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(71) Applicant *(for all designated States except US)*: **MERCK SHARP & DOHME CORP.** [US/US]; 1700 Owens Street, 4th Floor, San Francisco, CA 94158 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **STRAPPS, Walter** [CA/US]; 4th Floor, 1700 Owens Street, San Francisco, CA 94158 (US). **PICKERING, Victoria** [US/US]; 4th Floor, 1700 Owens Street, San Francisco, CA 94158 (US). **SHAH, Jyoti** [IN/US]; 4th Floor, 1700 Owens Street, San Francisco, CA 94158 (US).

(74) Common Representative: **MERCK SHARP & DOHME CORP.**; 4th Floor, 1700 Owens Street, San Francisco, CA 94158 (US).

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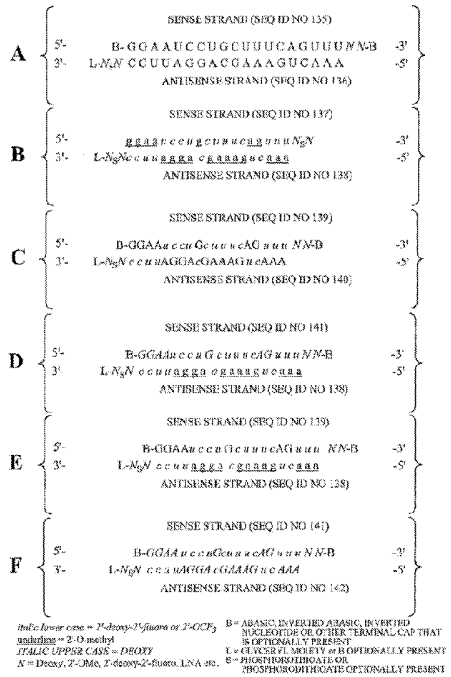
(54) Title: RNA INTERFERENCE MEDIATED INHIBITION OF BTB AND CNC HOMOLOGY 1, BASIC LEUCINE ZIPPER TRANSCRIPTION FACTOR 1 (BACH 1) GENE EXPRESSION USING SHORT INTERFERING NUCLEIC ACID (siNA) SEQUENCE LISTING

Figure 3



(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 Bach1 gene expression and/or activity, and/or modulate a Bach1 gene expression pathway. 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 that are capable of mediating or that mediate RNA interference (RNAi) against Bach1 gene expression.

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RNA INTERFERENCE MEDIATED INHIBITION OF BTB AND CNC HOMOLOGY 1, BASIC LEUCINE ZIPPER TRANSCRIPTION FACTOR 1 (Bach1) GENE EXPRESSION USING SHORT INTERFERING NUCLEIC ACID (siNA)

[0001] This application claims the benefit of U.S. Provisional Application No. 61/161,699, filed March 19, 2009. The above listed application is hereby incorporated by reference herein in its entirety, including the drawings.

SEQUENCE LISTING

[0002] The sequence listing submitted via EFS, in compliance with 37 CFR §1.52(e)(5), is incorporated herein by reference. The sequence listing text file submitted via EFS contains the file "SequenceListing75WPCT", created on February 25, 2010, which is 109,874 bytes in size.

BACKGROUND OF THE INVENTION

[0003] Bach1 is a transcriptional repressor of Heme Oxygenase-1 (HO-1) and other Nrf2-dependent phase II genes. The transcription factor Bach1 belongs to the cap'n'collar (CNC) and basic region leucine zipper factor superfamily of transcriptional regulators (known as CNC-bZip). In addition, Bach1 presents a so-called broad complex, tram-track, bric-a-brac (BTB) domain (also known as poxvirus and zinc finger (POZ) domain) in its N-terminal region. The BTB/POZ domain is involved in transcriptional repression by interacting with co-repressors; these BTB/POZ domains facilitate protein-protein interactions and formation of homo- and/or hetero-oligomers with other proteins. Bach1 and Nrf2 (another member of the CNC-bZip family, also known as NF-E2 related factor-2, nuclear factor (erythroid-derived 2)-like-2) form heterodimers with members of the Maf family of proteins; these heterodimers bind to Maf recognition elements (MARE, Maf-related Antioxidant Response Elements) in gene promoter regions and regulate gene transcription [Dhakshinamoorthy, S. et al (2005) J. Biol. Chem. 280, pp. 16891-16900; Ogawa,K. et al (2001), EMBO J. 20, pp. 2835-2843; Reichard,J.F. et al (2007) Nucleic Acids Res. 35, pp. 7074-7086; Reichard,J.F. et al (2008) J. Biol. Chem. 283, pp. 22363-22370]. While Nrf2 is a positive regulator of Phase II gene transcription (e.g., Heme Oxygenase-1 (HO-1), NAD(P)H quinone oxidoreductase-1 (NQO1), Glutamate Cysteine Ligase, Modifier subunit (GCLM), glutathione S-transferase, glutathione peroxidase, thioredoxin, and others), Bach1 acts as a repressor of many of these

genes. The ability of Bach1 to repress HO-1 seems dominant over transcriptional stimulators, including Nrf2; the balance between Bach1 and Nrf2 in the nucleus modulates ARE-dependent gene transcription. Overall, available data suggest that suppressing Bach1 repression is sufficient to induce HO-1 expression, even in the absence of stimulus.

[0004] Bach1 is expressed in many cells and tissues, including lung epithelial and endothelial cells, as well as macrophages. As a consequence of Bach1 widespread expression, the expression of HO-1 (the inducible form of HO) is generally low in basal conditions, either in cell culture or in normal, uninjured tissues *in vivo*; however, HO-1 expression is up-regulated by injury or stress in most tissues. A reduced level of expression of HO-1 (and other phase II genes) has been detected in lungs of COPD patients, suggesting that HO-1 is insufficiently up-regulated in *chronic* lung disease. More recently, several groups have reported differential levels of expression of Nrf2 and its regulators Keap1, DJ-1 and Bach1 in healthy vs. COPD subjects [Slebos,D.J et al, (2004) Eur. Respir. J. 23, pp. 652-653; Maestrelli,P et al, (2003) Eur. Respir. J. 21, pp. 971-976]. The expression of Nrf2-dependent phase II genes was significantly different in normal vs. disease lungs, with significantly lower levels of expression of HO-1, NQO1 and GLCM observed in severe emphysema lungs [Goven, D. et al, (2008) Thorax, 63, pp.916-924; Malhotra,D. et al (2008), Am. J. Respir. Crit Care Med., 178, pp. 592-604; Suzuki,M. et al (2008) Am. J. Respir. Cell Mol. Biol. 39, pp. 673-682.]. Overall, the human lung expression data support the hypothesis that altered equilibrium between modulators of Nrf2 activity, including positive (DJ-1) and negative (Keap1 and Bach1) factors, in COPD lungs results in loss of Nrf2-dependent anti-oxidants. As part of this abnormal response, Bach1 expression is elevated and HO-1 expression is concomitantly down-regulated in advanced COPD or severe emphysema patients.

[0005] The primary indication for this target is COPD; secondary indications for this target include severe asthma and cystic fibrosis, and other respiratory diseases such as respiratory disease such as, for example, but not limitation, eosinophilic cough, bronchitis, sarcoidosis, pulmonary fibrosis, rhinitis, and sinusitis given the potential role of oxidative stress in the pathogenesis of these diseases. The importance of anti-oxidant mechanisms is highlighted by the increased inflammation, airway hyperresponsiveness and Th2 cytokine production observed in asthma models using Nrf2-deficient mice. Furthermore, pharmacological up-regulation of HO-1 has shown beneficial effects in animal models of allergic disease and in cytoprotection of cystic fibrosis cells [Rangasamy,T. et al (2005) J. Exp. Med. 202, pp. 47-59; Williams,M.A et al, (2008) J. Immunol. 181, pp. 4545-4559;

Xia,Z.W. et al (2006) J. Immunol. 177, pp. 5936-5945; Xia,Z.W. et al (2007) Am. J. Pathol. 171, pp. 1904-1914; Zhou,H. et al (2004) Am. J. Respir. Crit Care Med. 170, pp. 633-640]. Various mechanisms, including inflammation, protease/antiprotease imbalance and oxidative stress-induced cell apoptosis (epithelial and endothelial cells), contribute to alveolar destruction and lung damage in COPD. Oxidative stress (defined as an imbalance between the generation of oxidant species and the cellular anti-oxidant capacity) is increased in COPD patients, particularly during exacerbations, and the contribution of ROS to COPD pathophysiology is well established [Demedts,I.K. et al (2006) Respir. Res. 7, pp. 53-57; Macnee,W (2007) Clin. Chest Med. 28, pp. 479-513; Barnes,P.J (2008) Proc. Am. Thorac. Soc. 5, pp. 857-864]. Activation of the Nrf2 pathway is suggested as a feasible approach to restoration of anti-oxidant defenses and reduction in oxidative stress burden, which would provide clinical benefit through reduction in inflammation and possibly augmentation of the effect of steroid therapy in these patients.

[0006] In the absence of oxidative stress, Bach1 binds to Maf recognition elements (MAREs) as a heterodimer with a member of the small Maf protein family (MafK or MafG), and suppresses the expression of HO-1 and other phase II genes. Bach1/MafK heterodimers bind with high affinity to clusters of MARE sequences present in the HO-1 promoter; these sequences, which contain 2-3 MARE motifs, are located within the enhancers E1 (268 bp, located approximately at –4 kb) and E2 (161 bp, located at -10 kb) of the HO-1 promoter. The transcriptional activator Nrf2 is normally (i.e. basal conditions) retained in the cytoplasm by the redox regulated protein Keap1; oxidative stress results in dissociation of the Nrf2-Keap1 complex and subsequent translocation of Nrf2 to the nucleus. Nrf2/Maf heterodimers then bind to MARE/ARE sequences and induce transcription of phase II genes. In conditions of oxidative stress, or if intracellular free heme concentration is elevated, binding of heme to Bach1 will cause the dissociation of Bach1/MafK from MARE sequences and induce nuclear export and degradation of Bach1 [Ozono,R. (2006) Curr. Pharm. Biotechnol. 7, pp. 87-93]. As a result, Nrf2/MafK binds to MAREs and HO-1 and other phase II gene transcription is turned on.

[0007] Small molecule activators of the Nrf2 pathway, in particular by disrupting the interaction of Nrf2/Keap1 and thus allowing translocation of Nrf2 into the nucleus to initiate gene transcription, has been shown to be protective against cigarette smoke-induced lung destruction. Specifically, the triterpenoid CDDO-Im (which strongly up-regulates HO-1 and other phase II Nrf2-dependent genes both *in vivo* and *in vitro*) has been shown to significantly reduce lung oxidative stress markers, alveolar cell apoptosis and the subsequent

lung destruction, and pulmonary hypertension [Sussan, T.E. et al (2009) Proc. Nat. Acad. Sci. 106, pp. 250 to 255].

[0008] Targeting Bach1 would restore the mechanisms of protection against excessive oxidative stress burden in the COPD lung, by increasing expression and activity of HO-1 (and to a lesser extent other phase II genes). Specifically, this should result in decreased apoptosis of structural cells (including alveolar epithelial and endothelial cells) and increased resolution of inflammation. Altogether, these activities would increase cytoprotection, preserve lung structure and favor repair in the COPD lung, thus slowing disease progression. Thus, there is a need for new therapeutics that target Bach1.

[0009] Alteration of gene expression, specifically Bach1 gene expression, through RNA interference (hereinafter "RNAi") is a one approach for meeting this need. RNAi is induced by short double-stranded RNA ("dsRNA") molecules. The short dsRNA molecules, called "short interfering RNA" or "siRNA" or "RNAi inhibitors" silence the expresssion of messenger RNAs ("mRNAs") that share sequence homology to the siRNA. This can occur via cleavage of the mRNA mediated by an endonuclease complex containing a siRNA, commonly referred to as an RNA-induced silencing complex (RISC). Cleavage of the target RNA typically 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).

SUMMARY OF THE INVENTION

[0010] The present invention provides compounds, compositions, and methods useful for modulating the expression of Bach1 genes, specifically those Bach1 genes associated with the development or maintenance of inflammatory and/or respiratory diseases and conditions by RNA interference (RNAi) using small nucleic acid molecules.

[0011] In particular, the instant invention features small nucleic acid molecules, i.e., short interfering nucleic acid (siNA) molecules including, but not limited to, short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA

(shRNA) and circular RNA molecules and methods used to modulate the expression of Bach1 genes and/or other genes involved in pathways of Bach1 gene expression and/or activity.

[0012] In one aspect, the present invention provides a double-stranded short interfering nucleic acid (siNA) molecule comprising a first strand and a second strand having complementary to each other, wherein at least one strand comprises at least 15 nucleotides of:

5'-GGAAUCCUGCUUUCAGUUU -3' (SEQ ID NO: 1);

5'-AAACUGAAAGCAGGAUUCC-3' (SEQ ID NO: 143);

5'-GUCUGAGUGUCCGUGGUUA-3' (SEQ ID NO: 10);

5'-UAACCACGGACACUCAGAC-3' (SEQ ID NO: 144);

5'-GCAGUUACUUCCACUCAAG -3' (SEQ ID NO: 11);

5'-CUUGAGUGGAAGUAACUGC-3' (SEQ ID NO: 145);

5'-CUACACUGCUAAACUGAUU-3' (SEQ ID NO: 15);

5'-AAUCAGUUUAGCAGUGUAG-3' (SEQ ID NO: 146);

5'-GAUUUGCAGGUGAUGUUAA-3' (SEQ ID NO: 18);

5'-UUAACAUCACCUGCAAAUC-3' (SEQ ID NO: 147);

5'-AUUUGAACCUUUAAUUCAG-3' (SEQ ID NO: 42);

5'- CUGAAUUAAAGGUUCAAAU-3' (SEQ ID NO: 148);

5'-GUUAAAGGAUUUGAACCUU-3' (SEQ ID NO: 38); or

5'-AAGGUUCAAAUCCUUUAAC -3' (SEQ ID NO: 150); and

wherein one or more of the nucleotides are optionally chemically modified.

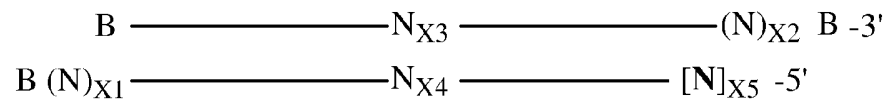
[0013] In some embodiments of the invention, all of the nucleotides are unmodified. In other embodiments, one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19. 20 or 21 modified nucleotides) of the nucleotide positions in one or both strands of an siNA molecule are modified. Modifications include nucleic acid sugar modifications, base modifications, backbone (internucleotide linkage) modifications, non-nucleotide modifications, and/or any combination thereof. In certain instances, purine and pyrimidine nucleotides are differentially modified. For example, purine and pyrimidine nucleotides can

be differentally modified at the 2'-sugar position (i.e., at least one purine has a different modification from at least one pyrimidine in the same or different strand at the 2'-sugar position). In other instances, at least one modified nucleotide is a 2'-deoxy-2'-fluoro nucleotide, a 2'-deoxy nucleotide, or a 2'-O-alkyl nucleotide

[0014] In certain embodiments, the siNA molecule has 3' overhangs of one, two, three, or four nucleotide(s) on one or both of the strands. In other embodiments, the siNA lacks overhangs (i.e., has blunt ends). Preferably, the siNA molecule has 3' overhangs of two nucleotides on both the sense and antisense strands. The overhangs can be modified or unmodified. Examples of modified nucleotides in the overhangs include, but are not limited to, 2'-O-alkyl nucleotides, 2'-deoxy-2'-fluoro nucleotides, or 2'-deoxy nucleotides. The overhang nucleotides in the antisense strand can comprise nucleotides that are complementary to nucleotides in the Bach1 target sequence. Likewise, the overhangs in the sense stand can comprise nucleotides that are in the Bach1 target sequence. In certain instances, the siNA molecules of the invention have two 3' overhang nucleotides on the antisense stand that are 2'-O-alkyl nucleotides and two 3' overhang nucleotides on the sense stand that are 2'-deoxy nucleotides.

[0015] In some embodiments, the siNA molecule has caps (also referred to herein as "terminal caps" The cap can be present at the 5'-terminus (5'-cap) or at the 3'- terminus (3'-cap) or can be present on both termini, such as at the 5' and 3' termini of the sense strand of the siNA.

[0016] In certain embodiments, double-stranded short interfering nucleic acid (siNA) molecules are provided, wherein the molecule has a sense strand and an antisense strand and comprises formula (A):



(A)

wherein, the upper strand is the sense strand and the lower strand is the antisense strand of the double-stranded nucleic acid molecule; wherein the antisense strand comprises at least 15 nucleotides of SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, or SEQ ID NO: 150, and the sense strand comprises a sequence having complementarity to the antisense strand;

each N is independently a nucleotide which is unmodified or chemically modified;

each B is a terminal cap that is present or absent;

(N) represents overhanging nucleotides, each of which is independently unmodified chemically modified;

[N] represents nucleotides that are ribonucleotides;

X1 and X2 are independently integers from 0 to 4;

X3 is an integer from 17 to 36;

X4 is an integer from 11 to 35; and

X5 is an integer from 1 to 6, provided that the sum of X4 and X5 is 17-36;

[0017] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) of formula (A); wherein

(a) one or more pyrimidine nucleotides in N_{X4} positions are independently 2'-deoxy-2'-fluoro nucleotides, 2'-O-alkyl nucleotides, 2'-deoxy nucleotides, ribonucleotides, or any combination thereof;

(b) one or more purine nucleotides in N_{X4} positions are independently 2'-deoxy-2'-fluoro nucleotides, 2'-O-alkyl nucleotides, 2'-deoxy nucleotides, ribonucleotides, or any combination thereof;

(c) one or more pyrimidine nucleotides in N_{X3} positions are independently 2'-deoxy-2'-fluoro nucleotides, 2'-O-alkyl nucleotides, 2'-deoxy nucleotides, ribonucleotides, or any combination thereof; and

(d) one or more purine nucleotides in N_{X3} positions are independently 2'-deoxy-2'-fluoro nucleotides, 2'-O-alkyl nucleotides , 2'-deoxy nucleotides, ribonucleotides, or any combination thereof.

[0018] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) of formula (A); wherein

(a) each pyrimidine nucleotide in N_{X4} positions is independently a 2'-deoxy-2'-fluoro nucleotide, 2'-O-alkyl nucleotide, 2'-deoxy nucleotide, or ribonucleotide;

(b) each purine nucleotide in N_{X4} positions is independently a 2'-deoxy-2'-fluoro nucleotide, 2'-O-alkyl nucleotide, 2'-deoxy nucleotide, or ribonucleotide;

(c) each pyrimidine nucleotide in N_{X3} positions is independently a 2'-deoxy-2'-fluoro nucleotide, 2'-O-alkyl nucleotide, 2'-deoxy nucleotide, or ribonucleotide; and

(d) each purine nucleotides in N_{X3} positions is independently a 2'-deoxy-2'-fluoro nucleotide, 2'-O-alkyl nucleotide, 2'-deoxy nucleotide, or ribonucleotide.

[0019] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) of formula (A); wherein

(a) each pyrimidine nucleotide in N_{X4} positions is independently a 2'-deoxy-2'-fluoro nucleotide;

(b) each purine nucleotide in N_{X4} positions is independently a 2'-O-alkyl nucleotide;

(c) each pyrimidine nucleotide in N_{X3} positions is independently a 2'-deoxy-2'-fluoro nucleotide; and

(d) each purine nucleotide in N_{X3} positions is independently a 2'-deoxy nucleotide.

[0020] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) of formula (A); wherein

(a) each pyrimidine nucleotide in N_{X4} positions is independently a 2'-deoxy-2'-fluoro nucleotide;

(b) each purine nucleotide in N_{X4} positions is independently a 2'-O-alkyl nucleotide;

(c) each pyrimidine nucleotide in N_{X3} positions is independently a 2'-deoxy-2'-fluoro nucleotide; and

(d) each purine nucleotide in N_{X3} positions is independently a ribonucleotide.

[0021] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) of formula (A); wherein

(a) each pyrimidine nucleotide in N_{X4} positions is independently a 2'-deoxy-2'-fluoro nucleotide;

(b) each purine nucleotide in N_{X4} positions is independently a ribonucleotide;

(c) each pyrimidine nucleotide in N_{X3} positions is independently a 2'-deoxy-2'-fluoro nucleotide; and

(d) each purine nucleotide in N_{X3} positions is independently a ribonucleotide.

[0022] In yet another embodiment, the invention provides a double stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

```
5'-  BGGAAuccuGcuuucAGuuuTTB  -3'      (Sense)     (SEQ ID NO: 43)
       ||||||||||||||||||||
3'- UUccuuAGGAcGAAAGucAAA     -5'      (Antisense) (SEQ ID NO: 44)
```

wherein:

each B is an inverted abasic cap moiety as shown in Fig. 10;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'-deoxyguanosine;

T is thymidine;

A is adenosine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

[0023] In yet another embodiment, the invention provides a double stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

```
5'- BGucuGAGuGuccGuGGuuATTB  -3'      (Sense)     (SEQ ID NO:61)
       ||||||||||||||||||||
3' UUcAGAcucAcAGGcAccAAU     -5'      (Antisense) (SEQ ID NO:62)
```

wherein:

each B is an inverted abasic cap as shown in Fig. 10;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

A is adenosine;

U is uridine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

[0024] In yet another embodiment, the invention provides a double stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

```
5'  BGcAGuuAcuuccAcucAAGTTB 3'(Sense) (SEQ ID NO:63)
      ||||||||||||||||||||
3' UUcGucAAuGAAGGuGAGUUC    5'(Antisense) (SEQ ID NO:64)
```

wherein:

each B is an inverted abasic cap moiety as shown in Fig. 10;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

C is cytidine;

U is uridine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

[0025] In yet another embodiment, the invention provides a double stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

```
5'- BcuAcAcuGcuAAAcuGAuuTTB -3'     (Sense)     (SEQ ID NO:71)
      ||||||||||||||||||||
3' UUGAuGuGAcGAuuuGAcUAA    -5'     (Antisense) (SEQ ID NO:72)
```

wherein:

each B is an inverted abasic cap moiety as shown in Fig. 10;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

A is adenosine;

U is uridine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

[0026] In yet another embodiment, the invention provides a double stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

```
5'- BGAuuuGcAGGuGAuGuuAATTB -3'     (Sense)     (SEQ ID NO:77)
      ||||||||||||||||||||
3' UUcuAAAcGuccAcuAcAAUU    -5'     (Antisense) (SEQ ID NO:78)
```

wherein:

each B is an inverted abasic cap moiety as shown in Fig. 10;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

A is adenosine;

U is uridine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

[0027] In yet another embodiment, the invention provides a double stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

```
5'- BAuuuGAAccuuuAAuucAGTTB -3'     (Sense)     (SEQ ID NO:125)
      ||||||||||||||||||||
```

3′ UUuAAAcuuGGAAAuuAAGUC −5′ (Antisense) (SEQ ID NO:126)

wherein:

each B is an inverted abasic cap moiety as shown in Fig. 10;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

G is guanosine;

U is uridine;

C is cytidine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

[0028] In still another embodiment, the invention provides a double stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

5′- BGuu*AAAGGA*uuu*GAA*ccuuTTB −3′ (Sense) (SEQ ID NO:117)
3′ UUcAAuuuccuAAAcuuGGAA −5′ (Antisense) (SEQ ID NO:118)

wherein:

each B is an inverted abasic cap moiety as shown in Figure 10;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

G is guanosine;

A is adenosine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

[0029] The present invention further provides pharmaceutical compositions comprising the double-stranded nucleic acids molecules described herein and optionally a pharmaceutically acceptable carrier.

[0030] The administration of the pharmaceutical composition may be carried out by known methods, wherein the nucleic acid is introduced into a desired target cell in vitro or in vivo.

[0031] Commonly used techniques for introduction of the nucleic acid molecules of the invention into cells, tissues, and organisms include the use of various carrier systems, reagents and vectors. Non-limiting examples of such carrier systems suitable for use in the present invention include nucleic-acid-lipid particles, lipid nanoparticles (LNP), liposomes, lipoplexes, micelles, virosomes, virus like particles (VLP), nucleic acid complexes, and mixtures thereof.

[0032] The pharmaceutical compositions may be in the form of an aerosol, dispersion, solution (e.g., an injectable solution), a cream, ointment, tablet, powder, suspension or the like. These compositions may be administered in any suitable way, e.g. orally, sublingually, buccally, parenterally, nasally, or topically. In some embodiments, the compositions are aerosolized and delivered via inhalation.

[0033] The molecules and pharmaceutical compositions of the present invention have utility over a broad range of therapeutic applications, accordingly another aspect of this invention relates to the use of the compounds and pharmaceutical compositions of the invention in treating a subject. The invention thus provides a method for treating a subject, such as a human, suffering from a condition which is mediated by the action, or by the loss of action, of Bach1, wherein the method comprises administering to the subject an effective amount of a double-stranded short interfering nucleic acid (siNA) molecule of the invention. In certain embodiments, the condition is a respiratory disease such as, for example, but not limitation, COPD, cystic fibrosis, asthma, eosinophilic cough, bronchitis, sarcoidosis, pulmonary fibrosis, rhinitis, and sinusitis.

[0034] These and other aspects of the invention will be apparent upon reference to the following detailed description and attached figures. To that end, patents, patent applications, and other documents are cited throughout the specification to describe and more specifically set forth various aspects of this invention. Each of these references cited herein is hereby incorporated by reference in its entirety, including the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] **Figure 1** 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.

[0036] **Figure 2A-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 **Figure 4C**). Furthermore, although not depicted on the Figure, the sequences shown in **Figure 2** can optionally include a ribonucleotide at the 9^{th} position from the 5'-end of the sense strand or the 11^{th} 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 **Figure 4C**). The antisense strand of constructs A-F comprises 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 **Figure 2 A-F**, the modified internucleotide linkage is optional.

[0037] **Figure 2A:** 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, phosphonoacetate, thiophosphonoacetate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

[0038] **Figure 2B:** The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that can be present are 2'deoxy-2'-fluoro modified nucleotides and all purine nucleotides that can 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 can be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that can 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.

[0039] **Figure 2C:** The sense strand comprises 21 nucleotides having 5'- and 3'- terminal caps wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that can 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 can 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.

[0040] **Figure 2D:** The sense strand comprises 21 nucleotides having 5'- and 3'- terminal caps wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that can 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 can 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 can be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that can 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.

[0041] **Figure 2E:** The sense strand comprises 21 nucleotides having 5'- and 3'- terminal caps wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that can 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 can be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that can 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.

[0042] **Figure 2F:** The sense strand comprises 21 nucleotides having 5'- and 3'- terminal caps wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that can 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 can 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 can be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that can 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.

[0043] **Figure 3A-F** shows non-limiting examples of specific chemically modified siNA sequences of the invention. **A-F** applies the chemical modifications described in **Figure 2A-F** to an exemplary Bach1 siNA sequence. Such chemical modifications can be applied to any Bach1 sequence. Furthermore, although this is not depicted on **Figure 3**, the sequences shown in **Figure 3** 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 **Figure 4C**). In addition, the sequences shown in **Figure 3** can optionally include terminal ribonucleotides at up to about 6 positions at the 5'-end of the antisense strand (e.g., about 1, 2, 3, 4, 5, or 6 terminal ribonucleotides at the 5'-end of the antisense strand).

[0044] **Figure 4A-C** shows non-limiting examples of different siNA constructs of the invention.

[0045] The examples shown in **Figure 4A** (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*.

[0046] The examples shown in **Figure 4B** 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.

[0047] The example shown in **Figure 4C** 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 a 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.

[0048] **Figure 5** shows non-limiting examples of different stabilization chemistries (1-10) that can be used, for example, to stabilize the 5' and/or 3'-ends 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 sugar and base nucleotide modifications as described herein.

[0049] **Figure 6** 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 is 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.

[0050] **Figure 7** shows non-limiting examples of phosphorylated siNA molecules of the invention, including linear and duplex constructs and asymmetric derivatives thereof.

[0051] **Figure 8** shows non-limiting examples of chemically modified terminal phosphate groups of the invention.

[0052] **Figure 9** 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 an siNA molecule.

[0053] **Figure 10** depicts an embodiment of 5' and 3' inverted abasic cap linked to a nucleic acid strand.

[0054] **Figure 11** is TaqMan data from transfected TLR8-U2OS cells showing effect of siNAs on IL8 mRNA levels.

[0055] **Figure 12** is TaqMan data from transfected TLR7-U2OS cells showing effect of siNAs on IL8 mRNA levels.

[0056] **Figure 13** is data evidencing siNA increased HO-1 protein expression in a concentration-dependent manner in lung epithelial cells upon treatment with siNAs.

[0057] **Figure 14** shows that there is no significant increases in inflammatory cell influx or cytokine production following intra-tracheal administration of each of the target siNAs.

DETAILED DESCRIPTION OF THE INVENTION

A. Terms and Definitions

[0058] The following terminology and definitions apply as used in the present application.

[0059] The term "abasic" refers to 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.

[0060] The term "acyclic nucleotide" as used herein refers to any nucleotide having an acyclic ribose sugar, for example where any of the ribose carbon/carbon or carbon/oxygen bonds are independently or in combination absent from the nucleotide.

[0061] The term "alkyl" refers to a saturated or unsaturated hydrocarbons, including straight-chain, branched-chain, alkenyl, alkynyl groups and cyclic groups, but excludes aromatic groups. Notwithstanding the foregoing, alkyl also refers to non-aromatic heterocyclic 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, C1-C4alkoxy, =O, =S, NO_2 , $SH_{,,}$, NH_2, or NR_1R_2, where R_1 and R_2 independently are H or C1-C4 alkyl

[0062] The term "aryl" 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 can be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SII, OII, cyano, C1-C4alkoxy, C1-C4alkyl, C2-C4alkenyl, C2-C4alkynyl, NH_2, and NR_1R_2 groups, where R_1 and R_2 independently are H or C1-C4 alkyl. .

[0063] The term "alkylaryl" 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 examples of heterocyclic aryl groups having such heteroatoms include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. Preferably, the alkyl group is a C1-C4alkyl group.

[0064] The term "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen.

[0065] The phrase "antisense region" refers to a nucleotide sequence of an siNA molecule having complementarity to a target nucleic acid sequence. In addition, the antisense region of an 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.

[0066] The phrase "asymmetric hairpin" refers to 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.

[0067] The term "Bach1" refers to the BTB and CNC Homology 1, Basci Leucine Zipper Transcription Factor 1 gene, or to the genes that encode Bach1 proteins, Bach1 peptides, Bach1 polypeptides, Bach1 regulatory polynucleotides (*e.g.*, Bach1 miRNAs and siRNAs), mutant Bach1 genes, and splice variants of Bach1 genes, as well as other genes involved in Bach1 pathways of gene expression and/or activity. Thus, each of the embodiments described herein with reference to the term "Bach1" are applicable to all of the protein, peptide, polypeptide, and/or polynucleotide molecules covered by the term "Bach1", as that term is defined herein. Comprehensively, such gene targets are also referred to herein generally as "target" sequences (including **Table 10**).

[0068] The term "biodegradable" refers to degradation in a biological system, for example, enzymatic degradation or chemical degradation.

[0069] The term "biodegradable linker" refers to a nucleic acid or non-nucleic acid linker molecule that is designed to connect one molecule to another molecule, for example, a biologically active molecule to an siNA molecule of the invention or the sense and antisense strands of an siNA molecule of the invention, and is biodegradable.. 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.

[0070] The phrase "biologically active molecule" refers to compounds or molecules that are capable of eliciting or modifying a biological response in a system and/or are capable of modulating the pharmacokinetics and/or pharmacodynamics of other biologically active molecules, Non-limiting examples of biologically active molecules, include siNA molecules alone or in combination with other molecules including, but not limited to 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, polyamines, polyamides, polyethylene glycol, other polyethers, .2-5A chimeras, siNA, dsRNA, allozymes, aptamers, decoys and analogs thereof.

[0071] The phrase "biological system" refers to 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. Thus, the phrase includes, for example, a cell, tissue, subject, or organism, or extract thereof. The term also includes reconstituted material from a biological source.

[0072] The phrase "blunt end" refers to a 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.

[0073] The term "cap" also refered to herein as "terminal cap," refers to chemical modifications, which can be incorporated at either 5' or 3' terminus of the oligonucleotide of either the sense or the antisense strand (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 can help in delivery and/or localization within a cell. The cap can be present at the 5'-terminus (5'-cap) or at the 3'-terminal (3'-cap) or can 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 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). **Figure 5** shows some non-limiting examples of various caps.

