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(57) Abstract: The present disclosure provides single- or double-stranded interfering RNA molecules (e.g., siRNA) that target a SCN9A gene. The interfering RNA molecules may contain specific patterns of nucleoside modifications and internucleoside linkage modifications, as pharmaceutical compositions including the same. The siRNA molecules may be branched siRNA molecules, such as di-branched, tri¬ branched, ortetra-branched siRNA molecules. The disclosed siRNA molecules may further feature a 5' phosphorus stabilizing moiety and/or a hydrophobic moiety. Additionally, the disclosure provides methods for delivering the siRNA molecule of the disclosure to the central nervous system of a subject, such as a subject experiencing pain or identified as having a pain disorder.

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COMPOSITIONS AND METHODS FOR TREATMENT OF PAIN

Technical Field

This disclosure relates to small interfering RNA (siRNA) molecules, and compositions containing the same, that target RNA transcripts (e.g., mRNA) of a sodium voltage-gated channel alpha subunit 9 (*SCN9A*) gene. The disclosure further describes methods for the treatment of pain (e.g., chronic or acute pain) by delivering *SCN9A*-targeting siRNA molecules to the central nervous system of a subject in need.

Background

Pain indications represent a substantial unmet medical need. Among the existing therapeutics for pain, pregabalin and duloxetine have quite limited efficacy, and opioids are effective against some forms of acute or persistent pain but come with severe respiratory, gastrointestinal, and addiction liabilities. Other pharmacological treatments are sometimes used off-label for neuropathic or chronic pain but by and large have weak efficacy and prohibitive side effects. Accordingly, much interest has focused on developing new treatments for pain, particularly on making inhibitors of the Nav1.7 voltage-gated sodium ion channel protein encoded by the voltage-gated sodium channel alpha subunit 9 (*SCN9A*) gene.

However, Nav1.7 protein has proven difficult to target. One significant difficulty stems from the selectivity required for an Nav1.7 inhibitor to be an effective therapeutic. While Nav1.7 itself is not anticipated to have prohibitive on-target liability to inhibition, among eight other sodium channel paralogs are those governing cellular excitability in brain, cardiac muscle, and skeletal muscle. Since the functional areas of different sodium channels are highly conserved, few small molecule inhibitors have been reported that have meaningful selectivity for Nav1.7 among sodium channel isoforms. Achieving central nervous system penetrance of a small molecule Nav1.7-selective inhibitor has also been challenging.

Accordingly, there remains a need for therapeutics capable of selectively diminishing Nav1.7 activity among other sodium channels in a manner that provides effective relief from various forms of pain.

Summary of the Disclosure

The present disclosure provides compositions and methods for reduction of voltage-gated sodium channel alpha subunit 9 expression by way of small interfering RNA (siRNA)-mediated silencing of sodium voltage-gated channel alpha subunit 9 (*SCN9A)* transcripts. The compositions and methods provide the benefit of exhibiting high selectivity toward *SCN9A* over other central nervous system (CNS) genes, including those that encode other sodium channel paralogs.

The siRNA molecules of the disclosure can be used to silence the *SCN9A* gene, thereby preventing the translation of the corresponding mRNA transcript and reducing SCN9A expression. This reduction of SCN9A levels thus prevents transmission of noxious stimuli that result in pain. The siRNA molecules of the disclosure can be administered to individuals with a pain syndrome or to individuals identified as having a gain-of-function *SCN9A* mutation. The siRNA molecules of the disclosure can be delivered directly to the CNS or neurons of a subject in need of *SCN9A* silencing by way of, for example, injection intrathecally, intracerebroventricularly, intrastriatally, intraparenchymally, direct injection into a specific nerve or ganglion(ganglia) (e.g., trigeminal or dorsal root ganglia), intra-cisterna magna injection, such as by catheterization, intravenous injection, subcutaneous injection, or intramuscular injection..

In an aspect, the disclosure provides a siRNA molecule containing an antisense strand and sense strand having complementarity to the antisense strand. The antisense strand has complementarity sufficient to hybridize to a region within an *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152. In some embodiments, the antisense strand has complementarity sufficient to hybridize to a region within an *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576. The antisense strand may be, for example, from 10 to 50 nucleotides in length (e.g., from 10 to 45 nucleotides in length, from 10 to 40 nucleotides in length, from 10 to 35 nucleotides in length, from 10 to 30 nucleotides in length, from 10 to 29 nucleotides in length, from 10 to 28 nucleotides in length, from 10 to 27 nucleotides in length, from 10 to 26 nucleotides in length, from 10 to 25 nucleotides in length, from 10 to 24 nucleotides in length, from 10 to 23 nucleotides in length, from 10 to 22 nucleotides in length, from 10 to 21 nucleotides in length, or from 10 to 20 nucleotides in length). In some embodiments, the antisense strand is 10 nucleotides in length, 11 nucleotides in length, 12 nucleotides in length, 13 nucleotides in length, 14 nucleotides in length, 15 nucleotides in length, 16 nucleotides in length, 17 nucleotides in length, 18 nucleotides in length, 19 nucleotides in length, 20 nucleotides in length, 21 nucleotides in length, 22 nucleotides in length, 23 nucleotides in length, 24 nucleotides in length, 25 nucleotides in length, 26 nucleotides in length, 27 nucleotides in length, 28 nucleotides in length, 29 nucleotides in length, 30 nucleotides in length, or more.

In some embodiments of any of the foregoing aspects, the antisense strand has at least 70% (e.g., at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76, at least 77, at least 78, at least 79, at least 80, at least 81, at least 82, at least 83, at least 84, at least 85, at least 86, at least 87, at least 88, at least 89, at least 90, at least 91, at least 92, at least 93, at least 94, at least 95, at least 96, at least 97, at least 98, at least 99, or 100%) complementarity to a region of 15 contiguous nucleobases within the *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152. In some embodiments, the antisense strand has at least 70% (e.g., at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76, at least 77, at least 78, at least 79, at least 80, at least 81, at least 82, at least 83, at least 84, at least 85, at least 86, at least 87, at least 88, at least 89, at least 90, at least 91, at least 92, at least 93, at least 94, at least 95, at least 96, at least 97, at least 98, at least 99, or 100%) complementarity to a region of 16 contiguous nucleobases within the *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152. In some embodiments, the antisense strand has at least 70% (e.g., at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76, at least 77, at least 78, at least 79, at least 80, at least 81, at least 82, at least 83, at least 84, at least 85, at least 86, at least 87, at least 88, at least 89, at least 90, at least 91, at least 92, at least 93, at least 94, at least 95, at least 96, at least 97, at least 98, at least 99, or 100%) complementarity to a region of 17 contiguous nucleobases within the *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152. In some embodiments, the antisense strand has at least 70% (e.g., at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76, at least 77, at least 78, at least 79, at least 80, at least 81, at least 82, at least 83, at least 84, at least 85, at least 86, at least 87, at least 88, at least 89, at least 90, at least 91, at least 92, at least 93, at least 94, at least 95, at least 96, at least 97, at least 98, at least 99, or 100%) complementarity to a region of 18 contiguous nucleobases within the *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576

and 961-1152. In some embodiments, the antisense strand has at least 70% (e.g., at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76, at least 77, at least 78, at least 79, at least 80, at least 81, at least 82, at least 83, at least 84, at least 85, at least 86, at least 87, at least 88, at least 89, at least 90, at least 91, at least 92, at least 93, at least 94, at least 95, at least 96, at least 97, at least 98, at least 99, or 100%) complementarity to a region of 19 contiguous nucleobases within the *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152. In some embodiments, the antisense strand has at least 70% (e.g., at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76, at least 77, at least 78, at least 79, at least 80, at least 81, at least 82, at least 83, at least 84, at least 85, at least 86, at least 87, at least 88, at least 89, at least 90, at least 91, at least 92, at least 93, at least 94, at least 95, at least 96, at least 97, at least 98, at least 99, or 100%) complementarity to a region of 20 contiguous nucleobases within the *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152. In some embodiments, the antisense strand has at least 70% (e.g., at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76, at least 77, at least 78, at least 79, at least 80, at least 81, at least 82, at least 83, at least 84, at least 85, at least 86, at least 87, at least 88, at least 89, at least 90, at least 91, at least 92, at least 93, at least 94, at least 95, at least 96, at least 97, at least 98, at least 99, or 100%) complementarity to a region of 21 contiguous nucleobases within the *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

In some embodiments, the antisense strand has at least 70% (e.g., at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) complementarity to the region within the *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

In some embodiments, the antisense strand has at least 75% complementarity to the region within the *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152. For example, the antisense strand may have at least 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementarity to the region within the *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID Nos: 385-576 and 961-1152.

In some embodiments, the antisense strand has at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or 30 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

In some embodiments, the antisense strand has from 10 to 30 contiguous nucleotides (e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides) that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the

SCN9A RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

In some embodiments, the antisense strand has from 12 to 30 contiguous nucleotides (e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides) that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

In some embodiments, the antisense strand has from 15 to 30 contiguous nucleotides (e.g., 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides) that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

In some embodiments, the antisense strand has from 18 to 30 contiguous nucleotides (e.g., 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides) that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

In some embodiments, the antisense strand has from 18 to 25 contiguous nucleotides (e.g., 18, 19, 20, 21, 22, 23, 24, or 25 contiguous nucleotides) that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID Nos: 385-576 and 961-1152.

In some embodiments, the antisense strand has from 18 to 21 contiguous nucleotides (e.g., 18, 19, 20, or 21 contiguous nucleotides) that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

In some embodiments, the antisense strand has 21 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

In some embodiments, the antisense strand has from 21 to 30 contiguous nucleotides (e.g., 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides) that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID Nos: 385-576 and 961-1152.

In some embodiments, the antisense strand has from 24 to 30 contiguous nucleotides (e.g., 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides) that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

In some embodiments, the antisense strand has 30 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

In some embodiments, the antisense strand has 9 or fewer nucleotide mismatches relative to the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152, optionally wherein the antisense strand contains 8 or fewer, 7 or fewer, 6 or fewer, 5 or

fewer, 4 or fewer, 3 or fewer, 2 or fewer, or only 1 mismatch relative to the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

In some embodiments of any of the foregoing aspects or embodiments of the disclosure, the region of the *SCN9A* RNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 385-576. In some embodiments of any of the foregoing aspects or embodiments of the disclosure, the region of the *SCN9A* RNA transcript has the nucleic acid sequence of SEQ ID NO: 970 or 1072.

In some embodiments, the antisense strand has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 1-192 and 577-768. In some embodiments, the antisense strand has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 1-192. In some embodiments, the antisense strand has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 586 or 688.

In some embodiments, the antisense strand has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 1-192 and 577-768. In some embodiments, the antisense strand has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 1-192. In some embodiments, the antisense strand has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 586 or 688.

In some embodiments, the antisense strand has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 1-192 and 577-768, optionally wherein the antisense strand has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of any one of SEQ ID NOs: 1-192 and 577-768. In some embodiments, the antisense strand has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 1-192, optionally wherein the antisense strand has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of any one of SEQ ID NOs: 1-192. In some embodiments, the antisense strand has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 586 or 688, optionally wherein the antisense strand has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of SEQ ID NO: 586 or 688.

In some embodiments, the antisense strand has the nucleic acid sequence of any one of SEQ ID NOs: 1-192 and 577-768. In some embodiments, the antisense strand has the nucleic acid sequence of any one of SEQ ID NOs: 1-192. In some embodiments, the antisense strand has the nucleic acid sequence of SEQ ID NO: 586 or 688.

In some embodiments, the sense strand has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%

identical) to the nucleic acid sequence of any one of SEQ ID NOs: 193-384 and 769-960. In some embodiments, the sense strand has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 193-384. In some embodiments, the sense strand has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 778 or 880.

In some embodiments, the sense strand has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 193-384 and 769-960. In some embodiments, the sense strand has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 193-384. In some embodiments, the sense strand has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 778 or 880.

In some embodiments, the sense strand has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 193-384 and 769-960, optionally wherein the sense strand has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of any one of SEQ ID NOs: 193-384 and 769-960. In some embodiments, the sense strand has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 193-384, optionally wherein the sense strand has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of any one of SEQ ID NOs: 193-384. In some embodiments, the sense strand has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 778 or 880, optionally wherein the sense strand has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of SEQ ID NO: 778 or 880.

In some embodiments, the siRNA molecule has a sense strand having the nucleic acid sequence of any one of SEQ ID NOs: 193-384 and 769-960. In some embodiments, the siRNA molecule has a sense strand having the nucleic acid sequence of any one of SEQ ID NOs: 193-384. In some embodiments, the siRNA molecule has a sense strand having the nucleic acid sequence of SEQ ID NO: 778 or 880.

In some embodiments, the antisense strand has a structure represented by Formula I, wherein Formula I is, in the 5'-to-3' direction:

$$A\text{-}B\text{-}(A')_j\text{-}C\text{-}P^2\text{-}D\text{-}P^1\text{-}(C'\text{-}P^1)_k\text{-}C'$$

Formula I;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$;

each A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$;

B is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2$;

each C is a 2'-O-methyl (2'-O-Me) ribonucleoside;

each C', independently, is a 2'-O-Me ribonucleoside or a 2'-fluoro (2'-F) ribonucleoside;
each D is a 2'-F ribonucleoside;
each P^1 is a phosphorothioate internucleoside linkage;
each P^2 is a phosphodiester internucleoside linkage;
j is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); and
k is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7).

In some embodiments, the antisense strand has a structure represented by Formula A1, wherein Formula A1 is, in the 5'-to-3' direction:

A-S-B-S-A-O-B-O-B-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-A-S-A-S-B-S-A

Formula A1;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments, the antisense strand has a structure represented by Formula II, wherein Formula II is, in the 5'-to-3' direction:

$$A\text{-}B\text{-}(A')_j\text{-}C\text{-}P^2\text{-}D\text{-}P^1\text{-}(C\text{-}P^1)_k\text{-}C'$$

Formula II;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$;
each A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$;
B is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2$;
each C is a 2'-O-methyl (2'-O-Me) ribonucleoside;
each C', independently, is a 2'-O-Me ribonucleoside or a 2'-fluoro (2'-F) ribonucleoside;
each D is a 2'-F ribonucleoside;
each P^1 is a phosphorothioate internucleoside linkage;
each P^2 is a phosphodiester internucleoside linkage;
j is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); and
k is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7).

In some embodiments, antisense strand has a structure represented by Formula A2, wherein Formula A2 is, in the 5'-to-3' direction:

A-S-B-S-A-O-B-O-B-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-A-S-A-S-A-S-A

Formula A2;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments, the sense strand has a structure represented by Formula III, wherein Formula III is, in the 5'-to-3' direction:

$E\text{-}(A')_m\text{-}F$

Formula III;

wherein E is represented by the formula $(C\text{-}P^1)_2$;

F is represented by the formula $(C\text{-}P^2)_3\text{-}D\text{-}P^1\text{-}C\text{-}P^1\text{-}C$, $(C\text{-}P^2)_3\text{-}D\text{-}P^2\text{-}C\text{-}P^2\text{-}C$, $(C\text{-}P^2)_3\text{-}D\text{-}P^1\text{-}C\text{-}P^1\text{-}D$, or $(C\text{-}P^2)_3\text{-}D\text{-}P^2\text{-}C\text{-}P^2\text{-}D$;

A', C, D, P^1, and P^2 are as defined in Formula II; and

m is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7).

In some embodiments, the sense strand has a structure represented by Formula S1, wherein Formula S1 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A-O-A-O-B-S-A-S-A

Formula S1;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments, the sense strand has a structure represented by Formula S2, wherein Formula S2 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A-O-A-O-B-O-A-O-A

Formula S2;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments, the sense strand has a structure represented by Formula S3, wherein Formula S3 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A-O-A-O-B-S-A-S-B

Formula S3;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments, the sense strand has a structure represented by Formula S4, wherein Formula S4 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A-O-A-O-B-O-A-O-B

Formula S4;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments, the antisense strand has a structure represented by Formula IV, wherein Formula IV is, in the 5'-to-3' direction:

$$A\text{-}(A')_j\text{-}C\text{-}P^2\text{-}B\text{-}(C\text{-}P^1)_k\text{-}C'$$

Formula IV;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$;
each A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$;
B is represented by the formula $D\text{-}P^1\text{-}C\text{-}P^1\text{-}D\text{-}P^1$;
each C is a 2'-O-Me ribonucleoside;
each C', independently, is a 2'-O-Me ribonucleoside or a 2'-F ribonucleoside;
each D is a 2'-F ribonucleoside;
each P^1 is a phosphorothioate internucleoside linkage;
each P^2 is a phosphodiester internucleoside linkage;
j is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); and
k is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7).

In some embodiments, the antisense strand has a structure represented by Formula A3, wherein Formula A3 is, in the 5'-to-3' direction:

A-S-B-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-B-S-A-S-A-S-A

Formula A3;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments, the sense strand has a structure represented by Formula V, wherein Formula V is, in the 5'-to-3' direction:

$$E\text{-}(A')_m\text{-}C\text{-}P^2\text{-}F$$

Formula V;

wherein E is represented by the formula $(C\text{-}P^1)_2$;
F is represented by the formula $D\text{-}P^1\text{-}C\text{-}P^1\text{-}C$, $D\text{-}P^2\text{-}C\text{-}P^2\text{-}C$, $D\text{-}P^1\text{-}C\text{-}P^1\text{-}D$, or $D\text{-}P^2\text{-}C\text{-}P^2\text{-}D$;
A', C, D, P^1 and P^2 are as defined in Formula IV; and
m is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7).

In some embodiments, the sense strand has a structure represented by Formula S5, wherein Formula S5 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-A

Formula S5;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments, the sense strand has a structure represented by Formula S6, wherein Formula S6 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A

Formula S6;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments, the sense strand has a structure represented by Formula S7, wherein Formula S7 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-B

Formula S7;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments, the sense strand has a structure represented by Formula S8, wherein Formula S8 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B

Formula S8;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments, the antisense strand has a structure represented by Formula VI, wherein Formula VI is, in the 5'-to-3' direction:

$A\text{-}B_j\text{-}E\text{-}B_k\text{-}E\text{-}F\text{-}G_l\text{-}D\text{-}P^1\text{-}C'$

Formula VI;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$;

each B is represented by the formula $C\text{-}P^2$;

each C is a 2'-O-Me ribonucleoside;

each C', independently, is a 2'-O-Me ribonucleoside or a 2'-F ribonucleoside;

each D is a 2'-F ribonucleoside;

each E is represented by the formula $D\text{-}P^2\text{-}C\text{-}P^2$;

F is represented by the formula $D\text{-}P^1\text{-}C\text{-}P^1$;

each G is represented by the formula $C\text{-}P^1$;

each P^1 is a phosphorothioate internucleoside linkage;

each P^2 is a phosphodiester internucleoside linkage;
j is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7);
k is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); and
l is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7).

In some embodiments, the antisense strand has a structure represented by Formula A4, wherein Formula A4 is, in the 5'-to-3' direction:

A-S-B-S-A-O-A-O-A-O-B-O-A-O-A-O-A-O-A-O-A-O-A-O-A-O-B-O-A-O-B-S-A-S-A-S-A-S-B-S-A

Formula A4;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments, the sense strand has a structure represented by Formula VII, wherein Formula VII is, in the 5'-to-3' direction:

$$H\text{-}B_m\text{-}I_n\text{-}A'\text{-}B_o\text{-}H\text{-}C$$

Formula VII;

wherein A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$;
each H is represented by the formula $(C\text{-}P^1)_2$;
each I is represented by the formula $(D\text{-}P^2)$;
B, C, D, P^1 and P^2 are as defined in Formula VI;
m is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7);
n is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); and
o is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7).

In some embodiments, the sense strand has a structure represented by Formula S9, wherein Formula S9 is, in the 5'-to-3' direction:

A-S-A-S-A-O-A-O-A-O-B-O-B-O-B-O-A-O-B-O-A-O-A-O-A-O-A-S-A-S-A

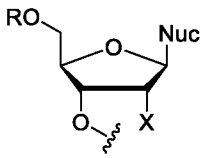
Formula S9;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

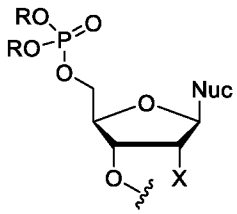
In some embodiments, the antisense strand also has a 5' phosphorus stabilizing moiety at the 5' end of the antisense strand.

In some embodiments, the sense strand also has a 5' phosphorus stabilizing moiety at the 5' end of the sense strand.

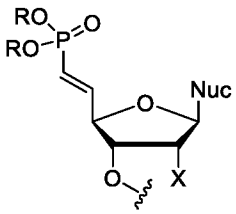
In some embodiments, each 5' phosphorus stabilizing moiety is, independently, represented by any one of Formulas IX, XX, XI, XII, XIII, XIV, XV, or XVI:



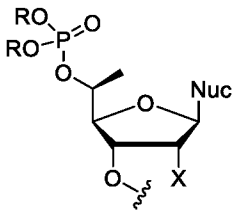
Formula IX



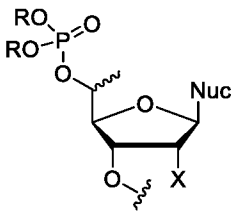
Formula X



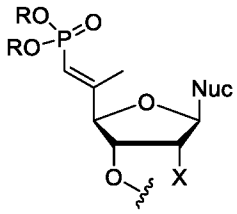
Formula XI



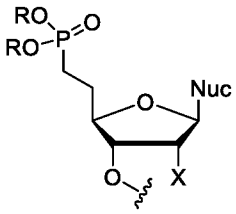
Formula XII



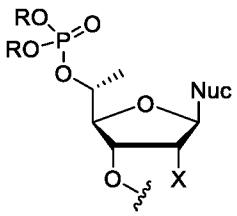
Formula XIII



Formula XIV



Formula XV



Formula XVI

wherein Nuc represents a nucleobase, optionally wherein the nucleobase is selected from the group consisting of adenine, uracil, guanine, thymine, and cytosine, and R represents an optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, phenyl, benzyl, a cation (e.g., a monovalent cation), or hydrogen.

In some embodiments, the nucleobase is an adenine, uracil, guanine, thymine, or cytosine.

In some embodiments, the 5' phosphorus stabilizing moiety is (E)-vinylphosphonate represented by Formula XI.

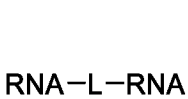
In some embodiments, the siRNA molecule also has a hydrophobic moiety at the 5' or the 3' end of the siRNA molecule.

In some embodiments, the hydrophobic moiety is selected from a group consisting of cholesterol, vitamin D, or tocopherol.

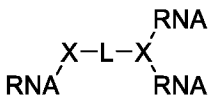
In some embodiments, the siRNA molecule is a branched siRNA molecule.

In some embodiments, the branched siRNA molecule is di-branched, tri-branched, or tetra-branched.

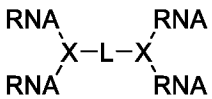
In some embodiments, the siRNA molecule is di-branched, optionally wherein the di-branched siRNA molecule is represented by any one of Formulas XVII, XVIII, or XIX:



Formula XVII;



Formula XVIII;

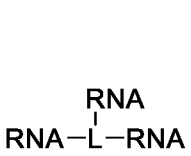


Formula XIX;

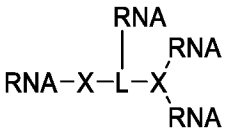
wherein each RNA is, independently, an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

In some embodiments, the di-branched siRNA molecule is represented by Formula XVII. In some embodiments, the di-branched siRNA molecule is represented by Formula XVIII. In some embodiments, the di-branched siRNA molecule is represented by Formula XIX.

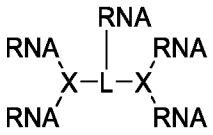
In some embodiments, the siRNA molecule is tri-branched, optionally wherein the tri-branched siRNA molecule is represented by any one of Formulas XX, XXI, XXII, or XXIII:



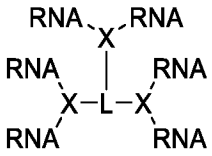
Formula XX;



Formula XXI;



Formula XXII;

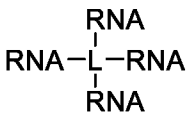


Formula XXIII;

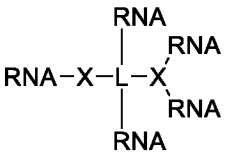
wherein each RNA is, independently, an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

In some embodiments, the tri-branched siRNA molecule is represented by Formula XX. In some embodiments, the tri-branched siRNA molecule is represented by Formula XXI. In some embodiments, the tri-branched siRNA molecule is represented by Formula XXII. In some embodiments, the tri-branched siRNA molecule is represented by Formula XXIII.

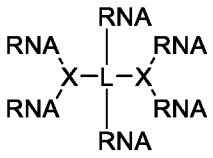
In some embodiments, the siRNA molecule is tetra-branched, optionally wherein the tetra-branched siRNA molecule is represented by any one of Formulas XXIV, XXV, XXVI, XXVII, or XXVIII:



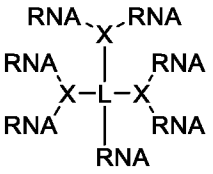
Formula XXIV;



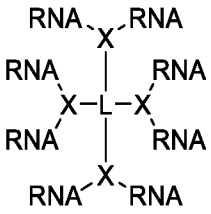
Formula XXV;



Formula XXVI;



Formula XXVII;



Formula XXVIII;

wherein each RNA is, independently, an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

In some embodiments, the tetra-branched siRNA molecule is represented by Formula XXIV. In some embodiments, the tetra-branched siRNA molecule is represented by Formula XXV. In some embodiments, the tetra-branched siRNA molecule is represented by Formula XXVI. In some embodiments, the tetra-branched siRNA molecule is represented by Formula XXVII. In some embodiments, the tetra-branched siRNA molecule is represented by Formula XXVIII.

In some embodiments of the branched siRNA, the linker is selected from a group consisting of one or more contiguous subunits of an ethylene glycol (e.g., polyethylene glycol (PEG), such as, e.g., triethylene glycol (TrEG) or tetraethylene glycol (TEG)), alkyl, carbohydrate, block copolymer, peptide, RNA, and DNA.

In some embodiments, the linker is an ethylene glycol oligomer. In some embodiments, the linker is an alkyl oligomer. In some embodiments, the linker is a carbohydrate oligomer. In some embodiments, the linker is a block copolymer. In some embodiments, the linker is a peptide oligomer. In some embodiments, the linker is an RNA oligomer. In some embodiments, the linker is a DNA oligomer.

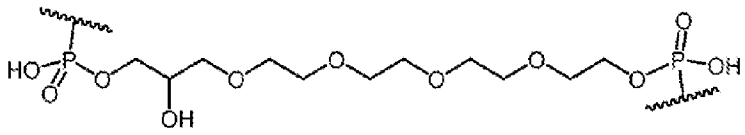
In some embodiments, the ethylene glycol oligomer is a PEG. In some embodiments, the PEG is a TrEG. In some embodiments, the PEG is a TEG.

In some embodiments, the oligomer or copolymer contains 2 to 20 contiguous subunits (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous subunits).

In some embodiments, the linker attaches one or more (e.g., 1, 2, 3, 4, or more) siRNA molecules by way of a covalent bond-forming moiety.

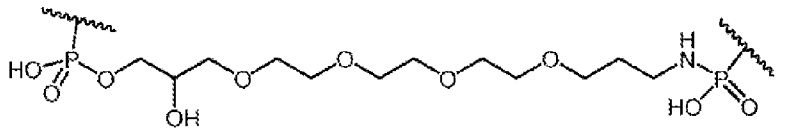
In some embodiments, the covalent bond-forming moiety is selected from the group consisting of an alkyl, ester, amide, carbamate, phosphonate, phosphate, phosphorothioate, phosphoroamidate, triazole, urea, and formacetal.

In some embodiments, the linker includes a structure of Formula L1:



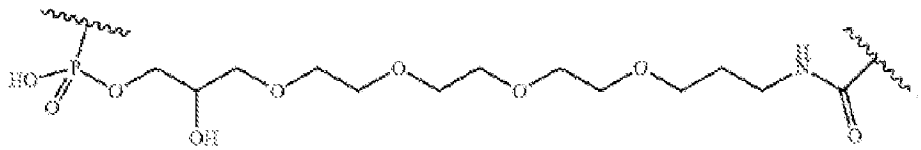
(**Formula L1**)

In some embodiments, the linker includes a structure of Formula L2:



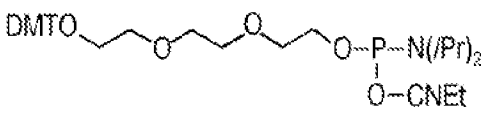
(**Formula L2**)

In some embodiments, the linker includes a structure of Formula L3:



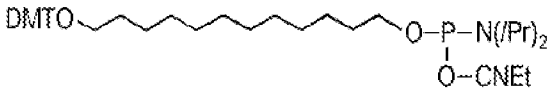
(**Formula L3**)

In some embodiments, the linker includes a structure of Formula L4:



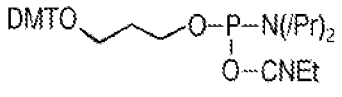
(**Formula L4**)

In some embodiments, the linker includes a structure of Formula L5:



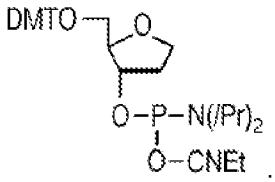
(**Formula L5**)

In some embodiments, the linker includes a structure of Formula L6:



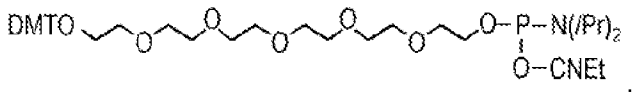
(**Formula L6**)

In some embodiments, the linker includes a structure of Formula L7:



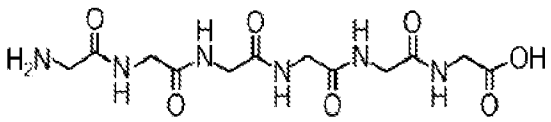
(**Formula L7**)

In some embodiments, the linker includes a structure of Formula L8:



(**Formula L8**)

In some embodiments, the linker includes a structure of Formula L9:



(**Formula L9**)

In some embodiments of any of the siRNA molecules described herein, 50% or more of the ribonucleotides in the antisense strand are 2′-O-Me ribonucleotides (e.g., 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the ribonucleotides in the antisense strand may be 2′-O-Me ribonucleotides).

