UGUGGGCUGUGCGCUUCUC	589
1272	GCCAUUGAGAAGCGCUACC	263	1272	GCCAUUGAGAAGCGCUACC	263	1290	GGUAGCGCUUCUCAAUGGC	590
1360	AAUCUGCUGUCUUGCGCAA	264	1360	AAUCUGCUGUCUUGCGCAA	264	1378	UUGCGCAAGACAGCAGAUU	591
1363	CUGCUGUCUUGCGCAAGGC	265	1363	CUGCUGUCUUGCGCAAGGC	265	1381	GCCUUGCGCAAGACAGCAG	592
1737	CGCUCCCGCCUGGCCCUGU	266	1737	CGCUCCCGCCUGGCCCUGU	266	1755	ACAGGGCCAGGCGGGAGCG	593
1348	CAAAGCUGAAUAAAUCUGC	267	1348	CAAAGCUGAAUAAAUCUGC	267	1366	GCAGAUUUAUUCAGCUUUG	594
1362	UCUGCUGUCUUGCGCAAGG	268	1362	UCUGCUGUCUUGCGCAAGG	268	1380	CCUUGCGCAAGACAGCAGA	595
1729	UGCUGGACCGCUCCCGCCU	269	1729	UGCUGGACCGCUCCCGCCU	269	1747	AGGCGGGAGCGGUCCAGCA	596
1735	ACCGCUCCCGCCUGGCCCU	270	1735	ACCGCUCCCGCCUGGCCCU	270	1753	AGGGCCAGGCGGGAGCGGU	597
2300	CUACCAUAAGCUGCACCAG	271	2300	CUACCAUAAGCUGCACCAG	271	2318	CUGGUGCAGCUUAUGGUAG	598
3121	AGAAGGCCAGUGGGUACCU	272	3121	AGAAGGCCAGUGGGUACCU	272	3139	AGGUACCCACUGGCCUUCU	599
1726	GCAUGCUGGACCGCUCCCG	273	1726	GCAUGCUGGACCGCUCCCG	273	1744	CGGGAGCGGUCCAGCAUGC	600
2204	GGUGGGCCGCUGGCUGGCA	274	2204	GGUGGGCCGCUGGCUGGCA	274	2222	UGCCAGCCAGCGGCCCACC	601
1012	ACUUCAUCAAGGCAGACUC	275	1012	ACUUCAUCAAGGCAGACUC	275	1030	GAGUCUGCCUUGAUGAAGU	602
1727	CAUGCUGGACCGCUCCCGC	276	1727	CAUGCUGGACCGCUCCCGC	276	1745	GCGGGAGCGGUCCAGCAUG	603
3047	CUCCUUCAAGGCUGCCCGG	277	3047	CUCCUUCAAGGCUGCCCGG	277	3065	CCGGGCAGCCUUGAAGGAG	604
3116	CUGUGAGAAGGCCAGUGGG	278	3116	CUGUGAGAAGGCCAGUGGG	278	3134	CCCACUGGCCUUCUCACAG	605
1730	GCUGGACCGCUCCCGCCUG	279	1730	GCUGGACCGCUCCCGCCUG	279	1748	CAGGCGGGAGCGGUCCAGC	606
2561	CAUGCAGUGGCUCUGCCAC	280	2561	CAUGCAGUGGCUCUGCCAC	280	2579	GUGGCAGAGCCACUGCAUG	607
2563	UGCAGUGGCUCUGCCACCC	281	2563	UGCAGUGGCUCUGCCACCC	281	2581	GGGUGGCAGAGCCACUGCA	608
1273	CCAUUGAGAAGCGCUACCG	282	1273	CCAUUGAGAAGCGCUACCG	282	1291	CGGUAGCGCUUCUCAAUGG	609
1350	AAGCUGAAUAAAUCUGCUG	283	1350	AAGCUGAAUAAAUCUGCUG	283	1368	CAGCAGAUUUAUUCAGCUU	610
1733	GGACCGCUCCCGCCUGGCC	284	1733	GGACCGCUCCCGCCUGGCC	284	1751	GGCCAGGCGGGAGCGGUCC	611

TABLE II-continued

		SREBP1 siNA and Target Sequences							
Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
1734	GACCGCUCCCGCCUGGCCC	285	1734	GACCGCUCCCGCCUGGCCC	285	1752	GGGCCAGGCGGGAGCGGUC	612
2298	GUCUACCAUAAGCUGCACC	286	2298	GUCUACCAUAAGCUGCACC	286	2316	GGUGCAGCUUAUGGUAGAC	613
3113	CAUCUGUGAGAAGGCCAGU	287	3113	CAUCUGUGAGAAGGCCAGU	287	3131	ACUGGCCUUCUCACAGAUG	614
1736	CCGCUCCCGCCUGGCCCUG	288	1736	CCGCUCCCGCCUGGCCCUG	288	1754	CAGGGCCAGGCGGGAGCGG	615
3115	UCUGUGAGAAGGCCAGUGG	289	3115	UCUGUGAGAAGGCCAGUGG	289	3133	CCACUGGCCUUCUCACAGA	616
3355	UGGCACAGAGCUUCCGGCC	290	3355	UGGCACAGAGCUUCCGGCC	290	3373	GGCCGGAAGCUCUGUGCCA	617
1368	GUCUUGCGCAAGGCCAUCG	291	1368	GUCUUGCGCAAGGCCAUCG	291	1386	CGAUGGCCUUGCGCAAGAC	618
1725	GGCAUGCUGGACCGCUCCC	292	1725	GGCAUGCUGGACCGCUCCC	292	1743	GGGAGCGGUCCAGCAUGCC	619
2203	GGGUGGGCCGCUGGCUGGC	293	2203	GGGUGGGCCGCUGGCUGGC	293	2221	GCCAGCCAGCGGCCCACCC	620
2899	UGGCCAAGUGGUGGGCCUC	294	2899	UGGCCAAGUGGUGGGCCUC	294	2917	GAGGCCCACCACUUGGCCA	621
3049	CCUUCAAGGCUGCCCGGGC	295	3049	CCUUCAAGGCUGCCCGGGC	295	3067	GCCCGGGCAGCCUUGAAGG	622
3120	GAGAAGGCCAGUGGGUACC	296	3120	GAGAAGGCCAGUGGGUACC	296	3138	GGUACCCACUGGCCUUCUC	623
1358	UAAAUCUGCUGUCUUGCGC	297	1358	UAAAUCUGCUGUCUUGCGC	297	1376	GCGCAAGACAGCAGAUUUA	624
1359	AAAUCUGCUGUCUUGCGCA	298	1359	AAAUCUGCUGUCUUGCGCA	298	1377	UGCGCAAGACAGCAGAUUU	625
1364	UGCUGUCUUGCGCAAGGCC	299	1364	UGCUGUCUUGCGCAAGGCC	299	1382	GGCCUUGCGCAAGACAGCA	626
2202	UGGGUGGGCCGCUGGCUGG	300	2202	UGGGUGGGCCGCUGGCUGG	300	2220	CCAGCCAGCGGCCCACCCA	627
2206	UGGGCCGCUGGCUGGCAGG	301	2206	UGGGCCGCUGGCUGGCAGG	301	2224	CCUGCCAGCCAGCGGCCCA	628
2303	CCAUAAGCUGCACCAGCUG	302	2303	CCAUAAGCUGCACCAGCUG	302	2321	CAGCUGGUGCAGCUUAUGG	629
2558	UGCCAUGCAGUGGCUCUGC	303	2558	UGCCAUGCAGUGGCUCUGC	303	2576	GCAGAGCCACUGCAUGGCA	630
3123	AAGGCCAGUGGGUACCUGC	304	3123	AAGGCCAGUGGGUACCUGC	304	3141	GCAGGUACCCACUGGCCUU	631
2207	GGGCCGCUGGCUGGCAGGC	305	2207	GGGCCGCUGGCUGGCAGGC	305	2225	GCCUGCCAGCCAGCGGCCC	632
2208	GGCCGCUGGCUGGCAGGCC	306	2208	GGCCGCUGGCUGGCAGGCC	306	2226	GGCCUGCCAGCCAGCGGCC	633
3114	AUCUGUGAGAAGGCCAGUG	307	3114	AUCUGUGAGAAGGCCAGUG	307	3132	CACUGGCCUUCUCACAGAU	634
396	AACCAAGACAGUGACUUCC	308	396	AACCAAGACAGUGACUUCC	308	414	GGAAGUCACUGUCUUGGUU	635
1732	UGGACCGCUCCCGCCUGGC	309	1732	UGGACCGCUCCCGCCUGGC	309	1750	GCCAGGCGGGAGCGGUCCA	636
397	ACCAAGACAGUGACUUCCC	310	397	ACCAAGACAGUGACUUCCC	310	415	GGGAAGUCACUGUCUUGGU	637
3119	UGAGAAGGCCAGUGGGUAC	311	3119	UGAGAAGGCCAGUGGGUAC	311	3137	GUACCCACUGGCCUUCUCA	638
390	AUCAACAACCAAGACAGUG	312	390	AUCAACAACCAAGACAGUG	312	408	CACUGUCUUGGUUGUUGAU	639
1361	AUCUGCUGUCUUGCGCAAG	313	1361	AUCUGCUGUCUUGCGCAAG	313	1379	CUUGCGCAAGACAGCAGAU	640
2304	CAUAAGCUGCACCAGCUGC	314	2304	CAUAAGCUGCACCAGCUGC	314	2322	GCAGCUGGUGCAGCUUAUG	641
1728	AUGCUGGACCGCUCCCGCC	315	1728	AUGCUGGACCGCUCCCGCC	315	1746	GGCGGGAGCGGUCCAGCAU	642
1367	UGUCUUGCGCAAGGCCAUC	316	1367	UGUCUUGCGCAAGGCCAUC	316	1385	GAUGGCCUUGCGCAAGACA	643
1356	AAUAAAUCUGCUGUCUUGC	317	1356	AAUAAAUCUGCUGUCUUGC	317	1374	GCAAGACAGCAGAUUUAUU	644
1357	AUAAAUCUGCUGUCUUGCG	318	1357	AUAAAUCUGCUGUCUUGCG	318	1375	CGCAAGACAGCAGAUUUAU	645
2301	UACCAUAAGCUGCACCAGC	319	2301	UACCAUAAGCUGCACCAGC	319	2319	GCUGGUGCAGCUUAUGGUA	646