[0074] The term "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 being. 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.

[0075] The phrase "chemical modification" refer to any modification of the 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 at the sugar, base, or internucleotide linkage, as described herein or as is otherwise known in the art. See for example, USSN 12/064,015 for non-limiting examples of

chemical modifications that are compatible with the nucleic acid molecules of the present invention.

[0076] The term "complementarity" refers to the formation of hydrogen bond(s) between one nucleic acid sequence and another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types of bonding as described herein. 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). Perfect 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. Partial complementarity can include various mismatches or non-based paired nucleotides (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more mismatches or non-based paired nucleotides) within the nucleic acid molecule, 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 nucleic acid molecule or between the antisense strand or antisense region of the nucleic acid molecule and a corresponding target nucleic acid molecule.

[0077] The term "gene" or phrase "target gene" refer to a nucleic acid (e.g., DNA or RNA) sequence that comprises partial length or entire length coding sequences necessary for the production of 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. Aberrant 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.

[0078] The phrase "homologous sequence" refers to 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 sequence regions shared by more than one polynucleotide sequence. Homology does not need to be perfect identity (100%), as partially homologous sequences are also contemplated by and within the scope of the instant invention (*e.g.*, at least 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80% etc.). Percent homology is the number of matching nucleotides between two sequences divided by the total length being compared multiplied by 100.

[0079] The phrase "improved RNAi activity" refer to an increase in 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 an 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.*

[0080] The terms "inhibit", "down-regulate", or "reduce", refer to the reduction in 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, below that observed in the absence of the nucleic acid molecules (*e.g.*, siNA) of the invention. Down-regulation can also be associated with post-transcriptional silencing, such as, RNAi mediated cleavage or by alteration in DNA methylation patterns or DNA chromatin structure. Inhibition, down-regulation or reduction with an siNA molecule can be in reference to an inactive molecule, an attenuated molecule, an siNA molecule with a scrambled sequence, or an siNA molecule with mismatches or alternatively, it can be in reference to the system in the absence of the nucleic acid.

[0081] The terms "mammalian" or "mammal" refer to any warm blooded vertebrate species, such as a human, mouse, rat, dog, cat, hamster, guinea pig, rabbit, livestock, and the like.

[0082] The phrase "metered dose inhaler" or MDI refers to a unit comprising a can, a secured cap covering the can and a formulation metering valve situated in the cap. MDI systems includes a suitable channeling device. Suitable channeling devices comprise for example, a valve actuator and a cylindrical or cone-like passage through which medicament can be delivered from the filled canister via the metering valve to the nose or mouth of a patient such as a mouthpiece actuator.

[0083] The term "microRNA" or "miRNA" refers to 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).

[0084] The term "modulate" means 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.

[0085] The phrase "modified nucleotide" refers to a nucleotide, which contains a modification in the chemical structure of the base, sugar and/or phosphate of the unmodified

(or natural) nucleotide. Non-limiting examples of modified nucleotides are described herein and in USSN 12/064,015.

[0086] The phrase "non-base paired" refers to nucleotides that are not base paired between the sense strand or sense region and the antisense strand or antisense region of an double-stranded siNA molecule.; and can include for example, but not limitation, mismatches, overhangs, single stranded loops, etc.

[0087] The term "non-nucleotide" refers to any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, such as abasic moieties. 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 nucleobase at the 1'-position.

[0088] The term "nucleotide" is used as is recognized in the art. Nucleotides generally comprise a base, a sugar, and a phosphate moiety.. The base can be a. natural bases (standard) or modified bases as are well known in the art. Such bases are generally located at the 1' position of a nucleotide sugar moiety. Additionally, 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, USSN 12/064,015.

[0089] The term "overhang" refers to the 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, **Figure 4**).

[0090] The term "parenteral" refers administered in a manner other than through the digestive tract, and includes epicutaneous, subcutaneous, intravascular (e.g., intravenous), intramuscular, or intrathecal injection or infusion techniques and the like.

[0091] The phrase "pathway target" refers to any target involved in pathways of gene expression or activity. For example, any given target can have related pathway targets that can include upstream, downstream, or modifier genes in a biologic pathway. These pathway target genes can provide additive or synergistic effects in the treatment of diseases, conditions, and traits herein.

[0092] A "pharmaceutical composition" or "pharmaceutical 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, inhalation, 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, pharmaceutical 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. As used herein, pharmaceutical formulations include formulations for human and veterinary use. 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, DF *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, 4910-4916; and Tyler *et al.*, 1999, *PNAS USA.*, 96, 7053-7058. A "pharmaceutically acceptable composition" or "pharmaceutically acceptable formulation" refer to 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.

[0093] The term "phosphorothioate" refers to an internucleotide phosphate linkage comprising one or more sulfur atoms in place of an oxygen atom. Hence, the term phosphorothioate refers to both phosphorothioate and phosphorodithioate internucleotide linkages.

[0094] The term "ribonucleotide" refers to a nucleotide with a hydroxyl group at the 2' position of a β-D-ribofuranose moiety.

[0095] The term "RNA" refers to a molecule comprising at least one ribofuranoside moiety. The term includes 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.

[0096] The phrase "RNA interference" or term "RNAi" refer to the 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). Additionally, 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 either 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 via translational inhibition, as is known in the art or modulation can result from transcriptional inhibition (see for example Janowski *et al.*, 2005, *Nature Chemical Biology*, 1, 216-222).

[0097] The phrase "RNAi inhibitor" refers to 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 an siNA molecule, an antisense molecule, an aptamer, or a small molecule that interacts with or interferes with the function of RISC, a miRNA, or an 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.

[0098] The phrase "sense region" refers to nucleotide sequence of an siNA molecule having complementarity to an antisense region of the siNA molecule. In addition, the sense region of an siNA molecule can comprise a nucleic acid sequence having homology with a target nucleic acid sequence. The sense region of the siNA molecule can also refer to as the sense strand or passenger strand.

[0099] The phrases "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" refer 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 strands, wherein the antisense strand comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises a nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. 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 a nucleotide sequence that is complementary to a nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region comprises a 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 a nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region comprises a 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 a 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 a 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.

[0100] The term "subject" 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. The term also refers to an organism, which is a donor or recipient of explanted cells or the cells themselves.

[0101] The phrase "systemic administration" refers to in vivo systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body.

[0102] The term "target" as it refers to Bach1 refers to any Bach1 target protein, peptide, or polypeptide, such as encoded by Genbank Accession Nos. shown in **Table 10**. The term also refers to nucleic acid sequences or target polynucleotide sequence encoding any target protein, peptide, or polypeptide, such as proteins, peptides, or polypeptides encoded by sequences having Genbank Accession Nos. shown in **Table 10**. 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, sRNA) or other regulatory polynucleotide sequences as described herein.

[0103] The phrase "target site" refers to a sequence within a target RNA that is "targeted" for cleavage mediated by an siNA construct, which contains sequences within its antisense region that are complementary to the target sequence.

[0104] The phrase "therapeutically effective amount" refers to the amount of the compound or pharmaceutical composition that will elicit the biological or medical response of a cell, tissue, system, animal or human that is be sought by the researcher, veterinarian, medical doctor or other clinician.

[0105] The phrase "universal base" 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).

[0106] The phrase "unmodified nucleoside" refers to one of the bases, adenine, cytosine, guanine, thymine, or uracil, joined to the 1' carbon of β-D-ribo-furanose.

[0107] The term "up-regulate" refers to an increase in the expression of a 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, above that observed in the absence of the nucleic acid molecules (*e.g.*, siNA) of the invention. In certain instances, 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 other instances, 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 still other instances, 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 some instances, 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.

[0108] The term "vectors" refers to any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

B. siNAs Molecules of the Invention

[0109] The present invention provides compositions and methods comprising siNAs targeted to Bach1 that can be used to treat diseases, e.g., respiratory or inflammatory, associated with Bach1. In particular aspects and embodiments of the invention, the nucleic acid molecules of the invention comprise sequences shown in **Table 1a-1b** and/or **Figures 2-3**. The siNAs can be provided in several forms. For example, the siNA can be isolated as one or more siNA compounds, or it may be in the form of a transcriptional cassette in a DNA plasmid. The siNA may also be chemically synthesized and can include modifications.. The siNAs can be administered alone or co-administered with other siNA molecules or with conventional agents that treat a Bach1 related disease or condition.

[0110] The siNA molecules of the invention can be used to mediate gene silencing, specifically Bach1, 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 such as, for example, but not limited to, RNAi or through cellular processes that modulate the chromatin structure or methylation patterns of the target and prevent transcription of the target gene, with the nucleotide sequence of the target thereby mediating silencing. More specifically, the target is any of Bach1 RNA, DNA, mRNA, miRNA, siRNA, or a portion thereof.

[0111] In one aspect, the present invention provides a double-stranded short interfering nucleic acid (siNA) molecule comprising a first strand and a second strand having complementarity to each other, wherein at least one strand comprises at least 15 nucleotides of:

5'-GGAAUCCUGCUUUCAGUUU -3' (SEQ ID NO: 1);

5'-AAACUGAAAGCAGGAUUCC-3' (SEQ ID NO: 143);

5'-GUCUGAGUGUCCGUGGUUA-3' (SEQ ID NO: 10);

5'-UAACCACGGACACUCAGAC-3' (SEQ ID NO: 144);

5'-GCAGUUACUUCCACUCAAG -3' (SEQ ID NO: 11);

5'-CUUGAGUGGAAGUAACUGC-3' (SEQ ID NO: 145);

5'-CUACACUGCUAAACUGAUU-3' (SEQ ID NO: 15);

5'-AAUCAGUUUAGCAGUGUAG-3' (SEQ ID NO: 146);

5'-GAUUUGCAGGUGAUGUUAA-3' (SEQ ID NO: 18);

5'-UUAACAUCACCUGCAAAUC-3' (SEQ ID NO: 147);

5'-AUUUGAACCUUUAAUUCAG-3' (SEQ ID NO: 42);

5'- CUGAAUUAAAGGUUCAAAU-3' (SEQ ID NO: 148);

5'-GUUAAAGGAUUUGAACCUU-3' (SEQ ID NO: 38); or

5'-AAGGUUCAAAUCCUUUAAC -3' (SEQ ID NO: 150); and

wherein one or more of the nucleotides are optionally chemically modified.

[0112] In certain embodiments the 15 nucleotides form a contiguous stretch of nucleotides.

[0113] In other embodiments, the siNA molecule can contain one or more nucleotide deletions, substitutions, mismatches and/or additions to SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 10, SEQ ID NO: 144, SEQ ID NO: 11, SEQ ID NO: 145, SEQ ID NO: 15, SEQ ID NO: 146, SEQ ID NO: 18, SEQ ID NO: 147, SEQ ID NO: 42, SEQ ID NO: 148; SEQ ID NO: 38, or SEQ ID NO: 150; provided, however, that the siNA molecule maintains its activity, for example, to mediate RNAi. In a non-limiting example, the deletion, substitution,

mismatch and/or addition can result in a loop or buldge, or alternately a wobble or other alternative (non Watson-Crick) base pair.

[0114] These siNA molecules can comprise short double-stranded regions of RNA. The double stranded RNA molecules of the invention can comprise two distinct and separate strands that can be symmetric or asymmetric and are complementary, i.e., two single-stranded RNA molecules, or can comprise one single-stranded molecule in which two complementary portions, e.g., a sense region and an antisense region, are base-paired, and are covalently linked by one or more single-stranded "hairpin" areas (i.e. loops) resulting in, for example, a single-stranded short-hairpin polynucleotide or a circular single-stranded polynucleotide.

[0115] The linker can be polynucleotide linker or a non-nucleotide linker. In some embodiments, the linker is a non-nucleotide linker. In some embodiments, a hairpin or circular siNA molecule of the invention contains one or more loop motifs, wherein at least one of the loop portion of the siNA molecule is biodegradable. For example, a single-stranded 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 1, 2, 3 or 4 nucleotides. Or alternatively, 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.

[0116] In symmetric siNA molecules of the invention, each strand, the sense (passenger) strand and antisense (guide) strand, are independently about 15 to about 40 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30,31,32, 33, 34, 35, 36, 37, 38, 39, or 40) nucleotides in length

[0117] In asymmetric siNA molecules, the antisense region or strand of the molecule 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.

[0118] In yet other embodiments, siNA molecules of the invention comprise single stranded hairpin siNA molecules, wherein the siNA molecules are about 25 to about 70 (*e.g.*,

about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length.

[0119] In still other embodiments, siNA molecules of the invention comprise single-stranded circular siNA molecules, wherein the siNA molecules are about 38 to about 70 (*e.g.*, about 38, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length.

[0120] In various symmetric embodiments, the siNA duplexes of the invention independently comprise about 15 to about 40 base pairs (*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).

[0121] In yet other embodiments, where the siNA molecules of the invention are asymmetric, the siNA molecules comprise about 3 to 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).

[0122] In still other embodiments, where the siNA molecules of the invention are hairpin or circular structures, the siNA molecules comprise about 3 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.

[0123] The sense strand and antisense strands or sense region and antisense regions of the siNA molecules of the invention can be complementary. Also, the antisense strand or antisense region can be complementary to a nucleotide sequence or a portion thereof of the Bach1 target RNA. The sense strand or sense region if the siNA can comprise a nucleotide sequence of a Bach1 gene or a portion thereof. In certain embodiments, the sense region or sense strand of an 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 Bach1 target polynucleotide sequence, such as for example, but not limited to, those sequences represented by GENBANK Accession Nos. shown in **Table 10.**

[0124] In some embodiments, siNA molecules of the invention have perfect complementarity between the sense strand or sense region and the antisense strand or antisense region of the siNA molecule. In other or the same embodiments, siNA molecules of the invention are perfectly complementary to a corresponding target nucleic acid molecule.

[0125] In yet other embodiments, siNA molecules of the invention have 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. Thus, in some embodiments, the double-stranded nucleic acid molecules of the invention, have between 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, 32, 33, 34, 35, 36, 37, 38, 39, or 40) nucleotides in one strand that are complementary to the nucleotides of the other strand. In other embodiments, the molecules have between 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, 32, 33, 34, 35, 36, 37, 38, 39, or 40) nucleotides in the sense region that are complementary to the nucleotides of the antisense region. of the double-stranded nucleic acid molecule. In yet other embodiments, the double-stranded nucleic acid molecules of the invention have between 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, 32, 33, 34, 35, 36, 37, 38, 39, or 40) nucleotides in the antisense strand that are complementary to a nucleotide sequence of its corresponding target nucleic acid molecule.

[0126] In some embodiments, the double-stranded nucleic acid molecules of the invention, have 1 or more (*e.g.*, 1, 2, 3, 4, 5, or 6) nucleotides, in one strand or region that are mismatches or non-base-paired with the other strand or region.. In other embodiments, the double-stranded nucleic acid molecules of the invention, have 1 or more (*e.g.*, 1, 2, 3, 4, 5, or 6) nucleotides in each strand or region that are mismatches or non-base-paired with the other strand or region.

[0127] The invention also comprises double-stranded nucleic acid (siNA) molecules as otherwise described hereinabove in which the first strand and second strand are complementary to each other and wherein at least one strand is hybridisable to the polynucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 10, SEQ ID NO: 144, SEQ ID NO: 11, SEQ ID NO: 145, SEQ ID NO: 15, SEQ ID NO: 146, SEQ ID NO: 18, SEQ ID NO: 147, SEQ ID NO: 42, SEQ ID NO: 148, SEQ ID NO: 38, or SEQ ID NO: 150 under conditions of high stringency, and wherein any of the nucleotides is unmodified or chemically modified.

[0128] Hybridization techniques are well known to the skilled artisan (see for instance, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). Preferred stringent hybridization conditions include overnight incubation at 42°C in a solution comprising: 50% formamide,

5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA; followed by washing the filters in 0.1x SSC at about 65°C.

[0129] In one specific embodiment, the first strand has about 15, 16, 17, 18, 19, 20 or 21 nucleotides that are complementary to the nucleotides of the other strand and at least one strand is hybridisable to the polynucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 10, SEQ ID NO: 144, SEQ ID NO: 11, SEQ ID NO: 145, SEQ ID NO: 15, SEQ ID NO: 146, SEQ ID NO: 18, SEQ ID NO: 147, SEQ ID NO: 42, SEQ ID NO: 148, SEQ ID NO: 38, or SEQ ID NO: 150 under conditions of high stringency, and wherein any of the nucleotides is unmodified or chemically modified.

[0130] In certain embodiments, the siNA molecules of the invention comprise overhangs of about 1 to about 4 (*e.g.*, about 1, 2, 3 or 4) nucleotides. The nucleotides in the overhangs can be the same or different nucleotides. In some embodiments, the overhangs occur at the 3'-end at 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.

[0131] In some embodiments, the nucleotides comprising the overhang portion of an siNA molecule of the invention comprise sequences based on the Bach1 target polynucleotide sequence in which nucleotides comprising the overhang portion of the guide strand or antisense strand/region of an siNA molecule of the invention can be complementary to nucleotides in the Bach1 target polynucleotide sequence and/or nucleotides comprising the overhang portion of the passenger strand or sense strand/region of an siNA molecule of the invention can comprise the nucleotides in the Bach1 target polynucleotide sequence. Thus, in some embodiments, the overhang comprises a two nucleotide overhang that is complementary to a portion of the Bach1 target polynucleotide sequence. In other embodiments, however, the overhang comprises a two nucleotide overhang that is not complementary to a portion of the Bach1 target polynucleotide sequence. In certain embodiments, the overhang comprises a 3'-UU overhang that is not complementary to a portion of the Bach1 target polynucleotide sequence. In other embodiments, the overhang

comprises a UU overhang at the 3' end of the antisense strand and a TT overhang at the 3' end of the sense strand.

[0132] In any of the embodiments of the siNA molecules described herein having 3'-terminal nucleotide overhangs, the overhangs are optionally chemically modified at one or more nucleic acid sugar, base, or backbone positions. Representative, but not limiting examples of modified nucleotides in the overhang portion of a double-stranded nucleic acid (siNA) molecule of the invention include 2'-O-alkyl (e.g., 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 more preferred embodiments, the overhang nucleotides are each independently, a 2'-O-alkyl nucleotide, 2'-O-methyl nucleotide, 2'-dexoy-2-fluoro nucleotide, or 2'-dexoyribonucleotide

[0133] In yet other embodiments, siNA molecules of the invention comprise duplex nucleic acid molecules with blunt ends (i.e., does not have any nucleotide overhangs), where both ends are blunt, or alternatively, where one of the ends is blunt.. In some embodiments, the siNA molecules of the invention can 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 other embodiments, siNA molecules of the invention comprise 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.

[0134] In any of the embodiments or aspects of the siNA molecules of the invention, the sense strand and/or the antisense strand can further have a cap, such as described herein or as known in the art, at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand and/or antisense strand. Or as in the case of a hairpin siNA molecule, the cap can be at either one or both of the terminal nucleotides of the polynucleotide. In some embodiments, the cap is at one of both of the ends of the sense strand of a double-stranded siNA molecule. In other embodiments, the cap is at the at the 5'-end and 3'-end of antisense (guide) strand. In preferred embodiments, the caps are at the 3'-end of the sense strand and the 5' end of the sense strand.

[0135] Representative, but non-limiting examples of such terminal caps include an inverted abasic nucleotide, an inverted deoxy abasic nucleotide, an inverted nucleotide moiety, a group shown in **Figure 5,** a glyceryl modification, an alkyl or cycloalkyl group, a heterocycle, or any other group that prevents RNAi activity.

[0136] Any of the embodiments of the siNA molecules of the invention can have a 5' phosphate termini. In some embodiments, the siNA molecules lack terminal phosphates.

[0137] Any siNA molecule or construct of the invention can comprise one or more chemical modifications. Modifications can be used to improve *in vitro* or *in vivo* characteristics such as stability, activity, toxicity, immune response (e.g., prevent stimulation of an interferon response, an inflammatory or pro-inflammatory cytokine response, or a Toll-like Receptor (TlF) response.), and/or bioavailability.

[0138] 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 pharmacokinetic properties suitable for use in therapeutic applications.

[0139] In various embodiments, the siNA molecules of the invention comprise modifications wherein any (*e.g.,* one or more or all) nucleotides present in the sense and/or antisense strand are modified nucleotides (*e.g.*, wherein one nucleotide is modified or all nucleotides are modified nucleotides or alternately a plurality (i.e. more than one) of the nucleotides are modified nucleotides. In some embodiments, the siNA molecules of the invention are partially modified (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, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 55, 60, 65, 70, 75, 80 nucleotides are modified) with chemical modifications. In other embodiments, the siNA molecules of the invention are completely modified (e.g., 100% modified) with chemical modifications, i.e., the siNA molecule does not contain any ribonucleotides. In other embodiments, an siNA molecule of the invention comprises at least about 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78 nucleotides that are modified nucleotides. In

some of embodiments, 1 or more of the nucleotides in the sense strand of the siNA molecules of the invention are modified. In the same or other embodiments, 1 or more of the nucleotides in the antisense strand of the siNA molecules of the invention are modified.

[0140] The chemical modification within a single siNA molecule can be the same or different. In some embodiments, at least one strand has at least one chemical modification. In other embodiments, each strand has at least one chemical modifications, which can be the same or different, such as, sugar, base, or backbone (i.e., internucleotide linkage) modifications. In other embodiments, siNA molecules of the invention contains at least 2, 3, 4, 5, or more different chemical modifications..

[0141] Non-limiting examples of chemical modifications that are suitable for use in the present invention, are disclosed in USSN 10/444,853, USSN 10/981,966, USSN 12/064,015 and in references cited therein and include sugar, base, and phosphate, non-nucleotide modifications, and/or any combination thereof.

[0142] In various embodiments, a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification. In yet other embodiments, a majority of the purine nucleotides present in the double-stranded siNA molecule comprises a sugar modification. In certain instances, the purines and pyrimidines are differentially modified at the 2'-sugar position (i.e., at least one purine has a different modification from at least one pyrimidine in the same or different strand at the 2'-sugar position).

[0143] In certain specific embodiments of this aspect of the invention, at least one modified nucleotide is a 2'-deoxy-2-fluoro nucleotide, a 2'-deoxy nucleotide, or a 2'-O-alkyl (e.g., 2'-O-methyl) nucleotide.

[0144] In yet other embodiments of the invention, at least one nucleotide has a ribo-like, Northern or A form helix configuration (see e.g., Saenger, Principles of Nucleic Acid Structure, Springer-Verlag ed., 1984). 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 nucleotides, 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.

[0145] In certain embodiments of the invention, all the pyrimidine nucleotides in the complementary region on the sense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In certain embodiments, all of the pyrimindine nucleotides in the complementary region of the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In certain embodiments, all the purine nucleotides in the complementary region on the sense strand are 2'-deoxy purine nucleotides. In certain embodiments, all of the purines in the complementary region on the antisense strand are 2'-O-methyl purine nucleotides. In certain embodiments, all of the pyrimidine nucleotides in the complementary regions on the sense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides; all of the pyrimidine nucleotides in the complementary region of the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides; all the purine nucleotides in the complementary region on the sense strand are 2'-deoxy purine nucleotides and all of the purines in the complementary region on the antisense strand are 2'-O-methyl purine nucleotides.

[0146] Any of the above described modifications, or combinations thereof, including those in the references cited, can be applied to any of the siNA molecules of the invention.

[0147] The modified siNA molecules of the invention can comprise modifications at various locations within the siNA molecule. In some embodiments, the double-stranded siNA molecule of the invention comprises modified nucleotides at internal base paired positions within the siNA duplex. In other embodiments, a double-stranded siNA molecule of the invention comprises modified nucleotides at non-base paired or overhang regions of the siNA molecule. In yet other embodiments, 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 and/or 5'-position of the sense and/or antisense strand or region of the siNA molecule. Additionally, any of the modified siNA molecules of the invention can have a modification in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. Moreover, with regard to chemical modifications of the siNA molecules of the invention, each strand of the double-stranded siNA molecules of the invention can have one or more chemical modifications, such that each strand comprises a different pattern of chemical modifications.

[0148] In certain embodiments 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 11**) modification patterns herein or any

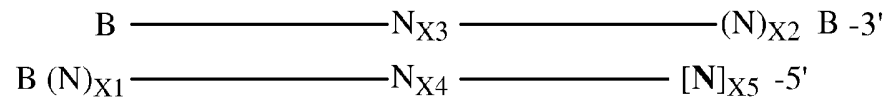
combination thereof. Further, non-limiting examples of modification schemes that could give rise to different patterns of modifications are shown in **Table 11.** The stabilization chemistries referred to in **Table 11** as Stab, can be combined in any combination of Sense/Antisense chemistries, such 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 11.** For example, "Stab 7/8" refers to both Stab 7/8 and Stab 7F/8F etc.

[0149] In other embodiments, 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.

[0150] In some embodiments, the pyrimidine nucleotides in the antisense strand are 2'-O-methyl or 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the antisense strand are 2'-O-methyl nucleotides or 2'-deoxy nucleotides. In other embodiments, the pyrimidine nucleotides in the sense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense strand are 2'-O-methyl or 2'-deoxy purine nucleotides.

[0151] Further non-limiting examples of sense and antisense strands of such siNA molecules having various modification patterns are shown in **Figures 2 and 3**.

[0152] In certain embodiments of the invention, double-stranded siNA molecules are provided, wherein the molecule has a sense strand and an antisense strand and comprises the following formula (A):



(A)

wherein, the upper strand is the sense strand and the lower strand is the antisense strand of the double-stranded nucleic acid molecule; wherein the antisense strand comprises at least 15 nucleotides of SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, or SEQ ID NO: 150; and the sense strand comprises a sequence having complementarity to the antisense strand;

each N is independently a nucleotide which is unmodified or chemically modified;

each B is a terminal cap that is present or absent;

(N) represents overhanging nucleotides, each of which is independently unmodified chemically modified;

[N] represents nucleotides that are ribonucleotides;

X1 and X2 are independently integers from 0 to 4;

X3 is an integer from 17 to 36;

X4 is an integer from 11 to 35; and

X5 is an integer from 1 to 6, provided that the sum of X4 and X5 is 17-36.

[0153] In certain embodiments, the at least 15 nucleotides form a contiguous stretch of nucleotides.

[0154] In other embodiments, the siNA molecule can contain one or more nucleotide deletions, substitutions, mismatches and/or additions to SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, or SEQ ID NO: 150, provided however, that the siNA molecule maintains its activity, for example, to mediate RNAi. In a non-limiting example, the deletion, substitution, mismatch and/or addition can result in a loop or bulge, or alternately a wobble or other alternative (non Watson-Crick) base pair.

[0155] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) of formula (A); wherein

(a) one or more pyrimidine nucleotides in N_{X4} positions are independently 2'-deoxy-2'-fluoro nucleotides, 2'-O-alkyl nucleotides, 2'-deoxy nucleotides, ribonucleotides, or any combination thereof;

(b) one or more purine nucleotides in N_{X4} positions are independently 2'-deoxy-2'-fluoro nucleotides, 2'-O-alkyl nucleotides, 2'-deoxy nucleotides, ribonucleotides, or any combination thereof;

(c) one or more pyrimidine nucleotides in N_{X3} positions are independently 2'-deoxy-2'-fluoro nucleotides, 2'-O-alkyl nucleotides, 2'-deoxy nucleotides, ribonucleotides, or any combination thereof; and

(d) one or more purine nucleotides in N_{X3} positions are independently 2'-deoxy-2'-fluoro nucleotides, 2'-O-alkyl nucleotides, 2'-deoxy nucleotides, ribonucleotides, or any combination thereof.

[0156] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) of formula (A); wherein

(a) each pyrimidine nucleotide in N_{X4} positions is independently a 2'-deoxy-2'-fluoro nucleotide, 2'-O-alkyl nucleotide, 2'-deoxy nucleotide, or ribonucleotide;

(b) each purine nucleotide in N_{X4} positions is independently a 2'-deoxy-2'-fluoro nucleotide, 2'-O-alkyl nucleotide, 2'-deoxy nucleotide, or ribonucleotide;

(c) each pyrimidine nucleotide in N_{X3} positions is independently a 2'-deoxy-2'-fluoro nucleotide, 2'-O-alkyl nucleotide, 2'-deoxy nucleotide, or ribonucleotide; and

(d) each purine nucleotides in N_{X3} positions is independently a 2'-deoxy-2'-fluoro nucleotide, 2'-O-alkyl nucleotide, 2'-deoxy nucleotide, or ribonucleotide.

[0157] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) of formula (A); wherein

(a) each pyrimidine nucleotide in N_{X4} positions is independently a 2'-deoxy-2'-fluoro nucleotide;

(b) each purine nucleotide in N_{X4} positions is independently a 2'-O-alkyl nucleotide;

(c) each pyrimidine nucleotide in N_{X3} positions is independently a 2'-deoxy-2'-fluoro nucleotide; and

(d) each purine nucleotide in N_{X3} positions is independently a 2'-deoxy nucleotide.

[0158] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) of formula (A); wherein

(a) each pyrimidine nucleotide in N_{X4} positions is independently a 2'-deoxy-2'-fluoro nucleotide;

(b) each purine nucleotide in N_{X4} positions is independently a 2'-O-alkyl nucleotide;

(c) each pyrimidine nucleotide in N_{X3} positions is independently a 2'-deoxy-2'-fluoro nucleotide; and

(d) each purine nucleotide in N_{X3} positions is independently a ribonucleotide.

[0159] In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) of formula (A); wherein

(a) each pyrimidine nucleotide in N_{X4} positions is independently a 2'-deoxy-2'-fluoro nucleotide;

(b) each purine nucleotide in N_{X4} positions is independently a ribonucleotide;

(c) each pyrimidine nucleotide in N_{X3} positions is independently a 2'-deoxy-2'-fluoro nucleotide; and

(d) each purine nucleotide in N_{X3} positions is independently a ribonucleotide.

[0160] In some embodiments, siNA molecules having formula A comprise a terminal phosphate group at the 5'-end of the antisense strand or antisense region of the nucleic acid molecule.

[0161] In various embodiments, siNA molecules having formula A comprise X5 = 1, 2, or 3; each X1 and X2 = 1 or 2; X3 = 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.

[0162] In one specific embodiment, an siNA molecule having formula A comprises X5 = 1; each X1 and X2 = 2; X3 = 19, and X4 = 18.

[0163] In another specific embodiment, an siNA molecule having formula A comprises X5 = 2; each X1 and X2 = 2; X3 = 19, and X4 = 17

[0164] In yet another embodiment, an siNA molecule having formula A comprises X5 = 3; each X1 and X2 = 2; X3 = 19, and X4 = 16.

[0165] In certain embodiments, siNA molecules having formula A comprise caps (B) at the 3' and 5' ends of the sense strand or sense region.

[0166] In certain embodiments, siNA molecules having formula A comprise caps (B) at the 3'-end of the antisense strand or antisense region.

[0167] In various embodiments, siNA molecules having formula A comprise caps (B) at the 3' and 5' ends of the sense strand or sense region and caps (B) at the 3'-end of the antisense strand or antisense region.

[0168] In yet other embodiments, siNA molecules having formula A comprise caps (B) only at the 5'-end of the sense (upper) strand of the double-stranded nucleic acid molecule.

[0169] In some embodiments, siNA molecules having formula A further comprise one or more phosphorothioate internucleotide linkages between the first terminal (N) and the adjacent nucleotide 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.

[0170] In some embodiments, siNA molecules having formula A comprises (N) nucleotides in the antisense strand (lower strand) that are complementary to nucleotides in a Bach1 target polynucleotide sequence which also has complementarity to the N and [**N**] nucleotides of the antisense (lower) strand.