In some embodiments, 60% or more of the ribonucleotides in the antisense strand are 2′-O-Me ribonucleotides (e.g., 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the ribonucleotides in the antisense strand may be 2′-O-Me ribonucleotides).

In some embodiments, 70% or more of the ribonucleotides in the antisense strand are 2′-O-Me ribonucleotides (e.g., 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the ribonucleotides in the antisense strand may be 2′-O-Me ribonucleotides).

In some embodiments, 80% or more of the ribonucleotides in the antisense strand are 2′-O-Me ribonucleotides (e.g., 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the ribonucleotides in the antisense strand may be 2′-O-Me ribonucleotides).

In some embodiments, 90% or more of the ribonucleotides in the antisense strand are 2′-O-Me ribonucleotides (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the ribonucleotides in the antisense strand may be 2′-O-Me ribonucleotides).

In some embodiments, 10% or less of the internucleoside linkages are phosphodiester linkages or phosphorothioate linkages. In some embodiments, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the internucleoside linkages are phosphodiester linkages or phosphorothioate linkages. In some embodiments, 100% of the internucleoside linkages are phosphodiester linkages or phosphorothioate linkages.

In some embodiments, 9 internucleoside linkages are phosphodiester linkages or phosphorothioate linkages.

In some embodiments, the length of the antisense strand is between 10 and 30 nucleotides (e.g., 10 nucleotides, 11 nucleotides, 12 nucleotides, 13 nucleotides, 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides), 15 and 25 nucleotides (e.g., 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, or 25 nucleotides), or 18 and 23 nucleotides (e.g., 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, or 23 nucleotides). In some embodiments, the length of the antisense strand is 20 nucleotides. In some embodiments, the length of the antisense strand is 21 nucleotides. In some embodiments, the length of the antisense strand is 22 nucleotides. In some embodiments, the length of the antisense strand is 23 nucleotides. In some embodiments, the length of the antisense strand is 24 nucleotides. In some embodiments, the length of the antisense strand is 25 nucleotides. In some embodiments, the length of the antisense strand is 26 nucleotides. In some embodiments, the length of the antisense strand is 27 nucleotides. In some embodiments, the length of the antisense strand is 28 nucleotides. In some embodiments, the length of the antisense strand is 29 nucleotides. In some embodiments, the length of the antisense strand is 30 nucleotides.

In some embodiments, the siRNA molecules of the branched compound are joined to one another by way of a linker (e.g., an ethylene glycol oligomer, such as tetraethylene glycol). In some embodiments, the siRNA molecules of the branched compound are joined to one another by way of a linker between the sense strand of one siRNA molecule and the sense strand of the other siRNA molecule. In some embodiments, the siRNA molecules are joined by way of linkers between the antisense strand of one

siRNA molecule and the antisense strand of the other siRNA molecule. In some embodiments, the siRNA molecules of the branched compound are joined to one another by way of a linker between the sense strand of one siRNA molecule and the antisense strand of the other siRNA molecule.

In some embodiments, the length of the sense strand is between 12 and 30 nucleotides (e.g., 12 nucleotides, 13 nucleotides, 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides), or 14 and 18 nucleotides (e.g., 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, or 18 nucleotides). In some embodiments, the length of the sense strand is 15 nucleotides. In some embodiments, the length of the sense strand is 16 nucleotides. In some embodiments, the length of the sense strand is 17 nucleotides. In some embodiments, the length of the sense strand is 18 nucleotides. In some embodiments, the length of the sense strand is 19 nucleotides. In some embodiments, the length of the sense strand is 20 nucleotides. In some embodiments, the length of the sense strand is 21 nucleotides. In some embodiments, the length of the sense strand is 22 nucleotides. In some embodiments, the length of the sense strand is 23 nucleotides. In some embodiments, the length of the sense strand is 24 nucleotides. In some embodiments, the length of the sense strand is 25 nucleotides. In some embodiments, the length of the sense strand is 26 nucleotides. In some embodiments, the length of the sense strand is 27 nucleotides. In some embodiments, the length of the sense strand is 28 nucleotides. In some embodiments, the length of the sense strand is 29 nucleotides. In some embodiments, the length of the sense strand is 30 nucleotides.

In some embodiments, four internucleoside linkages are phosphorothioate linkages.

In some embodiments of the siRNA molecules described herein, the antisense strand is 18 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 18 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 18 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 18 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 18 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 19 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 19 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 19 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 19 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 19 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 19 nucleotides in length and the sense strand is 19 nucleotides in length. In some embodiments, the antisense strand is 20 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 20 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 20 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 20 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 20 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 20 nucleotides in length and the

sense strand is 19 nucleotides in length. In some embodiments, the antisense strand is 20 nucleotides in length and the sense strand is 20 nucleotides in length. In some embodiments, the antisense strand is 21 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 21 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 21 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 21 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 21 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 21 nucleotides in length and the sense strand is 19 nucleotides in length. In some embodiments, the antisense strand is 21 nucleotides in length and the sense strand is 20 nucleotides in length. In some embodiments, the antisense strand is 21 nucleotides in length and the sense strand is 21 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 19 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 20 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 21 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 22 nucleotides in length. In some embodiments, the antisense strand is 23 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 23 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 23 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 23 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 23 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 23 nucleotides in length and the sense strand is 19 nucleotides in length. In some embodiments, the antisense strand is 23 nucleotides in length and the sense strand is 20 nucleotides in length. In some embodiments, the antisense strand is 23 nucleotides in length and the sense strand is 21 nucleotides in length. In some embodiments, the antisense strand is 23 nucleotides in length and the sense strand is 22 nucleotides in length. In some embodiments, the antisense strand is 23 nucleotides in length and the sense strand is 23 nucleotides in length. In some embodiments, the antisense strand is 24 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 24 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 24 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 24 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 24 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 24 nucleotides in length and the sense strand is 19 nucleotides in length. In some embodiments, the antisense strand is 24 nucleotides in length and the sense strand is 20

nucleotides in length. In some embodiments, the antisense strand is 24 nucleotides in length and the sense strand is 21 nucleotides in length. In some embodiments, the antisense strand is 24 nucleotides in length and the sense strand is 22 nucleotides in length. In some embodiments, the antisense strand is 24 nucleotides in length and the sense strand is 23 nucleotides in length. In some embodiments, the antisense strand is 24 nucleotides in length and the sense strand is 24 nucleotides in length. In some embodiments, the antisense strand is 25 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 25 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 25 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 25 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 25 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 25 nucleotides in length and the sense strand is 19 nucleotides in length. In some embodiments, the antisense strand is 25 nucleotides in length and the sense strand is 20 nucleotides in length. In some embodiments, the antisense strand is 25 nucleotides in length and the sense strand is 21 nucleotides in length. In some embodiments, the antisense strand is 25 nucleotides in length and the sense strand is 22 nucleotides in length. In some embodiments, the antisense strand is 25 nucleotides in length and the sense strand is 23 nucleotides in length. In some embodiments, the antisense strand is 25 nucleotides in length and the sense strand is 24 nucleotides in length. In some embodiments, the antisense strand is 25 nucleotides in length and the sense strand is 25 nucleotides in length. In some embodiments, the antisense strand is 26 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 26 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 26 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 26 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 26 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 26 nucleotides in length and the sense strand is 19 nucleotides in length. In some embodiments, the antisense strand is 26 nucleotides in length and the sense strand is 20 nucleotides in length. In some embodiments, the antisense strand is 26 nucleotides in length and the sense strand is 21 nucleotides in length. In some embodiments, the antisense strand is 26 nucleotides in length and the sense strand is 22 nucleotides in length. In some embodiments, the antisense strand is 26 nucleotides in length and the sense strand is 23 nucleotides in length. In some embodiments, the antisense strand is 26 nucleotides in length and the sense strand is 24 nucleotides in length. In some embodiments, the antisense strand is 26 nucleotides in length and the sense strand is 25 nucleotides in length. In some embodiments, the antisense strand is 26 nucleotides in length and the sense strand is 26 nucleotides in length. In some embodiments, the antisense strand is 27 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 27 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 27 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 27 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 27 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 27 nucleotides in length and the sense strand is 19 nucleotides in

length. In some embodiments, the antisense strand is 27 nucleotides in length and the sense strand is 20 nucleotides in length. In some embodiments, the antisense strand is 27 nucleotides in length and the sense strand is 21 nucleotides in length. In some embodiments, the antisense strand is 27 nucleotides in length and the sense strand is 22 nucleotides in length. In some embodiments, the antisense strand is 27 nucleotides in length and the sense strand is 23 nucleotides in length. In some embodiments, the antisense strand is 27 nucleotides in length and the sense strand is 24 nucleotides in length. In some embodiments, the antisense strand is 27 nucleotides in length and the sense strand is 25 nucleotides in length. In some embodiments, the antisense strand is 27 nucleotides in length and the sense strand is 26 nucleotides in length. In some embodiments, the antisense strand is 27 nucleotides in length and the sense strand is 27 nucleotides in length. In some embodiments, the antisense strand is 28 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 28 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 28 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 28 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 28 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 28 nucleotides in length and the sense strand is 19 nucleotides in length. In some embodiments, the antisense strand is 28 nucleotides in length and the sense strand is 20 nucleotides in length. In some embodiments, the antisense strand is 28 nucleotides in length and the sense strand is 21 nucleotides in length. In some embodiments, the antisense strand is 28 nucleotides in length and the sense strand is 22 nucleotides in length. In some embodiments, the antisense strand is 28 nucleotides in length and the sense strand is 23 nucleotides in length. In some embodiments, the antisense strand is 28 nucleotides in length and the sense strand is 24 nucleotides in length. In some embodiments, the antisense strand is 28 nucleotides in length and the sense strand is 25 nucleotides in length. In some embodiments, the antisense strand is 28 nucleotides in length and the sense strand is 26 nucleotides in length. In some embodiments, the antisense strand is 28 nucleotides in length and the sense strand is 27 nucleotides in length. In some embodiments, the antisense strand is 28 nucleotides in length and the sense strand is 28 nucleotides in length. In some embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 19 nucleotides in length. In some embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 20 nucleotides in length. In some embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 21 nucleotides in length. In some embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 22 nucleotides in length. In some embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 23 nucleotides in length. In some embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 24 nucleotides in length. In some embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 25 nucleotides in length. In some

embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 26 nucleotides in length. In some embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 27 nucleotides in length. In some embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 28 nucleotides in length. In some embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 29 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 19 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 20 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 21 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 22 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 23 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 24 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 25 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 26 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 27 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 28 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 29 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 30 nucleotides in length.

In a further aspect, the disclosure provides a pharmaceutical composition containing an siRNA molecule of any of the preceding aspects or embodiments of the disclosure, and a pharmaceutically acceptable excipient, carrier, or diluent.

In a further aspect, the disclosure provides a method of delivering an siRNA molecule to the CNS or neurons of a subject experiencing pain or diagnosed as having pain or a pain disorder by administering a therapeutically effective amount of the siRNA molecule or a pharmaceutical composition of any of the preceding aspects or embodiments of the disclosure to the subject.

In a further aspect, the disclosure provides a method of treating pain or a pain disorder in a subject in need thereof by administering a therapeutically effective amount of an siRNA molecule or a pharmaceutical composition of any of the preceding aspects or embodiments of the disclosure to the CNS or neurons of the subject.

In some embodiments, the pain is neuropathic pain.

In some embodiments, the pain is nociceptive pain.

In some embodiments, the pain is post-operative pain. In some embodiments, the pain is persistent pain. In some embodiments, the pain is inflammatory pain.

In some embodiments, the pain disorder is Gerhardt disease, Mitchell disease, or Weir-Mitchell disease. In some embodiments, the subject has been diagnosed with erythromelalgia.

In another aspect, the disclosure provides a method of reducing *SCN9A* expression in a subject in need thereof by administering a therapeutically effective amount of an siRNA or pharmaceutical composition of any of the preceding aspects or embodiments of the disclosure to the CNS or neurons of the subject.

In some embodiments, the subject exhibits selective reduction in *SCN9A* expression compared to reduction in expression of one or more other voltage-gated sodium ion channel genes upon administration of an siRNA molecule or pharmaceutical composition of any of the preceding aspects or embodiments of the disclosure.

In some embodiments, the siRNA molecule or the pharmaceutical composition is administered to the subject by way of intrathecal injection or other delivery into the central nervous system.

In some embodiments, the subject is a human.

In another aspect, the disclosure provides a kit having an siRNA molecule or pharmaceutical composition of any of the preceding aspects or embodiments of the disclosure, and a package insert that instructs a user of the kit to perform the method of any of the preceding aspects or embodiments of the disclosure.

Brief Description of the Figure

FIG. 1 is a graph showing the IC50 determination of two exemplary siRNA molecules of the disclosure having (1) an antisense strand of SEQ ID NO: 688 and a sense strand of SEQ ID NO: 880, having an IC50 of 0.0334 nM, and (2) an siRNA molecule having an antisense strand of SEQ ID NO: 586 and a sense strand of SEQ ID NO: 778, having an IC50 of 0.0166 nM.

Definitions

Unless otherwise defined herein, scientific, and technical terms used herein have the meanings that are commonly understood by those of ordinary skill in the art. In the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The use of "or" means "and/or" unless stated otherwise. The use of the term "including," as well as other forms, such as "includes" and "included," is not limiting.

As used herein, the term "nucleic acids" refers to RNA or DNA molecules consisting of a chain of ribonucleotides or deoxyribonucleotides, respectively.

As used herein, the term "therapeutic nucleic acid" refers to a nucleic acid molecule (e.g., ribonucleic acid) that has partial or complete complementarity to, and interacts with, a disease-associated target mRNA and mediates silencing of expression of the mRNA.

As used herein, the term "carrier nucleic acid" refers to a nucleic acid molecule (e.g., ribonucleic acid) that has sequence complementarity with, and hybridizes with, a therapeutic nucleic acid. As used herein, the term "3' end" refers to the end of the nucleic acid that contains an unmodified hydroxyl group at the 3' carbon of the ribose ring.

As used herein, the term "nucleoside" refers to a molecule made up of a heterocyclic base and its sugar.

As used herein, the term "nucleotide" refers to a nucleoside having a phosphate group on its 3' or 5' sugar hydroxyl group.

In the context of this disclosure, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring (e.g., modified) portions that function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases.

As used herein, the term "siRNA" refers to small interfering RNA duplexes that induce the RNA interference (RNAi) pathway. siRNA molecules may vary in length (generally, between 10 and 30 base pairs) and may contain varying degrees of complementarity to their target mRNA. The term "siRNA" includes duplexes of two separate strands, as well as single strands that optionally form hairpin structures including a duplex region.

As used herein, the term "antisense strand" refers to the strand of the siRNA duplex that contains some degree of complementarity to the target gene.

As used herein, the term "sense strand" refers to the strand of the siRNA duplex that contains complementarity to the antisense strand.

The term “interfering RNA molecule” refers to an RNA molecule, such as a small interfering RNA (siRNA), microRNA (miRNA), short hairpin RNA (shRNA), or an antisense oligonucleotide (ASO) that suppresses the endogenous function of a target RNA transcript.

As used herein, the terms "express" and “expression” refer to one or more of the following events: (1) production of an RNA template from a DNA sequence (e.g., by transcription); (2) processing of an RNA transcript (e.g., by splicing, editing, 5' cap formation, and/or 3' end processing); and (3) translation of an RNA into a polypeptide or protein. In the context of a gene that encodes a protein product, the terms “gene expression” and the like are used interchangeably with the terms “protein expression” and the like. Expression of a gene or protein of interest in a patient can manifest, for example, by detecting: an increase in the quantity or concentration of mRNA encoding corresponding protein (as assessed, e.g., using RNA detection procedures described herein or known in the art, such as quantitative polymerase chain reaction (qPCR) and RNA seq techniques), an increase in the quantity or concentration of the corresponding protein (as assessed, e.g., using protein detection methods described herein or known in the art, such as enzyme-linked immunosorbent assays (ELISA), among others), and/or an increase in the activity of the corresponding protein (e.g., in the case of an enzyme, as assessed using an enzymatic activity assay described herein or known in the art) in a sample obtained from the patient. As used herein, a cell is considered to “express” a gene or protein of interest if one or more, or all, of the above events can be detected in the cell or in a medium in which the cell resides. For example, a gene or protein of interest is considered to be “expressed” by a cell or population of cells if one can detect (i) production of a corresponding RNA transcript, such as an mRNA template, by the cell or population of cells (e.g., using RNA detection procedures described herein); (ii) processing of the RNA transcript (e.g., splicing, editing, 5’

cap formation, and/or 3' end processing, such as using RNA detection procedures described herein); (iii) translation of the RNA template into a protein product (e.g., using protein detection procedures described herein); and/or (iv) post-translational modification of the protein product (e.g., using protein detection procedures described herein).

As used herein, the terms "target," "targeting," and "targeted," in the context of the design of an siRNA, refers to generating an antisense strand so as to anneal the antisense strand to a region within the mRNA transcript of interest in a manner that results in a reduction in translation of the mRNA into the protein product.

As used herein, the terms "chemically modified nucleotide," "nucleotide analog," "altered nucleotide," and "modified nucleotide" refer to a non-standard nucleotide, including non-naturally occurring ribonucleotides or deoxyribonucleotides. Exemplary nucleotide analogs are modified at any position so as to alter certain chemical properties of the nucleotide yet retain the ability of the nucleotide analog to perform its intended function.

As used herein, the term "metabolically stabilized" refers to RNA molecules that contain ribonucleotides that have been chemically modified in order to decrease the rate of metabolism of an RNA molecule that is administered to a subject. Exemplary modifications include 2'-hydroxy to 2'-O-methoxy or 2'-fluoro, and phosphodiester to phosphorothioate.

As used herein, the term "phosphorothioate" refers to a phosphate group of a nucleotide that is modified by substituting one or more of the oxygens of the phosphate group with sulfur.

As used herein, the terms "internucleoside" and "internucleotide" refer to the bonds between nucleosides and nucleotides, respectively.

As used herein, the term "antagomirs" refers to nucleic acids that can function as inhibitors of miRNA activity.

As used herein, the term "gapmers" refers to chimeric antisense nucleic acids that contain a central block of deoxynucleotide monomers sufficiently long to induce RNase H cleavage. The deoxynucleotide block is flanked by ribonucleotide monomers or ribonucleotide monomers containing modifications.

As used herein, the term "mixmers" refers to nucleic acids that contain a mix of locked nucleic acids (LNAs) and DNA.

As used herein, the term "guide RNAs" refers to nucleic acids that have sequence complementarity to a specific sequence in the genome immediately or 1 base pair upstream of the protospacer adjacent motif (PAM) sequence as used in CRISPR/Cas9 gene editing systems. Alternatively, "guide RNAs" may refer to nucleic acids that have sequence complementarity (e.g., are antisense) to a specific messenger RNA (mRNA) sequence. In this context, a guide RNA may also have sequence complementarity to a "passenger RNA" sequence of equal or shorter length, which is identical or substantially identical to the sequence of mRNA to which the guide RNA hybridizes.

As used herein, the term "branched siRNA" refers to a compound containing two or more double-stranded siRNA molecules covalently bound to one another. Branched siRNA molecules may be "di-branched," also referred to herein as "di-siRNA," wherein the siRNA molecule includes 2 siRNA molecules covalently bound to one another, e.g., by way of a linker. Branched siRNA molecules may be "tri-branched," also referred to herein as "tri-siRNA," wherein the siRNA molecule includes 3 siRNA molecules

covalently bound to one another, e.g., by way of a linker. Branched siRNA molecules may be "tetra-branched," also referred to herein as "tetra-siRNA," wherein the siRNA molecule includes 4 siRNA molecules covalently bound to one another, e.g., by way of a linker.

As used herein, the term "branch point moiety" refers to a chemical moiety of a branched siRNA structure of the disclosure that may be covalently linked to a 5' end or a 3' end of an antisense strand or a sense strand of an siRNA molecule and which may support the attachment of additional single- or double-stranded siRNA molecules. Non-limiting examples of branch point moieties suitable for use in conjunction with the disclosed methods and compositions include, e.g., phosphoroamidite, tosylated solketal, 1,3-diaminopropanol, pentaerythritol, and any one of the branch point moieties described in US 10,478,503.

The term "phosphate moiety" as used herein, refers to a terminal phosphate group that includes phosphates as well as modified phosphates. The phosphate moiety may be located at either terminus but is preferred at the 5′-terminal nucleoside. In one aspect, the terminal phosphate is unmodified having the formula —O—P(═O)(OH)OH. In another aspect, the terminal phosphate is modified such that one or more of the O and OH groups are replaced with H, O, S, N(R') or alkyl where R' is H, an amino protecting group or unsubstituted or substituted alkyl. In some embodiments, the 5′ and or 3′ terminal group may include from 1 to 3 phosphate moieties that are each, independently, unmodified (di- or tri-phosphates) or modified.

As used herein, the term "5′ phosphorus stabilizing moiety" refers to a terminal phosphate group that includes phosphates as well as modified phosphates (e.g., phosphorothioates, phosphodiesters, phosphonates). The phosphate moiety may be located at either terminus but is preferred at the 5′-terminal nucleoside. In one aspect, the terminal phosphate is unmodified having the formula –O–P(=O)(OH)OH. In another aspect, the terminal phosphate is modified such that one or more of the O and OH groups are replaced with H, O, S, N(R'), or alkyl where R' is H, an amino protecting group, or unsubstituted or substituted alkyl. In some embodiments, the 5′ and or 3′ terminal group may include from 1 to 3 phosphate moieties that are each, independently, unmodified (di- or tri-phosphates) or modified.

The phosphate group of the nucleotide may also be modified, e.g., by substituting one or more of the oxygens of the phosphate group with sulfur (e.g., phosphorothioates), or by making other substitutions which allow the nucleotide to perform its intended function such as described in, for example, Eckstein, *Antisense Nucleic Acid Drug Dev.* 10:117-21, 2000; Rusckowski et al., *Antisense Nucleic Acid Drug Dev.* 10:333-45, 2000; Stein, *Antisense Nucleic Acid Drug Dev.* 11:317-25, 2001; Vorobjev et al., *Antisense Nucleic Acid Drug Dev.* 11:77-85, 2001; and US 5,684,143.

As used herein, the term "complementary" refers to two nucleotides that form canonical Watson-Crick base pairs. For the avoidance of doubt, Watson-Crick base pairs in the context of the present disclosure include adenine-thymine, adenine-uracil, and cytosine-guanine base pairs. A proper Watson-Crick base pair is referred to in this context as a "match," while each unpaired nucleotide, and each incorrectly paired nucleotide, is referred to as a "mismatch." Alignment for purposes of determining percent nucleic acid sequence complementarity can be achieved in various ways that are within the capabilities of one of skill in the art, for example, using publicly available computer software such as BLAST, BLAST-2, or Megalign software.

"Percent (%) sequence complementarity" with respect to a reference polynucleotide sequence is defined as the percentage of nucleic acids in a candidate sequence that are complementary to the nucleic acids in the reference polynucleotide sequence, after aligning the sequences and introducing gaps, if

necessary, to achieve the maximum percent sequence complementarity. A given nucleotide is considered to be "complementary" to a reference nucleotide as described herein if the two nucleotides form canonical Watson-Crick base pairs. For the avoidance of doubt, Watson-Crick base pairs in the context of the present disclosure include adenine-thymine, adenine-uracil, and cytosine-guanine base pairs. A proper Watson-Crick base pair is referred to in this context as a "match," while each unpaired nucleotide, and each incorrectly paired nucleotide, is referred to as a "mismatch." Alignment for purposes of determining percent nucleic acid sequence complementarity can be achieved in various ways that are within the capabilities of one of skill in the art, for example, using publicly available computer software such as BLAST, BLAST-2, or Megalign software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal complementarity over the full length of the sequences being compared. As an illustration, the percent sequence complementarity of a given nucleic acid sequence, A, to a given nucleic acid sequence, B, (which can alternatively be phrased as a given nucleic acid sequence, A that has a certain percent complementarity to a given nucleic acid sequence, B) is calculated as follows:

100 multiplied by (the fraction X/Y)

where X is the number of complementary base pairs in an alignment (e.g., as executed by computer software, such as BLAST) in that program's alignment of A and B, and where Y is the total number of nucleic acids in B. It will be appreciated that where the length of nucleic acid sequence A is not equal to the length of nucleic acid sequence B, the percent sequence complementarity of A to B will not equal the percent sequence complementarity of B to A. As used herein, a query nucleic acid sequence is considered to be "completely complementary" to a reference nucleic acid sequence if the query nucleic acid sequence has 100% sequence complementarity to the reference nucleic acid sequence.

"Percent (%) sequence identity" with respect to a reference polynucleotide or polypeptide sequence is defined as the percentage of nucleic acids or amino acids in a candidate sequence that are identical to the nucleic acids or amino acids in the reference polynucleotide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid or amino acid sequence identity can be achieved in various ways that are within the capabilities of one of skill in the art, for example, using publicly available computer software such as BLAST, BLAST-2, or Megalign software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, percent sequence identity values may be generated using the sequence comparison computer program BLAST. As an illustration, the percent sequence identity of a given nucleic acid or amino acid sequence, A, to, with, or against a given nucleic acid or amino acid sequence, B, (which can alternatively be phrased as a given nucleic acid or amino acid sequence, A that has a certain percent sequence identity to, with, or against a given nucleic acid or amino acid sequence, B) is calculated as follows:

100 multiplied by (the fraction X/Y)

where X is the number of nucleotides or amino acids scored as identical matches by a sequence alignment program (e.g., BLAST) in that program's alignment of A and B, and where Y is the total number of nucleic acids in B. It will be appreciated that where the length of nucleic acid or amino acid sequence A is not equal to the length of nucleic acid or amino acid sequence B, the percent sequence identity of A to B will not equal the percent sequence identity of B to A.

The term "complementarity sufficient to hybridize," as used herein, refers to a nucleic acid sequence or a portion thereof that need not be fully complementary (e.g., 100% complementary) to a target region or a nucleic acid sequence or a portion thereof that has one or more nucleotide mismatches relative to the target region but that is still capable of hybridizing to the target region under specified conditions. For example, the nucleic acid may be, e.g., 95% complementary, 90%, complementary, 85% complementary, 80% complementary, 75% complementary, 70% complementary, 65% complementary, 60% complementary, 55% complementary, 50% complementary, or less, but still form sufficient base pairs with the target so as to hybridize across its length.

"Hybridization" or "annealing" of nucleic acids is achieved when one or more nucleoside residues within a polynucleotide base pairs with one or more complementary nucleosides to form a stable duplex. The base pairing is typically driven by hydrogen bonding events. Hybridization includes Watson-Crick base pairs formed from natural and/or modified nucleobases. The hybridization can also include non-Watson-Crick base pairs, such as wobble base pairs (guanosine-uracil, hypoxanthine-uracil, hypoxanthine-adenine, and hypoxanthine-cytosine) and Hoogsteen base pairs. Nucleic acids need not be 100% complementary to undergo hybridization. For example, one nucleic acid may be, e.g., 95% complementary, 90%, complementary, 85% complementary, 80% complementary, 75% complementary, 70% complementary, 65% complementary, 60% complementary, 55% complementary, 50% complementary, or less, relative to another nucleic acid, but the two nucleic acids may still form sufficient base pairs with one another so as to hybridize.

The "stable duplex" formed upon the annealing/hybridization of one nucleic acid to another is a duplex structure that is not denatured by a stringent wash. Exemplary stringent wash conditions are known in the art and include temperatures of about 5° C less than the melting temperature of an individual strand of the duplex and low concentrations of monovalent salts, such as monovalent salt concentrations (e.g., NaCl concentrations) of less than 0.2 M (e.g., 0.2 M, 0.19 M, 0.18 M, 0.17 M, 0.16 M, 0.15 M, 0.14 M, 0.13 M, 0.12 M, 0.11 M, 0.1 M, 0.09 M, 0.08 M, 0.07 M, 0.06 M, 0.05 M, 0.04 M, 0.03 M, 0.02 M, 0.01 M, or less).

The term "gene silencing" refers to the suppression of gene expression, e.g., endogenous gene expression of *SCN9A*, which may be mediated through processes that affect transcription and/or through processes that affect post-transcriptional mechanisms. In some embodiments, gene silencing occurs when an RNAi molecule initiates the inhibition or degradation of the mRNA transcribed from a gene of interest in a sequence-specific manner by way of RNA interference, thereby preventing translation of the gene's product.