TABLE II-continued

SREBP1 siNA and Target Sequences

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
2562	AUGCAGUGGCUCUGCCACC	320	2562	AUGCAGUGGCUCUGCCACC	320	2580	GGUGGCAGAGCCACUGCAU	647
3048	UCCUUCAAGGCUGCCCGGG	321	3048	UCCUUCAAGGCUGCCCGGG	321	3066	CCCGGGCAGCCUUGAAGGA	648
2122	GCCGGCCCCUGCCCACCUC	322	2122	GCCGGCCCCUGCCCACCUC	322	2140	GAGGUGGGCAGGGGCCGGC	649
2121	GGCCGGCCCCUGCCCACCU	323	2121	GGCCGGCCCCUGCCCACCU	323	2139	AGGUGGGCAGGGGCCGGCC	650
2118	CUGGGCCGGCCCCUGCCCA	324	2118	CUGGGCCGGCCCCUGCCCA	324	2136	UGGGCAGGGGCCGGCCCAG	651
2119	UGGGCCGGCCCCUGCCCAC	325	2119	UGGGCCGGCCCCUGCCCAC	325	2137	GUGGGCAGGGGCCGGCCCA	652
2120	GGGCCGGCCCCUGCCCACC	326	2120	GGGCCGGCCCCUGCCCACC	326	2138	GGUGGGCAGGGGCCGGCCC	653
3715	UCACUUCCAGCUAGACCCC	327	3715	UCACUUCCAGCUAGACCCC	327	3733	GGGGUCUAGCUGGAAGUGA	654

The 3'-ends of the Upper sequence and the Lower sequence of the siNA construct can include an overhang sequence, for example about 1, 2, 3, or 4 nucleotides in length, preferably 2 nucleotides in length, wherein the overhanging sequence of the lower sequence is optionally complementary to a portion of the target sequence. The upper sequence is also referred to as the sense strand, whereas the lower sequence is also referred to as the antisense strand. The upper and lower sequences in the Table can further comprise a chemical modification having Formulae I-VII, such as exemplary siNA constructs shown in FiGS. 4 and 5, or having modifications described in Table IV or any combination thereof.

TABLE III

SREBP1 Synthetic Modified siNA constructs

Target Pos	Target	Seq ID	Cmpd #	Aliases	Sequence	Seq ID
1375	GCGCAAGGCCAUCGACUACAUUC	655	5	SREBP1: 1375U21 siRNA	GCAAGGCCAUCGACUACAUTT	663
1374	UGCGCAAGGCCAUCGACUACAUU	656	6	SREBP1:CGCAAGGCCAUCGACUACATT 1374U21 siRNA	664	
391	UAUCAACAACCAAGACAGUGACU	657	7	SREBP1:UCAACAACCAAGACAGUGATT 391U21 siRNA	665	
395	AACAACCAAGACAGUGACUUCCC	658	8	SREBP1:CAACCAAGACAGUGACUUCTT 395U21 siRNA	666	
1351	AAAGCUGAAUAAAUCUGCUGUCU	659	9	SREBP1:AGCUGAAUAAAUCUGCUGUTT 1351U21 siRNA	667	
1352	AAGCUGAAUAAAUCUGCUGUCUU	660	10	SREBP1:GCUGAAUAAAUCUGCUGUCTT 1352U21 siRNA	668	
1366	UGCUGUCUUGCGCAAGGCCAUCG	661	11	SREBP1:CUGUCUUGCGCAAGGCCAUTT 1366U21 siRNA	669	
1369	UGUCUUGCGCAAGGCCAUCGACU	662	12	SREBP1:UCUUGCGCAAGGCCAUCGATT 1369U21 siRNA	670	
1375	GCGCAAGGCCAUCGACUACAUUC	663	13	SREBP1:AUGUAGUCGAUGGCCUUGCTT 1393L21 siRNA (1375C)	671	

TABLE III-continued

SREBP1 Synthetic Modified siNA constructs

Target Pos	Target	Seq ID	Cmpd #	Aliases	Sequence	Seq ID
1374	UGCGCAAGGCCAUCGACUACAUU	655	5	SREBP1:UGUAGUCGAUGGCCUUGCGTT 1392L21 siRNA (1374C)		672
391	UAUCAACAACCAAGACAGUGACU	656	5	SREBP1:UCACUGUCUUGGUUGUUGATT 409L21 siRNA (391C)		673
395	AACAACCAAGACAGUGACUUCCC	657	5	SREBP1:GAAGUCACUGUCUUGGUUGTT 413L21 siRNA (395C)		674
1351	AAAGCUGAAUAAAUCUGCUGUCU	658	5	SREBP1:ACAGCAGAUUUAUUCAGCUTT 1369L21 siRNA (1351C)		675
1352	AAGCUGAAUAAAUCUGCUGUCUU	659	5	SREBP1:GACAGCAGAUUUAUUCAGCTT 1370L21 siRNA (1352C)		676
1366	UGCUGUCUUGCGCAAGGCCAUCG	660	5	SREBP1:AUGGCCUUGCGCAAGACAGTT 1384L21 siRNA (1366C)		677
1369	UGUCUUGCGCAAGGCCAUCGACU	661	5	SREBP1:UCGAUGGCCUUGCGCAAGATT 1387L21 siRNA (1369C)		678
1375	GCGCAAGGCCAUCGACUACAUUC	662	5	SREBP1:B GcAAGGccAucGAcuAcAuTT B 1375U21 siRNA stab04		679
1374	UGCGCAAGGCCAUCGACUACAUU	663	5	SREBP1:B cGcAAGGccAucGAcuAcATT B 1374U21 siRNA stab04		680
391	UAUCAACAACCAAGACAGUGACU	664	5	SREBP1:B ucAAcAAccAAGAcAGuGATT B 391U21 siRNA stab04		681
395	AACAACCAAGACAGUGACUUCCC	665	5	SREBP1:B cAAccAAGAcAGuGAcuucTT B 395U21 siRNA stab04		682
1351	AAAGCUGAAUAAAUCUGCUGUCU	666	5	SREBP1:B AGcuGAAuAAAucuGcuGuTT B 1351U21 siRNA stab04		683
1352	AAGCUGAAUAAAUCUGCUGUCUU	667	5	SREBP1:B GcuGAAuAAAucuGcuGucTT B 1352U21 siRNA stab04		684
1366	UGCUGUCUUGCGCAAGGCCAUCG	668	5	SREBP1:B cuGucuuGcGcAAGGccAuTT B 1366U21 siRNA stab04		685