[0171] In yet another embodiment, the invention provides a double stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

5'- B*GGAA*uccu*G*cuuuc*AG*uuuTTB –3' (Sense) (SEQ ID NO: 43)
3'- UUccuuAGGAcGAAAGucAAA –5' (Antisense) (SEQ ID NO: 44)

wherein:

each B is an inverted abasic cap moiety as shown in Fig. 10;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-dcoxyadcnosinc;

G is 2'deoxyguanosine;

T is thymidine;

A is adenosine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

[0172] In yet another embodiment, the invention provides a double stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

```
5'- BGucuGAGuGuccGuGGuuATTB -3'      (Sense)      (SEQ ID NO:61)
      ||||||||||||||||||||
3' UUcAGAcucAcAGGcAccAAU     -5'      (Antisense)  (SEQ ID NO:62)
```

wherein:

each B is an inverted abasic cap as shown in Fig. 10;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

A is adenosine;

U is uridine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

[0173] In yet another embodiment, the invention provides a double stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

```
5'  BGcAGuuAcuuccAcucAAGTTB 3'(Sense) (SEQ ID NO:63)
      ||||||||||||||||||||
3' UUcGucAAuGAAGGuGAGUUC     5'(Antisense) (SEQ ID NO:64)
```

wherein:

each B is an inverted abasic cap moiety as shown in Fig. 10;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

C is cytidine;

U is uridine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

[0174] In yet another embodiment, the invention provides a double stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

```
5'- BcuAcAcuGcuAAAcuGAuuTTB -3'     (Sense)     (SEQ ID NO:71)
     ||||||||||||||||||||
3' UUGAuGuGAcGAuuuGAcUAA    -5'     (Antisense) (SEQ ID NO:72)
```

wherein:

each B is an inverted abasic cap moiety as shown in Fig. 10;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

A is adenosine;

U is uridine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

[0175] In yet another embodiment, the invention provides a double stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

```
5'- BGAuuuGcAGGuGAuGuuAATTB -3'     (Sense)     (SEQ ID NO:77)
     ||||||||||||||||||||
3' UUcuAAAcGuccAcuAcAAUU    -5'     (Antisense) (SEQ ID NO:78)
```

wherein:

each B is an inverted abasic cap moiety as shown in Fig. 10;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

A is adenosine;

U is uridine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

[0176] In yet another embodiment, the invention provides a double stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

```
5'- BAuuuGAAccuuuAAuucAGTTB -3'     (Sense)     (SEQ ID NO:125)
      ||||||||||||||||||||
3' UUuAAAcuuGGAAAuuAAGUC    -5'     (Antisense) (SEQ ID NO:126)
```

wherein:

each B is an inverted abasic cap moiety as shown in Fig. 10;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

G is guanosine;

U is uridine;

C is cytidine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

[0171] In still another embodiment, the invention provides a double stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

```
5'- BGuuAAAGGAuuuGAAccuuTTB -3'     (Sense)     (SEQ ID NO:117)
     ||||||||||||||||||||
3' UUcAAuuuccuAAAcuuGGAA    -5'     (Antisense) (SEQ ID NO:118)
```

wherein:

each B is an inverted abasic cap moiety as shown in Figure 10;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

G is guanosine;

A is adenosine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

C. Generation/Synthesis of siNA Molecules

[0172] The siNAs of the invention can be obtained using a number of techniques known to those of skill in the art. For example the siNA can be chemically synthesized or may be encoded by plasmid (e.g., transcribed as sequences that automatically fold into duplexes with hairpin loops.). siNA can also be generated by cleavage of longer dsRNA (e.g., dsRNA greater than about 25 nucleotides in length) by the E coli RNase II or Dicer. These enzymes process the dsRNA into biologically active siRNA (see, e.g., Yang et al., PNAS USA 99:9942-9947 (2002); Calegari et al. PNAS USA 99:14236 (2002) Byron et al. Ambion Tech Notes; 10 (1):4-6 (2009); Kawaski et al., Nucleic Acids Res., 31:981-987 (2003), Knight and Bass, Science, 293:2269-2271 (2001) and Roberston et al., J. Biol. Chem 243:82(1969).

1. Chemical Synthesis

[0173] Preferably, siNA of the invention are chemically synthesized. Oligonucleotides (*e.g.*, certain modified oligonucleotides or portions of oligonucleotides lacking ribonucleotides) 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. 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.

[0174] siNA molecules without modifications are synthesized using procedures as described in Usman *et al.*, 1987, *J. Am. Chem. Soc.*, 109, 7845; Scaringe *et al.*, 1990, *Nucleic Acids Res.*, 18, 5433. These which makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end, can be used for certain siNA molecules of the invention.

[0175] In certain embodiments, the siNA molecules of the invention are synthesized, deprotected, and analyzed according to methods described in U.S. Patent Nos. 6,995,259, 6,686,463, 6,673,918, 6,649,751, 6,989,442, and USSN 10/190,359

[0176] In a non-limiting synthesis 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 12** outlines the amounts and the contact times of the reagents used in the synthesis cycle.

[0177] Alternatively, the siNA 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.

[0178] Various siNA molecules of the invention can also be synthesized using the teachings of Scaringe *et al.*, US Patent Nos. 5,889,136; 6,008,400; and 6,111,086.

2. Vector Expression

[0179] Alternatively, siNA molecules of the invention that interact with and down-regulate gene encoding target Bach1 molecules can be expressed and delivered 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.

[0180] In some embodiments, pol III based constructs are used to express nucleic acid molecules of the invention 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). (see for example Thompson, U.S. Patent. Nos. 5,902,880 and 6,146,886). (See also, 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. 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. U S A*, 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. U S A*, 89, 10802-6; Chen *et al.,* 1992, *Nucleic Acids Res.*, 20, 4581-9; Yu *et al.,* 1993, *Proc. Natl. Acad. Sci. U S A*, 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).*

[0181] Vectors used to express the siNA molecules of the invention can encode one or both strands of an siNA duplex, or a single self-complementary strand that self hybridizes into an 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).

D. Carrier/Delivery Systems

[0182] The siNA molecules of the invention are added directly, or can be complexed with cationic lipids, packaged within liposomes, or as a recombinant plasmid or viral vectors which express the siNA molecules, or otherwise delivered to target cells or tissues. 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. 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 US Patent 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).

[0183] In one aspect, the present invention provides carrier systems containing the siNA molecules described herein. In some embodiments, the carrier system is a lipid-based carrier system, cationic lipid, or liposome nucleic acid complexes, a liposome, a micelle, a virosome, a lipid nanoparticle or a mixture thereof. In other embodiments, the carrier system is a polymer-based carrier system such as a cationic polymer-nucleic acid complex. In additional embodiments, the carrier system is a cyclodextrin-based carrier system such as a cyclodextrin polymer-nucleic acid complex. In further embodiments, the carrier system is a protein-based carrier system such as a cationic peptide-nucleic acid complex. Preferably, the carrier system in a lipid nanoparticle formulation. Lipid nanoparticle ("LNP") formulations described in **Table 13** can be applied to any siNA molecule or combination of siNA molecules herein.

[0184] In certain embodiment, the siNA molecules of the invention are formulated as a lipid nanoparticle composition such as is described in USSN 11/353,630 and USSN 11/586,102.

[0185] In some embodiments, the invention features a composition comprising an siNA molecule 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 13**).

[0186] In other embodiments, 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. Non-limiting, examples of such conjugates are described in USSN 10/427,160 and USSN 10/201,394; and U.S. Patent Nos. 6,528,631; 6,335,434; 6, 235,886; 6,153,737; 5,214,136; 5,138,045.

[0187] In various embodiments, 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).

[0188] In yet other embodiments, the invention features compositions or formulations comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes) and siNA molecules of the

invention, such as is disclosed in for example, 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).

[0189] In some embodiments, the siNA 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 U.S. Patent Application Publication No. 20030077829.

[0190] In other embodiments, siNA molecules of the invention are complexed with membrane disruptive agents such as those described in U.S. Patent Application Publication No. 20010007666. In still other embodiments, 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. Patent No. 6,235,310.

[0191] In certain embodiments, siNA molecules of the invention are complexed with delivery systems as described in U.S. Patent Application Publication Nos. 2003077829; 20050287551; 20050164220; 20050191627; 20050118594; 20050153919; 20050085486; and 20030158133; and International PCT Publication Nos. WO 00/03683 and WO 02/087541.

[0192] In some embodiments, a liposomal formulation of the invention comprises an siNA molecule of the invention (e.g., siNA) formulated or complexed with compounds and compositions described in U.S. Patent Nos 6,858,224; 6,534,484; 6,287,591; 6,835,395; 6,586,410; 6,858,225; 6,815,432; 6,586,001; 6,120,798; 6,977,223; 6,998,115; 5,981,501; 5,976,567; 5,705,385; and U.S. Patent Application Publication Nos. 2006/0019912; 2006/0019258; 2006/0008909; 2005/0255153; 2005/0079212; 2005/0008689; 2003/0077829, 2005/0064595, 2005/0175682, 2005/0118253; 2004/0071654; 2005/0244504; 2005/0265961 and 2003/0077829.

[0193] Alternatively, recombinant plasmids and viral vectors, as discussed above, which express siRNA of the invention can be used to deliver the molecules of the invention. Delivery of siNA molecule expressing vectors can be systemic, such as by intravenous or intra-muscular 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). Such recombinant plasmids can also be administered directly or in conjunction with a suitable delivery reagents, including, for example, the Mirus Transit LT1 lipophilic reagent; lipofectin; lipofectamine; cellfectin; polycations (e.g., polylysine) or liposomes lipid-based carrier system, cationic lipid, or liposome nucleic acid complexes, , a micelle, a virosome, a lipid nanoparticle.

E. Kits

[0194] The present invention also provides nucleic acids in kit form. The kit may comprise a container. The kit typically contains a nucleic acid of the invention with instructions for its administration. In certain instances, the nucleic acids may have a targeting moiety attached. Methods of attaching targeting moieties (e.g. antibodies, proteins) are known to those of skill in the art. In certain instances the nucleic acids is chemically modified. In other embodiments, the kit contains more than one siNA molecule of the invention. The kits may comprise an siNA molecule of the invention with a pharmaceutically acceptable carrier or diluent. The kits may further comprise excipients.

F. Therapeutic Uses/Pharmaceutical Compositions

[0195] The present body of knowledge in Bach1 research indicates the need for methods to assay Bach1 activity and for compounds that can regulate Bach1 expression for research, diagnostic, and therapeutic use. As described *infra*, the nucleic acid molecules of the present invention can be used in assays to diagnose disease state related of Bach1 levels. In addition, the nucleic acid molecules and pharmaceutical compositions can be used to treat disease states related to Bach1 levels

1. Disease States Associated with Bach1

[0196] Particular disease states that can be associated with Bach1 expression modulation include, but are not limited to, respiratory, inflammatory, and autoimmune disease, traits, conditions, and phenotypes. Non-limiting examples of such disease states or indications include Chronic Obstructive Pulmonary Disease (COPD), asthma, eosinophilic cough, bronchitis, acute and chronic rejection of lung allograft, sarcoidosis, pulmonary fibrosis, rhinitis and sinusitis. Each of the inflammatory respiratory diseases are all characterized by the presence of mediators that recruit and activate different inflammatory cells, which release

enzymes or oxygen radicals causing symptoms, the persistence of inflammation and when chronic, destruction or disruption of normal tissue.

[0197] It is understood that the siNA molecules of the invention can degrade the target Bach1 mRNA (and thus inhibit the diseases stated above). Inhibition of a disease can be evaluated by directly measuring the progress of the disease in a subject. It can also be inferred through observing a change or reversal in a condition associated with the disease. Additionally, the siNA molecules of the invention can be used as a prophylaxis. Thus, the use of the nucleic acid molecules and pharmaceutical compositions of the invention can be used to ameliorate, treat, prevent, and/or cure these diseases and others associated with regulation of Bach1.

2. Pharmaceutical Compositions

[0198] The siNA molecules of the instant invention provide useful reagents and methods for a variety of therapeutic, prophylactic, cosmetic, veterinary, diagnostic, target validation, genomic discovery, genetic engineering, and pharmacogenomic applications.

a. Formulations

[0199] Thus, the present invention, in one aspect, also provides for pharmaceutical compositions of the siNA molecules described. These pharmaceutical compositions include salts of the above compounds, *e.g.*, acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid. These pharmaceutical formulations or pharmaceutical compositions can comprise a pharmaceutically acceptable carrier or diluent.

[0200] In one embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising at least 15 nucleotides of SEQ ID NO: 1. In another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising at least 15 nucleotides of SEQ ID NO: 143. In yet another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising at least 15 nucleotides of SEQ ID NO: 10. In still another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising at least 15 nucleotides of SEQ ID NO: 144. In another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising at least 15 nucleotides of SEQ ID NO: 11. In another embodiment, the invention features a

pharmaceutical composition comprising an siNA molecule comprising at least 15 nucleotides of SEQ ID NO: 145. In another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising at least 15 nucleotides of SEQ ID NO: 15. In yet another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising at least 15 nucleotides of SEQ ID NO: 146. In another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising at least 15 nucleotides of SEQ ID NO: 18. In yet another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising at least 15 nucleotides of SEQ ID NO: 147. In another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising at least 15 nucleotides of SEQ ID NO: 42. In yet another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising at least 15 nucleotides of SEQ ID NO: 148. In still another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising at least 15 nucleotides of SEQ ID NO: 38. In yet another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising at least 15 nucleotides of SEQ ID NO: 150. In another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising SEQ ID NO: 43 and SEQ ID NO: 44. In still another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising SEQ ID NO: 61 and SEQ ID NO: 62. In yet another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising SEQ ID NO: 63 and SEQ ID NO: 64. In yet another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising SEQ ID NO: 71 and SEQ ID NO: 72. In yet another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising SEQ ID NO: 77 and SEQ ID NO: 78. In still another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising SEQ ID NO: 125 and SEQ ID NO: 126. In yet another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising SEQ ID NO: 117 and SEQ ID NO: 118. In still another embodiment, the invention features a pharmaceutical composition comprising an siNA molecule comprising formula (A).

[0201] The siNA molecules of the invention are preferably formulated as pharmaceutical compositions prior to administering to a subject, according to techniques known in the art.

Pharmaceutical compositions of the present invention are characterized as being at least sterile and pyrogen-free. Methods for preparing pharmaceutical composition of the invention are within the skill in the art for example as described in *Remington's Pharmaceutical Science*, 17th ed., Mack Publishing Company, Easton, Pa. (1985).

[0202] In some embodiments, pharmaceutical compositions of the invention (e.g. siNA and/or LNP formulations thereof) further comprise conventional pharmaceutical excipients and/or additives. Suitable pharmaceutical excipients include preservatives, flavoring agents, stabilizers, antioxidants, osmolality adjusting agents, buffers, and pH adjusting agents. Suitable additives include physiologically biocompatible buffers (e.g., trimethylamine hydrochloride), addition of chelants (such as, for example, DTPA or DTPA-bisamide) or calcium chelate complexes (as for example calcium DTPA, CaNaDTPA-bisamide), or, optionally, additions of calcium or sodium salts (for example, calcium chloride, calcium ascorbate, calcium gluconate or calcium lactate). In addition, antioxidants and suspending agents can be used.

[0203] Non-limiting examples of various types of formulations for local administration include ointments, lotions, creams, gels, foams, preparations for delivery by transdermal patches, powders, sprays, aerosols, capsules or cartridges for use in an inhaler or insufflator or drops (for example eye or nose drops), solutions/suspensions for nebulization, suppositories, pessaries, retention enemas and chewable or suckable tablets or pellets (for example for the treatment of aphthous ulcers) or liposome or microencapsulation preparations.

[0204] Ointments, creams and gels, can, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agent and/or solvents. Non limiting examples of such bases can thus, for example, include water and/or an oil such as liquid paraffin or a vegetable oil such as arachis oil or castor oil, or a solvent such as polyethylene glycol. Thickening agents and gelling agents which can be used according to the nature of the base. Non-limiting examples of such agents include soft paraffin, aluminum stearate, cetostearyl alcohol, polyethylene glycols, woolfat, beeswax, carboxypolymethylene and cellulose derivatives, and/or glyceryl monostearate and/or non-ionic emulsifying agents.

[0205] In one embodiment lotions can be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents or thickening agents.

[0206] In one embodiment powders for external application can be formed with the aid of any suitable powder base, for example, talc, lactose or starch. Drops can be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents, suspending agents or preservatives.

[0207] 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.

[0208] 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.

[0209] 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.

[0210] 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

[0211] 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.

[0212] 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.

[0213] 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.

[0214] 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.

[0215] In other embodiments, 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, lysophosphatidylethanolamine, 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 lamellar 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 can 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.

b. Combinations

[0216] The compound and pharmaceutical formulations according to the invention can be administered to a s subject alone or used in combination with or include one or more other therapeutic agents, for example selected from anti-inflammatory agents, anticholinergic agents (particularly an $M_1/M_2/M_3$ receptor antagonist), β_2-adrenoreceptor agonists, antiinfective agents, such as antibiotics, antivirals, or antihistamines. The invention thus provides, in a further embodiment, a combination comprising an siNA molecule of the invention, such as for example, but not limitation, an siNA molecule comprising at least 15 nucleotides of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 10, SEQ ID NO: 144, SEQ ID NO: 11, SEQ ID NO: 145, SEQ ID NO: 15, SEQ ID NO: 146, SEQ ID NO: 18, SEQ ID NO: 147, SEQ ID NO: 42, SEQ ID NO: 148, SEQ ID NO: 38, or SEQ ID NO: 150; or comprising SEQ ID NO: 43 and SEQ ID NO: 44, or SEQ ID NO: 61 and SEQ ID NO: 62, or SEQ ID NO: 63 and SEQ ID NO: 64, or SEQ ID NO: 71 and SEQ ID NO: 72, or SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 125 and SEQ ID NO: 126, or SEQ ID NO: 117 and SEQ ID NO: 118, or formula (A), or a pharmaceutically acceptable salt, solvate or physiologically functional derivative thereof together with one or more other therapeutically active agents, for example selected from an anti-inflammatory agent, such as a corticosteroid or an NSAID, an anticholinergic agent, a β_2-adrenoreceptor agonist, an antiinfective agent, such as an antibiotic or an antiviral, or an antihistamine. Other embodiments of the invention encompasses combinations comprising an siNA molecule of the invention comprising at least 15 nucleotides of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 10, SEQ ID NO: 144, SEQ ID NO: 11, SEQ ID NO: 145, SEQ ID NO: 15, SEQ ID NO: 146, SEQ ID NO: 18, SEQ ID NO: 147, SEQ ID NO: 42, SEQ ID NO: 148, SEQ ID NO: 38, or SEQ ID NO: 150; or comprising SEQ ID NO: 43 and SEQ ID NO: 44, or SEQ ID NO: 61 and SEQ ID NO: 62, or SEQ ID NO: 63 and SEQ ID NO: 64, or SEQ ID NO: 71 and SEQ ID NO: 72, or SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 125 and SEQ ID NO: 126, or SEQ ID NO: 117 and SEQ ID NO: 118, or formula (A), or a pharmaceutically acceptable salt, solvate or physiologically functional derivative thereof together with a β_2-adrenoreceptor agonist, and/or an anticholinergic, and/or a Bach1 inhibitor, and/or an antihistamine.

[0217] In one embodiment, the invention encompasses a combination comprising a siNA molecule of the invention together with a β2-adrenoreceptor agonist. Non-limiting examples of β2-adrenoreceptor agonists include salmeterol (which can be a racemate or a single enantiomer such as the R-enantiomer), salbutamol (which can be a racemate or a single enantiomer such as the R-enantiomer), formoterol (which can be a racemate or a single diastereomer such as the R,R-diastereomer), salmefamol, fenoterol, carmoterol, etanterol, naminterol, clenbuterol, pirbuterol, flerbuterol, reproterol, bambuterol, indacaterol, terbutaline and salts thereof, for example the xinafoate (1-hydroxy-2-naphthalenecarboxylate) salt of salmeterol, the sulphate salt or free base of salbutamol or the fumarate salt of formoterol. In one embodiment the β2-adrenoreceptor agonists are long-acting β2-adrenoreceptor agonists, for example, compounds which provide effective bronchodilation for about 12 hours or longer.

[0218] Other β2-adrenoreceptor agonists include those described in WO 02/066422, WO 02/070490, WO 02/076933, WO 03/024439, WO 03/072539, WO 03/091204, WO 04/016578, WO 2004/022547, WO 2004/037807, WO 2004/037773, WO 2004/037768, WO 2004/039762, WO 2004/039766, WO01/42193 and WO03/042160.

[0219] Further examples of β2-adrenoreceptor agonists include 3-(4-{[6-({(2R)-2-hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl}amino) hexyl] oxy} butyl) benzenesulfonamide; 3-(3-{[7-({(2R)-2-hydroxy-2-[4-hydroxy-3-hydroxymethyl) phenyl] ethyl}-amino) heptyl] oxy} propyl) benzenesulfonamide; 4-{(1R)-2-[(6-{2-[(2, 6-dichlorobenzyl) oxy] ethoxy} hexyl) amino]-1-hydroxyethyl}-2-(hydroxymethyl)phenol;4-{(1R)-2-[(6-{4-[3-(cyclopentylsulfonyl) phenyl]butoxy}hexyl)amino]-1-hydroxyethyl}-2-(hydroxymethyl)phenol; N-[2-hydroxyl-5-[(1R)-1-hydroxy-2-[[2-4-[[(2R)-2-hydroxy-2phenylethyl]amino]phenyl]ethyl] amino]ethyl] phenyl]formamide; N-2{2-[4-(3-phenyl-4-methoxyphenyl)aminophenyl]ethyl}-2-hydroxy-2-(8-hydroxy-2(1H)-quinolinon-5-yl)ethylamine; and 5-[(R)-2-(2-{4-[4-(2-amino-2-methyl-propoxy)-phenylamino]-phenyl}-ethylamino)-1-hydroxy-ethyl]-8-hydroxy-1H-quinolin-2-one.

[0220] In one embodiment, the β2-adrenoreceptor agonist can be in the form of a salt formed with a pharmaceutically acceptable acid selected from sulphuric, hydrochloric, fumaric, hydroxynaphthoic (for example 1- or 3-hydroxy-2-naphthoic), cinnamic, substituted cinnamic, triphenylacetic, sulphamic, naphthaleneacrylic, benzoic, 4-methoxybenzoic, 2- or 4-hydroxybenzoic, 4-chlorobenzoic and 4-phenylbenzoic acid.

[0221] Suitable anti-inflammatory agents also include corticosteroids. Examples of corticosteroids which can be used in combination with the compounds of the invention are those oral and inhaled corticosteroids and their pro-drugs which have anti-inflammatory activity. Non-limiting examples include methyl prednisolone, prednisolone, dexamethasone, fluticasone propionate,6α,9α-difluoro-11β-hydroxy-16α-methyl-17α-[(4-methyl-1,3-thiazole-5-carbonyl) oxy] -3-oxo-androsta-1,4-diene-17β-carbothioic acid S-fluoromethyl ester, 6α,9α-difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid S-fluoromethyl ester (fluticasone furoate), 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxy- androsta-1,4-diene-17β-carbothioic acid S-(2-oxo-tetrahydro-furan-3S-yl)ester,6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-(2,2,3,3 tetramethycyclopropyl-carbonyl)oxy-androsta-1,4-diene-17β-carbothioic acid S-cyanomethyl ester and 6α,9α-difluoro-11β-hydroxy-16α-methyl-17α-(1-methycyclopropylcarbonyl)oxy-3-oxo-androsta-1,4-diene-17β -carbothioic acid S-fluoromethyl ester, beclomethasone esters (for example the 17-propionate ester or the 17,21-dipropionate ester), budesonide, flunisolide, mometasone esters (for example mometasone furoate), triamcinolone acetonide, rofleponide, ciclesonide (16α,17-[[(R)-cyclohexylmethylene]bis(oxy)]-11β,21-dihydroxy-pregna-1,4-diene-3,20-dione), butixocort propionate, RPR-106541, and ST-126. In one embodiment corticosteroids include fluticasone propionate, 6α,9α-difluoro-11β-hydroxy-16α-methyl-17α-[(4-methyl-1,3-thiazole-5-carbonyl) oxy]-3-oxo-androsta-1,4-diene-17β-carbothioic acid S-fluoromethyl ester, 6α,9α-difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid S-fluoromethyl ester, 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-(2,2,3,3- tetramethycyclopropylcarbonyl)oxy-androsta-1,4-diene-17β-carbothioic acid S-cyanomethyl ester and 6α,9α-difluoro-11β-hydroxy-16α-methyl-17α-(1-methylcyclo-propylcarbonyl)oxy-3-oxo-androsta-1,4-diene-17β-carbothioic acid S-fluoromethyl ester. In one embodiment the corticosteroid is 6α,9α-difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid S-fluoromethyl ester. Non-limiting examples of corticosteroids include those described in the following published patent applications and patents: WO02/088167, WO02/100879, WO02/12265, WO02/12266, WO05/005451, WO05/005452, WO06/072599 and WO06/072600.

[0222] In one embodiment, are combinations comprising siNA molecules of the invention and non-steroidal compounds having glucocorticoid agonism that can possess selectivity for transrepression over transactivation such as non-steroidal compounds disclosed in the following published patent applications and patents: WO03/082827, WO98/54159, WO04/005229, WO04/009017, WO04/018429, WO03/104195, WO03/082787, WO03/082280, WO03/059899, WO03/101932, WO02/02565, WO01/16128, WO00/66590, WO03/086294, WO04/026248, WO03/061651, WO03/08277, WO06/000401, WO06/000398 and WO06/015870.

[0223] Non-limiting examples of other anti-inflammatory agents that can be used in combination with the siNA molecules of the invention include non-steroidal anti-inflammatory drugs (NSAID's).

[0224] Non-limiting examples of NSAID's include sodium cromoglycate, nedocromil sodium, phosphodiesterase (PDE) inhibitors (for example, theophylline, PDE4 inhibitors or mixed PDE3/PDE4 inhibitors), leukotriene antagonists, inhibitors of leukotriene synthesis (for example montelukast), iNOS inhibitors, tryptase and elastase inhibitors, beta-2 integrin antagonists and adenosine receptor agonists or antagonists (e.g. adenosine 2a agonists), cytokine antagonists (for example chemokine antagonists, such as a CCR3 antagonist) or inhibitors of cytokine synthesis, or 5-lipoxygenase inhibitors. In one embodiment, the invention encompasses iNOS (inducible nitric oxide synthase) inhibitors for oral administration. Examples of iNOS inhibitors include those disclosed in the following published international patents and patent applications: WO93/13055, WO98/30537, WO02/50021, WO95/34534 and WO99/62875. Examples of CCR3 inhibitors include those disclosed in WO02/26722.

[0225] Compounds include cis-4-cyano-4-(3-cyclopentyloxy-4-methoxyphenyl)cyclohexan-1-carboxylic acid, 2-carbomethoxy-4-cyano-4-(3-cyclopropylmethoxy-4-difluoromethoxy-phenyl)cyclohexan-1-one and cis-[4-cyano-4-(3-cyclopropylmethoxy-4-difluoromethoxy-phenyl)cyclohexan-1-ol]. Also, cis-4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]cyclo-hexane-1-carboxylic acid (also known as cilomilast) and its salts, esters, pro-drugs or physical forms, which is described in U.S. patent 5,552,438

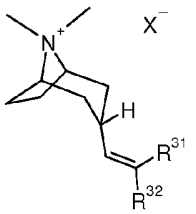
[0226] Other compounds include AWD-12-281 from Elbion (Hofgen, N. et al. 15th EFMC Int Symp Med Chem (Sept 6-10, Edinburgh) 1998, Abst P.98; CAS reference No. 247584020-9); a 9-benzyladenine derivative nominated NCS-613 (INSERM); D-4418 from Chiroscience and Schering-Plough; a benzodiazepine PDE4 inhibitor identified as CI-1018 (PD-168787) and attributed to Pfizer; a benzodioxole derivative disclosed by Kyowa Hakko in WO99/16766; K-34 from Kyowa Hakko; V-11294A from Napp (Landells, L.J. et al. Eur Resp J [Annu Cong Eur Resp Soc (Sept 19-23, Geneva) 1998] 1998, 12 (Suppl. 28): Abst P2393); roflumilast (CAS reference No 162401-32-3) and a pthalazinone (WO99/47505, the disclosure of which is hereby incorporated by reference) from Byk-Gulden; Pumafentrine, (-)-p-[(4aR*,10bS*)-9-ethoxy-1,2,3,4,4a,10b-hexahydro-8-methoxy-2-methylbenzo[c][1,6]naphthyridin-6-yl]-N,N-diisopropyl-benzamide which is a mixed PDE3/PDE4 inhibitor which has been prepared and published on by Byk-Gulden, now Altana; arofylline under development by Almirall-Prodesfarma; VM554/UM565 from Vernalis; or T-440 (Tanabe Seiyaku; Fuji, K. et al. J Pharmacol Exp Ther,1998, 284(1): 162), and T2585. Further compounds are disclosed in the published international patent applications WO04/024728 (Glaxo Group Ltd), WO04/056823 (Glaxo Group Ltd) and WO04/103998 (Glaxo Group Ltd).

[0227] Example of cystic fibrous agents that can be use in combination with the compounds of the invention include, but are not limited to, compounds such as Tobi® and Pulmozyme®.

[0228] Examples of anticholinergic agents that can be used in combination with the compounds of the invention are those compounds that act as antagonists at the muscarinic receptors, in particular those compounds which are antagonists of the M1 or M3 receptors, dual antagonists of the M1/M3 or M2/M3, receptors or pan-antagonists of the M1/M2/M3 receptors. Exemplary compounds for administration via inhalation include ipratropium (for example, as the bromide, CAS 22254-24-6, sold under the name Atrovent), oxitropium (for example, as the bromide, CAS 30286-75-0) and tiotropium (for example, as the bromide, CAS 136310-93-5, sold under the name Spiriva). Also of interest are revatropate (for example, as the hydrobromide, CAS 262586-79-8) and LAS-34273 which is disclosed in WO01/04118. Exemplary compounds for oral administration include pirenzepine (CAS 28797-61-7), darifenacin (CAS 133099-04-4, or CAS 133099-07-7 for the hydrobromide sold under the name Enablex), oxybutynin (CAS 5633-20-5, sold under the name Ditropan),

terodiline (CAS 15793-40-5), tolterodine (CAS 124937-51-5, or CAS 124937-52-6 for the tartrate, sold under the name Detrol), otilonium (for example, as the bromide, CAS 26095-59-0, sold under the name Spasmomen), trospium chloride (CAS 10405-02-4) and solifenacin (CAS 242478-37-1, or CAS 242478-38-2 for the succinate also known as YM-905 and sold under the name Vesicare).

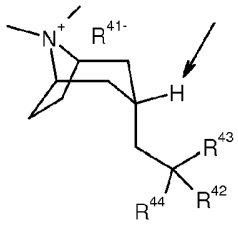
[0229] Other anticholinergic agents include compounds of formula (XXI), which are disclosed in US patent application 60/487981:



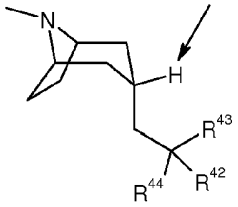
(XXI)

in which the preferred orientation of the alkyl chain attached to the tropane ring is *endo*; R^{31} and R^{32} are, independently, selected from the group consisting of straight or branched chain lower alkyl groups having preferably from 1 to 6 carbon atoms, cycloalkyl groups having from 5 to 6 carbon atoms, cycloalkyl-alkyl having 6 to 10 carbon atoms, 2-thienyl, 2-pyridyl, phenyl, phenyl substituted with an alkyl group having not in excess of 4 carbon atoms and phenyl substituted with an alkoxy group having not in excess of 4 carbon atoms; X^- represents an anion associated with the positive charge of the N atom. X^- can be but is not limited to chloride, bromide, iodide, sulfate, benzene sulfonate, and toluene sulfonate. Examples of formula XXI include, but are not limited to, (3-*endo*)-3-(2,2-di-2-thienylethenyl)-8,8-dimethyl-8-azoniabicyclo[3.2.1] octane bromide; (3-*endo*)-3-(2,2-diphenylethenyl)-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane bromide; (3-*endo*)-3-(2,2-diphenylethenyl)-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane 4-methylbenzene-sulfonate; (3-*endo*)-8,8-dimethyl-3-[2-phenyl-2-(2-thienyl)ethenyl]-8-azoniabicyclo[3.2.1]octane bromide; and/or (3-*endo*)-8,8-dimethyl-3-[2-phenyl-2-(2-pyridinyl)ethenyl]-8-azoniabicyclo [3.2.1]octane bromide.