The phrase "overactive disease driver gene," as used herein, refers to a gene having increased activity and/or expression that contributes to or causes a disease state in a subject (e.g., a human). The disease state may be caused or exacerbated by the overactive disease driver gene directly or by way of an intermediate gene(s).

As used herein, the term "ethylene glycol chain" refers to a carbon chain with the formula $((CH_2OH)_2)$.

As used herein, “alkyl” refers to a saturated hydrocarbon group. Alkyl groups may be acyclic or cyclic and contain only C and H when unsubstituted. When an alkyl residue having a specific number of carbons is named, all geometric isomers having that number of carbons are intended to be encompassed and described; thus, for example, “butyl” is meant to include *n*-butyl, *sec*-butyl, and *iso*-butyl. Examples of alkyl include ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, and the like. In some embodiments, alkyl may be substituted. Suitable substituents that may be introduced into an alkyl group include, for example, hydroxy, alkoxy, amino, alkylamino, and halo, among others.

As used herein, “alkenyl” refers to an acyclic or cyclic unsaturated hydrocarbon group having at least one site of olefinic unsaturation (i.e., having at least one moiety of the formula C=C). Alkenyl groups contain only C and H when unsubstituted. When an alkenyl residue having a specific number of carbons is named, all geometric isomers having that number of carbons are intended to be encompassed and described; thus, for example, “butenyl” is meant to include *n*-butenyl, *sec*-butenyl, and *iso*-butenyl. Examples of alkenyl include $–CH=CH_2$, $–CH_2-CH=CH_2$, and $–CH_2-CH=CH-CH=CH_2$. In some embodiments, alkenyl may be substituted. Suitable substituents that may be introduced into an alkenyl group include, for example, hydroxy, alkoxy, amino, alkylamino, and halo, among others.

As used herein, “alkynyl” refers to an acyclic or cyclic unsaturated hydrocarbon group having at least one site of acetylenic unsaturation (i.e., having at least one moiety of the formula C≡C). Alkynyl groups contain only C and H when unsubstituted. When an alkynyl residue having a specific number of carbons is named, all geometric isomers having that number of carbons are intended to be encompassed and described; thus, for example, “pentynyl” is meant to include *n*-pentynyl, *sec*-pentynyl, *iso*-pentynyl, and *tert*-pentynyl. Examples of alkynyl include $–C≡CH$ and $–C≡C-CH_3$. In some embodiments, alkynyl may be substituted. Suitable substituents that may be introduced into an alkynyl group include, for example, hydroxy, alkoxy, amino, alkylamino, and halo, among others.

As used herein the term "phenyl" denotes a monocyclic arene in which one hydrogen atom from a carbon atom of the ring has been removed. A phenyl group may be unsubstituted or substituted with one or more suitable substituents, wherein the substituent replaces an H of the phenyl group.

As used herein, the term “benzyl” refers to monovalent radical obtained when a hydrogen atom attached to the methyl group of toluene is removed. A benzyl generally has the formula of phenyl-CH_2-. A benzyl group may be unsubstituted or substituted with one or more suitable substituents. For example, the substituent may replace an H of the phenyl component and/or an H of the methylene (-CH_2-) component.

As used herein, the term "amide" refers to an alkyl, alkenyl, alkynyl, or aromatic group that is attached to an amino-carbonyl functional group.

As used herein, the term "triazole" refers to heterocyclic compounds with the formula $(C_2H_3N_3)$, having a five-membered ring of two carbons and three nitrogens, the positions of which can change resulting in multiple isomers.

As used herein, the term "terminal group" refers to the group at which a carbon chain or nucleic acid ends.

As used herein, an "amino acid" refers to a molecule containing amine and carboxyl functional groups and a side chain specific to the amino acid.

In some embodiments the amino acid is chosen from the group of proteinogenic amino acids. In some embodiments, the amino acid is an L-amino acid or a D-amino acid. In some embodiments, the amino acid is a synthetic amino acid (e.g., a beta-amino acid).

As used herein, the term "lipophilic amino acid" refers to an amino acid including a hydrophobic moiety (e.g., an alkyl chain or an aromatic ring).

As used herein, the term "target of delivery" refers to the organ or part of the body to which it is desired to deliver the branched oligonucleotide compositions.

As used herein, the term “between X and Y” is inclusive of the values of X and Y. For example, “between X and Y” refers to the range of values between the value of X and the value of Y, as well as the value of X and the value of Y.

As used herein, the terms “subject’ and “patient” are used interchangeably and refer to an organism, such as a mammal (e.g., a human) that receives treatment for acute or chronic pain and/or contains a gain-of-function *SCN9A* variant gene. Examples of subjects and patients may also include those diagnosed with a pain disorder, such as Gerhardt disease, Mitchell disease, Weir-Mitchell disease, and/or exhibit symptoms of erythromelalgia.

As use herein, the term “pain” includes any and all forms of chronic and acute pain, including neuropathic pain and nociceptive pain, among others recited herein.

As used herein, the term “*SCN9A*” refers to the gene encoding the Nav1.7 voltage-gated sodium ion channel protein, including any native *SCN9A* gene from any source. The term encompasses “full-length,” unprocessed *SCN9A* as well as any form of *SCN9A* that results from processing in the cell. The term also encompasses naturally occurring variants of *SCN9A*, e.g., splice variants or allelic variants. The nucleic acid sequence of an exemplary *SCN9A* gene is shown in European Nucleotide Archive (ENA) Accession No. DQ857292.1. The amino acid sequence of an exemplary protein encoded by a *SCN9A* gene is shown in UNIPROT™ Accession No. Q15858.

As used herein, the terms “treat,” “treated,” and “treating” mean both therapeutic treatment and prophylactic or preventative measures wherein the object is to prevent, ameliorate, or slow down (lessen) an undesired physiological condition, disorder, or disease, or obtain beneficial or desired clinical results. Beneficial or desired clinical results include, but are not limited to, a reduction in a patient’s reliance on analgesics; alleviation of symptoms; diminishment of the extent of a condition, disorder, or disease; stabilized (i.e., not worsening) state of condition, disorder, or disease; delay in onset or slowing of condition, disorder, or disease progression; amelioration of the condition, disorder, or disease state or remission (whether partial or total), whether detectable or undetectable; an amelioration of at least one measurable physical parameter, not necessarily discernible by the patient; or enhancement or improvement of condition, disorder, or disease. Treatment includes eliciting a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment.

As used herein, the terms “benefit” and “response” are used interchangeably in the context of a subject undergoing therapy for the treatment of, for example, acute pain, chronic pain, nociceptive pain, neuropathic pain, post-operative pain, inflammatory pain, erythromelalgia, primary erythromelalgia,

secondary erythromelalgia, a pain disorder, Gerhardt disease, Mitchell disease, or Weir-Mitchell disease. For example, clinical benefits in the context of a subject administered an siRNA molecule or siRNA composition of the disclosure include, without limitation, a reduction of acute pain, chronic pain, reliance on analgesics, symptoms of erythromelalgia, wild type *SCN9A* transcripts, mutant *SCN9A* transcripts, variant *SCN9A* transcripts, splice isoforms of *SCN9A* transcripts, and/or overexpressed *SCN9A* transcripts thereof (relative to a healthy subject).

Detailed Description

The present disclosure provides compositions of small interfering RNA (siRNA) molecules with sequence homology to a sodium voltage-gated channel alpha subunit 9 (*SCN9A*) gene and methods for administering said siRNA molecules to the central nervous system of a subject. Furthermore, the siRNA molecules described herein may be composed as branched siRNA structures, such as di-branched, tri-branched, and tetra-branched siRNA structures and may further include specific patterns of chemical modifications (e.g., 2' ribose modifications or internucleoside linkage modifications) to improve resistance against nuclease enzymes, toxicity profile, and physicochemical properties (e.g., thermostability). Small interfering RNA molecules are short, double-stranded RNA molecules. They are capable of mediating RNA interference (RNAi) by degrading mRNA with a complementary nucleotide sequence, thus preventing the translation of the target gene.

The siRNA molecules of the disclosure may exhibit, for example, robust gene-specific suppression of *SCN9A*, relative to other genes in the SCN family (e.g., *SCN1A*, *SCN2A*, *SCN3A*, *SCN4A*, *SCN5A*, *SCN8A*, *SCN10A*, and *SCN11A)*. The siRNA sequences of the disclosure also avoid gain-of-function variants in *SCN9A* that cause spontaneous pain (primary erythromelalgia), thereby preserving the efficacy of siRNAs to produce analgesia in this genetically-defined population.

The siRNA molecules of the disclosure may feature an antisense strand having a nucleic acid sequence that is complementary to a region of a *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152. The degree of complementarity of the antisense strand to the region of the *SCN9A* mRNA transcript may be sufficient for the antisense strand to anneal over the full length of the region of the *SCN9A* mRNA transcript. For example, the antisense strand may have a nucleic acid sequence that is at least 60% complementary (e.g., 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%,74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary) to the region of the *SCN9A* mRNA transcript.

In some embodiments, the siRNA molecules of the disclosure feature an antisense strand having the nucleic acid sequence of any one of SEQ ID NOs: 1-192 and 577-768, or a nucleic acid sequence that is at least 60% identical thereto. For example, the siRNA molecules of the disclosure may feature an antisense strand having a nucleic acid sequence that is at least 60% identical (e.g., 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%,74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 1-192 and 577-768.

In some embodiments, the siRNA molecules of the disclosure feature a sense strand having the nucleic acid sequence of any one of SEQ ID NOs: 193-384 and 769-960, or a nucleic acid sequence that is

at least 60% identical thereto. For example, the siRNA molecules of the disclosure may feature a sense strand having a nucleic acid sequence that is at least 60% identical (e.g., 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%,74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 193-384 and 769-960.

Exemplary siRNA molecules of the disclosure are those shown in Table 1, below. Table 1 summarizes the antisense strands, sense strands, and corresponding regions of a *SCN9A* mRNA transcript that are targeted by each antisense strand.

Table 1. Nucleotide sequences for gene-specific *SCN9A*-targeting siRNA

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
1	UGUUCAAU GAGGGCAA GAGAC	193	UUGCCCUC AUUGAACA	385	GUCUCUUG CCCUCAUU GAACA
2	UUGUUCAAU GAGGGCAA GAGA	194	UGCCCUCA UUGAACAA	386	UCUCUUGC CCUCAUUGA ACAA
3	UCCUUUGU UCAAUACUA UGAA	195	AGUAUUGAA CAAAGGA	387	UUCAUAGUA UUGAACAAA GGG
4	UCCCUUUG UUCAAUACU AUGA	196	GUAUUGAAC AAAGGGA	388	UCAUAGUAU UGAACAAAG GGA
5	UUCCCUUU GUUCAAUAC UAUG	197	UAUUGAACA AAGGGAA	389	CAUAGUAUU GAACAAAGG GAA
6	UUUCCCUU UGUUCAAUA CUAU	198	AUUGAACAA AGGGAAA	390	AUAGUAUUG AACAAAGGG AAA
7	UUUUCCCU UUGUUCAAU ACUA	199	UUGAACAAA GGGAAAA	391	UAGUAUUGA ACAAAGGGA AAA
8	UUUGAAACG GAAGAUUG UUUU	200	AAUCUUCCG UUUCAAA	392	AAAACAAUC UUCCGUUU CAAU
9	UUUCUUAGA GGACUGAAA GGA	201	UCAGUCCU CUAAGAAA	393	UCCUUUCA GUCCUCUAA GAAG
10	UCUUCUUA GAGGACUG AAAGG	202	CAGUCCUC UAAGAAGA	394	CCUUUCAG UCCUCUAAG AAGA
11	UUUCUUCU UAGAGGAC UGAAA	203	GUCCUCUAA GAAGAAA	395	UUUCAGUC CUCUAAGAA GAAU
12	UAUUCUUCU UAGAGGAC UGAA	204	UCCUCUAAG AAGAAUA	396	UUCAGUCC UCUAAGAAG AAUA
13	UAUAUUCUU CUUAGAGG ACUG	205	CUCUAAGAA GAAUAUA	397	CAGUCCUC UAAGAAGAA UAUC

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
14	UGAUAUUCU UCUUAGAG GACU	206	UCUAAGAAG AAUAUCA	398	AGUCCUCUA AGAAGAAUA UCU
15	UAGAUAUUC UUCUUAGA GGAC	207	CUAAGAAGA AUAUCUA	399	GUCCUCUAA GAAGAAUAU CUA
16	UUAGAUAUU CUUCUUAGA GGA	208	UAAGAAGAA UAUCUAA	400	UCCUCUAAG AAGAAUAUC UAU
17	UAUAGAUAU UCUUCUUA GAGG	209	AAGAAGAAU AUCUAUA	401	CCUCUAAGA AGAAUAUCU AUU
18	UUGCUGAA UAAGGAGU GUACU	210	ACUCCUUAU UCAGCAA	402	AGUACACUC CUUAUUCAG CAU
19	UAUGCUGAA UAAGGAGU GUAC	211	CUCCUUAUU CAGCAUA	403	GUACACUCC UUAUUCAGC AUG
20	UCAUGCUG AAUAAGGAG UGUA	212	UCCUUAUUC AGCAUGA	404	UACACUCCU UAUUCAGCA UGC
21	UGCAUGCU GAAUAAGGA GUGU	213	CCUUAUUCA GCAUGCA	405	ACACUCCUU AUUCAGCAU GCU
22	UAGCAUGC UGAAUAAGG AGUG	214	CUUAUUCAG CAUGCUA	406	CACUCCUUA UUCAGCAU GCUC
23	UCAGUAAAA GUGUACUC GACA	215	AGUACACUU UUACUGA	407	UGUCGAGU ACACUUUUA CUGG
24	UCCAGUAAA AGUGUACU CGAC	216	GUACACUUU UACUGGA	408	GUCGAGUA CACUUUUAC UGGA
25	UAAGCCUCU UGCAAGGA UUUU	217	CCUUGCAA GAGGCUUA	409	AAAAUCCUU GCAAGAGG CUUC
26	UCAAAUUCU GUUAAAUAC GCA	218	AUUUAACAG AAUUUGA	410	UGCGUAUU UAACAGAAU UUGU
27	UACAAAUUC UGUUAAAUA CGC	219	UUUAACAGA AUUUGUA	411	GCGUAUUU AACAGAAUU UGUA
28	UGGAUUACA GAAAUAGUU UUC	220	CUAUUUCU GUAAUCCA	412	GAAAACUAU UUCUGUAAU CCC
29	UGGGAUUA CAGAAAUAG UUUU	221	UAUUUCUG UAAUCCCA	413	AAAACUAUU UCUGUAAUC CCA
30	UUUAGUGC AAACACACU CAGA	222	GUGUGUUU GCACUAAA	414	UCUGAGUG UGUUUGCA CUAAU
31	UAUUAGUG CAAACACAC UCAG	223	UGUGUUUG CACUAAUA	415	CUGAGUGU GUUUGCAC UAAUU

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
32	UCAAUUAGU GCAAACACA CUC	224	UGUUUGCA CUAAUUGA	416	GAGUGUGU UUGCACUAA UUGG
33	UCCAAUUAG UGCAAACAC ACU	225	GUUUGCAC UAAUUGGA	417	AGUGUGUU UGCACUAAU UGGA
34	UUAGUCCAA UUAGUGCAA ACA	226	GCACUAAUU GGACUAA	418	UGUUUGCA CUAAUUGGA CUAC
35	UGUAGUCC AAUUAGUGC AAAC	227	CACUAAUUG GACUACA	419	GUUUGCAC UAAUUGGAC UACA
36	UUGUAGUC CAAUUAGUG CAAA	228	ACUAAUUGG ACUACAA	420	UUUGCACUA AUUGGACUA CAG
37	UCUGUAGU CCAAUUAGU GCAA	229	CUAAUUGGA CUACAGA	421	UUGCACUAA UUGGACUA CAGC
38	UGCUGUAG UCCAAUUAG UGCA	230	UAAUUGGAC UACAGCA	422	UGCACUAAU UGGACUACA GCU
39	UCAGCUGU AGUCCAAUU AGUG	231	AUUGGACUA CAGCUGA	423	CACUAAUUG GACUACAGC UGU
40	UGAGCAUC UUUGGAUC CUUCC	232	GAUCCAAAG AUGCUCA	424	GGAAGGAU CCAAAGAUG CUCU
41	UAGAGCAUC UUUGGAUC CUUC	233	AUCCAAAGA UGCUCUA	425	GAAGGAUC CAAAGAUGC UCUC
42	UCUGAAUCU GUGCUGAA ACCA	234	UCAGCACAG AUUCAGA	426	UGGUUUCA GCACAGAUU CAGG
43	UCCUGAAUC UGUGCUGA AACC	235	CAGCACAGA UUCAGGA	427	GGUUUCAG CACAGAUUC AGGU
44	UGCUCGUG UAGCCAUAA UCAG	236	UAUGGCUA CACGAGCA	428	CUGAUUAU GGCUACAC GAGCU
45	UUCUGUUU AGCUUCUU CAAUG	237	AAGAAGCUA AACAGAA	429	CAUUGAAGA AGCUAAACA GAA
46	UUUCUGUU UAGCUUCU UCAAU	238	AGAAGCUAA ACAGAAA	430	AUUGAAGAA GCUAAACAG AAA
47	UUUUCUGU UUAGCUUC UUCAA	239	GAAGCUAAA CAGAAAA	431	UUGAAGAAG CUAAACAGA AAG
48	UCUUUCUG UUUAGCUU CUUCA	240	AAGCUAAAC AGAAAGA	432	UGAAGAAGC UAAACAGAA AGA
49	UCACGAAUG CUGAGUGG UGAC	241	CACUCAGCA UUCGUGA	433	GUCACCACU CAGCAUUC GUGG

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
50	UCCACGAAU GCUGAGUG GUGA	242	ACUCAGCAU UCGUGGA	434	UCACCACUC AGCAUUCG UGGC
51	UUGAUGUU ACUGCUGC GUCGC	243	GCAGCAGU AACAUCAA	435	GCGACGCA GCAGUAACA UCAG
52	UGAGCAUG AGGGCUGA GCGUC	244	UCAGCCCU CAUGCUCA	436	GACGCUCA GCCCUCAU GCUCC
53	UGGAGCAU GAGGGCUG AGCGU	245	CAGCCCUCA UGCUCCA	437	ACGCUCAG CCCUCAUG CUCCC
54	UGGGAGCA UGAGGGCU GAGCG	246	AGCCCUCAU GCUCCCA	438	CGCUCAGC CCUCAUGC UCCCC
55	UCAAGUUCU UCCACAGU GUUU	247	CUGUGGAA GAACUUGA	439	AAACACUGU GGAAGAACU UGA
56	UUCAAGUUC UUCCACAGU GUU	248	UGUGGAAG AACUUGAA	440	AACACUGUG GAAGAACUU GAA
57	UCAAAUCUG UACCACCAA GGU	249	GGUGGUAC AGAUUUGA	441	ACCUUGGU GGUACAGA UUUGC
58	UGCAAAUCU GUACCACCA AGG	250	GUGGUACA GAUUUGCA	442	CCUUGGUG GUACAGAUU UGCA
59	UGAGAGCAA UUCCAGAUC AAG	251	UCUGGAAU UGCUCUCA	443	CUUGAUCU GGAAUUGC UCUCC
60	UGGAGAGC AAUUCCAGA UCAA	252	CUGGAAUU GCUCUCCA	444	UUGAUCUG GAAUUGCU CUCCA
61	UAUGGAGA GCAAUUCCA GAUC	253	GGAAUUGC UCUCCAUA	445	GAUCUGGA AUUGCUCU CCAUA
62	UUAUGGAG AGCAAUUCC AGAU	254	GAAUUGCU CUCCAUAA	446	AUCUGGAAU UGCUCUCC AUAU
63	UAUAUGGA GAGCAAUUC CAGA	255	AAUUGCUCU CCAUAUA	447	UCUGGAAU UGCUCUCC AUAUU
64	UAAUAUGGA GAGCAAUUC CAG	256	AUUGCUCU CCAUAUUA	448	CUGGAAUU GCUCUCCA UAUUG
65	UCAAUAUGG AGAGCAAUU CCA	257	UUGCUCUC CAUAUUGA	449	UGGAAUUG CUCUCCAUA UUGG
66	UCCAAUAUG GAGAGCAAU UCC	258	UGCUCUCC AUAUUGGA	450	GGAAUUGC UCUCCAUAU UGGA
67	UAUCCAAUA UGGAGAGC AAUU	259	CUCUCCAUA UUGGAUA	451	AAUUGCUCU CCAUAUUG GAUA

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
68	UUAUCCAAU AUGGAGAG CAAU	260	UCUCCAUAU UGGAUAA	452	AUUGCUCU CCAUAUUG GAUAA
69	UUUAUCCAA UAUGGAGA GCAA	261	CUCCAUAUU GGAUAAA	453	UUGCUCUC CAUAUUGGA UAAA
70	UUUUAUCCA AUAUGGAGA GCA	262	UCCAUAUUG GAUAAAA	454	UGCUCUCC AUAUUGGAU AAAA
71	UAUUUUAUC CAAUAUGGA GAG	263	CAUAUUGGA UAAAAUA	455	CUCUCCAUA UUGGAUAAA AUU
72	UGAAUUUUA UCCAAUAUG GAG	264	UAUUGGAUA AAAUUCA	456	CUCCAUAUU GGAUAAAAU UCA
73	UAGAUCUAC AAAAGGAUC CAU	265	UCCUUUUG UAGAUCUA	457	AUGGAUCC UUUUGUAG AUCUU
74	UAAGAUCUA CAAAAGGAU CCA	266	CCUUUUGU AGAUCUUA	458	UGGAUCCU UUUGUAGA UCUUG
75	UCAAGAUCU ACAAAAGGA UCC	267	CUUUUGUA GAUCUUGA	459	GGAUCCUU UUGUAGAU CUUGC
76	UGCAAGAUC UACAAAAGG AUC	268	UUUUGUAG AUCUUGCA	460	GAUCCUUU UGUAGAUC UUGCA
77	UUGCAAGAU CUACAAAAG GAU	269	UUUGUAGA UCUUGCAA	461	AUCCUUUU GUAGAUCU UGCAA
78	UUUGCAAGA UCUACAAAA GGA	270	UUGUAGAU CUUGCAAA	462	UCCUUUUG UAGAUCUU GCAAU
79	UAUUGCAAG AUCUACAAA AGG	271	UGUAGAUC UUGCAAUA	463	CCUUUUGU AGAUCUUG CAAUU
80	UAAUUGCAA GAUCUACAA AAG	272	GUAGAUCU UGCAAUUA	464	CUUUUGUA GAUCUUGC AAUUA
81	UGUAAUUG CAAGAUCUA CAAA	273	AGAUCUUG CAAUUACA	465	UUUGUAGA UCUUGCAAU UACC
82	UGGUAAUU GCAAGAUCU ACAA	274	GAUCUUGC AAUUACCA	466	UUGUAGAU CUUGCAAUU ACCA
83	UUGGUAAU UGCAAGAUC UACA	275	AUCUUGCAA UUACCAA	467	UGUAGAUC UUGCAAUUA CCAU
84	UAUGGUAAU UGCAAGAUC UAC	276	UCUUGCAAU UACCAUA	468	GUAGAUCU UGCAAUUAC CAUU
85	UAAUGGUAA UUGCAAGAU CUA	277	CUUGCAAUU ACCAUUA	469	UAGAUCUU GCAAUUACC AUUU

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
86	UAAAUGGUAAUUGCAAGAUCU	278	UUGCAAUUACCAUUUA	470	AGAUCUUGCAAUUACCAUUUG
87	UCAAAUGGUAAUUGCAAGAUC	279	UGCAAUUACCAUUUGA	471	GAUCUUGCAAUUACCAUUUGC
88	UUAUGCAAAUGGUAAUUGCAA	280	AUUACCAUUUGCAUAA	472	UUGCAAUUACCAUUUGCAUAG
89	UCUAUGCAAAUGGUAAUUGCA	281	UUACCAUUUGCAUAGA	473	UGCAAUUACCAUUUGCAUAGU
90	UAACUAUGCAAAUGGUAAUUG	282	ACCAUUUGCAUAGUUA	474	CAAUUACCAUUUGCAUAGUUU
91	UGUUUAAAACUAUGCAAAUGG	283	UGCAUAGUUUUAAACA	475	CCAUUUGCAUAGUUUUAAACA
92	UCUUGGAAAUACUCAUAUGGA	284	AUGAGUAUUUCCAAGA	476	UCCAUAUGAGUAUUUCCAAGU
93	UACUUGGAAAUACUCAUAUGG	285	UGAGUAUUUCCAAGUA	477	CCAUAUGAGUAUUUCCAAGUA
94	UUACUUGGAAAUACUCAUAUG	286	GAGUAUUUCCAAGUAA	478	CAUAUGAGUAUUUCCAAGUAG
95	UCUACUUGGAAAUACUCAUAU	287	AGUAUUUCCAAGUAGA	479	AUAUGAGUAUUUCCAAGUAGG
96	UCCUACUUGGAAAUACUCAUA	288	GUAUUUCCAAGUAGGA	480	UAUGAGUAUUUCCAAGUAGGC
97	UCCUUCCACAUCUGCUAGAAA	289	AGCAGAUGUGGAAGGA	481	UUUCUAGCAGAUGUGGAAGGA
98	UUCCUUCCACAUCUGCUAGAA	290	GCAGAUGUGGAAGGAA	482	UUCUAGCAGAUGUGGAAGGAU
99	UCUUGAAGACUCGGAGCAGUC	291	CUCCGAGUCUUCAAGA	483	GACUGCUCCGAGUCUUCAAGU
100	UACUUGAAGACUCGGAGCAGU	292	UCCGAGUCUUCAAGUA	484	ACUGCUCCGAGUCUUCAAGUU
101	UAACUUGAAGACUCGGAGCAG	293	CCGAGUCUUCAAGUUA	485	CUGCUCCGAGUCUUCAAGUUG
102	UCAACUUGAAGACUCGGAGCA	294	CGAGUCUUCAAGUUGA	486	UGCUCCGAGUCUUCAAGUUGG
103	UUAAACAAUAAGGCACAUAGC	295	GUGCCUUAUUGUUUAA	487	GCUAUGUGCCUUAUUGUUUAC

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
104	UGUAAACAA UAAGGCACA UAG	296	UGCCUUAU UGUUUACA	488	CUAUGUGC CUUAUUGU UUACA
105	UAUGUAAAC AAUAAGGCA CAU	297	CCUUAUUG UUUACAUA	489	AUGUGCCU UAUUGUUUA CAUG
106	UCAUGUAAA CAAUAAGGC ACA	298	CUUAUUGU UUACAUGA	490	UGUGCCUU AUUGUUUAC AUGA
107	UUCAUGUAA ACAAUAAGG CAC	299	UUAUUGUU UACAUGAA	491	GUGCCUUA UUGUUUACA UGAU
108	UAUCAUGUA AACAAUAAG GCA	300	UAUUGUUUA CAUGAUA	492	UGCCUUAU UGUUUACAU GAUG
109	UCAUCAUGU AAACAAUAA GGC	301	AUUGUUUAC AUGAUGA	493	GCCUUAUU GUUUACAU GAUGG
110	UCCAUCAUG UAAACAAUA AGG	302	UUGUUUACA UGAUGGA	494	CCUUAUUG UUUACAUGA UGGU
111	UACCAUCAU GUAAACAAU AAG	303	UGUUUACAU GAUGGUA	495	CUUAUUGU UUACAUGAU GGUC
112	UGACCAUCA UGUAAACAA UAA	304	GUUUACAU GAUGGUCA	496	UUAUUGUU UACAUGAUG GUCA
113	UUGACCAUC AUGUAAACA AUA	305	UUUACAUGA UGGUCAA	497	UAUUGUUUA CAUGAUGG UCAU
114	UUUGCUGU AAGAUUGUC UGAA	306	ACAAUCUUA CAGCAAA	498	UUCAGACAA UCUUACAGC AAU
115	UAUUGCUG UAAGAUUGU CUGA	307	CAAUCUUAC AGCAAUA	499	UCAGACAAU CUUACAGCA AUU
116	UAAUUGCU GUAAGAUU GUCUG	308	AAUCUUACA GCAAUUA	500	CAGACAAUC UUACAGCAA UUG
117	UCAAUUGCU GUAAGAUU GUCU	309	AUCUUACAG CAAUUGA	501	AGACAAUCU UACAGCAAU UGA
118	UUCAAUUGC UGUAAGAUU GUC	310	UCUUACAGC AAUUGAA	502	GACAAUCUU ACAGCAAUU GAA
119	UGGAGGUU GUUUGCAU CAGGG	311	AUGCAAACA ACCUCCA	503	CCCUGAUG CAAACAACC UCCA
120	UCUGGAGG UUGUUUGC AUCAG	312	GCAAACAAC CUCCAGA	504	CUGAUGCAA ACAACCUCC AGA
121	UUCUGGAG GUUGUUUG CAUCA	313	CAAACAACC UCCAGAA	505	UGAUGCAAA CAACCUCCA GAU