TABLE III-continued

SREBP1 Synthetic Modified siNA constructs

Target Pos	Target	Seq ID	Cmpd #	Aliases	Sequence	Seq ID
1369	UGUCUUGCGCAAGGCCAUCGACU	662	1369U21 siRNA stab04	SREBP1:B ucuuGcGcAAGGccAucGATT B		686
1375	GCGCAAGGCCAUCGACUACAUUC	655	1393L21 siRNA (1375C) stab05	SREBP1:AuGuAGucGAuGGccuuGcTsT		687
1374	UGCGCAAGGCCAUCGACUACAUU	656	1392L21 siRNA (1374C) stab05	SREBP1:uGuAGucGAuGGccuuGcGTsT		688
391	UAUCAACAACCAAGACAGUGACU	657	409L21 siRNA (391C) stab05	SREBP1:ucAcuGucuuGGuuGuuGATsT		689
395	AACAACCAAGACAGUGACUUCCC	658	413L21 siRNA (395C) stab05	SREBP1:GAAGucAcuGucuuGGuuGTsT		690
1351	AAAGCUGAAUAAAUCUGCUGUCU	659	1369L21 siRNA (1351C) stab05	SREBP1:AcAGcAGAuuuAuucAGcuTsT		691
1352	AAGCUGAAUAAAUCUGCUGUCUU	660	1370L21 siRNA (1352C) stab05	SREBP1:GAcAGcAGAuuuAuucAGcTsT		692
1366	UGCUGUCUUGCGCAAGGCCAUCG	661	1384L21 siRNA (1366C) stab05	SREBP1:AuGGccuuGcGcAAGAcAGTsT		693
1369	UGUCUUGCGCAAGGCCAUCGACU	662	1387L21 siRNA (1369C) stab05	SREBP1:ucGAuGGccuuGcGcAAGATsT		694
1375	GCGCAAGGCCAUCGACUACAUUC	655	1375U21 siRNA stab07	SREBP1:B GcAAGGccAucGAcuAcAuTT B		695
1374	UGCGCAAGGCCAUCGACUACAUU	656	1374U21 siRNA stab07	SREBP1:B cGcAAGGccAucGAcuAcATT B		696
391	UAUCAACAACCAAGACAGUGACU	657	391U21 siRNA stab07	SREBP1:B ucAAcAAccAAGAcAGuGATT B		697
395	AACAACCAAGACAGUGACUUCCC	658	395U21 siRNA stab07	SREBP1:B cAAccAAGAcAGuGAcuucTT B		698

TABLE III-continued

SREBP1 Synthetic Modified siNA constructs

Target Pos	Target	Seq ID	Cmpd #	Aliases	Sequence	Seq ID
1351	AAAGCUGAAUAAAUCUGCUGUCU	659	59	SREBP1:B AGcuGAAuAAAucuGcuGuTT B 1351U21 siRNA stab07		699
1352	AAGCUGAAUAAAUCUGCUGUCUU	660	60	SREBP1:B GcuGAAuAAAucuGcuGucTT B 1352U21 siRNA stab07		700
1366	UGCUGUCUUGCGCAAGGCCAUCG	661	61	SREBP1:B cuGucuuGcGcAAGGccAuTT B 1366U21 siRNA stab07		701
1369	UGUCUUGCGCAAGGCCAUCGACU	662	62	SREBP1:B ucuuGcGcAAGGccAucGATT B 1369U21 siRNA stab07		702
1375	GCGCAAGGCCAUCGACUACAUUC	663	63	SREBP1:AuGuAGucGAuGGccuuGcTsT 1393L21 siRNA (1375C) stab11		703
1374	UGCGCAAGGCCAUCGACUACAUU	664	64	SREBP1:uGuAGucGAuGGccuuGcGTsT 1392L21 siRNA (1374C) stab11		704
391	UAUCAACAACCAAGACAGUGACU	665	65	SREBP1:ucAcuGucuuGGuuGuuGATsT 409L21 siRNA (391C) stab11		705
395	AACAACCAAGACAGUGACUUCCC	666	66	SREBP1:GAAGucAcuGucuuGGuuGTsT 413L21 siRNA (395C) stab11		706
1351	AAAGCUGAAUAAAUCUGCUGUCU	667	67	SREBP1:AcAGcAGAuuuAuucAGcuTsT 1369L21 siRNA (1351C) stab11		707
1352	AAGCUGAAUAAAUCUGCUGUCUU	668	68	SREBP1:GAcAGcAGAuuuAuucAGcTsT 1370L21 siRNA (1352C) stab11		708
1366	UGCUGUCUUGCGCAAGGCCAUCG	669	69	SREBP1:AuGGccuuGcGcAAGAcAGTsT 1384L21 siRNA (1366C) stab11		709
1369	UGUCUUGCGCAAGGCCAUCGACU	670	70	SREBP1:ucGAuGGccuuGcGcAAGATsT 1387L21 siRNA (1369C) stab11		710

TABLE III-continued

SREBP1 Synthetic Modified siNA constructs

Target Pos	Target	Seq ID	Cmpd #	Aliases	Sequence	Seq ID
1375	GCGCAAGGCCAUCGACUACAUUC	655	55	SREBP1:B 1375U21 siRNA stab18	GcAAGGccAucGAcuAcAuTT B	711
1374	UGCGCAAGGCCAUCGACUACAUU	656	56	SREBP1:B 1374U21 siRNA stab18	cGcAAGGccAucGAcuAcATT B	712
391	UAUCAACAACCAAGACAGUGACU	657	57	SREBP1:B 391U21 siRNA stab18	ucAAcAAccAAGAcAGuGATT B	713
395	AACAACCAAGACAGUGACUUCCC	658	58	SREBP1:B 395U21 siRNA stab18	cAAccAAGAcAGuGAcuucTT B	714
1351	AAAGCUGAAUAAAUCUGCUGUCU	659	59	SREBP1:B 1351U21 siR- NAs- tab18	AGcuGAAuAAAucuGcuGuTT B	715
1352	AAGCUGAAUAAAUCUGCUGUCUU	660	60	SREBP1:B 1352U21 siR- NAs- tab18	GcuGAAuAAAucuGcuGucTT B	716
1366	UGCUGUCUUGCGCAAGGCCAUCG	661	61	SREBP1:B 1366U21 siR- NAs- tab18	cuGucuuGcGcAAGGccAuTT B	717
1369	UGUCUUGCGCAAGGCCAUCGACU	662	62	SREBP1:B 1369U21 siRNA stab18	ucuuGcGcAAGGccAucGATT B	718
1375	GCGCAAGGCCAUCGACUACAUUC	655	58	SREBP1:AuGuAGucGAuGGccuuGcTsT 1393L21 siRNA (1376C) stab08		719
1374	UGCGCAAGGCCAUCGACUACAUU	656	59	SREBP1:uGuAGucGAuGGccuuGcGTsT 1392L21 siRNA (1374C) stab08		720
391	UAUCAACAACCAAGACAGUGACU	657	57	SREBP1:ucAcuGucuuGGuuGuuGATsT 409L21 siRNA (391C) stab08		721
395	AACAACCAAGACAGUGACUUCCC	658	58	SREBP1:GAAGucAcuGucuuGGuuGTsT 413L21 siRNA (395C) stab08		722
1351	AAAGCUGAAUAAAUCUGCUGUCU	659	59	SREBP1:AcAGcAGAuuuAuucAGcuTsT 1369L21 siRNA (1351C) stab08		723