[0230] Further anticholinergic agents include compounds of formula (XXII) or (XXIII), which are disclosed in US patent application 60/511009:



(XXII)



(XXIII)

wherein: the H atom indicated is in the exo position; R^{41} represents an anion associated with the positive charge of the N atom. R^{41} can be, but is not limited to, chloride, bromide, iodide, sulfate, benzene sulfonate and toluene sulfonate; R^{42} and R^{43} are independently selected from the group consisting of straight or branched chain lower alkyl groups (having preferably from 1 to 6 carbon atoms), cycloalkyl groups (having from 5 to 6 carbon atoms), cycloalkyl-alkyl (having 6 to 10 carbon atoms), heterocycloalkyl (having 5 to 6 carbon atoms) and N or O as the heteroatom, heterocycloalkyl-alkyl (having 6 to10 carbon atoms) and N or O as the heteroatom, aryl, optionally substituted aryl, heteroaryl, and optionally substituted heteroaryl; R^{44} is selected from the group consisting of $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_{12})$cycloalkyl, $(C_3\text{-}C_7)$heterocycloalkyl, $(C_1\text{-}C_6)$alkyl$(C_3\text{-}C_{12})$cycloalkyl, $(C_1\text{-}C_6)$alkyl$(C_3\text{-}C_7)$heterocycloalkyl, aryl, heteroaryl, $(C_1\text{-}C_6)$alkyl-aryl, $(C_1\text{-}C_6)$alkyl-heteroaryl, $-OR^{45}$, $-CH_2OR^{45}$, $-CH_2OH$, $-CN$, $-CF_3$, $-CH_2O(CO)R^{46}$, $-CO_2R^{47}$, $-CH_2NH_2$, $-CH_2N(R^{47})SO_2R^{45}$, $-SO_2N(R^{47})(R^{48})$, $-CON(R^{47})(R^{48})$, $-CH_2N(R^{48})CO(R^{46})$, $-CH_2N(R^{48})SO_2(R^{46})$, $-CH_2N(R^{48})CO_2(R^{45})$, $-CH_2N(R^{48})CONH(R^{47})$; R^{45} is selected from the group consisting of $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkyl$(C_3\text{-}C_{12})$cycloalkyl, $(C_1\text{-}C_6)$alkyl$(C_3\text{-}C_7)$heterocycloalkyl, $(C_1\text{-}C_6)$alkyl-aryl, $(C_1\text{-}C_6)$alkyl-heteroaryl; R^{46} is selected from the group consisting of $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_{12})$cycloalkyl, $(C_3\text{-}C_7)$heterocycloalkyl, $(C_1\text{-}C_6)$alkyl$(C_3\text{-}C_{12})$cycloalkyl, $(C_1\text{-}C_6)$alkyl$(C_3\text{-}C_7)$heterocycloalkyl, aryl, heteroaryl, $(C_1\text{-}C_6)$alkyl-aryl, $(C_1\text{-}C_6)$alkyl-heteroaryl; R^{47} and R^{48} are, independently, selected from the group consisting of H, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_{12})$cycloalkyl, $(C_3\text{-}C_7)$heterocycloalkyl, $(C_1\text{-}C_6)$alkyl$(C_3\text{-}C_{12})$cycloalkyl, $(C_1\text{-}C_6)$alkyl$(C_3\text{-}C_7)$heterocycloalkyl, $(C_1\text{-}C_6)$alkyl-aryl, and $(C_1\text{-}C_6)$alkyl-heteroaryl, representative, but non-limiting, examples include: (*endo*)-3-(2-methoxy-2,2-di-thiophen-2-yl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide; 3-((*endo*)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionitrile;(*endo*)-8-methyl-3-(2,2,2-triphenyl-ethyl)-8-azabicyclo[3.2.1] oct-ane; 3-((*endo*)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenylpropionamide; 3-((*endo*)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionic acid; (*endo*)-3-(2-cyano-2,2-di-

phenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide; (*endo*)-3-(2-cyano-2,2-dipheny l-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane bromide; 3-((*endo*)-8-methyl-8-aza-bicyclo [3.2.1]oct-3-yl)-2,2-diphenyl-propan-1-ol; *N*-benzyl-3-((*endo*)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionamide; (*endo*)-3-(2-carbamoyl-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide; 1-benzyl-3-[3-((*endo*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,2-diphcnyl-propyl]-urea;1-ethyl-3-[3-((*endo*)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-di -phenyl-propyl]-urea; *N*-[3-((*endo*)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-acetamide; *N*-[3-((*endo*)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-benzamide ; 3-((*endo*)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-di-thiophen-2-yl-propionitrile; (*endo*)-3-(2-cyano-2,2-di-thiophen-2-yl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octaneiodide; *N*-[3-((*endo*)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-benzenesulfonamide; [3-((*endo*)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-urea; *N*-[3-((*endo*)-8-methyl 8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-methanesulfonamide; and/or (*endo*)-3-{2,2-diphenyl-3-[(1-phenyl-methanoyl)-amino]-propyl}-8,8-dimethyl-8-azoniabicyclo [3.2.1] octane bromide.

[0231] Further compounds include: (endo)-3-(2-methoxy-2,2-di-thiophen-2-yl-ethyl)-8,8-di-methyl-8-azonia-bicyclo[3.2.1]octane iodide; (endo)-3-(2-cyano-2,2-diphenyl-ethyl)-8,8-di- methyl-8-azonia-bicyclo[3.2.1]octane iodide; (endo)-3-(2-cyano-2,2-diphenyl-ethyl)-8,8-di-methyl-8-azonia-bicyclo[3.2.1]octane bromide; (endo)-3-(2-carbamoyl-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide; (endo)-3-(2-cyano-2,2-di-thiophen-2-yl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide; and/or (endo)-3-{2,2-diphenyl-3-[(1-phenyl-methanoyl)-amino]-propyl}-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane bromide.

[0232] In certain embodiments, the invention provides a combination comprising an siNA molecule of the invention comprising at least 15 nucleotides of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 10, SEQ ID NO: 144, SEQ ID NO: 11, SEQ ID NO: 145, SEQ ID NO: 15, SEQ ID NO: 146, SEQ ID NO: 18, SEQ ID NO: 147, SEQ ID NO: 42, SEQ ID NO: 148, SEQ ID NO: 38, or SEQ ID NO: 150; or comprising SEQ ID NO: 43 and SEQ ID NO: 44, or SEQ ID NO: 61 and SEQ ID NO: 62, or SEQ ID NO: 63 and SEQ ID NO: 64, or SEQ ID NO: 71 and SEQ ID NO: 72, or SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 125 and SEQ ID NO: 126, or SEQ ID NO: 117 and SEQ ID NO: 118, or formula (A), or a pharmaceutically acceptable salt thereof together with an H1 antagonist. Examples of H1

antagonists include, without limitation, amelexanox, astemizole, azatadine, azelastine, acrivastine, brompheniramine, cetirizine, levocetirizine, efletirizine, chlorpheniramine, clemastine, cyclizine, carebastine, cyproheptadine, carbinoxamine, descarboethoxyloratadine, doxylamine, dimethindene, ebastine, epinastine, efletirizine, fexofenadine, hydroxyzine, ketotifen, loratadine, levocabastine, mizolastine, mequitazine, mianserin, noberastine, meclizine, norastemizole, olopatadine, picumast, pyrilamine, promethazine, terfenadine, tripelennamine, temelastine, trimeprazine and triprolidine, particularly cetirizine, levocetirizine, efletirizine and fexofenadine.

[0233] In other embodiments, the invention provides a combination comprising an siNA molecule of the invention comprising at least 15 nucleotides of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 10, SEQ ID NO: 144, SEQ ID NO: 11, SEQ ID NO: 145, SEQ ID NO: 15, SEQ ID NO: 146, SEQ ID NO: 18, SEQ ID NO: 147, SEQ ID NO: 42, SEQ ID NO: 148, SEQ ID NO: 38, or SEQ ID NO: 150; or comprising SEQ ID NO: 43 and SEQ ID NO: 44, or SEQ ID NO: 61 and SEQ ID NO: 62, or SEQ ID NO: 63 and SEQ ID NO: 64, or SEQ ID NO: 71 and SEQ ID NO: 72, or SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 125 and SEQ ID NO: 126, or SEQ ID NO: 117 and SEQ ID NO: 118, or formula (A), or a pharmaceutically acceptable salt thereof together with an H3 antagonist (and/or inverse agonist). Examples of H3 antagonists include, for example, those compounds disclosed in WO2004/035556 and in WO2006/045416. Other histamine receptor antagonists which can be used in combination with the compounds of the present invention include antagonists (and/or inverse agonists) of the H4 receptor, for example, the compounds disclosed in Jablonowski et al., J. Med. Chem. 46:3957-3960 (2003).

[0234] The invention thus provides a combination comprising an siNA molecule of the invention comprising at least 15 nucleotides of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 10, SEQ ID NO: 144, SEQ ID NO: 11, SEQ ID NO: 145, SEQ ID NO: 15, SEQ ID NO: 146, SEQ ID NO: 18, SEQ ID NO: 147, SEQ ID NO: 42, SEQ ID NO: 148, SEQ ID NO: 38, or SEQ ID NO: 150; or comprising SEQ ID NO: 43 and SEQ ID NO: 44, or SEQ ID NO: 61 and SEQ ID NO: 62, or SEQ ID NO: 63 and SEQ ID NO: 64, or SEQ ID NO: 71 and SEQ ID NO: 72, or SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 125 and SEQ ID NO: 126, or SEQ ID NO: 117 and SEQ ID NO: 118, or formula (A), and/or a pharmaceutically acceptable salt, solvate or physiologically functional derivative thereof together with a Bach1 inhibitor.

[0235] The invention also provides, in a further embodiments, combinations comprising an siNA molecule of the invention comprising at least 15 nucleotides of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 10, SEQ ID NO: 144, SEQ ID NO: 11, SEQ ID NO: 145, SEQ ID NO: 15, SEQ ID NO: 146, SEQ ID NO: 18, SEQ ID NO: 147, SEQ ID NO: 42, SEQ ID NO: 148, SEQ ID NO: 38, or SEQ ID NO: 150; or comprising SEQ ID NO: 43 and SEQ ID NO: 44, or SEQ ID NO: 61 and SEQ ID NO: 62, or SEQ ID NO: 63 and SEQ ID NO: 64, or SEQ ID NO: 71 and SEQ ID NO: 72, or SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 125 and SEQ ID NO: 126, or SEQ ID NO: 117 and SEQ ID NO: 118, or formula (A), and/or a pharmaceutically acceptable salt, solvate or physiologically functional derivative thereof together with a β2-adrenoreceptor agonist.

[0236] The invention also provides, in a further embodiments, combinations comprising an siNA molecule of the invention comprising at least 15 nucleotides of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 10, SEQ ID NO: 144, SEQ ID NO: 11, SEQ ID NO: 145, SEQ ID NO: 15, SEQ ID NO: 146, SEQ ID NO: 18, SEQ ID NO: 147, SEQ ID NO: 42, SEQ ID NO: 148, SEQ ID NO: 38, or SEQ ID NO: 150; or comprising SEQ ID NO: 43 and SEQ ID NO: 44, or SEQ ID NO: 61 and SEQ ID NO: 62, or SEQ ID NO: 63 and SEQ ID NO: 64, or SEQ ID NO: 71 and SEQ ID NO: 72, or SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 125 and SEQ ID NO: 126, or SEQ ID NO: 117 and SEQ ID NO: 118, or formula (A), and/or a pharmaceutically acceptable salt, solvate or physiologically functional derivative thereof together with a corticosteroid.

[0237] The invention also provides, in a further embodiments, combinations comprising an siNA molecule of the invention comprising at least 15 nucleotides of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 10, SEQ ID NO: 144, SEQ ID NO: 11, SEQ ID NO: 145, SEQ ID NO: 15, SEQ ID NO: 146, SEQ ID NO: 18, SEQ ID NO: 147, SEQ ID NO: 42, SEQ ID NO: 148, SEQ ID NO: 38, or SEQ ID NO: 150; or comprising SEQ ID NO: 43 and SEQ ID NO: 44, or SEQ ID NO: 61 and SEQ ID NO: 62, or SEQ ID NO: 63 and SEQ ID NO: 64, or SEQ ID NO: 71 and SEQ ID NO: 72, or SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 125 and SEQ ID NO: 126, or SEQ ID NO: 117 and SEQ ID NO: 118, or formula (A), and/or a pharmaceutically acceptable salt, solvate or physiologically functional derivative thereof together with an anticholinergic.

[0238] The invention provides, in a further aspect, combinations comprising an siNA molecule of the invention comprising at least 15 nucleotides of SEQ ID NO: 1, SEQ ID NO:

143, SEQ ID NO: 10, SEQ ID NO: 144, SEQ ID NO: 11, SEQ ID NO: 145, SEQ ID NO: 15, SEQ ID NO: 146, SEQ ID NO: 18, SEQ ID NO: 147, SEQ ID NO: 42, SEQ ID NO: 148, SEQ ID NO: 38, or SEQ ID NO: 150; or comprising SEQ ID NO: 43 and SEQ ID NO: 44, or SEQ ID NO: 61 and SEQ ID NO: 62, or SEQ ID NO: 63 and SEQ ID NO: 64, or SEQ ID NO: 71 and SEQ ID NO: 72, or SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 125 and SEQ ID NO: 126, or SEQ ID NO: 117 and SEQ ID NO: 118, or formula (A), and/or a pharmaceutically acceptable salt, solvate or physiologically functional derivative thereof together with an antihistamine.

[0239] The invention provides, in yet a further aspect, combinations comprising an siNA molecule of the invention comprising at least 15 nucleotides of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 10, SEQ ID NO: 144, SEQ ID NO: 11, SEQ ID NO: 145, SEQ ID NO: 15, SEQ ID NO: 146, SEQ ID NO: 18, SEQ ID NO: 147, SEQ ID NO: 42, SEQ ID NO: 148, SEQ ID NO: 38, or SEQ ID NO: 150; or comprising SEQ ID NO: 43 and SEQ ID NO: 44, or SEQ ID NO: 61 and SEQ ID NO: 62, or SEQ ID NO: 63 and SEQ ID NO: 64, or SEQ ID NO: 71 and SEQ ID NO: 72, or SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 125 and SEQ ID NO: 126, or SEQ ID NO: 117 and SEQ ID NO: 118, or formula (A), and/or a pharmaceutically acceptable salt, solvate or physiologically functional derivative thereof together with an Bach1 inhibitor and a β2-adrenoreceptor agonist.

[0240] The invention thus provides, in a further aspect, combinations comprising an siNA molecule of the invention comprising at least 15 nucleotides of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 10, SEQ ID NO: 144, SEQ ID NO: 11, SEQ ID NO: 145, SEQ ID NO: 15, SEQ ID NO: 146, SEQ ID NO: 18, SEQ ID NO: 147, SEQ ID NO: 42, SEQ ID NO: 148, SEQ ID NO: 38, or SEQ ID NO: 150; or comprising SEQ ID NO: 43 and SEQ ID NO: 44, or SEQ ID NO: 61 and SEQ ID NO: 62, or SEQ ID NO: 63 and SEQ ID NO: 64, or SEQ ID NO: 71 and SEQ ID NO: 72, or SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 125 and SEQ ID NO: 126, or SEQ ID NO: 117 and SEQ ID NO: 118, or formula (A), and/or a pharmaceutically acceptable salt, solvate or physiologically functional derivative thereof together with an anticholinergic and a Bach1 inhibitor.

[0241] The combinations referred to above can conveniently be presented for use in the form of a pharmaceutical formulation and thus pharmaceutical compositions comprising a combination as defined above together with a pharmaceutically acceptable diluent or carrier represent a further aspect of the invention.

[0242] The individual compounds of such combinations can be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations. In one embodiment, the individual compounds will be administered simultaneously in a combined pharmaceutical formulation.

[0243] 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 siNa molecules of the invention can be used with additional airway hydration therapies such as hypertonic saline, denufosol, bronchitol; CFTR gene therapy; protein assist/repair such as CFTR correctors, eg. VX-809 (Vertex), CFTR potentiators, eg. VX-770 (Vertex); mucus treatments such as pulmozyme; anti-inflammatory treatments such as oral N-acetylcysteine, sildenafil, inhaled glutathione, pioglitazone, hydroxychloroquine, simvastatin; anti-infective therapies such as azithromycin, arikace; transplant drugs such as inhaled cyclosporin; and nutritional supplements such as aquADEKs, pancrelipase products, trizytek. Thus, 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, such as other Bach1 inhibitors.

3. Therapeutic Applications

[0244] The present body of knowledge in Bach1 research indicates the need for methods that can regulate Bach1 expression for therapeutic use.

[0245] Thus, one aspect of the invention comprises a method of treating a subject including, but not limited to, a human suffering from a condition which is mediated by the action, or by loss of action, of Bach1, which method comprises administering to said subject an effective amount of a double-stranded siNA molecule of the invention. In one embodiment of this aspect, the siNA molecules comprises at least 15 nucleotides of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 10, SEQ ID NO: 144, SEQ ID NO: 11, SEQ ID NO: 145, SEQ ID NO: 15, SEQ ID NO: 146, SEQ ID NO: 18, SEQ ID NO: 147, SEQ ID NO: 42, SEQ ID NO: 148, SEQ ID NO: 38, or SEQ ID NO: 150; or comprising SEQ ID NO: 43 and SEQ ID NO: 44, or SEQ ID NO: 61 and SEQ ID NO: 62, or SEQ ID NO: 63 and SEQ ID NO: 64, or SEQ ID NO: 71 and SEQ ID NO: 72, or SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 125 and SEQ ID NO: 126, or SEQ ID NO: 117 and SEQ ID NO: 118, or

formula (A). In another embodiment of this aspect, the condition is or is caused by a respiratory disease. Respiratory diseases treatable according to this aspect of the invention include COPD, asthma, eosinophilic cough, bronchitis, sarcoidosis, pulmonary fibrosis, rhinitis, sinusitis.. In a particular embodiment, the use is for the treatment of a respiratory disease selected from the group consisting of COPD, cystic fibrosis, and asthma. In certain embodiments, the administration of the siNA molecule is via local administration or systemic administration. In other embodiments, the invention features contacting the subject or organism with an siNA molecule of the invention via local administration to relevant tissues or cells, such as lung cells and tissues, such as via pulmonary delivery. In yet other embodiments the invention features contacting the subject or organism with an siNA molecule of the invention via systemic administration (such as via intravenous or subcutaneous administration of siNA) to relevant tissues or cells, such as tissues or cells involved in the maintenance or development of the inflammatory disease, trait, or condition in a subject or organism.

[0246] siNA molecules of the invention are also 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 Bach1 target cells from a patient are extracted. These extracted cells are contacted with Bach1 siNAs 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.

[0247] For therapeutic applications, a pharmaceutically effective dose of the siNA molecules or pharmaceutical compositions of the invention is administered to the subject. 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. One skilled in the art can readily determine a therapeutically effective dose of the siNA of the invention to be administer to a given subject, by taking into account factors, such as the size

and weight of the subject, the extent of the disease progression or penetration, the age, health, and sex of the subject, the route of administration m and whether the administration is regional or systemic. 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. The siNA molecules of the invention can be administered in a single dose or in multiple doses.

G. Administration

[0248] Compositions or formulations can be administered in a variety of ways. Non-limiting examples of administration methods of the invention include oral, buccal, sublingual, parenteral (i.e., intraarticularly, intravenously, intraperitoneally, subcutaneously, or intramuscularly), local rectal administration or other local administration. In one embodiment, the composition of the invention can be administered by insufflation and inhalation. Administration can be accomplished via single or divided doses. In some embodiments, the pharmaceutical compositions are administered intravenously or intraperitoneally by a bolus injection (see, e.g., U.S. Pat. No. 5,286,634). The lipid nucleic acid particles can be administered by direct injection at the site of disease or by injection at a site distal from the site of disease (see, e.g., Culver, HUMAN GENE THERAPY, MaryAnn Liebert, Inc., Publishers, New York. pp. 70-71(1994)). 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.

1. In Vivo Administration

[0249] 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.

[0250] 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, patches, 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.

[0251] The compounds of the invention can in general be given by internal administration in cases wherein systemic glucocorticoid receptor agonist therapy is indicated.

[0252] 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).

[0253] In one embodiment, the invention features the use of methods to deliver the siNA molecules of the instant invention to hematopoietic cells, including monocytes and lymphocytes. These methods are described in detail by Hartmann *et al.*, 1998, *J. Phamacol. Exp. Ther.*, 285(2), 920-928; Kronenwett *et al.*, 1998, *Blood*, 91(3), 852-862; Filion and Phillips, 1997, *Biochim. Biophys. Acta.*, 1329(2), 345-356; Ma and Wei, 1996, *Leuk. Res.*, 20(11/12), 925-930; and Bongartz *et al.*, 1994, *Nucleic Acids Research*, 22(22), 4681-8.

[0254] In one embodiment, the siNA molecules of the invention and formulations or compositions thereof are administered directly or topically (e.g., locally) to the dermis or follicles as is generally known in the art (see for example Brand, 2001, *Curr. Opin. Mol. Ther.*, 3, 244-8; Regnier *et al.*, 1998, *J. Drug Target*, 5, 275-89; Kanikkannan, 2002, *BioDrugs*, 16, 339-47; Wraight *et al.*, 2001, *Pharmacol. Ther.*, 90, 89-104; and Preat and Dujardin, 2001, STP PharmaSciences, 11, 57-68). In one embodiment, the siNA molecules of the invention and formulations or compositions thereof are administered directly or topically using a hydroalcoholic gel formulation comprising an alcohol (e.g., ethanol or isopropanol), water, and optionally including additional agents such isopropyl myristate and carbomer 980.. In other embodiments, the siNA are formulated to be administered topically to the nasal cavity.. Topical preparations can be administered by one or more applications per day to the affected area; over skin areas occlusive dressings can advantageously be used. Continuous or prolonged delivery can be achieved by an adhesive reservoir system.

[0255] In one embodiment, an 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.

[0256] In one embodiment, the siNA molecules of the invention and formulations or compositions thereof are administered to the lung as is described herein and as is generally known in the art. In another embodiment, the siNA molecules of the invention and formulations or compositions thereof are administered to lung tissues and cells as is described in U.S. Patent Publication Nos. 2006/0062758; 2006/0014289; and 2004/0077540.

2. Aerosols and Delivery Devices

a. Aerosol Formulations

[0257] The compositions of the present invention, either alone or in combination with other suitable components, can be made into aerosol formulations (i.e., they can be "nebulized") to be administered via inhalation (e.g., intranasally or intratracheally) (see, Brigham et al., *Am. J. Sci.,* 298:278 (1989)). Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

[0258] In one embodiment, the siNA molecules of the invention and formulations thereof 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 siNA 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.

[0259] Spray compositions comprising siNA molecules or compositions of the invention can, for example, be formulated as aqueous solutions or suspensions or as aerosols delivered from pressurized packs, such as a metered dose inhaler, with the use of a suitable liquefied propellant. In one embodiment, aerosol compositions of the invention suitable for inhalation can be either a suspension or a solution and generally contain an siNA molecule comprising at least 15 nucleotides of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 10, SEQ ID NO: 144, SEQ ID NO: 11, SEQ ID NO: 145, SEQ ID NO: 15, SEQ ID NO: 146, SEQ ID NO: 18, SEQ ID NO: 147, SEQ ID NO: 42, SEQ ID NO: 148, SEQ ID NO: 38, or SEQ ID NO: 150; or comprising SEQ ID NO: 43 and SEQ ID NO: 44, or SEQ ID NO: 61 and SEQ ID NO: 62, or SEQ ID NO: 63 and SEQ ID NO: 64, or SEQ ID NO: 71 and SEQ ID NO: 72, or SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 125 and SEQ ID NO: 126, or SEQ ID NO: 117 and SEQ ID NO: 118, or formula (A), and a suitable propellant such as a fluorocarbon or hydrogen-containing chlorofluorocarbon or mixtures thereof, particularly hydrofluoroalkanes, especially 1,1,1,2-tetrafluoroethane, 1,1,1,2,3,3,3-heptafluoro-n-propane or a mixture thereof. The aerosol composition can optionally contain additional formulation excipients well known in the art such as surfactants. Non-limiting examples include oleic acid, lecithin or an oligolactic acid or derivative such as those described in WO94/21229 and WO98/34596 and co-solvents for example ethanol. In one embodiment a pharmaceutical aerosol formulation of the invention comprising a compound of the invention and a fluorocarbon or hydrogen-containing chlorofluorocarbon or mixtures thereof as propellant, optionally in combination with a surfactant and/or a co-solvent.

[0260] The aerosol formulations of the invention can be buffered by the addition of suitable buffering agents.

[0261] Aerosol formulations can include optional additives including preservatives if the formulation is not prepared sterile. Non-limiting examples include, methyl hydroxybenzoate, anti-oxidants, flavorings, volatile oils, buffering agents and emulsifiers and other formulation surfactants. In one embodiment, fluorocarbon or perfluorocarbon carriers are used to reduce degradation and provide safer biocompatible non-liquid particulate suspension compositions of the invention (e.g., siNA and/or LNP formulations thereof). In another embodiment, a device comprising a nebulizer delivers a composition of the invention (e.g., siNA and/or LNP formulations thereof) comprising fluorochemicals that are bacteriostatic thereby decreasing the potential for microbial growth in compatible devices.

[0262] Capsules and cartridges comprising the composition of the invention for use in an inhaler or insufflator, of for example gelatine, can be formulated containing a powder mix for inhalation of a compound of the invention and a suitable powder base such as lactose or starch. In one embodiment, each capsule or cartridge contain an siNA molecule comprising at least 15 nucleotides of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 10, SEQ ID NO: 144, SEQ ID NO: 11, SEQ ID NO: 145, SEQ ID NO: 15, SEQ ID NO: 146, SEQ ID NO: 18, SEQ ID NO: 147, SEQ ID NO: 42, SEQ ID NO: 148, SEQ ID NO: 38, or SEQ ID NO: 150; or comprising SEQ ID NO: 43 and SEQ ID NO: 44, or SEQ ID NO: 61 and SEQ ID NO: 62, or SEQ ID NO: 63 and SEQ ID NO: 64, or SEQ ID NO: 71 and SEQ ID NO: 72, or SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 125 and SEQ ID NO: 126, or SEQ ID NO: 117 and SEQ ID NO: 118, or formula (A),and one or more excipients. In another embodiment, the compound of the invention can be presented without excipients such as lactose

[0263] The aerosol compositions of the present invention can 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. In one embodiment, the particulate range can be from 1 to 5 microns. In another embodiment, the particulate range can be from 2 to 3 microns. 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.

[0264] In some embodiments, an siNA composition of the invention is administered topically to the nose for example, for the treatment of rhinitis, via pressurized aerosol formulations, aqueous formulations administered to the nose by pressurized pump or by nebulization. Suitable formulations contain water as the diluent or carrier for this purpose. In certain embodiments, the aqueous formulations for administration of the composition of the invention to the lung or nose can be provided with conventional excipients such as buffering agents, tonicity modifying agents and the like.

b. Devices

[0265] The siNA molecules of the invention can be formulated and delivered as particles and/or aerosols as discussed above and dispensed from various aerosolization devices known by those of skill in the art.

[0266] Aerosols of liquid or non-liquid particles comprising an siNA molecule or formulation of the invention can be produced by any suitable means, such as with a device comprising a nebulizer (see for example US 4,501,729) such as ultrasonic or air jet nebulizers. In one embodiment, the nebulizer for administering an siNA molecule of the invention, relies on oscillation signals to drive a piezoelectric ceramic oscillator for producing high energy ultrasonic waves which mechanically agitate a composition of the invention (e.g., siNA and/or LNP formulations thereof) generating a medicament aerosol cloud. (See for example, U.S. Pat. Nos. 7,129, 619 B2 and 7,131,439 B2). In another embodiment, the nebulizer relies on air jet mixing of compressed air with a composition of the invention (e.g., siNA and/or LNP formulations thereof) to form droplets in an aerosol cloud.

[0267] Nebulizer devices used with the siNA molecules or formulations of the invention can use carriers, typically water or a dilute aqueous or non-aqueous solution comprising siNA molecules of the invention.. One embodiment of the invention is a device comprising a nebulizer that uses an alcoholic solution, preferably made isotonic with body fluids by the addition of, for example, sodium chloride or other suitable salts which comprises an siNA molecule or formulation of the invention. In another embodiment, the nebulizer devices comprises one or more non-aqueous fluorochemical carriers comprising an siNA molecule or formulation of the invention.

[0268] Solid particle aerosols comprising an siNA molecule or formulation of the invention and surfactant can be produced with any solid particulate aerosol generator. In one embodiment, aerosol generators are used for administering solid particulate agents to a subject. These generators produce particles which are respirable, as explained below, as a predetermined metered dose of a composition. Certain embodiments of the invention comprise an aerosol comprising a combination of particulates having at least one siNA molecule or formulation of the invention with a pre-determined volume of suspension medium or surfactant to provide a respiratory blend. Other embodiments of the invention, comprise an aerosol generator that comprises an siNA molecule or formulation of the invention.

[0269] One type of solid particle aerosol generator used with the siNA molecules 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. A second type of illustrative aerosol generator comprises a metered dose inhaler ("MDI")

[0270] MDIs are pressurized aerosol dispensers, typically containing a suspension or solution formulation of the active ingredient in a liquefied 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 US Patent Nos. 6,592,904; 6,582,728; 6,565,885..

[0271] The canisters of a MDI typically comprise a container capable of withstanding the vapor pressure of the propellant used, such as a plastic or plastic-coated glass bottle or preferably a metal can, for example, aluminum or an alloy thereof which can optionally be anodized, lacquer-coated and/or plastic-coated (for example incorporated herein by reference WO96/32099 wherein part or all of the internal surfaces are coated with one or more fluorocarbon polymers optionally in combination with one or more non-fluorocarbon polymers, such as for example, but not limitation, a polymer blend of polytetrafluoroethylene (PTFE) and polyethersulfone (PES)), which container is closed with a metering valve. The metering valves are designed to deliver a metered amount of the formulation per actuation and incorporate a gasket to prevent leakage of propellant through the valve. The gasket can comprise any suitable elastomeric material such as, for example, low density polyethylene, chlorobutyl, bromobutyl, EPDM, black and white butadiene-acrylonitrile rubbers, butyl

rubber and neoprene. Suitable valves are commercially available from manufacturers well known in the aerosol industry, for example, from Valois, France (e.g. DF10, DF30, DF60), Bespak plc, UK (e.g. BK300, BK357) and 3M-Neotechnic Ltd, UK (e.g. SpraymiserTM).

[0272] MDIs containing siNA molecules or formulations taught herein can be prepared by methods of the art (for example, see Byron, above and WO96/32099).

[0273] The MDIs used with the siNA molecules of the invention can also be used in conjunction with other structures such as, without limitation, overwrap packages for storing and containing the MDIs, including those described in U.S. Patent Nos. 6,119,853; 6,179,118; 6,315,112; 6,352,152; 6,390,291; and 6,679,374, as well as dose counter units such as, but not limited to, those described in U.S. Patent Nos. 6,360,739 and 6,431,168.

[0274] The siNA molecules can also be formulated as a fluid formulation for delivery from a fluid dispenser, for example a fluid dispenser having a dispensing nozzle or dispensing orifice through which a metered dose of the fluid formulation is dispensed upon the application of a user-applied force to a pump mechanism of the fluid dispenser. In one embodiment of the invention are provided fluid dispensers, which use reservoirs of multiple metered doses of a fluid formulation, the doses being dispensable upon sequential pump actuations, and which comprise siNA molecules or formulations of the invention. In certain embodiments, the dispensing nozzle or orifice of the dispenser can be configured for insertion into the nostrils of the user for spray dispensing of the fluid formulation comprising siNA molecules or formulations into the nasal cavity. A fluid dispenser of the aforementioned type is described and illustrated in WO05/044354,. The dispenser has a housing which houses a fluid discharge device having a compression pump mounted on a container for containing a fluid formulation. In various embodiments, the housing of the dispenser has at least one finger-operable side lever which is movable inwardly with respect to the housing to cam the container upwardly in the housing to cause the pump to compress and pump a metered dose of the formulation out of a pump stem through a nasal nozzle of the housing. In another embodiment, the fluid dispenser is of the general type illustrated in Figures 30-40 of WO05/044354.

[0275] In certain embodiments of the invention, nebulizer devices are used in applications for conscious, spontaneously breathing subjects, and for controlled ventilated subjects of all ages. The nebulizer devices can be used for targeted topical and systemic drug delivery to

the lung. In one embodiment, a device comprising a nebulizer is used to deliver an siNA molecule or formulation of the invention locally to lung or pulmonary tissues. In another embodiment, a device comprising a nebulizer is used to deliver a an siNA molecule or formulation of the invention systemically.

[0276] In other embodiments, nebulizer devices are used to deliver respiratory dispersions comprising emulsions, microemulsions, or submicron and nanoparticulate suspensions of at least one active agent. (See for example U.S. Pat. No. 7128,897 and 7,090,830 B2,).