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
122	UAUCUGGA GGUUGUUU GCAUC	314	AAACAACCU CCAGAUA	506	GAUGCAAAC AACCUCCAG AUU
123	UUCACUGU GAGGCUGG GAUUG	315	CCAGCCUCA CAGUGAA	507	CAAUCCCAG CCUCACAGU GAC
124	UGUCACUG UGAGGCUG GGAUU	316	CAGCCUCAC AGUGACA	508	AAUCCCAGC CUCACAGU GACA
125	UUGAAGCU UUCAAACCA ACUG	317	GGUUUGAA AGCUUCAA	509	CAGUUGGU UUGAAAGCU UCAU
126	UAUGAAGCU UUCAAACCA ACU	318	GUUUGAAA GCUUCAUA	510	AGUUGGUU UGAAAGCUU CAUU
127	UAGGAUAAU CUUAAUGG UCUU	319	CAUUAAGAU UAUCCUA	511	AAGACCAUU AAGAUUAUC CUG
128	UCAGGAUAA UCUUAAUG GUCU	320	AUUAAGAUU AUCCUGA	512	AGACCAUUA AGAUUAUCC UGG
129	UCCAGGAUA AUCUUAAUG GUC	321	UUAAGAUUA UCCUGGA	513	GACCAUUAA GAUUAUCCU GGA
130	UUCCAGGA UAAUCUUAA UGGU	322	UAAGAUUAU CCUGGAA	514	ACCAUUAAG AUUAUCCUG GAG
131	UCUCCAGG AUAAUCUUA AUGG	323	AAGAUUAUC CUGGAGA	515	CCAUUAAGA UUAUCCUG GAGU
132	UACUCCAG GAUAAUCUU AAUG	324	AGAUUAUCC UGGAGUA	516	CAUUAAGAU UAUCCUGG AGUA
133	UUACUCCAG GAUAAUCUU AAU	325	GAUUAUCCU GGAGUAA	517	AUUAAGAUU AUCCUGGA GUAU
134	UAUACUCCA GGAUAAUCU UAA	326	AUUAUCCUG GAGUAUA	518	UUAAGAUUA UCCUGGAG UAUG
135	UGCAUACUC CAGGAUAAU CUU	327	UAUCCUGG AGUAUGCA	519	AAGAUUAUC CUGGAGUA UGCA
136	UUUCCAGAA UGAAGAUG UAAG	328	AUCUUCAUU CUGGAAA	520	CUUACAUCU UCAUUCUG GAAA
137	UAGUAGCCA AGAGUGUU UGCC	329	ACACUCUUG GCUACUA	521	GGCAAACAC UCUUGGCU ACUC
138	UGAGUAGC CAAGAGUG UUUGC	330	CACUCUUG GCUACUCA	522	GCAAACACU CUUGGCUA CUCA
139	UUGAGUAG CCAAGAGU GUUUG	331	ACUCUUGG CUACUCAA	523	CAAACACUC UUGGCUAC UCAG

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
140	UCUGAGUA GCCAAGAG UGUUU	332	CUCUUGGC UACUCAGA	524	AAACACUCU UGGCUACU CAGA
141	UUUCCUUCA AAUCUAGAU AAG	333	CUAGAUUU GAAGGAAA	525	CUUAUCUAG AUUUGAAG GAAU
142	UCAUUCCUU CAAAUCUAG AUA	334	AGAUUUGAA GGAAUGA	526	UAUCUAGAU UUGAAGGAA UGA
143	UUCAUUCCU UCAAAUCUA GAU	335	GAUUUGAA GGAAUGAA	527	AUCUAGAUU UGAAGGAAU GAG
144	UUUGCUCC UAUGAGUG CAUUC	336	CACUCAUAG GAGCAAA	528	GAAUGCACU CAUAGGAG CAAU
145	UAUUGCUC CUAUGAGU GCAUU	337	ACUCAUAGG AGCAAUA	529	AAUGCACUC AUAGGAGCA AUU
146	UCUCAUAGA ACUUGCCA GCAA	338	GGCAAGUU CUAUGAGA	530	UUGCUGGC AAGUUCUAU GAGU
147	UACUCAUAG AACUUGCCA GCA	339	GCAAGUUC UAUGAGUA	531	UGCUGGCA AGUUCUAU GAGUG
148	UCACUCAUA GAACUUGC CAGC	340	CAAGUUCUA UGAGUGA	532	GCUGGCAA GUUCUAUG AGUGU
149	UACACUCAU AGAACUUGC CAG	341	AAGUUCUAU GAGUGUA	533	CUGGCAAG UUCUAUGA GUGUA
150	UUUUCCAUC GCACAUUUU GAC	342	AAUGUGCG AUGGAAAA	534	GUCAAAAUG UGCGAUGG AAAA
151	UUUGCAACU UGAAGCAGA GAU	343	UGCUUCAA GUUGCAAA	535	AUCUCUGC UUCAAGUU GCAAC
152	UGUUGCAA CUUGAAGCA GAGA	344	GCUUCAAG UUGCAACA	536	UCUCUGCU UCAAGUUG CAACU
153	UCUGCUGC AUACAUAAU AAUC	345	UUAUGUAU GCAGCAGA	537	GAUUAUUAU GUAUGCAG CAGU
154	UACUGCUG CAUACAUAA UAAU	346	UAUGUAUG CAGCAGUA	538	AUUAUUAUG UAUGCAGCA GUG
155	UGUUGGUU GAAAUUAUC UAUG	347	AUAAUUUCA ACCAACA	539	CAUAGAUAA UUUCAACCA ACA
156	UCUGUUGG UUGAAAUUA UCUA	348	AAUUUCAAC CAACAGA	540	UAGAUAAUU UCAACCAAC AGA
157	UUCUGUUG GUUGAAAUU AUCU	349	AUUUCAACC AACAGAA	541	AGAUAAUUU CAACCAACA GAA

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
158	UUUCUGUU GGUUGAAA UUAUC	350	UUUCAACCA ACAGAAA	542	GAUAAUUUC AACCAACAG AAA
159	UCUUGACC UCCAAGCUU CUUU	351	AGCUUGGA GGUCAAGA	543	AAAGAAGCU UGGAGGUC AAGA
160	UUCUUGAC CUCCAAGCU UCUU	352	GCUUGGAG GUCAAGAA	544	AAGAAGCUU GGAGGUCA AGAC
161	UCAAAUAUA CAUCCUUG GAUU	353	AAGGAUGUA UAUUUGA	545	AAUCCAAGG AUGUAUAUU UGA
162	UGUCAAAUA UACAUCCUU GGA	354	GGAUGUAU AUUUGACA	546	UCCAAGGAU GUAUAUUU GACC
163	UUGGUUAC CAUGUUGA GACAG	355	UCAACAUGG UAACCAA	547	CUGUCUCAA CAUGGUAAC CAU
164	UAUGGUUA CCAUGUUG AGACA	356	CAACAUGGU AACCAUA	548	UGUCUCAAC AUGGUAACC AUG
165	UUUCUACCA UCAUGGUU ACCA	357	ACCAUGAUG GUAGAAA	549	UGGUAACCA UGAUGGUA GAAA
166	UUUUCUACC AUCAUGGU UACC	358	CCAUGAUG GUAGAAAA	550	GGUAACCAU GAUGGUAG AAAA
167	UCAUGUCAU UAAUUCCAU CUU	359	GGAAUUAAU GACAUGA	551	AAGAUGGAA UUAAUGACA UGU
168	UACAUGUCA UUAAUUCCA UCU	360	GAAUUAAUG ACAUGUA	552	AGAUGGAAU UAAUGACAU GUU
169	UAACAUGUC AUUAAUUCC AUC	361	AAUUAAUGA CAUGUUA	553	GAUGGAAU UAAUGACAU GUUC
170	UGAACAUGU CAUUAAUUC CAU	362	AUUAAUGAC AUGUUCA	554	AUGGAAUUA AUGACAUGU UCA
171	UUGAACAUG UCAUUAAUU CCA	363	UUAAUGACA UGUUCAA	555	UGGAAUUAA UGACAUGU UCAA
172	UUUGAACAU GUCAUUAAU UCC	364	UAAUGACAU GUUCAAA	556	GGAAUUAAU GACAUGUU CAAU
173	UACGAAGAG AAUCCAUCU CCC	365	AUGGAUUC UCUUCGUA	557	GGGAGAUG GAUUCUCU UCGUU
174	UAACGAAGA GAAUCCAUC UCC	366	UGGAUUCU CUUCGUUA	558	GGAGAUGG AUUCUCUUC GUUC
175	UGAACGAAG AGAAUCCAU CUC	367	GGAUUCUC UUCGUUCA	559	GAGAUGGA UUCUCUUC GUUCA

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
176	UUGAACGAAGAGAAUCCAUCU	368	GAUUCUCUUCGUUCAA	560	AGAUGGAUUCUCUUCGUUCAC
177	UGUGAACGAAGAGAAUCCAUC	369	AUUCUCUUCGUUCACA	561	GAUGGAUUCUCUUCGUUCACA
178	UUGUGAACGAAGAGAAUCCAU	370	UUCUCUUCGUUCACAA	562	AUGGAUUCUCUUCGUUCACAG
179	UCCAUCUGUGAACGAAGAGAA	371	UUCGUUCACAGAUGGA	563	UUCUCUUCGUUCACAGAUGGA
180	UUCCAUCUGUGAACGAAGAGA	372	UCGUUCACAGAUGGAA	564	UCUCUUCGUUCACAGAUGGAA
181	UUUCCAUCUGUGAACGAAGAG	373	CGUUCACAGAUGGAAA	565	CUCUUCGUUCACAGAUGGAAG
182	UCUUCCAUCUGUGAACGAAGA	374	GUUCACAGAUGGAAGA	566	UCUUCGUUCACAGAUGGAAGA
183	UUCUUCCAUCUGUGAACGAAG	375	UUCACAGAUGGAAGAA	567	CUUCGUUCACAGAUGGAAGAA
184	UUUUCUUCCAUCUGUGAACGA	376	CACAGAUGGAAGAAAA	568	UCGUUCACAGAUGGAAGAAAG
185	UCUUUCUUCCAUCUGUGAACG	377	ACAGAUGGAAGAAAGA	569	CGUUCACAGAUGGAAGAAAGG
186	UCCUUUCUUCCAUCUGUGAAC	378	CAGAUGGAAGAAAGGA	570	GUUCACAGAUGGAAGAAAGGU
187	UACCUUUCUUCCAUCUGUGAA	379	AGAUGGAAGAAAGGUA	571	UUCACAGAUGGAAGAAAGGUU
188	UAACCUUUCUUCCAUCUGUGA	380	GAUGGAAGAAAGGUUA	572	UCACAGAUGGAAGAAAGGUUC
189	UGAACCUUUCUUCCAUCUGUG	381	AUGGAAGAAAGGUUCA	573	CACAGAUGGAAGAAAGGUUCA
190	UAUGAACCUUUCUUCCAUCUG	382	GGAAGAAAGGUUCAUA	574	CAGAUGGAAGAAAGGUUCAUG
191	UACAUGAACCUUUCUUCCAUC	383	AAGAAAGGUUCAUGUA	575	GAUGGAAGAAAGGUUCAUGUC
192	UGUGAUGGGUUCAUAGGACAC	384	CUAUGAACCCAUCACA	576	GUGUCCUAUGAACCCAUCACA
577	UUCUUUUCAUCCUGUAUAUUU	769	UACAGGAUGAAAAGAA	961	AAAUAUACAGGAUGAAAAGAU

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
578	UUUGUUCAAUGAGGGCAAGAG	770	GCCCUCAUUGAACAAA	962	CUCUUGCCCUCAUUGAACAAC
579	UGUUGUUCAAUGAGGGCAAGA	771	CCCUCAUUGAACAACA	963	UCUUGCCCUCAUUGAACAACG
580	UCGUUGUUCAAUGAGGGCAAG	772	CCUCAUUGAACAACGA	964	CUUGCCCUCAUUGAACAACGC
581	UUGCGUUGUUCAAUGAGGGCA	773	UCAUUGAACAACGCAA	965	UGCCCUCAUUGAACAACGCAU
582	UUUUGUUCAAUACUAUGAAAG	774	AUAGUAUUGAACAAAA	966	CUUUCAUAGUAUUGAACAAAG
583	UCUUUGUUCAAUACUAUGAAA	775	UAGUAUUGAACAAAGA	967	UUUCAUAGUAUUGAACAAAGG
584	UGUUUUCCCUUUGUUCAAUAC	776	GAACAAAGGGAAAACA	968	GUAUUGAACAAAGGGAAAACA
585	UUGUUUUCCCUUUGUUCAAUA	777	AACAAAGGGAAAACAA	969	UAUUGAACAAAGGGAAAACAA
586	UAACGGAAGAUUGUUUUCCCU	778	AAACAAUCUUCCGUUA	970	AGGGAAAACAAUCUUCCGUUU
587	UAAACGGAAGAUUGUUUUCCC	779	AACAAUCUUCCGUUUA	971	GGGAAAACAAUCUUCCGUUUC
588	UGAAACGGAAGAUUGUUUUCC	780	ACAAUCUUCCGUUUCA	972	GGAAAACAAUCUUCCGUUUCA
589	UUGAAACGGAAGAUUGUUUUC	781	CAAUCUUCCGUUUCAA	973	GAAAACAAUCUUCCGUUUCAA
590	UAUUGAAACGGAAGAUUGUUU	782	AUCUUCCGUUUCAAUA	974	AAACAAUCUUCCGUUUCAAUG
591	UCAUUGAAACGGAAGAUUGUU	783	UCUUCCGUUUCAAUGA	975	AACAAUCUUCCGUUUCAAUGC
592	UGCAUUGAAACGGAAGAUUGU	784	CUUCCGUUUCAAUGCA	976	ACAAUCUUCCGUUUCAAUGCC
593	UGGCAUUGAAACGGAAGAUUG	785	UUCCGUUUCAAUGCCA	977	CAAUCUUCCGUUUCAAUGCCA
594	UUAGAGGACUGAAAGGAGAAA	786	CCUUUCAGUCCUCUAA	978	UUUCUCCUUUCAGUCCUCUAA
595	UUUAGAGGACUGAAAGGAGAA	787	CUUUCAGUCCUCUAAA	979	UUCUCCUUUCAGUCCUCUAAG

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
596	UCUUAGAG GACUGAAAG GAGA	788	UUUCAGUC CUCUAAGA	980	UCUCCUUU CAGUCCUC UAAGA
597	UUCUUAGA GGACUGAAA GGAG	789	UUCAGUCC UCUAAGAA	981	CUCCUUUCA GUCCUCUAA GAA
598	UUCUUCUUA GAGGACUG AAAG	790	AGUCCUCUA AGAAGAA	982	CUUUCAGU CCUCUAAGA AGAA
599	UUAUUCUUC UUAGAGGA CUGA	791	CCUCUAAGA AGAAUAA	983	UCAGUCCU CUAAGAAGA AUAU
600	UAAUAGAUA UUCUUCUUA GAG	792	AGAAGAAUA UCUAUUA	984	CUCUAAGAA GAAUAUCUA UUA
601	UUAAUAGAU AUUCUUCUU AGA	793	GAAGAAUAU CUAUUAA	985	UCUAAGAAG AAUAUCUAU UAA
602	UUUAAUAGA UAUUCUUCU UAG	794	AAGAAUAUC UAUUAAA	986	CUAAGAAGA AUAUCUAUU AAG
603	UCUUAAUAG AUAUUCUUC UUA	795	AGAAUAUCU AUUAAGA	987	UAAGAAGAA UAUCUAUUA AGA
604	UUCUUAAUA GAUAUUCUU CUU	796	GAAUAUCUA UUAAGAA	988	AAGAAGAAU AUCUAUUAA GAU
605	UAUCUUAAU AGAUAUUCU UCU	797	AAUAUCUAU UAAGAUA	989	AGAAGAAUA UCUAUUAAG AUU
606	UAAUCUUAA UAGAUAUUC UUC	798	AUAUCUAUU AAGAUUA	990	GAAGAAUAU CUAUUAAGA UUU
607	UCUAAAAUC UUAAUAGAU AUU	799	CUAUUAAGA UUUUAGA	991	AAUAUCUAU UAAGAUUUU AGU
608	UUAUGCAG UUUGUCAG AAUAG	800	CUGACAAAC UGCAUAA	992	CUAUUCUGA CAAACUGCA UAU
609	UAUAUGCAG UUUGUCAG AAUA	801	UGACAAACU GCAUAUA	993	UAUUCUGAC AAACUGCAU AUU
610	UAAAAGUGU ACUCGACAU UUU	802	GUCGAGUA CACUUUUA	994	AAAAUGUCG AGUACACUU UUA
611	UUAAAAGUG UACUCGACA UUU	803	UCGAGUACA CUUUUAA	995	AAAUGUCGA GUACACUUU UAC
612	UGUAAAAGU GUACUCGA CAUU	804	CGAGUACAC UUUUACA	996	AAUGUCGA GUACACUUU UACU
613	UAGUAAAAG UGUACUCG ACAU	805	GAGUACACU UUUACUA	997	AUGUCGAG UACACUUUU ACUG

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
614	UUCCAGUAAAAGUGUACUCGA	806	UACACUUUUACUGGAA	998	UCGAGUACACUUUUACUGGAA
615	UUUCCAGUAAAAGUGUACUCG	807	ACACUUUUACUGGAAA	999	CGAGUACACUUUUACUGGAAU
616	UAUUCCAGUAAAAGUGUACUC	808	CACUUUUACUGGAAUA	1000	GAGUACACUUUUACUGGAAUA
617	UUAUUCCAGUAAAAGUGUACU	809	ACUUUUACUGGAAUAA	1001	AGUACACUUUUACUGGAAUAU
618	UAUUCUGUUAAAUACGCAAAA	810	CGUAUUUAACAGAAUA	1002	UUUUGCGUAUUUAACAGAAUU
619	UAAUUCUGUUAAAUACGCAAA	811	GUAUUUAACAGAAUUA	1003	UUUGCGUAUUUAACAGAAUUU
620	UAAAUUCUGUUAAAUACGCAA	812	UAUUUAACAGAAUUUA	1004	UUGCGUAUUUAACAGAAUUUG
621	UUACAAAUUCUGUUAAAUACG	813	UUAACAGAAUUUGUAA	1005	CGUAUUUAACAGAAUUUGUAA
622	UUUACAAAUUCUGUUAAAUAC	814	UAACAGAAUUUGUAAA	1006	GUAUUUAACAGAAUUUGUAAA
623	UGAUUACAGAAAUAGUUUUCA	815	ACUAUUUCUGUAAUCA	1007	UGAAAACUAUUUCUGUAAUCC
624	UCUGGGAUUACAGAAAUAGUU	816	UUUCUGUAAUCCCAGA	1008	AACUAUUUCUGUAAUCCCAGG
625	UUCCAAUUAGUGCAAACACAC	817	UUUGCACUAAUUGGAA	1009	GUGUGUUUGCACUAAUUGGAC
626	UGUCCAAUUAGUGCAAACACA	818	UUGCACUAAUUGGACA	1010	UGUGUUUGCACUAAUUGGACU
627	UAGUCCAAUUAGUGCAAACAC	819	UGCACUAAUUGGACUA	1011	GUGUUUGCACUAAUUGGACUA
628	UACAGCUGUAGUCCAAUUAGU	820	UUGGACUACAGCUGUA	1012	ACUAAUUGGACUACAGCUGUU
629	UCUAAUGUUUCAUUAUUUUCA	821	AUAAUGAAACAUUAGA	1013	UGAAAAUAAUGAAACAUUAGA
630	UCUUUCUAAUGUUUCAUUAUU	822	UGAAACAUUAGAAAGA	1014	AAUAAUGAAACAUUAGAAAGC
631	UUGCUUUCUAAUGUUUCAUUA	823	AAACAUUAGAAAGCAA	1015	UAAUGAAACAUUAGAAAGCAU

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
632	UAUGCUUU CUAAUGUUU CAUU	824	AACAUUAGA AAGCAUA	1016	AAUGAAACA UUAGAAAGC AUA
633	UAAUCUGU GCUGAAACC ACAA	825	GUUUCAGC ACAGAUUA	1017	UUGUGGUU UCAGCACAG AUUC
634	UGUGUAGC CAUAAUCAG GGUU	826	UGAUUAUG GCUACACA	1018	AACCCUGAU UAUGGCUA CACG
635	UCGUGUAG CCAUAAUCA GGGU	827	GAUUAUGG CUACACGA	1019	ACCCUGAUU AUGGCUACA CGA
636	UUCGUGUA GCCAUAAUC AGGG	828	AUUAUGGC UACACGAA	1020	CCCUGAUUA UGGCUACA CGAG
637	UCUCGUGU AGCCAUAAU CAGG	829	UUAUGGCU ACACGAGA	1021	CCUGAUUAU GGCUACAC GAGC
638	UAGUGUCAA AGCUCGUG UAGC	830	ACGAGCUU UGACACUA	1022	GCUACACGA GCUUUGAC ACUU
639	UAAGUGUCA AAGCUCGU GUAG	831	CGAGCUUU GACACUUA	1023	CUACACGAG CUUUGACAC UUU
640	UAAAGUGUC AAAGCUCGU GUA	832	GAGCUUUG ACACUUUA	1024	UACACGAGC UUUGACACU UUC
641	UGAAAGUG UCAAAGCUC GUGU	833	AGCUUUGA CACUUUCA	1025	ACACGAGCU UUGACACUU UCA
642	UGACUUGU UCUGCUGC UUCGC	834	GCAGCAGAA CAAGUCA	1026	GCGAAGCA GCAGAACAA GUCU
643	UAGACUUG UUCUGCUG CUUCG	835	CAGCAGAAC AAGUCUA	1027	CGAAGCAG CAGAACAAG UCUU
644	UAAGUUCUU CCACAGUG UUUG	836	ACUGUGGA AGAACUUA	1028	CAAACACUG UGGAAGAAC UUG
645	UUCCAAUAU GGAGAGCA AUUC	837	GCUCUCCA UAUUGGAA	1029	GAAUUGCU CUCCAUAUU GGAU
646	UAAUUUUAU CCAAUAUGG AGA	838	AUAUUGGAU AAAAUUA	1030	UCUCCAUAU UGGAUAAAA UUC
647	UUGAAUUUU AUCCAAUAU GGA	839	AUUGGAUAA AAUUCAA	1031	UCCAUAUUG GAUAAAAUU CAA
648	UUUGAAUUU UAUCCAAUA UGG	840	UUGGAUAAA AUUCAAA	1032	CCAUAUUG GAUAAAAUU CAAA
649	UUUUGAAUU UUAUCCAAU AUG	841	UGGAUAAAA UUCAAAA	1033	CAUAUUGGA UAAAAUUCA AAA

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
650	UAAUAAAAU AGAUACACU UUU	842	UGUAUCUAU UUUAUUA	1034	AAAAGUGUA UCUAUUUUA UUG
651	UUUACAAUA AAAUAGAUA CAC	843	UCUAUUUUA UUGUAAA	1035	GUGUAUCU AUUUUAUUG UAAU
652	UAUUACAAU AAAAUAGAU ACA	844	CUAUUUUAU UGUAAUA	1036	UGUAUCUAU UUUAUUGUA AUG
653	UCCAUUACA AUAAAAUAG AUA	845	AUUUUAUUG UAAUGGA	1037	UAUCUAUUU UAUUGUAAU GGA
654	UUCCAUUAC AAUAAAAUA GAU	846	UUUUAUUG UAAUGGAA	1038	AUCUAUUUU AUUGUAAUG GAU
655	UAUCCAUUA CAAUAAAAU AGA	847	UUUAUUGUA AUGGAUA	1039	UCUAUUUUA UUGUAAUG GAUC
656	UGAUCCAUU ACAAUAAAA UAG	848	UUAUUGUAA UGGAUCA	1040	CUAUUUUAU UGUAAUGG AUCC
657	UGGAUCCA UUACAAUAA AAUA	849	UAUUGUAAU GGAUCCA	1041	UAUUUUAUU GUAAUGGA UCCU
658	UAGGAUCCA UUACAAUAA AAU	850	AUUGUAAUG GAUCCUA	1042	AUUUUAUUG UAAUGGAUC CUU
659	UAAGGAUCC AUUACAAUA AAA	851	UUGUAAUG GAUCCUUA	1043	UUUUAUUG UAAUGGAUC CUUU
660	UAAAGGAUC CAUUACAAU AAA	852	UGUAAUGG AUCCUUUA	1044	UUUAUUGUA AUGGAUCC UUUU
661	UAAAAGGAU CCAUUACAA UAA	853	GUAAUGGA UCCUUUUA	1045	UUAUUGUAA UGGAUCCU UUUG
662	UCAAAAGGA UCCAUUACA AUA	854	UAAUGGAUC CUUUUGA	1046	UAUUGUAAU GGAUCCUU UUGU
663	UACAAAAGG AUCCAUUAC AAU	855	AAUGGAUCC UUUUGUA	1047	AUUGUAAUG GAUCCUUU UGUA
664	UUACAAAAG GAUCCAUUA CAA	856	AUGGAUCC UUUUGUAA	1048	UUGUAAUG GAUCCUUU UGUAG
665	UUAAUUGCA AGAUCUACA AAA	857	UAGAUCUU GCAAUUAA	1049	UUUUGUAG AUCUUGCAA UUAC
666	UAAACUAUG CAAAUGGUA AUU	858	CCAUUUGCA UAGUUUA	1050	AAUUACCAU UUGCAUAG UUUU
667	UAAAACUAU GCAAAUGG UAAU	859	CAUUUGCAU AGUUUUA	1051	AUUACCAUU UGCAUAGU UUUA

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
668	UUAAAACUAUGCAAAUGGUAA	860	AUUUGCAUAGUUUUAA	1052	UUACCAUUUGCAUAGUUUUAA
669	UUUAAAACUAUGCAAAUGGUA	861	UUUGCAUAGUUUUAAA	1053	UACCAUUUGCAUAGUUUUAAA
670	UUUUAAAACUAUGCAAAUGGU	862	UUGCAUAGUUUUAAAA	1054	ACCAUUUGCAUAGUUUUAAAC
671	UUGUUUAAAACUAUGCAAAUG	863	GCAUAGUUUUAAACAA	1055	CAUUUGCAUAGUUUUAAACAC
672	UGUGUUUAAAACUAUGCAAAU	864	CAUAGUUUUAAACACA	1056	AUUUGCAUAGUUUUAAACACA
673	UGGAAAUACUCAUAUGGAUCC	865	CAUAUGAGUAUUUCCA	1057	GGAUCCAUAUGAGUAUUUCCA
674	UUGGAAAUACUCAUAUGGAUC	866	AUAUGAGUAUUUCCAA	1058	GAUCCAUAUGAGUAUUUCCAA
675	UUUGGAAAUACUCAUAUGGAU	867	UAUGAGUAUUUCCAAA	1059	AUCCAUAUGAGUAUUUCCAAG
676	UGCCUACUUGGAAAUACUCAU	868	UAUUUCCAAGUAGGCA	1060	AUGAGUAUUUCCAAGUAGGCU
677	UACAAUAAGGCACAUAGCUUG	869	UAUGUGCCUUAUUGUA	1061	CAAGCUAUGUGCCUUAUUGUU
678	UAACAAUAAGGCACAUAGCUU	870	AUGUGCCUUAUUGUUA	1062	AAGCUAUGUGCCUUAUUGUUU
679	UGUCUUCUUCAAUUGCUGUAA	871	GCAAUUGAAGAAGACA	1063	UUACAGCAAUUGAAGAAGACC
680	UACUGUGAGGCUGGGAUUGUG	872	UCCCAGCCUCACAGUA	1064	CACAAUCCCAGCCUCACAGUG
681	UAGGGUUAUCAACUGUGCUGC	873	ACAGUUGAUAACCCUA	1065	GCAGCACAGUUGAUAACCCUU
682	UUGUUAACUUGGCAGCAUGAG	874	GCUGCCAAGUUAACAA	1066	CUCAUGCUGCCAAGUUAACAU
683	UAUGUUAACUUGGCAGCAUGA	875	CUGCCAAGUUAACAUA	1067	UCAUGCUGCCAAGUUAACAUA
684	UUCUAUGUUAACUUGGCAGCA	876	CCAAGUUAACAUAGAA	1068	UGCUGCCAAGUUAACAUAGAG
685	UAGCUUUCAAACCAACUGUGU	877	GUUGGUUUGAAAGCUA	1069	ACACAGUUGGUUUGAAAGCUU