TABLE III-continued

SREBP1 Synthetic Modified siNA constructs

Target Pos	Target	Seq ID	Cmpd #	Aliases	Sequence	Seq ID
1352	AAGCUGAAUAAAUCUGCUGUCUU	668	1370	SREBP1:GAcAGcAGAuuuAuucAGcTsT siRNA (1352C) stab08 L21		724
1366	UGCUGUCUUGCGCAAGGCCAUCG	669	1384	SREBP1:AuGGccuuGcGcAAGAcAGTsT siRNA (1366C) stab08 L21		725
1369	UGUCUUGCGCAAGGCCAUCGACU	670	1387	SREBP1:ucGAuGGccuuGcGcAAGATsT siRNA (1369C) stab08 L21		726
1375	GCGCAAGGCCAUCGACUACAUUC	671	1375	SREBP1:B GCAAGGCCAUCGACUACAUTT B siRNA stab09 U21		727
1374	UGCGCAAGGCCAUCGACUACAUU	672	1374	SREBP1:B CGCAAGGCCAUCGACUACATT B siRNA stab09 U21		728
391	UAUCAACAACCAAGACAGUGACU	673	391	SREBP1:B UCAACAACCAAGACAGUGATT B siRNA stab09 U21		729
395	AACAACCAAGACAGUGACUUCCC	674	395	SREBP1:B CAACCAAGACAGUGACUUCTT B siRNA stab09 U21		730
1351	AAAGCUGAAUAAAUCUGCUGUCU	675	1351	SREBP1:B AGCUGAAUAAAUCUGCUGUTT B siRNA stab09 U21		731
1352	AAGCUGAAUAAAUCUGCUGUCUU	676	1352	SREBP1:B GCUGAAUAAAUCUGCUGUCTT B siRNA stab09 U21		732
1366	UGCUGUCUUGCGCAAGGCCAUCG	677	1366	SREBP1:B CUGUCUUGCGCAAGGCCAUTT B siRNA stab09 U21		733
1369	UGUCUUGCGCAAGGCCAUCGACU	678	1369	SREBP1:B UCUUGCGCAAGGCCAUCGATT B siRNA stab09 U21		734
1375	GCGCAAGGCCAUCGACUACAUUC	679	1393	SREBP1:AUGUAGUCGAUGGCCUUGCTsT siRNA (1375C) stab10 L21		735
1374	UGCGCAAGGCCAUCGACUACAUU	680	1392	SREBP1:UGUAGUCGAUGGCCUUGCGTsT siRNA (1374C) stab10 L21		736

TABLE III-continued

SREBP1 Synthetic Modified siNA constructs

Target Pos	Target	Seq ID	Cmpd #	Aliases	Sequence	Seq ID
391	UAUCAACAACCAAGACAGUGACU	659	57	SREBP1:UCACUGUCUUGGUUGUUGATsT 409L21 siRNA (391C) stab10		737
395	AACAACCAAGACAGUGACUUCCC	659	58	SREBP1:GAAGUCACUGUCUUGGUUGTsT 413L21 siRNA (395C) stab10		738
1351	AAAGCUGAAUAAAUCUGCUGUCU	659	59	SREBP1:ACAGCAGAUUUAUUCAGCUTsT 1369L21 siRNA (1351C) stab10		739
1352	AAGCUGAAUAAAUCUGCUGUCUU	659	60	SREBP1:GACAGCAGAUUUAUUCAGCTsT 1370L21 siRNA (1352C) stab10		740
1366	UGCUGUCUUGCGCAAGGCCAUCG	659	61	SREBP1:AUGGCCUUGCGCAAGACAGTsT 1384L21 siRNA (1366C) stab10		741
1369	UGUCUUGCGCAAGGCCAUCGACU	659	62	SREBP1:UCGAUGGCCUUGCGCAAGATsT 1387L21 siRNA (1369C) stab10		742
1375	GCGCAAGGCCAUCGACUACAUUC	659	63	SREBP1:AuGuAGucGAuGGccuuGcTT B 1393L21 siRNA (1375C) stab19		743
1374	UGCGCAAGGCCAUCGACUACAUU	659	64	SREBP1:uGuAGucGAuGGccuuGcGTT B 1392L21 siRNA (1374C) stab19		744
391	UAUCAACAACCAAGACAGUGACU	659	65	SREBP1:ucAcuGucuuGGuuGuuGATT B 409L21 siRNA (391C) stab19		745
395	AACAACCAAGACAGUGACUUCCC	659	66	SREBP1:GAAGucAcuGucuuGGuuGTT B 413L21 siRNA (395C) stab19		746
1351	AAAGCUGAAUAAAUCUGCUGUCU	659	67	SREBP1:AcAGcAGAuuuAuucAGcuTT B 1369L21 siRNA (1351C) stab19		747
1352	AAGCUGAAUAAAUCUGCUGUCUU	659	68	SREBP1:GAcAGcAGAuuuAuucAGcTT B 1370L21 siRNA (1352C) stab19		748

TABLE III-continued

SREBP1 Synthetic Modified siNA constructs

Target Pos	Target	Seq ID	Cmpd #	Aliases	Sequence	Seq ID
1366	UGCUGUCUUGCGCAAGGCCAUCG	661	1384	SREBP1:AuGGccuuGcGcAAGAcAGTT L21 siRNA (1366C) stab19 B		749
1369	UGUCUUGCGCAAGGCCAUCGACU	662	1387	SREBP1:ucGAuGGccuuGcGcAAGATT L21 siRNA (1369C) stab19 B		750
1375	GCGCAAGGCCAUCGACUACAUUC	663	1393	SREBP1:AUGUAGUCGAUGGCCUUGCTT L21 siRNA (1375C) stab22 B		751
1374	UGCGCAAGGCCAUCGACUACAUU	664	1392	SREBP1:UGUAGUCGAUGGCCUUGCGTT L21 siRNA (1374C) stab22 B		752
391	UAUCAACAACCAAGACAGUGACU	665	409	SREBP1:UCACUGUCUUGGUUGUUGATT L21 siRNA (391C) stab22 B		753
395	AACAACCAAGACAGUGACUUCCC	666	413	SREBP1:GAAGUCACUGUCUUGGUUGTT L21 siRNA (395C) stab22 B		754
1351	AAAGCUGAAUAAAUCUGCUGUCU	667	1369	SREBP1:ACAGCAGAUUUAUUCAGCUTT L21 siRNA (1351C) stab22 B		755
1352	AAGCUGAAUAAAUCUGCUGUCUU	668	1370	SREBP1:GACAGCAGAUUUAUUCAGCTT L21 siRNA (1352C) stab22 B		756
1366	UGCUGUCUUGCGCAAGGCCAUCG	669	1384	SREBP1:AUGGCCUUGCGCAAGACAGTT L21 siRNA (1366C) stab22 B		757
1369	UGUCUUGCGCAAGGCCAUCGACU	670	1387	SREBP1:UCGAUGGCCUUGCGCAAGATT L21 siRNA (1369C) stab22 B		758
1375	GCGCAAGGCCAUCGACUACAUUC	671	1393	SREBP1:AUGuAGucGAuGGccuuGcTsT L21 siRNA (1375C) stab25		759

TABLE III-continued

SREBP1 Synthetic Modified siNA constructs

Target Pos	Target	Seq ID	Cmpd #	Aliases	Sequence	Seq ID
1374	UGCGCAAGGCCAUCGACUACAUU	659	58	SREBP1:UGUAGucGAuGGccuuGcGTsT 1392L21 siRNA (1374C) stab25		760
391	UAUCAACAACCAAGACAGUGACU	659	59	SREBP1:UCAcuGucuuGGuuGuuGATsT 409L21 siRNA (391C) stab25		761
395	AACAACCAAGACAGUGACUUCCC	659	59	SREBP1:GAAGucAcuGucuuGGuuGTsT 413L21 siRNA (395C) stab25		762
1351	AAAGCUGAAUAAAUCUGCUGUCU	659	59	SREBP1:ACAGcAGAuuuAuucAGcuTsT 1369L21 siRNA (1351C) stab25		763
1352	AAGCUGAAUAAAUCUGCUGUCUU	660	60	SREBP1:GACAGcAGAuuuAuucAGcTsT 1370L21 siRNA (1352C) stab25		764
1366	UGCUGUCUUGCGCAAGGCCAUCG	661	61	SREBP1:AUGGccuuGcGcAAGAcAGTsT 1384L21 siRNA (1366C) stab25		765
1369	UGUCUUGCGCAAGGCCAUCGACU	662	62	SREBP1:UCGAuGGccuuGcGcAAGATsT 1387L21 siRNA (1369C) stab25		766