[0277] Nebulizer devices can be used to administer aerosols comprising as siNA molecule or formulation of the invention continuously or periodically and can be regulated manually, automatically, or in coordination with a patient's breathing. (See U.S. Pat. No. 3,812,854, WO 92/11050). For example, periodical administer a siNA molecule of the invention can given as a single-bolus via a microchannel extrusion chamber or via cyclic pressurization. Administration can be once daily or several times daily, for example 2, 3, 4 or 8 times, giving for example 1, 2 or 3 doses each time. The overall daily dose and the metered dose delivered by capsules and cartridges in an inhaler or insufflator will generally be double that delivered with aerosol formulations.

H. Other Applications/Uses of siNA Molecules of the Invention

[0278] The siNA molecules of the invention can also be used for diagnostic applications, research applications, and/or manufacture of medicants.

[0279] In one aspect, 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.

[0280] In one embodiment, siNA molecules of the invention are used to down regulate or inhibit the expression of Bach1 proteins arising from haplotype polymorphisms that are associated with a trait, disease or condition in a subject or organism. Analysis of Bach1 genes, or Bach1 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 Bach1 protein or RNA levels directly or

indirectly can be used to determine treatment type and the course of therapy in treating a subject. Monitoring of Bach1 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 Bach1 proteins associated with a trait, disorder, condition, or disease.

[0281] In another embodiment, the invention comprises use of a double-stranded nucleic acid according to the invention for use in the manufacture of a medicament. In an embodiment, the medicament is for use in treating a condition that is mediated by the action, or by loss of action, of Bach1. In one embodiment, the medicament is for use for the treatment of a respiratory disease. In an embodiment the medicament is for use for the treatment of a respiratory disease selected from the group consisting of COPD, cystic fibrosis, asthma, eosinophilic cough, bronchitis, sarcoidosis, pulmonary fibrosis, rhinitis, and sinusitis. In a particular embodiment, the use is for the treatment of a respiratory disease selected from the group consisting of COPD, cystic fibrosis, and asthma.

[0282] In certain embodiments, siNAs 29961-DC, 29964-DC, 29947-DC, 29988-DC, 29984-DC, 29956-DC, and 29957-DC, and siNAs wherein at least one strand comprises at least 15 nucleotides of SEQ ID NO: 1, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID NO: 38, SEQ ID NO: 42, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145; SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, or SEQ ID NO: 150, and the siNAs comprising Formula A are for use in a method for treating respiratory disease, such as, for example but not limitation, COPD, cystic fibrosis, asthma, eosinophilic cough, bronchitis, sarcoidosis, pulmonary fibrosis, rhinitis, and sinusitis

I. Examples

[0283] The invention will now be illustrated with the following non-limiting examples. Those of skill in the art will readily recognize a variety of non-critical parameters which can be changed or modified to yield essential the same results.

Example 1: Design, Synthesis, and Identification of siNAs Active Against Bach1

Bach1 siNA Synthesis

[0284] A series of 42 siNA molecules were designed, synthesized and evaluated for efficacy against Bach1. The primary criteria for design of Bach1 for human siNAs were (i) homology between two species (human and mouse) and (ii) high efficacy scores as determined by a proprietary algorithm. Mouse sequences were also looked at for use in animal models. The effects of the siNAs on Bach1 RNA levels and their effect on the level of heme oxygenase-1 (HMOX-1) mRNA were also examined. The sequences of the siNAs that were designed, synthesized, and evaluated for efficacy against Bach1 are described in **Table 1a** (target sequences) and **Table 1b** (modified sequences).

Table 1a: Bach1 Target Sequences, noting target sites. The Homology column indicates perfect homology of the siRNA with the human transcript (h), with only the mouse transcript (m) to both the human and mouse transcript (hm) or with the number of mismatches (1 or 2 mm m) to a specific transcript (e.g., h 1 mm m, means perfect homology to the human transcript with one mismatch to the mouse)..

Duplex ID	Target Sequence	Target Site	Homology	SEQ ID NO:
29947-DC	GGAAUCCUGCUUUCAGUUU	472	h 1mm m	1
29948-DC	GCGAGAAGUGGCAGAACAC	1285	h 2mm m	2
29949-DC	GUGUAAACUCCGCAGGUAU	664	h	3
29950-DC	GAGGAAUCCUGCUUUCAGU	470	h 1mm m	4
29951-DC	GCCUUUGUCAGGUACAGAC	1162	h	5
29952-DC	CAUAUGAGUCCAUGUGCUU	732	h	6
29953-DC	CACAUAUGACCAAUAUGGU	1096	h	7
29954-DC	CAGAACAGAUCUCACAGAA	1389	h	8
29955-DC	GAGUCCAUGUGCUUAGAGA	737	h	9
29956-DC	GUCUGAGUGUCCGUGGUUA	1411	h	10
29957-DC	GCAGUUACUUCCACUCAAG	282	h 2mm m	11
29958-DC	GUCAAAGACAUUCAUGCUU	851	h	12
29959-DC	GAGUGUCCGUGGUUAGGUA	1415	h 2mm m	13
29960-DC	CCAAAGAUGGCUCAGAACA	1377	h 2mm m	14
29961-DC	CUACACUGCUAAACUGAUU	388	h 2mm m	15
29962-DC	CAGGGAAGAUAGUAGUGUU	1240	h	16
29963-DC	GGAUUUGAACCUUUAAUUC	362	h	17
29964-DC	GAUUUGCAGGUGAUGUUAA	929	h	18
29965-DC	GACCAGAGGGAUCUAGAAA	596	h	19
29966-DC	GGCCAAAGAUGGCUCAGAA	1375	h 2mm m	20
29967-DC	GAGUUUCUAAGCGUACACA	494	m	21
29968-DC	GCAGAUGAAUUCUUGGAAA	671	m	22
29969-DC	GAUUUCCAAUCCUUGUUGA	1790	m	23
29970-DC	GAGUGUCCCUGGUUGGGUA	1472	m 2mm h	24
29971-DC	GUAGCUUUCUGUUGAGGGA	2488	m	25
29972-DC	GAUUUAUAUCUGAAGUCUA	1262	m	26
29973-DC	CUCACGAAAUGAUUUCCAA	1780	m	27
29974-DC	GAGGUGAAGCUGCCAUUCA	1739	m 2mm h	28

29975-DC	GUCGCAAGAGGAAACUUGA	1893	m 1mm h	29
29976-DC	CUGCUCAAGCAACUUGGAA	1585	m 2mm h	30
29977-DC	GAGCCUUGCCCGUAUGCUU	1640	m 2mm h	31
29978-DC	GGUUAAAGGAUUUGAACCU	403	m 1mm h	32
29979-DC	CGGACUUUCACAACUCUCA	1523	m	33
29980-DC	CGAGAAGCUGCAAAGUGAA	1939	m 1mm h	34
29981-DC	CAGCUACUUCCACUCGAGA	331	m 2mm h	35
29982-DC	CAUUCAAUGCCCAACGGAU	1752	m 1mm h	36
29983-DC	GAUUUGAACCUUUAAUUCA	363	hm	37
29984-DC	GUUAAAGGAUUUGAACCUU	356	hm	38
29985-DC	AGGCUUCUGGAGUGACAUU	1312	hm	39
29986-DC	GCUUCUGGAGUGACAUUUG	1314	hm	40
29987-DC	GGCUUCUGGAGUGACAUUU	1313	hm	41
29988-DC	AUUUGAACCUUUAAUUCAG	364	hm	42

[0285] For each oligonucleotide of a target sequence, the two individual, complementary strands of the siRNA were synthesized separately using solid phase synthesis, then purified separately by reversed phase solid phase extraction (SPE). The complementary strands were annealed to form the double strand (duplex) and delivered in the desired concentration and buffer of choice.

[0286] Briefly, the single strand oligonucleotides were synthesized using phosphoramidite chemistry on an automated solid-phase synthesizer, as is generally known in the art (see for example USSN 12/064,015). A synthesis column was packed with solid support derivatized with the first nucleoside residue. Synthesis was initiated by detritylation of the acid labile 5'-O-dimethoxytrityl group to release the 5'-hydroxyl. Phosphoramidite and a suitable activator in acetonitrile were delivered simultaneously to the synthesis column resulting in coupling of the amidite to the 5'-hydroxyl. The column was then washed with acetonitrile. Iodine solution was pumped through the column to oxidize the phosphite triester linkage P(III) to its phosphotriester P(V) analog. Unreacted 5'-hydroxyl groups were capped using reagents such as acetic anhydride in the presence of 2,6-lutidine and N-methylimidazole. The elongation cycle was resumed with the detritylation step for the next phosphoramidite incorporation. This process was repeated until the desired sequence was synthesized. The synthesis concluded with the final 5'-terminus protecting group (trityl or 5'-O-dimethoxytrityl).

[0287] Upon completion of the synthesis, the solid-support and associated oligonucleotide was dried under argon pressure or vacuum. Aqueous base was added and the mixture was

heated to effect cleavage of the succinyl linkage, removal of the cyanoethyl phosphate protecting group, and deprotection of the exocyclic amine protection.

[0288] The following process is performed on single strands that do not contain ribonucleotides. After treating the solid support with the aqueous base, the mixture is filtered to separate the solid support from the deprotected crude synthesis material. The solid support is then rinsed with water, which is combined with the filtrate. The resultant basic solution allows for retention of the 5'-O-dimethoxytrityl group to remain on the 5' terminal position (trityl-on).

[0289] For single strands that contain ribonucleotides, the following process was performed . After treating the solid support with the aqueous base, the mixture was filtered to separate the solid support from the deprotected crude synthesis material. The solid support was then rinsed with dimethylsulfoxide (DMSO), which was combined with the filtrate. Fluoride reagent, such as triethylamine trihydrofluoride, was added to the mixture, and the solution was heated. The reaction was quenched with suitable buffer to provide a solution of crude single strand with the 5'-O-dimethoxytrityl group on the final 5' terminal position.

[0290] The trityl–on solution of each crude single strand was purified using chromatographic purification, such as SPE RPC purification. The hydrophobic nature of the trityl group permits stronger retention of the desired full-length oligo than the non-tritylated truncated failure sequences. The failure sequences were selectively washed from the resin with a suitable solvent, such as low percent acetonitrile. Retained oligonucleotides were then detritylated on-column with trifluoroacetic acid to remove the acid-labile trityl group. Residual acid was washed from the column, a salt exchange was performed, and a final desalting of the material commenced . The full-length oligo was recovered in a purified form with an aqueous-organic solvent. The final product was then analyzed for purity (HPLC), identity (Maldi-TOF MS), and yield (UV A_{260}). The oligos were dried via lyophilization or vacuum condensation.

[0291] *Annealing:* Based on the analysis of the product, the dried oligos were dissolved in appropriate buffers followed by mixing equal molar amounts (calculated using the theoretical extinction coefficient) of the sense and antisense oligonucleotide strands. The solution was then analyzed for purity of duplex by chromatographic methods and desired final concentration. If the analysis indicated an excess of either strand, then the additional non-

excess strand was titrated until duplexing was complete. When analysis indicated that the target product purity has been achieved the material was delivered and ready for use.

[0292] Below is a table showing various siNAs synthesized using this protocol.

Table 1b. Bach1 siNA Strands Synthesized

Duplex ID	Target Site	SEQ ID NO:	Target Sequence	Modified Sequence	SEQ ID NO:
29947-DC	472	1	GGAAUCCUGCUUUCAGUUU	B *GGAA*uccu*G*cuuuc*AG*uuu *TT*B	43
29947-DC	472	1	GGAAUCCUGCUUUCAGUUU	AAAcuGAAAGcAGGAuuccUU	44
29948-DC	1285	2	GCGAGAAGUGGCAGAACAC	B *G*c*GAGAAG*u*GG*c*AGAA*cAc *TT*B	45
29948-DC	1285	2	GCGAGAAGUGGCAGAACAC	GUGuucuGccACuuCuCGcUU	46
29949-DC	664	3	GUGUAAACUCCGCAGGUAU	B *G*u*GuAAA*cucc*GcAGGuA*u *TT*B	47
29949-DC	664	3	GUGUAAACUCCGCAGGUAU	AUAccuGcGGAGuuuAcAcUU	48
29950-DC	470	4	GAGGAAUCCUGCUUUCAGU	B *GAGGAA*uccu*G*cuuuc*AG*u *TT*B	49
29950-DC	470	4	GAGGAAUCCUGCUUUCAGU	ACUGAAAGcAGGAuuccucUU	50
29951-DC	1162	5	GCCUUUGUCAGGUACAGAC	B *G*ccuuu*G*uc*AGGuAcAGA*c *TT*B	51
29951-DC	1162	5	GCCUUUGUCAGGUACAGAC	GUCuGuAccuGAcAAAGGcUU	52
29952-DC	732	6	CAUAUGAGUCCAUGUGCUU	B c*A*u*AuGAG*ucc*A*u*G*u*G*cuu *TT*B	53
29952-DC	732	6	CAUAUGAGUCCAUGUGCUU	AAGcAcAuGGAcucAuAuGUU	54
29953-DC	1096	7	CACAUAUGACCAAUAUGGU	B c*A*c*AuAuGAcc*A*AuAuGG*u *TT*B	55
29953-DC	1096	7	CACAUAUGACCAAUAUGGU	ACCAuAuuGGucAuAuGuGUU	56
29954-DC	1389	8	CAGAACAGAUCUCACAGAA	B c*AGAAcAGA*ucucA*cAGAA *TT*B	57
29954-DC	1389	8	CAGAACAGAUCUCACAGAA	UCCuGuGAGAucuGuucuGUU	58
29955-DC	737	9	GAGUCCAUGUGCUUAGAGA	B GAGucc*A*u*G*u*G*cuu*AGAGA *TT*B	59
29955-DC	737	9	GAGUCCAUGUGCUUAGAGA	UCUcuAAGcAcAuGGAcucUU	60
29956-DC	1411	10	GUCUGAGUGUCCGUGGUUA	B *G*ucu*GAG*u*G*ucc*G*u*GG*uu*A *TT*B	61
29956-DC	1411	10	GUCUGAGUGUCCGUGGUUA	UAAccAcGGAcAcucAGAcUU	62
29957-DC	282	11	GCAGUUACUUCCACUCAAG	B *G*c*AG*uuAcuucc*A*cuc*AAG *TT*B	63
29957-DC	282	11	GCAGUUACUUCCACUCAAG	CUUGAGuGGAAGuAAcuGcUU	64
29958-DC	851	12	GUCAAAGACAUUCAUGCUU	B *G*uc*AAAGA*c*A*uuc*A*u*G*cuu *TT*B	65
29958-DC	851	12	GUCAAAGACAUUCAUGCUU	AAGcAuGAAuGucuuuGAcUU	66
29959-DC	1415	13	GAGUGUCCGUGGUUAGGUA	B *GAG*u*G*ucc*G*u*GG*uu*AGG*u*A *TT*B	67
29959-DC	1415	13	GAGUGUCCGUGGUUAGGUA	UACcuAAccAcGGAcAcuCUU	68
29960-DC	1377	14	CCAAAGAUGGCUCAGAACA	B cc*AAAGA*u*GG*cuc*AGAAcA *TT*B	69
29960-DC	1377	14	CCAAAGAUGGCUCAGAACA	UGUucuGAGccAucuuuGGUU	70
29961-DC	388	15	CUACACUGCUAAACUGAUU	B cu*A*c*A*cu*G*cu*AAA*cu*GA*uu *TT*B	71
29961-DC	388	15	CUACACUGCUAAACUGAUU	AAUcAGuuuAGcAGuGuAGUU	72
29962-DC	1240	16	CAGGGAAGAUAGUAGUGUU	B c*AGGGAAGA*u*AG*u*AG*u*G*uu *TT*B	73
29962-DC	1240	16	CAGGGAAGAUAGUAGUGUU	AACAcuAcuAucuucccuGUU	74
29963-DC	362	17	GGAUUUGAACCUUUAAUUC	B *GGA*uuu*GAA*ccuuu*AA*uuc *TT*B	75
29963-DC	362	17	GGAUUUGAACCUUUAAUUC	GAAuuAAAGGuucAAAuccUU	76
29964-DC	929	18	GAUUUGCAGGUGAUGUUAA	B *GA*uuu*G*c*AGGuGA*u*G*uu*AA *TT*B	77
29964-DC	929	18	GAUUUGCAGGUGAUGUUAA	UUAAcAucAccuGcAAAucUU	78
29965-DC	596	19	GACCAGAGGGAUCUAGAAA	B *GA*cc*AGAGGGA*ucu*AGAAA *TT*B	79
29965-DC	596	19	GACCAGAGGGAUCUAGAAA	UUUcuAGAucccucuGGucUU	80
29966-DC	1375	20	GGCCAAAGAUGGCUCAGAA	B *GG*cc*AAAGA*u*GG*cuc*AGAA *TT*B	81

29966-DC	1375	20	GGCCAAAGAUGGCUCAGAA	UUCuGAGccAucuuuGGccUU	82
29967-DC	494	21	GAGUUUCUAAGCGUACACA	B *GAG*uuucu*AAGcGuAcAcA TT*B	83
29967-DC	494	21	GAGUUUCUAAGCGUACACA	UGUGuAcGcuuAGAAAcucUU	84
29968-DC	671	22	GCAGAUGAAUUCUUGGAAA	B *GcAGAuGAA*uucuu*GGAAA TT*B	85
29968-DC	671	22	GCAGAUGAAUUCUUGGAAA	UUUccAAGAAuucAucuGcUU	86
29969-DC	1790	23	GAUUUCCAAUCCUUGUUGA	B *GA*uuucc*AA*ucc*uu*G*uuGA TT*B	87
29969-DC	1790	23	GAUUUCCAAUCCUUGUUGA	UCAAcAAGGAuuGGAAAucUU	88
29970-DC	1472	24	GAGUGUCCCUGGUUGGGUA	B *GAGuGucccuGGuuGGGuA TT*B	89
29970-DC	1472	24	GAGUGUCCCUGGUUGGGUA	UACccAAccAGGGAcAcucUU	90
29971-DC	2488	25	GUAGCUUUCUGUUGAGGGA	B *GuAG*cuuucu*Guu GAGGGA TT*B	91
29971-DC	2488	25	GUAGCUUUCUGUUGAGGGA	UCCcucAAcAGAAAGcuAcUU	92
29972-DC	1262	26	GAUUUAUAUCUGAAGUCUA	B *GA*uuu*Au*Aucu*GAAG*ucu*A TT*B	93
29972-DC	1262	26	GAUUUAUAUCUGAAGUCUA	UAGAcuucAGAuAuAAAucUU	94
29973-DC	1780	27	CUCACGAAAUGAUUUCCAA	B cuc*Ac GAAAuGA*uuuccc*AA TT*B	95
29973-DC	1780	27	CUCACGAAAUGAUUUCCAA	UUGGAAAucAuuucGuGAGUU	96
29974-DC	1739	28	GAGGUGAAGCUGCCAUUCA	B *GAGGuGAAGcuGccAuucA TT*B	97
29974-DC	1739	28	GAGGUGAAGCUGCCAUUCA	UGAAuGGcAGcuucAccucUU	98
29975-DC	1893	29	GUCGCAAGAGGAAACUUGA	B *GucGcAAGAGGAAA*cuu*GA TT*B	99
29975-DC	1893	29	GUCGCAAGAGGAAACUUGA	UCAAGuuuccucuuGcGAcUU	100
29976-DC	1585	30	CUGCUCAAGCAACUUGGAA	B cu*Gcuc AAGcAAcuu GGAA TT*B	101
29976-DC	1585	30	CUGCUCAAGCAACUUGGAA	UUCcAAGuuGcuuGAGcAGUU	102
29977-DC	1640	31	GAGCCUUGCCCGUAUGCUU	B *GAG*ccuu*GcccGuAuGcuu TT*B	103
29977-DC	1640	31	GAGCCUUGCCCGUAUGCUU	AAGcAuAcGGGcAAGGcucUU	104
29978-DC	403	32	GGUUAAAGGAUUUGAACCU	B *GGuuAAAGGA*uuu*GAAcc u TT*B	105
29978-DC	403	32	GGUUAAAGGAUUUGAACCU	AGGuucAAAuccuuuAAccUU	106
29979-DC	1523	33	CGGACUUUCACAACUCUCA	B c*GGA*cuuuc*AcAA*cucuc*A TT*B	107
29979-DC	1523	33	CGGACUUUCACAACUCUCA	UGAGAGuuGuGAAAGuccGUU	108
29980-DC	1939	34	CGAGAAGCUGCAAAGUGAA	B c*GAGAAGcuGcAAAGuGAA TT*B	109
29980-DC	1939	34	CGAGAAGCUGCAAAGUGAA	UUCAcuuuGcAGcuucucGUU	110
29981-DC	331	35	CAGCUACUUCCACUCGAGA	B c*AGcuAcuucc Acuc GAGA TT*B	111
29981-DC	331	35	CAGCUACUUCCACUCGAGA	UCUcGAGuGGAAGuAGcuGUU	112
29982-DC	1752	36	CAUUCAAUGCCCAACGGAU	B c*AuucAAuGcccAAcGGA*u *TT*B	113
29982-DC	1752	36	CAUUCAAUGCCCAACGGAU	AUCcGuuGGGcAuuGAAuGUU	114
29983-DC	363	37	GAUUUGAACCUUUAAUUCA	B *GA*uuu*GAA*ccuuu*AA*uuc*A TT*B	115
29983-DC	363	37	GAUUUGAACCUUUAAUUCA	UGAAuuAAAGGuucAAAucUU	116
29984-DC	356	38	GUUAAAGGAUUUGAACCUU	B *GuuAAAGGA*uuu*GAA*ccuu *TT*B	117
29984-DC	356	38	GUUAAAGGAUUUGAACCUU	AAGGuucAAAuccuuuAAcUU	118
29985-DC	1312	39	AGGCUUCUGGAGUGACAUU	B *AGG*cuucu*GGA*Gu*GA*cAuu *TT*B	119
29985-DC	1312	39	AGGCUUCUGGAGUGACAUU	AAUGucAcuccAGAAGccuUU	120
29986-DC	1314	40	GCUUCUGGAGUGACAUUUG	B *G*cuucu*GGA*Gu*GA*cAuuu *TT*B	121
29986-DC	1314	40	GCUUCUGGAGUGACAUUUG	CAAAuGucAcuccAGAAGcUU	122
29987-DC	1313	41	GGCUUCUGGAGUGACAUUU	B *GG*cuucu*GGA*Gu*GA*cAuuu *TT*B	123
29987-DC	1313	41	GGCUUCUGGAGUGACAUUU	AAAuGucAcuccAGAAGccUU	124
29988-DC	364	42	AUUUGAACCUUUAAUUCAG	B *A*uuu*GAA*ccuuu*AA*uuc*AG TT*B	125
29988-DC	364	42	AUUUGAACCUUUAAUUCAG	CUGAAuuAAAGGuucAAAuUU	126

wherein:
A, C, G, and U = ribose A, C, G or U
c and u = 2'-deoxy-2'-fluoro C or U
A, U and G = 2'-O-methyl (2'-OMe) A U or G

A and *G* = deoxy A or G
B = inverted abasic
T = thymidine

Further Synthesis Steps for Commercial Preparation

[0293] Once analysis indicates that the desired product purity has been achieved after the annealing step, the material is transferred to the tangential flow filtration (TFF) system for concentration and desalting, as opposed to doing this prior to the annealing step.

[0294] Ultrafiltration: The annealed product solution is concentrated using a TFF system containing an appropriate molecular weight cut-off membrane. Following concentration, the product solution is desalted via diafiltration using Milli-Q water until the conductivity of the filtrate is that of water.

[0295] Lyophilization: The concentrated solution is transferred to a bottle, flash frozen and attached to a lyophilizer. The product is then freeze-dried to a powder. The bottle is removed from the lyophilizer and is now ready for use.

Initial Screening Protocol (96-Well Plate Transfections)

Cell Culture Preparation:

[0296] All cells were obtained from ATCC (Manassas, VA) unless otherwise indicated. Cells were grown and transfected under standard conditions, which are detailed below for each cell line.

[0297] A549 (human; ATCC cat# CCL-185): Cells were cultured at 37°C in the presence of 5% CO_2 and grown in Ham's F12K medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and supplemented with fetal bovine serum at a final concentration of 10%, 100µg/mL of streptomycin, and 100U/mL penicillin.

[0298] NIH 3T3 (mouse; ATCC cat# CRL-1658): Cells were cultured at 37°C in the presence of 5% CO_2 and grown in Dulbecco's modified Eagle's medium (DMEM) with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose and supplemented with fetal bovine serum at a final concentration of 10%, 100µg/mL of streptomycin, and 100U/mL penicillin.

Transfection and Screening

[0299] Cells were plated in all wells of a tissue-culture treated, 96-well plate at a final count of 5000 cells/well in 100µL of the appropriate culture media. The cells were cultured for 24 hours after plating at 37°C in the presence of 5% CO_2.

[0300] After 24 hours, complexes containing siNA and RNAiMax were created as follows: A solution of RNAiMax diluted 33-fold in OPTI-MEM was prepared. In parallel, solutions of the siNAs for testing were prepared to a final concentration of 120 nM in OPTI-MEM. After incubation of RNAiMax/OPTI-MEM solution at room temperature for 5 min, an equal volume of the siNA solution and the RNAiMax solution were added together for each of the siNAs.

[0301] Mixing resulted in a solution of siNA/RNAiMax where the concentration of siNA was 60 nM. This solution was incubated at room temperature for 20 minutes. After incubation, 20 uL of the solution was added to each of the relevant wells. The final concentration of siNA in each well was 10 nM and the final volume of RNAiMax in each well was 0.3ul.

[0302] The time of incubation with the RNAiMax-siNA complexes was 48 hours and there was no change in media between transfection and harvesting, unless otherwise indicated.

RNA Isolation and Reverse Transcription(96-Well Plate)

[0303] RNA was extracted from a 96-well plate using the TaqMan® Gene Expression Cells-to-CT™ Kit (Cat# 4399002) with a modified protocol. Briefly, a 60uL (1 plate) or 110uL (2 plates) of the Lysis Solution with DNase I was dispensed into each well of the Lysis Buffer Plate (twin.tec full skirt plate). The lysis buffer and stop plates were stored at 4°C until the cells were washed.

[0304] The plate was spun at 1100 rpm for 5 minutes. The culture medium was aspirated and discarded from the wells of the culture plate. The lysis was performed automatically using a BioMek FX instrument and method. After the Biomek method was completed, the lysis plate was incubated for 2 min. at room temperature. The lysis plate can be stored for 2 hours at 4°C, or at -20 °C or -80 °C for two months.

[0305] Each well of the reverse transcription plate required 10uL of 2X reverse transcriptase Buffer, 1uL of 20X reverse transcription enzyme and 2uL of nuclease-free water. The reverse transcription master mix was prepared by mixing 2X reverse transcription buffer, 20X reverse transcription enzyme mix, and nuclease-free water. 13uL of the reverse transcription master mix was dispensed into each well of the reverse transcription plate (semi-skirted). A separate reverse transcription plate was prepared for each cell plate. The plate was loaded onto a Biomek NX or Biomek FX Dual -96 and the Biomek method was run. The program is programmed to automatically added 7uL of lysate from the cell lysis procedure described above into each well of the reverse transcription plate. The plate is sealed and spun on a centrifuge (1000rpm for 30 seconds) to settle the contents to the bottom of the reverse transcription plate. The plate is placed in a thermocycler at 37 °C for 60 min, 95 °C for 5 min, and 4 °C until the plate is removed from the thermocycler. Upon removal, if not used immediately, the plate was frozen at -20 °C.

Quantitative RT-PCR (Taqman)

[0306] A series of probes and primers were used to detect the various mRNA transcripts of the genes of Bach1, HMOX-1, and GAPDH in mouse and human cell lines. The assays were performed on an ABI 7900 instrument, according to the manufacturer's instructions. A TaqMan Gene Expression Master Mix (provided in the Cells-to-CT™, Applied Biosystems, Cat # 4399002) was used. The PCR reactions were carried out at 50 °C for 2 min, 95 °C for 20 min followed by 40 cycles at 95 °C for 15 secs and 60 °C for 1 min.

[0307] Within each experiment, the baseline was set in the exponential phase of the amplification curve, and based on the intersection point of the baselines with the amplification curve, a Ct value was assigned by the instrument.

Calculations

[0308] The expression level of the gene of interest and % knock-down was calculated using Comparative Ct method:

$$\Delta Ct = Ct_{Target} - Ct_{GAPDH}$$

$$\Delta\Delta Ct = \Delta Ct_{(Target\ siRNA)} - \Delta Ct_{(NTC)}$$

$$\text{Relative expression level} = 2^{-\Delta\Delta Ct}$$

$$\% \text{ KD} = 100 \times (1 - 2^{-\Delta\Delta Ct})$$

[0309] To evaluate the expression level and % knock-down of GAPDH, STAT 4 gene was used as an endogenous control to calculate ΔCt for siNA treated samples and the universal control:

$$\Delta Ct = Ct_{GAPDH} - Ct_{STAT4}$$

[0310] STAT 4 gene was selected as an internal normalizer based on its relatively consistent expression across 49 test samples in three independent experiments. ΔΔCt, Relative expression level and %KD were calculated as described above.

[0311] The non-targeting control siNA was, unless otherwise indicated, chosen as the value against which to calculate the % knock-down, because it is the most relevant control.

[0312] Additionally, only normalized data, which reflects the general health of the cell and quality of the RNA extraction, was examined. This was done by looking at the level of two different mRNAs in the treated cells, the first being the target mRNA and the second being the normalizer mRNA. This allowed for elimination of siNAs that might be potentially toxic to cells rather than solely knocking down the gene of interest. This was done by comparing the Ct for GAPDH in each well relative to the Ct for the entire plate.

[0313] All calculations of IC_{50}s were performed using SigmaPlot 10.0 software. The data were analyzed using the sigmoidal dose-response (variable slope) equation for simple ligand binding. In all of the calculations of the % knock-down, the calculation was made relative to the normalized level of expression of the gene of interest in the samples treated with the non-targeting control (Ctrl siNA) unless otherwise indicated.

[0314] For the statistical measures of significance for the HMOX-1 mRNA increase in response to Bach1 siNA treatment, a two-tailed Student's t-test on the dCt values for wells treated with the non-targeting control siNA compared with the dCt values for wells treated with the highest concentration of each respective active siNA, was performed..

Results:

[0315] The Bach1 siNAs were designed and synthesized as described previously. The siNAs were screened in two cell lines. Human A549 cells and mouse NIH 3T3 cells. The

data from the screen of Bach1 siNAs in these species is shown in **Tables 2 and 3.** Each screen was performed at 24 hrs post-transfection.

Table 2: Summary of screening data (10nM) in human A549 Cells (n = 2). Quantitative RT-PCR was used to assess the level of Bach1 mRNA and the data were normalized to the expression level of GAPDH (a ubiquitously expressed 'house-keeping' gene), and each treatment was normalized against the non-targeting control. % KD is represented as mean ± S.D.

Duplex ID	Target Site	Homology	% KD
29947-DC	472	h 1mm m	83.4 ± 6.9
29948-DC	1285	h 2mm m	51.9 ± 3
29949-DC	664	h	25 ± 1.4
29950-DC	470	h 1mm m	90.6 ± 1.7
29951-DC	1162	h	39 ± 11.2
29952-DC	732	h	75 ± 0.9
29953-DC	1096	h	24 ± 14.1
29954-DC	1389	h	63.4 ± 0.9
29955-DC	737	h	14.6 ± 0.2
29956-DC	1411	h	75.8 ± 7.7
29957-DC	282	h 2mm m	84.6 ± 5.4
29958-DC	851	h	75.7 ± 8.6
29959-DC	1415	h 2mm m	68 ± 14.9
29960-DC	1377	h 2mm m	34 ± 3
29961-DC	388	h 2mm m	90 ± 1.3
29962-DC	1240	h	88 ± 0.7
29963-DC	362	h	32.9 ± 3.4
29964-DC	929	h	90.1 ± 0.8
29965-DC	596	h	34.4 ± 3.6
29966-DC	1375	h 2mm m	57.3 ± 5.9
29967-DC	494	m	34.6 ± 6.7
29968-DC	671	m	28.2 ± 3.7
29969-DC	1790	m	36 ± 7.3
29970-DC	1472	m 2mm h	46.8 ± 5.1
29971-DC	2488	m	23.9 ± 3.8
29972-DC	1262	m	-3.3 ± 27.4
29973-DC	1780	m	-8 ± 11.1
29974-DC	1739	m 2mm h	-4.3 ± 15.5
29975-DC	1893	m 1mm h	59 ± 8.3
29976-DC	1585	m 2mm h	17.5 ± 1.7
29977-DC	1640	m 2mm h	18.9 ± 0.3
29978-DC	403	m 1mm h	14.4 ± 0
29979-DC	1523	m	-18.1 ± 18
29980-DC	1939	m 1mm h	91.3 ± 0.9
29981-DC	331	m 2mm h	1 ± 0.9
29982-DC	1752	m 1mm h	-9.2 ± 4.6

29983-DC	363	hm	74.7 ± 1.8
29984-DC	356	hm	66.2 ± 15.3
29985-DC	1312	hm	18.6 ± 6.5
29986-DC	1314	hm	8.2 ± 2.3
29987-DC	1313	hm	16 ± 0.2
29988-DC	364	hm	86.2 ± 3.1

Table 3: Summary of screening data in mouse NIH3T3 Cells (n = 2).