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
686	UAAGCUUUC AAACCAACU GUG	878	UUGGUUUG AAAGCUUA	1070	CACAGUUG GUUUGAAA GCUUC
687	UGAAGCUU UCAAACCAA CUGU	879	UGGUUUGA AAGCUUCA	1071	ACAGUUGG UUUGAAAGC UUCA
688	UAAUGAAGC UUUCAAACC AAC	880	UUUGAAAGC UUCAUUA	1072	GUUGGUUU GAAAGCUUC AUUG
689	UCAAUGAAG CUUUCAAAC CAA	881	UUGAAAGCU UCAUUGA	1073	UUGGUUUG AAAGCUUCA UUGU
690	UACAAUGAA GCUUUCAAA CCA	882	UGAAAGCUU CAUUGUA	1074	UGGUUUGA AAGCUUCAU UGUC
691	UGACAAUGA AGCUUUCAA ACC	883	GAAAGCUUC AUUGUCA	1075	GGUUUGAA AGCUUCAUU GUCC
692	UGAUAAUCU UAAUGGUC UUUU	884	ACCAUUAAG AUUAUCA	1076	AAAAGACCA UUAAGAUUA UCC
693	UCACAUUCA UGAUGGAA GGAA	885	UCCAUCAUG AAUGUGA	1077	UUCCUUCCA UCAUGAAUG UGC
694	UUACACUCA UAGAACUUG CCA	886	AGUUCUAU GAGUGUAA	1078	UGGCAAGU UCUAUGAG UGUAU
695	UAUACACUC AUAGAACUU GCC	887	GUUCUAUG AGUGUAUA	1079	GGCAAGUU CUAUGAGU GUAUU
696	UAAUACACU CAUAGAACU UGC	888	UUCUAUGA GUGUAUUA	1080	GCAAGUUC UAUGAGUG UAUUA
697	UCAUCGCAC AUUUUGACU AAC	889	UCAAAAUGU GCGAUGA	1081	GUUAGUCAA AAUGUGCG AUGG
698	UCCAUCGCA CAUUUUGAC UAA	890	CAAAAUGUG CGAUGGA	1082	UUAGUCAAA AUGUGCGA UGGA
699	UUCCAUCG CACAUUUUG ACUA	891	AAAAUGUGC GAUGGAA	1083	UAGUCAAAA UGUGCGAU GGAA
700	UUUCCAUC GCACAUUUU GACU	892	AAAUGUGC GAUGGAAA	1084	AGUCAAAAU GUGCGAUG GAAA
701	UCAACUUGA AGCAGAGAU AGG	893	CUCUGCUU CAAGUUGA	1085	CCUAUCUCU GCUUCAAG UUGC
702	UGCAACUU GAAGCAGA GAUAG	894	UCUGCUUC AAGUUGCA	1086	CUAUCUCU GCUUCAAG UUGCA
703	UUGCAACUU GAAGCAGA GAUA	895	CUGCUUCAA GUUGCAA	1087	UAUCUCUG CUUCAAGUU GCAA

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
704	UAAGUUGCA ACUUGAAGC AGA	896	UUCAAGUU GCAACUUA	1088	UCUGCUUC AAGUUGCAA CUUU
705	UAAAGUUGC AACUUGAAG CAG	897	UCAAGUUG CAACUUUA	1089	CUGCUUCAA GUUGCAAC UUUU
706	UAAAAGUUG CAACUUGAA GCA	898	CAAGUUGCA ACUUUUA	1090	UGCUUCAA GUUGCAAC UUUUA
707	UCAAAGUGA AGAAUGACC CAA	899	UCAUUCUUC ACUUUGA	1091	UUGGGUCA UUCUUCACU UUGA
708	UUCAAAGUG AAGAAUGAC CCA	900	CAUUCUUCA CUUUGAA	1092	UGGGUCAU UCUUCACUU UGAA
709	UUUCAAAGU GAAGAAUGA CCC	901	AUUCUUCAC UUUGAAA	1093	GGGUCAUU CUUCACUUU GAAC
710	UGUUCAAAG UGAAGAAUG ACC	902	UUCUUCACU UUGAACA	1094	GGUCAUUC UUCACUUU GAACU
711	UCAAGUUCA AAGUGAAGA AUG	903	UUCACUUU GAACUUGA	1095	CAUUCUUCA CUUUGAACU UGU
712	UACAAGUUC AAAGUGAAG AAU	904	UCACUUUGA ACUUGUA	1096	AUUCUUCAC UUUGAACUU GUU
713	UAAUGAACA AGUUCAAAG UGA	905	UUGAACUU GUUCAUUA	1097	UCACUUUGA ACUUGUUCA UUG
714	UCCAAUGAA CAAGUUCAA AGU	906	GAACUUGU UCAUUGGA	1098	ACUUUGAAC UUGUUCAU UGGU
715	UACACCAAU GAACAAGUU CAA	907	CUUGUUCA UUGGUGUA	1099	UUGAACUU GUUCAUUG GUGUC
716	UUUGGUUG AAAUUAUCU AUGA	908	GAUAAUUUC AACCAAA	1100	UCAUAGAUA AUUUCAACC AAC
717	UUUUCUGU UGGUUGAA AUUAU	909	UUCAACCAA CAGAAAA	1101	AUAAUUUCA ACCAACAGA AAA
718	UAAUAUACA UCCUUGGA UUUU	910	CCAAGGAU GUAUAUUA	1102	AAAAUCCAA GGAUGUAU AUUU
719	UAAAUAUAC AUCCUUGG AUUU	911	CAAGGAUG UAUAUUUA	1103	AAAUCCAAG GAUGUAUAU UUG
720	UUCAAAUAU ACAUCCUUG GAU	912	AGGAUGUA UAUUUGAA	1104	AUCCAAGGA UGUAUAUUU GAC
721	UGGUCAAAU AUACAUCCU UGG	913	GAUGUAUAU UUGACCA	1105	CCAAGGAU GUAUAUUU GACCU

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
722	UAGGUCAAA UAUACAUCC UUG	914	AUGUAUAUU UGACCUA	1106	CAAGGAUG UAUAUUUGA CCUA
723	UUAGGUCAA AUAUACAUC CUU	915	UGUAUAUUU GACCUAA	1107	AAGGAUGUA UAUUUGACC UAG
724	UCUAGGUC AAAUAUACA UCCU	916	GUAUAUUU GACCUAGA	1108	AGGAUGUA UAUUUGACC UAGU
725	UAGAUAAGA ACCAUGAUA CUA	917	UCAUGGUU CUUAUCUA	1109	UAGUAUCAU GGUUCUUA UCUG
726	UUCAUGGU UACCAUGUU GAGA	918	ACAUGGUAA CCAUGAA	1110	UCUCAACAU GGUAACCAU GAU
727	UAUCAUGG UUACCAUGU UGAG	919	CAUGGUAAC CAUGAUA	1111	CUCAACAUG GUAACCAUG AUG
728	UCAUCAUG GUUACCAU GUUGA	920	AUGGUAACC AUGAUGA	1112	UCAACAUGG UAACCAUGA UGG
729	UCCAUCAUG GUUACCAU GUUG	921	UGGUAACCA UGAUGGA	1113	CAACAUGGU AACCAUGAU GGU
730	UUACCAUCA UGGUUACC AUGU	922	GUAACCAUG AUGGUAA	1114	ACAUGGUAA CCAUGAUG GUAG
731	UCUACCAUC AUGGUUAC CAUG	923	UAACCAUGA UGGUAGA	1115	CAUGGUAAC CAUGAUGG UAGA
732	UUGUCAUUA AUUCCAUCU UCC	924	AUGGAAUUA AUGACAA	1116	GGAAGAUG GAAUUAAUG ACAU
733	UAUGUCAUU AAUUCCAUC UUC	925	UGGAAUUAA UGACAUA	1117	GAAGAUGG AAUUAAUGA CAUG
734	UAUUGAACA UGUCAUUAA UUC	926	AAUGACAUG UUCAAUA	1118	GAAUUAAUG ACAUGUUCA AUU
735	UAAAUUGAA CAUGUCAUU AAU	927	UGACAUGU UCAAUUUA	1119	AUUAAUGAC AUGUUCAAU UUU
736	UAAAAUUGA ACAUGUCAU UAA	928	GACAUGUU CAAUUUUA	1120	UUAAUGACA UGUUCAAUU UUG
737	UCUGUGAA CGAAGAGAA UCCA	929	UCUCUUCG UUCACAGA	1121	UGGAUUCU CUUCGUUC ACAGA
738	UUCUGUGA ACGAAGAGA AUCC	930	CUCUUCGU UCACAGAA	1122	GGAUUCUC UUCGUUCA CAGAU
739	UAUCUGUG AACGAAGAG AAUC	931	UCUUCGUU CACAGAUA	1123	GAUUCUCU UCGUUCACA GAUG

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
740	UCAUCUGU GAACGAAGA GAAU	932	CUUCGUUC ACAGAUGA	1124	AUUCUCUUC GUUCACAGA UGG
741	UCAUGAACC UUUCUUCCA UCU	933	GAAGAAAGG UUCAUGA	1125	AGAUGGAA GAAAGGUU CAUGU
742	UGACAUGAA CCUUUCUU CCAU	934	AGAAAGGUU CAUGUCA	1126	AUGGAAGAA AGGUUCAU GUCU
743	UAGACAUGA ACCUUUCUU CCA	935	GAAAGGUU CAUGUCUA	1127	UGGAAGAAA GGUUCAUG UCUG
744	UCUUUUAU GUAUAUACU UGAU	936	GUAUAUACA UAAAAGA	1128	AUCAAGUAU AUACAUAAA AGA
745	UUCUUUUAU GUAUAUACU UGA	937	UAUAUACAU AAAAGAA	1129	UCAAGUAUA UACAUAAAA GAU
746	UAUCUUUUA UGUAUAUAC UUG	938	AUAUACAUA AAAGAUA	1130	CAAGUAUAU ACAUAAAAG AUG
747	UCAUCUUUU AUGUAUAUA CUU	939	UAUACAUAA AAGAUGA	1131	AAGUAUAUA CAUAAAAGA UGG
748	UCCAUCUUU UAUGUAUAU ACU	940	AUACAUAAA AGAUGGA	1132	AGUAUAUAC AUAAAAGAU GGA
749	UUCCAUCUU UUAUGUAUA UAC	941	UACAUAAAA GAUGGAA	1133	GUAUAUACA UAAAAGAUG GAG
750	UCUCCAUCU UUUAUGUAU AUA	942	ACAUAAAAG AUGGAGA	1134	UAUAUACAU AAAAGAUGG AGA
751	UGUCUCCA UCUUUUAU GUAUA	943	AUAAAAGAU GGAGACA	1135	UAUACAUAA AAGAUGGA GACA
752	UCUGGACU UGAGUUCU CAUUA	944	AGAACUCAA GUCCAGA	1136	UAAUGAGAA CUCAAGUCC AGA
753	UUCUGGAC UUGAGUUC UCAUU	945	GAACUCAAG UCCAGAA	1137	AAUGAGAAC UCAAGUCCA GAA
754	UUUCUGGA CUUGAGUU CUCAU	946	AACUCAAGU CCAGAAA	1138	AUGAGAACU CAAGUCCAG AAA
755	UGUGGUUA UUCUAUGAA CAAA	947	UCAUAGAAU AACCACA	1139	UUUGUUCA UAGAAUAAC CACA
756	UAGGAGUG UAAUAUGUA CUUA	948	ACAUAUUAC ACUCCUA	1140	UAAGUACAU AUUACACUC CUC
757	UUUAUUAUA UAAAUGUAA UUU	949	ACAUUUAUA UAAUAAA	1141	AAAUUACAU UUAUAUAAU AAA

Antisense SEQ ID NO:	Antisense Strand	Sense SEQ ID NO:	Sense Strand	Targeting Region SEQ ID NO:	*SCN9A* mRNA targeted sequence
758	UCUUAAUUG AAUGUAUUU AAA	950	AUACAUUCA AUUAAGA	1142	UUUAAAUAC AUUCAAUUA AGA
759	UUUAAUAGA AAAUUACAC ACG	951	GUAAUUUUC UAUUAAA	1143	CGUGUGUA AUUUUCUAU UAAU
760	UUUAAUAUG ACAGUGCC UUCU	952	GCACUGUC AUAUUAAA	1144	AGAAGGCAC UGUCAUAUU AAU
761	UAAACUAGG UAACUAUCA AAA	953	AUAGUUACC UAGUUUA	1145	UUUUGAUA GUUACCUA GUUUG
762	UGAAAUAGC UAUUUAGAA CUG	954	CUAAAUAGC UAUUUCA	1146	CAGUUCUAA AUAGCUAUU UCA
763	UUGUAAAGA AUCCUAUGU AAA	955	AUAGGAUUC UUUACAA	1147	UUUACAUAG GAUUCUUUA CAA
764	UAACAUUUU GAGCAUUCA UAG	956	AAUGCUCAA AAUGUUA	1148	CUAUGAAUG CUCAAAAUG UUU
765	UAAACAUUU UGAGCAUU CAUA	957	AUGCUCAAA AUGUUUA	1149	UAUGAAUGC UCAAAAUGU UUG
766	UCAAACAUU UUGAGCAU UCAU	958	UGCUCAAAA UGUUUGA	1150	AUGAAUGCU CAAAAUGUU UGA
767	UCAAGUAUA ACUACAAUA UAA	959	UUGUAGUU AUACUUGA	1151	UUAUAUUGU AGUUAUACU UGA
768	UUCAAGUAU AACUACAAU AUA	960	UGUAGUUA UACUUGAA	1152	UAUAUUGUA GUUAUACUU GAG

siRNA Structure

The siRNA molecules of the disclosure may be in the form of a single-stranded (ss) or double-stranded (ds) oligonucleotide structure. In some embodiments, the siRNA molecules may be di-branched, tri-branched, or tetra-branched molecules. Furthermore, the siRNA molecules of the disclosure may contain one or more phosphodiester internucleoside linkages and/or an analog thereof, such as a phosphorothioate internucleoside linkage. The siRNA molecules of the disclosure may further contain chemically modified nucleosides having 2' sugar modifications.

The simplest siRNAs consist of a ribonucleic acid, including a ss- or ds- structure, formed by a first strand (i.e., antisense strand), and in the case of a ds-siRNA, a second strand (i.e., sense strand). The first strand includes a stretch of contiguous nucleotides that is at least partially complementary to a target nucleic acid. The second strand also includes a stretch of contiguous nucleotides where the second stretch is at least partially identical to a target nucleic acid. The first strand and said second strand may be hybridized to each other to form a double-stranded structure. The hybridization typically occurs by Watson Crick base pairing.

Depending on the sequence of the first and second strand, the hybridization or base pairing is not necessarily complete or perfect, which means that the first and second strand are not 100% base-paired due to mismatches. One or more mismatches may also be present within the duplex without necessarily impacting the siRNA RNAi activity.

The first strand contains a stretch of contiguous nucleotides which is essentially complementary to a target nucleic acid. Typically, the target nucleic acid sequence is, in accordance with the mode of action of interfering ribonucleic acids, a ss-RNA, preferably an mRNA. Such hybridization occurs most likely through Watson Crick base pairing but is not necessarily limited thereto. The extent to which the first strand has a complementary stretch of contiguous nucleotides to a target nucleic acid sequence may be between 80% and 100%, e.g., 80%, 85%, 90%, 95%, or 100% complementary.

The siRNA molecules described herein may employ modifications to the nucleobase, phosphate backbone, ribose core, 5′- and 3′-ends, and branching, wherein multiple strands of siRNA may be covalently linked.

Lengths of Small Interfering RNA Molecules

It is within the scope of the disclosure that any length, known and previously unknown in the art, may be employed for the current invention. As described herein, potential lengths for an antisense strand of the siRNA molecules of the present disclosure is between 10 and 30 nucleotides (e.g., 10 nucleotides, 11 nucleotides, 12 nucleotides, 13 nucleotides, 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides), 15 and 25 nucleotides (e.g., 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, or 25 nucleotides), or 18 and 23 nucleotides (e.g., 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, or 23 nucleotides). In some embodiments, the antisense strand is 20 nucleotides. In some embodiments, the antisense strand is 21 nucleotides. In some embodiments, the antisense strand is 22 nucleotides. In some embodiments, the antisense strand is 23 nucleotides. In some embodiments, the antisense strand is 24 nucleotides. In some embodiments, the antisense strand is 25 nucleotides. In some embodiments, the antisense strand is 26 nucleotides. In some embodiments, the antisense strand is 27 nucleotides. In some embodiments, the antisense strand is 28 nucleotides. In some embodiments, the antisense strand is 29 nucleotides. In some embodiments, the antisense strand is 30 nucleotides.

In some embodiments, the sense strand of the siRNA molecules of the present disclosure is between 12 and 30 nucleotides (e.g., 12 nucleotides, 13 nucleotides, 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides), or 14 and 23 nucleotides (e.g., 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, or 23 nucleotides). In some embodiments, the sense strand is 15 nucleotides. In some embodiments, the sense strand is 16 nucleotides. In some embodiments, the sense strand is 17 nucleotides. In some embodiments, the sense strand is 18 nucleotides. In some embodiments, the sense strand is 19

nucleotides. In some embodiments, the sense strand is 20 nucleotides. In some embodiments, the sense strand is 21 nucleotides. In some embodiments, the sense strand is 22 nucleotides. In some embodiments, the sense strand is 23 nucleotides. In some embodiments, the sense strand is 24 nucleotides. In some embodiments, the sense strand is 25 nucleotides. In some embodiments, the sense strand is 26 nucleotides. In some embodiments, the sense strand is 27 nucleotides. In some embodiments, the sense strand is 28 nucleotides. In some embodiments, the sense strand is 29 nucleotides. In some embodiments, the sense strand is 30 nucleotides.

2′ Sugar Modifications

The present disclosure may include ss- and ds- siRNA molecule compositions including at least one (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or more) nucleosides having 2' sugar modifications. Possible 2′-modifications include all possible orientations of OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C1 to C10 alkyl or C2 to C10 alkenyl and alkynyl. In some embodiments, the modification includes a 2'-O-methyl (2'-O-Me) modification. Other potential sugar substituent groups include: C1 to C10 lower alkyl, substituted lower alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. In some embodiments, the modification includes 2′-methoxyethoxy (2′-O-$CH_2CH_2OCH_3$, also known as 2′-O-(2-methoxyethyl) or 2′-MOE). In some embodiments, the modification includes 2′-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2′-DMAOE, and 2′-dimethylaminoethoxyethoxy (also known in the art as 2′-O-dimethylamino-ethoxy-ethyl or 2′-DMAEOE), i.e., 2′-O-$CH_2OCH_2N(CH_3)_2$. Other potential sugar substituent groups include, e.g., aminopropoxy (-$OCH_2CH_2CH_2NH_2$), allyl (-CH_2-CH=CH_2), -O-allyl (-O-CH_2-CH=CH_2) and fluoro (F). 2′-sugar substituent groups may be in the arabino (up) position or ribo (down) position. In some embodiments, the 2′-arabino modification is 2′-F. Similar modifications may also be made at other positions on the siRNA molecule, particularly the 3′ position of the sugar on the 3′ terminal nucleoside or in 2′-5′ linked oligonucleotides and the 5′ position of 5′ terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar.

Nucleobase Modifications

The siRNA molecules of the disclosure may also include nucleosides or other surrogate or mimetic monomeric subunits that include a nucleobase (often referred to in the art simply as "base" or "heterocyclic base moiety"). The nucleobase is another moiety that has been extensively modified or substituted and such modified and or substituted nucleobases are amenable to the present disclosure. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases also referred herein as heterocyclic base moieties include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-

thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (-C=C-CH3) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Further nucleobases include those disclosed in US 3,687,808, those disclosed in Kroschwitz, J.I., ed. *The Concise Encyclopedia of Polymer Science and Engineering,* New York, John Wiley & Sons, 1990, pp. 858-859; those disclosed by Englisch et al., *Angewandte Chemie,* International Edition 30:613, 1991; and those disclosed by Sanghvi, Y.S., Chapter 16, *Antisense Research and Applications,* CRC Press, Gait, M.J. ed., 1993, pp. 289-302. The siRNA molecules of the present disclosure may also include polycyclic heterocyclic compounds in place of one or more heterocyclic base moieties. A number of tricyclic heterocyclic compounds have been previously reported. These compounds are routinely used in antisense applications to increase the binding properties of the modified strand to a target strand.

Representative cytosine analogs that make three hydrogen bonds with a guanosine in a second strand include 1,3-diazaphenoxazine-2-one (Kurchavov *et al., Nucleosides and Nucleotides,* 16:1837-46, 1997), 1,3-diazaphenothiazine-2-one (Lin et al. *Am. Chem. Soc.,* 117:3873-4, 1995), and 6,7,8,9-tetrafluoro-l,3-diazaphenoxazine-2-one (Wang et al., *Tetrahedron Lett.,* 39:8385-8, 1998). Incorporated into oligonucleotides, these base modifications were shown to hybridize with complementary guanine and the latter was also shown to hybridize with adenine and to enhance helical thermal stability by extended stacking interactions (also see US 10/155,920 and US 10/013,295, both of which are herein incorporated by reference in their entirety). Further helix-stabilizing properties have been observed when a cytosine analog/substitute has an aminoethoxy moiety attached to the rigid 1,3-diazaphenoxazine-2-one scaffold (Lin et al., *Am. Chem. Soc.,* 120:8531-2, 1998).

Internucleoside Linkage Modifications

Another variable in the design of the present disclosure is the internucleoside linkage making up the phosphate backbone of the siRNA molecule. Although the natural RNA phosphate backbone may be employed here, derivatives thereof may be used which enhance desirable characteristics of the siRNA molecule. Although not limiting, of particular importance in the present disclosure is protecting parts, or the whole, of the siRNA molecule from hydrolysis. One example of a modification that decreases the rate of hydrolysis is phosphorothioates. Any portion or the whole of the backbone may contain phosphate substitutions (e.g., phosphorothioates). For instance, the internucleoside linkages may be between 0 and 100% phosphorothioate, e.g., between 0 and 100%, 10 and 100%, 20 and 100%, 30 and 100%, 40 and 100%, 50 and 100%, 60 and 100%, 70 and 100%, 80 and 100%, 90 and 100%, 0 and 90%, 0 and 80%, 0 and 70%, 0 and 60%, 0 and 50%, 0 and 40%, 0 and 30%, 0 and 20%, 0 and 10%, 10 and 90%, 20 and 80%, 30 and 70%, 40 and 60%, 10 and 40%, 20 and 50%, 30 and 60%, 40 and 70%, 50 and 80%, or 60 and 90% phosphorothioate linkages. Similarly, the internucleoside linkages may be between 0 and 100% phosphodiester linkages, e.g., between 0 and 100%, 10 and 100%, 20 and 100%, 30 and 100%, 40 and

100%, 50 and 100%, 60 and 100% 70 and 100%, 80 and 100%, 90 and 100%, 0 and 90%, 0 and 80%, 0 and 70%, 0 and 60%, 0 and 50%, 0 and 40%, 0 and 30%, 0 and 20%, 0 and 10%, 10 and 90%, 20 and 80%, 30 and 70%, 40 and 60%, 10 and 40%, 20 and 50%, 30 and 60%, 40 and 70%, 50 and 80%, or 60 and 90% phosphodiester linkages.

Specific examples of some potential siRNA molecules useful in this invention include oligonucleotides containing modified e.g., non-naturally occurring internucleoside linkages. As defined in this specification, oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom and internucleoside linkages that do not have a phosphorus atom. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. A preferred phosphorus containing modified internucleoside linkage is the phosphorothioate internucleoside linkage. In some embodiments, the modified oligonucleotide backbones containing a phosphorus atom therein include, for example, phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, selenophosphates, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein one or more internucleotide linkages is a 3' to 3', 5' to 5' or 2' to 2' linkage. Exemplary U.S. patents describing the preparation of phosphorus-containing linkages include but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6,239,265; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; and U.S. Pat. RE39464, the entire contents of each of which are hereby incorporated herein by reference.

In some embodiments, the modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; riboacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts. Non-limiting examples of U.S. patents that teach the preparation of non-phosphorus backbones include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, the entire contents of each of which are hereby incorporated herein by reference.

Patterns of Modifications of siRNA Molecules

The following section provides a set of exemplary scaffolds into which the siRNA molecules of the disclosure may be incorporated.

In some embodiments of the disclosure, the siRNA may contain an antisense strand including a region represented by Formula I, wherein Formula I is, in the 5'-to-3' direction:

$$A\text{-}B\text{-}(A')_j\text{-}C\text{-}P^2\text{-}D\text{-}P^1\text{-}(C'\text{-}P^1)_k\text{-}C'$$

Formula I;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$; each A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$; B is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2$; each C is a 2'-O-methyl (2'-O-Me) ribonucleoside; each C', independently, is a 2'-O-Me ribonucleoside or a 2'-fluoro (2'-F) ribonucleoside; each D is a 2'-F ribonucleoside; each P^1 is a phosphorothioate internucleoside linkage; each P^2 is a phosphodiester internucleoside linkage; j is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); and k is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7). In some embodiments, j is 4. In some embodiments, k is 4. In some embodiments, j is 4 and k is 4. The antisense is complementary (e.g., fully or partially complementary) to a target nucleic acid sequence.

In some embodiments, the antisense strand includes a structure represented by Formula A1, wherein Formula A1 is, in the 5'-to-3' direction:

A-S-B-S-A-O-B-O-B-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-A-S-A-S-B-S-A

Formula A1;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the siRNA may contain an antisense strand including a region represented by Formula II, wherein Formula II is, in the 5'-to-3' direction:

$$A\text{-}B\text{-}(A')_j\text{-}C\text{-}P^2\text{-}D\text{-}P^1\text{-}(C\text{-}P^1)_k\text{-}C'$$

Formula II;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$; each A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$; B is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2$; each C is a 2'-O-methyl (2'-O-Me) ribonucleoside; each C', independently, is a 2'-O-Me ribonucleoside or a 2'-fluoro (2'-F) ribonucleoside; each D is a 2'-F ribonucleoside; each P^1 is a phosphorothioate internucleoside linkage; each P^2 is a phosphodiester internucleoside linkage; j is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); and k is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7). In some embodiments, j is 4. In some embodiments, k is 4. In some embodiments, j is 4 and k is 4. The antisense is complementary (e.g., fully or partially complementary) to a target nucleic acid sequence.

In some embodiments of the disclosure, the antisense strand includes a structure represented by Formula A2, wherein Formula A2 is, in the 5'-to-3' direction:

A-S-B-S-A-O-B-O-B-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-A-S-A-S-A-S-A

Formula A2;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the sense strand includes a structure represented by Formula III, wherein Formula III is, in the 5'-to-3' direction:

$$E\text{-}(A')_m\text{-}F$$

Formula III;

wherein E is represented by the formula $(C\text{-}P^1)_2$; F is represented by the formula $(C\text{-}P^2)_3\text{-}D\text{-}P^1\text{-}C\text{-}P^1\text{-}C$, $(C\text{-}P^2)_3\text{-}D\text{-}P^2\text{-}C\text{-}P^2\text{-}C$, $(C\text{-}P^2)_3\text{-}D\text{-}P^1\text{-}C\text{-}P^1\text{-}D$, or $(C\text{-}P^2)_3\text{-}D\text{-}P^2\text{-}C\text{-}P^2\text{-}D$; A', C, D, P^1, and P^2 are as defined in Formula I; and m is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7). In some embodiments, m is 4. The sense strand is complementary (e.g., fully or partially complementary) to the antisense strand.

In some embodiments of the disclosure, the sense strand includes a structure represented by Formula S1, wherein Formula S1 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A-O-A-O-B-S-A-S-A

Formula S1;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the sense strand includes a structure represented by Formula S2, wherein Formula S2 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A-O-A-O-B-O-A-O-A

Formula S2;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the sense strand includes a structure represented by Formula S3, wherein Formula S3 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A-O-A-O-B-S-A-S-B

Formula S3;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the sense strand includes a structure represented by Formula S4, wherein Formula S4 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A-O-A-O-B-O-A-O-B

Formula S4;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the siRNA may contain an antisense strand including a region represented by Formula IV, wherein Formula IV is, in the 5'-to-3' direction:

$A\text{-}(A')_j\text{-}C\text{-}P^2\text{-}B\text{-}(C\text{-}P^1)_k\text{-}C'$

Formula IV;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$; each A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$; B is represented by the formula $D\text{-}P^1\text{-}C\text{-}P^1\text{-}D\text{-}P^1$; each C is a 2'-O-Me ribonucleoside; each C', independently, is a 2'-O-Me ribonucleoside or a 2'-F ribonucleoside; each D is a 2'-F ribonucleoside; each P^1 is a phosphorothioate internucleoside linkage; each P^2 is a phosphodiester internucleoside linkage; j is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); and k is an integer from 1 to7 (e.g., 1, 2, 3, 4, 5, 6, or 7). In some embodiments, j is 6. In some embodiments, k is 4. In some embodiments, j is 6 and k is 4. The antisense strand is complementary (e.g., fully or partially complementary) to a target nucleic acid.

In some embodiments of the disclosure, the antisense strand includes a structure represented by Formula A3, wherein Formula A3 is, in the 5'-to-3' direction:

A-S-B-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-B-S-A-S-A-S-A

Formula A3;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the siRNA of the disclosure may have a sense strand represented by Formula V, wherein Formula V is, in the 5'-to-3' direction:

$E\text{-}(A')_m\text{-}C\text{-}P^2\text{-}F$

Formula V;

wherein E is represented by the formula $(C\text{-}P^1)_2$; F is represented by the formula $D\text{-}P^1\text{-}C\text{-}P^1\text{-}C$, $D\text{-}P^2\text{-}C\text{-}P^2\text{-}C$, $D\text{-}P^1\text{-}C\text{-}P^1\text{-}D$, or $D\text{-}P^2\text{-}C\text{-}P^2\text{-}D$; A', C, D, P^1, and P^2 are as defined in Formula IV; and m is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7). In some embodiments, m is 5. The sense strand is complementary (e.g., fully or partially complementary) to the antisense strand.