Uppercase = ribonucleotide
u, c = 2'-deoxy-2'-fluoro U, C
T = thymidine
B = inverted deoxy abasic
s = phosphorothioate linkage
A = deoxy Adenosine
G = deoxy Guanosine
G = 2'-O-methyl Guanosine
A = 2'-O-methyl Adenosine

TABLE IV

Non-limiting examples of Stabilization Chemistries for chemically modified siNA constructs

Chemistry	pyrimidine	Purine	cap	p = S	Strand
"Stab 00"	Ribo	Ribo	TT at 3'-ends		S/AS
"Stab 1"	Ribo	Ribo	—	5 at 5'-end 1 at 3'-end	S/AS
"Stab 2"	Ribo	Ribo	—	All linkages	Usually AS
"Stab 3"	2'-fluoro	Ribo	—	4 at 5'-end 4 at 3'-end	Usually S
"Stab 4"	2'-fluoro	Ribo	5' and 3'-ends	—	Usually S
"Stab 5"	2'-fluoro	Ribo	—	1 at 3'-end	Usually AS
"Stab 6"	2'-O-Methyl	Ribo	5' and 3'-ends	—	Usually S
"Stab 7"	2'-fluoro	2'-deoxy	5' and 3'-ends	—	Usually S

TABLE IV-continued

	Non-limiting examples of Stabilization Chemistries for chemically modified siNA constructs				
Chemistry	pyrimidine	Purine	cap	p = S	Strand
"Stab 8"	2'-fluoro	2'-O-Methyl	—	1 at 3'-end	S/AS
"Stab 9"	Ribo	Ribo	5' and 3'-ends	—	Usually S
"Stab 10"	Ribo	Ribo	—	1 at 3'-end	Usually AS
"Stab 11"	2'-fluoro	2'-deoxy	—	1 at 3'-end	Usually AS
"Stab 12"	2'-fluoro	LNA	5' and 3'-ends		Usually S
"Stab 13"	2'-fluoro	LNA		1 at 3'-end	Usually AS
"Stab 14"	2'-fluoro	2'-deoxy		2 at 5'-end 1 at 3'-end	Usually AS
"Stab 15"	2'-deoxy	2'-deoxy		2 at 5'-end 1 at 3'-end	Usually AS
"Stab 16"	Ribo	2'-O-Methyl	5' and 3'-ends		Usually S
"Stab 17"	2'-O-Methyl	2'-O-Methyl	5' and 3'-ends		Usually S
"Stab 18"	2'-fluoro	2'-O-Methyl	5' and 3'-ends		Usually S
"Stab 19"	2'-fluoro	2'-O-Methyl	3'-end		S/AS
"Stab 20"	2'-fluoro	2'-deoxy	3'-end		Usually AS
"Stab 21"	2'-fluoro	Ribo	3'-end		Usually AS
"Stab 22"	Ribo	Ribo	3'-end		Usually AS
"Stab 23"	2'-fluoro*	2'-deoxy*	5' and 3'-ends		Usually S
"Stab 24"	2'-fluoro*	2'-O-Methyl*	—	1 at 3'-end	S/AS
"Stab 25"	2'-fluoro*	2'-O-Methyl*	—	1 at 3'-end	S/AS
"Stab 26"	2'-fluoro*	2'-O-Methyl*	—		S/AS
"Stab 27"	2'-fluoro*	2'-O-Methyl*	3'-end		S/AS
"Stab 28"	2'-fluoro*	2'-O-Methyl*	3'-end		S/AS
"Stab 29"	2'-fluoro*	2'-O-Methyl*		1 at 3'-end	S/AS
"Stab 30"	2'-fluoro*	2'-O-Methyl*			S/AS
"Stab 31"	2'-fluoro*	2'-O-Methyl*	3'-end		S/AS
"Stab 32"	2'-fluoro	2'-O-Methyl			S/AS
"Stab 33"	2'-fluoro	2'-deoxy*	5' and 3'-ends	—	Usually S
"Stab 34"	2'-fluoro	2'-O-Methyl*	5' and 3'-ends		Usually S
"Stab 35"	2'-fluoro	2'-O-Methyl**			Usually AS
"Stab 36"	2'-fluoro	2'-O-Methyl**			Usually AS
"Stab 3F"	2'-OCF3	Ribo	—	4 at 5'-end 4 at 3'-end	Usually S
"Stab 4F"	2'-OCF3	Ribo	5' and 3'-ends	—	Usually S
"Stab 5F"	2'-OCF3	Ribo	—	1 at 3'-end	Usually AS
"Stab 7F"	2'-OCF3	2'-deoxy	5' and 3'-ends	—	Usually S
"Stab 8F"	2'-OCF3	2'-O-Methyl	—	1 at 3'-end	S/AS
"Stab 11F"	2'-OCF3	2'-deoxy	—	1 at 3'-end	Usually AS
"Stab 12F"	2'-OCF3	LNA	5' and 3'-ends		Usually S
"Stab 13F"	2'-OCF3	LNA		1 at 3'-end	Usually AS
"Stab 14F"	2'-OCF3	2'-deoxy		2 at 5'-end 1 at 3'-end	Usually AS
"Stab 15F"	2'-OCF3	2'-deoxy		2 at 5'-end 1 at 3'-end	Usually AS
"Stab 18F"	2'-OCF3	2'-O-Methyl	5' and 3'-ends		Usually S
"Stab 19F"	2'-OCF3	2'-O-Methyl	3'-end		S/AS
"Stab 20F"	2'-OCF3	2'-deoxy	3'-end		Usually AS

TABLE IV-continued

Chemistry	pyrimidine	Purine	cap	p = S	Strand
	Non-limiting examples of Stabilization Chemistries for chemically modified siNA constructs				
"Stab 21F"	2'-OCF3	Ribo	3'-end		Usually AS
"Stab 23F"	2'-OCF3*	2'-deoxy*	5' and 3'-ends		Usually S
"Stab 24F"	2'-OCF3*	2'-O-Methyl*	—	1 at 3'-end	S/AS
"Stab 25F"	2'-OCF3*	2'-O-Methyl*	—	1 at 3'-end	S/AS
"Stab 26F"	2'-OCF3*	2'-O-Methyl*	—		S/AS
"Stab 27F"	2'-OCF3*	2'-O-Methyl*	3'-end		S/AS
"Stab 28F"	2'-OCF3*	2'-O-Methyl*	3'-end		S/AS
"Stab 29F"	2'-OCF3*	2'-O-Methyl*		1 at 3'-end	S/AS
"Stab 30F"	2'-OCF3*	2'-O-Methyl*			S/AS
"Stab 31F"	2'-OCF3*	2'-O-Methyl*	3'-end		S/AS
"Stab 32F"	2'-OCF3	2'-O-Methyl			S/AS
"Stab 33F"	2'-OCF3	2'-deoxy*	5' and 3'-ends	—	Usually S
"Stab 34F"	2'-OCF3	2'-O-Methyl*	5' and 3'-ends		Usually S
"Stab 35F"	2'-OCF3	2'-O-Methyl**			Usually AS
"Stab 36F"	2'-OCF3	2'-O-Methyl**			Usually AS

CAP = any terminal cap, see for example FIG. 10.
All Stab 00-36 chemistries can comprise 3'-terminal thymidine (TT) residues
All Stab 00-36 chemistries typically comprise about 21 nucleotides, but can vary as described herein.
All Stab 00-36 chemistries can also include a single ribonucleotide in the sense or passenger strand at the 11th base paired position of the double stranded nucleic acid duplex as determined from the 5'-end of the antisense or guide strand (see FIG. 6C)
S = sense strand
AS = antisense strand
*Stab 23 has a single ribonucleotide adjacent to 3'-CAP
*Stab 24 and Stab 28 have a single ribonucleotide at 5'-terminus
*Stab 25, Stab 26, and Stab 27 have three ribonucleotides at 5'-terminus
*Stab 29, Stab 30, Stab 31, Stab 33, and Stab 34 any purine at first three nucleotide positions from 5'-terminus are ribonucleotides
p = phosphorothioate linkage
**Stab 35 has 2'-O-methyl U at 3'-overhangs
**Stab 36 has 2'-O-methyl overhangs that are complementary to the target sequence (naturally occurring overhangs)