Duplex ID	Target Site	Homology	% KD
29947-DC	472	h 1mm m	46 ± 5.8
29948-DC	1285	h 2mm m	-16.3 ± 12.6
29949-DC	664	h	-7.1 ± 5.2
29950-DC	470	h 1mm m	42.5 ± 6.4
29951-DC	1162	h	-22 ± 13.2
29952-DC	732	h	-15.7 ± 1.8
29953-DC	1096	h	-11 ± 3.3
29954-DC	1389	h	-10.2 ± 10.1
29955-DC	737	h	-12.2 ± 4.3
29956-DC	1411	h	-14.5 ± 8.4
29957-DC	282	h 2mm m	42.4 ± 1.2
29958-DC	851	h	-50.4 ± 6.8
29959-DC	1415	h 2mm m	-9.9 ± 3.8
29960-DC	1377	h 2mm m	5.9 ± 6.4
29961-DC	388	h 2mm m	-23.5 ± 4.6
29962-DC	1240	h	21.3 ± 2.2
29963-DC	362	h	18.5 ± 2.2
29964-DC	929	h	19.6 ± 3.9
29965-DC	596	h	4.8 ± 6.3
29966-DC	1375	h 2mm m	-12.7 ± 7.4
29967-DC	494	m	34.7 ± 8.4
29968-DC	671	m	71 ± 8.7
29969-DC	1790	m	72.7 ± 4.5
29970-DC	1472	m 2mm h	69.4 ± 4.8
29971-DC	2488	m	75 ± 4.7
29972-DC	1262	m	71.7 ± 5.5
29973-DC	1780	m	76.4 ± 5.3
29974-DC	1739	m 2mm h	-12 ± 6.9
29975-DC	1893	m 1mm h	40.4 ± 0.4
29976-DC	1585	m 2mm h	-1.2 ± 11.6
29977-DC	1640	m 2mm h	53 ± 2.9
29978-DC	403	m 1mm h	3.3 ± 3.8
29979-DC	1523	m	62.4 ± 2.6
29980-DC	1939	m 1mm h	49.2 ± 1.1
29981-DC	331	m 2mm h	3.5 ± 5
29982-DC	1752	m 1mm h	9 ± 8.9
29983-DC	363	hm	49 ± 2
29984-DC	356	hm	50.5 ± 0.8

29985-DC	1312	hm	1.3 ± 4.5
29986-DC	1314	hm	-8.7 ± 5.2
29987-DC	1313	hm	-16.1 ± 11.6
29988-DC	364	hm	71.3 ± 8.9

[0316] Summary data, as to potency and efficacy for Bach1 mRNA knock-down in human cells for certain siNA molecules, is presented in **Table 4**. Cells for the experiments were treated with 12 concentrations of siNA ranging from 0.083fM to 30nM. All of the data is presented as % knockdown of Bach1 normalized against the expression of GAPDH mRNA expression level. Then the data was determined as a % knockdown relative to the non-targeting control siNA.

Table 4. Summary of efficacy (%KD) and potency (IC_{50}) of the Bach1 mRNA knock-down by siRNA leads in human A549 cells. Data are from three separate experiments Values are mean ± standard deviation.

Duplex ID	**Homology**	**Maximum % KD in DRC**	**IC50 (pM)**
29947-DC	h (1 mm m)	86 ± 0.5	27 ± 5
29956-DC	h	80 ± 7.7	28 ± 18
29957-DC	h (2 mm m)	87 ± 0.9	39 ± 13
29961-DC	h (2 mm m)	89 ± 2.2	10 ± 4
29964-DC	h	87 ± 2.8	27 ± 15
29988-DC	hm	84 ± 0.9	63 ± 19

[0317] The specificity of these siNAs was also assessed. These siNAs were tested at a lower concentration (1 nM versus the original screening concentration of 10 nM) to determine the effect of the Bach1 siNAs on the mRNA transcript of human GAPDH. No significant reduction in GAPDH was observed in response to any of the Bach1 lead siNAs relative to changes seen in cells treated with universal control siNA. The data is shown in **Table 5.**

Table 5. Summary of Bach1 mRNA Screening Data and GAPDH Specificity Screen. This is data from three experiments.

siNA ID	**Homology**	**% KD Bach1**	**% KD GAPDH**
29947-DC	h (1 mm m)	83.4 ± 6.9	-15 ± 29

29956-DC	h	75.8 ± 7.7	-18 ± 15
29957-DC	h (2 mm m)	84.6 ± 5.4	-10 ± 9
29961-DC	h (2 mm m)	90 ± 1.3	7 ± 7
29964-DC	h	90.1 ± 0.8	14 ± 2
29988-DC	hm	86.2 ± 3.1	23 ± 5

[0318] Various siNAs were also evaluated for their potential to knock-down Bach1 protein. As no validated reagents were available to directly track changes in protein expression., this was done by assessing the increase in HMOX-1 mRNA in response to increasing doses of the active Bach1 siNAs.

[0319] The siNAs were tested at a single time point post-transfection (24 hrs). This was the time point at which the Bach1 mRNA levels had previously been determined. The non-targeting control siNA was also tested on each plate of samples and was found, across the six plates to show a maximum of 29 ± 12% increase in the level of HMOX-1.

[0320] The comparison of the maximum percentage increase in HMOX-1 and EC_{50} and the maximum percentage decrease of Bach1 and IC_{50} are shown in **Table 6**. **Table 6** also includes the measure of statistical significance for the increase in HMOX-1 mRNA levels in response to treatment with the respective Bach1 siNA.

Table 6. Comparison between maximum Bach1 mRNA reduction and IC_{50} and maximum HMOX-1 mRNA increase and EC_{50}. Values are mean ± SD.

Duplex ID	Homology	Maximum % KD in DRC	IC50 (pM) Bach1 mRNA	Maximum % Hmox-1 Increase in DRC	EC50 (pM) Hmox-1 mRNA	Hmox-1 increase p-value (by dCt)
29947-DC	h (1 mm m)	86 ± 0.5	27 ± 5	187 ± 71	37.7 ± 21.5	0.00094
29956-DC	h	80 ± 7.7	28 ± 18	308 ± 87	30.9 ± 19.9	0.00012
29957-DC	h (2 mm m)	87 ± 0.9	39 ± 13	213 ± 29	33.6 ± 20	0.00042
29961-DC	h (2 mm m)	89 ± 2.2	10 ± 4	262 ± 87	10.4 ± 10.0	0.00041
29964-DC	h	87 ± 2.8	27 ± 15	240 ± 30	21.8 ± 15.5	0.00046
29988-DC	hm	84 ± 0.9	63 ± 19	190 ± 62	38.2 ± 29.8	0.00058

Example 2: In Vitro Assessment of siNAs in Human Bronchial Epithelial Cells

[0321] The siNAs with Duplex ID numbers corresponding to 29961-DC, 29964-DC, 29984-DC, 29988-DC, 29957-DC, 29947-DC, and 29956-DC were tested for maximum

Bach1 mRNA knockdown and for potency in human bronchial epithelial cells as follows.

Cell Culture Preparation:

[0322] Human Bronchial Epithelial cells (NHBE cells) obtained from Lonza (Cat. No. CC-2540) were grown at 37deg in the presence of 5% CO2 and cultured in BEBM basal medium (Lonza,Cat.No. CC-3171) on Biocoat Collagen1 coated flasks (Becton Dickinson).

Transfection:

[0323] NHBE cells were plated in collagen 1 coated plates and cultured in appropriate culture media. The cells were cultured for 24 hours after plating at 37 degrees in the presence of 5% CO2. siNAs were diluted in OptiMEM 1 to 1uM and the the transfection agent to 25ug/ml. For formulation of the siNAs equal volumes of the diluted siNA and delivery lipid were combined and incubated for 20 minutes at room temperature. Cells were meanwhile trypsinised and resuspended in antibiotic free BEBM media at 150,000 cells/ml. 20ul of the formulated siNA and 80ul of BEBM media was added per well of a 96 well plate (6 replicates/data point/siNA concentration) so as to give a nine point dose range of the siNAs (100nM, 30nM, 10nM, 3nM, 1nM, 0.3nM, 0.1nM, 0.03nM, 0.01nM). The time of incubation with the transfection-siNA complexes were 48 hours with one change of media at 24 hours.

RNA Isolation 96 well Plate:

[0324] Total RNA was isolated from the cells in the 96-well plate format using the Automated SV96 Total RNA Isolation System (Promega) according to the manufacturer's instructions. The Biomek 2000 Laboratory Automation Workstation (Beckman Coulter) was used to apply the transfected cell lysates to a silica membrane. RNase-Free DNase I was then applied directly to the silica membrane to digest contaminating genomic DNA. The bound total RNA was further purified from contaminating salts, proteins and cellular impurities by simple washing steps. Finally, total RNA was eluted from the membrane by the addition of Nuclease-Free Water.

Quantitative RT-PCR (TaqMan):

[0325] A series of probes and primers were used for the detection of mRNA transcripts of Bach1, OAS1, IL8 and GAPDH (as control/normalisation) in the human cell lines. The assays were performed on an ABI 7900HT instrument according to the manufacturer's instructions. Primer Probe sets used:

Table 7: Primer Probe Sets

Human Target	Primer Probe Sequence	SEQ ID NO:
GAPDH	Forward 5'-CAAGGTCATCCATGACAACTTTG-3'	151
GAPDH	Reverse 5'-GGGCCATCCACAGTCTTCT-3'	152
GAPDH	Probe 5'd FAM-ACCACAgTCCATgCCATCACTgCCA-TAMRA 3'	153
BACH1	Forward 5'- TGTGCGATGTCACCATCTTTG-3'	154
BACH1	Reverse 5'-CTTGAGTGGAAGTAACTGCTGCAT -3'	155
BACH1	Probe 5'd FAM-ACAGCGGTTCCGCGCTCACC -TAMRA 3'	156
HMOX1	Forward 5'-CCGCTCCCAGGCTCCGCTTC -3'	157
HMOX1	Reverse 5'-AGGGAAGCCCCCACTCAAC -3'	158
HMOX1	Probe 5'd FAM- ACTGTCGCCACCAGAAAGCT-TAMRA 3'	159
OAS1	Forward 5'-ACCTAACCCCCAAATCTATGTCAA-3'	160
OAS1	Reverse 5'-TGGAGAACTCGCCCTCTTTC-3'	161
OAS1	Probe 5'd FAM-CTCATCgAggAgTgCACCgACCTg-TAMRA 3'	162
IL8	Forward 5'-CTGGCCGTGGCTCTCTTG-3'	163
IL8	Reverse 5'-CCTTGGCAAAACTGCACCTT-3'	164
IL8	Probe 5'd FAM- CAGCCTTCCTGATTTCTGCAGTCTGTG-TAMRA 3'	165

TAMRA (Tetramethyl-6-carboxyrhodamine) is a quencher dye
FAM (carboxyfluorescein) is a reporter dye

Calculations:

[0326] With Taqman data critical threshold values (Ct) were converted to copy numbers corresponding to the particular gene analysed in each well of the 384 well plate. Six identical wells were prepared in each plate for a given treatment. Hence, an average gene copy number and standard deviation were calculated. Determination of the percentage coefficient of variation (CV) (%C.V.=[standard deviation/average]*100) allowed the omitting of wells whose value was an outlier

(so that %C.V.<25). Relative abundance (aka relative expression) of a gene was determined by dividing the mean copy number of that gene with its GAPDH counterpart in that particular sample.

Statistical Analysis of data:

[0327] EC50 values were calculated from the data using sigma plot. All calculations of the efficacy and potency of the siNAs were done relative to the non-targeting control siNA. Percentage knockdown was compared between the four lead siNA's. The data was analysed using an ANOVA test and then the p-values were corrected for multiple comparisons using the False Discovery Rate correction (FDR). A 95% confidence interval plot was also produced to show graphically where leads were significantly different from each other.

Results:

[0328] siNAs 29961-DC, 29964-DC, 29984-DC, 29988-DC, 29957-DC, 29947-DC, and 29956-DC (target sites 388, 929, 356, 364, 282, 472, and 1411, respectively) showed a maximum as well as a dose dependent knock-down (KD) of Bach1 mRNA in human normal bronchial epithelial cells (NHBEs), evidencing high efficacy and potency. The same siNAs were used to demonstrate a Hem Oxygenase (HO-1) mRNA up-regulation in relation to Bach1 mRNA knockdown. **Table 8** shows the mean data from three individual donors of NHBEs with siNAs targeting Bach1 at 48 hours post transfection.

Table 8: Knockdown and potency of Bach1 siNAs in human bronchial epithelial cells,(EC50 data, maximum mRNA knockdown data, EC50 HMOX1 data, and Maximum HMOX1 data each is a mean of 3 donors).

siNA ID	Target site	EC50	Maximum mRNA knockdown	EC50 HMOX1 upregulation	Maximum HMOX1 upregulation
29961-DC	388	7.87nM	80%	50.43nM	10266%
29964-DC	929	9.52nM	72%	30.27nM	4800%
29984-DC	356	23.16nM	65%	388nM	153%
29988-DC	364	3.84nM	70%	34.46nM	550%
29957-DC	282	0.95nM	70%	29.82nM	290%

29947-DC	472	4.31nM	65%	575nM	1566%
29956-DC	1411	2.16nM	68%	33.09nM	2416%

Example 3: Testing of siNAs for TLR3, TLR7 and TLR8 Mediated Immunostimulation

[0329] NHBE cell were treated as describe above in Example 2 and used for the measurement of endosomal TLR3 mediated immunostimulation, with the inclusion of polyI:C as a positive control for OAS1 mRNA upregulation. For the measurement of membrane bound TLR3 mediated immunostimulation, the NHBE cells were cultured at 1200 cells/ per 96 well and siNAs administered in PBS in the absence of a delivery vehicle at (100nM, 30nM, 10nM, 3nM, 1nM, 0.3nM, 0.1nM, 0.03nM, 0.01nM).

[0330] Cell surface TLR3 mediated immunostimulation responses were measured by recording the % increase in OAS1 mRNA levels when the NHBE cells were treated with the siNAs in the absence of a delivery vehicle. (% increase in OAS1 mRNA levels relative to the PBS dilutant).

[0331] To determine immunostimulation TLR3 mediated % increase in OAS1(immunostimulatory biomarker) mRNA levels, a nine point dose response was measured using 0.01-100nM concentration of the seven siNAs 29961-DC, 29964-DC, 29947-DC, 29988-DC, 29984-DC, 29956-DC and 29957-DC.

[0332] Endosomal TLR3 mediated immunostimulation was measured by recording the % increase in OAS1 mRNA levels when the NHBE cells were transfected with the siNAs (% increase in OAS1 mRNA levels relative to the transfection agent control). The TLR3 agonist Poly I:C is used as a positive control for OAS1 mRNA induction.

[0333] Human U2OS-TLR7 and Human U2OS-TLR8 cells were grown at 37 degrees in the presence of 5% CO2 and were cultured in Dulbeco's modified Eagle's Medium (DMEM) , 1% non essential amino acids, supplemented with fetal bovine

serum at 10% and 100ug/ml streptomycin and 100u/ml penicillin. Stable expression of TLR7 and TLR8 was maintained by the addition of 300ug/ml Gentamycin.

[0334] TLR7 and TLR8 expressing U2OS osteosarcoma cells were seeded in 96-well plate format at a concentration of $2x10^4$cells/well 24 hours prior to transfection. Cells were transfected with the siNAs using DharmaFECT1 lipid transfection reagent using Resiquimod (R848) and the LyoVec-complexed, GU-rich oligonucleotide ssRNA40 respectively as controls (100µl/well). (DharmaFECT1 was used as the delivery agent for the siNAs as it combines low immunostimulatory effects with high delivery efficiency.) The treatment media were replaced after 6 hours with antibiotic containing DMEM. Cells were harvested 24 hours following transfection. R848 and ssRNA40 are characterised agonists of the two receptors upon stimulation; the transformed osteosarcoma cells exhibited an increased IL8 expression in a dose response manner. An agonist concentration range of 4-10µg/ml was established since it caused optimal levels of IL8 mRNA expression for the assay. No significant IL8 expression was observed in the native U2OS cell line lacking TLRs following treatment with the two agonists, R848 and ssRNA40.

[0335] TLR7 and TLR8 mediated immunostimulation was measured by the increase in IL8 mRNA levels when the siNAs were formulated with DharmaFect1 (Gibco BRL) and delivered to U2OS cells that were engineered to stably express TLR7 or TLR8. The cells were treated with the TLR7 and TLR8 agonists Resiquimod (R848) and ssRNA40/LyoVec respectively to act as positive controls for IL8 mRNA induction. IL8 mRNA levels were used as a biomarker of TLR7 and TLR8 mediated immunostimulation.

Results:

[0336] As shown in Figure 11, five of the seven siNAs tested, specifically 29961-DC, 29964-DC, 29947-DC, 29988-DC, 29984-DC (target sites 388, 929, 472, 364, and 356 respectively) showed no TLR8 mediated immunostimulatory activity as demonstrated by the absence of IL8 mRNA induction 48 hours post delivery relative to the agonist control ssRNA40.

[0337] Likewise, these same five siNAs, 29961-DC, 29964-DC, 29947-DC, 29988-DC, 29984-DC (target sites 388, 929, 472, 364, and 356 respectively) showed no or very low TLR7 mediated immunostimulatory activity, as demonstrated by the absence of IL8 mRNA induction 48 hours post delivery relative to the agonist control R484 (see Figure 12).

[0338] The immunostimulatory activity data of the Bach1 siNAs tested is summarized in **Table 9** below.

Table 9. Summary of TLR3, TLR7 and TLR8 immunostimulatory activity of Bach1 siNAs

siNA ID	Target Site	Immunostimulation TLR3 mediated NHBEs OAS1 mRNA increase n=3 donors	Immunostimulation TLR7 mediated in Human U2OS- TLR7 cells TLR8 mediated in Human U2OS-TLR8 cells IL8 mRNA increase n=4 individual expts
29961-DC	388	No significant effect up to 100nM	No significant effect up to 100nM
29964-DC	929	No significant effect up to 100nM	No significant effect up to 100nM
29984-DC	356	No significant effect up to 100nM	No significant effect up to 100nM
29988-DC	364	No significant effect up to 100nM	No significant effect up to 100nM
29957-DC	282	No significant effect up to 100nM	Upregulation up to 100nM
29947-DC	472	No significant effect up to 100nM	No significant effect up to 100nM
29956-DC	1411	No significant effect up to 100nM	No significant effect up to 100nM

Example 4: Evidence of the Role of Bach1 in COPD.

[0339] Human airway epithelial cells were obtained by bronchoscopy from a 62-year old male subject with COPD (35-pack years). The cells were seeded at a density of 1 x 10^5 cells in a 24-well plate and incubated at 37°C and 5%CO_2 overnight. The cells were transfected with Dharmacon's BACH1 siNA SmartPool for 72 hours in Bronchial Epithelial Basal Media with supplements (Lonza). Whole cell lysates were created to detect Heme Oxygenase-1 (HO-1) protein expression via Western Blot.

[0340] Whole cell lysates were quantified and 5 micrograms of protein per lane were loaded into a 12.5% Tris-HCL gel. A 1:5000 dilution of Anti-Human Heme Oxygenase-1 polyclonal antibody (Assay Designs) was used to detect HO-1 protein levels. The blot was stripped and reprobed with a beta actin antibody to confirm equal loading.

[0341] As shown in Figure 13, siNA obtained from Dharmacon's BACH1 SmartPool increased HO-1 protein expression in a concentration-dependent manner in lung epithelial cells obtained from the subject diagnosed with COPD. The positive controls Sulforaphane (a known KEAP1 inhibitor) and hemin (a common BACH1 inactivator) also increased HO-1 protein expression following an 18-hour treatment period. The Non-targeting, GAPDH, or HO-1 Dharmacon siNA SmartPools were used as negative controls.

Example 5: In Vivo Immunostimulation Testing in the Airways of Male CD Rats

[0342] Male CD rats were anaesthetised and intra-tracheally dosed with 200ul of either vehicle, Poly I:C (1mg/kg) or siNA (10mg/kg). 24 hours later the rats were euthanized and the lungs lavaged 3 times with 5ml of heparinised PBS. Total and differential cell counts as well as cytokine analysis were performed on the lung lavage fluid.

[0343] As shown in Figure 14, no significant increases in inflammatory cell (neutrophils and macrophages) influx or cytokine production was observed following intra-tracheal administration of siNAs 29961-DC, 29964-DC, 29947-DC, and 29988-DC.

Example 6: In Vivo Assessment of Actions of siNAs Administered Topically to the Airway

[0344] Following identification of active siNA constructs in vitro, the activities of the siNAs following topical administration to the airway can be assessed in a variety of laboratory species - a typical example is rat, using the methodology summarised below. siNA, an appropriate scrambled control, or vehicle are injected in 200µl volume into the trachea, via a cannula placed trans-orally, whilst the animals are anaesthetised briefly using isoflurane (4.5% in oxygen) and nitrous oxide (anaesthetics delivered in a ratio of 1:3). In

order to facilitate administration of material, animals are supine and placed on a dosing table at an angle of approximately 45° in order to facilitate visualisation of the airway via a cold light source placed over the throat. Alternatively, the anaesthetised animals are dosed intranasally via a pipette (dosing volume 25µl per nostril). In other studies, conscious rodents are placed in a circular Perspex chamber and exposed to an aerosol of nebulised test material for at least 20 min. When each dosing procedure is completed, the animals are returned to standard holding cages and allowed free access to food and water. Groups of animals (typically n=4-6) are then humanely euthanatized by i.p. injection of pentobarbital at set intervals post dose. Samples of airway cells and tissue are removed immediately and placed in Trizol or RNAlater for subsequent mRNA extraction and analysis. In some studies airway tissue is fixed in 4% paraformaldehyde for subsequent histological analysis. In other experiments the airways are lavaged for analysis of infiltrating leukocyte populations and/or cytokine/ mediator content. RNA extraction is carried out using standard methods and QRT-PCR used to quantify the expression of the target mRNA of interest between animals treated with active and control siRNA and to determine whether target knockdown had been achieved. In some cases, mRNA expression levels are normalized relative to either the housekeeping gene, GAPDH, or the epithelial specific marker, E-cadherin.

Preparation of materials

[0345] Solutions of unformulated siNAs and scrambled controls are prepared in phosphate-buffered saline. A range of formulated materials can also been used – in each case the effects of an siNA are compared to that of an equivalent volume of scrambled control.

Example 7: Preparation of Nanoparticle Encapsulated siNA/Carrier Formulations

General LNP Preparation

[0346] siNA nanoparticle solutions are prepared by dissolving siNAs and/or carrier molecules in 25 mM citrate buffer (pH 4.0) at a concentration of 0.9 mg/mL. Lipid solutions are prepared by dissolving a mixture of cationic lipid (e.g., CLinDMA or DOBMA, see structures and ratios for Formulations in **Table 13**), DSPC, Cholesterol, and PEG-DMG (ratios shown in **Table 13**) in absolute ethanol at a concentration of about 15 mg/mL. The nitrogen to phosphate ratio is approximate to 3:1.

[0347] Equal volume of siNA/carrier and lipid solutions are delivered with two FPLC pumps at the same flow rates to a mixing T connector. A back pressure valve is used to adjust

to the desired particle size. The resulting milky mixture is collected in a sterile glass bottle. This mixture is then diluted slowly with an equal volume of citrate buffer, and filtered through an ion-exchange membrane to remove any free siNA/carrier in the mixture. Ultra filtration against citrate buffer (pH 4.0) is employed to remove ethanol (test stick from ALCO screen), and against PBS (pH 7.4) to exchange buffer. The final LNP is obtained by concentrating to a desired volume and sterile filtered through a 0.2 μm filter. The obtained LNPs are characterized in term of particle size, Zeta potential, alcohol content, total lipid content, nucleic acid encapsulated, and total nucleic acid concentration

LNP Manufacture Process

[0348] In a non-limiting example, a LNP-086 siNA/carrier formulation is prepared in bulk as follows. The process consists of (1) preparing a lipid solution; (2) preparing an siNA/carrier solution; (3) mixing/particle formation; (4) incubation; (5) dilution; (6) ultrafiltration and concentration.

1. Preparation of Lipid Solution

[0349] A 3-necked 2L round bottom flask, a condenser, measuring cylinders, and two 10L conical glass vessels are depyrogenated. The lipids are warmed to room temperature. Into the3-necked round bottom flask is transferred 50.44g of CLinDMA with a pipette and 43.32g of DSPC, 5.32g of Cholesterol , 6.96g of PEG-DMG, and 2.64g of linoleyl alcohol are added. To the mixture is added 1L of ethanol.. The round bottom flask is placed in a heating mantle that is connected to a J-CHEM process controller. The lipid suspension is stirred under Argon with a stir bar and a condenser on top. A thermocouple probe is put into the suspension through one neck of the round bottom flask with a sealed adapter. The suspension is heated at 30 °C until it became clear. The solution is allowed to cool to room temperature and transferred to a conical glass vessel and sealed with a cap.

2. Preparation of siNA/Carrier Solution

[0350] Into a sterile container, such as the Corning storage bottle., is weighed 3.6 g times the water correction factor (approximately 1.2) of siNA-1 powder. The siNA is transferred to a depyrogenated 5 L glass vessel. The weighing container is rinsed 3x with citrate buffer (25mM, pH 4.0, and 100mM NaCl) and the rinses are placed into the 5 L vessel, QS with citrate buffer to 4 L. The concentration of the siNA solution is determined with a UV spectrometer using the following procedure. 20μL is removed from the solution, diluted 50

times to 1000μL, and the UV reading recorded at A260 nm after blanking with citrate buffer. This is repeated. If the readings for the two samples are consistent, an average is taken and the concentration is calculated based on the extinction coefficients of the siNAs. If the final concentration are out of the range of 0.90 ± 0.01 mg/mL, the concentration is adjusted by adding more siNA/carrier powder, or adding more citrate buffer. This process is repeated for the second siNA, siNA-2.. Into a depyrogenated 10L glass vessel, 4 L of each 0.9 mg/mL siNA solution is transferred.

[0351] Alternatively, if the siNA/carrier solution comprised a single siNA duplex and or carrier instead of a cocktail of two or more siNA duplexes and/or carriers, then the siNA/carrier is dissolved in 25 mM citrate buffer (pH 4.0, 100 mM of NaCl) to give a final concentration of 0.9 mg/mL.

[0352] The lipid/ethanol solution is then sterile/filtered through a Pall Acropak 20 0.8/0.2 μm sterile filter PN 12203 into a depyrogenated glass vessel using a Master Flex Peristaltic Pump Model 7520-40 to provide a sterile starting material for the encapsulation process. The filtration process is run at an 80 mL scale with a membrane area of 20 cm^2. The flow rate is 280 mL/min. This process is scaleable by increasing the tubing diameter and the filtration area.

3. Particle formation – Mixing step

[0353] An AKTA P900 pump is turned on and sanitized by placing 1000 mL of 1 N NaOH into a 1 L glass vessel and 1000 mL of 70% ethanol into a 1 L glass vessel and attaching the pump with a pressure lid to each vessel. A 2000 mL glass vessel is placed below the pump outlet and the flow rate is set to 40 mL/min for a 40 minute time period with argon flushing the system at 10 psi. When the sanitation is complete, the gas is turned off and the pump is stored in the solutions until ready for use. Prior to use, the pump flow is verified by using 200 mL of ethanol and 200 mL of sterile citrate buffer.

[0354] To the AKTA pump is attached the sterile lipid/ethanol solution, the sterile siNA/carrier or siNA/carrier cocktail /citrate buffer solution and a depyrogenated receiving vessel (2x batch size) with lid. The gas is turned on and the pressure maintained between 5 to10 psi during mixing.

4. Incubation

[0355] The solution is held after mixing for a 22 ± 2 hour incubation. The incubation is done at room temperature (20 – 25°C) and the in-process solution was protected from light.

5. Dilution

[0356] The lipid siNA solution is diluted with an equal volume of Citrate buffer using a dual head peristaltic pump, Master Flex Peristaltic Pump, Model 7520-40 that is set up with equal lengths of tubing and a Tee connection and a flow rate of 360 mL/minute.

6. Ultrafiltration and Concentration

[0357] The ultrafiltration process is a timed process and the flow rates must be monitored carefully. This is a two step process; the first is a concentration step taking the diluted material from 32 liters to 3600 mLs and to a concentration of approximately 2 mg/mL.

[0358] In the first step, a Flexstand with a ultrafiltration membrane GE PN UFP-100-C-35A installed is attached to the quatroflow pump. 200 mL of WFI is added to the reservoir followed by 3 liters of 0.5 N sodium hydroxide which is then flushed through the retentate to waste. This process is repeated three times. Then 3 L WFI are flushed through the system twice followed by 3 L of citrate buffer. The pump is then drained.

[0359] The diluted LNP solution is placed into the reservoir to the 4 liter mark. The pump is turned on and the pump speed adjusted so the permeate flow rate is 300 mL/min. and the liquid level is constant at 4L in the reservoir. .The pump is stopped when all the diluted LNP solution has been transferred to the reservoir. The diluted LNP solution is concentrated to 3600 mL in 240 minutes by adjusting the pump speed as necessary.

[0360] The second step is a diafiltration step exchanging the ethanol citrate buffer to phosphate buffered saline. The diafiltration step takes 3 hours and again the flow rates must be carefully monitored. During this step, the ethanol concentration is monitored by head space GC. After 3 hours (20 diafiltration volumes), a second concentration is undertaken to concentrate the solution to approximately 6 mg/mL or a volume of 1.2 liters. This material is collected into a depyrogenated glass vessel. The system is rinsed with 400 mL of PBS at high flow rate and the permeate line closed. This material is collected and added to the first

collection. The expected concentration at this point is 4.5 mg/mL. The concentration and volume are determined.

[0361] The feed tubing of the peristaltic pump is placed into a container containing 72 L of PBS (0.05 μm filtered) and the flow rate is adjusted initially to maintain a constant volume of 3600 mL in the reservoir and then increased to 400 mL/min. The LNP solution is diafiltered with PBS (20 volumes) for 180 minutes.

[0362] The LNP solution is concentrated to the 1.2 liter mark and collected into a depyrogenated 2 L graduated cylinder. 400 mL of PBS are added to the reservoir and the pump is recirculated for 2 minutes. The rinse is collected and added to the collected LNP solution in the graduated cylinder.

[0363] The obtained LNPs are characterized in terms of particle size, Zeta potential, alcohol content, total lipid content, nucleic acid encapsulated, and total nucleic acid concentration.

[0364] 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.