In some embodiments of the disclosure, the sense strand includes a structure represented by Formula S5, wherein Formula S5 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-A

Formula S5;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the sense strand includes a structure represented by Formula S6, wherein Formula S6 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A

Formula S6;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the sense strand includes a structure represented by Formula S7, wherein Formula S7 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-B

Formula S7;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the sense strand includes a structure represented by Formula S8, wherein Formula S8 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B

Formula S8;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the siRNA may contain an antisense strand including a region represented by Formula VI, wherein Formula VI is, in the 5'-to-3' direction:

$A\text{-}B_j\text{-}E\text{-}B_k\text{-}E\text{-}F\text{-}G_l\text{-}D\text{-}P^1\text{-}C'$

Formula VI;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$; each B is represented by the formula $C\text{-}P^2$; each C is a 2'-O-Me ribonucleoside; each C', independently, is a 2'-O-Me ribonucleoside or a 2'-F ribonucleoside; each D is a 2'-F ribonucleoside; each E is represented by the formula $D\text{-}P^2\text{-}C\text{-}P^2$; F is represented by the formula $D\text{-}P^1\text{-}C\text{-}P^1$; each G is represented by the formula $C\text{-}P^1$; each P^1 is a phosphorothioate internucleoside linkage; each P^2 is a phosphodiester internucleoside linkage; j is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); k is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); and l is an integer from 1 to

7 (e.g., 1, 2, 3, 4, 5, 6, or 7). In some embodiments, j is 3. In some embodiments, k is 6. In some embodiments, l is 2. In some embodiments, j is 3, k is 6, and l is 2. The antisense strand is complementary (e.g., fully or partially complementary) to a target nucleic acid.

In some embodiments of the disclosure, the antisense strand includes a structure represented by Formula A4, wherein Formula A4 is, in the 5'-to-3' direction:

A-S-B-S-A-O-A-O-A-O-B-O-A-O-A-O-A-O-A-O-A-O-A-O-A-O-B-O-A-O-B-S-A-S-A-S-A-S-B-S-A

Formula A4;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the siRNA may contain a sense strand including a region represented by Formula VII, wherein Formula VII is, in the 5'-to-3' direction:

$$H\text{-}B_m\text{-}I_n\text{-}A'\text{-}B_o\text{-}H\text{-}C$$

Formula VII;

wherein A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$; each H is represented by the formula $(C\text{-}P^1)_2$; each I is represented by the formula $(D\text{-}P^2)$; B, C, D, P^1, and P^2 are as defined in Formula VI; m is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); n is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); and o is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7). In some embodiments, m is 3. In some embodiments, n is 3. In some embodiments, o is 3. In some embodiments, m is 3, n is 3, and o is 3. The sense strand is complementary (e.g., fully or partially complementary) to the antisense strand.

In some embodiments of the disclosure, the sense strand includes a structure represented by Formula S9, wherein Formula S9 is, in the 5'-to-3' direction:

A-S-A-S-A-O-A-O-A-O-B-O-B-O-B-O-A-O-B-O-A-O-A-O-A-O-A-S-A-S-A

Formula S9;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the siRNA may contain an antisense strand including a region that is represented by Formula VIII:

$$Z\text{-}((A\text{-}P\text{-})_n(B\text{-}P\text{-})_m)_q;$$

Formula VIII

wherein Z is a 5' phosphorus stabilizing moiety; each A is a 2'-O-methyl (2'-O-Me) ribonucleoside; each B is a 2'-fluoro-ribonucleoside; each P is, independently, an internucleoside linkage selected from a phosphodiester linkage and a phosphorothioate linkage; n is an integer from 1 to 5 (e.g., 1, 2, 3, 4, or 5); m

is an integer from 1 to 5 (e.g., 1, 2, 3, 4, or 5); and q is an integer between 1 and 30 (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30).

Methods of siRNA Synthesis

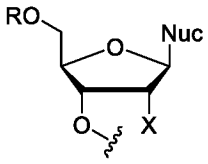
The siRNA molecules of the disclosure can be synthesized by standard methods known in the art as further discussed below, e.g., by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc.

The siRNA agent can be prepared using solution-phase or solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligonucleotide including unnatural or modified nucleotides can be easily prepared. siRNA molecules of the disclosure can be prepared using solution-phase or solid-phase organic synthesis or both.

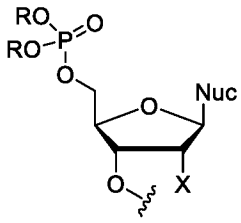
Further, it is contemplated that for any siRNA agent disclosed herein, further optimization could be achieved by systematically either adding or removing linked nucleosides to generate longer or shorter sequences. Further still, such optimized sequences can be adjusted by, e.g., the introduction of modified nucleosides, and/or modified internucleoside linkages as described herein or as known in the art, including alternative nucleosides, alternative sugar moieties, and/or alternative internucleoside linkages as known in the art and/or discussed herein to further optimize the molecule (e.g., increasing serum stability or circulating half-life, increasing thermal stability, enhancing transmembrane delivery, and/or targeting to a particular location or cell type).

5′ Phosphorus Stabilizing Moieties

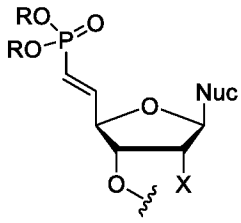
To further protect the siRNA molecules of this disclosure from degradation, a 5′-phosphorus stabilizing moiety may be employed. A 5′-phosphorus stabilizing moiety replaces the 5′-phosphate to prevent hydrolysis of the phosphate. Hydrolysis of the 5′-phosphate prevents binding to RISC, a necessary step in gene silencing. Any replacement for phosphate that does not impede binding to RISC is contemplated in this disclosure. In some embodiments, the replacement for the 5′-phosphate is also stable to *in vivo* hydrolysis. Each strand of a siRNA molecule may independently and optionally employ any suitable 5′-phosphorus stabilizing moiety.



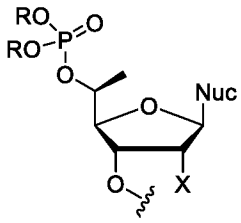
Formula IX



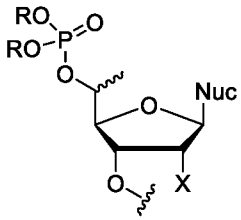
Formula X



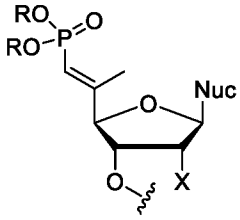
Formula XI



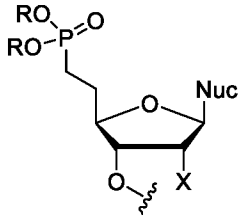
Formula XII



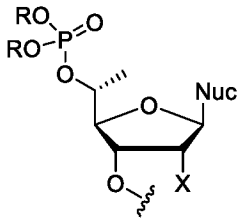
Formula XIII



Formula XIV



Formula XV



Formula XVI

Some exemplary endcaps are demonstrated in Formulas IX-XVI. Nuc in Formulas IX-XVI represents a nucleobase or nucleobase derivative or replacement as described herein. X in formula IX-XVI represents a 2'-modification as described herein. Some embodiments employ hydroxy as in Formula IX, phosphate as in Formula X, vinylphosphonates as in Formula XI and XIV, 5'-methyl-substitued phosphates as in Formula XII, XIII, and XVI, methylenephosphonates as in Formula XV, or vinyl 5′-vinylphsophonate as a 5′-phosphorus stabilizing moiety as demonstrated in Formula XI.

Hydrophobic Moieties

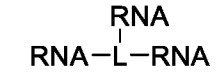
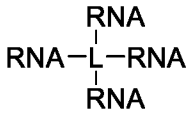
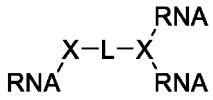
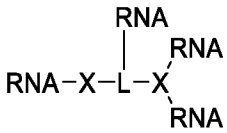
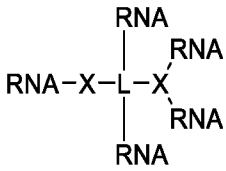
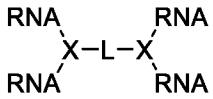
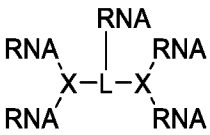
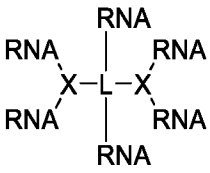
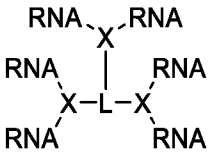
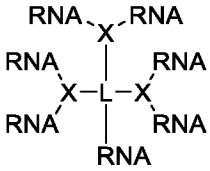
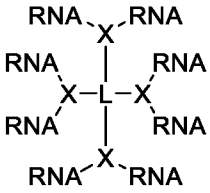
The present disclosure further provides siRNA molecules having one or more hydrophobic moieties attached thereto. The hydrophobic moiety may be covalently attached to the 5' end or the 3' end of the siRNA molecules of the disclosure. Non-limiting examples of hydrophobic moieties suitable for use with the siRNA molecules of the disclosure may include cholesterol, vitamin D, tocopherol, phosphatidylcholine (PC), docosahexaenoic acid, docosanoic acid, PC-docosanoic acid, eicosapentaenoic acid, lithocholic acid or any combination of the aforementioned hydrophobic moieties with PC.

siRNA Branching

The siRNA molecules of the disclosure may be branched. For example, the siRNA molecules of the disclosure may have one of several branching patterns, as described herein.

According to the present disclosure, the siRNA molecules disclosed herein may be branched siRNA molecules. The siRNA molecule may not be branched, or may be di-branched, tri-branched, or tetra-branched, connected through a linker. Each main branch may be further branched to allow for 2, 3, 4, 5, 6, 7, or 8 separate RNA single- or double-strands. The branch points on the linker may stem from the same atom, or separate atoms along the linker. Some exemplary embodiments are listed in Table 2.

Table 2. Branched siRNA structures

Di-branched	Tri-branched	Tetra-branched
RNA−L−RNA **Formula XVII**	 **Formula XX**	 **Formula XXIV**
 **Formula XVIII**	 **Formula XXI**	 **Formula XXV**
 **Formula XIX**	 **Formula XXII**	 **Formula XXVI**
	 **Formula XXIII**	 **Formula XXVII**
		 **Formula XXVIII**

In some embodiments, the siRNA molecule is a branched siRNA molecule. In some embodiments, the branched siRNA molecule is di-branched, tri-branched, or tetra-branched. In some embodiments, the di-branched siRNA molecule is represented by any one of Formulas XVII-XIX, wherein each RNA, independently, is an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety (e.g., phosphoroamidite, tosylated solketal, 1,3-diaminopropanol, pentaerythritol, or any one of the branch point moieties described in US 10,478,503).

In some embodiments, the tri-branched siRNA molecule represented by any one of Formulas XX-XXIII, wherein each RNA, independently, is an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

In some embodiments, the tetra-branched siRNA molecule represented by any one of Formulas XXIV-XXVIII, wherein each RNA, independently, is an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

Linkers

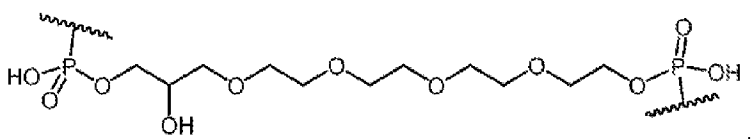
Multiple strands of siRNA described herein may be covalently attached by way of a linker. The effect of this branching improves, *inter alia*, cell permeability allowing better access into cells (e.g., neurons or glial cells) in the CNS. Any linking moiety may be employed which is not incompatible with the siRNAs of the present invention. Linkers include ethylene glycol chains of 2 to 10 subunits (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 subunits), alkyl chains, carbohydrate chains, block copolymers, peptides, RNA, DNA, and others. In some embodiments, any carbon or oxygen atom of the linker is optionally replaced with a nitrogen atom, bears a hydroxyl substituent, or bears an oxo substituent. In some embodiments, the linker is a poly-ethylene glycol (PEG) linker. The PEG linkers suitable for use with the disclosed compositions and methods include linear or non-linear PEG linkers. Examples of non-linear PEG linkers include branched PEGs, linear forked PEGs, or branched forked PEGs.

PEG linkers of various weights may be used with the disclosed compositions and methods. For example, the PEG linker may have a weight that is between 5 and 500 Daltons. In some embodiments, a PEG linker having a weight that is between 500 and 1,000 Dalton may be used. In some embodiments, a PEG linker having a weight that is between 1,000 and 10,000 Dalton may be used. In some embodiments, a PEG linker having a weight that is between 200 and 20,000 Dalton may be used. In some embodiments, the linker is covalently attached to a sense strand of the siRNA. In some embodiments, the linker is covalently attached to an antisense strand of the siRNA. In some embodiments, the PEG linker is a triethylene glycol (TrEG) linker. In some embodiments, the PEG linker is a tetraethylene linker (TEG).

In some embodiments, the linker is an alkyl chain linker. In some embodiments, the linker is a peptide linker. In some embodiments, the linker is an RNA linker. In some embodiments, the linker is a DNA linker.

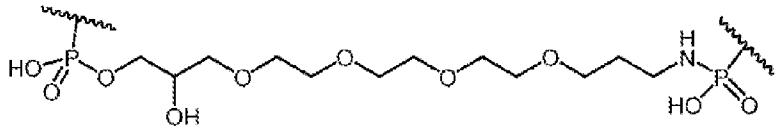
Linkers may covalently link 2, 3, 4, or 5 unique siRNA strands. The linker may covalently bind to any part of the siRNA oligomer. In some embodiments, the linker attaches to the 3′ end of nucleosides of each siRNA strand. In some embodiments, the linker attaches to the 5′ end of nucleosides of each siRNA strand. In some embodiments, the linker attaches to a nucleoside of an siRNA strand (e.g., sense or antisense strand) by way of a covalent bond-forming moiety. In some embodiments, the covalent-bond-forming moiety is selected from the group consisting of an alkyl, ester, amide, carbonate, carbamate, triazole, urea, formacetal, phosphonate, phosphate, and phosphate derivative (e.g., phosphorothioate, phosphoramidate, etc.).

In some embodiments, the linker has a structure of Formula L1:



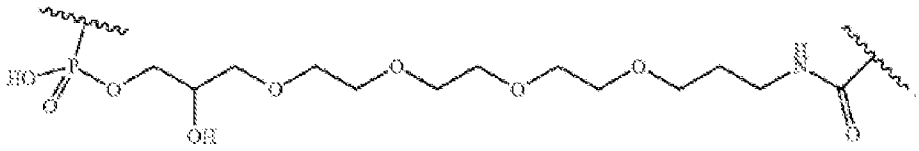
(**Formula L1**)

In some embodiments, the linker has a structure of Formula L2:



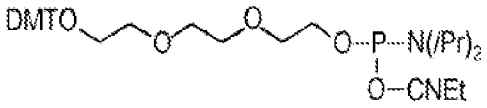
(**Formula L2**)

In some embodiments, the linker has a structure of Formula L3:



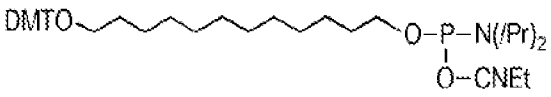
(**Formula L3**)

In some embodiments, the linker has a structure of Formula L4:



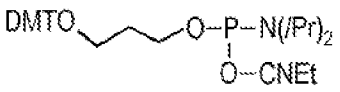
(**Formula L4**)

In some embodiments, the linker has a structure of Formula L5:



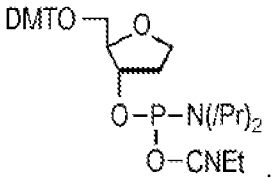
(**Formula L5**)

In some embodiments, the linker has a structure of Formula L6:



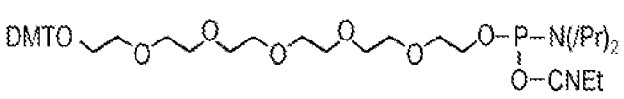
(**Formula L6**)

In some embodiments, the linker has a structure of Formula L7:



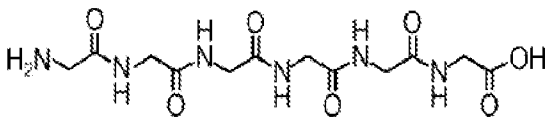
(**Formula L7**)

In some embodiments, the linker has a structure of Formula L8:



(**Formula L8**)

In some embodiments, the linker has a structure of Formula L9:



(**Formula L9**)

In some embodiments, the selection of a linker for use with one or more of the branched siRNA molecules disclosed herein may be based on the hydrophobicity of the linker, such that, e.g., desirable hydrophobicity is achieved for the one or more branched siRNA molecules of the disclosure. For example, a linker containing an alkyl chain may be used to increase the hydrophobicity of the branched siRNA molecule as compared to a branched siRNA molecule having a less hydrophobic linker or a hydrophilic linker.

The siRNA agents disclosed herein may be synthesized and/or modified by methods well established in the art, such as those described in Beaucage, S. L. et al. (edrs.), *Current Protocols in Nucleic Acid Chemistry*, John Wiley & Sons, Inc., New York, N.Y., 2000, which is hereby incorporated herein by reference.

Methods of Treatment

The *SCN9A*-targeting siRNA molecules of the disclosure may be delivered to a subject, for example, as an analgesic effective against multiple forms of acute or chronic pain (e.g., nociceptive pain or neuropathic pain). Furthermore, the siRNA molecules of the disclosure may also be delivered to a subject having a gain-of-function variant of the *SCN9A* gene (e.g., primary erythromelalgia) for which siRNA-mediated gene silencing of the *SCN9A* variant gene reduces the expression level of *SCN9A* transcript, thereby reducing the level of pain experienced by the subject and/or mitigating symptoms of a pain disorder, such as Gerhardt disease, Mitchell disease, or Weir-Mitchell disease.

The disclosure provides methods of treating a subject by way of *SCN9A* gene silencing with one or more of the small interfering RNA (siRNA) molecules described herein. The gene silencing may be performed in a subject to silence wild type *SCN9A* transcripts, mutant *SCN9A* transcripts, splice isoforms of *SCN9A* transcripts, and/or overexpressed *SCN9A* transcripts thereof, relative to a healthy subject. The method may include delivering to the CNS or neurons of the subject (e.g., a human) the siRNA molecules of the disclosure or a pharmaceutical composition containing the same by any appropriate route of administration (e.g., intrathecal injection, direct injection into a specific nerve or ganglion(ganglia), such as trigenminal or dorsal root ganglia, or by intra-cisterna magna injection by catheterization). The active compound can be administered in any suitable dose. The actual dosage amount of a composition of the present disclosure administered to a patient can be determined by physical and physiological factors such as body weight, severity of condition, previous or concurrent therapeutic interventions, idiopathy of the patient and on the route of administration. Depending upon the dosage and the route of administration, the number of administrations of a preferred dosage and/or an effective amount may vary according to the response of the subject. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject.

Administration may occur any suitable number of times per day, and for as long as necessary. Subjects may be adult or pediatric humans, with or without comorbid diseases.

Selection of Subjects

Subjects that may be treated with the small interfering RNA (siRNA) molecules disclosed herein are subjects in need of treatment for chronic, persistent, or acute symptoms of pain. Such symptoms of pain may be neuropathic or nociceptive in nature. Additionally, subjects in need of treatment of pain may be characterized as having spontaneous pain (e.g., primary erythromelalgia or secondary erythromelalgia) or may be diagnosed with a pain disorder (e.g., Gerhardt disease, Mitchell disease, or Weir-Mitchell disease). Subjects that may be treated with the siRNA molecules disclosed herein may include, for example, humans, monkeys, rats, mice, pigs, and other mammals containing at least one orthologous copy of the *SCN9A* gene. Subjects may be adult or pediatric humans, with or without comorbid diseases.

Pharmaceutical Compositions

The siRNA molecules in the present disclosure may be formulated into a pharmaceutical composition for administration to a subject in a biologically compatible form suitable for administration *in vivo*. Accordingly, the present disclosure provides a pharmaceutical composition containing a siRNA molecule of the disclosure in admixture with a suitable diluent, carrier, or excipient. The siRNA molecules may be administered, for example, directly into the CNS or affected tissues or neurons of the subject (e.g., by way of intracerebroventricular injection, intrastriatal injection, intrathecal injection, intra-cisterna magna injection by catheterization, intraparenchymal injection, direct injection into a specific nerve or ganglion(ganglia) (e.g., trigenminal or dorsal root ganglia), intravenous injection, subcutaneous injection, or intramuscular injection)..

Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington, J.P. *The Science and Practice of Pharmacy*, Easton, PA. Mack Publishers, 2012, 22nd ed. and in The United States Pharmacopeial Convention, *The National Formulary*, United States Pharmacopeial, 2015, USP 38 NF 33).

Under ordinary conditions of storage and use, a pharmaceutical composition may contain a preservative, e.g., to prevent the growth of microorganisms. Pharmaceutical compositions may include sterile aqueous solutions, dispersions, or powders, e.g., for the extemporaneous preparation of sterile solutions or dispersions. In all cases the form may be sterilized using techniques known in the art and may be fluidized to the extent that may be easily administered to a subject in need of treatment.

A pharmaceutical composition may be administered to a subject, e.g., a human subject, alone or in combination with pharmaceutically acceptable carriers, as noted herein, the proportion of which may be determined by the solubility and/or chemical nature of the compound, chosen route of administration, and standard pharmaceutical practice.

Dosing Regimens

A physician having ordinary skill in the art can readily determine an effective amount of the siRNA molecule for administration to a mammalian subject (e.g., a human) in need thereof. For example, a physician could start prescribing doses of one the siRNA molecules of the disclosure at levels lower than

that required to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. Alternatively, a physician may begin a treatment regimen by administering one of the siRNA molecules of the disclosure at a high dose and subsequently administer progressively lower doses until reaching a minimal dosage at which a therapeutic effect is achieved (e.g., a reduction in expression of a target gene sequence). In general, a suitable daily dose of one of the siRNA molecules of the disclosure will be an amount of the siRNA molecule which is the lowest dose effective to produce a therapeutic effect. The ss- or ds-siRNA molecules of the disclosure may be administered by injection, e.g., intrathecally, intracerebroventricularly, by intra-cisterna magna injection by catheterization, intraparenchymally, by direct injection into a specific nerve or ganglion(ganglia) (e.g., trigeminal or dorsal root ganglia), intravenously, subcutaneously, or intramuscularly. A daily dose of a therapeutic composition of the siRNA molecules of the disclosure may be administered as a single dose or as two, three, four, five, six or more doses administered separately at appropriate intervals throughout the day, week, month, or year, optionally, in unit dosage forms. While it is possible for the siRNA molecules of the disclosure to be administered alone, it may also be administered as a pharmaceutical formulation in combination with excipients, carriers, and optionally, additional therapeutic agents.

Routes of Administration

The method of the disclosure contemplates any route of administration tolerated by the therapeutic composition. Some embodiments of the method include injection intrathecally or by intra-cisterna magna injection by catheterization. Some embodiments of the method include direct injection into a specific nerve or ganglion(ganglia) (e.g., trigeminal or dorsal root ganglia).

Intrathecal injection is the direct injection into the spinal column or subarachnoid space. By injecting directly into the CSF of the spinal column the siRNA molecules of the disclosure have direct access to cells (e.g., neurons and glial cells) in the spinal column and a route to access the cells in the brain by bypassing the blood brain barrier, or a route to access cell bodies of those neurons that are outside the blood brain barrier.

Intracerebroventricular (ICV) injection is a method to directly inject into the CSF of the cerebral ventricles. Similar to intrathecal injection, ICV is a method of injection which bypasses the blood brain barrier. Using ICV allows the advantage of access to the cells of the brain and spinal column without the danger of the therapeutic being degraded in the blood.

Intrastriatal injection is the direct injection into the striatum, or corpus striatum. The striatum is an area in the subcortical basal ganglia in the brain. Injecting into the striatum bypasses the blood brain barrier and the pharmacokinetic challenges of injection into the blood stream and allows for direct access to the cells of the brain.

Intraparenchymal administration is the direct injection into the parenchyma (e.g., the brain parenchyma). Injection into the brain parenchyma allows for injection directly into brain regions affected by a disease or disorder while bypassing the blood brain barrier.

Intra-cisterna magna injection by catheterization is the direct injection into the cisterna magna. The cisterna magna is the area of the brain located between the cerebellum and the dorsal surface of the medulla oblongata. Injecting into the cisterna magna results in more direct delivery to the cells of the cerebellum, brainstem, and spinal cord.

In some embodiments of the methods described herein, the therapeutic composition may be delivered to the subject by way of systemic administration, e.g., intravenously, intramuscularly, or subcutaneously.

Intravenous (IV) injection is a method to directly inject into the bloodstream of a subject. The IV administration may be in the form of a bolus dose or by way of continuous infusion, or any other method tolerated by the therapeutic composition.

Intramuscular (IM) injection is injection into a muscle of a subject, such as the deltoid muscle or gluteal muscle. IM may allow for rapid absorption of the therapeutic composition.

Subcutaneous injection is injection into subcutaneous tissue. Absorption of compositions delivered subcutaneously may be slower than IV or IM injection, which may be beneficial for compositions requiring continuous absorption.

Examples

The following examples are put forth so as to provide those of ordinary skill in the art with a description of how the compositions and methods described herein may be used, made, and evaluated, and are intended to be purely exemplary of the disclosure and are not intended to limit the scope of what the inventors regard as their disclosure.

Example 1. Knockdown of *SCN9A* with siRNA Molecules of the Disclosure

SCN9A-targeting siRNA molecules of the disclosure were screened for activity. G402 cells were cultured in the presence of an siRNA molecule of the disclosure at either 2 μM or 0.5 μM concentration. After 72 hours, cells were lysed, and mRNA levels of SCN9A and a housekeeping gene (GAPDH) were assessed via reverse transcription-quantitative polymerase chain reaction (RT-qPCR), using standard reagents and Applied Biosystems TaqMan Assays. Results are presented in Table 3, below, as the percent residual SCN9A mRNA relative to untreated control cells in the same assay, corrected for any changes in expression due to housekeeping gene (% UNT SCN9A mRNA). STDEV = standard deviation; ND = not determined.