TABLE V

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time* RNA
		A. 2.5 μmol Synthesis Cycle ABI 394 Instrument			
Phosphoramidites	6.5	163 μL	45 sec	2.5 min	7.5 min
S-Ethyl Tetrazole	23.8	238 μL	45 sec	2.5 min	7.5 min
Acetic Anhydride	100	233 μL	5 sec	5 sec	5 sec
N-Methyl Imidazole	186	233 μL	5 sec	5 sec	5 sec
TCA	176	2.3 mL	21 sec	21 sec	21 sec
Iodine	11.2	1.7 mL	45 sec	45 sec	45 sec
Beaucage	12.9	645 μL	100 sec	300 sec	300 sec
Acetonitrile	NA	6.67 mL	NA	NA	NA

TABLE V-continued

B. 0.2 μmol Synthesis Cycle ABI 394 Instrument

Phosphoramidites	15	31 μL	45 sec	233 sec	465 sec
S-Ethyl Tetrazole	38.7	31 μL	45 sec	233 min	465 sec
Acetic Anhydride	655	124 μL	5 sec	5 sec	5 sec
N-Methyl Imidazole	1245	124 μL	5 sec	5 sec	5 sec
TCA	700	732 μL	10 sec	10 sec	10 sec
Iodine	20.6	244 μL	15 sec	15 sec	15 sec
Beaucage	7.7	232 μL	100 sec	300 sec	300 sec
Acetonitrile	NA	2.64 mL	NA	NA	NA

C. 0.2 μmol Synthesis Cycle 96 well Instrument

Reagent	Equivalents: DNA/ 2'-O-methyl/Ribo	Amount: DNA/ 2'-O-methyl/Ribo	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time* Ribo
Phosphoramidites	22/33/66	40/60/120 μL	60 sec	180 sec	360 sec
S-Ethyl Tetrazole	70/105/210	40/60/120 μL	60 sec	180 min	360 sec
Acetic Anhydride	265/265/265	50/50/50 μL	10 sec	10 sec	10 sec
N-Methyl Imidazole	502/502/502	50/50/50 μL	10 sec	10 sec	10 sec
TCA	238/475/475	250/500/500 μL	15 sec	15 sec	15 sec
Iodine	6.8/6.8/6.8	80/80/80 μL	30 sec	30 sec	30 sec
Beaucage	34/51/51	80/120/120	100 sec	200 sec	200 sec
Acetonitrile	NA	1150/1150/1150 μL	NA	NA	NA

Wait time does not include contact time during delivery.
Tandem synthesis utilizes double coupling of linker molecule

TABLE VI

Lipid Nanoparticle (LNP) Formulations

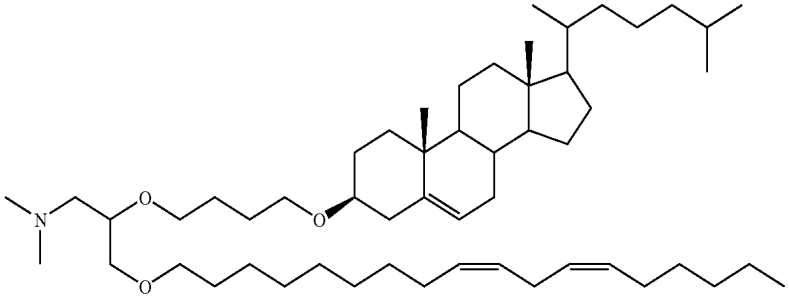
Formulation #	Composition	Molar Ratio
L051	CLinDMA/DSPC/Chol/PEG-n-DMG	48/40/10/2
L053	DMOBA/DSPC/Chol/PEG-n-DMG	30/20/48/2
L054	DMOBA/DSPC/Chol/PEG-n-DMG	50/20/28/2
L069	CLinDMA/DSPC/Cholesterol/PEG-Cholesterol	48/40/10/2
L073	pCLinDMA or CLin DMA/DMOBA/DSPC/Chol/PEG-n-DMG	25/25/20/28/2
L077	eCLinDMA/DSPC/Cholesterol/2KPEG-Chol	48/40/10/2
L080	eCLinDMA/DSPC/Cholesterol/2KPEG-DMG	48/40/10/2
L082	pCLinDMA/DSPC/Cholesterol/2KPEG-DMG	48/40/10/2
L083	pCLinDMA/DSPC/Cholesterol/2KPEG-Chol	48/40/10/2
L086	CLinDMA/DSPC/Cholesterol/2KPEG-DMG/Linoleyl alcohol	43/38/10/2/7
L061	DMLBA/Cholesterol/2KPEG-DMG	52/45/3
L060	DMOBA/Cholesterol/2KPEG-DMG N/P ratio of 5	52/45/3
L097	DMLBA/DSPC/Cholesterol/2KPEG-DMG	50/20/28
L098	DMOBA/Cholesterol/2KPEG-DMG, N/P ratio of 3	52/45/3
L099	DMOBA/Cholesterol/2KPEG-DMG, N/P ratio of 4	52/45/3
L100	DMOBA/DOBA/3% PEG-DMG, N/P ratio of 3	52/45/3
L101	DMOBA/Cholesterol/2KPEG-Cholesterol	52/45/3
L102	DMOBA/Cholesterol/2KPEG-Cholesterol, N/P ratio of 5	52/45/3
L103	DMLBA/Cholesterol/2KPEG-Cholesterol	52/45/3

TABLE VI-continued

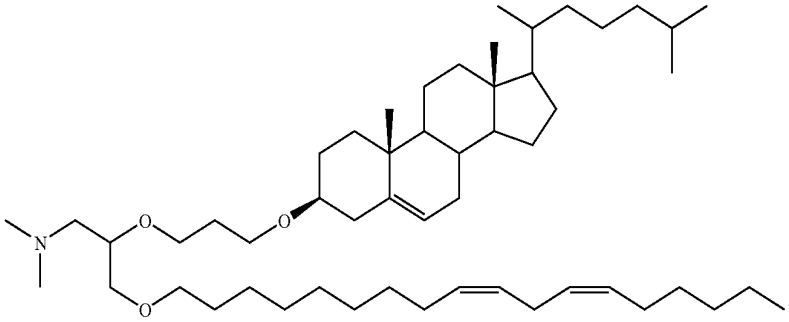
Lipid Nanoparticle (LNP) Formulations

Formulation #	Composition	Molar Ratio
L104	CLinDMA/DSPC/Cholesterol/2KPEG-cholesterol/Linoleyl alcohol	43/38/10/2/7
L105	DMOBA/Cholesterol/2KPEG-Chol, N/P ratio of 2	52/45/3
L106	DMOBA/Cholesterol/2KPEG-Chol, N/P ratio of 3	67/30/3
L107	DMOBA/Cholesterol/2KPEG-Chol, N/P ratio of 1.5	52/45/3
L108	DMOBA/Cholesterol/2KPEG-Chol, N/P ratio of 2	67/30/3
L109	DMOBA/DSPC/Cholesterol/2KPEG-Chol, N/P ratio of 2	50/20/28/2
L110	DMOBA/Cholesterol/2KPEG-DMG, N/P ratio of 1.5	52/45/3
L111	DMOBA/Cholesterol/2KPEG-DMG, N/P ratio of 1.5	67/30/3
L112	DMLBA/Cholesterol/2KPEG-DMG, N/P ratio of 1.5	52/45/3
L113	DMLBA/Cholesterol/2KPEG-DMG, N/P ratio of 1.5	67/30/3
L114	DMOBA/Cholesterol/2KPEG-DMG, N/P ratio of 2	52/45/3
L115	DMOBA/Cholesterol/2KPEG-DMG, N/P ratio of 2	67/30/3
L116	DMLBA/Cholesterol/2KPEG-DMG, N/P ratio of 2	52/45/3
L117	DMLBA/Cholesterol/2KPEG-DMG, N/P ratio of 2	52/45/3