Table 10: Bach1 Accession Numbers

NM_001186– SEQ ID NO: 149
Homo sapiens BTB and CNC homology 1, basic leucine zipper transcription factor 1 (BACH1), transcript variant 2, mRNA
gi|45827688|ref|NM_001186.2|[45827688]

NM_206866
Homo sapiens BTB and CNC homology 1, basic leucine zipper transcription factor 1 (BACH1), transcript variant 1, mRNA
gi|45827689|ref|NM_206866.1|[45827689]

NM_001011545
Homo sapiens BTB and CNC homology 1, basic leucine zipper transcription factor 1 (BACH1), transcript variant 3, mRNA
gi|59559716|ref|NM_001011545.1|[59559716]

AF124731
Homo sapiens chromosome 21q22.1 PAC A20292 containing BACH-1 gene, complete sequence
gi|5306199|gb|AF124731.2|AF124731[5306199]

NM_007520
Mus musculus BTB and CNC homology 1 (Bach1), mRNA
gi|82659113|ref|NM_007520|

Table 11

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
"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
"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 **Figure 5**.
All Stab 00-34 chemistries can comprise 3'-terminal thymidine (TT) residues
All Stab 00-34 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 **Figure 4C**)
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, Stab 27, Stab 35 and Stab 36 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 and three ribonucleotides at 5'-terminus
†Stab 36 has 2'-O-methyl overhangs that are complementary to the target sequence (naturally occurring overhangs) and three ribonucleotides at 5'-terminus

Table 12

A. 2.5 µmol Synthesis Cycle ABI 394 Instrument

Reagent	**Equivalents**	**Amount**	**Wait Time* DNA**	**Wait Time* 2'-O-methyl**	**Wait Time*RNA**
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

B. 0.2 µmol Synthesis Cycle ABI 394 Instrument

Reagent	**Equivalents**	**Amount**	**Wait Time* DNA**	**Wait Time* 2'-O-methyl**	**Wait Time*RNA**
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	360sec
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 13

Lipid Nanoparticle (LNP) Formulations

Formulation #	Composition	Mole 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 /2
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
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
L118	LinCDMA/DSPC/Cholesterol/2KPEG-DMG/Linoleyl alcohol, N/P ratio of 2.85	43 / 38 / 10 / 2 / 7
L121	2-CLIM/DSPC/Cholesterol/2KPEG-DMG/, N/P ratio of 3	48 / 40 / 10 / 2
L122	2-CLIM/ Cholesterol/2KPEG-DMG/, N/P ratio of 3	68 / 30 / 2
L123	CLinDMA/DSPC/Cholesterol/2KPEG-DMG/Linoleyl alcohol, N/P ratio of 2.85	43 / 37 / 10 / 3 / 7
L124	CLinDMA/DSPC/Cholesterol/2KPEG-DMG/Linoleyl alcohol, N/P ratio of 2.85	43 / 36 / 10 / 4 / 7
L130	CLinDMA / DOPC / Chol / PEG-n-DMG, N/P ratio of 3	48 / 39 / 10 / 3
L131	DMLBA/Cholesterol/2KPEG-DMG, N/Pratio of 3	52 / 43 / 5
L132	DMOBA/Cholesterol/2KPEG-DMG, N/Pratio of 3	52 / 43 / 5
L133	CLinDMA / DOPC / Chol / PEG-n-DMG, N/P ratio of 3	48 / 40 / 10 / 2
L134	CLinDMA / DOPC / Chol / PEG-n-DMG, N/P ratio of 3	48 / 37 / 10 / 5
L149	COIM/DSPC/Cholesterol/2KPEG-DMG/, N/P ratio of 3	48 / 40 / 10 / 2
L155	CLinDMA/DOPC/Cholesterol/2KPEG-DMG/Linoleyl alcohol, N/P ratio of 2.85	43 / 38 / 10 / 2 / 7
L156	CLinDMA/DOPC/Cholesterol/2KPEG-DMG, N/P ratio of 2.85	45 / 43 / 10 / 2
L162	CLinDMA/DOPC/Cholesterol/2KPEG-DMG, N/P ratio of 2.5	45 / 43 / 10 / 2
L163	CLinDMA/DOPC/Cholesterol/2KPEG-DMG, N/P ratio of 2	45 / 43 / 10 / 2
L164	CLinDMA/DOPC/Cholesterol/2KPEG-DMG, N/P ratio of 2.25	45 / 43 / 10 / 2
L165	CLinDMA/DOPC/Cholesterol/2KPEG-DMG,	40 / 43 / 15 / 2

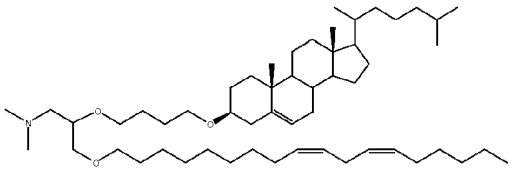
	N/P ratio of 2.25	
L166	CLinDMA/DOPC/Cholesterol/2KPEG-DMG, N/P ratio of 2.5	40 / 43 / 15 / 2
L167	CLinDMA/DOPC/Cholesterol/2KPEG-DMG, N/P ratio of 2	40 / 43 / 15 / 2
L174	CLinDMA/DSPC/DOPC/Cholesterol/2KPEG-DMG/Linoleyl alcohol, N/P ratio of 2.85	43 / 9 / 27 / 10 / 4/ 7
L175	CLinDMA/DSPC/DOPC/Cholesterol/2KPEG-DMG/Linoleyl alcohol, N/P ratio of 2.85	43 / 27 / 9 / 10 / 4/ 7
L176	CLinDMA/DOPC/Cholesterol/2KPEG-DMG/Linoleyl alcohol, N/P ratio of 2.85	43 / 36 / 10 / 4 / 7
L180	CLinDMA/DOPC/Cholesterol/2KPEG-DMG/Linoleyl alcohol, N/P ratio of 2.25	43 / 36 / 10 / 4 / 7
L181	CLinDMA/DOPC/Cholesterol/2KPEG-DMG/Linoleyl alcohol, N/P ratio of 2	43 / 36 / 10/ 4 / 7
L182	CLinDMA/DOPC/Cholesterol/2KPEG-DMG, N/P ratio of 2.25	45 / 41 / 10 / 4
L197	CODMA/DOPC/Cholesterol/2KPEG-DMG, N/P ratio of 2.85	43 / 36 / 10 / 4 / 7
L198	CLinDMA/DOPC/Cholesterol/2KPEG-DMG/2KPEG-DSG/Linoleyl alcohol, N/P ratio of 2.85	43 / 34 / 10 / 4 / 2 / 7
L199	CLinDMA/DOPC/Cholesterol/2KPEG-DMG/Linoleyl alcohol, N/P ratio of 2.85	43 / 34 / 10 / 6 / 7
L200	CLinDMA/Cholesterol/2KPEG-DMG, N/P ratio of 3.0	50 / 46 / 4
L201	CLinDMA/Cholesterol/2KPEG-DMG, N/P ratio of 3.0	50 / 44 / 6
L206	CLinDMA/Cholesterol/2KPEG-DMG, N/P ratio of 3.0	40 / 56 / 4
L207	CLinDMA/Cholesterol/2KPEG-DMG, N/P ratio of 3.0	60 / 36 / 4
L208	CLinDMA/DOPC/Cholesterol/2KPEG-DMG, N/P ratio of 3.0	40 / 10 / 46 / 4
L209	CLinDMA/DOPC/Cholesterol/2KPEG-DMG, N/P ratio of 3.0	60 / 10 / 26 / 4

N/P ratio = Nitrogen:Phosphorous ratio between cationic lipid and nucleic acid

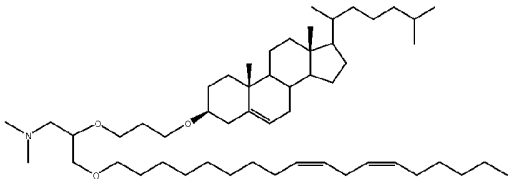
The 2KPEG utilized is PEG2000, a polydispersion which can typically vary from ~1500 to ~3000 Da (i.e., where PEG(n) is about 33 to about 67, or on average ~45).

Table 14

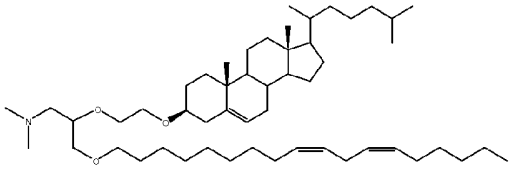
CLinDMA structure



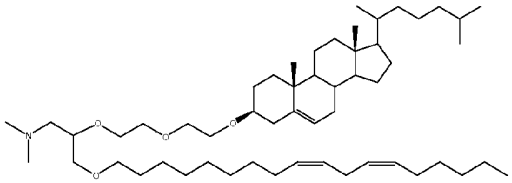
pCLinDMA structure



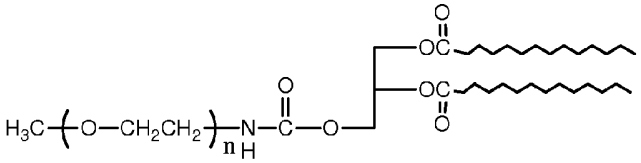
eCLinDMA structure



DEGCLinDMA structure

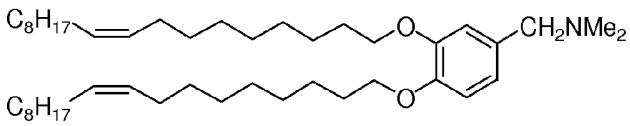


PEG-n-DMG structure

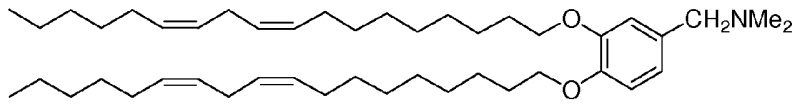


n = about 33 to 67, average = 45 for 2KPEG/PEG2000

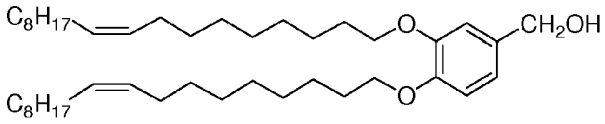
DMOBA structure



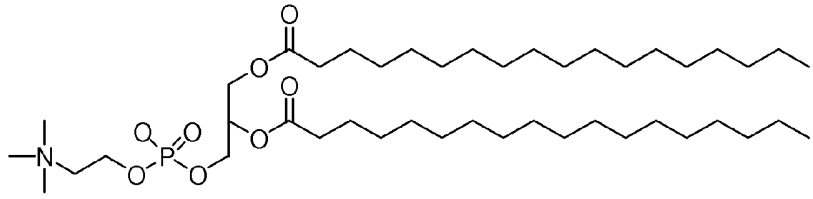
DMLBA structure



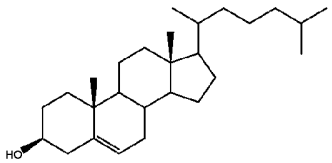
DOBA structure



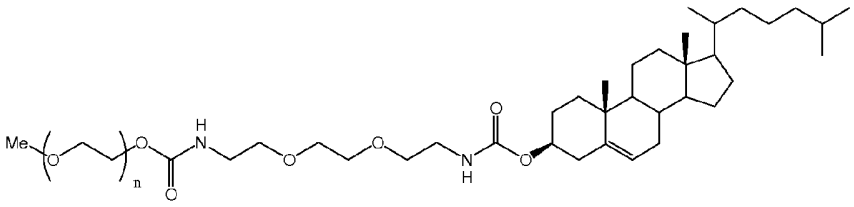
DSPC structure



Cholesterol structure

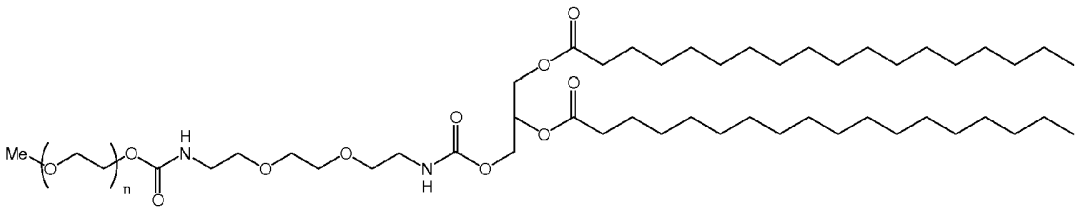


2KPEG-*Cholesterol structure*



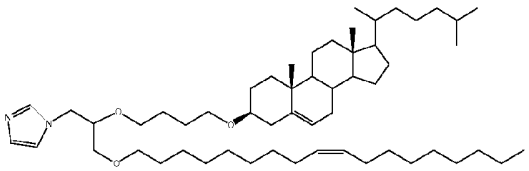
n = about 33 to 67, average = 45 for 2KPEG/PEG2000

2KPEG-*DMG structure*

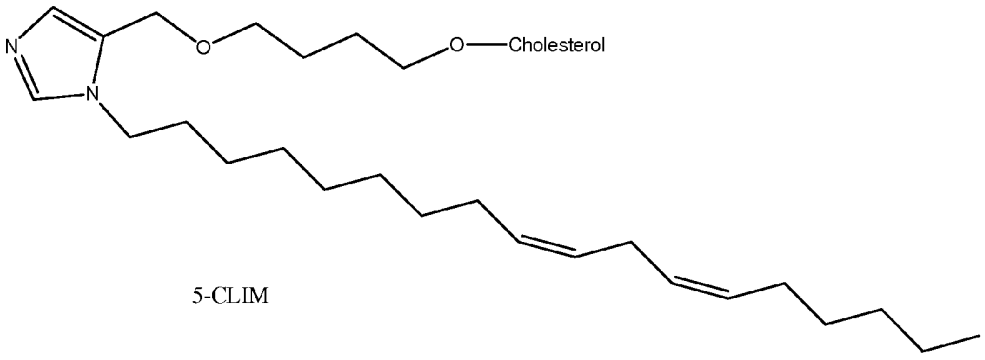


n = about 33 to 67, average = 45 for 2KPEG/PEG2000

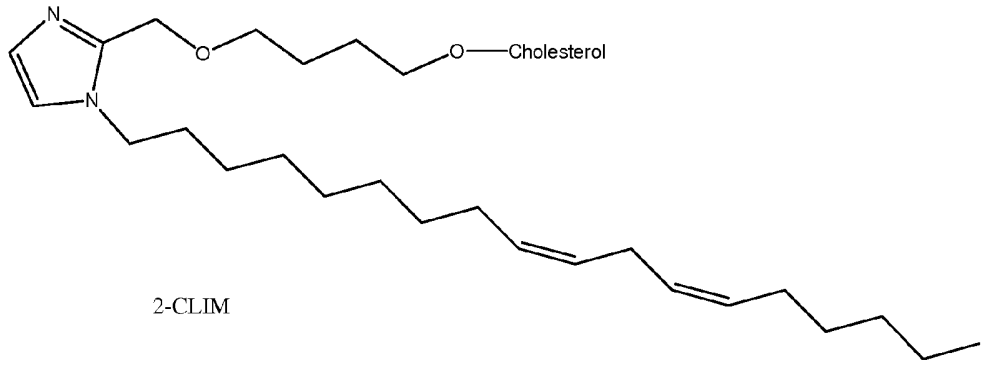
COIM STRUCTURE



5-CLIM AND 2-CLIM STRUCTURE



5-CLIM



2-CLIM

CLAIMS

What we claim is:

1. A double-stranded short interfering nucleic acid (siNA) molecule comprising a first strand and a second strand having complementarity to each other, wherein at least one strand comprises at least 15 nucleotides of:

5'-GGAAUCCUGCUUUCAGUUU -3' (SEQ ID NO: 1);

5'-AAACUGAAAGCAGGAUUCC-3' (SEQ ID NO: 143);

5'-GUCUGAGUGUCCGUGGUUA-3' (SEQ ID NO: 10);

5'-UAACCACGGACACUCAGAC-3' (SEQ ID NO: 144);

5'-GCAGUUACUUCCACUCAAG -3' (SEQ ID NO: 11);

5'-CUUGAGUGGAAGUAACUGC-3' (SEQ ID NO: 145);

5'-CUACACUGCUAAACUGAUU-3' (SEQ ID NO: 15);

5'-AAUCAGUUUAGCAGUGUAG-3' (SEQ ID NO: 146);

5'-GAUUUGCAGGUGAUGUUAA-3' (SEQ ID NO: 18);

5'-UUAACAUCACCUGCAAAUC-3' (SEQ ID NO: 147);

5'-AUUUGAACCUUUAAUUCAG-3' (SEQ ID NO: 42);

5'- CUGAAUUAAAGGUUCAAAU-3' (SEQ ID NO: 148);

5'-GUUAAAGGAUUUGAACCUU-3' (SEQ ID NO: 38); or

5'-AAGGUUCAAAUCCUUUAAC -3' (SEQ ID NO: 150); and

wherein one or more of the nucleotides are optionally chemically modified.

2. The double-stranded short interfering nucleic acid (siNA) molecule of claim 1 wherein all the nucleotides are unmodified.

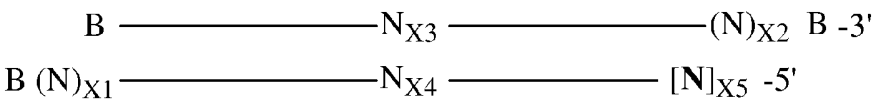
3. The double-stranded short interfering nucleic acid (siNA) molecule of claim 1 wherein at least one nucleotide is a chemically modified nucleotide.

4. The double-stranded short interfering nucleic acid (siNA) molecule of claim 3, wherein the chemically modified nucleotide is a 2'-deoxy-2'-fluoronucleotide.

5. The double-stranded short interfering nucleic acid (siNA) molecule of claim 3, wherein the chemically modified nucleotide is a 2'-deoxynucleotide.

6. The double-stranded short interfering nucleic acid (siNA) molecule of claim 3, wherein the chemically modified nucleotide is a 2'-O-alkyl nucleotide.

7. A double-stranded short interfering nucleic acid (siNA) molecule, comprising formula (A) having a sense strand and an antisense strand:



(A)

wherein, the upper strand is the sense strand and the lower strand is the antisense strand of the double-stranded nucleic acid molecule; wherein the antisense strand comprises at least 15 nucleotides of SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, or SEQ ID NO: 150, and the sense strand comprises a sequence having complementarity to the antisense strand;

each N is independently a nucleotide which is unmodified or chemically modified;

each B is a terminal cap that is present or absent;

(N) represents overhanging nucleotides, each of which is independently unmodified chemically modified;

[N] represents nucleotides that are ribonucleotides;

X1 and X2 are independently integers from 0 to 4;

X3 is an integer from 17 to 36;

X4 is an integer from 11 to 35; and

X5 is an integer from 1 to 6, provided that the sum of X4 and X5 is 17-36.

8. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 7; wherein

(a) one or more pyrimidine nucleotides in N_{X4} positions are independently 2'-deoxy-2'-fluoro nucleotides, 2'-O-alkyl nucleotides, 2'-deoxy nucleotides, ribonucleotides, or any combination thereof;

(b) one or more purine nucleotides in N_{X4} positions are independently 2'-deoxy-2'-fluoro nucleotides, 2'-O-alkyl nucleotides, 2'-deoxy nucleotides, ribonucleotides, or any combination thereof;

(c) one or more pyrimidine nucleotides in N_{X3} positions are independently 2'-deoxy-2'-fluoro nucleotides, 2'-O-alkyl nucleotides, 2'-deoxy nucleotides, ribonucleotides, or any combination thereof; and

(d) one or more purine nucleotides in N_{X3} positions are independently 2'-deoxy-2'-fluoro nucleotides, 2'-O-alkyl nucleotides, 2'-deoxy nucleotides, ribonucleotides, or any combination thereof.

9. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 7; wherein

(a) each pyrimidine nucleotide in NX4 positions is independently a 2'-deoxy-2'-fluoro nucleotide, 2'-O-alkyl nucleotide, 2'-deoxy nucleotide, or ribonucleotide;

(b) each purine nucleotide in N_{X4} positions is independently a 2'-deoxy-2'-fluoro nucleotide, 2'-O-alkyl nucleotide, 2'-deoxy nucleotide, or ribonucleotide;

(c) each pyrimidine nucleotide in N_{X3} positions is independently a 2'-deoxy-2'-fluoro nucleotide, 2'-O-alkyl nucleotide, 2'-deoxy nucleotide, or ribonucleotide; and

(d) each purine nucleotides in N_{X3} positions is independently a 2'-deoxy-2'-fluoro nucleotide, 2'-O-alkyl nucleotide, 2'-deoxy nucleotide, or ribonucleotide.

10. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 7; wherein

(a) each pyrimidine nucleotide in N_{X4} positions is independently a 2'-deoxy-2'-fluoro nucleotide;

(b) each purine nucleotide in N_{X4} positions is independently a 2'-O-alkyl nucleotide;

(c) each pyrimidine nucleotide in N_{X3} positions is independently a 2'-deoxy-2'-fluoro nucleotide; and

(d) each purine nucleotide in N_{X3} positions is independently a 2'-deoxy nucleotide.

11. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 7; wherein

(a) each pyrimidine nucleotide in N_{X4} positions is independently a 2'-deoxy-2'-fluoro nucleotide;

(b) each purine nucleotide in N_{X4} positions is independently a 2'-O-alkyl nucleotide;

(c) each pyrimidine nucleotide in N_{X3} positions is independently a 2'-deoxy-2'-fluoro nucleotide; and

(d) each purine nucleotide in N_{X3} positions is independently a ribonucleotide.

12. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 7; wherein

(a) each pyrimidine nucleotide in N_{X4} positions is independently a 2'-deoxy-2'-fluoro nucleotide;

(b) each purine nucleotide in N_{X4} positions is independently a ribonucleotide;

(c) each pyrimidine nucleotide in N_{X3} positions is independently a 2'-deoxy-2'-fluoro nucleotide; and

(d) each purine nucleotide in N_{X3} positions is independently a ribonucleotide.

13. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 7, wherein X5 is 3.

14. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 7, wherein X1 is 2 and X2 is 2.

15. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 7, wherein X5 is 3, X1 is 2 and X2 is 2.

16. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 7, wherein X5 = 1, 2, or 3; each X1 and X2 = 1 or 2; X3 = 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.

17. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 7, wherein X5 = 1; each X1 and X2 = 2; X3 = 19, and X4 = 18.

18. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 7, wherein X5 = 2; each X1 and X2 = 2; X3 = 19, and X4 = 17.

19. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 7, wherein X5 is 3, X1 is 2, X2 is 2, X3 is 19 and X4 is 16.

20. A double-stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

5'- BGGAAuccuGcuuucAGuuuTTB -3' (Sense) (SEQ ID NO: 43)
3'- UUccuuAGGAcGAAAGucAAA -5' (Antisense) (SEQ ID NO: 44)

wherein:

each B is an inverted abasic cap moiety;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

A is adenosine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

21. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 20, wherein the internucleotide linkages are unmodified.

22. A double-stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

5'- BGucuGAGuGuccGuGGuuATTB -3' (Sense) (SEQ ID NO:61)
3' UUcAGAcucAcAGGcAccAAU -5' (Antisense) (SEQ ID NO:62)

wherein:

each B is an inverted abasic cap;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

A is adenosine;

U is uridine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

23. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 22, wherein the internucleotide linkages are unmodified.

24. A double-stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

```
5'  BGcAGuuAcuuccAcucAAGTTB 3'(Sense) (SEQ ID NO:63)
     ||||||||||||||||||||
3' UUcGucAAuGAAGGuGAGUUC     5'(Antisense) (SEQ ID NO:64)
```

wherein:

each B is an inverted abasic cap moiety;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

C is cytidine;

U is uridine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

25. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 24, wherein the internucleotide linkages are unmodified.

26. A double-stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

5′- BcuAcAcuGcuAAAcuGAuuTTB -3′ (Sense) (SEQ ID NO:71)
3′ UUGAuGuGAcGAuuuGAcUAA -5′ (Antisense) (SEQ ID NO:72)

wherein:

each B is an inverted abasic cap moiety;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

A is adenosine;

U is uridine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

27. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 26, wherein the internucleotide linkages are unmodified.

28. A double-stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

5′- BGAuuuGcAGGuGAuGuuAATTB -3′ (Sense) (SEQ ID NO:77)
3′ UUcuAAAcGuccAcuAcAAUU -5′ (Antisense) (SEQ ID NO:78)

wherein:

each B is an inverted abasic cap moiety;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

A is adenosine;

U is uridine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

29. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 28, wherein the internucleotide linkages are unmodified.

30. A double-stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

```
5'- BAuuuGAAccuuuAAuucAGTTB -3'      (Sense)     (SEQ ID NO:125)
     ||||||||||||||||||||
3' UUuAAAcuuGGAAAuuAAGUC    -5'      (Antisense) (SEQ ID NO:126)
```

wherein:

each B is an inverted abasic cap moiety;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

G is guanosine;

U is uridine;

C is cytidine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

31. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 30, wherein the internucleotide linkages are unmodified.

32. A double-stranded short interfering nucleic acid (siNA) molecule wherein the siNA is:

```
5'- BGuuAAAGGAuuuGAAccuuTTB -3'      (Sense)     (SEQ ID NO:117)
     ||||||||||||||||||||
3' UUcAAuuuccuAAAcuuGGAA    -5'      (Antisense) (SEQ ID NO:118)
```

wherein:

each B is an inverted abasic cap moiety;

c is 2'-deoxy-2'fluorocytidine;

u is 2'-deoxy-2'fluorouridine;

A is 2'-deoxyadenosine;

G is 2'deoxyguanosine;

T is thymidine;

G is guanosine;

A is adenosine;

A is 2'-O-methyl-adenosine;

G is 2'-O-methyl-guanosine;

U is 2'-O-methyl-uridine; and

the internucleotide linkages are chemically modified or unmodified.

33. The double-stranded short interfering nucleic acid (siNA) molecule according to claim 32, wherein the internucleotide linkages are unmodified.

34. A pharmaceutical composition comprising the double-stranded short interfering nucleic acid (siNA) of any of claims 1, 7, 20, 22, 24, 26, 28, 30, or 32 in a pharmaceutically acceptable carrier or diluent.

35. A pharmaceutical composition comprising the double-stranded short interfering nucleic acid (siNA) molecule of claim 1, 7, 20, 22, 24, 26, 28, 30, or 32 in an aerosol formulation.

36. A method of treating a human subject suffering from a condition which is mediated by the action, or by loss of action, of Bach1 which comprises administering to said subject an effective amount of the double-stranded short interfering nucleic acid (siNA) molecule of claim 7.

37. A method of treating a human subject suffering from a condition which is mediated by the action, or by loss of action, of Bach1 which comprises administering to said subject an effective amount of the double-stranded short interfering nucleic acid (siNA) molecule of claim 20, 22, 24, 26, 28, 30, or 32.

38. The method according to claim 36, wherein the condition is a respiratory disease.

39. The method according to claim 37, wherein the condition is a respiratory disease

40. The method according to claim 38, wherein the respiratory disease is selected from the group consisting of COPD, cystic fibrosis, asthma, eosinophilic cough, bronchitis, sarcoidosis, pulmonary fibrosis, rhinitis, and sinusitis.

41. The method according to claim 39, wherein the respiratory disease is selected from the group consisting of COPD, cystic fibrosis, asthma, eosinophilic cough, bronchitis, sarcoidosis, pulmonary fibrosis, rhinitis, and sinusitis.

Figure 1

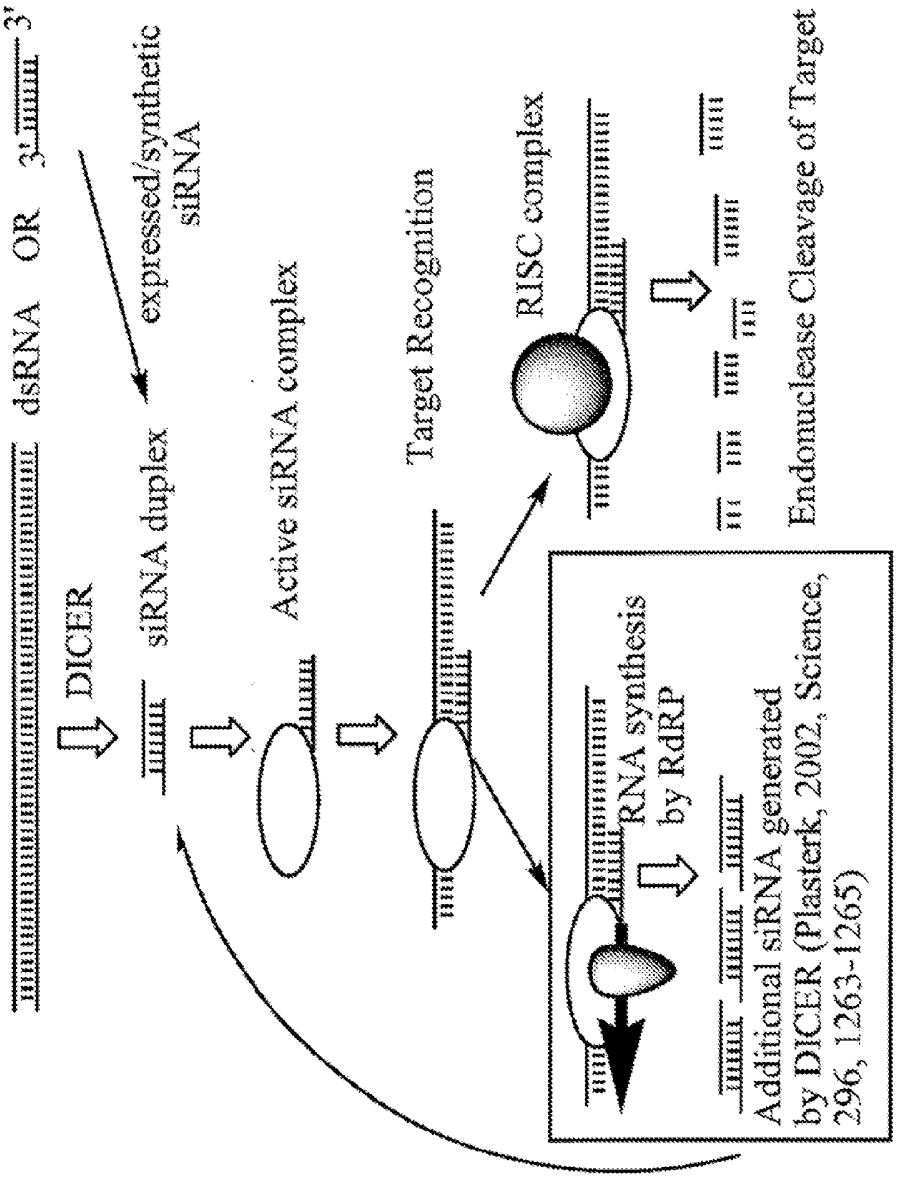
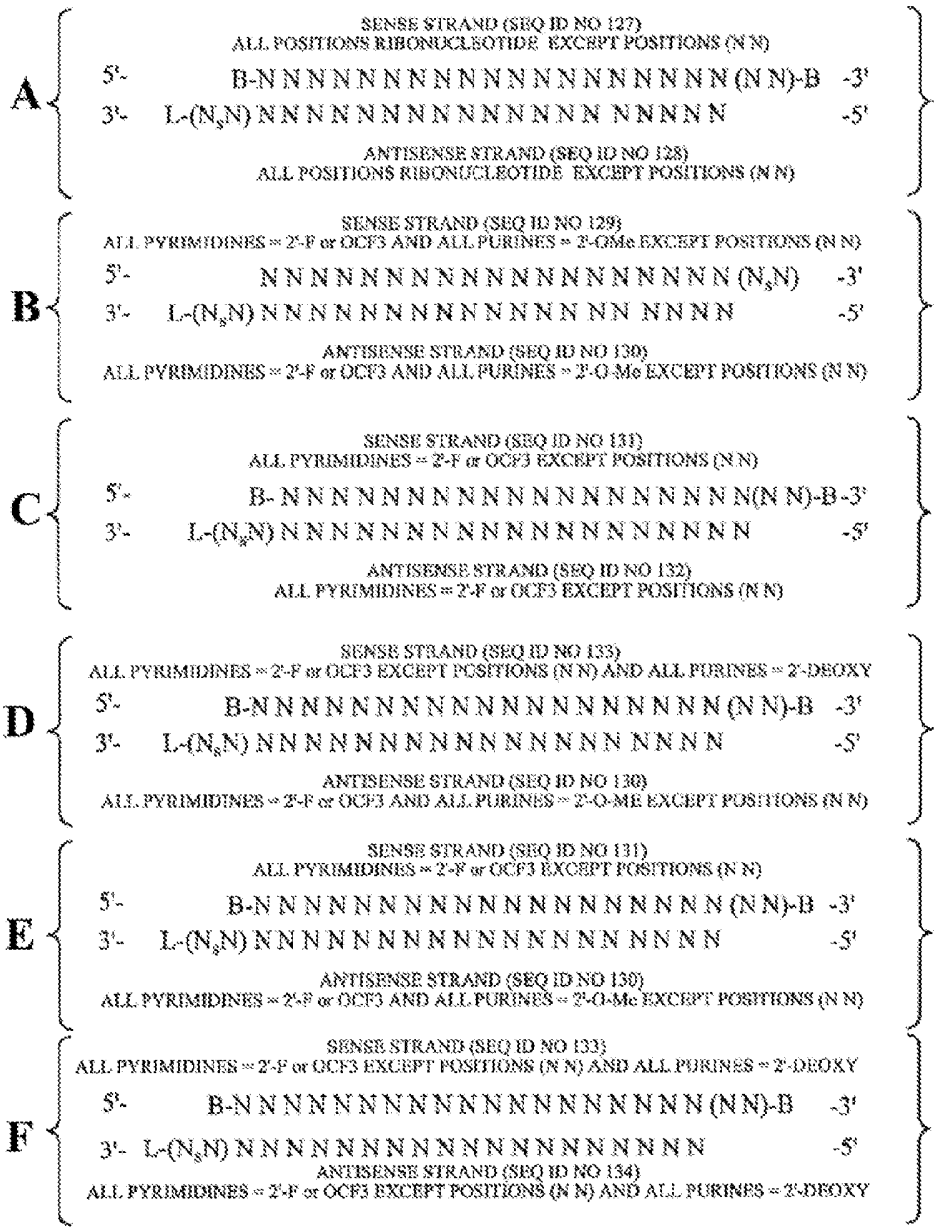

dsRNA OR 3' 3'
DICER
siRNA duplex
expressed/synthetic siRNA
Active siRNA complex
Target Recognition
RISC complex
RNA synthesis by RdRP
Additional siRNA generated by DICER (Plasterk, 2002, Science, 296, 1263-1265)
Endonuclease Cleavage of Target