Table 3. SCN9A Knockdown with siRNA molecules of the disclosure

Antisense SEQ ID NO:	Sense SEQ ID NO:	Targeting Region SEQ ID NO:	% UNT SCN9A mRNA expression at 2 uM	STDEV	% UNT SCN9A mRNA expression at 0.5 uM	STDEV
1	193	385	97.75	4.60	78.02	10.08
2	194	386	95.30	5.36	72.92	8.20
3	195	387	72.19	0.50	73.81	9.75
4	196	388	64.58	6.07	64.63	3.74
5	197	389	70.36	1.62	63.42	6.54
6	198	390	49.09	4.41	49.93	1.53
7	199	391	63.58	0.95	61.83	1.64
8	200	392	77.79	6.72	59.82	2.11

Antisense SEQ ID NO:	Sense SEQ ID NO:	Targeting Region SEQ ID NO:	% UNT SCN9A mRNA expression at 2 uM	STDEV	% UNT SCN9A mRNA expression at 0.5 uM	STDEV
9	201	393	68.79	7.38	53.20	1.45
10	202	394	67.28	4.62	58.97	1.25
11	203	395	73.45	7.45	74.97	3.80
12	204	396	98.13	6.89	77.09	1.98
13	205	397	62.29	5.38	71.24	10.14
14	206	398	80.10	3.88	71.22	2.23
15	207	399	55.55	5.00	47.45	1.85
16	208	400	62.43	11.29	54.07	2.72
17	209	401	67.18	0.51	47.44	0.19
18	210	402	122.71	20.27	90.03	5.76
19	211	403	121.62	20.39	80.27	3.51
20	212	404	91.57	11.67	82.61	0.49
21	213	405	76.30	5.63	74.56	3.83
22	214	406	78.28	3.63	82.45	4.97
23	215	407	54.27	2.51	63.68	3.61
24	216	408	56.02	2.79	58.23	1.28
25	217	409	81.89	4.02	83.16	3.13
26	218	410	59.70	7.24	65.56	3.68
27	219	411	38.42	1.35	ND	ND
28	220	412	44.02	0.60	ND	ND
29	221	413	43.56	4.35	ND	ND
30	222	414	47.98	7.04	ND	ND
31	223	415	55.36	10.21	ND	ND
32	224	416	49.58	0.76	ND	ND
33	225	417	61.08	5.04	ND	ND
34	226	418	83.07	ND	ND	ND
35	227	419	66.59	ND	ND	ND
36	228	420	63.84	11.90	ND	ND
37	229	421	75.49	ND	ND	ND
38	230	422	50.87	0.48	ND	ND
39	231	423	43.55	1.32	ND	ND
40	232	424	66.43	5.84	ND	ND
41	233	425	47.88	4.76	ND	ND
42	234	426	72.32	4.70	ND	ND
43	235	427	71.45	0.76	ND	ND
44	236	428	55.65	2.60	ND	ND
45	237	429	65.68	1.20	ND	ND
46	238	430	62.46	4.95	ND	ND
47	239	431	38.70	1.70	ND	ND
48	240	432	49.94	0.62	ND	ND
49	241	433	60.63	5.37	ND	ND

Antisense SEQ ID NO:	Sense SEQ ID NO:	Targeting Region SEQ ID NO:	% UNT SCN9A mRNA expression at 2 uM	STDEV	% UNT SCN9A mRNA expression at 0.5 uM	STDEV
50	242	434	69.32	3.81	ND	ND
51	243	435	65.15	5.70	ND	ND
52	244	436	52.22	3.63	ND	ND
53	245	437	72.33	3.17	80.69	0.78
54	246	438	82.98	22.17	82.58	2.12
55	247	439	60.28	13.26	65.74	4.62
56	248	440	66.24	15.36	70.83	0.85
57	249	441	65.77	11.67	81.48	0.35
58	250	442	83.93	4.71	103.29	10.63
59	251	443	65.17	4.85	90.97	3.31
60	252	444	69.48	7.42	77.44	13.14
61	253	445	112.83	12.66	84.17	1.09
62	254	446	90.53	5.61	76.38	2.32
63	255	447	75.85	8.91	69.48	3.15
64	256	448	90.38	22.85	74.11	4.55
65	257	449	79.42	8.11	86.33	4.37
66	258	450	94.93	9.49	91.71	2.22
67	259	451	102.34	13.30	83.65	0.04
68	260	452	74.62	13.07	92.60	1.25
69	261	453	88.11	11.67	89.12	ND
70	262	454	93.23	3.72	79.22	1.37
71	263	455	84.10	8.42	73.71	0.88
72	264	456	66.32	5.08	60.34	5.05
73	265	457	50.76	ND	53.12	0.72
74	266	458	47.81	ND	47.64	2.33
75	267	459	92.44	ND	78.42	3.07
76	268	460	95.37	4.44	72.88	1.41
77	269	461	107.93	0.28	78.90	2.15
78	270	462	97.77	20.85	73.13	3.50
79	271	463	85.28	6.27	47.18	0.92
80	272	464	87.02	4.72	53.62	1.44
81	273	465	66.14	3.41	47.10	3.28
82	274	466	71.90	10.13	49.18	2.51
83	275	467	92.18	2.95	60.76	1.71
84	276	468	45.59	1.28	39.73	1.75
85	277	469	113.79	7.71	95.14	0.22
86	278	470	114.44	15.18	89.92	6.57
87	279	471	97.89	6.79	79.19	3.99
88	280	472	66.65	4.44	62.64	2.69
89	281	473	129.28	29.89	58.68	0.05
90	282	474	94.19	21.08	48.91	0.98

Antisense SEQ ID NO:	Sense SEQ ID NO:	Targeting Region SEQ ID NO:	% UNT SCN9A mRNA expression at 2 uM	STDEV	% UNT SCN9A mRNA expression at 0.5 uM	STDEV
91	283	475	112.42	19.71	57.64	1.92
92	284	476	116.73	8.20	57.56	0.40
93	285	477	85.67	2.64	47.13	1.08
94	286	478	115.46	12.08	65.76	3.67
95	287	479	143.28	23.78	84.64	7.76
96	288	480	103.23	0.49	44.29	ND
97	289	481	117.10	11.48	75.20	1.17
98	290	482	86.54	18.94	66.58	1.22
99	291	483	176.69	11.84	65.61	0.21
100	292	484	171.74	8.26	67.17	3.73
101	293	485	133.89	2.24	68.27	1.82
102	294	486	139.48	20.02	68.38	2.96
103	295	487	113.23	9.20	66.23	1.61
104	296	488	123.02	11.23	77.37	1.26
105	297	489	64.53	8.01	71.64	2.45
106	298	490	70.10	11.61	74.34	4.09
107	299	491	73.77	3.02	74.08	1.64
108	300	492	75.30	13.09	71.57	1.05
109	301	493	71.15	3.46	77.65	1.98
110	302	494	88.07	20.37	77.67	4.19
111	303	495	83.52	23.63	72.19	1.57
112	304	496	86.43	11.92	72.12	1.91
113	305	497	87.97	9.82	77.85	1.50
114	306	498	80.76	17.45	75.02	7.43
115	307	499	74.04	14.48	91.67	11.04
116	308	500	107.62	32.98	97.13	10.03
117	309	501	102.20	22.34	100.43	16.37
118	310	502	113.14	39.17	86.36	5.01
119	311	503	133.82	56.67	85.60	5.27
120	312	504	159.34	70.28	87.67	6.83
121	313	505	100.33	37.26	80.38	1.20
122	314	506	72.18	ND	90.58	0.79
123	315	507	68.49	ND	89.58	0.64
124	316	508	158.43	85.10	87.89	1.22
125	317	509	78.04	15.74	107.01	19.24
126	318	510	64.63	13.42	78.75	20.57
127	319	511	76.63	9.96	72.40	6.91
128	320	512	99.49	0.95	84.58	10.46
129	321	513	80.03	3.47	71.94	0.33
130	322	514	77.92	9.38	92.70	6.31
131	323	515	88.26	4.83	90.89	22.17

Antisense SEQ ID NO:	Sense SEQ ID NO:	Targeting Region SEQ ID NO:	% UNT SCN9A mRNA expression at 2 uM	STDEV	% UNT SCN9A mRNA expression at 0.5 uM	STDEV
132	324	516	56.05	11.39	65.27	1.42
133	325	517	72.67	16.79	76.33	1.31
134	326	518	69.70	11.12	72.00	1.95
135	327	519	60.83	5.43	75.66	6.54
136	328	520	61.32	7.42	83.96	11.17
137	329	521	54.88	7.97	73.16	4.78
138	330	522	54.95	15.55	79.24	5.04
139	331	523	48.12	6.61	75.85	12.07
140	332	524	65.27	18.62	76.84	4.96
141	333	525	140.03	27.31	108.67	19.63
142	334	526	127.28	17.88	93.16	5.81
143	335	527	102.44	3.95	95.25	9.83
144	336	528	90.33	1.13	88.77	5.05
145	337	529	74.49	0.80	91.46	6.88
146	338	530	74.33	2.47	90.54	4.69
147	339	531	57.39	1.95	83.08	1.08
148	340	532	59.82	2.79	85.74	0.63
149	341	533	56.71	4.84	93.14	0.27
150	342	534	45.93	1.20	70.91	1.80
151	343	535	95.78	6.79	75.01	2.62
152	344	536	94.45	3.93	77.96	2.11
153	345	537	81.80	2.94	76.72	4.09
154	346	538	82.65	3.64	89.10	7.48
155	347	539	74.74	10.87	87.82	5.29
156	348	540	84.39	0.35	92.81	11.19
157	349	541	124.73	16.18	68.61	3.46
158	350	542	116.59	8.60	63.38	4.79
159	351	543	162.66	38.43	74.34	4.98
160	352	544	129.01	14.45	71.74	5.11
161	353	545	115.11	17.75	73.97	5.44
162	354	546	81.32	13.59	69.88	2.43
163	355	547	105.04	12.07	79.94	5.11
164	356	548	116.78	11.57	81.16	12.58
165	357	549	97.71	28.43	72.36	8.12
166	358	550	92.75	33.63	67.75	11.80
167	359	551	156.27	13.66	66.67	0.02
168	360	552	102.33	ND	53.48	4.74
169	361	553	76.16	4.45	45.54	1.31
170	362	554	83.17	11.09	45.85	2.70
171	363	555	79.65	19.67	54.93	6.13
172	364	556	89.79	29.47	60.12	6.57

Antisense SEQ ID NO:	Sense SEQ ID NO:	Targeting Region SEQ ID NO:	% UNT SCN9A mRNA expression at 2 uM	STDEV	% UNT SCN9A mRNA expression at 0.5 uM	STDEV
173	365	557	96.00	7.81	67.77	2.81
174	366	558	75.92	8.03	57.73	1.32
175	367	559	64.69	0.56	57.88	1.54
176	368	560	64.83	2.72	66.19	6.35
177	369	561	164.71	3.31	57.47	2.05
178	370	562	104.77	ND	63.78	0.07
179	371	563	141.99	17.38	79.39	1.38
180	372	564	112.24	20.83	63.32	0.76
181	373	565	169.81	42.18	86.72	0.61
182	374	566	168.14	ND	74.26	0.65
183	375	567	177.67	24.06	64.57	3.12
184	376	568	144.64	19.10	57.26	1.66
185	377	569	94.39	15.46	52.90	2.03
186	378	570	126.24	8.32	70.27	2.50
187	379	571	118.08	7.58	58.69	6.29
188	380	572	91.30	21.91	53.46	1.62
189	381	573	73.79	6.42	62.02	3.80
190	382	574	62.78	8.96	53.87	2.70
191	383	575	94.62	6.56	61.55	0.98
192	384	576	86.90	4.16	76.45	0.17
577	769	961	92.46	3.86	84.03	0.51
578	770	962	105.19	0.27	95.47	2.52
579	771	963	105.75	2.05	91.36	0.43
580	772	964	89.39	6.99	82.97	2.03
581	773	965	86.10	4.31	78.54	4.86
582	774	966	90.10	9.74	75.13	0.02
583	775	967	105.49	7.70	84.84	2.07
584	776	968	71.49	0.02	68.46	0.67
585	777	969	94.69	2.34	83.47	1.96
586	778	970	51.90	1.27	49.09	0.89
587	779	971	67.35	ND	67.86	3.80
588	780	972	72.86	ND	75.25	10.55
589	781	973	65.82	ND	62.84	5.76
590	782	974	64.47	ND	59.67	7.18
591	783	975	73.47	ND	73.00	4.56
592	784	976	85.24	ND	77.89	9.56
593	785	977	72.70	ND	70.63	7.59
594	786	978	79.46	ND	87.21	1.68
595	787	979	79.36	ND	73.70	10.85
596	788	980	77.07	ND	67.62	2.40
597	789	981	110.12	2.28	99.46	2.21

Antisense SEQ ID NO:	Sense SEQ ID NO:	Targeting Region SEQ ID NO:	% UNT SCN9A mRNA expression at 2 uM	STDEV	% UNT SCN9A mRNA expression at 0.5 uM	STDEV
598	790	982	93.05	2.87	88.72	0.04
599	791	983	87.34	2.23	90.55	4.50
600	792	984	79.18	3.35	79.73	2.65
601	793	985	68.06	0.37	75.22	1.26
602	794	986	69.15	1.12	73.42	6.50
603	795	987	53.41	1.31	56.03	1.13
604	796	988	56.66	2.51	61.28	3.59
605	797	989	58.58	0.04	61.53	0.47
606	798	990	63.45	0.92	68.47	1.59
607	799	991	78.69	1.94	83.13	1.32
608	800	992	90.63	1.15	90.29	2.97
609	801	993	66.76	3.08	72.37	0.09
610	802	994	88.88	2.27	86.28	0.35
611	803	995	73.02	3.98	77.79	0.03
612	804	996	75.95	0.58	76.05	2.13
613	805	997	71.56	0.69	84.87	2.85
614	806	998	71.05	3.46	79.09	2.17
615	807	999	60.62	3.21	63.79	4.57
616	808	1000	60.75	2.67	64.72	5.60
617	809	1001	71.05	5.18	64.70	8.00
618	810	1002	84.09	ND	71.16	5.03
619	811	1003	71.30	3.88	64.39	6.51
620	812	1004	76.67	2.79	71.60	5.32
621	813	1005	68.82	6.12	63.58	6.13
622	814	1006	77.82	3.12	71.47	4.97
623	815	1007	87.13	1.83	98.91	0.07
624	816	1008	87.22	1.89	88.45	3.72
625	817	1009	64.99	4.22	73.08	4.06
626	818	1010	79.77	4.04	91.95	0.48
627	819	1011	85.95	4.34	101.60	2.29
628	820	1012	89.00	1.49	97.59	0.80
629	821	1013	99.94	3.47	93.27	3.94
630	822	1014	80.09	0.87	89.56	2.49
631	823	1015	98.01	4.12	84.39	4.80
632	824	1016	84.89	4.28	86.46	3.15
633	825	1017	105.65	6.82	95.39	1.27
634	826	1018	99.48	3.64	92.33	0.34
635	827	1019	126.39	1.80	94.03	0.23
636	828	1020	123.75	4.93	85.65	11.77
637	829	1021	76.65	2.09	73.60	6.19
638	830	1022	61.01	0.90	105.53	ND

Antisense SEQ ID NO:	Sense SEQ ID NO:	Targeting Region SEQ ID NO:	% UNT SCN9A mRNA expression at 2 uM	STDEV	% UNT SCN9A mRNA expression at 0.5 uM	STDEV
639	831	1023	69.40	3.19	85.20	1.17
640	832	1024	93.89	0.50	97.24	1.61
641	833	1025	104.96	6.66	96.06	1.24
642	834	1026	94.76	3.43	99.36	7.73
643	835	1027	87.00	4.17	93.87	0.41
644	836	1028	94.30	5.23	95.28	6.77
645	837	1029	87.79	1.07	99.08	3.70
646	838	1030	124.33	28.51	89.23	6.39
647	839	1031	107.46	24.49	76.77	3.97
648	840	1032	127.66	32.41	83.70	9.45
649	841	1033	100.95	1.99	108.87	11.52
650	842	1034	77.74	4.09	85.23	6.90
651	843	1035	69.63	1.25	80.95	2.44
652	844	1036	80.12	0.52	88.88	0.34
653	845	1037	84.68	5.47	90.49	3.01
654	846	1038	81.17	2.54	91.83	9.67
655	847	1039	118.45	3.66	90.02	2.94
656	848	1040	126.80	5.13	90.48	3.85
657	849	1041	148.19	2.45	91.46	3.42
658	850	1042	144.20	6.84	80.26	0.81
659	851	1043	142.70	6.65	79.29	3.46
660	852	1044	116.40	7.99	81.51	3.38
661	853	1045	100.66	5.89	81.85	2.41
662	854	1046	112.13	3.29	80.45	1.32
663	855	1047	110.57	6.63	64.25	2.31
664	856	1048	71.66	1.10	79.78	5.37
665	857	1049	112.61	0.78	89.03	0.07
666	858	1050	128.70	6.17	75.78	0.10
667	859	1051	101.14	0.01	82.02	1.96
668	860	1052	101.47	2.55	82.91	1.03
669	861	1053	104.09	3.51	68.45	1.67
670	862	1054	79.31	7.17	86.54	1.28
671	863	1055	116.84	5.13	80.55	3.46
672	864	1056	107.43	3.22	83.11	2.65
673	865	1057	85.84	4.36	80.71	0.64
674	866	1058	116.38	8.51	80.54	0.13
675	867	1059	96.36	0.08	94.82	4.36
676	868	1060	86.00	2.53	101.63	7.24
677	869	1061	115.82	2.13	92.81	2.22
678	870	1062	129.61	2.23	88.71	2.07
679	871	1063	118.20	3.61	99.26	6.01

Antisense SEQ ID NO:	Sense SEQ ID NO:	Targeting Region SEQ ID NO:	% UNT SCN9A mRNA expression at 2 uM	STDEV	% UNT SCN9A mRNA expression at 0.5 uM	STDEV
680	872	1064	98.36	0.45	103.85	7.75
681	873	1065	126.28	ND	88.21	1.32
682	874	1066	84.03	5.83	89.72	2.58
683	875	1067	128.37	ND	93.00	0.72
684	876	1068	81.50	7.21	90.83	3.53
685	877	1069	62.09	1.95	74.06	2.81
686	878	1070	62.23	6.72	77.04	0.24
687	879	1071	60.84	1.29	75.82	0.08
688	880	1072	58.74	0.86	73.38	0.89
689	881	1073	70.34	4.92	86.12	0.29
690	882	1074	68.16	0.70	76.82	0.04
691	883	1075	71.48	ND	73.10	2.04
692	884	1076	97.75	ND	86.30	5.82
693	885	1077	116.90	ND	97.90	11.95
694	886	1078	81.04	ND	82.40	2.96
695	887	1079	76.17	ND	79.68	6.47
696	888	1080	86.54	ND	85.94	0.41
697	889	1081	72.90	ND	73.39	0.75
698	890	1082	79.80	ND	79.23	3.94
699	891	1083	80.35	ND	82.03	2.98
700	892	1084	86.43	ND	84.53	7.44
701	893	1085	85.38	0.92	85.15	3.69
702	894	1086	71.14	8.76	79.87	3.57
703	895	1087	91.42	4.11	96.30	0.84
704	896	1088	85.58	4.21	100.36	2.91
705	897	1089	72.46	2.18	83.78	3.74
706	898	1090	74.44	4.91	91.55	2.60
707	899	1091	70.26	ND	68.31	0.86
708	900	1092	64.67	2.72	74.73	2.37
709	901	1093	60.10	1.66	70.06	1.20
710	902	1094	77.68	2.71	79.86	1.70
711	903	1095	80.87	6.93	88.45	0.26
712	904	1096	71.77	2.82	77.15	2.49
713	905	1097	74.06	6.45	71.78	ND
714	906	1098	95.36	2.53	90.70	0.12
715	907	1099	69.22	3.55	73.53	1.78
716	908	1100	75.88	3.79	83.50	3.68
717	909	1101	88.08	3.45	88.50	1.56
718	910	1102	86.90	4.00	80.60	2.21
719	911	1103	75.91	1.03	78.74	0.67
720	912	1104	73.73	1.54	81.50	7.00

Antisense SEQ ID NO:	Sense SEQ ID NO:	Targeting Region SEQ ID NO:	% UNT SCN9A mRNA expression at 2 uM	STDEV	% UNT SCN9A mRNA expression at 0.5 uM	STDEV
721	913	1105	82.27	0.19	84.35	ND
722	914	1106	89.62	2.49	85.72	ND
723	915	1107	86.04	1.61	89.23	5.61
724	916	1108	73.72	3.32	78.28	4.53
725	917	1109	68.99	1.56	74.19	4.12
726	918	1110	94.34	3.29	84.91	11.00
727	919	1111	103.47	2.56	104.13	1.91
728	920	1112	100.31	7.79	97.07	0.88
729	921	1113	97.91	5.02	100.13	2.09
730	922	1114	84.68	3.18	93.19	2.70
731	923	1115	81.38	7.98	95.51	3.67
732	924	1116	81.85	2.73	93.07	2.15
733	925	1117	84.52	2.37	84.81	4.04
734	926	1118	93.08	3.32	88.28	1.77
735	927	1119	95.30	0.64	81.68	0.81
736	928	1120	106.28	6.53	92.75	3.38
737	929	1121	90.21	6.73	86.80	0.68
738	930	1122	101.80	3.65	92.58	0.34
739	931	1123	93.95	6.69	82.79	0.30
740	932	1124	93.12	8.07	83.03	1.30
741	933	1125	67.53	3.34	69.37	2.83
742	934	1126	83.72	6.03	86.19	1.20
743	935	1127	58.26	0.61	66.88	0.04
744	936	1128	72.73	3.24	78.72	2.13
745	937	1129	80.74	1.94	82.42	0.76
746	938	1130	70.86	0.42	75.36	1.10
747	939	1131	78.97	7.40	76.39	0.62
748	940	1132	93.04	2.05	82.68	4.05
749	941	1133	90.99	3.24	86.48	0.83
750	942	1134	72.37	0.89	76.62	0.76
751	943	1135	81.25	0.50	88.27	1.91
752	944	1136	84.21	1.77	85.36	2.46
753	945	1137	91.49	8.94	95.76	5.34
754	946	1138	94.32	12.14	93.06	2.32
755	947	1139	91.12	6.78	85.98	2.61
756	948	1140	86.35	8.68	83.18	5.45
757	949	1141	91.59	6.48	87.31	3.57
758	950	1142	90.14	1.65	86.73	2.14
759	951	1143	68.86	0.07	67.06	ND
760	952	1144	91.97	3.07	80.84	ND
761	953	1145	88.44	3.85	80.32	1.04

Antisense SEQ ID NO:	Sense SEQ ID NO:	Targeting Region SEQ ID NO:	% UNT SCN9A mRNA expression at 2 uM	STDEV	% UNT SCN9A mRNA expression at 0.5 uM	STDEV
762	954	1146	81.95	7.60	69.60	3.18
763	955	1147	86.70	5.34	82.01	2.86
764	956	1148	72.72	4.57	69.85	0.13
765	957	1149	77.28	2.85	70.08	1.55
766	958	1150	74.82	3.42	76.48	2.07
767	959	1151	83.21	0.43	77.47	1.13
768	960	1152	80.25	5.26	87.11	2.08

Example 2. IC50 Potency Determination of siRNA Molecules of the Disclosure.

For analysis of compound potency, G402 cells were actively transfected with SCN9A-targeting siRNA at concentrations of 1 fM to 100 nM. Expression of SCN9A mRNA was assessed at 72 hours using RT-qPCR as described above in Example 1, and the IC50 of each compound was calculated. Two siRNA molecules were tested in this assay: (1) an siRNA molecule having an antisense strand of SEQ ID NO: 688 and a sense strand of SEQ ID NO: 880, having an IC50 of 0.0334 nM, and (2) an siRNA molecule having an antisense strand of SEQ ID NO: 586 and a sense strand of SEQ ID NO: 778, having an IC50 of 0.0166 nM. The IC50 curves are shown in FIG. 1.

Example 3. Generating *SCN9A*-targeting siRNA Molecules

The small interfering RNA (siRNA) molecules of the disclosure can be synthesized by standard methods known in the art as further discussed below, e.g., by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc.

The siRNA agent can be prepared using solution-phase or solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligonucleotide including unnatural or modified nucleotides can be easily prepared. Specific examples of siRNA molecules, with the nucleotide sequence of the sense and antisense strand, as well as the sodium voltage-gated channel alpha subunit 9 (*SCN9A)* mRNA target sequence, are shown above in Table 1. It is appreciated that one of skill in the art could anneal the antisense (AS) strand to the corresponding sense (S) strand to yield a ds-siRNA molecule. Alternatively, one of skill in the art could derive a ss-siRNA molecule using antisense strand only.

Example 4. Optimizing *SCN9A*-targeting siRNA Molecules

It is contemplated that for siRNA agent disclosed herein, modifications to the siRNA may further optimize the molecule's efficacy or biophysical properties (e.g., increasing serum stability or circulating half-life, increasing thermal stability, enhancing transmembrane delivery, and/or targeting to a particular location or cell type). Such optimization could be achieved by systematically either adding or removing linked nucleosides to generate longer or shorter sequences. Further siRNA optimization could include the incorporation of, for example, one or more alternative nucleosides, alternative 2' sugar moieties, and/or

alternative internucleoside linkages. Further still, such optimized siRNA molecules may include the introduction of hydrophobic and/or stabilizing moieties at the 5' and/or 3' ends.

siRNA Optimization with Alternative Nucleosides

Optimization of the siRNA molecules of the disclosure may include one or more of the following nucleoside modifications: 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (-C=C-CH3) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine, and/or 3-deazaguanine and 3-deazaadenine. The siRNA molecules may also include nucleobases in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine, and/or 2-pyridone. Further optimization of the siRNA molecules of the disclosure may include nucleobases disclosed in US 3,687,808; Kroschwitz, J.I., ed. *The Concise Encyclopedia of Polymer Science and Engineering,* New York, John Wiley & Sons, 1990, pp. 858-859; Englisch et al., *Angewandte Chemie,* International Edition 30:613, 1991; and Sanghvi, Y.S., Chapter 16, *Antisense Research and Applications,* CRC Press, Gait, M.J. ed., 1993, pp. 289-302.

siRNA Optimization with Alternative Sugar Modifications

Optimization of the siRNA molecules of the disclosure may include one or more of the following 2' sugar modifications: 2'-O-methyl (2'-O-Me), 2'-methoxyethoxy (2'-O-$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE), 2'-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, and/or 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylamino-ethoxy-ethyl or 2'-DMAEOE), i.e., 2'-O-$CH_2OCH_2N(CH_3)_2$. Other possible 2'-modifications that can optimize the siRNA molecules of the disclosure include all possible orientations of OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C1 to C10 alkyl or C2 to C10 alkenyl and alkynyl. Other potential sugar substituent groups include, e.g., aminopropoxy (-$OCH_2CH_2CH_2NH_2$), allyl (-CH_2-CH=CH_2), -O-allyl (-O-CH_2-CH=CH_2) and fluoro (F). 2'-sugar substituent groups may be in the arabino (up) position or ribo (down) position. In some embodiments, the 2'-arabino modification is 2'-F. Similar modifications may also be made at other positions on the siRNA molecule, particularly the 3' position of the sugar on the 3' terminal nucleoside or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar.

siRNA Optimization with Alternative Internucleoside Linkages

Optimization of the siRNA molecules of the disclosure may include one or more of the following internucleoside modifications: phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-

alkylene phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, selenophosphates, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein one or more internucleotide linkages is a 3' to 3', 5' to 5' or 2' to 2' linkage.

siRNA Optimization with Hydrophobic Moieties

Optimization of the siRNA molecules of the disclosure may include hydrophobic moieties covalently attached to the 5' end or the 3' end. Non-limiting examples of hydrophobic moieties suitable for use with the siRNA molecules of the disclosure may include cholesterol, vitamin D, tocopherol, phosphatidylcholine (PC), docohexaenoic acid, docosanoic acid, PC-docosanoic acid, eicosapentaenoic acid, lithocholic acid or any combination of the aforementioned hydrophobic moieties with PC.

siRNA Optimization with Stabilizing Moieties

Optimization of the siRNA molecules of the disclosure may include a 5'-phosphorous stabilizing moiety that protects the siRNA molecules from degradation. A 5′-phosphorus stabilizing moiety replaces the 5′-phosphate to prevent hydrolysis of the phosphate. Hydrolysis of the 5′-phosphate prevents binding to RISC, a necessary step in gene silencing. Any replacement for phosphate that does not impede binding to RISC is contemplated in this disclosure. In some embodiments, the replacement for the 5′-phosphate is also stable to *in vivo* hydrolysis. Each siRNA strand may independently and optionally employ any suitable 5′-phosphorus stabilizing moiety. Non-limiting examples of 5' stabilizing moieties suitable for use with the siRNA molecules of the disclosure may include those demonstrated by Formulas IX-XVI above.

siRNA Optimization with Branched siRNA

Optimization of the siRNA molecules of the disclosure may include the incorporation of branching patterns, such as, for example, di-branched, tri-branched, or tetra-branched siRNAs connected by way of a linker. Each main branch may be further branched to allow for 2, 3, 4, 5, 6, 7, or 8 separate RNA single- or double-strands. The branch points on the linker may stem from the same atom, or separate atoms along the linker. Some exemplary embodiments are listed in Table 2, above.

The siRNA composition of the disclosure may be optimized to be in the form of: di-branched siRNA molecules, as represented by any one of Formulas XVII-XIX; tri-branched siRNA molecules, as represented by any one of Formulas XX-XXIII; and/or tetra-branched siRNA molecules, as represented by any one of Formulas XXIV-XXVIII, wherein each RNA, independently, is an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety (e.g., phosphoroamidite, tosylated solketal, 1,3-diaminopropanol, pentaerythritol, or any one of the branch point moieties described in US 10,478,503).

Example 5. Preparation and Administrating *SCN9A*-targeting siRNA Molecules

The siRNA molecules in the present disclosure may be formulated into a pharmaceutical composition for administration to a subject in a biologically compatible form suitable for administration *in vivo*. For example, the siRNA molecules of the disclosure may be administered in a suitable diluent, carrier, or excipient, and may further contain a preservative, e.g., to prevent the growth of microorganisms.

Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington, J.P. *The Science and Practice of Pharmacy*, Easton, PA. Mack Publishers, 2012, 22nd ed. and in The United States Pharmacopeial Convention, *The National Formulary*, United States Pharmacopeial, 2015, USP 38 NF 33).

The method of the disclosure contemplates any route of administration to the subject's CNS or neurons that is tolerated by the siRNA compositions of the disclosure. Non-limiting examples of siRNA injections into the CNS or neurons include intrathecal injection, intra-cisterna magna injection by catheterization, or direct injection into a specific nerve or ganglion (ganglia) (e.g., trigeminal or dorsal root ganglia). A physician having ordinary skill in the art can readily determine an effective route of administration.

Example 6. Methods for the Treatment of Pain Using *SCN9A*-targeting siRNA Molecules

A subject in need of treatment for chronic, persistent, or acute symptoms of pain, including pain that is nociceptive or neuropathic in nature, is treated with a dosage of the siRNA molecule or siRNA composition of the disclosure, formulated as a salt, at frequency determined by a practitioner. A physician having ordinary skill in the art can readily determine an effective amount of the siRNA molecule for administration to a mammalian subject (e.g., a human) in need thereof. For example, a physician could start prescribing doses of one of the siRNA molecules of the disclosure at levels lower than that required to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. Alternatively, a physician may begin a treatment regimen by administering one of the siRNA molecules of the disclosure at a high dose and subsequently administer progressively lower doses until a therapeutic effect is achieved (e.g., a reduction in expression of *SCN9A* mRNA). In general, a suitable daily dose of one of one of the siRNA molecules of the disclosure will be an amount which is the lowest dose effective to produce a therapeutic effect. The ss- or ds-siRNA molecules of the disclosure may be administered by injection, e.g., intrathecally, directly into a specific nerve or ganglion (ganglia) (e.g., trigeminal or dorsal root ganglia), or by intra-cisterna magna injection via catheterization. A daily dose of a therapeutic composition of one of the siRNA molecules of the disclosure may be administered as a single dose or as two, three, four, five, six or more doses administered separately at appropriate intervals throughout the day, week, month, or year, optionally, in unit dosage forms. While it is possible for any of the siRNA molecules of the disclosure to be administered alone, it may also be administered as a pharmaceutical formulation in combination with excipients, carriers, and optionally, additional therapeutic agents. Dosage and frequency are determined based on the subject's height, weight, age, sex, and other disorders.