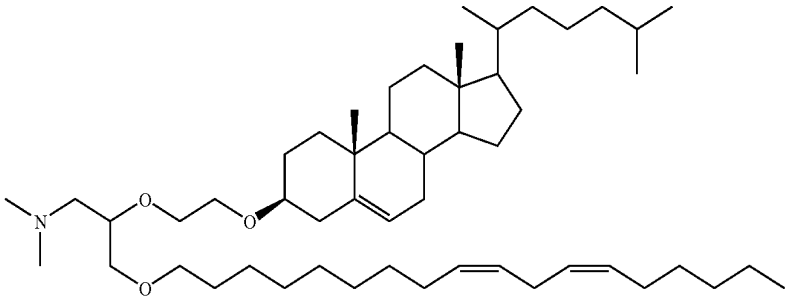
N/P ratio = Nitrogen:Phosphorous ratio between cationic lipid and nucleic acid



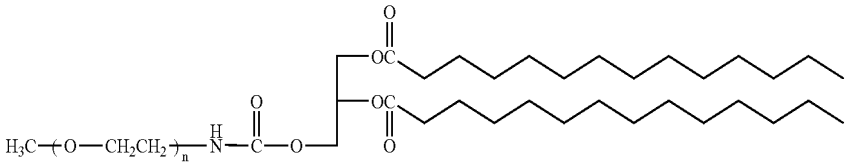
CLinDMA structure



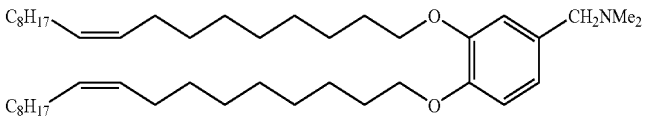
pCLinDMA structure



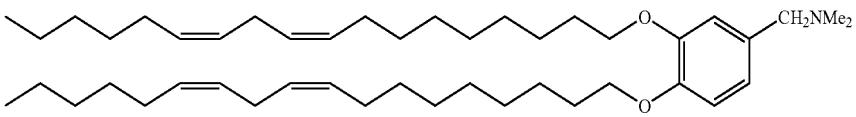
eCLinDMA structure



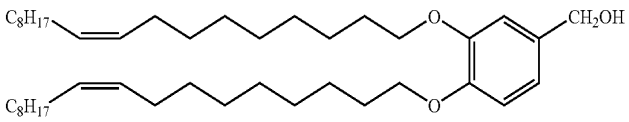
PEG-n-DMG structure



DMOBA structure

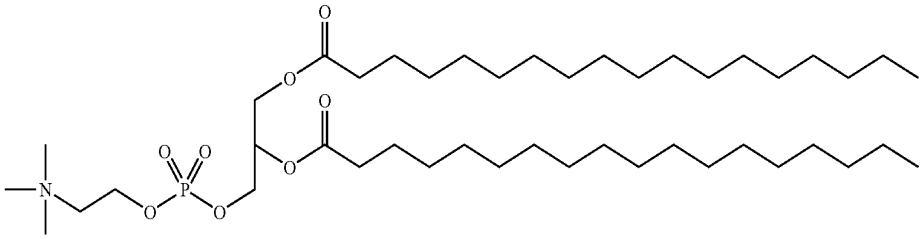


DMLBA structure

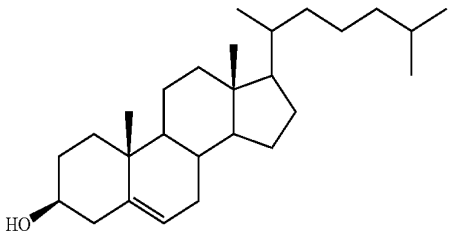


DOBA structure

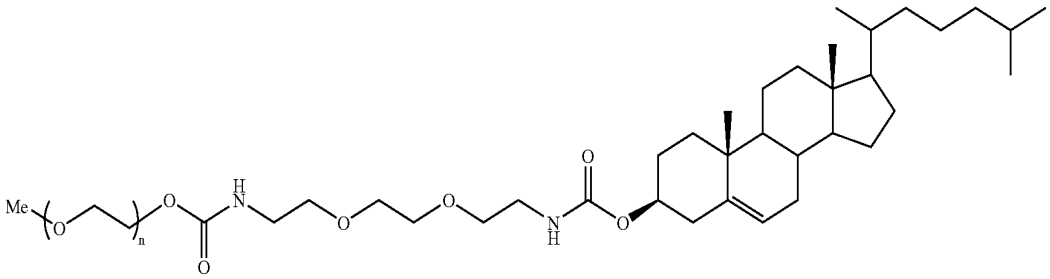
-continued



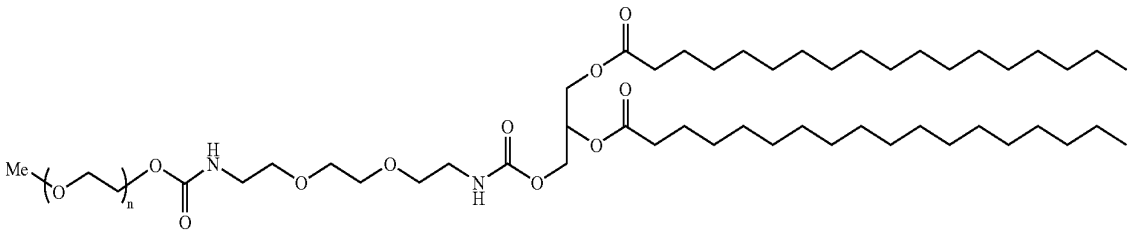
DSPC



Cholesterol



2KPEG-Cholesterol



2KPEG-DMG

TABLE VII

Table VII: Sirna algorithm describing patterns with their relative score for predicting hyper-active siNAs. All the positions given are for the sense strand of 19-mer siNA.

Description of pattern	Pattern #	Score
G or C at position 1	1	5
A or U at position 19	2	10
A/U rich between position 15-19	3	10
String of 4 Gs or 4 Cs (not preferred)	4	−100
G/C rich between position 1-5	5	10
A or U at position 18	6	5

TABLE VII-continued

Table VII: Sirna algorithm describing patterns with their relative score for predicting hyper-active siNAs. All the positions given are for the sense strand of 19-mer siNA.

Description of pattern	Pattern #	Score
A or U at position 10	7	10
G at position 13 (not preferred)	8	−3
A at position 13	9	3
G at position 9 (not preferred)	10	−3
A at position 9	11	3
A or U at position 14	12	10

SEQUENCE LISTING

The patent application contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US20080249040A1). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

What we claim is:

1. A double stranded nucleic acid molecule having structure SIX comprising a sense strand and an antisense strand:

$$\begin{array}{l} B—N_{X3}—(N)_{X2}\ B\text{-}3' \\ B\ (N)_{X1}—N_{X4}—[N]_{X5}\text{-}5' \end{array} \quad \text{SIX}$$

wherein the upper strand is the sense strand and the lower strand is the antisense strand of the double stranded nucleic acid molecule; said antisense strand comprises sequence complementary to a Sterol Regulatory Element-Binding Protein 1 (SREBP1) RNA; each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides which can be unmodified or chemically modified; [N] represents nucleotide positions that are ribonucleotides; X1 and X2 are independently integers from about 0 to about 4; X3 is an integer from about 9 to about 30; X4 is an integer from about 11 to about 30, provided that the sum of X4 and X5 is about 17-36; X5 is an integer from about 1 to about 6; and

(a) any pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the antisense strand other than the purines nucleotides in the [N] nucleotide positions, are independently 2'-O-methyl nucleotides, 2'-deoxyribonucleotides or a combination of 2'-deoxyribonucleotides and 2'-O-methyl nucleotides;

(b) any pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the sense strand are independently 2'-deoxyribonucleotides, 2'-O-methyl nucleotides or a combination of 2'-deoxyribonucleotides and 2'-O-methyl nucleotides; and

(c) any (N) nucleotides are optionally 2'-O-methyl, 2'-deoxy-2'-fluoro, or deoxyribonucleotides.