Figure 2



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 3

A

SENSE STRAND (SEQ ID NO 135)

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

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

ANTISENSE STRAND (SEQ ID NO 136)

B

SENSE STRAND (SEQ ID NO 137)

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

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

ANTISENSE STRAND (SEQ ID NO 138)

C

SENSE STRAND (SEQ ID NO 139)

5'- B-GGAA*u c cu* Gc *u u u c*AG *u u u N N*-B -3'

3'- L-N_S*N c c u u*AGGA*c*GAAAG *u c*AAA -5'

ANTISENSE STRAND (SEQ ID NO 140)

D

SENSE STRAND (SEQ ID NO 141)

5'- B-*GGAAu c c u G c u u u cAG u u u N N*-B -3'

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

ANTISENSE STRAND (SEQ ID NO 138)

E

SENSE STRAND (SEQ ID NO 139)

5'- B-GGAA*u c c u* G *c u u u c*AG *u u u N N*-B -3'

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

ANTISENSE STRAND (SEQ ID NO 138)

F

SENSE STRAND (SEQ ID NO 141)

5'- B-*GGAA u c c uGcu u u cAG u u u N N*-B -3'

3'- L-N_S*N c c u uAGGA cGAAAG u c AAA* -5'

ANTISENSE STRAND (SEQ ID NO 142)

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 4A

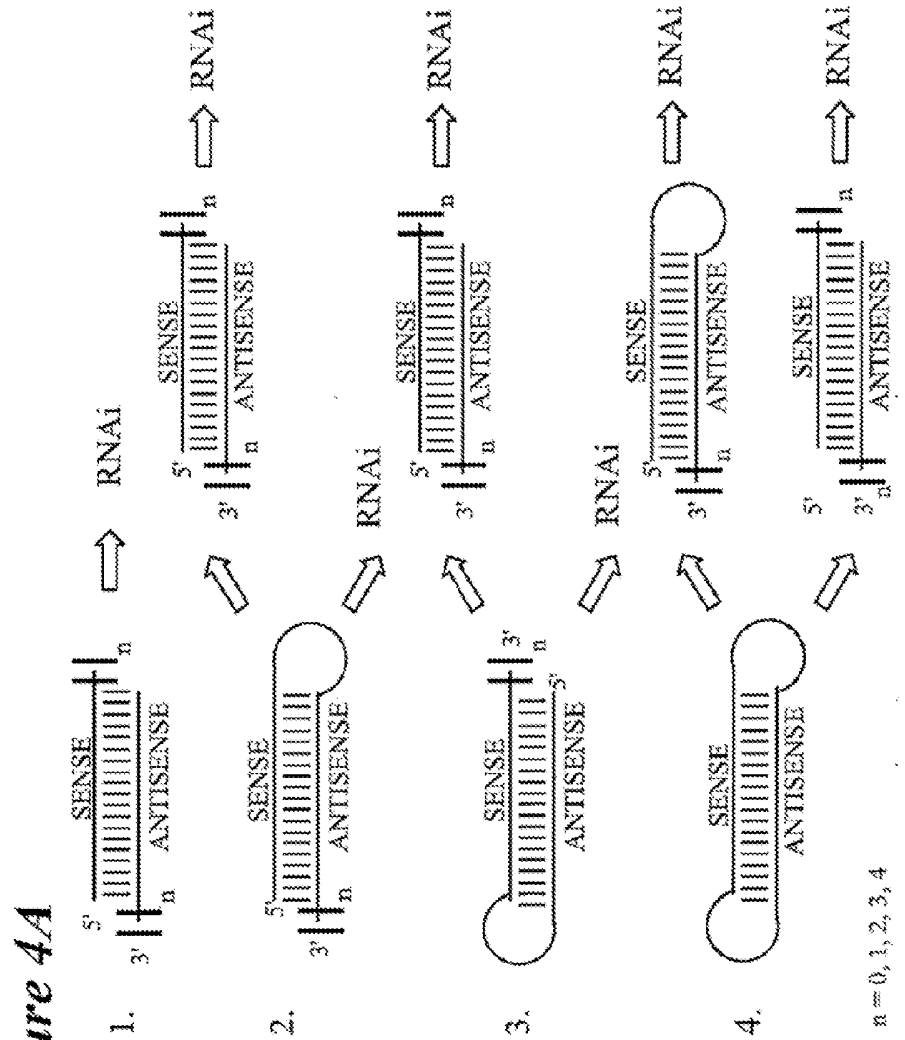


Figure 4B

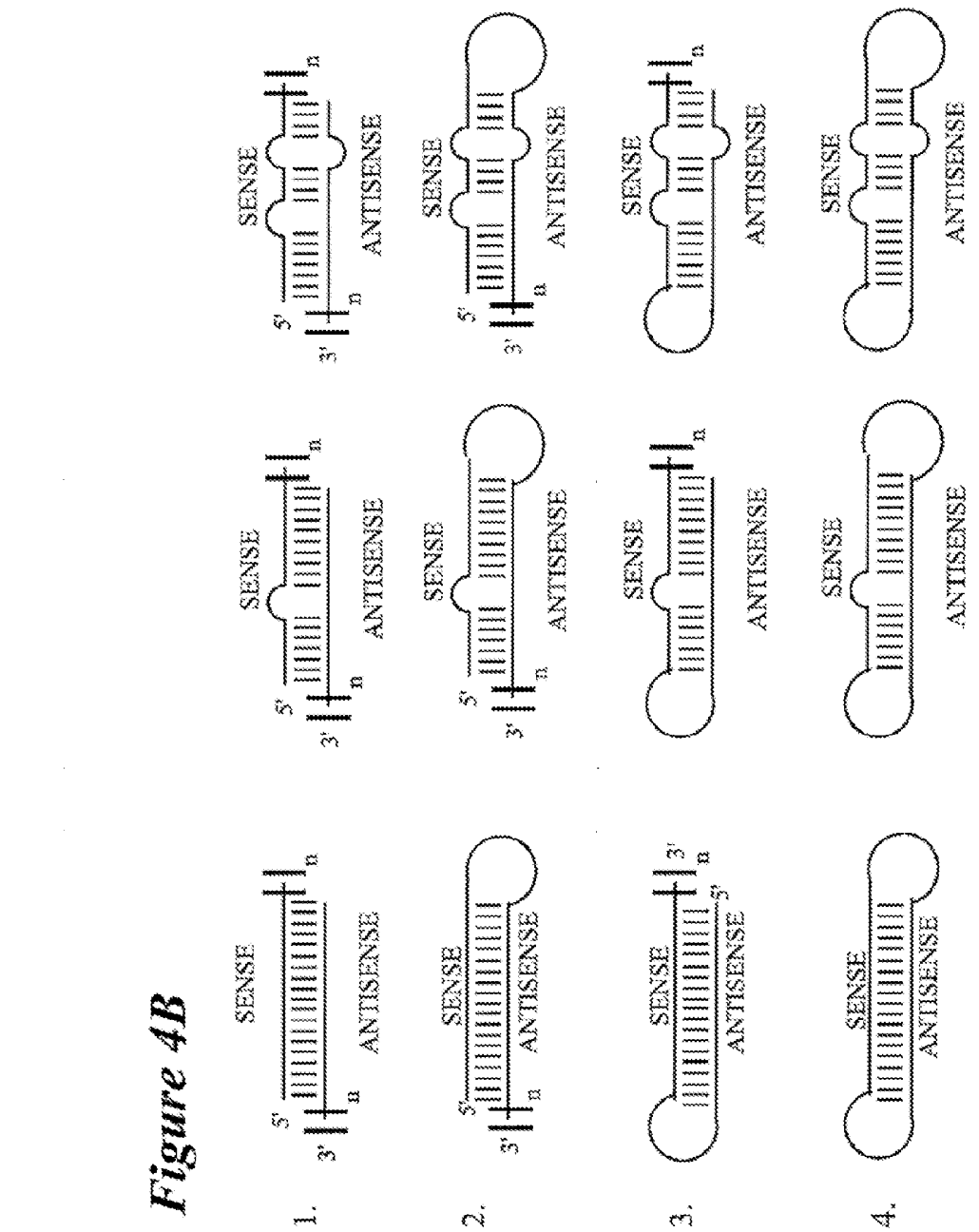
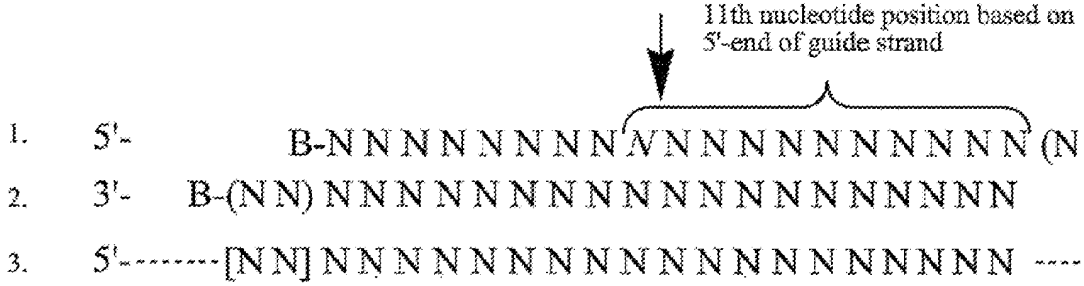


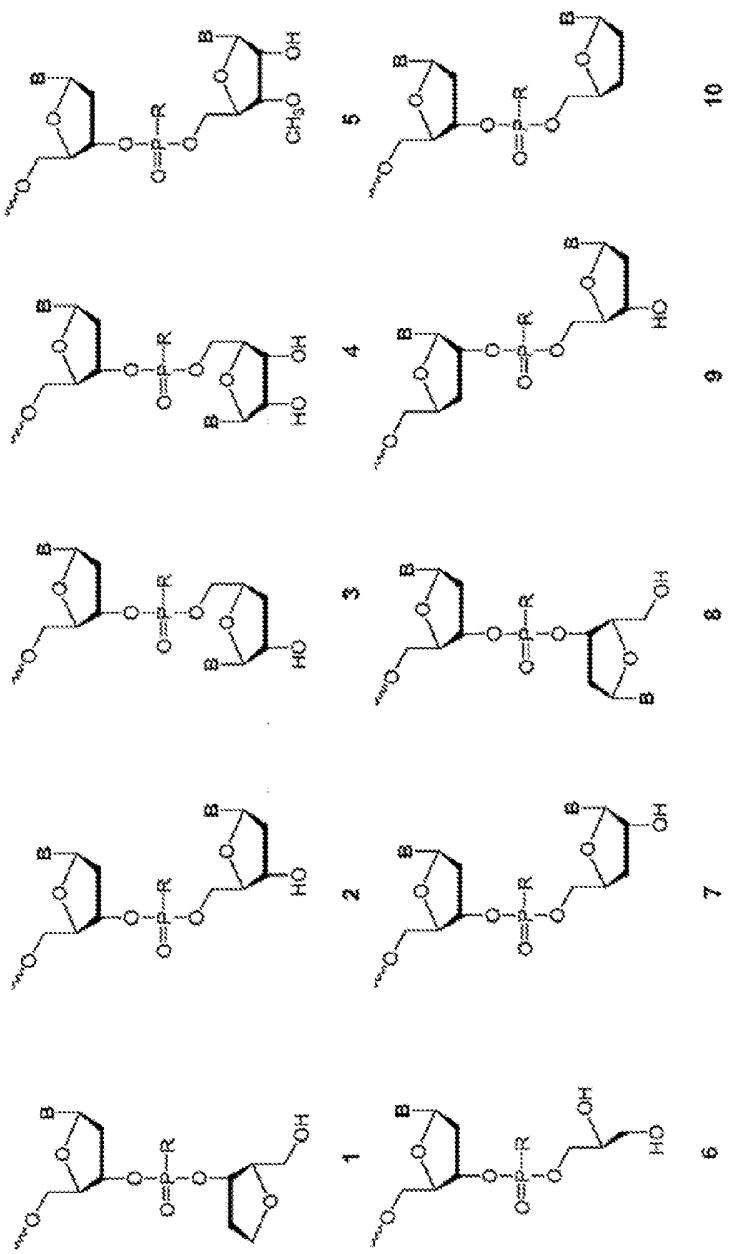
Figure 4C



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 5



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 6: Modification Strategy

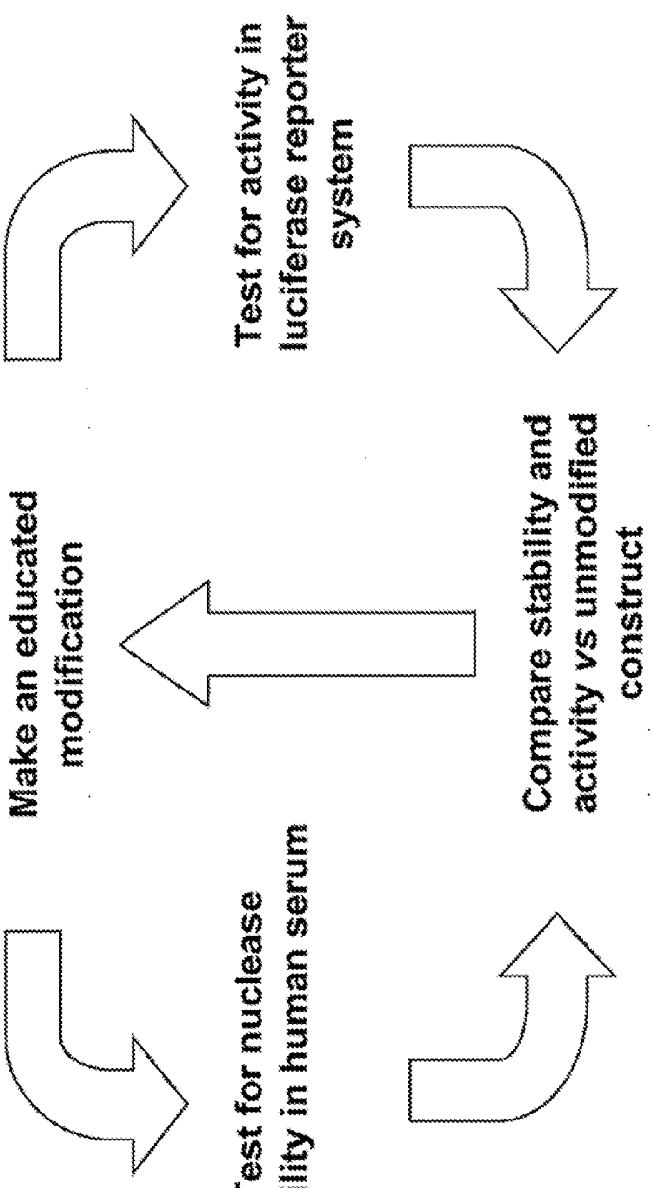


Figure 7: Phosphorylated siNA constructs

Figure 8: 5'-phosphate modifications

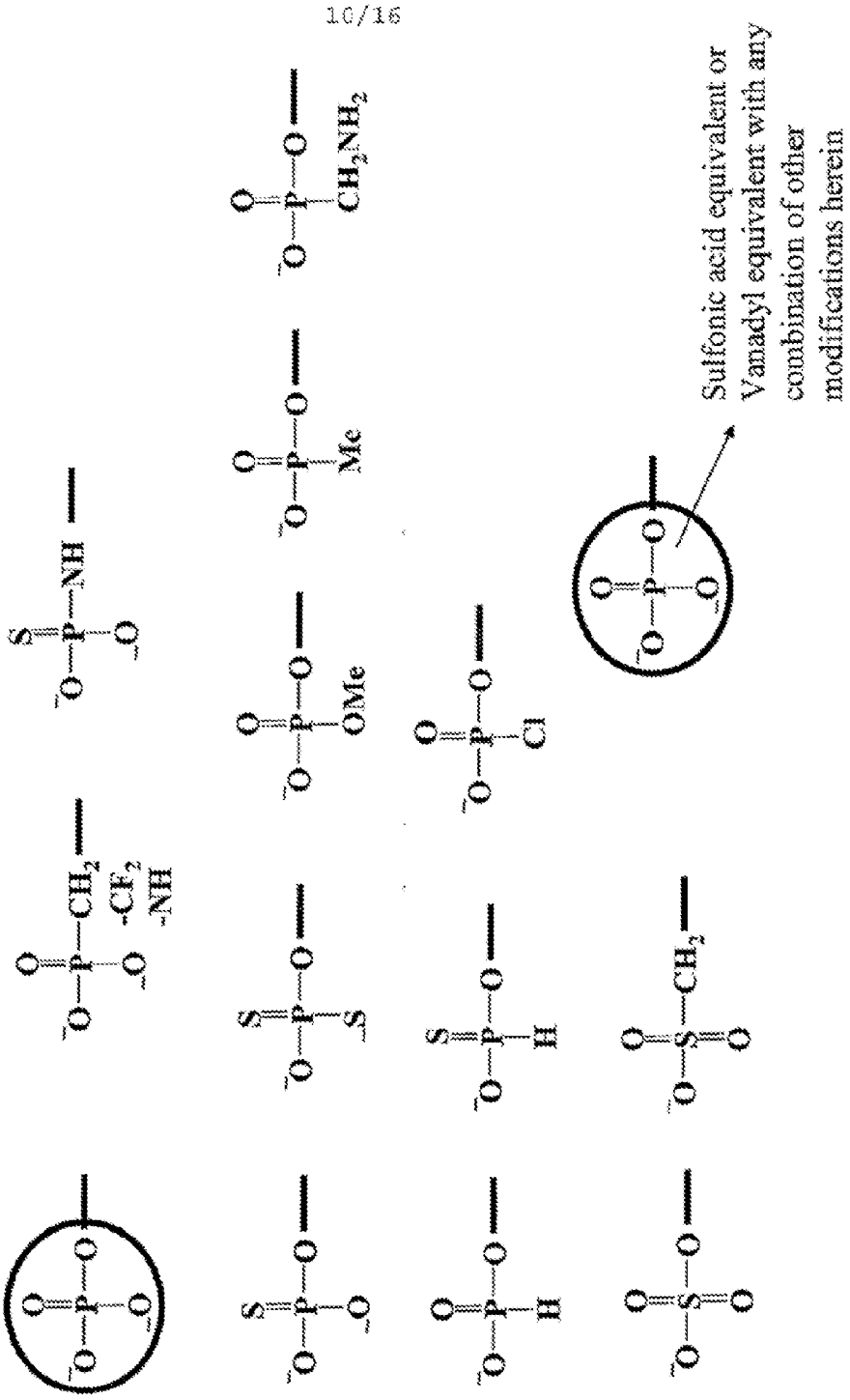
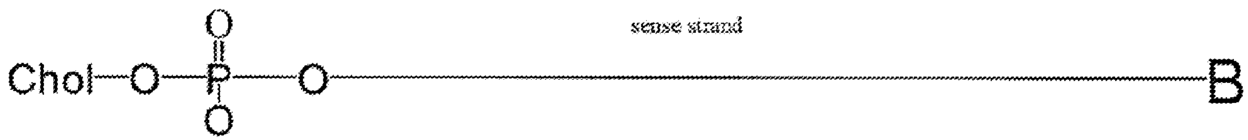
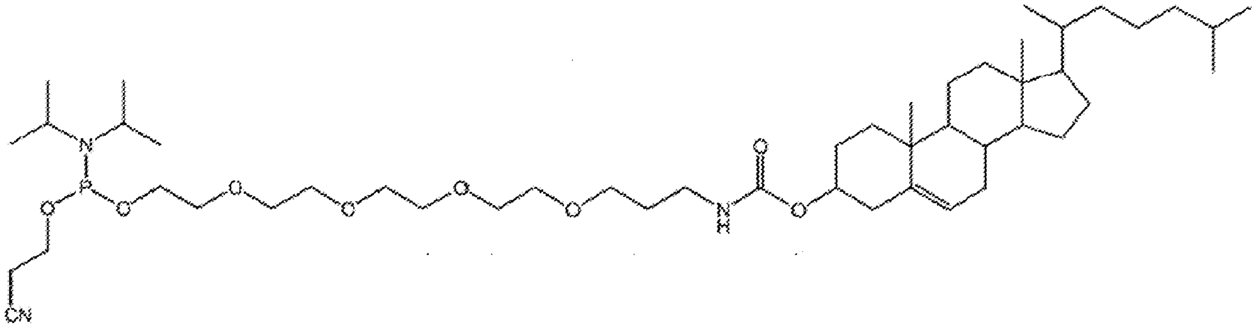


Figure 9: Cholesterol Conjugate Approach





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

Figure 10

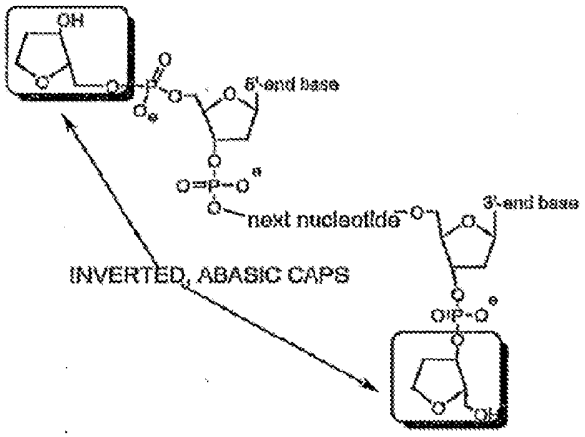
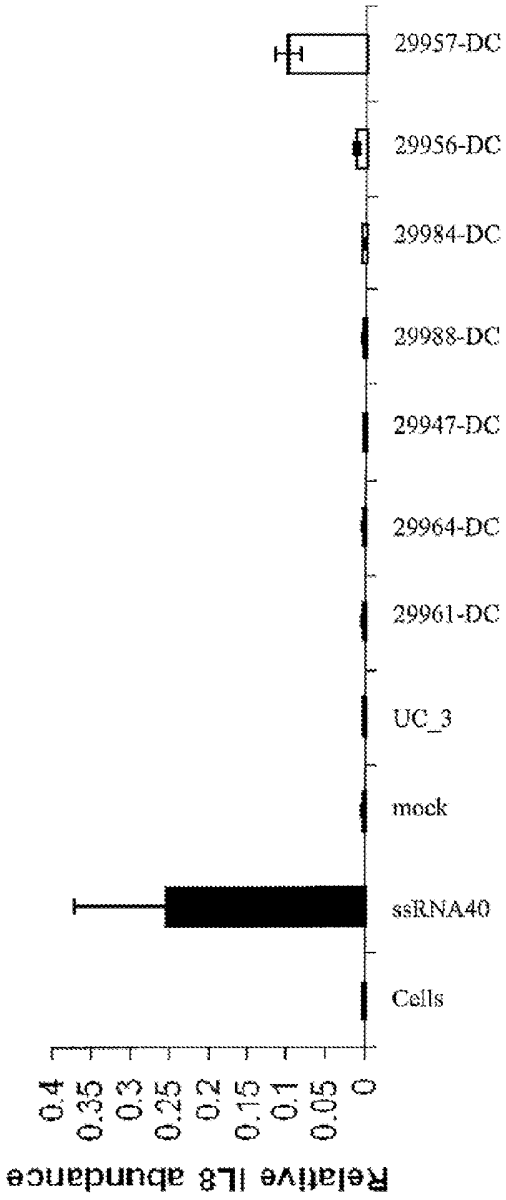

OH
5'-end base
next nucleotide
3'-end base
INVERTED ABASIC CAPS
OH

Figure 11: IL8 expression in TLR8 U2OS



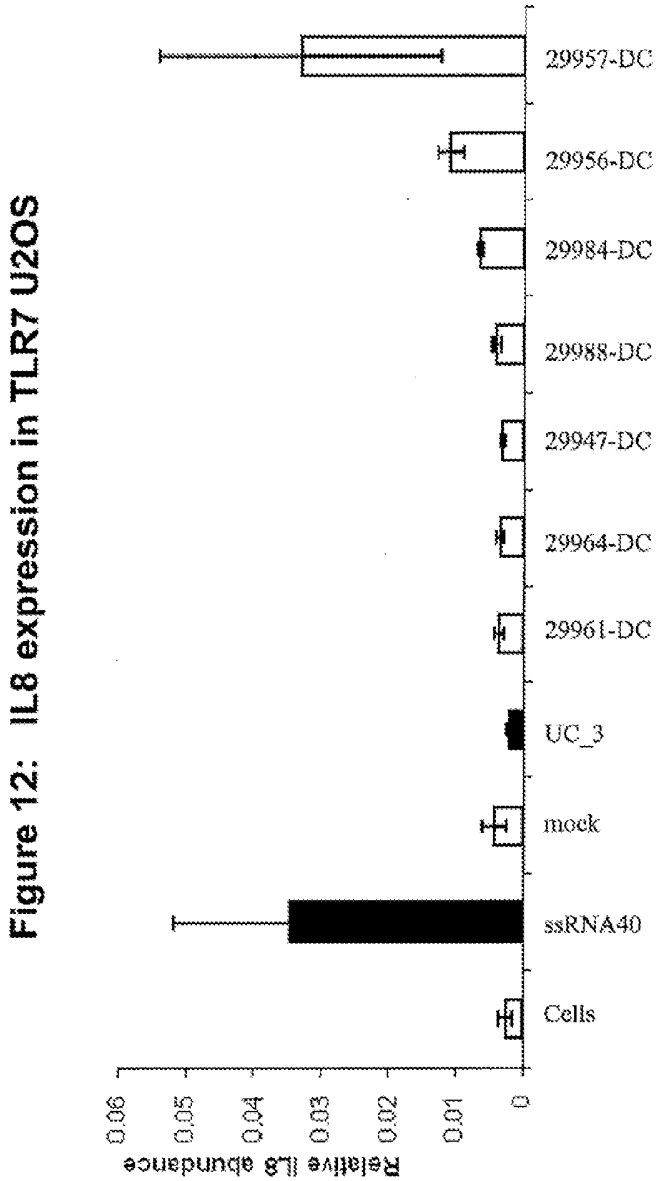


Figure 12: IL8 expression in TLR7 U2OS

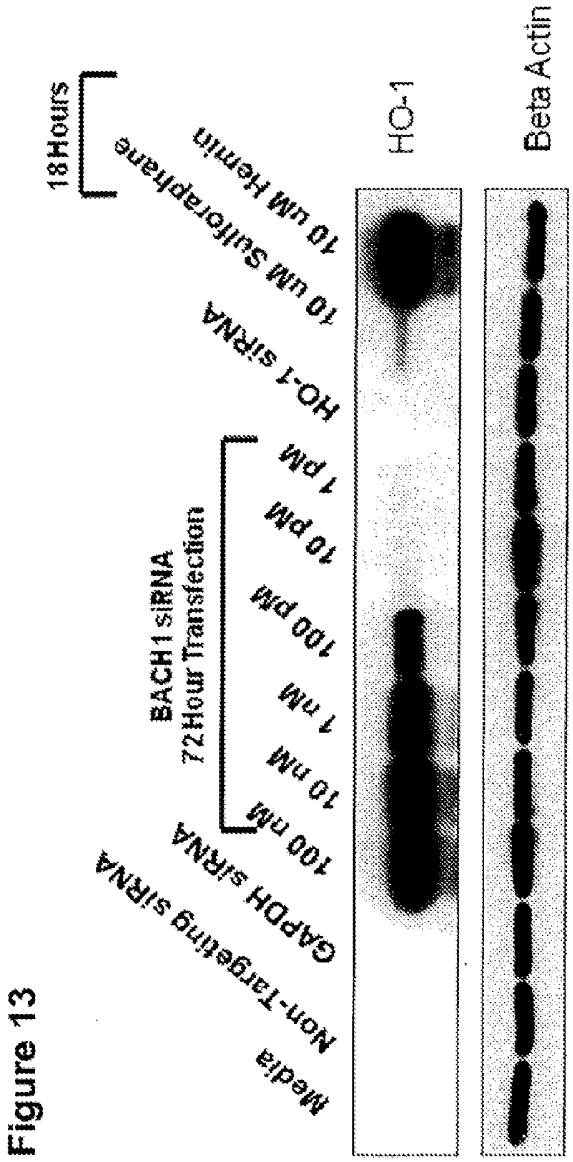

HO-1
Beta Actin
18Hours
10 uM Hemin
10 uM Sulforaphane
HO-1 siRNA
BACH1 siRNA
72Hour Transfection
1 pM
10 pM
100 pM
1 nM
10 nM
100 nM
GAPDH siRNA
Non-Targeting siRNA
Media

Figure 13

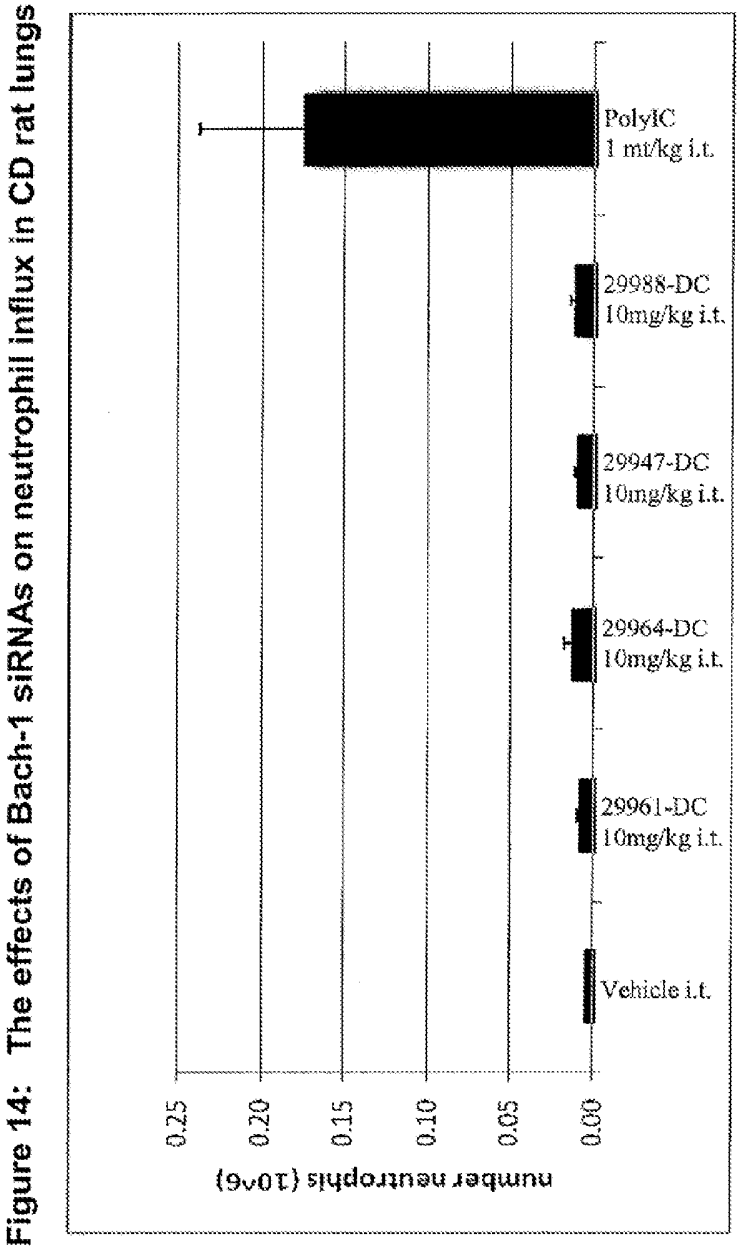


Figure 14: The effects of Bach-1 siRNAs on neutrophil influx in CD rat lungs