The siRNA molecule(s) of the disclosure is selected by the practitioner for compatibility with the subject. Single- or double-stranded siRNA molecules (e.g., non-branched siRNA, di-branched siRNA, tri-branched siRNA, tetra-branched siRNA) are available for selection. The siRNA molecule chosen has an antisense strand and may have a sense strand with a sequence and RNA modifications (e.g., natural and non-natural internucleoside linkages, modified sugars, 5′-phosphorus stabilizing moieties, hydrophobic moieties, and/or branching sructures) best suited to the patient.

The siRNA molecule is delivered by the route best suited the patient (e.g., intrathecally, intracerebroventricularly, intrastriatally, by direct injection into a specific nerve or ganglion (ganglia) such as

trigeminal or dorsal root ganglia, or by intra-cisterna magna injection via catheterization) and condition at a rate tolerable to the patient until the subject has reached a maximum tolerated dose, or until the symptoms of pain are ameliorated satisfactorily.

Example 7. Methods for the Treatment of Pain Associated with a Pain Disorder

The small interfering RNA (siRNA) molecules of the disclosure can be used for the treatment of pain disorders, such as those characterized as erythromelalgia (e.g., episodes of pain, redness, and swelling, typically at the extremities) and/or those induced by gain-of-function *SCN9A* gene variants. Non-limiting examples of clinical diagnoses suitable for treatment with the siRNA molecules of the disclosure include Gerhardt disease, Mitchell disease, or Weir-Mitchell disease.

A subject with a condition of erythromelalgia is treated with a dosage of the siRNA molecule or composition of the disclosure, formulated as a salt, at frequency determined by a practitioner. A physician having ordinary skill in the art can readily determine an effective amount of the siRNA molecule for administration to a mammalian subject (e.g., a human) in need thereof. For example, a physician could start prescribing doses of one of the siRNA molecules of the disclosure at levels lower than that required to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. Alternatively, a physician may begin a treatment regimen by administering one of the siRNA molecules of the disclosure at a high dose and subsequently administer progressively lower doses until a therapeutic effect is achieved (e.g., a reduction in expression of *SCN9A* mRNA). In general, a suitable daily dose of one of one of the siRNA molecules of the disclosure will be an amount which is the lowest dose effective to produce a therapeutic effect. The ss- or ds-interfering RNA molecules of the disclosure may be administered by injection, e.g., intrathecally, by direct injection into a specific nerve or ganglion (ganglia) (e.g., trigeminal or dorsal root ganglia) or by intra-cisterna magna injection via catheterization. A daily dose of a therapeutic composition of one of the siRNA molecules of the disclosure may be administered as a single dose or as two, three, four, five, six or more doses administered separately at appropriate intervals throughout the day, week, month, or year, optionally, in unit dosage forms. While it is possible for any of the siRNA molecules of the disclosure to be administered alone, it may also be administered as a pharmaceutical formulation in combination with excipients, carriers, and optionally, additional therapeutic agents. Dosage and frequency are determined based on the subject's height, weight, age, sex, and other disorders.

The siRNA molecule(s) of the disclosure is selected by the practitioner for compatibility with the subject. Single- or double-stranded siRNA molecules (e.g., non-branched siRNA, di-branched siRNA, tri-branched siRNA, tetra-branched siRNA) are available for selection. The siRNA molecule chosen has an antisense strand and may have a sense strand with a sequence and RNA modifications (e.g., natural and non-natural internucleoside linkages, modified sugars, 5′-phosphorus stabilizing moieties, hydrophobic moieties, and/or branching sructures) best suited to the patient.

The siRNA molecule is delivered by the route best suited the patient (e.g., intrathecally, by direct injection into a specific nerve or ganglion (ganglia), or by intra-cisterna magna injection via catheterization) and condition at a rate tolerable to the patient until the subject has reached a maximum tolerated dose, or until the symptoms of pain are ameliorated satisfactorily.

Other Embodiments

All publications, patents, and patent applications mentioned in this specification are incorporated herein by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the invention that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the claims.

Other embodiments are within the claims.

Claims

1. A small interfering RNA (siRNA) molecule comprising an antisense strand and sense strand having complementarity to the antisense strand, wherein the antisense strand has complementarity sufficient to hybridize to a region within a sodium voltage-gated channel alpha subunit 9 (*SCN9A*) mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

2. The siRNA molecule of claim 1, wherein the antisense strand has at least 70% complementarity to a region of 19, 20, 21, or more contiguous nucleobases within the *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152, optionally wherein the antisense strand has at least 70% complementarity to the *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

3. The siRNA molecule of claim 2, wherein the antisense strand has at least 75% complementarity to the region within the *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152, optionally wherein the antisense strand has at least 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementarity to the region within the *SCN9A* mRNA transcript having the nucleic acid sequence of any one of SEQ ID Nos: 385-576 and 961-1152.

4. The siRNA molecule of any one of claims 1-3, wherein the antisense strand comprises at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or 30 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

5. The siRNA molecule of claim 4, wherein the antisense strand comprises from 10 to 30 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

6. The siRNA molecule of claim 5, wherein the antisense strand comprises from 12 to 30 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

7. The siRNA molecule of claim 6, wherein the antisense strand comprises from 15 to 30 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

8. The siRNA molecule of claim 7, wherein the antisense strand comprises from 18 to 30 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

9. The siRNA molecule of claim 8, wherein the antisense strand comprises from 18 to 25 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID Nos: 385-576 and 961-1152.

10. The siRNA molecule of claim 9, wherein the antisense strand comprises from 18 to 21 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

11. The siRNA molecule of claim 10, wherein the antisense strand comprises 21 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

12. The siRNA molecule of any one of claims 1-11, wherein the antisense strand comprises 9 or fewer nucleotide mismatches relative to the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152, optionally wherein the antisense strand comprises 8 or fewer, 7 or fewer, 6 or fewer, 5 or fewer, 4 or fewer, 3 or fewer, 2 or fewer, or only 1 mismatch relative to the region of the *SCN9A* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 385-576 and 961-1152.

13. The siRNA molecule of any one of claims 1-12, wherein the antisense strand has a nucleic acid sequence that is at least 85% identical to the nucleic acid sequence of any one of SEQ ID NOs: 1-192 and 577-768.

14. The siRNA molecule of claim 13, wherein the antisense strand has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of any one of SEQ ID NOs: 1-192 and 577-768.

15. The siRNA molecule of claim 14, wherein the antisense strand has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NOs: 1-192 and 577-768, optionally wherein the antisense strand has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of any one of SEQ ID NOs: 1-192 and 577-768.

16. The siRNA molecule of claim 15, wherein the antisense strand has the nucleic acid sequence of any one of SEQ ID NOs: 1-192 and 577-768.

17. The siRNA molecule of any one of claims 1-16, wherein the sense strand has a nucleic acid sequence that is at least 85% identical to the nucleic acid sequence of any one of SEQ ID NOs: 193-384 and 769-960.

18. The siRNA molecule of claim 17, wherein the sense strand has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of any one of SEQ ID NOs: 193-384 and 769-960.

19. The siRNA molecule of claim 18, wherein the sense strand has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NOs: 193-384 and 769-960, optionally wherein the sense strand has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of any one of SEQ ID NOs: 193-384 and 769-960.

20. The siRNA molecule of claim 19, wherein the sense strand has the nucleic acid sequence of any one of SEQ ID NOs: 193-384 and 769-960.

21. The siRNA molecule of any one of claims 1-20, wherein the antisense strand comprises a structure represented by Formula I, wherein Formula I is, in the 5'-to-3' direction:

$$A\text{-}B\text{-}(A')_j\text{-}C\text{-}P^2\text{-}D\text{-}P^1\text{-}(C'\text{-}P^1)_k\text{-}C'$$

Formula I;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$;
each A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$;
B is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2$;
each C is a 2'-O-methyl (2'-O-Me) ribonucleoside;
each C', independently, is a 2'-O-Me ribonucleoside or a 2'-fluoro (2'-F) ribonucleoside;
each D is a 2'-F ribonucleoside;
each P^1 is a phosphorothioate internucleoside linkage;
each P^2 is a phosphodiester internucleoside linkage;
j is an integer from 1 to 7; and
k is an integer from 1 to 7.

22. The siRNA molecule of claim 21, wherein the antisense strand comprises a structure represented by Formula A1, wherein Formula A1 is, in the 5'-to-3' direction:

A-S-B-S-A-O-B-O-B-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-A-S-A-S-B-S-A

Formula A1;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

23. The siRNA molecule of any one of claims 1-20, wherein the antisense strand comprises a structure represented by Formula II, wherein Formula II is, in the 5'-to-3' direction:

$$A\text{-}B\text{-}(A')_j\text{-}C\text{-}P^2\text{-}D\text{-}P^1\text{-}(C\text{-}P^1)_k\text{-}C'$$

Formula II;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$;
each A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$;
B is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2$;
each C is a 2'-O-methyl (2'-O-Me) ribonucleoside;
each C', independently, is a 2'-O-Me ribonucleoside or a 2'-fluoro (2'-F) ribonucleoside;
each D is a 2'-F ribonucleoside;
each P^1 is a phosphorothioate internucleoside linkage;
each P^2 is a phosphodiester internucleoside linkage;
j is an integer from 1 to 7; and
k is an integer from 1 to 7.

24. The siRNA molecule of claim 23, wherein the antisense strand comprises a structure represented by Formula A2, wherein Formula A2 is, in the 5'-to-3' direction:

A-S-B-S-A-O-B-O-B-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-A-S-A-S-A-S-A

Formula A2;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

25. The siRNA molecule of any one of claims 1-24, wherein the sense strand comprises a structure represented by Formula III, wherein Formula III is, in the 5'-to-3' direction:

$$E\text{-}(A')_m\text{-}F$$

Formula III;

wherein E is represented by the formula $(C\text{-}P^1)_2$;
F is represented by the formula $(C\text{-}P^2)_3\text{-}D\text{-}P^1\text{-}C\text{-}P^1\text{-}C$, $(C\text{-}P^2)_3\text{-}D\text{-}P^2\text{-}C\text{-}P^2\text{-}C$, $(C\text{-}P^2)_3\text{-}D\text{-}P^1\text{-}C\text{-}P^1\text{-}D$, or $(C\text{-}P^2)_3\text{-}D\text{-}P^2\text{-}C\text{-}P^2\text{-}D$;
A', C, D, P^1, and P^2 are as defined in Formula II; and
m is an integer from 1 to 7.

26. The siRNA molecule of claim 25, wherein the sense strand comprises a structure represented by Formula S1, wherein Formula S1 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A-O-A-O-B-S-A-S-A

Formula S1;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

27. The siRNA molecule of claim 25, wherein the sense strand comprises a structure represented by Formula S2, wherein Formula S2 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A-O-A-O-B-O-A-O-A

Formula S2;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

28. The siRNA molecule of claim 25, wherein the sense strand comprises a structure represented by Formula S3, wherein Formula S3 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A-O-A-O-B-S-A-S-B

Formula S3;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

29. The siRNA molecule of claim 25, wherein the sense strand comprises a structure represented by Formula S4, wherein Formula S4 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A-O-A-O-B-O-A-O-B

Formula S4;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

30. The siRNA molecule of any one of claims 1-20 and 25-29, wherein the antisense strand comprises a structure represented by Formula IV, wherein Formula IV is, in the 5'-to-3' direction:

$A\text{-}(A')_j\text{-}C\text{-}P^2\text{-}B\text{-}(C\text{-}P^1)_k\text{-}C'$

Formula IV;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$;
each A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$;
B is represented by the formula $D\text{-}P^1\text{-}C\text{-}P^1\text{-}D\text{-}P^1$;

each C is a 2'-O-Me ribonucleoside;
each C', independently, is a 2'-O-Me ribonucleoside or a 2'-F ribonucleoside;
each D is a 2'-F ribonucleoside;
each P^1 is a phosphorothioate internucleoside linkage;
each P^2 is a phosphodiester internucleoside linkage;
j is an integer from 1 to 7; and
k is an integer from 1 to 7.

31. The siRNA molecule of claim 30, wherein the antisense strand comprises a structure represented by Formula A3, wherein Formula A3 is, in the 5'-to-3' direction:

A-S-B-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-B-S-A-S-A-S-A
Formula A3;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

32. The siRNA molecule of any one of claims 1-24, 30, and 31, wherein the sense strand comprises a structure represented by Formula V, wherein Formula V is, in the 5'-to-3' direction:

$E\text{-}(A')_m\text{-}C\text{-}P^2\text{-}F$
Formula V;

wherein E is represented by the formula $(C\text{-}P^1)_2$;
F is represented by the formula $D\text{-}P^1\text{-}C\text{-}P^1\text{-}C$, $D\text{-}P^2\text{-}C\text{-}P^2\text{-}C$, $D\text{-}P^1\text{-}C\text{-}P^1\text{-}D$, or $D\text{-}P^2\text{-}C\text{-}P^2\text{-}D$;
A', C, D, P^1 and P^2 are as defined in Formula IV; and
m is an integer from 1 to 7.

33. The siRNA molecule of claim 32, wherein the sense strand comprises a structure represented by Formula S5, wherein Formula S5 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-A
Formula S5;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

34. The siRNA molecule of claim 32, wherein the sense strand comprises a structure represented by Formula S6, wherein Formula S6 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A
Formula S6;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

35. The siRNA molecule of claim 32, wherein the sense strand comprises a structure represented by Formula S7, wherein Formula S7 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-B

Formula S7;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

36. The siRNA molecule of claim 32, wherein the sense strand comprises a structure represented by Formula S8, wherein Formula S8 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B

Formula S8;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

37. The siRNA molecule of any one of claims 1-20, 25-29, and 32-36, wherein the antisense strand comprises a structure represented by Formula VI, wherein Formula VI is, in the 5'-to-3' direction:

$A\text{-}B_j\text{-}E\text{-}B_k\text{-}E\text{-}F\text{-}G_l\text{-}D\text{-}P^1\text{-}C'$

Formula VI;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$;
each B is represented by the formula $C\text{-}P^2$;
each C is a 2'-O-Me ribonucleoside;
each C', independently, is a 2'-O-Me ribonucleoside or a 2'-F ribonucleoside;
each D is a 2'-F ribonucleoside;
each E is represented by the formula $D\text{-}P^2\text{-}C\text{-}P^2$;
F is represented by the formula $D\text{-}P^1\text{-}C\text{-}P^1$;
each G is represented by the formula $C\text{-}P^1$;
each P^1 is a phosphorothioate internucleoside linkage;
each P^2 is a phosphodiester internucleoside linkage;
j is an integer from 1 to 7;
k is an integer from 1 to 7; and
l is an integer from 1 to 7.

38. The siRNA molecule of claim 37, wherein the antisense strand comprises a structure represented by Formula A4, wherein Formula A4 is, in the 5'-to-3' direction:

A-S-B-S-A-O-A-O-A-O-B-O-A-O-A-O-A-O-A-O-A-O-A-O-A-O-B-O-A-O-B-S-A-S-A-S-A-S-B-S-A

Formula A4;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

39. The siRNA molecule of any one of claims 1-24, 30, 31, 37, and 38, wherein the sense strand comprises a structure represented by Formula VII, wherein Formula VII is, in the 5'-to-3' direction:

$H\text{-}B_m\text{-}I_n\text{-}A'\text{-}B_o\text{-}H\text{-}C$

Formula VII;

wherein A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$;
each H is represented by the formula $(C\text{-}P^1)_2$;
each I is represented by the formula $(D\text{-}P^2)$;
B, C, D, P^1 and P^2 are as defined in Formula VI;
m is an integer from 1 to 7;
n is an integer from 1 to 7; and
o is an integer from 1 to 7.

40. The siRNA molecule of claim 39, wherein the sense strand comprises a structure represented by Formula S9, wherein Formula S9 is, in the 5'-to-3' direction:

A-S-A-S-A-O-A-O-A-O-B-O-B-O-B-O-A-O-B-O-A-O-A-O-A-O-A-S-A-S-A

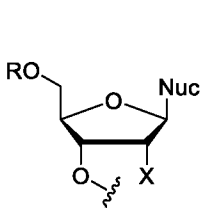
Formula S9;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

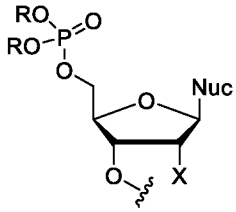
41. The siRNA molecule of any one of claims 1-40, wherein the antisense strand further comprises a 5' phosphorus stabilizing moiety at the 5' end of the antisense strand.

42. The siRNA molecule of any one of claims 1-41, wherein the sense strand further comprises a 5' phosphorus stabilizing moiety at the 5' end of the sense strand.

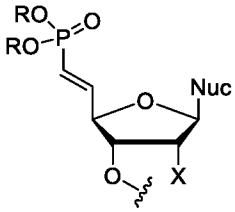
43. The siRNA molecule of claim 41 or 42, wherein each 5' phosphorus stabilizing moiety is, independently, represented by any one of Formulas IX-XVI:



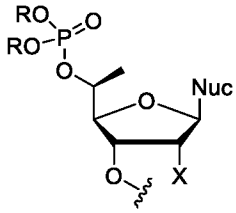
Formula IX



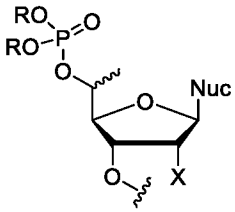
Formula X



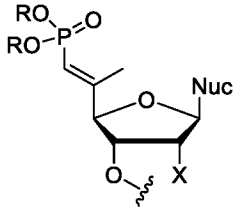
Formula XI



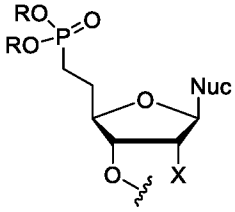
Formula XII



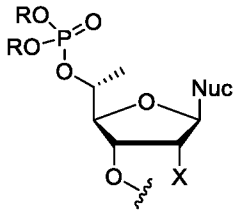
Formula XIII



Formula XIV



Formula XV



Formula XVI

wherein Nuc represents a nucleobase selected from the group consisting of adenine, uracil, guanine, thymine, and cytosine, and R represents an optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, phenyl, benzyl, hydroxy, or hydrogen.

44. The siRNA molecule of claim 43, wherein the nucleobase is an adenine, uracil, guanine, thymine, or cytosine.

45. The siRNA molecule of any one of claims 41-44, wherein the 5' phosphorus stabilizing moiety is (E)-vinylphosphonate represented by Formula XI.

46. The siRNA molecule of any one of claims 1-45, wherein the siRNA molecule further comprises a hydrophobic moiety at the 5' or the 3' end of the siRNA molecule.

47. The siRNA molecule of claim 46, wherein the hydrophobic moiety is selected from a group consisting of cholesterol, vitamin D, or tocopherol.

48. The siRNA molecule of any one of claims 1-47, wherein the length of the sense strand is between 10 and 30 nucleotides.

49. The siRNA molecule of claim 48, wherein the length of the sense strand is between 10 and 25 nucleotides.

50. The siRNA molecule of claim 49, wherein the length of the sense strand is between 12 and 25 nucleotides.

51. The siRNA molecule of claim 50, wherein the length of the sense strand is between 12 and 20 nucleotides.

52. The siRNA molecule of claim 51, wherein the length of the sense strand is between 12 and 19 nucleotides.

53. The siRNA molecule of claim 52, wherein the length of the sense strand is 15 nucleotides.

54. The siRNA molecule of claim 52, wherein the length of the sense strand is 16 nucleotides.

55. The siRNA molecule of claim 52, wherein the length of the sense strand is 18 nucleotides.

56. The siRNA molecule of any one of claims 1-55, wherein the length of the antisense strand is between 10 and 30 nucleotides.

57. The siRNA molecule of claim 56, wherein the length of the antisense strand is between 12 and 30 nucleotides.

58. The siRNA molecule of claim 57, wherein the length of the antisense strand is between 15 and 30 nucleotides.

59. The siRNA molecule of claim 58, wherein the length of the antisense strand is between 18 and 30 nucleotides.

60. The siRNA molecule of claim 59, wherein the length of the antisense strand is between 18 and 25 nucleotides.

61. The siRNA molecule of claim 60, wherein the length of the antisense strand is between 18 and 21 nucleotides.

62. The siRNA molecule of claim 61, wherein the length of the antisense strand is 18 nucleotides.

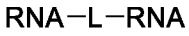
63. The siRNA molecule of claim 61, wherein the length of the antisense strand is 20 nucleotides.

64. The siRNA molecule of claim 61, wherein the length of the antisense strand is 21 nucleotides.

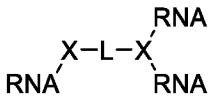
65. The siRNA molecule of any one of claims 1-64, wherein the siRNA molecule is a branched siRNA molecule.

66. The siRNA molecule of claim 65, wherein the branched siRNA molecule is di-branched, tri-branched, or tetra-branched.

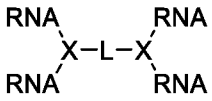
67. The siRNA molecule of claim 66, wherein the siRNA molecule is a di-branched siRNA molecule, optionally wherein the di-branched siRNA molecule is represented by any one of Formulas XVII-XIX:



Formula XVII;



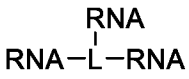
Formula XVIII;



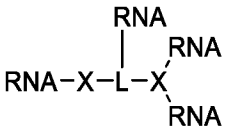
Formula XIX;

wherein each RNA is, independently, an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

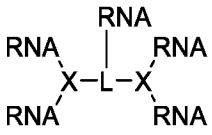
68. The siRNA molecule of claim 66, wherein the siRNA molecule is a tri-branched siRNA molecule, optionally wherein the tri-branched siRNA molecule is represented by any one of Formulas XX-XXIII:



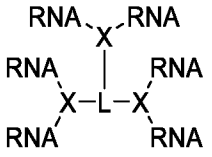
Formula XX;



Formula XXI;



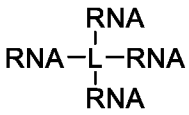
Formula XXII;



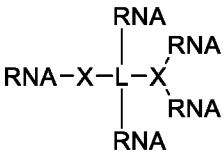
Formula XXIII;

wherein each RNA is, independently, an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

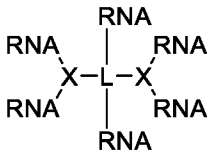
69. The siRNA molecule of claim 66, wherein the siRNA molecule is a tetra-branched siRNA molecule, optionally wherein the tetra-branched siRNA molecule is represented by any one of Formulas XXIV-XXVIII:



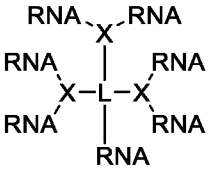
Formula XXIV;



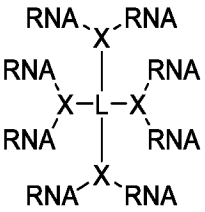
Formula XXV;



Formula XXVI;



Formula XXVII;



Formula XXVIII;

wherein each RNA is, independently, an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

70. The siRNA molecule of any one of claims 67-69, wherein the linker is selected from a group consisting of one or more contiguous subunits of an ethylene glycol, alkyl, carbohydrate, block copolymer, peptide, RNA, and DNA.

71. The siRNA molecule of claim 70, wherein the one or more contiguous subunits is 2 to 20 contiguous subunits.

72. A pharmaceutical composition comprising the siRNA molecule of any one of claims 1-71 and a pharmaceutically acceptable excipient, carrier, or diluent.

73. A method of delivering an siRNA molecule to the central nervous system (CNS) or neurons of a subject experiencing pain or diagnosed as having a pain disorder, the method comprising administering a therapeutically effective amount of the siRNA molecule of any one of claims 1-71 or the pharmaceutical composition of claim 72 to the subject.

74. A method of treating pain or a pain disorder in a subject in need thereof, the method comprising administering a therapeutically effective amount of the siRNA molecule of any one of claims 1-71 or the pharmaceutical composition of claim 72 to the subject.

75. The method of claim 73 or 74, wherein the pain is neuropathic pain.

76. The method of claim 73 or 74, wherein the pain is nociceptive pain.

77. The method of claim 73 or 74, wherein the pain is post-operative pain.

78. The method of claim 73 or 74, wherein the pain is persistent pain.

79. The method of claim 73 or 74, wherein the pain is inflammatory pain.

80. The method of claim 73 or 74, wherein the pain disorder is Gerhardt disease, Mitchell disease, or Weir-Mitchell disease.

81. The method of claim 73 or 74, wherein the subject has been diagnosed with erythromelalgia.

82. A method of reducing *SCN9A* expression in a subject in need thereof, the method comprising administering a therapeutically effective amount of the siRNA molecule of any one of claims 1-71 or the pharmaceutical composition of claim 72 to the CNS of the subject.

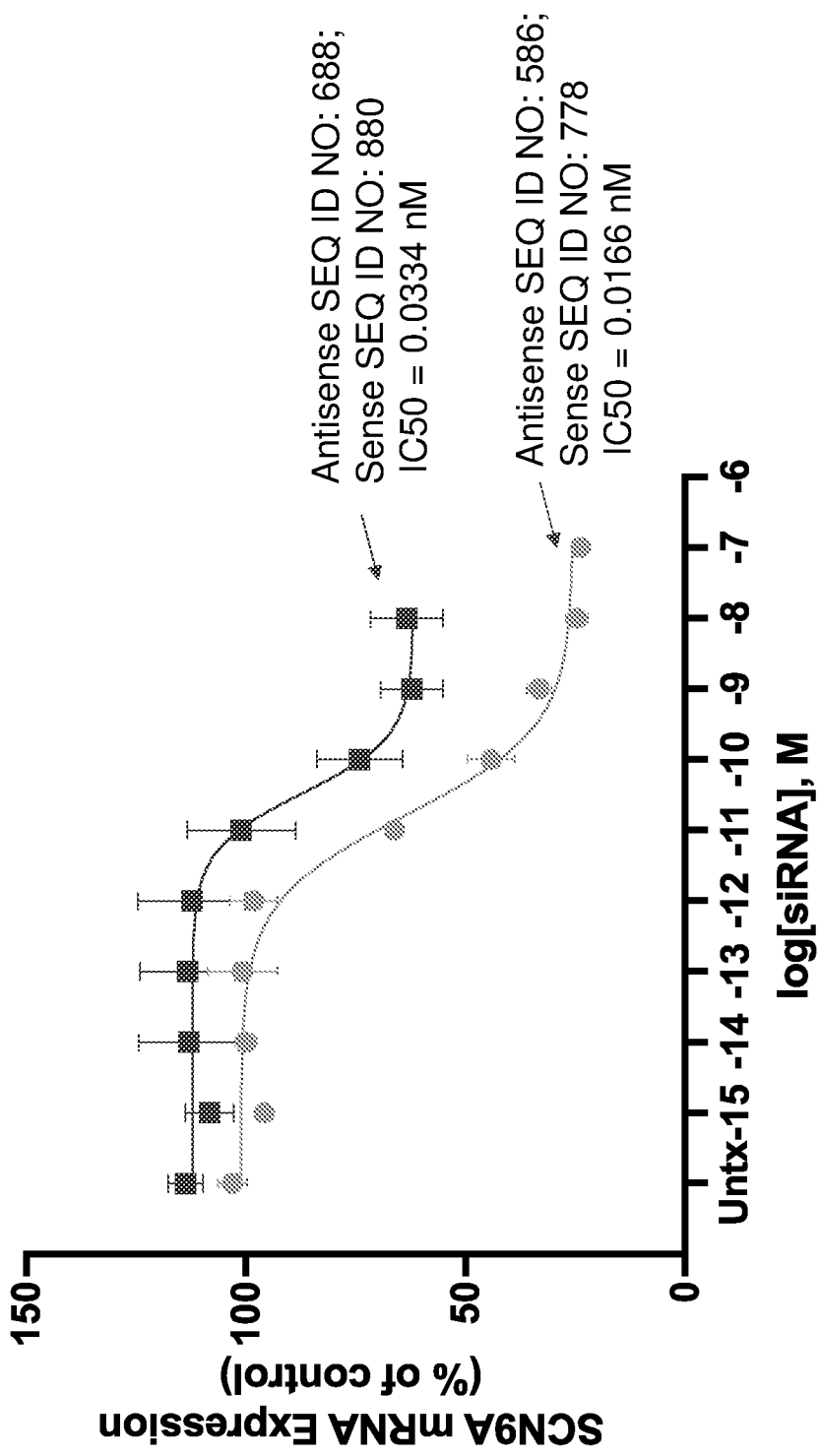
83. The method of claim 82, wherein, upon administration of the siRNA molecule or pharmaceutical composition to the subject, the subject exhibits selective reduction in *SCN9A* expression over expression of one or more other voltage-gated sodium ion channel genes.

84. The method of any one of claims 73-83 wherein the siRNA molecule or the pharmaceutical composition is administered to the subject by way of intrathecal injection or by direct injection into a specific nerve or ganglion.

85. The method of any one of claims 73-84, wherein the subject is a human.

86. A kit comprising the siRNA molecule of any one of claims 1-71, or the pharmaceutical composition of claim 72, and a package insert, wherein the package insert instructs a user of the kit to perform the method of any one of claims 73-85.

FIG. 1


SCN9A mRNA Expression
(% of control)
150
100
50
0
Untx-15 -14 -13 -12 -11 -10 -9 -8 -7 -6
log[siRNA], M
Antisense SEQ ID NO: 688;
Sense SEQ ID NO: 880
IC50 = 0.0334 nM
Antisense SEQ ID NO: 586;
Sense SEQ ID NO: 778
IC50 = 0.0166 nM