2. A double stranded nucleic acid molecule having structure SX comprising a sense strand and an antisense strand:

$$\begin{array}{l} B—N_{X3}—(N)_{X2}\ B\text{-}3' \\ B\ (N)_{X1}—N_{X4}—[N]_{X5}\text{-}5' \end{array} \quad \text{SX}$$

wherein the upper strand is the sense strand and the lower strand is the antisense strand of the double stranded nucleic acid molecule; said antisense strand comprises sequence complementary to a Sterol Regulatory Element-Binding Protein 1 (SREBP1) RNA; each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides which can be unmodified or chemically modified; [N] represents nucleotide positions that are ribonucleotides; X1 and X2 are independently integers from about 0 to about 4; X3 is an integer from about 9 to about 30; X4 is an integer from about 11 to about 30, provided that the sum of X4 and X5 is about 17-36; X5 is an integer from about 1 to about 6; and

(a) any pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the antisense strand other than the purines nucleotides in the [N] nucleotide positions, are 2'-O-methyl nucleotides;

(b) any pyrimidine nucleotides present in the sense strand are ribonucleotides; any purine nucleotides present in the sense strand are ribonucleotides; and

(c) any (N) nucleotides are optionally 2'-O-methyl, 2'-deoxy-2'-fluoro, or deoxyribonucleotides.

3. A double stranded nucleic acid molecule having structure SXI comprising a sense strand and an antisense strand:

$$\begin{array}{l} B—N_{X3}—(N)_{X2}\ B\text{-}3' \\ B\ (N)_{X1}—N_{X4}—[N]_{X5}\text{-}5' \end{array} \quad \text{SXI}$$

wherein the upper strand is the sense strand and the lower strand is the antisense strand of the double stranded nucleic acid molecule; said antisense strand comprises sequence complementary to a Sterol Regulatory Element-Binding Protein 1 (SREBP1) RNA; each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides which can be unmodified or chemically modified; [N] represents nucleotide positions that are ribonucleotides; X1 and X2 are independently integers from about 0 to about 4; X3 is an integer from about 9 to about 30; X4 is an integer from about 11 to about 30, provided that the sum of X4 and X5 is about 17-36; X5 is an integer from about 1 to about 6; and

(a) any pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the antisense strand other than the purines nucleotides in the [N] nucleotide positions, are 2'-O-methyl nucleotides;

(b) any pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the sense strand are ribonucleotides; and

(c) any (N) nucleotides are optionally 2'-O-methyl, 2'-deoxy-2'-fluoro, or deoxyribonucleotides.

4. A double stranded nucleic acid molecule having structure SXII comprising a sense strand and an antisense strand:

SXII

$$B—N_{X3}—(N)_{X2}\ B\text{-}3'$$
$$B\ (N)_{X1}—N_{X4}—[N]_{X5}\text{-}5'$$

wherein the upper strand is the sense strand and the lower strand is the antisense strand of the double stranded nucleic acid molecule; said antisense strand comprises sequence complementary to a Sterol Regulatory Element-Binding Protein 1 (SREBP1) RNA; each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides which can be unmodified or chemically modified; [N] represents nucleotide positions that are ribonucleotides; X1 and X2 are independently integers from about 0 to about 4; X3 is an integer from about 9 to about 30; X4 is an integer from about 11 to about 30, provided that the sum of X4 and X5 is about 17-36; X5 is an integer from about 1 to about 6; and

(a) any pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the antisense strand other than the purines nucleotides in the [N] nucleotide positions, are 2'-O-methyl nucleotides;

(b) any pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'-fluoro nucleotides; any purine nucleotides present in the sense strand are deoxyribonucleotides; and

(c) any (N) nucleotides are optionally 2'-O-methyl, 2'-deoxy-2'-fluoro, or deoxyribonucleotides.

5. A double stranded nucleic acid molecule having structure SXIII comprising a sense strand and an antisense strand:

SXIII

$$B—N_{X3}—(N)_{X2}\ B\text{-}3'$$
$$B\ (N)_{X1}—N_{X4}—[N]_{X5}\text{-}5'$$

wherein the upper strand is the sense strand and the lower strand is the antisense strand of the double stranded nucleic acid molecule; said antisense strand comprises sequence complementary to a Sterol Regulatory Element-Binding Protein 1 (SREBP1) RNA; each N is independently a nucleotide; each B is a terminal cap moiety that can be present or absent; (N) represents non-base paired or overhanging nucleotides which can be unmodified or chemically modified; [N] represents nucleotide that are ribonucleotides; X1 and X2 are independently integers from about 0 to about 4; X3 is an integer from about 9 to about 30; X4 is an integer from about 11 to about 30, provided that the sum of X4 and X5 is about 17-36; X5 is an integer from about 1 to about 6; and

(a) any pyrimidine nucleotides present in the antisense strand are nucleotides having a ribo-like, Northern or A-form helix configuration; any purine nucleotides present in the antisense strand other than the purines nucleotides in the [N] nucleotide positions, are 2'-O-methyl nucleotides;

(b) any pyrimidine nucleotides present in the sense strand are nucleotides having a ribo-like, Northern or A-form helix configuration; any purine nucleotides present in the sense strand are 2'-O-methyl nucleotides; and

(c) any (N) nucleotides are optionally 2'-O-methyl, 2'-deoxy-2'-fluoro, or deoxyribonucleotides.

6. The double stranded nucleic acid molecule of claim **1**, wherein X5=1, 2, or 3; each X1 and X2=1 or 2; X3=12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, and X4=15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30.

7. The double stranded nucleic acid molecule of claim **2**, wherein X5=1, 2, or 3; each X1 and X2=1 or 2; X3=12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, and X4=15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30.

8. The double stranded nucleic acid molecule of claim **3**, wherein X5=1, 2, or 3; each X1 and X2=1 or 2; X3=12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, and X4=15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30.

9. The double stranded nucleic acid molecule of claim **4**, wherein X5=1, 2, or 3; each X1 and X2=1 or 2; X3=12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, and X4=15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30.

10. The double stranded nucleic acid molecule of claim **5**, wherein X5=1, 2, or 3; each X1 and X2=1 or 2; X3=12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, and X4=15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30.

11. The double stranded nucleic acid molecule of claim **1**, wherein B is present at the 3' and 5' ends of the sense strand and at the 3'-end of the antisense strand.

12. The double stranded nucleic acid molecule of claim **2**, wherein B is present at the 3' and 5' ends of the sense strand and at the 3'-end of the antisense strand.

13. The double stranded nucleic acid molecule of claim **3**, wherein B is present at the 3' and 5' ends of the sense strand and at the 3'-end of the antisense strand.

14. The double stranded nucleic acid molecule of claim **4**, wherein B is present at the 3' and 5' ends of the sense strand and at the 3'-end of the antisense strand.

15. The double stranded nucleic acid molecule of claim **5**, wherein B is present at the 3' and 5' ends of the sense strand and at the 3'-end of the antisense strand.

16. The double stranded nucleic acid molecule of claim **1**, comprising one or more phosphorothioate internucleotide linkages at the first terminal (N) on the 3' end of the sense strand, antisense strand, or both sense strand and antisense strands of the siNA molecule.

17. The double stranded nucleic acid molecule of claim **2**, comprising one or more phosphorothioate internucleotide linkages at the first terminal (N) on the 3' end of the sense strand, antisense strand, or both sense strand and antisense strands of the siNA molecule.

18. The double stranded nucleic acid molecule of claim **3**, comprising one or more phosphorothioate internucleotide linkages at the first terminal (N) on the 3' end of the sense strand, antisense strand, or both sense strand and antisense strands of the siNA molecule.

19. The double stranded nucleic acid molecule of claim **4**, comprising one or more phosphorothioate internucleotide linkages at the first terminal (N) on the 3' end of the sense strand, antisense strand, or both sense strand and antisense strands of the siNA molecule.

20. The double stranded nucleic acid molecule of claim **5**, comprising one or more phosphorothioate internucleotide linkages at the first terminal (N) on the 3' end of the sense strand, antisense strand, or both sense strand and antisense strands of the siNA molecule.

21. A composition comprising the double stranded nucleic acid molecule of claim **1** in a pharmaceutically acceptable carrier or diluent.

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